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GC/TCD Analysis of A Natural Gas Sample on A Single HP-PLOT Q Column

Application Note
228-387

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Key Word

GC/TCD
Natural gas
PLOT Q column

Abstract

An Agilent 6890 series gas chromatograph (GC) equipped with a TCD (thermal conductivity detector) was used with and HP-PLOT Q capillary column for the analysis of a natural gas sample. Over 70 sequential runs showed good separation for a wide variety of analytes with good method reproducibility.

Introduction

Natural gas is an important energy source and widely used as a starting material for many chemical processes. It contains mainly methane and different levels of other hydrocarbons and fixed gases such as nitrogen, helium, and carbon dioxide. Hydrocarbons heavier than C7 are usually present at ppm levels. Hydrogen sulfide and other sulfur compounds may be present, either naturally or as added odorants. Additional components may include polar compounds, such as low levels of water, and small amounts of methanol and/or glycol which may have been added for processing purposes [1,2]. Natural gases from different sources usually have the same composition but different concentration levels.

In GC analyses, the variety of components in natural gas requires the separation of both polar/non-polar compounds. Multi-dimensional GC is often required since no single column can separate this wide variety of natural gas constituents. Nor can a single detector detect all compounds satisfactorily. Specifically, the separation of fixed gases and water from hydrocarbons is very difficult to obtain on most wall-coated open-tubular (WCOT) columns; and TCD has a limited sensitivity for trace level compounds and odorant compounds.

Multi-dimensional GC coupled with switching valves requires the use of several different types of PLOT columns [2-4]. The HP-PLOT Al_2O_3 column [3-4] is often used for hydrocarbon separations and the determination of BTUs. The HP-PLOT MoleSieve column is used for the separation of fixed gases such as oxygen, nitrogen and helium, and even argon, [3-4] from methane. And the separation of polar and active compound—such as water, CO_2, and odorants—is obtained using a porous polymer PLOT column, mostly Q type [3].

All three columns are connected by one or more multiple port valves and the complete separation is obtained by time switching eluents to each column and detector. Backflushing hydrocarbon compounds heavier than C_7 is necessary in most cases. Clearly the column interchange and connection as well as the valve and time switching make this a difficult technique to use for routine analyses.

The ideal approach would be one-dimensional GC. Natural gas analysis using two parallel connected PLOT columns has been done with the successful separation of hydrocarbons and oxygen and nitrogen [4]. However, this method is limited because the separation of polar compounds from hydrocarbons cannot be

achieved on these two kinds of PLOT columns. Additionally, water, CO_2, and odorants deactivate Al_2O_3 and molesieve PLOT column coatings. Therefore, these interactions cause shifting of retention times thereby affecting the repeatability, reliability, and accuracy of the natural gas analysis.

Porous polymer, Q-type PLOT columns combine the separation features of the Al_2O_3 PLOT and molesieve PLOT columns when separating features of the Al_2O_3 PLOT and molesieve PLOT columns when separating alkanes and fixed gases. The PLOT-Q coating overcomes the reproducibility problem caused by polar compounds deactivating Al_2O_3 and molesieve absorbent coatings in natural gas samples.

Additionally, PLOT-Q columns can separate CO_2, water, and odorants from an alkanes matrix. Thus, the analysis of natural gas on PLOT-Q columns will satisfy most the separation requirements from BTUs through hydrocarbon components and polar compound determinations.

However, there are also some problems associated with the use of PLOT-Q columns for natural gas analysis. Fixed gas (such as air, CO, and noble gases) cannot be separated on PLOT-Q columns at above ambient temperatures. The upper temperature limits are usually low (250°C) for most commercial PLOT-Q columns.

For some commercial PLOT-Q columns, loose particle binding in the coating, high column bleed, and the limited resolution of nitrogen/air from methane are major problems restricting their usefulness in natural gas analyses. Loose particle binding in the coating causes baseline spiking when the sampling valve is operated or fast temperature ramping is used.

High column bleed makes these columns useful only at temperatures below 250°C and this situation prolongs the analysis time for hydrocarbons heavier than C_7 and/or requires backflushing of the hydrocarbons. The limited resolution of nitrogen/air from methane requires low starting temperatures (@ 40°C) which increases analysis time and affects the accuracy of the analysis. Since a fraction of the nitrogen, carbon dioxide, and methane peaks overlap, the concentration of methane will be incorrectly quantified.

New HP PLOT-Q columns overcome some of these problems making them suitable for natural gas analyses. This application note examines a simple GC/TCD mehtod for the analysis of natural gas using a new HP porous polymer, Q-type, PLOT column. The resolution of nitrogen and carbon dioxide from methane on different commercially available PLOT columns is compared and reproducibility and reliability are evaluated.

Experimental

Gas chromatography analysis of a natural gas sample was done using an Agilent 6890 series gas chromatograph (GC) with electronic pneumatics control (EPC) and a Thermal Conductivity Detector (TCD). For conventional gas analysis, a six-port valve with an 0.25 cc sampling loop was used to introduce natural gas sample onto the HP-PLOT Q column in split mode (split ratio 18:1). The GC parameters are listed in **Table 1**.

A natural gas sample supplied by Scott Specialty Gases, Inc, (Plumsteadville, PA) was used and the original compounds and concentrations are listed in **Table 2**. This sample was modified by adding methanol, water, and hydrogen sulfide. During analysis, the possible leaking of some air in the sampling loop may also have caused some change in concentrations.
Analyses were run using an HP-PLOT Q porous polymer column (part number 19095P-QO4) with two other brands (X and Y) of Q-type PLOT columns used for resolution comparisons. All columns were conditioned at 250°C overnight per manufacturer recommendation to reduce column bleed.

Table 2. Natural Gas Sample

Compound	Concentration (v/v%)
Nitrogen	2.500
Methane	88.660
Carbon Dioxide	3.000
Ethane	3.520
Propane	1.050
iso-Butane	0.400
n-Butane	0.400
neo-Pentane	0.100
iso-Pentane	0.150
n-Pentane	0.150
Hexane	0.050
Heptane	0.020

Table 1. GC Experimental Conditions

GC	6890 GC with EPC
Columns	0.53 mm x 30 m PLOT-Q columns
Carrier	Helium 8.6 ml/min @ 60°C, Constant flow mode
Oven	60°C (2 min) 30°C/min to 240°C (1 min)
Injection	Split mode, 250°C, 0.25 cc sampling loope
Split flow	150 ml/min
Valve	Valco 6-port valve, 0.25 cc sampling loop
Detector	TCD
Reference flow	Helium, 30 ml/min
Auxilary gas flow	Helium, 3 ml/min

Results and Discussions

HP-PLOT Q type columns are coated with porous polymer particles made of divinylbenzene and ethylvinylbenzene and can separate hydrocarbons up to C_{14} as well as some polar compounds. Their upper isothermal and programming temperature limits are 270°C and 290°C, respectively.

The separation of the constitutents in the natural gas sample was done using a porous polymer HP-PLOT Q column as shown in **Figure 1**. The analysis time for this run was 9 minutes. Hydrogen sulfide, water, and methanol were well-separated from ethane, propane and iso-butane. Although baseline spiking is commonly associated with this analysis for some commercially available columns, no baseline spiking was observed with the HP-PLOT Q column, indicating that the stationary phase of this PLOT column provides excellent immobilization that can withstand: fast oven temperature ramping (30°C/min), a pressure pulse generated from valve actuation, and carrier gas pressure ramping at constant flow mode. Resultant column bleed was very low.

Limited resolution of nitrogen and carbon dixoide from methane is obtained using most commercial PLOT-Q columns. To evaluate the resolution of the new HP-PLOT Q column (**Figure 1**), an HP-PLOT Q column and two other brands of PLOT-Q columns (brand X brand Y) were compared. All columns were 0.53 mm internal diameter. The natural gas sample size was 0.25 cc with a split ratio of 18:1. Peak resolutions (R_S) were calculated based on the formulae in (1) and the results listed in **Table 3**.

$$R_s\,(A/B) = \frac{2^*\,(t_b - t_a)}{1.7^*\,(W_{a(1/2)} + W_{b(1/2)})}$$

- Where t_a and t_b are the retention times of peaks A and B

Table 3. Resolution Comparisons (Sample and size, natural gas, 0.25 cc)

Resolution R_S	HP-PLOT Q	Brand X	Brand Y
R_S (N_2-Air/Methane, 40°C)	2.560	2.380	1.760
R_S (N_2-Air/Methane, 60°C)	1.760	1.600	1.340
R_S (CO_2/Methane, 40°C)	11.200	8.100	10.100
R_S (CO_2/Methane, 60°C)	7.200	5.100	5.300

- $W_{a(1/2)}$ and $W_{b(1/2)}$ are their peak widths at half height, respectively.

Peak resolution for N_2-air/methane using the HP-PLOT Q column was greater than 1.5 which is the conventional requirement for base line separation, even at a 60°C initial oven temperature. The resolution of carbon dioxide from methane at 60°C on the HP-PLOT Q column is 40% higher than the same resolution on the other two brands of PLOT-Q columns tested. This separation capability of the HP-PLOT Q column also can sufficiently resolve nitrogen and carbon dioxide from methane, even if the methane peak is tailing due to sample overload. The starting temperature of 60°C also results in a 30% reduction in GC cycle time.

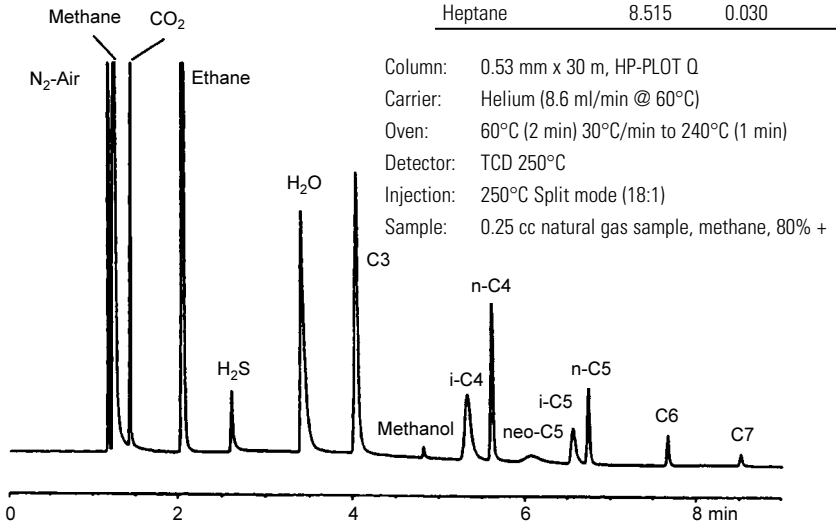
One of the concerns associated with using PLOT columns for natural gas analysis is reproducibility. It is well known that — when using alumina PLOT and molesieve PLOT columns — the retention times for hydrocarbons shift due to deactivation of column absorbants from sample components such as water and CO_2 during repeated runs. Retention time shifting makes the sample timing switch more difficult and sometimes incorrect. To investigate this problem, 70 sequential runs were performed over three days. **Tables 4** and **5** list the relative standard deviations (RSDs) for the retention time averages as well as the peak ratios.

Table 4 shows that the change in retention time is very small for most components. The largest variation, up to 0.6%, was observed for water. This indicates that column performance

Table 4. Sequential Runs of Natural Gas: Retention Time (min)

Compound	Average	RSD%
Nitrogen	1.165	0.190
Methane	1.220	0.490
Carbon Dioxide	1.429	0.200
Ethane	2.036	0.220
Water	3.413	0.560
Propane	4.027	0.130
i-Butane	5.313	0.060
n-Butane	5.600	0.060
i-Pentane	6.563	0.040
n-Pentane	6.740	0.030
Hexane	7.671	0.030
Heptane	8.515	0.030

Figure 1. Separation of Natural Gas



will not be affected by water. **Table 5** shows some larger variations in methane and heptane peak areas.

The larger variation in peak area ratio in comparison to those for retention time can be caused by two factors. First, sample size changed due to sample loop leakage with a resultant change in water amount. Second, the integration of peak areas for methane and heptane was not very accurate. The methane peak is relatively sharp but tailing, which affects the baseline determination for the integral peak area, while the heptane peak is very small. Tight control of the sample size should minimize the variation in the peak area ratios.

The chromatograms obtained at the beginning and the end of the sequential runs are shown in **Figure 2.** This figure demonstrates that the retention time, elution order and peak shape do not change after repeat runs.

For safety reasons, the analysis of natural gas containing mercaptans (added to natural gas as odorants) was not carried out in this experiment. However, **Figure 3** shows the GC separation of four kinds of mercaptans, carbonyl sulfide and hydrogen sulfide, starting from 60°C. They are well separated and resolved. Their elution positions still fall in between those for ethane and i-butane using the same conditions as those listed in **Table 1**.

Although backflushing heavier compounds in natural gas analysis is very common for all PLOT columns, this technique may not be needed for HP-PLOT Q columns. **Figure 4** shows this possibility, where heavier alkanes up to C_{14} were eluted on HP-PLOT Q column at 300°C, at such a high temperature, the column maintained relatively low bleed.

Conclusion

Natural gas analysis by GC/TCD operation on a single porous polymer HP-PLOT Q column gives satisfactory separation using a very simple GC/TCD configuration and operation. The reproducibility of the analysis is very good. Backflush may not be needed for hydrocarbons up to C_{14}, which can be eluted at 300°C temperatures.

Table 5. Sequential Runs of Natural Gas: Peak Area Ratio

Ratio	Average	RSD%
Methane/Ethane	10.280	5.899
CO_2/Ethane	0.846	1.867
Propane/Ethane	0.359	2.800
Heptane/Ethane	0.011	12.712

Figure 2. Sequential Runs of Natural Gas

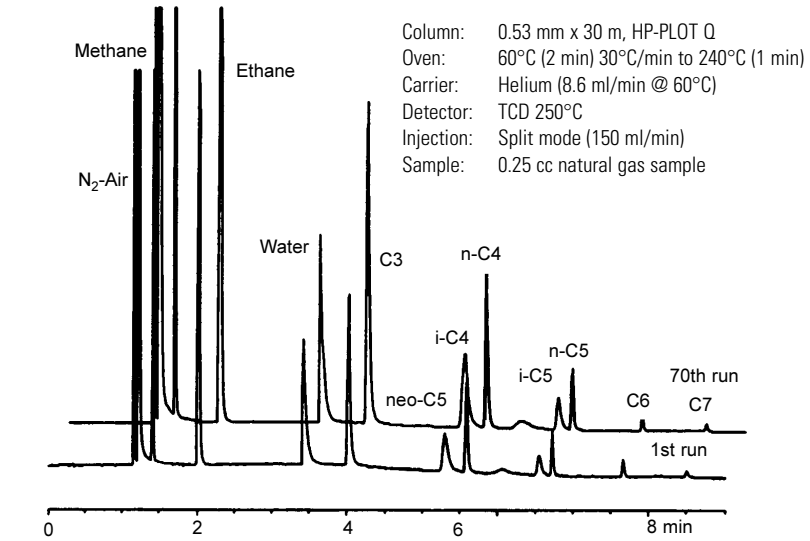


Figure 3. Sulfur Compound Separation

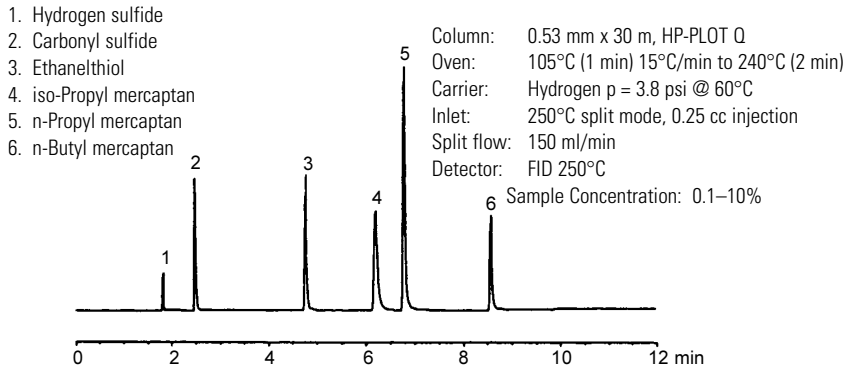
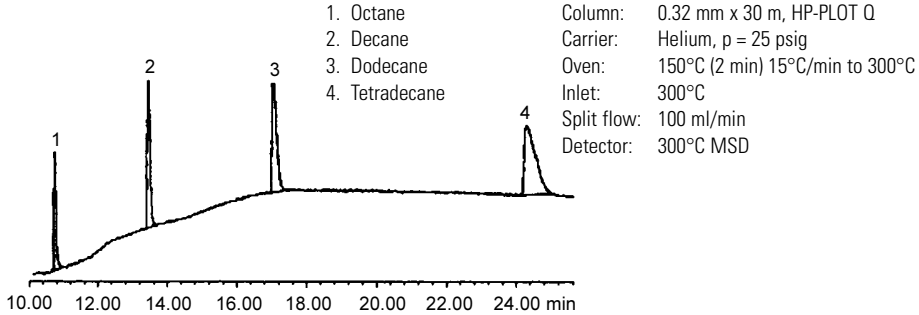


Figure 4. Elution of C8 to C14 on HP-PLOT Q column



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Analysis of Permanent Gases and Methane with the Agilent 6820 Gas Chromatograph

Application

Petrochemical

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Abstract

The analysis of permanent gases using the Agilent 6820 equipped with a single filament Thermal Conductivity Detector is described. For these applications, the Agilent 6820 gas chromatography system was configured with a gas sampling valve, isolation valve, and purged-packed inlet. Agilent Cerity for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and date analysis. HP-PLOT Q and HP-Molsieve 5A columns were used for separation of permanent gases including carbon monoxide, carbon dioxide, oxygen, nitrogen, hydrogen, and methane. Carbon dioxide, oxygen, nitrogen, and methane were analyzed at the level of 10 ppm. In this application note, benefits of the Agilent 6820 Thermal Conductivity Detector are also discussed.

Introduction

Permanent gas analysis finds wide application in the fields of petrochemical, chemical, and energy industries. Permanent gases such as carbon monoxide, CO_2, O_2, N_2, and methane are common in refinery gases, natural gas, fuel cell gases, and many other industrial processes. Understanding the concentration of these components can be important for controlling manufacturing processes and production quality. For example, impurities such as carbon monoxide and CO_2 in polymer grade propylene and ethylene are deleterious to certain catalysts.

Several methods for permanent gas analysis based on packed columns are standardized. For example, the American Society for Testing and Materials (ASTM) D2504 covers the determination of H_2, N_2, O_2, and carbon monoxide at the parts-per-million (ppm) (v/v) level in C_2 and lighter hydrocarbon products [1]. ASTM D1946 analyzes permanent gases, methane, ethane, and ethylene [2]. The Chinese domestic standard method GB/T3394 determines carbon monoxide and CO_2 in polymer grade ethylene and propylene using a nickel catalyst accessory and Flame Ionization Detector (FID) [3]. This application offers a highly flexible system assembled with three porous layer open tubular (PLOT) capillary columns and rotary valves for analysis of permanent gases and light hydrocarbons. Compared to packed columns, PLOT columns offer many advantages including separation power, temperature range, stability, low bleed, and the ability to achieve lower detection limits.

Experimental

Experiments were performed on the Agilent 6820 GC equipped with a purged packed inlet and single filament Thermal Conductivity Detector (TCD). The valving diagram for the configuration used is presented in Figure 1, which shows two analysis systems. System 2 is used for analyzing hydrocarbons (spit/splitless inlet and FID) and is discussed in a separate application note. System 1 is used for analyzing permanent gases. This application is based on a 10-port valve (Valve 1) for gas sampling and backflush of the precolumn to the detector. Two HP-PLOT Q columns are associated with the 10-port valve. A 6-port column isolation valve (Valve 2), with adjustable restrictor, is used to switch the Molesieve 5A column in and out of the carrier stream. Valve 2 is switched to the OFF position to allow unresolved peaks containing air, carbon monoxide, and methane to enter the Molesieve 5A PLOT as they elute from the PLOT Q column. Once these components are in the Molesieve 5A column, it is isolated (Valve 2=ON). After heavier components and CO_2 elute from the PLOT column and are detected, Valve 2 is turned OFF to elute the trapped components to the single filament TCD through the 5A PLOT. The purged packed inlet is interfaced directly to the valve to provide a source of carrier gas.

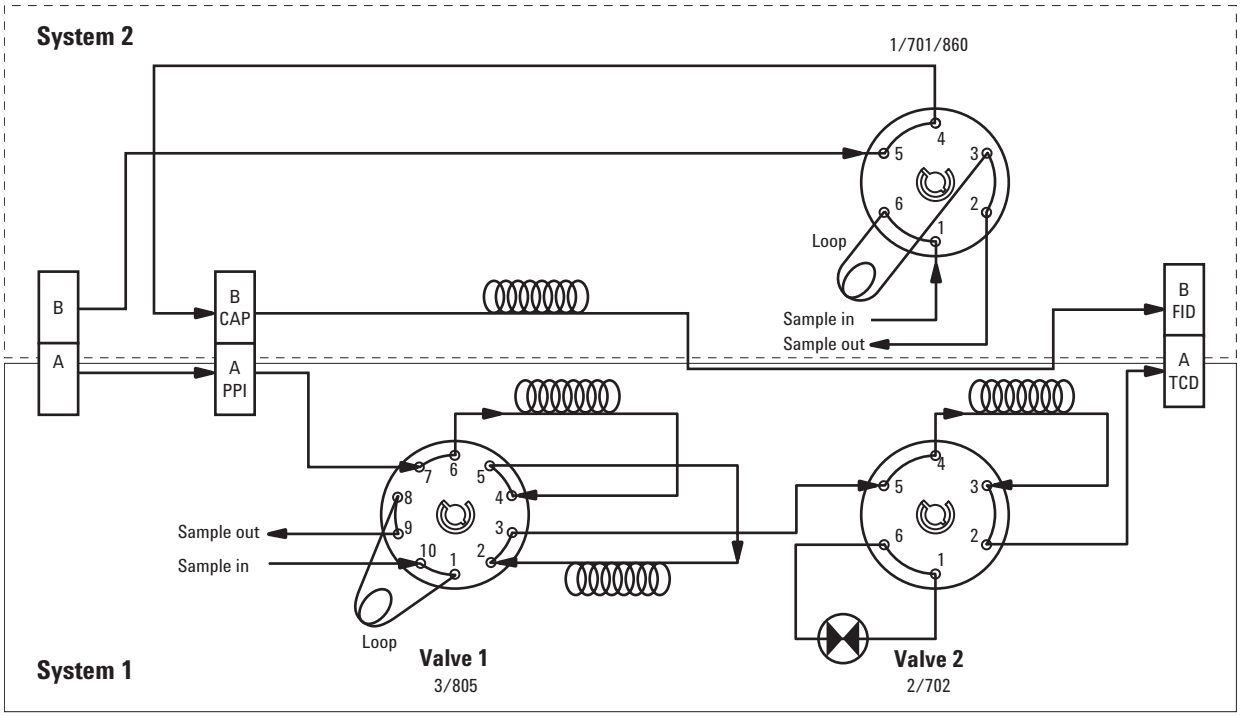

Figure 1. Valve diagram.

The analysis was performed by separating the gas sample into a two-column system. An HP-PLOT Q 15 m × 0.53 mm × 40 µm was used to separate hydrocarbons in the gas sample. An HP PLOT Molesieve 5A 30 m × 0.53 mm × 50 µm was used to separate O_2, N_2, carbon monoxide, and methane. An additional column, the HP PLOT Q 30 m × 0.53 mm × 40 µm, was used as the precolumn in a blackflush to detector configuration. The GC parameters are listed in Table 1.

Table 1. Gas Chromatographic Conditions: System 1

GC	Agilent 6820 Gas Chromatograph
Data system	Agilent NDS Cerity for QA/QC
Purged packed Inlet	50 °C
Valve temperature	80 °C
Sample loop	0.25 mL
Column flow (H_2)	4.8 mL/min
Column	HP-PLOT Q 15 m × 0.53 mm × 40 µm (p/n: 19095P-Q03)
	HP-PLOT Q 30 m × 0.53 mm × 40 µm (p/n: 19095P-Q04)
	HP PLOT Molesieve 5A 30 m × 0.53 mm × 50 µm (p/n: 19095P-MSO)
Oven	50 °C Isothermal
Detector	TCD, 180 °C
Reference	40 mL/min
Make up	10 mL/min

Special fused silica adapters and bulkhead fittings were used in this application to connect the megabore columns to the 1/16-inch tubing from the valves. These provide a reliable, airtight, low internal volume connection system for optimal chromatography. This connection is also important for ppm level gas applications. The fused silica adapter was used to help to decrease the leak risk from the column connection and to provide a zero dead volume connection of a capillary column to a valve. This adapter includes: a polyamide ferrule (p/n 0100-1512), counter-bored nut (p/n 0100-1511), polyamide liner (p/n 0100-1513 for 0.53 mm column), and a clear slotted tube (p/n 18900-20850). Figure 2 illustrates the parts used to attach the column to the bulkhead fitting in the oven.

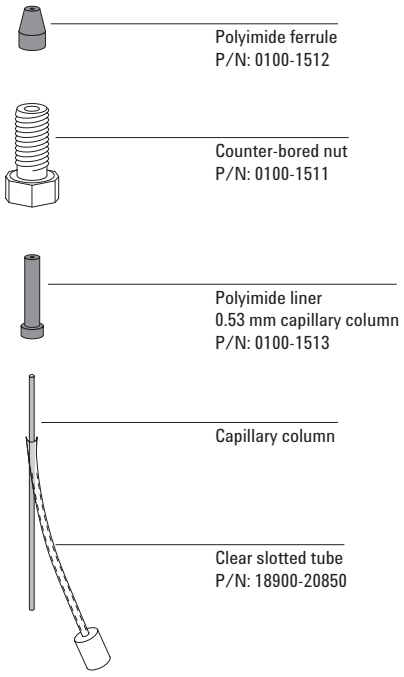

Figure 2. Installing capillary columns using fused silica adapters.

A fixed gas mix standard, supplied by Scott Specialty Gases Inc., was used in this application. This sample was dynamically blended or diluted to achieve concentrations down to 10 ppm per component [4]. The compounds and concentrations are listed in Table 2.

Table 2. Standard Mix Gas

Compound	Concentrations (ppm)
Carbon dioxide	100.6
Methane	100.8
Nitrogen	100.8
Oxygen	101.2

Agilent Cerity Networked Data System for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and reporting. Cerity was operated at a data acquisition rate of 5 Hz/0.04 min.

Results and Discussion

PLOT Columns

PLOT columns have an advantage of low bleed, which is important for trace analysis. A PLOT Molecular sieve 5A column exhibits a high retention for permanent gases. This makes permanent gas separations possible at starting oven temperatures of 50 °C. The PLOT Q column is excellent for the separation of CO_2 and hydrocarbons through C6, depending on the GC oven program used [5].

Agilent 6820 TCD

The TCD is a concentration sensitive detector. It is a simple, easy to use, low-cost detector suitable for the analysis of permanent gases, hydrocarbons, and many other gases. The single-filament flow-switching design eliminates the need for a reference column. This unique design alternately exposes the filament to column effluent and reference flows at a frequency of 10 Hz. Digital processing is used throughout. See Figure 3 for a cross-sectional diagram of the Agilent TCD.

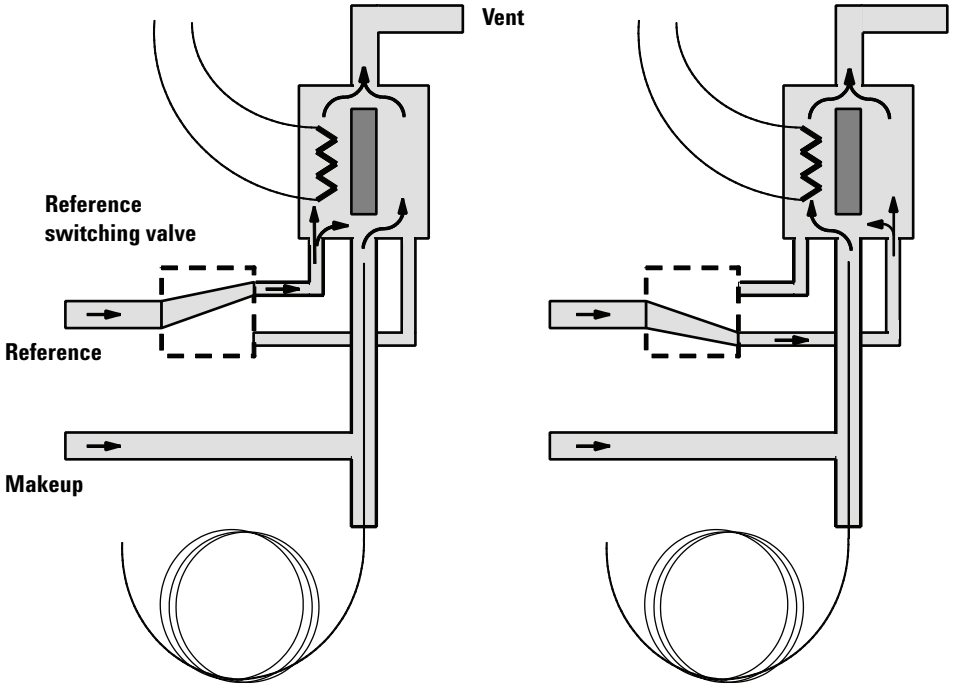

Figure 3. Pneumatic diagram of the Agilent single-filament TCD.

The most common TCD design is still based on the typical 4-element tungsten filament incorporated in a Wheatstone bridge design. This filament design requires dual channels (an analytical column and a blank column). The variation and column bleed in each channel can cause response changes, baseline noise, and drift. It is not an ideal approach for a capillary column application due to the large dead volume and the long time needed for stabilization. For low ppm level permanent gas applications such as N_2, carbon monoxide and CO_2, a dual-channel traditional TCD may not offer enough sensitivity and stability.

The Agilent single-filament TCD is optimized for use with capillary columns, improving the performance in sensitivity and stability. The cell volume is only 3.5 µL for fast response. The single-filament design eliminates the need to "match" the resistance or temperature coefficients of the filament, resulting in reduced noise and drift. These improved performance features contribute to chromatographic fidelity and sensitivity in many low-level gas analysis problems.

Low Level Permanent Gases

Figure 4 shows the chromatogram of a 100 ppm permanent gas mix. Hydrogen was used as the carrier gas and is a common choice for TCDs in China. CO_2, O_2, N_2, and methane gave a good response in this experiment. Because He is the balance gas in the standard sample (at a high concentration), O_2 separated on the tailing of the He peak.

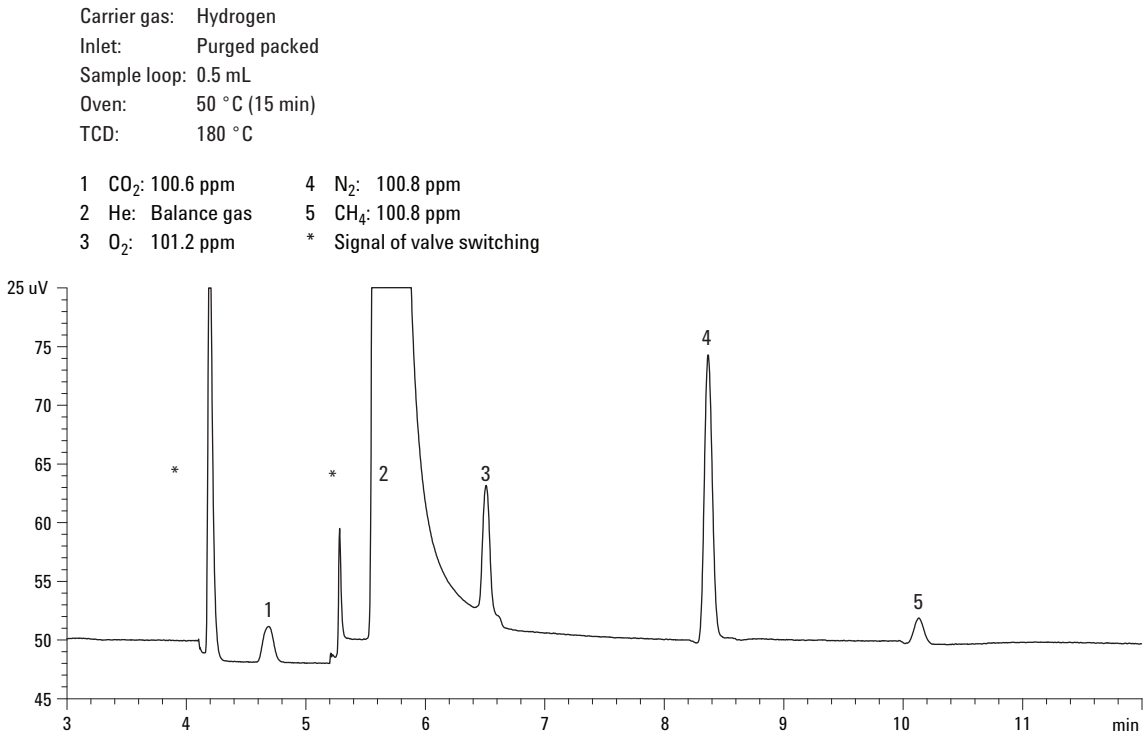

Figure 4. Chromatogram of 100 ppm permanent gas calibration standard, Carrier gas: Hydrogen.

Figure 5 shows the chromatogram of a permanent gas mix at 10 ppm. Dynamic blending was used to dilute the standard to the 10 ppm level. Chemical traps were used to efficiently condition carrier and diluent gas streams. An oxygen scrubber and second-level gas filter was used to remove other foreign material. This high level of contaminant removal is required when analyzing low level concentrations. A blank run was done to verify that the dilute gas was clean. The sample was diluted with H_2 from the 100 ppm level to 10 ppm. CO_2, O_2, N_2, and methane were easily detected at a good signal-to-noise ratio. The baseline was also very stable, making low-level analysis possible.

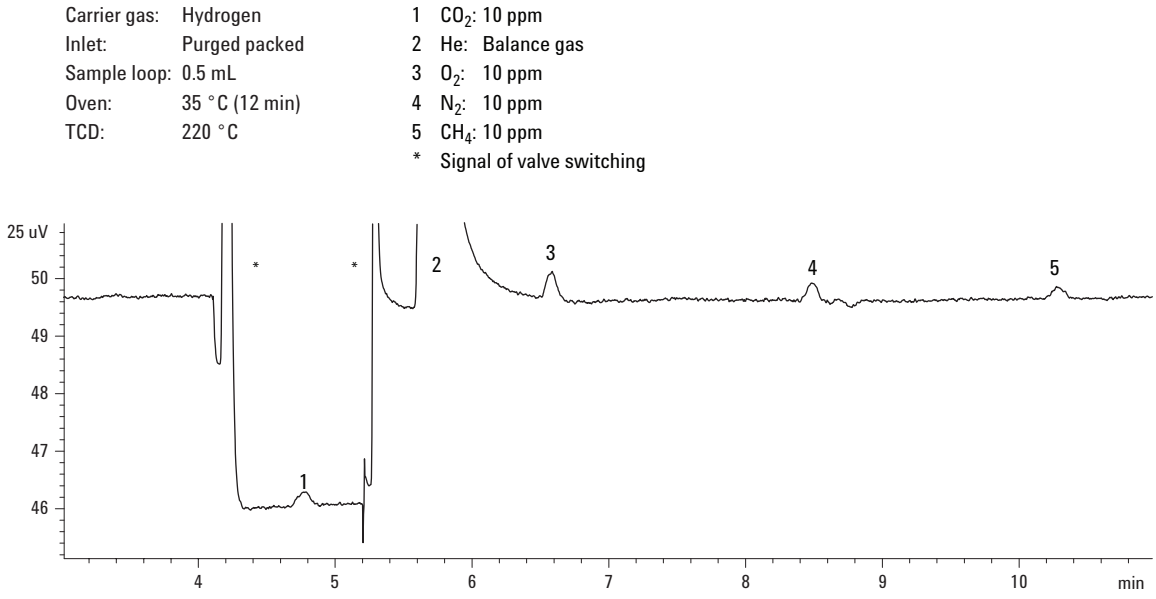

Figure 5. Ten ppm permanent gases by dynamic blending.

Analysis of Fuel Cell Gases

Figure 6 shows the chromatogram of the fuel cell mix. The composition of the mix is typical of the gases that need to be measured during the development of fuel cell systems. Baseline separation is achieved for all the permanent gases and methane. Hydrogen is detected as a negative peak because helium is used as the carrier gas in order to achieve desirable sensitivity for most gases. By setting TCD polarity in the run table, the hydrogen signal can be reversed from a negative peak to a positive one, as shown in the chromatogram. Argon is a good carrier gas if hydrogen analysis over a wide concentration range is required.

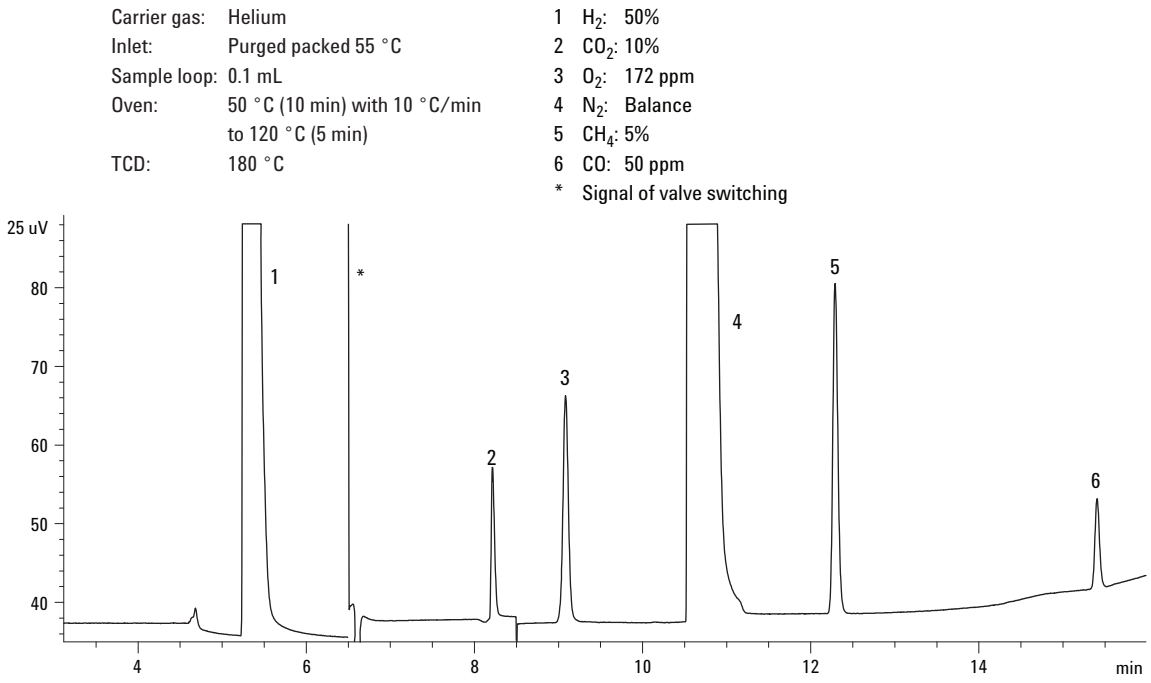

Figure 6. Chromatogram of fuel cell gas standard.

Conclusions

The Agilent 6820 Gas Chromatograph equipped with TCD detector and two valves was used to analyze permanent gases and methane. An HP-Molsieve 5A was used for the separation of O_2, N_2, carbon monoxide, H_2, and methane. The combination of an Agilent HP- PLOT Q column and isolation valve was used for the separation of CO_2 from the other gases. Higher hydrocarbons, such as ethane and propane, could also be separated and measured with the HP-PLOT Q. Of course, if only the permanent gases and methane need to be measured, the 10-port valve with PLOT Q columns would not be needed. The Agilent 6820 single filament TCD demonstrated excellent sensitivity; even 10 ppm permanent gases can be detected reliably. This system offers excellent flexibility. When light hydrocarbon analysis is required (C1 to C8), System 2 (see Figure 1) with alumina PLOT column and FID can be used. This system is suitable for a variety of applications in the petrochemical and energy industries, including natural and refinery gases, fuel cell gas, propylene, and ethylene.

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3. China National Standards GB/T3394-93 “Ethylene and Propylene for Industrial Use – Determination of Trace of Carbon Monoxide and Carbon Dioxide, – Gas chromatographic method” (1996) Chemical Industrial Standard Collection: Organic Chemicals No.16 Shanlihe North Street, FuxinMenwai, Beijing China, Postcode 100045
4. Roger Firor, “Automated Dynamic Blending System for the Agilent 6890 Gas Chromatograph: Low Level Sulfur Detection,” Agilent Technologies, publication 5988-2465EN www.agilent.com/chem
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Printed in the USA
May 7, 2003
5988-9260EN



Analysis of Permanent Gases and Methane with the Agilent 6820 Gas Chromatograph

Application

Petrochemical

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Abstract

The analysis of permanent gases using the Agilent 6820 equipped with a single filament Thermal Conductivity Detector is described. For these applications, the Agilent 6820 gas chromatography system was configured with a gas sampling valve, isolation valve, and purged-packed inlet. Agilent Cerity for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and date analysis. HP-PLOT Q and HP-Molsieve 5A columns were used for separation of permanent gases including carbon monoxide, carbon dioxide, oxygen, nitrogen, hydrogen, and methane. Carbon dioxide, oxygen, nitrogen, and methane were analyzed at the level of 10 ppm. In this application note, benefits of the Agilent 6820 Thermal Conductivity Detector are also discussed.

Introduction

Permanent gas analysis finds wide application in the fields of petrochemical, chemical, and energy industries. Permanent gases such as carbon monoxide, CO_2, O_2, N_2, and methane are common in refinery gases, natural gas, fuel cell gases, and many other industrial processes. Understanding the concentration of these components can be important for controlling manufacturing processes and production quality. For example, impurities such as carbon monoxide and CO_2 in polymer grade propylene and ethylene are deleterious to certain catalysts.

Several methods for permanent gas analysis based on packed columns are standardized. For example, the American Society for Testing and Materials (ASTM) D2504 covers the determination of H_2, N_2, O_2, and carbon monoxide at the parts-per-million (ppm) (v/v) level in C_2 and lighter hydrocarbon products [1]. ASTM D1946 analyzes permanent gases, methane, ethane, and ethylene [2]. The Chinese domestic standard method GB/T3394 determines carbon monoxide and CO_2 in polymer grade ethylene and propylene using a nickel catalyst accessory and Flame Ionization Detector (FID) [3]. This application offers a highly flexible system assembled with three porous layer open tubular (PLOT) capillary columns and rotary valves for analysis of permanent gases and light hydrocarbons. Compared to packed columns, PLOT columns offer many advantages including separation power, temperature range, stability, low bleed, and the ability to achieve lower detection limits.

Experimental

Experiments were performed on the Agilent 6820 GC equipped with a purged packed inlet and single filament Thermal Conductivity Detector (TCD). The valving diagram for the configuration used is presented in Figure 1, which shows two analysis systems. System 2 is used for analyzing hydrocarbons (spit/splitless inlet and FID) and is discussed in a separate application note. System 1 is used for analyzing permanent gases. This application is based on a 10-port valve (Valve 1) for gas sampling and backflush of the precolumn to the detector. Two HP-PLOT Q columns are associated with the 10-port valve. A 6-port column isolation valve (Valve 2), with adjustable restrictor, is used to switch the Molesieve 5A column in and out of the carrier stream. Valve 2 is switched to the OFF position to allow unresolved peaks containing air, carbon monoxide, and methane to enter the Molesieve 5A PLOT as they elute from the PLOT Q column. Once these components are in the Molesieve 5A column, it is isolated (Valve 2=ON). After heavier components and CO_2 elute from the PLOT column and are detected, Valve 2 is turned OFF to elute the trapped components to the single filament TCD through the 5A PLOT. The purged packed inlet is interfaced directly to the valve to provide a source of carrier gas.

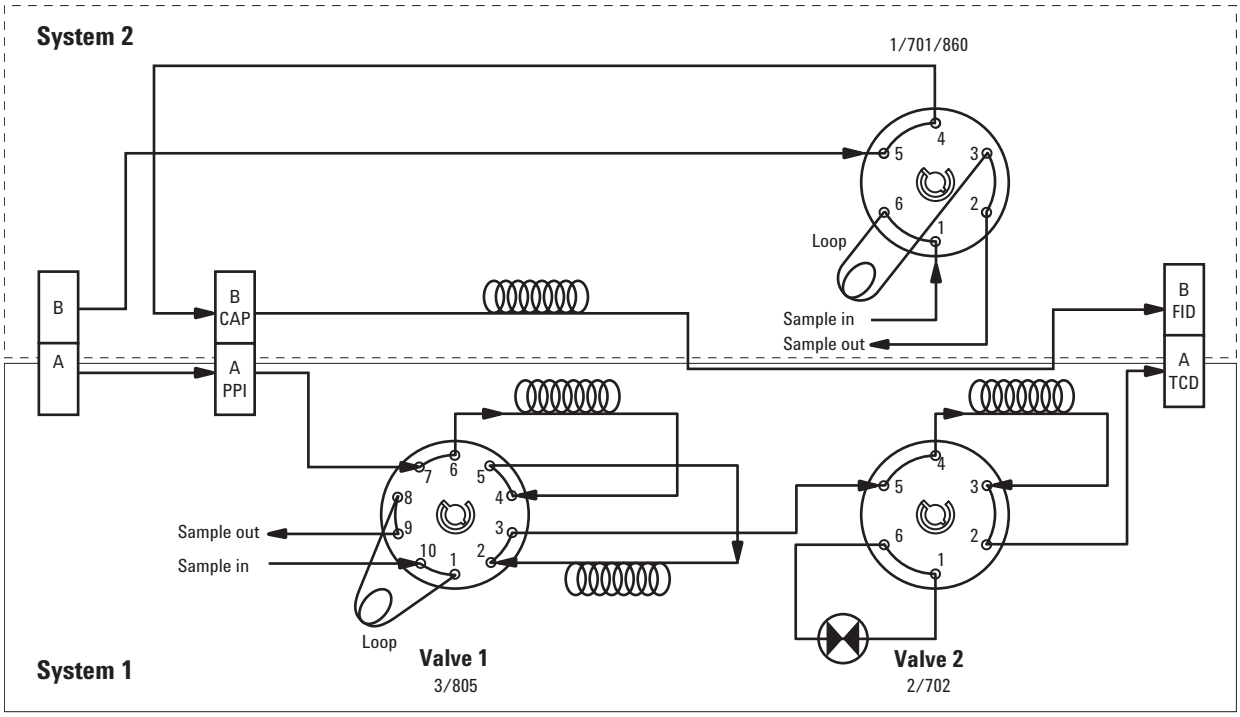

Figure 1. Valve diagram.

The analysis was performed by separating the gas sample into a two-column system. An HP-PLOT Q 15 m × 0.53 mm × 40 µm was used to separate hydrocarbons in the gas sample. An HP PLOT Molesieve 5A 30 m × 0.53 mm × 50 µm was used to separate O_2, N_2, carbon monoxide, and methane. An additional column, the HP PLOT Q 30 m × 0.53 mm × 40 µm, was used as the precolumn in a blackflush to detector configuration. The GC parameters are listed in Table 1.

Table 1. Gas Chromatographic Conditions: System 1

GC	Agilent 6820 Gas Chromatograph
Data system	Agilent NDS Cerity for QA/QC
Purged packed Inlet	50 °C
Valve temperature	80 °C
Sample loop	0.25 mL
Column flow (H_2)	4.8 mL/min
Column	HP-PLOT Q 15 m × 0.53 mm × 40 µm (p/n: 19095P-Q03)
	HP-PLOT Q 30 m × 0.53 mm × 40 µm (p/n: 19095P-Q04)
	HP PLOT Molesieve 5A 30 m × 0.53 mm × 50 µm (p/n: 19095P-MSO)
Oven	50 °C Isothermal
Detector	TCD, 180 °C
Reference	40 mL/min
Make up	10 mL/min

Special fused silica adapters and bulkhead fittings were used in this application to connect the megabore columns to the 1/16-inch tubing from the valves. These provide a reliable, airtight, low internal volume connection system for optimal chromatography. This connection is also important for ppm level gas applications. The fused silica adapter was used to help to decrease the leak risk from the column connection and to provide a zero dead volume connection of a capillary column to a valve. This adapter includes: a polyamide ferrule (p/n 0100-1512), counter-bored nut (p/n 0100-1511), polyamide liner (p/n 0100-1513 for 0.53 mm column), and a clear slotted tube (p/n 18900-20850). Figure 2 illustrates the parts used to attach the column to the bulkhead fitting in the oven.

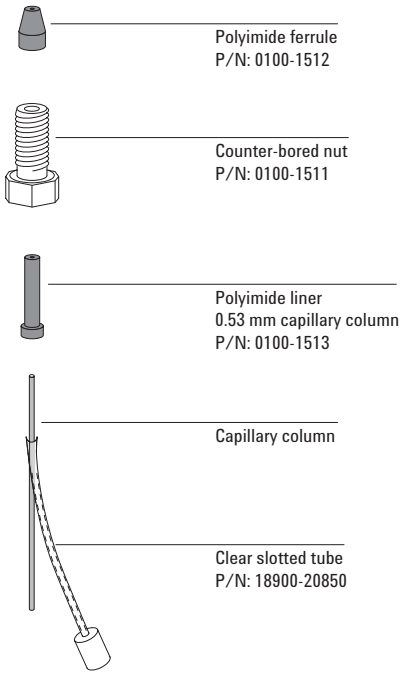

Figure 2. Installing capillary columns using fused silica adapters.

A fixed gas mix standard, supplied by Scott Specialty Gases Inc., was used in this application. This sample was dynamically blended or diluted to achieve concentrations down to 10 ppm per component [4]. The compounds and concentrations are listed in Table 2.

Table 2. Standard Mix Gas

Compound	Concentrations (ppm)
Carbon dioxide	100.6
Methane	100.8
Nitrogen	100.8
Oxygen	101.2

Agilent Cerity Networked Data System for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and reporting. Cerity was operated at a data acquisition rate of 5 Hz/0.04 min.

Results and Discussion

PLOT Columns

PLOT columns have an advantage of low bleed, which is important for trace analysis. A PLOT Molecular sieve 5A column exhibits a high retention for permanent gases. This makes permanent gas separations possible at starting oven temperatures of 50 °C. The PLOT Q column is excellent for the separation of CO_2 and hydrocarbons through C6, depending on the GC oven program used [5].

Agilent 6820 TCD

The TCD is a concentration sensitive detector. It is a simple, easy to use, low-cost detector suitable for the analysis of permanent gases, hydrocarbons, and many other gases. The single-filament flow-switching design eliminates the need for a reference column. This unique design alternately exposes the filament to column effluent and reference flows at a frequency of 10 Hz. Digital processing is used throughout. See Figure 3 for a cross-sectional diagram of the Agilent TCD.

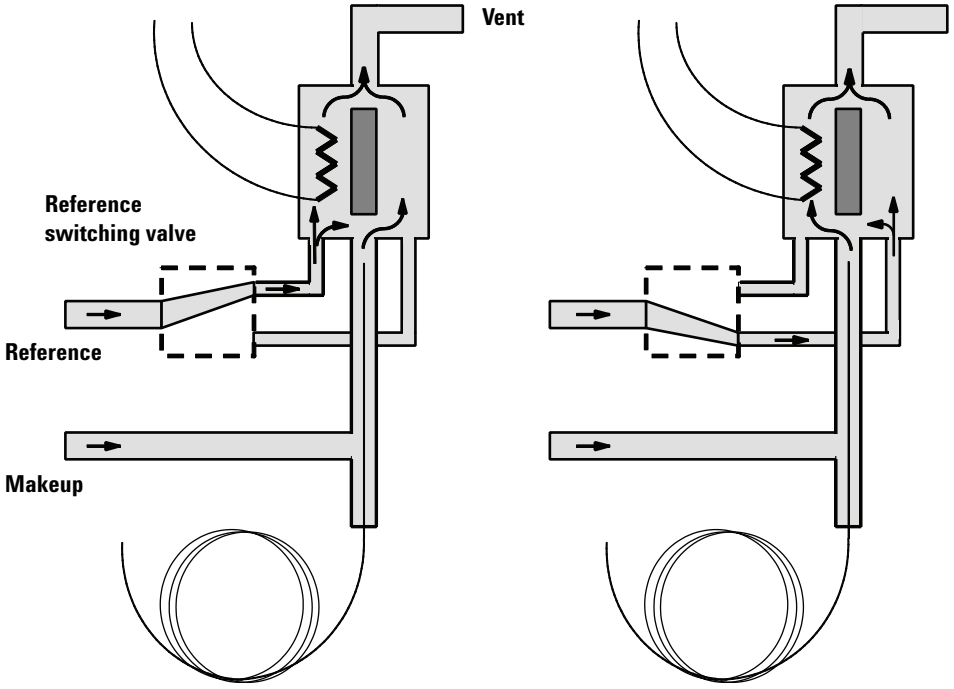

Figure 3. Pneumatic diagram of the Agilent single-filament TCD.

The most common TCD design is still based on the typical 4-element tungsten filament incorporated in a Wheatstone bridge design. This filament design requires dual channels (an analytical column and a blank column). The variation and column bleed in each channel can cause response changes, baseline noise, and drift. It is not an ideal approach for a capillary column application due to the large dead volume and the long time needed for stabilization. For low ppm level permanent gas applications such as N_2, carbon monoxide and CO_2, a dual-channel traditional TCD may not offer enough sensitivity and stability.

The Agilent single-filament TCD is optimized for use with capillary columns, improving the performance in sensitivity and stability. The cell volume is only 3.5 µL for fast response. The single-filament design eliminates the need to "match" the resistance or temperature coefficients of the filament, resulting in reduced noise and drift. These improved performance features contribute to chromatographic fidelity and sensitivity in many low-level gas analysis problems.

Low Level Permanent Gases

Figure 4 shows the chromatogram of a 100 ppm permanent gas mix. Hydrogen was used as the carrier gas and is a common choice for TCDs in China. CO_2, O_2, N_2, and methane gave a good response in this experiment. Because He is the balance gas in the standard sample (at a high concentration), O_2 separated on the tailing of the He peak.

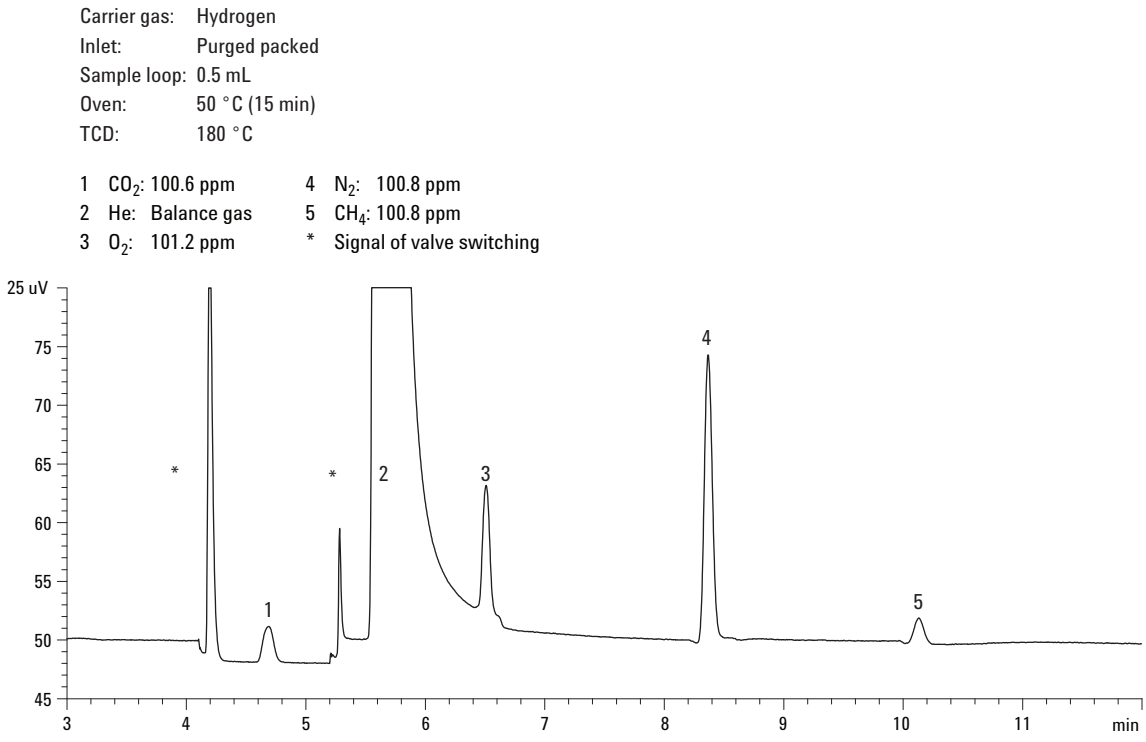

Figure 4. Chromatogram of 100 ppm permanent gas calibration standard, Carrier gas: Hydrogen.

Figure 5 shows the chromatogram of a permanent gas mix at 10 ppm. Dynamic blending was used to dilute the standard to the 10 ppm level. Chemical traps were used to efficiently condition carrier and diluent gas streams. An oxygen scrubber and second-level gas filter was used to remove other foreign material. This high level of contaminant removal is required when analyzing low level concentrations. A blank run was done to verify that the dilute gas was clean. The sample was diluted with H_2 from the 100 ppm level to 10 ppm. CO_2, O_2, N_2, and methane were easily detected at a good signal-to-noise ratio. The baseline was also very stable, making low-level analysis possible.

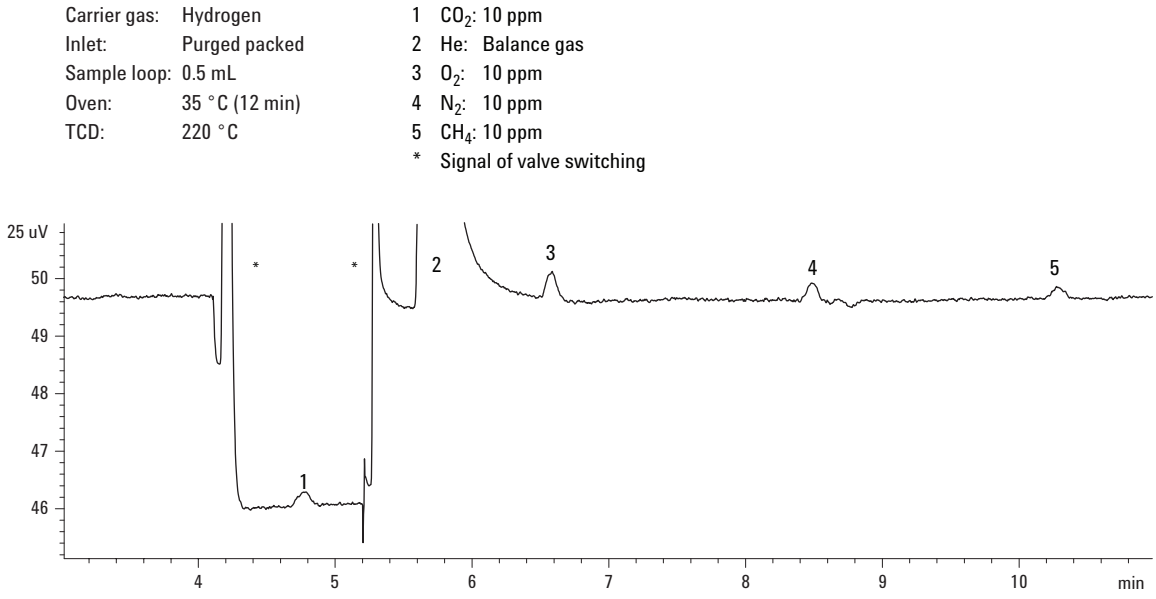

Figure 5. Ten ppm permanent gases by dynamic blending.

Analysis of Fuel Cell Gases

Figure 6 shows the chromatogram of the fuel cell mix. The composition of the mix is typical of the gases that need to be measured during the development of fuel cell systems. Baseline separation is achieved for all the permanent gases and methane. Hydrogen is detected as a negative peak because helium is used as the carrier gas in order to achieve desirable sensitivity for most gases. By setting TCD polarity in the run table, the hydrogen signal can be reversed from a negative peak to a positive one, as shown in the chromatogram. Argon is a good carrier gas if hydrogen analysis over a wide concentration range is required.

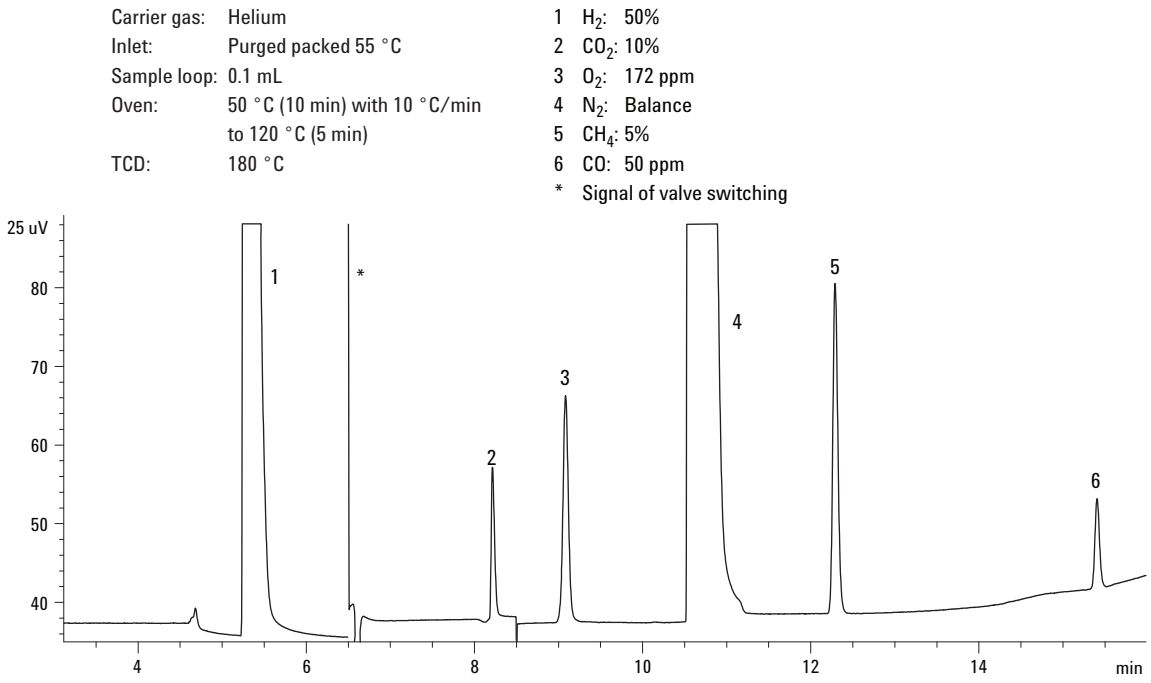

Figure 6. Chromatogram of fuel cell gas standard.

Conclusions

The Agilent 6820 Gas Chromatograph equipped with TCD detector and two valves was used to analyze permanent gases and methane. An HP-Molsieve 5A was used for the separation of O_2, N_2, carbon monoxide, H_2, and methane. The combination of an Agilent HP- PLOT Q column and isolation valve was used for the separation of CO_2 from the other gases. Higher hydrocarbons, such as ethane and propane, could also be separated and measured with the HP-PLOT Q. Of course, if only the permanent gases and methane need to be measured, the 10-port valve with PLOT Q columns would not be needed. The Agilent 6820 single filament TCD demonstrated excellent sensitivity; even 10 ppm permanent gases can be detected reliably. This system offers excellent flexibility. When light hydrocarbon analysis is required (C1 to C8), System 2 (see Figure 1) with alumina PLOT column and FID can be used. This system is suitable for a variety of applications in the petrochemical and energy industries, including natural and refinery gases, fuel cell gas, propylene, and ethylene.

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Printed in the USA
May 7, 2003
5988-9260EN



High-Pressure Liquid Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Application

Hydrocarbon Processing

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Abstract

In gas chromatography, sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Introduction

There are several known techniques for injecting volatile liquefied hydrocarbons in gas chromatographs. The simplest tools are high-pressure syringes. However, the pressure limit is not high enough to analyze light hydrocarbons such as liquefied natural gas and ethylene. The traditional methods [1, 2] include the use of vaporizing regulators and rotary sampling valves. During sampling, discrimination of the analytes will take place for samples with wide boiling points due to condensing of heavy components and selective vaporization of light components in transfer lines. Recently, piston sampling valves were introduced and are commercially available [3]. These can suffer from discrimination and short service lifetimes at high vaporization temperatures or high sample pressures.

Combining the advantages of simple syringes and high-pressure rotary valves, a unique sample injection device has been designed. The system consists of a 4-port liquid sampling valve, a Siltek deactivated needle, and a split/splitless inlet. This compact device is installed directly over the GC inlet. This unit is operated just like a typical liquid autosampler; however, the needle is not withdrawn. The maximum limit of sample pressure is 5,000 psig. Various pressurized gas samples have been evaluated on this device such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with 0.47% to 1.09% RSD in quantitative analyses. Wide boiling point hydrocarbon samples (C5 to C40) have also been analyzed using this injector, with excellent quantitative results.

Experimental

Injection Device

The high-pressure liquid injection (HPLI) device consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Co. The valve works under 75 °C and 5,000 psi.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

The following components are recommended. These are not supplied in the option or accessory kit.

- **Filter:** To remove particles from samples, it is necessary to install a filter between the sample line and port S.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in option or accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

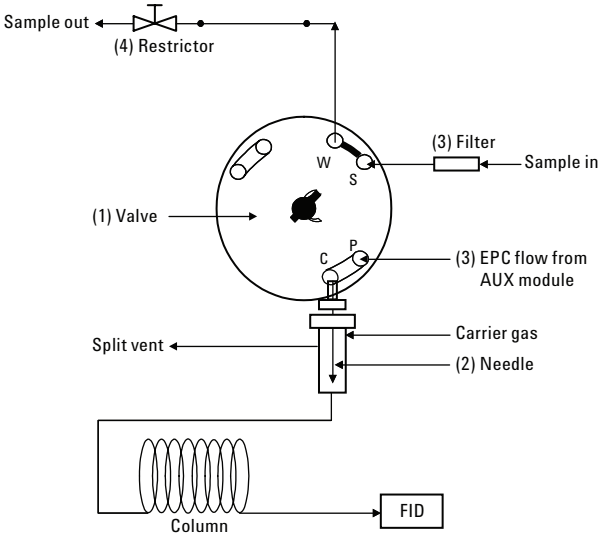


Figure 1. Flow diagram of the HPLI device.

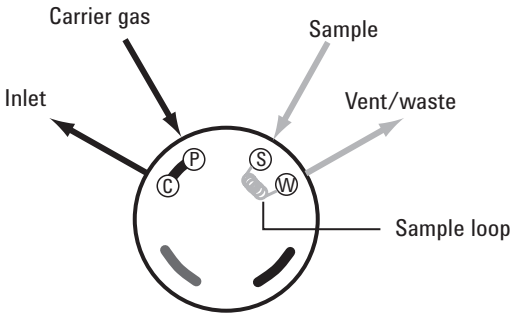
Samples for System Evaluation

- Liquefied natural gas: Calibration standard, 1,200 psi, with nC7-nC9 (0.102%–0.0503%)
- Liquefied ethylene: Purity 99.5, 1,200 psi
- Pressurized propylene: Grade C. P., purity 99.0%, 200 psi
- Pressurized propane + n-butane: 50.0%:50.0%, 200 psi
- Pressurized 1, 3-butadiene: Purity 99.5%, 180 psi
- n-Hexane + 1.0 % 2# BP standard (Agilent PN 5080-8768, nC5–nC18)
- nC5–nC40 D2887 1# BP standard (Agilent PN 5080-8716, diluted by CS_2)
- Glycols, including monoethylene glycol, diethylene glycol, and triethylene glycol
- C8 to C16 hydrocarbons at 100 ppm each

Operating Process

The valve is operated with an Agilent pneumatic air actuator. To load the sample, the valve is set at the OFF position (Figure 1). The sample is loaded from port S and vented to port W. The pneumatic and sample paths in load and inject positions are shown in Figure 2. To maintain the sample in the liquid phase and to avoid “bubbles” in the sample line, it is important to adjust resistance of the metering valve and check for possible leaks at the connections. To inject, the valve is switched to the

Load



Inject

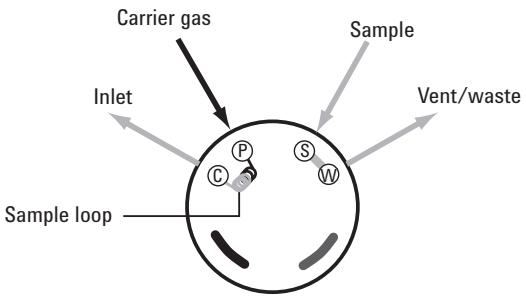


Figure 2. Pneumatic and sample paths in load and inject positions.

ON position. A 2- to 3-second injection time should be used.

The system should always be carefully checked for leaks before introduction of high-pressure hydrocarbons. Instrumental conditions and application-specific columns are shown in Table 1 and Table 2, respectively.

When the valve is actuated, a stream of carrier gas from the Aux EPC or PCM will enter the inlet and combine with the inlet carrier flow; the combined flow will vent through the split vent. Therefore, the actual split ratio will be higher than the value set from ChemStation. The actual split ratio can be calculated by measuring the split vent flow.



Figure 3. Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Table 1. Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	HPLI device at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.5-µL (0.2 µL for C5–C40) device supplied with 0.06-µL rotor
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

Table 2. Columns and Parameters

Samples	Columns	Column flow mL/min	Split ratio	Temperature program	Sample pressure psig
Natural gas	30 m × 0.53 mm × 0.5 µm DB-1 #125-1037	8	40:1	35 °C, 1 min 20 °C/min to 180 °C, 1 min	1200
Ethylene	50 m × 0.53 mm × 15 µm AL2O3 PLOT/KCL + 30 m × 0.53 mm × 5 µm DB-1, #19095P-K25 and #125-1035	8	20:1	35 °C, 2 min 4 °C/min to 160 °C, 3.8 min	1100
Propylene	50 m × 0.53 mm HP AL2O3 PLOT + 30 m × 0.53 mm × 5 µm DB-1	7	25:1	35 °C, 2 min 4 °C/min to 160 °C, 1.8 min	180
Propane + n-butane	30 m × 0.53 mm × 1.0 µm DB-1, #125-103J	5	50:1	35 °C	150
1,3-Butadiene	50 m × 0.53 mm AL2O3 PLOT/KCL	10	15:1	35 °C, 2 min 10 °C/min to 195 °C, 15 min	180
n-Hexane	30 m × 0.53 mm × 1.0 µm DB-1	5	50:1	45 °C	N/A
nC5-nC40	10 m × 0.53 mm × 0.88 µm HP-1, #19095Z-021	10	15:1	35 °C, 1 min 15 °C/min to 350 °C, 5 min	N/A
Glycols	30 m × 0.25 mm × 1.0 µm HP-1 ms	1.8	15:1	50 °C, 3 min 15 °C/min to 250 °C, 2 min	

Results and Discussion

Check for Carryover

A set of normal hydrocarbons was used to perform a basic check of the system, looking for good peak shape and lack of carryover.

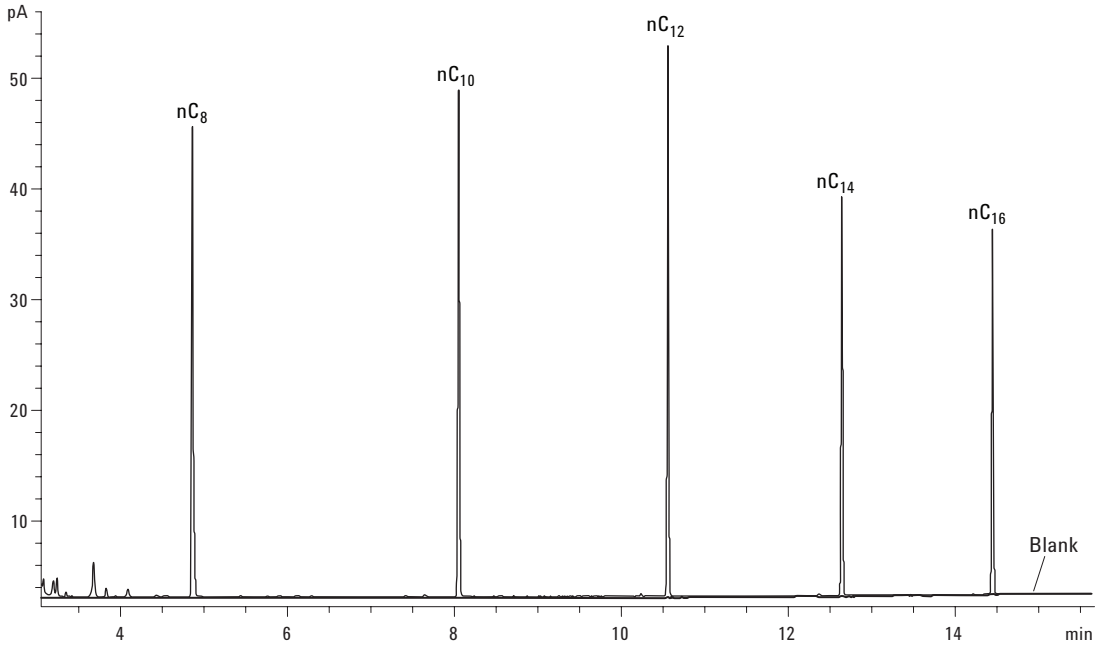


Figure 4. Overlay of standard versus blank (100 ppm each in cyclohexane).

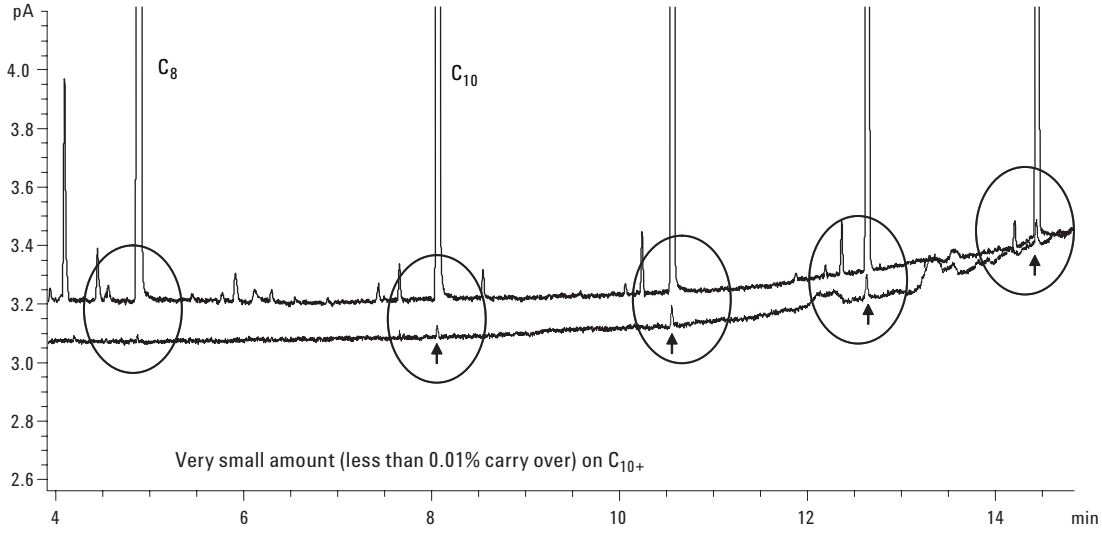


Figure 5. Carryover less than 0.01% on C_{10+}.

Sample Analysis

A series of glycols was used to model performance of the device for highly polar analytes. Minimal peak tailing is seen, due in part to the inertness of the needle interface. Also, carryover is very low.

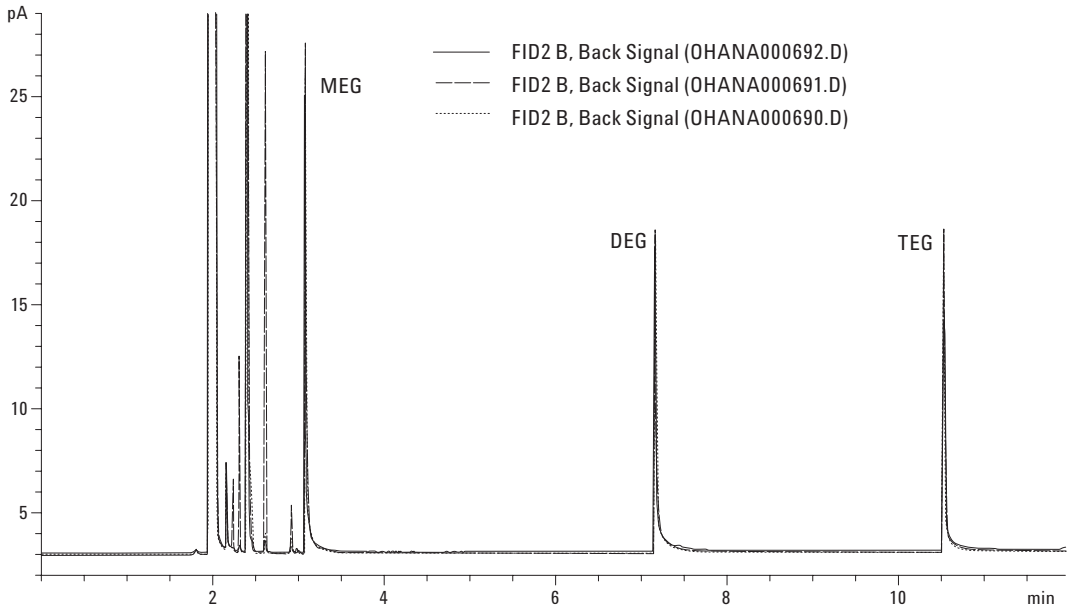


Figure 6. Triplicate run of 100 ppm each of MEG, DEG, and TEG in IPA.

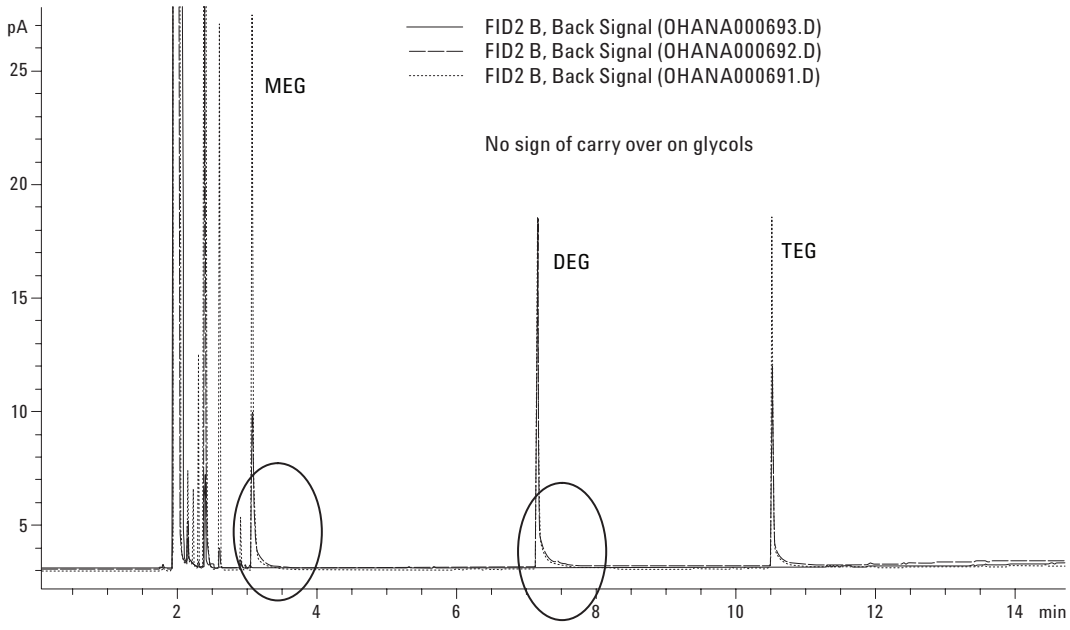


Figure 7. Glycols versus blank. Two standard duplicates, blank run immediately after injection of standard.

A. Liquefied Natural Gas

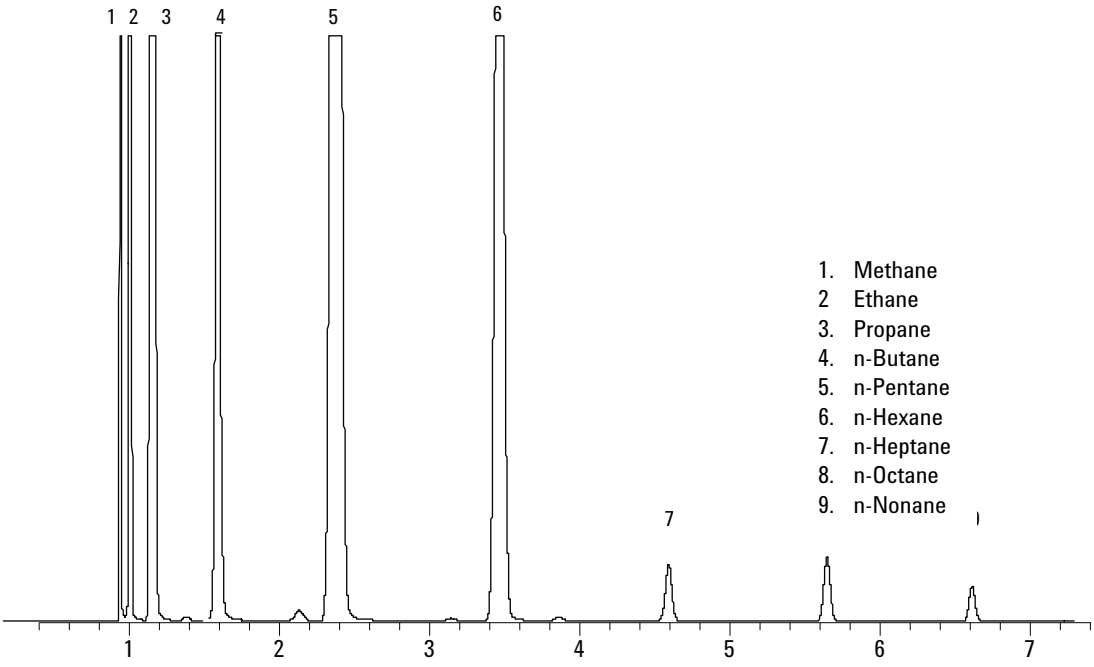


Figure 8. Chromatogram of liquefied natual gas (calibration standard).

Low discrimination is seen in Figure 8 for liquefied natural gas (LNG). Excellent repeatability is obtained with RSDs of less than 1%.

B. Liquefied Ethylene

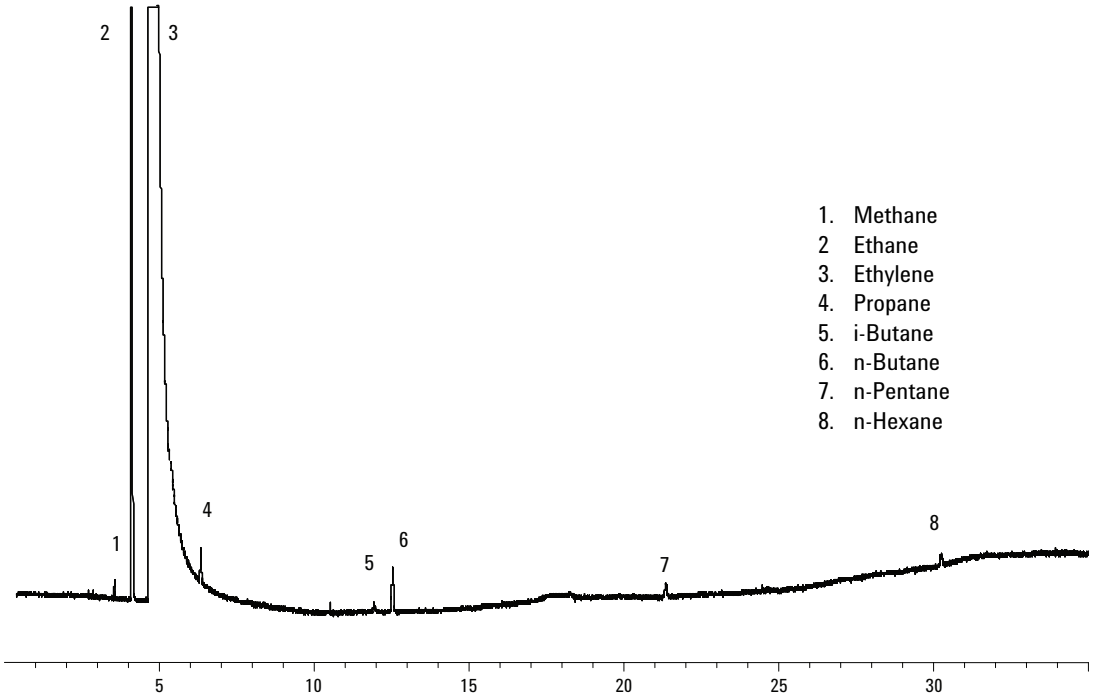


Figure 9. Chromatogram of liquefied ethylene.

The sample in Figure 9 is analyzed by ASTM D6159, "Standard Test Method for Impurities in Ethylene by Gas Chromatography." The method detection limits (MDLs) for the two methods are listed in Table 3.

The MDL using the HPLI device is 10 times lower than reported in the ASTM method due largely to the lack of peak tailing.

Table 3. MDLs (ppm V) by ASTM D6159 and HPLI

Components	ASTM D6159	HPLI
Methane	5.57–62.3	0.27
Ethane	35.1–338	0.78
Propane	8.07–59.7	0.88
i-Butane	7.74–48.4	0.38
Butane	4.97–56.1	1.61
n-Pentane		0.61
n-Hexane		0.74

C. Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159 (above method for ethylene analysis). The chromatogram is shown in Figure 10.

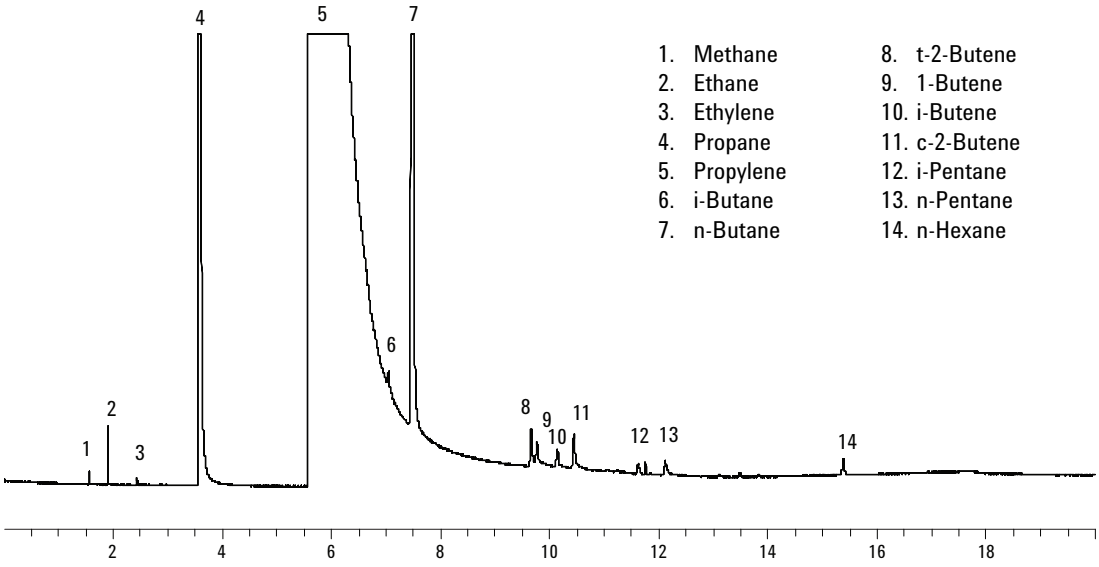


Figure 10. Chromatogram of pressurized propylene.

D. Pressurized 1,3-Butadiene

As an example of C4 hydrocarbons analysis,
Figure 11 shows a typical result for 1,3-Butadiene.

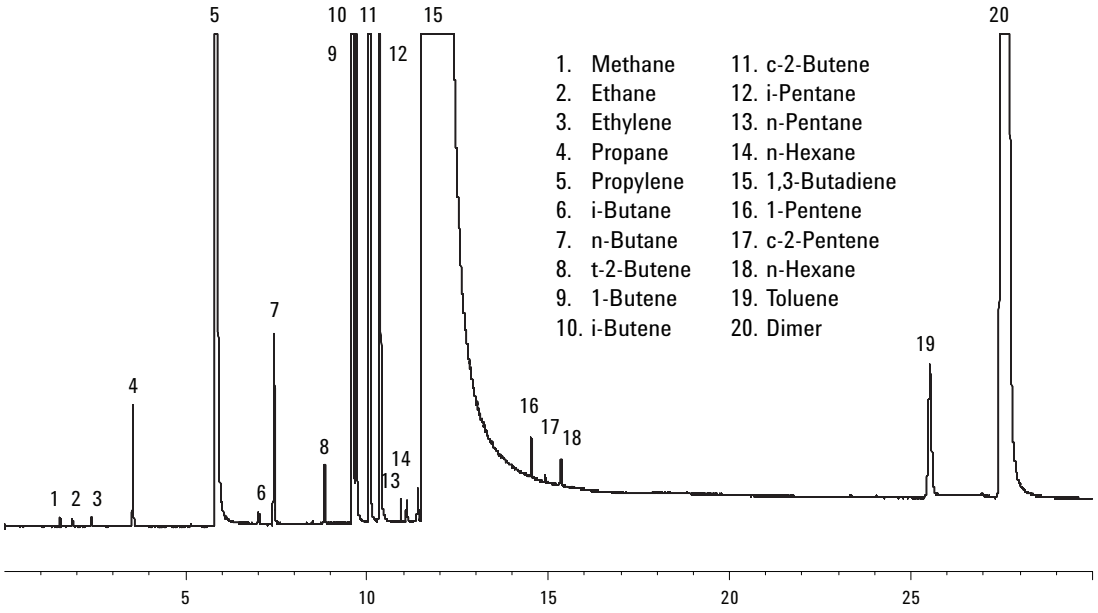


Figure 11. Chromatogram of pressurized 1,3-butadiene.

E. Pressurized Propane + n-Butane

This is a quantitative calibration sample:
Propane:n-Butane = 50%:50%.
The chromatogram is shown in Figure 12 with the results of a quantitative analysis shown in Table 4.

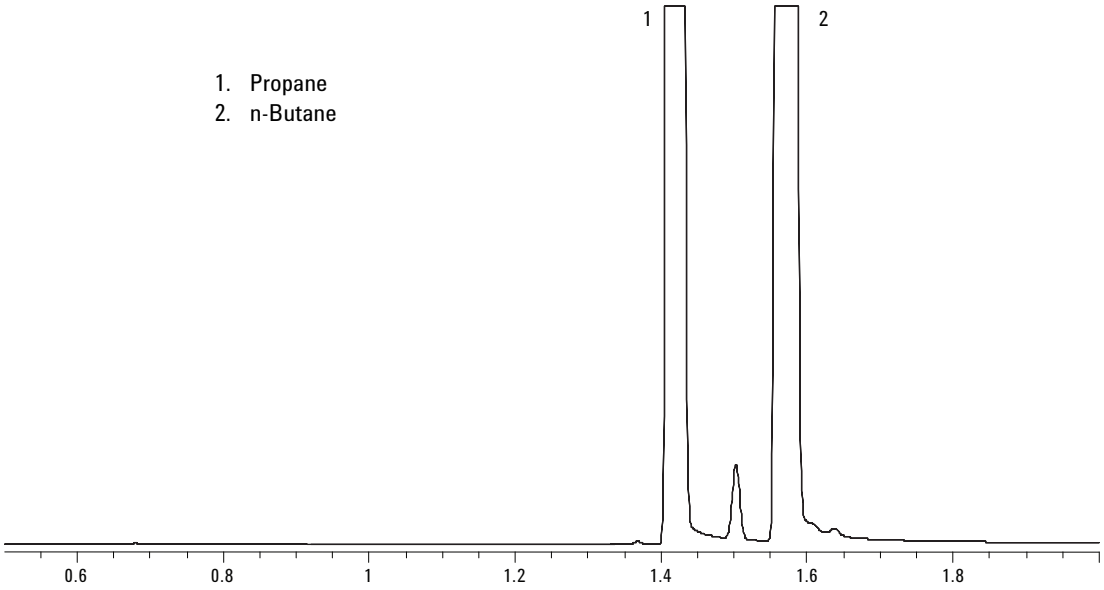


Figure 12. Chromatogram of pressurized propane + n-butane.

Table 4. Quantitative Analysis of Pressurized Propane 50.0% + n-Butane 50.0%. One Percent Difference Between the Blend (actual) and the Analysis Result

	Propane	n-Butane
Response factor	1.03	1.01
Density	0.5139	0.5788
Blend by V%	50.0	50.0
By wt%	47.031	52.969
Analysis		
By area%	45.441	54.559
By wt%	45.927	54.073

F. n-Hexane + 1.0% BP Standard (C5-C18)

To check the quantitative results, a small amount (1.0% BP standard) of C5 to C18 hydrocarbons was added to n-hexane (Figure 13). Table 5 shows the analytical results obtained by adding the C5 to C18 hydrocarbons with both the HPLI device and the automatic liquid sampler (ALS). In Figure 14, chromatograms by HPLI (top) and by ALS (bottom) are shown.

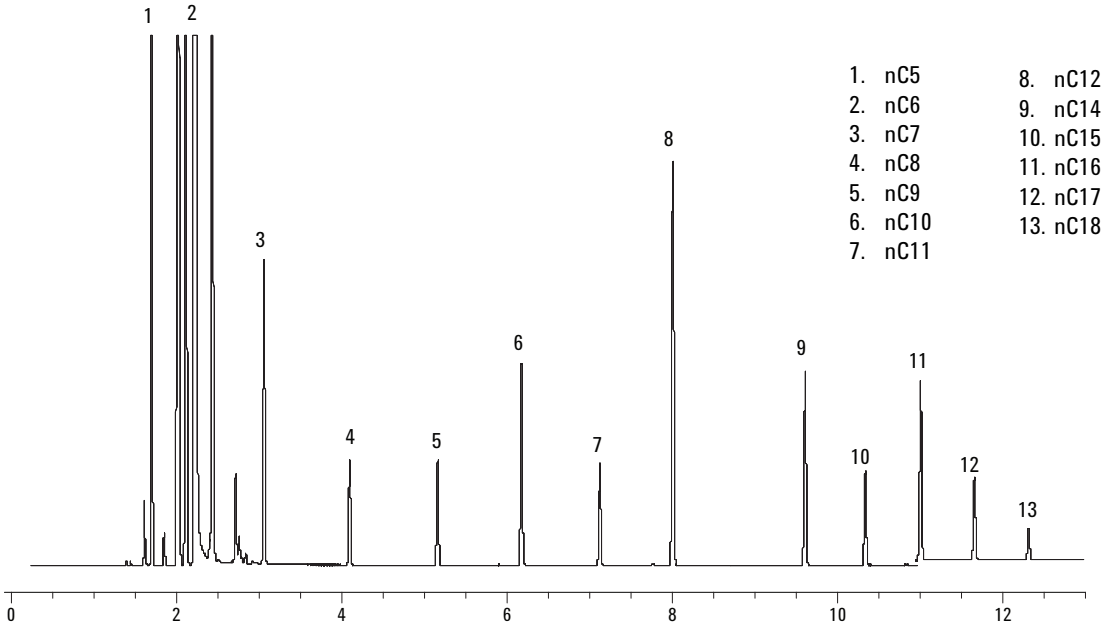


Figure 13. Chromatogram of n-hexane + 1.0% BP standard.

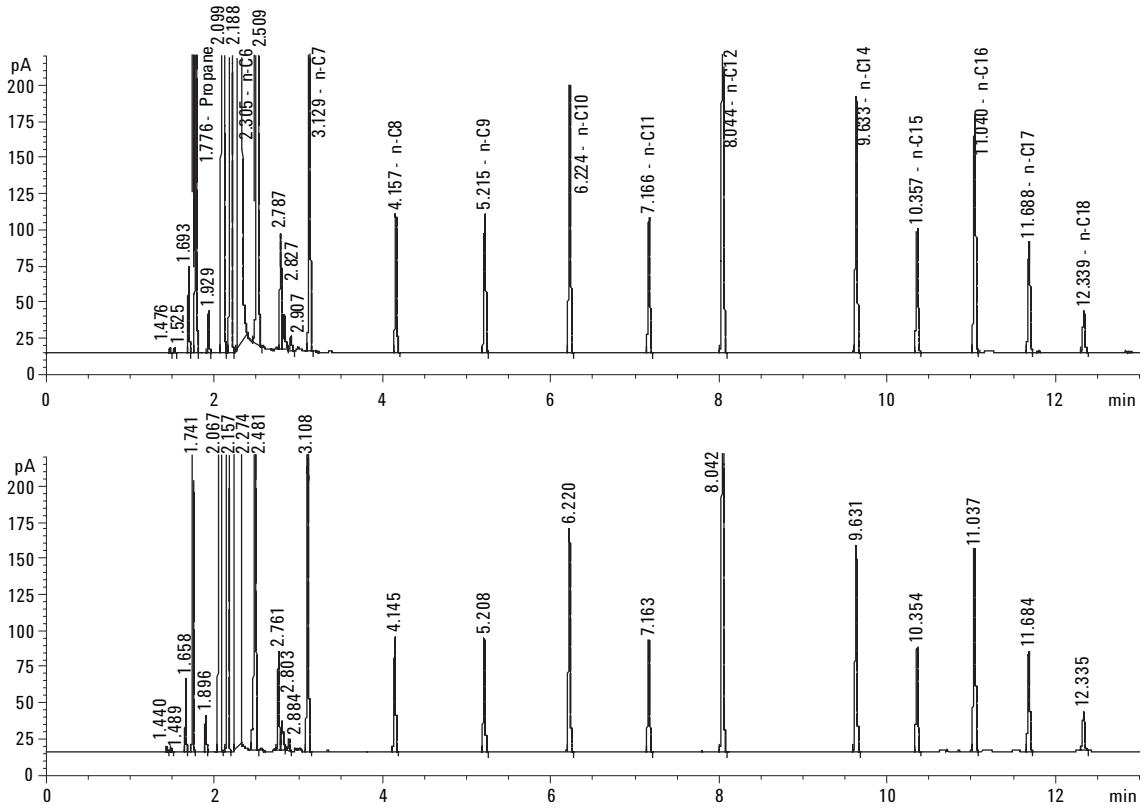


Figure 14. Chromatograms of n-hexane + 1.0% BP standard. Top: HPLI. Bottom: ALS (syringe).

Table 5. Analytical Results for C5-C18 by HPLI and ALS

	HPLI		AUTO INJECTOR	
COMPONENTS	**Area %**	**Width (min)**	**Area %**	**Width (min)**
nC5	0.282		0.279	
nC6	96.950	0.0209	96.922	0.0195
nC7	0.146		0.148	
nC8	0.0524		0.0532	
nC9	0.0537		0.0548	
nC10	0.109		0.111	
nC11	0.0550		0.0559	
nC12	0.219		0.221	
nC14	0.109		0.110	
nC15	0.0532		0.0547	
nC16	0.102		0.109	
nC17	0.0484		0.0546	
nC18	0.0203		0.0239	

The peak width of hexane at top: 0.0209 min
The peak width of hexane at bottom: 0.0195 min

There are no significant differences in quantitative results up to nC14. Compared with the results from an ALS injection, the HPLI device yields results about 10% lower in response above approximately nC16.

G. nC5-nC40 (D2887 BP Standard Diluted by CS_2)

A sample with hydrocarbons (nC5-nC40 D2887 1# BP standard diluted by CS_2) is also run on HPLI. The chromatogram is shown in Figure 15.

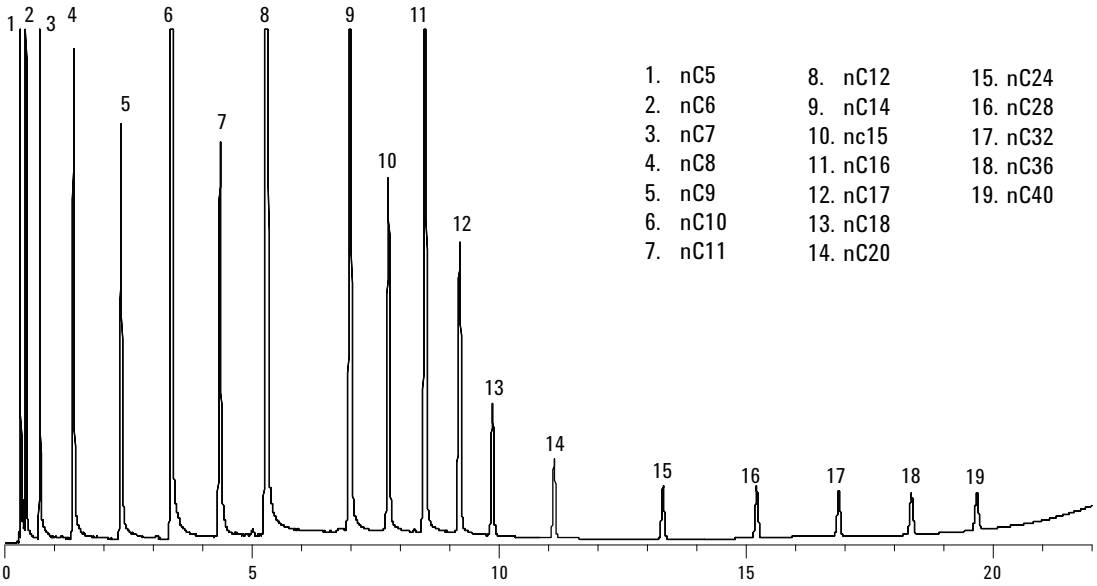


Figure 15. Chromatogram of nC5-nC40 (D2887 BP standard diluted by CS_2).

A lack of discrimination is seen with the HPLI device. In the future, it would be interesting to run some unstable condensates for evaluating the device.

From the above GC evaluation, excellent analytical results could be obtained using the HPLI device. These are summarized below.

1. Excellent repeatability
2. Capable of quantitative results
3. No significant peak width broadening
4. The wide boil point hydrocarbon samples could be analyzed by this device with minimal discrimination.

Conclusions

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC inlet. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

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Acknowledgement

Figures 1 through 4 are courtesy of Ronda Gras and Jim Luong, Dow Chemical Canada, Analytical Sciences.

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Printed in the USA
February 26, 2008
5989-6081EN



Agilent Technologies

Parallel GC for Complete Refinery Gas Analysis

Application

Hydrocarbon Processing

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Abstract

An Agilent 7890A gas chromatograph configured with three parallel channels with simultaneous operation provides a complete, high-resolution analysis for refinery gas in six minutes. The system uses an optimized combination of several packed columns and PLOT alumina columns to allow fast separation of light hydrocarbons and permanent gases with the same oven temperature program. A third channel with TCD with nitrogen (or argon) carrier gas improves the hydrogen sensitivity and linearity. This application also shows the excellent performance for natural gas analysis.

Introduction

Refinery gas is a mixture of various gas streams produced in refinery processes. It can be used as a fuel gas, a final product, or a feedstock for further processing. An exact and fast analysis of the components is essential for optimizing refinery processes and controlling product quality. Refinery gas stream composition is very complex, typically containing hydrocarbons, permanent gases, sulfur compounds, and so on. Successful separation of such a complex gas mixture is often difficult using a single-channel GC system. Three parallel channel analyses allow a separation problem to be divided into three sections. Each channel can optimize a particular part of the separation. TCD with helium carrier gas can be used for permanent gases analysis like O_2, N_2, CO, CO_2, H_2S, and COS. However, hydrogen has only a small difference in thermal conductivity compared to helium, making analysis by TCD using helium carrier gas difficult. To achieve full-range capability for hydrogen, an additional TCD with nitrogen or argon as a carrier is required. Light hydrocarbons are separated on an alumina PLOT column and detected on a FID.

The Agilent 7890A GC now supports an optional third detector (TCD), allowing simultaneous detection across three channels; this provides a complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and hydrocarbons to nC_5, C_6+ fraction within six minutes.

Experimental

A single Agilent 7890A GC is configured with three channels, including one FID, and two TCDs. Light hydrocarbons are determined on the FID channel. One TCD with nitrogen or argon carrier is used for the determination of hydrogen and helium. The other TCD with helium carrier is used for the detection of all other required permanent gases. Figure 1 shows the valve drawing. The system conforms to published methods such as ASTM D1945 [1], D1946 [2], and UOP 539 [3].

The FID channel is for light hydrocarbon analysis. The sample from valve 4 is injected via the capillary injector into valve 3 to permit an early back-

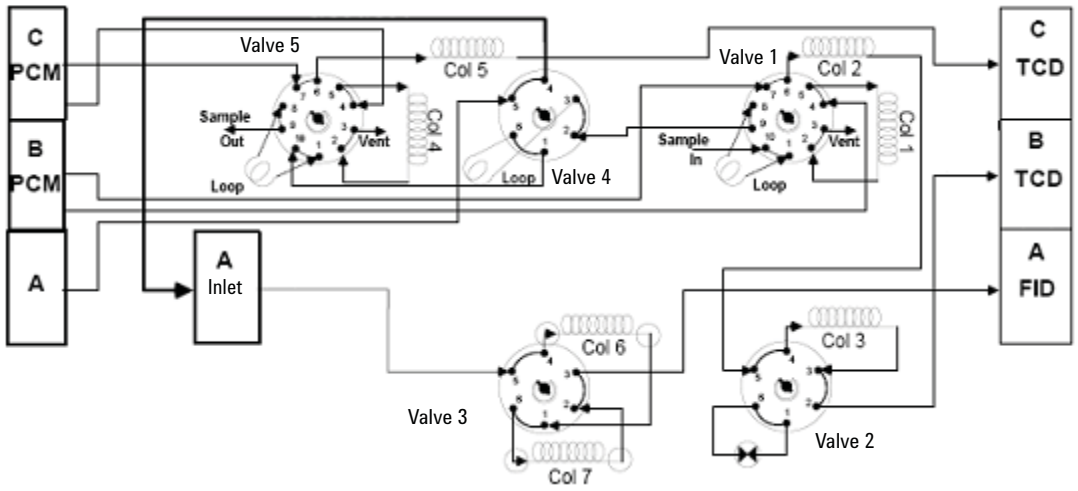
flush of the grouped heavier hydrocarbons (normally C_6+). Valve 3 is a sequence reversal with a short DB1 (column 6) for separating the hexane plus fraction (C_6+) from the lighter components. C_1 through C_5 hydrocarbons are separated on a PLOT alumina column. As soon as the light components C_1 through C_5 pass through the DB1column, valve 3 is switched to reverse the sequence of the DB1 and PLOT aluminum column so that components heavier than nC_6, including nC_6, are backflushed early. As a result, group C_6+ is followed by the individual hydrocarbons from the PLOT alumina column.

A new tube connector based on capillary flow technology is used to connect the valve to the capillary column to enhance the hydrocarbons analysis by improving the peak shape.

The second TCD channel (B TCD) employs three packed columns and two valves for the separation of permanent gases including O_2, N_2, CO, and CO_2 using helium as a carrier gas. Valve 1 is a 10-port valve used for gas sampling and backflushing heavier components; normally components heavier than ethylene are backflushed to vent when H_2S is not required to be analyzed. A six-port isolation valve (valve 2) with adjustable restrictor is used to switch the molecular sieve 5A column in and out of the carrier stream. Initially, the isolated valve is in the OFF position so that unresolved components air, CO, and CH_4 pass quickly through the HayeSep Q (column 2) onto the molecular sieve (column 3). The valve is then switched to the ON position to trap them in column 3 and allow the CO_2 to bypass this column. When the CO_2 has eluted, valve 2 is switched back into the flow path to allow O_2, N_2, CH_4, and CO to elute from the molecular sieve column.

The third TCD channel (C TCD) is for the analysis of H_2. Sample from the 10-port valve (valve 5) is injected into a precolumn (column 4, HayeSep Q) when H_2 with its coeluted compounds O_2, N_2, and CO pass through the short precolumn HayeSep Q onto the molecular sieve 5A column (column 5). Valve 5 is switched so that CO_2 and other compounds will be backflushed to vent, while H_2 is separated on the molecular sieve 5A.

Typical GC conditions for fast refinery gas analysis are listed in Table 1. The refinery gas standard mixture that was used for the method develoment is listed in Table 2.



Column 1 HayeSep Q 80/100 mesh
Column 2 HayeSep Q 80/100 mesh
Column 3 Molsieve 5A 60/80 mesh
Column 4 HayeSep Q 80/100 mesh
Column 5 Molsieve 5A 60/80 mesh
Column 6 DB-1
Column 7 HP-PLOT Al_2O_3
PCM: Electronic pneumatics control (EPC) module

Figure 1. RGA valve system.

Table 1. Typical GC Conditions for Fast Refinery Gas Analysis

Valve temperature	120 °C
Oven temperature program	60 °C hold 1 min, to 80 °C at 20°C/min, to 190 °C at 30 °C/min
FID channel	
Front inlet	150°C, split ratio: 30:1 (uses higher or lower split ratio according to the concentrations of hydrocarbons)
Column	6: DB-1 7: HP-PLOT Al2O3 S
Column flow (He)	3.3 mL/min (12.7 psi at 60 °C), constant flow mode
FID	
Temperature	200 °C
H_2 flow	40 mL/min
Air flow	400 mL/min
Make up (N_2)	40 mL/min
Second TCD channel	
Column	1: HayeSep Q 80/100 mesh 2: HayeSep Q, 80/100 mesh 3: Molecular sieve 5A, 60/80 mesh
Column flow (He)	25 mL/min (36 psi at 60 °C), constant flow mode
Procolumn flow (He)	22 mL/min at 60 °C (7 psi), constant pressure mode
TCD	
Temperature	200 °C
Reference flow	45 mL/min
Make up	2 mL/min
Third TCD channel	
Column	4: HayeSep Q 80/100, mesh 5: Molecular sieve 5A, 60/80, mesh
Column flow (N_2)	24 mL/min, (26 psi at 60 °C), constant flow mode
Procolumn flow (N_2)	7 psi, (24 mL/min at 60 °C), constant pressure mode
TCD	
Temperature	200 °C
Reference flow	30 mL/min
Make up	2 mL/min

Table 2. RGA Calibration Gas Standards

	Compound	% (V/V)		Compound	% (V/V)
1	Methane	5.98	15	i-Pentane	0.101
2	Ethane	5.07	16	n-pentane	0.146
3	Ethylene	2.99	17	1,3-Butadiene	1.46
4	Propane	8.04	18	Propyne	0.476
5	Cyclopropane	0.50	19	t-2-Pentene	0.195
6	Propylene	3.04	20	2-Methyl-2-butene	0.149
7	i-Butane	2.71	21	1-Pentene	0.094
8	n-Butane	2.11	22	c-2-Pentene	0.146
9	Propadiene	0.94	23	n-Hexane	0.099
10	Acetylene	1.72	24	H_2	15.00
11	t-2-Butene	1.55	25	O_2	2.00
12	1-Butene	1.00	26	CO	1.50
13	i-Butene	0.808	27	CO_2	3.00
14	c-2-Butene	1.230	28	N_2	BL

Results and Discussion

Enhance Gas Analysis with Union Connector

The system uses the new union connector based on capillary flow technology for connecting the capillary column to the valve, enhancing the peak shapes in gas analysis and making the connections easier. Figure 2 shows the comparison of peak shapes obtained from a traditional polyamide connector and the new union connecter. With the new union connecter the improvement in peak shape is readily apparent.

Fast Refinery Gas Analysis (RGA)

Use of an optimized combination of several packed columns and a PLOT alumina column allows fast separation of light hydrocarbons and permanent gases with the same oven temperature program without the need of an additional oven.

The separation results from each channel are illustrated in Figure 3.

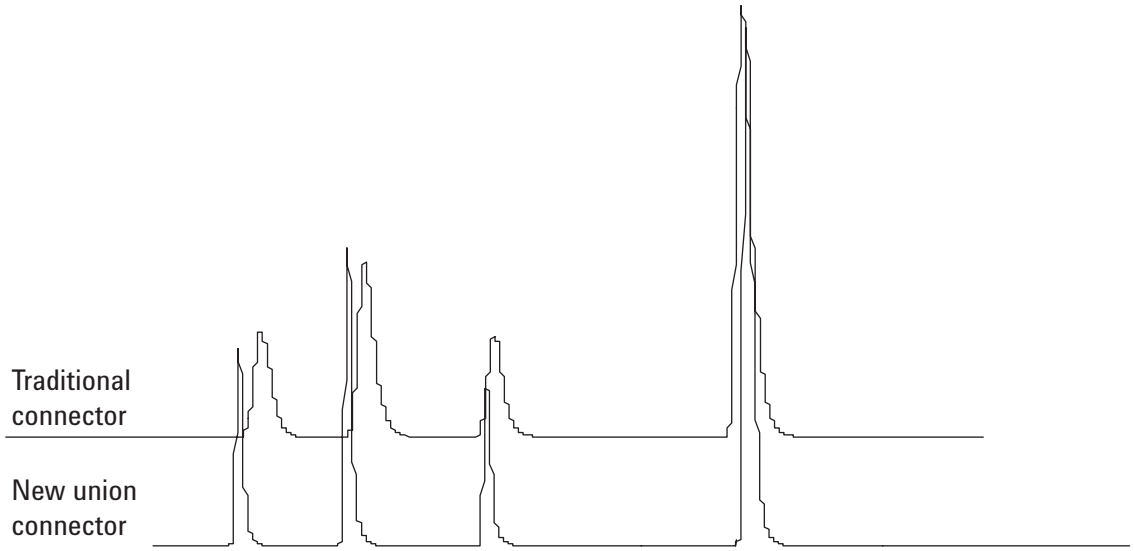


Figure 2. Hydrocarbon peaks obtained from traditional tube connector and new union connector.

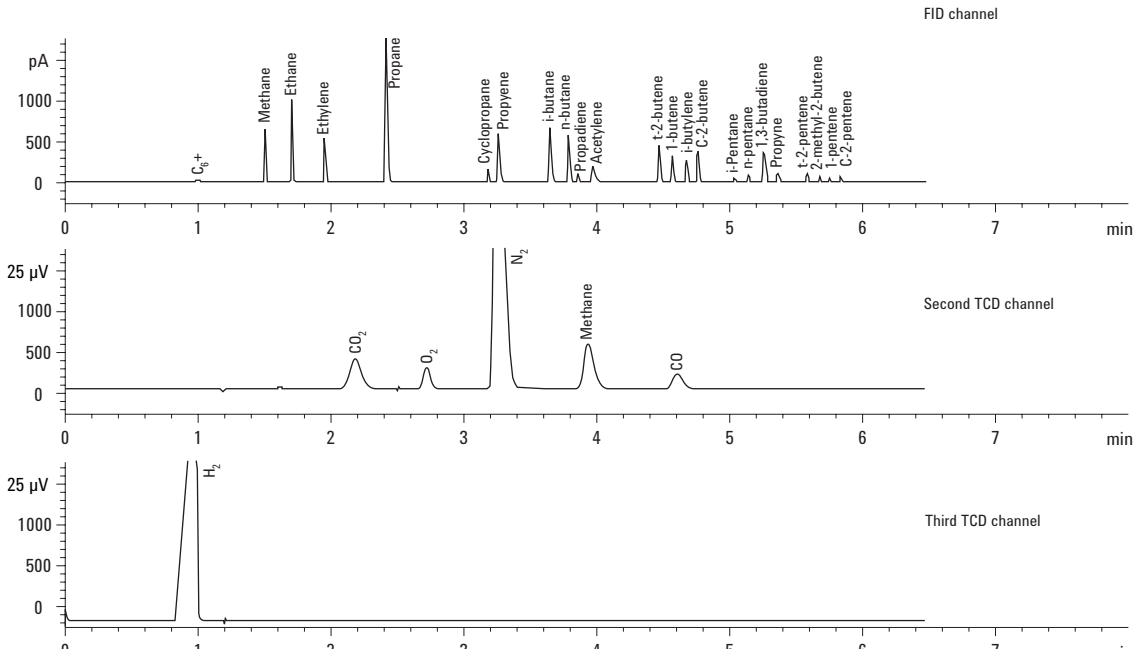


Figure 3. Refinery gas calibration standards analysis. The concentrations for each compound are shown in Table 2.

The top chromatogram (FID channel) is the hydrocarbon analysis. The PLOT alumina column provides excellent separation of hydrocarbons from C_1 to nC_5, including 22 isomers. Components heavier than nC_6 are backflushed early as a group (C_6+) through the precolumn. The middle chromatogram (second TCD channel) is the separation of permanent gases using helium as a carrier gas. The bottom chromatogram (third TCD channel) is the separation of hydrogen, since hydrogen has only a little difference in thermal conductivity compared to helium. Use of an additional TCD with nitrogen (or argon) as a carrier gas improves the hydrogen detectability and linearity.

Table 3 shows very good repeatability for both retention time and area for analysis of the refinery gas standard.

Table3. Repeatability-Refinery Gas Analysis (6 runs) with 1 Run Excluded

	Retention time			Area		
Compounds	**Average**	**Std. dev.**	**RSD%**	**Average**	**Std. dev.**	**RSD%**
C_6+	0.99648	0.00031	0.03	59.01	1.10	1.86
Methane	1.50780	0.00046	0.03	490.02	1.45	0.30
Ethane	1.70788	0.00052	0.03	807.40	2.35	0.29
Ethylene	1.95732	0.00071	0.04	472.31	1.31	0.28
Propane	2.41706	0.00075	0.03	1950.35	5.96	0.31
Cyclopropane	3.18506	0.00075	0.02	145.62	0.45	0.31
Propyene	3.26195	0.00072	0.02	732.90	2.01	0.27
i-butane	3.64883	0.00055	0.02	885.04	3.15	0.36
n-butane	3.79161	0.00070	0.02	682.13	2.59	0.38
Propadiene	3.86098	0.00095	0.02	109.08	0.65	0.60
Acetylene	3.96990	0.00120	0.03	348.17	2.39	0.69
t-2-butene	4.47301	0.00106	0.02	507.88	2.59	0.51
1-butene	4.57118	0.00110	0.02	332.39	2.03	0.61
i-butylene	4.67529	0.00121	0.03	260.95	1.95	0.75
c-2-butene	4.76367	0.00112	0.02	403.80	3.47	0.86
i-pentane	5.03923	0.00090	0.02	45.03	0.05	0.11
n-pentane	5.14583	0.00099	0.02	69.23	0.40	0.58
1,3-butadiene	5.25906	0.00122	0.02	485.49	3.66	0.75
Propyne	5.36385	0.00155	0.03	101.08	0.41	0.40
t-2-pentene	5.58664	0.00121	0.02	82.85	0.66	0.79
2-methyl-2-butene	5.68220	0.00117	0.02	62.54	0.61	0.98
1-pentene	5.75553	0.00126	0.02	39.57	0.38	0.96
c-2-pentene	5.83970	0.00131	0.02	59.08	0.50	0.85
CO_2	2.18561	0.00221	0.10	2040.33	2.37	0.12
O_2	2.72634	0.00060	0.02	930.68	6.53	0.70
N^2	3.25170	0.00044	0.01	22500.18	68.87	0.31
CO	4.61692	0.00083	0.02	903.09	2.77	0.31
H_2	0.9869	0.00099	0.10	16097.38	106.53	0.66

Typical natural gas also can be characterized with the system using the same conditions for the fast RGA. The chromatograms of natural gas on the three channels are shown in Figure 4; hydrogen (3% Mol) and helium (1% Mol) are separated on the third TCD channel.

Flexibility for Hydrocarbon Analysis

The system is very flexible for hydrocarbon analysis. By setting up different valve (valve 3) switch times, the early backflush group can be C_6+ followed by individual C_1 to C_5 hydrocarbons as mentioned in fast RGA, or C_7+ followed by individual C_1 to C_6 hydrocarbons, or no backflush to separate C_1 to C_9 individual hydrocarbons. The top chromatogram in Figure 5 is the result with backflush group of C_6+, the middle one is that of C_7+, and the bottom one is that of no backflush. With such flexibility, a wide range of refinery gas and natural gas compositions can be measured reliably without hardware or column changes.

H_2S and COS Analysis

H_2S and COS (methyl-mercaptan) can be analyzed on the rear TCD channel by adding an additional delay to the backflush time (valve 1) to allow H_2S and COS to elute onto column 2 (HayeSep Q). The analysis time is extended an additional 3 to 4 minutes, and requires a sample containing no water. Figure 6 shows the chromatogram of H_2S at approximately 500 ppm and COS 300 ppm with 1 mL sample size. The Nickel tubing packed columns and Hastelloy-C valves can be chosen for high concentration of H_2S analysis to minimize corrosion.

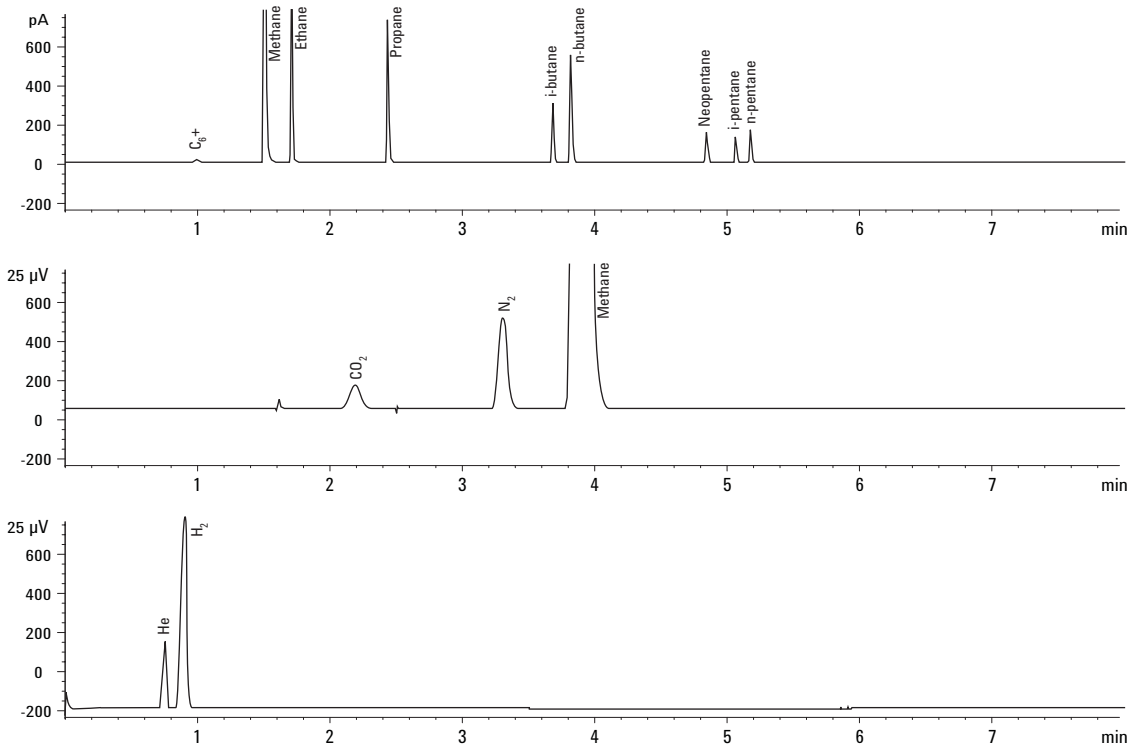


Figure 4. Natural gas analysis of a calibration gas.

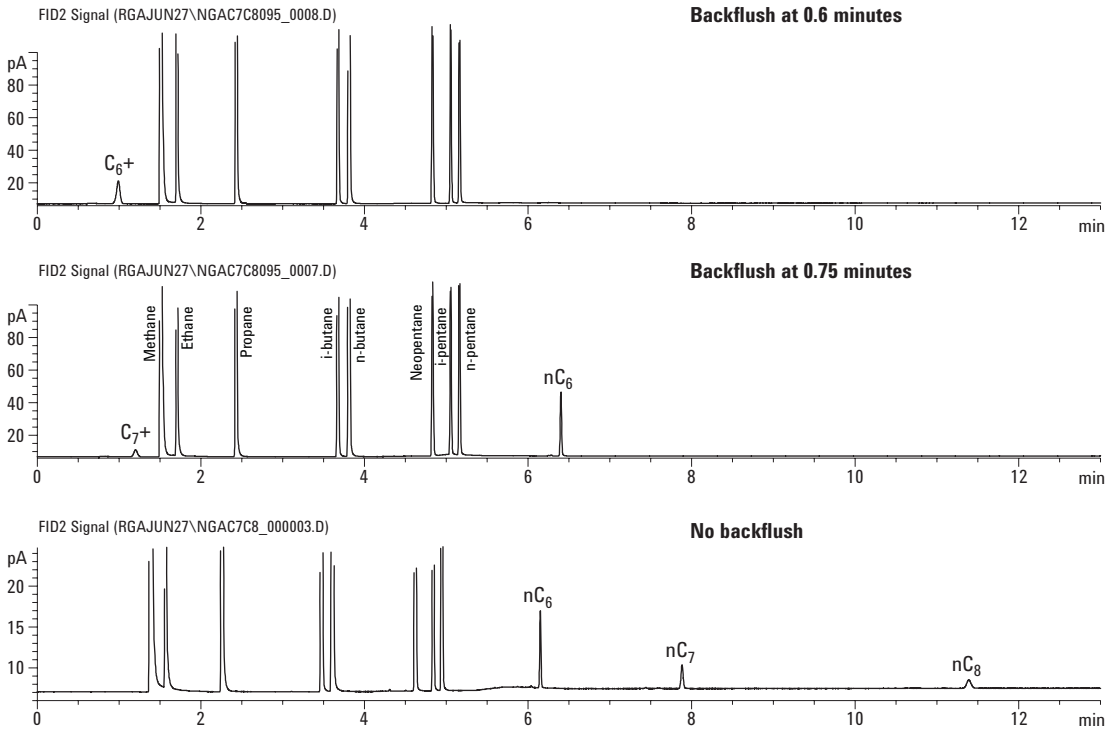


Figure 5. Chromatograms of light hydrocarbons on FID channel with different backflush times .

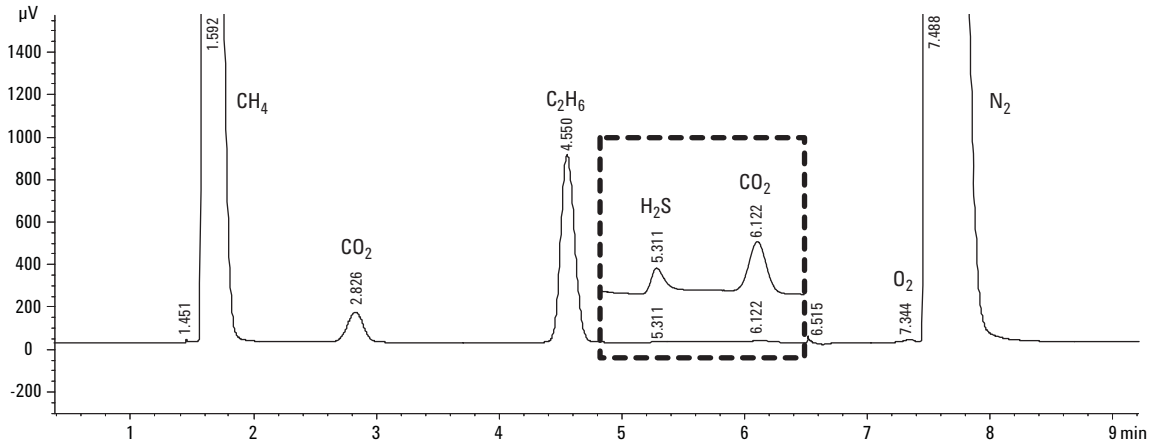


Figure 6. H_2S at approximately 500 ppm and COS 300 ppm on second TCD channel.

Oven program: 50 hold 2 minutes, to 150 °C at 30 °C/min, hold 3 minutes, to 190 °C at 30 °C/min, hold 1 minute
Sample loop: 1 mL

Reporting

A macro program provides automated gas properties calculation. It gives a report in mole %, weight %, volume %, or any combination of the three. If required, heat values for the gas analyzed and other standard calculations are also available. Reports can be calculated using formulas given in the ASTM/GPA or ISO standards.

Conclusions

An exact and fast analysis of the components in refinery gas is essential for optimizing refinery processes and controlling product quality.

One 7890A GC configured with three parallel channels with simultaneous operation provides complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and all hydrocarbons to C_5 and C_6+ as a group within six minutes. A second TCD with nitrogen or argon as a carrier gas improves the hydrogen sensitivity and linearity.

The configuration is very flexible for hydrocarbon analysis, different backflush times may be set to obtain the early backflush group for C_6+ or C_7+, or no backflush to separate C_1 to C_{10} individual hydrocarbons. In these cases, the analysis time is increased by 6 minutes. H_2S and COS can be analyzed on the same GC configuration; it requires 3 to 4 minutes of additional time.

A macro program provides automated gas properties calculation. Reports can be calculated using formulas given in the ASTM/GPA or ISO standards. It gives a report in mole %, weight %, volume %, or any combination of the three.

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Printed in the USA
September 26, 2007
5989-7437EN



Agilent Technologies

Parallel GC for Complete RGA Analysis

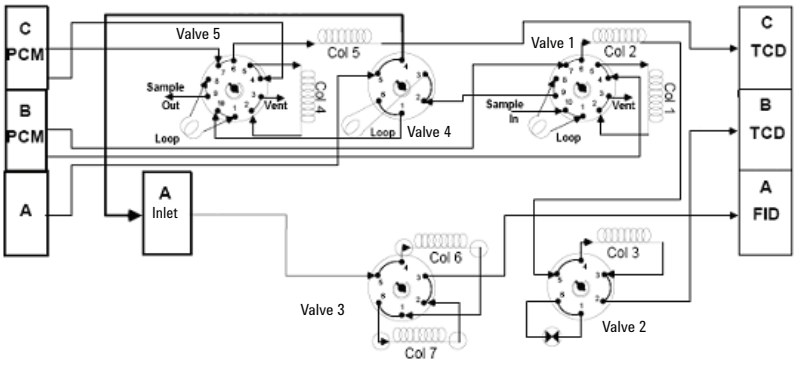
Application Brief

Chunxiao Wang

A previous application brief [1] has shown that a 7890A GC configured with three parallel channels provides a complete refinery gas analysis (RGA) within six minutes. The configuration for fast RGA in the brief has been updated by adding a fifth valve, which can now be supported by the 7890A GC. The updated configuration is almost the same as the previous one except for the third channel (TCD) for H_2 analysis using N_2 or Ar as carrier gas to improve H_2 detectability and linearity. The updated configuration uses a 10-port valve with a pre-column for backflushing late-eluting components while H_2 is separating on the molsieve column instead of a three-way splitter plus split/splitless inlet.

Refinery gases are mixtures of various gas streams produced in refinery processes. They can be used as a fuel gas, a final product, or a feedstock for further processing. The composition of refinery gas streams is very complex, typically containing hydrocarbons, permanent gases, sulfur compounds, etc. An exact and fast analysis of the components is essential for optimizing refinery processes and controlling product quality.

The Agilent 7890A GC now supports an optional detector (TCD), allowing simultaneous detection across three channels. This provides a complete analysis of permanent gases, including nitrogen, hydrogen, oxygen, carbon monoxide,



Column 1 HayeSep Q 80/100 mesh
Column 2 HayeSep Q 80/100 mesh
Column 3 Molsieve 5A 60/80 mesh
Column 4 HayeSep Q 80/100 mesh
Column 5 Molsieve 5A 60/80 mesh
Column 6 DB-1
Column 7 HP-PLOT Al_2O_3
PCM: Electronic pneumatics control (EPC) module

Figure1. RGA valve system.

Highlights

- One 7890A GC configured with three parallel channels with simultaneous detection provides a comprehensive, fast, and high-resolution analysis of refinery gas in 6 minutes.
- Use of optimized columns allows faster analysis of hydrocarbons and permanent gases using a single oven temperature program without the need for an additional column oven.
- A third TCD channel can be used for improving hydrogen detection and linearity by using nitrogen (or argon) as carrier gas.
- A new, easy-to-use union tubing connector based on capillary flow technology is used to connect valves and capillary columns to improve the chromatographic performance, including peak shape.
- Excellent results are achieved. The lowest detection limit is 50 ppm for all compounds, 500 ppm for hydrogen sulfide.
- ChemStation macro program is supplied for RGA reporting.
- The system can be obtained by ordering option SP1 7890-0322 for the standard fast RGA and 7890-0338 for the fast RGA with Hastelloy valves and nickel tubing for H_2S containing samples on the 7890A.

carbon dioxide, and hydrocarbons to nC6. The total run time is less than 6 minutes. The configuration is suitable for most refinery gas streams such as atmospheric overhead, FCC overhead, fuel gas, and recycle gases.

In this analysis, a single Agilent 7890A GC is configured with three channels, including an FID channel and 2 TCD channels. Light hydrocarbons are determined on the FID channel using an alumina column. One TCD is used with nitrogen or argon carrier gas for improved determination of hydrogen and helium; the other TCD is used with helium carrier for the detection of all other required permanent gases. The configuration is shown in Figure 1. An Agilent union tube connector, based on capillary flow technology, is used to quickly and easily connect the valve and capillary column for improved performance. The system conforms to published methods such as ASTM D1945 [2], D1946 [3], and UOP 539 [4].

Separation resulting from each channel is illustrated in Figure 2. The top chromatogram shows the hydrocarbon analysis. A PLOT AL2O3 column provides excellent separation of hydrocarbons from C1 to nC5 containing 22 isomers. Components heavier than nC6 are backflushed early in the run as a group (C6+) through a short DB-1 pre-column.The middle chromatogram shows the separation of permanent gases using helium as the carrier gas on the second TCD channel (B TCD). H_2S and COS can be analyzed on the second TCD channel as well, requiring 3 to 4 additional minutes. The bottom chromatogram shows the separation of hydrogen. Because hydrogen has only a small difference in thermal conductivity compared to helium, it requires an additional TCD with nitrogen or argon as the carrier gas to improve the hydrogen detectability and linearity. All channels operate simultaneously to provide a comprehensive, fast analysis with high resolution of components. A macro program automatically provides the calculation of gas properties. Reports can be generated using formulas specified in the ASTM/GPA and/or ISO standards. Reports in mole%, weight%, volume%, or any combination of the three are available.

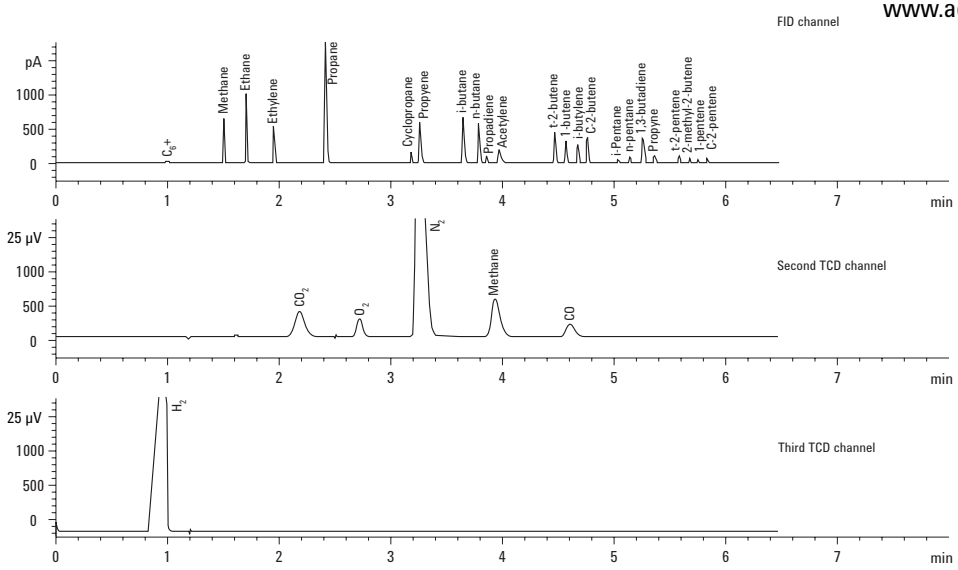


Figure 2. Refinery gas calibration standards analysis.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Reference

1. Chunxiao Wang, "Parallel GC for Complete RGA Analysis,"Agilent application brief, 5989-6103EN, January 19, 2007
2. ASTM D1945-03, "Standard Test Method for Analysis of Natural Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. ASTM D1946-90 (2006), "Standard Practice for Analysis of Reformed Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
4. UOP Method 539, "Refinery Gas Analysis by Gas Chromatography,"ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428, USA.

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Published in the USA
December 12, 2008
5989-7438EN

High-Pressure Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Accessory G3505A

Introduction

Gas chromatography sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve, has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and an auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Injection Device

The high-pressure injection device (HPLI) consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Company.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

Ordering Information

Order accessory G3505A. The accessory is compatible with both the 7890A and 6890 series GCs.

The following components are recommended. These are not supplied in the accessory kit.

- **Filter:** To remove particles from samples.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

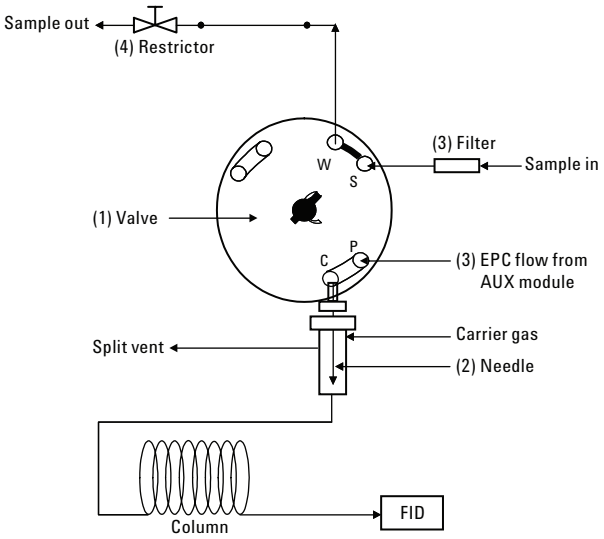


Figure 1. Flow diagram of the high-pressure injection device (HPLI).

Typical Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	High-pressure injection device (HPLI) at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.06 µL
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

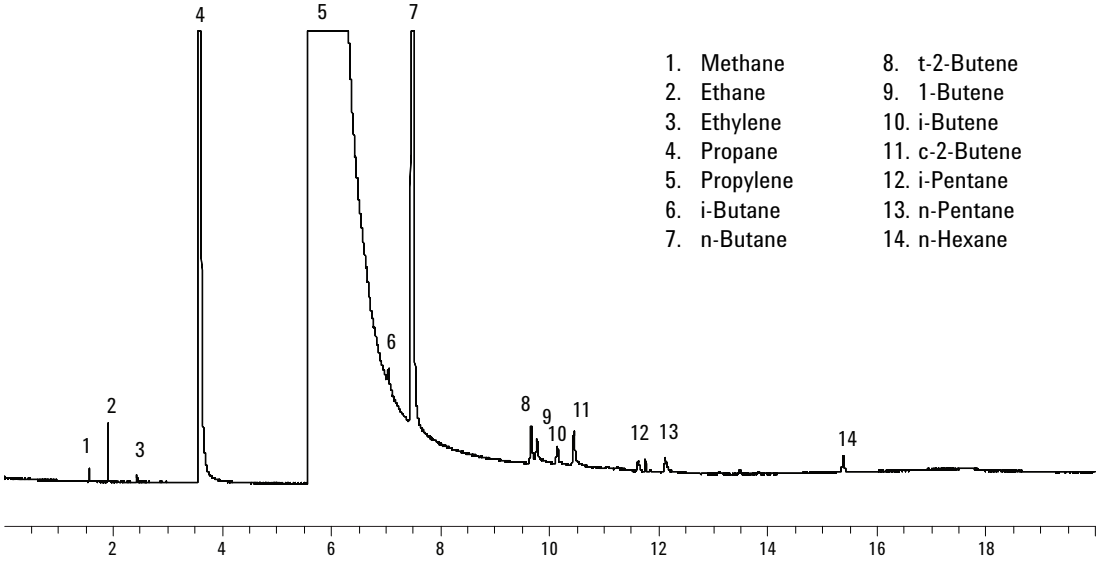


Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Sample Chromatograms

Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159. A typical chromatogram is shown in Figure 2.



1. Methane
2. Ethane
3. Ethylene
4. Propane
5. Propylene
6. i-Butane
7. n-Butane
8. t-2-Butene
9. 1-Butene
10. i-Butene
11. c-2-Butene
12. i-Pentane
13. n-Pentane
14. n-Hexane

Figure 2. Chromatogram of pressurized propylene.

Pressurized 1,3-Butadiene

Figure 3 is an example of C4 hydrocarbons analysis showing 1.3 butadiene purity.

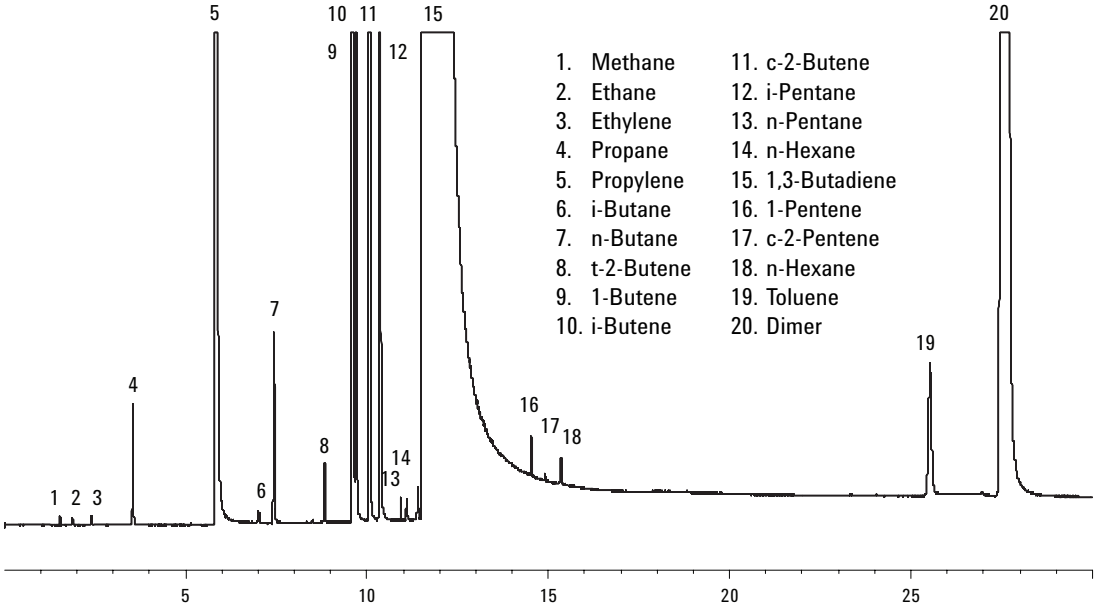


Figure 3. Chromatogram of pressurized 1,3-butadiene.

Summary

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC split/splitless inlet in a few minutes. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

Competitive Advantages

The HPLI can be used with a wide variety of sample streams or pressurized vessels. Because the sampling valve is interfaced directly to the inlet with an inert needle, loss or adsorption of analytes is minimized compared to conventional liquid sample valve systems. Compared to other gas chromatographic vaporizers for handling pressurized or nonpressurized samples, the Agilent HPLI has the following advantages:

- Better results with polar analytes such as glycols
- Superior inertness
- Low discrimination (no discrimination up to C_{16})
- Flexibility: Install or uninstall in less than 10 minutes
- Good for trace impurity analysis with 0.5 µL rotor
- Excellent repeatability, typically RSDs below 1 %

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Printed in the USA
February 25, 2008
5989-8037EN



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Crude Oil & Natural Gas

Sulfur & Odorants

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Volatile Sulfur in Natural Gas, Refinery Gas, and Liquified Petroleum Gas

Application

Gas Chromatography

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USA

Abstract

An Agilent 6890 gas chromatograph equipped with an FPD (flame photometric detector) is used to characterize low level sulfur compounds in natural gas, refinery gas, and liquified petroleum gas (LPG) using a J&W 105 m × 0.53 mm × 5.0 μm DB-1 column. Analysis of volatile sulfur to less than 100 ppb can easily be performed with a volatiles interface (VI) connected to a 6-port gas-sampling valve. The system as configured provides a cost-effective solution for the determination of odorants in natural gas. Coelutions of hydrocarbons and sulfur compounds that result in signal quenching are documented.

Introduction

Monitoring of low-level volatile sulfur compounds in light hydrocarbon streams such as refinery gas, and in fuels including natural gas and LPG, persist as measurement challenges. To highlight one application area, odorant monitoring is an essential measurement need of the natural gas industry. Table 1 lists a number of the commonly used additives in the United States and Europe. Europe currently favors t-butyl mercaptan, methyl ethyl sulfide, ethyl mercaptan and tetrahydrothiophene. Odorant quality, including characterization of contaminants and possible reaction byproducts, is also important. Optimal odorization is also dependent on the quality of the natural gas stream, making measurement of the naturally occurring sulfur important for optimal metering of odorant addition and for monitoring odor threshold.

Table 1. Common Odorizers in Natural Gas

Methyl mercaptan	Ethyl mercaptan
Dimethyl sulfide	Isopropyl mercaptan
Methyl ethyl sulfide	Normal propyl mercaptan
Tertiary butyl mercaptan	Secondary butyl mercaptan
Diethyl sulfide	Tetrahydrothiophene
Ethyl isopropyl sulfide	

Sulfur selective measurements can assist in blending operations to assure proper ratios of components are injected into the pipeline, and to ensure that effects such as pipeline oxidation are understood.

Natural gas and other light hydrocarbon streams are finding use as fuel feedstocks for a variety of fuel cell technologies. Fuel cells and the reformer catalysts are generally not sulfur tolerant. Depending on the technology employed, sulfur can be a poison at single digit ppm levels. The need for low level sulfur measurement in the fuel cell industry will continue to grow as the various technologies see wider deployment. Odorant monitoring at various locations within a gas distribution system can also be important.

Gas chromatography plays an important role in sulfur measurement. The flame photometric detector (FPD) is ideal for many of these applications, given its low cost and ease-of-use, provided coelution can be avoided. Selection of the appropriate column, temperature program, and sample introduction system are key to the deployment of a successful system. This work illustrates what can be done with a methyl silicone column (105 m × 0.53 mm × 5.0 µm DB-1) without use of cryogenic oven cooling.

ASTM method D 5504-94 details a chemiluminescence approach to the analysis of sulfur in various gaseous streams including natural gas. Other sulfur selective detectors are not excluded from the method provided they meet criteria for sensitivity and hydrocarbon interference. The system described in this paper generally follows the method, pointing out situations where particular hydrocarbon matrices can cause quantitative problems. A subset of the sulfur compounds listed in the ASTM method is used.

Experimental

An Agilent 6890 gas chromatograph equipped with a FPD operating in a hydrogen rich mode for optimal sensitivity was used in this work. Sample introduction consisted of a 6-port Hastelloy C gas sample valve (GSV) interfaced directly to the volatiles interface (VI) with Sulfinert tubing (Figure 1). Instrument conditions are given in Table 2. A point-of-use gas blending system was used for preparation of ppb level sulfur compounds in the hydrocarbon matrices. Figure 2 illustrates the basic components and configuration of the gas blending hardware. The details of this system have been described

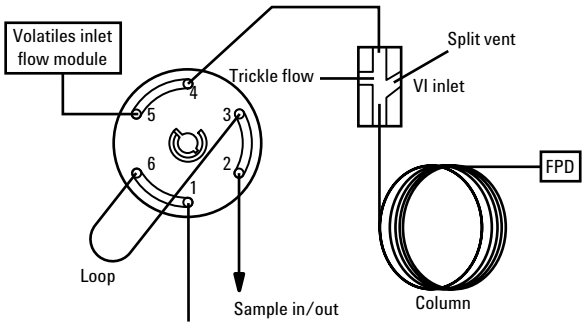
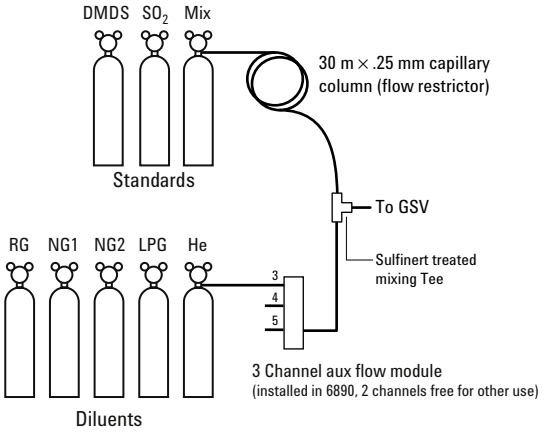


Figure 1. Sample introduction scheme.

Table 2. Instrument Conditions

Agilent 6890 Gas Chromatograph	
Detector	**Flame Photometric**
Temperature	250 °C
Hydrogen Flow	50 mL/min
Air Flow	60 mL/min
Makeup	N_2 60 mL/min, constant mode
Filter	393 nm
Injection Source	**6-port Gas Sampling Valve**, Hastelloy C
Temperature	120 °C
Loop	0.50 cc Sulfinert treated (replaces standard loop)
Injection Port	**Volatiles Interface**
Temperature	120 °C
Split Ratio	0.2 to 1 typical
Carrier Gas	Helium
Constant Flow Mode	
Columns	**105 m × 0.53 mm × 5.0 µm DB-1, J&W Cat. No. 125-10B5**
Oven Program	
Initial Temperature	35 °C
Initial Time	5 min
Temperature Ramp	25 °C/min
Final Temperature	240 °C
Final Time	5 min
Aux EPC	
Restrictor	Medium flow resistance frits, Part no. 19231-60700



Cylinder codes:
- RG - Refinery gas
- NG1 - High methane natural gas
- NG2 - High ethane natural gas
- LPG - Liquified petroleum gas
- Mix - 8 component sulfur mix
- DMDS Dimethyl disulfide

Figure 2. Point-of-use automated blending system.

previously.[1] Sulfur components and their respective cylinder concentrations in the calibration mix are listed in Table 3. The mix was obtained from DCG Partnership 1, LTD., Pearland, TX, 281-648-1894, The GPA natural gas mixtures were purchased from Scott Specialty Gases.

Table 3. Sulfur Calibration Mix

Number	Compound	Conc.(ppm)
1	Hydrogen sulfide	5.00
2	Carbonyl sulfide	4.96
3	Methyl mercaptan	5.00
4	Ethyl mercaptan	5.04
5	Dimethyl sulfide	4.91
6	Carbon disulfide	5.01
7	t-Butyl mercaptan	5.04
8	Tetrahydrothiophene	5.05

Results

Sensitivity will always be the first and perhaps most important attribute of a selective detector. This should be well understood prior to tackling complex application problems. First, note that the FPD is a non-linear detector due to the mechanism of S2 formation from sulfur atoms in the flame. Excited S2 is responsible for light emission at approximately 393 nm, which gives the detector its selectivity. A comprehensive review of various sulfur selective detectors and applications have been previously discussed.[2] To establish the performance potential of the 6890-FPD system, specifically in terms of sensitivity, a dilution study was conducted where the 8 component mix was systematically diluted in helium to obtain concentrations from 50 ppbv to approximately 400 ppbv. Programming the Aux EPC over a pressure range from 60 psig to 10 psig automatically does this at a sulfur calibration mix flow of 0.9 mL/min. The pressure needed to achieve this mix flow is set from the cylinder regulator. Four methods were setup, each with a different Aux pressure setting, and subsequently used in the ChemStation sequence table. The resulting calibration curve in log-log format for one of the components, ethyl mercaptan, is shown in Figure 3. Figure 4 shows an FPD chromatogram of the sulfur in helium mix at 78 ppbv per component, obtained from one of the methods used in the automatic dilution sequence. Good signal to noise is seen even at this low sulfur level.

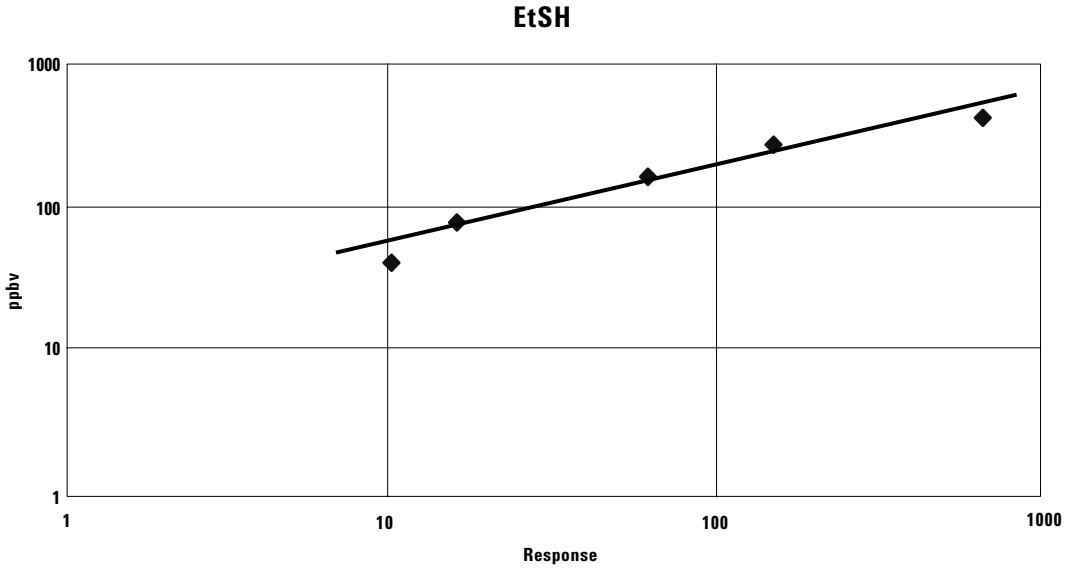


Figure 3. Calibration of ethyl mercaptan in helium from 400 ppbv to 50 ppbv.

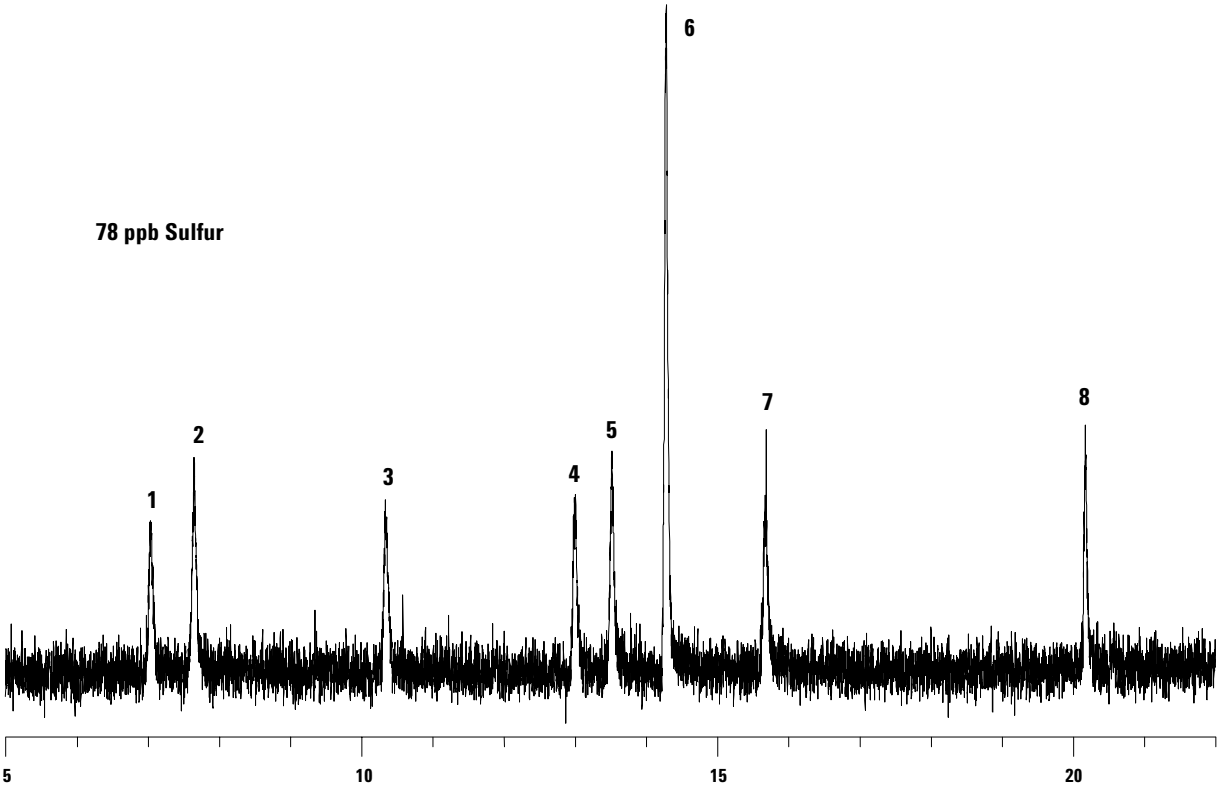


Figure 4. Eight volatile sulfur compounds at 78 ppbv per component. Detector - Flame Photometric. See Table 3 for peak id's.

Prior to use of the FPD, the Agilent atomic emission detector (AED) was used to characterize potential hydrocarbon-sulfur coelutions and false hydrocarbon responses that result when the selectivity of the detector is exceeded. Both carbon and sulfur chromatograms can be collected simultaneously, allowing potential interferences that would lead to signal quenching on the FPD to be quickly identified. Figures 5, 6 and 7 show overlaid sulfur and carbon chromatograms for high ethane natural gas, high methane natural gas, and refinery gas, respectively.

These chromatograms were produced by blending the sulfur mix into the hydrocarbon matrices to produce sulfur levels of 145 ppbv per component. The AED carbon chromatograms shown are due to the hydrocarbon matrix since the contribution of the carbon in the sulfur compounds is exceedingly small.

Coelutions of carbonyl sulfide/propane and methyl mercaptan/t-2-butene are clearly identified. Therefore, in natural gas streams, analysis of low level COS will be problematic on the FPD when using the methyl silicone column. This is not a major limitation since COS is normally not found in natural gas streams beyond the well head. However, most other volatile sulfur compounds found naturally or added as odorants should be quantifiable over the sensitivity range of the detector. For more complex hydrocarbon streams such as refinery gas, the additional coelution of methyl mercaptan/ t-2-butene must be watched.

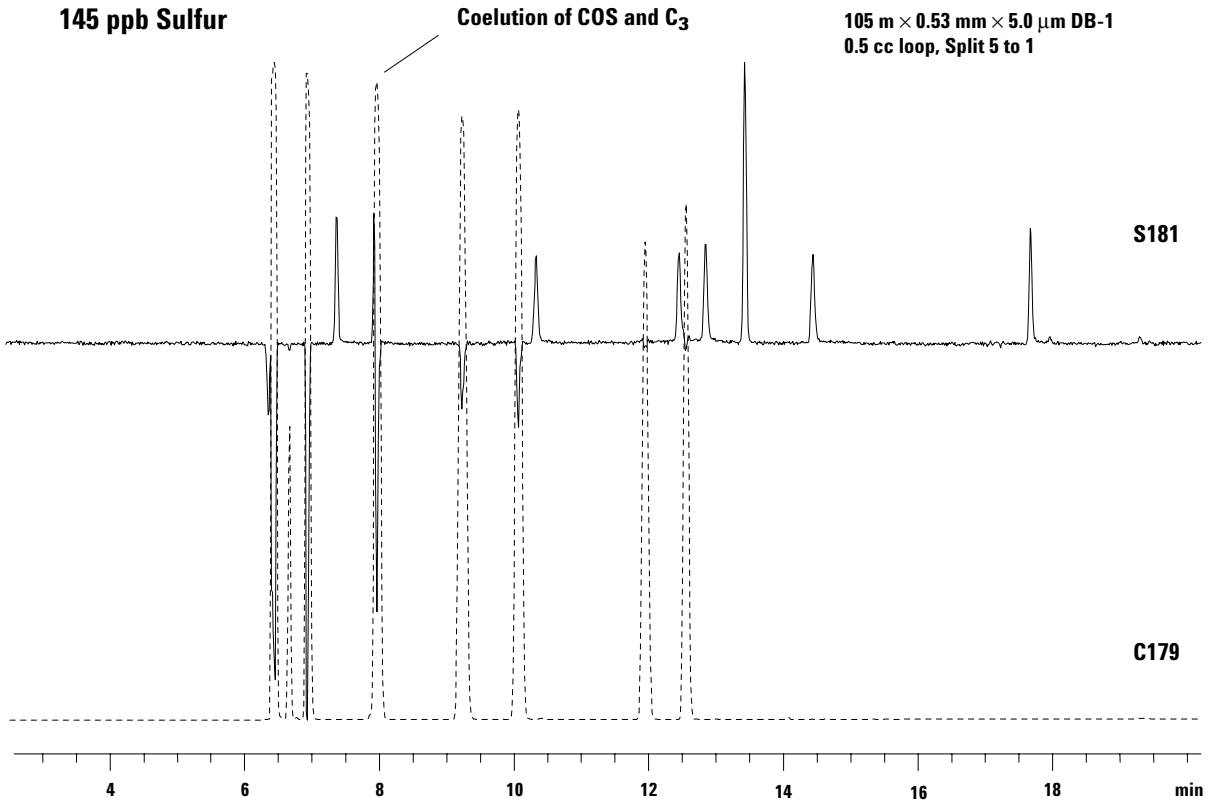


Figure 5. AED carbon and sulfur chromatograms of high ethane (9%) natural gas blended with the eight component sulfur mix. Dashed line is carbon. The carbon and sulfur chromatograms are not drawn to the same scale.

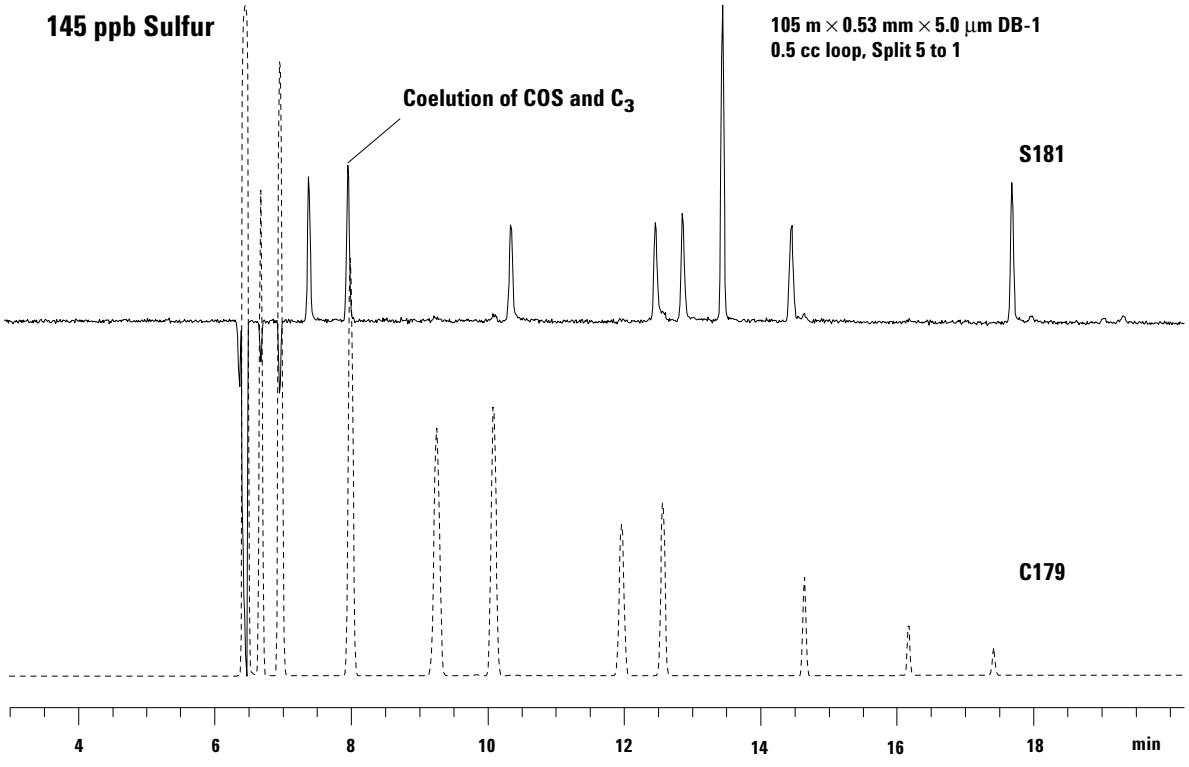


Figure 6. AED chromatograms of high methane natural gas blended with the sulfur calibration mix. Dashed line is carbon.

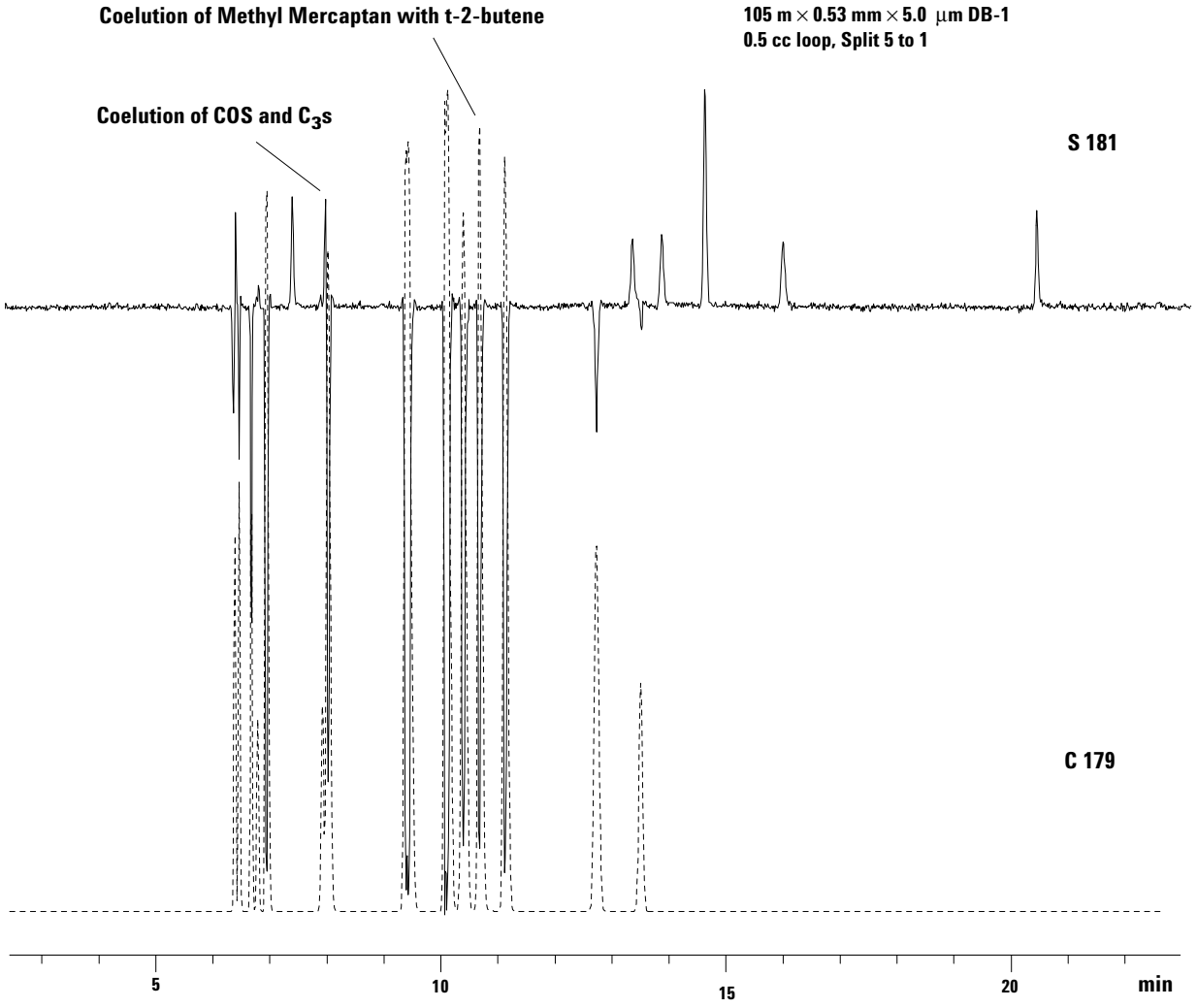


Figure 7. AED chromatograms of refinery gas blended with the sulfur calibration mix. Dashed line is carbon.

Once potential interferences have been characterized, the dynamic blending system can be used to mix various hydrocarbon matrices with the sulfur calibration mix to simulate real world samples and analytical problems. With this information in hand, the method developer and routine user can confidently use the system to identify and quantify a variety of low-level volatile sulfur compounds.

Examples of sulfur compounds blended with high methane (2 ppm and 410 ppb/sulfur compound) and high ethane natural gas (120 ppb/sulfur compound) are shown in Figures 8 and 9, respectively. The upper chromatogram in Figure 8 shows sulfur components at 2 ppm in high methane natural gas. This is representative of a typical range of odorant addition. Only COS cannot be reliably quantified at these levels due to quenching. All common natural gas odorants are cleanly separated from hydrocarbons and should be easily quantified with the FPD.

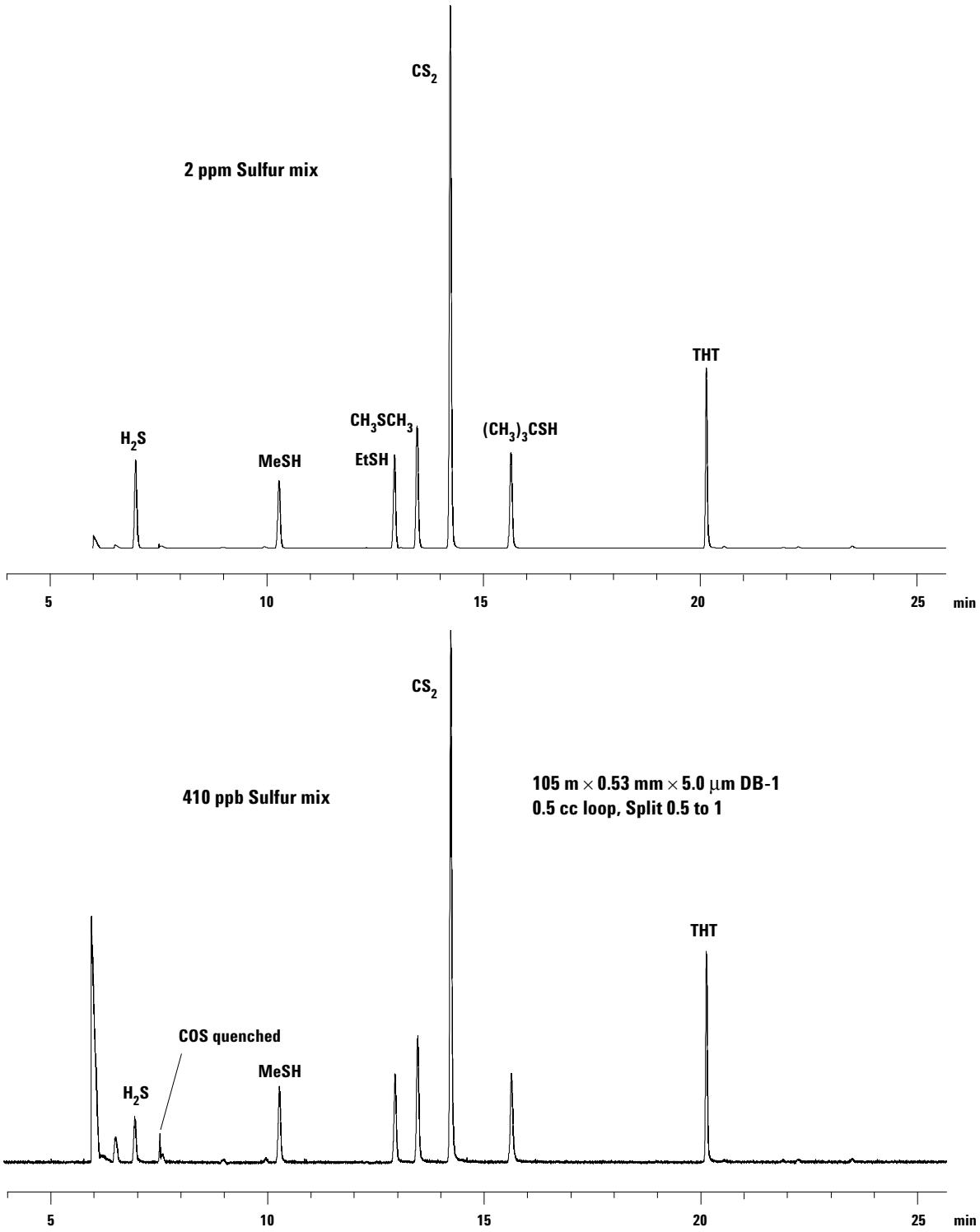


Figure 8. Sulfur mix blended with high methane natural gas at 2 ppm and 410 ppb. Detector - Flame Photometric.

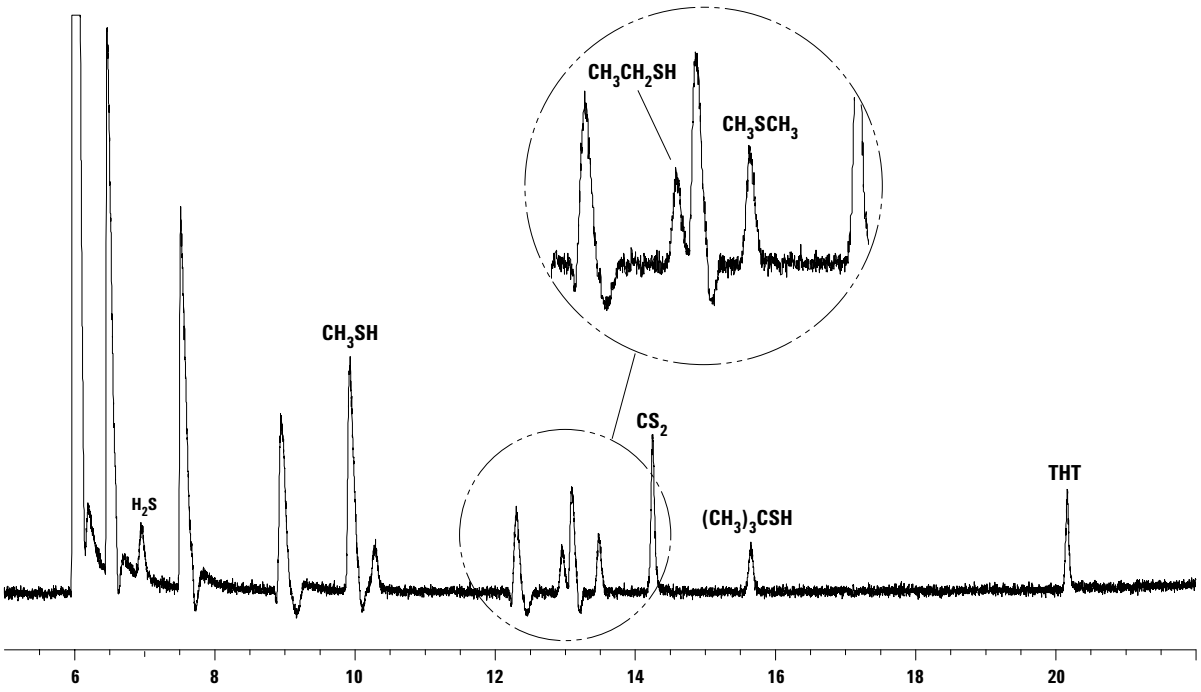


Figure 9. Sulfur mix blended with high ethane natural gas. Sulfur level: 120 ppb. Detector - Flame Photometric.

Refinery gas presents a more challenging matrix from potential sulfur coelutions with the relatively large number of C4 and C5 isomers. In Figure 10, 120 ppb sulfur mix in a refinery gas qualitative standard is shown. From the AED work, measurement of COS and CH_3SH at these 100 ppb sulfur levels is expected to be erroneous. Peaks labeled 1, 4, 5, 6, 7, and 8 (see figure) identifies the six sulfur compounds from among the false hydrocarbon response peaks. These six sulfur species can be easily quantified.

The last example shown in Figure 11, illustrates the measurement of sulfur in LPG. Ethyl mercaptan, the most common odorant used in LPG is seen at approximately 2.5 ppm. The presence of methyl mercaptan seen at approximately 10 minutes RT may be naturally occurring in origin. Two peaks at 20.5 and 22.0 minutes are unidentified sulfur compounds.

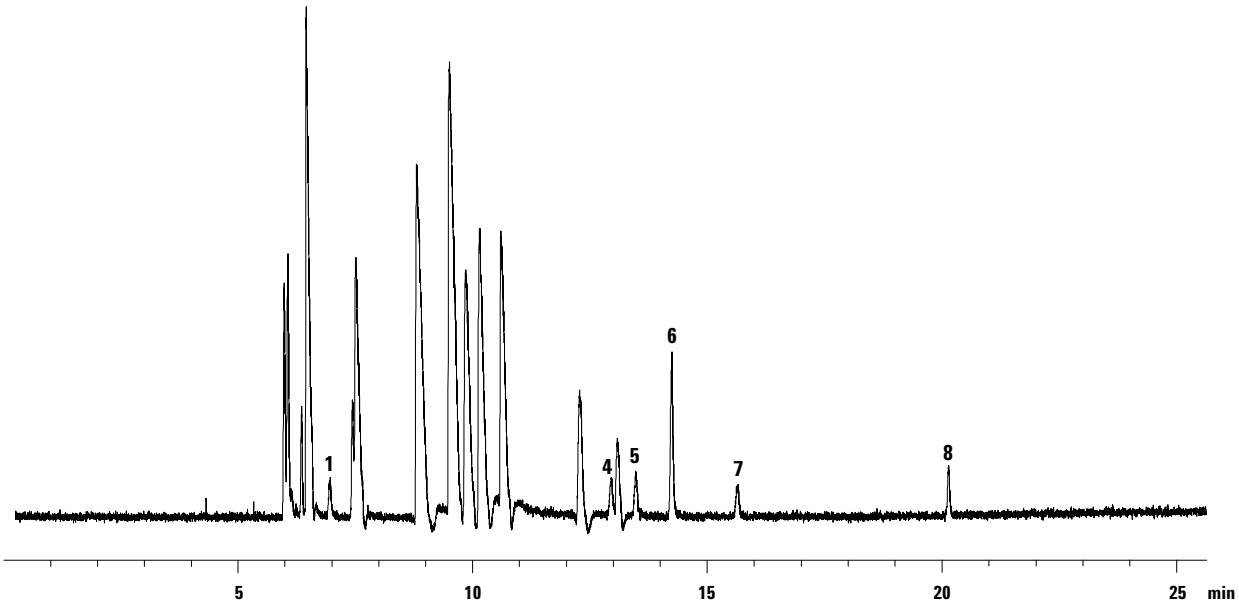


Figure 10. Sulfur mix blended with refinery gas. Sulfur level: 120 ppb. Detector - Flame Photometric. See Table 3 for peak id's.

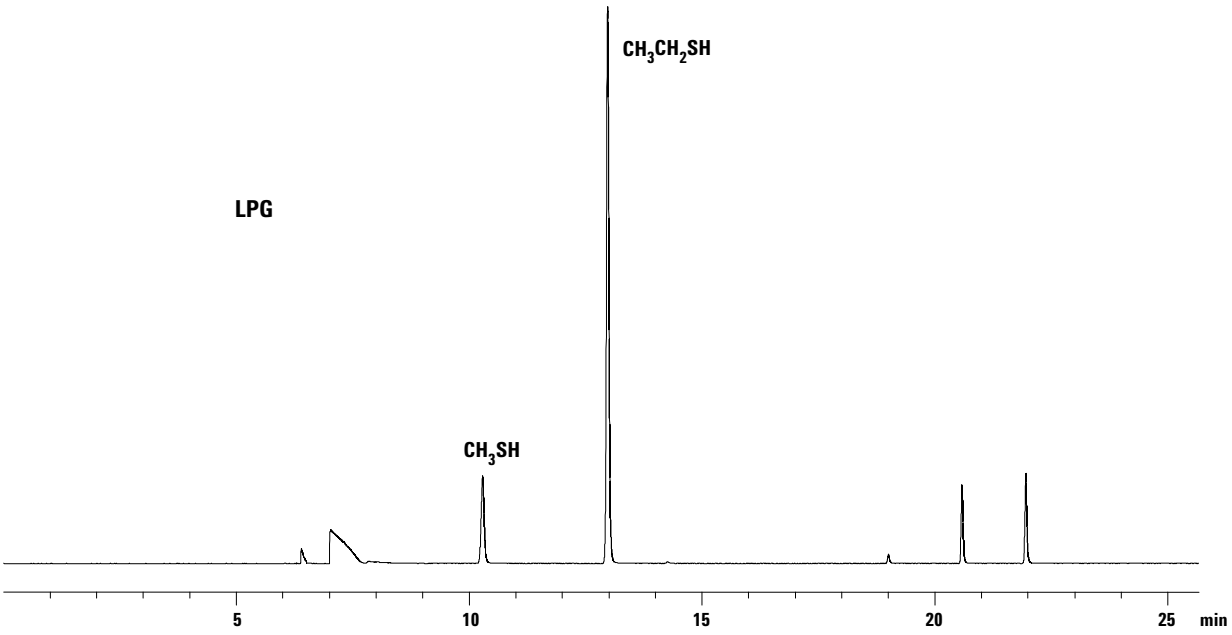


Figure 11. FPD analysis of LPG showing ethyl mercaptan at 2.5 ppm.

Conclusions

Many sulfur selective detectors cannot be characterized as easy to use or low maintenance instruments. The FPD is an exception to this rule with setup, operation, and stability on par with a standard FID. When the gas chromatograph is carefully configured with inert sample path components and optimized sample introduction hardware, reliable routine detection of volatile sulfur components under 50 ppb is achievable. Although the FPD is subject to quenching by high hydrocarbon concentrations, careful selection of the column can largely eliminate the problem. The 105 m × 0.53 mm × 5.0 µm DB-1 offers a high level of inertness, capacity, and efficiency for volatile sulfur analysis. Dynamic blending, controlled by the Aux EPC offers an automatable means of system calibration.

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2. R. L. Firor and B.D. Quimby, *A Comparison of Sulfur Selective Detectors for Low Level Analysis in Gaseous Streams*, Publication Number 5988-2426, April 2001 (Downloadable from www.agilent.com)

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Printed in the USA
May 17, 2001
5988-2791EN



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Use of GC/MSD for Determination of Volatile Sulfur: Application in Natural Gas Fuel Cell Systems and Other Gaseous Streams

Application

Fuel Cells, Petrochemicals

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Abstract

The mass selective detector is an ideal tool for the analysis of trace level volatile sulfur compounds. It is differentiated from other sulfur-selective detectors in that structural information is provided. When operated in the Selected Ion Monitoring mode, excellent sensitivity and selectivity is obtained. Eight volatile sulfur compounds are used to demonstrate low parts-per-billion analysis in a variety of hydrocarbon matrices. The system is well suited for fuel cell developers for characterization of fuel feedstocks and the analysis of impurities that can poison critical catalytic processes. Measurement of carbonyl sulfide in propylene is also demonstrated.

Introduction

Sulfur detectors are finding widespread use in a broad range of applications that cut across many industries. Demand for low-level sulfur detection will only increase in the future in response to more stringent quality control and regulation. The significance and need for low level sulfur measurements have been detailed in previous Agilent application literature [1, 2, 3, 4].

Emerging needs are found in alternative energy applications such as the fuel cell industry. Fuel processors serve a vital role in many fuel cell systems and are sensitive to feedstock composition and impurities. Potential fuels include hydrogen, natural gas, propane, methanol, gasoline and other hydrocarbon streams. Near-term development is concentrating on reformed hydrocarbon fuels creating a need to monitor composition and impurities. Fuel contaminants can adversely affect performance of the fuel cell system. This is especially true for Polymer Electrolyte Membrane (PEMFC) and Molten Carbonate (MCFC) types, although Phosphoric Acid (PAFC) and Solid Oxide (SOFC) technologies are also subject to sulfur poisoning. For example, natural gas feeds to external or internal catalytic reformers need to be desulfurized since low ppm sulfur levels can poison the reformer catalyst and fuel cell stack. Potential breakthrough of sulfur from the desulfurization beds needs to be closely monitored.

The mass selective detector (MSD) is usually not considered when the need for low-level volatile sulfur quantitation and speciation arises in the analytical laboratory or plant. Selective detectors such as the flame photometric (FPD), pulsed flame photometric (PFPD), and sulfur chemiluminescence (SCD) have traditionally dominated these applications [1]. The 6890N/5973N GC/MSD system is a very capable alternative to these detectors providing the added benefit of positive compound identification. Details on how to set up the

system for optimum sensitivity and selectivity are discussed in this paper. The specific hardware configuration reviewed is applicable to a wide range of applications.

MSDs are now widely used in many routine applications including QA/QC environments. The 5973N is easy to use, compact in size, and stable over long time periods. Tuning is software controlled and automatic, a significantly easier task than what is needed for some sulfur-selective detectors.

A common problem with many sulfur-selective detectors is hydrocarbon interference, especially from chromatographic coelution [4]. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene. In most cases, an accurate determination of the sulfur compound is not possible. However, the use of the MSD and selected ion monitoring (SIM) can largely overcome the coelution problem for many applications.

Experimental

Networked versions of the 6890 and 5973, designated by the N following the product number were used in this work; replacing the previously HPIB-interfaced models. Well known benefits of LAN include reliability, lack of distance limitations and simple configuration.

The sulfur calibration mix consisted of the following components at 5 ppm each: Hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, carbonyl sulfide, t-butyl mercaptan, and tetrahydrothiophene. The blend in helium was purchased from DCG Partnership, Pearland, TX.

A 6-port gas-sampling valve was connected directly to the volatiles interface on the 6890N with Sulfinert 1/16-inch tubing. See the sample introduction diagram in Figure 1. The sample loop, tubing and inlet are either Sulfinert or Silcosteel treated for inertness. Table 1 contains the instrument conditions.

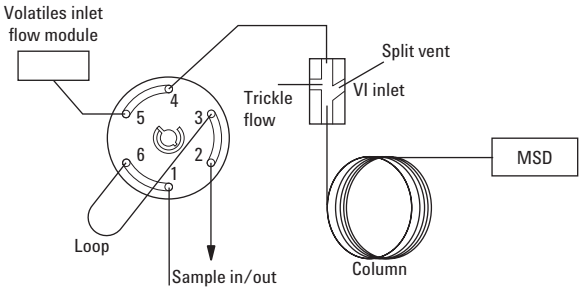

Figure 1. Sample introduction scheme.

Table 1. Instrument Conditions

Injection port	**Volatiles interface**
Temperature	150 °C
Split ratios	1 : 1 up to 50 : 1
Carrier gas	Helium
Constant flow mode	1.9 mL/min
Injection source	**6-Port sampling valve**
Material	Hastelloy C
Temperature	150 °C
Loop	Sulfinert, 0.5 cc
Column	**60 m × 0.320 mm × 5.0 µm DB-1**
Initial temperature	40 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	270 °C
Final time	2 min
5973 MSD	
Mass range	33 to 100 and 12 to 100
Scans	13.1/sec and 15.9/sec
Samples	2
Threshold	150
EM voltage	BFB.u tune voltage
Solvent delay	3 min
Source temperature	230 °C
Quad temperature	150 °C
Transfer line	280 °C

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases are mixed with the calibration standard using an Aux EPC module on the 6890N GC. This system and the hardware employed were described in detail previously [2].

Positioning of the column in the MSD must be carefully done to avoid loss of sulfur sensitivity. To position the column just inside the source, 2 to 3 millimeters of the column should be visible at the MSD end of the transfer line. See reference 5 for installation details.

Results and Discussion

System Calibration

First, the system was calibrated and checked for linearity by analyzing the sulfur mix at various concentrations. The dynamic blending system was used to prepare seven and five level calibrations using helium and natural gas as diluents, respectively. Table 2 lists the concentrations used. Calibrations were focused in the ppb range since this is where most analytical problems for sulfur analysis are found. SIM acquisition mode, discussed later in this section, was used.

Table 2. Calibration levels for checking system linearity. Sulfur concentrations in ppbv.

Cal Level	1	2	3	4	5	6	7
Conc. in helium	21	35	46	57	95	1600	3600
Conc. in nat gas	88	242	475	880	1170	--	--

Calibrations are linear in both matrices for all eight sulfur compounds. This is seen in Table 3 where the regression coefficient, r^2 values appear. This is an indication not only that the system response is linear but also that calculated concentrations from the blending system are accurate. Unlike some sulfur-selective detectors, the MSD does not show equimolar response. Each compound will have its own response characteristics, requiring each component's response factor to be determined.

Table 3. Calibration Regression Coefficient r^2 Values

Compound	Helium	Natural gas
H_2S	0.998	0.998
COS	0.998	0.999
CH_3SH	0.997	0.999
EtSH	0.996	0.998
DMS	0.998	0.998
CS_2	0.998	0.998
t-ButylSH	0.996	0.993
THT	0.996	0.992

One of the calibration plots as produced by the MSD Chemstation is shown in Figure 2 for the calibration of H_2S in natural gas.

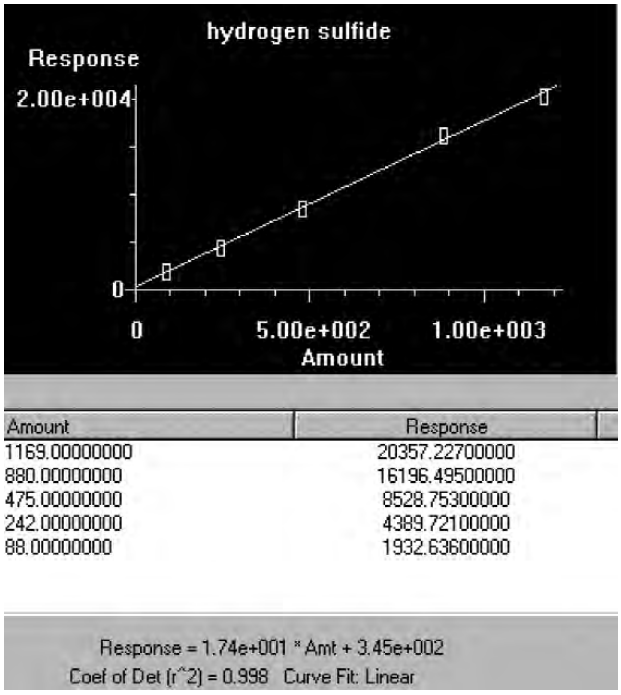

Figure 2. Five level calibration plot of H_2S using natural gas as the diluent. Calibration range is from 88 to 1170 ppb.

Scan Results

The total ion chromatogram (TIC) of the eight-component sulfur mix at 1.3 ppm in helium is shown in Figure 3 using a split ratio of 0.5 to 1. As is evident in the figure, H_2S is close to the minimum detectable limit (MDL) for this particular set of operating conditions. While operating in scan mode is useful for initial method development, unknown identification and retention time determinations, use of extracted ions from a scan and/or SIM are required to improve overall sensitivity and selectivity.

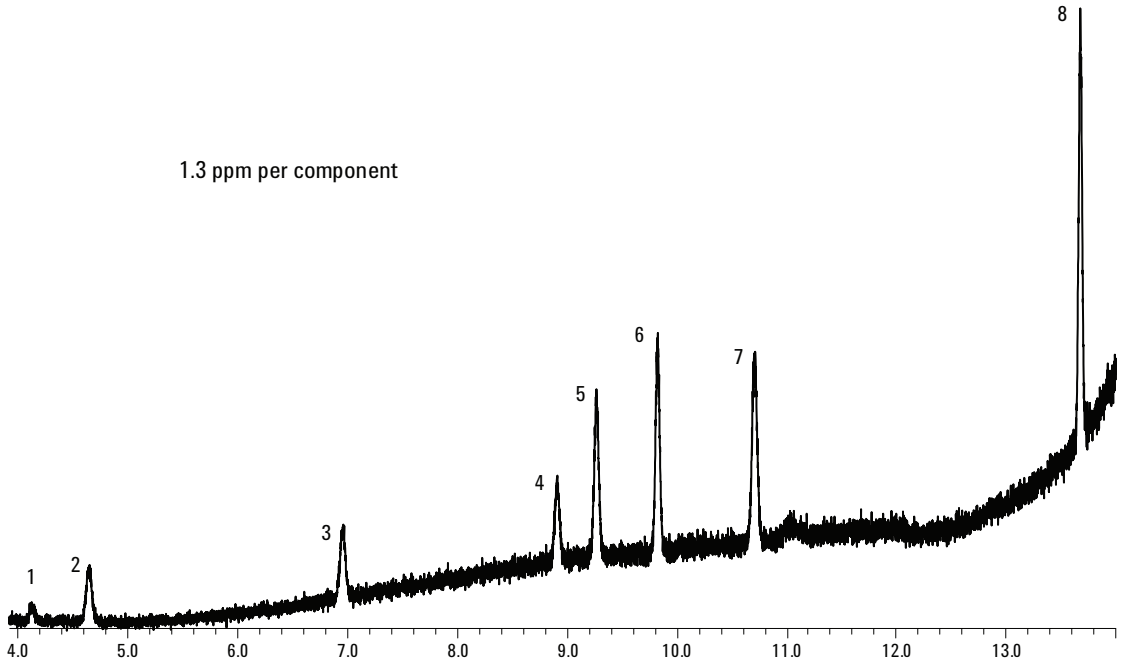

Figure 3. Total ion chromatogram of the eight-component sulfur mix at 1.3 ppm per component. Scan 33-100 amu. Peak labels: 1. hydrogen sulfide, 2. carbonyl sulfide, 3. methyl mercaptan, 4. ethyl mercaptan, 5. dimethyl sulfide, 6. carbon disulfide, 7. t-butyl sulfide, 8. tetrahydrothiophene.

Extracted Ion Results

In Figure 4, extracted ion chromatograms are shown for ions 60 and 62. Three of the eight sulfur compounds are found with these target ions. Ion 60 is present in COS and tetrahydrothiophene, and ion 62 is found in ethyl mercaptan and dimethyl sulfide. The concentration of the sample was 86 ppb per component in helium. Extracted ion chromatograms for the other sulfur compounds show similar signal to noise ratios at the concentration level. A considerable improvement in sensitivity is achieved by using extracted ions. In cases where this does not provide sufficient sensitivity, the next step should be SIM.

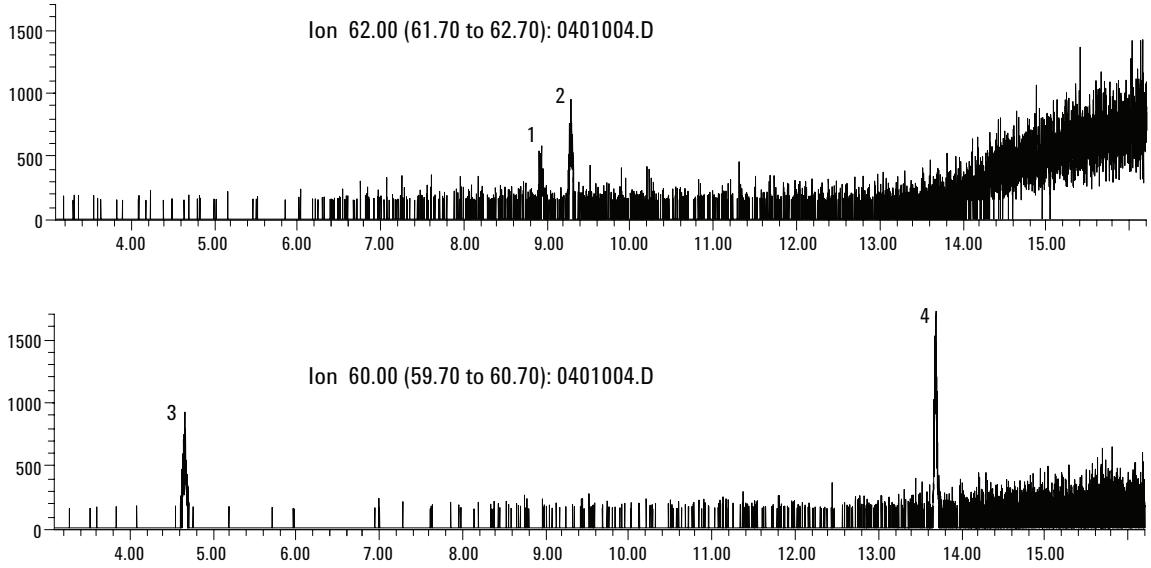

Figure 4. Extracted ion chromatograms for ions 60 and 62. Concentration is 86 ppb per component. Split ratio 1:1. Peak labels: 1. EtSH, 2. DMS, 3. COS, 4. THT.

Application of SIM

SIM provides superior sensitivity and selectivity. Since sulfur determinations will normally be done in hydrocarbon matrices, care must be taken to select ions that ideally have no hydrocarbon contribution. If this can be done, excellent selectivity can be achieved even in cases where coelution of sulfur species and hydrocarbon occur. This is an important distinction and advantage of the MSD compared to some of the common gas chromatographic sulfur-selective detectors. Both the FPD and PFPD will suffer from quenching if coelution occurs making accurate quantitation of low-level sulfur impossible [2]. Even the SCD will have problems measuring low ppm sulfur in the presence of a dominant coeluting hydrocarbon. In situations where a unique sulfur ion cannot be found, refinement of the method and chromatographic column/conditions to achieve separation from the interfering hydrocarbon should be tried [2]. Also, when operating the MSD in SIM mode, it is usually best to select low resolution for maximum sensitivity at the expense of some mass selectivity loss.

The SIM ions used for each sulfur compound are listed in Table 4. These ions were chosen to minimize interference from hydrocarbons. To arrive at the ions shown in the table, a scan of the sulfur mix in helium is acquired to identify target ions. Library spectra can also be consulted. Hydrocarbon mixes such as natural gas and refinery gas are then run separately using the SIM table to look for ions that may match those selected for the sulfur. The table may be further refined if hydrocarbon interferences appear. Retention times of the sulfur compounds are also needed to set up the time-programmed groups. These are not the only possible ions that can be used. For some of the compounds other choices or additional ions could be included in the SIM table. While not necessary for this relatively simple sulfur example, the use of second and third qualifier ions may give the analyst a higher level of confidence of a compound's identity by comparing ion ratios to library spectra for a particular compound.

Table 4. Optimized SIM table for selective sulfur detection in hydrocarbon streams. Dwell time for each ion is 100 ms.

GROUP	START TIME (min)	TARGET and QUALIFIER IONS	COMPOUND
1	3.00	33,34	H_2S
2	4.20	60	COS
3	6.00	45,47	MeSH
4	8.00	47	EtSH
5	9.10	45,47,62	DMS
6	9.70	44,76	CS_2
7	10.20	57,90	t-ButylSH
8	11.80	45,60,88	THT

Fuel Cell Natural Gas Feedstocks: Composition and Impurities

The TIC of a natural gas scan and sulfur mix SIM runs are overlaid for illustration purposes in Figure 5. Note that with the 60 m × 0.32 mm × 5.0 µm DB-1 column, all hydrocarbons and CO_2 are separated. Natural gas compounds in order of elution are: O_2/N_2, CH_4, CO_2, ethane, propane, i-butane, n-butane, i-pentane, and n-pentane. From the overlay, it can be seen that seven of the eight sulfurs do not coelute with natural gas components; only COS and propane show potential overlap. This also demonstrates the utility of the system for fuel cell feed streams, providing both hydrocarbon composition and gas impurity analysis.

The chromatogram shown in Figure 6 of the sulfur mix in helium was produced using the SIM parameters in Table 3. The offsets seen in the baseline are a result of the MSD switching from group to group and should not be interpreted as a chromatographic problem. Excellent signal to noise is seen for all components at the 46 ppb level. The sulfur mix was then further diluted to 16 ppb per component. The resulting chromatograms for H_2S and COS, the most challenging analytes, and tetrahydrothiophene are shown in Figure 7.

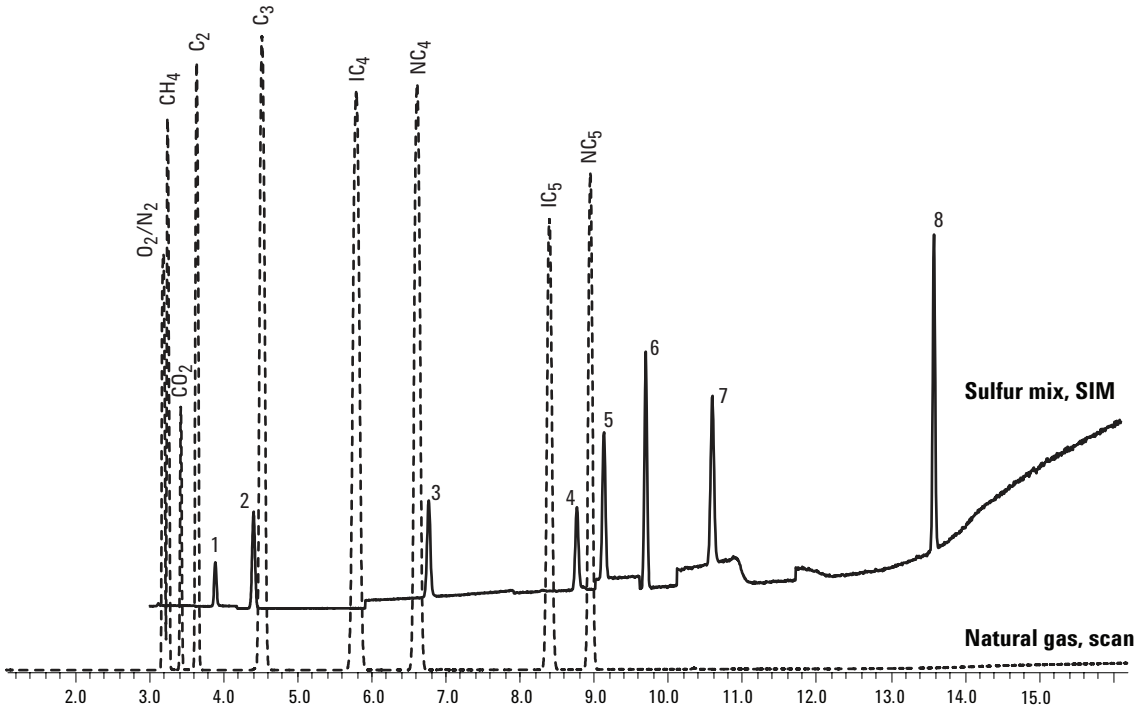

Figure 5. Overlay of two runs: natural gas scan (12 to 100 amu) and sulfur mix at 4.5 ppm in SIM mode. Split ratio 20:1. Sulfur peak labels same as in Figure 3.

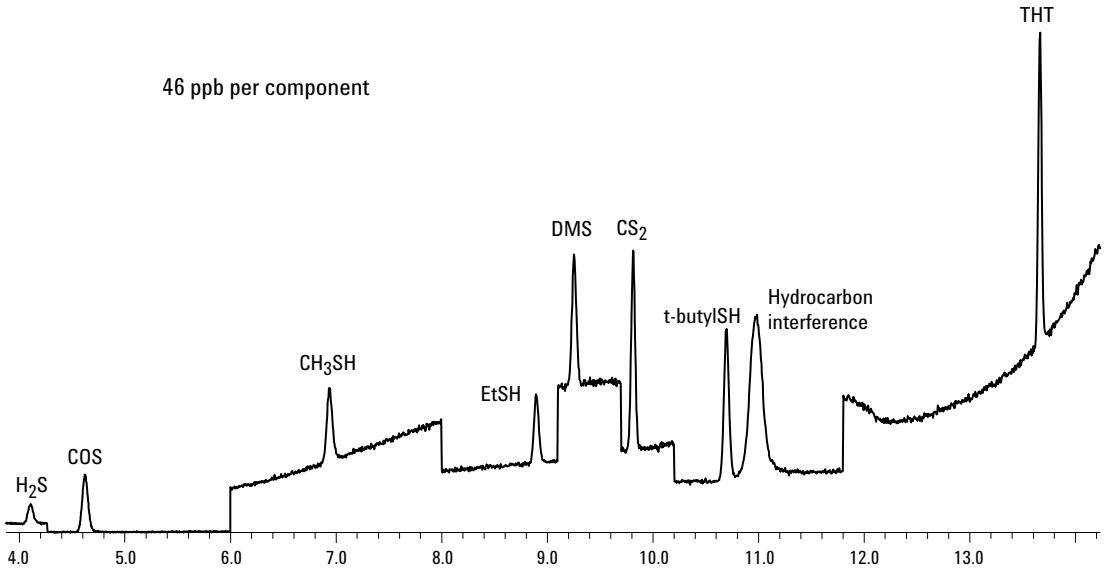

Figure 6. Eight-component sulfur mix in helium at 46 ppb per component in SIM mode. Split ratio 0.5:1.

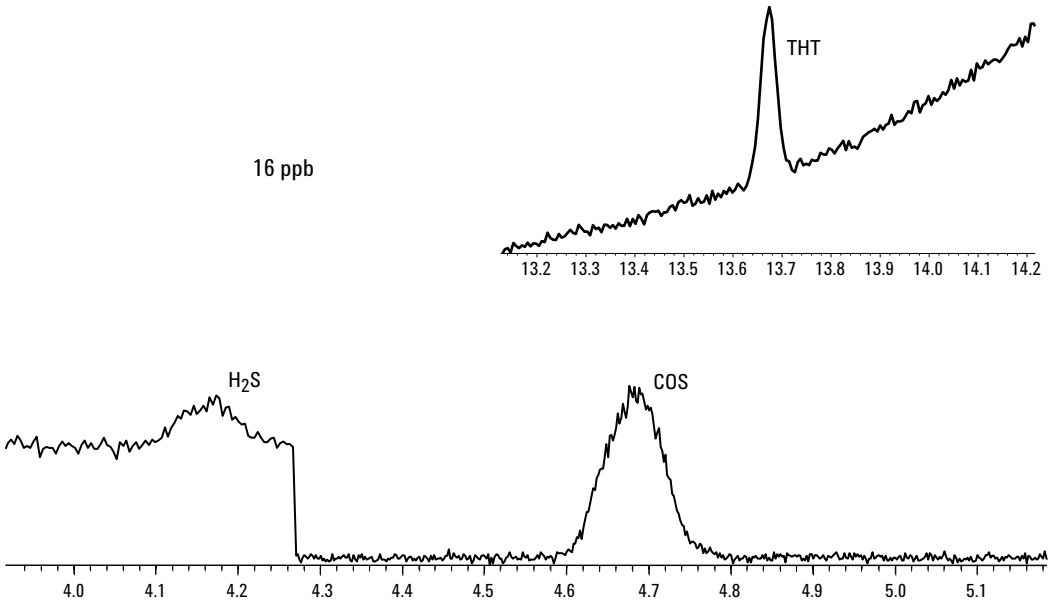

Figure 7. H_2S, COS and tetrahydrothiophene (insert) at 16 ppb each. SIM was used.

For ppb sulfur analysis, it is recommended that the pure matrix be run separately using the sulfur SIM acquisition parameters. Ideally, no response would be seen. If ions of the hydrocarbon matrix are seen, they can be noted and not mistaken for sulfur compounds. This is illustrated in Figure 8 for natural gas streams. Chromatograms of the sulfur mix in scan mode and pure natural gas in SIM mode are overlaid for illustrative purposes. Both are drawn to the same scale. This is a good practice to follow not only for sulfur but also for any impurity analysis using SIM.

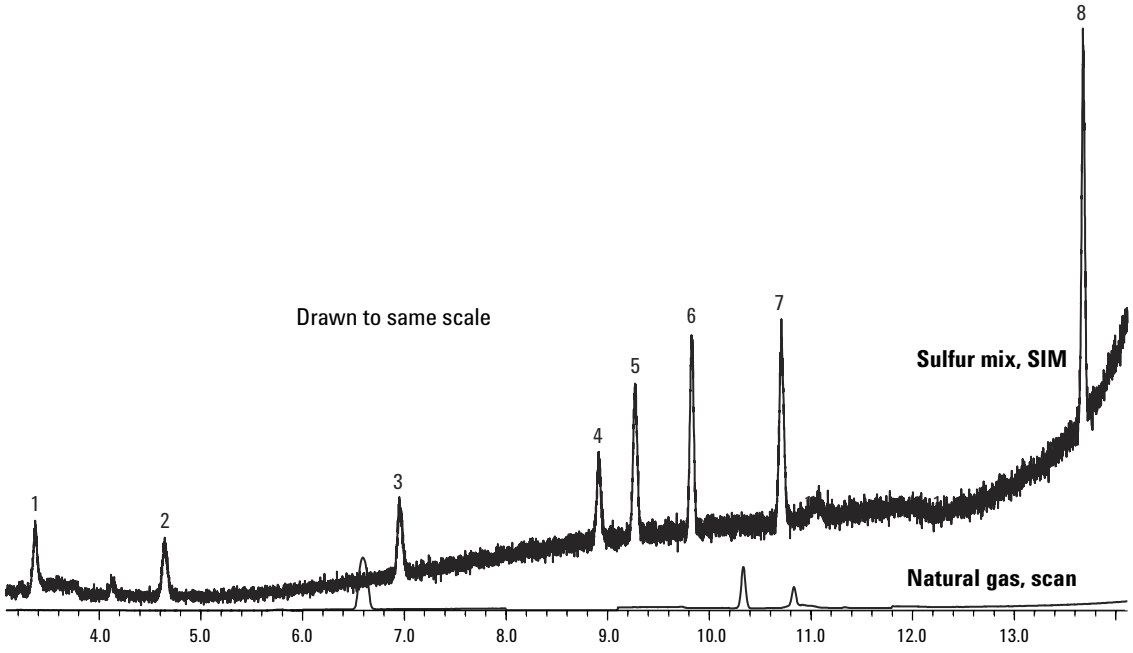

Figure 8. Overlay of sulfur mix in scan (33-100 amu) and natural gas (using sulfur SIM table). Ideally the natural gas chromatogram would be blank. Same scale used for both.

Analysis of COS in Propylene and Propane

Measurement of ppb COS in propylene and propane can be a challenging analytical problem due to coelution of COS/propylene on the preferred methyl silicone columns. This coelution is illustrated in Figure 9 where two independent (separate runs) are overlaid. Both the FPD and PFPD will be unsuccessful with this analysis due to quenching. The SCD's selectivity can also be exceeded for low ppb COS analysis

SIM (ion 60) was employed for the analysis of COS. To avoid overloading the source, the split ratio was increased to 50:1. To determine the effect of coeluting propylene on COS response, two runs were performed at identical concentrations of 105 ppb COS. The diluents for the first and second runs were helium and propylene, respectively. Chromatograms for both runs are shown in Figure 10. The helium chromatogram shows the true COS area unaffected by any other coeluting compound. This area is then compared to that of COS in propylene diluent using the area ratio (COS propylene/COS helium) to indicate how coelution has affected the MSD response. This ratio of 0.77 indicates that COS in propylene response is suppressed by only 23%, probably due to a reduction in ionization efficiency. Moreover, a subsequent experiment that constructed a five level calibration of COS in propylene showed linear behavior over the range of 20 to 1200 ppb. Therefore, using a carefully constructed SIM method, the MSD has the capability of quantifying ppb level COS in coeluting 99+% propylene. It follows, in the general case, that coeluting analytes do not preclude quantification even when concentration differences exceed 10^5 provided unique ions can be identified for the component of interest.

These results and conclusions are relevant to fuel cell developers who are using high propane (for example 50 to 99%) as a feedstock. The performance, chromatographic behavior, and minimal detectable impurity levels will be very similar. Under the conditions used the retention time of propane will differ by less than 0.1 minute from propylene (see propane retention time in Figure 5). Sulfur impurities other than COS can be easily measured.

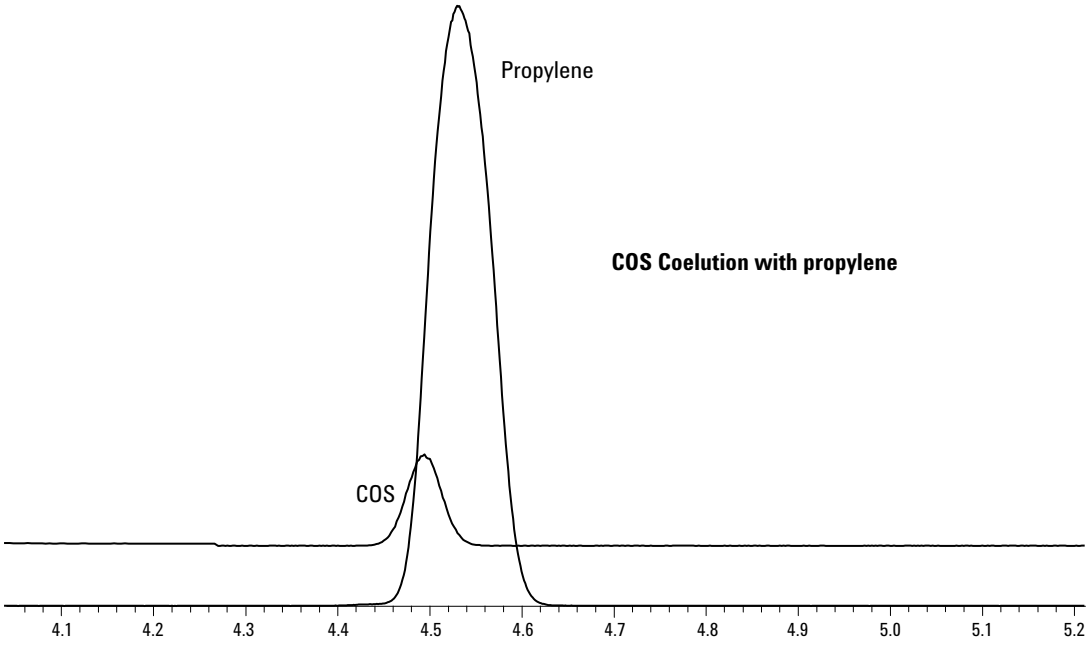

Figure 9. Two separate chromatograms (from separate runs) superimposed showing the coelution of COS with propylene. Split ratio 50:1.

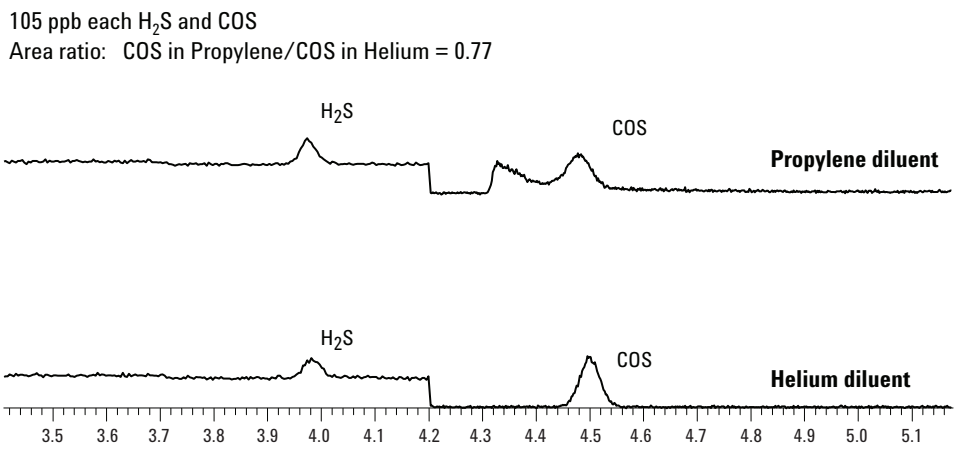

Figure 10. Comparison of COS response (SIM mode) in helium and propylene. Split ratio 50 to 1.

Conclusions

The hardware and associated methods outlined in this paper demonstrate the MSD's capabilities as a sensitive and selective detector for gaseous analytes. It has the added advantage of providing structural information. Sulfur detection at low ppb levels is easily achieved through use of a time programmed SIM table consisting of unique ions for the compounds of interest. This minimizes hydrocarbon interference making it possible to quantitate low-level analytes such as COS with coeluting propylene.

The 6890N/5973N system is also a powerful tool for fuel cell developers, providing detailed composition and impurity analyses of common fuels. The examples shown here demonstrate how natural gas feed could be characterized providing complete speciation of sulfur compounds including odorants or naturally occurring impurities such as H_2S. The system can also be used to monitor the performance of desulfurization beds and reformer output.

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Printed in the USA
November 14, 2001
5988-4453EN



Agilent Technologies

Dual-Channel Gas Chromatographic System for the Determination of Low-Level Sulfur in Hydrocarbon Gases

Application

Hydrocarbon Processing

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Abstract

A 6890N equipped with dual flame photometric detectors is described for the analysis of ppb level volatile sulfur compounds in a variety of hydrocarbons using thick film DB-1 and GS-GasPro columns. Enhanced performance flame photometric detectors are employed that can achieve detection of sulfur compounds below 20 ppb. Examples of arsine and phosphine analysis with the same hardware are also discussed.

Introduction

Gas chromatography with sulfur selective detection is finding widespread application in many segments of the petroleum, petrochemical, and specialty chemical industries. Demand for low-level sulfur detection will increase in the future in response to more stringent regulations and tighter quality control.

Sulfur compounds can be significant poisons for various catalytic processes involved in hydrocarbon conversion. Monitoring these low-level poisons can lead to considerable saving in terms of improved yields, increased catalyst lifetime, and higher quality products. In looking at the future of fuel cells, fuel contaminants can adversely affect performance of fuel cell systems and fuel processors that are powered by natural gas or other fossil fuels. Finally, environmental regulatory issues in certain regions will continue, necessitating the need to monitor fuel impurities.

A common problem with many gas chromatographic sulfur selective detectors is hydrocarbon interference, especially from co-elution. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene, or sulfur in natural gas [1, 2]. In most cases, an accurate determination of the sulfur compound is difficult or not possible even with highly selective sulfur detectors. However, the use of a dual-channel system employing two very different separation columns (in terms of selectivity) largely avoids the interference problem. The configuration is shown in Figure 1. Sulfur compounds that have a severe interference on one column are likely to be separated from that interference on the other column. By assuring that a given sulfur compound will be separated on at least one of the columns, the system can use a reliable, stable, and relatively inexpensive flame photometric detector (FPD) for detection. If the hydrocarbons can be chromatographically separated from the sulfur compounds of interest, enhanced FPDs can quantitate sulfur to less than 20 ppb.

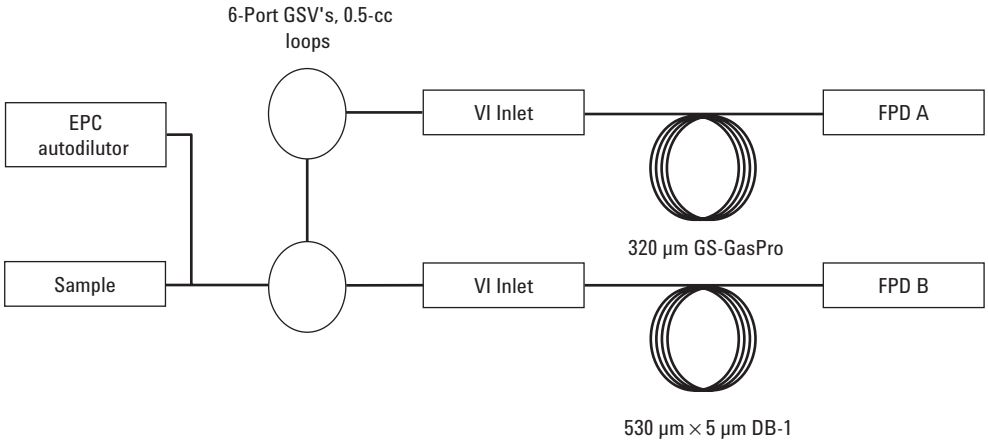

Figure 1. System configuration on the Agilent 6890N. Valves (plumbed in series) are Hastelloy C and all plumbing is Silcosteel® or Sulfinert™ treated.

Experimental

Selection of the appropriate capillary column is often key to the solution of a particular analysis problem, and this is especially true for this system. Four columns are employed (two for any given analysis) as described in Table 1.

Table 1. Recommended Column Combinations by Application

Applications	Column set
Natural gas, fuel cell gases	60 m × 530 µm × 5.0 µm DB-1
	30 m × 320 µm GS-GasPro
Ethylene, propylene, C4 streams	105 m × 530 µm × 5.0 µm DB-1
	60 m × 320 µm GS-GasPro

Recommended GC oven programs are 40 °C (5 min) to 290 °C (5 min) at 25 °C/min for natural gas, fuel cell gases and ethylene, and 35 °C (7 min) to 290 °C (5 min) at 20 °C/min for propylene. Somewhat lower detection limits can be achieved for sulfur in a propylene stream by employing cryo oven programs such as: –35 °C (7 min) to 290 °C (5 min) at 20 °C/min. Split ratios, as set in the GC method, vary from 0.5:1 to 2:1.

Each valve was interfaced to a specialized inert (Silcosteel treated) volatiles interface for accurate sample introduction at low split ratios into a capillary column. Due to the tendency for organosulfur compounds (especially H_2S) to adsorb to metal surfaces, great care must be used in selecting and constructing the chromatographic sample introduction system. The sample loop, tubing, and inlet are either Sulfinert or Silcosteel treated for inertness.

A factory modified FPD, with enhanced sensitivity, was used for each channel. The FPD is optimized for the analysis of trace sulfur gases, arsine, and phosphine in gaseous samples. See Table 2 for appropriate gas flow settings. These detectors achieve detection limits that are roughly four times better than standard. The sensitivity advantage is illustrated in Figure 2, where standard and modified FPDs are compared using a standard calibration blend. Minimum detection level (MDL) calculated on methyl mercaptan using linearized data and the 60 m DB-1 column is better than 15 ppb.

Table 2. FPD Gas Flow Settings

Analysis	Gas	Flow rate (mL/min)
Sulfur	Air	60
	Hydrogen	50
	Makeup	58
Arsine	Air	150
	Hydrogen	50
	Makeup	100
Phosphine	Air	110
	Hydrogen	150
	Makeup	58

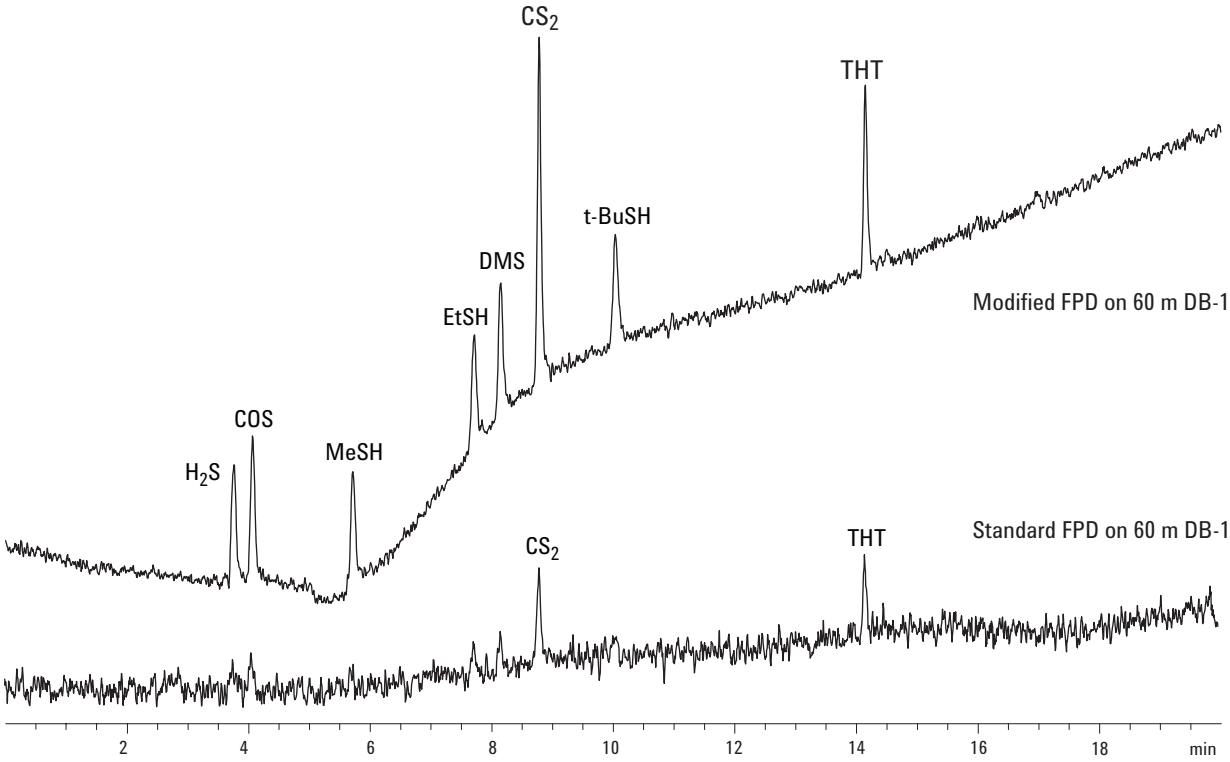

Figure 2. Sensitivity comparison of standard and enhanced FPDs. Concentrations are 33 ppb per component (v/v) in helium.

Due to the use of all available heated zones on the 6890N GC for either inlet or detector heating, the 6-port sample valves are not actively heated. This does not pose a problem for the light gaseous streams studied in this work. However, if desired, the valves can be heated by an auxiliary stand-alone temperature controller (Agilent model 19265B). The system is designed only for gaseous samples containing significant concentrations of hydrocarbons of C_6 or below.

Discussion

Channel 1 employs the GS-GasPro column, using a unique bonded PLOT technology, where COS is separated from C_2 and C_3 hydrocarbons, allowing measurement at trace levels. However, H_2S and the C_3s coelute. Channel 2 uses a thick film DB-1 column where H_2S is well separated from C_2s and C_3s, making low-level measurements of this sulfur impurity possible. COS and C_3s will coelute on this column. In summary, using a dual-column approach with the unique separation capabilities of GS-GasPro and thick film DB-1, both COS and H_2S can be measured in one chromatographic analysis at low ppb levels regardless of the concentrations of light hydrocarbons present in the sample. The elution order difference between the two columns is illustrated in Figure 3.

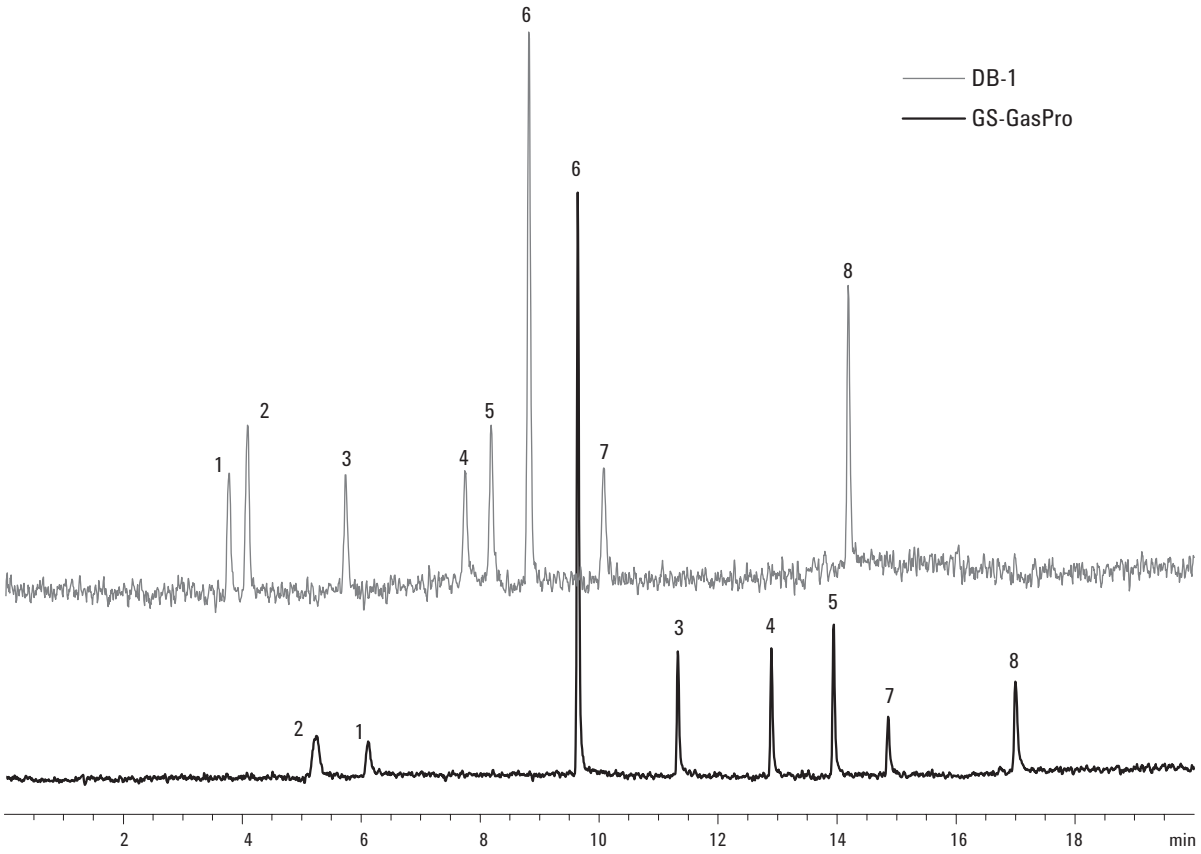

Figure 3. The dual-column advantage. Sulfur mix at 90 ppb per component in helium. 1. H_2S, 2. COS, 3. MeSH, 4. EtSH, 5. DMS, 6. CS_2, 7. t-BuSH, 8. THT.

Other potential interferences or coelutions between light sulfur compounds and hydrocarbons are avoided with this approach. A coeluting pair on one column will likely be separated on the other. Split ratios were set depending on the application from 0.5:1 to 2:1 in order to achieve the reported detection limits.

The sulfur calibration mix consisted of the following components at 5 ppm each: Hydrogen sulfide (H_2S), carbonyl sulfide (COS), methyl mercaptan (MeSH), ethyl mercaptan (EtSH), dimethyl sulfide, carbonyl sulfide (DMS), t-butyl mercaptan (t-BuSH), and tetrahydrothiophene (THT). The blend in helium was purchased from DCG Partnership, Pearland, TX. These compounds are representative of the most common light sulfur species encountered in gaseous fuels or petrochemical feedstocks.

Some adsorption of H_2S on the GS-GasPro column is possible. Priming the system a few times with a low ppm sulfur stream such as the calibration mix described here can largely eliminate the loss in sensitivity that can result from adsorption. This priming is usually only necessary for low ppb analyses where the active sites in the column could adsorb most of the sulfur present in the sample during an initial run.

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propane, liquidfied petroleum gas (LPG), propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases were mixed with the sulfur calibration standard using an Aux EPC module on the 6890N GC. Accurate concentrations from low ppb to ppm levels can be easily prepared by knowing the flow rates of the two streams as they mix in a Tee fitting prior to the gas sampling valves on the GC. This system and the hardware employed were described previously in detail [3].

Sulfur in Fuel Cell Gases, Natural Gas, and Proypylene

Figure 4 shows the chromatograms from the eight-component sulfur standard diluted with a fuel cell mix to 45 ppb (v/v) each component. The fuel cell

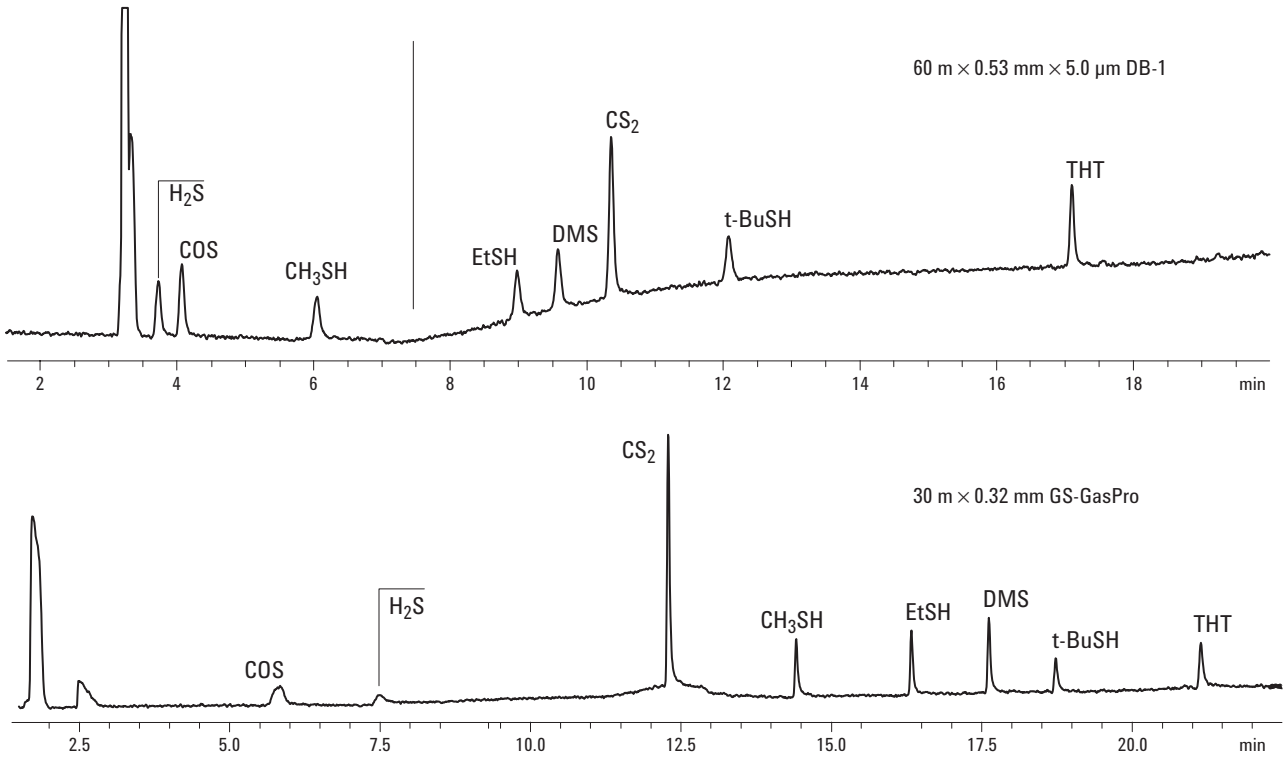

Figure 4. Simultaneous dual column analysis of fuel cell mix containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

mix is 50% hydrogen, 10% carbon dioxide, and 5% methane. This mix is often used to simulate the output stream of a natural gas reformer used as the feed to a fuel cell. This matrix is one of the easier ones because the large hydrocarbon (methane) elutes before all of the sulfurs on both columns. Note that elution order of the sulfurs is significantly different on the GS-GasPro column compared to the DB-1 (see Figure 3). All eight compounds are clearly detectable at 45 ppb.

Natural gas is a much more challenging matrix because of the high concentrations of several hydrocarbons. These interferences extend out into the retention time range of the sulfur compounds.

Figure 5 shows the chromatograms from the eight-component sulfur standard diluted with sulfur free natural gas to 45 ppb (v/v) each component. There are more peaks evident in these chromatograms than just the eight sulfur compounds. The additional peaks are interference responses from the large hydrocarbons in the natural gas.

In the DB-1 chromatogram, H_2S is clear but COS is lost to a severe overlap with a large C_3 peak. Ethyl mercaptan is also overlapped with n-pentane. On the GS-GasPro column, however, only the H_2S is occluded by interference. The COS and EtSH are free from interferences. With the dual-column approach, all eight compounds can be measured down to 45 ppb.

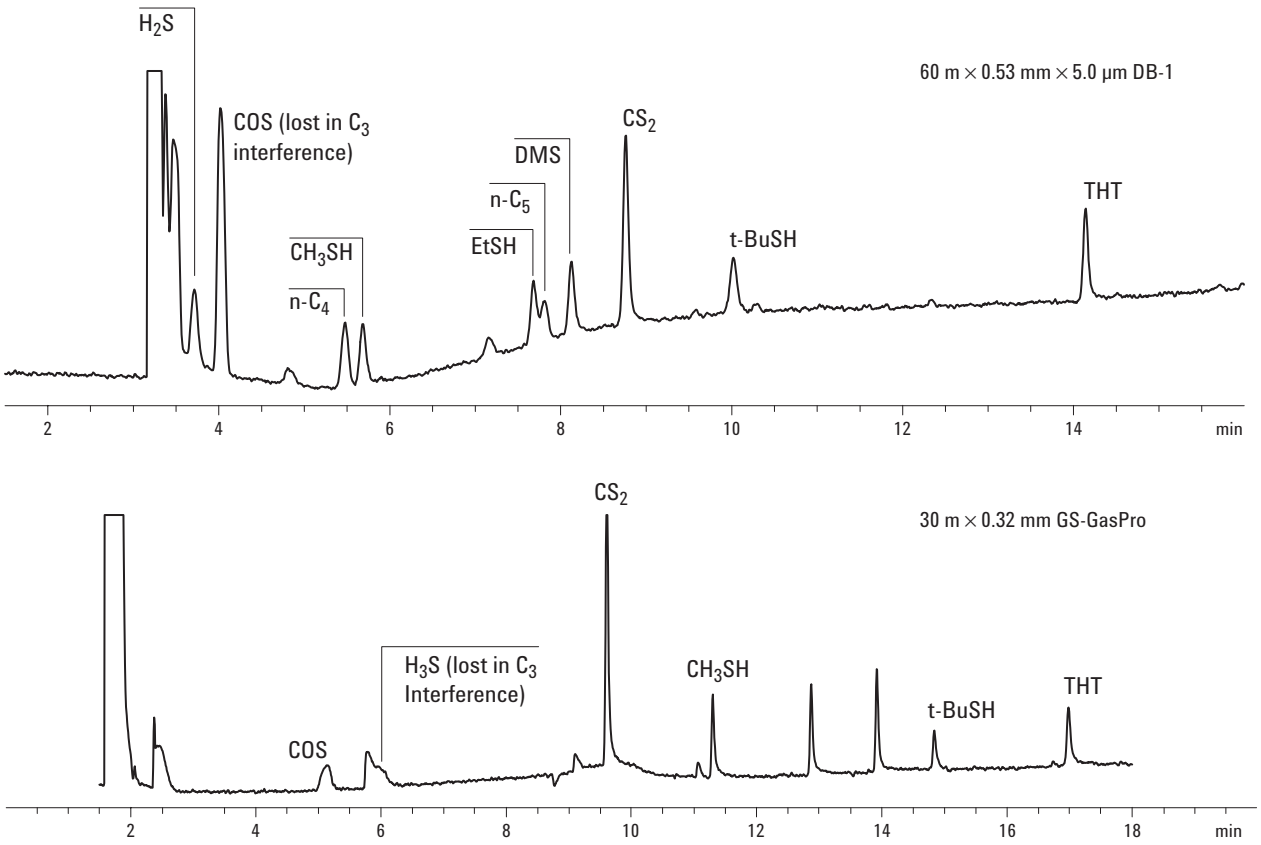

Figure 5. Natural gas blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

Propylene monomer offers another interesting challenge. The huge C_3 peaks interfere with both the H_2S and COS on both columns used above. To address this, longer versions of the same columns were used (Table 1). The oven temperature and split ratio are also modified (see Experimental on page 2) to improve resolution of the H_2S and COS from the C_3s.

Figure 6 shows the chromatograms from the eight-component sulfur standard diluted with polymer-grade propylene to 45 ppb (v/v) each component. By using longer DB-1 and GS-GasPro columns, lower oven temperature, and a higher split ratio, the H_2S and COS can be measured with somewhat poorer detection limits.

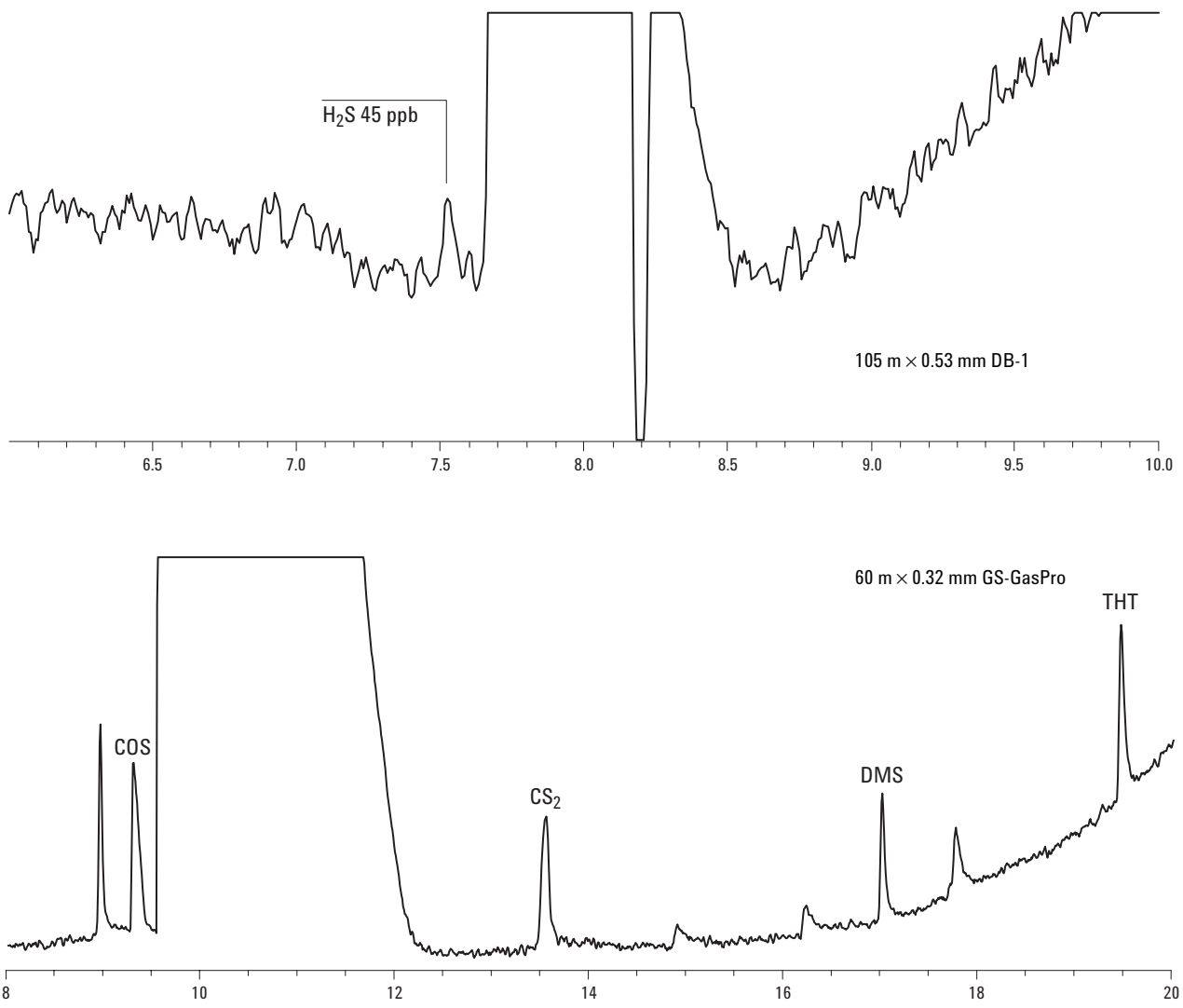

Figure 6. Polymer-grade propylene blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 2:1. Top chromatogram: 105 m × 530 µm DB-1 showing only H_2S, bottom: 60 m GS-GasPro.

Cryogenic oven temperatures were evaluated to see if the separation of H_2S and COS could be improved enough to allow use of the more sensitive 0.5:1 split ratio. The oven program tested was: -35 °C for 7 min, 20 °C/min to 300 °C, hold for 5 min. The separation was improved enough to allow the analysis of H_2S on the DB-1 column with the 0.5:1 split ratio, but COS was still occluded by the C_3s on the GS-GasPro. A DB-1 chromatogram illustrating the increased separation between H_2S and propylene is given in Figure 7.

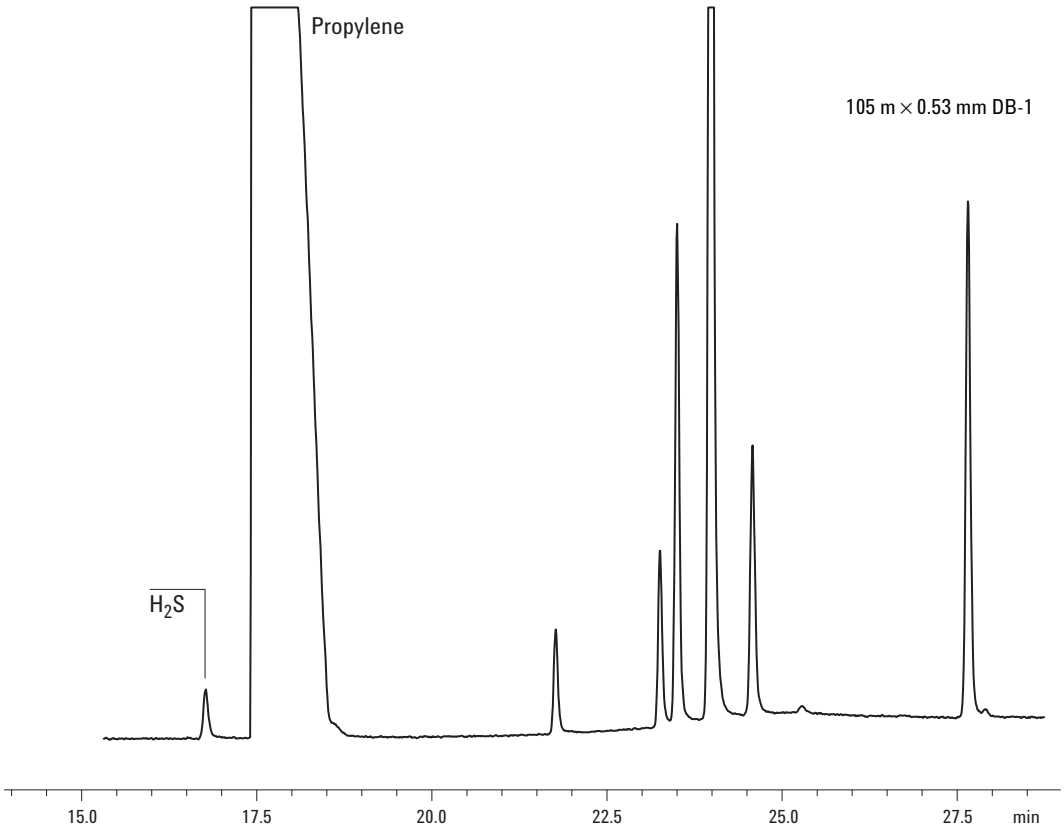

Figure 7. Use of cryogenic oven temperatures for analysis of H_2S (400 ppb) in propylene at 0.5:1 split.

Phosphorus and Arsenic on the Same System

One interesting characteristic of the modified FPD is that the filter used also passes the emissions for phosphorus and arsenic. This means that the same detectors can also be used to measure arsine and phosphine in polymer grade ethylene and propylene. A change of detector gas flows to that optimum for each element, followed by a rerun of the sample is all that is required. Since the 6890N detector flows are controlled by EPC, these reruns can be automated.

Figure 8 shows the chromatograms from an arsine and phosphine standard (DCG Partnership) diluted with polymer grade propylene to 36 ppb (v/v) each component. These are run under the same chromatographic conditions as in Figure 6, except that the FPD detector flows are set to those listed for phosphorus detection and the split ratio is back to 0.5:1. The detection limit under these conditions for phosphine in helium is under 5 ppb. If the detector flows are set to those listed for arsenic detection, the detection limit for arsine is about 60 ppb measured in helium. This system is well suited for gas analysis, however it is not really applicable to pesticide analysis due to the lack of selectivity between sulfur, phosphorus, and arsenic.

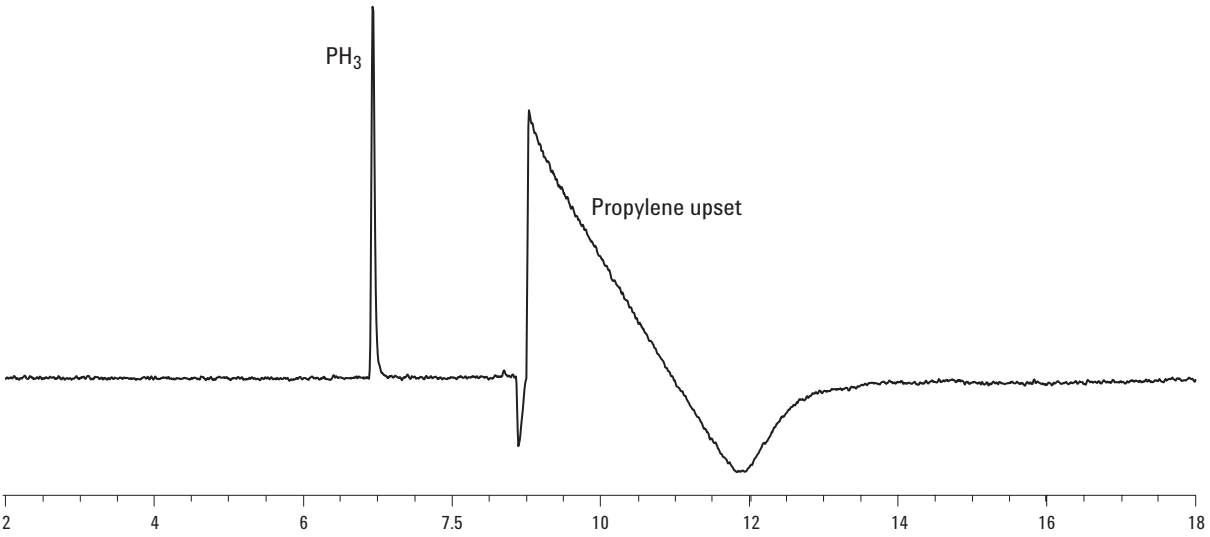

Figure 8. Polymer-grade propylene blend containing 36 ppb (v/v) each of arsine and phosphine. Split ratio is 0.5:1. Note longer 105 m DB-1 columns are used.

An example of arsine detection in propylene is shown in Figure 9.

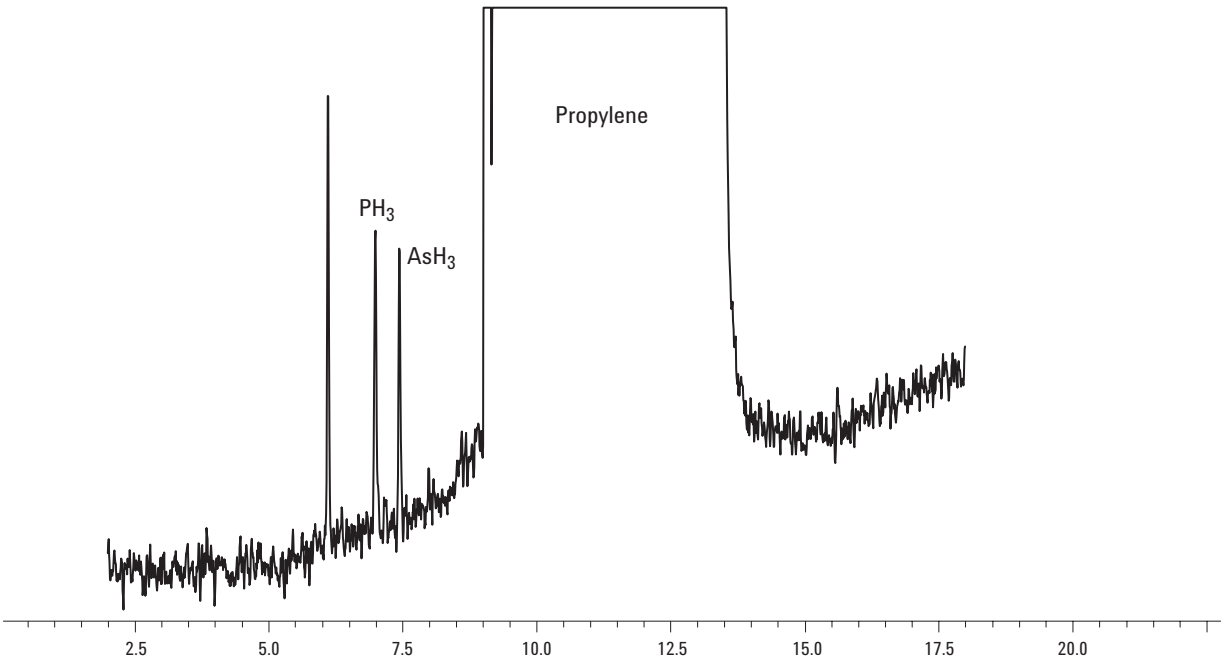

Figure 9. Arsine optimized FPD flows. H_2: 50 mL/min, air: 150 mL/min. 60 m × 0.32 mm GS-GasPro, 0.5 to 1 split. 90 ppb each of AsH_3 and PH_3.

How to Order and Configure a Dual-Channel FPD System

The Dual-Channel FPD System, including columns and valves, can be ordered as a special (SP-1) option on any new Agilent 6890N GC. This special also includes the enhanced performance FPD. Contact your local Agilent representative for more information.

Learn more about low-level sulfur detection from these application notes available from any Agilent sales office or Agilent's Web site at www.agilent.com/chem. Just click "Library" in the menu listing, and type "sulfur" in the keyword field.

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Printed in the USA
March 17, 2003
5988-8904EN



Agilent Technologies

Detection of Sulfur Compounds in Natural Gas According to ASTM D5504 with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) on the 7890A Gas Chromatograph

Application

Hydrocarbon Processing

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Abstract

An Agilent dual plasma sulfur chemiluminescence detector (DP SCD) combined with an online dilutor was used for the analysis of sulfur compounds. By using this method, the detection limits of the sulfur compounds achieved the ppb level. The stability of the DP SCD was also investigated. The long-term and short-term stability show that the performance of DP SCD is stable, and no hydrocarbon interference was found during the analysis of natural gas samples.

Introduction

Many sources of natural gas and petroleum gases contain varying amounts and types of sulfur compounds. The analysis of gaseous sulfur compounds is difficult because they are polar, reactive, and present at trace levels. Sulfur compounds pose problems both in sampling and analysis. Analysis of sulfur compounds many times requires special treatment to sample pathways to ensure inertness to the reactive sulfur species. Sampling must be done using containers proven to be nonreactive. Laboratory equipment must also be inert and well conditioned to ensure reliable results. Frequent calibration using stable standards is required in sulfur analysis [1].

GC SCD configuration with inert plumbing is one of the best methods to detect sulfur compounds in different hydrocarbon matrices. Sulfur compounds elute from the gas chromatographic column and are combusted within the SCD burner. These combustion products are transferred to the SCD detector box via vacuum to a reaction cell for ozone mixing. This detection technique provides a highly sensitive, selective, and linear response to volatile sulfur compounds.

Agilent Technologies DP technology is the detector of choice for sulfur analysis when dealing with a hydrocarbon matrix. The burner easily mounts on the 6890 and 7890A GCs and incorporates features for easier and less frequent maintenance. In this application, the Agilent 355 DP SCD was used to analyze the gaseous sulfur compounds in natural gas. Detection limits, stability and linearity were investigated.

Experimental

An Agilent 7890A GC configured with a split/splitless inlet (Sulfinert-treated), and an Agilent 355 DP SCD were used. Sample introduction was through a six-port Hastelloy C gas sample valve (GSV) interfaced directly to the sulfur-treated inlet with Sulfinert tubing. An online dilutor was used for preparation of ppb-level sulfur compounds in

different matrices. Two four-port valves were used — one for sample introduction and one for static sample injection. The valves were installed sequentially prior to the GSV. Figure 1 illustrates the configuration of the gas blending system and GC SCD.

The sulfur standards were blended in helium at 1 ppm (V/V) and were purchased from Praxair, Inc. (Geismar, LA). See Table 1 for component details.

Table 1. Sulfur Standards in Helium

1.	Hydrogen sulfide	H_2S
2.	Carbonyl sulfide	COS
3.	Methyl mercaptan	CH_3SH
4.	Ethyl mercaptan	CH_4CH_3SH
5.	Dimethyl sulfide	CH_3SCH_3
6.	Carbon disulfide	CS_2
7.	2-propanethiol	$CH_3SHC_2H_5$
8.	Tert-butyl mercaptan	$(CH_3)_3CSH$
9.	1-propanethiol	$CH_3(CH_2)_2SH$
10.	Thiophene	C_4H_4S
11.	n-butanethiol	$CH_3(CH_2)_3SH$
12.	Diethyl sulfide	$CH_3CH_2SCH_2CH_3$
13.	Methyl ethyl sulfide	$CH_3SCH_2CH_3$
14.	2-methyl-1-propanethiol	$(CH_3)_2CHCH_2SH$
15.	1-methyl-1-propanethiol	$CH_3CH_2CHSHCH_3$

Experimental Conditions

GC Conditions

Front Inlet	Split/splitless (Sulfinert-treated capillary inlet system)
Heater	150 °C
Pressure	14.5 psi
Septum purge flow	3 mL/min
Mode	Splitless
Gas saver	20 mL/min after 2 min
Sample loop	1 mL
Oven	30 °C (1.5 min), 15 °C/min 200 °C (3 min)
Column	HP-1 60 m × 0.53 mm × 5 µm
Injection mode	Static flow and dynamic flow modes

SCD Conditions

Burner temperature	800 °C
Vacuum of burner	372 torr
Vacuum of reaction cell	5 torr
H_2	40 mL/min
Air	53 mL/min

Results and Discussion

From the comparative results of the sulfur detectors' sensitivity, it could be seen that SCD is the best detector for sulfur components, especially at low levels [3]. The Agilent DP technology is the most sensitive and selective detector for sulfur-containing gaseous hydrocarbon samples.

Figure 2 is the chromatogram of low-level sulfur compounds at 1.35 ppb (H_2S), which is prepared by the point-of-use gas blending system. Table 2 is the calculated signal to noise (S/N) of each compound, from the achieved data. It can be seen that DP SCD can detect low-level sulfur compounds.

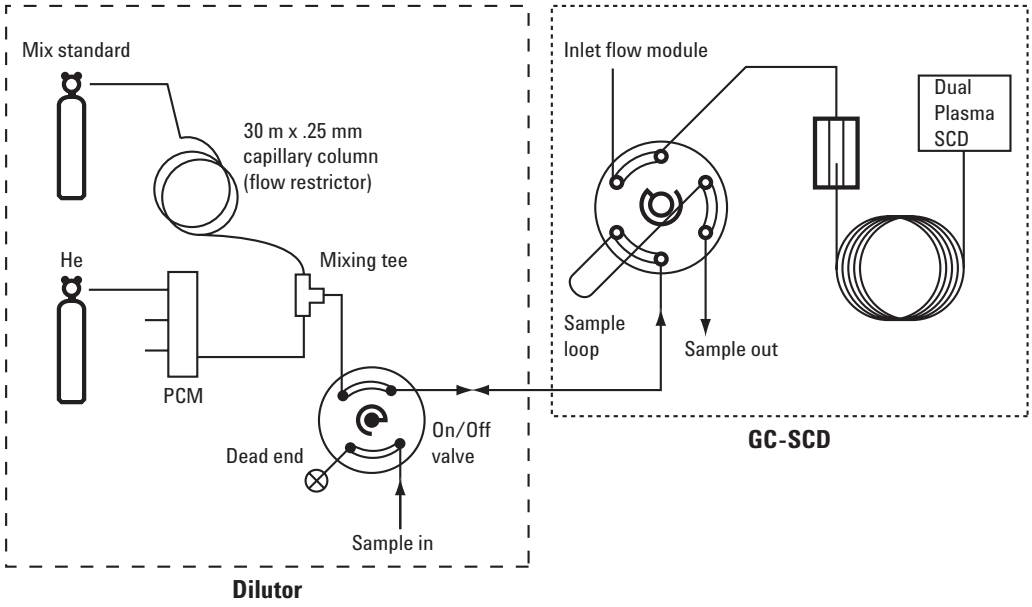

Figure 1. Diagram of online dilutor GC-DP SCD.

Table 2. S/N of Each Sulfur Component at 1.35 ppb (Refer to Table 1 for peak identification.)

Peak No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S/N	12.0	5.0	2.1	2.6	4.9	11.5	4.0	2.7	3.7	9.1	7.6	2.3	5.7	1.0	1.1

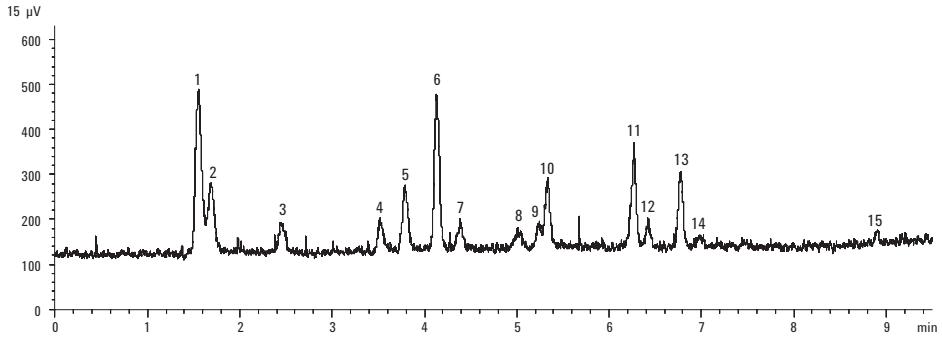

Figure 2. Chromatogram of sulfur compounds in helium at 1.35 ppb. (Refer to Table 1 for peak identification.)

Because the low-level sulfur components were prepared by the online dilutor system, which was prepared by adjusting the aux EPC to get appropriate diluent flow, high diluent flow could have the potential to cause high pressure in the sample loop, which results in the amount of the sample in the loop being different when the diluent flow changes from low to high. In this application, two sample injection modes, static and dynamic, were investigated. The mode is actuated by the on/off valve installed prior to GSV. When using static injection mode, the valve is switched to the off position, the pressure in the sample loop balances to ambient pressure, and then the sample is injected into the GC.

Table 3 shows the linear ranges of the two injection modes. The two injection modes have no difference from a linearity perspective, which means that the two injection modes are both suitable when using the 1-mL sample loop. The 1-mL sample loop's resistance is not high enough to cause variation in the sample injection amount.

Table 3 Linear Ranges of Two Injection Modes (Refer to Table 1 for peak identification.)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Linear range (ppb)				**6.24-544.5**				
Static mode	1	0.99996	0.99995	0.99999	0.99996	0.99999	0.99996	0.99999
Dynamic mode	1	0.99996	0.99997	0.99997	0.99996	0.99999	0.99998	0.99998
	9	**10**	**11**	**12**	**13**	**14**	**15**	
Linear range (ppb)				**6.24-544.5**				
Static mode	0.99995	0.99994	0.99996	0.99996	0.99996	0.99998	0.99998	
Dynamic mode	0.99998	0.99997	0.99998	0.99998	0.99998	1	0.99998	

Table 4 shows the long-term (72 hours) and short-term (8 hours) stability of the SCD at different concentration levels.

In an effort to investigate the coelution of hydrocarbon and sulfur, the same sulfur standards in natural gas were analyzed on the SCD. Figure 3 shows the chromatogram; no quenching was found.

Table 4 The Long-Term and Short-Term Stability of SCD (Refer to Table 1 for peak identification.)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
20.79 ppb	2.7	2.6	2.9	3.0	0.9	1.4	2.8	4.0	2.6	1.7	1.7	3.3	3.2	8.6	7.9
S.T. RSD (%)															
L.T. RSD (%)	3.0	2.7	2.4	2.5	1.4	1.5	2.6	4.3	3.8	2.7	2.0	4.9	3.2	7.9	6.9
1.38 ppb	6.6	10.1	11.7	22.8	30.4	4.1	6.9	18.7	10.7	25.1	5.1	11.1	5.8	29.6	24.1
S.T. RSD (%)															
L.T. RSD (%)	14.4	7.5	16.3	20.8	21.7	4.6	6.1	27.7	23.7	25.3	12.2	24.6	6.1	35.7	38.4

ST: Short term (8 hours); LT: Long term (72 hours)

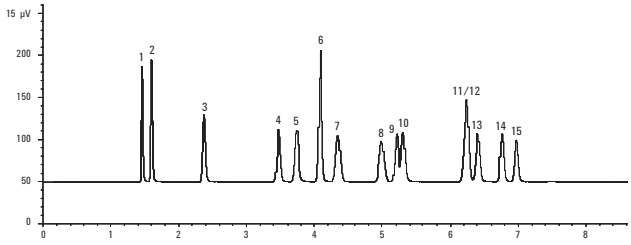

Figure 3. Chromatogram of sulfurs in natural gas. (Refer to Table 1 for peak identification.)

Natural Gas Sample Analysis

Three natural gas samples were analyzed by using the GC DP SCD system. Because the concentration of the target compounds is at ppm level, split mode was used and the method was recalibrated at ppm level. Table 5 shows the result of the three gas samples.

Table 5. Result of the Three Real Samples

Samples		H_2S	COS	Methyl Mercaptan
BLEND AL	Conc. (ppm, v/v)	2.3	2.0	2.0
	RSD (%, n = 5)	2.3	0.3	1.4
BLEND 6	Conc. (ppm, v/v)	27.1	21.9	17.3
	RSD (%, n = 5)	1.2	0.4	2.3
BLEND 12	Conc. (ppm, v/v)	15.0	9.2	10.1
	RSD (%, n = 5)	0.7	0.6	0.6
Standard	Conc. (ppm, v/v)	2.0	0.8	0.9
natural gas	RSD (%, n = 5)	1.7	2.5	1.7

Conclusions

An online dilutor combined with a GC DP SCD is suitable for gaseous sulfur components analysis, especially for the low-level components. The online dilutor offers an automatable means of system calibration and the detection limits for the trace sulfur detection are down to ppb level. By using an on/off valve prior to the GSV, both the static and dynamic injection modes of the sample gas blending system can be used. The static injection mode is important when a small sample loop with a large resistance is used. The diluter system with GC/SCD is available as an Agilent SP1, please refer to SP1 7890-0375 for order information.

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Printed in the USA
August 12, 2008
5989-9234EN

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Crude Oil & Natural Gas

Crude Oil

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Using a New Gas Phase Micro-Fluidic Deans Switch for the 2-D GC Analysis of Trace Methanol in Crude Oil by ASTM Method D7059

Application

Petrochemical

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Abstract

A new ASTM method was developed for the analysis of trace methanol in crude oil samples. This method relies on the use of two-dimensional heart-cutting gas chromatography to separate methanol from the complex matrix. A new microfluidic Deans switch was developed for the Agilent 6890N GC system that improves the performance of heart-cutting two-dimensional gas chromatography. This system was used to perform the analysis of methanol in crude oil with results that exceed the performance requirements of the ASTM method.

Introduction

The chemical characterization of crude oils present a real challenge to analytical chemists due to the varied and complex nature of the sample matrix. This is especially true when trying to separate and quantify trace amounts of low boiling contaminants or additives that cannot be separated using conventional capillary gas chromatography (GC). For such analyses, two-dimensional (2-D) GC offers a relatively simple yet powerful solution. Recently, ASTM Committee D2 has developed a heart-cutting 2-D GC method for the analysis of methanol in crude between 15 ppm (m/m) and 900 ppm (m/m) [1]. Methanol is added to crude oil to prevent the formation of gas hydrates, but it must be removed since the oxygen can cause problems with further refining processes.

Heart-cutting 2-D GC using a Deans switch has recently experienced a revival due to the advanced technology of modern columns and instruments [2]. The latest GC instruments make heart-cutting GC much easier to set-up, more reliable, and precise. However, the actual hardware used to perform heart-cutting has not kept pace with the advances offered by today's instruments. A typical 2-D manifold still consists of a collection of individual plumbing pieces such as tees, stainless tubing, and graphite/vespel ferrules that are assembled by hand. While this plumbing works well for some applications, especially those with packed columns, it is not optimized for modern capillary chromatography.

The large thermal mass of the device can be difficult to heat uniformly, introducing cold spots in the plumbing resulting in reduced chromatographic performance for higher boiling compounds. While the fittings are machined to reduce dead volume and minimize flow paths, there are still significant plumbing problems that contribute to peak broadening within the device. Capillary columns are also difficult to connect to these fittings and must rely on a graphite/vespel ferrule and sleeve combination to make a tight seal. This connection is difficult to make and can leak with repeated oven temperature cycling from <80 °C to >250 °C. Additionally, the graphite/vespel ferrules

can adsorb solvents and analytical components, resulting in reduced sensitivity, increased peak tailing, and elevated baselines.

To overcome these difficulties, a new micro-fluidic Deans switch was designed that combines the individual switch components into a smaller, single device (Figure 1).

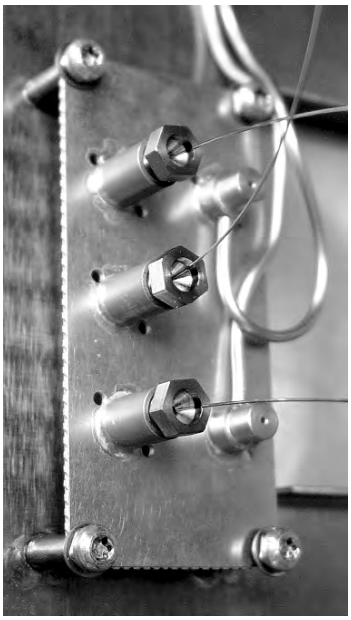

Figure 1. A close-up view of the new micro-fluidic Deans switch in the 6890N GC.

The switch's flow paths and connections are laid out and etched onto a small, thin, stainless steel plate using photolithography and chem-milling technologies. The plate is diffusion bonded, mounted with column connectors, and surface deactivated, resulting in an integrated, micro-fluidic switch that has a number of advantages for heart-cutting 2-D GC. The 4-times smaller thermal mass does not act as a heat sink; therefore, the device works optimally with modern GC ovens, especially for faster applications. The micro-fluidic switch also has far fewer connections, greatly reducing leak potential. Metal ferrules are used to interface capillary columns to the device that are also leak-free in high-temperature cycling applications. These metal ferrules will also not adsorb solvents or sample matrix, improving sensitivity for trace analysis applications. This application note describes the use of the micro-fluidic Deans switch in the analysis of trace methanol in crude oil with ASTM method D7059.

Experimental

An Agilent 6890N gas chromatograph was equipped with a split/splitless injector, a pneumatics control module (PCM), two flame ionization detectors (FIDs), and an automatic liquid sampler (ALS). A DB-1 (polydimethylsiloxane) column was used as the primary column and a CP-Lowox (Chrompack International BV) was used as the secondary column. The two columns were linked using a micro-fluidic Deans switch. Table 1 lists the details of the hardware configuration. The instrument operating conditions for this analysis are outlined in Table 2.

Table 1. Hardware Configuration

6890N GC Hardware	
G1540N	Agilent 6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210 (2 of each)	FID with EPC control
Option 309	Pneumatics control module with EPC control
G2855B	Micro-fluidic Deans switch kit
G2613A	Agilent 7683 Autoinjector
Columns	
Primary column	DB-1 column, 5.00-µm film, 10 m x 0.53-mm id (Agilent part no. 125-10H5)
Secondary column	CP-Lowox column, 10 m x 0.53-mm id (Chrompack International BV)
Fixed restrictor	Deactivated fused silica tubing, 0.5 m x 0.25-mm id (Agilent part no. 160-2255-10)
Data System	
G2070A	Agilent Multitechnique ChemStation
Other Consumables	
Agilent part no. 5181-1267	10-µL fixed tapered needle autoinjector syringe
Agilent part no. 5183-4647	Inlet liner optimized for splitless operation
Agilent part no. 5183-4759	Advanced green septa

Table 2. Instrument Conditions

Injection port	Split mode, 7:1 ratio
Temperature	325 °C
EPC pressure	3.51 psi helium, constant pressure mode
Injection size	1 µL
DB-1 column flow	3 mL/min
Pneumatics control module (PCM)	5.07 psi helium, constant pressure mode
CP-Lowox column flow	5 mL/min
FID temperatures	350 °C
Oven temperature program	
Initial temp	150 °C for 3 min
Ramp #1	20 °C to 300 °C for 5 min

Electronic pneumatics control (EPC) pressures, flow rates, and the fixed restrictor dimensions were determined using a Deans switch calculator software program that was designed for this system. This calculator program is included with the Deans switch hardware option for the Agilent 6890N GC.

Crude oil samples spiked with methanol were obtained from Spectrum Quality Standards (Houston, TX, USA). Each sample was prepared according to ASTM Method D7059 by mixing 5.0 g of crude oil sample with 5 mL of ACS grade toluene containing 1000 µg/g of 1-propanol. The 1-propanol was used as an internal standard (ISTD). If the samples were not analyzed immediately, they were stored in glass vials with TFE-fluorocarbon lined caps below 5 °C. During storage there was little or no headspace in the vials to reduce the partition of methanol into the headspace. Seven calibration standards were prepared containing 5 to 1000 ppm (m/m) of methanol in toluene, and each containing 500 ppm (m/m) of 1-propanol. The calibration standards should be used immediately after preparation since the methanol concentration is not stable in toluene. The standards can be stored for several days below 5 °C in glass vials with little or no headspace.

Results and Discussion

Heart-cut times were determined by injecting the 1000-ppm methanol standard onto the primary DB-1 column with no cutting to the Lowox column. The retention time for methanol was 1.82 min and 2.11 min for 1-propanol. Using this data, the cut-time for all standards and samples was 1.70 to 2.35 min. The 1000-ppm standard was then analyzed using this cut time to evaluate the separation of the alcohols on the Lowox column after cutting. The methanol and 1-propanol were easily separated on the Lowox column with retention times of 4.72 and 6.38 min, respectively (Figure 2).

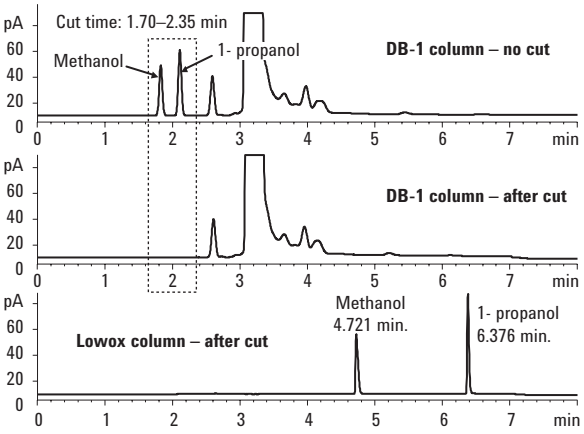

Figure 2. Setting the heart-cut times for the 2-D GC analysis of methanol in crude oil.

Calibration of the systems was performed using seven standards of methanol in toluene at concentrations of 5, 25, 75, 125, 250, 500, and 1000 ppm with 500 ppm of 1-propanol as the ISTD. The ChemStation was used to develop a calibration curve (Figure 3). This calibration exceeded the correlation coefficient of 0.99 required by the ASTM method. The detectability of the system was also checked using a 1-ppm standard of methanol in toluene, with no ISTD. This sample was analyzed and the signal-to-noise of the methanol peak on the second column (Lowox) was found to be 5:1, which exceeded the method requirement of a 5:1 signal to noise for a 2-ppm standard.

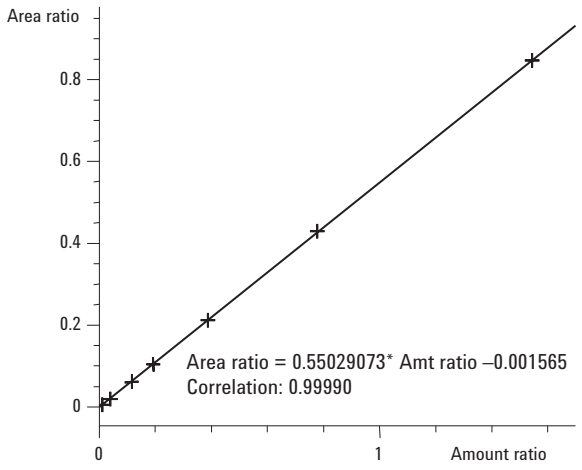

Figure 3. Calibration of methanol from 5 ppm (m/m) to 1000 ppm (m/m) using 2-D heart-cutting GC.

A quality control check of the system was also made using two crude oil samples; one contained 15-ppm methanol, and the other 670 ppm. For the 15-ppm sample, the reported result must be within ±5 ppm and for the 670-ppm sample, within ± 35 ppm. Figure 4 shows the data obtained from the analysis of the crude oil sample containing 15 ppm of methanol in crude oil. Two replicates of the 15-ppm sample yielded results of 10 ppm and 17 ppm. For the 670-ppm samples, the replicates yielded results of 670 ppm and 667 ppm.

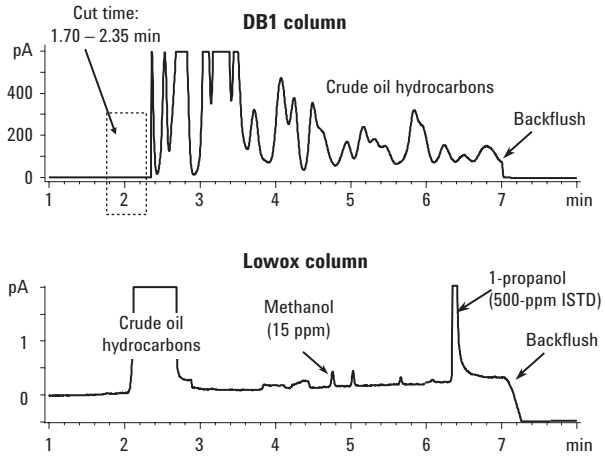

Figure 4. The 2-D GC analysis of 15 ppm (m/m) of methanol in crude oil using a micro-fluidic Deans switch.

The analysis time of the method was reduced by backflushing the primary column to quickly remove the higher boiling crude oil components from the DB-1 column. Backflushing was done after the elution of the 1-propanol peak from the Lowox column. At 7 min, the split/splitless inlet pressure was reduced to 0.5 psi while the PCM pressure was increased to 35 psi. This reversed the flow in the primary DB-1 column so that any remaining compounds at the head of the column were eluted through the split vent (Figure 5).

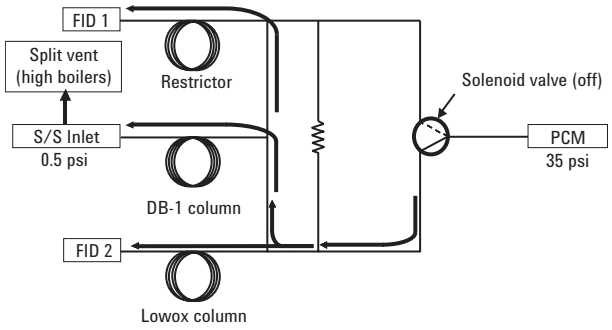

Figure 5. Backflushing the DB-1 column can be done to reduce the analysis time using the EPC on the 6890N Deans system.

Crude oil analysis also requires more maintenance than with more volatile samples. Since crude contains a wide range of compounds, from low boiling to nonvolatile, the inlet liner will need more frequent replacement. It is recommended that the liner be changed after 50 injections. Additionally, one should also inspect the top of the split/splitless inlet body to evaluate any contamination of crude oil tars that can accumulate at the top of the inlet and at the outlet of the split vent line. Depending on the samples, the inlet body may need to be cleaned after 100 injections.

Conclusions

The analysis of any components in crude oil presents a number of challenges due to the difficult nature of the sample matrix. The recently developed ASTM method D7059 uses heart-cutting 2-D GC to separate and quantify trace levels of methanol in crude oil samples. A new micro-fluidic Deans switch designed for the 6890N was shown to be ideally suited to this difficult application. It has 4-times less thermal mass so that it is effectively and uniformly heated, avoiding cold spots where high-boiling crude oil components could be condensed. The shorter flow paths, inert surfaces, and capillary optimized fittings ensure that active compounds like methanol can be separated and detected at trace levels in crude oil.

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Printed in the USA
November 3, 2004
5989-1840EN



Agilent Technologies

The Use Of Automated Backflush on the 7890A/5975A GC-MS System

Setup Example Using Biomarkers

Application

Hydrocarbon Processing

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Abstract

The use of column backflushing in capillary gas chromatography has been sparingly used over the years, primarily due to its added complexity and demands on data system control for use in automated/routine laboratories. The potential of backflushing has been demonstrated in a gamut of applications from environmental, refining, and residues in food where high boiling point and complex matrices are commonplace. This application describes the setup, use and tricks and tips for implementing backflushing on the 7890A/5975A GC-MS system, with the specific example of monitoring biomarkers in crude oil.

Introduction

Until recently, the implementation of capillary column backflush has required a cumbersome conglomeration of parts and separate controllers. The nonintuitive combination of manual pressure regulators, timers, stand-alone valve controllers, and experimentally determined GC setpoints conspired against chromatographers with interest in attempting it. The few who were successful on a given system would rarely consider implementing backflush routinely, even if their efforts met with success the first time. Considerable improvements in implementation of backflush became available with the 6890 GC and 6890/5973 GC MSD systems [1–6]. With the release of the Agilent 7890A/5975A GC-MS system with ChemStation version E.01.00, implementation of capillary column backflush has never been easier. Full electronic control of all backflush parameters is possible in a manner never before offered in a GC-MS system. At the same time, major advancements in fluidic devices now greatly improve the mechanical aspects of implementing routine capillary column backflush.

The benefits of backflush in capillary gas chromatography are myriad:

- More samples/day/instrument
- Better quality data
- Lower operating costs
- Less frequent and faster GC and MSD maintenance
- Longer column life
- Less chemical background

When a mass spectrometer (MS) is employed, a key additional benefit is that backflushing high-boiling components from the capillary column and out of the inlet to waste (usually via a split/splitless inlet or PTV) prevents them from being deposited in the ion source. This improves detection limits for sub-

sequent samples (less background) and greatly increases the number of samples that can be run before ion source cleaning is required.

As illustrated by the many prior examples (see references), backflush technology is relevant in many areas, including the geochemical/hydrocarbon area, wherein samples generally span a large boiling point range and analyses are typically long yet contain only one or two compounds of specific interest. Biomarker determination in crude oils is such an example where backflush can provide several significant benefits. Analytical run times are greatly reduced; high-boiling, less important components are removed from the system and prevented from reaching the mass spectrometer; and the column is exposed to much lower final oven temperatures. In this application, backflushing on a 7890A/5875 system is presented to show the new setup screens and increased ease of setting up backflush conditions.

Experimental

Table 1 shows the analytical conditions used in a traditional GC-MS analysis of crude oil. The boiling point range of this oil sample is very wide (spanning C_4 to C_{50}), with the target components of interest eluting around 30 minutes in a 74-minute analysis (see Results and Discussion).

Table 1. Original Analytical Method Conditions

Column	HP5-MS 30 m × 0.25 µm × 0.25 µm; part number 19091S-433
Carrier gas	Helium, constant flow mode; 1.2 mL/min
Split/splitless inlet	340 °C, split 30:1
Oven	50 °C (1 min) → 320 °C at 5 °C/min hold for 20 minutes
Analysis time	74 min
Sample	Crude oil in CS_2, 1-µL injection
MSD	Scan = 35–700 u Samples = 2^2 Source = 300 °C Quad = 150 °C Transfer line = 320 °C

A 3-way purged splitter (Agilent part number G3183B) Capillary Flow Technology device was used for this application, in part to demonstrate its flexibility. The device has a purge and four connections (Figure 1). As used herein, only two of the ports were used, one for the column outlet (port 3) and the other for the restrictor to the MSD (port 4). The other two ports (1 and 2) were plugged with solid wire instead of column connections. Very reliable connections are a feature of Capillary Flow Technology devices because of the use of soft metal ferrules. Care needs to be taken when making these connections, but the process is very straightforward and easily learned. The manuals provided with the various Capillary Flow Technology devices contain explicit instructions.

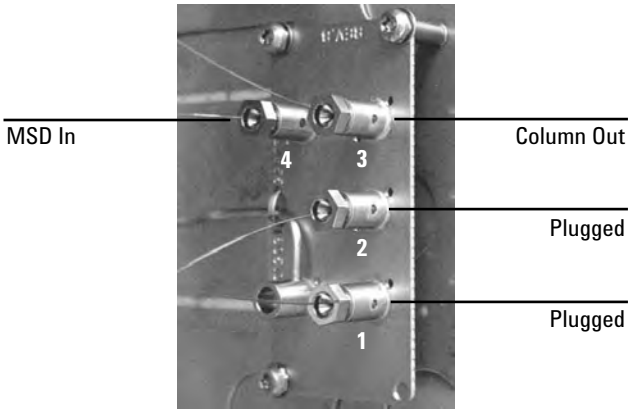

Figure 1. 3-way purged splitter. The column outlet was attached to port 3 and the MSD restrictor was attached to port 4. Ports 1 and 2 were plugged.

Careful consideration must be made before a restrictor internal diameter (ID) and length are chosen for a backflush application. Parameters such as detector type (atmospheric pressure versus vacuum), vacuum pumping capacity (for example, diffusion pump, standard and performance turbo molecular pumps), and Capillary Flow Device pressure and desired split ratio (if splitting detector effluent to multiple detectors) must all be taken into consideration. Such considerations are described in detail in a previous application [1].

In this example with a 5975A MSD, a deactivated restrictor of 1 m × 0.18 mm id (such as Agilent part number 160-2615-1) provided a balanced match for this application.

Table 2 shows the analytical conditions used for this backflush application, and Figures 2 to 7 show the software setup screens for the 7890A/5975A GC-MS system with MSD ChemStation revision E.01.00 software.

Table 2. Backflush Analytical Method Conditions

Column	HP5-MS 30 m × 0.25 µm × 0.25 µm part number 19091S-433
Carrier gas	Helium, constant flow mode; 1.2 mL/min
Split/splitless inlet	340 °C, split 30:1
Oven	50 °C (2 min) → 205 °C at 5 °C/min no hold
Backflush restrictor	1m × 0.18 mm deactivated capillary column tubing
Aux 3 pressure	1 psi
Backflush pressure	75 psi
Analysis time	31 min + 5.47 post run at 205 °C Total time = 36.47 min
Sample	Crude oil in CS_2, 1-µL injection
MSD	Scan = 45–700 u Samples = 2^2 Source = 300 °C Quad = 150 °C Transfer line = 320 °C

By setting up the required analytical column and restrictor with the correct inlet and outlet connections (Figures 2 to 4), the software automatically calculates the inlet pressure required to maintain analytical column flow. By selecting the "evaluate" button (Figure 5), the backflush pressure required for a predetermined number of column "sweeps" or "void volumes" is calculated, displayed for review, and uploaded to the analytical method along with the GC oven hold time (Figures 6 to 8). As a general guide, 10 void volumes is effective for most applications. As few as two void times can effectively backflush a capillary column under certain conditions (for example, high oven ramp rates prior to backflush). However, some applications may require more than 10 void volumes to backflush everything, so the onus is on the user to validate appropriately backflush times for a given application. A blank run (that is, pure solvent as sample) following a sample run with backflush is helpful during method validation to see that all components are effectively removed from the analytical column by the chosen backflush conditions.

In this application, a 75 psi backflush pressure resulted in a backflush flow of approximately 6 mL/min through the capillary column and 75 mL/min into the performance turbo molecular pump. A figure shown later in this application illustrates that these backflush conditions were effective.

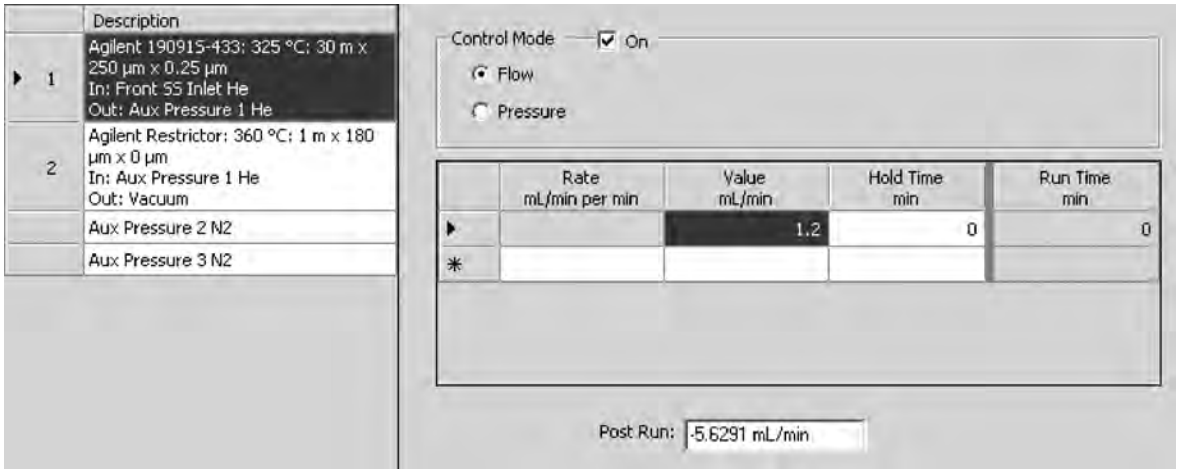

Figure 2. Software setpoints for the analytical column.

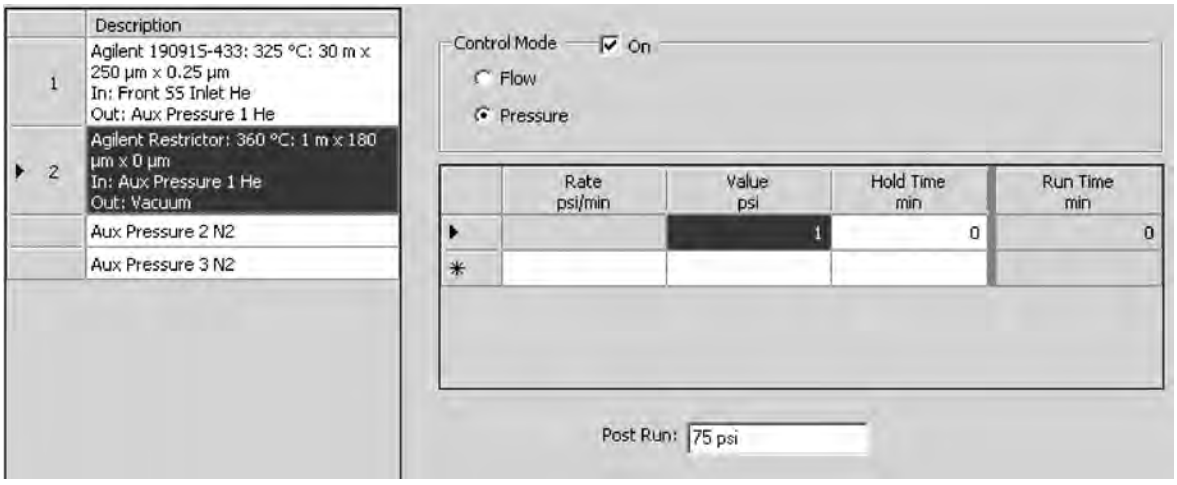

Figure 3. Software setpoints for the restrictor to the MSD.

	Description
▶ 1	Agilent 19091S-433: 325 °C: 30 m x 250 µm x 0.25 µm In: Front SS Inlet He Out: Aux Pressure 1 He
2	Agilent Restrictor: 360 °C: 1 m x 180 µm x 0 µm In: Aux Pressure 1 He Out: Vacuum

Figure 4. Column inlet and outlet conditions.

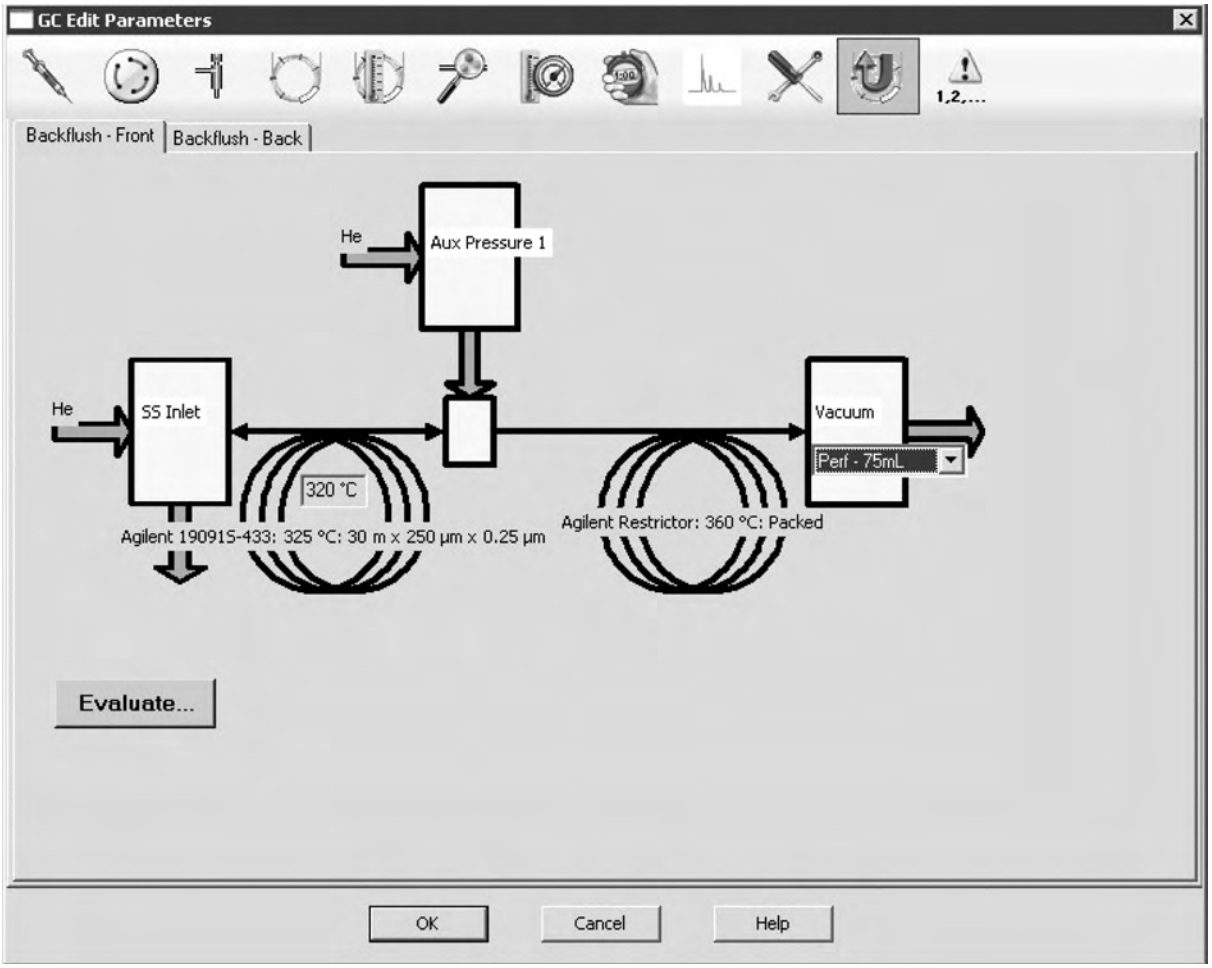

Figure 5. Interactive setup for backflush conditions in ChemStation.

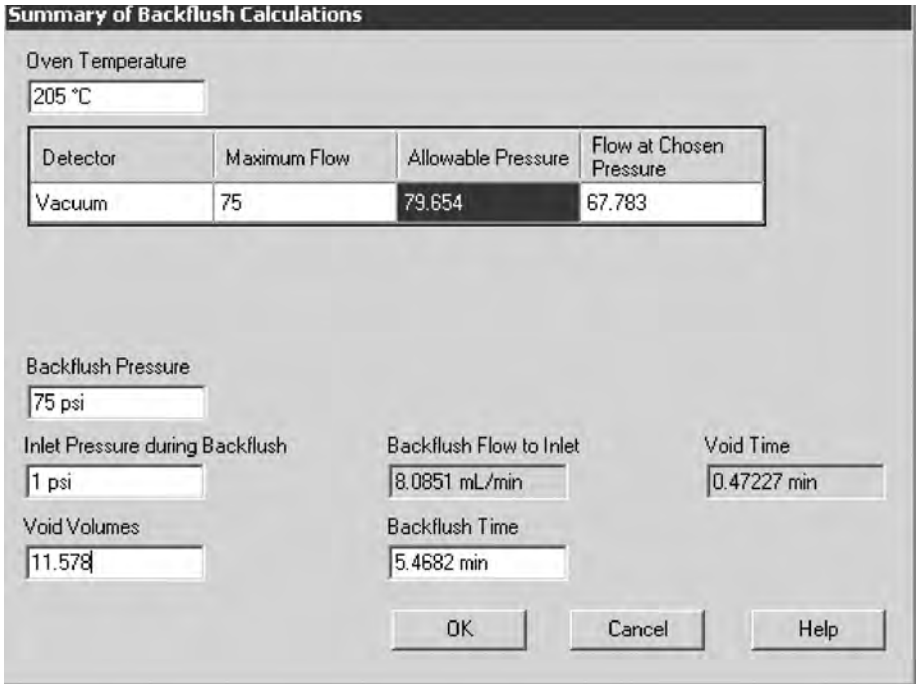

Figure 6. Conditions uploaded to method setpoints.

Results and Discussion

The profile seen in Figure 9 is typical of many crude oils with complex distribution over a large boiling point range, with a large number of unresolved components. Another feature is the long tail of high-boiling components that must be eluted after the compounds of interest. Figure 10 illustrates the three components of interest: a series of three methylbenzothiophenes through an extracted ion chromatogram (EIC) of *m/z* 198.

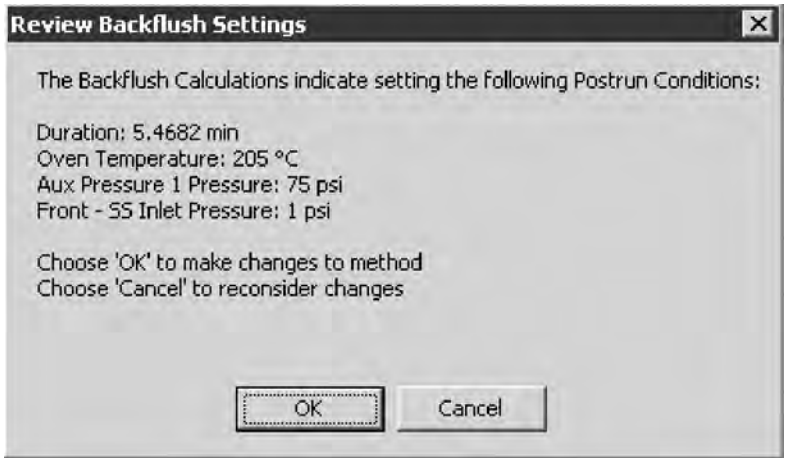

Figure 7. Conditions uploaded to method setpoints.

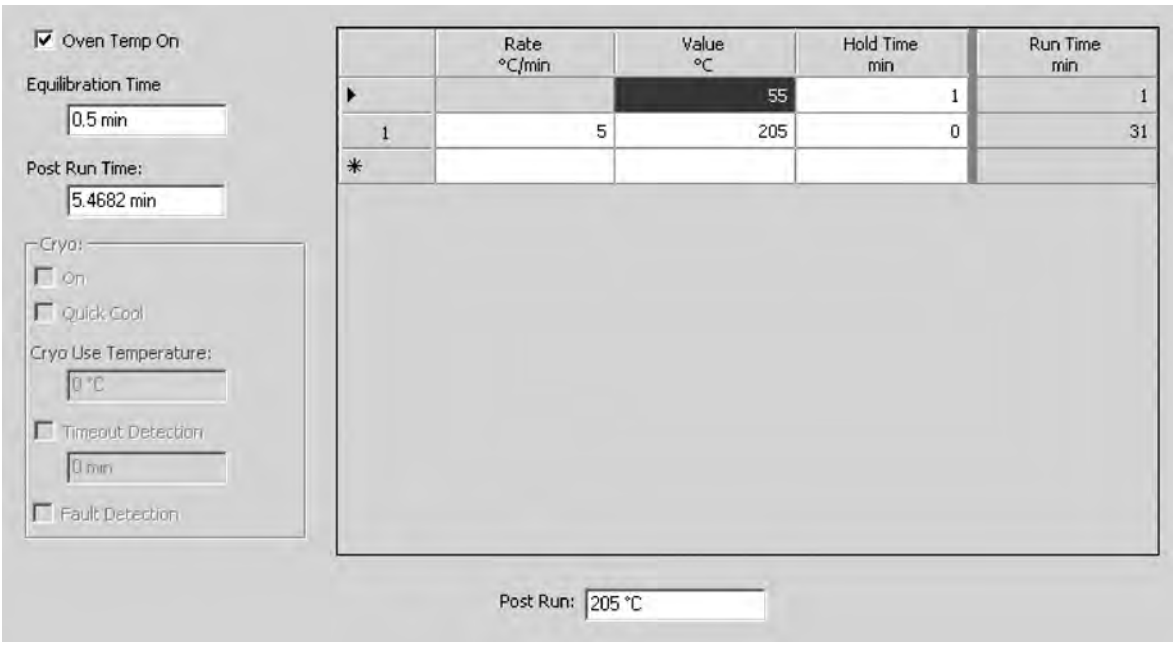

Figure 8. Note that the post-run time has been updated automatically.

Figure 11 shows the chromatogram from another run that includes a backflush immediately after the benzothiophenes had eluted.

In order to validate the efficacy of the backflush, a full-length analysis was undertaken with pure solvent immediately after the backflush run. It

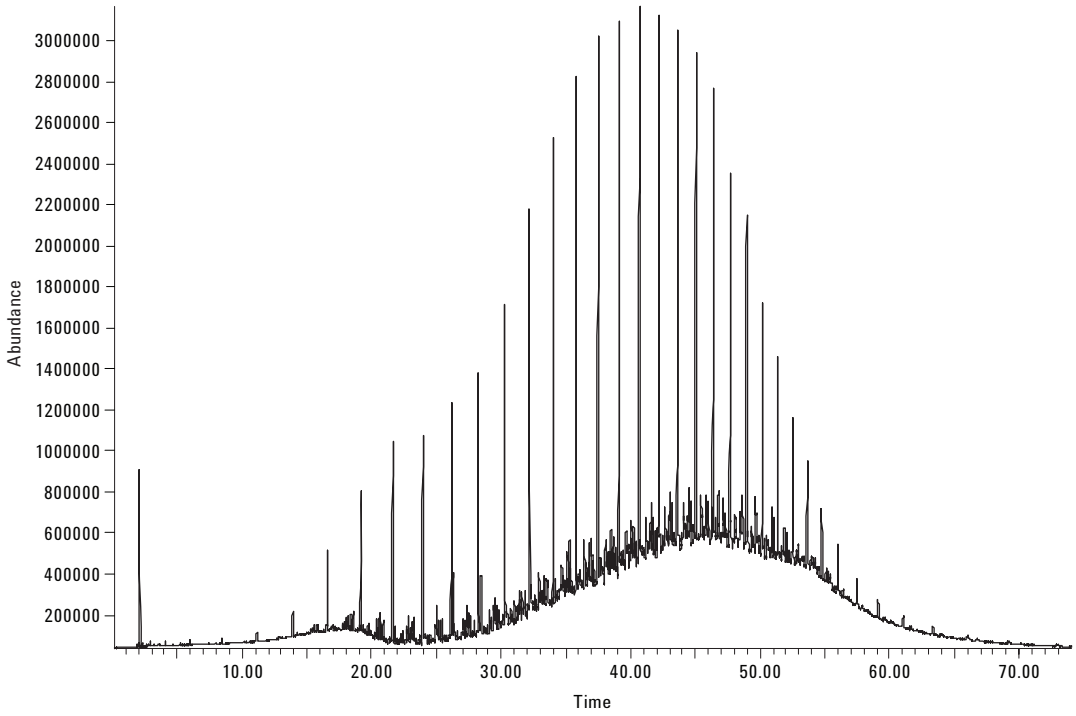

Figure 9. Total ion chromatogram (TIC) of normal analysis. Peaks of interest (benzothiophenes) are obscured by the high concentration of hydrocarbons.

can be seen from Figure 12 that no residual high-boiling components remained in the capillary column after the backflush from this blank solvent injection. Also, there are no residual biomarkers at *m/z* 198. All material (representing over 50% of the sample introduced into the column) eluting after 31 minutes was effectively backflushed.

Figure 13 shows the EIC (*m/z* = 198) for both the normal run and the backflushed runs, showing that no material was lost and retention times were not changed by implementing the backflush.

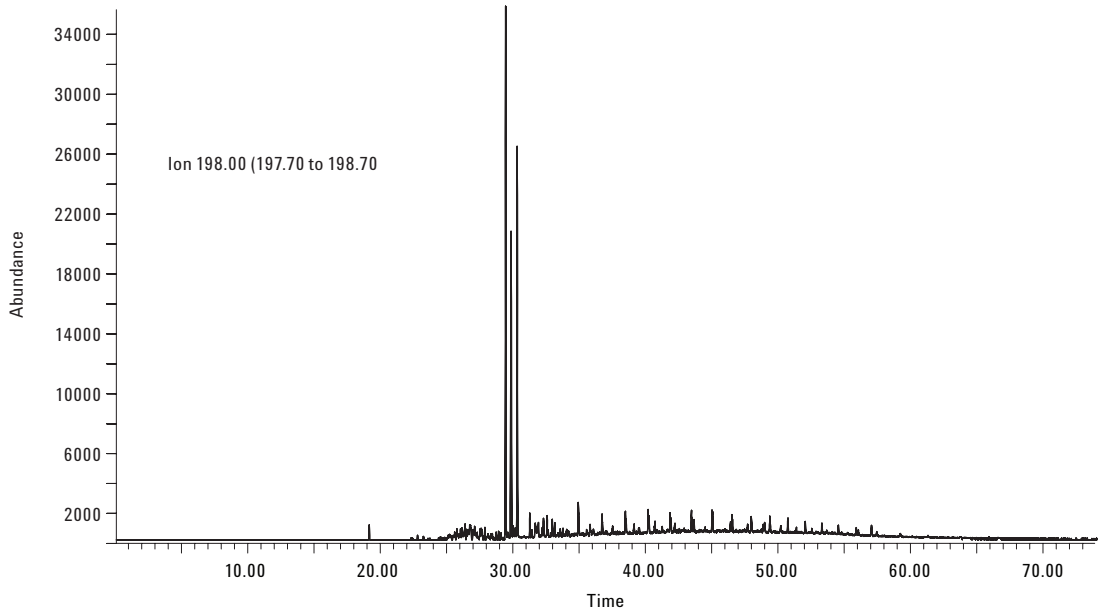

Figure 10. EIC of *m/z* 198 ion. The three methylbenzothiophene peaks of interest at approximately 30 minutes are easily visualized.

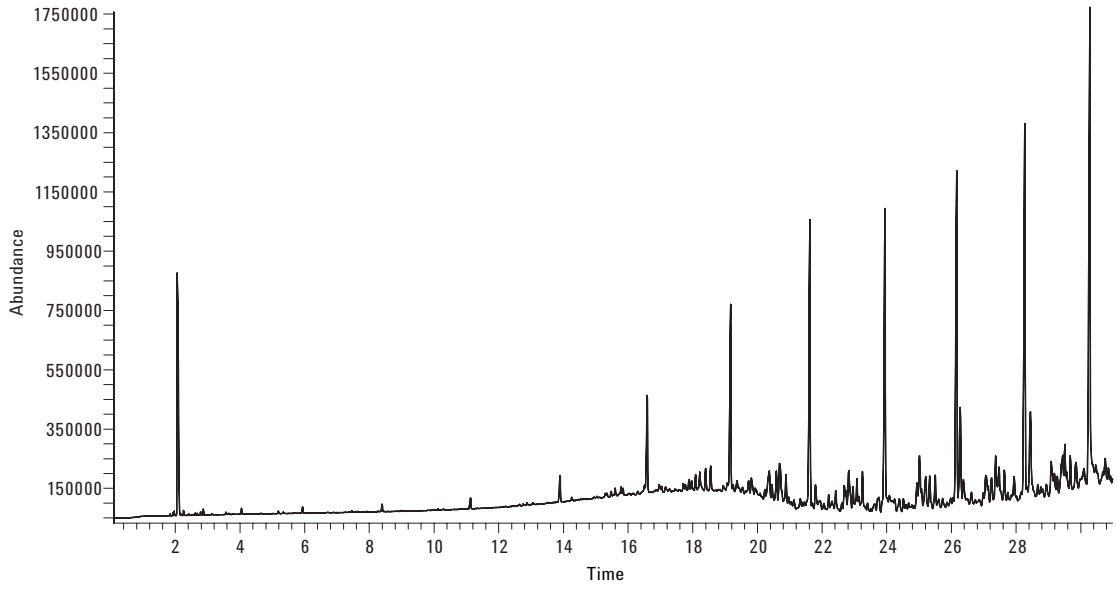

Figure 11. TIC of backflush run; run switched to backflush mode at 31 minutes.

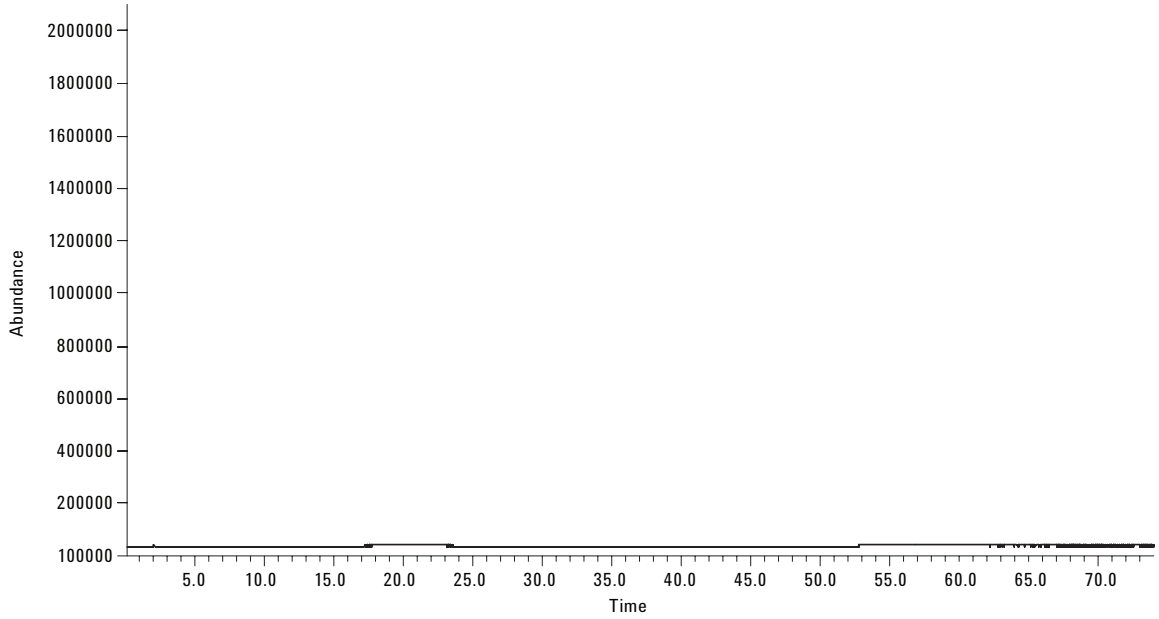

Figure 12. TIC of full run after the backflush with inset of the EIC of *m/z* 198.

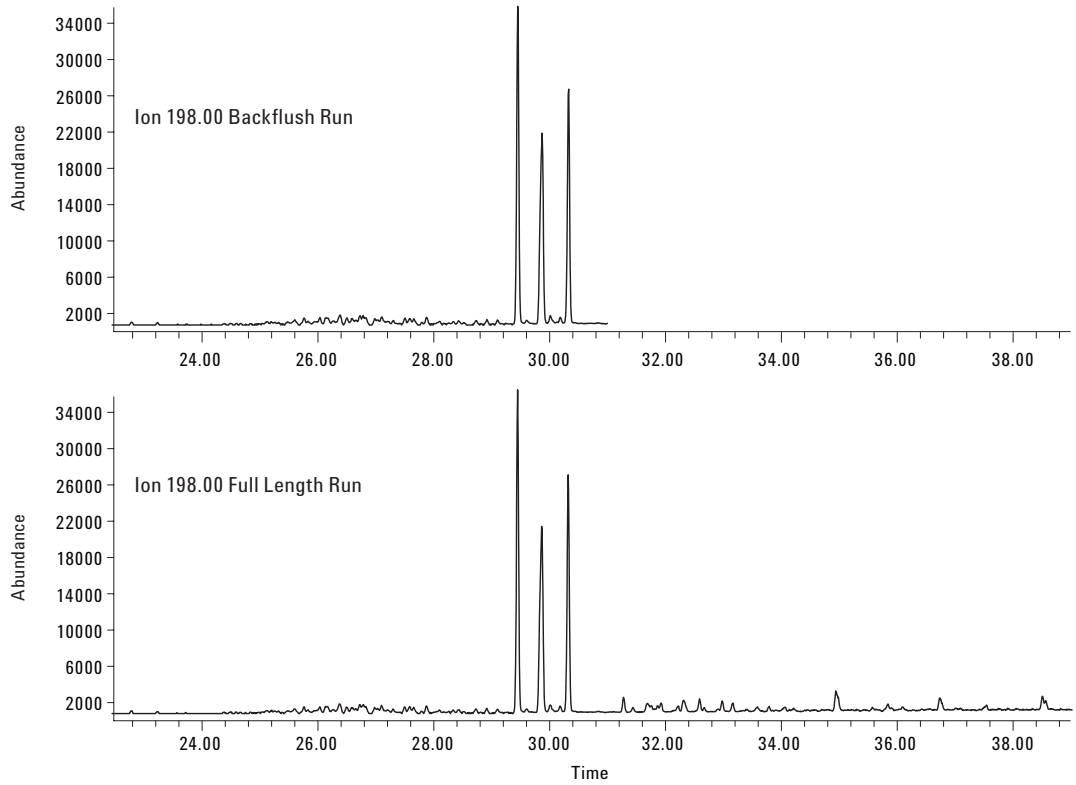

Figure 13. Overlay of EIC of *m/z* 198 from full run and backflush run, showing the exact matching of the analytical portion of each run for the three methylbenzothiophene biomarkers.

Conclusions

This application demonstrates the ease with which backflush can be set up and executed with the 7890A/5975A GC-MS system with EA 01.00 MSD ChemStation. In this example, a total run time saving of 37.5 minutes effectively halved the run time of the original run while ensuring that the analytical column was free from sample carryover. A confirmatory blank run following backflushing substantiates the efficacy of the backflush, verifying removal of all remaining sample components.

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Printed in the USA
July 11, 2008
5989-8588EN



Agilent Technologies

Investigation of the Unique Selectivity and Stability of Agilent GS-OxyPLOT Columns

Application

Gas Chromatography

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Abstract

The stationary phase of a GS-OxyPLOT column is a proprietary, salt deactivated adsorbent. GS-OxyPLOT columns show unique selectivity to oxygenated hydrocarbons, excellent stability and reproducibility, long column lifetime, and a wide application range.

Introduction

The determination of oxygenated hydrocarbons in different sample matrices is very important for the petrochemical industry, because oxygenates directly influence product quality. Presence of such oxygenates may cause the catalysts to be poisoned and deactivated, resulting in more downtime and higher costs. ASTM has developed several methods for analysis of oxygenates, such as ASTM D7059, D4815, and D5599. The oxygenates include ethers, esters, ketones, alcohols, and aldehydes.

Methanol is one of the oxygenates that often present in light hydrocarbon streams. For example, it is added to natural gas and production of crude oil to prevent hydration of hydrocarbons during transportation via pipelines. Therefore, it is important to accurately measure the content of methanol from light hydrocarbons at different concentrations, including at trace levels.

To achieve this, a new porous layer open tubular (PLOT) capillary column, the GS-OxyPLOT column, was used. The stationary phase of the GS-OxyPLOT is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons, while having virtually no interactions with saturated hydrocarbon solutes [1].

Using Capillary Flow Technology, such as backflush or Deans switch, GS-OxyPLOT columns can provide a turnkey solution for the analysis of trace level oxygenate impurities in complex matrices, such as motor fuels, crude oil, and gaseous hydrocarbon [2]. Meanwhile, a GS-OxyPLOT column can be used as a single analytical column to separate oxygenates for some samples. In this application, methanol was set as an example to investigate the performance of the GS-OxyPLOT column.

Experimental

The experiments were performed on an Agilent 7890A GC system and a 6890N GC system equipped with split/splitless capillary inlet, flame ionization detector (FID), and Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with long-lifetime septa (Agilent p/n 5183-4761) and spilt/splitless injection liners (Agilent p/n 5183-4711). Injections were done using 10-µL syringes (Agilent p/n 9301-0714). A glass indicating moisture trap (Agilent p/n LGMT-2-HP), an oxygen trap (Agilent p/n BOT-2), and a

hydrocarbon trap (Agilent p/n 5060-9096) were installed. Agilent ChemStation was used for all instrument control, data acquisition, and data analysis.

Results and Discussion

Analysis of Normal Hydrocarbons and Methanol

A mixture of normal hydrocarbons and methanol was prepared with the following approximate concentrations %(w/w): 34.8% n-pentane, 12.8% n-hexane, 1.8% n-heptane, 1.9% n-octane, 2.1% n-nonane, 3.9% n-decane, 2.1% n-undecane, 9.8% n-dodecane, 11.8% n-tridecane, 4.7% n-tetradecane, 2.4% n-pentadecane, 4.5% n-hexadecane, 2.4% n-heptadecane, 1.0% n-octadecane, 0.9% n-eicosane, 0.9% n-docosane, 1.1% n-tetracosane, and 0.8% methanol.

The analytical conditions are summarized in Table 1. The normal hydrocarbons and methanol analysis was performed on a GS-OxyPLOT column (Agilent p/n 115-4912). The GC chromatogram is shown in Figure 1.

Table 1. Conditions for Normal Hydrocarbons and Methanol Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm (Agilent p/n 115-4912)
Carrier gas	Helium, constant flow mode, 40 cm/s @ 50 °C
Inlet	Split/splitless at 325 °C
Split ratio	80:1
Oven temperature	50 °C (2 min); 10 °C/min to 290 °C (2 min)
Post-run	300 °C (2 min)
Detector	FID at 325 °C
Injection size	0.2 µL

In Figure 1, the GS-OxyPLOT column shows unique retention characteristics for methanol. The lower boiling point hydrocarbons were not strongly retained on the stationary phase and eluted through the FID very rapidly. The methanol eluted after n-C14, allowing it to be quantified without any interference from the hydrocarbon matrix, and making it feasible for trace-level methanol analysis in a range of hydrocarbon streams.

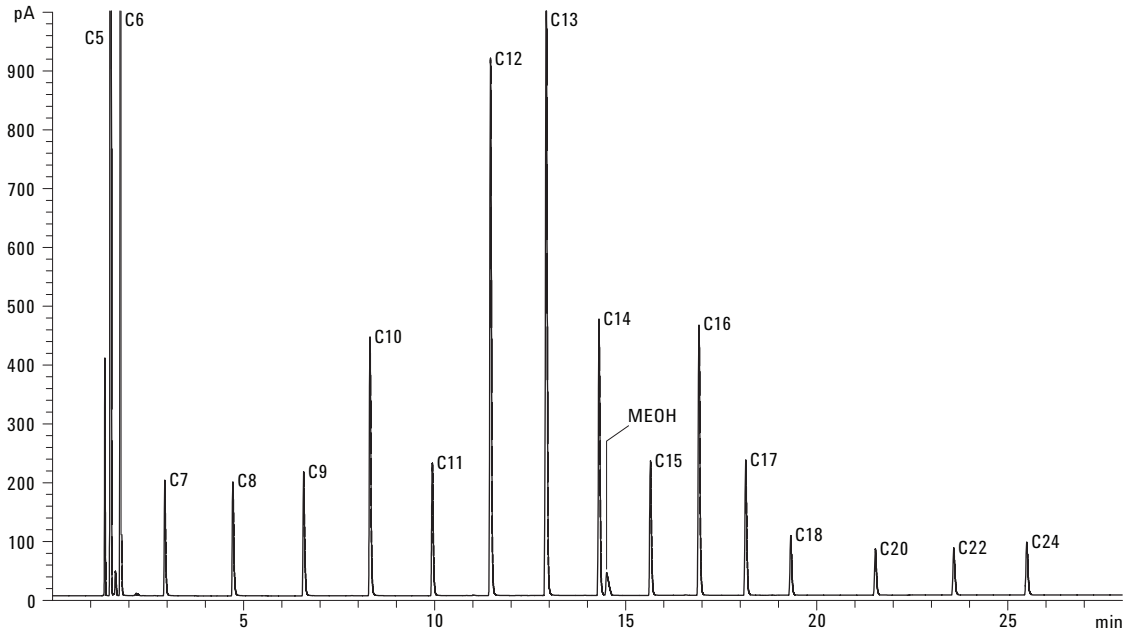

Figure1. Analysis of methanol and normal hydrocarbons on a GS-OxyPLOT column, 10 m × 0.53 mm × 10 µm.

In addition, the baseline was quite smooth, even when the oven temperature was up to 290 °C. GS-OxyPLOT has an upper temperature limit of 350 °C and exhibits virtually no bleed, making it widely applicable for long-term reliable analysis.

Analysis of Alcohols

A mixture containing a range of primary alcohols from methanol to lauryl alcohol was analyzed on a GS-OxyPLOT column using a temperature-programmed method. Table 2 lists conditions for alcohols separation, and the resulting chromatogram is shown in Figure 2.

Sample

The sample had an approximate concentration (v/v) of 1% methanol, ethanol, propanol, butanol, amyl-alcohol, heptanol, octanol, nonanol, decyl alcohol, and lauryl alcohol in toluene.

As can be seen in Figure 2, all of the alcohols are separated and eluted with good peak shape within an analysis time of 15 min. In this experiment, oven temperature was set up to 300 °C. Thanks to its advanced dynamic coating process, Agilent's GS-OxyPLOT stationary phase exhibits virtually no detector spiking due to particle generation from the phase coating [3].

Due to the high viscosity of alcohols, especially decyl alcohol and lauryl alcohol, it is necessary to wash the needle after each injection in case of carryover problems.

Table 2. Conditions for Alcohols Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier Gas	Helium, constant flow mode, 40 cm/s at 150 °C
Inlet	Split/splitless at 325 °C
Split ratio	50:1
Oven temperature	150 °C (0 min); 10 °C/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	0.2 µL

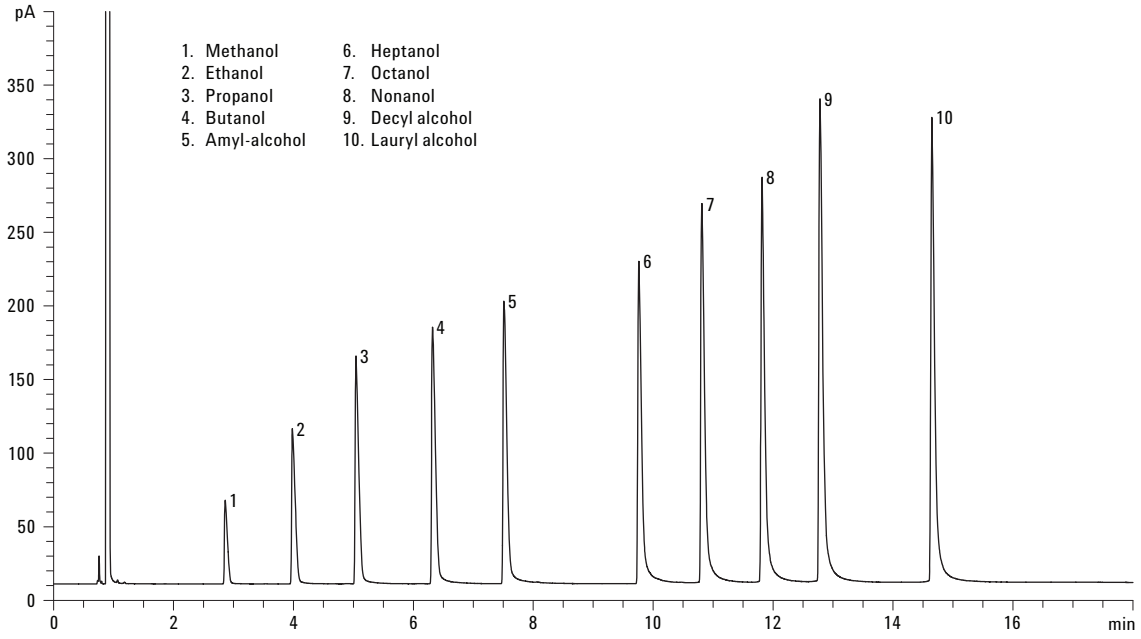

Figure 2. Separation of alcohols using GS-OxyPLOT, 10 m × 0.53 mm × 10 µm.

Influence of Temperature on the Selectivity of GS-OxyPLOT

To polar stationary phases, the temperature has a direct influence on the selectivity. GS-OxyPLOT offers extremely high polarity. The analysis of normal hydrocarbons and methanol demonstrated that methanol elutes after n-C14. Using a mixture containing methanol, n-tetradecane, and n-pentadecane, isothermal Kovats retention indices were tested at isothermal oven temperatures of 150, 200, 220 and 250 °C, respectively (Table 3). The relationship between Kovats retention indices and oven temperature is shown in Table 4.

Table 3. Conditions for Kovats Retention Indices Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 30 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temperature	150, 200, 220, and 250 °C, respectively; isothermal
Detector	FID at 250 °C
Injection size	0.2 µL

Table 4. Kovats Retention Indices and Oven Temperature (n > 3)

Oven temp.	150 °C	200 °C	220 °C	250 °C
LOT1	1419	1418	1418	1413
LOT2	1420	1421	1419	1417

Retention index, Ix, was calculated using the following equation:

$$Ix = 100n + 100[\log(t_x) - \log(t_n)]/[\log(t_{n+1}) - \log(t_n)]$$

Where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound X; t_x is the retention time of compound X. Here compound X is methanol, the reference n-alkane hydrocarbons are n-tetradecane and n-pentadecane, respectively.

Table 4 shows good repeatability of Kovats rentention indices for two different lots of GS-OxyPLOT columns. The retention index for methanol only changed by less than 10 index units over 100 °C temperature difference. Therefore, when the oven temperature changes from 150 to 250 °C, it has little influence on the selectivity of GS-OxyPLOT.

Influence of Moisture on GS-OxyPLOT

Some PLOT columns can adsorb water, which can lead to changes in retention times and selectivity for analytes. Therefore, column performance will be influenced greatly in the presence of water. Although cumbersome solvent-extraction procedures can be performed before injection, injecting sample that contains water is, in some cases, unavoidable.

From a GC point of view, water is a less-than-ideal solvent. The problems associated with water include large vapor expansion volume, poor wet ability and solubility in many stationary phases, detector problems, and perceived chemical damage to the stationary phase. In order to test the effect of water, a GS-OxyPLOT column that had gone through about 1,500 runs was tested before and after injecting 100% aqueous samples.

Water has a large vapor expansion volume; the vapor volume of water (assuming a 1-µL injection) can easily exceed the physical volume of the injection liner (typically 200 to 900 µL). The volume for the liner used in this experiment (Agilent p/n 5183-4711) is 870 µL, so the injection volume was set as 0.2 µL. Table 5 lists the conditions for the moisture testing, and the resulting chromatograms are shown in Figure 3.

Table 5. Conditions for Moisture Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 38 cm/s at 150 °C
Inlet	Split/splitless at 300 °C 15:1 split ratio
Oven temperature	150 °C isothermal, post-run: 300 °C (5 min)
Detector	FID at 300 °C, H2:45mL/min, air: 400 mL/min, makeup: 30 mL/min
Injection size	0.2 µL
Sample	0.1% n-Dodecane, Methyl tert-butyl ether, n-Tridecane, Iso-Butyraldehyde, n-Tetradecane, Methanol, Acetone, and n-Pentadecane

As shown in Figure 3, the area of n-pentadecane remained the same before and after 100 injections of water. However, compared with the area before injecting water, the area of methanol (peak 6) decreased by 50%, and the area of acetone (peak 7) decreased by14.4% after 100 injections of water (see Table 6). It demonstrated that water can affect the activity of GS-OxyPLOT, especially for the analysis of those relatively low molecular weight oxygenated compounds, such as methanol and acetone.

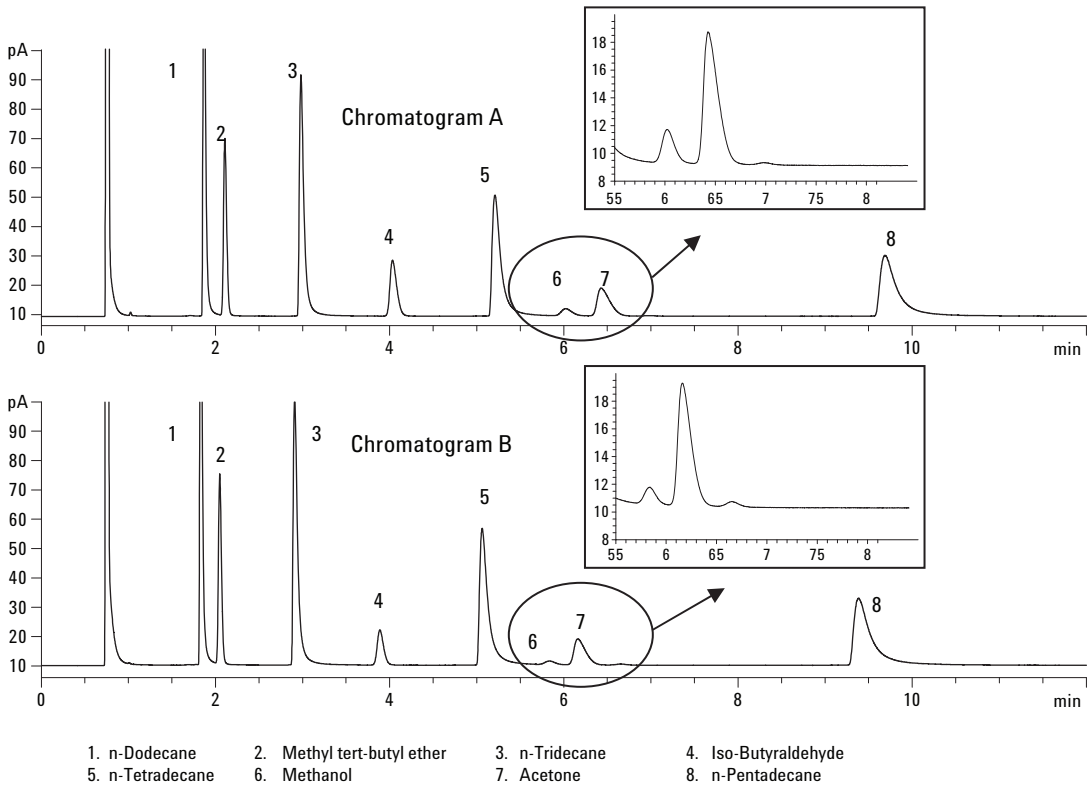

Figure 3. Comparison of test mixture separation before (A) and after (B) 100 injections of water.

As for retention times and column efficiency, they are not strongly influenced. After 100 injections of water, the retention time of C15 changed from 9.689 min to 9.384 min, and the column efficiency of C15 changed from 14,792 to 14,781.

Condition the column at 300 °C for two hours, followed by 12 hours at 250 °C. As shown in Figure 4 and Table 6, it is obvious that GS-OxyPLOT phase can be regenerated by conditioning.

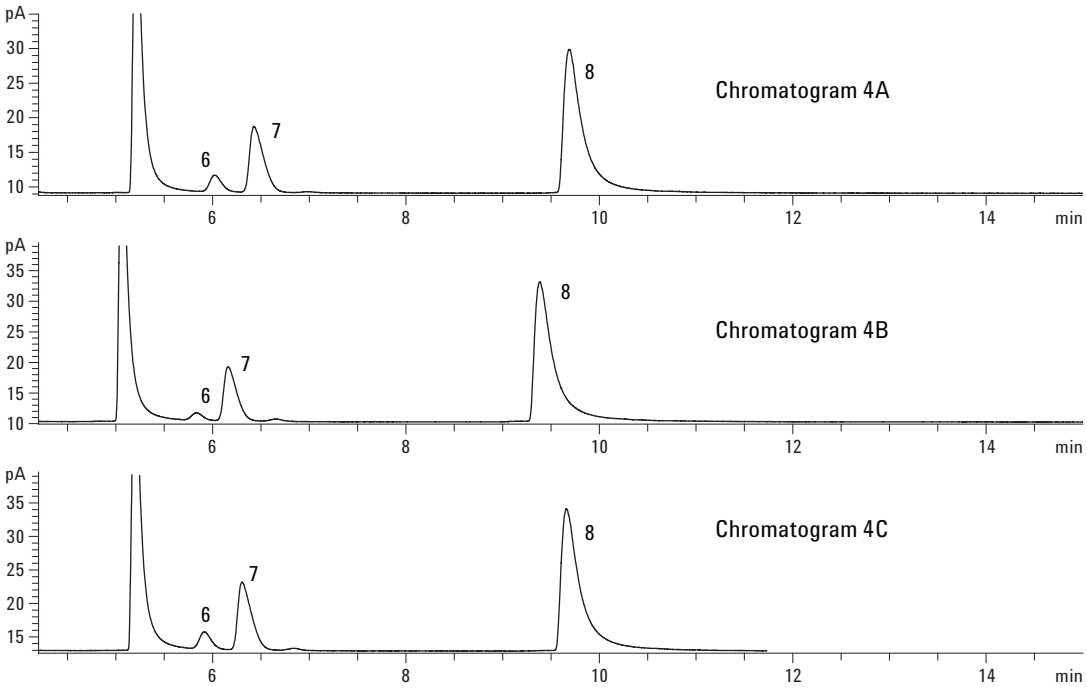

Figure 4. Expanded view shows comparison of test mixture separation on GS-OxyPLOT.
4A. Before injection of water. 4B. After 100 injections of water. 4C. After conditioning the column.

Table 6. Comparison of Test Mixture Separation

	Methanol			Acetone			n-Pentadecane		
	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column
RT (min)	6.022	5.835	5.915	6.429	6.160	6.305	9.689	9.384	9.658
Area	20.23	9.18	20.88	94.53	80.92	98.07	277.79	287.7	287.9
Plates	11887	12920	11616	9532	10357	9573	14792	14781	15100

After conditioning the GS-OxyPLOT column, the peak area and retention time reproducibility were determined. Figure 5 and Table 7 show excellent RT precision, lower than 0.6% over five test mixture runs on this GS-OxyPLOT column. The peak area has a relative standard deviation (RSD%) below 2.5%. It proved that column performance can be restored via conditioning.

Determination of Methanol

The following analysis of methanol followed ASTM D7059 [4]: "Standard Test Method for Determination of Methanol in Crude Oils by Multidimensional Gas Chromatography." Methanol was determined by gas chromatography with FID using internal standard method with GS-OxyPLOT column.

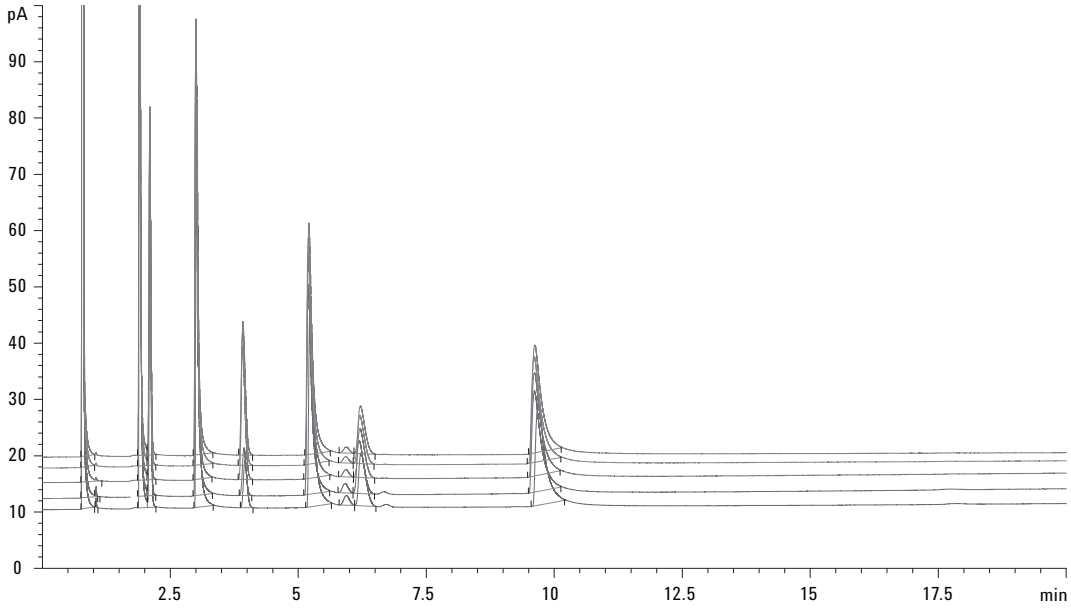

Figure 5. Fifth run overlaid using GS-OxyPLOT (after conditioning column).

Table 7. Peak Area Reproducibility and Retention Time Reproducibility on GS-OxyPLOT (after conditioning column)

Compound (by eluted order)	Dodecane	MTBE	Tridecane	Iso-Butyraldehyde	Tetradecane	MeOH	Acetone	n-C15
Area RSD% (N = 5)	1.18	1.58	1.59	2.49	1.15	2.12	1.98	1.82
RT RSD% (N = 5)	0.18	0.12	0.26	0.55	0.29	0.16	0.19	0.33

Reagents and Materials

Carrier gas, Helium, > 99.95% purity
Methanol, > 99.9% purity
1-propanol, > 99.9% purity, and containing < 500 ppm methanol
Toluene, > 99.9% purity, and containing < 0.5 ppm methanol

A set of calibration standards 5, 25, 125, 250, 500, 1,000 and 1,500 ppm (m/m) of methanol, and each containing 500 ppm (m/m) of 1-propanol internal standard, were prepared in toluene.

The calibration standard solutions should be stored in tightly sealed bottles in a dark place below 5 °C.

Linearity

Under the conditions listed in Table 8, the methanol calibration standards were analyzed. The linearity is shown by plotting the response ratio of methanol and internal standard 1-propanol against their amount ratio (see Figure 6). For methanol, good linearity was gained ranging from 5 to 1,500 ppm. The correlation r^2 value for the calibration curve is higher than 0.999.

Figure 7 and Figure 8 are chromatograms of methanol at a level of 5 ppm and 1500 ppm, respectively. At a relatively high concentration of 1500 ppm, methanol still could get a sharp peak. The limit of quantification (LOQ) was calculated to be 1 ppm using the chromatogram of 5 ppm methanol.

Table 8. System Settings for the Calibration Curve

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 50 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 10:1 split ratio
Oven temperature	150 °C (3 min); 20/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	1 µL

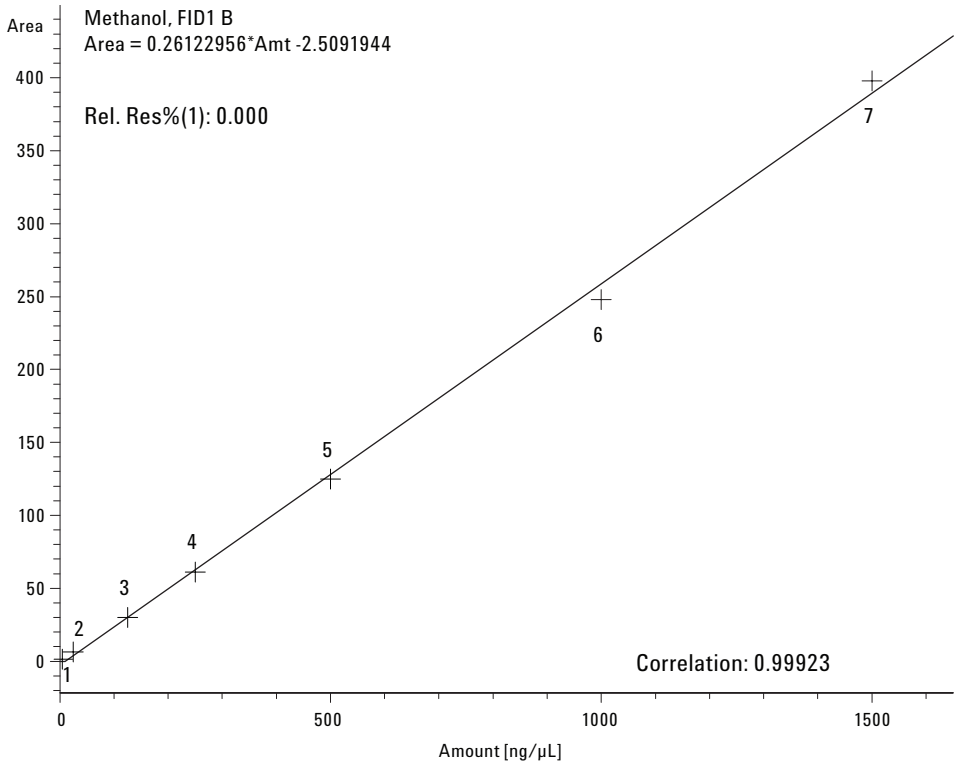

Figure 6. The calibration curve of methanol in toluene.

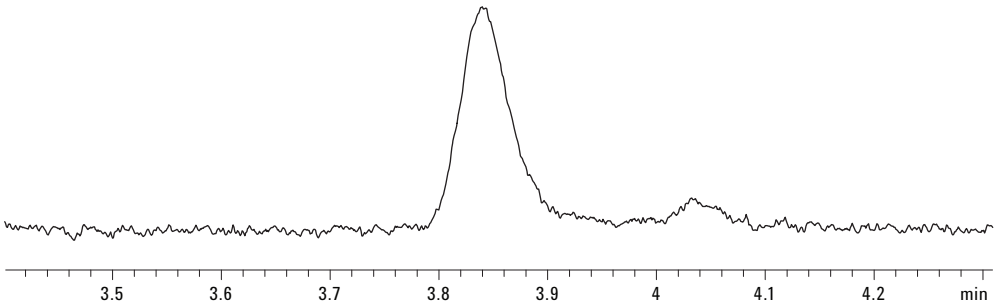

Figure 7. Test mixture of 5 ppm methanol in toluene.

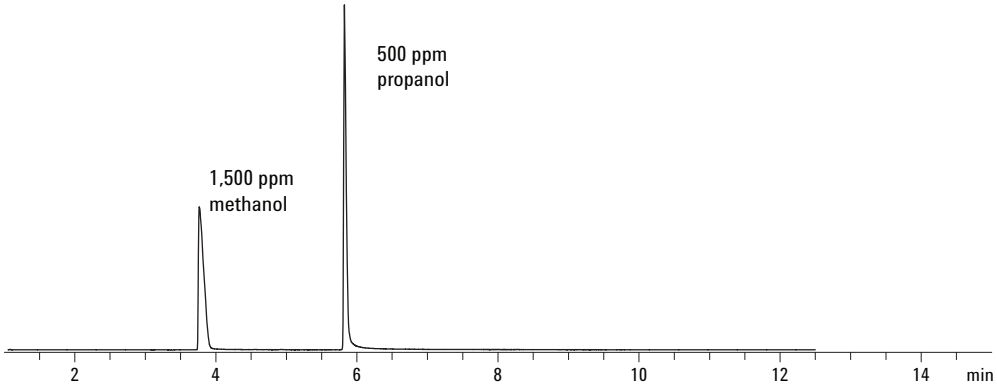

Figure 8. Test mixture of 1,500 ppm methanol in toluene.

Repeatability

The reproducibility of the GS-OxyPLOT is given in Table 9. Those values were obtained by the replicate analysis of different methanol levels (25, 125, and 1,500 ppm) in different days. The injection was done by ALS with RSD no less than 3% either intraday or interday analysis, which was very low for this type of determination.

Life Span

Under the conditions in Table 5, a mixture was analyzed with a GS-OxyPLOT column which went through 1,500 injections of methanol. It shows that the column has a long lifetime. The GS-OxyPLOT column still has good resolution for each compound and high efficiency of 1,482 plates per meter for n-pentadecane (see Figure 9).

Table 9. Relative Standard Deviations Intraday and Interday at Different Levels (25, 125, and 1,500 ppm) of Methanol

Day	25 ppm (average)	RSD (%)	125 ppm (average)	RSD (%)	1,500 ppm (average)	RSD (%)
D 1	25.2	0.46	123.9	0.45	1507.3	0.55
D 2	25.3	1.53	123.2	0.79	1494.4	0.45
D 3	24.4	0.36	125.4	1.71	1523.5	0.35
D 4	25.9	1.06	123.0	0.90	1537.8	0.51
D 5	23.9	0.44	121.1	0.76	1502.4	1.03
Stand. dev.	0.7		1.70		17.4	
Average	24.97		123.6		1513.1	
RSD (%)	2.8		1.37		1.15	

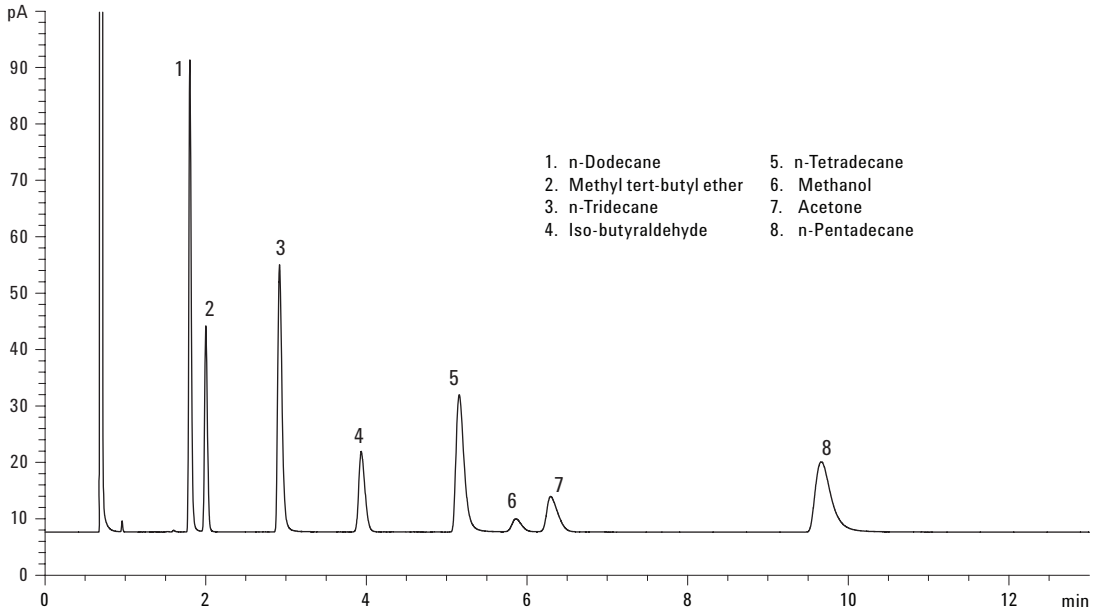

Figure 9. Chromatogram of performance mixture after 1,500 injections.

Conclusions

GS-OxyPLOT provides good retention and selectivity for oxygenated compounds. Normal alkanes up to C24 and primary alcohols up to lauryl alcohol can elute from GS-OxyPLOT within its program temperature maximum limit of 350 °C. Methanol elutes after n-C14 with retention index higher than 1,400; the retention index is quite stable from 150 to 250 °C, allowing methanol to be measured at low levels in a wide range of hydrocarbon streams.

Methanol has to be measured usually at specs as low as 5 ppm. From 5 to 1,500 ppm, it shows good linearity on GS-OxyPLOT. And the column has proven extremely stable with long lifetime.

GS-OxyPLOT can tolerate a little amount of water in samples, and column performance can be restored via conditioning.

GS-OxyPLOT can be used for a single-column system or in multidimensional GC systems. It offers a unique solution for the analysis of oxygenates in the chemical and petrochemical industries.

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Printed in the USA
June 17, 2008
5989-8771EN



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Analysis of Permanent Gases and Light Hydrocarbons Using Agilent 7820A GC With 3-Valve System

Application Note

HPI

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Highlights

- Agilent 7820A GC 3-valve system provides a low-cost but powerful platform for analysis of permanent gases and light hydrocarbons.
- Full electronic pneumatics control (EPC) provides an easy-to-use operation for the end user and ensures excellent repeatability for both retention time and peak area.
- This application work can also be used as a reference in the analysis of natural gas, petroleum gas, synthesis gas, purified gas, water gas, blast furnace gas, stack gas, and so on.

Abstract

A new economical solution is provided to test permanent gases and light hydrocarbons. An Agilent 7820A Gas Chromatograph equipped with three valves, a flame ionization detector (FID), and a thermal conductivity detector (TCD), is configured for analysis of permanent gases and light hydrocarbons. The TCD channel with packed columns is used to measure H_2,CO_2,O_2, N_2,CH_4 and CO. A capillary column (Al_2O_3 PLOT: 50 m × 0.53 mm) is used to measure all hydrocarbons (C1~C6) including CH_4.

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Introduction

Analysis of permanent gases and light hydrocarbons has been widely employed in the petrochemical, chemical and energy industries. These permanent gases, such as O_2, N_2, CH_4, CO, and CO_2 are the common target compounds in natural gas, petroleum gas, synthesis gas, purified gas, water gas, blast furnace gas, stack gas, and so on. Understanding the concentrations of these components is important for petrochemical, chemical and energy industrial processes. The 7820A 3-valve system offers an easy-to-use and powerful platform for the analysis of these kinds of samples.

This work illustrates one typical application of the 7820A 3-valve system for the analysis of permanent gases and light hydrocarbons.

Experimental

Three valves were used in this 7820A system: six-port gas sampling, ten-port gas sampling with back-flush to vent, and another six-port column isolation. The valve diagram and columns configuration are shown in Figure 1. Normally, the valve sample loops are connected in series for simultaneous dual-channel injection. Valve control is handled by EZChrom Elite compact software. Chromatographic conditions and valve time events are listed in Tables 1 and 2.

Table 1. Gas Chromatographic Conditions

Sample loop size	0.25 mL
FID channel flow	5 mL/min
FID temp	300 °C
FID channel carrier	N_2
Capillary splitter temp	200 °C
Split ratio	25:1
TCD channel flow	30 mL/min
TCD temp	250 °C
TCD channel carrier	He
Valve box temp	120 °C
Oven program	45 °C (6 min) >180 °C (2.25 min) at 20 °C/min

Table 2. Time Events

Events	**Time (min)**
Valve 1 ON*	0.01
TCD Negative Polarity ON	0.6
TCD Negative Polarity OFF	1.4
Valve 2 ON	1.7
Valve 1 OFF*	2.5
Valve 2 OFF	3.2

*Time events of valve 3 are the same as valve 1.

A fixed gas mix standard, (Jiliang Standard Gas Inc., Shanghai), was used in this application test. The components and concentrations are listed in Table 3.

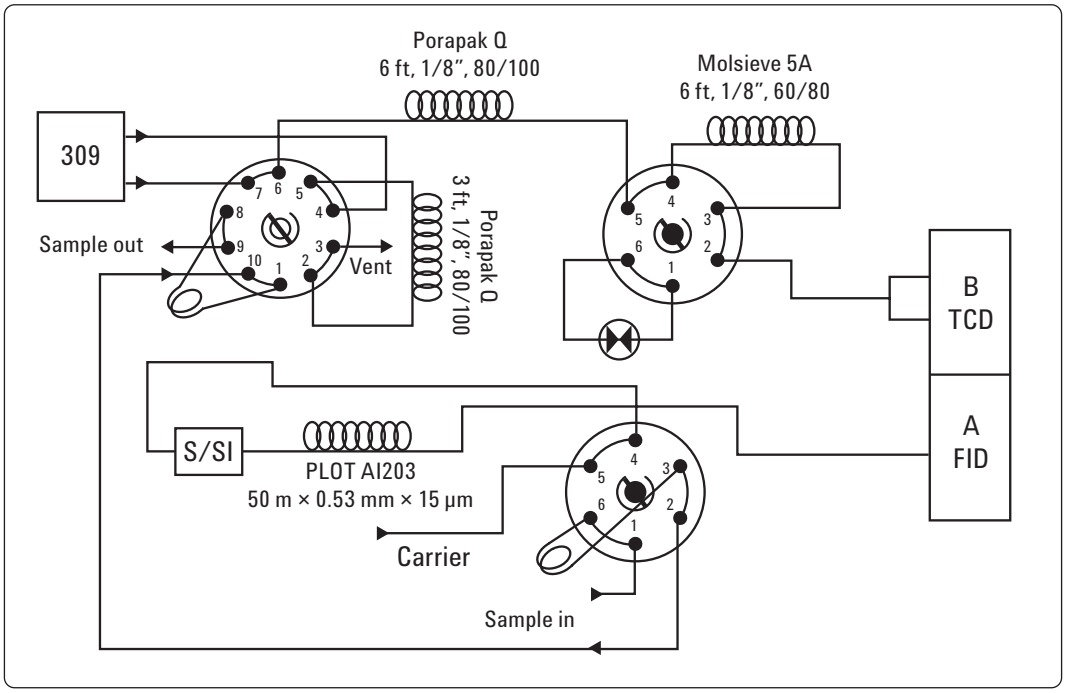

Figure 1. Valve diagram for dual-channel natural gas analysis.

Table 3. Concentrations of the Standard Gases

Components	H_2	O_2	N_2	CO	CO_2	CH_4	C_2H_6	C_3H_8	iC_4	nC_4	iC_5	nC_5	nC_6
Conc. (%)	6.09	3.00	9.97	1.99	3.48	71.92	2.00	0.99	0.11	0.10	0.12	0.12	0.11

Results

Chromatograms

Chromatograms for the FID and TCD channels of standard gas are shown in Figures 2 and 3. Hydrocarbons from C1 to C6 are separated by a PLOT Al_2O_3 column in approximately 15 minutes. For natural gas samples containing hydrocarbons higher than C6, the final temperatures of the oven program can be modified to 220 °C for the elution of hydrocarbons up to C11.

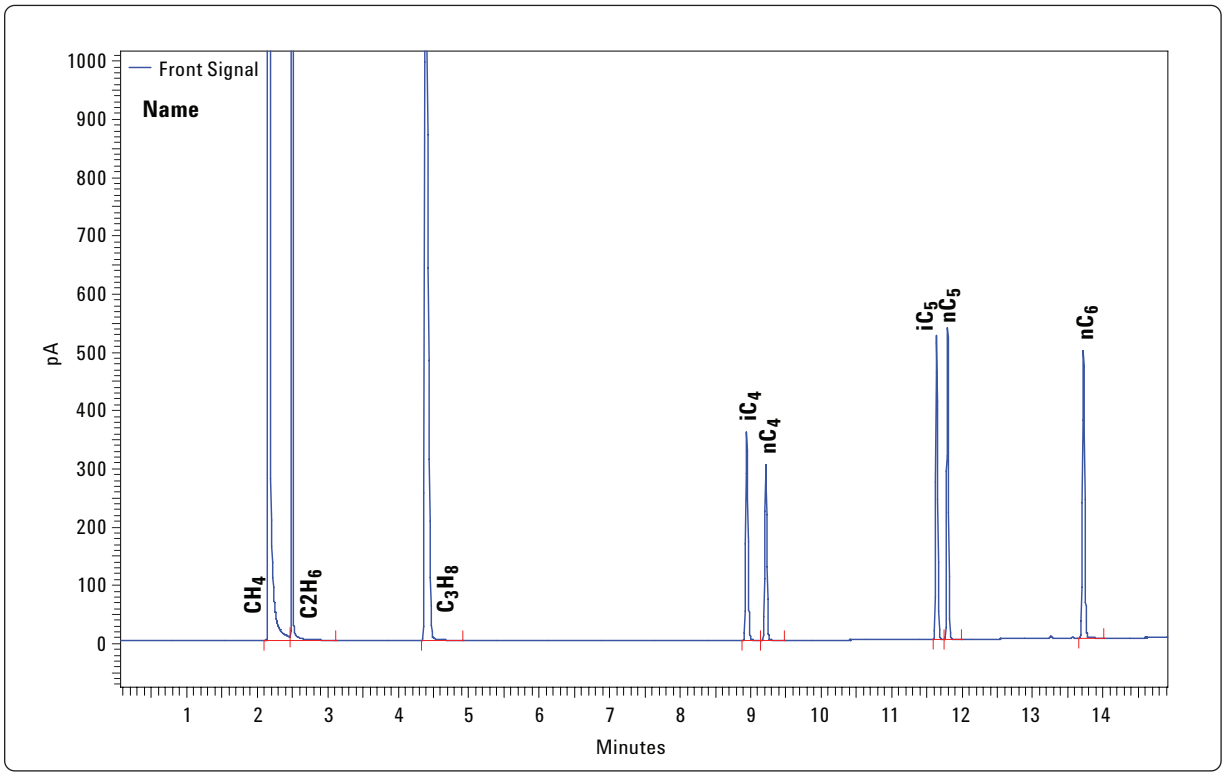

Figure 2. FID Channel chromatogram of CH_4, C_2H_6, C_3H_8, iC_4, nC_4, iC_5, nC_5, and nC_6.

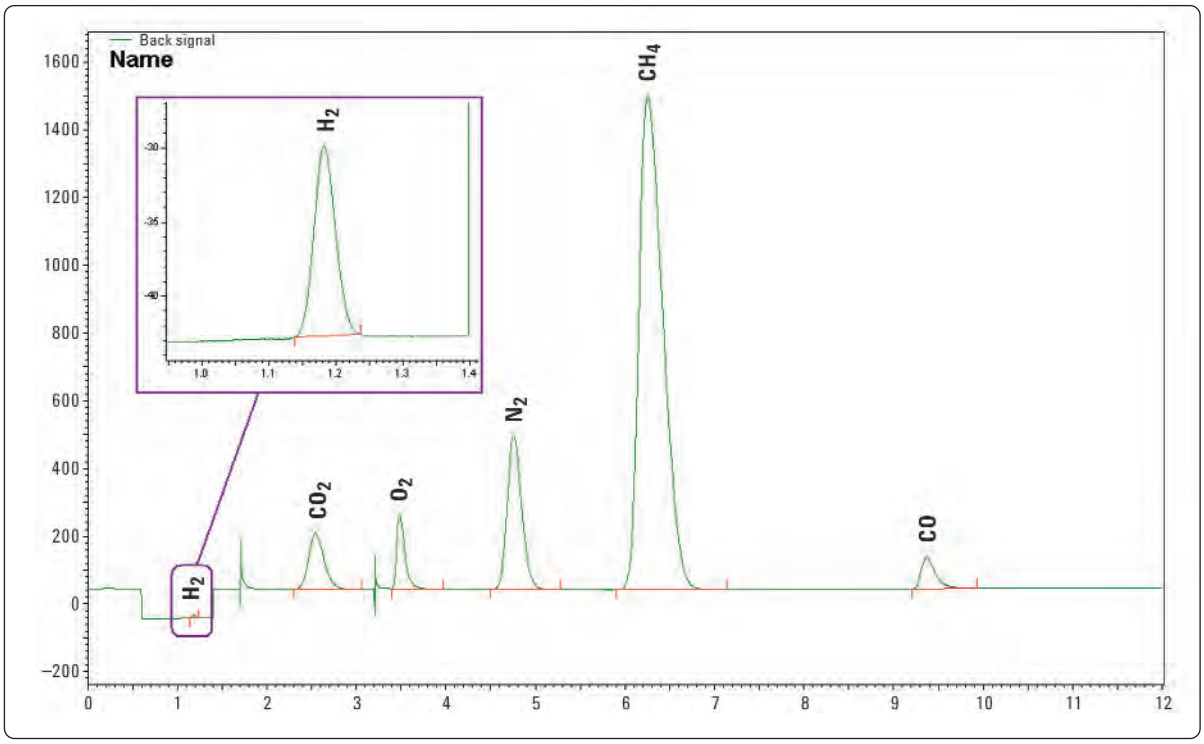

Figure 3. TCD Channel chromatogram of H_2, O_2, CO_2, N_2, CH_4, CO.

Linearity

The mixed standard was dynamically diluted to five different lower-concentration levels for calibration. The linearity results of all the permanent gas components are listed in Table 4.

Table 4. Linearity Results of TCD Channel

%	H_2	CO_2	O_2	N_2	CH_4	CO
Level 1	0.305	0.174	0.150	0.500	3.596	0.100
Level 2	0.609	0.348	0.300	0.997	7.192	0.199
Level 3	1.523	0.870	0.750	2.493	17.98	0.498
Level 4	3.045	1.740	1.500	4.985	35.96	0.995
Level 5	6.090	3.480	3.000	9.970	71.92	1.990
R^2	0.999	0.999	0.998	1.000	0.999	0.999

Repeatability

The relative standard deviations (RSD) for all hydrocarbon components were lower than 0.8% by using split injection on the FID channel. This was due to the full electronic pneumatics control (EPC) from injector to detector on 7820A. Results of the TCD channel also show excellent repeatability (Table 5). Component concentrations were 0.305%, 0.174%, 0.15%, 0.5%, 3.596%, and 0.1%, respectively for H_2, CO_2, O_2, N_2, CH_4, and CO.

Table 5. TCD Channel Repeatability

Runs	H_2	CO_2	O_2	N_2	CH_4	CO
1	10389	753601	137865	2180997	10904896	370250
2	10630	750304	142332	2191591	10947696	378184
3	10498	749748	140281	2156911	10926314	379868
4	10595	745289	139133	2168986	10822886	374996
5	10358	744909	140300	2172639	10826691	371749
RSD%	1.15	0.49	1.18	0.6	0.53	1.09

Low Level Permanent Gases

Another standard gas cylinder (Jiliang Standard Gas Inc., Shanghai) was tested by the 7820A 3-valve system to check low level response and repeatability. Figure 4 shows the chromatogram of the low level permanent gas mix and Figure 5 shows the overlapped chromatograms of five runs. Chromatogram conditions and concentrations of each compound are listed as follows:

Carrier gas:	He	
Sample loop:	1 mL	
Oven:	45 °C (6 min) >180 °C (2.25 min) at 20°C/min	
TCD:	250 °C	
1.	CO_2	200 ppm
2.	O_2	176 ppm
3.	N_2	Balance gas
4.	CH_4	810 ppm
*	Signal of valve switching	

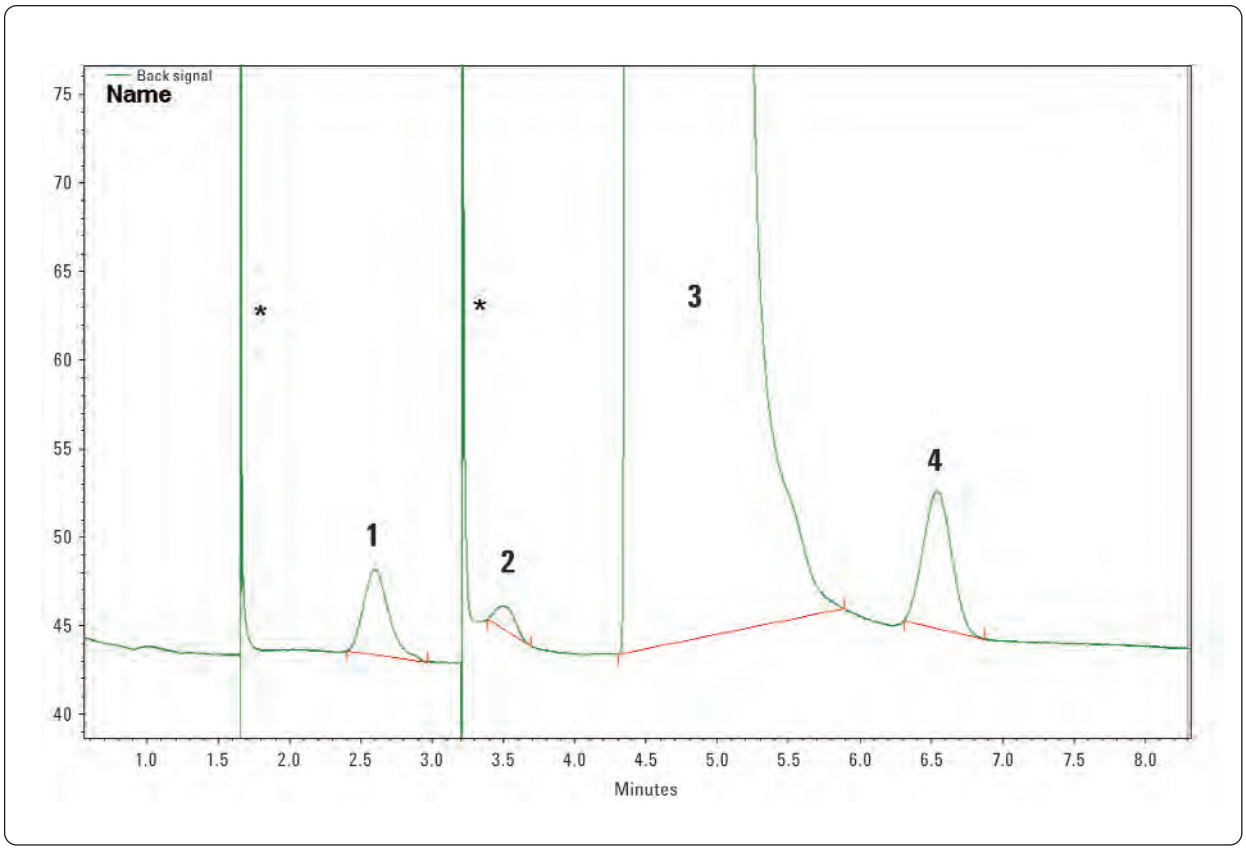

Figure 4. Chromatogram of low level permanent gas standard mix.

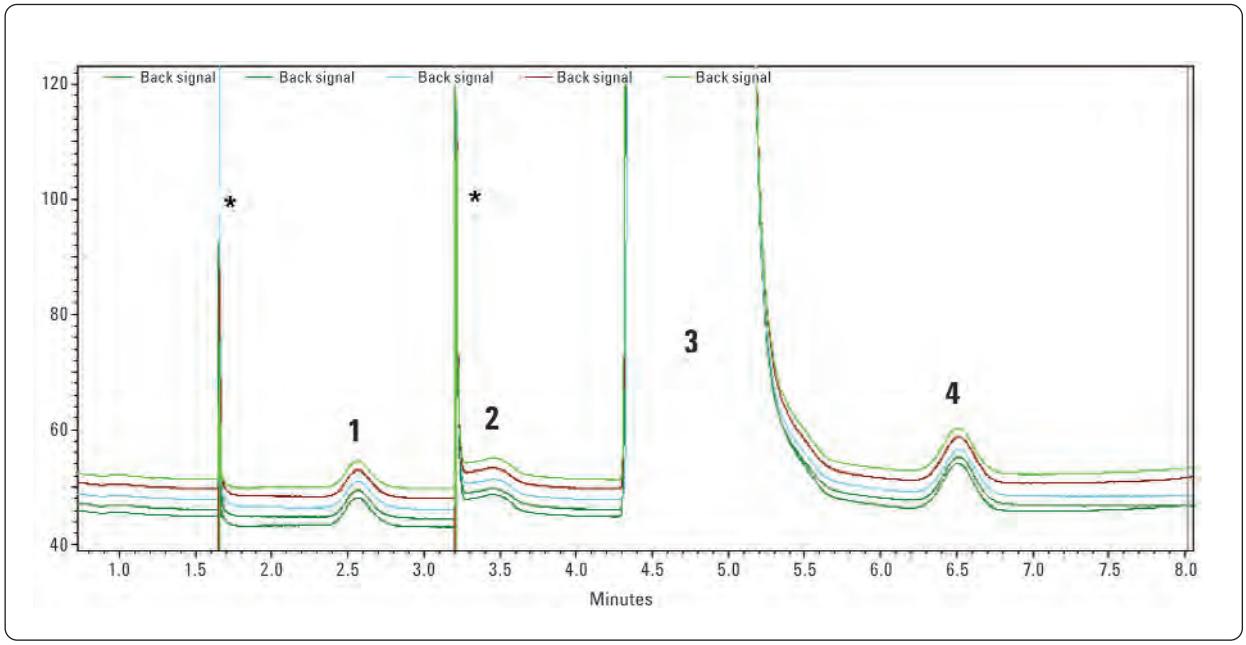

Figure 5. Overlapped chromatograms of five runs.

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Printed in the USA
October 13, 2009
5990-4667EN



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Enhanced Sensitivity for Biomarker Characterization in Petroleum Using Triple Quadrupole GC/MS and Backflushing

Application Note

Environmental

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Abstract

A rapid, reliable method for the routine detection and quantification of biomarkers in petroleum was developed using the Agilent 7890A/7000A Series Triple Quadrupole GC/MS with backflushing using a Pressure Controlled Tee configuration. In a single run, diverse biomarkers from several transitions can be detected, confirmed, and quantified at levels as low as 2 ppm, with RSDs well below 5%. This method is suitable for "fingerprinting" of petroleum samples and the deconvolution of oil mixtures in complex, multisource petroleum systems.

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Introduction

Petroleum biomarkers are complex molecular fossils derived from once living organisms [1]. These compounds provide unique clues to the identity of source rocks from which petroleum samples are derived. This information includes the biological source organisms which generated the organic matter, the environmental conditions that prevailed in the water column and sediment at the time, the thermal history of both the rock and the oil, and the degree of microbial biodegradation. Biomarkers are used in conjunction with other geochemical parameters to help solve oil exploration, development, and production problems. They provide much more detailed information about petroleum source and history than nonbiomarker analysis (bulk isotopes, elemental analysis, and so forth) alone.

High resolution mass spectrometry (HRMS) is often used to analyze biomarkers in petroleum, due to its ability to provide quantitative data for compounds present in complex mixtures. However, HRMS requires a significant financial investment as well as highly trained operators to assure valid results. Triple Quadrupole GC/MS offers a viable alternative for the rapid, routine analysis of biomarkers in petroleum, providing excellent precision, sensitivity, selectivity, and dynamic range. Implementing GC backflushing in the acquisition method improves data quality robustness, due to the very complex and varied nature of petroleum samples.

Experimental

Standards and Samples

STANFORD-1 is a new external standard for quantitative biomarker analysis. It is a mixture of pure biomarker standards and paraffin-free saturate fractions from Paleozoic, Mesozoic, Cenozoic, biodegraded, terrestrially-influenced, carbonate/evaporate-sourced, and open-marine sourced petroleum samples. It contains known quantities of most, if not all, commonly used biomarkers and two internal standards, BTI-6 and 5-β cholane, which are useful for quantifying hopane and sterane biomarkers across diverse GC/MS systems.

C30 sterane fractions were prepared using standard normal phase liquid chromatography techniques, n-alkane removal, and proprietary molecular sieve and HPLC techniques for the final enrichment of target compounds. Two compounds which coelute with n-propylcholestane (4-methylstigmastane and hopane) were completely removed from the sample to avoid known interference with the *m/z* 414→217 transition.

Instruments

The experiments were performed on an Agilent 7890A gas chromatograph equipped with a split/splitless inlet, an Agilent 7000A Triple Quadrupole GC/MS with Triple-Axis Detector, and an Agilent 7683B automatic liquid sampler (ALS). The split/splitless inlet is fitted with a deactivated, helical double taper injection liner (p/n 5188-5398). Injections were made using a 10-µL syringe (p/n 9301-0713). A variety of configurations was explored to examine possible improvements in analysis time. Ultimately, two configurations were used for the experiments, and the instrument conditions and specific configurations are listed in Table 1.

MS SRM Parameters

The MS/MS parameters used in the analysis of the petroleum samples are shown in Tables 2 and 3 and in the Figure 6 legend. Experience with HRMS metastable transitions was used to select these precursor and product ions, and an extensive study of product ions was not performed.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	60 m Configuration	40 m Configuration
Column	Two 30 m x 0.25 mm x 0.25 µm DB-1MS Ultra Inert columns (p/n123-0132UI)	Two 20 m x 0.18 mm x 0.18 µm DB-1MS Ultra Inert columns (p/n 121-0122UI)
Inlet temperature	325 °C	325 °C
Inlet pressure	19.197 psi	17.13 psi
Carrier gas	Helium, constant flow mode	Hydrogen, constant flow mode
Flow rate	Column 1: 1.15 mL/min; Column 2: 1.20 mL/min	Column 1: 0.95 mL/min; Column 2: 1.0 mL/min
Injection mode	Pulsed splitless (50 psi until 1 min)	Pulsed splitless (50 psi until 0.75 min)
Oven program	50 °C (1 min hold), then 40 °C/min to 140 °C for 0 min, then 2 °C/min to 313.5 °C for 0 min	40 °C (0.6 min hold), then 40 °C/min to 140 °C for 0.5 min, then 3.4 °C/min to 300 °C for 1 min
Column velocity	Column 1: 27.636; Column 2: 39.923 cm/s	Column 1: 45.449; Column 2: 65.944 cm/s
Injection volume	1 µL	1 µL
Transfer line temperature	325 °C	325 °C
GC Post-Run Conditions		
Backflush device	Purged Ultimate Union (p/n G3186-60580) controlled by a Electronic Pneumatic Control (EPC) (p/n G3470A)	Purged Ultimate Union (p/n G3186-60580) controlled by a Electronic Pneumatic Control (EPC) (p/n G3471A)
Backflush conditions	–4 mL/min at 325 °C for 7 min	–4 mL/min at 325 °C for 5 min
MS Conditions		
Tune	Autotune	Autotune
Delta EMV	70 eV	70 eV
Acquisition parameters	EI; selected reaction monitoring	EI; selected reaction monitoring
Solvent delay	5 min	3 min
MS temperatures	Source 250 °C; Quadrupoles 150 °C	Source 250°C; Quadrupoles 150 °C

Table 2. Analysis Parameters for Precision Experiments*

Compound	Transition (*m/z*)
Stigmastane	400.4 → 217.2
Homohopane (22S)	426.4 → 191.2
n-propylcholestane	414.4 → 217.2
27-nordiacholestane (13β,17α(H),20S)	358.4 → 217.2
27-norcholestane	358.4 → 217.2
4-methylstigmastane	414.4 → 231.2
Dinosterane	414.4 → 98.1
Hopane	412.4 → 369.4
5β-Cholane (ISTD)	330.3 → 217.2

*The method contained 17 transitions in total. The dwell time and collision energy used for each transition was 50 msec and 5 eV, respectively, using the 60 m configuration.

Results and Discussion

Backflushing with a Pressure Controlled Tee Configuration

Backflushing was used to remove higher boiling substances from the column prior to each subsequent run. Using this technique, late eluting peaks are flushed out of the inlet split flow vent instead of driving them through the entire length of column and into the mass spectrometer. Backflushing reduces accumulated chemical noise due to carryover (which can be observed even in SRM mode as a rising baseline) and the cycle time of the analysis, thus increasing throughput. System uptime is also increased, due to reduced maintenance of the columns and MS detector. The suite of Agilent Capillary

Flow Technology modules comprises a proprietary solution that enables easy and rapid backflushing with minimal dead volumes for maintaining chromatographic resolution. It also uses ferrules and fittings that eliminate leaks. All Capillary Flow Technology modules require the use of an Auxiliary Electronic Pneumatic Control (EPC) module or a Pneumatic Control Module (PCM) to provide a precisely-controlled second source of gas that directs the column flow to the appropriate column or detector. During analysis, the EPC module supplies a pressure slightly above the pressure of the carrier gas through the column. When backflushing, the inlet pressure is dropped and the EPC module pressure is increased, forcing the flow to reverse through the column and out the split vent.

A quick and simple approach to backflushing is to use a Capillary Flow Technology device in the middle of the analytical column [2–4]. As an example employed here, instead of using a 40-m column, two 20-m columns are used and connected by an ultralow dead volume Purged Ultimate Union in a Pressure Controlled Tee (PCT) configuration (Figure 1). The EPC module adds just enough makeup gas to match that from the first column, so there is very little flow addition and subsequent decrease in sensitivity due to suboptimal carrier gas flows into the mass spectrometer. As a general rule, the flow for column 2 is set to be 0.02 to 0.05 mL/min greater than that for column 1. Backflushing in this configuration is accomplished simply by reducing the flow or pressure in the first column and increasing it in the second column.

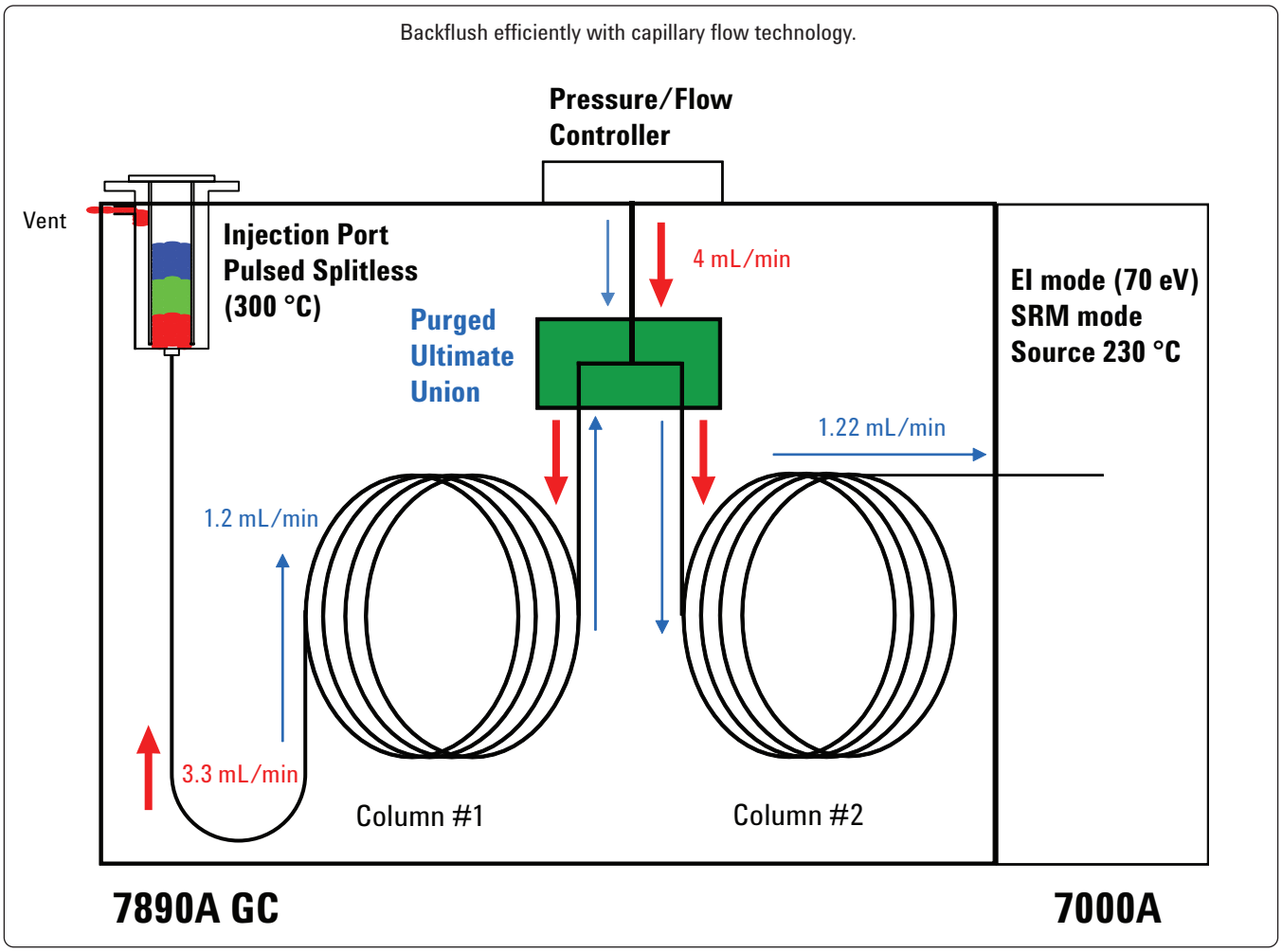

Figure 1. Schematic of the Pressure Controlled Tee GC/MS configuration. The narrower (blue) lines indicate the forward flow during analysis and the thicker (red) lines indicate the backflushing post-run state.

Figure 2 illustrates the advantages of backflushing with the PCT configuration. Typical hydrocarbon GC/MS analysis requires long cycle times due to long hold times at high oven temperatures to avoid contaminating subsequent analyses with carryover of high-boiling components (top chromatogram). Using backflush, targeted volatile components, in this case those eluting within 25 minutes, can be analyzed with significantly shorter cycle times, eliminating the need for column baking and extended GC run times (bottom chromatogram). High boiling hydrocarbons are not retained and column degradation by "permanently" absorbed components and high temperature hold times is decreased. In the example shown, cycle times are reduced from over 100 minutes to less than 30 minutes, and a blank injection after backflushing reveals no high-boiling components and only the baseline rise associated with column bleed.

Faster Analysis of Biomarkers

Run times can be accelerated 30 minutes per cycle without loss in chromatographic resolution or substantial loss in signal by switching from a 60-m (0.25-mm id) column with helium carrier gas to a 40-m (0.18-mm id) column with hydrogen carrier gas (Figure 3). The speed of the 7000A Triple Quadrupole mass spectrometer in SRM mode required only a change in dwell time from 50 to 20 msec to record the required 17 transitions with the same number of scans over the peaks. Because the 7000A Triple Quadrupole MS allows dwell times as short as 1 msec, even faster analysis is possible. An experimental comparison with an uninterrupted 60-m column (results not shown) demonstrated that the insertion of the PCT configuration results in no degradation in chromatography due to the low dead-volume of the Purged Ultimate Union.

Backflush for rapid and robust analyses.

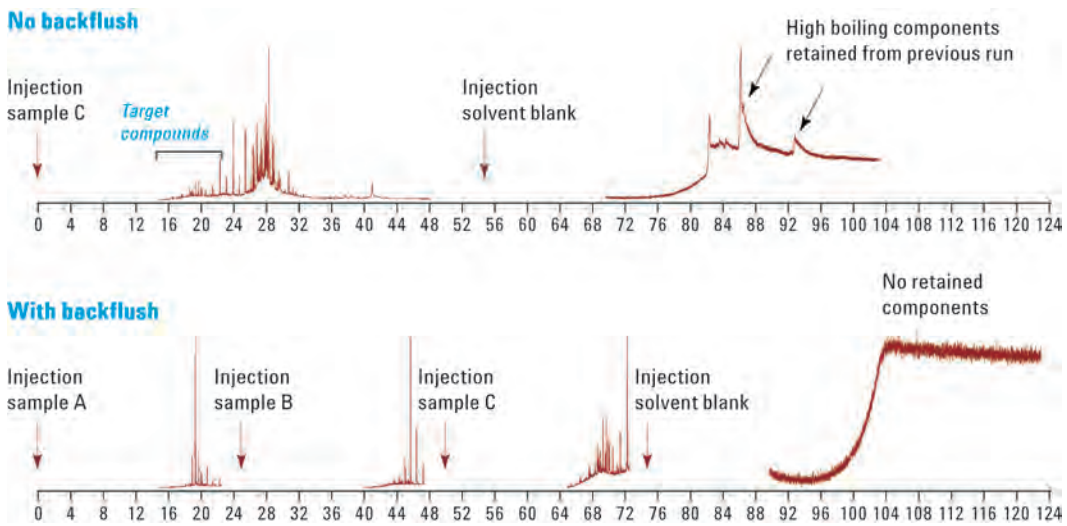

Figure 2. Petroleum samples, including one from Williston Basin source rocks (Sample C) which contains many late eluting, high molecular weight hydrocarbons, were analyzed without (top) and with (bottom) backflushing (40 m configuration). The target compounds comprise a subset of the total number of possible compounds in any injected sample and are indicated by brackets in the top chromatogram. As in a typical analysis, a sequence of samples was analyzed from three sources using the backflushing method in the bottom trace, followed by a solvent blank injection which demonstrated the lack of retained components.

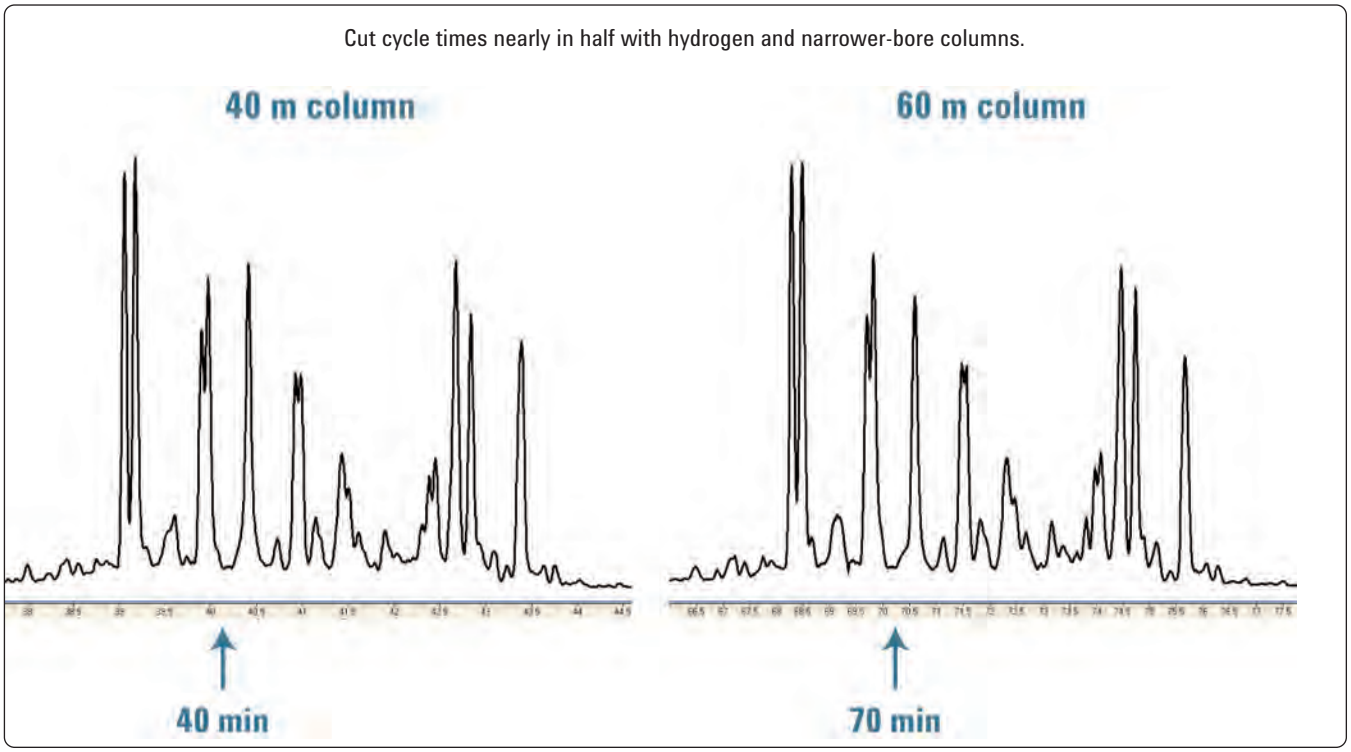

Figure 3. C28 steranes were analyzed using m/z transition 386→217 on either a 60 meter, 0.25 µm column and helium carrier gas, or a 40 meter, 0.18 µm column with hydrogen carrier gas. Employing hydrogen and the smaller bore column reduces analytical time significantly without loss in compound resolution.

Sensitivity, Selectivity and Precision

Routine biomarker analysis in petroleum samples requires precise determination of the abundance of a large number of individual compounds which can vary over a large range of concentrations in these complex mixtures. This precision allows the distinction of differences between petroleum samples with subtly different source or post-generation history. Results for ten sequential runs of the STANFORD-1 standard demonstrate that calculated concentrations of eight different compounds using several different transitions with widely varying concentrations is quite precise (Table 2, Figure 4a).

Most relative standard deviations (RSDs) were well below 5%. The only compound that gave an RSD higher than 5% (dinosterane) was present at a very low concentration (~2 ppm) and required manual integration for quantification. In addition, the calculated concentrations of the compounds were within a few percent of the expected concentration across all ten runs, except for the manually integrated dinosterane (Figure 4b). This precision demonstrates the ability of the Triple Quadrupole GC/MS system to distinguish subtle variations in petroleum composition for traditional biomarker studies, reservoir partitioning studies, and three-dimensional basin modeling.

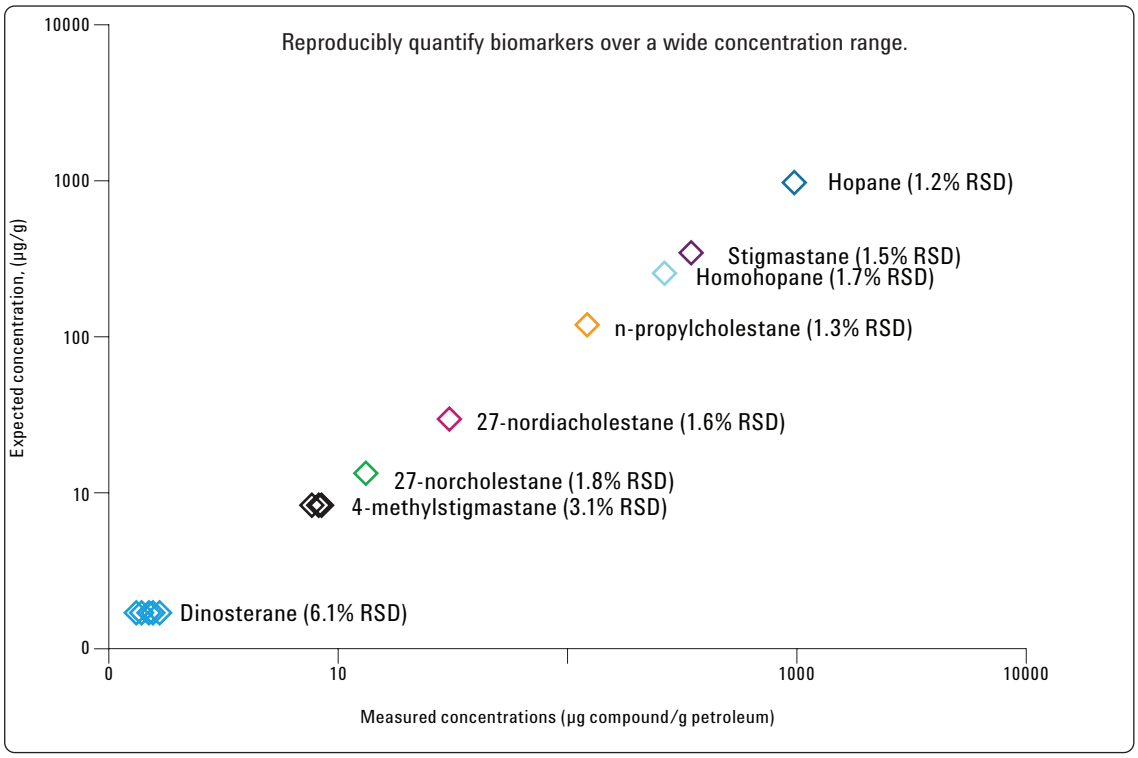

Figure 4a. Precision experiment results for eight biomarkers of widely varying concentrations contained within the STANFORD-1 standard. Ten sequential analyses were performed over a 15 hour period using the 60-m column PCT configuration. See Table 3 for transitions.

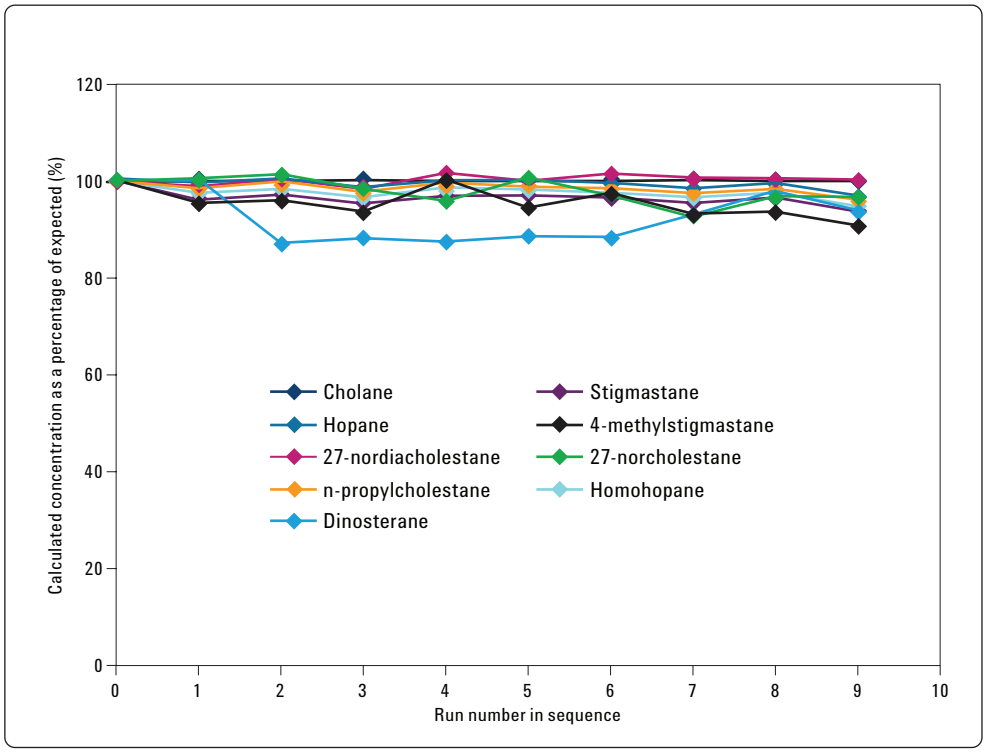

Figure 4b. The data from the analysis described in Figure 4a were plotted as calculated concentration of each biomarker versus the expected concentration over 10 analyses.

Deconvolving Oil Mixtures

A sophisticated understanding of petroleum systems requires the recognition and deconvolution of oil samples derived from more than one source rock. This problem is common where stacked source rocks exist in sedimentary basins (Figure 5). For this work a series of laboratory mixtures consisting of a marine petroleum endmember and a lacustrine endmember were analyzed for stigmastane, a ubiquitous component present in petroleum from both sources, and n-propylcholestane, a compound unique to oil from marine source rock.

As the ubiquitous component must be measured on a different SRM transition and is an order of magnitude more abundant in the marine oil, transition ratio stability and a large instrumental dynamic range are necessary to accurately identify small marine petroleum inputs in lacustrine source rock samples. The data demonstrate that mixtures as low as 0.2% (v/v) in the minor marine component can be accurately determined (Figure 6).

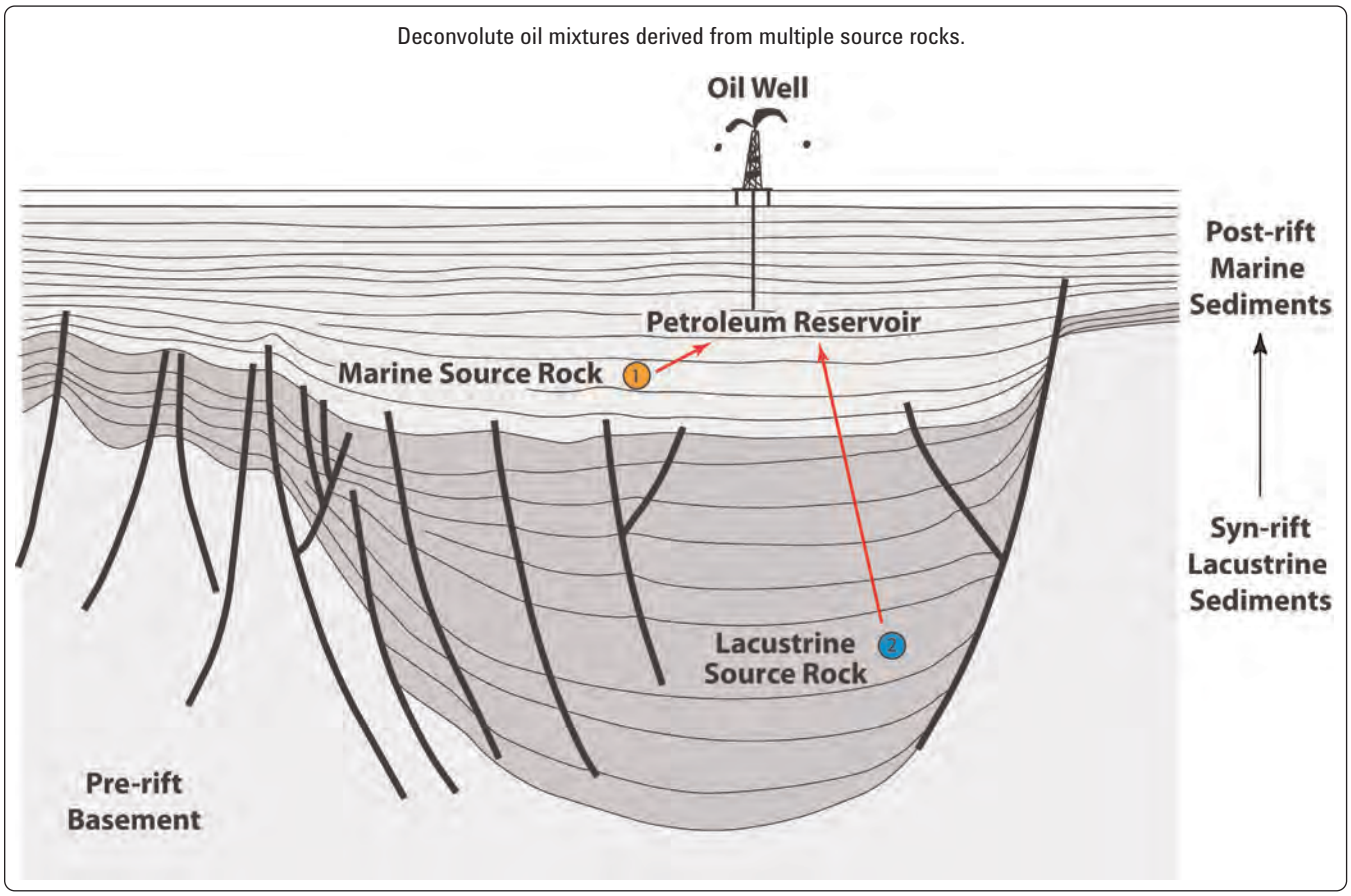

Figure 5. Diagram of an oil deposit containing source rocks from both marine (1) and lacustrine (2) sources.

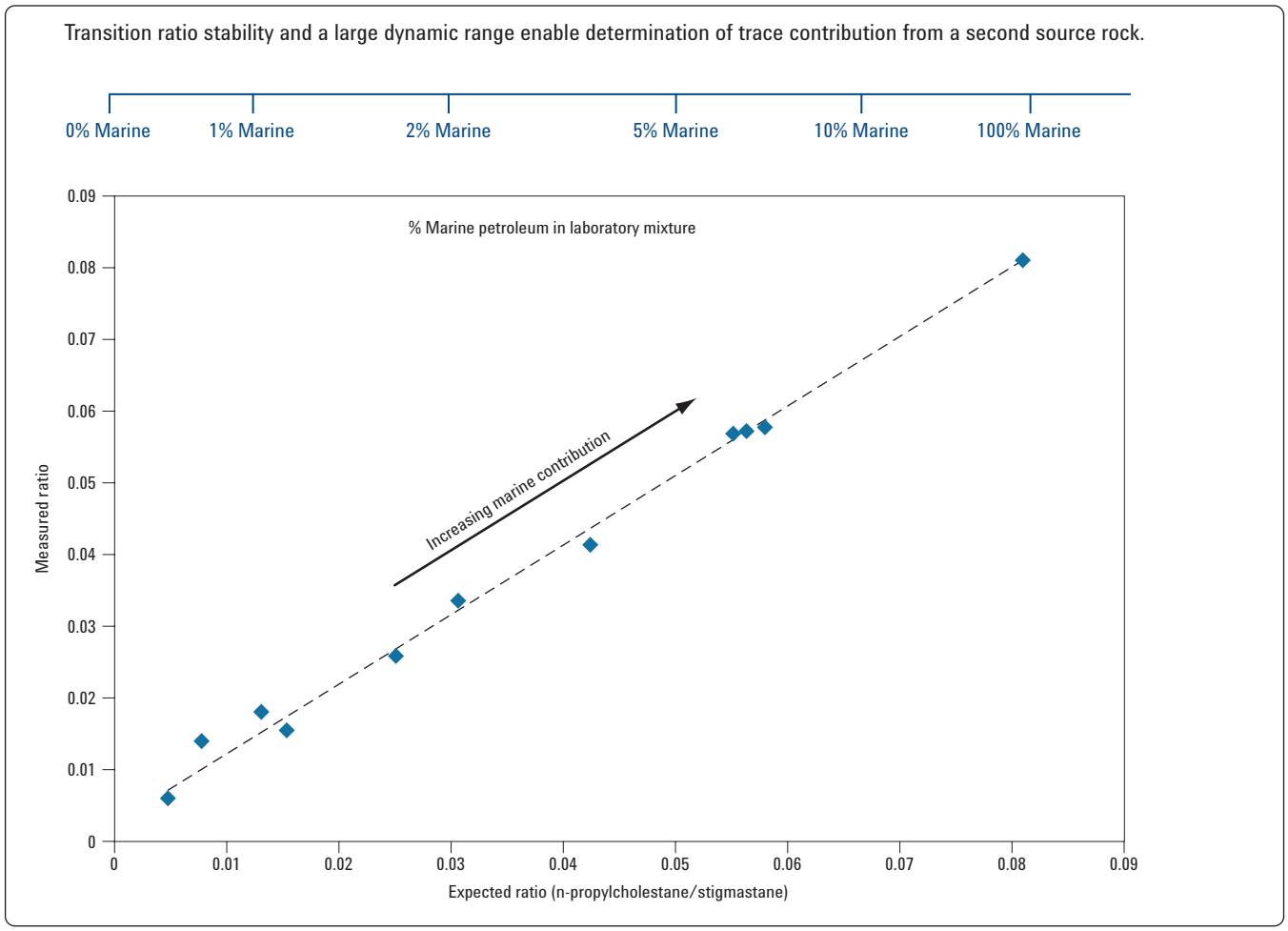

Figure 6. A series of laboratory mixtures consisting of various percentages of a marine petroleum sample in a lacustrine sample were analyzed for stigmastane, a ubiquitous component present in petroleum from both sources, and n-propylcholestane, a compound unique to oil from lacustrine source rock. The measured ratio of the two compounds was then plotted versus the expected ratio. Transitions monitored were: n-propylcholestane, m/z 414.4→217.2; stigmastane, m/z 400.4→217.2.

Conclusions

The Agilent 7000A Triple Quadrupole MS with 7890 GC using backflushing is a viable approach to the routine analysis of petroleum biomarkers, providing increased sensitivity, better selectivity and the potential to greatly reduce analysis time versus traditional GC/MS analysis. Column backflush provides higher sample throughput with lower carryover and source maintenance, and the use of hydrogen carrier gas and narrower bore columns reduces run times nearly two-fold at no significant loss in chromatographic resolution. The SRM speed, linearity, dynamic range and transition ratio stability of the 7000A Triple Quadrupole mass spectrometer enable quantitative characterization for the fingerprinting of petroleum samples and the deconvolution of complex petroleum mixtures.

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3. H. Prest, Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination, Agilent Technologies Publication 5989-8664EN.
4. H. Prest, The Pressure Controlled Tee (PCT): Configurations, Installation and Use, Agilent Technologies Technical Document G1472-90001.

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Printed in the USA
October 15, 2009
5990-4754EN

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Crude Oil & Natural Gas

Technical Overviews

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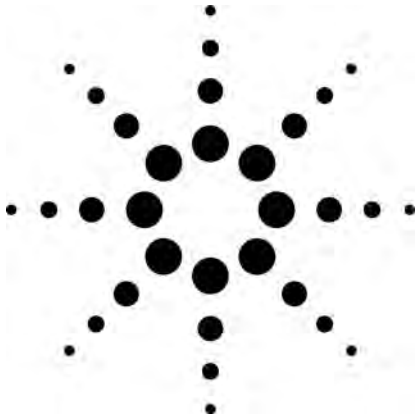
GS-OxyPLOT: A PLOT Column for the GC Analysis of Oxygenated Hydrocarbons

Technical Overview



Allen Vickers

Introduction

GS-OxyPLOT is a porous layer open tubular (PLOT) column. The stationary phase is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons. It is designed for and ideally suited for application in the ASTM methods listed in Table 1. It is an appropriate replacement for Varian's CP-LowOx column, usually with little to no changes in analytical parameters. This column is particularly useful for the trace analysis of oxygenates such as those listed in Table 2. Other oxygenated hydrocarbons are also suitable for analysis with this column subject to limitations given below.

The column can be used as a single, primary analytical separation column for oxygenated compounds. In complex sample matrices that have high molecular weight species (ca. 300 mol. wt. and higher) and/or species with insufficiently high vapor pressure to migrate through the GS-OxyPLOT, this column can be used in multidimensional GC systems with other columns that have vastly different polarity and lower selectivity toward oxygenated hydrocarbons. For example, a nonpolar DB-1 column can be used as an injection precolumn to retain low volatility solutes, allowing the less retained, polar oxygenated solutes to move into the GS-OxyPLOT. Since the stationary phase of GS-OxyPLOT is an oxygenate adsorbent phase, the oxygenates that enter the column are trapped. As the GC oven temperature is increased, the oxygenates will begin to migrate and are separated in the column prior to detection.

When first installed, the GS-OxyPLOT should be conditioned at 300 °C for at least 3 hours. Experience has shown that this column has an infinite shelf life, but when the column has not been in use for extended periods of time, longer conditioning times of 8 hours or more may be required to obtain retention time stability. The column can be stored with septa placed over the ends of the column, returned to the original column box, and stored at normal ambient temperatures for future use.

GS-OxyPLOT has a minimum temperature limit of 0 °C, an isothermal maximum temperature limit of 300 °C, and an oven program maximum temperature of 350 °C. Because the stationary phase is a strong adsorbent for polar compounds, especially water, it is recommended that when the column is installed in a GC, but idle, that the GC oven be set to an isothermal temperature of 220 °C with normal carrier gas flow, so that the instrument can be brought back into operation quickly when samples are ready to be analyzed. Otherwise, if the column is left at low oven temperatures, it may require reconditioning at 300 °C for several hours to obtain stable retention times.

Saturated hydrocarbon solutes have virtually no interaction with the GS-OxyPLOT and elute from the column so long as the column temperature is hot enough to induce a high enough vapor pressure for the solute to move in the carrier gas. Normal alkanes up to C_{18} will elute from GS-OxyPLOT within the program temperature maximum limit of the column. Because of the highly polar character of the GS-OxyPLOT phase, as would be

expected for oxygenate-selective PLOT column, the column has a relatively low sample load capacity for these nonpolar solutes. The low sample loading capacity is manifested chromatographically as a tailing peaking, indicative of phase overload in GS-OxyPLOT columns. Unsaturated hydrocarbons and aromatic hydrocarbons have relatively high retention. Injection of these organic compound classes should be limited to organic compounds with 11 carbons or less to prevent the column from fouling. As with the normal alkanes, the alkyl benzenes will show phase overloading at relatively low concentrations.

While GS-OxyPLOT is an ideal analytical solution for low molecular weight, oxygenated hydrocarbons, like all other similar oxygenate-selective PLOT columns,it is not recommended for higher molecular weight alkenals (e.g., 1-hexenal and 1-ocetenal). The combined interaction of the unsaturated and carbonyl functional groups can instigate tailing due to strong interactions and in some cases reaction between the phase and solutes.

Table 1. ASTM Standardized Methods for Which GS-OxyPLOT Is Specifically Designed

ASTM Method D7059	Determination of Methanol in Crude Oils by Gas Chromatography with Flame Ionization Detection
Proposed ASTM Method	Determination of C_1 to C_5 Oxygenates at Trace Levels in High Ethanol Content Gasoline Streams by Multidimensional Chromatography with Flame Ionization Detection*
Proposed ASTM Method	Determination of Oxygenates in Ethene, Propene, and C_4 and C_5 Hydrocarbon Matrices by Gas Chromatography and Flame Ionization Detection*

*These are "proposed methods" (i.e., do not have method designation numbers) that are destined for approval by ASTM Committee D2. These methods have already been accepted by, and are being implemented in, petrochemical refineries around the world.

Table 2. Examples of Oxygenated Compounds Suitable for GC Analysis Using the GS-OxyPLOT Column

1. Dimethyl Ether	13. Acetone
2. Diethyl Ether	14. Isovaleraldehyde
3. Acetaldehyde	15. Valeraldehyde
4. Ethyl t-Butyl Ether	16. Methyl Ethyl Ketone
5. Methyl t-Butyl Ether	17. Ethanol
6. Diisopropyl Ether	18. 1-Propanol
7. Propionaldehyde	19. Isopropyl Alcohol
8. tert-Amyl Methyl Ether	20. Allyl Alcohol
9. Propyl Ether	21. Isobutyl Alcohol
10. Isobutraldehyde	22. tert-Butyl Alcohol
11. Butylaldehyde	23. sec-Butyl Alcohol
12. Methanol	24. n-Butyl Alcohol
	25. 2-Methyl-2-Pentanol

Ordering Information for the GS-OxyPLOT Column

ID (mm)	Length (m)	Film Thickness (µm)	Temperature Limit (°C)	Cage Size	Part Number
0.53	10	10	350	7″	115-4912
0.53	10	10	350	5″	115-4912E

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1. A. K. Vickers, “A ‘Solid’ Alternative for Analyzing Oxygenated Hydrocarbons—Agilent’s New Capillary GC PLOT Column,” Agilent Technologies publication 5989-6323EN, Feb 2006.
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Printed in the USA
March 9, 2007
5989-6447EN



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Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Odorants and Other Sulfur Compounds in Liquefied Petroleum and Natural Gases

Technical Overview

Introduction

This technical overview briefly describes the analysis of liquefied petroleum gases as fully defined in ASTM D 5504-95. The method provides for the determination of individual volatile sulfur containing compounds, as well as the determination of total sulfur content in gaseous fuels, including natural gas.

Gas chromatography with sulfur chemiluminescence detection provides a rapid means to identify and quantify various sulfur compounds that may be present in petroleum feeds and products, such as liquefied petroleum gases (LPGs) and natural gas liquids (NGLs). These samples can contain many different amounts and types of sulfur compounds. Many sulfur compounds are corrosive to equipment, inhibit or destroy catalysts employed in downstream processing, and impart other undesirable properties to products. However, odorous sulfur compounds such as ethyl mercaptan, tetrahydrothiophene, and occasionally thiophane are intentionally added to propane as warning agents for detecting LPG leaks. The ability to speciate sulfur compounds in these liquids is useful for quality assurance of odorant addition and for better control of the sulfur compounds in finished products. The following chromatogram illustrates the ability of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) to speciate sulfur compounds in an NGL sample.

This particular NGL sample contained approximately 100 ppm wt total sulfur. The chromatographic conditions for the illustrated analysis were as follows: Agilent 5890 Series II gas chromatograph; Agilent 355 SCD operated according to standard conditions; and 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica column. The temperature program was: –10° C for three minutes to the final required temperature at a rate of 10° C per minute.

Evolving column technology allows for the analysis of liquefied petroleum gases or natural gas liquids with separation of H_2S and COS at ambient temperatures using capillary columns such as the Chrompack CP Sil 5 CB (0.32 mm), Chrompack CP SilicaPLOT (30 m 0.32 mm id), or the Astec Gaspro (15 m 0.32 mm id).

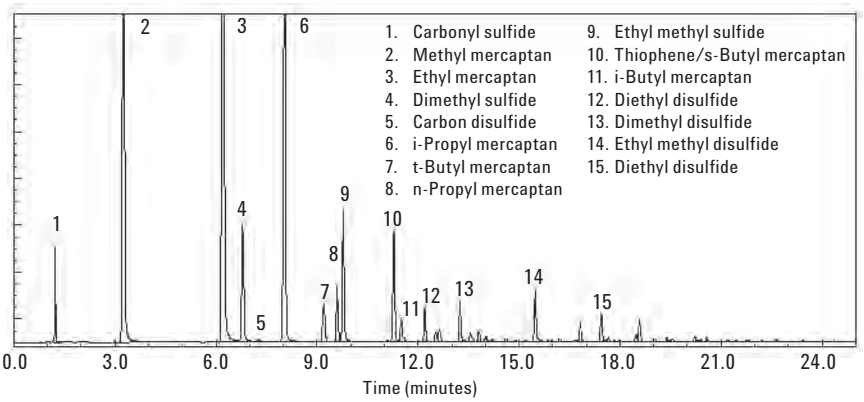

Figure 1. Sulfur compounds in natural gas liquids.

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Printed in the USA
June 8, 2007
5989-6788EN

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Refining

Refinery Gas

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Trace Level Hydrocarbon Impurities in Ethylene and Propylene

Application

Gas Chromatography

March 1997

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Abstract

An Agilent 6890 Series gas chromatography system was used to determine trace (low ppm) levels of hydrocarbon impurities in high-purity ethylene and propylene. The gas chromatograph (GC) was equipped with a heated gas sample valve, split/splitless inlet, and flame ionization detector (FID). An Agilent HP-PLOT Al_2O_3 column was used for separation of the trace hydrocarbons. Impurity levels below 10 ppm (mole) were easily detected in both ethylene and propylene.

Introduction

High-purity ethylene and propylene are commonly used as feedstocks for production of polyethylene, polypropylene, and other chemicals. Typically, these low molecular weight monomers are of very high purity (99.9+ percent). However, hydrocarbons, sulfur compounds, and other impurities in feed streams can cause such problems as reduced catalyst lifetime and changes to product quality. Process yields can also be adversely affected. Many impurities have been identified as potential contaminants (1,2).

Recently, ASTM has proposed several procedures to determine trace hydrocarbon impurities in both ethylene and propylene (3). These methods, currently in the investigation stage, use alumina porous layer open tubular (PLOT) columns. This application note describes the suggested Agilent configuration for such methods and illustrates resulting separations of both quantitative calibration blends and actual process samples. These proposed methods should be valuable in meeting commercial specifications.

Experimental

All experiments were performed on an 6890 Series gas chromatograph (GC) equipped with a split/splitless inlet and capillary optimized flame ionization detector (FID). All gas flows and pressures within the GC were controlled electronically. Gas sample injections were made using an automated sample valve placed in the 6890 valve oven (80 °C). The gas sample valve was interfaced to the capillary inlet using an aluminum tube (1/8-in.) that jacketed the stainless steel transfer line (option 860). The inlet was fitted with a split/splitless liner (part no. 19241-60540). All injections were made in the split mode.

A 50-m × 0.53-mm, HP-PLOT Al_2O_3 "M" column was used for separation. For ethylene analysis only, a 30-m × 0.53-mm, 5-µm HP-1 column was placed directly behind the HP-PLOT column. The two columns were joined using a glass press-fit connector.

The Agilent ChemStation was used to control the 6890 Series GC and to provide data acquisition and peak integration. The ChemStation was operated at a data acquisition rate of 10 Hz.

Standards for retention time and response factor calculation were obtained from DCG Partnership (Houston, Texas, USA 77061). Samples used for this work were obtained from commercial sources.

Table 1 lists the entire set of equipment and conditions.

Results and Discussion

Ethylene

The configuration used for ethylene analysis is found in figure 1.

The HP-PLOT Al_2O_3 column was used for hydrocarbon separation. The use of HP-PLOT Al_2O_3 columns for light hydrocarbon analyses has been previously described (4). These columns exhibit excellent separation characteristics for the C_1 through C_5 isomers.

The proposed method for ethylene specifies the use of a second nonpolar column placed after the HP-PLOT alumina column to improve the separation of impurity peaks eluting on the tail of ethylene. This nonpolar column gains importance for trace level analysis, where higher concentrations of ethylene (99.9 percent and higher) exhibit increased tailing. No attempt was made to compare separations without the nonpolar Agilent HP-1 column.

Table 1. Instrument Configuration and Operating Conditions

Item	Description
Gas Chromatograph	
G1540A	6890 Series GC
Option 112	Split/splitless inlet
Option 211	Capillary optimized FID
Option 701	6-port gas sample valve and automation
Option 751	Valve oven
Option 860	Valve to inlet interface
Column	• 50-m x 0.53-mm HP-PLOT Al_2O_3 "M" (part no. 19095P-M25) • 30-m x 0.53-mm, 5-µm HP-1 (part no. 19091Z-236), used for ethylene analysis only
Data Acquisition	
G2070AA	Agilent ChemStation
Operating Parameters	
Injection port temperature	200 °C
Detector temperature	250 °C
Split ratio	10/1 to 50/1 depending on sample
FID conditions	30 mL/min hydrogen, 350 mL/min air, nitrogen make-up (25 mL/min column + makeup)
Temperature program	• Ethylene: 35 °C (2 min), 4 °C/min to 190 °C • Propylene: 40 °C (2 min), 4 °C/min to 190 °C
Injection volume	• Ethylene: 0.5 mL • Propylene: 0.25 mL
Column flow	• Ethylene: 6 mL/min constant flow (10 psi) • Propylene: 3.5 mL/min constant flow (4 psi)
Valve temperature	80 °C

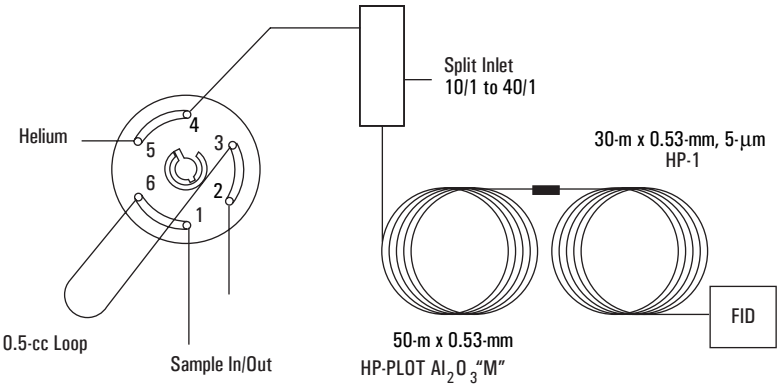

Figure 1. Valve drawing for impurities in ethylene.

Figure 2 shows the chromatogram of an ethylene calibration blend containing most of the major hydrocarbon impurities. This sample was analyzed at a split ratio of 10/1. The concentration of most components (except for ethane) ranges from 8 to 12 ppm (mole). For this analysis, baseline separation is achieved for all the impurities except for propane. Total analysis time is approximately 30 minutes. Because this separation is more than adequate, analysis time can be reduced by increasing the temperature program rate. Based upon conditions used for this analysis, most components can be detected at the 1-ppm level.

Chromatographic results for two process ethylene samples are given in figures 3 and 4. The sample presented in figure 3 contains only methane, ethane, and propylene as impurities. Less than 1-ppm methane was detected. The ethylene sample in figure 4 shows a high concentration of methane, with trace amounts of ethane, propane, and propylene.

1. Methane (10 ppm)
2. Ethane (219 ppm)
3. Ethylene
4. Propane (12 ppm)
5. Propylene (9 ppm),
6. Isobutane (10 ppm)
7. n-Butane (10 ppm)
8. Propadiene (10 ppm)
9. Acetylene (10 ppm)
10. t-2-Butene (8 ppm)
11. 1-Butene (8 ppm)
12. Isobutylene (9 ppm)
13. c-2-Butene (9 ppm)
14. 1,3-Butadiene (10 ppm)
15. Methylacetylene (9 ppm)

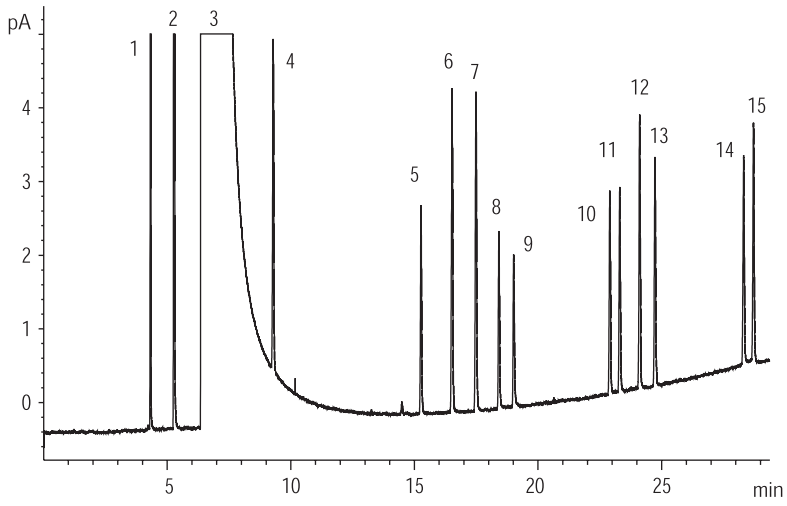

Figure 2. Chromatogram of ethylene calibration blend, split ratio 10/1.

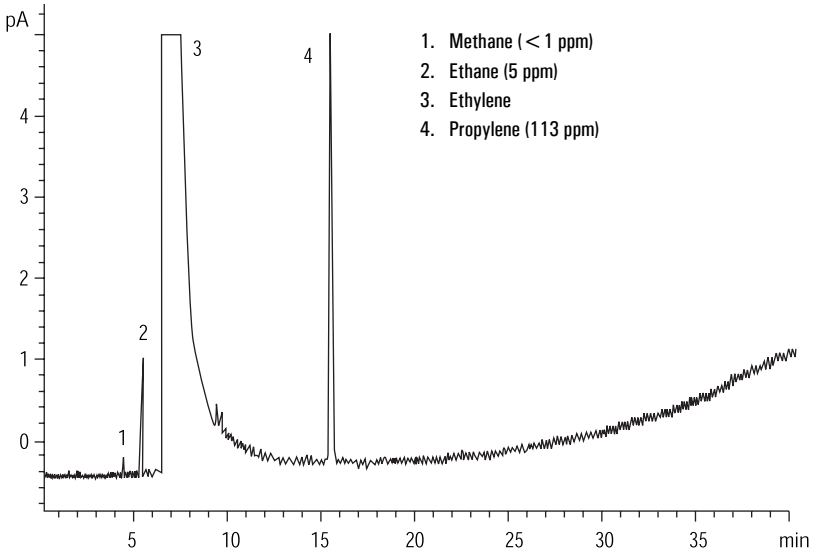

Figure 3. Chromatogram of process ethylene sample, split ratio 20/1.

Propylene

The configuration used for propylene analysis is illustrated in figure 5. This configuration is essentially the same as for ethylene, but without the HP-1 column. The sample volume was reduced to 0.25 mL. Propylene was sampled in the gas state.

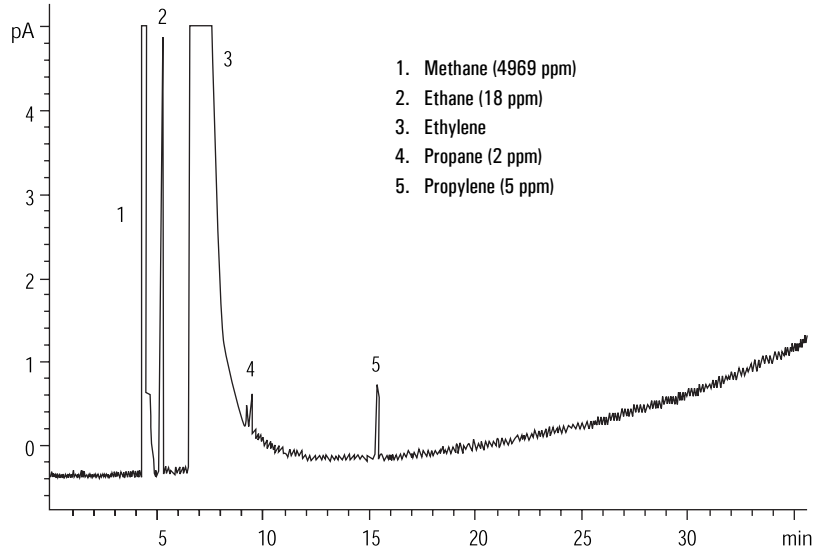

Figure 4. Chromatogram of process ethylene sample.

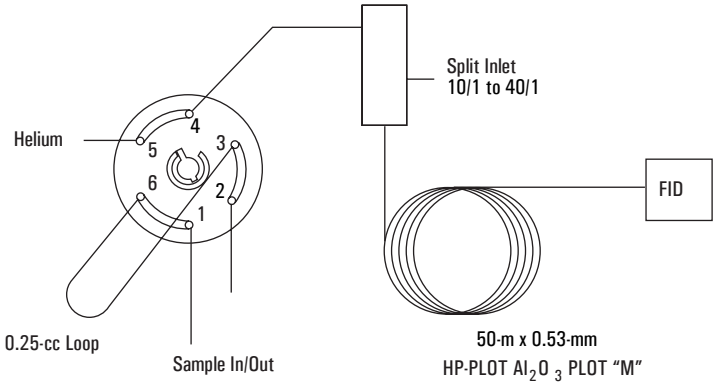

Figure 5. Valve drawing for impurities in propylene.

A chromatogram representing the trace hydrocarbon impurities in propylene is shown in figure 6. This sample was analyzed at a split ratio of 20/1. The concentration of most impurities range from 8 to 20 ppm. Ethylene is present at a higher concentration level. Most of the impurities in the sample are well separated using the conditions described in table 1. Cyclopropane elutes just before propylene and is baseline separated under these conditions. Several of the C_4 hydrocarbons elute on the tail of the high-purity propylene. This affects the lower limit of detection for these peaks, compared to those components that are baseline separated. The remainder of the C_4 and C_5 impurities are well separated.

1. Methane
2. Ethane (10 ppm)
3. Ethylene (50 ppm)
4. Propane
5. Cyclopropane (10 ppm)
6. Propylene
7. Isobutane (10 ppm)
8. n-Butane (7 ppm)
9. Propadiene
10. Acetylene
11. t-2-Butene (10 ppm)
12. 1-Butene
13. neo-Pentane
14. Isobutylene (9 ppm)
15. Isopentane
16. c-2-Butene (9 ppm)
17. n-Pentane (10 ppm)
18. 1,3-Butadiene (9 ppm)

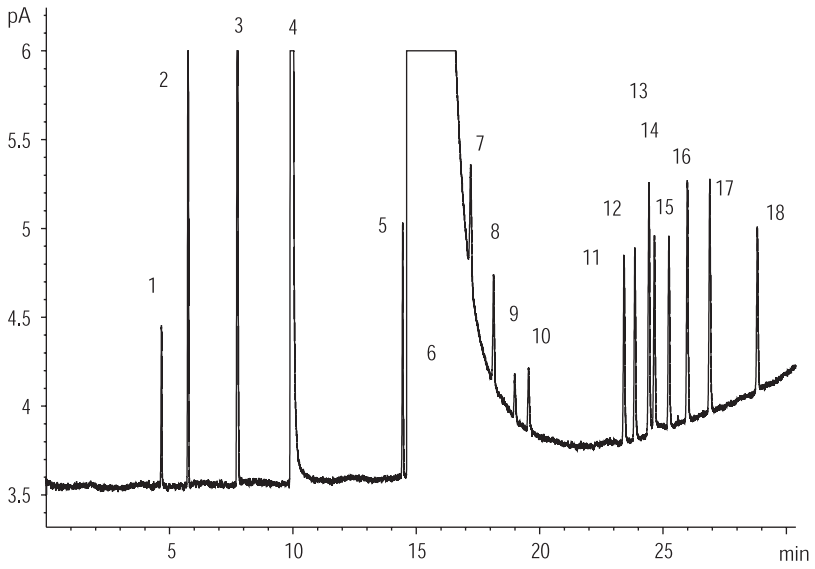

Figure 6. Chromatogram of propylene calibration standard.

For comparison, figure 7 shows the analysis of a second calibration blend containing a higher level of impurities (50 to 1000 ppm).

Figure 8 presents the chromatographic results for a high-purity propylene process sample. This sample contains only ethane and propane impurities.

Summary

This application note describes two methods for analyzing trace hydrocarbon impurities in ethylene and propylene. These methods use a gas sample valve with split injection, an Agilent HP-PLOT Al_2O_3 and HP-1 (for ethylene only) column, and an FID. Impurities below the 10-ppm mole level can be easily quantitated using these methods. For some impurities, especially those that are well separated from the large ethylene or propylene peaks, detection limits were estimated to be about 1 ppm.

1. Methane
2. Ethane
3. Ethylene
4. Propane (988 ppm)
5. Cyclopropane (100 ppm)
6. Propylene
7. Isobutane (129 ppm)
8. n-Butane
9. Propadiene (62 ppm)
10. Acetylene (48 ppm)
11. t-2-Butene
12. 1-Butene
13. Isobutylene
15. Isopentane
16. c-2-Butene
17. 1,3-Butadiene
18. Methylacetylene (100 ppm)

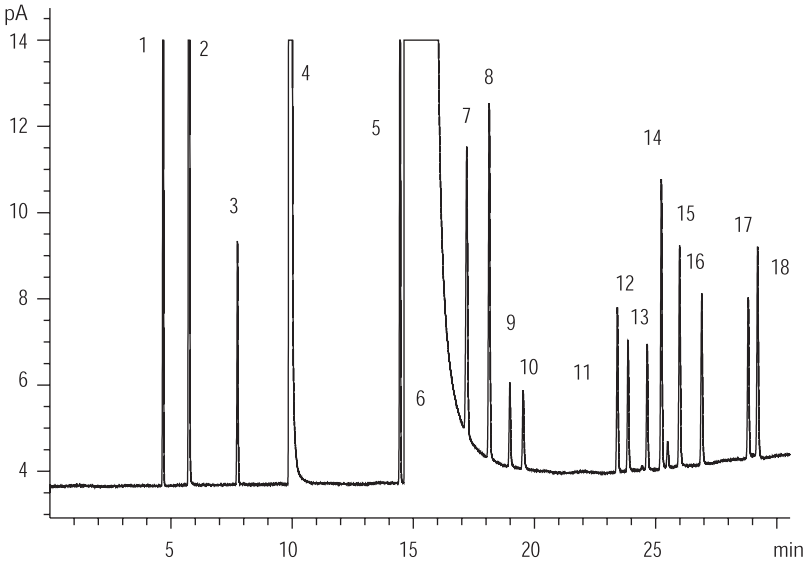

Figure 7. Chromatogram of propylene calibration blend containing higher levels of impurities.

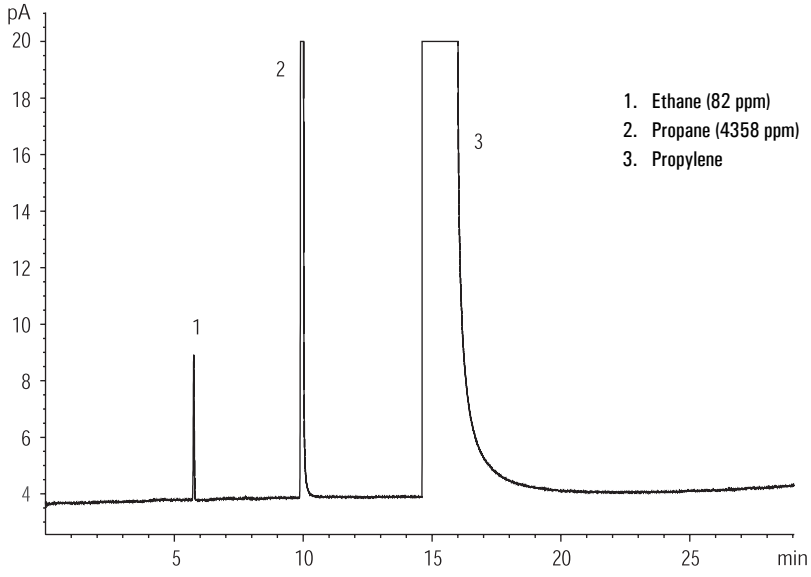

Figure 8. Chromatogram of process propylene sample.

References

1. ASTM Method D 5325, “Standard Guide for the Analysis of Ethylene Product,” Annual Book of Standards, Volume 5, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
2. ASTM Method D 5273, “Standard Guide for the Analysis of Propylene Concentrates,” Annual Book of Standards, Volume 5, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. Proposed methods for hydrocarbon impurities in ethylene and propylene by gas chromatography are being investigated under ASTM committee D-2, subcommittee D.
4. “Optimized Determination of C_1–C_6 Impurities in Propylene and Ethylene Using HP-PLOT/Al_2O_3 Columns,” Agilent Technologies, Inc. Publication (43) 5062-8417E, March 1994.

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5965-7824E



Agilent Technologies
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Analysis of Trace Sulfur Compounds in Beverage Grade Carbon Dioxide

Application

Gas Chromatography

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Abstract

An inert valving system using the volatiles interface (VI) on the Agilent 6890 is described for the analysis of ppb level volatile sulfur compounds in food grade carbon dioxide. Examples using the Agilent AED, FPD, and Sievers SCD are shown. The blending of CO2 with an eight component sulfur mix provides an easy to use means of calibrating the system for low level sulfur determinations.

Introduction

The food and beverage industry has strict guidelines on the quality of CO_2 required for use in products for human consumption. Food grade CO_2 (> 99.95%) can contain a number of impurities. Impurities can produce off-odors or flavors and in some rare instances a health hazard can result. Guidelines[1] have been established recently for allowable levels of impurities by chemical type. These have been published by the International Society of Beverage Technologists (ISBT).

The most common impurities are acetaldehyde, methanol, ethanol, hydrogen sulfide, carbonyl sulfide, and sulfur dioxide. For example, the guideline concentrations for total sulfur and total volatile hydrocarbons is 0.1 ppm v/v and 50 ppm v/v, respectively. A chromatographic system based on the Agilent 6890 equipped with a sulfur selective detector can easily quantify sulfur at levels well below those recommended for food grade CO_2. Strict adherence to sample introduction hardware recommendations is required for reliable operation.

Experimental

The sulfur selective detectors (one per GC) were mounted on the Agilent 6890 gas chromatograph equipped with the volatiles interface. The GC was configured for 220 V operation in order to achieve high oven ramp rates. All GC gas flows and pressures were controlled electronically. A 6-port gas-sampling valve (GSV) in Hastelloy C was interfaced directly to the Silcosteel treated VI with 1/16-inch Silcosteel tubing, Restek part no. 20595. All lines coming in contact with the sample including the 1 cc sample loop were Silcosteel treated to provide inertness necessary for low level sulfur analysis. A diagram of the sample introduction system is shown in Figure 1.

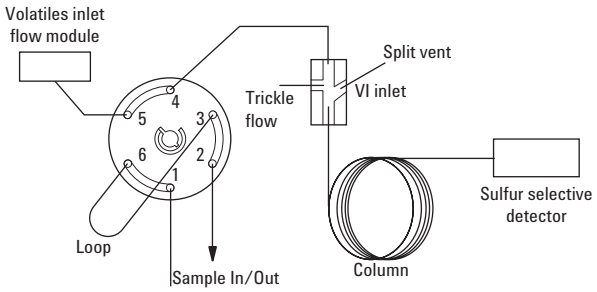

Figure 1. Sample introduction system.

All samples were introduced to the GC in the gas phase. Chromatographic conditions employed are given below.

Gas sampling valve:	1 cc loop at 140 °C, Hastelloy C material
Column:	105 m × 0.53 mm × 5 µm DB-1
Typical oven:	-20 °C (9.5 min), 10 °C/min to 250 °C, 80 °C/min to 280 °C
VI Inlet:	5 mL/min, constant flow mode Temp 150 °C, Split ratio 0.1:1

A dynamic blending system was used to add sulfur calibration mix, in a controlled fashion, to the pure CO_2 matrix, producing a stream of known sulfur concentrations for delivery to the GSV. This system is shown in Figure 2. The sulfur calibration mix (DCG Partnership I, LTD., Pearland, TX, 281-648-1894) consisted of the components listed in Table 1 at certified concentration levels of 5 ppm each.

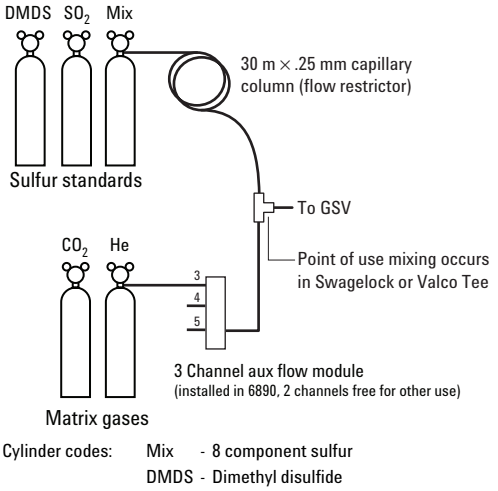

Figure 2. Dynamic blending scheme.

Table 1. Sulfur Mix Components

Number	Compound
1	Hydrogen sulfide
2	Carbonyl sulfide
3	Methyl mercaptan
4	Ethyl mercaptan
5	Dimethyl sulfide
6	Carbon disulfide
7	t-Butyl mercaptan
8	Tetrahydrothiophene

The AED, FPD, and Sievers SCD (Sulfur Chemiluminescent Detector) were used in the study. For maximum sensitivity, the FPD was setup in hydrogen rich mode. Detector conditions are shown in Table 2.

Table 2 Detector Settings

Detector	Temperatures	Pressures and Flows
Agilent	250 °C	H_2 50 mL/min
Flame Photometric		Air 60 mL/min
		N_2 makeup 50 mL/min
Agilent G2350A	Cavity 245 °C	O_2 55 psi
Atomic Emission	Transfer line 245 °C	H_2 45 psi
Sulfur 181 nm		He makeup 100 mL/min
Sievers 355	803 °C Furnace	P1 5.4 Detector
Sulfur Chemiluminescent		P2 171 Furnace
		H_2 100 mL/min
		O_2 8 mL/min
		O_3 5.6 psi

Results

The FPD demonstrated excellent sensitivity to a 25 ppbv/component sulfur mix in pure CO_2 as shown in Figure 3. This level of sample mix was prepared using the dynamic blending system by

mixing 1 mL/min sulfur calibration standard with 199 mL/min CO_2. Quantitation of all components except for H_2S is possible at this level. Hydrogen sulfide at levels of approximately 50 ppbv should be quantifiable with the FPD when using the sample introduction system described here.

In Figure 4, AED chromatograms of the sulfur 181 nm line are shown at sulfur mix concentrations of 5 ppm (no dilution) and 35 ppb (dilution in CO_2). All eight sulfur compounds show good signal to noise at the 35 ppb level.

Next, pure CO_2 was analyzed without addition of the sulfur calibration mix. Care was taken to ensure that carryover of residual sulfur compounds was not mistakenly identified as components present in the CO_2. Helium was used to purge the lines thoroughly for approximately 1 hour. Injections of helium were made into the GC-AED system to monitor any potential carryover. It is not uncommon to see residual levels of some sulfur mix compounds present for one or two GSV injections after the mix is disconnected.

Figure 5 shows a SCD chromatogram of pure CO_2 blended with the sulfur mix to 25 ppb. The SCD was able to detect down to levels of approximately 2 ppb for COS when using a 5 mL sample loop. Carbonyl sulfide and carbon disulfide in pure CO_2 can be seen at levels of 2 ppb and 1 ppb, respectively, on the AED in Figure 6.

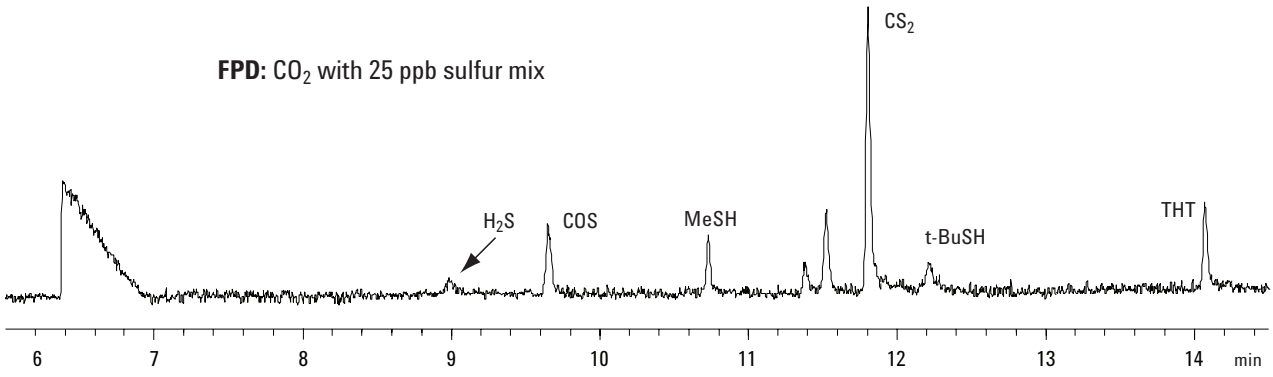

Figure 3. Carbon dioxide with 25 ppb sulfur calibration mix. Detector: Agilent Flame Photometric. One cc sample loop, no split.

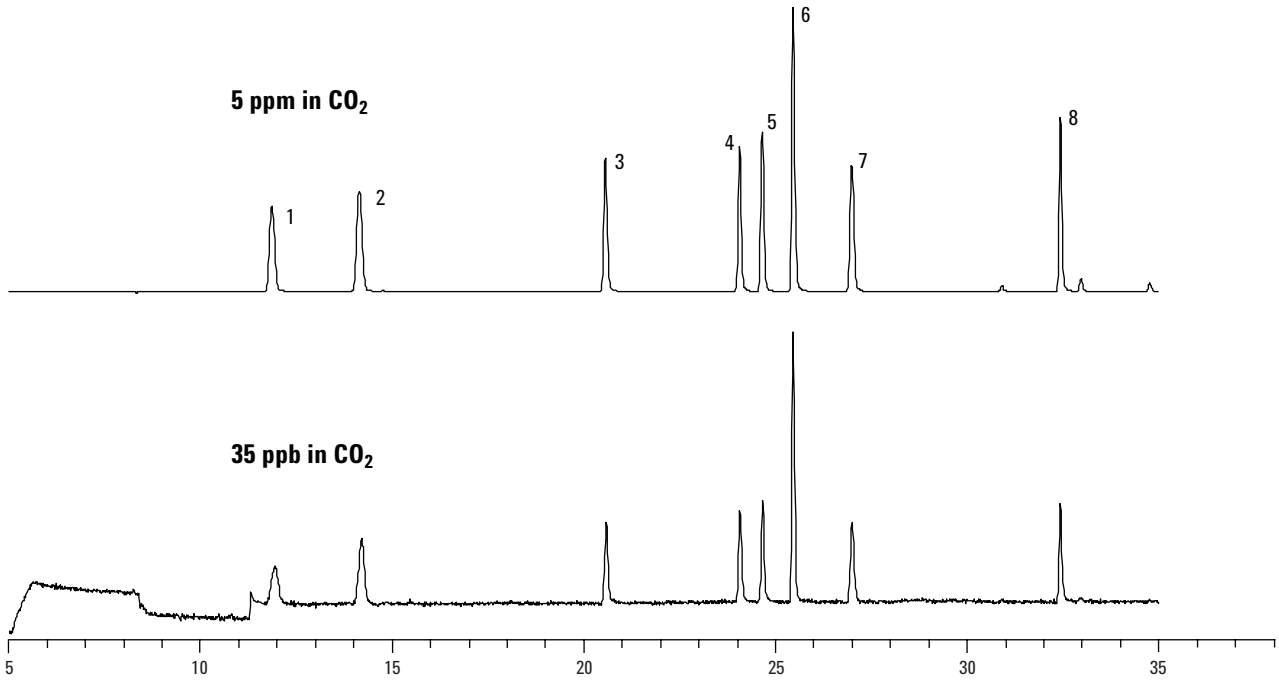

Figure 4. Sulfur calibration mix in CO_2 at levels of 5 ppm and 35 ppb. Detector: Agilent Atomic Emission.

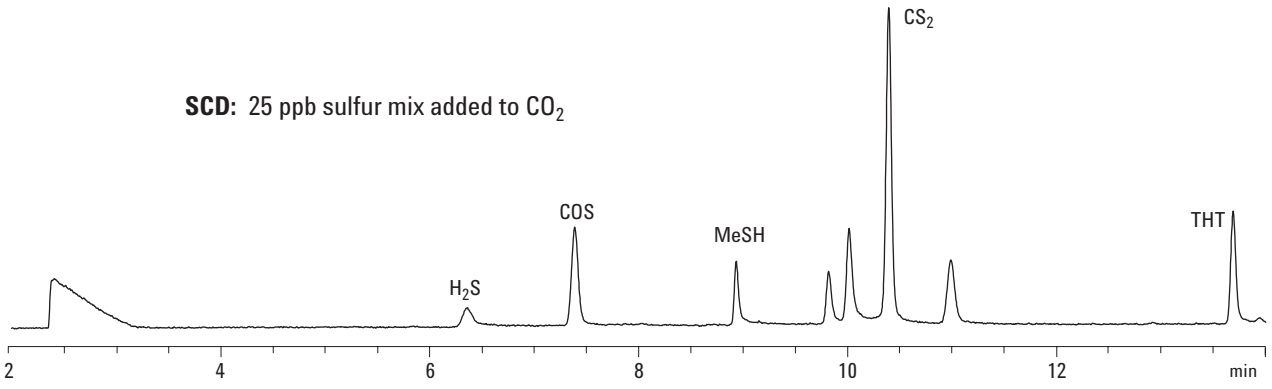

Figure 5. Analysis of food grade CO_2. Detector: Sievers SCD.

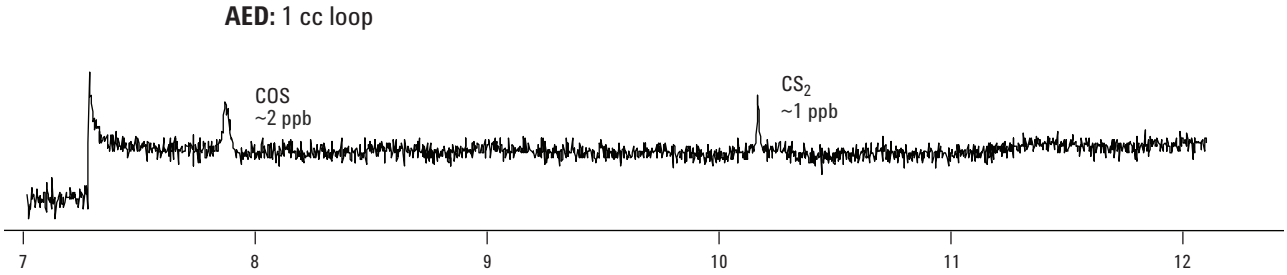

Figure 6. Analysis of food grade CO_2. Detector: Agilent AED.

A number of non-sulfur impurities can also be present as outlined in the ISBT guide. Although not shown in this application note, the AED could be used to look at the S 181 nm and C 193 nm lines simultaneously. Thus, many of the hydrocarbon impurities could easily be separated and quantified at low ppb levels using the 105 m × 0.53 mm × 5.0 µm DB1 column in an Agilent 6890/AED system. The carbon 193 nm line is approximately five times more sensitive than the FID for those compounds that respond well on a FID. Therefore detectability of the hydrocarbon impurities would not be difficult at the levels required.

Conclusions

For detection of sulfur impurities in beverage grade CO_2 at levels down to 25 ppb, the FPD offers an easy-to-use low cost solution when coupled to the optimized sample introduction system presented here. The sulfur chemiluminescent detector (SCD) offers better sensitivity at a higher cost and somewhat more difficult setup/tuning compared to the FPD. The AED offers the most complete solution when analysis of hydrocarbons is also desired with a single valve-column-detector combination.

References

1. Carbon Dioxide Quality Guidelines and Analytical Procedure Bibliography, International Society of Beverage Technologists, Homosassa, FL, (1999)

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Printed in the USA
April 2, 2001
5988-2464EN

Automated Dynamic Blending System for the Agilent 6890 Gas Chromatograph: Low Level Sulfur Detection

Application

Gas Chromatography

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Abstract

A point-of-use dynamic blending system for gaseous sample preparation and calibration has been developed for the Agilent 6890. Gaseous samples in the low ppb range can be easily prepared in numerous matrices. The analysis of low level sulfur gases including H_2S, COS, and mercaptans is used to demonstrate the system.

Introduction

Calibration of gas chromatographic systems for gaseous analytes in the low ppm to single digit ppb range has always presented challenges. This is especially true when reactive compounds are involved. With the growing use of selective detectors such as the flame photometic detector (FPD), atomic emission detector (AED), pulsed flame photometric detector (PFPD), sulfur chemiluminescent detector (SCD), and mass selective detector (MSD) for trace level analysis of volatile sulfur and other small molecules, a clear need has emerged for simple and reliable calibration/blending systems. Accurate trace level gas analysis is critical in many diverse industries including hydrocarbon processing, semiconductor fab, and food/beverage.

Permeation tubes offer one solution that is both accurate and reliable, emitting a stable concentration under controlled carrier flow. Well over 300 compounds are available. However, this approach has several disadvantages. Individual tubes, with precise temperature control, must be used for each gas requiring calibration. Also, special hardware such as gas standard generators is normally required to control multiple tubes for preparation of multi-component calibrations.

Cylinder based gas standards are convenient allowing custom calibration mixes to be made to order. Use of cylinder standards also have some limitations:

1. It is difficult or impossible to prepare accurate standards below a few ppm for many gases.
2. Calibration over a concentration range requires purchase of a different cylinder for each analyte level.
3. Adsorption of analytes on the cylinder walls.

Many problems with cylinder standards can be overcome by using a point-of-use dynamic blending system. This application describes such a system for the 6890 that is inexpensive, reliable, inert to active compounds, and automatable.

Experimental

An Agilent 6890 gas chromatograph equipped with an AED served as the system core. Sample introduction consisted of a 6-port Hastelloy C GSV interfaced directly to the Volatiles Inlet (VI) with Sulfinert tubing. Any 6890/detector combination could be used as appropriate for the application. The FPD, for example, would be an excellent choice for low level sulfur applications.
The point-of-use preparation of ppb level volatile sulfur compounds and subsequent analysis was chosen as an industrially significant application to demonstrate use and performance of the blending system. Figure 1 illustrates the basic components and configuration of the blending hardware. Table 1 lists some of the key options used on the 6890. The recommended non-GC hardware needed to construct the blending system is as follows:

1. 10 to 20 ft of 1/16-inch OD Restek Silcosteel or Sulfinert tubing. Silcosteel part no. 20595, Sulfinert part no. 22505.
2. One ss Swagelok or Valco 1/16-inch Tee, Swagelok Sulfinert Tee available from Restek, part no. 22526,
3. One 30 m × 0.25 mm ID column or equivalent retention gap tubing 30 m × 0.25 mm ID deactivated fused silica tubing J&W part no. 160-2255-30. Equivalent in metal tubing can also be used: Restek Sulfinert tubing part no. 22502.
4. Appropriate fitting to connect flow restrictor to the Standards cylinder regulator.
5. 1/16-inch ss union for connection to the GSV sample in line.
6. 1/8-inch OD line connecting GSV sample out to laboratory external vent for safety.

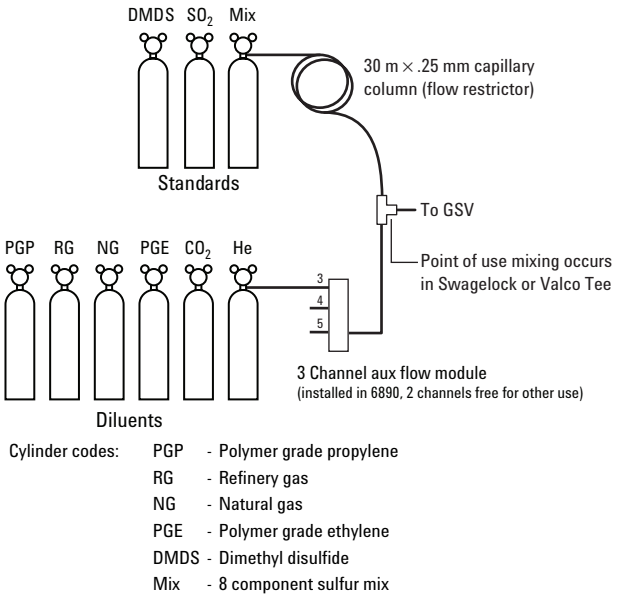

Figure 1. Layout of the 6890 dynamic blending system.

Table 1. 6890 Options and Parts

Option or part number	Description
142	Volatiles interface w/EPC
301	3 Channels of aux EPC for AED
312	N_2 cryo cool
207	AED interface
751	Install and automation of 1 valve
701	Gas sampling, 0.25 cc loop
868	Substitute Hastelloy C Valve
No option required	Interface GSV to VI inlet
Restek No.	
22846 and 22847	Sulfinert sample loops 0.25 cc and 0.50 cc (Restek Corp.), replaces loop from option 701

The column used as the flow restrictor is inserted directly into the Tee and sealed using a graphite/vespel ferrule. Detail of how the connections are made is shown in Figure 2. Note how the column tubing is inserted into the 1/16-inch line inside the Tee. For typical chromatographic conditions, see Table 2.

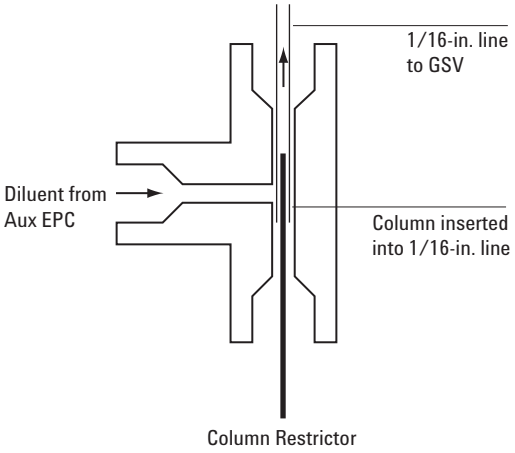

Figure 2. Detail of connections at the blending Tee.

Table 2. Instrument Conditions

Gas Chromatograph	**Agilent 6890**
Injection Source	**6-port Gas Sampling Valve, Hastelloy C**
Temperature	120 °C
Loop	0.25 cc, 0.50 cc, 1.0 cc Sulfinert treated
Injection Port	**Volatiles Interface**
Temperature	120 °C
Split ratios	0.1 to 1, 0.5 to 1 typical
Carrier gas	Helium
Constant flow mode	
Columns	105 m × 0.53 mm × 5.0 µm DB-1, J&W Cat. No. 125-10B5 60 m × 0.32 mm GS-GasPro, J&W Cat. No. 113-4362
DB-1 Program	
Initial temperature	35 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	240 °C
Final time	5 min
GasPro Program	
Initial temperature	40 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	290 °C
Final time	10 min
Aux EPC	
	Medium flow resistance frits, Part no. 19231-60700
Agilent FPD	
Temperature	250 °C
Flows	H_2 50 mL/min Air 60 mL/min N_2 makeup 50 mL/min
Agilent AED	
Cavity	245 °C
Transfer line	245 °C
Settings	O_2 55 psi H_2 45 psi He makeup 100 mL/min

Actual concentrations of the standard analytes in any given matrix are determined from the ratio of the volumetric flow rates for the calibration mix and diluent. The Flow calculator program[1] (free download from the Chemical Analysis Group web site) is used to calculate flow exiting from the column flow restrictor for a given inlet pressure. The Aux EPC module must also be calibrated for each diluent gas; this is described later in the Results section. Starting with a sulfur calibration mix with a certified concentration of 5 ppm per component (DCG Partnership 1, LTD., Pearland, TX, 281-648-1894), the resulting concentrations delivered to the gas chromatograph in ppb by volume for a number of mix/diluent combinations are shown in Table 3. The actual components in the sulfur calibration mix and their concentrations are given in Table 4. In Figures 3 and 4, chromatographic results of an automated dilution of the sulfur mix are shown where each successive run is overlaid.

Table 3. Dilution Table. Starting Calibration Mix at 5.0 ppm per Component. Calibration Mix Restrictor: 30 m × 0.25 mm

Calibration Mix		**EPC AUX (He)**		**Concentration**
Pressure Setting psig	**Flow mL/min**	**Pressure Setting psig**	**Flow mL/min**	**ppbV**
29	4	10	10	1400
29	4	40	57	330
29	4	60	105	183
29	4	90	215	91
18	2	70	142	70
18	2	85	193	51
10	0.9	70	142	31
10	0.9	90	215	21

Table 4. Sulfur Calibration Mix

Number	**Compound**	**Conc.(ppm)**
1	Hydrogen sulfide	5.00
2	Carbonyl sulfide	4.96
3	Methyl mercaptan	5.00
4	Ethyl mercaptan	5.04
5	Dimethyl sulfide	4.91
6	Carbondisulfide	5.01
7	t-Butyl mercaptan	5.04
8	Tetrahydrothiophene	5.05

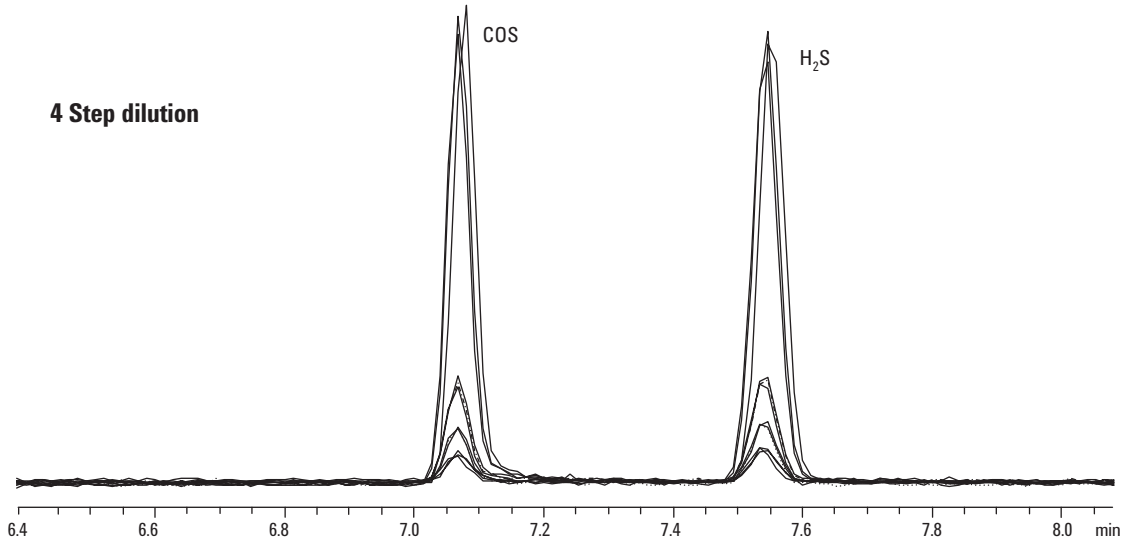

Figure 3. Four step dilution of COS and H_2S.

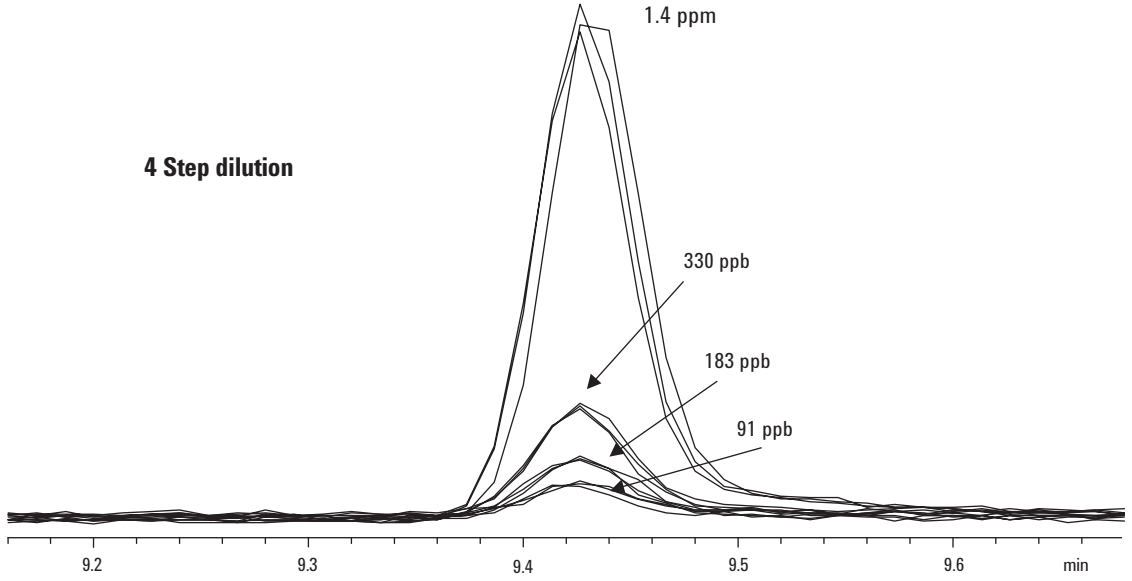

Figure 4. Four step dilution of methyl mercaptan.

Results

Repeatability is an important attribute of any analytical system, and essential for quantitative trace level work. The dynamic blending system was set up to blend flows of 2 mL/min sulfur mix and 105 mL/min helium in an experiment to determine precision. These flows yielded a concentration of 90 ppb for each sulfur component. Table 5 shows the integrated areas for all eight of the sulfur compounds present in the mix for a total of 11 runs along with standard deviations. Results are excellent with the exception of tetrahydrothiophene. Using higher GSV and VI temperatures would probably improve the RSD for this sulfur compound.

Due to the adsorptive behavior of many light sulfur compounds on various surfaces, take care to ensure that a stable concentration of analyte is delivered to the gas chromatographic column. It is best to allow the sulfur calibration mix to flow through the sample loop to vent for approximately 20 minutes before sampling into the GC.

Table 5. System Repeatability, Replicate Runs at 90 ppbv. Numbers in Table are Integrated Areas.

	RT	1	2	3	4	5	6	7	8	9	10	11	STDDEV	AVG	%RSD
1	7.11	2.94	2.86	2.79	2.80	2.69	2.66	2.81	2.72	3.01	2.70	2.93	0.12	2.81	4.10%
2	7.58	2.95	2.91	2.85	2.93	2.93	2.91	3.07	2.84	3.06	3.03	2.85	0.08	2.94	2.76%
3	9.46	2.37	2.31	2.20	2.59	2.33	2.47	2.26	2.37	2.46	2.48	2.66	0.14	2.41	5.72%
4	11.15	2.24	2.39	2.19	2.24	2.39	2.35	2.27	2.30	2.23	2.35	2.38	0.07	2.30	3.10%
5	11.47	2.65	2.45	2.38	2.50	2.44	2.35	2.43	2.39	2.59	2.49	2.39	0.09	2.46	3.81%
6	11.98	5.93	5.86	5.76	5.78	5.88	5.76	5.88	5.80	5.96	5.90	5.79	0.07	5.85	1.20%
7	12.80	2.60	2.31	2.84	2.41	2.38	2.38	2.48	2.34	2.43	2.32	2.54	0.16	2.46	6.36%
8	15.76	2.84	2.68	2.69	2.81	3.12	2.75	3.80	3.50	3.93	2.55	2.70	0.61	2.98	15.6%

Once in the column, additional loss of sulfur can occur on the stationary phase. This effect is minimal on methyl silicone columns, making them a good choice to access the performance of the dynamic blending system.

In Figures 5 and 6, sequential AED runs are overlaid showing an increase in peak area for each successive run until a stable peak height is obtained by the third run. This indicates that some minor adsorption of sulfur does occur in the blending hardware and/or GC inlet during initial exposure to sulfur analytes. Some adsorption may occur in the column flow restrictor until equilibrium is established and consistent concentration results. As expected, the effect is most pronounced for highly reactive species such as H_2S and heavier analytes such as tetrahydothiophene. Therefore, several “priming runs” need to be performed when the system is initially exposed to sulfur before the results are used for quantitative purposes. Note that t-butyl mercaptan and tetrahydrothiophene do not appear in the first run. As a general rule, perform this procedure whenever the system has been idle for more than 36 hours since desorption of sulfur analytes occurs when exposed to a constant carrier gas flow. Setting up a sequence on the ChemStation performs this conditioning when the first few runs are designated as priming samples.

Keep the behavior described above in mind when running unknown samples. It is advisable to begin using data from the second or third run if quantitative results are required, disregarding the first injection.

The consistency of response factors for the eight sulfur compounds over a wide dilution range was also studied. All response factors for the sulfur compounds should be approximately the same since the AED is characterized by equimolar response. In addition, as the calibration mix is diluted, normalized response factors should not

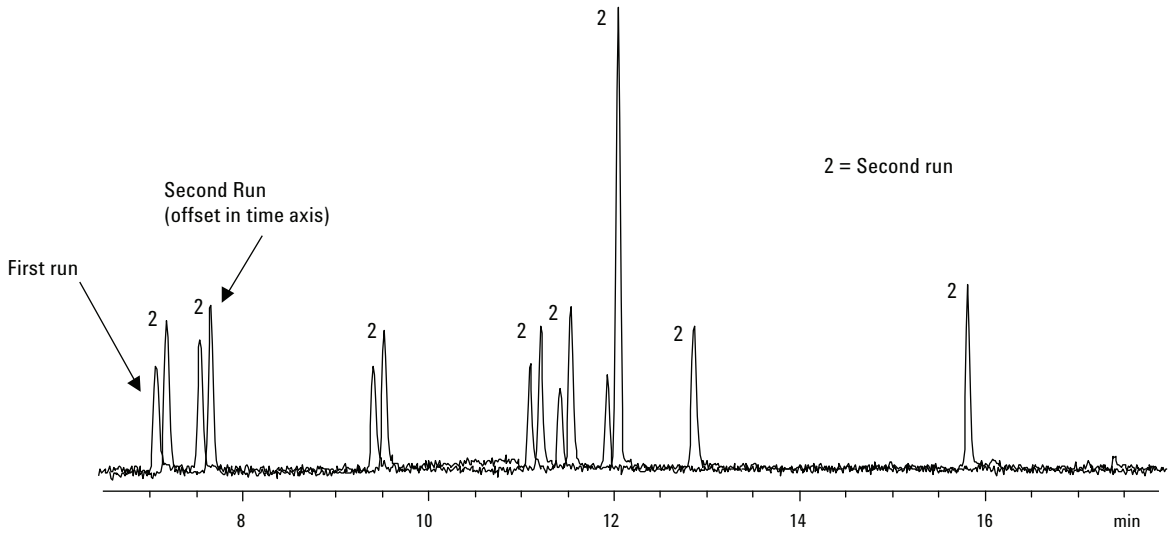

Figure 5. System startup behavior. Eight component sulfur mix. Concentration of each component is 90 ppb. Loop size 0.25 cc.

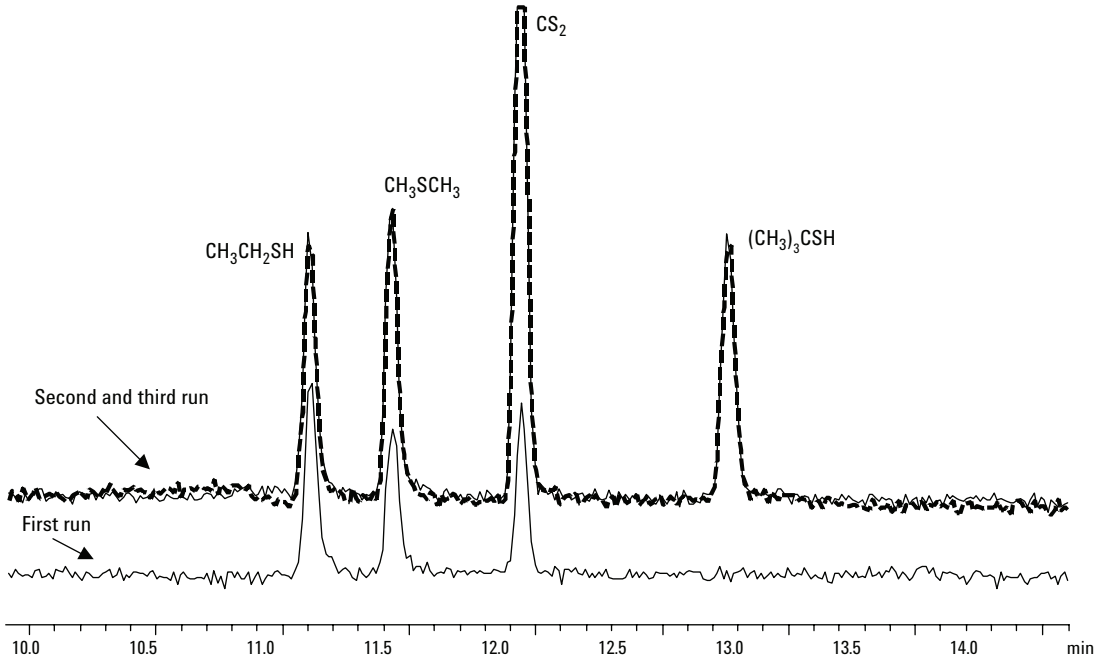

Figure 6. System startup behavior showing selected components.

vary widely. Significant variation may suggest that adsorption is occurring someplace in the chromatographic system. In particular, watch carefully hydrogen sulfide and polar compounds. Problems will tend to manifest themselves as dilutions increase. See Table 6 for a check of normalized response factors at three concentration levels. The lack of any major deviations from unity indicates consistent performance without loss of analytes as analyte concentrations decrease.

Matrix gases such as ethylene and propylene are easily added and blended with the calibration mix. This capability can be very useful during method development when the GC column type and temperature program are being optimized to eliminate or minimize coelution of sulfur components with hydrocarbons. Response factors can be checked for selectivity or quenching. For example, when method developing with the FPD, use the blending system to optimize a given analysis by quickly identifying coelutions. First run the calibration mix diluted in helium to the appropriate level to check sensitivity. Then run the pure matrix to check false responses. Finally run the calibration mix diluted in the matrix.

Since the system employs point-of-use blending, reactive and cylinder-incompatible gases can be combined just prior to analysis. In Figure 7, an FPD chromatogram is shown where sulfur dioxide is blended into the eight component sulfur mix and diluted in helium. Such a mix would not be available from a supplier in a single cylinder. To maximize sensitivity at the expense of chromatographic peak shape, a 5 cc sample loop was used in this example.

Table 6. Normalized Response Factors at Several Concentrations

Component	Starting Conc. ppm	ng S/pk 0.25 mL inj (5 ppm)	Normalized RF 1400 ppb	Normalized RF 91 ppb	Normalized RF 46 ppb
H_2S	5.00	1.78	1.04	1.00	1.03
COS	4.96	1.78	1.07	1.16	1.10
CH_3SH	5.00	1.78	0.92	0.91	0.84
CH_3CH_2SH	5.04	1.78	0.89	0.83	0.95
CH_3SCH_3	4.91	1.78	1.05	0.97	0.90
CS_2	5.01	3.56	1.11	1.21	1.14
$(CH_3)_3CSH$	5.04	1.78	0.93	0.95	0.83
C_4H_8S	5.05	1.78	0.98	0.96	1.21

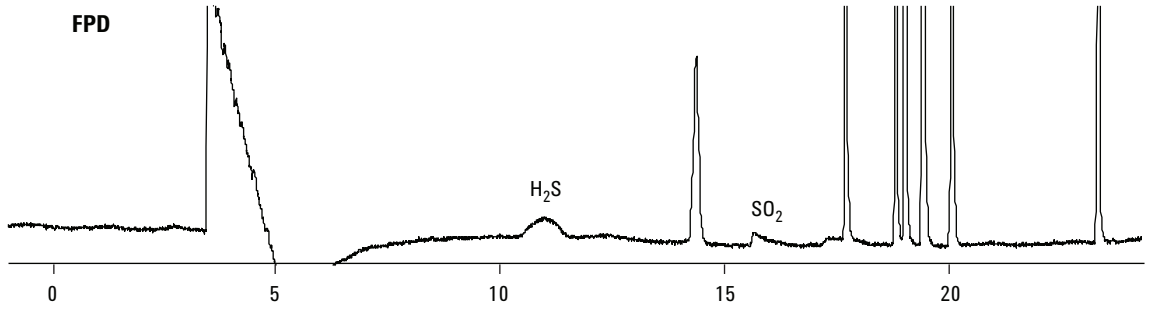

Figure 7. FPD chromatogram of the sulfur mix with SO_2 added. 50 ppb sulfur per compound in ethylene. Five cc sample loop, no split.

A powerful feature of the system is the ability to automate calibration. Four to six ChemStation methods are created differing only in the Aux EPC pressure setting. For example, methods may be created with Aux settings of 10, 30, 60, and 90 psig. A ChemStation sequence table is then constructed, calling out each method from low to high Aux pressure settings (decreasing analyte concentrations). Running three to four replicates in the sequence table at each concentration is recommended. Figure 8 illustrates the result of such an automated calibration for carbon disulfide where the concentrations shown are those calculated from the flow ratios. The linear relationship shown in the graph indicates excellent performance of the blending system.

Each diluent or matrix gas that is plumbed to the Aux EPC channel will have its own unique pressure vs. flow calibration curve due to differences in viscosity and molecular weight. Consequently, a curve of pressure vs. flow for each diluent gas should be constructed and saved. To create this curve, first turn off the calibration gas flow and then measure the diluent gas flow from the GSV sample out vent at several settings of EPC diluent pressure. Be sure to note which frit is installed in the Aux EPC as this will affect the calibration. Figure 9 shows the values characteristic of ethylene and helium. For most diluents or matrix gases, the flow rate differences compared to helium will not be large, For semi-quantitative purposes independent calibration of the Aux channel for each diluent may not be necessary.

An example chromatogram of ethylene blended with the calibration mix is shown in Figure 10. The 60 meter GasPro column was installed in the 6890 for this evaluation. This column has considerable separation power for the analysis of volatile sulfur compounds in light hydrocarbon streams, making it a good choice for many petrochemical applications. All volatile sulfur compounds can be easily separated from the ethylene, which is solvent vented on the AED a full minute before elution of H_2S.

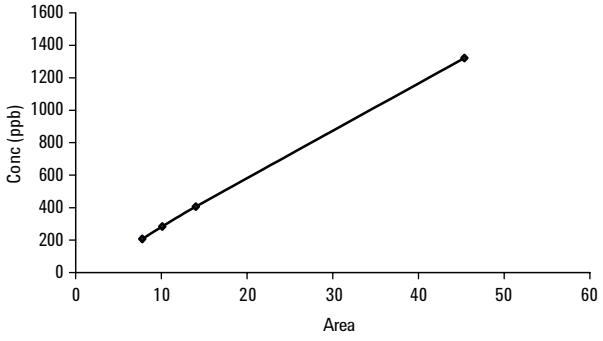

Figure 8. Calibration result for carbon disulfide. Four-step automated dilution. Detector: AED.

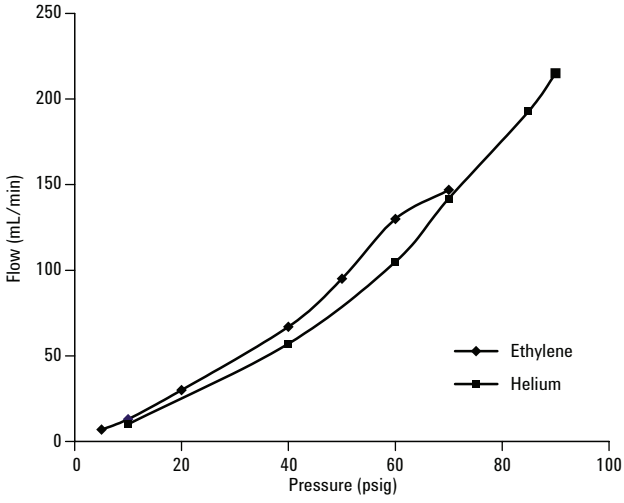

Figure 9. Calibration of Aux EPC channel for helium and ethylene.

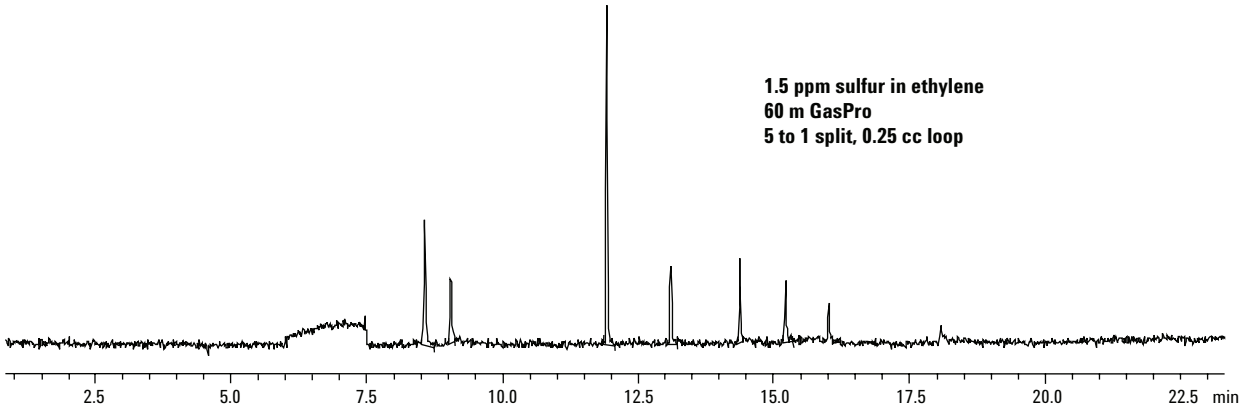

Figure 10. 1.5 ppm sulfur in ethylene using the 60 m GasPro column. Detector: AED.

Conclusions

Dynamic blending for point-of-use preparation of low-level gaseous calibration standards is inexpensive and automatable with the Agilent 6890 GC. The system can be assembled quickly with a few readily available components and one channel of an Aux EPC module. Calibrations to single digit ppbV levels are attainable with active components such as H_2S, COS, sulfides, and light mercaptans when the system is constructed with Silcosteel or Sulfinert tubing and fittings. When coupled with selective detectors such as the Flame Photometric or Atomic Emission, a powerful system is available for routine low-level gas analysis.

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1. Agilent's FlowCalc 2.0 software (available free from the Agilent Technologies Web site, **http://www.chem.agilent.com/cag/servsup/usersoft/main.html**

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Printed in the USA
April 2, 2001
5988-2465EN

Agilent Technologies

Volatile Sulfur in Natural Gas, Refinery Gas, and Liquified Petroleum Gas

Application

Gas Chromatography

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Abstract

An Agilent 6890 gas chromatograph equipped with an FPD (flame photometric detector) is used to characterize low level sulfur compounds in natural gas, refinery gas, and liquified petroleum gas (LPG) using a J&W 105 m × 0.53 mm × 5.0 μm DB-1 column. Analysis of volatile sulfur to less than 100 ppb can easily be performed with a volatiles interface (VI) connected to a 6-port gas-sampling valve. The system as configured provides a cost-effective solution for the determination of odorants in natural gas. Coelutions of hydrocarbons and sulfur compounds that result in signal quenching are documented.

Introduction

Monitoring of low-level volatile sulfur compounds in light hydrocarbon streams such as refinery gas, and in fuels including natural gas and LPG, persist as measurement challenges. To highlight one application area, odorant monitoring is an essential measurement need of the natural gas industry. Table 1 lists a number of the commonly used additives in the United States and Europe. Europe currently favors t-butyl mercaptan, methyl ethyl sulfide, ethyl mercaptan and tetrahydrothiophene. Odorant quality, including characterization of contaminants and possible reaction byproducts, is also important. Optimal odorization is also dependent on the quality of the natural gas stream, making measurement of the naturally occurring sulfur important for optimal metering of odorant addition and for monitoring odor threshold.

Table 1. Common Odorizers in Natural Gas

Methyl mercaptan	Ethyl mercaptan
Dimethyl sulfide	Isopropyl mercaptan
Methyl ethyl sulfide	Normal propyl mercaptan
Tertiary butyl mercaptan	Secondary butyl mercaptan
Diethyl sulfide	Tetrahydrothiophene
Ethyl isopropyl sulfide	

Sulfur selective measurements can assist in blending operations to assure proper ratios of components are injected into the pipeline, and to ensure that effects such as pipeline oxidation are understood.

Natural gas and other light hydrocarbon streams are finding use as fuel feedstocks for a variety of fuel cell technologies. Fuel cells and the reformer catalysts are generally not sulfur tolerant. Depending on the technology employed, sulfur can be a poison at single digit ppm levels. The need for low level sulfur measurement in the fuel cell industry will continue to grow as the various technologies see wider deployment. Odorant monitoring at various locations within a gas distribution system can also be important.

Gas chromatography plays an important role in sulfur measurement. The flame photometric detector (FPD) is ideal for many of these applications, given its low cost and ease-of-use, provided coelution can be avoided. Selection of the appropriate column, temperature program, and sample introduction system are key to the deployment of a successful system. This work illustrates what can be done with a methyl silicone column (105 m × 0.53 mm × 5.0 µm DB-1) without use of cryogenic oven cooling.

ASTM method D 5504-94 details a chemiluminescence approach to the analysis of sulfur in various gaseous streams including natural gas. Other sulfur selective detectors are not excluded from the method provided they meet criteria for sensitivity and hydrocarbon interference. The system described in this paper generally follows the method, pointing out situations where particular hydrocarbon matrices can cause quantitative problems. A subset of the sulfur compounds listed in the ASTM method is used.

Experimental

An Agilent 6890 gas chromatograph equipped with a FPD operating in a hydrogen rich mode for optimal sensitivity was used in this work. Sample introduction consisted of a 6-port Hastelloy C gas sample valve (GSV) interfaced directly to the volatiles interface (VI) with Sulfinert tubing (Figure 1). Instrument conditions are given in Table 2. A point-of-use gas blending system was used for preparation of ppb level sulfur compounds in the hydrocarbon matrices. Figure 2 illustrates the basic components and configuration of the gas blending hardware. The details of this system have been described

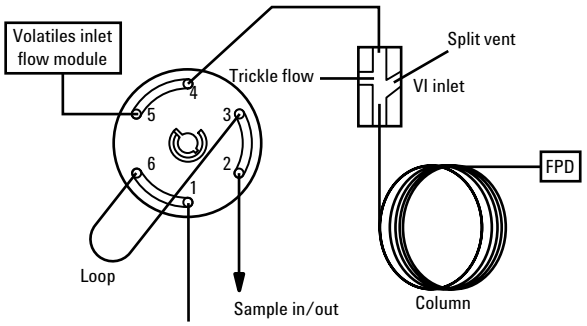
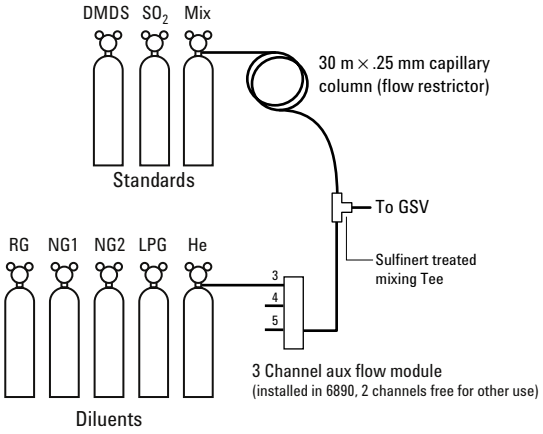


Figure 1. Sample introduction scheme.

Table 2. Instrument Conditions

Agilent 6890 Gas Chromatograph	
Detector	**Flame Photometric**
Temperature	250 °C
Hydrogen Flow	50 mL/min
Air Flow	60 mL/min
Makeup	N_2 60 mL/min, constant mode
Filter	393 nm
Injection Source	**6-port Gas Sampling Valve**, Hastelloy C
Temperature	120 °C
Loop	0.50 cc Sulfinert treated (replaces standard loop)
Injection Port	**Volatiles Interface**
Temperature	120 °C
Split Ratio	0.2 to 1 typical
Carrier Gas	Helium
Constant Flow Mode	
Columns	**105 m × 0.53 mm × 5.0 µm DB-1, J&W Cat. No. 125-10B5**
Oven Program	
Initial Temperature	35 °C
Initial Time	5 min
Temperature Ramp	25 °C/min
Final Temperature	240 °C
Final Time	5 min
Aux EPC	
Restrictor	Medium flow resistance frits, Part no. 19231-60700



Cylinder codes:
- RG - Refinery gas
- NG1 - High methane natural gas
- NG2 - High ethane natural gas
- LPG - Liquified petroleum gas
- Mix - 8 component sulfur mix
- DMDS Dimethyl disulfide

Figure 2. Point-of-use automated blending system.

previously.[1] Sulfur components and their respective cylinder concentrations in the calibration mix are listed in Table 3. The mix was obtained from DCG Partnership 1, LTD., Pearland, TX, 281-648-1894, The GPA natural gas mixtures were purchased from Scott Specialty Gases.

Table 3. Sulfur Calibration Mix

Number	Compound	Conc.(ppm)
1	Hydrogen sulfide	5.00
2	Carbonyl sulfide	4.96
3	Methyl mercaptan	5.00
4	Ethyl mercaptan	5.04
5	Dimethyl sulfide	4.91
6	Carbon disulfide	5.01
7	t-Butyl mercaptan	5.04
8	Tetrahydrothiophene	5.05

Results

Sensitivity will always be the first and perhaps most important attribute of a selective detector. This should be well understood prior to tackling complex application problems. First, note that the FPD is a non-linear detector due to the mechanism of S2 formation from sulfur atoms in the flame. Excited S2 is responsible for light emission at approximately 393 nm, which gives the detector its selectivity. A comprehensive review of various sulfur selective detectors and applications have been previously discussed.[2] To establish the performance potential of the 6890-FPD system, specifically in terms of sensitivity, a dilution study was conducted where the 8 component mix was systematically diluted in helium to obtain concentrations from 50 ppbv to approximately 400 ppbv. Programming the Aux EPC over a pressure range from 60 psig to 10 psig automatically does this at a sulfur calibration mix flow of 0.9 mL/min. The pressure needed to achieve this mix flow is set from the cylinder regulator. Four methods were setup, each with a different Aux pressure setting, and subsequently used in the ChemStation sequence table. The resulting calibration curve in log-log format for one of the components, ethyl mercaptan, is shown in Figure 3. Figure 4 shows an FPD chromatogram of the sulfur in helium mix at 78 ppbv per component, obtained from one of the methods used in the automatic dilution sequence. Good signal to noise is seen even at this low sulfur level.

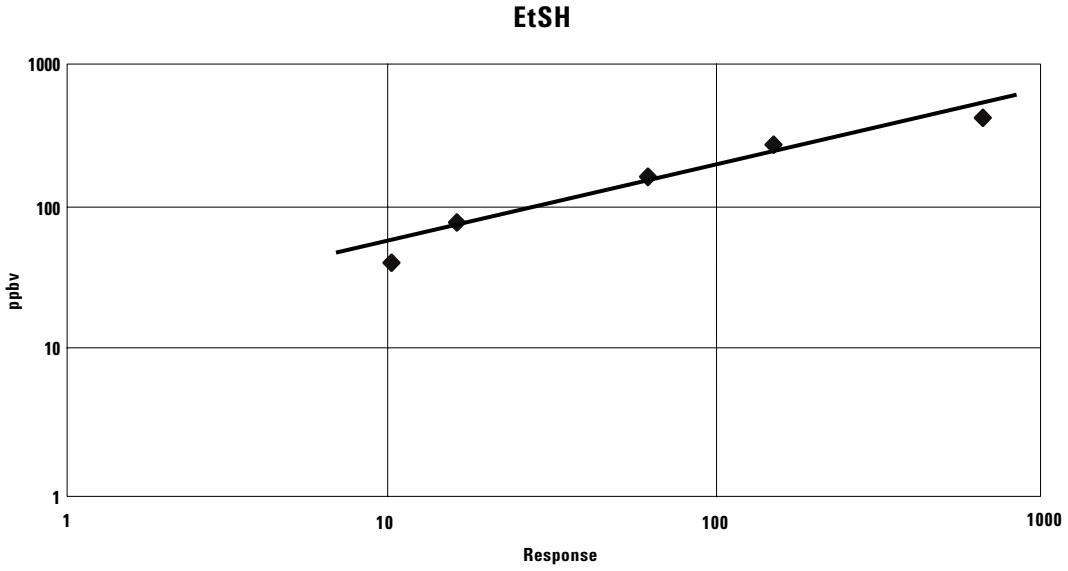


Figure 3. Calibration of ethyl mercaptan in helium from 400 ppbv to 50 ppbv.

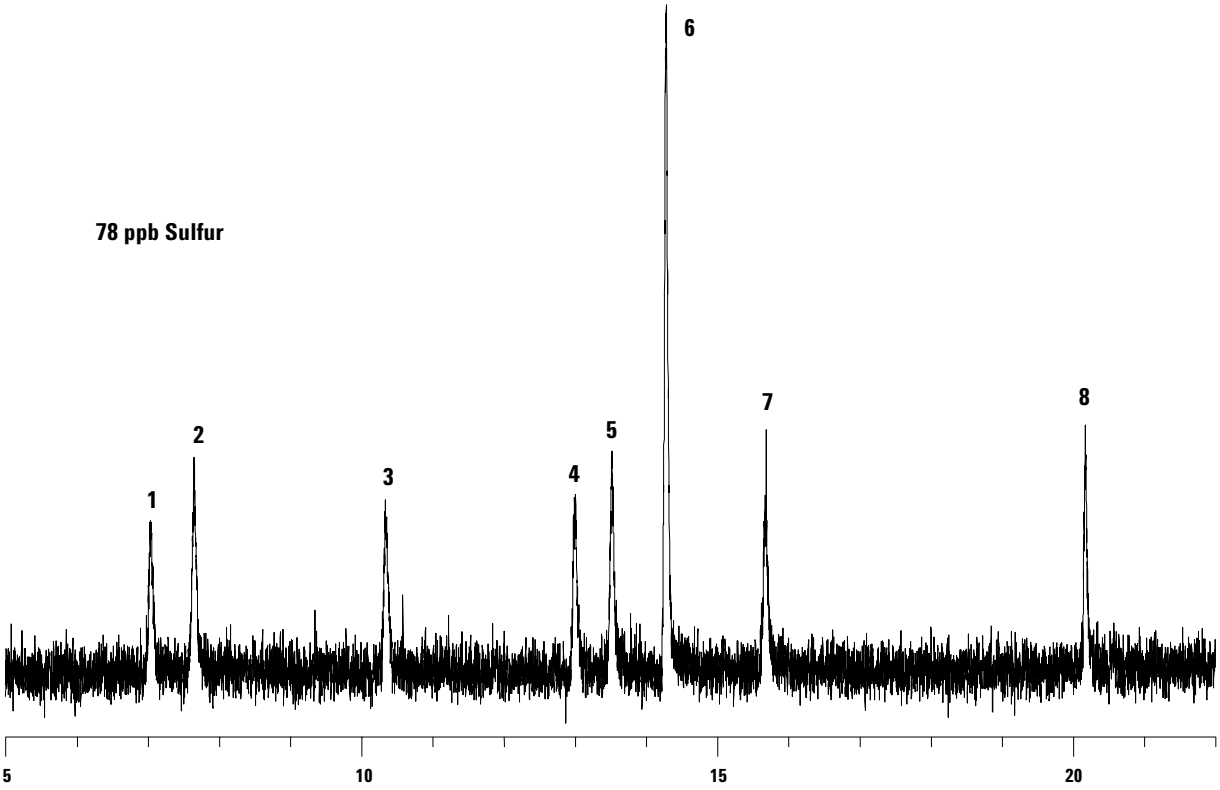


Figure 4. Eight volatile sulfur compounds at 78 ppbv per component. Detector - Flame Photometric. See Table 3 for peak id's.

Prior to use of the FPD, the Agilent atomic emission detector (AED) was used to characterize potential hydrocarbon-sulfur coelutions and false hydrocarbon responses that result when the selectivity of the detector is exceeded. Both carbon and sulfur chromatograms can be collected simultaneously, allowing potential interferences that would lead to signal quenching on the FPD to be quickly identified. Figures 5, 6 and 7 show overlaid sulfur and carbon chromatograms for high ethane natural gas, high methane natural gas, and refinery gas, respectively.

These chromatograms were produced by blending the sulfur mix into the hydrocarbon matrices to produce sulfur levels of 145 ppbv per component. The AED carbon chromatograms shown are due to the hydrocarbon matrix since the contribution of the carbon in the sulfur compounds is exceedingly small.

Coelutions of carbonyl sulfide/propane and methyl mercaptan/t-2-butene are clearly identified. Therefore, in natural gas streams, analysis of low level COS will be problematic on the FPD when using the methyl silicone column. This is not a major limitation since COS is normally not found in natural gas streams beyond the well head. However, most other volatile sulfur compounds found naturally or added as odorants should be quantifiable over the sensitivity range of the detector. For more complex hydrocarbon streams such as refinery gas, the additional coelution of methyl mercaptan/ t-2-butene must be watched.

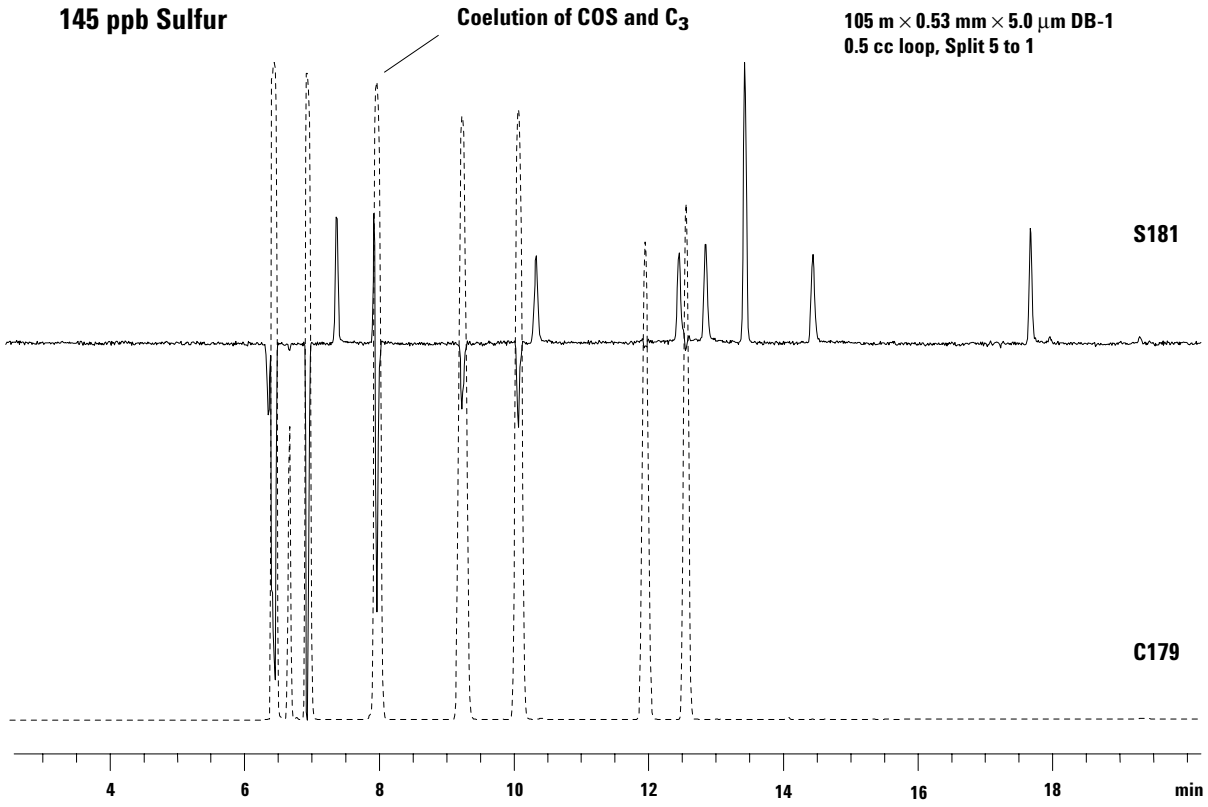


Figure 5. AED carbon and sulfur chromatograms of high ethane (9%) natural gas blended with the eight component sulfur mix. Dashed line is carbon. The carbon and sulfur chromatograms are not drawn to the same scale.

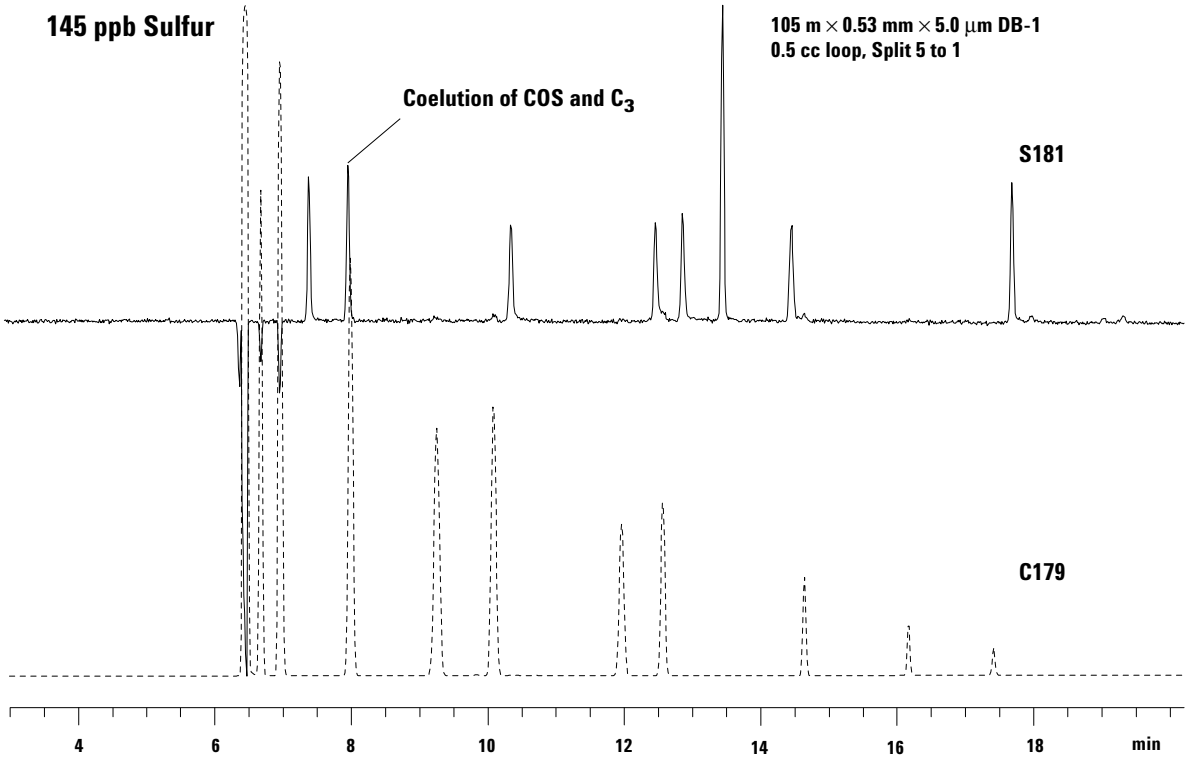


Figure 6. AED chromatograms of high methane natural gas blended with the sulfur calibration mix. Dashed line is carbon.

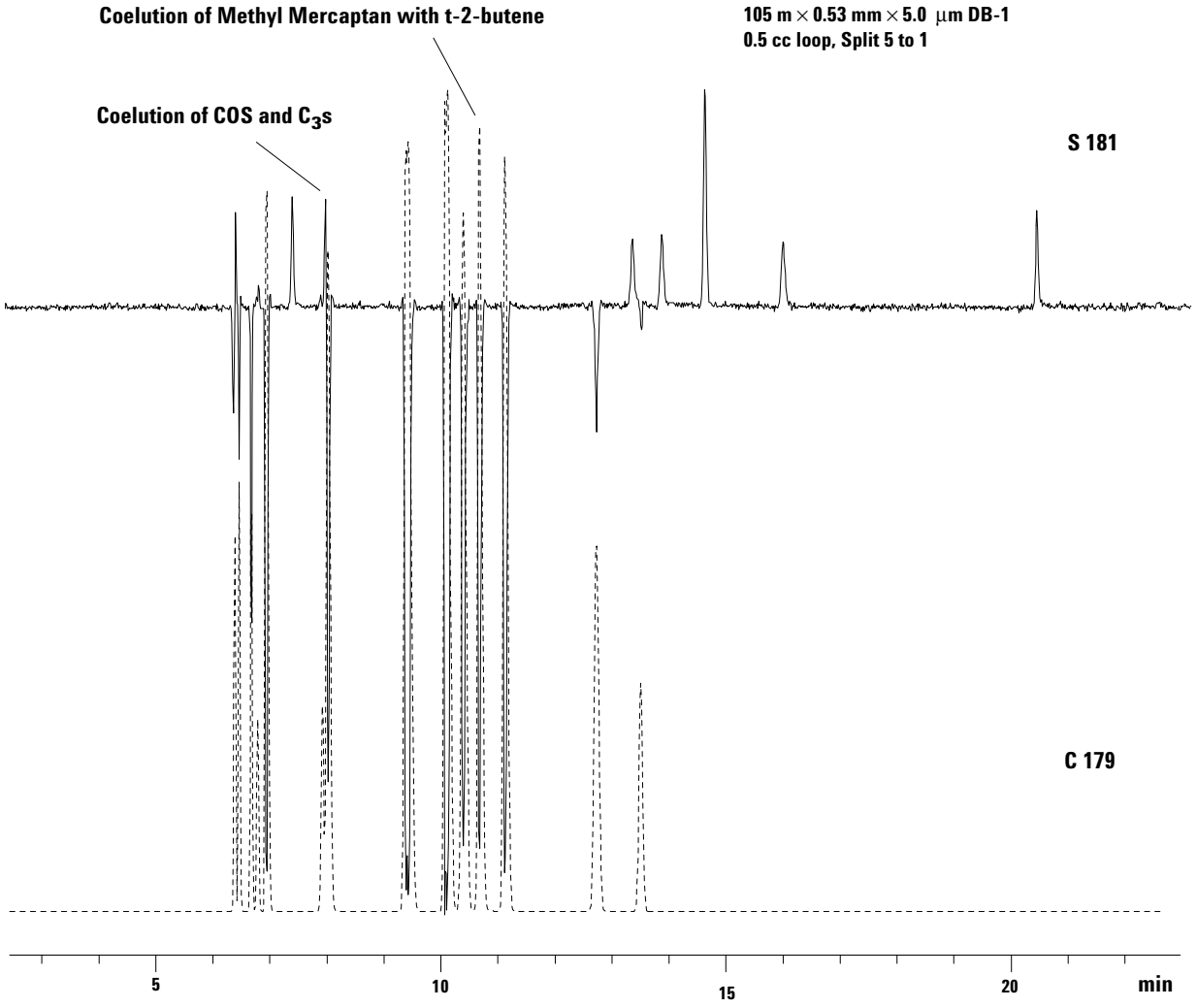


Figure 7. AED chromatograms of refinery gas blended with the sulfur calibration mix. Dashed line is carbon.

Once potential interferences have been characterized, the dynamic blending system can be used to mix various hydrocarbon matrices with the sulfur calibration mix to simulate real world samples and analytical problems. With this information in hand, the method developer and routine user can confidently use the system to identify and quantify a variety of low-level volatile sulfur compounds.

Examples of sulfur compounds blended with high methane (2 ppm and 410 ppb/sulfur compound) and high ethane natural gas (120 ppb/sulfur compound) are shown in Figures 8 and 9, respectively. The upper chromatogram in Figure 8 shows sulfur components at 2 ppm in high methane natural gas. This is representative of a typical range of odorant addition. Only COS cannot be reliably quantified at these levels due to quenching. All common natural gas odorants are cleanly separated from hydrocarbons and should be easily quantified with the FPD.

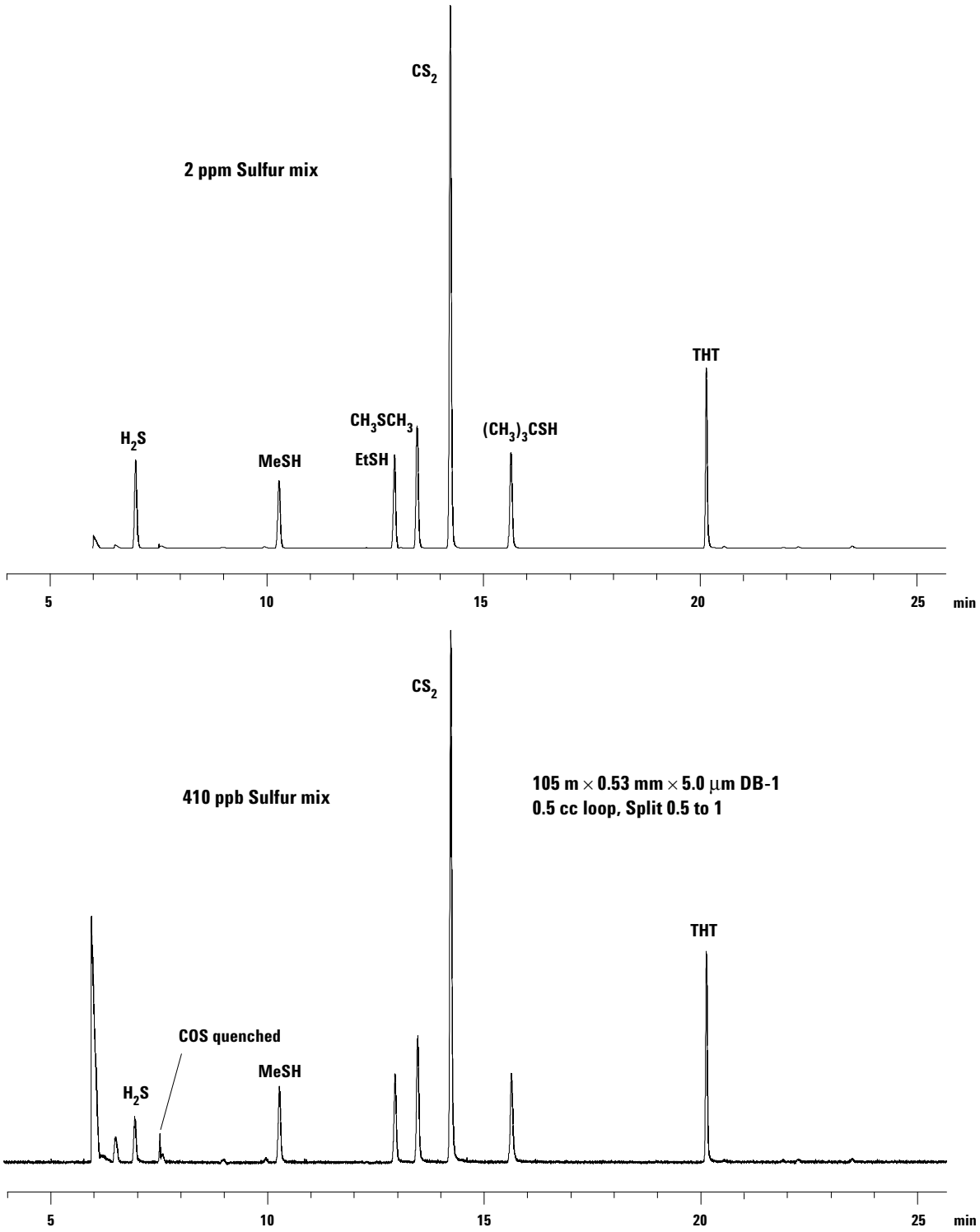


Figure 8. Sulfur mix blended with high methane natural gas at 2 ppm and 410 ppb. Detector - Flame Photometric.

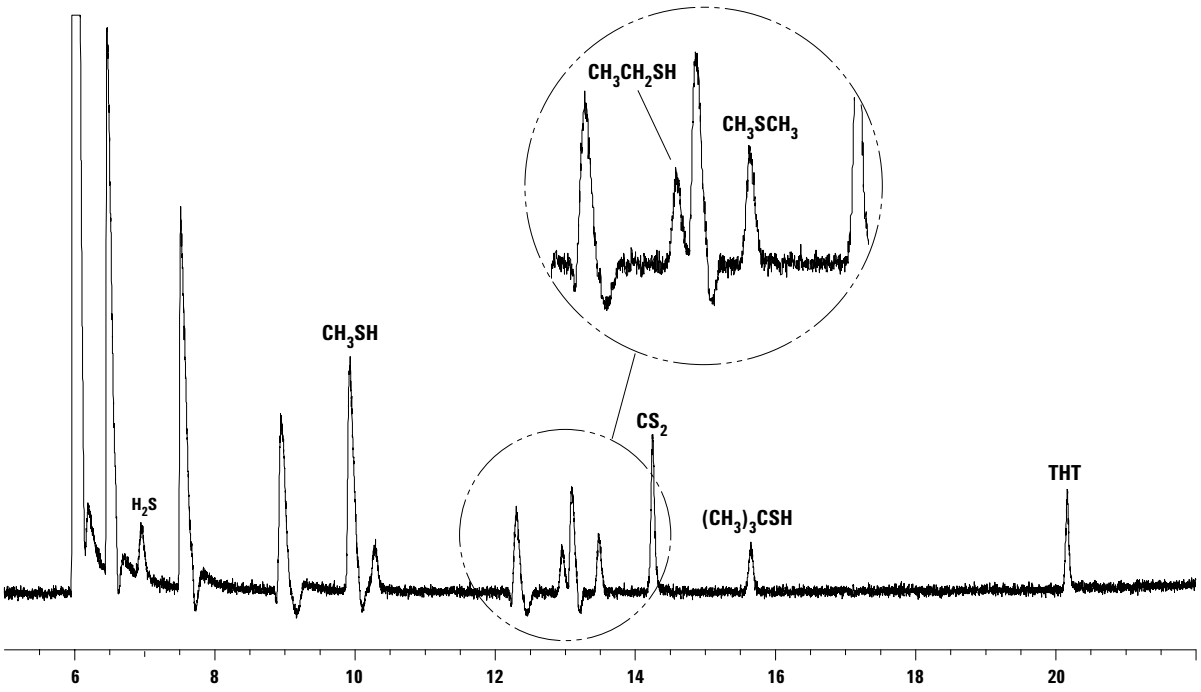


Figure 9. Sulfur mix blended with high ethane natural gas. Sulfur level: 120 ppb. Detector - Flame Photometric.

Refinery gas presents a more challenging matrix from potential sulfur coelutions with the relatively large number of C4 and C5 isomers. In Figure 10, 120 ppb sulfur mix in a refinery gas qualitative standard is shown. From the AED work, measurement of COS and CH_3SH at these 100 ppb sulfur levels is expected to be erroneous. Peaks labeled 1, 4, 5, 6, 7, and 8 (see figure) identifies the six sulfur compounds from among the false hydrocarbon response peaks. These six sulfur species can be easily quantified.

The last example shown in Figure 11, illustrates the measurement of sulfur in LPG. Ethyl mercaptan, the most common odorant used in LPG is seen at approximately 2.5 ppm. The presence of methyl mercaptan seen at approximately 10 minutes RT may be naturally occurring in origin. Two peaks at 20.5 and 22.0 minutes are unidentified sulfur compounds.

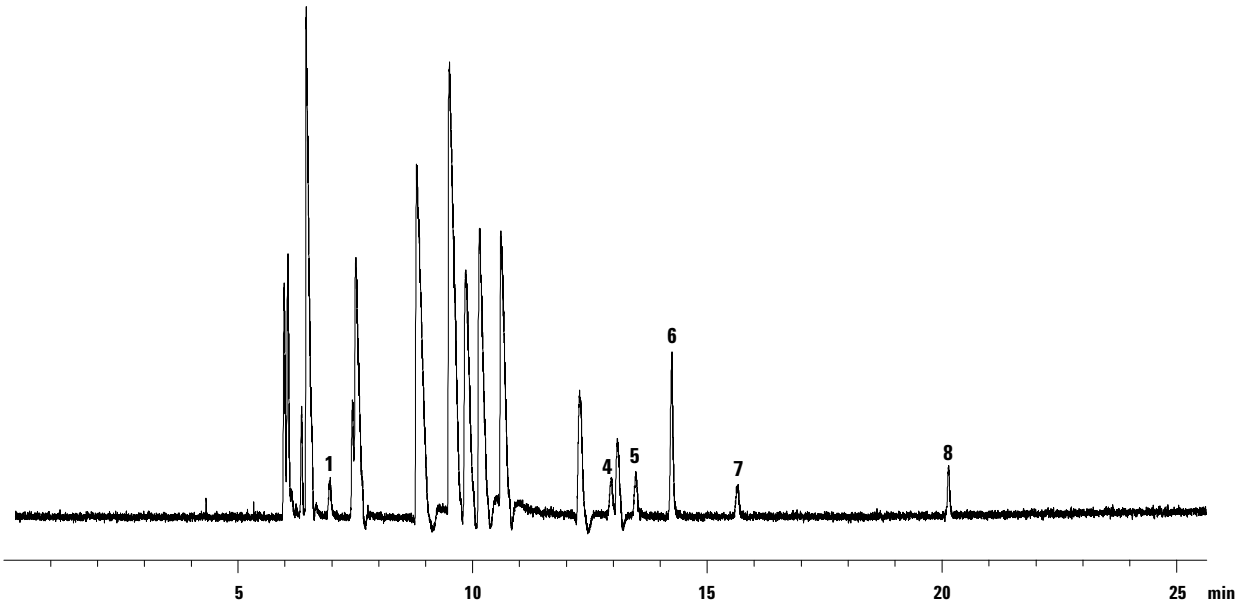


Figure 10. Sulfur mix blended with refinery gas. Sulfur level: 120 ppb. Detector - Flame Photometric. See Table 3 for peak id's.

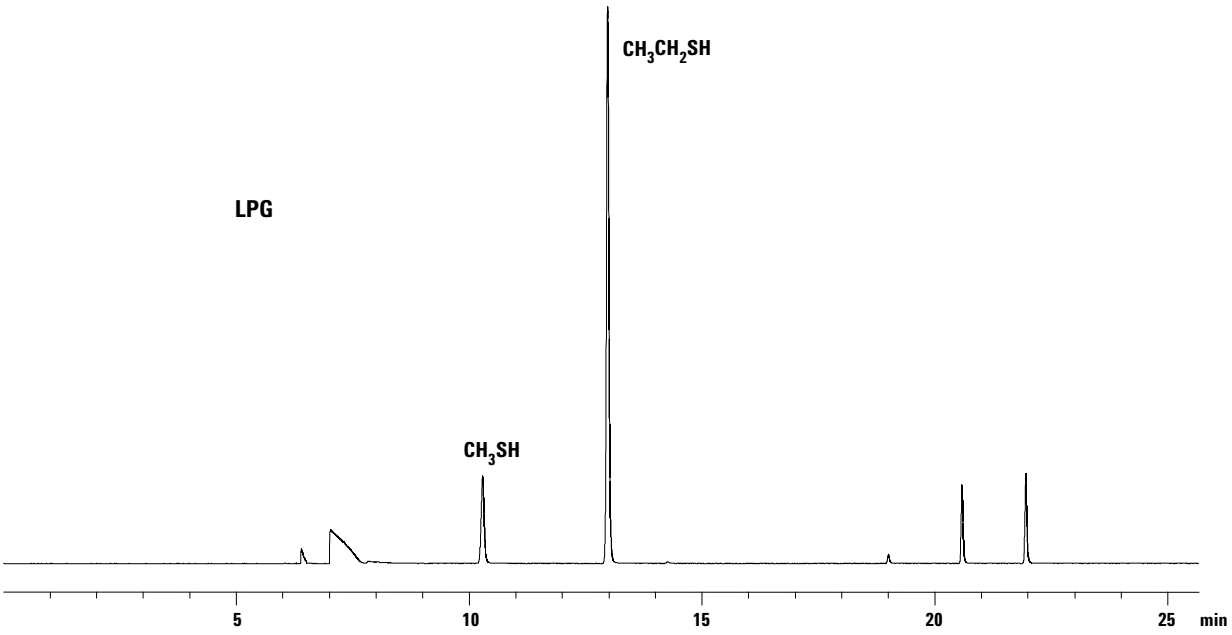


Figure 11. FPD analysis of LPG showing ethyl mercaptan at 2.5 ppm.

Conclusions

Many sulfur selective detectors cannot be characterized as easy to use or low maintenance instruments. The FPD is an exception to this rule with setup, operation, and stability on par with a standard FID. When the gas chromatograph is carefully configured with inert sample path components and optimized sample introduction hardware, reliable routine detection of volatile sulfur components under 50 ppb is achievable. Although the FPD is subject to quenching by high hydrocarbon concentrations, careful selection of the column can largely eliminate the problem. The 105 m × 0.53 mm × 5.0 µm DB-1 offers a high level of inertness, capacity, and efficiency for volatile sulfur analysis. Dynamic blending, controlled by the Aux EPC offers an automatable means of system calibration.

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2. R. L. Firor and B.D. Quimby, *A Comparison of Sulfur Selective Detectors for Low Level Analysis in Gaseous Streams*, Publication Number 5988-2426, April 2001 (Downloadable from www.agilent.com)

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5988-2791EN



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Use of GC/MSD for Determination of Volatile Sulfur: Application in Natural Gas Fuel Cell Systems and Other Gaseous Streams

Application

Fuel Cells, Petrochemicals

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Abstract

The mass selective detector is an ideal tool for the analysis of trace level volatile sulfur compounds. It is differentiated from other sulfur-selective detectors in that structural information is provided. When operated in the Selected Ion Monitoring mode, excellent sensitivity and selectivity is obtained. Eight volatile sulfur compounds are used to demonstrate low parts-per-billion analysis in a variety of hydrocarbon matrices. The system is well suited for fuel cell developers for characterization of fuel feedstocks and the analysis of impurities that can poison critical catalytic processes. Measurement of carbonyl sulfide in propylene is also demonstrated.

Introduction

Sulfur detectors are finding widespread use in a broad range of applications that cut across many industries. Demand for low-level sulfur detection will only increase in the future in response to more stringent quality control and regulation. The significance and need for low level sulfur measurements have been detailed in previous Agilent application literature [1, 2, 3, 4].

Emerging needs are found in alternative energy applications such as the fuel cell industry. Fuel processors serve a vital role in many fuel cell systems and are sensitive to feedstock composition and impurities. Potential fuels include hydrogen, natural gas, propane, methanol, gasoline and other hydrocarbon streams. Near-term development is concentrating on reformed hydrocarbon fuels creating a need to monitor composition and impurities. Fuel contaminants can adversely affect performance of the fuel cell system. This is especially true for Polymer Electrolyte Membrane (PEMFC) and Molten Carbonate (MCFC) types, although Phosphoric Acid (PAFC) and Solid Oxide (SOFC) technologies are also subject to sulfur poisoning. For example, natural gas feeds to external or internal catalytic reformers need to be desulfurized since low ppm sulfur levels can poison the reformer catalyst and fuel cell stack. Potential breakthrough of sulfur from the desulfurization beds needs to be closely monitored.

The mass selective detector (MSD) is usually not considered when the need for low-level volatile sulfur quantitation and speciation arises in the analytical laboratory or plant. Selective detectors such as the flame photometric (FPD), pulsed flame photometric (PFPD), and sulfur chemiluminescence (SCD) have traditionally dominated these applications [1]. The 6890N/5973N GC/MSD system is a very capable alternative to these detectors providing the added benefit of positive compound identification. Details on how to set up the

system for optimum sensitivity and selectivity are discussed in this paper. The specific hardware configuration reviewed is applicable to a wide range of applications.

MSDs are now widely used in many routine applications including QA/QC environments. The 5973N is easy to use, compact in size, and stable over long time periods. Tuning is software controlled and automatic, a significantly easier task than what is needed for some sulfur-selective detectors.

A common problem with many sulfur-selective detectors is hydrocarbon interference, especially from chromatographic coelution [4]. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene. In most cases, an accurate determination of the sulfur compound is not possible. However, the use of the MSD and selected ion monitoring (SIM) can largely overcome the coelution problem for many applications.

Experimental

Networked versions of the 6890 and 5973, designated by the N following the product number were used in this work; replacing the previously HPIB-interfaced models. Well known benefits of LAN include reliability, lack of distance limitations and simple configuration.

The sulfur calibration mix consisted of the following components at 5 ppm each: Hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, carbonyl sulfide, t-butyl mercaptan, and tetrahydrothiophene. The blend in helium was purchased from DCG Partnership, Pearland, TX.

A 6-port gas-sampling valve was connected directly to the volatiles interface on the 6890N with Sulfinert 1/16-inch tubing. See the sample introduction diagram in Figure 1. The sample loop, tubing and inlet are either Sulfinert or Silcosteel treated for inertness. Table 1 contains the instrument conditions.

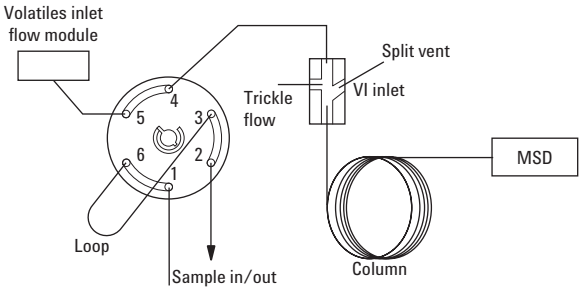

Figure 1. Sample introduction scheme.

Table 1. Instrument Conditions

Injection port	**Volatiles interface**
Temperature	150 °C
Split ratios	1 : 1 up to 50 : 1
Carrier gas	Helium
Constant flow mode	1.9 mL/min
Injection source	**6-Port sampling valve**
Material	Hastelloy C
Temperature	150 °C
Loop	Sulfinert, 0.5 cc
Column	**60 m × 0.320 mm × 5.0 µm DB-1**
Initial temperature	40 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	270 °C
Final time	2 min
5973 MSD	
Mass range	33 to 100 and 12 to 100
Scans	13.1/sec and 15.9/sec
Samples	2
Threshold	150
EM voltage	BFB.u tune voltage
Solvent delay	3 min
Source temperature	230 °C
Quad temperature	150 °C
Transfer line	280 °C

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases are mixed with the calibration standard using an Aux EPC module on the 6890N GC. This system and the hardware employed were described in detail previously [2].

Positioning of the column in the MSD must be carefully done to avoid loss of sulfur sensitivity. To position the column just inside the source, 2 to 3 millimeters of the column should be visible at the MSD end of the transfer line. See reference 5 for installation details.

Results and Discussion

System Calibration

First, the system was calibrated and checked for linearity by analyzing the sulfur mix at various concentrations. The dynamic blending system was used to prepare seven and five level calibrations using helium and natural gas as diluents, respectively. Table 2 lists the concentrations used. Calibrations were focused in the ppb range since this is where most analytical problems for sulfur analysis are found. SIM acquisition mode, discussed later in this section, was used.

Table 2. Calibration levels for checking system linearity. Sulfur concentrations in ppbv.

Cal Level	1	2	3	4	5	6	7
Conc. in helium	21	35	46	57	95	1600	3600
Conc. in nat gas	88	242	475	880	1170	--	--

Calibrations are linear in both matrices for all eight sulfur compounds. This is seen in Table 3 where the regression coefficient, r^2 values appear. This is an indication not only that the system response is linear but also that calculated concentrations from the blending system are accurate. Unlike some sulfur-selective detectors, the MSD does not show equimolar response. Each compound will have its own response characteristics, requiring each component's response factor to be determined.

Table 3. Calibration Regression Coefficient r^2 Values

Compound	Helium	Natural gas
H_2S	0.998	0.998
COS	0.998	0.999
CH_3SH	0.997	0.999
EtSH	0.996	0.998
DMS	0.998	0.998
CS_2	0.998	0.998
t-ButylSH	0.996	0.993
THT	0.996	0.992

One of the calibration plots as produced by the MSD Chemstation is shown in Figure 2 for the calibration of H_2S in natural gas.

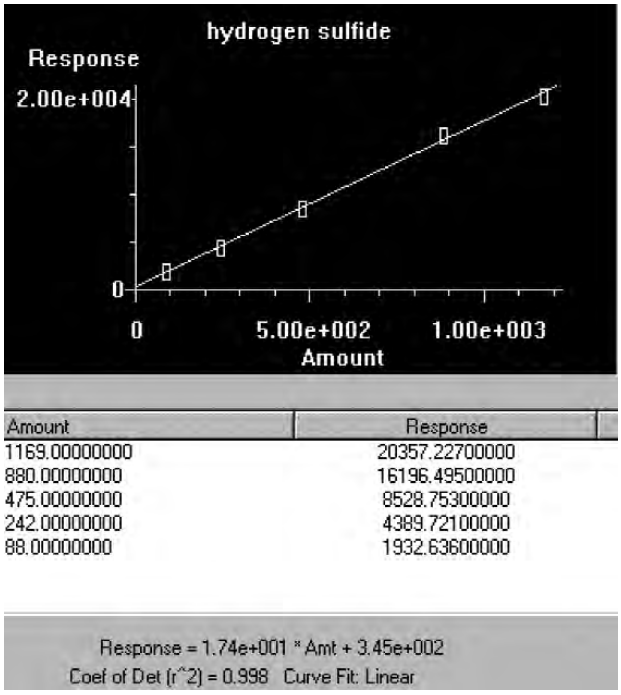

Figure 2. Five level calibration plot of H_2S using natural gas as the diluent. Calibration range is from 88 to 1170 ppb.

Scan Results

The total ion chromatogram (TIC) of the eight-component sulfur mix at 1.3 ppm in helium is shown in Figure 3 using a split ratio of 0.5 to 1. As is evident in the figure, H_2S is close to the minimum detectable limit (MDL) for this particular set of operating conditions. While operating in scan mode is useful for initial method development, unknown identification and retention time determinations, use of extracted ions from a scan and/or SIM are required to improve overall sensitivity and selectivity.

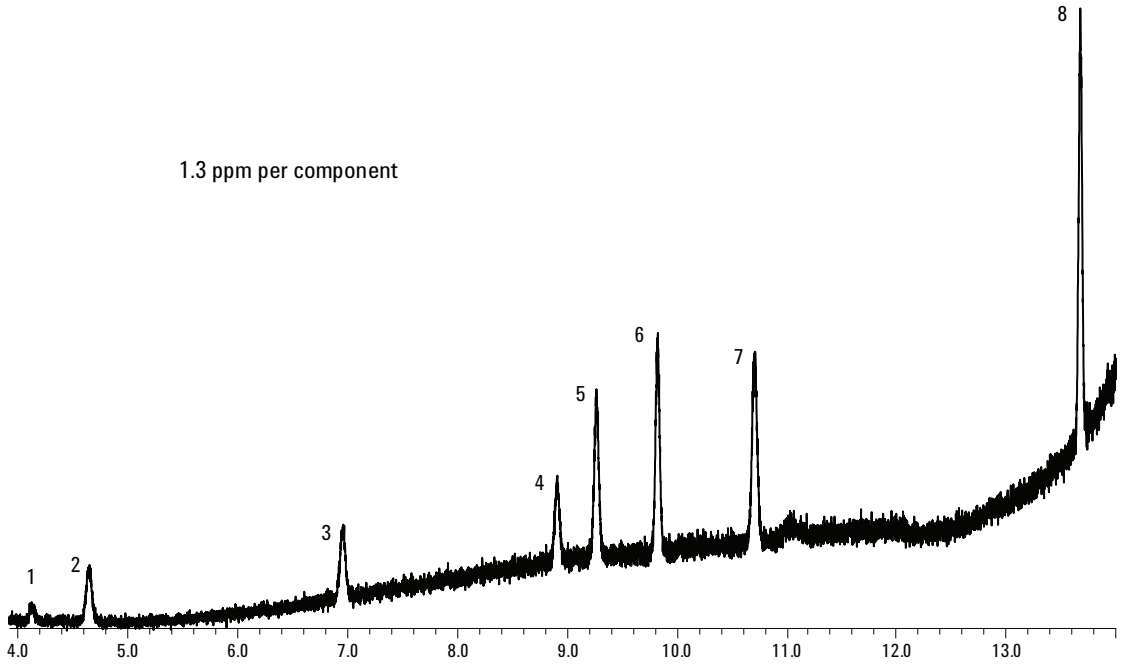

Figure 3. Total ion chromatogram of the eight-component sulfur mix at 1.3 ppm per component. Scan 33-100 amu. Peak labels: 1. hydrogen sulfide, 2. carbonyl sulfide, 3. methyl mercaptan, 4. ethyl mercaptan, 5. dimethyl sulfide, 6. carbon disulfide, 7. t-butyl sulfide, 8. tetrahydrothiophene.

Extracted Ion Results

In Figure 4, extracted ion chromatograms are shown for ions 60 and 62. Three of the eight sulfur compounds are found with these target ions. Ion 60 is present in COS and tetrahydrothiophene, and ion 62 is found in ethyl mercaptan and dimethyl sulfide. The concentration of the sample was 86 ppb per component in helium. Extracted ion chromatograms for the other sulfur compounds show similar signal to noise ratios at the concentration level. A considerable improvement in sensitivity is achieved by using extracted ions. In cases where this does not provide sufficient sensitivity, the next step should be SIM.

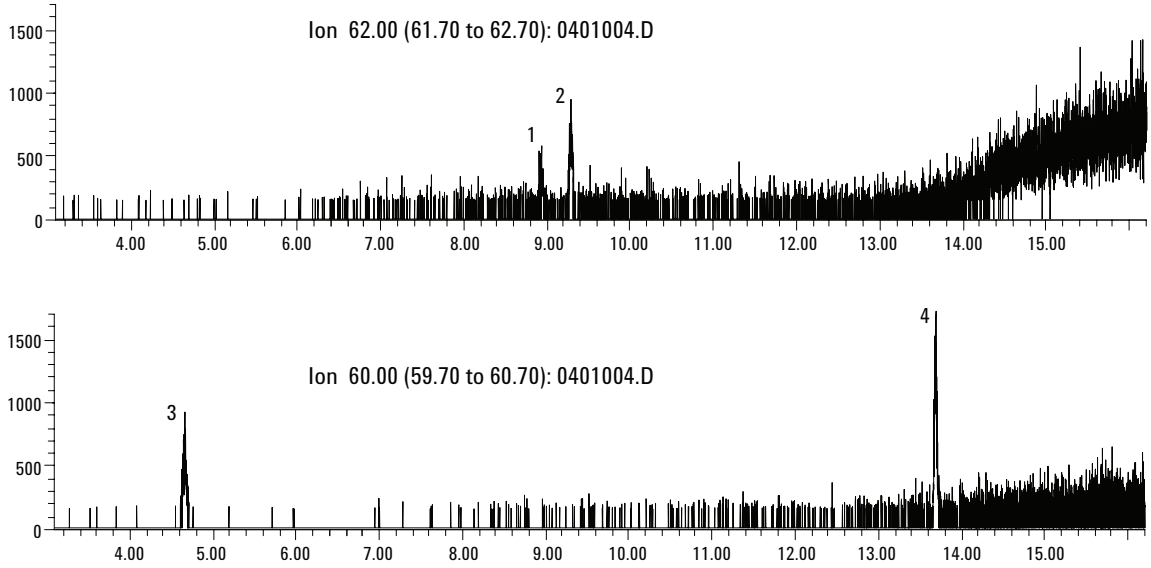

Figure 4. Extracted ion chromatograms for ions 60 and 62. Concentration is 86 ppb per component. Split ratio 1:1. Peak labels: 1. EtSH, 2. DMS, 3. COS, 4. THT.

Application of SIM

SIM provides superior sensitivity and selectivity. Since sulfur determinations will normally be done in hydrocarbon matrices, care must be taken to select ions that ideally have no hydrocarbon contribution. If this can be done, excellent selectivity can be achieved even in cases where coelution of sulfur species and hydrocarbon occur. This is an important distinction and advantage of the MSD compared to some of the common gas chromatographic sulfur-selective detectors. Both the FPD and PFPD will suffer from quenching if coelution occurs making accurate quantitation of low-level sulfur impossible [2]. Even the SCD will have problems measuring low ppm sulfur in the presence of a dominant coeluting hydrocarbon. In situations where a unique sulfur ion cannot be found, refinement of the method and chromatographic column/conditions to achieve separation from the interfering hydrocarbon should be tried [2]. Also, when operating the MSD in SIM mode, it is usually best to select low resolution for maximum sensitivity at the expense of some mass selectivity loss.

The SIM ions used for each sulfur compound are listed in Table 4. These ions were chosen to minimize interference from hydrocarbons. To arrive at the ions shown in the table, a scan of the sulfur mix in helium is acquired to identify target ions. Library spectra can also be consulted. Hydrocarbon mixes such as natural gas and refinery gas are then run separately using the SIM table to look for ions that may match those selected for the sulfur. The table may be further refined if hydrocarbon interferences appear. Retention times of the sulfur compounds are also needed to set up the time-programmed groups. These are not the only possible ions that can be used. For some of the compounds other choices or additional ions could be included in the SIM table. While not necessary for this relatively simple sulfur example, the use of second and third qualifier ions may give the analyst a higher level of confidence of a compound's identity by comparing ion ratios to library spectra for a particular compound.

Table 4. Optimized SIM table for selective sulfur detection in hydrocarbon streams. Dwell time for each ion is 100 ms.

GROUP	START TIME (min)	TARGET and QUALIFIER IONS	COMPOUND
1	3.00	33,34	H_2S
2	4.20	60	COS
3	6.00	45,47	MeSH
4	8.00	47	EtSH
5	9.10	45,47,62	DMS
6	9.70	44,76	CS_2
7	10.20	57,90	t-ButylSH
8	11.80	45,60,88	THT

Fuel Cell Natural Gas Feedstocks: Composition and Impurities

The TIC of a natural gas scan and sulfur mix SIM runs are overlaid for illustration purposes in Figure 5. Note that with the 60 m × 0.32 mm × 5.0 µm DB-1 column, all hydrocarbons and CO_2 are separated. Natural gas compounds in order of elution are: O_2/N_2, CH_4, CO_2, ethane, propane, i-butane, n-butane, i-pentane, and n-pentane. From the overlay, it can be seen that seven of the eight sulfurs do not coelute with natural gas components; only COS and propane show potential overlap. This also demonstrates the utility of the system for fuel cell feed streams, providing both hydrocarbon composition and gas impurity analysis.

The chromatogram shown in Figure 6 of the sulfur mix in helium was produced using the SIM parameters in Table 3. The offsets seen in the baseline are a result of the MSD switching from group to group and should not be interpreted as a chromatographic problem. Excellent signal to noise is seen for all components at the 46 ppb level. The sulfur mix was then further diluted to 16 ppb per component. The resulting chromatograms for H_2S and COS, the most challenging analytes, and tetrahydrothiophene are shown in Figure 7.

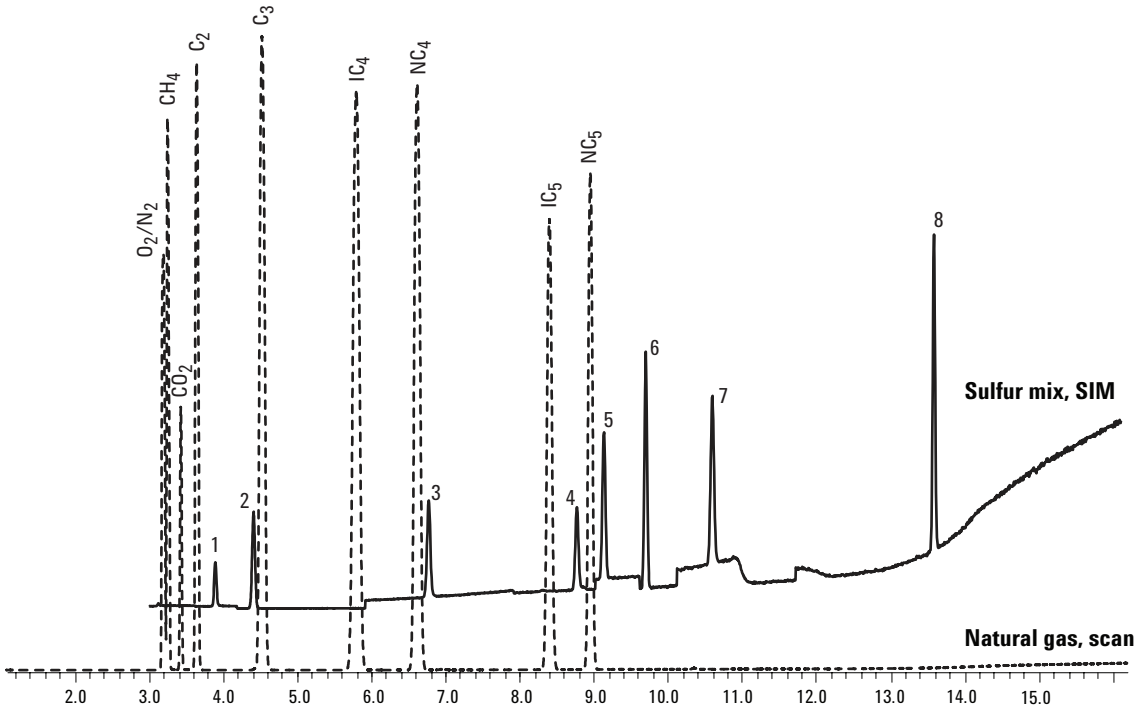

Figure 5. Overlay of two runs: natural gas scan (12 to 100 amu) and sulfur mix at 4.5 ppm in SIM mode. Split ratio 20:1. Sulfur peak labels same as in Figure 3.

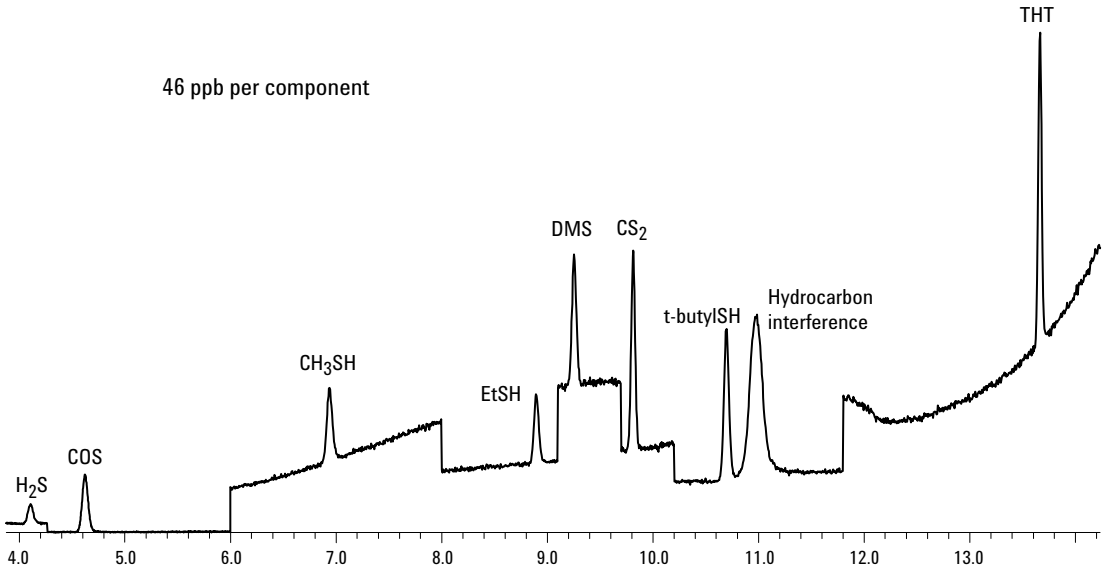

Figure 6. Eight-component sulfur mix in helium at 46 ppb per component in SIM mode. Split ratio 0.5:1.

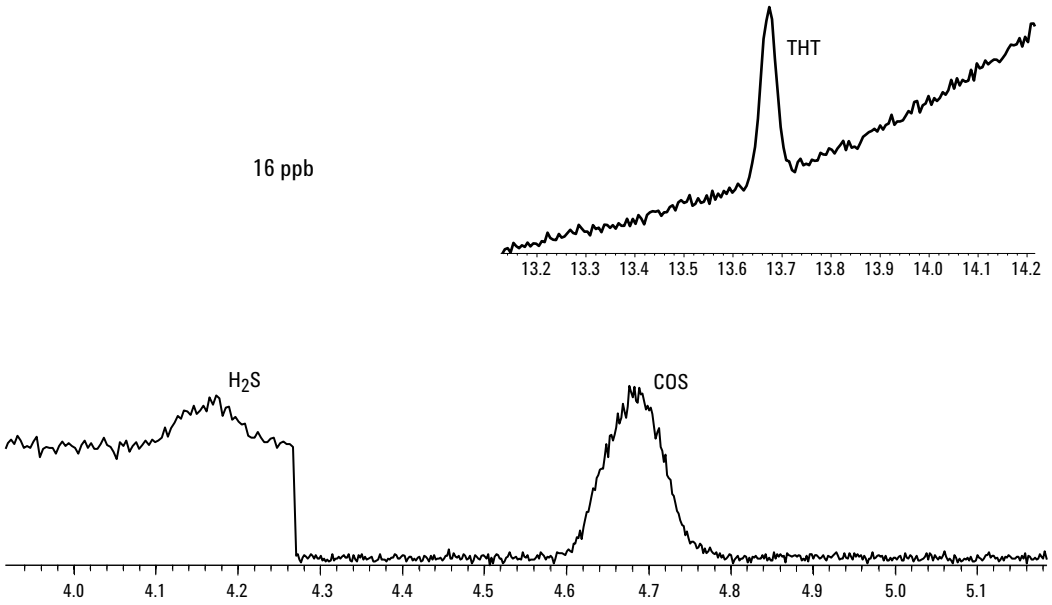

Figure 7. H_2S, COS and tetrahydrothiophene (insert) at 16 ppb each. SIM was used.

For ppb sulfur analysis, it is recommended that the pure matrix be run separately using the sulfur SIM acquisition parameters. Ideally, no response would be seen. If ions of the hydrocarbon matrix are seen, they can be noted and not mistaken for sulfur compounds. This is illustrated in Figure 8 for natural gas streams. Chromatograms of the sulfur mix in scan mode and pure natural gas in SIM mode are overlaid for illustrative purposes. Both are drawn to the same scale. This is a good practice to follow not only for sulfur but also for any impurity analysis using SIM.

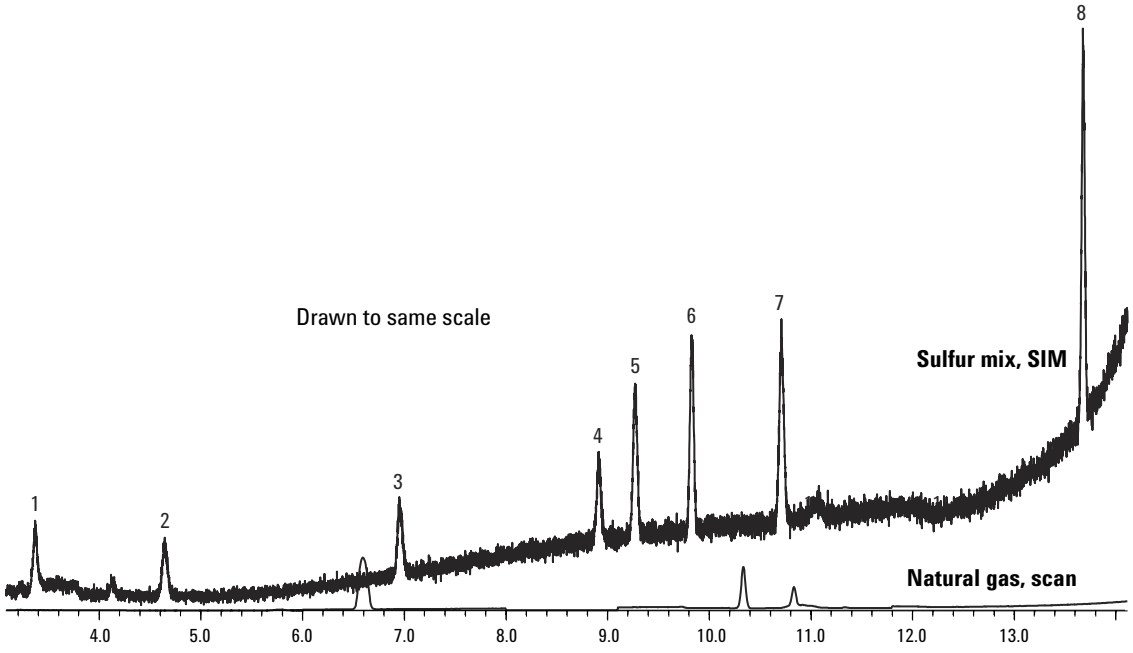

Figure 8. Overlay of sulfur mix in scan (33-100 amu) and natural gas (using sulfur SIM table). Ideally the natural gas chromatogram would be blank. Same scale used for both.

Analysis of COS in Propylene and Propane

Measurement of ppb COS in propylene and propane can be a challenging analytical problem due to coelution of COS/propylene on the preferred methyl silicone columns. This coelution is illustrated in Figure 9 where two independent (separate runs) are overlaid. Both the FPD and PFPD will be unsuccessful with this analysis due to quenching. The SCD's selectivity can also be exceeded for low ppb COS analysis

SIM (ion 60) was employed for the analysis of COS. To avoid overloading the source, the split ratio was increased to 50:1. To determine the effect of coeluting propylene on COS response, two runs were performed at identical concentrations of 105 ppb COS. The diluents for the first and second runs were helium and propylene, respectively. Chromatograms for both runs are shown in Figure 10. The helium chromatogram shows the true COS area unaffected by any other coeluting compound. This area is then compared to that of COS in propylene diluent using the area ratio (COS propylene/COS helium) to indicate how coelution has affected the MSD response. This ratio of 0.77 indicates that COS in propylene response is suppressed by only 23%, probably due to a reduction in ionization efficiency. Moreover, a subsequent experiment that constructed a five level calibration of COS in propylene showed linear behavior over the range of 20 to 1200 ppb. Therefore, using a carefully constructed SIM method, the MSD has the capability of quantifying ppb level COS in coeluting 99+% propylene. It follows, in the general case, that coeluting analytes do not preclude quantification even when concentration differences exceed 10^5 provided unique ions can be identified for the component of interest.

These results and conclusions are relevant to fuel cell developers who are using high propane (for example 50 to 99%) as a feedstock. The performance, chromatographic behavior, and minimal detectable impurity levels will be very similar. Under the conditions used the retention time of propane will differ by less than 0.1 minute from propylene (see propane retention time in Figure 5). Sulfur impurities other than COS can be easily measured.

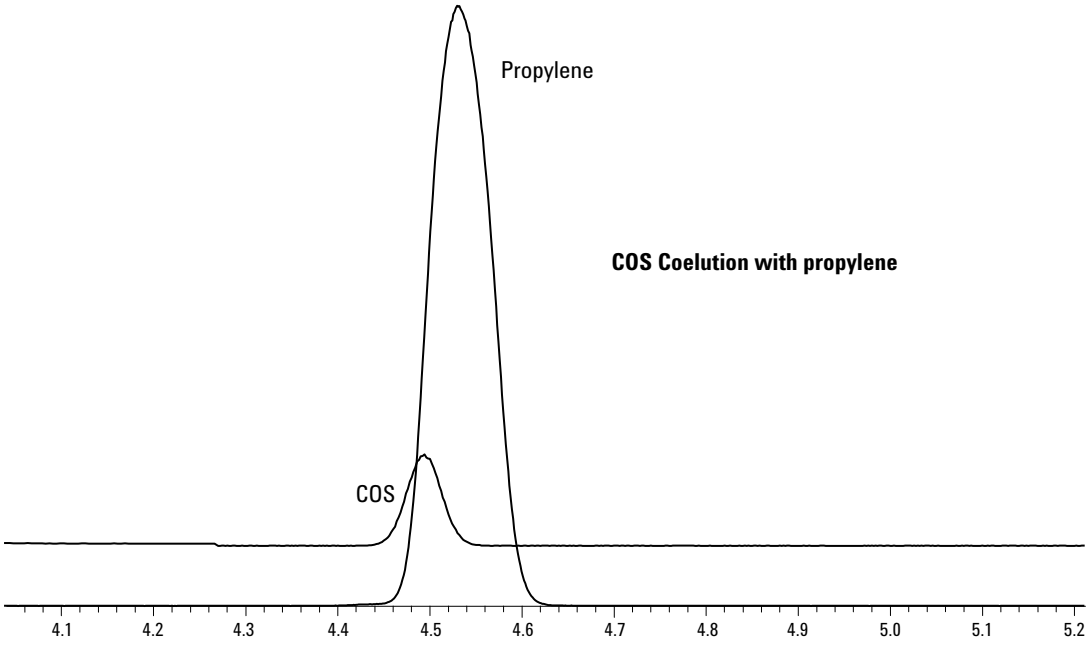

Figure 9. Two separate chromatograms (from separate runs) superimposed showing the coelution of COS with propylene. Split ratio 50:1.

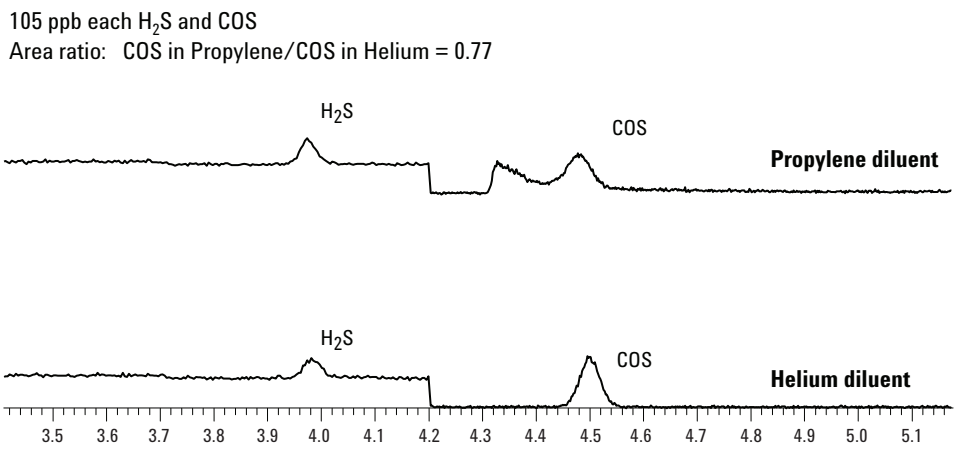

Figure 10. Comparison of COS response (SIM mode) in helium and propylene. Split ratio 50 to 1.

Conclusions

The hardware and associated methods outlined in this paper demonstrate the MSD's capabilities as a sensitive and selective detector for gaseous analytes. It has the added advantage of providing structural information. Sulfur detection at low ppb levels is easily achieved through use of a time programmed SIM table consisting of unique ions for the compounds of interest. This minimizes hydrocarbon interference making it possible to quantitate low-level analytes such as COS with coeluting propylene.

The 6890N/5973N system is also a powerful tool for fuel cell developers, providing detailed composition and impurity analyses of common fuels. The examples shown here demonstrate how natural gas feed could be characterized providing complete speciation of sulfur compounds including odorants or naturally occurring impurities such as H_2S. The system can also be used to monitor the performance of desulfurization beds and reformer output.

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Printed in the USA
November 14, 2001
5988-4453EN



Agilent Technologies

Dual-Channel Gas Chromatographic System for the Determination of Low-Level Sulfur in Hydrocarbon Gases

Application

Hydrocarbon Processing

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Abstract

A 6890N equipped with dual flame photometric detectors is described for the analysis of ppb level volatile sulfur compounds in a variety of hydrocarbons using thick film DB-1 and GS-GasPro columns. Enhanced performance flame photometric detectors are employed that can achieve detection of sulfur compounds below 20 ppb. Examples of arsine and phosphine analysis with the same hardware are also discussed.

Introduction

Gas chromatography with sulfur selective detection is finding widespread application in many segments of the petroleum, petrochemical, and specialty chemical industries. Demand for low-level sulfur detection will increase in the future in response to more stringent regulations and tighter quality control.

Sulfur compounds can be significant poisons for various catalytic processes involved in hydrocarbon conversion. Monitoring these low-level poisons can lead to considerable saving in terms of improved yields, increased catalyst lifetime, and higher quality products. In looking at the future of fuel cells, fuel contaminants can adversely affect performance of fuel cell systems and fuel processors that are powered by natural gas or other fossil fuels. Finally, environmental regulatory issues in certain regions will continue, necessitating the need to monitor fuel impurities.

A common problem with many gas chromatographic sulfur selective detectors is hydrocarbon interference, especially from co-elution. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene, or sulfur in natural gas [1, 2]. In most cases, an accurate determination of the sulfur compound is difficult or not possible even with highly selective sulfur detectors. However, the use of a dual-channel system employing two very different separation columns (in terms of selectivity) largely avoids the interference problem. The configuration is shown in Figure 1. Sulfur compounds that have a severe interference on one column are likely to be separated from that interference on the other column. By assuring that a given sulfur compound will be separated on at least one of the columns, the system can use a reliable, stable, and relatively inexpensive flame photometric detector (FPD) for detection. If the hydrocarbons can be chromatographically separated from the sulfur compounds of interest, enhanced FPDs can quantitate sulfur to less than 20 ppb.

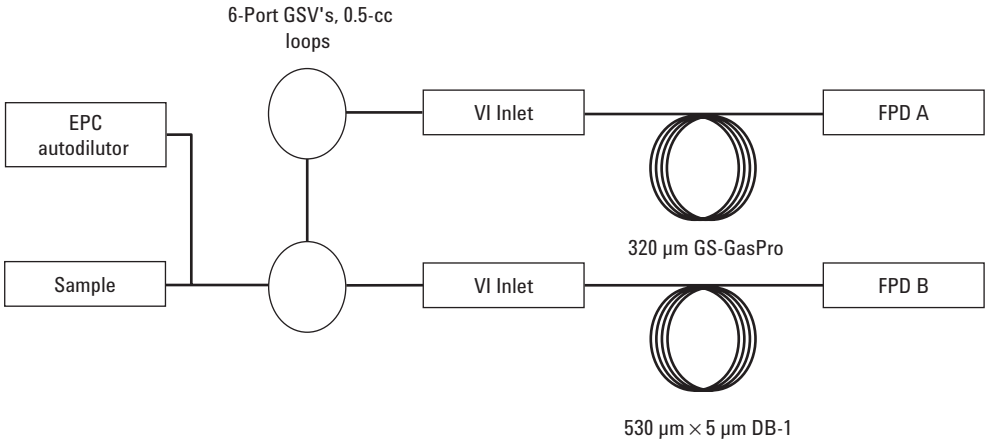

Figure 1. System configuration on the Agilent 6890N. Valves (plumbed in series) are Hastelloy C and all plumbing is Silcosteel® or Sulfinert™ treated.

Experimental

Selection of the appropriate capillary column is often key to the solution of a particular analysis problem, and this is especially true for this system. Four columns are employed (two for any given analysis) as described in Table 1.

Table 1. Recommended Column Combinations by Application

Applications	Column set
Natural gas, fuel cell gases	60 m × 530 µm × 5.0 µm DB-1
	30 m × 320 µm GS-GasPro
Ethylene, propylene, C4 streams	105 m × 530 µm × 5.0 µm DB-1
	60 m × 320 µm GS-GasPro

Recommended GC oven programs are 40 °C (5 min) to 290 °C (5 min) at 25 °C/min for natural gas, fuel cell gases and ethylene, and 35 °C (7 min) to 290 °C (5 min) at 20 °C/min for propylene. Somewhat lower detection limits can be achieved for sulfur in a propylene stream by employing cryo oven programs such as: –35 °C (7 min) to 290 °C (5 min) at 20 °C/min. Split ratios, as set in the GC method, vary from 0.5:1 to 2:1.

Each valve was interfaced to a specialized inert (Silcosteel treated) volatiles interface for accurate sample introduction at low split ratios into a capillary column. Due to the tendency for organosulfur compounds (especially H_2S) to adsorb to metal surfaces, great care must be used in selecting and constructing the chromatographic sample introduction system. The sample loop, tubing, and inlet are either Sulfinert or Silcosteel treated for inertness.

A factory modified FPD, with enhanced sensitivity, was used for each channel. The FPD is optimized for the analysis of trace sulfur gases, arsine, and phosphine in gaseous samples. See Table 2 for appropriate gas flow settings. These detectors achieve detection limits that are roughly four times better than standard. The sensitivity advantage is illustrated in Figure 2, where standard and modified FPDs are compared using a standard calibration blend. Minimum detection level (MDL) calculated on methyl mercaptan using linearized data and the 60 m DB-1 column is better than 15 ppb.

Table 2. FPD Gas Flow Settings

Analysis	Gas	Flow rate (mL/min)
Sulfur	Air	60
	Hydrogen	50
	Makeup	58
Arsine	Air	150
	Hydrogen	50
	Makeup	100
Phosphine	Air	110
	Hydrogen	150
	Makeup	58

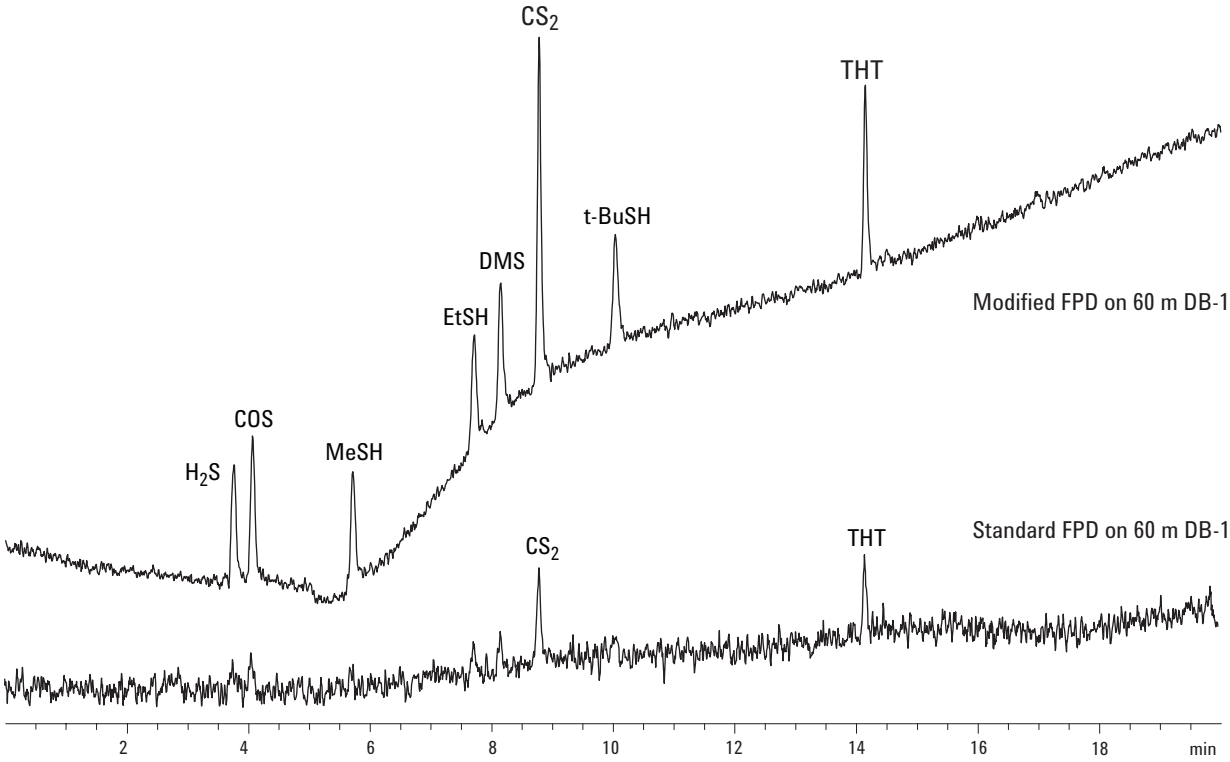

Figure 2. Sensitivity comparison of standard and enhanced FPDs. Concentrations are 33 ppb per component (v/v) in helium.

Due to the use of all available heated zones on the 6890N GC for either inlet or detector heating, the 6-port sample valves are not actively heated. This does not pose a problem for the light gaseous streams studied in this work. However, if desired, the valves can be heated by an auxiliary stand-alone temperature controller (Agilent model 19265B). The system is designed only for gaseous samples containing significant concentrations of hydrocarbons of C_6 or below.

Discussion

Channel 1 employs the GS-GasPro column, using a unique bonded PLOT technology, where COS is separated from C_2 and C_3 hydrocarbons, allowing measurement at trace levels. However, H_2S and the C_3s coelute. Channel 2 uses a thick film DB-1 column where H_2S is well separated from C_2s and C_3s, making low-level measurements of this sulfur impurity possible. COS and C_3s will coelute on this column. In summary, using a dual-column approach with the unique separation capabilities of GS-GasPro and thick film DB-1, both COS and H_2S can be measured in one chromatographic analysis at low ppb levels regardless of the concentrations of light hydrocarbons present in the sample. The elution order difference between the two columns is illustrated in Figure 3.

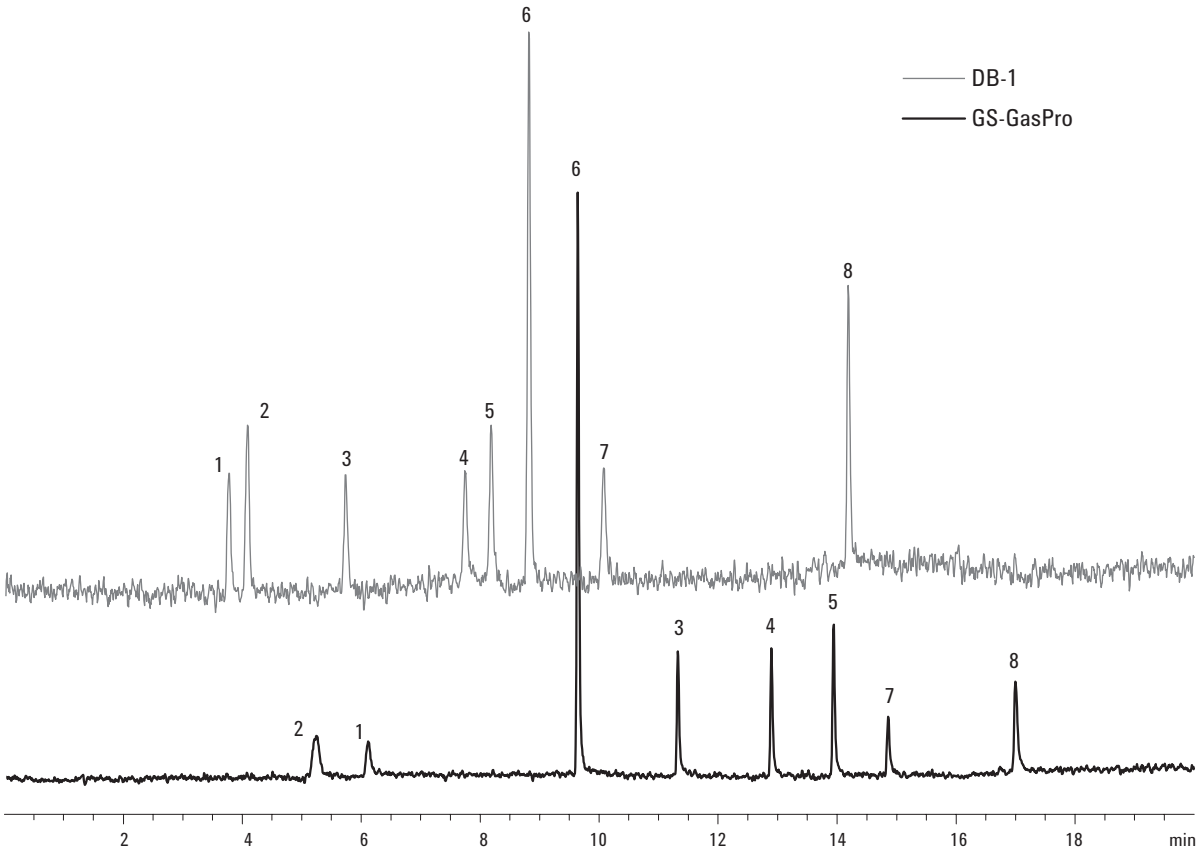

Figure 3. The dual-column advantage. Sulfur mix at 90 ppb per component in helium. 1. H_2S, 2. COS, 3. MeSH, 4. EtSH, 5. DMS, 6. CS_2, 7. t-BuSH, 8. THT.

Other potential interferences or coelutions between light sulfur compounds and hydrocarbons are avoided with this approach. A coeluting pair on one column will likely be separated on the other. Split ratios were set depending on the application from 0.5:1 to 2:1 in order to achieve the reported detection limits.

The sulfur calibration mix consisted of the following components at 5 ppm each: Hydrogen sulfide (H_2S), carbonyl sulfide (COS), methyl mercaptan (MeSH), ethyl mercaptan (EtSH), dimethyl sulfide, carbonyl sulfide (DMS), t-butyl mercaptan (t-BuSH), and tetrahydrothiophene (THT). The blend in helium was purchased from DCG Partnership, Pearland, TX. These compounds are representative of the most common light sulfur species encountered in gaseous fuels or petrochemical feedstocks.

Some adsorption of H_2S on the GS-GasPro column is possible. Priming the system a few times with a low ppm sulfur stream such as the calibration mix described here can largely eliminate the loss in sensitivity that can result from adsorption. This priming is usually only necessary for low ppb analyses where the active sites in the column could adsorb most of the sulfur present in the sample during an initial run.

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propane, liquidfied petroleum gas (LPG), propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases were mixed with the sulfur calibration standard using an Aux EPC module on the 6890N GC. Accurate concentrations from low ppb to ppm levels can be easily prepared by knowing the flow rates of the two streams as they mix in a Tee fitting prior to the gas sampling valves on the GC. This system and the hardware employed were described previously in detail [3].

Sulfur in Fuel Cell Gases, Natural Gas, and Proypylene

Figure 4 shows the chromatograms from the eight-component sulfur standard diluted with a fuel cell mix to 45 ppb (v/v) each component. The fuel cell

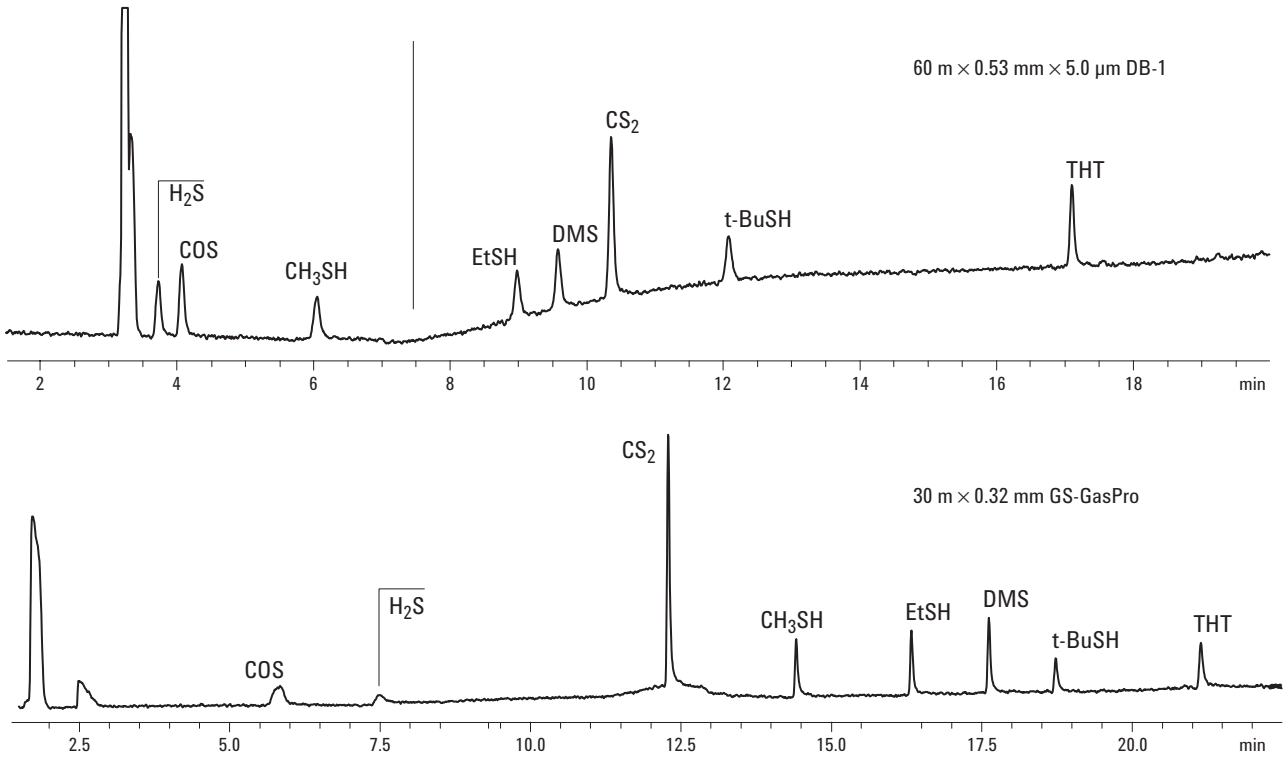

Figure 4. Simultaneous dual column analysis of fuel cell mix containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

mix is 50% hydrogen, 10% carbon dioxide, and 5% methane. This mix is often used to simulate the output stream of a natural gas reformer used as the feed to a fuel cell. This matrix is one of the easier ones because the large hydrocarbon (methane) elutes before all of the sulfurs on both columns. Note that elution order of the sulfurs is significantly different on the GS-GasPro column compared to the DB-1 (see Figure 3). All eight compounds are clearly detectable at 45 ppb.

Natural gas is a much more challenging matrix because of the high concentrations of several hydrocarbons. These interferences extend out into the retention time range of the sulfur compounds.

Figure 5 shows the chromatograms from the eight-component sulfur standard diluted with sulfur free natural gas to 45 ppb (v/v) each component. There are more peaks evident in these chromatograms than just the eight sulfur compounds. The additional peaks are interference responses from the large hydrocarbons in the natural gas.

In the DB-1 chromatogram, H_2S is clear but COS is lost to a severe overlap with a large C_3 peak. Ethyl mercaptan is also overlapped with n-pentane. On the GS-GasPro column, however, only the H_2S is occluded by interference. The COS and EtSH are free from interferences. With the dual-column approach, all eight compounds can be measured down to 45 ppb.

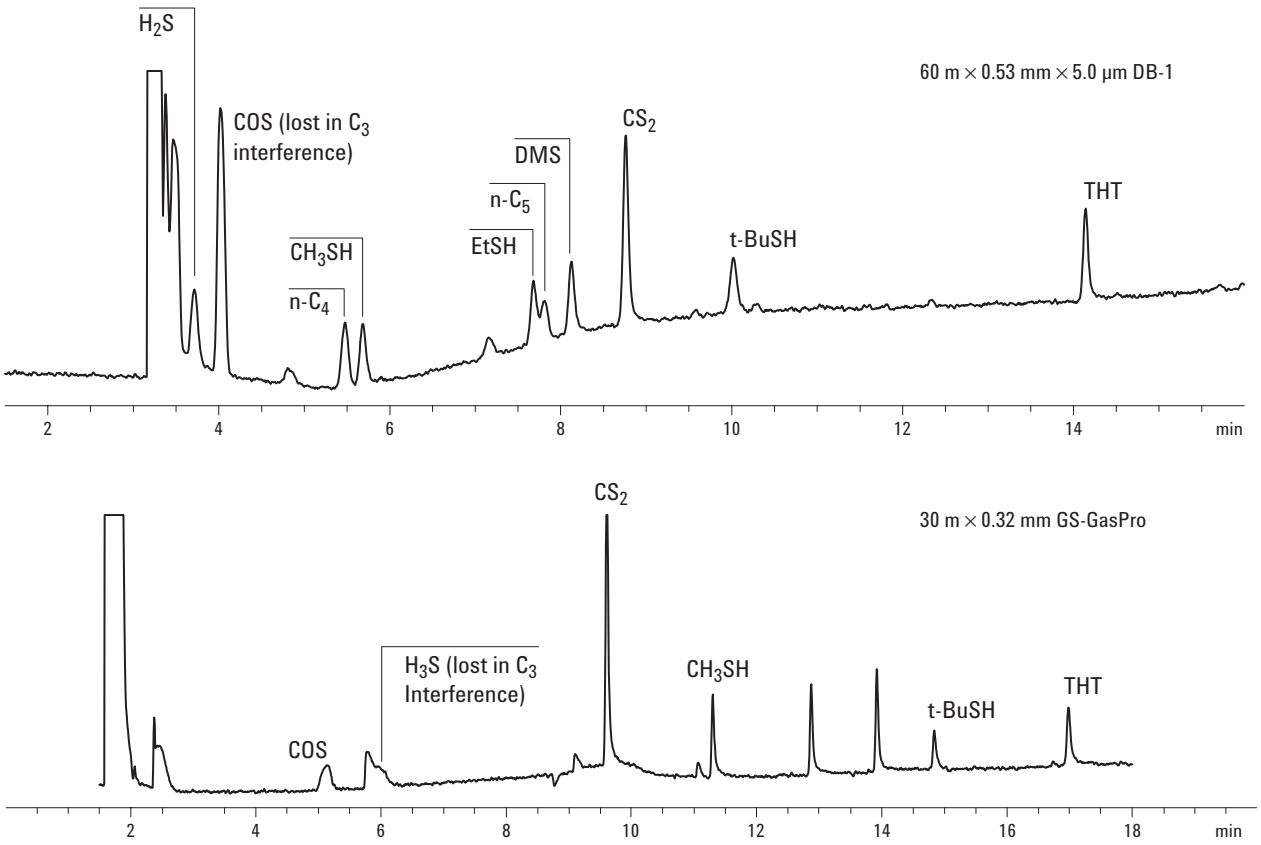

Figure 5. Natural gas blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

Propylene monomer offers another interesting challenge. The huge C_3 peaks interfere with both the H_2S and COS on both columns used above. To address this, longer versions of the same columns were used (Table 1). The oven temperature and split ratio are also modified (see Experimental on page 2) to improve resolution of the H_2S and COS from the C_3s.

Figure 6 shows the chromatograms from the eight-component sulfur standard diluted with polymer-grade propylene to 45 ppb (v/v) each component. By using longer DB-1 and GS-GasPro columns, lower oven temperature, and a higher split ratio, the H_2S and COS can be measured with somewhat poorer detection limits.

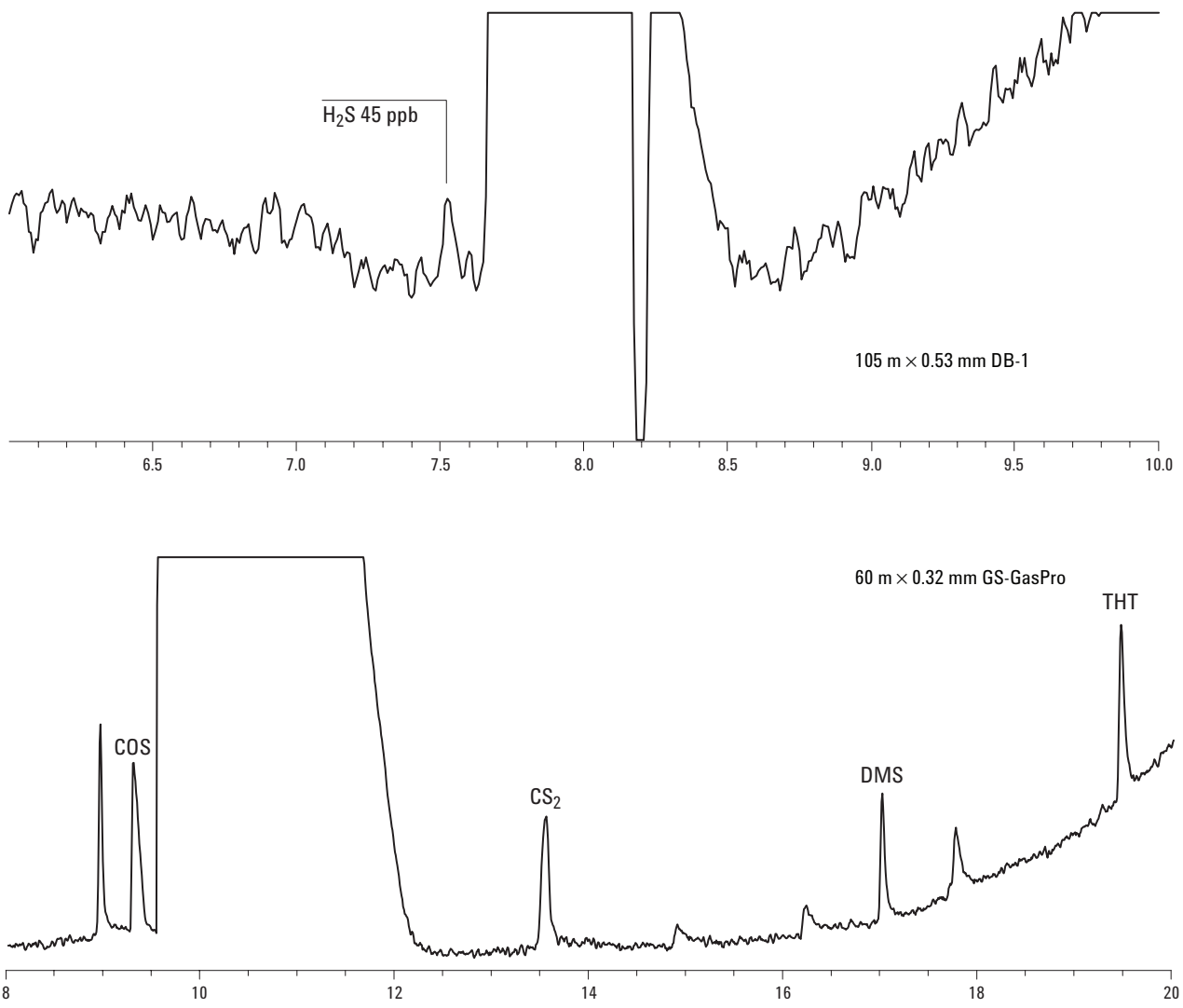

Figure 6. Polymer-grade propylene blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 2:1. Top chromatogram: 105 m × 530 µm DB-1 showing only H_2S, bottom: 60 m GS-GasPro.

Cryogenic oven temperatures were evaluated to see if the separation of H_2S and COS could be improved enough to allow use of the more sensitive 0.5:1 split ratio. The oven program tested was: -35 °C for 7 min, 20 °C/min to 300 °C, hold for 5 min. The separation was improved enough to allow the analysis of H_2S on the DB-1 column with the 0.5:1 split ratio, but COS was still occluded by the C_3s on the GS-GasPro. A DB-1 chromatogram illustrating the increased separation between H_2S and propylene is given in Figure 7.

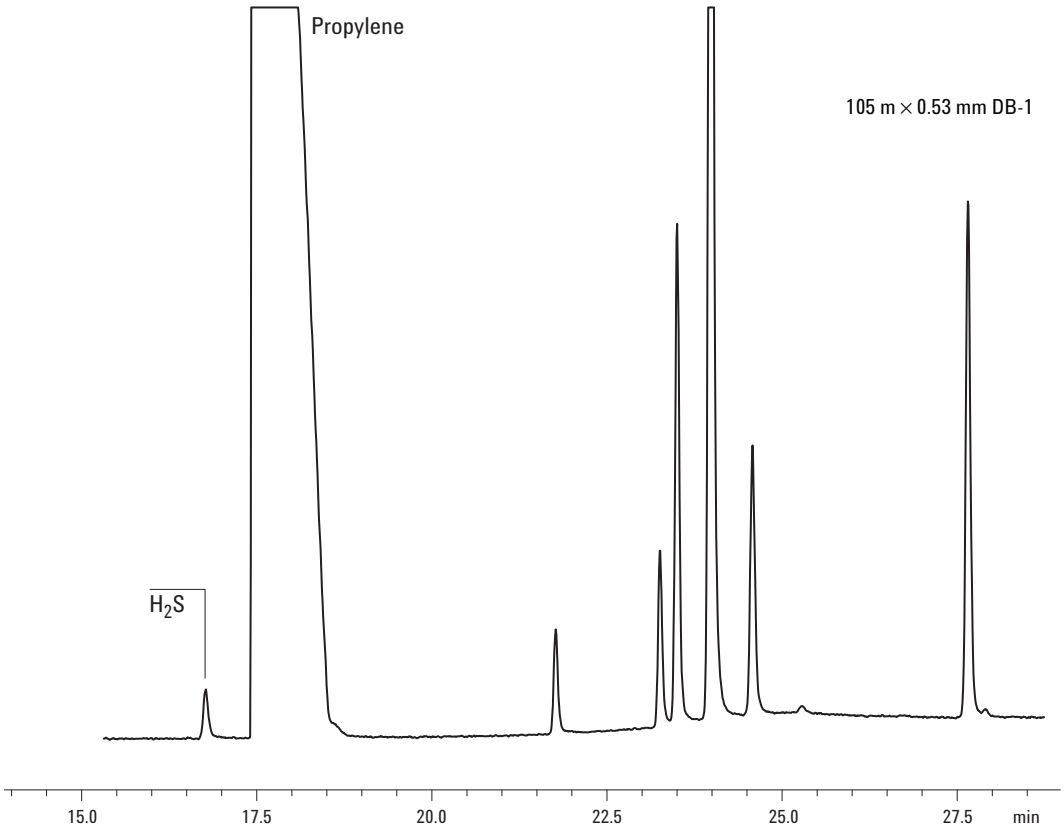

Figure 7. Use of cryogenic oven temperatures for analysis of H_2S (400 ppb) in propylene at 0.5:1 split.

Phosphorus and Arsenic on the Same System

One interesting characteristic of the modified FPD is that the filter used also passes the emissions for phosphorus and arsenic. This means that the same detectors can also be used to measure arsine and phosphine in polymer grade ethylene and propylene. A change of detector gas flows to that optimum for each element, followed by a rerun of the sample is all that is required. Since the 6890N detector flows are controlled by EPC, these reruns can be automated.

Figure 8 shows the chromatograms from an arsine and phosphine standard (DCG Partnership) diluted with polymer grade propylene to 36 ppb (v/v) each component. These are run under the same chromatographic conditions as in Figure 6, except that the FPD detector flows are set to those listed for phosphorus detection and the split ratio is back to 0.5:1. The detection limit under these conditions for phosphine in helium is under 5 ppb. If the detector flows are set to those listed for arsenic detection, the detection limit for arsine is about 60 ppb measured in helium. This system is well suited for gas analysis, however it is not really applicable to pesticide analysis due to the lack of selectivity between sulfur, phosphorus, and arsenic.

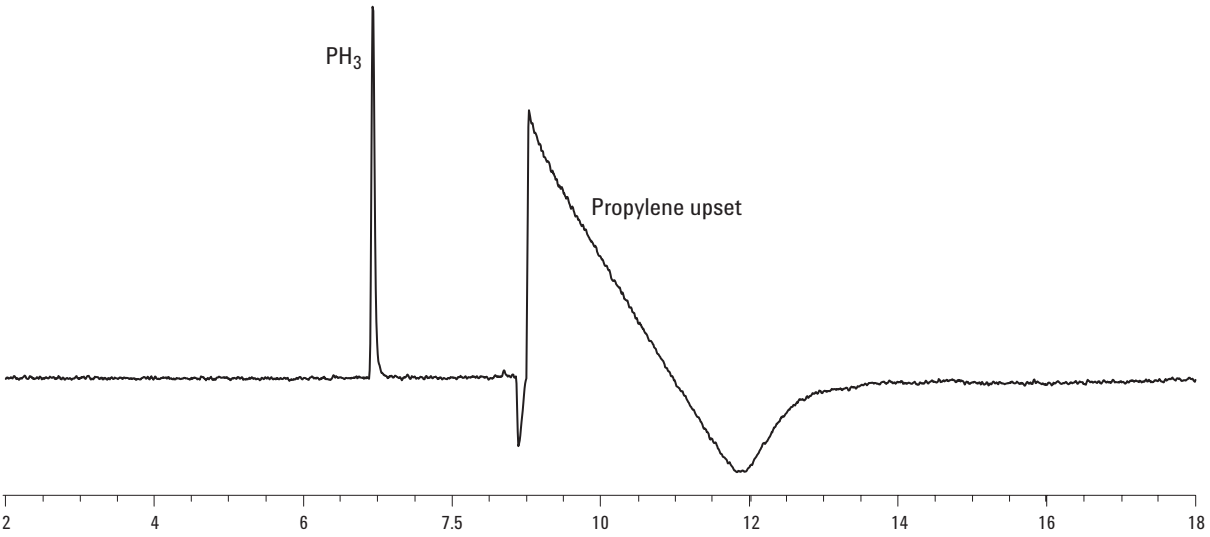

Figure 8. Polymer-grade propylene blend containing 36 ppb (v/v) each of arsine and phosphine. Split ratio is 0.5:1. Note longer 105 m DB-1 columns are used.

An example of arsine detection in propylene is shown in Figure 9.

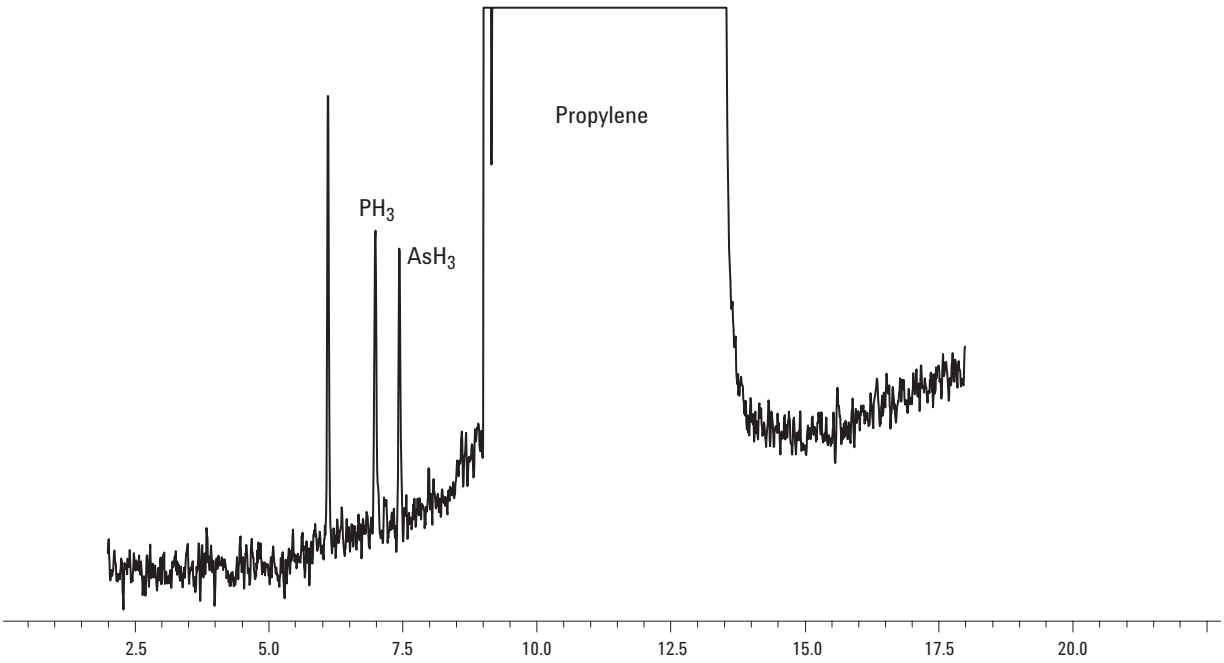

Figure 9. Arsine optimized FPD flows. H_2: 50 mL/min, air: 150 mL/min. 60 m × 0.32 mm GS-GasPro, 0.5 to 1 split. 90 ppb each of AsH_3 and PH_3.

How to Order and Configure a Dual-Channel FPD System

The Dual-Channel FPD System, including columns and valves, can be ordered as a special (SP-1) option on any new Agilent 6890N GC. This special also includes the enhanced performance FPD. Contact your local Agilent representative for more information.

Learn more about low-level sulfur detection from these application notes available from any Agilent sales office or Agilent's Web site at www.agilent.com/chem. Just click "Library" in the menu listing, and type "sulfur" in the keyword field.

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Printed in the USA
March 17, 2003
5988-8904EN



Agilent Technologies

The Analysis of Light Hydrocarbons Using the Agilent 6820 Gas Chromatograph with "M" Deactivated Alumina PLOT Column

Application

Petrochemical

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Abstract

This application note describes methods for analyzing C1-C6 light hydrocarbons. An Agilent 6820 Gas Chromatograph configured with a 6-port gas-sampling valve interfaced to a split/splitless inlet and flame ionization detector was used. The Agilent HP-AL_2O_3 "M" deactivated 50 m PLOT column was used for separation.

Introduction

Measurement of light hydrocarbons is necessary in refinery gas streams, liquefied petroleum gas (LPG), and natural gas. Knowing the concentration and distribution of hydrocarbons in these gases is critical for controlling manufacturing processes and production quality. Light hydrocarbon measurements are also useful in the development of processes for new plant production. Those who produce, transport, or consume natural gas also require accurate analyses to monitor and to calculate ratios of different gas streams in order to optimize furnace performance. Detailed information about natural gas hydrocarbon content is also used to establish market pricing.

Both packed and capillary columns are used individually for analyzing light hydrocarbons. In packed columns, hydrocarbons heavier than C5 are usually backflushed ($C6^{+)}$ by a switching valve [1]. Compared to packed columns, capillary PLOT columns have superior separation power with much lower bleed. Alumina columns can easily separate C4 and C5 isomers that are difficult or impossible to separate well with packed columns. They can also elute C6 hydrocarbons in most applications [2]. In this application, the HP-PLOT AL_2O_3 "M" deactivated column was used.

Experimental

The Agilent 6820 Gas Chromatograph was used for this work using helium as the carrier gas. The system is configured with a split/splitless capillary inlet and flame ionization detector (FID). An automatic 6-port gas-sampling valve heated to 80 °C injects gas samples. The gas sample valve is interfaced to the carrier line of the split inlet using a 1/16-inch stainless steel tubing. An aluminum tube is used to jacket the transfer line for improved thermal performance. A valving diagram for the system is shown in Figure 1.

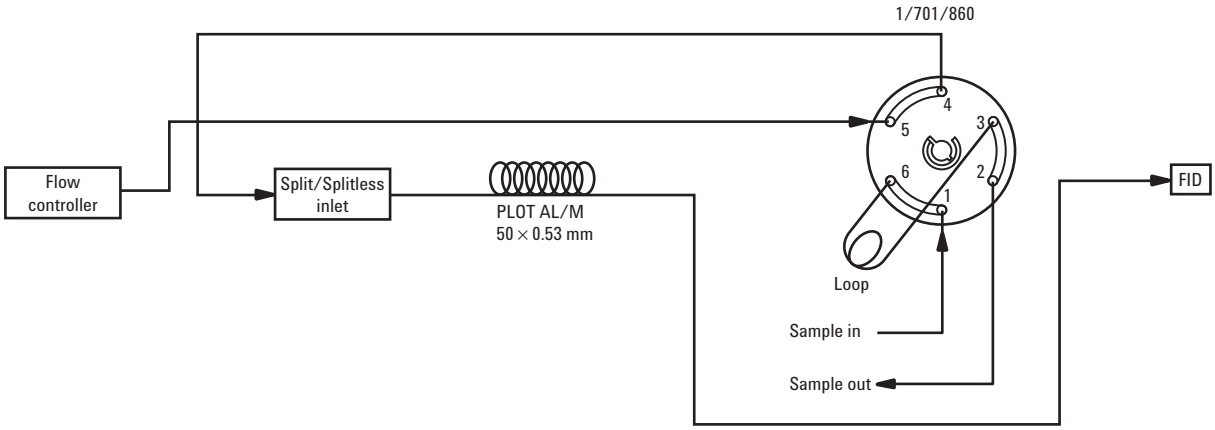

Figure 1. System configuration.

PLOT Alumina 50 m × 0.53 mm "M" deactivated column was used to separate hydrocarbons, including saturated, unsaturated, and aromatic compounds. An Agilent split liner (p/n 19251-60540) and bleed/temperature optimized Agilent advanced green septa (p/n 5183-4759) were used. Agilent Cerity NDS for chemical QA/QC was used for data acquisition and data analysis. Various temperature programs were used depending on sample complexity. Table 1 lists conditions representative for the hydrocarbon separations. Variation in the oven program, sample loop size, and split ratio are necessary to optimize the system for a given application. Care must be taken to optimize sample loop size to avoid overload of the alumina PLOT column.

Table 1. Gas Chromatographic Conditions

GC	Agilent 6820 Gas Chromatograph
Data system	Agilent Cerity NDS for chemical QA/QC, option 335
S/S Inlet	175 °C, 30:1 Split ratio typical
Valve	Gas sampling valve, 6-Port, option 701
Valve temperature	80 °C
Sample loop	0.1 mL typical
Column flow (He)	5 mL/min
Column	PLOT Alumina "M" 50 m × 0.53 mm × 0.25 µm (p/n: 19095P-M25)
Oven	40 °C (2 min) to 140 °C (5 min) at 4 °C/min
Detector	FID, 300 °C
H_2	35 mL/min
Air	350 mL/min
Column and make up	26 mL/min

Results and Discussion

This system is suitable for analyzing light hydrocarbons, including C4 isomers, in a typical C1-C5 refinery gas stream. The FID is used for wide dynamic range hydrocarbon measurements, while for fixed gas determinations such as carbon dioxide, hydrogen, oxygen, and nitrogen, a second channel using a Thermal Conductivity Detector (TCD) is needed. The fully digital signal path employed in the 6820 allows measurement of ppm level impurities and very high percent level determinations in a single run. This is not possible for many gas chromatographs that use an analog signal path. Split ratios and sample loop sizes were modified as needed, according to the concentrations present in the sample. This application demonstrates a simple but powerful 6820 configuration suitable for a wide variety of hydrocarbon samples. Figure 2 and Figure 3 show chromatograms for refinery gas standards. For some of the more complicated hydrocarbon analyses (compounds heavier than approximately C8), a 10-port valve with precolumn and backflush to vent can be employed to protect the PLOT column. As Figure 3 shows, 21 hydrocarbon components and isomers present in a standard sample achieve baseline separation.

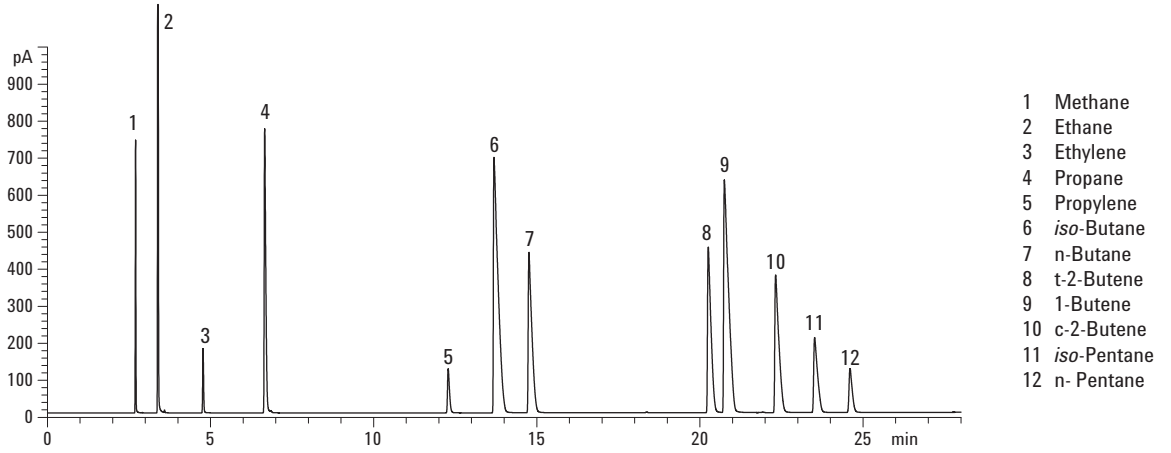

Figure 2. Chromatogram of refinery gas test sample Split ratio: 30:1, Sample loop 0.1 mL.

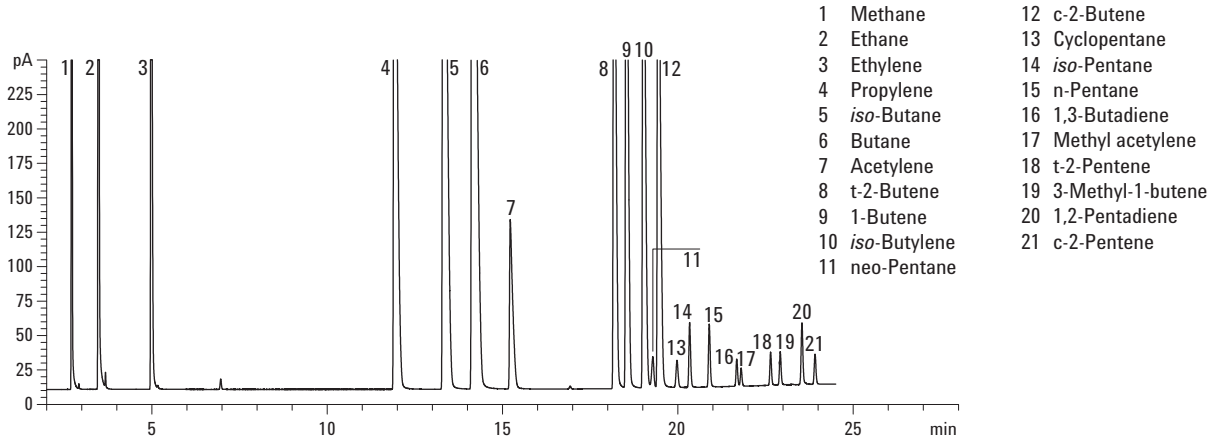

Figure 3. Chromatogram of hydrocarbon standard sample Split ratio: 30:1, Sample loop 0.05 mL.

Conclusions

The Agilent 6820 gas chromatograph configured with a 6-port gas sampling valve, split/splitless inlet, and FID was used for detecting light hydrocarbons. The Agilent AL_2O_3 "M" PLOT column exhibited low bleed and baseline separation of C4 isomers. This system offers an easy-to-use configuration where the valve is interfaced directly to the split inlet for accurate sample transfer. Applications include refinery gas, LPG, natural gas, and C4 streams.

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Printed in the USA
May 7, 2003
5988-9257EN

Determination of Low Level Hydrocarbon Impurities in Propylene Using the Agilent 6820 Gas Chromatograph

Application

Petrochemical

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Abstract

A method for analyzing trace hydrocarbon impurities in propylene is described. The method employs an Agilent 6820 gas chromatography (GC) system configured with a gas sampling valve, split/splitless inlet, and flame ionization detector. The Agilent Cerity Networked Data System for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and data analysis. An Agilent HP-Al_2O_3 column was used for separation of the trace hydrocarbons. Impurity levels at 1 ppm were easily detected in propylene. This method does not determine all possible impurities such as CO, CO_2, H_2O, alcohols, nitrogen oxides, and carbonyl sulfide, or hydrocarbons larger than decane.

Introduction

High purity propylene is commonly used as the feedstock for production of polypropylene, and the quality of this monomer is critical to successful polymerization. The presence of trace amounts of certain hydrocarbon impurities can have deleterious effects on the catalyst. For example, acetylene can be adsorbed at the active center of the catalyst, resulting in catalyst deactivation. Dienes may reduce the rate of polymerization and adversely affect product quality. To maintain catalytic efficiency, most propylene processes require that alkyne and diene contaminants in the monomer be less than 10 ppm. The availability of a suitable method for the determination of impurities in propylene is critical to setting specifications, controlling internal quality, and doing development or research work.

Some propylene producers use their own standard method in which packed columns are used. It is difficult to detect trace level impurities by packed column. Presently, the American Society of Testing and Materials (ASTM) has published Method D2712 for the determination of trace hydrocarbon impurities in propylene streams [1]. In this method, an alumina porous layer open tubular (PLOT) column is used. The improved efficiency of the PLOT column provides better resolution and increases effective sensitivity.

Experimental

An Agilent 6820 GC system was used for this work. It was configured with a split/splitless capillary inlet and a flame ionization detector (FID). Gas samples were injected using an automatic gas sample valve that was heated to 80 °C. The sample loop volume was 0.25 mL. The gas sample valve was connected to the inlet using an aluminum-jacketed stainless steel tube that maintains the sample temperature during transfer from the sample loop. The configuration used for propylene analysis is shown in Figure 1 and the instrument conditions are given in Table 1.

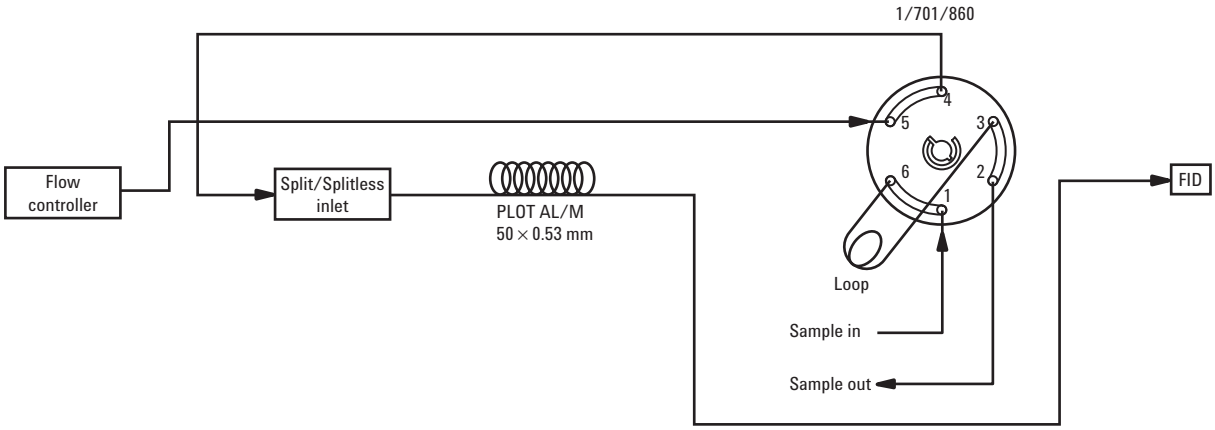

Figure 1. Configuration diagram.

An Agilent 50 m × 0.53 mm PLOT Al_2O_3 "M" deactivated column was used. The sample was run in the split mode using an Agilent split liner (Agilent part number 19251-60540).

The Agilent Cerity NDS for Chemical QA/QC was used for instrument control, data acquisition, and data analysis. Data was acquired at 20 Hz.

Table 1. Instrument Conditions

Split/Splitless inlet	175 °C, Split mode, with 15:1 and ~4:1 Split ratio
Valve	Gas sample valve, 6-Port, option 701
Valve temperature	80 °C
Sample loop	0.25 mL
Column flow (He)	4 mL/min
Column	PLOT Al_2O_3 "M" 50 m × 0.53 mm × 0.25 µm (p/n: 19095P-M25)
Oven	40 °C for 2 min, 4 °C/min to 190 °C for 5 min
Detector	FID, 300 °C
H_2	35 mL/min
Air	350 mL/min
Makeup gas (N_2)	22 mL/min

A propylene standard mix (DCG Partnership I, LTD., Pearland, TX 77581) consisting of the components listed in Table 2 at the certified concentrations shown (mole fraction) was used.

Table 2. Propylene Sample Mix Component Concentrations

Compound	Concentrations (ppm)	Compound	Concentrations (ppm)
1. Methane	10	10 Acetylene	9.8
2. Ethane	27	11. *trans*-2-Butene	9.92
3. Ethylene	10	12. 1-Butene	9.89
4. Propane	3526	13. neo-Pentane	9.86
5. Cyclopropane	10	14. *iso*-Butylene	9.87
6. Propylene	Balance gas	15. *iso*-Pentane	9.83
7. *iso*-Butane	9.94	16. *cis*-2-Butene	9.91
8. n-Butane	9.85	17. n-Pentane	9.86
9. Propadiene	9.84	18. 1,3-Butadiene	9.96

A dynamic blending system (Figure 2) was used to quantitatively dilute the sample with helium.

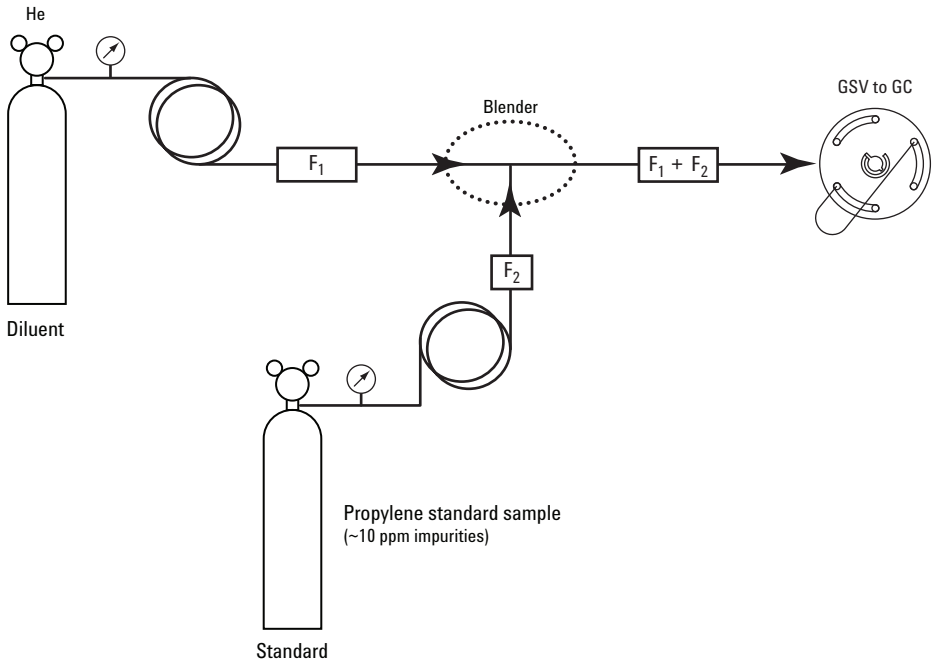

Figure 2. Dynamic blending scheme.

Diluted standard blend concentration is calculated by the following formula:

$C = C_o * F_2/(F_1+F_2)$

Where:
C: is diluted component concentration in ppm
C_o: original component concentration in standard blend in ppm
F_1: helium flow (mL/min)
F_2: propylene standard blend flow (mL/min)

Results and Discussion

Repeatability of 10 ppm Level Impurities in Propylene Analyses

Figure 3 shows the chromatogram from the undiluted sample. The PLOT AL_2O_3 column provides excellent separation for the C1 through C5 isomers [2]. The concentrations of most components are about 10 ppm. These trace level hydrocarbon impurities have a good FID response and are easily detected with baseline separation for most. Because the concentration of propylene is very high, some of the impurities such as *iso*-butane, n-butane, propadiene, and acetylene appear on the tail of the propylene peak. Even so, the Agilent 6820 GC system demonstrated very good repeatability, as shown in Table 3.

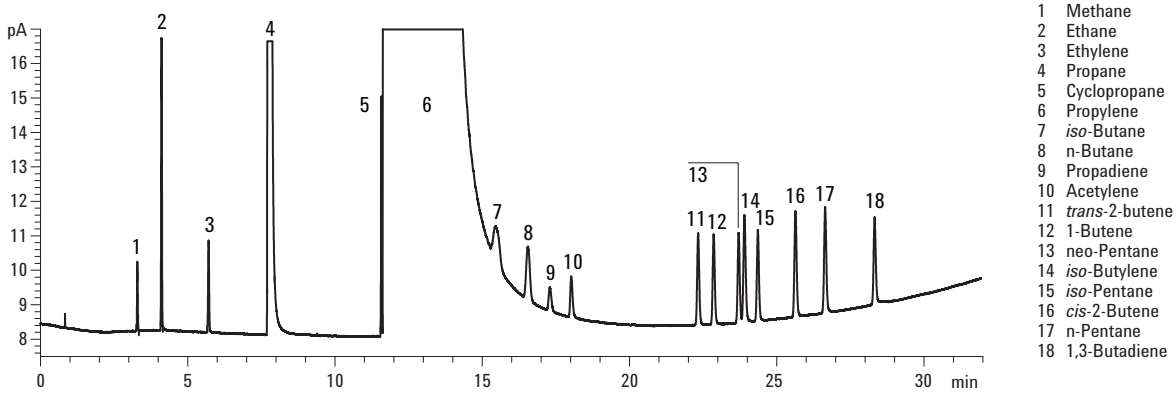

Figure 3. Propylene standard mix. Concentrations are given in Table 2. Split ratio: 15:1.

Table 3. System Repeatability of Three Propylene Standard Runs

Component	Amt1 (ppm)	Amt2 (ppm)	Amt3 (ppm)	Avg. (ppm)	RSD (%)
Methane	9.96	9.91	10.13	10.00	1.17
Ethane	26.95	26.78	27.27	27.00	0.92
Ethylene	10.21	9.90	9.90	10.00	1.76
Propane	3522	3503	3553	3526	0.73
Cyclopropane	9.99	9.95	10.07	10.00	0.61
Propylene	995642	988398	1004994	996344	0.84
iso-Butane	10.02	9.77	10.04	9.94	1.54
n-Butane	9.75	9.69	10.12	9.85	2.40
Propadiene	9.71	9.91	9.91	9.84	1.19
Acetylene	9.71	9.88	9.82	9.80	0.90
t-2-Butane	9.91	9.84	10.01	9.92	0.89
1-Butene	9.89	9.80	9.98	9.89	0.95
neo-Pentane	9.86	9.76	9.96	9.86	1.03
iso-Butylene	9.88	9.76	9.98	9.87	1.12
iso-Pentane	9.80	9.76	9.94	9.83	0.95
c-2-Butane	9.90	9.84	9.99	9.91	0.72
n-Pentane	9.84	9.76	9.98	9.86	1.16
1,3-Butadiene	9.97	9.87	10.04	9.96	0.85

Full Dynamic Range Data

One of the advantages of the Agilent 6820 GC system is its ability to obtain full dynamic range data. The signal "range" setting is not required because the Cerity/ChemStation uses digital data that goes from the noise level all the way to 100% samples. Without this feature, the propylene peak would be flat at the top as soon as the range was exceeded, making accurate integration impossible. In many cases without digital signal processing, users would have to run the sample at two different ranges in order to quantitate both large and small peaks. The Agilent GC system with Cerity/ChemStation can simultaneously acquire both large and small peaks in one run without setting different ranges. This feature helps quantitate 100% and ppm compounds at the same time. Figures 4 and 5 separately illustrate scaling the small peaks and one large peak to demonstrate acquisition of ppm level peaks and high percent level peaks.

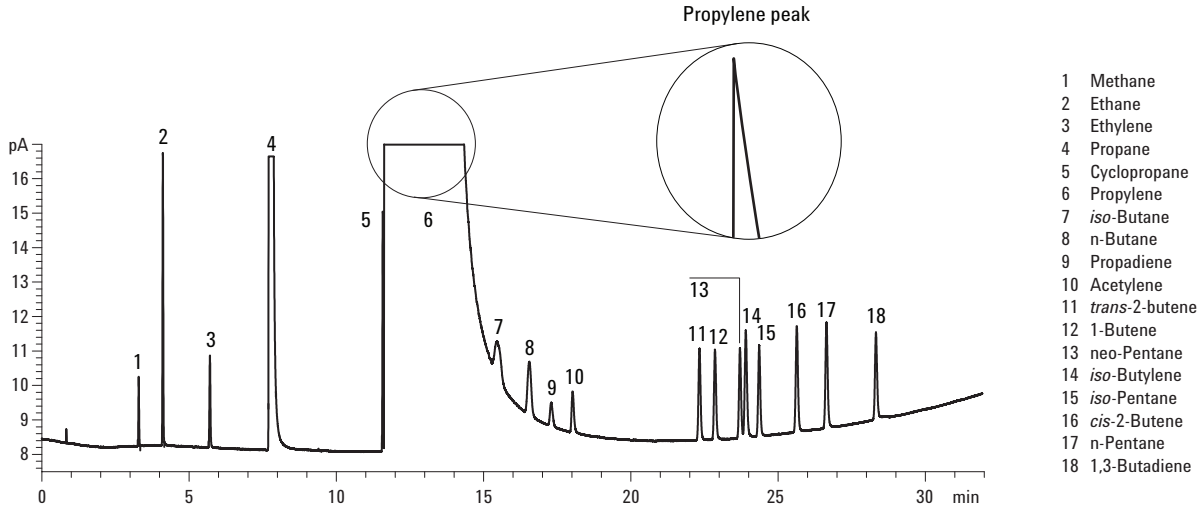

Figure 4. Propylene standard mix shown on small scale. Propylene peak looks flat due to graphic scaling.

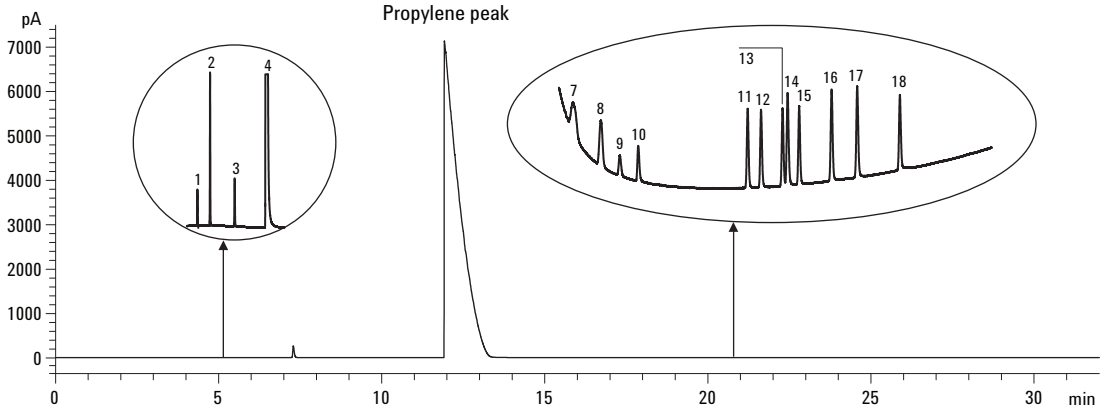

Figure 5. Propylene standard mix shown on high scale. Zooming in shows good resolution, identification, and integration.

Sensitive to 1 ppm Impurities

Figure 6 illustrates the chromatogram of less than 1 ppm impurities in propylene. The injected sample was prepared by a 10X dilution of the standard mix sample using helium; the impurities level decreased to 1 ppm as well as the 10:1 dilution of the propylene peak. In this analysis, the method was modified to use a split ratio of 4:1 instead of 15:1 in order to achieve the 1 ppm impurities detection. The sample presented in Figure 6 shows *iso*-butane, n-butane, propadiene, and acetylene clearly detected on the tail of the propylene peak. Other impurities show baseline separation with excellent signal to noise as well. This demonstrates the performance of the Agilent 6820 GC for sensitive and quantitative detection of 1 ppm hydrocarbon impurities in propylene.

Conclusions

The Agilent 6820 configured with a 6-port gas sampling valve interfaced directly to a split/splitless inlet was used to analyze trace hydrocarbon impurities in propylene with FID. Impurities below the 10 ppm mole % level can be easily quantitated. This system was able to detect 1 ppm level hydrocarbon impurities with excellent signal to noise. The Agilent 6820 system with Cerity can simultaneously acquire and quantitate both large concentrations (99 + mole %) and trace (low ppm) levels in a single run due to the use of a full dynamic range digital signal path. Manual range changes are not required. The feature of full dynamic range allows for accurate quantitation of near 100% propylene and ppm level compounds in one analysis. The system is simple and convenient to set up and use for routine QA/QC labs in the petrochemical and chemical industries.

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2. Roger Firor, “Trace Level Hydrocarbon Impurities in Ethylene and Propylene,” Agilent Technologies, publication 5965-7824E www.agilent.com/chem

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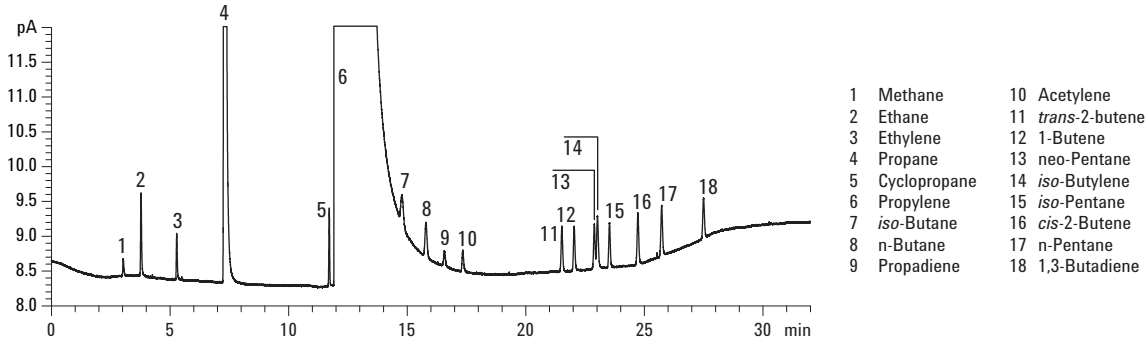

Figure 6. One ppm level impurities in the propylene standard mix.

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Printed in the USA
May 7, 2003
5988-9258EN

Agilent Technologies

Use of the New 5973 inert for Determination of Low-Level Volatile Sulfur in Gaseous Streams

Application

Gas Chromatography

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Abstract

The enhanced inertness of the new 5973 inert MSD improves analysis of trace level volatile sulfur compounds. When operated in scan or selected ion monitoring mode, excellent sensitivity, selectivity, and peak symmetry are obtained for active compounds. Eight volatile sulfur species are used to demonstrate these attributes in a variety of hydrocarbon matrices. The system is well suited for the characterization of fuel feedstocks and basic petrochemicals, where impurities can poison critical catalytic processes or affect product quality.

Introduction

Sulfur detectors find widespread use in a broad range of applications that span across many industries. Demand for low-level sulfur detection will only increase in the future in response to more stringent quality control and regulation. The significance and need for low-level sulfur measurements are detailed in previous Agilent application literature [1, 2, 3, 4].

The mass selective detector (MSD) is usually not considered first when the need for low-level volatile sulfur quantitation and speciation arises in the analytical laboratory. Selective detectors such as the flame photometric detector (FPD), pulsed flame photometric detector (PFPD), and sulfur chemilumiscence detector (SCD) have traditionally dominated these applications [1]. The 6890N/5973 inert GC/MSD system is a very capable alternative to these detectors, providing optimized inertness and the benefit of positive compound identification. This applicaton note details how to set up the system for optimum sensitivity and selectivity. The specific hardware configuration is applicable to a wide range of applications where ppb detection of gaseous analytes is required.

A common problem with many sulfur selective detectors is hydrocarbon interference, especially from co-elution [4]. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene. In most cases, an accurate determination of the sulfur compound is not possible. However, the use of the 5973 inert in selected ion monitoring (SIM) mode can largely overcome quenching caused by co-elution for many applications.

Experimental

The 5973 inert equipped with a new deactivated source was used for all experiments. The 3-mm drawout lens was used to achieve low ppb sensitivity while maintaining linearity over the ppb to low ppm concentration range needed for most sulfur measurements.

The sulfur calibration mix consisted of the following components at 5 ppm each: hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, carbonyl sulfide, t-butyl mercaptan, and tetrahydrothiophene. The blend in helium was purchased from DCG Partnership, Pearland, TX.

A 6-port gas-sampling valve was connected directly to the volatiles interface on the 6890N with Siltek™ 1/16-inch tubing. See the sample introduction diagram in Figure 1. The sample loop, tubing, and inlet are Siltek treated for inertness.

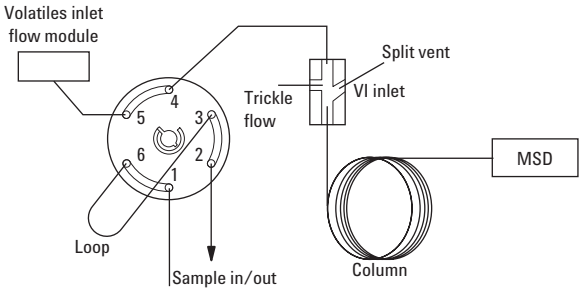

Figure 1. Sample introduction scheme.

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases are mixed with the calibration standard using an Aux EPC module on the 6890N GC. This system and the hardware employed have been described in detail [2].

Positioning of the column in the MSD must be carefully done to avoid loss of sulfur sensitivity. To position the column just inside the source, 2 mm to 3 mm of the column should be visible at the MSD end of the transfer line. See Reference 5 for installation details. See Table 1 for instrument conditions.

Results and Discussion

System Calibration

First, the system was calibrated and checked for linearity by analyzing the sulfur mix at various concentrations. The dynamic blending system was used to prepare seven and five level calibrations using helium and natural gas as diluents, respectively. Table 2 lists the concentrations used. Calibrations were focused in the ppb range since this is where most analytical problems for sulfur analysis are found. SIM acquisition mode was used.

Table 1. Instrument Conditions

6890N GC	
Injection port	**Volatiles interface**
Temperature	150 °C
Split ratios	1:1 up to 50:1
Carrier gas	Helium
Constant Flow Mode	1.9 mL/min
Injection source	**6-port gas sampling valve**
Material	Hastelloy C
Temperature	150 °C
Loop	Siltek, 0.5 cc
Column	**60 m × 0.320 mm × 5.0 µm DB-1**
Initial temperature	40 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	270 °C
Final time	2 min

5973 *inert* MSD	
Mass range	33–100 and 12–100 amu
Scans	13.1/s and 15.9/s
Samples	2
Threshold	150
EM Voltage	BFB.U tune voltage
Solvent delay	3.00 min
Source	Surface deactivated
Drawout lens	3 mm
Source temperature	230 °C
Quad temperature	150 °C
Transfer line	280 °C

Calibrations are linear in both matrices for all eight sulfur compounds. Refer to Table 3 where the regression coefficient r^2 values are shown. This is an indication that not only is the system response linear, but also that adsorption is not occurring in the GC or MSD from active sites. If adsorption were present, then one would expect a drop off at the lower end of the calibration curve. This is a direct benefit of the new inert MSD source.

Two calibration plots, as produced by the MSD ChemStation, are shown in Figures 2 and 3 for the calibration of H_2S and COS in natural gas, respectively. These are two challenging compounds with respect to activity, and they help illustrate the effectiveness of the inert system.

The 3-mm Drawout Lens

The 3-mm lens offers excellent sensitivity-optimized performance for this application. The 3-mm drawout was chosen for this work to meet the objective of reliable low ppb sulfur analysis. In addition, linearity over only a part of the MSD's dynamic range was required. Calibrations from 20 ppb to 5 ppm cover expected impurity ranges in real world samples and show excellent linearity with the 3-mm lens including samples run in a natural gas matrix where significant hydrocarbon fragmentation occurs.

Table 2. Calibration Levels for Checking System Linearity. Sulfur Concentrations in ppb.

Cal Level	1	2	3	4	5	6	7
Conc. in helium	21	35	46	57	95	1600	3600
Conc. in nat gas	88	242	475	880	1170	--	--

Table 3. Calibration Regression Coefficient r^2 Values

Compound	Helium	Natural gas
H_2S	0.998	0.998
COS	0.998	0.999
CH_3SH	0.997	0.999
EtSH	0.996	0.998
DMS	0.998	0.998
CS2	0.998	0.998
t-ButylSH	0.996	0.993
THT	0.996	0.992

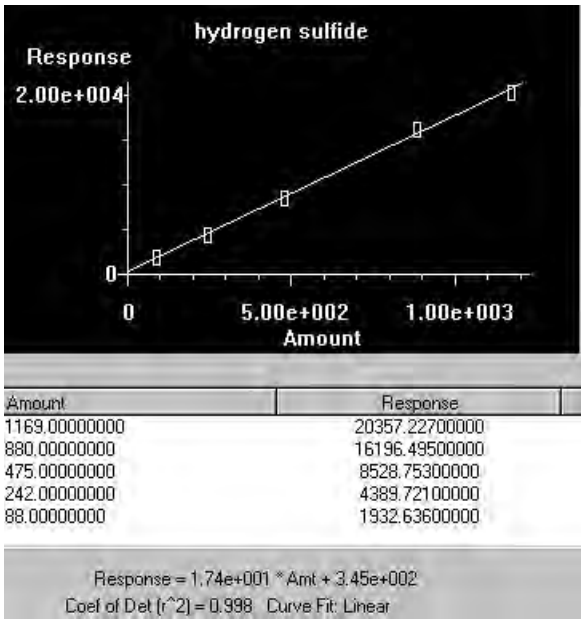

Figure 2. Five level calibration plot of H_2S in natural gas diluent. Calibration range is from 88 ppb to 1170 ppb.

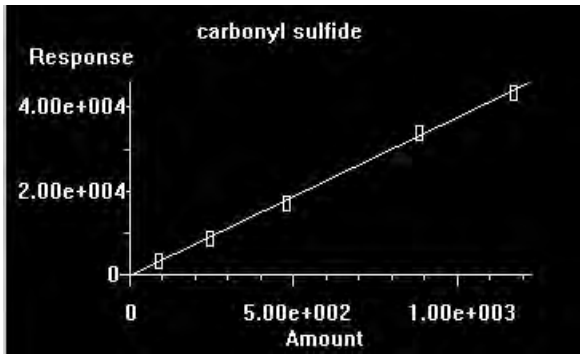

Figure 3. Five level calibration plot of COS in natural gas diluent. Calibraton range is from 88 ppb to 1170 ppb.

Scan Results

The total ion chromatogram (TIC) of the eight-component sulfur mix at 1.3 ppm in helium using a split ratio of 0.5 to 1 is shown in Figure 4. As is evident in the figure, H_2S is close to the minimum detection level (MDL) for this particular set of operating conditions. Symmetric peak shapes are seen for all components including adsorptive H_2S and COS.

Application of SIM

SIM provides the best sensitivity and selectivity for target analytes. Since sulfur determinations will normally be done in hydrocarbon matrices, care must be taken to select ions that ideally have no hydrocarbon contribution. If this can be done, excellent selectivity can be achieved even in cases where co-elution of sulfur species and hydrocarbon occur. This is an important distinction and advantage of the MSD compared to some of the common gas chromatographic sulfur selective detectors. Both the FPD and PFPD will suffer from quenching if co-elution occurs, making accurate quantitation of low-level sulfur problematic [2]. Even the SCD will have problems measuring low ppm sulfur in the presence of a dominant co-eluting hydrocarbon. In situations where a unique sulfur ion cannot be found, refinement of the method and chromatographic column/conditions to achieve separation from the interfering hydrocarbon should be attempted [2]. Also, when operating the MSD in SIM mode, it is usually best to select low resolution for maximum sensitivity at the expense of some resolution loss.

Refer to Reference 6 for guidelines for setting SIM parameters and instructions on using the AutoSIM feature available in the MSD ChemStation, G1701DA.

The SIM ions used for each sulfur compound are listed in Table 4. These ions were chosen to minimize interference from hydrocarbons. To arrive at the ions shown in the table, a scan of the sulfur mix in helium is acquired to identify target ions. Library spectra can also be consulted. Hydrocarbon mixes, such as natural gas and refinery gas, are then run separately using the SIM table to look for ions that may match those selected for sulfur. The table may be further refined if hydrocarbon interferences appear. These are not the only possible ions that can be used. For some of the compounds, other choices or additional ions could be included in the SIM table. While not necessary for this relatively simple sulfur example, the use of second and third qualifier ions may give the analyst a higher level of confidence of a compound's identity by comparing ion ratios to library spectra for a particular compound.

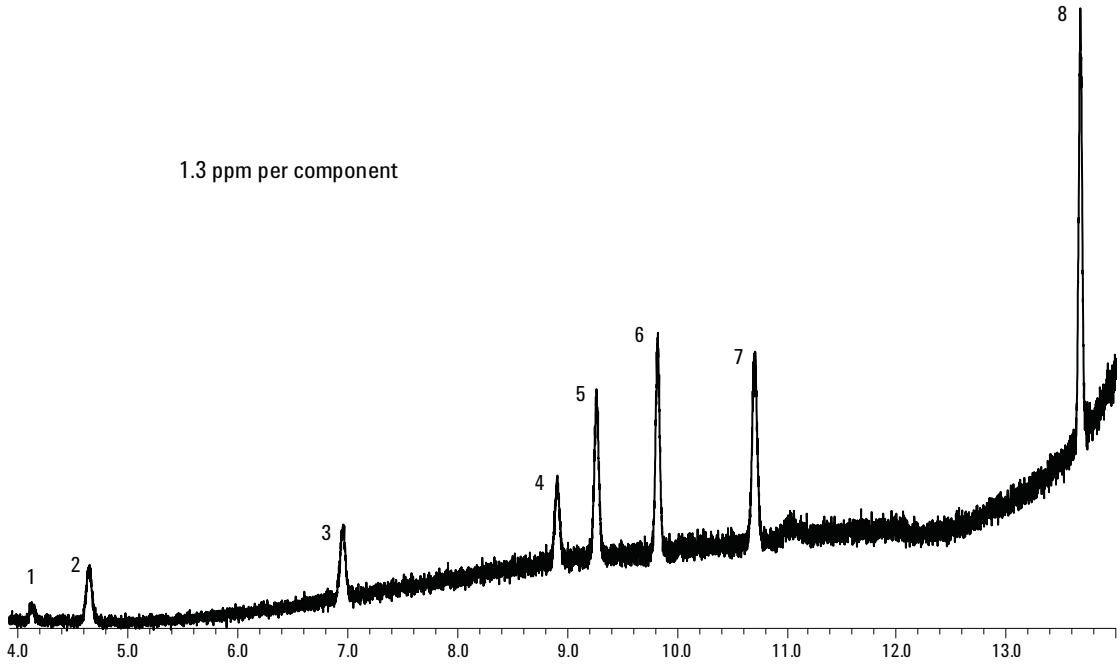

Figure 4. TIC of the eight-component sulfur mix at 1.3 ppm per component. Scan 33–100 amu. Peak labels: 1. hydrogen sulfide, 2. carbonyl sulfide, 3. methyl mercaptan, 4. ethyl mercaptan, 5. dimethyl sulfide, 6. carbon disulfide, 7. t-butyl sulfide, 8. tetrahydrothiophene.

Table 4. Optimized SIM Table for Selective Sulfur Detection in Hydrocarbon Streams. Dwell Time for Each Ion is 100 ms.

Group	Start time (min)	Target and qualifier ions	Compound
1	3.00	33,34	H_2S
2	4.20	60	COS
3	6.00	45,47	MeSH
4	8.00	47	EtSH
5	9.10	45,47,62	DMS
6	9.70	44,76	CS_2
7	10.20	57,90	t-ButylSH
8	11.80	45,60,88	THT

The sulfur mix chromatogram shown in Figure 5 was produced using the SIM parameters shown in Table 4. The offsets seen in the baseline are a result of the MSD switching from group to group and are not chromatographic. Excellent signal-to-noise and peak shape are seen for all components at the 46-ppb level. The sulfur mix was then further diluted to 16 ppb per component. The resulting chromatograms for H_2S, COS, and THT, the most challenging analytes, appear in Figure 6. At these levels, any problems with system or source activity would be evident. Sensitivity and peak shape are maintained, indicating excellent source inertness.

Natural Gas and Refinery Gas: Composition and Impurities

The TIC of a natural gas scan and sulfur mix SIM runs are overlaid for illustration purposes in Figure 7. Note that with the 60 m × 0.32 mm × 5.0 µm DB-1 all hydrocarbons and CO_2 are separated. Natural gas compounds in order of elution are: O_2/N_2, CH_4, CO_2, ethane, propane, I-butane, N-butane, I-pentane, and N-pentane. From the overlay, it can be seen that seven of the eight sulfurs do not co-elute with natural gas components; only COS and propane have nearly identical retention times. Even with co-elution, SIM makes it possible to quantify the COS; this will be addressed in the following section on propylene impurities.

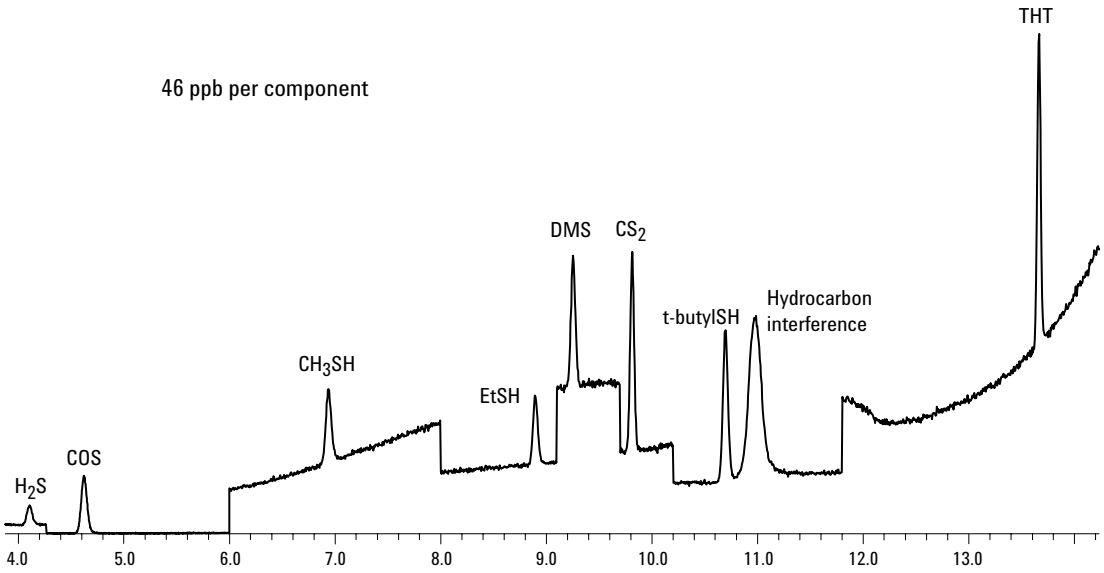

Figure 5. Eight-component sulfur mix in helium at 46 ppb per component in SIM mode. Split ratio 0.5:1. Refer to Figure 4 for peak identification.

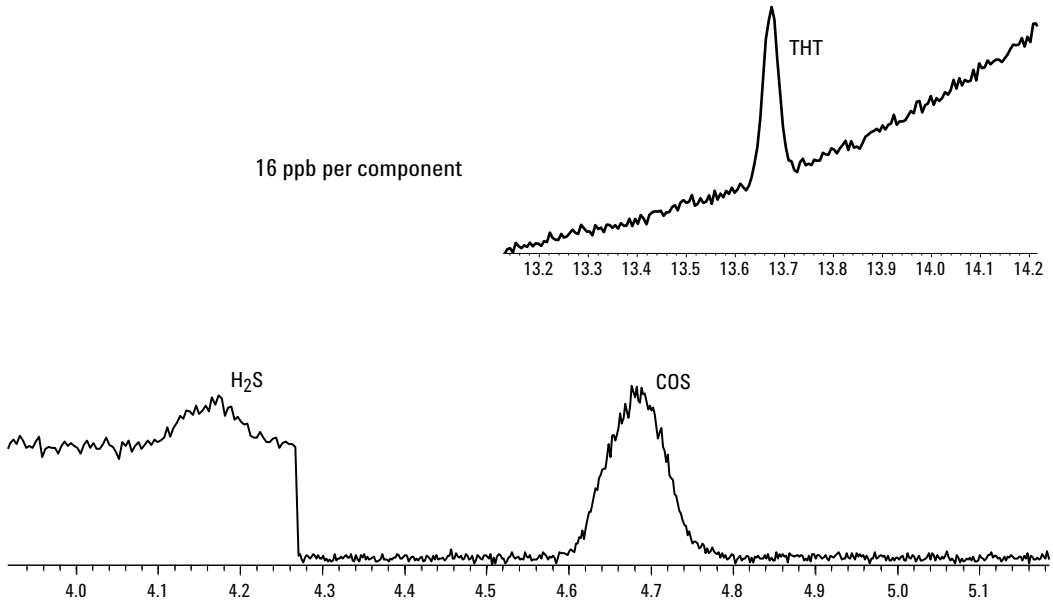

Figure 6. H_2S, COS and tetrahydrothiophene (insert) at 16 ppb each.

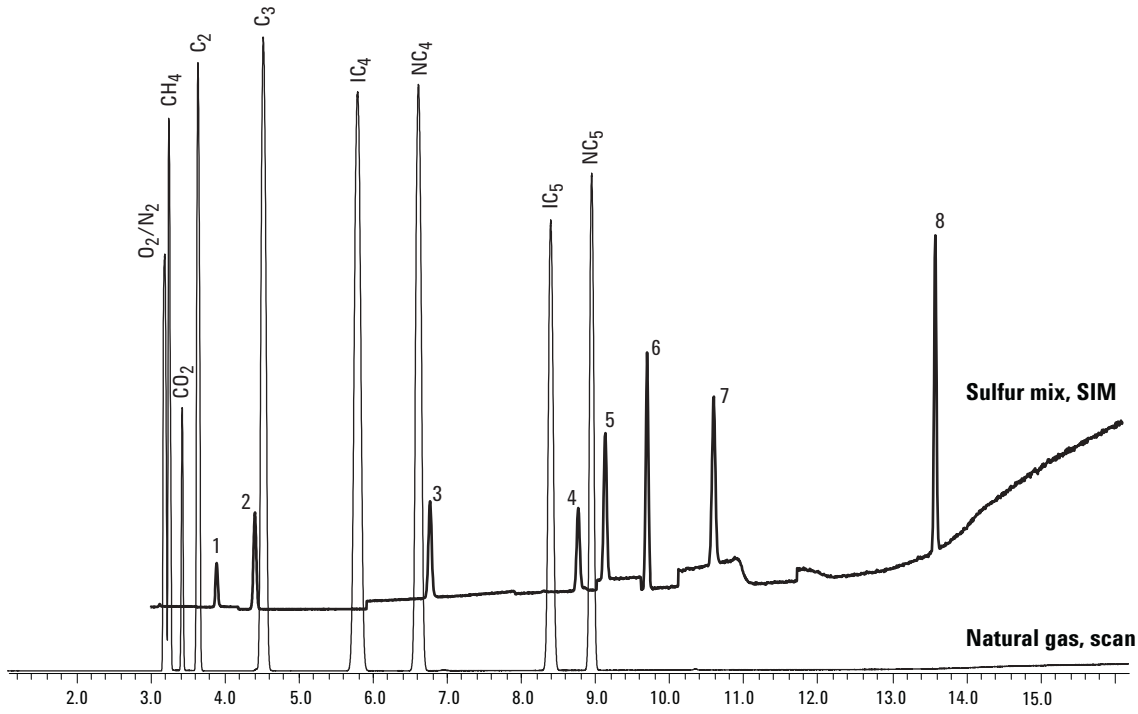

Figure 7. Overlay of two runs: natural gas scan (12–100 amu), and sulfur mix at 4.5 ppm in SIM mode. Split ratio 20:1. Peak numbering same as Figure 4.

Low level (350 ppb) sulfur gases in a representative refinery gas matrix are shown in Figure 8. Again, good peak shape and signal-to-noise are seen. Only methyl mercaptan is lost to hydrocarbon interference.

Analysis of COS in Propylene

Measurement of ppb COS and H_2S in propylene or propane can be challenging due to the co-elution of COS/propylene and the reactivity of H_2S. The COS co-elution is illustrated in Figure 9, where two independent separate runs are superimposed.

SIM (ion 60) was employed for the analysis of COS. To avoid overloading the source, the split ratio was increased to 50:1. To determine the effect of co-eluting propylene on COS response, two runs were performed at identical concentrations of 105 ppb COS. The diluents for the first and second runs were helium and propylene, respectively. Chromatograms for both runs are shown in Figure 10. The helium chromatogram shows the true COS area unaffected by co-elution. This area is then compared to that of COS in propylene diluent using the area ratio (COS propylene/COS helium) to indicate how co-elution has affected the 5973 inert response. This ratio of 0.77 indicates that COS in propylene response is suppressed by only 23% probably due to a reduction in ionization efficiency. Moreover, a subsequent experiment that constructed a five level calibration of COS in propylene showed linear behavior over the range of 20 ppb to 1200 ppb. Therefore, using a carefully constructed SIM method, the 5973 inert equipped with 3-mm drawout has the capability of quantifying ppb level COS in co-eluting propylene. Co-eluting active analytes do not preclude quantification even when concentration differences exceed 105 provided unique ions can be identified for the component of interest.

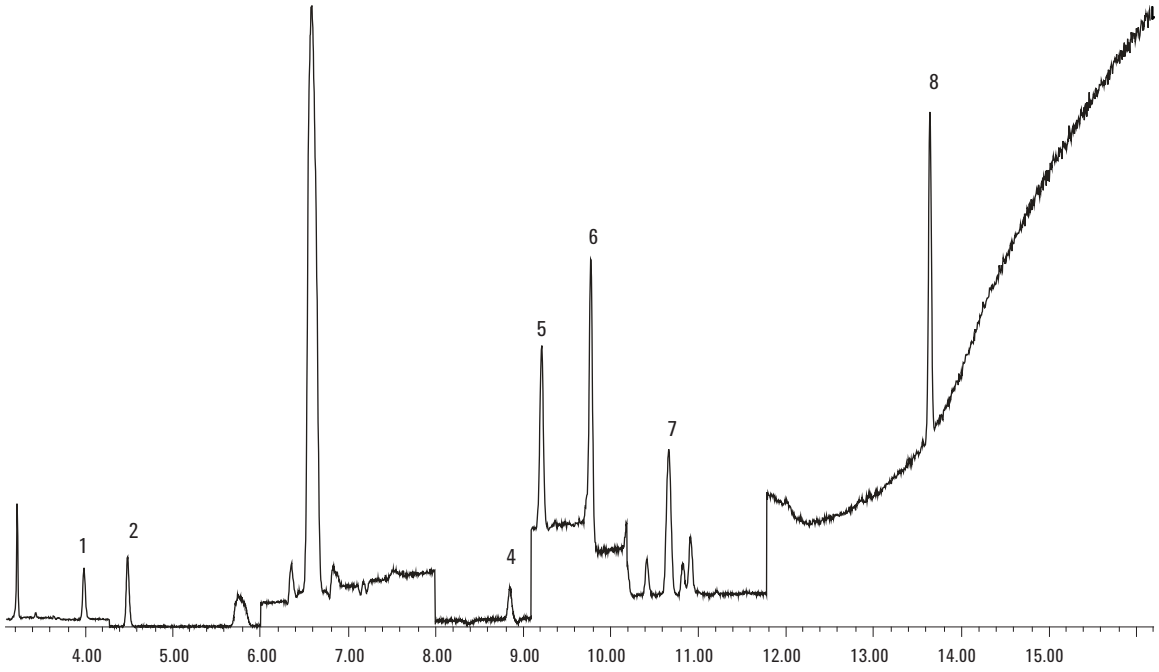

Figure 8. Three hundred fifty ppb sulfur mix in refinery gas. Peak identifications same as Figure 4. Good peak symmetry and sensitivity seen.

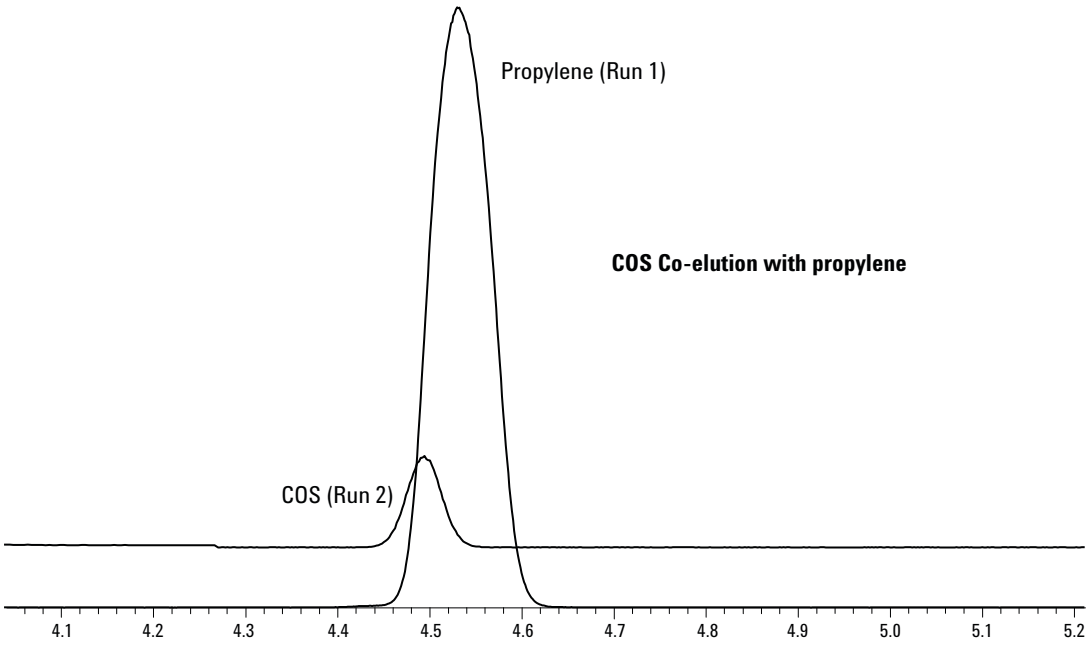

Figure 9. Two separate chromatograms superimposed showing the co-elution of COS with propylene. Split ratio 50:1.

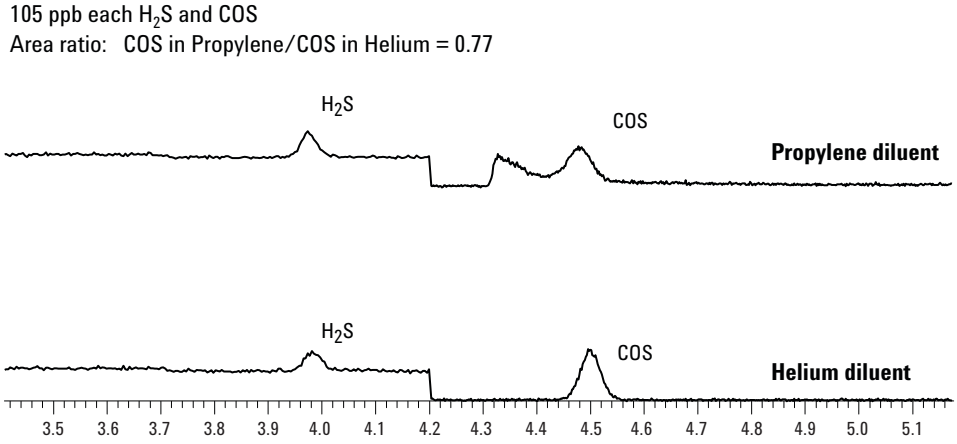

Figure 10. Comparison of COS response (SIM mode) in helium and propylene. Split ratio 50:1.

Conclusions

The importance of inertness cannot be over emphasized when analyzing and quantifying ppb level volatile sulfur compounds. The 5973 inert has excellent capabilities as a sensitive, repeatable, and selective detector for active gaseous analytes at low levels. Sulfur detection at low ppb levels is easily achieved through use of a time programmed SIM table consisting of unique ions for the compounds of interest. This minimizes hydrocarbon interference making it possible to quantitate low-level analytes such as COS with co-eluting propylene.

Use of the new inert source leads to excellent detection limits of active, adsorptive compounds with minimal peak tailing. Good peak symmetry is maintained at the ppm and ppb level for H_2S, COS, and other light organo-sulfur compounds. Detection of low-level polar analytes in general will improve with the 5973 inert.

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Printed in the USA
October 13, 2003
5988-9942EN



Agilent Technologies

High-Pressure Liquid Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Application

Hydrocarbon Processing

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Abstract

In gas chromatography, sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Introduction

There are several known techniques for injecting volatile liquefied hydrocarbons in gas chromatographs. The simplest tools are high-pressure syringes. However, the pressure limit is not high enough to analyze light hydrocarbons such as liquefied natural gas and ethylene. The traditional methods [1, 2] include the use of vaporizing regulators and rotary sampling valves. During sampling, discrimination of the analytes will take place for samples with wide boiling points due to condensing of heavy components and selective vaporization of light components in transfer lines. Recently, piston sampling valves were introduced and are commercially available [3]. These can suffer from discrimination and short service lifetimes at high vaporization temperatures or high sample pressures.

Combining the advantages of simple syringes and high-pressure rotary valves, a unique sample injection device has been designed. The system consists of a 4-port liquid sampling valve, a Siltek deactivated needle, and a split/splitless inlet. This compact device is installed directly over the GC inlet. This unit is operated just like a typical liquid autosampler; however, the needle is not withdrawn. The maximum limit of sample pressure is 5,000 psig. Various pressurized gas samples have been evaluated on this device such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with 0.47% to 1.09% RSD in quantitative analyses. Wide boiling point hydrocarbon samples (C5 to C40) have also been analyzed using this injector, with excellent quantitative results.

Experimental

Injection Device

The high-pressure liquid injection (HPLI) device consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Co. The valve works under 75 °C and 5,000 psi.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

The following components are recommended. These are not supplied in the option or accessory kit.

- **Filter:** To remove particles from samples, it is necessary to install a filter between the sample line and port S.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in option or accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

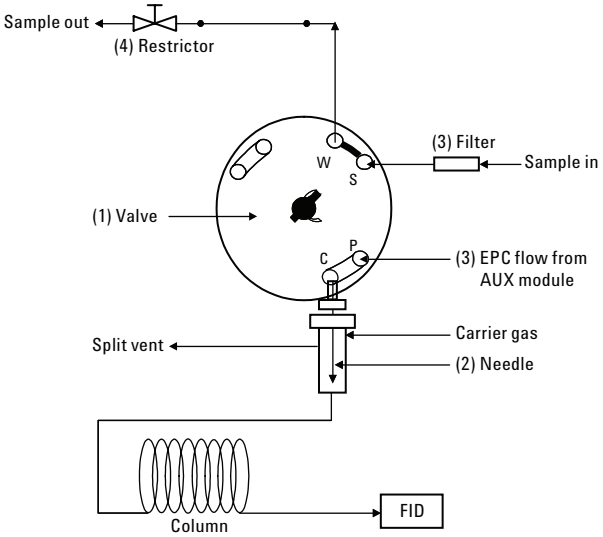


Figure 1. Flow diagram of the HPLI device.

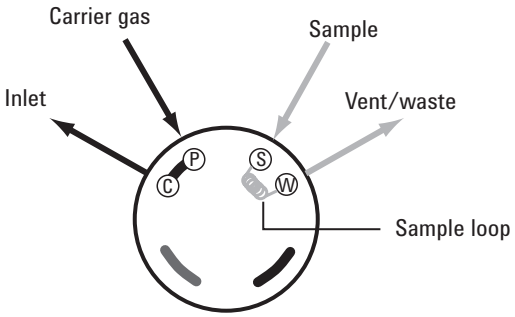
Samples for System Evaluation

- Liquefied natural gas: Calibration standard, 1,200 psi, with nC7-nC9 (0.102%–0.0503%)
- Liquefied ethylene: Purity 99.5, 1,200 psi
- Pressurized propylene: Grade C. P., purity 99.0%, 200 psi
- Pressurized propane + n-butane: 50.0%:50.0%, 200 psi
- Pressurized 1, 3-butadiene: Purity 99.5%, 180 psi
- n-Hexane + 1.0 % 2# BP standard (Agilent PN 5080-8768, nC5–nC18)
- nC5–nC40 D2887 1# BP standard (Agilent PN 5080-8716, diluted by CS_2)
- Glycols, including monoethylene glycol, diethylene glycol, and triethylene glycol
- C8 to C16 hydrocarbons at 100 ppm each

Operating Process

The valve is operated with an Agilent pneumatic air actuator. To load the sample, the valve is set at the OFF position (Figure 1). The sample is loaded from port S and vented to port W. The pneumatic and sample paths in load and inject positions are shown in Figure 2. To maintain the sample in the liquid phase and to avoid “bubbles” in the sample line, it is important to adjust resistance of the metering valve and check for possible leaks at the connections. To inject, the valve is switched to the

Load



Inject

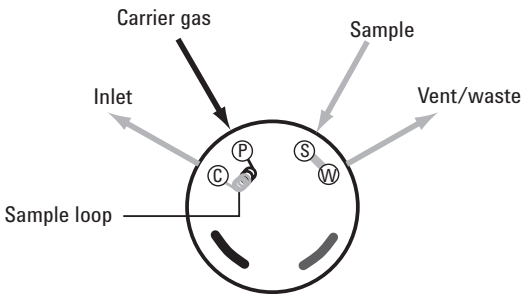


Figure 2. Pneumatic and sample paths in load and inject positions.

ON position. A 2- to 3-second injection time should be used.

The system should always be carefully checked for leaks before introduction of high-pressure hydrocarbons. Instrumental conditions and application-specific columns are shown in Table 1 and Table 2, respectively.

When the valve is actuated, a stream of carrier gas from the Aux EPC or PCM will enter the inlet and combine with the inlet carrier flow; the combined flow will vent through the split vent. Therefore, the actual split ratio will be higher than the value set from ChemStation. The actual split ratio can be calculated by measuring the split vent flow.



Figure 3. Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Table 1. Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	HPLI device at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.5-µL (0.2 µL for C5–C40) device supplied with 0.06-µL rotor
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

Table 2. Columns and Parameters

Samples	Columns	Column flow mL/min	Split ratio	Temperature program	Sample pressure psig
Natural gas	30 m × 0.53 mm × 0.5 µm DB-1 #125-1037	8	40:1	35 °C, 1 min 20 °C/min to 180 °C, 1 min	1200
Ethylene	50 m × 0.53 mm × 15 µm AL2O3 PLOT/KCL + 30 m × 0.53 mm × 5 µm DB-1, #19095P-K25 and #125-1035	8	20:1	35 °C, 2 min 4 °C/min to 160 °C, 3.8 min	1100
Propylene	50 m × 0.53 mm HP AL2O3 PLOT + 30 m × 0.53 mm × 5 µm DB-1	7	25:1	35 °C, 2 min 4 °C/min to 160 °C, 1.8 min	180
Propane + n-butane	30 m × 0.53 mm × 1.0 µm DB-1, #125-103J	5	50:1	35 °C	150
1,3-Butadiene	50 m × 0.53 mm AL2O3 PLOT/KCL	10	15:1	35 °C, 2 min 10 °C/min to 195 °C, 15 min	180
n-Hexane	30 m × 0.53 mm × 1.0 µm DB-1	5	50:1	45 °C	N/A
nC5-nC40	10 m × 0.53 mm × 0.88 µm HP-1, #19095Z-021	10	15:1	35 °C, 1 min 15 °C/min to 350 °C, 5 min	N/A
Glycols	30 m × 0.25 mm × 1.0 µm HP-1 ms	1.8	15:1	50 °C, 3 min 15 °C/min to 250 °C, 2 min	

Results and Discussion

Check for Carryover

A set of normal hydrocarbons was used to perform a basic check of the system, looking for good peak shape and lack of carryover.

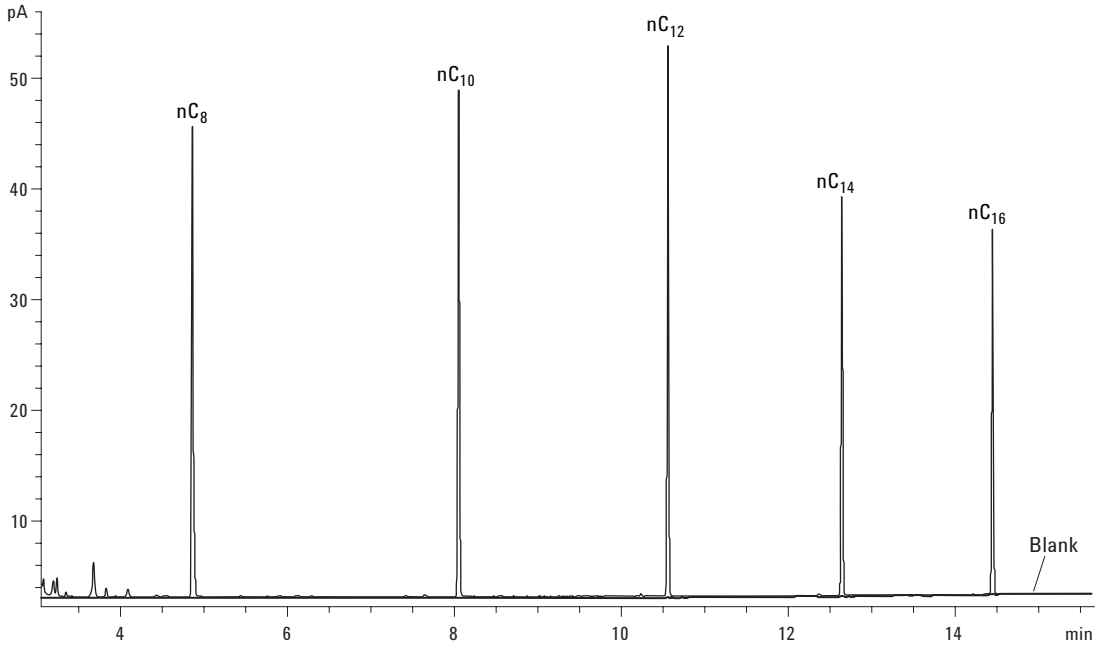


Figure 4. Overlay of standard versus blank (100 ppm each in cyclohexane).

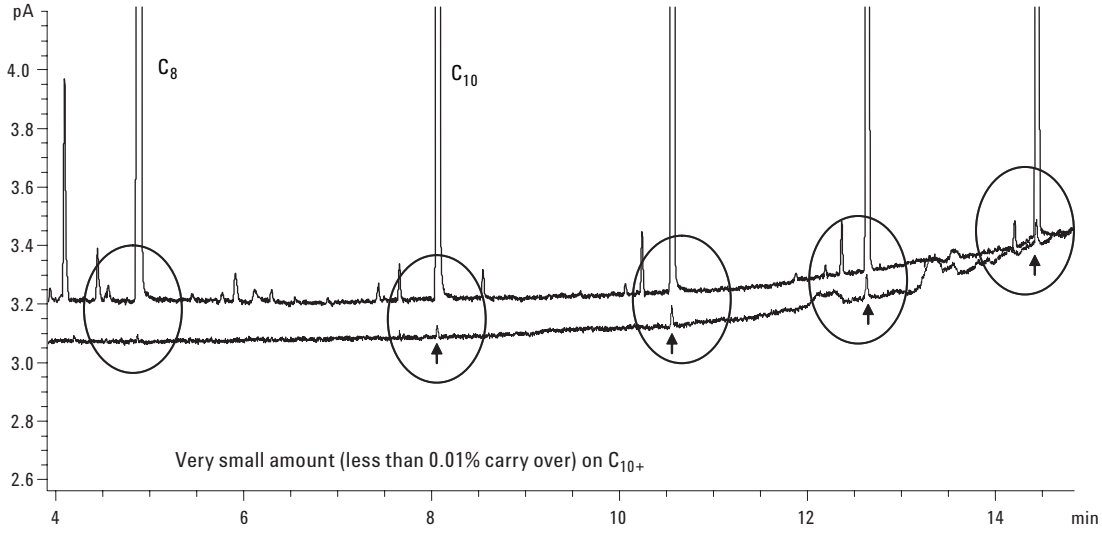


Figure 5. Carryover less than 0.01% on C_{10+}.

Sample Analysis

A series of glycols was used to model performance of the device for highly polar analytes. Minimal peak tailing is seen, due in part to the inertness of the needle interface. Also, carryover is very low.

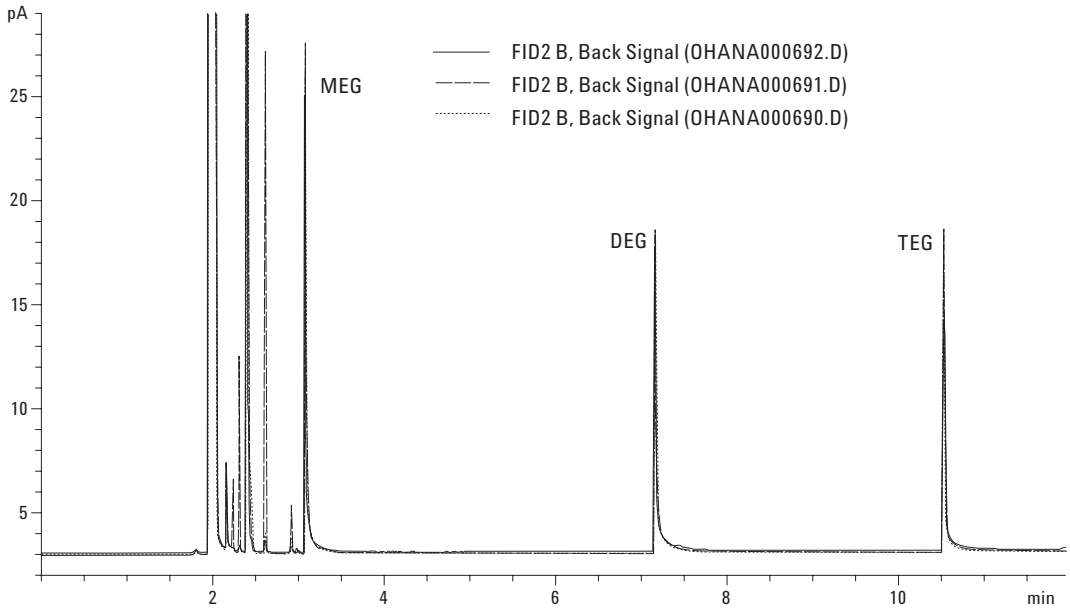


Figure 6. Triplicate run of 100 ppm each of MEG, DEG, and TEG in IPA.

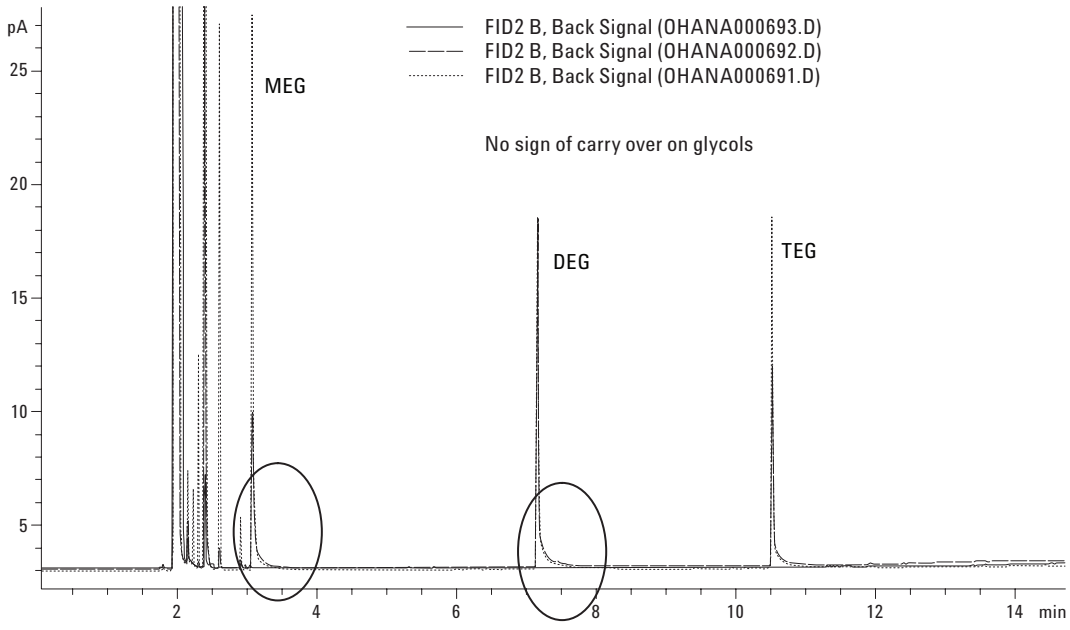


Figure 7. Glycols versus blank. Two standard duplicates, blank run immediately after injection of standard.

A. Liquefied Natural Gas

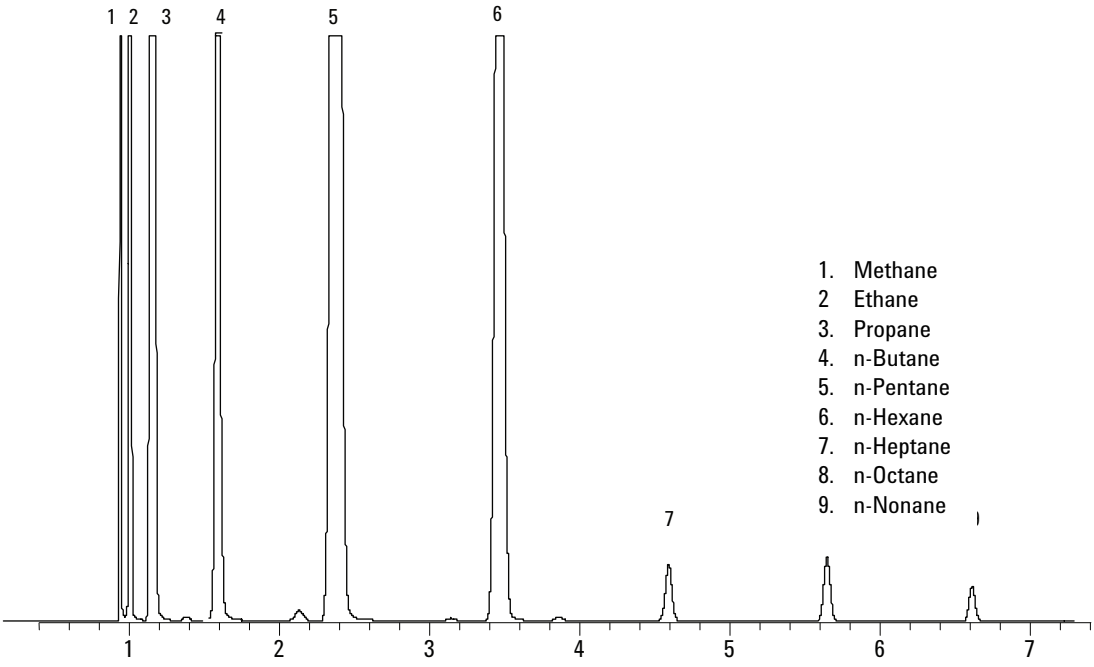


Figure 8. Chromatogram of liquefied natual gas (calibration standard).

Low discrimination is seen in Figure 8 for liquefied natural gas (LNG). Excellent repeatability is obtained with RSDs of less than 1%.

B. Liquefied Ethylene

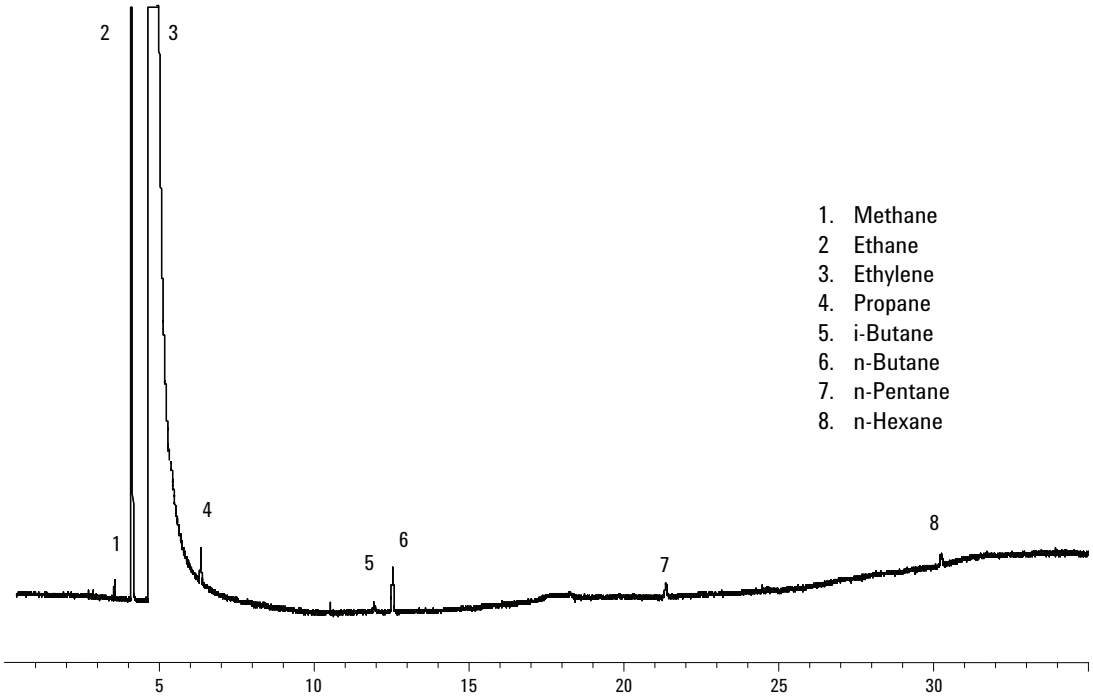


Figure 9. Chromatogram of liquefied ethylene.

The sample in Figure 9 is analyzed by ASTM D6159, "Standard Test Method for Impurities in Ethylene by Gas Chromatography." The method detection limits (MDLs) for the two methods are listed in Table 3.

The MDL using the HPLI device is 10 times lower than reported in the ASTM method due largely to the lack of peak tailing.

Table 3. MDLs (ppm V) by ASTM D6159 and HPLI

Components	ASTM D6159	HPLI
Methane	5.57–62.3	0.27
Ethane	35.1–338	0.78
Propane	8.07–59.7	0.88
i-Butane	7.74–48.4	0.38
Butane	4.97–56.1	1.61
n-Pentane		0.61
n-Hexane		0.74

C. Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159 (above method for ethylene analysis). The chromatogram is shown in Figure 10.

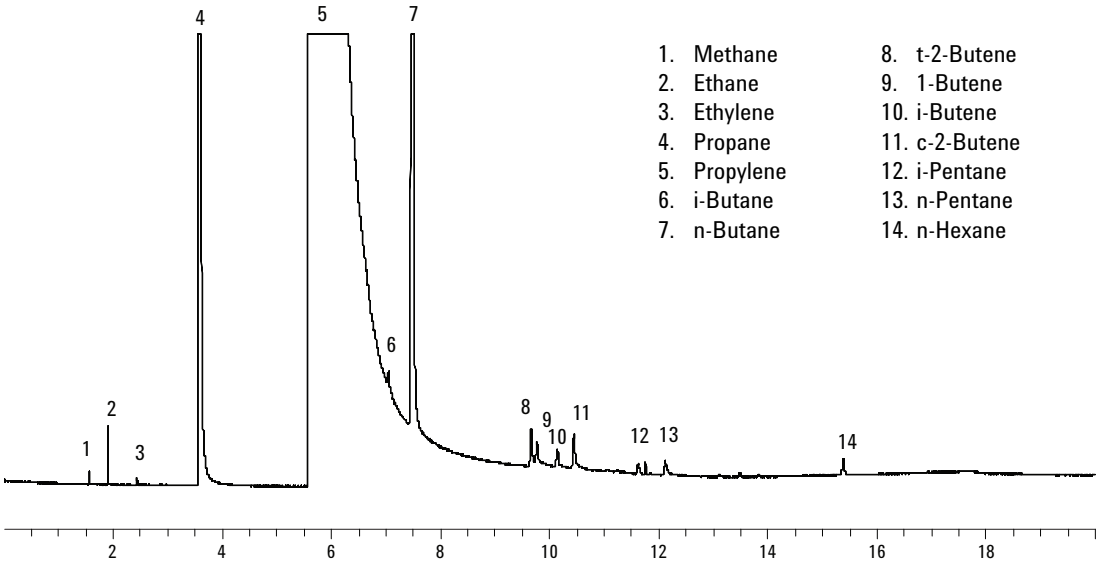


Figure 10. Chromatogram of pressurized propylene.

D. Pressurized 1,3-Butadiene

As an example of C4 hydrocarbons analysis,
Figure 11 shows a typical result for 1,3-Butadiene.

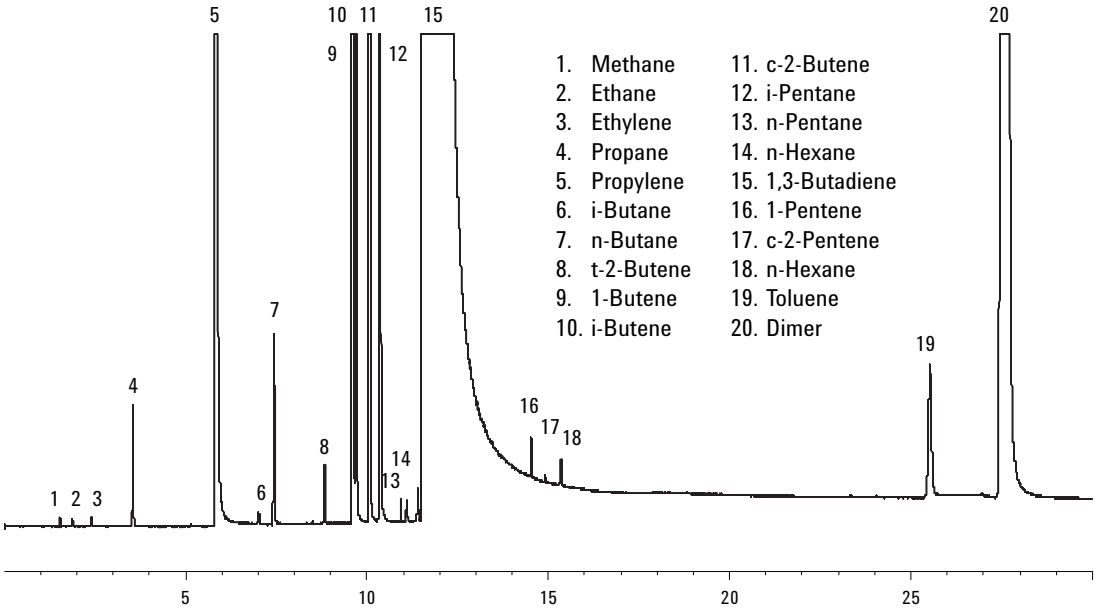


Figure 11. Chromatogram of pressurized 1,3-butadiene.

E. Pressurized Propane + n-Butane

This is a quantitative calibration sample:
Propane:n-Butane = 50%:50%.
The chromatogram is shown in Figure 12 with the results of a quantitative analysis shown in Table 4.

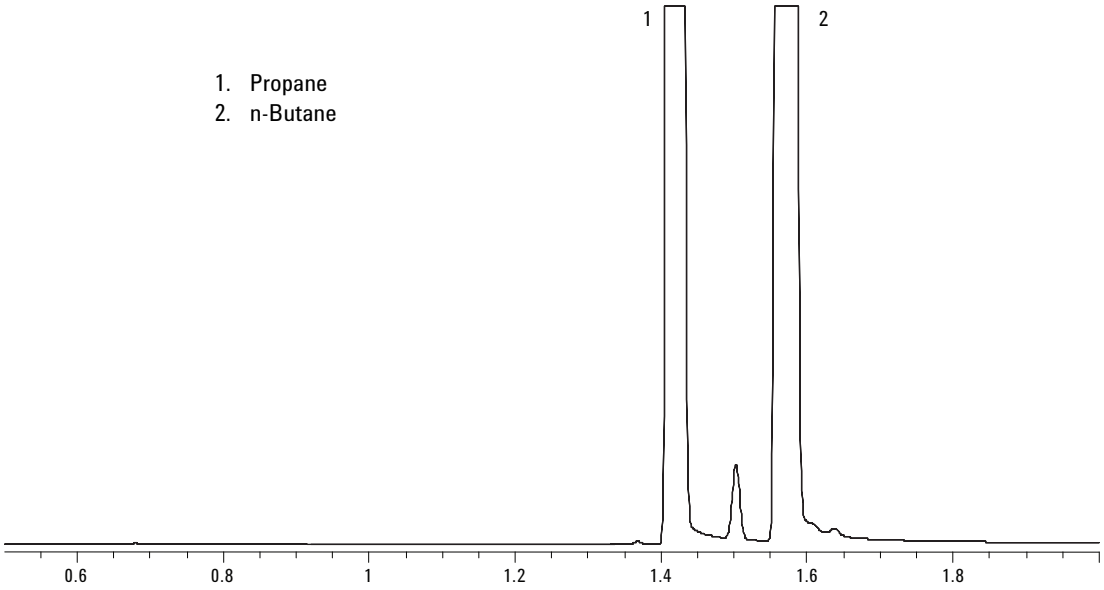


Figure 12. Chromatogram of pressurized propane + n-butane.

Table 4. Quantitative Analysis of Pressurized Propane 50.0% + n-Butane 50.0%. One Percent Difference Between the Blend (actual) and the Analysis Result

	Propane	n-Butane
Response factor	1.03	1.01
Density	0.5139	0.5788
Blend by V%	50.0	50.0
By wt%	47.031	52.969
Analysis		
By area%	45.441	54.559
By wt%	45.927	54.073

F. n-Hexane + 1.0% BP Standard (C5-C18)

To check the quantitative results, a small amount (1.0% BP standard) of C5 to C18 hydrocarbons was added to n-hexane (Figure 13). Table 5 shows the analytical results obtained by adding the C5 to C18 hydrocarbons with both the HPLI device and the automatic liquid sampler (ALS). In Figure 14, chromatograms by HPLI (top) and by ALS (bottom) are shown.

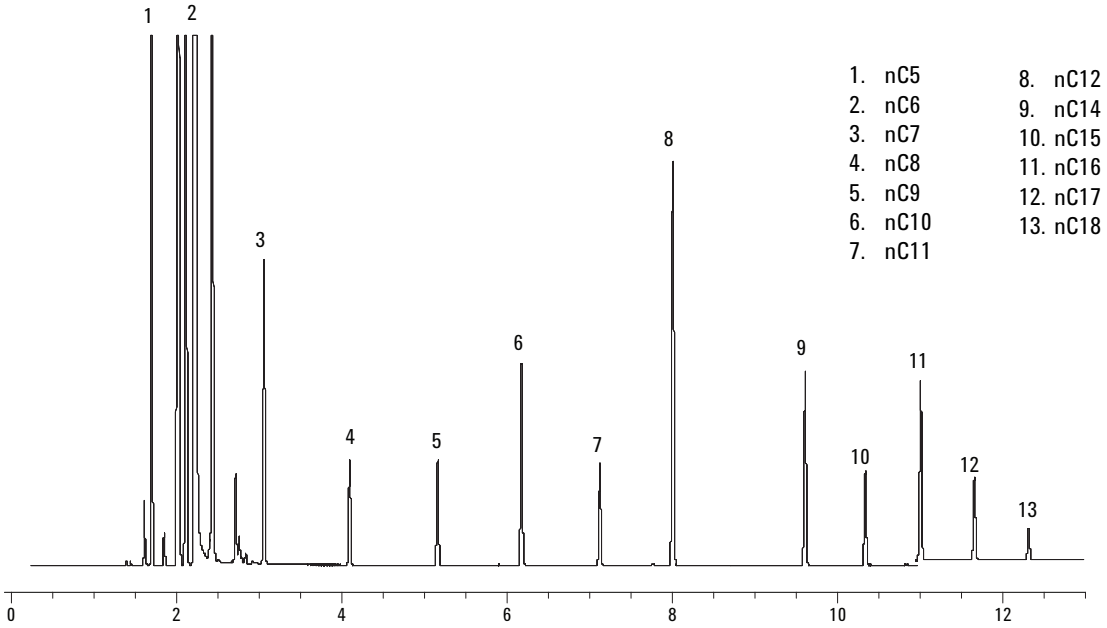


Figure 13. Chromatogram of n-hexane + 1.0% BP standard.

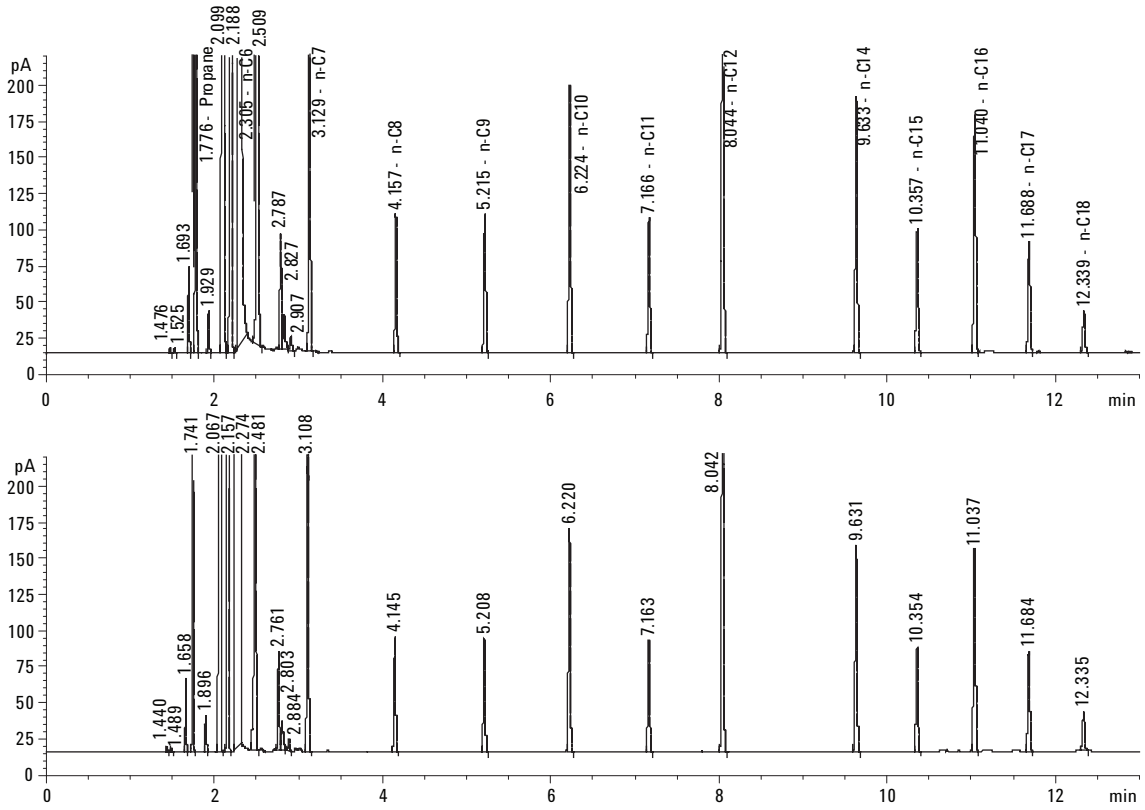


Figure 14. Chromatograms of n-hexane + 1.0% BP standard. Top: HPLI. Bottom: ALS (syringe).

Table 5. Analytical Results for C5-C18 by HPLI and ALS

	HPLI		AUTO INJECTOR	
COMPONENTS	**Area %**	**Width (min)**	**Area %**	**Width (min)**
nC5	0.282		0.279	
nC6	96.950	0.0209	96.922	0.0195
nC7	0.146		0.148	
nC8	0.0524		0.0532	
nC9	0.0537		0.0548	
nC10	0.109		0.111	
nC11	0.0550		0.0559	
nC12	0.219		0.221	
nC14	0.109		0.110	
nC15	0.0532		0.0547	
nC16	0.102		0.109	
nC17	0.0484		0.0546	
nC18	0.0203		0.0239	

The peak width of hexane at top: 0.0209 min
The peak width of hexane at bottom: 0.0195 min

There are no significant differences in quantitative results up to nC14. Compared with the results from an ALS injection, the HPLI device yields results about 10% lower in response above approximately nC16.

G. nC5-nC40 (D2887 BP Standard Diluted by CS_2)

A sample with hydrocarbons (nC5-nC40 D2887 1# BP standard diluted by CS_2) is also run on HPLI. The chromatogram is shown in Figure 15.

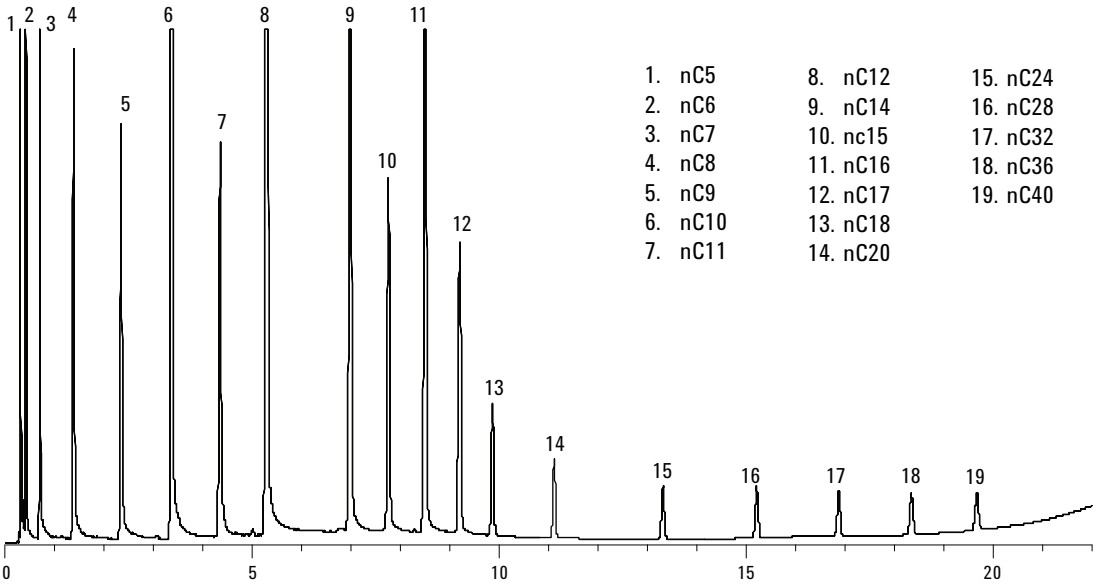


Figure 15. Chromatogram of nC5-nC40 (D2887 BP standard diluted by CS_2).

A lack of discrimination is seen with the HPLI device. In the future, it would be interesting to run some unstable condensates for evaluating the device.

From the above GC evaluation, excellent analytical results could be obtained using the HPLI device. These are summarized below.

1. Excellent repeatability
2. Capable of quantitative results
3. No significant peak width broadening
4. The wide boil point hydrocarbon samples could be analyzed by this device with minimal discrimination.

Conclusions

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC inlet. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

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2. K. J. Rygle, G. P. Feulmer, and R. F. Scheideman, *J. Chromatogr. Sci.,* 22 (1984) 514–519.
3. Jim Luong, Ronda Gras, and Richard Tymko, *J. Chromatogr. Sci.,* 41 (2003) 550–5.

Acknowledgement

Figures 1 through 4 are courtesy of Ronda Gras and Jim Luong, Dow Chemical Canada, Analytical Sciences.

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Printed in the USA
February 26, 2008
5989-6081EN



Agilent Technologies

Simultaneous Analysis of Trace Oxygenates and Hydrocarbons in Ethylene Feedstocks Using Agilent 7890A GC Capillary Flow Technology

Application Brief

James McCurry

The presence of trace hydrocarbons in ethylene can have damaging effects on both the process catalysts and the final polymer products. Test methods such as ASTM D6159 are used to ensure the quality of these feedstocks [1]. However, the analysis of other key contaminants, such as oxygenates, requires GC methods that run on separate instruments. This can be time consuming and expensive for the process analysis lab.

The Agilent 7890A GC serves as the ideal platform when analyzing different classes of trace compounds in ethylene. Maximum productivity can be realized by:

- Using Capillary Flow Technology to perform analysis of trace oxygenates and hydrocarbons in a single run through 2-D Deans switch chromatography.
- Automating the preparation of multilevel calibration standards using the new auxiliary electronic pneumatics control (EPC) modules.
- Protecting the sensitive and expensive alumina PLOT column by preventing polar oxygenates from entering the column.

Enhancing ASTM Method D6159 with Capillary Flow Technology 2-D GC

ASTM Method D6159 uses a methyl silicon column in series with an alumina PLOT column to resolve light hydrocarbons in ethylene. Polar oxygenated compounds cannot be analyzed on this column set because methyl silicon has insufficient selectivity and the alumina column will adsorb oxygenates, resulting in column damage. Wax-type liquid phases such as HP-INNOWax can easily separate polar compounds from light hydrocarbons using 2-D GC [2]. A wax column placed before an alumina column will retain polar compounds while the light hydrocarbons elute near the void volume. Therefore, if a Deans switch is placed between the columns, the hydrocarbons can be heart-cut from the wax to the alumina columns while oxygenates are held by the wax column. The optimized thermal and pneumatic performance of the Agilent 7890A Deans switch is a result of Capillary Flow Technology. This provides the high levels of retention time precision and narrow peak shape needed for optimal heart-cutting 2-D GC (Figure 1).

Highlights

- The Agilent 7890A GC Capillary Flow Technology combined with enhanced electronic pneumatics control (EPC) provide greater productivity and flexibility in the analysis of trace contaminants in ethylene.
- Multiple auxiliary EPC channels provide the ability to automatically generate gas calibration standards for trace level impurities.
- Enhancement of ASTM D6159 method with 2-D GC Deans switching measures trace oxygenates and hydrocarbons in a single run.

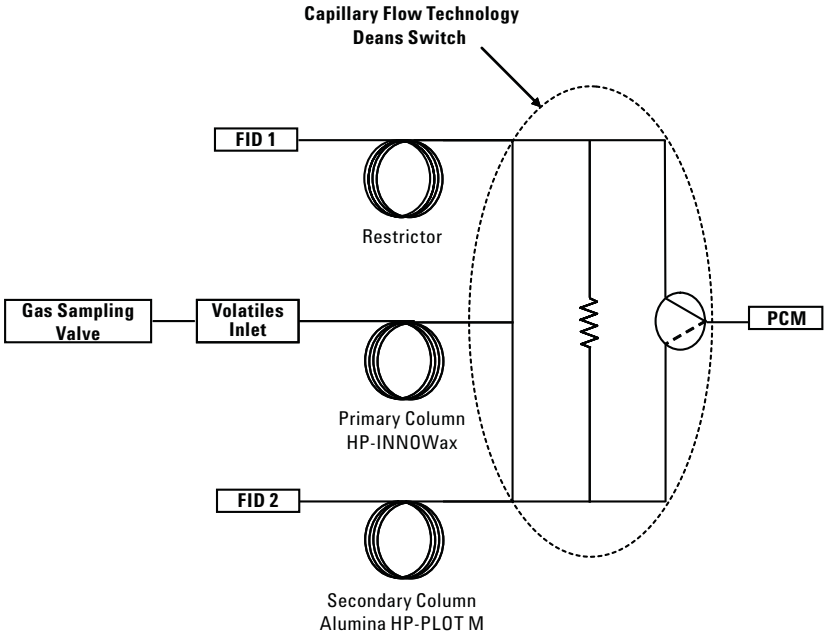

Figure 1. Configuration of Agilent 7890A for the 2-D GC analysis of trace oxygenates and hydrocarbons in ethylene.

Method Parameters for Enhanced ASTM D6159 Method

Primary column:	HP-INNOWax, 30 m × 0.32 mm id × 0.5 µm film (19091N-213)
Primary column flow:	Helium at 2.5 mL/min
Secondary column:	Alumina HP-PLOT M, 30 m × 0.53 mm id × 15 µm (19095P-M23)
Secondary column flow:	Helium at 6 mL/min
Oven temperature program:	40 °C for 6 min, 4 °C/min to 125 °C
Volatiles inlet conditions:	150 °C, 5:1 split
Sample loop:	250 µL at 65 °C
Detector temperature:	250 °C
Capillary Flow Technology: Deans switch cut time	2.3 to 4.5 min

Automating the Preparation of Trace-Level Calibration Standards

Another advantage of the Agilent 7890A GC is the expanded capabilities in EPC. These extra channels of auxiliary EPC are used with the dynamic blending system hardware to allow automated preparation of ppmV gas standards for calibration. This approach has been described for the automated preparation of trace sulfur compounds in various gas matrices [3].

Results

Figure 2 shows the 2-D GC analysis of methanol and C1 to C4 hydrocarbons in a sample of technical grade ethylene. The HP-INNOWax column first separates the polar methanol from the unresolved hydrocarbon peaks. The Deans switch transfers the hydrocarbons to the Agilent alumina HP-PLOT M column, where the C1 to C4 hydrocarbons are easily separated. This column is also shown to provide better separation of trace hydrocarbons from the large ethylene peaks, while maintaining excellent peak shape and intensity for the acetylene. The performance of this alumina column is maintained over many injections since the HP-INNOWax column prevents polar oxygenates (water, alcohols) from damaging the sensitive stationary phase. Table 1 shows very good precision using this method for a sample containing approximately 2 ppmV.

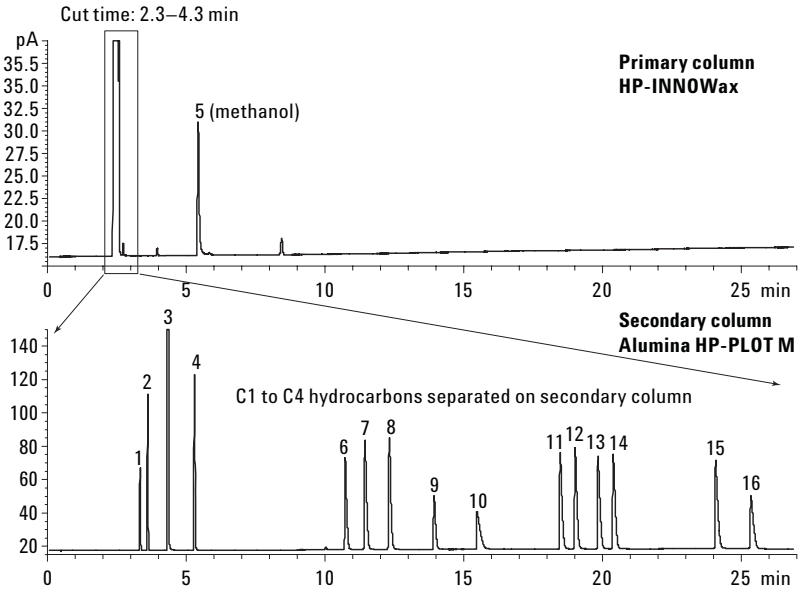

Figure 2. Capillary Flow Technology Deans switch used to separate 100 ppmV oxygenate and hydrocarbon impurities in ethylene.

Table 1. Method Precision for 2-D GC Analysis of Ethylene Impurities

Peak No.	Name	Avg. (ppmV)*	Std Dev*	%RSD*
1	Methane	2.1	0.011	0.5
2	Ethane	21.5	0.049	0.2
3	Ethylene	Balance	Balance	Balance
4	Propane	2.1	0.062	3.0
5	Methanol	2.1	0.081	3.8
6	Propylene	2.1	0.023	1.1
7	Isobutane	2.1	0.015	0.7
8	n-Butane	2.0	0.011	0.5
9	Propadiene	2.1	0.025	1.2
10	Acetylene	1.9	0.036	1.9
11	Tran-2-butene	2.1	0.011	0.5
12	1-Butene	2.0	0.013	0.7
13	Isobutylene	2.1	0.016	0.8
14	cis-2-butene	2.1	0.017	0.8
15	1,3-Butadiene	2.1	0.018	0.9
16	Methylacetylene	2.0	0.015	0.7

*Sample run 20 times

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Printed in the USA
January 5, 2007
5989-6082EN



Agilent Technologies

Parallel GC for Complete Refinery Gas Analysis

Application

Hydrocarbon Processing

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Abstract

An Agilent 7890A gas chromatograph configured with three parallel channels with simultaneous operation provides a complete, high-resolution analysis for refinery gas in six minutes. The system uses an optimized combination of several packed columns and PLOT alumina columns to allow fast separation of light hydrocarbons and permanent gases with the same oven temperature program. A third channel with TCD with nitrogen (or argon) carrier gas improves the hydrogen sensitivity and linearity. This application also shows the excellent performance for natural gas analysis.

Introduction

Refinery gas is a mixture of various gas streams produced in refinery processes. It can be used as a fuel gas, a final product, or a feedstock for further processing. An exact and fast analysis of the components is essential for optimizing refinery processes and controlling product quality. Refinery gas stream composition is very complex, typically containing hydrocarbons, permanent gases, sulfur compounds, and so on. Successful separation of such a complex gas mixture is often difficult using a single-channel GC system. Three parallel channel analyses allow a separation problem to be divided into three sections. Each channel can optimize a particular part of the separation. TCD with helium carrier gas can be used for permanent gases analysis like O_2, N_2, CO, CO_2, H_2S, and COS. However, hydrogen has only a small difference in thermal conductivity compared to helium, making analysis by TCD using helium carrier gas difficult. To achieve full-range capability for hydrogen, an additional TCD with nitrogen or argon as a carrier is required. Light hydrocarbons are separated on an alumina PLOT column and detected on a FID.

The Agilent 7890A GC now supports an optional third detector (TCD), allowing simultaneous detection across three channels; this provides a complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and hydrocarbons to nC_5, C_6+ fraction within six minutes.

Experimental

A single Agilent 7890A GC is configured with three channels, including one FID, and two TCDs. Light hydrocarbons are determined on the FID channel. One TCD with nitrogen or argon carrier is used for the determination of hydrogen and helium. The other TCD with helium carrier is used for the detection of all other required permanent gases. Figure 1 shows the valve drawing. The system conforms to published methods such as ASTM D1945 [1], D1946 [2], and UOP 539 [3].

The FID channel is for light hydrocarbon analysis. The sample from valve 4 is injected via the capillary injector into valve 3 to permit an early back-

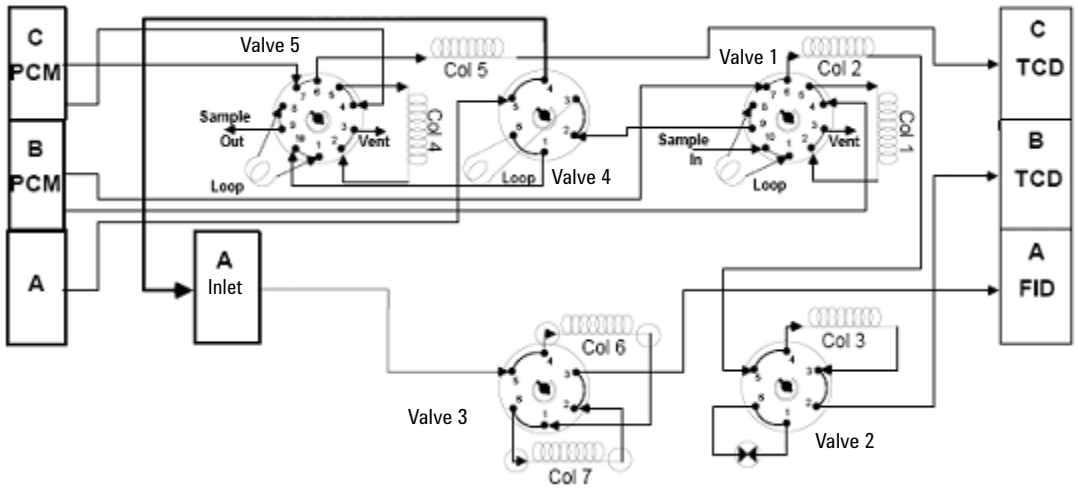
flush of the grouped heavier hydrocarbons (normally C_6+). Valve 3 is a sequence reversal with a short DB1 (column 6) for separating the hexane plus fraction (C_6+) from the lighter components. C_1 through C_5 hydrocarbons are separated on a PLOT alumina column. As soon as the light components C_1 through C_5 pass through the DB1column, valve 3 is switched to reverse the sequence of the DB1 and PLOT aluminum column so that components heavier than nC_6, including nC_6, are backflushed early. As a result, group C_6+ is followed by the individual hydrocarbons from the PLOT alumina column.

A new tube connector based on capillary flow technology is used to connect the valve to the capillary column to enhance the hydrocarbons analysis by improving the peak shape.

The second TCD channel (B TCD) employs three packed columns and two valves for the separation of permanent gases including O_2, N_2, CO, and CO_2 using helium as a carrier gas. Valve 1 is a 10-port valve used for gas sampling and backflushing heavier components; normally components heavier than ethylene are backflushed to vent when H_2S is not required to be analyzed. A six-port isolation valve (valve 2) with adjustable restrictor is used to switch the molecular sieve 5A column in and out of the carrier stream. Initially, the isolated valve is in the OFF position so that unresolved components air, CO, and CH_4 pass quickly through the HayeSep Q (column 2) onto the molecular sieve (column 3). The valve is then switched to the ON position to trap them in column 3 and allow the CO_2 to bypass this column. When the CO_2 has eluted, valve 2 is switched back into the flow path to allow O_2, N_2, CH_4, and CO to elute from the molecular sieve column.

The third TCD channel (C TCD) is for the analysis of H_2. Sample from the 10-port valve (valve 5) is injected into a precolumn (column 4, HayeSep Q) when H_2 with its coeluted compounds O_2, N_2, and CO pass through the short precolumn HayeSep Q onto the molecular sieve 5A column (column 5). Valve 5 is switched so that CO_2 and other compounds will be backflushed to vent, while H_2 is separated on the molecular sieve 5A.

Typical GC conditions for fast refinery gas analysis are listed in Table 1. The refinery gas standard mixture that was used for the method develoment is listed in Table 2.



Column 1 HayeSep Q 80/100 mesh
Column 2 HayeSep Q 80/100 mesh
Column 3 Molsieve 5A 60/80 mesh
Column 4 HayeSep Q 80/100 mesh
Column 5 Molsieve 5A 60/80 mesh
Column 6 DB-1
Column 7 HP-PLOT Al_2O_3
PCM: Electronic pneumatics control (EPC) module

Figure 1. RGA valve system.

Table 1. Typical GC Conditions for Fast Refinery Gas Analysis

Valve temperature	120 °C
Oven temperature program	60 °C hold 1 min, to 80 °C at 20°C/min, to 190 °C at 30 °C/min
FID channel	
Front inlet	150°C, split ratio: 30:1 (uses higher or lower split ratio according to the concentrations of hydrocarbons)
Column	6: DB-1 7: HP-PLOT Al2O3 S
Column flow (He)	3.3 mL/min (12.7 psi at 60 °C), constant flow mode
FID	
Temperature	200 °C
H_2 flow	40 mL/min
Air flow	400 mL/min
Make up (N_2)	40 mL/min
Second TCD channel	
Column	1: HayeSep Q 80/100 mesh 2: HayeSep Q, 80/100 mesh 3: Molecular sieve 5A, 60/80 mesh
Column flow (He)	25 mL/min (36 psi at 60 °C), constant flow mode
Procolumn flow (He)	22 mL/min at 60 °C (7 psi), constant pressure mode
TCD	
Temperature	200 °C
Reference flow	45 mL/min
Make up	2 mL/min
Third TCD channel	
Column	4: HayeSep Q 80/100, mesh 5: Molecular sieve 5A, 60/80, mesh
Column flow (N_2)	24 mL/min, (26 psi at 60 °C), constant flow mode
Procolumn flow (N_2)	7 psi, (24 mL/min at 60 °C), constant pressure mode
TCD	
Temperature	200 °C
Reference flow	30 mL/min
Make up	2 mL/min

Table 2. RGA Calibration Gas Standards

	Compound	% (V/V)		Compound	% (V/V)
1	Methane	5.98	15	i-Pentane	0.101
2	Ethane	5.07	16	n-pentane	0.146
3	Ethylene	2.99	17	1,3-Butadiene	1.46
4	Propane	8.04	18	Propyne	0.476
5	Cyclopropane	0.50	19	t-2-Pentene	0.195
6	Propylene	3.04	20	2-Methyl-2-butene	0.149
7	i-Butane	2.71	21	1-Pentene	0.094
8	n-Butane	2.11	22	c-2-Pentene	0.146
9	Propadiene	0.94	23	n-Hexane	0.099
10	Acetylene	1.72	24	H_2	15.00
11	t-2-Butene	1.55	25	O_2	2.00
12	1-Butene	1.00	26	CO	1.50
13	i-Butene	0.808	27	CO_2	3.00
14	c-2-Butene	1.230	28	N_2	BL

Results and Discussion

Enhance Gas Analysis with Union Connector

The system uses the new union connector based on capillary flow technology for connecting the capillary column to the valve, enhancing the peak shapes in gas analysis and making the connections easier. Figure 2 shows the comparison of peak shapes obtained from a traditional polyamide connector and the new union connecter. With the new union connecter the improvement in peak shape is readily apparent.

Fast Refinery Gas Analysis (RGA)

Use of an optimized combination of several packed columns and a PLOT alumina column allows fast separation of light hydrocarbons and permanent gases with the same oven temperature program without the need of an additional oven.

The separation results from each channel are illustrated in Figure 3.

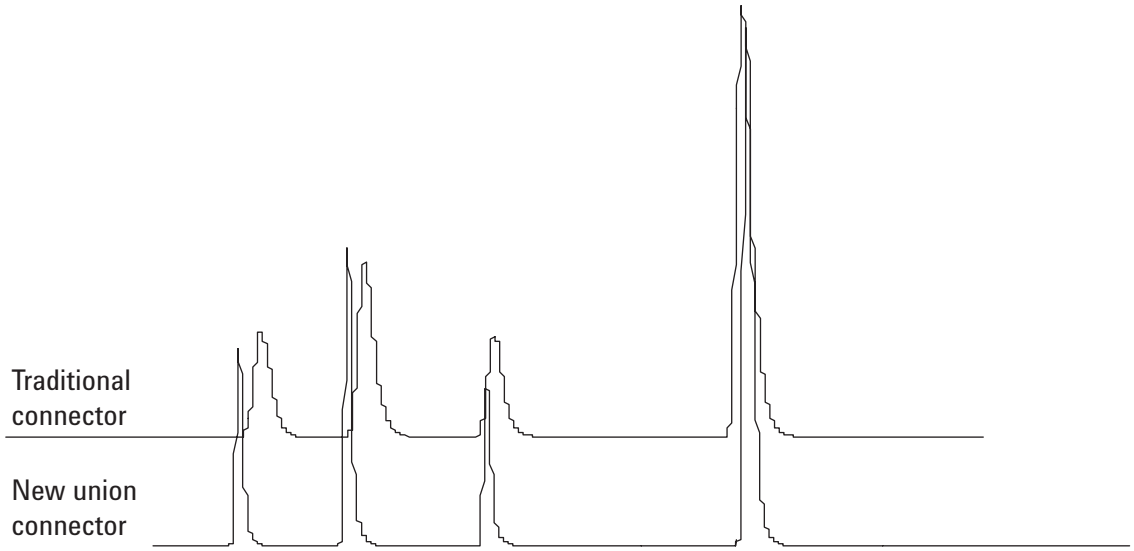


Figure 2. Hydrocarbon peaks obtained from traditional tube connector and new union connector.

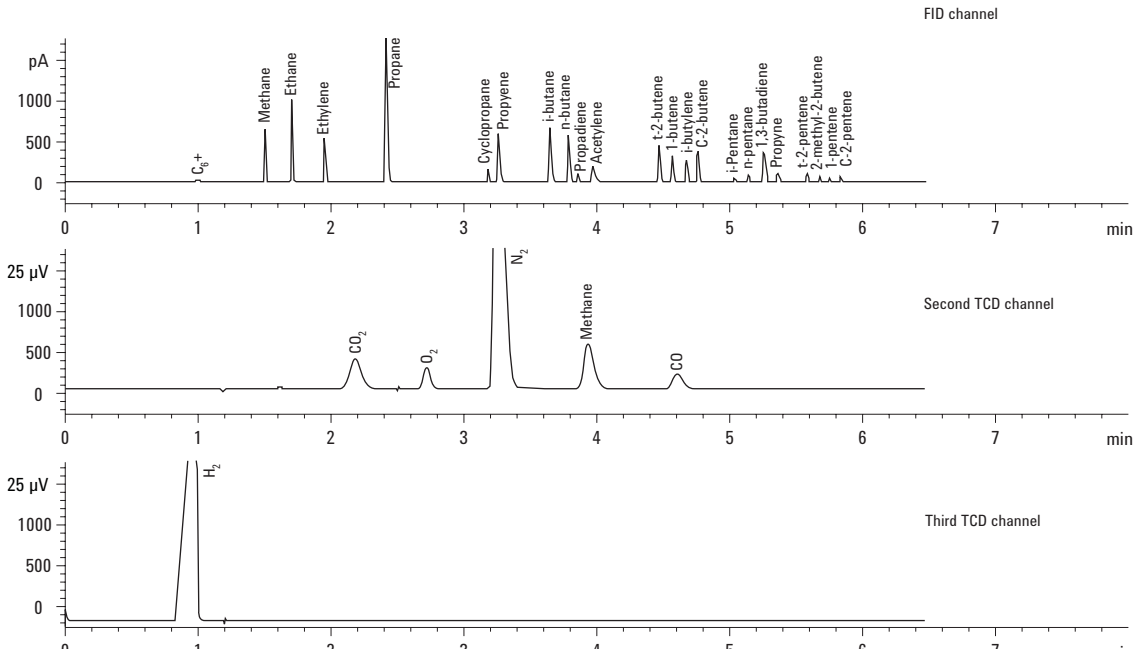


Figure 3. Refinery gas calibration standards analysis. The concentrations for each compound are shown in Table 2.

The top chromatogram (FID channel) is the hydrocarbon analysis. The PLOT alumina column provides excellent separation of hydrocarbons from C_1 to nC_5, including 22 isomers. Components heavier than nC_6 are backflushed early as a group (C_6+) through the precolumn. The middle chromatogram (second TCD channel) is the separation of permanent gases using helium as a carrier gas. The bottom chromatogram (third TCD channel) is the separation of hydrogen, since hydrogen has only a little difference in thermal conductivity compared to helium. Use of an additional TCD with nitrogen (or argon) as a carrier gas improves the hydrogen detectability and linearity.

Table 3 shows very good repeatability for both retention time and area for analysis of the refinery gas standard.

Table3. Repeatability-Refinery Gas Analysis (6 runs) with 1 Run Excluded

	Retention time			Area		
Compounds	**Average**	**Std. dev.**	**RSD%**	**Average**	**Std. dev.**	**RSD%**
C_6+	0.99648	0.00031	0.03	59.01	1.10	1.86
Methane	1.50780	0.00046	0.03	490.02	1.45	0.30
Ethane	1.70788	0.00052	0.03	807.40	2.35	0.29
Ethylene	1.95732	0.00071	0.04	472.31	1.31	0.28
Propane	2.41706	0.00075	0.03	1950.35	5.96	0.31
Cyclopropane	3.18506	0.00075	0.02	145.62	0.45	0.31
Propyene	3.26195	0.00072	0.02	732.90	2.01	0.27
i-butane	3.64883	0.00055	0.02	885.04	3.15	0.36
n-butane	3.79161	0.00070	0.02	682.13	2.59	0.38
Propadiene	3.86098	0.00095	0.02	109.08	0.65	0.60
Acetylene	3.96990	0.00120	0.03	348.17	2.39	0.69
t-2-butene	4.47301	0.00106	0.02	507.88	2.59	0.51
1-butene	4.57118	0.00110	0.02	332.39	2.03	0.61
i-butylene	4.67529	0.00121	0.03	260.95	1.95	0.75
c-2-butene	4.76367	0.00112	0.02	403.80	3.47	0.86
i-pentane	5.03923	0.00090	0.02	45.03	0.05	0.11
n-pentane	5.14583	0.00099	0.02	69.23	0.40	0.58
1,3-butadiene	5.25906	0.00122	0.02	485.49	3.66	0.75
Propyne	5.36385	0.00155	0.03	101.08	0.41	0.40
t-2-pentene	5.58664	0.00121	0.02	82.85	0.66	0.79
2-methyl-2-butene	5.68220	0.00117	0.02	62.54	0.61	0.98
1-pentene	5.75553	0.00126	0.02	39.57	0.38	0.96
c-2-pentene	5.83970	0.00131	0.02	59.08	0.50	0.85
CO_2	2.18561	0.00221	0.10	2040.33	2.37	0.12
O_2	2.72634	0.00060	0.02	930.68	6.53	0.70
N^2	3.25170	0.00044	0.01	22500.18	68.87	0.31
CO	4.61692	0.00083	0.02	903.09	2.77	0.31
H_2	0.9869	0.00099	0.10	16097.38	106.53	0.66

Typical natural gas also can be characterized with the system using the same conditions for the fast RGA. The chromatograms of natural gas on the three channels are shown in Figure 4; hydrogen (3% Mol) and helium (1% Mol) are separated on the third TCD channel.

Flexibility for Hydrocarbon Analysis

The system is very flexible for hydrocarbon analysis. By setting up different valve (valve 3) switch times, the early backflush group can be C_6+ followed by individual C_1 to C_5 hydrocarbons as mentioned in fast RGA, or C_7+ followed by individual C_1 to C_6 hydrocarbons, or no backflush to separate C_1 to C_9 individual hydrocarbons. The top chromatogram in Figure 5 is the result with backflush group of C_6+, the middle one is that of C_7+, and the bottom one is that of no backflush. With such flexibility, a wide range of refinery gas and natural gas compositions can be measured reliably without hardware or column changes.

H_2S and COS Analysis

H_2S and COS (methyl-mercaptan) can be analyzed on the rear TCD channel by adding an additional delay to the backflush time (valve 1) to allow H_2S and COS to elute onto column 2 (HayeSep Q). The analysis time is extended an additional 3 to 4 minutes, and requires a sample containing no water. Figure 6 shows the chromatogram of H_2S at approximately 500 ppm and COS 300 ppm with 1 mL sample size. The Nickel tubing packed columns and Hastelloy-C valves can be chosen for high concentration of H_2S analysis to minimize corrosion.

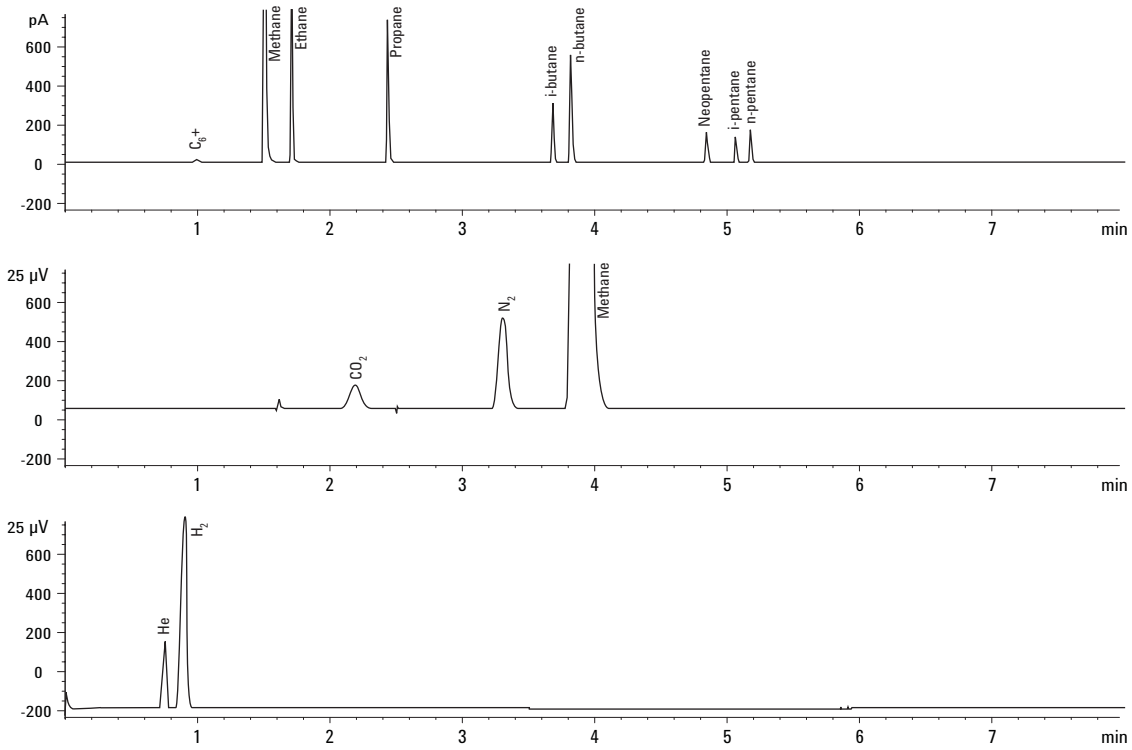


Figure 4. Natural gas analysis of a calibration gas.

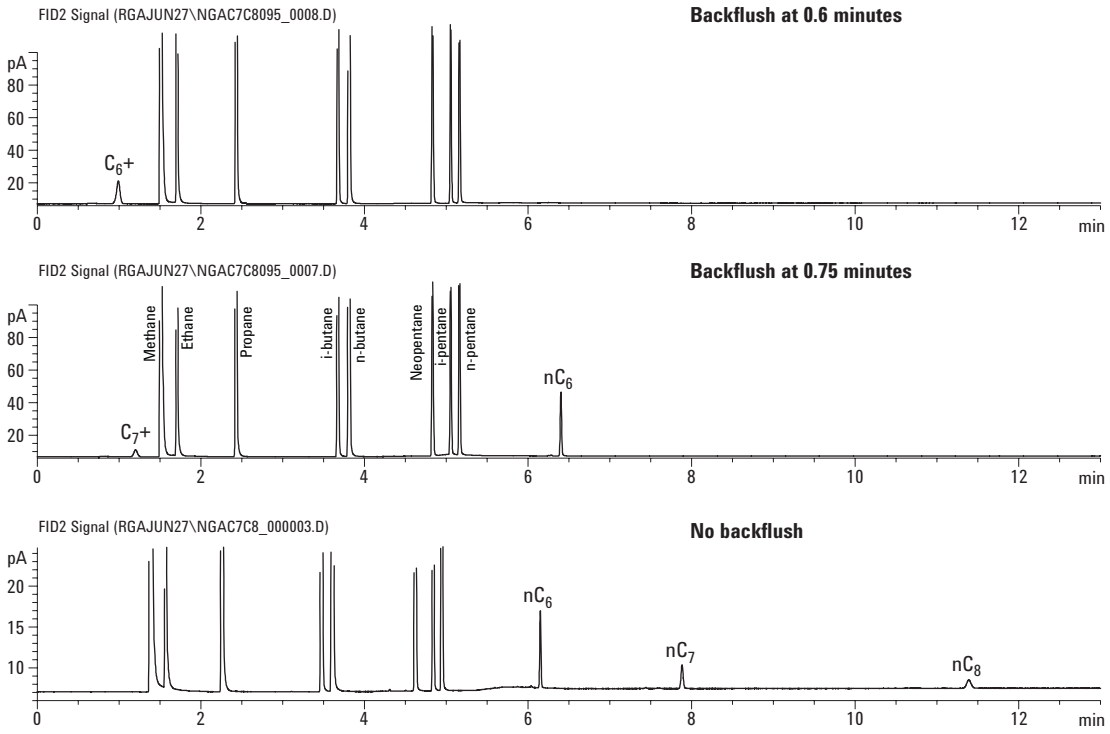


Figure 5. Chromatograms of light hydrocarbons on FID channel with different backflush times .

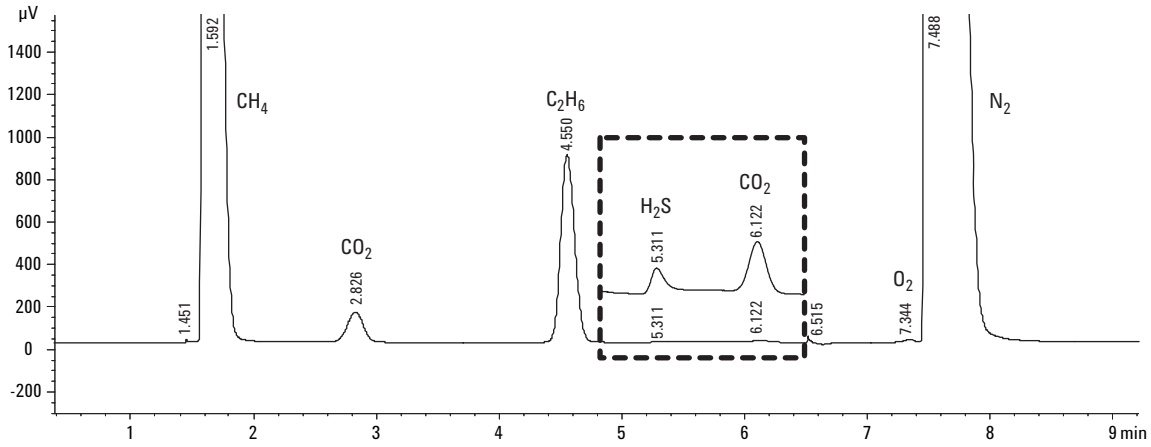


Figure 6. H_2S at approximately 500 ppm and COS 300 ppm on second TCD channel.

Oven program: 50 hold 2 minutes, to 150 °C at 30 °C/min, hold 3 minutes, to 190 °C at 30 °C/min, hold 1 minute
Sample loop: 1 mL

Reporting

A macro program provides automated gas properties calculation. It gives a report in mole %, weight %, volume %, or any combination of the three. If required, heat values for the gas analyzed and other standard calculations are also available. Reports can be calculated using formulas given in the ASTM/GPA or ISO standards.

Conclusions

An exact and fast analysis of the components in refinery gas is essential for optimizing refinery processes and controlling product quality.

One 7890A GC configured with three parallel channels with simultaneous operation provides complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and all hydrocarbons to C_5 and C_6+ as a group within six minutes. A second TCD with nitrogen or argon as a carrier gas improves the hydrogen sensitivity and linearity.

The configuration is very flexible for hydrocarbon analysis, different backflush times may be set to obtain the early backflush group for C_6+ or C_7+, or no backflush to separate C_1 to C_{10} individual hydrocarbons. In these cases, the analysis time is increased by 6 minutes. H_2S and COS can be analyzed on the same GC configuration; it requires 3 to 4 minutes of additional time.

A macro program provides automated gas properties calculation. Reports can be calculated using formulas given in the ASTM/GPA or ISO standards. It gives a report in mole %, weight %, volume %, or any combination of the three.

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References

1. ASTM D1945-03, “Standard Test Method for Analysis of Natural Gas by Gas Chromatography,” ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
2. ASTM D1946-90 (2006), “Standard Practice for Analysis of Reformed Gas by Gas Chromatography,” ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. UOP Method 539, “Refinery Gas Analysis by Gas Chromatography,” ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428, USA.

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Printed in the USA
September 26, 2007
5989-7437EN



Agilent Technologies

Parallel GC for Complete RGA Analysis

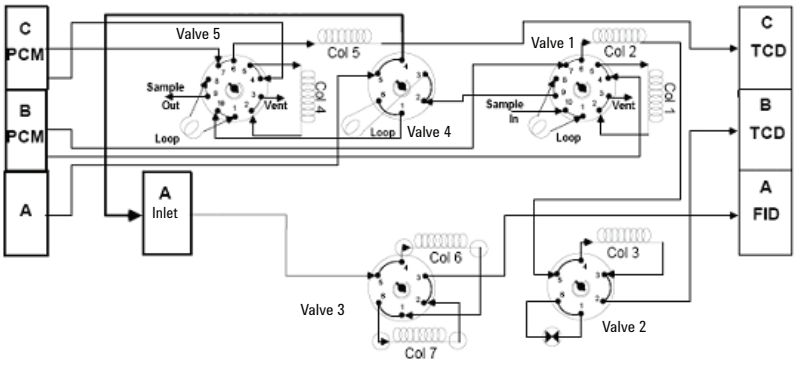
Application Brief

Chunxiao Wang

A previous application brief [1] has shown that a 7890A GC configured with three parallel channels provides a complete refinery gas analysis (RGA) within six minutes. The configuration for fast RGA in the brief has been updated by adding a fifth valve, which can now be supported by the 7890A GC. The updated configuration is almost the same as the previous one except for the third channel (TCD) for H_2 analysis using N_2 or Ar as carrier gas to improve H_2 detectability and linearity. The updated configuration uses a 10-port valve with a pre-column for backflushing late-eluting components while H_2 is separating on the molsieve column instead of a three-way splitter plus split/splitless inlet.

Refinery gases are mixtures of various gas streams produced in refinery processes. They can be used as a fuel gas, a final product, or a feedstock for further processing. The composition of refinery gas streams is very complex, typically containing hydrocarbons, permanent gases, sulfur compounds, etc. An exact and fast analysis of the components is essential for optimizing refinery processes and controlling product quality.

The Agilent 7890A GC now supports an optional detector (TCD), allowing simultaneous detection across three channels. This provides a complete analysis of permanent gases, including nitrogen, hydrogen, oxygen, carbon monoxide,



Column 1 HayeSep Q 80/100 mesh
Column 2 HayeSep Q 80/100 mesh
Column 3 Molsieve 5A 60/80 mesh
Column 4 HayeSep Q 80/100 mesh
Column 5 Molsieve 5A 60/80 mesh
Column 6 DB-1
Column 7 HP-PLOT Al_2O_3
PCM: Electronic pneumatics control (EPC) module

Figure1. RGA valve system.

Highlights

- One 7890A GC configured with three parallel channels with simultaneous detection provides a comprehensive, fast, and high-resolution analysis of refinery gas in 6 minutes.
- Use of optimized columns allows faster analysis of hydrocarbons and permanent gases using a single oven temperature program without the need for an additional column oven.
- A third TCD channel can be used for improving hydrogen detection and linearity by using nitrogen (or argon) as carrier gas.
- A new, easy-to-use union tubing connector based on capillary flow technology is used to connect valves and capillary columns to improve the chromatographic performance, including peak shape.
- Excellent results are achieved. The lowest detection limit is 50 ppm for all compounds, 500 ppm for hydrogen sulfide.
- ChemStation macro program is supplied for RGA reporting.
- The system can be obtained by ordering option SP1 7890-0322 for the standard fast RGA and 7890-0338 for the fast RGA with Hastelloy valves and nickel tubing for H_2S containing samples on the 7890A.

carbon dioxide, and hydrocarbons to nC6. The total run time is less than 6 minutes. The configuration is suitable for most refinery gas streams such as atmospheric overhead, FCC overhead, fuel gas, and recycle gases.

In this analysis, a single Agilent 7890A GC is configured with three channels, including an FID channel and 2 TCD channels. Light hydrocarbons are determined on the FID channel using an alumina column. One TCD is used with nitrogen or argon carrier gas for improved determination of hydrogen and helium; the other TCD is used with helium carrier for the detection of all other required permanent gases. The configuration is shown in Figure 1. An Agilent union tube connector, based on capillary flow technology, is used to quickly and easily connect the valve and capillary column for improved performance. The system conforms to published methods such as ASTM D1945 [2], D1946 [3], and UOP 539 [4].

Separation resulting from each channel is illustrated in Figure 2. The top chromatogram shows the hydrocarbon analysis. A PLOT AL2O3 column provides excellent separation of hydrocarbons from C1 to nC5 containing 22 isomers. Components heavier than nC6 are backflushed early in the run as a group (C6+) through a short DB-1 pre-column.The middle chromatogram shows the separation of permanent gases using helium as the carrier gas on the second TCD channel (B TCD). H_2S and COS can be analyzed on the second TCD channel as well, requiring 3 to 4 additional minutes. The bottom chromatogram shows the separation of hydrogen. Because hydrogen has only a small difference in thermal conductivity compared to helium, it requires an additional TCD with nitrogen or argon as the carrier gas to improve the hydrogen detectability and linearity. All channels operate simultaneously to provide a comprehensive, fast analysis with high resolution of components. A macro program automatically provides the calculation of gas properties. Reports can be generated using formulas specified in the ASTM/GPA and/or ISO standards. Reports in mole%, weight%, volume%, or any combination of the three are available.

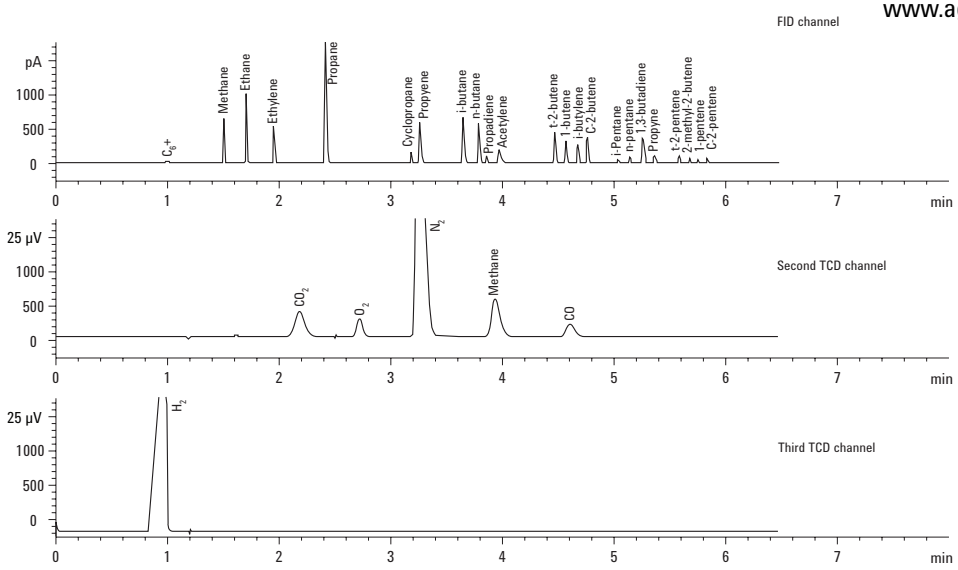


Figure 2. Refinery gas calibration standards analysis.

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Reference

1. Chunxiao Wang, "Parallel GC for Complete RGA Analysis,"Agilent application brief, 5989-6103EN, January 19, 2007
2. ASTM D1945-03, "Standard Test Method for Analysis of Natural Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. ASTM D1946-90 (2006), "Standard Practice for Analysis of Reformed Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
4. UOP Method 539, "Refinery Gas Analysis by Gas Chromatography,"ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428, USA.

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Published in the USA
December 12, 2008
5989-7438EN

High-Pressure Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Accessory G3505A

Introduction

Gas chromatography sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve, has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and an auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Injection Device

The high-pressure injection device (HPLI) consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Company.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

Ordering Information

Order accessory G3505A. The accessory is compatible with both the 7890A and 6890 series GCs.

The following components are recommended. These are not supplied in the accessory kit.

- **Filter:** To remove particles from samples.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

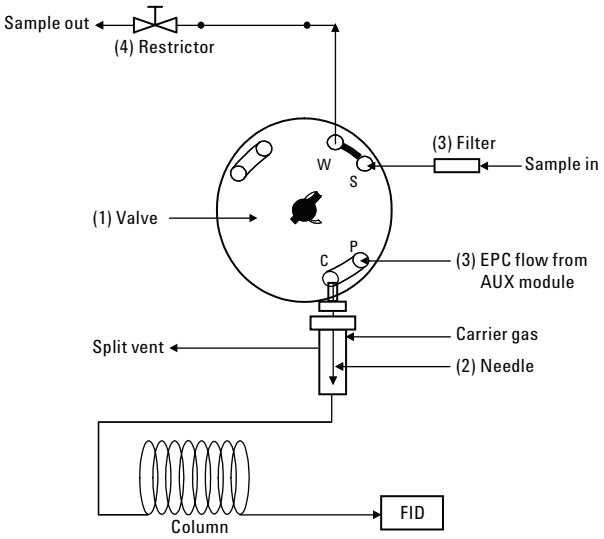


Figure 1. Flow diagram of the high-pressure injection device (HPLI).

Typical Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	High-pressure injection device (HPLI) at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.06 µL
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

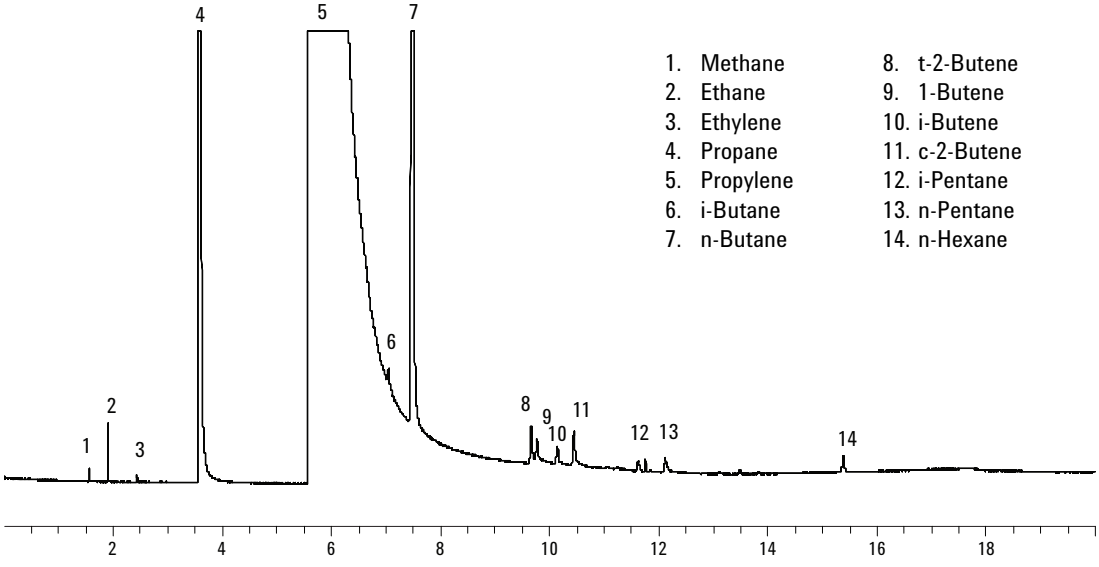


Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Sample Chromatograms

Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159. A typical chromatogram is shown in Figure 2.



1. Methane
2. Ethane
3. Ethylene
4. Propane
5. Propylene
6. i-Butane
7. n-Butane
8. t-2-Butene
9. 1-Butene
10. i-Butene
11. c-2-Butene
12. i-Pentane
13. n-Pentane
14. n-Hexane

Figure 2. Chromatogram of pressurized propylene.

Pressurized 1,3-Butadiene

Figure 3 is an example of C4 hydrocarbons analysis showing 1.3 butadiene purity.

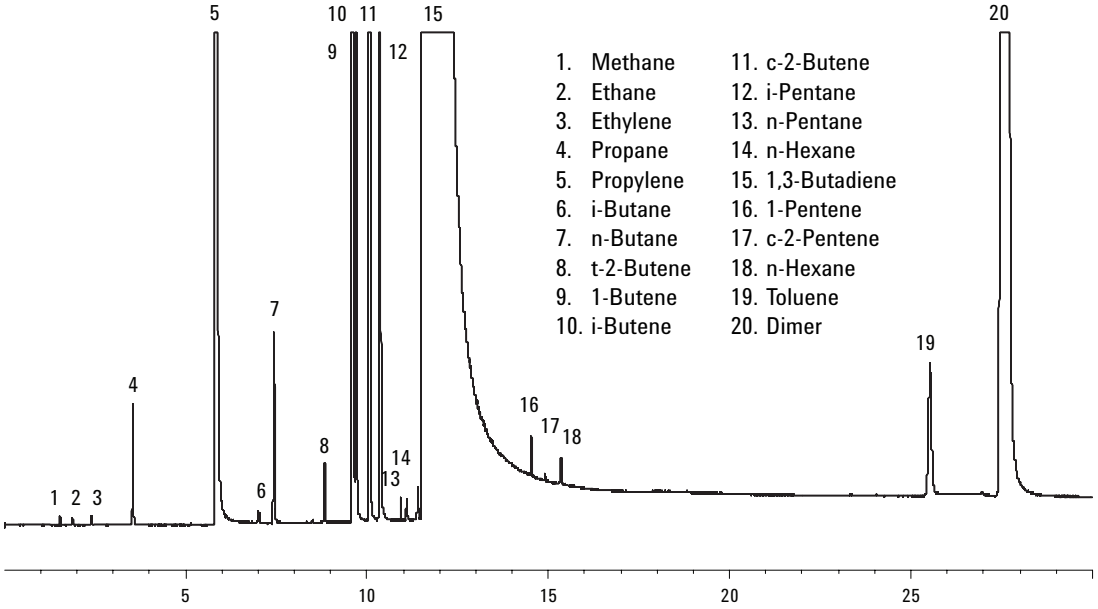


Figure 3. Chromatogram of pressurized 1,3-butadiene.

Summary

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC split/splitless inlet in a few minutes. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

Competitive Advantages

The HPLI can be used with a wide variety of sample streams or pressurized vessels. Because the sampling valve is interfaced directly to the inlet with an inert needle, loss or adsorption of analytes is minimized compared to conventional liquid sample valve systems. Compared to other gas chromatographic vaporizers for handling pressurized or nonpressurized samples, the Agilent HPLI has the following advantages:

- Better results with polar analytes such as glycols
- Superior inertness
- Low discrimination (no discrimination up to C_{16})
- Flexibility: Install or uninstall in less than 10 minutes
- Good for trace impurity analysis with 0.5 µL rotor
- Excellent repeatability, typically RSDs below 1 %

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Printed in the USA
February 25, 2008
5989-8037EN



Agilent Technologies

Detection of Sulfur Compounds in Natural Gas According to ASTM D5504 with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) on the 7890A Gas Chromatograph

Application

Hydrocarbon Processing

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Abstract

An Agilent dual plasma sulfur chemiluminescence detector (DP SCD) combined with an online dilutor was used for the analysis of sulfur compounds. By using this method, the detection limits of the sulfur compounds achieved the ppb level. The stability of the DP SCD was also investigated. The long-term and short-term stability show that the performance of DP SCD is stable, and no hydrocarbon interference was found during the analysis of natural gas samples.

Introduction

Many sources of natural gas and petroleum gases contain varying amounts and types of sulfur compounds. The analysis of gaseous sulfur compounds is difficult because they are polar, reactive, and present at trace levels. Sulfur compounds pose problems both in sampling and analysis. Analysis of sulfur compounds many times requires special treatment to sample pathways to ensure inertness to the reactive sulfur species. Sampling must be done using containers proven to be nonreactive. Laboratory equipment must also be inert and well conditioned to ensure reliable results. Frequent calibration using stable standards is required in sulfur analysis [1].

GC SCD configuration with inert plumbing is one of the best methods to detect sulfur compounds in different hydrocarbon matrices. Sulfur compounds elute from the gas chromatographic column and are combusted within the SCD burner. These combustion products are transferred to the SCD detector box via vacuum to a reaction cell for ozone mixing. This detection technique provides a highly sensitive, selective, and linear response to volatile sulfur compounds.

Agilent Technologies DP technology is the detector of choice for sulfur analysis when dealing with a hydrocarbon matrix. The burner easily mounts on the 6890 and 7890A GCs and incorporates features for easier and less frequent maintenance. In this application, the Agilent 355 DP SCD was used to analyze the gaseous sulfur compounds in natural gas. Detection limits, stability and linearity were investigated.

Experimental

An Agilent 7890A GC configured with a split/splitless inlet (Sulfinert-treated), and an Agilent 355 DP SCD were used. Sample introduction was through a six-port Hastelloy C gas sample valve (GSV) interfaced directly to the sulfur-treated inlet with Sulfinert tubing. An online dilutor was used for preparation of ppb-level sulfur compounds in

different matrices. Two four-port valves were used — one for sample introduction and one for static sample injection. The valves were installed sequentially prior to the GSV. Figure 1 illustrates the configuration of the gas blending system and GC SCD.

The sulfur standards were blended in helium at 1 ppm (V/V) and were purchased from Praxair, Inc. (Geismar, LA). See Table 1 for component details.

Table 1. Sulfur Standards in Helium

1.	Hydrogen sulfide	H_2S
2.	Carbonyl sulfide	COS
3.	Methyl mercaptan	CH_3SH
4.	Ethyl mercaptan	CH_4CH_3SH
5.	Dimethyl sulfide	CH_3SCH_3
6.	Carbon disulfide	CS_2
7.	2-propanethiol	$CH_3SHC_2H_5$
8.	Tert-butyl mercaptan	$(CH_3)_3CSH$
9.	1-propanethiol	$CH_3(CH_2)_2SH$
10.	Thiophene	C_4H_4S
11.	n-butanethiol	$CH_3(CH_2)_3SH$
12.	Diethyl sulfide	$CH_3CH_2SCH_2CH_3$
13.	Methyl ethyl sulfide	$CH_3SCH_2CH_3$
14.	2-methyl-1-propanethiol	$(CH_3)_2CHCH_2SH$
15.	1-methyl-1-propanethiol	$CH_3CH_2CHSHCH_3$

Experimental Conditions

GC Conditions

Front Inlet	Split/splitless (Sulfinert-treated capillary inlet system)
Heater	150 °C
Pressure	14.5 psi
Septum purge flow	3 mL/min
Mode	Splitless
Gas saver	20 mL/min after 2 min
Sample loop	1 mL
Oven	30 °C (1.5 min), 15 °C/min 200 °C (3 min)
Column	HP-1 60 m × 0.53 mm × 5 µm
Injection mode	Static flow and dynamic flow modes

SCD Conditions

Burner temperature	800 °C
Vacuum of burner	372 torr
Vacuum of reaction cell	5 torr
H_2	40 mL/min
Air	53 mL/min

Results and Discussion

From the comparative results of the sulfur detectors' sensitivity, it could be seen that SCD is the best detector for sulfur components, especially at low levels [3]. The Agilent DP technology is the most sensitive and selective detector for sulfur-containing gaseous hydrocarbon samples.

Figure 2 is the chromatogram of low-level sulfur compounds at 1.35 ppb (H_2S), which is prepared by the point-of-use gas blending system. Table 2 is the calculated signal to noise (S/N) of each compound, from the achieved data. It can be seen that DP SCD can detect low-level sulfur compounds.

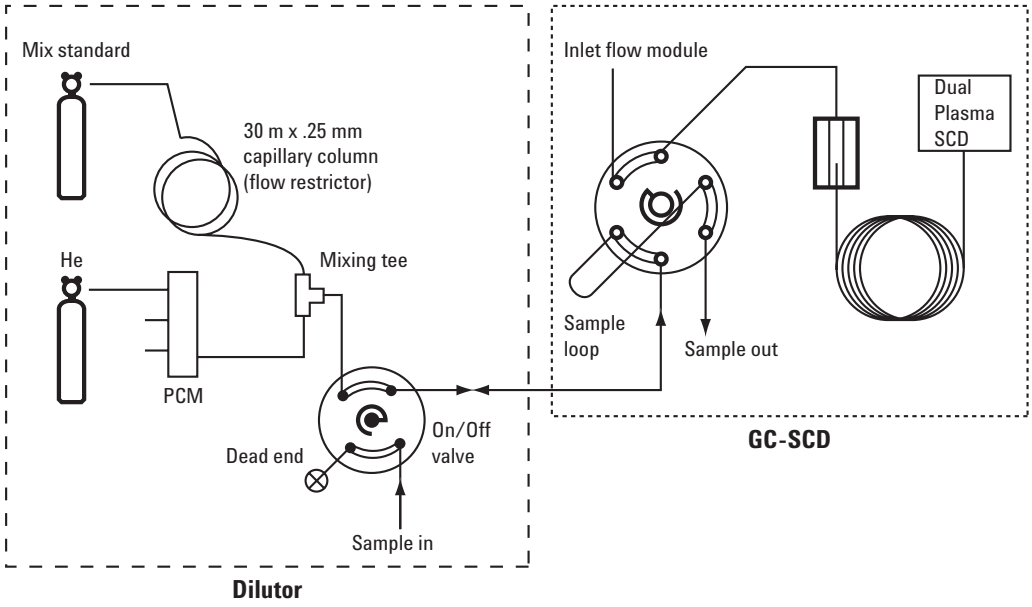

Figure 1. Diagram of online dilutor GC-DP SCD.

Table 2. S/N of Each Sulfur Component at 1.35 ppb (Refer to Table 1 for peak identification.)

Peak No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S/N	12.0	5.0	2.1	2.6	4.9	11.5	4.0	2.7	3.7	9.1	7.6	2.3	5.7	1.0	1.1

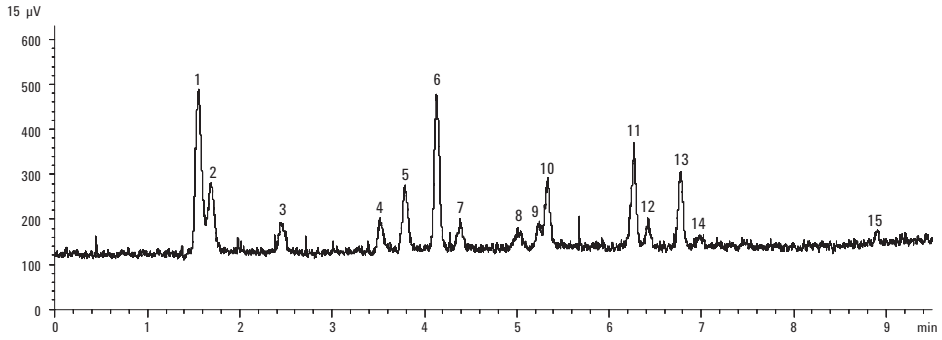

Figure 2. Chromatogram of sulfur compounds in helium at 1.35 ppb. (Refer to Table 1 for peak identification.)

Because the low-level sulfur components were prepared by the online dilutor system, which was prepared by adjusting the aux EPC to get appropriate diluent flow, high diluent flow could have the potential to cause high pressure in the sample loop, which results in the amount of the sample in the loop being different when the diluent flow changes from low to high. In this application, two sample injection modes, static and dynamic, were investigated. The mode is actuated by the on/off valve installed prior to GSV. When using static injection mode, the valve is switched to the off position, the pressure in the sample loop balances to ambient pressure, and then the sample is injected into the GC.

Table 3 shows the linear ranges of the two injection modes. The two injection modes have no difference from a linearity perspective, which means that the two injection modes are both suitable when using the 1-mL sample loop. The 1-mL sample loop's resistance is not high enough to cause variation in the sample injection amount.

Table 3 Linear Ranges of Two Injection Modes (Refer to Table 1 for peak identification.)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Linear range (ppb)				**6.24-544.5**				
Static mode	1	0.99996	0.99995	0.99999	0.99996	0.99999	0.99996	0.99999
Dynamic mode	1	0.99996	0.99997	0.99997	0.99996	0.99999	0.99998	0.99998
	9	**10**	**11**	**12**	**13**	**14**	**15**	
Linear range (ppb)				**6.24-544.5**				
Static mode	0.99995	0.99994	0.99996	0.99996	0.99996	0.99998	0.99998	
Dynamic mode	0.99998	0.99997	0.99998	0.99998	0.99998	1	0.99998	

Table 4 shows the long-term (72 hours) and short-term (8 hours) stability of the SCD at different concentration levels.

In an effort to investigate the coelution of hydrocarbon and sulfur, the same sulfur standards in natural gas were analyzed on the SCD. Figure 3 shows the chromatogram; no quenching was found.

Table 4 The Long-Term and Short-Term Stability of SCD (Refer to Table 1 for peak identification.)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
20.79 ppb	2.7	2.6	2.9	3.0	0.9	1.4	2.8	4.0	2.6	1.7	1.7	3.3	3.2	8.6	7.9
S.T. RSD (%)															
L.T. RSD (%)	3.0	2.7	2.4	2.5	1.4	1.5	2.6	4.3	3.8	2.7	2.0	4.9	3.2	7.9	6.9
1.38 ppb	6.6	10.1	11.7	22.8	30.4	4.1	6.9	18.7	10.7	25.1	5.1	11.1	5.8	29.6	24.1
S.T. RSD (%)															
L.T. RSD (%)	14.4	7.5	16.3	20.8	21.7	4.6	6.1	27.7	23.7	25.3	12.2	24.6	6.1	35.7	38.4

ST: Short term (8 hours); LT: Long term (72 hours)

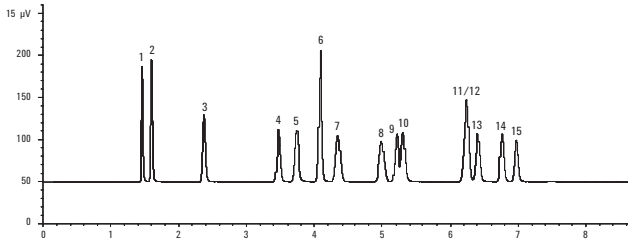

Figure 3. Chromatogram of sulfurs in natural gas. (Refer to Table 1 for peak identification.)

Natural Gas Sample Analysis

Three natural gas samples were analyzed by using the GC DP SCD system. Because the concentration of the target compounds is at ppm level, split mode was used and the method was recalibrated at ppm level. Table 5 shows the result of the three gas samples.

Table 5. Result of the Three Real Samples

Samples		H_2S	COS	Methyl Mercaptan
BLEND AL	Conc. (ppm, v/v)	2.3	2.0	2.0
	RSD (%, n = 5)	2.3	0.3	1.4
BLEND 6	Conc. (ppm, v/v)	27.1	21.9	17.3
	RSD (%, n = 5)	1.2	0.4	2.3
BLEND 12	Conc. (ppm, v/v)	15.0	9.2	10.1
	RSD (%, n = 5)	0.7	0.6	0.6
Standard	Conc. (ppm, v/v)	2.0	0.8	0.9
natural gas	RSD (%, n = 5)	1.7	2.5	1.7

Conclusions

An online dilutor combined with a GC DP SCD is suitable for gaseous sulfur components analysis, especially for the low-level components. The online dilutor offers an automatable means of system calibration and the detection limits for the trace sulfur detection are down to ppb level. By using an on/off valve prior to the GSV, both the static and dynamic injection modes of the sample gas blending system can be used. The static injection mode is important when a small sample loop with a large resistance is used. The diluter system with GC/SCD is available as an Agilent SP1, please refer to SP1 7890-0375 for order information.

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Printed in the USA
August 12, 2008
5989-9234EN

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Optimized Analysis of Gasoline (BTEX) in Water and Soil Using GC/FID with Purge and Trap

Application Note
228-324

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Abstract

Gas chromatography with purge and trap analysis using their HP-1 capillary column and the Agilent 5890 Series II gas chromatograph/flame ionization detector was done to determine gasoline components in contaminated water and soil in accordance with modified EPA Methods 8015/8020. Purge and trap and gas chromatograph parameters were optimized for accurate quantitation of gasoline range organics (aliphatics, aromatics, and oxygenates) and to increase analysis speed.

Key Words

- EPA Methods 8015/8020
- Gasoline
- BTEX
- Purge and Trap
- GROs
- GC/FID analysis
- LUST

Introduction

Modified EPA Methods 8015/8020 are used to determine gasoline and gasoline components in water and soil by capillary gas chromatography (GC) with a flame ionization detector (FID) or photoionization detector (PID). The hydrocarbons in gasoline encompass a wide range, from butane to decane and benzene to naphthalene, and cover a boiling point range of 50°C to 281°C. For such complex mixtures, an efficient purge and trap (P and T) system is required to concentrate samples for high-resolution gas chromatography. Detection is achieved using an FID, and quantitation is based on FID response to a gasoline standard. Other light petroleum products that can be determined in the same manner include paint stripper, Stoddard solvent, mineral spirits, petroleum naphtha, and aviation jet fuels using the pattern recognition technique.

The analysis of gasoline components, e.g., gasoline range organics (GROs), and benzene, toluene, ethylbenzene, and xylenes (BTEX) in particular is of great importance because BTEX is frequently used as a marker in the identification of gasoline-type products. Subsequently, the analysis of BTEX is often used to determine the composition and the origin of such products including weathered fuels leaking from underground storage tanks (LUST), spills in pipe lines, and run-off from surface transportation.

For the analysis of gasoline with BTEX, the sample is introduced into a sparge tube on the P and T autosampler or purge vessel or the P and T unit. The P and T concentrates the volatiles in the sample and transfers them onto the capillary column.

Parameters affecting the efficiency of P and T sample concentration include time and temperature for sample purge, dry purge, desorption of trapped volatile organics and trap baking. Most P and T system manufacturers recommend 11 minutes of purge or a total of 440 ml purge gas through the sample. Many laboratories use the manufacturer's set purge flow of 40 ml/min which corresponds to 11 minutes of purge time, to achieve a minimum of 440 ml purge gas through the sample. In this study a Vocarb-3000 trap was used because it can provide higher trapping efficiency and allow for higher desorption and baking temperature.

A typical analysis can usually be completed in 35 to 40 minutes. In this application both P and T parameters and GC conditions were optimized for accurate quantitation and analysis speed.

Experimental

Samples were concentrated using an Agilent 7695A P and T system with a Vocarb-3000 trap (part no. 5182-0775) and a 5-ml frit sparger (part no. 5182-0852). Using an HP-1 column (30 m x 0.53 mm x 5.0 µm, (part no. 19095Z-623), hydrocarbons were analyzed on an Agilent 5890 Series II GC with EPC and FID. Instrument requirements and optimal GC and P and T conditions are listed in **Table 1**.

Working solutions were prepared from diluting commercial gasoline, LUST-modified GROs (part no. 5182-0860), and internal standard and surrogate (part no. 8500-6007) with GC-grade methanol (Burdick and Jackson). Concentrations of GROs, gasoline, and jet fuel standards are listed in **Table 2**.

Samples were prepared from spiking 5 ml of organic-free reagent water using a 5-ml sample syringe with a luer connector (part no. 9301-1185) with standard solutions using 5-µl to l00-µl fixed needle syringes (HP part nos. 9301-0810, 9301-0818, 9301-0059, 9301-0063, respectively).

Results and Discussion

To obtain accurate and reproducible results, complete sample purging, managing water adequately from the P and T system, and preventing carry-over from the trap are essential. Many environmental laboratories analyze gasoline with BTEX using long sample purge (11 to 15 minutes), dry purge (2 to 4 minutes), trap desorb (2 to 4 minutes), and trap bake (10 to 20 minutes) times. Therefore, a typical run usually takes 40 to 48 minutes including 3 to 5 minutes for trap cool-down.

Figure 1 shows a GC/FID analysis of a gasoline standard and a GC/PID chromatogram of a GROs standard using an OI 4460A P and T system with a BTX trap and DB-1 column (30 m x 0.53 mm x 5 µm). GC and P and T conditions are listed in **Table 3**. Although the GC runs were completed in 27 minutes, the actual cycle time for each run was 37 to 40 minutes.

Table 1. Instrument Requirements and Optimized Conditions

A. Recommended Instrumentation

Gas chromatograph:	5890 Series II
Injection port:	Split/splitless inlet
Column:	HP-1, 30 m x 0.53 mm x 5.0 µm (Part no. 19095Z-623)
Detector:	FID
Injection technique:	7695A P and T
Data system:	3365 ChemStation and HP Vectra 486/100MX

B. Experimental Conditions

GC Parameters

Inlet:	220°C, split injection (split ratio 5:1)
Carrier:	Helium, 10 ml/min, constant flow (6.5 psi at 40°C)
Oven parameters:	40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min
Detector:	FID, 300°C; nitrogen makeup gas, 25 ml/min; H_2, 30 ml/min; and air, 350 ml/min PID, 250°C

P and T Parameters

Line temperature:	200°C	Purge time:	11 min
Valve temperature:	200°C	Dry purge time:	1 min
Mount temperature:	40°C	Desorb time:	2 min
MCS line temperature:	100°C	Bake time:	5 min
Purge ready temperature:	30°C	BGB time:	2 min
MCS desorb temperature	40°C		
Desorb preheat temperature:	245°C		
Desorb temperature:	250°C		
Bake temperature:	265°C		
MCS bake temperature:	300°C		

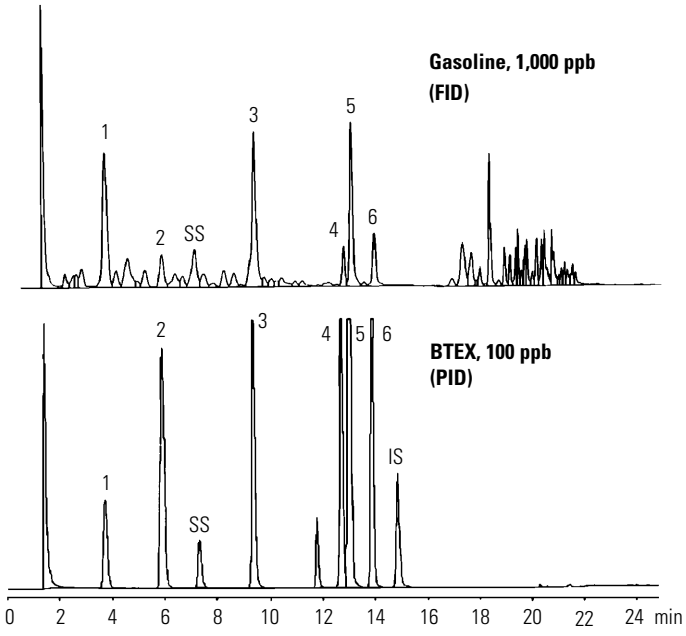


Figure 1. Typical chromatograms of gasoline and GROs standards using a DB-1 column under the GC and P and T conditions (Table 3) used in environmental testing laboratories (see Table 2 for peak identification).

Optimized GC Run Time

With the HP-1 column (30 m x 0.53 mm id x 5 µm) and a faster oven temperature, the GC run time was initially reduced to 21 minutes for GROs and gasoline (see **Figure 2**). Good baseline separations and sharp symmetric peaks **(Figure 2B)** were obtained for all GROs, including surrogate (α,α,α-trifluorotoluene) and internal (4-bromofluorobenzene) standard. The oven temperature program used was 40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min and a constant carrier flow of 10 ml/min. Under these conditions (**Table 1**), both pentane and MtBE were clearly separated from the large solvent peak (menthanol).

Even though the last GROs component (naphthalene) eluted below 200°C at 17.8 minutes, the oven temperature was increased to 250°C to bake out the high-boiling material purged from the sample. As a result, no carryovers were found even with repeated injections of gasoline standard in the 23,000-ppb level.

GC run times were further lowered by using a thinner-film HP-1 column and/or faster oven temperature programs. **Table 4** shows the benefits of using various column thicknesses, temperature ramps, and carrier flows to achieve the optimal GC run time of 17 minutes. Analytes generally elute faster from a thin-film column (**Figure 4**). In **Figure 3**, the thick-film column retained hydrocarbons longer initially until the faster oven temperature ramp (15°C/min) sped up the elution of all GROs components from the column. To avoid potential coelution (peaks 4 and 5), a comparative smaller carrier flow (4.5 ml/min) was used instead of the optimal 10 ml/min carrier flow. Reducing the GC run time, however, would be counterproductive because the total run time is dependent on the P and T cycle.

Table 2. Analytes in Working Standards

Standards	Peak No.	Components	Concentration
GROS mix	1	MtBE	100 ppm each
	2	Benzene	
	3	Toluene	
	4	Ethylbenzene	
	5	m-/p-Xylene	
	6	o-Xylene	
	7	1,2,4-Trimethylbenzene	
	8	1,3,5-Trimethylbenzene	
	9	Naphthalene	
	10	α,α,α-Trifluorotoluene (SS)	
	11	4-Bromofluorobenzene (IS)	
Gasoline standard		Gasoline	500 ppm
Gasoline		Gasoline	2,500 ppm
Jet fuel		Aviation jet fuel	1,000 ppm

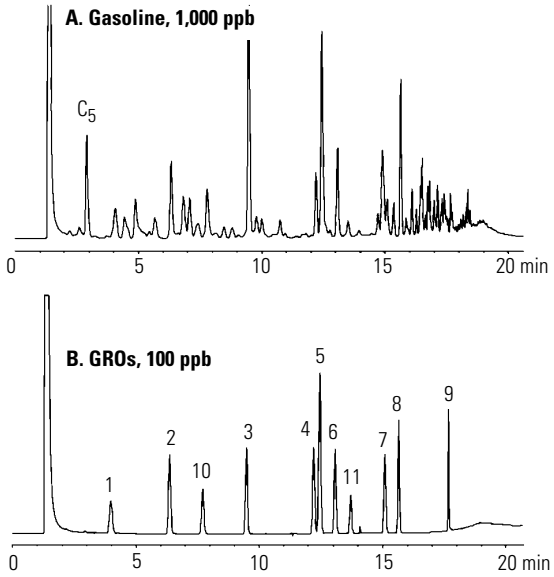


Figure 2. Chromatograms for gasoline and GROs standards using an HP-1 column under the optimal GC and P and T conditions listed in Table 1. (See Table 2 for peak identification.)

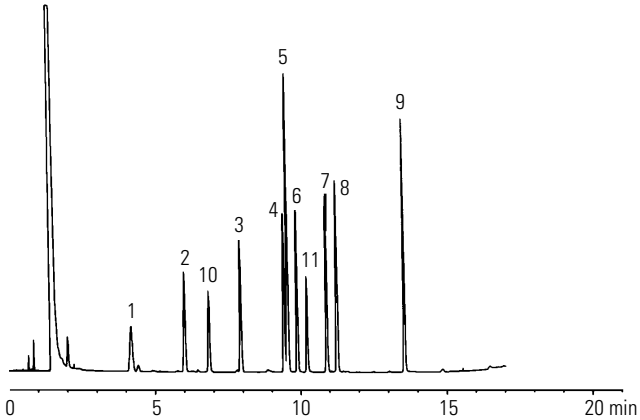
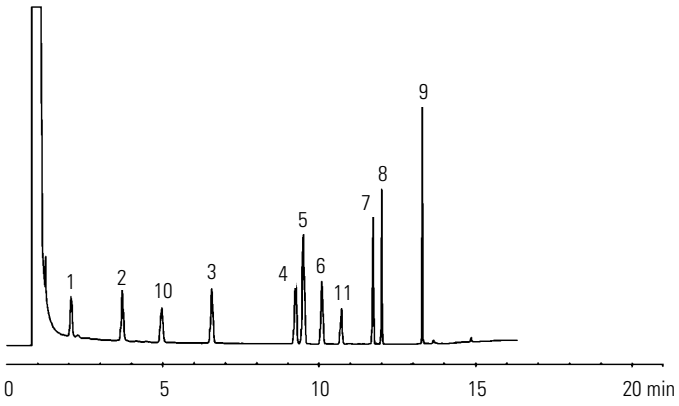


Figure 3. Chromatogram of GROs standards using a thick-film HP-1 (30 m x 0.53 mm x 5 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)

Table 3. Typical GC and P and T Conditions for Gasoline and BTEX Analysis

GC Parameters			
Injection:	Direct injection		
Carrier flow:	Initially 10 ml/min, constant pressure mode		
Oven temperature:	50°C (hold 3 min) to 125°C at 5°C/min to 240°C (5 min) at 45°C/min		
Detector:	PID (250°C) in series with FID (300°C)		
P and T Parameters			
Trap:	BTX trap		
Purge temperature:	Ambient	Purge time:	11 min
Dry purge temperature	22°C	Dry purge time:	2 min
Desorb preheat temperature	150°C	Desorb time:	4 min
Desorb temperature	180°C	Bake time:	15 min
Bake temperature:	200°C		

Figure 4. Chromatogram of GROs standards using a thin-film HP-1 (30 m x 0.53 mm x 3 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)



Optimized P and T Cycle Time

Further optimization of the run was dependent on obtaining the most efficient parameters for the P and T cycle. Each aspect of the cycle was optimized as follows.

Sample Purge

Experimentation showed 11 minutes of purge time, or 440 ml of helium purge gas, to be the most efficient time for analyses of gasoline and GROs because shorter purge times (8 minutes or 320 ml of purge gas) were not sufficient to purge all GROs from the sample solution. **Figure 5** shows a comparative analysis of the same GROs standard shown in **Figure 2B** using 8 minutes of purge time instead of 11 minutes of purge time. The conditions for both analyses were the same and are shown in **Table 1**. By comparison, hydrocarbon recoveries (including aromatics) for the GC runs with 8 minutes of sample purge were not as good particularly for the high-boiling fractions, such as trimethylbenzenes and naphthalene (compare peaks 7, 8, and 9 in **Figure 5** and **Figure 2B**). The naphthalene peak in **Figure 5** (8 minutes of purge) was remarkably small, and area counts were lower than 1% of that recovered in **Figure 2B**. Based on this finding, 11 minutes is the optimal sample purge time for the determination of gasoline with BTEX.

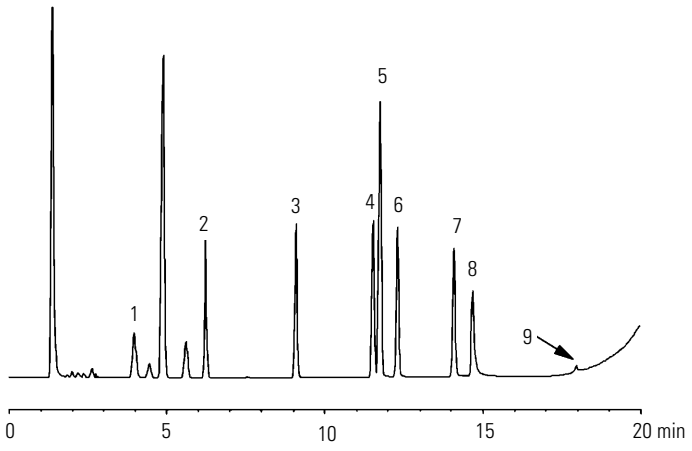
Dry Purge

During sample purge, a larger amount of water is purged along with the volatile organics and is collected on the trap sorbent. Sorbent material in the Vocarb-3000 trap is designed to minimize water trapping and reduce the release of excessive water onto the GC column during the thermal desorption process. A 1-minute dry purge of the Vocarb trap was selected because the early-eluting peaks (such as pentane, MtBE, and benzene in **Figure 2**) were not skewed by water released from the trap onto the column.

Desorption

According to Klee[1], a fast and reproducible desorption temperature is the key to good chromatography using the P and T concentration technique. The higher the desorption temperature and desorption rate, the faster the volatile analytes can be moved to the GC column, and the narrower the peak widths of the early-eluting analytes. Therefore, a short desorption time is preferred. In addition,

Figure 5. Chromatogram of GROs standard using an 8-minute sample purge. (See Table 2 for peak identification and Table 1 for GC and P and T conditions.)



Doherty[2] reported that peak heights and peak areas of volatile organics, including those in the GROs mix, were virtually unchanged when the desorb time changed from 4 minutes to 1 minute. Several manufacturers of P and T systems also recommend a 1-minute desorb time for the routine analysis of volatile organics. However, experimentation (**Figure 2**) using a 2-minute desorb time at 250°C accommodated sharp initial peaks as well as good separation. This study applied a 2-minute desorption time at 250°C to all analyses.

Trap Baking

Three different bake times were evaluated for the Vocarb-3000 trap (used a bake temperature of 265°C, recommended for the Vocarb-3000 trap): 10, 8, and 5 minutes. At each bake time, the gasoline sample (1000-ppb concentration) was run using an 11-minute purge time followed by a run of reagent water with no sample purge. Chromatograms of these two runs were evaluated for carryover. In all three cases (bake times of 5, 8, and 10 minutes), no carryover was observed for any gasoline component. Therefore, a 5-minute bake time at 265°C was selected as an optimal bake time for the analysis of gasoline and GROs aromatics.

For samples containing 46,000 ppb of gasoline, no carry over from the trapped analytes was observed at the 5-minute bake time. This is based on the comparison of chromatograms of reagent water (0-minute purge) run immediately after each sample. However, carry over from the purge vessel was found. Repeated rinsing of the purge vessel with reagent water reduced the amount of carry over but did not eliminate it. Therefore, after a high level sample is run, it is advisable to remove and clean the purge vessel prior to the next run.

Heavier petroleum products, such as diesel and jet fuel (**Figure 6**), that often contain volatile components are also detectable by this method. Again, carry over is a problem. Carry over was observed in the reagent water (used an 11-minute purge) run immediately after the jet fuel sample.

Carry over ranged from 10 ppb to 60 ppb jet fuel and was high enough to cause a false-positive identification in subsequent runs.

As demonstrated by **Figure 7B** (a chromatogram of reagent water, 0-minute purge, run immediately after a jet fuel sample), carry over from the Vocarb trap was found to be negiligible. Clearly the carry over was the result of contamination from the purge vessel (see **Figure 7A**). Although repeated rinsing reduced the amount of carry over, it did not eliminate it completely. Purge vessel carry over was eliminated completely when the purge vessel and the purge needle were removed and cleaned (see **Figure 7C**).

Table 4. GC Run Time of 17 Minutes

HP-1 Column Thickness	Oven Ramp	Carrier Flow Time
30 m x 0.53 mm x 5 µm	40°C (3 min) at 15°C/min to 250°C	4.5 ml/min (see **Figure 3**)
30 m x 0.53 mm x 3 µm	40°C (3 min) at 7°C/min to 95°C to 250°C (2 min) at 45°C/min	10 ml/min (see **Figure 4**)

Figure 6. Chromatogram of 1,000-ppb aviation jet fuel standard. (See Table 1 for GC and P and T conditions.)

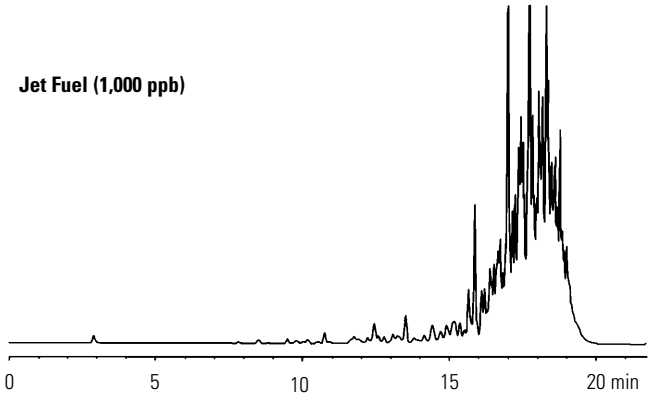
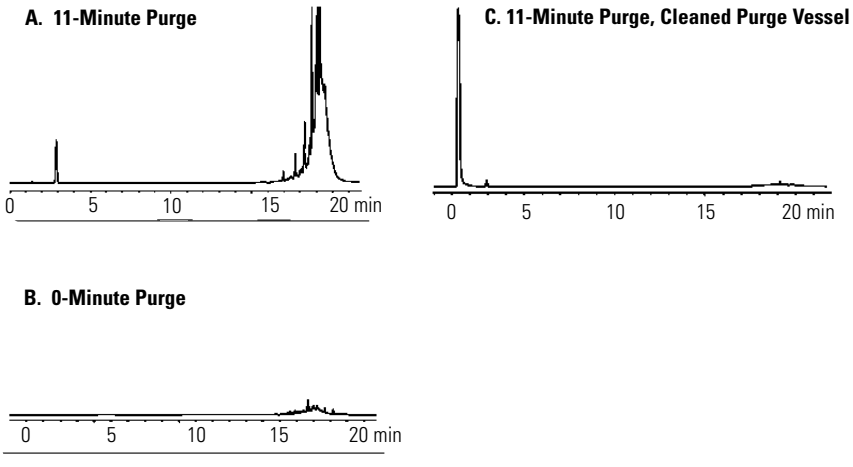


Figure 7. Chromatograms of reagent water following the analysis of the 1,000-ppb aviation jet fuel sample. (See Table 1 for GC and P and T conditions.) Note: The chromatograms were plotted on the same FID response scale.



Conclusion

Determination of optimized P and T parameters is critical in establishing optimized run times for the analysis of gasoline/BTEX. By reducing the P and T bake time to 5 minutes and selecting shorter dry purge (1 minute) and desorption times (2 minutes), the overall P and T cycle was shortened to 25-26 minutes. This is compatible with the run time of 21-22 minutes established for optimized GC conditions. When carry over from the purge vessel is controlled, this same application can be used successfully for the analysis of samples containing in excess of 46,000 ppb of gasoline and other volatile organics in light petroleum products.

Acknowledgment

The authors wish to than Mr. Robert Remlinger at the Pace Laboratory in Petaluma, California for providing his GC/FID and GC/PID chromatograms.

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Publication Number 5963-9968E



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Determining Aromatics in Finished Gasoline: ASTM Method D 5580

Application

Gas Chromatography

June 1995

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Abstract

An Agilent 6890 Series gas chromatography system, including the Agilent ChemStation and automatic liquid sampler, was optimized to perform aromatics analysis according to ASTM Method D 5580-94. This standard method is for the analysis of benzene, toluene, ethylbenzene, p/m-xylene, o-xylene (BTEX), C_9+ aromatics, and total aromatics in finished gasolines and some gasoline-related streams. A description of a guaranteed analyzer for D 5580 is given, with which analysis through full report takes approximately 45 minutes. The configuration, conditions, and sample chromatograms are presented.

Introduction

In 1990 the United States initiated the trend to improve air quality with the Clean Air Act (CAA). Legislation similar to the CAA is pending in other states in the U.S. and in other countries. The CAA is expected to serve as a guideline for setting pollutant limits and the corresponding test methods.

The CAA established guidelines for reformulated gasoline (RFG) in order to control ground-level ozone and toxins formation. The U.S. Environmental Protection Agency (USEPA) also established standards for the maximum allowable concentration of ground-level ozone. When areas exceed that allowable concentration, they are in nonattainment of the ozone standard and suffer specific consequences.

Beginning on January 1, 1995, the CAA requires certification of all RFG sold in ozone nonattainment areas and specifies a maximum concentration level of benzene (benzene is a health hazard and carcinogen) and a maximum allowable concentration of aromatics (aromatics lead to poor combustion and increased exhaust emissions).

The USEPA identified test methods for seven properties of RFG and requires testing of each batch of RFG for these. Of the identified test methods, however, no single method is suitable for addressing all requirements for reformulated gasoline. The sole USEPA regulatory method for measuring benzene concentrations in finished motor and aviation gasoline is ASTM D 3606-92[1]. However, if the gasoline contains oxygenates, they interfere with the determination of benzene and toluene. Similarly, ASTM Method D 4815-94[2], for the determination of oxygenated components, does not allow quantification of benzene.

ASTM D 5580-94[3, 4] eliminates the interference caused by the presence of ethanol and methanol when analyzing aromatics. In addition, ASTM is currently working on a revision of D 5580-94 to further improve the method. The California Air Resource Board (CARB) requires D 5580 as the analytical method for benzene quantitation in gasolines sold in California. The method requires two separate analyses; the first analysis quantifies benzene and toluene, and the second analysis determines ethyl-benzene, o-xylene, p/m-xylene, and the C_9 and heavier aromatics content. Summing the results of bothanalyses determines the total aromatics content.

The **AC Aromatics Analyzer** is a guaranteed analyzer for ASTM D 5580, is based on the Agilent 6890 Series gas chromatograph (GC), and is available through and supported by Analytical Controls[5], an Agilent Channel Partner. The AC Aromatics Analyzer consists of a 6890 Series GC configured with a split/splitless inlet, a ten-port valve, two chromatographic columns, and a single flame ionization detector (FID).

Experimental

The complete configuration for the AC Aromatics Analyzer appears in table 1. The Agilent ChemStation performs system control, data analysis, and reporting.

A micropacked TCEP column preseparates aromatics from non-aromatics in the same boiling point range. The capillary column then separates the aromatics. It is possible to use an extra detector, a thermal conductivity detector (TCD), to facilitate the determination of the valve backflush and to reset times and monitor the separation of the polar TCEP precolumn. See figure 1 for the flow diagram of the system.

ASTM D 5580 requires all standards and samples to be prepared by mass using 2-hexanone as an internal standard.

The Agilent ChemStation in a Microsoft® Windows™ environment controls the AC Aromatics Analyzer and automates all aspects of the calibration, sample analysis, and data reporting.

Table 1. AC Aromatics Analyzer Configuration

Hardware and Software	
G1540A	6890 Series GC
Opt 112	Capillary split/splitless inlet with EPC control
Opt 210	FID with EPC control
Opt 301	Three channels of auxiliary EPC
Opt 403	GPIB communication cable
G1916A	6890 Series automatic liquid sampler
G1875AA	Single-instrument GC ChemStation
Columns	
Methyl silicone fused silica capillary column	30 m x 0.53 mm, 5-μm HP-1
Micropacked TCEP precolumn	560 mm x 0.38 mm id, 20% on chromosorb PAW 80/100
Valves	
Ten-port rotary valve	
AC Application	
Calibration standards	
Reference gasoline	
Method development	
Software including methods	
Calibration and certification data	
Operating manual	
Performance guarantee	

Method Description

For the first of two analyses, a reproducible volume of sample containing the internal standard, 2-hexanone, is injected onto a pre-column containing a polar liquid phase (TCEP). C_9 and lighter non-aromatics elute from the pre-column and are vented, including 1-methylcyclopentene and ethers such as MTBE, ETBE, and TAME. It is possible to use a TCD before the vent to monitor the progress of this preliminary separation. Figure 1 shows the valve configuration and flow path during injection.

The TCEP precolumn backflushes just before the elution of benzene and directs the remaining portion of the sample onto a nonpolar capillary column for separation and FID quantitation. Benzene, toluene, and the internal standard elute in the order of their boiling points and are detected by an FID. Figure 2 shows the flow path during backflush.

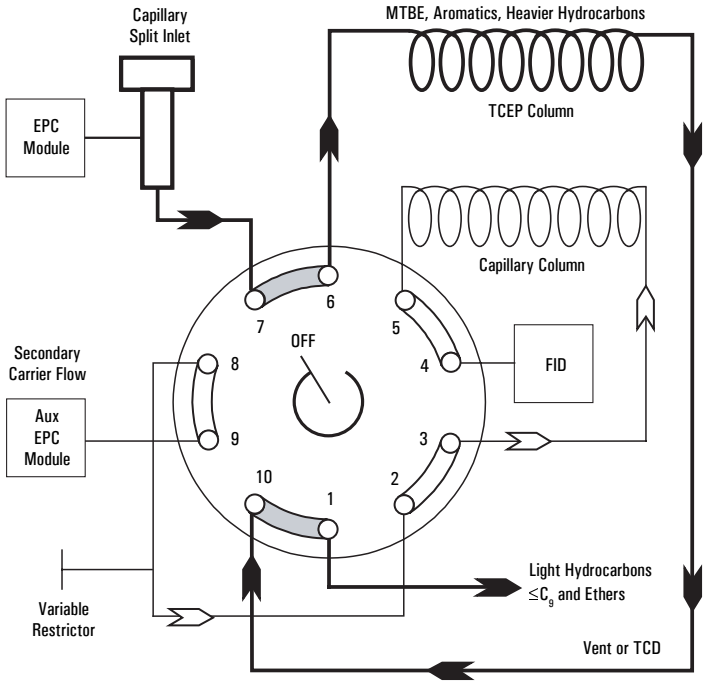

Figure 1. Sample flow path during sample injection.

Immediately after the elution of the internal standard from the capillary column, the flow through it is reversed to backflush the remainder of the sample (a mixture of hydrocarbons with boiling points above that of C_9) from the column to the FID. Although detected, this peak is not quantitated because it contains both aromatic and non-aromatic hydrocarbons. Figure 3 illustrates the valve position during backflush from the capillary column.

The analysis repeats a second time allowing the C_{12} and lighter non-aromatics, benzene and toluene, to elute from the polar TCEP precolumn to vent. A TCD is recommended to monitor this separation. The TCEP precolumn backflushes just before the elution of ethyl-benzene and directs the remaining aromatic portion into the capillary column. The internal standard and C_8 aromatics components elute in the order of their boiling points and are detected by an FID. Immediately after o-xylene elutes, the flow through the nonpolar WCOT column reverses to backflush the C_9 and heavier aromatic components to the FID. During backflush, pressure is increased to minimize the time required to remove all components from the column. Table 2 gives the chromatographic operating parameters.

Results

Figure 4 is a chromatogram of the first portion of a gasoline analysis-elution of benzene, toluene, 2-hexanone, and backflushed hydrocarbons. Figure 5 is a chromatogram of the second separation-elution of 2-hexanone, ethylbenzene, p/m-xylene, and C_9 + higher-boiling aromatic components. The oxygenates contained in the gasoline did not interfere with the aromatics analysis.

Electronic pneumatics control (EPC) helps minimize analysis time by speeding up the elution of higher-boiling components during capillary column backflush. Total analysis time (two separations plus the report) is approximately 45 minutes.

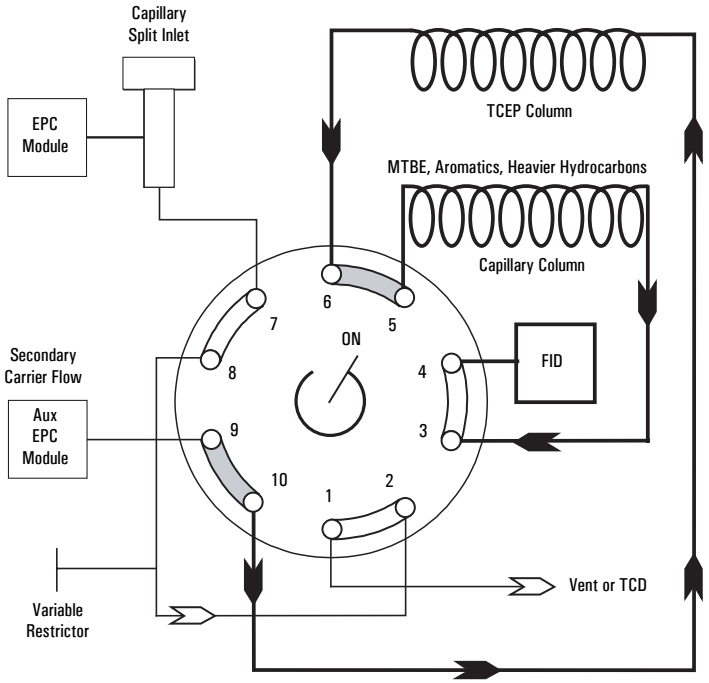

Figure 2. Backflush of micropacked TCEP column onto nonpolar capillary column for analysis of aromatics.

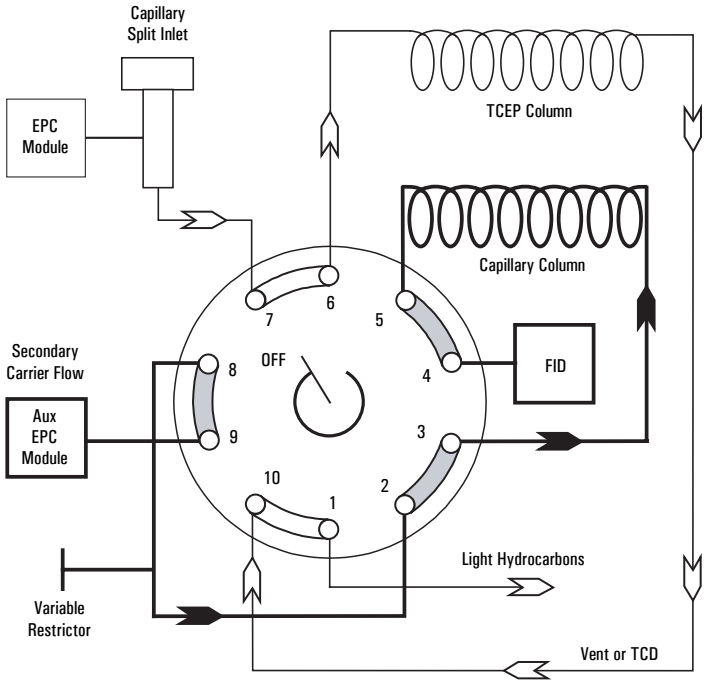

Figure 3. Backflush of the capillary column to elute >C_8 aromatics and >C_{10} hydrocarbons (first analysis) or >C_9 + aromatics (second analysis) to the FID.

Conclusion

The AC Aromatics ASTM D 5580 Analyzer is applicable for analysis of all finished motor gasolines and some gasoline-related refinery streams. The analyzer provides a simple approach to determining the benzene content in gasoline with-out interference from oxygenates. Capillary column technology provides excellent separation capability. The optimal configuration of the 6890 Series GC system reduces operator involvement and improves the accuracy of the results.

Table 2. Gas Chromatographic Operating Parameters

Injection port temperature:		200°C
FID temperature:		250°C
TCD temperature:		200°C
Oven temperature (both packed TCEP and WCOT column temperature	*Initial*	60°C (6 min)
	Program rate	2°C/min
	Final	120°C
Valve temperature:		80°C
Carrier gas:		Helium
Split ratio:		11:1
Sample size:		1 µL
Flow to TCEP precolumn:		10 mL/min
Flow to WCOT capillary column:		10 mL/min
Split vent flow:		100 mL/min

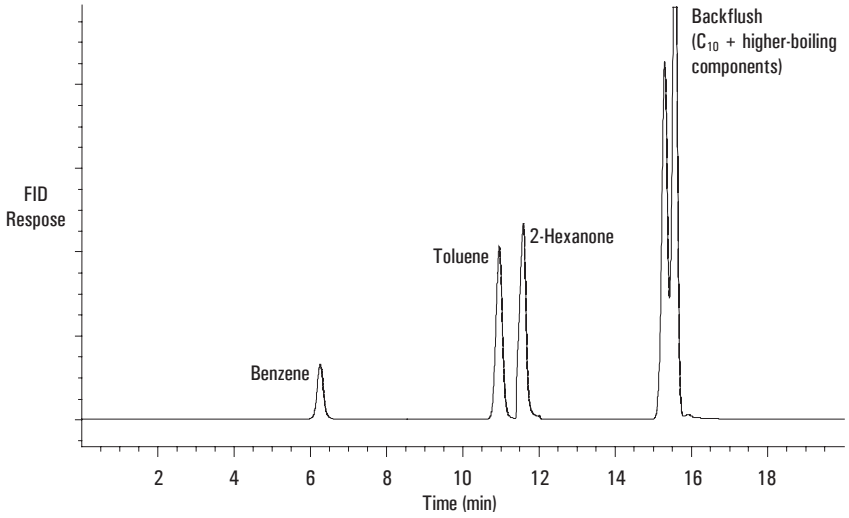

Figure 4. First analysis: separation of early-eluting aromatics.

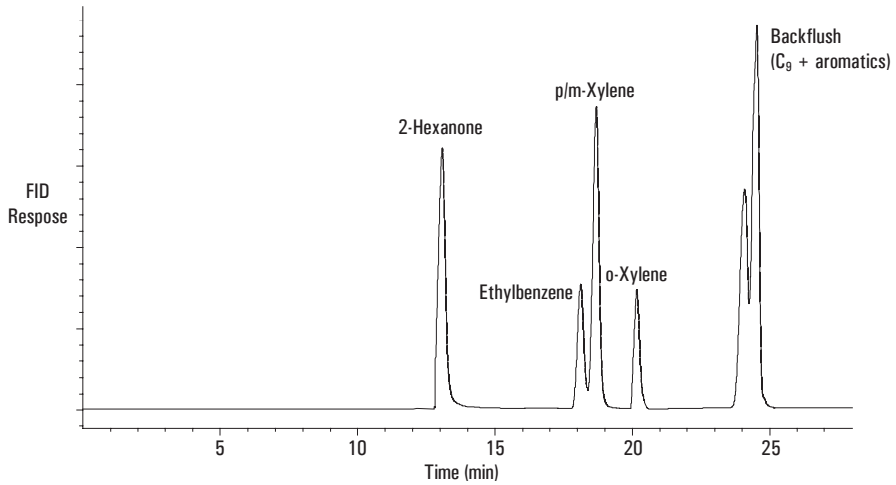

Figure 5. Second analysis: separation of later-eluting aromatics.

References

1. ASTM D 3606-92; *Test Method for the Determination of Benzene and Toluene in Finished Motor and Aviation Gasoline by Gas Chromatography*, American Society for Testing and Materials, Philadelphia, Pennsylvania.

2. ASTM D 4815-94; *Test Method for Determination of MTBE, ETBE, TAME, DIPE, tertiary-Amyl Alcohol and C_1 to C_4 Alcohols in Gasoline by Gas Chromatography*, American Society for Testing and Materials, Philadelphia, Pennsylvania.

3. ASTM D 5580-94; *Test Method for Determination of Benzene, Toluene, Ethylbenzene, p/m-Xylene, o-Xylene, C_9 and Heavier Aromatics and Total Aromatics in Finished Gasoline by Gas Chromatography*, American Society for Testing and Materials, Philadelphia, Pennsylvania.

4. Vince Giarrocco, "Analysis of Benzene, Toluene, C_8 Aromatics, and Total Aromatics in Finished Gasoline by GC Using ASTM D 5580-94," Agilent Technologies, Inc., Application Note 228-293, Publication No. (43) 5963-5048E, November 1994.

5. AC Analytical Controls, Innsbruckweg 35, 3047 AG Rotterdam, The Netherlands, and 3494 Progress Drive, Bensalem, Pennsylvania 19020 USA.

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Printed in the USA 3/2000
5964-0394E



Agilent Technologies
Innovating the HP Way

Determining Oxygenates in Gasoline: ASTM Method D 4815

Application

Gas Chromatography

June 1995

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Abstract

An Agilent 6890 Series gas chromatographic system (GC, ChemStation, and automatic liquid sampler) was used as the foundation of the AC Oxygenate Analyzer. The analyzer determines individual ethers and alcohols in finished gasolines according to ASTM Method D 4815-94[1] and takes advantage of the unique features of the 6890 Series GC. An analysis through full report takes 15 minutes. A complete description of the instrument configuration, analysis conditions, and chromatograms is given.

Introduction

The 1990 Clean Air Act Amendments (CAA) establish guidelines for fuel composition with the intent of reducing atmospheric levels of carbon monoxide, nitrogen oxides, and hydrocarbons. The Amendments include a reformulated gasoline (RFG) program that is in effect from January 1995 to March 1997. RFG is specifically targeted to reduce levels of ozone. The simple model on which the regulations are based requires a minimum content of 2 wt% oxygen in RFG. The California Air Resources Board (CARB) has designated ASTM D 4815-94 as the test method for oxygenate quantification in all gasoline sold in California.

The AC Oxygenate Analyzer from AC Analytical Controls is an analyzer based on the 6890 Series GC system and designed to perform ASTM D 4815. It tests all finished motor gasolines for oxygenates listed in table 1 to determine regulatory compliance and to ensure uniform product quality. Individual ethers are quantified from 0.1 to 20.0 mass % and individual alcohols from 0.1 to 12.0 mass % in one analysis.

Table 1. Scope of the AC Oxygenate Analyzer

Ethers	Alcohols
Methyl tert-butylether (MTBE)	Methanol
Diisopropylether (DIPE)	Ethanol
Ethyl tert-butylether (ETBE)	Isopropanol
tert-Amylmethylether (TAME)	n-Propanol
	Isobutanol
	tert-Butanol
	sec-Butanol
	n-Butanol
	tert-Pentanol
	(tert-amyl alcohol)

Experimental

The AC Oxygenate Analyzer consists of the 6890 Series GC configured with two GC columns, a split/splitless inlet, a ten-port sampling valve, and a flame ionization detector (FID). The complete configuration is given in table 2.

Table 2. AC Oxygenate Analyzer System Configuration

Hardware and Software	
G1540A	6890 Series GC
Opt 112	Capillary split/splitless inlet with EPC control
Opt 210	FID with EPC control
Opt 301	Three channels of auxiliary EPC
Opt 403	GPIB communication cable
G1916A	6890 Series automatic liquid sampler
G1875AA	Single-instrument GC ChemStation
Columns	
Methyl silicone fused silica column (30 m x 530 µm id, 2.65 µm HP-1)	
Micropacked TCEP precolumn (560 mm x 0.38 mm id, 20% on chromosorb PAW 80/100)	
Valves	
Ten-port rotary valve	
AC Application-Specific Components	
Calibration standards	
Reference gasoline	
Optimized Methods	
Software including methods	
Calibration and certification data	
Operating manual	
Performance guarantee	
Instrument Control and Data Acquisition	
ChemStation	

The micropacked TCEP column preseparates the low-boiling non-polar components from the polar and higher-boiling nonpolar components. The nonpolar capillary column then separates the polar components, including the oxygenates, according to their boiling points. Electronic pneumatics control (EPC) is used for faster backflush of the TCEP column. This reduces the analysis time by approximately 40%.

The Agilent ChemStation runs in a Microsoft® Windows™ environment, controls the AC Oxygenate Analyzer, and automates all aspects of data handling and data reporting.

Calibration

Detector response is calibrated using several levels of the oxygenates to be analyzed and a single level of an internal standard such as 1,2-dimethoxyethane (ethylene glycol dimethyl ether). After analyzing this standard, the analyzer generates a standard curve automatically that includes detection limits and linearity. The internal standard is also added to the samples to be analyzed, and their results are reported in mass percent.

Method Description

Figure 1 shows the valve configuration and sample flow path during injection. The sample first passes into the TCEP column where the low-boiling and nonpolar components, those eluting before methyl-cyclopentane, pass directly to vent. The column retains polar compounds, beginning with DIPE and MTBE, and higher-boiling components.

The valve then switches, and components trapped in the TCEP column backflush to the nonpolar capillary column for separation and analysis, as shown in figure 2. This column performs a boiling point separation on components through benzene and TAME. After TAME elutes from the nonpolar column, the valve switches to backflush the components remaining on the capillary column to the

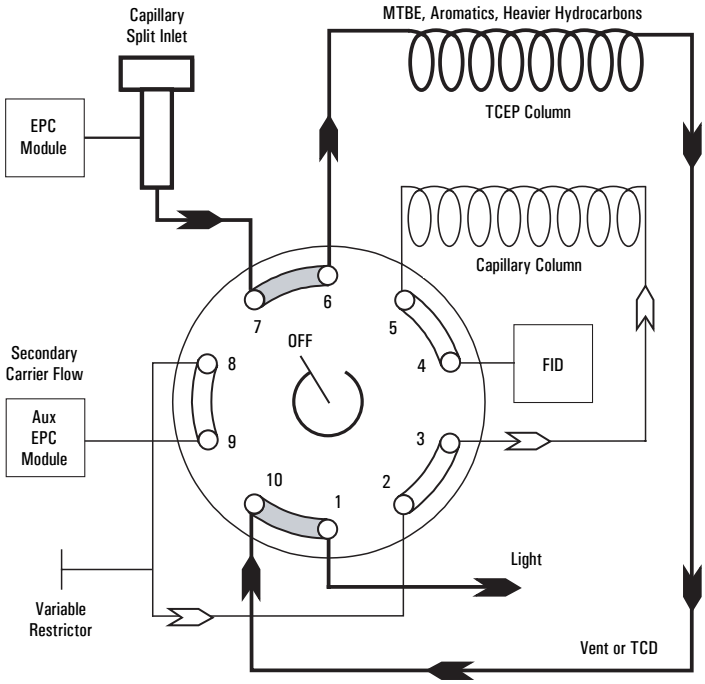

Figure 1. Sample flow path during and directly after sample injection. Low- polarity volatiles pass through the polar TCEP column and are vented. Polar components and higher-boiling apolar components are trapped in the TCEP column.

detector as one unresolved peak at increased head pressure (higher flow). Although detected, this backflush fraction is not quantified. By using EPC to increase pressure during the last backflush, total analysis time is reduced by approximately 40%. Figure 3 illustrates the valve position during the backflush from the capillary column when using pressure programming. Table 3 gives the chromatographic operating parameters.

Results

A report listing results in mass percent is generated automatically at the end of each run. Targeted ethers are reported from 0.1 to 20.0 mass % and targeted alcohols from 0.1 to 12.0 mass %. Figure 4 shows a typical chromatogram for an ether blend. Figure 5 is a typical chromatogram of an alcohol blend, showing good resolution and lack of interferences.

The AC Oxygenate Analyzer meets and exceeds the repeatability and reproducibility standards set by ASTM D 4815-94. A summary of analysis precision is shown in table 4.

Conclusion

The AC Oxygenate ASTM D 4815 Analyzer provides a rapid, accurate analysis of oxygenates in finished gasolines including RFGs, taking advantage of 6890 Series GC system automation to reduce operator involvement, increase sample throughput, and improve accuracy of the results. The electronic pneumatics control of the 6890 Series GC helps achieve a 40% reduction in analysis time through the use of pressure programming. Capillary column technology yields excellent separation of targeted oxygentates.

References

1. *ASTM D 4814-94, Standard Test Method for Determination of MTBE, ETBE, TAME, DIPE, tertiary-Amyl Alcohol, and C1 to C4 Alcohols in Gasoline by Gas Chromatography*, American Society of Testing and Materials, Philadelphia, Pennsylvania.

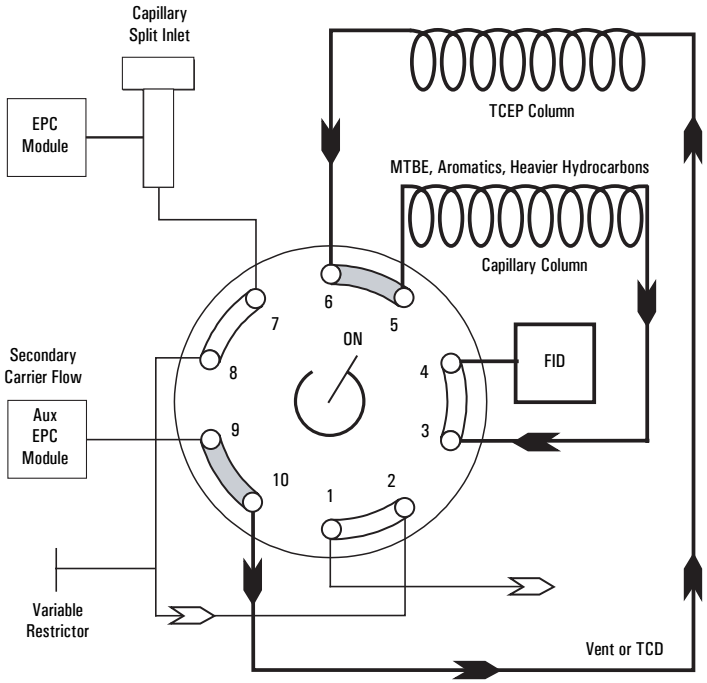

Figure 2. Flow path of polar and medium-volatility compounds backflushed from the TCEP column to the capillary column for separation and analysis.

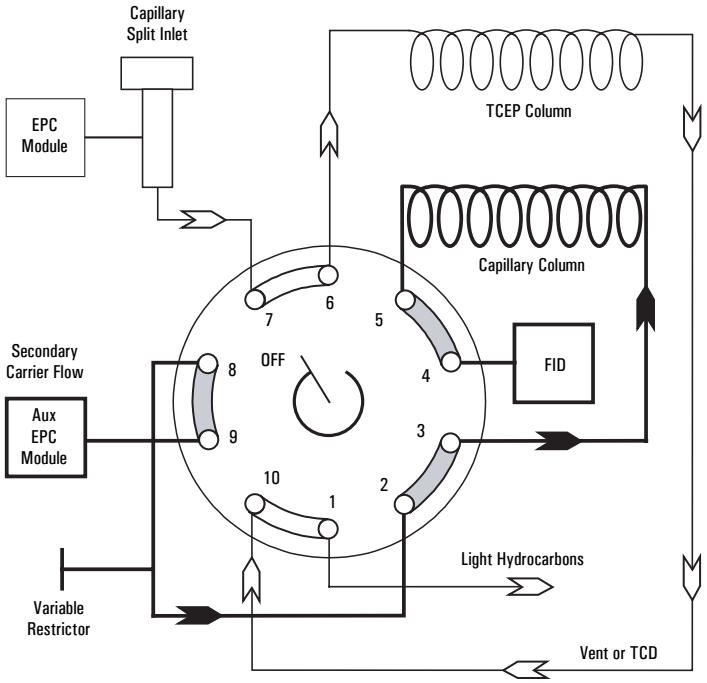

Figure 3. Final backflush of heavy hydrocarbons from the capillary column. Column head pressure is increased through programming via EPC to reduce run time.

Table 3. Gas Chromatographic Operating Parameters

Injection port temperature:		200°C
FID temperature:		250°C
TCD temperature:		200°C
Nonpolar WCOT capillary column temperature:	Initial:	60°C (6 min)
	Program rate:	2°C/min
	Final:	115°C
Polar TCEP precolumn temperature (isothermal):		60°C
Valve temperature:		80°C
Carrier gas:		Helium
Split ratio:		15:1
Sample size:		1.0-3.0 µL
Flow to TCEP precolumn:		5 mL/min
Flow to WCOT capillary column:		5 mL/min
Split vent flow:		100 mL/min
Makeup flow:		18 mL/min

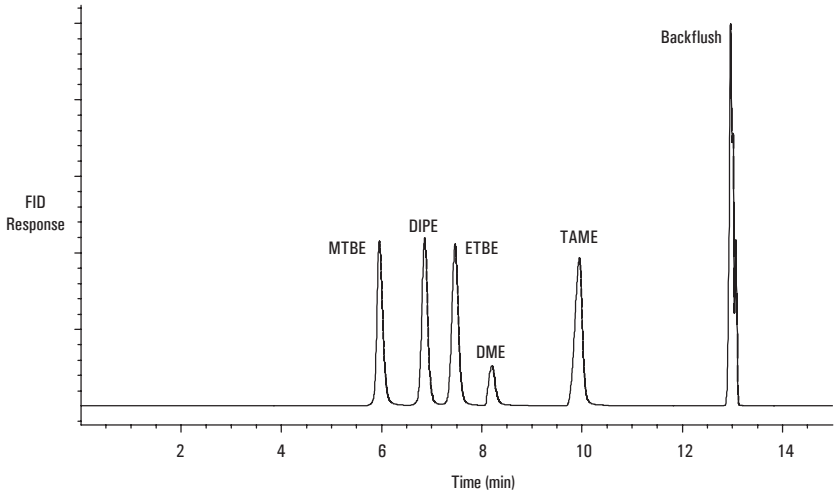

Figure 4. Typical chromatogram of an ether gasoline blend.

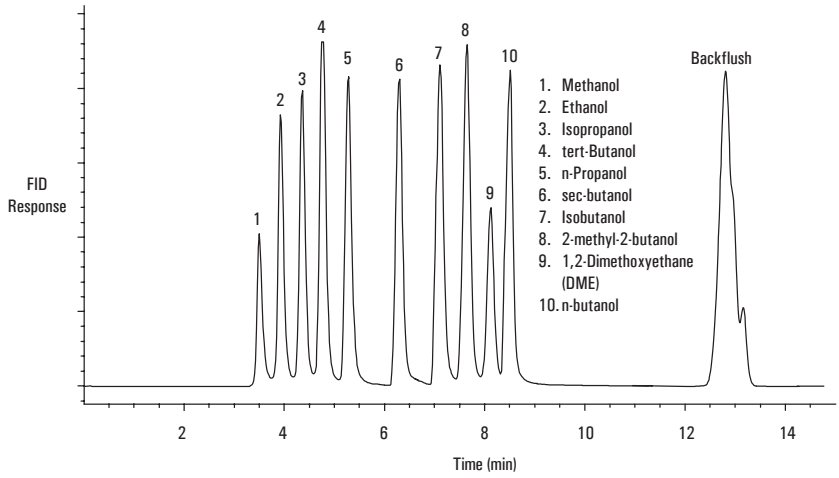

Figure 5. Typical chromatogram for alcohol gasoline blend.

Table 4. ASTM D 4815-94 Precision with the AC Oxygenate Analyzer (X = Oxygenate Concentration in Mass %)

Component	Repeatability	Reproducibility
Methanol (MeOH)	0.09 ($X^{0.59}$)	0.37 ($X^{0.61}$)
Ethanol (EtOH)	0.06 ($X^{0.61}$)	0.23 ($X^{0.57}$)
Isopropanol (iPA)	0.04 ($X^{0.56}$)	0.42 ($X^{0.67}$)
tert-Butanol (tBA)	0.04 ($X^{0.56}$)	0.19 ($X^{0.67}$)
n-Propanol (nPA)	0.003 ($X^{0.57}$)	0.11 ($X^{0.57}$)
MTBE	0.05 ($X^{0.56}$)	0.12 ($X^{0.67}$)
sec-Butanol (sBA)	0.003 ($X^{0.61}$)	0.44 ($X^{0.67}$)
DIPE	0.08 ($X^{0.56}$)	0.42 ($X^{0.67}$)
Isobutanol (iBA)	0.08 ($X^{0.56}$)	0.42 ($X^{0.67}$)
ETBE	0.05 ($X^{0.82}$)	0.36 ($X^{0.76}$)
tert-Pentanol (tAA)	0.04 ($X^{0.61}$)	0.15 ($X^{0.57}$)
n-Butanol (nBA)	0.06 ($X^{0.61}$)	0.22 ($X^{0.57}$)
TAME	0.05 ($X^{0.70}$)	0.31 ($X^{0.51}$)
Total Oxygen	0.02 ($X^{1.26}$)	0.09 ($X^{1.27}$)

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5964-1729E



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Determining Benzene in Finished Gasoline and Refinery Streams to Comply with CAA Requirements: ASTM Method D 3606

Application

Gas Chromatography

June 1995

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Abstract

An Agilent 6890 Series gas chromatographic system (GC, Agilent ChemStation and automatic liquid sampler) was used as the foundation of the AC Benzene/Toluene in Gasoline Analyzer. The analyzer separates and detects benzene, methyl ethyl ketone (MEK, an internal standard), and toluene in finished gasoline. Sample analysis through full report takes approximately 10 minutes. A complete description of the instrument configuration, analysis conditions, and results are given.

Introduction

The United States Clean Air Act of 1990 (CAA) establishes guidelines for fuel composition. To meet the requirements of the CAA, the U.S. Environmental Protection Agency (USEPA) introduced programs aimed at reducing atmospheric levels of carbon monoxide, nitrogen oxide, ozone, and hydrocarbons. Petroleum refiners around the world are adapting their operations to accommodate the U.S. environmental regulations.

To reduce emissions of toxic compounds by motor vehicles, one program mandates the quantification of toxic compounds found in gasoline. The reformulated gasoline (RFG) program requires lowering the total aromatics, benzene, and olefin content in the gasoline pool. The CAA classifies benzene as a toxic air pollutant; the level of benzene in RFG must not exceed 1 volume percent. To meet the stipulated limits of benzene, a quantitative determination of benzene at all process stages is essential.

The EPA prescribed ASTM Method D 3606-92[1] as the single regulatory test method for determining benzene concentrations in gasoline. The AC Analytical Controls Benzene/Toluene in Gasoline Analyzer separates benzene from both light and heavy hydrocarbons, the internal standard, and toluene in one analysis, and meets all the specifications of ASTM D 3606-92. The analyzer may also be used to measure benzene and toluene concentrations in other gasoline-related refinery streams. Knowledge of benzene and toluene concentrations in refinery streams allows refiners to optimize their production processes.

The AC Benzene/Toluene Analyzer consists of the Agilent 6890 Series GC configured with two GC columns and a single thermal conductivity detector (TCD).

Experimental

The complete configuration for the AC Benzene/Toluene Analyzer is given in table 1. The ChemStation performs system control and data analysis.

Electronic pneumatics control (EPC) is used to control carrier gas to both columns and gases to the TCD. The 6890 Series automatic liquid sampler is used to maximize precision in sample introduction into the packed column injection port. The Agilent ChemStation provides instrument control, data acquisition and report generation.

Table 1. AC Benzene/Toluene in Gasoline Analyzer Configuration

Hardware and Software	
G1540A	6890 Series GC
Opt 102	Packed column inlet with EPC control
Opt 220	TCD with EPC control
Opt 301	Three channels of auxiliary EPC
Opt 403	GPIB communication cable
G1916A	6890 Series automatic liquid sampler
G1875AA	Single-instrument GC ChemStation

Columns
Methyl silicone packed column (0.8 m x 3.2 mm od)
Micropacked TCEP column (4.6 m x 3.2 mm od)

Valves
Six-port rotary switching valve

AC Application
Calibration standards
Reference gasoline
Optimized methods
Software including methods
Calibration and certification data
Operating manual
Performance guarantee

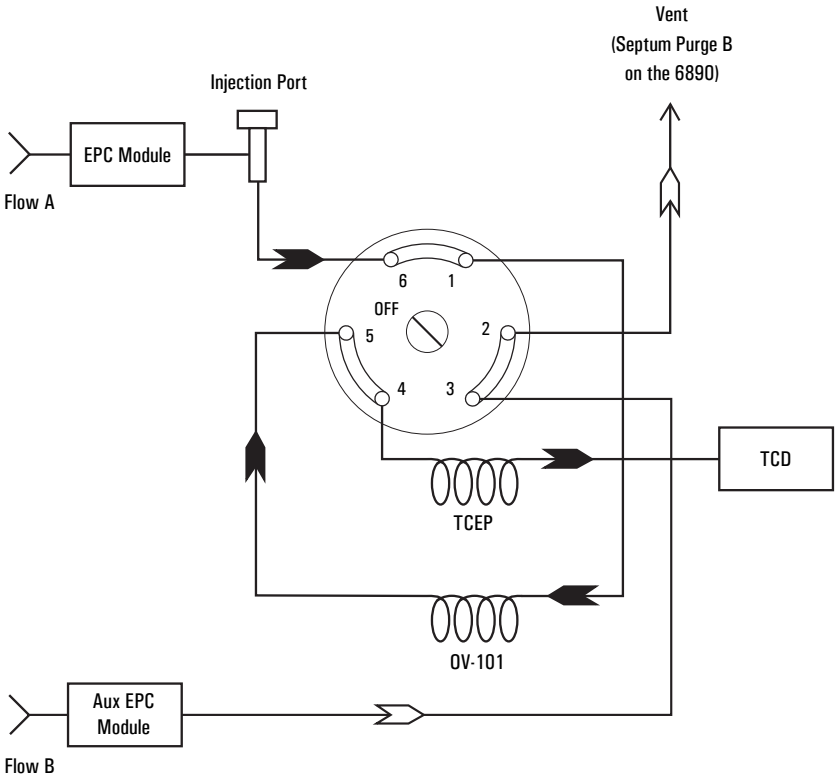

Figure 1. Flow path during and immediately after sample introduction; the six-port switching valve is in the OFF position. Sample is separated by boiling point in the first column. Lower-boiling components, including benzene, toluene, the internal standard MEK, and hydrocarbons up through the boiling point of e-octane, transfer to the the TCEP column.

Method Description

During analysis, the sample flows from the inlet to the nonpolar column, as illustrated in figure 1, with the six-port switching valve in the "OFF" position. This nonpolar column separates components according to their boiling points; the lighter components, including benzene, toluene, and non-aromatics up to n-octane, elute first from this column and transfer into the polar TCEP column. After elution of n-octane from the first column, the valve switches to the "ON" position, as illustrated in figure 2. With the valve ON, heavier components backflush from the OV-101 column to vent while separation of the components of interest continues in the TCEP column. Flow to the TCEP column is controlled by the B channel of auxiliary EPC.

Table 2 provides a list of analysis conditions.

Table 2. Gas Chromatographic Operating Parameters

Injection port temperature:	200°C
TCD temperature:	200°C
Packed column temperature:	145°C
Carrier gas:	Helium
Linear gas rate:	6 cm/s
Sample size:	2 µL
Volume flow rate:	Approx 30 mL/min
Column head pressure:	Approx 200 kPa (30 psi)
Total cycle time:	10 min
Valve switch:	Approx 0.75 min

Calibration

The ASTM D 3606-92 test method requires calibration before routine sample preparation and analysis. Seven levels of benzene within the range of 0.1 and 5.0 volume percent, and seven levels of toluene within the range of 0.5 and 20.0 volume percent, are required to calibrate response including detection limits and linearity. Methyl ethyl ketone (MEK) is used as the the internal standard for calibration standards and samples. Results are reported in volume percent relative to the internal standard.

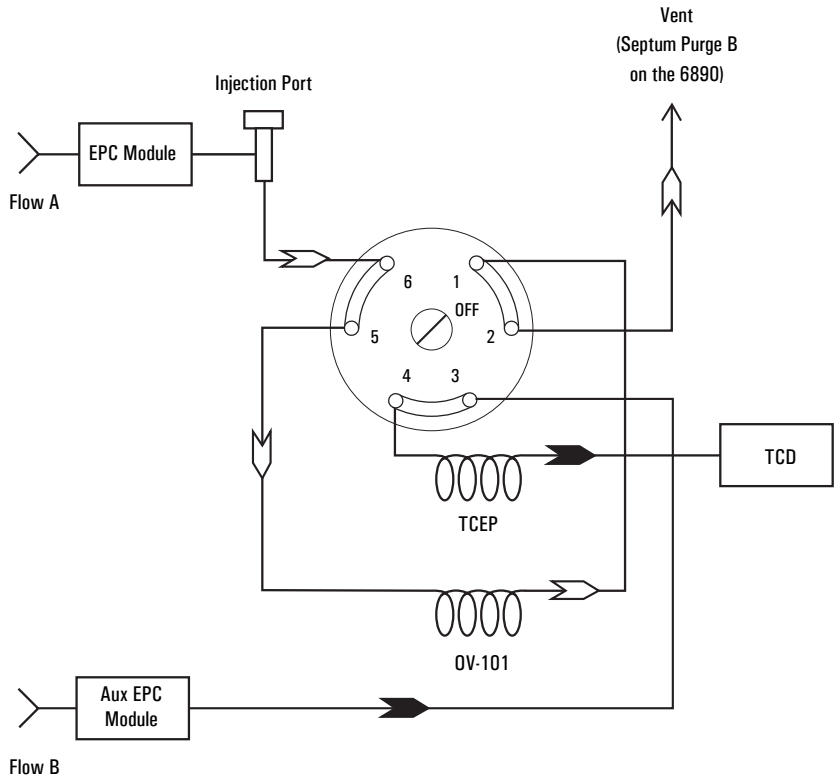

Figure 2. **Flow path after the six-port switching valve has been turned ON. Flow through the first column is reversed to backflush higher-boiling sample components. Auxiliary EPC provides flow to the second column that separates the components of interest.**

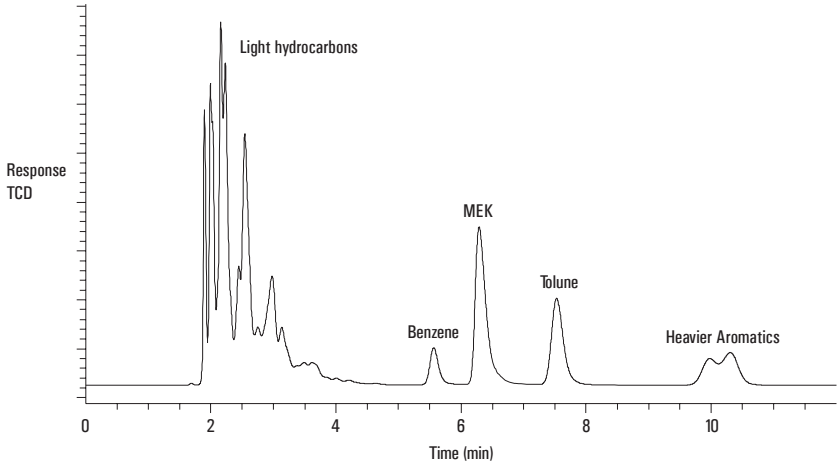

Figure 3. **A typical chromatogram that shows the separation of benzene, toluene, and MEK from other potential interferences.**

Results

Figure 3 illustrates a typical chromatogram for the analysis of benzene in gasoline, derived by the AC Benzene/Toluene in Gasoline Analyzer. The analyzer meets or exceeds the repeatability and reproducibility requirements of ASTM D 3606-92 as shown in table 3.

Table 3. Repeatability and Reproducibility of the AC Benzene/Toluene Analyzer

Component	Repeatability	Reproducibility
Benzene	0.03 liquid	0.23 x measured value
Toluene	0.62 liquid volume%	1.15 liquid volume volume %

Conclusion

The AC Benzene/Toluene in Gasoline Analyzer complies with the sole test approved by the USEPA for benzene analysis in gasoline. Its robust components (e.g., packed columns) and low maintenance configuration ensure trouble-free routine operation. The repeatability and reproducibility of the analyzer meet or exceed USEPA standards.

The AC Benzene/Toluene in Gasoline Analyzer is applicable for all finished motor gasolines and most gasoline-related refinery streams. The analyzer provides a simple approach for the determination of benzene content in gasoline and makes use of the features available on the Agilent 6890 Series GC.

References

1. ASTM 3606-92, *Standard Test Method for Determination of Benzene and Toluene in Finished Motor and Aviation Gasoline by Gas Chromatography*, American Society for Testing Materials, Race Street, Philadelphia, Pennsylvania.

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Printed in the USA 3/2000
5964-1730E



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Latest Enhancements to the GC/MS Analysis of Gasoline by ASTM Method D-5769

Application Note

Agilent Technologies
6890/5973 GC/MSD System

Author

Roger J. Leibrand
Agilent Technologies

Abstract

The 6890/5973 GC/MSD system and its latest ChemStation software each contain enhancements that refine the analysis of aromatics in gasoline by ASTM Method D-5769. The improvements in the MSD hardware are the addition of a high energy dynode electron multiplier and increased scanning speed. The improvements in the software are easy entry of weights, easy signal to noise calculation, weight and volume percent calculated in the customized report, use of API index number, and expanded Help files. The improvements in the GC/MS method are fast temperature programming, carrier gas programming, high split ratio, narrow mass range, and electron multiplier programming. The performance of the 6890/5973 GC/MSD system with respect to ASTM Method D-5769 has been characterized. It was found that excellent toluene linearity, fast 20 minute analysis times, and excellent precision on duplicates could be achieved.

Keywords

Benzene, toluene, aromatics, gasoline, reformulated gasoline, RFG, gas chromatography/mass spectrometry, GC/MS, ASTM D-5769

Introduction

ASTM Method D-5769 is for analysts who have to certify the aromatic content in finished motor gasoline. These analysts are gasoline producers, manufacturers, blenders, distributors, independent testing laboratories, and regulatory bodies. A recent review by Vince Giarrocco of Agilent Technologies details the background of the reformulated gasoline analytical methodology.[1] An earlier Agilent Technologies Applications Brief describes the use of the 5972 MSD as applied to the first version of this ASTM method.[2]

The latest enhancements afforded by using the 6890/5973 GC/MSD here provide an improved means to validate compliance with government regulations and enhance productivity, and are more specific to the latest version of D-5769. The hardware and software recommendations are listed in Table 1.

Table 1. Hardware and software recommendations

GC:	6890
GC Consumables:	60 m × 0.25 mm ID, 1.0µm HP-1 P/N 19091Z-236
	Split/Splitless liner with glass wool P/N 19251-60540
	Merlin Microseal P/N 5181-8833
ALS:	7673 with nanoliter adapter
MS:	5973A Mass Selective Detector
MSD ChemStation:	Aromatics in Gasoline mode MSD Productivity ChemStation Software

Software Enhancements

The improvements in the software are easy entry of sample weights, internal standard weights, density, easy signal to noise calculation, inclusion of uncalibrated compounds in the calculations, weight and volume percent calculated in the customized report, use of API index number, and expanded Help files that are specific to this analysis.

When recalibration utilizes a new batch of calibration standards, the individual amounts defining the calibration curve will need to be updated. This process is automated if the calibration standards are purchased from a vendor that also supplies an update disk. The individual amounts will be inserted for each compound that has a name and a CAS number that matches the one in the file.

The gasoline analysis method ASTM D5769 calls for a signal to noise check on the extracted ion chromatogram from the GC/MS analysis of a 0.01 weight % solution of 1,4-diethylbenzene. With a few simple click and drag operations the check is made and a special report is printed out.

The calibrated components do not account for all the aromatics in gasoline. These components are identified by the existence of peaks with characteristic ions in specified retention time ranges. The quantitation of the uncalibrated components utilizes the calibration curve of an appropriate calibrated component.

The ASTM method also calls for a report of the weight % and volume % for each aromatic compound in the sample. These are provided by the software via a standard internal standard report that is enhanced with sample weight, sample density, ISTD weight, weight %, and volume %. Calculation of these quantities requires sample specific information. The sample weight and sample density need to be input by the operator.

There are also several editing features that are enhancements beyond the standard quantitation software, such as use of sample density or API index number and the y-intercept test for each calibration curve.

GC/MS Enhancements

There are two approaches to GC analysis of gasoline. One requires long analysis times, high resolution columns, and universal detectors. The other uses mass selective detectors and can result in shorter analysis times with less stringent chromatographic resolution requirements. Detailed hydrocarbon analysis (DHA) is an example of the former approach while D-5769 and its enhancements described here is an example of the later. This ASTM method has only a few mandated conditions. The refinement of the final method is at the individual laboratory discretion. The major requirements are that a methylsilicone column is used and the mass spectrometer is operated in full scan mode generating electron impact ions at 70 electron volts. There are also requirements of minimum column resolution, sensitivity, and data collection rate; these will be discussed later.

Experimental

In this work several chromatographic method enhancements were employed to enhance throughput. In the past for Method D-5769 run times of 30 to 50 minutes have been common in some laboratories. In order to attain a more desirable 20 minute run time, a starting oven temperature of 120° was used with a relatively fast two-level temperature program.[3] Holding the helium carrier gas flow at a constant 35 cm/sec during the run was also employed. This is 40% above the flow used for maximum efficiency, i.e., maximum chromatographic resolution. The details are shown in Table 2.

Even with 0.1 microliter injected and split the suggested 250 to 1, column overloading and toluene nonlinearity were observed. By raising the split ratio to 1100 to 1, the GC peaks shapes improved markedly. But at 15% toluene levels a nonlinear curve was still observed. Method D-5769 calls for the use of m/z 91 for toluene quantitation. This is the base peak. However, use of the carbon 13 isotope peak of m/z 92, which is 54% of m/z 91 produces a linear calibration curve.[4] Use of m/z 65, which is 10% of m/z 91 also, gives a linear calibration curve. Of the three possible mass peaks, the use of m/z 92 gives the best signal to noise along with a linear calibration curve. The high use of helium for the 1100 to 1 split ratio is

Table 2. Recommended Instrumental Parameters

GC Injection Port:	250°C Split 1100:1
GC Oven Ramp:	120°C for 0.5 minutes 3°C/minute to 140°C 10°C/minute to 250°C 0 minutes hold at 250°C
Carrier Gas:	Helium
GC Pressure Program:	0.3 mL/minute for 1.0 minutes 1.0 mL/min at 1.0 minutes 35 cm/second constant flow Vacuum compensation ON Gas saver ON after 1 minute
ALS:	5µL syringe Nanoliter adapter, stop = 1 (0.1µL injection size)
MSD:	Transfer Line: 280°C Ion Source: 200°C Quadrupole: 150°C Solvent Delay: 3 minutes Electron Energy: 70 eV Emission current: 35 µamps Mass Range: 60 to 170 Daltons Scan Speed: 2^3 A/D samples Autotune: Standard Multiplier Voltage: Autotune value (1153 volts) +200 volts after 6.0 minutes

somewhat wasteful and so the "gas saver" mode is used to reduce the split flow at times other than injection.

The 5973 uses a high energy dynode electron multiplier that incorporates enhanced ion optics to reduce noise and increase sensitivity. Special ion focusing elements concentrate the ion beam exiting the quadrupole on the 10 kV high energy dynode where a conversion to electrons takes place. This gives higher sensitivity and longer multiplier life.

The high energy dynode is 90° off-axis to minimize noise. Further detector noise reduction is accomplished by careful shielding of the electron multiplier and high energy dynode. This type of detector has increasing sensitivity with mass, which improves the detection of the heavier gasoline components.

The electron multiplier voltage obtained with Autotune is used for benzene and toluene. It is then raised programmatically 200 volts higher for the remaining components. This assures that the very highest toluene levels that are in some gasoline blending stocks will not overload the detector electronics and that there is sufficient signal for the low level components containing ten carbons and above.

Method D-5769 requires at least five mass spectral scans to be taken over the top half of the GC peak. According to Mathews and Hayes[5] if the worst case error is to be kept below 1%, at least nine scans must be taken. There is a real quantitation benefit to having as many scans as possible over the GC peak. However, the signal to noise ratio is adversely affected. Therefore, a compromise is needed. The 5973 scans twice as fast as its predecessor, the 5972. In real terms this means that with an A/D setting of 2^3 selected, 6.5 scans per second are taken. For the 1,4-diethylbenzene peak this represents 27 scans, reducing the sampling error to 0.1%, which is quite acceptable. These conditions yield a signal to noise ratio of 71 to 1 for the 0.01 wt % sample of 1,4-diethylbenzene.

Additionally, the mass range to use was explored. While Method D-5769 suggests that a mass range of 50 to 350 could be used, it is important to note that the highest mass required in the quantitation is m/z 148. Some of the higher uncalibrated compounds that may need to be quantitated separately have ions no higher than m/z 168.[6] Therefore, a narrow mass range of m/z 60 to 170 was used to maximize the number of scans.

Results

Figure 1 is the total ion chromatogram of a gasoline test sample, RFA003 QC. Note all compounds elute between 3 and 20 minutes. Figure 2 is a typical report for an RFA sample using the Aromatics in Gasoline software. Figure 3 shows the final linear calibration curve for toluene utilizing the high split ratio, electron multiplier voltage programming, and change of quant ion. The analysis of gasoline by Method D-5769 is not simple and requires careful attention to operational parameters as well as sample preparation and further method refinements.[4] It was found that excellent toluene linearity, fast 20 minute analysis times, and excellent precision on duplicates could be achieved with the 6890/5973 GC/MSD.

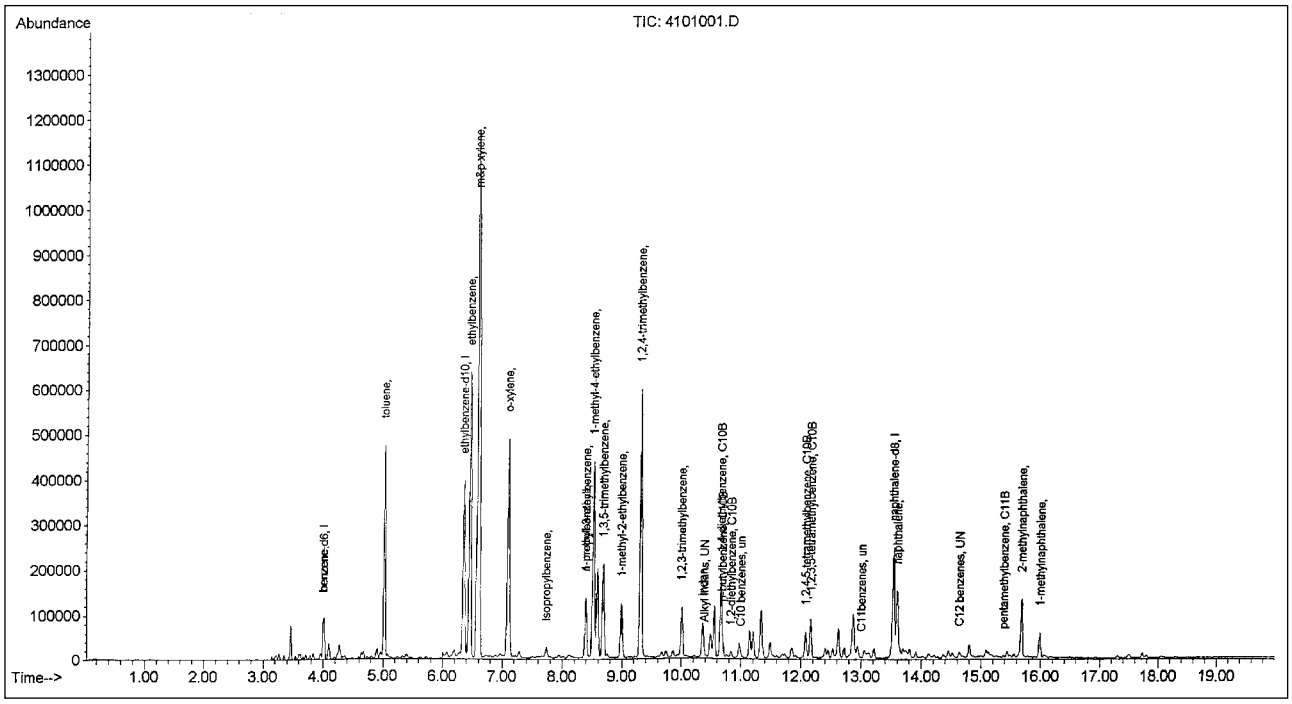

Figure 1. Total ion chromatogram of a gasoline test sample, RFA003 QC.

Aromatics Quantitation Report (Not Reviewed)

Data File : C:\HPCHEM\2\DATA\92\1801006.D Vial: 18
Acq On : 2 Sep 96 17:27 Operator: R Leibrand
Sample : rfa3 Inst : 5973
Misc : 5973 Multiplr: 1.00
MS Integration Params: RTEINT.P
Quant Time: Sep 2 18:53 1996 Quant Results File: RFG6890B.RES
SampWt: 1.2818 SampDens: 0.7501 IstdWt: 0.0569

Quant Method : C:\HPCHEM\2\METHODS\RFG6890B.M (RTE Integrator)
Title : Gasoline Aromatics System
Last Update : Mon Sep 02 18:45:01 1996
Response via : Initial Calibration
DataAcq Meth : RFG6890B

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)	
1) benzene-d6	3.94	84	143289	22.76	mg	0.02	
3) ethylbenzene-d10	6.32	116	191828	22.76	mg	0.03	
17) naphthalene-d8	13.52	136	519787	11.38	mg	0.02	
System Monitoring Compounds							
Aromatic Compounds				Wt%	Vol%		
2) benzene	3.96	78	106819	1.33	1.13		
4) toluene	4.98	92	492998	9.21	7.92		
5) ethylbenzene	6.43	106	321130	2.45	2.10		
6) m&p xylene	6.57	106	978127	6.13	5.29		
7) o-xylene	7.08	106	335231	2.17	1.84		
8) Isopropylbenzene	7.72	120	9665	0.08	0.07		
9) n-propylbenzene	8.37	120	64838	0.52	0.45	#	
10) 1-methyl-3-ethylbenzene	8.37	120	64838	0.52	0.45	#	
11) 1-methyl-4-ethylbenzene	8.51	120	237310	1.67	1.45		
12) 1,3,5-trimethylbenzene	8.67	120	157941	0.84	0.73		
13) 1-methyl-2-ethylbenzene	8.97	120	67186	0.49	0.42		
14) 1,2,4-trimethylbenzene	9.30	120	434304	2.49	2.12		
15) 1,2,3-trimethylbenzene	10.00	120	79801	0.47	0.40		
16) indan	10.35	117	110913	0.30	0.23		
18) 1,4-diethylbenzene	10.65	119	197255	0.73	0.63	#	
19) n-butylbenzene	10.68	91	36861	0.09	0.07	#	
20) 1,2-diethylbenzene	10.82	105	6707	0.03	0.03		
21) 1,2,4,5-tetramethylbenzene	12.07	134	43624	0.21	0.18		
22) 1,2,3,5-tetramethylbenzene	12.15	134	59607	0.30	0.25		
23) naphthalene	13.59	128	285796	0.47	0.40		
24) pentamethylbenzene	15.36	148	3758	0.02	0.02		
25) 2-methylnaphthalene	15.66	142	176980	0.42	0.35		
26) 1-methylnaphthalene	15.97	142	71005	0.18	0.15		
27) C10 benzenes	11.00	134	238008	1.21	1.01		
28) C11benzenes	13.00	148	117323	0.56	0.46		
29) C12 benzenes	14.64	162	27702	0.13	0.11		
30) Alkyl Indans	10.39	117	299343	0.78	0.60		
			Total:	33.80	28.86		

(#) = qualifier out of range (m) = manual integration
1801006.D RFG6890B.M Mon Sep 02 18:53:55 1996 Page 1

Figure 2. RFA sample using the Aromatics in Gasoline software.

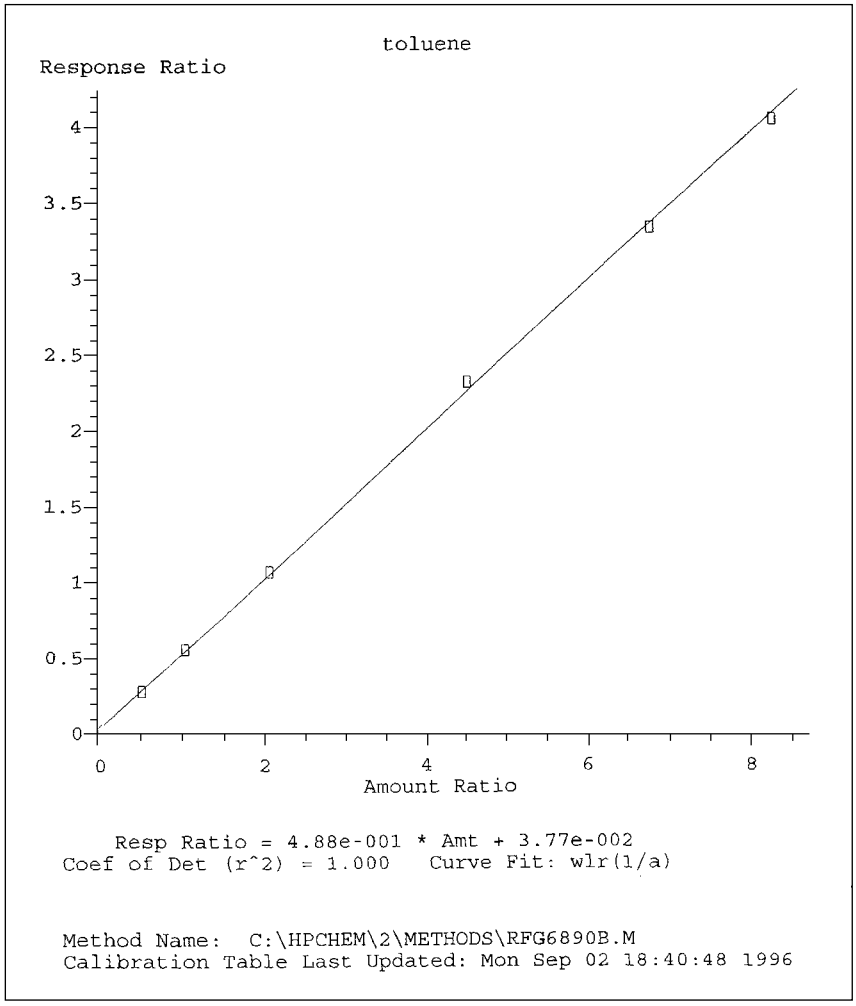

Figure 3. Final linear calibration curve for toluene utilizing the high split ratio, electron multiplier voltage programming and change of quant ion.

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6. Personal communication with Axel Lubeck, Marathon Oil.

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Printed in the U.S.A. December 1999
(23) 5966-2798E

Improved Analysis of Sulfur, Nitrogen, and Other Heteroatomic Compounds in Gasoline- and Diesel-Range Materials Using GC/Atomic Emission Detection

Application

Gas Chromatography

January 1998

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Abstract

An optimized procedure was developed for the analysis of sulfur and nitrogen compounds in gasoline- and diesel range materials. The analysis is performed using an Agilent 6890 gas chromatograph (GC) configured with an Agilent G2350A atomic emission detection (AED) system. A 30-m × 0.32-mm, 1-µm HP-1 MS capillary column is used for separation. The method is applicable to low-sulfur gasoline and diesel fuels. Nitrogen compounds can also be determined. The method can be applied to other light gas oil fractions with final boiling points of approximately C25 (402 °C, 756 °F). The configuration meets the requirements for determining sulfur in gasoline by ASTM method D 5623. The procedure can be extended to also measure oxygenates, organolead, organomanganese, and fluorine compounds.

Introduction

Determination of the sulfur content in petroleum feed streams is useful for improving and optimizing refinery catalytic processes. With the trend toward manufacturing of low-sulfur fuel products, such as gasoline and diesel fuel, accurate and precise measurements are important for obtaining product quality and meeting regulatory requirements. Additionally, knowledge of the individual sulfur types gives valuable insight into processing characteristics.

The measurement of nitrogen compounds is also of interest as these compounds are suspected of causing problems such as color and gum formation, engine deposits, and poisoning of some catalysts. The quantitation of the nitrogen compounds in petroleum materials is quite challenging, however, because of the low levels of nitrogen present and the potential for interference from the petroleum matrix.

Gas chromatography-atomic emission detection (GC/AED) has been used to measure the sulfur and nitrogen content in many types of petroleum samples.[1,2,3] The AED technique is well suited for these measurements because it has:

- High sensitivity required to measure low amounts of sulfur and nitrogen
- High selectivity required to reject hydrocarbon interferences
- No quenching
- Linear response
- Compound-independent element (equimolar) response factors

The ASTM method D 5623-94 (Standard Test Method for Sulfur Compounds in Light Petroleum Liquids by Gas Chromatography and Sulfur Selective Detection)[4] recommends the use of the sulfur chemiluminescence detector (SCD) or the AED.

Flame photometric detectors, both conventional and pulsed,[5] are not recommended because of problems with quenching of the sulfur signal by coeluting hydrocarbons.

With the development of the G2350A GC/AED system and further improvements in spectrometer design and element detection algorithms, the AED is well suited for sulfur and nitrogen measurements in petroleum liquids.[6]

The AED is a multi-element detector that can be used to measure twenty-three different elements. In addition to sulfur and nitrogen, elements such as carbon, hydrogen, oxygen, lead, manganese, fluorine, and silicon, can all be detected. A single instrument setup can then be used for a wide range of petroleum analyses.

This application note describes a procedure for the analysis of sulfur and nitrogen compounds in gasoline- and diesel-range materials. This method can also be extended to materials boiling just beyond the diesel fuel range (for example, light gas and cycle oils). The experimental conditions used in this study, including AED reagent gas flows, were adjusted to maximize the sulfur and nitrogen selectivity over carbon (hydrocarbons). Using these conditions, the GC/AED can be used to perform ASTM D 5623-94. Examples of detecting other elements such as oxygen, lead, manganese, and fluorine are also given.

Experimental

A 6890 GC coupled to a G2350A AED was used for this study. The GC was equipped with a split/splitless injection port operated in split mode. All gas flows and pressures within the GC were controlled electronically. An auxiliary pressure control module on the 6890 GC was used to set the AED reagent gas pressures. Sample injections were made by a 6890 Series automatic liquid sampler and a 5-µL syringe.

The inlet was fitted with a single-tapered liner (part no. 5181-3316) containing a 1-cm piece of glass wool. The glass wool plug was transferred directly from the standard split/splitless liner (part no. 19251-60540) to the single-tapered liner and positioned so that the top of the plug was 16 to 18 mm from the top of the liner. This liner acted to minimize discrimination across the boiling range and improve sample repeatability by "wiping" the sample droplet from the end of the syringe. The inlet end of the capillary column was positioned in the center of the small diameter section of the liner at about 4 mm above the tip of the column ferrule. A 30-m × 0.32-mm id × 1-µm film thickness HP-1 MS capillary column was used for separation (part no. 19091S-713). This column was chosen because it has a lower specified rate of column bleed than conventional methyl silicone capillary columns. The inlet ferrule used is graphitized polyimide to reduce diffusion of air into the inlet. Note that the ferrule should be retightened after the first few runs to compensate for shrinkage.

The Agilent AED ChemStation was used to control the GC/AED system and to provide for data acquisition and peak integration. The ChemStation was operated using a Microsoft® Windows™ environment, and the AED ChemStation automated the entire process. Two injections were made to determine carbon, sulfur, and nitrogen in the samples. Hydrogen and oxygen were used as reagent gases for both carbon (179 nm) and sulfur (181 nm). In the case of nitrogen (388 nm), methane was added as the third reagent gas.

To further improve sulfur and nitrogen selectivity over carbon, the AED gas flows (hydrogen, oxygen, and helium makeup) were optimized to minimize interferences from hydrocarbons. In addition to the reagent gases, the amount injected was optimum for each analysis. The AED ChemStation method automated this process for routine laboratory use. The makeup gas flow was set to 100 mL/min when the high makeup flow valve turned on for the nitrogen and metals analysis. With this setting, the makeup flow is raised to about 230 mL/min. With electronic pressure control (EPC) of the reagent gases, all methods shown here can be run in sequence without intervention except for the oxygen analysis. This requires the source of auxiliary gas to the AED to be changed from methane (used for nitrogen analysis) to 10% methane in nitrogen.

To obtain the best performance for nitrogen detection, it is important to use helium carrier and makeup gas that is nitrogen-free. A heated getter-type helium purifier (model GC50, SAES Pure Gas Inc.) was used in the present setup. This type of purifier removes N_2 and many other contaminants in helium down to ppb levels.

Quantitation of sulfur and nitrogen was based on ASTM D 5623-94 with

both internal and external standardization used. External standard quantitation was based on the response of a t-butyl disulfide (TBDS) solution. For internal standard calculations, a solution of TBDS in iso-octane was gravimetrically spiked into the gasoline. The samples used for internal standard calculations were determined to be free of TBDS. Nitrogen was quantitated externally based on the response of a nitrobenzene standard.

CARB Low Sulfur RFG and Conventional Gasoline quality control samples were obtained from AccuStandard. RFA gasoline with 14.9% methyltertiarybutylether was purchased from Scott Specialty Gases. Tertiary-butyldissulfide (TBDS), nitrobenzene, and methylcyclopentadienyl manganese tricarbonyl (MMT) were from Aldrich Chemical. The standard reference materials with certified sulfur content and the tetraethyl lead were obtained from National Institute of Standards Technology (NIST). The remainder of the samples were provided by various petroleum laboratories.

Table 1 lists the entire set of equipment and conditions used for this study.

Results and Discussion

Gasoline Range Analysis

Figure 1 shows the carbon, sulfur, and nitrogen chromatograms from

Table 1 Experimental Setup and Conditions

Gas Chromatograph and Detector	
Agilent G1530A	6890 Series GC
Option 002	Power supply for fast oven ramps
Option 112	Split/splitless inlet
Option 207	GC-AED interface
Option 308	Aux EPC for AED reagent gas control
G2350A AED	Atomic emission detector
Column	30-m x 0.32-mm, 1.0 μm HP-1MS (part no. 19091S-713)
Automatic Liquid Sampler	
Agilent G1916A	6890 Series ALS with tray
Data Acquisition/Data Analysis	
Agilent G2371AA	AED ChemStation
Operating Parameters, GC	
Injection port temperature	280 °C
AED transfer line/cavity temperature	310 °C
Oven temperature program	40 °C to 300 °C at 10 °C/min, hold 10 min
Column pressure (constant pressure mode)	12 psi
Carrier gas	Helium
Split ratio (Carbon, Sulfur)	50/1
Split ratio (Lead, Manganese, Oxygen)	100/1
Split ratio (Nitrogen)	10/1 (Gas Saver 200 mL/min at 0.3 min)
Split ratio (Fluorine)	20/1
Injection volume	1 μL (except as noted)
Operating Parameters, AED	
Data rate	2.5 Hz
Method 1, carbon 179 nm and sulfur 181 nm	
Reagent gases	Oxygen 55 psi, hydrogen 45 psi
Makeup flow	100 mL/min
Method 2, nitrogen 388 nm	
Reagent gases	Oxygen 80 psi, hydrogen 40 psi, methane 50 psi
Makeup flow	230 mL/min
Method 3, Oxygen 171 nm	
Reagent gases	Hydrogen 40 psi, 10% methane in nitrogen 25 psi
Makeup flow	100 mL/min
Method 4, lead 261 nm and manganese 259 nm	
Reagent gases	Oxygen 55 psi, hydrogen 45 psi
Makeup flow	230 mL/min
Method 4, fluorine 690 nm	
Reagent gases	Hydrogen 15 psi
Makeup flow	100 mL/min

the analysis of the conventional gasoline sample. The carbon and sulfur data are collected simultaneously in the first method and the nitrogen in a second. For comparison The sulfur chromatogram of the sample after addition of internal standard tertiarybutyldisulfide, is also shown. This sample was analyzed according to ASTM D 5623 using both internal and external standard quantitation. The total sulfur content was measured to be 330 ppm (weight) by both methods. Two trace level nitrogen peaks were also detected, each containing about 0.30 ppm of nitrogen. The lack of any significant response on the sulfur and nitrogen chromatograms from the large hydrocarbons in the gasoline demonstrates the high selectivity afforded by the AED.

A gasoline range product from a catalytic cracking unit is shown in figure 2. Most of the sulfur compounds observed in figure 1 are also present in this sample. Unlike the gasoline in figure 1, the sulfur content of this material is made up mostly of benzothiophene and alkyl-substituted benzothiophenes and the level of nitrogen is much higher (about 560 ppm). The nitrogen profile in figure 2 is typical of that observed in gasolines. The tentative identifications of the nitrogen compounds are taken from the literature.7 We have observed that for most gasolines having measurable nitrogen content, the pattern of nitrogens is very similar to that in figure 2, with only the total amount varying significantly. The AED responds uniformly to nitrogen in all forms, including N2. The large peak at the beginning of the nitrogen chromatogram results from small amounts of air injected with the sample. This peak is excluded from the total nitrogen measurement.

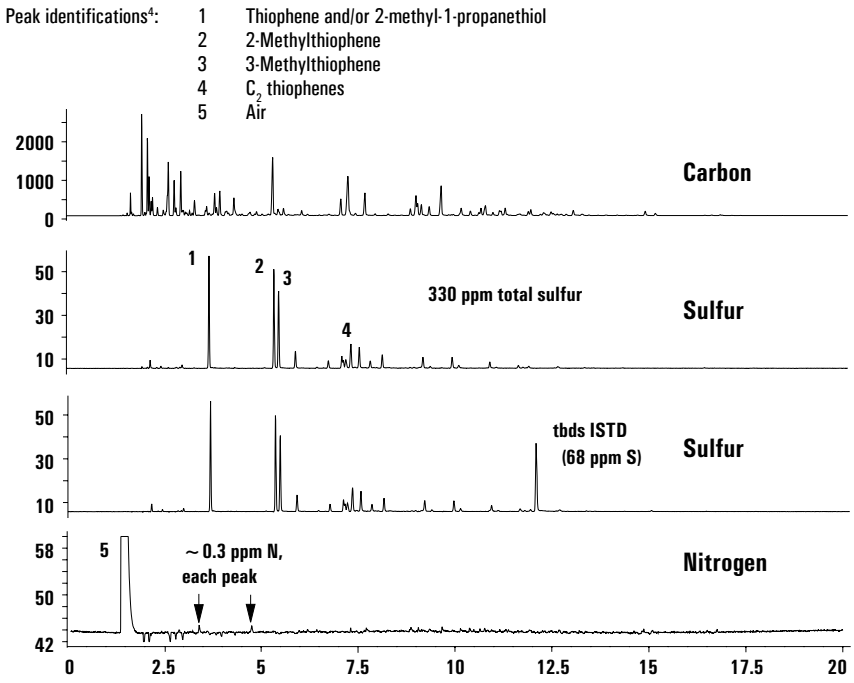

Figure 1. Conventional gasoline quality control sample. Upper sulfur trace is without added internal standard (TBDS), lower trace is with 68 ppm S as TBDS.

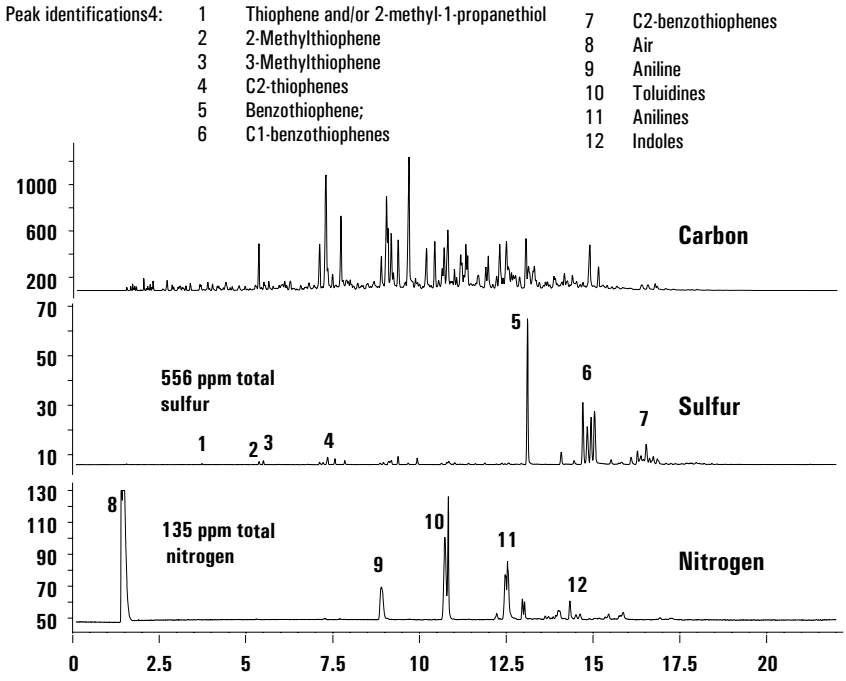

Figure 2. FCC Gasoline

The analysis of a low sulfur (California Phase II) gasoline sample is presented in figure 3. The detection limit for any one component was found to be approximately 0.7 ppm S. This sample contains about 42 ppm of sulfur by ASTM D 5623. The first two peaks in the sulfur chromatograph are not sulfur compounds, but are slight interferences from the largest hydrocarbons in the gasoline. These responses should not be included in the total sulfur calculation. If they are included, the error introduced is minimal because their total area corresponds to only approximately 3 ppm sulfur.

The nitrogen chromatogram in figure 3 was made with a 2-mL injection to increase sensitivity. The largest hydrocarbons at the front of the chromatogram produce small negative responses, but the actual nitrogen peaks are easily identified, even at the relatively low level of 25 ppm total nitrogen.

Figure 4 represents the analysis of a reference gasoline, RFA, containing methyl tertiary-butyl ether (MTBE). The large amount, 14.9 volume %, of MTBE does not cause an interference in the sulfur channel. As in the previous sample, the nitrogen chromatogram was collected with a 2-mL injection. The nitrogen peaks are still visible at 13 ppm total nitrogen. Peak 9 contains approximately 2.5 ppm N.

Figure 5 shows the oxygen chromatogram for RFA. Measuring additive oxygenates in gasoline is simplified greatly by using oxygen selective detection.[3,8] With the

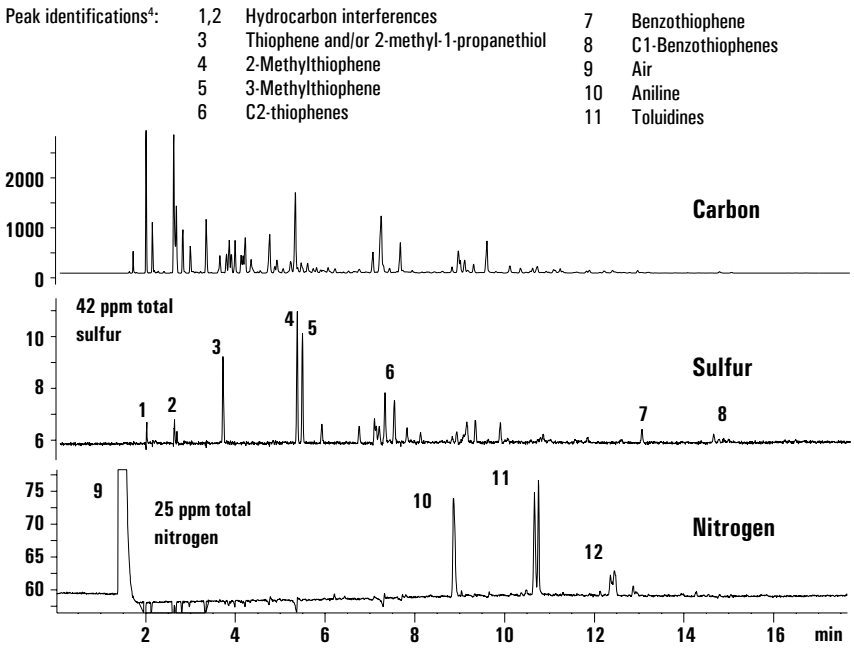

Figure 3. CARB Low-sulfur RFG quality control sample

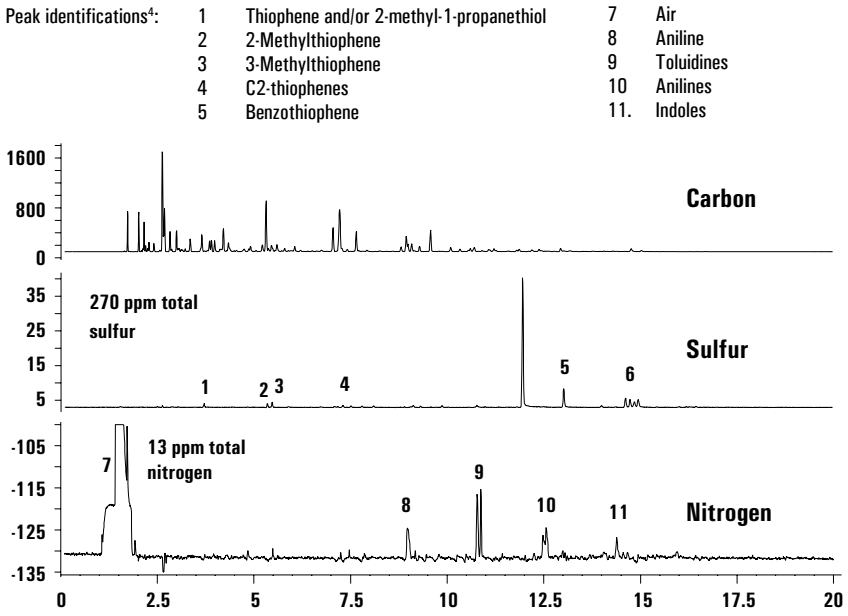

Figure 4. RFA Gasoline with 14.9% MTBE

conditions used here, the AED can detect individual oxygenates down to approximately 0.05%. The detection limit (signal-to-noise ratio = 2) for MTBE in figure 5 is 0.02%, and its performance is comparable to, or exceeds that of, the oxygen flame ionization detector (OFID).

Atomic emission with a helium plasma is a very sensitive and selective means of detecting volatile organometalic compounds. Also shown in figure 5 are the lead and manganese chromatograms from tetraethyl lead (TEL) and methylcyclopentadienyl manganese tricarbonyl (MMT) spiked into RFA. Lead compounds have been used worldwide as antiknock additives for gasoline but are currently being phased out of use in most countries. MMT has been used in the past in the U.S. and Canada.

Note that both the lead and manganese chromatograms exhibit little or no peak tailing and no hydrocarbon interference. Under the chromatographic conditions used here, the detection limit for TEL is approximately 1 ppm. Manganese detection is also very sensitive, allowing measurement of MMT well below 1 ppm. The small peaks before and after the MMT peak are not interferences, but are manganese containing impurities in the MMT.

An important capability of the AED is the ability to confirm the presence of an element in an unknown peak. Figure 6 shows the emission spectra obtained on the MMT peak and on the impurity peak (cymantrene) in figure 5. The three emission lines are

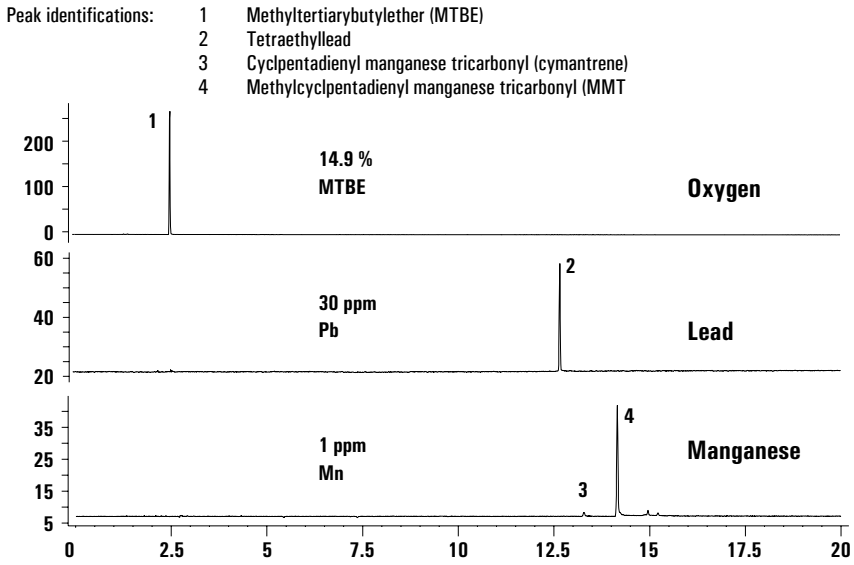

Figure 5. RFA Gasoline with 14.9% MTBE

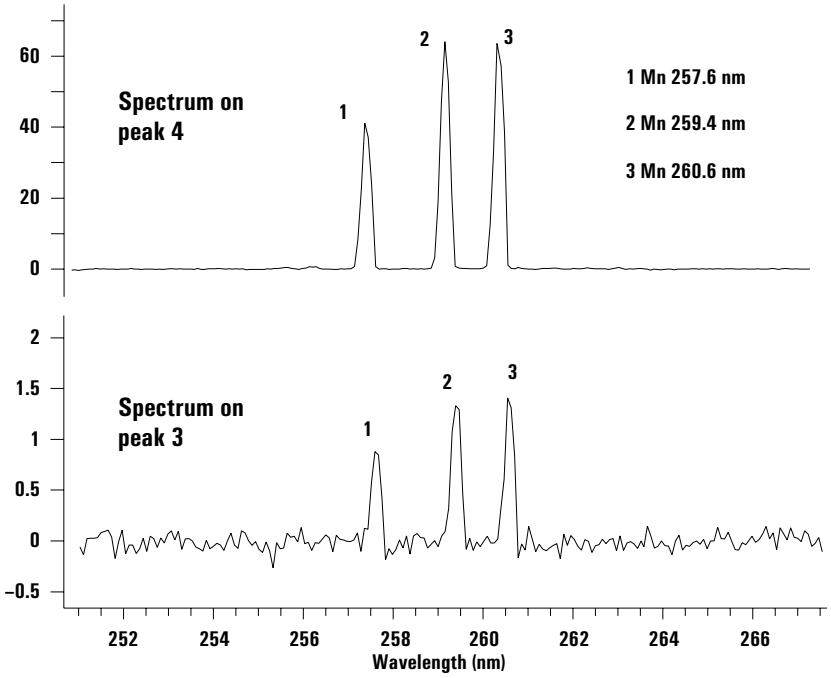

Figure 6. AED Emission spectra used to confirm presence of manganese in peaks 3 (cymantrene) and 4 (MMT) from figure 5

a fingerprint for the element manganese. If the spectrum of a peak contains these three emission lines in the relative intensities shown, then the presence of manganese is confirmed. The cymantrene peak only contains 40 ppb manganese. At this low level, the spectrum clearly shows the three manganese emission lines as seen in figure 6.

Figure 7 shows the chromatogram of a product from an alkylation unit. In this process, a C4 stream undergoes alkylation using hydrofluoric acid as the catalyst. In this example, the process is out of specification, with the level of organofluorides in the product at about 1000 ppm total fluorine. The AED fluorine chromatogram clearly shows the organofluoride impurities elute as compared to the hydrocarbons. From the relative patterns of the carbon and fluorine chromatograms, it appears that the fluorinated compounds are analogues of each of the main hydrocarbons in the sample. The detection limit (signal-to-noise ratio = 2) for a single fluorinated compound is about 0.6 ppm F under the conditions used here.

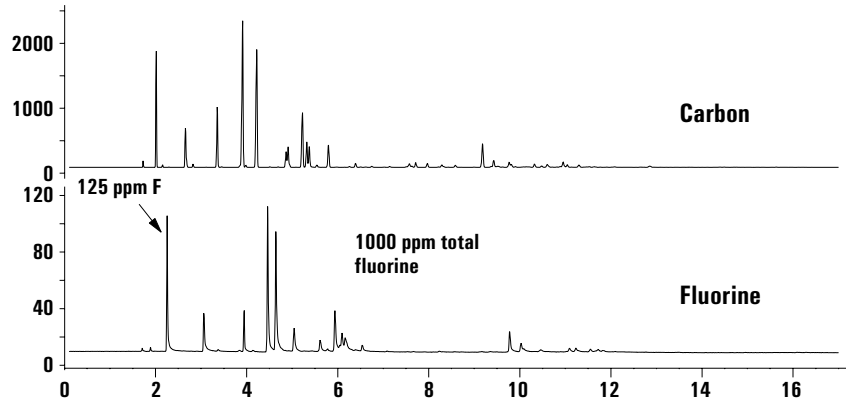

Figure 7. Off-specification product of HF alkylation unit

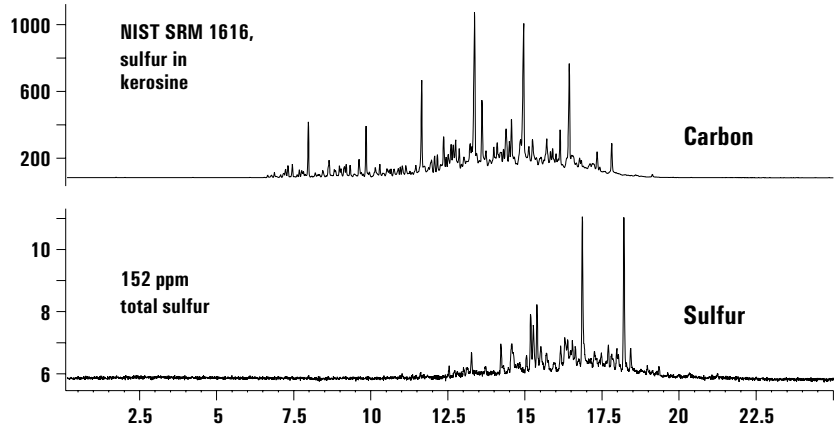

Figure 8. NIST SRM 1616 Sulfur in 1-k kerosine standard

Kerosine Analysis

The analysis of NIST sulfur in kerosine Standard Reference Materials (SRMs) is shown in figures 8 and 9. The SRM 1616 is a 1-k low-sulfur kerosine with a certified value of 152 ppm total sulfur. The retention time region where TBDS elutes is free from sulfur peaks, so the internal standard technique could be used.

The SRM 1617 is a high-sulfur kerosine standard, with a certified value of 1690 ppm total sulfur. It is interesting to note that the standard appears to have been constructed from a low-sulfur, 1-k kerosine with TBDS added

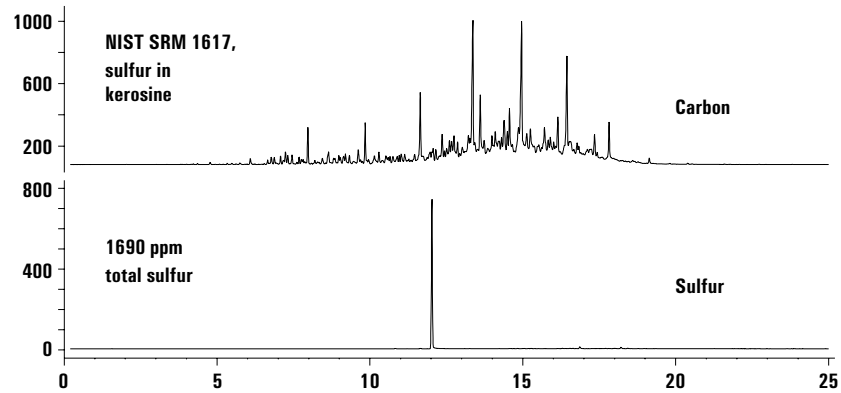

Figure 9. NIST SRM 1617 Sulfur in kerosine standard

to increase the sulfur content. Because TBDS is already in the standard, it could not be used as the internal standard for analysis.

There were no detectable nitrogen compounds observed in either kerosine.

Diesel Analysis

Figures 10 and 11 show the sulfur and nitrogen analysis of NIST sulfur in diesel fuel SRM 2724 and SRM 1624b.The SRM 2724 is a commercial no. 2-D distillate fuel oil as defined by ASTM. The certified total sulfur content is 425 ppm. The SRM 1624b is a similar fuel with a certified total sulfur content of 3320 ppm. These standards are very useful for diesel analysis because they bracket the range of sulfur normally encountered in these fuels.

The distribution and relative concentrations of sulfur compounds are similar in both SRMs, with only the total sulfur concentration being significantly different. The reverse is observed in the nitrogen chromatograms—the total nitrogen level is similar, but the profiles are noticeably different, especially in the earlier sections of the chromatograms.

A high-sulfur, high-nitrogen Light Cycle Oil (LCO) is shown in figure 12. Here the sulfur and nitrogen profiles are similar to the other diesels, but the carbon profile is clearly different. This sample contains higher-boiling material and requires an extra 10 minutes of hold time at the end of the chromatographic run to elute the sample completely.

Two samples of commercial low-sulfur diesel fuels from California are

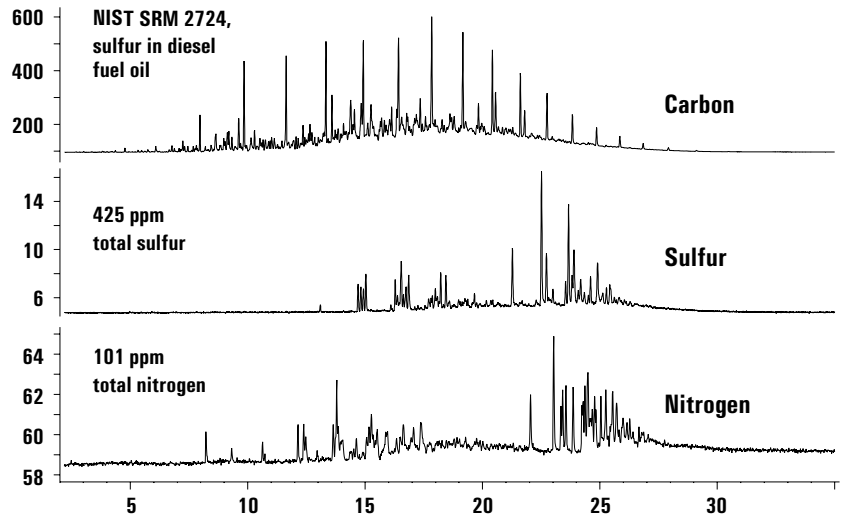

Figure 10. NIST SRM 2724 Sulfur in distillate fuel (diesel) standard

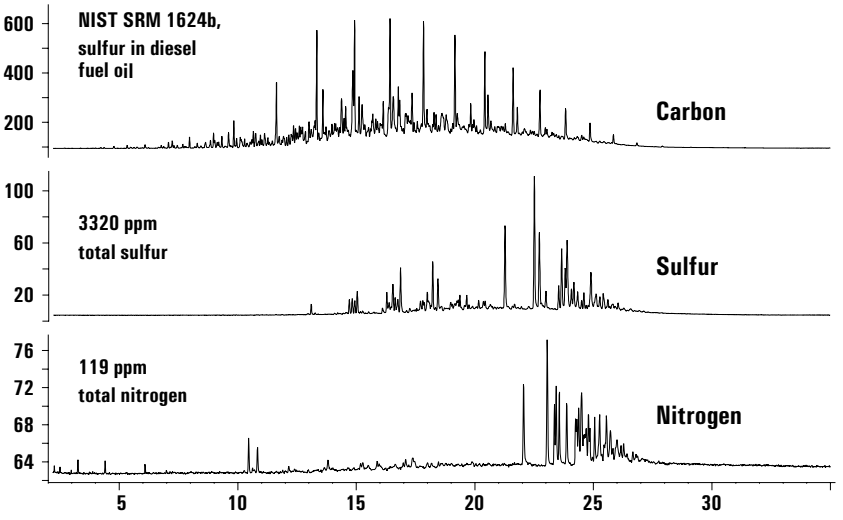

Figure 11. NIST SRM 1624b Sulfur in distillate fuel (diesel) standard

shown in figures 13 and 14. Comparison of the chromatograms illustrates how different the composition of sulfur and nitrogen can be at similar total concentrations. In the first sample, the total nitrogen is contained in a broad, unresolved distribution. In the second sample, the total nitrogen content arises from a few discrete peaks. It was thought that some of the nitrogen peaks in sample 2 could be nitro compounds added as cetane improvers. Both samples were then analyzed for oxygen to see if any of the nitrogen peaks were also oxygenated (i.e., nitro

compounds). While there were no oxygenates found in sample 2, an oxygen-containing peak that did not contain nitrogen was observed in sample 1. The oxygenate was identified as MTBE, and the concentration was measured to be at 0.26%.

In all the diesel-range samples analyzed, the time range where TBDS elutes had little or no sulfur signal, allowing its use as an internal standard.

Precision and Accuracy

The precision of the GC/AED system was evaluated by analyzing the "conventional" gasoline sample, discussed above, fifteen times over a 5-day period. A section of the chromatogram is shown in figure 15. The chromatograms are all offset to zero and are drawn overlaid. The precision of the retention time and sulfur response for the system is clearly evident. The percent relative standard deviation (%RSD) of the total sulfur area is 1.8%. The %RSD of the total carbon area measured simultaneously was 1.1%. The standard deviation of the retention time was 0.001 min for the peak at 8.6 min in the carbon chromatogram of figure 1.

The long-term precision observed here is significantly improved over that of 5890/5921 AED systems. Improvements in the 6890 EPC, such as atmospheric pressure correction, provides more precise control of column flow and split ratio. When used with the split liner described here, the amount injected into the column is very repeatable. The G2350 AED uses the 6890 Aux EPC module to control the reagent gases. The more precise control of reagents, combined with spectrometer and detection algorithm improvements, greatly increases the precision of the AED detector. The sum of the

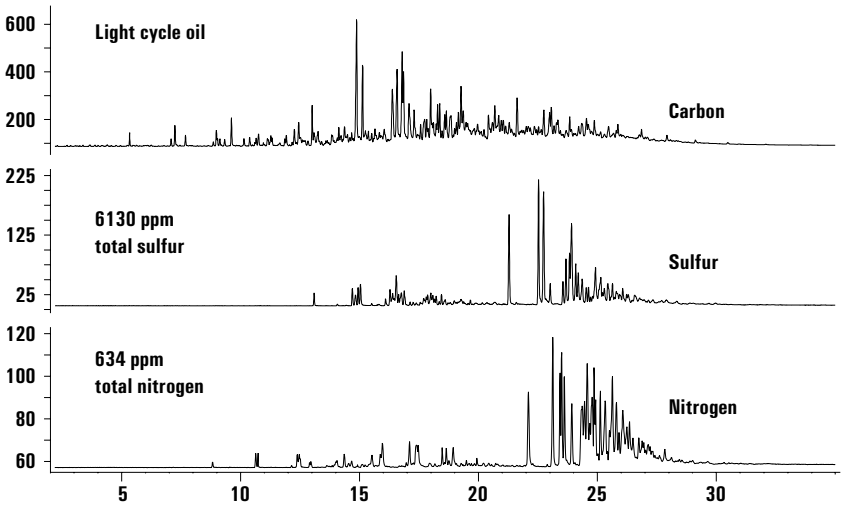

Figure 12. Refinery Light Cycle Oil (LCO) sample

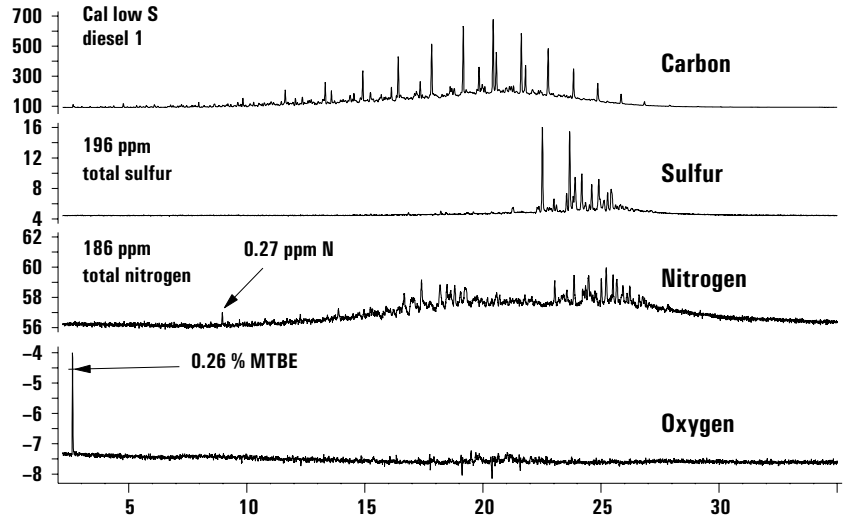

Figure 13. Commercial California low-sulfur diesel fuel, sample 1

improvements in both the GC and the AED using the conditions described here result in a measured response factor drift for sulfur and carbon of less then 3% in a week.

Seven of the samples shown here were analyzed for total sulfur using both the external and internal standard calculations from ASTM D 5623. The results agreed within 2%, indicating that with the precision observed with the AED, acceptable results can be obtained with the simpler external standard method. The external standard analysis is simpler to perform because there is less sample preparation required, and the problem of finding a suitable internal standard compound for each sample is eliminated. The internal standard approach is most useful with sulfur detectors or GC systems that have response factor drift problems. The

system described here does not exhibit these problems, thus, simplifying the analysis.

The NIST kerosine and diesel fuel SRMs were used to assess the accuracy (bias) of the method. Using the method as described here, the measured values for total sulfur agreed within 2% of the certified value.

Conclusions

The technique of GC/AED has many valuable uses in petroleum analyses. The ability to speciate of sulfur, nitrogen, oxygen, fluorine, lead, and manganese compounds in one instrument provides a very cost-effective solution to a broad range of analytical needs.

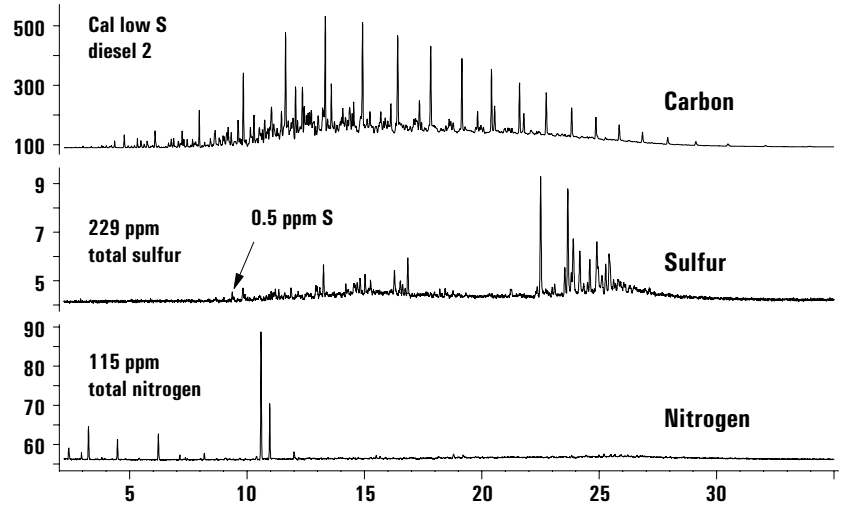

Figure 14. Commercial California low-sulfur diesel fuel, sample 2

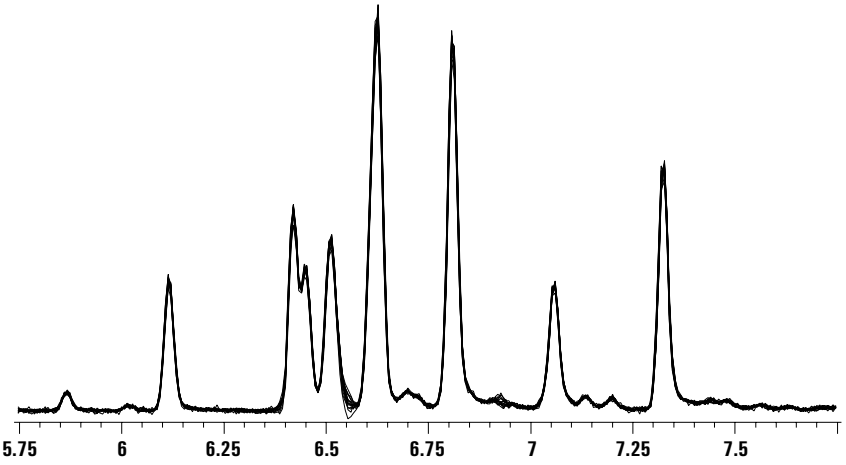

Figure 15. Chromatograms from 15 analyses of conventional gasoline sample run over a 1-week period. Chromatograms are offset to zero and drawn overlaid.

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Printed in the USA 3/2000
5966-3089E



Agilent Technologies
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Agilent 6850 Sub-Ambient Oven Performance for ASTM D3710 Simulated Distillation of Gasoline

Application

Gas Chromatography

September 2000

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Abstract

The Agilent 6850 gas chromatograph is now offered with sub-ambient column oven capabilities using liquid carbon dioxide as the cryogenic gas. This extends the 6850 oven temperature from -20 °C to 350 °C. The sub-ambient oven performance of the 6850 was tested using the ASTM D3710 method for simulated distillation of gasoline range materials. This method requires starting oven temperatures of -20 °C followed by programming to 180 °C. Four chromatogaphic performance tests required by the ASTM method were used to evaluate 6850 system performance. The same tests were also run on the Agilent 6890 GC to offer a comparison with an industry standard system. Both the 6850 and the 6890 results exceeded the specifications for peak shape, resolution, and retention time repeatability and area percent repeatability. In most cases both GCs showed performance that was 10 times better than the ASTM requirements. The 6850 and the 6890 also showed nearly identical performance when compared to each other. Additionally, the 6850 used about one-half of the CO_2 cryogenic gas compared to the 6890 when running this method.

Introduction

Most gas chromatographic separations can be performed with GC oven temperatures starting above ambient conditions. However, there are some instances that require GC oven temperatures that are below ambient. For example, separation of gases, low boiling components, and solvent focusing are all examples where the chromatographer needs to use cold oven temperatures. For modern instruments, sub-ambient temperatures are achieved by controlled introduction of a cryogenic gas into the oven. Typically, liquid carbon dioxide (CO_2) or liquid nitrogen are used as the cryogenic gas.

The Agilent 6850 gas chromatograph is a rugged, easy-to-use, single channel instrument especially suited for production laboratories where space is a premium. The 6850 occupies 50% of the linear bench space compared to the Agilent 6890, while still providing the same chromatographic performance. For applications requiring sub-ambient oven temperatures down to -20 °C, the 6850 now offers a CO_2 cryogenic cooling option.

The 6850 cryogenic oven performance was evaluated using the ASTM D3710 Method for Boiling Range Distribution of Gasoline and Gasoline Fractions by Gas Chromatography[1]. This simulated distillation method was slightly modified by using a fused silica capillary column in place of a packed column. Instrument conditions for this method are listed in table 1. The sample run on these instruments was a D3710 Qualitative Calibration Mix (part # 506427, Supelco, Bellefonte, PA, USA) which contains the nineteen compounds required for system performance evaluation. Five consecutive runs of this mix were made on each GC. The quantity of CO_2 cryogenic gas used for each run was also measured to compare consumption differences between the 6850 and 6890.

Table 1. Instrument conditions for ASTM D3710 Simulated Distillation

Autoinjector	Agilent 7683 ALS 10 µL Teflon-tipped plunger syringe
Inlet	Split/splitless operated in split mode Temperature: 200 °C Liner part # 5183-4647
Column	HP-1, 3.0 µm film thickness, 15 m × 530 µm (part # 19095Z-421E)
Oven	-20 °C for 2 min, 20 °C/min to 180 °C, hold 2 min
Detector	Flame ionization detector (FID) Temperature: 300 °C FID hydrogen flow rate: 40 mL/min FID air flow rate: 450 mL/min FID make-up flow rate: 45 mL/min nitrogen Data acquisition rate: 10 Hz
Data system	Agilent Chemstation

This ASTM D3710 method is useful for evaluating GC cryogenic oven performance. It is a widely used application and is familiar to many GC analysts. D3710 also has stringent chromatographic performance specifications that can be used to assess the sub-ambient oven performance. These requirements are listed below:

1. *Resolution* - compounds lighter than isopentane must be separated such that the valley above the baseline is less than 5% of the height of the smaller peak.
2. *Peak Shape* (Skew) - peak skew must be not less than 0.5 and not more than 2.0.
3. *Retention Time Repeatability* - the retention time difference between consecutive runs must not be not more than 3 seconds for isopentane and lighter compounds. For compounds heavier than n-pentane, the maximum difference in retention time between successive runs must not be greater than the time equivalent of 3 °C. Additionally, the minimum retention time of the propane must be greater than 15 seconds.
4. *Area Percent Repeatability* - duplicate area percent results for each compound from consecutive runs must not differ by more than 0.1 area percent.

Results

Resolution. Figure 1 shows the chromatograms of the D3710 qualitative calibration sample run on both the 6850 and 6890 GCs. The nineteen compounds in this sample are baseline resolved on both GCs in approximately 12 minutes. Figure 2 shows the resolution of the compounds that are lighter than isopentane. For all four peaks, the valley above the baseline is much less than 5% of the height of the smaller peak.

Peak Shape. Table 2 lists the symmetry of each peak in the chromatograms shown in figure 1. A skew value of 1.0 would indicate perfectly symmetrical peaks. Skews of less than 0.5 would indicate tailing, and values greater than 2.0 would indicate fronting (overload). Both the 6850 and 6890 show peaks that are almost all perfectly symmetrical.

Retention Time Repeatability. Both the 6850 and 6890 show excellent retention time repeatability as shown in table 3. For isopentane and lighter compounds, the retention time repeatability on both GCs is about ten times better than the ASTM specification. For compounds heavier than n-pentane, the ASTM specification calls for repeatability of less than the time equivalent of 3 °C. For this method, that translates into a value of ≤ 2.0 seconds. Both the 6850 and the 6890 show retention time repeatability that is 20 times better. Additionally, the retention time of the propane on each GC is greater than 15 seconds (figure 2).

Area Percent Repeatability. Table 4 shows the area percent repeatability of the 6850 and 6890. Both instruments show 5-10 times better performance than what is required by the ASTM specification.

CO_2 Cryogenic Gas Usage. The 6850 required about 1.5 lbs. of CO_2 cryogen for each run of D3710. The 6890 GC used about 3 lbs. of carbon dioxide. The smaller oven design of the 6850 makes it easier to cool below ambient conditions and hold that temperature. Oven cycle times after temperature programming were approximately the same for both instruments (10 minutes).

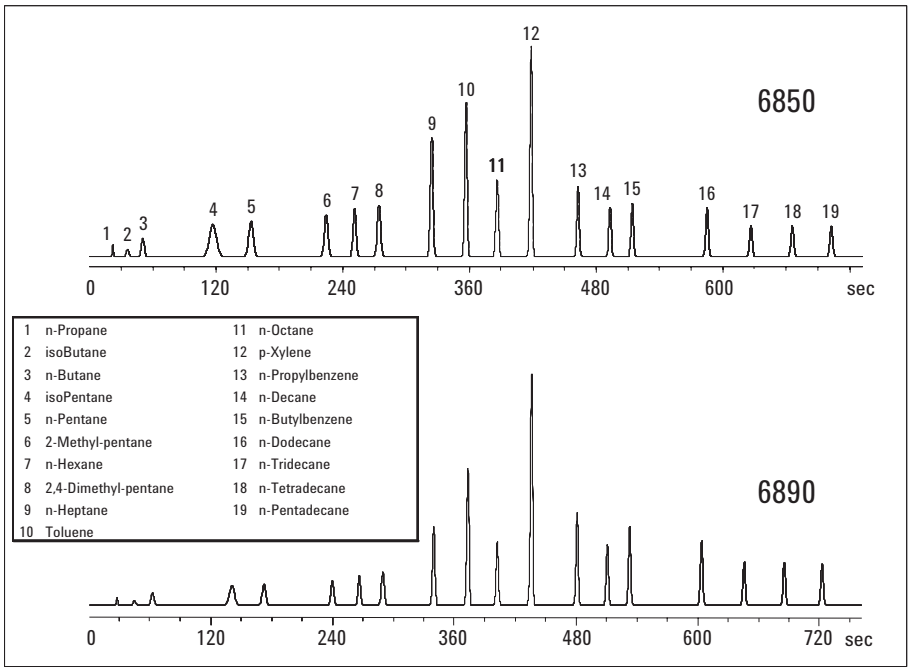

Figure 1. Separation of nineteen compounds used to evaluate GC system performance of the ASTM D3710 method for the simulated distillation of gasoline. Each run was made using CO_2 as the cryogenic gas for sub-ambient oven temperatures on both the 6850 and 6890.

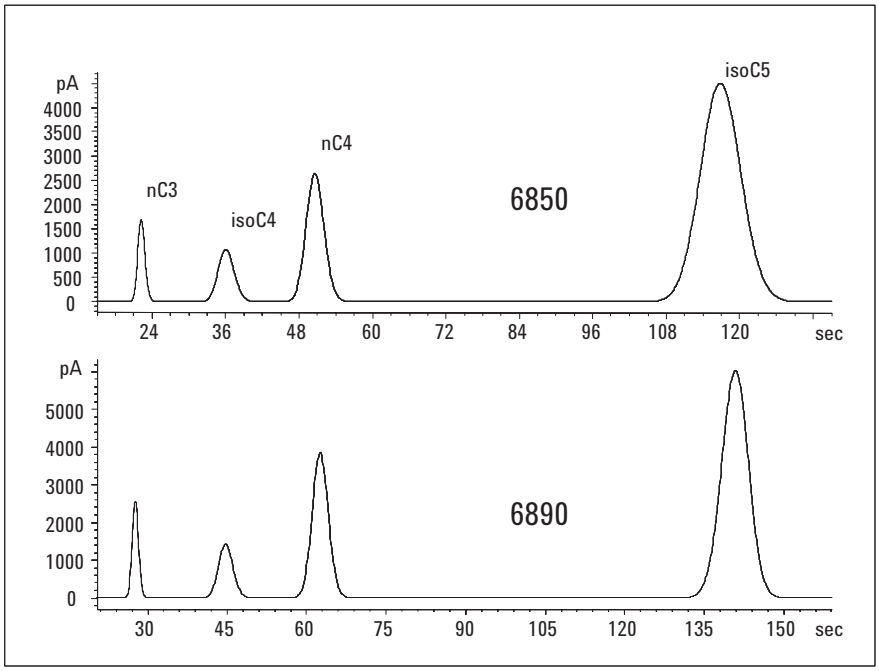

Figure 2. Baseline resolution is achieved for isopentane and lighter compounds on both the 6850 and 6890.

Table 2. ASTM D3710 Peak Symmetry Test (Skew)

Compound	ASTM Specification	Observed* 6890	Observed* 6850
n-Propane	0.5-2.0	0.9	0.9
IsoButane	0.5-2.0	0.9	0.9
n-Butane	0.5-2.0	1.0	0.9
isoPentane	0.5-2.0	1.0	1.0
n-Pentane	0.5-2.0	1.0	1.0
2-Methyl-pentane	0.5-2.0	1.0	1.0
n-Hexane	0.5-2.0	1.0	1.0
2,4-Dimethyl-pentane	0.5-2.0	1.0	1.0
n-Heptane	0.5-2.0	1.1	1.1
Toluene	0.5-2.0	1.2	1.1
n-Octane	0.5-2.0	1.1	1.1
p-Xylene	0.5-2.0	1.5	1.2
n-Propylbenzene	0.5-2.0	1.2	1.1
n-Decane	0.5-2.0	1.1	1.0
n-Butylbenzene	0.5-2.0	1.2	1.1
n-Dodecane	0.5-2.0	1.2	1.1
n-Tridecane	0.5-2.0	1.1	1.0
n-Tetradecane	0.5-2.0	1.1	1.0
n-Pentadecane	0.5-2.0	1.1	1.0

*Average from 5 consecutive runs

Table 3. ASTM D3710 Retention Time Repeatability Test

Compound	ASTM Specification	Observed (sec)* 6890	Observed (sec)* 6850
n-Propane	≤ 3.0s	0.1	0.1
isoButane	≤ 3.0s	0.1	0.1
n-Butane	≤ 3.0s	0.1	0.0
isoPentane	≤ 3.0s	0.2	0.3
n-Pentane	≤ 2.0s	0.2	0.2
2-Methyl-pentane	≤ 2.0s	0.2	0.1
n-Hexane	≤ 2.0s	0.2	0.1
2,4-Dimethyl-pentane	≤ 2.0s	0.1	0.1
n-Heptane	≤ 2.0s	0.1	0.1
Toluene	≤ 2.0s	0.1	0.1
n-Octane	≤ 2.0s	0.1	0.1
p-Xylene	≤ 2.0s	0.1	0.1
n-Propylbenzene	≤ 2.0s	0.1	0.1
n-Decane	≤ 2.0s	0.1	0.1
n-Butylbenzene	≤ 2.0s	0.1	0.1
n-Dodecane	≤ 2.0s	0.1	0.1
n-Tridecane	≤ 2.0s	0.1	0.1
n-Tetradecane	≤ 2.0s	0.1	0.1
n- Pentadecane	≤ 2.0s	0.1	0.1

*Average from 5 consecutive runs

Table 4. ASTM D3710 Area Percent Repeatability Test

Compound	ASTM Specification	Observed (area%)* 6890	6850
n-Propane	≤ 0.10%	<0.01	<0.01
isoButane	≤ 0.10%	<0.01	<0.01
n-Butane	≤ 0.10%	0.01	<0.01
isoPentane	≤ 0.10%	0.02	<0.01
n-Pentane	≤ 0.10%	0.01	<0.01
2-Methyl-pentane	≤ 0.10%	0.01	<0.01
n-Hexane	≤ 0.10%	0.01	<0.01
2,4-Dimethyl-pentane	≤ 0.10%	0.01	<0.01
n-Heptane	≤ 0.10%	0.02	<0.01
Toluene	≤ 0.10%	<0.01	0.01
n-Octane	≤ 0.10%	<0.01	<0.01
p-Xylene	≤ 0.10%	0.03	0.01
n-Propylbenzene	≤ 0.10%	0.02	<0.01
n-Decane	≤ 0.10%	0.01	<0.01
n-Butylbenzene	≤ 0.10%	0.02	<0.01
n-Dodecane	≤ 0.10%	0.02	<0.01
n-Tridecane	≤ 0.10%	0.01	<0.01
n-Tetradecane	≤ 0.10%	0.02	<0.01
n- Pentadecane	≤ 0.10%	0.02	<0.01

*Average from 5 consecutive runs

Summary

The Agilent 6850 gas chromatograph can now use liquid carbon dioxide to achieve sub-ambient column oven temperatures down to -20 °C. The CO_2 cryogenic performance characteristics of the 6850 were tested using the ASTM D3710 method for simulated distillation of gasoline. The 6850 showed ten-times better chromatographic performance. These results were also identical to the Agilent 6890 equipped with CO_2 cryogenic oven cooling. The 6850 also used 50% less CO_2 for each run of D3710 when compared to the 6890.

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Printed in the USA 9/2000
5980-0250E



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A Comparison of Sulfur Selective Detectors for Low Level Analysis in Gaseous Streams

Application

Gas Chromatography

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Abstract

A comparison of four selective detectors for analysis of single-digit ppb level sulfur is described. A dynamic blending system is used for point of use preparation of low level gaseous mixtures of eight sulfur compounds including H_2S, COS, CS_2 and selected mercaptans in helium, ethylene, propylene, and carbon dioxide. The criteria to consider for selecting the best detector for an application depends on many factors including, sensitivity, selectivity, stability, and ease of use. Guidelines for making the best detector choice are discussed. The Atomic Emission, Flame Photometric, Pulsed Flame Photometric, and Sulfur Chemiluminescent detectors are included in the study.

Introduction

The use of sulfur selective detectors in gas chromatography has grown in recent years with the increasing industrial demands for sulfur measurement. A major driving force has come from the hydrocarbon processing industry where the need for lower level more reliable sulfur measurements is intensifying. Regulations on sulfur levels in fuels continue to tighten. The need for low level sulfur measurements exists in nearly all segments of the chemical industry. Examples include the analysis of beverage grade CO_2 and the determination of ordorants in natural gas. In this paper, the characteristics and performance of four types of sulfur detectors will be compared with example applications used to demonstrate their operation under real-world conditions. The detectors include the AED (Atomic Emission Detector), SCD (Sulfur Chemiluminescent Detector), PFPD (Pulsed Flame Photometric Detector), and FPD (Flame Photometric Detector).

The Significance of Sulfur

Most sources of light hydrocarbons contain sulfur compounds that can be corrosive to pipes and equipment. The emission of undesirable odors caused by volatile sulfur compounds in intermediates and final products can have serious economic impact. Perhaps the most important consequence of sulfur is its' detrimental effect on a variety of catalysts used for processing and conversion of light hydrocarbon streams. Also, full compositional determinations of LPG (Liquefied Petroleum Gases) and propene mixes are required frequently where pricing of the end product is based on quality.

The presence of sulfur does not always come from natural sources. Sulfur is added to natural gas and LPG as a safety measure. In addition to the importance of monitoring concentration, some odorants tend to react over time forming compounds with different odor thresholds. Therefore, the need for measurement is important for a variety of reasons.

High purity ethylene and propylene are feedstocks for a wide variety of chemicals and end products. For example, ethylene is the base material for products such as styrene monomer, ethylene oxide/ethylene glycol, and low and high-density polyethylene. In polyolefin production, sulfur impurities commonly found include H_2S and COS. The removal of such impurities are required in order to prevent deactivation of polymerization catalysts, eliminate polymer with poor color or off-odor, enhance reaction rates, and provide consistent physical properties. The economic impact of any decrease in yield or catalyst life is quite high given the commodity nature of these processes. Strict quality control of hydrocarbon and sulfur impurities is, therefore, required. As measurement technologies advance, the acceptable levels decrease. However, the measurement methods must be reliable, stable, and easy to use before widespread acceptance is possible. The demand from the petrochemical industry is clearly growing.

Environmental concerns are also present. Allowable sulfur levels emitted into the atmosphere from both liquid and gaseous streams are constantly under scrutiny to reduce damage caused by precipitation of sulfuric acid in rain, for example.

Experimental

All detectors were interfaced to the Agilent 6890 gas chromatograph equipped with the volatiles interface (VI). The volatiles inlet was interfaced directly to a 6-port Hastelloy C automated gas-sampling valve (GSV). Additional insulation was used to cover the exposed length of line connecting the valve to the VI. Silcosteel tubing was used for all lines that had contact with sample. This tubing included use of Silcosteel treated sample loops. Sample loop size was typically 1.0 mL to maximize sensitivity. The use of 0.25 and 0.50 mL loops will result in better overall chromatography and peak shape for most applications where maximum sensitivities are not required. It is extremely important that all lines in contact with the sample are deactivated for successful detection of low level sulfur. All GC gas flows and pressures were controlled electronically. The Aux EPC module was plumbed so that Aux 5 was assigned as the diluent stream for the dynamic blending system described later in this section. The basic plumbing scheme employed for sample introduction is shown in Figure 1. A cross sectional view of the volatiles inlet is depicted in Figure 2. This inlet has an internal volume of only 35 µL and is also Silcosteel treated for inertness.

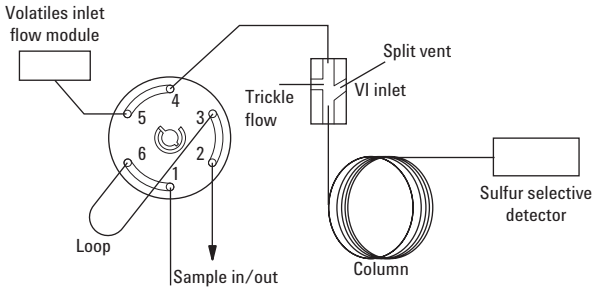

Figure 1. Silcosteel treated sample introduction system for ppb sulfur detection.

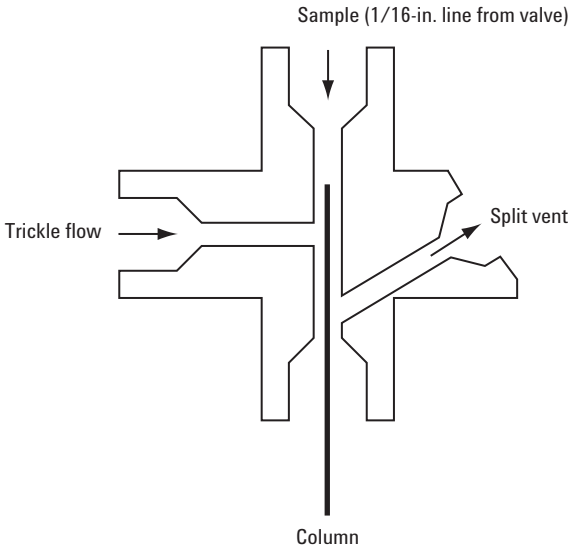

Figure 2. Cross-section of the Volatiles Inlet (not drawn to scale) depicting flow paths. All internal surfaces Silcosteel treated.

Calibration cylinders of light sulfur gases generally cannot be certified at levels below approximately 5 ppm per component due to the difficulty of preparation and the adsorption of sulfur species on the cylinder walls. As a result, calibration of sulfur selective detectors at ppb levels can be challenging. Point of use sample preparation is one relatively simple approach to the problem. This approach can be used for calibration by addition of a diluent to the high-level calibration standard. The diluent may be just helium or some other matrix such as ethylene or propylene. The ratio of total volumetric flow (diluent Aux 5 plus calibration mix) to the calibration mix flow will determine the dilution factor. When switching gas mixtures, take care to ensure that all lines are flushed to avoid carry over. In Figure 3, a simplified diagram of the point-of-use plumbing scheme

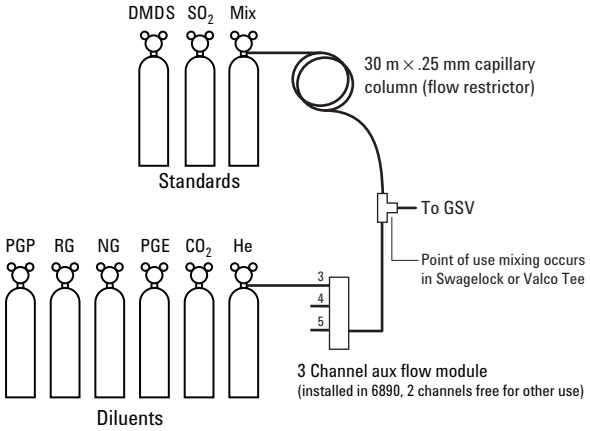

Figure 3. Plumbing scheme for dynamic blending.

is shown. The restrictor employed on the calibration mix flow path was a 30 m × 0.25 mm capillary column. The Agilent flow calculator program[1] is then used to determine flow rate at a given inlet pressure. Components in the sulfur mix used in this study are listed in Table 1. Peaks in the chromatograms (Figures 4 through 9) can be identified by number with reference to this table. The sulfur calibration mix was obtained from DCG Partnership I, LTD., Pearland, TX, 281-648-1894. Sulfur dioxide was used from a separate cylinder due to its reactivity.

Table 1. Sulfur Mix Components

Number	Compound
1	Hydrogen sulfide
2	Carbonyl sulfide
3	Methyl mercaptan
4	Ethyl mercaptan
5	Dimethyl sulfide
6	Carbon disulfide
7	t-Butyl mercaptan
8	Tetrahydrothiophene

Representative detector settings are given in Table 2.

Table 2 Detector Settings

Detector	Temperatures	Pressures and Flows
Agilent Flame Photometric	250 °C	H_2 50 mL/min Air 60 mL/min N_2 makeup 50 mL/min
Agilent G2350A Atomic Emission Carbon 193 nm, Sulfur 181nm	Cavity 245 °C Transfer line 245 °C	O_2 55 psi H_2 45 psi He makeup 100 mL/min
Antek 7090 Sulfur Chemiluminescent	950 °C Furnace	P1 38 detector P2 176 furnace H_2 200 mL/min O_2 8 mL/min O_3 25 mL/min
Sievers 355 Sulfur Chemiluminescent	803 °C Furnace	P1 5.4 detector P2 171 furnace H_2 100 mL/min O_2 8 mL/min O_3 5.6 psi
OI 5380 Pulsed Flame Photometric	250 °C	H_2 11.5 mL/min Air(1) 12.5 mL/min Air(2) 11.0 mL/min (makeup)

Unless otherwise stated, the following gas chromatographic conditions were used.

Gas Sampling Valve:	1 cc loop at 140 °C, Hastelloy C valve material
Column:	105 m × 0.53 mm × 5 µm DB-1
Oven:	-20 °C (9.5 min), 10 °C/min to 250 °C, 80 °C/min to 255 °C
VI Inlet:	5 mL/min constant flow mode
	Split ratio 0.1:1 typical
	Temp 225 °C

Results and Discussion

Gas chromatographic sulfur selective detectors can be extremely powerful tools to characterize and quantify a wide range of low level sulfur species in gaseous streams. The FID, while sensitive, will not detect some sulfur gases such as H_2S, COS, CS_2, and SO_2. Thermal Conductivity Detectors have traditionally found application for sulfur analysis; however, ppb sensitivities required for many applications are not realistic.

Many factors must be considered when deciding on the best sulfur selective detector to employ for a given application. Attributes such as selectivity, response factor behavior, quenching, column compatibility, and sensitivity should be considered and matched to the application. All detectors studied in this work have at least three orders of magnitude dynamic range, making them suitable for a relatively wide concentration band. A summary of the detector characteristics used in this work is given in Table 3. The values for sensitivity, selectivity, and dynamic range are those claimed by the respective suppliers. In this study, instrumental conditions were generally optimized for maximum sensitivity. These conditions will not always lead to the best chromatography.

The sulfur mix diluted with helium as per the point of use sample preparation scheme was used to access practical limits of detection. A methyl silicone column (105 m × 0.53 mm × 5 µm DB-1) was used for the comparison because very little adsorption of sulfur species occurs on the stationary phase, eliminating the potential for inaccurate MDL calculations. Adsorption of certain sulfur compounds is known to occur on Plot or silica columns, making their use somewhat more problematic. Chromatograms of the sulfur mix are shown in Figure 4 for each of the four detectors. Note that the time scales for the five GC traces do not line up, the chromatograms have been adjusted either right or left to make the figure easier to read. As is evident from the figure, the AED, PFPD, and SCD's are all capable of sulfur detection in the sub 10 ppb range with reasonable signal to noise ratios. Even the FPD can show good results at 50 ppb when the system is maximized for sensitivity.

The dynamic blending system provides a convenient means of looking at detector selectivity by mixing in real world samples (matrix) with the low-level sulfur calibration mix. It also allows looking at just the pure matrix once potential hydrocarbon or other gas interferences/coelutions are understood with any given column and temperature program

Table 3. Summary of Detector Characteristics

Detector characteristics				
Detector	FPD	PFPD	SCD	AED
Supplier	Agilent	OI	Sievers/Antek	Agilent
MDL sulfur	20 pg/sec	1 pg/sec	0.5 pg/sec	2 pg/sec
Selectivity	10^5	10^6	10^6	10^5
Dynamic range	10^3	10^3	10^5, linear	10^4, linear
Quenching	Yes	Yes	No	No
Equimolar response	Approximate	Yes	Yes	Yes
Packed column compatible	Yes	No, 1 mL/min flow	Yes	Yes
Other elements	P, Sn	P	N	C,H,N,O,Cl, etc., total 26
Approximate relative cost	$	$$	$$$	$$$$

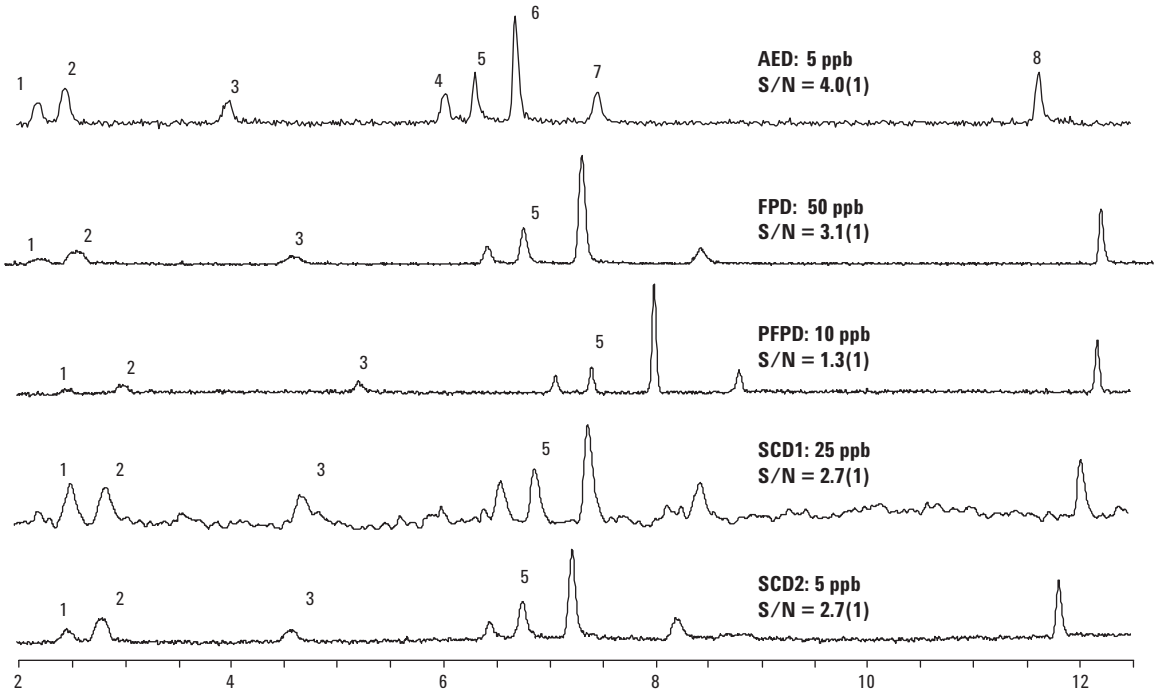

Figure 4. Detection limits for the four sulfur selective detectors using the eight component calibration mix.

regime. In Figure 5, FPD chromatograms are shown where polymer grade ethylene (PGE) was blended into the sulfur calibration mix. Sulfur levels of 50 ppb and 10 ppb per component are shown in the top and bottom traces, respectively. Because the sulfur compounds are well separated from the ethylene on the DB-1 column, most can still be quantified at 10 ppb. Only H_2S and SO_2, two of the more reactive; less well chromatographically behaved compounds disappear at 10 ppb. In Figures 6 through 9, chromatograms are shown for the AED, PFPD, SCD (Antek), and SCD (Sievers),

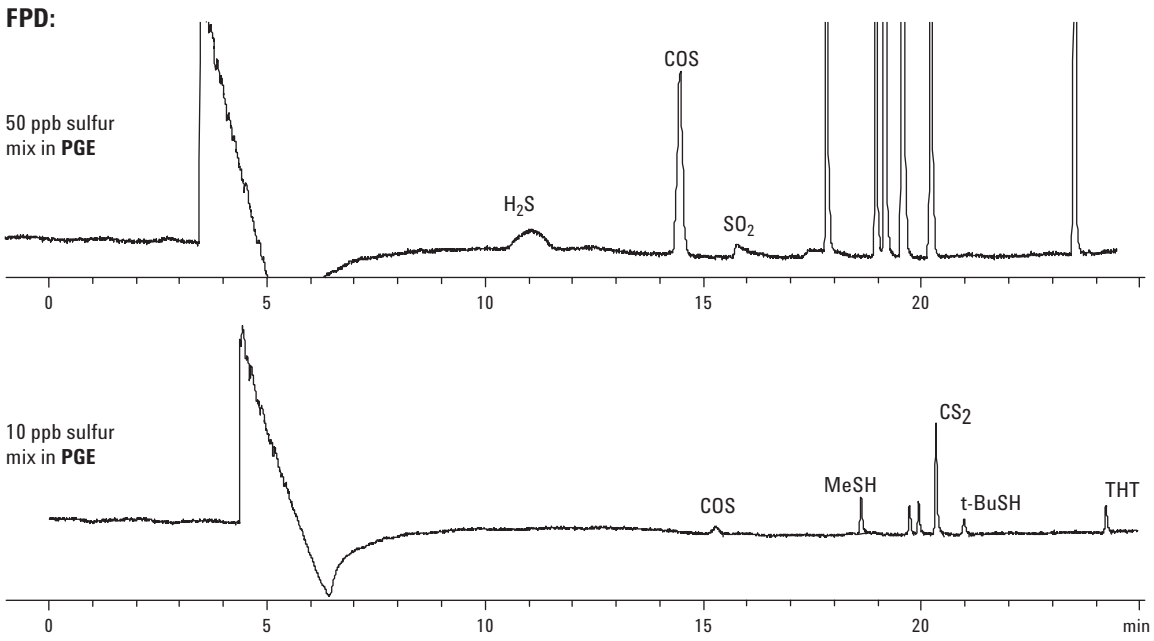

Figure 5. Agilent FPD, ppb level sulfur mix in polymer grade ethylene. Five cc sample loop.

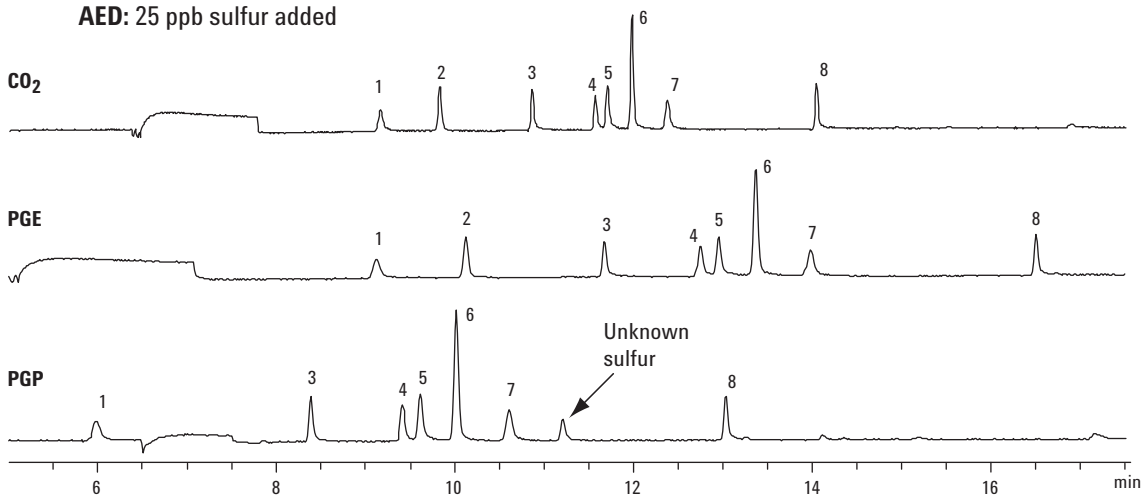

Figure 6. Agilent AED, ppb level sulfur mix in food grade CO_2, polymer grade ethylene, and polymer grade propylene.

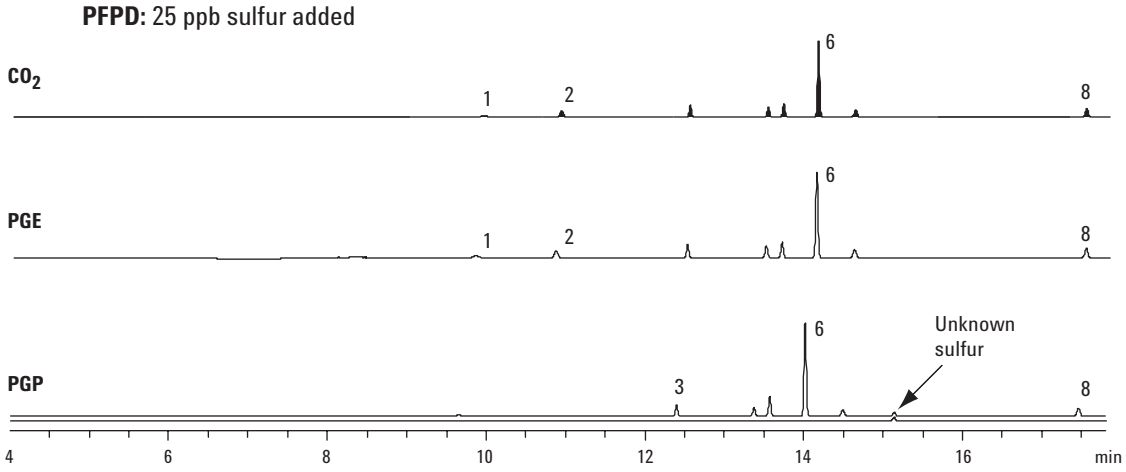

Figure 7. OI Analytical PFPD, ppb level sulfur mix in food grade CO_2, polymer grade ethylene, and polymer grade propylene.

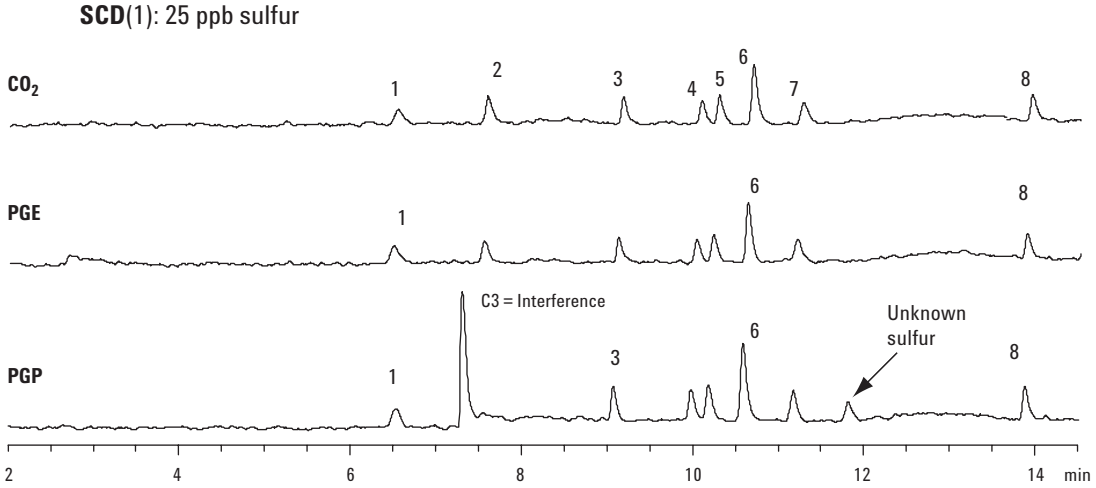

Figure 8. Antek SCD, ppb level sulfur mix in food grade CO_2, polymer grade ethylene, and polymer grade propylene.

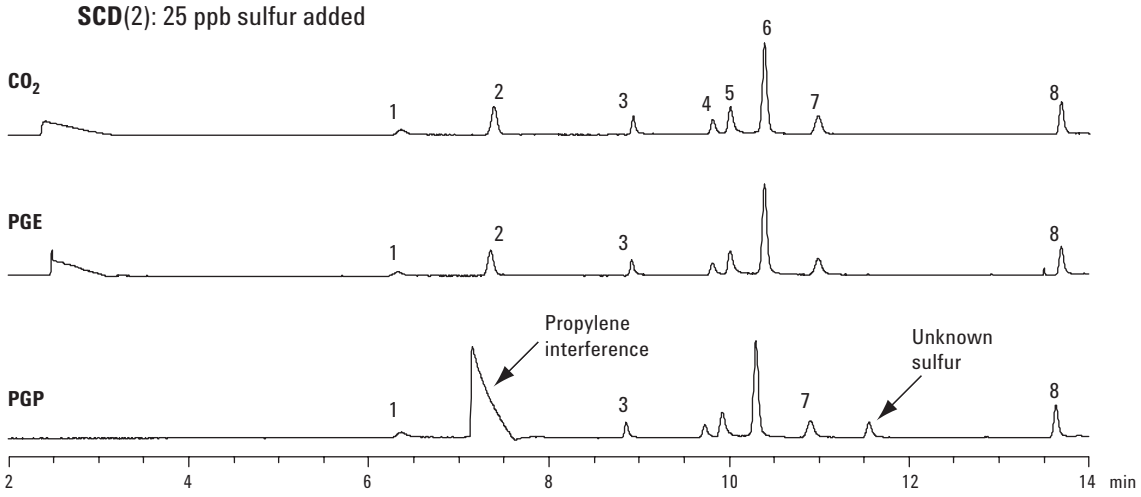

Figure 9. Sievers SCD, ppb level sulfur mix in food grade CO_2, polymer grade ethylene, and polymer grade propylene.

respectively, with 25 ppb per sulfur component added into matrices of CO_2, polymer grade ethylene (PGE), and polymer grade propylene (PGP). Note that with all four detectors, COS cannot be analyzed in propylene on the methyl silicone column due to hydrocarbon coelution. The method developer should consider use of the J&W GasPro column for situations where COS must be quantified.

Detector Considerations

FPD

A good over-all choice for sulfur levels above approximately 100 ppb when using the configuration described here. Sulfur response is determined by a square root relationship since the flame reaction converts sulfur atoms to an S_2 complex. The FPD has ease-of-use equivalent to a FID and compatibility with many column types. However, quenching can be an issue; hence the need for complete separation of the sulfur analytes from the matrix for ppb analyses. Maximum sensitivity is obtained by running a hydrogen rich flame. Cost is also the lowest of all detectors in this comparison.

SCD

A good detector for low-level sulfur, reaching 5 to 10 ppb in ideal cases. Quenching is not a problem with this detector, however, response factors can drift over a period of just a few days. Coking in the reaction chamber can also occur and care must be taken to avoid overload. When well tuned 10^6 selectivity is possible.

PFPD

Setup can be time consuming to achieve best performance. Once operational, sensitivity can be 5X to 7X better than the FPD. Very good specificity and equimolar sulfur response are among the detector's strong points. Quenching problems may be somewhat less severe than the FPD if ideally tuned. The column flow is limited to approximately 1 mL/min with a margin of only ±10%, thus column choices are very limited.

AED

The AED is the ultimate sulfur detector with low ppb sensitivity, no quenching, equimolar response and compatibility with many column types. Reliability, stability and ease of use are all proven attributes of the AED. The ability to perform compound independent calibration and determine molecular formulas empirically are useful features.[2] For many hydrocarbon streams, the ability to simultaneously collect carbon chromatograms (C 179 nm for high levels and C 193 nm for low levels) and sulfur is extremely useful. For example, quantitation of hydrocarbons, CO, CO_2, and sulfur in propylene is possible in one run, followed by a second run to obtain trace arsine and phosphine. These features come at the expense of a higher price point than other sulfur selective detectors. However, versatility with detection of 26 elements can make the AED a very cost-effective solution for many petrochemical applications.

Conclusions and Application Guidelines

When choosing a chromatographic system for trace sulfur detection, first look at your sensitivity requirements; this may eliminate some choices. Always employ Silcosteel or Sulfinert tubing and fitting where possible in the sample path. The low volume inert volatiles interface is a real asset for trace work providing a convenient low volume valve/column interface. If the matrix is reasonably simple and sulfur levels are in the low ppm to 50 ppb range then the FPD will usually be a good choice. In fact, with careful attention paid to sample introduction hardware and column choice, the FPD is surprisingly sensitive (sub 50 ppb sensitivity). For samples where it is not feasible to completely separate sulfur components from the hydrocarbon matrix and low ppb sensitivities are required, the SCD can be considered if experienced users are available. The PFPD also is capable of excellent sensitivity to low ppb levels. Because of quenching, it is best suited for light sulfur and hydrocarbon streams where good separation is achievable. Dynamic range must be considered as well. The AED and SCD can handle the widest concentration range from low ppb to high ppm levels. In terms of over all performance, the AED will handle the widest range of applications obtaining carbon and sulfur chromatograms simultaneously without time consuming “tuning” for optimal operation. Analysis down to 5 ppb in complex matrices is easily accomplished.

References

1. Agilent's FlowCalc 2.0 software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html
2. M .J. Szelewski, *Empirical Formula Determinations and Compound-Independent Calibration Using a GC-AED System*, Application Note 228-382, Publication Number 5965-8144E, May 1997 (Downloadable from agilent.com).

Statement Concerning Use of Third Party Detectors

The information presented here for non-Agilent sulfur selective detectors is based on one detector of each model number. The SCD's and PFPD were setup and operated per the manufacturers' written documentation. Agilent does not claim to be an expert in the operation of these detectors.

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Printed in the USA
August 16, 2001
5988-2426EN



Agilent Technologies

Enhancements in the Operation and Precision of an ASTM D4815 Analyzer for the Determination of Oxygenates in Gasoline

Application

Hydrocarbon Processing, Transportation Fuels

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Abstract

American Society for Testing and Materials method D4815 is widely used for the analysis of oxygenated additives in gasoline. This two-dimensional gas chromatographic method uses a micro-packed precolumn to separate hydrocarbon interference from the oxygenated compounds prior to analytical separation on a capillary column. This application note describes improvements made to the precolumn assembly that result in a method that is easier to use and more precise in operation. Analyzers built on the Agilent 6890 Series gas chromatograph platform include this new column and are shown to meet or exceed American Society for Testing and Materials performance specifications for the D4815 method.

Introduction

The reformulated gasoline (RFG) program was established to meet the goals of the 1990 Clean Air Act by reducing the amount of volatile organic compounds and other toxics emitted during combustion. To achieve the targeted reduction of ozone precursor and carbon monoxide emissions, the oxygen content in reformulated gasoline must be at least 2 percent by weight. Refiners blend combustible oxygenated compounds such as alcohols or ethers into gasoline to meet this requirement. Table 1 lists the oxygenated compounds used as reformulated gasoline additives. Although refiners may choose which of these compounds to blend with gasoline, methyl tert-butyl ether (MTBE) and ethanol are the most commonly used oxygenated additives in RFG.

Table 1. Scope of ASTM D4815 Method for Oxygenates in Gasoline

Alcohols	Range (wt. percent)	Ethers	Range (wt. percent)
methanol	0.1 to 12.0	methyl tert-butyl ether (MTBE)	0.1 to 20.0
ethanol	0.1 to 12.0	diisopropyl ether (DIPE)	0.1 to 20.0
isopropanol	0.1 to 12.0	ethyl tert-butyl ether (ETBE)	0.1 to 20.0
n-propanol	0.1 to 12.0	tert-amylmethyl ether (TAME)	0.1 to 20.0
isobutanol	0.1 to 12.0		
tert-butanol	0.1 to 12.0		
sec-butanol	0.1 to 12.0		
n-butanol	0.1 to 12.0		
tert-pentanol	0.1 to 12.0		

With the implementation of the RFG program, analytical methods were required for regulatory compliance and quality control measurements. The American Society for Testing and Materials (ASTM) developed D4815; a two-dimensional gas chromatographic method used to measure volatile oxygenates in gasoline [1]. Configuration of this method on the Agilent 6890 Series gas chromatograph (GC) is shown in Figure 1.

Chromatographic separation of oxygenates from the complex hydrocarbon matrix in gasoline is difficult to achieve with a single capillary column; therefore the D4815 method employs a two-column configuration with a 10-port column-switching valve. At the time of sample introduction, the valve is in the OFF position and the sample passes onto a polar TCEP [1,2,3-tris-(2-cyanoethoxy)propane] precolumn where oxygenates and heavy hydrocarbons are retained while the light hydrocarbons are eluted to vent (Figure 1).

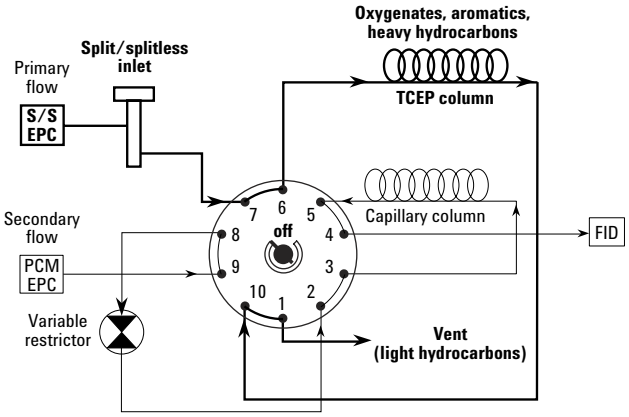

Figure 1. Valve in the OFF position at time of injection. The polar TCEP precolumn retains the oxygenates, aromatics, and heavier hydrocarbons. Lighter hydrocarbons are not retained and are discarded.

After the elution of light hydrocarbons, the valve is switched to the ON position. Oxygenates and heavy hydrocarbons are backflushed onto a methyl silicone capillary column for a boiling point separation (Figure 2).

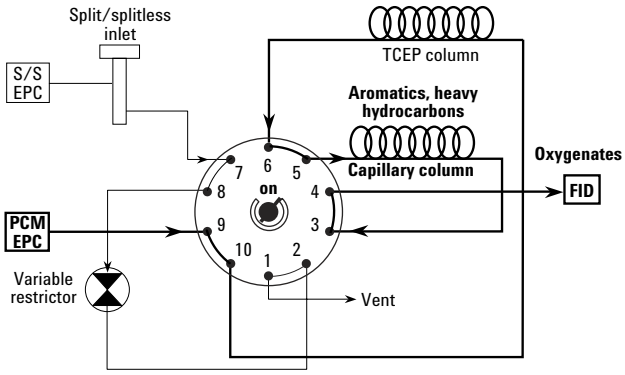

Figure 2. Valve in the ON position after backflush time. The reversed carrier gas flow through the TCEP column (supplied by the pneumatics control module) backflushes oxygenates, aromatics, and heavy hydrocarbons to the capillary column for boiling point separation and detection by the flame ionization detector (FID).

Once oxygenates of interest are eluted from the capillary column, the valve is reset to the OFF position and the heavy hydrocarbons are backflushed to the detector (Figure 3).

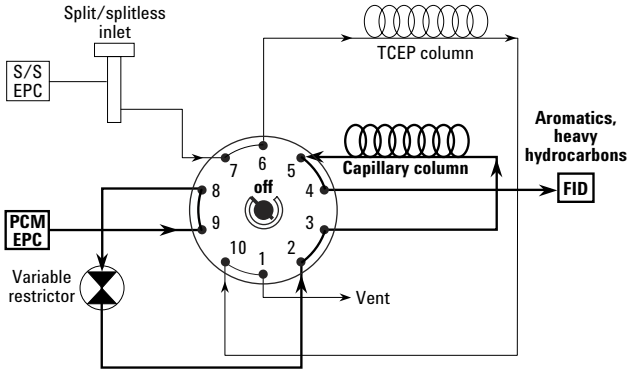

Figure 3. Valve reset to the OFF position after oxygenates detection. Reversed flow through the capillary column backflushes aromatics and heavy hydrocarbons to the FID. Increased carrier flow speeds the process.

While backflushing the heavy hydrocarbons, the capillary column pressure is automatically increased using the electronic pneumatics control feature of the 6890 GC. The increased column flow speeds the backflush of the heavy hydrocarbons and reduces the method run time.

The key to successful use of this method is determining the initial backflush time so that all of the light hydrocarbons are vented but oxygenates are still fully retained on the TCEP precolumn. If this time is too short, the light hydrocarbons will be backflushed onto the capillary column causing chromatographic interference with oxygenates. Excessively long backflush times result in a loss of the oxygenated compounds from the TCEP column with a corresponding decrease in sensitivity and less quantitative precision. This application note describes improvements to the TCEP column along with enhancements to the Agilent 6890 hardware that result in easier, more precise backflush time setting.

Experimental

Apparatus and Materials

Five Agilent 6890 Plus Series GCs were configured to run the ASTM D4815 method. Table 2 lists the hardware components of these systems. The micropacked TCEP precolumn was installed in the heated valve box.

Instrument Conditions

Conditions set on each GC are listed in Table 2. Flow setting and flow balance between the two EPC channels was performed according to the procedure outlined in section 11.3 of the ASTM D4815 method [1]. The backflush time was initially set to 0.23 minutes and the GC conditions were tested with a 1-mL injection of the AccuStandard Retention Time Mix.

Table 2. Agilent 6890 Hardware Configuration for D4815 Oxygenates Analysis

Standard 6890 GC hardware	
G1540N	6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210	FID with EPC control
Option 309	Pneumatics control module with EPC control
Option 751	Temperature controlled valve box for one valve
Option 800	10-port valve
Option 872 (2 of each)	Zero dead volume bulkhead unions
SP1 2310-0049	Special product configuration which includes: Polar pre-column (see below) Capillary column (see below) Analyzer manuals Alcohol in gasoline test mix (part no. 18900-60640)
G2613A	Agilent 7683 autoinjector
Columns	
Polar pre-column	Micropacked 20% TCEP on Chromosorb PAW 80/100, stainless steel 560 mm × 0.38 mm id
Capillary column	2.65 µm film HP-1, 30 m × 0.53 mm id (Agilent part no. 19095Z-123)
Data system	
G2070A	Agilent Multitechnique ChemStation Rev. A.08.01
Optional consumables	
5182-342	Merlin Microseal septum
5181-8809	10 mL fixed straight needle autoinjector syringe for Merlin Microseal
5183-4647	Inlet liner optimized for spit operation
Standards and samples	
D-4815-RT[a]	AccuStandard Qualitative Retention Time Mix
D-4815-VT[a]	AccuStandard Valve Timing Mixture
D-4815/IS-SET[a]	AccuStandard Quantitative Calibration Mixture
M-GRO-EPA-CC/IS-5ML[a]	AccuStandard Quantitative Check Standard
SRM 2294[b]	Reformulated gasoline with 11% (w/w) MTBE

[a]Available from AccuStandard, 125 Market St., New Haven, CT 06513, USA

[b]Available from NIST Standard Reference Material Program 100 Bureau Dr., Room 204, Build. 202, Gaithersburg, MD 20899-2322, USA

Backflush Time Optimization

The Valve Timing Mixture from AccuStandard was used to optimize the backflush time for each instrument. This mixture contains 10% w/w each of MTBE, ETBE, DIPE, and methylcyclopentane (MCP) in hexane. MCP serves as a marker to indicate the complete elution of light hydrocarbons from the TCEP precolumn. The mixture is run at 0.02-minute intervals between backflush times of 0.20 minutes to 0.38 minutes. At each interval the data is evaluated to determine the optimal backflush time for that particular system. After the backflush time is determined, a sample of RFG containing several oxygenates and the internal standard (1,2-dimethoxyethane) was used to test the system and, if necessary, make fine adjustments to the backflush time.

Analyzer Performance

The analyzers were calibrated using the AccuStandard Quantitative Calibration Mix. After calibration, the analyzers' quantitative accuracy was measured using two samples, the AccuStandard Quantitative Check Standard and the National Institute of Science and Technology (NIST) Standard Reference Material number 2294. The quantitative precision of the systems was evaluated using the ASTM repeatability and reproducibility measures outlined in the D4815 method [1].

Results and Discussion

Initial Setup

Figure 4 shows a chromatogram of the AccuStandard Qualitative Retention Time Mixture using the instrument conditions in Table 3 and a backflush time of 0.23 minutes. Running this test mix is useful to establish approximate retention times and elution order of the various oxygenated compounds. One can also assess the system inertness for oxygenates by observing the peak shapes in the chromatogram. In this example, highly polar compounds like methanol and ethanol exhibit excellent peak shape.

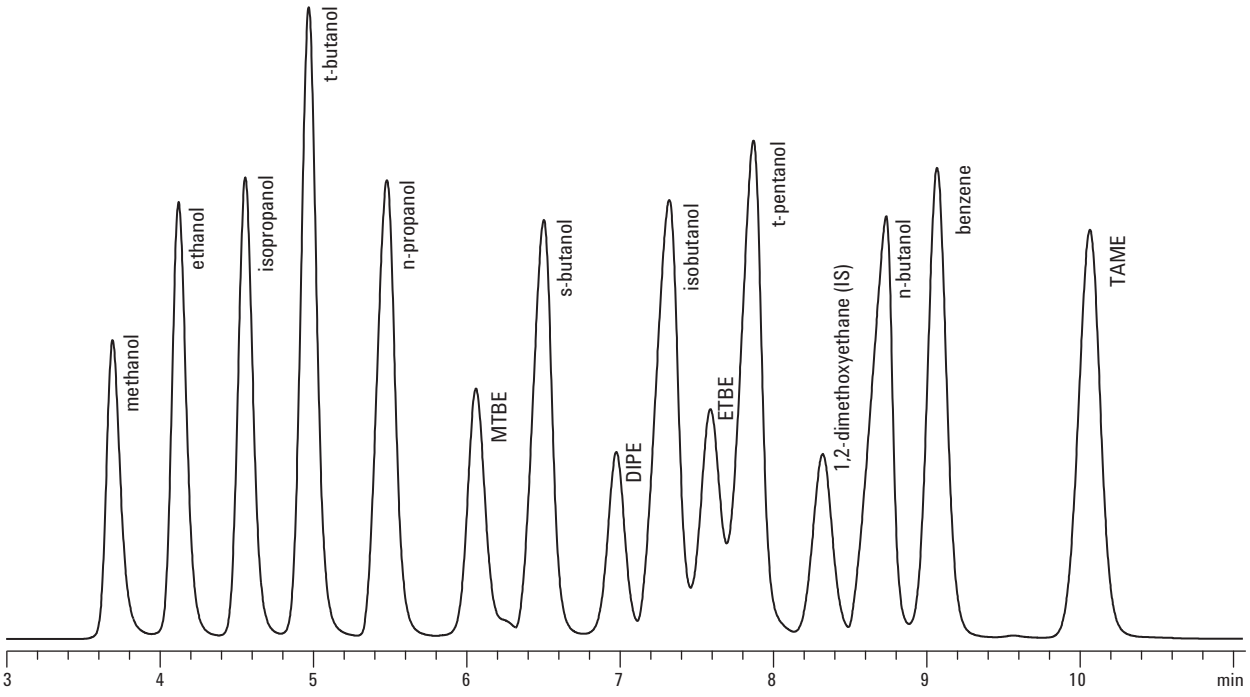

Figure 4. Retention time mix run. Backflush time 0.23 minutes.

Table 3. Instrument Conditions for D4815 Oxygenate Analysis

Split/splitless injection port	
Temperature	200 °C
Pressure	14 psi helium
Split vent flow	70 mL/min
Split ratio	14:1
TCEP Precolumn flow	5 mL/min
Pneumatics control module pressure program	9 psi helium for 12 min; 40 psi for 3 min
HP-1 capillary column flow	3.1 mL/min
FID temperature	250 °C
Valve temperature	60 °C
Oven temperature	60 °C isothermal

Backflush Time Optimization

Backflush time optimization was performed for each of the five 6890 gas chromatographs. Figure 5 shows the data collected from one of these instruments during backflush time optimization. The compounds elute in order of decreasing polarity; MTBE is the most polar compound in the mix and MCP is the least polar compound. Therefore, the MCP serves as a marker to indicate the complete elution of light hydrocarbons from the TCEP precolumn.

Visual inspection of these chromatograms indicates that this TCEP precolumn removes the MCP marker after 0.24 minutes. A better way of

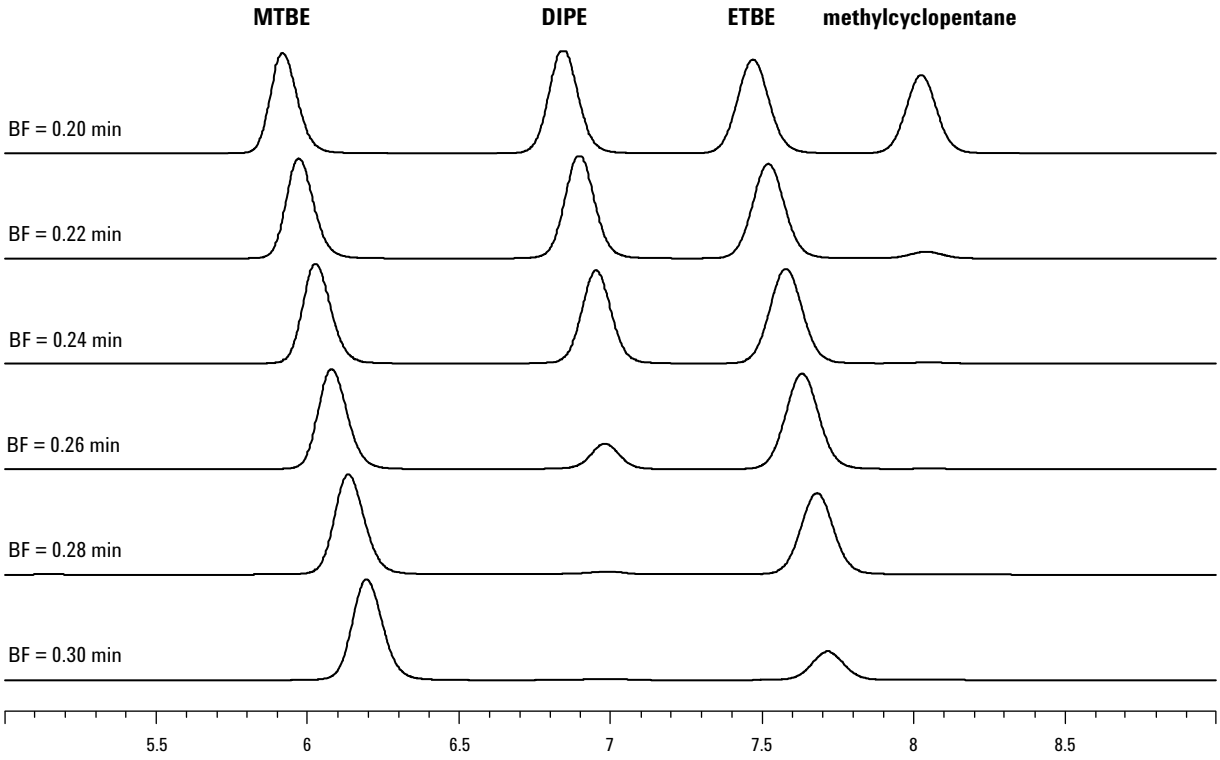

Figure 5. Backflush time optimization experiment. Methylcyclopentane is completely vented from the TCEP column after a backflush time of 0.24 minutes.

examining this data is shown in Figure 6 where the detector response of each peak is plotted against the backflush time. This graph confirms that this TCEP column removes the MCP after 0.24 minutes while the MTBE and ETBE are completely recovered and the less polar DIPE response is starting to decrease. Therefore, for this instrument, a backflush time of 0.24 minutes should be sufficient for any oxygenate other than DIPE. If the quantitiative analysis of DIPE is required, one may have to optimize the backflush time of the system for this compound.

After initial optimization of the backflush time, the system should be tested by running a check sample containing known concentrations of oxygenates in reformulated gasoline. Figure 7 shows the results of a system test using the AccuStandard Quantitative Check Standard listed in Table 2. The upper chromatogram shows this check standard run with a backflush time of 0.24 minutes. One observes a co-eluting interference with the internal standard in this sample that will result in artificially low results. The graph in Figure 6 shows that the backflush time can be increased up to 0.26 minutes without loss of oxygenated compounds from the TCEP column. The lower chromatogram in Figure 7 shows that a 0.26-minute backflush time removes the interference while maintaining the response of the oxygenated compounds.

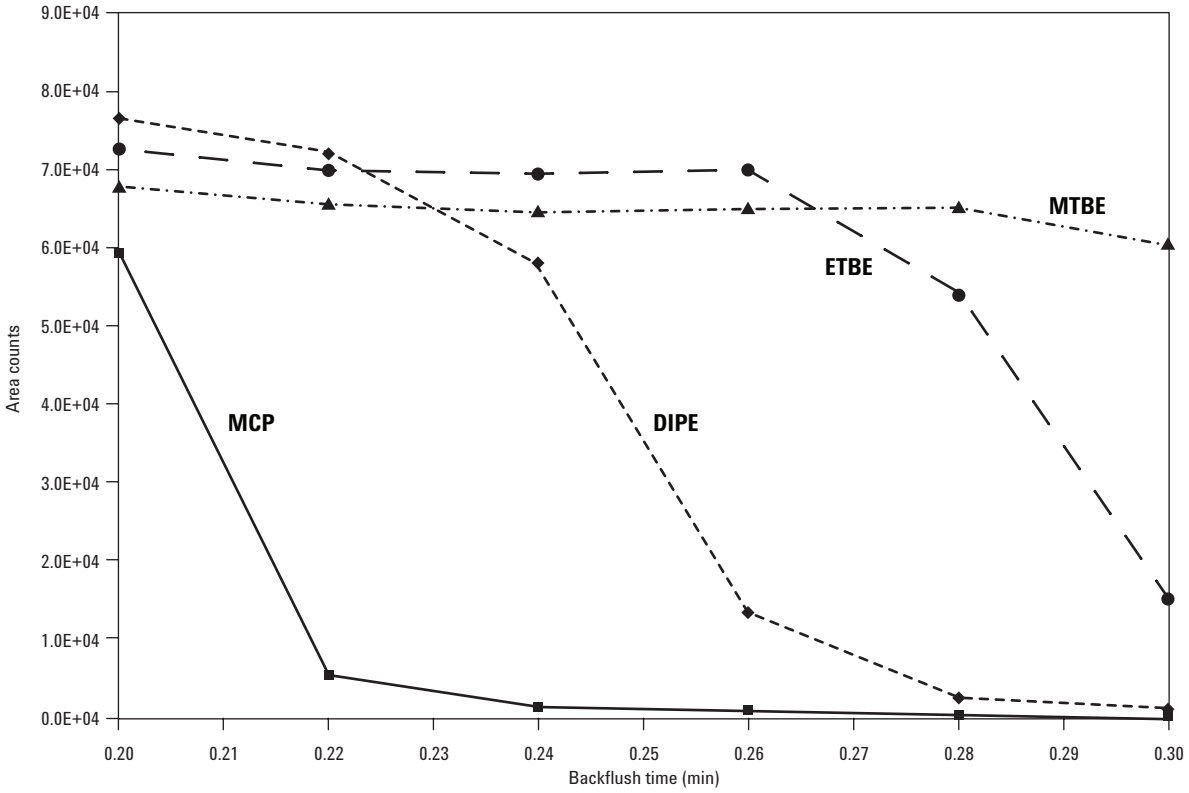

Figure 6. Data from Figure 5. After 0.24 minutes, MCP and the lighter hydrocarbons are eluted from the TCEP column, while MTBE and ETBE are completely retained. Reduced DIPE response indicates some compound loss.

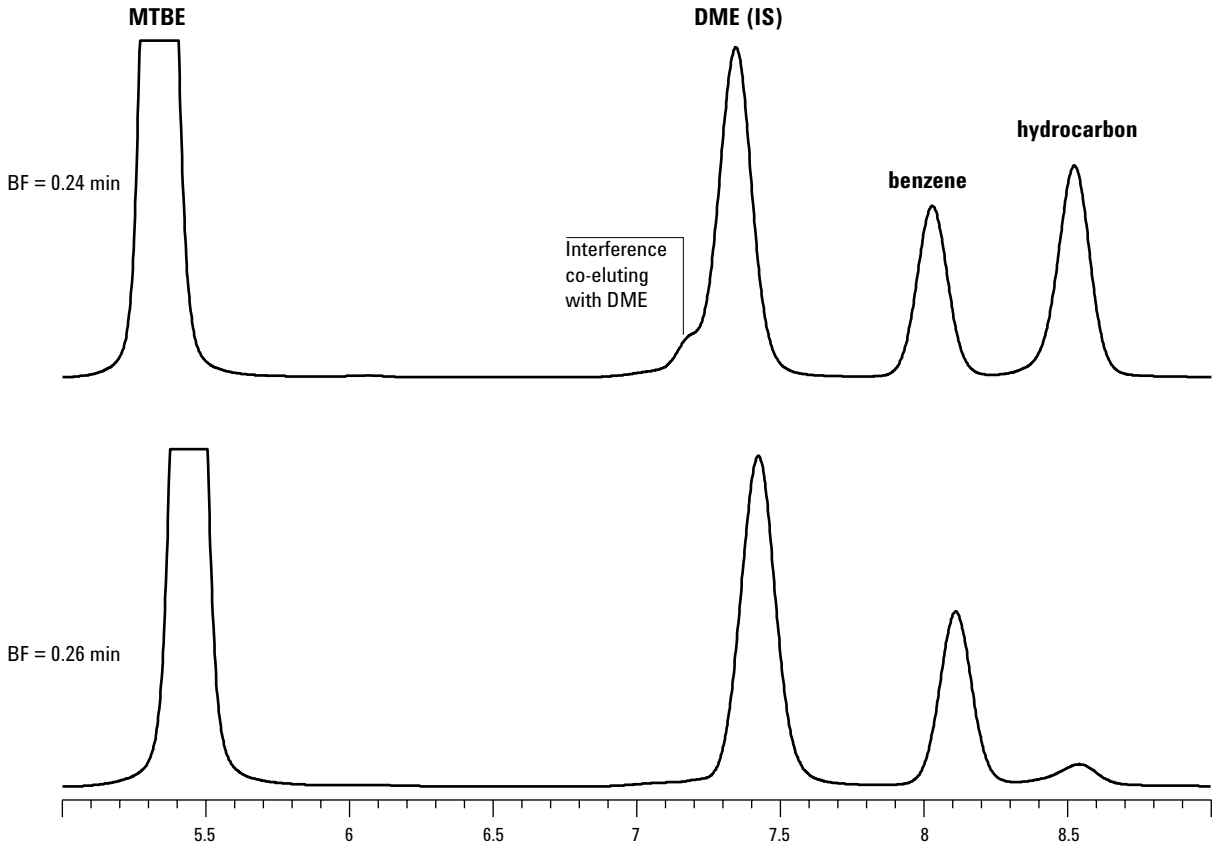

Figure 7. System check of the 0.24 minute backflush time. The upper chromatogram shows an interference co-eluting with the internal standard. The lower chromatogram shows that increasing the backflush time to 0.26 minutes eliminates the interference while retaining the oxygenate response.

Nine TCEP precolumns from two different manufacturers were tested in the five GC systems to measure the variation in backflush times. Table 4 shows the results of these tests. Three of the columns could not be used due to blockage of the column flow by the steel wire used as end plugs. This resulted in head pressures in excess of 60 psi and no measurable carrier gas flows. Of the six remaining columns, the backflush times ranged from 0.10 minutes to 0.34 minutes, with a standard deviation of 0.09 minutes. This large variation is due to two different factors. First, preparation of uniform TCEP columns is difficult due to the small size and the small amount of packing in the column. The result is columns with large differences in ability to separate oxygenate from hydrocarbons. Second, the column is installed inside the valve box but is not directly attached to the heated valve block. This results in column temperature variations and can contribute to differences in separation.

Table 4. Variations in Optimized Backflush Times for Nine TCEP Precolumns

Column	Head pressure[a] (psi)	Backflush time (min)
Mfg A #1	5.3	0.10
Mfg A #2	15.6	0.28
Mfg A #3	9.9	0.18
Mfg B #1	16.3	0.30
Mfg B #2	11.0	0.24
Mfg B #3	>60.0	na
Mfg B #4	>60.0	na
Mfg B #5	18.6	0.34
Mfg B #6	>60.0	na

[a]Head pressure needed for required 5 mL/min flow

These problems can be easily overcome. Instead of using steel or silver wire as column end plugs, a union equipped with a 2-micron frit is installed at the column ends to contain the packing (Figure 8).

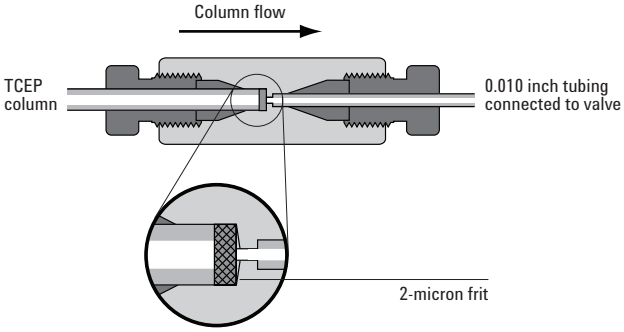

Figure 8. TCEP column fitting using a 2-micron frit as the end plug.

The advantages of this more uniform configuration are easier, reproducible column preparation and very consistent head pressures needed to produce the required column flow rate of 5 mL/min. Direct heating of the column is accomplished by wrapping it onto a cylindrical metal mandrel which is directly attached to the valve block heater. This assembly is shown in Figure 9.

The five 6890 GC systems were equipped with this improved TCEP column assembly and the backflush times were optimized for each system. The results are listed in Table 5. For the improved TCEP columns, the head pressures are much more consistent and the backflush time variations are reduced. The backflush time range is now 0.28 to 0.30 minutes and the standard deviation is 0.01 minutes. This is a significant improvement over the original results listed in Table 4. The GC systems with the improved TCEP precolumn were used to measure analyzer performance.

Table 5. Variations in Optimized Backflush Times for Five Improved TCEP Column Assemblies

Instrument	Head pressure[a] (psi)	Backflush time (min)
GC1	14.4	0.28
GC2	14.0	0.28
GC3	17.0	0.30
GC4	13.9	0.28
GC5	15.8	0.30

[a]Head pressure needed for required 5 mL/min flow

Analyzer Performance

The ASTM D4815 method uses a correlation coefficient test and a y-intercept test to measure calibration performance for the analyzers [1]. Figure 10 shows the calibration curves and calibration performance results for ethanol and MTBE. The five-point calibration range for ethanol was between 0.1 wt% and 11.4 wt%. For MTBE the five-point calibration range was from 0.1 wt% to 19.0 wt%. For both calibrations the ASTM correlation coefficient and y-intercept tests were either met or exceeded using the Agilent 6890 analyzers. Similar results were observed for the other oxygenates.

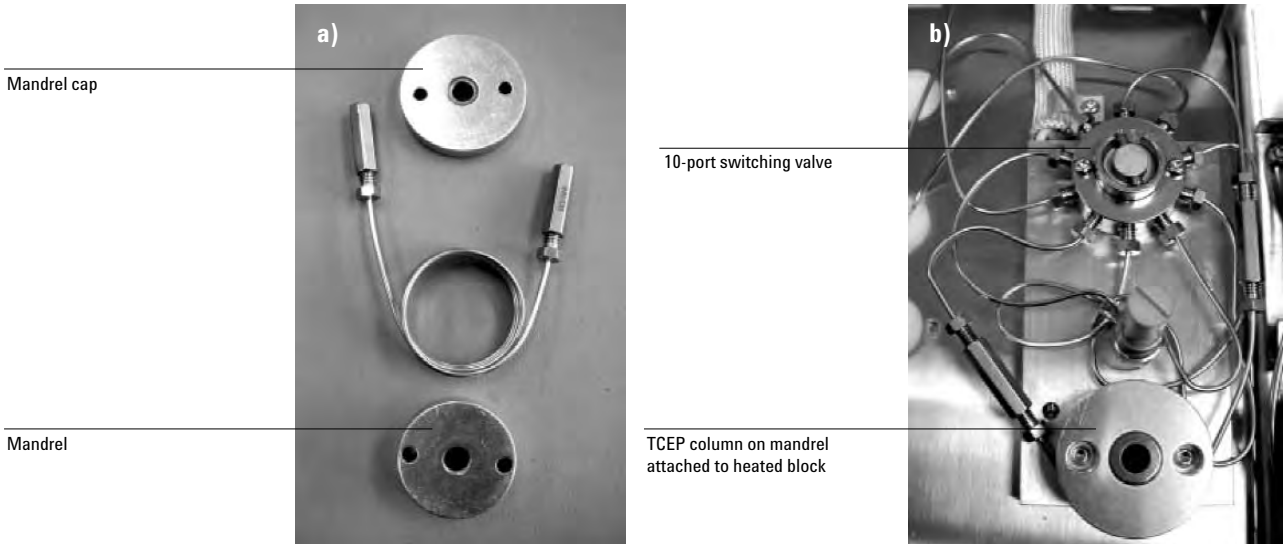

Figure 9. a) The TCEP precolumn with end fittings containing 2-µm frits along with the mandrel and cap. b) The TCEP precolumn wrapped on the mandrel and directly connected to the heated valve block.

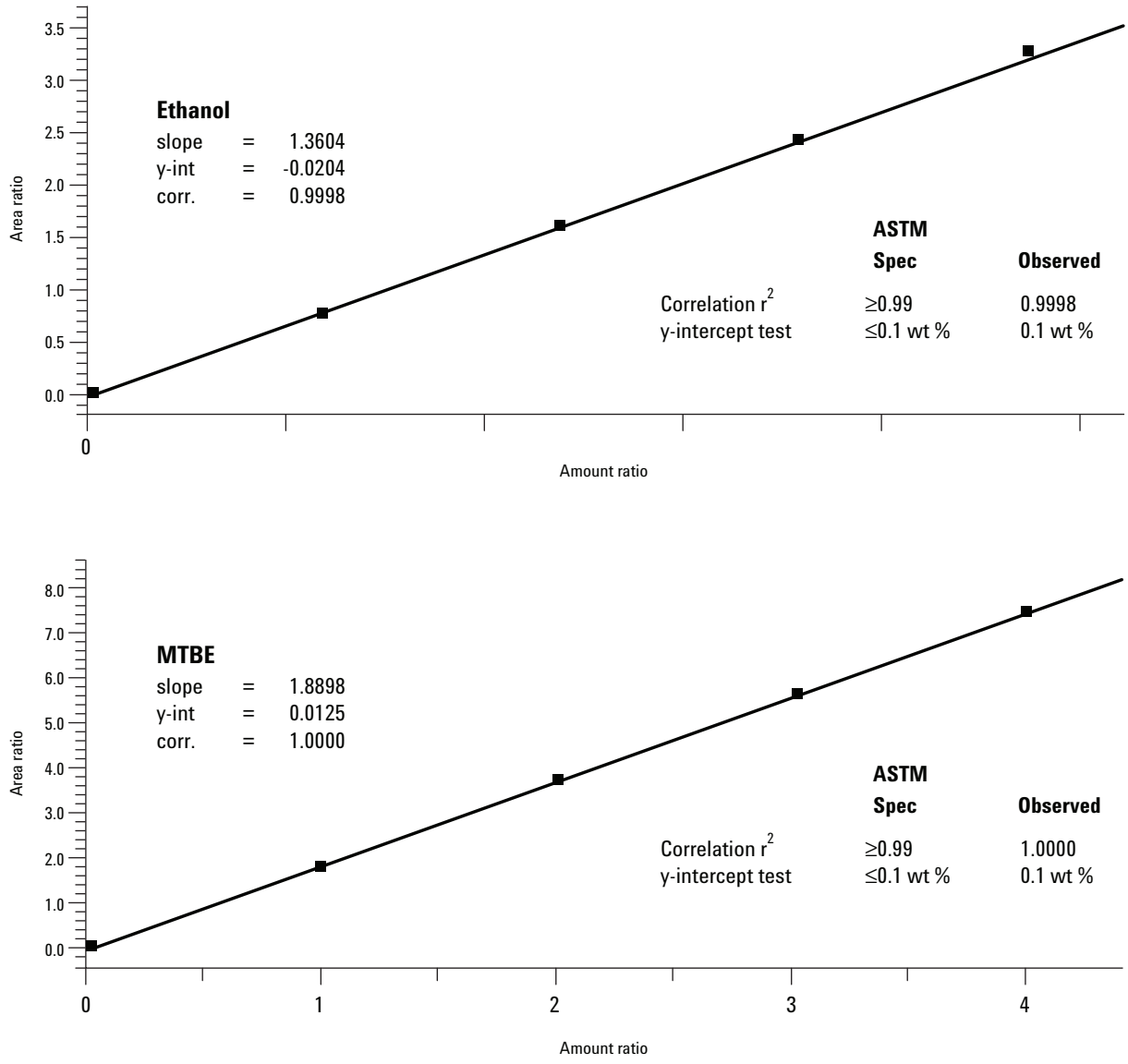

Figure 10. Calibration performance of the Agilent 6890 GC using the improved TCEP column assembly. For both ethanol and MTBE, the system meets or exceeds ASTM calibration specifications.

Quantitative precision for the D4815 method is evaluated using the ASTM repeatability and reproducibility measures. Four samples were prepared with a low or high concentration of either ethanol or MTBE spiked in gasoline. Blank gasoline samples were also run. Table 6 shows the precision performance of the analysis. For all four samples, the Agilent 6890 D4815 analyzers exceeded the ASTM specifications for method precision.

Table 6. Precision Performance of Agilent 6890 Analyzer Using ASTM Repeatability and Reproducibility Measurements

		Repeatability (wt%)		Reproducibility (wt%)	
Compound	**Wt%**	**ASTM Spec**	**Observed**	**ASTM Spec**	**Observed**
ethanol	0.99	<0.06	0.01	<0.23	0.01
ethanol	6.63	<0.19	0.03	<0.68	0.04
MTBE	2.10	<0.08	0.01	<0.2	0.01
MTBE	11.29	<0.19	0.05	<0.61	0.08

Quantitative accuracy was evaluated using the AccuStandard Check Standard and the NIST Standard Reference Material #2294 (Figure 11). The AccuStandard sample contains known quantities of methanol, ethanol, t-butanol and MTBE in reformulated gasoline. The NIST sample contains a known quantity of MTBE in reformulated gasoline. With both of these samples, dimethoxyethane (DME) was added as an internal standard to the bulk samples.

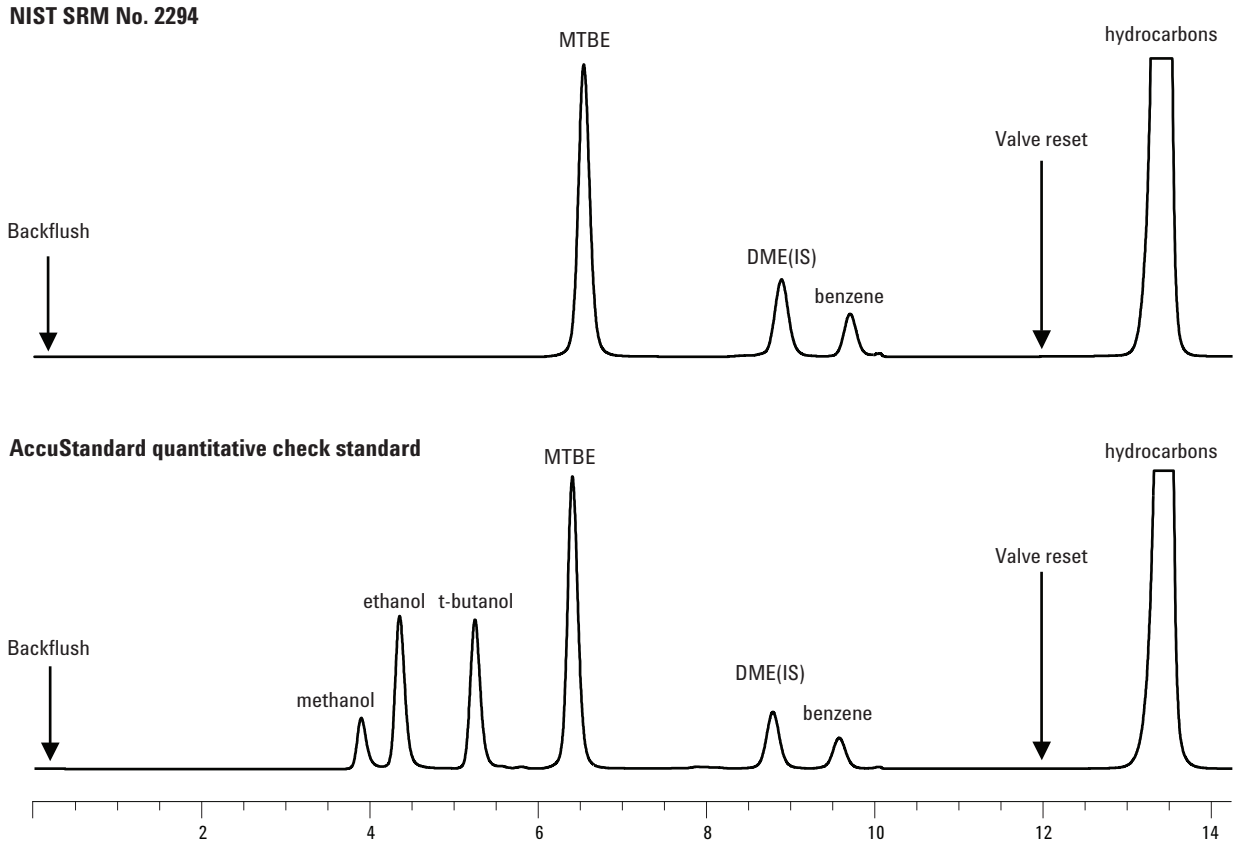

Figure 11. Analysis of NIST Standard Reference Material and AccuStandard Check Standard containing oxygenates in gasoline. Dimethoxyethane (DME) is the internal standard.

Table 7 lists the results of this test. For each compound, the analyzers recovered nearly 100 percent of the oxygenates in the sample. However, the results for each compound are slightly lower than the known quantity. This is often observed with this method due to evaporative losses during sample handling and internal standard addition.

Conclusion

The improved TCEP precolumn used for the D4815 method is shown to provide more precise and easier backflush time setting by providing more consistent carrier flow and column heating. Analytical performance of the D4815 method using Agilent 6890 based analyzers was shown to meet or exceed ASTM specification for calibration and quantitative precision. These improvements have now been incorporated in a standard SP1 configuration for D4815 analyzers on the Agilent 6890 Series Gas Chromatograph.

References

1. Annual Book of ASTM Standards, Vol. 05.03 *Petroleum Products and Lubricants (III), D 4928 - D 5950*, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.

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Table 7. Accuracy Evaluation of D4815 Analysis

Sample	Methanol wt%		Ethanol wt%		t-Butanol wt%		MTBE wt%	
	Known	Found	Known	Found	Known	Found	Known	Found
AccuStandard Check Std	3.8	3.6	7.6	7.5	4.8	4.6	11.8	11.4
NIST SRM2294	na	na	na	na	na	na	10.9	10.6

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Printed in the USA
November 29, 2001
5988-4475EN



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Two-Dimensional Gas Chromatographic Analysis of Oxygenates and Aromatics in Gasoline Using a Heart-Cutting Technique

Application

Hydrocarbon Processing, Production-QA/QC

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Abstract

A two-dimensional GC method for the analysis of oxygenated additives and aromatics in gasoline is described. This method employs an Agilent 6890N GC system equipped with a Deans switch device to dynamically heart-cut the analytes from the gasoline matrix to a second GC column. This technique provides enhanced resolution so that oxygenate and aromatic compounds can be completely separated from the hydrocarbon matrix. The unique design of the heart-cutting device allows fast and easy method setup when determining cut times. The Agilent 6890N electronic pneumatics control (EPC) used for this system provides better retention time precision that allows narrower cut times for better resolution and quantitative precision. This design also greatly reduces system activity that can lead to carry-over and poor peak shape, thereby allowing greater confidence in results for low concentrations of polar additives. Excellent calibration and quantitative performance of this system is demonstrated for several commonly used oxygenate additives and aromatic compounds. The EPC of the Agilent 6890N GC is shown to enable greater productivity by decreasing analysis times with a backflushing technique.

Introduction

Gasoline is a complex chemical mixture that contains low-boiling aromatic compounds and polar additives, such as ethers or alcohols, used to improve performance and reduce emissions. Quantitative measurement of these additives by gas chromatography is difficult because there is no single column that can completely and quickly separate the target compounds from the complex hydrocarbon matrix. Two-dimensional gas chromatography can solve this problem by using two GC columns with different selectivity to increase the overall resolving power of the method. The two columns are coupled together with a switching device that transfers an unresolved portion of the chromatogram eluting from the first column to the second column, where complete separation can be achieved.

The American Society of Testing and Materials (ASTM) has developed several methods based on two-dimensional GC for the analysis of oxygenates and aromatics in gasoline [1, 2]. The transfer device used by these methods is a gas-switching valve. These robust and widely used methods require an expensive, high-quality valve that will maintain good chromatographic peak shape. These valves also need regular maintenance, ultimately requiring system shutdown for repair or replacement.

An alternative to a valve-based device is to use a fluidic switch that will selectively divert the

primary column flow from the detector to a secondary column without allowing the sample to contact the switching hardware. This type of device is called a Deans switch and is the basis of two methods published by the European Committee for Standardization (CEN) for determination of oxygenates and benzene in gasoline [3, 4]. The scope of these methods covers benzene concentration of 0.05% (v/v) to 6% (v/v) and individual organic oxygenates from 0.17% (m/m) to 15% (m/m).

Experimental

System Design

Figure 1 shows the Agilent 6890N GC System configured with the Deans switch hardware. The gasoline sample is injected into the split/splitless inlet and is initially separated on the TCEP primary column. With the fluidic switch in the off position, the pneumatics control module (PCM) delivers 4 mL/min of helium through the lower flow path. This supplies 3 mL/min of helium to the DB-1 column. The remaining 1 mL/min is diverted to the TCEP column outlet so that the TCEP column eluent is directed to FID A.

Figure 2 shows the flow path when heart cutting with the Deans switch. Just before the peaks of interest elute from the TCEP column, the fluidic switch is set to the "on" position. Helium from the PCM is now directed through the upper flow path so that 3 mL/min goes through the restrictor and 1 mL/min diverts unresolved peaks from the TCEP column to the DB-1 column for separation and detection on FID B. After these peaks are loaded onto the DB-1 column, the fluidic switch is returned to the "off" position so that any peaks still on the TCEP column are eluted to FID A. This process of switching can be repeated many times during the run so that other unresolved peaks are diverted from the TCEP column to the DB-1 column. All of the fluidic switching times are automatically controlled through the Agilent 6890N timed events table.

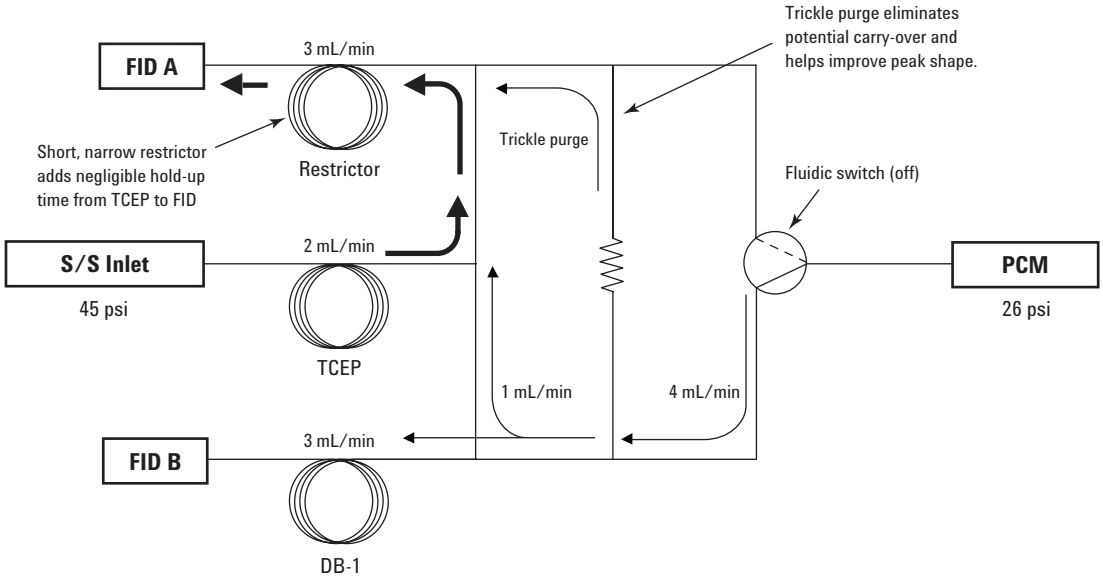

Figure 1. Deans switch configuration on the Agilent 6890N gas chromatograph for the analysis of benzene and oxygenated additives in gasoline according to CEN methods EN12177 and EN13132. In this mode, the fluidic switch is in the off position so that no heart cutting will occur. The bold arrows show the flow path of the sample.

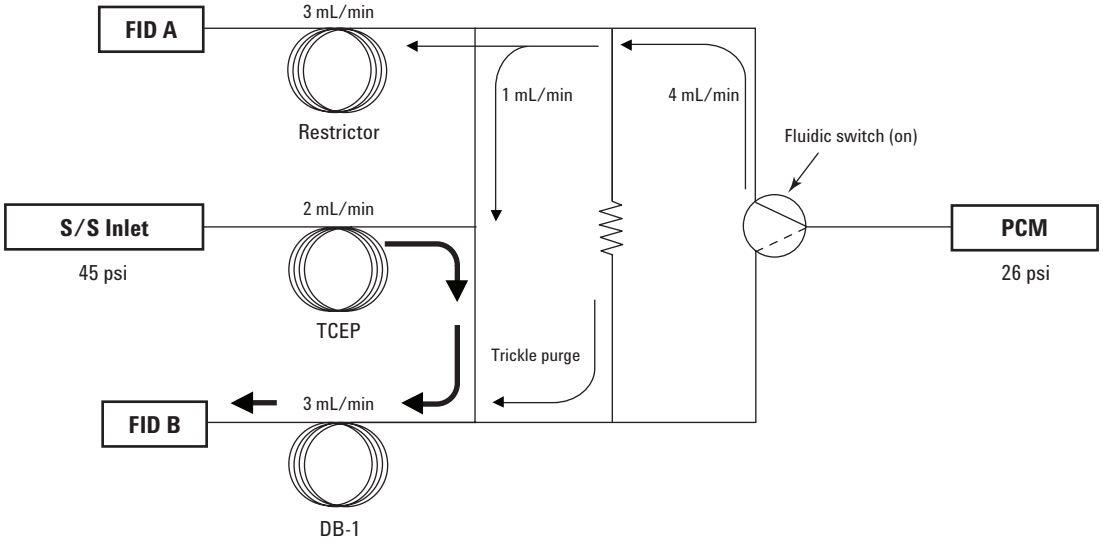

Figure 2. In this mode, the fluidic switch is in the on position so that the flow from the PCM causes the TCEP column flow to be diverted to the DB-1 column. The bold arrows show the flow path of the sample.

Oxygenates and Aromatics in Gasoline

The details of the system hardware for this analysis are listed in Table 1, and the instrument conditions are listed in Table 2. Retention times on the TCEP column and cut times for individual oxygenates and aromatics were determined by injecting a sample of the Supelco D4815 Qualitative Peak ID Mix with the fluidic switch set in the off position during the entire run. Table 3 lists the uncut retention times of each peak on the TCEP column; the retention times of each peak cut to the DB-1 column and the cut start and stop times. When setting the cut times, it is also important to use a standard that contains the highest concentration of target compounds that will be expected in the samples without overloading the column. If the column is overloaded, then the split ratio, injection size, or both should be adjusted to maintain good peak shape. This assures that the cut times will accommodate the maximum peak widths that will be encountered.

Table 1. Agilent 6890 Hardware Configuration

Standard 6890 GC Hardware	
G1540N	Agilent 6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210 (two of each)	FID with EPC control
Option 309	Pneumatics control module (PCM) with EPC control
SP1 2310-0098	Special product configuration that includes: Primary column (see below) Secondary column (see below) Pre-installed specialized plumbing and hardware Analyzer manuals Method Check-out test mix
G2613A	Agilent 7683 autoinjector
Columns	
Primary column	TCEP capillary column, 0.4 µm film, 60 m × 0.25 mm id
Secondary column	DB-1 capillary column, 0.5 µm film, 30 m × 0.25 mm id
Data system	
G2070A	Agilent multi-technique ChemStation rev. A.08.01
Optional consumables	
5182-3442	Merlin Microseal high-pressure septum
5182-0875	5 µL fixed straight needle autoinjector syringe for Merlin Microseal
5183-4647	Inlet liner optimized for split operation
Standards and samples	
47213#	D4815 Qualitative peak ID mix
4815-OXYCAL*	4815 Oxygenate calibration set
DCK-2-(10)*	MTBE in RFA check sample
DCK-3- (10)*	Ethanol in RFA check sample
5580CAL*	5580 Aromatic calibration set
5580DCK*	5580 Aromatic check sample

#Available from Supelco, Supelco Park, Bellefonte, PA 16823-0048, USA

*Available from Spectrum Quality Standards Inc., P.O. Box 2346, Sugarland, TX 77487-2346, USA

Table 2. Instrument Conditions

Split/splitless injection port	
Temperature	225 °C
Pressure	45 psi helium
Split ratio	300:1
TCEP column flow	2.0 mL/min, constant pressure mode
Pneumatics control module (PCM)	26 psi helium, constant pressure mode
HP-1 column flow	3.0 mL/min
FID temperatures	250 °C
Oven temperature program	40 °C for 6 min, 5 °C/min to 120 °C, 120 °C for 20 min

Once the retention times and cut times for an analyte are determined, a matrix blank is run using these cut times. This will determine any potential interference from the matrix that is not resolved by the secondary column. For this work, RFA gasoline was used as the matrix for the oxygenates and aromatics. Samples of this gasoline without any oxygenates were run using each of the cut times listed in Table 3.

Quantitative analyses for oxygenates and aromatics in RFA gasoline were performed by running the commercially prepared calibration standards listed in Table 2. For the oxygenate analyses, 5 weight percent of dimethoxyethane (DME) was added as the internal standard. The internal standard used for the aromatic analyses was 10 weight percent of 2-hexanone. Quantitative performance of the method was measured using commercially prepared check standards with known concentrations of MTBE, ethanol, benzene, toluene, ethylbenzene, and o-xylene in RFA gasoline.

Table 3 Retention Times and Heart-Cut Times of Oxygenates and Aromatics in Gasoline

Compound	TCEP RT(min)	Cut start(min)	Cut end(min)	DB-1 RT(min)
DIPE	5.209	5.15	5.44	7.117
ETBE	5.388	5.35	5.61	7.448
MTBE	5.533	5.48	5.76	7.158
TAME	6.436	6.37	6.72	8.896
Methanol	9.295	9.21	9.72	10.339
t-Butanol	9.358	9.26	9.64	10.592
Acetone	9.698	9.57	10.10	10.850
Isopropanol	9.986	9.89	10.34	11.147
Benzene	10.251	10.14	10.60	11.996
Ethanol	10.302	10.22	10.58	11.400
t-Amyl alcohol	12.676	12.55	13.17	14.128
s-Butanol	12.873	12.77	13.28	14.191
DME (Int Std)	13.393	13.31	13.62	14.937
n-Propanol	13.414	13.30	13.75	14.635
Toluene	13.521	13.41	13.90	15.587
i-Butanol	14.740	14.63	15.05	16.076
MIBK	14.946	14.82	15.32	16.636
n-Butanol	16.964	16.86	17.30	18.309

Results and Discussion

The Deans switch plumbing design used for this work offers several advantages for method development and performance. First, the restrictor used between the TCEP column and FID A is designed to provide the same backpressure to the TCEP column as the DB-1 column. This allows for consistent retention times on the TCEP column when the fluidic switch is in both the off and on positions. This helps maintain reproducible start and stop times when heart cutting. The restrictor is also designed to provide negligible hold-up time from the TCEP column to FID A. This allows for easier determination of cut times. One simply needs to run a test mix containing the analytes on the TCEP column with the fluidic switch in the off position (no heart cutting). The start and end times for elution of each peak from the TCEP column can then be used as the start and stop times for heart cutting. Figure 3 shows a typical result when heart cutting MTBE from the TCEP column to the DB-1 column. The upper chromatogram shows that MTBE elutes from the TCEP between 5.48 and 5.76 minutes and quickly passes through the restrictor to FID A. Using this elution time as the heart-cut time, the middle chromatogram shows that all of the MTBE is diverted to the DB-1 column to complete the chromatography (lower chromatogram).

Another advantage in the plumbing design is the "trickle purge" between the upper and lower legs of the PCM flow path. This low flow of helium eliminates any unswept volumes that can adversely affect the analysis. For polar compounds, such as methanol and ethanol, system activity and unswept volumes can cause severe peak tailing that will contribute to quantitative errors, especially at lower concentrations. A check was made on the peak shape for these two alcohols and for MTBE, the most widely used oxygenate additive in gasoline. Figure 4 shows the peak shape for these three compounds at the 0.1 wt% level, which is the lowest quantitative limit of the method's scope. Methanol and ethanol exhibited some peak tailing at this concentration, but this will not cause any problems for quantitation. The peak shape for methanol and ethanol was expected because these compounds are known to exhibit slight tailing on methyl silicone columns. The MTBE peak does not appear to have any significant peak tailing and exhibits a nearly perfect gaussian shape. From these results, reproducible quantitative analysis can be easily performed for the concentration ranges specified by the European Standards.

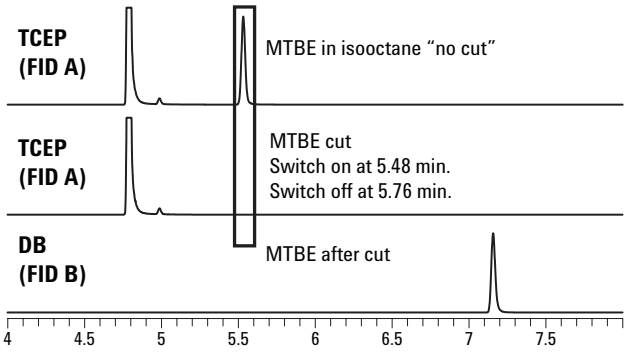

Figure 3. Results of heart cutting MTBE from the TCEP primary column to the DB-1 secondary column. The fluidic switch is on between 5.48 and 5.76 minutes. During this time, eluent from the TCEP column containing the MTBE peak is transferred to the DB-1 column.

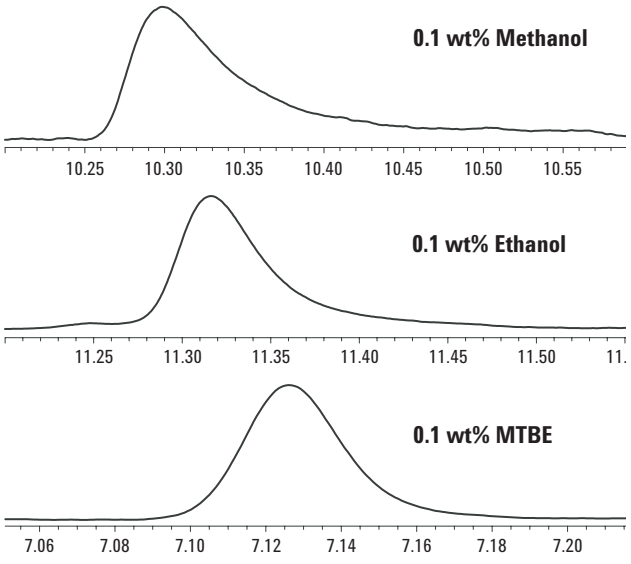

Figure 4. Peak shape of widely used oxygenate additives at low-level concentrations.

No matrix interference from the RFA gasoline was found using the cut times for all the oxygenates and aromatics listed in Table 3. Figure 5 shows a typical result when performing the matrix interference evaluation. In this example, the cut time for ETBE was between 5.35 to 5.61 minutes. The hydrocarbons that co-elute with the ETBE on the TCEP column become well resolved from the ETBE on the DB-1 column after the cut.

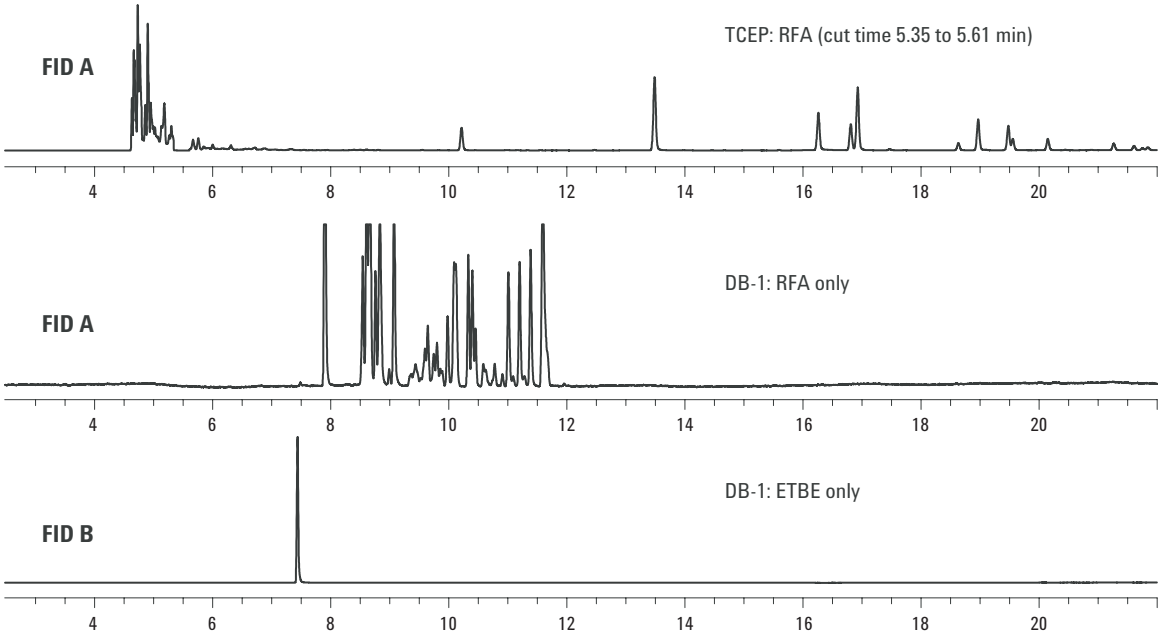

Figure 5. In the upper chromatogram, a sample of RFA gasoline is run using the cut time for ETBE. The middle chromatogram shows the separation of gasoline hydrocarbons that are cut from the matrix to the DB-1 column. The lower chromatogram is a cut of ETBE from a standard. This shows that any hydrocarbons cut with ETBE from the gasoline sample are well resolved from the ETBE peak.

The five-point linear calibration curves for several oxygenate and aromatics were made using commercially available standards prepared in RFA gasoline. These curves and the regression statistics are shown in Figures 6 and 7 and were used to calculate the concentrations of these compounds. The quantitative performance of the system was measured using gasoline samples containing known quantities of several oxygenate and aromatic compounds.

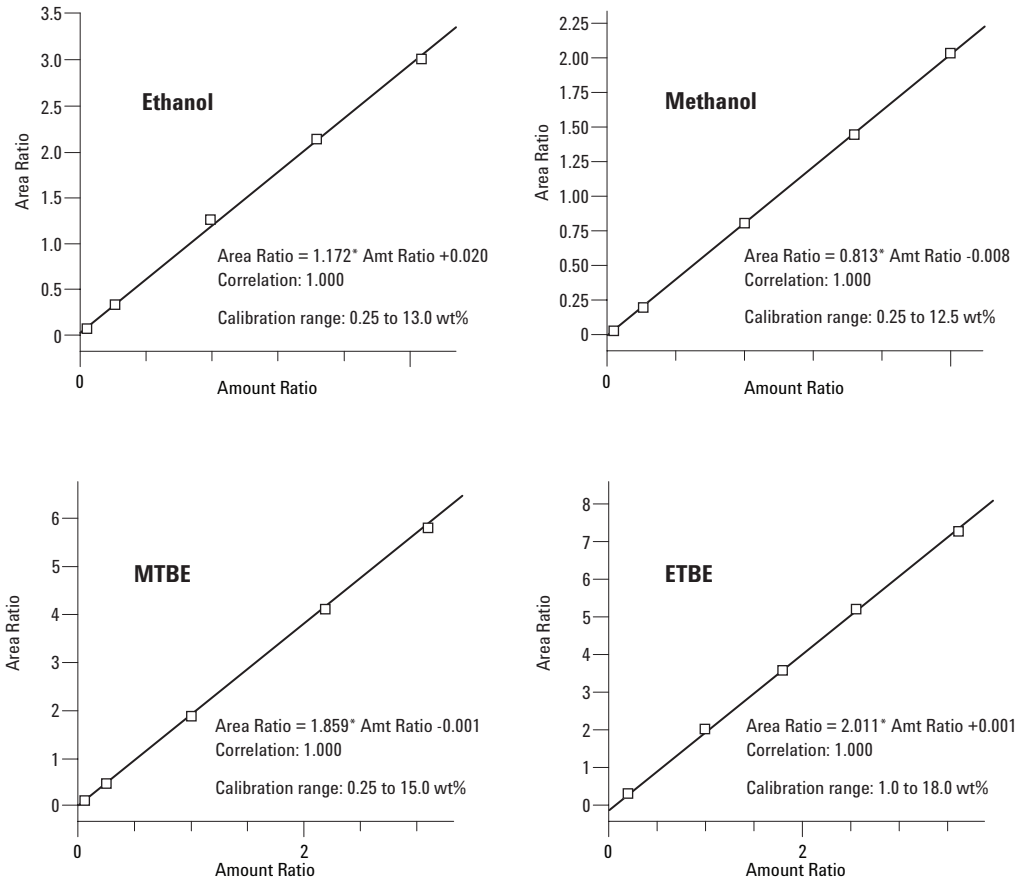

Figure 6. Five-point internal standard calibration curves of the most commonly used oxygenate additives in gasoline analyzed with the heart-cutting GC technique.

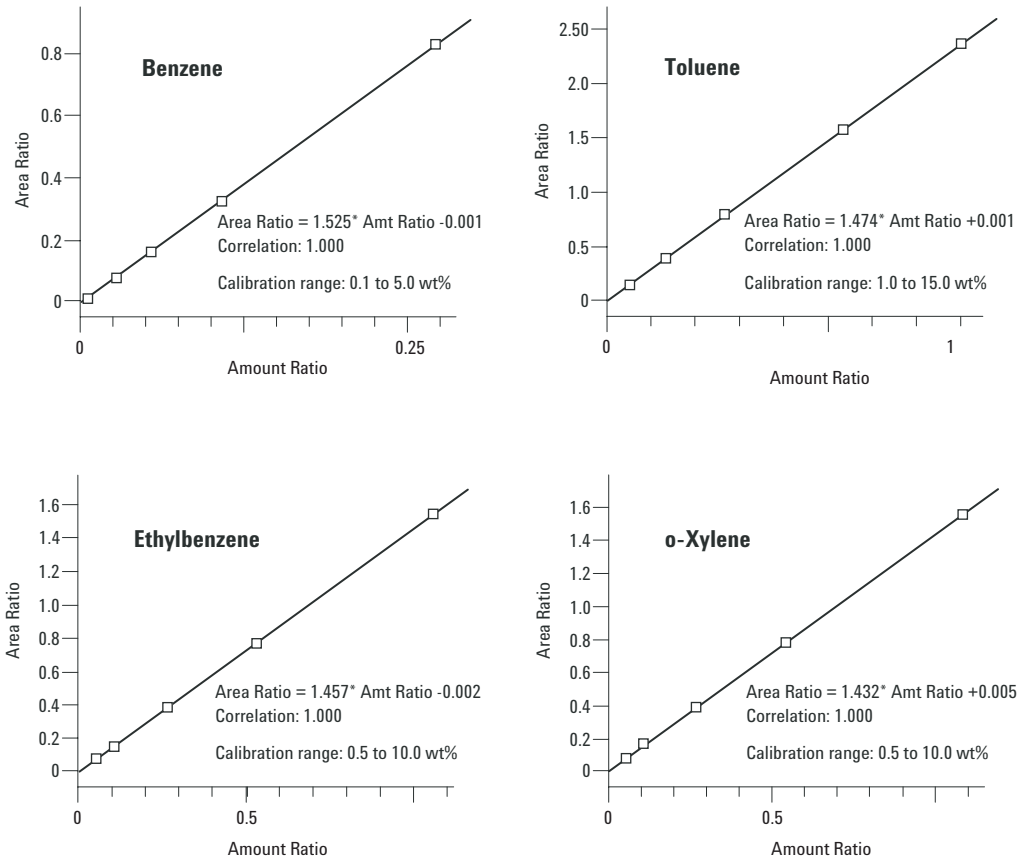

Figure 7. Five-point internal standard calibration curves of four commonly measured aromatic compounds in gasoline using the heart-cutting technique.

For MTBE and ethanol, the results of this test are shown in Figures 8 and 9. Both samples showed excellent quantitative accuracy for these two compounds at concentrations typically used in reformulated gasoline.

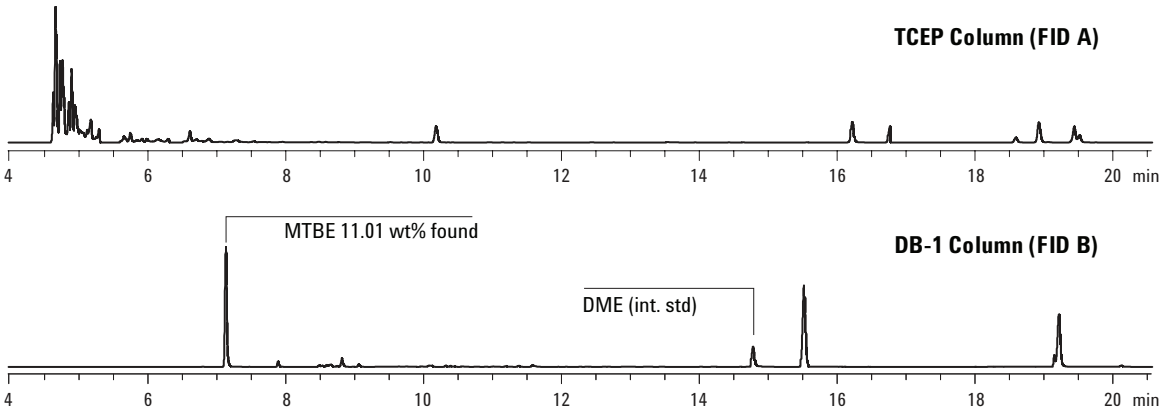

Figure 8. An analysis of a gasoline sample containing 11.10 wt% MTBE. Using the heart-cutting technique, 11.01 wt% was measured.

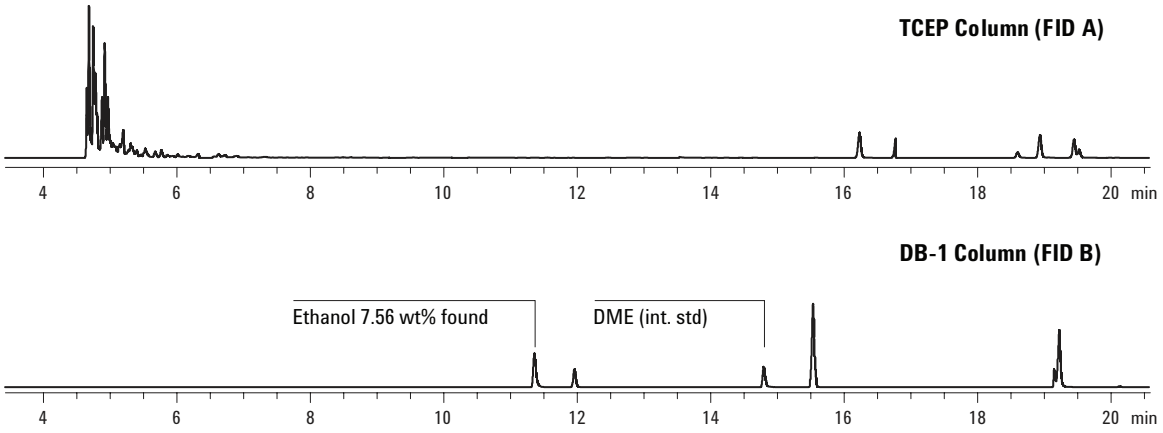

Figure 9. An analysis of a gasoline sample containing 7.77 wt% of ethanol. The GC heart-cutting analysis found 7.56 wt%.

Figure 10 shows the results for the aromatic compound analysis. Again, the heart-cutting system design illustrates excellent performance for separating and quantitating compounds in a complex matrix.

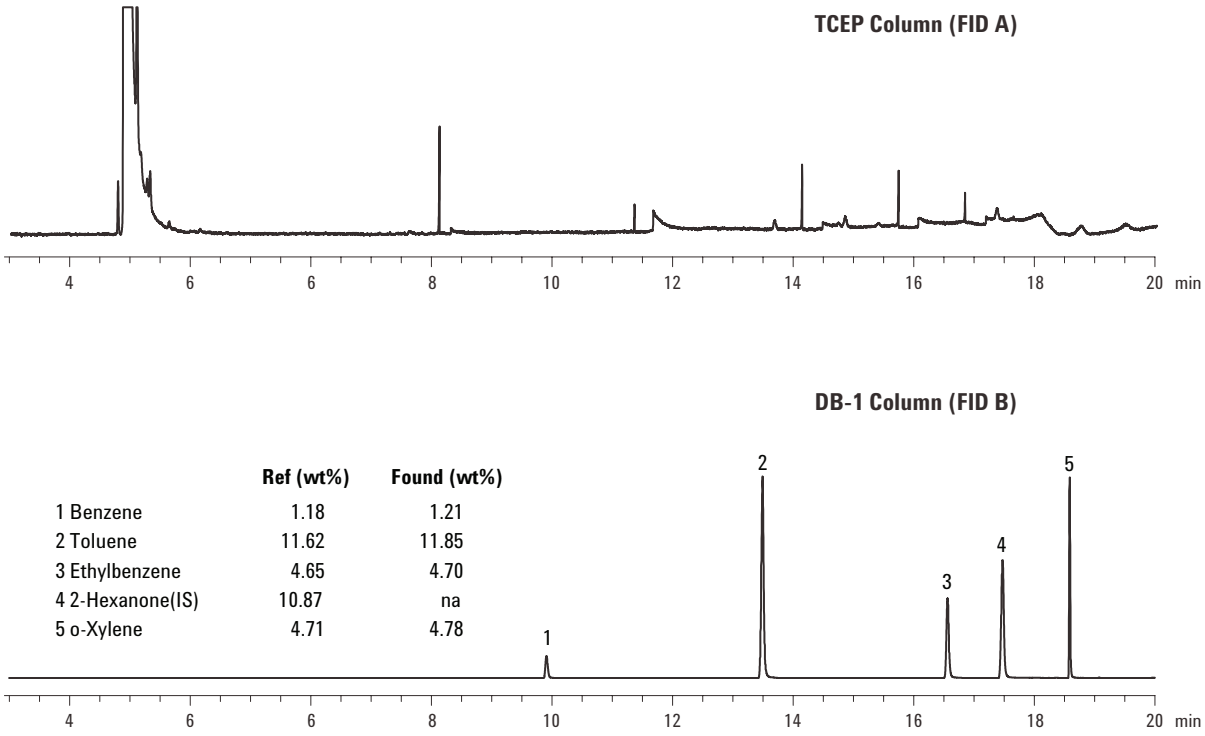

Figure 10. An RFA gasoline sample, containing known quantities of four aromatic compounds, was analyzed using the heart-cutting GC technique.

One disadvantage of this method is the analysis run time, which can be as long as 70 minutes for some gasoline samples. Based on the retention times listed in Table 3, the analysis can be completed in 20 minutes or less. The long run time is mostly attributed to waiting for high-boiling hydrocarbons to elute from the TCEP column. Because the upper temperature limit of TCEP is 120 °C, increasing the oven temperature is not an option for reducing run time. However, because the system design uses EPC for both the split/splitless inlet and the PCM, the primary column (TCEP) can be backflushed with a high flow rate after the last analyte peak elutes. Figure 11 shows how backflushing can be easily performed with this design on the Agilent 6890 GC. After the last peak of interest elutes, the fluidic switch is in the off position and the split/splitless inlet pressure is automatically decreased to 0.5 psi and the PCM pressure increased to 60 psi. Any high-boiling compounds remaining on the TCEP column will be backflushed to the split vent, and those on the DB-1 column will be quickly eluted with high flow to FID B. This backflushing technique was used for all standards and samples discussed in this paper.

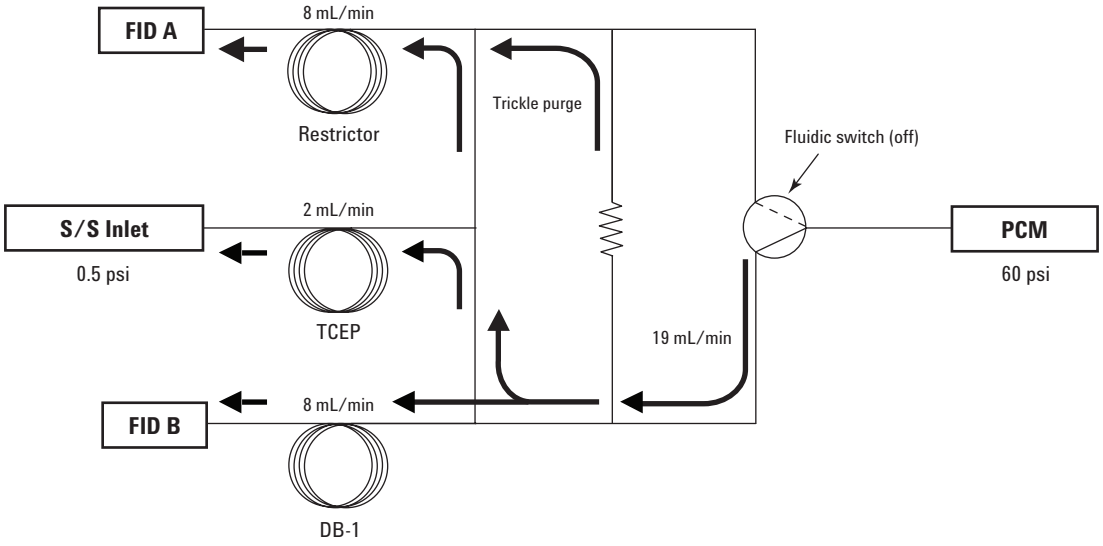

Figure 11. A significant reduction in analysis time was made by backflushing the system after the peaks of interest had eluted from the DB-1 column. This was possible because the system design used the Agilent 6890 electronic pneumatics control (EPC) for both the split/splitless inlet and the PCM.

Conclusions

A two-dimensional GC method was shown to comply with European Standards EN 13132 for oxygenate additives in gasoline and EN 12177 for benzene in gasoline. A Deans switch device was used to heart cut unresolved analytes from one column to another so that complete chromatographic separation was achieved for all target compounds from hydrocarbons in the matrix. The unique plumbing design used on the Agilent 6890N GC systems provided easier method setup and reliable performance by eliminating carry-over and minimizing peak tailing for very polar compounds. System calibration and quantitative results using real gasoline samples demonstrated excellent performance with this system. Additionally, enhanced productivity was shown by using the Agilent 6890N EPC modules to allow faster analysis time using a backflushing technique.

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Printed in the USA
May 15, 2002
5988-6696EN



Agilent Technologies

Running ASTM Methods D4815 and D5580 on a Single Agilent 6890N Gas Chromatograph with Nitrogen Carrier Gas

Application

Petrochemical

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Abstract

An Agilent 6890N GC system is described that allows both ASTM methods D4815 and D5580 to run on single hardware configuration for the analysis of oxygenates and aromatics in gasoline. Additionally, the methods were implemented with nitrogen as the carrier gas instead of helium. Nitrogen is sometimes preferred because it is cheaper and easier to obtain in chromatographic grades in some locations. The 6890N based system offers a significant improvement in laboratory efficiency and a reduction in laboratory operating expenses. This application note outlines the details of the GC hardware, including columns and instrument conditions for performing these analyses. Setup and optimization of the methods are described in detail. The system was extensively tested with several gasoline samples and was shown to give excellent analytical precision for both retention time and detector response.

Introduction

Chromatographic separation of oxygenated and aromatic compounds from the complex hydrocarbon matrix in gasoline is difficult to achieve with a single capillary column; therefore, ASTM methods D4815 and D5580 use a two-column configuration with a 10-port column-switching valve [1,2]. At the time of sample introduction, the valve is in the "Off" position, and the sample is injected onto a polar TCEP pre-column where oxygenates, aromatics, and heavy hydrocarbons are retained while the light hydrocarbons are eluted to a thermal conductivity detector (TCD) (Figure 1). After the elution of light hydrocarbons, the valve is switched to the "On" position and oxygenates, aromatics, and heavy hydrocarbons are backflushed onto the methyl silicone capillary column for a boiling point separation (Figure 2). Once the compounds of interest are eluted from the capillary column, the valve is reset to the "Off" position so those heavier compounds remaining on the capillary column are backflushed to the flame ionization detector (FID) (Figure 3). While backflushing, the capillary column pressure is automatically increased using the electronic pneumatics control of the 6890N GC. The increased column flow will speed the elution of the heavy compounds, thus reducing the run time for the method.

Two separate gas chromatographs are usually needed to run ASTM methods D4815 and D5890 for the analysis of oxygenated and aromatic compounds in gasoline. Although these two methods use the same GC hardware configuration, the column configurations differ slightly. Each method uses the same TCEP [1,2,3-*tris*-(2-cyanoethoxy) propane] micro-packed column to remove matrix interference, however, the analytical capillary columns are somewhat different for each method. D4815 uses a methyl silicone column (HP-1 or DB-1)

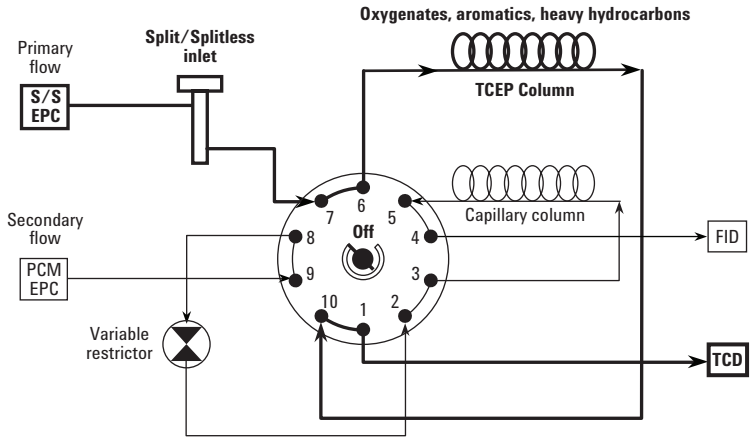

Figure 1. The valve is in the "off" position at the time of injection. The sample flow path is shown in bold. The polar TCEP pre-column retains the oxygenates, aromatics and heavier hydrocarbons. The lighter hydrocarbons are not retained and are eluted to vent.

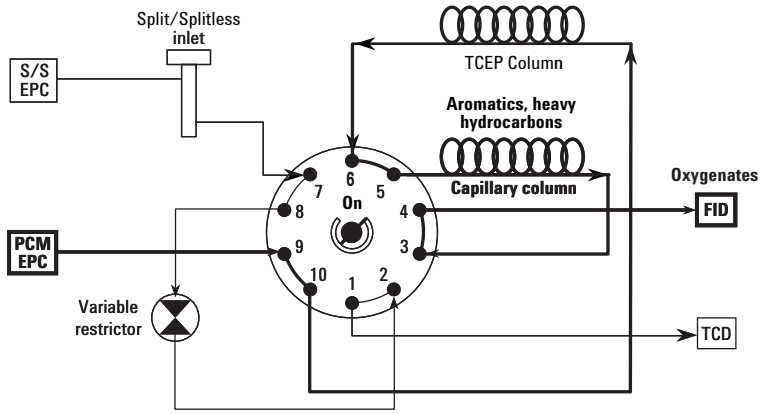

Figure 2. After the light hydrocarbons have eluted from the TCEP column the valve is switched to the "on" position. The reversed carrier gas flow through the TCEP column is supplied by the pneumatics control module (PCM). The oxygenates, aromatics and heavy hydrocarbons are backflushed from the TCEP column to the capillary column for boiling point separation and detection by the flame ionization detector (FID).

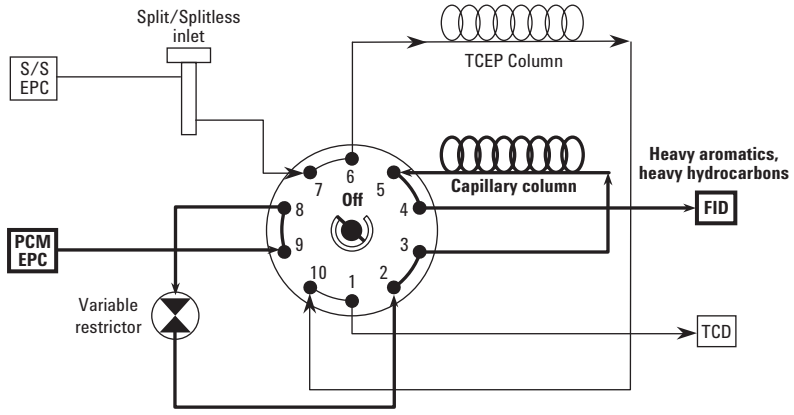

Figure 3. The valve is in the "off" position after detection of the oxygenates. The sample flow path is shown in bold. The capillary column flow is reversed and the heavy hydrocarbons are backflushed to the FID. At this time, the capillary column pressure is increased using the PCM electronic pneumatics control. The increase column flow elutes these compounds faster.

that is 30 meters long, 530 microns internal diameter, and a 2.65-micron film thickness. The D5580 method uses a methyl silicone column with the same column dimensions, but with a film thickness of 5 microns.

This application note describes a 6890N GC configuration along with optimized methods that allow the analysis of both oxygenated and aromatic compounds in gasoline using a single instrument equipped with a single pair of columns. Additionally, these methods use nitrogen as the carrier gas instead of helium. In some regions, chromatographic grade helium is difficult and expensive to acquire for use as a carrier gas, whereas nitrogen is much easier and less expensive to obtain as a carrier gas choice.

Experimental

The GC hardware configuration used for this work is outlined in Table 1. The capillary column used for the combined system was the 5 µm film HP-1 because this column could supply the necessary separation for all of the oxygenated additives, as well as the aromatics found in gasoline.

Table 1. Agilent 6890 Hardware Configuration for the Combined D4815/D5580 Method

Standard 6890 GC hardware	
G1540N	6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210	FID with EPC control
Option 220	TCD with EPC controls
Option 309	PCM module with EPC controls
Option 751	Temperature controlled valve box for one valve
Option 800	10-Port valve
Option 872 (2 of each)	Zero dead volume bulkhead unions
G2613A	Agilent 7683 Autoinjector
Columns	
Polar pre-column	Micro-packed 20% TCEP on chromosorb PAW 80/100, stainless steel 560 mm × 0.38 mm id
Capillary column	5 µm film HP-1, 30 m × 0.53 mm id (Agilent part number. 19095Z-623)
Data system	
G2070A	Agilent multi-technique ChemStation
Optional consumables	
5181-8809	10 µL fixed straight needle autoinjector syringe for Merlin Microseal
5183-4647	Inlet liner optimized for split operation
Standards and samples	
D-4815-VT*	AccuStandard valve timing mixture
D-4815/IS-SET*	AccuStandard quantitative calibration mixture
M-GRO-EPA-CC/IS-5ML*	AccuStandard quantitative check standard
SRM 2294**	Reformulated gasoline with 11%(w/w) MTBE

*Available from Accustandard, 125 Market St., New Haven, CT 06513, USA

**Available from NIST Standard Reference Material Program

100 Bureau Dr., Room 204, Build. 202, Gaithersburg, MD 20899-2322, USA

D4815 - Oxygenates TCEP Column Backflush Time Setting

Table 2 lists the method conditions used for the analysis of oxygenated compounds in gasoline. Since the capillary column used for this work has a thicker film than the standard D4815 column, the oven temperature was set to 80 °C instead of 60 °C. This higher temperature gave run times that were approximately the same as those in the original D4815 method. The pressure and flow settings in Table 2 were typical for this analysis, but they must be checked and adjusted for each individual instrument.

A valve timing mixture was prepared to optimize the backflush time in this system. This mixture contains 10% w/w each of MtBE, EtBE, DIPE and methylcyclopentane (MCP) in hexane. The MCP served as a marker to indicate the complete elution of light hydrocarbons from the TCEP pre-column. The valve timing mixture was run at 0.02-minute intervals between backflush times of 0.16 minutes to 0.30 minutes. At each interval the data was evaluated to determine the optimal backflush time for that particular system.

D5580 Aromatics TCEP Column Backflush Time Setting

Table 3 lists the GC instrument conditions used for the analysis of aromatics in gasoline. The pressure and flow settings in Table 3 were typical for this analysis, but they must be checked and adjusted for each individual instrument.

Table 2. Instrument Conditions for D4815 Oxygenate Analysis

Split/Splitless injection port	
Temperature	200 °C
Pressure	9 psi Nitrogen (constant pressure mode)
Split vent flow	70 mL/min
Split ratio	15:1
TCEP Pre-column flow	5 mL/min
Pneumatics control module	13 psi Nitrogen (ramped pressure mode)
Pressure program	13 psi for 14 min 99 psi/min to 40 psi
HP-1 Capillary column flow	3 mL/min
FID Temperature	250 °C
Valve temperature	60 °C
Oven temperature	80 °C Isothermal

Table 3. Instrument Conditions for D5580 Oxygenate Analysis

Split/Splitless injection port	
Temperature	200 °C
Pressure	25 psi Nitrogen (constant pressure mode)
Split vent flow	100 mL/min
Split ratio	100:1
TCEP Pre-column flow	10 mL/min
Pneumatics control module	23 psi Nitrogen (ramped pressure mode)
Pressure program (benzene/toluene)	23 psi for 12.1 min 99 psi/min to 40 psi
Pressure program (C8/C9 Plus)	23 psi for 22 min 99 psi/min to 40 psi
HP-1 Capillary column flow	10 mL/min
FID Temperature	250 °C
Valve temperature	60 °C
Oven temperature	60 °C for 6 min, 2 °C/min to 115 °C, hold at 115 °C for 1.5 min

For a complete aromatic analysis, ASTM method D5580 must be run two times with two different TCEP column backflush times. The first backflush time, T1, was used for the analysis of benzene and toluene. The second backflush time, T2, was used for the analysis of C8 and C9 plus aromatics. Each of these times was determined by running a valve timing test mixture that contains 5% w/w each of benzene, toluene, ethylbenzene, o-xylene, and 2-hexanone in isooctane (2,2,4-trimethylpentane). A 1 µL volume of this mixture was injected into the GC with the valve set to the "Off" position for the entire run so that the sample was separated on the TCEP column and detected with the TCD. This chromatogram was used to select backflush times, T1 and T2.

Evaluation of System Precision when Performing Sequential Oxygenate and Aromatic Analyses

After determining the backflush and reset times for D4815 and D5580, the system was evaluated by running two separate gasoline samples. These samples were a pump gasoline and a Renewable Fuels Association (RFA) gasoline, each one containing MtBE. These samples were run in a sequence so that the 6890N GC would have to perform an oxygenate analysis followed by a complete aromatics analysis. This sequence was repeated five consecutive times in order to evaluate the robustness and precision of the system when switching between the different setpoints (flow, pressure, temperature, valve timing) required by each method.

Results and Discussion

D4815 - Oxygenates TCEP Column Backflush Time Setting

The data obtained from the backflush time setting experiment appears in Figure 4. Inspection of this data shows that the hydrocarbons, represented by MCP, were completely eluted from the TCEP column after 0.20 minutes. This data is graphically represented in Figure 5. For this system, a backflush time of 0.22 minutes was used for the analysis of MtBE and ethanol. This backflush time was specific to the system used for this work and must be optimized for each individual GC system. This method was saved as "D4815" on the Agilent ChemStation.

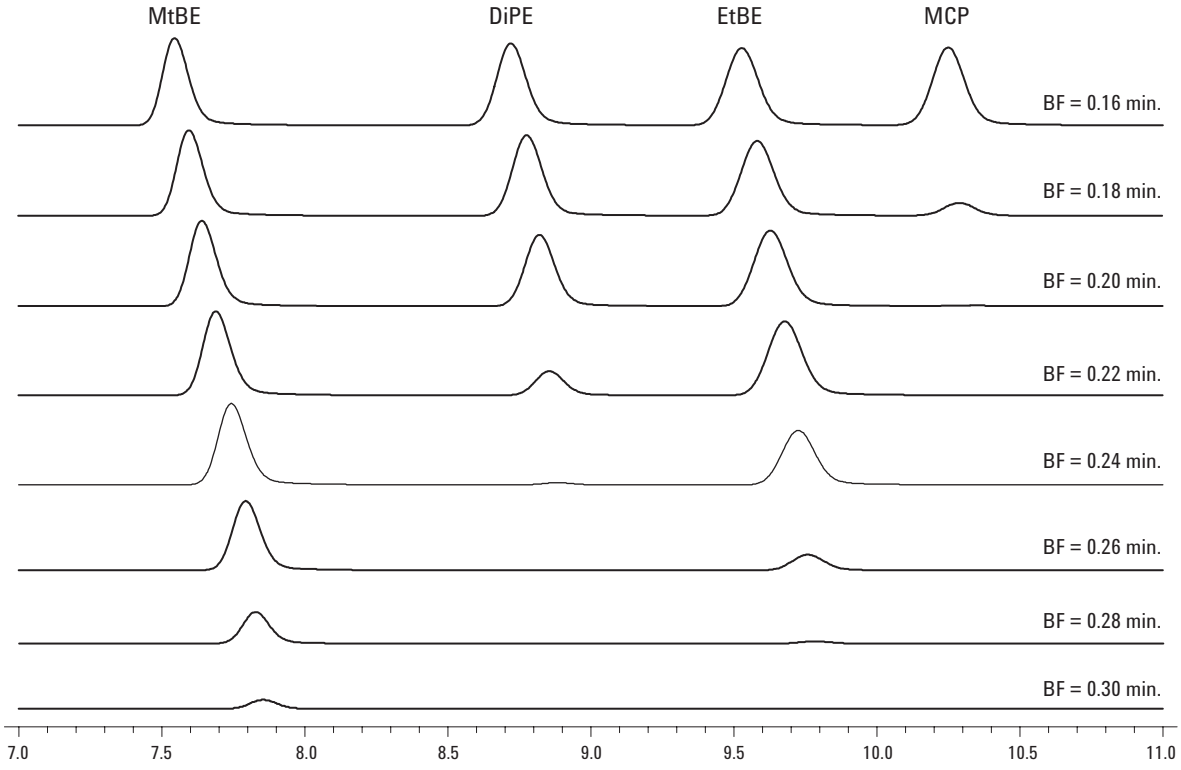

Figure 4. Chromatographic data from backflush time optimization experiment for D4815 analysis of oxygenates. In this example, the MCP is completely vented from the TCEP column after a backflush time of 0.20 minutes.

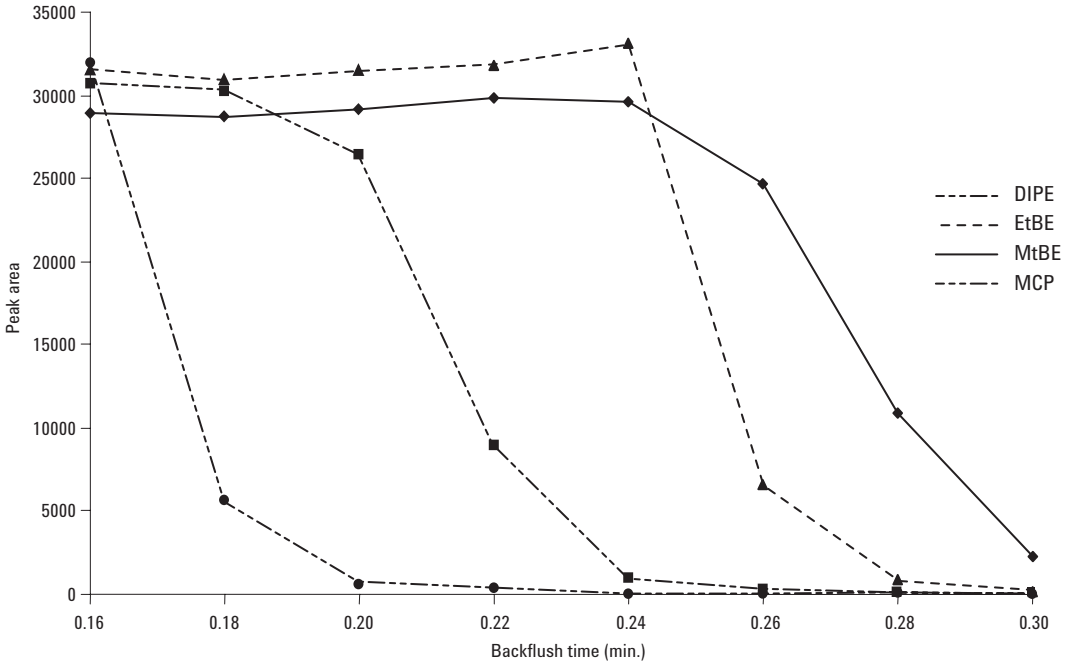

Figure 5. This shows a graphic presentation of the data from Figure 4. After 0.20 minutes the hydrocarbons represented by MCP are vented from the TCEP column, while the MtBE and EtBE are completely retained. The DIPE response is reduced indicating some loss from the column.

Using the GC conditions listed in Table 2 and with a backflush time of 0.22 minutes, a gasoline sample containing 11 wt% MtBE and another containing 8 wt% ethanol were run on this system to evaluate the chromatography. Figure 6 shows the results of these analyses. After the elution of the benzene from the capillary column, the valve was reset to the "Off" position and the PCM pressure increased to 40 psi to quickly elute any aromatic and heavy nonaromatic hydrocarbons from the capillary column.

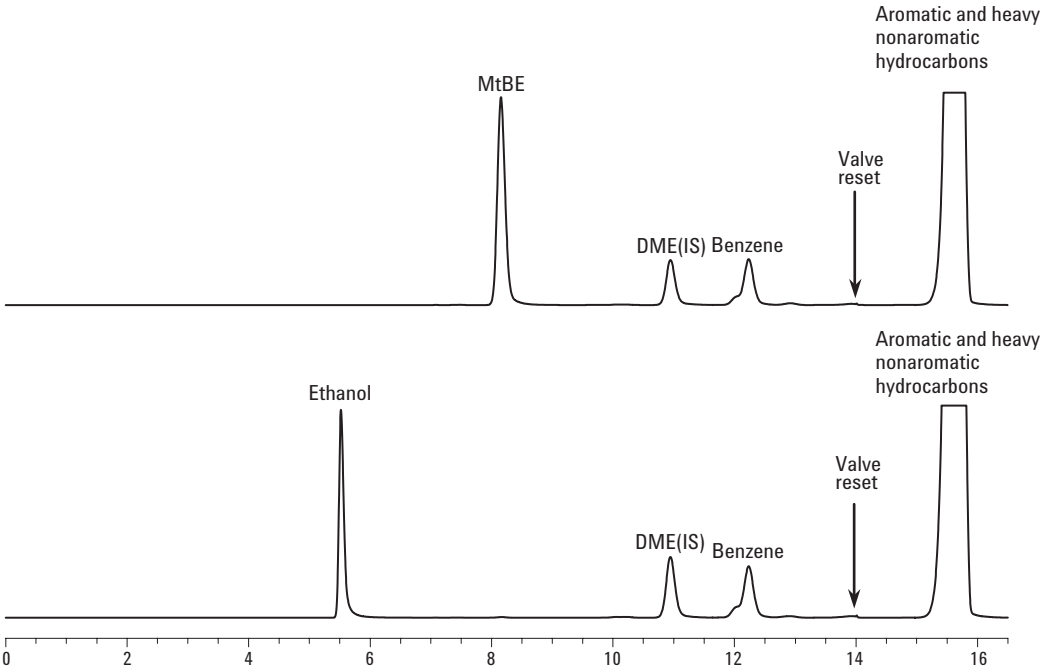

Figure 6. Analysis of two gasoline samples containing 11 wt% MtBE (top) and 8 wt% ethanol (bottom) using a backflush time of 0.22 minutes. Each sample contains 5 wt% of the internal standard, dimethoxyethanol (DME). At 14 minutes, the valve was reset to the "Off" position and the PCM module pressure increased to quickly elute any aromatic and nonaromatic hydrocarbons still on the capillary column.

D5580 Aromatics TCEP Column Backflush Time Setting

Setting the correct backflush times for the aromatics analysis was more complicated because D5580 requires two different times for a complete aromatics analysis. Figure 7 shows the chromatogram of the D5580 valve timing mix separated on the TCEP column and detected by the TCD. From this chromatogram, the benzene/toluene backflush time (T1) was determined by subtracting 6 seconds from the start of the benzene peak. For this system, 0.58 minutes was used for T1. The second backflush time (T2), for C8 and C9 plus aromatics, was determined by subtracting 6 seconds from the start of the ethylbenzene peak. For this system a value of 1.68 minutes was used for T2. The benzene/toluene method was saved on the ChemStation as "D5580A" and the C8/C9 plus aromatics method was saved as "D5580B".

With these two backflush times and the GC instrument conditions listed in Table 3, the chromatography of the system was evaluated by running a sample of pump gasoline (89 octane) that contained 10 wt% of the internal standard, 2-hexanone. Figure 8 shows the two chromatograms obtained with this method. This data was also used to determine the valve reset times for backflushing the capillary column after the last peak of interest has eluted. For the benzene/toluene analysis, 12.1 minutes was used for the valve reset time (T3). For the C8/C9 plus aromatics, the valve rest time (T4) was set to 22 minutes. After each reset time, the PCM pressure was increased to 40 psi so that any aromatic and nonaromatic hydrocarbons remaining on the capillary column will be quickly eluted to the FID for detection.

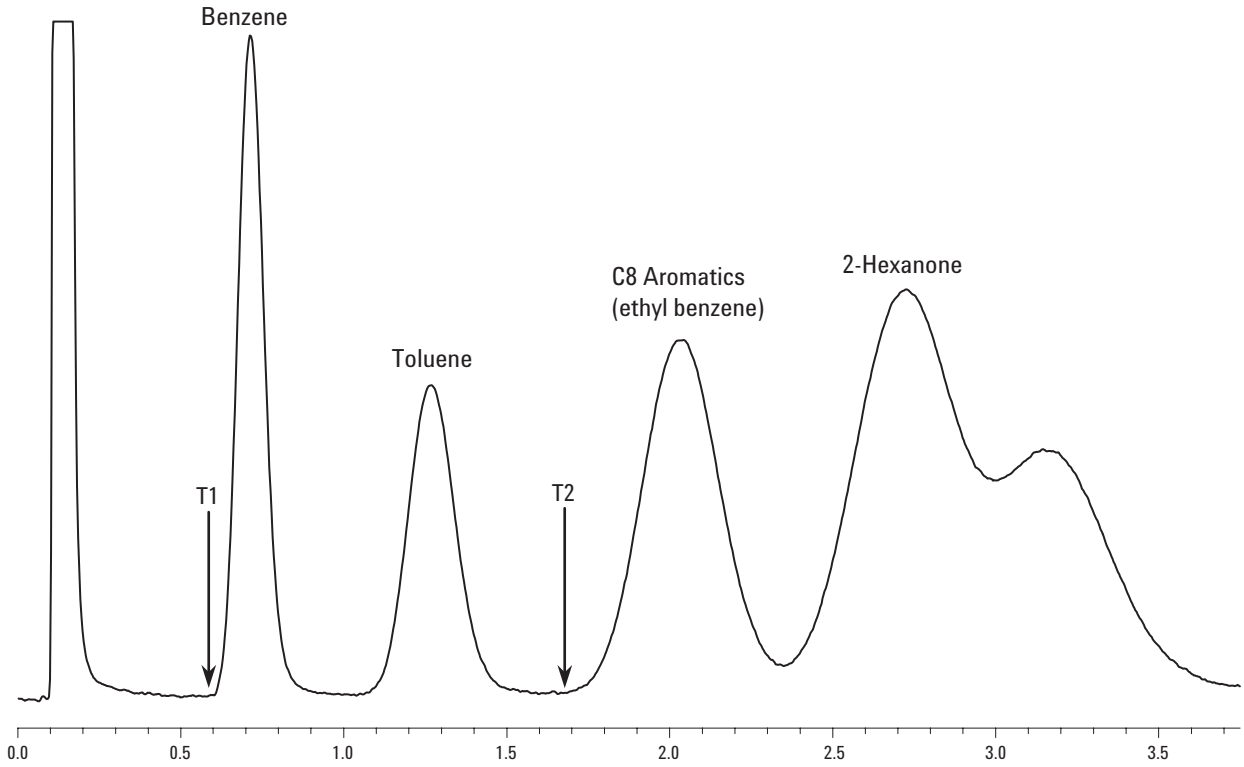

Figure 7. D5580 valve timing mix showing the two backflush times for this system. Backflush time T1 (0.58 min.) is set just before the elution of benzene from the TCEP column. This time is used for the analysis of benzene and toluene. Backflush time T2 (1.68 min.) is set just before the elution of ethylbenzene and is used for the analysis of C8 and C9 plus aromatic compounds.

Evaluation of System Precision when Performing Sequential Oxygenate and Aromatic Analyses

Although this application note describes a common 6890N GC configuration for both oxygenate and aromatic analysis, the three methods that perform these measurements used different instrument conditions such as oven temperature, column pressures, column flows, and valve timing. Many analysts using this system will want to perform a complete analysis, (oxygenate, benzene/toluene, and C8/C9 plus) for each gasoline sample before going on to the next sample. This means that these three different methods will run consecutively, and the GC system must be stable enough to ensure retention time and quantitative precision as the methods change between analyses. To test the system, a ChemStation sequence was created so that a sample was run by the D4815 method followed by the D5580A method (benzene/toluene) and the D5580B method (C8 and C9 plus aromatic). This sequence of three analyses was repeated five times for each sample.

Operating parameters (such as pneumatics stability, oven stability, and valve timing) combine to affect the overall retention time precision of this system, especially when changing methods between sample runs. Tables 4 and 5 show the retention time precision of this system for a pump gasoline sample and an RFA gasoline sample, both containing MtBE. From this data one can see that the retention time precision remains high for all compounds, even while alternating GC conditions for each sample.

Tables 6 and 7 show the detector response precision for these two samples as the three methods are alternated. The system provides excellent detector precision for all compounds analyzed by these methods.

Table 4. Retention Time Precision for Sequential Analysis of a Pump Gasoline Sample

		Retention Time (minutes)					
Run no.	**Method**	**MtBE**	**Benzene**	**Toluene**	**Ethylbenzene**	**m, p-Xylene**	**o-Xylene**
1	D4815	8.119					
2	D5580A		6.527	11.147			
3	D5580B				18.251	18.794	20.285
4	D4815	8.148					
5	D5580A		6.528	11.145			
6	D5580B				18.242	18.787	20.277
7	D4815	8.155					
8	D5580A		6.534	11.153			
9	D5580B				18.235	18.776	20.272
10	D4815	8.157					
11	D5580A		6.539	11.156			
12	D5580B				18.230	18.771	20.269
13	D4815	8.156					
14	D5580A		6.539	11.158			
15	D5580B				18.235	18.772	20.270
	Average	8.147	6.533	11.152	18.238	18.780	20.275
	Std Dev	0.016	0.005	0.006	0.008	0.010	0.007
	%RSD	0.195	0.083	0.050	0.045	0.053	0.033

Table 5. Retention Time Precision for Sequential Analysis of a RFA Gasoline Sample

		Retention Time (minutes)					
Run no.	**Method**	**MtBE**	**Benzene**	**Toluene**	**Ethylbenzene**	**m, p-Xylene**	**o-Xylene**
16	D4815	8.156					
17	D5580A		6.539	11.160			
18	D5580B				18.241	18.786	20.281
19	D4815	8.154					
20	D5580A		6.540	11.160			
21	D5580B				18.243	18.791	20.281
22	D4815	8.154					
23	D5580A		6.542	11.162			
24	D5580B				18.242	18.788	20.280
25	D4815	8.153					
26	D5580A		6.541	11.162			
27	D5580B				18.242	18.788	20.283
28	D4815	8.151					
29	D5580A		6.539	11.160			
30	D5580B				18.244	18.790	20.282
	Average	8.154	6.540	11.161	18.242	18.788	20.281
	Std Dev	0.002	0.001	0.001	0.001	0.002	0.001
	%RSD	0.025	0.017	0.010	0.006	0.010	0.005

Table 6. Detector Response Precision for Sequential Analysis of a Pump Gasoline Sample

		FID Response (area counts)					
Run no.	**Method**	**MtBE**	**Benzene**	**Toluene**	**Ethylbenzene**	**m, p-Xylene**	**o-Xylene**
1	D4815	24077					
2	D5580A		3306	19427			
3	D5580B				5402	21825	8069
4	D4815	23384					
5	D5580A		3244	19436			
6	D5580B				5402	21956	8105
7	D4815	22807					
8	D5580A		3178	19580			
9	D5580B				5422	22342	8232
10	D4815	22915					
11	D5580A		3048	19344			
12	D5580B				5432	22263	8201
13	D4815	23053					
14	D5580A		3099	19529			
15	D5580B				5422	22253	8199
	Average	23247	3175	19463	5416	22128	8161
	Std Dev	512	105	92	14	224	70
	%RSD	2.2	3.3	0.5	0.2	1.0	0.9

Table 7. Detector Response Precision for Sequential Analysis of a RGA Gasoline Sample

		FID Response (area counts)					
Run no.	**Method**	**MtBE**	**Benzene**	**Toluene**	**Ethylbenzene**	**m, p-Xylene**	**o-Xylene**
16	D4815	32456					
17	D5580A		8384	39783			
18	D5580B				11860	35424	12723
19	D4815	32762					
20	D5580A		8393	40300			
21	D5580B				11925	35583	35583
22	D4815	32998					
23	D5580A		8383	40107			
24	D5580B				11833	35272	35272
25	D4815	32715					
26	D5580A		8464	40387			
27	D5580B				11780	35205	35205
28	D4815	33339					
29	D5580A		8566	40068			
30	D5580B				11998	35841	35841
	Average	32854	8438	40129	11879	35465	12738
	Std Dev	332	79	234	84	256	93
	%RSD	1.0	0.9	0.6	0.7	0.7	0.7

Optimizing Chromatographic Resolution for Ethylbenzene and m, p-Xylene

In Figure 8, the resolution between the ethylbenzene peak and the m, p-xylene peak is not baseline, but it is acceptable for good quantitative analysis. This is due to the nitrogen carrier gas used at higher than optimum linear velocity in the capillary column. This high-linear velocity was used to give the same run times as shown in the original ASTM method, which uses helium as the carrier gas. Occasionally, some combinations of TCEP precolumns and HP-1 capillary columns will provide less retention with nitrogen carrier gas due to variations in the preparation of the TCEP column.

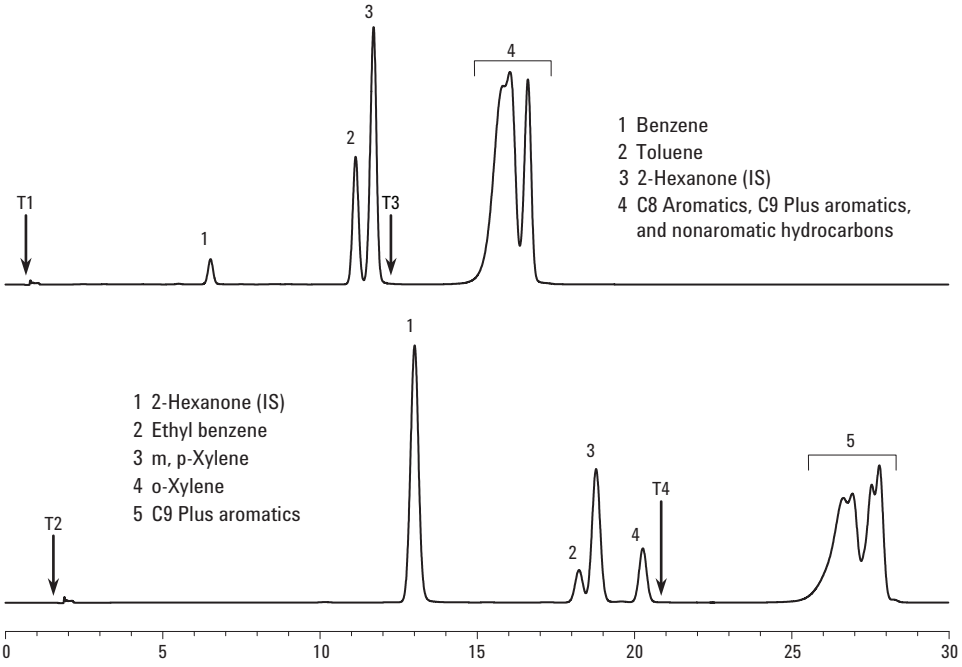

Figure 8. D5580 valve timing mix showing the analysis of aromatic compounds in gasoline. The top chromatogram uses backflush time T1 (0.58 min.) for the analysis of benzene and toluene. After elution of 2-hexanone, the valve is reset at 12.1 minutes (T3) to quickly elute the C8/C9 aromatics and any heavy hydrocarbons from the capillary column. The bottom chromatogram measures C8 and C9 plus aromatics using backflush time T2 (1.68 min.). The valve is reset at 22 minutes (T4) to elute the C9 plus aromatics.

In Figure 9 (upper chromatogram), one GC system was found to have less peak retention and resolution due to these column conditions. One can also see that the overall retention times were about 25% faster. Potential solutions to this problem include: replacing the TCEP column, lowering column flows, or using helium as the carrier gas. However, the quickest and easiest solution is to slightly change the GC oven temperature program. In Figure 9, after the initial oven temperature was lowered to 50 °C, the peak retention and resolution were returned to the levels shown in Figure 8.

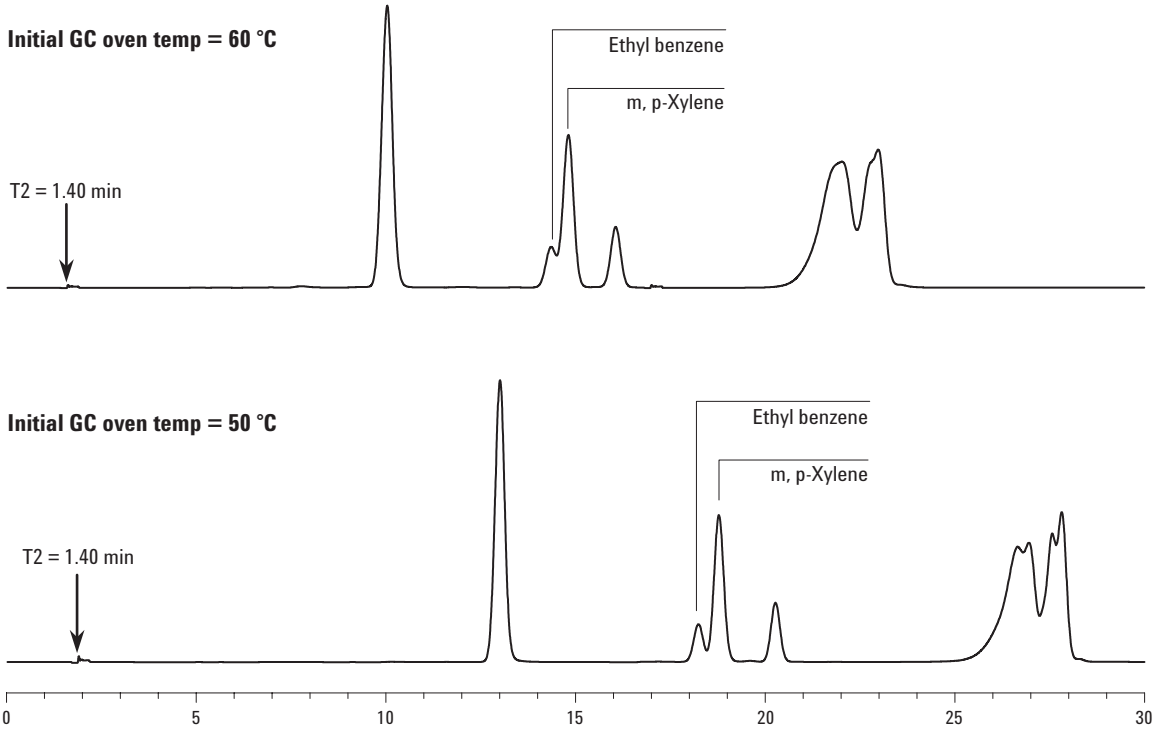

Figure 9. The upper chromatogram shows the analysis of C8/C9 plus aromatics in gasoline where the overall peak retention is faster and the ethylbenzene/m, p-xylene resolution is reduced. This is due to a variation in the TCEP micropacked column prepration. This can be corrected by simply lowering the initial GC oven temperature until the desired retention and resolution is obtained (lower chromatogram).

Conclusions

A GC hardware configuration was developed that can run ASTM methods D4815 and D5580 on a single instrument for the analysis of oxygenated and aromatic compounds in gasoline. These methods were also used with nitrogen (instead of helium) as the carrier gas, with no changes in column-flow settings and minimal loss of chromatographic resolution. Using both a single instrument and nitrogen carrier gas offers a significant improvement in laboratory efficiency and laboratory operating expenses. Real gasoline samples were used to demonstrate that this system could also provide exceptionally good performance for the analysis of these important components in modern fuels.

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Printed in the USA
March 17, 2003
5988-9153EN

Analysis of Aromatic Hydrocarbons in Gasoline and Naphtha with the Agilent 6820 Series Gas Chromatograph and a Single Polar Capillary Column

Application

Petrochemical

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Abstract

The aromatic hydrocarbons in finished gasoline, gasoline blending feedstock, and straight-run naphtha were quantitatively analyzed using an Agilent 6820 gas chromatograph equipped with a flame ionization detector and a single polar capillary column. An Internal standardization method, using n-tridecane as the internal standard, yielded quantitative concentration data for 57 aromatic target compounds, with a relative standard deviation of less than 1% for almost all compounds.

Introduction

Motorization in China is growing rapidly, resulting in an increasing demand for gasoline. Since carcinogenic components exist within gasoline, there is a need to monitor and control its composition. Many regulatory standards were created worldwide for this purpose.

The People's Republic of China National Standards GB 17930-1999 [1] describes the quality of unleaded finished gasoline for motor vehicles. Maximum allowable concentrations for benzene and total aromatic hydrocarbon are 2.5 and 40 weight percent (wt %) respectively. This latter method uses a gas chromatograph (GC) equipped with a valve system and two packed columns (OV-101 and TCEP) to analyze benzene and toluene in gasoline.

In Europe and Japan, the benzene content of gasoline must be 1 wt % or less, and the total aromatic hydrocarbon content is limited by regulation.

There are approved methods for the analysis of aromatic hydrocarbons in gasoline from the American Society for Testing and Materials (ASTM). ASTM D5769 [2] is the standard test method for determining benzene, toluene, and total aromatics in finished gasoline by gas chromatography/mass spectrometry (GC/MS). This method requires a GC/MS system.

ASTM D5580 [3] is the standard gas chromatography (GC) test method for determining benzene, toluene, ethylbenzene, p/m-xylene, o-xylene, C9 and heavier aromatics, and total aromatics in finished gasoline. This method uses a valve system, two packed columns, and requires two separate analyses to completely measure the aromatic content in gasoline.

ASTM D3606 [4] is the standard test method for determining benzene and toluene in finished motor and aviation gasoline by GC. Although this method can analyze for benzene and toluene, it is unable to analyze for C8 or heavier aromatic hydrocarbons.

Japanese Industrial Standards (JIS) K 2536-1996 [5] describes the use of a single TCEP or PEG-20M capillary column to analyze aromatic hydrocarbons in gasoline. This method requires no valve system or modification of the GC. While both columns are non-cross-linked liquid phase columns, they cannot withstand high-temperature conditions.

In this application note, the described method uses a single cross-linked polyethylene glycol capillary column for the GC analysis of aromatic hydrocarbons in finished gasoline and gasoline blending feedstock.

Experimental

In this work, an Agilent 6820 GC equipped with a split/splitless inlet and a flame ionization detector (FID) appear in Table 1.

Table 1. Analytical Conditions

GC	Agilent 6820 Gas Chromatograph
Inlet	Split/Splitless; 240 °C, split mode, split ratio 50:1
Carrier	Helium, 1.4 mL/min at 40 °C
Column	60 m, 0.25 mm id, 0.25 µm film HP-INNOWax (p/n 19091N-136)
Oven	40 °C (5 min); 5 °C /min; 240 °C (15 min)
Detector	FID, 240 °C; Makeup gas: Helium, 40 mL/min

Samples analyzed in this work include: Chinese finished gasoline (RON = 93), Japanese finished regular gasoline (RON = 90), Japanese finished premium gasoline (RON = 100), reformate gasoline, heavy fluid catalytic cracking (FCC) gasoline, and straight-run heavy naphtha.

JIS K 2536-1996 describes a quantitative method for aromatic hydrocarbons using n-undecane as the internal standard. Since Chinese gasoline includes some n-undecane, a different internal standard (IS) was required. The alternate, n-tridecane, was used and about 2 wt % was added to each sample.

Each aromatic hydrocarbon component was identified using an Agilent 6890N/5973N GC/MS and analytical conditions were determined by method translation.

By concensus, theoretical response factors are used for the correction of the FID detector response of hydrocarbons (as determined by this method). The response of an FID to hydrocarbons is determined by the ratio of the molecular weight of the carbon in the analyte to the total molecular weight of analyte. The response factors, as listed in Table 2, are relative to those calculated for heptane. Calculations are based on the following equation:

$$Fh = \frac{(C_{aw} \times {}_{Cn}) + (H_{aw} \times H_n)}{C_{aw} \times C_n} \times 0.83905$$

Where: Fh = Relative response factor for a hydrocarbon type group of a particular carbon number

C_{aw} = Atomic weight of carbon, 12.011

C_n = Number of carbon molecules in the group

H_{aw} = Atomic weight of hydrogen, 1.008

H_n = Number of hydrogen molecules in the group; 0.83905 is the correction factor with heptane as unity (1.0000). 0.7487 is used with methane as unity.

Table 2. Theoretical FID Relative Response Factors

Carbon number	Saturated paraffins	Olefins	Saturated naphthenes	Unsaturated naphthenes	Aromatics
1	1.1207	–	–	–	–
2	1.0503	–	–	–	–
3	1.0268	0.9799	–	–	–
4	1.0151	0.9799	–	–	–
5	1.0080	0.9799	0.9799	0.9517	–
6	1.0034	0.9799	0.9799	0.9564	0.9095
7	1.0000	0.9799	0.9799	0.9598	0.9195
8	0.9975	0.9799	0.9799	0.9623	0.9271
9	0.9955	0.9799	0.9799	0.9642	0.9329
10	0.9940	0.9799	0.9799	0.9658	0.9376
11	0.9927	0.9799	0.9799	0.9671	0.9415
12	0.9916	0.9799	0.9799	0.9681	0.9447
13	0.9907	0.9799	0.9799	0.9690	0.9474
14	0.9899	0.9799	0.9799	0.9698	0.9497
15	0.9893	0.9799	0.9799	0.9705	0.9517

Results and Discussion

A chromatogram of reformate gasoline is shown in Figure 1. Reformate is a high concentration, aromatic hydrocarbon gasoline feedstock from the reformation process. There are low boiling point nonaromatic hydrocarbons eluted before benzene. High boiling point nonaromatic hydrocarbons were not found in reformate, and all peaks after benzene were aromatic hydrocarbons. A peak of n-tridecane (peak number 19) elutes at 19.45 minutes, between 1,2,4-trimethylbenzene and 1,4-diethylbenzene. These peaks separate at the base line. The n-tridecane peak does not overlap with any aromatic hydrocarbon peak.

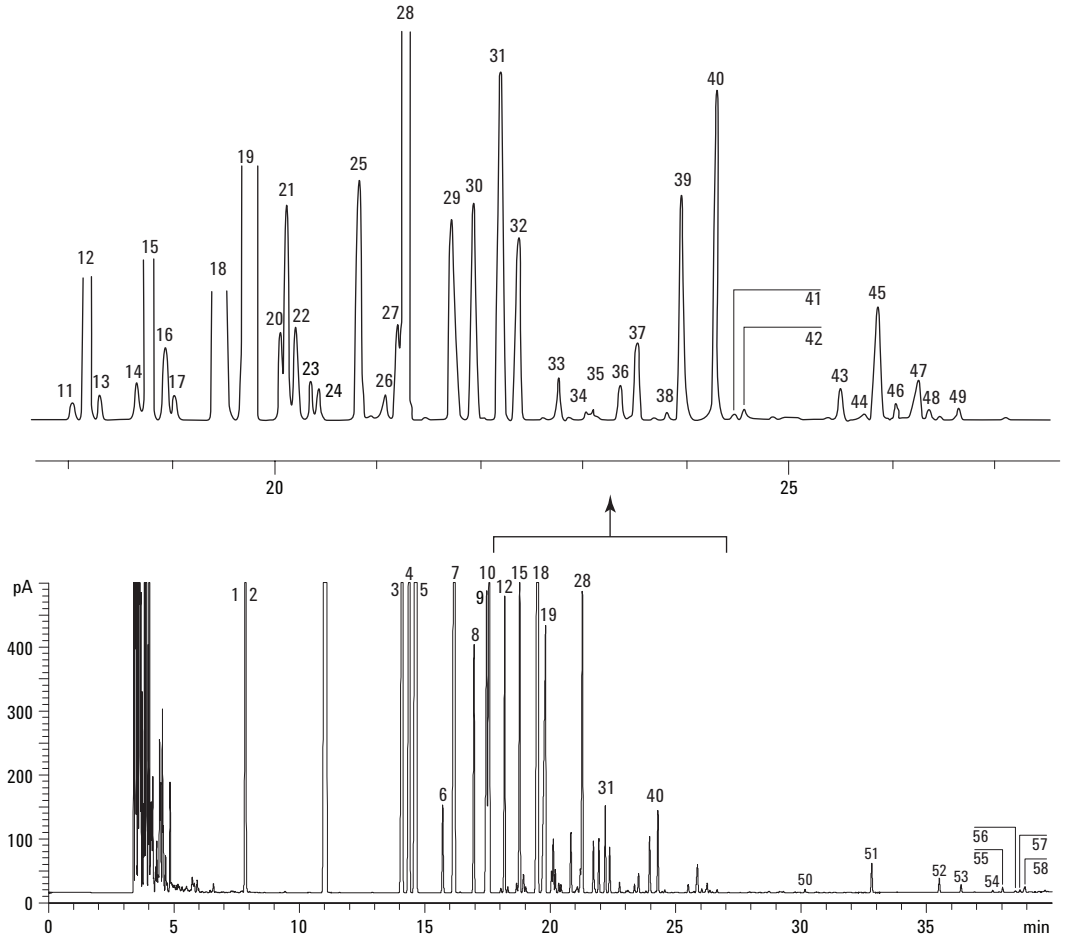

Figure 1. Chromatogram of reformate.

A chromatogram of heavy FCC gasoline is shown in Figure 2. FCC gasoline contains paraffin, olefin, naphthene, and aromatic hydrocarbons. There are some small interfering nonaromatic hydrocarbon peaks among benzene and ethylbenzene. It also seems that benzene and toluene overlap another nonaromatic hydrocarbon, but the peak area of this interference is relatively small.

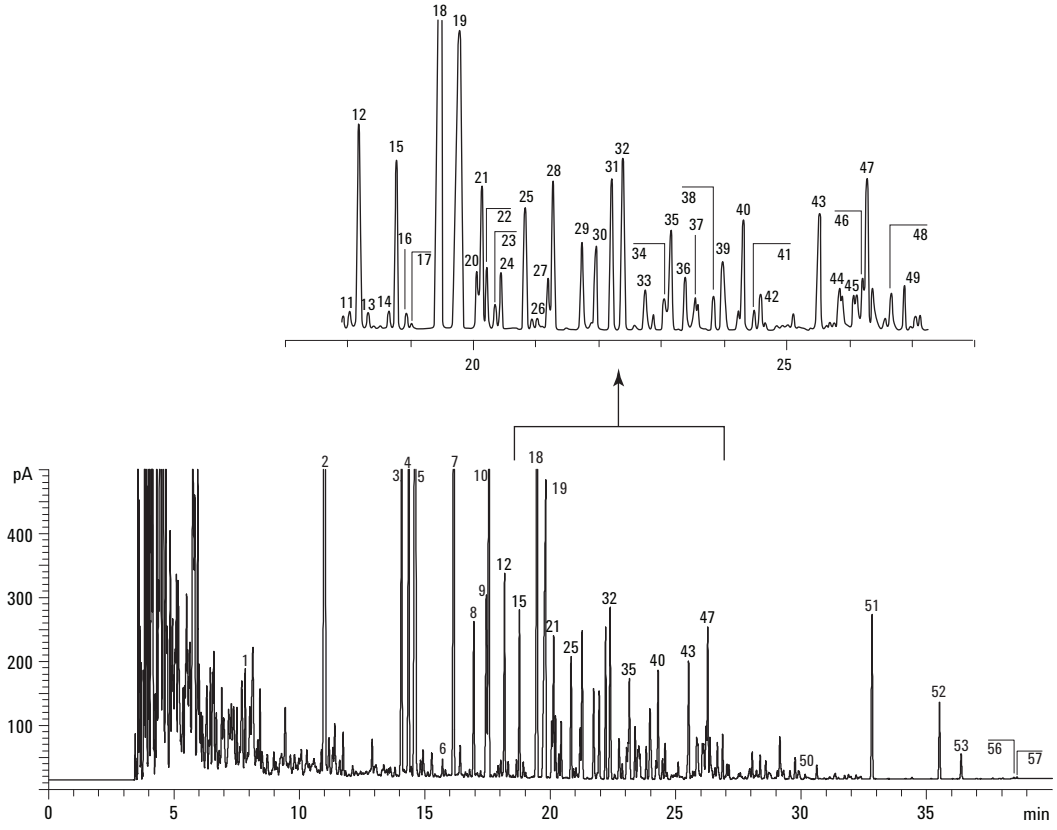

Figure 2. Chromatogram of heavy FCC gasoline.

A chromatogram of heavy naphtha is shown in Figure 3. Naphtha contains paraffin, naphthene, and aromatic hydrocarbons. There are some nonaromatic hydrocarbon peaks near benzene and few after toluene.

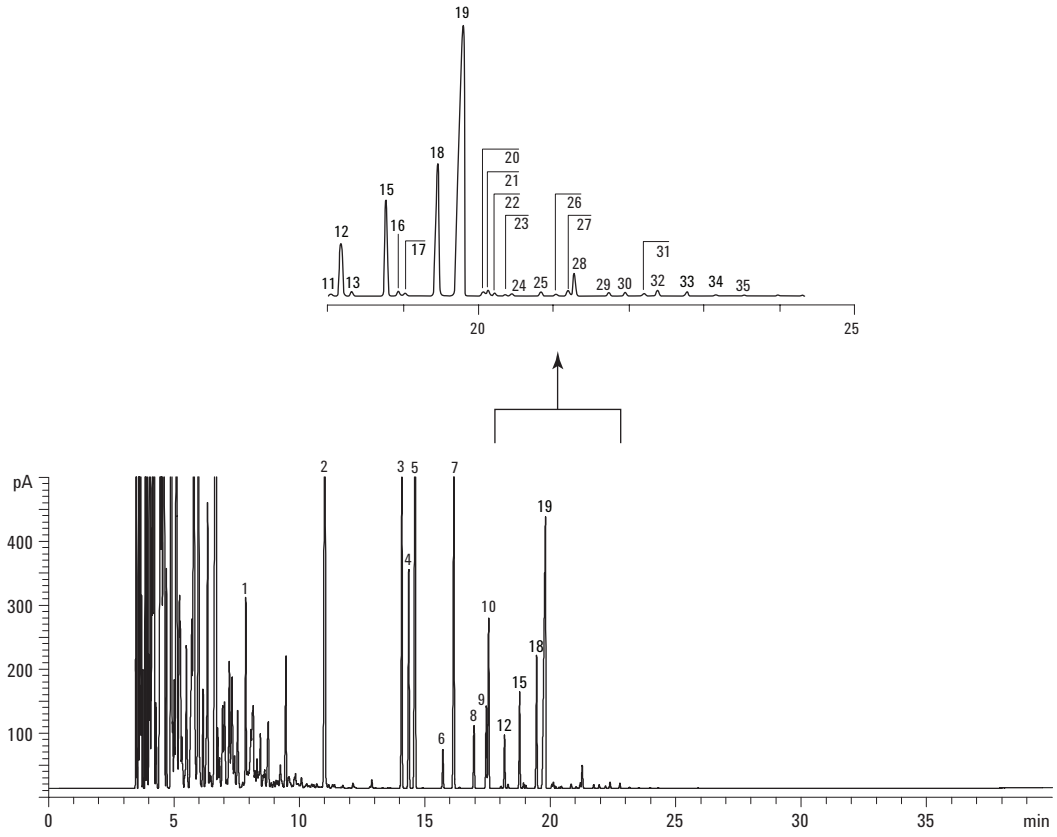

Figure 3. Chromatogram of heavy straight-run naphtha.

A chromatogram of Japanese finished premium gasoline (RON = 100) is shown in Figure 4. The premium gasoline is normally blended with reformate, light FCC gasoline, alkylate, isomerate, butane, butene, and some oxygenates. The premium gasoline does not contain a heavy fraction of nonaromatic hydrocarbons. Only a few small peaks of nonaromatic hydrocarbons are found near the benzene peak.

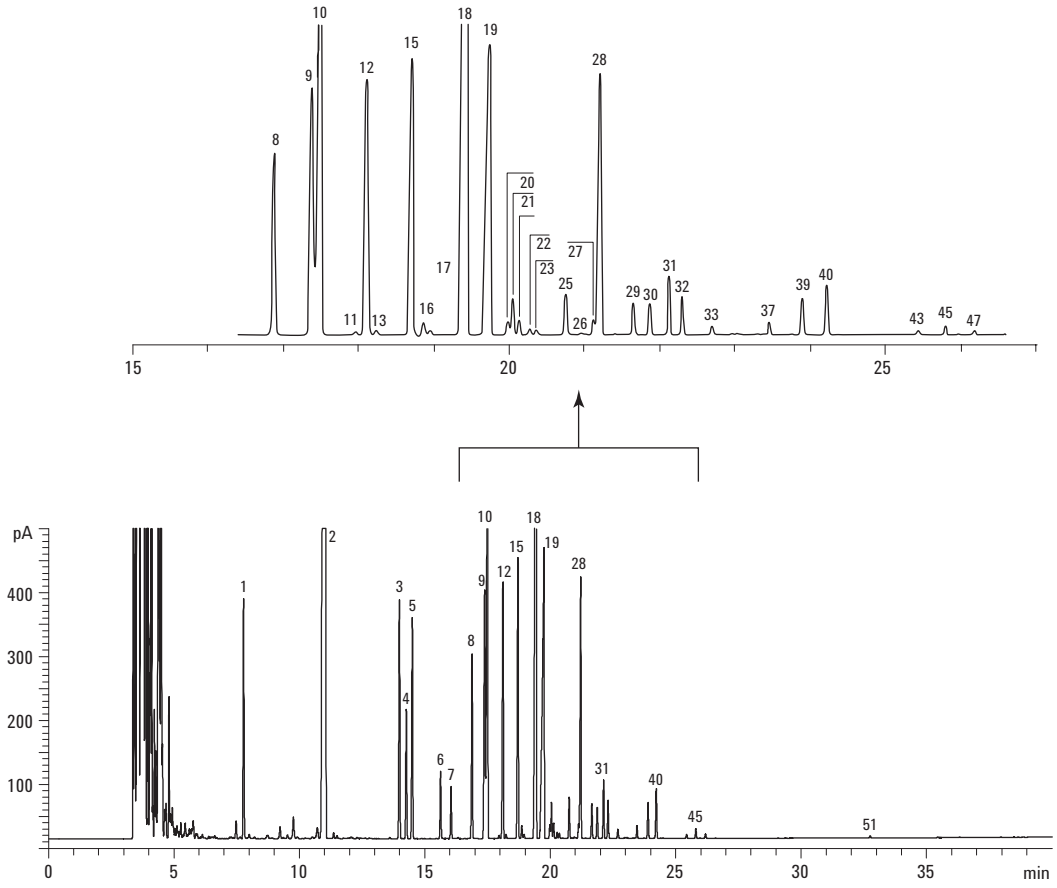

Figure 4. Chromatogram of Japanese finished premium gasoline.

A chromatogram of Japanese finished regular gasoline (RON = 90) is shown in Figure 5. The regular gasoline is normally blended with reformate, heavy FCC gasoline, light naphtha, butane, and butene. In this case, there are some heavy nonaromatic hydrocarbons. These peaks elute between benzene and o-xylene. There is no nonaromatic hydrocarbon peak after o-xylene.

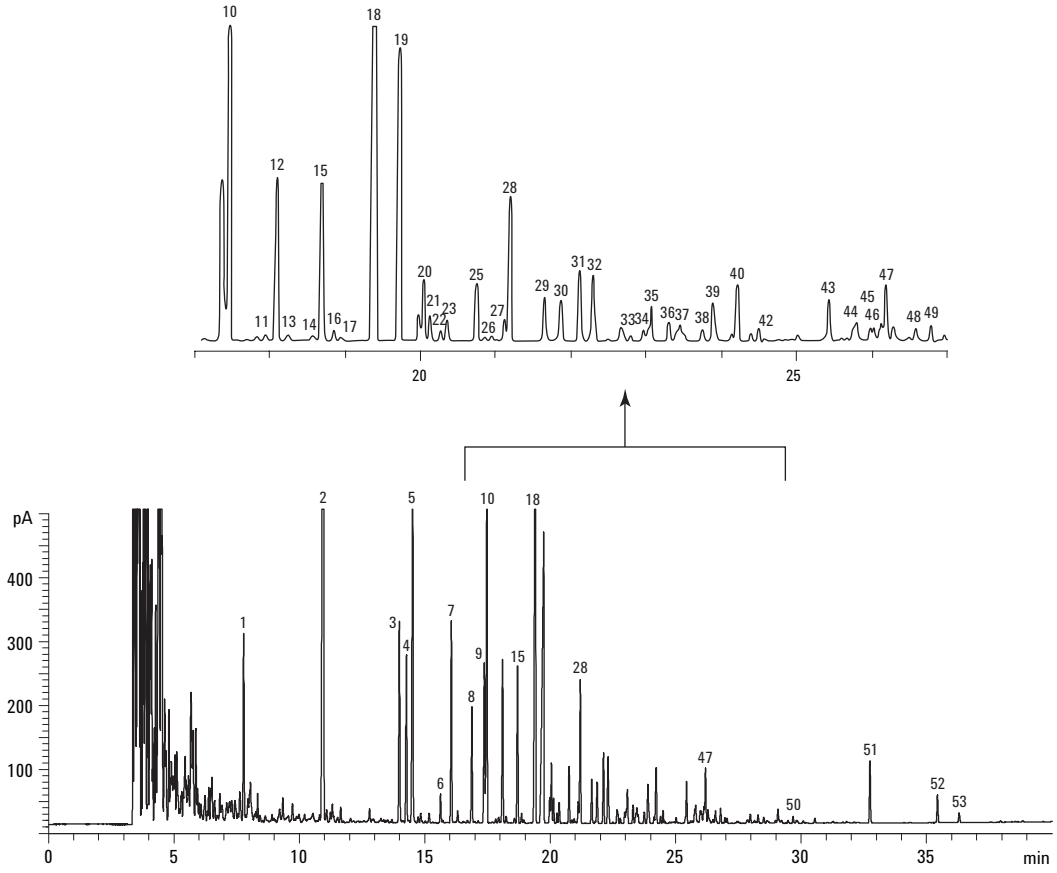

Figure 5. Chromatogram of Japanese finished regular gasoline.

A chromatogram of Chinese finished gasoline (RON = 93) is shown in Figure 6. This gasoline contains heavy fractions of nonaromatic hydrocarbons. The peak at 12.9 min is n-undecane. In this case, if n-undecane is used as an internal standard, the quantitative results will be incorrect. There is no nonaromatic hydrocarbon near n-tridecane.

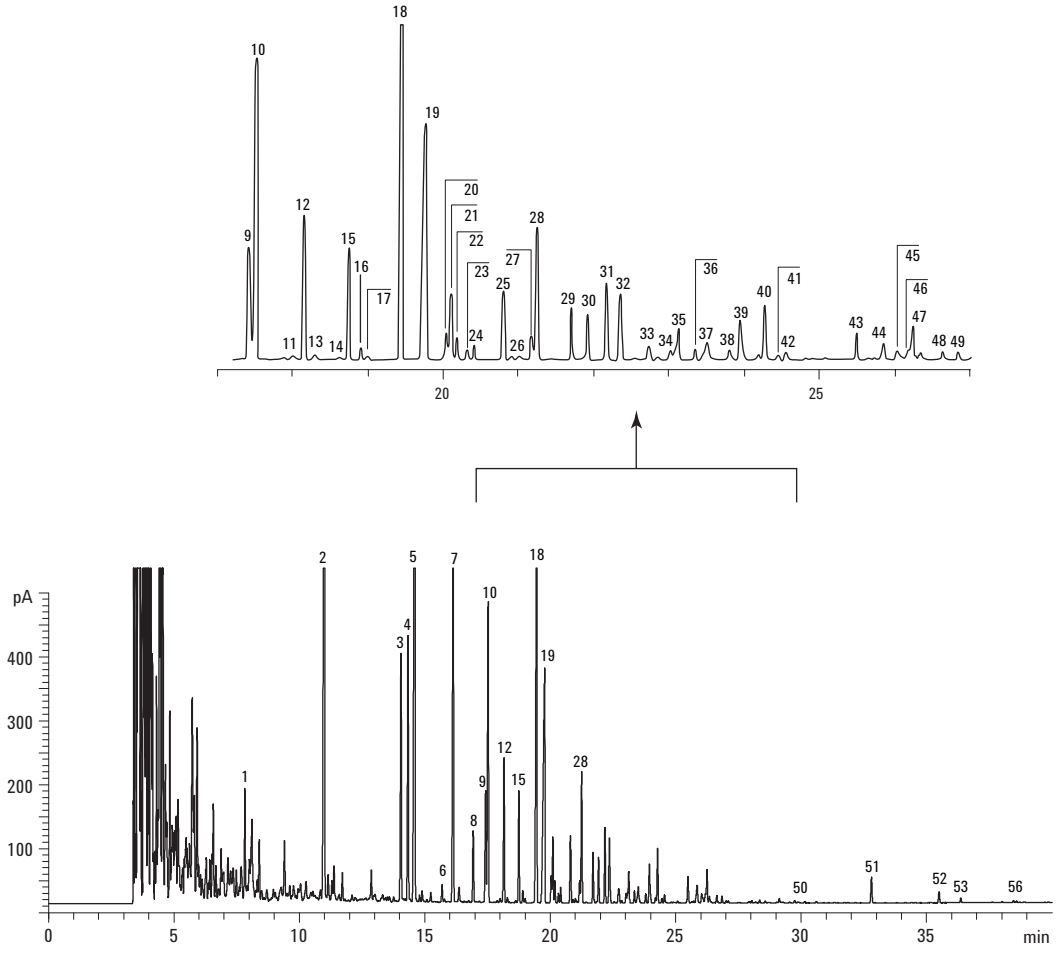

Figure 6. Chromatogram of Chinese finished gasoline.

Quantitative results for finished gasoline and gasoline feedstock are shown in Table 3. Reformate and heavy FCC gasoline contain heavy aromatic hydrocarbons such as biphenyl and dimethylnaphthalene. However, heavy straight-run naphtha does not contain any heavy fraction; tetramethylbenzenes and naphthalene are not detected.

Table 3. Quantitative Results for Finished Gasoline and Blending Feedstock

Peak number	Compound name	Reformate Wt %	Heavy FCC gasoline Wt %	Heavy naphtha Wt %	Japanese premium gasoline Wt %	Japanese regular gasoline Wt %	Chinese gasoline Wt %
1	Benzene	4.716	0.347	0.667	0.729	0.604	0.371
2	Toluene	15.705	6.141	3.466	22.345	5.744	3.550
3	Ethylbenzene	4.227	1.988	1.286	0.828	0.714	0.909
4	p-Xylene	3.794	1.797	0.878	0.439	0.582	0.951
5	m-Xylene	8.260	4.706	2.213	0.765	1.487	2.669
6	*iso*-Propylbenzene	0.332	0.068	0.152	0.229	0.101	0.061
7	o-Xylene	5.506	2.201	1.360	0.174	0.697	1.444
8	n-Propylbenzene	0.865	0.590	0.247	0.667	0.405	0.248
9	1-Methyl-4-ethylbenzene	1.350	0.819	0.332	1.094	0.630	0.411
10	1-Methyl-3-ethylbenzene	2.935	2.070	0.660	2.396	1.463	1.108
11	*iso*-Butylbenzene	0.018	0.070	0.009	0.013	0.025	0.019
12	1,3,5-Trimethylbenzene	1.069	0.812	0.210	0.938	0.577	0.521
13	*sec*-Butylbenzene	0.020	0.074	0.017	0.015	0.028	0.019
14	Styrene	0.036	0.073	0.000	0.000	0.020	0.009
15	1-Methyl-2-ethylbenzene	1.336	0.629	0.375	1.043	0.555	0.390
16	1-Methyl-4-*iso*-propylbenzene	0.071	0.061	0.022	0.049	0.036	0.044
17	1-Methyl-3-*iso*-propylbenzene	0.023	0.027	0.012	0.017	0.014	0.016
18	1,2,4-Trimethylbenzene	4.501	2.599	0.530	3.932	2.185	1.954
19	n-Tridecane (IS)	1.854	2.127	2.107	2.168	2.043	1.556
20	1,4-Diethylbenzene	0.073	0.241	0.018	0.122	0.211	0.099
21	1-Methyl-4-n-propylbenzene	0.179	0.534	0.025	0.050	0.081	0.232
22	1-Methyl-3-n-propylbenzene	0.073	0.201	0.009	0.020	0.034	0.072
23	1,3-Diethylbenzene	0.031	0.087	0.005	0.017	0.069	0.032
24	n-Butylbenzene	0.027	0.200	0.009	0.000	0.000	0.054
25	1,3-Dimetyl-5-ethylbenzene	0.212	0.471	0.016	0.148	0.012	0.246
26	*alpha*-Methylstyrene	0.031	0.013	0.007	0.006	0.005	0.006
27	1-Methyl-2-n-propylbenzene	0.069	0.196	0.022	0.044	0.077	0.079
28	1,2,3-Trimethylbenzene	1.120	0.566	0.095	0.963	0.516	0.484
29	1,4-Dimetyl-2-ethylbenzene	0.181	0.352	0.014	0.119	0.155	0.180
30	1,3-Dimetyl-4-ethylbenzene	0.188	0.373	0.013	0.105	0.156	0.167
31	1,2-Dimetyl-4-ethylbenzene	0.283	0.539	0.010	0.197	0.245	0.261

Table 3. Quantitative Results for Finished Gasoline and Blending Feedstock (continued)

Peak number	Compound name	Reformate Wt %	Heavy FCC gasoline Wt %	Heavy naphtha Wt %	Japanese premium gasoline Wt %	Japanese regular gasoline Wt %	Chinese gasoline Wt %
32	Indane	0.154	0.744	0.025	0.135	0.276	0.261
33	1,3-Dimethyl-2-ethylbenzene	0.035	0.190	0.020	0.032	0.018	0.077
34	Diethylmethylbenzene	0.006	0.120	0.000	0.000	0.040	0.034
35	1-Methyl-4-*iso*-butylbenzene	0.010	0.495	0.005	0.000	0.160	0.148
36	*iso*-Propenylbenzene	0.026	0.195	0.000	0.000	0.062	0.043
37	1,2-Dimethyl-3-ethylbenzene	0.062	0.122	0.000	0.043	0.024	0.087
38	1-Methyl-4-*iso*-butylbenzene	0.007	0.131	0.000	0.000	0.044	0.036
39	1,2,3,4-Tetramethylbenzene	0.192	0.382	0.000	0.126	0.174	0.170
40	1,2,3,5-Tetramethylbenzene	0.284	0.449	0.000	0.177	0.217	0.209
41	2,4-Diethyl-1-methylbenzene	0.004	0.081	0.000	0.000	0.044	0.016
42	1-Methyl-4-*tert*-butylbenzene	0.008	0.131	0.000	0.000	0.008	0.028
43	5-Methylindane	0.028	0.496	0.000	0.015	0.161	0.100
44	C11-Aromatic	0.004	0.030	0.000	0.000	0.009	0.005
45	1,2,4,5-Tetramethylbenzene	0.128	0.143	0.000	0.034	0.120	0.087
46	C11-Aromatic	0.013	0.137	0.000	0.000	0.048	0.051
47	4-Methylindane	0.039	0.610	0.000	0.016	0.206	0.123
48	C11-Aromatic	0.006	0.234	0.000	0.000	0.074	0.027
49	C11-Aromatic	0.009	0.173	0.000	0.000	0.057	0.028
50	Pentamethylbenzene	0.012	0.042	0.000	0.000	0.007	0.006
51	Naphthalene	0.105	0.664	0.000	0.008	0.233	0.097
52	2-Methylnaphthalene	0.051	0.309	0.000	0.000	0.106	0.043
53	1-Methylnaphthalene	0.029	0.104	0.000	0.000	0.038	0.019
54	2-Ethylnaphthalene	0.007	0.000	0.000	0.000	0.000	0.000
55	Dimethylnaphthalene	0.017	0.000	0.000	0.000	0.000	0.000
56	Biphenyl	0.005	0.006	0.000	0.000	0.000	0.007
57	Dimethylnaphthalene	0.009	0.008	0.000	0.000	0.000	0.000
58	Dimethylnaphthalene	0.029	0.000	0.000	0.000	0.000	0.000

Chinese finished gasoline and reformate were investigated for reproducibility of quantitative results. These data are shown in Tables 4 and 5, respectively. Most percent RSD are within 1%, except for a few peaks in very small concentration.

Table 4. Reproducibility Data for Chinese Finished Gasoline

Peak	Compound name	RT, min	Ave wt %	%RSD
1	Benzene	7.83	0.373	0.658
2	Toluene	10.99	3.567	0.658
3	Ethylbenzene	14.05	0.911	0.481
4	p-Xylene	14.33	0.953	0.438
5	m-Xylene	14.59	2.679	0.548
6	*iso*-Propylbenzene	15.68	0.061	0.259
7	o-Xylene	16.13	1.448	0.498
8	n-Propylbenzene	16.93	0.249	0.406
9	1-Methyl-4-ethylbenzene	17.42	0.412	0.526
10	1-Methyl-3-ethylbenzene	17.52	1.111	0.485
11	*iso*-Butylbenzene	18.00	0.019	0.285
12	1,3,5-Trimethylbenzene	18.15	0.523	0.454
13	*sec*-Butylbenzene	18.29	0.019	1.776
14	Styrene	18.62	0.009	0.754
15	1-Methyl-2-ethylbenzene	18.75	0.391	0.405
16	1-Methyl-4-*iso*-propylbenzene	18.91	0.044	0.333
17	1-Methyl-3-*iso*-propylbenzene	19.00	0.016	0.819
18	1,2,4-Trimetylbenzene	19.45	1.959	0.426
19	n-Tridecane (IS)	19.77	1.556	0.000
20	1,4-Diethylbenzene	20.03	0.099	0.308
21	1-Methyl-4-n-propylbenzene	20.10	0.232	0.393
22	1-Methyl-3-n-propylbenzene	20.19	0.072	0.377
23	1,3-Diethylbenzene	20.33	0.033	0.474
24	n-Butylbenzene	20.41	0.054	0.435
25	1,3-Dimetyl-5-ethylbenzene	20.81	0.246	0.369
26	*alpha*-Methylstyrene	21.00	0.017	0.315
27	1-Methyl-2-n-propylbenzene	21.17	0.079	0.367
28	1,2,3-Trimethylbenzene	21.25	0.485	0.345
29	1,4-Dimetyl-2-ethylbenzene	21.71	0.180	0.417
30	1,3-Dimetyl-4-ethylbenzene	21.93	0.168	0.342
31	1,2-Dimetyl-4-ethylbenzene	22.18	0.261	0.354
32	Indane	22.36	0.261	0.446
33	1,3-Dimethyl-2-ethylbenzene	22.72	0.077	0.412
34	Diethylmethylbenzene	23.02	0.034	0.282
35	1-Methyl-4-*iso*-butylbenzene	23.13	0.148	0.451
36	*iso*-Propenylbenzene	23.36	0.043	0.320
37	1,2-Dimethyl-3-ethylbenzene	23.51	0.087	0.496
38	1-Methyl-4-*iso*-butylbenzene	23.80	0.036	0.952
39	1,2,3,4-Tetramethylbenzene	23.95	0.170	0.245
40	1,2,3,5-Tetramethylbenzene	24.28	0.209	0.304
41	2,4-Diethyl-1-methylbenzene	24.45	0.016	0.268

Table 4. Reproducibility Data for Chinese Finished Gasoline (continued)

Peak	Compound name	RT, min	Ave wt %	%RSD
42	1-Methyl-4-*tert*-butylbenzene	24.55	0.028	0.527
43	5-Methylindane	25.49	0.100	0.356
44	C11-Aromatic	25.73	0.005	4.640
45	1,2,4,5-Tetramethylbenzene	25.86	0.088	0.602
46	C11-Aromatic	26.03	0.052	0.764
47	4-Methylindane	26.25	0.123	0.472
48	C11-Aromatic	26.34	0.028	1.321
49	C11-Aromatic	26.64	0.028	0.674
50	Pentamethylbenzene	30.14	0.006	1.083
51	Naphthalene	32.80	0.097	0.374
52	2-Methylnaphthalene	35.50	0.043	0.299
53	1-Methylnaphthalene	36.36	0.020	1.206
54	2-Ethylnaphthalene	37.64	–	–
55	Dimethylnaphthalene	38.03	–	–
56	Biphenyl	38.47	0.007	5.155
57	Dimethylnaphthalene	38.73	–	–
58	Dimethylnaphthalene	38.92	–	–

Table 5. Reproducibility Data for Reformate

Peak	Compound name	RT, min	Ave wt %	%RSD
1	Benzene	7.862	4.659	0.805
2	Toluene	11.086	15.556	0.581
3	Ethylbenzene	14.110	4.201	0.386
4	p-Xylene	14.395	3.772	0.369
5	m-Xylene	14.677	8.215	0.346
6	*iso*-Propylbenzene	15.716	0.331	0.375
7	o-Xylene	16.200	5.477	0.333
8	n-Propylbenzene	16.962	0.861	0.297
9	1-Methyl-4-ethylbenzene	17.465	1.347	0.235
10	1-Methyl-3-ethylbenzene	17.580	2.924	0.271
11	*iso*-Butylbenzene	18.032	0.018	0.771
12	1,3,5-Trimethylbenzene	18.188	1.065	0.283
13	*sec*-Butylbenzene	18.307	0.020	0.892
14	Styrene	18.661	0.035	1.926
15	1-Methyl-2-ethylbenzene	18.789	1.328	0.339
16	1-Methyl-4-iso-propylbenzene	18.933	0.070	0.555
17	1-Methyl-3-iso-propylbenzene	19.025	0.023	0.870
18	1,2,4-Trimethylbenzene	19.512	4.478	0.271
19	n-Tridecane (IS)	19.815	1.854	0.000
20	1,4-Diethylbenzene	20.052	0.073	0.490
21	1-Methyl-4-n-propylbenzene	20.120	0.179	0.662
22	1-Methyl-3-n-propylbenzene	20.203	0.073	1.586
23	1,3-Diethylbenzene	20.344	0.031	1.174
24	n-Butylbenzene	20.429	0.026	0.402
25	1,3-Dimetyl-5-ethylbenzene	20.823	0.212	0.276
26	*alpha*-Methylstyrene	21.076	0.031	0.853
27	1-Methyl-2-n-propylbenzene	21.196	0.069	0.548
28	1,2,3-Trimethylbenzene	21.282	1.114	0.322
29	1,4-Dimetyl-2-ethylbenzene	21.721	0.180	0.348
30	1,3-Dimetyl-4-ethylbenzene	21.939	0.187	0.281
31	1,2-Dimetyl-4-ethylbenzene	22.195	0.281	0.314
32	Indane	22.368	0.153	0.419
33	1,3-Dimethyl-2-ethylbenzene	22.763	0.035	1.199
34	Diethylmethylbenzene	23.032	0.006	4.721
35	1-Methyl-4-*iso*-butylbenzene	23.089	0.010	4.312
36	*iso*-Propenylbenzene	23.360	0.028	6.343
37	1,2-Dimethyl-3-ethylbenzene	23.520	0.064	2.826
38	1-Methyl-4-iso-butylbenzene	23.815	0.007	1.099
39	1,2,3,4-Tetramethylbenzene	23.962	0.192	0.325
40	1,2,3,5-Tetramethylbenzene	24.294	0.283	0.268
41	2,4-Diethyl-1-methylbenzene	24.463	0.004	0.785
42	1-Methyl-4-tert-butylbenzene	24.564	0.008	3.870
43	5-Methylindane	25.500	0.028	0.453
44	C11-Aromatic	25.732	0.004	3.307
45	1,2,4,5-Tetramethylbenzene	25.866	0.127	0.428
46	C11-Aromatic	26.042	0.013	1.102
47	4-Methylindane	26.255	0.039	0.295
48	C11-Aromatic	26.363	0.005	5.996

Table 5. Reproducibility Data for Reformate (continued)

Peak	Compound name	RT, min	Ave wt %	%RSD
49	C11-Aromatic	26.651	0.009	0.708
50	Pentamethylbenzene	30.158	0.012	0.550
51	Naphthalene	32.815	0.104	0.435
52	2-Methylnaphthalene	35.507	0.051	0.370
53	1-Methylnaphthalene	36.376	0.029	0.394
54	2-Ethylnaphthalene	37.636	0.007	0.544
55	Dimethylnaphthalene	38.033	0.017	0.957
56	Biphenyl	38.549	0.005	1.054
57	Dimethylnaphthalene	38.725	0.009	1.276
58	Dimethylnaphthalene	38.921	0.028	0.710

This method requires no valve or precolumn. It only requires a normal GC equipped with split/splitless inlet, FID, and a single, polar HP-INNOWax capillary column. Quantitative results are improved using n-tridecane, instead of n-undecane, as the internal standard.

Conclusion

The aromatic hydrocarbons in finished gasoline, gasoline blending feedstock, and straight-run naphtha were quantitatively analyzed using an Agilent 6820 GC equipped with FID and a single polar capillary column. An internal standardization method, using n-tridecane as the internal standard, yielded quantitative concentration data for 57 aromatic target compounds, with a relative standard deviation of less than 1% for almost all compounds.

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4. ASTM D3606 ; standard test method for determination of benzene and toluene in finished motor and aviation gasoline by GC.
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Printed in the USA
May 7, 2003
5988-9261EN



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Quantification and Characterization of Sulfur in Low-Sulfur Reformulated Gasolines by GC-ICP-MS

Application

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Abstract

Reducing total sulfur in motor fuels has become a critical air pollution control goal worldwide. As countries mandate ever lower permissible levels of total sulfur in fuels, the environmental monitoring and fuel manufacturing industries have been forced to find more sensitive techniques for analysis. Gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICP-MS) has the capability to meet current and projected detection limits for both total sulfur in reformulated gasolines and individual sulfur species. Additionally, GC-ICP-MS can identify and quantify other volatile organometallic species in fuels. This application note examines the quantification of total sulfur levels (using compound independent calibration) and sulfur speciation in reformulated gasoline.

Introduction

Sulfur in motor fuels has been implicated as a contributing factor to global warming and acid rain. It is also a catalyst poison for automobile catalytic converters [1] and refinery catalytic crackers [2]. As a result, the trend in Europe and the US has been to reduce the acceptable level of total sulfur in motor fuels. The US Environmental Protection Agency (EPA) tier-2 guidelines (to take effect in 2004) will create an average sulfur standard of 30 parts per million (ppm) and a cap of 80 ppm total sulfur by 2007 [3]. The California Air Resources Board (CARB) has established similar standards that are currently in effect [4]. Both New York and Massachusetts have adopted these requirements. The European Union announced, in December of 2002, that new regulations will require full market availability of sulfur-free fuels, defined as containing less than 10 ppm sulfur content, by January 1, 2005 [5]. The phase-in is to be complete by January 1, 2009. The EU committed under the U.N. Kyoto protocol of 1997, that it would reduce emissions 8 percent from 1990 levels by 2010.

To achieve these goals, it is necessary to be able to measure total sulfur in motor fuels at ever lower levels. It is also necessary to be able to determine the form and distribution of sulfur species to help facilitate their removal. The predominant sulfur containing compounds in gasoline consist of various substituted thiophenes ranging from thiophene to dimethylbenzothiophenes. Typically, total sulfur has been measured by x-ray fluorescence and sulfur species by gas chromatography using a sulfur-specific detector such as sulfur chemiluminescence detector (SCD), flame photometric detector (FPD), or atomic emission detector (AED). However, as the requirement for lower detection limits (DL) increases, there is need for a more sensitive sulfur-specific detector. Inductively

coupled plasma mass spectrometry (ICP-MS) is known to be a very sensitive element-specific detector. When coupled to a gas chromatograph, ICP-MS has the potential to quantify very low concentrations of sulfur species in hydrocarbon fuels. Additionally, since ICP-MS is not limited to detecting only sulfur, it can be used for the simultaneous detection of other elements and volatile organometallics in fuels which may also contribute to air pollution or catalyst poisoning.

Experimental

In this study, the applicability of GC-ICP-MS to quantify and characterize total sulfur and sulfur species in low-sulfur reformulated gasoline (RFG) is examined. An Agilent 6890 gas chromatograph with split/splitless injector was coupled to an Agilent 7500a ICP-MS using the Agilent GC-ICP-MS interface (Figure 1). GC and ICP-MS conditions are summarized in Table 1. Calibration was based on a multilevel analysis of thiophene and 2-methyl-thiophene spiked into 3:1 isooctane:toluene obtained from Ultra Scientific [http://www.ultrasci.com].

Calibration levels ranged from 2.5 ppm per compound to 500 ppm per compound. Because GC-ICP-MS is capable of compound-independent calibration (Wilbur et. al. 2002 [6]), it was not necessary to calibrate every possible sulfur compound separately. The sulfur response factor for any compound(s) can be determined from a single compound. In this case, the response factors from thiophene were used and confirmed by those from 2-methylthiophene.

Figure 2 depicts calibration curves for thiophene from 2.5–150 ppm sulfur and 2-methylthiophene from 2.5–50 ppm sulfur. Figures 3, 4, and 5 depict three different gasoline samples, a low level RFG with approximately 55 ppm total sulfur, a conventional gasoline with approximately 330 ppm total sulfur and an ASTM gasoline sample which is not certified for total sulfur. Note the very different profile of sulfur compounds in each sample, especially with respect to the relative abundance of the higher boiling methyl and dimethylbenzothiophenes.

Table 1. Instrumental Conditions, GC and ICP-MS

Instrumentation	
Chromatographic system	Agilent 6890 GC
Inlet	Split/Splitless
Detector	Agilent 7500a ICP-MS
Liner	Splitless liner (part number 5062-3587)
Column	30 M × 0.25 mm id × 0.25 µ HP-5
GC Conditions	
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Split 1:50
Carrier gas	Helium
Carrier gas flow	2.5 mL/min (constant flow mode)
Transfer line temperature	250 °C
Oven temperature program	40 °C/4 minutes, 20 °C/min to 250 °C, hold for 1 min
ICP-MS Conditions	
ShieldTorch	Long-life shield installed
Forward power	700 W
Sample depth	13 mm
Carrier gas flow	1.1 L/min
Extract 1	–150 V
Extract 2	–75 V
Auxiliary gas	He, 10 mL/min added to Ar carrier
Injector temperature	260 °C

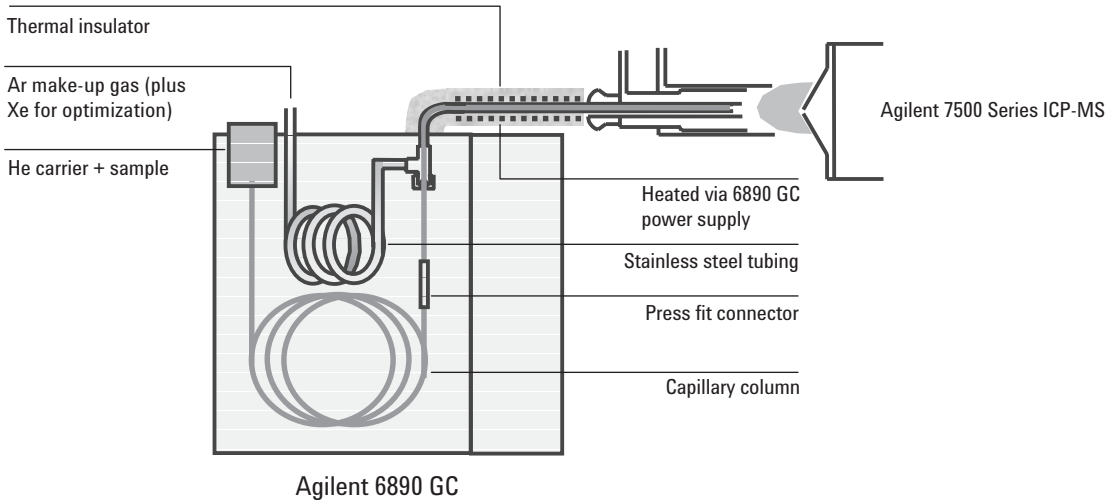

Figure 1. Schematic diagram of Agilent GC-ICP-MS system.

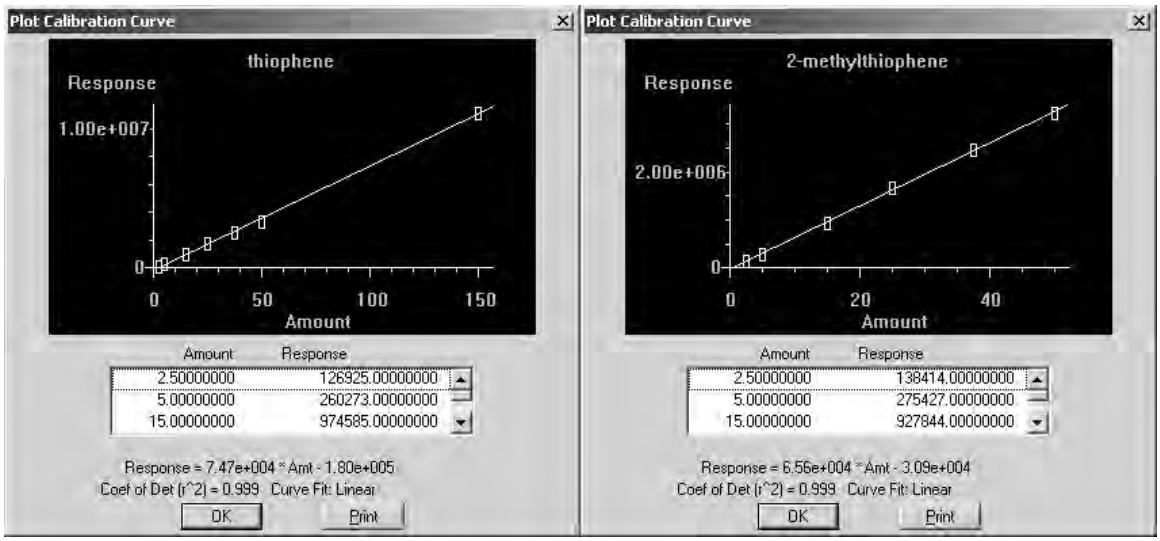

Figure 2. Calibration curves, thiophene and 2-methylthiophene in 3:1 isooctane:toluene.

Analytical Conditions

All analysis were performed using an Agilent 6890 gas chromatograph with split/splitless injector coupled to an Agilent 7500a ICP-MS with Shield-Torch system. GC-ICP-MS coupling was via the Agilent GC interface (model G3158A).

Results and Discussion

Sensitivity

The method as tested exhibits excellent sensitivity compared with other sulfur specific GC detectors such as the FPD, SCD, and AED. Single compound DL based on peak-to-peak signal-to-noise measurements for thiophene and 2-methylthiophene are in the 3–10 ppb range based on peak-to-peak signal-to-noise of 2:1. When translated to total sulfur in gasoline, assuming approximately 40 significant sulfur-containing compounds as detected by the ChemStation integrator, the DL for total sulfur is approximately 0.1 ppm to 0.5 ppm (Figure 6). This is in good agreement with visual inspection of overall signal-to-noise for the 55 ppm gasoline standard (Figure 3). In fact, examining the peak-to-peak signal-to-noise for 2-methylthiophene in the 50-ppm RFG standard gives a signal-to-noise of about 300:1 which is in agreement with the estimated DL of about 1/100 of the 55-ppm standard or 0.5 ppm. The analysis of certified, lower sulfur natural gasoline standards is needed in order to verify these assumptions about the absolute DL for total sulfur.

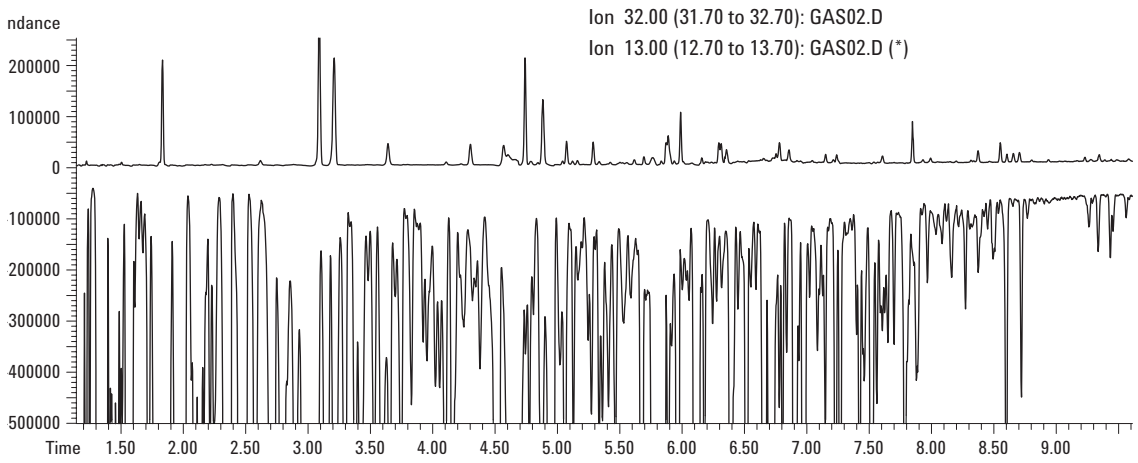

Figure 3. CARB low sulfur RFG with ~55 ppm total sulfur. Sulfur chromatogram (top), inverted carbon-13 chromatogram (below).

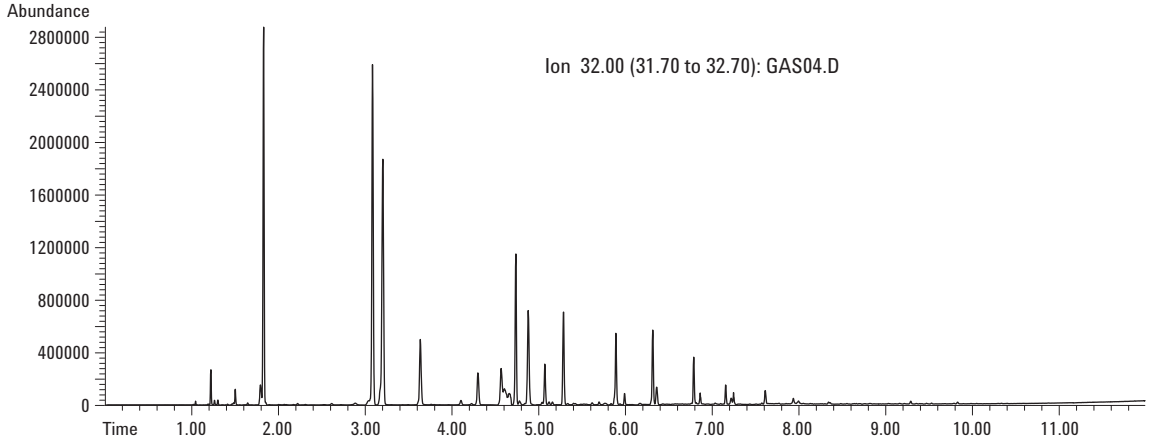

Figure 4. ASTM-Fuel-QCS-02, Conventional gasoline QC sample, ~330 ppm total sulfur.

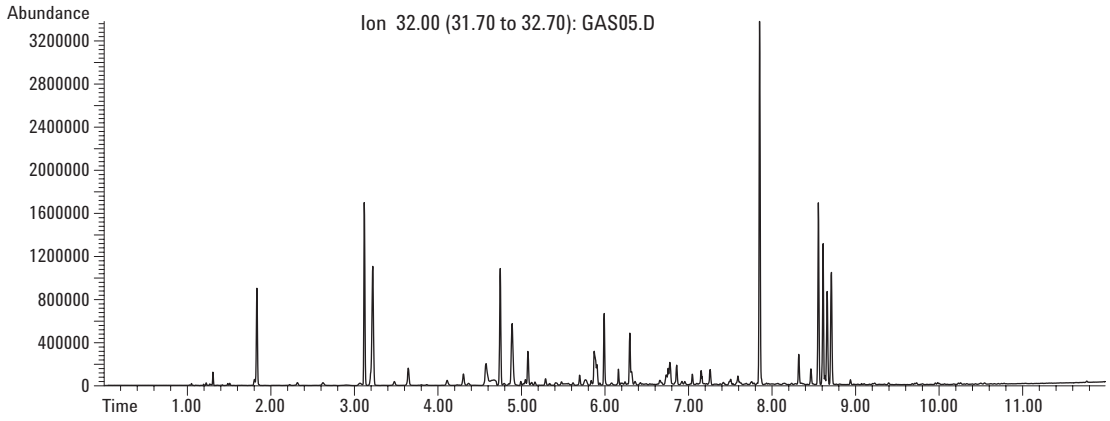

Figure 5. ASTM Round robin gasoline standard #2, not certified for sulfur.

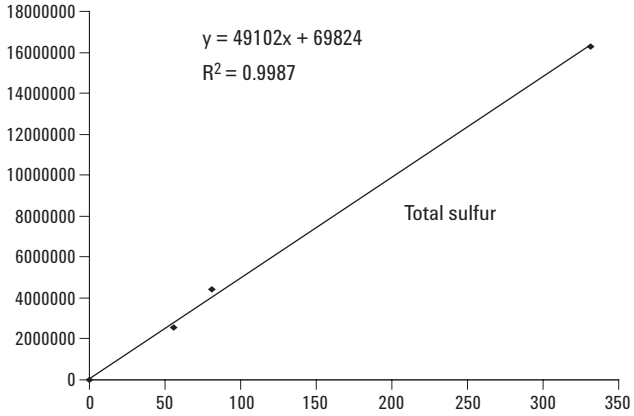

Figure 6. Calibration, total sulfur in gasoline calculated as area sum of sulfur-32 peaks.

Compound Independent Calibration

When calculated against thiophene using the area sum of all sulfur containing peaks (*m/z* = 32), the ASTM QCS02 conventional gasoline standard and CARB low sulfur RFG sample showed excellent recovery (see Table 2).

Advantages of ICP-MS

No Signal Suppression: In addition to excellent sensitivity and selectivity for sulfur, ICP-MS offers significant additional advantages over other detectors for the analysis of fuels. Because of the size and robustness of the ICP plasma, signal suppression of the analyte signal, in this case sulfur, by coeluting hydrocarbon compounds is virtually eliminated in gasoline samples. Figure 3 shows the sulfur peaks in the CARB low-sulfur RFG standard contrasted with the hydrocarbon peaks. The hydrocarbons are displayed as the C13 elemental chromatogram for scaling and dynamic range purposes. In this figure, the C13 chromatogram has been displayed inverted for ease of viewing. Note that while the C13 trace shows only 1% of the actual carbon abundance (C13/C12 ratio is 0.01), there is no apparent suppression of the sulfur signal. Very slight suppression was observed in the sulfur baseline when analyzing standards made up in a non-natural hydrocarbon solvent such as 3:1 isooctane:toluene. In this case, the entire carbon signal is concentrated into two very large peaks unlike natural fuels where each hydrocarbon peak is a much smaller fraction of the total carbon concentration. The slight depressions in baseline in Figure 7 immediately after thiophene and before 2-methylthiophene are due to suppression from isooctane and toluene respectively.

Faster Run Times: Since GC-ICP-MS does not suffer from significant analyte signal suppression due to coeluting hydrocarbons, the GC separation does not need to be compromised to separate analyte and hydrocarbon peaks. As a result, much faster run times can be achieved. Typical run times for sulfur species in gasoline by GC using conventional detectors are 25 to 30 minutes. In this work, the total run time was 12 minutes, with good separation of all compounds of interest (see Figure 8).

Table 2. Calculated Recovery of Total Sulfur in ASTM and CARB Gasoline Samples by Compound Independent Calibration Against Thiophene Standard

Sample	Area sum	Thiophene RF	Certified concentration	Measured concentration	% Recovery
ASTM QCS 02 gasoline	16299680	50958 cts/ppm	330 ppm	319 ppm	96.7
ASTM #2 Round robin gasoline	20050794	50958 cts/ppm	NA	393 ppm	NA
CARB low sulfur RFG	2755986	50958 cts/ppm	55	54.08 ppm	98.3

NA Not applicable

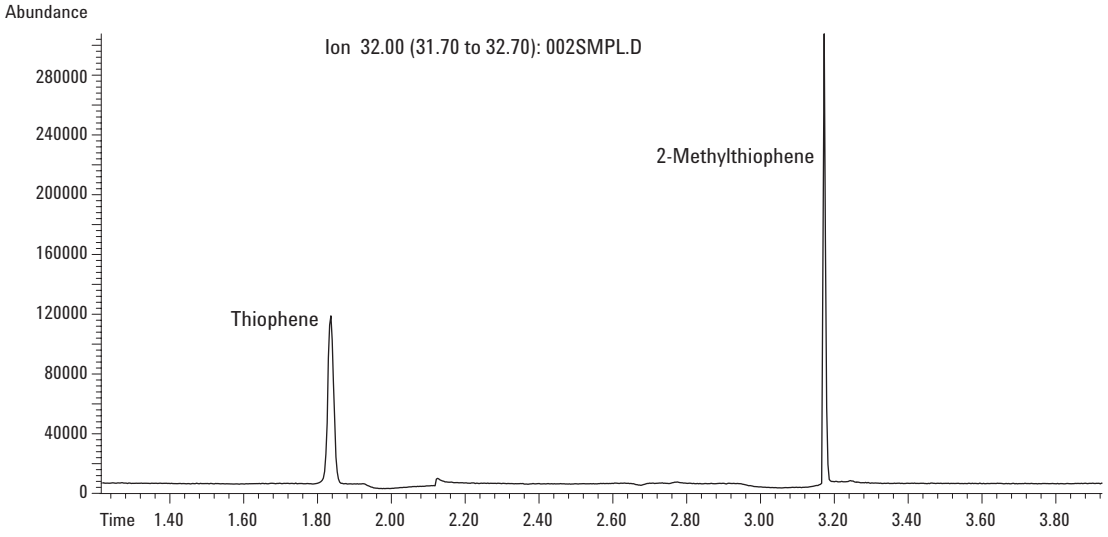

Figure 7. Calibration standard, 2.5 ppm each thiophene and 2-methylthiophene.

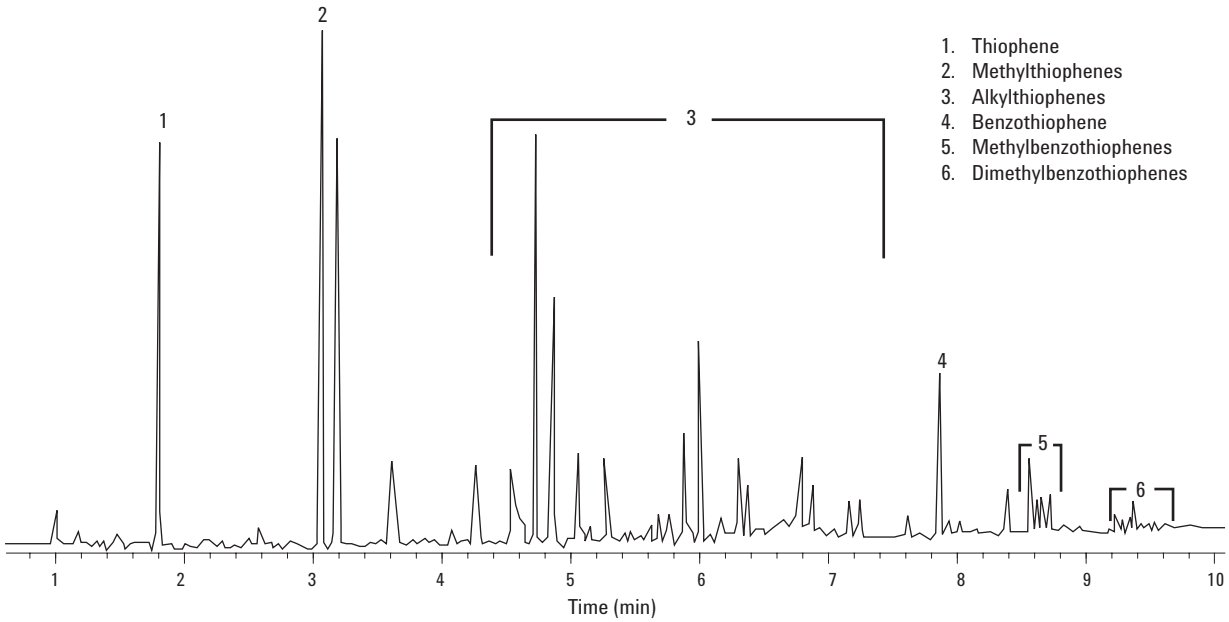

Figure 8. Typical distribution of sulfur containing compounds in low-sulfur RFG.

Additional Elements: Since ICP-MS is a scanning, elemental detector, the detection of additional elements such as vanadium, manganese, lead, mercury, or others can be performed simultaneously with DL similar to those for sulfur. This can give important additional information about fuel quality and process control without additional investment in time or equipment.

Ease of Use

Since the ICP-MS is actually designed to handle much higher matrix loads of aqueous liquid samples, it exhibits exceptional stability and robustness when used as a GC detector. As a result, the need for frequent tuning, cleaning and calibration is minimized and the use of internal standards is not necessary. The large dynamic range of the detector means that accurate quantification can be achieved over several orders of magnitude without the need for dilution or preconcentration of samples.

Conclusions

GC-ICP-MS offers a simple, sensitive, selective technique for the characterization of sulfur in fuels at the levels needed to meet new US and EU regulations. With DL for individual sulfur species in the low ppb range and total sulfur at less than 1 ppm, it is the only technique currently available which can measure both individual sulfur species and total sulfur at newly regulated levels. With the ability to do both, GC-ICP-MS can offer the hydrocarbon processor both regulatory compliance information and process control information simultaneously.

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Printed in the USA
August 1, 2003
5988-9880EN



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Optimizing the Agilent Technologies 6890 Series GC for High Performance MS Analysis

Technical Overview

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Abstract

Trace level GC/MS analysis requires a system that is performing at its best. Without a properly optimized GC, the mass spectrometer may not give the sensitivity expected. In other words, when more of the sample gets from the injection port to the ion source, the more likely a detector will produce a signal. Also, if the chemical noise from the GC is too high, the signal-to-noise ratio and ability to detect small analyte concentrations will be reduced. This note is a "how-to" guide for improving the GC performance. This will, in many instances, improve the overall performance of a GC/MS system. This guide is specific for the Agilent Technologies 6890 Series GC used with the Agilent Technologies 5973 MSD.

Supplies

In order to improve the system performance, some supplies should be on hand. These may not give significant improvements by themselves, but when installed together, they will give the best results. Many of the referenced supplies have changed over the past few years and will continue to be improved. It is important to stay informed and purchase the most recently updated versions of the consumables. Refer to www.agilent.com/chem for the latest comsumables available.

The Carrier Gas Line

The GC carrier gas should be at least 99.999% helium. Lower grades of helium are available and may contain impurities that can damage the GC column (for example, oxygen) and contribute to the chemical noise background. Even with a high purity gas there may be trace water, oxygen, and hydrocarbons. Putting a trap in the carrier line will eliminate these contaminants (see Figure 1). The mass spectrometer (MS) gas purifier trap is recommended and shipped with all new mass selective detectors (MSDs). It must be installed diagonally or vertically for optimum performance. Do not install it horizontally. High-purity helium also increases the effective lifetime of traps.

Precleaned, refrigeration grade 1/8-inch copper tubing should be used with high quality carrier gas. Other tubing can be cleaned by running solvents (methanol, ethyl acetate, hexane) through it in a water aspirator vacuum setup. The use of chlorinated solvents is not recommended due to

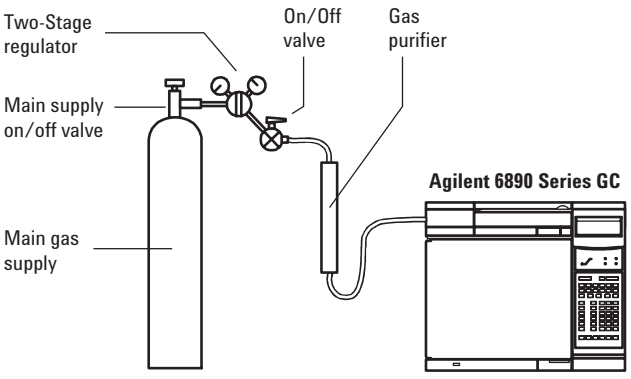

Figure 1. General gas plumbing assembly. The MS gas purifier must be installed diagonally or vertically.

possible long term contamination of flow lines and controllers. Another commonly used cleaning technique is to heat the copper tubing with a Bunsen burner, propane torch, or heat gun, while helium is flowing through the tubing. This is done after connecting the tubing to the helium supply but before connecting it to the GC. This process bakes off all the volatile contaminants. Take proper safety precautions while heating the tubing. Laboratory manifold systems, especially when new, tend to have hydrocarbon contaminants. Purging the new lines, before connecting the clean tubing to analytical instruments, is essential. The supplies needed for the carrier gas line are:

- ≥99.999% He: Gas supplier
- Clean copper tubing (50 ft): p/n 5180-4196
- Mass Spectrometer gas purifier: p/n RMSH-2
- Bracket to mount the gas purifier: p/n UMC-5-2

Splitless Inlet Consumables

The capillary inlet (Figure 2) has many consumable parts that should be kept on hand. Many of these consumables, such as liners (5), come in a variety of designs (Appendix A). The proper liner to use is dependent on the application. For trace level analysis, the single tapered, deactivated liner is recommended. The Viton O-ring (4) holding the liner in place should be replaced periodically to reduce the chance of leaking. The seal and the washer in the bottom of the injection port (10, 11) should be replaced whenever the reducing nut (12) is removed. The recommended seal is gold plated to reduce metal catalyzed thermal degradation of analytes. Septa (2) should be replaced quite frequently: for example, every 100 injections. The low bleed, precored, red septa should be used. Keeping a beaker of septa in an oven at 250 °C at all times will eliminate the need to condition the septum once it is in place. A Merlin Microseal™ is highly recommended over a conventional septum nut and septum (1, 2). The Microseal eliminates the need for septa and lasts for tens of thousands of injections without leaking. It is most appropriate with the Automatic Liquid Sampler (ALS) injection tower and only works with untapered, blunt tip syringe needles. The Microseal has been improved this past year. Older seals have a maximum pressure rating of 30 psi. The new seals are rated to 100 psi. The new seal and nut are recommended. The gold nuts (stamped “303C”) are not compatible with the new seals. A gray nut, stamped “221B,” should be installed.

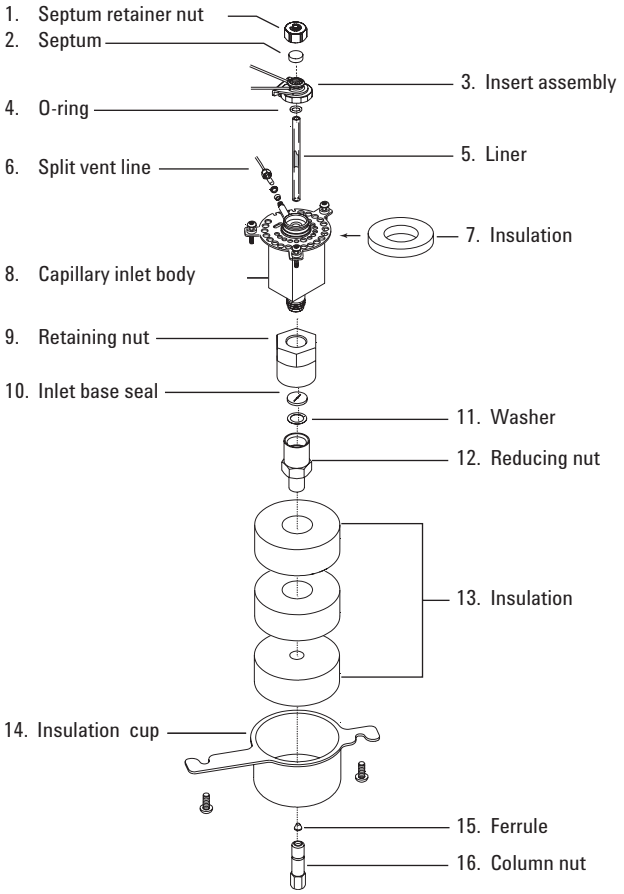

Figure 2. Capillary inlet assembly.

Electronic pneumatic control (EPC) is an integral part of the Agilent 6890 Series GC. The EPC version of the Agilent 6890 is required when using an Agilent 5973 MSD. The manual version of the Agilent 6890 will not work with the Agilent 5973 MSD. Electronic control gives the best repeatability in retention time and area counts. Using the electronic pulsed splitless mode allows for complete transfer of larger volume injections (up to 5 µL) onto the column. Even larger volume injections (>5 µL) may result in more inlet maintenance, especially with dirty samples.

The Agilent 6890 does have an inlet that accommodates injections up to 250 µL. It is called the programmable temperature vaporizer (PTV). It works with the ALS to deliver large volume injections (LVI). The inlet works by venting the solvent before analysis. The analyte is trapped and concentrated. It is then delivered to the column as a single plug.

The list of splitless inlet consumables is:

- Molded Septa (11 mm, red, 25/pk): p/n 5181-3383

Or

- High Pressure Merlin Microseal starter kit: p/n 5182-3442
- Merlin Microseal septum: p/n 5182-3444
- Merlin Microseal septum nut: p/n 5182-3445
- Liner, single taper, deactivated, no glass wool: p/n 5181-3316
- Viton O-ring (12/pk): p/n 5180-4182
- Gold plated seal: p/n 18740-20885
- Washer (to go with seal): p/n 5061-5869
- 10-µL Blunt needle syringe: p/n 9301-0713

Column Consumables

The optimal choice of column is once again dependent on the application. For trace-level, high-sensitivity applications, a column with a thin film and low bleed is best. A 30 m, 0.25-mm id, 0.25-µm film, 5% phenyl–95% methyl silicone column is a versatile column used for many applications. Special low-bleed MS columns cost more but give better results.

The proper column nut and ferrule combination are critical for a leaktight seal. Newer column nuts may not be compatible with all ferrules. The proper ferrule will be dependent on column outer diameter (od) and is specified below. The ferrule should only be slightly larger than the column od. The use of 100% graphite ferrules is not recommended, as they are easily over-tightened causing graphite to extrude into the injection port. This will be apparent when disassembling the injection port. If there are pieces of graphite in the bottom of the injection port, the ferrule(s) was/were overtightened. The presence of graphite in a hot injection port can cause thermally labile compounds to degrade. It can also affect the chromatography and cause tailing. Thus, 10% graphite, 90% Vespel ferrules are highly recommended. Vespel® ferrules will shrink as they are heated. Conditioning them for 4 hours in a 250 °C oven will preshrink them before use. Alternatively, the column nuts (Figure 2, number 16) can be retightened after the column oven cycles a few times.

The column, column nut, and ferrules supplies include:

- 30-m column, 0.25-mm id, 0.25 µm, low bleed (HP-5MS): p/n 19091S-433
- Column nuts (wrench-tighten only) 2/pk: p/n 5181-8830
- Ferrules for 0.2-mm id columns, 10/pk: p/n 5062-3516
- Ferrules for 0.25-mm id columns, 10/pk: p/n 5181-3323
- Ferrules for 0.32-mm id columns, 10/pk: p/n 5062-3514
- Ceramic scoring wafer (column cutter), 4/pk: p/n 5181-8836

A sharp column-cutting tool is needed for making clean cuts. The ceramic scoring wafers or sapphire square edge pens are desirable. The diamond point pens are harder to use. Ceramic scoring wafers are extremely sharp. They should be used with care. An X-ACTO® or Swiss Army knife is not a column cutting tool.

Use a 10× magnifier to assure that the cut is clean and no column shards are lodged inside the column.

Interfacing the Column to the MS

The column is connected to the MS through an interface that is sealed with a column nut and ferrule. The specific ferrule used depends on the column diameter. Never use a 100% graphite ferrule. Similar to the injection port, pieces of graphite may extrude into the interface and contaminate the MS. The ferrules required are 15% graphite, 85% Vespel. The column nut listed is brass; stainless steel nuts should never be substituted. Stainless nuts may damage the threads on the interface. Damaged threads cause air leaks and replacement of the entire interface.

The MS interface supplies include:

- Brass column nut: p/n 05988-20066
- Ferrules for 0.2 and 0.25-mm id columns, 10/pk: p/n 5062-3508
- Ferrules for 0.32-mm id columns, 10/pk: p/n 5062-3506

Installation of Consumables

This section assumes that you are going to perform preventative maintenance (PM) on your Agilent 6890 Series GC. If this is a new gas chromatography/mass selective detector (GC/MSD) system, many of these steps will be completed by a Customer Engineer during installation. Before beginning the PM, please read this section carefully. The necessary manuals will be referenced frequently. These manuals can be downloaded from our Web site at www.agilent.com/chem.

The manuals necessary include:

- Agilent 6890N GC User Information Manual: G1530-90210
- Agilent 6890 Series GC Service Manual: G1530-90220
- Agilent 5973 Series MSD Hardware Installation Manual: G2589-90006
- Agilent 5973 inert MSD Hardware Manual: G2589-90071
- Agilent 5973 Series MSD Site Preparation Guide: G2589-90070

With all of the consumable supplies previously mentioned at hand, a proper PM can be completed. To begin a PM, it is necessary to cool the GC zones (oven, inlet, MS interface). Vent the Agilent 6890 Series MSD. Please refer to the MS hardware manuals for venting instructions.

Installation of Gas Supplies

Follow the directions in the GC site preparation/installation manual and install the gas line supplies. Take care in making the Swagelok connections. The trap can break if too much stress is placed on the connection during tightening. Leak check all connections with a helium leak detector. (No Snoop please!) Make sure to purge all lines with helium before connecting them to the GC.

Installation of Capillary Inlet Supplies

Before handling any of the injection port supplies, wash hands and/or wear lint-free gloves. Oils on the hands will be transferred to these parts and become background in the system, requiring extra bakeout time. Following the instructions in the GC operating manual, remove the septum nut, septum, and liner. Discard the septum, liner, and liner O-ring. Open the oven door, loosen the 1/16-inch column nut, and remove the column and nut.

Remove the insulation cup and any necessary insulation (Figure 2, number 14) to provide access to the reducing nut (Figure 2, number 12). If the lower insulation cup was not in place, find it, because this piece improves the inlet temperature profile.

With a 1/2-inch wrench, remove the reducing nut (Figure 2, number 12). Due to heat cycling of the GC, the reducing nut will be very tight. Remove the seal and the washer (Figure 2, number 10, 11) and discard. Place a new washer in the reducing nut and a new seal (flat side with groove up). Hand-tighten the reducing nut back into place within the retaining nut and then wrench-tighten until very tight. Replace the insulation cup. Insert a new liner and O-ring. The single taper liners are installed with the taper down, toward the column end of the inlet. Hand-tighten the insert assembly (Figure 2, number 3). Add the Merlin Microseal or proper preconditioned septum and septum nut. The molded septum is installed with the hole up. Follow the directions supplied with the Merlin Microseal to insure proper installation. If the green septum nut is used, wrench-tighten the weldment and septum nut with the septum nut wrench until the C-ring lifts off the top of the green septum nut.

At this point, the inlet should be leak checked. Follow the directions in the GC maintenance and troubleshooting section of the manual.

Column Installation

Working with fused silica columns may be dangerous. Wear proper eye protection. Inspect the column for damage or breakage. Unweave 0.5–1 coil of the column from its basket to make it easier to install. Push a septum onto the inlet end of the column about 10 cm. Put the column nut and appropriate ferrule on the column. Cut 5–10 cm off the inlet end of the column. Check the cut with a 10× magnifier; the cut should be straight, not jagged, with no column shards within the column. If the cut is jagged or shards are inside, try again. After a clean cut is obtained, mark the proper column position with the septum (Figure 3). The septum will hold the column nut and ferrule in place. Place the column on the column hanger. Insert the column nut into the inlet reducing nut and finger-tighten. Wrench-tighten the column nut. The column should be stationary in the ferrule. Carefully slide the septum down away from the nut without disturbing the column positioning. The septum can be left in place if desired. Using the GC keypad or the MS ChemStation software, input the

Table 1. Head Pressures and Calculated Flowrates for a Splitless Inlet at an Oven Temperature of 25 °C with the Outlet Pressure set to Vacuum

Column id (mm)	Length (meters)	Head pressure (psi)	Linear velocity (cm/s)	Column flow (mL/min)
0.20	12	6.0	57	1.0
0.20	25	15.0	39	1.0
0.20	50	28.0	28	1.0
0.25	30	6.2	36	1.0
0.32	30	3.4	50	2.0
0.32	50	5.5	34	1.5

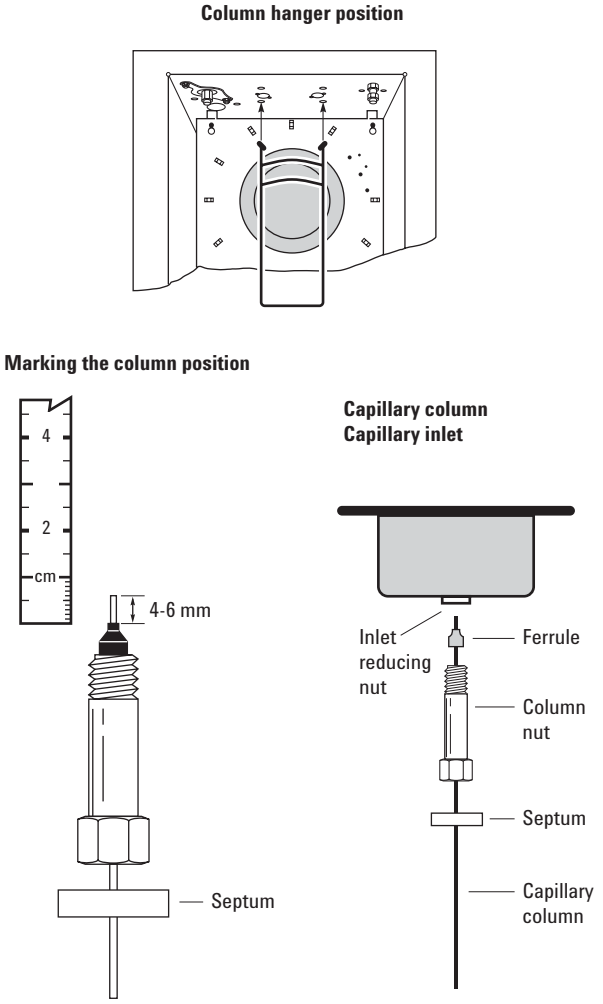

Figure 3. Proper installation of capillary columns in a capillary inlet.

Column Dimensions, set Outlet Pressure to Vacuum and Column Flow between 1 and 1.5 mL/min helium (Table 1). The Split Vent Flow should be approximately 50 mL/min. These parameters are accessed through the inlet and column screens. Place the detector end of the column into a beaker of water and check for bubbles to show helium flow. Heat the injection port. When the injection port reaches the setpoint temperature, retighten the column nut in case it loosened. Check once again for column flow. Remove the end of the column from the beaker and close the oven door. Condition the column by slowly (5 °C/min) ramping it to its maximum operating temperature. Leave it at that temperature for least 2 hours; overnight is preferable. The maximum operating temperature for an HP-5MS column is 325 °C. Cool the oven to ambient and insert the interface nut and ferrule onto the column. Properly cut off 5–10 cm of the column. Properly place the column into the interface by following the directions in the MS hardware manual for the Agilent 5973 and for the Agilent 6890 series MSD. Hand-tighten the interface nut and then wrench-tighten the nut. The nut should be tightened only until you hear two squeaks. This is a firm seal. Pump down the detector as directed by the MSD manual (for the Agilent 5973 and for the Agilent 6890 Series MSD). Keep the oven at ambient temperature until the source is hot. Check the interface connection after the interface is heated. The interface nut may need additional tightening.

Tips for Better Method Performance

Numerous splitless parameters need to be optimized for the best splitless injection. Those parameters include:

- Injection port temperature
- Column flow
- Liner design
- Solvent
- Sample volume
- Sample volatility
- Splitless valve time
- Injection speed

The proper inlet temperature is needed to evaporate high boiling point compounds without thermally degrading other compounds. Normally, the inlet temperature is a compromise between these two factors. A good starting point is 250 °C.

Liner design is one of the most difficult choices simply because of the variety of liners available. The features that are most important in a liner are the volume, whether it is deactivated or not, and whether or not it contains deactivated glass wool. As a general choice for high sensitivity work, a 4-mm single tapered, deactivated liner with no glass wool is recommended. For large volume injections (≥2 µL) and for the highest repeatability (especially with small volume injections of ≤0.5 µL [1]), deactivated glass wool is necessary. For dirty samples, deactivated glass wool helps to keep the nonvolatiles from getting to the column, but too much deactivated glass wool can greatly decrease sensitivity. Often, the most appropriate liner must be determined through experimentation. Please note: Removing and/or breaking deactivated glass wool creates active sites.

Splitless valve timing is critical. The ON time (splitless mode) should to be long enough to assure that all of the injected sample reaches the column.

A textbook splitless injection has the liner volume swept at least two times (during the "ON" time). A 4-mm liner has an approximate volume of 1 mL. With a GC/MS flow rate of 1 mL/min, a 2-min splitless injection would be necessary.

However, this long splitless time has not been common. There are two reasons for this:

- Conventional (manually controlled) capillary inlets were pressure regulated (constant pressure, regardless of oven temperature) and not flow regulated (changing pressure with oven temperature), so a higher-than-optimal flow was set initially so that the flow did not go to zero at high oven temperatures. Thus, a typical splitless or "OFF" time has been between 0.5 to 1.5 min.
- Liner volumes smaller than the textbook examples have typically been used. Since a 2-mm liner (250 µL volume) was more commonly employed, the splitless time was proportionally shorter.

Finally, with the programmable control afforded by EPC, flows can be reliably pulsed during the injection process. With pulsed splitless injections, flows during the splitless time can be 2-6 mL/min, resulting in splitless times less than 2 min for a 4-mm id liner. The pulsed splitless injection mode on the Agilent 6890 is recommended for GC/MS work. After the injection pulse, the system returns to analytical flow rates of 1–4 mL/min He. The highest flow allowable depends on the MSD. Refer to the appropriate MS hardware manual for your detector's limit.

Unless all analytes have high boiling points, the initial oven temperature should be set to take advantage of the solvent effect. The solvent effect focuses the analytes on the head of the column. The oven temperature should typically be set to ≥10 °C below the boiling point of the solvent used (Table 2). On the other hand, the MS interface temperature should be hot enough to avoid loss of analytes on cold spots. The interface should be set to the maximum oven temperature for the analysis or 10 °C–15 °C higher if the upper temperature limit for the column is not exceeded. The default method temperature is 280 °C; the interface temperature should be optimized as part of method development.

Finally, the rate of auto-injection of a sample has been studied for splitless injections. It has been found that fast injections, such as with the ALS, tend to give the most repeatable and non-discriminating results.

Table 2. Boling and Initial Oven Temperatures for Common Solvents

Solvent	Boiling point (°C)	Initial oven temperature (°C)
Diethyl ether	36	10 to 25
n-Pentane	36	10 to 25
Methylene chloride	40	10 to 35
Carbon disulfide	46	10 to 35
Acetone	56	25 to 45
Chloroform	61	25 to 50
Methanol	65	35 to 55
n-Hexane	69	40 to 60
Ethyl acetate	77	45 to 65
Acetonitrile	82	50 to 70
n-Heptane	98	70 to 90
Isooctane	99	70 to 90
Toluene	111	80 to 100

Using Pulsed Splitless Injections

Pulsed splitless injections are the best way to do splitless injections. EPC of the splitless inlet allows for high flow rates initially, followed by more typical GC/MS flow rates. A pulsed splitless injection transfers more of the sample onto the column and allows for increased injection volumes up to 5 µL. When the injected volume is flash vaporized, the required expansion volume for the solvent is greatly increased (solvent choice also effects expansion volume). The use of higher initial inlet pressures reduces the volume ($P_1V_1 = P_2V_2$) so the entire injected volume can move to the column. The higher pressure also decreases the likelihood that highly volatile compounds will escape out the top of the injection port through the septum purge vent (Figures 4 and 5). In the case of thermally labile compounds, the faster they leave the hot injection port the less likely they are to degrade [2, 3]. The flow rate is then reduced to the value desired for the chromatographic separation. This flow is held constant by increasing the pressure as the oven temperature increases. Figure 6 is a graphical representation of the pulsed splitless technique with constant flow. Pulsed splitless injections should always be used when sensitivity and/or repeatability are critical. Refer to the GC operating manual for how to set up a pulsed splitless injection.

Electronic pressure and flow control of carrier gases not only help with larger volumes, they also help to decrease run times and maintain stable MS sensitivity by keeping the carrier gas flow constant. These lead to shorter analyses, higher sensitivity, and higher reproducibility. The benefits for the analyst are more chromatographic analyses per shift, better data, and higher revenues per instrument.

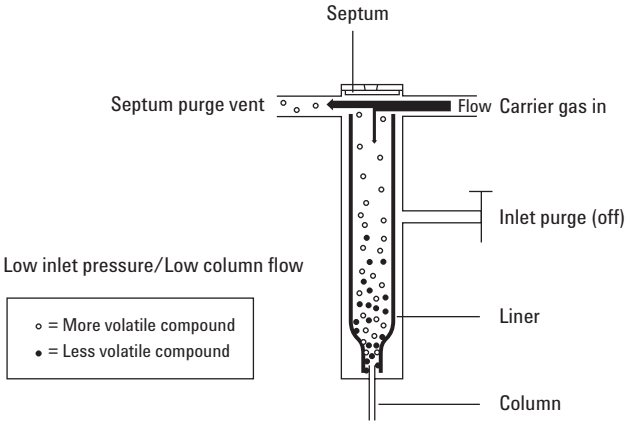

Figure 4. A low initial inlet pressure causes loss of volatile compounds.

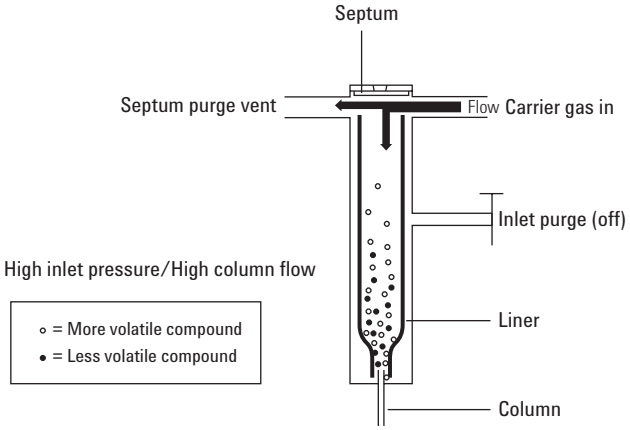

Figure 5. With the correct inlet pressure there is no loss of volatile compounds.

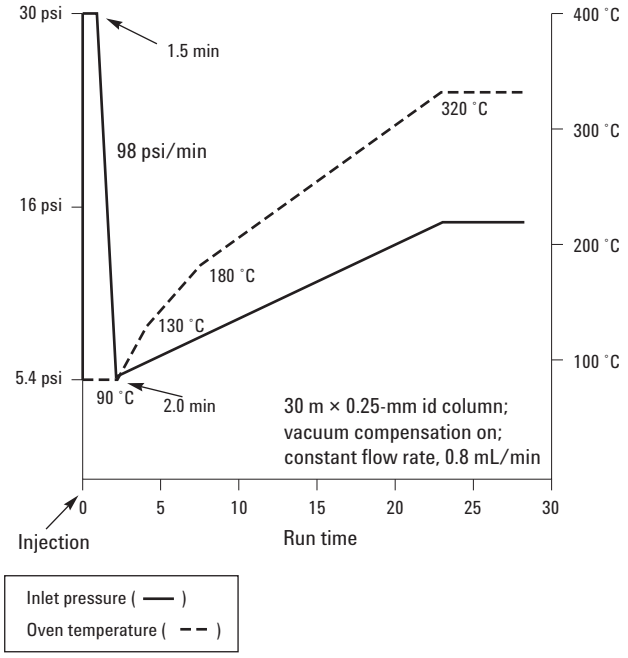

Figure 6. Pulsed splitless injection technique employing constant flow with EPC. This technique allows larger injection volumes and inhibits the loss of volatile compounds out of the septum purge vent.

Summary

Following the instructions in this guide will improve analytical results with your GC/MSD system. Contamination interferring with the determination of your analytes will be minimized and sample transfer optimized, both improving sensitivity.

A final note: the choice of vials and septa will affect your results. Screw cap vials are not recommended for GC/MS analyses. Application note "Effects of Vial Septa Used in GC/ECD Analysis of Trace Organics" Agilent Technologies publication 5091-8980E www.agilent.com/chem will help you make the right choice.

References

1. "Analyzing aromatics in reformulated gasoline by GC/MS", Agilent Technologies, publication (23) 5964-0116E.
2. Philip L. Wylie et al. Improving the Analysis of Pesticides by Optimizing Splitless Injections. 1995 Pittsburgh Conference paper number 347.
3. Philip L. Wylie and Katsura Uchiyama. Improved Gas Chromatogaphic Analysis of Organophoshorous Pesticides with Pulsed Splitless Injection. (1996) *JAOAC* **79**: 571-577.

Appendix A

Capillary Inlet System Liners

Liner	Part no.	Price	Configuration: ID volume	Glass type	Deactivated	Glass wool packing**	Applications: Fast injection (7673 ALS) Split	Fast injection (7673 ALS) Split-less	Slow & manual injection Split	Slow & manual injection Split-less	Linear seals: Fluoro-carbon (Max 350 °C)	Linear seals: Graphite (350 °C and above)
Single-Taper liner	5062-3587	34	4 mm (0.8-mm end) 900 L	Borosilicate	YES	YES**	C	●	C	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Single-Taper liner	5181-3316	31	4 mm (0.8-mm end) 900 L	Borosilicate	YES	NO	—	A	—	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Double-Taper liner	5181-3315	34	4 mm (0.8-mm end) 800 L	Borosilicate	YES	NO	—	A	—	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Capillary liner	19251-60540	26	4 mm 990 L	Borosilicate	NO	YES**	●	D	●	D	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Split liner	18740-60840	46	4 mm with cup	Borosilicate	NO	YES + column packing	—	—	E	—	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Split liner	18740-80190	41	4 mm with cup	Borosilicate	NO	NO	—	—	●	—	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Splitless liner	18740-80220	27	2 mm 250 L	Quartz (Purity 8)	NO	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Splitless liner	5181-8818	30	2 mm 250 L	Quartz (Purity 8)	YES	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Direct liner	18740-80200	17	1.5 mm 140 L	Borosilicate	NO	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48

● General recommendation

○ See notes at left regarding use

* Quality discounts available; please inquire.

** Silanized glass wool, 10 gm, (pesticide grade) (Agilent p/n 5181-3317).

A. Can be used without the glass wool in some applications, but liners with glass wool are generally recommended for best reproducibility in fast injections.

B. Recommended only for small (< 0.5 µL) volumes, depending on solvent and conditions.

C. The glass wool packing as supplied may not provide adequate mixing for good precision in split injections. Liners can be packed with silanized glass wool positioned as in the straight 4-mm capillary liner, part number 19251-60540.

D. Taper liners are recommended for best performance in this application, particularly with labile samples and wide-boilingrange mixtures.

E. Not recommended for use with EPC.

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Printed in the USA
November 14, 2003
5988-9944EN



Agilent Technologies

Improved Performance for the Analysis of Aromatics in Gasoline by ASTM Method D5769 Using the Agilent 5973 inert Gas Chromatography/Mass Spectrometry System

Application

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Abstract

The new Agilent 5973 inert gas chromatography/mass spectrometry system can be used to measure high concentrations of aromatic compounds in gasoline using American Society of Testing and Materials Method D5769. This system provides improved linear calibrations when equipped with the optional extended linearity ion source kit. This makes Method D5769 easier and faster to set up, with more reliable results. The Agilent 5973 inert with the extended linearity source was also shown to quickly and easily pass the D5769 method performance tests for resolution, sensitivity, and spectral integrity.

Introduction

The levels of aromatic compounds in gasoline are adjusted upward or downward by refiners to comply with environmental regulations and to improve the properties and performance of the finished product. Due to the complex hydrocarbon matrix, it is difficult to chromatographically separate all aromatics from the other nonaromatic hydrocarbons. The American Society of Testing and Materials (ASTM International) has developed Method D5769 that uses gas chromatography/mass spectrometry (GC/MS) to quickly separate, identify, and quantitate benzene, toluene, other specific and total aromatic hydrocarbons in gasoline [1]. This method is designed to measure these compounds between 0.1 and 42 weight percent.

One difficulty encountered when using Method D5769 is obtaining linear calibrations for compounds at concentrations higher than 10 weight percent. Modern GC/MS systems are designed to provide extremely low analytical sensitivity (ppm to ppb), with linear response over several orders of magnitude. However, when trying to analyze compounds at percent levels, the mass spectrometer electron ionization (EI) source can be saturated with sample, resulting in a nonlinear response over a relatively narrow concentration range.

There are a number of remedies to this issue that include custom mass spectrometer tuning, time programmed interface splitters, very small injections (<1 µL), very large split ratios (>1500:1), nonlinear curve fitting, and a specialized inlet pressure program [2]. These can be used individually or in combinations to meet the calibration requirements of the ASTM method. However, these approaches significantly add to the complexity of a method that is already very complex to successfully set up and run. The best solution to a detector response problem would be to have an EI source that is designed specifically for enhanced linearity.

The new Agilent Technologies 5973 inert GC/MS system has an EI source, constructed of a solid inert material, to provide high sensitivity for difficult samples containing pesticides, nitrophenols, and active sulfur compounds. At first glance, an instrument with this degree of sensitivity would not have the required linear dynamic range necessary to analyze aromatics in gasoline using Method D5769. However, the Agilent 5973 inert GC/MS system offers an optional extended linearity source kit that provides greater linear dynamic range for major compound analysis. This application note will demonstrate the improved performance of Method D5769 using this system.

Experimental

The Agilent 5973 inert GC/MS system components used for this work are outlined in Table 1. Analysis conditions were set exactly as described by Method D5769 and are shown in Table 2. The ChemStation Autotune program was used to tune the Agilent 5973 inert. No changes were made to the electron multiplier voltage obtained by Autotune.

Method D5769 calibration standards were obtained from Accustandard (125 Market Street, New Haven, CT, USA). These standards contained all 23 aromatic compounds specified by the ASTM method along with four deuterated internal standards; benzene-d6, toluene-d9, ethylbenzene-d10, and naphthalene-d8. The calibration standards were used to construct a five-point calibration curve using the quantitation ions specified for each compound by the ASTM method. Other system performance tests for chromatographic resolution, mass spectral integrity, and sensitivity were performed using samples obtained from Accustandard that met ASTM Method D5769 requirements.

Table 1. Agilent 5973*inert* Hardware Configuration for ASTM Method D5769

Agilent 5973*inert* GC/MS hardware	
G1778A	5973*inert* with standard turbo pump and ChemStation
G2589-20045	Ultra large aperture drawout plate for extended linearity source
G1942N	6890N GC with capillary inlet and mass spectrometer direct capillary interface
G2613A	Agilent 7683 Automatic Liquid Injector (ALS) with nanoliter adapter
G2614A	100-sample tray for ALS
Columns	
Capillary column	1 µm film HP-1, 60 m × 0.25 mm id (Agilent part number 19091Z-236)
Consumables	
Syringe	10 µL fixed needle (Agilent part number 5181-1267)
Inlet liner	Split optimized, low pressure drop liner (Agilent part number 5183-4647)

Table 2. Conditions for the GC/MS Analysis of Aromatics in Gasoline by ASTM Method D5769

GC Conditions	
Carrier gas	Helium at 28.5 psig constant pressure mode 35 cm/s average linear velocity at 50 °C
Inlet	Capillary at 250 °C 250:1 split ratio
Oven temp	60 °C (0 min) 3 °C/min to 120 °C (0 min) 10 °C/min to 250 °C (0 min)
Injection size	0.2 µL using a 10-µL syringe
Mass Spectrometer Conditions	
GC/MS Interface	Direct capillary at 280 °C
Ion source	250 °C
Quadrupole	150 °C
Solvent delay	3 min.
Electron energy	70 eV
Emission current	35 amps
Scan range	45 to 300 Daltons
Sampling rate	2^3 A/D
Scan speed	2.89 scans/s
Autotune	Standard

Results and Discussion

Since ASTM Method D5769 is a complex application, there are a number of system measures that must be met before calibration and sample analysis. First, the chromatographic resolution was tested under normal operating conditions using 1-methyl-2-ethylbenzene and 1,3,5-trimethylbenzene at 3 mass % each. The resolution between these two peaks must be greater than 2. Figure 1 shows that the resolution between these two compounds was 6.5 using the Agilent 5973 inert system, therefore exceeding the method requirements.

For a 0.01 mass % sample of 1,4-diethylbenzene, the signal-to-noise ratio must be at least 5 at mass 134. This sample was run with the Agilent 5973 inert system and the signal-to-noise ratio (peak-to-peak) was found to be 21.

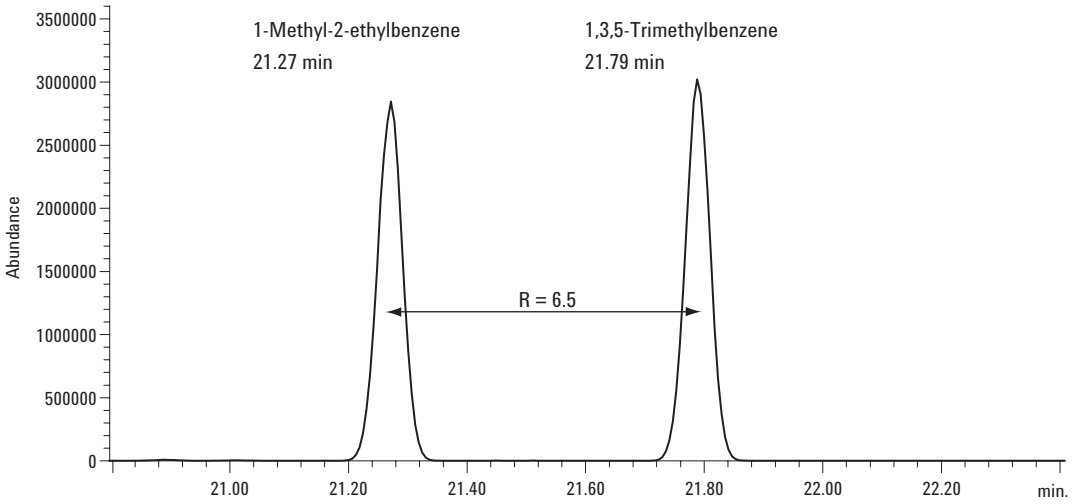


Figure 1. The chromatographic resolution between 1-Methyl-2-ethybenzene and 1,3,5-Trimethylbenzene exceeds the minimum requirement of 2.0 specified by ASTM method D5769.

The mass spectral integrity performance was measured by running a 3 mass % sample of 1,2,3-trimethybenzene. The spectrum of this compound must meet certain mass and relative abundance criteria to assure that the instrument is properly tuned and functioning. Figure 2 shows that this test was successfully met using the enhanced linearity Agilent 5973 inert.

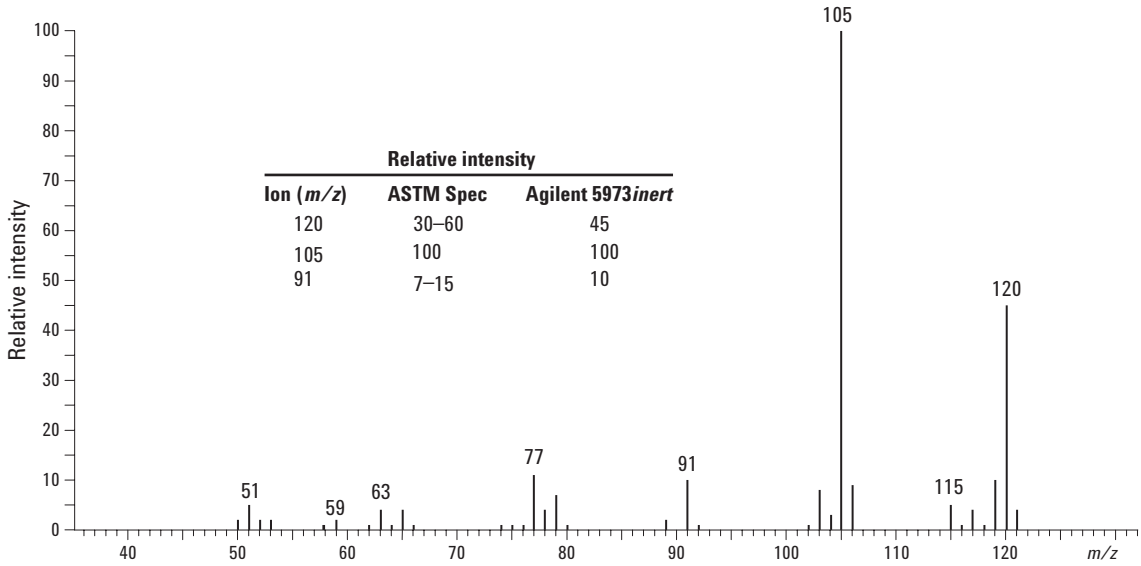


Figure 2. For a 3 mass % of 1,2,3-trimethylbenzene sample, the spectral integrity requirements of the ASTM D5769 method are met using the Agilent 5973*inert* enhanced linearity source.

Calibration of the system was performed with standards containing 23 analytical compounds and four deuterated internal standards. The resulting five-point calibration curves for each aromatic compound spanned concentrations in gasoline of 0.1 to 42 weight percent. Figure 3 shows a total ion chromatogram (TIC) for one of these standard mixes.

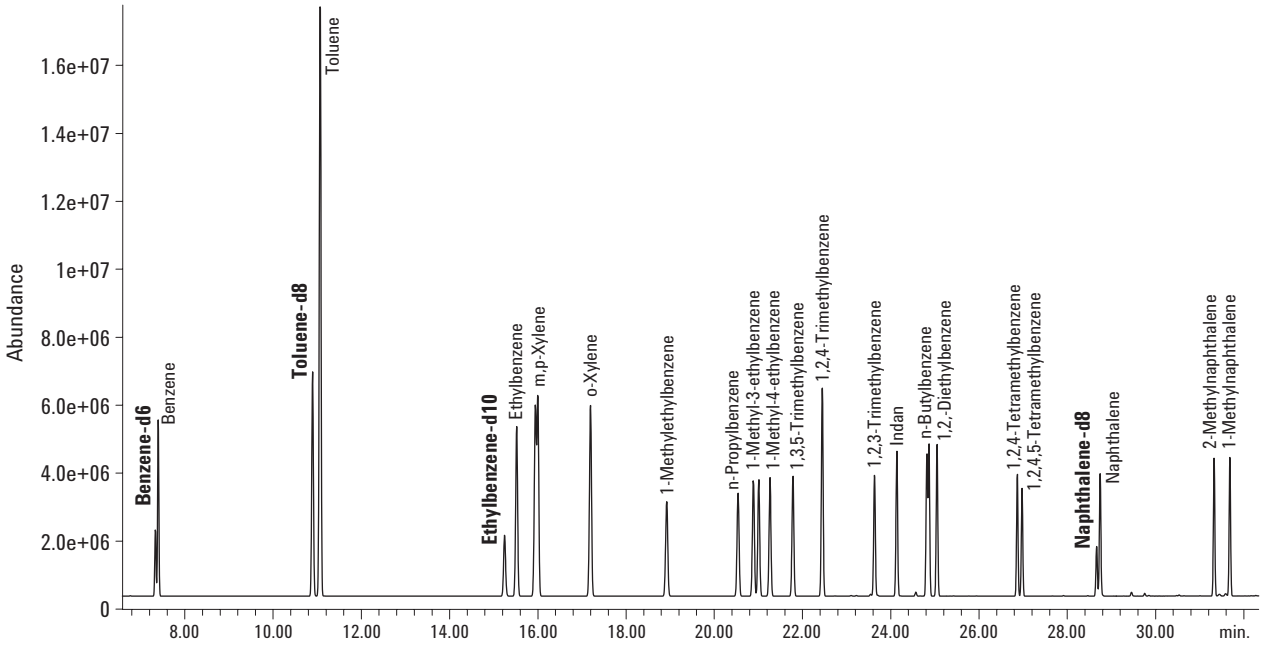


Figure 3. TIC of a calibration standard containing all 23 analytical compounds and four internal standards for the analysis of aromatics in gasoline.

Reconstructed ion chromatograms (RIC) were made from characteristic ions for each calibrated compound. These RICs were integrated and the responses used to construct the calibration curves for each compound. Using conventional, high-sensitivity ion sources, the high-concentration standards can saturate the ion source, resulting in a "flat" or non-linear response for that compound. This effect is shown in Figure 4 for a 10 weight percent standard of toluene. In the upper chromatogram, the toluene RIC peak (*m/z* 91) shows a "flat" top, indicating that the ion source contains too much toluene. However, by simply switching to the Agilent 5973 inert extended linearity source, the same toluene standard has the expected peak shape and response. This is shown in the lower chromatogram of Figure 4.

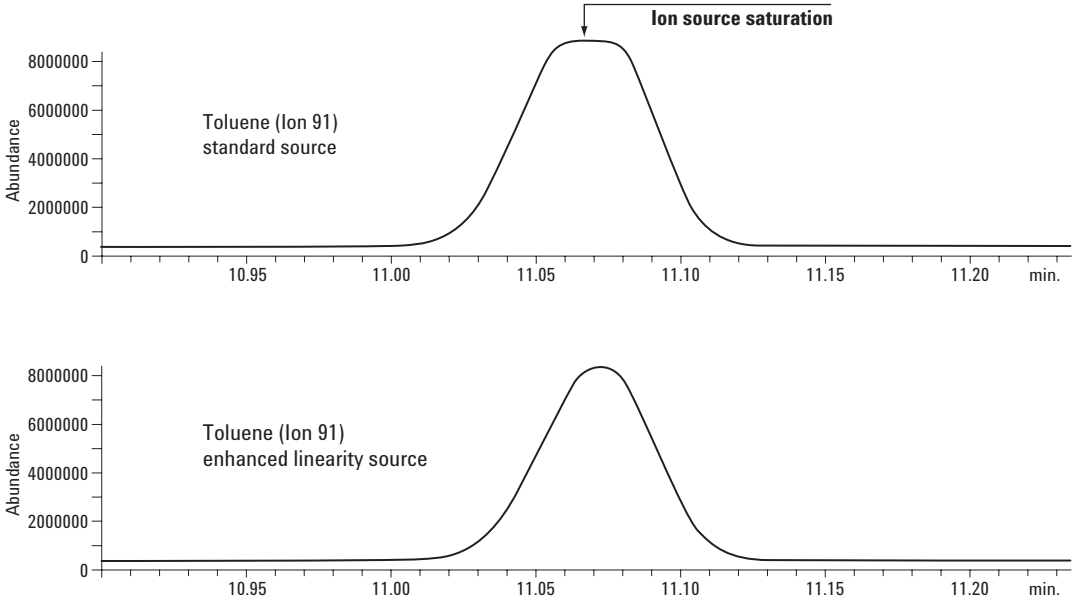
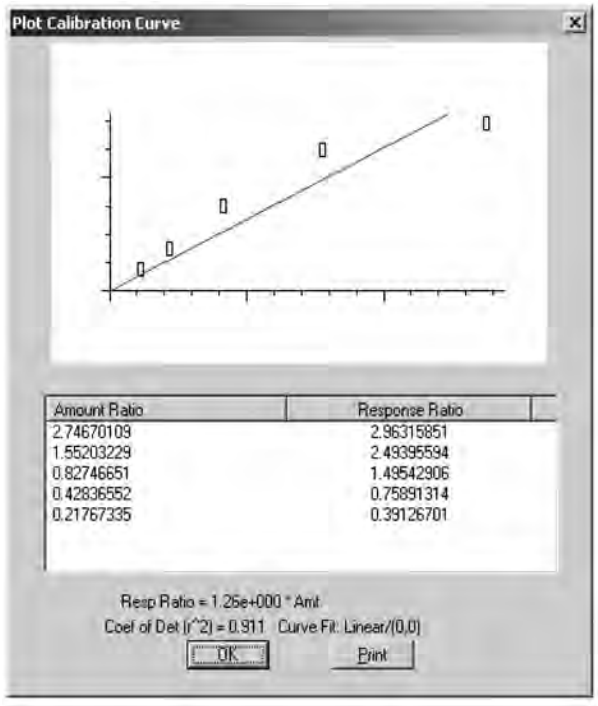


Figure 4. RIC of *m/z* 91 for the analysis of toluene. The upper chromatogram was obtained with the high sensitivity source of the Agilent 5973*inert* system. The lower chromatogram shows the same standard run using an Agilent 5973*inert* equipped with an enhanced linearity source.

The result of this ion source saturation was calibration curves that do not meet the ASTM Method D5769 linearity specification. This problem was completely overcome when using the Agilent 5973 inert extended linearity source. Figure 5 shows a comparison of toluene calibration for the two different source designs on the Agilent 5973 inert.

A. Standard Agilent 5973*inert* source



B. Enhanced linearity Agilent 5973*inert* source

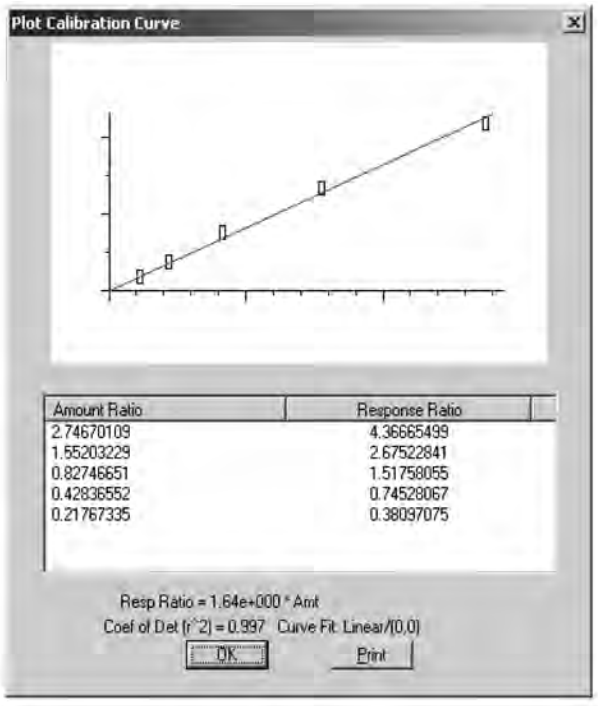


Figure 5. Two toluene calibrations for ASTM Method D5769. A) the standard source does not meet the ASTM method requirements for linearity. B) using the enhanced linearity source, the requirements are easily achieved for toluene.

Conclusion

The new Agilent 5973 inert equipped with the optional extended linearity source kit was shown to meet or exceed all performance measures for the analysis of aromatics in gasoline by ASTM Method D5769. By quickly and easily obtaining linear calibration curves for percent level components, analysts using this method do not have to apply complex remedies such as specialized tuning, time programmed interface splitters, very small injections, very large split ratios, non-linear curve fitting, and specialized inlet pressure programs. More time can be spent running samples and producing analytical results.

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Printed in the USA
October 13, 2003
5989-0093EN



Agilent Technologies

Determining Aromatics in Finished Gasoline Using the Agilent 6820 GC System

Application

Gasoline Analysis and Environmental Protection

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Abstract

A two-column Agilent 6820 gas chromatograph (GC), equipped with two flame ionization detectors (FID) and a 10-port valve, is used for the analysis of benzene, toluene, ethylbenzene, p/m-xylene, o-xylene, C9 and heavier aromatics, and total aromatics in finished gasoline. The internal standard used is 2-hexanone. This method requires two runs; the first is for benzene and toluene, and the second is for ethylbenzene, p/m-xylene, o-xylene, the C9 and heavier aromatics.

Introduction

Benzene and aromatics are classified as carcinogens. Regulations limiting their concentration in finished gasoline are established worldwide [1]. In order to assess product quality and to meet the clean fuel regulations, it is necessary to create the required analytical methods for benzene and other aromatics. There are several methods available for the analysis of aromatics in gasoline. One method employs a single polar HP-INNOWax capillary column [2], and another uses a single nonpolar capillary column [3]. The latter method takes 90 minutes. The American Society for Testing and Materials (ASTM) has published analytical methods for the analysis of aromatics in gasoline. ASTM D5769 [4] employs gas chromatography/mass spectroscopy (GC/MS) for the analysis of benzene, toluene, and total aromatic content in gasoline. ASTM D3606 [5] is a GC method for the analysis of benzene and toluene in finished motor and aviation gasoline, but this method is unable to analyze for C8 or heavier aromatics. ASTM D5580 [6] is another GC method for determining benzene, toluene, ethylbenzene, p/m-xylene, o-xylene, C9, heavier aromatics, and total aromatics in finished gasoline.

This application follows the ASTM D5580 method but is run using an Agilent 6820 GC. Another Agilent application note [8] cites that ASTM D4815 [7] and D5580 [6] were run on a single Agilent 6890N GC system.

Experimental

The configuration used for this application is listed in Table 1. A column micropacked with 1,2,3-*tris*-2-cyanoethoxypropane (TCEP) is used for preseparating aromatics from nonaromatics in the same boiling point range. A nonpolar HP-1 column is used for separating the aromatics.

Table 1. Configuration

Hardware and software		
G1180A	6820 GC	1
161	Split/Splitless inlet	1
215	Flame ionization detector (FID)	2
302	One manual controller	1
751	Installation and automation of one valve	1
800	10-port switch valve	1
872	Zero dead volume (ZDV) connection of a capillary column to a valve	2
870	Adjustable restrictor	1
874	Install valve downstream from the capillary inlet capillary inlet	1
335	Cerity NDS software	1
Columns		
19095Z-623	Capillary column: HP-1, 30 m, 0.53 mm, 5-µm	1
	micropacked column: 56 cm × 1/16-inch TCEP (20%)	1
Standard		
	Aromatics standards	

Method Description

This method requires two runs for a complete aromatics analysis, as described below:

First Run

The sample containing the internal standard (ISTD) (2-hexanone) is injected into a precolumn containing micropacked polar TCEP. With the valve in the "OFF" position, C9 and lighter nonaromatics elute from this column and are vented. FID B is used to monitor this separation. See Figure 1A.

Before the elution of benzene from the TCEP column, immediately switch the valve to the "ON" position (switch valve at T_1). This backflushes the TCEP column and transfers the remaining compounds to the nonpolar HP-1 column. Benzene, toluene, and the ISTD elute in boiling point order and are detected by FID A. See Figure 1B.

After elution of the ISTD from the HP-1 column, switch the valve to the "OFF" position (switch valve at T_3). The HP-1 column-flow is now reversed to backflush the remainder of sample components (C8 and heavier aromatics, plus C10 and heavier nonaromatics) to FID A. See Figure 1C.

Second Run

The run is repeated a second time, allowing the C12 and lighter nonaromatics, benzene and toluene to elute from the TCEP column to the vent. FID B is used to monitor this separation. See Figure 1A.

Before the elution of ethylbenzene, immediately backflush the TCEP column, allowing the remaining sample components to enter the HP-1 column (switch valve at T_2). The ISTD and C8 aromatics are thereby separated in boiling point order and are detected by FID A. See Figure 1B.

After the elution of o-xylene from the HP-1 column, immediately reverse the flow (switch valve at T_4) to backflush C9 and heavier aromatics to FID A for detection. See Figure 1C.

Instrument Conditions

The instrument conditions are listed in Table 2.

Table 2. Instrument Conditions

Inlet	Split/Splitless, 200 °C, split ratio: 11:1
Detector	FID, 250 °C, makeup: N_2,15 mL/min
Oven program	50 °C (10 min), 2 °C/min, 120 °C
Valve temperature	80 °C
HP-1 column flow	N_2, 7 mL/min
TCEP precolumn flow	N_2, 8 mL/min
Air flow	350 mL/min
H_2 flow	40 mL/min
Valve switch times	T_1 = 0.5 min, T_2 = 1.6 min, T_3 = 18.5 min, T_4 = 29.5 min*
Precolumn	TCEP micropacked, 560 mm,1.6-mm od, 0.38-mm id
Analytic column	HP-1, 30 m, 0.53 mm, 5 µm

* Switch times should be determined for each GC system.

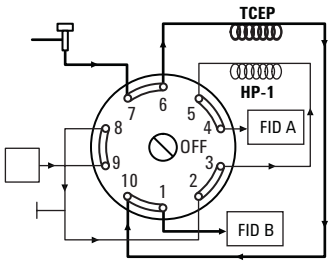


Figure 1A. Valve.

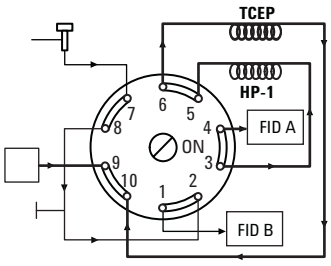


Figure 1B. Valve.

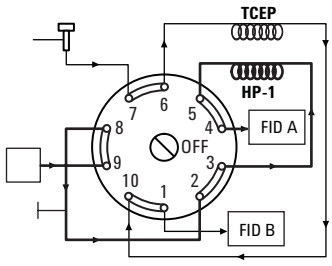


Figure 1C. Valve.

Results and Discussion

Valve Switch Time Setting

Appropriate valve switch time T_1 and T_2 must be determined experimentally. If switch time T_1 and T_2 are not set correctly (for example, switched too late), it is possible that part of the benzene and ethylbenzene will be vented. If switched too early, it may allow interfering compounds to enter the analytical column.

Setting valve switch time T_1 and T_2

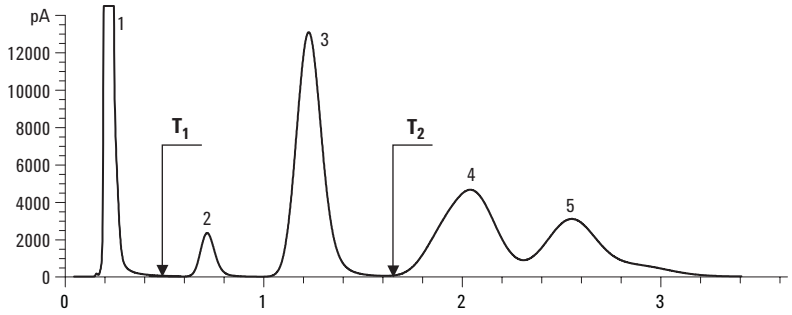
Set valve to the "OFF" position, and then inject 1.0 µL of a calibration blend containing approximately 5% benzene, ethylbenzene, o-xylene, and 2-hexanone, respectively, in isooctane (the exact concentration need not be known). The correct time for T_1 and T_2 is just prior to the elution of benzene and ethylbenzene from the TCEP precolumn. The chromatogram is shown in Figure 2.

Setting valve switch time T_3

Re-inject the calibration blend and turn the valve to backflush at time T_1. The time when the ISTD returns to baseline is called T_3, shown in Figure 3.

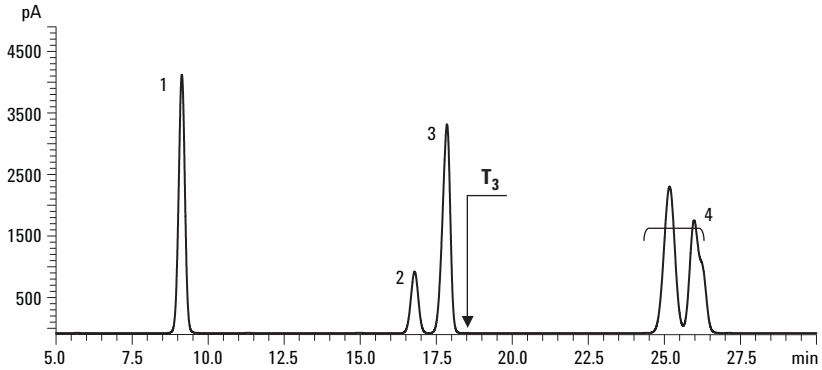
Setting valve switch time T_4

Re-inject the calibration blend and backflush at time T_2. The time when the o-xylene peak returns to the baseline is called T_4, as shown in Figure 4.



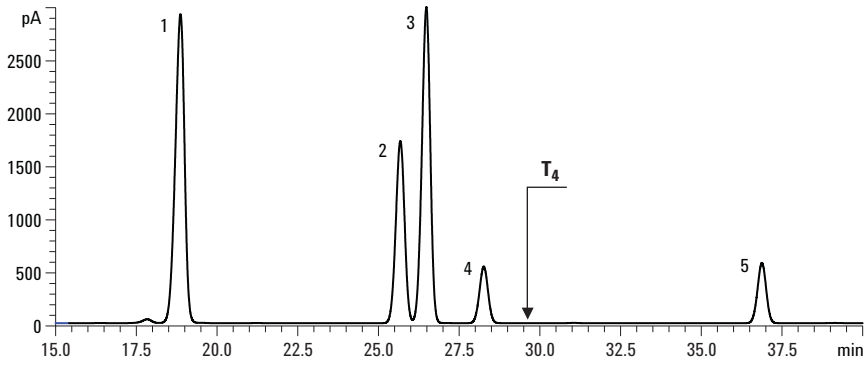
1. Isooctane
2. Benzene
3. Toluene
4. Ethylbenzene
5. 2-Hexanone

Figure 2. Setting precolumn backflush times, T_1 and T_2. The compounds are detected by FID B.



1. Benzene
2. Toluene
3. 2-Hexanone
4. Backflushed peaks (C8+ aromatics and C10+ nonaromatics)

Figure 3. Setting valve switch time T_3.



1. 2-Hexanone
2. Ethylbenzene
3. p/m-Xylene
4. o-Xylene
5. 1,2,4-Trimethylbenzene

Figure 4. Setting valve switch time T_4.

Calibration

The experimental data yields correlation coefficients (R^2) >0.999 for each aromatic, accompanied by very low y-axis intercepts. A typical calibration curve for ethylbenzene is shown in Figure 5.

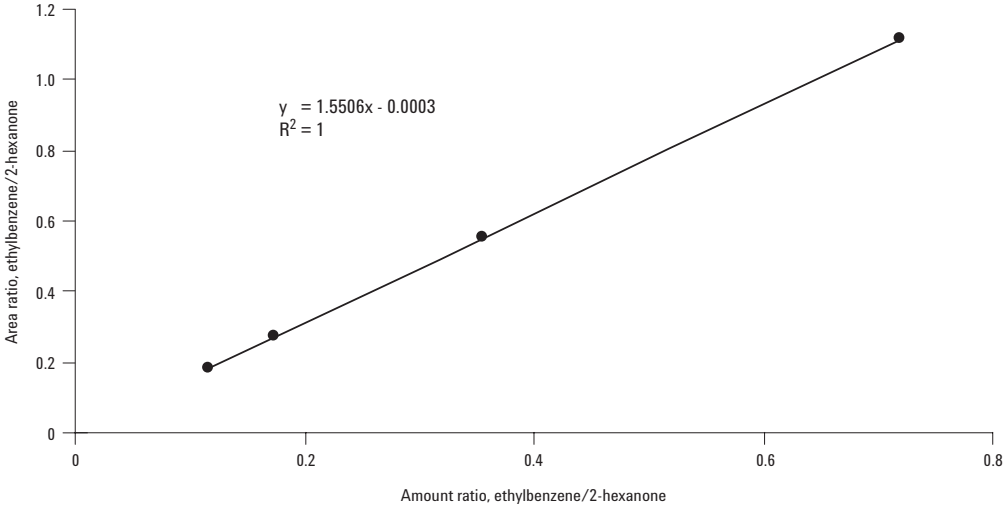


Figure 5. Calibration curve for ethylbenzene.

For an optimum calibration, the absolute value of the y-intercept (b_i) must be at minimum. In this case, A_i approaches zero when w_i is less than 0.1 mass%. This means the mass% (w_i) calculated for an aromatic with zero peak area must be close to zero. The y-intercept can be tested by using Equation 1 below:

$$w_i = (b_i/m_i) \times (w_s/w_g) \times 100\%$$

where:

w_i = Mass% of aromatic compound i
w_s = Mass of ISTD added, g
w_g = Mass of gasoline sample, g
m_i = Slope
b_i = Intercept
A_i = Area of the internal standard

The ASTM D5580 standard specifies that w_i must be <0.02 mass% for benzene and 0.2 mass% for the other aromatics.

The following is an example of the y-intercept test using data from Figure 5.

For aromatic i (ethylbenzene), b_i = 0.0003, m_i = 1.5506, a typical sample preparation may contain approximately w_s = 0.8 g (1 mL) of ISTD and w_g = 7 g (9 mL) of a gasoline sample. Substituting these values into Equation 1 yields:

$w_i = (0.0003/1.5506) \times (0.8\ g/7\ g) \times 100\% = 0.0022$ mass%

Since w_i = 0.0022 mass% is less than 0.2 mass% (the ASTM D5580 specification), the y-intercept has an acceptable value for ethylbenzene.

Repeatability

Table 3 shows that observed repeatability meet or exceed ASTM D5580 specifications.

Table 3. Repeatability

	Observed		ASTM D5580 specification	
Compound	**Mean, (mass%)**	**Repeatability ***	**Range, (mass%)**	**Repeatability**
Benzene	0.852	0.009	0.14-1.79	$0.0265(X^{0.65}) = 0.024$
Toluene	6.229	0.014	2.11-10.08	$0.0301(X^{0.5}) = 0.077$
Ethylbenzene	1.614	0.009	1.57-2.65	0.029
o-Xylene	3.370	0.049	0.77-3.92	$0.0296(X^{0.5}) = 0.055$

X = mass% of mean.

* Difference between two runs

Conclusion

A two-column Agilent 6820 GC system equipped with 10-port switch valve, two FIDs, and Cerity NDS software is used for analysis of aromatics in gasoline. This system provides a simple approach for determining benzene, toluene, ethylbenzene, p/m-xylene, o-xylene, C9 , heavier aromatics, and total aromatics. The Cerity NDS software is very easy to use for instrument control and data analysis. The results demonstrate that the observed data meet or exceed the calibration and repeatability specifications of the ASTM D5580 standard method.

References

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Printed in the USA
July 12, 2004
5989-1336EN



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Running ASTM Method D4815 for Determining Oxygenates in Gasoline on the Agilent 6820 GC

Application

Gasoline Analysis and Environmental Protection

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Abstract

The analytical method is designed for the determination of ethers and alcohols in gasolines by using the Agilent 6820 gas chromatography system. This method employs one 10-port switch valve and two columns. Specific compounds determined are: methyl tert-butylether (MTBE), ethyl tert-butylether(ETBE), tert-amylmethylether (TAME), diisopropylether (DIPE), methanol, ethanol, isopropanol, n-propanol, isobutanol, tert-butanol, sec-butanol, n-butanol and tert-pentanol. For quantitative analysis, 1,2-dimethoxyethane (DME) is used as the internal standard.

Introduction

Unleaded gasoline is produced to reduce environmental pollution. Ethers, alcohols, and other oxygenates can be added to gasoline to increase the octane number and to reduce the amount of carbon monoxide in exhaust. Type and concentration of various oxygenates are specified and regulated to ensure acceptable commercial gasoline quality. It is therefore important to develop test methods for the analysis of these oxygenates. The American Society for Testing and Materials (ASTM) has published method ASTM D4815 for the analysis of oxygenates [1]. This application follows the ASTM D4815 method. Another application note shows running methods ASTM D4815 and D5580 on a single Agilent 6890N GC [2].

Experimental

The experiments are performed on an Agilent 6820 GC system equipped with 10-port switch valve, split/splitless inlet, and flame ionization detector (FID). A micropacked column packed with polar 1,2,3 -*tris*-2-cyanothoxypropane (TECP) is used for prefraction; a nonpolar HP-1 capillary column is used for separating components of interest. In order to obtain steady gas flows, the auxiliary 2-channel gas pressure regulator (G4323A) is suggested. The configuration is listed in Table 1.

Table 1. Configuration

Hardware		
G1180A	6820 GC	1
161	Split/Splitless inlet	1
215	Flame ionization detector (FID)	2
302	One manual controller	1
751	Installation and automation of one valve	1
800	10-port switch valve	1
872	Zero dead volume (ZDV) connection of a capillary column to a valve	2
870	Adjustable restrictor	1
874	Install valve downstream from the capillary inlet capillary inlet	1
335	Cerity NDS software	1
G4323A	Auxiliary 2-channel gas pressure regulator	1
Columns		
19095Z-123	Capillary column HP-1, 30 m, 0.53 mm, 2.65 µm	1
	Micropacked column: 56 cm x 1/16 inch, TCEP (20%)	1
Standards		
	Ethers and alcohols standards	

Method Description

1. The sample containing the internal standard (ISTD) 1,2-dimethoxyethane (DME) is injected into the GC and passes onto a polar TCEP column. The lighter hydrocarbons elute first from the column and are vented, while oxygenates and heavier hydrocarbons are retained. The switch valve is in the "OFF" position, as shown in Figure 1A.
2. After methylcyclopentane (MCP), but before DIPE and MTBE elute from the TCEP column, switch the valve to the "ON" position, as shown in Figure 1B. The components trapped in the TCEP column backflush to the nonpolar HP-1 column for separation in boiling point order.
3. After TAME elutes from the nonpolar HP-1 column, switch the valve to its original "OFF" position to backflush the heavier remainder, as shown in Figure 1C.

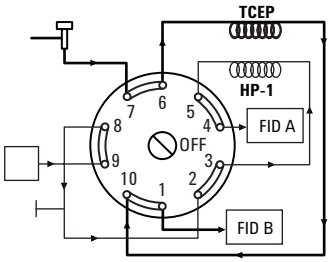


Figure 1A. Valve.

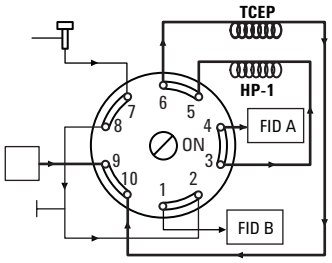


Figure 1B. Valve.

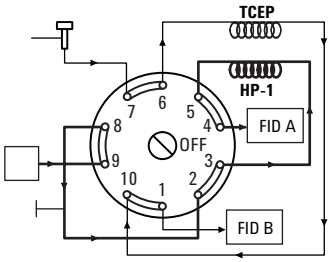


Figure 1C. Valve.

Backflush Time Setting

Setting the correct backflush time is key to this method. If valve switching is too short, C5 and lighter hydrocarbons are backflushed and interfere with the analysis of C4 alcohols. If the switching time is too long, all of the ether components are vented, resulting in an incorrect ether measurement.

Backflush time is determined experimentally by using switching times between 0.18 and 0.34 minutes. The data obtained from the backflush time setting experiment appear in Figure 2. Inspection of this data shows that the hydrocarbons, represented by MCP, are completely eluted from the TCEP column after 0.22 minutes. For this system, a backflush time of 0.22 minutes is used for analysis of DIPE, MTBE, other ethers, and alcohols. If analysis of DIPE is not required, the backflush time will be slightly longer. For this system, a backflush time of 0.28 minutes is used for the analysis of MTBE and alcohols. The valve switch, with the preferred backflush time, is controlled by the Agilent Cerity NDS software.

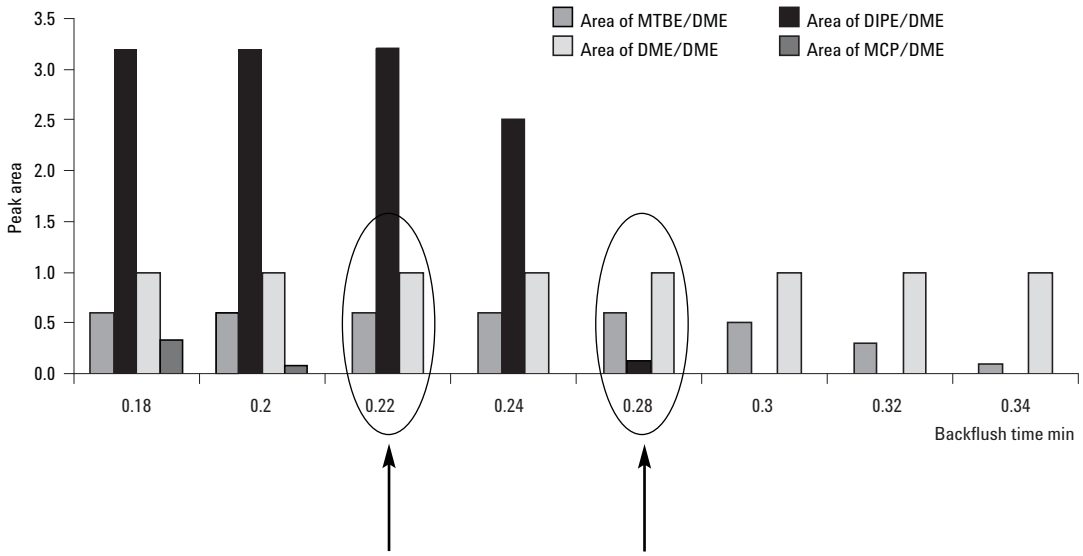
GC Conditions

The instrument conditions are listed in Table 2.

Table 2. Instrument Conditions

Inlet	Split/Splitless, 200 °C, split ratio: 15:1
Detector	FID, 250 °C, makeup: N_2,18 mL/min
Oven program	60 °C (6 min), 2 °C/min, 75 °C, 5 °C/min, 115 °C
Valve temperature	60 °C
HP-1 column flow	N_2, 3 mL/min
TCEP precolumn flow	N_2, 3.5 mL/min
Air flow	350 mL/min
H_2 flow	40 mL/min
Backflush time	0.22 min*
Precolumn	TCEP micropacked, 56 cm,1.6 mm od, 0.38-mm id
Analytic column	HP-1, 30 m, 0.53 mm, 5 µm

* Switch times should be determined for each GC system.



Backflush time of 0.22-min for analysis of DIPE. At this point, hydrocarbons represented by MCP are completely eluted from the TCEP, while the MTBE and DIPE are completely retained.

Backflush time of 0.28-min for analysis of MTBE when DIPE is not required.
At this point, very little DIPE is retained in TCEP, while the MTBE is completely retained.

Figure 2. Graphic presentation of the data from backflush time setting experiment.

Results and Discussion

Calibration and Standardization

Prepare multicomponent calibration standards of the oxygenates and concentration ranges of 0.1, 0.5, 2, 5, 10, 15, and 20 mass percent; for each oxygenate, prepare a minimum of five calibration standards. Run the calibration standard and establish the calibration curve for each oxygenate. It is easy to build the multicalibration by using Cerity NDS software. The experimental data demonstrated excellent calibration linearity; the correlation R^2 exceeds 0.99 and the y-axis intercept is at a minimum for each oxygenate. Figure 3 shows the calibration plot for tert-butanol, as an example.

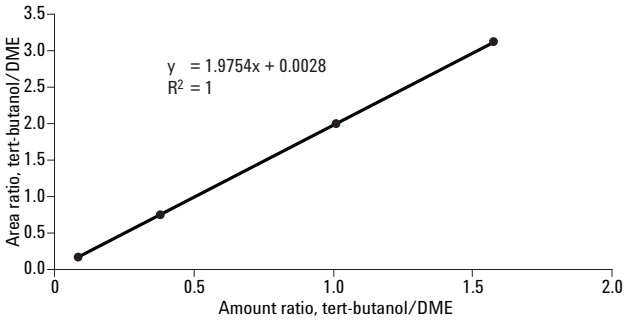


Figure 3. Calibration curve of tert-butanol.

For an optimum calibration, the absolute value of the y-intercept, b_i, must be at a minimum. In this case, when w_i is <0.1 mass%, A_i approaches zero. The equation to determine the mass percent oxygenate i or w_i, reduces to Equation 1. The y-intercept can be tested using Equation 1 below:

$$w_i = (b_i/m_i) \times (w_s/w_g) \times 100$$

where:

w_i = mass% oxygenate i, where w_i is <0.1 mass%,

w_s = mass of ISTD added to the gasoline sample, g

w_g = mass of gasoline sample, g.

b_i = absolute value of the y-intercept for component i

m_i = mass ratio of component i in sample relative mass of standard

The following is an example of the calculation for the y-intercept (b_i) test using Figure 3 for tert-butanol, for which $b_i = 0.0028$ and $m_i = 1.9754$. A typical sample preparation may contain approximately 0.5 mL of ISTD ($w_s = 0.4$ g) and approximately 9.5 mL of gasoline sample ($w_g = 7$ g). Substituting these values into Equation 1 yields:

$$w_i = (0.0028/1.9754) \times (0.4\text{ g}/7\text{ g}) \times 100 = 0.008 \text{ mass\%}$$

Since w_i (0.008 mass%) is less than 0.1 mass%, the y-intercept b_i has an acceptable value for tert-butanol. Similarly, determine w_i for all other oxygenates. The results show that for all oxygenates, w_i is less than 0.1 mass%. (Data not shown for all compounds, only tert-butanol is shown as an example.)

Oxygenate Mixture Analysis and Repeatability

Figure 4 shows the analytical results for an oxygenate mixture. Oxygenates including MTBE, TAME, DIPE, tert-pentanol, and the C1–C4 alcohols are all well separated.

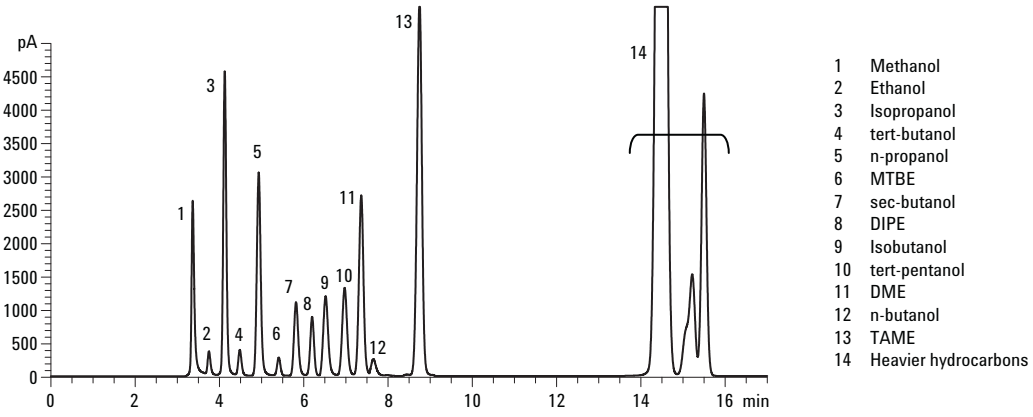


Figure 4. Chromatogram of oxygenates mix.

Table 3 shows very good repeatability, exceeding the specification of ASTM D4815.

Table 3. Repeatability for Five Runs

	Observed		ASTM D4815 specification
Compound	**Average, (mass%)**	**Repeatability***	**Repeatability**
Methanol	7.974	0.225	$0.09(X^{0.59}) = 0.306$
Ethanol	0.5391	0.026	$0.06(X^{0.61}) = 0.041$
iso-Propanol	7.958	0.012	$0.04(X^{0.56}) = 0.128$
tert-Butanol	0.4912	0.023	$0.04(X^{0.56}) = 0.027$
n-Propanol	5.0080	0.033	$0.03(X^{0.57}) = 0.075$
sec-Butanol	2.008	0.004	$0.03(X^{0.61}) = 0.046$
iso-Butanol	1.996	0.008	$0.08(X^{0.56}) = 0.119$
tert-Butanol	1.851	0.015	$0.04(X^{0.61}) = 0.058$
n-Butanol	0.488	0.004	$0.06(X^{0.61}) = 0.039$
MTBE	0.499	0.031	$0.05(X^{0.56}) = 0.034$
DIPE	1.867	0.021	$0.08(X^{0.56}) = 0.114$
TAME	16.668	0.278	$0.05(X^{0.70}) = 0.358$

* Repeatability: Difference between maximum and minimum mass%
X = mass percent of the component.

Conclusion

The Agilent 6820 GC system configured with FID, 10-port valve, and two columns is used for analysis of oxygenates including MTBE, TAME, DIPE, tert-pentanol, and C1–C4 alcohols in gasoline, following the ASTM D4815 standard method. In this GC system, an independently heated valve system ensures the heavy components in the sample are not condensed. It is very easy for the GC to control and run data analysis by using the Cerity NDS software. The experimental data demonstrates that the results exceed the ASTM D4815 calibration and repeatability specifications.

References

1. ASTM D4815, Standard Test Method for Determination of MTBE, TAME, DIPE, tertiary-Amyl Alcohol, and C1 to C4 Alcohols in Gasoline by Gas Chromatography
2. James D. McCurry, “Running ASTM Methods D4815 and D5580 on a Single Agilent 6890N Gas Chromatograph With Nitrogen Carrier Gas”, Agilent Technologies, publication 5988-9153EN www.agilent.com/chem

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Printed in the USA
July 7, 2004
5989-1337EN



Agilent Technologies

Determination of Benzene and Toluene in Finished Motor and Aviation Gasoline with Agilent 6820 GC System Using ASTM Method D3606

Application

Gasoline Analysis and Environmental Protection

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Abstract

The Agilent 6820 gas chromatographic (GC) system configured with flame ionization detector and 6-port valve is used to analyze benzene and toluene in motor and aviation gasoline. In this method, the methyl silicone packed column OV-101 and a TCEP [1,2,3-*tris*(2-cyanoethoxy) propane) are used as precolumn and analytic column respectively. The concentration of each component is calculated by reference to the internal standard (methyl ethyl ketone). Agilent Cerity NDS software is used for 6820 GC control and data analysis. This application demonstrates that calibration and precision meet or exceed the ASTM D3606 specifications.

Introduction

Benzene is classified as a toxic air pollutant. A knowledge of the concentration of benzene compounds can aid in evaluating possible health hazards to persons handling and using gasoline.

Some worldwide regulatory standards were created for monitoring and controlling fuel composition. The United States Clean Air Act (CAA) requires that the level of benzene in reformulated gasoline (RFG) must not exceed 1 volume percent. In China, Nation Standard GB17930 requires that the level of benzene in finished gasoline must not exceed 2.5 volume percent [1]. To meet the stipulated limits of benzene, quantitative determinations of benzene at all process stages is essential.

The American Society for Testing and Materials (ASTM) has published method ASTM D3606 for determining benzene and toluene in finished motor and aviation gasoline [2]. The only difference between ASTM D3606 and this application is the use of a different detector. The detector in D3606 is a thermal conductivity detector (TCD), but in this work the easier-to-maintain, flame ionization detector (FID) is used.

Experimental

Configuration

The GC system is configured with packed inlet, FID, and 6-port valve. Packed columns OV-101 and TCEP are precolumn and analytic column respectively. The configurations are listed in Table 1.

Table 1. Agilent 6820 Configuration

Standard hardware and software	
G1180A	Agilent 6820 GC
152	Packed inlet system with septum purge
215	FID
302	One manual flow controller
751	Installation and automation of one valve
700	6-port valve
335	NDS Cerity chemical software
Columns	
	Stainless Steel packed column, 2.6' × 1/8-inch OV-101 (10%) on Chromosorb WAW 80/100 mesh
	Steel packed column, 15' × 1/8-inch TCEP (20%) on Chromosorb PAW 80/100

Method Description

Methyl ethyl ketone (MEK) is added as an internal standard (ISTD) to the sample which is then introduced into a GC equipped with two columns connected in series. The sample passes through the nonpolar OV-101 precolumn first, as illustrated in Figure 1A. The six-port switching valve is in the "OFF" position. On the OV-101 column, the components are separated according to their boiling points. The lighter components including benzene, toluene, and nonaromatics up to n-octane, elute first from the OV-101 column and transfer into the polar TCEP column. After octane has eluted, the valve switches to the "ON" position, as illustrated in Figure 1B. The components heavier than octane backflush from the OV-101 to vent, while the components of interest are separated on the TCEP column. The eluted components are detected by an FID.

Instrument Conditions

Table 2 provides a list of analysis conditions.

Table 2. GC Conditions

Inlet	Packed inlet, 250 °C
Detector	FID, 250 °C
Oven temperature	90 °C
Valve temperature	80 °C
TCEP flow	N_2, 40 mL/min (~44 psi)
OV-101 flow	N_2, 40 mL/min (~44 psi)
Air flow	350 mL/min
H_2 flow	40 mL/min
Valve switch	3.02 min*
Precolumn	Stainless Steel packed column, 2.6' × 1/8-inch OV-101 (10%) on Chromosorb WAW 80/100 mesh
Analytical column	Steel packed column, 15' × 1/8-inch TCEP (20%) on Chromosorb PAW 80/100

* The time to backfush must be determined for each column system.

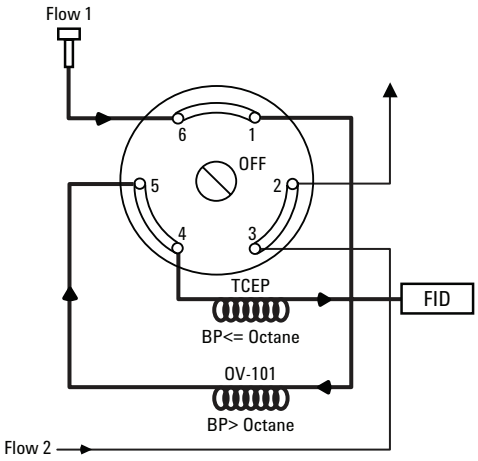

Figure 1A. Valve "OFF" Forward flow mode.

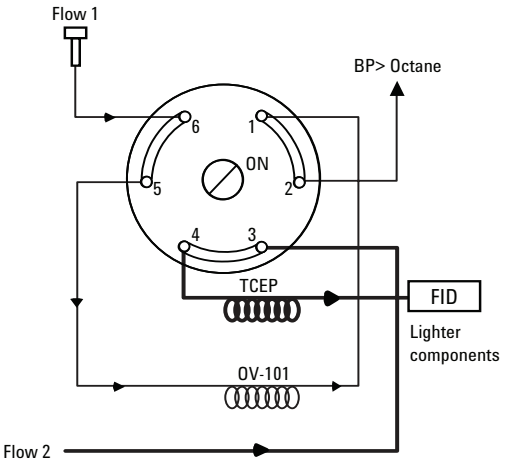

Figure 1B. Valve "ON" Backflush flow mode.

Calibration

Standard sample: seven standard samples covering the range of 0.06 to 5 volume% benzene and 0.5 to 20 volume% toluene are shown in Table 3. For each standard, the volumes of benzene and toluene listed below were added into a 100-mL volumetric flask, and diluted with isooctane.

Table 3. Range of Standard Samples

Benzene		Toluene	
Volume%	**mL**	**Volume%**	**mL**
5.00	5.00	20.00	20.00
2.50	2.50	15.00	15.00
1.25	1.25	10.00	10.00
0.67	0.67	5.00	5.00
0.33	0.33	2.50	2.50
0.12	0.12	1.00	1.00
0.06	0.06	0.50	0.50

Calibration blends: Accurately add 1.0 mL of MEK into a 25-mL volumetric flask, and fill to the mark with the first standard sample. Repeat this procedure until all blends are prepared.

Results and Discussion

Calibration

The calibration is shown to be linear. The correlation (R^2) of both benzene and toluene exceeds 0.999. Figure 2 shows a calibration curve for benzene, as an example.

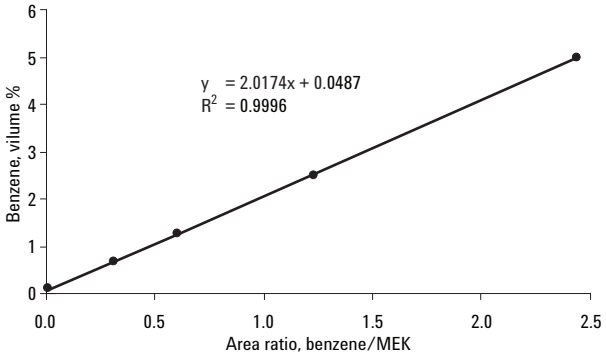

Figure 2. Calibration of curve for benzene.

Repeatability

The good separation of benzene, toluene, and MEK from other potential interferences is shown in Figure 3.

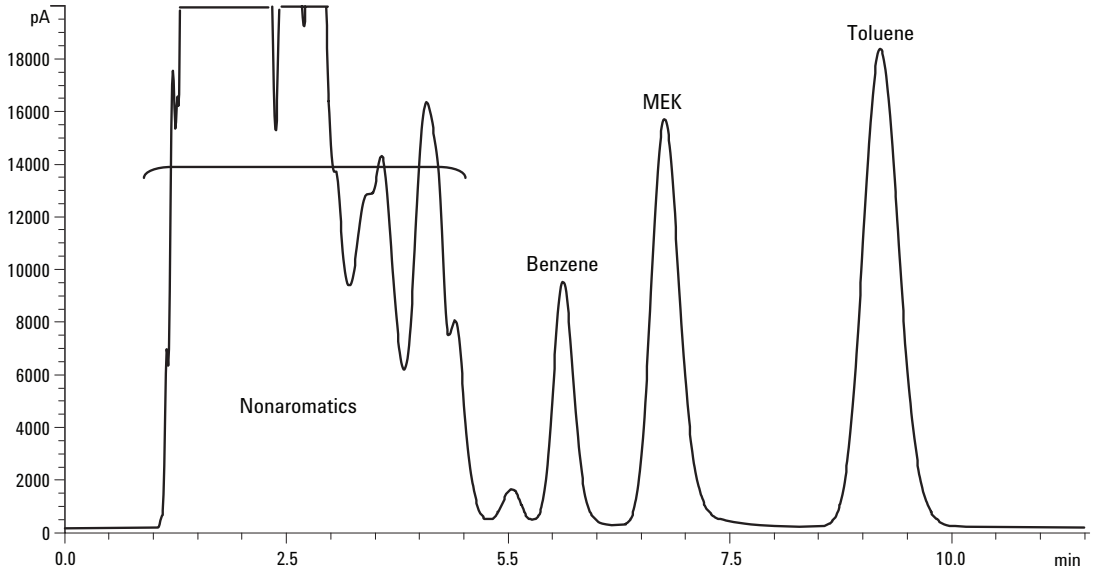

Figure 3. Chromatogram of gasoline.

Table 4 shows very good repeatability, meeting or exceeding ASTM D3606 specifications.

Table 4. Repeatability of %(V/V) for Five Runs

	Observed		ASTM D3606 specifications	
Compound	Average %(V/V)	Difference* %(V/V)	Range %(V/V)	Repeatability
Benzene	1.24	0.01	0.1–1.5	0.03 (x) + 0.01 = 0.04
	2.54	0.01	>1.5	0.03
Toluene	4.78	0.08	1.7–9	0.03 (x) + 0.02 = 0.16
	9.11	0.16	9	0.62

Note: X is the mean volume% of the component.
* Difference between maximum and minimum volume% for five runs.

Sample Injection

In this application, a manual injection technique is used. The following process is necessary so that sharp symmetrical peaks are obtained.

1. Flush the micro-syringe at least five times with sample mixture and then fill with 3 µL of sample. (Avoid any air bubbles in the syringe.)
2. Slowly inject the sample until 2.0 µL remains in the syringe.
3. Wipe the needle with tissue and draw back the plunger to admit 1 to 2 µL of air into the syringe.
4. Insert the needle of the syringe through the septum cap of the chromatograph and push until the barrel of the syringe is resting against the septum cap.
5. Push the plunger completely down and remove the syringe immediately from the chromatograph.

Cerity NDS

With Cerity NDS, it is very easy to control the instrument. It can switch the valve automatically and build the multi-calibration.

Conclusions

The Agilent 6820 GC configured FID and 6-port switch valve is used to determine benzene and toluene in finished motor and aviation gasoline. An independently heated valve system ensures heavy components in the sample not to be condensed. The result demonstrates the correlation of calibration curve for benzene and toluene exceed 0.999 and the repeatability meets or exceeds the specification of ASTM D3606.

References

1. People's Republic of China National Standards GB17930-1999.
2. ASTM D3606-99, Standard Test Method for Determination of Benzene and Toluene in Finished Motor and Aviation Gasoline by Gas Chromatography.

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Printed in the USA
July 7, 2004
5989-1338EN

Direct Elemental Analysis of Gasoline by Agilent 7500ce ORS ICP-MS

Application

Petrochemical

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Abstract

The Agilent 7500ce ICP-MS is shown to be an excellent analytical tool for the elemental analysis of gasoline. It offers the best detection limits of any multi-element technique and a full elemental analysis suite that takes less than 5 minutes to acquire. The Octopole Reaction System (ORS), integral to the 7500ce, ensures that a single method and set of conditions can be used to directly determine all elements of interest in the sample. Comparison data for selected elements in both gas mode and no-gas mode highlight the effectiveness of the ORS for interference removal. Results showing the excellent long-term stability of the 7500ce ORS are also given.

Introduction

Traditionally ICP-MS (inductively coupled plasma-mass spectrometry) has not been considered as the most appropriate technique for the measurement of trace metals in organic matrices due to perceived problems with sample introduction, plasma stability, carbon deposition on the interface and lenses, and the formation of carbon-based interferences. These problems can be difficult to overcome without the appropriate hardware setup and methodology, even for the easiest of organic matrices, that is, those with low vapor pressure.

Direct aspiration of gasoline provides a more unique problem due to its relatively high vapor pressure and aggressive nature. The high vapor pressure would typically cause plasma instability, even to the extent that the plasma is extinguished and the aggressive nature of the solvent precludes the use of most types of peristaltic pump tubing.

However, these obstacles are relatively simple to overcome by intelligent system design, choice of sample introduction system hardware, and plasma optimization. The 7500 Series features the only digital drive solid-state plasma RF (radio frequency) generator available in a commercial ICP-MS. The crystal controlled fixed frequency (27.12 MHz) generator features a high-speed real-time matching network maintaining plasma stability when analyzing volatile organic matrices. The 27.12 MHz plasma has been shown to generate a higher ionization temperature than higher frequency plasmas [1], which improves matrix decomposition and also gives lower metal oxide ratios compared to 40.68 MHz plasmas [2].

To help reduce vapor pressure effects on the plasma, the spray chamber must be cooled to improve the condensation of excess solvent vapor produced during nebulization. All 7500 ICP-MS systems feature a Peltier cooled spray chamber that can be set to temperatures below 0 °C, thus reducing the solvent loading on the plasma. To further reduce the effects of high vapor pressure, a narrow bore torch injector was used (1.0-mm internal diameter (id) in place of the standard 2.5-mm id injector torch). The narrow injector minimizes the effect of solvent droplets rapidly expanding into vapor.

Sample introduction can be facilitated by any solvent-resistant uptake tubing and nebulizer, however, to reduce the likelihood of contamination, avoid Viton uptake pump tubing. In this study, a quartz concentric nebulizer was used under free aspiration to avoid contamination problems.

To eliminate the effects of carbon deposition on the interface and ion lenses, oxygen needs to be introduced into the plasma. For water-soluble solvents, it is often sufficient to dilute the solvent in water as this can provide enough oxygen to convert the carbon into CO or CO_2. However for hydrophobic solvents, it is necessary and easier to introduce the solvent directly and add oxygen gas to the plasma. The 7500 can be fitted with an additional mass flow controller that allows the addition of oxygen to the sample stream after the nebulizer and spray chamber. This configuration allows complete control over plasma conditions facilitating easy optimization regardless of the matrix.

Trace Impurities in Gasoline

The requirement to measure metal impurities in organic matrices varies depending upon the end use. Impurities in gasoline must be controlled to ensure the final quality of the product for example, to ensure reliable burn characteristics. Some metallic impurities, such as vanadium and lead, are catalyst poisons and must be kept under control while other impurities have corrosive properties. Additionally, the metallic impurities must be controlled in order to reduce their environmental impact and meet legislative requirements. The levels and type of impurity also dictate the removal process; some aqueous soluble salts are relatively easy to remove but organic soluble metals (usually porphyrin-metal complexes) are more difficult. An economical approach is often to dilute a product gasoline with high metallic impurities with a cleaner gasoline batch.

Sample Preparation and Instrumental Conditions

The samples were taken directly from the gas station pump of a leading petroleum company and analyzed using a standard Agilent 7500ce ICP-MS fitted with the Agilent quartz concentric nebulizer and Scott-type double-pass quartz spray chamber; no desolvation device was necessary. Platinum interface cones were used to allow the addition of O_2 (as a 50% mix in Ar), which was added after the spray chamber from a separate mass flow controlled channel. The system was fitted with a taper torch with a 1-mm id injector to further improve plasma stability.

Instrumental conditions are given in Table 1.

Table 1. Agilent 7500ce ORS-ICP-MS Operating Conditions

Parameter	Value
Forward power	1200 W
Plasma gas flow	15.0 L min^{-1}
Auxiliary gas flow	1.0 L min^{-1}
Nebulizer gas flow	0.70 L min^{-1}
Make up gas flow	0.13 L min^{-1}
50% O_2 in Ar flow	7% open (~0.14 L min^{-1})
Sampling depth	9.0 mm
Spray chamber temperature	–5 °C

All instrumental conditions were optimized from the system's ChemStation PC including all gases, torch position, and sample introduction parameters. The ORS cell gas conditions were essentially identical to those conditions used for aqueous samples, with the exception of a slightly higher hydrogen flow rate (2 mL min^{-1} higher) for the removal of C_2 interference on Mg.

Calibration was prepared by weight-weight dilution of the stock standard (Conostan oil-based standards) into the gasoline. Additionally, an internal standard mix (containing Be, Y, In, and Bi) was added to compensate for any sample preparation or minor viscosity differences. The four internal standard elements were added in case any of these elements were present in the sample, thus giving enough flexibility for data processing and method development.

Data Acquisition

Data was acquired operating the ORS in helium mode [He], hydrogen mode [H_2], and no-gas mode [No Gas]. The use of the different gasses generally depends upon the analyte.

Helium is used for the removal of matrix-based interferences regardless of the interference source and is applicable to a single interference-analyte pair, for example, ArC on ^{52}Cr, as well as the removal of interferences on those masses suffering several different interferences, for example, ArCl, CaCl, ArSH, on As. Because He is inert, no reaction products are formed and polyatomic interferences are removed by energy discrimination (the interfering ion loses more energy compared to the analyte ion and is ejected from the ion beam). He mode is also applicable to reducing argon-oxide, metal-oxide, and metal-halide interferences such as CaO, CeO, CaCl, improving the measurement of Fe, As, and interfered rare earth elements (REE) in complex matrices.

H_2 reaction mode is used to remove a small number of very large interferences: N_2 and CO on Si, Ar on Ca, C_2 on Mg (in gasoline), and Ar_2 on Se. These are all large, stable and well known interferences, and with the exception of C_2 (in organics), these are all plasma-based.

Switching between ORS modes is automatic within a single acquisition, so there is no need to repeat each sample analysis for each mode. Furthermore, no interference equations are necessary as the appropriate gas mode removes all interferences.

Several interfered elements were also determined without cell gas for comparison of cell performance.

On-Mass Measurements

ORS cell mode allows the determination of isotopes that would not normally be available in many sample types. For example the iron 56 isotope (^{56}Fe suffers a severe overlap from ArO and CaO) has higher % relative abundance than the 57 isotope (suffers from ArOH and CaOH interferences), so analyzing the 56 isotope provides better sensitivity and better detection limits (DLs) once the interferences are eliminated using the ORS cell in He mode.

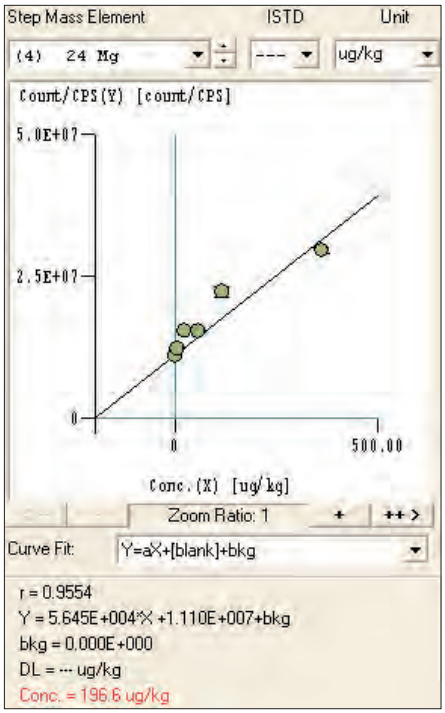
Results and Discussion

Table 2 displays the quantitation and DL data for the base gasoline. Notably the effect of the C-based interferences can be observed in the data for Cr and Mg with no cell gas compared to gas mode. The data presented in bold typeface is for information only.

Comparative data has been provided for Cr and Cu in gas and no-gas mode for two free isotopes and also several other elements although during "real-life" analyses, this would not be necessary. Measuring the most abundant isotope in He is the default analysis mode.

Calibrations for several elements are given in Figures 1 to 5. Note the calibrations for Mg (Figure 1a) and Cr (Figure 2a) in no-gas mode; although relatively linear, there is a large offset due to the interferences in the organic matrix (C_2 and ArCl, respectively). Figures 1b and 2b show that the respective interferences have been significantly reduced.

1a



1b

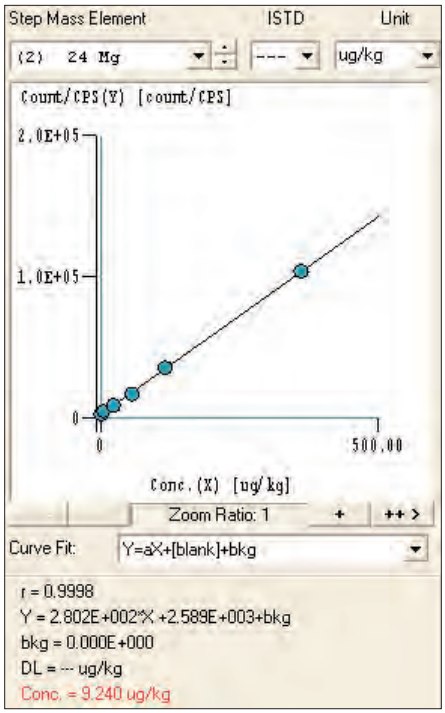
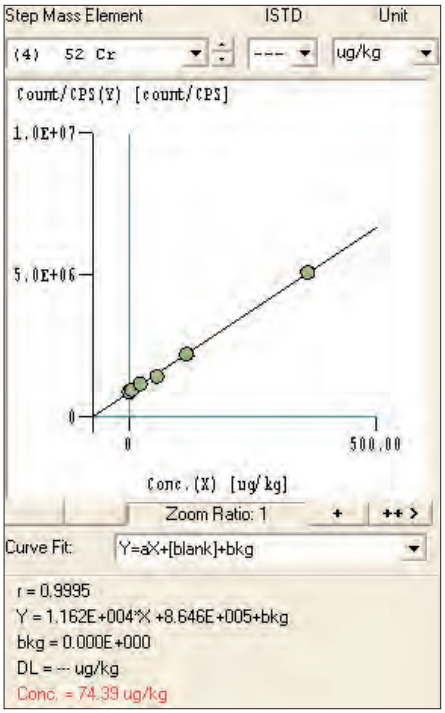


Figure 1. 1a) Magnesium calibration (No-gas mode). Note apparent concentration of 196.6 µg/kg due to C_2 interference (left). 1b) Magnesium calibration (H_2 Mode). Note concentration of 9.24 µg/kg (right).

2a



2b

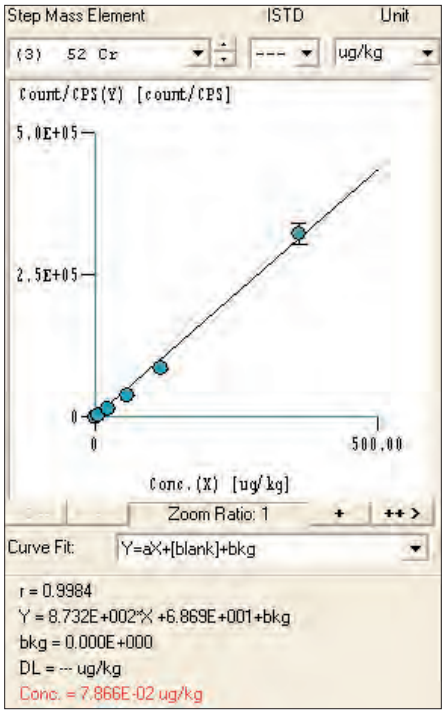


Figure 2. 2a) Chromium calibration (No-gas mode). Note apparent concentration of 74.4 µg/kg due to Ar C interference. 2b) Chromium calibration (He Mode). Note concentration of 0.078 µg/kg.

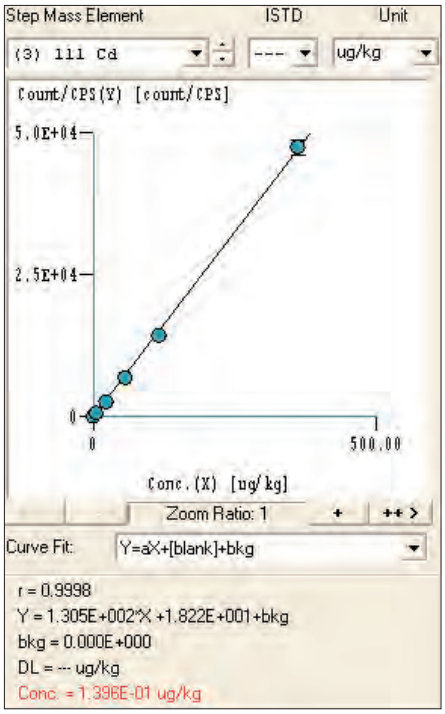


Figure 3. Cadmium calibration (He mode).

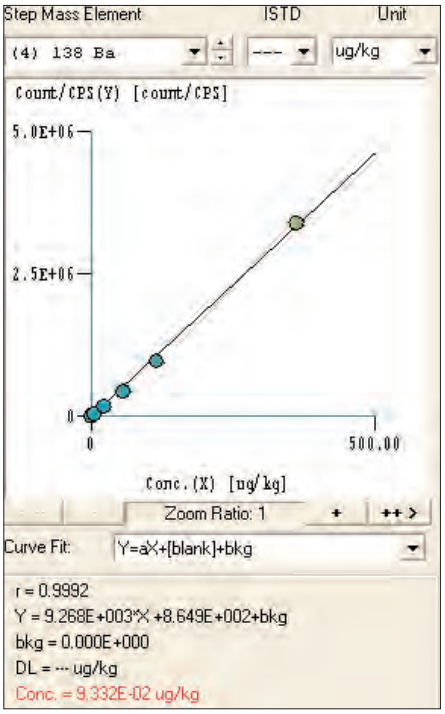


Figure 4. Barium calibration (No-gas mode).

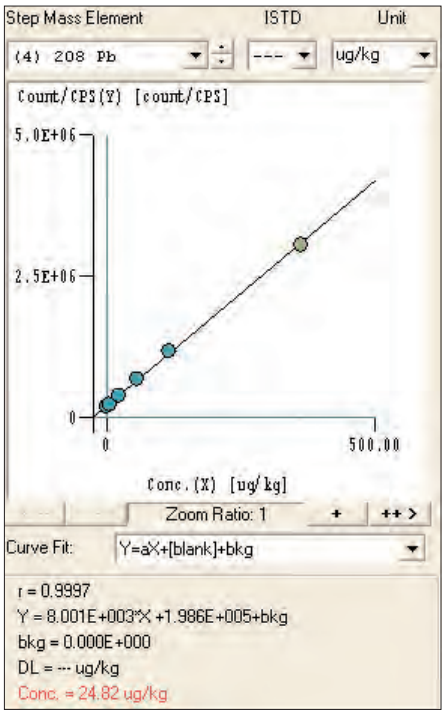


Figure 5. Lead calibration (No-gas mode).

It should be noted that the DL data for phosphorus and silicon proved higher than usual in organic matrices due to relatively high concentrations in the base gasoline.

The effect of interferences from the sample matrix can be observed for all the interfered elements in no-gas mode particularly those suffering from carbon-based overlaps. For example, Al suffers from a CN interference while Cr suffers from an ArC interference in no-gas mode; this is borne out in the differences between the quantitation results once a gas is applied to the cell. Data for Cd also improved in He mode, due to the removal of MoO interference on this element.

Table 2. Quantitation and DL Data for the Gasoline.*

Element	Mass	Tune	Conc. (µg/kg)	SD	3σ DL
B	11	H_2	2.11	0.2584	0.775
Na	23	No gas	1.57	0.009118	0.027
Mg	24	H_2	9.24	0.08363	0.251
Mg	**24**	**No gas**	**196.60**	**1.565**	**4.695**
Al	27	He	1.36	0.2526	0.758
Al	**27**	**No gas**	**56.86**	**0.1229**	**0.369**
Si	28	H_2	316.80	10.91	32.730
P	31	No gas	310.40	0.9064	2.719
Ca	40	H_2	5.31	0.1445	0.434
Ti	47	He	0.18	0.1515	0.455
V	51	He	0.04	0.004013	0.012
Cr	52	He	0.08	0.001157	0.003
Cr	**52**	**No gas**	**74.39**	**0.275**	**0.825**
Cr	53	He	0.05	0.005534	0.017
Cr	**53**	**No gas**	**32.61**	**1.035**	**3.105**
Mn	55	He	0.33	0.01104	0.033
Fe	56	He	2.85	0.03929	0.118
Fe	**56**	**No gas**	**8.35**	**0.1046**	**0.314**
Ni	60	He	0.17	0.02741	0.082
Cu	63	He	2.10	0.04912	0.147
Cu	65	He	2.16	0.04511	0.135
Zn	66	He	12.18	0.5757	1.727
Mo	95	No gas	1.76	0.02916	0.087
Ag	107	No gas	1.03	0.01434	0.043
Cd	111	He	0.14	0.03079	0.092
Cd	**111**	**No gas**	**0.88**	**0.04549**	**0.136**
Sn	118	No gas	0.51	0.003098	0.009
Ba	138	No gas	0.09	0.003994	0.012
Pb	208	No gas	24.82	0.0648	0.194

*Data was acquired in appropriate cell mode with comparative data for selected interfered elements with no cell gas (shown in bold type) to highlight the incorrect results that would be reported by a non-cell ICP-MS. All data presented as µg/kg.

Note that the data acquired for the elements with more than one isotope (in cell mode) was reproducible. This is a good indication that the cell is efficiently removing matrix and plasma-based interferences.

As He is a totally inert medium, no side reactions can occur which would form interferences in another region of the mass spectrum. In addition helium will not react with analyte ions – it is essentially acting as a simple molecular filter removing all polyatomic interferences.

Spike Recoveries

There are no commercially available reference materials for inorganic constituents in gasoline so a spike recovery was performed in the sample to test the performance of the method. The % recoveries for this sample are shown in Table 3. Recoveries were very good; although Pb displayed a higher recovery than the other elements, which was probably due to the relatively high concentration already in this sample. P and Si were spiked at a higher concentration due to the higher natural concentration in the sample.

Table 3. Spike Recoveries in the Gasoline Matrix

	Spike (56.2 µg/kg)	**% Recovery**
Na/23 [No gas]	57.4569	102.2
Mg/24 [H_2]	58.435	103.9
Al/27 [He]	55.7417	99.2
Ca/40 [H_2]	59.7195	106.2
Ti/47 [He]	59.566	106.0
V/51 [He]	52.5743	93.5
Cr/52 [He]	54.9369	97.7
Cr/53 [He]	55.508	98.7
Mn/55 [He]	56.1541	99.9
Fe/56 [He]	57.075	101.5
Ni/60 [He]	58.916	104.8
Cu/63 [He]	54.745	97.4
Cu/65 [He]	55.647	99.0
Zn/66 [He]	56.38	100.3
Mo/95 [No gas]	50.02	89.0
Ag/107 [No gas]	58.948	104.9
Cd/111 [He]	61.739	109.8
Sn/118 [No gas]	54.4923	96.9
Ba/138 [No gas]	51.775	92.1
Pb/208 [No gas]	67.24	119.6
	Spike (360.82 µg/kg)	**% Recovery**
Si/28 [H_2]	388.39	107.6
P/31 [No gas]	396.62	109.9

Instrument Stability

The results show that the 7500ce ORS method is excellent for this sample matrix. However if it were not reproducible or proved to have poor stability over an extended sample batch then the technique would be of less value in a routine environment. To demonstrate the stability of the method a sample was spiked and run continuously for almost 5 hours. Figure 6 displays the recovery for the spiked gasoline sample over the analysis time, normalized to the first repeat. The 7500ce stability proved to be excellent indicating its applicability as a routine technique.

Conclusions

The results showed that many of the isotopes, which would not normally be available to ICP-MS at trace levels, become readily accessible when using the ORS cell in gas mode. Examples include the removal of all C-based interferences: $^{12}C_2$ on Mg; $^{12}C^{15}N$ on Al; $^{12}C^{16}O$ on ^{28}Si; $^{40}Ar^{12}C$ on ^{52}Cr; $^{40}Ar^{13}C$ on ^{53}Cr as well as interferences arising from other elemental species: $^{23}Na^{40}Ar$ on ^{63}Cu; $^{40}Ar^{16}O$ and $^{40}Ca^{16}O$ on ^{56}Fe; $^{40}Ar^{16}OH$ and $^{40}Ca^{16}OH$ on ^{57}Fe; $^{32}S^{34}S$ and $^{34}S(^{16}O)_2$ on ^{66}Zn, and many more potential interferences in variable matrices. Importantly, energy and physical processes govern the interference removal for the majority of interferences with a reactive gas (H_2) only being needed for the most intense interferences (Mg, Ca, and Si). This makes setup and operation simple. Method development is quick and easy as only a single set of cell conditions is required, no prior knowledge of the samples is needed, and no interference correction equations need to be set up or updated daily.

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1. B. Capelle, J. M. Mermet, J. P. Robin; (1982) *Appl Spectrosc* **36**, 102–106.
2. C. J. Park, K. W. Lee; (1991) *J Anal At Spectrom*; **6**, 431–437.

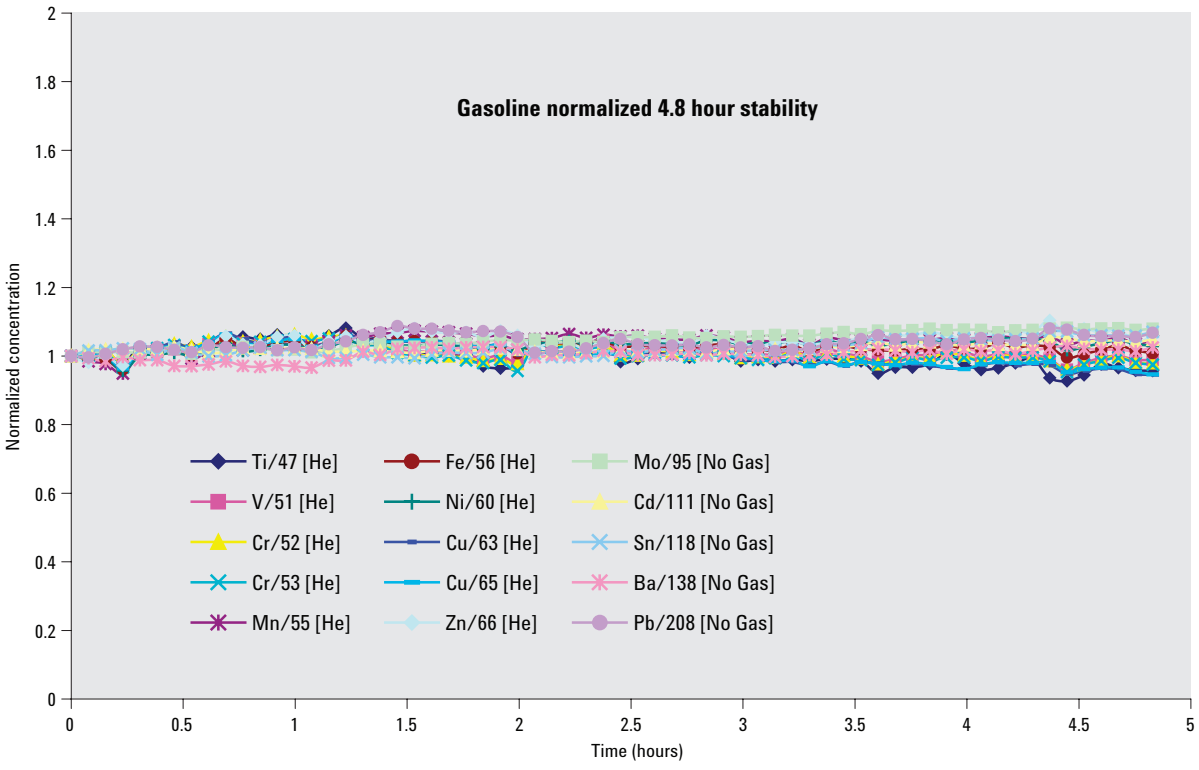

Figure 6. Normalized (to the first analysis) 4.8 hour stability of a continuously aspirated spiked gasoline sample.

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Printed in the USA
November 4, 2005
5989-4147EN



Agilent Technologies

Comprehensive GC System Based on Flow Modulation for the 7890A GC

Application Brief

Introduction

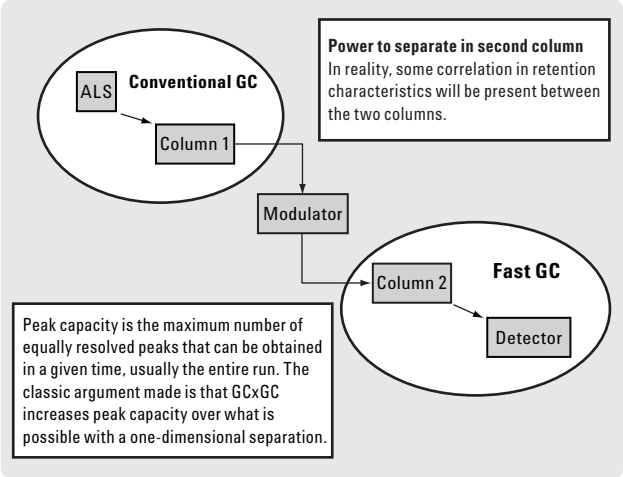
A hardware solution is available on the 7890A for the practice of comprehensive GC. The system uses a capillary flow modulator controlled by the 7890A GC. The system is offered with factory checkout using an FID detector. Other detectors, preferably those operating at 50 Hz or greater, can be used.

Comprehensive two-dimensional (2D) GC, or GCxGC, is a powerful technique that can be used to separate very complex mixtures, such as those found in the hydrocarbon processing, environmental, and food/fragrance industries.

The method uses two columns, typically of very different polarities, installed in series with a modulator in between. The second column is much shorter than the first column to effect a fast separation. The entire assembly is located inside the GC oven.

The modulator performs three functions:

1. It collects effluent from the first column for a fraction of the time equal to peak width. For example, if a peak from column one is six seconds wide, the modulator will accumulate material every two to three seconds, thereby dividing the peak from the first column into two or three "cuts."
2. It focuses the material collected from each cut into a very narrow band through flow compression.
3. It introduces the bands sequentially onto the second column, resulting in additional separation for each band injected onto the second column.



Comprehensive 2D GC uses a primary column (conventional separation), a flow modulator, a second column (very fast separation), and a fast detector.

This technique provides a second dimension of information that can increase the peak resolution and capacity.

A number of different modulator designs have been described in the literature, most relying on thermal cycling to focus the bands from the first column and release them into the second column. Some disadvantages to this approach are:

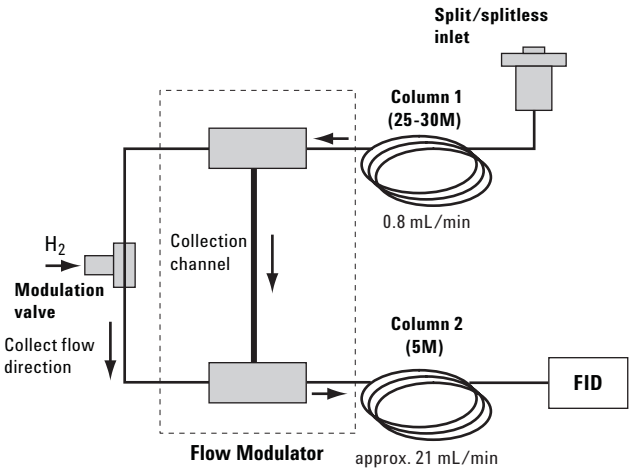
- Large usage of expensive cryogenic gases leading to a high cost of analysis
- Complexity of the hardware
- Longer analysis times

Agilent's proprietary Capillary Flow Technology and fourth-generation Electronic Pneumatics Control (EPC) enable the use of a differential flow modulator to conduct comprehensive 2D-GC without the use of cryogenic gases or complex hardware.

The key to operation is the flow differential (typically 20 to 1) between the second and first columns, respectively. This compresses and focuses the analytes present in any given modulation "inject" pulse into the second column. Precise timing of the modulator is made possible by installing a driver board in the Aux det 2 detector slot of the 7890A mainframe.

The Capillary Flow Technology modulator uses a deactivated, stainless steel structure with all flow splitters and the collector channel incorporated internally in the device. It has low thermal mass so it can track the oven temperature very closely, and its GC oven location allows precise temperature control without lag during programmed runs. All external connections are made using Agilent's Ultimate Union technology for leak-free operation and extremely small, well-swept volumes. A micro three-way solenoid valve, installed on the side of the gas chromatograph, connects to a pneumatics control module (PCM) to accurately and precisely control the flows through the modulator.

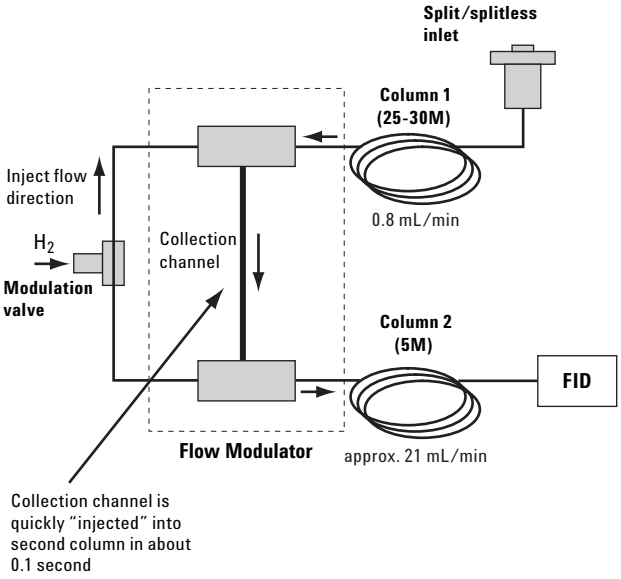
The figures below illustrate the modulator. A three-way solenoid valve receives a controlled supply of hydrogen gas from a PCM. The periodic switching of this three-way valve drives the modulator. The precisely timed and synchronized switching between the collect and inject states directs discrete sample pulses continuously to the second column for additional fast separation throughout the chromatographic run. Both columns are run in constant flow mode. For optimal performance, injection size and split ratio should be carefully adjusted to avoid overloading, which can lead to excessive peak tailing.



Flow rates and flow directions during the load or collect portion of the modulation cycle

Load or collect state (above): At the beginning of this state, the collection channel is filled with hydrogen gas from a previous injection cycle flush.

The primary column effluent enters the modulator's top tee connection and flows into the collection channel. The analytes from this column enter one end of the collection channel. Hydrogen flow from the PCM/three-way micro valve exits the modulator at the bottom tee and is sent to the second column.



Flow rates and flow directions during the transfer or inject portion of the modulation cycle

Inject or flush state (above): Hydrogen gas flow from the three-way solenoid valve is directed to the top tee. A high flow of typically 20 mL/min for about 0.1 second rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes collected in the channel undergo rapid separation.

What is required:

- Agilent 7890A GC with firmware version A.04.06 or higher
- FID with 200 Hz data collection rate or other fast detector
- Split/splitless inlet
- Capillary Flow Technology modulator option or accessory
- Capillary Flow Technology modulator checkout kit
- Pneumatics control module (PCM)
- Agilent GC ChemStation B.03.02 or other data collection and analysis system that can control the flow modulator cycle
- 30-m × 0.25-mm × 0.25-µm DB-5ms column (included with option or accessory)
- 5-m × 0.25-mm × 0.15-µm INNOWax column (included with option or accessory)
- 2D data analysis software, GC Image recommended (not provided by Agilent)
- Internal column nuts and SilTite ferrules

Ordering Information

Description	Part number
7890A GC with Capillary Flow Technology Modulator (requires checkout kit)	G3440A Option 887 or accessory G3486A
7890A GC with 200 Hz FID	G3440A Option 211 or accessory G3462A
7890A GC with split/splitless inlet	G3440A Option 112 or accessory G3452A
Capillary Flow Technology modulator checkout kit	G3487A
PCM for 7890A GC	G3440A Option 309 or accessory G3471A
SilTite metal ferrules, 1/16-in × 0.4-mm id, 10/pk, includes 2 column nuts	5184-3569
Agilent 32-bit ChemStation for 1 GC	G2070BA
Agilent 32-bit ChemStation Bundle for 1 GC includes: – G2070BA 32-bit ChemStation software – Computer with monitor and Windows operating system – Printer	G1875BA
2D GC software Recommend GC Image software, which can be purchased from Zoex Corporation	www.zoex.com

Application Examples

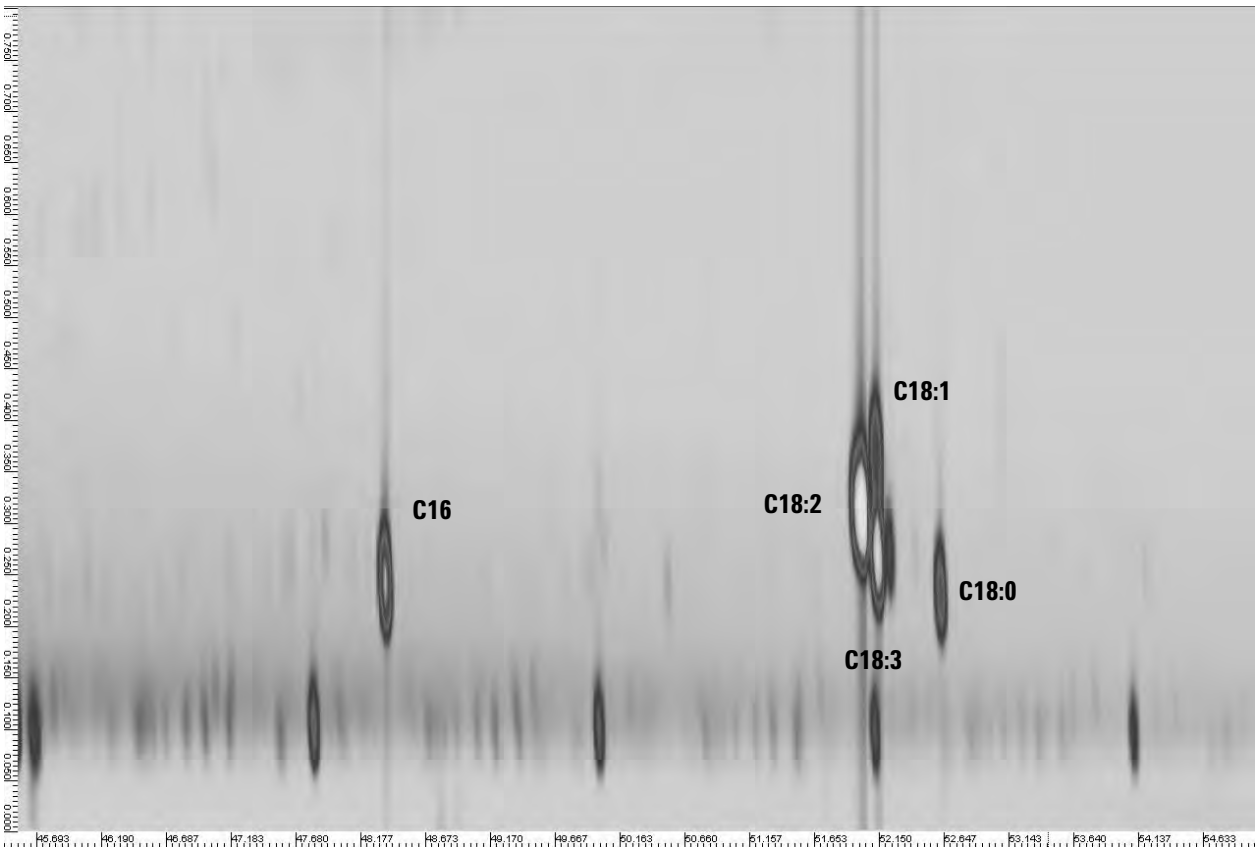
Several applications are shown. Note that primary column lengths have been chosen to give optimal results. While the 30M column that is shipped with the system is an excellent choice for a wide range of applications, other lengths can be used to optimize a given separation. Various columns have been used in these examples to illustrate some of the possibilities. The GC Image software package was used for processing the ChemStation data.

1. B20 biodiesel based on soy FAMES. Section of the 2D image showing the C16 and C18 FAMES is shown.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

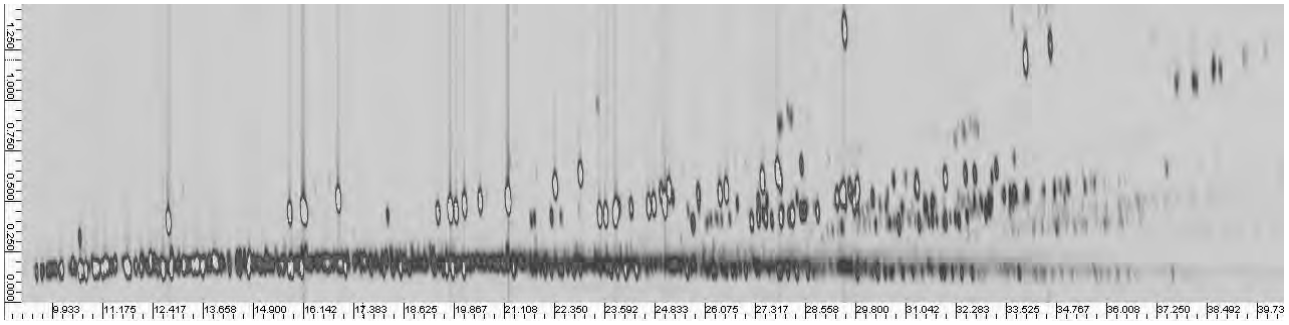


2. Complete 2D image of a sample of heavy gasoline. Each series of substituted 1-ring aromatics is well separated, making hydrocarbon class grouping possible.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

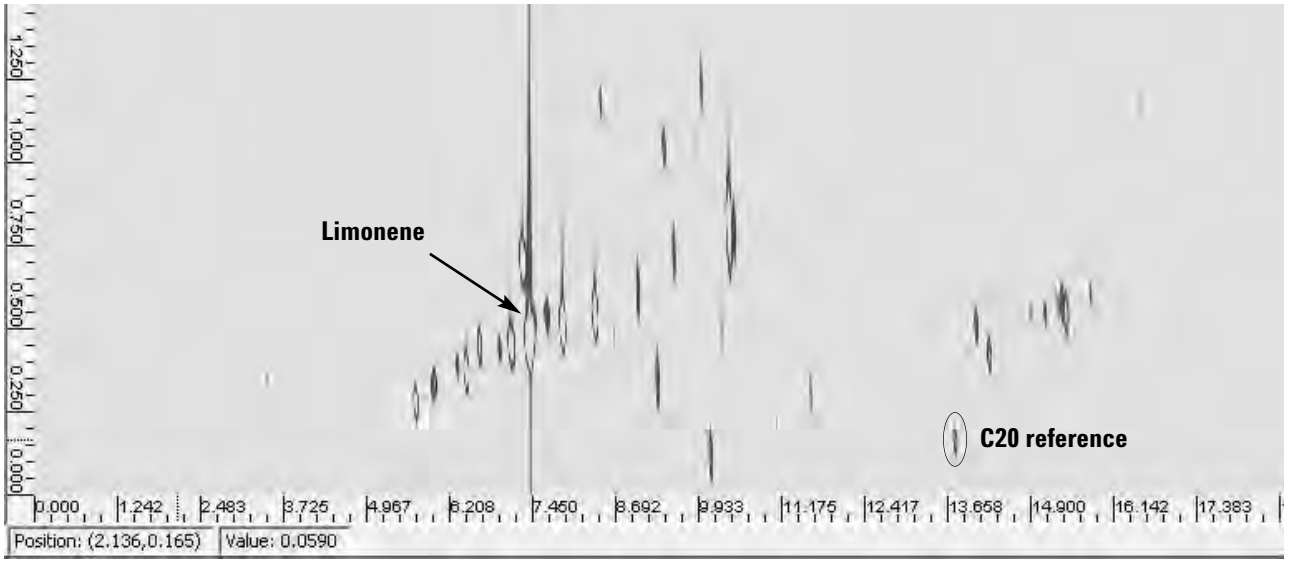


3. Lime oil 2D image.

   Column 1: 15 m × 0.25 m × 0.25 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm DB-17HT

   Modulation: 1.40 s load, 0.10 s inject



Thermal vs. Flow Modulation

Since competitors offer only systems based on thermal modulation, the following table summarizes the key points about the respective approaches of thermal vs. flow modulation.

Thermal modulation	Differential Flow modulation
Cryo-focusing provides potentially narrower peaks in second dimension	Peak widths comparable to thermal. Usually no more than 20% wider. Many users want to sum regions of peaks where peak width is not as critical
Lower flows – Can be used with high-vacuum detectors (TOF)	MSD can be used with a splitter over limited scan range
Large consumption of cryogen	No cryogen required
Complex hardware design, set-up, and maintenance	Simple, reliable Capillary Flow Technology based hardware; small thermal foot print
Long chromatographic runs required for best performance	Run times comparable to a 1D separation
System price (estimate) $60 to $70K	Agilent system approximately $60K (list)

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Printed in the USA
April 1, 2008
5989-8060EN



Agilent Technologies

Detection of Sulfur Compounds According to ASTM D5623 in Gasoline with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) and an Agilent 7890A Gas Chromatograph

Application

Hydrocarbon Processing

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Abstract

Sulfur components in gasoline samples are detected utilizing ASTM D 5623 with an Agilent 7890A GC configured with Agilent 355 dual plasma sulfur chemiluminescence detector (DP SCD). The 355 SCD response is linear, equimolar, and provides a linear range from 0.1 to 10 ppm. The coelution of hydrocarbon and sulfur peaks does not present a problem for the detector. The detection limits of sulfur compounds in gasoline are down to 20 ppb, while no quenching was found in the gasoline sample analysis.

Introduction

Gas chromatography with sulfur chemiluminescence detection (SCD) provides a rapid and highly specific means to identify and quantify various sulfur compounds that may be present in petroleum feed stocks and products, such as gasoline.

Frequently, petroleum feeds and products contain varying amounts and types of sulfur compounds. Many sulfur compounds can be corrosive to equipment, can inhibit or destroy catalysts employed in downstream processing, and can impart undesirable odors to products. The ability to speciate sulfur compounds in various petroleum liquids is useful in controlling sulfur compounds in finished products and is frequently more important than the ability to simply measure total sulfur content alone.

The SCD burner easily mounts on the 6890 and 7890A GCs, and incorporates features for easier and less frequent maintenance. The DP technology harnesses the power of dual flame plasma combustion, optimizing combustion of the sample matrix and formation of sulfur monoxide (SO).

The 355 DP SCD response is inherently linear, equimolar, and far less susceptible to hydrocarbon interference. These advantages eliminate the need for linearizing data or determining separate response factors for individual sulfur compounds. Furthermore, hydrocarbons are virtually invisible to the DP SCD. The coelution of hydrocarbon and sulfur peaks does not show quenching. Frequent column changes are required for analysis of various hydrocarbon products by flame photometric detectors (FPD) to avoid serious quenching and inaccurate results. ASTM Method D5623 utilizes the sulfur chemiluminescence detector (SCD) for the detection of sulfur compounds in gasoline.

Experimental

An Agilent 7890A GC configured with split/splitless inlet (Sulfinert-treated), 7683B autosampler, and Agilent 355 DP SCD were used. The sulfur standards in toluene and iso-octane (10:90) were purchased from Supelco (Bellefonte, PA). The component information is in Table 1.

Table 1. Sulfur Standards Components

	Components	Formula	Concentration (ppm) (w/w)
1	Ethyl mercaptan	CH_3CH_3SH	11.62
2	Dimethyl sulfide	$(CH_3)_2S$	11.92
3	Carbon disulfide	C_2S	17.84
4	Isopropyl mercaptan	$(CH_3)_2CHSH$	34.32
5	T-butyl mercaptan	$(CH_3)_3CSH$	11.28
6	n-propyl mercaptan	$CH_3CH_2CH_2SH$	5.93
7	Methylethyl sulfide	$CH_3CH_2SCH_3$	11.87
8	Thiophene	C_4H_4S	14.81
9	Sec-butyl mercaptan	$CH_3CH_2CH(SH)CH_3$	23.26
10	n-butyl mercaptan	$CH_3(CH_2)_3SH$	5.89
11	Dimethyl disulfide	CH_3SSCH_3	14.75
12	2-methyl thiophene	C_5H_6S	14.29
13	3-metyl thiophene	C_5H_6S	21.35
14	Diethyl disulfide	$(C_2H_5)_2S_2$	27.99
15	Benzo(b)thiophene	C_8H_6S	40.49

NIST Standard Reference Material 2299, Sulfur in Gasoline, was used. The total sulfur in gasoline is 13.6 ± 1.5 µg/g based on analyses by isotope dilution thermal ionization mass spectrometry (ID-TIMS). Homogeneity testing was performed using X-ray fluorescence spectrometry.

Experimental Conditions

7890A GC Conditions

Front inlet	Split/splitless (Sulfinert-treated capillary inlet system)
Heater	275 °C
Pressure	10.951 psi
Septum purge flow	3 mL/min
Mode	Split
Gas saver	20 mL/min after 2 min
Split ratio	10 :1
Split flow	15 mL/min
Oven	30 °C (1 min) 10 °C/min 250 °C (1 min)
Column	HP-1 30 m × 0.32 mm × 4 µm (P/N 19091Z-613)

SCD Conditions – Agilent G6603A

Burner temperature	800 °C
Vacuum of burner	372 torr
Vacuum of reaction cell	5 torr
H_2	40 mL/min
Air	53 mL/min

Results and Discussion

Several commercially available sulfur detectors are available for the determination of sulfur compounds in various matrices. When compared to flame photometric detection (FPD), pulsed flame photometric detection (PFPD), atomic emission detection (AED), and inductively coupled plasma-mass spectrometry (ICP-MS), the SCD shows the best performance based on stability, cost, and quantification. [2,3]

With DP technology, the performance of Agilent 355 SCD has been further enhanced, for unsurpassed stability, selectivity, and lack of quenching. Table 2 lists the DP SCD stability at different sulfur levels.

Table 2. Repeatability of Sulfur Compounds at Different Concentrations (Refer to Table 1 for peak identification.)

	1	2	3	4	5	6	7	8	10	11	12	13	14	15
Con. ppm	1.16	1.79	1.78	3.42	1.13	0.59	1.19	3.80	0.59	1.47	1.43	2.14	2.80	4.0
RSD (%)	2.8	3.6	3.1	1.9	3.0	2.7	3.9	3.9	2.9	2.1	2.2	2.9	0.4	3.7
Con. ppm	0.12	0.18	0.18	0.34	0.11	0.06	0.12	0.38	0.06	0.15	0.14	0.21	0.28	0.4
RSD (%)	5.7	7.4	3.4	3.7	6.6	4.8	5.7	4.8	8.0	4.0	3.3	4.7	7.3	3.1

Correlation coefficients of the tested sulfurs over three orders of magnitude were better than 0.99 (R^2). Table 3 shows the linearity of each sulfur compound. Figure 1 shows the chromatogram of sulfurs in a hydrocarbon matrix without interference (1 to approximately 2 µg/kg).

Table 3. Linear Ranges of Tested Sulfurs (Refer to Table 1 for peak identification.)

Analytes	Concentration Range	Linearity (R^2)
1	0.1ppm to 10ppm	0.9975
2	0.1ppm to 10ppm	0.9982
3	0.1ppm to 10ppm	0.9991
4	0.1ppm to 10ppm	0.9992
5	0.1ppm to 10ppm	0.9995
6	0.1ppm to 10ppm	0.9996
7	0.1ppm to 10ppm	0.9998
8/9	0.1ppm to 10ppm	0.9998
10	0.1ppm to 10ppm	0.9994
11	0.1ppm to 10ppm	0.9999
12	0.1ppm to 10ppm	0.9997
13	0.1ppm to 10ppm	0.9995
14	0.1ppm to 10ppm	0.9997
15	0.1ppm to 10ppm	0.9999

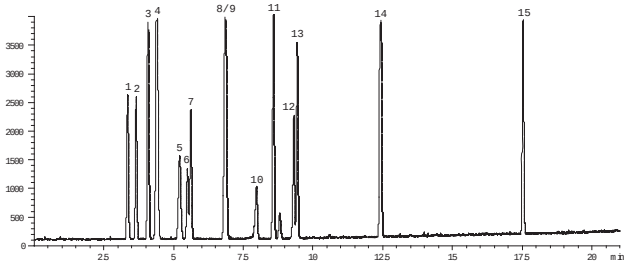

Figure 1. Chromatogram of sulfur standard in hydrocarbon matrix. (Refer to Table 1 for peak identification.)

The data in Table 4 illustrate the sensitivity (S/N) of 355 SCD for trace-level analysis (approximately 20 ng/kg) of sulfurs in a hydrocarbon matrix. Figure 2 shows the chromatogram of trace sulfurs, which also indicates no interferences in the analysis.

Table 4. Sulfur Sensitivity (Refer to Table 1 for peak identification.)

Peak No.	1	2	3	4	5	6	7	8/9	10	11	12	13	14	15
S/N	2.0	2.5	5.0	4.6	1.8	1.6	2.4	5.0	1.5	3.6	2.0	4.6	3.2	5.2

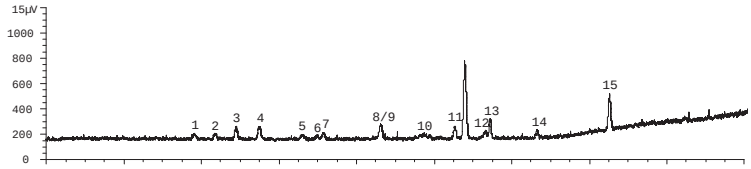

Figure 2. Chromatogram of sulfurs at trace levels (20 ng/g). (Refer to Table 1 for peak identification.)

Gasoline Samples Analysis

NIST Standard Reference Material 2299 was detected on an Agilent SCD and the mass concentration of total sulfur in sample was calculated by summing the sulfur content of all sulfur components (known and unknown) in the sample to arrive at its total sulfur value as recommended by ASTM 5623.

Figure 3A shows the chromatogram of the sulfur standard and Figure 3B shows the chromatogram of the standard gasoline sample. The total amount of sulfur is calculated by summing all the peak areas in Figure 3B and quantitated as thiophene 11.8 µg/g of total sulfur with an RSD 2.7% (n = 5) was calculated in the gasoline sample. This was within the specified range of 13.6 ± 1.5 µg/g.

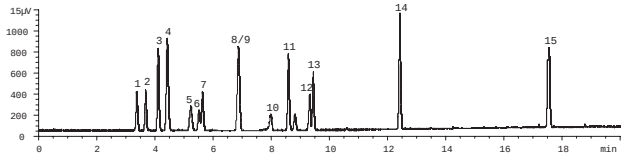

Figure 3A. Chromatogram of sulfur standard.

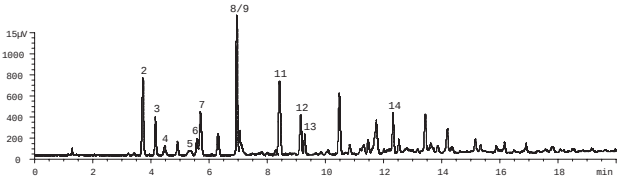

Figure 3B. Chromatogram of gasoline standard sample. (Refer to Table 1 for peak identification.)

Conclusions

The Agilent DP SCD is used for the detection of sulfur compounds in a complex hydrocarbon matrix. The results show that the DP SCD has linear response to sulfur compounds without quenching, yielding MDLs down to 20 ng/g. This solution is available as an Agilent preconfigured system; please refer to Agilent SP1 7890-0365 for ordering information.

References

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Printed in the USA
August 12, 2008
5989-9233EN

Agilent Technologies

An Alternative Application for a Highly Oxygen-Selective Capillary GC Plot Column

Application Note

Hydrocarbons

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USA

Abstract

This application note successfully shows selective retention and resolution of low molecular weight sulfur containing species on an Agilent J&W GS-OxyPLOT column. The selective retention for sulfur species enables enough of a retention shift away from hydrocarbon matrices such as base gasoline and n-butane to demonstrate the feasibility of effective quantification of sulfur species in process streams. Using an Agilent J&W GS-OxyPLOT column it is possible to shift the retention of sulfur containing analytes away from a hydrocarbon matrix to achieve effective quantification in the low ppm range.

Agilent Technologies

Introduction

The Agilent J&W GS-OxyPLOT column is highly selective for oxygen-containing species. Most of the initial applications for this column focus on the determination of trace level oxygenates in hydrocarbon matrices [1,2]. Example applications include testing and quantification of species containing oxygen in complex matrixes such as reformulated gasoline and C1-C4 hydrocarbon process streams. ASTM method 7059 is in effect for the analysis of methanol in crude oil by GC [3]. A recently approved method (June 2009, subcommittee D02.D04) D7423 for the analysis of trace oxygenates in light hydrocarbon matrices is also an application of the Agilent J&W GS-OxyPLOT column. Sub 10 ppm quantifications of alcohols, aldehydes and ethers in these feed stocks are important analyses to avoid poisoning of the catalysts used in processing these materials.

Oxygen and Sulfur Chemical Similarity

Chemical species that contain oxygen and sulfur share similar chemical behaviors often undergoing similar reactions to form similar products [4]. Affinity and retention on a highly oxygen selective PLOT column by sulfur-containing species is no exception. This application note demonstrates that the chromatographic behaviors of species containing sulfur on an Agilent J&W GS-OxyPLOT are quite similar to the behaviors demonstrated by oxygen-containing species on the same phase.

Selective retention and resolution of species containing sulfur from complex hydrocarbon matrices can help facilitate trace level analysis of low boiling mercaptans, thiols and sulfides. Monitoring levels of these compounds at low ppm levels has become increasingly important as stack emission and fuel content regulations have stiffened. In hydrocarbon processing, analyses of the sulfur content in feedstocks are used to make processing decisions that hopefully avoid sulfur poisoning of expensive catalysts and enhance refinery throughput.

Experimental

Three different sulfur standards, from liquid to gas, were tested using the Agilent J&W GS-OxyPLOT column.

For the test of a liquid sample, a standard mix of 14 sulfur containing compounds in base gasoline was purchased from Spectrum Quality Standards, Houston TX. Class A volumetric flasks and pipettes were used for dilutions. The liquid sample was analyzed at Agilent Technologies Little Falls Site in Wilmington DE.

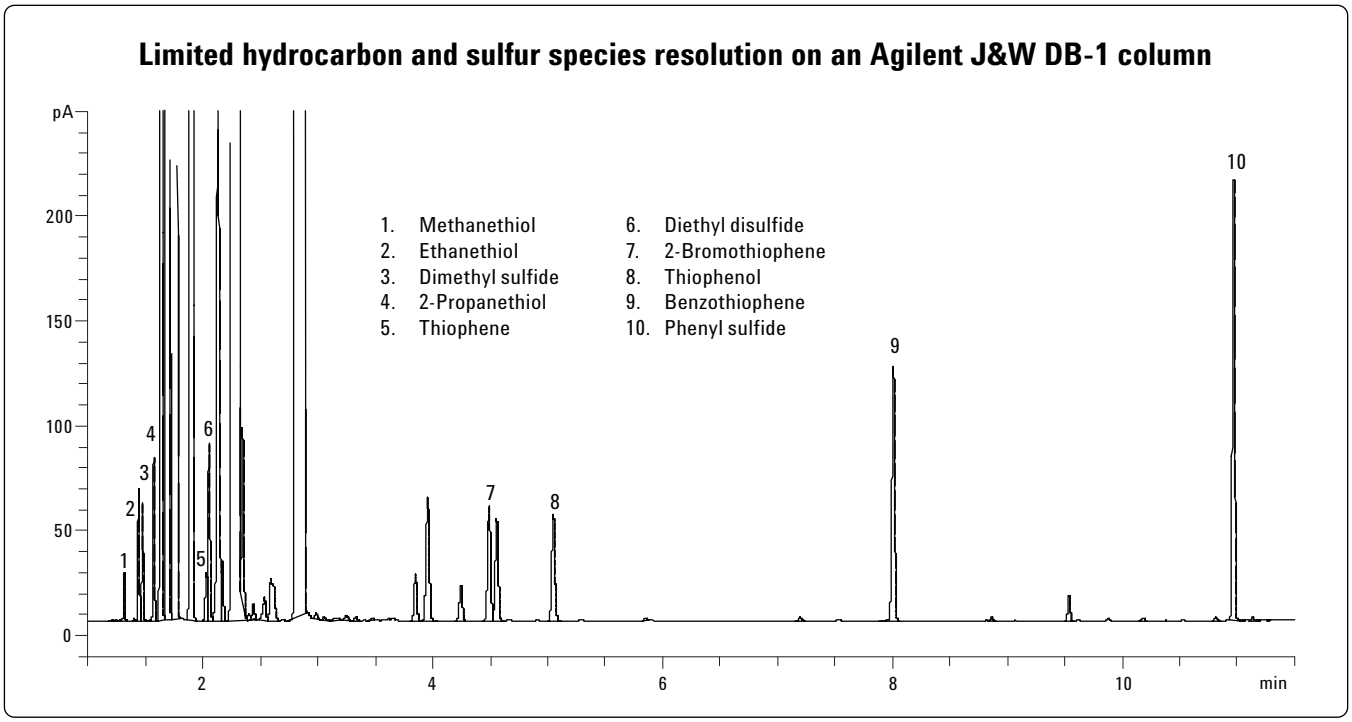
For the test of a gaseous sample, a sulfur mix of 11 sulfur compounds in N_2 was prepared and analyzed in DCG Partnership, Pearland TX.

For the test of a liquefied gas-liquid sample, a sulfur standard mix of 5 sulfur compounds in n-butane was prepared and analyzed in DCG Partnership, Pearland TX.

Chromatographic conditions appear beneath each figure.

Results and Discussion

Figure 1 shows the injection of a liquid sulfur standard in a base gasoline matrix on an Agilent J&W DB-1 column. Most of the sulfur species of interest elute early in the chromatogram along with the hydrocarbon species in the gasoline matrix. This figure illustrates the difficulty in separating sulfur species commonly found in gasoline from hydrocarbons in the gasoline matrix with a primarily boiling point separation mechanism. Higher selectivity and retention for the lighter sulfur species is necessary to resolve the peaks containing sulfur from the hydrocarbon matrix. This type of application is where the selective retention of sulfur species on the Agilent J&W GS-OxyPLOT is most useful.



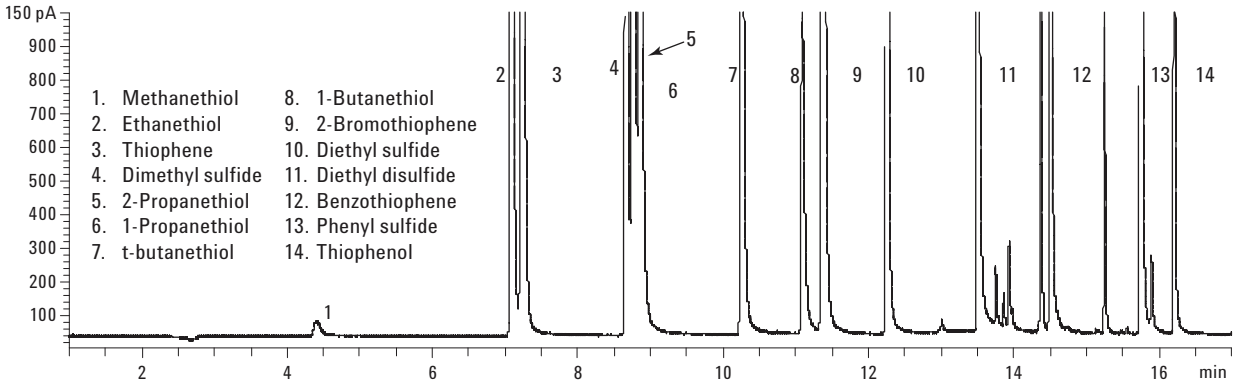
GC:	Agilent 6890N network GC system
Column:	Agilent J&W DB-1 0.53 mm × 25 m, 1.0 µm (Agilent part number 125-102J)
Oven:	60 °C (0.5 min), 10 °C/min to 120 °C, then 20 °C/min to 300 °C (3 min)
Injection:	250 °C, 1 µL, 25:1 split
Carrier:	He, constant flow, 30 cm/s at 60 °C (3.9 mL/min)
Detection:	FID 350 °C, H_2 40 mL/min, Air 450 mL/min, N_2 makeup 30 mL/min

Figure 1. Chromatogram of a liquid sulfur standard mix in base gasoline on an Agilent J&W DB-1 column.

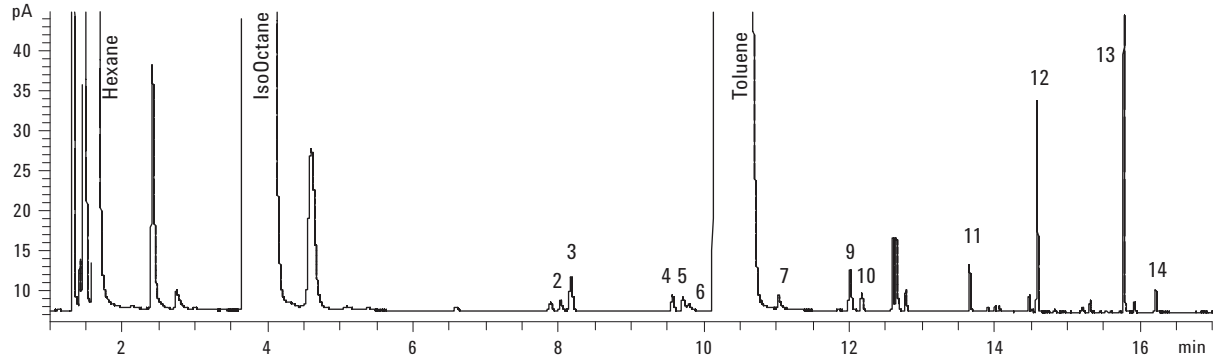
Figure 2 illustrates the selective retention of the sulfur-containing species versus the components in a base gasoline standard. The FPD signal in Figure 2A shows the elution of the sulfur-containing species and helps with peak identification. The FID signal in Figure 2B shows the elution of both the hydrocarbons and the sulfur species as they elute from the Agilent J&W GS-OxyPLOT. Most of the hydrocarbon components of the gasoline matrix, with the exception of toluene, elute early in the chromatogram and are resolved from the sulfur species of interest.

Selective sulfur species resolution from hydrocarbons on an Agilent J&W GS-OxyPLOT

A) 25 ppm Liquid Std OxyPLOT/FPD



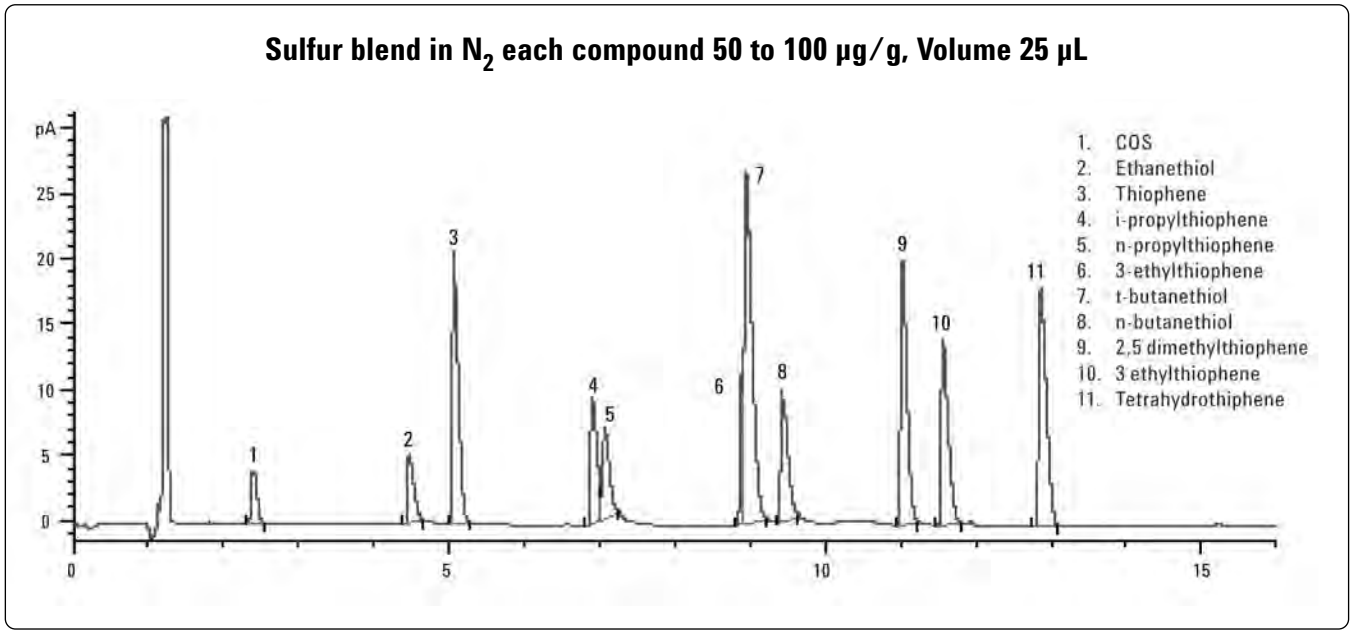
B) 25 ppm Liquid Std OxyPLOT/FID



GC:	Agilent 6890N network GC system, Dual Injection
Columns:	Agilent J&W GS-OxyPLOT 0.53 mm × 10 m, 10 µm (Agilent part number 115-4912)
Oven:	40 °C (3 min), 8 °C/min to 110 °C, then 35 °C/min to 300 °C (3 min)
Injection:	250 °C, 1 µL, 25:1 split, gas saver 20 mL/min at 2min
Carrier :	He (Col 1 to FID), 4.7 mL/min constant flow
	He (Col 2 to FPD), 3.15 mL/min constant flow
Detection:	FID 350 °C, H_2 40 mL/min, Air 450 mL/min, N_2 makeup 30 mL/min
	FPD 250°C, H_2 50 mL/min, Air 60 mL/min, N_2 makeup 60 mL/min

Figure 2. Chromatogram of a liquid sulfur standard mix in base gasoline on an Agilent J&W GS-OxyPLOT columns with simultaneous injection. Figure 2a (top) is the FPD signal and Figure 2b (bottom) is the FID signal.

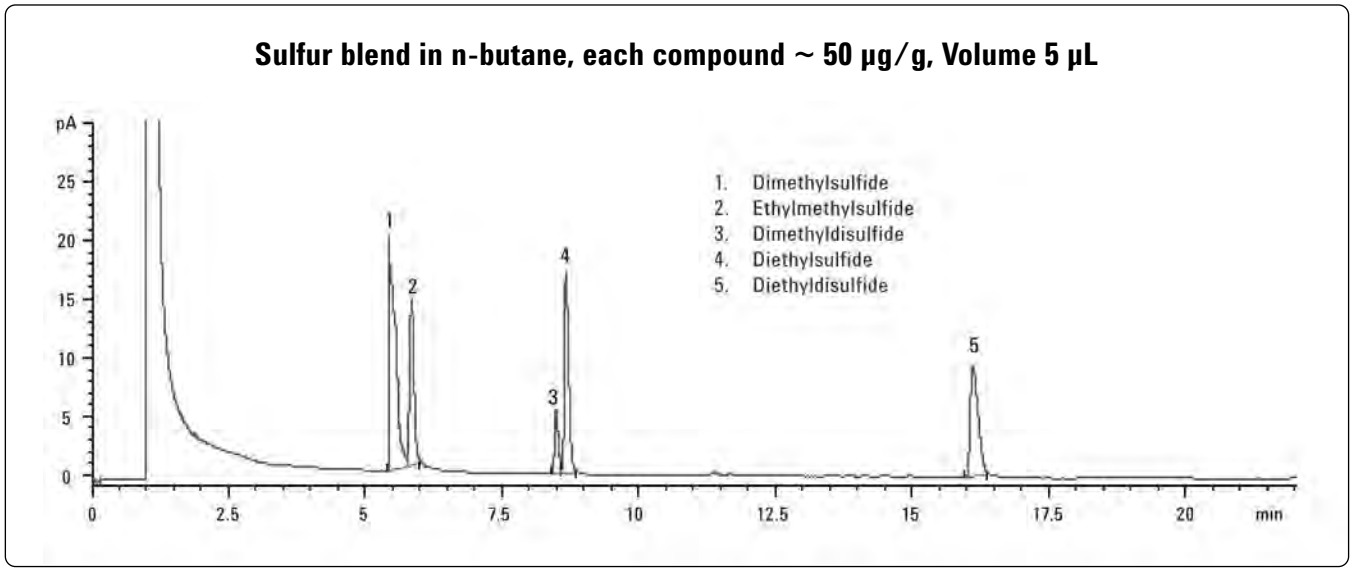
Figure 3 shows the injection of a gaseous sulfur species blend in N_2 on a combination of an Agilent J&W DB-1 connected by a glass insert with an Agilent J&W GS-OxyPLOT. The sulfur species in this sample are well retained. The selective retention of the Agilent J&W GS-OxyPLOT was again useful in separating the sulfur species of interest and achieving retention for these low boiling sulfur compounds.



GC:	Agilent 6890N network GC system
Column:	Agilent J&W DB-1 0.53 mm × 10 m, 0.5 µm (Agilent part number 125-1017)
	+ Agilent J&W GS-OxyPLOT 0.53 mm × 10 m, 10 µm (Agilent part number 115-4912)
Oven:	40 °C (0.1 min), 10 °C/min to 300 °C (5 min)
Injection:	250 °C, 25 µL, 10:1 split
Carrier:	H_2, 0.9 mL/min constant flow
Detection:	FID 250 °C, H_2 30 mL/min, Air 300 mL/min, N_2 makeup 15 mL/min

Figure 3. Chromatogram of a gaseous sulfur standard blend in N_2 on an Agilent J&W GS-OxyPLOT column.

Figure 4 shows an injection of a low molecular weight sulfur standard blend in a liquid n-butane matrix on a combination of an Agilent J&W DB-1 column connected by a glass insert with an Agilent J&W GS-OxyPLOT column. Excellent retention and resolution for the sulfur species from the n-butane were observed. An FID detector was used for this test. Figure 4 shows evidence of overloading of the trace level components in this detector, due to the large sample size. The large volume of injection caused no baseline problem, because of the effective separation away from the non-polar matrix of the analytes.



GC:	Agilent 6890N network GC system
Column:	Agilent J&W DB-1 0.53 mm × 10 m, 0.5 µm (Agilent part number 125-1017)
	+ Agilent J&W GS-OxyPLOT 0.53 mm × 10 m, 10 µm
	(Agilent part number 115-4912)
Oven:	40 °C (0.1 min), 10 °C/min to 300 °C (5 min)
Injection:	250 °C, 5 µL, 10:1 split
Carrier:	H_2, 0.9 mL/min constant flow
Detection:	FID 250 °C, H_2 30 mL/min, Air 300 mL/min, N_2 makeup 15 mL/min

Figure 4. Chromatogram of a liquefied gas-liquid sulfur standard blend in n-butane on an Agilent J&W GS-OxyPLOT.

Conclusions

This application note successfully demonstrates selective retention and resolution of low molecular weight sulfur containing species on an Agilent J&W GS-OxyPLOT column. The selective retention for sulfur species enables enough of a retention shift away from hydrocarbon matrices such as base gasoline and n-butane to suggest that effective quantification of sulfur species in process streams is quite feasible.

The Agilent J&W GS-OxyPLOT column also retained toluene in the base gasoline sample where co-elution was observed between toluene and tert-butanethiol in the liquid standard mix. Potential interferences between analytes of interest and aromatic species are a possibility with the Agilent J&W GS-OxyPLOT column that should be manageable with careful planning and method design.

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Printed in the USA
August 28, 2009
5990-4493EN



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Analysis of Sulfur and Phosphorus Compounds with a Flame Photometric Detector on the Agilent 6890 Series Gas Chromatograph

Application

Gas Chromatography

February 1997

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Abstract

A gas chromatograph (GC) with a flame photometric detector (FPD) is frequently employed in analyzing complex samples for specific compounds. The wavelength filter of the FPD can be set to select for many elements, but it is most commonly used to detect sulfur and phosphorus. This application note discusses the uses of the FPD in gas chromatography, demonstrates the linearity and method detection limits (MDL) of the 6890 Series GC with an FPD, and gives examples of analyses of organophosphorus pesticides using the 6890 GC with an FPD.

Key Words

Gas chromatography, flame photometric detector, FPD, sulfur analysis, phosphate analysis, pesticides, organophosphorus pesticides, EPA method 1618, EPA method 622.

Introduction

The flame photometric detector is one of the most widely used selective detectors in gas chromatography. The FPD consists of a reducing flame that produces chemiluminescent species. These species emit characteristic light that is optically filtered for the desired wavelength; the wavelength selection determines which compound is detected. The filtered light is measured by a photomultiplier and transduced into a signal. A second photomultiplier can be added, which allows simultaneous detection of a second signal.

FPD filters can be selected for many different compounds, but the most common uses are for the selective detection of sulfur and phosphorus compounds in complex mixtures. The selectivity of classical FPDs is typically (as a ratio by weight to carbon) 10^5 for sulfur and 10^6 for phosphorus. The FPD operates over a dynamic range of 1×10^3 for sulfur and 1×10^4 for phosphorus.[1]

Gas chromatography with an FPD can be used to detect sulfur compounds in crude oil and sulfur contaminants in natural gas.

In food analysis it is used to detect off-flavors resulting from the liberation of volatile sulfur compounds. It is also used to simultaneously detect sulfur and phosphorus in chemical warfare agents. In the environmental area, the FPD is used for detection of organophosphorus pesticides and herbicides. Several EPA methods for pesticide detection, including EPA methods 1618[2] and 622[3], specify the use of an FPD.

A schematic of a single FPD for the 6890 Series GC is shown in figure 1. A dual wavelength version is available that has a second photomultiplier mounted perpendicular to the first for simultaneous detection of a second wavelength. The 6890 GC is available with either a single or dual FPD.

The sensitivity of any FPD is affected by detector temperature, flame chemistry, and filter wavelength.

- **Detector temperature**. To protect the photomultiplier, the maximum temperature limit for the 6890 FPD is 250 °C. Photomultiplier tube (PMT) noise increases with setpoint temperature, so the detector temperature should be as low as possible. Generally, the temperature should be set about 25 °C above the highest temperature reached in the oven program. To prevent water condensation and clouding of the window, the minimum operating temperature is 120 °C.[4]

- **Flame chemistry**. FPD sensitivity is highly dependent on detector gas flows. On the 6890 GC, the gas flows are electronically controlled. This allows rapid and precise optimization of flow rates. Sulfur and phosphorus modes have different optimum flow requirements, so the ability to easily set and reset flows increases the quality of results and saves time.
- **Filter wavelength**. For the FPD, filters of specific wavelength are physically installed in the detector. A 394-nm filter is used for sulfur detection, and 526-nm filter for phosphorus detection.

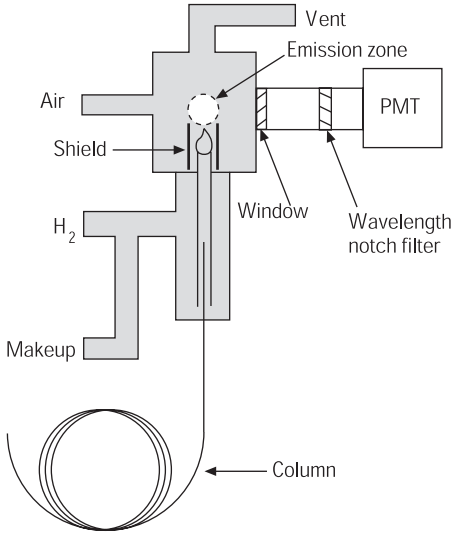


Figure 1. Single photomultiplier tube FPD for 6890 Series GC

Experimental

All experiments were performed on a 6890 Series GC with electronic pneumatics control (EPC) and an Agilent 7673 automatic liquid sampler (ALS). An Agilent 1707A ChemStation was used for instrument control and data acquisition. Chromatography conditions are shown with the individual chromatograms in figures 2, 3, and 4.

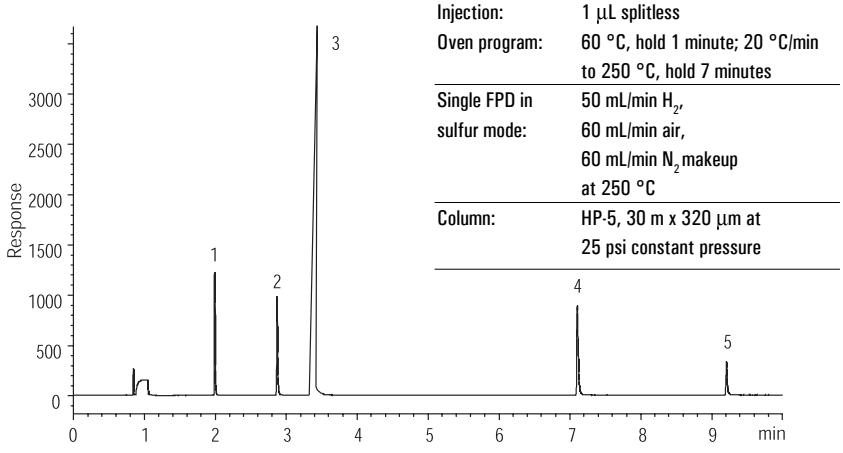
Table 1. MDL and Linearity over 10^2 Range Sulfur Mix on the FPD

Peak Number	Compound Name	MDL pgS/sec n = 11	Linearity r^2 n = 15
1	2,5-dimethylthiophene	26.22	0.9986
2	sec-butylsulfide	20.10	0.9983
3	1,4-butanedithiol	22.27	0.9972
4	dodecanethiol	16.90	0.9985
5	octyl sulfide	16.14	0.9979

Results and Discussion

Linearity and MDL

In sulfur mode, the response of the FPD is proportional to analyte concentration squared. The calculated MDL and r^2 values from linearity experiments for a single photomultiplier in sulfur mode are listed in table 1, and the chromatogram for a 20–40 ppb sample from the experiment is shown in figure 2. The square of the concentration was used to calculate regression statistics. When using a ChemStation for data analysis, a quadratic calibration fit is used for sulfur.



Conditions

Injection:	1 µL splitless
Oven program:	60 °C, hold 1 minute; 20 °C/min to 250 °C, hold 7 minutes
Single FPD in sulfur mode:	50 mL/min H_2, 60 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	HP-5, 30 m x 320 µm at 25 psi constant pressure

Figure 2. 1 µL of 1.5 ppm sulfur standard, FPD in sulfur mode (The peaks are identified in table 1.)

The response of the FPD is linear in phosphorus mode. Table 2 shows the r^2 values for an organo-phosphorus pesticide mixture and the MDL calculated from the study. Figure 3 shows the chromatogram. A standard linear curve fit is used for phosphorus when using a ChemStation for data analysis.

Analysis of EPA Method 1618

Figure 4 shows the chromatogram obtained from the analysis of organophosphorus pesticides according to EPA method 1618. The injected concentration of each compound was 1–2 ppm.

Table 2. MDL and Linearity over 10^3 Range for Organophosphorus Pesticides on the FPD

Peak Number	Compound Name	MDL pgPesticide/sec n = 11	Linearity r^2 n = 15
1	phorate	1.85	0.9996
2	demeton	1.13	> 0.9998
3	disulfoton	1.31	> 0.9999
4	diazinon	1.74	> 0.9999
5	malathion	1.74	> 0.9999
6	fenthion	1.75	> 0.9999
7	parathion	1.84	> 0.9999
8	trichloronate	2.27	> 0.9999
9	tokuthion	2.51	> 0.9999
10	fensulfothion	—	> 0.9999
11	ethion	1.29	> 0.9999
12	sulprofos	2.36	> 0.9999
13	guthion	1.24	> 0.9999
14	coumaphos	2.08	> 0.9999

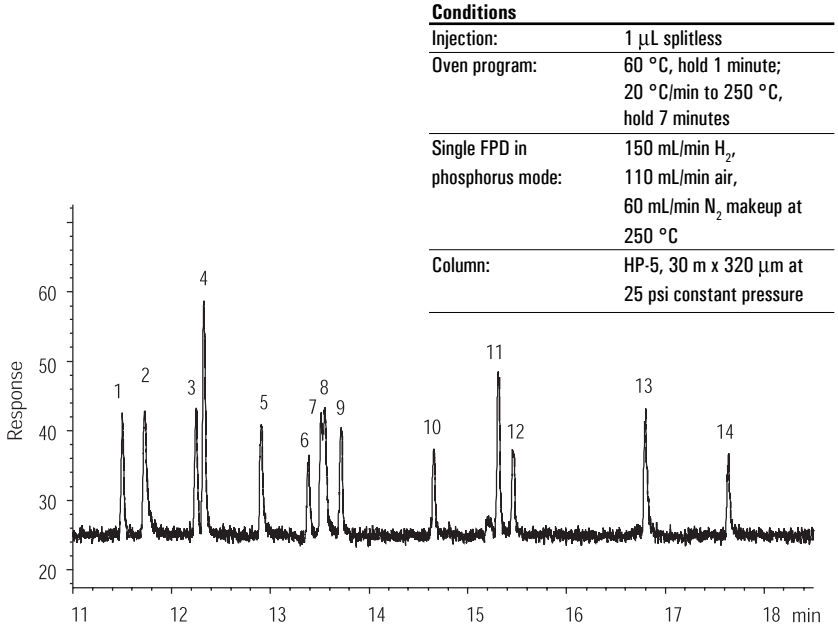


Figure 3. 1 µL Splitless injection of 20–40 ppb organophosphorus pesticide standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conditions	
Injection:	1 µL cool on-column
Oven program:	60 °C, hold 0.5 minute; 25 °C/min to 110 °C, 3 °C/min to 250 °C, hold 10 minutes
Single FPD in phosphorus mode:	75 mL/min H_2, 100 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	1.5 µm HP-1, 30 m x 530 µm, 7 mL/min He constant flow

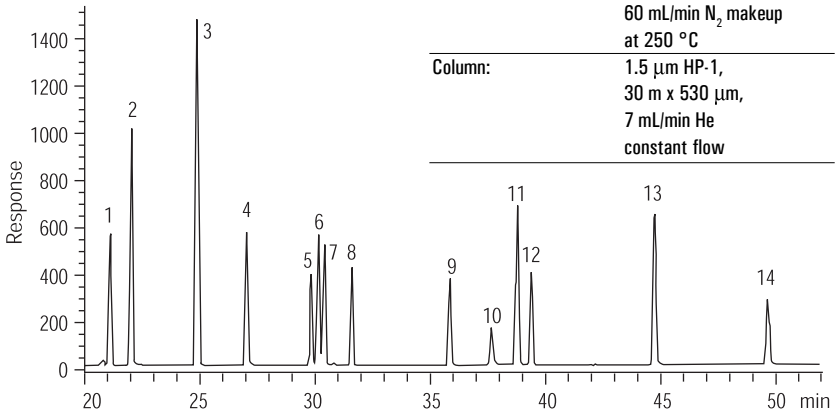


Figure 4. Analysis of organophosphorus pesticides according to EPA method 1618, 1 µL on-column injection of 1–2 ppm standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conclusions

The Agilent 6890 Series GC with an FPD can be used for the sensitive, and selective measurement of sulfur- and phosphorus-containing compounds in complex mixtures. The electronic pneumatics control on the Agilent 6890 GC ensures rapid and accurate gas flow control, provides for easier method setup and documentation, and simplifies optimization.

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Printed in the USA 3/2000
5965-7442E

Predictable Translation of Capillary Gas Chromatography Methods for Fast GC

Application

Gas Chromatography

March 1997

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Abstract

The time required for capillary gas chromatography (GC) runs is often the major factor in sample turnaround time. Fast GC can significantly improve laboratory productivity by decreasing analysis time. However, converting established capillary GC methods to fast GC can be a daunting task.

The Agilent GC method translation software is a freeware program that logically and predictably translates traditional capillary GC methods. The translation program preserves the elution order for each compound, so that identification of each peak does not have to be repeated. Validation for the new method is made easier. The method translation software will instantly calculate temperature and flow conditions to meet various requirements including retention time locking, best theoretical efficiency, and decreased run time.

This application note discusses considerations involved in choosing fast GC over conventional GC, describes the Agilent GC method translation software, and demonstrates translating methods for faster analysis of solvents, styrene monomers, reformate gasoline, hydrocarbon emissions, semivolatile mixtures, and a chemical process intermediate.

Key Words

Capillary GC, fast GC, gas chromatography, laboratory productivity, method translation, solvent analysis, styrene monomer analysis, reformate gasoline analysis, hydrocarbon emission analysis, semivolatile analysis.

Introduction

A goal of many laboratories is to decrease the turnaround time for each sample. Shorter turnaround times mean quicker analytical results, lower operating costs, increased laboratory productivity, and higher revenues. Fast gas chromatography (GC) promises faster sample analysis, and is thus appealing as a component of increased productivity.

Fast GC is only one possible way to improve productivity. Many of the contributions to the total time required for sample analysis are not affected by the GC run time.

A complete analysis involves sample preparation, sample introduction, the GC cycle time (including run time and oven cool-down), data analysis, report generation, and the time required to document and track each sample. If the GC run time is short compared to the other steps, changing to fast GC may not result in concomitant productivity improvement. Fast GC is most appropriate when GC run times are a major contribution to total sample turnaround time. The laboratory should streamline as many operations as possible as part of a total productivity improvement program.

Once the GC run time is targeted as an area to improve, there are a

Table 1. Benefits and Drawbacks of Changing to Fast GC Methods

Parameter	Benefit	Detriment
Increase speed of injection process	Faster, more reproducible injections; automation	Cost of purchasing an automatic injector
Decrease column id (50 mm = current practical limit)	Faster optimum carrier gas velocity; shorter columns (higher plates/m); fastest possible separations	Lower column capacity; requires high inlet pressure; requires high split ratios or low injection volume
Increase carrier gas velocity	No new equipment/supplies required	Lower resolution; may degrade detector performance
Shorten column	Standard id columns (capacity); low pressure drops; standard hardware	Low separation power (resolution)
Perform isothermal analysis	No cool down cycle; short time until elution of early peaks of interest	No focusing possible; no splitless or cool on-column possible; applicable to narrow volatility range of solutes unless using multiple columns; contamination by late eluters
Increase oven program rate	Elutes full volatility range in minimum time	Lower resolution; limited by instrument capabilities; peak reversals possible
Change carrier gas (H_2 > He > N_2)	Same efficiency in shorter time with H_2; cost savings	Safety issues with H_2

number of ways to speed up the GC analysis time. However, there are tradeoffs in any attempt to decrease analysis time. A balance between speed, sensitivity, and resolution must be selected for each analysis to meet the laboratory goals. Table 1 lists the major benefits and potential disadvantages of optimizing each run parameter for speed. Adapting methods for fast GC can be complicated because peak reversals are common, and some fast GC methods decrease separation efficiency. This is illustrated in figure 1.

Figure 1, chromatogram A, shows a standard GC chromatogram for a semivolatile mixture using splitless injection. The run time is 45 minutes. Figure 1, chromatogram B, shows the same sample using fast GC. The run time has been decreased from 45 minutes to 12.5 minutes, but the oven temperature program was changed with little regard for possible peak shifting or reversals.

Although the chromatograms are similar and most peaks are adequately separated, a closer examination reveals problems typical of method adaptation. Figure 1, chromatograms C and D, compare the center sections of the two chromatograms A and B, respectively.

The three peaks labeled “1” in chromatogram C are reversed in chromatogram D. The peak pair at “2” is reversed in the fast GC run, and the two peaks at “3” in chromatogram C co-elute in chromatogram D.

These changes mean that validating the new method will be a time-consuming process. The Agilent GC method translation software avoids these problems by making the change from traditional capillary GC methods to fast GC methods predictable.

It locks the elution order for each compound, so peaks do not have to be painstakingly identified. This decreases the time necessary to validate the new method. The method translation software instantly calculates the correct oven temperature program and column head pressure as a function of the new column dimensions, phase ratio, and carrier gas type.

When using the method translation software to calculate conditions for a different column, the new column should have a stationary phase identical to the original column. Columns of 100 percent methyl and 5 percent phenyl/95 percent methyl can often be used interchangeably from different manufacturers, but more polar columns can vary significantly between manufacturers. Method translation may not preserve the elution order for phases that are chemically different. As a column ages, the stationary phase may decompose or become contaminated. This can also affect peak elution order initially and over time.

Method Translation Software

The Agilent GC method translation software, version 2.0, is a freeware program that simplifies fast GC method development. (For the web site address to download the freeware, refer to the last page of this publication.) The main data screen of the software is shown in figure 2.

The tool offers selection of several modes of method translation:

- **Translate Only**. Translates the current method to a new one based on a change of column dimensions, carrier gas type,

outlet pressure, and/or phase ratio. The relative retention times of the peaks are locked, so the order of elution is preserved. In "Translate Only" mode, the relative efficiency of the current method is maintained. This is useful if you need to convert a method from the literature, to convert an established method to use a column with different dimensions or a different phase ratio, or to change to a detector with a different outlet pressure.

- **Best Efficiency**. Calculates new conditions (using your current column) that correspond to the theoretical optimum gas flow rate for the gratest separation efficiency for most compounds. Simply enter your current conditions, and the program adjusts the temperatures and flow rate to match the theoretical optimum. The elution order of the peaks will stay the same, but the retention time will probably change.
- **Fast Analysis**. Calculates the temperature and pressure for your current column and carrier gas for a run that has an outlet carrier gas flow that is twice as fast as the "Best Efficiency" mode. Depending on the pressure drop across the column, run time will decrease 1.5 to 2 times. As always with method translation, the elution order of your current method will hold constant.

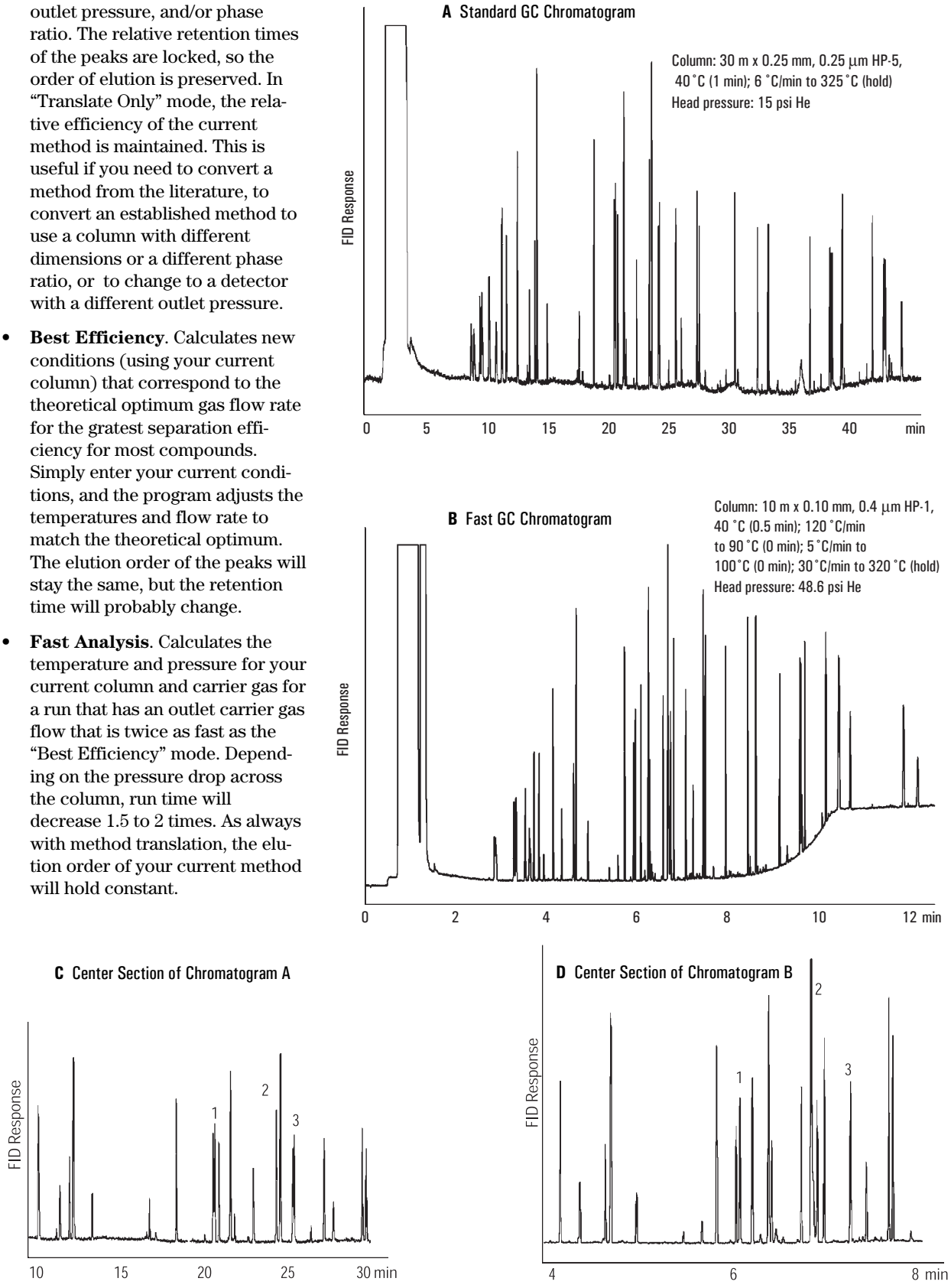


Figure 1. Comparison of traditional GC and fast GC for a splitless injection of a semivolatile mixture. (Concentration: 1 ppm; injection size: 0.5 mL)

- **None**. Allows you to change any run parameter of interest. You can decrease method development time by examining the effects of various parameters on the speed, head pressure, and oven temperature ramp of your method before you do any chromatography. This mode is also useful to try out changes to your current method. For instance, if your current method is already at maximum efficiency and you need to maintain separation power, you can enter a smaller column id to see the exact impact on run time. If you have excess separation power, you can calculate conditions for a shorter column or faster gas flow rate. The method translation software allows you to rapidly develop a feel for the effects f each parameter on speed of analysis, pressure, and oven temperature program rate.

Separation power (resolution) is a function of column dimensions, flow, and oven temperature. When a new column is selected to maintain separation efficiency, the GC method translation software will make sure that the flow and oven temperature ramp rates are scaled appropriately.

Experimental

All experiments were performed using an Agilent 6890 Series gas chromatograph (GC) with the 240-volt option. The 6890 Series GC has many features specifically designed for the successful migration to faster GC methods. These include:

- 100- and 150-psi EPC split/splitless inlet
- Automated split ratios to 7500:1
- Fast detector sampling rates (0.1–200 Hz) for flame ionization detection (FID) and nitrogen-phosphorus detection (NPD) with a ChemStation

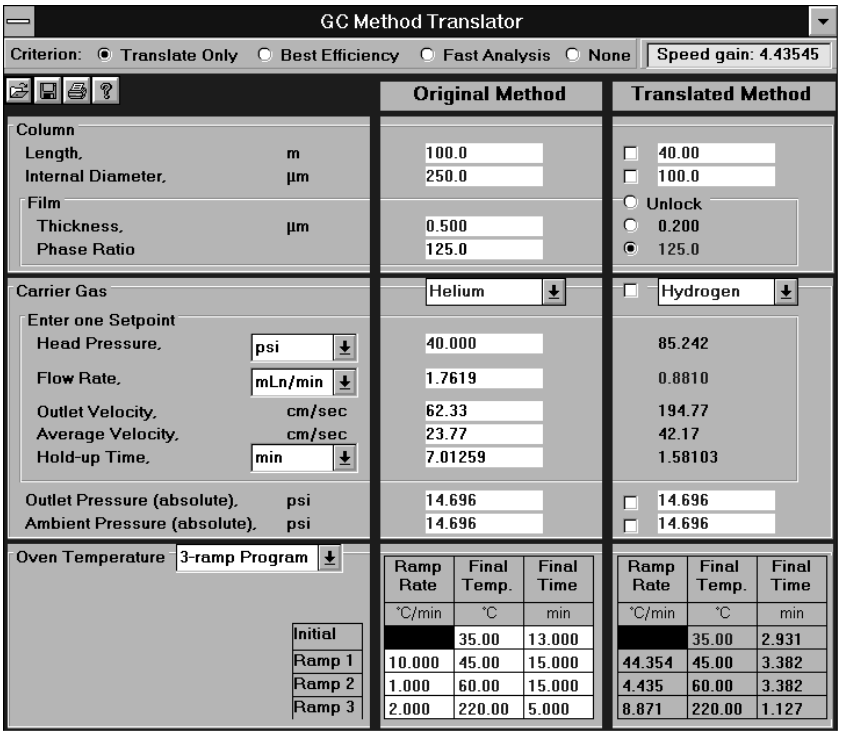


Figure 2. The Agilent GC Method Translation Software, Version 2.0a.

- Fast oven program rates (up to 120 °C/min, depending on the temperature range)

A G1916A automatic liquid sampler (ALS) was used for sample introduction, and an Agilent ChemStation was used for instrument control and data acquisition. Refer to the individual chromatograms for details about columns, temperatures, split ratios, carrier gas, gas flows, and oven ramps.

Injection Liner

To maximize sensitivity and resolution with split injections, it is important to obtain good peak shape. Using a different column size may require a different size liner to maintain good peak shape. Figure 3 demonstrates the difference in peak shape obtained with a 2-mm liner versus a 4-mm liner with glass wool plugs. The smaller diameter liner improves the peak shape for the 0.05-mm id column in this example.

Results and Discussion

Examples of fast GC for analysis of hydrocarbons in gasoline and simulated distillation are discussed in other Agilent publications.[1,2] This application note demonstrates the time savings achieved for various other analyses.

Chemical Process Intermediate

Figure 4 shows the development of a fast GC analysis for a chemical process intermediate. Figure 4, chromatogram A, shows the original method; figure 4, chromatogram B, shows the translated method with the column dimensions decreased by a factor of three and the relative retention times of the peaks constant. For even faster analysis, the column was shortened, as shown in figure 4, chromatogram C.

The relative retention times are still correct, and the run is almost eight

times as fast. In figure 4, chromatogram C, some resolution is lost due to the shorter column. Scaling the column to 10 m × 0.05 mm (0.2 mm) would have provided the identical resolution because of the higher efficiency per unit length of the smaller id column.

Solvent Analysis

A traditional capillary GC method for analysis of solvent from a commercial paint thinner is shown in figure 5, chromatogram A. Using the method translation software with the criterion of "fast analysis" selected, the carrier gas outlet flow rate was doubled. The tool calculated the oven temperature ramp that would maintain relative peak retention times at the faster flow rate. The resulting chromatogram is shown in figure 5, chromatogram B. The run time has decreased from 9 minutes to 6 minutes, and the peaks are still well separated.

Figure 6 shows the effect of changing carrier gas from helium to hydrogen in a similar analysis using smaller id columns. Because the theoretical optimum flow rate is faster for hydrogen than for helium, changing to hydrogen can significantly decrease the run time while not requiring high head pressures.

In figure 6, the flow rate of hydrogen was increased beyond that used for helium to reduce analysis time even

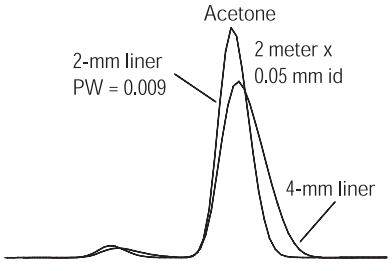


Figure 3. Peak shape vs liner diameter.

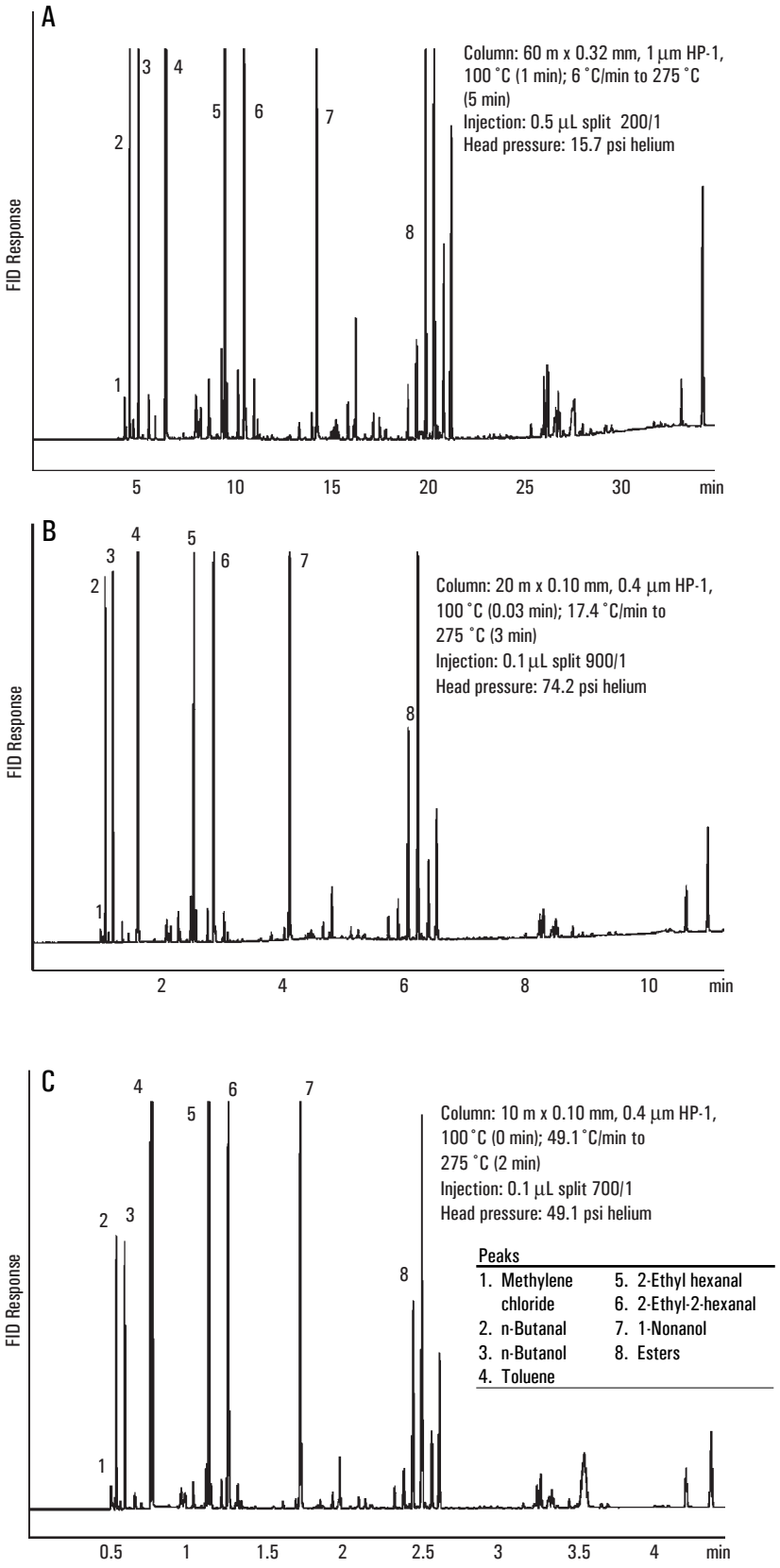


Figure 4. A chemical process intermediate on an HP-1 column at three different column sizes and phase ratios.

more. The conditions were not "translated." In the analyses shown in figures 5 and 6 (less than 10 highly resolved peaks), the use of method translation is optional. This is not the case with more complex samples.

Styrene Monomer Impurities

A standard GC analysis for impurities in styrene monomer takes about 12 minutes, as shown in figure 7. Using a smaller column with a faster oven program rate and slower carrier gas flow rate, an equivalent analysis was obtained in 3.6 minutes.

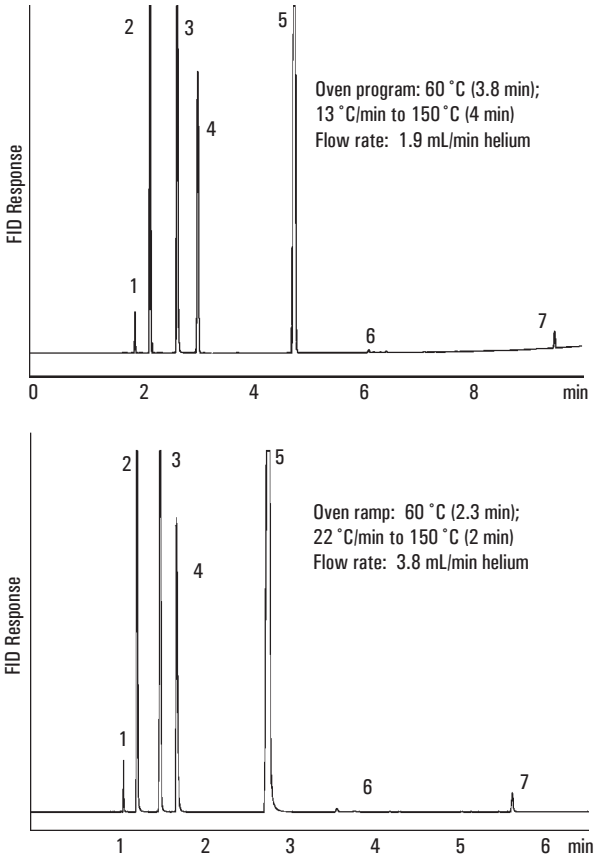
Reformate Gasoline Analysis

The development of a fast GC method for reformate gasoline analysis is shown in figure 8, chromatogram A, which shows a standard GC analysis that takes about 20 minutes. In figure 8, chromatogram B, a smaller column decreased the run time to 6 minutes. In figure 8, chromatogram C, the carrier gas was changed from helium to hydrogen, and the run time decreased to less than 4 minutes. The peaks are still well resolved and the order of elution is maintained. Table 2 shows the conditions used for the chromatograms in figure 8.

Conclusions

Despite the improvements in instrumentation, fast chromatography will always involve tradeoffs among speed, sensitivity, and resolution. The discussion here details considerations involved in choosing fast chromatography, discusses Agilent method translation software, and gives specific examples of some types of mixtures that are amenable to fast GC.

Use of Agilent method translation software eases the migration to faster methods by providing the conditions that will maintain the current order of elution. It can also highlight the potential instrument limitations (head

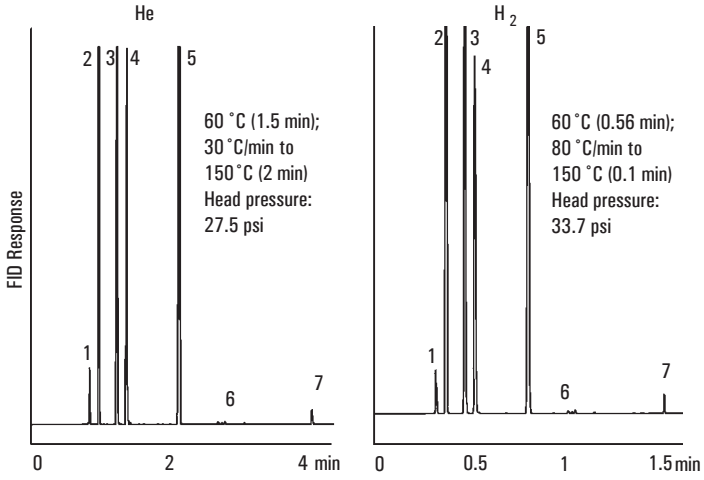


Peaks

1. Propylene oxide
2. Acetone
3. Methanol
4. Methylene chloride
5. Toluene
6. C_8 aromatics
7. 4-Hydroxy-4-methyl-2-pentanone

Column: 30 m x 0.32 mm, 0.5-mm HP-INNOWax
Injection: 0.5 µL
Split ratio: 200:1

Figure 5. Comparison of solvent analysis from a commercial paint remover at two flow rates



Column: 10 m x 0.10 µm HP-Wax
Injection size: 0.1 µL
Split ratio: 1000:1

Figure 6. Comparison of using helium and hydrogen carrier gas for analysis of a commercial paint remover

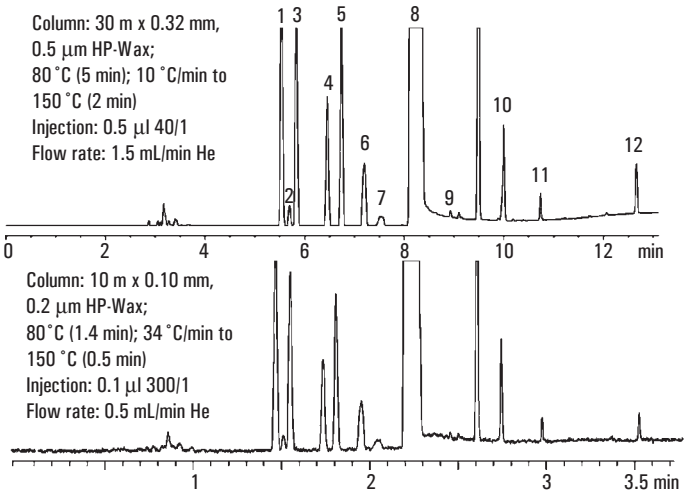
pressure or oven temperature program rate) that would be associated with translating a current method for use on a smaller id column.

To Obtain Agilent Method Translation Freeware

The Agilent method translation software can be downloaded from the Chemical Analysis Group (CAG) section of the Agilent home page on the World Wide Web at http://www.chem.agilent.com/cag/servsup/usersoft/main.html.

Table 2. Conditions for Fast GC of Reformate Gasoline

	A	B	C
Column	30 m x 0.32 mm	10 m x 0.32 mm	10 m x 0.10 mm
df	0.5 µm	0.2 µm	0.2 µm
Phase	HP-Wax	HP-Wax	HP-Wax
Carrier	Helium	Helium	Hydrogen
Pressure	6.4 psi	37.3 psi	21.9 psi
Flow	1.2 mL/min	0.37 mL/min	0.33 mL/min
Temperature	60 °C (hold 4 min)	60 °C (hold 1 min)	60 °C (hold 0.7 min)
Rate 1	10 °C/min	36.7 °C/min	55.1 °C/min
Final Temp	140 °C	140 °C	140 °C
Rate 2	15 °C/min	55.1 °C/min	70 °C/min
Final Temp	200 °C (hold 4 min)	200 °C (hold 2 min)	200 °C (hold 1 min)
Injection Volume	0.5 µL; split 200:1	0.1 µL; split 800:1	0.1 µL; split 800:1



Peaks

1. Ethylbenzene	7. p/m-Ethyl toluene
2. p-Xylene	8. Styrene
3. m-Xylene	9. a-Methylstyrene
4. Isopropyl benzene	10. Phenylacetylene
5. o-Xylene	11. b-Methylstyrene
6. n-Propylbenxene	12. Benzaldehyde

Figure 7. Comparison of traditional capillary GC and fast GC for the analysis of impurities in styrene monomer.

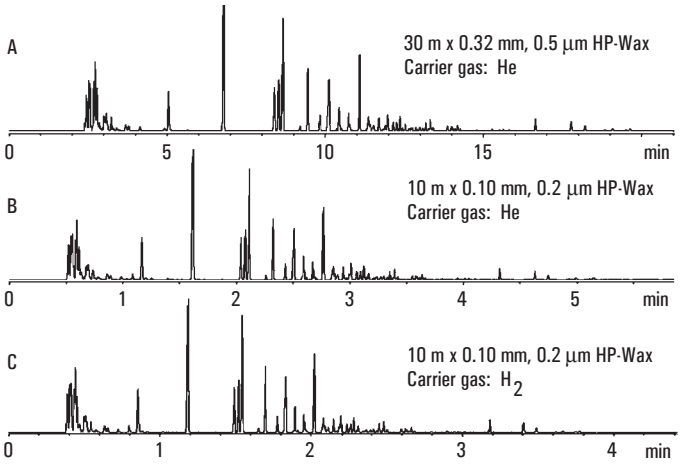


Figure 8. Development of fast GC for reformate gasoline.

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1. B. Quimby, V. Giarrocco, and M. Klee. "Speed Improvements in Detailed Hydrocarbon Analysis of Gasoline Using 100-µm Capillary Columns," Agilent Technologies, Inc., Application Note 228-294, Publication Number (23) 5963-5190E, February 1995.

2. V. Giarrocco. "Two-Minute Simulated Distillation Analysis of Gasoline-Range Materials—A Preliminary Investigation," Agilent Technologies, Inc., Application Note 228-370, Publication Number (23) 5965-6461E, January 1997.

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Analysis of Aromatic Hydrocarbons in Middle Distillates with HPLC using IP Standard Method 391/95

Angelika Gratzfeld-Huesgen

Petrochemicals

Abstract

The performance of diesel fuel is predominantly determined by its ignition quality. This parameter is known as the Cetan number. The Cetan number describes how much Vol.% Cetan (hexadecane) is present in a mixture of Cetan and 1-Methylnaphthalene. Generally, in order to provide the best performance and lifetime of an engine, the amount of aromatics in diesel should be as low as possible.

For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range 150 °C to 400 °C, there exists an IP Method (391/95), which uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) were separated using normal phase HPLC and a column which has little affinity for non-aromatic but pronounced selectivity for aromatic hydrocarbons. In order to avoid long retention times due to late elution of polyaromatic hydrocarbons (PAHs) the column was backflushed after the elution of the di-aromatic hydrocarbons to elute the PAHs as a single sharp band. The refractive index detector was used because this detector responds to both non-aromatic and aromatic hydrocarbons.

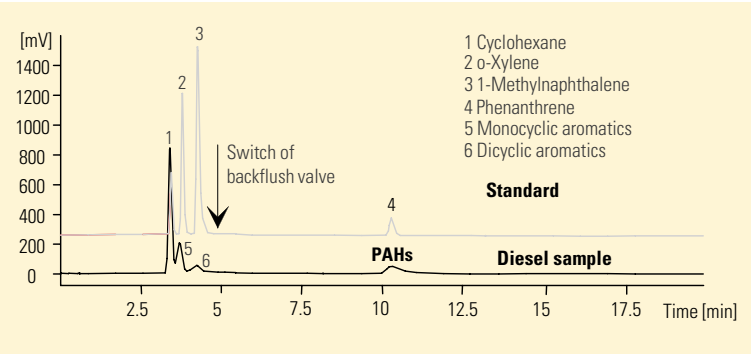

Figure 1
Standard chromatogram

Conditions

Column 200 x 4.6 mm AP NH_2, 5 µm
Mobile Phase Heptane
Flow Rate 0.8 ml/min
Injection Vol 2 µl
Oven Temp 20 °C
Backflush on 5 min
Refractive index detector

Sample preparation
1 g diesel sample filled to 10 ml with heptane

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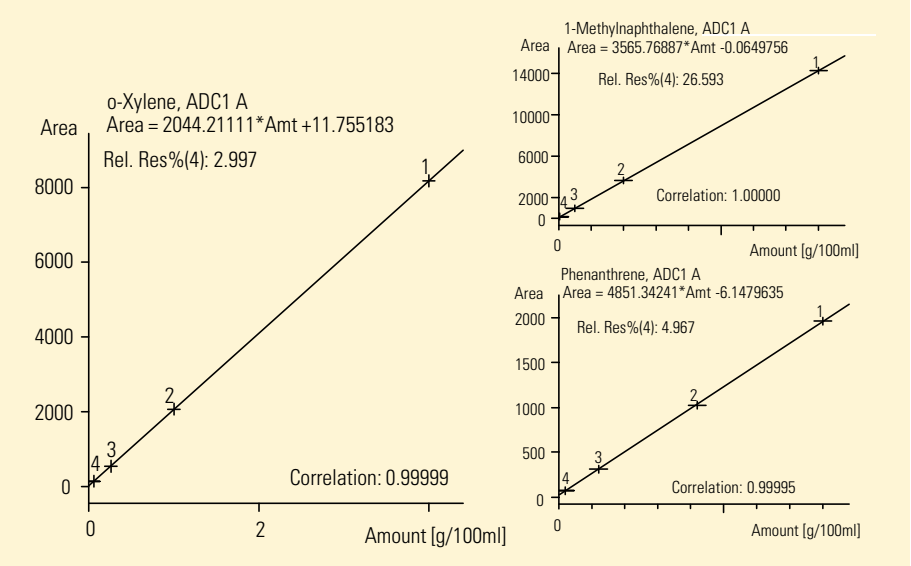

Figure 2
Linearity

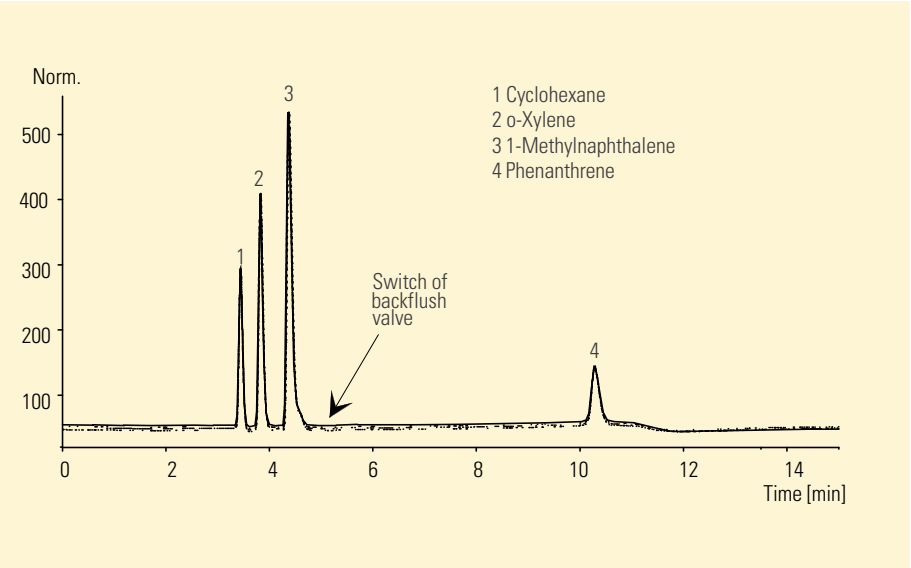

Figure 3
Repeatability—overlay of 5 runs

Method performance

Application range:
4–40 % m/m monocyclic aromatics
0–20 % m/m dicyclic aromatics
0–6 % m/m polyaromatics
4–65 % m/m total aromatic hydrocarbons
Precision of amounts:
rsd = 0.35 % for monocyclic aromatics
rsd = 0.23 % for dicyclic aromatics
rsd = 0.59 % for PAHs

Equipment

Agilent 1100 Series
- vacuum degasser
- isocratic pump head seal for normal phase: Agilent Part number 0905–1420
- autosampler
- thermostatted column compartment and backflush valve HP 1047 refractive index detector

Agilent ChemStation + HPLC software

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Released 06/97
Publication Number 5965-9044E

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Analysis of Sudan Red in Diesel Oil using HPLC

Angelika Gratzfeld-Huesgen

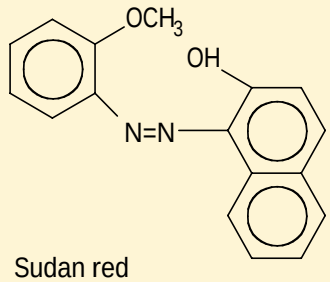
Polymer/ chemical industry

Abstract

Sudan red is an azo-dye used as the coloring agent for diesel. The coloring of mineral oil products is done to prevent misuse, for example, the prevention of fuel oil from being used in diesel motors. In Germany, fuel oil is less expensive than diesel because of added taxes. Governmental institutions have created legislation which has forced manufacturers to color the more expensive diesel so that it can be identified with a visual inspection. In some cases, the diesel fuel may have darkened , making visual inspection impossible. In these cases HPLC can be used to identify and quantify sudan red.

Method Performance

Figure 1 shows the HPLC chromatogram for the dieselsample with sudan red. The visual inspection was impossible, as the diesel oil color was nearly black. For additional identification, a spectrum can be taken which can be compared to the standard spectrum. In order to



Sudan red

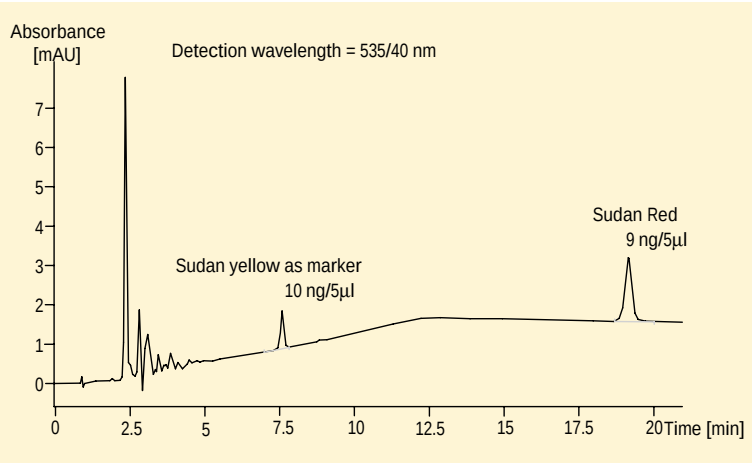


Figure 1
Analysis of sudan red in diesel oil

Conditions

Column 100 x 4 mm Hypersil BDS, 3 µm
Mobile Phase
A = Water, pH = 2.16 with H_2SO_4,
B = Acetonitrile
Gradient
at start 70 % B, at 10 min 99 %B
Post Time 4 min
Flow Rate 0.8 ml/min
Oven Temp 35 °C
Injection Vol 5 µl
Diode array 535/40 nm
Detector Reference 700/100 nm

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obtain a complete spectrum (figure 2), and to set a reasonable reference wavelength, the wavelength range of the diode array detector used should be greater than 600 nm.

Method performance

LOD: 0.8 to 0.9 ng for signal/noise = 2
red RT ~0.2 %
rsd area < 1 %
linearity = min 1 ng to 1 ug

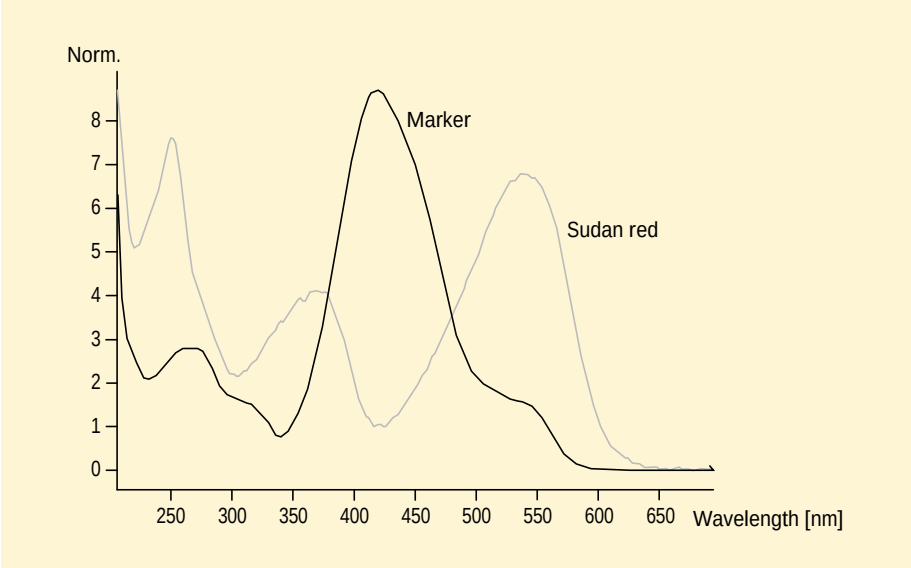
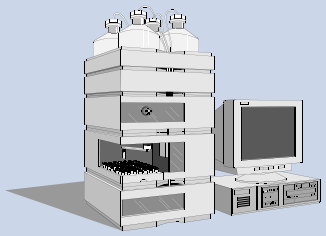


Figure 2
Spectra of sudan colors

Equipment

Agilent 1100 Series
- degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Released 06/97
Publication Number 5965-9057E

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Applications of Retention Time Locking in Simulated Distillation

Application

Gas Chromatography

September 1997

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Abstract

Retention time locking is used to lock retention times when transferring methods between chromatographic instruments, between columns, and when changing to a different type of detector. The retention times for a simulated distillation calibration standard analyzed on a second GC system matched the original retention times on the first system within 0.02 minutes after locking. Thus, using retention time locking, retention time reproducibility between GC systems meets typical ASTM simulated distillation specifications for reproducibility on a single system.

Key Words

Retention time locking (RTL), method validation, ASTM D 2887, capillary gas chromatography, laboratory productivity, SimDis, simulated distillation.

Introduction

Simulated distillation (SimDis) is one of the most common analyses in the petroleum industry. Many laboratories using SimDis run duplicate methodology on several instruments. However, the retention times for each instrument differ slightly, which means that each instrument must have a separate calibration and integration event table. Differences in retention times also complicate comparison of data between instruments and over time. The short, large-diameter columns used for higher-boiling ("extended") SimDis are particularly prone to retention time differences due to the very thin stationary phase and low pressure drop.

Retention time locking (RTL) is the ability to match chromatographic retention times exactly in any system to those in another chromatographic system, with the same nominal column. Agilent RTL software allows rapid, accurate locking of all retention times. This application note examines the use of retention time locking with RTL software for a SimDis sample run according to an extended ASTM D 2887[1], which extends the ASTM method to include analysis of hydrocarbons boiling up to n-C_{60}.

Experimental

An Agilent 6890 Series gas chromatograph (GC) with electronic pneumatics control (EPC) was used for these experiments. Sample injection was accomplished with an Agilent G1916A automatic liquid sampler (ALS). The 6890 was equipped with a cool on-column inlet and a flame ionization detector (FID). A 10 m x 0.53 mm, 0.88 µm HP-1 column (part number 19095Z-021) was used for separation. An Agilent ChemStation was used for instrument control and data acquisition. The experimental conditions for the GC methods are given by each chromatogram.

Results and Discussion

Figure 1 shows a SimDis sample run according to an extended ASTM D 2887 procedure. The top chromatogram represents the n-paraffin calibration mixture containing C_5–C_{60} hydrocarbons. The bottom chromatogram is of an actual sample.

The same analysis was run under identical conditions on a new system (GC system 2), and the chromatograms are compared in figure 2. The retention times are shifted 0.15 to 0.3 minutes, which is typical when changing columns or systems.

After adjusting the inlet pressure of GC system 2 using Agilent RTL software, the retention time deviation was only 0.04 to 0.08 minutes (figure 3). The retention times for GC system 2 now meet ASTM D 2887 criteria for retention time on a single system, let alone two different systems.

After a second iteration of inlet pressure adjustment, retention times match even better as shown in figure 4. The retention times locked within 0.02 minutes.

The most accurate results for SimDis analyses would still be obtained from standard calibrations on a given system. However, these RTL experiments demonstrate that using a common (universal) boiling point calibration curve based on locked GC systems can give results that easily meet ASTM criteria for a large number of locked systems. This, in turn, would allow easier review of data from multiple instruments and over extended periods of time. This capability clearly saves time, increases the value of the data, and provides more useful historical records on sample results.

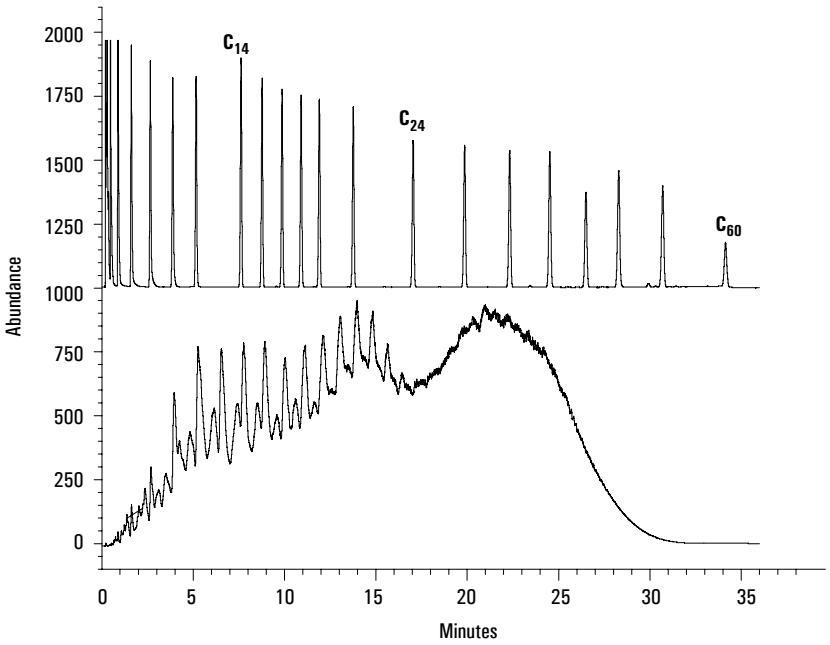

Figure 1. GC Simulated Distillation, C_5–C_{60} range. Conditions: column, 10 m x 0.53 mm, 0.88 µm HP-1; carrier gas: 19 mL/min helium, 4.3 psi constant flow; injection: 0.1 µL on-column; oven program: 35 °C to 375 °C, 10 °C/min.

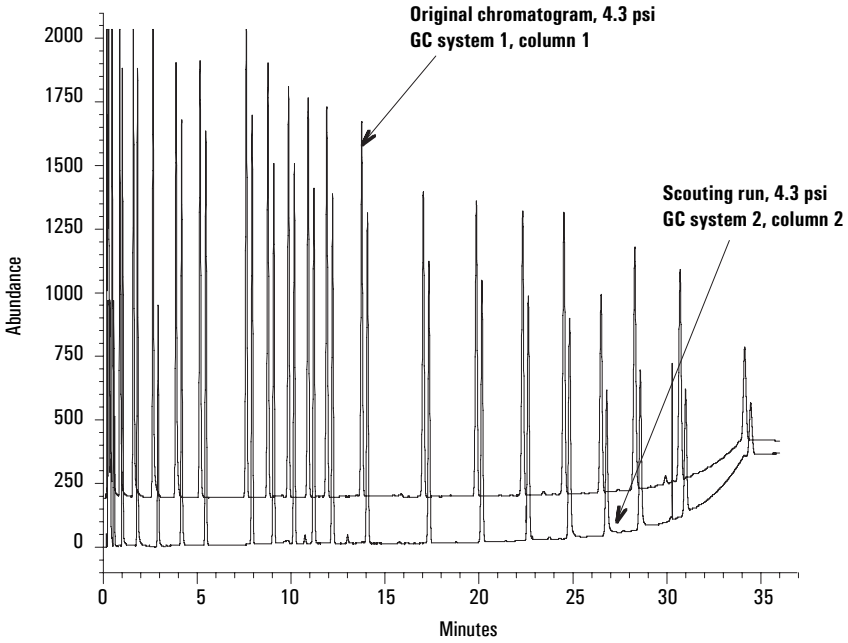

Figure 2. Comparison of the original chromatogram and a scouting run on GC System 2. The retention time shift is 0.15–0.30 minutes.

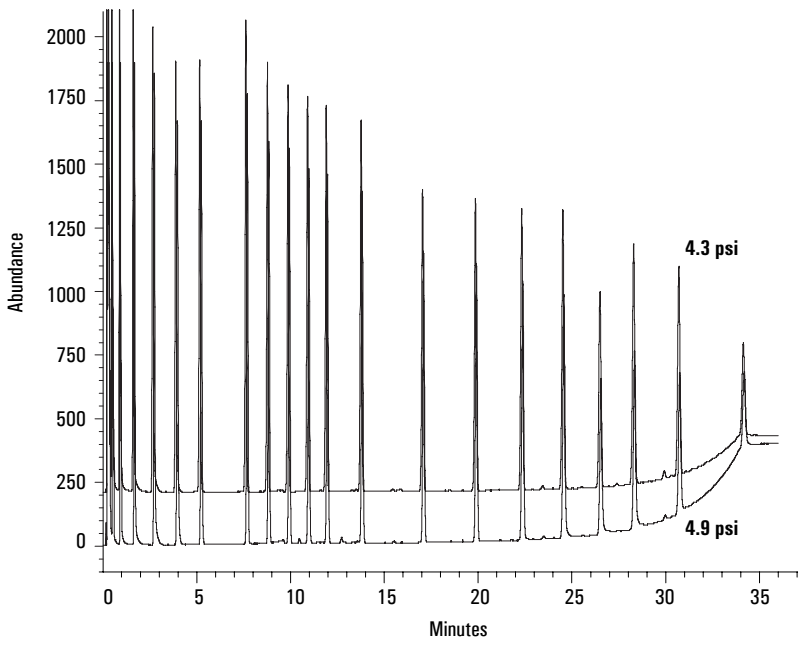

Figure 3. Comparison of original chromatogram and chromatogram obtained on GC System 2 after pressure adjustment based upon retention time vs. pressure calibration. The retention time differences between the two systems are 0.04–0.08 minutes.

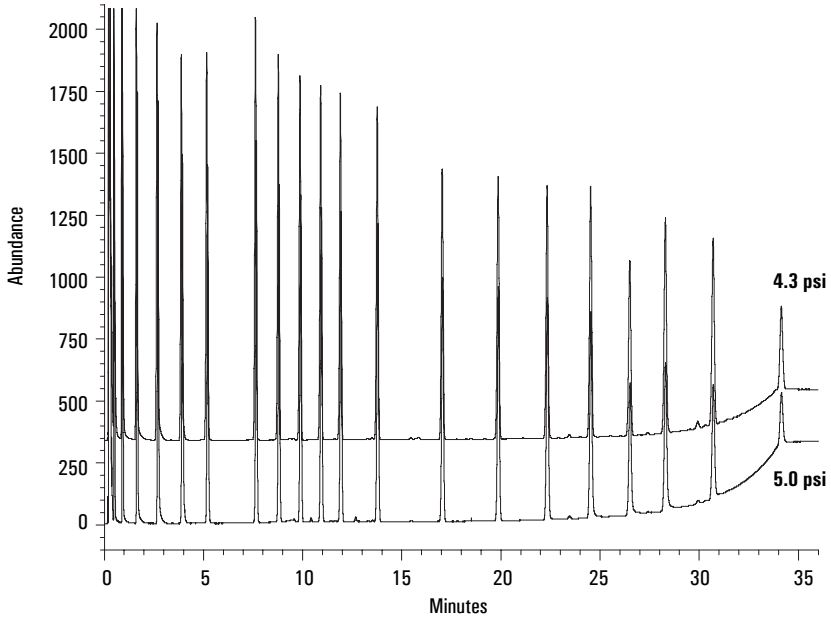

Figure 4. Comparison of original chromatogram and chromatogram obtained on GC System 2 after a second pressure adjustment based upon retention time results obtained from the first pressure correction. The largest retention time deviation between the two systems is now 0.02 minutes.

Conclusions

The Agilent RTL software was used to lock retention times for a simulated distillation sample analyzed on a second GC system. The retention times on the second system matched the original retention times on the first system within 0.02 minutes, meeting ASTM specifications for reproducibility on one system. This not only demonstrates the exceptional retention time reproducibility of the 6890 Series GC, but also the potential of RTL to improve quality, productivity, and intercomparability of capillary GC analyses.

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Retention Time Locking: Concepts and Applications

Application

Gas Chromatography

December 1997

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Abstract

The concepts and applications of retention time locking (RTL) are described. RTL simplifies the process of transferring methods from chromatographic instrument to chromatographic instrument, column to column, and detector to detector. The analysis of impurities in styrene according to ASTM D 5135 is used to demonstrate the efficacy of the approach. Using RTL, the retention times matched within an average of 0.16% (0.02–0.03 minute) in constant pressure modes.

Key Words

Retention time locking, method validation, styrene analysis, ASTM D 5135, capillary gas chromatography, laboratory productivity

Introduction

Retention time is the fundamental qualitative measurement of chromatography. Most peak identification is performed by comparing the retention time of the unknown peak with that of a standard. It is much easier to identify peaks and validate methods if there is no variation in the retention time of each analyte.

However, shifts in retention time occur frequently. Routine maintenance procedures such as column trimming alter retention times. In a multi-instrument laboratory running duplicate methods, the retention times for each instrument will differ from each other, even when run under nominally identical conditions. These differences in retention times mean that each instrument must have a separate calibration and integration event table, making it time-consuming to transfer methods from one instrument to another. Differences in retention time also complicate comparison of data between instruments and over time.

Retention time locking (RTL) is the ability to very closely match chromatographic retention times in any Agilent 6890 gas chromatograph (GC) system to those in another 6890 GC system with the same nominal column.

There are several subtle effects that combine to cause retention time differences between similarly configured GC systems. Columns of the same part number can vary slightly in length, diameter, and film thickness.

GC pneumatics can have small variations in the actual inlet pressure applied at a given setpoint. The actual temperature of the GC oven also has minute but real deviations from the indicated value. The sum of these and other effects result in the observed retention time differences between similarly configured GC systems.

The pneumatics and oven temperature control of the 6890 GC have advanced the state of the art in GC hardware accuracy and precision. Agilent's advances in fused silica capillary column technology have resulted in highly reproducible column-to-column retention characteristics. With these advances, retention time precision for a given peak in a single GC setup is usually better than 0.01 minute. However, even with these advances in columns and instrument hardware, the sum of the effects mentioned above can cause retention time differences between identically configured GC systems of as much as 0.4 minute.

It would be impractical to control all of the instrument and column variables to a degree where retention time differences between similarly configured GC systems are removed. There is, however, a means of greatly reducing these differences. By making an adjustment in the inlet pressure, the retention times on a given GC setup can be closely matched to those of a similarly configured GC system. RTL is based on this principle. The process of RTL is to determine what adjustment in inlet pressure is necessary to achieve the desired match in retention times. Agilent RTL software (G2080AA), which integrates into the Agilent GC ChemStation (version A.05.02 or later), provides the tool required to determine the correct inlet pressure quickly and simply.

There are several advantages gained by using RTL in the laboratory. Peak identification becomes easier and more reliable. It is easier to compare data both between instruments and over time. Comparison of data when using different detectors for analyte identification is simplified. Transferring methods from instrument to instrument or laboratory to laboratory is easier because calibration time windows normally will not require readjustment. Validation of system performance is easier. With "locked" GC methods, the development and use of retention time data bases for unknown identification is much more straightforward.

To maintain a locked method, RTL should be performed whenever:

- The column is changed or trimmed
- The method is installed on a new instrument
- A detector of different outlet pressure is used
- System performance is validated
- Troubleshooting chromatographic problems

To lock a given method for the first-time or for the reasons below, one must first develop a retention time versus pressure (RT vs. P) calibration.

Even when using columns with the same part number (same id, stationary phase type, phase ratio, and same nominal length), separate/different locking calibration curves are needed when using:

- Systems with different column outlet pressures (FID/atmospheric, MSD/vacuum, AED/elevated)
- Columns differing from the "nominal" length by more than 15% (e.g., due to trimming)
- Systems where the predicted locking pressure falls outside the range of the current calibration

A specific solute (usually one found in the normal method calibration standard) must be chosen and then used for both developing the locking calibration and locking all future systems. The solute, or target peak, should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Solutes that are very polar or subject to degradation should be avoided.

Once the target solute has been chosen and all other chromatographic parameters of the method have been determined, five calibration runs are performed. The runs are made at conditions identical to the nominal method except that four of the runs are made at different pressures. The pressures used are typically:

- Target pressure – 20%
- Target pressure – 10%
- Target pressure (nominal method pressure)
- Target pressure + 10%
- Target pressure + 20%

The retention time of the target compound is determined for each run. The resulting five pairs of inlet pressures and corresponding retention times are entered into the ChemStation software to generate an RTL calibration file.

Figure 1 shows the dialog box used to enter the calibration data. After the data is entered, a plot is displayed, as shown in figure 2. The maximum departure of the fitted curve from the data is given for both time and pressure. If the fit is acceptable, the retention time versus pressure calibration is stored and becomes part of the GC

method. This calibration need only be generated once. Subsequent users of the method can use this calibration when running the method on a similar instrument setup, regardless of location.

To relock a system or lock a new one:

1. Set up the method conditions and run a standard containing the target compound.
2. Enter the actual retention time of the target compound into the "(Re)Lock current method" dialog box (see figure 3).
3. Update the 6890 method with the new calculated pressure, and save the method.
4. Validate the retention time lock by injecting the standard at the new pressure, and compare the retention time obtained to the desired retention time.
5. Repeat steps 2 to 4, if necessary.

A Note on Constant Flow versus Constant Pressure Modes of EPC Operation

Many GC chromatographers prefer to use the "constant flow mode" of EPC operation. In this mode, inlet pressure increases automatically to maintain constant outlet flow rate as the oven temperature increases during the run. Constant flow mode reduces run time and ensures that flow-sensitive detectors see a constant column effluent flow.

The "constant pressure" mode of EPC operation is also popular. In this mode, the pressure remains constant during the run (outlet flow will decrease as temperature increases). For those wishing to reduce run time in constant pressure mode, a higher pressure can be chosen. For

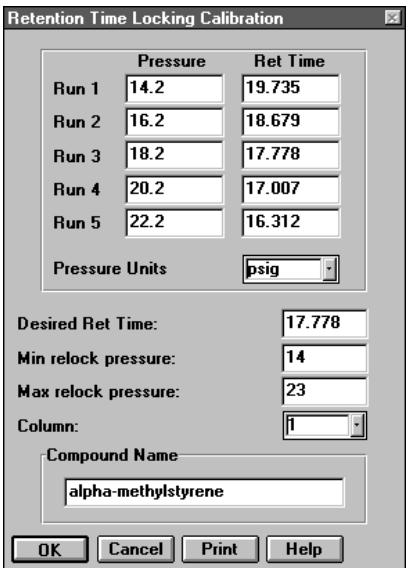

Figure 1. Dialog box used for entering retention time locking calibration data

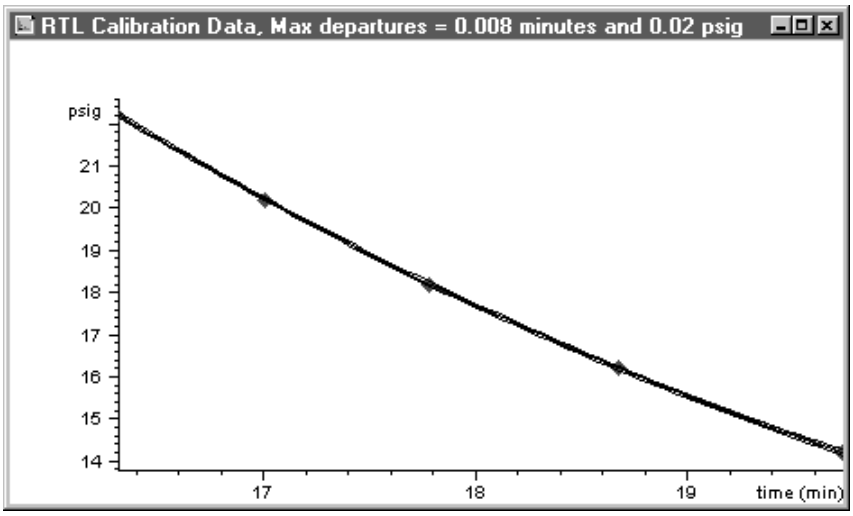

Figure 2. Plot of calibration data as displayed by RTL software

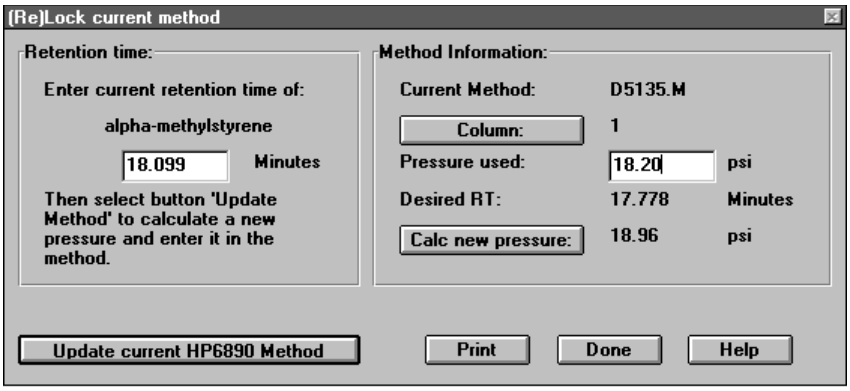

Figure 3. Dialog box used to calculate locking pressure and update the 6890 method

flow-sensitive detectors, one can set "constant column flow + makeup" via the 6890 keyboard or ChemStation. In this mode, the makeup flow is increased as the column flow decreases to keep the sum of the two constant.

The underlying theory of RTL predicts that constant pressure mode of EPC provides the closest matching of retention times. If one desires to compare data from systems with very different configurations, such as GC/FID to GC/MSD, it is best to use constant pressure mode. As can be seen from the styrene analysis data herein, retention time matching between systems of the same configuration (GC/FID, in this case) is still quite good in the constant flow mode.

This application note shows the use of RTL to lock retention times between multiple chromatographic instruments, columns, and detector types and demonstrates RTL in both constant flow and constant pressure modes.

Experimental

Two 6890 Series GC systems were used. Each system was equipped with:

- Electronic pneumatics control (EPC)
- Split/splitless inlet (250 °C, He carrier gas, split 80:1)
- Automatic liquid sampler
- GC ChemStation (version A.05.02)
- Flame ionization detector (FID)
- 60 m × 0.32 mm, 0.5 mm HP-INNOWax column (part no. 19091N-216)
- Temperature program: 80 °C (9 min), 5 °C/min to 150 °C

The inlet pressures/flows used are indicated with each chromatogram.

A third 6890 Series GC was also used. This system was equipped with an Agilent 5973 mass selective detector (MSD) and was used for peak identification. The GC-MSD chromatographic parameters used were the same as the GC systems noted above except for the inlet pressures as indicated.

Results and Discussion

GC-FID to GC-FID Locking

Figure 4 shows the original chromatogram (GC system 1) obtained from running a styrene sample under the conditions specified in ASTM D 5135.[1] Many of the typical impurities found in styrene are found here. The phenylacetylene peak represents about 60 ppm. The peaks are identified in table 1.

The sample was then run at four other pressures to collect the five data pairs for RTL calibration. Because this method was run in constant flow mode, the pressures entered into the RTL software were the initial pressures. The α-methylstyrene peak (peak 10) was chosen as the target compound. The calibration data are shown in figure 1.

The method conditions and RTL calibration were then moved to GC system 2, a different GC and column. The sample was run at the original method inlet pressure of 18.2 psi. The chromatogram obtained using this scouting run is overlaid on the original chromatogram in figure 5. The retention times shifted about 0.3 minute on the second GC. This is a typical result obtained when trying to replicate an analysis on a second instrument or with a second column.

The retention time of α-methylstyrene was entered into the RTL software

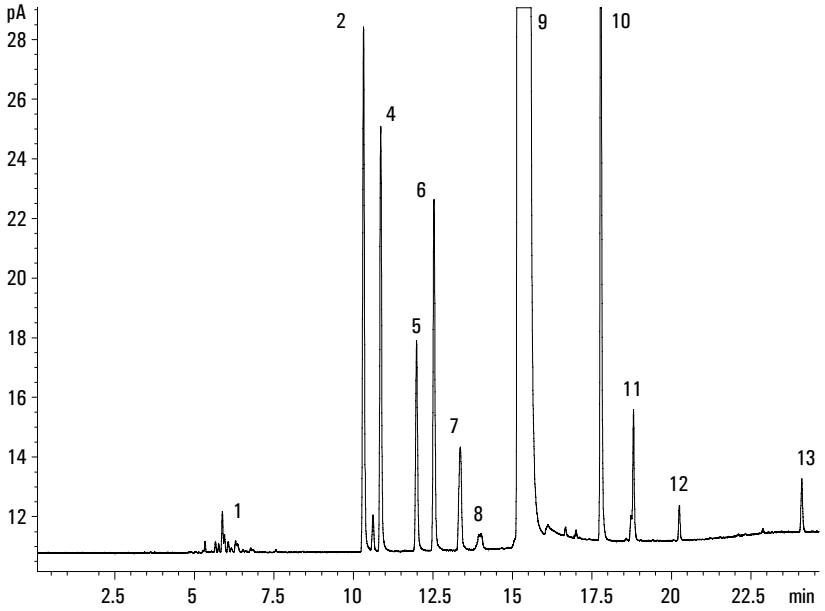

Figure 4. Styrene sample run on GC system 1 at 18.2 psi initial pressure, constant flow mode

dialog box on GC system 2, as shown in figure 3. The RTL software indicated the initial pressure should be modified from 18.2 psi to 18.96 psi. The new initial pressure was entered into the method and saved.

Figure 6 compares the chromatograms obtained from the original run and after locking retention times using the α-methylstyrene. Table 2 compares the retention times before and after using this approach. The retention times are now closely matched.

GC-FID to GC-MSD Locking

A second experiment was conducted to lock the original method from GC system 1 to the GC-MSD. This is useful for identification of unknown impurities that show up in the FID chromatogram. For example, there is a shoulder evident on the front side of the phenylacetylene peak in figure 4. It would simplify locating the impurity in the GC-MSD data if the retention times closely matched that of the GC-FID.

Because constant pressure mode is preferred when comparing data from FID and MSD systems, constant pressure mode was chosen, and the styrene sample was re-run on GC system 1 at 18.2 psi for reference.

The next step was to determine the chromatographic conditions to be used on the GC-MSD. The Agilent method translation software tool was used to calculate the conditions necessary to have the peaks elute in the identical order on the two systems.[2,3] Because the retention times need to match, the dead time and temperature program used for running the GC-MSD must be the same as the GC

Table 1. Peak Identities for Figure 4

Peak #	Name	Peak #	Name
1	Nonaromatics	8	p/m-Ethyltoluene
2	Ethylbenzene	9	Styrene
3	p-Xylene	10	α-Methylstyrene
4	m-Xylene	11	Phenylacetylene
5	i-Propylbenzene	12	β-Methylstyrene
6	o-Xylene	13	Benzaldehyde
7	n-Propylbenzene		

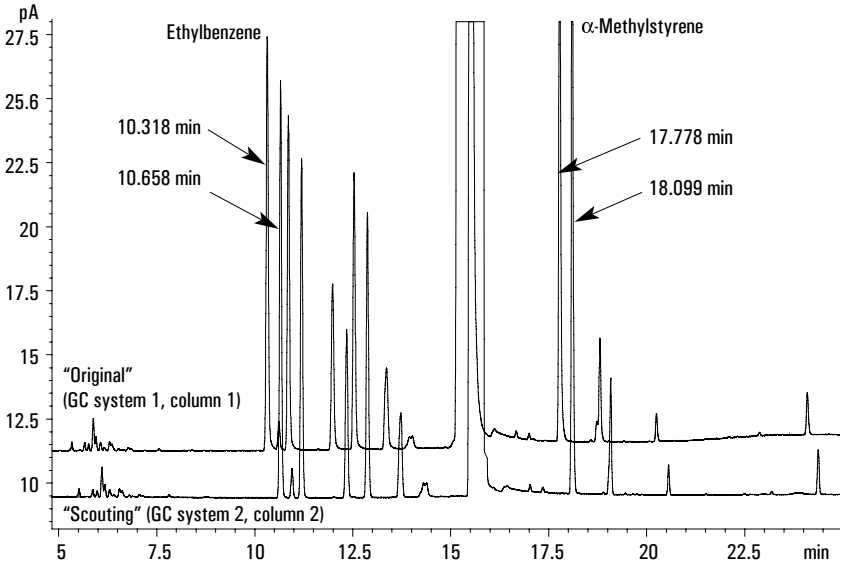

Figure 5. Comparison of original chromatogram on GC system 1 with GC system 2 before retention time locking

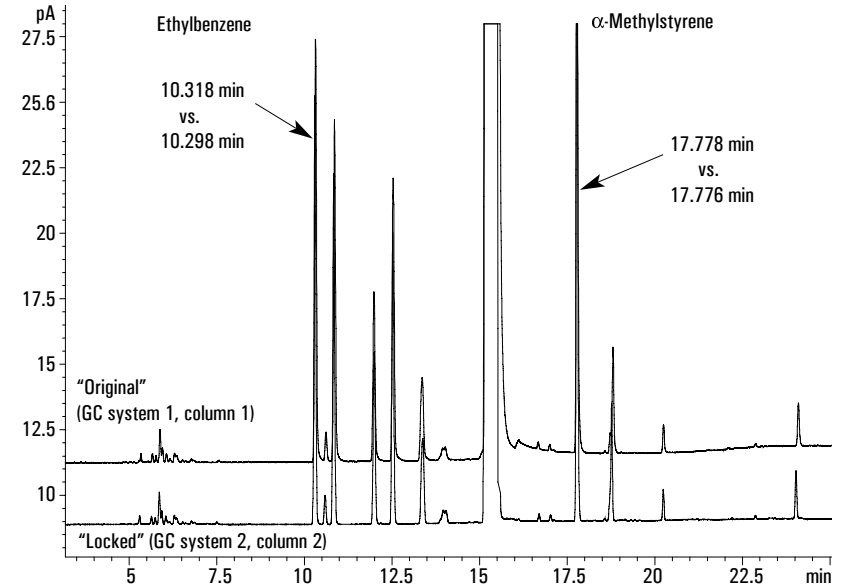

Figure 6. Comparison of original chromatogram on GC system 1 with GC System 2 after retention time locking

method. The pressure used, however, will be different due to the difference in column outlet pressure. The GC-MSD inlet pressure is calculated using the "none" mode of the method translation software (figure 7). In this mode, the holdup time between the two columns was forced to be identical to the GC-FID. This gives a speed gain of 1. The pressure calculated for use on the GC-MSD was 8.44 psi. Note that this calculated pressure is only the nominal pressure required to get similar retention times, not the exact locking pressure.

A different RTL calibration is required for GC-MSD because the outlet pressure is vacuum, and that of the FID is atmospheric pressure. Five runs were made on the GC-MSD system bracketing the 8.44 psi nominal method pressure. Because the GC-MSD used in this study was not equipped with RTL software, a dummy method was created in GC system 1 and the GC-MSD RTL calibration data was entered into it. A scouting run of the Styrene sample was made on the GC-MSD, and the α-methylstyrene retention time was used for locking. The locking inlet pressure calculated with the dummy method was 7.9 psi and was entered into the GC-MSD.

Figure 8 shows the resulting matched chromatograms from the GC-FID and GC-MSD. As seen in table 3, the retention times are now closely matched within 0.02 minute.

Figure 9 shows the MSD first choice of library search result of the impurity that created the shoulder on the front side of the Phenylacetylene peak. RTL ensured that this shoulder remained separated on the MSD system and eluted at the same time for easy comparison to the FID results.

Table 2. GC-FID Retention Times Before and After Locking for Styrene Impurities (Constant Flow Conditions). Chromatograms Shown in Figures 4, 5, and 6.

Component	Original Run GC 1/Column 1 18.2 psi	GC2–GC1 Before RTL	Scouting Run GC 2/Column 2 18.2 psi	GC2–GC1 After RTL	Locking Run GC 2/Column 2 19.0 psi
Ethylbenzene	10.318	0.340	10.658	–0.020	10.298
p-Xylene	10.616	0.333	10.949	–0.026	10.590
m-Xylene	10.858	0.337	11.195	–0.022	10.836
i-Propylbenzene	11.985	0.359	12.344	+0.005	11.990
o-Xylene	12.533	0.345	12.878	–0.012	12.521
n-Propylbenzene	13..360	0.364	13.724	–0.016	13.376
α-Methylstyrene*	17.778	0.321	18.099	–0.002	17.776
Phenylacetylene	18.806	0.275	19.081	–0.040	18.766
β-Methylstyrene	20.248	0.310	20.558	–0.006	20.242
Benzaldehyde	24.097	0.279	24.376	–0.069	24.028
Average Δ		0.326		0.028	

* Used in locking calculation

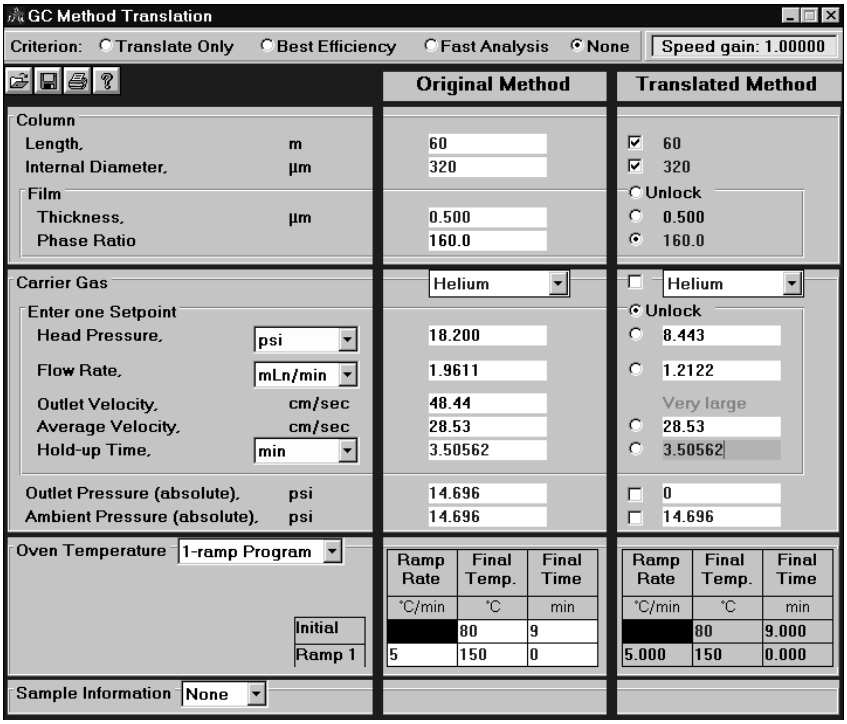

Figure 7. Method translation software provides scaled conditions for GC systems with different configurations

Conclusions

Retention time locking facilitates replicating results from instrument to instrument, from column to column, and from detector to detector by locking retention times. The retention times of a styrene sample analyzed according to ASTM D 5135 matched to within 0.06 minute after locking.

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2. M. Klee and V. Giarrocco, “Predictable Translation of Capillary GC Methods for Fast GC” Agilent Technologies, Inc., Application Note 228-373, Publication 5965-7673E, March 1997.
3. GC Pressure/Flow Calculator for Windows, Version 2.0 and Method Translation Tool Version 2.0. Available at http://www.chem.agilent.com/servsup/usersoft/main.html.

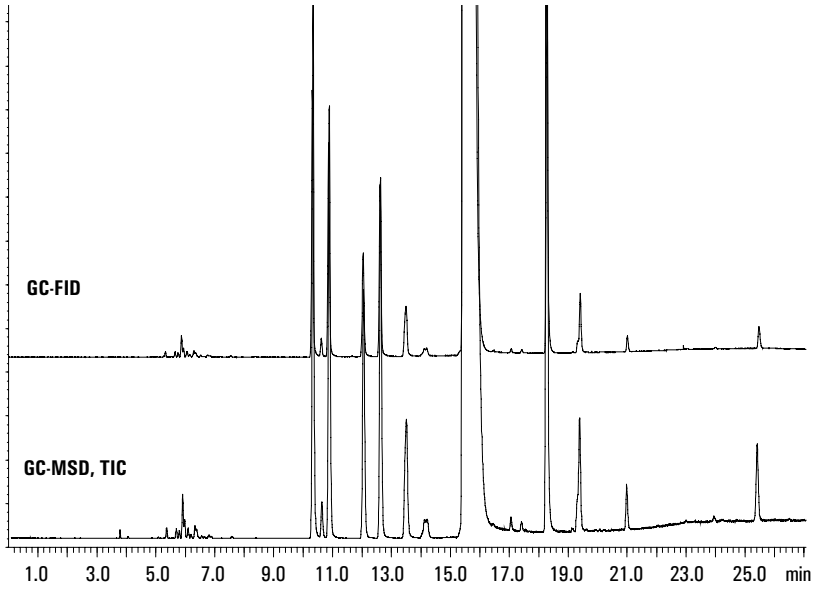

Figure 8. Comparison of chromatogram on GC system 1 with GC-MSD system after retention time locking, Constant Pressure Mode

Table 3. GC-FID vs. GC-MSD, Method Translated then Locked—Retention Times (Constant Pressure Conditions)

Component	GC-FID Original 18.2 psi	GC-MSD 7.9 psi	RT Difference min
Ethylbenzene	10.315	10.338	0.023
p-Xylene	10.620	10.642	0.022
m-Xylene	10.869	10.890	0.021
i-Propylbenzene	12.038	12.053	0.015
o-Xylene	12.613	12.630	0.017
n-Propylbenzene	13.492	13.508	0.016
a-Methylstyrene*	18.276	18.267	–0.009
Phenylacetylene	19.406	19.389	–0.017
b-Methylstyrene	21.008	20.987	–0.011
Benzaldehyde	25.475	25.415	–0.060
		Average	0.021

* Used in locking calculation

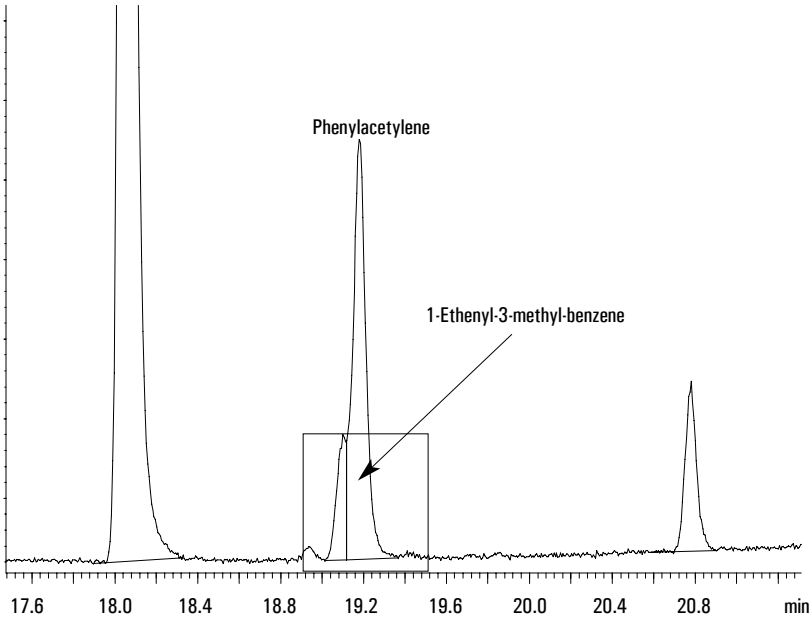

Figure 9. GC-MSD identification of impurity in shoulder of phenylacetylene peak

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Printed in the USA 3/2000
5966-2469E



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Improved Analysis of Sulfur, Nitrogen, and Other Heteroatomic Compounds in Gasoline- and Diesel-Range Materials Using GC/Atomic Emission Detection

Application

Gas Chromatography

January 1998

Authors

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Abstract

An optimized procedure was developed for the analysis of sulfur and nitrogen compounds in gasoline- and diesel range materials. The analysis is performed using an Agilent 6890 gas chromatograph (GC) configured with an Agilent G2350A atomic emission detection (AED) system. A 30-m × 0.32-mm, 1-µm HP-1 MS capillary column is used for separation. The method is applicable to low-sulfur gasoline and diesel fuels. Nitrogen compounds can also be determined. The method can be applied to other light gas oil fractions with final boiling points of approximately C25 (402 °C, 756 °F). The configuration meets the requirements for determining sulfur in gasoline by ASTM method D 5623. The procedure can be extended to also measure oxygenates, organolead, organomanganese, and fluorine compounds.

Introduction

Determination of the sulfur content in petroleum feed streams is useful for improving and optimizing refinery catalytic processes. With the trend toward manufacturing of low-sulfur fuel products, such as gasoline and diesel fuel, accurate and precise measurements are important for obtaining product quality and meeting regulatory requirements. Additionally, knowledge of the individual sulfur types gives valuable insight into processing characteristics.

The measurement of nitrogen compounds is also of interest as these compounds are suspected of causing problems such as color and gum formation, engine deposits, and poisoning of some catalysts. The quantitation of the nitrogen compounds in petroleum materials is quite challenging, however, because of the low levels of nitrogen present and the potential for interference from the petroleum matrix.

Gas chromatography-atomic emission detection (GC/AED) has been used to measure the sulfur and nitrogen content in many types of petroleum samples.[1,2,3] The AED technique is well suited for these measurements because it has:

- High sensitivity required to measure low amounts of sulfur and nitrogen
- High selectivity required to reject hydrocarbon interferences
- No quenching
- Linear response
- Compound-independent element (equimolar) response factors

The ASTM method D 5623-94 (Standard Test Method for Sulfur Compounds in Light Petroleum Liquids by Gas Chromatography and Sulfur Selective Detection)[4] recommends the use of the sulfur chemiluminescence detector (SCD) or the AED.

Flame photometric detectors, both conventional and pulsed,[5] are not recommended because of problems with quenching of the sulfur signal by coeluting hydrocarbons.

With the development of the G2350A GC/AED system and further improvements in spectrometer design and element detection algorithms, the AED is well suited for sulfur and nitrogen measurements in petroleum liquids.[6]

The AED is a multi-element detector that can be used to measure twenty-three different elements. In addition to sulfur and nitrogen, elements such as carbon, hydrogen, oxygen, lead, manganese, fluorine, and silicon, can all be detected. A single instrument setup can then be used for a wide range of petroleum analyses.

This application note describes a procedure for the analysis of sulfur and nitrogen compounds in gasoline- and diesel-range materials. This method can also be extended to materials boiling just beyond the diesel fuel range (for example, light gas and cycle oils). The experimental conditions used in this study, including AED reagent gas flows, were adjusted to maximize the sulfur and nitrogen selectivity over carbon (hydrocarbons). Using these conditions, the GC/AED can be used to perform ASTM D 5623-94. Examples of detecting other elements such as oxygen, lead, manganese, and fluorine are also given.

Experimental

A 6890 GC coupled to a G2350A AED was used for this study. The GC was equipped with a split/splitless injection port operated in split mode. All gas flows and pressures within the GC were controlled electronically. An auxiliary pressure control module on the 6890 GC was used to set the AED reagent gas pressures. Sample injections were made by a 6890 Series automatic liquid sampler and a 5-µL syringe.

The inlet was fitted with a single-tapered liner (part no. 5181-3316) containing a 1-cm piece of glass wool. The glass wool plug was transferred directly from the standard split/splitless liner (part no. 19251-60540) to the single-tapered liner and positioned so that the top of the plug was 16 to 18 mm from the top of the liner. This liner acted to minimize discrimination across the boiling range and improve sample repeatability by "wiping" the sample droplet from the end of the syringe. The inlet end of the capillary column was positioned in the center of the small diameter section of the liner at about 4 mm above the tip of the column ferrule. A 30-m × 0.32-mm id × 1-µm film thickness HP-1 MS capillary column was used for separation (part no. 19091S-713). This column was chosen because it has a lower specified rate of column bleed than conventional methyl silicone capillary columns. The inlet ferrule used is graphitized polyimide to reduce diffusion of air into the inlet. Note that the ferrule should be retightened after the first few runs to compensate for shrinkage.

The Agilent AED ChemStation was used to control the GC/AED system and to provide for data acquisition and peak integration. The ChemStation was operated using a Microsoft® Windows™ environment, and the AED ChemStation automated the entire process. Two injections were made to determine carbon, sulfur, and nitrogen in the samples. Hydrogen and oxygen were used as reagent gases for both carbon (179 nm) and sulfur (181 nm). In the case of nitrogen (388 nm), methane was added as the third reagent gas.

To further improve sulfur and nitrogen selectivity over carbon, the AED gas flows (hydrogen, oxygen, and helium makeup) were optimized to minimize interferences from hydrocarbons. In addition to the reagent gases, the amount injected was optimum for each analysis. The AED ChemStation method automated this process for routine laboratory use. The makeup gas flow was set to 100 mL/min when the high makeup flow valve turned on for the nitrogen and metals analysis. With this setting, the makeup flow is raised to about 230 mL/min. With electronic pressure control (EPC) of the reagent gases, all methods shown here can be run in sequence without intervention except for the oxygen analysis. This requires the source of auxiliary gas to the AED to be changed from methane (used for nitrogen analysis) to 10% methane in nitrogen.

To obtain the best performance for nitrogen detection, it is important to use helium carrier and makeup gas that is nitrogen-free. A heated getter-type helium purifier (model GC50, SAES Pure Gas Inc.) was used in the present setup. This type of purifier removes N_2 and many other contaminants in helium down to ppb levels.

Quantitation of sulfur and nitrogen was based on ASTM D 5623-94 with

both internal and external standardization used. External standard quantitation was based on the response of a t-butyl disulfide (TBDS) solution. For internal standard calculations, a solution of TBDS in iso-octane was gravimetrically spiked into the gasoline. The samples used for internal standard calculations were determined to be free of TBDS. Nitrogen was quantitated externally based on the response of a nitrobenzene standard.

CARB Low Sulfur RFG and Conventional Gasoline quality control samples were obtained from AccuStandard. RFA gasoline with 14.9% methyltertiarybutylether was purchased from Scott Specialty Gases. Tertiary-butyldissulfide (TBDS), nitrobenzene, and methylcyclopentadienyl manganese tricarbonyl (MMT) were from Aldrich Chemical. The standard reference materials with certified sulfur content and the tetraethyl lead were obtained from National Institute of Standards Technology (NIST). The remainder of the samples were provided by various petroleum laboratories.

Table 1 lists the entire set of equipment and conditions used for this study.

Results and Discussion

Gasoline Range Analysis

Figure 1 shows the carbon, sulfur, and nitrogen chromatograms from

Table 1 Experimental Setup and Conditions

Gas Chromatograph and Detector	
Agilent G1530A	6890 Series GC
Option 002	Power supply for fast oven ramps
Option 112	Split/splitless inlet
Option 207	GC-AED interface
Option 308	Aux EPC for AED reagent gas control
G2350A AED	Atomic emission detector
Column	30-m x 0.32-mm, 1.0 μm HP-1MS (part no. 19091S-713)
Automatic Liquid Sampler	
Agilent G1916A	6890 Series ALS with tray
Data Acquisition/Data Analysis	
Agilent G2371AA	AED ChemStation
Operating Parameters, GC	
Injection port temperature	280 °C
AED transfer line/cavity temperature	310 °C
Oven temperature program	40 °C to 300 °C at 10 °C/min, hold 10 min
Column pressure (constant pressure mode)	12 psi
Carrier gas	Helium
Split ratio (Carbon, Sulfur)	50/1
Split ratio (Lead, Manganese, Oxygen)	100/1
Split ratio (Nitrogen)	10/1 (Gas Saver 200 mL/min at 0.3 min)
Split ratio (Fluorine)	20/1
Injection volume	1 μL (except as noted)
Operating Parameters, AED	
Data rate	2.5 Hz
Method 1, carbon 179 nm and sulfur 181 nm	
Reagent gases	Oxygen 55 psi, hydrogen 45 psi
Makeup flow	100 mL/min
Method 2, nitrogen 388 nm	
Reagent gases	Oxygen 80 psi, hydrogen 40 psi, methane 50 psi
Makeup flow	230 mL/min
Method 3, Oxygen 171 nm	
Reagent gases	Hydrogen 40 psi, 10% methane in nitrogen 25 psi
Makeup flow	100 mL/min
Method 4, lead 261 nm and manganese 259 nm	
Reagent gases	Oxygen 55 psi, hydrogen 45 psi
Makeup flow	230 mL/min
Method 4, fluorine 690 nm	
Reagent gases	Hydrogen 15 psi
Makeup flow	100 mL/min

the analysis of the conventional gasoline sample. The carbon and sulfur data are collected simultaneously in the first method and the nitrogen in a second. For comparison The sulfur chromatogram of the sample after addition of internal standard tertiarybutyldisulfide, is also shown. This sample was analyzed according to ASTM D 5623 using both internal and external standard quantitation. The total sulfur content was measured to be 330 ppm (weight) by both methods. Two trace level nitrogen peaks were also detected, each containing about 0.30 ppm of nitrogen. The lack of any significant response on the sulfur and nitrogen chromatograms from the large hydrocarbons in the gasoline demonstrates the high selectivity afforded by the AED.

A gasoline range product from a catalytic cracking unit is shown in figure 2. Most of the sulfur compounds observed in figure 1 are also present in this sample. Unlike the gasoline in figure 1, the sulfur content of this material is made up mostly of benzothiophene and alkyl-substituted benzothiophenes and the level of nitrogen is much higher (about 560 ppm). The nitrogen profile in figure 2 is typical of that observed in gasolines. The tentative identifications of the nitrogen compounds are taken from the literature.7 We have observed that for most gasolines having measurable nitrogen content, the pattern of nitrogens is very similar to that in figure 2, with only the total amount varying significantly. The AED responds uniformly to nitrogen in all forms, including N2. The large peak at the beginning of the nitrogen chromatogram results from small amounts of air injected with the sample. This peak is excluded from the total nitrogen measurement.

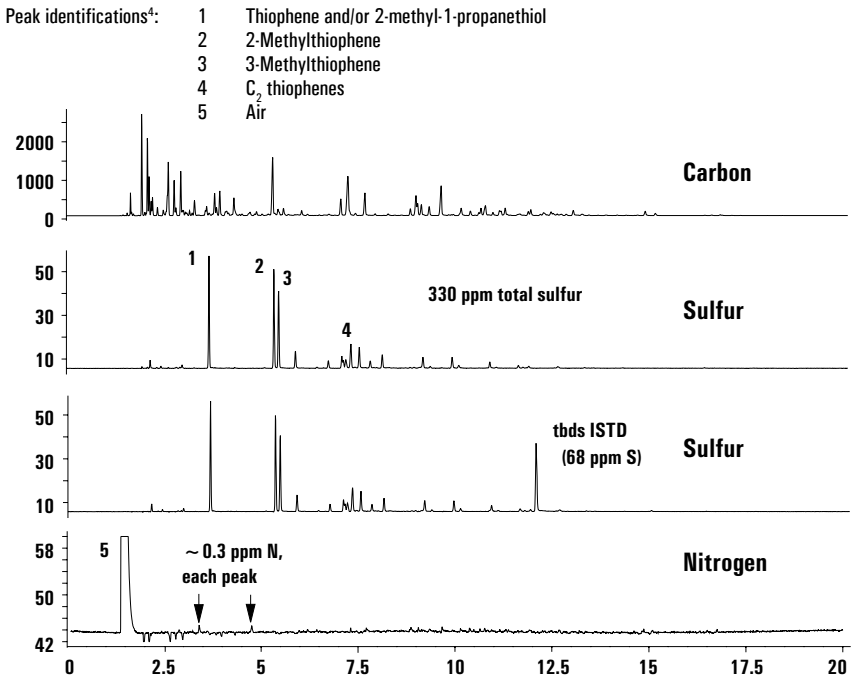

Figure 1. Conventional gasoline quality control sample. Upper sulfur trace is without added internal standard (TBDS), lower trace is with 68 ppm S as TBDS.

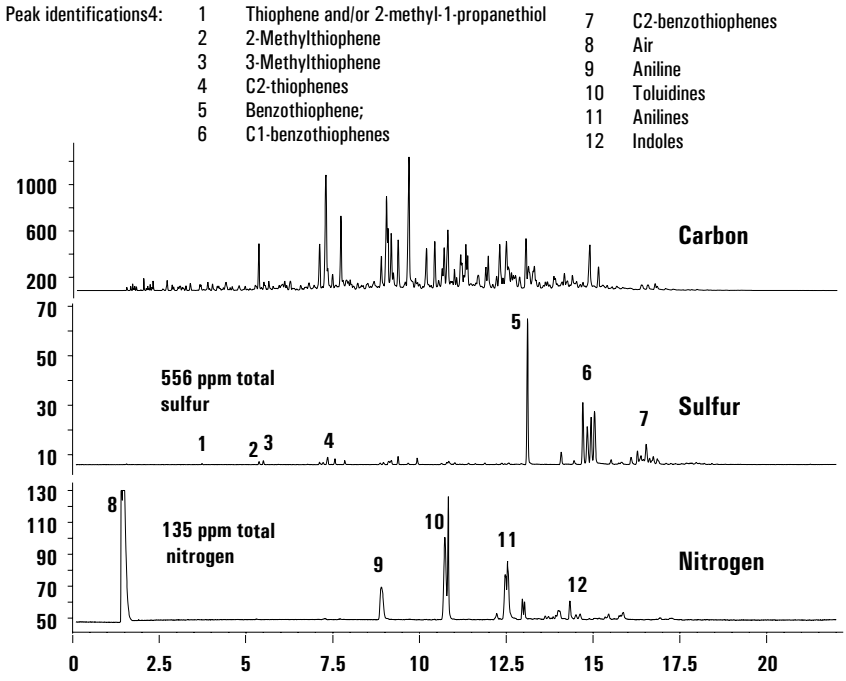

Figure 2. FCC Gasoline

The analysis of a low sulfur (California Phase II) gasoline sample is presented in figure 3. The detection limit for any one component was found to be approximately 0.7 ppm S. This sample contains about 42 ppm of sulfur by ASTM D 5623. The first two peaks in the sulfur chromatograph are not sulfur compounds, but are slight interferences from the largest hydrocarbons in the gasoline. These responses should not be included in the total sulfur calculation. If they are included, the error introduced is minimal because their total area corresponds to only approximately 3 ppm sulfur.

The nitrogen chromatogram in figure 3 was made with a 2-mL injection to increase sensitivity. The largest hydrocarbons at the front of the chromatogram produce small negative responses, but the actual nitrogen peaks are easily identified, even at the relatively low level of 25 ppm total nitrogen.

Figure 4 represents the analysis of a reference gasoline, RFA, containing methyl tertiary-butyl ether (MTBE). The large amount, 14.9 volume %, of MTBE does not cause an interference in the sulfur channel. As in the previous sample, the nitrogen chromatogram was collected with a 2-mL injection. The nitrogen peaks are still visible at 13 ppm total nitrogen. Peak 9 contains approximately 2.5 ppm N.

Figure 5 shows the oxygen chromatogram for RFA. Measuring additive oxygenates in gasoline is simplified greatly by using oxygen selective detection.[3,8] With the

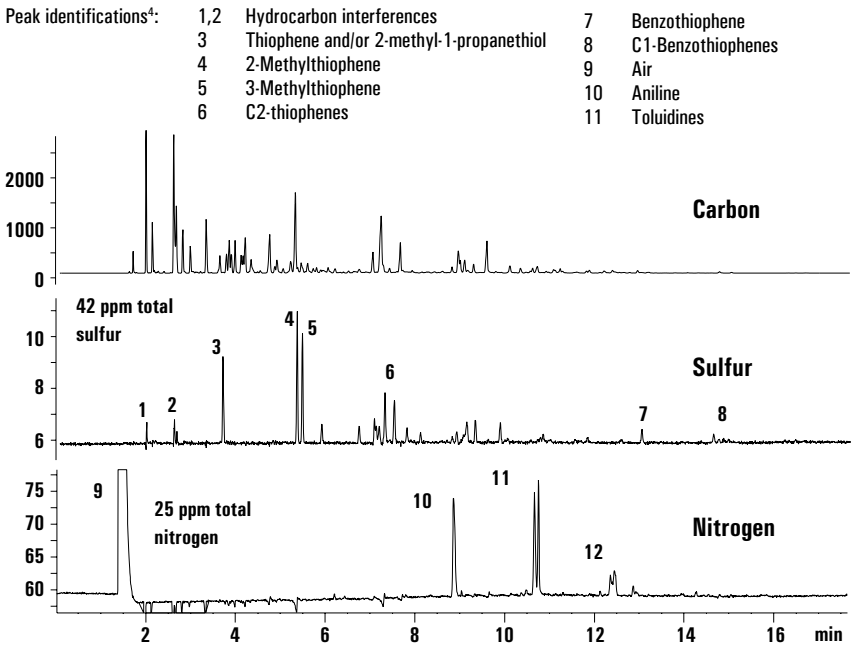

Figure 3. CARB Low-sulfur RFG quality control sample

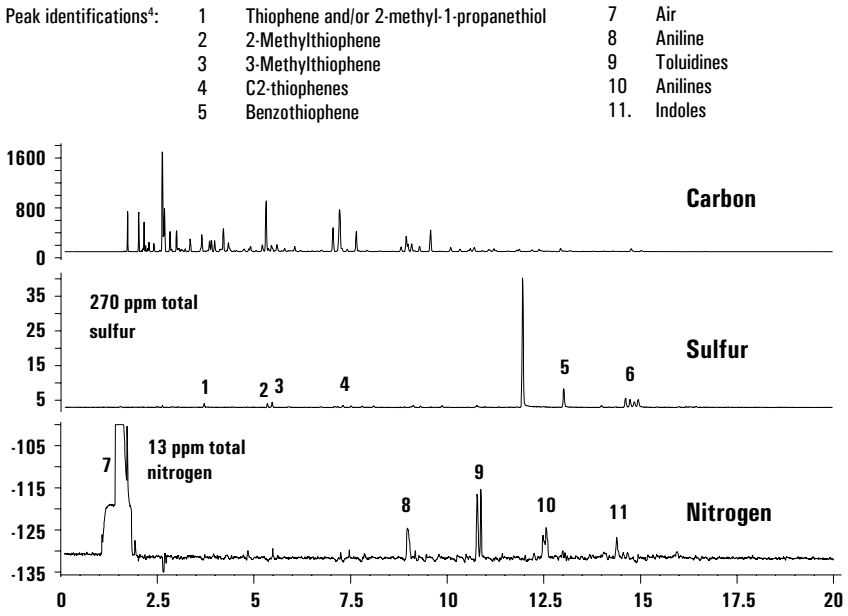

Figure 4. RFA Gasoline with 14.9% MTBE

conditions used here, the AED can detect individual oxygenates down to approximately 0.05%. The detection limit (signal-to-noise ratio = 2) for MTBE in figure 5 is 0.02%, and its performance is comparable to, or exceeds that of, the oxygen flame ionization detector (OFID).

Atomic emission with a helium plasma is a very sensitive and selective means of detecting volatile organometalic compounds. Also shown in figure 5 are the lead and manganese chromatograms from tetraethyl lead (TEL) and methylcyclopentadienyl manganese tricarbonyl (MMT) spiked into RFA. Lead compounds have been used worldwide as antiknock additives for gasoline but are currently being phased out of use in most countries. MMT has been used in the past in the U.S. and Canada.

Note that both the lead and manganese chromatograms exhibit little or no peak tailing and no hydrocarbon interference. Under the chromatographic conditions used here, the detection limit for TEL is approximately 1 ppm. Manganese detection is also very sensitive, allowing measurement of MMT well below 1 ppm. The small peaks before and after the MMT peak are not interferences, but are manganese containing impurities in the MMT.

An important capability of the AED is the ability to confirm the presence of an element in an unknown peak. Figure 6 shows the emission spectra obtained on the MMT peak and on the impurity peak (cymantrene) in figure 5. The three emission lines are

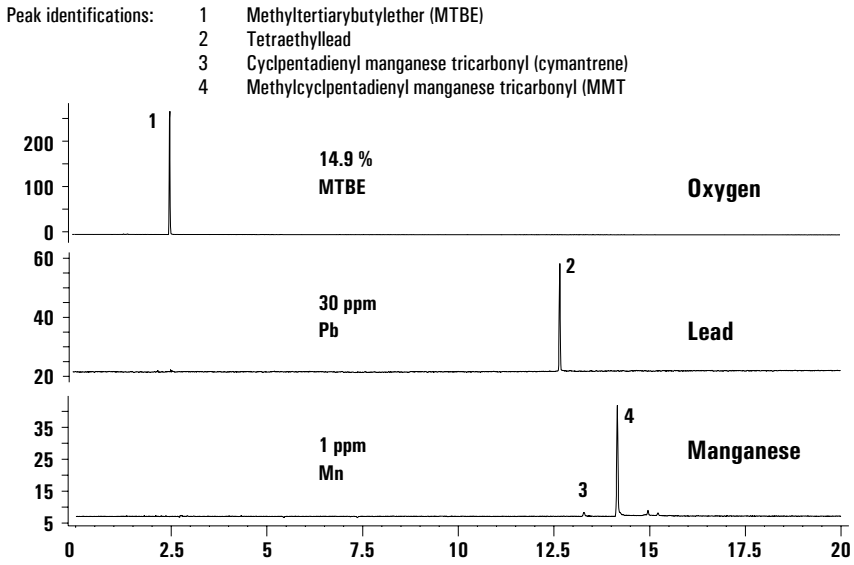

Figure 5. RFA Gasoline with 14.9% MTBE

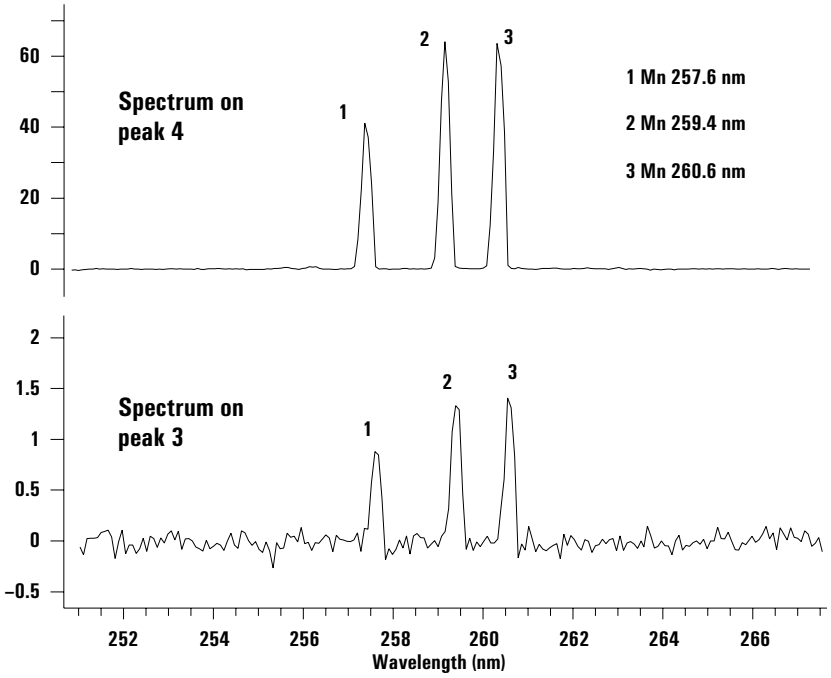

Figure 6. AED Emission spectra used to confirm presence of manganese in peaks 3 (cymantrene) and 4 (MMT) from figure 5

a fingerprint for the element manganese. If the spectrum of a peak contains these three emission lines in the relative intensities shown, then the presence of manganese is confirmed. The cymantrene peak only contains 40 ppb manganese. At this low level, the spectrum clearly shows the three manganese emission lines as seen in figure 6.

Figure 7 shows the chromatogram of a product from an alkylation unit. In this process, a C4 stream undergoes alkylation using hydrofluoric acid as the catalyst. In this example, the process is out of specification, with the level of organofluorides in the product at about 1000 ppm total fluorine. The AED fluorine chromatogram clearly shows the organofluoride impurities elute as compared to the hydrocarbons. From the relative patterns of the carbon and fluorine chromatograms, it appears that the fluorinated compounds are analogues of each of the main hydrocarbons in the sample. The detection limit (signal-to-noise ratio = 2) for a single fluorinated compound is about 0.6 ppm F under the conditions used here.

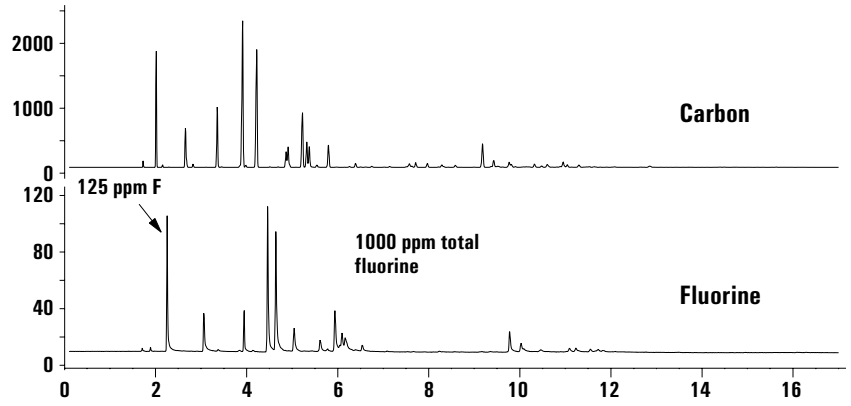

Figure 7. Off-specification product of HF alkylation unit

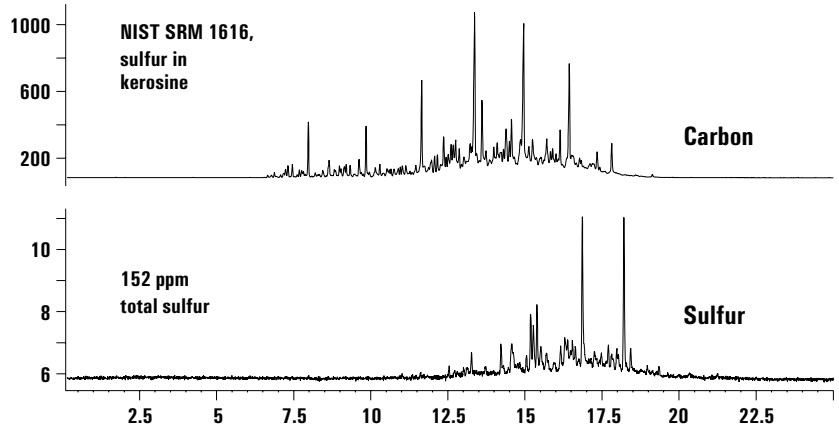

Figure 8. NIST SRM 1616 Sulfur in 1-k kerosine standard

Kerosine Analysis

The analysis of NIST sulfur in kerosine Standard Reference Materials (SRMs) is shown in figures 8 and 9. The SRM 1616 is a 1-k low-sulfur kerosine with a certified value of 152 ppm total sulfur. The retention time region where TBDS elutes is free from sulfur peaks, so the internal standard technique could be used.

The SRM 1617 is a high-sulfur kerosine standard, with a certified value of 1690 ppm total sulfur. It is interesting to note that the standard appears to have been constructed from a low-sulfur, 1-k kerosine with TBDS added

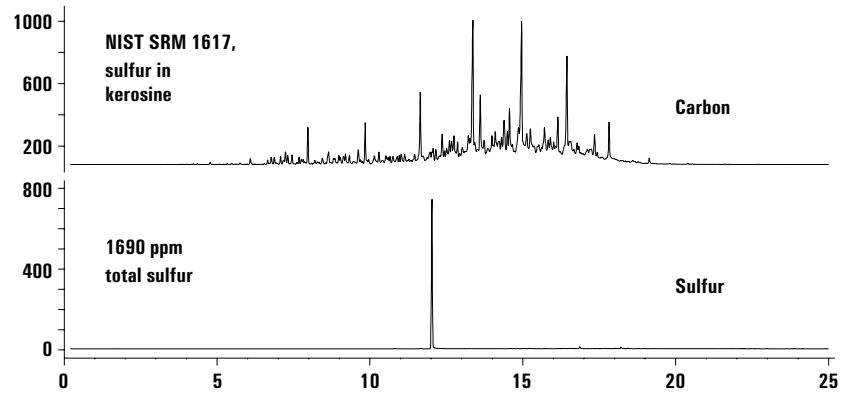

Figure 9. NIST SRM 1617 Sulfur in kerosine standard

to increase the sulfur content. Because TBDS is already in the standard, it could not be used as the internal standard for analysis.

There were no detectable nitrogen compounds observed in either kerosine.

Diesel Analysis

Figures 10 and 11 show the sulfur and nitrogen analysis of NIST sulfur in diesel fuel SRM 2724 and SRM 1624b.The SRM 2724 is a commercial no. 2-D distillate fuel oil as defined by ASTM. The certified total sulfur content is 425 ppm. The SRM 1624b is a similar fuel with a certified total sulfur content of 3320 ppm. These standards are very useful for diesel analysis because they bracket the range of sulfur normally encountered in these fuels.

The distribution and relative concentrations of sulfur compounds are similar in both SRMs, with only the total sulfur concentration being significantly different. The reverse is observed in the nitrogen chromatograms—the total nitrogen level is similar, but the profiles are noticeably different, especially in the earlier sections of the chromatograms.

A high-sulfur, high-nitrogen Light Cycle Oil (LCO) is shown in figure 12. Here the sulfur and nitrogen profiles are similar to the other diesels, but the carbon profile is clearly different. This sample contains higher-boiling material and requires an extra 10 minutes of hold time at the end of the chromatographic run to elute the sample completely.

Two samples of commercial low-sulfur diesel fuels from California are

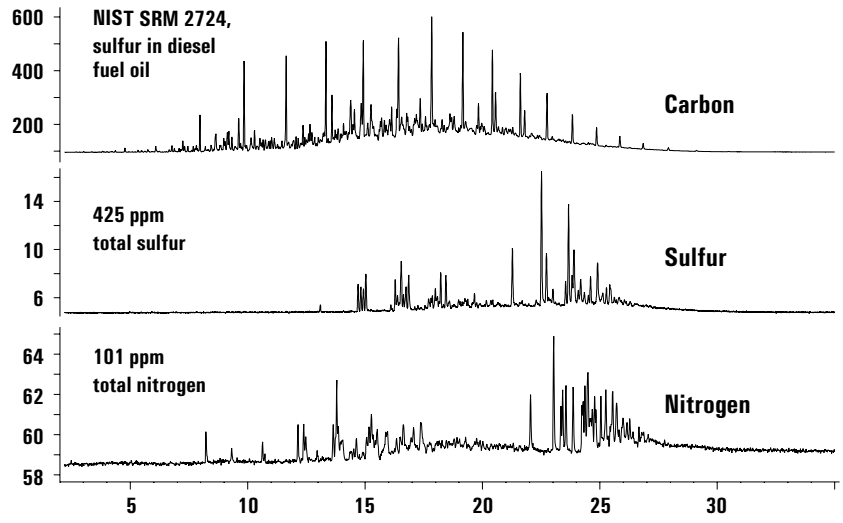

Figure 10. NIST SRM 2724 Sulfur in distillate fuel (diesel) standard

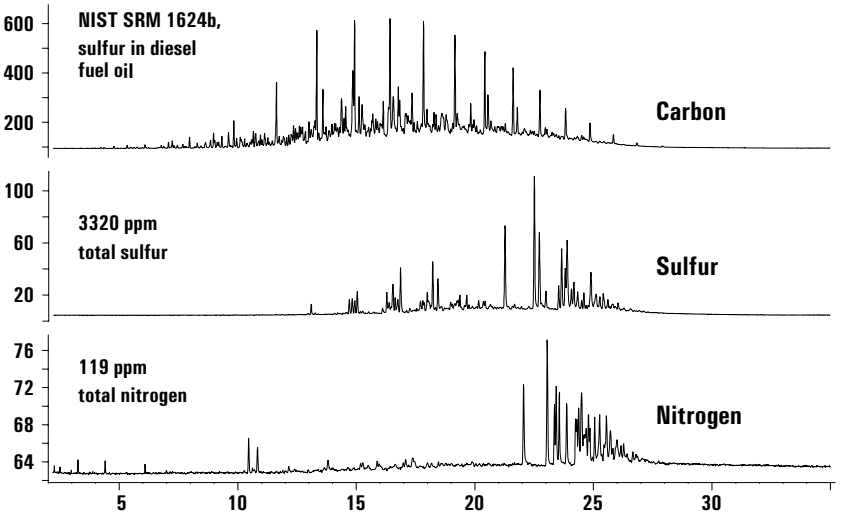

Figure 11. NIST SRM 1624b Sulfur in distillate fuel (diesel) standard

shown in figures 13 and 14. Comparison of the chromatograms illustrates how different the composition of sulfur and nitrogen can be at similar total concentrations. In the first sample, the total nitrogen is contained in a broad, unresolved distribution. In the second sample, the total nitrogen content arises from a few discrete peaks. It was thought that some of the nitrogen peaks in sample 2 could be nitro compounds added as cetane improvers. Both samples were then analyzed for oxygen to see if any of the nitrogen peaks were also oxygenated (i.e., nitro

compounds). While there were no oxygenates found in sample 2, an oxygen-containing peak that did not contain nitrogen was observed in sample 1. The oxygenate was identified as MTBE, and the concentration was measured to be at 0.26%.

In all the diesel-range samples analyzed, the time range where TBDS elutes had little or no sulfur signal, allowing its use as an internal standard.

Precision and Accuracy

The precision of the GC/AED system was evaluated by analyzing the "conventional" gasoline sample, discussed above, fifteen times over a 5-day period. A section of the chromatogram is shown in figure 15. The chromatograms are all offset to zero and are drawn overlaid. The precision of the retention time and sulfur response for the system is clearly evident. The percent relative standard deviation (%RSD) of the total sulfur area is 1.8%. The %RSD of the total carbon area measured simultaneously was 1.1%. The standard deviation of the retention time was 0.001 min for the peak at 8.6 min in the carbon chromatogram of figure 1.

The long-term precision observed here is significantly improved over that of 5890/5921 AED systems. Improvements in the 6890 EPC, such as atmospheric pressure correction, provides more precise control of column flow and split ratio. When used with the split liner described here, the amount injected into the column is very repeatable. The G2350 AED uses the 6890 Aux EPC module to control the reagent gases. The more precise control of reagents, combined with spectrometer and detection algorithm improvements, greatly increases the precision of the AED detector. The sum of the

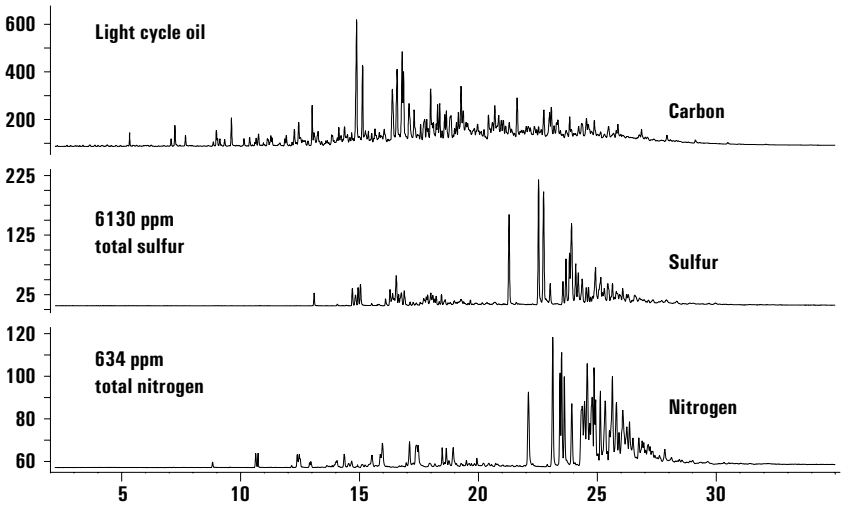

Figure 12. Refinery Light Cycle Oil (LCO) sample

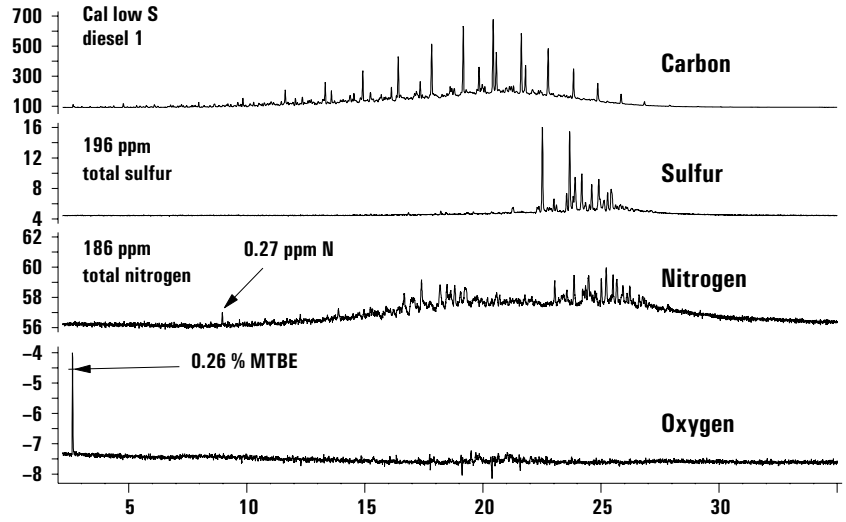

Figure 13. Commercial California low-sulfur diesel fuel, sample 1

improvements in both the GC and the AED using the conditions described here result in a measured response factor drift for sulfur and carbon of less then 3% in a week.

Seven of the samples shown here were analyzed for total sulfur using both the external and internal standard calculations from ASTM D 5623. The results agreed within 2%, indicating that with the precision observed with the AED, acceptable results can be obtained with the simpler external standard method. The external standard analysis is simpler to perform because there is less sample preparation required, and the problem of finding a suitable internal standard compound for each sample is eliminated. The internal standard approach is most useful with sulfur detectors or GC systems that have response factor drift problems. The

system described here does not exhibit these problems, thus, simplifying the analysis.

The NIST kerosine and diesel fuel SRMs were used to assess the accuracy (bias) of the method. Using the method as described here, the measured values for total sulfur agreed within 2% of the certified value.

Conclusions

The technique of GC/AED has many valuable uses in petroleum analyses. The ability to speciate of sulfur, nitrogen, oxygen, fluorine, lead, and manganese compounds in one instrument provides a very cost-effective solution to a broad range of analytical needs.

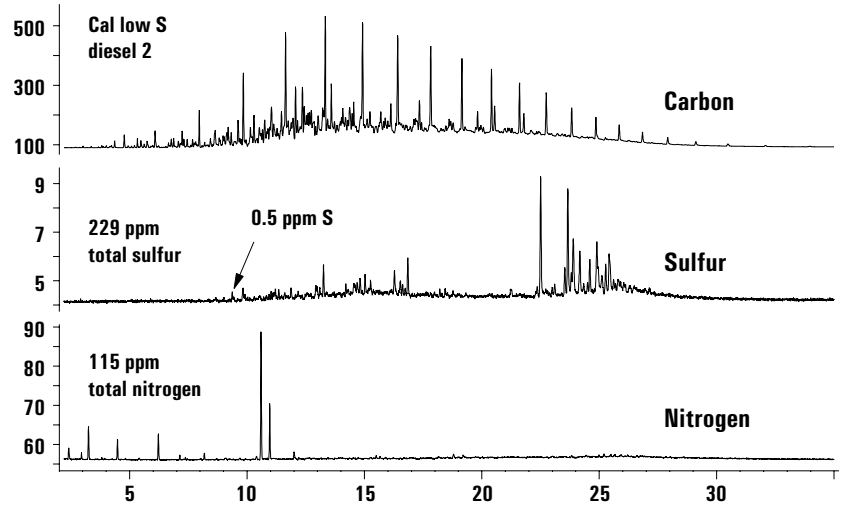

Figure 14. Commercial California low-sulfur diesel fuel, sample 2

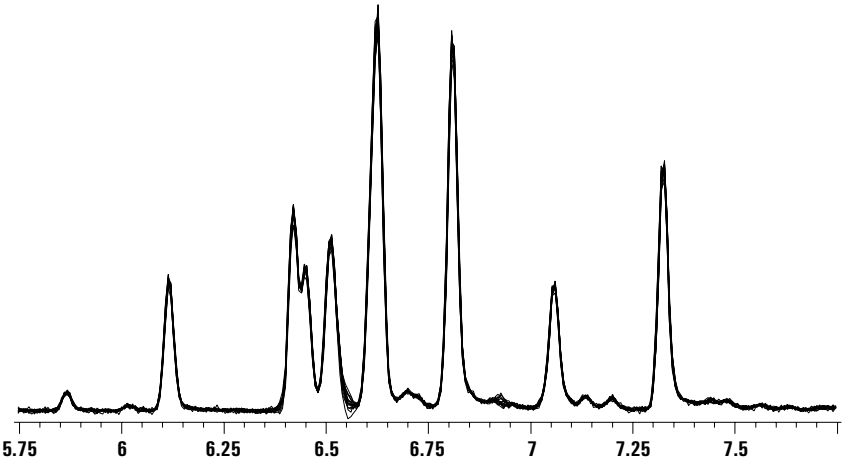

Figure 15. Chromatograms from 15 analyses of conventional gasoline sample run over a 1-week period. Chromatograms are offset to zero and drawn overlaid.

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Printed in the USA 3/2000
5966-3089E



Agilent Technologies
Innovating the HP Way

J&W Capillary Columns for GC/MS and Trace Analysis from Agilent Technologies

Technical Note

A letter from Walt Jennings

Dear Chromatographer,



Today's analysts are expected to detect and quantitate increasingly smaller quantities of more and more active solutes. Detectors like benchtop mass spectrometers and ion traps have become much more common. Our analytical potentials are now increasingly challenged by extraneous signals, resulting in an increased demand for lower bleed columns. Answering that demand gave rise to a new generation of thermally resistant columns capable of operation at higher temperatures and a much lower level of bleed signal. Once again, J&W was the industry leader, and our merger with Agilent Technologies will only strengthen that position.

Walt Jennings,
Cofounder,
J&W Scientific



Capillary Columns for Demanding Applications



There is a rapidly increasing population of benchtop GC/MS instruments in analytical laboratories that analyze a broadening range of trace level, higher temperature samples. These samples require increasingly inert, lower bleed, higher temperature columns. It seems logical and proper to apply the "ms" name to columns deliberately designed to chromatograph a broader range of low level samples and generating lower bleed at even higher temperatures.

What makes a J&W low bleed column from Agilent unique? Unique polymer chemistry and proprietary surface deactivation technology, both of which have contributed to columns that adhere to the tightest quality control specifications in the industry for bleed, inertness, selectivity and efficiency. Some column companies simply select the better performing capillary columns from batches of their standard columns. J&W "ms" columns utilize special surface deactivation and siloxane chemistries which enhance the chromatographic performance of siloxane polymers.

Four of the eight low bleed capillary columns from Agilent (DB™-5ms, DB-35ms, DB-17ms, DB-225ms) were designed to provide selectivities that are virtually identical to their conventional counterparts. As a result, in most cases all of the benefits of low bleed columns can be realized without any noticeable shift in analyte retention.

Not all low bleed phases are designed to mimic existing column selectivity. Because the polysiloxane backbone of the stationary phase was optimized to yield the lowest bleed column possible, DB-XLB has no conventional counterpart. Many users also gain sensitivity by replacing their general purpose, apolar columns such as 100% dimethylpolysiloxane and 5% diphenyl/95% dimethylpolysiloxane with DB-XLB.

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The Benefits of "ms" Grade Columns

Column bleed is the normal degradation of the stationary phase polymer. This degradation is accelerated at higher temperatures, hence the elevated baseline as temperature rises to the column's upper limit (Figure 1).

Analytical sensitivity and detection limits are ultimately a function of the ratio of signal to noise (S/N). Decrease the noise, and sensitivity is increased. Using low bleed stationary phases reduces the column contribution to background noise and the reduced column bleed results in improved mass spectral purity and more accurate library identification. In addition, the exteded upper temperature range of these more thermally stable phases results in shorter analysis times and extended column lifetime.

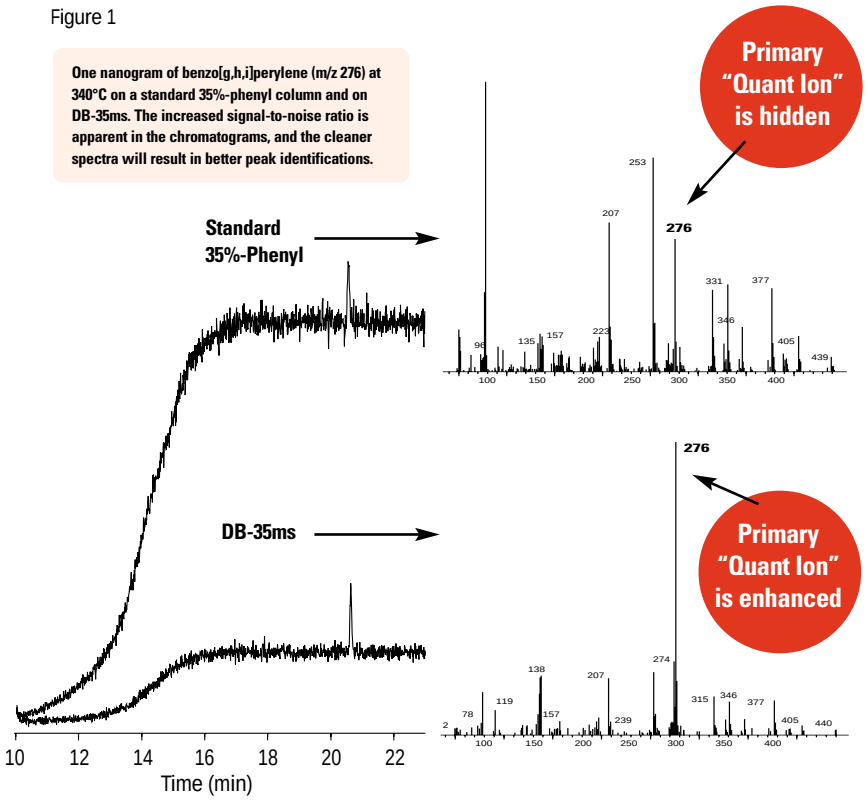
Low bleed columns are not just for the "ms" user. Anyone performing trace analysis where sensitivity and instrument performance are issues should be concerned about bleed. Many GC detectors (MS, FID, PID, ECD, NPD, SCD) are sensitive to contamination from bleed and will require less maintenance when using low bleed columns. Ion trap "ms" users, because of the unique relationship between ion storage capacity of the trap and sensitivity, will especially benefit from low bleed columns. Many users are already taking advantage of all the benefits that low bleed stationary phases provide. With improved sensitivity, shorter analysis times, excellent inertness and virtually identical selectivity these phases are clearly the superior choice for your GC and GC/MS applications.

Table 1
Retention Indices at 90°C

Compound	Column DB-5ms	DB-5	DB-XLB	DB-35ms	DB-35	DB-17ms	DB-17
3,5-dimethylpyridine	980.5	978.9	1016.8	1124.5	1104.3	1159.3	1160.3
1-nitrohexane	1088.9	1083.3	1053.6	1150.0	1164.9	1209.5	1208.3
1,4-diisopropylbenzene	1168.9	1168.7	1186.2	1255.1	1257.1	1291.0	1289.3
1-nonanol	1171.9	1173.0	1186.2	1247.7	1243.8	1274.4	1273.7
2-decanone	1190.0	1190.9	1215.4	1283.0	1276.6	1310.2	1309.2

Figure 1

One nanogram of benzo[g,h,i]perylene (m/z 276) at 340°C on a standard 35%-phenyl column and on DB-35ms. The increased signal-to-noise ratio is apparent in the chromatograms, and the cleaner spectra will result in better peak identifications.



Selectivity Options in GC/MS Columns

The special siloxane chemistry used by Agilent allows for the manufacture of low bleed columns over a range of polarity. The columns are highlighted on the following pages.

The data in Table 1 makes several important points. The first is the close agreement of the retention values for the arylene "ms" columns relative to the standard siloxane polymers. These differences will be transparent to the majority of all users. The second observation is that DB-XLB is a unique stationary phase with a relatively low polarity in the range of a (12% phenyl)-methylsiloxane. This selectivity, coupled with the attributes of extremely low bleed and a deactivation technology that yields a highly inert surface, makes DB-XLB an excellent choice for trace analytical work.

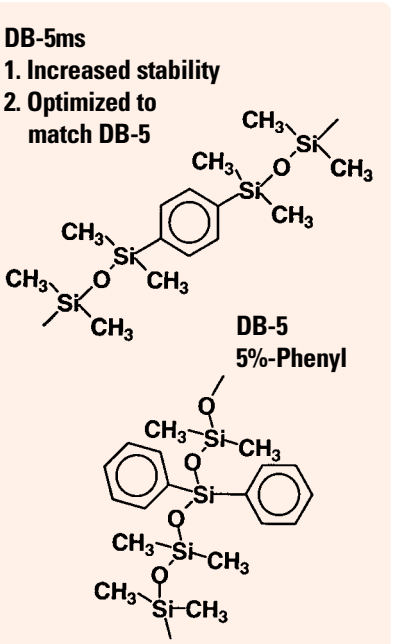
Two Phase Chemistries Provide Many Column Options

"ms" grade columns using two approaches to siloxane chemistry are available from Agilent. Both have advantages and yield columns of low bleed, high thermal stability and excellent inertness.

Low Bleed Arylene Phases

Arylene "ms" columns utilize special surface deactivation and siloxane chemistries, which enhance the chromatographic performance of siloxane polymers. Figure 2 is a representation of the arylene chemistry used to make DB-5ms. DB-XLB, DB-35ms, DB-17ms and DB-225ms use a proprietary second generation arylene technology to provide even better performance at high temperatures, expanding the range of commercially available low bleed columns. These polymers were designed to be virtually identical to their "parent" polymers, so selectivity differences are often very subtle (see Table 1). What one quickly notices with these columns is the low bleed signature of the column at elevated temperatures, as well as the improved thermal stability of the more polar "ms" columns such as DB-35ms, DB-17ms and DB-225ms. Higher temperature limits also mean extended column lifetime and shorter analysis times for many applications.

Figure 2



These columns are available in a wide range of configurations

DB-5ms (equivalent to HP-5TA):

Designed with the selectivity of a standard 5%-phenyl siloxane phase, DB-5ms incorporates arylene technology to provide a phase that not only bleeds less but also offers excellent inertness to both acidic and basic sample components. Available in a large selection of standard part numbers, DB-5ms has upper temperature limits of 325°C isothermal and 350°C on a program. DB-5ms is equivalent to HP-5TA.

DB-XLB:

XLB means eXceptionally Low Bleed. This is the lowest bleed column available anywhere. DB-XLB uses second generation arylene technology to provide a phase with excellent inertness, higher temperature limits (340/360°C) and a unique selectivity. Good for all general applications as well as those that push the limits of your existing GC/MS column, DB-XLB has a selectivity that makes it the first choice for the GC/MS analysis of specific PCB congeners.

DB-35ms (equivalent to HP-35ms):

Second generation arylene technology has also yielded phases with increasing polarity. DB-35ms has a selectivity that is virtually identical to a standard 35%-phenyl siloxane with increased upper temperature limits (340/360°C) and greatly reduced bleed. DB-35ms gives the GC/MS user a selectivity option for the resolution of high boiling, late eluting components. DB35ms is equivalent to HP-35ms.

DB-17ms:

DB-17ms uses second generation arylene polymer technology to give a selectivity that is virtually identical to standard 50%-phenyl siloxane columns. These columns have significantly higher upper temperature limits (320/340°C) with greatly reduced column bleed.

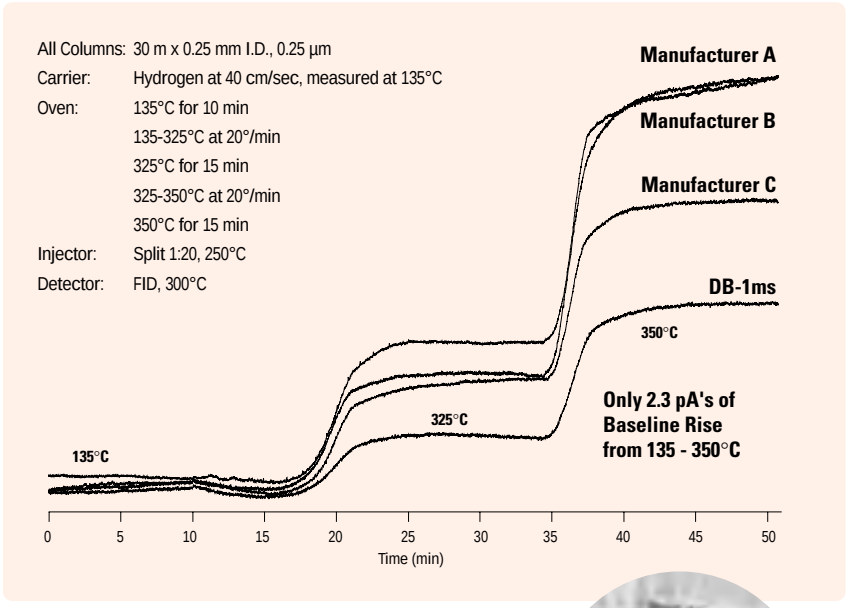
DB-225ms:

DB-225ms has been designed to provide lower bleed and a higher upper temperature limit (240/260°C) while maintaining selectivity which is virtually identical to that of the standard DB-225 (50% cyano-propylphenyl-methylpolysiloxane). The lower bleed allows for more sensitive analyses of trace components in bleed sensitive detectors and less detector fouling. DB-225ms is an excellent choice for the analysis of fatty acid methyl esters (FAMEs) and tetrachlorodibenzo-p-furans (TCDFs).

Optimized Siloxanes

Stationary phases such as DB-1 or HP-1, which are essentially 100% dimethylpolysiloxane, are not accurately mimicked with arylene inclusion to strengthen the polymer backbone. That's because the addition of other functional groups causes a significant change in the selectivity of the phase. While some manufacturers have taken this path, the resulting columns have a significantly different selectivity from the standard 100% dimethylpolysiloxane. J&W Scientific has utilized its years of experience as the worlds largest manufacturer of capillary GC columns to design phases that meet the requirements of trace analysis and retain the selectivity of the original phases. This translates to improved deactivation, new polymer synthesis and processing techniques and stringent quality control. One of these phases, DB-1ms, has a greatly reduced background level even at elevated temperatures (Figure 3).

Figure 3
Low Bleed Comparison of "1ms" Columns





These columns are available in a wide range of configurations

DB-1ms

Provides the same selectivity as the standard DB-1 column. Improved thermal stability is achieved through unique, proprietary techniques allowing for increased temperature limits (340/360°C). DB-1ms exhibits exceptional inertness for the analysis of acidic and basic compounds. Lower bleed, increased temperature limits and excellent inertness are the perfect combination for trace level analysis. Availability in a large selection of standard part numbers makes the DB-1ms a good choice for general applications that push the limits of your standard DB-1 column.

HP-1ms

Provides the same selectivity and inertness as the standard HP-1. An optimized manufacturing process ensures that each column meets ultra-low bleed specifications. Increased signal-to-noise ratios at maximum temperatures (325-350°C) and incredible inertness for acidic and basic compounds make HP-1ms a good choice for applications requiring low detection limits.

HP-5ms

HP-5ms uses the same stationary phase as the standard HP-5, providing low bleed without the use of arylene technology. HP-5ms exhibits the same selectivity as the standard HP-5 with reduced bleed at the maximum temperatures (325-350°C). A good choice for general GC/MS applications and trace level analysis.

Phase	Dimensions	Part Number
DB-1ms	30 m x 0.25 mm x 0.25 µm	122-0132
HP-1ms	30 m x 0.25 mm x 0.25 µm	19091S-933
DB-5ms	30 m x 0.25 mm x 0.25 µm	122-5532
HP-5ms	30 m x 0.25 mm x 0.25 µm	19091S-433
DB-XLB	30 m x 0.25 mm x 0.25 µm	122-1232
DB-35ms	30 m x 0.25 mm x 0.25 µm	122-3832
DB-17ms	30 m x 0.25 mm x 0.25 µm	122-4732
DB-225ms	30 m x 0.25 mm x 0.25 µm	122-2932

Each stationary phase is available in a variety of dimensions. A full listing of the low bleed columns are on the chemical analysis online store at ***www.agilent.com***

For further information, please contact your local Agilent Office or your authorized Agilent distributor. You can also logon to www.agilent.com/chem

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Increasing Sample Throughput with High-Speed Megabore Columns

Application

Greater than 20% More Plates Per Meter

Improved Resolution and/or Faster Run Times Compared to 0.53-mm ID Columns

No Special Hardware Required

Decreasing the diameter of a capillary column is an effective way of increasing column efficiency. This increase in the number of theoretical plates per meter (N/m) can be utilized to improve compound resolution. A significant decrease in analysis time can also be achieved by adjusting the analysis conditions or shortening the column length.

For the chromatographer using Megabore (that is, 0.53-mm ID) columns, going to smaller internal diameter columns has not always been an option, due in part to capacity issues and injector and/or detector hardware incompatibilities. The 0.45-mm ID, High-Speed Megabore column introduces the traditional Megabore chromatographer to a column that can increase the resolution of analytes and/or reduce some analysis times by as much as 45%. Because Agilent's High-Speed Megabore columns retain the same outer diameter as 0.53-mm ID columns, no special ferrules or adaptors are required.

High-Speed Megabore columns also have the same phase ratio (ß) as 0.53-mm ID columns, making it very easy to translate the method conditions. Methods can easily be optimized for speed or resolution using free method translation software available from the Agilent Web site or by speaking with our Technical Support Department (call 800-227-9770 in the U.S. or Canada or visit www.agilent.com/chem).

On average, the High-Speed Megabore provides 24% more theoretical plates per meter than the comparable 0.53-mm ID column (Table 1). At some point, increasing a column's length can begin to work against chromatographic efficiency gain due to high carrier gas pressure drop in long capillaries. This is exemplified with the 105 m, DB-502.2. Figure 1 compares the two DB-502.2 columns for the analysis of volatile organics by purge and trap (for example, EPA Method 502.2). Most notable in these chromatograms are the essentially identical resolution of analytes and the 23-minute decrease in run time with the High-Speed Megabore column.

High-Speed Megabore columns are ideally suited to applications where dual 0.53-mm columns are currently being used. Figure 2a and 2b show one such application.

Table 1. Column Efficiencies

Column phase	Column length	Internal diameter	Film thickness [1]	Plates/meter (% increase) [2]
DB-VRX	75 meters	0.449 mm	2.55 µm	1997 (28)
	75 meters	0.540 mm	3.00 µm	1447
DB-624	75 meters	0.446 mm	2.55 µm	1402 (22)
	75 meters	0.546 mm	3.00 µm	1090
DB-502.2	75 meters	0.453 mm	2.55 µm	1526 (20)
	105 meters	0.544 mm	3.00 µm	873
DB-WAX	30 meters	0.447 mm	0.85 µm	1656 (25)
	30 meters	0.544 mm	1.00 µm	1357
DB-1	30 meters	0.455 mm	1.30 µm	1477 (27)
	30 meters	0.551 mm	1.50 µm	1357
DB-5	30 meters	0.446 mm	1.30 µm	1895 (23)
	30 meters	0.540 mm	1.50 µm	1454
DB-608	30 meters	0.450 mm	0.71 µm	1477 (23)
	30 meters	0.535 mm	0.83 µm	1134

[1] Phase ratio (ß) held constant for all columns

[2] Average 24%

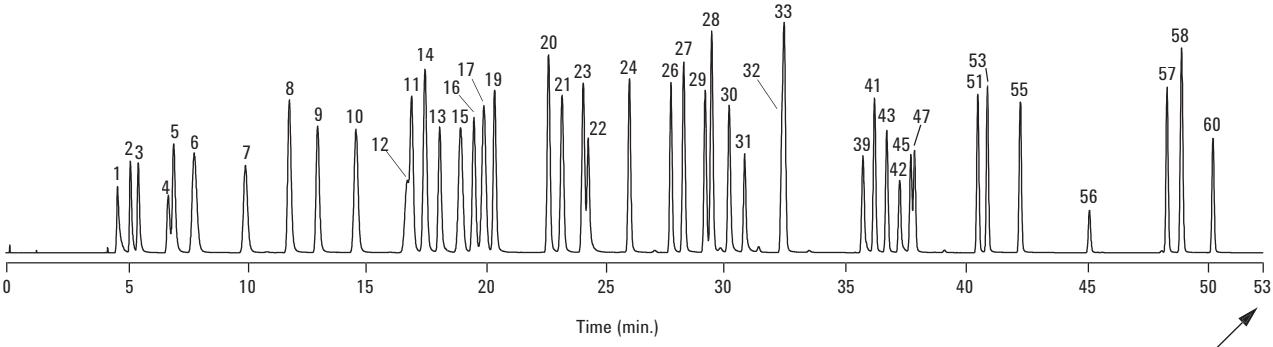
Compound List for all Chromatograms

1. Dichlorodifluoromethane
2. Chloromethane
3. Vinyl chloride
4. Bromomethane
5. Chloroethane
6. Trichlorofluoromethane
7. 1,1-Dichloroethene
8. Methylene chloride
9. trans-1,2-Dichloroethene
10. 1,1-Dichloroethane
11. cis-1,2-Dichlorethene
12. 2,2-Dichloropropane
13. Bromochloromethane
14. Chloroform
15. 1,1,1-Trichloroethane
16. 1,1-Dichloropropene
17. Carbon Tetrachloride
18. Benzene
19. 1,2-Dichloroethane
20. Silica trichloroethene
21. 1,2-Dichloropropane
22. Dibromomethane
23. Bromodichloromethane
24. cis-1,3-Dichloropropene
25. Toluene
26. trans-1,3-Dichloropropene
27. 1,1,2-Trichloroethane
28. Tetrachloroethene
29. 1,3-Dichloropropane
30. Dibromochloromethane
31. 1,2-Dibromomethane
32. Chlorobenzene
33. 1,1,1,2-Tetrachloroethane
34. Ethylbenzene
35. meta-Xylene
36. para-Xylene
37. ortho-Xylene
38. Styrene
39. Bromoform
40. Isopropylbenzene
41. 1,1,2,2-Tetrachloroethane
42. Bromobenzene
43. 1,2,3-Trichloropropane
44. n-Propylbenzene
45. 2-Chlorotoluene
46. 1,2,3-Trimethylbenzene
47. 4-Chlorotoluene
48. tert-Butylbenzene
49. 1,2,4-Trimethylbenzene
50. sec-Butylbenzene
51. 1,3-Dichlorobenzene
52. para-Isopropyltoluene
53. 1,4-Dichlorobenzene
54. n-Butylbenzene
55. 1,2-Dichlorobenzene
56. 1,2-Dibromo-3-chloropropane
57. 1,2,4-Trichlorobenzene
58. Hexachlorobutadiene
59. Naphthalene
60. 1,2,3-Trichlorobenzene

Conditions

Column: **DB-502.2, 105 m x 0.53-mm ID, 3.0 µm**
Part no.: 125-14A4
Carrier: Helium at 10 mL/min, measured at 35 °C
Oven: 35 °C for 10 min
35 °C - 200 °C at 4 °C/min
200 °C for 5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax™/Silica gel/Charcoal
Detector: Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat™ reaction tube in the halogen mode



Conditions

Column: **DB-502.2, 75 m x 0.45-mm ID, 2.55 µm**
Part no.: 124-1474
Carrier: Helium at 9 mL/min, measured at 35 °C
Oven: 35 °C for 6 min
35 °C - 200 °C at 8 °C/min
200 °C for 3.5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax/Silica gel/Charcoal
Detector: ELCD (OIA 4420) with NiCat reaction tube in the halogen mode

High-Speed Megabore saves 23 minutes!

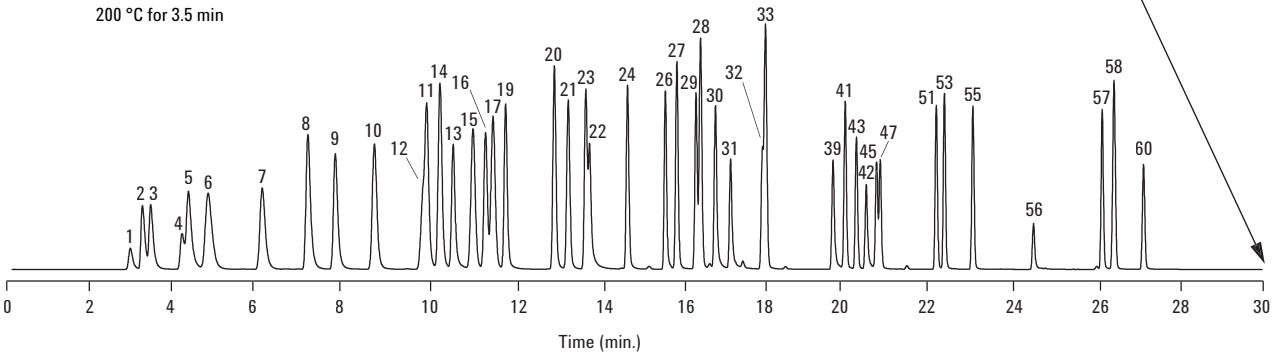


Figure 1. Analysis time comparison

Conditions
Figure 2a and 2b

Columns:	**DB-624** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1374 **DB-VRX** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1574
Guard Column:	5m x 0.53-mm ID deactivated fused silica tubing 3-way universal glass union
Carrier:	Helium at 9 mL/min (18 mL/min total), measured at 35 °C
Oven:	35 °C for 12 min 35 °C - 60 °C at 5 °C/min 60 °C for 1 min 60 °C - 200 °C at 17 °C/min 200 °C for 4 min
Injector:	Purge and trap (OIA 4560) 40 ppb per component in 5 mL water
Trap:	Tenax/Silica gel/Charcoal
Detector A:	Photoionization detector (PID) (OIA 4430) at 220 °C
Detector B:	Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat reaction tube in the halogen mode

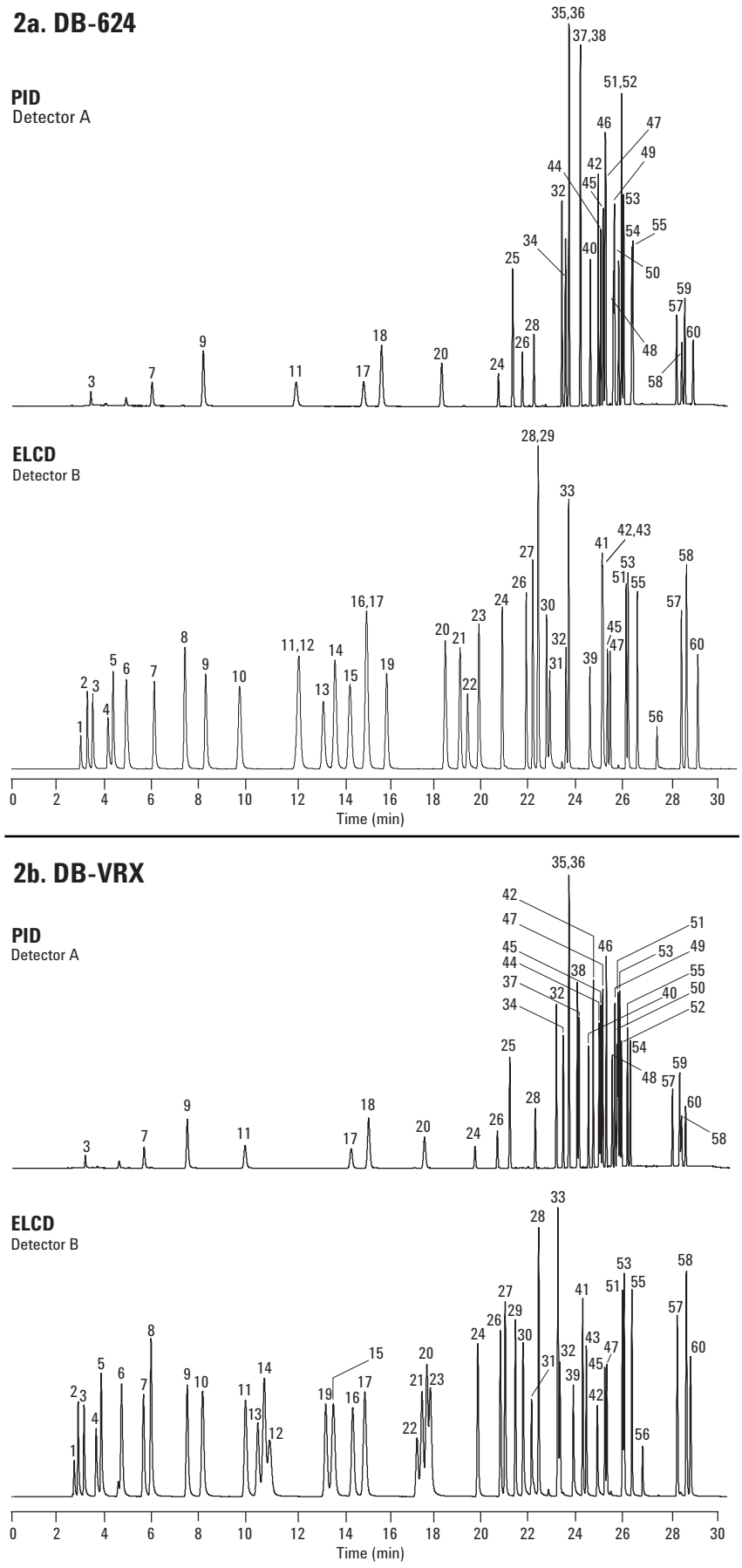


Figure 2a and 2b. High-Speed Megabore dual column applications.

0.45-mm ID High-Speed Megabore Column Order Guide

Phase[1]	Inner diameter (mm)	Length (meter)	Film thickness (µm)	Temperature limits (°C)	Part number
DB-1	0.45	15	1.27	-60 to 300/320	124-1012
DB-1	0.45	15	2.55	-60 to 260/280	124-1014
DB-1	0.45	30	0.42	-60 to 300/320	124-1037
DB-1	0.45	30	1.27	-60 to 300/320	124-1032
DB-1	0.45	30	2.55	-60 to 260/280	124-1034
DB-1	0.45	30	4.25	-60 to 260/280	124-1005
DB-1	0.45	60	1.27	-60 to 300/320	124-1062
DB-5	0.45	15	1.27	-60 to 300/320	124-5012
DB-5	0.45	30	0.42	-60 to 300/320	124-5037
DB-5	0.45	30	1.27	-60 to 300/320	124-5032
DB-5	0.45	30	4.25	-60 to 260/280	124-5035
DB-17	0.45	15	0.85	40 to 260/280	124-1712
DB-17	0.45	30	0.85	40 to 260/280	124-1732
DB-1701	0.45	30	0.42	-20 to 260/280	124-0737
DB-1701	0.45	30	0.85	-20 to 260/280	124-0732
DB-200	0.45	30	0.85	30 to 280/300	124-2032
DB-210	0.45	30	0.85	45 to 220/240	124-0232
DB-2887	0.45	10	2.55	-60 to 350	124-2814
DB-502.2	0.45	75	2.55	0 to 260/280	124-1474
DB-502.2	0.45	105	2.55	0 to 260/280	124-14A4
DB-608	0.45	30	0.42	40 to 260/280	124-6837
DB-608	0.45	30	0.70	40 to 260/280	124-1730
DB-624	0.45	30	2.55	-20 to 260	124-1334
DB-624	0.45	75	2.55	-20 to 260	124-1374
DB-FFAP	0.45	15	0.85	40 to 250/250	124-3212
DB-FFAP	0.45	30	0.85	40 to 250	124-3232
DB-MTBE	0.45	30	2.55	35 to 260/280	124-0034
DB-TPH	0.45	30	1.00	-10 to 290/290	124-1632
DB-VRX	0.45	30	2.55	-10 to 260	124-1534
DB-VRX	0.45	75	2.55	-10 to 260	124-1574
DB-WAX	0.45	60	0.85	20 to 230/240	124-7062
DB-WAX	0.45	15	0.85	20 to 230/240	124-7012
DB-WAX	0.45	30	0.85	20 to 230/240	124-7032
DB-WAXetr	0.45	5	1.70	50 to 230/250	124-7304
DB-XLB	0.45	30	1.27	30 to 320/340	124-1232

[1]Additional phases, lengths, and film thickness can be made with a 0.45-mm ID High-Speed Megabore column. If you do not find the column you are looking for, ask for a custom column quote (order part number 100-2000 and specify the phase, ID, length, and film thickness).

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Printed in the USA
February 13, 2002
5988-5271EN

Agilent Technologies

Using Statistical Peak Identification

Application

Agilent Cerity NDS for Chemical QA/QC

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Abstract

Quantitation demands correct identification for peaks of interest. Traditional peak identification is based upon correlating compounds with their retention times. This retention time correlation is complicated by shifts due to changes in concentration and changes caused by external forces. When peak identification algorithms cannot deal with these complications, they fail to identify peaks correctly. The Statistical Peak Identification feature addresses some of these complications in Agilent's Cerity NDS for Chemical QA/QC. This application shows how Statistical Peak Identification provides a more robust quantitation.

Introduction

There are many ways that retention times (RTs) of peaks can be shifted. Sources can be characterized in terms of the system and of the sample. System issues include effects of ambient temperature and pressure on the ability of the instrument to control column flow rates and zone temperatures. Instruments, such as the Agilent 6890, provide compensation for shifts in ambient temperature and pressure, and also provide precise control of column flow rates and heated zones on the chromatograph. This has reduced variation due to these factors. However, if method conditions are changed, peak identification may no longer be valid. Concentration of components in the sample can also dramatically affect their RTs and, thereby, the peak identification process. Sample concentration also can cause significant shifts in the RT due to peak overload.

In Figure 1 the RT shifts to earlier RTs using gas solid chromatography. Gas liquid chromatography can behave differently. With the overload of an acidic compound, which is not very soluble in the liquid phase, the RT shifts to longer times. The computed symmetries for these peaks are very different. The peaks can move towards other peaks and obscure them.

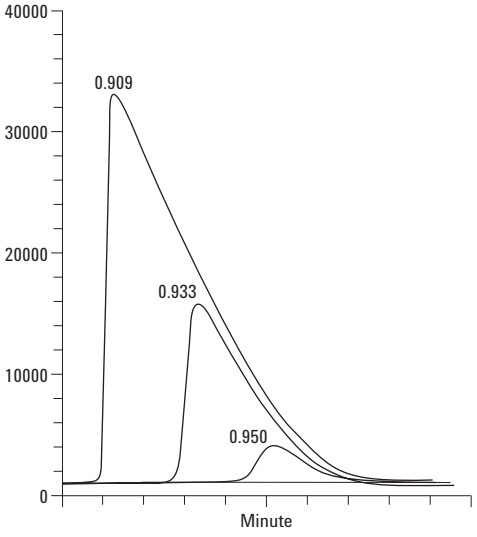


Figure 1. RT as a function of sample concentration on an Alumina column of a refinery gas analyzer (RGA).

The primary means for automated peak identification has been correlation of RTs with the RTs given for compounds listed in the method's calibration table. Focusing only on RT requires that RT windows be defined for identification purposes. When RT windows for adjacent peaks overlap, a decision rule for peak identification is necessary. The rule is normally based on the assumption that the named peak will be the largest peak in the window. For many samples run with gas chromatography (GC), this assumption is sufficient. However, sometimes, even subtle RT shifts can lead to peak misidentification for peaks which are closely eluting due to decision rules used. Figure 2 from the PlotU channel of a Natural Gas Analyzer (NGA) illustrates how a small shift can cause misidentification.

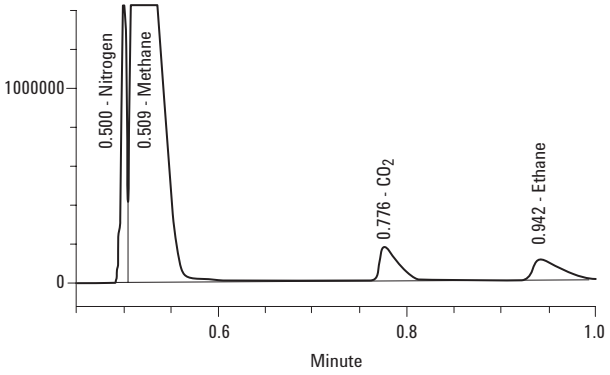


Figure 2. NGA Analysis on the PlotU channel with nitrogen and methane correctly identified.

Note that the RT for nitrogen is 0.500, and for methane is 0.509. A second run shows a 0.003-min shift for the peak which is actually the methane: identification is reversed because of the decision rules used (See Figure 3).

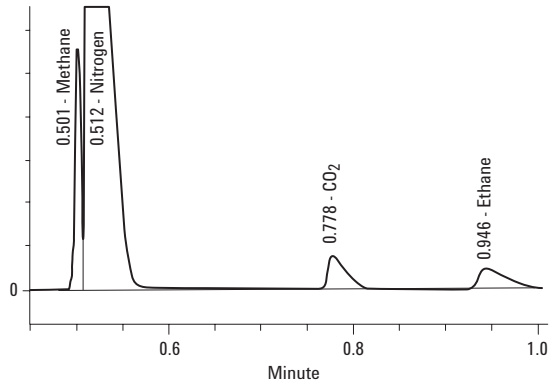


Figure 3. Small RT shift causing misidentification of the nitrogen and methane on the PlotU channel.

Prior to availability of the Statistical Peak Identification (SPID) algorithm, the only way to correct the misidentification in this case was to define one of the other peaks as a reference peak. With release of Cerity 4.03, the user is given a second choice.

Statistical Peak Identification

SPID is a feature added to the Calibration Options user interface (Figure 4) in Agilent's Cerity

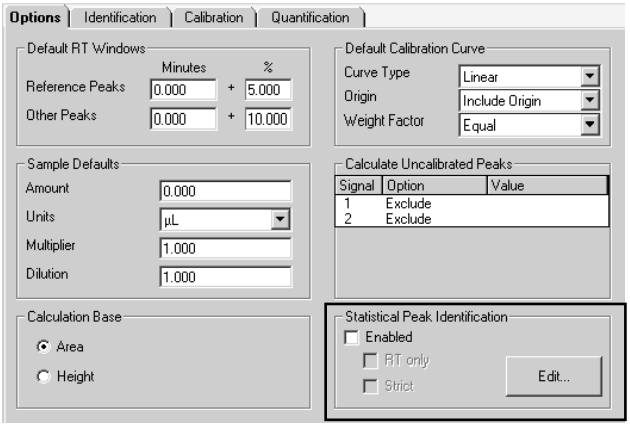


Figure 4. SPID on the calibration options screen.

networked data system (NDS). Using this feature requires the user to enter at least one correctly identified sample in the SPID Data Pool and to check the Enabled checkbox. The Data Pool enables the SPID algorithm to use peak symmetry and area information for each compound in the calibration table. The first set of results is added to the SPID Data Pool prior to enabling SPID in the Options screen for Calibration.

The Data Pool is generated by selecting a sample(s) having correct identification and reprocessing the sample(s) using the Group Reprocessing button at the extreme left of the Reprocess toolbar (Figure 5).

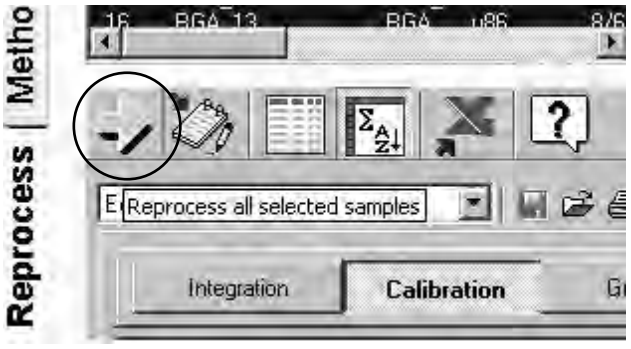
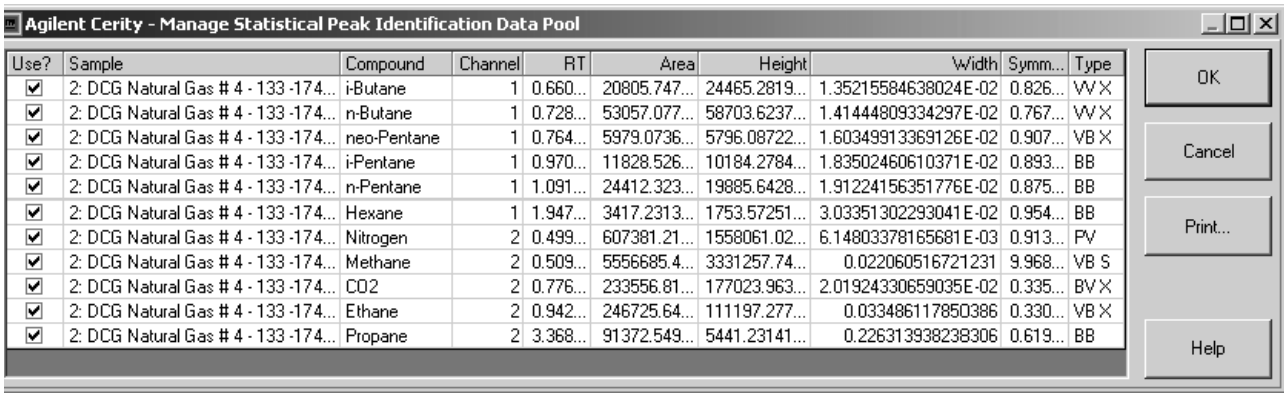


Figure 5. The toolbar for group reprocess on the reprocess view.

Next, results are added to the Data Pool by selecting the Add reprocessed data to SPID data button (Figure 5, adjacent on the right of the Reprocess all selected samples button). The user can then go to the Calibration page under Edit Analysis and click the Edit button. This brings up the following screen, Figure 6, which shows part of the data from the sample in Figure 2. The check box for enabling SPID can now be selected. Peak information is now in the Data Pool and can be used for peak identification (See Figure 7).

current method. The sample can then be reprocessed.

SPID has yet to be used to its fullest advantage. Referencing Figure 1, concentration changes cause large shifts in RT. Developing the SPID Data Pool to include correctly identified components at different concentrations gives a more robust peak identification. Using the Data Pool gives the method developer control of RT changes analogous to Multi-Level Calibration for Amount changes.



Use?	Sample	Compound	Channel	RT	Area	Height	Width	Symm...	Type
☑	2: DCG Natural Gas # 4 - 133 -174...	i-Butane	1	0.660...	20805.747...	24465.2819...	1.35215584638024E-02	0.826...	VV X
☑	2: DCG Natural Gas # 4 - 133 -174...	n-Butane	1	0.728...	53057.077...	58703.6237...	1.41444809334297E-02	0.767...	VV X
☑	2: DCG Natural Gas # 4 - 133 -174...	neo-Pentane	1	0.764...	5979.0736...	5796.08722...	1.60349913369126E-02	0.907...	VB X
☑	2: DCG Natural Gas # 4 - 133 -174...	i-Pentane	1	0.970...	11828.526...	10184.2784...	1.83502460610371E-02	0.893...	BB
☑	2: DCG Natural Gas # 4 - 133 -174...	n-Pentane	1	1.091...	24412.323...	19885.6428...	1.91224156351776E-02	0.875...	BB
☑	2: DCG Natural Gas # 4 - 133 -174...	Hexane	1	1.947...	3417.2313...	1753.57251...	3.03351302293041E-02	0.954...	BB
☑	2: DCG Natural Gas # 4 - 133 -174...	Nitrogen	2	0.499...	607381.21...	1558061.02...	6.14803378165681E-03	0.913...	PV
☑	2: DCG Natural Gas # 4 - 133 -174...	Methane	2	0.509...	5556685.4...	3331257.74...	0.022060516721231	9.968...	VB S
☑	2: DCG Natural Gas # 4 - 133 -174...	CO2	2	0.776...	233556.81...	177023.963...	2.01924330659035E-02	0.335...	BV X
☑	2: DCG Natural Gas # 4 - 133 -174...	Ethane	2	0.942...	246725.64...	111197.277...	0.033486117850386	0.330...	VB X
☑	2: DCG Natural Gas # 4 - 133 -174...	Propane	2	3.368...	91372.549...	5441.23141...	0.226313938238306	0.619...	BB

Figure 6. Manage SPID Data Pool screen.

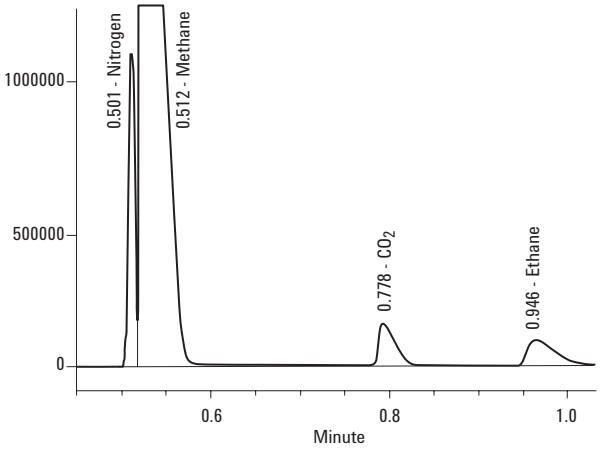


Figure 7. SPID correctly identifying peaks on the previously misidentified chromatogram (Figure 3).

Note that further editing is possible by choosing to use data for an individual sample's results for peak identification. The Data Pool provides means to include additional peak information such as area and symmetry for peak identification. Correcting RT misidentification in this NGA example only required the one sample shown in the Data Pool. From the reprocess screen, the method can be saved either as another version of the original method, or as a new method. However, the user could select a sample with the misidentification without saving, but then must select to retain the

The following example illustrates building a method covering the analytical range of interest. The method is developed using a Refinery Gas Calibration mixture on an Agilent 3000 micro Gas Chromatograph. This multicolumn system rapidly analyzes fixed gases and light hydrocarbons. The Alumina column, used primarily for the unsaturates, will be examined in detail. Table 1 lists the components quantitated on Alumina. Column temperature for the Alumina channel was set at 120 °C. Concentration was varied both by dilution and by injection time (5, 10, and 20 ms for the undiluted sample).

Table 1. Refinery Gas Components on Alumina in Elution Order

Analyte:	**Mole %**
Propane	5.0
Propylene	1.0
i-Butane	10.0
n-Butane	5.0
trans-2-Butene	**5.0**
1-Butene	**10.0**
cis-2-Butene	**5.0**
i-Pentane	2.0
n-Pentane	1.0

Of particular interest are the higher concentration components: 1-Butene and the two 2-Butene isomers. Overloading behavior of these components eventually limits quantitation, thereby establishing the high end of the Analytical range of interest for this analysis. To cover the Analytical range of interest, a series of successive dilutions of the RGA sample was analyzed using extremes as indicated in Figure 8. The range in the area depicted is about 190:1 for the 10% 1-Butene (somewhat different for other components).

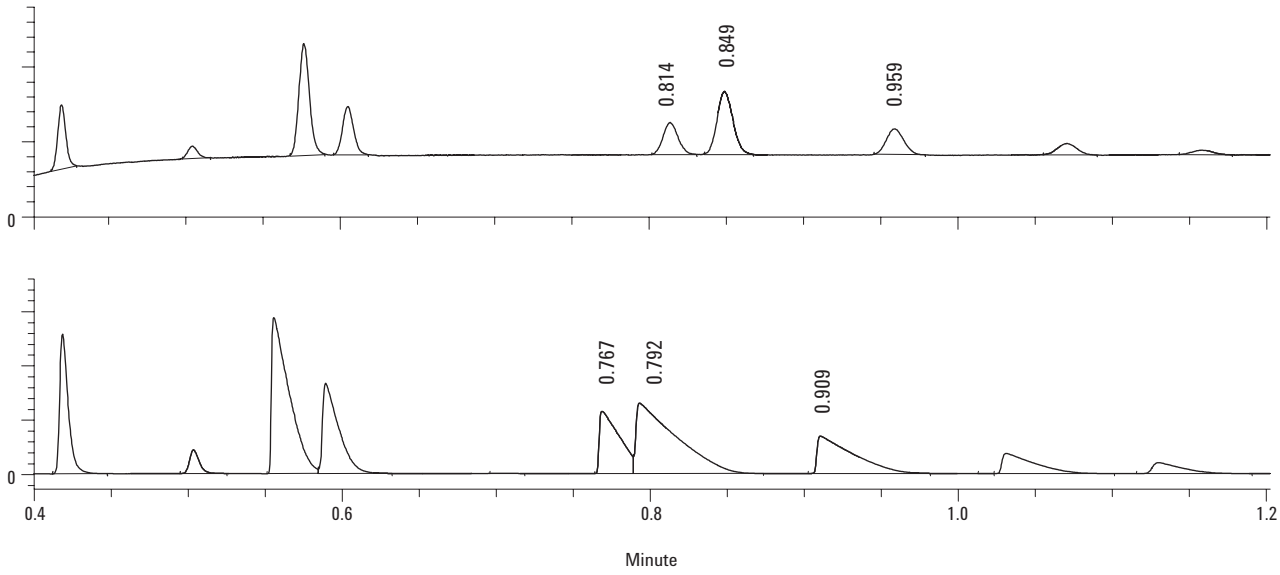
Calibrating from RTs of the dilute analysis, Figure 8 demonstrates results achieved on the overloaded sample. All the ID techniques correctly identify the first and second peaks, Propane and Propylene (not included in the Table). The ID technique in the Table [5% no ref] refers to the Default calibration settings: 5% RT time windows and no time reference peak.

The default technique fails badly. Only one of the seven remaining peaks is correctly identified. Five are missed and n-Butane is misidentified. Increasing the time window to 8% only helps to correctly identify iso-Pentane, but does not handle the 6.7% shift in 1-Butene. Adding Pentane [8% nC_5 ref] as a timed reference peak correctly identifies five of the seven peaks, but still cannot identify two of the Butenes.

Although the chromatogram looks simple, conventional identification methods using the "one peak to a time window" paradigm have significant difficulty. Correct identification of the components over the indicated concentration range is achieved using SPID.

This is a challenging analysis. We will describe how SPID was applied (refer to Table 2). One quick way to construct a SPID Data Pool, which spans a range of conditions, is to start with a center point and to then add the extremes. Once extremes are successfully identified, the bracketed samples should be much easier.

This "Go to Extremes" (GTX) approach has been employed on several other SPID samples with good results. It was not successful here, however, due, as in Table 1, to problems with the Butene components. The GTX approach was attempted for the extremes shown in Figure 8 using a pool from a single run. As the Report summary for the low extreme shows (Figure 9), this approach failed for



	Butanes		Butenes			Pentanes	
ID Technique	i-C_4	n-C_4	trans-2-C_4	1-C_4	cis-2-C_4	i-C_5	n-C_5
5%, No reference	N	I	N	N	N	N	C
8%, No reference	N	I	N	I	N	C	C
8%, n-C_5 reference	C	C	N	I	C	C	C
SPID	C	C	C	C	C	C	C

N = Not identified; I = Incorrectly identified; C = Correctly identified

Figure 8. RGA Chromatograms for the concentration extremes over the analytical range of interest.

Signal 2. The 1-Butene candidate is rejected on the basis of Symmetry and results in one less peak found for a total of eight. At this failure point, an experienced Cerity user could manually intervene via several steps in the Reprocess view (Table 3). We will continue without manual editing of the method.

Report summary:

Signal 1 : Found 5 peaks with fit error 2.854E-04 from 15 of 16 iterations

Signal 2 : Found 4 peaks with fit error 1.3271E-02 from 4 of 5 iterations

1-butene candidate rejected by: Symmetry 0.107/0.859

Signal 3 : Found 8 peaks with fit error 3.3917E-03 from 81 of 91 iterations

Signal 4 : Found 4 peaks with fit error 1.1020E-03 from 5 of 5 iterations

Figure 9. SPID information (Step G, Table 2) in the Report summary following quantitative results for the Low Extreme.

From Figure 10, the symmetry extremes (MIN and MAX values) from the complete 1-Butene results are both outside the single run Data Pool limit in Figure 9.

Only one extreme could be correctly identified from a single midpoint Data Pool. This halted the GTX approach at Step **H** (Table 2) and required a shift to the Maximum Inclusion approach shown to the right in Table 2. As noted in Steps **K-L** (Table 2), when the Data Pool was expanded to include a 9:1 concentration range for 1-Butene, this was adequate to correctly identify all nine components across the 190:1 concentration range. The GTX technique worked for 10 of the 12 samples in the analytical range of interest. The usage of Maximum Inclusion (always the most robust approach) was required for the extremes.

Table 2. Steps to Build a Complete SPID Data Pool

Using extremes and center point	Maximum Inclusion for all correctly identified samples
A. No entries in the Data Pool; SPID Off.	
B. Group Reprocess a mid-range sample.	
C. Verify correct identification.	
D. Add reprocessed data to Data Pool using the Add Reprocessed icon.	
E. A valid Data Pool now exists, so Enable SPID on Calibration|Options screen.	
F. Save the method with a single set of calibration values in the pool. From this point on SPID remains Enabled.	
G. Group Reprocess the extreme samples.	
H. Verify correct identification at extremes, then add the components to the pool.	
If halted by problems identifying extreme components, then use the Maximum Inclusion approach, OR manually edit the Data Pool as specified in Table 3 and then proceed.	
I. Save the method now with a bracketed set of calibration values in the pool.	**I.** Group Reprocess all remaining samples.
J. Group Reprocess, verify and add remaining samples to the pool.	**J.** Verify correctly identified samples and add all of them to the pool.
K. Save the method now with the complete data set of calibration values in the pool.	**Note: All intermediate concentrations, except extremes, were added.**
	K. Save the method with 10 calibration samples over a concentration range ~9:1.
	L. Group Reprocess the extremes.
	M. Verify correct identification at the extremes, then add the components to the pool.
	N. Save the method now with the complete data set of calibration values in the pool.

Table 3. Manual Edit of the Data Pool

1. Select RT only under SPID.
2. Manually edit retentions times.
3. Group Reprocess this manually modified method.
4. Use the Add Reprocessed data to SPID data to add this peak information to the Data Pool.
5. Unselect RT under SPID.
6. Replace the modified RTs with the original values.
7. Save the method. (Return to step I under using extremes and center point in Table 2.)

Results in Figure 10 are generated by selecting the samples and then group reprocessing the entire set following Step N (Table 2). The Cerity Spreadsheet view, requiring Excel to be installed, is selected from the Reprocess tool bar. Monitoring successful identification in these 12 samples is aided by reference to this display: note the extreme values found for 1-Butene. The MIN and MAX RTs are the extremes from Figure 8. Note that statistics for 1-Butene also display the full analytical range of several important quantitative properties: area, width, and symmetry.

Group Reprocess Summary

	C	D	E	F	G	H	I	J
1	Name	Cha	Time	Amount	Area	Height	Width	Symme
107	1-butene	3	0.791506	76.89899	116178.4	64659.11	0.022762	0.092478
112								
113	<NUMBER>		12	12	12	12	12	12
114	<AVERAGE>		0.832253	13.31598	20117.67	16519.32	0.013234	0.386476
115	<STD DEV>		0.014896	20.82839	31467.36	16843.94	0.003336	0.211417
116	<% STDEV>		1.789803	156.4165	156.4165	101.9651	25.20556	54.70366
117	<MIN>		0.791506	0.397567	600.641	843.4225	0.009508	0.092478
118	<MAX>		0.848871	76.89899	116178.4	64659.11	0.022762	0.873529
119								

Figure 10. Group reprocess results for 1-Butene in Cerity using Excel format.

Figure 11 shows the fit error reported for these cases. Fit error and total amount are shown in the log-log plot.

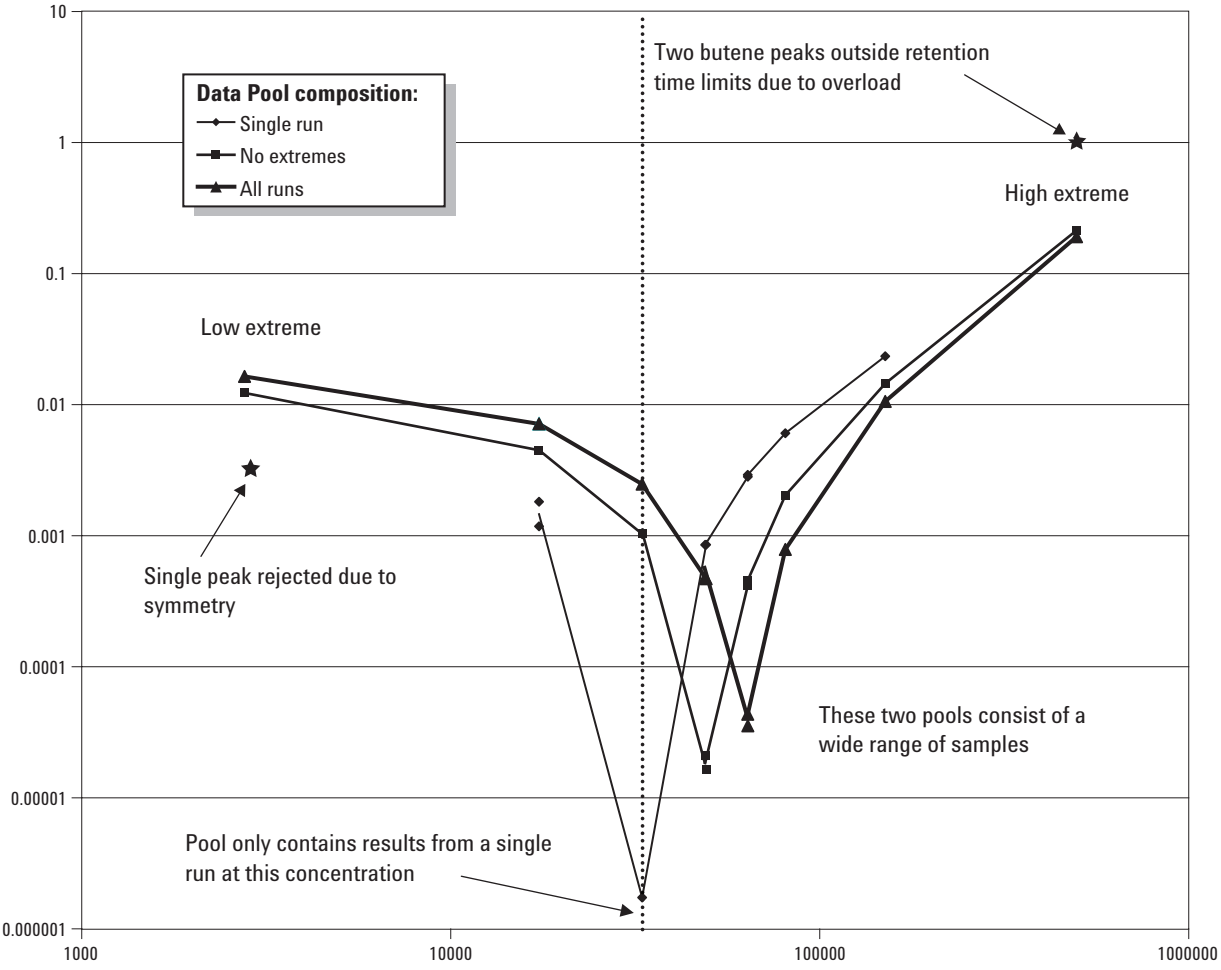


Figure 11. Log-Log plot: fit error vs. concentration of RGA sample for several Data Pool compositions. Increasing fit error correlates with increasing deviation of a given sample's RTs from those at the Data Pool mean.

Fit error is found on the report summary line associated with the Alumina column:

```
Signal 3 : found 9 peaks with fit error 1.7334E-06 from 71 of 71 iterations
```

This is the lowest value of fit error, and therefore the best fit, in the single run Data Pool plot displayed in Figure 11. Note that extreme values are not included because they are not successfully identified.

The fit error at the High Extreme is nearly six orders of magnitude greater than the lowest fit error for this single run Data Pool. This indicates difficulty with fit of the mean pool values for RTs to RTs for the High Extreme case. In this High Extreme case, overloading moves all peaks to lower times by varying degrees. There is no such increase in fit error at the Low Extreme since there is hardly any shift in RT in approaching infinite dilution.

As runs are added producing a “wider” pool with multiple concentrations, extremes are identified successfully.

Limitations

Figure 12 is an example, using temperature programming, demonstrating limitations of various peak identification techniques, and also illustrating the SPID reporting mechanism.

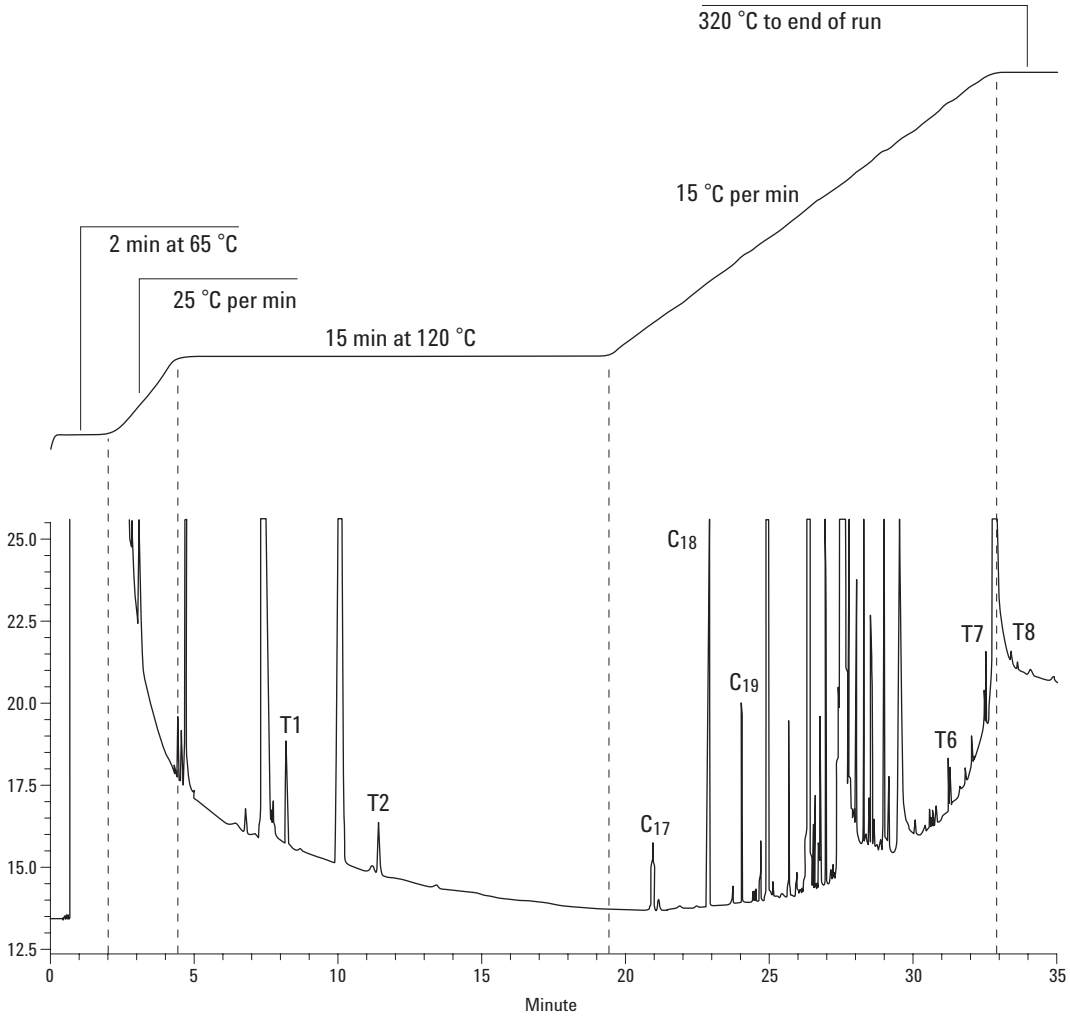


Figure 12. Some peak identities at a 6.0 mL/min column flow rate.

The analysis uses two oven ramps separated by isothermal holds. Flow rate changes are used to simulate effects of column aging, changes in column length, and transfers of analytical methods among instruments and laboratories. To challenge peak identification for this example, a flow rate change of 10% [from 6.1 to 5.5 mL/min] is necessary.

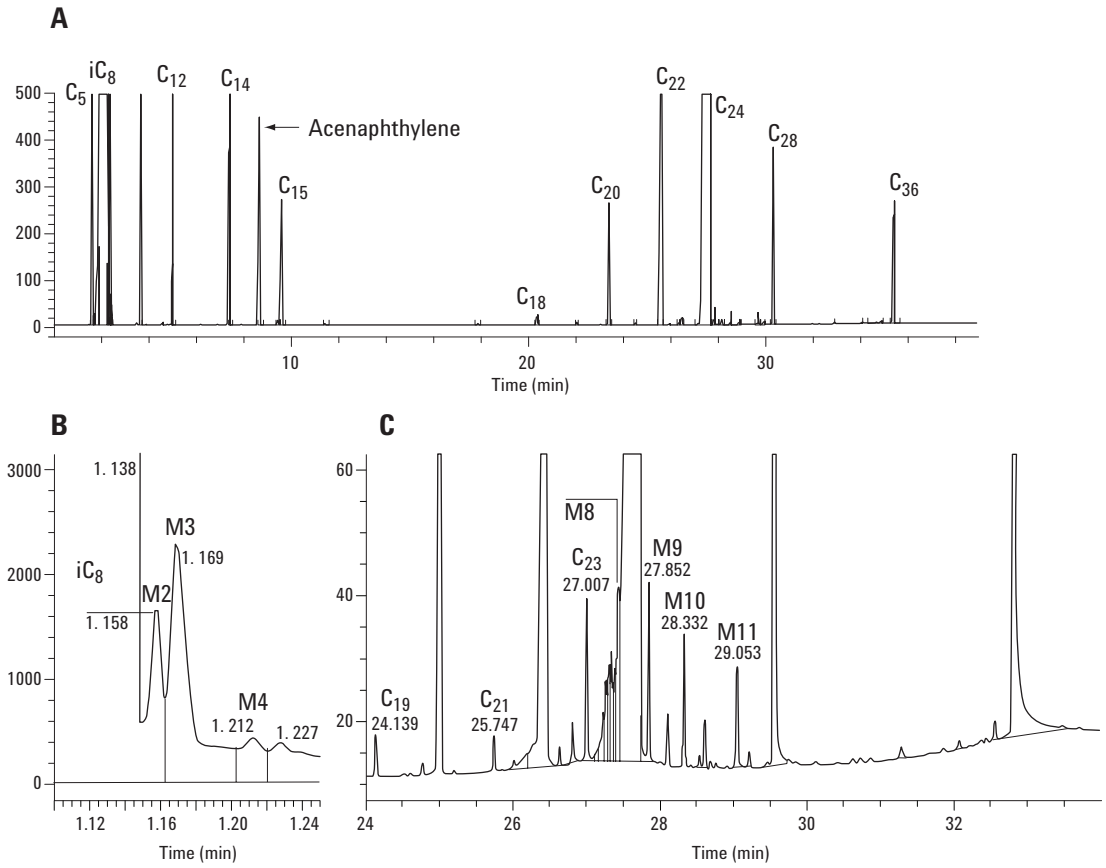


Figure 13. A) Original sample (from [1]); B) Additional peaks near the solvent peak i-C_8; C) Additional trace peaks in the C_{24} region.

The sample, used in an earlier paper [1], ran under different conditions (isothermal region at 140 °C), included 14 hydrocarbon components, including three small peaks, C_{17}, C_{18}, and C_{19}, which approached trace level. To increase SPID computational load, the number of calibrated components was doubled to 28 (close to the maximum of 30 handled per chromatographic signal by SPID) by including most of the trace constituents. These additions are shown in Figures 12 and 13 and include:

Unknown compounds M2, M3, and M4 which elute near the solvent isothermal hold at 65 °C.

Unknown compounds T1 and T2 from the second isothermal hold at 120 °C.

Hydrocarbon impurities C_{21} and C_{23}, and unknowns M8, M9, M10, and M11 from the second oven ramp.

Unknown compounds T6, T7, and T8 from the final hold at 320 °C.

The greatest flow rate induced RT shift occurs in the 15-minute isothermal hold at 120 °C. At just under 5%, the shift is within the default RT window setting. Figure 14 illustrates extremes of flow rate.

Even the most error-prone identification procedure manages to correctly identify C_{15}. If all the chromatographic peaks were this well resolved from possible interferences (resolution of approximately 17 from T1), there would be no need for SPID, or reference peaks, and/or possibly flow control. There is no opportunity to misidentify C_{15} when it is the only peak present in the window.

Less than 3 minutes earlier, at the C_{14}—Acenaphthylene pair, with resolution of 1.3, opportunities to misidentify are plentiful, and a more accurate identification technique can be appreciated.

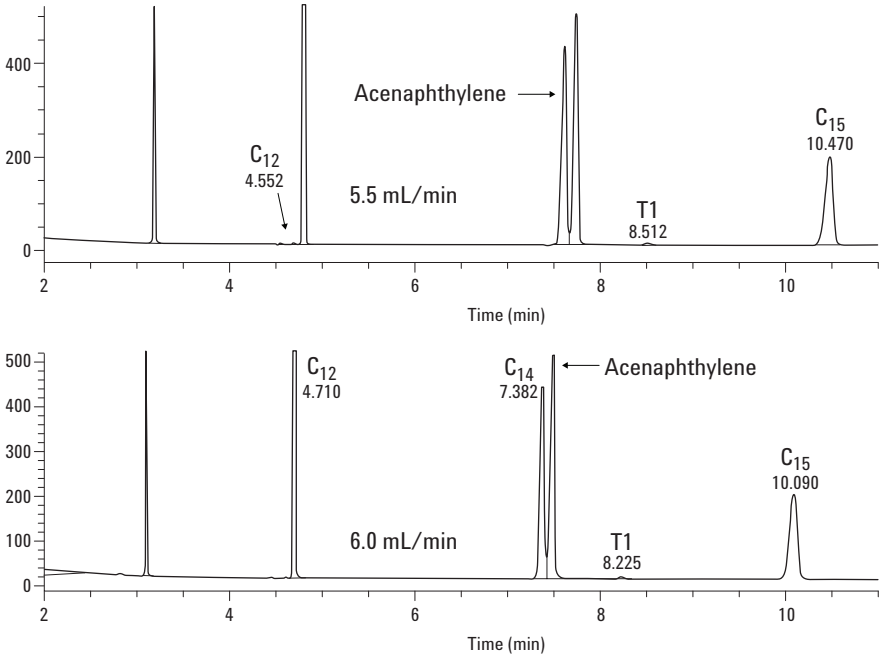


Figure 14. Identifications using 6.0 and 5.5 mL/min flow rate methods.

Table 4 shows procedures of varying robustness in attempting to identify an increasingly distorted chromatogram. Calibration of each identification procedure is done at or near a flow rate value of 6.0 mL/min. Interaction of column flow rate with this multi-ramp temperature programmed analysis produces a non-linear shift in observed RTs. RT shift rises steeply through the isothermal hold at 120 °C, then decreases to a near constant value. Four peak identification procedures are shown:

Least robust technique using fixed time windows and no reference peaks

Adjustment of time windows using a single time reference peak

SPID using a Data Pool composed of 10 runs at a flow rate of 6.0 mL/min (“deep” pool)

SPID using a Data Pool of four runs at flow rates of 5.8, 5.9, 6.0 and 6.1 mL/min (“wide” pool)

Not shown are results from identification using multiple time reference peaks. When multiple reference peaks are carefully chosen to deal with each of the regions in the distorted chromatogram, results improve versus the 5% window using a single reference. Since this is the traditional approach, with only a single iteration performed, there is no testing against statistical limits for RT, area, and symmetry, and no exception reporting for unidentified peaks.

Table 4. Comparative Robustness of Identification Procedures

Procedure	Column flow mL/minute	6.1	6	5.9	5.8	5.5
STD - no ref peaks	Peaks correct	23	28	25	23	14
5% windows	Peaks misidentified	3	0	2	3	7
	Not found	1	0	1	2	7
	Norm total amount	**27.549**	**28**	**4767.117**	**2103.815**	**12065.615**
	Area wrong (m+n)	4630831	0	4656362	4763423	4820000
	Misidentified	IC_8, M2, 3, 4		IC_8, M2, 3	IC_8, M3, 4, Acen, C_{14}	IC_8, M2 ,3 ,4 , $C_{10, 12, 14}$ Acen, T1, T2, C_{23} C_{24}, M8, 9
STD - reference at C_{36}	Peaks correct	23	28	25	23	18
5% windows	Peaks misidentified	3	0	2	3	4
	Not found	1	0	1	2	6
	Norm total amount	**27.549**	**28**	**4767.117**	**2103.972**	**2090.378**
	Area wrong	4630831	0	4656362	4763423	4765346
	Misidentified	IC_8, M2, 3, 4		IC_8, M2, 3	IC_8, M3, 4, C_{14}, Acen	IC_8, M2, 3, 4, $C_{10, 12, 14}$ Acen, T1, T2
SPID - pool: 10 at 6 mL	Peaks correct	26	28	26	23	21
SPID DEEP	Misidentified	0	0	1	4	5
	Not found	1	0	0	1	2
	Norm total amount	**24.806**	**28**	**26.0687**	**24.3037**	**21.9596**
	Fit error	8.9	0.13	3.9	48	284
	Area wrong	0	0	28	82	164
	Misidentified			M11	C_{19}, M10, 11, T6	$C_{18, 19, 21}$, M10, 11, T6
	Key peaks	3	0	3	3	4
SPID - pool : 6.1 - 5.8	Peaks correct	26	27	26	24	21
SPID WIDE	Misidentified	1	1	0	3	6
	Not found	1	0	2	1	1
	Norm total amount	**24.8174**	**28**	**27.51**	**26.041**	**23.2898**
	Fit error	15	1	0.6	27	212
	Area wrong	57	46	13	47	183
	Misidentified	C_{23}	C_{23}	M8	C_{19}, M11, T6	$C_{19, 21, 23}$, M9, 10, 11
	Key peaks	3	3	3	3	4

In this example, “deep” and “wide” pools are contrasted:

The “deep” pool consists of multiple runs under identical analytical conditions- at least eight samples were available for each of 28 calibrated peaks.

The “wide” pool extends across a broad range of flow conditions- computed mean flow rate for the this pool is 5.95 mL/min. Four samples were available for the majority of peaks, but only two samples were available for M3, and three samples were available for M4, C_{23}, M8, M9, T6, and T8.

Response factors provided a normalized contribution of 1.0000 for each of the 28 components at 6.0 mL/min. These contributions sum to 28.0000 when all are identified correctly. Misidentification of the solvent peak has dramatic effects on this summation. SPID's area and symmetry limits prevent this confusion.

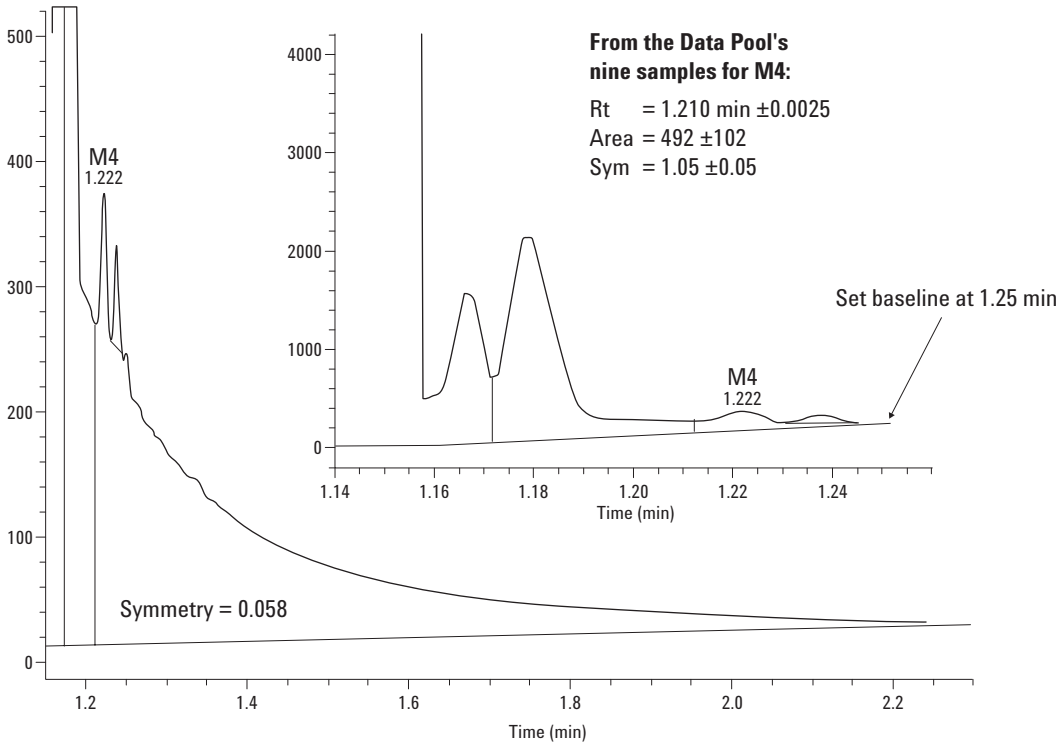


Figure 15. Repairing inconsistent quantitation of M4.

The smallest of the 28 peaks illustrates SPID limitations. The earliest peaks elute immediately following the solvent. Their peak shape is not consistent. No special care was taken to stabilize their detection or baseline treatment, so their respective integration results can vary.

Consider the 5.9 mL/min run (Figure 15) where the peak at 1.222 minutes is a candidate for compound M4. The peak at 1.222 is quantitated as a solvent, grafting onto a huge tail thereby increasing its area by more than an order of magnitude. Peak symmetry, computed as the ratio of frontal area to rear area, drops to 0.058 with addition of the tail. SPID compares this value with M4 symmetry limits, derived from the pool mean and standard deviation, and rejects 1.222 as a match to compound M4. Since there are no other candidates which can be matched to M4, the SPID report summary for this 5.9 mL/min run includes the message:

```
M4 candidate rejected by:Symmetry 0.489/1.695
```

which also lists acceptable limits. The inset (Figure 15) demonstrates how addition of a baseline timed event corrects spurious solvent treatment and permits SPID confirmation of 1.222 as M4 (with symmetry of ~0.84).

Similar, but less dramatic symmetry differences result in other rejections, such as shoulder peak M8. As peaks approach trace level, their quantitation becomes more difficult and results more variable. Examples include trace peaks T6, T7, and T8 which are just visible in the rising baseline around C_{36}. If this variability is not captured in the Data Pool, SPID cannot provide reliable identification.

SPID iterates through multiple choices of peak assignments to minimize fit error and to maximize the number of identified peaks. Fit error measures deviation of identified peak RTs from an “ideal” time, assigned from the mean value of the pool's times. Each SPID iteration begins with a selection of at least three peaks from the pool for use as initial time references. In this case, with half the pool dedicated to the smallest peaks, and with quite a surplus of them, opportunities for misidentification of references exist.

Rather than democratically selecting references at random from the pool, we can instruct SPID in selection of key peaks to serve as initial time references. We should choose at least three easily distinguished peaks covering regions where differences in distortion are likely. The criteria are analogous to that used in selecting conventional time reference peaks. Selecting key peaks was not necessary in the previous examples as their pools did not contain large numbers of trace level peaks.

Another incentive to select key peaks for this sample is to minimize the number of iterations performed. Suppose 10 of the compounds present have two alternatives to evaluate (for example, which peak is Acenaphthylene). This gives 2^{10} combinations (1024) to evaluate per iteration. Fortunately, the number of iterations and combinations is limited since the number of iterations and combinations determines SPID processing time. An estimate of the combination limit is given by:

Combination limit ≅ $2^{(\text{candidates present - 3})}$

For data shown in Table 4, key peaks are chosen in areas of greatest opportunity for mistakes, for example, the large clump of trace peaks between C_{22} and C_{28}. Sometimes, as distortion is increased, it is necessary to anchor identification across the entire chromatogram. For example, in the 5.8 mL/min flow rate case, the “deep” pool uses key peaks at i-C_8, C_{18}, and C_{28}. The “wide" pool substitutes C_{24} as the middle peak. Note that several peaks remain misidentified even using key peaks.

In Figure 16, shape of the fit error plot is similar to that seen previously with RGA results (Figure 11).

There is no value of fit error guaranteeing a good result. Fit error is specific to a particular chromatogram and its associated Data Pool. An error of approximately 0.1 is the best achieved for the 28 peaks in system test sample. For nine peaks on Alumina, errors are consistently below approximately 0.1.

When the analytical range of interest produces only small deviations, it is easiest to acquire replicate runs and to then construct a “deep” pool. More significant changes require capturing the variability and this demands a “wider” pool. This variability was more successfully captured for the RGA case than for the system test sample case.

By the time 5.5 mL/min is attained, all four approaches are seriously deficient. As seen earlier in the RGA case, fit error indicates good chromatogram-to-Data-Pool match. As fit error has increased several orders of magnitude, SPID results have deteriorated markedly, with trace peaks affected the most. Even in this case, however, larger peaks bounded by SPID's area and symmetry limits are correctly identified. This represents a limit to this particular unoptimized analysis.

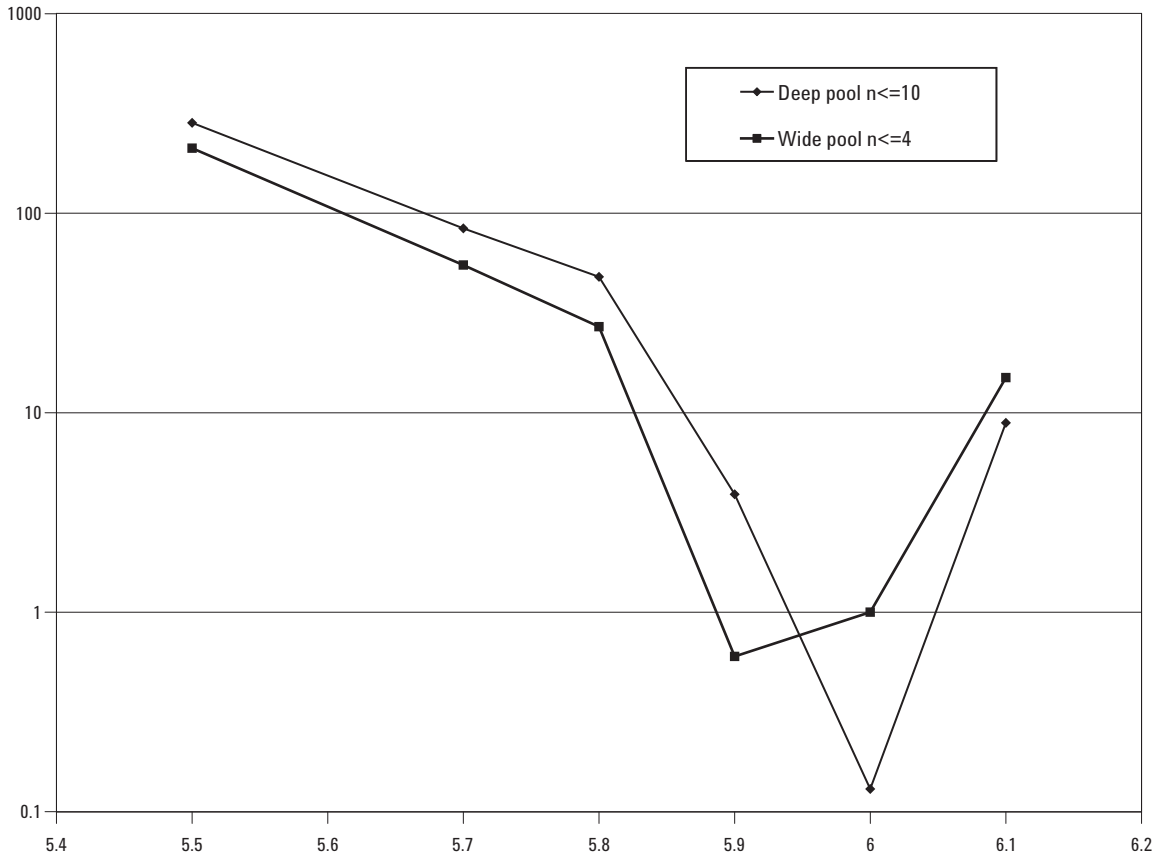


Figure 16. Log fit-error vs. flow rate for the system test sample.

Summary

SPID extends identification over a wider analytical range. Robustness of identification is enhanced for well characterized samples. The Report summary documents why peaks are not included. SPID briefly summarizes its performance on each signal with explicit criteria, peaks identified, fit error, and the number of iterations. Limitations of use were also discussed.

Acknowledgment

We thank Steve Lo for his assistance with the RGA sample discussed here.

References

1. Paul Larson, Robert Henderson, W. Dale Snyder, and Edwin E. Wikfors, “Effect of Environmental Factors on the HP 6890 Series Gas Chromatograph’s Performance Using Manual and Electronic Pneumatics,” Application note 228-321, Agilent Technologies, publication 5963-9807E. www.agilent.com/chem

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Printed in the USA
January 23, 2003
5988-8533EN



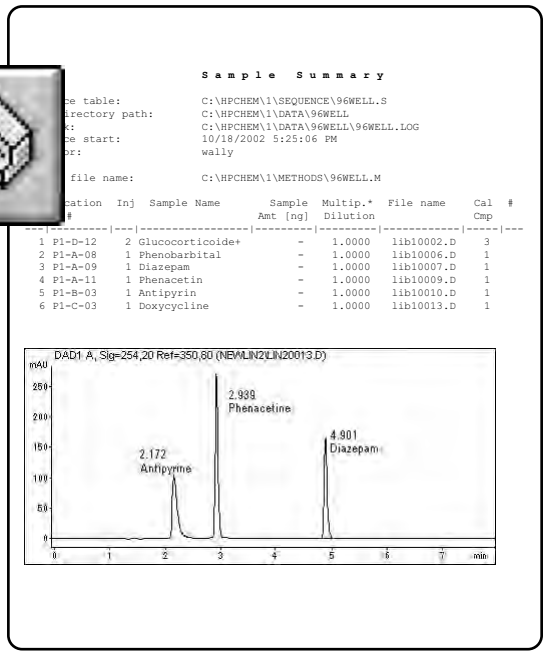
Agilent Technologies

Using Agilent ChemStation to generate summary reports for a single analysis or a sequence of analyses

Application

Angelika Gratzfeld-Huesgen





Sample Summary

ce table:	C:\HPCHEM\1\SEQUENCE\96WELL.S
irectory path:	C:\HPCHEM\1\DATA\96WELL
t:	C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
ce start:	10/18/2002 5:25:06 PM
or:	wally
file name:	C:\HPCHEM\1\METHODS\96WELL.M

cation #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal # Cmp
1 P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3
2 P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1
3 P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1
4 P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1
5 P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1
6 P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1

Introduction

The Agilent ChemStation base software includes a wide range of built-in report styles and types. For example, it provides standard reports such as area percent (AREA%), external standard (ESTD), internal standard (ISTD), and normalized (NORM) reports as well as system suitability reports and sequence summary reports with statistical evaluation of retention times, areas, heights and more.

For each type of report the user can determine the amount of information that is included in the report. The ChemStation base software also provides a report editor for customizing reports — a topic that is beyond the scope of this note.

This Application Note describes how to set up the different report types, explaining the software screens and giving example reports. The main objective is to give guidelines and to provide strategies on how to use the different built-in reports in the ChemStation base software.

Agilent Technologies

Equipment

The data for the report examples was generated using an Agilent 1100 Series HPLC system comprising the following modules.

- high pressure gradient pump
- micro-vacuum degasser
- well plate sampler
- thermostatted column compartment
- diode array detector

The Agilent ChemStation base software including the 3D data evaluation module, revision A.08.04, was used for instrument control, data acquisition, data handling, sample tracking, and reporting.

Report setup on ChemStation

The standard reporting function in the ChemStation base software provides for single run reports or sample-set reports for a full sequence of runs, whereby these so-called sequence summary reports can only be generated after completion of the sequence. The content of the sequence summary reports is defined by the acquisition sequence.

Further, the ChemStation base software includes a wide range of built-in standard reports that allow users to define the content and amount of printed information. Whereas this functionality meets the requirements of most standard applications to a large extent, it does not have the flexibility to create additional table elements for non-chromatographic information, charts or custom calculations.

If such extended reporting capabilities are required, it is recommended to use the ChemStation Plus data system including the ChemStore data organization module.

The ChemStation base software offers four types of report.

- Individual run reports, which can be generated automatically after each run or sequence, provide quick and easy printouts of results.

- Sequence summary reports provide comprehensive infomation for a full set of samples, including full GLP/GMP details. They are generated automatically at the end of a sequence and may include individual reports as well as statistical summary reports.

- Batch reports provide direct printouts of first-pass review modifications and results. They are generated during reprocessing of data from a complete sequence or of a subset of one sequence using ChemStation batch review.

- Advanced custom reports for requirements that go beyond the scope of the previous types. These include customized reports for individual runs or complete sequences and can also be obtained automatically after each run or sequence.

The following sections focus on the individual-run and sequence-summary report types, which are built-in as standard in the ChemStation base software, and explain in detail how to use and set up these report types.

Qualitative reports for individual runs

Qualitative reports are used mainly during the development of a separation or when a quick decision is needed as to whether a compound is present or not. Here the separation of peaks is of primary interest and a short AREA% report is sufficient. Particularly during method development it does not make sense to obtain reports with quantitative results.

Setup

To obtain an automated printout of an individual report such as a short AREA% report, the item *Standard Data Analysis* must be selected in the *Run Time Checklist*, which is part of the overall method for acquisition, data analysis and reporting, see figure 1. This screen is part of the *Edit Entire Method* dialog or can be accessed directly from the *Method* menu of the *Method and Run Control* view.

The item shown in figure 1 must be selected when the calculation of results is required, such as for printing reports, including sequence summary reports, with or without individual run reports.

Configuration

To obtain qualitative reports the item *Calculate* in the group *Quantitative Results* must be set to *Percent* as shown in figure 2.

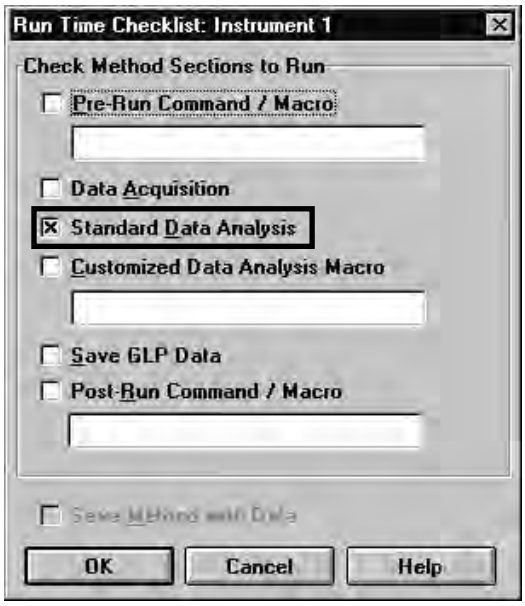


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

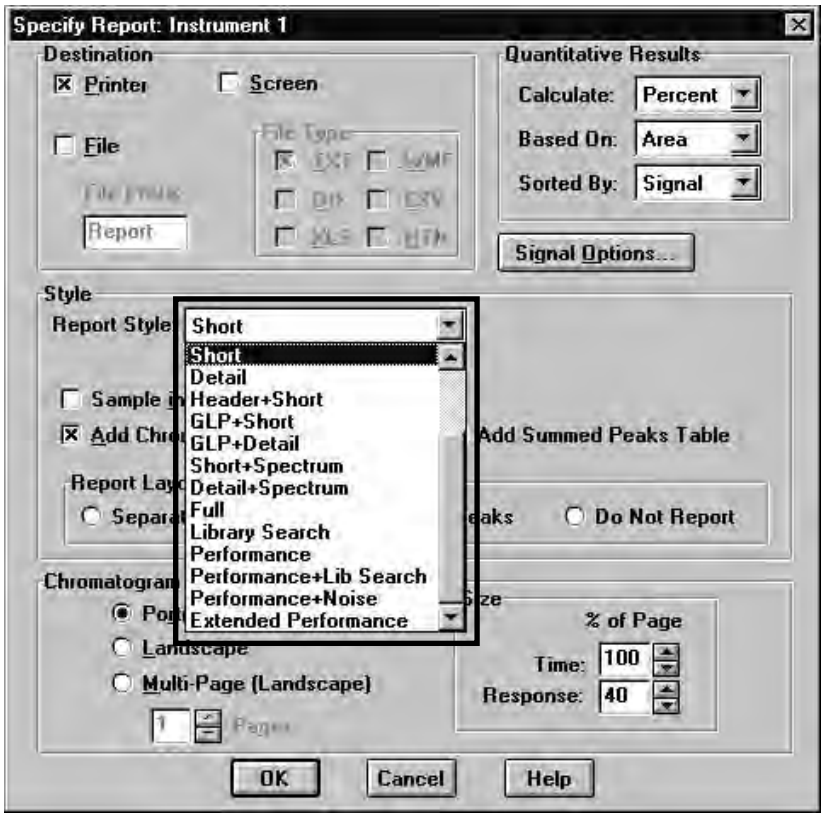


Figure 2
Specifying individual run reports

There are three ways to set up reports for individual runs.

1 Using the report smart icon in the *Method and Run Control* view.
2 Using part of the *Edit Entire Method* wizard
3 Using the *Data Analysis* view by selecting *Report* and then *Specify Report*.

Figure 2 shows the setup screen for run reports. Several report styles are available, covering a broad spectrum of report types. The report output can be sent to a printer, displayed on the screen or saved to a file. Multiple report destinations can be selected at a time. Other report parameters allow to include chromatograms, in landscape or portrait format or even distributed over several pages, and to define the way unknown compounds are reported.

An example of an AREA% report is given on page 12, containing information about the used method, data filename, time of injection, chromatogram and report.

The report styles that are available depend on the installed software modules. For example, the report styles Short+Spectrum, Detail+Spectrum and Library Serach are only available when the 3D data evaluation module is installed.

During method development the combination of *Percent* and *Performance* in reporting can be a valid tool to find out about k', resolution, selectivity, peak width and, for isocratic runs, the number of plates. An example is given on page 19.

Calculation procedures such as **Percent** (for others such as ESTD and ISTD, see section "Quantitative reports for individual runs") can be combined with any of the available standard reports shown in figure 2.

Qualitative reports can not use calculations based on standards such as ESTD and ISTD.

Quantitative reports for individual runs

Quantitative reports offer compound identification and compound quantification. They are mainly used with known samples or reference results in method optimization and quality control areas.

Setup

Before a quantitative report can be generated, standard samples with known compound concentrations have to be run and a calibration table has to be set up.

Peak integration should always be optimized before a peak is used as a reference in the calibration table and before the calibration tasks are done. To optimize integration, load a sample file with known sample concentration and then use the *Integration* tool set in the *Data Analysis* screen. When integration is optimized and saved, the calibration table can be created.

The calibration table is set up in *Data Analysis* from the *Calibration* menu, see figure 3.

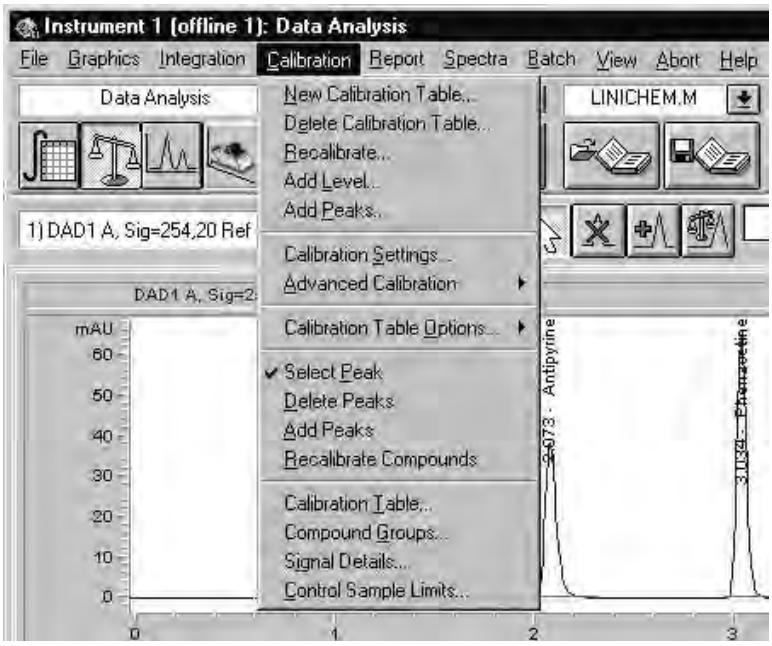


Figure 3
Calibration setup menu

In the following example we set up a multilevel calibration with four calibration levels. Multilevel calibrations use multiple files to complete the calibration. One file defines one level—completion of a four-level calibration thus requires four files. The steps involved are as follows.

1 Load the first file and click on *New Calibration Table*.

2 Calibrate each peak by selecting the peak (left mouse click), and filling in compound name and compound amount.

3 Repeat step 2 for all peaks.

4 When all peaks in the file are calibrated, load the next file with the next concentration. Use the *Add Level* tool to fill in the amounts for the next concentration level (level two).

5 Repeat step 4 for level three and four.

The calibration is stored as part of the ChemStation method. It is saved by simply saving the method. Every calibration update is easily accessible by loading the method, modifying (for example, updating) the calibration files and saving the new method revision.

Setup

When the calibration is complete all prerequisites for generating a quantitative report are met. The first step in generating a report is to specify the report style as described in the section "Qualitative reports for individual runs." The calibration of the method now offers access to all predefined report styles such as standards reports or normalized reports or, when running a sequence, to sequence summary reports (see separate section later.)

The calculation of results can be a normalized (NORM) area determination or based on an external standard (ESTD) or internal standard (ISTD). Result calculations can be based on area or height. Figure 4 shows selection of *External Standard Method* as calculation procedure and *Short* as *Report Style*. An example is given on page 13.

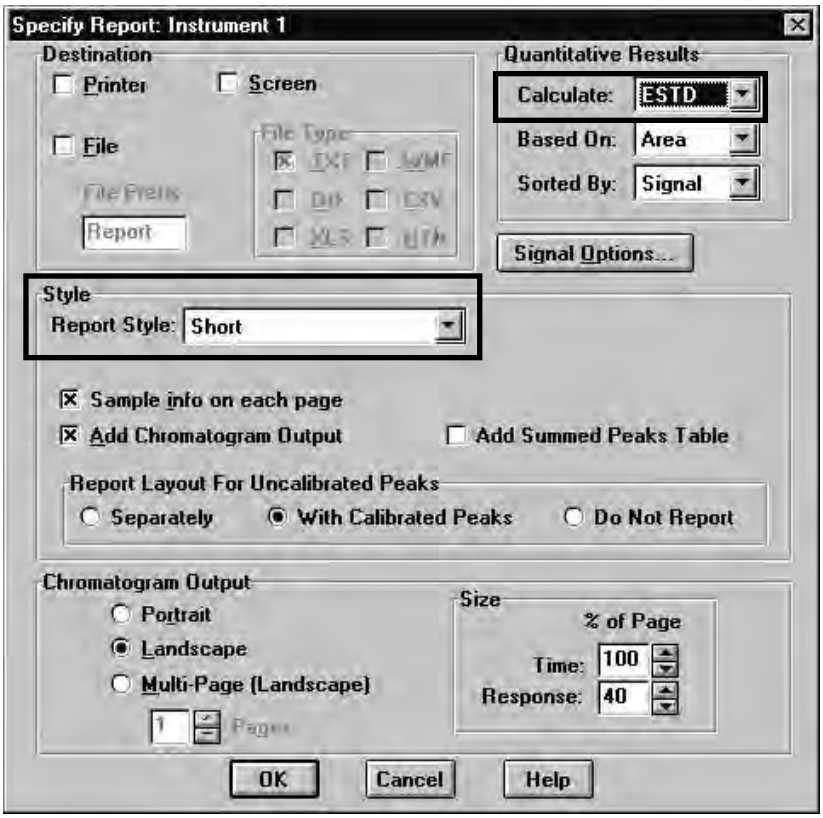


Figure 4
Selection of external standard report and short report style

Configuration

Additional report features can be specified such as output format for the chromatogram (including multipage outputs), picture size and the documentation of uncalibrated (which means unknown) peaks in the *Specify Report* screen as shown in figure 4. Any report style (see figure 2) can also be combined with any calculation procedure. Examples are given on pages 13 through 21.

- ESTD combined with report style *Short* (p 13)
- ESTD combined with report style *Library Search* (p 14)
- ESTD combined with report style *GLP+Short* (p 16)
- ESTD combined with report style *Performance* (p 19)
- ESTD combined with report style *Detail* (p 20)

Similar to the calibration, the report configuration is saved with the ChemStation method. Thus all data analysis steps for integration, calibration, result calculation and reporting are saved under one "umbrella" tool. Once setup, reuse of all steps is automated by simply reapplying the method to any sample under investigation.

The method that has been set up for data acquisition, integration, calibration and reporting has to be saved under a unique name to ensure that samples are analyzed and evaluated using the correct conditions.

Final report output

Final report outputs are quick and easy to obtain with ChemStation. Both qualitative and quantitative reports offer the same options and use identical tools to generate the final report.

Reports can be
- sent to a printer
- displayed on the screen for a quick review or preview when setting up report options
- saved to a file in HTML, CSV, XML, TXT, WMF, or DIF format

It is possible to combine all output types, for example, to get a printed copy on paper, an online report display on the screen and a file copy on the local hard disk.

The user can choose either
- automated report output at the end of each sample analysis (or reanalysis), or
- interactive report output at user request



Figure 5
Run method for automated method execution and result output

Automated report output

An automated report is output whenever the ChemStation method is executed and at least one report destination is selected in the *Specify Report* screen, see figure 4. If no report output is desired, simply leave all report destination check boxes blank.

Method execution typically is used to analyze a sample or to reapply changes in calculations or calibration during data analysis. To execute a method, simply press *F5* or select *Run method* from the ChemStation *Run control* menu as shown in figure 5.

If the user wants to re-analyze data without data acquisition, *Data Acquisition* must be disabled in the *Run Time Checklist*, see figure 1.

Interactive report printout

Manual report output is available from the ChemStation *Data Analysis* view. It is designed to preview report outputs on the screen during report configuration or to get an individual sample report during interactive result analysis or result review.

The *Data Analysis* view is designed to set up advanced reports such as library searches, detailed spectrum reports and others. It has a separate report menu and additional smart icons for report setup, preview and output to a printer as shown in figure 6.

When the user wants a report during their data review session, they simply press the preview or print button and immediately get the report on the screen or on paper.



Figure 6
Report menu and smart icons (far right) in ChemStation Data Analysis view

Sequence summary reports

In contrast to individual run reports, sequence summary reports can only be generated for a complete set of samples that have been analyzed in one continuos sequence. The sequence summary report (also referred to as a system suitability report) is designed to meet the specific needs of GLP and GMP regulations in the pharmaceutical industry as well as comparable ISO and DIN regulations in other industries.

In addition to result calculation and result documentation, all regulations require additional documentation on how the results have been obtained and how "well" the analytical system behaved during analysis. The sequence summary report is a single all-inclusive report style, combining the analytical result with full documentation of how the result was obtained and the system suitability information, thereby providing a comprehensive report that addresses all regulatory requirements.

Sequence summary reports are frequently used in quality control work. These reports include the analytical results along with documented evidence of the system's suitability for the analytical purpose. System suitability is defined in the various Pharmacopoeia guidelines and it typically includes system performance information based on parameters such as peak width, theoretical plate number, resolution and others.

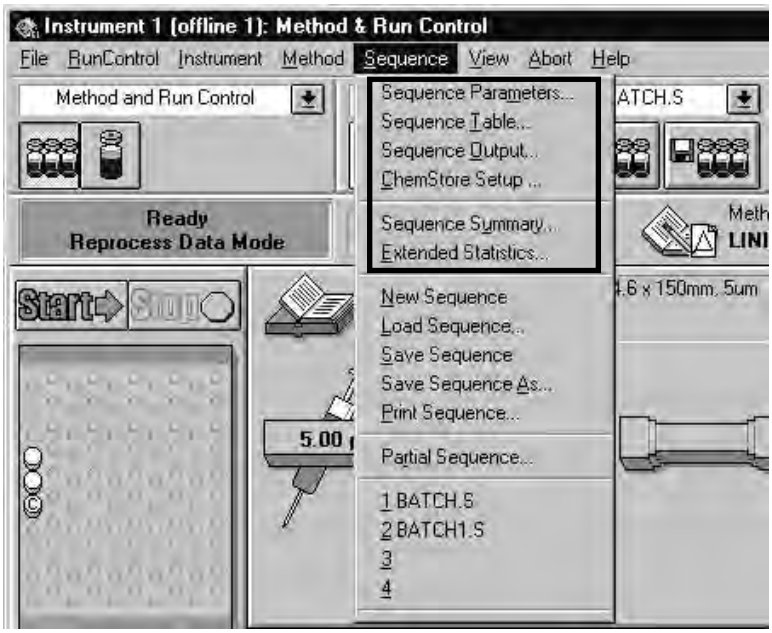


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

All these parameters are available in the report style, but the user must configure the report to suit their own specific needs. The following section describes setup and configuration of a sequence summary report in ChemStation.

Setup and configuration

After each sequence of runs a sequence summary report can be printed. Typically this is done to obtain statistical results and determine system suitability. In addition to the entries in the sequence table and before the report can be calculated and printed, several data inputs for sequence parameter and sequence output are required, see figure 7.

In the *Sequence Parameters* screen (figure 8) the item *Parts of Method to Run* must be set to *According to Runtime Checklist*. This entry determines which part of a method is executed during a sequence and *According to Runtime Checklist* refers to the run-time checklist configuration that was previously edited as part of the method in order to obtain integration and quantitative results.

If data acquisition is completed and the user wants to reanalyze a sequence of samples without data acquisition, the option *Reprocessing Only* allows to recalculate the sequence summary report easily.

In the *Sequence Output* screen the report destination and the content of a sequence summary report are defined by selecting the appropriate check boxes, see figure 9.

The content of the sequence summary report is defined by the items on the right side of the scrreen shown in figure 9. Selecting *Setup* in the *Sequence Output* dialog box accesses this configuration screen. The sequence summary report allows a variety of informations to be printed in one continuously enumerated report.

In addition to a wide selection of statistical results from sample and/or calibration runs, other items can be selected such as sample summary reports that list all acquired samples, com-



Figure 8
Sequence parameters screen

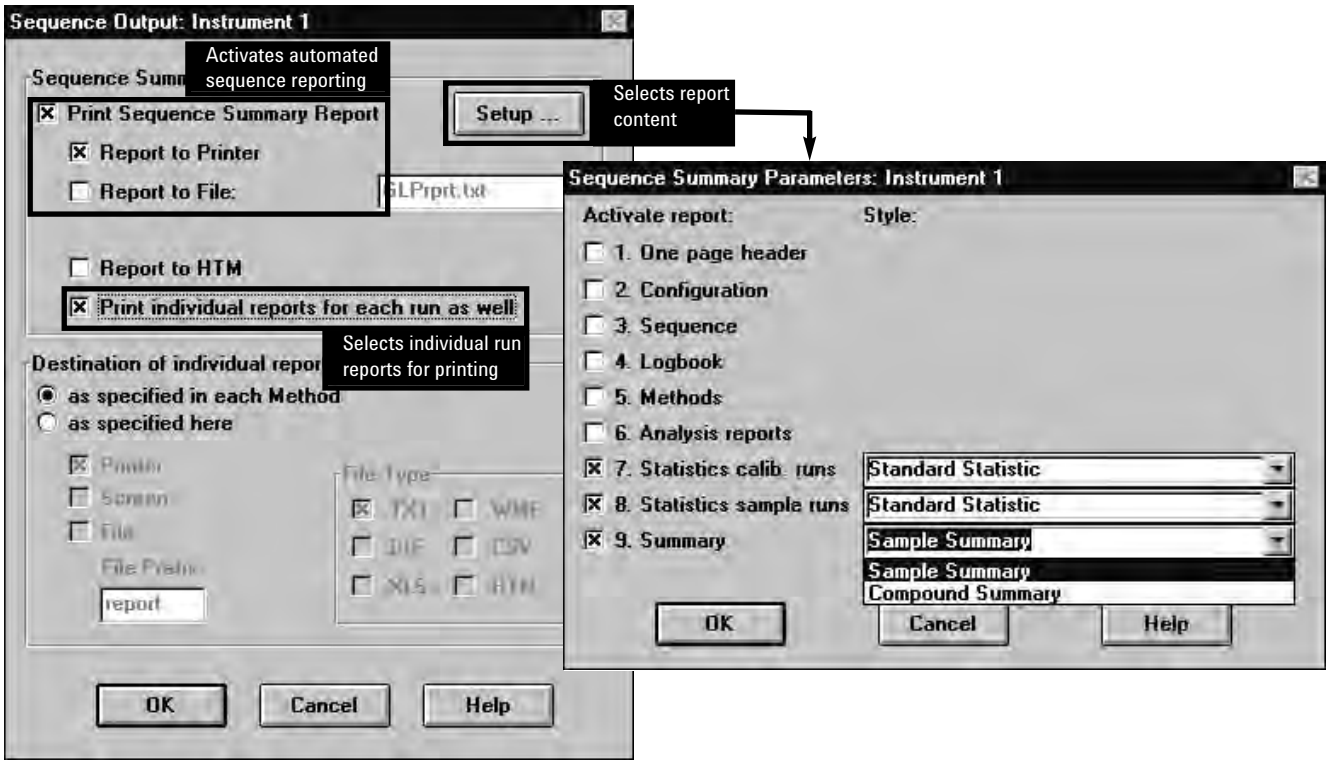


Figure 9
Selection of report destination and content of a sequence summary report

plete printouts of all parameters in the methods that were used, printouts of sequence logbooks and so on.

It is also possible to include the individual result reports for each run as part of the summary report instead of individual printouts after the end of each run.

The statistical evaluation of sequence runs is defined in the *Extended Statistic Parameter* screen, see figure 10. Statistical results can be obtained for all parameter shown in this dialog box. Either standard deviation or relative standard deviation or 95% confidence interval can be applied and upper/lower limits for each parameter can be specified.

A calibrated method is necessary to be able obtain statistical results.

Figure 11 shows the *Sequence Table* screen, in which it is important to ensure that the sample type is correctly set to *Sample*, *Calibration* or *Control Sample*, because statistical calculations can be selected based on sample type.

Figure 12 shows an example of a sequence summary report. It contains information about the analyzed samples such as location, sample name, filename, and so on. The header includes information such as operator name, the used chromatographic method, and date of acquisition.

Further report examples can be found on pages 11 through 35.

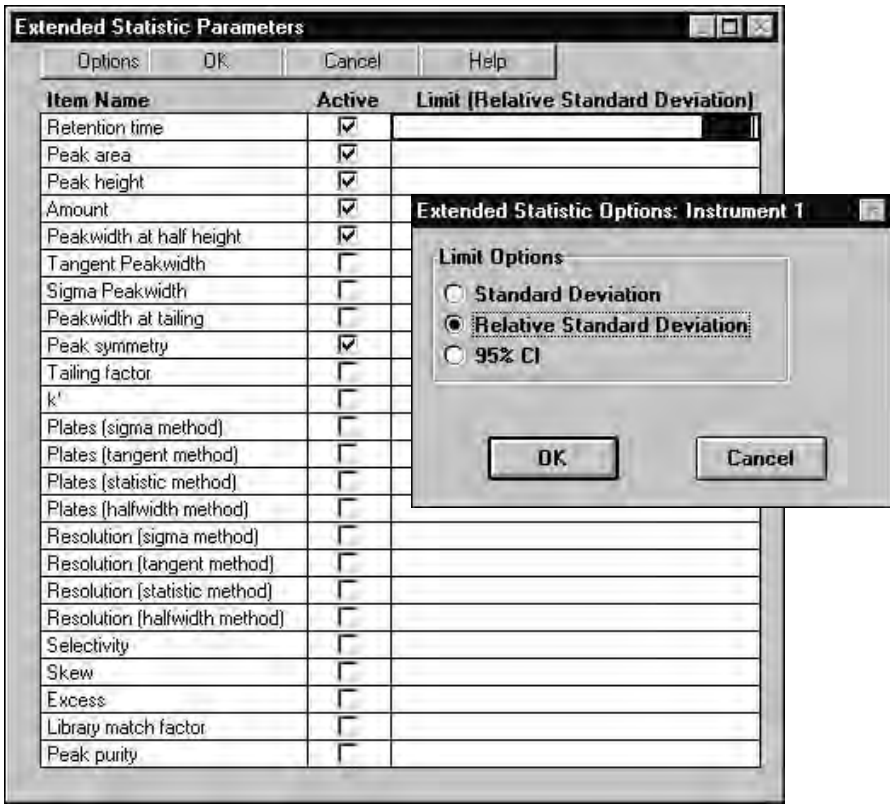


Figure 10
Setup of statistical calculations for sequence runs

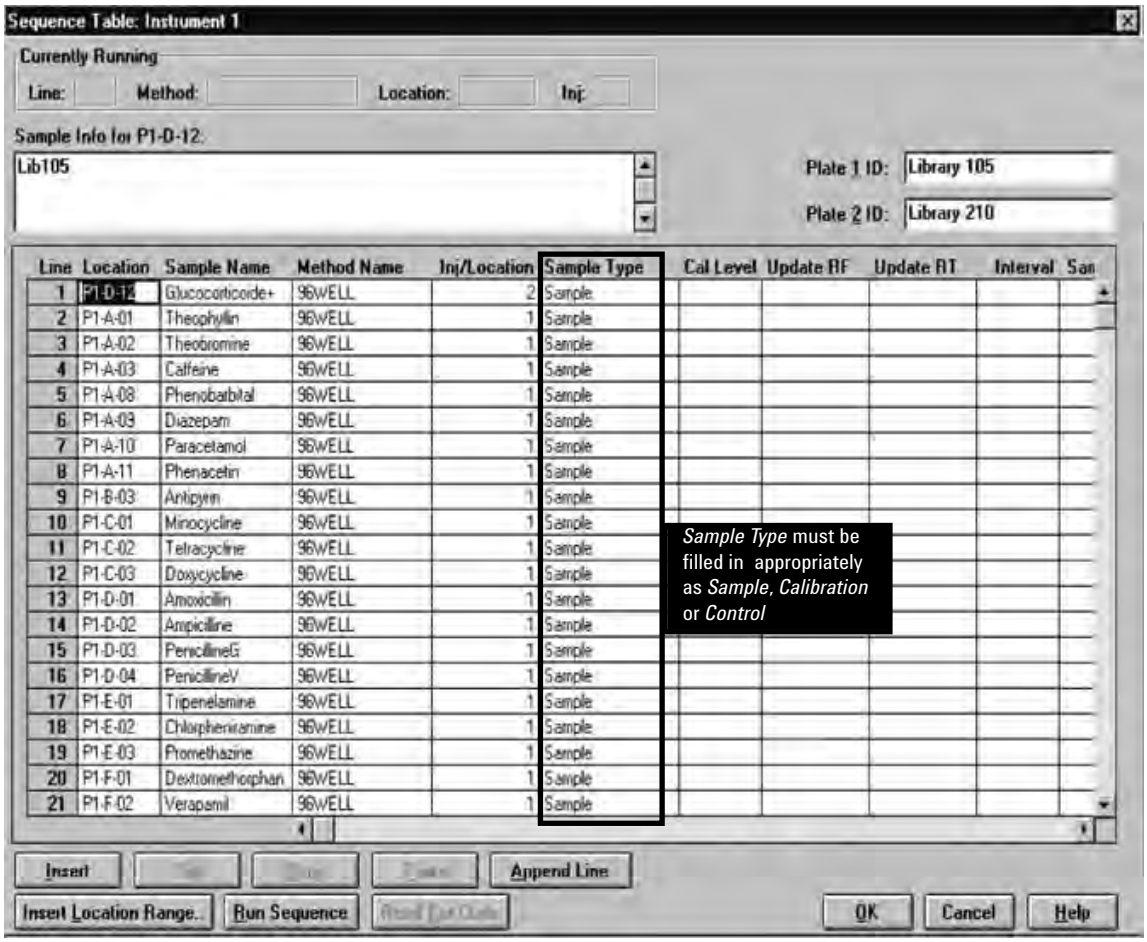


Figure 11
The Sequence Table screen

```
S a m p l e   S u m m a r y

Sequence table:          C:\HPCHEM\1\SEQUENCE\96WELL.S
Data directory path:     C:\HPCHEM\1\DATA\96WELL
Logbook:                 C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
Sequence start:          10/18/2002 5:25:06 PM
Operator:                agratz

Method file name:        C:\HPCHEM\1\METHODS\96WELL.M

Run Location Inj Sample Name       Sample    Multip.* File name    Cal #
 #     #                              Amt [ng]  Dilution               Cmp
---|---------|---|----------------|---------|---------|------------|-----|---
  1 P1-D-12    2 Glucocorticoide+     -      1.0000   lib10002.D    3
  2 P1-A-08    1 Phenobarbital        -      1.0000   lib10006.D    1
  3 P1-A-09    1 Diazepam             -      1.0000   lib10007.D    1
  4 P1-A-11    1 Phenacetin           -      1.0000   lib10009.D    1
  5 P1-B-03    1 Antipyrin            -      1.0000   lib10010.D    1
  6 P1-C-03    1 Doxycycline          -      1.0000   lib10013.D    1
```

Figure 12
Example of a sequence sample summary report

Conclusion

The built-in single-run and sequences summary reports that are available in the ChemStation base software offer a wide range of reporting capabilities. The various reports give access to all important sample-related information quickly and easily. For all report types the user can select the amount of information to be included, from a simple qualitative report on one page through detailed quantitative reports to comprehensive and powerful sequence summary reports. Knowledge of a report editor is not required to be able to set up the ChemStation reports.

Reports can be obtained after each run or at the end of a sequence. With the ChemStation Method concept users starting from scratch can have a printed result copy of any type in less than 10 minutes – once set up the report is available within seconds after run completion. ChemStation reports are easy to configure, fast to obtain and quickly stored and managed.

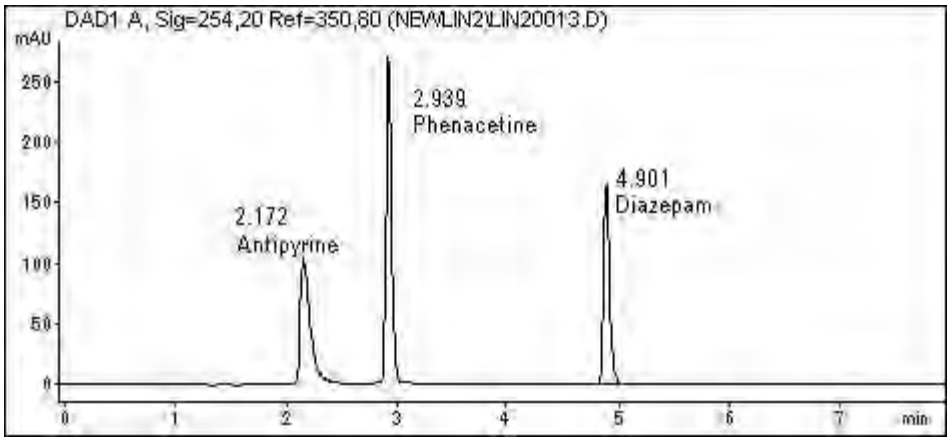
Appendix

The following pages show examples of summary reports that can be generated with the ChemStation base software. The examples were generated using the print-to-file function and may have different pagination than a report printed directly from the ChemStation. Reports shown include:

- Short Area Percent Report
- Short ESTD Report
- Spectral Library Search Report
- Short GLP Report
- Performance Report
- Detail Report
- Extended Performance Report
- Sequence Summary Report – Compound Summary
- Sequence Summary Report – Standard Statistics for Sample Runs

Short Area Percent Report

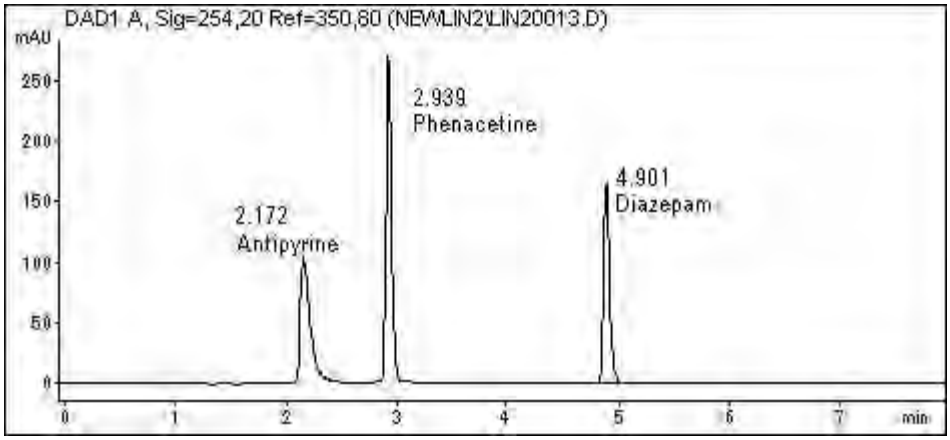
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 8:54:14 AM agratz

================================================================
Injection Date  : 10/25/00 8:47:20 AM            Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                             Inj :   1
                                                 Inj Volume :  1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 8:53:08 AM by agratz
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
================================================================
```




```
                          Area Percent Report
================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 8:52:20 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

Peak RetTime  Type    Width     Area      Area     Name
  #    [min]           [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
  1  1.424    BV    0.0829    10.51506   0.4743 ?
  2  2.172    BB    0.0933   661.70422  29.8443 Antipyrine
  3  2.939    BB    0.0535   934.32690  42.1402 Phenacetine
  4  4.901    BB    0.0566   610.64050  27.5412 Diazepam
Totals :                   2217.18669

================================================================
                          *** End of Report ***
```

Short ESTD Report

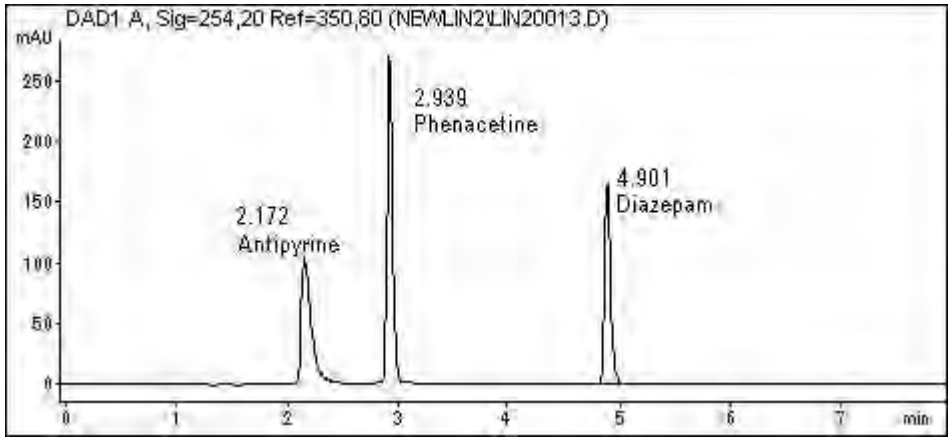
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:09:23 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                          Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !       Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:09:14 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                        External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB      661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB      934.32690    1.00317   937.28787     Phenacetine
  4.901 BB      610.64050 9.81915e-1   599.59734     Diazepam
Totals :                             1975.58590

=====================================================================
                            *** End of Report ***
```

Spectral Library Search Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:28:46 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:28:26 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                    External Standard Report
=====================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Library search mode:     Automatic library search

Library file No.       : 1
Library file name      : D:\HPCHEM\1\METHODS\LINICHEM.M\PHARMA.UVL
Match threshold        :     950        Purity threshold: Calculated
Time window  left [%] :    5.00         Case sensitive : No
Time window right [%] :    5.00             Whole word : No
Wavelength shift       :     0.0       Compare spectrum : Yes
Absorbance threshold   :     0.0           Search logic : OR
Search range           :     All
```

Spectral Library Search Report (continued)

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!
Calibrated compounds:

Meas. RetTime [min]	Library RetTime [min]	CalTbl RetTime [min]	Sig	Amount [ng]	Purity Factor	#	Library Match	Name
2.172	2.177	2.071	1	438.70069	1000	1	1000	Antipyrine
2.939	2.944	3.038	1	937.28787	1000	1	1000	Phenacetine
4.901	4.904	5.090	1	599.59734	1000	1	1000	Diazepam

Note(s):
u: compound identified at upslope. Purity factor exceeds threshold.
d: compound identified at downslope. Purity factor exceeds threshold.

==========

*** End of Report ***

Short GLP Report

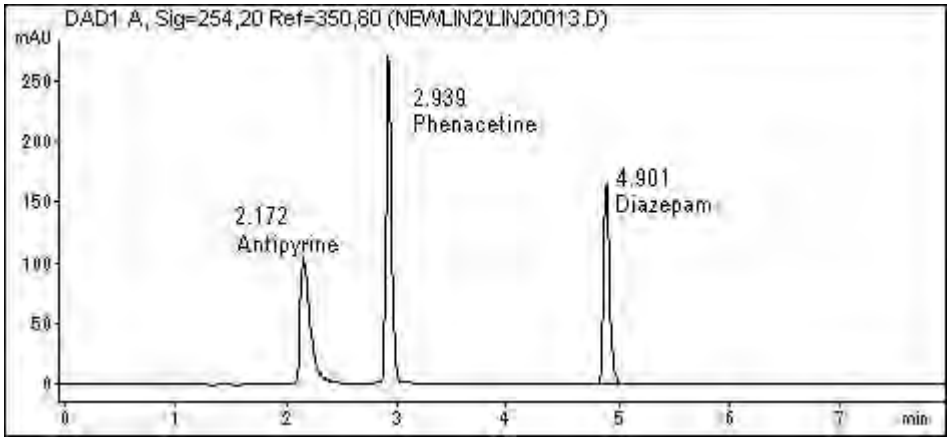
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:31:21 AM agratz

This is a special file, named RPTHEAD.TXT, in the directory of a method
which allows you to customize the report header page.
It can be used to identify the laboratory which uses the method.

This file is printed on the first page with the report styles:

   Header+Short, GLP+Short, GLP+Detail, Short+Spec, Detail+Spec, Full


                  XXXX    XXX
                 XX  XX    XX
                XX         XX        XXXXX    XXX XX
                XX         XX XXX   XX     X   XX X XX
                XX     X    XXX XX   XXXXXXX   XX X XX
                 XX  XX    XX  XX   XX         XX    XX
                  XXXX    XXX  XXX   XXXXX    XXX   XXX


    XXXXXX      X                   X          XX
   XX     X     XX                  XX
   XX          XXXXX     XXXXX     XXXXX      XXX       XXXX     XX XXX
    XXXXX      XX            X      XX          XX      XX  XX    XXX XX
        XX     XX       XXXXXX      XX          XX      XX  XX    XX  XX
   X    XX     XX XX   X    XX      XX XX       XX      XX  XX    XX  XX
   XXXXXX       XXX    XXXXX X      XXX       XXXX      XXXX     XX  XX


                                                              X
         XX XXX     XXXXX    XX XXX      XXXX    XX XXX     XXXXX
          XXX XX   XX     X    XX  XX    XX  XX    XXX XX     XX
          XX       XXXXXXX    XX  XX    XX  XX    XX         XX
          XX       XX          XXXXX     XX  XX    XX         XX XX
         XXXX       XXXXX     XX          XXXX    XXXX         XXX
                              XXXX


         XXX                                XXX
          XX                                 XX
          XX         XXXXX     XXXXX         XX     XXXXX    XX XXX
          XX XXX   XX     X         X      XXXXX    XX     X    XXX XX
          XXX XX   XXXXXXX   XXXXXX    XX  XX    XXXXXXX    XX
          XX  XX   XX         X    XX    XX  XX    XX          XX
         XXX  XXX   XXXXX    XXXXX X    XXXX X    XXXXX    XXXX
```

Short GLP Report (continued)

```
==================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                               Inj :   1
                                                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:31:10 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
Module                                Firmware revision  Serial number
-----------------------------------|------------------|---------------
1100 Wellplate Autosampler            A.04.08            DE02700294
1100 Column Thermostat                A.04.06            DE53400174
1100 Diode Array Detector             S.03.91            DE00900051
1100 Binary Pump                      A.04.06            DE53500104
1100 Sample Thermostat                n/a                DE82203241

Software Revisions for:
- Acquisition:   Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies
==================================================================

Instrument Conditions   :    At Start            At Stop
Air Temperature (Tray)  :      20.1   °C
Column Temp. (left)     :      40.0                40.0   °C
Column Temp. (right)    :      40.0                40.0   °C
Pressure                :      69.8                75.7   bar
Flow                    :       1.200               1.200 ml/min

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp          :       2.44              454.9   h
DAD 1, Visible Lamp     :       2.44              424.1   h

Solvent Description     :
PMP1, Solvent A         : Water
PMP1, Solvent B         : acn
==================================================================
```

Short GLP Report (continued)

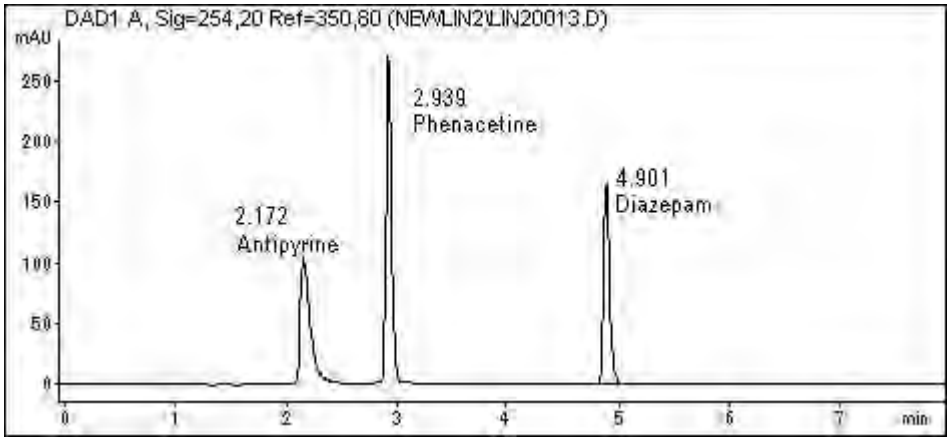
```
                                Run Logbook
=====================================================================
Method        Method started:  line# 7 vial# 2 inj# 1    10:46:18 10/25/00
Method        Instrument running sample Vial 2           10:46:18 10/25/00
1100 ALS    1 Air temperature (tray) = 20.1 °C           10:47:21 10/25/00
1100 PMP    1 Pressure = 69.8 bar                        10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:55:21 10/25/00
1100 PMP    1 Pressure = 75.7 bar                        10:55:21 10/25/00
Method        Instrument run completed                  10:55:23 10/25/00
Method        Method completed                           10:55:23 10/25/00
=====================================================================
=====================================================================
```




```
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1  438.70069     Antipyrine
  2.939 BB       934.32690    1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1  599.59734     Diazepam
Totals :                               1975.58590

=====================================================================
                           *** End of Report ***
```

Performance report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:36:38 AM agratz

======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                   Inj Volume : 1 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:36:32 AM by agratz (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150mm, 5µm
======================================================================
```

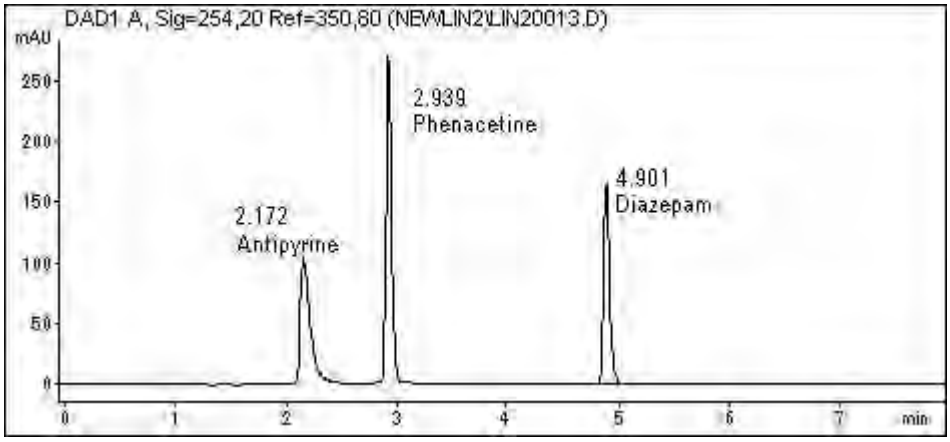



```
     External Standard Report with Performance
======================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!

RetTime     k'  Sig    Amount     Symm.  Width    Plates Resol  Name
 [min]                  [ng]             [min]           ution
-------|------|---|----------||-----|-------|-------|-----|----------
 2.172    0.81  1    438.70069    0.44  0.0883     3351  4.47 Antipyrine
 2.939    1.45  1    937.28787    0.83  0.0524    17435  6.40 Phenacetine
 4.901    3.08  1    599.59734    0.80  0.0550    43990 21.47 Diazepam

======================================================================
                          *** End of Report ***
```

Page 1 of 1

Detail report

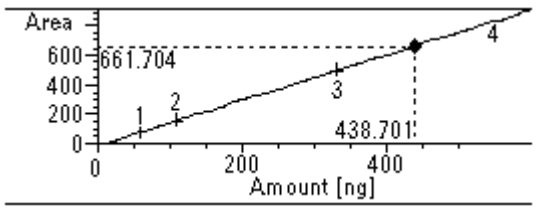
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:51:47 AM agratz

======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================
```




```
                  External Standard Report
======================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1  438.70069     Antipyrine
  2.939 BB       934.32690    1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1  599.59734     Diazepam
Totals :                               1975.58590
======================================================================
```

Detail report (continued)

```
======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================

======================================================================
----------------------------------------------------------------------
                        Calibration Curves
======================================================================
```




```
Antipyrine at exp. RT: 2.071
DAD1 A, Sig=254,20 Ref=350,80
Correlation:          1.00000
Residual Std. Dev.:   0.00000
Formula: y = ax^3 + bx^2 + cx + d
      a:     1.00818e-7
      b:     9.51014e-5
      c:     1.57593
      d:   -19.85331
      x: Amount (ng)
      y: Area
```

:
:
:

The header information and calibration curve is repeated for each peak

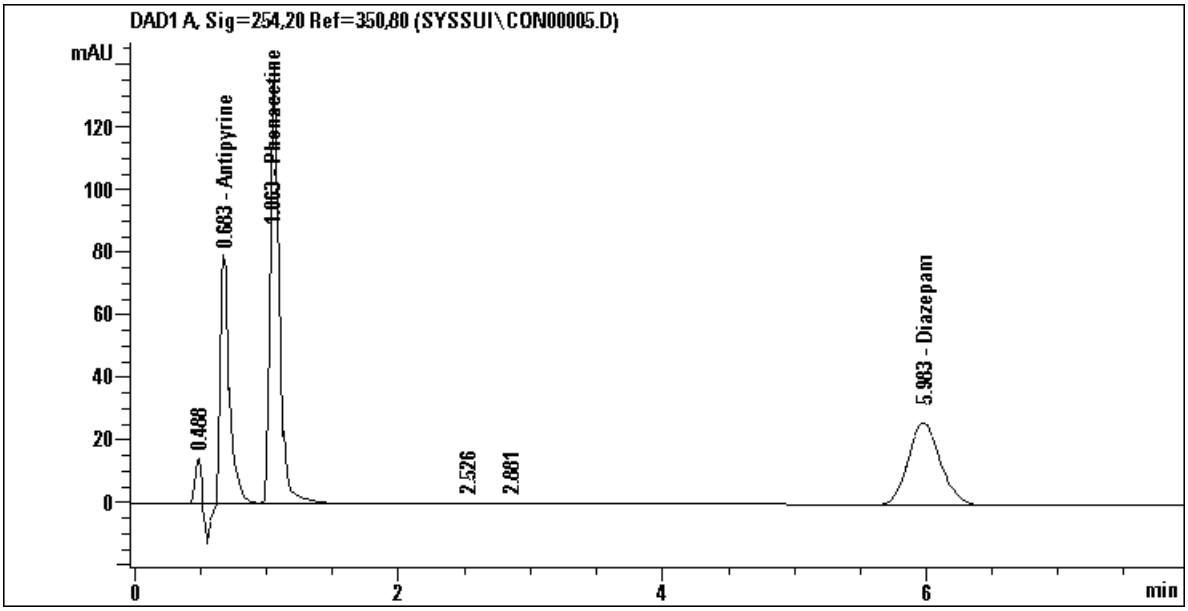
```
======================================================================
                        *** End of Report ***
```

Extended Performance Report

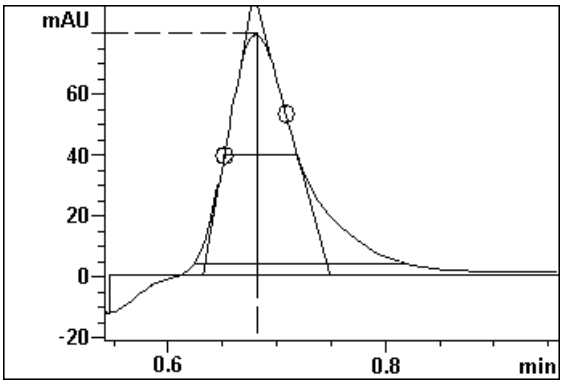
```
Data File D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D

                  Extended Performance Report

Instrument: Instrument 1

Module                          Firmware revision         Serial number
-------------------------------------------------------------------------
1100 Quaternary Pump            A.04.11                   DEl 1116042
1100 Wellplate Autosampler      A.04.13                   DE02700294
1100 Column Thermostat          A.04.11                   DE53400174
1100 Diode Array Detector       A.04.11                   DEO0900051
1100 Sample Thermostat          n/a                       DE82203241

Specials:
micro column switching valve installed in oven

Software Revisions for:
-Acquisition: Rev. A.08.04 [982] Copyright @ Agilent Technologies
-Data Analysis: Rev. A.08.04 [1008] Copyright @ Agilent Technologies

Column Description: XDB-C8
Product# Zorbax Batch#: b99024
Serial# USLLO00162
Diameter 2.1 mm Length: 30.0 mm
Particle size 3.5 mm Void volume 0.08 ml
Maximum Pressure 350 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment: system suitability

Analysis method: D:\HPCHEM\l\METHODS\SYSSUIP.M

Sample information for vial#: 21

  Sample Name:           calanti+     Multiplier:  1.00
  Injection#:            5            Dilution:    1.00
  Injection volume:      3 µl

Acquisition information:
  Operator:              agratz
  Date/Time:             2/11/029:06:34 AM
  Data file name:        D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D
  Method file name:      D:\HPCHEM\l\METHODS\SYSSUIP.M

  Flow:                  0.200 ml/min
  Pressure at start:     85 bar              Pressure at end:    88 bar
  Temperature at start:  25.1°C Temperature  at end:            25.0°C
```

Extended Performance Report (continued)

```
Solvents:              PMP1, Solvent A     water
                       PMP1, Solvent B     ACN
                       PMP1, Solvent C
                       PMP1, Solvent D

Signal description: DAD1 A, Sig=254,20 Ref=350,80
```




```
Compound# 2    :  Antipyrine
Amount [ng]:         51.1385

Peak description [min]:
      Signal: DAD1 A, Sig=254,20 Ref=350,80
      RetTime: 0.583      K':    0.706
      Height:  79.78  Area:      371.2
      Start:      0.546   End:   0.956
      Skew:    0.898   Excess:   1.643
      Width at half height:      0.067
            5 sigma:             0.196
            tangent:             0.117
            tailing:             0.190
      Symmetry:                  0.483
      USP Tailing:               1.657
      Integration type:            HV
      Time increment [macc]:     400.0
      Data points:                  66
```



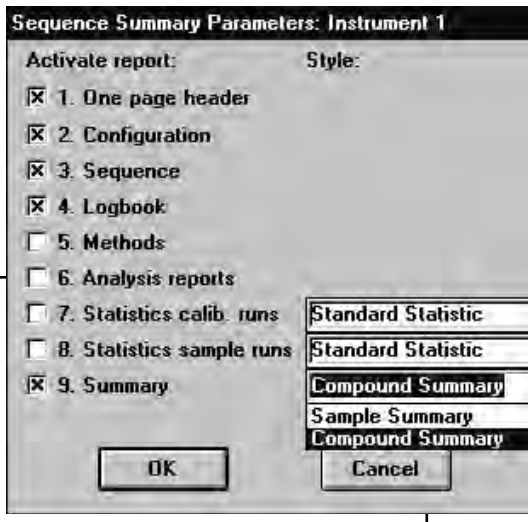
Extended Performance Report (continued)

```
Statistical moments (BB peak detection):    Efficiency:  Plates per ...
     M0:  514.1                                      column       meter
     M1:  0.699                Tangent method         541        18020
     M2:  0.00341              Halfwidth method       581        19360
     M3:  0.000179             5 sigma method         385        10153
     M4: 0.000054              Statistical            143        4782

Relationship to preceeding peak:             Selectivity: 3.217
Resolution Tangent method: 2.015             5 sigma method 1.700
Halfwidth method 2.034                       Statistical method 1.067



                                        :
                                        :
                                        :

==================================================================
                          *** End of Report ***
```

The peak description and statistical moments are repeated for each compound

Page 3 of 3

Sequence Summary Report – Compound Summary




```
  XXXXXX      XX        XXXXXX
 XX    XX     XX        XX    XX
 XX           XX        XX    XX
 XX           XX        XXXXXX
 XX    XX     XX        XX
 XX    XX     XX        XX
 XX    XX     XX        XX
  XXXXXX      XXXXXX    XX
```

S E Q U E N C E

S U M M A R Y

R E P O R T

A.G Huesgen

.....
Date/Signature

I n s t r u m e n t C o n f i g u r a t i o n

Instrument: Instrument 1

Module	Firmware revision	Serial number
1100 Wellplate Autosampler	A.04.08	DE02700294
1100 Column Thermostat	A.04.06	DE53400174
1100 Diode Array Detector	S.03.91	DE00900051
1100 Binary Pump	A.04.06	DE53500104
1100 Sample Thermostat	n/a	DE82203241

Software Revisions for:
- Acquisition: Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies

Sequence Summary Report – Compound Summary (continued)

S e q u e n c e

Sequence Parameters:

Operator:	agratz
Data File Naming:	Prefix/Counter
Signal 1 Prefix:	Lin2
Counter:	0001
Data Directory:	D:\HPCHEM\1\DATA\
Data Subdirectory:	NEWLIN2
Part of Methods to run:	Reprocessing only
	Use SAMPLE.MAC
Wait Time after loading Method:	0 min
Barcode Reader:	not used
Sequence Timeout:	0 min
Shutdown Cmd/Macro:	none
Sequence Comment:	Linearity Test

Sequence Table:

Sample Information Part:

Line	Location	Sample Information
1	Vial 1	1:10 diluted stock solution
2	Vial 1	1:10 diluted stock solution
3	Vial 1	1:10 diluted stock solution
4	Vial 1	1:10 diluted stock solution
5	Vial 1	1:10 diluted stock solution
6	Vial 1	1:10 diluted stock solution
7	Vial 2	1:100 diluted stock solution
8	Vial 2	1:100 diluted stock solution
9	Vial 2	1:100 diluted stock solution
10	Vial 2	1:100 diluted stock solution
11	Vial 2	1:100 diluted stock solution

Sequence Summary Report – Compound Summary (continued)

```
     Method and Injection Info Part:

     Line Location SampleName        Method   Inj SampleType InjVolume DataFile
     ==== ======== ================ ======== === ========== ========= ==========

     1    Vial 1   1:10dil.         LINICHEM 2   Sample        0.1
     2    Vial 1   1:10dil.         LINICHEM 2   Sample        0.5
     3    Vial 1   1:10dil.         LINICHEM 2   Sample        1
     4    Vial 1   1:10dil.         LINICHEM 2   Sample        3
     5    Vial 1   1:10dil.         LINICHEM 2   Sample        5
     6    Vial 1   1:10dil.         LINICHEM 2   Sample        10
     7    Vial 2   1:100dil.        LINICHEM 2   Sample        25
     8    Vial 2   1:100dil.        LINICHEM 2   Sample        50
     9    Vial 2   1:100dil.        LINICHEM 2   Sample        75
     10   Vial 2   1:100dil.        LINICHEM 2   Sample        100
     11   Vial 2   1:100dil.        LINICHEM 2   Sample        0.1


     Calibration Part:

     Line Location SampleName        Method   CalLev Update RF Update RT Interval
     ==== ======== ================ ======== ====== ========= ========= =========



     Quantification Part:

     Line Location SampleName        SampleAmount ISTDAmt   Multiplier Dilution
     ==== ======== ================ ============ =======   ========== =========

     1    Vial 1   1:10dil.
     2    Vial 1   1:10dil.
     3    Vial 1   1:10dil.
     4    Vial 1   1:10dil.
     5    Vial 1   1:10dil.
     6    Vial 1   1:10dil.
     7    Vial 2   1:100dil.
     8    Vial 2   1:100dil.
     9    Vial 2   1:100dil.
     10   Vial 2   1:100dil.
     11   Vial 2   1:100dil.


Sequence Output Parameters:

      Print Sequence Summary Report (SSR):              Yes
           SSR to Printer:                              Yes
           SSR to File:                                 Yes
           SSR File Name:                               GLPrprt.txt
           SSR to HTML:                                 No
           Print individual reports for each run:       No
```

Sequence Summary Report – Compound Summary (continued)

```
Sequence Summary Parameters:

One page header:                        Yes
Print Configuration:                    Yes
Print Sequence:                         Yes
Print Logbook:                          Yes
Print Method(s):                        No
Print Analysis reports:                 No
Print Statistics for Calib. runs:       No
Statistic Sample runs style:            No
Summary style:                          Compound Summary


                              L o g b o o k
24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                          Time      Date
-------------------------------------------------------------------------------------
Sequence      LIN2.S started                                         10:47:06 01/24/02
Method        Loading Method LINICHEM.M                              10:47:07 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 1                10:47:08 01/24/02
CP Macro      Analyzing rawdata Lin20001.D                           10:47:08 01/24/02
Method        Method completed                                       10:47:10 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 2                10:47:11 01/24/02
CP Macro      Analyzing rawdata Lin20002.D                           10:47:11 01/24/02
Method        Method completed                                       10:47:13 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 1                10:47:14 01/24/02
CP Macro      Analyzing rawdata Lin20003.D                           10:47:14 01/24/02
Method        Method completed                                       10:47:16 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 2                10:47:17 01/24/02
CP Macro      Analyzing rawdata Lin20004.D                           10:47:18 01/24/02
Method        Method completed                                       10:47:19 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 1                10:47:21 01/24/02
CP Macro      Analyzing rawdata Lin20005.D                           10:47:21 01/24/02
Method        Method completed                                       10:47:22 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 2                10:47:24 01/24/02
CP Macro      Analyzing rawdata Lin20006.D                           10:47:24 01/24/02
Method        Method completed                                       10:47:26 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 1                10:47:27 01/24/02
CP Macro      Analyzing rawdata Lin20007.D                           10:47:27 01/24/02
Method        Method completed                                       10:47:29 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 2                10:47:30 01/24/02
CP Macro      Analyzing rawdata Lin20008.D                           10:47:30 01/24/02
Method        Method completed                                       10:47:32 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 1                10:47:33 01/24/02
CP Macro      Analyzing rawdata Lin20009.D                           10:47:34 01/24/02
Method        Method completed                                       10:47:35 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 2                10:47:37 01/24/02
CP Macro      Analyzing rawdata Lin20010.D                           10:47:37 01/24/02
Method        Method completed                                       10:47:39 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 1                10:47:40 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

```
CP Macro      Analyzing rawdata Lin20011.D                          10:47:40 01/24/02
Method        Method completed                                      10:47:42 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 2               10:47:43 01/24/02
CP Macro      Analyzing rawdata Lin20012.D                          10:47:43 01/24/02
Method        Method completed                                      10:47:45 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 1               10:47:46 01/24/02
CP Macro      Analyzing rawdata Lin20013.D                          10:47:47 01/24/02
Method        Method completed                                      10:47:48 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 2               10:47:50 01/24/02
CP Macro      Analyzing rawdata Lin20014.D                          10:47:50 01/24/02

24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                         Time      Date
-------------------------------------------------------------------------------------
Method        Method completed                                      10:47:51 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 1               10:47:53 01/24/02
CP Macro      Analyzing rawdata Lin20015.D                          10:47:53 01/24/02
Method        Method completed                                      10:47:55 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 2               10:47:56 01/24/02
CP Macro      Analyzing rawdata Lin20016.D                          10:47:56 01/24/02
Method        Method completed                                      10:47:58 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 1               10:47:59 01/24/02
CP Macro      Analyzing rawdata Lin20017.D                          10:47:59 01/24/02
Method        Method completed                                      10:48:01 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 2               10:48:02 01/24/02
CP Macro      Analyzing rawdata Lin20018.D                          10:48:03 01/24/02
Method        Method completed                                      10:48:04 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 1              10:48:06 01/24/02
CP Macro      Analyzing rawdata Lin20019.D                          10:48:06 01/24/02
Method        Method completed                                      10:48:08 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 2              10:48:09 01/24/02
CP Macro      Analyzing rawdata Lin20020.D                          10:48:09 01/24/02
Method        Method completed                                      10:48:11 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 1              10:48:12 01/24/02
CP Macro      Analyzing rawdata Lin20021.D                          10:48:13 01/24/02
Method        Method completed                                      10:48:14 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 2              10:48:16 01/24/02
CP Macro      Analyzing rawdata Lin20022.D                          10:48:16 01/24/02
Method        Method completed                                      10:48:18 01/24/02
Sequence      LIN2.S completed                                      10:48:19 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

C o m p o u n d S u m m a r y

Sequence table: D:\HPCHEM\CORE\LIN2.S
Data directory path: D:\HPCHEM\1\DATA\NEWLIN2
Logbook: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG
Sequence start: 10/25/00 6:58:26 AM
Operator: agratz

Method file name: D:\HPCHEM\1\METHODS\LINICHEM.M

Sample Name	Sample Amt [ng]	Multip.* Dilution	FileName .D	RetTime [min]	Amount [ng]	Compound
sample1	0.00000	1.0000	Lin20001	2.071	-	-
				3.005	41.80740	Phenacetine
				5.061	27.57288	Diazepam
sample2	0.00000	1.0000	Lin20002	2.071	-	-
				2.927	37.71584	Phenacetine
				4.931	24.68503	Diazepam
sample3	0.00000	1.0000	Lin20003	2.159	113.94044	Antipyrine
				2.921	249.65462	Phenacetine
				4.927	162.09926	Diazepam
sample4	0.00000	1.0000	Lin20004	2.138	115.89423	Antipyrine
				2.888	254.19389	Phenacetine
				4.893	167.32050	Diazepam
sample5	0.00000	1.0000	Lin20005	2.071	-	-
				2.967	533.16102	Phenacetine
				4.977	350.64724	Diazepam
sample6	0.00000	1.0000	Lin20006	2.071	-	-
				2.935	555.34634	Phenacetine
				4.885	359.02135	Diazepam
sample7	0.00000	1.0000	Lin20007	2.120	770.88338	Antipyrine
				2.932	1659.61614	Phenacetine
				4.939	1090.77773	Diazepam
sample8	0.00000	1.0000	Lin20008	2.156	766.86882	Antipyrine
				2.978	1658.25754	Phenacetine
				4.990	1088.46781	Diazepam
sample9	0.00000	1.0000	Lin20009	2.112	1298.20959	Antipyrine
				2.956	2780.26621	Phenacetine
				4.874	1801.76061	Diazepam
sample10	0.00000	1.0000	Lin20010	2.125	1265.65752	Antipyrine
				2.931	2753.00356	Phenacetine
				4.917	1784.44912	Diazepam
sample11	0.00000	1.0000	Lin20011	2.070	2206.34622	Antipyrine
				2.928	4737.72659	Phenacetine
				4.931	3055.52966	Diazepam
sample12	0.00000	1.0000	Lin20012	2.157	2219.77978	Antipyrine
				2.959	4771.25573	Phenacetine
				4.905	3043.14819	Diazepam
sample13	0.00000	1.0000	Lin20013	2.172	438.70069	Antipyrine
				2.939	937.28787	Phenacetine
				4.901	599.59734	Diazepam

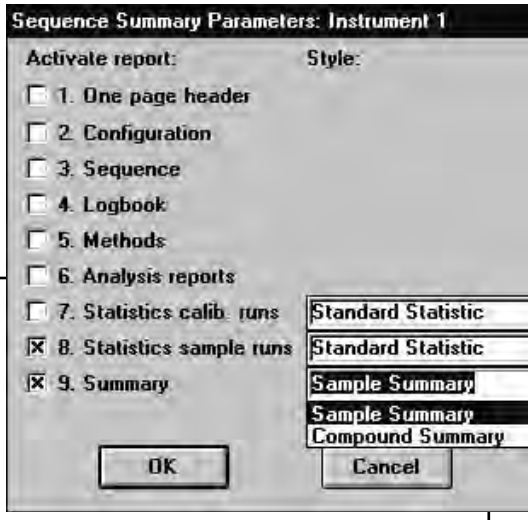
Sequence Summary Report – Compound Summary (continued)

sample14	0.00000	1.0000	Lin20014	2.137	431.19756	Antipyrine
				2.920	922.41613	Phenacetine
				4.914	598.82718	Diazepam
sample15	0.00000	1.0000	Lin20015	2.130	1050.21043	Antipyrine
				2.956	2257.23577	Phenacetine
				4.946	1454.09021	Diazepam
sample16	0.00000	1.0000	Lin20016	2.071	-	-
				3.062	2266.63554	Phenacetine
				4.914	1450.54300	Diazepam
sample17	0.00000	1.0000	Lin20017	2.112	1860.82017	Antipyrine
				2.958	4083.57167	Phenacetine
				4.943	2601.71134	Diazepam
sample18	0.00000	1.0000	Lin20018	2.114	1846.79895	Antipyrine
				2.970	4045.19575	Phenacetine
				4.970	2576.86650	Diazepam
sample19	0.00000	1.0000	Lin20019	2.152	2485.47770	Antipyrine
				3.019	5268.86688	Phenacetine
				4.973	3410.01754	Diazepam
sample20	0.00000	1.0000	Lin20020	2.135	2489.66113	Antipyrine
				2.975	5298.02094	Phenacetine
				4.943	3415.39103	Diazepam
sample21	0.00000	1.0000	Lin20021	2.155	2961.16799	Antipyrine
				3.010	6013.24563	Phenacetine
				5.003	4037.60722	Diazepam
sample22	0.00000	1.0000	Lin20022	2.156	2983.41614	Antipyrine
				3.042	6012.35737	Phenacetine
				4.988	4010.73532	Diazepam

==========

*** End of Report ***

Sequence Summary Report – Standard Statistics for Sample Runs




```
                    S t a t i s t i c    R e p o r t

Sequence table:          D:\HPCHEM\1\SEQUENCE\NEWLIN.S
Data directory path:     D:\HPCHEM\1\DATA\NEWLIN
Operator:                agratz

Method file name:        D:\HPCHEM\1\METHODS\LINI2.M
```

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	Vial 2	1	8/24/00 12:42:04 AM	new00061.D	sample1
2	Vial 2	2	8/24/00 12:51:09 AM	new00062.D	sample2
3	Vial 2	3	8/24/00 1:00:14 AM	new00063.D	sample3
4	Vial 2	4	8/24/00 1:09:18 AM	new00064.D	sample4
5	Vial 2	5	8/24/00 1:18:21 AM	new00065.D	sample5
6	Vial 2	6	8/24/00 1:27:25 AM	new00066.D	sample6
7	Vial 2	7	8/24/00 1:36:30 AM	new00067.D	sample7
8	Vial 2	8	8/24/00 1:45:34 AM	new00068.D	sample8
9	Vial 2	9	8/24/00 1:54:38 AM	new00069.D	sample9
10	Vial 2	10	8/24/00 2:03:42 AM	new00070.D	sample10

Compound: Antipyrine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BV	2.071	26.23064	834.52417	215.75279	0.0594	0.74
2	BV	2.071	26.28149	836.14185	216.26503	0.0594	0.74
3	BV	2.070	26.22879	834.46539	215.85945	0.0594	0.74
4	BV	2.070	26.27553	835.95233	216.52124	0.0594	0.74
5	BV	2.070	26.21720	834.09644	215.51944	0.0594	0.74
6	BV	2.070	26.19317	833.33203	216.02470	0.0593	0.74
7	BV	2.070	26.27779	836.02423	216.93185	0.0592	0.74
8	BV	2.072	26.29524	836.57941	216.89178	0.0593	0.74
9	BV	2.072	26.22549	834.36017	216.09763	0.0593	0.74
10	BV	2.071	26.21184	833.92590	216.06882	0.0593	0.74
Mean:		2.071	26.24372	834.94019	216.19327	0.0594	0.74
S.D.:		6.81e-4	3.53636e-2	1.12509	4.66512e-1	6.63e-5	1e-3
RSD :		0.033	1.34751e-1	1.34751e-1	2.15784e-1	0.1117	0.20
95% CI:		4.87e-4	2.52976e-2	8.04838e-1	3.33722e-1	4.74e-5	1e-3

Sequence Summary Report – Standard Statistics for Sample Runs

Compound: Phenacetine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	3.035	12.05932	1203.01074	357.49438	0.0528	0.88
2	BB	3.035	12.07862	1204.93591	357.76285	0.0527	0.87
3	BB	3.035	12.05487	1202.56653	357.16501	0.0527	0.88
4	BB	3.035	12.07567	1204.64221	357.80615	0.0527	0.88
5	BB	3.036	12.05951	1203.02979	356.62448	0.0528	0.87
6	BB	3.036	12.02965	1200.05090	356.52957	0.0528	0.88
7	BB	3.037	12.08083	1205.15625	357.92139	0.0527	0.88
8	BB	3.037	12.06433	1203.51099	357.60211	0.0527	0.88
9	BB	3.039	12.05340	1202.42065	356.89868	0.0527	0.87
10	BB	3.038	12.04430	1201.51282	356.41678	0.0528	0.88
Mean:		3.036	12.06005	1203.08368	357.22214	0.0527	0.88
S.D.:		1.35e-3	1.59266e-2	1.58880	5.70986e-1	3.70e-5	6e-3
RSD :		0.045	1.32061e-1	1.32061e-1	1.59840e-1	0.0702	0.68
95% CI:		9.69e-4	1.13932e-2	1.13656	4.08458e-1	2.65e-5	4e-3

Compound: Diazepam (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	5.085	17.51478	820.56067	228.97469	0.0556	0.84
2	BB	5.086	17.54309	821.88702	229.58243	0.0557	0.84
3	BB	5.085	17.51162	820.41229	229.04759	0.0557	0.84
4	BB	5.084	17.54478	821.96600	229.60602	0.0557	0.84
5	BB	5.086	17.51105	820.38562	229.37668	0.0556	0.84
6	BB	5.087	17.47411	818.65503	228.69946	0.0556	0.85
7	BB	5.088	17.54951	822.18774	229.63567	0.0556	0.84
8	BB	5.088	17.51423	820.53491	229.10289	0.0556	0.84
9	BB	5.090	17.51381	820.51508	229.17131	0.0557	0.84
10	BB	5.090	17.50570	820.13525	228.79688	0.0556	0.84
Mean:		5.087	17.51827	820.72396	229.19936	0.0556	0.84
S.D.:		2.12e-3	2.24801e-2	1.05318	3.38200e-1	3.77e-5	2e-3
RSD :		0.042	1.28324e-1	1.28324e-1	1.47557e-1	0.0678	0.29
95% CI:		1.52e-3	1.60813e-2	7.53401e-1	2.41934e-1	2.70e-5	2e-3

Sequence Summary Report – Standard Statistics for Sample Runs

```
                  S a m p l e    S u m m a r y


Sequence table:                          D:\HPCHEM\1\SEQUENCE\NEWLIN.S
Data directory path:                     D:\HPCHEM\1\DATA\NEWLIN
Logbook:                                 D:\HPCHEM\1\DATA\NEWLIN\NEWLIN.LOG
Sequence start:                          8/24/00 12:42:04 AM
Statistic report on calibration runs:  1
Operator:                                agratz

Method file name:                        D:\HPCHEM\1\METHODS\LINI2.M

Run Location Inj Sample Name Sample Amt Multip.* File name Cal # Page
 #             #                    [ng]    Dilution               Cmp  #
---|--------|---|----------------|----------|--------|----------|-|---|---
  1 Vial 2    1  sample1               -    1.0000  new00061.D *   3  -
  2 Vial 2    2  sample2               -    1.0000  new00062.D *   3  -
  3 Vial 2    3  sample3               -    1.0000  new00063.D *   3  -
  4 Vial 2    4  sample4               -    1.0000  new00064.D *   3  -
  5 Vial 2    5  sample5               -    1.0000  new00065.D *   3  -
  6 Vial 2    6  sample6               -    1.0000  new00066.D *   3  -
  7 Vial 2    7  sample7               -    1.0000  new00067.D *   3  -
  8 Vial 2    8  sample8               -    1.0000  new00068.D *   3  -
  9 Vial 2    9  sample9               -    1.0000  new00069.D *   3  -
 10 Vial 2   10  sample10              -    1.0000  new00070.D *   3  -


================================================================================
                            *** End of Report ***
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Published March 1, 2003
Publication Number 5988-9011EN



Agilent Technologies

Simulated Distillation System for ASTM D2887, Based on the Agilent 6890N GC

Application

Petroleum

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Abstract

An Agilent Simulated Distillation (SIMDIS) system is described for quality control and monitoring of refinery processes. The system uses an Agilent 6890N gas chromatograph (GC) equipped with a programmable temperature vaporizer (PTV) inlet designed specifically for high temperature analysis. A simulated distillation software program that couples with the Agilent GC ChemStation provides an easy-to-use solution for simulated distillation. The system allows analysts to quickly determine the boiling point distribution and cut points of petroleum products. This application note describes in detail the system needed to perform simulated distillation by ASTM method D2887. This method is applicable to petroleum products and fractions having an initial boiling point greater than 55 °C and a final boiling point less than 538 °C.

Introduction

The boiling range distribution of petroleum fractions provides insight into to the composition of feedstocks and products related to petroleum refining. Both physical distillation and simulated distillation methods can be used to determine boiling point (BP). However, physical distillation methods are time-consuming and require a high level of operator intervention compared to simulated distillation, resulting in wide specification ranges for reproducibility and repeatability. Simulated distillation, in contrast, allows accurate and fast determination of the BP distribution of petroleum fractions.

ASTM D2887 is widely used in the refinery industry [1]. It is designed to determine the boiling range distribution of petroleum products and fractions having a final boiling point of 538 °C or lower. The Agilent SIMDIS system offers a cost effective and easy-to-use package to practice ASTM method D2887.

Experimental

The SIMDIS application uses an Agilent 6890N GC with electronic pneumatics control (EPC), configured with a flame ionization detector (FID) and a 10 m × 530 µm × 2.65 µm DB-1 capillary column. The Agilent 7683B automatic liquid injector, equipped with either an SGE 0.5 µL or Agilent 5-µL syringe was used for sample introduction into a JAS UNIS 3100 high temperature, air-cooled PTV inlet. A qualitative mixture of normal paraffins C5

to C44 (Supelco Part no. 500658) or C5 to C40 (Agilent part number 5080-8716) was used to determine the BP versus retention time (RT) relationship over the range required by method D2887.

High Temperature PTV Inlet

This high temperature PTV inlet (HT PTV), developed by Joint Analytical Systems GmbH (Moers, Germany), provides efficient transfer of sample to the column without discrimination. It integrates a coiled wire cartridge for heating in a carefully constructed arrangement for optimized thermal gradient. The low mass design allows for rapid heating/cool-down rates using only the GC inlet fan for cool-down. For simulated distillation applications, the inlet is operated in a split/hot injection mode. The Agilent 7683B series injector operating in the fast injection mode is used with control and parameter setting of the inlet integrated into the ChemStation. Typically, the inlet is ramped rapidly in temperature at the time of sample introduction, held at the maximum programmed temperature for a few minutes and then cooled back uncontrolled to the starting temperature, all during the first half of the GC run. High program rates of 400 °C or 500 °C/min can be used for thermally stable analytes as normally encountered in simulated distillation.

General instrumental conditions used for performing method D2887 are listed in Table 1.

The process of SIMDIS analysis includes blank analysis (for baseline subtraction), calibration (C5 to C40 or C5 to C44), validation (reference gas oil No.1 for ASTM D2887) and sample analysis. These analyses can be automated through the GC ChemStation coupled to the Agilent SIMDIS software.

SIMDIS Software

The SIMDIS application software can operate standalone or be called to automatically execute as a post run event from ChemStation. The software is structured in functional modules including: Browse, Setup, SimDis, Report, and Automation. Each module provides specific functions to rapidly view data files, setup SIMDIS parameters, perform SIMDIS calculations, customize reporting, and track files used in automation.

The Agilent SIMDIS module provides a number of functions to correct artifacts such as solvent peaks and contributions from baseline, including a number of options for baseline treatment that optimize the SIMDIS analysis. Once optimized this collection of parameters can be saved and applied to all future sample analyses. The parameter selection and calculation functions are located at left of the menu for the SIMDIS module as shown in Figure 1. Also, icons for Start Elute, End Elute, initial-BP (IBP) display, and final-BP (FBP) display at the top left of the menu can be toggled to show the current selections graphically on the raw signal.

Table 1. Gas Chromatographic Conditions

PTV inlet typical temperature program options	1) 300 °C to 400 °C at 500 °C/min (4 min.), to 300 °C at 200 °C/min 2) 225 °C to 400 °C at 200 °C/min (4 min.), to 225 °C at 100 °C/min 3) 350 °C (no ramping)
Split ratio	2:1
Injection volume	0.05 µL to 0.1 µL
Liner	HT PTV SimDis liner
Column	DB-1, 10 m × 530 µm × 2.65 µm (part number 125-10HB) or DB-2887 (part number 125-2814)
Column flow (He)	14 mL/min, constant flow mode
FID temperatures	350 °C
H_2 flow	40 mL/min
Air flow	450 mL/min
Make up (N_2)	45 mL/min
Oven program	40 °C to 350 °C at 20 °C/min, hold 8 min
Data acquisition rate	5 Hz typical

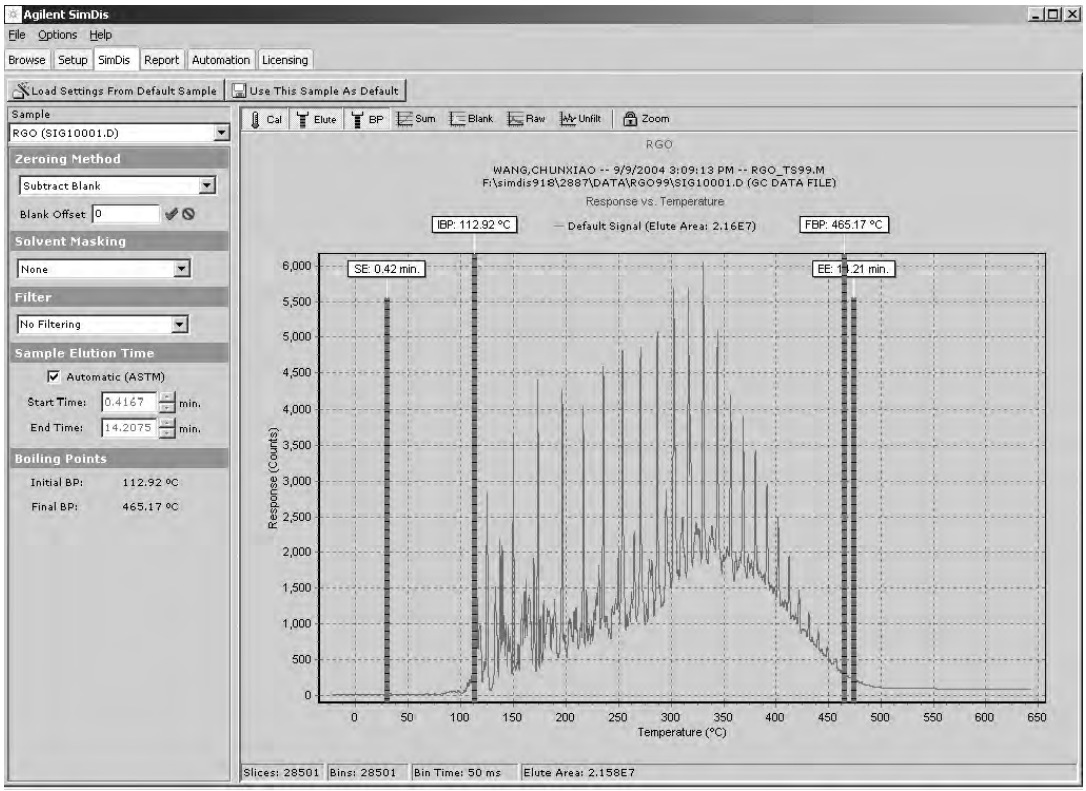

Figure 1. SIMDIS analysis setup pane showing start (IBP) and end (FBP) values.

The SIMDIS software Report tab offers several different report options for the results that can be displayed with a simple mouse click. The report list presents the available report formats and data treatment for output such as “calibration report”, “Engineering report”, “complete percentage yield”, “standard cut point”, “custom cut point”, and “D2887 to D86 correlation”. Figures 2a and 2b show examples of signature and custom cut point reports, respectively.

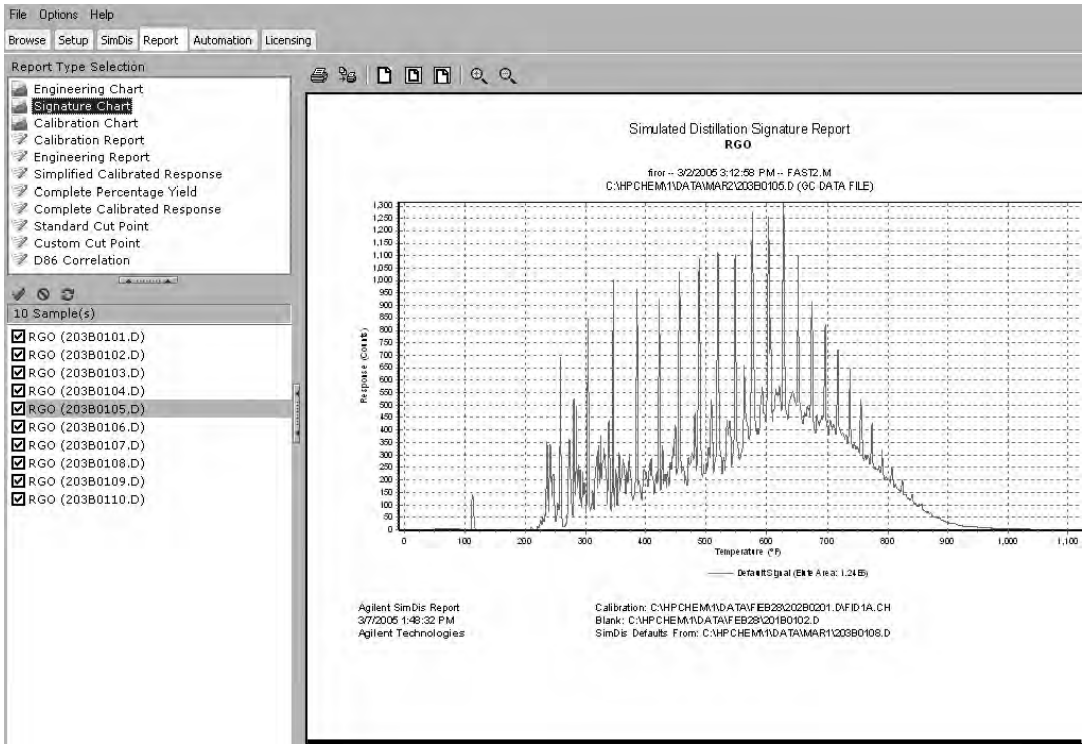

Figure 2a. Signature report for reference gas oil (RGO).

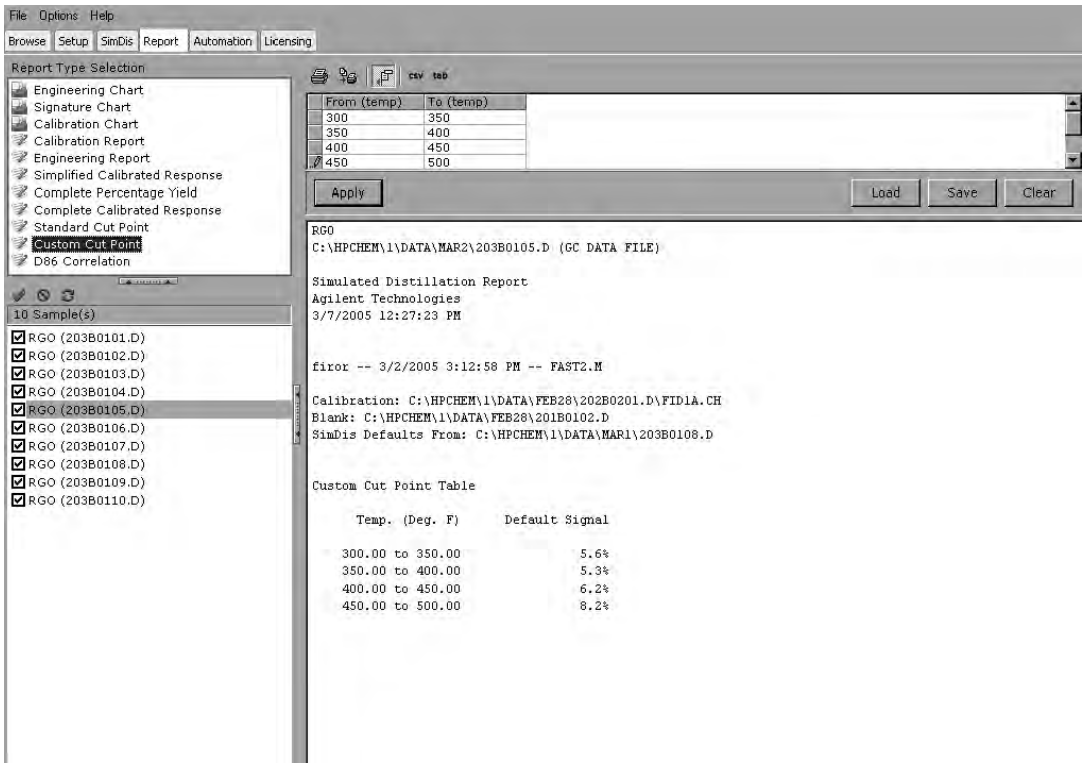

Figure 2b. Report pane showing a custom cut point table.

Results and Discussion

Blank Analysis

Two types of blank analyses are commonly performed; blank run without injection, and blank run with injection of solvent only. Blank analysis is performed with a method identical to the one used for sample and calibration runs. However, if the samples are too viscous, waxy, or require a reduction in concentration, dilution is required. The solvent often used is carbon disulfide (CS_2). It is injected neat as a blank run for subsequent subtraction from the sample runs. Thus, these blank analyses are used to correct for any baseline changes that may influence the baseline offset, such as column bleed. They are also useful to check for possible carryover from a previous overloaded injection. Also note in Figure 3, the overlay of two consecutive blank runs. It is always good practice to perform multiple blanks until a high degree of overlap is seen indicating a stable system ready to run test samples. An example of a valid calibration/CS_2 blank setup, as displayed by the software, is shown in Figure 4 (blank in bottom box).

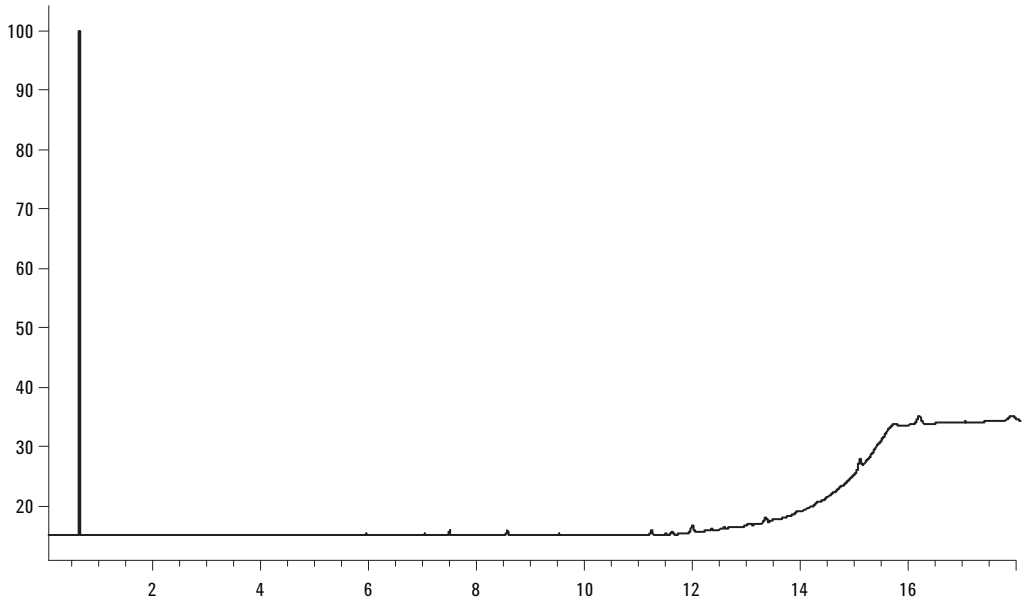

Figure 3. Overlay of two consecutive blank runs showing stable baseline profile.

Calibration

Initially, the ChemStation calibration table functions are used to set up a calibration between BP and RT. Calibration mixtures containing a series of known n-alkanes from C5 to C40 or C5 to C44, are used to establish the BP-RT correlation. The SIMDIS software has the capability to edit the calibration file, adding peaks, deleting peaks, and assigning/changing carbon number, all accomplished easily using the mouse. An example of a complete BP calibration (C5 to C40) is shown in Figure 4 (top box). A second example (C5-C44) using the Sulpelco calibration mix (p/n 500658) is shown in Figure 5.

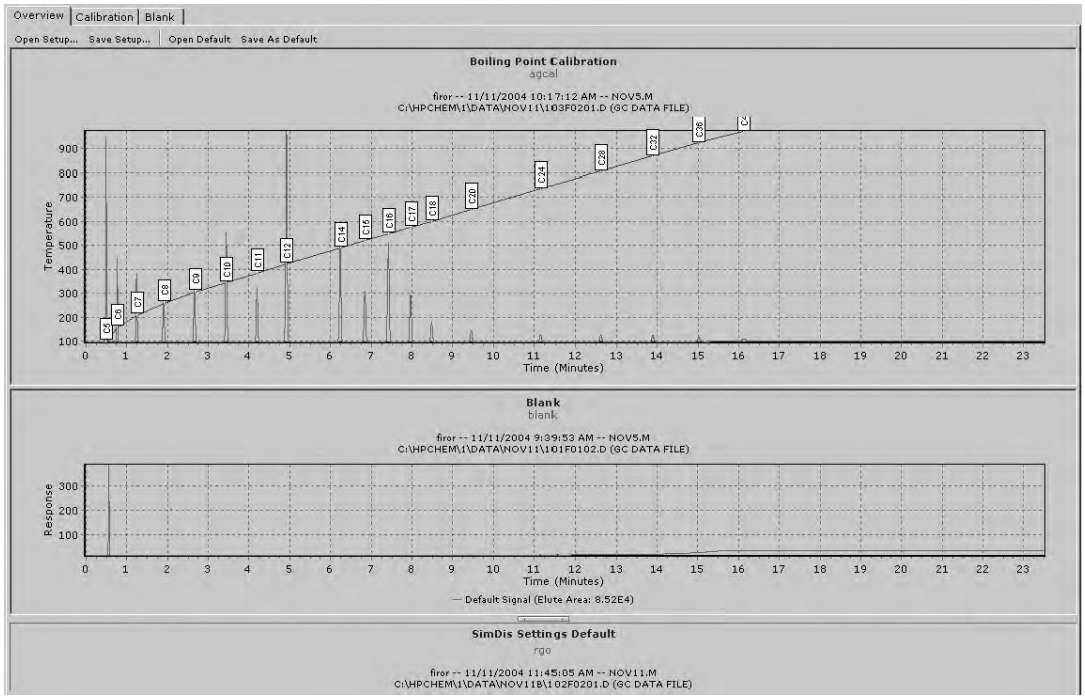

Figure 4. Calibration overview pane showing calibration and blank runs selected for subsequent use in SIMDIS calculations.

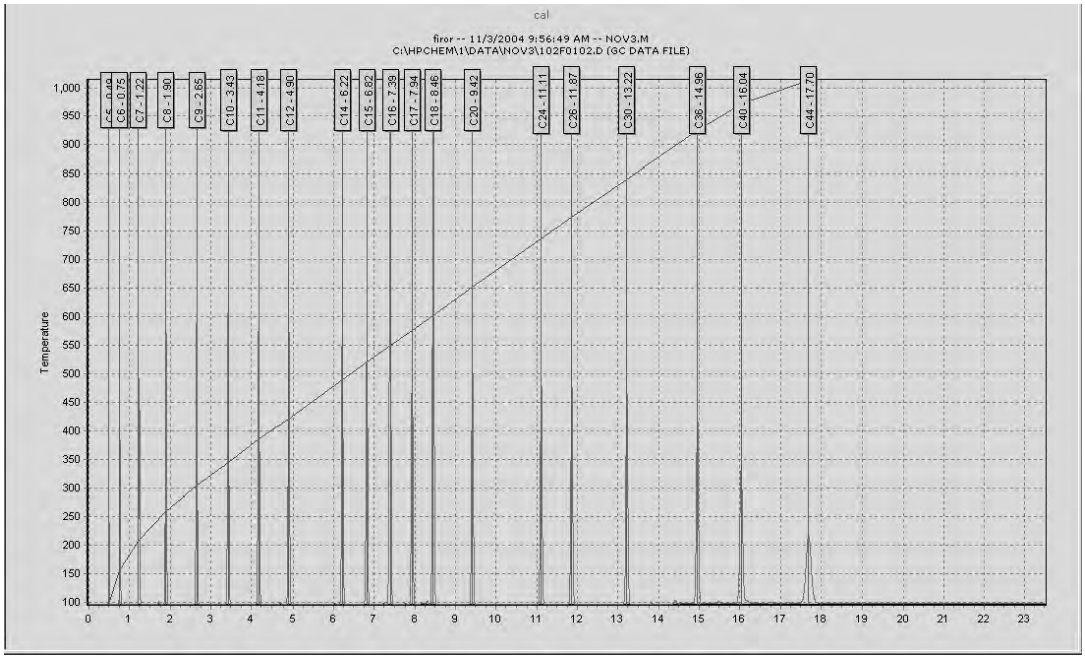

Figure 5. Calibration pane showing C5 to C44 peak assignments.

RGO Analysis

To meet the requirements of ASTM D2887, the RGO sample analysis must be performed to verify both the chromatographic and calculation algorithms involved in this test method.

Figure 6 shows the chromatographic overlay of RGO and blank for a typical D2887 analysis. Table 2 shows the results for five runs of RGO analysis. The data demonstrates that the observed BP values agree with the ASTM D2887 consensus BP values within the allowable difference range.

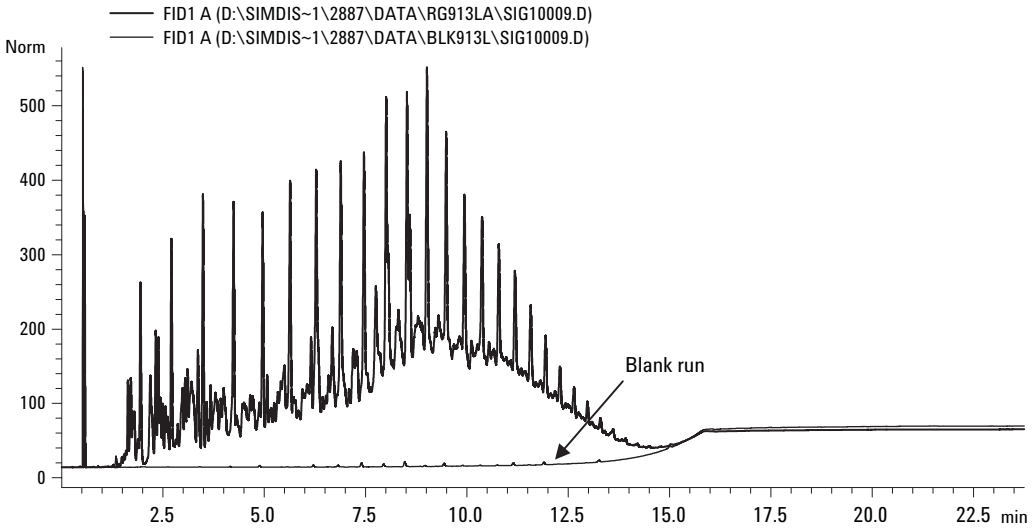

Figure 6. Chromatographic overlay of RGO and CS_2 blank.
Sample: ASTM RGO No. 1. Injection volume: 0.01 µL (no dilution). GC conditions are given in Table 1.

Table 2. Typical Results for the RGO (°C)

ASTM D2887 Values			Observed values	
OFF%	BP	Allowable difference	BP	Difference
IBP	115	7.6	113.3	1.7
10	176	4.1	177.8	–1.8
20	224	4.9	227.2	–3.2
30	259	4.7	262.8	–3.8
40	289	4.3	292.2	–3.2
50	312	4.3	314.4	–2.4
60	332	4.3	332.8	–0.8
70	354	4.3	355.6	–1.6
80	378	4.3	379.4	–1.4
90	407	4.3	409.4	–2.4
FBP	475	11.8	466.7	8.3

In Figure 7, ten consecutive runs of the RGO are overlaid without normalization. Run to run deviations over the entire runtime are small, indicating good system performance (injector, syringe, inlet, column and detector).

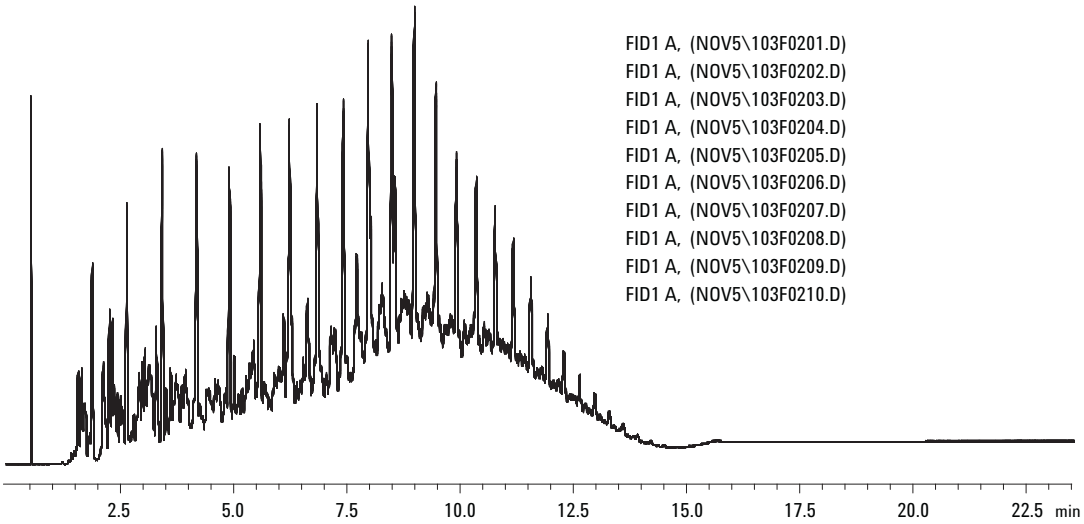

Figure 7. Overlay of 10 consecutive runs of RGO. Less than 3% run to run variation in the area of any given slice across the entire chromatogram.

An example of the engineering report format for analysis of the RGO is shown in Figure 8. The report indicates specific blank and calibration files used in calculating the results.

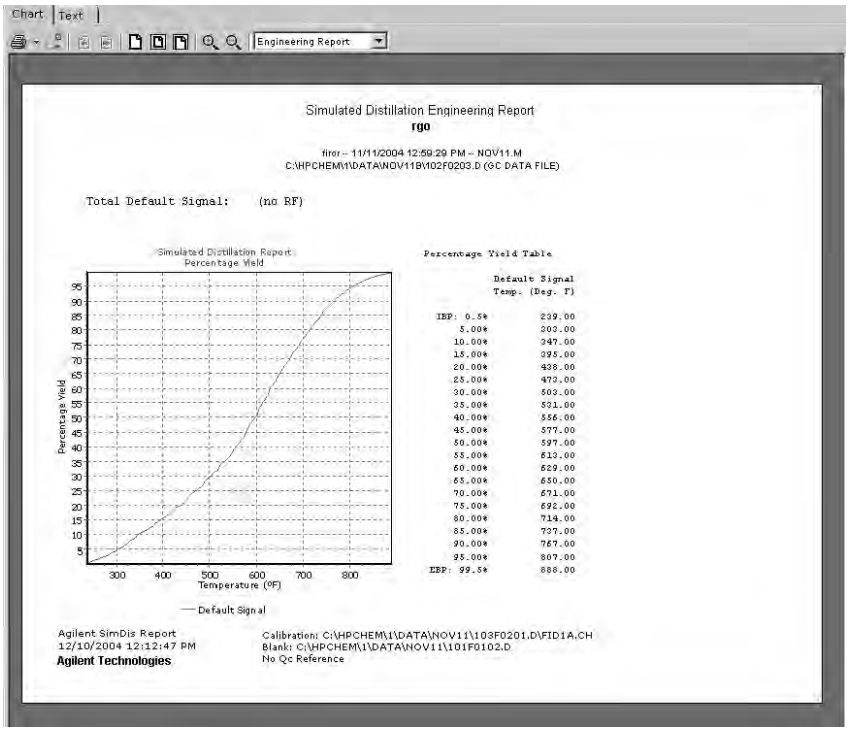

Figure 8. Engineering report for a RGO sample.

Cracked Gas Oil

Figure 9 shows the chromatographic overlay of five runs of cracked gas oil for simulated distillation analysis. The overlays of the chromatograms guarantee reliable and repeatable results. Calculated results are shown in Table 3.

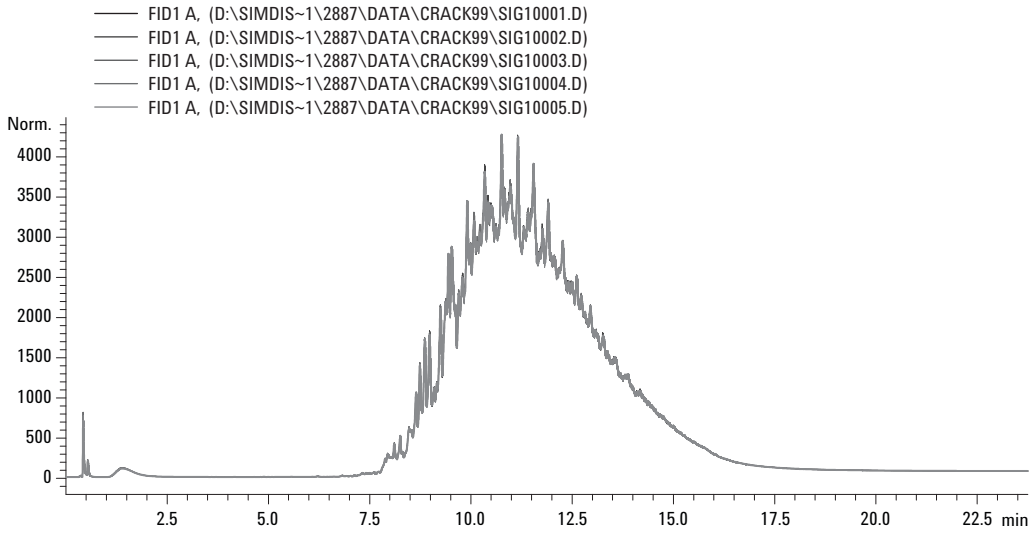

Figure 9. Chromatographic overlay of five runs of a cracked gas oil analysis.
Injection volume: 0.1 µL (4:1 approximate dilution in CS_2). GC conditions are given in Table 1.

Table 3. Results for Cracked Gas Oil Samples in °C

Observed BP OFF%	BP °C											SD
	1	2	3	4	5	6	7	8	9	10	Average	
IBP	297	297	298	298	299	298	298	298	298	297	297	0.2
10	344	344	344	344	344	344	344	344	344	344	344	0
20	360	361	361	361	361	361	361	361	361	361	360	0.1
30	373	373	373	373	373	373	373	373	373	373	373	0
40	384	385	385	385	385	385	385	385	386	386	385	0.1
50	397	397	397	397	397	397	397	397	398	398	397	0.1
60	410	411	411	411	411	411	411	411	411	411	411	0.1
65	417	417	417	417	417	417	417	417	418	417	417	0.1
70	424	424	424	424	424	424	424	424	425	425	424	0.1
80	442	442	442	442	442	442	442	442	443	442	442	0.1
90	467	468	468	468	468	468	468	468	469	468	468	0.1
FBP	516	516	517	516	516	517	515	516	517	519	517	0.2

Conclusions

Knowledge of the BP distribution of petroleum fractions and feedstocks as input to catalytic crackers is essential to ensure a uniform quality of finished product and to optimize refinery processes. Simulated distillation provides a fast and reliable technique to determine the BP distributions with a high degree of confidence. The Agilent SIMDIS system consisting of a 6890N GC equipped with the HT PTV inlet, FID, ChemStation, and simulated distillation application software allows analysts to quickly and easily perform simulated distillation analysis according to ASTM D2887. The system and software can also perform the proposed D2887x (extended D2887), and D6352 (high temperature simulated distillation) methods. These latter two methods are described in a separate application note [2].

This simulated distillation system is available complete with hardware and software, including verification of performance using the RGO as option 651 to the 6890N.

References

1. ASTM D2887-97a,"Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography", Annual Book of Standards, Volume 05.02, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA.
2. C. Wang and R. Firor, "High-Temperature Simulated Distillation System Based on the 6890N GC", Agilent Technologies, publication 5989-2727EN www.agilent.com/chem

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Printed in the USA
May 9, 2005
5989-2726EN



Agilent Technologies

High-Temperature Simulated Distillation System Based on the 6890N GC

Application

Petroleum

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Abstract

An Agilent Simulated Distillation (SimDis) system is described for characterizing petroleum fractions for quality control and monitoring of refinery feedstocks and products. The system uses a 6890N gas chromatograph (GC) equipped with a high temperature programmable temperature vaporizer (HT PTV) inlet designed specifically for simulated distillation analysis. A SimDis software program that couples with the Agilent GC ChemStation provides an easy-to-use solution for simulated distillation analysis. The system allows for a quick determination of the boiling point distribution and cut points of higher boiling petroleum fractions. This application note describes in detail the system designed to perform simulated distillation by ASTM method D6352, and by the proposed ASTM method for petroleum fractions boiling between 100 °C and 615 °C, commonly referred to as D2887X.

Introduction

Determining the boiling point (BP) distribution of mid- and heavy petroleum distillate fractions is essential in monitoring and controlling petroleum refining processes, such as hydrocracking, hydrotreating, visbreaking, or deasphalting. The gas chromatographic simulation of this BP determination can be used to replace difficult and costly conventional distillation methods. Also, this method can be used for product specification testing.

The American Society for Testing and Materials (ASTM) has established several methods for simulated distillation specifying various BP ranges for petroleum fractions. Among them, ASTM D3710 is used to determine the BP distribution of gasoline and gasoline fractions with a final boiling point (FBP) up to 260 °C (500 °F), and ASTM D2887 for a BP distribution between 55 °C and 538 °C 1000 °F). See reference for details on the Agilent system for D2887 [1]. For the analysis of heavier fractions, ASTM D6352 [2] is applicable for distributions with an initial boiling point (IBP) greater than 174 °C (345 °F) and a FBP of less than 700 °C (1292 °F). Lastly, another procedure, commonly known as extended D2887 or D2887X, has been proposed by ASTM for determining the BP range of 100 °C to 615 °C (1114 °F) materials. The Agilent SimDis system is designed to perform any of the aforementioned methods: D2887, D2887X, and D6352.

This application note describes the setup and performance of the Agilent SimDis system for the ASTM D6352 and D2887X methods.

Experimental

The SimDis system uses the Agilent 6890N GC with electronic pneumatic control (EPC) configured with a flame ionization detector (FID) and a metal capillary column (DBHT-SIMD, 5 m × 530-µm id × 0.15-µm film of 100% dimethylpolysiloxane). The Agilent 7683B automatic liquid injector equipped with an SGE 0.5-µL micro-syringe was used for sample introduction into a JAS UNIS 3100 high temperature air-cooled PTV (HT PTV), with appropriate dilution levels, the Agilent 5-µL syringe can also be used. Polywaxes were used to determine the BP versus retention time (RT) relationship over the BP range required by the methods. To determine the BP versus RT relationships, a qualitative mixture of approximately C10–C62 was used for D2887X, while for D6352, a mixture of approximately C10–C90 was used. The Agilent SimDis software module performed the D6352 and D2887X method calculations.

The instrument conditions listed in Table 1 for both methods discussed above are the same except for the PTV inlet program, calibration standards and oven temperature profile.

HT PTV Inlet

This HT PTV inlet, developed by Joint Analytical Systems, GmbH, (Moers, Germany) provides efficient transfer of sample to the column without discrimination. It integrates a coiled wire cartridge for heating in a carefully constructed arrangement for an optimized thermal gradient. The low mass design allows for rapid heating/cool-down rates using only the GC inlet fan for cool down. For simulated distillation applications, the inlet is operated in a split/hot injection mode. The inlet has minimal seals providing a leak-tight system essential when operating the column at temperatures above 350 °C. The Agilent 7683B series injector operating in the fast injection mode is also used with control of the inlet integrated into the ChemStation. Typically, the inlet is ramped rapidly in temperature at the time of sample introduction, held at the maximum programmed temperature for a number of minutes and then cooled back (uncontrolled) to the starting temperature, all during the first half or two-thirds of the GC runs. Sufficient "hold time" at the highest programmed inlet temperature is critical for good peak shape of the higher carbon number peaks. High program rates of 400–500 °C/min can be used for thermally stable analytes such as the hydrocarbons normally encountered in simulated distillation.

The basic SimDis analysis includes column conditioning, blank analysis, calibration, system validation sample analysis, and percent off reporting.

Calibration Mixture

Method D6352 requires a qualitative calibration mixture of n-paraffins from approximately C10 to C90 dissolved in a suitable solvent. In this application note, the calibration is obtained through two steps: first, dissolution of polywax 655 in carbon disulfide (CS_2) followed by the addition of a suitable mixture of n-paraffins from C5 to C18 (Agilent SimDis calibration mix No.2). The final concentration should be approximately one part of n-paraffin to 50 (or 100) parts of CS_2.

Table 1. Gas Chromatographic Conditions for HT Simulated Distillation

HT PTV Temperature programs	40 °C to 440 °C at 200 °C/min, hold 11 min, cool to 40 °C at 100 °C/min (D6352) 50 °C to 420 °C at 200 °C/min, hold 10 min., cool to 50 °C at 100 °C/min (2887X)
Split ratio	2:1 typical
Injection volume for calibration	0.5 µL to 2.0 µL
Injection volume for sample	0.05 µL to 0.10 µL
Column	5 m × 530 µm × 0.15 µm, DBHT-SIMD, part no. 145-1001
Column flow (He)	16 mL/min, constant flow mode
FID temperatures	400 °C
H_2 flow	40 mL/min
Air flow	450 mL/min
Make up (N_2)	45 mL/min
Oven programs	40 °C to 435 °C at 20 °C/min, hold 3 min (ASTM D6352); 40 °C to 420 °C at 20 °C/min, hold 6 min (D2887x); 45 °C to 440 °C at 15 °C/min.
Data acquisition rate	5 Hz

Table 2. Calibration Standards

Standard	Carbon range *	Solvents	Part no.
Polywax 500	C20-C72	CS_2, Toluene, p-xylene	5188-5316
Polywax 655	C20-C100	CS_2, Toluene, p-xylene	5188-5317
Low Boiling Std	C5-C18	Added to PW 500 or 655	5080-8768

* Approximate ranges for Polywaxes

Method D2887X calibration uses Polywax 500. See Table 2 for more details on the BP-RT calibration standards.

Sample Size and Preparation

Careful consideration of the HT PTV inlet and column capacity is necessary for proper sample injection volume and concentration to avoid carry-over and column overload. In this application, the injected sample size ranges from 0.05 µL to 0.1 µL. One part of sample is diluted with about 10–50 parts of CS_2. Injected volumes of the Polywax standards are higher due to the very low solubilities encountered. In this work, CS_2 was used exclusively as the solvent, however; reasonably good solubilities of the Polywaxes in toluene and p-xylene were found, making these an acceptable less toxic alternative to CS_2.

SimDis Software

The SimDis application software can operate standalone or be called to automatically execute as a post-run event from the ChemStation. The software includes the functional modules: Browse, Setup, SimDis, Report, and Automation. Each module provides specific functions to rapidly view data files, set up SimDis parameters, perform SimDis calculations, customize reporting, and track automation.

The Agilent SimDis module provides a number of functions to correct artifacts such as solvent peaks and contributions from baseline, including options for baseline treatment that optimize the SimDis analysis. Once optimized, this collection of parameters can be saved and applied to all future sample analyses.

As an example, the Setup module used for configuring the BP calibration selection (Polywax 655) and Blank (CS_2) selection files is shown in Figure 1. Each chromatogram can be moved about the window and zoomed in or out using simple (left - right button) mouse functions.

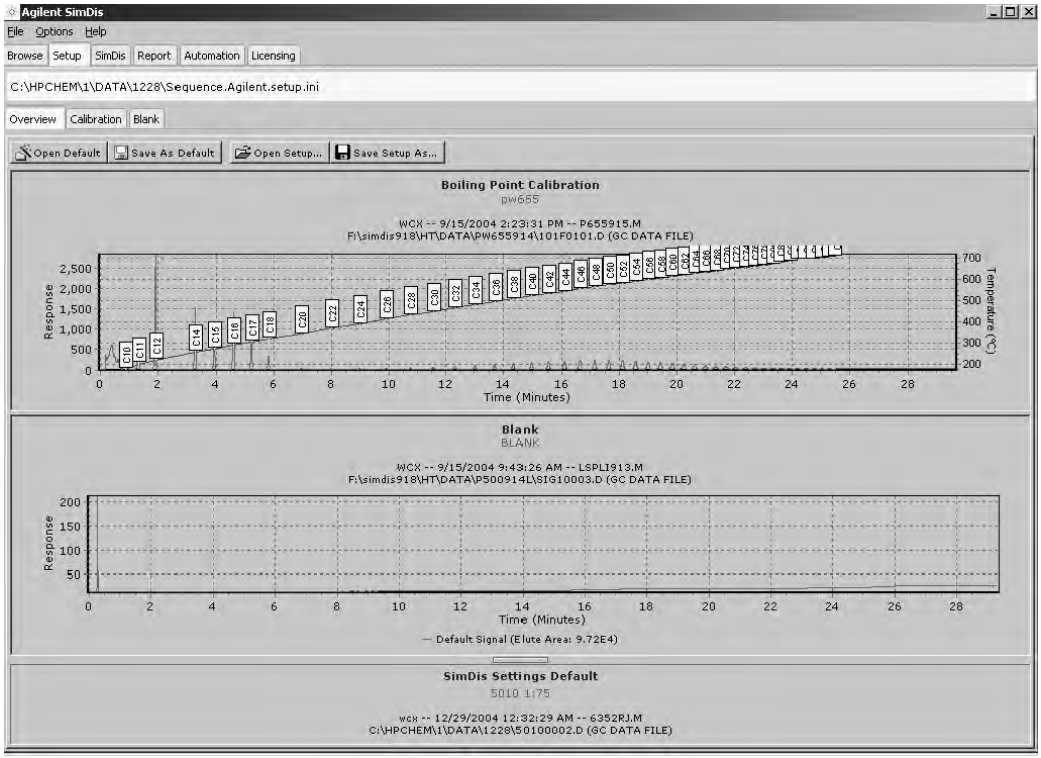

Figure 1. Examples of calibration and blank software panels after editing and selection.

The SimDis module is the core of the software where parameters dealing with the actual simulated distillation calculation are defined and set. For example, this module can set the calculation parameters used for setting the baseline, determining the start-elute (SE) and end-elute (EE) for calculations, eliminating solvent effects, and removing extraneous peaks. Typically, a reference or QA sample is run first to establish appropriate parameters. This setup is then saved (use this sample as default). Now all sequences run from the ChemStation will automatically use these parameters. Figure 2 shows an example software panel where selected parameters are displayed graphically, overlaid on the sample chromatogram. In this screen, the presence of a solvent or extraneous peak that may interfere with the accuracy of SimDis calculations can be factored out. The Sample Elution time function can correct inaccurate calculations by setting the SE time after the extraneous peak (see the marked regions). Also, icons of Elute, EE, the IBP and the FBP at the top left of the menu can be toggled to show the current calculations on the raw signal.

The Report module offers a number of ways to customize reporting of the SimDis analytical results. The dropdown report list presents available report formats and data treatment options for output such as “Calibration Report”, “Engineering Report”, “Complete Percentage Yield”, “Standard Cut Point”, and “Custom Cut Point.”

Result and Discussion

Blank Analysis

Blank analysis is performed to establish and record a baseline profile for subsequent subtraction during simulated distillation calculations. It is done using an identical method as used for sample analysis, usually with the pure solvent that is being used for sample dilution. In this application, an injection of carbon disulfide was used. The CS_2 injection volume was 0.1 µL. Blanks are typically performed prior to sample analysis, and subtracted from the sample signal to remove effects of column bleed that influence baseline offset. It can also be used to check for possible carryover resulting from previous too large injections. See Figure 6.

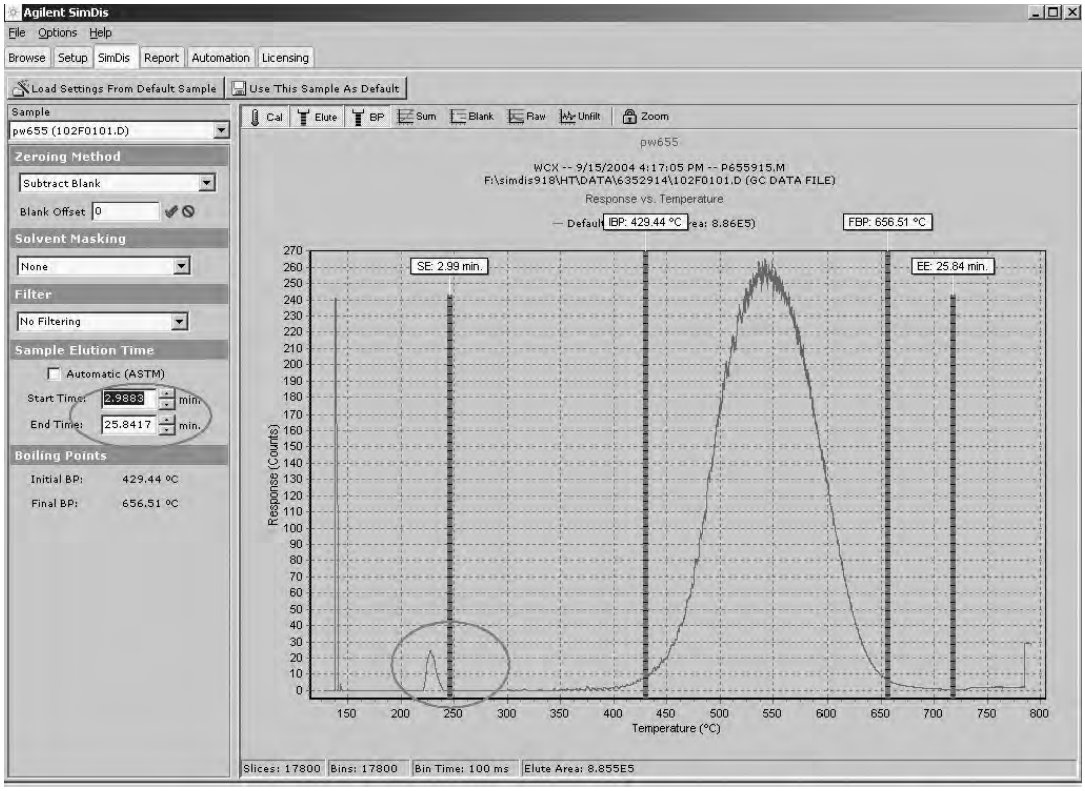

Figure 2. Parameter setup screen example from the SimDis module graphically showing SE, EE, IBP, and FBP.

Calibration

A calibration mixture containing a series of known n-alkanes was used to establish the BP-RT correlation. Polywax 655 plus C5–C18 is used for method D6352. A representative chromatogram is shown in Figure 3.

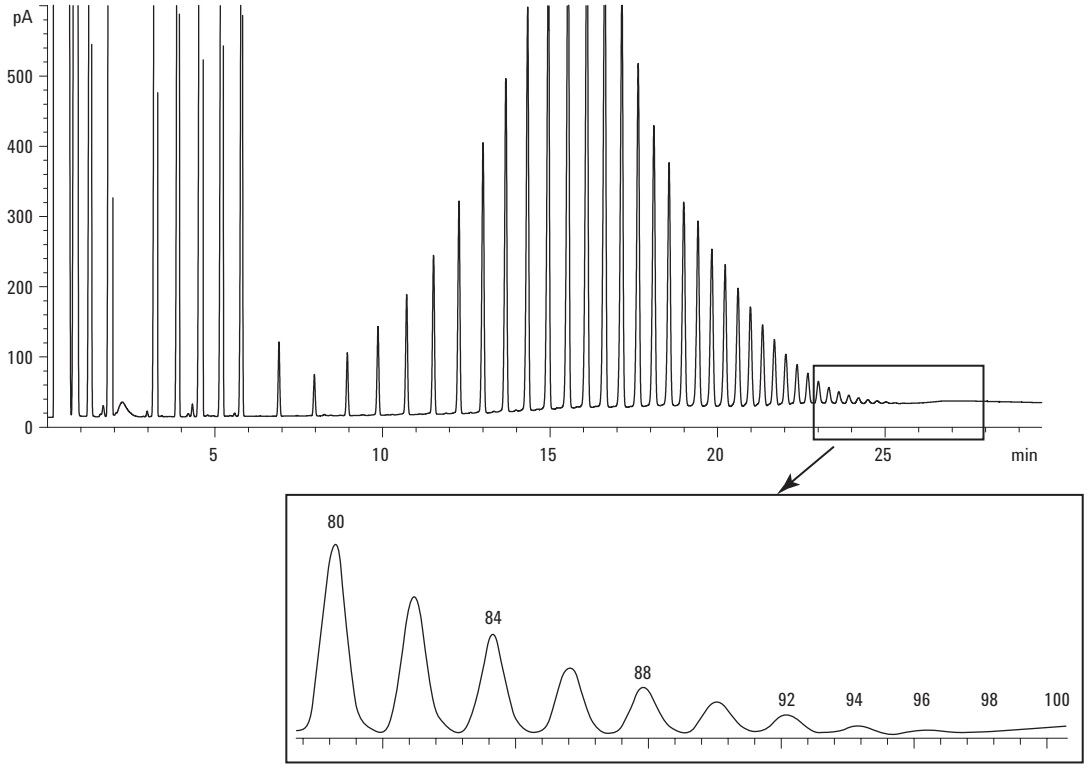

Figure 3. Chromatogram of Polywax 655 + C5–C18. Injected 0.1 µL. GC conditions given in Table 1.

Figure 4 shows the corresponding BP-RT plot. Polywax 500 plus C5–C18 was used for the calibration of method D2887X.

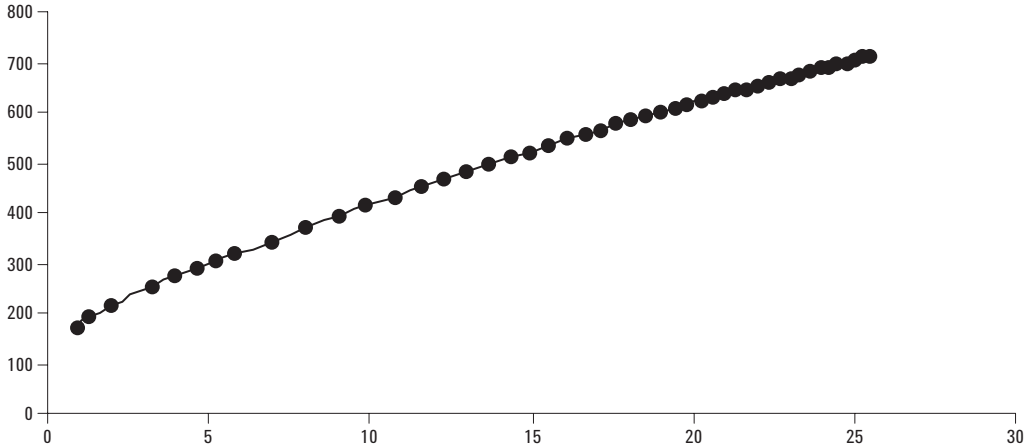

Figure 4. Plot of BP versus RT (min).

The chromatogram of PW500 shown in Figure 5 demonstrates excellent peak shape, a result of the focusing characteristics of the HT PTV inlet equipped with a specialized multirestricted liner.

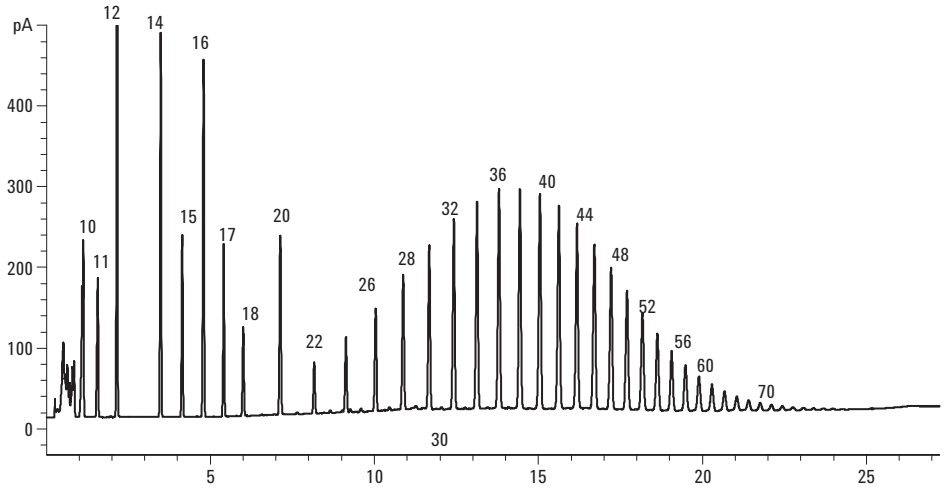

Figure 5. Chromatogram of Polywax 500 plus C5-C18 added. Injected volume 0.1 µL. GC conditions are given in Table 1.

Reference Sample Analysis

Reference sample 5010, as published in method D6352, can be analyzed to verify both chromatographic system performance and the calculation algorithms used. A chromatogram of standard 5010 is shown in Figure 6A. The chromatogram shows a return to baseline by the end of the temperature program, indicating that the sample has completely eluted from the column as required by the method. An engineering report and results are shown in Figure 6B and Table 3, respectively. The data demonstrate that the observed BP values agree with the D6352 consensus values within the allowable difference range published in the method.

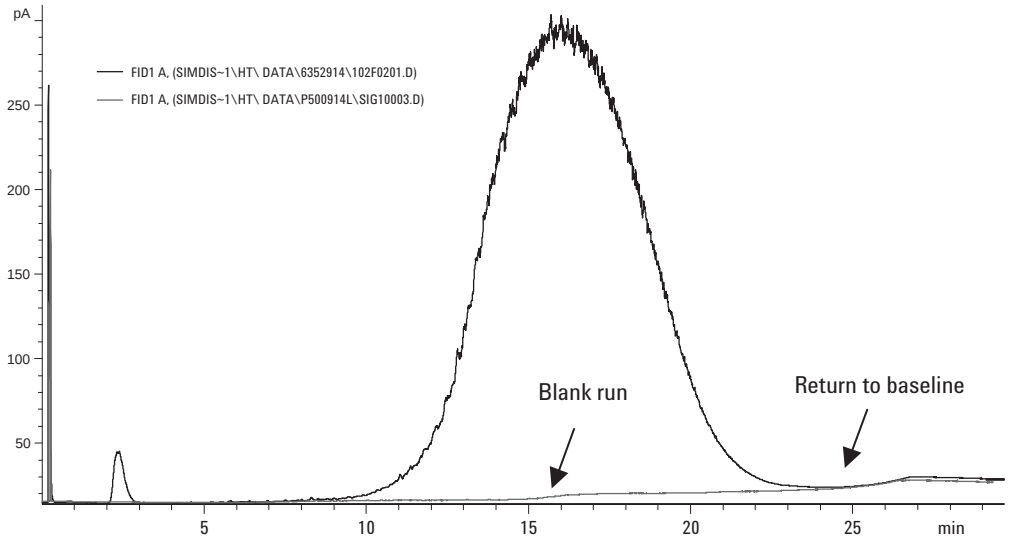

Figure 6A. Chromatographic overlay of reference sample 5010 and blank. Injected volume 0.1 µL (50:1 approximate dilution in CS_2). GC conditions given in Table 1. Polywax 655 was used for calibration.

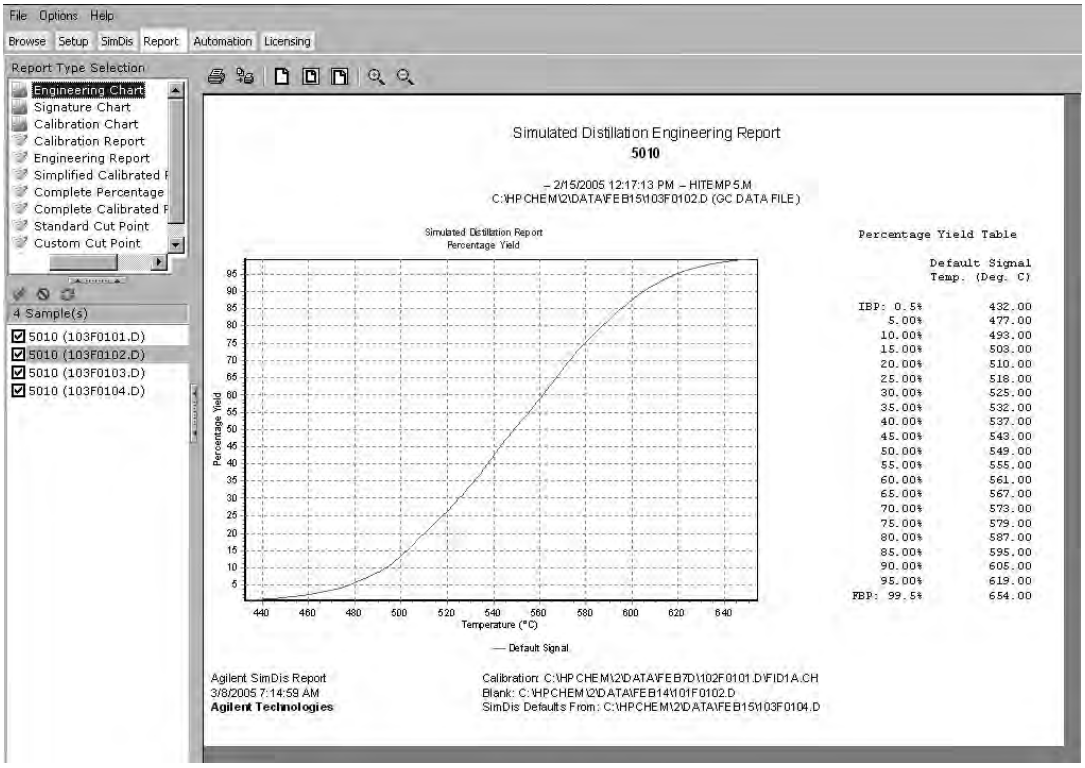

Figure 6B. Engineering report for reference sample 5010.

Table 3. Reference Sample 5010. Results in °C.

ASTM D6352 Values			Typical observed values	
OFF%	IBP	Allowable difference	FBP	Difference
IBP	428	9	429.4	–1.4
5.0	477	3	479.4	–2.4
10.0	493	3	493.3	–0.3
15.0	502	3	502.8	–0.8
20.0	510	3	510.6	–0.6
25.0	518	4	517.8	0.2
30.0	524	4	524.4	–0.4
35.0	531	4	530.6	0.4
40.0	537	4	536.1	0.9
45.0	543	4	542.2	0.8
50.0	548	5	547.8	0.2
55.0	554	4	553.9	0.1
60.0	560	4	560.0	0
65.0	566	4	566.1	–0.1
70.0	572	4	572.2	–0.2
75.0	578	5	578.3	–0.3
80.0	585	4	585.6	–0.6
85.0	593	4	593.9	–0.9
90.0	602	4	603.9	–1.9
95.0	616	4	620.0	–4.0
FBP	655	18	657.2	–2.2

Repeatability of Reference Sample 5010

Although injected volumes are very small (typically 0.05 µL to 0.1 µL), performance of the 7683B injector and HT PTV are exceptional. An overlay of eight sample runs of standard 5010 and two blanks are shown in Figure 7. Results as calculated from the SimDis module are given in Table 4.

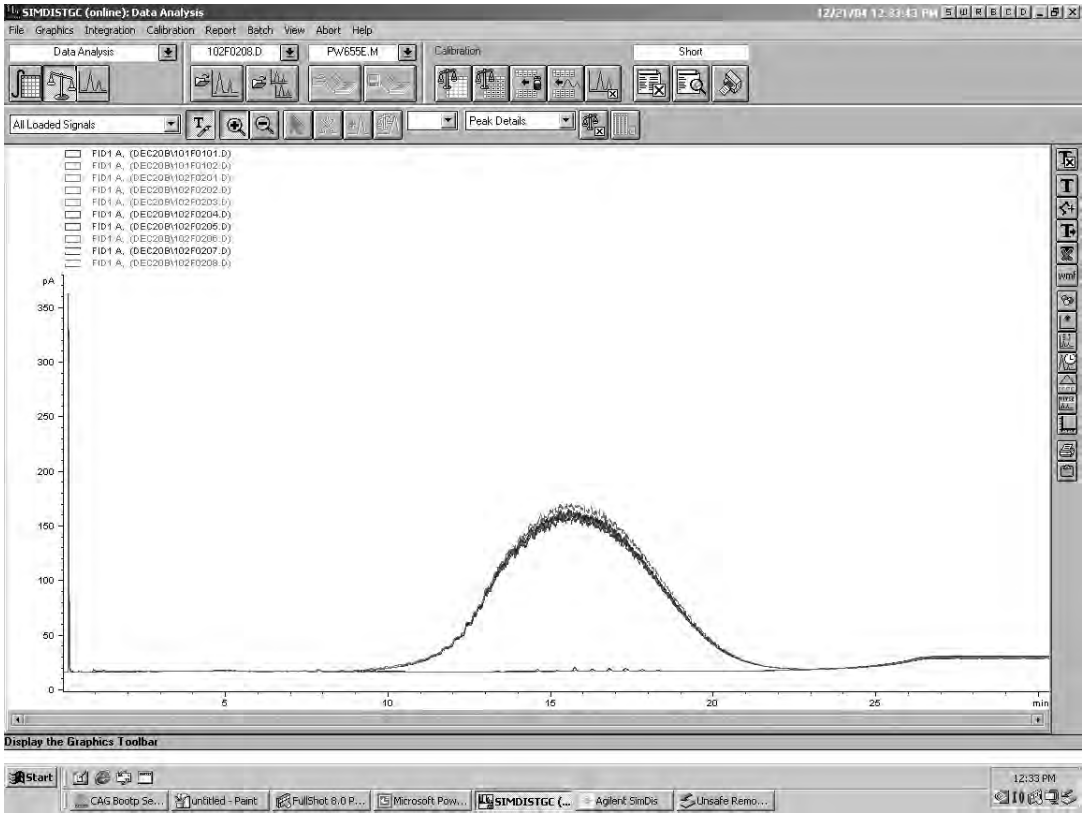

Figure 7. Eight runs of sample 5010 and two blanks are overlaid. Oven program: 45 °C (0 min) to 440 °C (4 min) at 15 °C/min.

Table 4. Values for Eight Sample Runs of D6352 Reference Material 5010 (% Off Numbers Listed)

Run	IPB	20%	40%	50%	60%	80%	FPB
1	428	508	535	546	558	583	648
2	427	508	535	546	558	584	649
3	426	509	535	547	559	584	645
4	427	508	535	546	558	584	649
5	426	508	535	546	558	584	649
6	423	507	534	546	558	584	650
7	430	509	535	546	558	583	646
8	428	508	535	546	558	583	648

Heavy Vacuum Gas Oil Analysis

Figure 8 shows the chromatographic overlay of five runs of heavy vacuum gas oil (HVGO) simulated distillation analysis. This sample falls within the requirements of the proposed method D2887X. The overlays of five chromatographic runs demonstrate that repeatable results are obtained. Table 5 lists numeric results for this HVGO sample.

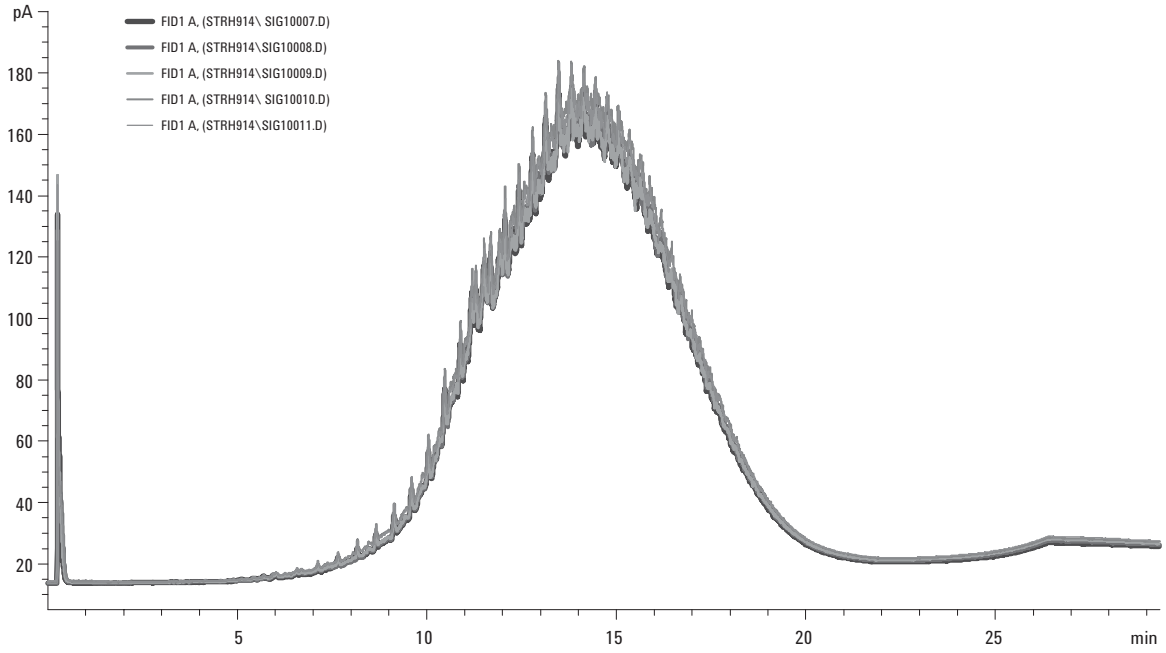

Figure 8. Chromatographic overlay of five runs of HVGO. Injected 0.1 µL (55:1 approximate dilution in CS_2). GC conditions given in Table 1.

Table 5. HVG0 Results

OFF%	Observed BP °C					Repeatability
	1	2	3	4	5	
IBP	140	141	141	141	141	1.1
5.0	282	282	282	286	284	3.9
10.0	369	369	368	371	369	3.3
20.0	433	433	432	433	432	1.1
30.0	458	459	458	458	458	0.6
40.0	478	479	478	478	478	1.1
50.0	496	496	496	495	496	1.1
60.0	511	511	511	510	511	1.1
70.0	527	528	527	527	527	1.1
80.0	546	546	545	544	545	1.7
90.0	569	571	569	568	569	2.2
95.0	587	588	586	584	586	3.9
FBP	606	607	604	601	604	6.1

Conclusions

Accurate determination of the cut point distribution of heavy petroleum fractions is essential in optimizing the efficiency of refining operations as the use of heavier feedstocks become more prevalent. The Agilent SimDis System provides an easy-to-use and efficient means to automate simulated distillation through the GC ChemStation. Analysts can quickly set up and perform simulated distillation analysis using the Agilent SimDis software module. The system supports ASTM D6352, D2887 and the proposed extended D2887 methods. The HT PTV inlet provides efficient and reproducible transfer of the high boiling calibration samples and petroleum fractions to the column without discrimination. Agilent's 5-meter metal thin-film column offers a stable HT stationary phase with long life even when programmed to 440 °C.

References

1. C. Wang and R. Firor, "Simulated Distillation System for ASTM D2887" Agilent Technologies, publication 5989-2726EN, www.agilent.com/chem.
2. ASTM D6352-02, "Standard Test Method for Boiling Range Distribution of Petroleum Distillates in Boiling Range from 174 to 700 °C by Gas Chromatography" Fractions by Gas Chromatography", Annual Book of Standards, Volume 05.02, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.

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Printed in the USA
April 27, 2005
5989-2727EN



Agilent Technologies

Fast Simulated Distillation Based on Agilent 6890N Gas Chromatograph

Application

Petroleum

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Abstract

A rapid simulated distillation method is described for analysis of petroleum fractions boiling within the range of ASTM D2887 [1]. The method employs an Agilent 6890N gas chromatograph (GC) equipped with a split/splitless inlet, flame ionization detector (FID), oven insert, and 3-meter 180-µm id capillary column. A SIMDIS software package is partially integrated into the GC ChemStation for data analysis and SimDis calculations. Compared to conventional ASTM D2887, the accelerated method yields run times at least two times faster. It yields results comparable to traditional D2887, and agrees with the guidelines of ASTM method D2887 based on published results for the reference gas oil (RGO).

Introduction

ASTM D2887 is widely used in the refining industry. The method is designed to determine the boiling range distribution of petroleum products and fractions having a final boiling point of 538 °C or lower. Agilent has developed a SimDis system including an easy-to-use software package. For details see reference [2]. High-throughput labs can benefit from the faster cycle times possible when short, narrow-bore capillary columns are used. This application employs 180-µm id columns as a good compromise between speed and ease of use. Faster D2887 runs (greater than 2X speed gain) are certainly possible, however, high oven ramp rates, available only with a 220 V GC and/or 100-µm id columns, are needed. For example, using the 220 V GC with oven insert will allow oven ramps of up to 65 °C/min when programmed from 30 °C to 450 °C. This configuration is recommended if faster analyses are required. Use the Agilent method translation software available as a download from www.chem.agilent.com to investigate other column/oven ramp rate scenarios.

Experimental

Experiments were performed on an Agilent 6890N GC (120 V version) with electronic pneumatics control configured with a split/splitless inlet, 7683 automatic liquid injector, FID, and a 3 m × 180 µm × 0.4 µm DB-1 capillary column. An oven insert (Agilent part/number G2646-60500) was used for fast chromatography in order to reduce the oven volume so that the column and sample are heated more quickly and yield faster separation and chromatography. Furthermore, the smaller volume oven cools faster than a full-sized one, thus reducing the overall analytical cycle time. A qualitative mixture of normal paraffins, C5 to C40, is used to

determine the boiling point (BP) versus retention time (RT) relationship over the range required by method D2887. In this application it is critical to choose the appropriate liner for the split/splitless inlet. A single taper liner (Agilent part/number 5183-4647) with glass wool positioned to "wipe" the syringe needle is required. The column is prepared by measuring and cutting a 3-meter piece of a 10-meter column (Agilent part/number 121-1013).

General instrument conditions used for this method are listed in Table 1.

Table 1. Gas Chromatographic Conditions

Inlet temperature	325 °C
Split ratio	50:1
Injection volume	0.1 µL
Column	DB-1, 3 m × 180 µm × 0.4 µm
Column flow program (He)	1.5 mL/min, hold 0.5 min, 80 °C /min, 5 mL/min, hold 8 min
FID temperature	350 °C
H_2 flow	40 mL/min
Air flow	450 mL/min
Make up (N_2)	45 mL/min
Oven program	35 °C to 350 °C at 30 °C/min, hold 0.5 min
Data acquisition rate	5 Hz

The process of SimDis analysis includes blank analysis, calibration (C5–C40), validation (reference gas oil No.1 for ASTM D2887) and sample analysis. The analysis process can be automated through the GC ChemStation coupled to Agilent SIMDIS software (G2887-90020).

SIMDIS Software

The SIMDIS application software can be operated in a stand-alone mode for post-run processing or called automatically to execute as a post-run event from on-line ChemStation. Its function is described in greater detail in other application notes. For details see references [2] and [3].

Result and Discussion

Calibration

Calibration mixtures containing a series of known n-alkanes from C5 to C40 are used to establish the BP-RT correlation. The 3-meter column provides a fast separation of hydrocarbons while maintaining good separation between C5 and C6 alkanes. The Agilent 6890 GC (120 V) configured with oven insert for fast temperature programming allows an oven ramp rate of 30 °C/min over the temperature range 35 °C to 350 °C, resulting in a twofold speed gain over a conventional D2887 analysis. An oven ramp rate of 30 °C/min is approximately the maximum possible using a 120 V 6890 with oven insert over the temperature range required for D2887.

Figures 1 and 2 compare the calibration standard analyzed using the fast SIMDIS method and the conventional D2887 method respectively. Conventional D2887 is performed on the 6890 GC configured with a 10 m × 530 µm × 2.65 µm DB-1 column [2].

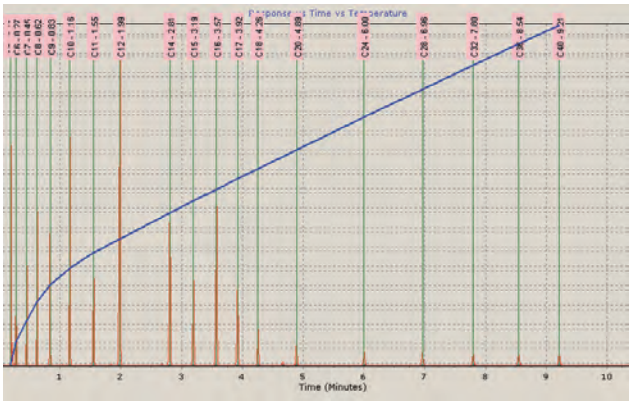

Figure 1. Calibration standard analysis using the fast SimDis method, showing C5–C40 peak assignments. Column: DB-1, 3 m × 180 µm × 0.4 µm. Oven program: 35 °C to 350 °C at 30 °C/min, and hold 0.5 min.

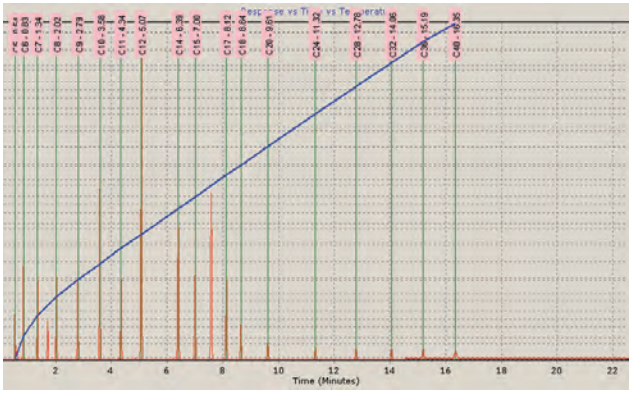

Figure 2. Calibration standard analysis using conventional D2887 method, showing C5–C40 peak assignments. Column: DB-1, 10 m × 530 µm × 2.65 µm. Oven program: 40 °C to 350 °C at 20 °C/min, and hold 8 min.

RGO Analysis

Figure 3 shows a chromatographic overlay of 20 consecutive injections of RGO. Run-to-run deviations over the entire chromatographic time range are small, indicating good system performance. Statistics for these runs are shown in Table 2.

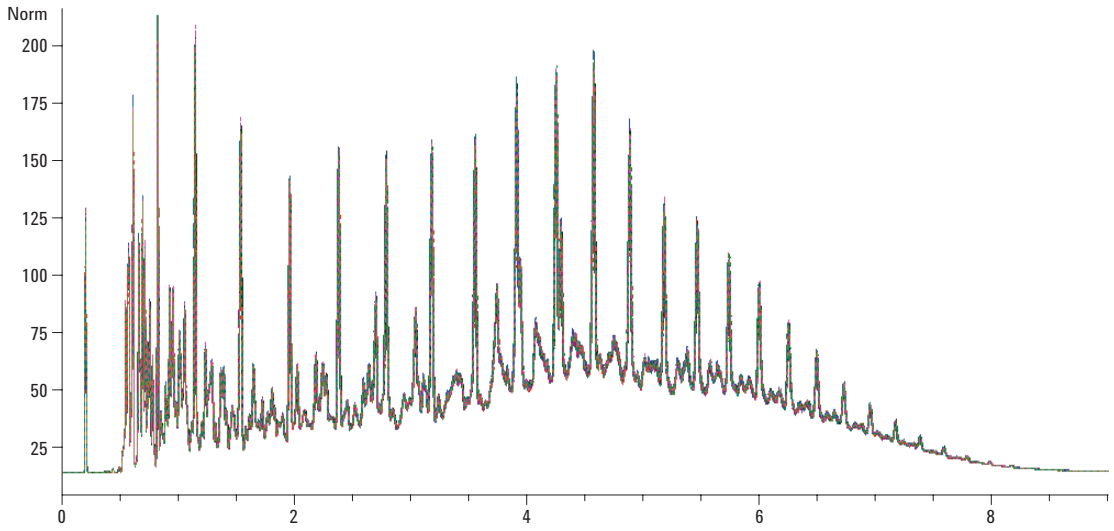

Figure 3. Chromatographic overlay of 20-run RGO fast analysis. Column: DB-1, 3 m × 180 µm × 0.4 µm Oven program: 35 °C to 350 °C at 30 °C/min, hold 0.5 min.

Table 2. Statistics for 20 Consecutive Runs of RGO

% OFF	Average	STDEV	% RSD
IPB: 0.5	118.0	0.00	0.00
5	150.0	0.00	0.00
10	174.0	0.00	0.00
15	200.8	0.37	0.18
20	224.3	0.57	0.25
25	243.0	0.51	0.21
30	258.6	0.50	0.19
35	272.6	0.50	0.18
40	287.4	0.50	0.17
45	301.6	0.49	0.16
50	310.1	0.31	0.10
55	318.0	0.00	0.00
60	329.1	0.37	0.11
65	339.5	0.51	0.15
70	350.6	0.49	0.14
75	362.6	0.50	0.14
80	374.9	0.31	0.08
85	388.3	0.49	0.13
90	403.0	0.39	0.10
95	424.0	0.60	0.14
FBP: 99.5	466.1	1.02	0.22

Table 3 shows results for the 20 RGO analyses. The data demonstrate that observed BP values agree with the ASTM consensus BP values within the allowable difference range.

Table 3. Results for RGO Fast SimDis. Rsults in °C

ASTM D2887 values			Observed values	
OFF%	BP	Allowable difference	BP (Average n = 20)	Difference
IBP	115	7.6	118.0	–3
10%	176	4.1	174.0	+2
20%	224	4.9	224.3	–0.3
30%	259	4.7	258.6	+0.4
40%	289	4.3	287.4	+1.6
50%	312	4.3	310.1	+1.9
60%	332	4.3	329.2	+2.8
70%	354	4.3	350.7	+3.3
80%	378	4.3	374.9	+3.1
90%	407	4.3	403.1	+3.9
FBP	475	11.8	466.1	+8.9

Table 4 shows a comparison of RGO fast analysis to conventional D2887 analysis. The results indicate good consistency between the two methods.

Table 4. Comparison of RGO Fast SimDis to Conventional D2887. Results in °C

	ASTM D2887 values	Fast	D2887
OFF%	BP	(Ave BP, n=10)	(Ave BP, n=10)
IBP	115	118.0	113.3
10	176	174.0	177.8
20	224	224.0	227.2
30	259	258.3	262.8
40	289	287.2	292.2
50	312	310.0	314.4
60	332	329.1	332.8
70	354	350.5	355.6
80	378	374.9	379.4
90	407	403.2	409.4
FBP	475	466.5	466.7

Cat Cracker Feed Analysis

Table 5 lists the results for 10 consecutive runs of cat cracker feed. The results show excellent repeatability.

Table 5. Repeatability of Cat Cracker Feed Analysis. Results in °C

OFF%	1	2	3	4	5	6	7	8	9	10	Average	RSD%
IBP	196	196	196	196	196	197	197	197	197	200	196.8	0.27
10	299	299	299	299	300	300	300	300	300	301	299.7	0.18
20	333	333	334	333	334	334	334	334	334	335	333.8	0.15
30	358	358	359	358	359	359	359	359	359	360	358.8	0.14
40	379	380	380	379	379	380	380	380	380	380	379.7	0.13
50	399	399	399	399	399	399	399	399	399	400	399.1	0
60	417	418	418	417	418	418	418	418	418	418	417.8	0.11
70	437	437	437	437	437	437	437	437	437	437	437.0	0
80	459	460	460	460	460	460	460	460	460	460	459.9	0.07
90	493	493	494	493	493	494	494	493	494	494	493.5	0.11
FBP	566	568	568	569	568	568	569	568	568	568	568.0	0.15

Conclusions

A 10-min simulated distillation method was demonstrated on the Agilent SimDis system. The fast method is suitable for petroleum fractions in the range covered by ASTM D2887. The procedure using a short, small-diameter capillary column, 3 m × 180 µm × 0.4 µm, achieves a two-fold speed gain over a conventional D2887 analysis while maintaining the same ease-of-use associated with 530-µm id columns. The results obtained from the fast SimDis method are comparable to that from D2887, and agree with the ASTM D2887 consensus BP values within the allowable percent off windows. The method can easily be deployed in routine labs where high throughput is required. Additional time savings can be achieved using the 220 V 6890 GC with oven insert. This configuration allows oven ramp rates up to 65 °C/min (35°C to 450°C).

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Printed in the USA
October 13, 2005
5989-4073EN



Agilent Technologies

Evaluation of Total Petroleum Hydrocarbon in Soil Using LC with Fraction Collector and GC/MS

Application

Environmental

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Abstract

Normal-phase liquid chromatography (NPLC) and capillary gas chromatography with mass spectrometry are employed to evaluate the total petroleum hydrocarbon (TPH) in the soil contaminated by crude oil. In this paper, paraffins and mono-aromatic and multi-aromatic compounds present in the sample were first separated by NPLC into different classes of compounds according to their individual polarities, and fractions were collected for subsequent analysis by GC/MS, separated by boiling point, and identified by their unique mass spectra.

Introduction

Pollution due to oil spills happens frequently all over the world. Positive identification of the source is a critical part of establishing liability for cleanup costs and environmental damages. Because the spill is subject to time-based alteration by weathering (dissolution or evaporation), chemical degradation (effects of sunlight, heat, air, and soil chemistry), and biological alteration (impact of microorganisms) it has become more and more important to map these effects. Scientists have developed diverse technologies to perform the comprehensive evaluation analysis of TPH in environmental matrices. DIN 38 409 H18 [1] is the official method using infrared spectrometry. Robert [2] introduced a comprehensive two-dimensional gas chromatography to track the weathering of an oil spill. A portable GC/MS method was presented to determine the concentration of TPH from unresolved signals in short test runs in the field.

Sjaak [3] introduced group-type characterization of mineral oil samples by two-dimensional comprehensively coupled LC x GC-ToF MS. The interface between LC and GC/MS consisted of a 100-µL syringe, with two side entrances/exits in the upper part of the barrel, installed in an injection robot. A stop-flow mode of LC was adopted during the GC/MS analysis.

In this paper, we employed a fraction collector to replace the complex interface between HPLC and GC/MS and applied a combination of NPLC and GC/MS to evaluate the TPH in the soil contaminated by crude oil.

Experimental

Instrumentation and Conditions

Agilent 1200 Series LC, consisting of:

G1379B	Micro vacuum degasser
G1312B	Binary pump SL
G1367C	High-performance autosampler SL
G1316B	Thermostatted column compartment SL with 6- or 10-port 2-position switching valve
G1315C	UV/VIS diode array detector SL
G1364C	Fraction collector (analytical scale)

ChemStation 32-bit version B.02.01-SR1

Agilent 6890GC with 5975B MSD, consisting of:

G1540N	6890N network GC system with options: 201 MSD interface
G3243A	5975B inert MSD/DS perf turbo EI bundle
G3397A	Ion gauge/controller for use with 5975 MSD
G2913A	7683B autoinjector module
G2614A	7683 autosampler tray module

MSD Chemstation version D.03.00 with NIST 05 MS Library version 2.0d

The LC and GC/MS operating conditions are listed in Table 1.

Sample Preparation

The crude oil sample was from the Daqing, China, oil field and contributed by Sinopec Shanghai Gaoqiao Petrochemical Corporation.

The sample was prepared by mixing a 1 g oil sample with a blank soil sample and depositing the mixture in a fume hood for 2 days. Next, 50 mL of hexanes was added and the sample was extracted in an ultrasonic water bath for 1 hour. The extract was filtered, and 10 mL of filtrate was pipetted and then evaporated under a nitrogen stream to less than 1 mL. The extract was then made up with hexanes to 1 mL, and the solution was injected into NPLC for analysis.

Operation of Column Switching Valve and Fraction Collector of NPLC

The crude oil sample was so complex that a column switching valve was employed to backflush the analysis column in the NPLC system. To approximately evaluate the retention time of every group of compounds, a system calibration standard was used, which was composed of cyclohexane, o-xylene, dibenzothiophene and 9-methylanthracene, as generally outlined in ASTM Methods D6379 and D6591. The separation of the system calibration standard is shown in Figure 1. To minimize the total analysis time, the LC eluate of the first 3 min was sent to waste. Afterwards, fractions were collected every 0.5 min by the fraction collector. After collecting the fractions that contained the compounds of interest, the column was switched to backflushing mode for cleaning and the LC run was closed after the baseline stabilized.

Results and Discussion

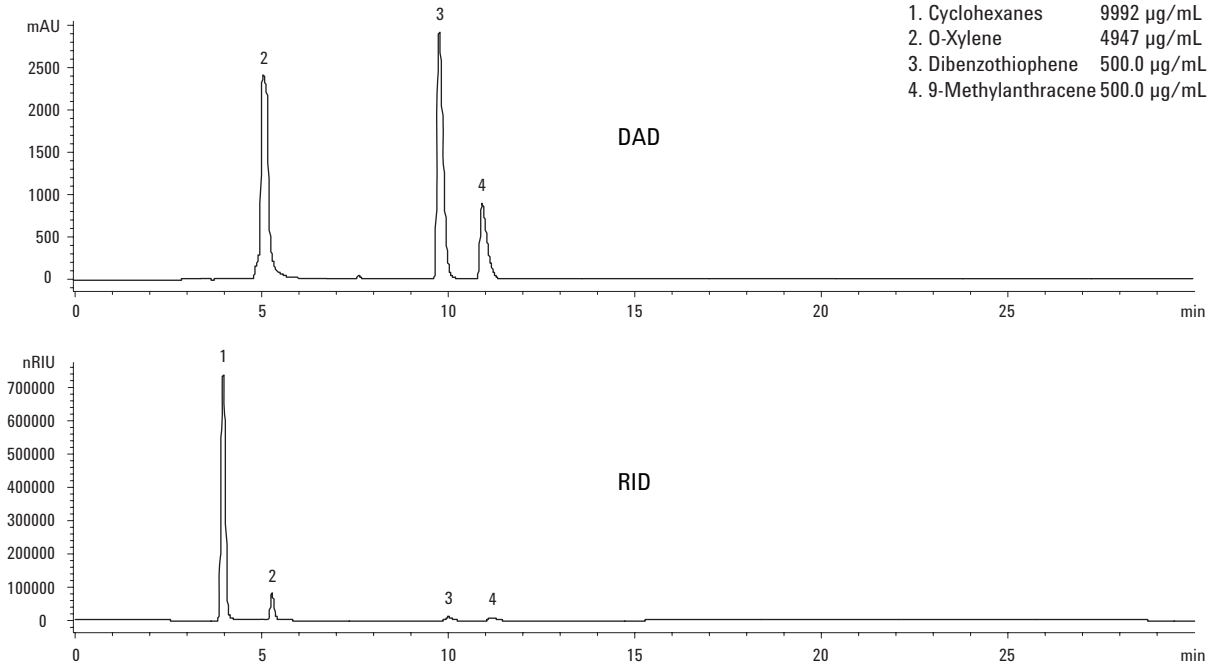
The soil sample extract was separated into different groups by normal phase liquid chromatography according to their polarities, as displayed in Figure 2. A total of 23 fractions were collected, which were injected into the GC/MS system for subsequent separation according to their boiling point and identification according to their characteristic mass fragments. A total ion chromatogram (TIC) of typical paraffins and mono-aromatic, bi-aromatic, and tri-aromatic compounds is depicted, respectively, in Figure 3. Through the identification by mass spectra, the first group with a retention time range of 3.7 to 4.7 min in LC chromatography contained paraffins; the second group, with a retention time range of 4.7 to 6.2 min, contained mono-aromatic compounds; the third group, with a retention time range of 6.2 to 11.2 min, contained bi-aromatic compounds; and the fourth group, with a retention time range of 11.2 to 13.7 min, contained tri-aromatic compounds. No aromatic compounds eluted at the retention time range from 13.7 min to the end.

Table 1. LC and GC/MS Operating Conditions

LC	**Agilent Technologies 1200SL**		
Mobile phase	Hexanes		
Flow rate	0.8 mL/min		
Wavelength	210 nm		
Injection volume	100 µL		
Mode	Isocratic		
Column	Agilent ZORBAX NH_2		
	4.6 mm x 250 mm, 5 µm		
Analysis time	30 min		
Column temperature	35 °C		
Column switching valve	Backflushing off		
Column switching timetable	Time	Column	
	15.00 min	Backflushing on	
	30.00 min	Backflushing off	
Fraction trigger mode	Use timetable		
Fraction collector timetable	Time	Trigger mode	Time slices
	3.70 min	Time-based	0.5 min
	15.00 min	Off	–

GC	**Agilent Technologies 6890N**
	7683 autoinjector and tray
Autoinjector	
Sample washes	3
Sample pumps	6
Injection volume	1 µL
Syringe size	5 µL
Preinjection solvent A	0
Preinjection solvent B	3
Post-injection solvent A	0
Post-injection solvent B	3
Viscosity delay	0 s
Plunger speed	Fast
Preinjection dwell	0 min
Post-injection dwell	0 min
Sampling depth	Disable

Inlet	**EPC**
Injection type	Splitless
Inlet temperature	250 °C
Pressure	7.61 psi
Purge flow	50.0 mL/min
Purge time	0.75 min
Total flow	54.0 mL/min
Gas saver	On
Saver flow	20.0 mL/min
Saver time	2.00 min
Gas type	Helium
Oven	
Initial temperature	50 °C
Initial time	1.00 min
Ramp rate	30.00 °C/min
Final temperature	300 °C
Final hold	2.00 min
Total run time	11.33 min
Equilibration time	0.5 min
Column	
Type	HP 5-ms
Length	30 m
Diameter	0.25 mm
Film thickness	0.25 µm
Mode	Constant flow
Initial flow	1.0 mL/min
MSD	**Agilent Technologies 5975B inert**
Solvent delay	4 min
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45.0 amu
High mass	450.0 amu
Threshold	150
Sampling	2
Scans	3.54
Quad temperature	150 °C
Source temperature	230 °C
Transfer line temperature	280 °C



Mobile phase	Hexanes
Flow rate	0.8 mL/min
Detection	DAD and RID
UV wavelength	210 nm
Analysis time	30 min (backflush @ 15 min)
Column	Agilent ZORBAX NH_2 4.6 x 250 mm, 5 µm
Method	Follow ASTM D6379 (or D6591)
Sample	System Calibration Standard
Sample size	10 µL
Column temperature	35 °C

Figure 1. Chromatogram of standard solution.

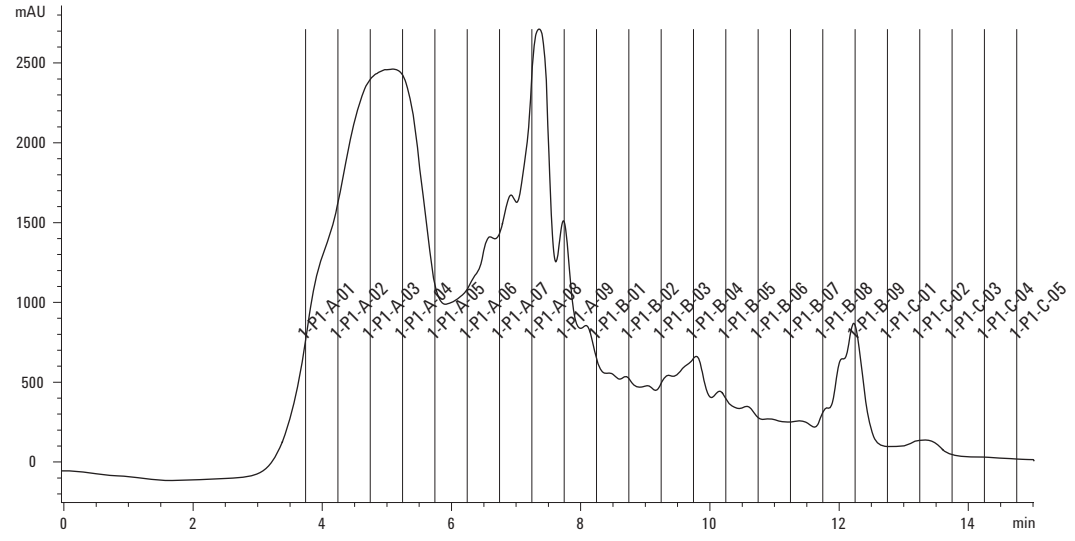


Figure 2. Chromatogram of soil sample extract and factions collected in different vials.

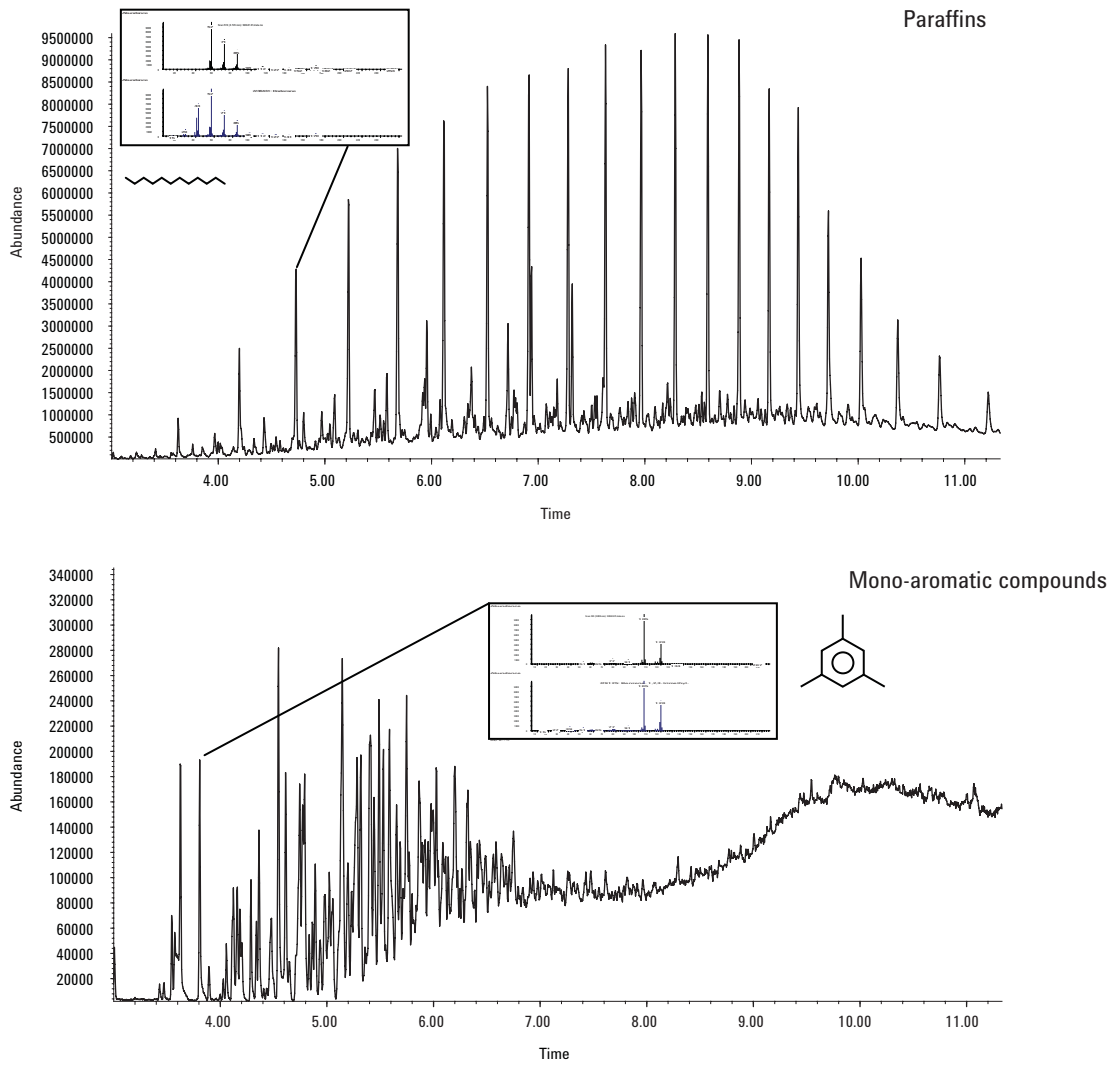


Figure 3. Total ion chromatogram of typical fractions including paraffins and mono-, bi-, and tri-aromatic compounds.

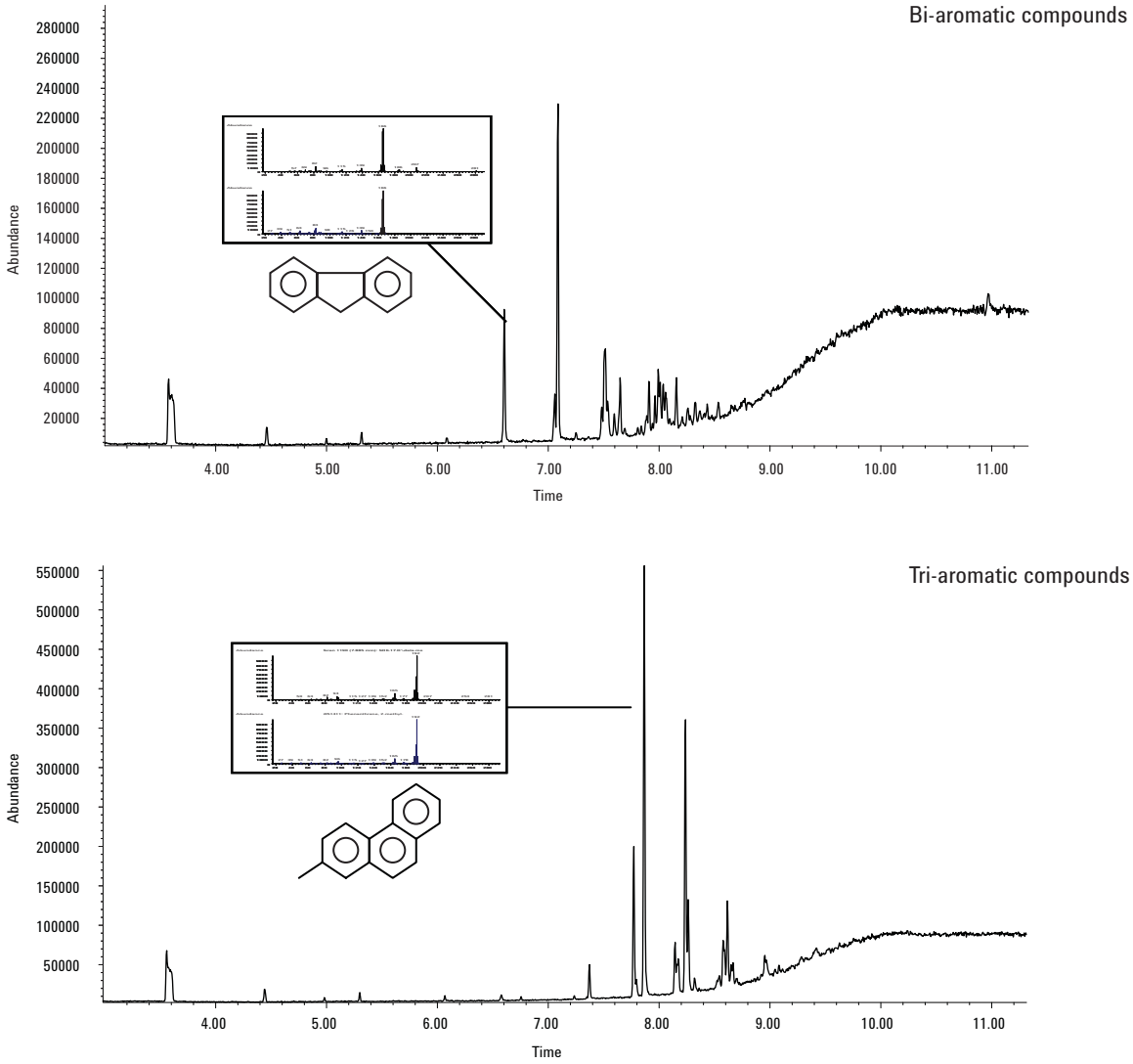


Figure 3. Total ion chromatogram of typical fractions including paraffins and mono-, bi-, and tri-aromatic compounds. (continued)

	Vial Position	Time Slices
Paraffin	1-P1-A-01	3.7 to 4.2 min
Mono-aromatic compounds	1-P1-A-04	5.2 to 5.7 min
Bi-aromatic compounds	1-P1-B-04	9.7 to 10.2 min
Tri-aromatic compounds	1-P1-B-08	11.7 to 12.2 min

Conclusions

The separations by NPLC and GC are based on polarity and boiling point, respectively. Mass spectra could provide the information on the molecular structure; therefore, the combination of NPLC and GC/MS could be used to evaluate the complex matrix. In this work, an LC with a fraction collector performed the separation of classes of paraffins and mono-, bi-, and tri-aromatic compounds and collected time-based fractions into individual sample vials. The fractions were injected into the GC/MS for identification. A soil sample contaminated by crude oil was analyzed by this method and the results showed the detailed component information of every typical class, based on fractionation by polarity, to evaluate the total petroleum hydrocarbon in soil.

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Printed in the USA
April 9, 2007
5989-6012EN



Agilent Technologies

Improving Productivity and Extending Column Life with Backflush

Application Brief

Chin-Kai Meng

All Industries

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, μECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

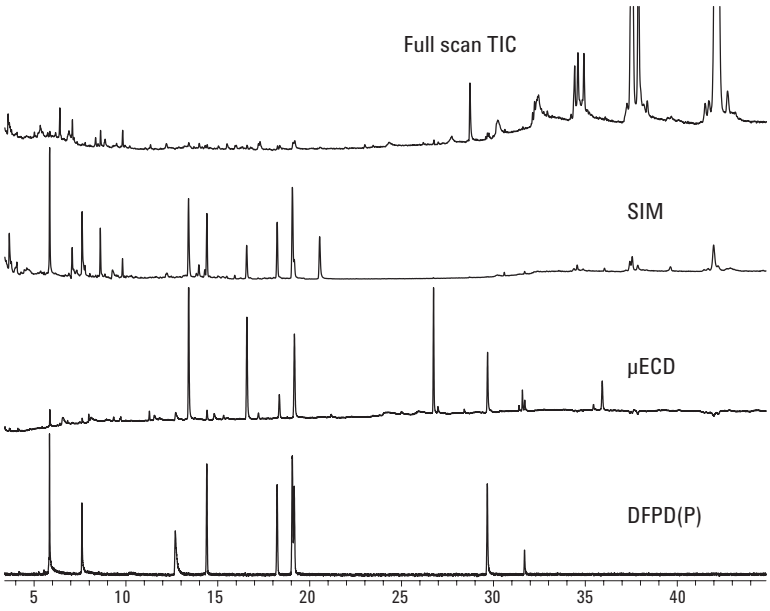

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

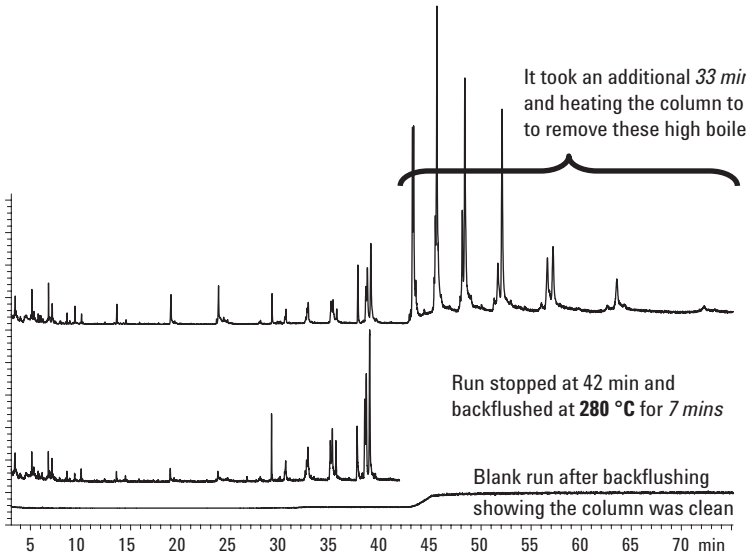

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

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Acknowledgement

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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December 26, 2006
5989-6018EN

Comprehensive Flow Modulated Two-Dimensional Gas Chromatography System

Application Brief

Roger L. Firor

Two-dimensional GC (GC x GC), a relatively new gas chromatographic technique, is being recognized for its powerful separation capabilities for the analysis of complex mixtures. The methodology involves the use of two capillary columns of usually very different polarities installed in series. Between the two columns a device known simply as a flow modulator is installed and interfaced to an auxillary programmable control module (PCM) on an Agilent 7890A gas chromatograph through a three-way solenoid valve. In the flow modulator, analyte bands from the first column are collected in a fixed volume channel and successively injected very quickly into the short second column in very narrow bands.[1] Any separation that occurs on the first column is preserved during transfer to the second column. In summary, GC x GC can greatly increase peak resolution and peak capacity.

The unique flow modulator used in this system is based on Agilent's Capillary Flow Technology hardware and does not require the use of cryogenics for focusing. The modulator consists of a planar structure where flow splitters and collector channel are all incorporated internally to the device. All external connections are made through Agilent CPM fittings (ultimate union technology) incorporated into the plate for zero unswept volumes and leak proof seals. A three-way solenoid valve is installed on top of the GC oven and is interfaced to a PCM module. Experimental conditions used are shown below.

Experimental

GC:	Agilent 7890A, FID at 200 Hz data collection rate, split/splitless inlet
Carrier:	Hydrogen
Column 1:	30 m x 0.25 mm x 0.25 µm HP-5ms, 19091S-433
Column 1 Pressure:	21.5 psig at 50 °C, constant flow mode
Column 2:	5 m x 0.25 mm x 0.15 µm HP-INNOWax
Column 2 Flow:	20 mL/min, constant flow mode
Oven Program:	50 °C (1.0 min) to 260 °C (4 min) @ 8 °C/min.
Modulator Period:	1.4 seconds collect, 0.12 seconds flush typical

GC x GC analysis software: GC Image[2]

An illustration of the modulator is given in Figure 1. The precisely timed and synchronized periodic switching between collect and flush states directs sample pulses continuously to the second column for additional separation during the

Highlights

- Flow modulation offers a viable alternative to thermal modulation without the burdens imposed by cryo requirements for comprehensive GC x GC.
- Modulation, timing (collect and inject), and synchronization are all integrated into the 7890A GC system for easy setup and operation.
- Agilent's fifth-generation electronic pneumatic control with setting to three decimal points combined with Capillary Flow Technology hardware forms the basis for an easy-to-use GC x GC system.

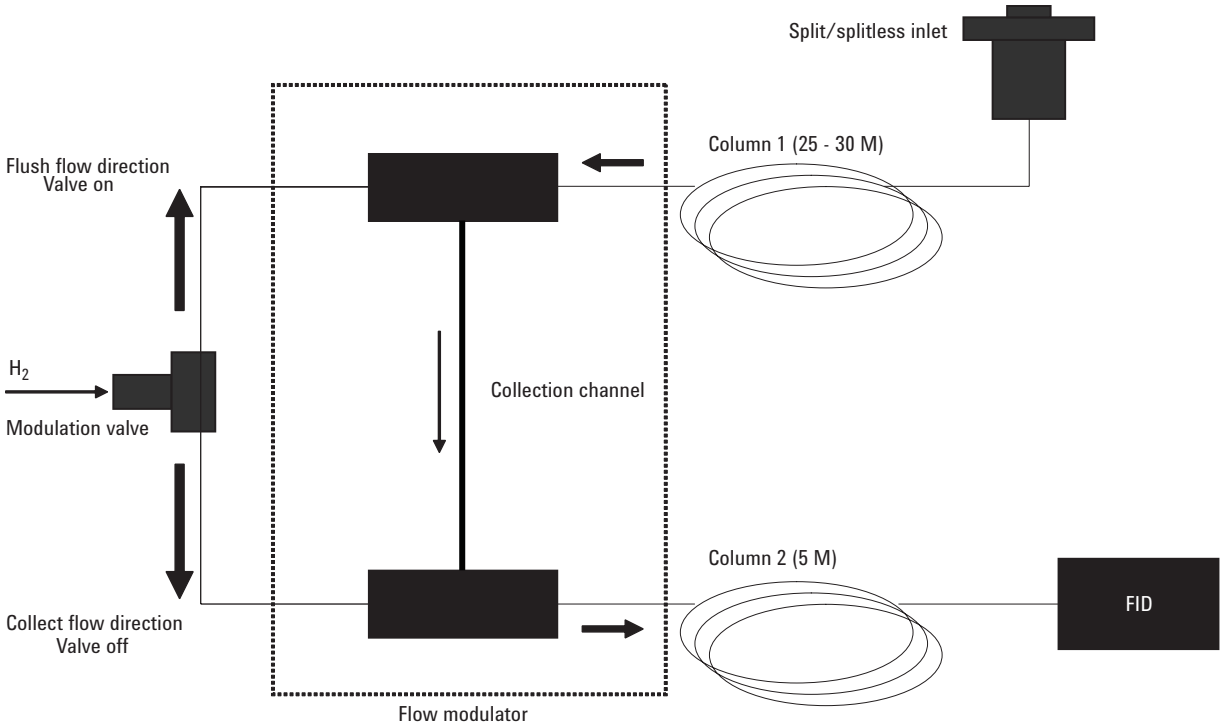

Figure 1. Flow modulator design – differential flow system.

length of the chromatographic run.

Discussion

Figures 2a and 2b show unmodulated and modulated peaks, respectively, of a pure analyte. In this example, n-butylbenzene is shown with approximately three modulations across the first-column peak. The areas of the modulated peaks should ideally equal the area of the un-modulated peak. In other words, no material should be lost in the transfer to the second column. Area agreement was within 3% for this test. Peak widths at half height for modulated butylbenzene are approximately 100 ms. Very narrow peaks as required for the technique are

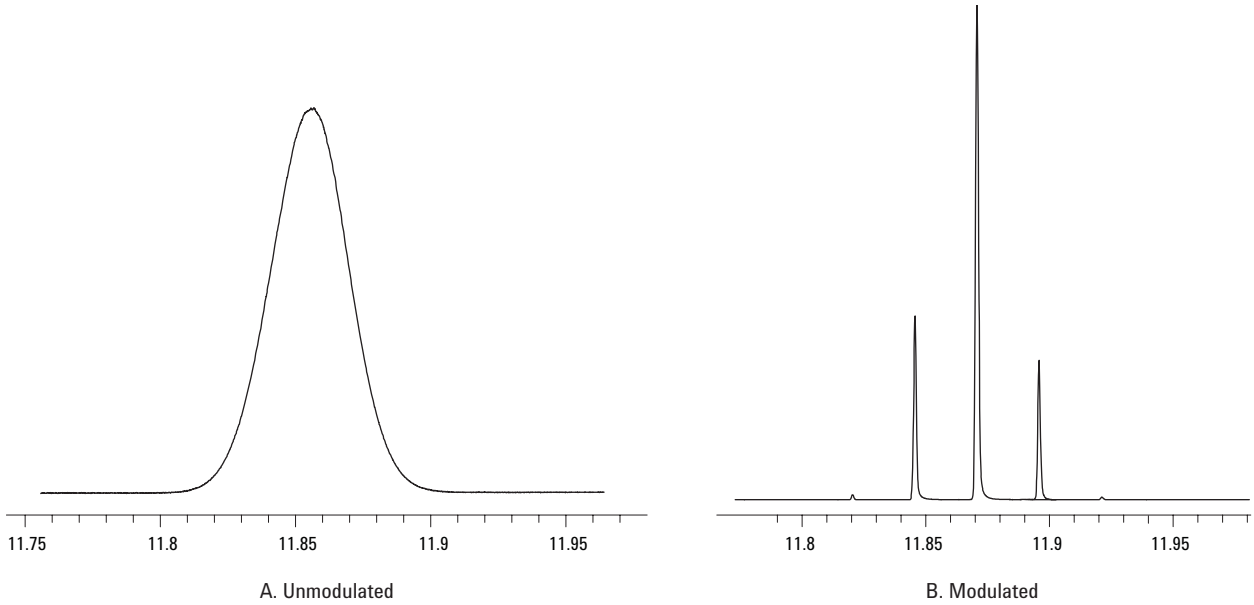

Figure 2. Modulated and unmodulated n-butylbenzene (not to same scale).

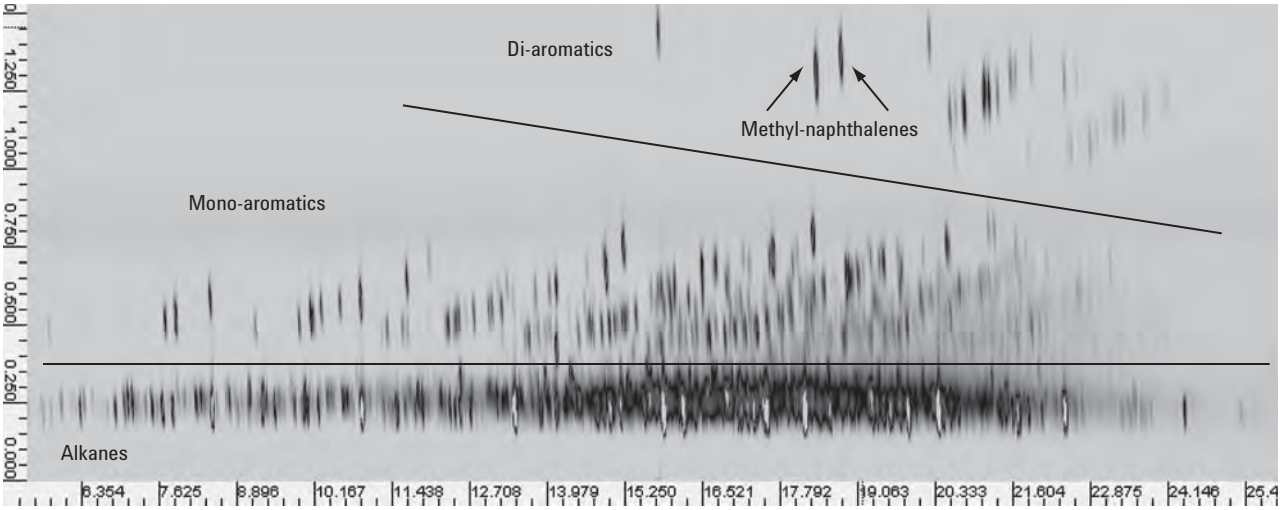

Figure 3. A 2D image of No. 2 kerosene.

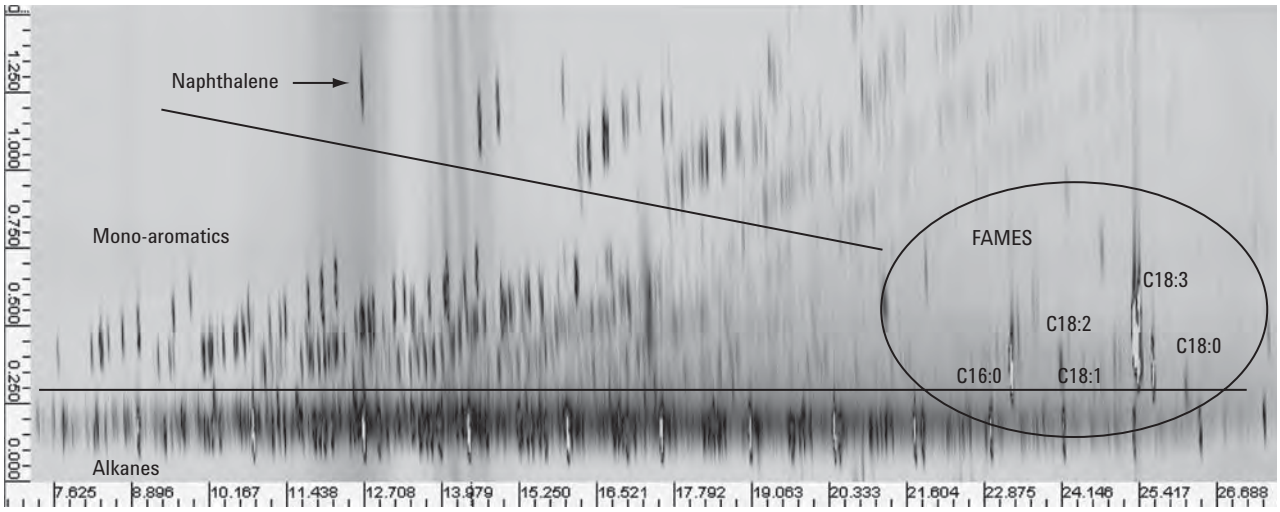

Figure 4. A B20 soy-based biodiesel (20% methyl ester, 80% diesel).

seen that approach the peak widths obtained by thermal modulation systems. Flow modulation has the distinct advantage of not requiring cryo fluid for focusing.

Use of a nonpolar column followed by a polar column produces hydrocarbon-type retention in the following order: alkanes, cyclic alkanes, olefins, single-ring aromatics, and multi-ring aromatics. An example of a 2D image of No. 2 kerosene is shown in Figure 3. Chemical classes are clearly discernable, with good resolution seen for the aromatics. Another example, B20 soy-based biodiesel (20% methyl ester, 80% diesel) is shown in Figure 4. Here the C16 and C18 fatty acid methyl esters are indicated. Data processing for all samples was performed using GC Image[2].

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Printed in the USA
January 17, 2007
5989-6078EN

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Handbook of Hyphenated ICP-MS Applications

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Introduction

Hyphenated techniques involving ICP-MS are among the fastest growing research and application areas in atomic spectroscopy. This is because, by itself, ICP-MS does not give information on the chemical or structural form of the analytes present (since all forms of the analytes are converted to positively charged atomic ions in the plasma). However, as an excellent elemental analyzer, it also performs as a superb detector for chromatography. Hyphenated ICP-MS is achieved through the coupling of the ICP-MS to a separation technique – normally a chromatographic separation. In this way, target analytes are separated into their constituent chemical forms or oxidation states before elemental analysis (Figure 1). The most common separation techniques are gas chromatography (GC) and high-performance liquid chromatography (HPLC), which includes ion chromatography (IC), but, other separation techniques, such as capillary electrophoresis (CE) and field flow fractionation (FFF), are also used.

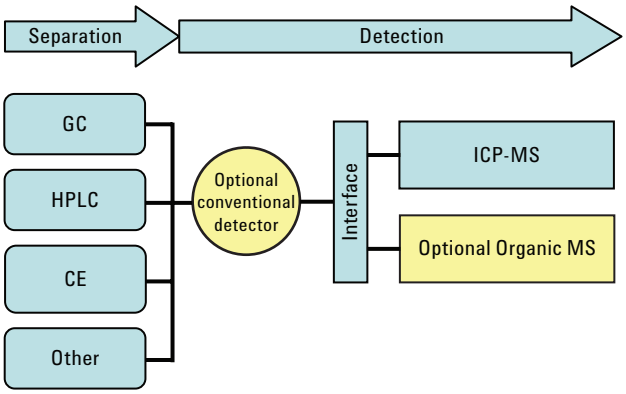

Figure 1. *Schematic of generic hyphenated system.*

This handbook specifically addresses the use of ICP-MS as an elemental detector for GC, LC, IC, and CE, though the same principles would apply to other similar techniques. Because of its ability to accurately distinguish isotopes of the same element, particularly now that collision/reaction cell (CRC) technology has all but eliminated interferences, ICP-MS is also capable of isotope dilution (ID) quantification.

Applications of hyphenated ICP-MS fall into the general category termed speciation analysis. In all cases, the fractionation device (chromatograph or other) is used to separate the species from each other and the matrix, and the ICP-MS is used to detect the species of interest. The analyte species may be as simple as elemental ions of various oxidation states in solution, or as complex as mixtures of pesticides or biomolecules. In all cases though, the ICP-MS is simply acting as an elemental detector. The fractionation device serves to separate the various components in the sample before detection as well as providing additional information in the form of retention time. Often this combination is sufficient to identify and quantify the target analytes. However where accurate retention time data is not available, analysis of standards or the use of additional mass spectrometric techniques can provide further confirmation of identification.

Elemental speciation is important in many application areas and is becoming particularly important in the environmental, food, and clinical industries. This is because, for many elements, properties such as those listed below depend on the species or chemical form of the element present in the sample.

- Toxicity or nutritional value
- Environmental mobility and persistence
- Bioavailability
- Volatility
- Chemical reactivity

A common example would be the measurement of Cr(VI) (toxic) and Cr(III) (essential nutrient) as opposed to total Cr in environmental samples. Similar examples of elemental speciation include As(III)/As(V), Se(IV)/ Se(VI), and other elements that can exist at different stable oxidation states. Furthermore, arsenic and selenium in particular also commonly exist in various organic forms which can significantly affect the traits listed above.

In the case of more complex molecules such as pesticides or biomolecules, the ICP-MS is able to identify and quantify the presence of a particular element or elements in molecular chromatographic peaks. When used in conjunction with organic MS techniques, this technique can permit quick screening for molecules (peaks) containing specific elements in a complex mixture, prior to analysis by organic MS. With modern, integrated systems and software, simultaneous analysis by ICP-MS and organic (for example, electrospray ionization [ESI]) MS is also possible, using a split flow from a single chromatographic device.

In addition to the more conventional liquid phase separations (HPLC and IC, for example), ICP-MS is also an excellent detector for separations carried out by GC. While other element-specific detectors exist for GC, none posses the elemental coverage, sensitivity, or specificity of ICP-MS. Examples of ICP-MS in molecular speciation are many and cover a broad variety of applications:

- Total sulfur and sulfur species in hydrocarbon fuels
- Organotin species in marine sediments and biota, consumer goods, and drinking water
- Mercury species in fish, industrial discharges, and petroleum processing
- Arsenic species in marine algae, food products, and drinking water
- Brominated and phosphorus-based flame retardants in consumer goods
- Phosphorus and sulfur in biological samples
- Protein- and peptide-bound metals

- Pesticides and herbicides
- Chemical warfare agents
- Volatile organohalides in air samples

In some cases, it is the presence of the target element that is important, (for example Cr(III) or Cr(VI)). In other cases, the element or elements are a simple way to identify and quantify a molecule present in a complex mixture (for example using P as a means of quantifying organophosphorus compounds).

This handbook is divided into sections based on the chromatographic component of the hyphenated ICP-MS system. Each section is composed of "application briefs" which outline typical or interesting applications for that technique. The application briefs are deliberately short, showing only general conditions and outlining results. Specific details for each application can be found in referenced publications in each section.

General Requirements

All hyphenated ICP-MS systems require that a few simple conditions are met.

- The connecting interface (transfer line) must transmit the fractionated sample quantitatively from the separation system (called a chromatograph from this point forward) to the plasma of the ICP-MS in a form that the plasma can tolerate.
- The temporal resolution of the sample components must not be unacceptably degraded.
- The chromatograph should communicate with the ICP-MS to allow synchronous separation and detection.
- The ICP-MS must be capable of transient signal acquisition at sufficient sampling frequency and over sufficient dynamic range to accommodate the resolution of the chromatograph and the required number of elements or isotopes per peak over their ranges of concentrations.

A good rule of thumb for chromatographic detectors applies here. In order to achieve accurate and precise peak integration, approximately 10 samples (scans) must be acquired for a typical Gaussian peak. Very narrow peaks will require a higher sampling frequency than wider peaks. As a quadrupole mass spectrometer, the ICP-MS sampling frequency is dependent on the scan speed of the quadrupole, the number of masses scanned, and the dwell time for each mass. Typically, since the number of elements or isotopes in hyphenated work is small, sufficient scan speed is not a problem. It must be possible to tune the ICP-MS under plasma conditions similar to those encountered during the chromatographic run. Generally, this entails introducing the tuning element(s) via the chromatographic interface. In general, using an ICP-MS as a detector for chromatography is a simple matter of connecting the outlet of the column to the sample introduction system of the ICP-MS. If the sample is gaseous, as in GC, the transfer line should be passivated and heated to eliminate sample degradation and condensation and will terminate directly into the ICP torch. If the sample is a liquid, the transfer line will likely terminate in a nebulizer in order to generate an aerosol compatible with the plasma. This may require either a split flow or makeup flow in order to match the chromatographic flow with the nebulizer and plasma requirements. Depending on the total sample flow and choice of nebulizers, the use of a spray chamber may or may not be necessary.

HPLC-ICP-MS



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HPLC-ICP-MS Introduction

High-performance liquid chromatography (HPLC) is used to describe any chromatographic technique where analytes dissolved in a liquid mobile phase are separated based on their interactions with the mobile phase and a stationary phase contained in a column. This would include both reverse- and normal-phase HPLC, size exclusion chromatography (SEC) and ion exchange chromatography. HPLC (or IC)-ICP-MS is used for the analysis of nonvolatile compounds or ions in solution. The solution can be aqueous, organic, or a mixture of both. It is this flexibility in the choice of both stationary and mobile phases, including gradient techniques where the mobile phase changes composition during the chromatographic run, which makes HPLC such a powerful separation technique for many applications. As an HPLC detector ICP-MS is the only universal, element-specific detector available for liquid chromatography and, as such, has many applications.

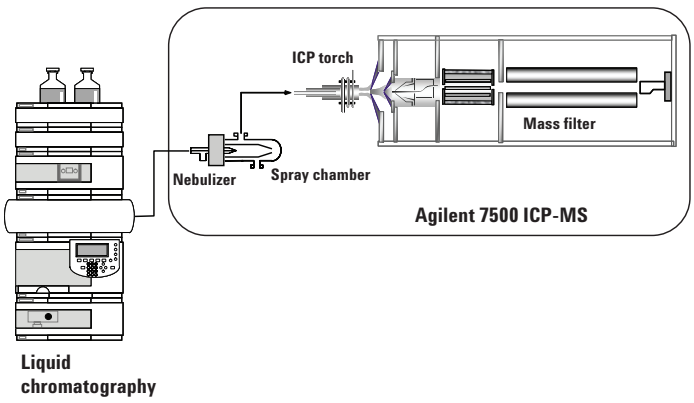
Combined with molecular mass spectrometry, ICP-MS can provide a powerful screening tool for metallic markers in biological compounds. It is also a powerful detector for specific nonmetals including sulfur and phosphorus.

Ion chromatography is a specialized form of HPLC designed to separate ionic species. It is typically used in the separation of cations (most metal ions in solution), though some metals exist as stable anions (usually oxyanions) in solution as well. The hardware is fundamentally similar to HPLC, though allowances are made for acidic or basic aqueous mobile phases, which could damage metal components in the HPLC. High background from dissolution of metal components can also be a problem. As a result, ion chromatographs rely on the use of polymeric or passivated components that are in contact with the mobile phase. Interfacing the IC to the ICP-MS is quite simple, since typical flows for IC are compatible with normal ICP-MS nebulizers. In addition, the sample handling components of the ICP-MS are already designed for acidic or caustic sample types. Since the ICP-MS is not a conductivity detector, special techniques to suppress the conductivity of the mobile phase that are required for normal ion chromatography are not necessary with IC-ICP-MS.

When used with ion chromatography, ICP-MS can provide positive elemental confirmation in addition to retention time. The Agilent LC connection kit supplies all the components and documentation necessary to interface an Agilent or other HPLC or IC to the Agilent 7500 Series ICP-MS.

Matching the Column Flow to the Nebulizer/ Spray Chamber

Matching the optimum column flow with the optimum nebulizer flow is critical to achieve both efficient separation and sample nebulization. Since the ICP-MS can tolerate nebulizer flow rates from near zero to in excess of 1 mL/minute, the nebulizer is generally selected to match the column flow. Any nebulizer has a range of flows over which it produces the highest proportion of fine droplets in the aerosol. This is critical since fine droplets are more efficiently transported through the spray chamber and atomized and ionized in the plasma. Therefore, a nebulizer that has an optimum flow rate at or near the optimum column flow should be selected. For typical HPLC flows of 100 µL/min to 1 mL/min, conventional concentric nebulizers, either in glass, quartz, or fluoropolymer work very well. At significantly higher flows, some of the sample will need to be split off prior to the nebulizer. This can be accomplished through the use of a low dead volume "Tee" near the nebulizer. In this case, a self-aspirating nebulizer must be used to avoid the need for a peristaltic pump, which would introduce unacceptable dead volume. As long as the column flow is larger than the nebulizer self-aspiration rate, there will be positive flow at the split outlet to drain. At the other extreme, if the column flow is extremely low as in the case of nano-LC, then a makeup flow may need to be added to the column flow in order to meet the flow requirements of the chosen nebulizer. While it may seem that this will result in a loss of sensitivity through dilution, in fact this is not necessarily the case. Since the ICP-MS is measuring analyte mass, not concentration, the presence of the makeup flow may not affect the result. There may be some loss, however, depending on the transport efficiency of the nebulizer/spray chamber selected. This configuration has the additional benefit of being able to add a post-column internal standard to the flow, which can be used to correct for instrument drift or matrix effects due to gradient elutions or for isotope dilution calculations. The makeup flow, with or without internal standard, can be supplied by a peristaltic pump, or, if higher precision is desired, by a piston-type LC pump or syringe pump.



HPLC-ICP-MS for Analysis of Chemical Warfare Agent Degradation Products

Douglas D. Richardson, Baki B.M. Sadi, and Joseph A. Caruso, Department of Chemistry,
University of Cincinnati, Cincinnati, OH, USA

Introduction

Phosphorus-containing nerve agents and their degradation products present difficulties for ultra-trace analysis due to their high polarity, low volatility, and lack of a good chromophore. Direct analysis of chemical warfare agent (CWA) degradation products can provide an indirect technique for CWA detection. Previous studies have successfully utilized methods such as GC-MS, ion mobility/mass spectrometry (IMMS), and LC-MS for the analysis of organophosphorus-containing degradation products with detection limits in the ng/mL range. However, considering the lethal doses, lower detection limits in the pg/mL range are desirable. When coupled to HPLC, ICP-MS can provide the necessary selectivity and sensitivity to meet the desired limits.

Table 1. *HPLC and ICP-MS operating parameters*

HPLC	
HPLC	Agilent 1100 with binary pump, vacuum degasser, diode array detector
LC column	Alltima C8, 100 Å, 3.2 mm x 150 mm, 5 µm
Guard column	Alltima C8, 7.5 mm x 3.0 mm, 5 µm
Buffer	50 mM ammonium acetate; 2% methanol; 5 mM myristyltrimethylammonium bromide, pH 4.85
Flow rate	0.5 mL/min
Injection volume	100 µL

ICP-MS	
ICP-MS	Agilent 7500ce
Forward power	1500 W
Nebulizer	Glass micro-concentric
Carrier gas flow	1.2 L/min
Aux gas flow	1.0 L/min
Spray chamber	2 °C
Sampling depth	6 mm
Dwell time	0.1 s
Isotopes	^{31}P and $^{47}PO^{+}$
ORS mode	He collision with KED

Hardware Setup

Reversed phase, ion-pairing HPLC coupled to collision/reaction cell ICP-MS was used. Conditions are listed in Table 1.

Standards and Reagents

The three chemical warfare degradation products (ethyl methylphosphonic acid [EMPA], isopropyl methylphosphonic acid [IMPA], and methylphosphonic acid [MPA]) used were obtained from Cerilliant (Austin, TX) as 1 mg/mL certified reference materials (CRMs). The remaining reagents were of analytical grade, prepared fresh daily through dilution of stock standards with DDI (18 MΩ) water.

Results

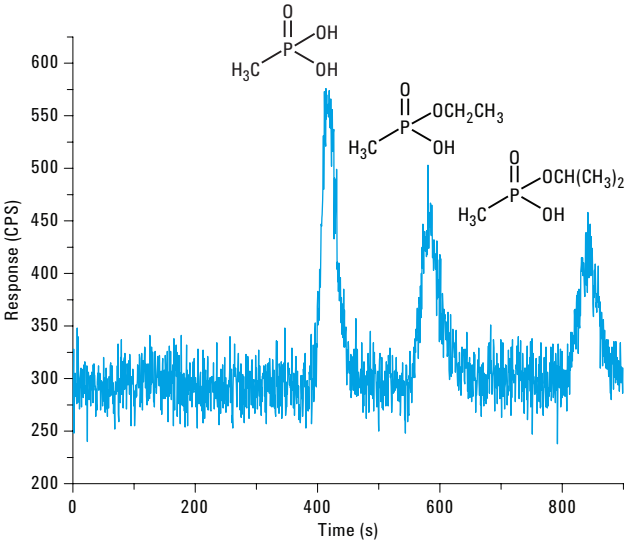
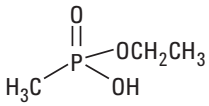
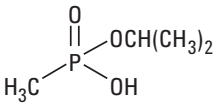
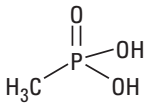


Figure 1. *Separation of MPA, EMPA, and IMPA in a standard mixture.*

Table 2. *Chemical warfare degradation product detection*

Chemical warfare degradation products	Analytical method	Detection limits ng/mL
 **EMPA**	Ion Mobility Mass Spectrometry[a]	560–1700[5]
	LC-ESI-TOF	80–1000[3]
 **IMPA**	Electrophoresis Microchip with Contactless Conductivity Detector	48–86
 **MPA**	RP-IP-HPLC-ICPMS	0.139–0.263

Conclusions

RP-IP-HPLC-ICPMS can provide rapid, sensitive detection of CWA degradation products at analytically useful levels superior to other techniques.

For Additional Information

Agilent Application Note: "Ultra-Trace Analysis of Organophosphorus Chemical Warfare Agent Degradation Products by HPLC-ICP-MS," 5989-5346EN.

First published in: *Journal of Analytical Atomic Spectrometry,* vol. 21, 396-403 (2006).

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Analysis of Glyphosate, Gluphosinate, and AMPA by Ion-Pairing LC-ICP-MS

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Introduction

Glyphosate (Roundup®) and the related compound gluphosinate are among the most widely used of nonselective herbicides. They act by inhibiting the synthesis of specific amino acids. AMPA (aminomethylphosphonic acid) is the major metabolite. While LC separation of these compounds is fairly straightforward, specific, sensitive detection has been problematic due to poor ionization characteristics in LC/MS. Detection of phosphorus using collision cell ICP-MS to eliminate the common interferences from NO^+ and NOH^+ when coupled to HPLC can provide a simple, highly sensitive method of analysis for these compounds. See Figure 1.

Hardware Setup

HPLC Conditions: Agilent 1100 liquid chromatograph equipped with a binary HPLC pump, autosampler, vacuum degasser, thermostatted column compartment and diode array detector. The HPLC system was connected to the ICP-MS using the Agilent LC connection kit (G1833-65200). A C8 column (ZORBAX SB-C8, 4.6 x 150 mm, 5 µm, Agilent Technologies) was used for separation. The column temperature was maintained at 30 °C for all experiments.

ICP-MS: Agilent 7500c ICP-MS was used for detection. Instrument operating conditions are shown under Methods.

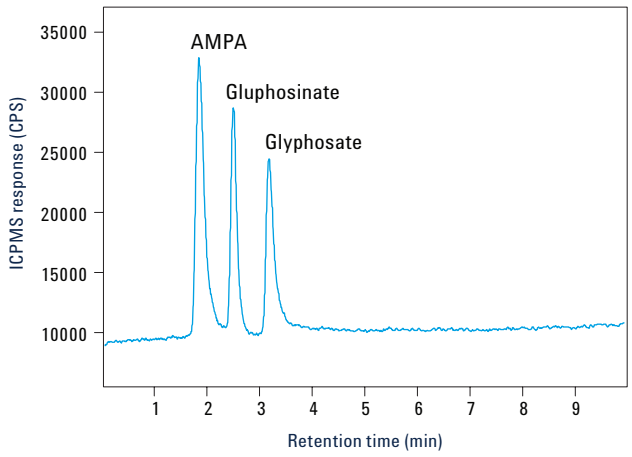

Figure 1. *Chromatogram of the herbicides gluphosinate, glyphosate and metabolite AMPA, (concentration x, y, z).*

Standards and Reagents

Deionized water (18 MΩ cm), NanoPure treatment system (Barnstead, Boston, MA, USA) was used in all standards and in buffer preparation. Commercial chemicals were of analytical reagent grade and were used without further purification. Aminomethyl-phosphonic acid (AMPA), N- (phosphonomethyl) glycine (glyphosate), gluphosinate, ammonium acetate, and tetrabutyl-ammonium hydroxide were purchased from Sigma.

Methods

Table 1. *HPLC and ICP-MS operating parameters.*

ICP-MS Parameters	
Forward power	1500 W
Plasma gas flow rate	15.0 L/min
Carrier gas flow rate	1.11 L/min
Sampling depth	6 mm
Sampling and skimmer cones	Nickel
Dwell time	0.1 s per isotope
Isotopes monitored	^{31}P
Nebulizer	Glass concentric
Spray chamber	Scott double-pass
Cell gas	He
Flow rate of cell gas	1.5 mL/min

HPLC Parameters	
Column	ZORBAX SB-C8, 4.6 x 150 mm, 5 µm
Mobile phase	50 mM ammonium acetate/acetic acid buffer 5 mM tetra-butylammonium as ion pairing reagent 1% methanol pH = 4.7
Flow rate	1.0 mL/min
Temperature	30 °C
Injection volume	100 µL

Results

Table 2. *Limits of detection for phosphorus in AMPA, gluphosinate, and glyphosate.*

	AMPA	Gluphosinate	Glyphosate
Regression coefficient	0.999	0.998	0.999
LOD (conc)	25 ppt	27 ppt	32 ppt
LOD (amount)	2.5 pg	2.7 pg	3.2 pg
RSD, retention time, n = 8	1.1 %	0.8 %	1.2 %

Conclusions

When coupled with ion-pairing HPLC, the Agilent 7500c ICP-MS, using ORS technology to remove interferences on phosphorus, can provide a superior detection system for the phosphorus-containing herbicides and their metabolites.

For Additional Information

Baki B.M. Sadi, Anne P. Vonderheide, and Joseph A. Caruso. Analysis of phosphorus herbicides by ion-pairing reversed-phase liquid chromatography coupled to inductively coupled plasma mass spectrometry with octopole reaction cell, *Journal of Chromatography A,* Volume 1050, Issue 1, 24 September 2004, Pages 95-101.

Analysis of Methyl Mercury in Water and Soil by HPLC-ICP-MS

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Introduction

Mercury can exist either in the elemental or alkylated form. Biological activity will typically methylate mercury to either methyl mercury (MeHg) or, less commonly, di-methyl mercury. The different chemical forms of mercury have different toxicities, with MeHg species being 10 to 100 times more toxic than inorganic mercury compounds. As a result, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently recommended that the Provisional Tolerable Weekly Intakes (PTWI) of MeHg be reduced to 1.6 µg per kg body weight per week, down from 3.3 µg per kg body weight per week.

The simultaneous determination of inorganic and organic mercury is difficult because the typical concentration of MeHg is much lower than that of inorganic mercury. The most common methods for mercury speciation are gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with a mercury-specific detector (fluorescence, photometry, or other elemental detector). The low concentration of mercury in natural waters leads to the need for very large sample volumes to be processed. A preconcentration step is usually necessary because the reporting limit required is often below the sensitivity of the detector used.

Instrumentation

The aim of this work was to evaluate HPLC-ICP-MS in terms of its sensitivity and specificity for the determination of MeHg. An Agilent 1100 LC was coupled to an Agilent 7500a ICP-MS using the LC-ICP-MS Connection Kit (G1833-65200).

HPLC Column

For best results, the HPLC column (ZORBAX Eclipse XDB-C18, 2.1 mm x 50 mm, 5 µm) should be preconditioned by pumping HPLC-grade methanol at 0.4 mL/min for at least 2 hours, and then conditioned with eluent at the same flow rate for at least half an hour.

Results and Discussion

A series of calibration standards was prepared from 10 ng/L to 100 µg/L by diluting a mixed Hg species stock solution (1.0 µg/mL Hg for Hg^{2+}, MeHg, and ethyl-Hg, in pure water). A 20-µL injection loop was used throughout except for the 10-ng/L data, which was obtained using a 100-µL loop. The peak areas were integrated for different concentration levels of three mixed Hg species. The linear range of the calibration curves (Figure 1) for Hg speciation by the HPLC-ICP-MS method was at least four orders. This range covers expected real sample levels, and so the method is appropriate for direct determination of water samples without the application of complicated preconcentration procedures.

Chromatographic Separation of Hg Species in 3% NaCl

In order to prove the applicability of the method to high-matrix sample analysis, the stock Hg species solution was also diluted into 3% NaCl (w/v in water) to obtain 100 ng/L MeHg, ethyl-Hg, and Hg^{2+}. The solution was filtered through a 0.45-µm membrane before analysis. A 20-µL injection loop was used for the measurement. The chromatogram was overlaid with the chromatogram of the pure water diluted solution at the same concentration, as shown in Figure 2. The peak areas of the Hg species in 3% NaCl were also integrated, and the recoveries were between 90% and 110% relative to standards in pure water. This demonstrates that the method is suitable for even high-matrix samples, such as seawater.

Application to Soil Samples

When the HPLC-ICP-MS method is applied to solid samples, such as tissues, soils, or sediments, sample preparation is necessary. The extraction of Hg species from the solid samples is a crucial step due to the presence of mercury in environmental samples at low levels, and the Hg species, especially MeHg is easy to lose or transform to other species. A simple extraction method based on dilute hydrochloric acid was used. The spike recoveries of the soil samples were between 80% and 120%. Further testing of the method and the MeHg-containing reference soil sample are planned for future work.

Table 1. *Working parameters of HPLC.*

HPLC parameters	
Column	ZORBAX Eclipse XDB-C18, 2.1 mm id x 50 mm, 5 µm
Mobile phase	0.06-mol/L ammonium acetate, 5% v/v methanol, 0.1% 2-mercaptoethanol, pH = 6.8
Flow rate	0.4 mL/min
Injection volume	100 µL

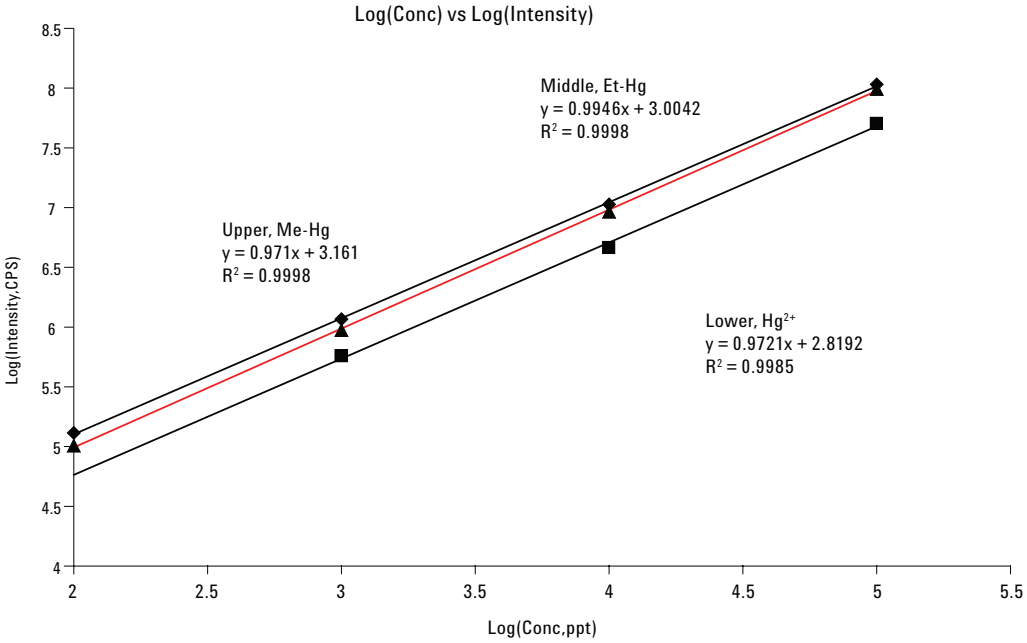

Figure 1. *Calibration curves for MeHg, Hg^{2+} and EtHg.*

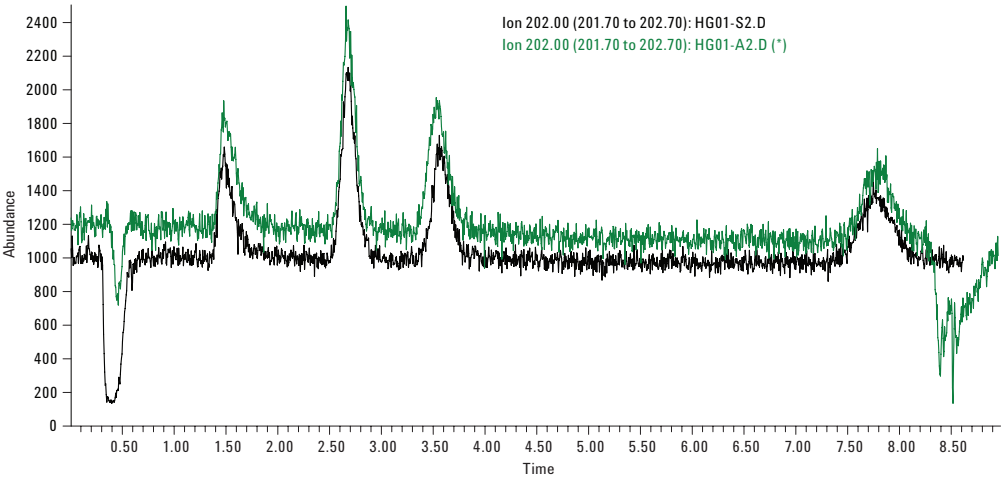

Figure 2. *Overlaid HPLC-ICP-MS ion chromatograms of 100 ng/L Hg species standards in pure water (upper) and in 3% NaCl (w/v, lower) (20 µL loop).*

Conclusions

HPLC-ICP-MS is appropriate for water samples analysis, even when the matrix in the water sample is high. The method detection limits for MeHg, ethyl-Hg, and Hg^{2+} are better than 10 ng/L and meet current regulatory requirements. When the method is applied to soil samples, Hg species extraction by 7.6% HCl is appropriate, with recoveries between 80% and 120%.

For Additional Information

Dengyun Chen, Miao Jing and Xiaoru Wang, "Determination of Methyl Mercury in Water and Soil by HPLC-ICP-MS," Agilent Technologies publication 5989-3572EN.

Agilent ICP-MS Journal May 2005, Issue 23, 5989-2950EN.

Determination of Ceruloplasmin in Human Serum by Immunoaffinity Chromatography and SEC-ICP-MS

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Orr Sharpe and William H. Robinson, Stanford University, Stanford, CA, USA

Introduction

Ceruloplasmin (Cp) is a blue alpha-2 glycoprotein with a molecular weight of 132 kilodaltons (kDa) that binds 90 to 95% of blood plasma copper (Cu) and has six to seven Cu atoms per molecule. The various functions of this protein include ferroxidase activity, amine oxidase activity, superoxidase activity, and involvement in Cu transport and homeostasis. At present there is no standardized reference method for Cp, and the immunologic methods cross-react with apoceruloplasmin (apoCp), which can bias data and deliver higher than expected concentrations for the target protein.

A method for the determination of Cp in human serum at biologically relevant concentrations > 0.01 mg/mL has been developed. Size-exclusion chromatography (SEC) is used to separate Cp from other proteins and from inorganic ions and ICP-MS, to detect Cu isotopes (m/z = 63, 65), and to confirm the identity of Cp using the $^{63}Cu/^{65}Cu$ ratio.

Experimental

Materials

Reconstituted, lyophilized Cp standards purified from human plasma were used in the study. (EMD Biosciences/Calbiochem, La Jolla, CA, USA, and Sigma, Saint Louis, MO, USA). Serum samples from patients with one of four different diseases, including myocardial infarction (MI), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and pulmonary embolism (PE), and normal controls (NC) were obtained from Stanford University (Stanford, CA). All samples were kept frozen at -20 °C until analysis. ERM DA470 is a human serum certified for 15 proteins, including Cp, and was purchased from RTC (Laramie, WY, USA).

Instrumentation

To eliminate possible interference from highly abundant proteins, some of which may bind Cu to form protein-Cu complexes, the serum sample is depleted of albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin by immunoaffinity chromatography using the Agilent 4.6 mm x 100 mm immunoaffinity column prior to SEC. An Agilent 1100 binary liquid chromatography (LC) system was used for the immunoaffinity work. Protein separation was achieved on a silica TSKGel column SW3000 from Tosoh Bioscience (Montgomeryville, PA, USA). All SEC analyses were performed on another Agilent 1100 Series binary HPLC system with diode array detector at 0.3 mL/min flow (0.1 M *tris* -pH 7). The exit from the diode array detector was connected directly to the Agilent 7500ce ICP-MS (MicroMist nebulizer) using polyetheretherketone (PEEK) tubing (60 cm length). The 7500ce was operated in helium collision mode using kinetic energy discrimination (KED) to remove the Na-, Mg- and P-based polyatomic interferences on ^{63}Cu and ^{65}Cu.

Determination of Cp by SEC-ICP-MS

SEC retention times were calibrated using a mixture of standard proteins. Cp eluted at 8.4 minutes, between albumin and IgG. However, its detection in real samples by UV is difficult due to overlap by other serum proteins. Using SEC-ICP-MS, the Cu containing Cp is easily identified. Cp-bound copper is easily distinguished from free Cu by retention time.

Method Performance

The performance of this assay was established with the reference human serum ERM DA470, which is certified for Cp at 0.205 mg/mL. The results, summarized in Table 1, illustrate excellent agreement with the certified values. Method performance data are included in Table 2. Total analysis time is approximately 95 min/sample from start to finish (15 min dilution and filtration, 30 min immunoaffinity chromatography, 20 to 30 min concentration, and 20 min SEC-ICP-MS analysis).

Table 1. *Concentration of Cp in the ERM DA470 reference serum.*

	Certified value (mg/mL)	Measured concentration (mg/mL)	$^{63}Cu/^{65}Cu$
ERM DA470 reference serum (freshly reconstituted)	0.205 (0.011)*	0.208 (5.4%)**	2.1 (3.6%)**

*Uncertainty (mg/mL)

**Average of three determinations; value given in parentheses is the percent coefficient of variation (CV%).

Table 2. *Determination of Cp by SEC-ICP-MS - method performance.*

Method indicator	Value
Detection limit (5-µL injection)	0.01 mg/mL
Dynamic range	0.01 to 5.0 mg/mL (tested only to 5 mg/mL)
Reproducibility	Overall CV: <10%
Accuracy	101% (ERM DA 470)
Cp identification	Retention time plus Cu 63/65 isotope ratio = 2.2 ± 0.1

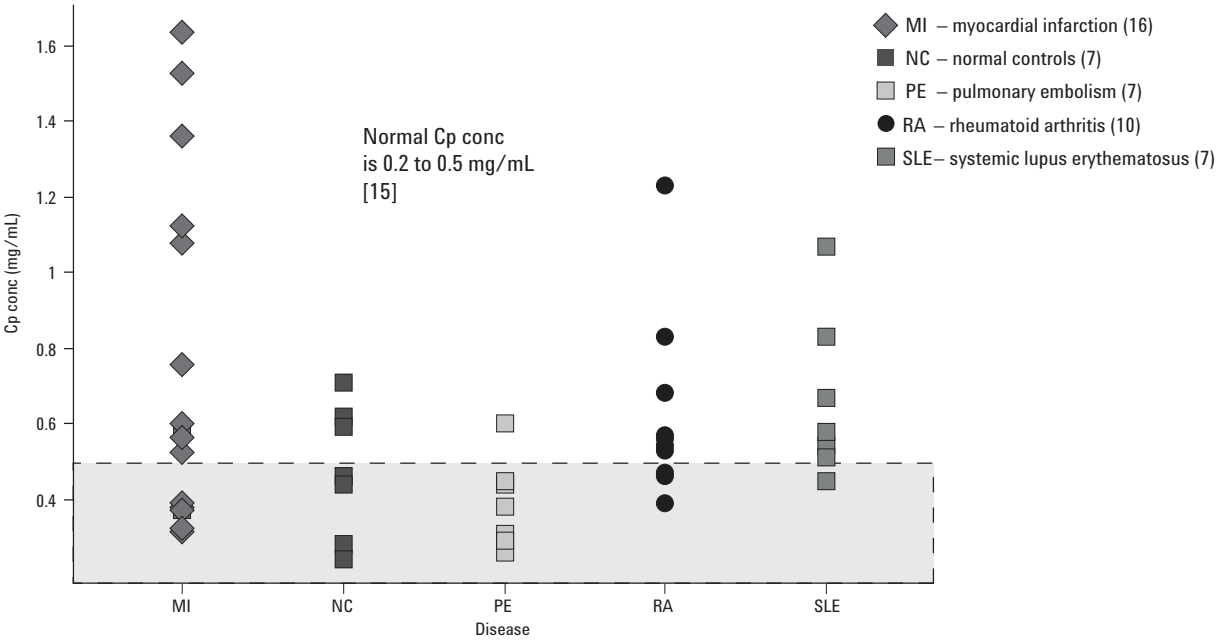

Figure 1. *Cp concentration in human sera from patients with four diseases and from normal controls; numbers in parentheses indicate the number of sera analyzed for Cp. Gray area shows Cp range reported for normal subjects (0.2 to 0.5 mg/mL).*

Results

Forty-seven human sera from patients with one of four different diseases and a set of normal controls were analyzed for Cp by the SEC-ICP-MS method (Figure 1).

Conclusions

Ceruloplasmin in human serum can be accurately determined at physiologically relevant levels using SEC-ICP-MS after cleanup by immunoaffinity chromatography as demonstrated using ERM DA470 reference serum. Initial application of the technique to sera of diseased patients shows a relationship between some diseases and elevated serum Cp concentrations.

For Additional Information

V. Lopez-Avila, O. Sharpe, and W. Robinson, "Determination of Ceruloplasmin in Human Serum by SEC-ICPMS," *Analytical and Bioanalytical Chemistry*, Volume 386, Number 1, Sept. 2006, pp 180-187.

V. Lopez-Avila, O. Sharpe, and W. Robinson,"Determination of Ceruloplasmin in Human Serum by Immunoaffinity Chromatography and Size-Exclusion Chromatography Coupled to ICP-MS," Agilent Technologies publication 5989-5304EN.

Iodine Speciation of Seaweed Using Different Chromatographic Techniques With ICP-MS Detection

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Introduction

Iodine is an essential micromineral for human nutrition, necessary for proper production of thyroid hormones. The recommended dietary allowance (RDA) of iodine is 150 mg per day (mg/d) in the United States and 150 to 200 mg/d in European and other countries. Iodine deficiency leads to various disorders associated with growth and development including "endemic goitres" and cretinism; while excessive iodine intake may cause hyperthyroidism which can also lead to the formation of a goitre, which in turn can lead to retarded brain development and functional impediment. Another important fact associated with consumption of iodine is that, like other elements, bioavailability and toxicity is species dependent. Inorganic forms of iodine, such as iodide and iodate, are less toxic than molecular iodine and some organically bound iodine. Likewise, the bioavailability of organically bound iodine, such as monoiodotyrosine (MIT) and diodotyrosine (DIT), is also less than that of mineral iodide.

Because supplementation of foodstuff with iodine is commonly practiced, total analysis and characterization of iodine species in food supplements is important. Sources include milk, iodized salt, and marine algae, including commercially available seaweed for example, Hizikia (Hiziki), Undaria (Wakame), Laminaria (Kombu) and Porphyra (Nori). Other marine algae used as a food supplement for iodine include Wakame (*Undaria pinnatifida pinnatifida*) and Kombu (*Laminaria digita japonica*).

The aim of this study is an initial characterization and identification of iodine species in commercially available seaweed samples using multidimensional chromatographic techniques coupled to ICP-MS.

Hardware Setup

Chromatographic separations were performed using an Agilent 1100 liquid chromatographic system equipped with an HPLC binary pump, an autosampler, a vacuum degasser, a thermostatted column compartment, and a diode array detector. Chromatographic conditions are summarized in Table 1.

An Agilent 7500ce ICP-MS equipped with a MicroMist nebulizer was used for iodine-specific detection. In order to connect the HPLC to the ICP-MS, the outlet of the UV detector was connected online to the liquid sample inlet of the ICP-MS nebulizer using 300 mm long by 0.25 mm PEEK tubing. For RP-HPLC, online dilution of the chromatographic eluent containing organic solvent was performed to reduce the organic solvent (methanol) load introduced into the plasma. Instrumental operating conditions are summarized in Table 1.

Table 1. *ICP-MS and chromatographic instrumental parameters.*

ICP-MS parameters	
Forward power	1500 W
Plasma gas flow rate	15.0 L/min
Auxiliary gas flow rate	0.87 L/min
Carrier gas flow rate	1.20 L/min
Dwell time	0.1 s per isotope
Isotopes monitored	^{127}I
SEC parameters	
Column	Superdex 75 HR 10/30
Mobile phase	0.03 mol/L Tris-HCl buffer, pH 8.0
Flow rate	0.6 mL/min
Injection volume	100 uL
Ion chromatography parameters	
Column	Ion Pac AS-11 anion exchange column (250 mm x 2.0 mm id x 13 µm)
Mobile phase	0.005 mol/L sodium hydroxide
Flow rate	0.3 mL/min
Injection volume	20 uL
RP-HPLC parameters	
Column	Alltima C18 (150 mm x 4.6 mm, 5 µm)
Mobile phase	(A) 0.01 mol/L Tris-HCl (pH 7.3) (B) 0.01 mol/L Tris-HCl (pH 7.3) and 50% MeOH
Flow rate	0.5 mL/min
Injection volume	50 uL
Make up solution	2% (v/v) HNO_3; 0.5 mL/min
Gradient	0-5 min-100% A to 45% B; 5-8 min-45% B to 85% B; 8-10 min-85% B to 100% B; and 10-40 min-100% B

Samples

Commercially available dried seaweed samples [marine algae Kombu (Laminaria japonica) and Wakame (Undaria pinnatifida)] were obtained from local Asian stores in the USA for total iodine analysis and speciation studies. The dried algae samples were ground in a household coffee grinder.

Results

- Total iodine concentration in samples and extracts – Both types of seaweeds were analyzed for total iodine content by ICP-MS after complete digestion using an MES 1000 closed vessel microwave digestion system (CEM Corp., Matthews, NC, USA). See Table 2.
- Size exclusion chromatography (SEC)-ICP-MS – SEC coupled to ICP-MS was used to investigate the association of iodine with various molecular weight fractions and to separate inorganic iodine from organically bound iodine.
- IC-ICP-MS for speciation of inorganic iodine – Anion exchange chromatography coupled to ICP-MS was used to separate iodide and iodate. See Table 2.
- RP-HPLC-ICP-MS for studying iodine species – Reversed-phase high-performance liquid chromatography (HPLC) coupled to ICP-MS was used for the separation and identification of low molecular weight iodine species in seaweed samples. Identification of iodine species was performed by matching the peak retention times with those of standards.

Table 2. *Inorganic iodine species present in seaweed samples.*

	Total content µg/g (% RSD)	Iodide µg/g	Iodate µg/g
Kombu	4170 (5.6)	3940	Not detectable
Wakame	226 (4.8)	140	4.16

Conclusions

In this study, the applicability of several chromatographic techniques, including SEC, IC-HPLC, and RP-HPLC, coupled to ICP-MS to iodine speciation in seaweed has been demonstrated. Moreover, the use of hyphenated techniques for iodine speciation in seaweed extracts allowed us to obtain important information on the association of iodine to the various matrix components of seaweed. Whereas iodide is about the most predominant species present in Kombu, a more complicated distribution of iodine is present in Wakame seaweed. This study shows that incorporation of iodine in different seaweeds follows different metabolic pathways, notwithstanding that both of them belong to the same class, *Phaeophyceae*. The presence of iodide was proved in Kombu, while in the case of Wakame, monoiodotyrosine and diiodotyrosine are also present and probably bound to the proteins. Since the bioavailability of iodide is better than any other form of iodine, Kombu seaweed would be preferred as a natural dietary supplement.

For Additional Information

Monika Shah, Rodolfo G. Wuilloud, Sasi S. Kannamkumarath, and Joseph A. Caruso. "Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection," *J. Anal. At. Spectrom.*, 2005, 20, 176-182.

Determination of Organic and Inorganic Selenium Species Using HPLC-ICP-MS

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Introduction

Selenium is important from an ecotoxicological point of view due to the narrow concentration range between its essential and toxic effects. Selenium compounds are distributed throughout the environment as a result of human activities (industrial and agricultural uses) and natural processes (weathering of minerals, erosion of soils, and volcanic activity). In waters, concentrations can vary from 2 ng/L to 1,900 µg/L depending on the system [1]. The natural cycle of selenium shows its existence in four oxidation states (-II, selenide; 0, elemental selenium; +IV, selenite; +VI, selenate) and in a variety of inorganic and organic compounds. The organically bound Se(-II) compounds include seleno-amino acids and volatile forms (dimethylselenide, dimethyldiselenide), which are less toxic relative to other species resulting from detoxification pathways.

Instrumentation

A standard 7500ce ICP-MS equipped with a concentric nebulizer (Meinhard Associates, California, USA) was used for this study. Chromatographic separation was carried out using the Agilent 1100 Series HPLC pump equipped with a variable volume sample loop. The analytical column was a Hamilton PRPX-100, 10 µm particle size, 25 cm length x 4.1 mm internal diameter (id). The chromatographic separation of selenocystine (SeCyst), selenomethionine (SeMet), selenite (SeIV), and selenate (SeVI) was adapted from [2] and performed using a 5 mmol/L ammonium citrate buffer, pH 5.2.

Injection volume was fixed at 100 µL. Methanol (2% v/v) was added to the mobile phase to improve sensitivity [3]. The mobile phase was delivered at 1 mL/min isocratically. The HPLC-ICP-MS interface consisted simply of polyetheretherketone (PEEK) tubing.

Experimental

Total selenium concentration (measured at ^{78}Se isotope) and selenium species concentrations were determined in different mineral and spring waters (Table 1). Results for certified simulated rain water (TM-Rain 95 from National Water Research Institute) are also given. The method was then applied to the mineral and spring water samples previously analyzed for their total selenium content.

Concentrations found in total and speciation analysis are in complete agreement showing the suitability of the method when applied to natural water samples. Although the bromine hydride interference on *m/z* 80 is present, it is separated chromatographically without overlapping with the selenium species. The chromatogram of water sample "C" (Figure 1) shows bromine elutes after the selenate peak.

Selenate, commonly found in oxygenated waters, was determined in commercial waters A through D. Selenite was identified in TM-Rain 95 water, which is only certified for its total selenium content. Only water E, a non-commercial ground water, contained both inorganic (selenite and selenate) species. See Figure 2.

Table 1. *Selenium concentrations determined in different natural waters (units: ng(Se)/L).*

Natural water sample	Elemental analysis	HPLC coupling			
	^{78}Se	^{78}Se		^{80}Se	
		SeIV	SeVI	SeIV	SeVI
TM-Rain 95	622 ± 19*	629 ± 7	< DL	615 ± 8	< DL
A	67 ± 1	< DL	69 ± 2	< DL	72 ± 6
B	142 ± 24	< DL	140 ± 9	< DL	143 ± 4
C	240 ± 20	< DL	232 ± 13	< DL	267 ± 13
D	467 ± 17	< DL	475 ± 4	< DL	492 ± 5
E	1890 ± 160	55 ± 2	1840 ± 30	57 ± 6	1920 ± 20

*Certified value 740 ± 290 ng(Se)/L

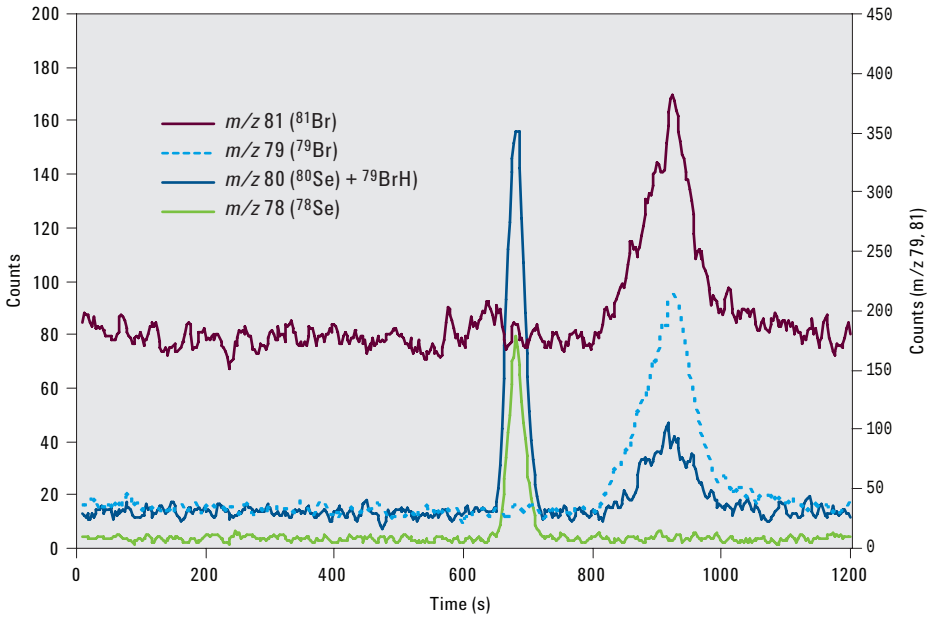

Figure 1. *Chromatogram of natural water "C" showing interference from bromine hydride elutes after the selenate peak.*

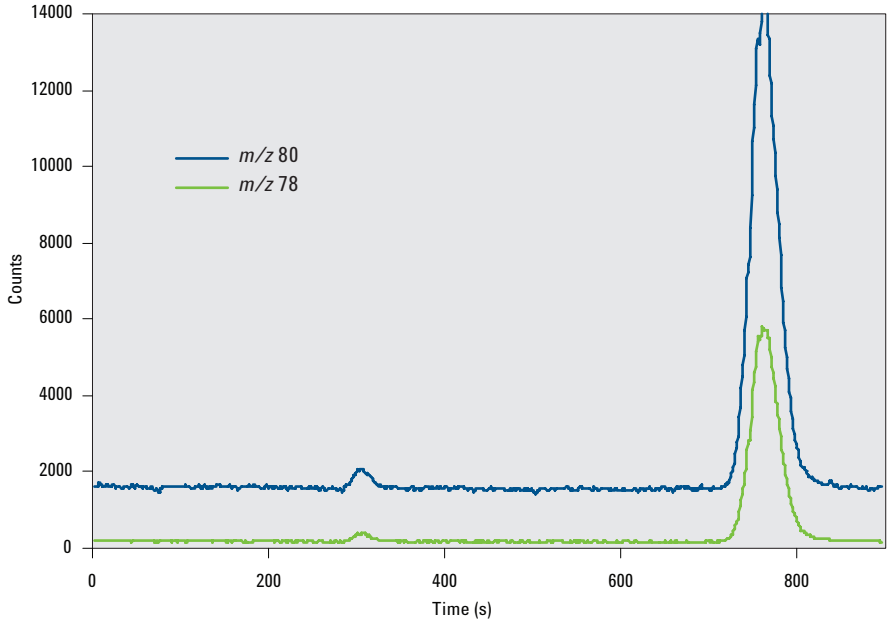

Figure 2. *Chromatogram of Natural Water "E," the only sample to contain both inorganic species. First peak is Se(IV), second peak is Se(VI).*

Conclusions

A hyphenated technique consisting of isocratic HPLC coupled to ICP-MS with optimized collision/reaction cell conditions allows for a quick and precise simultaneous analysis of organic and inorganic selenium species. Moreover as HPLC-ICP-MS coupling is easily automated, it can be considered a robust routine method to monitor selenium species levels in environmental and nutritional samples.

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HPLC-ICP-MS for Preliminary Identification and Determination of Methyl-Selenium Metabolites of Relevance to Health in Pharmaceutical Supplements

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Introduction

Selenium (Se) is an essential trace element with several functions that are relevant to health. While the nutritionally essential functions of Se are understood to be fulfilled by the selenoproteins, dietary Se can be metabolized to low molecular weight species (for example, methyl-Se compounds) that have more recently generated interest because of putative anticancer effects [1]. In contrast to such beneficial effects, at a sufficiently high dose level, Se metabolites can also cause toxicity. Since selenium is declining in the ordinary diet in Europe, efforts have been made to increase Se intake levels, mainly through biofortification of food and the production of pharmaceutical supplements.

Knowledge of speciation of selenium in food and food supplements will have implications with respect to the determination of Se requirements and to the investigation of relationships between Se status and health and disease. It will help in the development of safe and effective products and with future regulation of their production and use. Characterization of food and dietary supplements for Se speciation is challenging and demands the development of analytical techniques, such as hyphenated mass spectrometry methods, that allow the measurement and identification of Se chemical forms (species) in a complex sample matrix [2]. The combined application of element-specific MS (ICP-MS) and molecule-specific MS (ESI- or MALDI-MS) with HPLC has become an irreplaceable tool in this field.

In terms of quantifying Se compounds, the attractive features of ICP-MS, such as isotope specificity, versatility, high sensitivity, large dynamic range, and the virtual independence of the signal intensity of the structure of the biomolecule, makes this detector, in combination with a selective chromatographic separation, a potential and unique tool for quantitative Se speciation. ICP-MS, when used in combination with complementary HPLC separation methods, may allow preliminary identification of Se compounds, for which standards are available. Moreover, knowledge of the HPLC-ICP-MS retention times of minor Se-containing compounds in complex matrix samples (for example, food supplements) has been found to be essential to the identification of the Se isotope patterns in the total ion chromatogram (TIC) obtained by HPLC-ESI-MS [3].

In this paper, the potential of the coupling HPLC-ICP-MS for Se speciation analysis in complex samples (for example, dietary supplements) will be illustrated through its application to the measurement and preliminary identification of minor Se metabolites for example, γ-glutamyl-Se-methylselenocysteine (γ-glutamyl-SeMC) in selenized yeast used as the intervention agent in human cancer prevention trials [1].

Experimental

Instrumentation

Extraction of the water-soluble seleno-compounds from yeast was carried out by accelerated solvent extraction (ASE) using a Dionex ASE 200 system (Sunnyvale, CA, USA).

HPLC-ICP-MS measurements were performed using an Agilent Technologies 1100 Series HPLC system for chromatographic separations and an Agilent 7500i ICP-MS for element-specific detection. Reversed-phase HPLC was performed on an Agilent ZORBAX Rx-C8 column (250 mm x 4.6 mm ID, with a particle size of 5 µm). The HPLC column was directly connected to the 100 µL/min PFA microflow concentric nebulizer of the ICP-MS via PEEK tubing (30 cm x 0.1 mm ID). The Agilent Technologies ICP-MS chromatographic software (G1824C Version C.01.00) was used for integration of the chromatographic signal.

Reagents and Samples

Selenium standards (Figure 1) and other chemical substances were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. L-γ-glutamyl-Se-methylseleno-L-cysteine was purchased from PharmaSe (Lubbock, TX, USA). Single-standard stock solutions (1 mg/g) were prepared and stored as detailed elsewhere [4]. A standard solution of 10 µg/kg of Se in the corresponding mobile phase was prepared from a 1,000 mg/kg Se reference solution (Romil) and used for the daily optimization of the ICP-MS parameters (optimal settings: RF power: 1,300 W; make-up Ar flow rate: 0.31 L/min; nebulizer Ar flow rate: 0.85 L/min; isotopes: ^{77}Se, ^{82}Se, and ^{103}Rh; integration time per mass: 100 ms).

Samples from one batch of SelenoPrecise®, Lalmin™ Se2000 and SelenoExcell™ selenized yeast were supplied by Pharma Nord (Vejle, Denmark), Lallemand Inc. (Montréal, Canada) and Cypress Systems, Inc. (Fresno, CA, USA), respectively. These samples were stored at 4 °C in the dark under dry conditions and thoroughly mixed before sample treatment. The moisture content of these samples was determined using a procedure reported elsewhere [4].

Procedures

Extraction of Se species in water: 0.3 g of Se-yeast was extracted with degassed water using accelerated solvent extraction using the conditions described in a previous work [4].

Se speciation by RP HPLC-ICP-MS: A 50-µL portion of the 1:5 diluted extract was analyzed by ion pairing reversed phase HPLC-ICP-MS at the flow rate of 0.5 mL/min using a water-methanol (98 + 2, v/v) mixture containing 0.1% (v/v) formic acid as the mobile phase. For quantification, calibration was carried out by the standard addition technique at three concentration levels, using peak area measurements of the chromatographic signals by monitoring the ^{82}Se signal. The Se concentration of γ-glutamyl-SeMC in the water-soluble extracts is expressed as average ± SD (n = 3) and referred to as dry sample weight.

Total Se determination of the yeast samples was performed by ICP-MS after microwave acid digestion [4].

Results and Discussion

Two ion-pairing reversed phase HPLC methods (with trifluoroacetic acid [TFA] and formic acid as ion pairing reagents) coupled with ICP-MS were compared for preliminary identification of γ-glutamyl-SeMC in Se-yeast aqueous extracts. The method using formic acid with on-line ICP-MS detection (see conditions above) was preferred for further experiments due to its capability to provide enough retention of target Se species while offering good chromatographic/detection selectivity in the chromatographic region under investigation. Moreover, in comparison with TFA, the use of formic acid is preferable because of its higher compatibility with electrospray ionization (ESI). For chromatographic identification, retention time matching with an authentic standard was used. An alternative, standardless approach based on retention time matching with an aqueous extract from garlic, in which the major species of Se is known to be the γ-glutamyl-SeMC species, was also investigated [4].

The chromatograms of a standard mixture and of 1:5 diluted aqueous extracts from the Se-yeast samples are shown in Figure 1. Assignments based on retention times suggest that the samples seem to contain Se species such as SeMC, selenomethionine (SeMet), and γ-glutamyl-SeMC. For quantification of γ-glutamyl-SeMC, the 1:5 diluted extracts were spiked with γ-glutamyl-SeMC standard of a known Se concentration (see procedures) at three concentration levels. The recovery of added γ-glutamyl-SeMC was 98.9 ± 2.1%. Based on three times the standard deviation for 11 replicate determinations of the reagent blank, the detection limit for γ-glutamyl-SeMC was found to be 8.1 ng/kg.

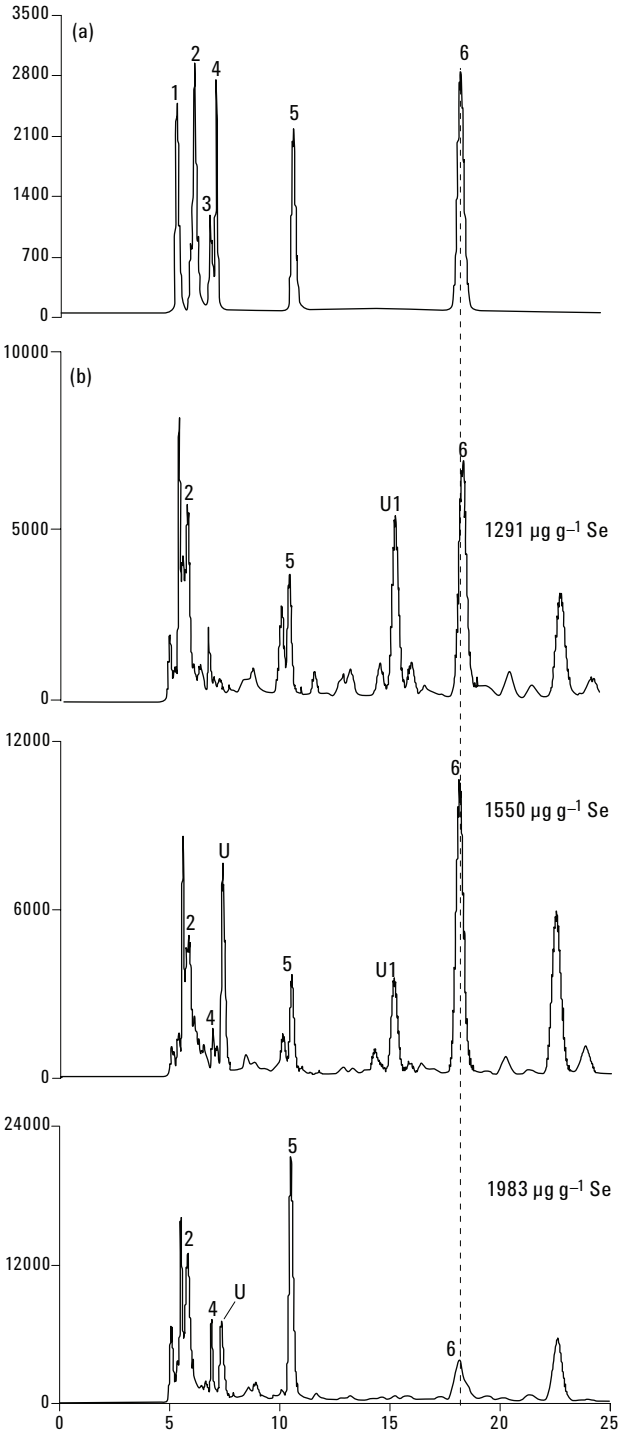

Figure 1. *RP HPLC-ICP-MS chromatograms of (a) a Se standard mixture containing 3 µg/L Se as Se(Cys)2 (peak 1), 2.5 µg/L Se as selenite (peak 2), 5 µg/L Se as SeMC (peaks 3 and 4) and SeMet (peak 5), and 25 µg/L Se as γ-glutamyl-SeMC (peak 6) and (b) the 1:5 diluted Se-yeast water extracts.*

Table 1 summarizes the percentage of the total Se in the water extract, which is associated with γ-glutamyl-SeMC as well as the concentration of Se found as γ-glutamyl-SeMC in the yeast samples analyzed. The results shown in Table 1 and Figure 1 suggest that there is a significant variation of the Se speciation in the water extracts with the variation of the total Se. The numbers in Table 1 also show that the concentration of γ-glutamyl-SeMC decreased not only relatively, but also in absolute terms between concentrations of 1,550 and 1,983 µ/g Se. Since Se-yeast samples with a wider range of total Se concentrations were not available, the results below, while intriguing, should be interpreted with caution regarding the change in selenium distribution upon increase in total Se content. Moreover, further studies should be pursued to elucidate whether or not the differences observed for the speciation of selenium may also be a result of the slightly different methods of yeast enrichment with selenium used by he different manufacturers.

Conclusions

Confirmation of the presence of γ-glutamyl-SeMC (a dipeptide of SeMC and glutamic acid) in the Se-yeast samples analyzed by HPLC-ICP-MS was achieved, for the first time, using the on-line coupling of the chromatographic method developed with ESI MS/MS in selected reaction monitoring mode without the need for extract pretreatment [4]. The presence of γ-glutamyl-SeMC might be relevant to the anticarcinogenic potential of selenized yeast since this species is believed to serve primarily as a carrier of SeMC, which appears to be easily converted in animals and possibly humans to methylselenol. This Se metabolite is thought to be an effective anticarcinogen.

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Table 1. *Percentage of the total Se in the yeast water extract and in the whole yeast sample associated with γ-glutamyl-SeMC and concentration of Se (µg/g) incorporated into γ-glutamyl-SeMC in the yeast sample, as found by RP HPLC-ICP-MS. Precisions are calculated for three independent chromatograms.*

Yeast source	Se concentration of γ-glutamyl-SeMC		
	Water extract (%)[a]	Yeast sample (%)[b]	µg/g
SelenoExcell™ (1291 µg/g Se)	4.4 ± 0.1	0.91 ± 0.04	11.2 ± 0.6
SelenoPrecise (1550 µg/g Se)	7.3 ± 0.2	1.00 ± 0.06	15.7 ± 0.9
Lalmin™ Se2000 (1983 µg/g Se)	2.4 ± 0.1	0.4 ± 0.02	7.2 ± 0.4

[a]Fraction of the total Se in the water extract

[b]Fraction of the total Se in yeast

Determination of Arsenic Species in Marine Samples Using Cation-Exchange HPLC-ICP-MS

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Introduction

A method for the determination of arsenic species in marine samples by cation-exchange HPLC ICP-MS was investigated. A three-step gradient elution of the arsenic species led to the detection of up to 23 different arsenic species in a single analytical run.

Sample Preparation

Freeze-dried samples of marine origin (0.25 g) were extracted three times by mechanical agitation with methanol/water (1 + 1). The three supernatants were combined, evaporated to dryness, and redissolved in 5 mL water prior to analysis.

Instrumentation

An Agilent 7500c ICP-MS was used as an element-specific detector connected to an Agilent 1100 Series HPLC system (degasser, autosampler, and quaternary pump).

A Chrompack Ionospher C column (100 x 3 mm id) was used as the stationary phase and a pyridine solution in 3% MeOH adjusted to pH = 2.7 with formic acid as the mobile phase.

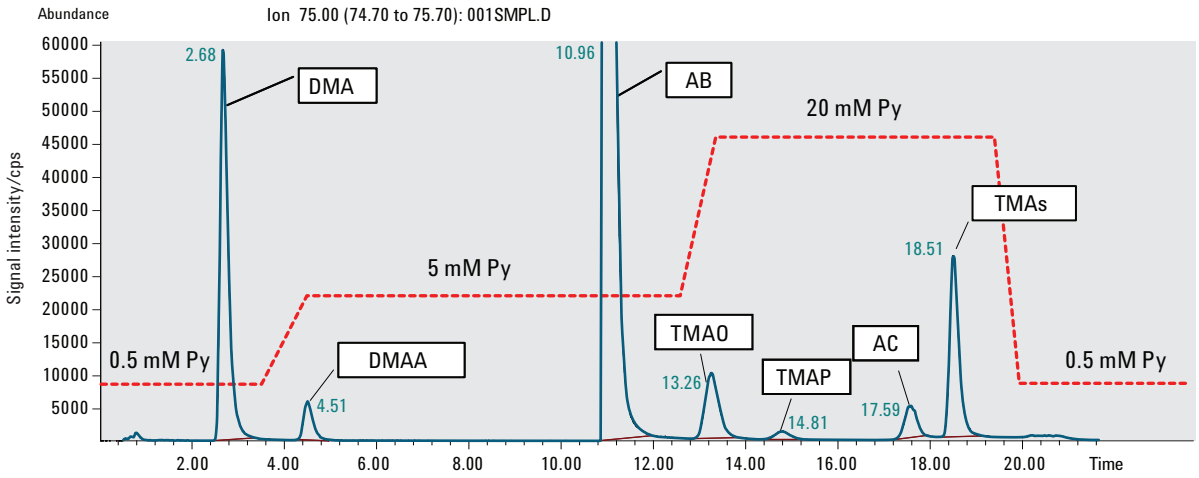
A three-step gradient elution was employed in order to achieve the best possible separation of the arsenic species. Figure 1 shows a chromatogram of a standard solution of the available arsenic species. The three-step gradient elution procedure is illustrated by the red dotted line.

Results and Discussion

The separation/detection capability of the methodology is illustrated in Figure 2, where 23 different arsenic species are detected in one analytical run (25 min) in a scallop kidney. Seven arsenic species have been identified by retention time matching with available standards. As can be seen from the chromatogram, unknown peaks remain. The majority of these peaks are probably arseno-riboside compounds (arsenosugars).

Analysis of Certified Reference Materials

To date, only two reference materials of marine origin have been certified for the content of arsenic species: NRCC DORM-2 Dogfish muscle and BCR627 Tuna. Table 1 shows the results from this work and the certified values. In all cases, good agreement between measured and target values was obtained.



Key:

DMA	Dimethylarsinic acid	TETRA	Tetramethylarsonium ion
AB	Arsenobetaine	DMAA	Dimethylarsinoylacetic acid
TMAO	Trimethylarsine oxide	TMAP	Trimethylarsoniopropionate
AC	Arsenocholine ion		

Figure 1. *Chromatogram of a standard solution. Dotted line illustrates the gradient elution of the arsenic species with the pyridinium mobile phase.*

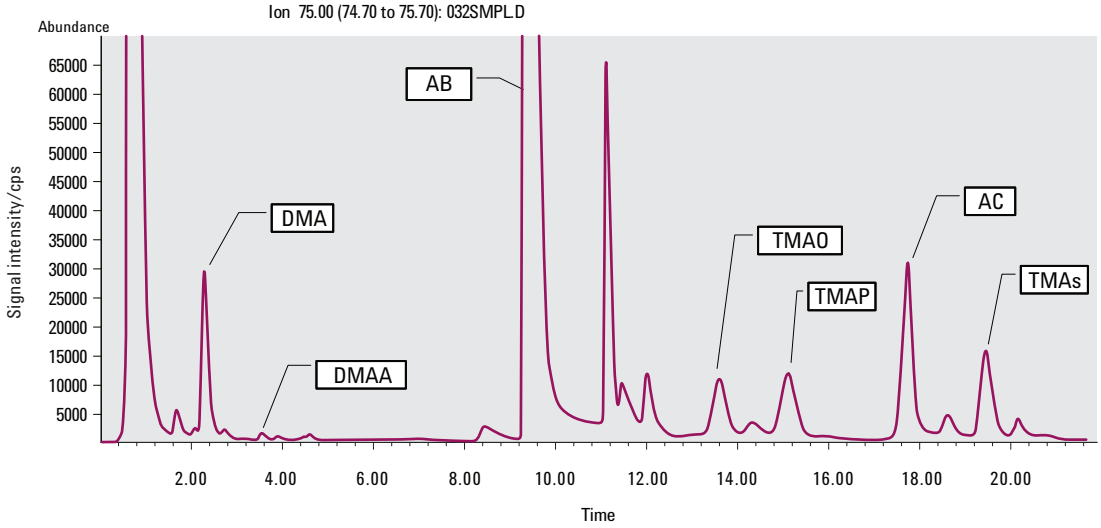


Figure 2. *Chromatogram of a kidney from a scallop.*

Conclusions

An HPLC-ICP-MS method capable of separating 23 arsenic species in one analytical run has been developed. The separation, which was based on cation exchange HPLC, employed a three-step gradient elution and resulted in excellent selectivity. The analysis of the CRMs DORM-2 and BCR627 Tuna fish tissue showed good agreement with certified values and provided a set of values for noncertified arsenic species. The method is useful for future studies of arsenic metabolism in biological samples of marine origin. Several naturally occurring arsenic species were detected but could not be identified in this study due to the lack of available standard substances. In order to characterize these unknowns, further investigation by, for example, ESI-MS/MS will be necessary. Identification of the unknown arsenic compounds will improve our understanding of arsenic-containing natural products and possibly help to elucidate the pathways of transformation of arsenic compounds in the environment.

Table 1. *Results from the analysis of the certified reference materials NRCC DORM-2 (Dogfish Muscle) and BCR627 Tuna. All results in mg (As)/kg ± 95% confidence interval.*

	DORM-2		BCR627 Tuna	
	Cert	Found	Cert	Found
AB	16.4 + 1.1	16.9 + 0.8	3.9 + 0.2	3.7 + 0.2
DMA	–	–	0.15 + 0.01	0.14 + 0.01
TETRA	0.248 + 0.054	0.26 + 0.01	–	–

For Additional Information

Jens J. Sloth, Erik H. Larsen, and Kåre Julshamn, "Determination of organoarsenic species in marine samples using gradient elution cation exchange HPLC-ICP-MS," *J. Anal. At. Spectrom.*, 2003, 18, 452-459.

Routine Determination of Toxic Arsenic Species in Urine Using HPLC-ICP-MS

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Introduction

Arsenic exposure may lead to cancer or other adverse effects, but the toxicity is strongly dependent on the species. Of the five As species most commonly found in human urine, the order of toxicity is: As(III) (arsenite) > As(V) (arsenate) > DMA (dimethylarsinic acid) = MMA (monomethyl arsonic acid) > AB (arsenobetaine).

While HPLC-ICP-MS is well accepted as the analytical technique of choice for As speciation in urine, some remaining difficulties have prevented the technique from becoming routine. These are:

- Finding chromatographic conditions that will separate the five most important species as well as inorganic chloride in a reasonable time, with good retention time reproducibility, dynamic range, and sensitivity.
- Resolving or eliminating the ArCl interference on As that is derived from the high NaCl concentration in urine samples.
- Avoiding clogging of the ICP-MS interface from total dissolved solids (TDS) contained in the urine and HPLC buffers.

Experimental

An Agilent 1100 Series HPLC isocratic pump with autosampler, thermostatted column compartment, and vacuum degasser was coupled to an Agilent 7500ce ICP-MS system fitted with an Agilent MicroMist glass concentric nebulizer. Typical ICP-MS conditions were used for As analysis, including forward power: 1,550 W; sample flow rate: 1 mL/min; and total carrier gas flow: 1.12 L/min. As was monitored at its elemental mass: m/z = 75.

Column Selection

A new anion exchange column was developed and manufactured by Agilent.

Column	G3288-80000 (4.6 mm x 250 mm)
Guard column	G3154-65002

The new Agilent column provides the advantages of excellent resolution of As(III) from both AB and DMA and good separation of MMA from Cl^- under isocratic conditions.

Mobile Phase

The basic mobile phase consisted of:

- 2 mM phosphate buffer solution (PBS), pH 11.0 adjusted with NaOH
- 10 mM, CH_3COONa
- 3.0 mM, $NaNO_3$
- 1% ethanol

Purging the mobile phase with argon during analysis minimized the effects of pH changes due to absorption of atmospheric carbon dioxide.

Interference Removal

The new Agilent G3288-80000 column provides the necessary chromatographic resolution to completely separate inorganic chloride from the arsenic species under isocratic conditions, thereby eliminating the ArCl interference on As. As a result, this method is also suitable for use with non-ORS 7500 ICP-MS systems.

Calculation of Detection Limits

Detection limits for each arsenic species were calculated as three times the chromatographic peak-to-peak signal-to-noise ratio. All species met the goal of < 0.1 µg/L (Table 1).

Table 1. *Calculation of detection limits.*

Species	Height counts	DL (S/N x 3) µg/L
Noise x 3 (average)	117.5	
AB*	2865	0.041
DMAA	3328	0.035
As(III)	2255	0.052
MMAA	1574	0.075
As(V)	1172	0.100

*Arsenobetaine, while well-separated from the four anionic species, elutes with the void volume and may coelute with other neutral or cationic species if present.

Results

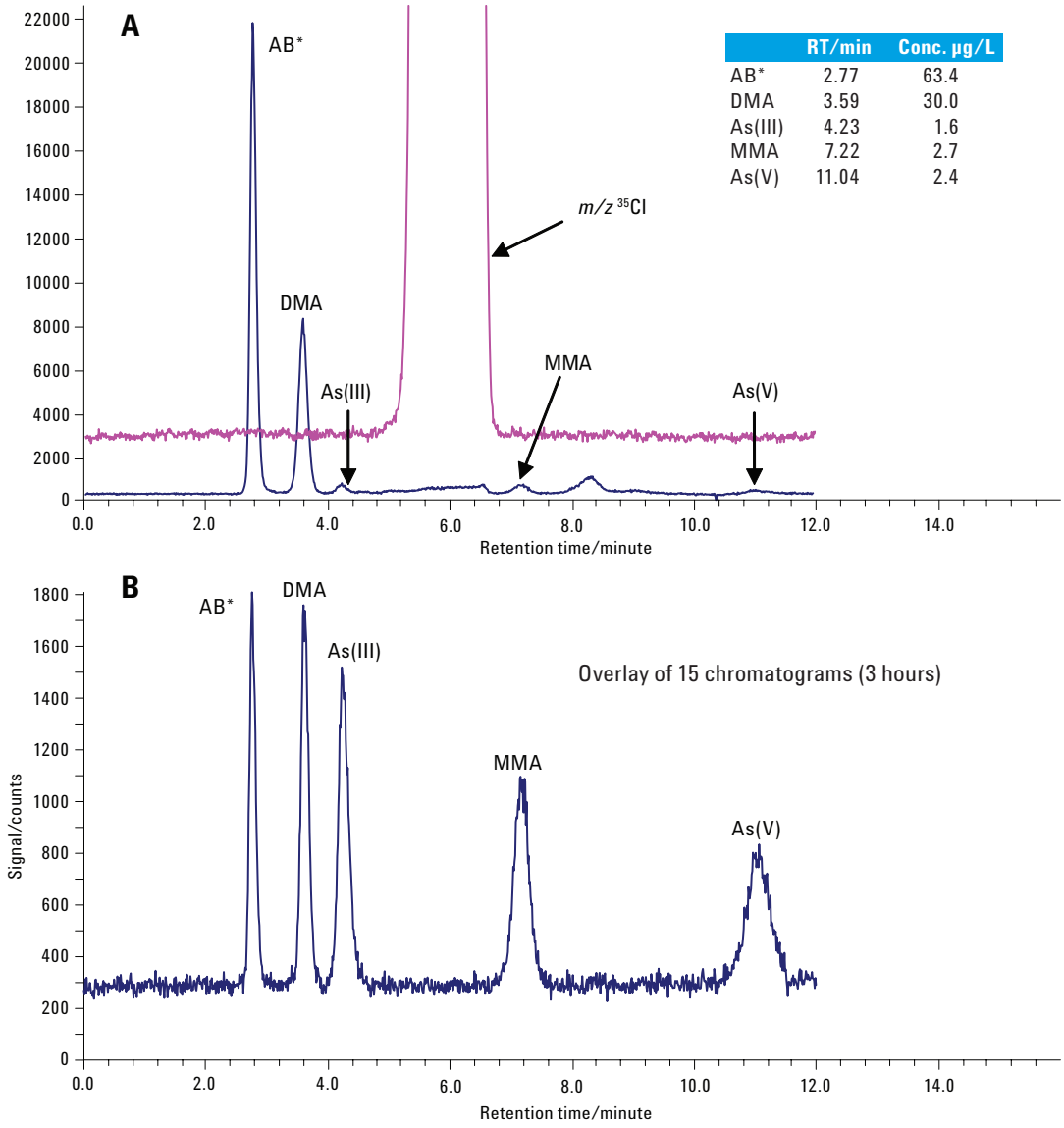
The new methodology was applied to the analysis of NIES CRM No.18 urine, using a 5-µL injection of the undiluted sample (Figure 1A). The results agree well with the certified values (AB 66.0 µg/L, DMA 31.0 µg/L). Repeated injections (n = 15) of a 1/10 diluted human urine sample spiked at 5 µg/L demonstrates good long-term stability and the robustness of the method (Figure 1B).

Conclusions

A new HPLC-ICP-MS method capable of separating all five important arsenic compounds in human urine within 12 minutes has been developed through careful, systematic optimization of all parameters, including the development and manufacture of a new column. The method is robust enough for the analysis of undiluted urine with limits of detection of 0.1 µg/L or less for the individual As species.

For Additional Information

Tetsushi Sakai, Steve Wilbur, "Routine Analysis of Toxic Arsenic Species in Urine Using HPLC with ICP-MS," Agilent Technologies publication, 5989-5505EN



*Arsenobetaine, while well-separated from the four anionic species, elutes with the void volume and may coelute with other neutral or cationic species if present.

Figure 1. *(a) Undiluted 5-µL injection of NIES CRM No.18 urine standard. (b) Reproducibility of 15 x 1/10 human urine samples (spiked 5 µg/L).*

Application of ICP-MS to the Analysis of Phospholipids

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Introduction

Phospholipids are the main constituents of membranes in all types of prokaryotic and eukariotic cells. Due to their complexity and heterogeneity in biological samples, qualitative and quantitative analyses of membrane phospholipids in cellular extracts represent major analytical challenges, mainly due to the requirement for suitable and sensitive detection methods. ICP-MS is a suitable detector for selective determination of phospholipids, which all contain phosphorus. However, the determination of phosphorus and its compounds by an ICP-MS is not an easy task because phosphorus has a high ionization potential and, consequently, is poorly ionized in the plasma. Additionally, it suffers from polyatomic interferences at *m/z* ratio 31 from $^{12}C^{1}H^{316}O+$, $^{15}N^{16}OH$, $^{15}N^{16}O$, and $^{14}N^{17}O$. Phospholipids are extractable with organic solvents; therefore, liquid chromatography with an organic mobile phase was used for separation of different lipid species.

Experimental

Reagents and Sample Preparation

A standard mixture of six phospholipids was prepared by diluting each standard in a chloroform/methanol mixture (2/1, v/v).

1,2-dioleoyl-phosphatidic acid monosodium salt ($C_{39}H_{72}O_8PNa$, DOPA)

1,2-dioleoyl-phosphatidylcholine ($C_{44}H_{84}NO_8P$, DOPC)

1,2-dioleoyl-phosphatidylethanolamine ($C_{41}H_{78}NO_8P$, DOPE)

1,2-dioleoyl-phosphatidylglycerol sodium salt ($C_{42}H_{78}O_{10}PNa$, DOPG)

1,2-dioleoyl-phosphatidylserine sodium salt ($C_{42}H_{77}NO_{10}PNa$, DOPS)

Phosphatidylinositol sodium salt isolated from bovine liver ($C_{47}H_{82}O_{13}PNa$, PI)

The concentrations of each expressed as phosphorus were as follows: 3.3 mg/L DOPA, 2.9 mg/L DOPG, 2.7 mg/L PI, 3.1 mg/L DOPE, 3.0 mg/L DOPS, and 2.9 mg/L DOPC.

Chromatographic System

HPLC separations were carried out using an Agilent 1100 chromatographic system equipped with a thermostatted autosampler (variable injection loop 0 to 100 µL), YMC Pack Diol-120 column (250 x 4.6 mm, 5 µm) (Kyoto, Japan) maintained at 50 °C and a flow rate of 0.6 mL/min. The composition of mobile phase A was acetone/hexane/acetic acid/triethlyamine (900/70/14/2 [v/v]) and the composition of mobile phase B was methanol/hexane/ acetic acid/triethlyamine (900/70/14/2 [v/v]). The following gradient elution program was used: 95% of A at 0 min, 82% of A at 40 min, 55% of A at 42 min, 40% of A at 44 min, 40% of A at 49 min, 95% of A at 49.5 min, and 95% of A at 58 min.

Detection System

The 0.6 mL/min flow from the HPLC column was split to approximately 130 µL/min before reaching the 7500c ICP-MS via self-aspiration using a PFA 100 nebulizer. To prevent deposition of carbon on the interface cones, an optional gas (20% oxygen in argon) was introduced. Since added oxygen promotes corrosion of interface cones, a platinum sampler cone was used. The detection was carried out by recording *m/z* ratio 31 at scan rate of 0.3 s per point.

The system was optimized by pumping mobile phase A containing 2 mg/L of phosphorus as a DOPE. The following optimized conditions were used for the detection of the phospholipids:

Plasma gas	15 L/min
Auxiliary gas	1.0 L/min
Carrier gas	0.50 L/min
Optional gas flow rate	24% (of carrier gas flow rate)
RF power	1600 W
ORS gas (helium)	4.0 mL/min
Spray chamber temperature	25 °C; and sample depth (torch-interface distance) 10 mm

All chromatograms were smoothed before integration.

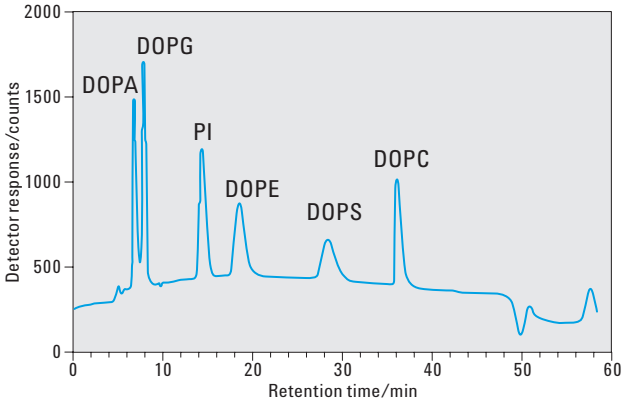

Figure 1. *Separation of six chemically defined phospholipids in standard mixture on YMC Pack Diol-120 column (250 x 4.6 mm, 5 µm) with ICP-MS detection of phosphorus at m/z ratio 31 (5 µL injected, 0.6 mL/min, each peak corresponds to ~15 ng of phosphorus).*

Results and Discussion

Chromatographic Separation

Successful chromatographic separation of six standard phospholipids was achieved by utilization of modified conditions described by Sas et al. [1]. A typical chromatogram is presented in Figure 1. Almost all six phospholipid standards were baseline separated.

The usefulness of the developed method for analysis of phospholipids was demonstrated on a complex lipid extract from yeast. Each identified compound was quantified by using the calibration curves given in Table 1. The results are presented in Table 2 as peak areas and as calculated masses and concentrations of each identified compound in the sample extract. It should be noted that all masses and concentrations are expressed as phosphorus and that peak coeluting with chemical class of phosphatitylcholine (PC) was integrated and considered as belonging to PC class.

Table 1. *Calibration parameters (expressed as mass of phosphorus).*

Compound	Retention time/min	Calibration curve	R	Linear range/ng	LOD/ng	Reproducibility* (%)
DOPA	6.7	A = 18000 x m – 830	0.9998	1.6-16	0.36	±6
DOPG	7.8	A = 23400 x m – 5650	0.9997	1.4-14	0.21	±5
PI	14.2	A = 25000 x m – 850	0.9999	1.4-55	0.54	±7
DOPE	18.3	A = 21000 x m – 10400	0.9999	3.0-61	1.2	±7
DOPS	28.1	A = 16500 x m – 18600	0.9998	3.0-59	1.2	±16
DOPC	35.9	A = 19500 x m – 230	0.9999	1.5-59	0.50	±14

* At lowest point of calibration curve.

Table 2. *Peak areas, calculated masses and concentrations, relative amounts of identified compounds and semi-quantitatively determined relative amounts of all phospholipids in yeast lipid extract (all values are expected as phosphorus).*

Class	Peak area/ 103 units	Mass/ng	Concentration/ mg 1^{-1}	Relative amounts* (%)	Semi-quantiative relative amounts** (%)
PA	65.5	3.7	0.74	1.6	1.2
PI	783	31	6.3	13	15
PE	1440	69	14	29	27
PS	150	10	2.0	4.3	2.8
PC	2380	120	24	51	44
X_1	400	–	–	–	7.6
X_2	45.6	–	–	–	0.9

* Relative amounts of identified compounds.

** Relative amounts determined bt semi-quantitative procedure.

Theoretically, the response of an ICP-MS is element dependant, meaning it is the same for all compounds regardless to their structure. The determined response factors, which are part of calibration curves in Table 1, have values from 16,500 to 25,000 peak area units per ng of phosphorus. Deviations from theory are expected, since we used a gradient elution program. This gives a different matrix composition for each compound, resulting in different nebulization and ionization efficiencies. Therefore, simplification of the quantification procedure by using a calibration curve based only on one compound should be used with caution and considered in the field of semi-quantitative analysis.

In cases when we are interested only in obtaining approximate ratios between classes of phospholipids in the sample, only peak areas without any calibration can be used. To show the usefulness of such a quick semiquantitative analysis, a yeast lipid extract was treated in that way. Peak areas of all peaks found in the chromatogram were summed; their relative amounts were calculated and are presented in Table 2. Compared to literature data [2], this semiquantitative approach gives good agreement.

Conclusions

Collision/reaction cell-ICP-MS has been shown to be a suitable detector for selective determination of phospholipids following separation of different lipids by LC. To reduce polyatomic interferences at *m/z* ratio 31 (for example, CH_3O^+) and to improve detection limits, helium was used as a collision gas within the ORS cell. The achieved absolute detection limits were between 0.21 and 1.2 ng of phosphorus and were superior to those obtained by an evaporative light-scattering detector, which provides an alternative detection system for lipid analysis.

The usefulness of the developed method was demonstrated by analysis of lipid extracts from the yeast Saccharomyces cerevisiae.

References

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Chromium Speciation in Natural Waters by IC-ICP-MS

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Introduction

Measurement of total chromium doesn't always tell the whole story. The anionic, hexavalent form of the element is toxic, while in its cationic trivalent oxidation state, chromium is an essential element for human nutrition. Methods to establish the potential toxicity of Cr must therefore determine the concentration of Cr(VI), rather than simply total Cr.

Separating and detecting Cr is challenging because the common forms of Cr in natural samples such as water are chromate (CrO_4^{2-}) for Cr(VI) and chromic ion (Cr^{3+}) for Cr(III). Chromate is an anion and the chromic ion is cationic, so a single ion exchange method will not work for both forms under the same conditions. A further problem is that Cr(III) is the most stable oxidation state in samples such as water, whereas Cr(VI) ions are strong oxidizing agents and are readily reduced to Cr(III) in the presence of acid or organic matter. Consequently, great care must be taken during sample collection, storage, and preparation, to ensure that the Cr species distribution present in the original sample is maintained up to the point of analysis.

This novel method, developed at Agilent, uses an optimized sample stabilization method, in which the samples were incubated at 40 °C with EDTA, which forms an anionic complex with the Cr(III), allowing a single chromatographic method to be used to separate the Cr(III)EDTA complex and the Cr(VI).

The Agilent 7500ce Octopole Reaction System (ORS) ICP-MS allows Cr to be measured with high accuracy and good sensitivity, using the main isotope at mass 52, by removing interferences from ArC and ClOH. The sample preparation method, column type, and chromatographic conditions used for Cr speciation are shown in Table 1.

The non-metal ion chromatography (IC) pump (Metrohm 818 IC Pump) was used to deliver the mobile phase, but the sample loop was filled and switched using the optional Integrated Sample Introduction System (ISIS) of the Agilent 7500ce ICP-MS. While this configuration maintains the high precision and relatively high pressure of the IC pump, it also provides a much simpler and lower cost alternative to a complete IC or HPLC system, since only the IC pump module is required in addition to the ICP-MS system.

Table 1. *Chromatographic conditions for Cr speciation.*

Sample Preparation	
Reaction temp	40 °C
Incubation time	3 h
EDTA concentration	5 ~ 15 mM pH 7 adjust by NaOH
Chromatographic conditions	
Cr column	Agilent p/n G3268A, 30 mm x 4.6 mm id
Mobile phase	5 mM EDTA (2Na), pH 7, adjust by NaOH
Flow rate	1.2 mL/min
Column temperature	Ambient
Injection volume	50 ~ 500 µL

Results and Discussion

Under the conditions described in Table 1, with ICP-MS detection using the Agilent 7500ce in H_2 cell gas mode to remove the ArC and ClOH interferences on Cr at mass 52, detection limits (DLs) of < 20 ng/L were obtained for the individual Cr species, as shown in Table 2.

Many international regulations for hexavalent Cr specify a maximum allowable concentration of 1 µg/L, with a required DL of one-tenth of this level (100 ng/L), and even the small sample volume injection of 100 µL easily meets these requirements. However, increasing the injection volume to 500 µL allowed the DLs to be reduced to 13.2 ng/L for Cr(III) and 15.8 ng/L for Cr(VI).

In order to test the suitability of the method for real-world sample types, the method was applied to the determination of both Cr species in spiked and unspiked mineral water samples.

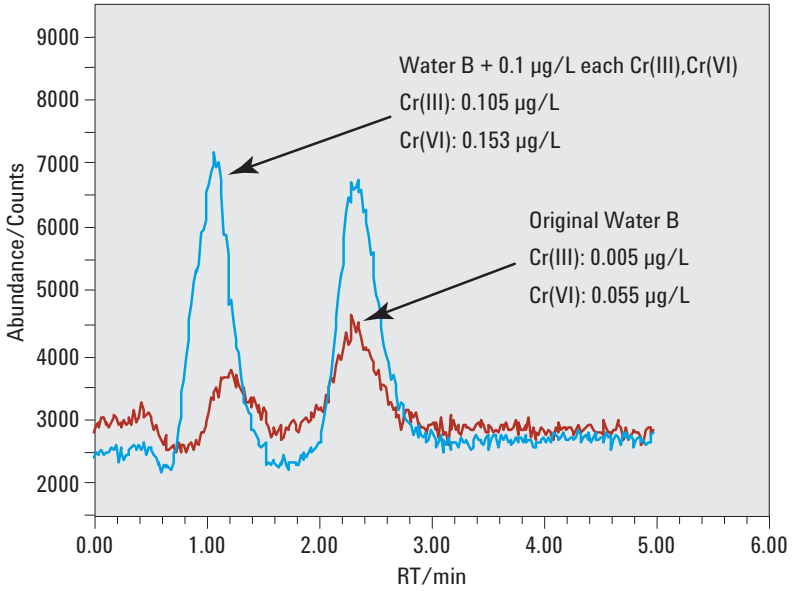
One mineral water sample analyzed was a French mineral water, referred to as mineral water B, which has among the highest levels of calcium and sulfates of any commonly available mineral water (over 450 mg/L Ca and more than 1,000 mg/L sulfates). Mineral water B was analyzed with and without a spike of the two Cr species and the spike recovery was assessed. The results

for the measured samples are shown in Figure 1. The major element composition of the mineral water is shown below the chromatogram, illustrating the very high mineral levels. Despite these high major element levels, the optimized sample prep and chromatographic method gave good chromatographic separation and identification for both Cr species.

The ability to recover low concentration spikes for both Cr species in such a high matrix sample indicates the effectiveness of the optimized method for sample stabilization, which ensures that a high enough concentration of EDTA is available for complete complexation of the Cr(III) species, even in the presence of a high level of competing ions. Furthermore, the accurate recovery of low concentration spikes of both species indicates that potential problems of species interconversion (reduction of Cr[VI] to Cr[III]) were avoided through the selection of an appropriate pH for the samples and the mobile phase, together with the use of EDTA in the mobile phase as well as for sample stabilization. See Table 2.

For Additional Information

For a full account of this application see: "Ion Chromatography (IC) ICP-MS for Chromium Speciation in Natural Samples," Agilent Technologies publication 5989-2481EN.



Na	7.3 mg/L
Ca	91.0 mg/L
Mg	19.9 mg/L
K	4.9 mg/L

Figure 1. *Major element composition (mg/L) and chromatogram for spiked mineral water B.*

Table 2. *Detection limits for Cr species by IC-ICP-MS.*

	Retention time/min		Peak area/counts		DL (S/N = 3) ng/L	
Inject/µL	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
50	0.79	2.09	1082295	914804	69.5	139.4
100	0.79	2.09	1704312	1525147	43.4	82.8
250	0.85	2.21	4939876	4546219	17.5	28.5
500	0.97	2.39	10268086	9398651	13.2	15.8

Multi-Element Speciation Using Ion Chromatography Coupled to ICP-MS

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Introduction

ICP-MS has been shown to be a powerful tool for the measurement of ionic species of single elements separated by ion chromatography (IC). However, simultaneous speciation of multiple elements has been challenging for a number of reasons. Chromatographic elution and separation of elements with widely varying ionic properties can be difficult, such as when some of the species exist as cations while others are anionic. Furthermore, detection limits can be compromised when polyatomic interferences overlap one or more of the analytes of interest. By combining simple anion chromatography with chelation using EDTA, species preservation and efficient separation of multiple species of 13 elements was achieved simultaneously under a single set of conditions. If necessary, polyatomic interferences that would normally interfere with the measurement of several elements, including Cr, As, and Se, can be eliminated using the Octopole Reaction System (ORS) of the Agilent 7500ce ICP-MS.

Hardware Setup

The IC-ICP-MS system (Figure 1) consists of a nonmetal IC pump (Metrohm), the Agilent Integrated Sample Introduction System (ISIS), and an Agilent 7500ce ICP-MS. The IC pump was used to deliver high-pressure mobile phase to the anion exchange column via the ISIS high-pressure 6-port valve and sample loop.

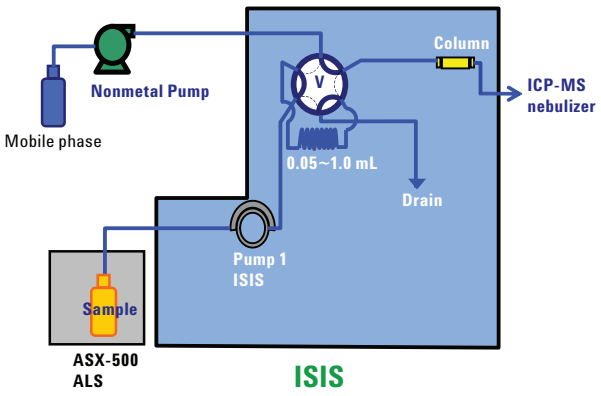

Figure 1. *Schematic of the IC-ICP-MS arrangement.*

Methods

Standards and samples were prepared by chelation with EDTA [1, 2, 3]. Chelation with EDTA serves two purposes. First, it converts all ionic species to anions, allowing separation by simple anion-exchange. Second, it helps to maintain the original species composition. This is particularly important for elements like chromium, which can exist as stable anions but are quite labile cations in nature. The rate of formation of stable Cr(III)-EDTA complex was found to be highly temperature dependent (Figure 2), and was determined to be 50 °C.

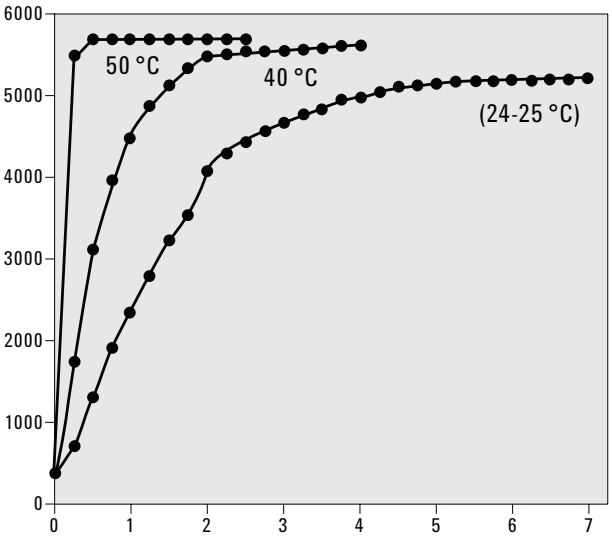

Figure 2. *Rate of formation of stable Cr(III)-EDTA complex as a function of time and temperature.*

Table 1. *Ion chromatography and ICP-MS conditions.*

Ion Chromatography	
IC column	Excelpack ICS-A23
Mobile phase	3.0 mM Na_2CO_3
Eluent flow	1 mL/min
Injection volume	50 µL
Run time	20 min
ICP-MS	
Isotopes acquired	^{31}P, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{66}Zn, ^{75}As, ^{78}Se, ^{79}Br, ^{127}I, ^{182}W, ^{208}Pb
Acq mode	Time resolved
Dwell time	0.1 s/point
RF power	1450 watts
Sample depth	8.5 mm
Carrier gas flow	1.1 L/min
Nebulizer	MicroMist
ORS mode	No gas mode

Results

Separation and detection of 20 species from 13 different elements was obtained in less than 20 minutes (Figure 3). Detection limits are approximately 0.5 µg/L for the most of elements.

Conclusions

Since ICP-MS is able to differentiate ionic species by their elemental mass spectra in addition to their retention time, it is not necessary to chromatographically resolve all species from each other as it would be with conductivity detection. This permits simultaneous analysis of multiple species under rapid, simple conditions that may not separate all species in time.

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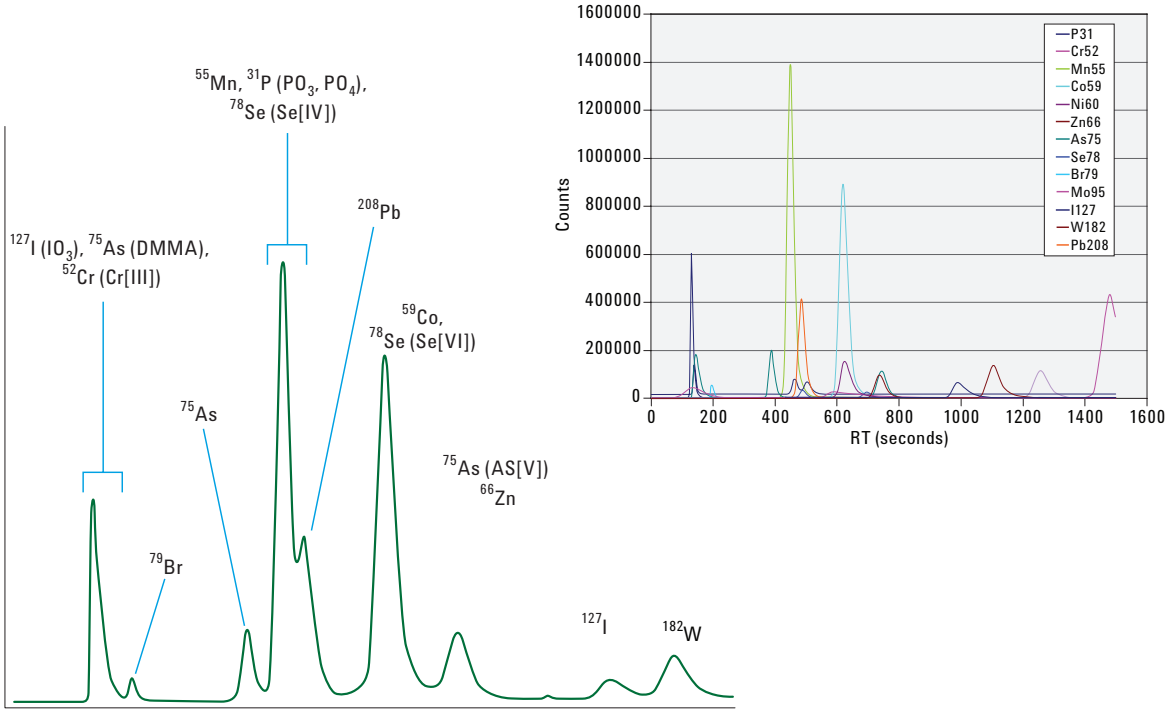

Figure 3. *Total ion chromatogram depicting simultaneous speciation of multiple elements. Inset shows extracted ion chromatograms of the 13 elements measured. Because of the elemental specificity of ICP-MS, it is not always necessary for each species to be chromatographically resolved.*

Determination of Trivalent and Hexavalent Chromium in Pharmaceutical, Nutraceutical, and Biological Matrices Using IC-ICP-MS

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Introduction

Chromium is a transition metal that can form complexes in four different valence states (CrIII, CrIV, CrV, and CrVI). However, the two most stable oxidation states, and therefore the most commonly detected, are complexes of trivalent and hexavalent chromium. Trivalent chromium (CrIII) has been characterized as an essential nutrient involved in carbohydrate and lipid metabolism, while hexavalent chromium (CrVI) is considered highly toxic.

Cr(VI) complexes, such as chromate $(CrO_4)^{2-}$, at physiological pH easily penetrate cell membranes through the sulfate and phosphate anion channels. It is accepted that for Cr(VI) to exert its cytotoxic and carcinogenic effects, it has to be reduced inside the cell. In contrast, cellular membranes are normally impermeable to Cr(III) cations, which can penetrate the cell membrane only under specific conditions.

After recent studies suggesting ingestion of Cr(VI) may cause chromate-induced cancers, there has been a renewed interest in the ability to separate, identify, and quantify trivalent and hexavalent chromium in a broad range of sample matrices. Additionally, the use of biological agents, such as bacteria or plants for bioremediation of chromium-polluted soils and water, is an active area of research. In the environment, Cr(VI) salts do not bind to constituents in the soil or readily precipitate from water. This creates the need to find naturally occurring organisms to reduce the Cr(VI) species to Cr(III) in the aqueous environment. Filamentous fungi have been shown to cause the complete reduction of Cr(VI) to Cr(III), without accumulating chromium in the biomass.

Experimental

This method exploits the ability of EDTA to form metal complexes. At pH 7, Cr(III) exists as a hydrated chromic cation (Cr^{3+}), while Cr(VI) exists as the chromate anion $(CrO_4)^{2-}$. Under these conditions a Cr(III)-EDTA complex is weakly retained on an anion exchange column amidst a strongly retained chromate anion, resulting in a resolution number greater than 1.5. Incubation times and temperatures were optimized for the greatest Cr(III) response, in conjunction with the concentration of the EDTA complexing solution, in an attempt to minimize sample preparation.

A powdered pharmaceutical sample was submitted to our laboratory for total chromium and chromium speciation analysis. The company wanted to determine which and how much of each chromium species, Cr(III) and Cr(IV), was present in its finished product. A similar determination was performed on over-the-counter nutraceutical samples from GNC (General Nutrition Center) consisting of powdered tablets containing 200 µg of chromium picolinate and over-the-counter gel caps (oil-based), also containing 200 µg of chromium picolinate. The tablets were manufactured in 1999, while the gel caps were manufactured in 2006. Standards and samples were prepared under the same conditions (see Table 1).

The pharmaceutical and nutraceutical samples were first dissolved in 200 mL of the 15 mM EDTA solution and allowed to incubate for 15 minutes in a 60 °C circulating water bath. Standards were prepared in double-distilled deionized water and diluted in a manner to maintain and minimize a change in the 15 mM EDTA concentration and incubated for 15 minutes in the 60 °C circulating water bath.

Fungal growth media were analyzed to illustrate the efficiency and reductive capability of the fungi to bioremediate 50 ppm of spiked Cr(VI) (ongoing research). Multiple samples of 500 mL aliquots of fungal growth medium solution were first frozen on dry ice and then lyophilized. After lyophilization, 500 µL of 15 mM EDTA solution was added to each sample and allowed to incubate for 30 minutes in a 60 °C circulating water bath for maximum Cr(III) response.

Monitoring chromium isotopes ^{50}Cr, ^{52}Cr, and ^{53}Cr, allowed for unambiguous chromium identification. ^{54}Cr was also monitored; however, it is a minor isotope (2.3%) and suffers from an isobaric interference from iron. Quantifications were attained by extracting the ^{52}Cr isotope trace and integrating peak areas with the chromatographic data analysis software (Figure 1). The Metrohm 818C isocratic pump and six-port valve were controlled by the Integrated Sample Introduction System (ISIS) software. Both the ISIS and chromatographic software options are integrated into the ICP-MS ChemStation software.

Table 1. *Chromatographic and ICP-MS conditions for Cr(III) and Cr(VI) speciation.*

IC Conditions	
LC pump	Metrohm 818C isocratic pump (PEEK)
Column	Agilent Technologies G3268A, 30 mm x 4.6 mm id
Mobile phase	5 mM (disodium) EDTA at pH 7 adjusted with NaOH
Flow rate	1.2 mL/min
Injection volume	100 µL PEEK sample loop
ICP-MS Conditions	
	MicroMist nebulizer (Glass Expansion) with Scott spray chamber 7500ce (Agilent Technologies) 3.5 mL/min H_2 collision gas Masses monitored (*m/z*): 50, 52, 53, 54
Sample Incubation	
Reaction temperature	60 °C (water bath or microwave)
Incubation time	15 min
EDTA concentration	15 mM at pH 7 adjusted with NaOH
Sample matrix	Pharmaceutical, nutraceutical, fungi (growth media)

Results and Discussion

Under the conditions previously stated, the LOD ($\sigma = 3$) for Cr(III) and Cr(VI) were 0.10 µg/L and 0.15 µg/L, respectively. The LODs are based upon a 100-µL PEEK sample loop and 15- and 30-minute incubation times (for drug samples and fungal samples, respectively). The LODs could easily be improved by using a larger sample loop. Table 2 illustrates the method's reproducibility for both response and retention time.

Table 2. *Cr(III) and Cr(VI) 50 ppb standard (n = 7).*

	Cr(III) Area	Cr(VI) Area	Cr(III) RT	Cr(VI) RT
Average	1452262	1965195	0.932 min	4.420 min
Std Dev	21065	42945	0.003 min	0.021 min
% RSD	1.5	2.2	0.3	0.5

Conclusions

By optimizing the incubation times and temperatures, along with the EDTA concentration, a sample preparation time of only 15 minutes for the drug samples and 30 minutes for the fungal samples was achieved. Stable and reproducible separation, identification, and quantification of Cr(III) and Cr(VI) were accomplished in under 7 minutes using this method. This brings the total time per sample to less than 25 minutes for the drug samples and less than 40 minutes for the fungal samples, while attaining low, reproducible LOD for each species.

For Additional Information

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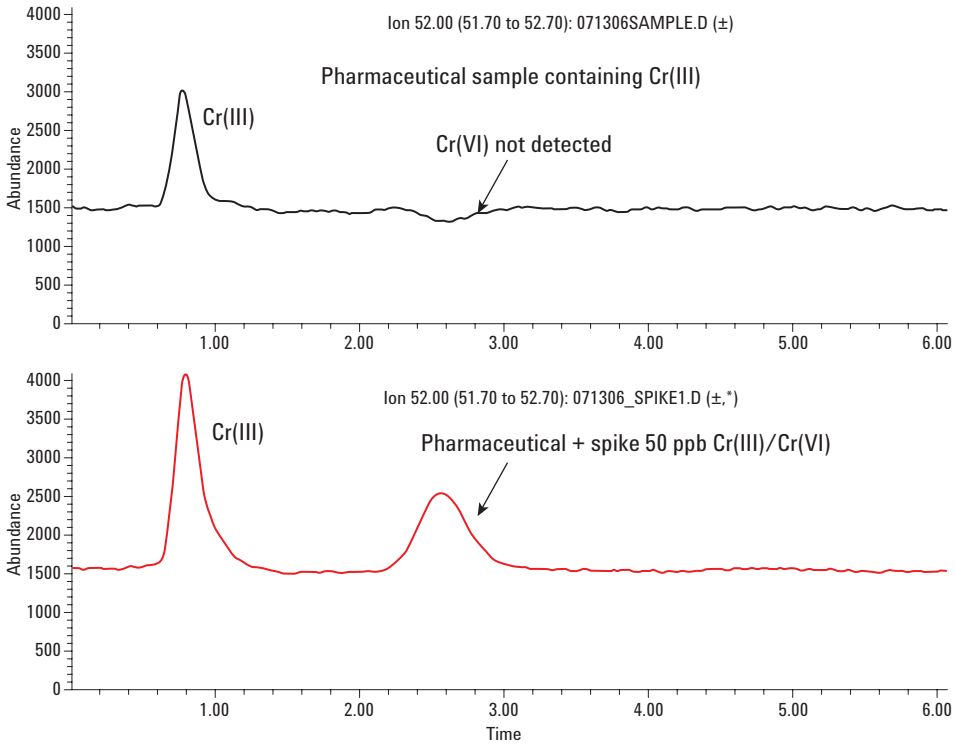

Figure 1. *Chromatograms showing separation and identification of Cr(III) and Cr(VI).*

Determination of Iodine Species Using IC-ICP-MS

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Introduction

Iodine is an essential micronutrient in mammals, necessary for proper production of thyroid hormones. A deficiency leads to various disorders associated with growth and development. Iodized salt is a common source of iodine, but excessive intake can lead to the development of high-iodine goiters, which in turn can lead to retarded brain development and functional impediment. The bioavailability and toxicity of iodine, like other essential elements, is species dependent. Inorganic iodide and iodate are less toxic than molecular iodine and some organically bound forms of the element. Likewise, the bioavailability of organically bound iodine is also lower than that of mineral iodine [1,2].

Instrumentation

An Agilent 7500a ICP-MS was coupled to an Agilent 1100 HPLC with ICS-A23 ion chromatography column (available only in Japan; Cr speciation column, G3268-80001, may work with some modified conditions) and ICS-A2G guard column. The mobile phase was 0.03 mol/L ammonium carbonate solution (pH 9.4).

Species Stability of Iodide and Iodate

In order to choose a suitable storage medium, pure water, the mobile phase, 0.01% KOH, and 0.1% KOH were tested. Interestingly, iodide was unstable and lost in pure water and the mobile phase medium when the stock solution was diluted or kept for a long time. Dilution factors from 10 to 10,000 and storage from one to five days were tested. However, iodide was found to be stable in 0.01% KOH and 0.1% KOH. No stability problems were observed with iodate in different media. In order to minimize any potential high-matrix effects, 0.01% KOH was selected as the storage medium for field sampling.

Linear Range and Detection Limits

The linear range of iodate was more than four orders of magnitude from 5 nmol/L to 50 µmol/L. Linear regression was investigated for all species and r^2 was found to be 0.9999. The repeatability (n = 7) for 50-nmol/L injections of iodate and iodide was 2.1% and 3.3%, respectively (Figure 1).

Application of Method

The method described is being used in conjunction with total iodine measurements by ICP-MS in a survey of various groundwater samples. Preliminary findings have identified that some samples contain iodine as iodide and iodate in which the sum of the two inorganic iodine species does not differ significantly from the total iodine concentration. In some cases, the sum of the two inorganic iodine species differs significantly from the total iodine concentration directly determined by ICP-MS. It is believed that this difference is due to the presence of organoiodine compounds. The theory was tested using SEC-ICP-MS.

A representative chromatogram is shown in Figure 2. The results suggest that organoiodine and iodide were the main species in these unusual groundwater samples.

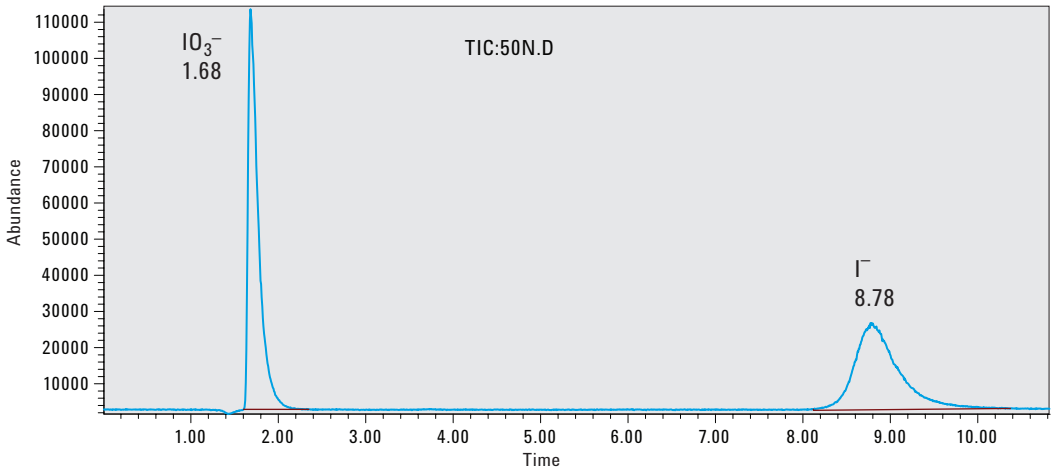

Figure 1. *IC-ICP-MS chromatogram of 50 nmol/L iodate and iodide standard solution (0.01% KOH medium, mobile: 0.03 mol/L ammonium carbonate).*

Conclusions

We have successfully developed a method to separate inorganic iodine anions using HPLC with detection using ICP-MS. The use of 0.01% KOH stabilizes the solutions and provides for species independence in terms of calibration. The method is being applied to a survey of ground-water samples, and for the majority of samples it is very successful. Some samples behave differently and there is evidence (using SEC-ICP-MS) that in these cases iodine is present in the form of organoiodine compounds.

References

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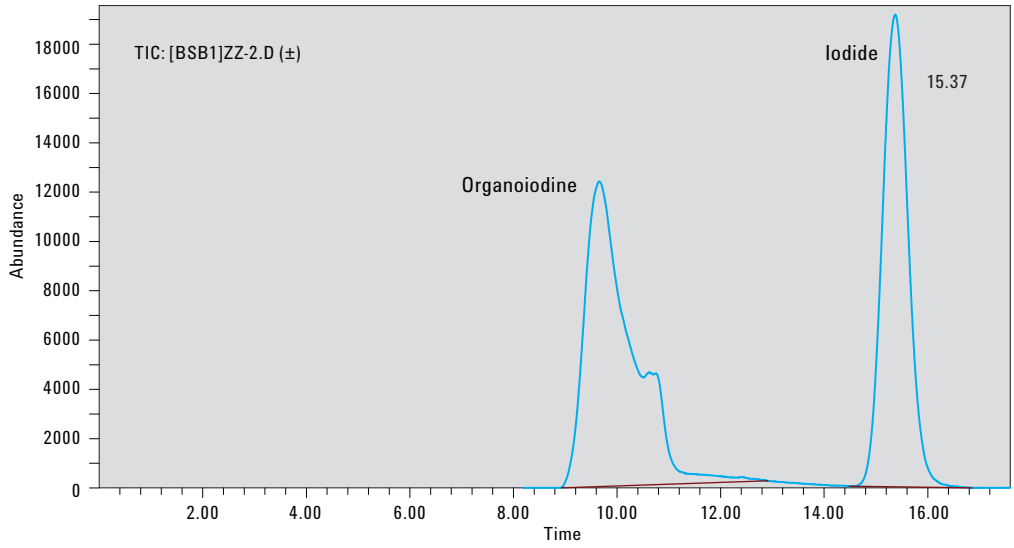

Figure 2. *SEC-ICP-MS chromatogram of organoiodine and iodide from a groundwater sample. Mobile phase: 0.03 mol/L ammonium carbonate, pH 9.4.*



GC-ICP-MS

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GC-ICP-MS Introduction

GC-ICP-MS is used for the analysis of volatile organic or organometallic compounds when no other GC detector can provide the required elemental or isotopic specificity or sensitivity. Furthermore, because of the generally higher resolution of GC compared with LC, it is sometimes advantageous to create volatile derivatives of otherwise non-volatile compounds for analysis by GC. When used as a detector for GC, ICP-MS provides several other advantages over alternative elemental detectors.

- ICP-MS is almost universal (only hydrogen, helium, argon, fluorine, and neon cannot be directly measured).
- ICP-MS can tolerate a wide range of GC carrier gases and flows.
- ICP-MS permits the use of compound independent calibration, which is useful for screening or when standards are expensive or unavailable.
- ICP-MS does not typically suffer from suppression of analyte response due to coeluting compounds.
- ICP-MS is capable of isotope dilution quantification.

The Agilent GC-ICP-MS interface consists of a heated, passivated transfer line and a special torch with a heated injector tube. In this way, the sample is maintained at constant high temperature from the end of the chromatographic column in the GC oven to the tip of the ICP injector (Figure 1).

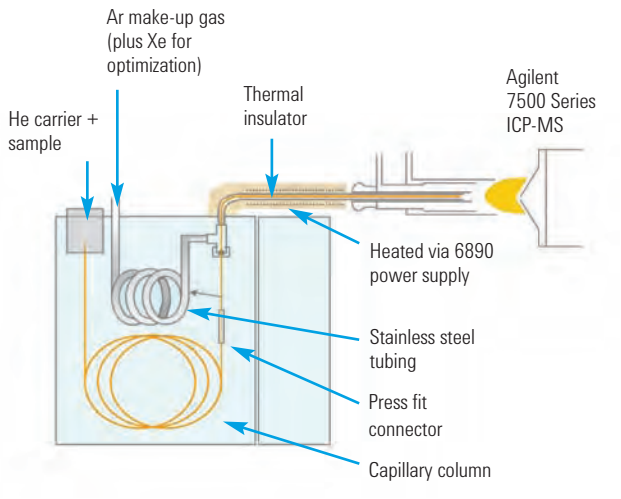

Figure 1. *Schematic diagram of Agilent GC-ICP-MS system.*

Tuning and Optimization of ICP-MS for GC Applications

Tuning the ICP-MS requires optimization of the plasma for efficient ion production, ion optics, and torch position for best sensitivity, octopole reaction cell (if equipped) for optimum interference removal, quadrupole for mass resolution and mass calibration, and detector for sensitivity and linear dynamic range. In conventional ICP-MS, these conditions are met by aspirating an aqueous tune solution containing several elements upon which the system is optimized. However in the case of GC-ICP-MS, the liquid sample introduction system is not fitted and the plasma conditions are sufficiently different that a solution-based tune would not be appropriate. In this case, tuning and optimization must be carried out using a gaseous tune sample. Normally, this is accomplished through the addition of 0.05% – 0.1% xenon in helium or argon, either in the GC carrier gas or in the argon makeup gas. Since Xe is composed of nine isotopes between masses 124 and 136 and ranging in abundance from ~0.1 to 26% relative abundance, it provides numerous good tuning points. Since it is introduced with the GC carrier gas, it is especially useful in optimizing the horizontal and vertical torch positions, which are critical to optimum sensitivity due to the narrow injector diameter of the GC torch. If a wider range of masses is needed, other tuning gases can be used, or, alternatively, plasma background masses such as 38 and 78 can be used.

Since the Agilent GC-ICP-MS interface does not rely on introduction of a wet aerosol for tuning or operation, optimization is somewhat different from typical wet plasma conditions. First, because no water or acids are being continuously introduced, polyatomic interferences, particularly oxides, are mostly eliminated. Second, without the introduction of cooling water, much lower plasma power is required for complete ionization, even of high IP elements. Typically, optimum performance is achieved with plasma power set between 600 and 700 watts.

Use of Optional Gases

In addition to Xe for tuning, the addition of other gases to the sample flow can have benefits. Adding a small amount of oxygen can be used to prevent carbon deposits on the interface components (primarily the cones) by oxidizing elemental carbon to carbon dioxide. Optional gases, including oxygen and nitrogen, have also been shown to enhance the sensitivity for several common analyte elements, including Sn, As, Se, and others. These gases are typically added via a Tee into the argon makeup gas line. Their flow can be controlled by either the auxiliary mass flow controller on the ICP-MS or by an optional mass flow control channel on the GC.

Analysis of Polybrominated Diphenyl Ether (PBDE) Flame Retardants by GC-ICP-MS

Steve Wilbur and Emmett Soffey, Agilent Technologies, Bellevue, WA, USA

Introduction

PBDEs are widely used flame retardants added to many common household products, including textiles, mattresses and furniture, and electronic devices. Their similarity in structure to PCBs and dioxins has raised concerns about health risks associated with their use. Recently some classes of these compounds have been banned in Europe in response to Restriction of Hazardous Substances (RoHS) regulations and and voluntarily removed from production in the US. However, some classes are still in use, and the compounds are widely distributed within the environment. Gas chromatography is typically used in the separation of these compounds, since the large number of possible congeners (209) makes LC separation impractical. However, the low volatility, high molecular weight, and fragile nature of some congeners make GC analysis difficult. In particular, identifying trace levels of PBDEs in the presence of other halogenated compounds is difficult with conventional GC detectors.

Hardware Setup

The analytical system consisted of an Agilent 6890N GC interfaced to an Agilent 7500a ICP-MS using the Agilent GC-ICP-MS interface. GC and ICP-MS conditions are summarized in Table 1. A short 5 M x 0.25 mm x 0.25 µm Agilent DB-5MS column was used. The GC was equipped with the optional three-channel auxiliary EPC module to control the addition of optional gases, including oxygen and helium. Oxygen is added to the plasma gas to burn off carbon deposits on the sample and skimmer cone. Helium is added to the carrier gas to enhance the sensitivity for bromine. 100 ppm Xe in He was used as an alternate GC carrier gas supplied to the GC via a manual switching valve to allow either pure He or Xe in He to be used. Xe is used to tune the ICP-MS for maximum sensitivity and can also be used as an online internal standard.

Standards and Reagents

PBDE standards were purchased from AccuStandard Inc. (New Haven, CT, USA) and diluted into either semiconductor-grade xylene or pesticide-grade isooctane. No certified standard reference materials are currently available for PBDE compounds in real matrices.

Method

Table 1. *Method parameters for the separation of PBDEs using the GC with ICP-MS detection.*

GC	
Injection	Split/splitless - 1µL
Oven program	80 °C (1 min), 20°/min –> 320 °C (5 min)
Carrier gas	He at 7 mL/min
Transfer line temp	250 °C
ICP injector temp	280 °C
ICP-MS	
Isotopes acquired	79, 81
Acq mode	Time resolved
Dwell time	0.1 s/point
RF power	650 watts
Sample depth	7 mm
Carrier gas	1.05 L/min
Extract 1	–180 V

Results

GC-ICP-MS as described is capable of rapid, sensitive detection of PBDEs, including the difficult-to-analyze deca-bromo congener (BDE-209), see Figure 1 and Table 2. Analysis times of less than 12 minutes with detection limits of ~150 fg on column (0.15 ppb) can be achieved.

Conclusions

ICP-MS is the ideal GC detector for PBDEs and other bromine-containing volatile organics. It is sensitive, selective, and can tolerate a wide range of GC carrier gases and flows. Very high GC flows allow rapid elution of deca-BDE, which improves recovery.

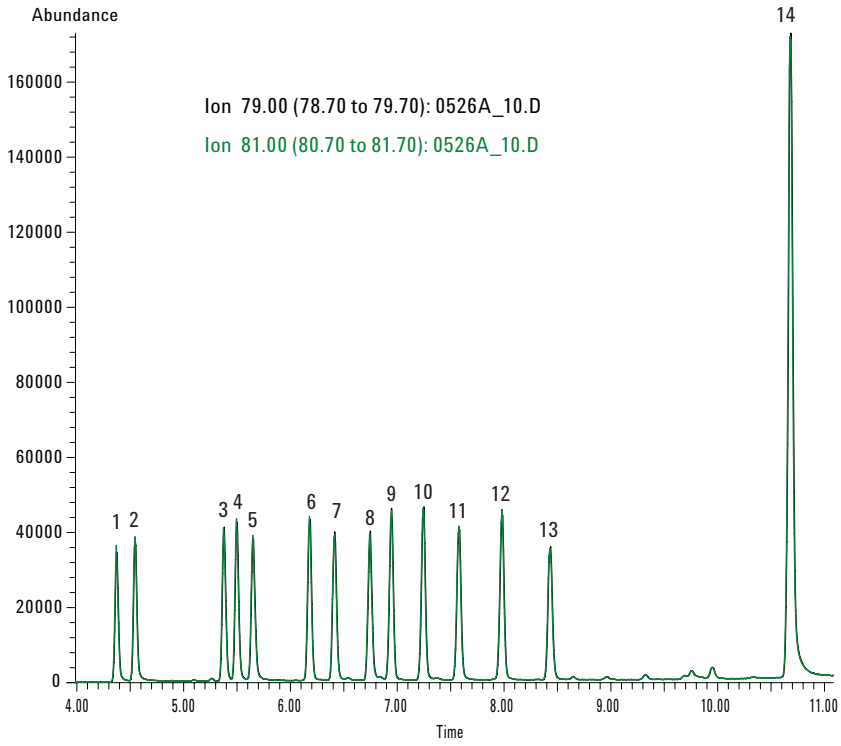

Figure 1. *GC-ICP-MS chromatogram of 10 ppb PBDE standard mix.*

Table 2. *PBDE peak identification.*

Peak	Congener (2.5 mg/mL)
1	2,2′,4-TriBDE (BDE-17)
2	2,4′,4-TriBDE (BDE-28)
3	2,3′,4′,6-TetraBDE (BDE-71)
4	2,2′,4,4′-TetraBDE (BDE-47)
5	2,3′,4,4′-TetraBDE (BDE-66)
6	2,2′,4,4′6-PentaBDE (BDE-100)
7	2,2′,4,4′5-PentaBDE (BDE-99)
8	2,2′,3,4,4′-PentaBDE (BDE-85)
9	2,2′,4,4′,5,6′-HexaBDE (BDE-154)
10	2,2′,4,4′,5,5′-HexaBDE (BDE-153)
11	2,2′,3,4,4′,5′-HexaBDE (BDE-138)
12	2,2′,3,4,4′,5′,6-HeptaBDE (BDE-183)
13	2,3,3′,4,4′,5,6-HeptaBDE (BDE-190)
14	DecaBDE (BDE-209) (12.5 mg/mL)

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Detecting the New PCBs using GC-ICP-MS – Challenges of PBDE Analysis, *Agilent ICP-MS Journal,* 18, January 2004, 5989-0588EN.

Analysis of Sulfur in Low-Sulfur Gasoline by GC-ICP-MS

Steve Wilbur and Emmett Soffey, Agilent Technologies, Bellevue, WA, USA

Introduction

Sulfur in motor fuels has been implicated in global warming and acid rain. It is also a catalyst poison for automobile catalytic converters and refinery catalytic crackers. Reducing total sulfur in motor fuels has become a critical air pollution control goal worldwide. The USEPA tier-2 guidelines beginning in 2004 mandate an average sulfur standard of 30 ppm and a cap of 80 ppm total sulfur by 2007. The European Union announced in December of 2002 that new regulations would require full market availability of sulfur-free fuels, defined as containing less than 10 parts per million (ppm) sulfur content, by January 1, 2005. GC-ICP-MS has the capability to meet current and projected detection limits for both total sulfur in reformulated gasolines and other motor fuels as well as individual sulfur species. Additionally, GC-ICP-MS can identify and quantify other volatile organometallic species in fuels.

Hardware Setup

An Agilent 6890 gas chromatograph with split/splitless injector was coupled to an Agilent 7500a ICP-MS using the Agilent GC-ICP-MS interface. GC and ICP-MS conditions are summarized in Table 1.

Standards and Reagents

Calibration was based on a multi-level analysis of thiophene and 2-methyl thiophene spiked into 3:1 isooctane/toluene obtained from Ultra Scientific. Calibration levels ranged from 2.5 ppm per compound to 500 ppm per compound (Figure 1). Because GC-ICP-MS is capable of compound-independent calibration, it was not necessary to calibrate every possible sulfur compound separately. The sulfur response factor for any compound(s) can be determined from a single compound. In this case, the response factors from thiophene were used and confirmed by those from 2-methylthiophene.

Table 1. *GC and ICP-MS operating parameters.*

Instrumentation	
GC	Agilent 6890 GC
Inlet	Split/splitless
Detector	Agilent 7500a ICP-MS
Column	30 M x 0.25 mm id x 0.25 µm HP-5
GC Conditions	
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Split 1:50
Carrier gas	Helium
Carrier gas flow	2.5 mL/min (constant flow mode)
Transfer line temperature	250 °C
Oven temperature	40 °C /4 minutes, 20 °C/min to 250 °C, program hold for 1 min
ICP-MS Conditions	
Forward power	700 watts
Sample depth	13 mm
Carrier gas flow	1.1 L/min
Extract 1	–150 V
Extract 2	–75 V
Aux gas	He, 10 mL/min added to Ar carrier
Injector temperature	260 °C

Results

Chromatograms of three standard reference gasolines and a standard reference diesel are shown in Figure 2. Comparison with quantitative results for total sulfur compared favorably with those obtained by x-ray fluorescence [1]. Single compound detection limits for thiophene and 2-methyl thiophene are less than 5 ppb. When translated to total sulfur in gasoline, the detection limit is approximately 0.1 to 0.5 ppm.

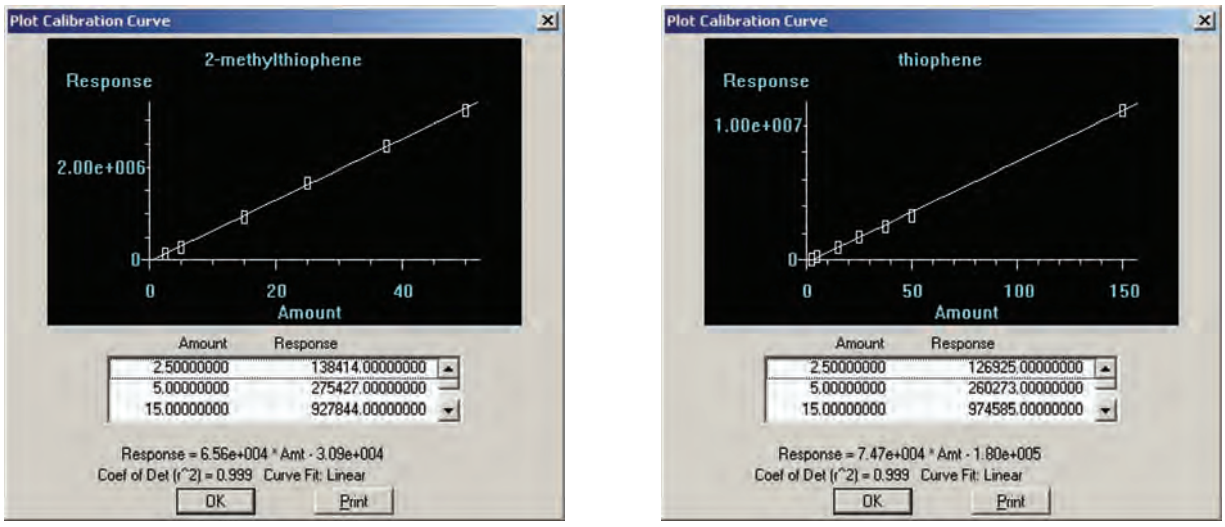

Figure 1. *Calibration curves, thiophene and 2-methylthiophene in 3:1 isooctane:toluene.*

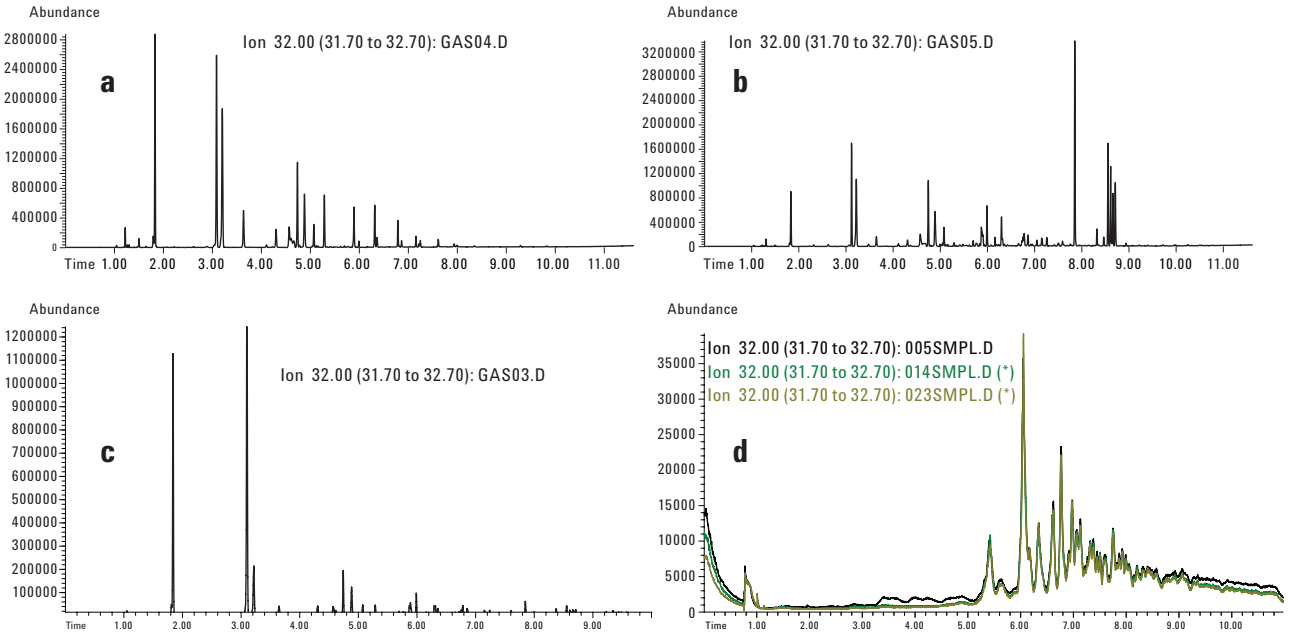

Figure 2. *a) ASTM-Fuel-QCS02, conventional gasoline QC sample, ~330 ppm total sulfur. b) ASTM Round Robin Gasoline Standard #2, not certified for sulfur. c) CARB low-sulfur reformulated gasoline, ~55 ppm total sulfur. d) Low-sulfur diesel diluted to ~5.6 ppm total sulfur, analyzed in triplicate.*

Conclusions

GC-ICP-MS offers significant advantages over other techniques for the analysis of total sulfur and sulfur species in motor fuels. These include high sensitivity, wide dynamic range, freedom from interferences and suppression, ability to use compound-independent calibration as well as the ability to simultaneously monitor other elements.

References

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For Additional Information

Quantification and Characterization of Sulfur in Low-Sulfur Reformulated Gasolines by GC-ICP-MS, Agilent Technologies publication 5988-9880EN.

Combining GC-ICP-MS and Species-Specific Isotope Dilution Mass-Spectrometry (SS-IDMS)

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Introduction

The toxic effects of organotin compounds in the environment have been well documented [1] and have led to extensive research into analytical methodologies for their determination in a variety of matrices. The widespread use of organotin compounds in pesticides and antifouling paints and as heat and light stabilizers in PVC products has resulted in their detection in most marine and fresh-water sediments and in open-ocean waters [2]. In recent years, the focus of research in organotin analysis has begun to include matrices with human health implications, such as seafood [3], artificial matrices such as PVC pipes used for drinking water distribution [4], and human blood [5] and liver samples [6]. Currently, a wide range of methods is being used for the extraction, separation, and detection of organotin compounds, and significant variation in the results can be obtained by different methodologies [7, 8].

Generally, the separation method of choice has been gas chromatography (GC), which allows for the analysis of many different groups of organotin compounds (for example, butyl-, phenyl-, octyl-, and propyl) in a single analysis after derivatisation [9]. GC separation has been successfully coupled to a variety of detectors, such as atomic absorption spectroscopy (AAS) [10, 11], atomic emission detection (AED) [12], microwave-induced plasma atomic emission detection (MIP-AED) [13, 14], and, more recently, inductively coupled plasma mass-spectrometry (ICP-MS) [15, 16]. All of these detectors can offer sufficient detection limits for organotin analysis.

However, the derivatization required for GC analysis can result in variation in yields between species and in terms of efficiency depending on matrix components. The use of SS-IDMS can effectively eliminate the bias that can be introduced by the derivatization step, and a number of high-throughput laboratories are now beginning to use the technique for this purpose. GC-ICP-MS has the unique potential to facilitate simultaneous multi-elemental speciation analysis, because species of several elements, such as Se [17], Pb [18], Hg [19], and Sn have volatile forms and could be analysed in a single analysis.

Species-specific isotope dilution mass-spectrometry (SS-IDMS) for organometallic speciation studies has been made possible by the synthesis of organometallic molecules containing an isotopically enriched hetero-atom [20]. Because IDMS has the capability to overcome shortfalls of analytical methods, such as analyte losses, analyte breakdown, or incomplete recovery (provided that complete equilibration between the spike and the inherent analyte has been achieved), the application to speciation methodologies can help reduce the uncertainties associated with such methods. SS-IDMS and GC-ICP-MS have previously been applied to the speciation of methylmercury [19] as well as organotin compounds [16].

The same approach for isotope dilution analysis was used for TBT and DBT in sediment as that described for nonspeciation IDMS measurements described by Catterick et al. [21]. This IDMS methodology relies on the approximate matching of the isotope ratios in both the sample (R'_B) and the calibration solution (R'_{Bc}). As described in reference 21 , this matching approach negates errors associated with mass-bias effects, detector dead-time, and the characterization of the spike. Because the concentration of the spike is eliminated from the calculation of the mass fraction in the sample (Equation 1), the time-consuming reverse isotope dilution mass spectrometry (RIDMS) used to characterize this value is not required.

Experimental

To carry out the IDMS analysis, alternating injections of the sample (SB) and calibration solutions (MB) were made. In order to obtain sufficient data for the calculation of meaningful uncertainty budgets, each sample extract was injected four times and bracketed by a total of five injections of the calibration blend. For each injection of the calibration blend, the measured isotope amount ratio (R'_{Bc}) was calculated from the ratio of the peak areas of ^{120}TBT and ^{117}TBT. The isotope amount ratio (R'_B) was also calculated in the same way for each sample injection. For the measured isotope amount ratio of the calibration blend (R'_{Bc}), the average of the two ratios measured before and after each sample blend isotope amount ratio (R'_B) were taken. The average of the four mass fractions was then reported as the mass fraction obtained for the blend analyzed. The final mass fraction was recalculated back to the original sample and corrected for moisture content.

The chromatographic peaks were integrated manually using the Agilent ICP-MS chromatographic software. The mass fraction obtained from the measurement of each sample blend injection was then calculated according to:

$$w'_X = w_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_{Zc}}{m_{Yc}} \cdot \frac{R_Y - R'_B \cdot \frac{R_{Bc}}{R'_{Bc}}}{R'_B \cdot \frac{R_{Bc}}{R'_{Bc}} - R_Z} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

R'_B	Measured isotope amount ratio of sample blend
R'_{Bc}	Measured isotope amount ratio of calibration blend
R_{Bc}	Gravimetric value of the isotope amount ratio of calibration blend
R_Z	Isotope amount ratio of primary standard Z (IUPAC value)
R_Y	Isotope amount ratio of spike Y (value from certificate)
w'_X	Mass fraction of Sn in sample X obtained from the measurement of one aliquot
w_Z	Mass fraction of Sn in primary standard Z
m_Y	Mass of spike Y added to the sample X to prepare the blend B
m_X	Mass of sample X added to the spike Y to prepare the blend B
m_{Zc}	Mass of primary standard solution Z added to the spike Y to make calibration blend Bc
m_{Yc}	Mass of spike Y added to the primary standard solution Z to make calibration blend Bc

The isotope amount ratios of the primary or natural TBTCl standard was calculated on the basis of the representative isotopic composition of Sn according to IUPAC. For the spike TBTCl, the isotopic composition was obtained from the certificate supplied with the ^{117}Sn enriched material from AEA Technology plc (UK).

Results

Table 1 shows the data obtained for the analysis of DBT and TBT in the CRM PACS-2 and Figure 1 shows a chromatogram obtained for this CRM. The comparison between the measured values and the certified concentrations for this reference material are in good agreement. A second evaluation of the GC-ICP-IDMS methodology was performed by comparing the data for the same samples when measured by LC-ICP-IDMS.

Table 1. *TBT and DBT determined in PACS-2 (fresh bottle) by ASE and GC-ICP-IDMS analysis.*

Analysis	DBT (ng/g Sn)	TBT (ng/g Sn)
1	1084	885
2	1081	879
3	1086	872
4	1097	869
Mean	1087	876
Expanded uncertainty (k = 2)	77	51
Certified concentration	1090	980
95% confidence interval	150	130

The comparative data provided for analysis of the same sediment extracts by HPLC-ICP-IDMS and GC-ICP-IDMS shows that the type of chromatographic separation used has no significant influence on the mass fractions determined. Therefore, the GC and HPLC methods can be used as an independent check on one another.

Table 2. *Comparative TBT data for the analysis of two different sediments (PACS-2 [old bottle] and P-18/HIPA-1) by both methods.*

All as ng/g Sn*	PACS-2 (old)	P-18/HIPA-1
LC-ICP-IDMS	828 ± 87	78.0 ± 9.7
GC-ICP-IDMS	848 ± 39	79.2 ± 3.8

*The values represent the mean result and their associated expanded uncertainty with a coverage factor of k = 2.

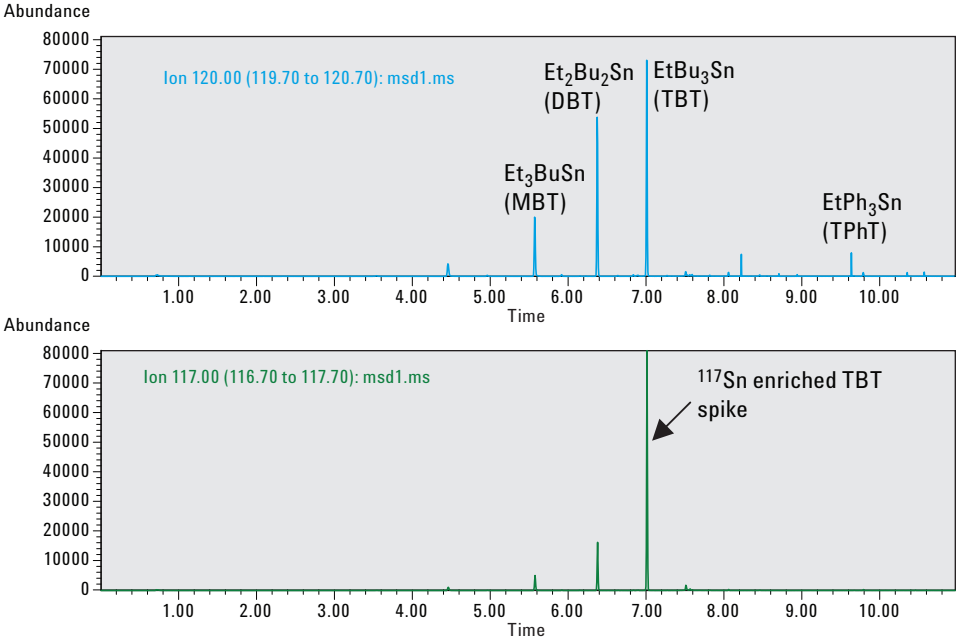

Figure 1. *GC-ICP-MS chromatogram of a sediment extract after ethylation.*

However, GC-ICP-MS analysis yields greater sensitivity with method detection limits based on sediment analysis (0.03 pg TBT [as Sn]) being two orders of magnitude better than achieved by HPLC-ICP-MS. Typical isotope amount ratio precisions achieved by GC-ICP-IDMS were superior by 1.5- to 2-fold compared to HPLC-ICP-IDMS. This difference is reflected in the expanded uncertainty associated with the final results, which are greater by HPLC-ICP-MS (10%) compared to GC-ICP-IDMS (5%) for analysis of the same extracts. It has been shown that the use of additional gases with GC-ICP-MS analysis can enhance the detection limits for a range of organotin compounds, and the addition of 0.1 L min^{-1} N_2 showed increases in peak areas of up to 150-fold compared to no gas addition.

The precision of the isotope amount ratio measurements for both methods can be compared for repeat injections of the mass-bias calibration blend solutions and the sample blends. Typical percent relative standard deviations achieved by HPLC-ICP-IDMS range from 0.3% to 1.4% for R'_B and from 0.7% to 1.3% for R'_{Bc}. For GC-ICP-IDMS the respective precisions achieved range from 0.5% to 1.0% for R'_B and 0.4% to 0.7% for R'_{Bc}. This data suggests that GC-ICP-MS can provide isotope amount ratios, which are 1.5- to 2-fold superior to HPLC-ICP-MS. This is partly explained by differences in the chromatographic separations and different plasma conditions used. The peak area integration by GC-ICP-MS is more reproducible than for HPLC-ICP-MS because the peaks are narrower, there is no significant background noise for the isotopes monitored, and the peaks do not suffer from tailing as much as the HPLC peaks. These differences in peak area integration are then reflected by the respective isotope amount ratio precision data for R'_B and R'_{Bc}, which shows GC-ICP-MS to have lower maximum RSD values (1.0% and 0.7%, respectively) compared to HPLC-ICP-MS (1.4% and 1.3%, respectively).

Conclusions

The combination of GC-ICP-MS and SS-IDMS can provide a number of advantages for organotin speciation analysis. On the one hand the GC-ICP-MS approach provides excellent sensitivity and chromatographic resolution for a wide range of organotin species whilst the SS-IDMS approach can either be used for high-accuracy high-precision analysis as shown above, or it can simply be used to eliminate the potential for bias due to differences in derivatization efficiency for different species or in different sample matrices that may otherwise lead to a significant bias in the result.

Acknowledgement

The data used for this article were first published in *Analytical and Bioanalytical Chemistry,* (2003), 377, 140-148 © LGC Limited 2003 and is used here with permission from LGC Limited.

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Determination of Phosphoric Acid Triesters in Human Plasma Using Solid-Phase Microextraction and GC-ICP-MS

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Introduction

Although phosphoric acid triesters are used as flame retardants and plasticizers in a variety of products, some of the alkyl phosphates like tris(2-chlororethyl) phosphate show neurotoxic and carcinogenic properties. Similarly, aryl phosphates such as triphenyl phosphate and 2-ethyl-hexyl diphenyl phosphate show allergenic and hemolytic effects. Analysis of these species in human blood plasma is gaining increasing attention due to their possible leaching from the plastic plasma collection bags.

Sample Preparation

Solid-phase microextraction (SPME) was utilized as a sample preparation step for extraction and preconcentration of phosphate esters from the human plasma samples stored in conventional polyvinylchloride plasma bags.

Instrumentation

An Agilent 6890 Series GC system for the separation of the species was connected via a heated GC-ICP-MS interface to an Agilent 7500cs ICP-MS with Octopole Reaction System collision/reaction cell. Separation of phosphoric acid esters was performed on a 30 M x 0.320 mm id x 0.25 µm DB-5 capillary column (Agilent Technologies, Folsom, CA, USA). The presence of phosphoric acid triesters in human plasma was further validated by SPME GC time-of-flight high-resolution mass spectrometry (GC-TOF-MS) using a Micromass GCT orthogonal time-of-flight mass spectrometer coupled to the Agilent 6890 GC.

Results and Discussion

To check the performance of the method, SPME analysis of spiked plasma samples containing known amounts of phosphoric acid ester standards was performed. The assay was linear ($r^2 > 0.993$) over a concentration range of 0.1 to 50 ng P/mL for each phosphoric ester studied (see Table 1). The detection limits were 50 ng/L for tripropyl phosphate, 17 ng/L for tributyl phosphate, 240 ng/L for *tris*(2-chloroethyl) phosphate, and 24 ng/L for triphenyl phosphate. Recovery of triphenyl phosphate increased from 5 to 66% after deproteinization of plasma samples while that for tripropyl, tributyl, and *tris*(2-chloroethyl) phosphates was in the range of 35%, 43%, and 49%, respectively, after sample deproteinization at 10 ng/mL of spiked concentration. Note that such a low analyte recovery is commonly encountered in drug determination from plasma due to considerable binding with the plasma proteins. The precision of the method was obtained by consecutive analysis of 10 replicate spiked plasma samples at 1 ng P/mL. The relative repeatability was below 15% for all the analytes. Validation of the method could not be performed due to lack of commercially available certified reference material for determination of analytes in the plasma matrix.

Application to Human Plasma Samples

Human plasma collected from a plasma bag was analyzed for the organophosphate esters. Presence of tributyl phosphate and tri-phenyl phosphate was detected in the plasma that was exposed to the polyvinyl chloride plasma collection bag for a two-week period (Figure 1b), while these compounds were absent in

Table 1. *Analytical performance characteristics of the phosphoric acid triester detection in human plasma.*

Phosphoric acid triesters	Limit of detection (ng P/L)	Retention time (min)	r^2	Method precision (%)	Recovery (%)
Tripropyl phosphate	50	3.36 ± 0.03	0.998	8	35
Tributyl phosphate	17	4.48 ± 0.01	0.999	11	43
Tris(2-chloro ethyl) phosphate	240	4.98 ± 0.01	0.993	7	49
Triphenyl phosphate	24	7.21 ± 0.04	0.995	14	66

the same plasma that had not been stored in the conventional plastic storage bags (Figure 1a). Levels of triphenyl phosphate were in the range of 0.2 ng P/mL, while that of tributyl phosphate was close to the detection limit of the method (0.02 ng P/mL). Both ^{31}P GC-ICP-MS and GC-TOF-MS chromatograms for the nonspiked human plasma are presented in Figure 1. Presence of triphenyl phosphate in natural plasma stored in polyvinylchloride bags was confirmed with high-resolution TOF-MS measurements. The identity of the triphenyl phosphate was verified by retention time matching, correct isotope pattern (M^{o+} and $[M-H]^+$) and accurate mass measurements (within 1 mDa accuracy) as seen in Figure 1. However, presence of tributyl phosphate could not be confirmed through GC-TOF-MS due to its very trace levels in the plasma samples.

Conclusions

Application of SPME in conjunction with GC-ICP-MS proved to be a very promising analytical method for determination of trace amounts of phosphoric acid esters in complex biological samples such as human plasma. The developed method is relatively simple, sensitive, reasonably fast, and solvent free.

Low detection limits obtained in the parts-per-trillion range also assisted in determination of triphenyl phosphate in human plasma previously stored in conventional plasma storage bags. Levels of this were found to be three orders of magnitude lower than its haemolytic EC20 value. Combination of GC-ICP-MS with GC-TOF-MS helped to confirm the presence of this species in plasma collected from the bag. The presence of this is attributed to the fact that triphenyl phosphate is applied as nonflammable plasticizer in polyvinyl chloride bags. Despite previous reports of large levels of ethylhexyl diphenyl phosphate in plasma stored in polyvinyl chloride bags, no evidence of this compound was found in our study.

For Additional Information

Monika Shah, Juris Meija, Baiba Cabovska, and Joseph A. Caruso, "Determination of Phosphoric Acid Triesters in Human Plasma Using Solid-Phase Microextraction and GC-ICP-MS," *Journal of Chromatography A*, 1103 (2006) 329-336.

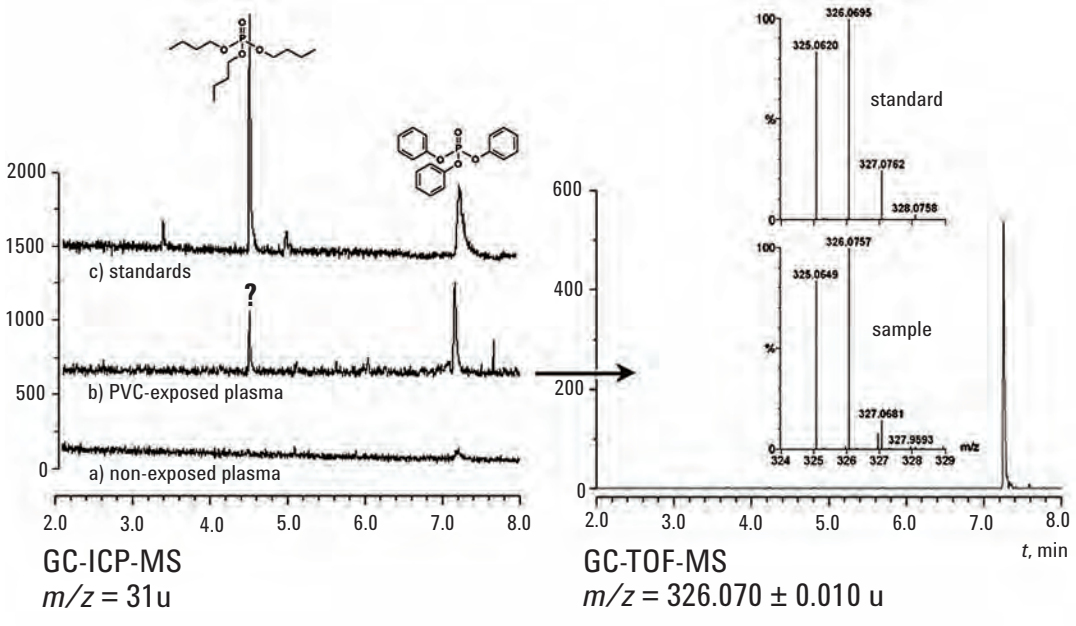

Figure 1. *Analysis of phosphoric acid triesters in human plasma with GC-ICP-MS (left) and GC-TOF-MS showing extracted ion chromatogram for triphenyl phosphate and Mo^+ and $[M-H]^+$ ions for sample and standard (right). (a) Native human plasma, (b) human plasma that has been stored in a polyvinyl chloride bag, and (c) human plasma spiked with 1 ng(P)/mL (1 ppb) of phosphoric acid triesters. SPME extraction was performed after sample deproteinization, and addition of 0.70 g NaCl at pH 7.0. Extraction was carried out with a 65-µm PDMS-DVB fiber for 30 min at 40 °C.*

Analysis of Methylmercury and Inorganic Mercury (Hg^{2+}) in Biological Tissue by Isotopic Dilution GC-ICP-MS

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Introduction

The predominant natural source of environmental mercury is from volcanic activity. However, human exposure to heavy metals, including mercury, has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. From a human toxicology perspective, methylmercury ($MeHg^+$) is the chemical species of greatest interest. The major form of Hg is elemental, which is oxidized to inorganic mercury, then methylated, and finally incorporated as $MeHg^+$ by fish. The principal sources of exposure to mercury in everyday life are from fish consumption, dental amalgams, skin-lightening cosmetic creams, and occupational environments. Once elemental Hg has been absorbed by the body, it is oxidized to inorganic divalent Hg.

Gas chromatography is typically used in the separation of Hg compounds in biological samples, since the high volatility and high fat matrix make analysis by LC difficult. Moreover, GC-ICP-MS, specifically when using a dry plasma (that is, no water vapor is introduced to the ICP), is a highly specific, high-sensitivity technique with negligible matrix effects.

Instrumentation

The analytical system consisted of an Agilent 6890N GC interfaced to an Agilent 7500ce ICP-MS using the Agilent GC-ICP-MS interface and high-sensitivity (cs) lenses. A 30 M x 0.25 mm x 0.25 µm Agilent HP-5 GC column was used. The GC was equipped with the optional three-channel auxiliary EPC module to control the addition of the optional gases. Oxygen was added to the plasma gas to burn off carbon deposits on the sample and skimmer cones (Pt). 50 ppm Xe in Ar was used as an additional GC carrier gas. It was supplied to the GC from a Tee connector positioned between the end of the capillary column and the start of the transfer line to increase the carrier gas velocity and minimize residence time in the transfer line. Xe was used to tune the ICP-MS for maximum sensitivity. It can also be used as an online internal standard.

Standards and Reagents

$MeHg^+$ and inorganic mercury (Hg^{2+}) standards (IRMM, Belgium, and Eurisotope, Paris), were diluted into Milli-Q water (18.2 MΩ), spiked with $^{202}MeHg^+$ and $^{199}Hg^{2+}$, derivatized with $NaBPr_4$ (Sigma-Aldrich, France) and then sonic-extracted into pesticide-grade hexane. Because there are no available certified standard reference materials (CRMs) for mercury speciation compounds in blood samples, DORM-2 fish (IRMM) was used as a CRM. Figure 1 shows chromatograms obtained following the analysis of DORM-2 using the operating parameters given in Table 1.

Method

Table 1. *GC and ICP-MS operating parameters.*

GC	
Injection	Split/splitless - 2 µL
Oven program	50 °C (1 min), 25°/min ' 220 °C (4 min)
Carrier gas	He at 2 mL/min
Transfer line temp	250 °C
GC injector temp	220 °C
ICP-MS	
Isotopes acquired	124, 198, 199, 200, 202
Acqired mode	Time resolved
Dwell time	0.03 sec/point
RF power	1050 watts
Sample depth	8.5 mm
Carrier gas	0.45 L/min
Extract 1	4 V

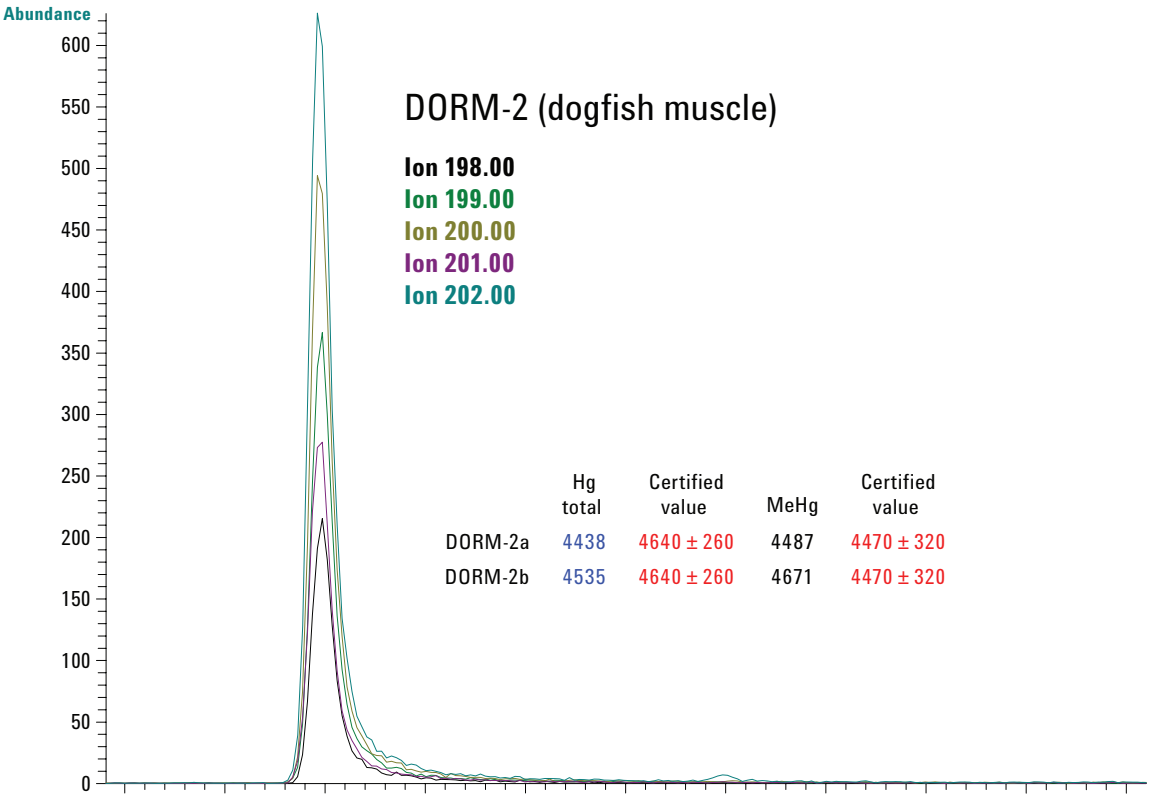

Figure 1. *Extracted ion chromatograms (overlaid) for mercury in DORM-2 dogfish muscle.*

Results

GC-ICP-MS is capable of rapid, sensitive detection of $MeHg^{+}$ and Hg^{2+}. Analysis times of less than 12 minutes with detection limits of approximately 3 fg on column for aqueous samples and 50 fg (0.05 ppb) for blood with a sample aliquot of 0.25 mL can be achieved.

Conclusions

ICP-MS is the ideal GC detector for elemental speciation of volatile and semivolatile organometallic compounds. It is sensitive, selective, and can tolerate a wide range of GC carrier gases and flows. Isotope dilution (ID) allows quality control and improves recovery. Limits of detection for GC-ID-ICP-MS are about 20 times better than HPLC and 100 times better than previous-generation GC-ICP-MS.

Reference

"Methylmercury ($MeHg^{+}$) and Inorganic Mercury (Hg^{2+}) Determination in Blood using GC-ICP-MS," Winter Plasma Conference, January 2005



CE-ICP-MS Introduction 52

Determination of Roxarsone and Its Transformation Products Using Capillary Electrophoresis Coupled to ICP-MS 53

CE-ICP-MS Introduction

While capillary electrophoresis (CE) (also known as Capillary Zone Elecrophoresis or CZE) is not strictly a chromatographic technique, it functions in much the same manner for the separation of mixtures into individual components (see Figure 1). CE differs from chromatography in how this is achieved. Chromatographic systems rely on partitioning of analytes between a stationary phase (usually a packed or coated column) and a flowing mobile phase (gas or liquid). CE uses a high-voltage electric potential applied across a narrow, fluid-filled capillary to induce the migration of charged species in the capillary according to their net charge, size etc.

CE is applicable to a wide range of analytes from small cations to large biomolecules. It can be coupled to ICP-MS in much the same way as nanoflow HPLC. The primary differences are the high voltages across the capillary, which must be taken into consideration, as well as the very low flow rate (nL/min) and lack of a high-pressure pump. Because of these, CE always requires the addition of a pumped makeup flow in order to achieve efficient nebulization. In order to minimize sample dilution and loss of analyte in a drained spray chamber, CE is most effectively coupled with a microflow nebulizer and direct coupling to the torch through an undrained spray chamber. Depending on the configuration, CE has the ability to achieve high-resolution separations based on charge, molecular weight, hydrophobicity, or other properties. In addition to potentially very high resolution, CE has the advantage over HPLC in being relatively simple and inexpensive. Disadvantages include very low capacity (nL sample size) and associated dynamic range limitations.

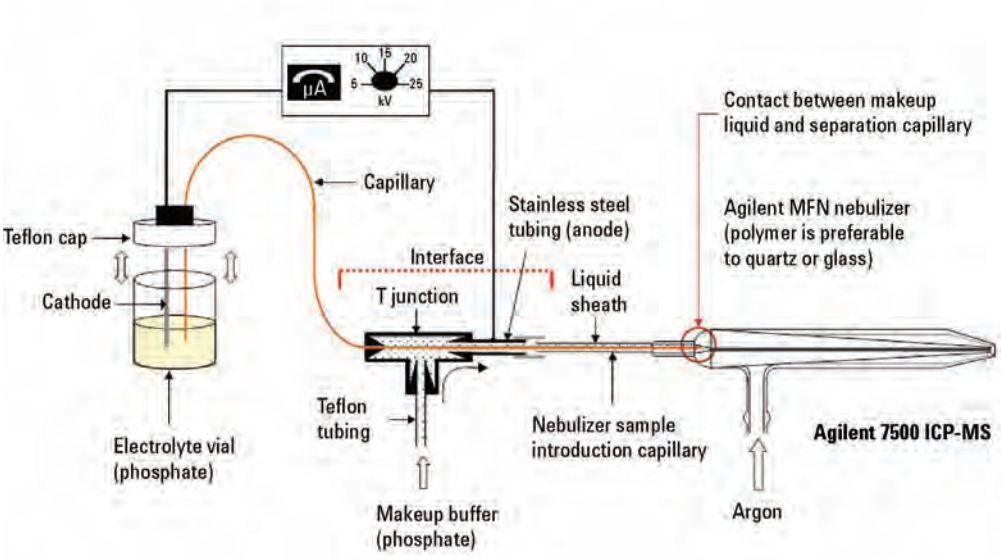

Figure 1. *Schematic of CE-ICP-MS.*

Determination of Roxarsone and Its Transformation Products Using Capillary Electrophoresis Coupled to ICP-MS

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Introduction

Roxarsone (3-nitro-4-hydroxyphenyl-arsonic acid) is one of the most widely used growth-promoting and disease-controlling feed additives in the United States. Many broiler chickens are fed roxarsone to promote weight gain and control parasites. Most of the roxarsone is believed to be excreted unchanged, and the resulting arsenic-containing waste is commonly recycled as fertilizer. Once in the environment, roxarsone can easily degrade into much more mobile and toxic arsenic (As) species. While HPLC coupled to ICP-MS has been used for the determination of As species including roxarsone degradation products, it is limited in its resolution. Capillary electrophoresis (CE) has the advantages of simple hardware and high efficiency. When coupled with ICP-MS for detection, CE-ICP-MS can provide a sensitive, highly selective method for the determination of roxarsone and its transformation products.

Instrumentation

A Beckman P/ACE 5500 Capillary Electrophoresis unit was coupled to an Agilent 7500c ICP-MS using the Burgener MiraMist CE nebulizer (Figure 1). 30 kV was applied to achieve electrophoretic separation through a 75-µm x 93-cm uncoated, fused silica capillary. Capillary temperature was set at 22 °C. Because of the very low CE flow (ca 181 nL/min), makeup flow is required to achieve efficient nebulization while providing closure of CE electrical circuit during separation. Makeup flow was set to 20 µL/min and consisted of 1% nitric acid with 3% methanol containing germanium as the internal standard at 100 ng/mL. CE and ICP-MS conditions are shown in Table 1.

Table 1. *CE-ICP-MS conditions.*

Capillary Electrophoresis	
Voltage	30kV
Capillary	75-µm id x 93-cm fused silica
Running buffer	20 mM sodium phosphate, pH 5.7
Pre-analysis rinse	0.1 M sodium hydroxide (3 min), running buffer (3 min)
Post-analysis rinse	0.1 M sodium hydroxide (3 min), DI water (3 min)
ICP-MS	
Forward power	1350 W
Nebulizer	Burgener MiraMist CE
Plasma gas flow	14.8 L/min
Auxiliary gas flow	0.92 L/min
Carrier gas flow	1 L/min
Make-up gas flow	0.25 L/min
Sample depth	6.3 mm
Make-up flow	20 µL/min
Masses monitored	72, 75
Integration time	0.70 sec per point

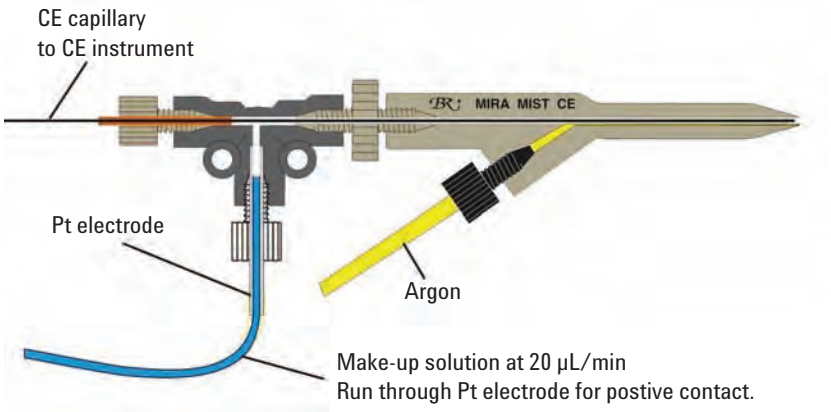

Figure 1. *Cutaway view of Burgener MiraMist CE interface.*

Standards and Reagents

Stock solutions (100 mg/L As) of arsenite (As(III)), arsenate (As(V)), dimethylarsinate (DMA), monomethylarsonate (MMA), 3-amino-4-hydroxyphenylarsonic acid (3-AHPAA), 4-hydroxyphenylarsonic acid (4-HPAA), ortho-arsanilic acid (o-ASA), and roxarsone were prepared in deionized water (18 MΩ resistivity) and diluted into working standards in 10% running buffer in DI water.

CE-ICP-MS Operation

Initially, the ICP-MS was tuned and optimized for response at *m/z* 75 with the CE disconnected by introducing a 10-µg/L solution of arsenate via a syringe pump. The CE capillary was preconditioned with 0.1 M NaOH followed by a rinse with DI water. After connecting the CE to the ICP-MS via the MiraMist CE nebulizer, the analytical run consisted of the following steps.

- Column is prerinsed for 3 minutes each with 0.1 M NaOH and 20 mM phosphate buffer
- 10-second pressure sample injection
- 30-minute separation at 30kV
- Nebulization and detection by ICP-MS

Results

Up to eight arsenic species in standards were separated by CE-ICP-MS in about 25 minutes with sensitivity superior to CE with UV detection (Figure 2). Absolute limits of detection (3 σ) based on a 30-nL injection volume were calculated as approximately 55 to 130 fg as arsenic.

Conclusions

Taking into account the small injection volume (low nL) when compared to other techniques such as HPLC (µL), the sensitivity using CE-ICP-MS is extremely high. However, migration time reproducibility, especially when running extracts of manure samples, was poorer than hoped. One cause is due to incomplete ionization of the silica capillary walls at the buffer pH of 5.7, which can affect the electro-osmotic flow. A second cause is matrix effects from high concentrations of dissolved solids in the samples. Additional sample cleanup, including protein precipitation, significantly improved the migration time reproducibility. Internal standard correction was also shown to improve retention time reliability.

For Additional Information

C. Rosal, G. Momplaisir, and E. Heithmar, "Roxarsone and transformation products in chicken manure: Determination by capillary electrophoresis-inductively coupled plasma-mass spectrometry," *Electrophor*esis, 2005, 26, 1606-1614.

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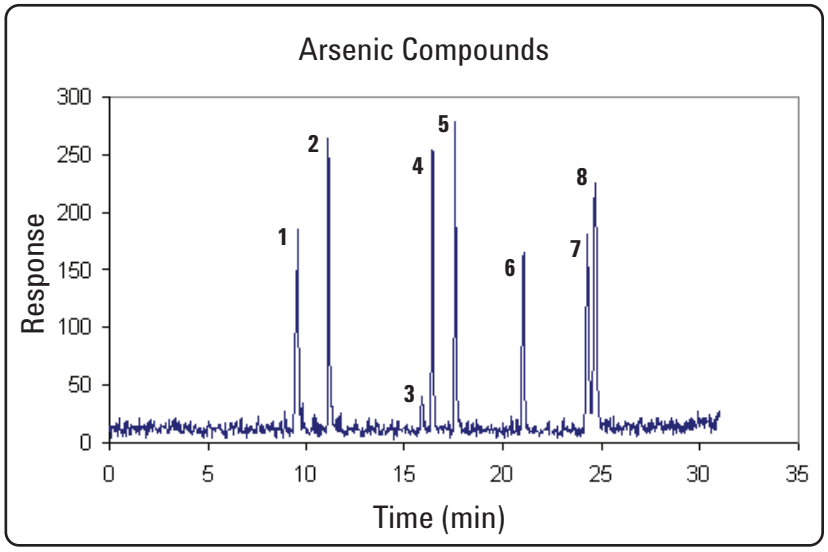

Arsenic Species
(in order of elution)

1. As(III)
2. DMA
3. 3-AHPAA
4. 4-HPAA
5. o-ASA
6. MMA
7. 3-NHPAA
8. As(V)

Figure 2. *Electropherogram of standard containing eight As species (20 ng/mL).*



Multi-MS

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Multi-MS Introduction

As a chromatographic detector, ICP-MS's strengths are also its weaknesses. As an elemental mass spectrometer, it possesses excellent sensitivity and specificity for individual isotopes. For the same reasons, by itself it is incapable of providing molecular or structural information. While the addition of retention or migration time data supplied by a coupled fractionation device can provide additional information, positive identification generally requires the analysis of standards, which may not always be available. Identification of unknown molecular species is the strength of molecular mass spectrometry including GC/MS and the various forms of LC/MS. These molecular mass spectrometers can provide information on structure via fragmentation information as well as molecular weight. However, due to the complexity of molecular mass spectra, particularly in the case of complex and incompletely resolved sample mixtures, it is often difficult to locate the compound(s) of interest in the resulting data. In this case, ICP-MS, with its ability to see the elemental needle in the molecular haystack, is a useful complement.

By coupling both ICP-MS and for example, Electrospray ionization (ESI)-MS to an LC, either in parallel (Figure 1) or as separate experiments, the ICP-MS can be used to locate target compounds based on unusual or unique elemental components. For example, ICP-MS can easily locate all the selenium-containing peaks from a complex mixture of peptides. Once the desired peaks have been located, the task of characterizing them becomes much simpler.

For these reasons, the simultaneous hyphenation of HPLC to both ICP-MS and ESI/MS has become a powerful tool in the identification and characterization of biomolecules.

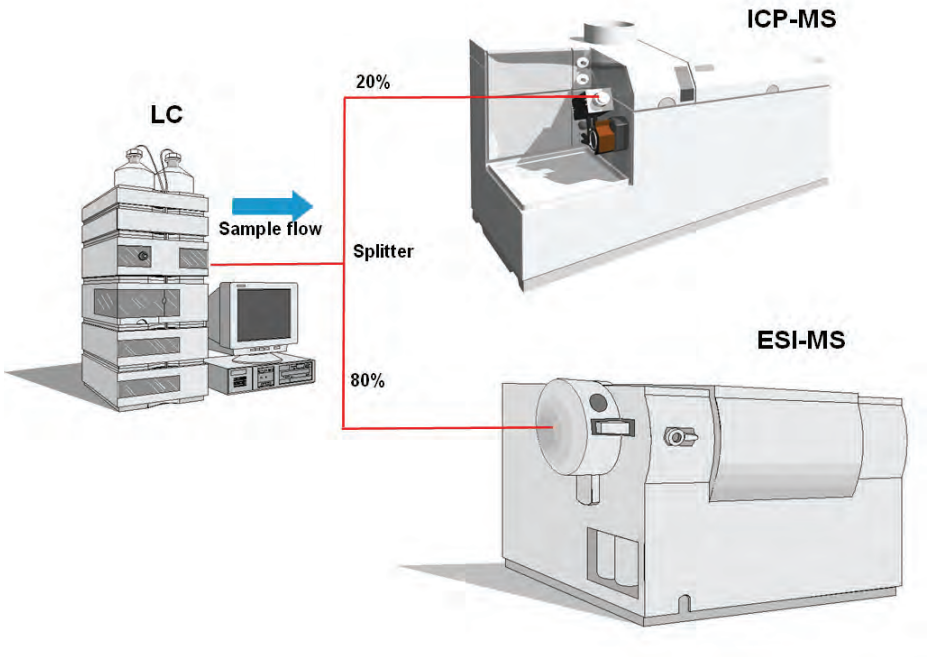

Figure 1. *Schematic showing a typical LC-multi-MS arrangement.*

Arsenic Metabolites in the Urine of Seaweed-Eating Sheep Using Simultaneous LC-ICP-MS/ES-MS

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Introduction

Sheep on a small island north of the Scottish mainland live almost entirely on seaweed, which contains enormous amounts of arsenic in the form of arsenosugars. They eat about 35 mg arsenic daily. Since the toxicity of arsenic in foodstuff depends on its molecular form or species, it is necessary to study the metabolism of arsenic in its many forms. Initial studies [1] showed that most of the arsenosugars are bioavailable and are metabolized to many different arsenic-containing species in urine. These studies were done by conventional speciation methods using HPLC-ICP-MS. However, only the main metabolite, dimethylarsinic acid, could be identified by retention time comparison with a standard. Although the Trace Element Speciation Laboratories Aberdeen (TESLA) group was in possession of more than 15 different arsenic standards, none of them gave exact retention time matches with the seven to eight unknown major metabolites. Fraction collection after anion exchange chromatography and the use of electrospray mass spectrometry (ES-MS) did not result in any successful identification.

When ICP-MS (Agilent 7500c) and ES-MS (Agilent 1100 Series) experiments were performed using identical chromatography, we were able to overlay the arsenic peaks from the ICP-MS (*m/z* 75) with that of certain *m/z* channels of the ES-MS; coeluting peaks could be identified easily. The ICP-MS signal gave the window in which an arsenic-containing compound must elute, and the ES-MS signal gave the possible molecular mass and fragmentation information.

Breakthrough

Real advances were made when the HPLC (Agilent 1100 Series) was simultaneously coupled online to the ES-qMS (Agilent 1100 Series) and the Agilent 7500c ICP-MS: HPLC-ICP-MS/ES-MS. The HPLC is connected to a microsplitter, which splits the flow into 75% ES-MS and 25% ICP-MS. The asymmetric split compensates for the differences in the sensitivity of the two detectors. The peaks and the exact time of the ICP-MS signal (*m/z* = 75) define the envelope in which molecular fragments from the arsenic metabolites are produced. This reduces the screening to less than 1/50 of the total chromatogram and makes it possible to identify arsenic-containing masses in the ESI spectrum. Otherwise (since arsenic is monoisotopic), no identifiable elemental isotope pattern can be recognized among the thousands of masses generated by the ES-MS. Using this technique, it was not long before most of the arsenic metabolites were identified and quantified [2,3]. Among the newly identified metabolites was the first arsenothiol compound found in a biological sample (dimethylarsenothioyl acetic acid), and also the new compound dimethylarsinoyl acetate (see Figure 1) [4,5,6,7]. Today the ES-qMS has been replaced by an Agilent ion trap-ES-MS, which has been used to identify larger molecules containing arsenic, such as arseno phytochelatin-3 or arsenic triglutathione in plants and for the identification of mercury and organomercury biomolecular species.

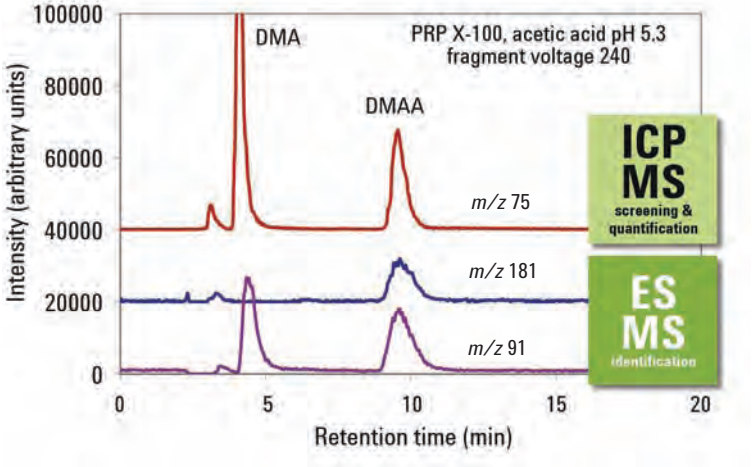

Figure 1. *First identification of dimethylarsinoyl acetate (DMAA) using HPLC-ICP-MS/ES-MS. The main metabolite dimethylarsinic acid (DMA) is also shown.*

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Determination of Unstable Arsenic Peptides in Plants Using Simultaneous Online Coupling of ES-MS and ICP-MS to HPLC

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Introduction

The determination of arsenic species in plants is necessary for various reasons. On the one hand, one would like to know the toxicity of arsenic when the plants are used as food or feed [1]. The Chinese government has released import guidelines for grains such as rice in which only inorganic arsenic is considered, which makes speciation analysis obligatory. On the other hand, the determination of arsenic species is key to understanding the uptake of arsenic from soil into the roots as well as the translocation of arsenic from the roots into the grains. In the past, only the traditional separation after a water-based extraction gave a hint that methylated arsenic species are only minor constituents while inorganic arsenic species (arsenite and arsenate) are paramount. These extractions are, however, only quantitative when rather strong extraction media are used, such as 1 *M* trifluoroacetic acid (TFA). The separation was classically done on a strong anion exchange column (PRP-X100). Although the uptake of those compounds from soil is dependent on chemical species, it cannot, however explain the variation of translocation rates in the different plants. Since plant physiologists proposed that weak, low-molecular-weight complexes with arsenic are transported into the vacuoles, it is important to detect such compounds in the plants. When this happens, the arsenic is no longer in a form that can be transported in the sap to the grain. In order to understand this process, the weak arsenic complexes need to be identified and quantified.

Experimental

Our studies identified that fresh plants can be extracted with 1 *M* formic acid at 4 °C and the analysis has to be performed within a few hours after extraction, then arsenic-polypepetides such as arsenic glutathione and arsenic phytochelatin complexes can be identified. This identification in a crude plant extract is only possible using mild chromatographic conditions such as size-exclusion or reverse-phase chromatography. Since the complexes are still of low molecular mass (< 1.5 kilodalton [kDa]), C_{18} columns using methanol gradients as mobile phase are ideal for the separation of those complexes from the inorganic and organometallic species. The identification can be achieved by coupling both mass spectrometers (ICP-MS and ES-MS) simultaneously online to the HPLC via a flow splitter from Upchurch. Approximately 20% of the flow goes to the ICP-MS (equipped with a microflow nebulizer) and the rest is transported to the ES-ion trap MS. When looking at the *m/z* 75 trace of the ICP-MS, it becomes apparent that the peptide-bound arsenics are well separated from the inorganic and methylated arsenic species, which elute near the void. Considering the slight variability of the arsenic sensitivity with the change of methanol in the eluent, the ICP-MS signal gives a good account of the quantities of the arsenic species in the extract.

Peptide Identification

If we used basic ES-MS with the crude plant extract, the identification of peptides would be a very laborious task. Having the ICP-MS signal available, only the retention times where arsenic signals appear have to be checked for coelution of any molecular mass (molecular fragments or protonated molecular mass in the positive mode). When the ES-MS was set to selected *m/z* of suspected complexes as seen in Figure 1, identification of the complexes was based on molecular mass coeluting with an arsenic signal of the ICP-MS (same retention time and peak shape).

However, unequivocal identification could only be obtained when full mass spectrum and MS/MS experiments were carried out on the $M\text{-}H^+$ signal (Figure 2). If the fragmentation pattern is consistent with that of the expected fragmentation of the peptides, the unstable arsenic peptide complex occurring in the plant extract can be positively assigned, as has been done for ferns, grass [2], and sunflower [3].

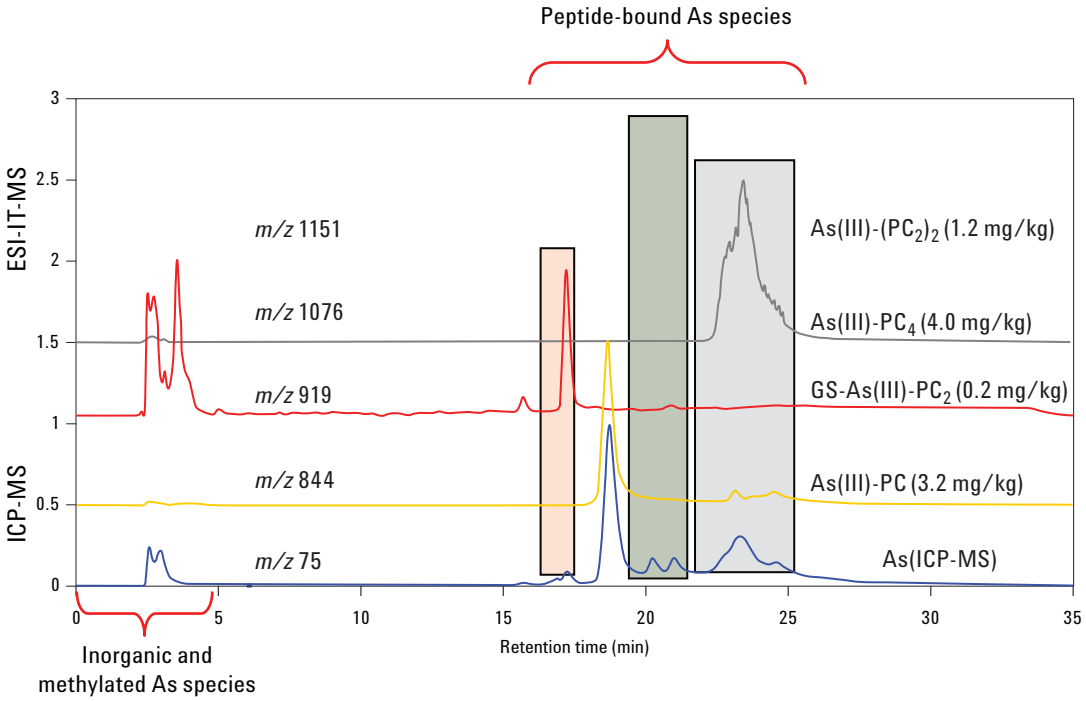

Figure 1. *Overlaid ion chromatograms of from electrospray MS and ICP-MS showing phytochelatin arsenic complexes.*

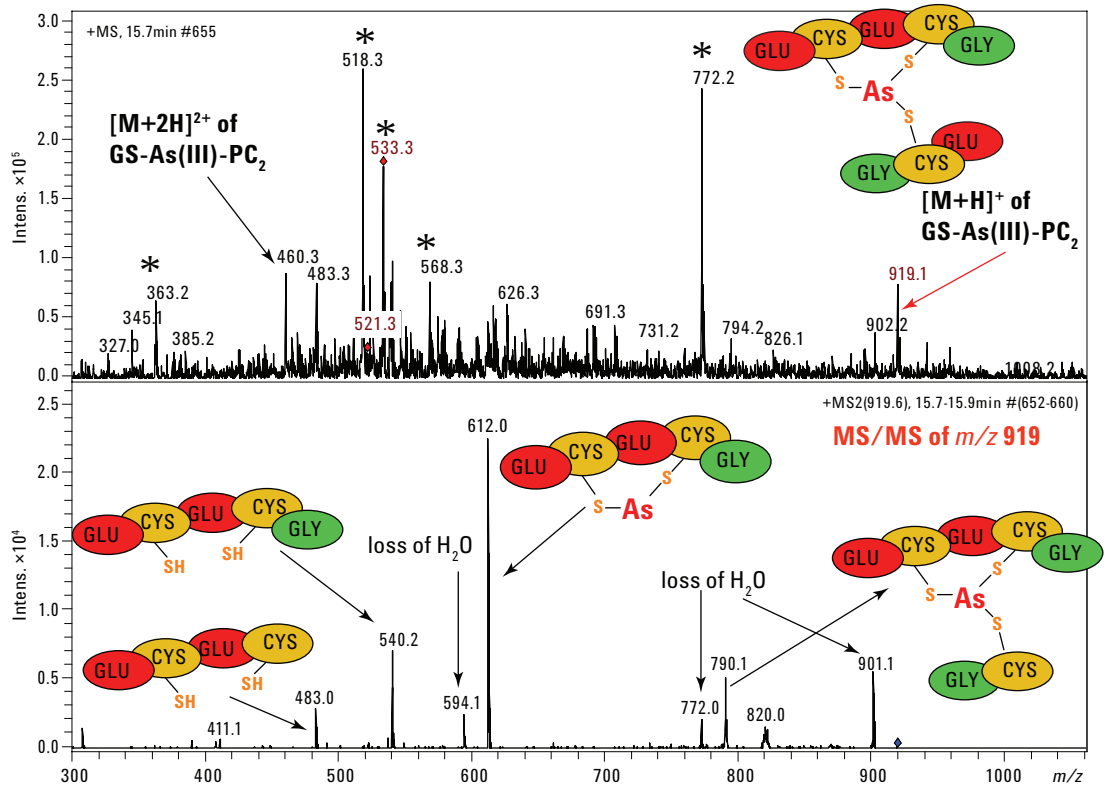

Figure 2. *ESI mass spectrum and MS/MS results from the M–H⁺ signal of GS-As(III)-PC₂.*

Conclusions

It is believed that arsenic peptide complexes trap arsenic in the roots and prevent them from being transported to the grain. The simultaneous online use of molecular and elemental mass spectrometry as detectors for HPLC has one disadvantage: the use of compromised conditions for the mobile phase, which can be used for ICP-MS and ES-MS. Otherwise, the simultaneous use saves analysis time, which is crucial when unstable compounds are investigated, and the mass spectra can be overlaid directly and ambiguities concerning a possible shift in retention time can be cancelled out [4].

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Phosphorylation Profiling of Tryptic Protein Digests Using Capillary LC Coupled to ICP-MS and ESI-MS

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Introduction

The reversible phosphorylation of proteins at the amino acid residues serine, threonine, and tyrosine is an important dynamic process in eukaryotic living systems that affects the structure of proteins, their catalytic activity during physiological processes, protein-protein interactions or the regulation of gene expression, and protein synthesis.

Traditional approaches to identifying protein phosphorylation have included incorporation of radioactive ^{32}P followed by gel electrophoreses or thin-layer chromatography, the use of phosphospecific antibodies, or high-resolution mass spectrometry. More recently, chromatographic or electrophoretic separation techniques such as liquid chromatography (LC) or capillary electrophoresis (CE) combined with element- or molecule-specific detection techniques such as ICP-MS or electrospray ionization (ESI) mass spectrometry have proven to be powerful complementary tools in biochemical analysis.

While ESI gives detailed information about the molecules under investigation, ICP-MS opens up the possibility of obtaining qualitative and quantitative information on the elemental stoichiometry of a molecule. Because of its high selectivity for elements and its nearly compound-independent ionization process, especially when using low-flow or nearly matrix-free separation techniques such as capillary-LC, ICP-MS is useful for rapid prescreening of tryptic digests for phosphorylated peptides before their final characterization by ESI-MS-MS or ESI-TOF-MS.

Handling the low solvent flow rate of capillary LC, which is normally around 4 µL/min, and controlling the background at the mass of phosphorus are the most critical issues in coupling capillary LC to ICP-MS for phosphorylation mapping. This approach offers the possibility of preselecting certain phosphorylated peptides for further analysis and detection of phosphorylated peptides, which have poor ionization properties in ESI.

Instrumentation

An Agilent 1100 Series capillary LC system was used for all experiments. Capillary-LC and nano-LC analysis were performed on an Agilent ZORBAX 300 SB-C18 column combined with a 300 SB-C18 precolumn. All connections were made of PEEK-coated silica tubing and zero dead-volume fittings. All LC modules were arranged to achieve the lowest possible dead volume between the cap-LC pump and the ICP-MS.

A modified capillary electrophoresis interface (CEI) 100 nebulizer (Cetac, Omaha, NB, USA) was used combined with a homemade 4-mL spray chamber with an injector tube extension used for direct nebulization inside the ICP torch (in-torch nebulization [ITN]).

An Agilent 7500cs ICP-MS system operating in helium collision mode was used as an element-specific detector. An Applied Biosystems API 4000 triple-quadrupole mass spectrometer was used as molecule-specific detector during the capillary LC-ESI-MS-MS experiments

Sample Preparation

The protein (beta-casein, 2 mg) was dissolved in 1 mL 50 mmol/L NH_4HCO_3 solution and used as stock solution for all further experiments. Trypsin was weighed and diluted with cold water (4 °C) to a final concentration of 100 µg/mL trypsin and 5 mmol/L $CaCl_2$. Before digestion, the proteins were denatured at 90 °C for 20 min. For digestion, 100 µL denatured protein stock solution and 50 µL trypsin solution were mixed in 1-mL vials. Digestion was carried out at 37 °C overnight. Formic acid (100%, 5 µL) was added to stop the activity of the enzyme. Finally, the digest was centrifuged at 25,000 g at 4 °C for about 5 min. The supernatant was pipetted into sealed 100-µL microvials that fit into the Agilent autosampler.

Results and Discussion

Figure 1 shows the comparison of the ^{31}P ICP-MS trace and the corresponding ESI-TIC of the beta-casein digestate. The ICP-MS peak for ^{31}P at approximately 40 minutes corresponds to the singly phosphorylated peptide FQ-pS-EEQQQTEDELQDK with a theoretical isotopic molecular weight of 2061.829 Da (as $[M+H]^+$), which is known from the literature.

Estimation of the Detection Limits for Phosphorus

To estimate the detection limit for phosphorus under Cap-LC conditions, flow-injection analysis of phosphorus standards of different concentrations was performed under isocratic conditions (90% A [0.05% TFA in water] and 10% B [0.05% TFA, 20% H_2O, 80% methanol]) at a flow rate of 4 µL/min. According to the IUPAC guidelines, a detection limit of 1.95 µg/L phosphorus corresponding to 1.95 pg P absolute was obtained.

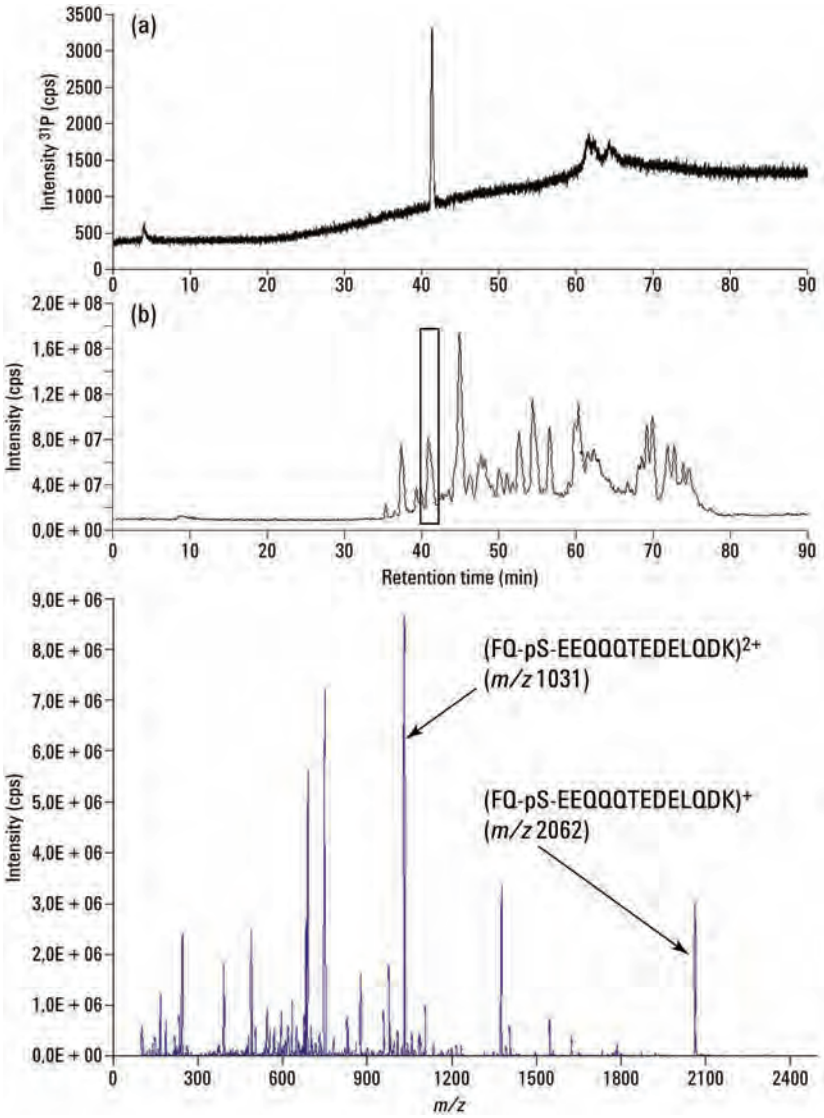

Figure 1. *(a) ^{31}P and ESI-TIC peaks of beta-case in separated by Cap-LC at 4 µL min-1 flow rate. (b) Data deconvolution of the ESI-TIC peak corresponding to the ^{31}P peak in Figure 1a. Reprinted with permission of ABC.*

Conclusions

The successful application of a new, high-efficiency capillary interface for collision cell ICP-MS has provided a sensitive complementary tool for use in connection with existing MS techniques in the field of protein phosphorylation analysis. The setup applied, consisting of a modified capillary electrophoresis nebulizer and a specially designed spray chamber that allows direct nebulization inside the ICP torch, takes into account the requirements when combining a low-flow separation technique with ICP-MS as element-specific detector, namely 100% transport efficiency, good nebulization stability, and minimized dead volumes. Because of the low solvent flow rates used and the high transport efficiency of the nebulizer, CC-ICP-MS has high compatibility with the organic gradient conditions usually used in protein and peptide analysis.

The optimized setup has been successfully applied to the phosphorylation screening of beta-casein, which has been used as a model protein. Additional ESI-MS-MS experiments proved the presence of a phosphorylated peptide under the identified peak.

The micronebulizer-based hyphenation of cap-LC to collision-cell ICP-MS, in general, opens up new application fields that are currently not accessible to element-specific detection techniques because of the use of highly organic mobile phases or the limited sensitivity or chromatographic resolution of conventional "large-bore" techniques. The proposed setup could be a sensitive alternative to already established HPLC techniques, especially for determination of other hetero-element-containing compounds, such as pesticides or pharmaceuticals.

More Information

D. Pröfrock, P. Leonhard, W. Ruck, and A. Prange, "Development and characterization of a new interface for coupling capillary LC with collision-cell ICP-MS and its application for phosphorylation profiling of tryptic protein digests," *Anal Bioanal Chem* (2005) 381:194-204.

Other Speciation Techniques



Other Hyphenated ICP-MS Techniques Used for Speciation	65
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Other Hyphenated ICP-MS Techniques Used for Speciation

In addition to the more common chromatographic techniques already described, many other possibilities exist and are (or will be) used to separate analyte species prior to elemental analysis by ICP-MS. In fact, any technique that can be used to separate a sample into its constituent components, either in time (as in chromatographic techniques), or in space (as in slab gel electrophoretic techniques), can probably be interfaced to an ICP-MS. Only the simple four requirements listed in the introduction need be met.

When the sample components elute from the fractionation system in a flowing stream of liquid such as in field flow fractionation (FFF), interfacing to the ICP-MS follows the same rules as any chromatographic technique (see General Requirements on page 5). In fact, FFF has shown itself to be a simple, versatile technique for the separation of components ranging from macromolecules to nanoparticles, based on a wide variety of physiochemical properties. Interfacing FFF to ICP-MS is straightforward and is beginning to see increased use, both as the versatility of FFF and the power of ICP-MS as a detector are realized.

Distillation is another category of simple fractionation techniques, commonly used in the chemical and hydrocarbon industries for separating components from a mixture based on differences in boiling point. Various forms include fractional distillation, steam distillation, vacuum distillation, azeotropic distillation, and others. Interfacing a distillation system to an ICP-MS varies with the size and type of system, but typically involves a simple transfer line (with or without flowing liquid makeup) between the outlet of the still and the ICP-MS nebulizer or torch.

Other techniques, where the sample is fractionated in space rather than in time, include solid-phase chromatographic and electrophoretic techniques. Solid-phase chromatographic techniques include paper and thin-layer chromatography, both one- and two-dimensional. Solid-phase electrophoretic techniques include slab gel electrophoresis and are commonly used for the separation of bio macro molecules, including peptides, and proteins, and nucleic acids. In these cases, the separated analytes are immobilized on or in a stationary medium (paper, silica, alumina or polyacrylamide or starch gels) after either one- or two-dimensional separation. Commonly, the "plate" is treated to render the analyte components visible through a staining procedure, allowing the analytes to be manually selected for transport to the ICP-MS. Transfer may involve simply removing the "spot" from the plate, dissolving or desorbing it in the appropriate buffer, and analyzing by conventional ICP-MS. More elegantly, the analyte spot(s) on the plate can be ablated or vaporized using a laser and transported to the ICP-MS as an aerosol in a flowing stream of argon using a technique called laser ablation. Alternatively, the entire plate can be "scanned" by slowly moving the laser across the surface while the ICP-MS measures and records the elemental composition constantly as a function of time. The result is a two-dimensional map of the elemental composition of the plate. In this case, staining is not necessary, since the entire plate is analyzed.

A related technique, while not strictly a separation technique, has been called metal imaging mass spectrometry (MIMS). In this case, the various metal "analytes" are fractionated biologically into various regions of a biological organ, depending on their biological function or state of disease. The organ is frozen and thin sections prepared, which can be analyzed by scanning laser ablation much the same way as a chromatographic or electrophoretic slab. The result is a two-dimensional "picture" of the elemental composition of the organ.

As old separation techniques find new applications and new separation techniques continue to emerge, interfacing them to ICP-MS as the "universal elemental detector" will continue to play an important role in the science of speciation analysis.

Analysis of Copper- and Zinc-Containing Superoxide Dismutase by Isoelectric Focusing Gel Electrophoresis Laser Ablation ICP-MS

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Introduction

Metals ions play an important role in biological systems. They can act as a catalytic center in numerous biochemical reactions and can be involved in enzyme regulation and gene expression. The characterization of metal-protein complexes is a challenging task that requires the development of analytical methodology capable of their separation and detection prior to identification by molecular mass spectrometry. For this purpose, the most powerful separation technique used in proteomics, that is, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) cannot be applied because of the noncovalent binding between metals and proteins. The objective can be achieved by combining a non-denaturing (to avoid metal-protein dissociation and metal loss) gel electrophoresis and a highly sensitive, quantitative and isotope-specific detection technique. ICP-MS using laser ablation micro-local sampling was investigated as a suitable detection technique for mapping trace elements in gel electrophoresis.

Sample

The Cu-Zn superoxide dismutase (SOD) was purchased from Sigma (Sigma-Aldrich, Saint Quentin Fallavier, France) and was dissolved in Milli-Q water. The SOD isoforms were separated using a nondenaturing separation protocol on a BioRad isoelectric focusing system. After separation, the proteins were photochemically stained according to the enzyme activity.

Instrumentation

A nanosecond laser CETAC LSX 100 (CETAC, Omaha, NE) was interfaced to an Agilent 7500cs ICP-MS (operating conditions are given in Table 1). Prior to introduction into the plasma, the aerosol produced via laser ablation was mixed with the nebulizer-induced aerosol (2% HNO_3) in a two-inlet torch. 5% O_2 was added into the spray chamber in order to prevent accumulation of carbon on the interface cones. Signal acquisition was performed in transient signal mode.

Table 1. *Experimental parameters for LA-ICP-MS.*

Laser	**Cetac LSX 100**
Wavelength	266 nm
Laser energy	0.7–0.8 mJ
Frequency	20 Hz
Pulses duration	8 ns
Scan speed	60 µm s^{-1}
Defocus	0
ICP-MS System	**Agilent 7500cs**
Nebulizer	MicroMist
Nebulizer gas flow rate	0.48 mL min^{-1}
Carrier gas flow rate (ablation cell)	0.5 mL min^{-1}
Optional gas	5% O_2 (spray chamber)
Cones	Pt
Torch	1 mm id
Isotoped monitored	^{63}Cu, ^{65}Cu, $^{6}4Cu$, ^{66}Cu
Dwell time	150 ms

Results

Staining the native isoelectric focusing (IEF) gel strip revealed the separation of different isoforms of the enzyme (Figure 1). Scanning the IEF strip using LA-ICP-MS identified Cu and Zn in one of these isoforms.

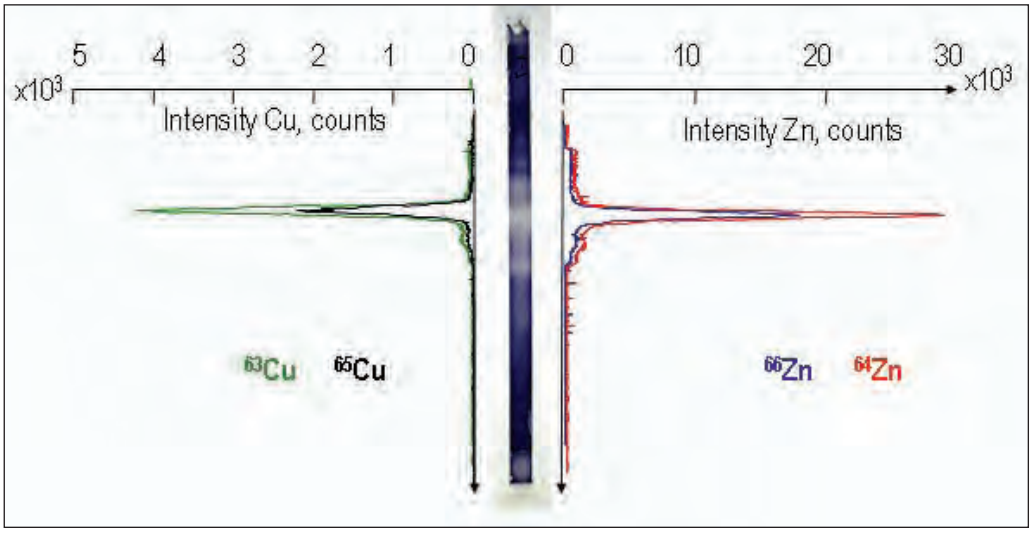

Figure 1. *Imaging of Cu and Zn in SOD separated by IEF and analyzed by LA-ICP-MS.*

Conclusions

Laser ablation ICP-MS is an attractive technique for the detection of metals present in gel electrophoresis owing to its sensitivity, speed, robustness, and multielement detection capability. It allows direct screening of trace elements in polyacrylamide gels without the need for a reaction/derivatization step. The signal obtained is directly proportional to the quantity of the element present in the gel.

Reference

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Acknowledgments

Agilent Technologies would like to acknowledge the following contributors, listed in alphabetical order by institute/company name.

- Dengyun Chen, Agilent Technologies, China
- Jérôme Darrouzes, Agilent Technologies, France
- Tetsushi Sakai, Agilent Technologies, Japan
- Ed McCurdy, Agilent Technologies, UK
- Steve Wilbur and Emmett Soffey, Agilent Technologies, USA
- Viorica Lopez-Avila, Agilent Technologies, USA
- Miao Jing and Xiaoru Wang, First Institute of Oceanography, China
- Daniel Pröfrock, Peter Leonhard, and Andreas Prange, GKSS Research Center, Geesthacht, Germany
- Hugues Preud'Homme, Sandra Mounicou, Christophe Pecheyran, and Ryszard Lobinski, Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE), France
- Heidi Goenaga-Infante, LGC Limited, UK
- Jens J. Sloth and Erik H. Larsen, National Food Institute, Denmark
- Jens J. Sloth and Kåre Julshamn, National Institute for Nutrition and Seafood Research (NIFES), Norway
- Miroslav Kovacevic, National Institute of Chemistry, Slovenia
- Li Bing, Liu Wei, Yang Hong-xia, and Liu Xiaoduan, National Research Center for Geoanalysis, China,
- Dr. Raimund Wahlen, RW Consulting, Hook Norton, UK
- Orr Sharpe and William H. Robinson, Stanford University, USA
- Maïté Bueno, Florence Pannier, and Martine Potin-Gautier, Université de Pau, France
- Regina Leber, Sepp D. Kohlwein, Walter Goessler, University Graz, Austria
- Jörg Feldmann, Andy Meharg, and Andrea Raab, University of Aberdeen, Scotland, UK
- Joseph A. Caruso, Douglas D. Richardson, Baki B.M. Sadi, Anne P. Vonderheide, Monika Shah, Sasi S. Kannamkumarath, Kirk E. Lokits, Juris Meija, and Rodolfo G. Wuilloud, University of Cincinnati, USA
- Wolfgang Ruck, University of Lüneburg, Germany
- Charlita Rosal and G. Momplaisir, US EPA/ORD/NERL-ESD, USA

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Printed in the USA
October 3, 2007
5989-6160EN

Agilent Technologies

Investigation of the Unique Selectivity and Stability of Agilent GS-OxyPLOT Columns

Application

Gas Chromatography

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Abstract

The stationary phase of a GS-OxyPLOT column is a proprietary, salt deactivated adsorbent. GS-OxyPLOT columns show unique selectivity to oxygenated hydrocarbons, excellent stability and reproducibility, long column lifetime, and a wide application range.

Introduction

The determination of oxygenated hydrocarbons in different sample matrices is very important for the petrochemical industry, because oxygenates directly influence product quality. Presence of such oxygenates may cause the catalysts to be poisoned and deactivated, resulting in more downtime and higher costs. ASTM has developed several methods for analysis of oxygenates, such as ASTM D7059, D4815, and D5599. The oxygenates include ethers, esters, ketones, alcohols, and aldehydes.

Methanol is one of the oxygenates that often present in light hydrocarbon streams. For example, it is added to natural gas and production of crude oil to prevent hydration of hydrocarbons during transportation via pipelines. Therefore, it is important to accurately measure the content of methanol from light hydrocarbons at different concentrations, including at trace levels.

To achieve this, a new porous layer open tubular (PLOT) capillary column, the GS-OxyPLOT column, was used. The stationary phase of the GS-OxyPLOT is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons, while having virtually no interactions with saturated hydrocarbon solutes [1].

Using Capillary Flow Technology, such as backflush or Deans switch, GS-OxyPLOT columns can provide a turnkey solution for the analysis of trace level oxygenate impurities in complex matrices, such as motor fuels, crude oil, and gaseous hydrocarbon [2]. Meanwhile, a GS-OxyPLOT column can be used as a single analytical column to separate oxygenates for some samples. In this application, methanol was set as an example to investigate the performance of the GS-OxyPLOT column.

Experimental

The experiments were performed on an Agilent 7890A GC system and a 6890N GC system equipped with split/splitless capillary inlet, flame ionization detector (FID), and Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with long-lifetime septa (Agilent p/n 5183-4761) and spilt/splitless injection liners (Agilent p/n 5183-4711). Injections were done using 10-µL syringes (Agilent p/n 9301-0714). A glass indicating moisture trap (Agilent p/n LGMT-2-HP), an oxygen trap (Agilent p/n BOT-2), and a

hydrocarbon trap (Agilent p/n 5060-9096) were installed. Agilent ChemStation was used for all instrument control, data acquisition, and data analysis.

Results and Discussion

Analysis of Normal Hydrocarbons and Methanol

A mixture of normal hydrocarbons and methanol was prepared with the following approximate concentrations %(w/w): 34.8% n-pentane, 12.8% n-hexane, 1.8% n-heptane, 1.9% n-octane, 2.1% n-nonane, 3.9% n-decane, 2.1% n-undecane, 9.8% n-dodecane, 11.8% n-tridecane, 4.7% n-tetradecane, 2.4% n-pentadecane, 4.5% n-hexadecane, 2.4% n-heptadecane, 1.0% n-octadecane, 0.9% n-eicosane, 0.9% n-docosane, 1.1% n-tetracosane, and 0.8% methanol.

The analytical conditions are summarized in Table 1. The normal hydrocarbons and methanol analysis was performed on a GS-OxyPLOT column (Agilent p/n 115-4912). The GC chromatogram is shown in Figure 1.

Table 1. Conditions for Normal Hydrocarbons and Methanol Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm (Agilent p/n 115-4912)
Carrier gas	Helium, constant flow mode, 40 cm/s @ 50 °C
Inlet	Split/splitless at 325 °C
Split ratio	80:1
Oven temperature	50 °C (2 min); 10 °C/min to 290 °C (2 min)
Post-run	300 °C (2 min)
Detector	FID at 325 °C
Injection size	0.2 µL

In Figure 1, the GS-OxyPLOT column shows unique retention characteristics for methanol. The lower boiling point hydrocarbons were not strongly retained on the stationary phase and eluted through the FID very rapidly. The methanol eluted after n-C14, allowing it to be quantified without any interference from the hydrocarbon matrix, and making it feasible for trace-level methanol analysis in a range of hydrocarbon streams.

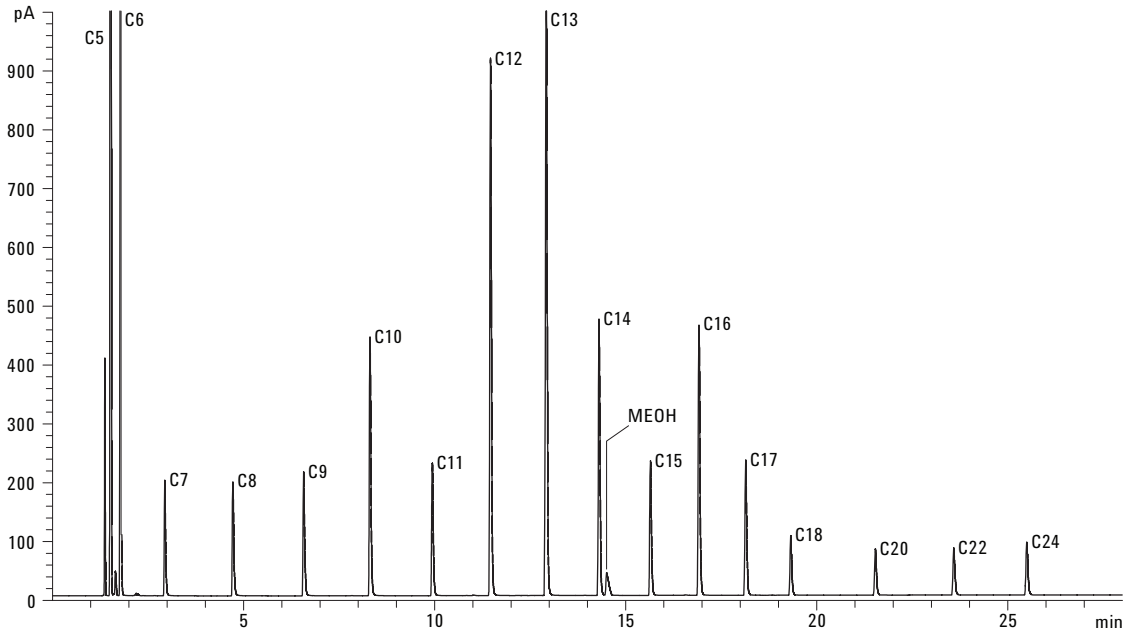

Figure1. Analysis of methanol and normal hydrocarbons on a GS-OxyPLOT column, 10 m × 0.53 mm × 10 µm.

In addition, the baseline was quite smooth, even when the oven temperature was up to 290 °C. GS-OxyPLOT has an upper temperature limit of 350 °C and exhibits virtually no bleed, making it widely applicable for long-term reliable analysis.

Analysis of Alcohols

A mixture containing a range of primary alcohols from methanol to lauryl alcohol was analyzed on a GS-OxyPLOT column using a temperature-programmed method. Table 2 lists conditions for alcohols separation, and the resulting chromatogram is shown in Figure 2.

Sample

The sample had an approximate concentration (v/v) of 1% methanol, ethanol, propanol, butanol, amyl-alcohol, heptanol, octanol, nonanol, decyl alcohol, and lauryl alcohol in toluene.

As can be seen in Figure 2, all of the alcohols are separated and eluted with good peak shape within an analysis time of 15 min. In this experiment, oven temperature was set up to 300 °C. Thanks to its advanced dynamic coating process, Agilent's GS-OxyPLOT stationary phase exhibits virtually no detector spiking due to particle generation from the phase coating [3].

Due to the high viscosity of alcohols, especially decyl alcohol and lauryl alcohol, it is necessary to wash the needle after each injection in case of carryover problems.

Table 2. Conditions for Alcohols Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier Gas	Helium, constant flow mode, 40 cm/s at 150 °C
Inlet	Split/splitless at 325 °C
Split ratio	50:1
Oven temperature	150 °C (0 min); 10 °C/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	0.2 µL

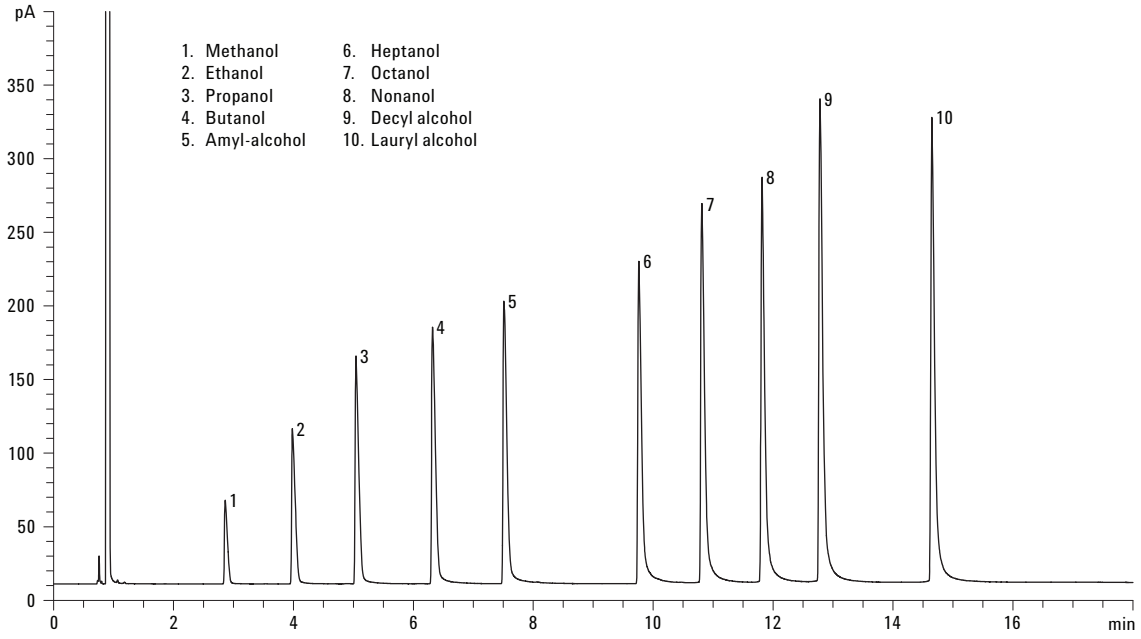

Figure 2. Separation of alcohols using GS-OxyPLOT, 10 m × 0.53 mm × 10 µm.

Influence of Temperature on the Selectivity of GS-OxyPLOT

To polar stationary phases, the temperature has a direct influence on the selectivity. GS-OxyPLOT offers extremely high polarity. The analysis of normal hydrocarbons and methanol demonstrated that methanol elutes after n-C14. Using a mixture containing methanol, n-tetradecane, and n-pentadecane, isothermal Kovats retention indices were tested at isothermal oven temperatures of 150, 200, 220 and 250 °C, respectively (Table 3). The relationship between Kovats retention indices and oven temperature is shown in Table 4.

Table 3. Conditions for Kovats Retention Indices Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 30 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temperature	150, 200, 220, and 250 °C, respectively; isothermal
Detector	FID at 250 °C
Injection size	0.2 µL

Table 4. Kovats Retention Indices and Oven Temperature (n > 3)

Oven temp.	150 °C	200 °C	220 °C	250 °C
LOT1	1419	1418	1418	1413
LOT2	1420	1421	1419	1417

Retention index, Ix, was calculated using the following equation:

$$Ix = 100n + 100[\log(t_x) - \log(t_n)]/[\log(t_{n+1}) - \log(t_n)]$$

Where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound X; t_x is the retention time of compound X. Here compound X is methanol, the reference n-alkane hydrocarbons are n-tetradecane and n-pentadecane, respectively.

Table 4 shows good repeatability of Kovats rentention indices for two different lots of GS-OxyPLOT columns. The retention index for methanol only changed by less than 10 index units over 100 °C temperature difference. Therefore, when the oven temperature changes from 150 to 250 °C, it has little influence on the selectivity of GS-OxyPLOT.

Influence of Moisture on GS-OxyPLOT

Some PLOT columns can adsorb water, which can lead to changes in retention times and selectivity for analytes. Therefore, column performance will be influenced greatly in the presence of water. Although cumbersome solvent-extraction procedures can be performed before injection, injecting sample that contains water is, in some cases, unavoidable.

From a GC point of view, water is a less-than-ideal solvent. The problems associated with water include large vapor expansion volume, poor wet ability and solubility in many stationary phases, detector problems, and perceived chemical damage to the stationary phase. In order to test the effect of water, a GS-OxyPLOT column that had gone through about 1,500 runs was tested before and after injecting 100% aqueous samples.

Water has a large vapor expansion volume; the vapor volume of water (assuming a 1-µL injection) can easily exceed the physical volume of the injection liner (typically 200 to 900 µL). The volume for the liner used in this experiment (Agilent p/n 5183-4711) is 870 µL, so the injection volume was set as 0.2 µL. Table 5 lists the conditions for the moisture testing, and the resulting chromatograms are shown in Figure 3.

Table 5. Conditions for Moisture Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 38 cm/s at 150 °C
Inlet	Split/splitless at 300 °C 15:1 split ratio
Oven temperature	150 °C isothermal, post-run: 300 °C (5 min)
Detector	FID at 300 °C, H2:45mL/min, air: 400 mL/min, makeup: 30 mL/min
Injection size	0.2 µL
Sample	0.1% n-Dodecane, Methyl tert-butyl ether, n-Tridecane, Iso-Butyraldehyde, n-Tetradecane, Methanol, Acetone, and n-Pentadecane

As shown in Figure 3, the area of n-pentadecane remained the same before and after 100 injections of water. However, compared with the area before injecting water, the area of methanol (peak 6) decreased by 50%, and the area of acetone (peak 7) decreased by14.4% after 100 injections of water (see Table 6). It demonstrated that water can affect the activity of GS-OxyPLOT, especially for the analysis of those relatively low molecular weight oxygenated compounds, such as methanol and acetone.

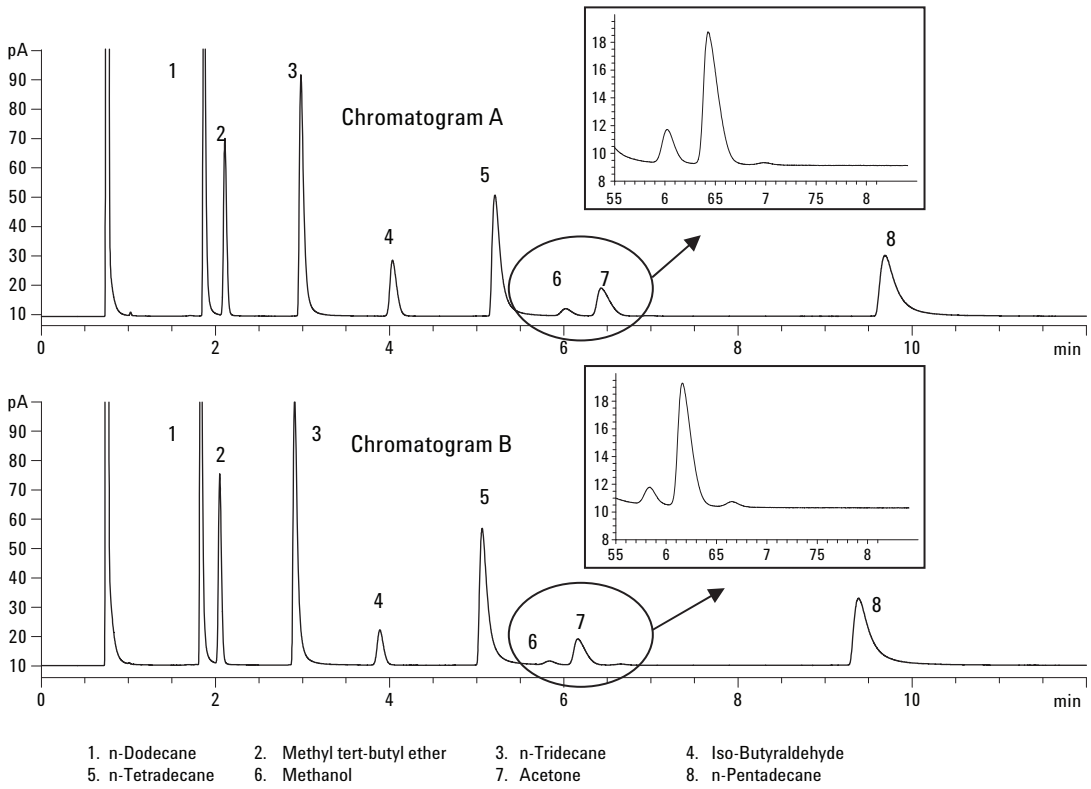

Figure 3. Comparison of test mixture separation before (A) and after (B) 100 injections of water.

As for retention times and column efficiency, they are not strongly influenced. After 100 injections of water, the retention time of C15 changed from 9.689 min to 9.384 min, and the column efficiency of C15 changed from 14,792 to 14,781.

Condition the column at 300 °C for two hours, followed by 12 hours at 250 °C. As shown in Figure 4 and Table 6, it is obvious that GS-OxyPLOT phase can be regenerated by conditioning.

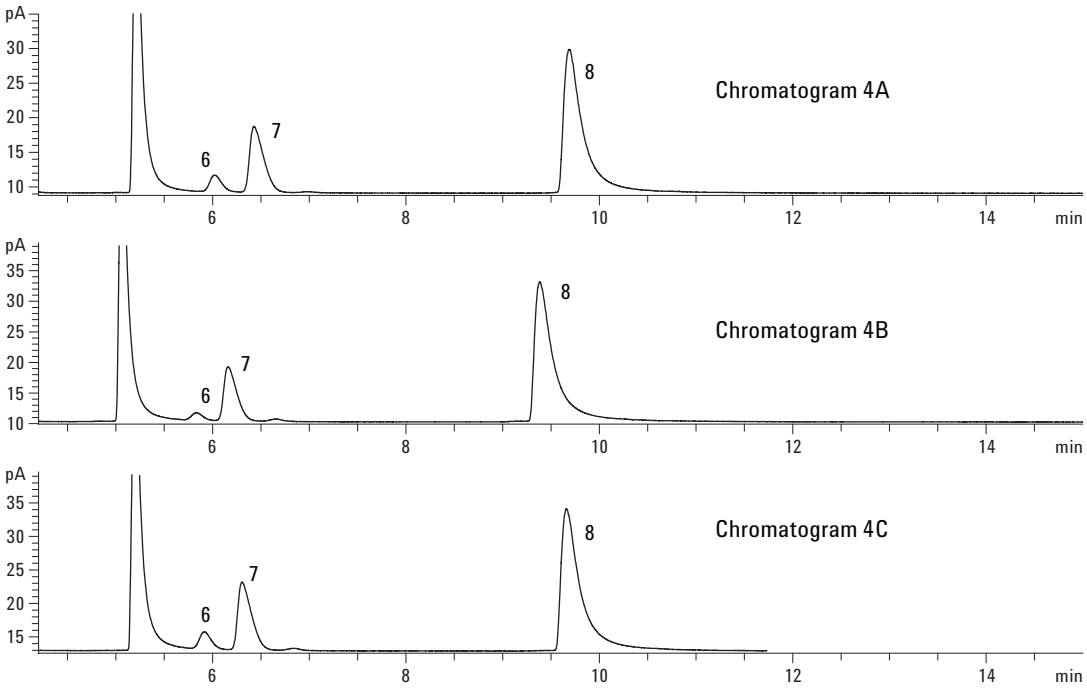

Figure 4. Expanded view shows comparison of test mixture separation on GS-OxyPLOT.
4A. Before injection of water. 4B. After 100 injections of water. 4C. After conditioning the column.

Table 6. Comparison of Test Mixture Separation

	Methanol			Acetone			n-Pentadecane		
	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column
RT (min)	6.022	5.835	5.915	6.429	6.160	6.305	9.689	9.384	9.658
Area	20.23	9.18	20.88	94.53	80.92	98.07	277.79	287.7	287.9
Plates	11887	12920	11616	9532	10357	9573	14792	14781	15100

After conditioning the GS-OxyPLOT column, the peak area and retention time reproducibility were determined. Figure 5 and Table 7 show excellent RT precision, lower than 0.6% over five test mixture runs on this GS-OxyPLOT column. The peak area has a relative standard deviation (RSD%) below 2.5%. It proved that column performance can be restored via conditioning.

Determination of Methanol

The following analysis of methanol followed ASTM D7059 [4]: "Standard Test Method for Determination of Methanol in Crude Oils by Multidimensional Gas Chromatography." Methanol was determined by gas chromatography with FID using internal standard method with GS-OxyPLOT column.

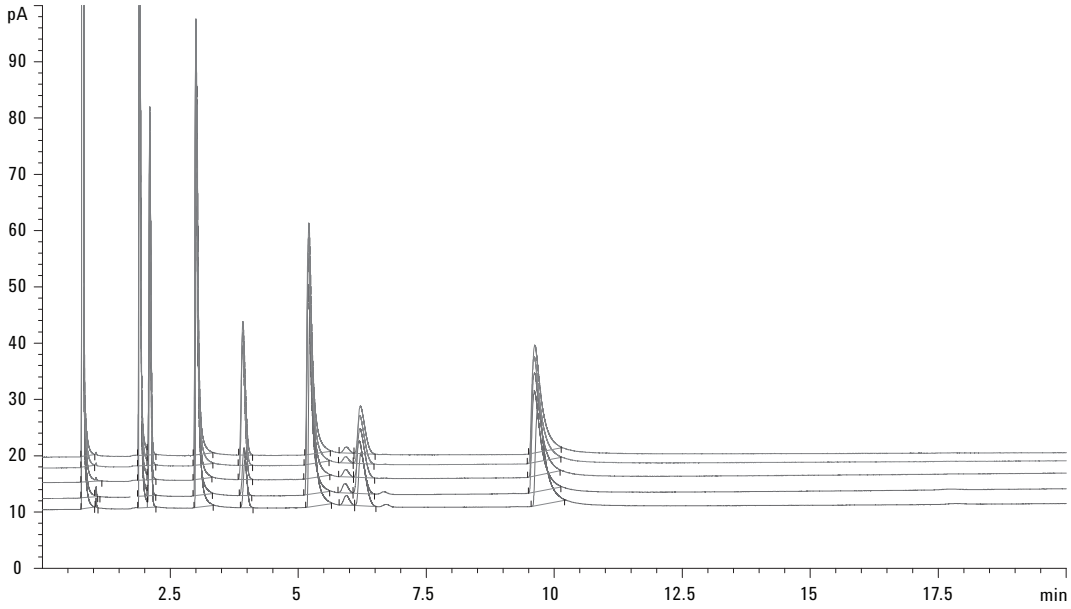

Figure 5. Fifth run overlaid using GS-OxyPLOT (after conditioning column).

Table 7. Peak Area Reproducibility and Retention Time Reproducibility on GS-OxyPLOT (after conditioning column)

Compound (by eluted order)	Dodecane	MTBE	Tridecane	Iso-Butyraldehyde	Tetradecane	MeOH	Acetone	n-C15
Area RSD% (N = 5)	1.18	1.58	1.59	2.49	1.15	2.12	1.98	1.82
RT RSD% (N = 5)	0.18	0.12	0.26	0.55	0.29	0.16	0.19	0.33

Reagents and Materials

Carrier gas, Helium, > 99.95% purity
Methanol, > 99.9% purity
1-propanol, > 99.9% purity, and containing < 500 ppm methanol
Toluene, > 99.9% purity, and containing < 0.5 ppm methanol

A set of calibration standards 5, 25, 125, 250, 500, 1,000 and 1,500 ppm (m/m) of methanol, and each containing 500 ppm (m/m) of 1-propanol internal standard, were prepared in toluene.

The calibration standard solutions should be stored in tightly sealed bottles in a dark place below 5 °C.

Linearity

Under the conditions listed in Table 8, the methanol calibration standards were analyzed. The linearity is shown by plotting the response ratio of methanol and internal standard 1-propanol against their amount ratio (see Figure 6). For methanol, good linearity was gained ranging from 5 to 1,500 ppm. The correlation r^2 value for the calibration curve is higher than 0.999.

Figure 7 and Figure 8 are chromatograms of methanol at a level of 5 ppm and 1500 ppm, respectively. At a relatively high concentration of 1500 ppm, methanol still could get a sharp peak. The limit of quantification (LOQ) was calculated to be 1 ppm using the chromatogram of 5 ppm methanol.

Table 8. System Settings for the Calibration Curve

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 50 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 10:1 split ratio
Oven temperature	150 °C (3 min); 20/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	1 µL

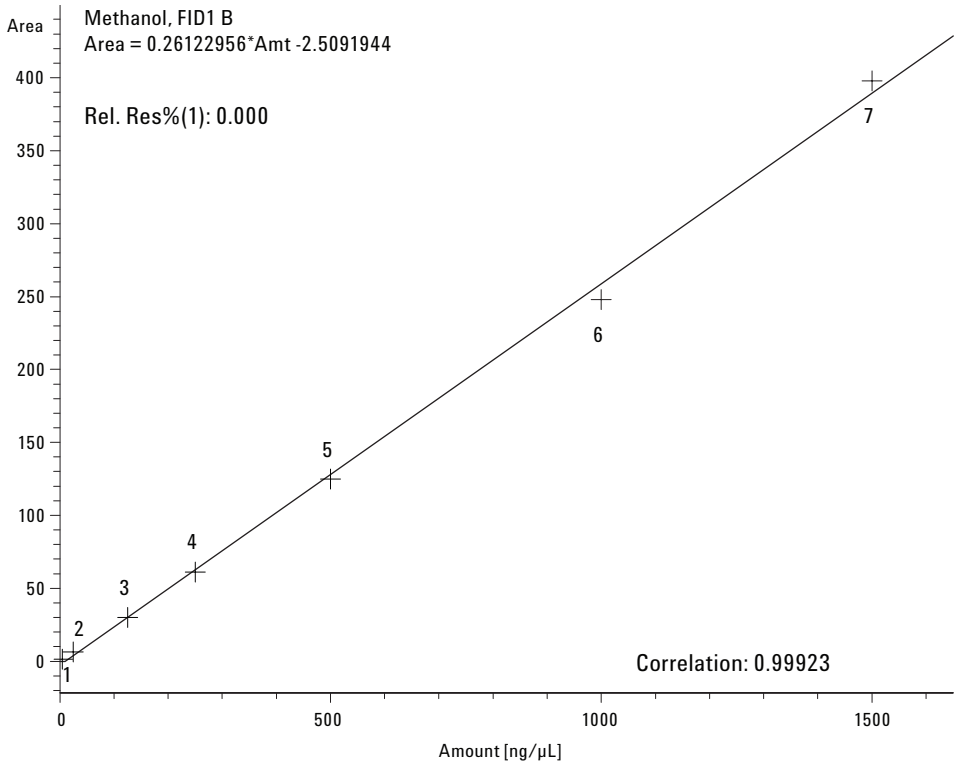

Figure 6. The calibration curve of methanol in toluene.

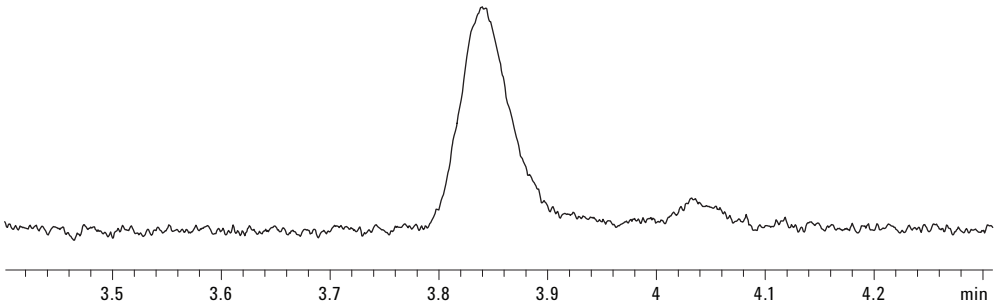

Figure 7. Test mixture of 5 ppm methanol in toluene.

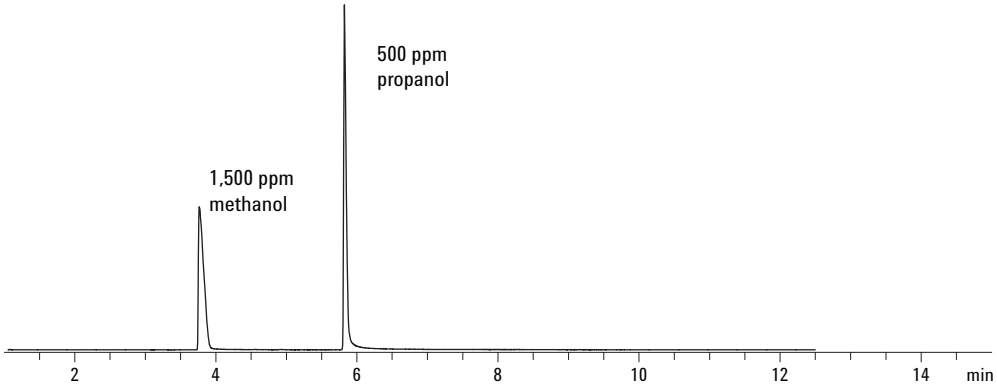

Figure 8. Test mixture of 1,500 ppm methanol in toluene.

Repeatability

The reproducibility of the GS-OxyPLOT is given in Table 9. Those values were obtained by the replicate analysis of different methanol levels (25, 125, and 1,500 ppm) in different days. The injection was done by ALS with RSD no less than 3% either intraday or interday analysis, which was very low for this type of determination.

Life Span

Under the conditions in Table 5, a mixture was analyzed with a GS-OxyPLOT column which went through 1,500 injections of methanol. It shows that the column has a long lifetime. The GS-OxyPLOT column still has good resolution for each compound and high efficiency of 1,482 plates per meter for n-pentadecane (see Figure 9).

Table 9. Relative Standard Deviations Intraday and Interday at Different Levels (25, 125, and 1,500 ppm) of Methanol

Day	25 ppm (average)	RSD (%)	125 ppm (average)	RSD (%)	1,500 ppm (average)	RSD (%)
D 1	25.2	0.46	123.9	0.45	1507.3	0.55
D 2	25.3	1.53	123.2	0.79	1494.4	0.45
D 3	24.4	0.36	125.4	1.71	1523.5	0.35
D 4	25.9	1.06	123.0	0.90	1537.8	0.51
D 5	23.9	0.44	121.1	0.76	1502.4	1.03
Stand. dev.	0.7		1.70		17.4	
Average	24.97		123.6		1513.1	
RSD (%)	2.8		1.37		1.15	

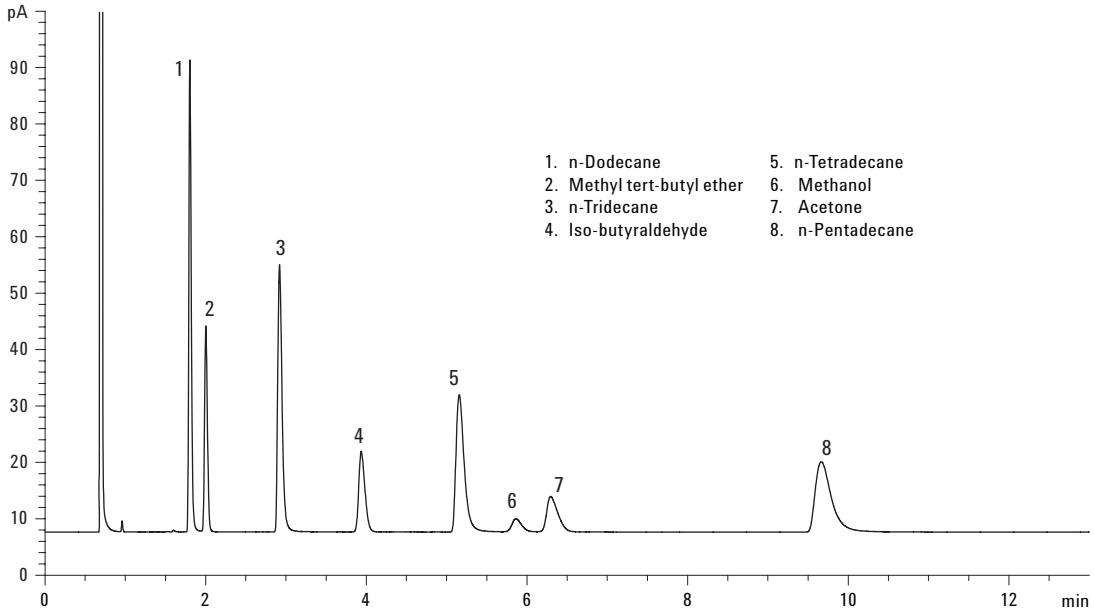

Figure 9. Chromatogram of performance mixture after 1,500 injections.

Conclusions

GS-OxyPLOT provides good retention and selectivity for oxygenated compounds. Normal alkanes up to C24 and primary alcohols up to lauryl alcohol can elute from GS-OxyPLOT within its program temperature maximum limit of 350 °C. Methanol elutes after n-C14 with retention index higher than 1,400; the retention index is quite stable from 150 to 250 °C, allowing methanol to be measured at low levels in a wide range of hydrocarbon streams.

Methanol has to be measured usually at specs as low as 5 ppm. From 5 to 1,500 ppm, it shows good linearity on GS-OxyPLOT. And the column has proven extremely stable with long lifetime.

GS-OxyPLOT can tolerate a little amount of water in samples, and column performance can be restored via conditioning.

GS-OxyPLOT can be used for a single-column system or in multidimensional GC systems. It offers a unique solution for the analysis of oxygenates in the chemical and petrochemical industries.

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Printed in the USA
June 17, 2008
5989-8771EN



Agilent Technologies

Fast Hydrocarbon and Sulfur Simulated Distillation Using the Agilent Low Thermal Mass (LTM) System on the 7890A GC and 355 Sulfur Chemiluminescence Detector

Application Note

Hydrocarbon Processing

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Abstract

A fast multielement simulated distillation method (SimDis) based on Low Thermal Mass (LTM) column technology is described. The LTM system technology using resistive heating allows rapid temperature programming with extremely fast column cool-down. Significantly shortened analytical cycle times as compared to conventional air bath GC ovens are achieved. The method combines hydrocarbon SimDis with an FID with sulfur SimDis using the Agilent 355 sulfur chemiluminescence detector (SCD) in a series configuration. The results show that the LTM method produces a run time for both hydrocarbon and sulfur boiling point distribution at least six times shorter than the conventional ASTM D2887 procedure [1]. The results agree with the specification in ASTM D2887 for the reference gas oil checkout sample.

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Introduction

Sulfur and carbon simulated distillation provide meaningful information to optimize refining processes and demonstrate compliance with petroleum product specifications. To meet the increasing needs for greater productivity, a fast sulfur and carbon SimDis method was developed using LTM technology. LTM technology was developed by RVM Scientific and acquired by Agilent Technologies in 2008 [2]. The Agilent 7890A system is designed to work with the LTM column module components to heat and cool the column very efficiently to significantly shorten analytical cycle times as compared to conventional air bath GC oven techniques involving much higher thermal mass. Aside from selectivity, temperature programming is an extremely useful feature of gas chromatography. When appropriate column dimensions and flow rates are choosen, high program rates (such as, 200 °C/min) can be used to great advantage. Agilent's method translation software can be useful in this regard [3]. For example, as temperature program rates increase, a corresponding increase in column flow rate should also be considered to achieve more optimal overall system performance. The system (except external power supply) is built into a replacement GC oven door, which is mounted as an add-on to an Agilent 7890A GC. The method described here combines hydrocarbon SimDis with sulfur selective SimDis using an FID and an SCD in series with simultaneous acquisition of both signals.

Experimental

This two-channel SimDis application uses the Agilent 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet and an SCD mounted in series with an FID by using a special adapter. A 5-inch format LTM column module is used for the analysis. Samples, such as boiling point (BP) calibration C5-C40, polywaxes, and resids, that are too viscous or waxy to sample with a syringe are heated to approximately 80 °C for a few minutes before injection.

The SimDis application software can process one or two channels of signal data (FID and SCD) from the GC ChemStation. The software is based on four modules: Browse, Setup, SimDis, and Report. Each module provides specific functions to rapidly perform multielement SimDis calculations. For example, the Setup module allows you to assign the files to use for BP Calibration, Blank, and QC reference. The detailed GC conditions used are listed in Table 1.

Table 1. Gas Chromatographic Conditions

HT-PTV inlet	
Temperature	350 °C
Split ratio	30:1
Injection volume	0.1 µL
7890/LTM	
Column (LTM)	DB-1 5 m × 320 mm × 1 µm
LTM temperature program	45 to 350 °C at 150 °C/min, hold 1 min (GC oven: 300 °C, held for duration)
Column flow (He)	Ramp pressure: 18 psi to 42 psi at 11.8 psi/min
FID temperatures	350 °C
H_2 flow	40 mL/min
Air flow	400 mL/min
Make up (N_2)	40 mL/min
Data rate	5 Hz
SCD	
Burner temperature	800 °C
Vacuum of burner	324 Torr
Vacuum of reaction cell	11.6 Torr
H_2	40 SCCM
Air	8.3 SCCM
Data rate (AIB)	5 Hz
LTM system	G6578A, bundle for 1-channel 5-inch modules

Processing Two-Channel Data in the SimDis Software

The Agilent SimDis System enables the use of gas chromatography to determine the boiling point range distribution and percent recovery of petroleum fractions. The SimDis software allows for reports to be generated in two ways: automated and manual. Both require the user to first manually set a blank, calibration, and optional QC reference chromatogram. When working with dual channels, the SimDis software requires that each channel be labeled by the detector type rather than the defaults used by the GC ChemStation. Since the SCD operates off the analog input board (AIB), its signal begins with "AIB." For this reason, the Post-Run command macro SCDnamer.mac must be run to rename the signal file. The macro renames the AIB2B.ch channel as SCD1.ch. If the channel name is not corrected, the software will switch the FID and SCD channels during analysis, giving faulty results. The macro code to do this is shown below. It assumes that the AIB is in the rear position (B).

```
! SCDNamer call this as a post run command when an SCD is
installed

! it renames the dual channel AIB2B.ch to SCD1.ch to allow
simdis to properly calibrate

NAME SCDNamer

! This macro renames the SCD files named as AIB2B.ch to
SCD1.ch
if filestat(mode,dadatapath$+dadatafile$+"\AIB2B.CH")=1
```

```
rename dadatapath$+dadatafile$+"\AIB2B.ch",dadata-
path$+dadatafile$+"\SCD1.ch"

print "File Renamed"

else

print "No AIB2B File found"

endif

RETURN

ENDMACRO
```

Under the Setup tab of SimDis, select the default chromatograms to be used for the calculation. All desired solvent masking should be done here. The calibration run should have major carbon peaks identified; this can be done with an imported calibration table from ChemStation or manually in SimDis. The QC reference should have concentrations and dilution factors entered. Now enter the SimDis pane and set the parameters to be used. Do not include the solvent peak in the calculations and account for the baseline and noise using a zeroing method. In order to get proper percent recovery values make sure Normalize to 100% is not selected and a proper dilution factor is entered. Set the settings as default. For automation, enter ChemStation and select SimDis > Setup > Use SimDis Defaults under Data Analysis and select a report to generate after each run. For manual operation, select the desired chromatogram under the Report pane in SimDis and enter the report pane to view and print results.

Results and Discussion

The LTM column module employes a unique direct resistive heating technology using ceramic-insulated heating wire where contact with the capillary column is maximized. This is packaged in a very low mass assembly. Accurate and precise temperature sensing is possible by incorporating the temperature sensor with the capillary GC column. This technology greatly reduces GC analytical cycle times, addressing demands for greater productivity. The result for the D2887 reference gas oil (RGO) shows that the run time using LTM is less than 2.5 minutes, or about six times faster compared to conventional ASTM D2887 procedures. The SimDis results agree with the specifications of ASTM D2887 with excellent repeatability (see Table 2). The upper chromatogram in Figure 1 shows the D2887 RGO analysis by conventional air bath GC oven and the lower chromatogram shows RGO by LTM GC. The run time is about 2.5 minutes and 15 minutes for LTM and conventional air bath GC, respectively.

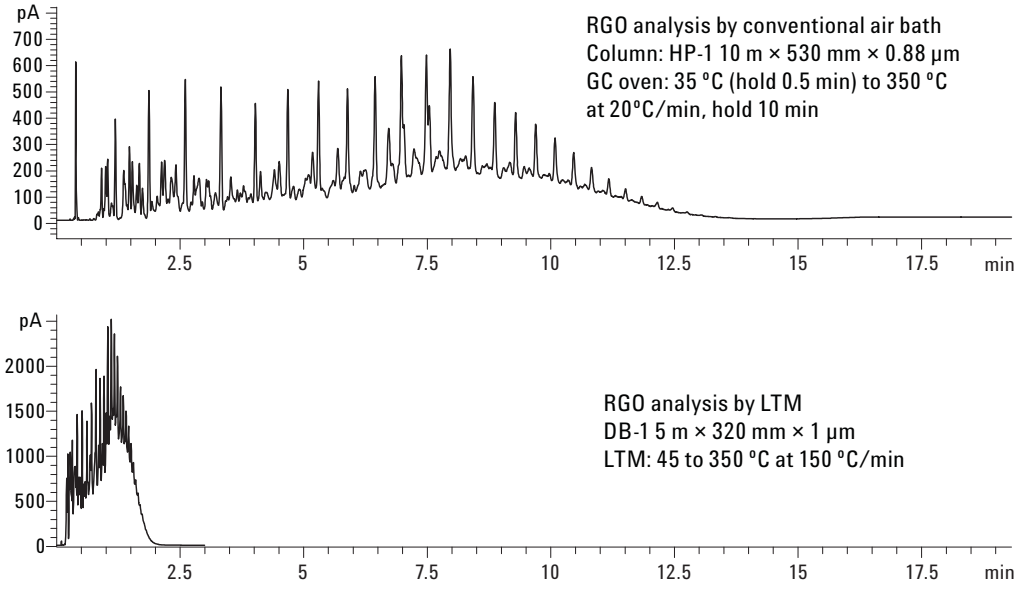

Figure 1. Comparison of RGO analysis by LTM and air bath GC ovens.

Table 2. RGO SimDis Results with the LTM System

	ASTM D2887 Values		Observed Value												
	BP, °C	Allowable difference	1	2	3	4	5	6	7	8	9	10	Average	Difference	RSD%
IBP	115	7.6	112	113	112	113	112	113	112	113	112	113	112.5	2.5	0.47
10%	176	4.1	173	174	173	174	173	174	173	174	173	174	173.5	2.5	0.30
20%	224	4.9	220	220	220	220	220	220	220	220	220	220	220	4	0.00
30%	259	4.7	254	255	255	255	255	255	255	255	255	255	254.9	4.1	0.12
40%	289	4.3	285	286	285	287	285	286	285	286	286	286	285.7	3.3	0.24
50%	312	4.3	309	310	309	310	309	309	309	309	309	309	309.2	2.8	0.14
60%	332	4.3	329	330	329	330	329	330	329	329	329	329	329.3	2.7	0.15
70%	354	4.3	350	352	351	352	351	352	351	352	351	352	351.4	2.6	0.20
80%	378	4.3	375	376	375	377	375	376	375	376	375	376	375.6	2.4	0.19
90%	407	4.3	404	405	405	406	404	406	404	405	404	405	404.8	2.2	0.19
FBP	475	11.8	474	475	475	476	475	476	475	475	475	475	475.1	–0.1	0.12

A mixture of C5 through C40 n-alkanes with known boiling points which are used for establishing the correlation between RT (min) and BP (°C) can be separated in less than 2.5 minutes with repeatability in retention time better than 0.1 percent relative standard deviation (RSD). Table 3 shows RT stability with RSD of approximately 0.02 to 0.15 percent (C40) using a rapid temperature program of 150 °C/min ramped from 45 to 350 °C. These results indicate a lack of cold spots and temperature nonuniformity in the LTM column module. Figure 2 is an overlay of five consecutive runs of the C5 to C40 calibration mixture.

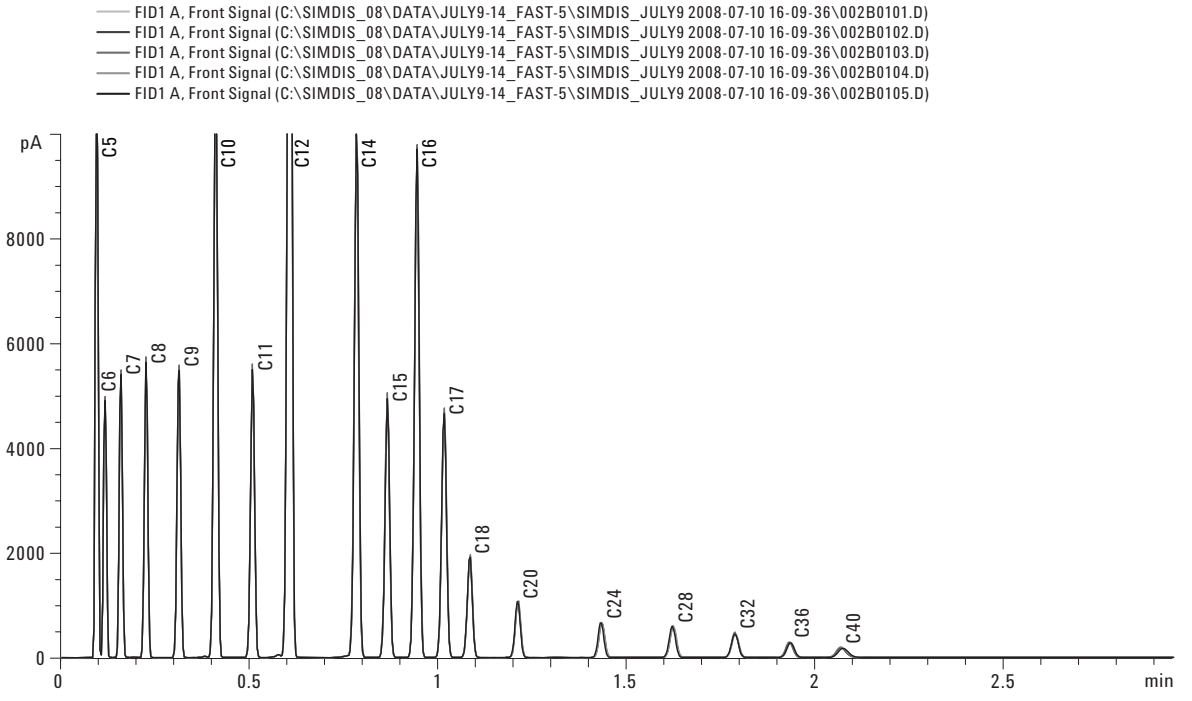

Figure 2. Overlays of five consecutive runs of the C5 to C40 calibration mixture. Chromatographic overlay shows outstanding repeatability. LTM: 45 to 350 °C at 150 °C/min.

Table3. Retention Time Repeatability of C5 to C40 Calibration Mixture (n = 5)

	1	2	3	4	5	Average	SD	RSD%
C5	0.09766	0.09760	0.09766	0.09763	0.09764	0.09764	2.33E-05	0.024
C6	0.11999	0.11992	0.11994	0.11989	0.11993	0.11994	3.67E-05	0.031
C7	0.16285	0.16274	0.16270	0.16263	0.16270	0.16272	8.07E-05	0.050
C8	0.23072	0.23051	0.23045	0.23036	0.23045	0.23050	0.000135	0.059
C9	0.31908	0.31883	0.31877	0.31867	0.31874	0.31882	0.000158	0.049
C10	0.41803	0.41775	0.41779	0.41777	0.41780	0.41783	0.000117	0.028
C11	0.51730	0.51707	0.51711	0.51716	0.51719	0.51717	8.61E-05	0.017
C12	0.61713	0.61688	0.61723	0.61716	0.61721	0.61712	0.000138	0.022
C14	0.79706	0.79676	0.79746	0.79707	0.79733	0.79714	0.000271	0.034
C15	0.87963	0.87964	0.88004	0.87985	0.88009	0.87985	0.000216	0.025
C16	0.95952	0.95940	0.95986	0.95941	0.95966	0.95957	0.000192	0.020
C17	1.03290	1.03263	1.03314	1.03279	1.03257	1.03281	0.000225	0.022
C18	1.10255	1.10190	1.10250	1.10236	1.10201	1.10226	0.00029	0.026
C20	1.23235	1.23131	1.23203	1.23151	1.23060	1.23156	0.000676	0.055
C24	1.45819	1.45721	1.45683	1.45772	1.45470	1.45693	0.001348	0.093
C28	1.65011	1.64884	1.64878	1.65001	1.64698	1.64895	0.001261	0.076
C32	1.81666	1.81587	1.81535	1.81739	1.81562	1.81618	0.000834	0.046
C36	1.96355	1.96259	1.96200	1.96611	1.96522	1.96389	0.001738	0.088
C40	2.10071	2.10110	2.10021	2.10619	2.10665	2.10297	0.003168	0.151

This work combines hydrocarbon SimDis with sulfur selective SimDis using an SCD mounted over the FID for simultaneous dual detector aquisition. In this configuration, about 20% of the FID exhaust gases enter the SCD burner, reducing sensitivity to 1/5 observed in an SCD-only configuration. This series configuration provides more than enough sensitivity for SimDis. A sample with known sulfur concentration is used as a QC reference (external reference sample) to calibrate response factors (RFs) for the sulfur channel needed for calculation of total sulfur. Sulfur linearity of the LTM method was checked by injecting the standard mixture at different concentration levels, ranging from 100 to 1,500 ng/µL (ppm). The calibration curve is displayed in Figure 3, giving a correlation coefficient above 0.999.

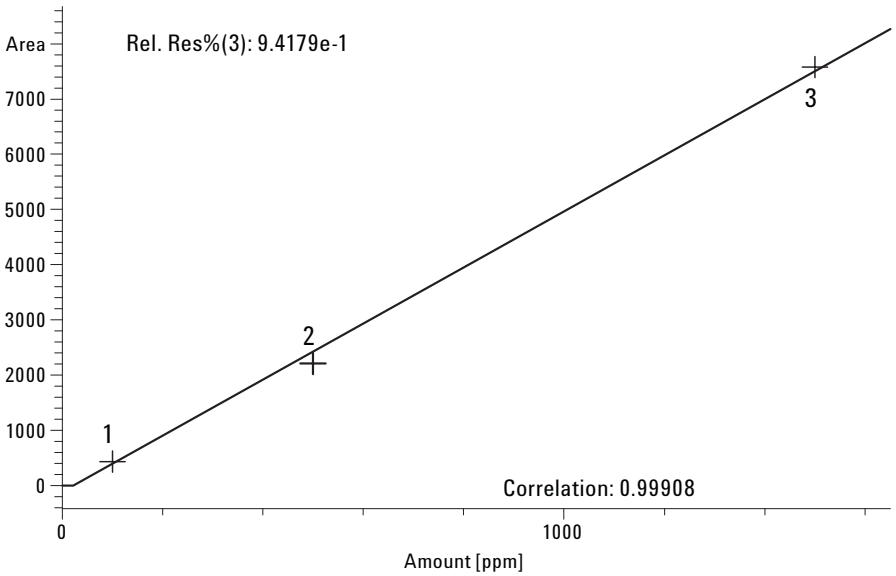

Figure 3. Linearity of sulfur with the SCD and LTM system.

A high-sulfur light cycle oil (LCO) sample was analyzed. Data were processed for hydrocarbon and sulfur simulated distillation, yielding excellent repeatability as shown in Table 4. The RSD is less than 0.4 percent for hydrocarbon and 0.5 percent except that of FBP for sulfur. The average total sulfur is 260.8 ppm with an RSD of 4.83 percent. Figure 4 shows the chromatograms for sulfur (top) and carbon (bottom).

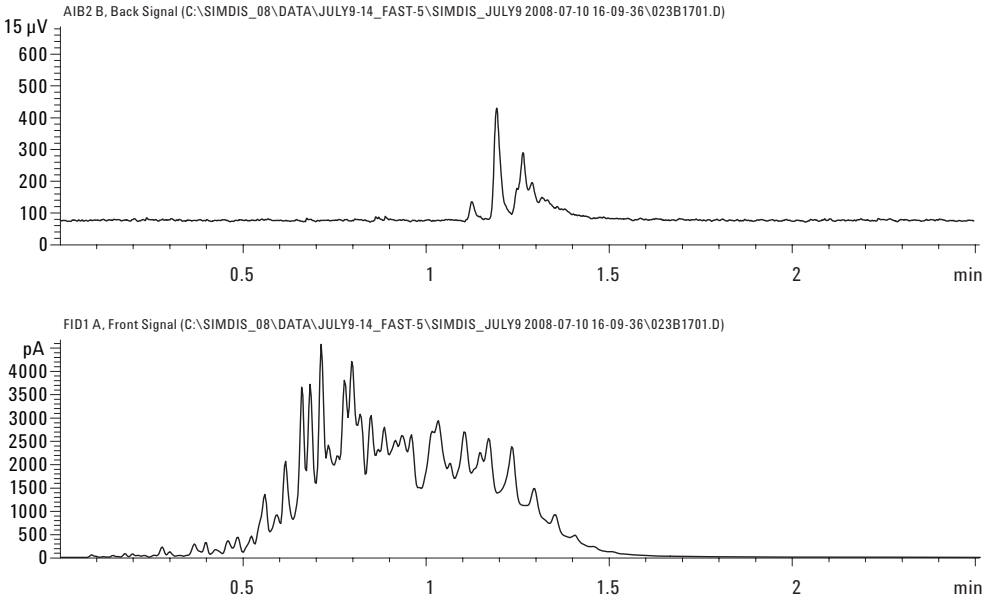

Figure 4. Chromatograms for sulfur and carbon. Sulfur top chromatogram.

Table 4. Hydrocarbon and Sulfur SimDis Results for Light Cycle Oil

Hydrocarbon SimDis												
	BP, °C										**Average**	**RSD%**
IBP	139	140	139	140	140	139	140	139	140	139	139.5	0.38
10%	222	223	222	223	223	222	223	222	224	223	222.7	0.30
20%	235	236	235	236	236	235	236	235	236	235	235.5	0.22
30%	249	250	249	250	250	249	250	249	250	249	249.5	0.21
40%	262	262	262	262	262	262	262	262	262	262	262.0	0.00
50%	276	277	277	277	277	277	277	276	277	277	276.8	0.15
60%	293	294	293	294	294	293	294	293	294	293	293.5	0.18
70%	308	309	308	309	309	308	309	308	309	308	308.5	0.17
80%	326	326	326	326	327	326	326	326	326	326	326.1	0.10
90%	347	347	347	347	347	347	347	347	347	347	347.0	0.00
FBP	411	411	411	412	413	412	412	412	412	412	411.8	0.15
Sulfur SimDis												
	BP, °C											
IBP	319	320	318	319	320	319	318	319	319	318	318.9	0.22
10%	334	334	334	334	334	334	334	334	334	334	334.0	0.00
20%	335	335	335	335	336	335	335	335	335	335	335.1	0.09
30%	337	337	337	337	337	337	337	337	337	337	337.0	0.00
40%	341	340	343	341	340	345	344	342	341	342	341.9	0.50
50%	349	349	349	349	349	350	349	349	349	349	349.1	0.09
60%	352	351	352	351	352	352	351	352	351	352	351.6	0.14
70%	355	354	355	355	355	355	355	355	355	355	354.9	0.08
80%	361	358	360	360	359	360	360	359	359	360	359.6	0.22
90%	368	365	367	367	366	369	368	367	367	367	367.1	0.30
FBP	399	384	385	386	383	407	392	384	395	395	391.0	2.05
Total sulfur (ppm)	260	237	255	262	252	281	273	254	263	273	260.8	4.83

Conclusions

A fast dual detector (FID and SCD in series) simulated distillation method using an LTM system on the 7890A GC provides a cycle time improvement over conventional systems of about 6X.

The SimDis result for ASTM D2887 RGO shows that the LTM system is equivalent to that of air-bath GCs. Results agree with the RGO specification of ASTM D2887 with RSDs of 0.12 to 0.47 percent across the reported percent off range. Wide boiling range hydrocarbons (C5 to C40 BP calibration mixtures) also show very good RT stability with RSD of 0.02 to 0.15 percent using a rapid column module temperature program of 150 ºC /min ramped from 45 to 350 ºC and sample injection using the HT-PTV. Sulfur linearity of the LTM/SCD system reveals an excellent correlation coefficient, above 0.999. SimDis of a high sulfur LCO sample shows an RSD of less than 0.4 percent for hydrocarbon and 0.5 percent except that of FBP for sulfur. The average total sulfur is 260.8 ppm, with an RSD of 4.83 percent.

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3. Method Translation Software. Download from Agilent.com: http://www.agilent.com/chem/mts

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Published in the USA
November 26, 2008
5990-3174EN

Agilent Technologies

Dual Channel Simulated Distillation of Carbon and Sulfur with the Agilent 7890A GC and 355 Sulfur Chemiluminescence Detector

Application Note

Hydrocarbon Processing

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Abstract

Two-channel simulated distillation by gas chromatography (GC) for both hydrocarbons and sulfur is described. The method utilizes a 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet and a sulfur chemiluminescence detector (SCD) mounted in series with a flame ionization detector (FID) by use of a special mounting adapter. A simulated distillation (SimDis) software program provides an easy-to-use solution for sulfur and hydrocarbon simulated distillation. The data show that observed boiling point (BP) values agree with the ASTM D2887 consensus BP values within the allowable differences. The system also demonstrates very good repeatability for both hydrocarbon and sulfur SimDis. An example of a light cycle oil (LCO) analyzed according to D2887 is also included.

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Introduction

Sulfur and hydrocarbon simulated distillation results provide meaningful information to optimize refining processes and ensure compliance with petroleum product specifications. A previous application note [1] describes a 6890 GC based system for hydrocarbon simulated distillation by ASTM D2887 [2]. Now with the highly selective Agilent Sulfur Chemiluminescence Detector (SCD), sulfur simulated distillation is possible. This 7890A GC based simulated distillation system consists of acquiring and analyzing simultaneously the specific detector data for hydrocarbon (FID) and sulfur (SCD).

Experimental

This two-channel SimDis application uses the Agilent 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet, and an SCD mounted onto an FID using a special adapter. Detailed GC conditions used are listed in Table 1.

Table 1. 7890A Gas Chromatographic Conditions (1) D2887, (2) D7213

HT-PTV inlet typical temperature programs	(1) 225 to 350 °C (hold 15 min) at 200 °C/ min to 225 °C at 100 °C /min (2) 50 to 420 °C (hold 15 min) at 200 °C /min to 50 °C at 100 °C /min
Split ratio	(1) 4:1 for diluted sample, 20:1 for nondiluted sample (2) 1:1
Injection volume	(1) 0.1 µL (2) 0.5 to 1 µL
Column	(1) HP-1 10 m × 530 mm × 0.88 µm (19095z-021) (2) DB-HT-SimDis 5 m × 530 mm × 0.15 µm (145-1001)
Column flow (He)	(1) 13 mL/min, constant flow mode (2) 16 mL/min, constant flow mode
FID temperatures	(1) 350 °C (2) 400 °C
H_2 flow	40 mL/min
Air flow	400 mL/min
Make up (N_2)	40mL/min
SCD	
Burner temperature	800 °C
Vacuum of burner	324 torr
Vacuum of reaction cell	11.6 torr
H_2	40 SCCM
Air	8.3 SCCM
Oven programs	(1) 35 °C (hold 0.5 min) to 350 °C at 20 °C/min , hold 10 min (2) 40 to 420 °C at 20 °C/min , hold 6 min
Data acquisition rate	5 Hz typical

SimDis Software

The processes of SimDis analysis include: blank analysis for baseline subtraction, calibration for establishing the relationship between boiling point and retention time (RT), validation for verifying both the chromatographic conditions and calculations in the method, and sample analysis. The Agilent SimDis software divides these functions under separate tabs that make navigation and data processing straightforward. The software is based on four modules: Browse, Setup, SimDis, and Report. For example, the Setup module allows you to configure the files to use for BP calibration, blank selection, and QC reference. Partial integration with the GC ChemStation sequence makes automated data analysis possible.

Processing Two Signals

The software can process one or two channels of signal data (FID and SCD for example) from GC ChemStation data files. When working with dual channels, the SimDis software requires that each channel be labeled by the detector type rather than the defaults used by the GC ChemStation. Since the SCD operates off the analog input board (AIB), its signal begins with "AIB." For this reason, the post-run command macro SCDnamer.mac must be run to rename the signal file. The macro renames the AIB2B.ch channel as SCD1.ch. If the channel name is not corrected, the software will switch the FID and SCD channels during analysis, giving faulty results. The macro code to do this is shown below. It assumes the AIB is in the rear position (B).

```
!=======================================
! SCDNamer call this as a post run command when an SCD is installed
! it renames the dual channel AIB2B.ch to SCD1.ch to allow simdis to
! properly calibrate
!=======================================
NAME SCDNamer
! This macro renames the SCD files named as AIB2B.ch to SCD1.ch
if filestat(mode,dadatapath$+dadatafile$+"\AIB2B.CH")=1
rename dadatapath$+dadatafile$+"\AIB2B.ch",dadatapath$+dadatafile$+"\SCD1.ch"
print "File Renamed"
else
print "No AIB2B File found"
endif
RETURN
ENDMACRO
```

Results and Discussion

Calibration

A calibration mixture containing a series of known n-alkanes can be used for establishing the relationship between BP and RT. C5 to C40 is used for ASTM D2887, and Polywax 500 dissolved in toluene is used to calibrate ASTM D7213 [3]. Since both are too viscous or waxy at ambient temperature to sample with a syringe, they need to be heated manually to approximately 80 °C before injection. RT repeatability is key for consistent correlation of BP and RT. Figure 1 and Figure 2 show overlays of consecutive runs of C5 to C40 and Polywax 500, respectively. Tables 2 and 3 show repeatability for both RT and area.

Polywax 500 Sample Preparation

Place approximately 80 mg of Polywax 500 in a 2-mL vial. Add about 1.5 mL toluene followed by the addition of a suitable mixture of n-paraffins from C5 to C18 (Agilent SimDis calibration No.2). The final concentration should be approximately one part of (C5–C18) to 20 parts of toluene. Initially heat the solution to 80 °C to dissolve the Polywax 500.

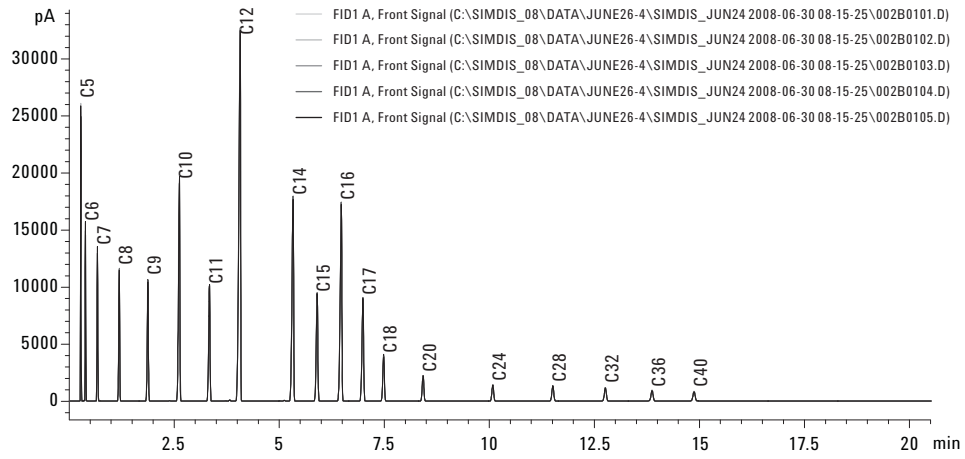

Figure 1. Overlay of five consecutive runs of C5 to C40 calibration mix, vial heated to 80 °C for 3 min prior to injection. GC conditions are listed in Table 1, items (1).

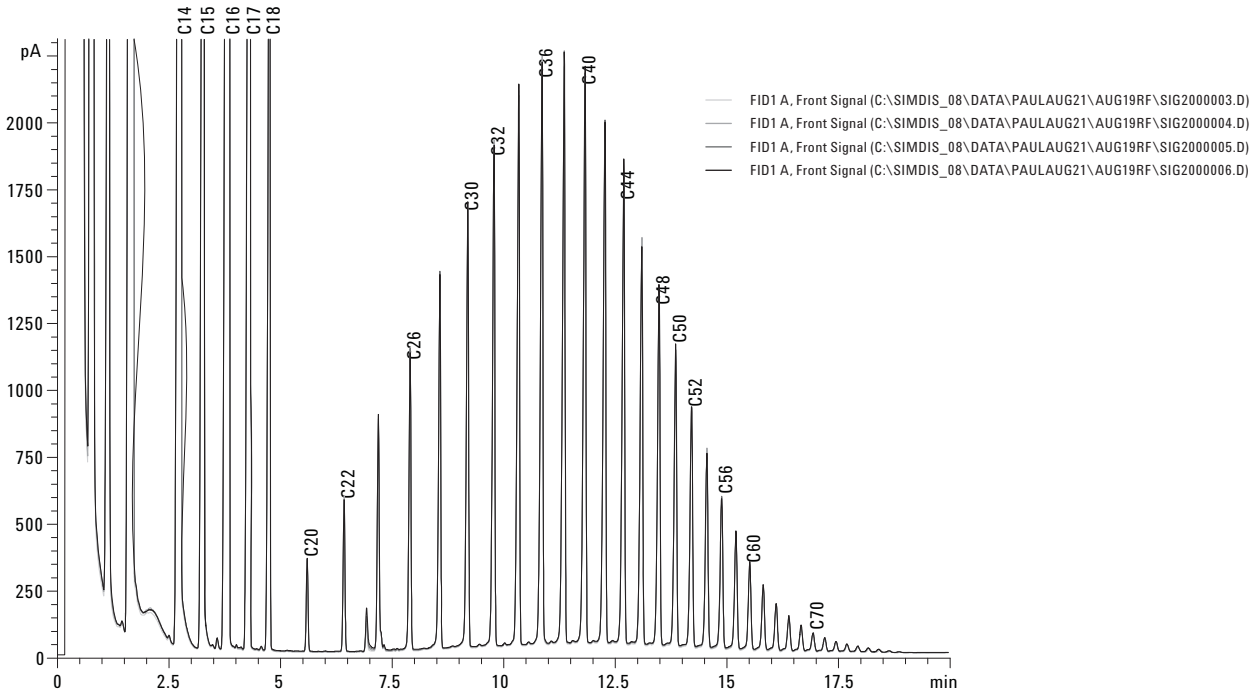

Figure 2. Overlay of four consecutive runs of Polywax 500 plus C5–C18. GC conditions are listed in Table 1, items (2).

Table 2. Repeatability for C5 to C40, n = 10

	Retention Time			Area		
	Average	STDEV	RSD%	Average	STDEV	RSD%
C5	0.275	0.000	0.06	19870	126	0.64
C6	0.388	0.000	0.09	14020	83	0.60
C7	0.673	0.001	0.17	16527	108	0.65
C8	1.192	0.002	0.16	18693	81	0.43
C9	1.874	0.002	0.12	20383	107	0.53
C10	2.622	0.003	0.10	43561	280	0.64
C11	3.338	0.002	0.07	22730	158	0.69
C12	4.068	0.002	0.05	94289	714	0.76
C14	5.327	0.002	0.03	48149	393	0.82
C15	5.902	0.002	0.03	24268	199	0.82
C16	6.477	0.001	0.02	49175	408	0.83
C17	6.991	0.001	0.02	24448	201	0.82
C18	7.485	0.000	0.00	10552	84	0.80
C20	8.424	0.001	0.01	6187	53	0.86
C24	10.083	0.000	0.00	4293	17	0.40
C28	11.512	0.001	0.01	4288	45	1.06
C32	12.762	0.002	0.01	3988	66	1.66
C36	13.874	0.001	0.01	3407	66	1.94
C40	14.874	0.002	0.01	3238	69	2.14

Table3. Repeatability of Polywax 500 Plus C5 to C18, n = 10

	Retention Time			Area		
	Average	STDEV	RSD%	Average	STDEV	RSD%
C14	2.769	0.002	0.07	49126	953	1.94
C15	3.278	0.002	0.05	24337	469	1.93
C16	3.847	0.002	0.05	49304	948	1.92
C17	4.311	0.002	0.05	24597	470	1.91
C18	4.753	0.001	0.03	11374	218	1.92
C20	5.596	0.001	0.01	952	17	1.80
C22	6.424	0.001	0.01	1635	30	1.81
C26	7.904	0.001	0.01	3615	62	1.71
C32	9.783	0.001	0.01	6856	105	1.53
C36	10.858	0.001	0.01	8418	137	1.63
C40	11.823	0.001	0.01	8432	128	1.52
C44	12.690	0.002	0.01	7037	137	1.95
C48	13.480	0.001	0.01	5288	104	1.98
C52	14.208	0.001	0.01	3677	67	1.83
C60	15.512	0.001	0.01	1353	19	1.40
C70	16.931	0.002	0.01	273	5	1.92

QC Reference

A QC reference sample is the basis for quantifying total sulfur and allows the direct entry of response factors for calculation based on total area and user-entered concentrations of sulfur. In this application, a diesel sample (SDF-1X-4, AccuStandard, Inc., New Haven, CT) with a sulfur concentration of 100 µg/g is used as the QC external reference for calibration of response factors for the SCD channel. This is needed for calculation of total sulfur in the sample. Figure 3 shows the graphic pane from the SimDis software for of the QC reference.

Reference Gas Oil Analysis

To meet the requirements of ASTM D2887, the reference gas oil (RGO) sample analysis must be performed to verify both the chromatographic performance and the calculation algo-

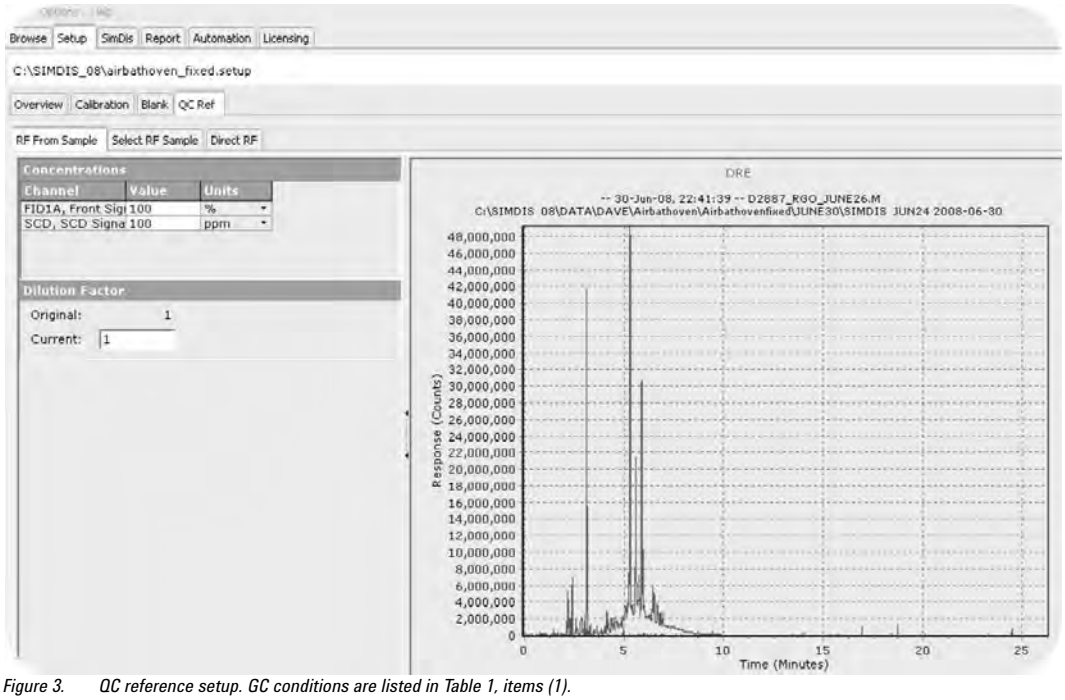

Figure 3. QC reference setup. GC conditions are listed in Table 1, items (1).

rithms involved in this test method. Figure 4 shows the chromatograms of RGO for both the hydrocarbon and sulfur channels. Tables 4 and 5 show the results for six runs of RGO analysis. The data show that observed BP values agree with the ASTM D2887 consensus BP values within the allowable differences and with good repeatability.

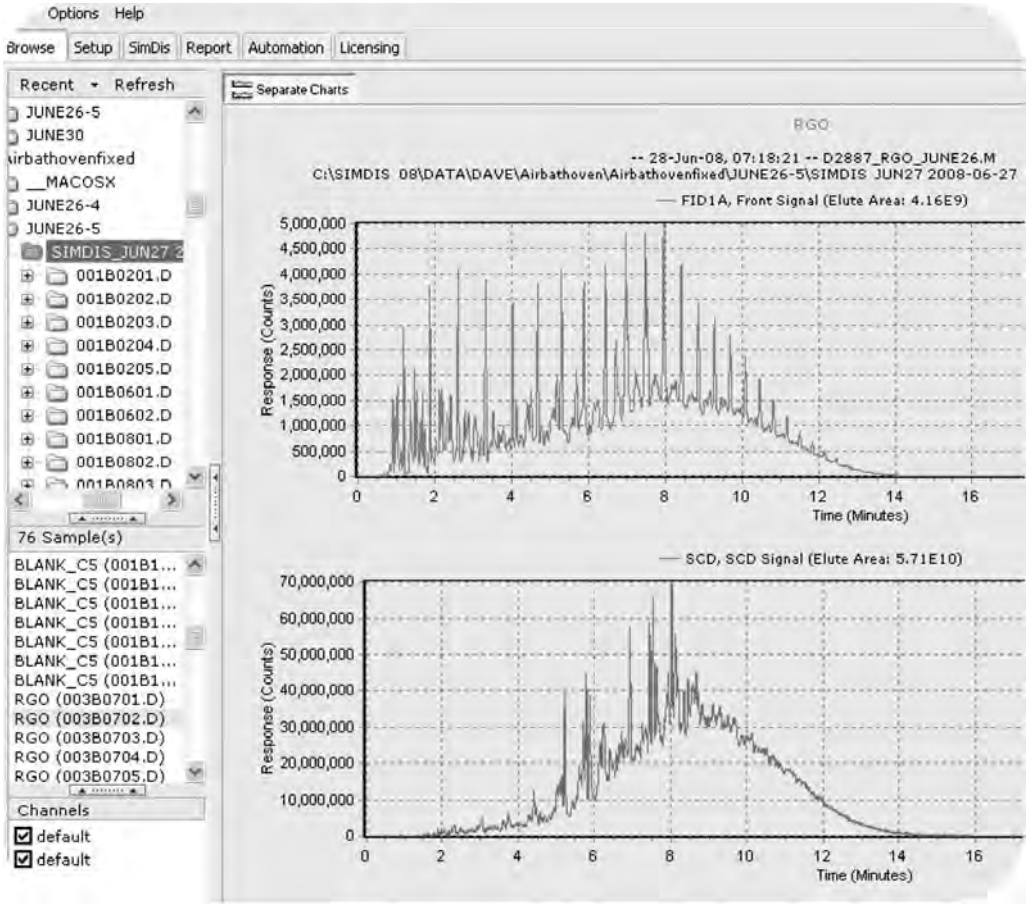

Figure 4, Chromatograms of RGO for hydrocarbon and sulfur channels. GC conditions are listed in Table 1, items (1).

Table 4. Hydrocarbon SimDis Results for Reference Gas Oil (Six runs shown.)

	ASTM D2887 Values										
OFF %	BP, °C	Allowable Difference	1	2	3	4	5	6	Average	Difference	RSD%
IBP	115	7.6	114	114	114	114	114	114	114	1	0.00
10%	176	4.1	174	174	174	174	174	174	174	2	0.00
20%	224	4.9	223	223	223	223	223	223	223	1	0.00
30%	259	4.7	258	258	258	258	258	258	258	1	0.00
40%	289	4.3	287	287	287	287	287	287	287	2	0.00
50%	312	4.3	311	311	311	311	311	311	311	1	0.00
60%	332	4.3	330	330	330	330	330	330	330	2	0.00
70%	354	4.3	352	352	351	352	352	352	352	2	0.12
80%	378	4.3	376	376	376	376	376	376	376	2	0.00
90%	407	4.3	405	405	405	405	405	405	405	2	0.00
FBP	475	11.8	471	471	471	471	471	471	471	4	0.00

Table 5. Sulfur SimDis Results for Reference Gas Oil, BP in °C

OFF%	1	2	3	4	5	6	Average	STDEV	RSD%
IBP	168	169	169	167	165	169	168	1.60	0.95
10%	265	265	265	265	265	265	265	0.00	0.00
20%	293	293	293	293	293	293	293	0.00	0.00
30%	314	314	314	314	314	314	314	0.00	0.00
40%	329	330	330	330	330	330	330	0.41	0.12
50%	344	344	344	344	344	345	344	0.41	0.12
60%	359	359	359	360	360	360	360	0.55	0.15
70%	376	376	377	377	377	377	377	0.52	0.14
80%	396	396	396	397	397	398	397	0.82	0.21
85%	408	408	408	409	409	409	409	0.55	0.13
90%	422	422	423	423	424	424	423	0.89	0.21
FBP	495	495	495	499	499	501	497	2.66	0.53

Light Cycle Oil Analysis

To illustrate repeatability, chromatographic overlays are shown in Figures 5a and 5b for an LCO sample. Tables 6 and 7 list the results for hydrocarbon and sulfur SimDis, respectively. The average total sulfur content calculated is 248 ppm with 3.5% RSD.

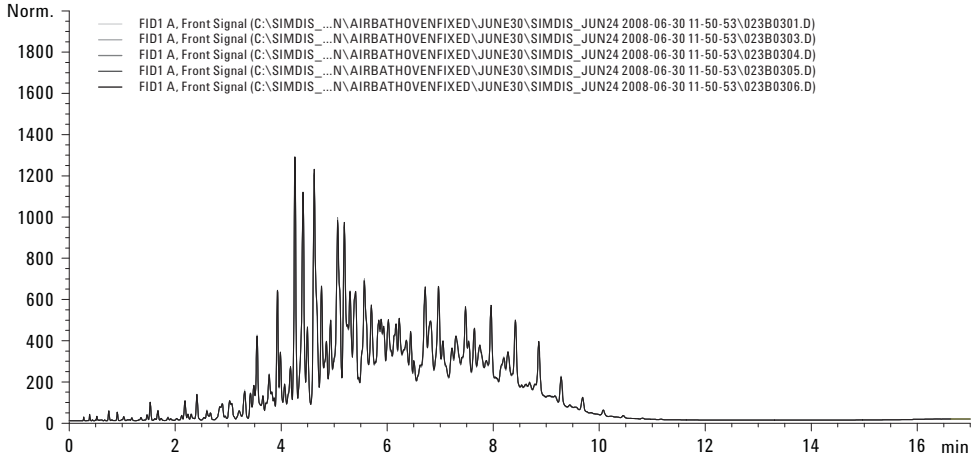

Figure 5a. Carbon SimDis of LCO. Five-run overlay.

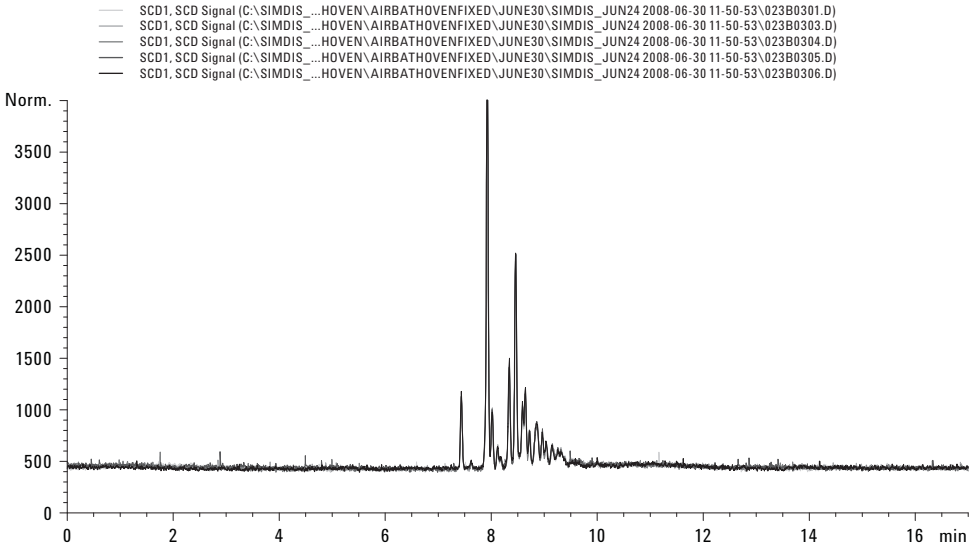

Figure 5b. Sulfur SimDis of LCO. Five-run overlay.

Table 6. Carbon SimDis Results for LCO, BP in °C

OFF%	1	2	3	4	5	Average	SD	RSD%
IBP	141	140	140	140	139	140	0.71	0.51
10%	221	221	221	221	221	221	0.00	0.00
20%	233	233	233	233	234	233	0.45	0.19
30%	247	247	247	247	247	247	0.00	0.00
40%	260	260	261	260	261	260	0.55	0.21
50%	274	275	275	275	275	275	0.45	0.16
60%	291	292	292	292	292	292	0.45	0.15
70%	306	307	307	307	307	307	0.45	0.15
80%	324	324	324	324	324	324	0.00	0.00
90%	344	344	344	344	344	344	0.00	0.00
FBP	391	391	391	391	392	391	0.45	0.11

Table 7. Sulfur SimDis Results for LCO, BP in °C

OFF%	1	2	3	4	5	Average	SD	RSD%
IBP	314	314	314	314	314	314	0.00	0.00
10%	328	329	328	328	328	328	0.45	0.14
20%	329	329	329	329	329	329	0.00	0.00
30%	329	329	329	329	329	329	0.00	0.00
40%	332	332	332	332	332	332	0.00	0.00
50%	342	342	342	342	342	342	0.00	0.00
60%	345	345	345	345	345	345	0.00	0.00
70%	347	347	346	346	347	347	0.55	0.16
80%	351	351	350	350	351	351	0.55	0.16
90%	359	359	357	359	358	358	0.89	0.25
FBP	375	375	371	374	371	373	2.05	0.55
Sulfur, ppm	254	250	240	238	258	248	8.62	3.48

Conclusions

This new SimDis procedure utilizes a 7890A GC configured with the HT-PTV inlet, and an SCD mounted in series with an FID. The Agilent SimDis software is capable of processing both FID and SCD data channels, providing a solution for hydrocarbon and sulfur simulated distillation.

Sulfur simulation distillation has been demonstrated using the Agilent 355 sulfur chemiluminescence detector. With a selectivity over carbon of approximately 10^6, reliable boiling point distributions of sulfur in petroleum fractions can be obtained.

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Published in the USA
December 5, 2008
5990-3237EN

Agilent Technologies

Automated Preparation of Simulated Distillation Samples for ASTM Methods D2887, D7213, D7398 and D6352 using a Dual Tower 7693A and Tray System

Application Note

Hydrocarbon Processing

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Abstract

A dual tower 7693A and tray system installed on the 7890A Gas Chromatograph was used for preparation of hydrocarbon calibration standards, solvent blanks, and actual petroleum samples for the purpose of analysis by simulated distillation (SimDis). The front tower is equipped with a 5 or 10 µL syringe while the back tower is equipped with a 250 or 500 µL syringe. A 150 sample tray with heater and mixer/barcode reader is also used. Procedures are described for sample preparation for ASTM D2887, D7213, D7398 and D6352. The Multimode Inlet, G3510, operated in a temperature programmed split mode was used for all samples.

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Introduction

Sample and calibration standard preparation for various simulated distillation methods is normally a manual process requiring dilution, mixing, and heating. Many procedures use volatile toxic solvents such as carbon disulfide. ASTM method D2887 commonly uses CS_2 for sample dilution while D6352 may use CS_2 or toluene for polywax calibration standard prepration. Sample heating is required for many of these procedures. Using the automation capabilities of the 7693A tower and tray system improves lab safety as well when working with CS_2 and other solvents by avoiding manual handling and uncontrolled heating of mixtures.

Experimental

For all experiments, the 7890A GC was equipped with dual 7693A towers and tray. The front tower used a standard 5 or 10 µl syringe and the rear tower was equipped with the optional large syringe carriage with either a 250 or 500 µL syringe. Sample prep procedures were done on the rear tower and sample injection occurred on the front tower. The 7890A was configured with the multimode inlet operated in temperature programmed split mode. Detection was with FID. In addition, two 7890A oven systems were used. The first configuration used the conventional air bath oven and the second used the Low Thermal Mass (LTM) system. Instrumental parameters for various configurations are listed in Table 1.

Table 1. 7890A SimDis parameters

LTM System for D2887	
LTM module	5M × 0.32 mm × 0.50 µm DB1, 5 inch format
7890A oven	300 °C isothermal
Inlet	Multimode, 270 °C (0 min) to 355 °C at 200 °C/min
Liner	Single taper with glass wool, 5183-4647
Split ratio	20:1
Pressure program (Inlet)	8 psi (0 min) – 42 psi (0.9 min) at 14 psi/min
LTM program	40 °C (0 sec) to 350 °C (30 sec) at 100 °C/min
Standard System for D2887	
Column	10M × 0.53 mm × 3.0 µm D2887
Oven	40 °C (0 min) to 350 °C (5 min) at 15 °C/min
Inlet	Multimode, G3510, 50 °C (0 min) to 330 °C (4 min) at 200 °C/min
Liner	Single taper with glass wool, 5183-4647
Split	4 to 1
Flow	3.2 psig at 40 °C, constant flow mode
7890A system for D7213 and D7398 (Polywax 500 calibration)	
LTM	
Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis 5-inch LTM format
Oven	LTM configuration, 7890A oven 325 °C isothermal, module 40 °C (0 min) to 400 °C (30 sec) at 50 °C/min
Inlet	Multimode, 270 °C (0 min) to 400 °C (3 min) at 300 °C/min
Split ratio	4 to 1 and 10 to 1
Pressure program	2.5 psi (0 min) to 9.5 psi (1.0 min) at 1 psi/min
Standard Air Bath Oven	
Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis
Oven program	40 °C (0 min) to 400 °C (5 min) at 15 °C/min
Inlet	Multimode, 210 °C (0 min) to 400 °C (10 min) at 200 °C/min
Split ratio	4 to 1
Flow	15 mL/min, constant flow mode
7890A system for D6352 (Polywax 655 calibration)	
Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis
Oven program	40 °C (0 min) to 430 °C (5 min) at 15 °C/min
Inlet	Multimode, 250 °C (0 min) to 430 °C (hold until end of run) at 200 °C/min
Split ratio	4 to 1
Flow	16 mL/min, constant flow mode
7693A System	
Front tower	5 or 10 µL syringe, G4513A
Back tower	250 or 500 µL syringe, G4521A syringe carriage
Tray	150 sample capacity with heater and mixer/barcode reader, G4520A
Inlet	G3510 Multimode, CO_2 cooled
ChemStation	B.04.01
7890A firmware	A.01.10 or greater

Discussion

A typical sample preparation program for D2887 setup is shown in Table 2. This illustrates just one way to program preparation of the calibration standard, reference gas oil (RGO), and blank that are necessary to set up a system for routine analyses. The commands can be assembled in other ways to produce the same end result. The following vials and tray locations are used with this program.

Tray position 1	Calibration mix, 0.5 µL of C5 to C40, Agilent part number 5080-8716
Tray position 2	1 mL RGO, Agilent part number 5060-9086
Tray position 3 to 5	Empty vials with 100 µL inserts, Agilent part number 5188-6592

When the procedure is complete, vial 3 will be the prepared RGO for injection, vial 4 will be the prepared calibration mix for injection, and vial 5 will be a CS_2 blank. Next, a three-line sequence is set up that starts with vial 4 (calibration mix). Vial 4 is run with the ChemStation method set with this procedure active, then vial 3 (RGO) and vial 5 (CS2 blank) are run using the same method but with the prep procedure inactive (unchecked in ChemStation's 7890A Injector Program pane under edit 7890A Parameters parameters menu because these samples are already prepared from the method in the first line of the sequence table). For all three samples, the core ChemStation method performs a sample preheat at 80 °C and a sample mix at 500 rpm for 20 seconds before injection. Lastly, the calibration, prepared RGO, and blank vials are fitted with 100 µL inserts so that the solvent amounts used for the procedure are minimized. Please note that when these inserts are used, mixing should be limited to speeds of approximately 500 rpm to avoid "spilling" liquid over the top of the insert into the bottom of the 2-mL vial.

Preparation of polywax standards for the higher temperature SimDis method is always challenging due to their low solubility. Solvents such as CS_2 and toluene are commonly used, and

Table 2. Sample prep procedure for D2887

Sampler program steps

Move vial from front sample vial offset by -3 vial(s) to back turret position #1
Dispense 750 µL from vial Wash A3 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to front sample vial offset by -3 vial(s)
Move vial from front sample vial offset by -1 vial(s) to back turret position #3
Move vial from front sample vial offset by 0 vial(s) to back turret position #2
Load 150 µl from vial Wash A1 with 0 µl airgap
Load 50 µl from vial Sample 3 with 0 µl airgap
Load 0 µl from vial Waste A1 with 0 µl airgap
Load 150 µl from vial Wash A1 with 0 µl airgap
Load 0 µl from vial Sample 2 with 0 µl airgap
Move vial from front sample vial offset by -3 vial(s) to heater
Heat vial at 80 degrees C for 300 seconds
Move vial from heater to back turret position #1
Load 5 µl from vial Sample 1 with 0 µl airgap
Load 0 µl from vial Sample 2 with 0 µl airgap
Load 150 µl from vial Wash A2 with 0 µl airgap
Wait for 1 minutes
Load 0 µl from vial Waste A3 with 0 µl airgap
Dispense 150 µL from vial Wash A3 to vial Waste A1 on the Back tower
Wash syringe in Back tower, drawing from Wash A2 dispensing into Waste B1 3 times
Move vial from back turret position #1 to front sample vial offset by -3 vial(s)
Move vial from back turret position #2 to front sample vial offset by 0 vial(s)
Move vial from front sample vial offset by -2 vial(s) to back turret position #1
Dispense 20 µL from vial Sample 1 to vial Sample 3 on the Back tower
Move vial from back turret position #3 to front sample vial offset by -1 vial(s)
Move vial from back turret position #1 to front sample vial offset by -2 vial(s)
Move vial from front sample vial offset by 1 vial(s) to back turret position #1
Wash syringe in Back tower, drawing from Wash B3 dispensing into Waste B2 3 times
Dispense 150 µL from vial Wash A3 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to front sample vial offset by 1 vial(s)
Wash syringe in Back tower, drawing from Wash A1 dispensing into Waste A1 2 times
Wash syringe in Front tower, drawing from Wash A1 dispensing into Waste A1 2 times

heating of the solvent/polywax vial is required just prior to injection. This entire procedure can be automated with the 7693A tower and tray system. The basic procedure for Polywax 500 is as follows:

- Place approximately 80–100 mg of Polywax 500 in a 2-mL vial and seal
- Add 125 µL of a C20/toluene solution to the polywax vial
- Add 1.25 mL of toluene to the polywax-C20 vial
- Mix the vial
- Heat the vial at 80 °C for 4 min
- Return to tray
- Heat one final time (3 min. typical) just prior to injection

Table 3 shows the basic prep procedure using a dual tower/tray system automating the steps shown above. The only manual step is adding the solid polywax to Vial 1. Vial 2 contains a C20/toluene mixture. Preparation of this sample could be automated as well. This procedure is applicable to D7213 SimDis and D7398 (Boiling Range Distribution of Fatty Acid Methyl Esters).

A resulting chromatogram from injection of the prepared Polywax 500 vial (vial 1) is shown in Figure 1. A symmetric distribution of the polywax fragments with good resolution to C80 can be seen.

Table 3. Preparation of Polywax 500

Sampler program steps
Wash syringe in Back tower, drawing from Wash A1 dispensing into Waste A1 2 times
Move vial from tray vial #1 to back turret position #1
Move vial from tray vial #2 to back turret position #2
Dispense 1000 µL from vial Wash A2 to vial Sample 1 on the Back tower
Dispense 5 µL from vial Sample 2 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to mixer
Move vial from back turret position #2 to tray vial #2
Mix at 2000 rpm 2 times for 10 seconds
Move vial from mixer to heater
Heat vial at 80 degrees C for 240 seconds
Move vial from heater to tray vial #1
Wash syringe in Back tower, drawing from Wash A2 dispensing into Waste B1 3 times

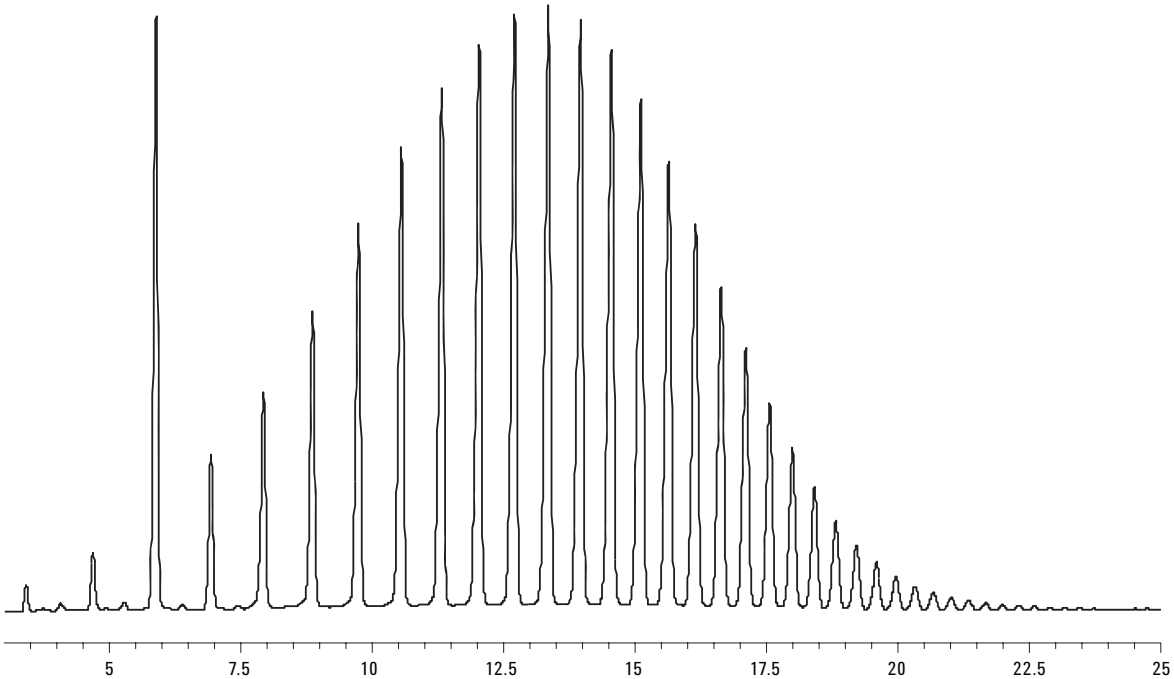

Figure 1. Polywax 500 with C20 marker. Multimode inlet with 7890A oven.

The preparation program for Polywax 655 is essentially the same as shown above for Polywax 500 except that heating is extended to 6 minutes, for better dissolution. Then just prior to injection, the prepared vial is heated for another 3 minutes. In the chromatogram shown below in Figure 2, a small amount (5 µL) of C5-C18 mix was added to the Polywax 655/ toluene solution as part of the automated procedure.

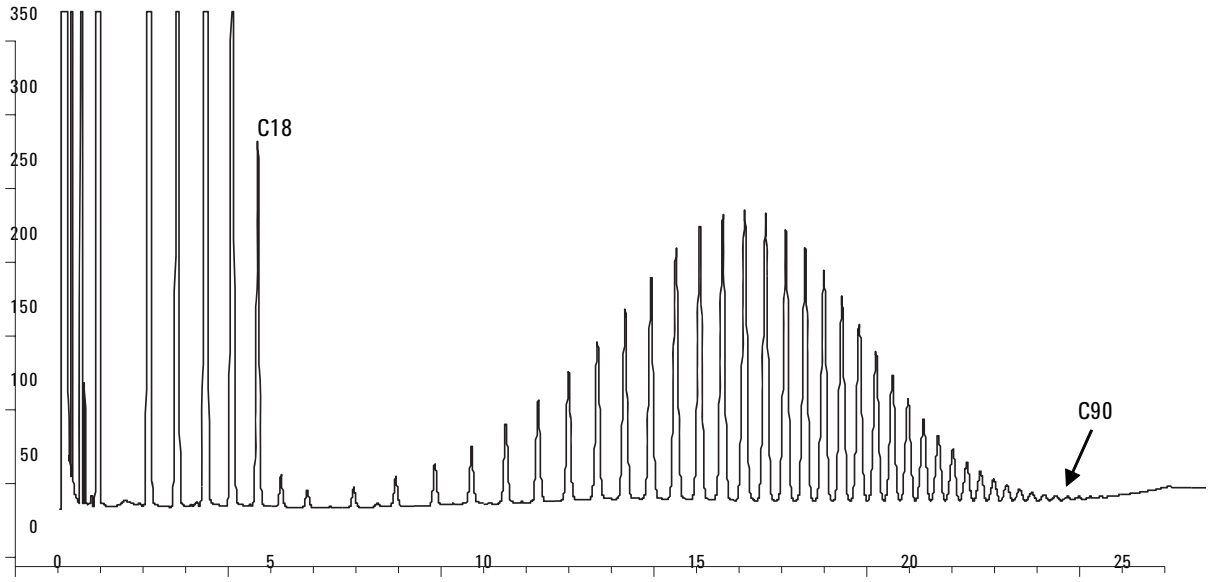

Figure 2. Chromatogram of Polywax 655.

The chromatogram was produced with the multimode inlet used in temperature-programmed split mode. Good definition of polyethylene fragmented to C110 is shown in Figure 3 where the last 5 minutes of the chromatogram are enlarged to show detail. Producing this detail out to C110 is extremely difficult for most chromatographic systems. The 7890A/7693A system produces excellent results with this sample.

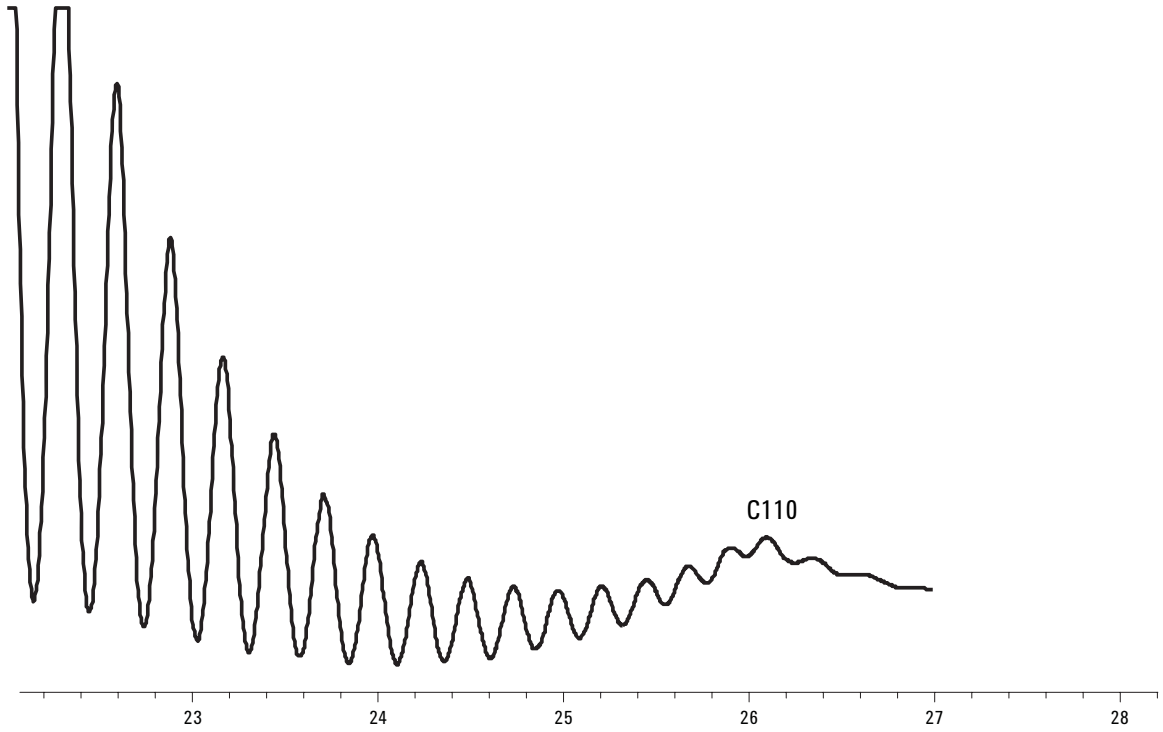

Figure 3. Polywax 655 to C110. Multimode inlet program: 150 °C (0 min) to 430 °C (hold until end of run) at 200 °C/min. 7890A oven: 40 °C (0 min) to 430 °C (5 min) at 15 °C/min. 3 µL injection. Solvent is toluene.

Reproducibility of the sample preparation steps is excellent as seen in Figure 4, for the dilution of a heavy vacuum gas oil sample (HVGO). The program steps that were followed to produce these chromatograms are given in Table 4. The back tower equipped with a 500-µL syringe, was used for sample preparation and the front tower with a 5-µL syringe was used for sample injection. Carbon disulfide was used for sample dilution. This program assumes a sequence is run using vial 2. Vial 1 is the stock HVGO sample that is first prepared by adding 0.5 g of the oil to a 2-mL vial. This material is extremely viscous and cannot be drawn into a syringe. Therefore the program performs a fully automated two-stage dilution prior to injection.

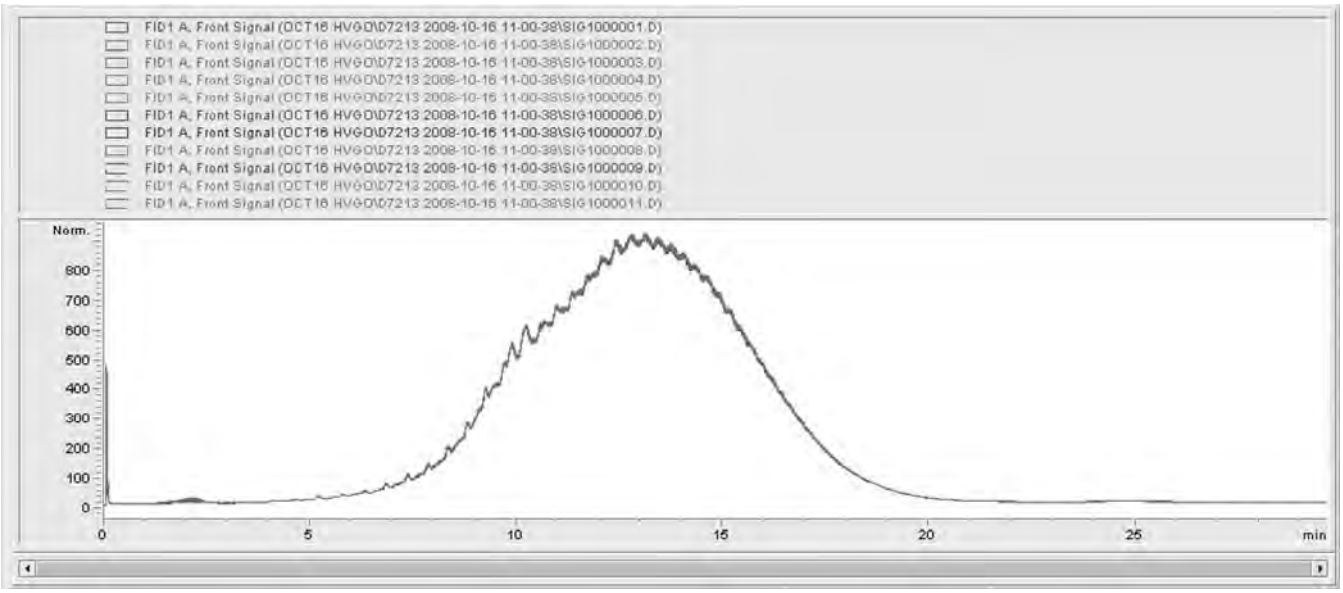

Figure 4. Overlay of 11 runs of HVGO, each prepared using 7693A towers and tray.

Table 4. Preparation of HVGO for injection. CS_2 is used as the solvent.

Sampler program steps
Move vial from front sample vial offset by -1 vial(s) to back turret position #1
Dispense 600 µL from vial Wash A3 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to heater
Move vial from front sample vial offset by 0 vial(s) to back turret position #1
Heat vial at 60 degrees C for 180 seconds
Mix at 3000 rpm 3 times for 20 seconds
Move vial from heater to back turret position #2
Dispense 250 µL from vial Sample 2 to vial Sample 1 on the Back tower
Wash syringe in Back tower, drawing from Wash A3 dispensing into Waste A1 3 times
Dispense 1000 µL from vial Wash A1 to vial Sample 1 on the Back tower
Move vial from back turret position #2 to front sample vial offset by -1 vial(s)
Move vial from back turret position #1 to mixer
Mix at 3000 rpm 4 times for 20 seconds
Move vial from mixer to front sample vial offset by 0 vial(s)

Conclusions

Difficult sample preparation procedures that are commonly used for petroleum and fuel samples can be easily automated with the 7693A tower and tray system for the 7890A and the 6890A. The system is particularly well suited for preparation of polywax calibration samples that are used for higher temperature methods. Tasks such as mixing, solid dissolution, dilution, heating, and internal standard addition are easily accomplished.

Chromatographic performance is enhanced through use of the multimode inlet. Using standard split injection liners, good sample capacity without carryover and with minimal discrimination of wide boiling samples is seen. The inlet was used in the temperature-programmed split mode for this work. Cryo cooling was not used, however, cryo can be used optionally to shorten inlet cool down between runs if desired.

For samples that fall within the boiling point range of D2887, D7213, and D7398, the Low Thermal Mass (LTM) system can be used to shorten typical analysis cycle times by 30 to 50% [1]. The high temperature method D6352 requires the standard 7890A oven.

The sample prep procedures listed here represent just one way of accomplishing a given task. Given the commands available with the system, there are many variants that will lead to the same end result.

Reference

1. C. Wang, R. Firor, and P. Tripp, “Fast Hydrocarbon and Sulfur Simulated Distillation Using the Agilent Low Thermal Mass (LTM) System on the 7890A GC and 355 Sulfur Chemiluminescence Detector,” November 2008, Agilent Technologies publication 5990-3174EN.

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Printed in the USA
March 26, 2009
5990-3778EN



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Analysis of Low Concentration Oxygenates in Environmental Water Samples Using Purge and Trap Concentration and Gas Chromatography/Mass Spectrometry

Environmental

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Abstract

The frequent use of oxygenate additives in gasoline to produce clean burning fuels has led to widespread and well documented contamination of ground water and drinking water supplies. The phasing out of methyl tert-butyl ether (MTBE) as the oxygenate of choice has led to an increased interest in testing for other common additives. These other additives may be ethers, other than MTBE, but also methanol or ethanol may be considered. Traditional techniques used for the analysis of volatile organic compounds in drinking and ground waters frequently employ the use of a purge and trap concentrator interfaced with a gas chromatograph. Detectors being used range from photoionization detectors (PID) and electrolytic conductivity detectors (ELCD) to mass selective detectors (MSD). Mass spectrometry is becoming the detection mode of choice for these additives, as it provides an additional level of confirmatory confidence in the presence of many potential matrix interferences. However, the challenge of extracting extremely polar analytes from an aqueous matrix requires modification and optimization of the purge and trap concentrator from its typical settings. As laboratories are seeking to determine these polar additives in the low part-per-billion (ppb) range, it is important that all aspects of the system be optimized. This application note will discuss system settings necessary for achieving low level quantitation of additives such as methanol and ethanol.

Experimental

This work was performed using a 6890 Plus gas chromatograph equipped with a 5973 mass spectrometer (Agilent Technologies, Inc. Wilmington DE). The purge and trap (P&T) used in the study was a model 3100 obtained from Tekmar/Dohrman (Cincinnati, OH). The J&W Scientific brand capillary column used, DB-VRX, was obtained from Agilent Technologies Inc. (Folsom, CA).

All standards used were prepared in-house from neat materials. Standard solutions were prepared in purified water.

Discussion

More and more frequently environmental laboratories are being asked to analyze for oxygenated analytes in drinking, ground and wastewater samples using pre-existing P&T GC/MS technology. Analytes such as acetone, ethyl ether, methyl-*tert*-butyl ether (MTBE), *tert*-butanol (TBA) and 2-butanone (MEK) are common, but now labs are beginning to receive an increasing number of requests for methanol and ethanol. As some laboratories are reporting very low method detection limits for these polar analytes, it is becoming apparent to others that not matching these low levels may eventually result in a loss of business. This work was performed for two primary reasons:

- To optimize P&T system conditions in order to achieve the best sensitivity possible for oxygenates in water
- To ascertain whether or not the low detection levels being reported by laboratories are realistic and achievable

The basic study design was as follows: Multiple replicates of an oxygenate standard (Table 1) were run using typical P&T conditions to establish a base response and to assure that reproducible results were being obtained. The %RSD for the multiple runs averaged 0.6%–0.8%. Once the base response was established analysts made modifications to the method parameters and charted any response changes for the same standard solution. Once the most significant modifications were defined they were combined to provide the best possible response enhancement. At this point a calibration curve was performed to establish that this technique was truly valid for quantitative work spanning a wide range of concentrations.

The oxygenate standard shown in Table 1 was prepared from neat materials in purified water and was used for all of the response enhancement work. Analytes are in solution at concentrations ranging from 5 to 500 µg/L. These concentrations were derived through experimentation using typical labratory P&T condtions so that all of the peaks of interest were on the same scale at the beginning of the study.

Table 1. Maximizing Oxygenate Response Analyte Concentrations in Purged Standard

Compound	Concentration (µg/L)
Methanol	500
Ethanol	500
Acetone	50
Ethyl ether	5
tert-Butanol (TBA)	50
Methyl-*tert*-butyl ether (MTBE)	5
Methyl ethyl ketone (MEK)	50

All stock solution prepared in purified water

Figure 1 shows the chromatogram obtained for the oxygenates standard using typical P&T concentrator conditions. Note that the abundance counts on the Y axis range up to approximately 35,000. This is an extracted ion chromatogram for *m/z* values of 31, 43, 73, and 59.

The primary variables considered in this work were:

- Purge gas flow
- Sample volume
- Sample temperature
- Matrix modification

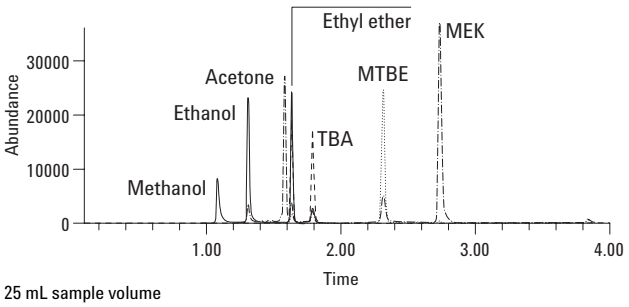


Figure 1. Oxygenates standard run using typical P&T conditions and DB-VRX capillary column.

Purge Gas Flow

The purge-gas flow was not adjusted or modified from typical settings as in most cases the laboratory will use the same P&T/GC/MS system for this analysis as well as for their standard 8260B and 524.2 work. Purge flow in most, if not all, P&T systems is a manual adjustment. Purge flow has a definite impact upon analyte recovery and if it is not kept constant calibration curves may become invalid and need to be rerun. If purge flow were manually increased for the oxygenate work and manually adjusted back down to return to 8260B or 524.2 work, it may jeopardize the current calibration curve. In addition, excessive purge flow can lead to trap breakthrough for some of the more volatile analytes contained in such methods. It was deemed more important that laboratories be able to easily adopt the changes suggested here without causing any loss in productivity for other methods of interest. As such, a purge flow of 40 mL/min for 11 minutes was maintained.

Sample Volume

It was shown in O.I. Analytical application note number 13271198 that utilization of a 25 mL sample volume vs. the typical 5 mL results in better sensitivity and improved calibration reproducibility. In its simplest form, five times the sample means five times the nanogram amount in solution. This does not mean five times the response will be achieved for all analytes, but for most a significant increase in response will be noted. All subsequent work was performed using a 25 mL sample size.

Sample Temperature

Multiple runs were performed with the sample temperature at ambient, 45 °C, 55 °C, 65 °C, 75 °C and 85 °C. Figure 2 shows the response enhancement with temperature increase for each analyte in the oxygenate standard. The responses graphed are all relative to the sample purged at ambient temperature, which is considered 100% recovery. There is a consistent response increase with temperature up to 75 °C but at 85 °C a dramatic increase is noted. As the response increase was so significant at 85 °C, this temperature was considered optimum. The sample preheat (heating prior to purge) was set for 1 minute for all temperatures. In reality, after 1 minute the purge temperature had only reached approximately 55 °C. After 2 minutes the temperature was around 65 °C, 3 minutes was 75 °C and not until the 4-minute mark did the vessel actually reach 85 °C. While the temperature was not at the set-point when purging began, it was deemed as acceptable as the heating rate of the sample was very consistent and even in the worst case, with an 85 °C set-point, the sample was purged for 8 minutes at full temperature. Increasing the preheat time to 3 and 4 minutes did not result in any significant response improvement, but definitely increased the overall purge and trap cycle time, thus reducing sample throughput. One minute of preheat gave excellent response with a minimal cycle time.

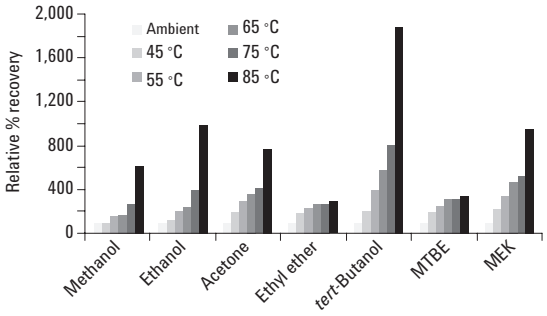


Figure 2. Analyte response vs. sample temperature.

When heating the sample to this degree, the amount of moisture transferred to the sorbent trap is significant. One benefit of the Vocarb™ 3000 trap used in this work is that it is dry-purgeable. Whether or not the water is actually purged from the trap during this step or simply purged completely into an appropriate sorbent was not explored, but a benefit in chromatographic performance is evident (Figure 3).

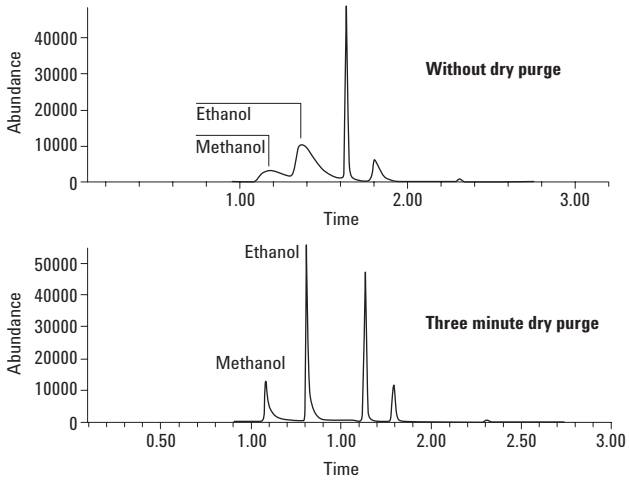


Figure 3. Modifying P&T conditions–secondary effects of increased sample temperature.

Matrix Modification

It is well understood that increasing the ion content of an aqueous solution can lead to improved recovery of non-ionic organic species from that solution. The process of increasing the ion content is generally termed 'Salting'. The primary considerations when salting a solution are what type of salt and how much salt to add. Table 2 refers to the benefits and drawbacks of several common salts. Considering the obvious drawbacks of sodium carbonate and potassium phosphate, this study focused on the use of sodium chloride and sodium sulfate. Figures 4 and 5 show the improved response achieved with different mass additions of both sodium chloride and sodium sulfate, respectively. Figure 6 gives a direct comparison of 'no salt' relative to optimum amounts of sodium chloride and sodium sulfate. It is obvious that sodium sulfate gives superior performance and, as it does not have the same corrosive characteristics as sodium chloride, it was chosen for all further work.

Table 2. The Benefits and Drawbacks of Several Common Salts

Compound	Results
Sodium chloride (NaCl)	Highly soluble in water (~8 g/25 mL), readily available, chlorine ion very reactive
Sodium sulfate (Na_2SO_4)	Highly soluble in water (~6 g/25 mL), neutral pH in solution, 2 sodium ions per molecule of salt
Sodium carbonate (Na_2CO_3)	Highly alkaline in solution
Potassium phosphate dibasic (K_2HPO_4)	Extremely soluble in water (~37 g/25 mL), difficult to work with

Figure 4 shows the effect of sodium chloride salt quantity on response relative to the 'no salt' standard P&T condtions. Above 6 g of NaCl the salt was not dissolving completely into solution and so addition was stopped here.

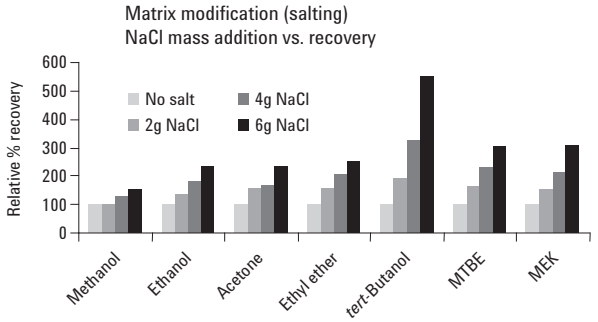


Figure 4. The effect of sodium chloride salt quantity on response relative to the 'no salt' standard P&T condtions.

Figure 5 shows the effect of sodium sulfate salt amount on response relative to the 'no salt' standard P&T conditions. There is a distinct rise in response at 6 g as we approach full saturation of solution.

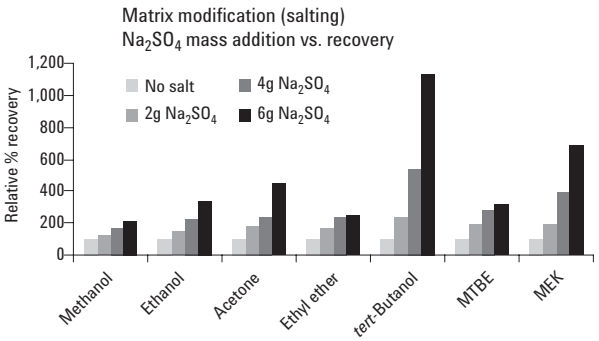


Figure 5. The effect of sodium sulfate salt amount on response relative to the 'no salt' standard P&T conditions.

In Figure 6, this combined bar-graph shows directly the response differences experienced with the two different types of salt (both at 6 g) relative to the 'no salt' standard P&T conditions. It is clear that there is a definite response advantage to using sodium sulfate vs. sodium chloride.

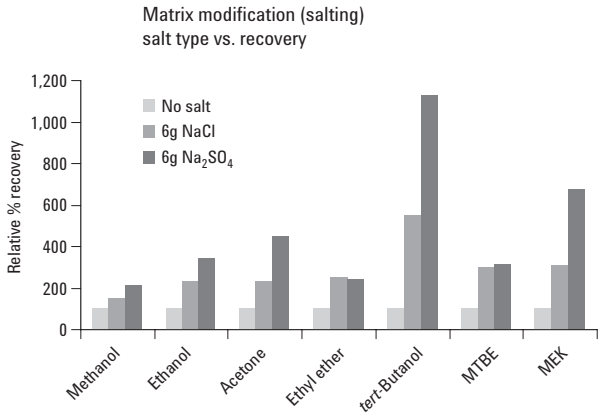


Figure 6. The response differences experienced with the two different types of salt relative to the 'no salt' standard P&T conditions.

Combining Parameters

Figure 7 shows the effect of temperature and salt addition alone, relative to standard conditions, but also shows how much more impact these modifications made once they were combined. For instance, *tert*-Butanol response was increased roughly 20 times using an 85 °C purge temperature and roughly 10 times using the addition of 6 g of sodium sulfate, but when these two modifications were combined it resulted in an overall response increase of over 75 times relative to standard P&T conditions.

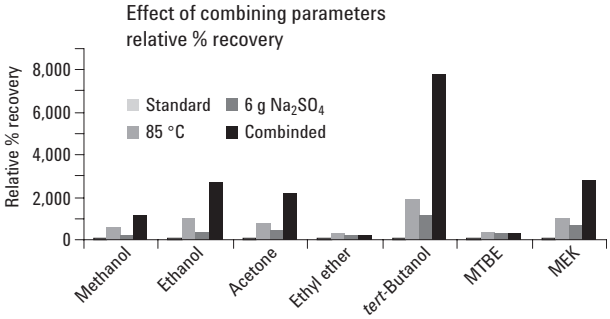


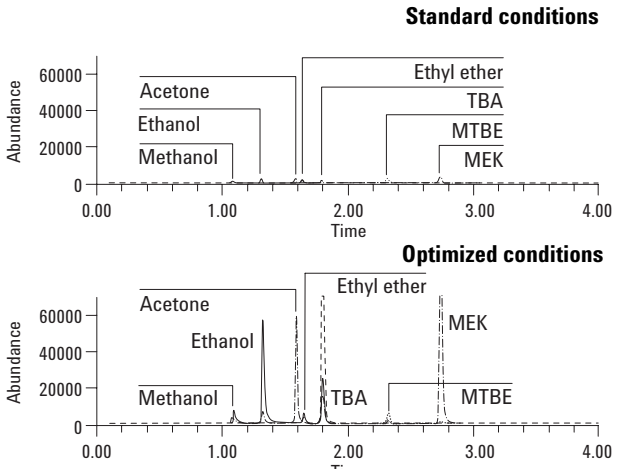
Figure 7. Effect of combining optimized purge and trap parameters.

Table 3 shows the final optimized run conditions for this analysis. Figure 8 is a direct visual comparison of standard vs. optimized P&T conditions. Again, methanol and ethanol are spiked into solution at 500 ppb. Note the much smaller response for ethyl ether and MTBE in the optimized chromatogram. Recall that they are in solution at 5 ppb vs. 50 ppb for acetone, TBA and MEK and 500 ppb for methanol and ethanol.

Table 3. Optimized Run Conditions

Column:	**DB-VRX**
P/N:	**121-1524**
Length:	20 m
Diameter:	0.18 mm
Film thickness:	1.0 µm film
Carrier:	Helium at 45 cm/sec (1.0 mL/min)
Oven:	45 °C for 3.5 minutes
	45–150 °C at 15 °C/min
Injector:	Tekmar 3100 Purge and Trap
Trap:	Vocarb 3000
Sample volume:	25 mL
Sample temp:	85 °C (1 minute preheat)
Purge:	11 Minutes
Dry purge:	3 Minutes
Desorb preheat:	245 °C
Desorb:	1 Minute at 250 °C
Bake:	10 Minutes at 260 °C
Line and valve temp:	125 °C
Interface:	Split injector at 200 °C,
	60:1 Split ratio
Gas saver:	150 mL/min at 1 minute
Agilent 5973 MSD	
Scan range:	29-260 amu
Scan rate:	3.17 scans/sec
Quad temperature:	150 °C
Source temperature:	230 °C
Transfer line temp:	250 °C
Matrix modification:	6 g Sodium sulfate

Figure 8 shows a chromatographic comparison between typical and optimized P&T conditions. The 'Y' scales are normalized for comparative purposes.



Abundance normalized for comparative reasons

Figure 8. A chromatographic comparison between typical and optimized P&T conditions.

Performing a Calibration Curve

Eight points were used with the realistic expectation that not all analytes were going to be linear from 0.5 ppb up to 200 ppb. It was expected that the methanol and ethanol may not achieve single digit ppb levels. As expected, methanol was not able to be calibrated down to 0.5 ppb or even 5 ppb. At these lower concentrations the signal-to-noise ratio was simply too low to be reliable. At 10 ppb it was approaching a more reasonable 10:1 ratio. Figure 9 shows the calibration curve for methanol. Using linear regression per EPA methodology and a calibration range of 10–200 ppb the R-squared value was 0.9987, which is well above the EPA required 0.990 needed for valid quantitative use. Ethanol was calibrated from 0.5–200 ppb with an R-squared value of 0.998 (Figure 10). Both methanol and ethanol calibration ranges could likely be extended to well above 200 ppb.

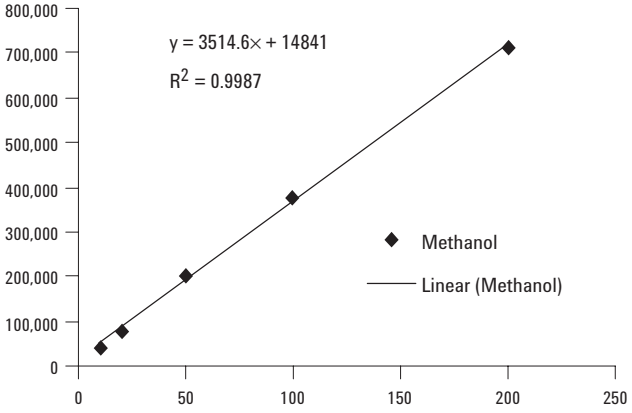


Figure 9. Calibration curve for methanol with a calibration range of 10–200 ppb

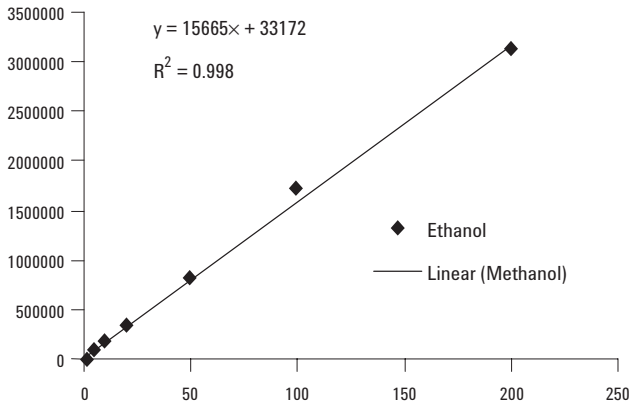


Figure 10. Calibration curve for ethanol with a calibration range of 0.5–200 ppb.

For all components, except methanol, a calibration range of 0.5–200 ppb was achieved with R-squared values ranging from 0.990–0.998 (Table 4), all at or above the Environmental Protective Agency (EPA) requirements for valid quantitation. Methanol as stated earlier was calibrated over a range of 10–200 ppb with an R-squared value of 0.9987.

Table 4. Calibration Curve Summary Using Optimized Analysis Conditions

Compound	Calibration range (ppb)	R^2 Value
Methanol	10–200	0.999
Ethanol	0.5–200	0.998
Acetone	0.5–200	0.993
Ethyl ether	0.5–200	0.994
TBA	0.5–200	0.990
MTBE	0.5–200	0.995
MEK	0.5–200	0.994

USEPA requires R^2 value of 0.990 or greater for quantitative use

Additional Considerations

Automated sampler systems, that accept full VOA vials to reduce sample handling, may require some special approaches to facilitate salt addition. It may be necessary to contact the manufacturer of the autosampler to find out the feasibility of salt addition.

Many laboratories attempt to run the low level oxygenates in conjunction with their 8260 or 524.2 methods. The conditions presented in this work likely will not work well with these standardized EPA methods, but this has not yet been confirmed. If it is desired to run the oxygenates together with the full VOC list these analysis conditions may need to be pared back somewhat, though this will reduce the sensitivity for methanol and ethanol. For example, heating the sample to 65 °C with no salt addition may work for full VOCs and will likely allow for calibration down to around 100 ppb for methanol and ethanol. If the selected ion monitoring (SIM) mode of the MSD is used, per EPA method 8260B section 7.5.12, increased sensitivity will be gained. This may allow for less aggressive conditions than those used in Table 3 while still achieving low ppb quantitation levels.

Salt should be baked to remove moisture and any possible contaminants.

Dry blanks should be run often and line/valve temperatures kept hot (125–150 °C) to reduce water build-up and carryover problems. If a single purge vessel is used for all samples it should be rinsed and/or baked thoroughly after every run.

Conclusion

With optimized P&T conditions it is possible to detect and accurately quantitate low ppb levels of oxygenated contaminants in aqueous sample matrices. This application note provides some of the tools needed if this type of work is to be considered.

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Printed in the USA
March 12, 2003
5988-8993EN

Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems

Application Note

Hydrocarbons

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Abstract

The performance of diesel fuel is predominantly determined by its ignition quality. This parameter is known as the Cetane number. The Cetane number describes the volume % Cetane (hexadecane) present in a mixture of Cetane and 1-Methylnaphthalene. Generally, in order to provide the best performance and lifetime of an engine, the amount of aromatics in diesel should be as low as possible. For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range 150 °C to 400 °C, there exists an IP Method (391/07), which uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) are separated using normal phase HPLC and a column which has little affinity for non-aromatic but pronounced selectivity for aromatic hydrocarbons [1]. Recent growth in biodiesel production created a demand for analysis of petrodiesel and petrodiesel/biodiesel blends. In this method revision, fatty acid methyl esters (FAME) originating from biodiesel sources must elute after a tetra-aromatic marker peak, chrysene, which facilitates improved accuracy of large PAH molecules without interference from FAME. The refractive index detector is used because this detector responds to both non-aromatic and aromatic hydrocarbons.

Agilent Technologies

About Standard Method IP391/07

"This European Standard specifies a test method for the determination of the concentration of mono-aromatic, di-aromatic and tri+-aromatic hydrocarbons in diesel fuels that may contain fatty acid methyl esters (FAME) up to 5 % (v/v) and petroleum distillates in the boiling range from 150 °C to 400 °C. The polycyclic aromatic hydrocarbon content is calculated from the sum of di-aromatic and tri+-aromatic hydrocarbons and the total content of aromatic compounds is calculated from the sum of the individual aromatic hydrocarbon types. Compounds containing sulfur, nitrogen and oxygen may interfere in the determination; mono-alkenes do not interfere, but conjugated di-alkenes and polyalkenes, if present, may do so.

The precision statement of the test method has been established for diesel fuels with and without FAME blending components, with a mono-aromatic content in the range from 6 % (m/m) to 30 % (m/m), a di-aromatic content from 1 % (m/m) to 10 % (m/m), a tri+-aromatic content from 0 % (m/m) to 2 % (m/m), a polycyclic aromatic content from 1 % (m/m) to 12 % (m/m), and a total aromatic content from 7 % (m/m) to 42 % (m/m)." [2]

This method, also known as EN12916:2006, is an official method of the Energy Institute (United Kingdom, www.energyinst.org.uk) which maintains IP (Institute of Petroleum) standards since their acquisition of the IP. Earlier IP391 revisions are similar to ASTM D-6591-06 and include a column backflush-capable instrument configuration and analysis scheme. This requirement was discontinued in the current IP391/07 revision due to erroneous reporting of tri+aromatic hydrocarbons when FAME were present. The main IP391 changes from earlier revisions include the elimination of the backflushing valve, allowing compatibility with biodiesel/petrodiesel fuel blends (up to 5% v/v FAME) and modifications to calibrants to improve data accuracy.

The various methods associated with middle distillate fuel analysis are shown in Table 1.

Equipment and Conditions

LC:	Agilent 1200 Series LC including
G1312B:	Binary pump, used isocratically with pump head seals for normal phase, Agilent p/n 0905-1420
G1367C:	Autosampler with needle wash
G1316C:	Thermostatted column compartment
G1362A:	Refractive index detector
Software:	Agilent ChemStation with version B.04.01 software
Columns:	ZORBAX NH_2 4.6 mm × 150 mm, 5 µm (p/n 883952-708) and ZORBAX SB-CN 4.6 mm × 150 mm, 5 µm (p/n 883975-905) connected in series using 0.12 × 70 mm ss connector tubing, Agilent p/n G1316-87303
Mobile phase:	n-heptane, HPLC grade
Flow rate:	1 mL/min
Injection volume:	10 µL
Oven temperature:	25 °C
Detection:	Refractive index

Sample preparation

Samples and standards were prepared according to Standard Method IP391/07, using heptane as the diluent. Final quantitative results were reported using Agilent IP391/07 standard mixtures (p/n 5190-0485 system calibration standards SCS1 and SCS2, and p/n 5190-0484 quantitative calibration standards A-D).

Results and Discussion

The first step in implementing of IP391/07 is the analysis of calibrants that establish overall separation selectivity and resolution, and confirmation of the elution order of the calibrant components. (Sections 8.6, 8.7, 8.9 IP391/07). Figure 1 shows the results of running these calibrants on the Agilent system.

Table 1. Middle Distillate Fuel Analysis Methods

IP method and revision	Method overview	Special parameters	ASTM method	Comments
IP391/07	150-400 °C diesel fuel petro/bio blends up to B-5	No backflush, amino and/or cyano column	No current equivalent available	Same as method EN12916:2006 *MAH, DAH, Tri+AH are reported
IP436/01	50-300 °C aviation fuel, kerosene	No backflush, amino and/or cyano column	D-6379-04	MAH and DAH reported not for samples with Tri+AH
IP548/06	150-400 °C diesel fuel	Backflush required, amino and/or cyano column	D-6591-06	MAH, DAH, Tri+AH reported FAME interferes with result

*MAH – monoaromatic hydrocarbon, DAH –- diaromatic hydrocarbon, Tri+AH – tri and higher ring aromatic hydrocarbons

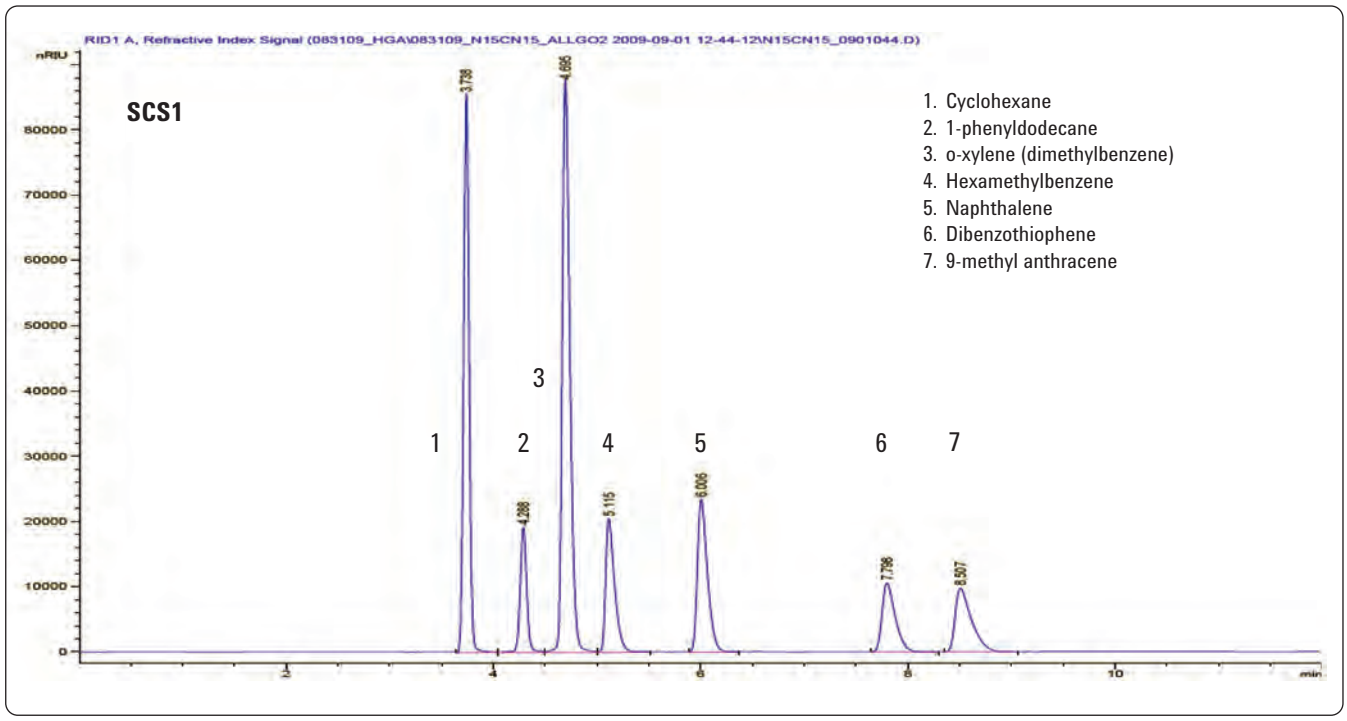

Figure 1. Standard chromatogram of SCS1.

The system calibration standard 1 (SCS1) determines selectivity and retention data for the saturate and aromatic markers used for method acceptance criteria. The SCS1 also determines retention time grouping parameters for sample reporting. Resolution between cyclohexane and o-xylene (1,2-dimethylbenzene) is part of the method specification and must conform to a minimum and maximum value.

System calibration standard 2 (SCS2) establishes the selectivity for components present in petro/bio diesel blends, demonstrating that there is no interference with tri+aromatic components by FAME. Petro/bio fuel blends require longer analysis time to elute all FAME peaks before the next analysis is begun. This applies whether there is an interest in quantifying the FAME or not. When FAME is present in the sample, but does not need to be quantified, it is possible to reduce analysis time by programming the flow rate through the column to increase with time. This will rapidly wash FAME components from the column.

The method requirements state that chrysene, a tetra-aromatic marker peak, must elute with or before the first FAME peak. As shown in Figure 2, the selected operating conditions provide ample separation of the chrysene from the first FAME peak (C16:0 and C18:0 partially resolved).

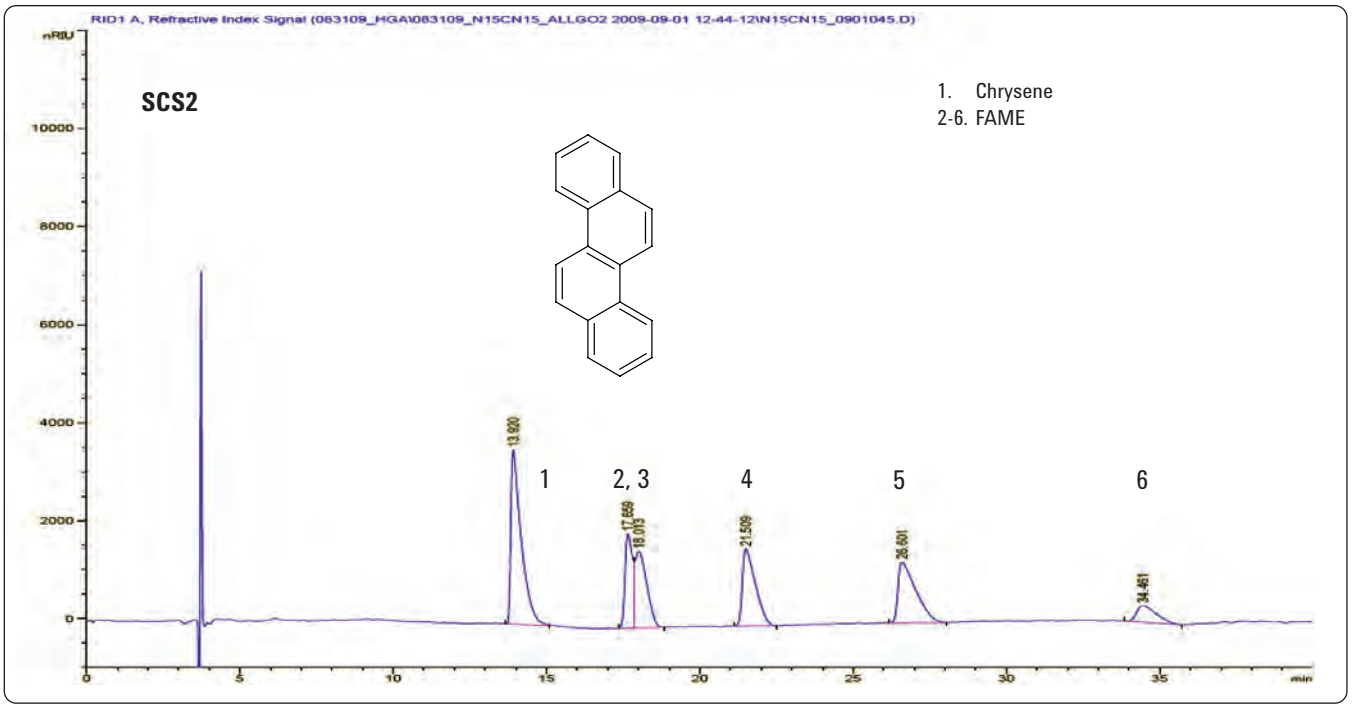

Figure 2. Standard chromatogram of SCS2.

With a genuine fuel sample, in this case retail quality petrodiesel, we can see greater complexity and overlapping of the various compound class regions (Figure 3). Within the method definitions there are specific "cut" points defining the grouping to be performed in the quantitative reports. These are calculated from retention and peak width data in SCS1.

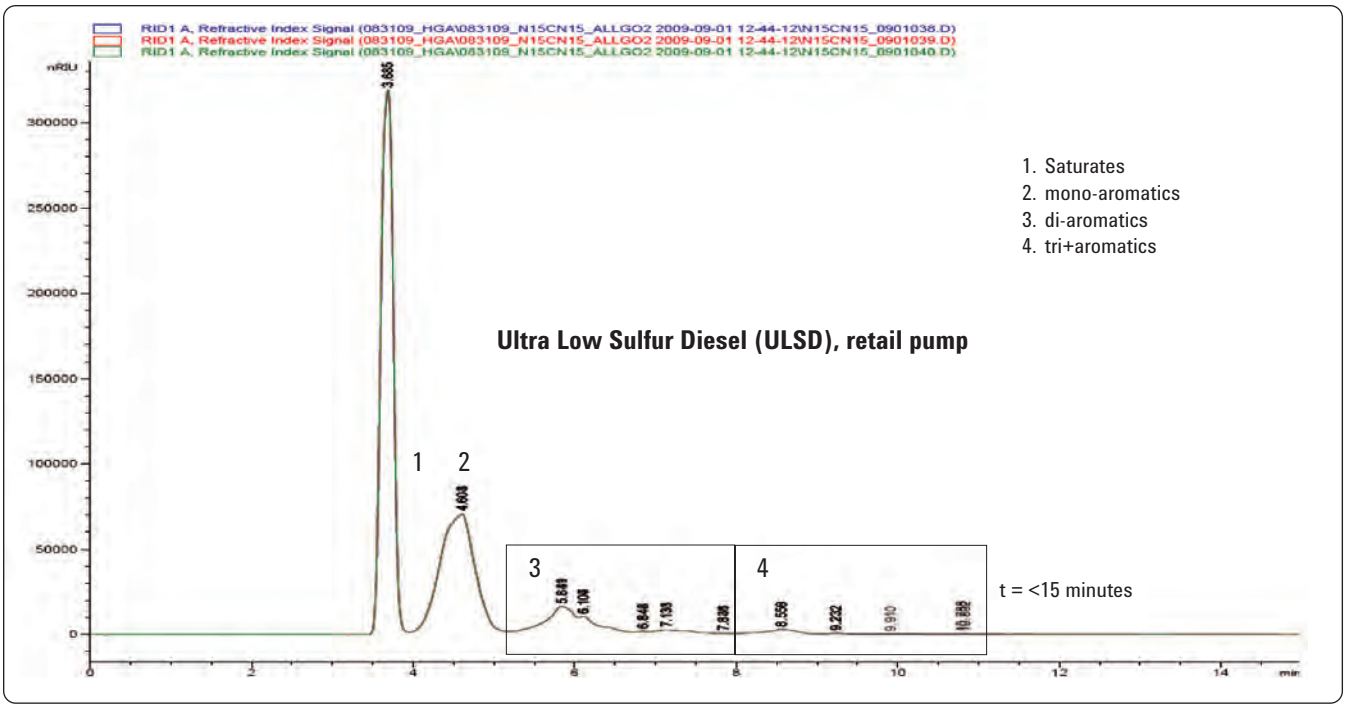

Figure 3. Petroleum diesel sample showing cut points for the various compound groups typically present in these samples.

Results and Discussion

Method Performance

As with most official methods, there are specific performance criteria that allow qualification of the separation system and its subsequent use for reporting quantitative results of diesel fuel analysis.

- 6.4 Column system, consisting of a stainless steel HPLC column(s) packed with a commercial 3 µm, 5 µm or 10 µm amino-bonded (or amino/cyano-bonded) silica stationary phase meeting the resolution requirements given in 8.6, 8.7 and 8.9.
- 8.6 Ensure the components of the SCS1 are eluted in the order: cyclohexane, phenyldodecane, 1,2, dimethylbenzene, hexamethylbenzene, naphthalene, dibenzothiophene and 9-methylanthracene.
- 8.7 Ensure that baseline separation is obtained between all components of the SCS1.
- 8.9 Ensure that the resolution between cyclohexane and 1,2 dimethylbenzene is between 5.7 and 10. [calculated as described in 11.2]
- 11.2 Column Resolution

Calculate the resolution, R, between cyclohexane and 1,2 dimethylbenzene using the following equation.

$$R = \frac{2(t3-t1)}{1.699(y1+y3)}$$

(difference in retention time)
(averaging of peak widths)

Name	Ret Time [min]	Resolution	W hh [min]	Cut ref
1. Cyclohexane	3.738	–	0.0558	t1
2. 1-phenyldodecane	4.288	–	0.0648	t2
3. 1,2-dimethylbenzene	4.695	8.57	0.0756	t3

- 8.11 Ensure the retention time peak of chrysene is higher than the ... 9-methylanthracene peak....and check that the chrysene peak elutes just before or with the first peak of FAME.

In Figure 4, we see that there is distinct separation between the markers specified in section 8.11 of the method. With this information in hand, it is possible to proceed to the evaluation of calibration standards.

- 9.4 R = >0.999, Intercept <0.01 g/100 mL)

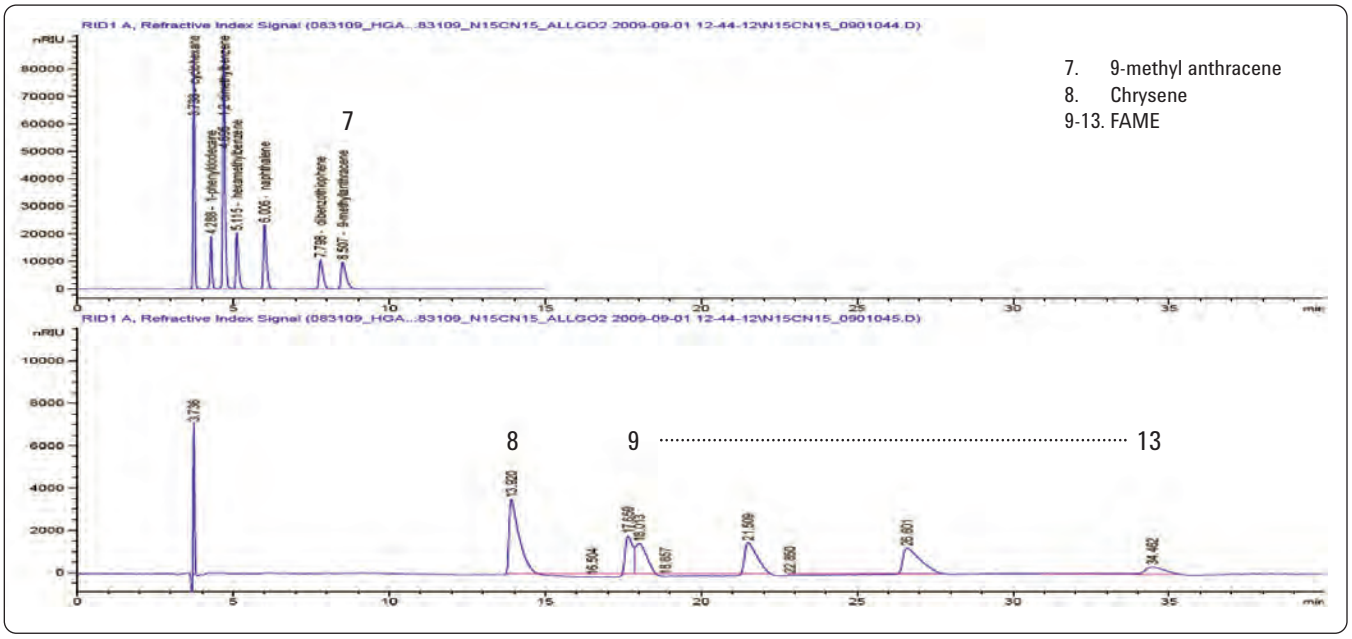

Figure 4. Stack plot of SCS1 and SCS2 showing proof for section 8.11.

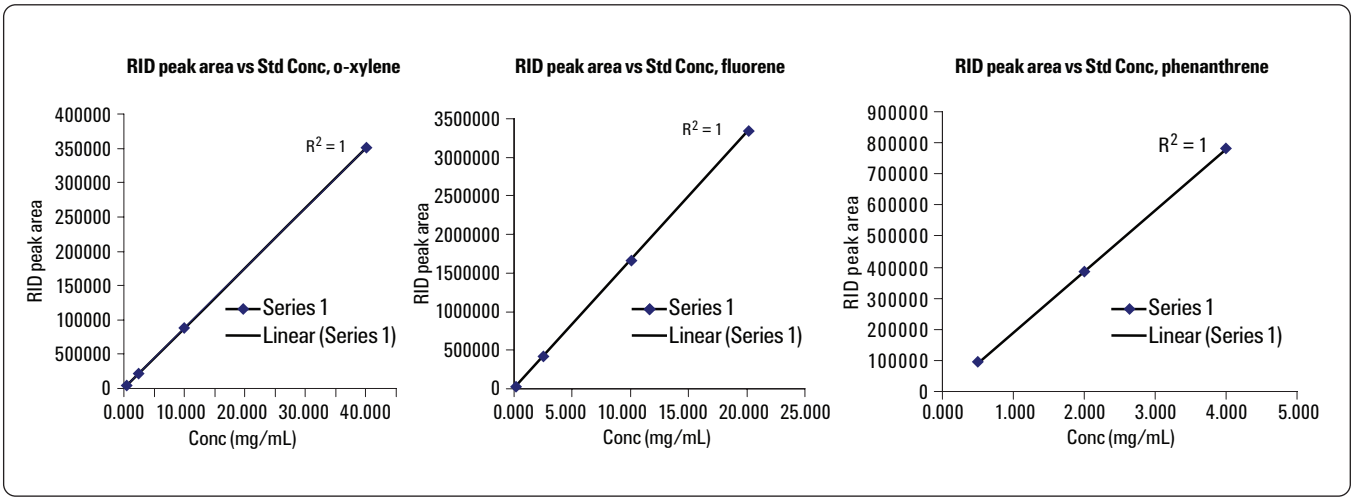

Figure 5. Calibration plots for o-xylene, fluorene and phenanthrene, the three components of the four calibration levels specified in the method.

In the calculated results, all calibration plots exceed linearity of 0.9999 and have calculated intercepts well below 0.01 g/100 mL, the method specification of section 9.4.

Retention time and peak area precision can be found in Table 2, in which we see that the overall performance of the method is excellent.

Table 2. Retention Time and Peak Area Precision

Calibrant A						
Analyte	**R.T. Avg, n=3**	**R.T. Std dev**	**R.T. RSD%**	**Area Avg, n=3**	**Area Std dev**	**Area RSD%**
Xylene	4.57	0.003	0.06	3.54E+06	5829.9	0.16
Fluorene	6.82	0.004	0.05	3.38E+06	2500.5	0.07
Phenanthrene	8.32	0.004	0.04	8.05E+05	594.03	0.07
Calibrant B						
Analyte	**R.T. Avg, n=3**	**R.T. Std dev**	**R.T. RSD%**	**Area Avg, n=3**	**Area Std dev**	**Area RSD%**
Xylene	4.65	0.001	0.02	9.23E+05	636.28	0.07
Fluorene	6.95	0.001	0.02	1.70E+06	1731.17	0.10
Phenanthrene	8.44	0	0.00	4.00E+05	473.79	0.12
Calibrant C						
Analyte	**R.T. Avg, n=3**	**R.T. Std dev**	**R.T. RSD%**	**Area Avg, n=3**	**Area Std dev**	**Area RSD%**
Xylene	4.70	0.002	0.03	2.24E+05	474.36	0.21
Fluorene	7.15	0.002	0.03	4.29E+05	507.38	0.12
Phenanthrene	8.62	0.002	0.02	1.00E+05	291.04	0.29
Calibrant D						
Analyte	**R.T. Avg, n=3**	**R.T. Std dev**	**R.T. RSD%**	**Area Avg, n=3**	**Area Std dev**	**Area RSD%**
Xylene	4.72	0.001	0.02	4.45E+04	321.90	0.72
Fluorene	7.36	0.002	0.02	1.70E+04	145.91	0.86
Phenanthrene	8.70	0.002	0.02	3.80E+04	551.43	1.45
Average RSD% All Runs			**0.029**			**0.355**

Results for Specific Petrodiesel and Petro/ Biodiesel Blends

Various samples were collected from local commercial and retail fuel delivery points. An overlay of four samples is shown in Figure 6.

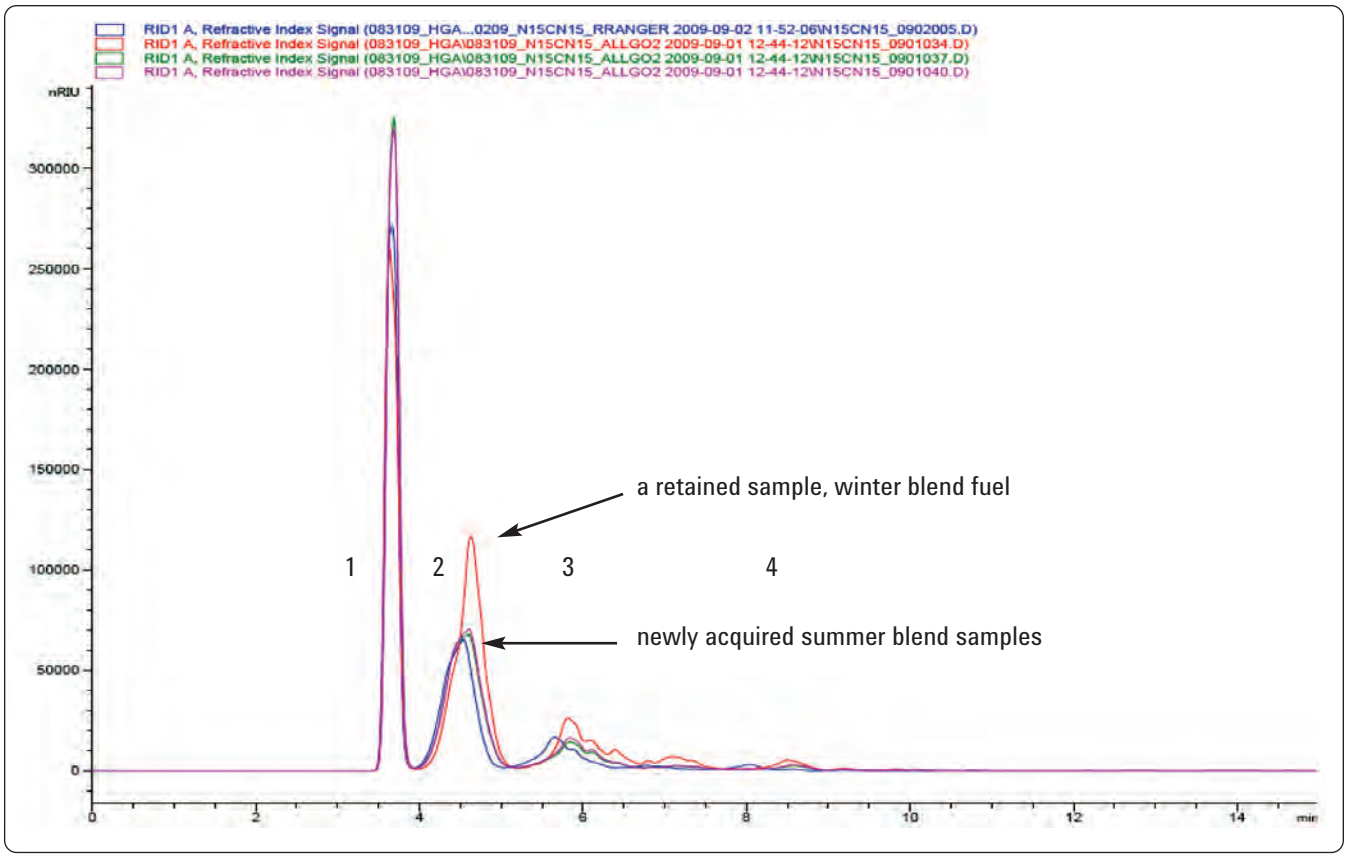

Figure 6. Overlay of four samples.

Results (n=3 each sample) and precision data are reported in Table 3, where vendor 4 results are n=3 based on one sampling of each of the three B-11 biodiesel delivery points (commercial heavy truck, commercial auto/light truck and retail auto/light truck).

Table 3. Results (n=3 each sample) and Precision Data for Four Vendors.

Vendor	Group	Avg, n=3		RSD%	Std dev
1	MAH	36.0	g/100 mL	0.06	0.022
	DAH	8.4	g/100 mL	0.09	0.008
	Tri+AH	1.2	g/100 mL	2.78	0.033
2	MAH	28.5	g/100 mL	0.05	0.016
	DAH	4.8	g/100 mL	0.48	0.023
	Tri+AH	0.6	g/100 mL	3.24	0.020
3	MAH	29.0	g/100 mL	0.07	0.021
	DAH	5.3	g/100 mL	0.37	0.020
	Tri+AH	0.7	g/100 mL	6.81	0.047
4	MAH	24.7	g/100 mL	5.06	1.252
	DAH	5.1	g/100 mL	3.38	0.174
	Tri+AH	0.7	g/100 mL	4.58	0.033

Because of very low level response and broadenend peaks, it is much more difficult to get high precision for the tri+aromatic hydrocarbons group of components. If no biodiesel components are present, it would be practical to consider using Method IP548/01, which uses a backflush valve to elute the tri+aromatic hydrocarbons group as a single peak via the backflush configuration.

Ruggedness and Stability of the IP391/07 Method

As with most normal phase methods the column is susceptible to the adsorption of highly polar components which can affect overall separation performance. Water present in samples or the mobile phase also adsorbs into the column and somewhat predictably causes reduced elution times for all sample components. Using a high quality HPLC grade mobile phase is essential, and the user may consider using a drying agent such as molecular sieve to dehydrate the mobile phase. While this is often done by adding molecular sieve to the solvent container, it is also possible and preferable to prepare a high pressure compatible column with pre-washed drying agent and placing it inline between the pump and injector.

Conclusion

The performance of the Agilent 1200 Series HPLC with normal phase separation and refractive index detection meets or exceeds the requirements of IP391/07 within the range of samples defined in the method. The user should take care identifying samples of petrodiesel that may contain biodiesel components to ensure adequate analysis time before proceeding to the next analytical run.

References

1. ASTM 6591-06 and ASTM 6379-04, www.astm.org
2. IP391/07, Energy Institute (formerly Institute of Petroleum Test Methods)
3. EN12916/06, Energy Institute (formerly Institute of Petroleum Test Methods)
4. Agilent Technologies publication 5965-9044EN, (1997)

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Printed in the USA
November 10, 2009
5990-4789EN

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Refining

Technical Overviews

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Lock in the Power of Gas Chromatography with RTL

- Improving Reliability and Precision Analyzing Oxygenates in Gasoline

The unique retention time locking (RTL) software from Agilent Technologies revolutionizes gas chromatography. With RTL, retention times (RT) can be reproduced within hundredths, even thousandths, of a minute from one Agilent GC based system to another. Be it between 6890 or 6850 GC systems or one 6890/5973 inert MSD system to another, RTs are virtually identical, regardless of inlet, detector, operator, or location.

This technique is an easy and economical way to improve confidence in results, reduce the risk of non-compliance, and reduce operating costs. All that is required is the RTL software, an Agilent 6890 or 6850 GC (equipped with Agilent's industry-leading electronic pneumatics control (EPC)) or an Agilent 6890/5973 inert MSD system, and the Agilent ChemStation for GC or MSD.

RTL - Real Performance Advantages

- Removes the need to update calibration table RTs, timed events, and integration events tables when a 6890/6850 method is transferred, a column installed or routine maintenance performed
- Ensures results that are easy to compare, document and explain
- Lock on a peak and reproduce exactly a 6890/6850 method developed in another laboratory
- Reproduce exactly the RTs from a published 6890/6850 method
- Allows fast setup and testing plus decreases method development time
- Delivers simplified method and system validation
- Provides fast, accurate identification of unknowns through retention time library searching

Improved Reliability and Precision for ASTM Method D4815

ASTM method D4815 is used to measure oxygenated additives in gasoline. The species of interest include ethers such as MtBE, EtBE DIPE, TAME at 0.1 - 20.0 wt% and alcohols - C1 to C4 and t-pentanol at 0.1 - 12.0 wt%. There are usually only 1 or 2 oxygenates in a sample. The scope of the original application was to improve this method, making it easier and to provide greater confidence in results using RTL.

The method calls for a polar TCEP micropacked column to provide preliminary separation and remove light hydrocarbons. Trapped oxygenates are back flushed to the capillary column and this is the key to success. Variations in the back flush time caused by the TCEP pre-column make RTL difficult to apply.

TCEP columns are generally difficult to prepare. The small dimensions of the column, the means in which wire is used as plugs and the use of crimping to hold in the wire lead to variations in column head pressure.

In addition, potential temperature variations can occur if the pre-column in the valve box is not uniformly heated, making it difficult to accurately set the back flush time.

Problems with head pressure can be overcome with the use of screen plugs, while wrapping the column and mounting it on a heated mandrill can address temperature variations. Once these problems have been solved, precise back flush times can be achieved and the RTL software can be used to accurately lock in the data. For ASTM method D4815, MtBE acted as the target peak for locking and RTL calibration was performed after back flush time optimization. Sample data for 5 different 6890 GC systems before and after RTL correction are summarized in Figures 1 (before) and 2 (after).

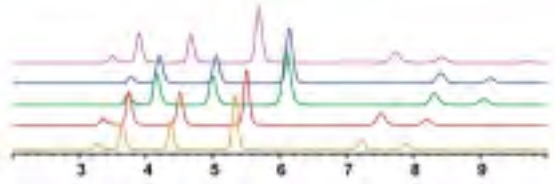

Figure 1 Chromatograms of oxygenates in gasoline from five different 6890 GC systems before locking

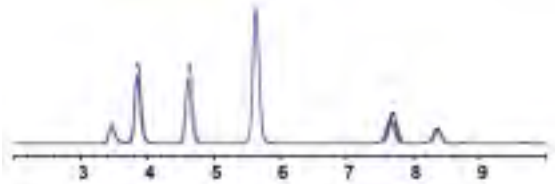

Figure 2 Chromatograms of oxygenates in gasoline from 5 diferent 6890 GC systems after locking

Table 1 summarizes results on replicate analyses using the modified method and illustrates the superb reproducibility. %RSDs exceed the ASTM requirements by a factor of 10.

		Repeatability		Reproducibility	
Compound	Mass%	Spec	Observed	Spec	Observed
Ethanol	0.99	0.06	0.01	0.23	0.01
Ethanol	6.63	0.19	0.03	0.68	0.04
MtBE	2.10	0.08	0.01	0.20	0.01
MtBE	11.29	0.19	0.05	0.61	0.08

Table 1 Replicate analyses of oxygenates in gasoline

Summary

Agilent's unique Retention Time Locking (RTL) software is an excellent means of improving RT reproducibility across various Agilent GC and GC/MS systems improving simplified method development and system validation. For more information on this and other Agilent Solutions for the petroleum/petrochemical industries log onto www.agilent.com/chem/hydrocarbon or contact your local Agilent Technologies, Life Science Chemical Analysis representative.

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Simplified Backflush Using Agilent 6890 GC Post Run Command

Application Note

Gas Chromatography

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Abstract

A simple way to perform backflush with gas-phase microfluidic devices using the Agilent 6890 gas chromatograph is described. By selecting the microfluidic pressure source as the "inlet" for "Column 2" in the Column Configuration screen, one can then set a backflush pressure and time in Post Run. Several advantages ensue, including the ability to backflush in methods that use Constant Flow mode.

Introduction

One way to significantly reduce cycle times in GC analysis is to backflush late-eluting compounds from the column. "Backflush" is a term used for the reversal of flow through a column such that sample components in the column are forced back out the inlet end of the column.

Applications where backflushing can provide the most benefits are:

- Methods where sample components of interest elute early, but a longer temperature ramp is required to remove later-eluting components from the column
- Methods where high-boiling matrix components contaminate the column, requiring frequent maintenance, such as trimming the head of the column

There are many ways that backflushing is beneficial:

- Cycle times (total time to run a sample and be ready to run the next sample) are improved
  - Run time is reduced
  - Cool down time is reduced (cooling down from a lower temperature)
- Data file sizes are smaller
- Data quality is better
  - Column bleed is reduced (from lower exposure to high temperatures)
  - Ghost peaks (due to carryover into subsequent runs) are eliminated
- Columns last longer
  - High-boilers do not build up on column
  - Column is spared exposure to highest temperatures
- Calibrations and system suitability are maintained for longer periods
- MSD source is not exposed to high-boiling components (less source cleaning required, a given tune will last longer, etc.)
- Columns can be conditioned in backflush mode to prevent MSD source contamination

- Electrical power is saved (lower temperature maximum and time at high temperatures, less air conditioning power required to keep room cool because GCs don't produce as much heat)
- Less gases are used (less time per sample = less carrier gas per sample)

There are several ways that one might program pressure changes with the Agilent 6890 and 6850 GCs to do backflush. The simplest is to use a Post Run program. There are several advantages to using the Post Run program:

- Data acquisition automatically ends (the MSD source and detector are turned OFF).
- Pressure changes are ballistic (as fast as they can be established) rather than at the 99.99 psi/min maximum controlled inlet pressure program rate.
- Constant Flow mode can be used during the analysis. Negative flows (such as what happens during backflush) are not accepted as set points by 6890 GC software and firmware, so only pressure programs can be used during the run. However, pressure programs are not allowed when using Constant Flow mode (mixed modes are not allowed).
- Changes can be made to the analytical part of the method (involving, for example, pressures, temperatures, times, and ramps) without needing to change Post Run backflush set-points.
- Post Run conditions are executed even if a run is stopped. Runs that are stopped will have remaining components backflushed, thereby quickly and effectively readying the column for subsequent use.

Backflushing can be accomplished with any of the microfluidic devices listed in Table 1.

Table 1. Microfluidic Devices

Microfluidic Device	Accessory Kit	Option
QuickSwap no-vent for MSD	G3185B	885 6890 or 6850 instruments
Purged 2-way splitter	G3180B	889 6890 or 6850 instruments
Purged 3-way splitter	G3183B	890
Dean's switch for multidimensional heart cutting	G2855B	888 6890 or 6850 instruments

For MSD users, QuickSwap is of a high interest and was the gas-phase microfluidics device chosen for the examples shown here. QuickSwap attaches to the column end of the MSD transfer line and is supplied by a secondary pressure source, typically Auxiliary Pressure channel #5. A restrictor resides within the MSD transfer line to limit total flow to the MSD. The restrictor size is chosen based on what is appropriate for the analysis at hand and the MSD system being used. The analytical column attaches to the GC-oven side of QuickSwap. See Figure 1.

Typically, operational pressure for QuickSwap is between 1 and 4 psig, depending on the application and the chosen restrictor. One configures the "outlet pressure" of Column 1 (the analytical column) to be the set point pressure of the microfluidic device (for example, QuickSwap, Aux #5). Note that the 6890 and 6850 GC firmware only allow outlet pressures ≤4 psig. Column flow will then be correctly reflected in the setup and display screens. A higher inlet pressure will be needed to

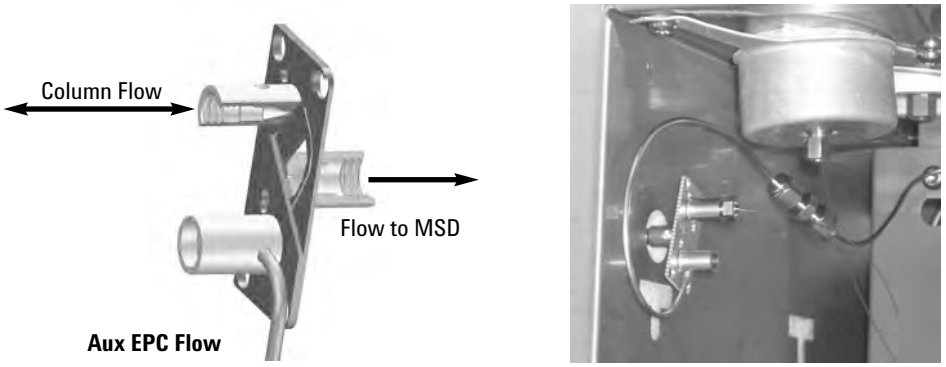

Figure 1. QuickSwap flow pattern on the left, and a QuickSwap installation in a 6890 GC on the right.

establish the same flow (similar retention time) as that of a column directly connected to the MSD because of the higher column outlet pressure.

To accomplish a backflush, the column outlet pressure must be raised higher than that of its inlet. For minimum backflush times, the inlet pressure should be minimized and the outlet pressure maximized. There are limits, however. The minimum controlled inlet pressure is limited by the backpressure created by the flow through the split vent trap (column backflush flow + inlet purge gas flow). 1.0 psig is usually achievable with a purge flow ≤50 mL/min under most backflush conditions, and was used in the examples shown here.

Column outlet pressure maximum during backflush is limited by the maximum allowable flow to the MSD when it is not acquiring data. The maximum allowable flow to the MSD depends on the type of vacuum pump. Because of the limited pumping capacity of diffusion pumps, backflush should only be done with turbopump systems. Practically, one must stay below an ion gauge reading of 10^{-3} torr. Table 2 provides estimates for maximum allowable flows based on pump type.

Table 2. Maximum Gas Flows for Turbopumps

Pump	Maximum He Flow
Standard turbo and performance turbo with dry pump	25 mL/min
Performance turbo	100 mL/min

In order to be allowed to set pressures for a backflush in Post Run, one must configure a second column (Column 2). One can simply repeat the configuration used for Column 1 (dimensions, column description, etc.) as Column 2, but instead of an inlet, one would select QuickSwap (Aux #5) as the inlet pressure source. Once this is done, one can set a high Post Run pressure for Column 2. The Post Run pressure for column 1 (the inlet) would be set low (for example, 1 psig).

How much time is needed to backflush?

It was found that for a typical column used for capillary GC-MS, the high-boilers ("late eluters") actually are backflushed first from the column, with the "least retained" components being the last to be backflushed. An example of this effect is shown in Figure 2. For this, ever-increasing backflush times were used, followed by reestablishment of forward flow to see what had not been backflushed from the column.

During the backflush experiments in Figure 2, the oven temperature was paused at 169 °C (the temperature at 10 min in the temperature program) for the duration of the backflush period. At the conclusion of each backflush, the temperature program was resumed. The ever increasing backflush periods are easily seen from the widening gaps starting at 10 min.

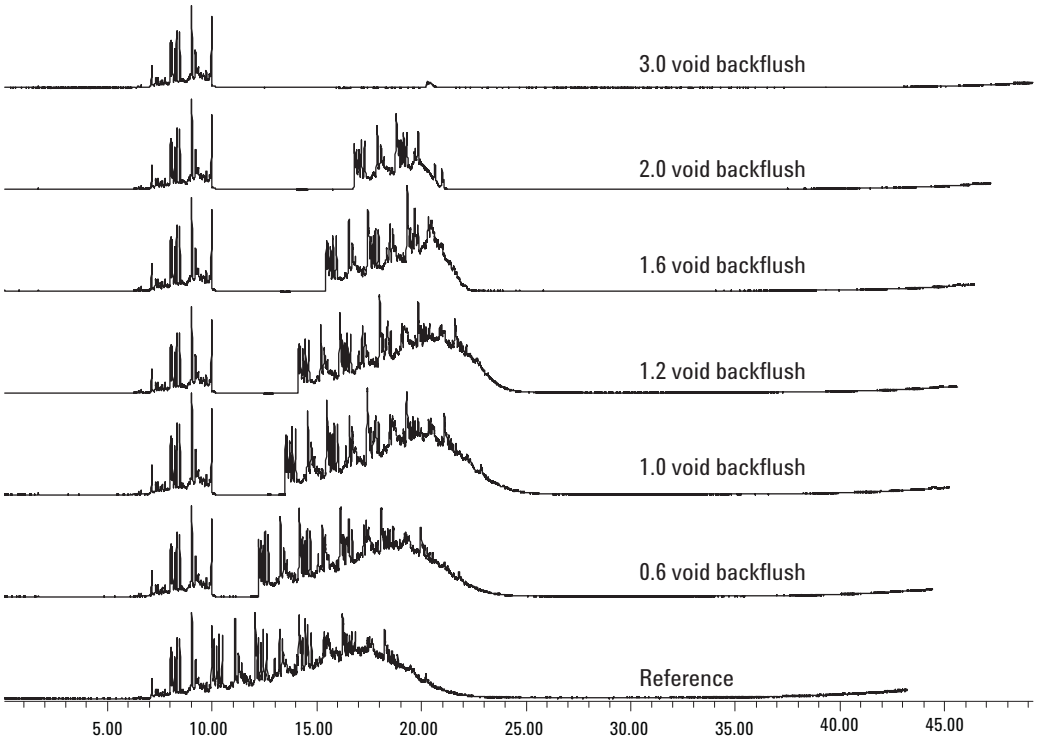

Figure 2. Successive chromatograms with increasing backflush times.

Actual void time was determined by injecting air under isothermal (169 °C) conditions, with inlet and outlet pressures the same as those used during backflush (only reversed).

As can be seen, a small amount of "least retained" components still remain after 3 void times. Something between 3 and 4 void times would be enough for the conditions used (10 °C/void time oven ramp rate). The minimum number of void times required to remove all components from the column depends on several factors, including, but not limited to:

- Temperature program rate
- Column dimensions
- Carrier gas type
- Pressure drop prior to backflush
- Pressure drop during backflush
- Manner of pressure change (programmed or ballistic)

Therefore, the simple rule for temperature-programmed analysis is that one should backflush for at least 5 void times. A void time (also known as holdup time) is determined using backflush conditions and a software tool such as Flow Calculator or Method Translator (available for download from Agilent website). Plug in the column dimensions, the oven temperature of the time backflush is started, the "inlet" pressure (that of the microfluidic device), and the "outlet" pressure (that of the inlet during backflush). Find the "holdup" or "void" time and multiply by 5. That is the time required to be sure all components have been
backflushed from the column.

First, set a Post Run time and temperature in the Oven screen. Shown in Figure 3, a temperature of 170 °C was set because that corresponded to the approximate oven temperature at 10 min in the original temperature program when the backflush was to occur. One and a half minutes was chosen for the backflush period because this corresponds to approximately 5 void times under the conditions chosen.

Once a Post Run time and temperature is established, one can set the Post Run pressures for both the inlet and QuickSwap from the Columns panel. See Figure 4. To set the backflush pressure for QuickSwap, select the radio button for Column 2 and set the pressure in the Post Run line at the bottom of the run table. Here, a pressure of 85 psi was selected because it will minimize the time required to backflush the column. Using Flow Calculator or Method Translator, one can input the value of 85 psi inlet pressure, 1 psi outlet pressure (15.496 absolute) and see that void time is approximately 0.3 min at an oven temperature of 170 °C.

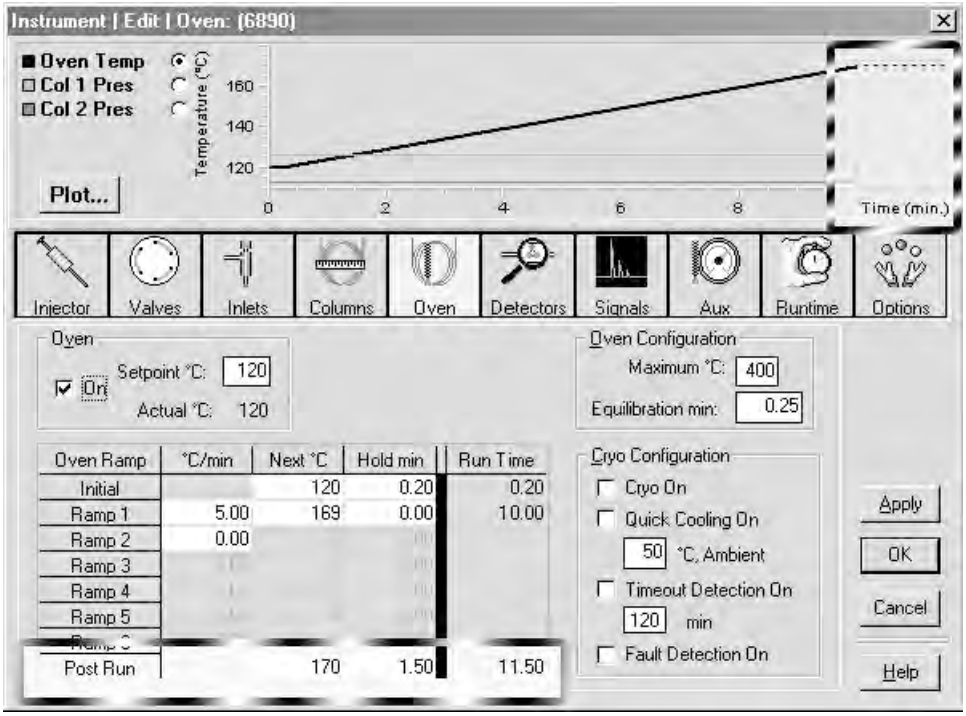

Figure 3. Oven screen, used to set up oven temperature ramp and Post Run time and temperature.

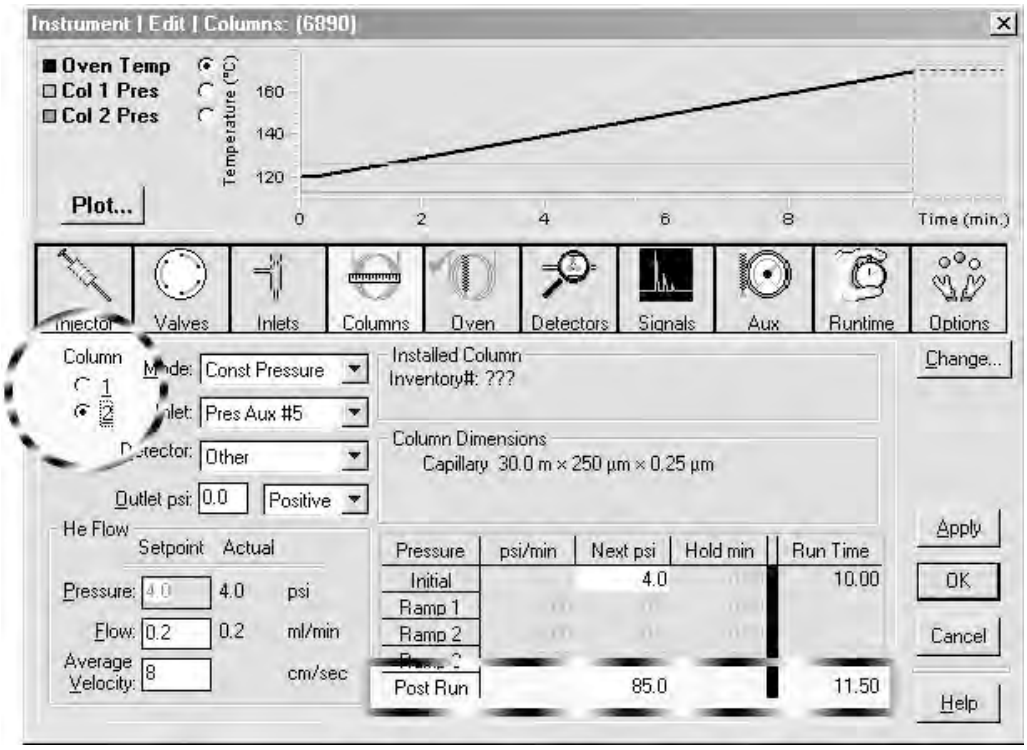

Figure 4. Columns screen, used to set up Post Run pressure and flow conditions.

Then, by clicking on the Column 1 radio button, one can set the low inlet pressure for backflush. See Figure 5. In this example, 1 psi was set. Remember to check that the low pressure can be controlled under backflush conditions and that sufficient split flow is set to sweep backflushed components out of the inlet to the split vent trap (30 < flow < 100 mL/min are recommended).

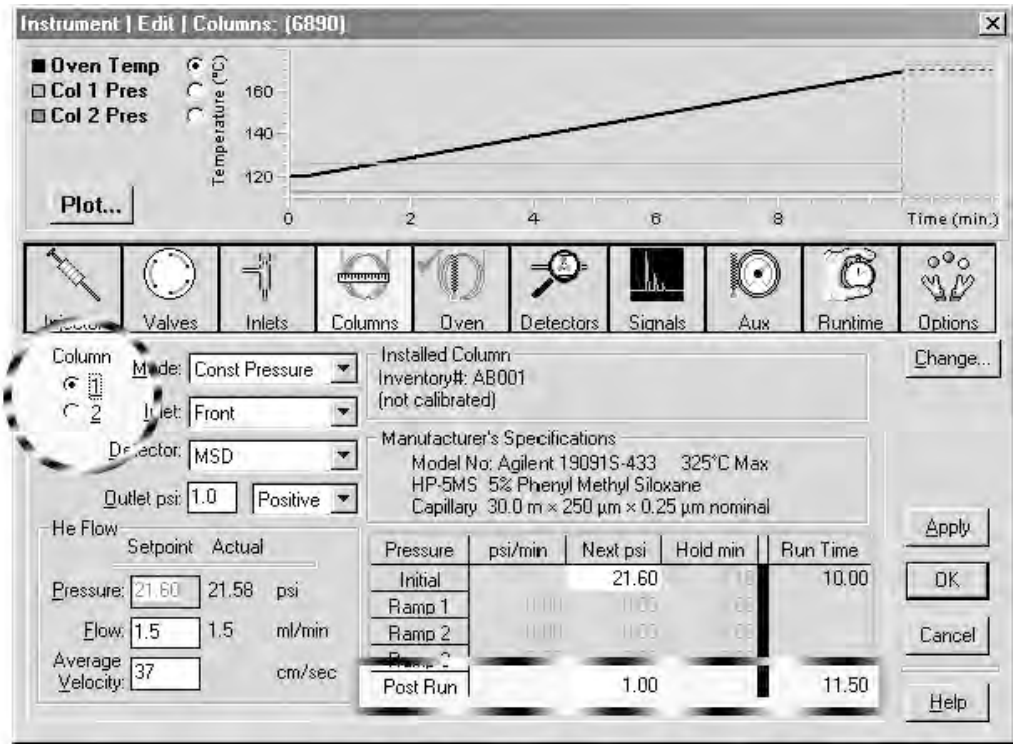

Figure 5. Columns screen, used to set a low inlet pressure for backflush.

Will backflushing through the inlet cause problems?

A common question from backflush neophytes is "Will I cause problems by backflushing high-boiling components back through the inlet?" Backflushing is only done with inlets that are purged, for example, split/splitless or PTV. These inlets have been designed to handle all sample components when doing split injections and when venting after splitless injection. To that end, these inlets have replaceable split-vent traps that are designed to handle whatever passes through the inlet. All sample components that make it into the column have already passed through the inlet once; there is no reason to believe that there would be a problem arising from them passing back through the inlet a second time, as long as a reasonable split vent (pure) gas flows to help backflushed components move to the split vent trap.

Gas Saver mode provides added independence to method

One way to ensure that a reasonable flow purges through the inlet during backflush is to use Gas Saver Mode. This achieves independence from both the conditions that are used during injection and the mode of injection (such as, splitless versus split). Here, we did a split injection at high split ratio with high associated split flow (617 mL/min). Gas Saver was activated at 3 minutes into the run, with a split flow reduced to 75 mL/min. If Gas Saver had not been used in this method, then the high vent flow associated with the high split ratio may have disallowed the pressure of the inlet to get down to the 1 psi set point (there is a small pressure drop through the split vent trap that might become notable at high split vent flow rates). See Figure 6.

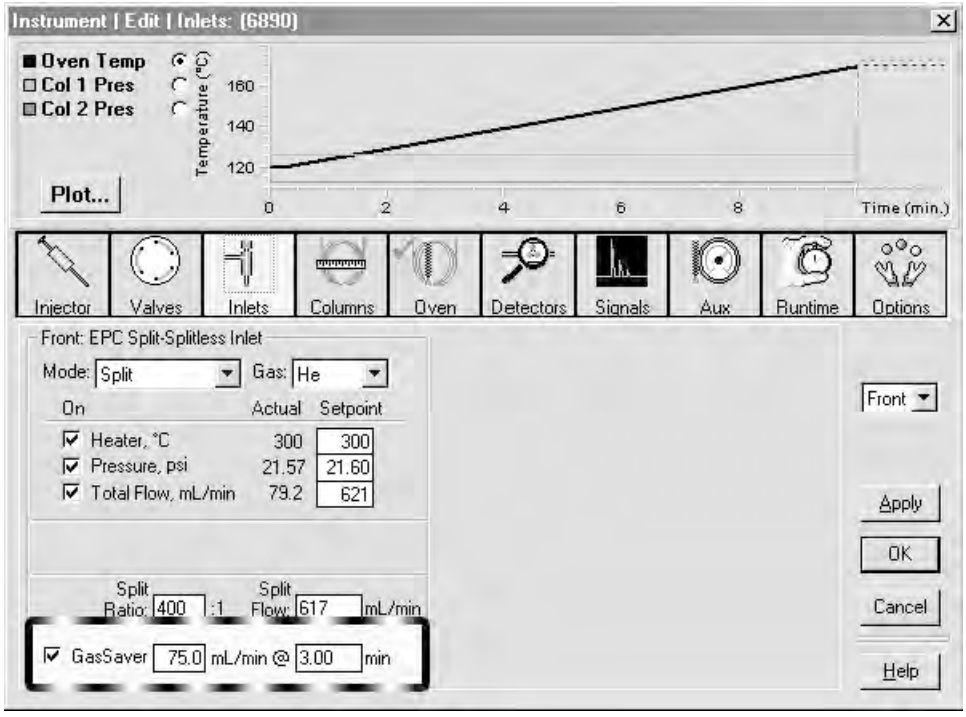

Figure 6. Inlets screen, displaying Gas Saver settings.

Instrumental

Below is an example listing of conditions used to perform a Post Run backflush (such as those shown in the Discussions section).

Oven

Initial temp:	120 °C (On)
Initial time:	0.20 min

Temperature Ramp for Original:

No.	Rate	Final temp	Final time
1	5.00	330	1.00
2	0.0 (Off)		

Post temp:	70 °C
Post time:	0.00 min
Run time:	43.20 min

Temperature Ramp for Backflush:

No.	Rate	Final temp	Final time
1	5.00	169	0.00
2	0.0 (Off)		

Post temp:	170 °C
Post time:	1.50 min
Run time:	10.00 min

Front Inlet (Split/Splitless)

Mode:	Split
Initial temp:	300 °C (On)
Pressure:	21.60 psi (On)
Split ratio:	400:1
Split flow:	617.1 mL/min
Total flow:	621.5 mL/min
Gas saver:	On
Saver flow:	75.0 mL/min
Saver time:	3.00 min
Gas type:	Helium

Column 1

Capillary column

Model number:	Agilent 19091S-433
	HP-5MS 5% phenyl methyl siloxane
Max temperature:	325 °C
Nominal length:	30.0 m
Nominal diameter:	250.00 µm
Nominal film thickness:	0.25 µm
Mode:	Constant pressure
Pressure:	21.60 psi
Nominal initial flow:	1.5 mL/min
Average velocity:	37 cm/sec
Inlet:	Front Inlet
Outlet:	MSD
Outlet pressure:	1.00 psi

Column 2

Capillary column

Nominal length:	30.0 m
Nominal diameter:	250.00 µm
Nominal film thickness:	0.25 µm
Mode:	Constant pressure
Pressure:	4.00 psi
Nominal initial flow:	0.2 mL/min
Average velocity:	8 cm/sec
Inlet:	Aux 5 Pressure Controller
Outlet:	(unspecified)
Outlet pressure:	0.00 psi

Aux Pressure 5

Description:	QuickSwap
Gas Type:	Helium
Driving Column 2	
Initial pressure:	4.00 psi (On)

Post Run

Post Time:	1.50 min
Oven Temperature:	170 °C
Column 1 Pressure:	1.0 psi
Column 2 Pressure:	85.0 psi

Front Injector

Sample washes:	2
Sample pumps:	3
Injection volume:	0.10 µL
Syringe size:	5.0 µL
Preinjection solvent A washes:	0
Preinjection solvent B washes:	0
Postinjection solvent A washes:	2
Postinjection solvent B washes:	0
Viscosity delay:	0 sec
Plunger speed:	Fast
Preinjection dwell:	0.00 min
Postinjection dwell:	0.00 min

5973 Performance Electronics MSD with Performance Turbo

General Information	
Tune file	atune.u
Acquisition mode	Scan
MS Information	
Solvent delay	0.00 min
EM absolute	False
EM offset	0
Resulting EM voltage	1776.5

Scan Parameters	
Low mass	28.4
High mass	800.0
Threshold	10
Sample number 1	A/D Samples 2
MSD Heated Zones	
MS quad	200 °C
MS source	300 °C

Results and Discussion

Two valved musical-instrument lubricants and a reference diesel fuel were analyzed. These were chosen for comparison because they had unique chromatographic and compositional features. A backflush time of 10 minutes was chosen. The analytical run ended at 10 min and was followed by a post-run backflush of 1.5 min. Based on the QuickSwap restrictor and MSD used, pressure conditions were determined that would minimize the time for 5 void times to complete. This was with QuickSwap pressure of 85 psi and inlet pressure of 1 psi.

For the following examples, a reference chromatogram was acquired with no backflush. Then, analysis was repeated with a backflush at 10 min. Finally, a blank was run under full analysis conditions to determine if any sample components were left on the column.

One valve oil product was unique in that it was a mixture of linear dimethyl siloxane homologs. As such, it was a nice reference sample for development of chromatographic method and backflush conditions. The backflush time of 10 min was chosen because it occurs before elution of the 7 silicon-unit siloxane homolog and is representative of analyses wherein only the volatile components are of analytical importance. See Figure 7.

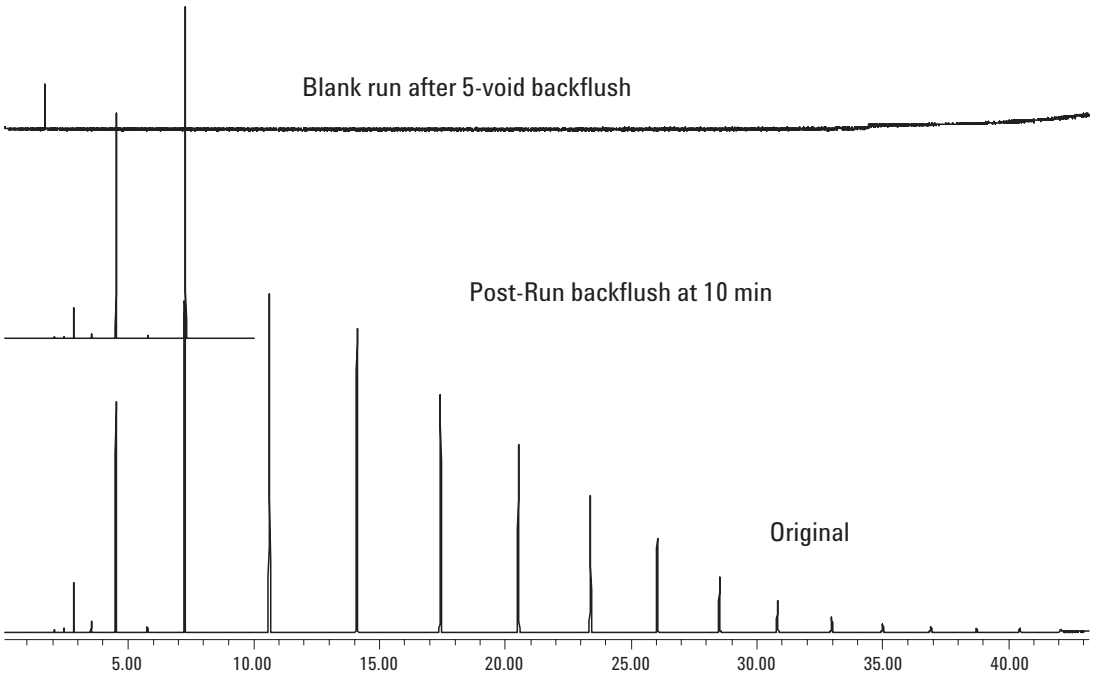

Figure 7. Example chromatograms of a valve oil composed of dimethyl siloxane homologs demonstrating backflush.

Notice from the blank run that the 1.5 min backflush (5 void times) was sufficient to remove all components that eluted after 10 min under the original conditions.

Another valve oil product was a petroleum distillation cut. The initiation of backflush at 10 minutes occurred in the thick of elution of sample components. As can be seen from the blank run, all remaining sample components were effectively removed from the column. See Figure 8.

A diesel sample was chosen to accentuate the removal of high boilers. The diesel had components eluting out past 25 min. The blank run after the backflush analysis demonstrates that all components were effectively removed during the backflush. See Figure 9.

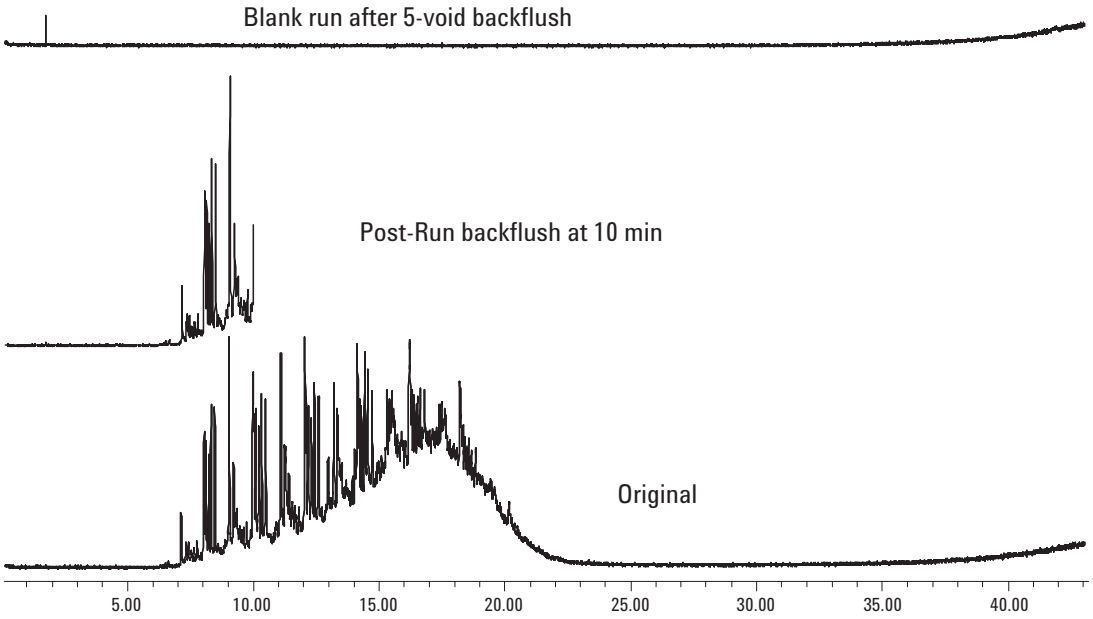

Figure 8. Chromatograms demonstrating backflush efficacy using a valve oil product composed of a petroleum distillation cut.

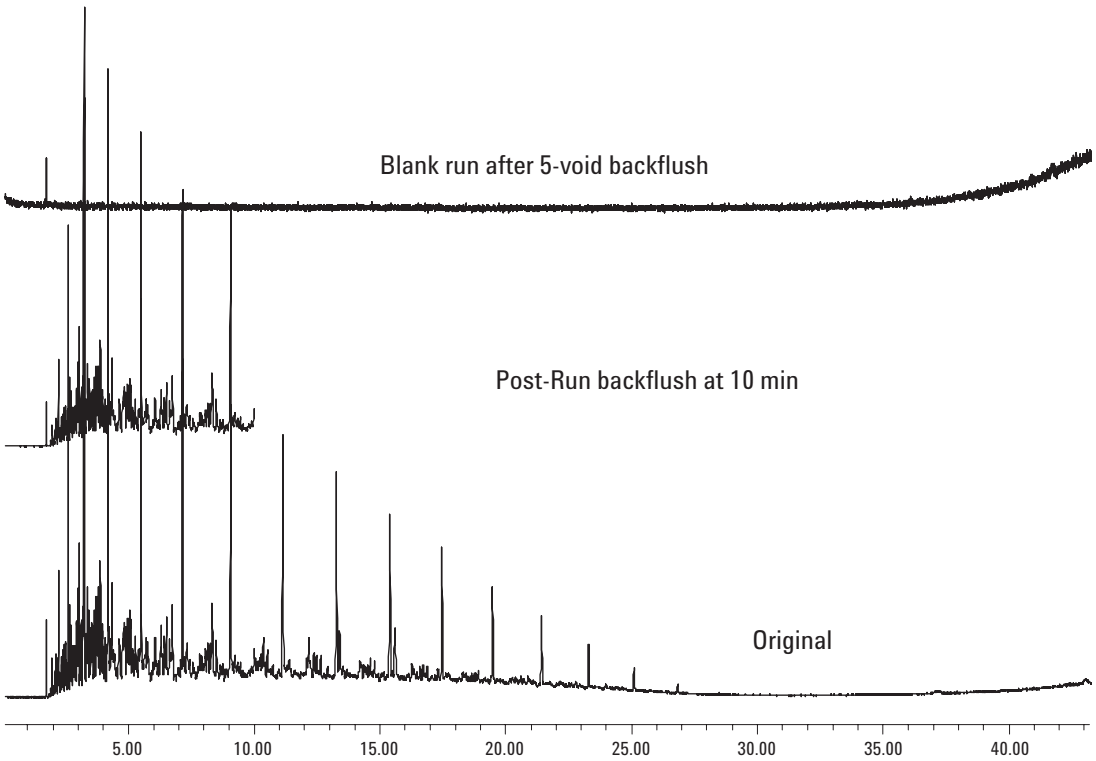

Figure 9. Example chromatograms of NIST 2724b reference diesel demonstrating backflush efficacy.

Time Savings

For the examples shown, cycle time was improved by both reducing run time and by stopping the oven temperature program at the time of backflush. This allowed the oven to cool from a lower temperature to starting conditions, saving time. See Table 3.

Table 3. Example of Time Saved Using Backflush

	Run time (min)	Cool down time (min)	Total (min)
Original	43.2	3.47	
Backflush	11.5	1.90	
Savings	31.7	1.57	33.27

Approximately 31.5 min run time was saved and another 1.5 min during cool down, for a total savings of approximately 33 min. If one were to need to run multiple samples, it is clear that lab productivity would significantly increase by incorporating a backflush.

Constant Flow Mode

A similar method was developed with basically the same conditions as those used earlier, except that 1 mL/min Constant Flow mode was employed. When in Constant Flow mode, the setup screens for Post Run backflush are slightly different than for Constant Pressure mode. The differences are highlighted below. See Figures 10–12.

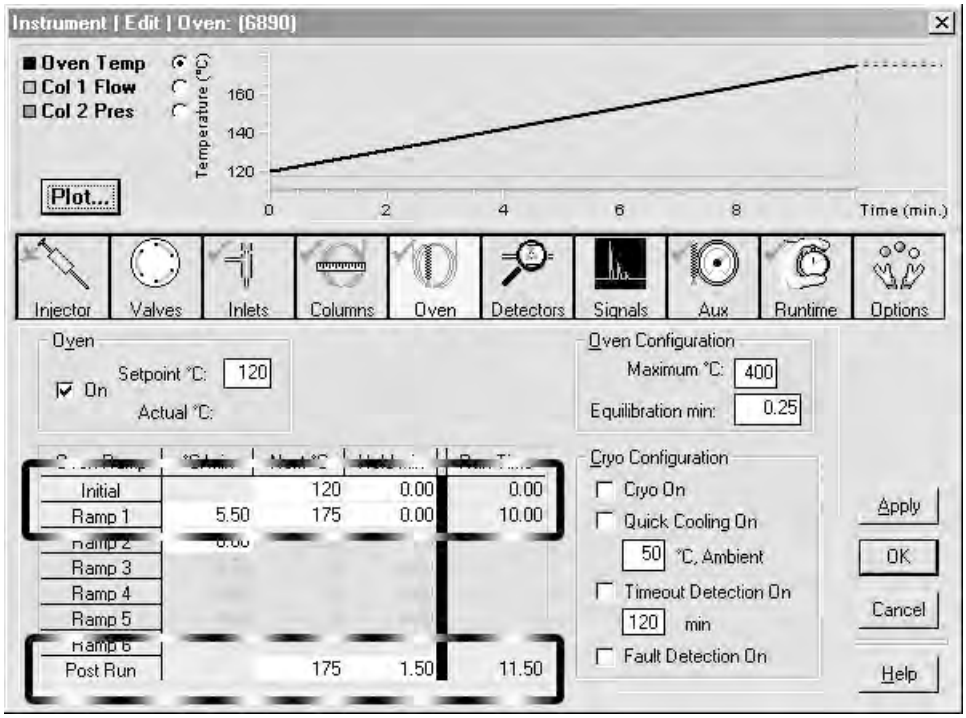

Figure 10. Oven setup screen for Constant Flow application.

First, the initial hold time was set to 0.0 min (0.2 min originally) for no other reason but that the initial hold was unnecessary. The temperature ramp rate was increased to 5.5 °C/min instead of the 5.0 °C/min used originally. This combination of change in initial hold and in ramp rate meant that the desired 10 min end of run/initiation of backflush corresponded to an oven temperature of 175 °C instead of the 169 °C in the Constant Pressure example.

For Post Run backflush in Constant Flow mode, Column 1 set points have a special requirement because the carrier gas terms are specified in flow instead of pressure. To ensure that the inlet pressure drops during backflush, one must ensure that the "column outlet pressure" is set to the ≤4.0 psi value you choose for the inlet backflush conditions (1 psi in this example) and set the value for column flow during backflush to 0.0. The system will then use the "Outlet" pressure set point as the inlet pressure during Post Run.

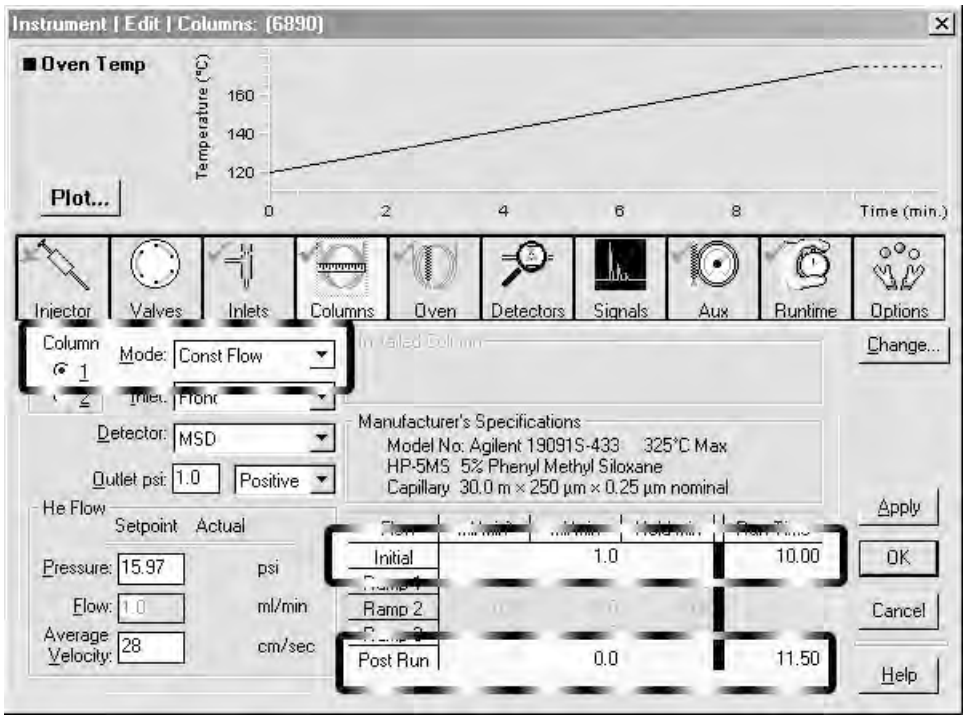

Figure 11. Columns screen for Constant Flow setup.

For Column 1, a 1 mL/min Constant Flow was used.

“Column 2” configuration is the same as in the Constant Pressure case. Constant Pressure mode should be selected, with QuickSwap as the Aux #5 inlet. This will allow a backflush pressure to be set during Post Run.

In this example, 1 psig was used as the pressure for analysis (the same as what was entered as Outlet Pressure for Column 1). A Post Run pressure of 85 psig was entered just as in the Constant Pressure example.

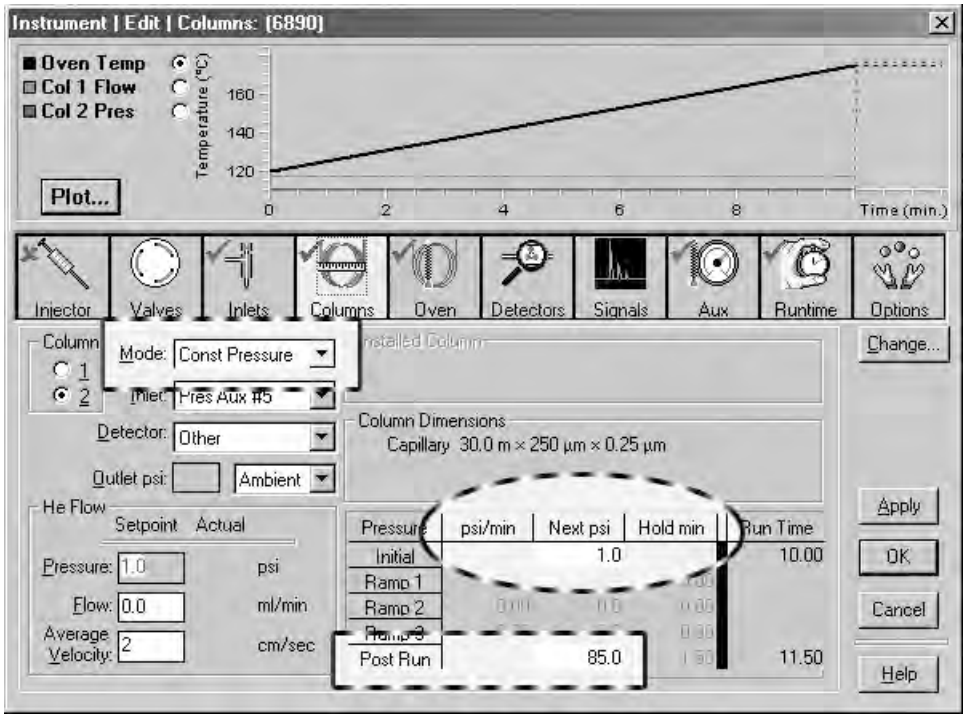

Figure 12. Columns screen for setting initial and Post Run pressure in Constant Pressure mode.

Some results from the above Constant Flow analysis and backflush conditions are shown in Figure 13.

For the homologous series of dimethyl siloxanes in Figure 13, the 1.5 min backflush (approximately 3.3 void times) was clearly sufficient to backflush all components remaining in the column at the conclusion of the 10 min run. This is confirmed in the following example of NIST 2724b diesel fuel. See Figure 14.

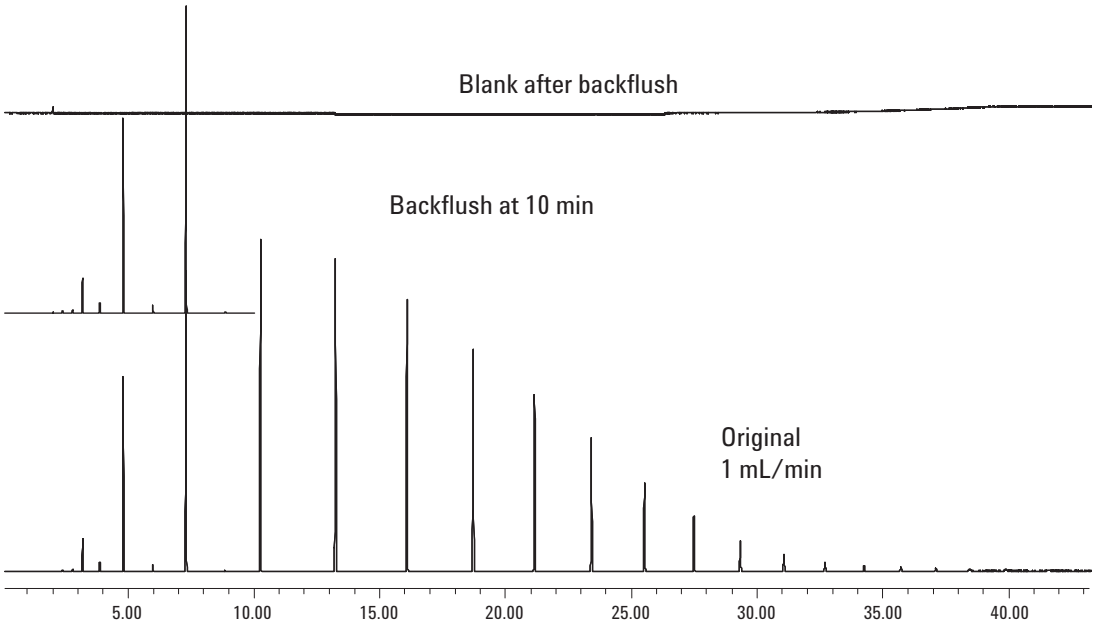

Figure 13. Chromatograms of dimethyl siloxane homologs analyzed under Constant Flow mode conditions and backflush.

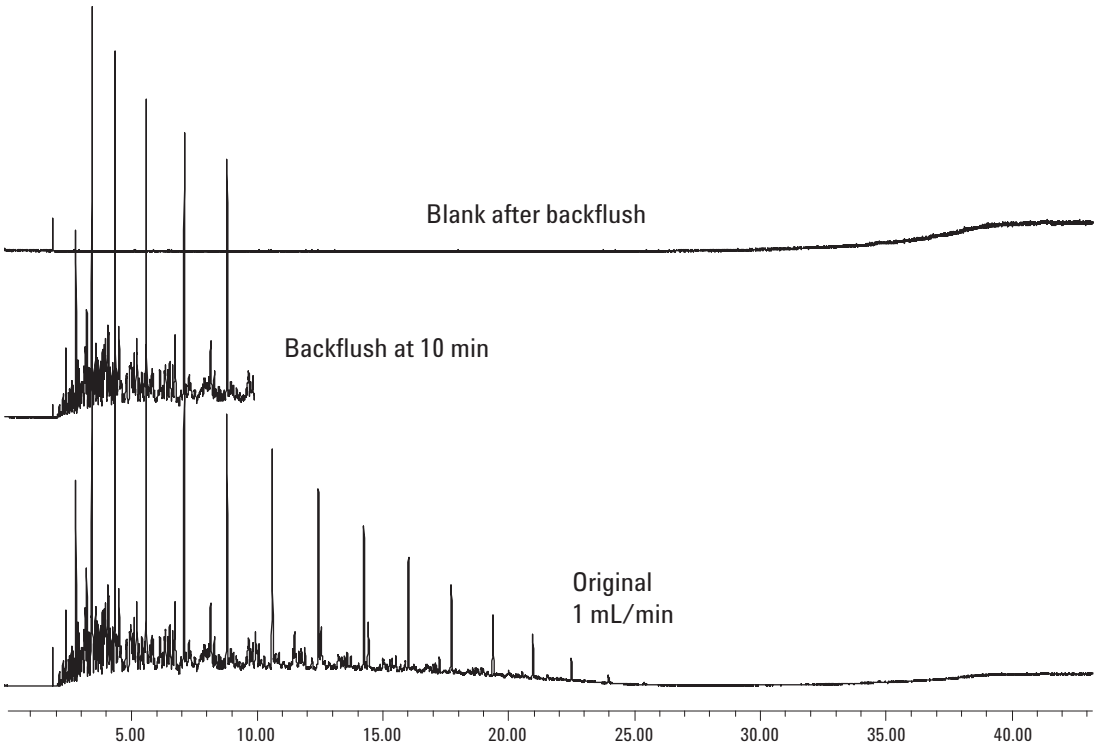

Figure 14. Backflush applied to diesel fuel sample NIST 2724b, analyzed under Constant Flow mode conditions.

Figure 15 is an expanded plot of the diesel fuel example shown in Figure 14. Notice that the baseline on the subsequent blank run after backflush is very clean. This shows that in addition to cycle time improvements, backflushing is an excellent way of improving overall analysis quality by removing all potential ghost peaks emanating from prior sample analyses.

Summary

A simple way to perform backflush with gas-phase microfluidic devices is described and demonstrated using the Agilent 6890 gas chromatograph.

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By selecting the microfluidic pressure source as the "inlet" for "Column 2" in the Column Configuration screen, one can then set a backflush pressure and time in Post Run. Several advantages ensue, including the ability to backflush in methods that use Constant Flow mode.

For More Information

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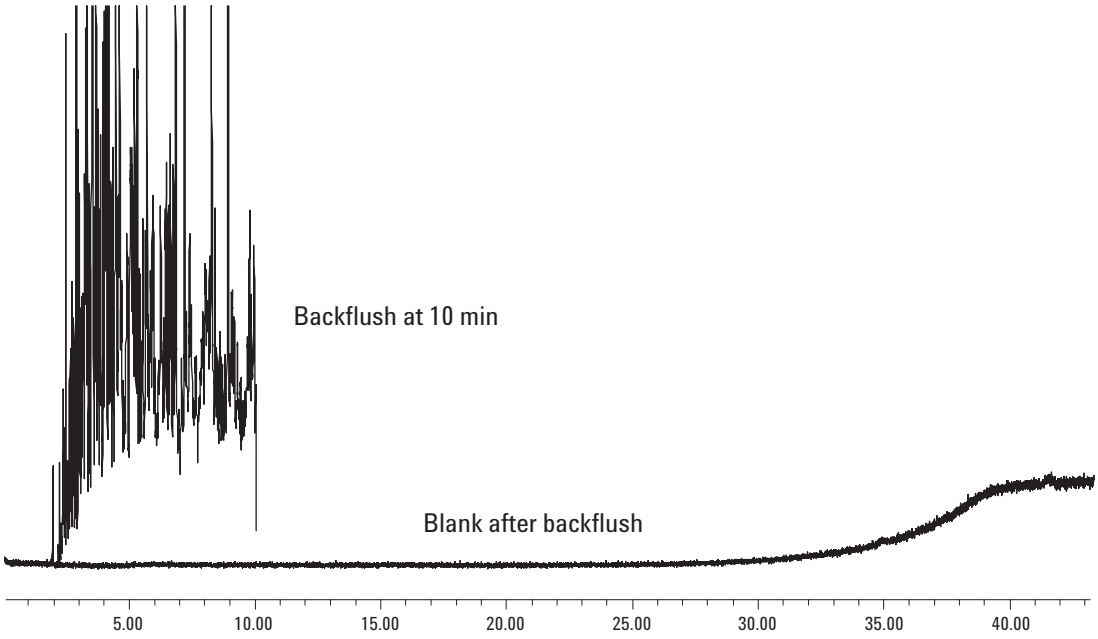

Figure 15. Expanded plot of the diesel fuel example showing excellent clean baseline after backflush.

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Printed in the USA
June 12, 2006
5989-5111EN

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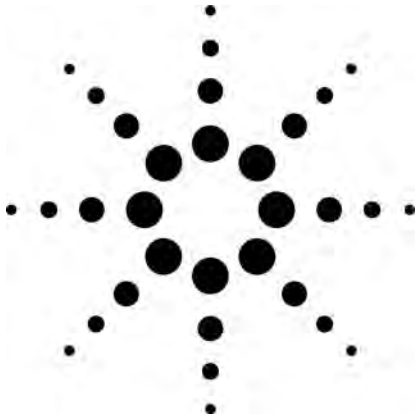
GS-OxyPLOT: A PLOT Column for the GC Analysis of Oxygenated Hydrocarbons

Technical Overview



Allen Vickers

Introduction

GS-OxyPLOT is a porous layer open tubular (PLOT) column. The stationary phase is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons. It is designed for and ideally suited for application in the ASTM methods listed in Table 1. It is an appropriate replacement for Varian's CP-LowOx column, usually with little to no changes in analytical parameters. This column is particularly useful for the trace analysis of oxygenates such as those listed in Table 2. Other oxygenated hydrocarbons are also suitable for analysis with this column subject to limitations given below.

The column can be used as a single, primary analytical separation column for oxygenated compounds. In complex sample matrices that have high molecular weight species (ca. 300 mol. wt. and higher) and/or species with insufficiently high vapor pressure to migrate through the GS-OxyPLOT, this column can be used in multidimensional GC systems with other columns that have vastly different polarity and lower selectivity toward oxygenated hydrocarbons. For example, a nonpolar DB-1 column can be used as an injection precolumn to retain low volatility solutes, allowing the less retained, polar oxygenated solutes to move into the GS-OxyPLOT. Since the stationary phase of GS-OxyPLOT is an oxygenate adsorbent phase, the oxygenates that enter the column are trapped. As the GC oven temperature is increased, the oxygenates will begin to migrate and are separated in the column prior to detection.

When first installed, the GS-OxyPLOT should be conditioned at 300 °C for at least 3 hours. Experience has shown that this column has an infinite shelf life, but when the column has not been in use for extended periods of time, longer conditioning times of 8 hours or more may be required to obtain retention time stability. The column can be stored with septa placed over the ends of the column, returned to the original column box, and stored at normal ambient temperatures for future use.

GS-OxyPLOT has a minimum temperature limit of 0 °C, an isothermal maximum temperature limit of 300 °C, and an oven program maximum temperature of 350 °C. Because the stationary phase is a strong adsorbent for polar compounds, especially water, it is recommended that when the column is installed in a GC, but idle, that the GC oven be set to an isothermal temperature of 220 °C with normal carrier gas flow, so that the instrument can be brought back into operation quickly when samples are ready to be analyzed. Otherwise, if the column is left at low oven temperatures, it may require reconditioning at 300 °C for several hours to obtain stable retention times.

Saturated hydrocarbon solutes have virtually no interaction with the GS-OxyPLOT and elute from the column so long as the column temperature is hot enough to induce a high enough vapor pressure for the solute to move in the carrier gas. Normal alkanes up to C_{18} will elute from GS-OxyPLOT within the program temperature maximum limit of the column. Because of the highly polar character of the GS-OxyPLOT phase, as would be

expected for oxygenate-selective PLOT column, the column has a relatively low sample load capacity for these nonpolar solutes. The low sample loading capacity is manifested chromatographically as a tailing peaking, indicative of phase overload in GS-OxyPLOT columns. Unsaturated hydrocarbons and aromatic hydrocarbons have relatively high retention. Injection of these organic compound classes should be limited to organic compounds with 11 carbons or less to prevent the column from fouling. As with the normal alkanes, the alkyl benzenes will show phase overloading at relatively low concentrations.

While GS-OxyPLOT is an ideal analytical solution for low molecular weight, oxygenated hydrocarbons, like all other similar oxygenate-selective PLOT columns,it is not recommended for higher molecular weight alkenals (e.g., 1-hexenal and 1-ocetenal). The combined interaction of the unsaturated and carbonyl functional groups can instigate tailing due to strong interactions and in some cases reaction between the phase and solutes.

Table 1. ASTM Standardized Methods for Which GS-OxyPLOT Is Specifically Designed

ASTM Method D7059	Determination of Methanol in Crude Oils by Gas Chromatography with Flame Ionization Detection
Proposed ASTM Method	Determination of C_1 to C_5 Oxygenates at Trace Levels in High Ethanol Content Gasoline Streams by Multidimensional Chromatography with Flame Ionization Detection*
Proposed ASTM Method	Determination of Oxygenates in Ethene, Propene, and C_4 and C_5 Hydrocarbon Matrices by Gas Chromatography and Flame Ionization Detection*

*These are "proposed methods" (i.e., do not have method designation numbers) that are destined for approval by ASTM Committee D2. These methods have already been accepted by, and are being implemented in, petrochemical refineries around the world.

Table 2. Examples of Oxygenated Compounds Suitable for GC Analysis Using the GS-OxyPLOT Column

1. Dimethyl Ether	13. Acetone
2. Diethyl Ether	14. Isovaleraldehyde
3. Acetaldehyde	15. Valeraldehyde
4. Ethyl t-Butyl Ether	16. Methyl Ethyl Ketone
5. Methyl t-Butyl Ether	17. Ethanol
6. Diisopropyl Ether	18. 1-Propanol
7. Propionaldehyde	19. Isopropyl Alcohol
8. tert-Amyl Methyl Ether	20. Allyl Alcohol
9. Propyl Ether	21. Isobutyl Alcohol
10. Isobutraldehyde	22. tert-Butyl Alcohol
11. Butylaldehyde	23. sec-Butyl Alcohol
12. Methanol	24. n-Butyl Alcohol
	25. 2-Methyl-2-Pentanol

Ordering Information for the GS-OxyPLOT Column

ID (mm)	Length (m)	Film Thickness (µm)	Temperature Limit (°C)	Cage Size	Part Number
0.53	10	10	350	7″	115-4912
0.53	10	10	350	5″	115-4912E

References

1. A. K. Vickers, “A ‘Solid’ Alternative for Analyzing Oxygenated Hydrocarbons—Agilent’s New Capillary GC PLOT Column,” Agilent Technologies publication 5989-6323EN, Feb 2006.
2. A New Megabore GC Column for the Adsorption and Chromatographic Separation of Oxygenates in Hydrocarbon Matrices, poster, Pittcon07-27.
3. Analysis and Chromatographic Separation of Oxygenates in Hydrocarbon Matrices, Power Point presentation, Pittcon07-20.

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Printed in the USA
March 9, 2007
5989-6447EN



Agilent Technologies

Successful Detection Using the Agilent 255 Nitrogen Chemiluminescence Detector (255 NCD)

Technical Overview

Introduction

The Agilent 255 Nitrogen Chemiluminescence Detector (255 NCD) can easily detect organic compounds containing nitrogen after conversion of the compounds to nitric oxide.

Frequently, chemists at Agilent are asked what nitrogen compounds are detected by the 255 NCD. Successful detection of nitrogen-containing compounds requires the conversion of these compounds to nitric oxide. The 255 NCD stainless steel burner converts nitrogen compounds to nitric oxide in a hydrogen and oxygen plasma at temperatures greater than 1800 °C.

The 255 NCD can easily detect organic compounds containing nitrogen after conversion of the compounds to nitric oxide. The stainless steel burner can also convert inorganic compounds such as ammonia and hydrazine to nitric oxide. The nitric oxide from the stainless steel burner reacts with ozone in the chemiluminescence reaction cell to produce a chemiluminescence reaction. A red optical filter allows transmission of the light from the chemiluminescence nitrogen reaction while suppressing chemiluminescence signals from other chemical species.

The selectivity of the 255 NCD results from the fact that not all compounds exhibit chemiluminescence when mixed with ozone. Also, the stainless burner cannot convert all compounds to nitric oxide.

Some compounds giving little or no response with the 255 NCD include carbon dioxide, water, nitrogen, oxygen, noble gases, and chlorinated hydrocarbons. These compounds represent the major constituents of many sample matrices. None of these compounds interfere significantly with the 255 NCD and determination of trace levels of the nitrogen-containing analytes.

Examples of Compounds Detected by the 255 NCD

- Amines
- Carbazoles
- Indoles
- Nitro-compounds
- Nitriles
- Nitrosamines
- Pyridines
- Quinolines
- Ammonia
- Hydrazine
- Hydrogen cyanide
- Nitric oxide, NO
- Nitrogen dioxide, NO_2
- NO_X

Compounds Not Detected by the 255 NCD

- Carbon dioxide
- Nitrogen gas
- Water
- Hydrocarbons

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May 17, 2007
5989-6770EN



Agilent Technologies

Agilent Model 255 Nitrogen Chemiluminescence Detector (NCD) Analysis of Adhesive Samples Using the NCD

Technical Overview

Introduction

The Agilent Model 255 NCD can easily replace a NPD for the analysis of 2-pyrrolidone and 1-vinyl-2-pyrrolidinone in adhesive samples.

A short-term evaluation was performed for the analysis of adhesive samples for two of the starting materials, 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. According to government regulations, the concentration of 1-vinyl-2-pyrrolidinone cannot exceed 800 ppm in the final product.

The primary objective for the analysis of adhesive was to demonstrate the repeatability and stability of the Model 255 NCD. The secondary objective of the study was to prove the NCD could replace the nitrogen-phosphorus detector (NPD) being used for the application. Table 1 compares the NCD and NPD.

The results from the evaluation demonstrated the Model 255 NCD could easily replace a NPD to monitor 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. The gas chromatograph and the Model 255 NCD were calibrated once and not recalibrated again during the 3-day demonstration. Representative precision data are shown in Tables 2 and 3. The gas chromatograph made over 160 injections of the adhesive samples during the 3-day evaluation with the percent relative standard deviation for the Model 255 NCD of less than 4%. The Model 255 was also linear from 20 to 3000 ppm. Figures 1 and 2 illustrate chromatographic response at high and low levels, respectively. Unlike an NPD, the sample matrix did not affect the detector performance.

For the 2-pyrrolidone and 1-vinyl-2-pyrrolidinone analysis, the NCD provides very good short-term and long-term precision. The NCD is unaffected by high levels of the sample matrix, and its use would require less day-to-day maintenance than an NPD. Use of the Model 255 NCD versus an NPD would result in more accurate and precise results and would reduce the level of instrument maintenance required.

Table 1. Comparison of Agilent NCD to NPD

	Agilent 255 NCD	NPD
Response	Equimolar	Non-equimolar
Quenching	No	Yes
Selectivity	> 107 gN/gC	105 gN/gC
Sensitivity	< 5 pg/sec	0.4 pg/sec
Ease of Use	Straightforward	Daily maintenance required

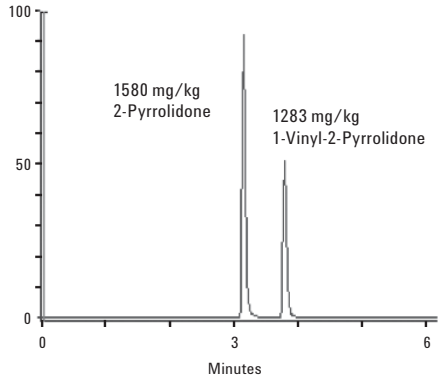

Figure 1. Agilent Model 255 analysis of adhesive standard.

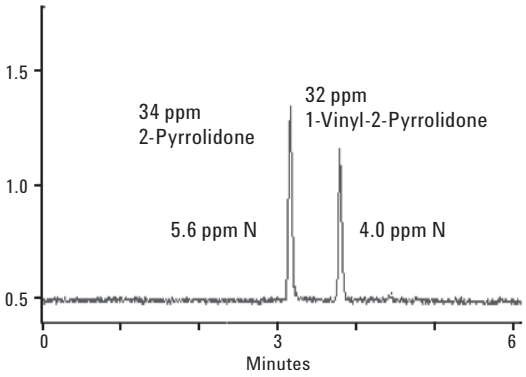

Figure 2. Agilent Model 255 NCD analysis of adhesive sample diluted in tetrahydrofuran.

GC Operating Conditions

(Agilent 6890 with EPC)

Temperature:	160 °C isothermal
Helium carrier:	2.2 mL/min
Split injection:	45.5:1 split
	200 °C
	1 µL injection volume

NCD Burner Conditions

Temperature:	800 °C
Hydrogen flow rate:	25 mL/min
Oxygen flow rate:	10 mL/min
Column:	20 Rtx-5, 0.32 mm id
	3 µm film thickness

Sample Preparation

Samples diluted in toluene
Dilution factors of 1:25 to 1:50

Table 2. Summary of 2-Pyrrolidone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	25.0	1094	2.2
Adhesive 2	43	1:41	28.1	1163	2.2
Adhesive 3	43	1:28	34.0	1405	2.2
Adhesive 4	43	1:45	20.3	838	1.7

Table 3. Summary of 1-Vinyl-2-Pyrrolidinone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	27.1	1188	2.2
Adhesive 2	43	1:41	15.1	624	2.2
Adhesive 3	43	1:28	32.5	1342	2.2
Adhesive 4	43	1:45	Not detected	Not detected	

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Printed in the USA
August 16, 2007
5989-6771EN

Agilent Technologies

Agilent Model 255 Nitrogen Chemiluminescence Detector (NCD) Simultaneous Hydrocarbon Analysis with the NCD and an FID

Technical Overview

Introduction

The nitrogen specificity of the Agilent Model 255 NCD and the universal detection of the flame ionization detector (FID) can provide a detailed analysis of a sample matrix. Chemiluminescence detection enables isolation of nitrogen-containing compounds, while the FID provides universal response for major components in many sample matrices.

Agilent has developed a detector interface to allow simultaneous universal and nitrogen specific detection for gas chromatography. An FID is used for universal detection and the Model 255 NCD is used for the specific detection of nitrogen compounds.

To perform simultaneous NCD and FID analysis, the capillary column is connected directly to the FID following the gas chromatograph manufacturer's installation instruction. The column effluent flows into the FID and then immediately continues through to the stainless steel burner of the NCD. The FID uses oxygen instead of air and the makeup gas is helium rather than nitrogen. Use of oxygen and helium avoids the formation of background nitric oxide in the flame of the detector.

The simultaneous mode is useful when the concentration of nitrogen in individual compounds is greater than 1 ppm. The detection scheme is also useful when the matrix of interest is unknown and there is a need for hydrocarbon data on the sample.

When necessary, the stainless steel burner is easily converted to Direct Analysis mode for better nitrogen sensitivity. The stainless steel burner was designed for use for either simultaneous NCD and FID analysis or specific nitrogen analysis only.

Figure 1 shows a chromatogram with nitrobenzene, 3-methylindole, and 9-methylcarbazole in toluene that demonstrates both the equimolar response and specificity of the NCD. The concentration of nitrogen is approximately 25 ppm for each compound. Also notice the lack of a solvent peak at the beginning of the NCD analysis. A benefit of the NCD is that non-nitrogen containing hydrocarbons in the sample are transparent to the NCD. The sample compounds flow from the column into the FID and the FID measures the hydrocarbon response. A portion of the FID effluent flows directly into the burner of the NCD.

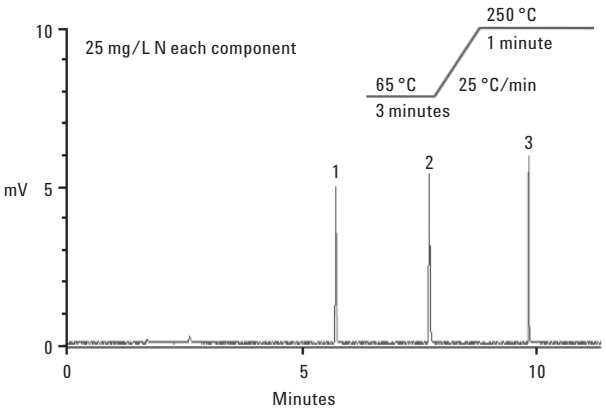

Figure 1. NCD analysis.

Table 1. Precision of Simultaneous NCD and FID Analysis

Number of runs	Compound concentration (ppm)	N concentration (ppm)	Model 255 NCD (% RSD)	FID (% RSD)
Nitrobenzene	218	25	2.3	1.5
3-methylindole	234	25	2.2	1.3
9-methylcarbazole	326	25	2.5	1.5

Notice the number of other small impurity peaks present in the FID chromatogram (Figure 2). The NCD did not detect these peaks since the NCD is specific only for nitrogen. If any of the small compounds contained nitrogen, the NCD would have detected them. Also, with the equimolar response of the NCD, it is possible to determine the concentration of nitrogen impurities in the sample.

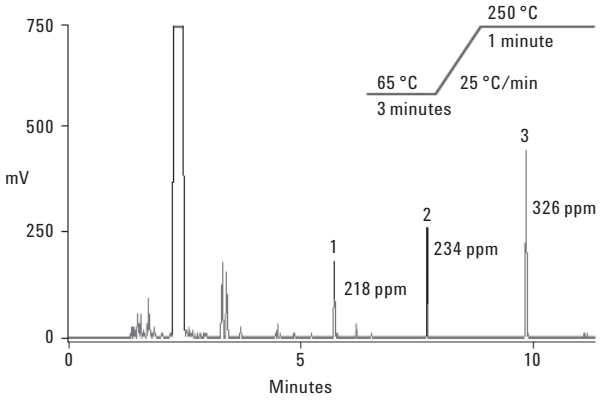

Figure 2. FID analysis.

GC Operating Conditions
(Agilent 6890 with EPC)

Initial temperature:	65 °C for 3 min
Temperature ramp:	25 °C/min
Final temperature:	250 °C for 1 min
Helium carrier:	2.2 mL/min
Split injection:	50:1 split
	250 °C
	2 µL injection volume

NCD Burner Condition

Temperature:	800 °C
Hydrogen flow rate:	25 mL/min
Oxygen flow rate:	10 mL/min
Column:	30 m HP-5, 0.32 mm id
	0.25 µm film thickness

Components

Peak 1:	Nitrobenzene
Peak 2:	Methylindole
Peak 3:	9-Methylcarbazole

The toluene sample with nitrobenzene, 3-methylindole, and 9-methylcarbazole was analyzed 143 times over a 3-day period to demonstrate the stability of the simultaneous NCD and FID analysis. The results of 2.5% relative standard deviation and less demonstrate the stability of the NCD when operated in tandem with the FID (see Table 1). The results also demonstrate that the tandem NCD/FID operation does not affect the performance of the FID. Furthermore, the results also demonstrate the consistency in transferring a fraction of the FID exhaust gases to the NCD.

The nitrogen specificity of the NCD and the universal detection of the FID can provide a detailed analysis. Chemiluminescence detection enables isolation of nitrogen-containing compounds in the sample, while the FID provides universal response for the major compounds of many sample matrices.

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Printed in the USA
May 17, 2007
5989-6772EN

Sulfur Compounds in Air – Agilent Model 355 SCD

Technical Overview

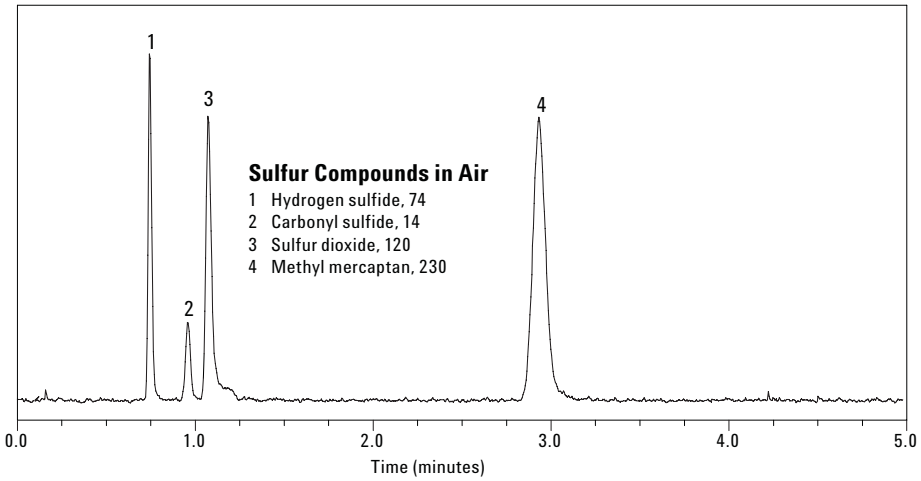
Introduction

The reliable measurement of sulfur gases in air is extremely important. Many sulfur compounds are toxic and notorious for their obnoxious odors even when present at only parts per billion levels. Gaseous sulfur compounds may be generated and emitted by various industrial processes, such as petroleum refining, ore smelting, and kraft paper pulping. Measurement of gaseous sulfur compounds aids in protection of the environment and human health. There are numerous natural sources of sulfur gases–vegetation, animals, soils, volcanoes, etc.–and measurement of sulfur gases is also of great importance in understanding atmospheric chemistry.

Gas chromatography with sulfur chemiluminescence detection (SCD) provides a rapid means to identify and quantify various sulfur compounds that may be present in air. Unlike other sulfur selective detectors, such as the flame photometric detector (FPD), the SCD produces a linear and equimolar response to sulfur compounds without significant hydrocarbon quenching or interferences. Furthermore, the Model 355 SCD is at least 10 times more sensitive and 100 times more selective than the FPD.

The following chromatogram illustrates the ability of the SCD to speciate and quantitate sulfur compounds at levels less than 1 ppm in an air sample without any sample preconcentration.

Conditions are as follows: Model 355 SCD operated according to standard conditions; 1 mL sample size; column: 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica; temperature program: –25 °C isothermal. The gas chromatograph was a Agilent Technologies Model 5890 Series II equipped with electronic pressure programming for compressing the initial bandwidth.



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Printed in the USA
May 22, 2007
5989-6778EN



Agilent Technologies

Agilent 355 Sulfur Chemiluminescence Detector (SCD): Sulfur Compounds in Light Petroleum Liquids Such as Gasoline

Technical Overview

Introduction

This technical overview briefly describes the analysis of light petroleum liquids as fully defined in ASTM D5623-94. The method is applicable to distillates, gasoline motor fuels (including those containing oxygenates), and other petroleum liquids with a final boiling point of approximately 230 °C (450 °F) or lower at atmospheric pressure.

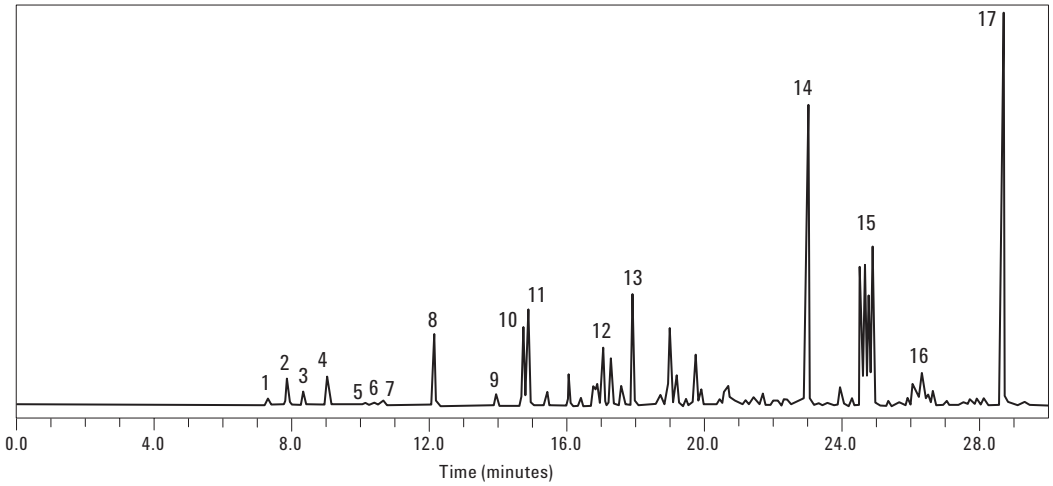
Gas chromatography with sulfur chemiluminescence detection (SCD) provides a rapid and highly specific means to identify and quantify various sulfur compounds that may be present in miscellaneous petroleum feeds and products, such as gasoline. Frequently, petroleum feeds and products contain varying amounts and types of sulfur compounds. Many sulfur compounds can be corrosive to equipment, inhibit or destroy catalysts employed in downstream processing, and impart undesirable odors to products. The ability to speciate sulfur compounds in various petroleum liquids is useful in controlling sulfur compounds in finished products and is frequently more important than the ability to simply measure total sulfur content alone.

The following chromatogram illustrates the ability of the Agilent 355 Sulfur Chemiluminescence Detector (355 SCD) to speciate sulfur compounds in a typical sample of gasoline. An internal standard (diphenyl sulfide) was added to the gasoline prior to analysis to aid in quantification.

This gasoline sample contained approximately 85 ppm wt total sulfur. Conditions are as follows: Agilent 355 SCD operated according to standard conditions: column: 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica; temperature program: –10 °C for three minutes to the final required temperature at a rate of 10 °C/minute.

Unlike the flame photometric detector or the pulsed flame photometric detector, the 355 SCD response is inherently linear, equimolar, and far less susceptible to hydrocarbon interference. These advantages eliminate the need for linearizing data or determining separate response factors for individual sulfur compounds.

Furthermore, hydrocarbons are virtually invisible to the 355 SCD. The coelution of hydrocarbon and sulfur peaks does not present a problem for the 355 SCD. Frequent column changes are required for analysis of various hydrocarbon products by flame photometric detectors to avoid serious quenching and inaccurate results. The linear and equimolar response, sensitivity, and selectivity of the 355 SCD is established and approved by the American Society of Testing and Measurements for sulfur in light petroleum liquids. (ASTM D5623-94).



1 Ethanethiol
2 Dimethyl Sulfide
3 Carbon Disulfide
4 2-Propanethiol
5 2-Methyl-2-Propanethiol
6 1-Propanethiol
7 Ethylmethyl Sulfide
8 Thiophene/2-Methyl-1-Propanethiol
9 Dimethyl Disulfide
10 2-Methylthiophene
11 3-Methylthiophene
12 C2-Thiophenes
13 Diethyl Disulfide
14 Benzothiophene
15 C1-Benzothiophene
16 C2-Benzothiophene
17 DiphenySulfide (Int Std)

Figure 1. Sulfur compounds in gasoline.

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June 5, 2007
5989-6782EN

Agilent Technologies

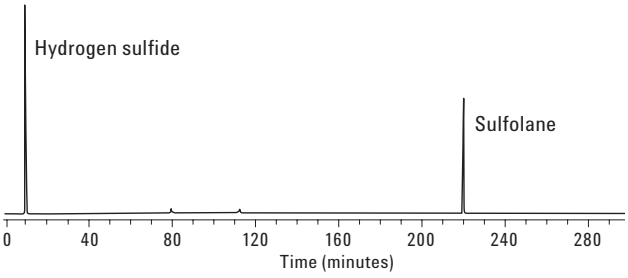
Agilent Model 355 Sulfur Chemiluminescence Detector (SCD): Sulfur Analysis Versus the Copper Strip Corrosion Test

Technical Overview

Introduction

The copper strip corrosion test (ASTM 1838) is commonly used as a product specification for liquid petroleum gases (LPGs). The test is designed to detect the presence of components that may be corrosive to copper and copper-alloy fittings that come into contact with the product. While the test is very simple to perform, its interpretation can be subjective. Furthermore, the test can produce erroneous results and it does not indicate the actual cause of corrosion, although it is generally assumed to be due to sulfur compounds.

Some situations where copper strip corrosion does not perform satisfactorily are as follows. While the presence of carbonyl sulfide does not cause corrosion in LPG systems, corrosive hydrogen sulfide is formed upon hydrolysis of carbonyl sulfide. Thus, an LPG could pass the copper strip corrosion test at its point of production and yet fail at its point of delivery or simply upon storage for a few days. [1] Different LPGs may each pass the copper strip corrosion test, but when commingled could fail. [2] This occurs because of hydrolysis as described or because one could contain a low level of hydrogen sulfide and the other could contain a low level of elemental sulfur. Hydrogen sulfide and elemental sulfur are known to have a synergistic effect on corrosivity. An LPG product could contain corrosive amounts of hydrogen sulfide and yet pass the copper strip corrosion test. This occurs when a masking agent is present in the LPG. Masking agents are polar compounds that preferentially chemisorb to the copper surface, preventing the formation of the copper tarnish. Amines, glycol amines, and other compounds such as sulfolane are known to have this effect. Mercaptans can also inhibit copper tarnish. The following chromatogram illustrates this masking effect. [3]



This particular LPG sample passed the copper strip corrosion test and yet contained over 40 ppm hydrogen sulfide by weight, according to stain tubes. Presence of polar compounds masked the corrosion effect of hydrogen sulfide. Their presence resulted from a unit upset in which gas treating chemicals were “burped” into a finished LPG storage jug. Presence of sulfolane also indicated the presence of amine treating compounds, since they are used simultaneously for sulfur compound removal.

Gas chromatography with a Model 355 sulfur chemiluminescence detector (SCD) was used to generate this chromatogram. Other chromatographic conditions were as follows: 0.5 µm liquid injection; column: 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica; temperature program: –10 °C for 3 minutes to the final required temperature at a rate of 10° C/minute. Gas chromatography with sulfur selective detection provides a rapid means to identify and quantify all of the various sulfur compounds that may be present in LPGs. This information is useful for explaining why a particular product passes or fails the copper strip corrosion test, and it provides the necessary information required to solve difficult product problems.

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June 5, 2007
5989-6783EN



Agilent Technologies

Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Separation of Carbonyl Sulfide and Hydrogen Sulfide

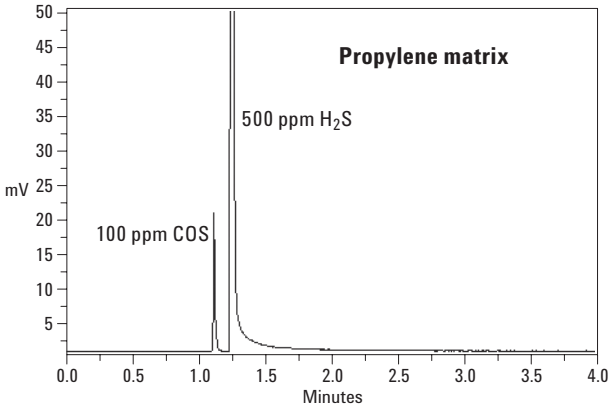
Technical Overview

Introduction

The Agilent 355 Sulfur Chemiluminescence Detector (SCD), in conjunction with the J&W GasPro silica-PLOT column for separation, can be used to detect trace levels of carbonyl sulfide (COS) in the presence of very high concentrations of hydrogen sulfide (H_2S).

Hydrogen and carbonyl sulfides are common sulfur gases. Their determination in gaseous samples, such as natural gas or various gaseous streams involved in sulfur recovery, is very important. H_2S and COS are readily separated on thick-film methyl silicone capillary columns, when present at nominal concentration levels. It is difficult, however, to separate and detect trace levels of COS in appreciably higher levels of H_2S because COS is eluted on the tail of H_2S (in the order of their boiling points). In some cases, cryogenic cooling of the column may help improve the separation, but when the level of H_2S is more than 100 to 500 times higher than COS, cryogenic cooling provides no benefit.

A solution to this difficult separation problem is to use a J&W GasPro silica-PLOT column. COS is eluted prior to H_2S without the use of cryogenics. The following chromatogram illustrates the successful separation and detection of trace COS and a high level of H_2S in a propylene matrix using a J&W GasPro column in conjunction with an Agilent 355 SCD.



Chromatographic Conditions

Injector temperature:	120 °C
Initial temperature:	50 °C (isothermal)
Injection type:	Splitless
Injection volume:	1 mL
Column type:	J&W GasPro
Column length:	15 m
Internal diameter:	0.32 mm
Head pressure:	5 psig

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June 5, 2007
5989-6784EN



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Agilent Model 355 Sulfur Chemiluminescence Detector (SCD): Thiophene in Benzene

Technical Overview

Introduction

This overview discusses the analysis of benzene for sulfur contamination. The sensitivity, selectivity, and linear response of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) in the analysis of trace levels of thiophene in benzene illustrates that the Model 355 is well-suited for low-level sulfur analysis.

Benzene is the basic unit of the aromatic class of compounds. The primary sources of benzene are from extraction of hydrocarbon crude distillates, refinery catalytic reforming, carbonization of coal, and the hydrodealkylation of a toluene charge stock. [1] The hydrodealkylation reaction results in the conversion of about 90% of the aromatics in the feed with a selectivity factor of about 95%. Thiophene, which also occurs in the light hydrocarbon fractions distilled from crude stocks and coal tar, is present in levels from 0.4 to 1.4 wt. %. [2] Traditionally, thiophene has been extracted by washing with H_2SO_4 to produce a sweeter product. However, this and other processes of thiophene removal still have difficulty reaching the level of purity required by many chemical markets. Consequently, it is often important to monitor trace levels of thiophene in benzene. The three main applications for benzene are production of ethylbenzene, cumene, and cyclohexane. These three products account for 80% of the benzene consumed as a chemical feedstock. [2]

These species can be classified as intermediates for a wide range of final products, including dyes, resins, solvents, and polymers, including nylon. The purity requirements for synthetic applications continue to become more confining, thereby increasing the need to monitor sulfur removal efficiency and to verify the purity of starting materials.

The data in Figure 1 illustrate the sensitivity of the 355 SCD for trace level analysis of sulfur in a hydrocarbon matrix without interference. Figure 2 displays the linear response of the SCD at trace levels. Correlation coefficients for five orders of magnitude were better than $R^2 = 0.999$. The selectivity of the 355 SCD for sulfur over hydrocarbon is shown in Figures 1 and 3, where sulfur chromatograms show no hydrocarbon interference from the eluting benzene.

The data in Figures 1 and 2 were collected on a Agilent 6890 gas chromatograph with a Agilent 355 SCD directly attached. The chromatograms in Figure 3 were collected simultaneously without column splitting, using the flame ionization detector attachment to the SCD. The chromatographic conditions for the trace analysis are summarized in Table 1.

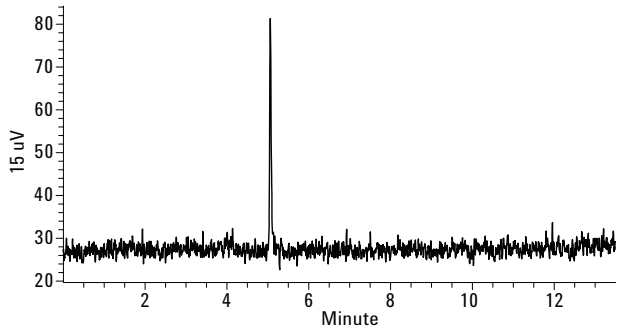

Figure 1. Chromatogram illustrating the analysis of 15 ppb thiophene (as sulfur, split 1:10).

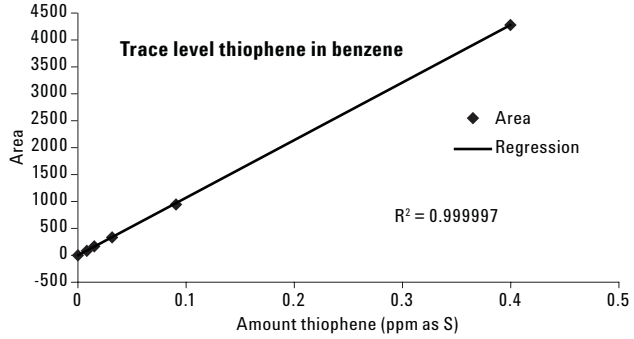

Figure 2. Linearity of trace level analysis of thiophene in benzene.

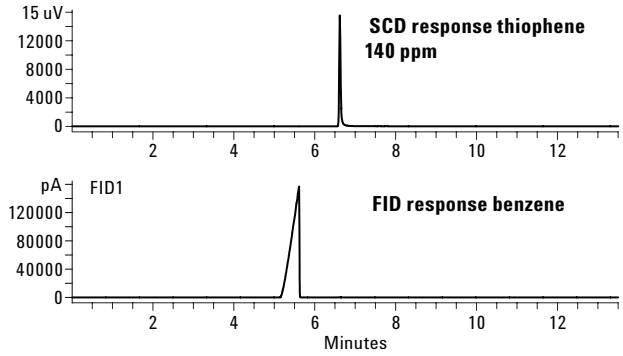

Figure 3. Simultaneous FID-SCD chromatogram on SUPELCOWAX-10.

Table 1. Chromatographic Conditions

Injection temperature	120 °C
Injection volume	1 µL
Initial temperature	30 °C
Initial time	2 min
Rate	10 °C/min
Final temperature	125 °C
Final time	2 min
Split ratio	1:10
Flow mode	Constant flow
Column flow	2 mL/min
Column type	SUPELCOWAX-10
Length	30 m
Internal diameter	0.32 mm
Film thickness	1 µm

References

1. James H. Gary and Glenn E. Handwerk, "Petroleum Refining Technology and Economics," Marcel Dekker Inc., New York, NY, 1984, Chapter 14.

2. Wolfgang Y. Gerhartz, Stephen Yamamoto, et. al. eds., "Ullmann's Encyclopedia of Industrial Chemistry," VCH Publisher, Deerfield Beach, FL, 1985, vol. A3.

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June 7, 2007
5989-6786EN

Agilent Model 355 Sulfur Chemiluminescence Detector (SCD): Sulfur Simulated Distillation

Technical Overview

Introduction

This technical overview briefly describes the determination of sulfur boiling point distribution and total sulfur content of petroleum and petrochemical products that fall within the scope of ASTM D 2887.

Sulfur simulated distillation is a useful tool to help meet sulfur specifications for numerous products through better process optimization and understanding. Sulfur simulated distillation may be carried out using various detectors and techniques, but utilization of sulfur simulated distillation has largely been limited to research laboratories. The fact that sulfur levels in crude oils are continually rising, coupled with the fact that environmental regulations require lower levels of sulfur in products, makes the implementation of sulfur simulated distillation in plant laboratories more desirable than ever before. Through the use of the right hardware and software, accurate and precise results from sulfur simulated distillation can be obtained for petrochemical products that fall within the scope of ASTM D 2887. For this broad range of samples, a gas chromatograph with electronic pressure control, a cool-on-column injector, D 2887 capillary column, flame ionization detector (FID), and a sulfur chemiluminescence detector with FID adapter produce the best results. Use of simulated distillation software, such as SimDis Expert from Separation Systems, is also required. The following examples are illustrative of the type of results that can be obtained. Additional details may be obtained from Agilent.

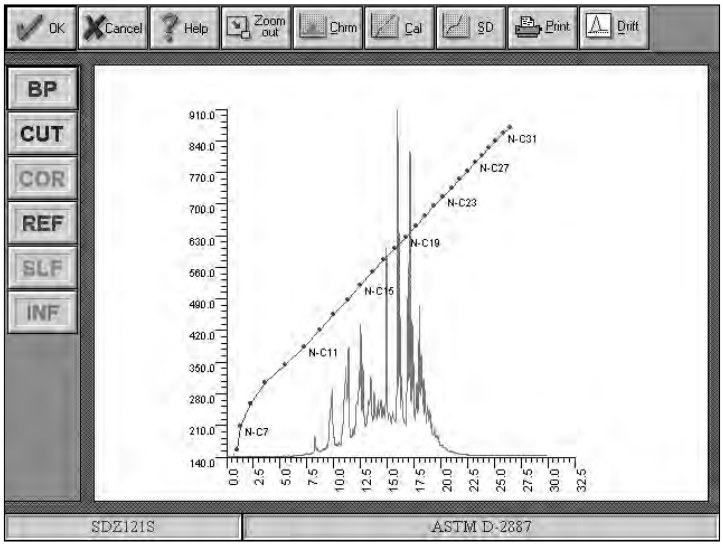

Figure 1. SCD simulated distillation chromatogram of NIST 1624b.

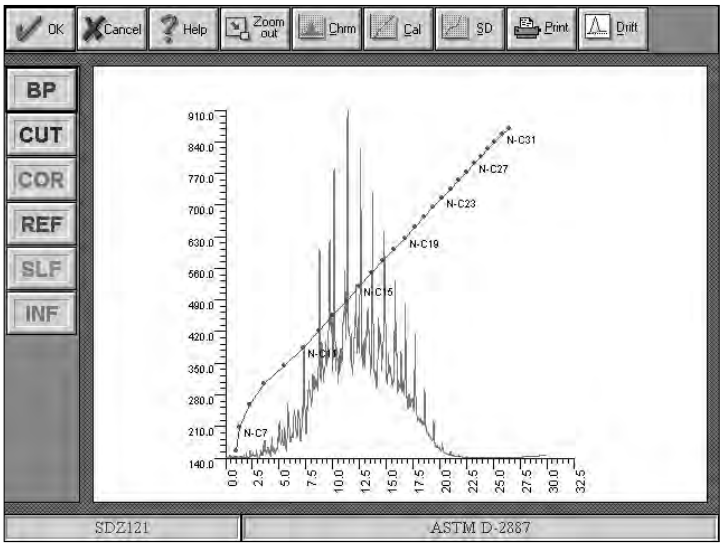

Figure 2. FID simulated distillation chromatogram of NIST 1624b.

Table 1. Simulated Distillation Data on Sulfur Boiling Point of NIST 1624b

Replicate	IBP (°C)	5% off (°C)	50% off (°C)	95% off (°C)	FBP (°C)
1	196.9	231.9	310.1	366.4	430.4
2	196.6	232.5	313.6	367.7	437.7
3	196.6	232.2	313.6	368.3	440.7

Table 2. Simulated Distillation Data on Hydrocarbon Boiling Point of NIST 1624b

Replicate	IBP (°C)	5% off (°C)	50% off (°C)	95% off (°C)	FBP (°C)
1	150.8	189.9	268.0	347.2	380.3
2	150.6	189.9	268.0	347.3	380.5
3	150.7	190.0	268.2	347.4	380.6

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June 7, 2007
5989-6787EN

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Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Odorants and Other Sulfur Compounds in Liquefied Petroleum and Natural Gases

Technical Overview

Introduction

This technical overview briefly describes the analysis of liquefied petroleum gases as fully defined in ASTM D 5504-95. The method provides for the determination of individual volatile sulfur containing compounds, as well as the determination of total sulfur content in gaseous fuels, including natural gas.

Gas chromatography with sulfur chemiluminescence detection provides a rapid means to identify and quantify various sulfur compounds that may be present in petroleum feeds and products, such as liquefied petroleum gases (LPGs) and natural gas liquids (NGLs). These samples can contain many different amounts and types of sulfur compounds. Many sulfur compounds are corrosive to equipment, inhibit or destroy catalysts employed in downstream processing, and impart other undesirable properties to products. However, odorous sulfur compounds such as ethyl mercaptan, tetrahydrothiophene, and occasionally thiophane are intentionally added to propane as warning agents for detecting LPG leaks. The ability to speciate sulfur compounds in these liquids is useful for quality assurance of odorant addition and for better control of the sulfur compounds in finished products. The following chromatogram illustrates the ability of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) to speciate sulfur compounds in an NGL sample.

This particular NGL sample contained approximately 100 ppm wt total sulfur. The chromatographic conditions for the illustrated analysis were as follows: Agilent 5890 Series II gas chromatograph; Agilent 355 SCD operated according to standard conditions; and 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica column. The temperature program was: –10° C for three minutes to the final required temperature at a rate of 10° C per minute.

Evolving column technology allows for the analysis of liquefied petroleum gases or natural gas liquids with separation of H_2S and COS at ambient temperatures using capillary columns such as the Chrompack CP Sil 5 CB (0.32 mm), Chrompack CP SilicaPLOT (30 m 0.32 mm id), or the Astec Gaspro (15 m 0.32 mm id).

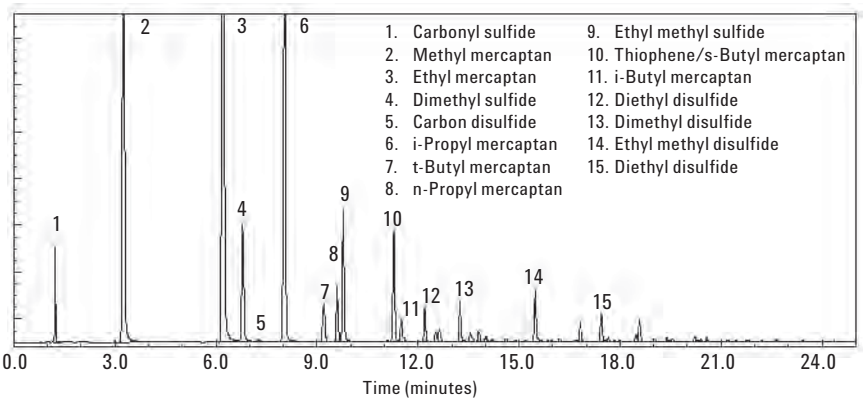

Figure 1. Sulfur compounds in natural gas liquids.

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June 8, 2007
5989-6788EN

Agilent Technologies

Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Sulfur Compounds in Distillate Fuels

Technical Overview

Introduction

This technical overview discusses the analysis of commercial jet fuels for sulfur using an Agilent 355 Sulfur Chemiluminescence Detector (SCD). The sensitivity, selectivity, and linear response of the 355 SCD coupled with the separation capabilities of the Chrompack CP Sil 5 CB capillary column demonstrate the power of the Agilent 355 SCD as the ideal detector for low-level sulfur analysis.

Distillate fuels are generally divided into three types: jet fuels, diesel fuels, and heating oils. Jet fuels are typically blended from low-sulfur or desulfurized kerosene, cracked blending stocks, and hydrocracked blending stocks. The two basic types of jet fuels are naptha and kerosene.

Naptha jet fuel is primarily produced for military uses and consists of wide boiling range stock that extends through both gasoline and diesel ranges. Military JP-4, as illustrated below, has restrictions similar to ASTM type B fuel, with limitations on freezing point (–60 °F to –70 °F maximum), gravity (45 to 70 API), Reid Vapor Pressure (2.0 psi to 3.0 psi), and aromatic content (20 to 25 percent maximum). [1] The wide boiling range of 150 to 550 °F provides for great flexibility in blending kerosene, naptha, and low-octane gasoline. Commercial jet fuels are regulated to a narrower boiling point range of 350 to 550 °F and are typically sold as JP-5.

Sulfur requirements as specified in ASTM D 910 specify that total sulfur levels of commercial jet fuels be regulated to less than 500 ppm. [2] Lower levels are common in military fuels to avoid corrosional wear of engine parts from sulfur combustion products and to avoid conditions that may promote gum formations, which lower storage life.

The data in Figure 1 illustrate the sensitivity of the 355 SCD for trace level analysis of sulfur in a hydrocarbon matrix without interference. The equimolar response of the 355 SCD allows the operator the flexibility to use either an external standard method or an internal standard method for quantification of both individual components and total sulfur content.

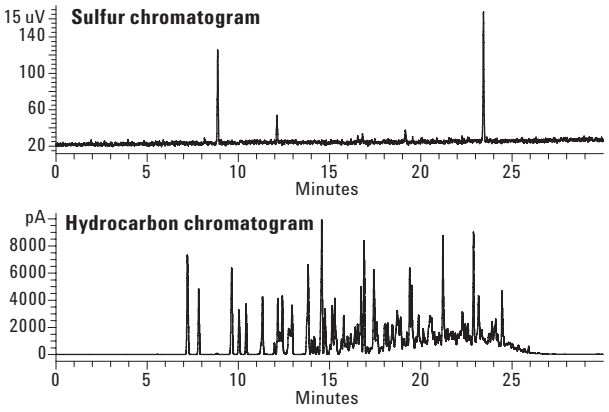

Figure 1. Simultaneous FID/SCD chromatogram of JP-4 (split 1:10).

Figure 2 illustrates the use of an internal standard, in this case ethyl methyl sulfide (EMS 10.6 min), to quantify each peak, as well as the total sulfur content. EMS was spiked into the sample at 2.8 ppm as sulfur. Individual component analysis is accomplished by comparison of the analyte area to the internal standard area, and total sulfur content is calculated through summation of the total area of the unknown peaks. The JP-4 sample analyzed was found to have 8.3 ppm total sulfur content. The peaks at 8.8 and 12.2 minutes were confirmed to be carbon disulfide, and thiophene at 3.34 ppm and 2.3 ppm respectively.

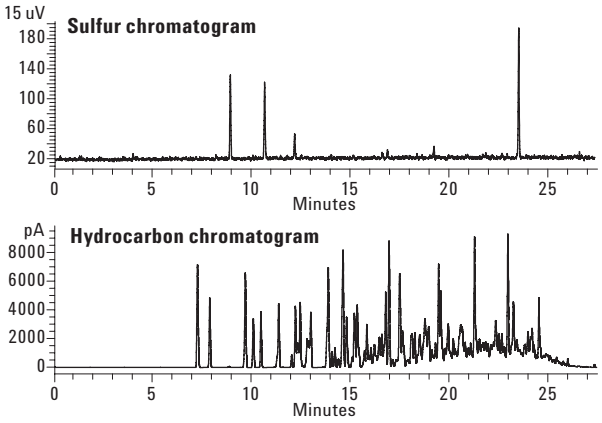

Figure 2. JP-4 FID/SCD chromatogram with internal standard (split 1:10).

All of the data were collected on a Agilent 6890 gas chromatograph with an Agilent 355 SCD in tandem on the flame ionization detector (FID) adaptor for the simultaneous collection of the hydrocarbon and sulfur chromatograms with no sample pretreatment, and no column splitting. This configuration provides the operator with the flexibility to monitor both the hydrocarbon and sulfur content of a sample simultaneously without requiring a separate detector port. The FID adapter samples approximately 10 percent of the effluent after the FID combustion, resulting in a 1:10 split to the SCD. In Figure 2, the 1:10 355 SCD split, coupled with the 1:10 injection split on the GC, result in a 1:100 sample split to the 355 SCD. The amount of sulfur arriving at the 355 SCD for the carbon disulfide peak is therefore 88 ppb, illustrating the extreme sensitivity of the 355 SCD. The column used was a Chrompack CP-Sil 5 CB, courtesy of Chrompack International. [3] The data were collected and analyzed by Agilent ChemStation version 4.02. Standard conditions for both analyses are summarized in Table 1.

Table 1. Chromatographic Conditions

Injection temperature	250 °C
Injection volume	1 µL
Initial temperature	40 °C
Initial time	2 min
Rate	10 °C/min
Final temperature	250 °C
Final time	2 min
Split rato	1:10
Flow mode	Constant flow
Column flow	2 mL/min
Column type	Chrompack CP-Sil 5 CB
Length	30 m
Internal diameter	0.32 mm
Film thickness	5 m

References

1. James H. Gary and Glenn E. Handwerk, “Petroleum Refining Technology and Economics,” Marcel Dekker Inc., New York, New York, 1984; Chapter 2.
2. Annual Book of ASTM Standards, Vol 05.01, ASTM D 910-90, Standard Specifications for Aviation Gasolines.
3. Chrompack International Herculesweg 8, P.O. Box 8033, 4330 EA Middleburg, The Netherlands.

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5989-6790EN

Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Sulfur Compounds in Ethylene and Propylene

Technical Overview

Introduction

This technical overview briefly describes the analysis of ethylene and propylene gases for trace amounts of hydrogen sulfide and carbonyl sulfide as well as other volatile sulfur compounds using gas chromatography and sulfur selective detection. The method provides for the determination of individual volatile sulfur-containing compounds, as well as the determination of total sulfur content in chemical feedstocks.

The measurement of trace amounts of volatile sulfur compounds in ethylene and proplyene is important because of the contaminant nature of these compounds in hydrocarbon feedstocks. Accurate gas chromatographic determinations of trace volatile sulfur compounds involve unique analytical difficulties due to the chemical nature of these compounds. Volatile sulfur compounds are particularly reactive and adsorptive in nature, making trace level analysis reliant on exceptionally good chromatographic technique, using inert sample handling systems and valving, and selective detection that is minimally affected by matrix interference. Because of their respective boiling point ranges, the measurement of hydrogen sulfide in ethylene and carbonyl sulfide in propylene is generally of great concern.

This analysis is especially difficult using detectors such as the flame photometric detectors, where coelution of the analyte and solvent contribute to hydrocarbon quenching and interference, which may result in erroneous results. The following chromatograms illustrate the ability of the SCD to selectively detect trace levels of volatile sulfur compounds in hydrocarbon gas samples without suffering from any quenching or interference from the hydrocarbon matrix.

The analyses presented here were performed on an Agilent 5890 Series II gas chromatograph equipped with a split/splitless injector. The Agilent Model 355 Sulfur Chemiluminescence Detector (SCD) was directly connected to an Astec Gaspro capillary column and operated according to standard conditions.

Figure 1 illustrates the power of the 355 SCD for the analysis of COS in propylene. A 1-mL propylene sample containing 60 ppb wt sulfur as COS was introduced to the GC with no pretreatment. The injector was operated with a split ratio of 1:6, and the linear velocity was approximately 38 cm sec^{-1}. Temperature programming for this analysis was as follows: 50 °C for 1 min to 100 °C at 10 °C/min. This chromatogram verifies the selectivity of the Agilent 355 SCD for sulfur over carbon, with no hydrocarbon response or anomalies visible in the baseline. Also evident is the sensitivity of the SCD to sulfur species, making it ideal for trace analysis.

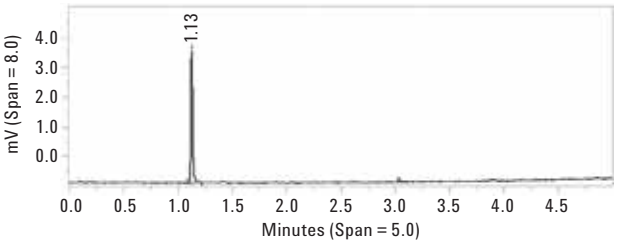

Figure 1. 60 ppb carbonyl sulfide in propylene.

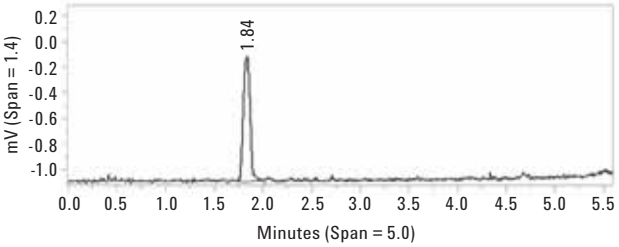

Figure 2. 100 ppb hydrogen sulfide in ethylene.

Figure 2 illustrates the analysis of 100 ppb hydrogen sulfide in ethylene using the GasPro column and SCD. As above, a 1-mL sample (gas) volume was introduced with a split 1:6. The oven temperature program started at 40 °C for 1 min and raised at 10 °C/min to the final temperature of 100 °C. In this case the temperature ramp for the analysis was deliberately set so that there would be a simultaneous elution of the ethylene and the hydrogen sulfide. As expected, this resulted in the exhibited band broadening; however, there was no evident quenching of the sulfur response and no hydrocarbon response or baseline anomalies. Although the SCD works well under these conditions, it is always recommended to separate the analyte from the matrix if at all possible to reduce solvent effects.

Recent developments in chromatographic column technology allow the ambient separation of hydrogen sulfide and carbonyl sulfide in hydrocarbon matrices. For ambient separation of H_2S and COS as well as light mercaptans and sulfides, capillary columns such as the Chrompack CP-SilicaPLOT (30 m 0.32 mm id) or the Astec Gaspro (15 m 0.32 mm id) are ideal. The retention characteristics of these columns are unique and seem to be best suited for light applications.

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5989-6791EN

Laboratory Total Sulfur Analysis by Gas Chromatography and Agilent Model 355 SCD

Technical Overview

Introduction

Gas chromatography with sulfur chemiluminescence detection (SCD) provides a relatively quick means to identify and quantify sulfur compounds that are present in petroleum and petrochemical feeds and products, such as natural gas, ethylene, propylene, liquefied petroleum gases, solvents, gasoline, and diesel fuels. In many cases, it is sufficient to simply measure the total sulfur content of a sample without the need for compound speciation. If that is the case, one can replace the GC column with an appropriate transfer line to connect the injector with the detector. This technical overview briefly covers the rapid determination of total sulfur using a GC as a sample introduction device and the SCD to quantitate the sulfur content of a sample.

Experimental conditions are as follows: Agilent Model 355 SCD operated according to standard conditions with FID adapter; 3-m high-purity stainless-steel transfer line from split/splitless injector to FID; oven, injector, and detector temperatures 200 °C; 1-L injector split 1:10; and 10 psi injector head pressure.

Figure 1 illustrates the measurement of 1 ppm of sulfur (thiophene) by weight in high-purity benzene. Note that the analysis is very rapid, requiring less than 15 seconds for the actual measurement. Analysis times will be longer than this of course because of sample handling, system calibration, and other requirements.

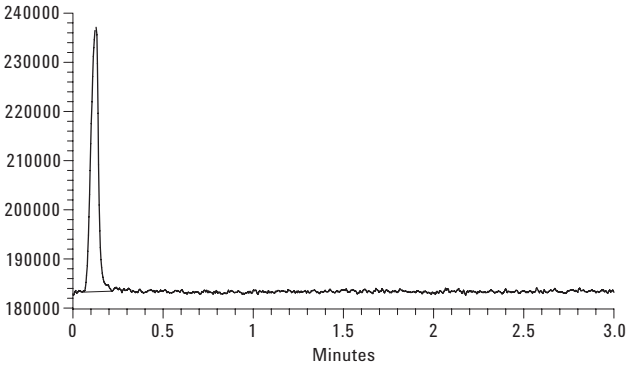

Figure 1. 1 ppm sulfur as thiophene.

Excellent sensitivity, selectivity, precision, and linearity are observed using this technique. For example, the detection limit of total sulfur in benzene is less than 100 ppb sulfur by weight.

The technique demonstrates very good precision at the 1 ppm wt level, around 2% relative standard deviation (RSD). For instance at 1 ppm wt thiophene (as sulfur) in benzene, the mean area was 157,600 area counts with a standard deviation of 3,080 (n = 5), yielding an RSD of 1.95%.

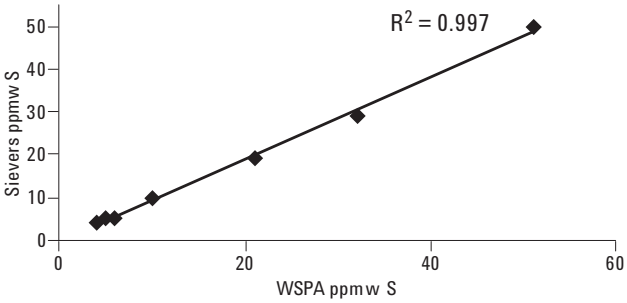

Figure 2. Relationship between the Agilent Model 355 SCD and WSPA results for total sulfur in gasoline.

Both linearity and accuracy are reflected in the results from comparing sulfur analyses obtained by this technique to the results determined by Western States Petroleum Association (WSPA) for seven gasoline samples. This is shown in Figure 2.

Relationship Between Sulfur Results by Laboratory

Chemiluminescent TSA and WSPA results calibration in this analytical technique is achieved through the use of appropriately generated standards. One should match the sample and standard matrices as closely as possible. For example, use a benzene standard matrix when measuring sulfur in a benzene sample. Since all sulfur compounds produce the same response, use of a single sulfur compound standard is sufficient for calibration. Please note that nonvolatile sulfur compounds will not be detected since they will remain in the injection port. Analyzer linearity is greater than three orders of magnitude, so generation of multiple calibration points may not be necessary. In summary, this technique is simple, accurate, and precise.

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June 26, 2007
5989-6792EN

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Gas Chromatography and Sulfur Chemiluminescence Detection for Low-Sulfur Diesel

Technical Overview

Introduction

Environmental regulations are driving down sulfur levels in fuels. These regulations place many challenges on the refining industry, not the least of which is low sulfur measurement. This technical overview briefly describes the ability of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) to help refiners meet some of the sulfur measurement challenges for low-sulfur diesel fuels.

Sulfur compounds occur naturally in petroleum and feedstocks for fuel production. Their presence creates processing challenges and impacts product quality. Many sulfur compounds are toxic, reactive, and corrosive to processing equipment and damage catalysts used in hydrocarbon processing. In terms of the product quality of fuels, sulfur compounds impart undesirable odors and contribute to gum formation in gasoline and diesel fuel, and turbine deposits in jet fuel, especially in military aircraft [1, 2].

From a pollution standpoint, all fuel sulfur contributes to acid rain. It is also known that sulfur compounds poison catalytic converters used in modern automobiles, contributing to other emission problems. In industrialized nations, strict environmental regulations directed toward sulfur have been critical to improving air quality. The U.S. EPA and other environmental regulatory bodies, particularly those in Europe, Japan, and other industrialized nations, have developed regulations aimed at further reducing air pollution from mobile sources. Sulfur plays a key role in this. The allowable levels of sulfur in liquid fuels, such as gasoline and diesel fuels, are being driven to lower levels.

Numerous instrumental methods are used to measure sulfur in gasoline and diesel fuels. [2] It is beyond the scope of this overview to cover these techniques. Nevertheless, it is safe to say that the sensitivity required for the fuels of tomorrow can only be achieved with a limited number of technologies, and only gas chromatography with sulfur-selective detection can provide the means to measure both trace total sulfur and sulfur speciation.

Total sulfur measurement alone provides very little information about the best manner to process a feed or even to determine whether a process is functioning as designed. For example, in a process that converts mercaptans to disulfides, total sulfur measurements would show no difference between the feed and the product, while a sulfur distribution measurement via gas chromatography and sulfur-selective detection tells if the intended chemical conversion is occurring. Simulated distillation methodologies can also tell how best to process a feed. Gas chromatographic techniques provide the means for determining individual sulfur species, types, and distribution, in addition to total sulfur.

This technical overview briefly illustrates the capability of gas chromatography with sulfur chemiluminescence detection using the Agilent 355 SCD for the analysis of sulfur in a low-sulfur diesel fuel. Figure 1 shows the response obtained from a diesel sample that contained approximately 6 mg/kg total sulfur, as determined by calibration with an NIST standard and independently by another total sulfur instrument. A brief description of the experimental details is given in the figure caption.

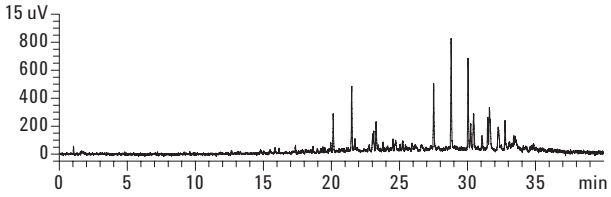

Figure 1. Low-sulfur diesel analysis by gas chromatography with the Agilent 355 SCD.

Experimental conditions:

Injection:	1 mL 1:8 split, 350 °C
Column:	30 m, 0.32 mm id, 0.25 mm HP-5
Oven temperature:	100 °C for 3 min to 250 °C at 4 °C/min.

References

1 K. H. Altgalt and M. M. Boduszynski, "Composition and Analysis of Heavy Petroleum Fractions," 1994, Marcel, Dekker, N.Y., Chapter 1.

2 R. A. Kishore Nadkarni, American Laboratory, 2000, 32, 16.

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Printed in the USA
June 14, 2007
5989-6794EN



Lock in the Power of Gas Chromatography with RTL

- Improving Reliability and Precision Analyzing Oxygenates in Gasoline

The unique retention time locking (RTL) software from Agilent Technologies revolutionizes gas chromatography. With RTL, retention times (RT) can be reproduced within hundredths, even thousandths, of a minute from one Agilent GC based system to another. Be it between 6890 or 6850 GC systems or one 6890/5973 inert MSD system to another, RTs are virtually identical, regardless of inlet, detector, operator, or location.

This technique is an easy and economical way to improve confidence in results, reduce the risk of non-compliance, and reduce operating costs. All that is required is the RTL software, an Agilent 6890 or 6850 GC (equipped with Agilent's industry-leading electronic pneumatics control (EPC)) or an Agilent 6890/5973 inert MSD system, and the Agilent ChemStation for GC or MSD.

RTL - Real Performance Advantages

- Removes the need to update calibration table RTs, timed events, and integration events tables when a 6890/6850 method is transferred, a column installed or routine maintenance performed
- Ensures results that are easy to compare, document and explain
- Lock on a peak and reproduce exactly a 6890/6850 method developed in another laboratory
- Reproduce exactly the RTs from a published 6890/6850 method
- Allows fast setup and testing plus decreases method development time
- Delivers simplified method and system validation
- Provides fast, accurate identification of unknowns through retention time library searching

Improved Reliability and Precision for ASTM Method D4815

ASTM method D4815 is used to measure oxygenated additives in gasoline. The species of interest include ethers such as MtBE, EtBE DIPE, TAME at 0.1 - 20.0 wt% and alcohols - C1 to C4 and t-pentanol at 0.1 - 12.0 wt%. There are usually only 1 or 2 oxygenates in a sample. The scope of the original application was to improve this method, making it easier and to provide greater confidence in results using RTL.

The method calls for a polar TCEP micropacked column to provide preliminary separation and remove light hydrocarbons. Trapped oxygenates are back flushed to the capillary column and this is the key to success. Variations in the back flush time caused by the TCEP pre-column make RTL difficult to apply.

TCEP columns are generally difficult to prepare. The small dimensions of the column, the means in which wire is used as plugs and the use of crimping to hold in the wire lead to variations in column head pressure.

In addition, potential temperature variations can occur if the pre-column in the valve box is not uniformly heated, making it difficult to accurately set the back flush time.

Problems with head pressure can be overcome with the use of screen plugs, while wrapping the column and mounting it on a heated mandrill can address temperature variations. Once these problems have been solved, precise back flush times can be achieved and the RTL software can be used to accurately lock in the data. For ASTM method D4815, MtBE acted as the target peak for locking and RTL calibration was performed after back flush time optimization. Sample data for 5 different 6890 GC systems before and after RTL correction are summarized in Figures 1 (before) and 2 (after).

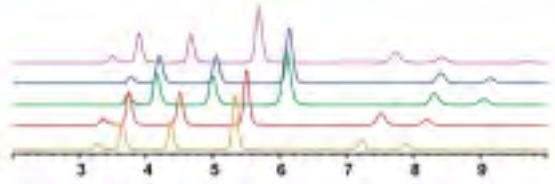

Figure 1 Chromatograms of oxygenates in gasoline from five different 6890 GC systems before locking

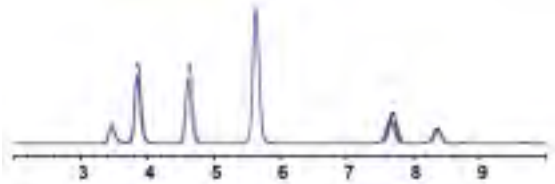

Figure 2 Chromatograms of oxygenates in gasoline from 5 diferent 6890 GC systems after locking

Table 1 summarizes results on replicate analyses using the modified method and illustrates the superb reproducibility. %RSDs exceed the ASTM requirements by a factor of 10.

		Repeatability		Reproducibility	
Compound	Mass%	Spec	Observed	Spec	Observed
Ethanol	0.99	0.06	0.01	0.23	0.01
Ethanol	6.63	0.19	0.03	0.68	0.04
MtBE	2.10	0.08	0.01	0.20	0.01
MtBE	11.29	0.19	0.05	0.61	0.08

Table 1 Replicate analyses of oxygenates in gasoline

Summary

Agilent's unique Retention Time Locking (RTL) software is an excellent means of improving RT reproducibility across various Agilent GC and GC/MS systems improving simplified method development and system validation. For more information on this and other Agilent Solutions for the petroleum/petrochemical industries log onto www.agilent.com/chem/hydrocarbon or contact your local Agilent Technologies, Life Science Chemical Analysis representative.

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Chemical and Specialty Gas

Ethylene/Propylene/ Light Hydrocarbons/ Fixed Gas

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Trace Level Hydrocarbon Impurities in Ethylene and Propylene

Application

Gas Chromatography

March 1997

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Abstract

An Agilent 6890 Series gas chromatography system was used to determine trace (low ppm) levels of hydrocarbon impurities in high-purity ethylene and propylene. The gas chromatograph (GC) was equipped with a heated gas sample valve, split/splitless inlet, and flame ionization detector (FID). An Agilent HP-PLOT Al_2O_3 column was used for separation of the trace hydrocarbons. Impurity levels below 10 ppm (mole) were easily detected in both ethylene and propylene.

Introduction

High-purity ethylene and propylene are commonly used as feedstocks for production of polyethylene, polypropylene, and other chemicals. Typically, these low molecular weight monomers are of very high purity (99.9+ percent). However, hydrocarbons, sulfur compounds, and other impurities in feed streams can cause such problems as reduced catalyst lifetime and changes to product quality. Process yields can also be adversely affected. Many impurities have been identified as potential contaminants (1,2).

Recently, ASTM has proposed several procedures to determine trace hydrocarbon impurities in both ethylene and propylene (3). These methods, currently in the investigation stage, use alumina porous layer open tubular (PLOT) columns. This application note describes the suggested Agilent configuration for such methods and illustrates resulting separations of both quantitative calibration blends and actual process samples. These proposed methods should be valuable in meeting commercial specifications.

Experimental

All experiments were performed on an 6890 Series gas chromatograph (GC) equipped with a split/splitless inlet and capillary optimized flame ionization detector (FID). All gas flows and pressures within the GC were controlled electronically. Gas sample injections were made using an automated sample valve placed in the 6890 valve oven (80 °C). The gas sample valve was interfaced to the capillary inlet using an aluminum tube (1/8-in.) that jacketed the stainless steel transfer line (option 860). The inlet was fitted with a split/splitless liner (part no. 19241-60540). All injections were made in the split mode.

A 50-m × 0.53-mm, HP-PLOT Al_2O_3 "M" column was used for separation. For ethylene analysis only, a 30-m × 0.53-mm, 5-µm HP-1 column was placed directly behind the HP-PLOT column. The two columns were joined using a glass press-fit connector.

The Agilent ChemStation was used to control the 6890 Series GC and to provide data acquisition and peak integration. The ChemStation was operated at a data acquisition rate of 10 Hz.

Standards for retention time and response factor calculation were obtained from DCG Partnership (Houston, Texas, USA 77061). Samples used for this work were obtained from commercial sources.

Table 1 lists the entire set of equipment and conditions.

Results and Discussion

Ethylene

The configuration used for ethylene analysis is found in figure 1.

The HP-PLOT Al_2O_3 column was used for hydrocarbon separation. The use of HP-PLOT Al_2O_3 columns for light hydrocarbon analyses has been previously described (4). These columns exhibit excellent separation characteristics for the C_1 through C_5 isomers.

The proposed method for ethylene specifies the use of a second nonpolar column placed after the HP-PLOT alumina column to improve the separation of impurity peaks eluting on the tail of ethylene. This nonpolar column gains importance for trace level analysis, where higher concentrations of ethylene (99.9 percent and higher) exhibit increased tailing. No attempt was made to compare separations without the nonpolar Agilent HP-1 column.

Table 1. Instrument Configuration and Operating Conditions

Item	Description
Gas Chromatograph	
G1540A	6890 Series GC
Option 112	Split/splitless inlet
Option 211	Capillary optimized FID
Option 701	6-port gas sample valve and automation
Option 751	Valve oven
Option 860	Valve to inlet interface
Column	• 50-m x 0.53-mm HP-PLOT Al_2O_3 "M" (part no. 19095P-M25) • 30-m x 0.53-mm, 5-µm HP-1 (part no. 19091Z-236), used for ethylene analysis only
Data Acquisition	
G2070AA	Agilent ChemStation
Operating Parameters	
Injection port temperature	200 °C
Detector temperature	250 °C
Split ratio	10/1 to 50/1 depending on sample
FID conditions	30 mL/min hydrogen, 350 mL/min air, nitrogen make-up (25 mL/min column + makeup)
Temperature program	• Ethylene: 35 °C (2 min), 4 °C/min to 190 °C • Propylene: 40 °C (2 min), 4 °C/min to 190 °C
Injection volume	• Ethylene: 0.5 mL • Propylene: 0.25 mL
Column flow	• Ethylene: 6 mL/min constant flow (10 psi) • Propylene: 3.5 mL/min constant flow (4 psi)
Valve temperature	80 °C

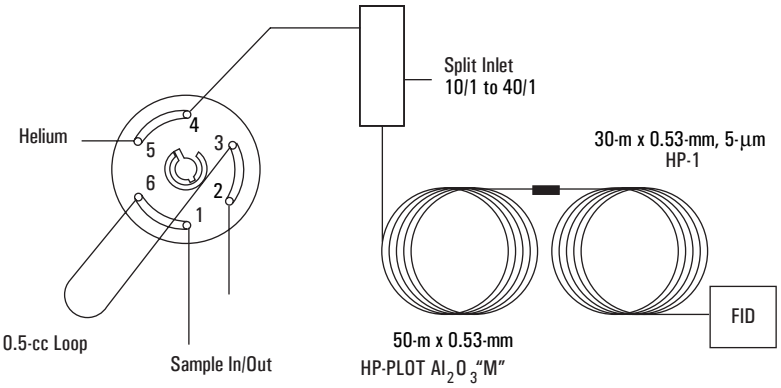

Figure 1. Valve drawing for impurities in ethylene.

Figure 2 shows the chromatogram of an ethylene calibration blend containing most of the major hydrocarbon impurities. This sample was analyzed at a split ratio of 10/1. The concentration of most components (except for ethane) ranges from 8 to 12 ppm (mole). For this analysis, baseline separation is achieved for all the impurities except for propane. Total analysis time is approximately 30 minutes. Because this separation is more than adequate, analysis time can be reduced by increasing the temperature program rate. Based upon conditions used for this analysis, most components can be detected at the 1-ppm level.

Chromatographic results for two process ethylene samples are given in figures 3 and 4. The sample presented in figure 3 contains only methane, ethane, and propylene as impurities. Less than 1-ppm methane was detected. The ethylene sample in figure 4 shows a high concentration of methane, with trace amounts of ethane, propane, and propylene.

1. Methane (10 ppm)
2. Ethane (219 ppm)
3. Ethylene
4. Propane (12 ppm)
5. Propylene (9 ppm),
6. Isobutane (10 ppm)
7. n-Butane (10 ppm)
8. Propadiene (10 ppm)
9. Acetylene (10 ppm)
10. t-2-Butene (8 ppm)
11. 1-Butene (8 ppm)
12. Isobutylene (9 ppm)
13. c-2-Butene (9 ppm)
14. 1,3-Butadiene (10 ppm)
15. Methylacetylene (9 ppm)

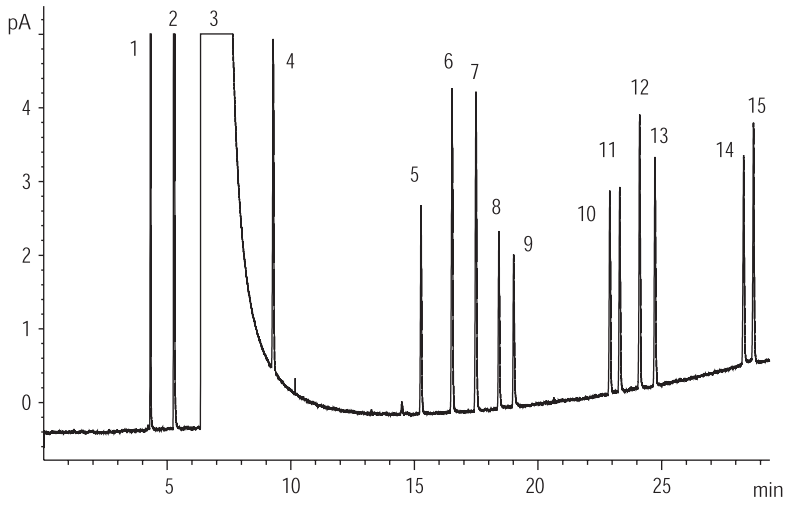

Figure 2. Chromatogram of ethylene calibration blend, split ratio 10/1.

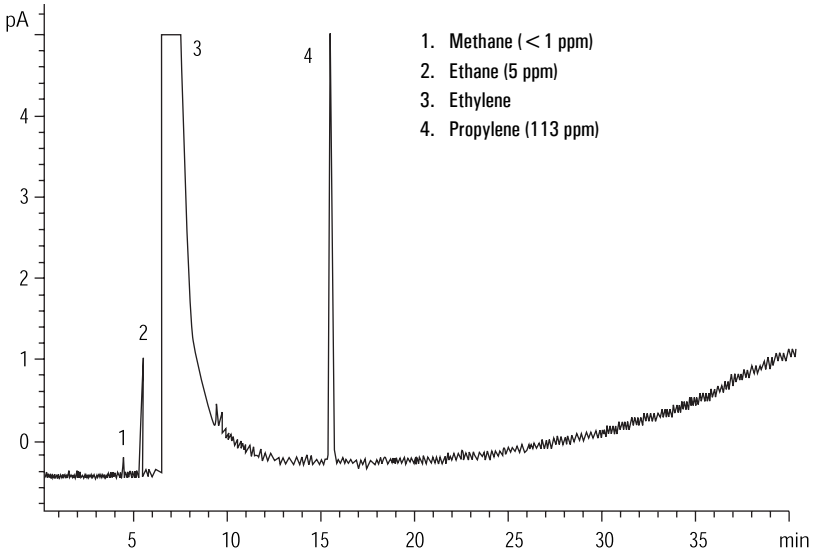

Figure 3. Chromatogram of process ethylene sample, split ratio 20/1.

Propylene

The configuration used for propylene analysis is illustrated in figure 5. This configuration is essentially the same as for ethylene, but without the HP-1 column. The sample volume was reduced to 0.25 mL. Propylene was sampled in the gas state.

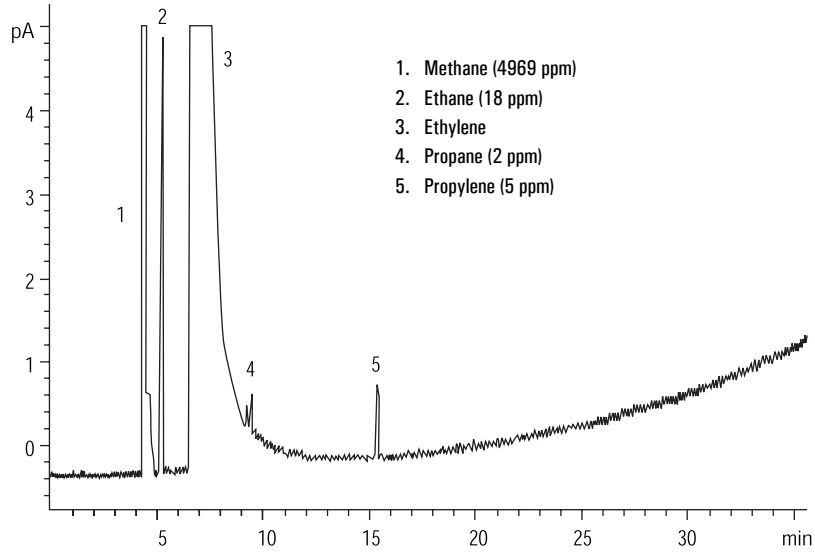

Figure 4. Chromatogram of process ethylene sample.

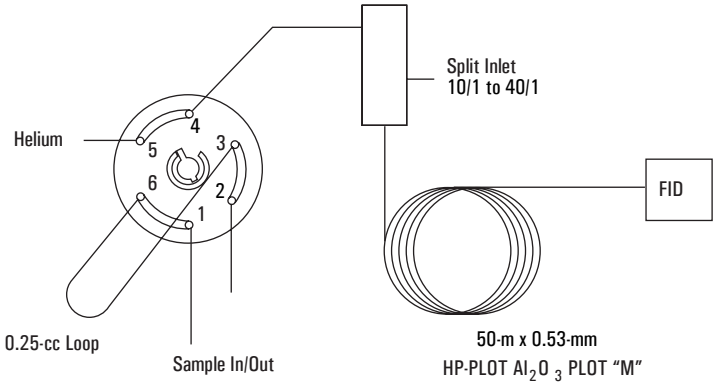

Figure 5. Valve drawing for impurities in propylene.

A chromatogram representing the trace hydrocarbon impurities in propylene is shown in figure 6. This sample was analyzed at a split ratio of 20/1. The concentration of most impurities range from 8 to 20 ppm. Ethylene is present at a higher concentration level. Most of the impurities in the sample are well separated using the conditions described in table 1. Cyclopropane elutes just before propylene and is baseline separated under these conditions. Several of the C_4 hydrocarbons elute on the tail of the high-purity propylene. This affects the lower limit of detection for these peaks, compared to those components that are baseline separated. The remainder of the C_4 and C_5 impurities are well separated.

1. Methane
2. Ethane (10 ppm)
3. Ethylene (50 ppm)
4. Propane
5. Cyclopropane (10 ppm)
6. Propylene
7. Isobutane (10 ppm)
8. n-Butane (7 ppm)
9. Propadiene
10. Acetylene
11. t-2-Butene (10 ppm)
12. 1-Butene
13. neo-Pentane
14. Isobutylene (9 ppm)
15. Isopentane
16. c-2-Butene (9 ppm)
17. n-Pentane (10 ppm)
18. 1,3-Butadiene (9 ppm)

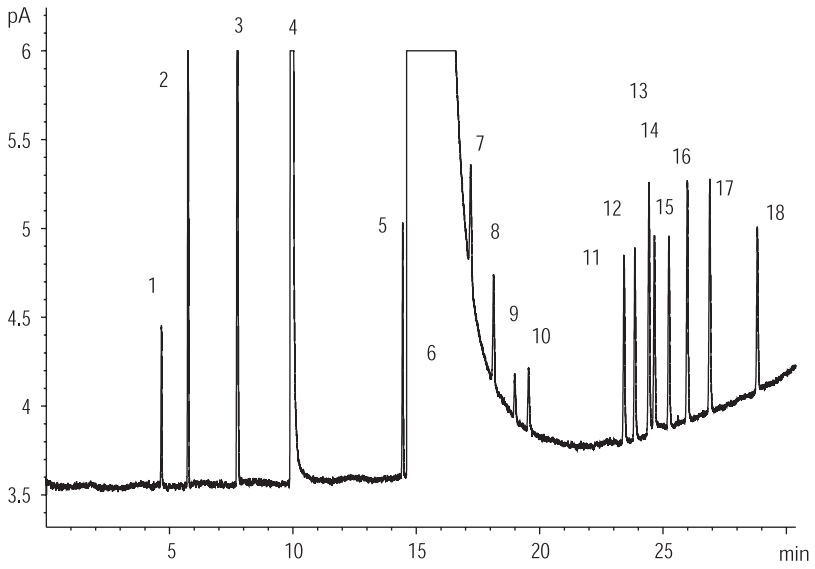

Figure 6. Chromatogram of propylene calibration standard.

For comparison, figure 7 shows the analysis of a second calibration blend containing a higher level of impurities (50 to 1000 ppm).

Figure 8 presents the chromatographic results for a high-purity propylene process sample. This sample contains only ethane and propane impurities.

Summary

This application note describes two methods for analyzing trace hydrocarbon impurities in ethylene and propylene. These methods use a gas sample valve with split injection, an Agilent HP-PLOT Al_2O_3 and HP-1 (for ethylene only) column, and an FID. Impurities below the 10-ppm mole level can be easily quantitated using these methods. For some impurities, especially those that are well separated from the large ethylene or propylene peaks, detection limits were estimated to be about 1 ppm.

1. Methane
2. Ethane
3. Ethylene
4. Propane (988 ppm)
5. Cyclopropane (100 ppm)
6. Propylene
7. Isobutane (129 ppm)
8. n-Butane
9. Propadiene (62 ppm)
10. Acetylene (48 ppm)
11. t-2-Butene
12. 1-Butene
13. Isobutylene
15. Isopentane
16. c-2-Butene
17. 1,3-Butadiene
18. Methylacetylene (100 ppm)

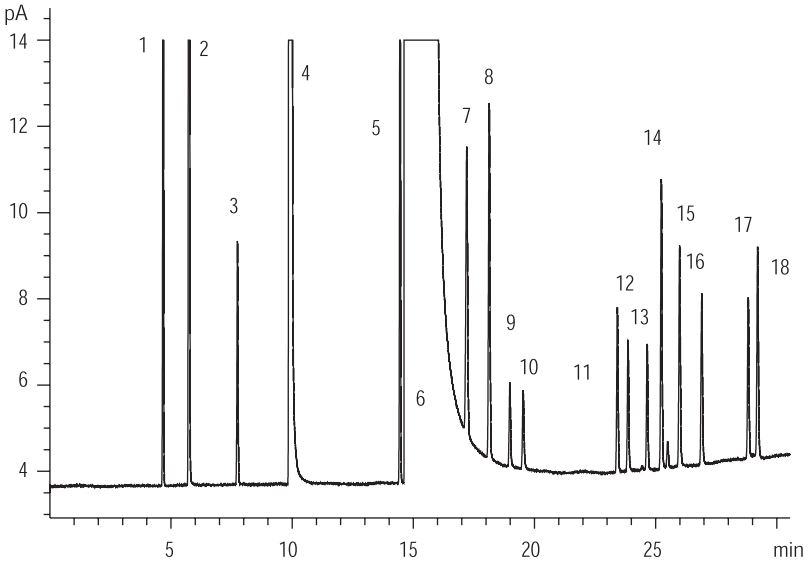

Figure 7. Chromatogram of propylene calibration blend containing higher levels of impurities.

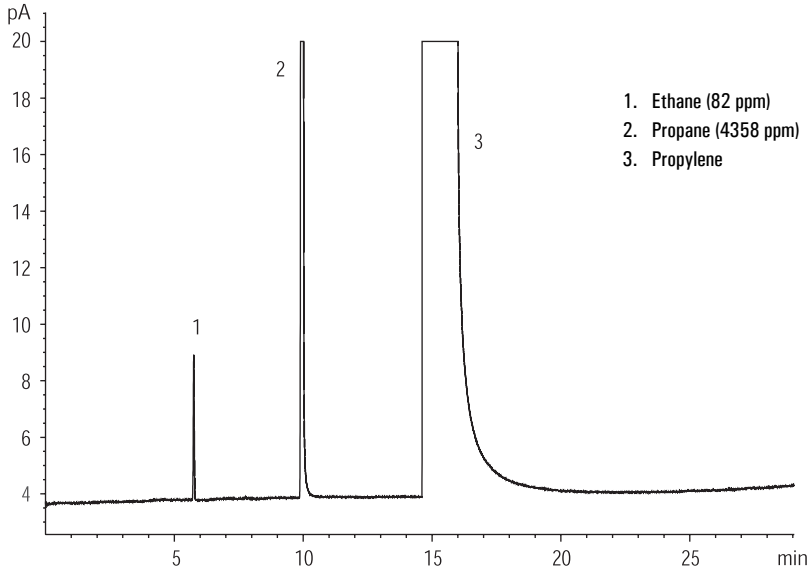

Figure 8. Chromatogram of process propylene sample.

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2. ASTM Method D 5273, “Standard Guide for the Analysis of Propylene Concentrates,” Annual Book of Standards, Volume 5, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. Proposed methods for hydrocarbon impurities in ethylene and propylene by gas chromatography are being investigated under ASTM committee D-2, subcommittee D.
4. “Optimized Determination of C_1–C_6 Impurities in Propylene and Ethylene Using HP-PLOT/Al_2O_3 Columns,” Agilent Technologies, Inc. Publication (43) 5062-8417E, March 1994.

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5965-7824E



Agilent Technologies
Innovating the HP Way

The Analysis of Trace Contaminants in High Purity Ethylene and Propylene Using GC/MS

Agilent Technologies/Wasson ECE Monomer Analyzer

Application

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Abstract

A new product (Application 460B-00) from Agilent Technologies/Wasson-ECE uses a 5973N GC/MSD (gas chromatograph/mass selective detector) for the determination of trace levels of impurities that are moderate-to-low carbon-content such as oxygenates, mercaptans, sulfides, arsine, and phosphine in ethylene and propylene. This work describes the performance of Application 460B-00 with respect to linearity, repeatability, and limits of detection (for most compounds, low parts-per-billion). Compared to determinations with GC/FID (flame ionization detection) or GC/TCD (thermal conductivity detection), the use of GC/MSD demonstrates comparable performance with respect to linearity and repeatability: for example, for mercaptans and sulfides (40–100 ppb) in the ethylene assay, correlation coefficients for calibration curves range from 0.992 to 1.000 and relative standard deviations range from 1.95% to 9.31% RSD. Compared to GC/FID for the range of contaminant analytes studied here, the sensitivity is increased 50-fold; compared to GC/TCD, the sensitivity is increased 5000-fold. While the sensitivity of MS detection is comparable to that of sulfur chemiluminescence detection for sulfur-containing compounds, MS has the same sensitivity for a broader range of compound types. Moreover, the use of MS detection provides specificity for positive identification of analytes. In Application 460B-00 the multipart assays are automated via "composite methods" [1]. The result is a tool that provides higher productivity and more key information about feedstock materials-both of which aid the polymer industry.

Introduction

For the polymer industry, the purity of ethylene and propylene monomer feedstocks is a high priority. Trace contaminants at the part-per-billion (ppb) concentration levels can affect yields dramatically by altering subsequent polymer properties and characteristics. Additionally, some trace impurities can irreversibly poison reactor catalysts. The competitive marketing strategies of monomer manufacturers include using new analytical technologies to guarantee lower and lower trace impurity levels.

Agilent Technologies/Wasson-ECE has focused on supplying integrated application products for the analysis of impurities in polymer feedstocks. One of the most recent products, Application 262-00, effectively combined the separate analyses for ethylene and propylene into a single analytical system [2].

Application 262-00 employs a gas chromatograph (GC) with two separate flame ionization detectors (FIDs), two capillary columns, appropriate valving, and analytical methods to quantitate very low levels of carbon monoxide and carbon dioxide by a methanizer FID and paraffins and olefins up to n-pentane by direct FID. Recently Agilent Technologies/ Wasson-ECE has expanded the capabilities of Application 262-00 by adding mass spectrometric (MS) detection in another product, Application 460B-00. This note describes applying GC/MS instrumentation and methods to analyze trace contaminants in polymer grade ethylene and propylene feedstocks.

Using GC/MS for Trace Contaminant Analyses

Mass Spectrometric Detection

Utilizing a mass spectrometer as the chromatographic detector provides significant benefits:

- Increased sensitivity
- Detection of analytes that do not produce a response with a FID
- Selectivity
- Positive identification through mass spectra

As the impact of trace levels of impurities becomes better understood, the need for increasing sensitivity in an analytical technique becomes more important. With flame ionization detection in Application 262-00, detection levels for carbon monoxide and carbon dioxide are on the order of 50 ppb while those for the paraffins and olefins up to n-pentane are about 1 ppm (parts-per-million). MS detection is equivalently sensitive for these compounds. However, MS detection offers gains in sensitivity for those compounds with little or no FID response factors, for example, oxygenates, sulfur-containing compounds, and compounds with no carbon-hydrogen bonds (hydrogen sulfide, carbonyl sulfide, arsine, phosphine). Moreover, the selectivity afforded by MS detection is important because it provides confirmation that an analyte in question is indeed present by examining its mass spectrum (scan mode) or monitoring of multiple, specific ions (selected ion monitoring - SIM mode).

The GC/MS methods described here address a range of analytes beyond the scope of Application 262-00.

Gas Chromatographic Separation

Even with the inherent strengths of a mass spectrometer, some chromatography must still be employed. The detector's selectivity is not absolute, especially when simultaneously detecting components at vastly different concentrations (ppb) versus bulk or percent levels). This means that the ppb contaminants must be reasonably separated from their ethylene or propylene matrix since coelution of a trace contaminant with the major matrix component yields a false positive with respect to the contaminant. Additionally, some of the analytes may interfere with each other by having the same molecular weight and/or fragment ions. Therefore, both chromatography and mass spectrometry are needed to provide identification.

For such a complex sample, no single gas chromatographic column provides adequate separation of the whole range of analytes and matrix components. Different selectivities of the capillary column stationary phases must be invoked to separate the various groups of very similar analytes. For this reason, Agilent Technologies/Wasson-ECE employs a multivalve, multicolumn approach to the chromatograph configuration.

Agilent Technologies/Wasson-ECE Application 460B-00

A new application product was developed to merge the advantages of MS detection with the necessary resolution afforded by appropriate chromatographic columns and automatic control of valved GC injections. The application includes multiple special inert capillary columns that do not irreversibly bind the analytes, addition of multiple valves, passivation of all components along the sample path (for example, valves, transfer lines), analytical methods for the GC/MS, and control software that coordinates the entire application.

In the multivalve, multicolumn approach, the sample (a gas or vaporized liquid) is purged through the injection valve. In this manner, the injection valve contains an aliquot of original sample, sized appropriately for the column to be used. The flexibility of programming both valve operation and chromatographic system pressures results in the sequential injection of the aliquot onto one of the multiple columns, the appropriate one being selected with a portion of the total analysis in mind. Each injection process defines a

method, resulting in a total of three sequential methods that comprise a full “composite method” to perform the selected assay. In executing a “composite method,” Agilent Technologies/ Wasson-ECE's Composite Analysis Control Software (CACS) automatically sequences the sampling of the three separate aliquots of a feedstock sample, applying the appropriate GC/MS methods to each, and produces a combined, final report.

A Modular Approach to the Analysis of Trace Contaminants: Building on Application 460B-00

Application 460B-00 is a building block in a modular approach to providing the degree of automation appropriate to the users' needs. As described just above, Application 460B-00 is suitable for use in the laboratory where an analyst connects one pressurized sample container at a time to the system. However, a sampling system that automatically and sequentially samples pressurized sample containers is available from Agilent Technologies/ Wasson-ECE; once the samples are installed, no user intervention is required.

Moreover, other products from Agilent Technologies/Wasson-ECE move Application 460B-00 from the laboratory to the process stream. In this configuration, there is a single sample source that is repetitively sampled. Since a “composite method” for Application 460B-00 takes about 1 hour per sample, knowledge of the bulk ethylene or propylene streams is available in a timely manner. This makes it possible to improve the quality of the product and reduce manufacturing costs associated with rework or waste disposal of large amounts of product that are out-of-specification due to contaminants in the bulk reactant.

Experimental

The instrumentation is outlined in Table 1. For the results presented here, an HP 5972 MSD was used. Table 2 outlines the impurities that were characterized for the evaluation of Application 460B-00. Note that the ethylene and propylene analyses each require three separate GC/MS methods; these methods are not the same.

Table 1. The Instrumentation and Control Software for the Ethylene and Propylene Analyses in the Agilent Technologies/Wasson-ECE Product, Application 460B-00

Mass spectrometer	5973 MSD and later models
Gas chromatograph	6890 GC
GC Valve configuration	Provided by Agilent Technologies /Wasson-ECE. Sample loops of 100 µL and 500 µL
Columns	Provided by Agilent Technologies/Wasson-ECE: Wasson Part No. KZA and Wasson Part No. KZB
Software	· G1701 DA · Composite Analysis Control Software from Agilent Technologies/Wasson-ECE
GC/MSD Methods	Provided by Agilent Technologies/Wasson-ECE

Table 2. Analytes Used to Characterize the Performance of Agilent Technologies/ Wasson-ECE Application 460B-00 for Impurities found in Ethylene

Component	Quant ion	Prepared analyte levels (ppb)
Methyl mercaptan	47	25, 35, 70, 115, 410
Ethyl mercaptan	62	15, 25, 55, 105, 315
Methyl-t-Butyl ether	73	10, 20, 38, 75, 223
Methanol	31	28, 55, 110, 205, 625
t-Butanol	59	12, 25, 45, 85, 265
Ethanol	31	18, 38, 75, 140, 433
isopropanol	45	15, 30, 78, 107, 335
sec-Butanol	45	15, 25, 45, 88, 270
1-Propanol	31	15, 30, 45, 110, 333
1-Butanol	56	12, 23, 58, 87, 270
Hydrogen sulfide	34	25, 100, 190, 575
Carbonyl sulfide*	60	15, 25, 75, 100, 310
Arsine	76	55, 115, 215, 440
Phosphine	34	50, 110, 205, 420

* For carbonyl sulfide in propylene, prepared analyte levels spanned 1–300 ppb

Standard mixtures of analytes in the bulk material were prepared by using permeation tubes from Agilent Technologies/Wasson-ECE; in this approach, concentration levels are determined by the flow rate of the bulk material. Mixtures of multiple trace analytes in the bulk material were prepared by plumbing multiple permeation tubes in series. The bulk ethylene was obtained from Alphagaz ("primary standard" grade). The analytes came from Chem Service.

Linearity for each analyte was determined in the usual manner (construction of calibration curves with the HP G1701 BA software) for the concentration levels noted in Table 2. For the repeatability studies, a single concentration level near the low end of the calibration range for each analyte was injected seven to eight times.

In general, detection limits (DLs) were determined in two ways. The first was to use the linearity and repeatability data to target the lowest concentrations at which the repeatability for five replicates would give 1% to 10% RSD (relative standard deviation). Samples at these concentrations were run in SIM mode (including automated peak detection and integration, and automated reporting) to provide actual minimum DLs.

The second was to run samples in SIM mode at concentrations estimated to yield responses having S/N (signal-to-noise) ratios of about 2.5. Integration was done manually, using the SIM mass chromatogram for each analyte.

Results

Figures 1 through 3 are examples taken from propylene and ethylene assays to show the type of output using the Agilent Technologies/Wasson-ECE product.

Tables 3 through 6 present the results of the experiments to characterize the performance of Application 460B-00 with respect to linearity, repeatability, and limits of detection (LOD).

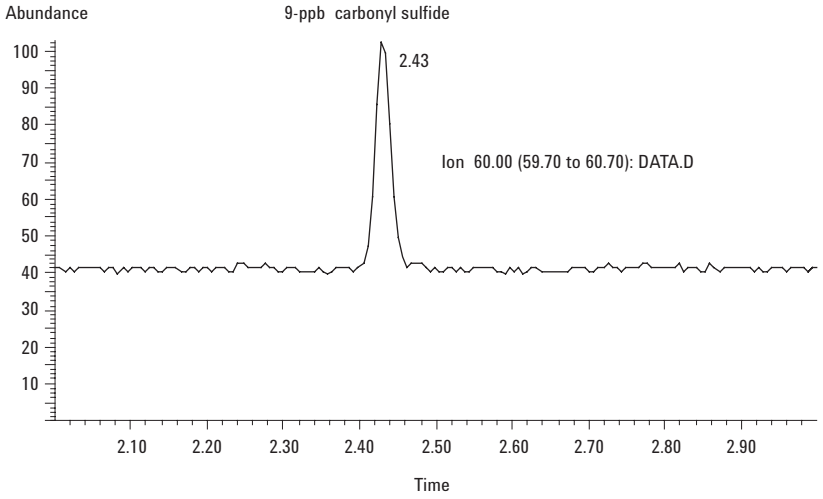

Figure 1. A section of chromatogram showing SIM detection for the determination of 9-ppb carbonyl sulfide in propylene at a customer's facility.

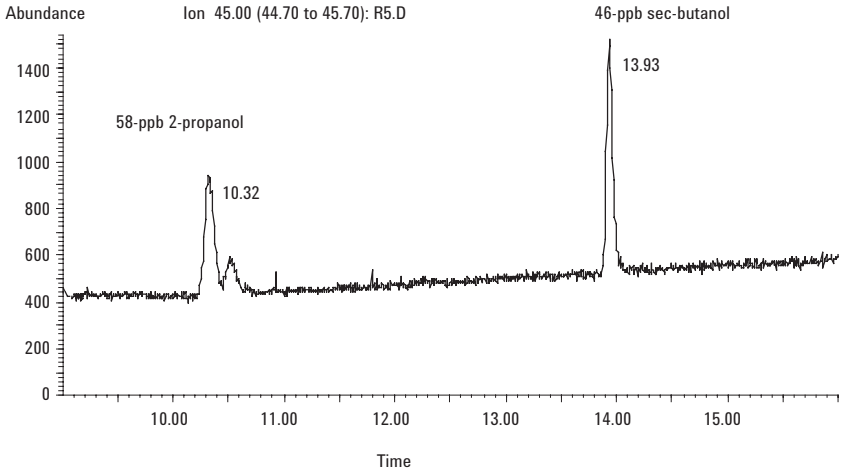

Figure 2. A section of chromatogram showing SIM detection for the determination of 58-ppb isopropanol and 46-ppb *sec*-Butanol in ethylene.

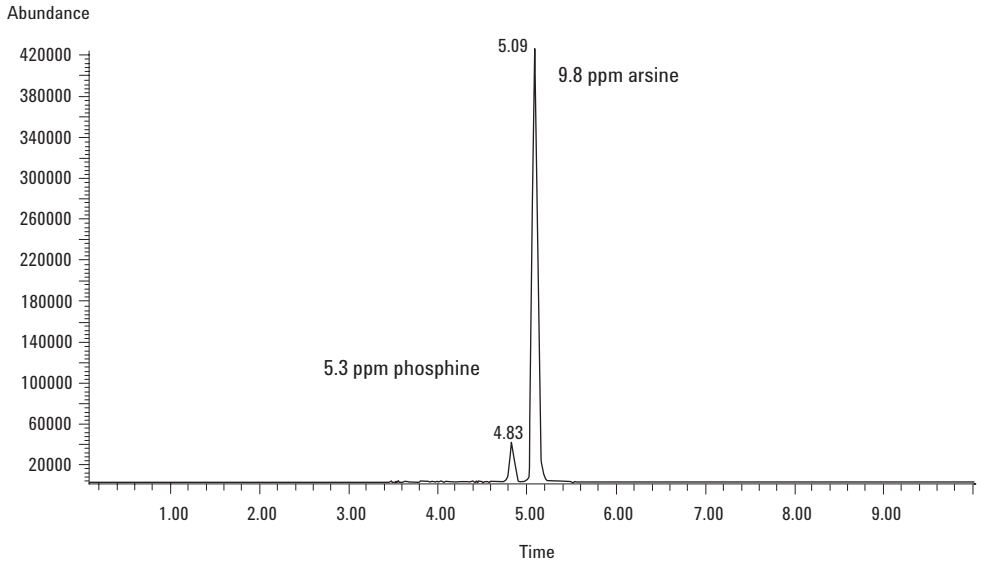

Figure 3. A section of chromatogram showing SIM detection for the determination of 240-ppb arsine in ethylene.

Table 3. Measures of Linearity for the Ethylene Impurity Analysis

	Concentration range	Number of calibration points	Correlation coefficient
Methyl mercaptan	25–410 ppb	5	0.999
Ethyl mercaptan	15–315 ppb	5	1.000
Methyl-t-Butyl-ether	10–223 ppb	5	1.000
Methanol	28–625 ppb	5	0.999
t-Butanol	12–265 ppb	5	1.000
Ethanol	18–433 ppb	5	0.999
isopropanol	15–335 ppb	5	1.000
sec-Butanol	15–270 ppb	5	1.000
1-Propanol	15–333 ppb	5	0.999
1-Butanol	12–270 ppb	5	0.998
Hydrogen sulfide	25–575 ppb	4	0.992
Carbonyl sulfide	15–310 ppb	5	1.000
Arsine	55–440 ppb	4	1.000
Phosphine	50–420 ppb	4	0.995

Table 4. Repeatability for Sulfur-Containing Compounds at ppb Concentrations Using the Agilent Technologies/Wasson-ECE Method for Determination of Ethylene Impurities

Sample number	Hydrogen sulfide	Carbonyl sulfide	Methyl mercaptan	Ethyl mercaptan
1	106.42	41.70	77.96	60.29
2	90.05	41.74	79.23	57.96
3	98.50	50.61	79.90	61.36
4	107.15	40.77	80.18	59.30
5	111.59	39.97	76.96	57.27
6	95.75	40.30	81.74	61.33
7	102.48	39.00	79.31	63.32
Average	101.71	42.01	79.33	60.12
Standard Deviation	7.44	3.91	1.55	2.11
% RSD	7.31	9.31	1.95	3.51

Table 5. Repeatability for Oxygenates at ppb Concentrations Using the Agilent Technologies/Wasson-ECE Method for Determination of Ethylene Impurities

Sample number	Methyl-t-Butyl ether	Methanol	t-Butanol	Isopropanol
1	48.25	160.91	54.62	73.03
2	46.33	141.46	51.91	65.61
3	48.58	79.02	49.19	61.98
4	48.38	122.15	48.99	62.01
5	44.91	98.72	51.29	70.76
6	46.52	126.15	53.50	67.15
7	48.92	127.03	54.32	66.80
8	45.35	127.09	52.54	64.90
Average	47.16	122.82	52.05	66.53
Standard Deviation	1.57	24.91	2.15	3.88
% RSD	3.33	20.29	4.12	5.83

Table 5 (continued). Repeatability for Oxygenates at ppb Concentrations Using the Agilent Technologies/Wasson-ECE Method for Determination of Ethylene Impurities

Sample number	Ethanol	*sec*-Butanol	1-Propanol	1-Butanol
1	86.10	52.38	63.47	58.22
2	81.44	54.94	67.02	60.33
3	82.82	53.65	62.64	59.24
4	75.90	52.05	67.34	54.88
5	95.45	51.00	68.12	59.47
6	80.51	54.19	66.98	59.89
7	85.32	54.20	65.37	58.13
8	84.86	53.50	71.87	55.85
Average	84.05	53.24	66.60	58.25
Standard Deviation	5.65	1.32	2.88	1.95
% RSD	6.73	2.47	4.32	3.35

Table 6. DLs for the Ethylene Impurity Analysis*

	Approach A** (ppb)	Approach B*** (ppb)
Methyl mercaptan	18	2
Ethyl mercaptan	14	3.5
Methyl-t-Butyl-ether	10	4
Methanol	28	20
t-Butanol	12	4
Ethanol	19	9
isopropanol	15	4
sec-Butanol	12	3
1-Propanol	15	4
1-Butanol	12	4
Hydrogen sulfide	26	10
Carbonyl sulfide	14†	10
Arsine	10	7
Phosphine	50	30

* All values are in mole ppb or mole ppm.

** The lower DL was determined by the ability of the established integration parameters to detect the peak and integrate the peak properly.

*** Peak height at 2.5 times the noise. This peak will typically be integrated manually. This value was extrapolated from lowest concentration analyzed for each component.

† The DL for carbonyl sulfide in propylene using Approach A was found to be 5 ppb.

Summary

The performance of Agilent Technologies/Wasson-ECE's Application 460B-00 was evaluated for linearity, repeatability, and LOD for trace contaminants in ethylene. The results show that impurities in ethylene can be precisely determined to the low ppb levels for compounds that are not amenable to either trace analysis by GC/FID (where the typical LOD for the cited compounds range from 50 ppb for carbon dioxide and carbon monoxide to ppm for paraffins and olefins) or by GC/TCD (where the sensitivity is 5000-fold lower).

Application 460B-00 can also be used to determine trace contaminants in propylene as demonstrated by the carbonyl sulfide in propylene results (Figure 1). Additionally, it should be applicable to other analytes that were not studied in this work: acetone, 4-methylcyclohexene, 4-ethyl-cyclohexane, and aromatics.

By having "composite methods" that automatically perform the appropriate sequences of sampling plus methods, manual intervention is minimized for each sample. In the laboratory, the operator needs only to install each pressurized sample source from one sample to another and start the composite method. A greater degree of automation with less intervention per sample is possible by adding an automatic sampler product.

The analysis times for the “composite method” are wellmatched to the needs of polymer manufacturers for feedstock assays. For example, the full composite method for the ethylene assay takes about 1 hour. Typically in an off-line analysis approach, manufacturing facilities obtain samples of the feedstock and submit them to the laboratory for analysis. The 1-hour analysis time is a fairly small part of the total turn-around-time (several hours) to get analytical information back to the production line.

By moving the Application 460B-00 online using other products from Agilent Technologies/ Wasson-ECE, the polymer manufacturer could analyze feedstock materials about once per hour to maintain maximum productivity and quality while minimizing loss of product and/or rework.

The Agilent Technologies/ Wasson-ECE product, Application 460B-00, provides reliable results in a timely manner for bulk ethylene and propylene feedstock fluids with a minimum of manual intervention for the determination of impurities that have minimal carbon content and/or are highly oxygenated.

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1. 460B-00
2. 262-00

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Printed in the USA
March 7, 2005
(23) 5966-3240E



Agilent Technologies

Dual-Channel Gas Chromatographic System for the Determination of Low-Level Sulfur in Hydrocarbon Gases

Application

Hydrocarbon Processing

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Abstract

A 6890N equipped with dual flame photometric detectors is described for the analysis of ppb level volatile sulfur compounds in a variety of hydrocarbons using thick film DB-1 and GS-GasPro columns. Enhanced performance flame photometric detectors are employed that can achieve detection of sulfur compounds below 20 ppb. Examples of arsine and phosphine analysis with the same hardware are also discussed.

Introduction

Gas chromatography with sulfur selective detection is finding widespread application in many segments of the petroleum, petrochemical, and specialty chemical industries. Demand for low-level sulfur detection will increase in the future in response to more stringent regulations and tighter quality control.

Sulfur compounds can be significant poisons for various catalytic processes involved in hydrocarbon conversion. Monitoring these low-level poisons can lead to considerable saving in terms of improved yields, increased catalyst lifetime, and higher quality products. In looking at the future of fuel cells, fuel contaminants can adversely affect performance of fuel cell systems and fuel processors that are powered by natural gas or other fossil fuels. Finally, environmental regulatory issues in certain regions will continue, necessitating the need to monitor fuel impurities.

A common problem with many gas chromatographic sulfur selective detectors is hydrocarbon interference, especially from co-elution. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene, or sulfur in natural gas [1, 2]. In most cases, an accurate determination of the sulfur compound is difficult or not possible even with highly selective sulfur detectors. However, the use of a dual-channel system employing two very different separation columns (in terms of selectivity) largely avoids the interference problem. The configuration is shown in Figure 1. Sulfur compounds that have a severe interference on one column are likely to be separated from that interference on the other column. By assuring that a given sulfur compound will be separated on at least one of the columns, the system can use a reliable, stable, and relatively inexpensive flame photometric detector (FPD) for detection. If the hydrocarbons can be chromatographically separated from the sulfur compounds of interest, enhanced FPDs can quantitate sulfur to less than 20 ppb.

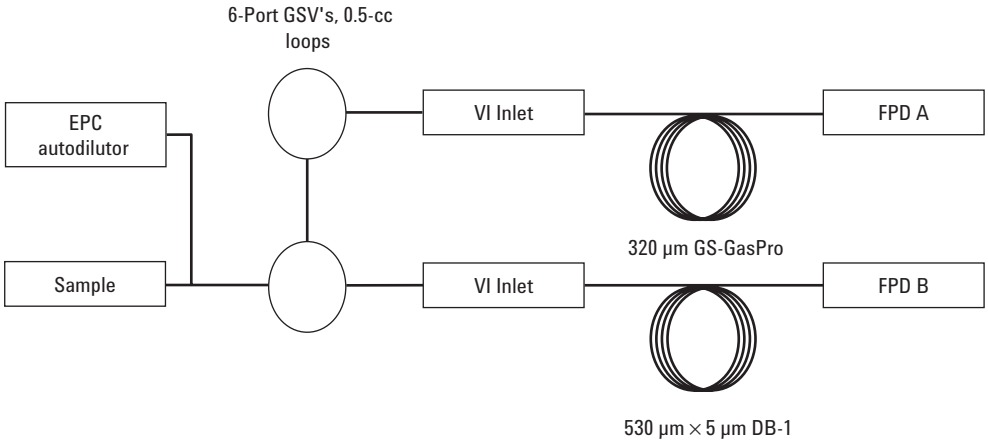

Figure 1. System configuration on the Agilent 6890N. Valves (plumbed in series) are Hastelloy C and all plumbing is Silcosteel® or Sulfinert™ treated.

Experimental

Selection of the appropriate capillary column is often key to the solution of a particular analysis problem, and this is especially true for this system. Four columns are employed (two for any given analysis) as described in Table 1.

Table 1. Recommended Column Combinations by Application

Applications	Column set
Natural gas, fuel cell gases	60 m × 530 µm × 5.0 µm DB-1
	30 m × 320 µm GS-GasPro
Ethylene, propylene, C4 streams	105 m × 530 µm × 5.0 µm DB-1
	60 m × 320 µm GS-GasPro

Recommended GC oven programs are 40 °C (5 min) to 290 °C (5 min) at 25 °C/min for natural gas, fuel cell gases and ethylene, and 35 °C (7 min) to 290 °C (5 min) at 20 °C/min for propylene. Somewhat lower detection limits can be achieved for sulfur in a propylene stream by employing cryo oven programs such as: –35 °C (7 min) to 290 °C (5 min) at 20 °C/min. Split ratios, as set in the GC method, vary from 0.5:1 to 2:1.

Each valve was interfaced to a specialized inert (Silcosteel treated) volatiles interface for accurate sample introduction at low split ratios into a capillary column. Due to the tendency for organosulfur compounds (especially H_2S) to adsorb to metal surfaces, great care must be used in selecting and constructing the chromatographic sample introduction system. The sample loop, tubing, and inlet are either Sulfinert or Silcosteel treated for inertness.

A factory modified FPD, with enhanced sensitivity, was used for each channel. The FPD is optimized for the analysis of trace sulfur gases, arsine, and phosphine in gaseous samples. See Table 2 for appropriate gas flow settings. These detectors achieve detection limits that are roughly four times better than standard. The sensitivity advantage is illustrated in Figure 2, where standard and modified FPDs are compared using a standard calibration blend. Minimum detection level (MDL) calculated on methyl mercaptan using linearized data and the 60 m DB-1 column is better than 15 ppb.

Table 2. FPD Gas Flow Settings

Analysis	Gas	Flow rate (mL/min)
Sulfur	Air	60
	Hydrogen	50
	Makeup	58
Arsine	Air	150
	Hydrogen	50
	Makeup	100
Phosphine	Air	110
	Hydrogen	150
	Makeup	58

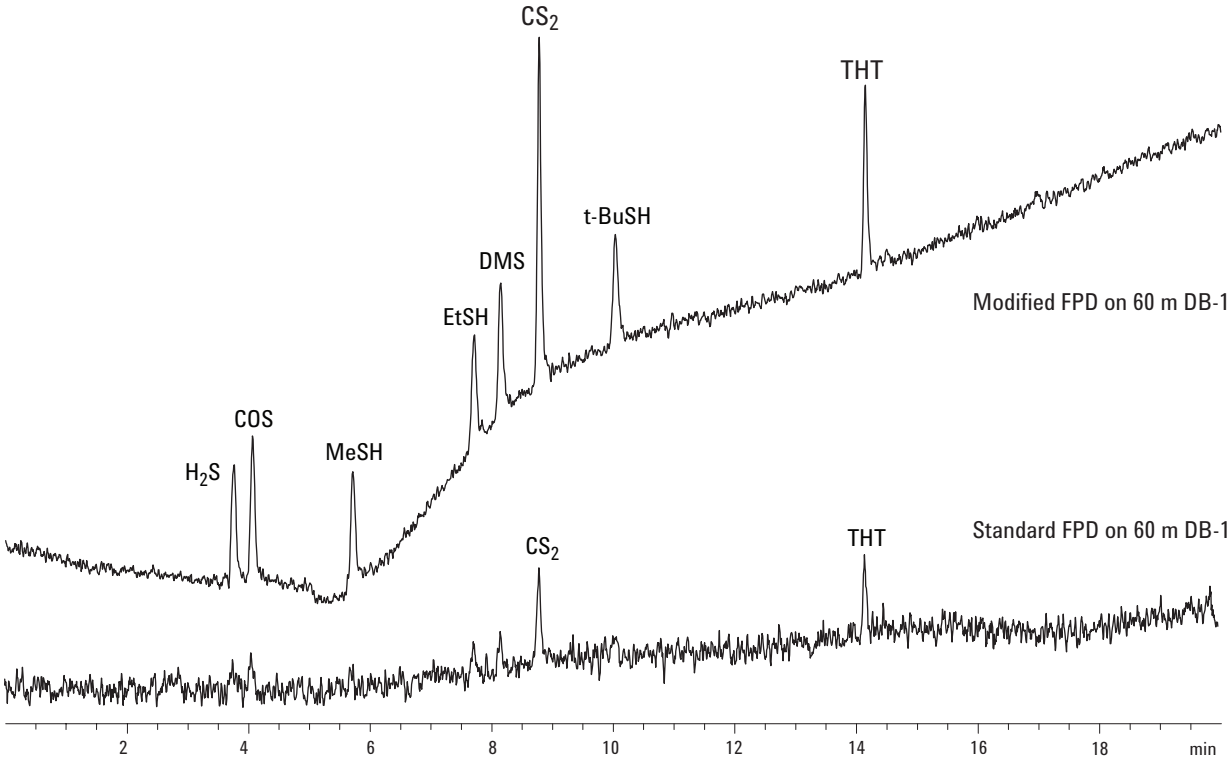

Figure 2. Sensitivity comparison of standard and enhanced FPDs. Concentrations are 33 ppb per component (v/v) in helium.

Due to the use of all available heated zones on the 6890N GC for either inlet or detector heating, the 6-port sample valves are not actively heated. This does not pose a problem for the light gaseous streams studied in this work. However, if desired, the valves can be heated by an auxiliary stand-alone temperature controller (Agilent model 19265B). The system is designed only for gaseous samples containing significant concentrations of hydrocarbons of C_6 or below.

Discussion

Channel 1 employs the GS-GasPro column, using a unique bonded PLOT technology, where COS is separated from C_2 and C_3 hydrocarbons, allowing measurement at trace levels. However, H_2S and the C_3s coelute. Channel 2 uses a thick film DB-1 column where H_2S is well separated from C_2s and C_3s, making low-level measurements of this sulfur impurity possible. COS and C_3s will coelute on this column. In summary, using a dual-column approach with the unique separation capabilities of GS-GasPro and thick film DB-1, both COS and H_2S can be measured in one chromatographic analysis at low ppb levels regardless of the concentrations of light hydrocarbons present in the sample. The elution order difference between the two columns is illustrated in Figure 3.

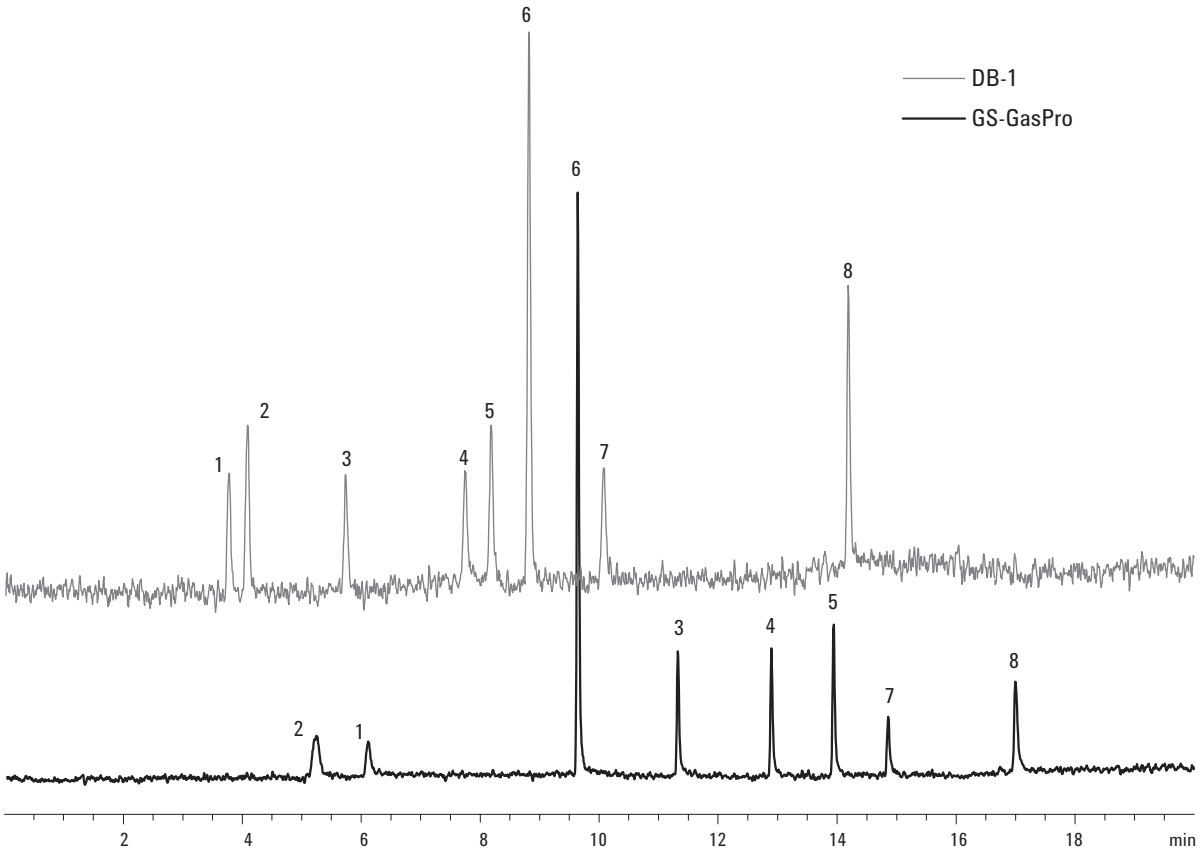

Figure 3. The dual-column advantage. Sulfur mix at 90 ppb per component in helium. 1. H_2S, 2. COS, 3. MeSH, 4. EtSH, 5. DMS, 6. CS_2, 7. t-BuSH, 8. THT.

Other potential interferences or coelutions between light sulfur compounds and hydrocarbons are avoided with this approach. A coeluting pair on one column will likely be separated on the other. Split ratios were set depending on the application from 0.5:1 to 2:1 in order to achieve the reported detection limits.

The sulfur calibration mix consisted of the following components at 5 ppm each: Hydrogen sulfide (H_2S), carbonyl sulfide (COS), methyl mercaptan (MeSH), ethyl mercaptan (EtSH), dimethyl sulfide, carbonyl sulfide (DMS), t-butyl mercaptan (t-BuSH), and tetrahydrothiophene (THT). The blend in helium was purchased from DCG Partnership, Pearland, TX. These compounds are representative of the most common light sulfur species encountered in gaseous fuels or petrochemical feedstocks.

Some adsorption of H_2S on the GS-GasPro column is possible. Priming the system a few times with a low ppm sulfur stream such as the calibration mix described here can largely eliminate the loss in sensitivity that can result from adsorption. This priming is usually only necessary for low ppb analyses where the active sites in the column could adsorb most of the sulfur present in the sample during an initial run.

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propane, liquidfied petroleum gas (LPG), propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases were mixed with the sulfur calibration standard using an Aux EPC module on the 6890N GC. Accurate concentrations from low ppb to ppm levels can be easily prepared by knowing the flow rates of the two streams as they mix in a Tee fitting prior to the gas sampling valves on the GC. This system and the hardware employed were described previously in detail [3].

Sulfur in Fuel Cell Gases, Natural Gas, and Proypylene

Figure 4 shows the chromatograms from the eight-component sulfur standard diluted with a fuel cell mix to 45 ppb (v/v) each component. The fuel cell

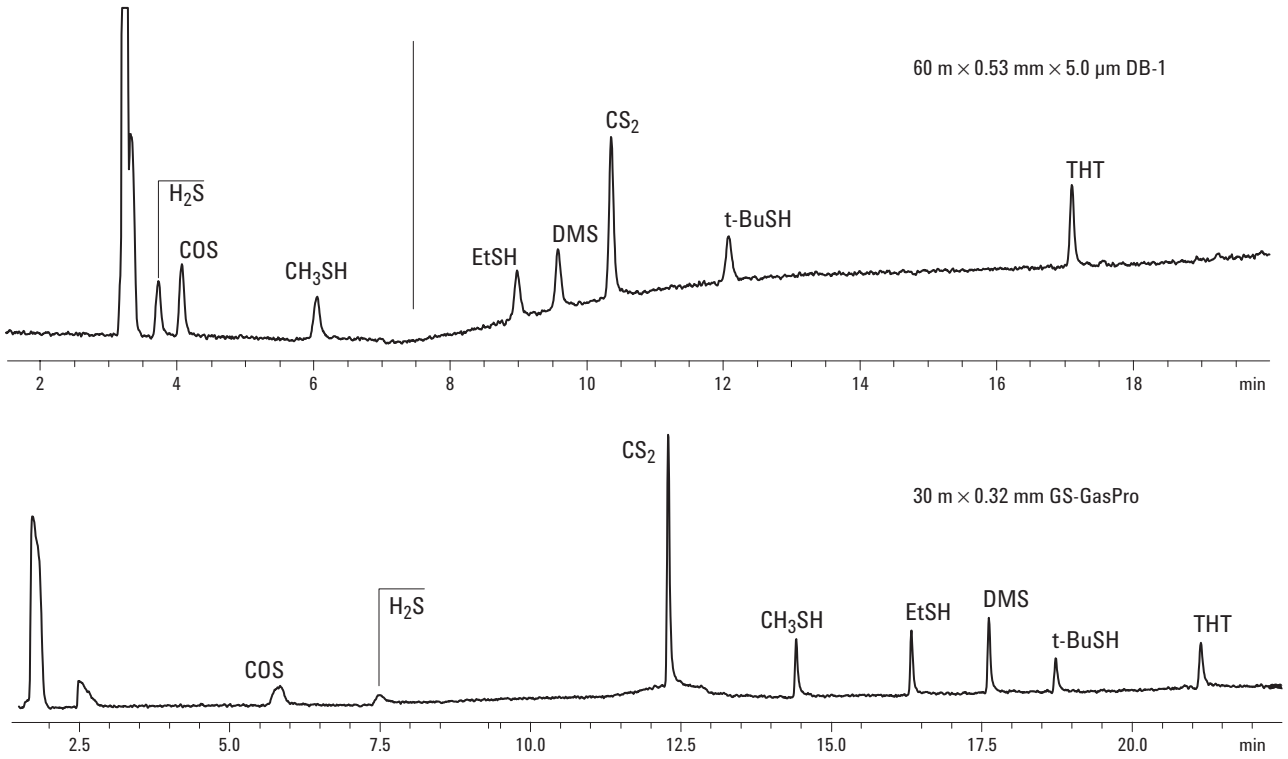

Figure 4. Simultaneous dual column analysis of fuel cell mix containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

mix is 50% hydrogen, 10% carbon dioxide, and 5% methane. This mix is often used to simulate the output stream of a natural gas reformer used as the feed to a fuel cell. This matrix is one of the easier ones because the large hydrocarbon (methane) elutes before all of the sulfurs on both columns. Note that elution order of the sulfurs is significantly different on the GS-GasPro column compared to the DB-1 (see Figure 3). All eight compounds are clearly detectable at 45 ppb.

Natural gas is a much more challenging matrix because of the high concentrations of several hydrocarbons. These interferences extend out into the retention time range of the sulfur compounds.

Figure 5 shows the chromatograms from the eight-component sulfur standard diluted with sulfur free natural gas to 45 ppb (v/v) each component. There are more peaks evident in these chromatograms than just the eight sulfur compounds. The additional peaks are interference responses from the large hydrocarbons in the natural gas.

In the DB-1 chromatogram, H_2S is clear but COS is lost to a severe overlap with a large C_3 peak. Ethyl mercaptan is also overlapped with n-pentane. On the GS-GasPro column, however, only the H_2S is occluded by interference. The COS and EtSH are free from interferences. With the dual-column approach, all eight compounds can be measured down to 45 ppb.

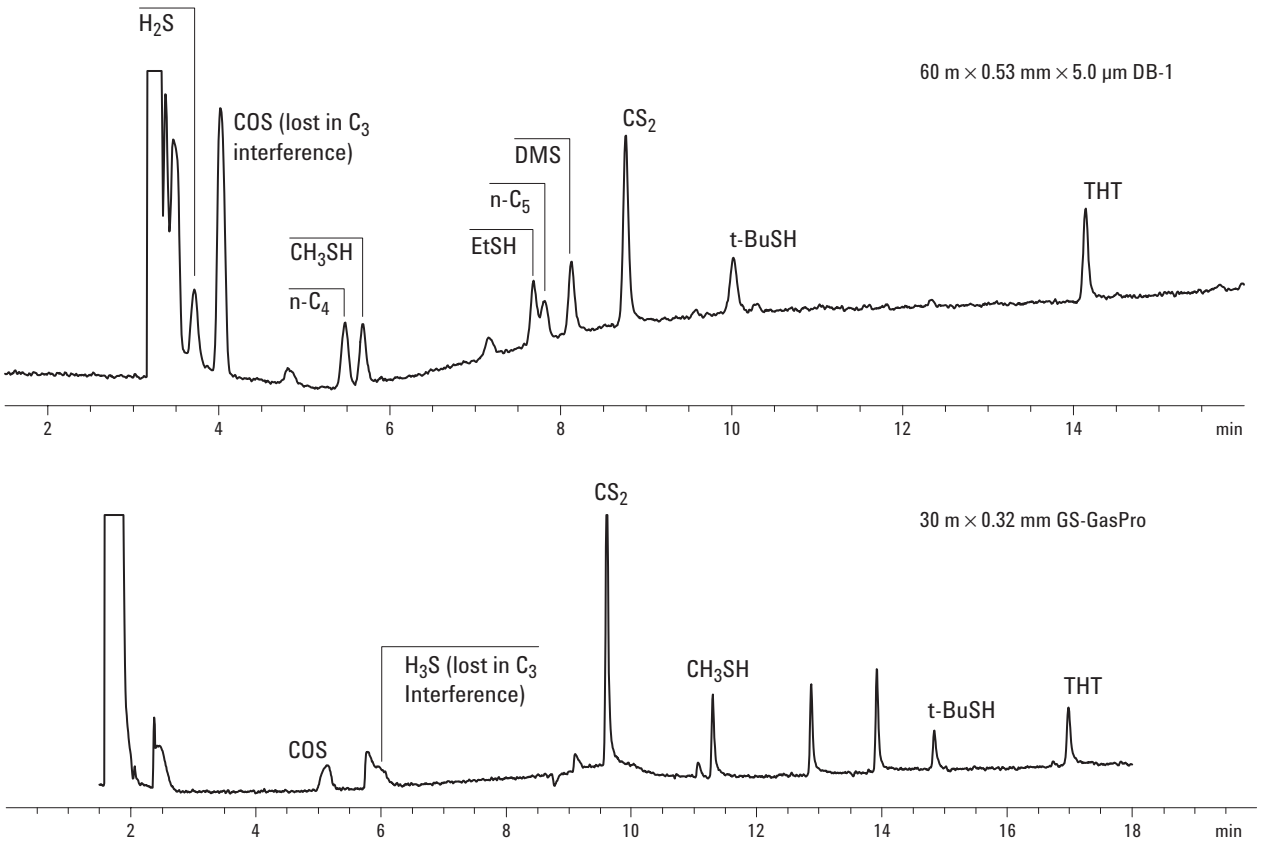

Figure 5. Natural gas blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 0.5:1.

Propylene monomer offers another interesting challenge. The huge C_3 peaks interfere with both the H_2S and COS on both columns used above. To address this, longer versions of the same columns were used (Table 1). The oven temperature and split ratio are also modified (see Experimental on page 2) to improve resolution of the H_2S and COS from the C_3s.

Figure 6 shows the chromatograms from the eight-component sulfur standard diluted with polymer-grade propylene to 45 ppb (v/v) each component. By using longer DB-1 and GS-GasPro columns, lower oven temperature, and a higher split ratio, the H_2S and COS can be measured with somewhat poorer detection limits.

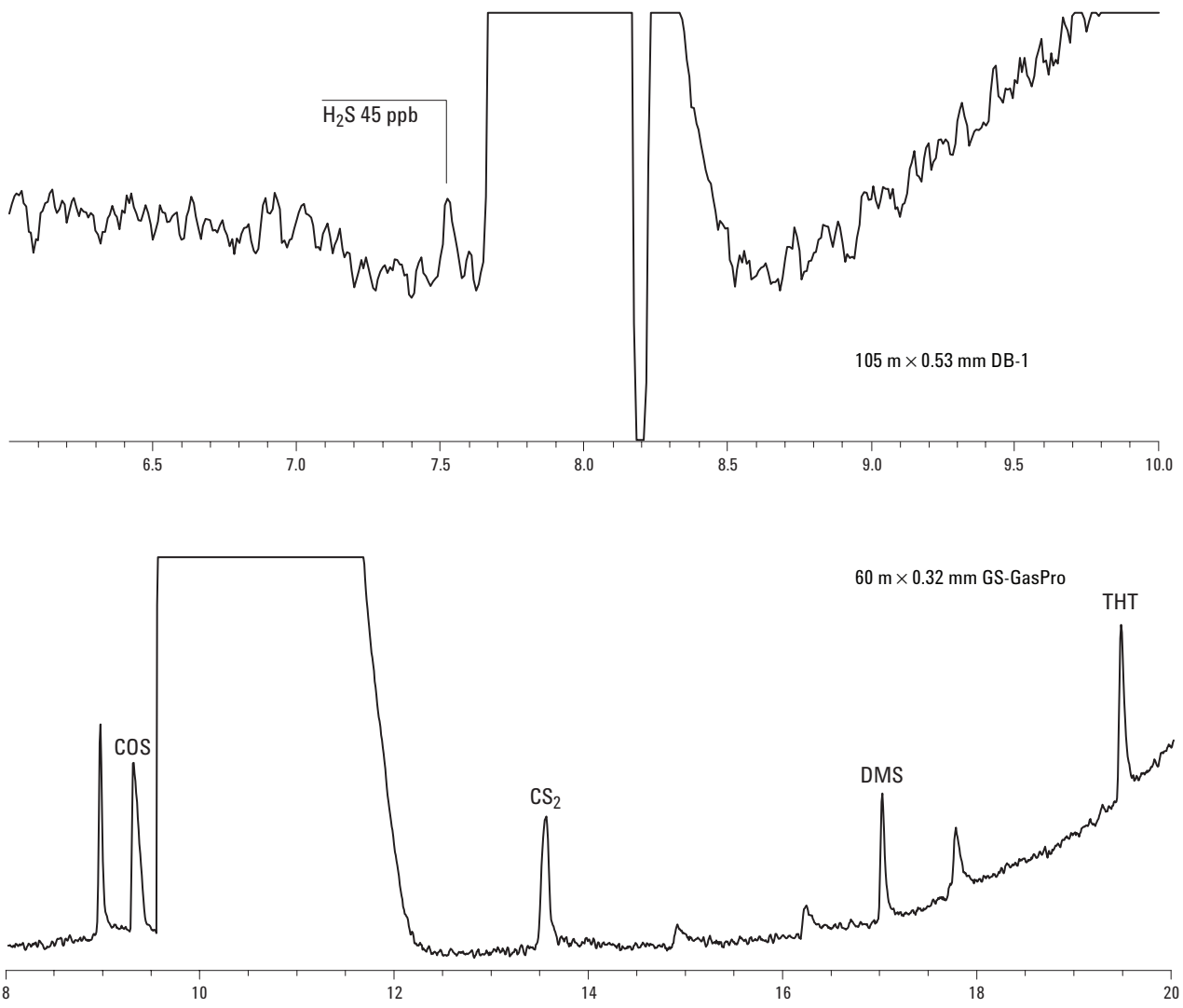

Figure 6. Polymer-grade propylene blend containing 45 ppb (v/v) each of the eight sulfur compounds. Split ratio is 2:1. Top chromatogram: 105 m × 530 µm DB-1 showing only H_2S, bottom: 60 m GS-GasPro.

Cryogenic oven temperatures were evaluated to see if the separation of H_2S and COS could be improved enough to allow use of the more sensitive 0.5:1 split ratio. The oven program tested was: -35 °C for 7 min, 20 °C/min to 300 °C, hold for 5 min. The separation was improved enough to allow the analysis of H_2S on the DB-1 column with the 0.5:1 split ratio, but COS was still occluded by the C_3s on the GS-GasPro. A DB-1 chromatogram illustrating the increased separation between H_2S and propylene is given in Figure 7.

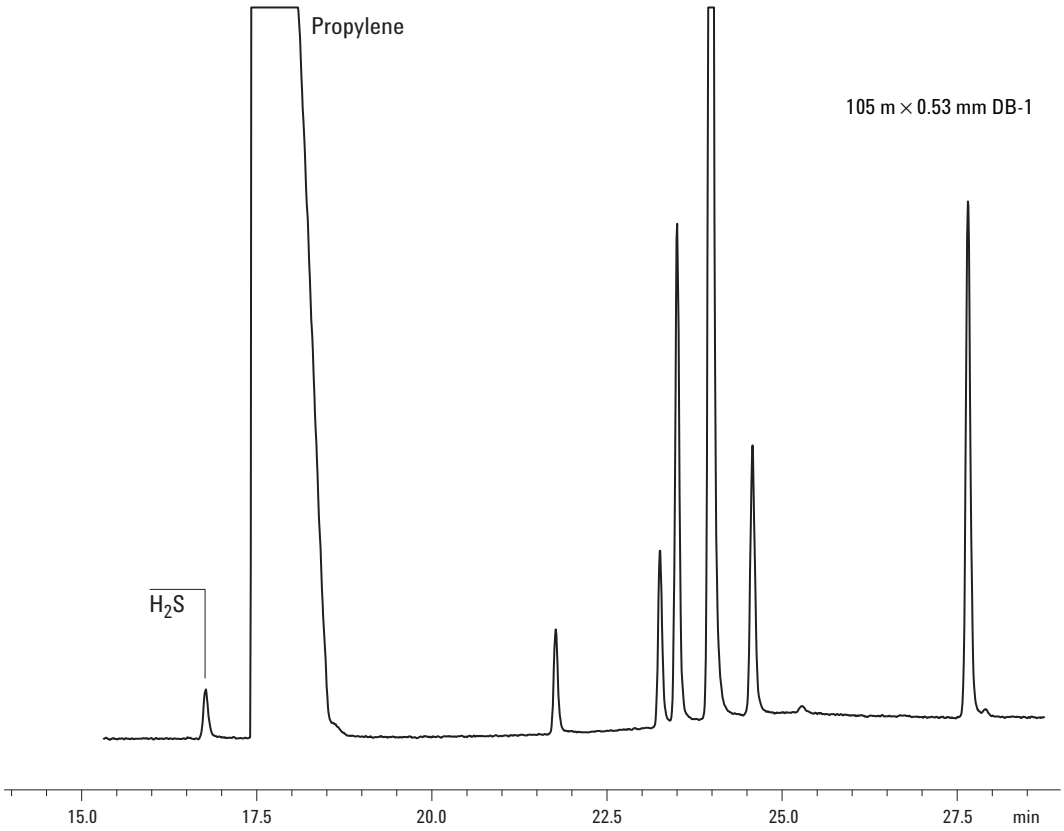

Figure 7. Use of cryogenic oven temperatures for analysis of H_2S (400 ppb) in propylene at 0.5:1 split.

Phosphorus and Arsenic on the Same System

One interesting characteristic of the modified FPD is that the filter used also passes the emissions for phosphorus and arsenic. This means that the same detectors can also be used to measure arsine and phosphine in polymer grade ethylene and propylene. A change of detector gas flows to that optimum for each element, followed by a rerun of the sample is all that is required. Since the 6890N detector flows are controlled by EPC, these reruns can be automated.

Figure 8 shows the chromatograms from an arsine and phosphine standard (DCG Partnership) diluted with polymer grade propylene to 36 ppb (v/v) each component. These are run under the same chromatographic conditions as in Figure 6, except that the FPD detector flows are set to those listed for phosphorus detection and the split ratio is back to 0.5:1. The detection limit under these conditions for phosphine in helium is under 5 ppb. If the detector flows are set to those listed for arsenic detection, the detection limit for arsine is about 60 ppb measured in helium. This system is well suited for gas analysis, however it is not really applicable to pesticide analysis due to the lack of selectivity between sulfur, phosphorus, and arsenic.

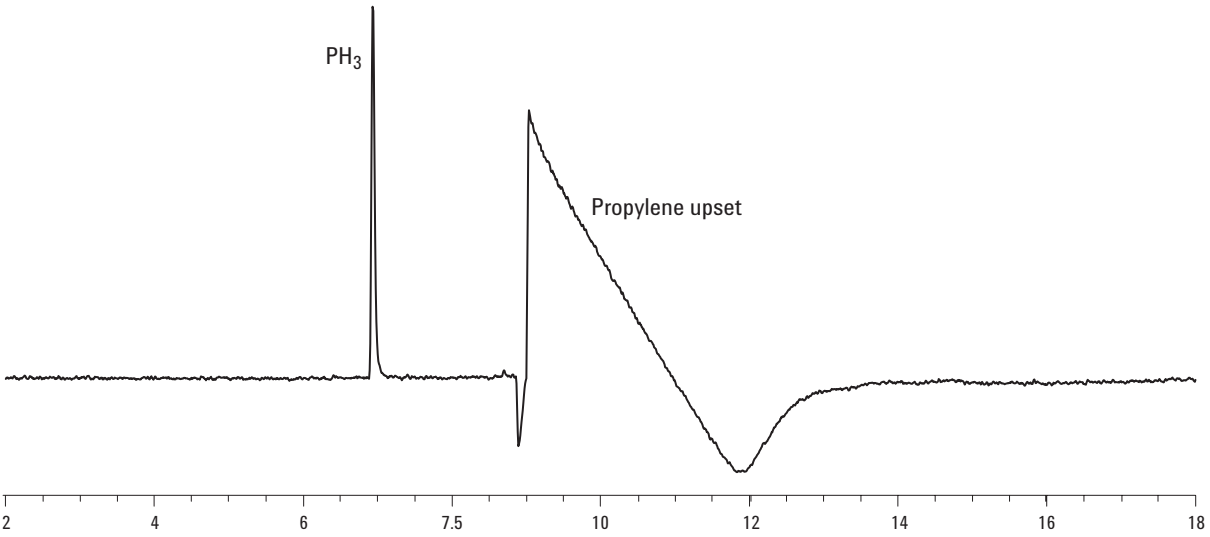

Figure 8. Polymer-grade propylene blend containing 36 ppb (v/v) each of arsine and phosphine. Split ratio is 0.5:1. Note longer 105 m DB-1 columns are used.

An example of arsine detection in propylene is shown in Figure 9.

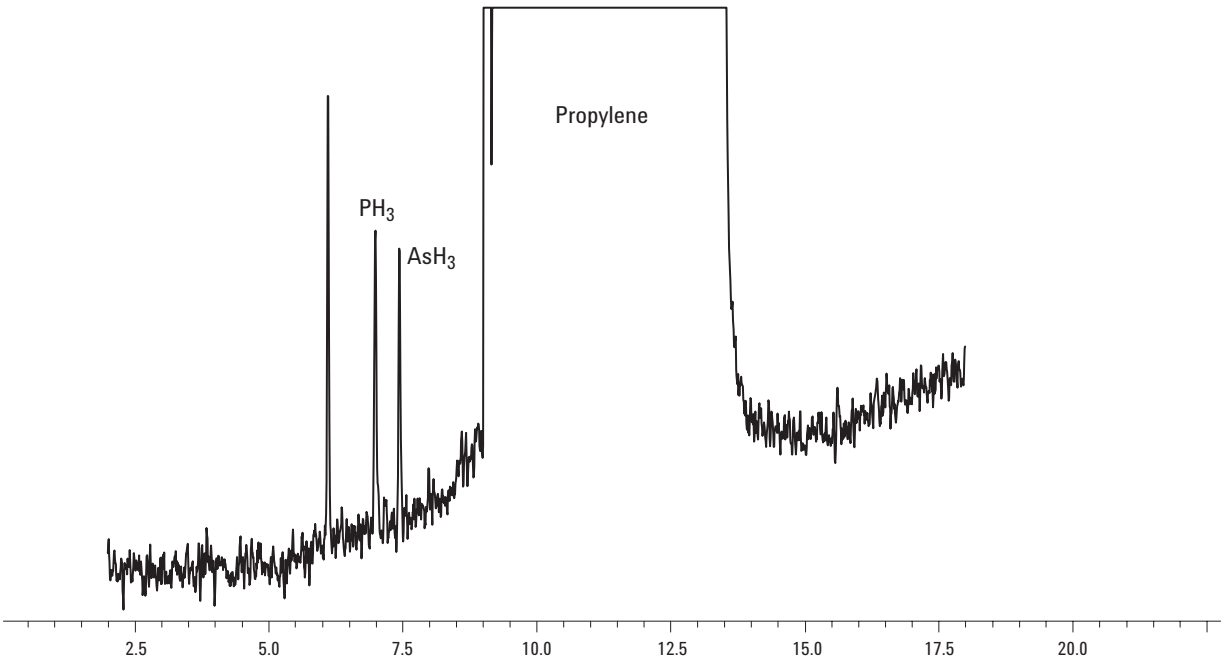

Figure 9. Arsine optimized FPD flows. H_2: 50 mL/min, air: 150 mL/min. 60 m × 0.32 mm GS-GasPro, 0.5 to 1 split. 90 ppb each of AsH_3 and PH_3.

How to Order and Configure a Dual-Channel FPD System

The Dual-Channel FPD System, including columns and valves, can be ordered as a special (SP-1) option on any new Agilent 6890N GC. This special also includes the enhanced performance FPD. Contact your local Agilent representative for more information.

Learn more about low-level sulfur detection from these application notes available from any Agilent sales office or Agilent's Web site at www.agilent.com/chem. Just click "Library" in the menu listing, and type "sulfur" in the keyword field.

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Printed in the USA
March 17, 2003
5988-8904EN



Agilent Technologies

Determination of Low Level Hydrocarbon Impurities in Propylene Using the Agilent 6820 Gas Chromatograph

Application

Petrochemical

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Abstract

A method for analyzing trace hydrocarbon impurities in propylene is described. The method employs an Agilent 6820 gas chromatography (GC) system configured with a gas sampling valve, split/splitless inlet, and flame ionization detector. The Agilent Cerity Networked Data System for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and data analysis. An Agilent HP-Al_2O_3 column was used for separation of the trace hydrocarbons. Impurity levels at 1 ppm were easily detected in propylene. This method does not determine all possible impurities such as CO, CO_2, H_2O, alcohols, nitrogen oxides, and carbonyl sulfide, or hydrocarbons larger than decane.

Introduction

High purity propylene is commonly used as the feedstock for production of polypropylene, and the quality of this monomer is critical to successful polymerization. The presence of trace amounts of certain hydrocarbon impurities can have deleterious effects on the catalyst. For example, acetylene can be adsorbed at the active center of the catalyst, resulting in catalyst deactivation. Dienes may reduce the rate of polymerization and adversely affect product quality. To maintain catalytic efficiency, most propylene processes require that alkyne and diene contaminants in the monomer be less than 10 ppm. The availability of a suitable method for the determination of impurities in propylene is critical to setting specifications, controlling internal quality, and doing development or research work.

Some propylene producers use their own standard method in which packed columns are used. It is difficult to detect trace level impurities by packed column. Presently, the American Society of Testing and Materials (ASTM) has published Method D2712 for the determination of trace hydrocarbon impurities in propylene streams [1]. In this method, an alumina porous layer open tubular (PLOT) column is used. The improved efficiency of the PLOT column provides better resolution and increases effective sensitivity.

Experimental

An Agilent 6820 GC system was used for this work. It was configured with a split/splitless capillary inlet and a flame ionization detector (FID). Gas samples were injected using an automatic gas sample valve that was heated to 80 °C. The sample loop volume was 0.25 mL. The gas sample valve was connected to the inlet using an aluminum-jacketed stainless steel tube that maintains the sample temperature during transfer from the sample loop. The configuration used for propylene analysis is shown in Figure 1 and the instrument conditions are given in Table 1.

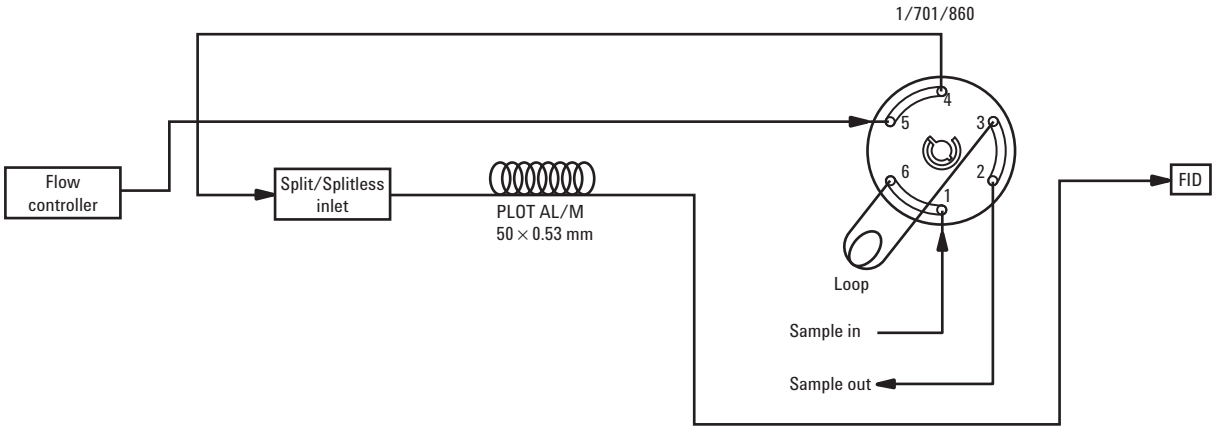

Figure 1. Configuration diagram.

An Agilent 50 m × 0.53 mm PLOT Al_2O_3 "M" deactivated column was used. The sample was run in the split mode using an Agilent split liner (Agilent part number 19251-60540).

The Agilent Cerity NDS for Chemical QA/QC was used for instrument control, data acquisition, and data analysis. Data was acquired at 20 Hz.

Table 1. Instrument Conditions

Split/Splitless inlet	175 °C, Split mode, with 15:1 and ~4:1 Split ratio
Valve	Gas sample valve, 6-Port, option 701
Valve temperature	80 °C
Sample loop	0.25 mL
Column flow (He)	4 mL/min
Column	PLOT Al_2O_3 "M" 50 m × 0.53 mm × 0.25 µm (p/n: 19095P-M25)
Oven	40 °C for 2 min, 4 °C/min to 190 °C for 5 min
Detector	FID, 300 °C
H_2	35 mL/min
Air	350 mL/min
Makeup gas (N_2)	22 mL/min

A propylene standard mix (DCG Partnership I, LTD., Pearland, TX 77581) consisting of the components listed in Table 2 at the certified concentrations shown (mole fraction) was used.

Table 2. Propylene Sample Mix Component Concentrations

Compound	Concentrations (ppm)	Compound	Concentrations (ppm)
1. Methane	10	10 Acetylene	9.8
2. Ethane	27	11. *trans*-2-Butene	9.92
3. Ethylene	10	12. 1-Butene	9.89
4. Propane	3526	13. neo-Pentane	9.86
5. Cyclopropane	10	14. *iso*-Butylene	9.87
6. Propylene	Balance gas	15. *iso*-Pentane	9.83
7. *iso*-Butane	9.94	16. *cis*-2-Butene	9.91
8. n-Butane	9.85	17. n-Pentane	9.86
9. Propadiene	9.84	18. 1,3-Butadiene	9.96

A dynamic blending system (Figure 2) was used to quantitatively dilute the sample with helium.

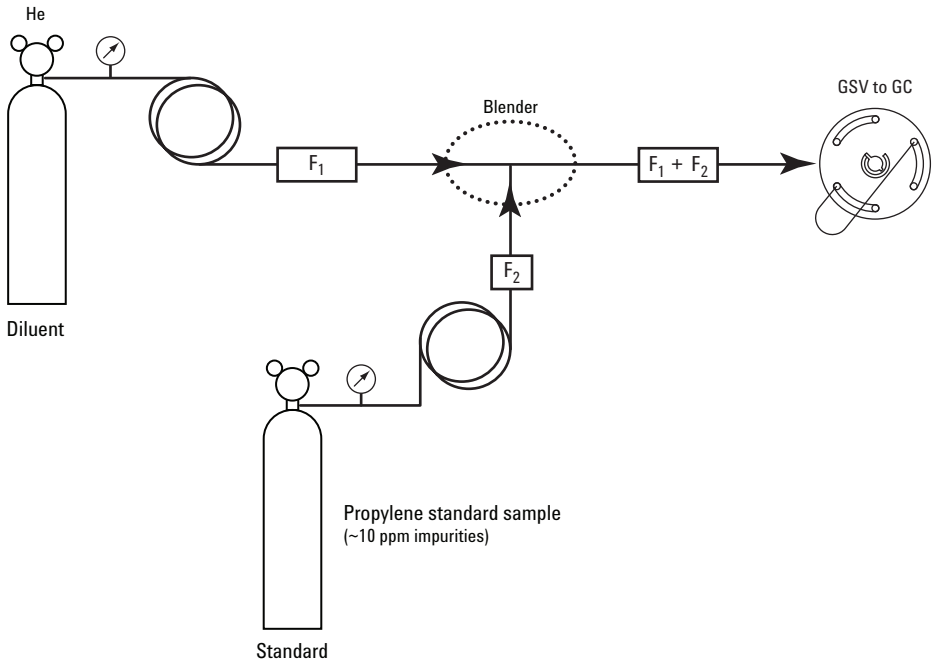

Figure 2. Dynamic blending scheme.

Diluted standard blend concentration is calculated by the following formula:

$C = C_o * F_2/(F_1+F_2)$

Where:
C: is diluted component concentration in ppm
C_o: original component concentration in standard blend in ppm
F_1: helium flow (mL/min)
F_2: propylene standard blend flow (mL/min)

Results and Discussion

Repeatability of 10 ppm Level Impurities in Propylene Analyses

Figure 3 shows the chromatogram from the undiluted sample. The PLOT AL_2O_3 column provides excellent separation for the C1 through C5 isomers [2]. The concentrations of most components are about 10 ppm. These trace level hydrocarbon impurities have a good FID response and are easily detected with baseline separation for most. Because the concentration of propylene is very high, some of the impurities such as *iso*-butane, n-butane, propadiene, and acetylene appear on the tail of the propylene peak. Even so, the Agilent 6820 GC system demonstrated very good repeatability, as shown in Table 3.

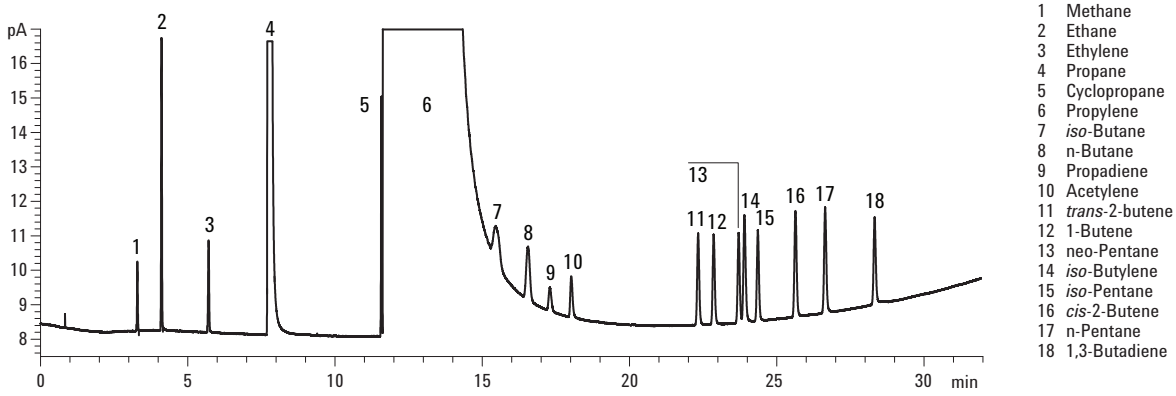

Figure 3. Propylene standard mix. Concentrations are given in Table 2. Split ratio: 15:1.

Table 3. System Repeatability of Three Propylene Standard Runs

Component	Amt1 (ppm)	Amt2 (ppm)	Amt3 (ppm)	Avg. (ppm)	RSD (%)
Methane	9.96	9.91	10.13	10.00	1.17
Ethane	26.95	26.78	27.27	27.00	0.92
Ethylene	10.21	9.90	9.90	10.00	1.76
Propane	3522	3503	3553	3526	0.73
Cyclopropane	9.99	9.95	10.07	10.00	0.61
Propylene	995642	988398	1004994	996344	0.84
iso-Butane	10.02	9.77	10.04	9.94	1.54
n-Butane	9.75	9.69	10.12	9.85	2.40
Propadiene	9.71	9.91	9.91	9.84	1.19
Acetylene	9.71	9.88	9.82	9.80	0.90
t-2-Butane	9.91	9.84	10.01	9.92	0.89
1-Butene	9.89	9.80	9.98	9.89	0.95
neo-Pentane	9.86	9.76	9.96	9.86	1.03
iso-Butylene	9.88	9.76	9.98	9.87	1.12
iso-Pentane	9.80	9.76	9.94	9.83	0.95
c-2-Butane	9.90	9.84	9.99	9.91	0.72
n-Pentane	9.84	9.76	9.98	9.86	1.16
1,3-Butadiene	9.97	9.87	10.04	9.96	0.85

Full Dynamic Range Data

One of the advantages of the Agilent 6820 GC system is its ability to obtain full dynamic range data. The signal "range" setting is not required because the Cerity/ChemStation uses digital data that goes from the noise level all the way to 100% samples. Without this feature, the propylene peak would be flat at the top as soon as the range was exceeded, making accurate integration impossible. In many cases without digital signal processing, users would have to run the sample at two different ranges in order to quantitate both large and small peaks. The Agilent GC system with Cerity/ChemStation can simultaneously acquire both large and small peaks in one run without setting different ranges. This feature helps quantitate 100% and ppm compounds at the same time. Figures 4 and 5 separately illustrate scaling the small peaks and one large peak to demonstrate acquisition of ppm level peaks and high percent level peaks.

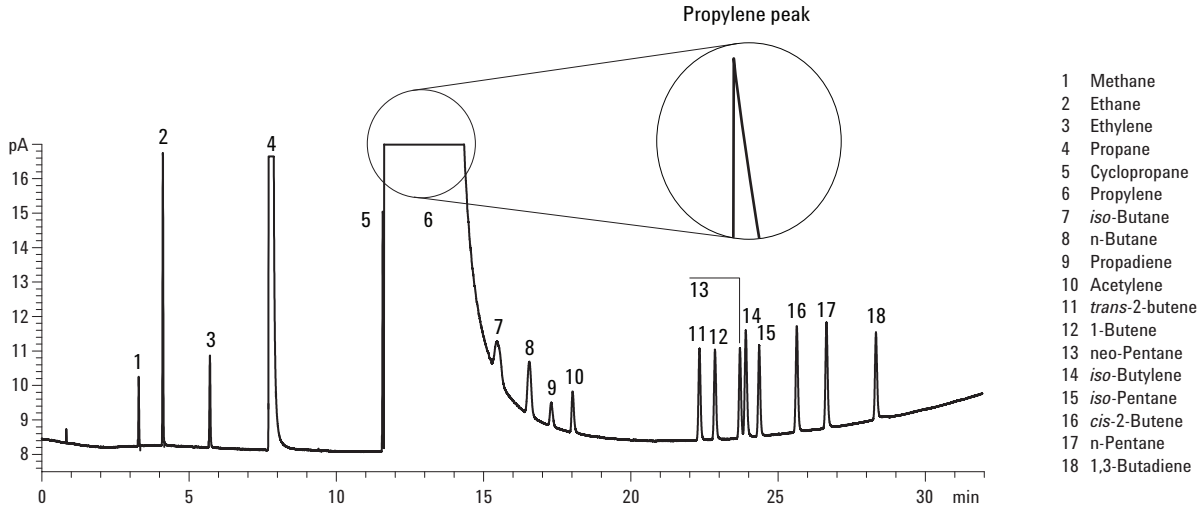

Figure 4. Propylene standard mix shown on small scale. Propylene peak looks flat due to graphic scaling.

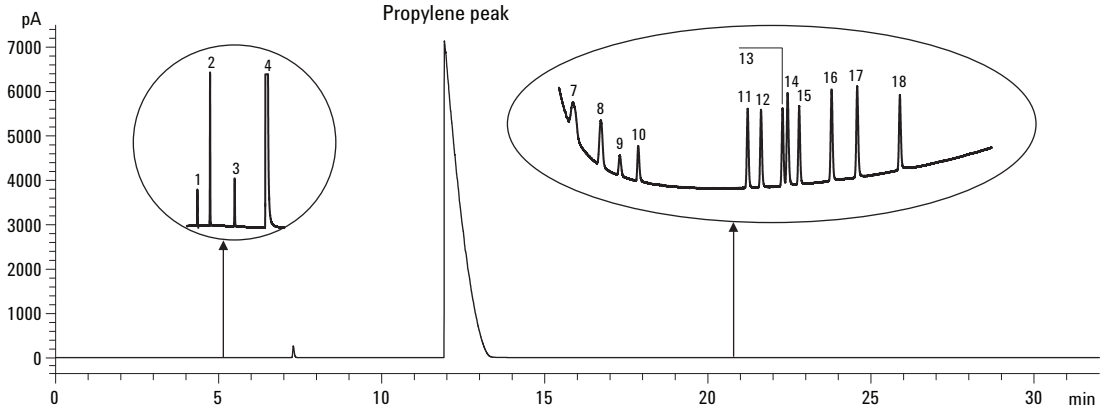

Figure 5. Propylene standard mix shown on high scale. Zooming in shows good resolution, identification, and integration.

Sensitive to 1 ppm Impurities

Figure 6 illustrates the chromatogram of less than 1 ppm impurities in propylene. The injected sample was prepared by a 10X dilution of the standard mix sample using helium; the impurities level decreased to 1 ppm as well as the 10:1 dilution of the propylene peak. In this analysis, the method was modified to use a split ratio of 4:1 instead of 15:1 in order to achieve the 1 ppm impurities detection. The sample presented in Figure 6 shows *iso*-butane, n-butane, propadiene, and acetylene clearly detected on the tail of the propylene peak. Other impurities show baseline separation with excellent signal to noise as well. This demonstrates the performance of the Agilent 6820 GC for sensitive and quantitative detection of 1 ppm hydrocarbon impurities in propylene.

Conclusions

The Agilent 6820 configured with a 6-port gas sampling valve interfaced directly to a split/splitless inlet was used to analyze trace hydrocarbon impurities in propylene with FID. Impurities below the 10 ppm mole % level can be easily quantitated. This system was able to detect 1 ppm level hydrocarbon impurities with excellent signal to noise. The Agilent 6820 system with Cerity can simultaneously acquire and quantitate both large concentrations (99 + mole %) and trace (low ppm) levels in a single run due to the use of a full dynamic range digital signal path. Manual range changes are not required. The feature of full dynamic range allows for accurate quantitation of near 100% propylene and ppm level compounds in one analysis. The system is simple and convenient to set up and use for routine QA/QC labs in the petrochemical and chemical industries.

References

1. ASTM Method D2712, “Standard Test Method for Hydrocarbon Traces in Propylene Concentrates By Gas Chromatography”.
2. Roger Firor, “Trace Level Hydrocarbon Impurities in Ethylene and Propylene,” Agilent Technologies, publication 5965-7824E www.agilent.com/chem

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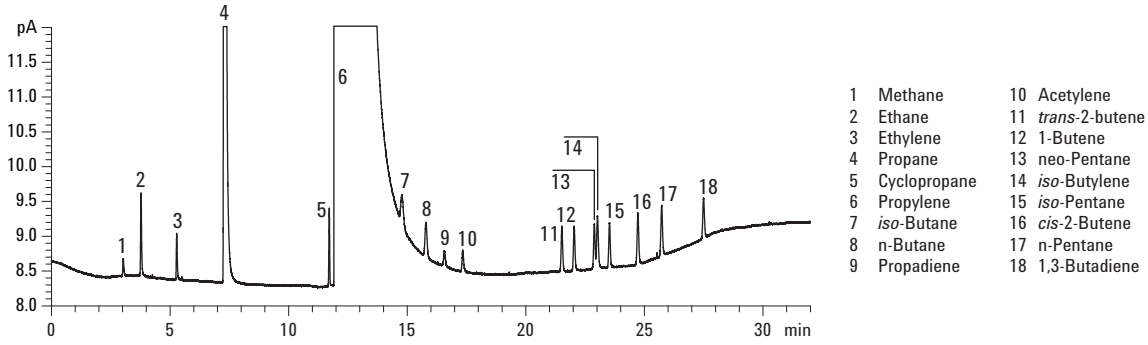

Figure 6. One ppm level impurities in the propylene standard mix.

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Printed in the USA
May 7, 2003
5988-9258EN

Agilent Technologies

Determination of Low Level Hydrocarbon Impurities in Ethylene Using the Agilent 6820 Gas Chromatograph

Application

Petrochemical

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Abstract

A method for analyzing trace hydrocarbon impurities in ethylene is described. The method employs an Agilent 6820 gas chromatography (GC) system configured with a gas sampling valve, split/splitless inlet, and flame ionization detector. The Agilent Cerity Networked Data System for Chemical QA/QC was used to control the 6820 GC and to provide data acquisition and data analysis. An Agilent PLOT Al_2O_3 megabore column was used for separation of the trace hydrocarbons. Impurity levels at about 15 ppm were easily detected in ethylene. This method does not determine all possible impurities such as CO, CO_2, H_2O, alcohols, nitrogen oxides and carbonyl sulfide, nor hydrocarbons larger than decane.

Introduction

High purity ethylene is required as a feedstock for several manufacturing processes. The presence of trace amounts of certain hydrocarbon impurities can have deleterious effects on the catalysts used for conversion. For example, acetylene can be adsorbed at the active sites of the catalyst, resulting in catalyst deactivation. Dienes may reduce the rate of polymerization and adversely affect product quality. The availability of a suitable method for the determination of impurities in ethylene is critical to setting specifications, controlling internal quality, and performing development or research work.

It is difficult to detect trace level impurities by packed column. Presently, the American Society of Testing and Materials (ASTM) has published Method D6159 for the determination of trace hydrocarbon impurities in ethylene streams [1]. In this method, both an alumina porous layer open tubular (PLOT) column and a methyl silicone megabore column are used to improve the separation of methyl acetylene, *iso*-pentane, and n-pentane. In this application, an Agilent 50 m × 0.53 mm PLOT Al_2O_3 "M" deactivated column is used to provide resolution and effective sensitivity.

Experimental

An Agilent 6820 GC system was used for this work. Configured with a split/splitless capillary inlet and a flame ionization detector (FID), gas samples were injected using an automatic gas sample valve heated to 80 °C. The sample loop volume was 0.25 mL. The gas sample valve was connected to the inlet using an aluminum-jacketed stainless steel tube that maintains the sample temperature during transfer from the sample loop. The configuration used for ethylene analysis is shown in Figure 1.

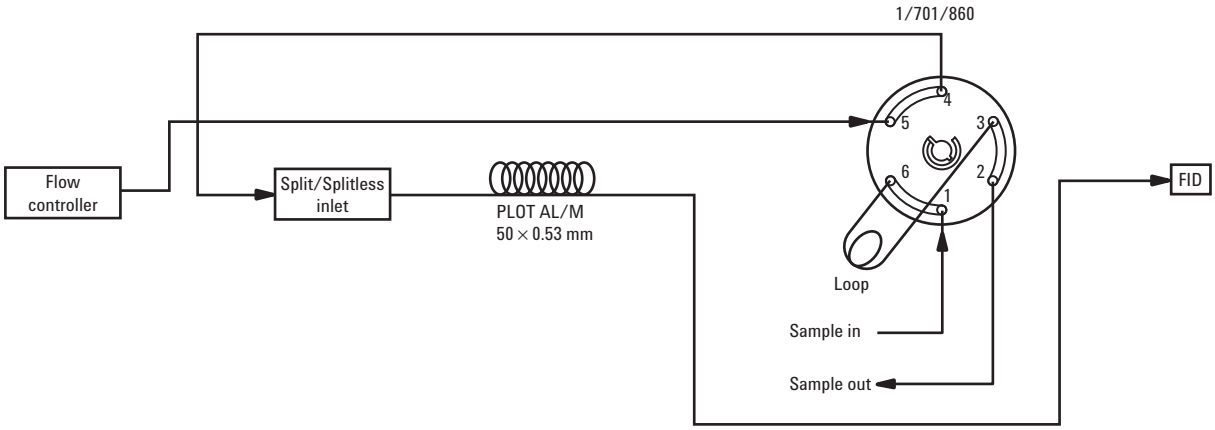

Figure 1. Configuration diagram.

An Agilent 50 m × 0.53 mm PLOT Al_2O_3 "M" deactivated column was used. The sample was run in the split mode using an Agilent split liner (Agilent part number 19251-60540) and an Agilent advanced green septum (Agilent part number 5183-4759).

The Agilent Cerity NDS for Chemical QA/QC was used for instrument control, data acquisition, and data analysis. Data was acquired at 20 Hz.

Table 1. Instrument Conditions

Split/Splitless inlet	175 °C, split mode, with 10:1 Split ratio
Valve	Gas sample valve, 6-Port, option 701
Valve temperature	80 °C
Sample loop	0.25 mL
Column flow (He)	6 mL/min
Column	PLOT Al_2O_3 "M" 50 m × 0.53 mm × 0.25 µm (p/n: 19095P-M25)
Oven	35 °C (2 min) with 4 °C/min to 140 °C (5 min)
Detector	FID, 300 °C
H_2	35 mL/min
Air	350 mL/min
Makeup gas (N_2)	19 mL/min

An ethylene standard mix (DCG Partnership I, LTD., Pearland, TX 77581) was used, consisting of the components listed in Table 2 at the certified concentrations shown (mole fraction).

Table 2. Ethylene Sample Mix Components

Compound	Concentrations (ppm)	Compound	Concentrations (ppm)
1. Methane	998	9. *trans*-2-butene	997
2. Ethane	981	10. Butene-1	1000
3. Propane	999	11. *iso*-Butylene	983
4. Propylene	1001	12. c*is*-2-Butene	986
5. Acetylene	981	13. Propyne	1006
6. *iso*-Butane	978	14. 1,3-Butadiene	1003
7. Allene	1001	Ethylene	Balance
8. n-Butane	1000		

A dynamic blending system (Figure 2) was used to quantitatively dilute the sample with helium.

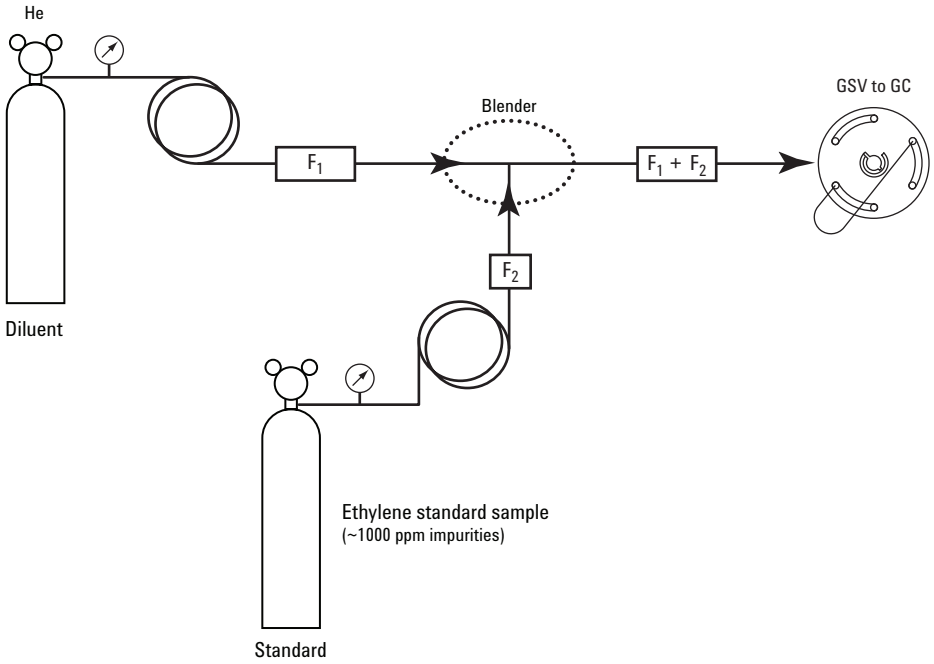

Figure 2. Dynamic blending scheme.

Diluted standard blend concentration is calculated by the following formula:

$C = C_o * F_2/(F_1+F_2)$

Where:
C: is diluted component concentration in ppm
C_o: original component concentration in standard blend in ppm
F_1: helium flow (mL/min)
F_2: propylene standard blend flow (mL/min)

Results and Discussion

Repeatability of Trace Level Impurities in Ethylene Analyses

Figure 3 shows the chromatogram from the blended sample. The PLOT Al_2O_3 column exhibits excellent separation for the C1 through C4 isomers [2]. The concentrations of most components are about 16 ppm. These trace level hydrocarbon impurities have a good FID response and are easily detected with baseline separation for most. In this method, the hydrocarbon impurities are determined and the total impurities are used to determine the ethylene content. Even in the trace level impurities, the Agilent 6820 GC system demonstrated good repeatability, as shown in Table 3.

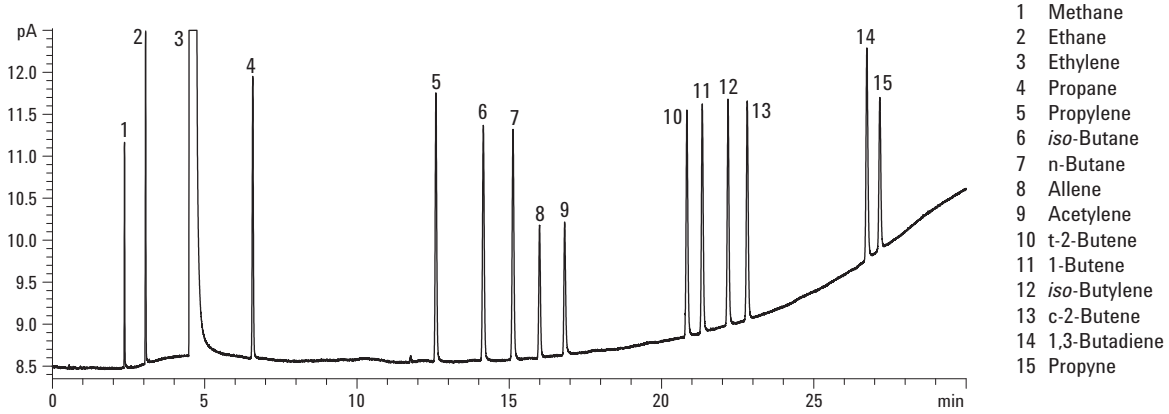

Figure 3. Sixteen ppm level impurities in the ethylene standard mix.

Table 3. System Repeatability of Three Runs for Diluted Ethylene Standard Mix

Component	Amt1	Amt2	Amt3	Avg.(ppmV)	RSD(%)	Repeatability criteria[1] (ASTM)
Methane	16.28	16.19	16.03	16.17	0.80	$0.02277 \times (ppmV^{0.6})$
Ethane	15.98	15.94	15.75	15.89	0.76	$0.03811 \times ppmV$
Propane	16.26	16.22	16.07	16.18	0.63	$0.03273 \times (ppmV + 21.23)$
Propylene	16.45	15.98	16.22	16.22	1.46	$0.04780 \times ppmV^{1.15}$
Isobutane	15.97	16.02	15.69	15.89	1.10	$0.04370 \times ppmV^{1.07}$
n-Butane	15.98	15.96	15.59	15.84	1.37	$0.1156 \times ppmV^{0.85}$
Allene	16.38	16.32	15.95	16.22	1.42	$0.05091 \times (ppmV + 0.7831)$
Acetylene	15.92	16.31	16.37	16.20	1.49	$0.1189 \times ppmV \times 0.8$
t-2-Butene	16.27	16.30	15.88	16.15	1.45	$0.063960 \times ppmV^{0.95}$
1-Butene	16.36	16.34	15.90	16.20	1.62	$0.03992 \times (ppmV + 17.14)$
iso-Butylene	16.12	16.05	15.60	15.92	1.77	$0.1229 \times ppmV^{0.85}$
c-2-Butene	16.17	16.07	15.68	15.97	1.62	$0.08350 \times ppmV^{0.93}$
1,3-Butadiene	16.51	16.43	15.95	16.30	1.85	$0.07518 \times ppmV^{0.9}$
Propyne	16.34	16.22	16.18	16.25	0.52	$0.05205 \times ppmV^{1.1}$

[1]Ethylene repeatability (ASTM)-The difference between successive results obtained by the same operator with the same apparatus under constant operating conditions on identical test materials would, in the long run and in the normal and correct operation of the test method, exceed the values in only 1 case in 20, where ppmV is the concentration of the component..

Conclusions

The Agilent 6820 gas chromatograph configured with a 6-port gas sampling valve interfaced directly to a split/splitless inlet was used to analyze trace hydrocarbon impurities in ethylene with FID. Impurities, about 15 ppm mole % level, can be easily quantitated and show good repeatability. The Agilent 6820 system with Cerity can simultaneously acquire and quantitate both large concentrations (99 + mole %) and trace (low ppm) levels in a single run due to the use of a full dynamic range digital signal path. Manual range changes are not required. The system is simple and convenient to set up and use for routine QA/QC lab in the petrochemical and chemical industries.

References

1. ASTM Method D6159, “Standard Test Method for Determination of Hydrocarbon Impurities in Ethylene By Gas Chromatography”
2. Roger Firor, “Trace Level Hydrocarbon Impurities in Ethylene and Propylene,” Agilent Technologies, publication 5965-7824E www.agilent.com/chem

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Printed in the USA
May 7, 2003
5988-9259EN



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Use of the New 5973 inert for Determination of Low-Level Volatile Sulfur in Gaseous Streams

Application

Gas Chromatography

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Abstract

The enhanced inertness of the new 5973 inert MSD improves analysis of trace level volatile sulfur compounds. When operated in scan or selected ion monitoring mode, excellent sensitivity, selectivity, and peak symmetry are obtained for active compounds. Eight volatile sulfur species are used to demonstrate these attributes in a variety of hydrocarbon matrices. The system is well suited for the characterization of fuel feedstocks and basic petrochemicals, where impurities can poison critical catalytic processes or affect product quality.

Introduction

Sulfur detectors find widespread use in a broad range of applications that span across many industries. Demand for low-level sulfur detection will only increase in the future in response to more stringent quality control and regulation. The significance and need for low-level sulfur measurements are detailed in previous Agilent application literature [1, 2, 3, 4].

The mass selective detector (MSD) is usually not considered first when the need for low-level volatile sulfur quantitation and speciation arises in the analytical laboratory. Selective detectors such as the flame photometric detector (FPD), pulsed flame photometric detector (PFPD), and sulfur chemilumiscence detector (SCD) have traditionally dominated these applications [1]. The 6890N/5973 inert GC/MSD system is a very capable alternative to these detectors, providing optimized inertness and the benefit of positive compound identification. This applicaton note details how to set up the system for optimum sensitivity and selectivity. The specific hardware configuration is applicable to a wide range of applications where ppb detection of gaseous analytes is required.

A common problem with many sulfur selective detectors is hydrocarbon interference, especially from co-elution [4]. The measurement challenge is acute when the interfering hydrocarbon comprises the majority of the sample, as in the analysis of impurities in ethylene and propylene. In most cases, an accurate determination of the sulfur compound is not possible. However, the use of the 5973 inert in selected ion monitoring (SIM) mode can largely overcome quenching caused by co-elution for many applications.

Experimental

The 5973 inert equipped with a new deactivated source was used for all experiments. The 3-mm drawout lens was used to achieve low ppb sensitivity while maintaining linearity over the ppb to low ppm concentration range needed for most sulfur measurements.

The sulfur calibration mix consisted of the following components at 5 ppm each: hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, carbonyl sulfide, t-butyl mercaptan, and tetrahydrothiophene. The blend in helium was purchased from DCG Partnership, Pearland, TX.

A 6-port gas-sampling valve was connected directly to the volatiles interface on the 6890N with Siltek™ 1/16-inch tubing. See the sample introduction diagram in Figure 1. The sample loop, tubing, and inlet are Siltek treated for inertness.

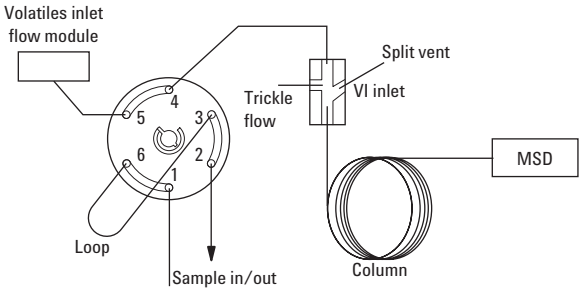

Figure 1. Sample introduction scheme.

Gaseous blends of the sulfur standard in helium or other matrices such as natural gas, propylene, and refinery gas were prepared using dynamic blending at the point and time of use. Diluent (matrix) gases are mixed with the calibration standard using an Aux EPC module on the 6890N GC. This system and the hardware employed have been described in detail [2].

Positioning of the column in the MSD must be carefully done to avoid loss of sulfur sensitivity. To position the column just inside the source, 2 mm to 3 mm of the column should be visible at the MSD end of the transfer line. See Reference 5 for installation details. See Table 1 for instrument conditions.

Results and Discussion

System Calibration

First, the system was calibrated and checked for linearity by analyzing the sulfur mix at various concentrations. The dynamic blending system was used to prepare seven and five level calibrations using helium and natural gas as diluents, respectively. Table 2 lists the concentrations used. Calibrations were focused in the ppb range since this is where most analytical problems for sulfur analysis are found. SIM acquisition mode was used.

Table 1. Instrument Conditions

6890N GC	
Injection port	**Volatiles interface**
Temperature	150 °C
Split ratios	1:1 up to 50:1
Carrier gas	Helium
Constant Flow Mode	1.9 mL/min
Injection source	**6-port gas sampling valve**
Material	Hastelloy C
Temperature	150 °C
Loop	Siltek, 0.5 cc
Column	**60 m × 0.320 mm × 5.0 µm DB-1**
Initial temperature	40 °C
Initial time	5 min
Temperature ramp	25 °C/min
Final temperature	270 °C
Final time	2 min

5973 *inert* MSD	
Mass range	33–100 and 12–100 amu
Scans	13.1/s and 15.9/s
Samples	2
Threshold	150
EM Voltage	BFB.U tune voltage
Solvent delay	3.00 min
Source	Surface deactivated
Drawout lens	3 mm
Source temperature	230 °C
Quad temperature	150 °C
Transfer line	280 °C

Calibrations are linear in both matrices for all eight sulfur compounds. Refer to Table 3 where the regression coefficient r^2 values are shown. This is an indication that not only is the system response linear, but also that adsorption is not occurring in the GC or MSD from active sites. If adsorption were present, then one would expect a drop off at the lower end of the calibration curve. This is a direct benefit of the new inert MSD source.

Two calibration plots, as produced by the MSD ChemStation, are shown in Figures 2 and 3 for the calibration of H_2S and COS in natural gas, respectively. These are two challenging compounds with respect to activity, and they help illustrate the effectiveness of the inert system.

The 3-mm Drawout Lens

The 3-mm lens offers excellent sensitivity-optimized performance for this application. The 3-mm drawout was chosen for this work to meet the objective of reliable low ppb sulfur analysis. In addition, linearity over only a part of the MSD's dynamic range was required. Calibrations from 20 ppb to 5 ppm cover expected impurity ranges in real world samples and show excellent linearity with the 3-mm lens including samples run in a natural gas matrix where significant hydrocarbon fragmentation occurs.

Table 2. Calibration Levels for Checking System Linearity. Sulfur Concentrations in ppb.

Cal Level	1	2	3	4	5	6	7
Conc. in helium	21	35	46	57	95	1600	3600
Conc. in nat gas	88	242	475	880	1170	--	--

Table 3. Calibration Regression Coefficient r^2 Values

Compound	Helium	Natural gas
H_2S	0.998	0.998
COS	0.998	0.999
CH_3SH	0.997	0.999
EtSH	0.996	0.998
DMS	0.998	0.998
CS2	0.998	0.998
t-ButylSH	0.996	0.993
THT	0.996	0.992

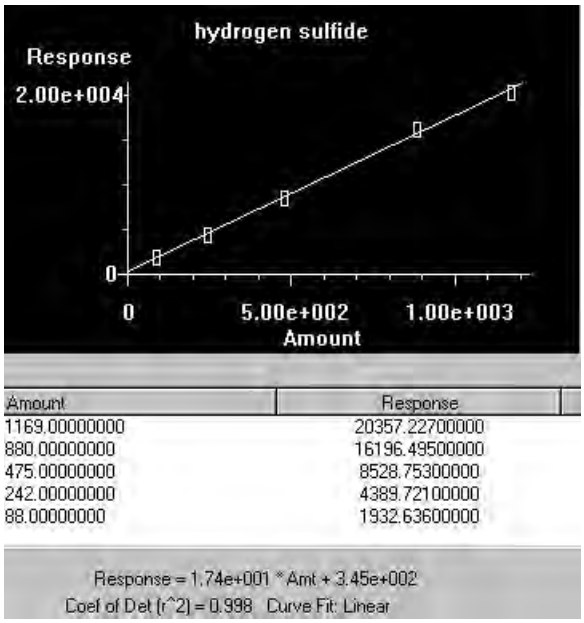

Figure 2. Five level calibration plot of H_2S in natural gas diluent. Calibration range is from 88 ppb to 1170 ppb.

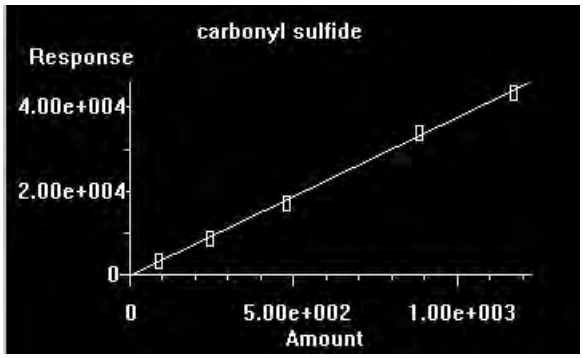

Figure 3. Five level calibration plot of COS in natural gas diluent. Calibraton range is from 88 ppb to 1170 ppb.

Scan Results

The total ion chromatogram (TIC) of the eight-component sulfur mix at 1.3 ppm in helium using a split ratio of 0.5 to 1 is shown in Figure 4. As is evident in the figure, H_2S is close to the minimum detection level (MDL) for this particular set of operating conditions. Symmetric peak shapes are seen for all components including adsorptive H_2S and COS.

Application of SIM

SIM provides the best sensitivity and selectivity for target analytes. Since sulfur determinations will normally be done in hydrocarbon matrices, care must be taken to select ions that ideally have no hydrocarbon contribution. If this can be done, excellent selectivity can be achieved even in cases where co-elution of sulfur species and hydrocarbon occur. This is an important distinction and advantage of the MSD compared to some of the common gas chromatographic sulfur selective detectors. Both the FPD and PFPD will suffer from quenching if co-elution occurs, making accurate quantitation of low-level sulfur problematic [2]. Even the SCD will have problems measuring low ppm sulfur in the presence of a dominant co-eluting hydrocarbon. In situations where a unique sulfur ion cannot be found, refinement of the method and chromatographic column/conditions to achieve separation from the interfering hydrocarbon should be attempted [2]. Also, when operating the MSD in SIM mode, it is usually best to select low resolution for maximum sensitivity at the expense of some resolution loss.

Refer to Reference 6 for guidelines for setting SIM parameters and instructions on using the AutoSIM feature available in the MSD ChemStation, G1701DA.

The SIM ions used for each sulfur compound are listed in Table 4. These ions were chosen to minimize interference from hydrocarbons. To arrive at the ions shown in the table, a scan of the sulfur mix in helium is acquired to identify target ions. Library spectra can also be consulted. Hydrocarbon mixes, such as natural gas and refinery gas, are then run separately using the SIM table to look for ions that may match those selected for sulfur. The table may be further refined if hydrocarbon interferences appear. These are not the only possible ions that can be used. For some of the compounds, other choices or additional ions could be included in the SIM table. While not necessary for this relatively simple sulfur example, the use of second and third qualifier ions may give the analyst a higher level of confidence of a compound's identity by comparing ion ratios to library spectra for a particular compound.

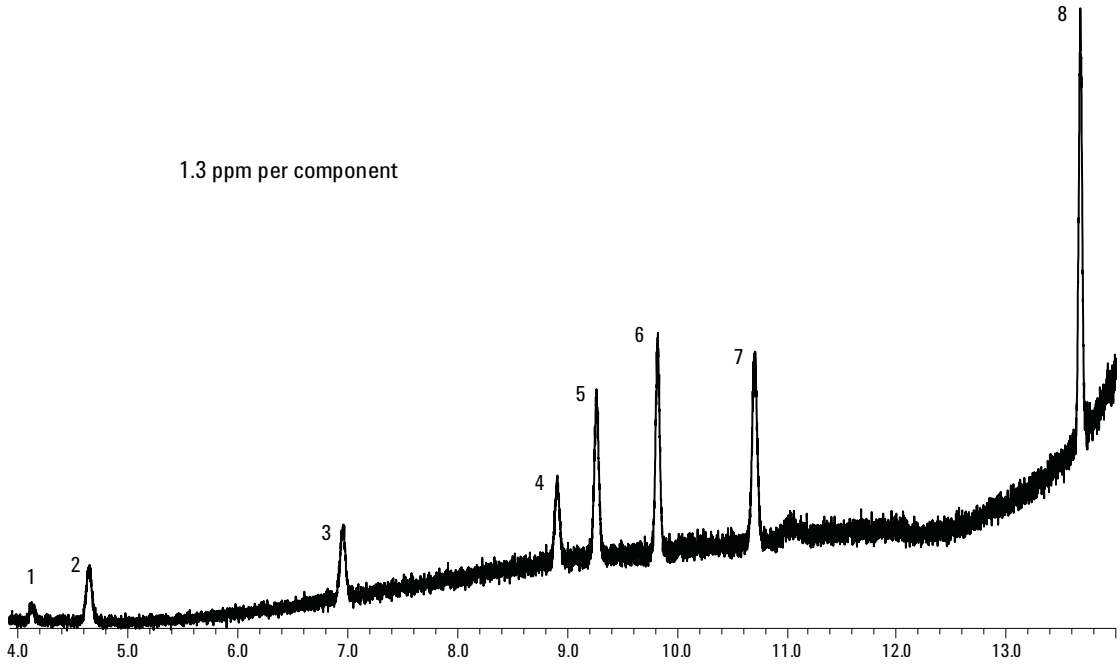

Figure 4. TIC of the eight-component sulfur mix at 1.3 ppm per component. Scan 33–100 amu. Peak labels: 1. hydrogen sulfide, 2. carbonyl sulfide, 3. methyl mercaptan, 4. ethyl mercaptan, 5. dimethyl sulfide, 6. carbon disulfide, 7. t-butyl sulfide, 8. tetrahydrothiophene.

Table 4. Optimized SIM Table for Selective Sulfur Detection in Hydrocarbon Streams. Dwell Time for Each Ion is 100 ms.

Group	Start time (min)	Target and qualifier ions	Compound
1	3.00	33,34	H_2S
2	4.20	60	COS
3	6.00	45,47	MeSH
4	8.00	47	EtSH
5	9.10	45,47,62	DMS
6	9.70	44,76	CS_2
7	10.20	57,90	t-ButylSH
8	11.80	45,60,88	THT

The sulfur mix chromatogram shown in Figure 5 was produced using the SIM parameters shown in Table 4. The offsets seen in the baseline are a result of the MSD switching from group to group and are not chromatographic. Excellent signal-to-noise and peak shape are seen for all components at the 46-ppb level. The sulfur mix was then further diluted to 16 ppb per component. The resulting chromatograms for H_2S, COS, and THT, the most challenging analytes, appear in Figure 6. At these levels, any problems with system or source activity would be evident. Sensitivity and peak shape are maintained, indicating excellent source inertness.

Natural Gas and Refinery Gas: Composition and Impurities

The TIC of a natural gas scan and sulfur mix SIM runs are overlaid for illustration purposes in Figure 7. Note that with the 60 m × 0.32 mm × 5.0 µm DB-1 all hydrocarbons and CO_2 are separated. Natural gas compounds in order of elution are: O_2/N_2, CH_4, CO_2, ethane, propane, I-butane, N-butane, I-pentane, and N-pentane. From the overlay, it can be seen that seven of the eight sulfurs do not co-elute with natural gas components; only COS and propane have nearly identical retention times. Even with co-elution, SIM makes it possible to quantify the COS; this will be addressed in the following section on propylene impurities.

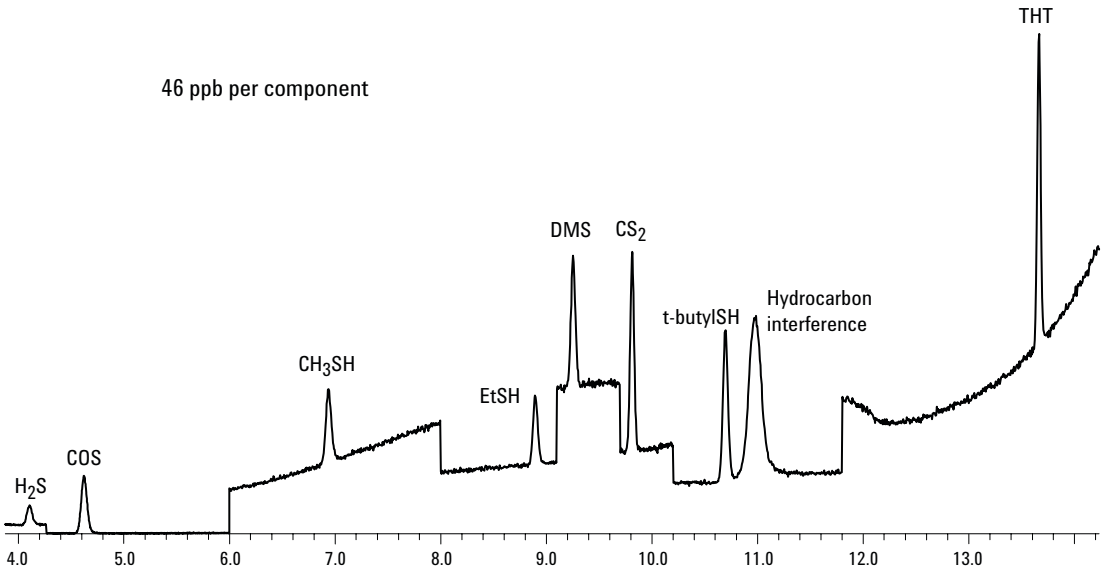

Figure 5. Eight-component sulfur mix in helium at 46 ppb per component in SIM mode. Split ratio 0.5:1. Refer to Figure 4 for peak identification.

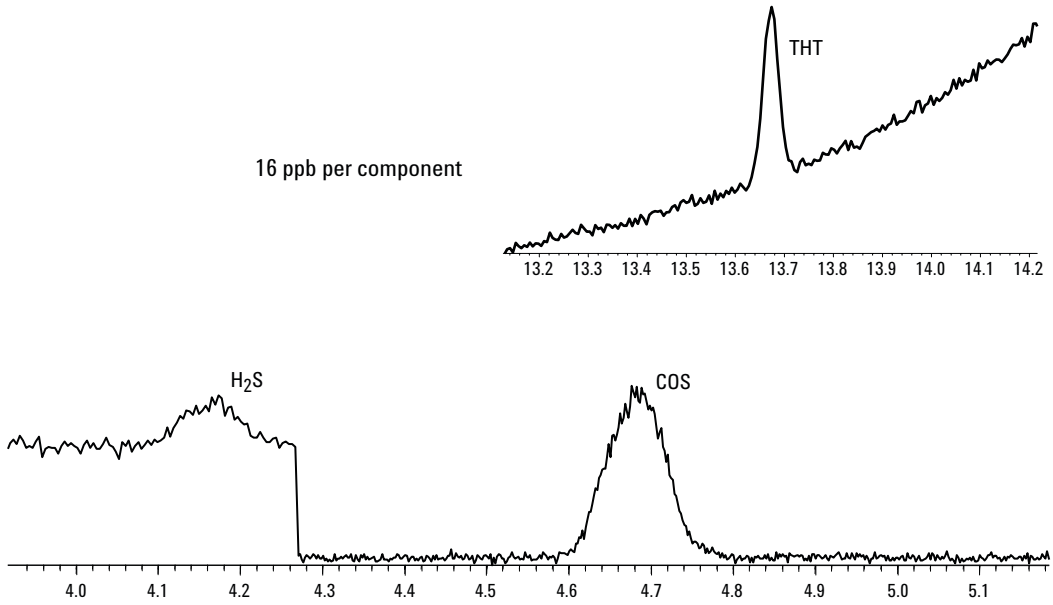

Figure 6. H_2S, COS and tetrahydrothiophene (insert) at 16 ppb each.

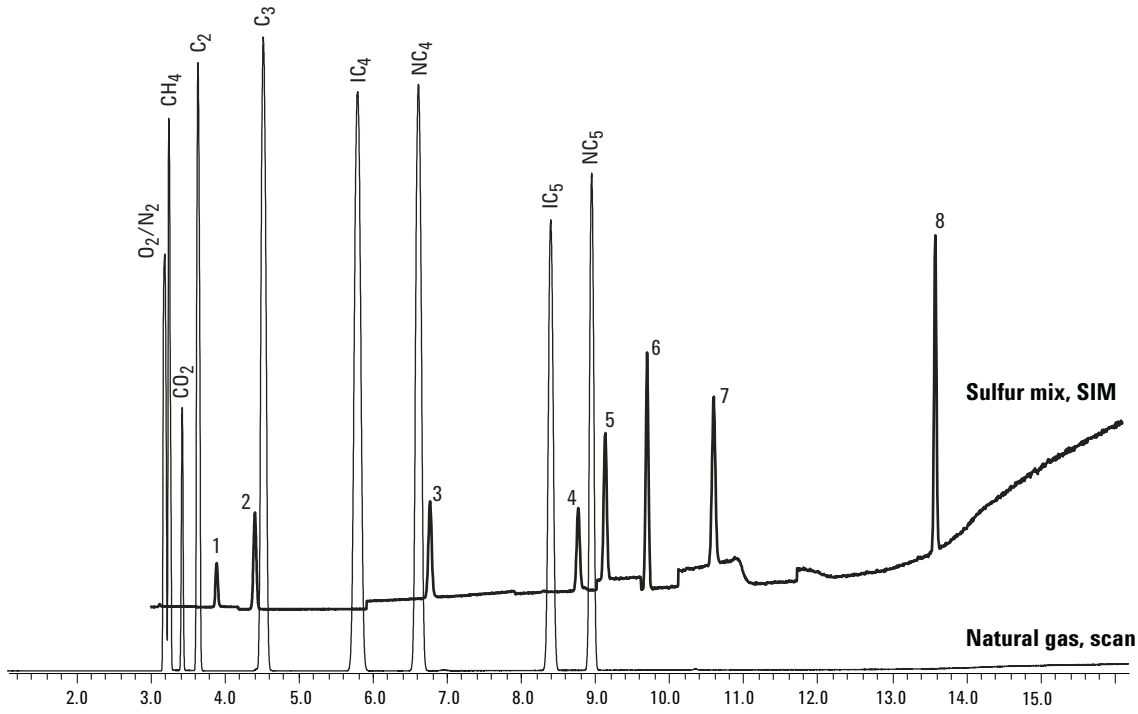

Figure 7. Overlay of two runs: natural gas scan (12–100 amu), and sulfur mix at 4.5 ppm in SIM mode. Split ratio 20:1. Peak numbering same as Figure 4.

Low level (350 ppb) sulfur gases in a representative refinery gas matrix are shown in Figure 8. Again, good peak shape and signal-to-noise are seen. Only methyl mercaptan is lost to hydrocarbon interference.

Analysis of COS in Propylene

Measurement of ppb COS and H_2S in propylene or propane can be challenging due to the co-elution of COS/propylene and the reactivity of H_2S. The COS co-elution is illustrated in Figure 9, where two independent separate runs are superimposed.

SIM (ion 60) was employed for the analysis of COS. To avoid overloading the source, the split ratio was increased to 50:1. To determine the effect of co-eluting propylene on COS response, two runs were performed at identical concentrations of 105 ppb COS. The diluents for the first and second runs were helium and propylene, respectively. Chromatograms for both runs are shown in Figure 10. The helium chromatogram shows the true COS area unaffected by co-elution. This area is then compared to that of COS in propylene diluent using the area ratio (COS propylene/COS helium) to indicate how co-elution has affected the 5973 inert response. This ratio of 0.77 indicates that COS in propylene response is suppressed by only 23% probably due to a reduction in ionization efficiency. Moreover, a subsequent experiment that constructed a five level calibration of COS in propylene showed linear behavior over the range of 20 ppb to 1200 ppb. Therefore, using a carefully constructed SIM method, the 5973 inert equipped with 3-mm drawout has the capability of quantifying ppb level COS in co-eluting propylene. Co-eluting active analytes do not preclude quantification even when concentration differences exceed 105 provided unique ions can be identified for the component of interest.

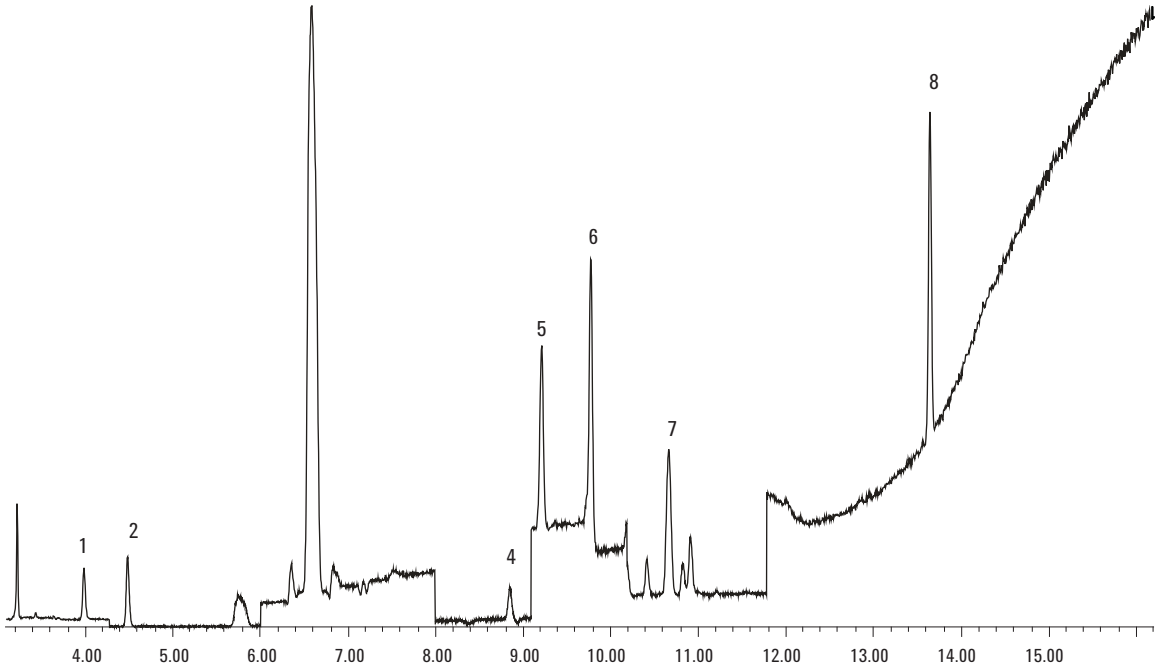

Figure 8. Three hundred fifty ppb sulfur mix in refinery gas. Peak identifications same as Figure 4. Good peak symmetry and sensitivity seen.

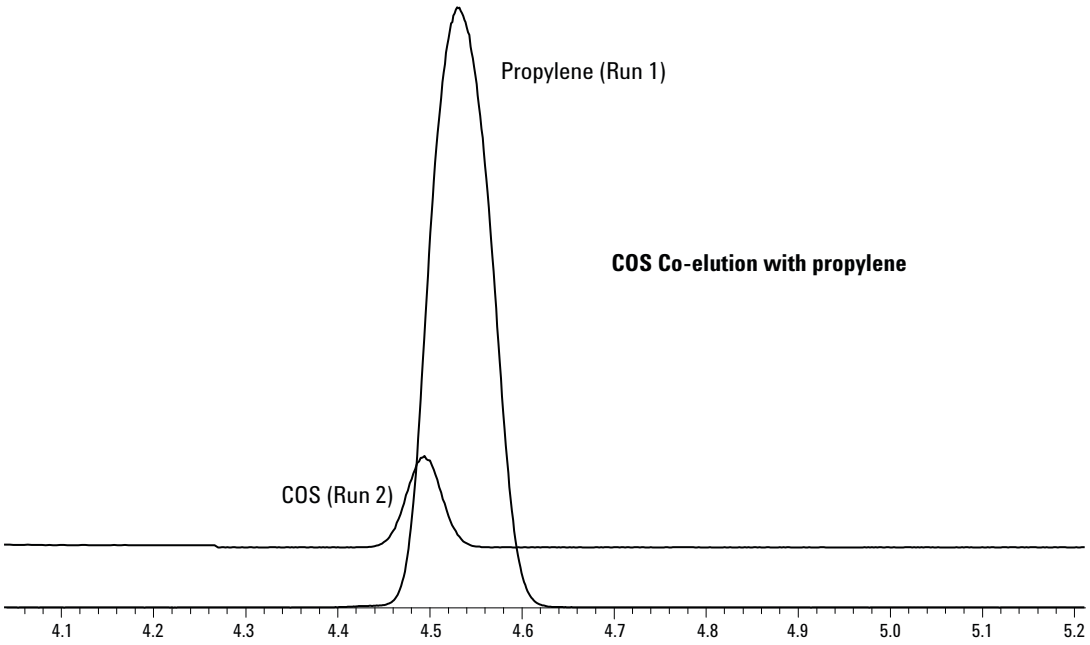

Figure 9. Two separate chromatograms superimposed showing the co-elution of COS with propylene. Split ratio 50:1.

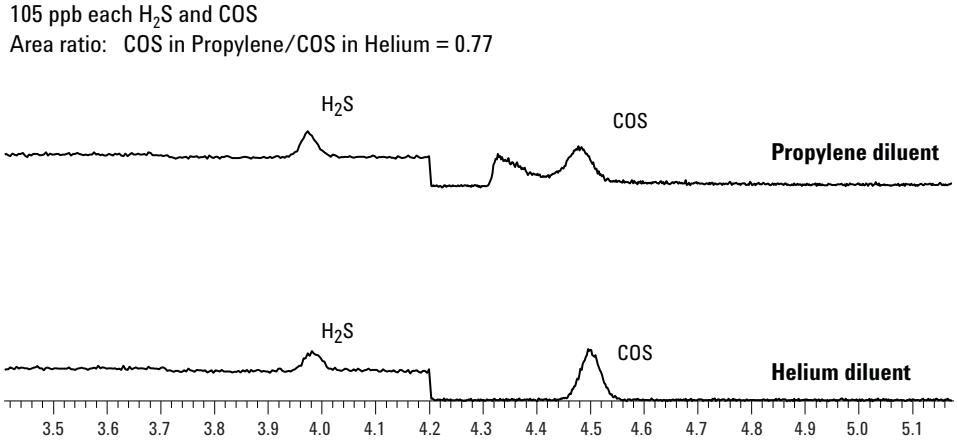

Figure 10. Comparison of COS response (SIM mode) in helium and propylene. Split ratio 50:1.

Conclusions

The importance of inertness cannot be over emphasized when analyzing and quantifying ppb level volatile sulfur compounds. The 5973 inert has excellent capabilities as a sensitive, repeatable, and selective detector for active gaseous analytes at low levels. Sulfur detection at low ppb levels is easily achieved through use of a time programmed SIM table consisting of unique ions for the compounds of interest. This minimizes hydrocarbon interference making it possible to quantitate low-level analytes such as COS with co-eluting propylene.

Use of the new inert source leads to excellent detection limits of active, adsorptive compounds with minimal peak tailing. Good peak symmetry is maintained at the ppm and ppb level for H_2S, COS, and other light organo-sulfur compounds. Detection of low-level polar analytes in general will improve with the 5973 inert.

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Printed in the USA
October 13, 2003
5988-9942EN



Agilent Technologies

High-Pressure Liquid Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Application

Hydrocarbon Processing

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Abstract

In gas chromatography, sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Introduction

There are several known techniques for injecting volatile liquefied hydrocarbons in gas chromatographs. The simplest tools are high-pressure syringes. However, the pressure limit is not high enough to analyze light hydrocarbons such as liquefied natural gas and ethylene. The traditional methods [1, 2] include the use of vaporizing regulators and rotary sampling valves. During sampling, discrimination of the analytes will take place for samples with wide boiling points due to condensing of heavy components and selective vaporization of light components in transfer lines. Recently, piston sampling valves were introduced and are commercially available [3]. These can suffer from discrimination and short service lifetimes at high vaporization temperatures or high sample pressures.

Combining the advantages of simple syringes and high-pressure rotary valves, a unique sample injection device has been designed. The system consists of a 4-port liquid sampling valve, a Siltek deactivated needle, and a split/splitless inlet. This compact device is installed directly over the GC inlet. This unit is operated just like a typical liquid autosampler; however, the needle is not withdrawn. The maximum limit of sample pressure is 5,000 psig. Various pressurized gas samples have been evaluated on this device such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with 0.47% to 1.09% RSD in quantitative analyses. Wide boiling point hydrocarbon samples (C5 to C40) have also been analyzed using this injector, with excellent quantitative results.

Experimental

Injection Device

The high-pressure liquid injection (HPLI) device consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Co. The valve works under 75 °C and 5,000 psi.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

The following components are recommended. These are not supplied in the option or accessory kit.

- **Filter:** To remove particles from samples, it is necessary to install a filter between the sample line and port S.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in option or accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

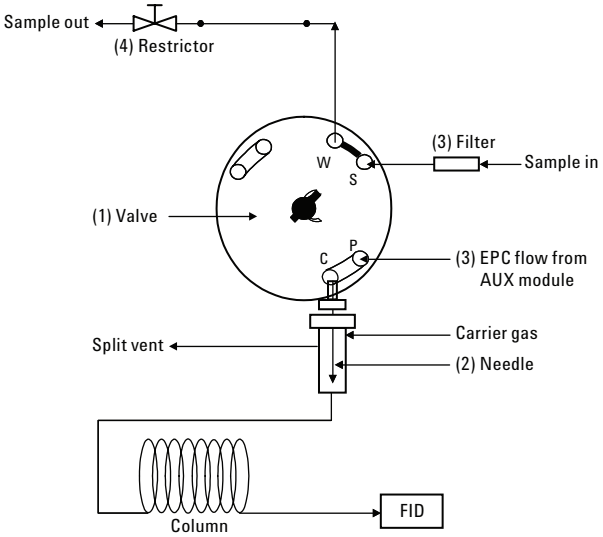


Figure 1. Flow diagram of the HPLI device.

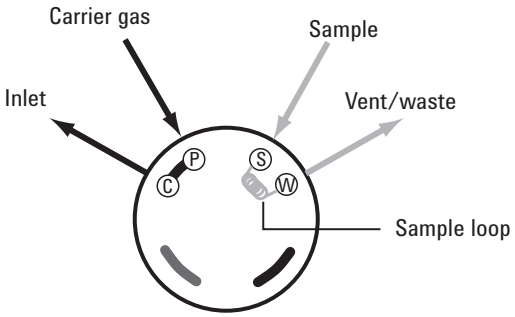
Samples for System Evaluation

- Liquefied natural gas: Calibration standard, 1,200 psi, with nC7-nC9 (0.102%–0.0503%)
- Liquefied ethylene: Purity 99.5, 1,200 psi
- Pressurized propylene: Grade C. P., purity 99.0%, 200 psi
- Pressurized propane + n-butane: 50.0%:50.0%, 200 psi
- Pressurized 1, 3-butadiene: Purity 99.5%, 180 psi
- n-Hexane + 1.0 % 2# BP standard (Agilent PN 5080-8768, nC5–nC18)
- nC5–nC40 D2887 1# BP standard (Agilent PN 5080-8716, diluted by CS_2)
- Glycols, including monoethylene glycol, diethylene glycol, and triethylene glycol
- C8 to C16 hydrocarbons at 100 ppm each

Operating Process

The valve is operated with an Agilent pneumatic air actuator. To load the sample, the valve is set at the OFF position (Figure 1). The sample is loaded from port S and vented to port W. The pneumatic and sample paths in load and inject positions are shown in Figure 2. To maintain the sample in the liquid phase and to avoid “bubbles” in the sample line, it is important to adjust resistance of the metering valve and check for possible leaks at the connections. To inject, the valve is switched to the

Load



Inject

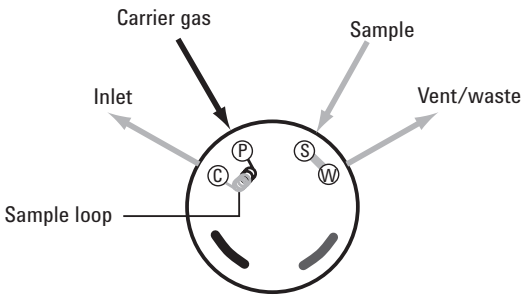


Figure 2. Pneumatic and sample paths in load and inject positions.

ON position. A 2- to 3-second injection time should be used.

The system should always be carefully checked for leaks before introduction of high-pressure hydrocarbons. Instrumental conditions and application-specific columns are shown in Table 1 and Table 2, respectively.

When the valve is actuated, a stream of carrier gas from the Aux EPC or PCM will enter the inlet and combine with the inlet carrier flow; the combined flow will vent through the split vent. Therefore, the actual split ratio will be higher than the value set from ChemStation. The actual split ratio can be calculated by measuring the split vent flow.



Figure 3. Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Table 1. Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	HPLI device at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.5-µL (0.2 µL for C5–C40) device supplied with 0.06-µL rotor
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

Table 2. Columns and Parameters

Samples	Columns	Column flow mL/min	Split ratio	Temperature program	Sample pressure psig
Natural gas	30 m × 0.53 mm × 0.5 µm DB-1 #125-1037	8	40:1	35 °C, 1 min 20 °C/min to 180 °C, 1 min	1200
Ethylene	50 m × 0.53 mm × 15 µm AL2O3 PLOT/KCL + 30 m × 0.53 mm × 5 µm DB-1, #19095P-K25 and #125-1035	8	20:1	35 °C, 2 min 4 °C/min to 160 °C, 3.8 min	1100
Propylene	50 m × 0.53 mm HP AL2O3 PLOT + 30 m × 0.53 mm × 5 µm DB-1	7	25:1	35 °C, 2 min 4 °C/min to 160 °C, 1.8 min	180
Propane + n-butane	30 m × 0.53 mm × 1.0 µm DB-1, #125-103J	5	50:1	35 °C	150
1,3-Butadiene	50 m × 0.53 mm AL2O3 PLOT/KCL	10	15:1	35 °C, 2 min 10 °C/min to 195 °C, 15 min	180
n-Hexane	30 m × 0.53 mm × 1.0 µm DB-1	5	50:1	45 °C	N/A
nC5-nC40	10 m × 0.53 mm × 0.88 µm HP-1, #19095Z-021	10	15:1	35 °C, 1 min 15 °C/min to 350 °C, 5 min	N/A
Glycols	30 m × 0.25 mm × 1.0 µm HP-1 ms	1.8	15:1	50 °C, 3 min 15 °C/min to 250 °C, 2 min	

Results and Discussion

Check for Carryover

A set of normal hydrocarbons was used to perform a basic check of the system, looking for good peak shape and lack of carryover.

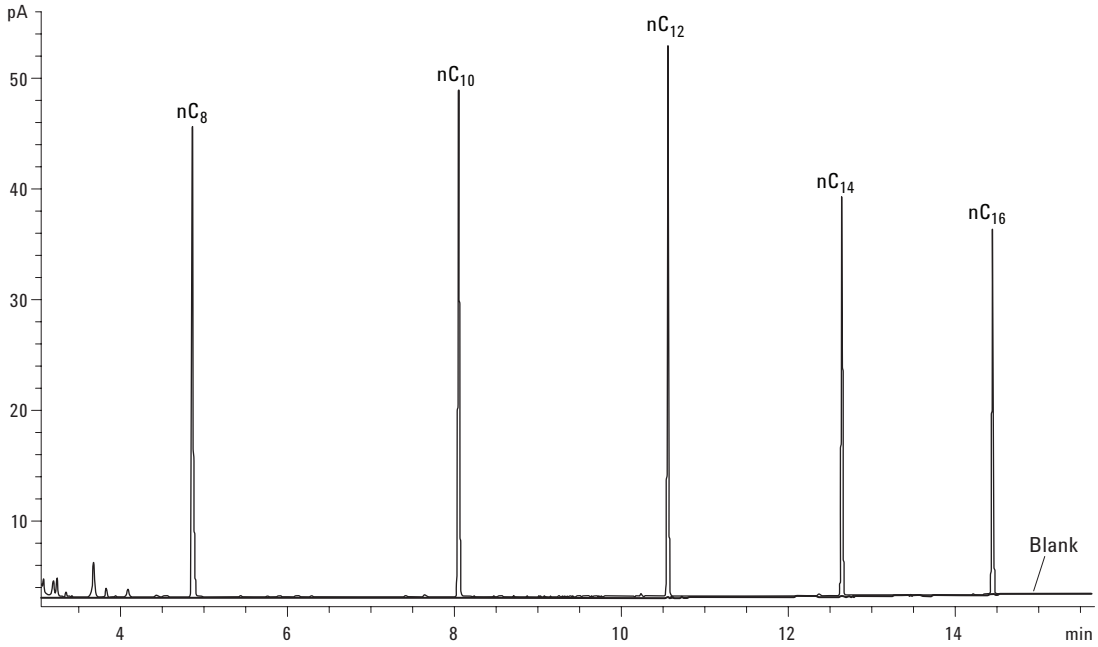


Figure 4. Overlay of standard versus blank (100 ppm each in cyclohexane).

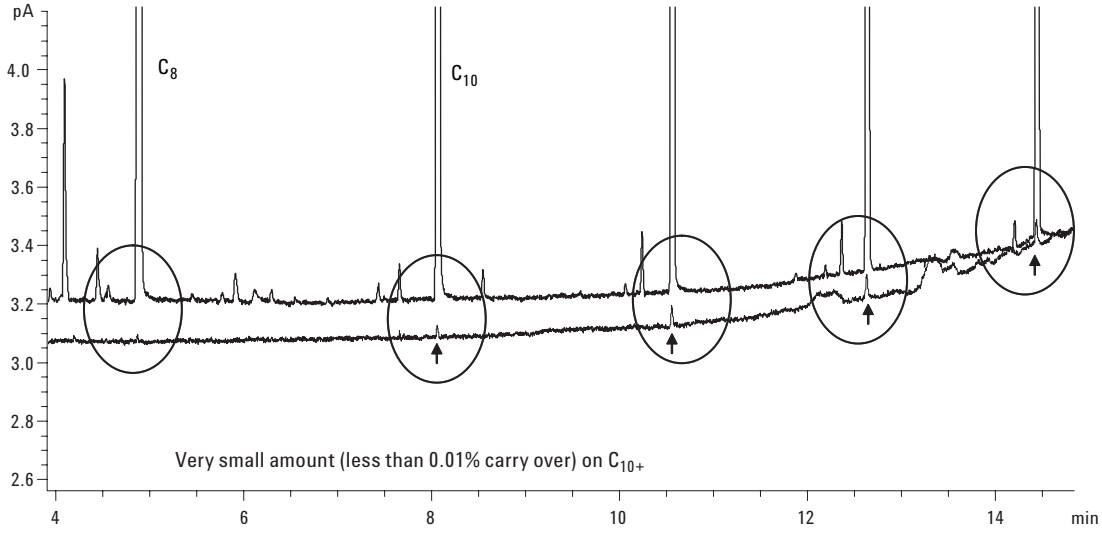


Figure 5. Carryover less than 0.01% on C_{10+}.

Sample Analysis

A series of glycols was used to model performance of the device for highly polar analytes. Minimal peak tailing is seen, due in part to the inertness of the needle interface. Also, carryover is very low.

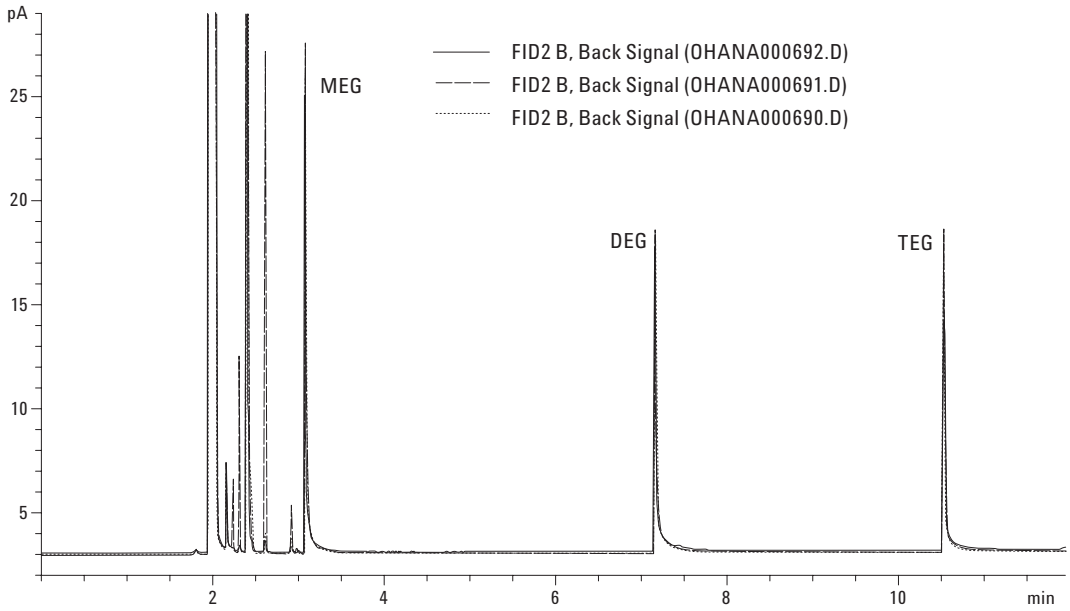


Figure 6. Triplicate run of 100 ppm each of MEG, DEG, and TEG in IPA.

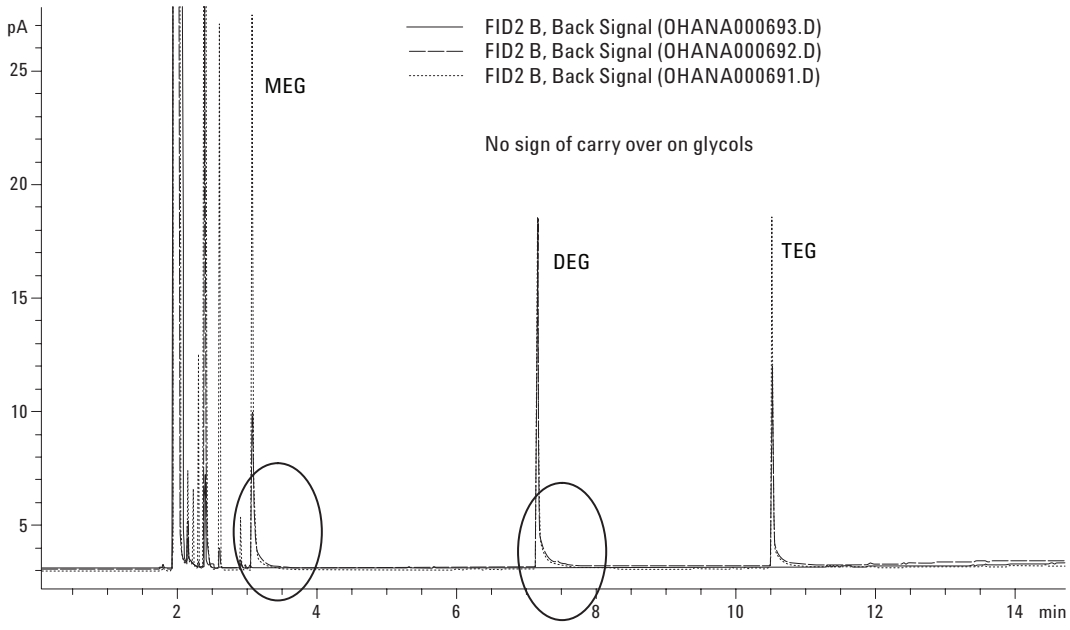


Figure 7. Glycols versus blank. Two standard duplicates, blank run immediately after injection of standard.

A. Liquefied Natural Gas

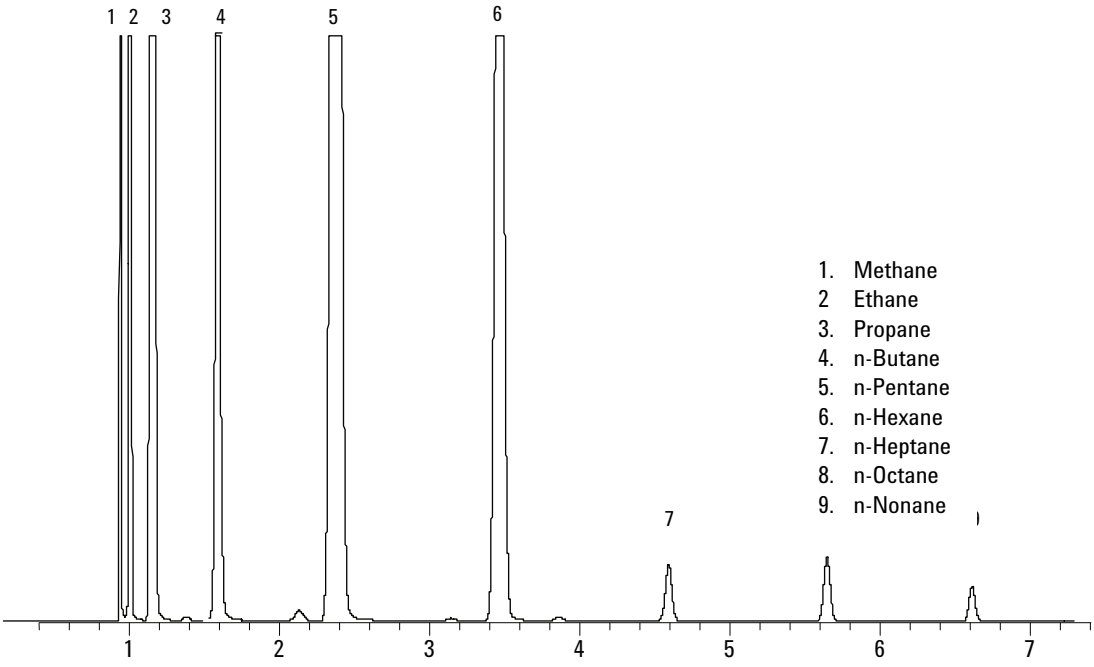


Figure 8. Chromatogram of liquefied natual gas (calibration standard).

Low discrimination is seen in Figure 8 for liquefied natural gas (LNG). Excellent repeatability is obtained with RSDs of less than 1%.

B. Liquefied Ethylene

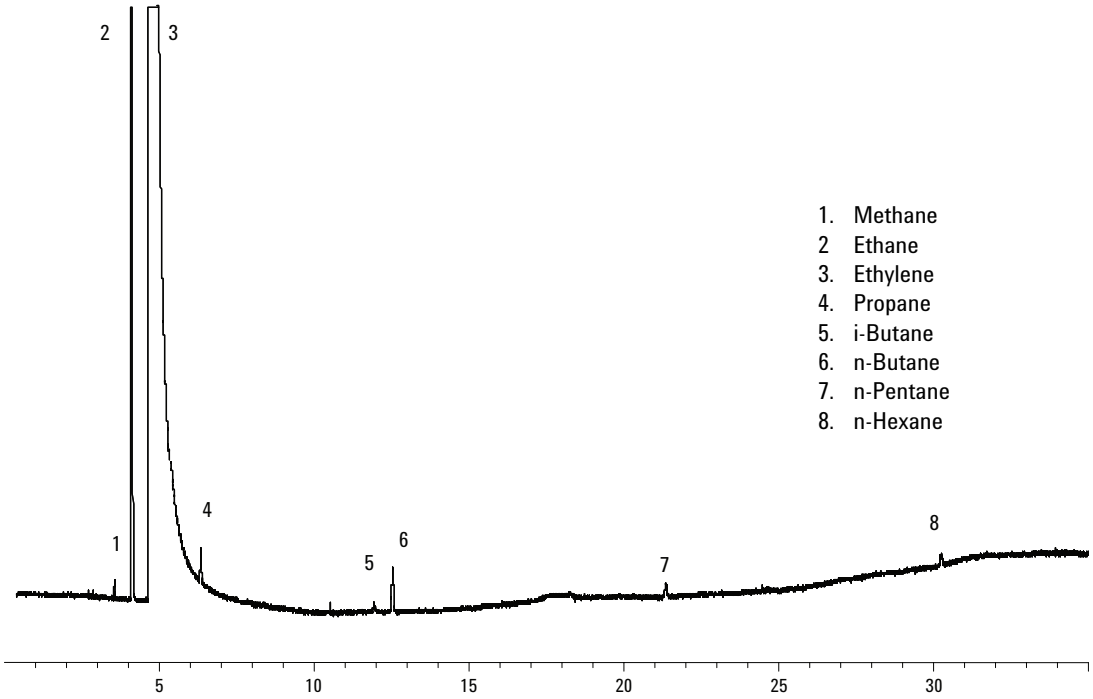


Figure 9. Chromatogram of liquefied ethylene.

The sample in Figure 9 is analyzed by ASTM D6159, "Standard Test Method for Impurities in Ethylene by Gas Chromatography." The method detection limits (MDLs) for the two methods are listed in Table 3.

The MDL using the HPLI device is 10 times lower than reported in the ASTM method due largely to the lack of peak tailing.

Table 3. MDLs (ppm V) by ASTM D6159 and HPLI

Components	ASTM D6159	HPLI
Methane	5.57–62.3	0.27
Ethane	35.1–338	0.78
Propane	8.07–59.7	0.88
i-Butane	7.74–48.4	0.38
Butane	4.97–56.1	1.61
n-Pentane		0.61
n-Hexane		0.74

C. Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159 (above method for ethylene analysis). The chromatogram is shown in Figure 10.

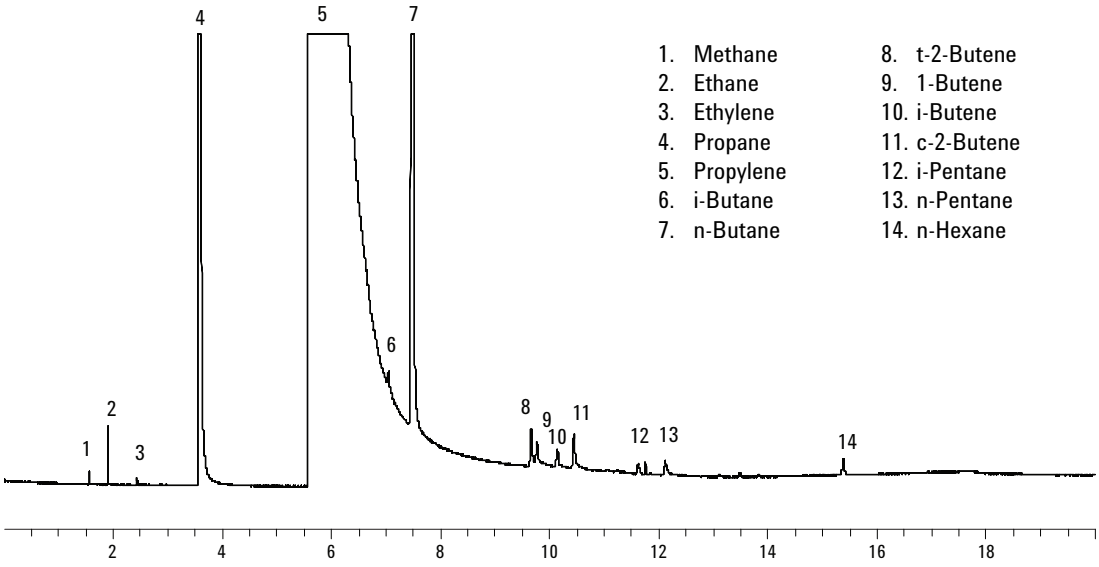


Figure 10. Chromatogram of pressurized propylene.

D. Pressurized 1,3-Butadiene

As an example of C4 hydrocarbons analysis,
Figure 11 shows a typical result for 1,3-Butadiene.

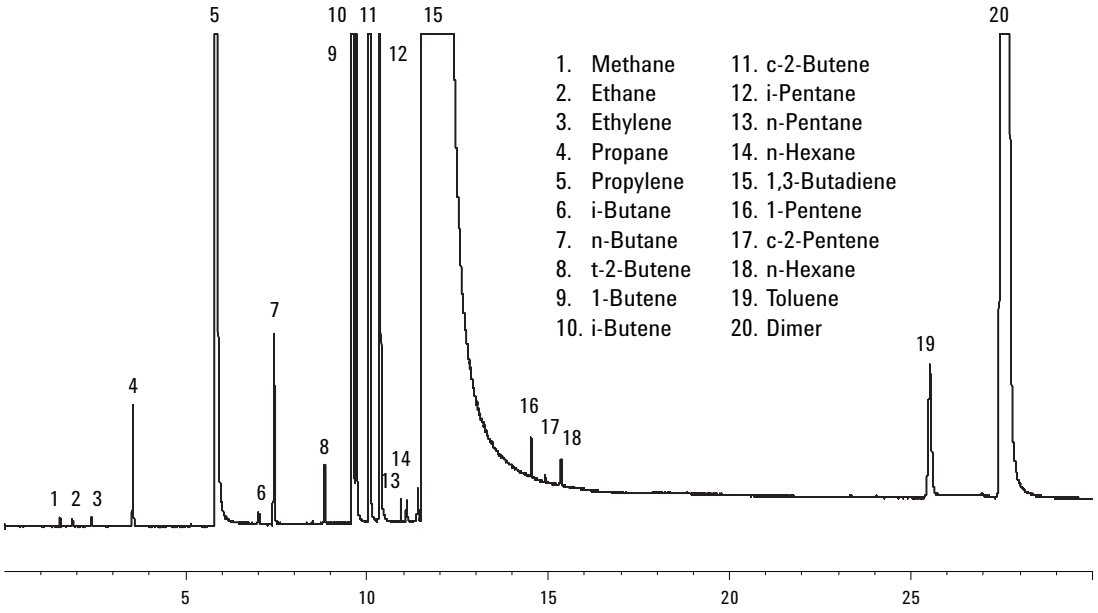


Figure 11. Chromatogram of pressurized 1,3-butadiene.

E. Pressurized Propane + n-Butane

This is a quantitative calibration sample:
Propane:n-Butane = 50%:50%.
The chromatogram is shown in Figure 12 with the results of a quantitative analysis shown in Table 4.

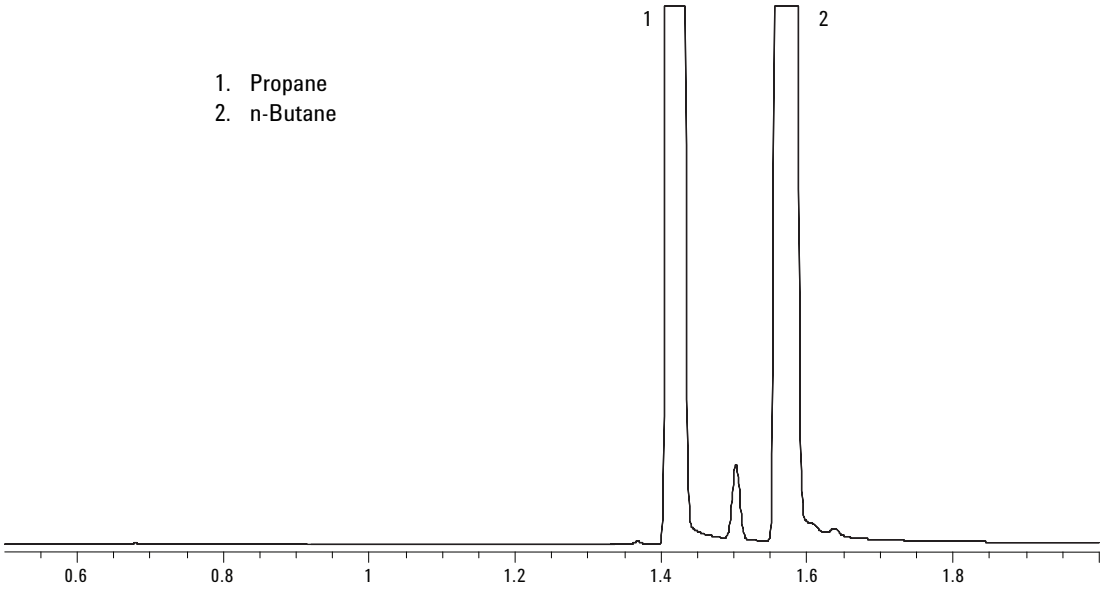


Figure 12. Chromatogram of pressurized propane + n-butane.

Table 4. Quantitative Analysis of Pressurized Propane 50.0% + n-Butane 50.0%. One Percent Difference Between the Blend (actual) and the Analysis Result

	Propane	n-Butane
Response factor	1.03	1.01
Density	0.5139	0.5788
Blend by V%	50.0	50.0
By wt%	47.031	52.969
Analysis		
By area%	45.441	54.559
By wt%	45.927	54.073

F. n-Hexane + 1.0% BP Standard (C5-C18)

To check the quantitative results, a small amount (1.0% BP standard) of C5 to C18 hydrocarbons was added to n-hexane (Figure 13). Table 5 shows the analytical results obtained by adding the C5 to C18 hydrocarbons with both the HPLI device and the automatic liquid sampler (ALS). In Figure 14, chromatograms by HPLI (top) and by ALS (bottom) are shown.

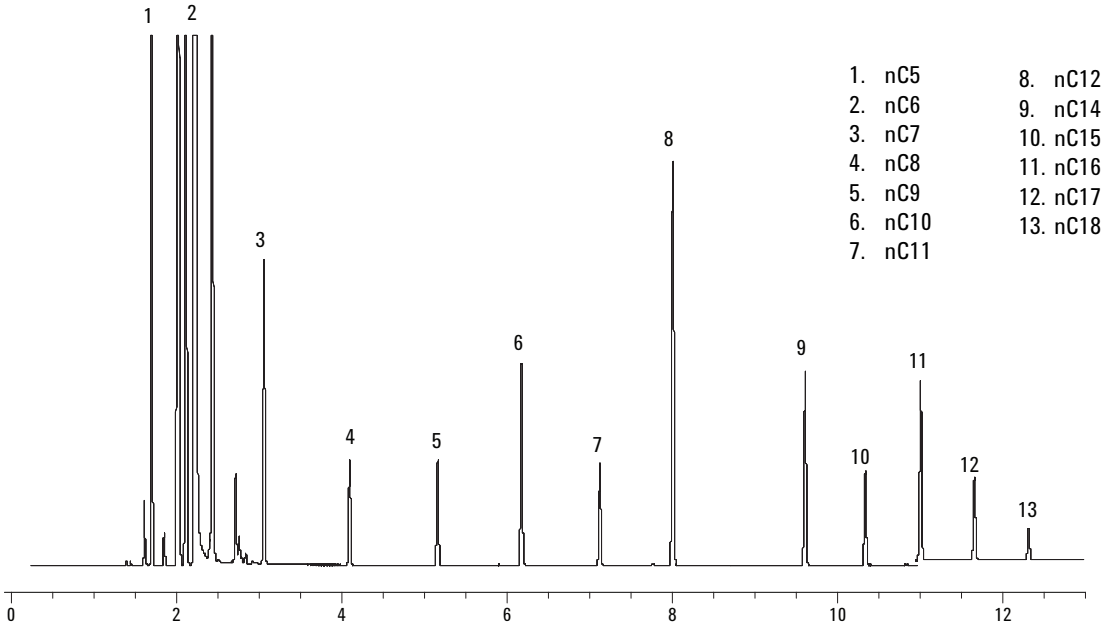


Figure 13. Chromatogram of n-hexane + 1.0% BP standard.

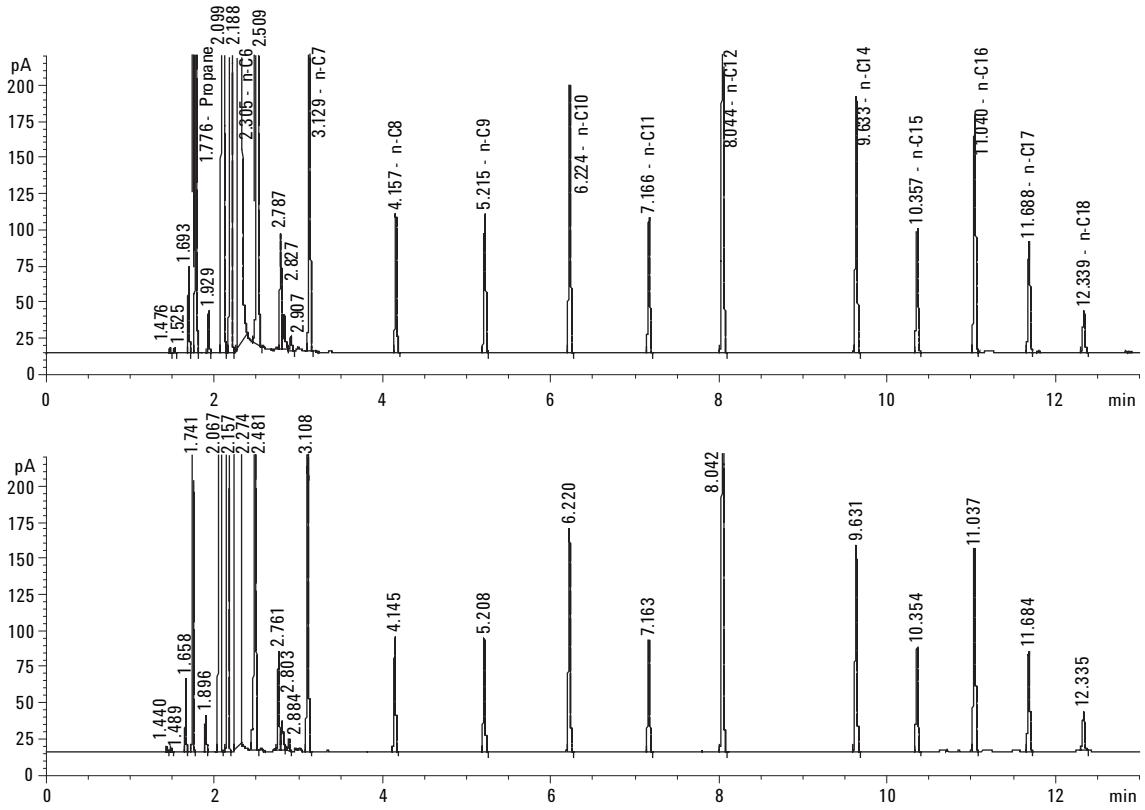


Figure 14. Chromatograms of n-hexane + 1.0% BP standard. Top: HPLI. Bottom: ALS (syringe).

Table 5. Analytical Results for C5-C18 by HPLI and ALS

	HPLI		AUTO INJECTOR	
COMPONENTS	**Area %**	**Width (min)**	**Area %**	**Width (min)**
nC5	0.282		0.279	
nC6	96.950	0.0209	96.922	0.0195
nC7	0.146		0.148	
nC8	0.0524		0.0532	
nC9	0.0537		0.0548	
nC10	0.109		0.111	
nC11	0.0550		0.0559	
nC12	0.219		0.221	
nC14	0.109		0.110	
nC15	0.0532		0.0547	
nC16	0.102		0.109	
nC17	0.0484		0.0546	
nC18	0.0203		0.0239	

The peak width of hexane at top: 0.0209 min
The peak width of hexane at bottom: 0.0195 min

There are no significant differences in quantitative results up to nC14. Compared with the results from an ALS injection, the HPLI device yields results about 10% lower in response above approximately nC16.

G. nC5-nC40 (D2887 BP Standard Diluted by CS_2)

A sample with hydrocarbons (nC5-nC40 D2887 1# BP standard diluted by CS_2) is also run on HPLI. The chromatogram is shown in Figure 15.

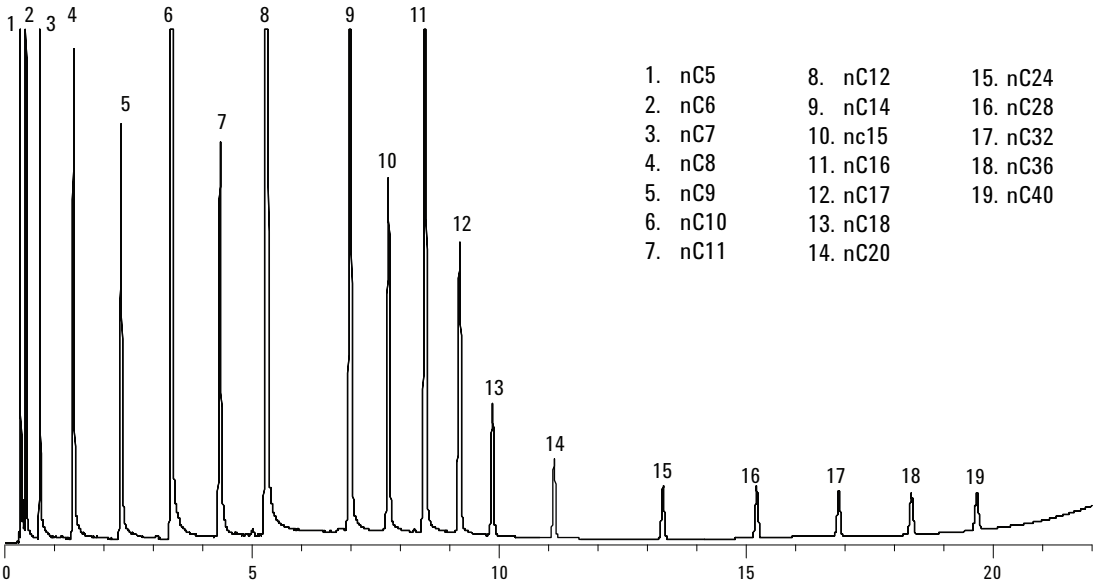


Figure 15. Chromatogram of nC5-nC40 (D2887 BP standard diluted by CS_2).

A lack of discrimination is seen with the HPLI device. In the future, it would be interesting to run some unstable condensates for evaluating the device.

From the above GC evaluation, excellent analytical results could be obtained using the HPLI device. These are summarized below.

1. Excellent repeatability
2. Capable of quantitative results
3. No significant peak width broadening
4. The wide boil point hydrocarbon samples could be analyzed by this device with minimal discrimination.

Conclusions

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC inlet. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

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Acknowledgement

Figures 1 through 4 are courtesy of Ronda Gras and Jim Luong, Dow Chemical Canada, Analytical Sciences.

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Printed in the USA
February 26, 2008
5989-6081EN



Agilent Technologies

Simultaneous Analysis of Trace Oxygenates and Hydrocarbons in Ethylene Feedstocks Using Agilent 7890A GC Capillary Flow Technology

Application Brief

James McCurry

The presence of trace hydrocarbons in ethylene can have damaging effects on both the process catalysts and the final polymer products. Test methods such as ASTM D6159 are used to ensure the quality of these feedstocks [1]. However, the analysis of other key contaminants, such as oxygenates, requires GC methods that run on separate instruments. This can be time consuming and expensive for the process analysis lab.

The Agilent 7890A GC serves as the ideal platform when analyzing different classes of trace compounds in ethylene. Maximum productivity can be realized by:

- Using Capillary Flow Technology to perform analysis of trace oxygenates and hydrocarbons in a single run through 2-D Deans switch chromatography.
- Automating the preparation of multilevel calibration standards using the new auxiliary electronic pneumatics control (EPC) modules.
- Protecting the sensitive and expensive alumina PLOT column by preventing polar oxygenates from entering the column.

Enhancing ASTM Method D6159 with Capillary Flow Technology 2-D GC

ASTM Method D6159 uses a methyl silicon column in series with an alumina PLOT column to resolve light hydrocarbons in ethylene. Polar oxygenated compounds cannot be analyzed on this column set because methyl silicon has insufficient selectivity and the alumina column will adsorb oxygenates, resulting in column damage. Wax-type liquid phases such as HP-INNOWax can easily separate polar compounds from light hydrocarbons using 2-D GC [2]. A wax column placed before an alumina column will retain polar compounds while the light hydrocarbons elute near the void volume. Therefore, if a Deans switch is placed between the columns, the hydrocarbons can be heart-cut from the wax to the alumina columns while oxygenates are held by the wax column. The optimized thermal and pneumatic performance of the Agilent 7890A Deans switch is a result of Capillary Flow Technology. This provides the high levels of retention time precision and narrow peak shape needed for optimal heart-cutting 2-D GC (Figure 1).

Highlights

- The Agilent 7890A GC Capillary Flow Technology combined with enhanced electronic pneumatics control (EPC) provide greater productivity and flexibility in the analysis of trace contaminants in ethylene.
- Multiple auxiliary EPC channels provide the ability to automatically generate gas calibration standards for trace level impurities.
- Enhancement of ASTM D6159 method with 2-D GC Deans switching measures trace oxygenates and hydrocarbons in a single run.

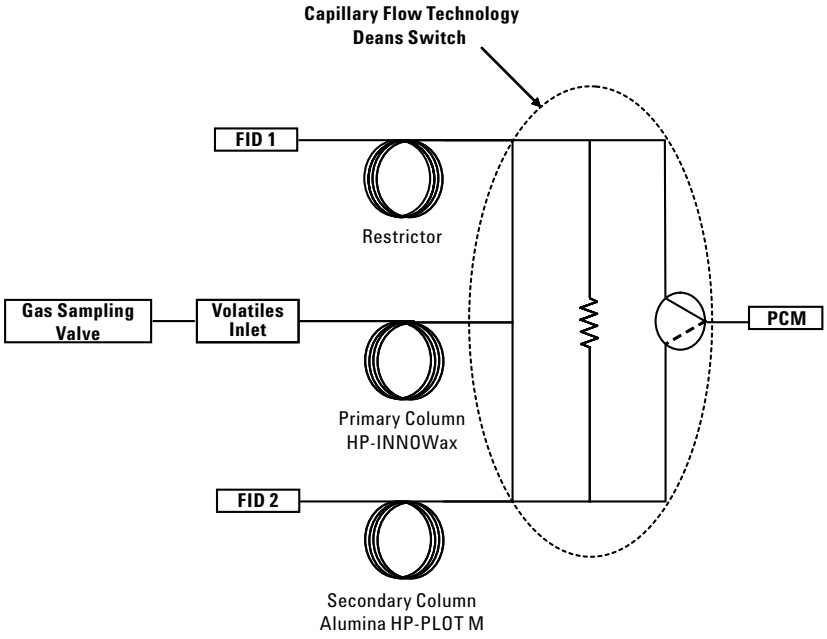

Figure 1. Configuration of Agilent 7890A for the 2-D GC analysis of trace oxygenates and hydrocarbons in ethylene.

Method Parameters for Enhanced ASTM D6159 Method

Primary column:	HP-INNOWax, 30 m × 0.32 mm id × 0.5 µm film (19091N-213)
Primary column flow:	Helium at 2.5 mL/min
Secondary column:	Alumina HP-PLOT M, 30 m × 0.53 mm id × 15 µm (19095P-M23)
Secondary column flow:	Helium at 6 mL/min
Oven temperature program:	40 °C for 6 min, 4 °C/min to 125 °C
Volatiles inlet conditions:	150 °C, 5:1 split
Sample loop:	250 µL at 65 °C
Detector temperature:	250 °C
Capillary Flow Technology: Deans switch cut time	2.3 to 4.5 min

Automating the Preparation of Trace-Level Calibration Standards

Another advantage of the Agilent 7890A GC is the expanded capabilities in EPC. These extra channels of auxiliary EPC are used with the dynamic blending system hardware to allow automated preparation of ppmV gas standards for calibration. This approach has been described for the automated preparation of trace sulfur compounds in various gas matrices [3].

Results

Figure 2 shows the 2-D GC analysis of methanol and C1 to C4 hydrocarbons in a sample of technical grade ethylene. The HP-INNOWax column first separates the polar methanol from the unresolved hydrocarbon peaks. The Deans switch transfers the hydrocarbons to the Agilent alumina HP-PLOT M column, where the C1 to C4 hydrocarbons are easily separated. This column is also shown to provide better separation of trace hydrocarbons from the large ethylene peaks, while maintaining excellent peak shape and intensity for the acetylene. The performance of this alumina column is maintained over many injections since the HP-INNOWax column prevents polar oxygenates (water, alcohols) from damaging the sensitive stationary phase. Table 1 shows very good precision using this method for a sample containing approximately 2 ppmV.

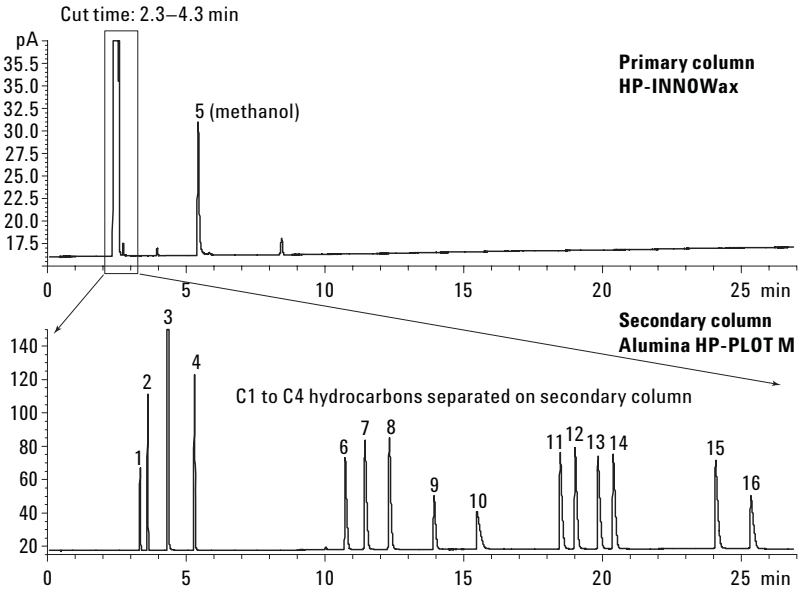

Figure 2. Capillary Flow Technology Deans switch used to separate 100 ppmV oxygenate and hydrocarbon impurities in ethylene.

Table 1. Method Precision for 2-D GC Analysis of Ethylene Impurities

Peak No.	Name	Avg. (ppmV)*	Std Dev*	%RSD*
1	Methane	2.1	0.011	0.5
2	Ethane	21.5	0.049	0.2
3	Ethylene	Balance	Balance	Balance
4	Propane	2.1	0.062	3.0
5	Methanol	2.1	0.081	3.8
6	Propylene	2.1	0.023	1.1
7	Isobutane	2.1	0.015	0.7
8	n-Butane	2.0	0.011	0.5
9	Propadiene	2.1	0.025	1.2
10	Acetylene	1.9	0.036	1.9
11	Tran-2-butene	2.1	0.011	0.5
12	1-Butene	2.0	0.013	0.7
13	Isobutylene	2.1	0.016	0.8
14	cis-2-butene	2.1	0.017	0.8
15	1,3-Butadiene	2.1	0.018	0.9
16	Methylacetylene	2.0	0.015	0.7

*Sample run 20 times

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Printed in the USA
January 5, 2007
5989-6082EN



Agilent Technologies

High-Pressure Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Accessory G3505A

Introduction

Gas chromatography sampling and representative analysis of highly volatile liquefied hydrocarbons with high precision and accuracy can be challenging. In the solution described here, a unique sample injection device based on a needle interface and liquid rotary valve, has been designed for sampling light petroleum matrices with broad boiling point distributions. The 7890A GC-based system consists of a 4-port liquid valve, a deactivated removable needle, and an auxiliary flow. The needle is directly installed on one port of the valve. This compact device is installed directly over the top of a split/splitless inlet. The unit is operated automatically just like a typical liquid autosampler; however, the needle is not withdrawn. Various pressurized liquid samples have been run on this device, such as liquefied natural gas (calibration standard), ethylene, propylene, and butadiene. Excellent repeatability is obtained with RSDs typically below 1% in quantitative analyses.

Injection Device

The high-pressure injection device (HPLI) consists of components as shown in Figure 1.

- **Valve:** Internal sample valve from Valco Instruments Co. Inc. 4-port equipped with a sample volume of 0.06 µL. Other rotor sizes are available from Valco Instruments Company.
- **EPC:** An auxiliary flow from a 7890A Aux module is connected to port P. In sample analysis, the flow can be set at 50 mL/min to 200 mL/min. The higher auxiliary flow gives better peak shape.

Ordering Information

Order accessory G3505A. The accessory is compatible with both the 7890A and 6890 series GCs.

The following components are recommended. These are not supplied in the accessory kit.

- **Filter:** To remove particles from samples.
- **Restrictor:** To maintain sample pressure, a metering valve (Agilent PN 101-0355) is connected to the end of the sample exit line tubing. Restrictor is not included in accessory kit.

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

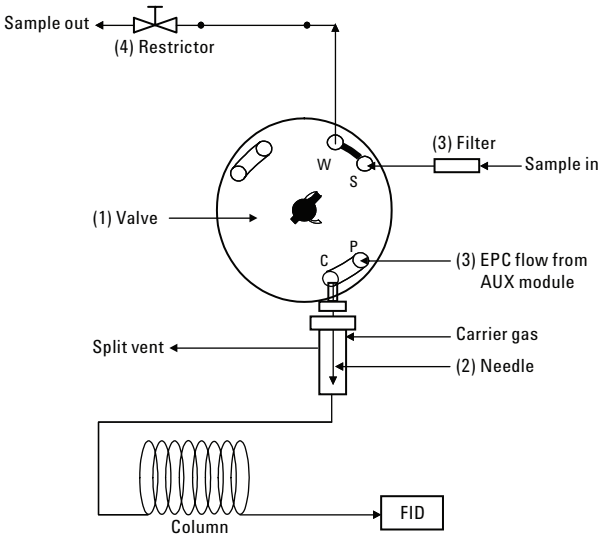


Figure 1. Flow diagram of the high-pressure injection device (HPLI).

Typical Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	High-pressure injection device (HPLI) at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.06 µL
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H_2, 35 mL/min Air, 400 mL/min

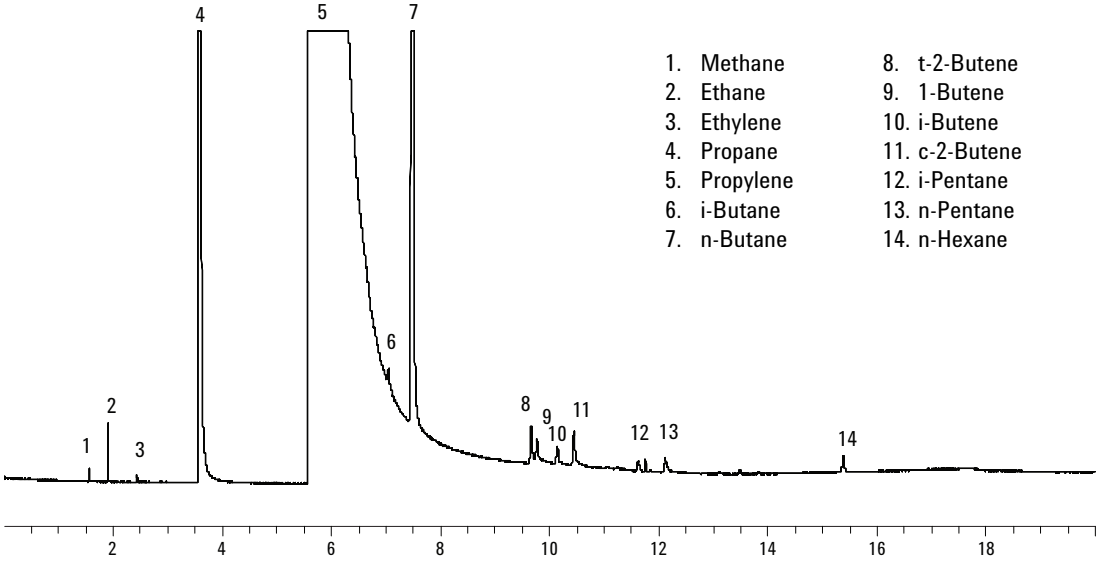


Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Sample Chromatograms

Pressurized Propylene

This sample is analyzed by the same conditions as in ASTM D6159. A typical chromatogram is shown in Figure 2.



1. Methane
2. Ethane
3. Ethylene
4. Propane
5. Propylene
6. i-Butane
7. n-Butane
8. t-2-Butene
9. 1-Butene
10. i-Butene
11. c-2-Butene
12. i-Pentane
13. n-Pentane
14. n-Hexane

Figure 2. Chromatogram of pressurized propylene.

Pressurized 1,3-Butadiene

Figure 3 is an example of C4 hydrocarbons analysis showing 1.3 butadiene purity.

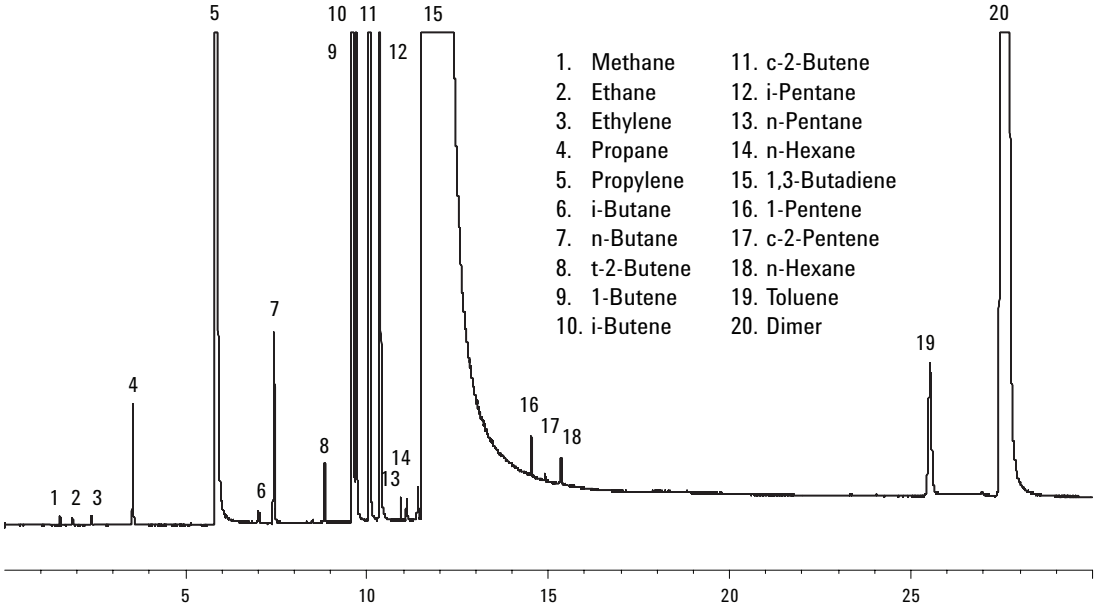


Figure 3. Chromatogram of pressurized 1,3-butadiene.

Summary

A unique sample injection device for the Agilent 7890A GC based on a unique deactivated interface and liquid rotary valve has been designed for sampling light petroleum matrices with broad boiling point distributions from methane to as high as C40. It is installed directly over a split/splitless GC split/splitless inlet in a few minutes. The maximum sample pressure is 3,000 psig, although typical samples will have pressures under 1,500 psig. Various pressurized liquid samples have been tested on this device with high accuracy and precision. The sampler is quick to install and easy to operate. As with all high-pressure sampling systems, appropriate safety precautions must be followed.

Competitive Advantages

The HPLI can be used with a wide variety of sample streams or pressurized vessels. Because the sampling valve is interfaced directly to the inlet with an inert needle, loss or adsorption of analytes is minimized compared to conventional liquid sample valve systems. Compared to other gas chromatographic vaporizers for handling pressurized or nonpressurized samples, the Agilent HPLI has the following advantages:

- Better results with polar analytes such as glycols
- Superior inertness
- Low discrimination (no discrimination up to C_{16})
- Flexibility: Install or uninstall in less than 10 minutes
- Good for trace impurity analysis with 0.5 µL rotor
- Excellent repeatability, typically RSDs below 1 %

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Printed in the USA
February 25, 2008
5989-8037EN



Agilent Technologies

Investigation of the Unique Selectivity and Stability of Agilent GS-OxyPLOT Columns

Application

Gas Chromatography

Authors

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Abstract

The stationary phase of a GS-OxyPLOT column is a proprietary, salt deactivated adsorbent. GS-OxyPLOT columns show unique selectivity to oxygenated hydrocarbons, excellent stability and reproducibility, long column lifetime, and a wide application range.

Introduction

The determination of oxygenated hydrocarbons in different sample matrices is very important for the petrochemical industry, because oxygenates directly influence product quality. Presence of such oxygenates may cause the catalysts to be poisoned and deactivated, resulting in more downtime and higher costs. ASTM has developed several methods for analysis of oxygenates, such as ASTM D7059, D4815, and D5599. The oxygenates include ethers, esters, ketones, alcohols, and aldehydes.

Methanol is one of the oxygenates that often present in light hydrocarbon streams. For example, it is added to natural gas and production of crude oil to prevent hydration of hydrocarbons during transportation via pipelines. Therefore, it is important to accurately measure the content of methanol from light hydrocarbons at different concentrations, including at trace levels.

To achieve this, a new porous layer open tubular (PLOT) capillary column, the GS-OxyPLOT column, was used. The stationary phase of the GS-OxyPLOT is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons, while having virtually no interactions with saturated hydrocarbon solutes [1].

Using Capillary Flow Technology, such as backflush or Deans switch, GS-OxyPLOT columns can provide a turnkey solution for the analysis of trace level oxygenate impurities in complex matrices, such as motor fuels, crude oil, and gaseous hydrocarbon [2]. Meanwhile, a GS-OxyPLOT column can be used as a single analytical column to separate oxygenates for some samples. In this application, methanol was set as an example to investigate the performance of the GS-OxyPLOT column.

Experimental

The experiments were performed on an Agilent 7890A GC system and a 6890N GC system equipped with split/splitless capillary inlet, flame ionization detector (FID), and Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with long-lifetime septa (Agilent p/n 5183-4761) and spilt/splitless injection liners (Agilent p/n 5183-4711). Injections were done using 10-µL syringes (Agilent p/n 9301-0714). A glass indicating moisture trap (Agilent p/n LGMT-2-HP), an oxygen trap (Agilent p/n BOT-2), and a

hydrocarbon trap (Agilent p/n 5060-9096) were installed. Agilent ChemStation was used for all instrument control, data acquisition, and data analysis.

Results and Discussion

Analysis of Normal Hydrocarbons and Methanol

A mixture of normal hydrocarbons and methanol was prepared with the following approximate concentrations %(w/w): 34.8% n-pentane, 12.8% n-hexane, 1.8% n-heptane, 1.9% n-octane, 2.1% n-nonane, 3.9% n-decane, 2.1% n-undecane, 9.8% n-dodecane, 11.8% n-tridecane, 4.7% n-tetradecane, 2.4% n-pentadecane, 4.5% n-hexadecane, 2.4% n-heptadecane, 1.0% n-octadecane, 0.9% n-eicosane, 0.9% n-docosane, 1.1% n-tetracosane, and 0.8% methanol.

The analytical conditions are summarized in Table 1. The normal hydrocarbons and methanol analysis was performed on a GS-OxyPLOT column (Agilent p/n 115-4912). The GC chromatogram is shown in Figure 1.

Table 1. Conditions for Normal Hydrocarbons and Methanol Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm (Agilent p/n 115-4912)
Carrier gas	Helium, constant flow mode, 40 cm/s @ 50 °C
Inlet	Split/splitless at 325 °C
Split ratio	80:1
Oven temperature	50 °C (2 min); 10 °C/min to 290 °C (2 min)
Post-run	300 °C (2 min)
Detector	FID at 325 °C
Injection size	0.2 µL

In Figure 1, the GS-OxyPLOT column shows unique retention characteristics for methanol. The lower boiling point hydrocarbons were not strongly retained on the stationary phase and eluted through the FID very rapidly. The methanol eluted after n-C14, allowing it to be quantified without any interference from the hydrocarbon matrix, and making it feasible for trace-level methanol analysis in a range of hydrocarbon streams.

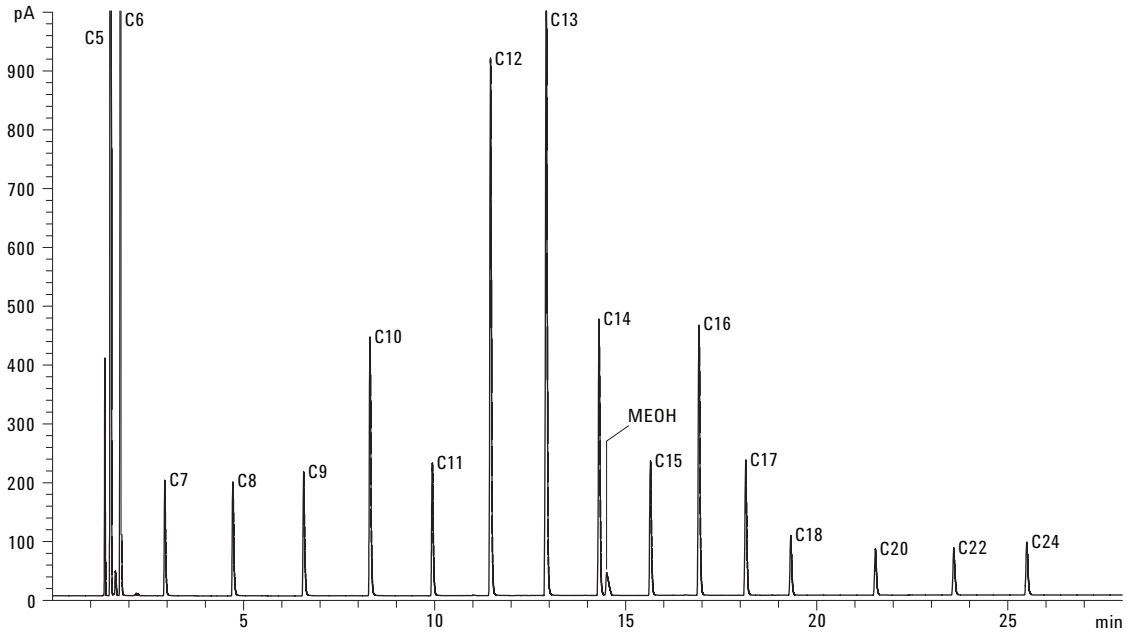

Figure1. Analysis of methanol and normal hydrocarbons on a GS-OxyPLOT column, 10 m × 0.53 mm × 10 µm.

In addition, the baseline was quite smooth, even when the oven temperature was up to 290 °C. GS-OxyPLOT has an upper temperature limit of 350 °C and exhibits virtually no bleed, making it widely applicable for long-term reliable analysis.

Analysis of Alcohols

A mixture containing a range of primary alcohols from methanol to lauryl alcohol was analyzed on a GS-OxyPLOT column using a temperature-programmed method. Table 2 lists conditions for alcohols separation, and the resulting chromatogram is shown in Figure 2.

Sample

The sample had an approximate concentration (v/v) of 1% methanol, ethanol, propanol, butanol, amyl-alcohol, heptanol, octanol, nonanol, decyl alcohol, and lauryl alcohol in toluene.

As can be seen in Figure 2, all of the alcohols are separated and eluted with good peak shape within an analysis time of 15 min. In this experiment, oven temperature was set up to 300 °C. Thanks to its advanced dynamic coating process, Agilent's GS-OxyPLOT stationary phase exhibits virtually no detector spiking due to particle generation from the phase coating [3].

Due to the high viscosity of alcohols, especially decyl alcohol and lauryl alcohol, it is necessary to wash the needle after each injection in case of carryover problems.

Table 2. Conditions for Alcohols Analysis

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier Gas	Helium, constant flow mode, 40 cm/s at 150 °C
Inlet	Split/splitless at 325 °C
Split ratio	50:1
Oven temperature	150 °C (0 min); 10 °C/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	0.2 µL

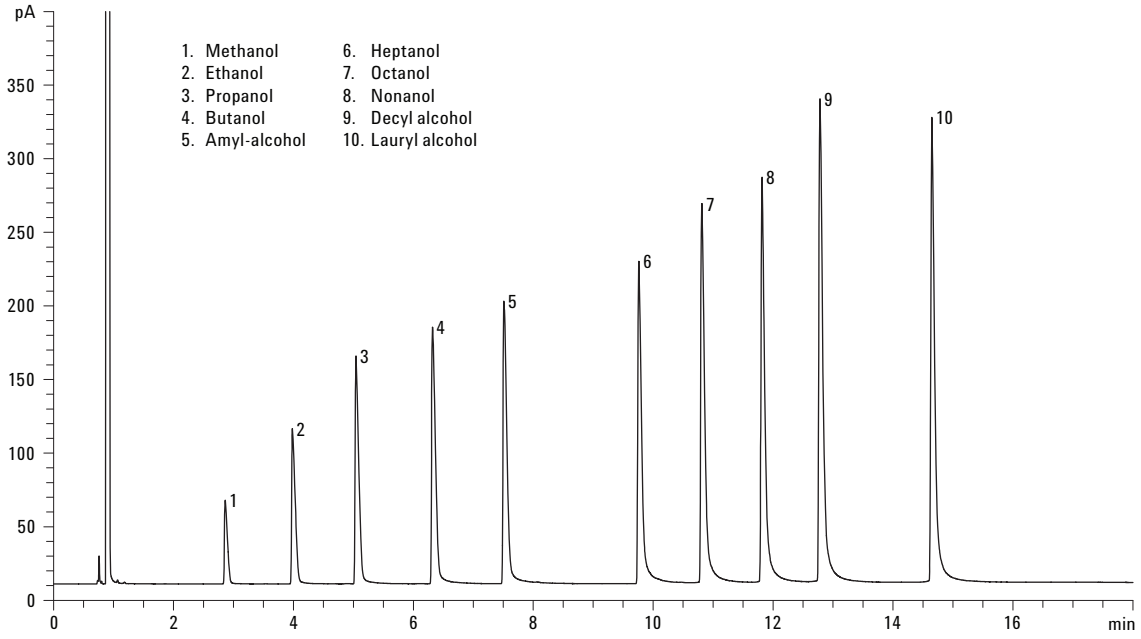

Figure 2. Separation of alcohols using GS-OxyPLOT, 10 m × 0.53 mm × 10 µm.

Influence of Temperature on the Selectivity of GS-OxyPLOT

To polar stationary phases, the temperature has a direct influence on the selectivity. GS-OxyPLOT offers extremely high polarity. The analysis of normal hydrocarbons and methanol demonstrated that methanol elutes after n-C14. Using a mixture containing methanol, n-tetradecane, and n-pentadecane, isothermal Kovats retention indices were tested at isothermal oven temperatures of 150, 200, 220 and 250 °C, respectively (Table 3). The relationship between Kovats retention indices and oven temperature is shown in Table 4.

Table 3. Conditions for Kovats Retention Indices Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 30 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temperature	150, 200, 220, and 250 °C, respectively; isothermal
Detector	FID at 250 °C
Injection size	0.2 µL

Table 4. Kovats Retention Indices and Oven Temperature (n > 3)

Oven temp.	150 °C	200 °C	220 °C	250 °C
LOT1	1419	1418	1418	1413
LOT2	1420	1421	1419	1417

Retention index, Ix, was calculated using the following equation:

$$Ix = 100n + 100[\log(t_x) - \log(t_n)]/[\log(t_{n+1}) - \log(t_n)]$$

Where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound X; t_x is the retention time of compound X. Here compound X is methanol, the reference n-alkane hydrocarbons are n-tetradecane and n-pentadecane, respectively.

Table 4 shows good repeatability of Kovats rentention indices for two different lots of GS-OxyPLOT columns. The retention index for methanol only changed by less than 10 index units over 100 °C temperature difference. Therefore, when the oven temperature changes from 150 to 250 °C, it has little influence on the selectivity of GS-OxyPLOT.

Influence of Moisture on GS-OxyPLOT

Some PLOT columns can adsorb water, which can lead to changes in retention times and selectivity for analytes. Therefore, column performance will be influenced greatly in the presence of water. Although cumbersome solvent-extraction procedures can be performed before injection, injecting sample that contains water is, in some cases, unavoidable.

From a GC point of view, water is a less-than-ideal solvent. The problems associated with water include large vapor expansion volume, poor wet ability and solubility in many stationary phases, detector problems, and perceived chemical damage to the stationary phase. In order to test the effect of water, a GS-OxyPLOT column that had gone through about 1,500 runs was tested before and after injecting 100% aqueous samples.

Water has a large vapor expansion volume; the vapor volume of water (assuming a 1-µL injection) can easily exceed the physical volume of the injection liner (typically 200 to 900 µL). The volume for the liner used in this experiment (Agilent p/n 5183-4711) is 870 µL, so the injection volume was set as 0.2 µL. Table 5 lists the conditions for the moisture testing, and the resulting chromatograms are shown in Figure 3.

Table 5. Conditions for Moisture Test

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 38 cm/s at 150 °C
Inlet	Split/splitless at 300 °C 15:1 split ratio
Oven temperature	150 °C isothermal, post-run: 300 °C (5 min)
Detector	FID at 300 °C, H2:45mL/min, air: 400 mL/min, makeup: 30 mL/min
Injection size	0.2 µL
Sample	0.1% n-Dodecane, Methyl tert-butyl ether, n-Tridecane, Iso-Butyraldehyde, n-Tetradecane, Methanol, Acetone, and n-Pentadecane

As shown in Figure 3, the area of n-pentadecane remained the same before and after 100 injections of water. However, compared with the area before injecting water, the area of methanol (peak 6) decreased by 50%, and the area of acetone (peak 7) decreased by14.4% after 100 injections of water (see Table 6). It demonstrated that water can affect the activity of GS-OxyPLOT, especially for the analysis of those relatively low molecular weight oxygenated compounds, such as methanol and acetone.

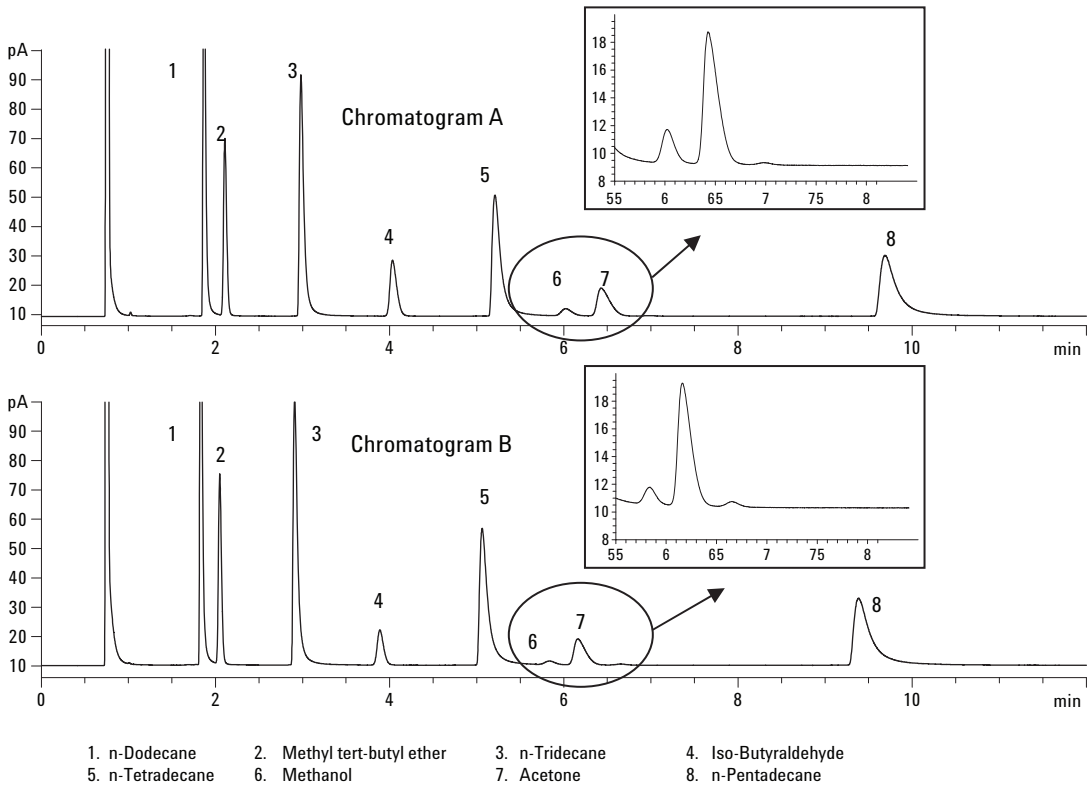

Figure 3. Comparison of test mixture separation before (A) and after (B) 100 injections of water.

As for retention times and column efficiency, they are not strongly influenced. After 100 injections of water, the retention time of C15 changed from 9.689 min to 9.384 min, and the column efficiency of C15 changed from 14,792 to 14,781.

Condition the column at 300 °C for two hours, followed by 12 hours at 250 °C. As shown in Figure 4 and Table 6, it is obvious that GS-OxyPLOT phase can be regenerated by conditioning.

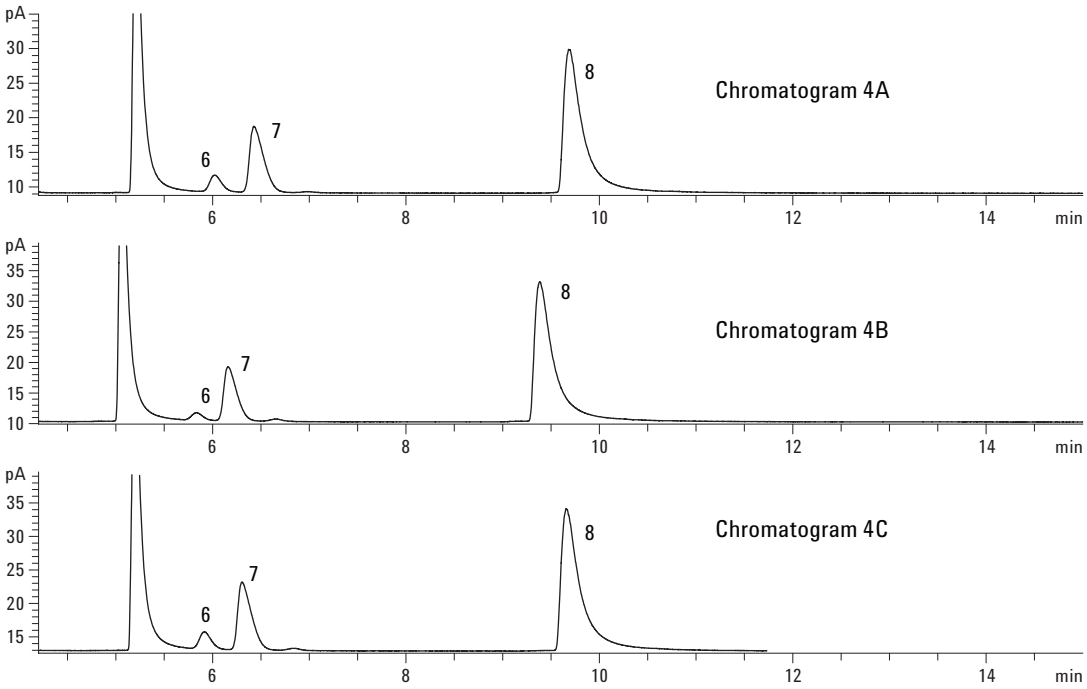

Figure 4. Expanded view shows comparison of test mixture separation on GS-OxyPLOT.
4A. Before injection of water. 4B. After 100 injections of water. 4C. After conditioning the column.

Table 6. Comparison of Test Mixture Separation

	Methanol			Acetone			n-Pentadecane		
	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column	Before injection of water	After 100 injections of water	After conditioning column
RT (min)	6.022	5.835	5.915	6.429	6.160	6.305	9.689	9.384	9.658
Area	20.23	9.18	20.88	94.53	80.92	98.07	277.79	287.7	287.9
Plates	11887	12920	11616	9532	10357	9573	14792	14781	15100

After conditioning the GS-OxyPLOT column, the peak area and retention time reproducibility were determined. Figure 5 and Table 7 show excellent RT precision, lower than 0.6% over five test mixture runs on this GS-OxyPLOT column. The peak area has a relative standard deviation (RSD%) below 2.5%. It proved that column performance can be restored via conditioning.

Determination of Methanol

The following analysis of methanol followed ASTM D7059 [4]: "Standard Test Method for Determination of Methanol in Crude Oils by Multidimensional Gas Chromatography." Methanol was determined by gas chromatography with FID using internal standard method with GS-OxyPLOT column.

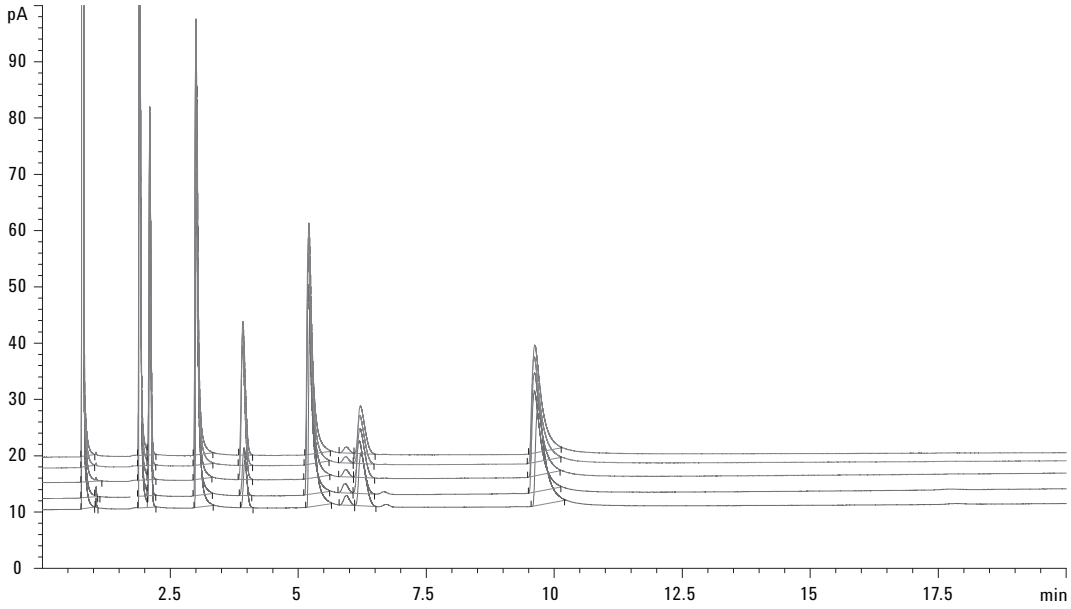

Figure 5. Fifth run overlaid using GS-OxyPLOT (after conditioning column).

Table 7. Peak Area Reproducibility and Retention Time Reproducibility on GS-OxyPLOT (after conditioning column)

Compound (by eluted order)	Dodecane	MTBE	Tridecane	Iso-Butyraldehyde	Tetradecane	MeOH	Acetone	n-C15
Area RSD% (N = 5)	1.18	1.58	1.59	2.49	1.15	2.12	1.98	1.82
RT RSD% (N = 5)	0.18	0.12	0.26	0.55	0.29	0.16	0.19	0.33

Reagents and Materials

Carrier gas, Helium, > 99.95% purity
Methanol, > 99.9% purity
1-propanol, > 99.9% purity, and containing < 500 ppm methanol
Toluene, > 99.9% purity, and containing < 0.5 ppm methanol

A set of calibration standards 5, 25, 125, 250, 500, 1,000 and 1,500 ppm (m/m) of methanol, and each containing 500 ppm (m/m) of 1-propanol internal standard, were prepared in toluene.

The calibration standard solutions should be stored in tightly sealed bottles in a dark place below 5 °C.

Linearity

Under the conditions listed in Table 8, the methanol calibration standards were analyzed. The linearity is shown by plotting the response ratio of methanol and internal standard 1-propanol against their amount ratio (see Figure 6). For methanol, good linearity was gained ranging from 5 to 1,500 ppm. The correlation r^2 value for the calibration curve is higher than 0.999.

Figure 7 and Figure 8 are chromatograms of methanol at a level of 5 ppm and 1500 ppm, respectively. At a relatively high concentration of 1500 ppm, methanol still could get a sharp peak. The limit of quantification (LOQ) was calculated to be 1 ppm using the chromatogram of 5 ppm methanol.

Table 8. System Settings for the Calibration Curve

Column	GS-OxyPLOT, 10 m × 0.53 mm × 10 µm
Carrier gas	Helium, constant flow mode, 50 cm/s at 150 °C
Inlet	Split/splitless at 250 °C 10:1 split ratio
Oven temperature	150 °C (3 min); 20/min to 300 °C (5 min)
Detector	FID at 325 °C
Injection size	1 µL

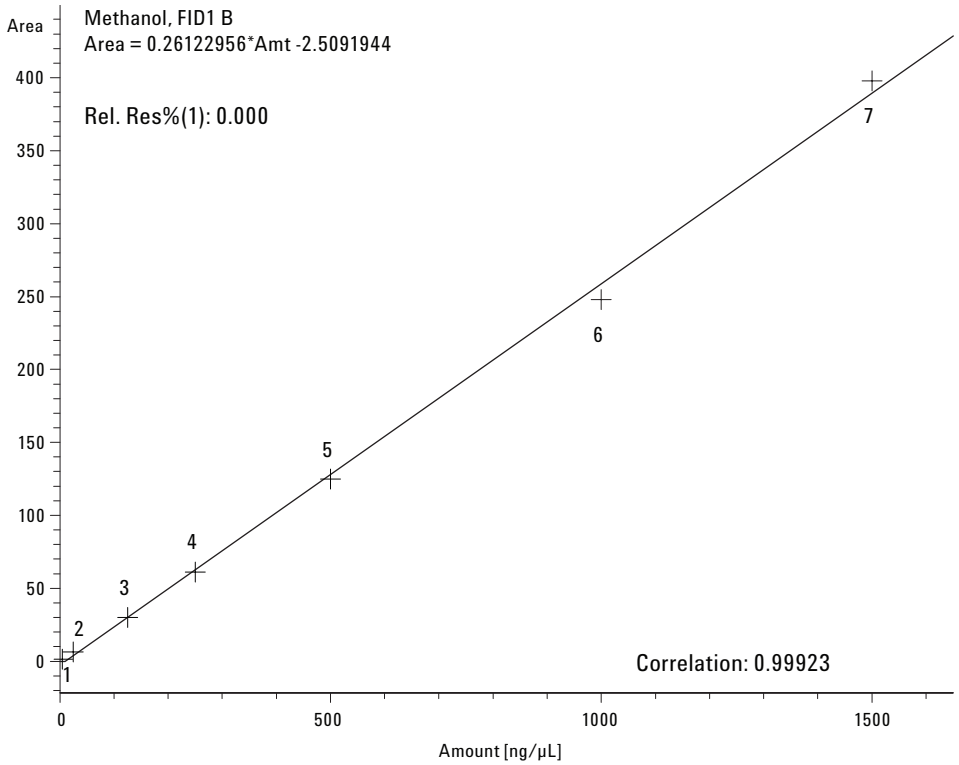

Figure 6. The calibration curve of methanol in toluene.

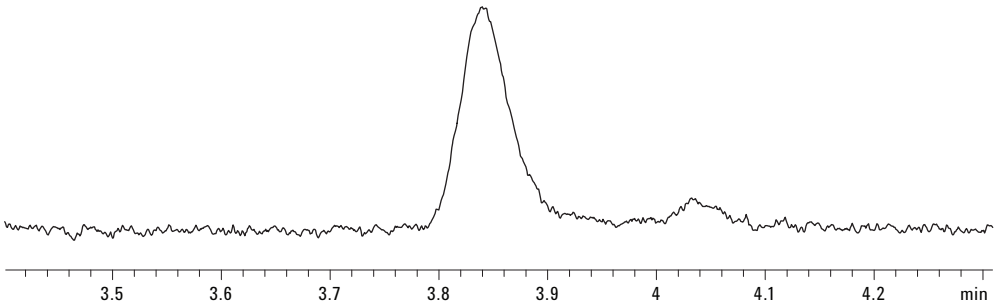

Figure 7. Test mixture of 5 ppm methanol in toluene.

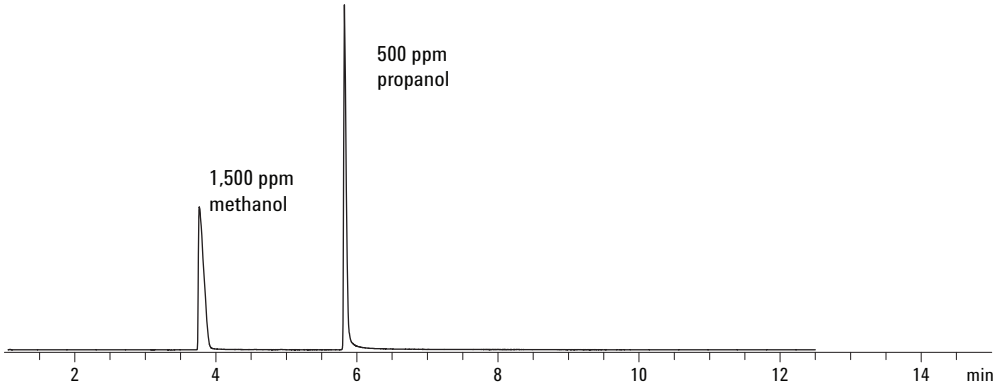

Figure 8. Test mixture of 1,500 ppm methanol in toluene.

Repeatability

The reproducibility of the GS-OxyPLOT is given in Table 9. Those values were obtained by the replicate analysis of different methanol levels (25, 125, and 1,500 ppm) in different days. The injection was done by ALS with RSD no less than 3% either intraday or interday analysis, which was very low for this type of determination.

Life Span

Under the conditions in Table 5, a mixture was analyzed with a GS-OxyPLOT column which went through 1,500 injections of methanol. It shows that the column has a long lifetime. The GS-OxyPLOT column still has good resolution for each compound and high efficiency of 1,482 plates per meter for n-pentadecane (see Figure 9).

Table 9. Relative Standard Deviations Intraday and Interday at Different Levels (25, 125, and 1,500 ppm) of Methanol

Day	25 ppm (average)	RSD (%)	125 ppm (average)	RSD (%)	1,500 ppm (average)	RSD (%)
D 1	25.2	0.46	123.9	0.45	1507.3	0.55
D 2	25.3	1.53	123.2	0.79	1494.4	0.45
D 3	24.4	0.36	125.4	1.71	1523.5	0.35
D 4	25.9	1.06	123.0	0.90	1537.8	0.51
D 5	23.9	0.44	121.1	0.76	1502.4	1.03
Stand. dev.	0.7		1.70		17.4	
Average	24.97		123.6		1513.1	
RSD (%)	2.8		1.37		1.15	

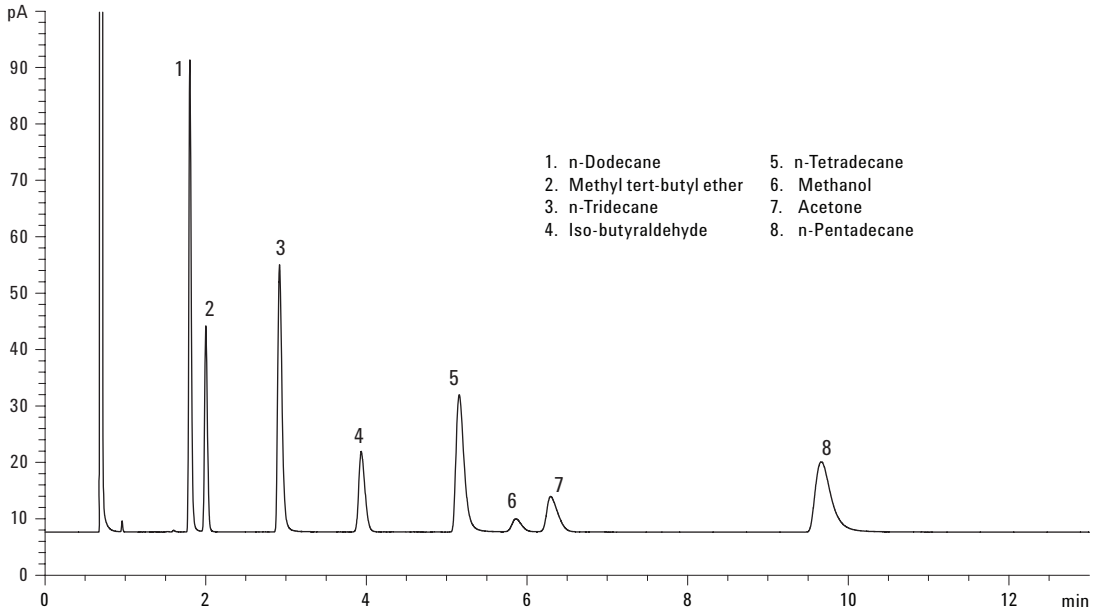

Figure 9. Chromatogram of performance mixture after 1,500 injections.

Conclusions

GS-OxyPLOT provides good retention and selectivity for oxygenated compounds. Normal alkanes up to C24 and primary alcohols up to lauryl alcohol can elute from GS-OxyPLOT within its program temperature maximum limit of 350 °C. Methanol elutes after n-C14 with retention index higher than 1,400; the retention index is quite stable from 150 to 250 °C, allowing methanol to be measured at low levels in a wide range of hydrocarbon streams.

Methanol has to be measured usually at specs as low as 5 ppm. From 5 to 1,500 ppm, it shows good linearity on GS-OxyPLOT. And the column has proven extremely stable with long lifetime.

GS-OxyPLOT can tolerate a little amount of water in samples, and column performance can be restored via conditioning.

GS-OxyPLOT can be used for a single-column system or in multidimensional GC systems. It offers a unique solution for the analysis of oxygenates in the chemical and petrochemical industries.

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4. Standard test method for the determination of methanol in crude oils by multidimensional gas chromatography, ASTM D7059-04, July 2004

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Printed in the USA
June 17, 2008
5989-8771EN



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Gradient LC analysis of herbicides and polyaromatic hydrocarbons by isocratic Capillary Electrochromatography

Gordon Ross,
Thomas Adam
and Monika Dittmann

Environmental/chemical

Abstract
Capillary Electrochromatography (CEC) combines the separation principle of HPLC (partitioning between mobile and stationary phases) with the high efficiency of capillary electroseparation methods. In CEC the electroosmotic flow (EOF) inherent in capillary electrophoretic separations is used to transport solute and mobile phase through a packed capillary column. The properties of the EOF provides higher efficiencies than can be realized with LC. This can be sufficient to allow the transfer of methods conventionally performed by gradient LC to be performed by isocratic CEC.

Experimental
All CEC experiments were performed using the Agilent CE system, equipped for CEC operation and with a built in diode array detector. The system includes an Agilent ChemStation for system control, data collection and data analysis. CEC columns were supplied by Agilent Technologies. Buffer salts were of the highest purity available and organic solvents were HPLC grade. All buffers were filtered and degassed prior to use. Buffers/mobile phase were adjusted to pH prior to the addition of organic modifiers.

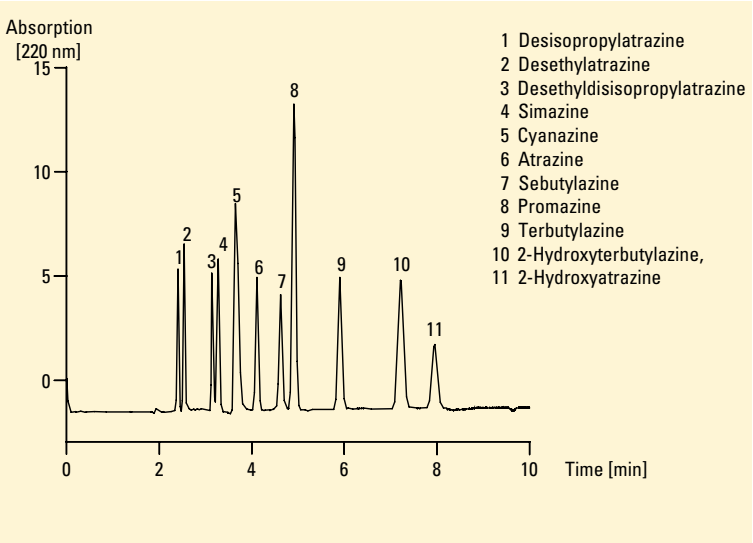

Figure 1
Isocratic CEC alternative to gradient HPLC separation of herbicides

Figure 1 shows the separation of a series of herbicides by CEC. The separation is normally achieved using gradient elution LC. The same is true for figure 2. Here the analysis is of polyaromatic hydrocarbons

Conditions

Column
250 mm × 100 µm; Sperisorb ODS1
Mobile Phase
60 % acetonitrile/40 % 25 mM TRIS pH 8
Voltage
30 kV
Temperature
15 °C

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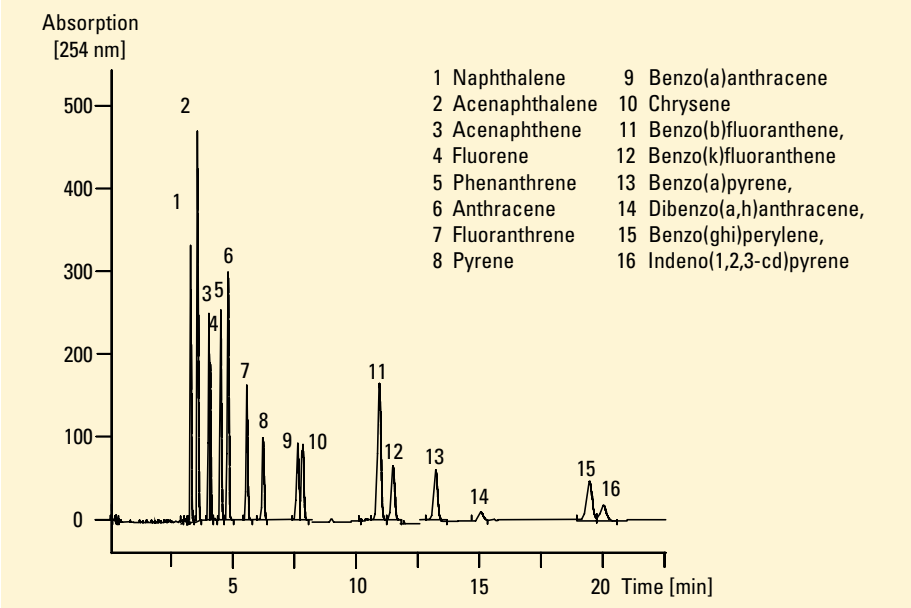


Figure 2
Fast CEC separation of EPA 16 PAH standard on CEC hypersil C18

which are of environmental significance and interest. Conventional analysis of these compounds can be achieved in a similar time however with isocratic CEC operation there is no inter-analysis time required for re-generation of the LC column.

Conclusions

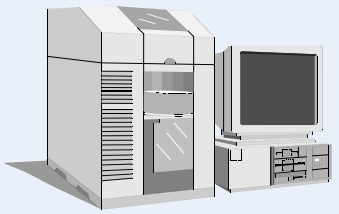
Some gradient LC separations can be succesfully performed using isocratic CEC. Very similar separations can be achieved in the same time frame. Time for re-equilibration of the LC column is not needed and therefore the overall analysis time is reduced.

Conditions

Column
CEC Hypersil C18, 250 mm (350 mm) × 0.1 mm i.d., 2.5 µm
Cell Standard
Eluent
90 % TRIS-HCl 50 mM, pH 8
Voltage 30 kV
Temperature 20 °C
Pressure 10 bar both sides

Equipment

- Agilent Capillary Electrophoresis System
- Agilent ChemStation + software



Monika Dittmann is an R&D chemist and Gordon Ross is an application chemists at Agilent Technologies, Waldbronn, Germany. Thomas Adam is at the University of Mainz, Germany.

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Released 06/97
Publication Number 5965-9032E

Analysis of Antioxidants and UV Stabilizers in Polymers using HPLC

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Additives are frequently used to protect polymers against thermo-oxidative degradation and destruction caused by UV irradiation. The following antioxidants and UV stabilizers were analyzed using reversed phase liquid chromatography and diode-array detection in technical styrene.

• Uvinol 3000 • Tinuvin P • Irganox 1098 • Uvinol 3008 • Lavinix BHT • Tinuvin 320 • Irganox 1010 • Irganox 1076 • Irgafos 168

Irganox 1010 for example, is a highly effective, non-discoloring stabilizer for organic substrates such as plastics, synthetic fibers, elastomers, waxes, oils and fats. Tinuvin P can be used to protect plastics against UV irradiation as it absorbs the UV light and transfers it into thermal energy which cannot destroy the polymer. Both compound classes have a wide ranging molecular structure and molecular weight. Irganox 1010 has a molecular weight of 1178 and its chemical structure is [3-(3,5-di-tert.butyl-4-hydroxyphenyl)-propionate]. Tinuvin P has a much lower molecular weight of 225 and its chemical structure is 2-(2'-hydroxy-5'-methyl-phenyl)-benzotriazol.

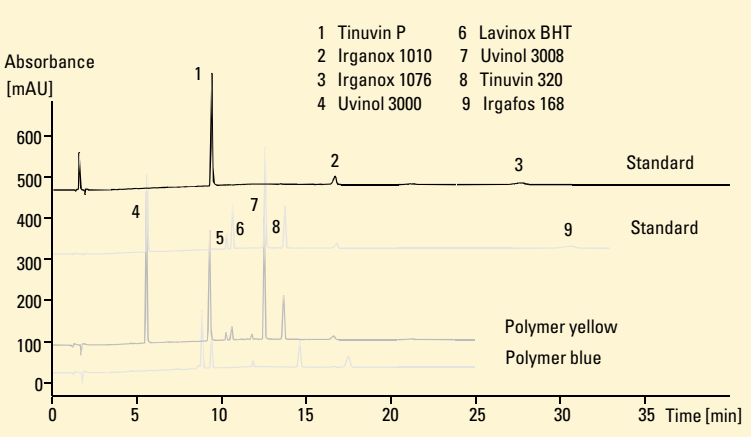

Figure 1
Analysis of antioxidants and stabilizers with the same conditions

Conditions

Column 125 x 3 mm BDS, 3 µm
Mobile Phase A = Water + 0.001 m Tetrabutylammoniumhydrogensulfate, pH = 3.0 with H_2SO_4, B = Acetonitrile
Gradient
Start with 30 % B, to 98 % B in 10 min
Flow Rate 0.5 ml/min
Injection Vol 5 µl
Oven Temp 40 ºC
UV-Detector DAD, 280/20 nm
Reference 900/50 nm

Sample preparation
Polymer samples were dissolved in Tetrahydrofurane and filtered after extraction with ultra-sonic bath for 30 min

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Antioxidants and UV stabilizers are typically added to polymers as a mixture of several compounds, which also includes costabilizers and antistatic agents. The application range of these additives is broad and can be found in most polymers.

All mentioned compounds are soluble in organic solvents and can be analyzed using reversed phase HPLC with ion-pairing modifier. In addition to the identification by retention time, UV spectra were used.

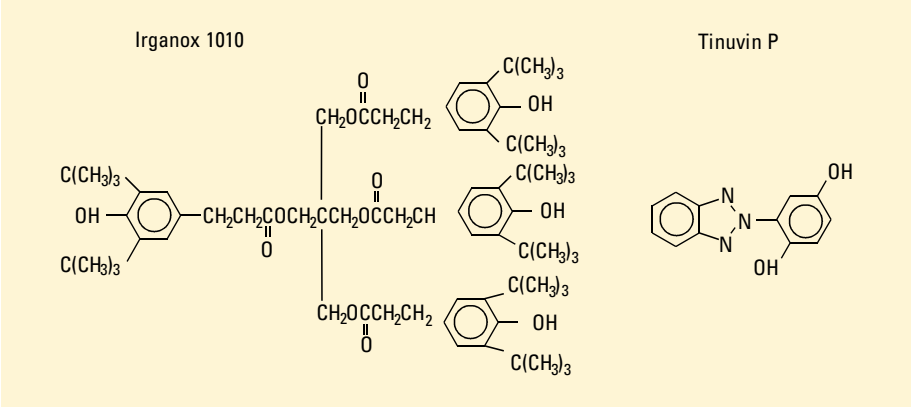


Figure 2
Formula of Antioxidants

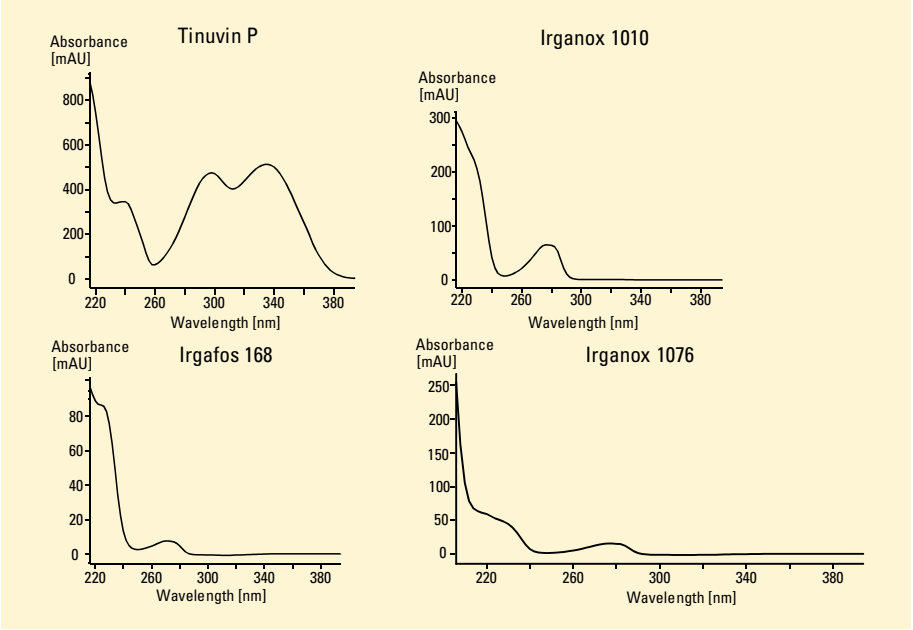


Figure 3
Spectra of antioxidants

Method performance

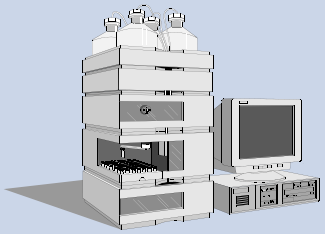
Limit of Detection (LOD) = < 1 ng
Precision of retention times (rsd) = 0.2 %
Precision of areas (rsd) = < 3 %

Equipment

Agilent 1100 Series

- degasser
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Analysis of Dyes in Plastics using HPLC

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Dyes which are used for coloring plastics have to fulfill special requirements. They have to be heat resistant, resistant against UV-irradiation and weatherproof. In addition, they should show strong coloring power and high brilliance. The analyzed colors in this application brief are soluble in organic solvents but are practically insoluble in water. This is important if the colored plastics are to be used in food packaging materials or in toys. Some of these colors are also used as coloring agents for polyamide fibers and other engineering plastics. The following colors were analyzed:

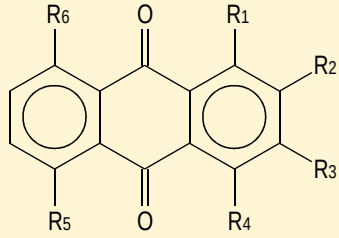

Figure 2
Chemical structure of dyes

Name	Color Index	Structure
Solvent yellow 21		Monoazo 1:2 chromo complex
Filamid violet RB		Monoazo 1:2 chromo complex
Disperse yellow 54	47020	Chinophthalon
Solvent red 52	68210	Anthraquinone
Macrolex blue 3R		Anthraquinone
Solvent blue 97		Anthraquinone

Table 1
Chemical structure of dyes

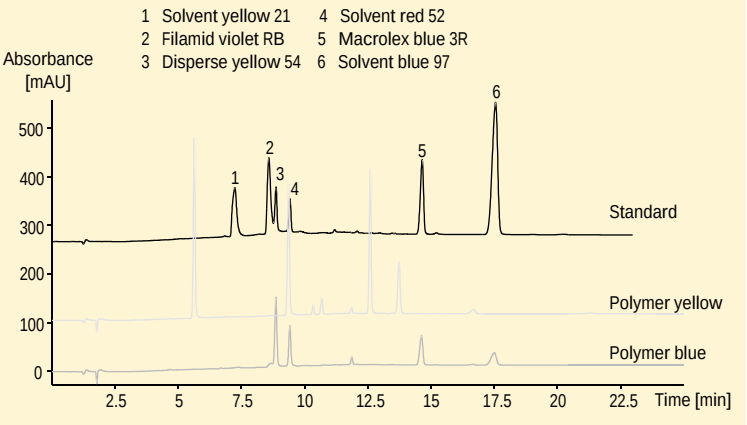

Figure 1
Standard chromatogram

Conditions

Column 125 x 3 mm BDS, 3 µm
Mobile Phase A = Water + 0.001 m Tetrabutylammoniumhydrogensulfate, pH = 3.0 with H_2SO_4, B = Acetonitrile
Gradient
Start with 30 % B, to 98 % B in 10 min
Flow Rate 0.5 ml/min
Injection Vol 5 µl
Oven Temp 40 °C
UV-Detector DAD,
280/20 nm Reference 900/50 nm
350/40 nm Reference 900/50 nm
465/40 nm Reference 900/50 nm
540/40 nm Reference 900/50 nm
600/40 nm Reference 900/50 nm
Sample preparation
Polymer samples were dissolved in Tetrahydrofurane and filtered after extraction with ultra-sonic bath for 30 min

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• Solvent yellow 21 • Filamid violet RB • Disperse yellow 54
• Solvent red • Macrolex blue 3R • Solvent blue 97

These dyes have different chemical compound classes, for example Anthraquinone type, Chinophthalon type and Monoazo-1:2-chromo complex type.

Method performance

The dyes in this analysis were analyzed using reversed phase HPLC with ion pairing compound in the mobile phase. A diode array detector was used as the detection system. Spectra which are very characteristic of this compound group were used as identification tools, in addition to the retention times.

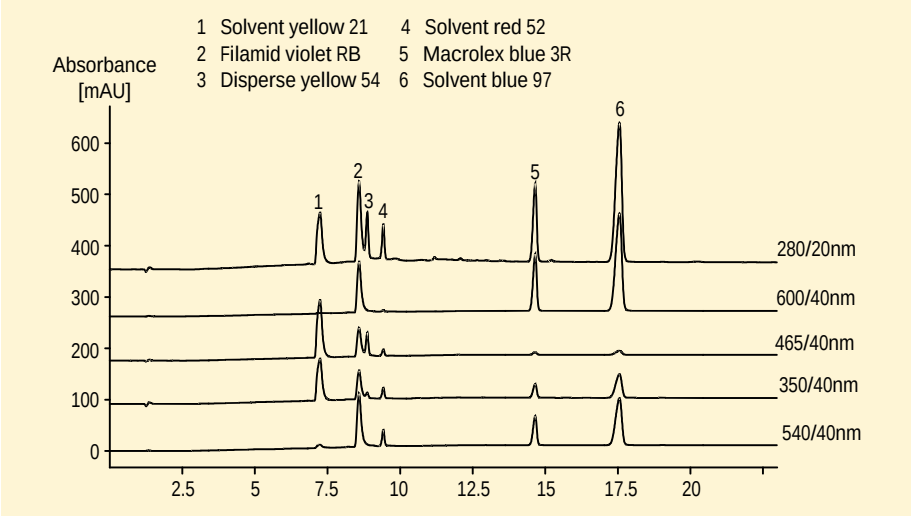


Figure 3
Analysis of dyes at different wavelengths

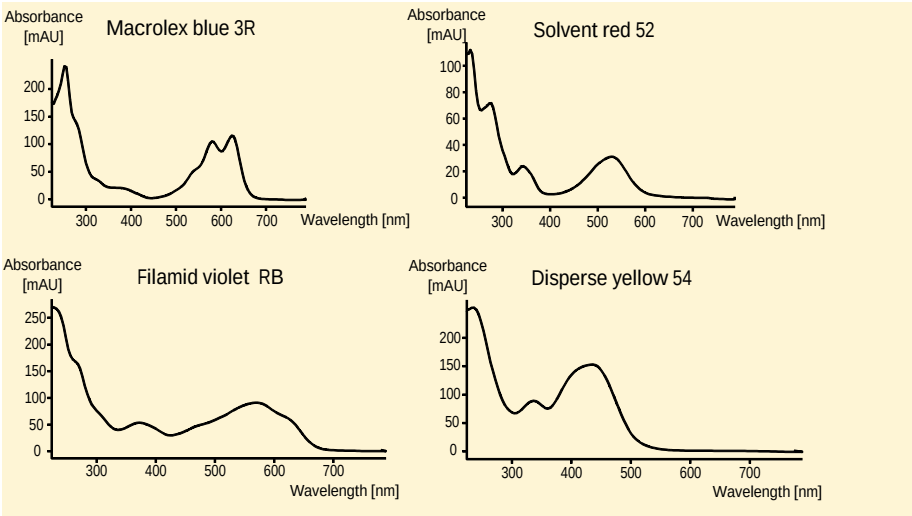


Figure 4
Spectra of polymer dyes

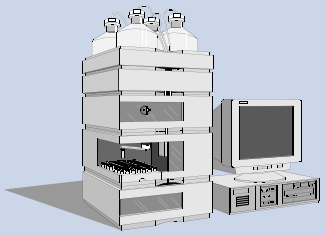
Method performance

Limit of Detection (LOD) = < 1 ng
Precision of retention times (rsd) = 0.2 %
Precision of areas (rsd) = < 3 %

Equipment

Agilent 1100 Series
- degasser
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Analysis of Polymethylmethacrylate (PMMA) using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polymethylmethacrylates are used as homo and co-polymers for the production of safety glasses, Plexiglas and glasses for optics, cars and dishes. The mol masses vary from 120000 to 180000 g/mol. In 1988 1.5 Mio.t. were used worldwide.

The performance of PMMA depends on the molecular weight of the polymer. To ensure quality, molecular weight (MW) data has to be evaluated for each batch of polymer that is produced. Gel Permeation Chromatography is an analytical tool for the characterization of polymers which are soluble in organic solvents. The separation is based on the differences in size of the polymer molecules, and provides primary result molecular weight distribution curves. This means that molecular weight data and quantitative data are calculated after calibration with standards of known molecular weight.

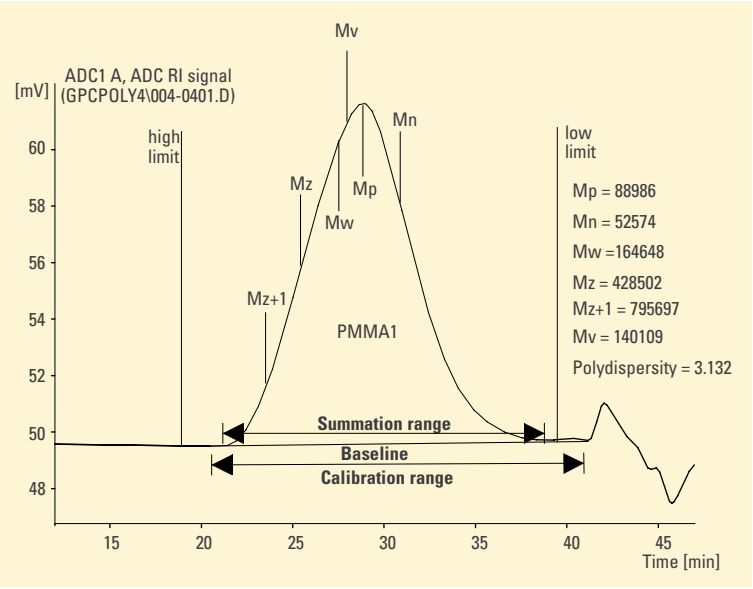

Figure 1
Standard Chromatogram

Conditions

Column
3 ˘ PSS GPC 8 ˘ 300 mm, 5 µm, 10^6, 10^5, 10^3 A
Mobile phase
Tetrahydrofurane (THF)
Flow rate
0.8 ml/min
Oven Temp
20 ºC
Injection vol 10 µl
Refractive index detector

Sample preparation
26 mg sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

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Method Performance

Having set up the chromatographic and GPC calculation procedures including the calibration, the polymer can be analyzed and MW and MWD (molecular weight distribution) data can be calculated. After analysis of the polymer, the baseline and summation range have to be defined. The baseline points should be selected within a flat part of the graph before and after the polymer peak. The summation range should be within the calibrated range and marked either side with lines indicating the high and low limits. The start and end points of the peak need to be carefully selected.

Method performance

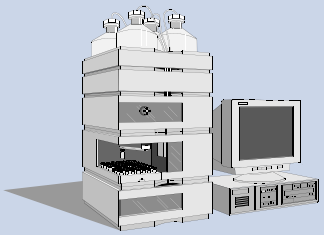
Precision of weight average molecular weight (rsd of Mw) = < 1 %
Precision of number weight average molecular weight (rsd of Mn) = < 2 %

Equipment

Agilent 1100 Series:

- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software



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Analysis of Polybutadiene using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polybutadiene is used as homo- and co-polymers for the production of tyres (70 %), moving belts and soles of shoes. In 1989 1.1 Mio.t. were used worldwide.

For example, the performance of tyres depends strongly on the molecular weight (MW) of the polybutadiene used and its additives. To ensure highest quality and consequently highest safety MW data need to be evaluated for each batch of produced polymer. Gel Permeation Chromatography (GPC) is an analytical tool used to characterize polymers which are soluble in organic solvents. In general an isocratic pump is sufficient for GPC analysis, however for ease of solvent change and rinsing, a pump with two or more channels would be advantageous. The pump should be able to pump the selected flow rate with a precision of typically < 0.15 %. Solvent degassing is recommended either offline or even better online with vacuum degassing. For high sample throughput the use of an autosampler would be beneficial. The temperature of the column oven needs to be very stable to avoid retention time shift and therefore MW errors—a peltier controlled thermostat is ideal for highest temperature stability especially at and below ambient temperatures

For detection a UV detector and/or a refractive index detector can be used. In this example we used both detectors to demonstrate, that results can be quite different, depending on the detection system used. The use of a diode array system enables the taking of spectra as an additional identification tool. This can help to identify for example remaining monomers (figure 1).

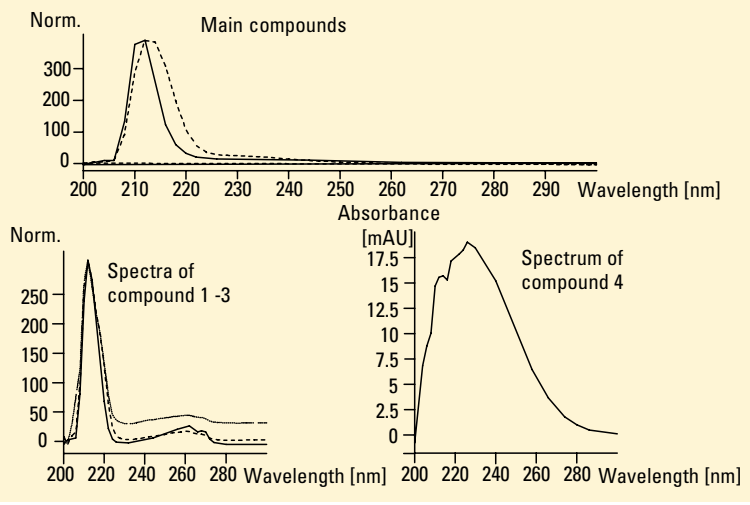

Figure 1
Spectra of oligomers and monomers

Method Performance

In figure 2 the different signal traces of UV-DAD and refractive index detection are shown. It can be seen that the calculated MW data differ significantly. In addition to MW data like Mw and Mn, GPC evaluation software also calculates molecular weight distribution curves (MWD),

which offer information about the relation between for example height percentage and log molecular weight or cumulative height percentage versus log molecular weight. (figure 3).

Method performance

Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight average: molecular weight (rsd of Mn) = < 2 %

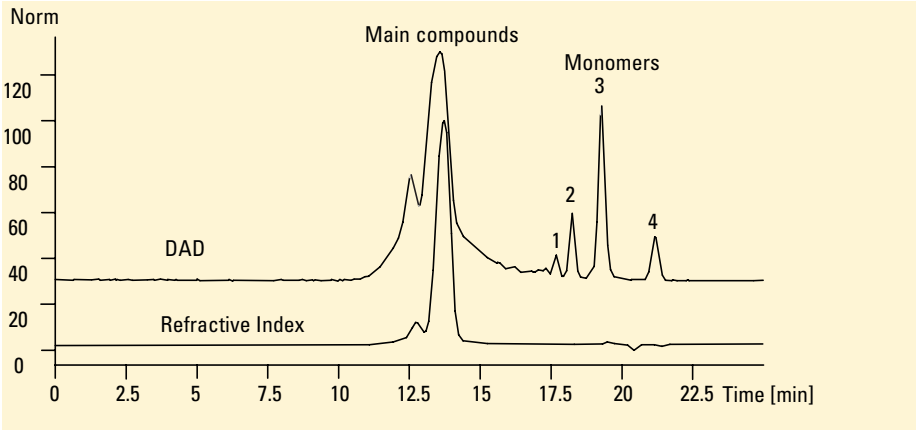

Figure 2
Analysis of polybutadiene using UV and refractive index

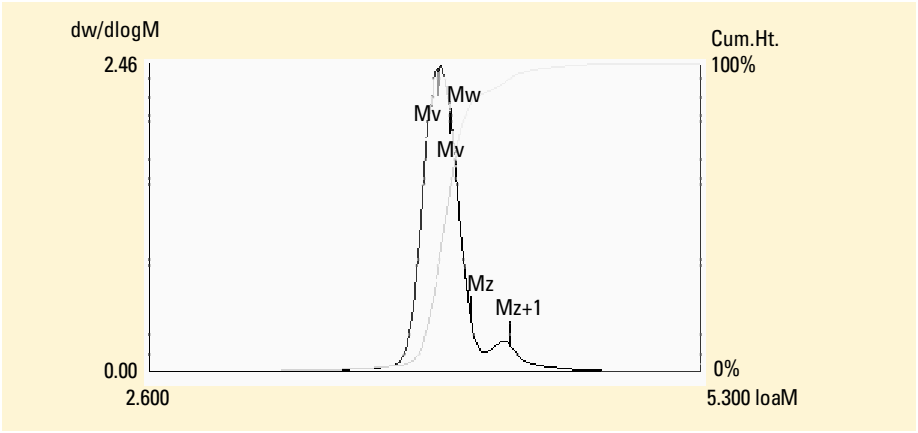

Figure 3
Molecular weight data based on refractive index detection

MW data	Refractive index	UV Detection
Mp	10283	10000
Mn	10543	6567
Mw	12054	13565
Polydispersity	1.143	2.066
Mz	14804	22037
Mz + 1	21860	35784
Mv	11780	12579

Table 1
Molecular weight data refactive index versus UV detection

Conditions

Column 2 × PLgel mixed-D, 7.5 × 300 mm, 5 µm
Mobile phase
Tetrahydrofurane (THF)
Flow rate 1 ml/min
Oven Temp 20 °C
Injection vol 20 µl
UV detector DAD 254/100 nm, reference 360/100 nm
Refractive index detector
Sample preparation
33 mg sample dissolved in 1 ml THF; Polystyrene standards from PSS were used for narrow standard calibration

Equipment

Agilent1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software

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Analysis of Polystyrene using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polystyrene is used as homo-, co-polymers, thermoplastic elastomers and foamed polystyrene (EPS) for the production of cabinets, housings, furniture, packing boxes and food packaging material. In 1988 7.1 million tons were used worldwide. The mol masses vary from 170000 to 1000000 g/mol.

Polystyrene has been around since 1839, but the current macromolecular structure of the molecules was discovered a lot later in 1920 by Staudinger. The first industrial product based on polystyrene was introduced in 1930, then in 1950, the first foamed polystyrene product was made commercially available. This was called styropor.

Since then the usage of this plastic has increased drastically and nowadays when used as co-polymer its application range is almost universal. To ensure the highest quality, molecular weight (MW) data have to be evaluated for each batch of produced polymer. Gel Permeation Chromatography (GPC) is an analytical tool used to charact-erize polymers which are soluble in organic solvents.

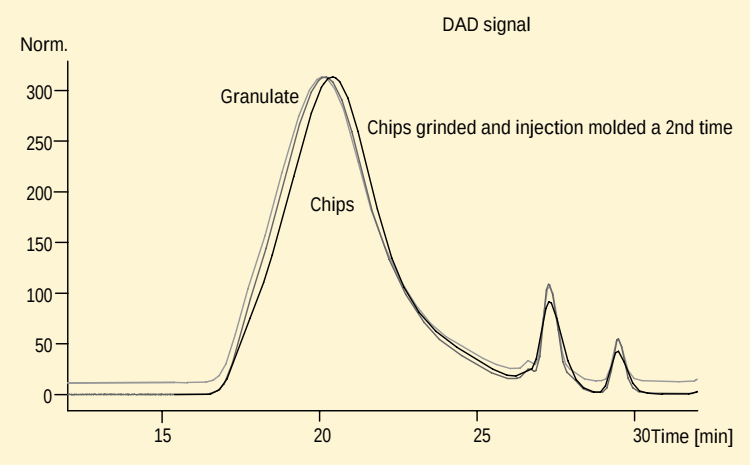

Figure 1
Different processed polystyrenes

Conditions

Column
3 ˘ PLgel mixed-B, 7.5 ˘ 300 mm, 5 µm
Mobile phase
Tetrahydrofurane (THF)
Flow rate 1 ml/min
Oven Temp 20 °C
Injection vol 10 µl
Refractive index detector

Sample preparation
Sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

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Method Performance

Figure 1 shows the signal traces of different treated polystyrenes. A granulate was used to produce colorless chips. These chips were grinded and injection molded a second time. The influence of these production procedures on the MW data are shown in table 1.

Method performance

Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 2 %

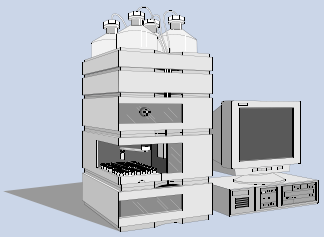
MW data	Granulate	Chips	Chips, grinded and injection molded a second time
Mp	109776	87563	103049
Mn	59152	49062	55036
Mw	159590	133565	149385
Polydispersity	2.698	2.722	2.714
Mz	327846	297500	311084
Mz + 1	545718	539583	533941
Mv	141380	117205	132243

Table 1
Molecular weight data

Equipment

Agilent 1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software



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Analysis of Polycarbonate using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polycarbonate is chemically a polyester of carbonic acid and aliphatic or aromatic hydroxy compounds that it is used for the production of cabinets, housings, packing boxes, light transparent roofs, noise protection walls, inside paneling and microwave compatible dishes. In 1989, 470000 tons were used worldwide, with the mol masses varying from 10000 to 200000 g/mol.

To ensure the highest quality, molecular weight (MW) data has to be evaluated for each batch of produced polymer. Gel Permeation Chromatography is an analytical tool used to characterize polymers which are soluble in organic solvents.

Method Performance

Figure 1 shows the signal traces of four different batches of polycarbonates. The differences in MW data for each of these four batches is shown in table 1. For one polycarbonate, MW data was determined by absolute methods. This data was used for a broad standard calibration.

The following explains the different calibration types.

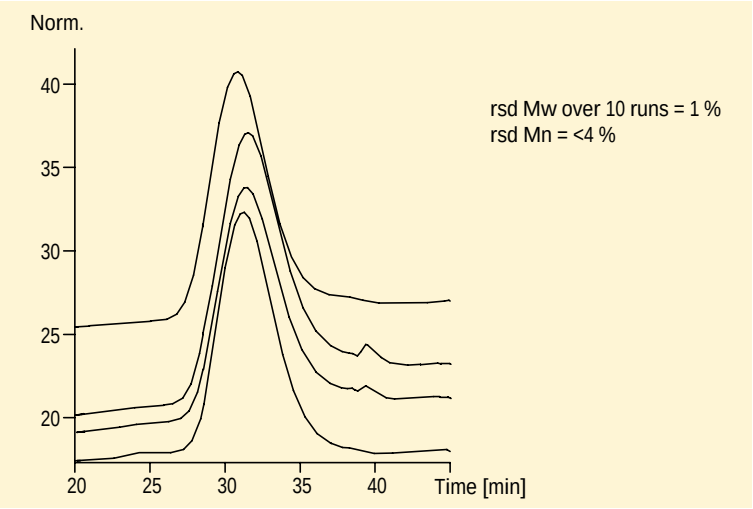

Figure 1
Molecular weight data analysis of four different polycarbonates

Conditions

Column 3 ˘ PSS GPC, 8 ˘ 300 mm, 5 µm, 10^6, 10^5, 10^3 A
Mobile phase Tetrahydrofurane (THF)
Flow rate 0.8 ml/min
Oven Temp 20 °C
Injection vol 10 µl
UV DAD 254/100 nm
Refractive index detector

Sample preparation
Sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

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The accuracy of MW data, is measured by conformity with data measured compared with absolute methods, and is mainly influenced by the calibration procedure used.

In an ideal situation narrow standards are available for the polymer of interest, however this is normally not true. In many cases where organic solvents are used narrow polystyrene standards are used for calibration. This means that the accuracy is often poor. This is seen when you look at the comparison with absolute MW from light scattering or viscometer measurements. A solution for this is to use the broad standard calibration, where a polymer of the same chemical structure and known Mw and Mn data is used as calibration compound (see figure 2). In this case broad standard calibration with a chemically identical polymer with known Mw and Mn would provide the best conformity.

On the software side, care should be taken in selecting the right calibration curve fit. Baseline setting and summation start and end points should be selected correctly.

Narrow standard calibration		Broad standard calibration	
Mw	**Mn**	**Mw**	**Mn**
44096	17996	30000	12000
33306	10709	22604	6697
34494	10787	23616	7176
38556	16446	26602	10547

Table 1
Molecular weight analysis of four different polycarbonates

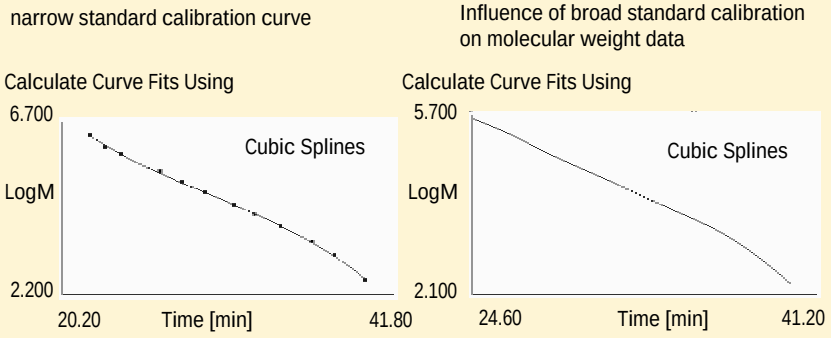


Figure 2
Influence of calibration on molecular weight data

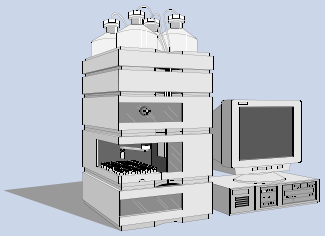
Method performance

Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 1 %

Equipment

Agilent 1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software



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Analysis of Polyvinylchloride using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polyvinylchloride (PVC) can be divided into two main groups—hard and soft PVC, which is used for the production of for example tubings, cables, cars, furniture, foils, artificial leather and the covering of wall papers. In 1990, 11.4 million tons were used worldwide. The mol masses vary from 30000 to 130000 g/mol. Chlorinated PVC with a maximum concentration of 73 % of chloride is used whenever the plastic material needs to have drastically increased solubility compared to normal PVC. Chlorination also improves thermal stability and mechanical stability. Examples of products made from this modified PVC are resins, foil and fibers. To ensure the highest quality, molecular weight (MW) data have to be evaluated for each batch of produced polymer. Gel Permeation Chromatography is an analytical tool used to characterize polymers which are soluble in organic solvents.

Method Performance

Figure 1 shows the signal traces of 3 different batches of polyvinylchloride. The production process started with a normal PVC. In the second step this PVC was chlorinated and in the final process it was formed into tubes. MW data from these 3 production processes were evaluated. The differences in MW data are shown in table 1. It is interesting to note that the UV absorption increases during the manufacturing process, see figure 2.

PVC type	Mw data	Mn data	Polydispersity
PVC	111852	53648	2.085
Chlorinated PVC	107355	52145	2.059
Chlorinated PVC as tube	124378	61005	2.039

Table 1
Analysis of PVC with refractive index detector

Influence of flow and temperature stability

The following in which PVC was analyzed, demonstrates the importance of stable flow rates and stable oven temperatures.

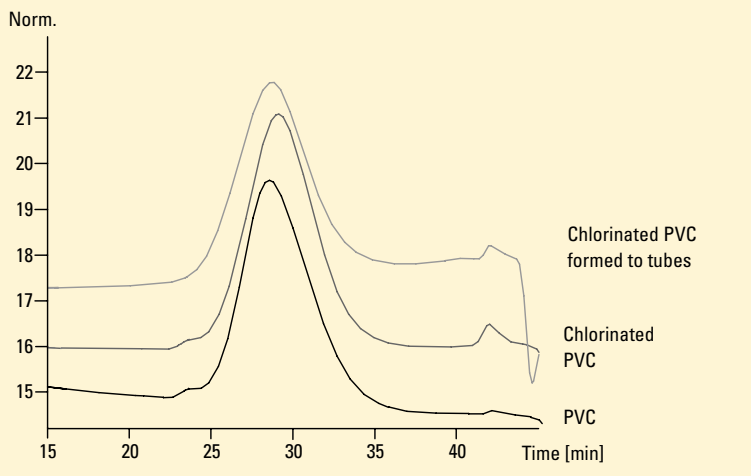

Figure 1
Analysis of PVC with refractive index detector

Conditions

Column 3 ˜ PSS GPC, 8 ˜ 300 mm, 5 µm 10^6,10^5,10^3 A
Mobile phase Tetrahydrofurane (THF)
Flow rate 0.8 ml/min
Oven Temp 20 °C
Injection vol 10 µl
UV DAD 254/100 nm
Refractive index detector
Sample preparation
Sample dissolved in 1 ml THF, filtered with 0.45 µm filter. Polystyrene standards from PSS were used for narrow standard calibration
Method performance
Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 1 %

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The precision of MW data, measured by relative standard deviation of for example Mw and Mn is mainly influenced by the stability of flow rate. To demonstrate the importance of stable flow rates and constant oven temperatures, experiments were done where for each parameter slight changes were made. It soon became obvious that flow changes even smaller than 0.5 % had an influence on the precision, whereas temperature changes below 1 °C did not have a major influence (see figure 3). Consequently the precision of the flow rate should be better than 0.1 %. 20 consecutive injections were made and the precision of the Mw data were < 0.4 %. (see figure 4.) Data was obtained using an HP 1090 Series HPLC system.

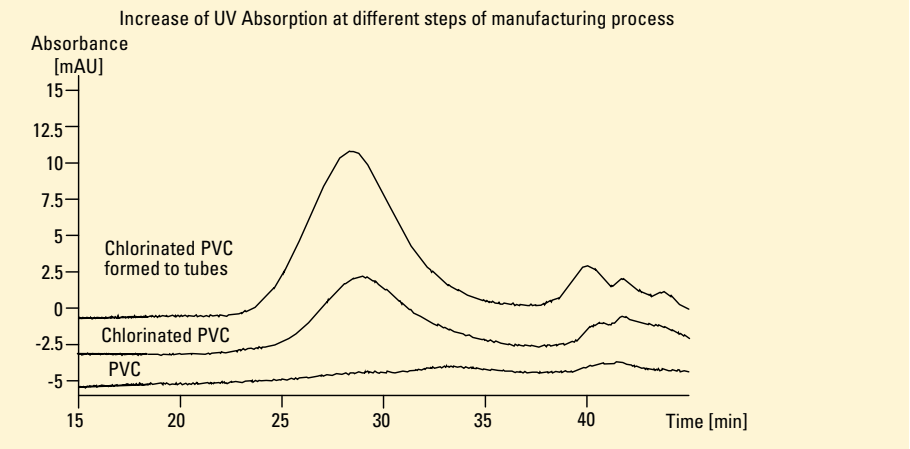

Figure 2
Different PVC types analyzed with UV DAD

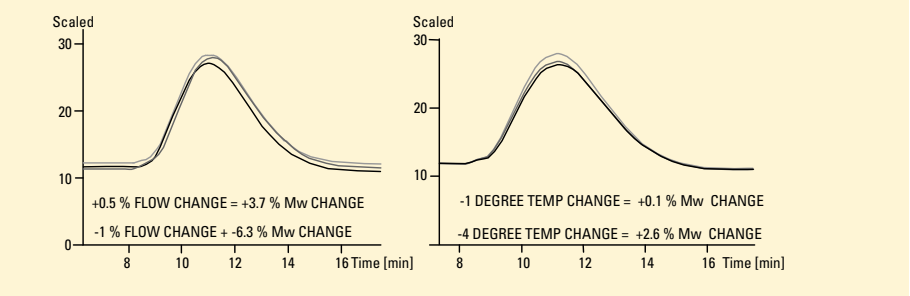

Figure 3
Influence of flow and temperature variations on precision of MW data

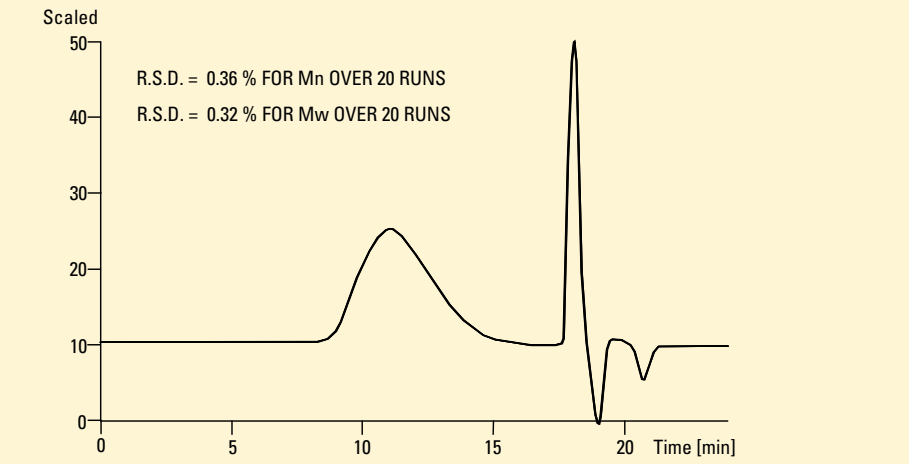

Figure 4
Precision for optimized conditions

Conditions

Column 7.5 ˜ 300 mm, 10^4 PLGel
Mobile phase
Tetrahydrofurane (THF)
Flow rate 0.2 ml/min
Oven Temp 40 °C
Injection vol 40 µl

Equipment

Agilent 1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector Agilent ChemStation + software + polymer labs GPC software

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Analysis of Acrylonitril-Butadiene-Styrene Copolymer using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer

Abstract

Acrylonitril-Butadiene-Styrene (ABS) copolymers are thermoplastic and elastic polymer blends that are used in the production of cars, housings , tubings, foils, sport kits and toys, where high impact strength, stability of shape and resistance against heat is required.

To ensure the highest quality, molecular weight (MW) data have to be evaluated for each batch of produced polymer. Gel Permeation Chromatography is an analytical tool used to characterize polymers which are soluble in organic solvents.

MW data	Colored product	Starting product
Mp	61665	80398
Mn	33321	46523
Mw	101677	108226
Polydispersity	30.51	2.326
Mz	279324	222124

Table 1
Molecular weight data of two ABS polymers

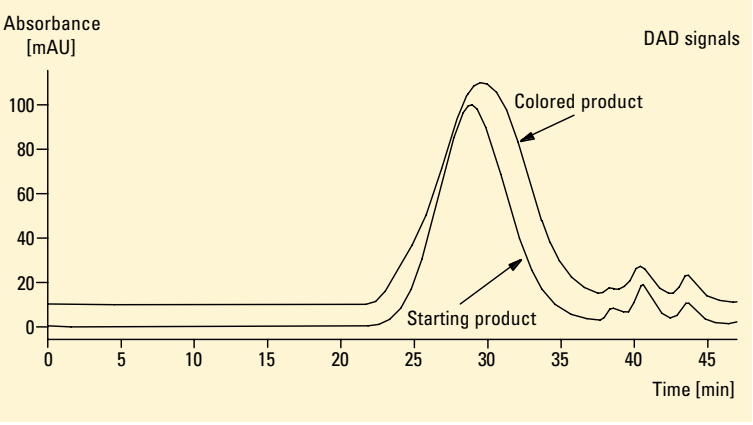

Figure 1
Molecular weight data of two ABS polymers

Method Performance

Figure 1 shows the overlay of a starting product and the respective colored end product. The differences in MW data are shown in table 1.

Conditions

Column
3 ˘ PSS GPC, 8 ˘ 300 mm, 5 µm
10^6, 10^5, 10^3 A
Mobile phase Tetrahydrofurane (THF)
Flow rate 0.8 ml/min
Oven Temp 20 ºC
Injection vol 10 µl
UV DAD 254/100 nm
Refractive index detector

Sample preparation
Sample dissolved in 1 ml THF, filtered with 0.45 µm filter
Polystyrene standards from PSS were used for narrow standard calibration

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Method performance

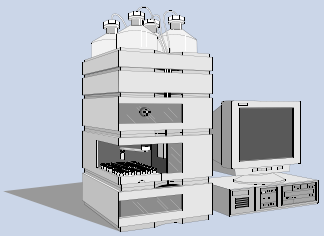
Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 2 %

Equipment

Agilent 1100 Series:

- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software



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Analysis of Styrene-Acrylonitrile-Copolymer using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer/ chemical industry

Abstract

Styrene-Acrylonitrile-Copolymer (SAN) contains 25 to 35 % acrylnitril, it is highly resistant against oil and fuel and used for the production of housings, show cases, food packaging, cosmetics and pharmaceutics. In 1989 65 000 tons were used in western Europe.

To ensure the highest quality, molecular weight (MW) data have to be evaluated for each batch of produced polymer. Gel Permeation Chromatography is an analytical tool used to characterize polymers which are soluble in organic solvents.

Method Performance

Figure 1 shows the signal traces of different treated SAN plastics. A granulate was used to produce colorless chips. The chips were then grinded and injection molded a second time. The influence of these production procedures on the MW data are shown in table 1.

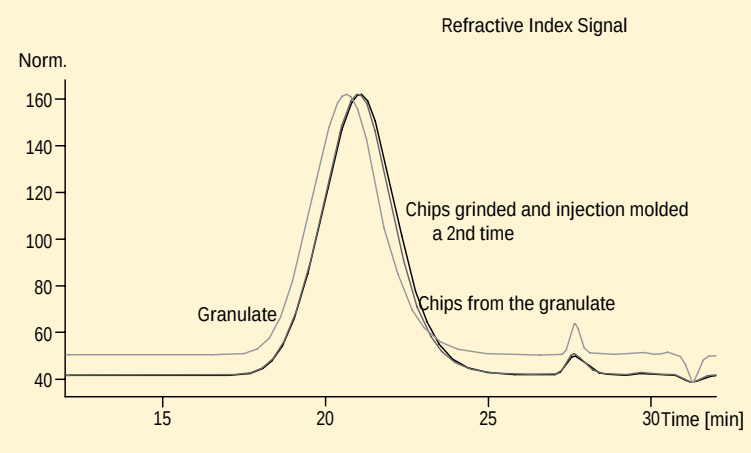


Figure 1
Styrene acrylonitrile from different production processes

Conditions

Column
3 ˘ PLgel mixed-B, 7.5 ˘ 300 mm, 5 µm
Mobile phase
Tetrahydrofurane (THF)
Flow rate
1 ml/min
Oven Temp
20 °C
Injection vol
10 µl
Refractive index detector

Sample preparation
Sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

Agilent Technologies
Innovating the HP Way

Method performance

Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 2 %

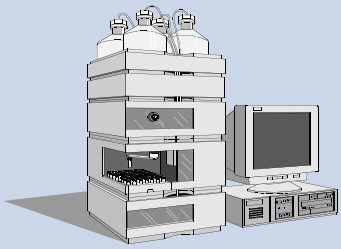
MW data	Granulate	Chips	Chips, grinded and injection molded a second time
Mp	86480	65812	62563
Mn	55525	42478	39616
Mw	117654	94559	91626
Polydispersity	2.119	2.226	2.313
Mz	224934	196436	195664
Mz + 1	408416	376650	393504
Mv	106412	84598	81671

Table 1
The influence of the production procedures on the molecular weight data

Equipment

Agilent 1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation
+ software
+ polymer labs GPC software



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Analysis of Epoxy Resins using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Epoxy resins are produced through the reaction of bisphenol A and epichlorohydrin to macromolecules, (figure 1). These products are then used in the production of duroplasts, which are highly resistant against oil and fuel. Examples of products made from this material include, casting resins for the electrical industry, laminates for cars and airplanes, inner coatings for containers and tubings for the chemical industry. In 1987 85 000 tons were used in Germany.

To ensure the highest quality, molecular weight (MW) data have to be evaluated for each batch of produced polymer. Gel Permeation Chromatography is an analytical tool used to characterize polymers which are soluble in organic solvents.

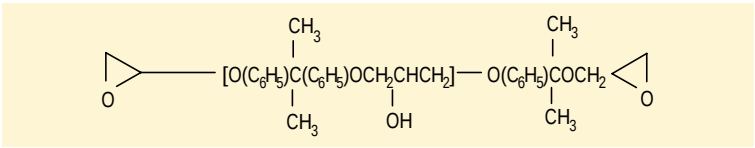

Figure 1
Epoxy resins

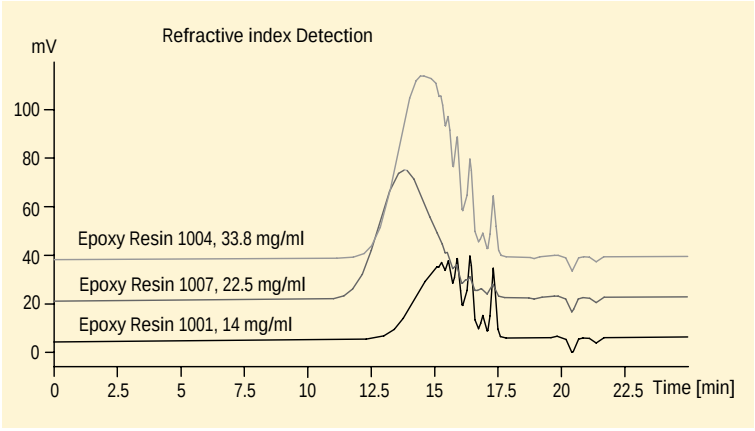

Figure 2
Analysis of three different epoxy resin qualities

Conditions

Column
2 ˜ PLgel mixed-D, 7.5 ˜ 300 mm, 5 µm
Mobile phase Tetrahydrofurane (THF)
Flow rate 1 ml/min
Oven Temp 20 °C
Injection vol 20 µl
UV detector
DAD 254/100 nm, reference 360/100 nm
Refractive index detector

Sample preparation
Sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

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Method Performance

Figure 2 shows an overlay of 3 different batches of epoxy resin. It can be seen that the epoxy resin 1001 has a relatively low molecular weight compared to the others. The epoxid 1007 has a high molecular weight whereas Epoxid 1004 falls in the middle. The molecular weight data of these three batches are combined in table 1. The precision of the molecular weight data was tested for batch 1001, and the MW data of 10 consecutive runs was evaluated. Figure 3 shows an overlay of the chromatograms. The rsd of Mw and Mn was calculated and found to be:

rsd of Mw over 10 runs = 0.66 %

rsd of Mn over 10 runs = 2.5 %

Method performance

Precision of weight: average molecular weight (rsd of Mw) = < 1 %
Precision of number weight: average molecular weight (rsd of Mn) = < 3 %

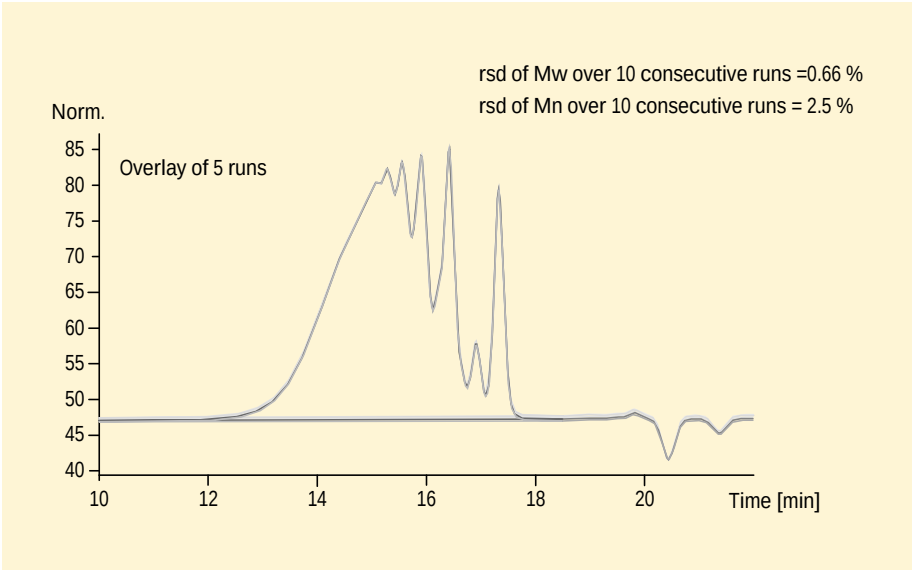
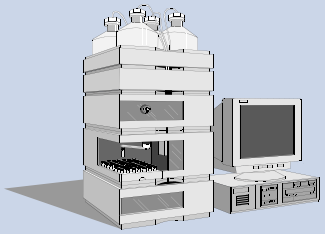


Figure 3
Precision of molecular weight data

Equipment

Agilent 1100 Series:
- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation
+ software
+ polymer labs GPC software



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Analysis of a Brenzcatechol Additive in Styrene using HPLC

Angelika Gratzfeld-Huesgen

Polymer/ chemical industry

Abstract

Brenzcatechol (TBC) or Benzene-1,2-diol is used as an antioxidant for polymers. The concentration range of Brenzcatechol used varies between 10 and 100 ppm.

Method Performance

Figure 1 shows the HPLC chromatogram of the analyzed styrene sample and the standard chromatogram of Brenzcatechol. For additional identification purposes, spectra can be taken and a comparison with the standard spectrum can be made. For this application, 2.1 mm columns were used in order to improve sensitivity so that the detection of 1 ng with signal to noise of 2, could be achieved.

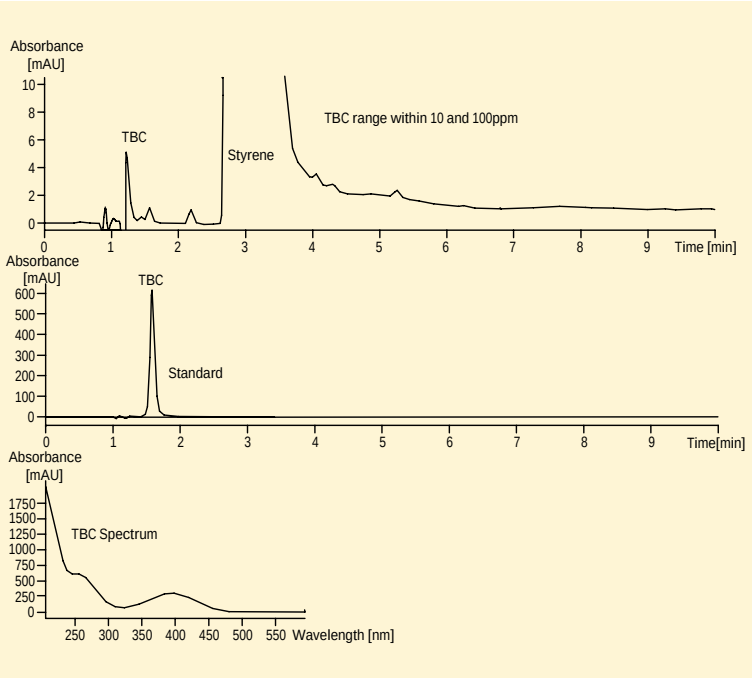

Figure 1
Analysis of brenzcatechine (TBC) additive in styrene

Conditions

Column
200 x 2.1 mm Hypersil ODS, 5 µm
Mobile Phase
A = Water, B = Acetonitrile
Gradient
at start 50 % B, at 10 min 99.9 %B, at 20 min 50 %B
Post Time 6 min
Flow Rate 0.5 ml/min
Oven Temp 40 °C
Injection Vol 1 µl
Diode array detector
280/30 nm; Reference 500/50 nm

Sample preparation
1 ml styrene sample was diluted with 1 ml Tetrahydrofurane (THF)

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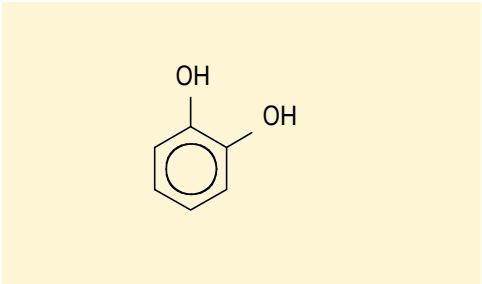


Figure 2
Brenzcatechin (Benzene-1,2-diol)

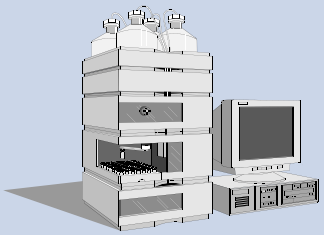
Method performance

LOD: 1 ng or 1 ppm with signal/noise = 2
red RT <0.2 %
rsd area <2 %

Equipment

Agilent 1100 Series
- degasser
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Measuring intraday and interday precision of GPC-SEC analysis data

Application

Heinz Goetz

Abstract

The daily (intraday) and day-to-day (interday) precision of M_n and M_w molecular weight data obtained by GPC-SEC has increased significantly over the past years. This Application Note describes what is possible nowadays in the area of intraday and interday precision of molecular weight data using state-of-the-art equipment. Typcial GPC conditions with organic eluents were chosen to obtain realistic-data.

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Introduction

Precision of molecular weight data obtained by GPC-SEC is of great interest to polymer chemists since the advent of the technique in the late 1960s.[1,2,3,4] Due to a special calibration procedure using a linear elution volume (retention time) on the x-axis versus a logarithmic molecular weight on the y-axis, each deviation of the elution volume has an exponential effect on the precision of the molecular weight data. Therefore, demands on the hardware are more stringent than in other HPLC modes.

Equipment

An Agilent 1100 Series GPC-SEC system consisting the following modules was used:

- Agilent 1100 Series vacuum degasser for efficient degassing of the mobile phase
- Agilent 1100 Series isocratic pump with large solvent cabinet
- Agilent 1100 Series autosampler with single valve design
- Agilent 1100 Series thermostatted column compartment for precise column temperatures
- Agilent 1100 Series refractive index detector with automatic recycle valve
- Agilent ChemStation Plus with GPC-SEC data analysis software

Results and discussion

Table 1 shows the strong influence of flow deviations on the weight average molecular weight M_w measured for a polystyrene sample. The system was calibrated at a flow rate of 1.0 mL/min. When analyzing the sample exactly at this flow rate the M_w value is 35400. Table 1 shows that, for example, for a flow deviation of only +0.60 % or +1.30 % errors of 11 % and even 23.6 % occur. The column temperature stability between calibration and sample run is also important. A 4 °C change, as it can easily occur if the column compartment is not thermostatted, will create an error of 2.6 %. Hardware and software parameter effects on precision of molecular weight data are discussed in references 4 and 5.

As outlined before an excellent inter- and intraday precision of the retention times (elution volumes) is a fundamental prerequisite. To measure the retention time precision we injected a technical poly(styreneacrylonitrile) (SAN) automatically every day over 20 days. Figure 1 shows the plot of the retention times versus the run number. Table 2 shows the calculated relative standard deviations for retention time, M_n and M_w. The very good interday (between days) precision from the 1st to the 20th day was 0.06 9%. The intraday (within day) precision was always below 0.05 % with

Flow [mL/min]	Flow deviation [%]	M_w	M_w deviation [%]
1.013	+1.30	43400	+23.6
1.006	+0.60	39300	+11.0
1.00	0	35400	-
0.992	-0.80	31100	-12.2
0.985	-1.50	27700	-21.80

Table 1
Influence of flow variations on M_w

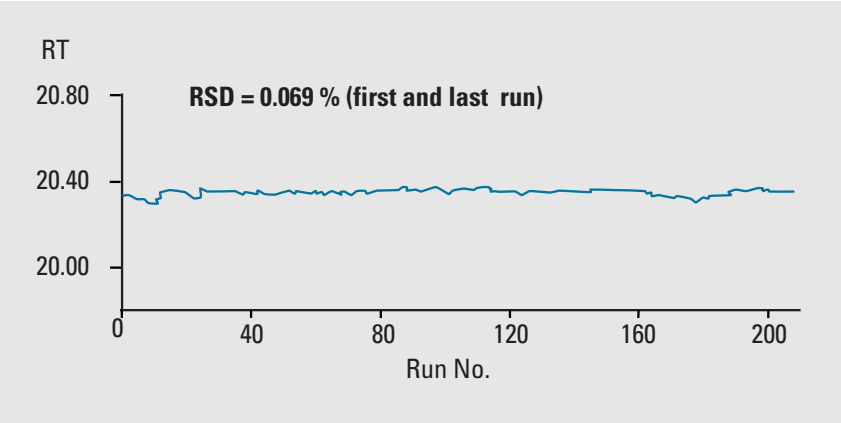

Figure 1
Intra-and interday precision of retention times for a poly(styreneacrylonitrile) copolymer (SAN) over 20 days

the exception of days 1, 2 and 15 but still below 0.08 %.

Figure 2 shows the precision of the styreneacrylonitrile analyses. It is an overlay of the injections made on days 1, 5, 10 and 20. The calculated relative standard deviations from day 1 to day 20 are shown for all injections. It should be pointed out that these very good data take almost all injections from day 1 to day 20 into account. Only about 10 injections had to be filtered out. They were stray points, for example, caused by a vial not filled correctly.

Conclusion

The intra- and interday precision of M_n and M_w molecular weight data obtained by GPC-SEC has increased significantly in recent years. With the Agilent 1100 Series GPC-SEC system intra-(within one day) and interday precision data (over 20 days) for M_n and M_w below 1.5 % were calculated in completely automated analyses for broad distributed polymers with THF as eluent. These results are mainly based on

- HPLC pumps with an intra- and interday flow stability better than 0.1 % (based on polymer retention time),
- column thermostats with a temperature precision better than 0.5 °C,
- automated eluent recycling after the analysis which results in a better conditioned system,

Day	% RSD retention time	% RSD for M_n	% RSD for M_w
1	0.071	1.16	1.12
2	0.075	1.43	0.78
3	0.020	0.92	0.72
4	0.032	0.82	0.83
5	0.030	1.18	0.97
6	0.038	0.95	0.78
7	0.037	1.13	1.08
8	0.030	0.58	0.81
9	0.043	0.91	0.66
10	0.025	0.73	0.32
11	0.022	1.43	0.43
12	0.021	0.81	0.35
13	0.016	0.89	0.59
14	0.029	0.88	1.19
15	0.065	1.08	1.27
16	0.002	0.68	0.70
17	0.045	0.99	0.85
18	0.038	0.94	0.78
19	0.041	0.95	0.80
20	0.009	0.70	0.71
Average %RSD per day	**0.035**	**0.96**	**0.78**

Table 2
Calculated relative standard deviations (intraday) for retention time, M_n and M_w

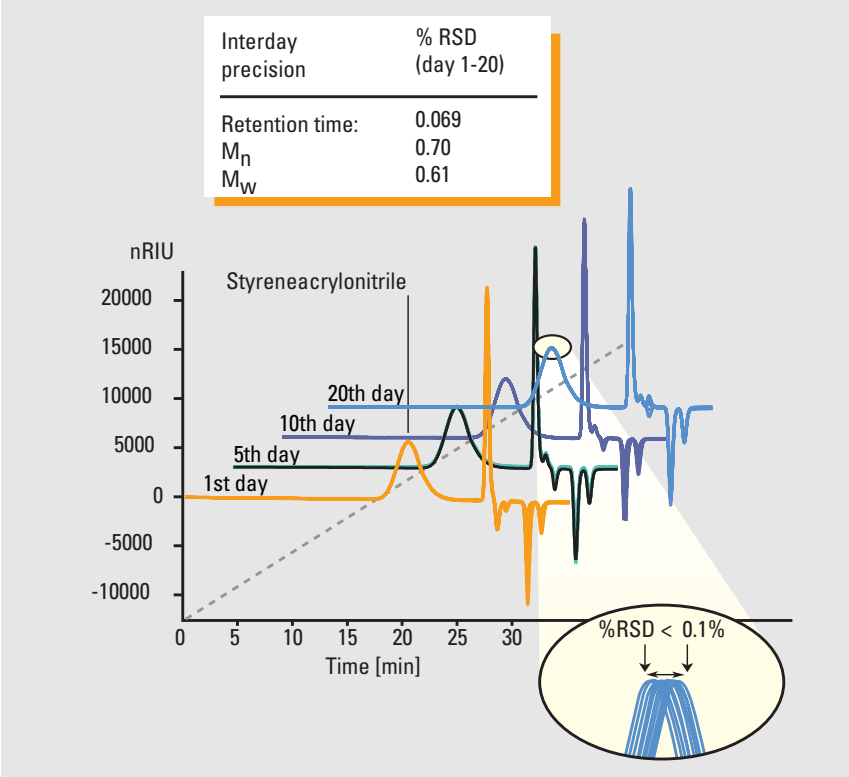

Figure 2
Overlay of all analyses of the poly(styreneacrylonitrile) sample on days 1, 5, 10 and 20. A zoom into the analyses of day 20 is shown in the bottom right.

- refractive index detectors with low noise (± 2.5×10^{-9} RIU)* and low drift (200×10^{-9} RIU/h)* for correct and repeat able baseline and integration window setting,
- software with flexible and repeatable integration and cal culation algorithms to adapt to broad polymer peaks, and
- full automation capabilities reducing human errors.

Good precision data not only improves the reliability of the results but also the productivity because less time-consuming recalibrations are needed.[5]

* According to ASTM E-1303-95 "Practice for Refractive Index Detectors used in Liquid Chromatography". Reference Conditions: response time 4 s, 35 °C, 1 ml/min water, restriction capillary

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"Investigation on Intra-and inter-day Precision of Molecular Weight Data Determined by GPC", **2001**

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Printed 11/2000
Publication Number 5988-0109EN



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Process control of polystyrenes

Application

Heinz Goetz and Angelika Gratzfeld-Huesgen

Polystyrenes are widely used for the production of packaging materials, household goods, cases of electronic equipment, toys, and insulation materials. Polymerization can be performed with pure styrene or by copolymerization with butadiene, acrylonitrile, rubber and methylstyrene. The properties of the product strongly depends on the monomers used, the molecular weights and the molecular weight distribution.

Figure 1 shows an overlay of 3 chromatograms of a technical polystyrene – the original granulate, one after 1st injection moulding and one after second injection moulding. After the first injection moulding there is almost no change in the chromatogram and therefore the molecular

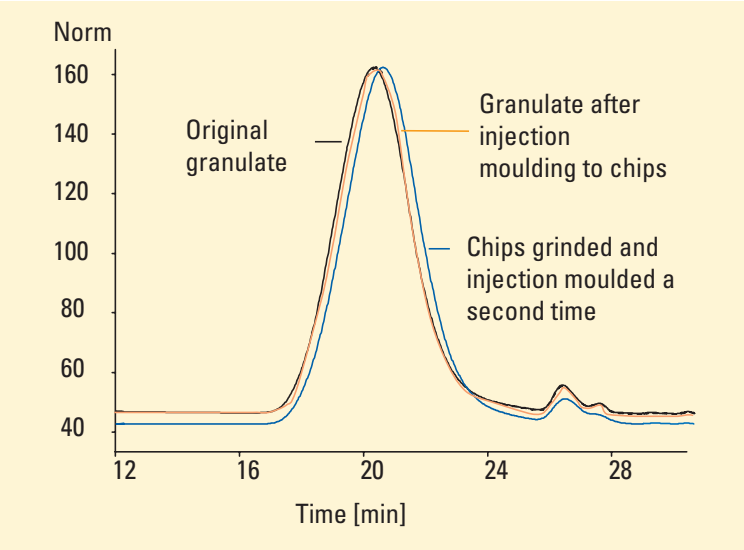

Figure 1
Overlay of three chromatograms of a technical polystyrene

Conditions

Sample preparation
Sample was dissolved in THF. Polystyrene EasyCal Vial standards (Agilent p/n 79911-60500 and 79911-60501) were used for narrow standard calibration.

Column
3 x PLgel mixed B, 7.5 x 300 mm, 10 µm (Agilent p/n 79911GP-MXB) in series

Mobile phase
Tetrahydrofuran

Flow rate
1.0 mL/min

Column compartment temperature
20° C

Injection volume
10 µL

Detector
Refractive index detector, alternatively VWD, 254 nm

weight distribution. After grinding the chips, and injection moulding a second time there is a significant change which will have an effect on the properties. The visual information is supported by the number average molecular weight, M_n, as calculated by the ChemStation data analysis software:

M_n (original granulate): 59000
M_n (after second process): 55000

To characterize such small differences in polymers reliably a GPC-SEC instrument with excellent precision, such as the Agilent 1100 Series GPC-SEC system, is required. Further information on the precision of this system is given in application brief “Precision in GPC-SEC analysis”, Agilent publication number 5988-0109EN.

HPLC performance

RSD of M_w	< 1%
RSD of M_n	< 2%

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

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Process control of polyamide-6,6

Application

Heinz Goetz
and R. Schewe

Polyamide-6,6 is a synthetic polyamide typically produced by polymerizing hexamethylendiamine and adipinic acid. It is widely used for the production of fibres, foils and raw materials. Typical applications are in the clothing industry for stockings and sports apparel, in the building industry for synthetic carpets and in the electronic industry for housings. The properties of polyamide-6,6 strongly depend on molecular weight and molecular weight distribution.

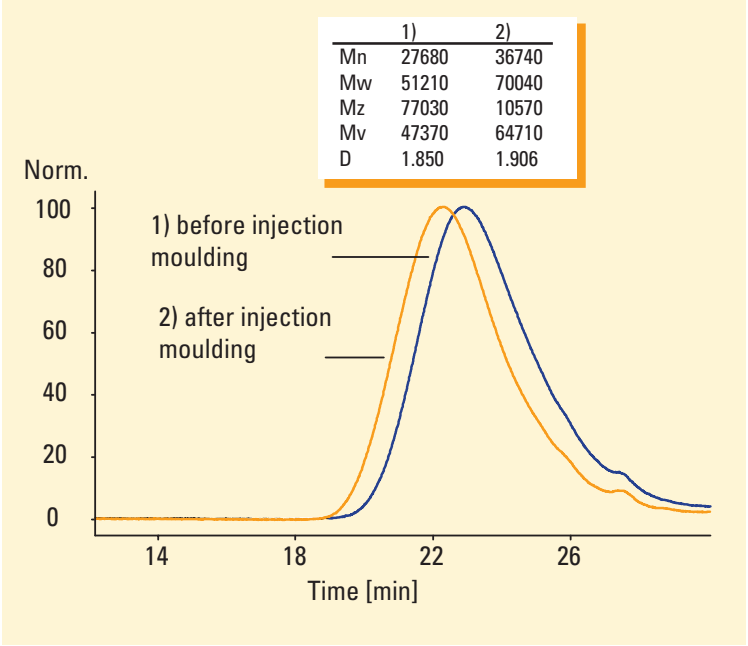

Figure 1
Overlay of two chromatograms of a technical polyamide-6,6 used for producing the housing of drilling machines

Conditions

Sample preparation
Samples were dissolved in the mobile phase and filtered (0.45 µm).
Polystyrene EasyCal vial standards (Agilent p/n 5064-8281) were used for narrow standard calibration.

Column
PFGgel 10^3 A, 8 x 300 mm, 5 µm in series with a PFGgel 300 Å, 8 x 300 mm, 5 µm and a PFGgel 100 A, 8 x 300 mm, 5 µm

Mobile phase
Trifluoroethanol and 1 g/l potassiumtrifluoroacetate

Flow rate
1.0 mL/min

Column compartment temperature
35 ° C

Injection volume
10 µL

Detector
Variable wavelength detector, 254 nm

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Figure 1 shows an overlay of two chromatograms of a technical polyamide-6,6 used for the production of the housing of drilling machines. One chromatogram was obtained with the original granulate and the other one after injection moulding. The picture and table clearly show that the moulding process changes the chromatograms and the molecular weight data. For a consistent product quality the moulding process needs to be optimized and controlled by GPC-SEC. Because of the insolubility of polyamide-6,6 in typical GPC-SEC eluents such as tetrahydofuran, toluene or dimethylformamide trifluoroethanol was used. To reduce the number of adsorptive sites on the stationary phase 1g/l of potassiumtrifluoroacetate was added to the mobile phase.

HPLC performance

RSD of M_w	< 1%
RSD of M_n	< 2%

Equipment

Agilent 1100 Series GPC-SEC system
consisting of
- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

Columns supplier:
Polymer Standards Service, Mainz, Germany

Heinz Goetz is an application chemist at Agilent Technologies, Waldbronn, Germany. R. Schewe is Laboratory Manager at Schumann GmbH, Kerpen, Germany.

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Quality control of paint resins

Application

Heinz Goetz

Resins such as alkyd or acrylic resins are essential ingredients of paints. The rapid determination of the resin quality is of particular interest. The capability to respond quickly to quality control requirements increases productivity and therefore profit. This example shows the quality control analysis of two resins used for high quality paints in the car industry. One resin showed good adhesion properties while the other one failed. The poor quality resin failed because the high molecular weight fraction was not present (figure 1, hatched area).

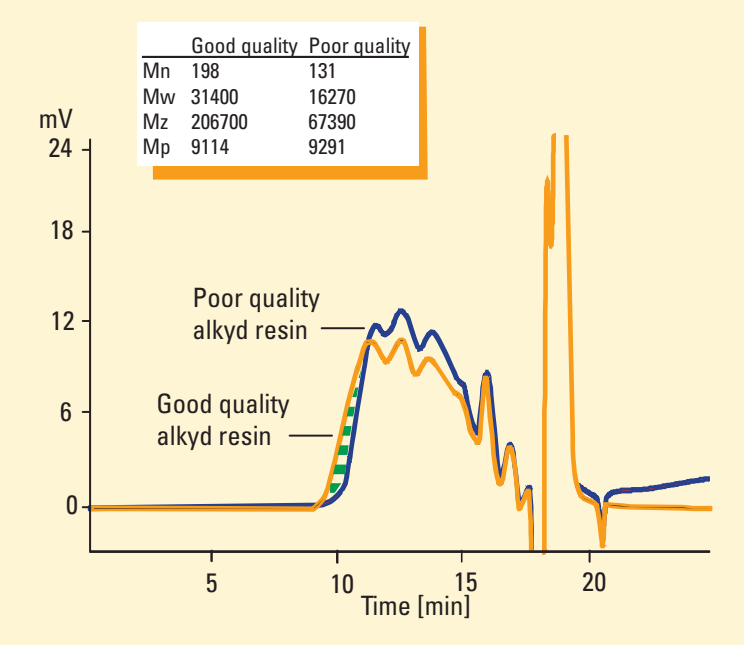

Figure 1
Quality control of two resins

Conditions

Sample preparation
Resins were dissolved in THF. Polystyrene EasyCal vial standards (Agilent p/n 5064-8281) were used for narrow standard calibration.

Column
PLgel 10^2 Å, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-501) in series with a PLgel 5 x 10^3, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-502) and a PLgel 10^4 Å, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-504)

Mobile phase
Tetrahydrofuran

Flow rate
1.5 mL/min

Column compartment temperature
20 °C

Injection volume
100 µL

Detector
Refractive index detector

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The ChemStation GPC data analysis software provides the conventional graphical information of the chromatograms with the additional rapid numeric data in form of molecular weight averages and the report subsets. With the report subsets we could easily determine that the high molecular weight fraction (between the arrows) was 22 % for the high quality polymer but only 14 % for the low quality polymer.

HPLC performance

RSD of M_w < 1%
RSD of M_n < 2%

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

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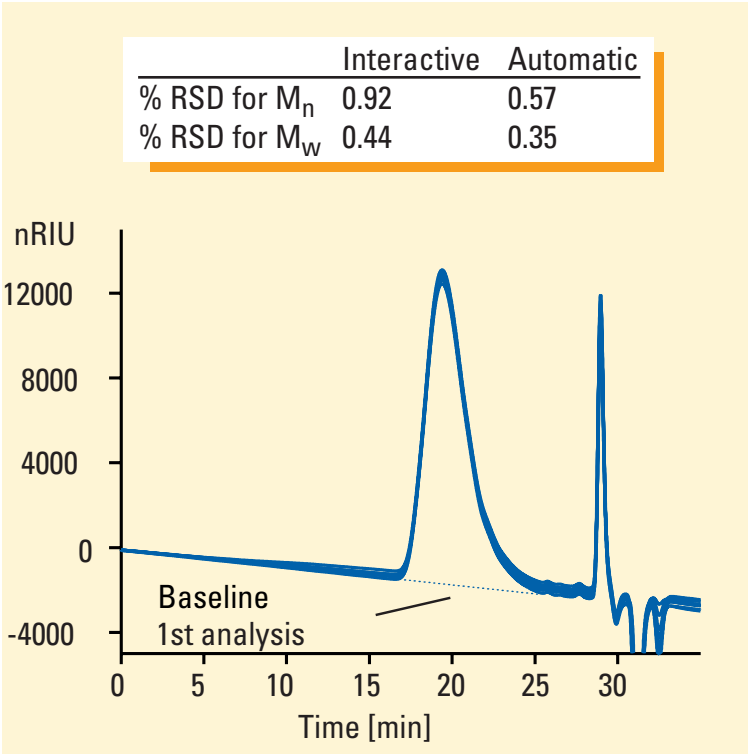
Automatic versus manual (interactive) data evaluation in GPC-SEC analysis

Application

Heinz Goetz

Abstract
It is widely accepted among polymer analysts that the data acquisition part of the analysis can be automated with modern, state-of-the-art GPC hardware without any loss of accuracy and precision. Regarding data evaluation there is still some discussion on whether automatic or interactive baseline setting should be used. In this note we have analyzed a technical polystyrene sample 10 times with interactive and another 10 times with fully automatic data evaluation. The figure shows an overlay of the 10 chromatograms for the technical polystyrene



Conditions

Sample
Technical polystyrene, 0.1 % in THF
Eluent
Tetrahydrofuran (HPLC grade)
Column
3 × PLGel mixed B in series, 7.8 × 300 mm, 10 µm (Agilent p/n 79911GP-MXC)
Flow rate
1 ml/min
Column compartment temperature
20° C
Injection Volume
100 µl
Polymer standards
Polystyrene EasyCal Vial standards (Agilent p/n 5064-8281)

Figure 1
Technical polystyrene sample analyzed with interactive and automatic baseline

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sample and the intraday precision data for automatic and interactive baseline setting. We see that the precision data for the automatic mode is slightly better than for the interactive mode for both M_n and M_w. We also found a similar superiority for other polymers. This is however not the case for every sample, such as a polymer with a strong tailing peak or when the peak height is small due to low sample concentration or low refractive index.

In most cases the ChemStation's *Enhanced Integrator* is perfectly suited to detect the start and end of a polymer peak correctly and to ensure reliable automation. It provides the following improved capabilities:

- optimized baseline tracking using parameters from the individual method and data files,
- better peak allocation,
- additional initial parameters to remove noise-generated peaks
- ease of use–the *Enhanced Integrator* algorithm has a new user interface based on tool bars and automatically focuses on key information.

Typical advantages of completely automated analysis (from data acquisition to reporting) are:

- often at least similar precision,
- less room for human interpretation and errors,
- higher traceability and consistency, and
- improvement of efficiency by freeing trained staff from time-consuming work

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- Isocratic pump with large solvent cabinet
- Autosampler with single valve design
- Thermostatted column compartment for precise column temperatures
- Refractive index detector with automatic recycle valve
- Variable wavelength detector, 254 nm, standard cell
- ChemStation Plus with GPC-SEC data analysis software

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Publication Number 5988-0115EN



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Molecular weight characterization of polyacrylamides

Application

Peter Kilz
and Heinz Goetz

The analyzed polyacrylamides are used for drag reduction effects of ships and submarines. They are sprayed onto the ship's surface and reduce drag and therefore noise and fuel consumption. A further application of polyacrylamides is in water clarification purposes as setting aids. The polymer acts as flocculants to help remove contaminants from the water stream. All three polyacrylamides have a very high weight average molecular weight M_w.The GPC-SEC method

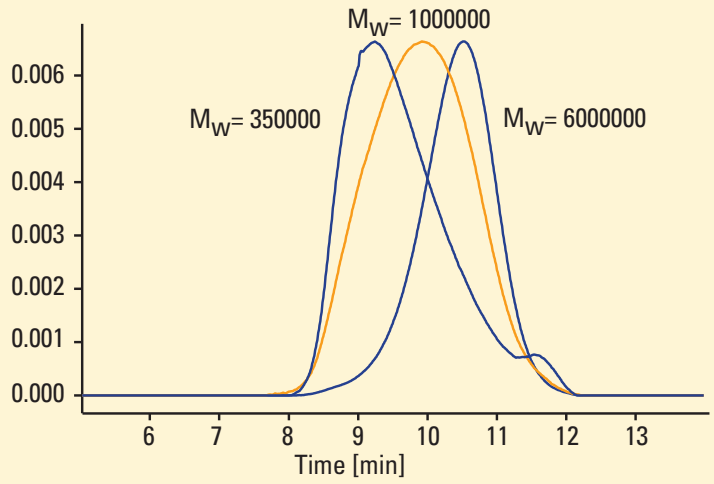

Figure 1
Overlay of high molecular weight polyacrylamides chromatograms

Conditions

Sample preparation
Sample was dissolved in mobile phase (concentration 0.1 %).
Column
PSS Suprema 10^4, 8 x 300 mm, 10 µm
Mobile phase
0.3 M $NaNO_3$
Flow rate
0.5 mL/min
Column compartment temperature
25 ° C
Injection volume
100 µL
Detector
Refractive index detector
Polymer standards
PSS broad polyacrylamide standards

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presented here shows an easy but reliable and precise analysis for the molecular weight characterization of polyacrylamides. Besides the weight average molecular weight M_w the ChemStation GPC-SEC data analysis software calculates data as Mn, Mz, Mp, Mv, polydispersity D, differential and integral molecular weight distribution. The software allows internal standard and detector delay corrections, and includes narrow, broad, universal and integral calibration.

HPLC performance

RSD of M_w	< 2%
RSD of M_n	< 5%

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

Columns supplier:
Polymer Standards Service, Mainz, Germany

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Released 09/2000
Publication Number 5988-0122EN

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Analysis of polyvinyl alcohol

Application

Heinz Goetz

Polyvinyl alcohols (PVA) are industrially synthesized by the catalytic reaction of polyvinyl acetates with alcohols, typically methanol. Due to properties such as excellent biological degradeability, water solubility , toxilogical harmlessness they are widely used as emulgators, binding agents in adhesives, salves and haircream. The properties can be varied with the molecular weight distribution and the molecular weight which ranges from 20000 to 100000 g/mol. Both parameters can be fast and reliably monitored by aqueous SEC. This is a convenient method for quality control analyis, and is more informative in production control and end-use performance evaluation than single-point viscosity measurements.

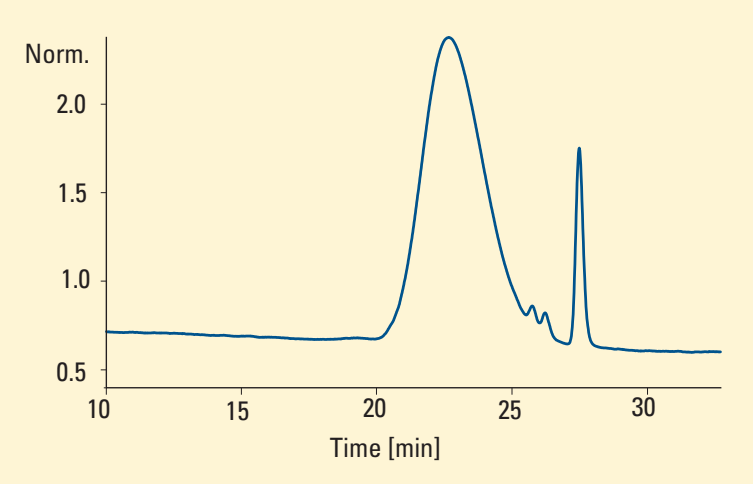

Figure 1
SEC chromatogram of polyvinyl alcohol

Conditions

Sample preparation
PVA was dissolved in the mobile phase (concentration 0.1 %)

Column
3 × PL aquagel-OH 30 in series, 7.5 × 300 mm, 8 µm (Agilent p/n 79911GF-MXA) in series with PL aquagel-OH 30, 7.5 × 300 mm, 8 µm (Agilent p/n 79911GF-083)

Mobile phase
0.2 M $NaNOH_3$, NaH_2PO_4, pH 7

Flow rate
1 mL/min

Column compartment temperature
25 ° C

Injection volume
100 µl

Detector
Refractive index detector

Polymer standards
Polyethylene oxide EasyCal standards in vials for calibration (Agilent p/n 5064-8280)

HPLC performance

RSD of M_w	< 1.5 %
RSD of M_n	< 3 %

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

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Released 09/2000
Publication Number 5988-0123EN

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Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection

Application

Consumer Products

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Abstract

Liquid chromatography with ultraviolet/visible spectroscopy and mass selective detection is a powerful approach to antioxidant analysis and identification. Examples illustrate that mobile-phase conditions affect the quality and usability of the acquired data. Unknown compounds can be identified with sufficient MS data and additive degradation can be quickly evaluated.

Introduction

Plastic products are an essential part of our lives today. Whether they are used for automotive components, CDs, toys, or biocompatible replacement parts for humans, they are the subjects of intense research into new and improved polymers and blends. Equally important is the selection and quantity of chemical additives which are used to provide color, density, opacity, stiffness, flexibility, resistance to heat, light and air, flame retardance, and to improve processing properties during pellet creation and final product fabrication.

This application note examines several antioxidant (AO) types, their chemical composition, and suitable high-performance liquid chromatography (HPLC) conditions for assessing their concentration and identity, as well as their degradation products.

AOs arise from various compound classes including small hindered phenols, large hydrophobic hindered phenols, and phosphite or phosphonate linked aromatics. Examples appear in Tables 1 and 2.

Table 1. AO Studied with Structures

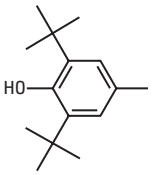
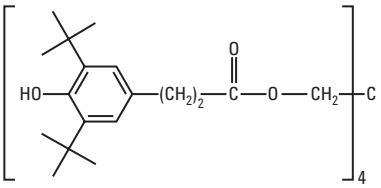
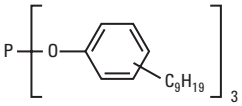
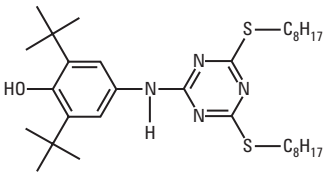
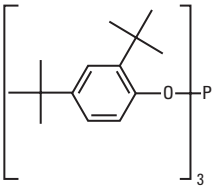
Name:	BHT	Butylated hydroxytoluene
Formula:	$C_{15}H_{24}O$	
Molecular Weight: (MW)	220.2	
Trade name:	Irganox 1010 (CibaGeigy)	Pentaerythritol tetrakis(3-(3,5-di-tert- butyl-4-hydroxyphenyl) propionate)
Formula:	$C_{73}H_{108}O_{12}$	
Molecular Weight: (MW)	1176.8	
Trade name:	Naugard P (Uniroyal)	Tris nonylphenyl phosphite
Formula:	$(C_{15}H_{23}O)_3P$	
Molecular Weight: (MW)	688.5	
Trade name:	Irganox 565 (CibaGeigy)	
Formula:	$C_{33}H_{56}N_4OS_2$	
Molecular Weight: (MW)	588.4	
Trade name:	Irgafos 168	
Formula:	$C_{42}H_{63}O_3P$	
Molecular Weight: (MW)	646.5	

Table 2. Other Common AOs

Name	Formula	MW
BHA	$C_{11}H_{16}O_2$	180.1
t-BHQ	$C_{10}H_{14}O_2$	166.1
Cyanox 1790	$C_{42}H_{57}N_3O_6$	699.4
Ethanox 330	$C_{54}H_{76}O_3$	772.6
Irganox 1076	$C_{35}H_{62}O_3$	530.5
Sandostab P-EPQ	$C_{68}H_{92}O_4P_2$	1034.6

Gas chromatographs with conventional detectors or mass spectrometers (MS) can readily analyze many small molecules; however, the increased molecular weight (MW) and decreased volatility of many AOs makes gas chromatography (GC) generally unsuitable. Liquid chromatography (LC) is a common choice because it can analyze materials exhibiting a wide MW range and varied solubility. Since LC is generally a nondestructive technique, it offers the possibility of compound isolation and recovery.

Many AOs contain functionalized aromatic groups and offer distinctive ultraviolet/visible spectroscopy (UV/VIS) spectral opportunities. This detector type is an essential part of an additive analysis system. Since UV/VIS detectors are relatively insensitive to the chromatographic mobile phase, they are readily compatible with gradient-elution separation methods.

The presence of functionalized aromatic rings, oxygen, nitrogen, phosphorous, and sulfur in many of the AOs also makes them ideal candidates for investigation by atmospheric pressure ionization mass spectrometry (API-MS). Compound identity can be supported by matching retention data, UV/VIS spectra, and from the MS, a molecular ion (essentially giving the molecular weight of the compound). Depending on the type of ionization and MS chosen, further identification can be made where higher energy is employed, causing fragmentation of the molecules. These fragments help experienced users propose chemical structures.

Instrumentation and General Method

Agilent 1100 LC system:

- Quaternary gradient pump with low volume degasser
- Binary gradient pump with degasser, for pre-MSD reagent addition
- ALS automatic sampler with 2-mL vial tray
- Thermostatted column compartment with automated 6-port, 2-position switching valve
- Diode array UV/VIS spectrophotometer

General chromatographic conditions:

- Gradient elution of increasing organic-solvent strength with combinations of:

  Water/Acetonitrile (ACN)
  Water/Methanol (MeOH)
  Water/Methanol/Tetrahydrofuran (THF), HPLC grade

- UV/VIS spectral-data collection from 200–400 nm, 1-nm slit, 4 nm resolution
- UV/VIS single-wavelength collection for 210 and 280 nm, at 4 nm resolution

ChemStation PC Data and Control System

Mass selective detector (MSD) SL single quadrupole MS with APCI interface

Fragmentor: 100 V, positive and negative ionization

Vaporizer: 400 °C

Nebulizer: 50 psi nitrogen

Drying gas: 6 LPM Nitrogen

Column: Zorbax XDB-C8, 4.6 mm id × 50 mm L, 3.5 µm particles

Gradients:

Method 1. "MeOH/THF", Column 30 °C, 25 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	50	0	10
1	15	0	90	0	10
1	20	0	90	0	10
1	21	40	50	0	10

Method 2. "MeOH", Column 40 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	60	0	0
1	10	0	100	0	0
1	15	0	100	0	0
1	16	40	60	0	0

Method 3. "ACN", column 50 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	0	60	0
1	10	0	0	100	0
1	15	0	0	100	0
1	16	40	0	60	0

Experimental Results

Figures 1 through 3 are overlaid UV chromatograms for nine AOs, using three different gradients.

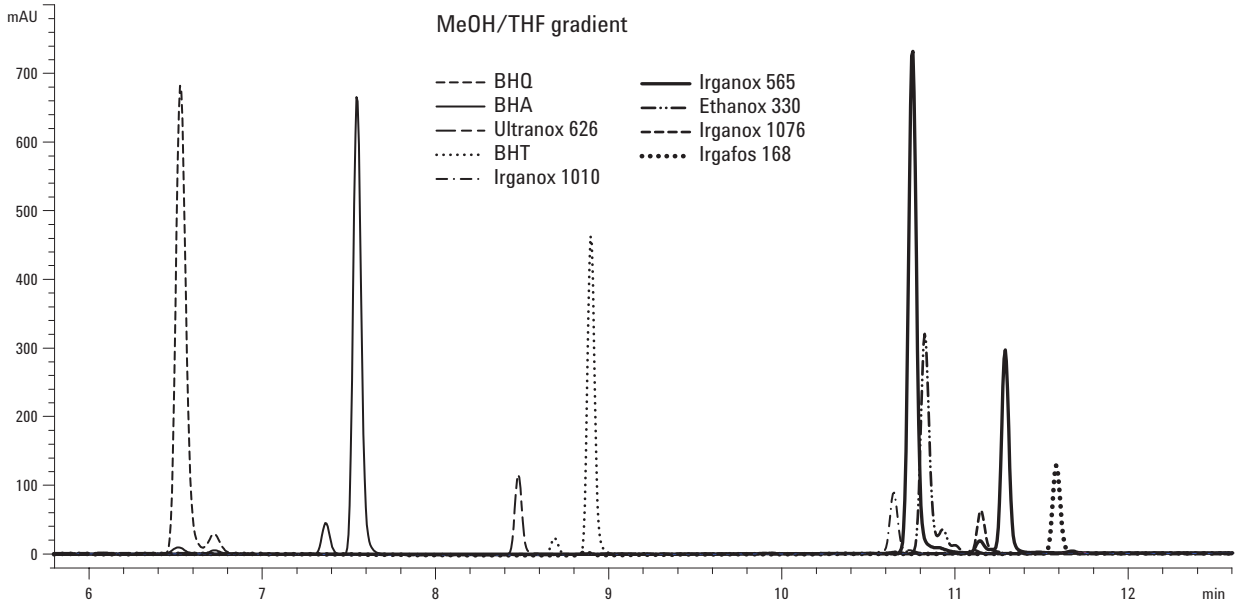


Figure 1. Overlaid UV chromatograms for the selected AOs using the methanol/THF gradient.

Many samples have minor peaks originating from impurities or degradation products having structures similar to the parent molecules. For the smaller molecules like BHA, BHQ, and BHT, there is no problem with resolution. For larger molecules, there is reduced resolution in the 10- to 12-minute region. These molecules have unique MWs, though, and can be analyzed using selective MS detection.

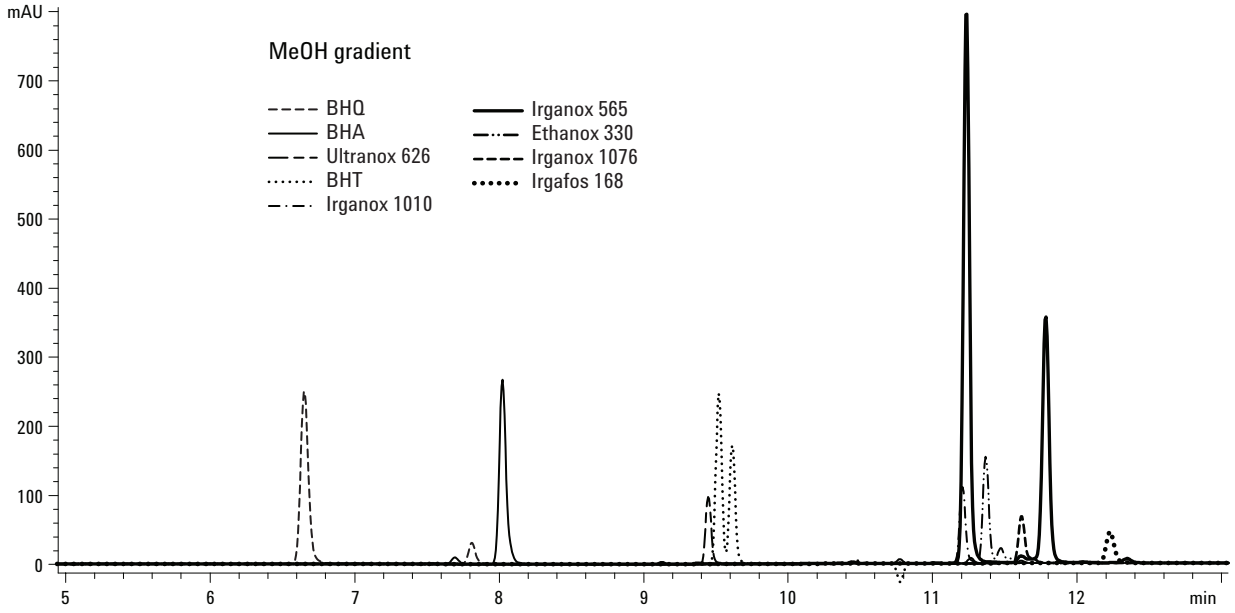


Figure 2. Separation of AOs using the MeOH gradient.

Using the MeOH gradient, relative separation is somewhat different, and as before, the smaller molecules are well resolved. The larger molecules in the 11- to12-minute region exhibit reduced resolution, but can be analyzed using selective MS detection.

Figure 3 shows the separation of the same AOs using the ACN gradient.

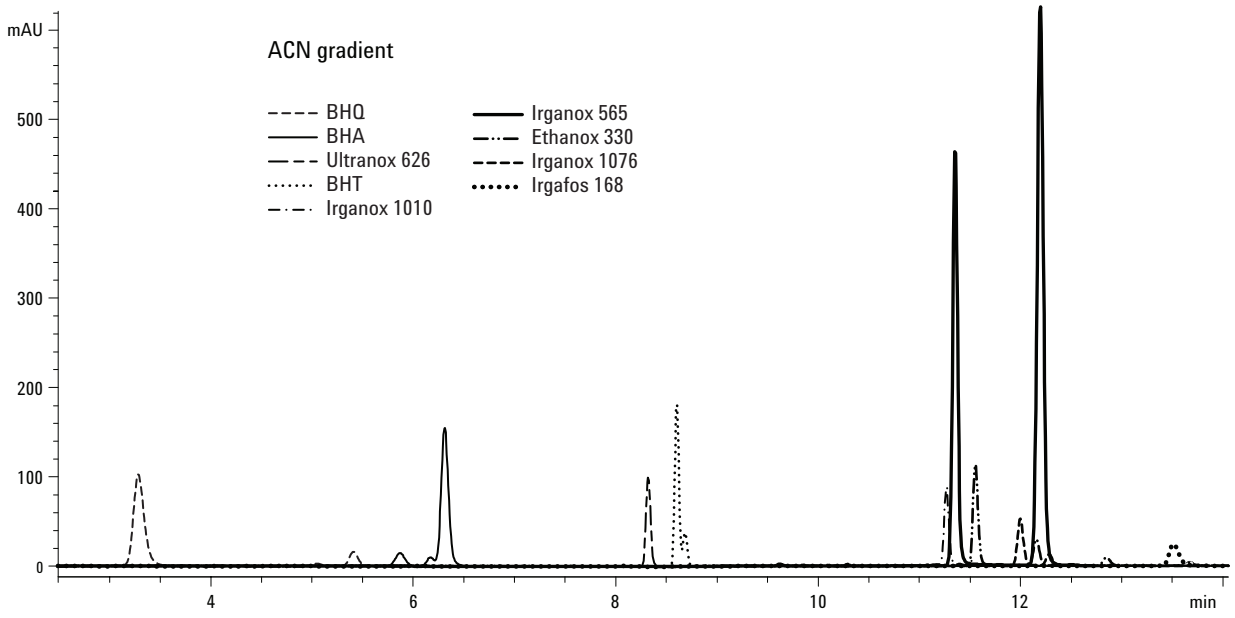


Figure 3. Separation of AOs using the ACN gradient.

Once again, no problem exists with resolution of the smaller molecules. For larger molecules in the 11- to 12-minute region there is somewhat better resolution. ACN has the best UV transparency at low wavelengths, maximizing baseline stability in the wavelength range where UV response would be observed for the AOs.

It is often attractive to use UV/VIS libraries to tentatively identify components in the sample mixture. This approach is especially useful when the various analytes have distinct spectra. Where many AOs have phenolic rings with characteristic UV/VIS spectra, distinguishing analytes by this approach is difficult and the user must rely on retention time data to support any identification attempt.

As we investigate various AO molecules, it is useful to note the general mass range for single- and multiple-ring structures. See Figure 4.

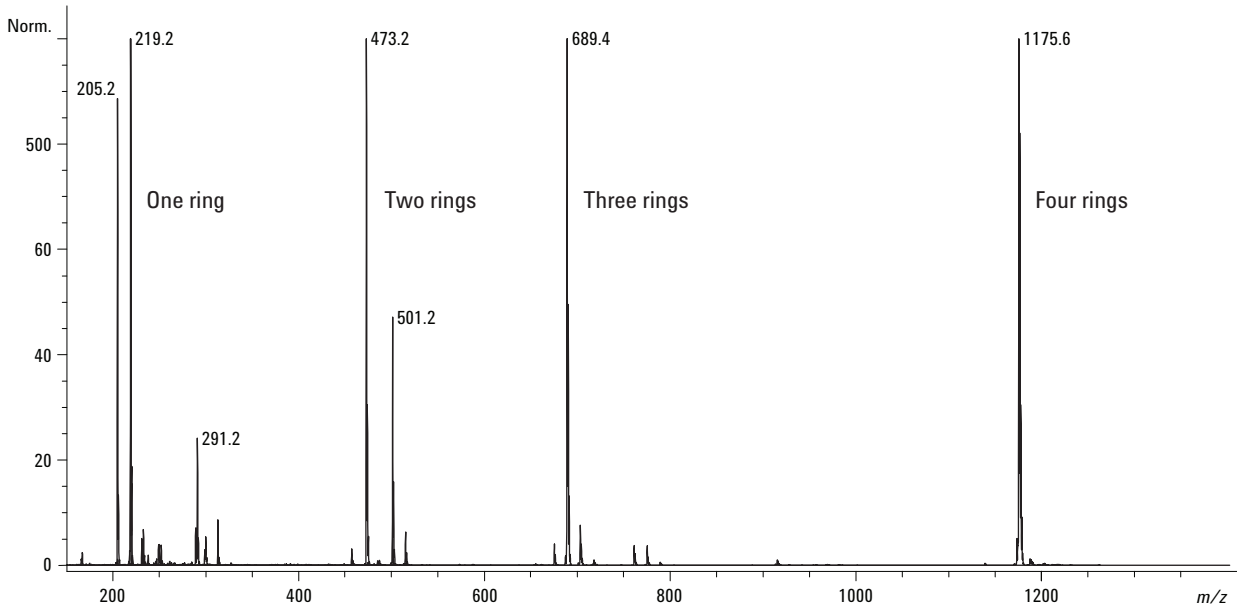


Figure 4. Overlaid AO mass spectra, illustrating effect of ring number on observed mass range.

In Figure 4 we see intact and fragmentation ions representing structures from one to four aromatic rings. The *m/z* 219 is $[M-H]^-$ for BHT while *m/z* 205, less one CH_2, is a fragmentation ion of a larger molecule having the hindered phenolic feature. The *m/z* 473 and *m/z* 501 are fragments discussed later in this text. The *m/z* 689 is Naugard P, $(C_{15}H_{23}O)_3P$. The *m/z* 1176, Irganox 1010, $(C_{73}H_{108}O_{12})$ has four rings and long alkyl chains that increase the mass and remind us that it is important to acquire mass data well over 1000 Da for general AO screening and analysis.

The mobile phase absorbance background invariably affects UV/VIS spectra. See Figure 5. In this example, the UV/VIS spectra for Irgafos 168 are shown for the three previously described solvent conditions.

Significant differences in response, especially in the important low UV range, are generally observed. This interference is also found with many ionic modifiers added to the mobile phase to control ionization of analytes, possibly improving the separation or enhancing ionization of the compounds in the MS.

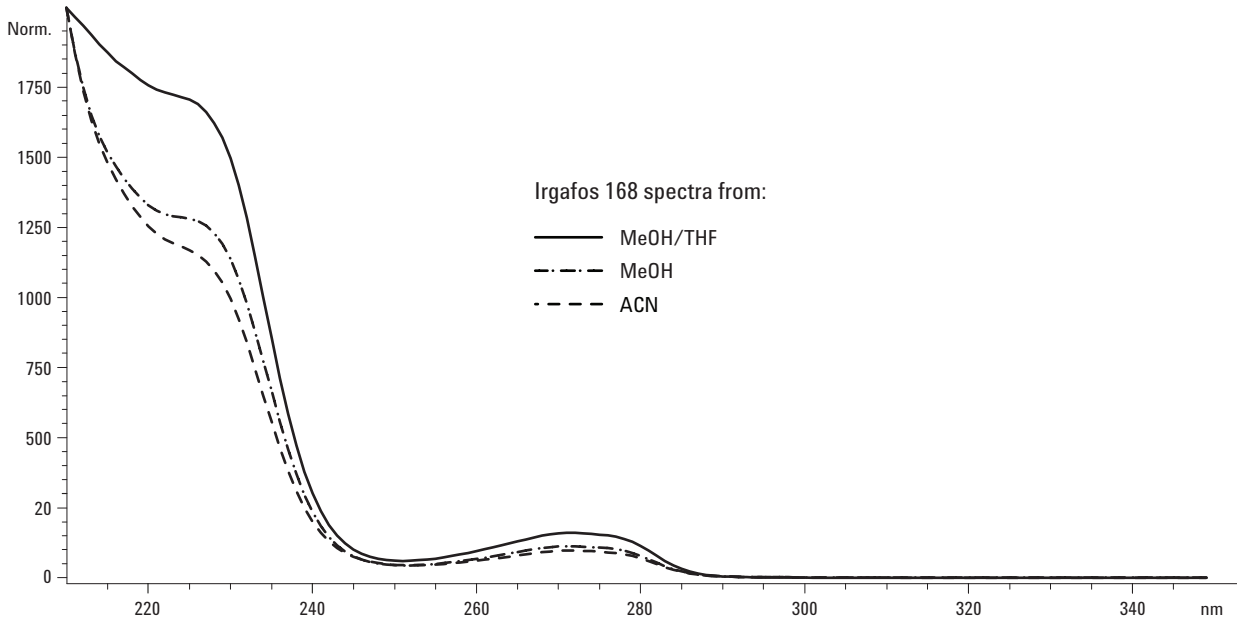


Figure 5. Solvent effects on UV/VIS spectra for Irgafos 168.

Ionization, and thus ion abundance in the MS, may also be affected by the mobile phase composition.

In Figure 6, the extracted positive-ion spectra for Irgafos 168 (molecular weight 646.5, detected as the $[M+H]^+$ ion) appear in the three previously described solvent conditions, where it elutes in high organic concentrations. Observe the significant differences in response, with the lowest response in ACN. Reduced response from the molecular ion may be from decreased ionization or increased fragmentation. It may be possible to add modifiers after the UV, and prior to the MSD inlet, to enhance MS response in circumstances where the solvent offers chromatographic or UV/VIS advantages but negatively impacts ionization in the MS.

The degree of fragmentation in the MS may also be affected by the mobile-phase composition. In Figure 7, the extracted negative-ion spectra for Irgafos 168 appear in the three previously described solvent conditions.

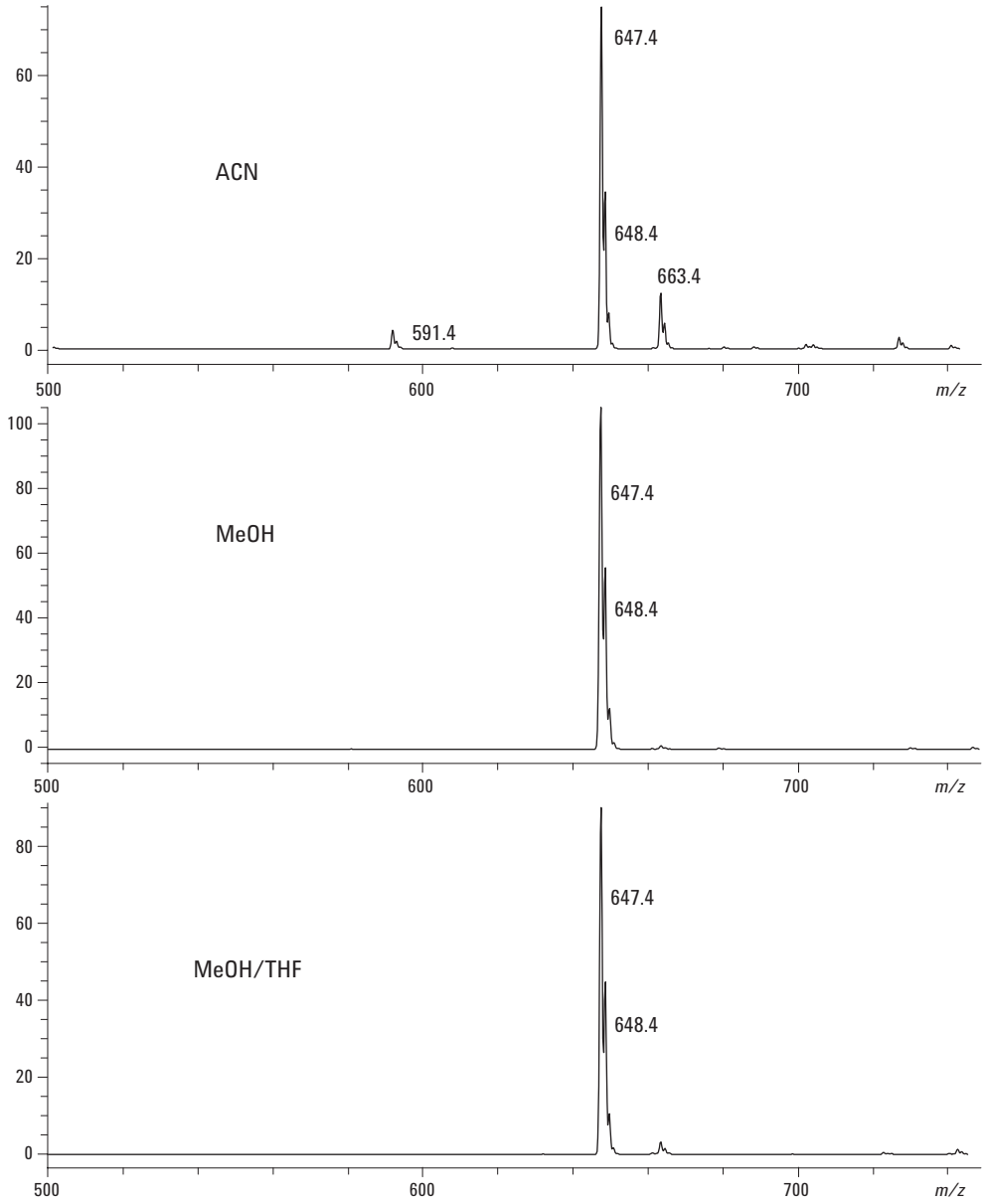


Figure 6. Solvent effects on positive-ion MSD spectra for Irgafos 168.

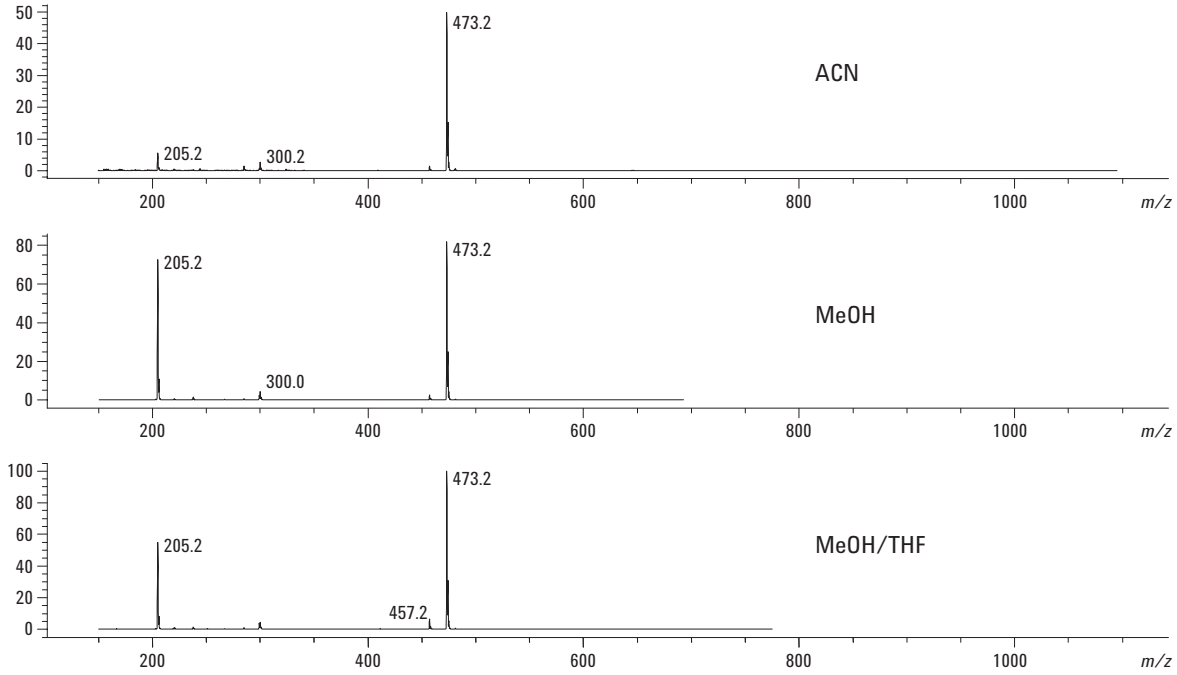


Figure 7. Solvent effects on negative-ion MSD spectra for Irgafos 168.

Note the significant differences in response with the lowest response in ACN. Reduced response for the molecular ion and fragment ions suggests that the ACN response is simply reduced ionization. Based on known degradation chemistry of Irgafos 168 and similar compounds, the *m/z* 473 fragment is likely $[C_{28}H_{42}O_4P]^-$ where an "arm" is lost (*m/z* 205) and an oxygen remains on phosphorous as P=O.

Identification of Unknowns

Retention data may allow experienced chromatographers to suggest how an unknown peak might differ structurally from a group of knowns run under the same conditions, but identification invariably takes far more resources than simple elution patterns provide. From UV/VIS data, we can often suggest molecule class, especially so in our discussion of compounds commonly having the phenoxy group in the chemical structure. UV/VIS spectra may be suggestive but, when used without significant prior knowledge, lack sufficient resolution to confirm identity. MS data, on the other hand, have the spectral resolution necessary to infer structural details leading to actual chemical identification. The following examples describe several situations in which either detector would be helpful.

In the simple case of an unknown containing either BHA or BHT, the UV spectra (Figure 8) are sufficiently unique to allow a reasonable identification along with characteristic retention data. Nearly 1.5 minutes separate these two peaks in the conditions above and little doubt would remain.

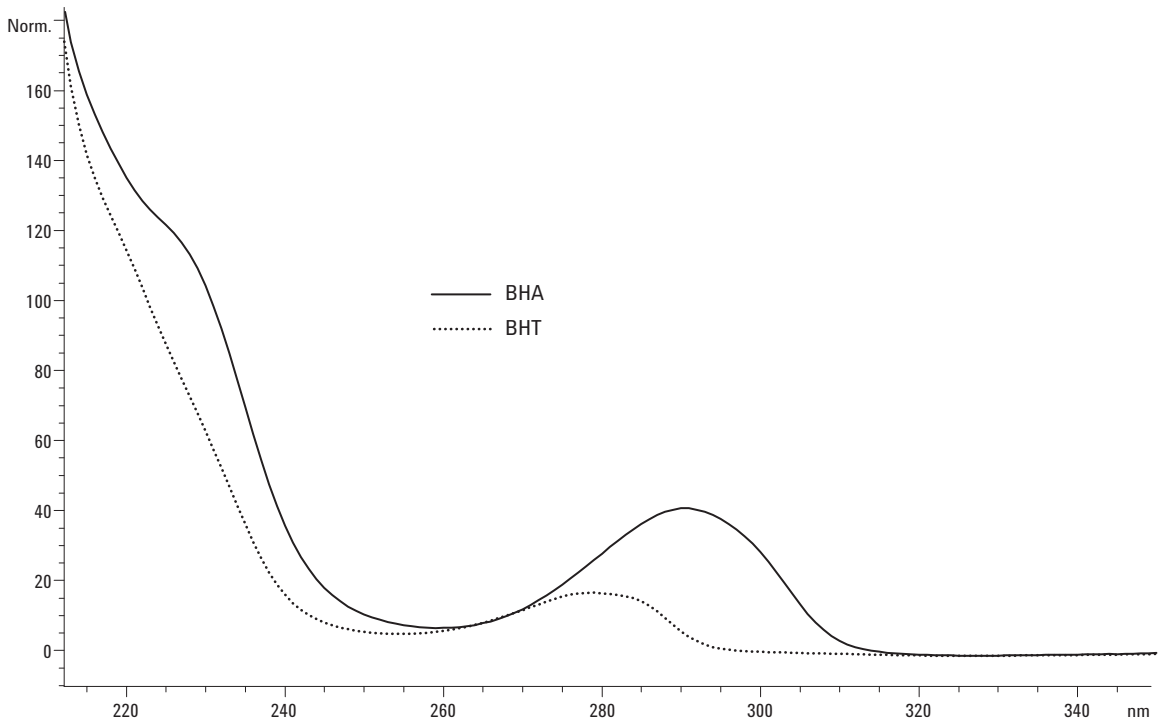


Figure 8. Extracted UV spectra from mixture containing only BHA and BHT.

Using MS data for the same sample, we would reach similar conclusions. See Figure 9.

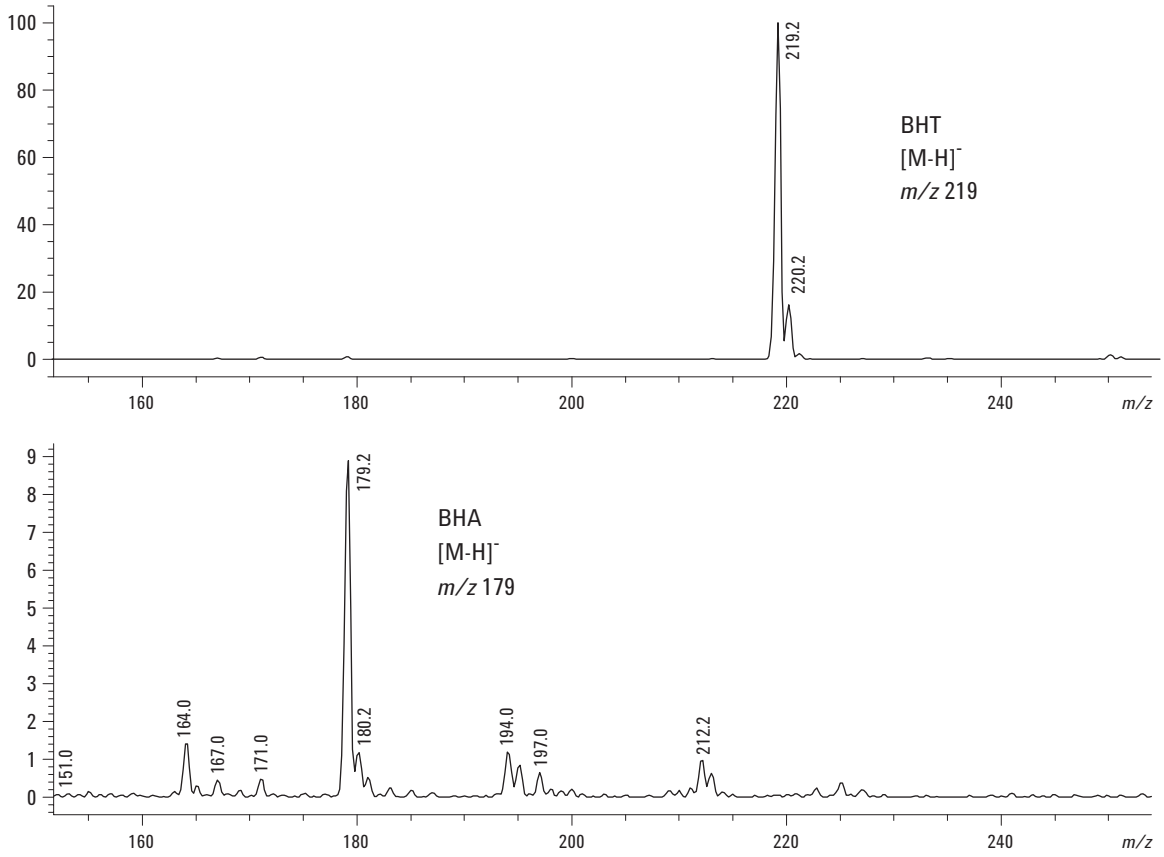


Figure 9. Extracted negative-ion MS spectra from mixture containing only BHA and BHT.

Retention data suggests two distinct molecules leading to an unambiguous identification without any need for MS fragmentation data.

When examining MS data, we generally expect to see classic molecular ions, either molecular mass+1 in positive-ion mode or mass-1 in negative-ion mode. These conditions, in the absence of significant adduct or fragment ion formation, often yield the best sensitivity and quantitative result. Such is the case in the Irganox 565 example shown in Figure 10.

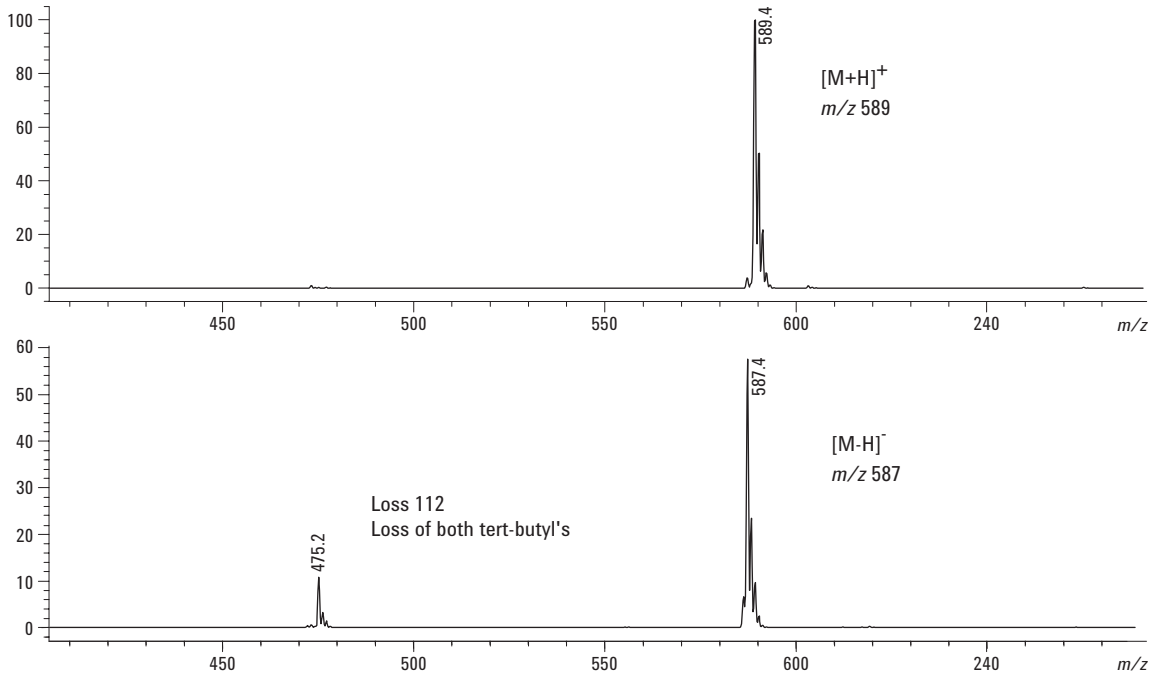


Figure 10. Positive- (upper) and negative- (lower) ion spectra for Irganox 565, using MeOH/THF gradient.

Only minor amounts of fragmentation are seen in the negative-ion spectrum, corresponding to the loss of both tert-butyl groups. In some cases, a radical ion is formed and the MS ion observed will correspond to the mass of the parent molecule. It is difficult to predict when this may occur, but the user must be prepared to interpret the spectral data with this situation in mind.

Irganox 1010 was run under the same conditions and produced minimal fragmentation in the negative-ion spectrum. An $[M\text{-}H]^-$ ion at *m/z* 1175.6 is detected for the expected MW 1176.8. See Figure 11.

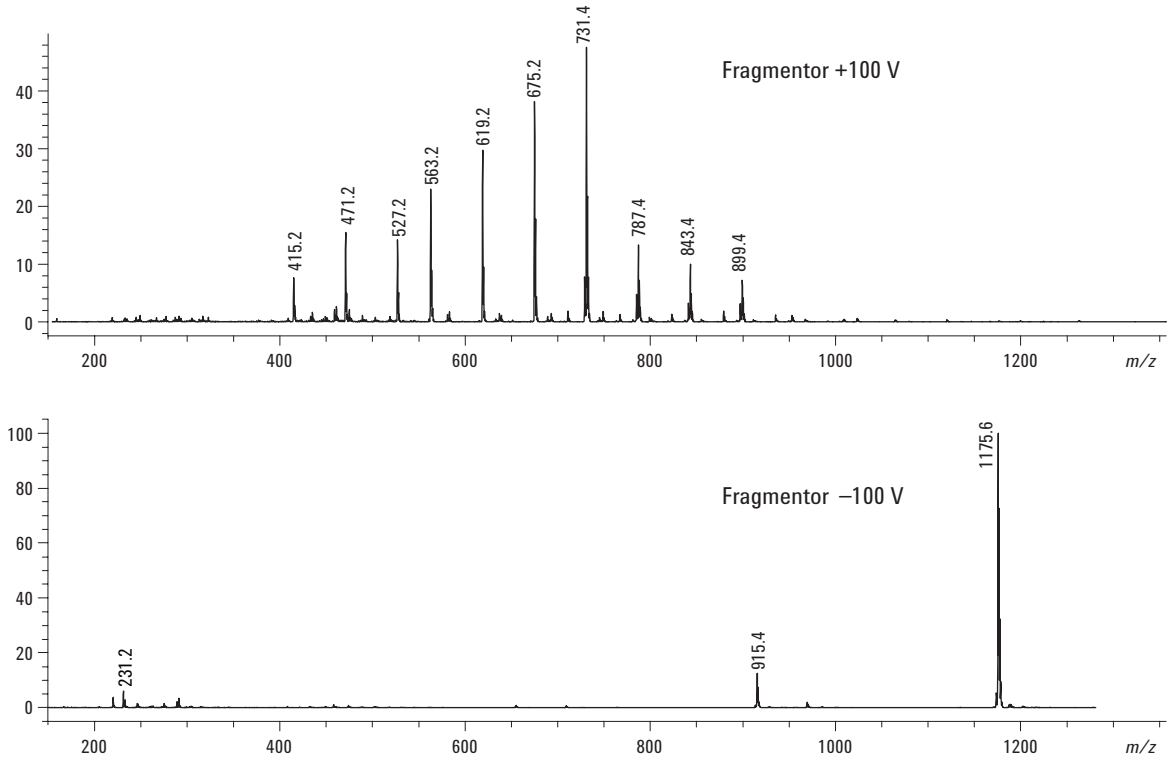


Figure 11. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH/THF gradient.

The positive-ion spectrum, however, is devoid of any useful amount of the molecular ion. The resulting fragmentation pattern suggests a molecule with a significant number of tert-butyl structures which, with the molecular ion from negative ionization, is consistent for a tentative identification for the named compound.

Little change is observed in the fragmentation pattern by reducing the fragmentor voltage to 25 V, though overall ion production is reduced from the 100 V experiments. See Figure 12.

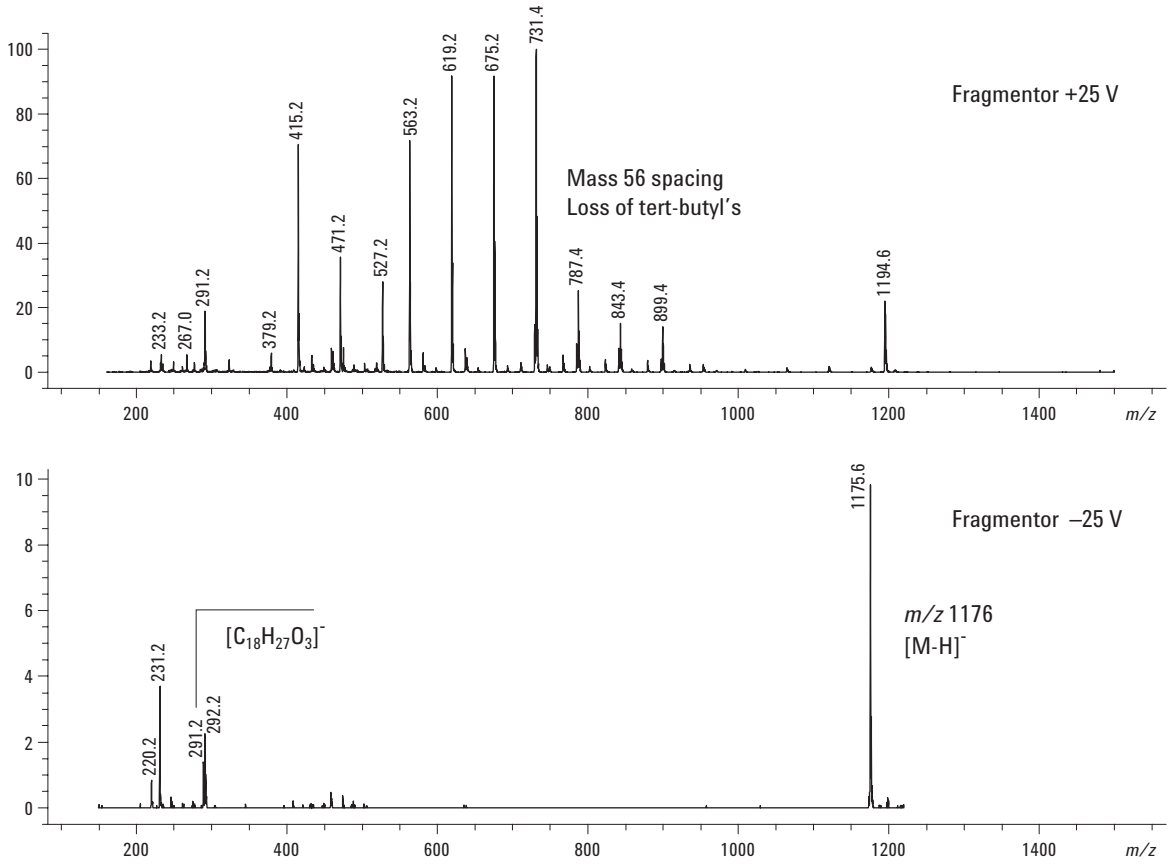


Figure 12. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH gradient.

An *m/z* 291 fragment ion can be observed, which corresponds to one of the symmetrical "arms" of the molecule.

The positive- and negative-ion spectra extracted from the main peak in a degraded standard of Naugard P appear in Figure 13. Naugard P responds comparably to the Irganox 1010 in positive-ion mode, yielding an easily observed molecular ion.

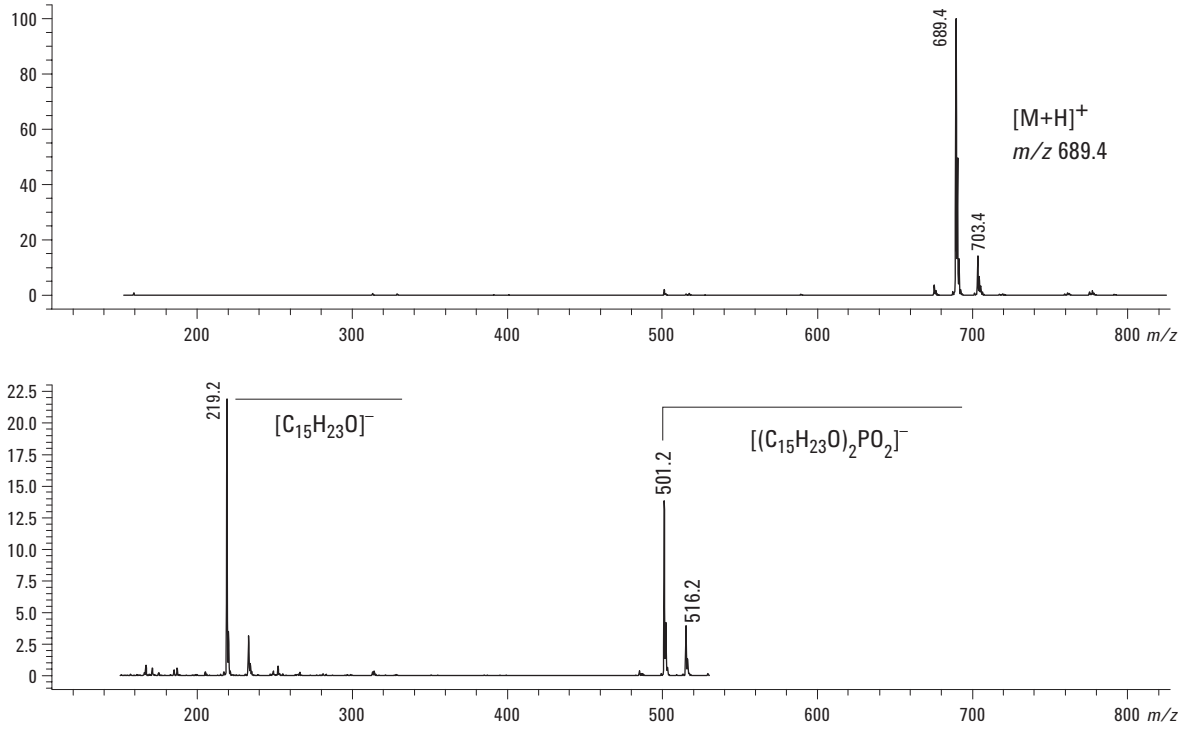


Figure 13. Extracted positive- (upper) and negative- (lower) ion spectra from the main peak in a degraded Naugard P standard.

Poor response in negative-ion mode is presumably due to excessive fragmentation, and no molecular ion is observed. Fragments and minor rearrangements found under these conditions are excellent markers for this sample type and would be good indicators if unknown samples were analyzed.

Peaks in the degraded Naugard P analysis have characteristic positive- and negative-ion spectra which could be studied to confirm typical or propose unknown degradation products. All the peaks seem to have the alkyl side chain present. The other variations presumably lie with the number of oxygen atoms attached to the phosphorous, as proposed in the spectra of the peak at 11.6 min in Figure 14.

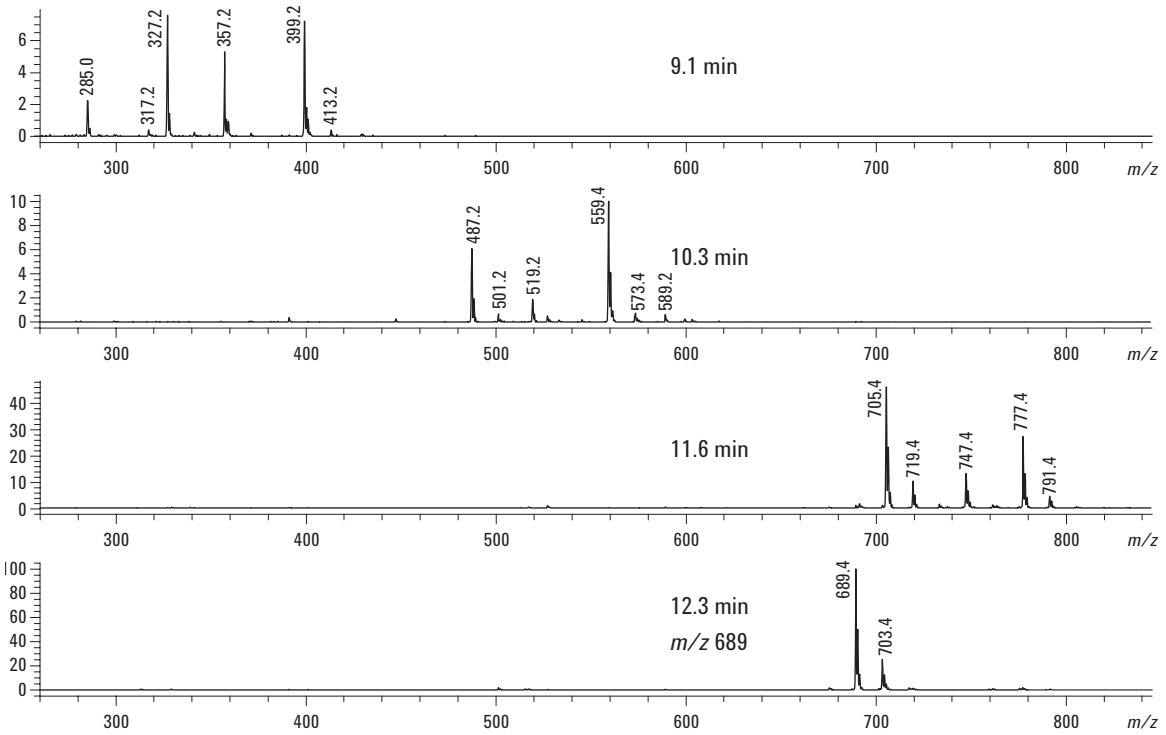


Figure 14. Extracted positive-ion spectra for Naugard P.

Likewise, the negative-ion fragmentation patterns shown in Figure 15 help simplify the investigation by showing differences in the alkyl chain or P-O bonds.

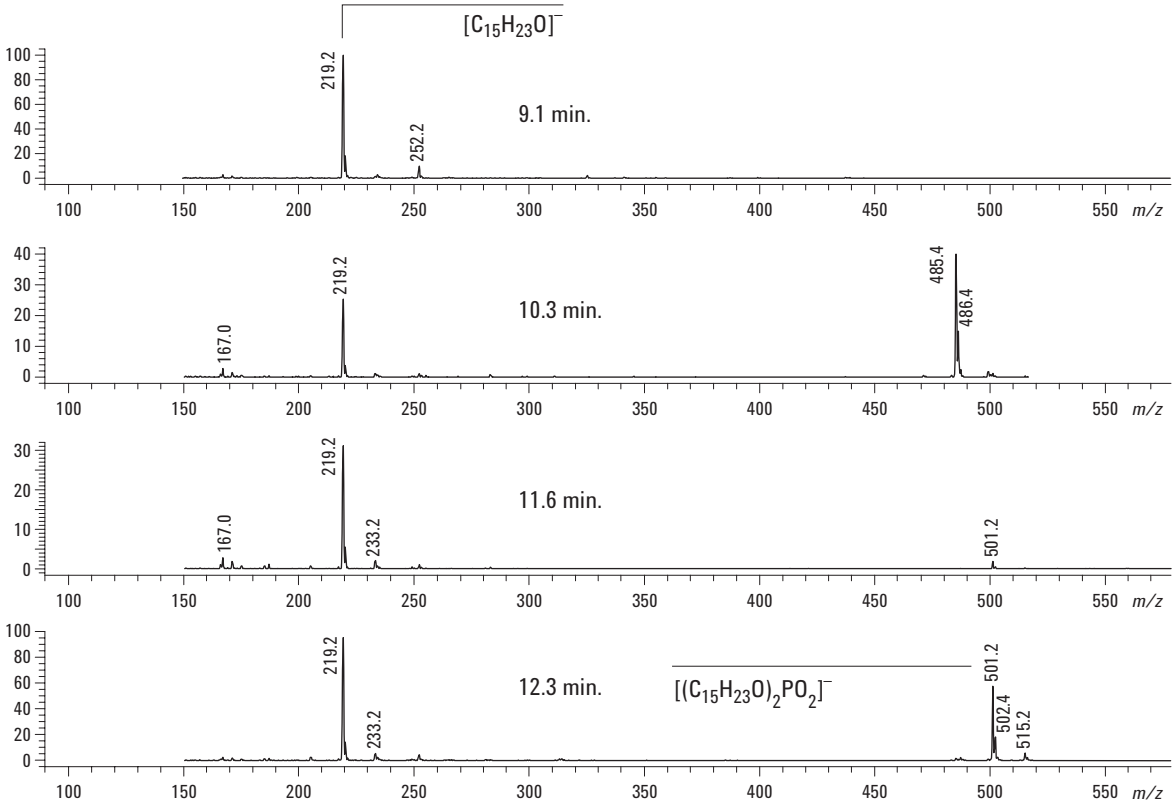


Figure 15. Extracted negative-ion spectra for Naugard P.

We received several unknown samples containing polymer additives. The prepared solutions were analyzed with a wide variety of known standards of AOs and other additive classes. Of all the analyzed standards, Naugard P chromatographic patterns, as shown in Figure 16, most closely matched the unknown samples. Additional spectral investigations followed.

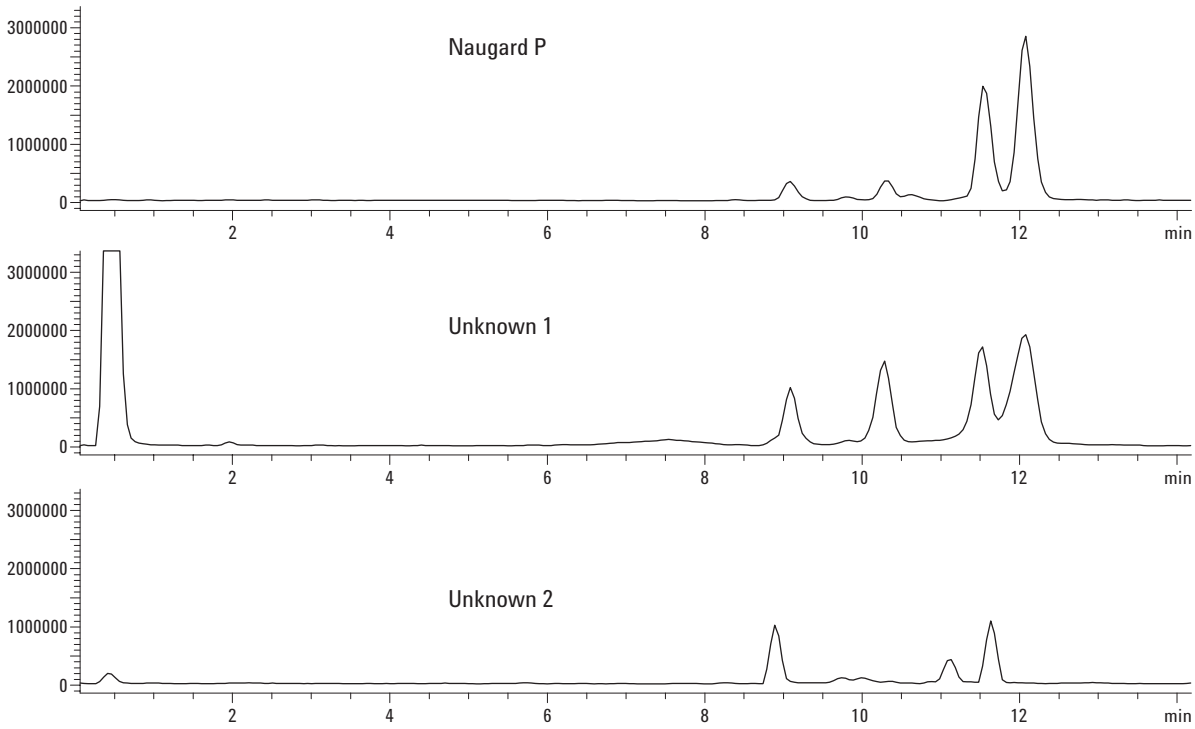


Figure 16. Total positive-ion chromatograms of Naugard P and two unknowns are compared.

The UV spectra for these same samples shown in Figure 17 are similar, though still generally characteristic of many aromatic compounds having minimal ring substitution. These data are interesting, but not conclusive.

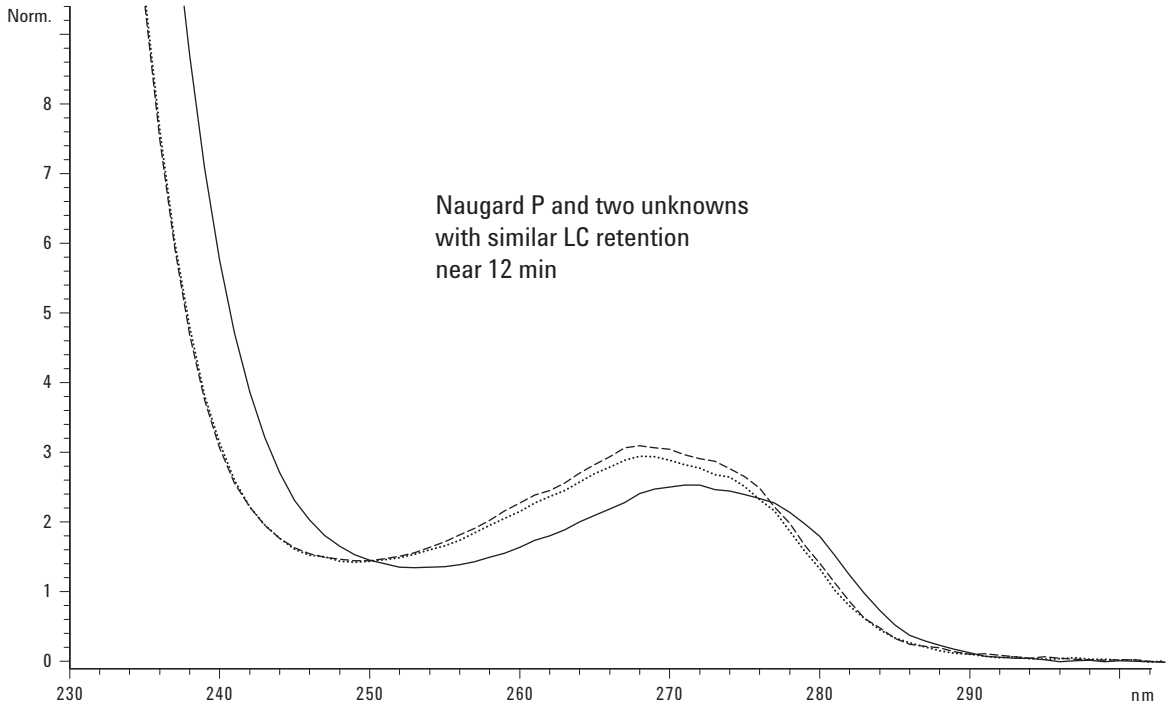


Figure 17. UV spectra of Naugard P and the two unknowns.

The positive-ion mass spectrum of Unknown 1, shown in Figure 18, is an excellent match to that of Naugard P, showing slightly more alkyl variation than the standard. This could be a different lot of Naugard P or a product from a different supplier. Unknown 2 has the primary positive-ion at *m/z* 647, reasonably due to a shorter alkyl chain, C_8H_{17}, compared to the C_9H_{19} alkyl chain on Naugard P.

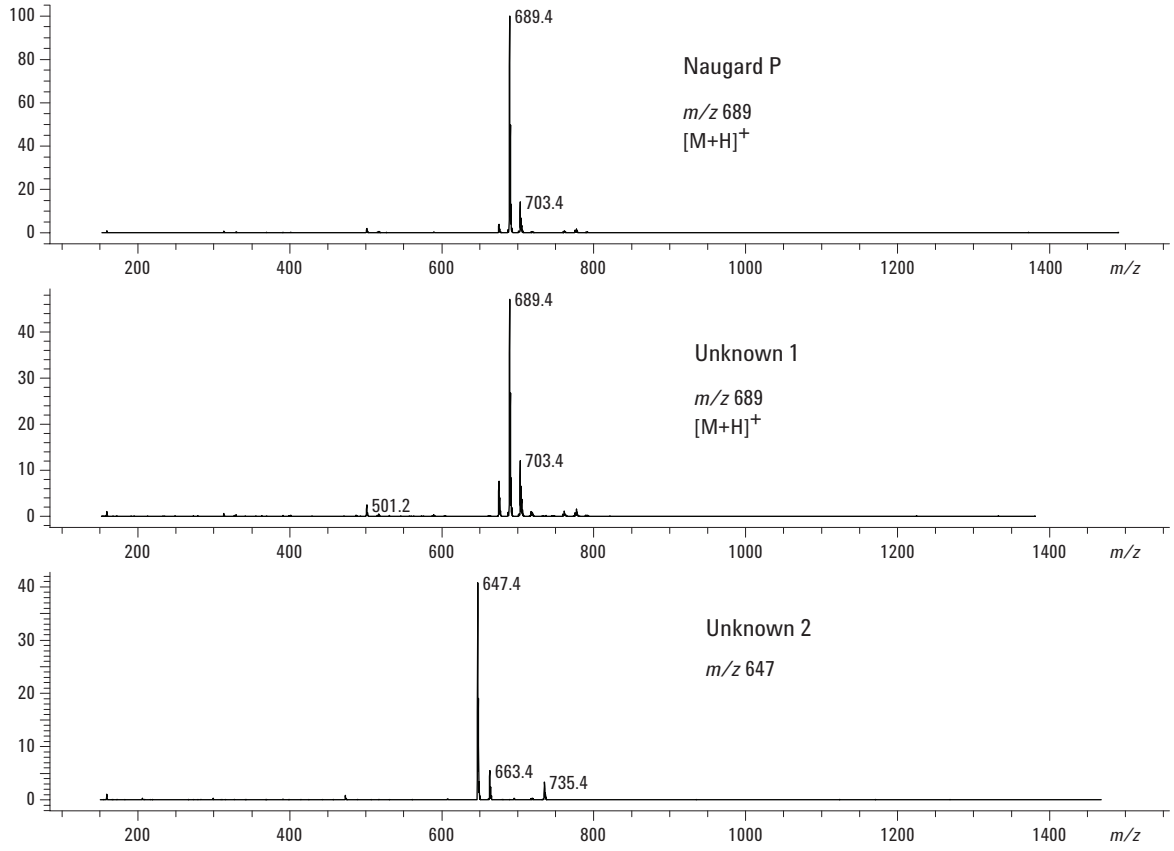


Figure 18. The positive-ion mass spectra of Naugard P and the two unknowns.

In negative-ion mass chromatograms, we see similarities to Naugard P in Unknown 1 and quite dissimilar data in Unknown 2. Recalling from earlier discussions that Naugard P is highly fragmented in negative-ion mode, the negative-ion mass spectra should be extremely helpful in supporting our initial thoughts taken from the positive-ion spectra. See Figure 19.

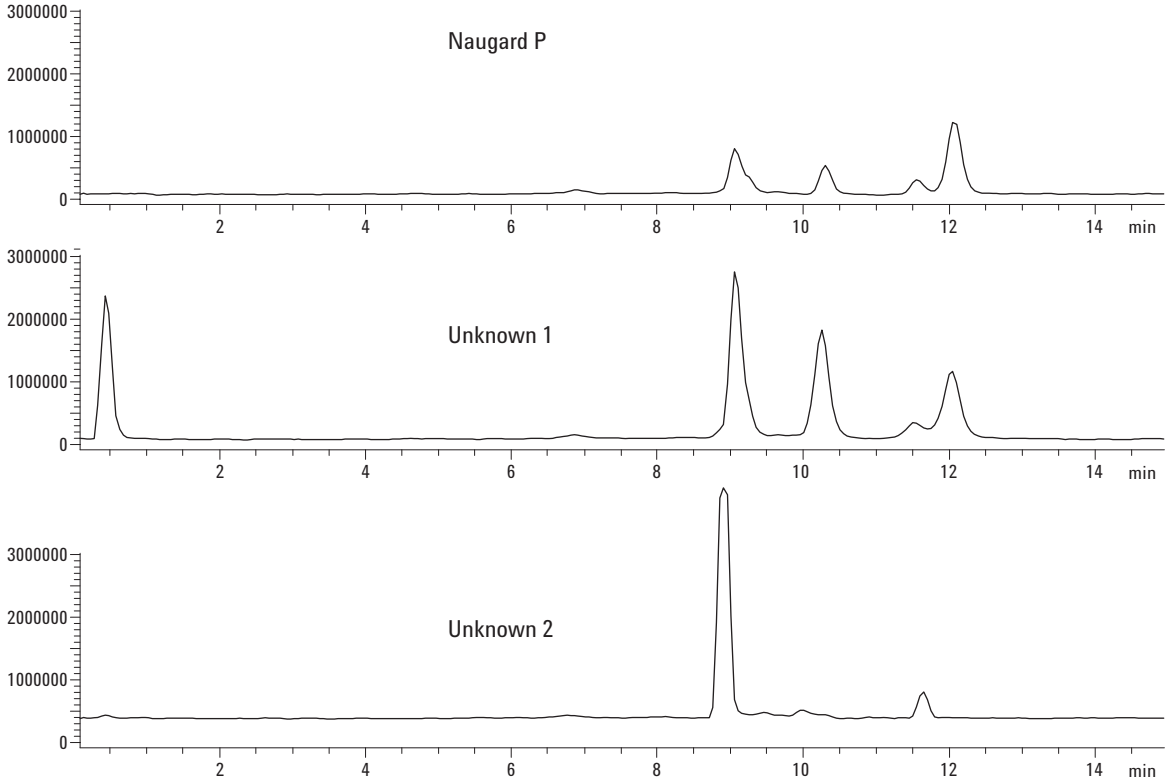


Figure 19. Total negative-ion chromatograms of Naugard P and two unknowns are compared.

The negative-ion spectra for Naugard P and Unknown 1 are an excellent match and probably offer the best support of that chemical identity and structural details. Unknown 2, however, speculatively presents two CH_2's less in the *m/z* 501 fragment and one CH_2 less the *m/z* 219 fragment. See Figure 20. This is highly supportive of the proposed structure from the positive-ion data and allows us to conclude that, while similar to Naugard P, it is a unique product whose structure is most likely $(C_6H_4\text{-}C_8H_{17}\text{-}O)_3P$.

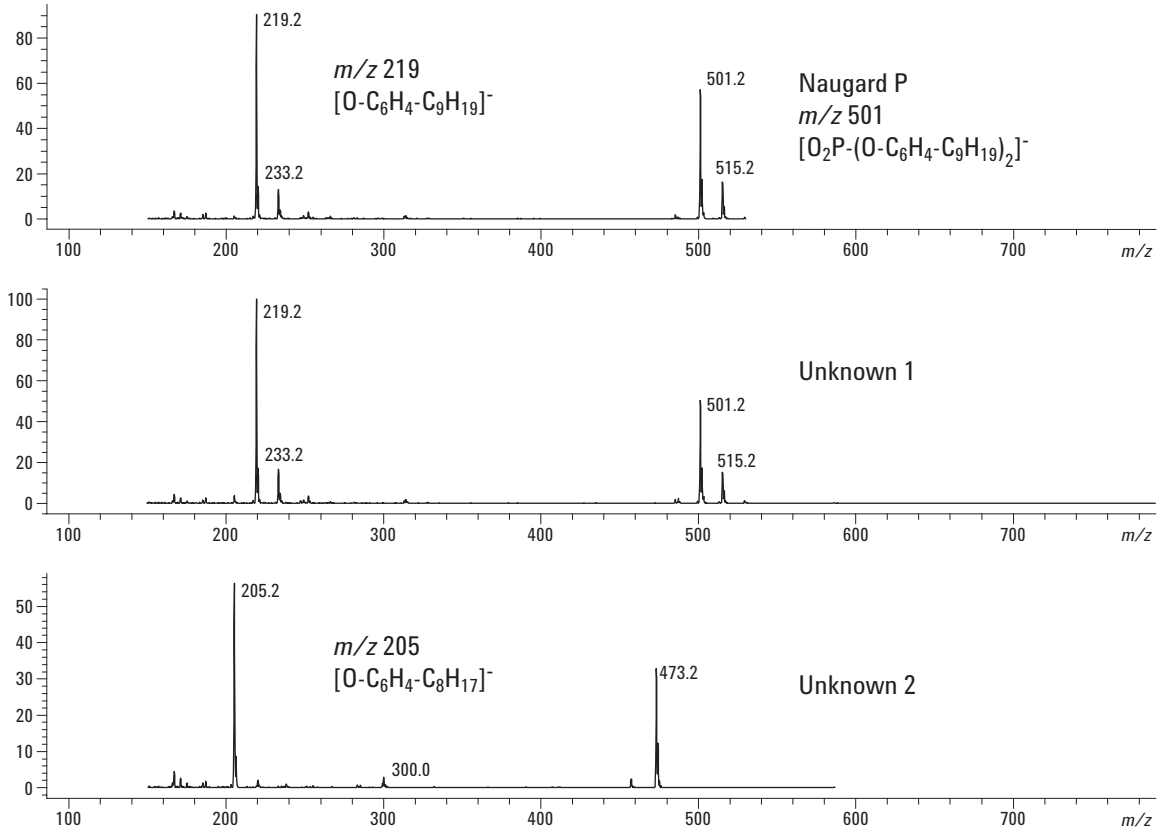


Figure 20. Negative-ion fragmentation mass spectra of Naugard P and the two unknowns.

Conclusions

- LC with UV/VIS and MSD detection is a powerful approach to compound analysis and identification.
- Mobile phase conditions affect the quality and usability of the acquired data.
- Unknown compounds can be tentatively identified with MS data.
- Additive degradation can be quickly evaluated to optimize formulations for better performance.

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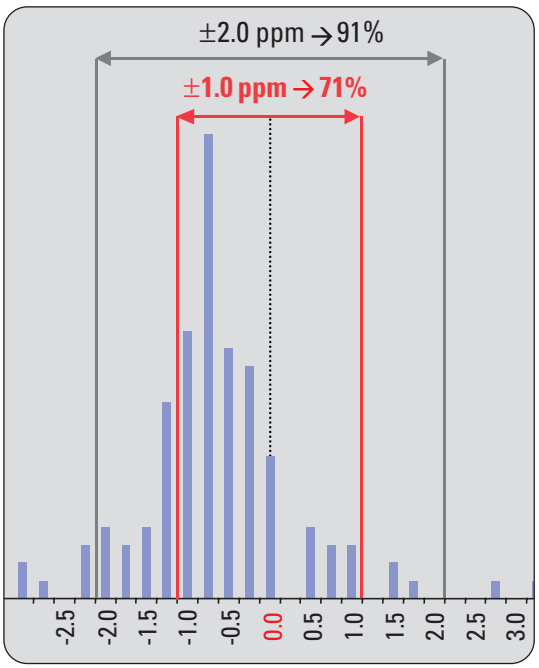
Printed in the USA
March 17, 2003
5988-8610EN



Agilent Technologies

Agilent 1200 Series Rapid Resolution LC system and the Agilent 6210 TOF MS – Highest data content with highest throughput

Application Note



Michael Frank

Abstract

Fast and unambiguous determination of purity and identity of compounds derived from screening libraries is a common task for many analytical labs in the pharmaceutical industriy. The method of choice to determine the identity of compounds is mass spectrometry, preferably with accurate mass. As yet, data quality was usually compromised by gaining higher throughput. This Application Note demonstrates how a daily throughput of far more than 1000 samples can be achieved together with full spectral data acquisition and accurate mass information with close to FT-MS mass accuracy.

Agilent Technologies

Introduction

In the quest to achieve highest throughput in LC/MS analyses, the quality of the data is often compromised. There are certain approaches to increase the throughput of LC/MS systems. One approach is to do flow injection analysis. This probably delivers the highest possible throughput, however since no chromatographic separation occurs, the probability to loose compounds by the ion suppression effect during the ionization process is high. Orthogonal detection methods like UV detection do not succeed at all in flow injection analysis as all compound signals are overlaid. Approaches to achieve at least minimal chromatographic separation by using very short columns with 5 µm particles and ballistic gradients are an improvement in view of data quality, however, not state-of-the-art. Some manufactures have established parallel working instrumentation with a shared mass spectrometer and shared UV detector. Obviously, this also compromises data quality as the full acquisition rate of each instrument has to be shared on each LC channel[1].

With the introduction of an LC/MS system which facilitates the use of columns with sub two micron particles it is now possible to achieve short analyses times as well as high chromatographic resolution. Furthermore the system is able to acquire full UV spectral data and mass spectral data with accurate masses.

Experimental

The Agilent 1200 Series Rapid Resolution LC system is set up for alternating column regeneration (ACR)[2] using 2.1-mm id columns. The pumps are in the low delay volume configuration with an internal volume of only ca. 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744) and the alternating column regeneration kit (G1316-68721). Consequently, from the injection valve on only capillaries of 0.12 mm id are used. In the thermostatted column compartment the newly introduced low dispersion heat exchangers consisting of 1.6 µL internal volume have been used as well as the high pressure rated 2-position/10-port valve.

The instrument set-up is shown in figure 1:

- Two Agilent 1200 Series binary pumps SL with the new Agilent 1200 Series micro vacuum degasser placed between the two pumps eliminates the need for long tubing to the pumps.
- Agilent 1200 Series high performance autosampler SL.
- An Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration.
- An Agilent 1200 Series diode-array detector SL allowing a data acquisition rate of 80 Hz and equipped with a 500 nano liter flow cell with 0.12-mm id connecting capillaries.
- Agilent 6210 Time-of-Flight mass spectrometer allowing a maximum data acquisition rate of 40 Hz and equipped with a dual ESI source for parallel ionization of the analyte and a reference mixture.
- Two ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm columns
- As mobile phase gradient grade water with 0.1 % trifluoro acetic acid and acetonitrile with 0.08 % trifluoro acetic acid was used. No additional filtering of the solvents was made.

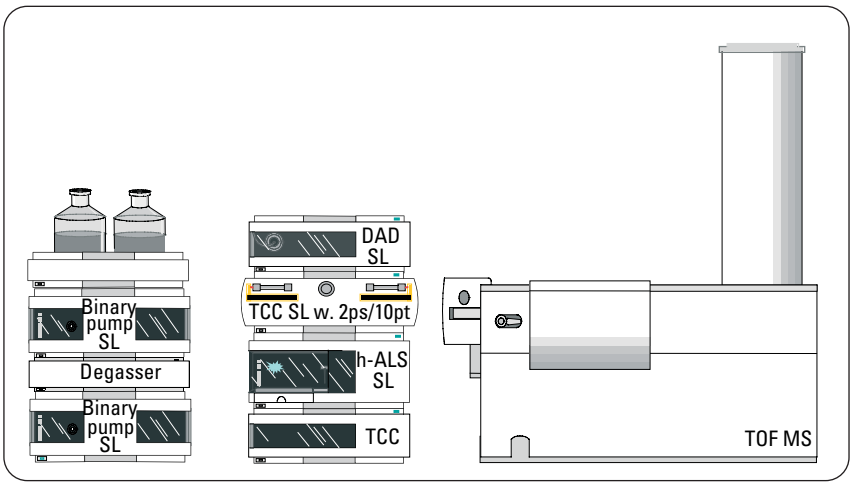


Figure 1
Agilent 1200 Series Rapid Resolution LC system with Agilent 6210 TOF-MS with low delay volume for high speed applications using 2.1-mm id columns with lengths ranging from 20 to 50 mm.

Instrument control and data acquisition was done by the Agilent TOF-software A02.01 running on a Hewlett-Packard xw 4300 workstation with an Intel dual core Pentium™ D840 CPU at 3.2 GHz.

Results and discussion

By applying elevated temperatures the viscosity of the solvent can be reduced which allows higher flow rates and therefore shorter gradient times. A maximum temperature of 80 °C was applied, which allowed a flow rate of 1.8 mL/min without hitting the pressure limit of the pump. This results in a linear velocity of approximately 11 mm/s for the 2.1 mm x 50 mm column (1.8 µm). With the help of the regeneration pump and the 2-position/10-port valve in the column compartment cycle times could be reduced significantly because one column is flushed with high organic content solvent and then re-equilibrated again with the starting composition of the gradient while on the second column the separation of a sample occurs. After this sequence the 10-port valve is switched and both columns are exchanged in the flow path. Details of alternating column regeneration and the correct setting of time points are described in another Application Note[2]. Despite the high flow rate (1.8 mL/min), the column effluent was not split prior to reaching the mass spectrometer. The standard ESI source specifies a maximum flow rate of up to 1 mL/min, however even these higher flows are tolerated if the drying gas temperature and flow rate are set to maximum and little condensation occurs. Condensation of water is practically eliminated when using ACR because equilibration is done on the column which

Data File	Sample Type	Inj Vol (µl)	Capillary	Fragmentor	Skimmer
opt_4000_215_60.wiff	Unknown	1	4000	210	60
opt_4000_215_60.wiff	Unknown	1	3000	210	60
opt_4000_215_60.wiff	Unknown	1	2000	210	60
opt_4000_215_60.wiff	Unknown	1	4000	180	60
opt_4000_215_60.wiff	Unknown	1	3000	180	60
opt_4000_215_60.wiff	Unknown	1	2000	180	60
opt_4000_215_60.wiff	Unknown	1	4000	150	60
opt_4000_215_60.wiff	Unknown	1	3000	150	60
opt_4000_215_60.wiff	Unknown	1	2000	150	60
opt_4000_215_60.wiff	Unknown	1	4000	210	40
opt_4000_215_60.wiff	Unknown	1	3000	210	40

Figure 2
Feature of the TOF software to modify the MS parameter from run to run.

Method:				
Solvent:	A = water (0.1% TFA), B = ACN (0.08% TFA)			
Temperature:	80 °C			
Flow:	1.8 mL/min			
Gradient:	0.00 min 5%B 0.50 min 90%B 0.51 min 5%B 0.65 min 5%B		**Regeneration:**	0.00 min 5%B 0.01 min 95%B 0.20 min 95%B 0.21 min 5%B 0.65 min 5%B
Stoptime:	0.65 min			no limit
Posttime:	off			off
DAD:	Wavelength:	210 nm (8), ref. off		
	Peak width:	>0.0025 min (0.05s responsetime), 80 Hz		
	Spectra:	no		
	Slit:	8 nm		
	Balance:	pre-run		
MS:	Scan range:	100-1000 m/z		
	Acquisition rate:	5, 20, 30 and 40 cycles/s		
	Data type:	profile data		
	Capillary voltage:	3000 V		
	Fragmentor:	180 V		
	Skimmer:	40V		
	Gas temperature:	350 °C		
	Gas flow:	13 L/min		
Injection volume:	1µL			
Injector:	Overlapped injection, Automatic delay volume reduction, Sample flush out factor = 10			
Valve position:	Next position			

Table 1
LC/MS method used for the data shown in figures 3-5. The method was also used to achieve the values in table 2.

is not connected to the detector. Generally the use of an Agilent multi mode source with a specified flow rate up to 2 mL/min even with pure water is recommended. The chromatographic conditions in table 1 were used to achieve gradient times of 0.5 min. Under these conditions, the peak capacity for the MS detection is in the range of >40 in 39 s. With the use of a 5-µm particle size column of the same dimension the peak capacity would only be half!

The detector of the Agilent 6210 TOF MS would be saturated if the compound concentrations used here to give also significant UV signals would be injected into the MS without special settings. Saturation of the MS detector would produce incorrect results in mass determination. The solution is to intentionally desensitize the TOF MS. This can be done quite easily by applying the functionality of the TOF software to alter the MS parameters from one run to the other,

simply by adding one or more "MS-parameter" columns to the worklist (figure 2). Select "add columns" from the worklist and then chose "MS-parameter" and the desired parameter. As the reference mixture is also affected by these settings, the concentration of the reference mixture was increased. Only the capillary voltage, the fragmentor voltage and the skimmer voltage were varied. The optimal conditions determined by this approach can be found in the method parameters in table 1.

In figure 3 the total ion chromatogram and the UV chromatogram achieved with conditions above (80 Hz DAD, 30 Hz TOF data acquisition rate) is shown for a five-component sample (58 ng/µL atenolol, 85 ng/µL primidon, 62 ng/µL metoprolol, 125 ng/µL verapamil and 75 ng/µL beclomethasone-dipropionat). The peaks of the total ion chromatogram are inherently broader than the peaks of the UV chromatogram because of additional extra column volume from the flow cell and also from connecting the capillary between the UV detector and ESI interface. But as can be seen in figure 3, the additional peak broadening of the MS peaks is only minor. The peak widths at half height of the MS peaks obtained under the highest data acquisition rate (40 Hz) are shown in figure 4 with values from as little as 0.34 to 0.42 s. The chromatograms shown in figure 5 were produced under the same chromatographic conditions, but with different data acquisition rates of the time-of-flight MS. The peak form and resolution are improved by having high data acquisition

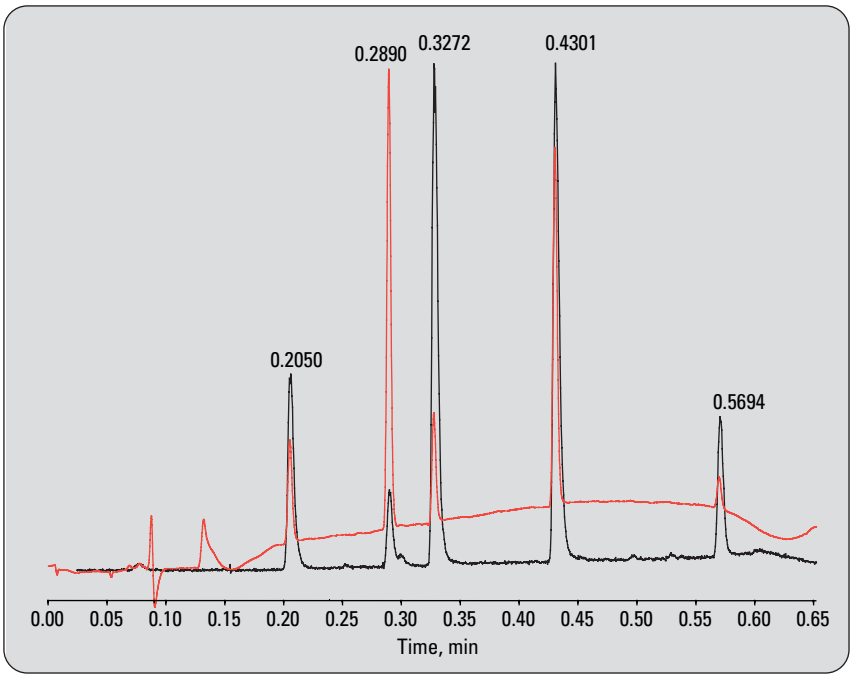


Figure 3
Comparison of corresponding peaks in the UV (red trace) and the MS detection (black trace).

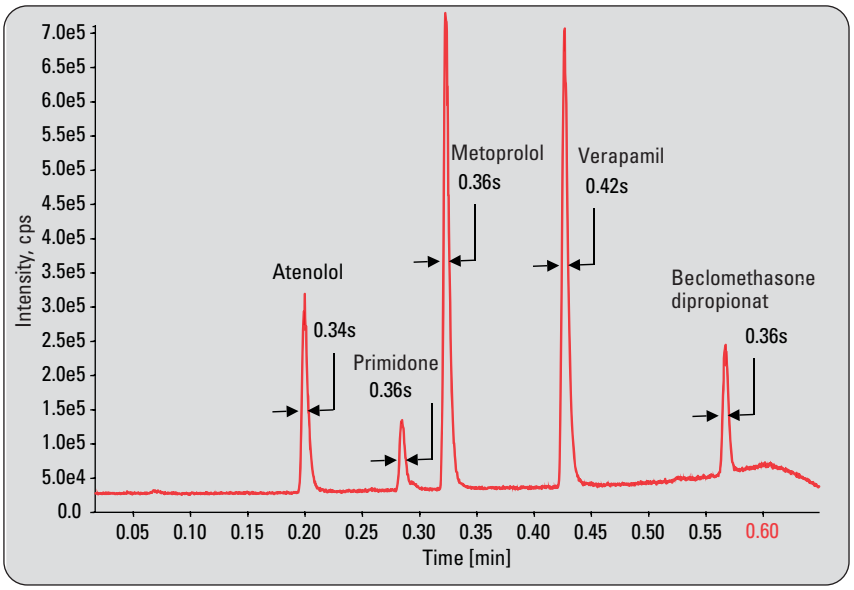


Figure 4
MS total ion chromatogram of highest speed LC-TOF-MS analysis (40 Hz TOF data acquisition rate).

rates in the MS which shows clearly in figure 5. The effect is nicely demonstrated on the little side peak next to the primidon peak – with 40-Hz data acquisition rate it is obvious that an additional compound shows up but with 5 Hz data acquisition rate this could not be differentiated from tailing of the primidon! The advantage, especially if MS quantization is necessary, is clear.

By applying the chromatographic conditions of table 1 and 80 Hz signal data acquisition of one wavelength and 30 Hz TOF centroid data, a cycle time of 49 s was achieved. The achievable cycle time is not only dependent on the used run time (that is the gradient time plus additional flush and re-equilibration times, or in Agilent terminology the stop time plus post time) but also very much dependent on the instrument overhead time. This is usually caused by communication between the data system and the individual LC/MS modules as well as the data system writing data to the hard disc and initiating certain processes. The overhead time caused by the data system can be significant if the computer's performance is not sufficient to handle the data amount or if other software programs or processes are consuming the power available. To decrease the cycle time it might be worth decreasing the amount of data acquired.

Table 2 shows the cycle times and the possible daily throughput depending on the DAD and MS settings. Since the MS data are constantly written to the hard disc during data acquisition, whereas the UV data are buffered and added to the data file after the stop time of

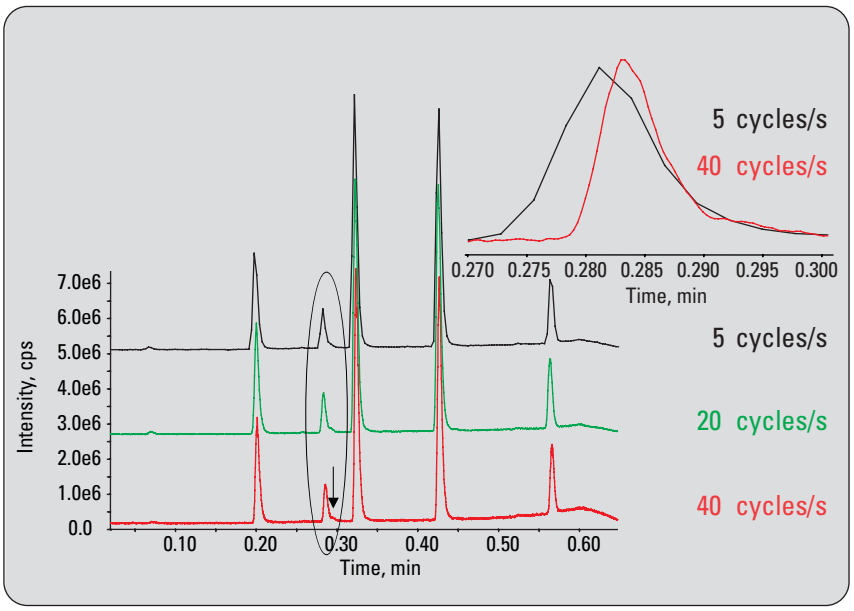


Figure 5
Total ion chromatograms recorded with varying data acquisition rates – dependence of the MS peak shape and resolution on the data acquisition rate.

DAD (80 Hz)		TOF (100 – 1000 Da)			Cycletime	Throughput
Type	Wavelength	Centroide	Profile	Data rate [Hz]	[s]	[Samples/day]
spectral	190-900 (1)		x	20	62	1394
spectral	190-900 (1)	x		20	62	1394
spectral	190-400 (2)		x	20	59	1464
spectral	190-400 (2)		x	40	59	1464
spectral	190-400 (2)		x	30	58	1490
signal	210/254		x	20	50	1728
signal	210	x		30	49	1763

Table 2
Dependence of the cycle time on the DAD and MS data acquisition settings, method stop-time was 0.65 min (39 s), pre-run balance was applied (ca. 2 s). The number in brackets for the DAD wavelength range stands for the scan width in nm.

the method, the cycle time depends more on the UV data amount than on the MS data amount. The cycle time was calculated from the time stamp each file gets assigned from the WindowsXP™ operating system after closing the file following data acquisition.

If using a TOF MS the attention is certainly focused on the accurate mass. The question may arise if the possibility to obtain low mass accuracy errors might suffer from these high speed conditions. Figure 6 shows the achieved mass accuracy errors of the analysis of 140 members of a chemical library used in a screening campaign by a pharmaceutical company. The shown error-values have been extracted from an automated empirical formula confirmation report and involved no manual interference. Sixteen of the compounds could not be ionized under positive ESI conditions and two compounds showed large mass errors of 11 and 15 ppm, probably caused by co-eluting isobaric impu-

rities. The cycle time was 90 s and was determined by a required injector program which allowed an on-line dilution of the samples directly prior to the analysis. Chromatographic conditions applied a 5-100 % water-acetonitrile (0.1 % TFA) gradient in 0.7 min at a flow rate of 1.5 mL/min and 60 °C column temperature. UV data acquisition to determine purity was done in the wavelength range of 210 to 500 nm with an acquisition rate of 80 Hz. The MS data acquisition rate was at 8 Hz to reduce the file size. The scan range was 120 – 1200 Da, capillary voltage 4000 V and the fragmentor voltage at 215 V. No ACR was applied and the flow to the MS was splitted in a 1:7.5 ratio.

More compelling is the histogram of the mass errors of these samples as shown in figure 7. More than 91 % of the ionizable compounds (outliers included) have a mass accuracy error in the range of ±2.0 ppm. Excluding the outliers even 93 % of the analyzed samples lie in-between the ±2.0 ppm range. In the ±1.0 ppm range which is FT-MS-like mass accuracy 71 % of the samples can be found (72 % excluding the outliers).

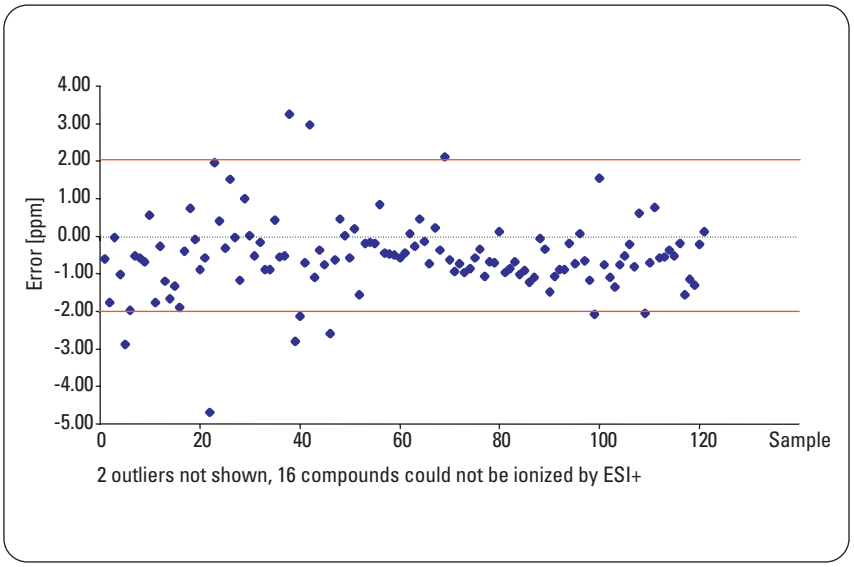


Figure 6
Mass accuracy errors of the analyses of a set of chemical library members under fast-LC conditions.

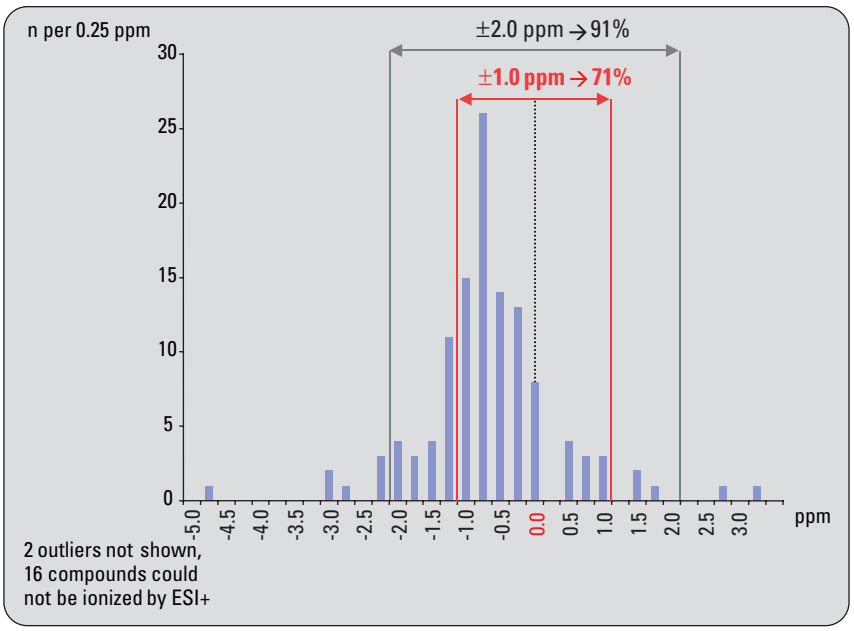


Figure 7
Histogram of the mass accuracy errors of the analyses of a set of chemical library members under fast LC conditions. The given populations of the ±1.0 ppm and ±2.0 ppm range include the outliers.

Conclusion

The Agilent 1200 Series Rapid Resolution LC system together with the Agilent 6210 Time-of-Flight mass spectrometer allows acquisition of a wealth of data to unambiguously determine the purity and identity of compounds in samples as they are typical for the high throughput analytical departments of pharmaceutical companies. In the time range of one minute high chromatographic resolution, full spectral diode-array data from 190-900 nm wavelength in a band width of 1 nm at an 80 Hz acquisition rate plus full MS spectral data from 100-1000 m/z with high acquisition rate and with an accurate mass with a mass error below ±2.0 ppm for more than 91 % of the samples could be acquired.

Using features like alternating column regeneration, overlapped injection, high temperatures, high flow rates together with highest data acquisition rates and most importantly stable and easy-to-use accurate mass, this system outperforms other high throughput LC/MS techniques used as yet in throughput and/or data quality. The linear velocities achieved were in the range of 11 mm/s and cycle times were as fast as 49 s for a run time of 41 s. Due to the columns with particle sizes of 1.8 µm, the UV peak capacities were still in the range of fifty and even the MS peak capacities were in the range of forty for a gradient time of 39 s.

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2.

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Most of the data herein was presented as a poster, titled "Non-multiplexed DAD-ToF Analysis of 1400 Samples/day" by Michael G. Frank, Edgar Naegele (Agilent Technologies, Waldbronn, Germany), Doug McIntyre (Agilent Technologies, Santa Clara, USA), Thilo A. Fligge, Stefan Buehler, Markus Christ (Boehringer-Ingelheim, Biberach, Germany), CO-1152, at the Pittcon conference 2006 in Orlando, Florida, USA.

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Published April 1, 2006
Publication Number 5989-4505EN



Agilent Technologies

Improving the Effectiveness of Method Translation for Fast and High Resolution Separations

Application

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Abstract

The increased availability of sub-2-micron (STM) columns and increased demand for methods friendly to mass spectrometers has led to strong trend toward conversion of existing HPLC methods to smaller diameter and smaller particle size columns. While the conversion is a simple mathematical exercise requiring the scaling flow rates, gradient times and injection volumes, many users observe less than perfect results. Here we look closely at the problem and propose calculations that improve the speed and/or resolution in a more predictable and beneficial way.

Introduction

Methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by replacing these columns with smaller dimension columns packed with smaller particle sizes. The potential benefits include reduced analysis time and solvent consumption, improved sensitivity and greater compatibility with mass spectrometer ionization sources.

Simplistically, a column of 250-mm length and containing 5-µm particles can be replaced by a 150-mm length column packed with 3-µm particles. If the ratio of length to particle size is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2, here about 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

LC/MS (Liquid Chromatography/Mass Spectrometry) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal LC/UV (UltraViolet UV/VIS optical detection) methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected on the smaller column, a proportional increase in peak response. We normally scale the injection mass to the size of the column,

though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column.

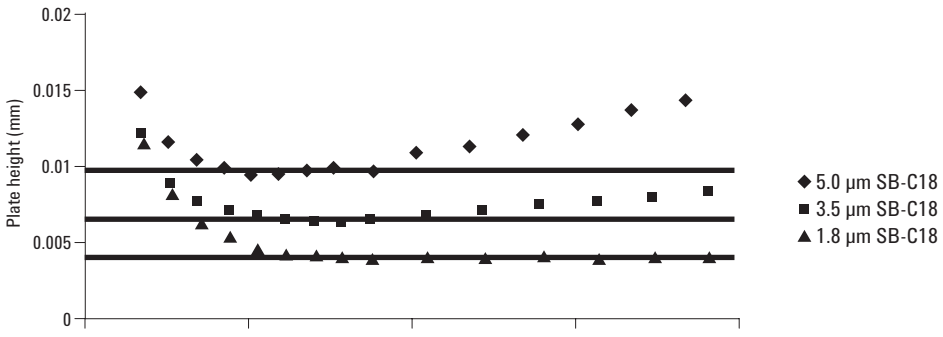
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. If one wishes to improve the outcome of the method conversion, though, there are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, or more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, this is often represented in van Deemter plots as shown in Figure 1, a modified version of the original plot [2].

In Figure 1 we observe that the linear velocity at which 5-µm materials are most efficient, under the conditions used by the authors, is about 1 mm/sec. For 3.5-µm materials the optimum linear velocity is about 1.7 mm/sec and has a less distinct optimum value, suggesting that 3.5-µm materials would give a more consistent column efficiency over a wider flow range. For the 1.8-µm materials, the minimum plate height, or maximum efficiency, is a broad range beginning at about 2 mm/sec and continuing past the range of the presented data. The practical application of this information is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity which results in a further reduction in analysis time. This increase in elution speed will decrease absolute peak width and may require the user to increase data acquisition rates and reduce signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings which will cause a measurable amount of bandspreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest



Lin. vel.	mm/sec	1	2	3	4	5
4.6 mm	mL/min	0.7	1.4	2.1	2.8	3.5
3 mm	mL/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	mL/min	0.14	0.29	0.44	0.58	0.73
1 mm	mL/min	0.033	0.066	0.1	0.133	0.166

Figure 1. van Deemter plot with various flow rates and particle sizes.

possible peak volumes. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of % change per minute, it is more useful to express it as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End}\% - \text{Start}\%)}{\#\text{Column volumes}} \right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C autosampler SL, with thermostatic temperature control
G1316B Thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 150 mm, 3.5 µm

Mobile phase conditions

Organic solvent:	Acetonitrile
Aqueous solvent:	25 mm phosphoric acid in Milli-Q water

Gradient Conditions

Gradient slope:	7.8% or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

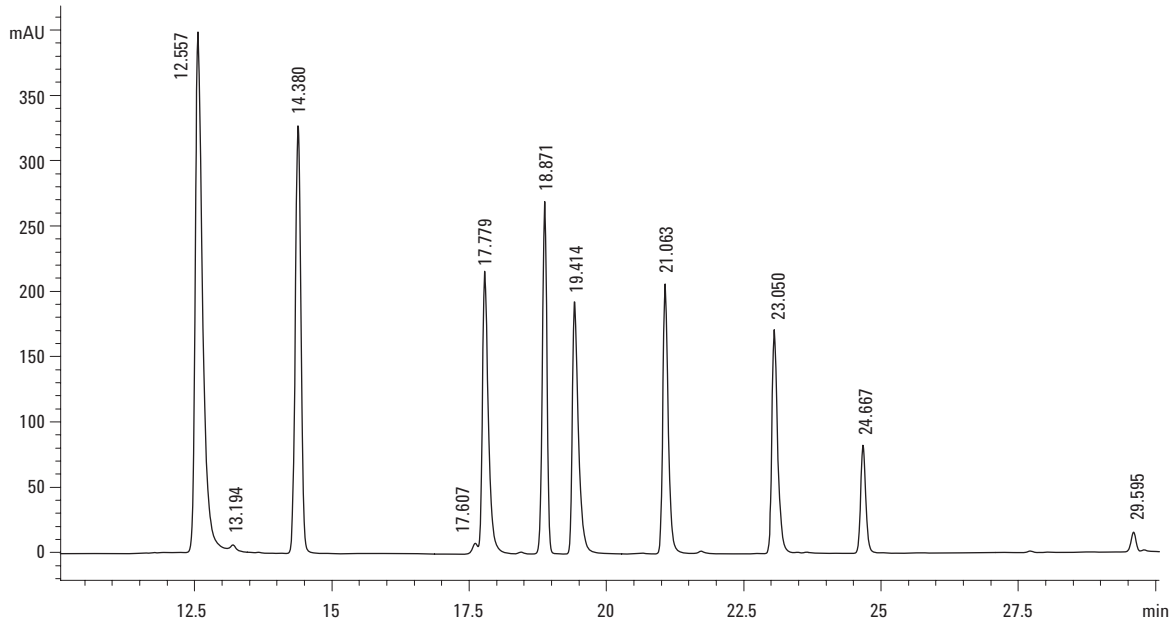
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

Results

The separation was initially performed on a standard 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column thermostatted to 25 °C (Figure 2) using conditions referenced in US EPA Method 555. The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm, 3.5-µm column (Figure 3). Solvent consumption is reduced from 60 mL to 15.5 mL per analysis.

The separation was then re-optimized for faster separation with the identical slope, 7.8%, by increasing the flow rate from 0.43 to 1.42 mL/min, and proportionately reducing the gradient time (Figure 4). Finally, increased resolution is demonstrated by keeping the original times used in Figure 3 with the increased flow rate (Figure 5). This yields a gradient with identical time but a reduced slope of 2.3%. The increased resolution of peaks 4 and 5 is readily apparent.

The conditions in Figure 4, 7.8% slope at increased linear velocity on 3.0 × 150 mm, 3.5-µm material, yield a separation with comparable resolution to the original 4.6 × 250 mm method, but with only a 12-minute total analysis time. This is excellent for

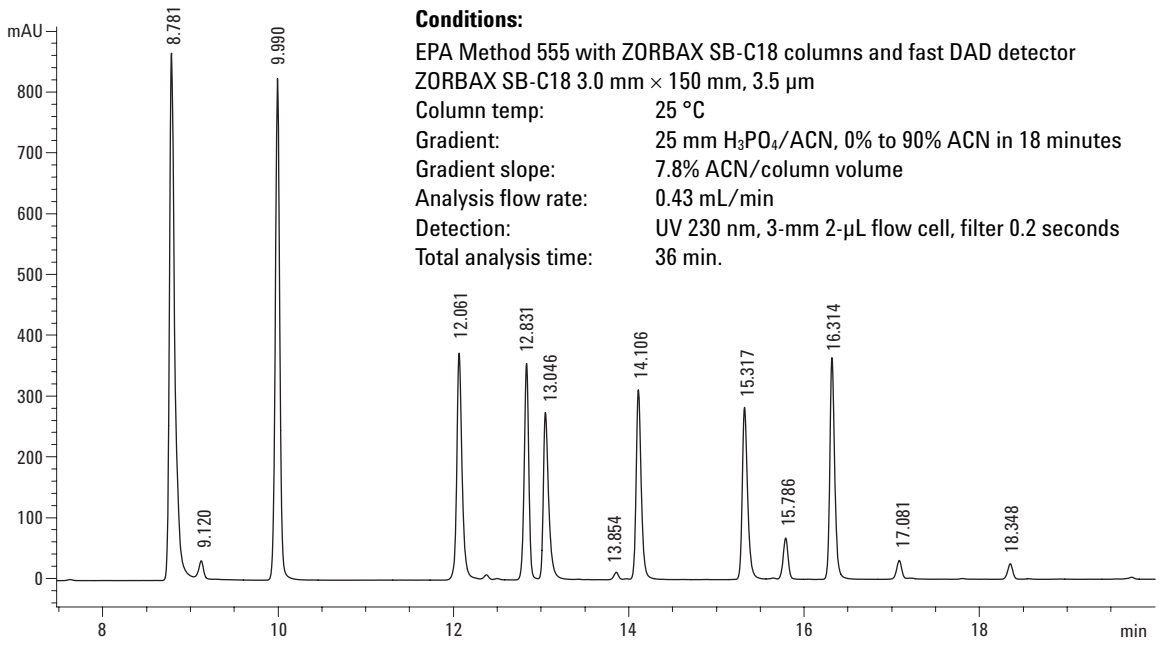


Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm	
Column temp:	25 °C
Gradient:	10% to 90% ACN vs. 25 mM H_3PO_4
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min
Group A Compounds	
Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 2. Gradient separation of herbicides on 4.6 × 250 mm 5-µm ZORBAX SB-C18.



Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 µm	
Column temp:	25 °C
Gradient:	25 mm H_3PO_4/ACN, 0% to 90% ACN in 18 minutes
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min.

Figure 3. Gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

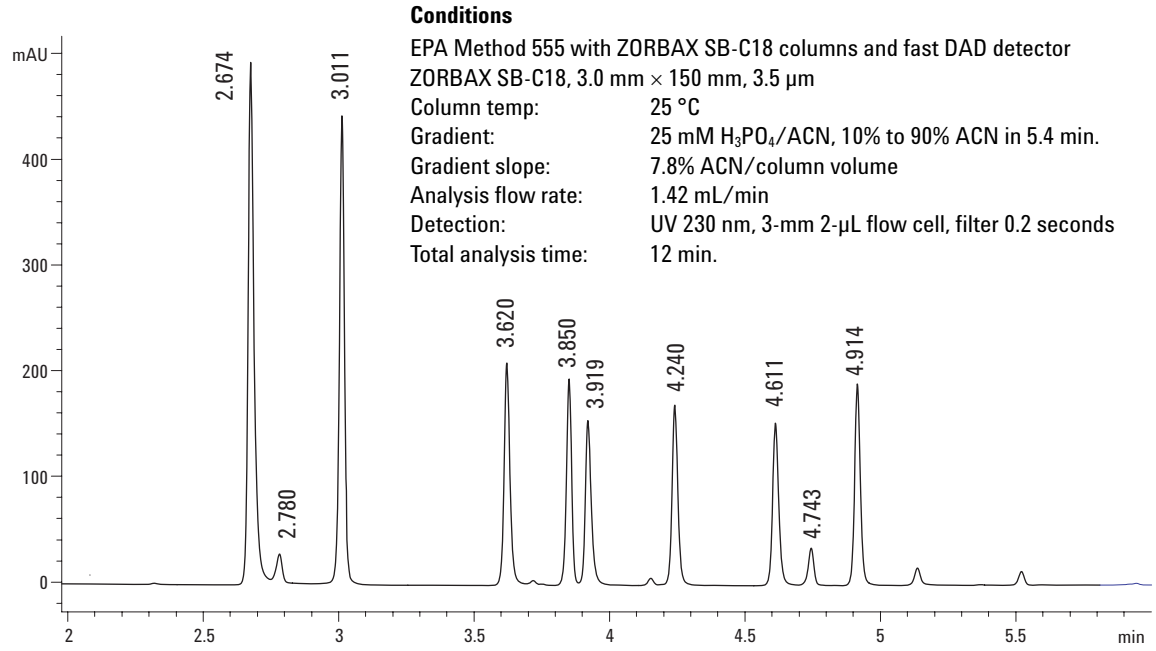


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

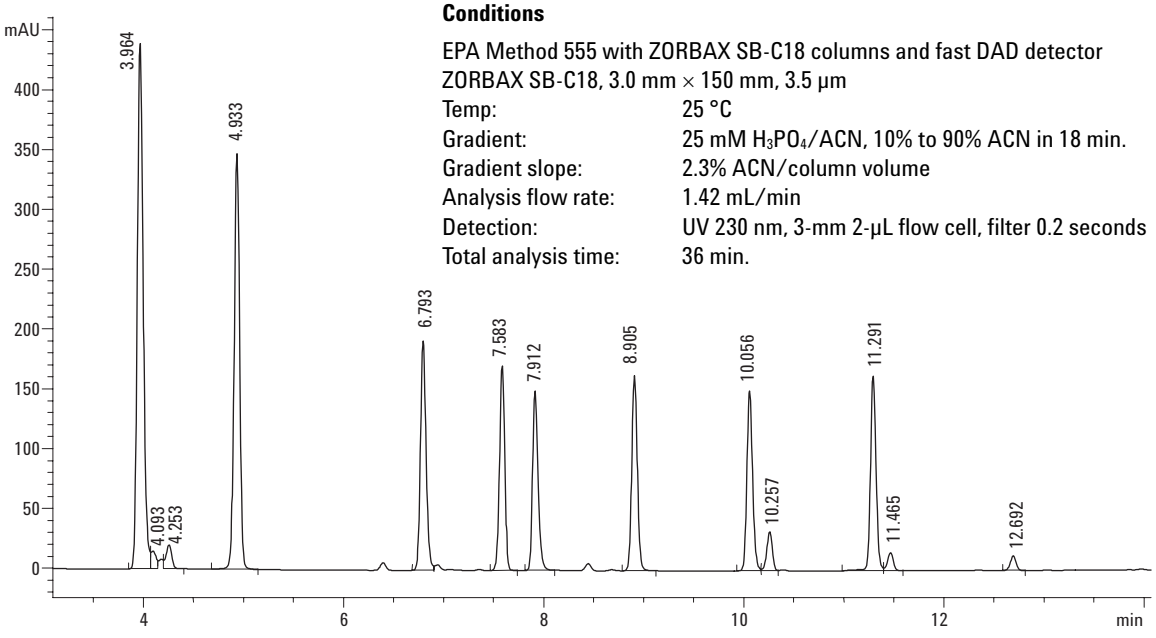


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

high throughput screening and quantitation of a large number of samples. Figure 5, with the gradient slope reduced to 2.3%, results in a high-resolution separation with a calculated R value of 3.3 vs. the standard 3.0 × 150 mm separation value of 1.9, for the critical pair seen in Figure 5 at 7.5 to 8 minutes.

In Table 1 the column has been replaced with a low dead volume connecting union in a system fitted with 0.12-mm id capillary tubing at all points of sample contact. A 1-µL injection of dilute actone is made to determine the bandspreading contribution of the system, with various flow cells. Multiple flow cells were tested, and the average result reported, where possible. The elution volume summarizes the total volume of all tubing in the system. While the absolute volume from the 2-µL to the 13-µL flow cells is 11 µL, we observe an increase of 15 to 16 µL because of the larger diameter inlet tubing integral to the larger volume flow cells.

Table 1. Volumetric Measurements of Various Flow Cells

Flow cell	Elution volume (µL)	Half height width (µL)	5 Sigma width (µL)
New SL 2 µL 3 mm	11	5	12
Micro 6 mm 1.7 µL (n = 2)	14	6	18
Semi-micro 6 mm 5 µL (n = 2)	13	6.5	18.5
Standard 10 mm 13 µL	26	11	26
New SL 10 mm 13 µL	27	11	25

Conclusion

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small and high resolution columns, will ensure good column efficiency which is critical to a successful translation of the method.

References

1. J. J. van Deemter, F. J. Zuiderweg, A. Klinkenberg, Chemical Engineering Science 1956, 5, 271–289
2. The Influence of Sub-Two Micron Particles on HPLC Performance, Agilent Technologies, application note 5989-9251EN, May 2003

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Printed in the USA
August 9, 2006
5989-5177EN

Process Monitoring of Bisphenol-A in Industrial Feedstock using High Throughput HPLC

Application

Process Control

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Abstract

The chromatographic method used to monitor the Bisphenol-A manufacturing process was improved using Agilent RRHT Eclipse XDB-C18 columns. These columns use 1.8-µm particles versus conventional 3.5-µm or 5-µm particles. The improved method allowed seven times faster analyses, improved resolution, and higher sensitivity.

Introduction

Bisphenol-A (Figure 1) is a highly versatile material used to manufacture many modern products. It is also known as 4,4"-Isopropylidenediphenol, 4,4"-(1-Methylethylidene) bisphenol, or simply BPA.

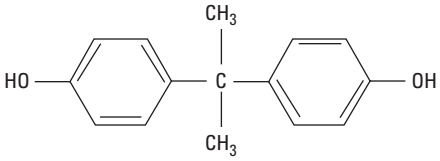

Figure 1. Bisphenol A

Every year, 2.8 million tons of BPA are produced. BPA is a building block for polycarbonate plastic and epoxy resins. Polycarbonate plastic is prized for its scratch resistance, optical clarity, and heat and electrical resistance. Because of these attributes, it is used for eyewear, CD/DVD disks, electronics, and food and drink containers. Epoxy resins are used for protective coatings because of their combination of inertness, chemical resistance, adhesion, and formability. For example, metal food cans are lined to protect taste. Epoxy resins are also used as a component in dental sealants and as a component in dental composites providing an alternative to mercury amalgam in veneers and fillings. Other uses include fungicides, polymer antioxidants, and components in automobiles and appliances.

BPA is produced through an acid-catalyzed condensation reaction of phenol with acetone. During condensation, a number of phenol-based byproducts are also formed. HPLC is used to determine the composition of many of the process streams in a commercial BPA plant.

Here we describe the use of new HPLC column technology for the possible improvement to one of the HPLC methods used in a commercial BPA facility.

Method Optimization and Scalability

The existing HPLC method was proven and robust; however, it was complicated. We sought a similar chromatogram, based on the original method, but using simpler method parameters. Because of the challenge of changing many chromatographic parameters, essentially redeveloping the method, we chose a 4.6 × 50 mm, 1.8-µm Eclipse XDB-C18 column for experiments to reduce the time required. Smaller particles packed in shorter columns increase the speed of analysis and still provide enough efficiency to maintain resolution equivalent to longer columns packed with larger particles. After several trials, we developed a method that produced a chromatogram similar to the original. The short analysis time is a major advantage of Rapid Resolution High Throughput (RRHT) technology. Whereas a handful of experimental runs would take an entire work day using a typical analytical-sized column (50 min/run), the series of runs took about an hour (7.5 min/run), using an RRHT column.

We incrementally scaled up to a 4.6 × 250 mm column. Figure 2 shows an overlay of the sample analyzed by three 4.6-mm id columns of different lengths and particle sizes. Injection volume was also changed proportionally to length. The smaller ZORBAX particles speed up the analysis while maintaining resolution. In fact, resolution increased when using the RRHT columns despite their shorter length.

One reason this method can be easily scaled (up or down) is the uniform spherical Eclipse XDB-C18 packing. It has a proprietary engineered particle size distribution, based on ZORBAX silica with a controlled surface area and pore size. The robust proprietary packing material and proven column manufacturing techniques consistently yield reproducible columns with similar chromatographic performance, independent of the column dimensions.

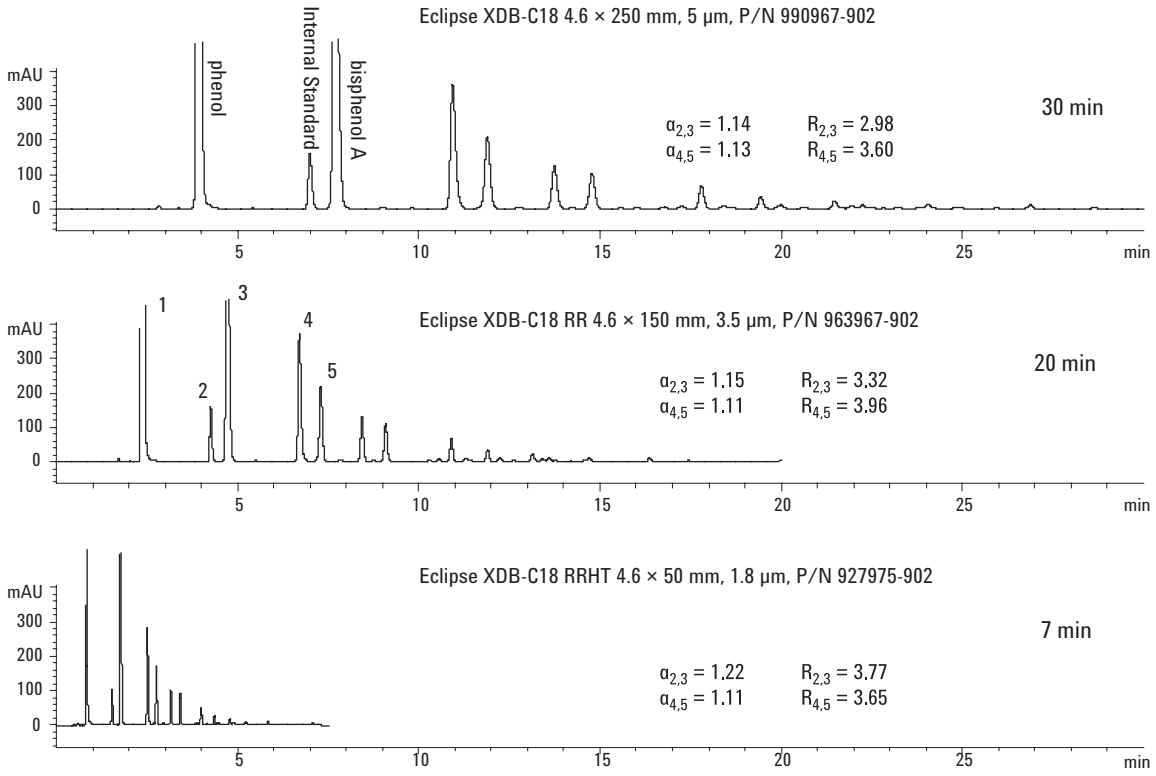

Figure 2. RRHT column configuration increased both speed and resolution.

Particle size does influence resolution. The influence can be noticed when comparing columns of identical dimensions, packed with three different particle sizes. Figure 3 shows the shortened Bisphenol-A analysis using different particle-sized Eclipse XDB-C18 columns. Resolution (Rs) is related to selectivity (α), efficiency (N) and retention (k´):

$$Rs = (1/4)(\alpha-1)\sqrt{N}\,[k'/(1+k')]$$

Factors affecting the selectivity term (stationary phase, mobile phase) and retention term (mobile phase, temperature) are constant for the three chromatograms. The efficiency term is influenced by column length, linear velocity of the mobile phase (both constant), and particle size (varied in Figure 3). N increases as particle size decreases. In Figure 3 the selectivity factors (α) and retention remain about the same, but resolution actually increases. The increase in resolution due to the decrease in particle size highlights the advantage of using smaller particles. The similar selectivity and retention highlight the suitability of ZORBAX Eclipse XDB-C18 columns for scaling methods, especially to more rapid, high-throughput methods.

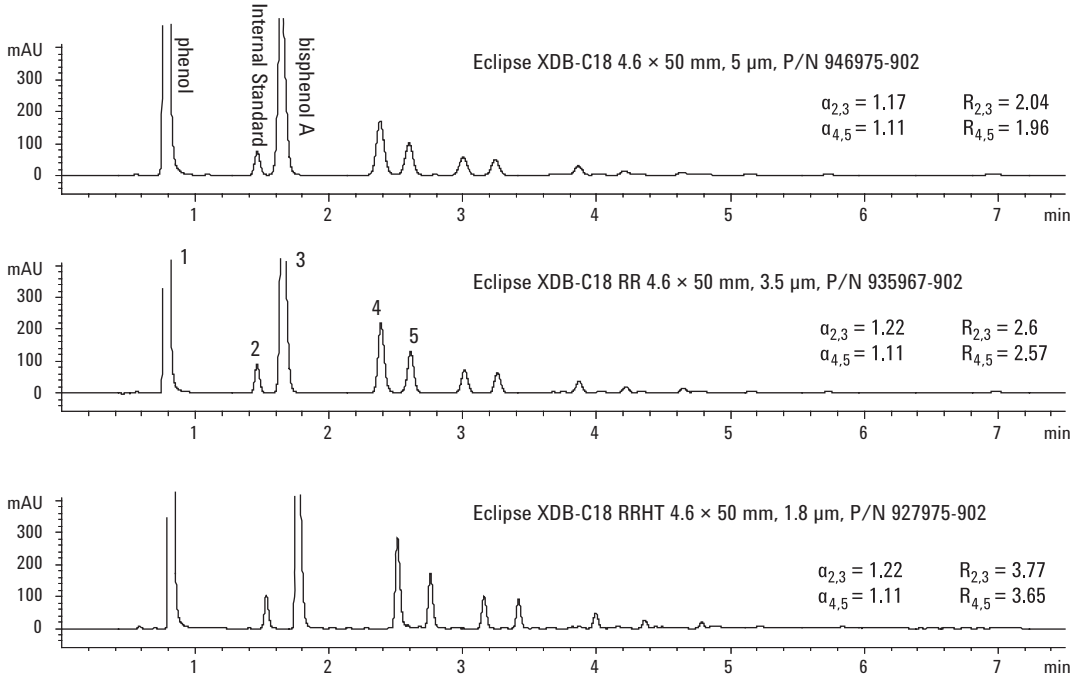

Figure 3. Effect of particle size on resolution and selectivity.

Comparing the Existing Method to the RRHT Method

Figure 4 compares the original BPA separation to the RRHT separation. The top chromatogram is an example of the analysis using the original commercial method, and the bottom is an example of the process sample analyzed with the RRHT method. The method developed with the new column technology clearly increases productivity.

Analysis time is reduced at least six-fold; solvent consumption is reduced about 12.5 times, from 100 mL/analysis to only 7.5 mL/analysis. Interestingly, the peak shape of Bisphenol-A is more symmetrical using Eclipse XDB-C18 as compared to the current C18 column used in the original analysis. The more Gaussian peak shape eluted by the Eclipse XDB-C18 column is important for accurate quantification. Other method improvements such as a simplified gradient and a binary mobile phase are listed in Table 1.

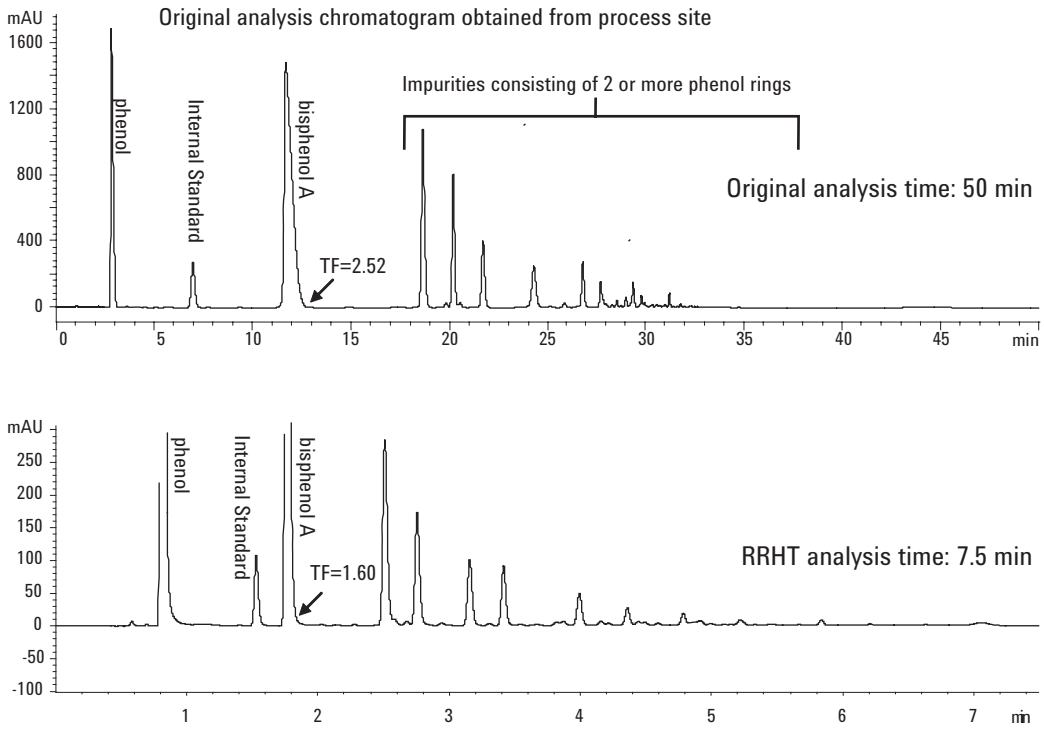

Figure 4. Comparison of methods; original to RRHT.

Table 1. Current and Improved Method Parameters

Original

- Column: Supelco LC-18, 4.6 × 250 mm, 5 µm
- Mobile phase: A: 0.025% H_3PO_4, B: ACN, C: MeOH
- Flow: 2 mL/min
- Temperature: 35 °C
- Sample size: 20 µL
- Gradient: segmented, has isocratic holds

Time	% A:B:C
0	65:25:10
13	65:25:10
18	50:40:10
23	50:40:10
27	30:50:20
32	0:70:30
35	0:70:30
36	0:60:40
40	0:50:50
43	0:20:80
48	65:25:10

RRHT

- Column: ZORBAX XDB-C18, 4.6 × 50 mm, 1.8 µm
- Mobile phase: A: 0.1% formic acid, B: ACN: MeOH (200:800)
- Flow: 1 mL/min
- Temperature: 25 °C
- Sample size: 2 µL
- Gradient: linear, no isocratic holds

Time	% B
0	60
6	95
6.01	60
8	60

Conclusion

Converting an existing method to a high-throughput method is one way to improve lab productivity. Using RRHT columns initially for method development also improves productivity. Eclipse XDB-C18 RRHT columns are a good choice for converting existing C18 methods into high-throughput methods. Smaller particles packed into shorter columns provide comparable resolution to larger particles packed into longer columns in a fraction of the time. RRHT columns are advantageous for gradient method development because gradient re-equilibration is time-consuming and often overlooked in the total analysis time. Methods developed on Agilent RRHT columns can be scaled easily because of the highly uniform particles, bonded phase chemistry, and column manufacturing techniques. An existing method developed on a "traditional analytical-sized" column was easily converted to a high throughput method using an Eclipse XDB-C18 RRHT column. The method was incrementally scaled up to an analytical-sized column, and it performed with predictable results on various column dimensions and particle sizes. The predictability of the results supports Eclipse XDB-C18 RRHT columns' ability to easily improve applications and transfer them into high-throughput and high-resolution applications.

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Printed in the USA
July 26, 2006
5989-5231EN



Agilent Technologies

Fast Analysis of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator

Application

Hydrocarbon Processing

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Abstract

Vitamin E (tocopherol), phenolic antioxidants and erucamide slip additives in polypropylene homopolymer formulations were resolved and detected using liquid chromatography with ultraviolet/visible detection, under guidelines suggested by ASTM Method D6042. Using the Agilent 1200 Rapid Resolution LC system with Agilent ZORBAX RRHT columns, the antioxidants could be rapidly separated with the same or improved resolution. The Agilent method translator was used to transfer the ASTM method into new methods based on the instrument parameters, column dimensions, and particle size in three modes: simple conversion and speed optimized and resolution optimized methods.

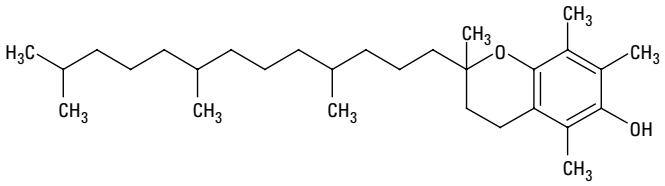
Introduction

Polymers are very popular all over the world owing to their unprecedented physical properties. Various additives are blended into polymeric materials to modify certain properties of the polymer formulation. Erucamide, Irganox 3114, Irganox 1010, Vitamin E (tocopherol), Irganox 1076, and Irgafos168 are often used as antioxidants to prevent the degradation of polypropylene homopolymer formulations by light, heat, and oxygen. In this work, with the goal to shorten the analysis time and reduce solvent consumption without losing separation quality, the existing ASTM method was recalculated for new operating conditions based on columns packed with smaller particle sizes. The chemical information of the antioxidants and Tinuvin P as internal standard is displayed in detail in Table 1.

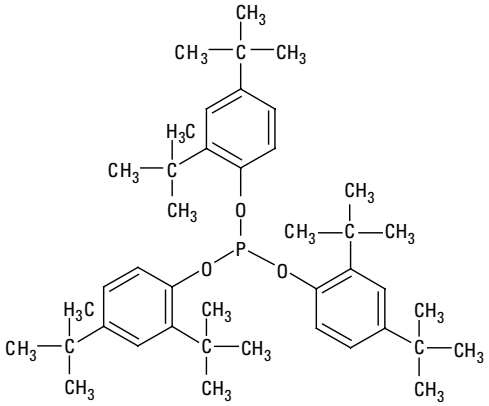
Specific additives and their concentrations in polymer formulations are critical to the properties of polymer, and careful analysis is required to ensure that the additives and levels are appropriate for the intended use. This application will compare two different stationary phases according to analyte retention characteristics and peak shape, show the influence of different injection volume of real sample on the peak shape, and then will focus on showing how to use the method translator. The latter is used to transfer the conventional method to new methods using smaller size columns to perform simple conversion and to extend the method to greater speed and higher resolution.

Table 1. Chemical Information of Antioxidants and Tinuvin P

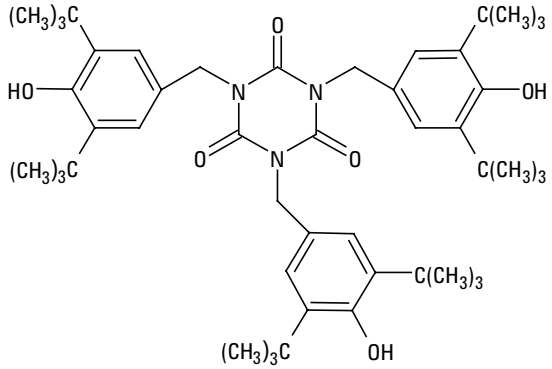
Name:	Vitamin E	DL-all-rac-α-Tocopherol
Formula:	$C_{29}H_{50}O_2$	
Molecular Weight:	430.71	
CAS No.:	10191-41-0	



Name:	Irgafos 168	Tris(2,4 di-tert-butylphenyl) phosphite
Formula:	$[[(CH_3)_3C]_2C_6H_3O]_3P$	
Molecular Weight:	646.92	
CAS No.:	31570-04-4	



Name:	Irganox 3114	Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate
Formula:	$C_{48}H_{69}N_3O_6$	
Molecular Weight:	784.08	
CAS No.:	27676-62-6	



Name:	Erucamide	cis-13-docosenamide
Formula:	$CH_3(CH_2)_7CH=CH(CH_2)_{11}CONH_2$	
Molecular Weight:	337.58	
CAS No.:	112-84-5	

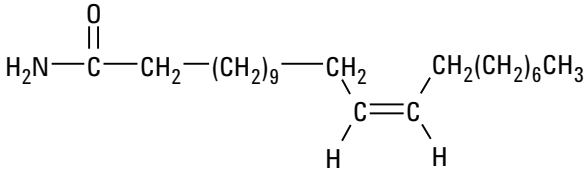
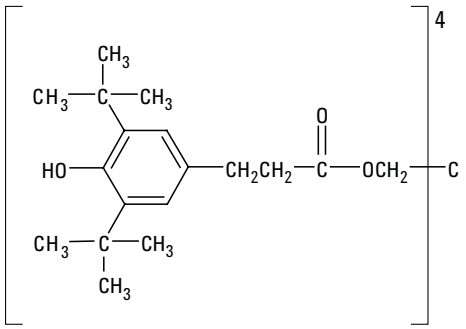
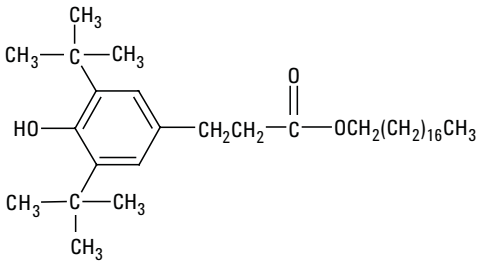
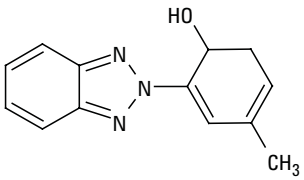


Table 1. Chemical Information of Antioxidants and Tinuvin P (Continued)

Name:	Irganox 1010	Pentaerythritol tetrakis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)
Formula:	$[HOC_6H_2[C(CH_3)_3]_2CH_2CH_2CO_2CH_2]_4C$	
Molecular Weight:	1177.63	
CAS No.:	6683-19-8	
Name:	Irganox 1076	Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate
Formula:	$[(CH_3)_3C]_2C_6H_2(OH)CH_2CH_2CO_2(CH_2)_{17}CH_3$	
Molecular Weight:	530.86	
CAS No.:	2082-79-3	
Name:	Tinuvin P	2-(2-hydroxy-5-methylphenyl)benzotriazole
Formula:	$C_{13}H_{11}N_3O$	
Molecular Weight:	225.25	
CAS No.:	2440-22-4	

Experimental

System

Agilent 1200 Series Rapid Resolution LC (RRLC), consisting of:
G1379B micro vacuum degasser
G1312B binary pump SL
G1367C high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL with 3 mm, 2 µL flow cell
ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 50 mm, 1.8 µm

Mobile Phase

Gradients:	A: water B: acetonitrile (ACN)
Gradient conditions:	See individual chromatograms
Column temperature	See individual chromatograms

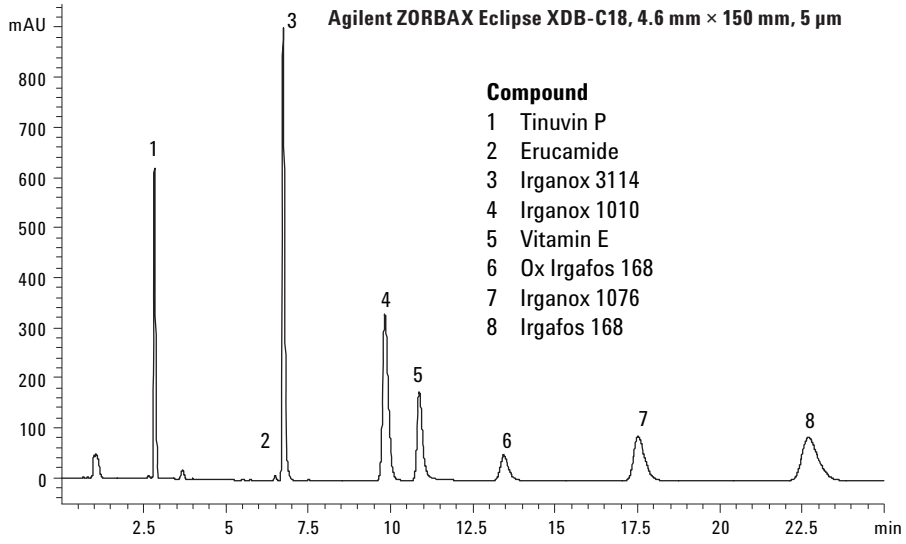
Samples

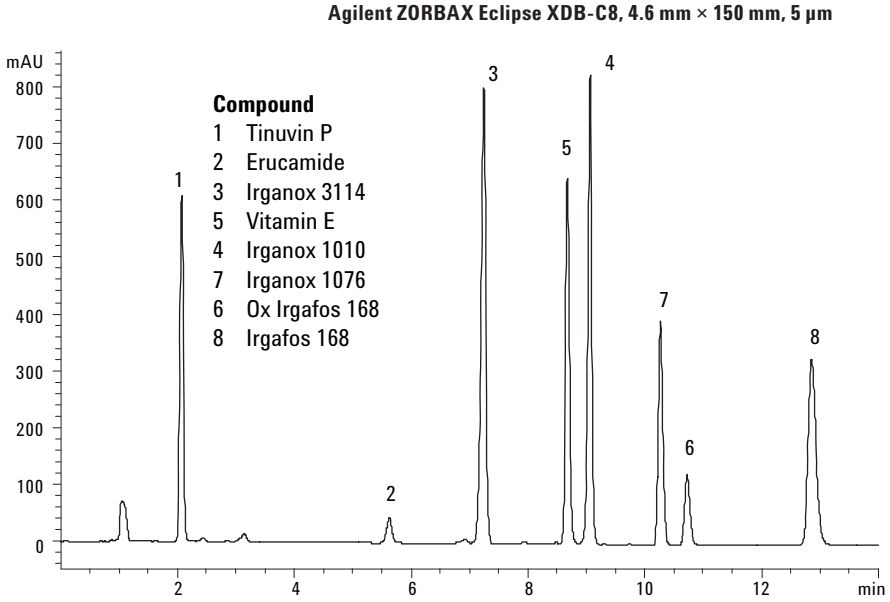
1. Standard mixture of Tinuvin P, Erucamide, Irganox 3114, Irganox 1010, Vitamin E, Irganox 1076, and Irgafos168, all 200 µg/mL in isopropanol
2. Polypropylene Homopolymer Formulation, from customer, extracted by ultrasonic according to the method ASTM D6042-04
3. Polypropylene extract spiked with 20 µg/mL standard mixture

Results and Discussion

Selection of Stationary Phase for the Separation of Antioxidants

It is desirable during method development to select a column that will provide the optimal analyte separation and shortest analysis time. Reversed phase C18 columns are recommended by ASTM D6042-04; however, in our application we determined that the retention characteristics of ZORBAX XDB-C18 columns were too strong for the specified solvents, resulting in broad peak shape and quantitation difficulties for late-eluting peaks. Compared with ZORBAX XDB-C18 columns, ZORBAX XDB-C8 columns showed better retention capability and peak shape. Therefore, we chose the ZORBAX XDB-C8 column for further method development. The different separations with ZORBAX XDB-C18 and ZORBAX XDB-C8 columns are shown in the Figure 1.





Conditions

		ZORBAX chemistry: Eclipse XDB-C18		Eclipse XDB-C8	
Mobile phase:	A: water; B: ACN	Gradient:	Min	%B	Min	%B
Flow rate:	1.5 mL/min		0.00	75	0.00	75
Wavelength:	200 nm		5.00	100	8.00	100
Injection volume:	10 µL		25.00	100	15.00	100
Column temperature:	50 °C		25.10	75	15.10	75
Column size:	4.6 mm × 150 mm, 5 µm		30.00	75	20.00	75
Sample:	Standard mixture, 200 µg/mL in isopropanol					

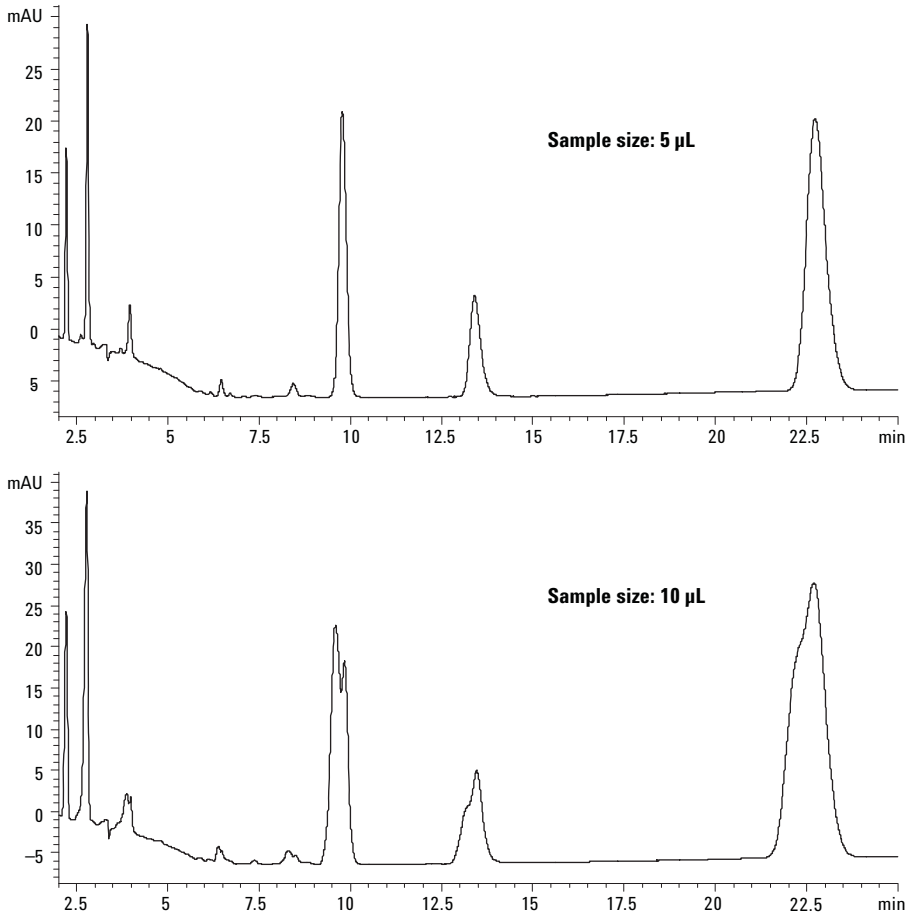
Figure 1. ZORBAX stationary phase comparison for antioxidants.

Injection Volume Influence of Real Sample Extraction Solution on the Peak Shape

According to ASTM D6042-04 [1], a solvent mixture of methylene chloride and cyclohexane (1/1 v/v) is used as the extraction solvent and, after filtration, the extracted solution is directly injected into the LC. Neither methylene chloride nor cyclohexane is miscible in the acetonitrile and water mobile phase. Peak splitting was observed when the injection volume was 10 µL. We decreased the sample size of real sample and found that the volume of 5 µL was suitable and free of solvent influence. The split and nonsplit peaks are shown in Figure 2. At the same time, the influence of injection volume was not found in the standard solution, which was dissolved in isopropanol per ASTM method guidance.

Fast Method Developed Based on New 1200 RRLC with Method Translator

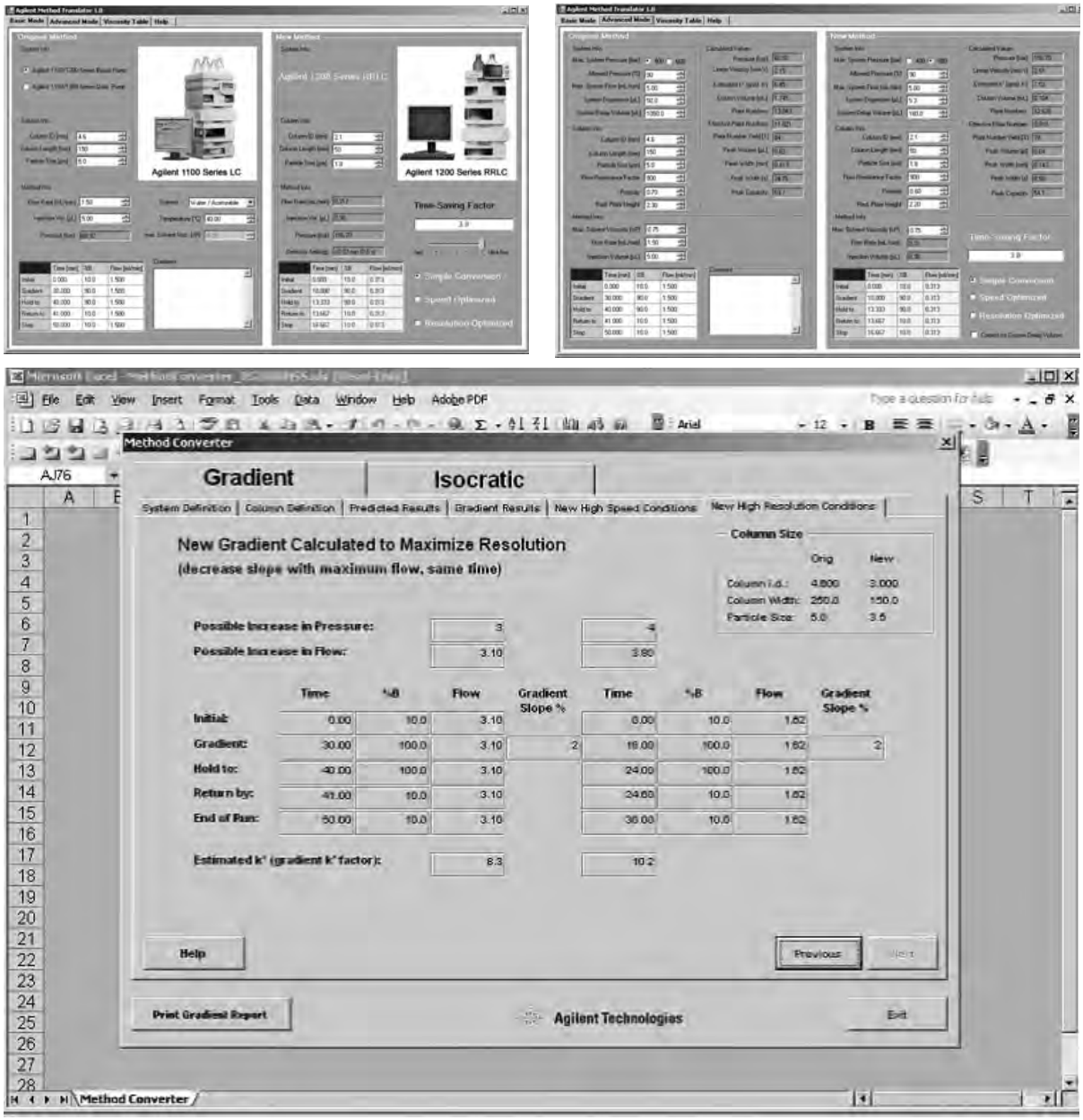
Due to the appearance of sub-two-micron columns and LC systems with higher pressure capabilities, the research of ultra-fast separation is more and more popular. Therefore, it is important to quickly and easily transfer conventional methods to fast or high-resolution methods. Agilent provides the users of RRLC systems with two versions of method translators; one is a Microsoft.net version, which requires that Net-Framework 2.0 be resident on the computer, the other is a Microsoft Excel version, which requires that Excel be resident on the PC. The interface of the two translators is displayed in Figure 3.



Conditions

Mobile phase:	A: water; B: ACN	**Gradient**:	
Flow rate:	1.5 mL/min	Min	%B
Wavelength:	200 nm	0.00	75
Injection volume:	5 or 10 µL	5.00	100
Column temperature:	50 °C	25.00	100
Column:	ZORBAX Eclipse XDB-C18 4.6 mm × 150 mm, 5 µm	25.10	75
Sample:	Polypropylene extraction solution	30.00	75

Figure 2. Injection volume influence of real sample extraction solution on the peak shape.



The upper one is the Microsoft.net version, the lower one is the Microsoft Excel version.

Figure 3. Two different method translators.

Sample Preparation

The two versions of method translators provide three modes of method conversion; the first is the simple conversion, which has the same gradient slope as the conventional method, and changes the flow rate according to equation 1:

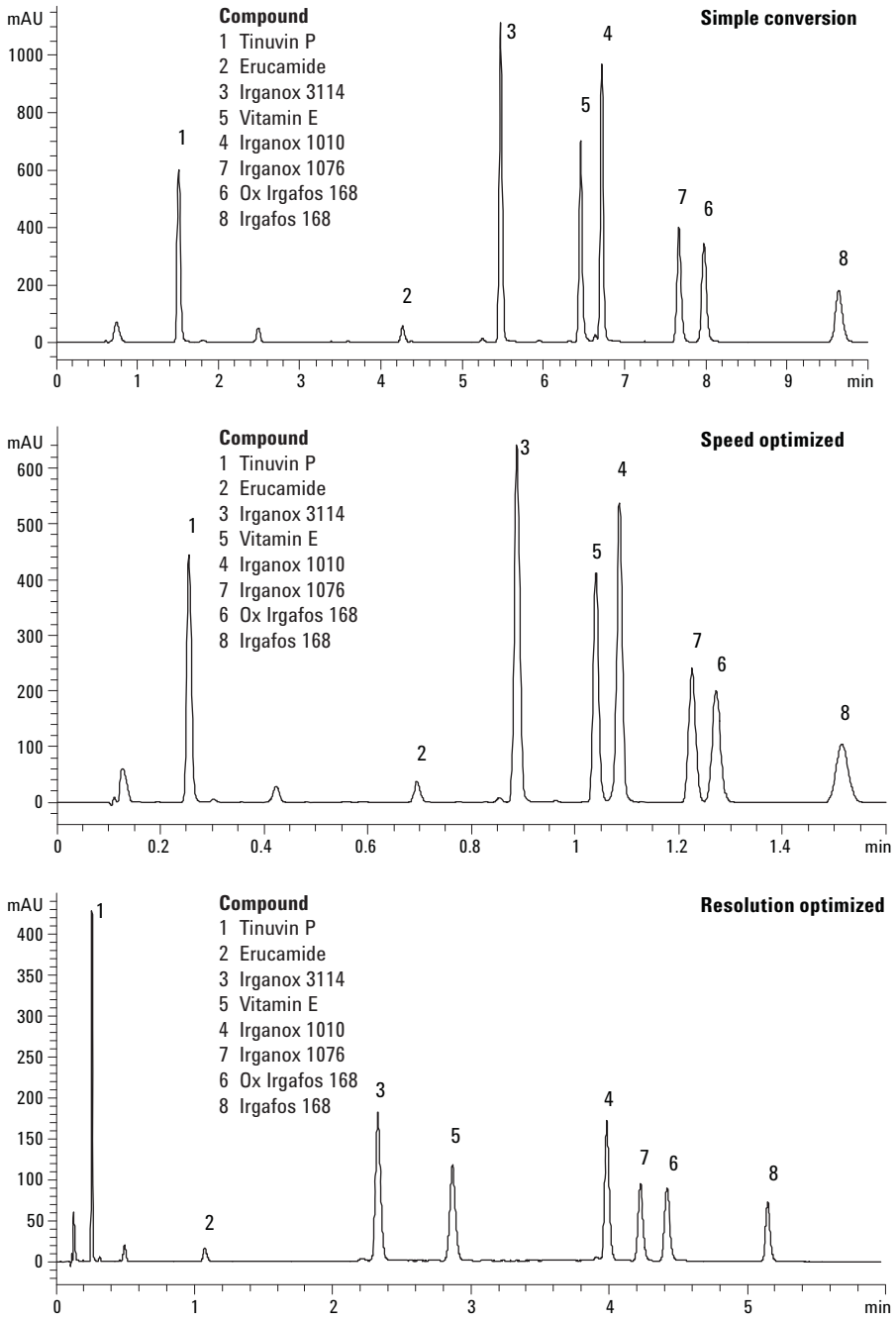
$$Flow_{Col.\,2} = \left[\frac{Diam_{Col.\,2}}{Diam_{Col.\,1}}\right]^2 \times Flow_{Col.\,1} \quad \text{(eq. 1)}$$

The second is the speed optimized conversion, which has the same gradient slope as the conventional method and maximizes the flow rate according the LC system pressure capability. The last is the resolution optimized conversion, which maximizes the flow rate according the LC system pressure capability and has the same gradient time as the simple converted mode, resulting in a reduced gradient slope that normally yields higher peak resolution. For the different columns, the injection volumes should be changed according to the relationship displayed in equation 2.

$$Inj.\ vol._{Col.\,2} = \left[\frac{Volume_{Col.\,2}}{Volume_{Col.\,1}}\right] \times Inj.\ vol._{Col.\,1} \quad \text{(eq. 2)}$$

As mentioned above, the method based on the ZORBAX Eclipse XDB-C8 4.6 mm x 150 mm, 5 µm, was selected as the initial method. Afterwards, the initial method was transferred with the method

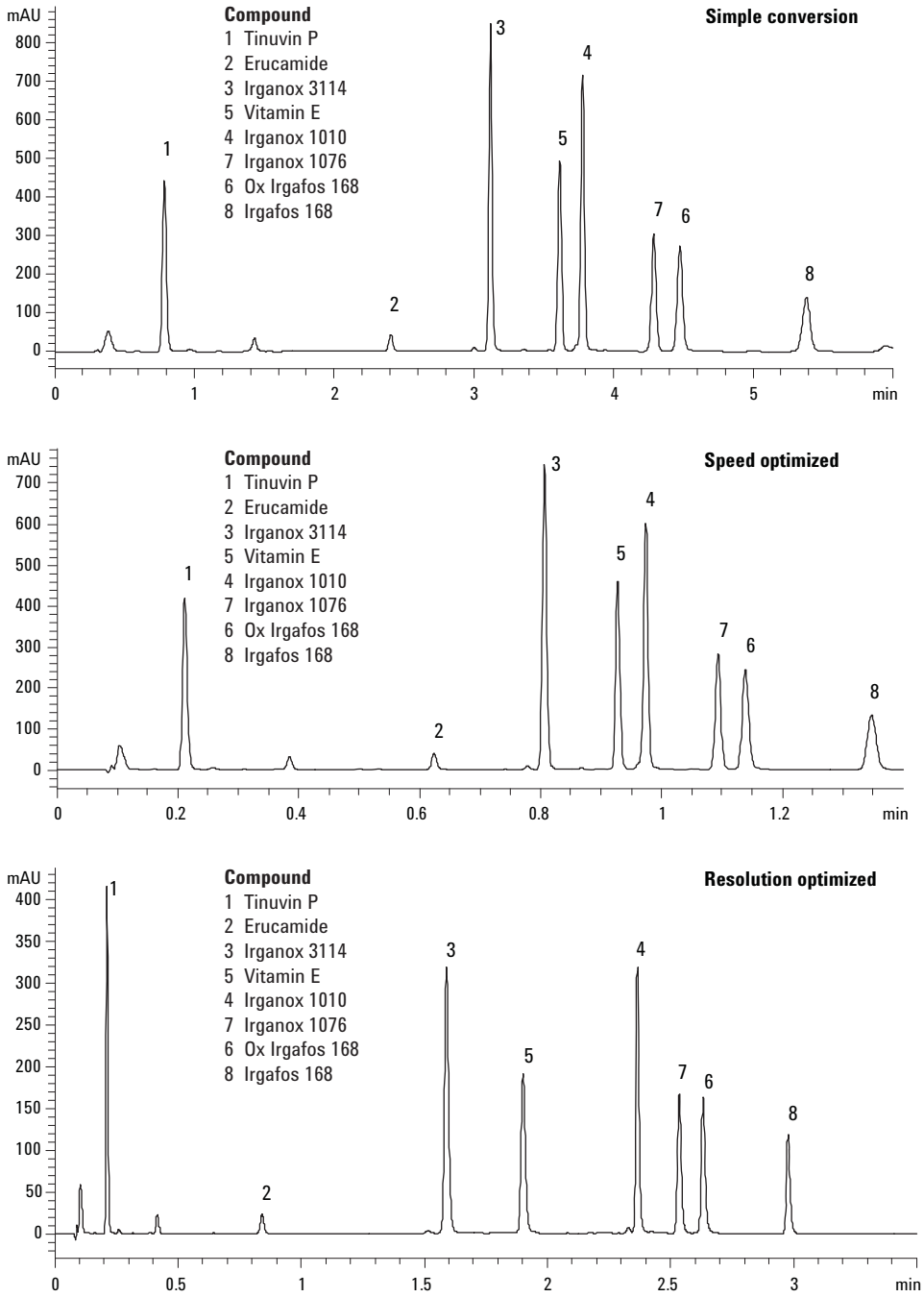
translator into three modes on different column lengths (100, 50 mm) and particle sizes (3.5, 1.8 µm), respectively. Figures 4 and 5 show the separation of antioxidants in smaller particle size columns with the recalculated methods.



Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol			
Mobile phase:	A: water; B: ACN			
Temperature:	50 °C			
Wavelength:	200 nm			
Injection volume:	6.7 µL			
Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 100 mm, 3.5 µm			
Mode:	Simple converted	Speed optimized	Resolution optimized	
Flow rate:	0.64 mL/min	4.00 mL/min	4.00 mL/min	
	Pressure:	110 bar	460 bar	460 bar
	Gradient slope:	3.1%	3.1%	0.5%
	Analysis time:	10 min	1.6 min	5.5 min

Figure 4. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 100 mm, 3.5 µm.

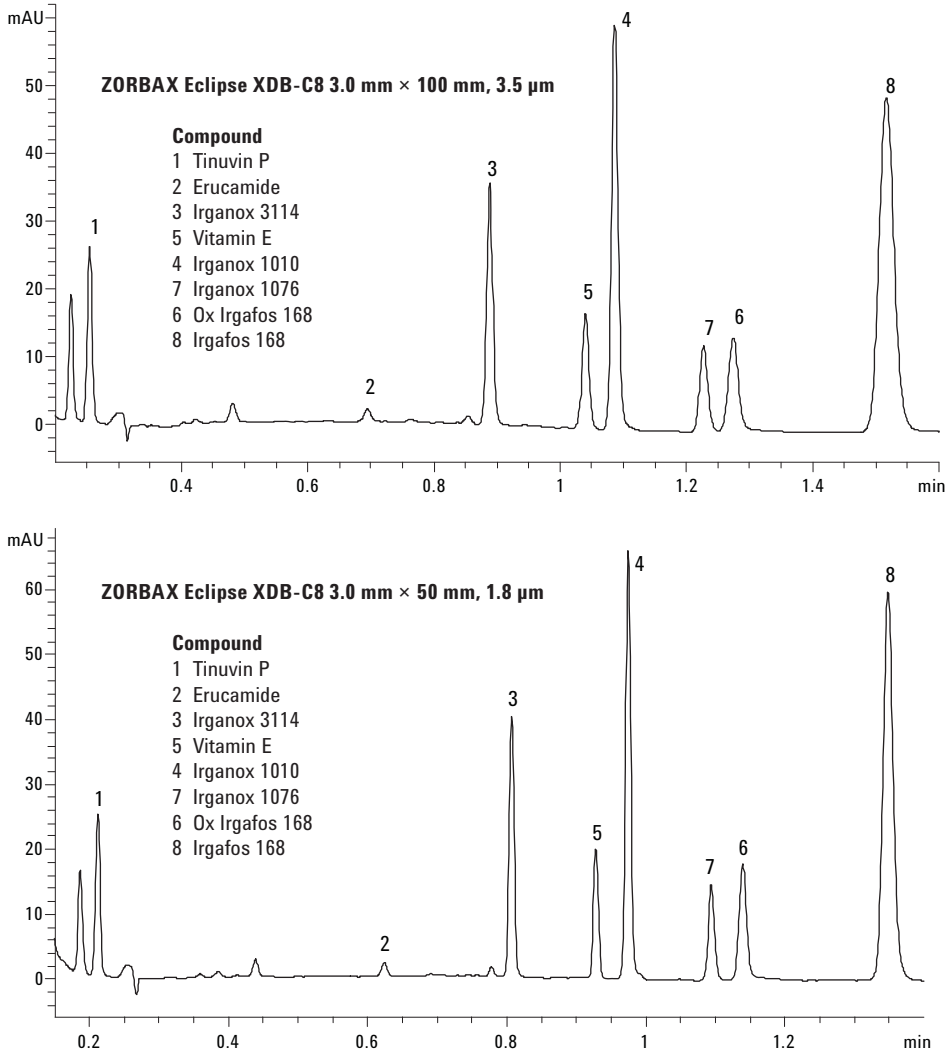


Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol		
Mobile phase:	A: water; B: ACN		
Temperature:	50 °C		
Wavelength:	200 nm		
Injection volume:	3.3 µL		
Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm		
Mode:	Simple converted	Speed optimized	Resolution optimized
Flow rate:	0.64 mL/min	2.50 mL/min	2.50 mL/min
Pressure:	160 bar	460 bar	460 bar
Gradient slope:	3.1%	3.1%	0.8%
Analysis time:	6 min	1.4 min	3 min

Figure 5. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm.

To identify the matrix influence on the separation, the polypropylene extract was spiked with 20 µg/mL standard mixture and injected into the LC system. Figure 6 depicts the separation of spiked sample with the speed optimized method, which shows a sufficient separation of antioxidant in polymer matrix with about 10 times faster speed than the conventional method mentioned above.



Conditions

Sample:	Polypropylene extract spiked with 20 µg/mL standard mixture	
Mobile phase:	A: water; B: ACN	
Temperature:	50 °C	
Wavelength:	200 nm	
Stationary phase:	ZORBAX Eclipse XDB-C8	
Column size:	3.0 mm × 100 mm, 3.5 µm	3.0 mm × 50 mm, 1.8 µm
Injection volume:	3 µL	1 µL
Mode:	Speed optimized	Speed optimized
Flow rate:	4.00 mL/min	2.50 mL/min
Pressure:	460 bar	460 bar
Gradient slope:	3.1%	3.1%
Analysis time:	1.6 min	1.4 min

Figure 6. Separation of spiked polypropylene extract by the speed optimized method.

Conclusions

As an important innovation in the advancement of liquid chromatography, the Agilent 1200 Rapid Resolution LC system provides the customer not only a rapid separation with the same or similar resolution, but also includes a method translator to convert any initial conventional method to a fast or high-resolution method according to the requirements of the user. This note applies the method translation tool in the separation of polymer additives and demonstrates the ease-of-use and power of the method translator using separations of a standard mixture and spiked real sample.

References

1. ASTM D6042-04, “Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using Liquid Chromatography (LC)”
2. Michael Woodman, “Improving the Effectiveness of Method Translation for Fast and High Resolution Separations”
3. Michael Woodman, “Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection”

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Printed in the USA
January 5, 2007
5989-5849EN



Agilent Technologies

Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC

Application

Hydrocarbon Processing

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Abstract

Liquid chromatography with ultraviolet/visible (UV/VIS) detection is a powerful approach for analyzing additives in polymer formulations. This application illustrates the use of the Agilent 1200 Series Rapid Resolution LC (RRLC) system for the separation of antioxidants and erucamide. The system can operate significantly faster than conventional HPLC without sacrificing resolution, precision, or sensitivity. The column chemistry and temperature influence on the separation and the sample preparation method are also discussed.

Introduction

Additives are incorporated into various polymeric materials to retard the degradation caused by ultraviolet light, heat, and oxygen or to modify processing characteristics. A rapid and accurate analytical method is required to ensure that the specified amount of an additive or combination of additives is incorporated into a polymer after the extrusion process. Conventional HPLC methods for additives [1,2] often require more then 30 minutes per analysis, while the application described here can achieve comparable results in as few as 3 minutes.

Agilent has developed an easy-to-use method conversion tool for transferring existing methods for higher speed and/or higher resolution. The tool was used for the method optimization in this application. [3]

This application examines additives mentioned in ASTM Methods D5815 and D1996. The chemical structures are shown in Table 1.

Table 1. Polymer Additives in ASTM Methods D5815 and D1996

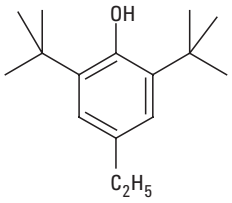
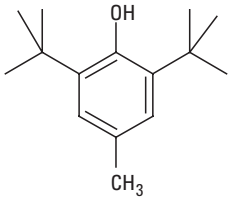
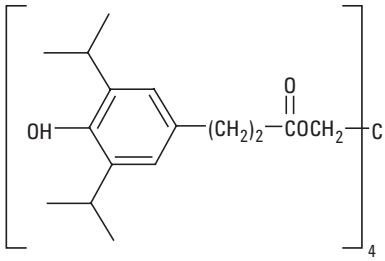
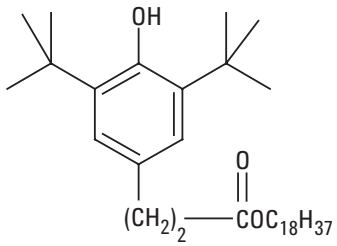
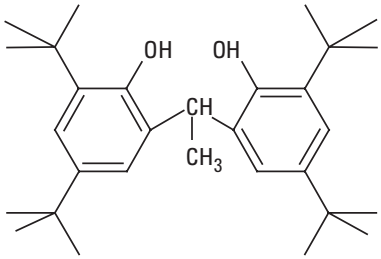
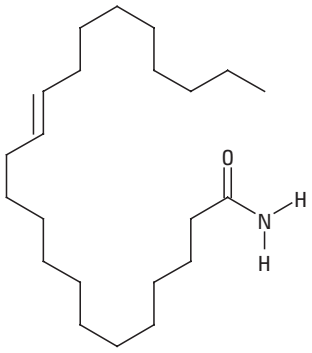
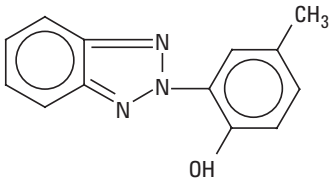
Registered trade name	CAS no.	Chemical name	Chemical structure
BHEB	4310-42-1	2,6-di-tert-butyl-4-ethyl-phenol or butylated hydroxyethyl benzene	
BHT	128-37-0	2,6-di-t-butyl-cresol or butylated hydroxy toluene	
Irganox 1010	6683-19-8	Tetrakis[methylene(3,5-di-t-butyl-4-hydroxy hydrocinnamate)] methane	
Irganox 1076	2082-79-3	Octadecyl-3,5-di-t-butyl-4-hydroxy hydrocinnamate	
Isonox 129	35958-30-6	2,2-ethylidene bis (4,6-di-t-butyl phenol)	

Table 1. Polymer Additives in ASTM Methods D5815 and D1996 (Continued)

Registered trade name	CAS no.	Chemical name	Chemical structure
Kemamide-E	112-84-5	Cis-13-docosenamide or Erucamide or Fatty acid amide ($C_{22}H_{43}NO$)	
Tinuvin P	2440-22-4	2(2'-hydroxy-5'-methyl phenyl) benzotriazole	

Experimental

System

Agilent 1200 Series rapid-resolution LC configured with
G1379B microvacuum degasser
G1312B binary pump SL
G1367B high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL
ChemStation 32-bit version B.02.01

Column

ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm
ZORBAX SB-C18, 4.6 mm × 150 mm, 5 µm
ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm

Mobile phase

Gradients:	A: water B: acetonitrile (ACN)
Gradient slope:	See individual chromatograms for flow rate and gradient time
Column temperature:	See individual chromatograms

Samples

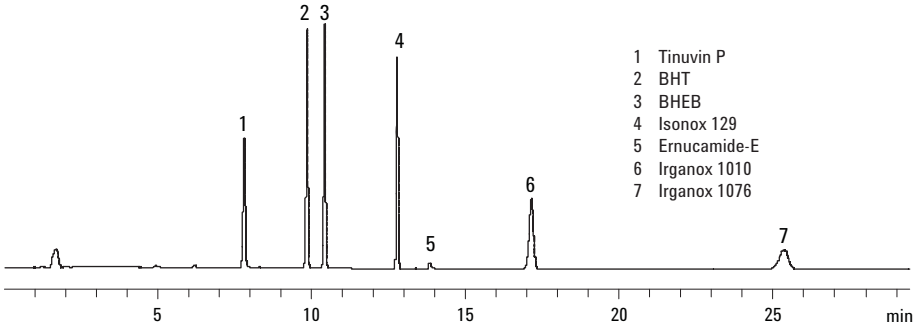
1. Standard mixture described in ASTM D5815 and D1996, 50 µg/mL, 200 µg/mL in isopropanol
2. Linear low-density polyethylene from customer, ground to 20 mesh, extracted by ultrasonic or reflux method

Results and Discussion

Fast Method Conversion

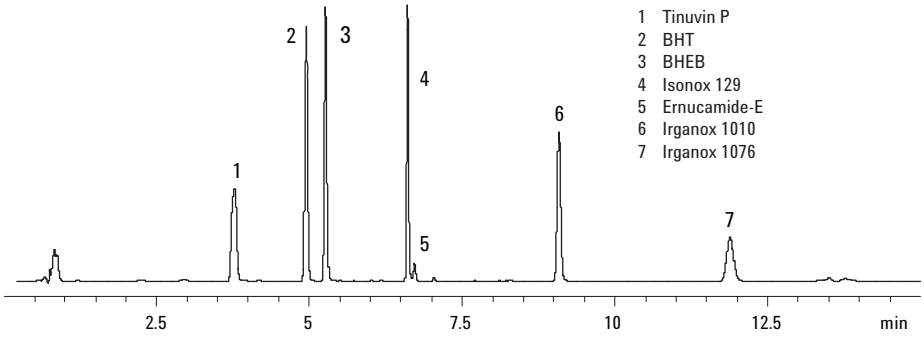
The separation was initially performed on a standard 4.6 mm × 150 mm, 5-µm ZORBAX Eclipse XDB-C18 column thermostatted to 60 °C (Figure 1) following the conditions in ASTM D5815 (or D1996). The method was then scaled in flow and time for exact translation to a 2.1 mm × 50 mm, 1.8-µm column (Figure 2). The analysis time was reduced from 25.5 to 12.5 minutes, and the solvent consumption was reduced from 25 to 2.5 mL.

The separation was then re-optimized for faster separation with the same gradient slope by increasing the flow rate from 0.21 to 0.9 mL/min and proportionately reducing the gradient time (Figure 3), achieving up to 10 times faster than conventional HPLC without sacrificing resolution, precision (showed in Table 2), or sensitivity. Figure 4 demonstrates that 1 ppm of additives can be determined with very good signal-to-noise response using the same condition in Figure 3, which exceeds the specification of 2 ppm of ASTM D5815 (or D1996). Peak 6, Irganox 1010, for example has a signal-to-noise response of 88 at 1 ppm.



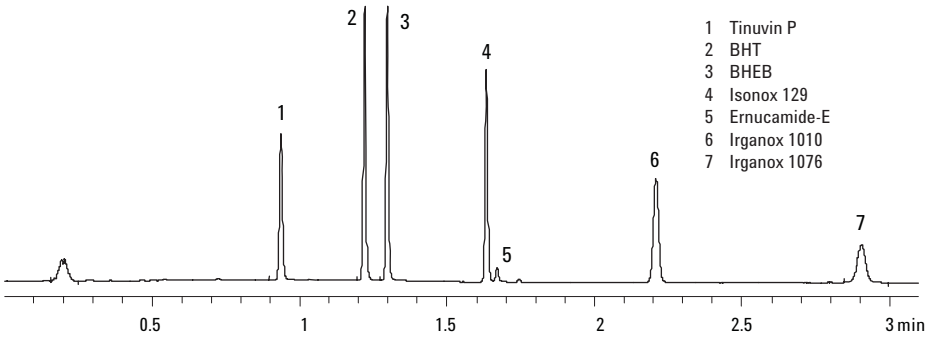
		Gradient	
Conventional method:	Follow ASTM D5815 (or D1996) method with		
	ZORBAX Eclipse XDB-C18,		%B
	4.6 mm × 150 mm, 5 µm	0	50
Sample:	Standard 50 µg/mL	11	100
Sample size:	10 µL	28	100
Detector:	UV 200 nm	28.1	50
Column temperature:	60 °C		
Mobile phase:	A: water		
	B: acetonitrile		
Flow rate:	1 mL/min		

Figure 1. Separation of additives standards on Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm.



		Gradient	
Simple-converted:	Translate the conventional method to a		
	ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm		%B
Sample:	Standard 50 µg/mL	0	50
Sample size:	2 µL	5.2	100
Detector:	UV 200 nm	12	100
Column temperature:	60 °C	12.1	50
Mobile phase:	A: water	15	50
	B: acetonitrile		
Flow rate:	0.21 mL/min (73 bar)		

Figure 2. Separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

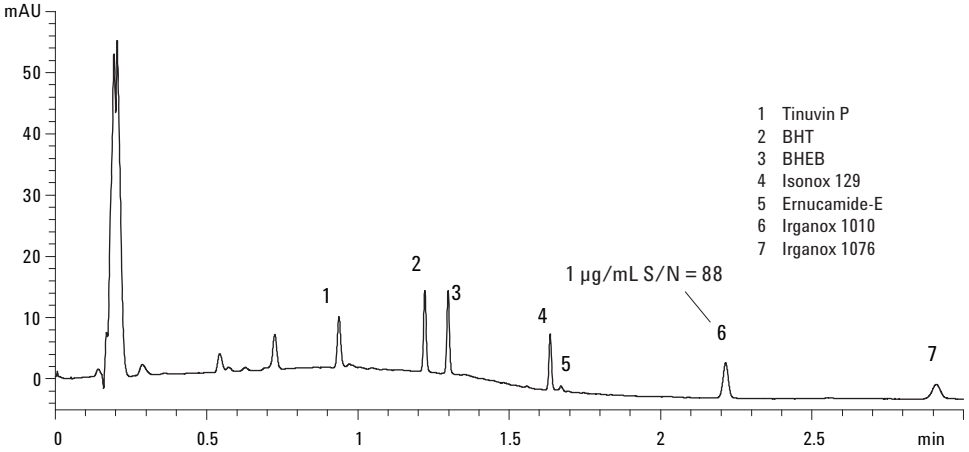


		Gradient	
Speed-optimized:	Optimize the conventional method for speed with ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm		%B
		0	50
Sample:	Standard 50 µg/mL	1.3	100
Sample size:	2 µL	3	100
Detector:	UV 200 nm	3.1	50
Column temperature:	60 °C	3.5	50
Mobile phase:	A: water B: acetonitrile		
Flow rate:	0.9 mL/min (357 bar)		

Figure 3. Fast separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Table 2. Repeatability for the Methods of Conventional, Simple-Converted, and Speed-Optimized Methods (n = 5)

	Area, RSD%		
Compounds (50 ppm)	**Conventional**	**Simple-converted**	**Speed-optimized**
Tinuvin P	0.37	0.39	0.09
Erucamide	0.40	0.57	0.13
Irganox 3114	0.44	0.49	0.22
Irganox 1010	0.38	0.39	0.26
Vitamin E	0.58	0.80	0.68
Irganox 1076	0.58	1.49	0.17
Irgafos 168	0.53	0.77	0.32



Speed-optimized method for analysis of additives standards with concentration of 1 µg/mL LC conditions is identical to that in Figure 3

Figure 4. Fast separation of 1 µg/mL additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Optimized Column Temperature

Increasing column temperature can lower both solvent viscosity and nonspecific column/analyte interactions. The new ZORBAX StableBond RRHT columns can operate at temperatures up to 90 °C. We tested operating temperatures at 60, 75, 85, and 90 °C with a ZORBAX SB-C8 4.6 mm × 150 mm, 5-µm column. The results (Figure 5) show that the analysis time obtained from 60 °C to 85 °C is reduced from 23.5 minutes to 17 minutes; at 90 °C, only an additional 0.5 minute is saved. Based on the combined speed reduction and optimized resolution of peaks 4 and 5, 85 °C is chosen as a suitable column temperature.

The method was then scaled in flow and time for exact translation to a 4.6 mm × 50 mm, 1.8-µm column (Figure 6). Finally, the separation was optimized for faster separation by increasing the flow rate from 1 mL/min to 3.5 mL/min, with only a 1.7-minute analysis time (Figure 7). This is really an excellent procedure for high-throughput screening and quantitation of a large number of samples. Figure 8, the separation of an extract of linear low-density polyethylene (LLDPE) spiked with 20 µg/mL of standard solution, shows excellent separation with real sample matrix.

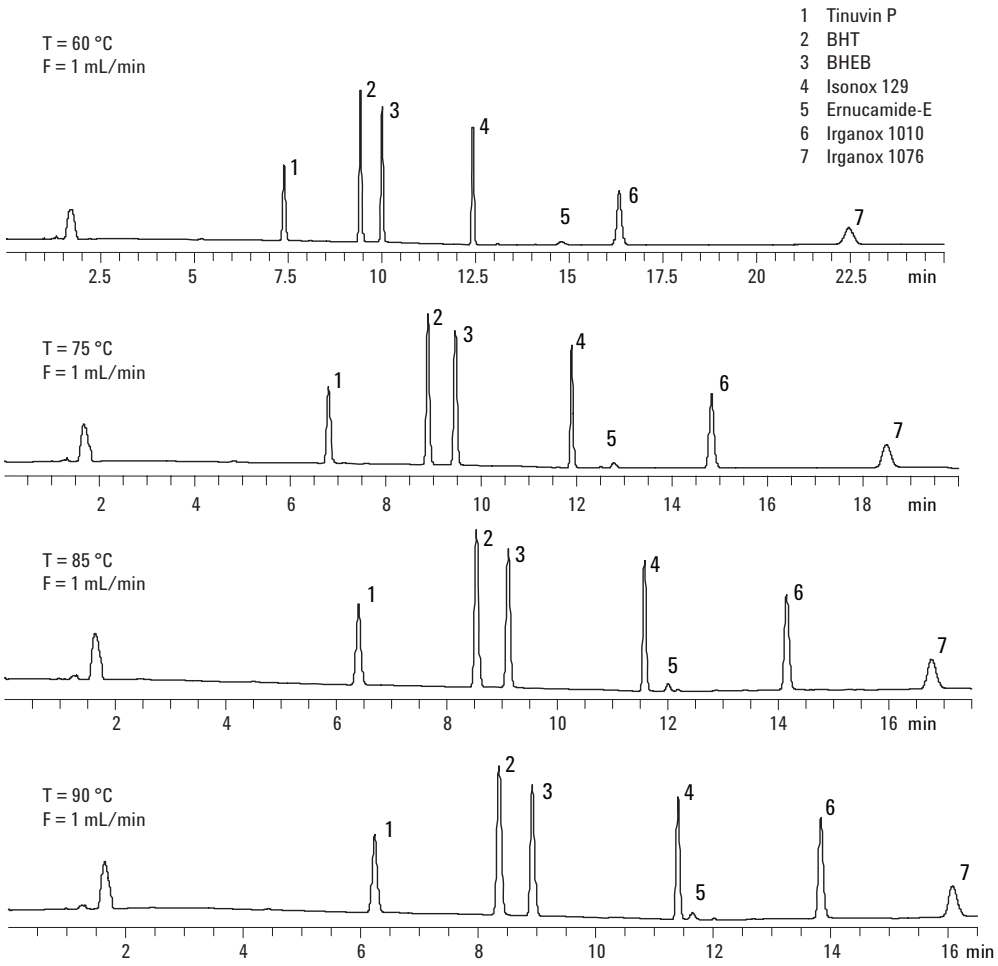
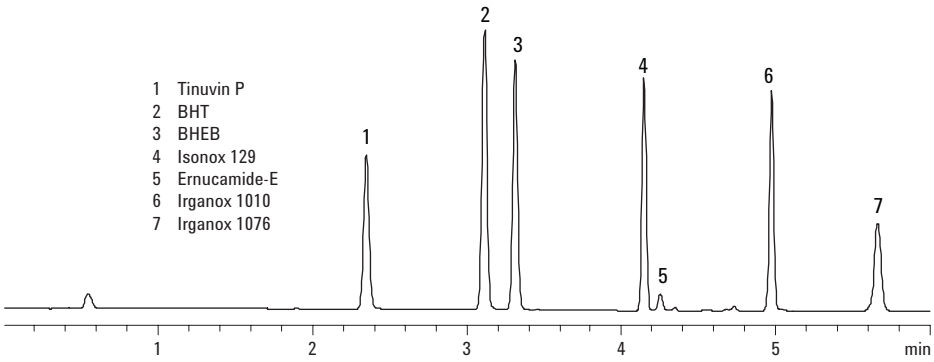
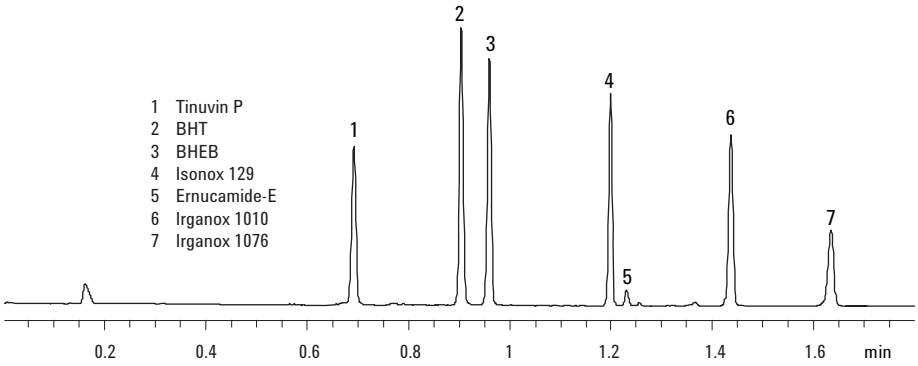


Figure 5. Separation of additives standards on ZORBAX StableBond RRHT SB-C18, 4.6 mm × 150 mm, 1.8 µm.



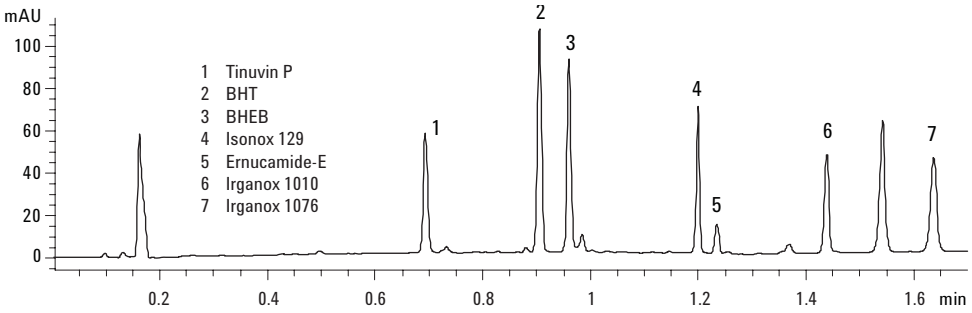
Sample:	Standard 200 mg/mL
Sample size:	2 µL
Detector:	UV 200 nm
Mobile phase:	A: water
	B: acetonitrile
Gradient slope:	6.8%
Flow rate:	1mL/min

Figure 6. Separation of additives standards on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.



Sample:	Standard_200 mg/mL
Sample size:	2 µL
Detector:	UV 200 nm
Mobile phase:	A: water
	B: acetonitrile
Gradient slope:	6.8%
Flow rate:	3.5 mL/min

Figure 7. Fast separation of additives standards on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.



LC conditions are identical with those in Figure 7.

Figure 8. Fast separation of spiked real sample-LLDPE (20 µg/mL) on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.

Sample Preparation

ASTM D5815 (or D1996) method recommends using a reflux apparatus for extracting additives in polymer. This requires periodic operator intervention over the 1.5-hour-long extraction period. To find a time-saving sample-preparation method, ultrasonic extraction was also tested, producing comparable results in 30 minutes. In terms of extraction efficiency, there is not much difference between these two methods. Figure 9 shows very good overlays of extractions by reflux and ultrasonic extraction methods for a LLDPE. Conditions are identical to those in Figure 1.

Conclusions

Liquid chromatography with ultraviolet/visible detection is an effective tool for analyzing additives in polymer formulations. The Agilent 1200 Series RRLC system equipped with RRHT 1.8-µm columns was used to achieve up to 10 times faster than the conventional HPLC method. The ultrasonic extraction method allowed fast extraction without user intervention for a significant reduction in overall analysis time. Total time saved was more than 80 minutes per sample when compared to the conventional analysis and extraction methods.

References

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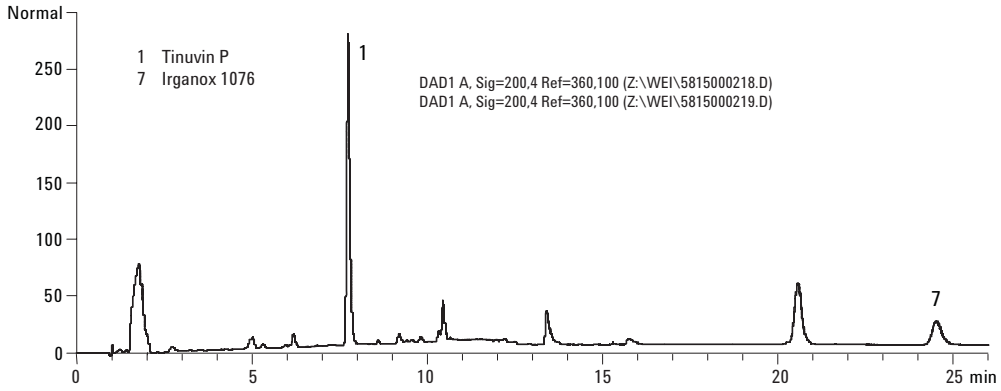

Figure 9. Chromatogram Overlays of extractions by reflux and ultrasonic extraction methods for LLDPE.

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Printed in the USA
January 17, 2007
5989-5850EN

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Fast Analysis Method for Rubber Chemical Antidegradants Using 1200 Rapid Resolution Liquid Chromatography (RRLC) Systems with Rapid Resolution High Throughput (RRHT) Columns

Application

Hydrocarbon Processing

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Abstract

***p*-Phenylenediamine (PPD), a chemical antidegradant for rubber, and its analogs were found to be optimally determined using liquid chromatography with ultraviolet/visible detection according to the guidelines of ASTM Method D5666. Using the Agilent 1200 RRLC system with ZORBAX 1.8-µm columns, the PPDs could be separated in one run with a total analysis time up to 6.4 times faster than the conventional method based on a 5-µm column.**

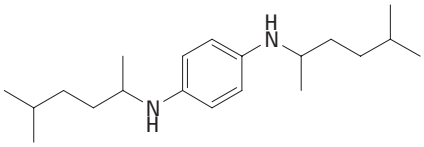
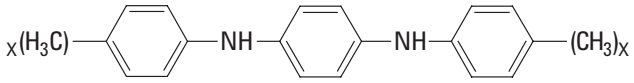
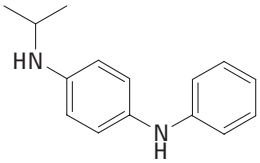
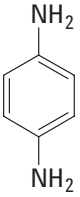
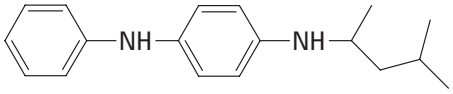
Introduction

Various additives are artificially incorporated into polymeric materials to modify certain properties of the polymer. Therefore, the additives and their concentration in the formulation are crucial to the properties of the end product. 77PD, DTPD, IPPD, PPD, and 6PPD (see Table 1) are often used as chemical antidegradants for rubber materials. The chemical information for five PPDs is displayed in detail in Table 1.

Liquid chromatography with ultraviolet\visible detection is a powerful approach to the qualitative and quantitative analysis of chemical antidegradants in rubber. The isocratic LC method for five PPDs is introduced by ASTM D5666. In this method, the five PPDs are divided into three groups and determined by three different methods (Table 1, ASTM Method D5666-95, 2004).

Agilent 1200 RRLC systems use conventional or sub-two-micron columns, in various lengths up to 300 mm, and can typically provide ultra-fast separations with the same or better resolution as the original method. This application will compare the retention capability and peak shape of the two different stationary phases and will focus on showing the separation of five PPDs in one run, within five minutes, using the 1200 RRLC system with Agilent RRHT reversed phase columns.

Table 1. Chemical Information of Five PPDs

Trade Name	CAS Number	Chemical Structure and Chemical Name
77PD	3081-14-9	N,N'-bis-(1,4-dimethylpentyl)-*p*-phenylenediamine 
DTPD	27417-40-9	N,N'-ditolyl-*p*-phenylenediamine 
IPPD	101-72-4	N-isopropyl-N'-phenyl-*p*-phenylenediamine 
PPD	106-50-3	*p*-phenylenediamine 
6PPD	793-24-8	N-(1,3 dimethylbutyl)-N'-phenyl-*p*-phenylenediamine 

Experimental

System

Agilent 1200 Series Rapid Resolution LC, consisting of:
G1379B micro vacuum degasser
G1312B binary pump SL
G1367C high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/Vis diode array detector SL with 3-mm, 2-µL flow cell
ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 µm

Mobile Phase Conditions

A: Water with 0.1 g/L ethanolamine
B: Acetonitrile (ACN) with 0.1 g/L ethanolamine

Samples

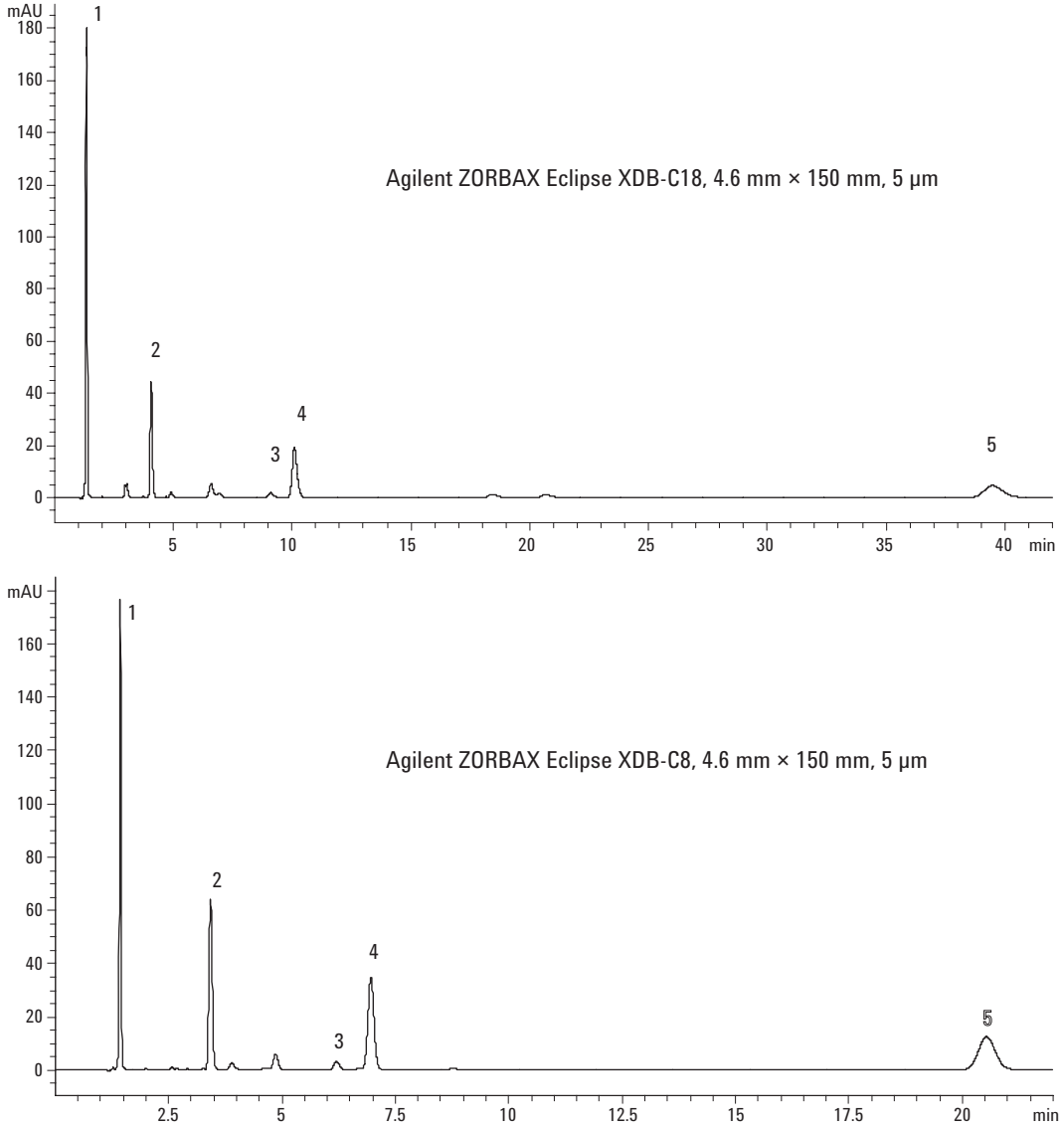
Mixture of 77PD, IPPD, PPD, DTPD, and 6PPD, all 50 µg/mL in acetonitrile. 77PD, IPPD, and PPD were standards from Sigma-Aldrich (St. Louis, Missouri, USA). DTPD and 6PPD were provided by a customer.

Results and Discussion

Selection of Stationary Phase for the Separation of Five PPDs

ASTM D5666-95 recommends a 10- to 15-cm long column packed with C18 grafted silica and 3- to 5-µm particle sizes. In our investigation, however, we observed that the retention characteristics of

ZORBAX Eclipse XDB-C18 were so strong that the total analysis time would be about 40 minutes or more. ZORBAX Eclipse XDB-C8 columns were found to have adequate resolution, and the retention time was only about half of the C18 column. Therefore, we chose the C8 column for further method development. The separations are shown in Figure 1.



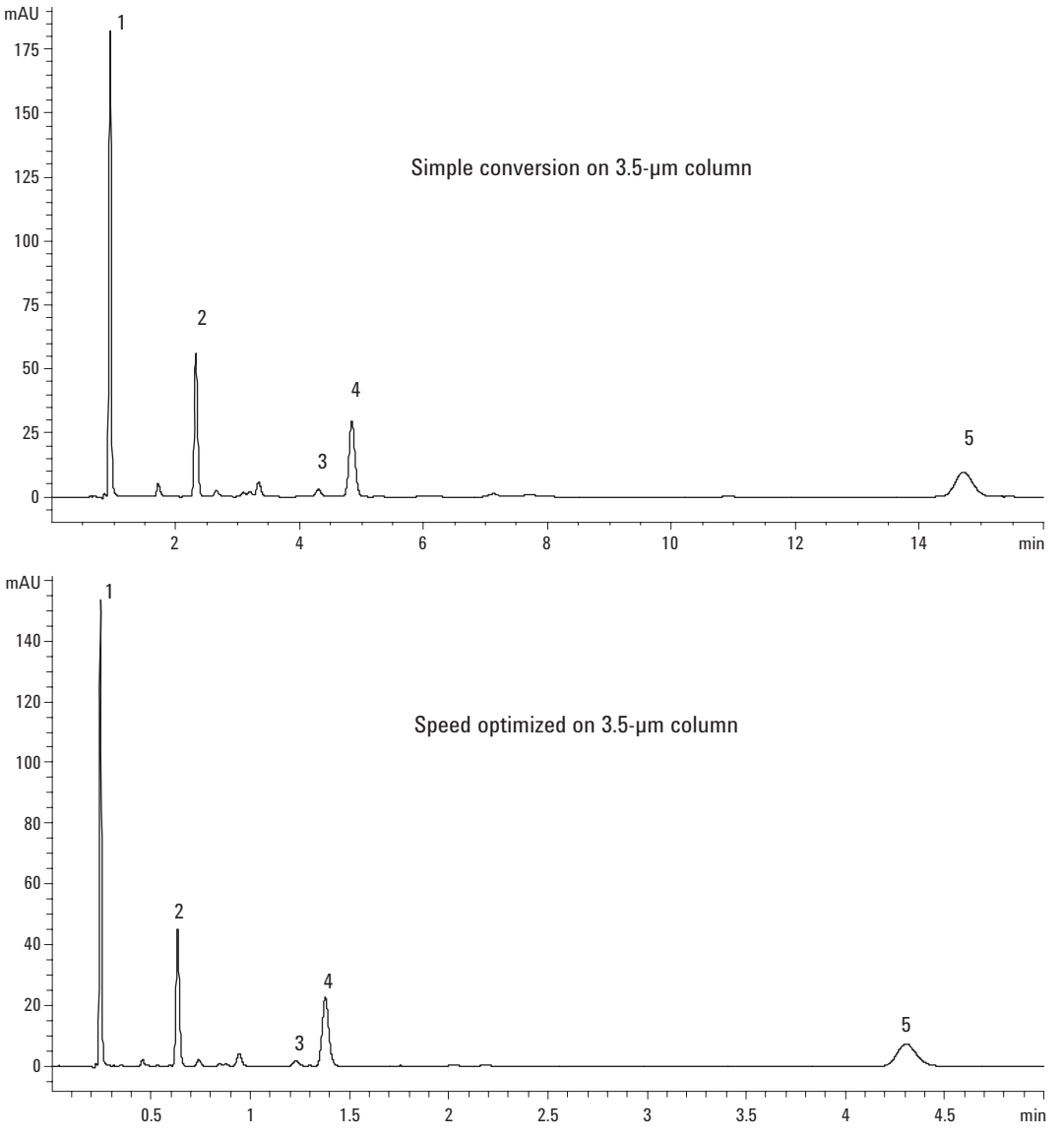
Compound	Conditions		
1 PPD	Mobile phase:	A: water with 0.1 g/L ethanolamine B: ACN with 0.1 g/L ethanolamine	
2 IPPD	Flow rate:	1.0 mL/min	
3 DTPD	Wavelength:	260 nm	
4 6PPD	Injection volume:	10 µL	
5 77PD	Column temperature:	35 °C	
	Sample:	Mixture, 50 µg/mL in ACN	
	Isocratic composition:	30% A, 70% B (v/v)	
	Column size:	4.6 mm × 150 mm, 5 µm	
	Stationary phase:	Agilent ZORBAX Eclipse XDB-C18	Agilent ZORBAX Eclipse XDB-C8
	Analysis time:	42 min	22.5 min

Figure 1. Column stationary phase comparison for five PPDs.

Fast Method Developed Based on New 1200 RRLC

The popular desire of chromatographers is to decrease the analysis time and increase the daily throughput with the same or similar resolution. Nowadays, the Agilent 1200 RRLC system with higher pressure capability and a higher temperature range can provide excellent chromatographic resolution with much shorter run times. Furthermore, a constant concern is how to quickly and easily transfer conventional methods to fast methods. Agilent provides two versions of method translators: one is a Microsoft.net version requiring that Net-Framework 2.0 be resident on the computer, and the other is a Microsoft Excel version requiring that Excel be resident on the PC. When the initial method is an isocratic method, the method translator can provide two modes of faster methods. One is simple conversion, with the scaled flow rate according to the column diameter; the other is speed optimized conversion, with the maximum flow rate and pressure. In gradient mode, an additional option is a resolution optimized conversion.

This application uses a set of Agilent ZORBAX Eclipse XDB-C8 columns, including 4.6 mm × 150 mm (5 µm), 4.6 mm × 100 mm (3.5 µm), and 4.6 mm × 50 mm (1.8 µm). The method translator is used to transfer the initial method on a 5-µm column to two fast methods on 3.5-µm and 1.8-µm columns, respectively. The resulting separation of five PPDs is depicted in Figure 2.



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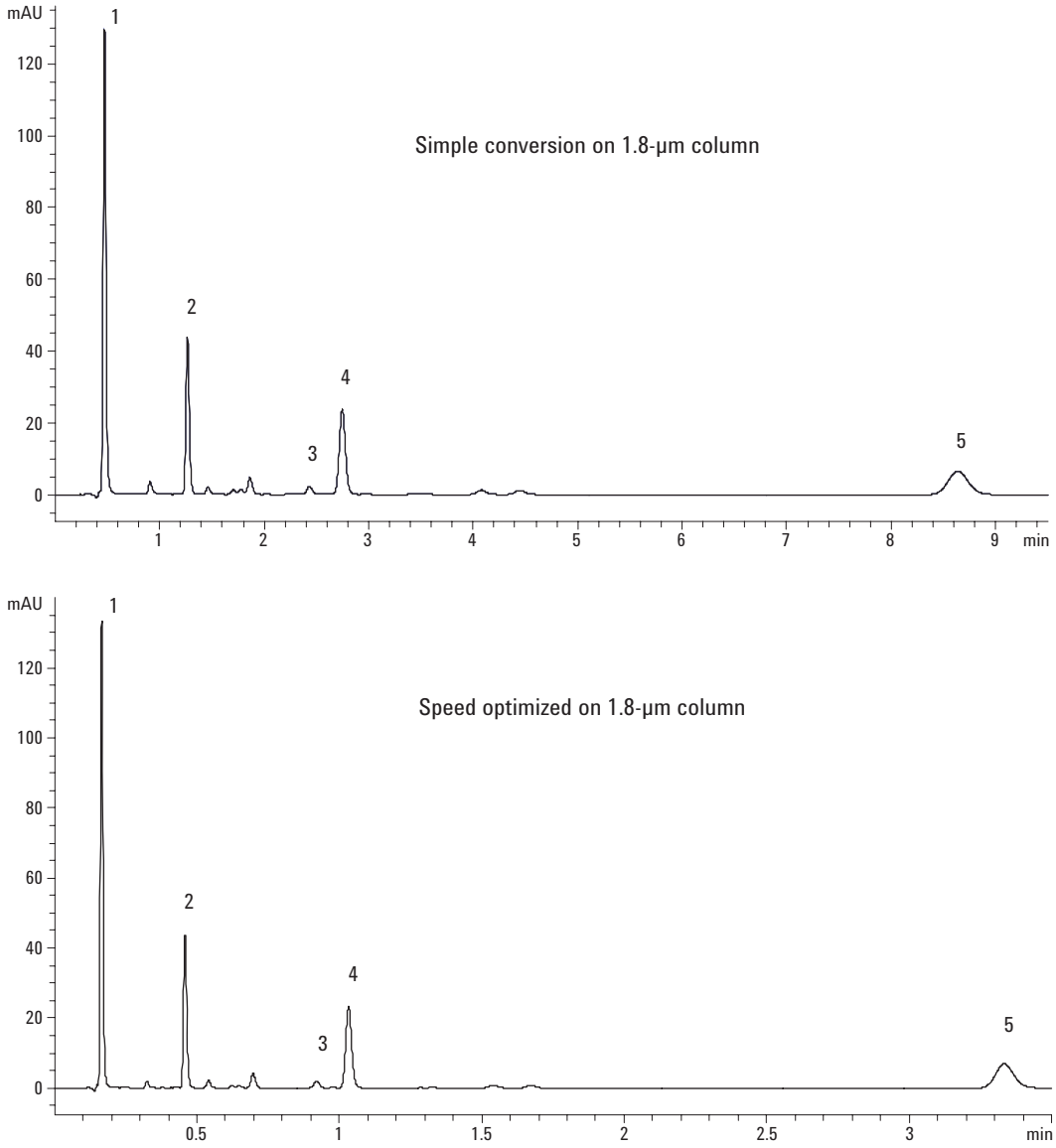

Compound	Conditions				
1 PPD	Stationary phase:	Agilent ZORBAX Eclipse XDB-C8			
2 IPPD	Mobile phase:	A: water with 0.1 g/L ethanolamine			
3 DTPD		B: ACN with 0.1 g/L ethanolamine			
4 6PPD	Isocratic composition:	30% A, 70% B (v/v)			
5 77PD	Column temperature:	35 °C			
	Wavelength:	260 nm			
	Column size	4.6 mm × 100 mm, 3.5 µm		4.6 mm × 50 mm, 1.8 µm	
	Conversion mode:	Simple	Speed optimized	Simple	Speed optimized
	Flow rate:	1.0 mL/min	4.0 mL/min	1.0 mL/min	3.0 mL/min
	Injection volume:	6.7 µL	6.7 µL	3.3 µL	3.3 µL
	Analysis time:	15 min	5 min	10 min	3.5 min

Figure 2. Separation of five PPDs on a smaller particle size column using the transferred methods.

Conclusions

As a powerful approach, liquid chromatography with ultraviolet\visible detection is often used to determine the chemical antidegradants in rubber. Agilent 1200 RRLC systems typically provide the customer with a rapid separation having the same or similar resolution. The method translator can convert any isocratic or gradient method to fast method according to customer requirements. This application details the selection of stationary phases for the separation of five PPDs, separates five PPDs with the RRLC system in one run, and applies the method translator to develop fast methods based on smaller particle size columns. With 1.8-µm column, the total analysis time of five PPDs in one run is about 6.4 times faster than the original 5-µm column method.

References

1. ASTM D5666-95 (Reapproved 2004) "Standard Test Method for Rubber Chemical Antidegradants - Purity of *p*-Phenylenediamine (PPD) Antidegradants by High Performance Liquid Chromatography"
2. Michael Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations," Agilent Technologies publication 5989-5177EN, 2006.

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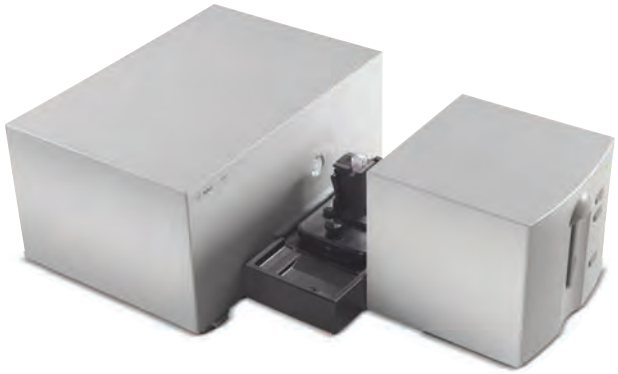
Printed in the USA
January 8, 2007
5989-6011EN



Agilent Technologies

Accurate Measurement of Particle Sizes of Polymers with the Agilent 7010 Particle Size Analyzer

Application Note



Abstract

Many properties of polymeric particles depend on particle size distribution (PSD), so it is important to be able to measure particle sizes in the nanometer to low micrometer range without error. When the PSD contains particles of more than one size population, traditional light scattering techniques are often unable to resolve them, and these instruments typically fail to detect a population of small particles in the presence of a population of large particles. The Agilent 7010 Particle Size Analyzer overcomes these limitations to quickly and accurately measure both particle sizes and concentrations in complex mixtures of fine polymeric particles.

Agilent Technologies

Introduction

Applications of fine polymeric particles are diverse. Coatings, paints, adhesives, drug delivery systems, and medical diagnostics are just a few examples. Unerring characterization of particle sizes is critical because many properties of these materials depend on their particle size distributions (PSDs). For example, the final attributes of a latex product include stability, film-forming ability, covering capacity, viscosity, opacity, texture, mechanical resistance, and processability. All of these properties are affected by its PSD. And in polymerization processes, controlling the PSD allows the synthesis of high-solids-content latexes with improved rheological properties and viscosity.

By correctly manipulating the PSD of the final product, concentrated polymer dispersions (over 65% on a volume basis) can be produced without overly increasing the viscosity of the dispersion. Small latex particle size (< 200 nm) gives best gloss, binding, and adhesion. Large particle size (> 500 nm) gives useful rheological properties (thixotropy, film build, etc), but less efficient binding and low gloss.

Agilent has developed an accurate particle size analyzer that is built on its robust UV-Visible (UV-Vis) spectrometric instrument platform. The Agilent 7010 Particle Size Analyzer is simple to use and maintain, and excels at characterizing the particle size distribution in polymer dispersions.

Measurement of reference monodisperse materials

Reference particles from several suppliers (Duke Scientific Corporation, Polymer Laboratories, Bangs Laboratories, Corpuscular Inc., Spherotech, Seragen Diagnostics) and ranging in size from 74 nm through 15 µm were extensively tested and measured with the Agilent 7010 Particle Size Analyzer. The particle sizes determined by the Agilent 7010 consistently agreed within 5% of the manufacturer-specified values and typically within 2%. The relative standard deviation for all these samples after five consecutive measurements was better than 1.5%. Figure 1 displays size analyses by the Agilent 7010 for a few of the many polystyrene reference sizes that were measured to check accuracy and precision in the range of 240 nm to 800 nm.

The Agilent 7010 accurately measures the particle size distribution of a variety of polymers, as shown in Figures 2 through 5. Fluorescent and dyed polystyrene particles commonly used for tagging applications in biotechnology are also measured easily; the absorptions of the dyes and fluorophores have a negligible effect on the scattering spectrum and do not alter the calculated particle size distribution, as shown in Figure 2. Figure 3 depicts the PSD for polymethylmethacrylate (PMMA) particles from Seragen Diagnostics. Various sizes of polybutadiene particles have also been tested. Figure 4 shows the particle size distribution for 160 nm, 400 nm and 590 nm polybutadiene particles dispersed in water. Melamine formaldehyde particles have been successfully measured, as shown in Figure 5 for 1.7 µm and 2.54 µm standards. Accurate measurement of polymer dispersions are not limited to dispersions in water, as is shown by measurement of polystyrene in 2-propanol in Figure 6.

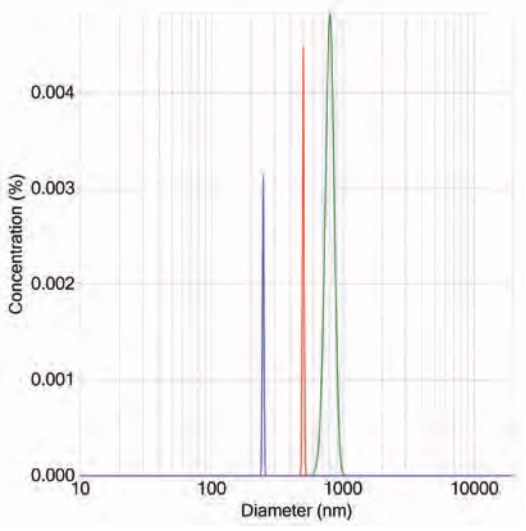


Figure 1. *Duke polystyrene 240 nm (blue), 500 nm (red), and 800 nm (green)*

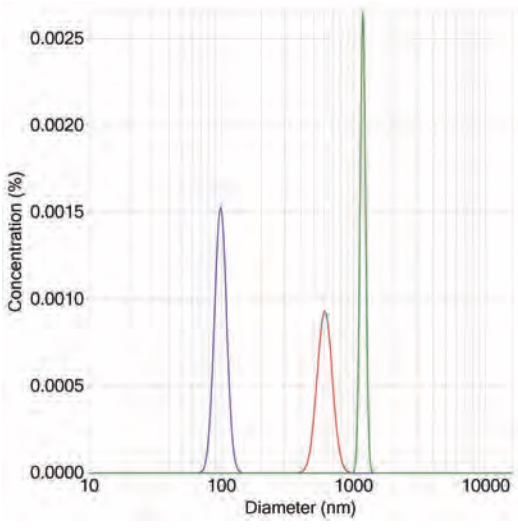


Figure 2. *Particle size distribution of blue-, red-, and green-dyed polystyrene.*

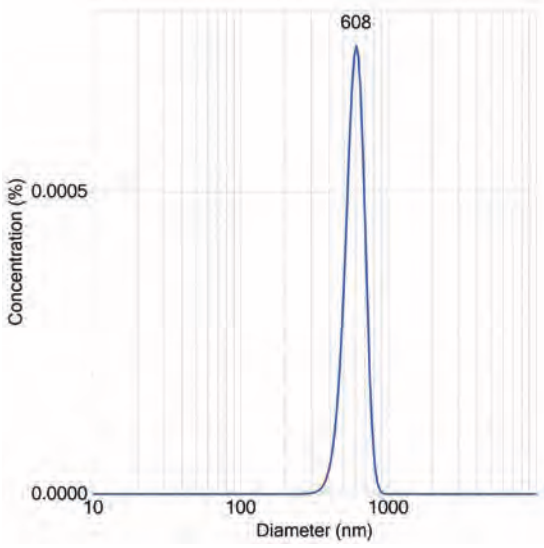


Figure 3. *Seragen polymethylmethacrylate (PMMA) at 586 nm*

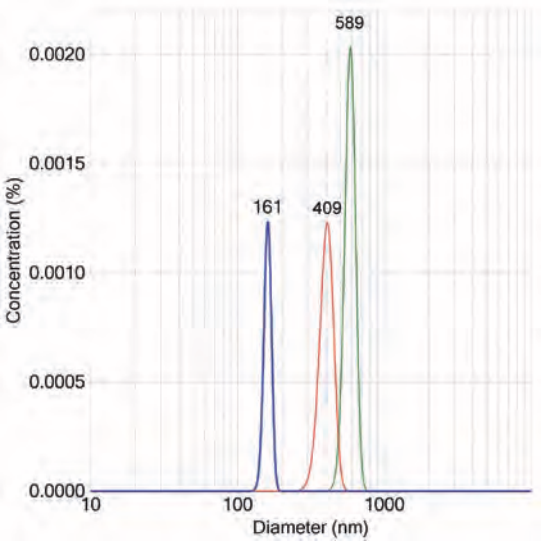


Figure 4. *Dow polybutadiene at 160 nm (blue), 400 nm (red), and 590 nm (green).*

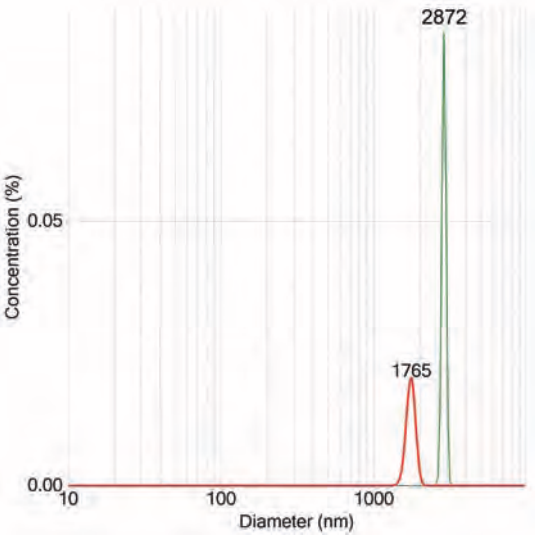


Figure 5. *Melamine formaldehyde at 1.7 µm (red) and 2.54 µm (green).*

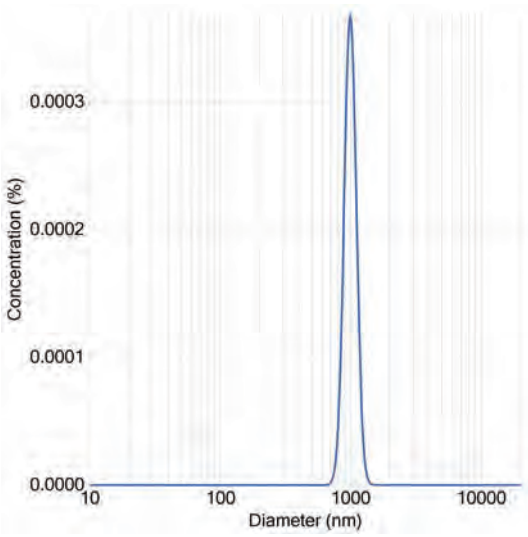


Figure 6. *1.2 µm polystyrene dispersed in 2-propanol.*

Coarse and fine populations mixed in different proportions

Figure 7 depicts a mixture of two polystyrene reference particles (Duke Scientific Corporation) of sizes 92 nm and 3000 nm. These images were obtained with the Hitachi S-4500 Field Emission Scanning Electron Microscope (FE-SEM). Samples were prepared with varying proportions of the fine 92 nm and coarse 3000 nm particles from a ratio of 2%/98% to 98%/2%, respectively.

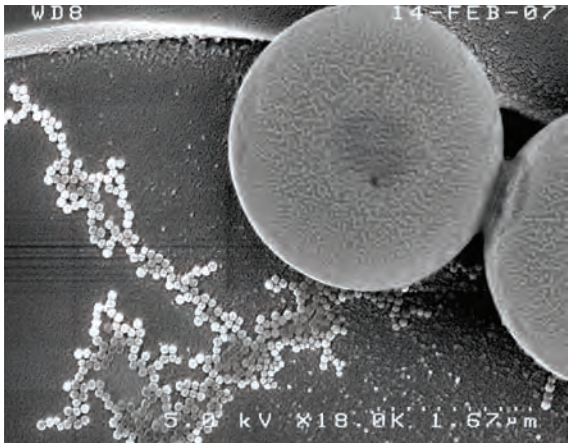


Figure 7. *Mixture of 92 nm and 3000 nm polystyrene particles (Hitachi S-4500 FE-SEM).*

Resolution of multimodal distributions is generally difficult for traditional light scattering techniques. Furthermore, the detection of fine particles in the presence of a large population of coarse particles is even more challenging since a few large particles can scatter too much light, masking the presence of the small particles. Figure 8 shows that the 7010 Particle Size Analyzer is able to detect even 9% of fine 92 nm particles in the presence of 91% of 3 µm particles.

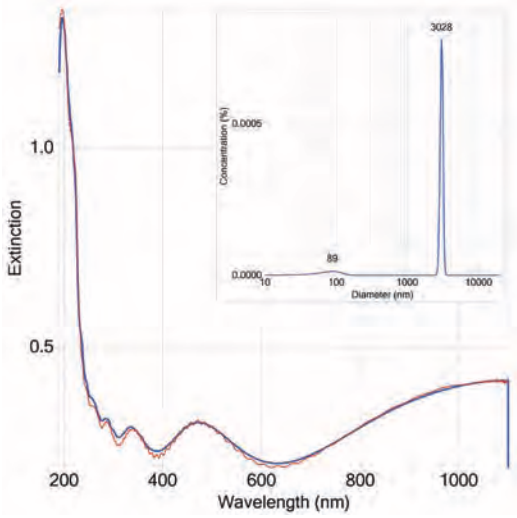


Figure 8. *Detection of 9% by volume of the fine 92 nm particles in a background of 91% of 3 µm particles.*

To understand how this UV-Vis spectroscopic technique is able to detect and resolve these complex dispersions, Figures 9 and 10 dissect the measurement. If the entire measured UV-near infrared (NIR) spectrum is to agree with theory within experimental accuracy, the instrument's size analysis must include the two populations. Static light scattering and dynamic light scattering instruments have difficulty detecting fine particles in the presence of a population of coarse particles because in visible wavelengths, the coarse particles scatter much more strongly than the fine particles. The Agilent 7010 does not have this difficulty because the respective signatures of the fine and coarse populations do not overlap throughout the wavelength range of the instrument.

Figures 9 and 10 assist in understanding why this instrument can discriminate two particle populations that are different in both size and concentration. In Figure 9, the 7010 Particle Size Analyzer was set to analyze the spectrum without considering the information in the wavelength range of 190 nm to 400 nm. The analysis delivered a peak at 3.028 µm. By analyzing the spectrum again and using only the wavelength range of 190 nm to 230 nm, Figure 10 shows a peak at 97 nm. Because those two peaks are very close to the actual sizes of the two populations, these measurements show that in the original spectrum, the signature associated with the fine population is predominantly in the UV region, while the signature associated with the coarse population is primarily in the visible region.

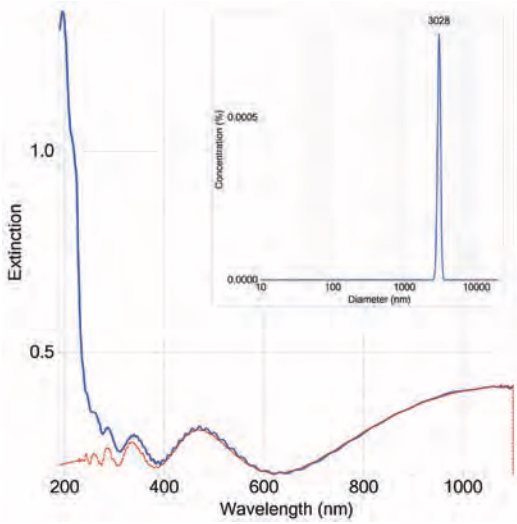


Figure 9. *Same measurement as in Figure 8, with elimination of information in the 190 nm to 400 nm range.*

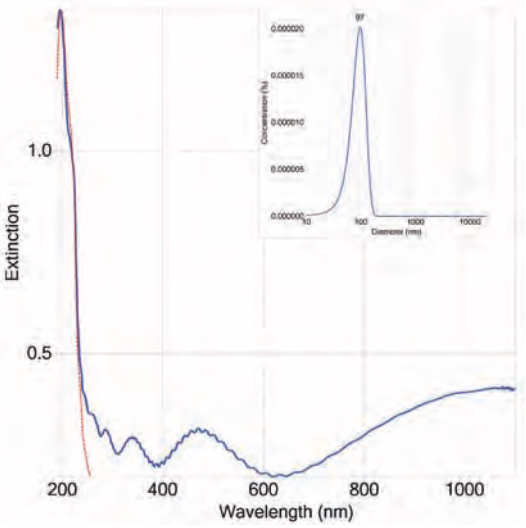


Figure 10. *Same measurement as in Figure 8, with elimination of information in the 230 nm to 1100 nm range.*

Figure 11 compares the size analysis for samples in which the volume percentage for the fine population was 4.8%, 9%, and 17%. From inspection of the spectra, it is apparent that as the proportion of fine particles increases, the spectral signature of the coarse particles becomes more and more insignificant in amplitude as the attenuation in the short wavelengths increases. The sharp decrease in extinction as a function of increasing wavelength in the UV range due to Rayleigh scattering of the fine particles is clearly observed. As the feature due to Rayleigh scattering of fine particles grows, the relative size of the signature for the coarse population decreases.

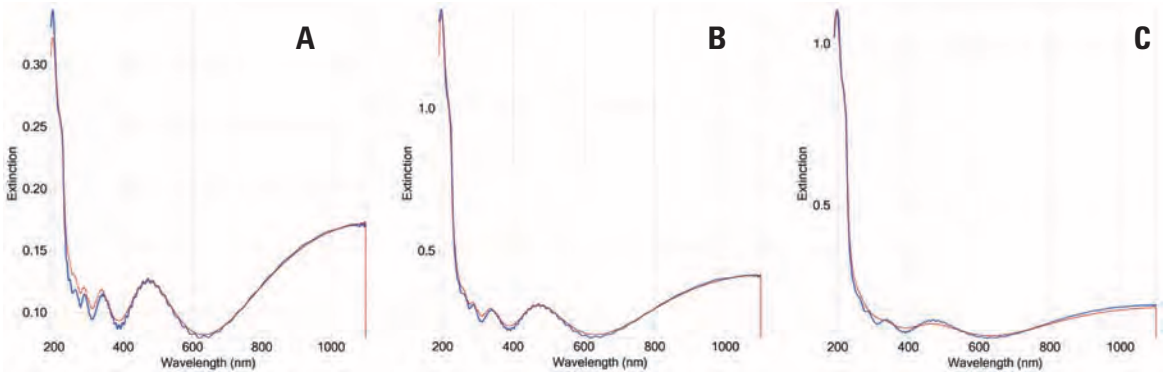


Figure 11. *A) 4.8% 92 nm and 95.2% 3 µm polystyrene. B) 9% 92 nm mixed with 91% 3 µm polystyrene. C) 17% 92 nm mixed with 83% 3 µm polystyrene.*

Resolution test

The results shown up to this point demonstrate that the 7010 Particle Size Analyzer can resolve populations that differ greatly in particle size, as well as in their volume proportions. The following examples demonstrate how close in size two populations can be while still being resolved by the Agilent 7010. Polystyrene at 200 nm was mixed first with 1 µm polystyrene, and then with incrementally smaller polystyrene particles to determine the population size limits that the Agilent 7010 can resolve. Figure 12 illustrates the size resolution of 50%/50% polystyrene mixtures: 200 nm/1000 nm (blue); 200 nm/800 nm (red); 200 nm/600 nm (green); and 200 nm/500 nm (black). In fact, the Agilent 7010 has resolved two different size populations that are as close as a ratio of 1:1.5. This is demonstrated in Figure 13 for 1 µm and 2 µm (blue), 2 µm and 3 µm (red) and 3 µm and 5 µm (green) polystyrene particles. Comparing these distributions, it appears that the broadening of the 1 µm peak in the mixture is an artifact of the fitting algorithm. There appears to be a trend in close bimodal samples of the smaller peak becoming artificially broadened.

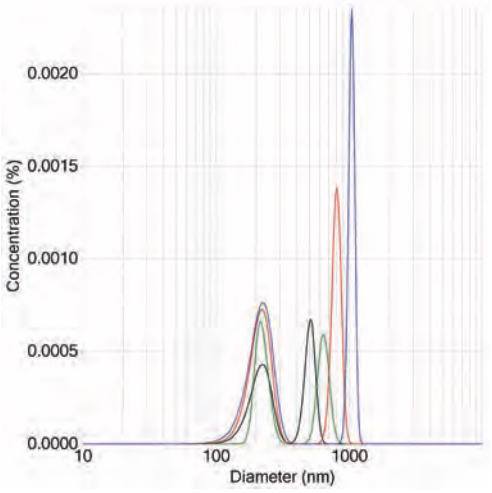


Figure 12. *Size resolution of 50% 200 nm polystyrene mixed with 50% 1 µm polystyrene (blue), 50% 800 nm polystyrene (red), 50% 600 nm polystyrene (green), and 50% 500 nm polystyrene (black).*

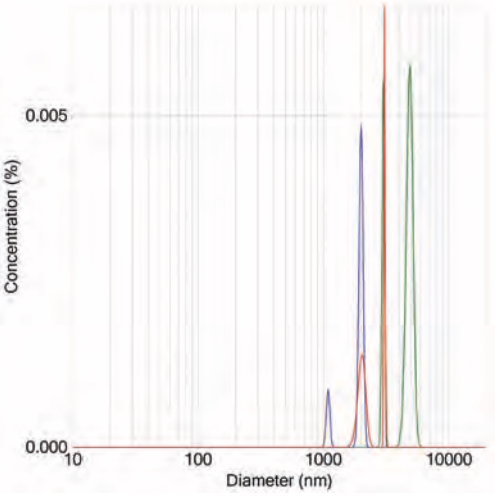


Figure 13. *Demonstration of 1:1.5 size resolution capability with polystyrene mixtures: 15% 1 µm mixed with 85% 2 µm (blue), 50% 2 µm mixed with 50% 3 µm (red), and 50% 3 µm mixed with 50% 5 µm (green).*

Polymer dispersions with UV-absorbing additives

Very often, dispersions of polymer particles in water contain additives that absorb in the UV range. For example, biocides may be added to reduce bacterial growth, surfactants may be added to promote colloidal stability, or ligands with specific chemical functionality may be attached. These UV-absorbing features must be either subtracted or ignored in order to produce the best particle size results. If the software tries to fit the UV-absorbing features to particle scattering, phantom small-particle peaks may be created in the PSD.

One method to produce results that are more accurate is to measure a blank using the exact suspending medium of the dispersion. This may be accomplished by separately preparing a solution with the correct concentration of additive, or if the particles are sufficiently large and not buoyant, by centrifuging the particles and using the supernatant as a blank. Note that if the centrifugation is not complete and some particles are present in the blank, it is still possible to get a very good measurement of particle size, but the particle concentration will be underestimated and the dynamic range for the sample will be drastically reduced.

A simpler procedure to reduce artifacts caused by UV-absorbing additives is to use pure water as a blank and to remove the UV wavelengths from the calculation of particle size. This is especially advisable if the particles are in the micron size range and the spectral scattering features are not in the UV range. To disregard the UV wavelengths in the calculation, the Agilent 7010 software has a feature called "UV Autocorrect."

Figures 14A and 14B give an example of a commercial latex emulsion with a broad distribution that maximizes around 7 µm. The correct particle size distribution, verified by comparison with SEM image analysis, is found with UV Autocorrect selected (Figure 14A). With UV Autocorrect off, a poor spectral fit (red and blue lines not corresponding) is found and the wrong particle size distribution is produced (Figure 14B).

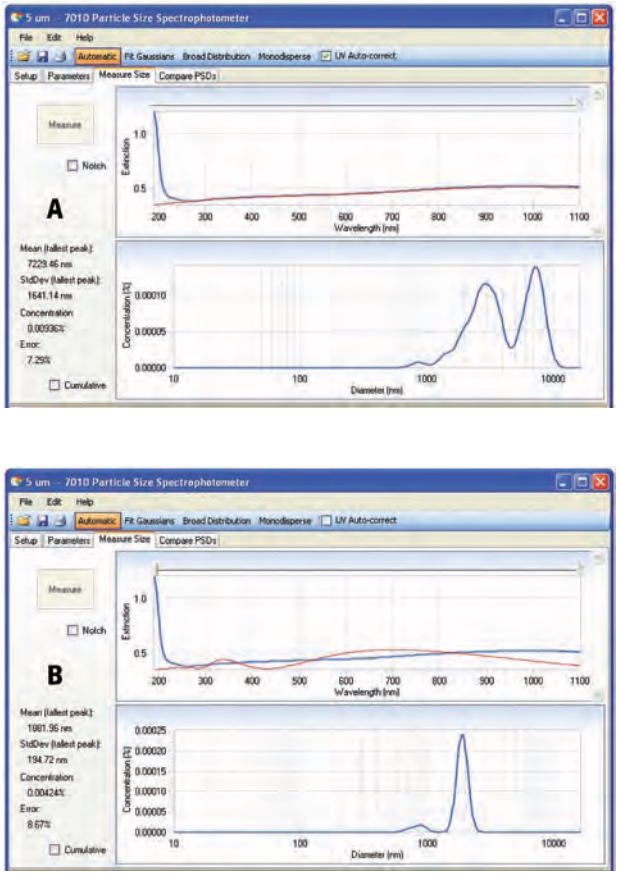


Figure 14. *Automatic mode measurement of commercial latex emulsion. A) Correct PSD found with UV Autocorrect on. B) Incorrect PSD found with UV Autocorrect off.*

The particle size distributions for several polystyrene reference standards in the range of 1 µm to 15 µm were also measured. Two of these are shown in Figures 15 and 16. Despite this, even if UV Autocorrect is off and pure water is used as the blank fluid, the size analysis in many cases will be accurate due to the high information content for monodisperse particles in this size range. However, the error will be higher than the advised range of 0 to 3% due to the discrepancy between measurement and theory in the UV wavelengths.

When *not* to use UV Autocorrect

Although the default setting of UV Autocorrect is on, it may be advisable to deselect this feature when measuring small particles (< 200 nm) of nonmetals. These particles scatter strongly in the UV range and may be entirely ignored by UV Autocorrect.

The screen shots in Figure 17 show a mixture of 100 nm and 400 nm polystyrene latex in a 1:3 mixture. With UV Autocorrect on (Figure 17A), the particle size distribution algorithms completely ignore the 100 nm particles. With UV Autocorrect deselected, as shown in Figures 17B and 17C, the software correctly finds both the 100 and 400 nm polystyrene peaks, and correctly determines their relative concentrations as well.

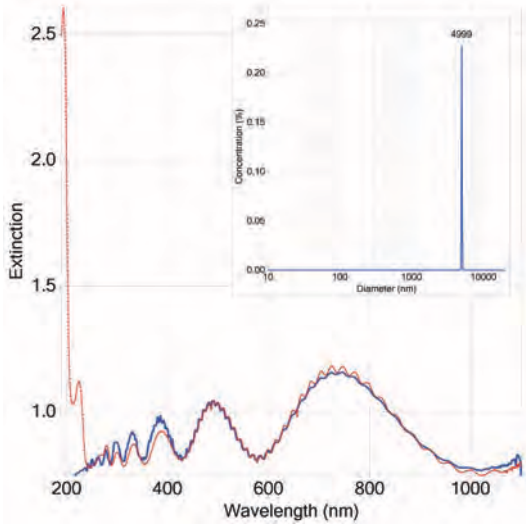


Figure 15. *5 µm polystyrene (Duke Scientific Corporation).*

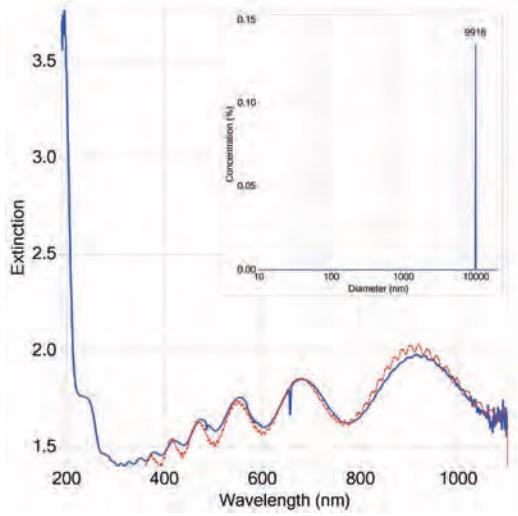


Figure 16. *10 µm polystyrene (Duke Scientific Corporation).*

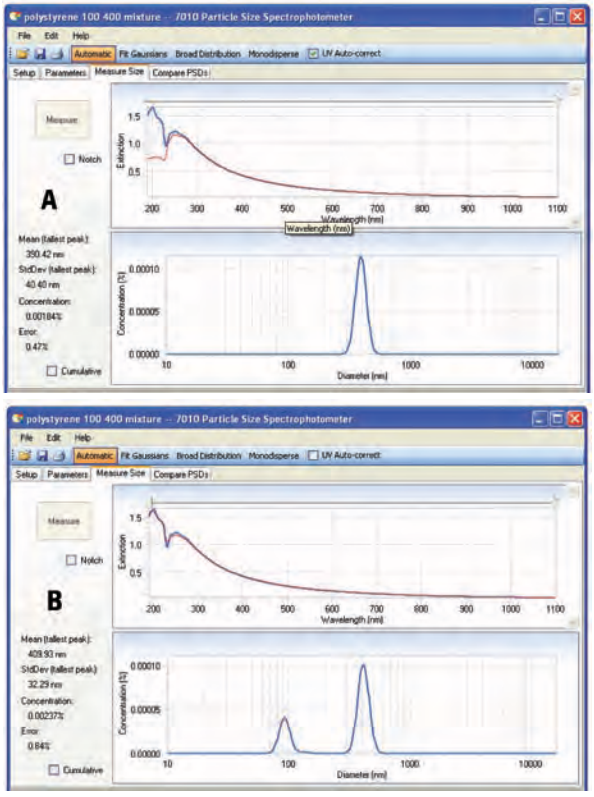


Figure 17. *Particle size distributions from mixture of 100 and 400 nm polystyrene size standards. A) Incorrect PSD with UV Autocorrect selected. B) Correct PSD with UV Autocorrect deselected. C) Same as B, except cumulative PSD is displayed.*

Conclusion

The Agilent 7010 Particle Size Analyzer provides accurate results for complex particle size distributions of polymer dispersions. The UV-Vis technology in the Agilent 7010 allows one to measure multimodal dispersions or mixtures of very small particles in the presence of very large particles, which are typically challenging for light scattering techniques (dynamic light scattering or laser diffraction). With its ease of use, ability to give concentration information as well as particle size, and fast measurement time (less than 10 seconds), the Agilent 7010 Particle Size Analyzer is an indispensable technology for characterizing polymer particles ranging in size from 50 nm to 15 µm.

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Printed in the U.S.A. January 9, 2009
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Agilent Technologies



Chemical and Specialty Gas

Technical Overviews

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ZORBAX Eclipse XDB HPLC Columns

The "Perfect Fit" for Developing Better HPLC Methods

Technical Overview

- Excellent peak shape for basic, acidic or neutral compounds
- High performance over a wide pH range
- Rugged, reproducible chromatography from column-to-column and lot-to-lot
- More selectivity options for method development

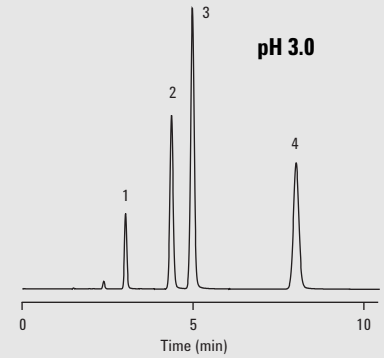
More and more chromatographers are developing their analytical and LC/MS separations on ZORBAX Eclipse XDB HPLC columns. Why? Because ZORBAX Eclipse XDB columns solve many of their separation challenges. In fact, Agilent Technologies includes a 4.6 x 150 mm, 5 µm ZORBAX Eclipse XDB-C8 column with every Agilent 1100 HPLC instrument it delivers. As Figure 1 shows, ZORBAX Eclipse XDB columns, specifically designed to extend column life and provide excellent peak shape for basic compounds in the pH range of 6 - 9, also deliver outstanding performance at low pH, as well.



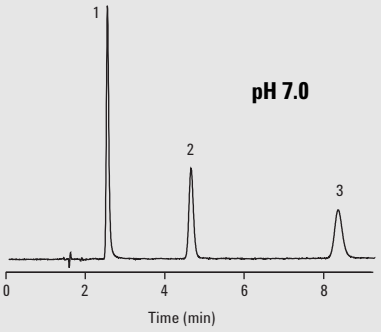
More detailed information about the benefits of the Eclipse column family can be found in this brochure.

Figure 1
Good Peak Shape for Acids, Bases and Neutrals at Low and Intermediate pH

ZORBAX Eclipse XDB-C18, 4.6 x 150 mm, 5 µm
(Agilent Part No. 993967-902)



Mobile Phase:	80% 25 mM Na_2HPO_4, pH 3.0 20% Methanol
Flow Rate:	1.0 mL/min
Temperature:	35°C
Sample:	1. Theobromine 2. Theophylline 3. 1,7-dimethylxanthine 4. Caffeine



Mobile Phase:	70% 25 mM Na_2HPO_4, pH 7.0 30% Methanol
Flow Rate:	1 mL/min
Temperature:	RT
Detection:	UV 254 nm
Sample:	1. Procainamide 2. N-acetylprocainamide 3. N-propionylprocainamide

Eclipse XDB HPLC Column Technology Provides:

Excellent Peak Shape



eXtra Dense Bonding + High Purity Silica = Excellent Peak Shape

eXtra Dense Bonding is key to the exceptional performance of ZORBAX Eclipse XDB columns at intermediate pH (Figure 2). This dense bonding is accomplished by adding an extra-dense monolayer of C18, C8 or Phenyl silane to the ultra-pure, fully-hydroxylated, ZORBAX Rx-silica surface. The packing is then endcapped not once, but twice, using two different and unique endcapping reagents. This combination of extra-dense surface coverage by the bonded phase and double endcapping produces a highly, deactivated stationary phase that virtually eliminates undesirable interactions between polar solutes and the silica surface. As a result, superior peak shape, high efficiency, and long-term chromatographic reproducibility are assured when using Eclipse XDB HPLC columns at both intermediate and low pH.

The exceptional and reproducible performance at low pH that you can expect from the Eclipse XDB is demonstrated in Figure 3. In this example, acetylsalicylic acid, and the base, dextromethorphan, are consistently separated with excellent peak shape at low pH on three different Eclipse XDB-C8 columns from three different lots of packing material.

Figure 2
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 7

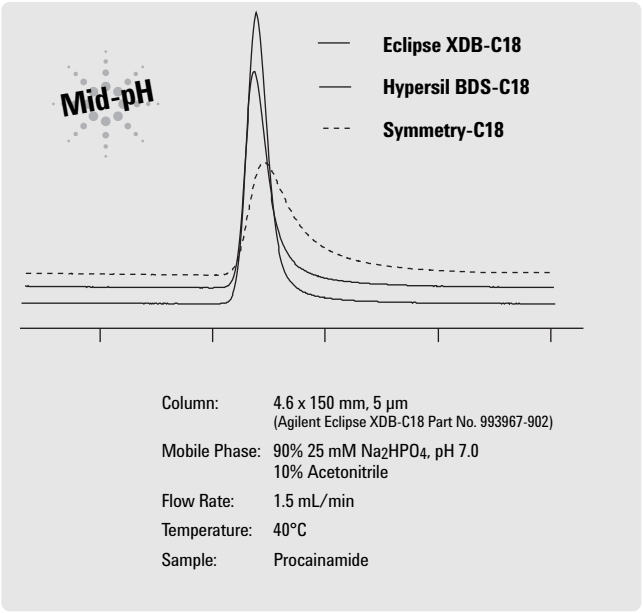
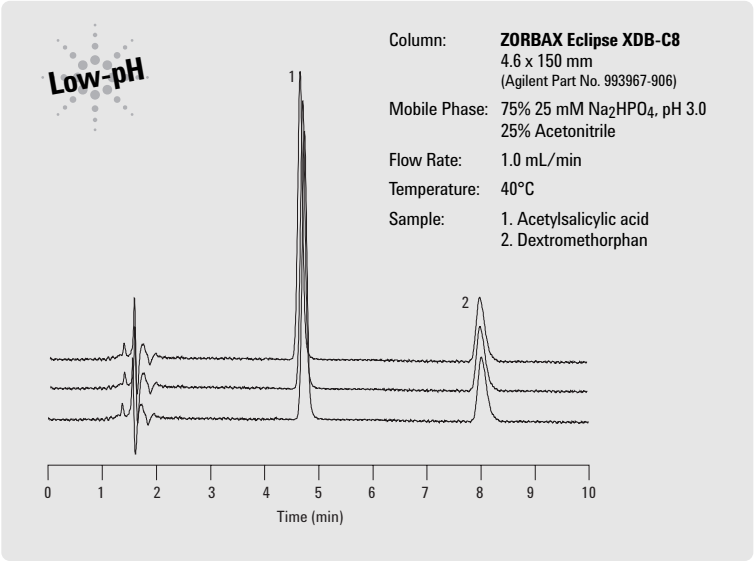


Figure 3
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 3



Eclipse XDB HPLC Column Technology Provides:

Long Column Life



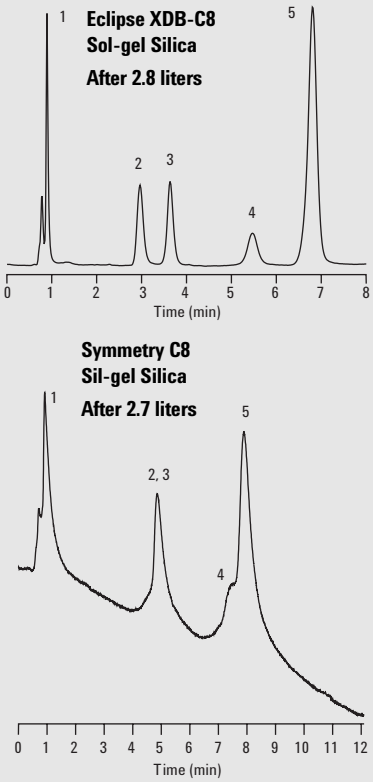
Eclipse XDB columns not only provide excellent peak shape but they are also exceptionally durable. In fact, the spherical ZORBAX Rx-SIL particles are the most durable, porous, 5 and 3.5 µm, silica particles commercially available. They are manufactured using a patented and proprietary process, forming thick, hard-walled silica, commonly referred to in the literature as "sol-gel" silica.

Because of the strength of the ZORBAX particle, all ZORBAX columns are consistently and reliably packed at pressures exceeding 8000 psi. The result is a durable column that can easily tolerate pressures up to 5000 psi in regular use without a loss in efficiency or a reduced lifetime.

Long Column Life at Intermediate pH

This thick, hard-walled "sol-gel" silica resists dissolution at intermediate pH and when densely bonded, the resultant Eclipse XDB column provides excellent column performance and increased column lifetime, even under the stressed intermediate pH conditions described in Figure 4.

Figure 4
Accelerated Column Aging Study Demonstrates the Durability of Eclipse XDB-C8 Over Waters' Symmetry C8



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase:
20% Acetonitrile
80% 250 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

Temperature: 60°C

Test Conditions:

Mobile Phase:
60% Acetonitrile
40% 10 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

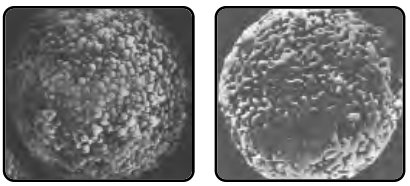
Temperature: 40°C

Sample: Tricyclic Antidepressants
1. Uracil 2. Nortriptyline 3. Doxepin
4. Amitriptyline 5. Trimipramine

Many commercial, base-deactivated, silica-based HPLC columns use manufacturing processes that produce a less robust "sil-gel" silica particle. The walls of these resulting high-surface area materials (typically 300 m^2/g for an 80-100Å pore material) are thinner and less uniform and can easily crush under high pressure conditions. Moreover, in many cases, they fail to withstand the high pressures of high flow rate LC/MS and High-Throughput methods.

The Eclipse XDB thick, hard-walled "sol-gel" is compared to the thin-walled "sil-gel" silica, used to make most of today's base-deactivated products, in electron micrographs shown in Figure 5.

Figure 5
Eclipse XDB is Based on Thick, Hard-Walled, Sol-gel Silica



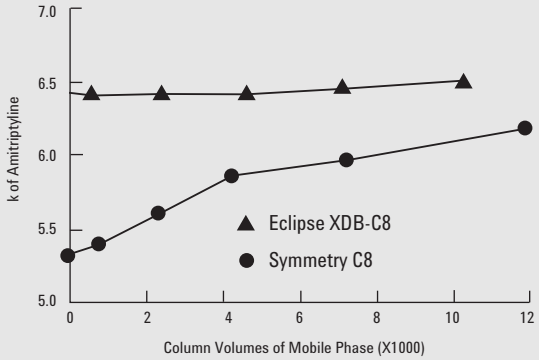
Sol-gel **Sil-gel**

Eclipse XDB columns are made with patented "hard wall" sol-gel silica particles. The thick walls of this silica are more resistant to dissolution than the "thin wall" sil-gel silica that is used to make most base-deactivated columns.

Long Column Life at Low pH

At low pH, Eclipse XDB columns provide better column life than most commercially available reversed-phase HPLC columns. Figure 6 summarizes the results from a low-pH accelerated aging study where loss in column performance is measured by a loss in bonded phase, indirectly measured by the change in retention of amitriptyline. The results show that less than 3% of the Eclipse XDB column performance is lost when exposed to 12,000 mL of a pH 3 mobile phase at 60°C. Under these same conditions more than 14% of column performance is lost on another popular "deactivated" reversed-phase HPLC column.

Figure 6
Accelerated Column Aging Study Demonstrates the Durability of ZORBAX Eclipse XDB-C8 Over Waters' Symmetry



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.5 mL/min

Temperature: 60°C

Retention Test Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.0 mL/min

Temperature: 60°C

Sample: Amitriptyline

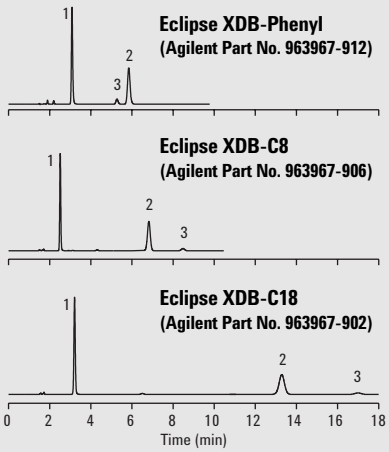
Eclipse XDB HPLC Column Technology Provides:

More Selectivity Options C18, C8 and Phenyl

Eclipse XDB HPLC columns are available as C18, C8 and Phenyl bonded phases. The Eclipse XDB-Phenyl phase complements both the most retentive Eclipse XDB-C18 and the moderately retentive Eclipse XDB-C8. The Eclipse XDB-Phenyl offers unique selectivity as well as reduced retention of non-polar and moderately polar compounds while maintaining retention of polar analytes.

These benefits are illustrated in Figure 7. Sunscreen components are well retained on the Eclipse XDB-C18, although the analysis time is long. Analysis time is reduced with more than acceptable resolution when using the Eclipse XDB-C8 or Eclipse XDB-Phenyl column, where analysis time is shortened by 50 or 61 percent, respectively.

Figure 7
Eclipse XDB-Phenyl Columns Offer Unique Selectivity and Short Analysis Times



Column: **ZORBAX Eclipse XDB**
4.6 x 150 mm, 3.5 µm

Mobile Phase: 85% Methanol
15% Water

Flow Rate: 1.0 mL/min

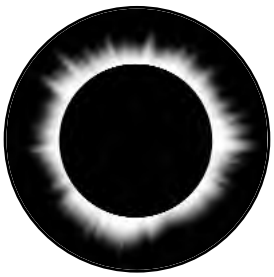
Temperature: 30°C

Detection: UV 310 nm

Sample: 1. Oxybenzone
2. Padimate O
3. Ethylhexyl Salicylate

Eclipse XDB HPLC Column Technology Provides:

Rapid Resolution

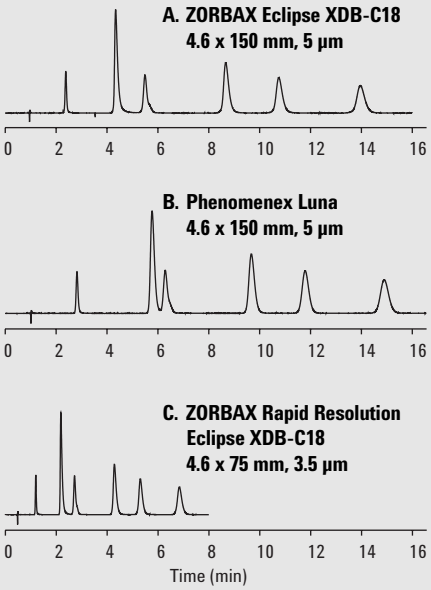


Eclipse XDB columns, available in 5 and 3.5 µm particle size packings, are highly efficient. In Figure 8A, six basic antidepressant compounds are well separated on a 5 µm, 4.6 x 150 mm, Eclipse XDB-C18 column using a methanol-phosphate mobile phase at pH 7. Peaks elute in sharp bands having average peak widths at half-height of 0.19 minutes. When the same sample was run on the Phenomenex Luna column, resolution between nortriptyline and doxepin ($R_{2,3}$) decreased significantly, with average peak widths at half-height being 47% wider, averaging 0.28 minutes for this assay.

For faster analysis, shorter Rapid Resolution Eclipse XDB columns, packed with 3.5 µm particles, provide equally efficient separations with reduced analysis times. This is demonstrated in Figure 8C, where analysis time is decreased by 50% — as retention decreases from 15.6 to 6.8 minutes — while resolution is maintained for this highly basic antidepressant sample.

If you have any questions about this or other applications in this bulletin, call 800-227-9770 select option 4 and ask for HPLC column technical support.

Figure 8
Eclipse XDB Provides High Efficiency and Rapid Resolution



Mobile Phase: 73% Methanol
27% 50 mM Phosphate, pH 7
Flow Rate: 1.5 mL/min.
Temperature: 40°C
Sample: Antidepressants
1. Imipramine
2. Nortriptyline
3. Doxepin
4. Doxylamine succinate
5. Amitriptyline
6. Cloripramine

Developing reliable reversed-phase methods for basic, acidic and neutral compounds just got easier . . .

Order Your ZORBAX Eclipse XDB HPLC Columns today!

ZORBAX Eclipse XDB Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse XDB-C18	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	10%
ZORBAX Eclipse XDB-C8	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.6%
ZORBAX Eclipse XDB-Phenyl	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.2%

Symmetry® is a registered trademark of Waters Corporation.
Luna® is a registered trademark of Phenomenex.

ZORBAX Eclipse XDB Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11
Standard Columns and Bulk Packings					
Semi-Prep	9.4 x 250	5	990967-202	990967-206	
Analytical	4.6 x 250	5	990967-902	990967-906	990967-912
Analytical	4.6 x 150	5	993967-902	993967-906	993967-912
Analytical	4.6 x 50	5	946975-902	946975-906	
Rapid Resolution	4.6 x 150	3.5	963967-902	963967-906	963967-912
Rapid Resolution	4.6 x 100	3.5	961967-902	961967-906	
Rapid Resolution	4.6 x 75	3.5	966967-902	966967-906	966967-912
Rapid Resolution	4.6 x 50	3.5	935967-902	935967-906	935967-912
Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312
Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312
Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312
Solvent Saver Plus	3.0 x 100	3.5	961967-302	961967-306	961967-312
Solvent Saver Plus	3.0 x 75	3.5	966954-302		
Narrow Bore	2.1 x 150	5	993700-902	993700-906	993700-912
Narrow Bore	2.1 x 50	5	960967-902	960967-906	960967-912
Narrow Bore RR*	2.1 x 150	3.5	930990-902	930990-906	
Narrow Bore RR	2.1 x 100	3.5	961753-902	961753-906	
Narrow Bore RR	2.1 x 75	3.5	966735-902		
Narrow Bore RR	2.1 x 50	3.5	971700-902	971700-906	
MicroBore RR	1.0 x 150	3.5	963600-902	963600-906	
MicroBore RR	1.0 x 50	3.5	965600-902	965600-906	
MicroBore RR	1.0 x 30	3.5	961600-902	961600-906	
Bulk Packing, 2 grams		5	920966-902		
Guard Cartridges, 4/pk	4.6 x12.5	5	820950-925	820950-926	820950-927
Guard Cartridges, 4/pk	2.1 x12.5	5	821125-926	821125-926	821125-926
Guard Hardware Kit			820777-901	820777-901	820777-901
Agilent Cartridge Columns					
Analytical	4.6 x 250	5	7995118-585	7995108-585	
Analytical	4.6 x 150	5	7995118-595	7995108-595	
Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344	
Solvent Saver	3.0 x 75	3.5	7995230-344		
Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504	
Cartridge Holder			5021-1845	5021-1845	
High Throughput Cartridge Columns (requires Hardware Kit 820222-901)					
Rapid Resolution Cartridge	4.6 x 30	3.5	933975-902	933975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	933975-932	933975-936	
Rapid Resolution Cartridge	4.6 x 15	3.5	931975-902	931975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	931975-932	931975-936	
Rapid Resolution Cartridge	2.1 x 30	3.5	973700-902	973700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	973700-932	973700-936	
Rapid Resolution Cartridge	2.1 x 15	3.5	975700-902	975700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	975700-932	975700-936	
Hardware Kit for High Throughput Columns			820222-901	820222-901	
CombiHT Columns (end fittings required)					
CombiHT	21.2 x 150	5	970150-902	970150-906	
CombiHT	21.2 x 100	5	970100-902	970100-906	
CombiHT	21.2 x 50	5	970050-902	970050-906	
CombiHT End Fittings (2) (required for use)			820400-901	820400-901	
Capillary Glass-lined Columns					
Capillary	0.5 x 250	5	5064-8286		
Capillary	0.5 x 150	5	5064-8287		
Capillary RR	0.5 x 150	3.5	5064-8288		
Capillary RR	0.5 x 35	3.5	5064-8298		
Capillary	0.3 x 250	5	5064-8269		
Capillary	0.3 x 150	5	5064-8291		
Capillary RR	0.3 x 150	3.5	5064-8271		
Guard Cartridges	0.5 x 35	5	5064-8296		
Guard Cartridges	0.3 x 35	5	5064-8297		

*RR: Rapid Resolution 3.5 µm columns.

For more information on these and other columns consult the Agilent web site at **www.agilent.com.**

Configurations not shown are available upon request.



Agilent Technologies

For the latest information on the complete line of Agilent Technologies columns and supplies for analytical instruments, see our online catalog at www.agilent.com/chem on the World Wide Web, or contact your local Agilent sales office. For all other areas contact Agilent or your local authorized distributor.

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Printed in the USA
December 3, 2002
5988-8367EN

Polymer analysis by GPC-SEC

Technical Note

Introduction

Gel Permeation Chromatography (GPC), also referred to as Size Exclusion Chromatography (SEC) is a mode of liquid chromatography in which the components of a mixture are separated on the basis of size. In GPC-SEC large molecules elute from the column first, followed by smaller molecules. It is an important tool for the analysis of polymers. The essential results are molecular weight data and molecular weight distribution curves which are needed to characterize a polymer with regard to differences in properties. GPC-SEC is mainly used for samples with a molecular weight above 2000 although it is also in use for oligomer separations. There is no upper limit in the molecular weight, even polymer analyses with molecular weights of several millions are possible. Demands on the instrumentation are very stringent due to a special calibration procedure using a linear elution volume on the x-axis versus a logarithmic molecular weight on the y-axis.

Agilent Technologies
Innovating the HP Way

Mechanism

The column packing for GPC-SEC is a rigid or semi-rigid totally porous material with pores of known size. Figure 1 illustrates the mechanism. The pores are conical in shape, which is not necessarily the case in reality. The example shows a mixture which contains three components A, B and C with A being the largest and C being the smallest. As the components are carried through the column by the mobile phase, component A cannot diffuse into the pores, (that is, it is excluded), component B may diffuse approximately halfway into the pores, (that is, it partially permeates) and component C may diffuse all the way into the pores (that is, it permeates totally). Thus the order of elution from the column would be A, then B, and then C.

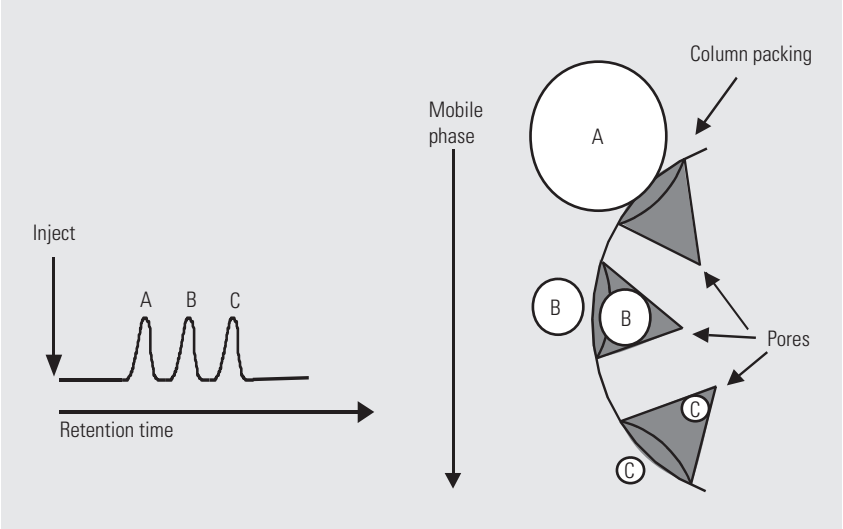

Figure 1
GPC-SEC separation mechanism

Molecular weight correlation: calibration

The separation mechanism in GPC-SEC is based on the size of the molecule when solvated by the mobile phase. A correlation can be made between size and molecular weight. Figure 2 shows that a plot of logM against retention volume is linear for components that selectively permeate the column packing pores. From a calibration plot and the retention volume of the sample, its molecular weight or molecular weight range can be determined.

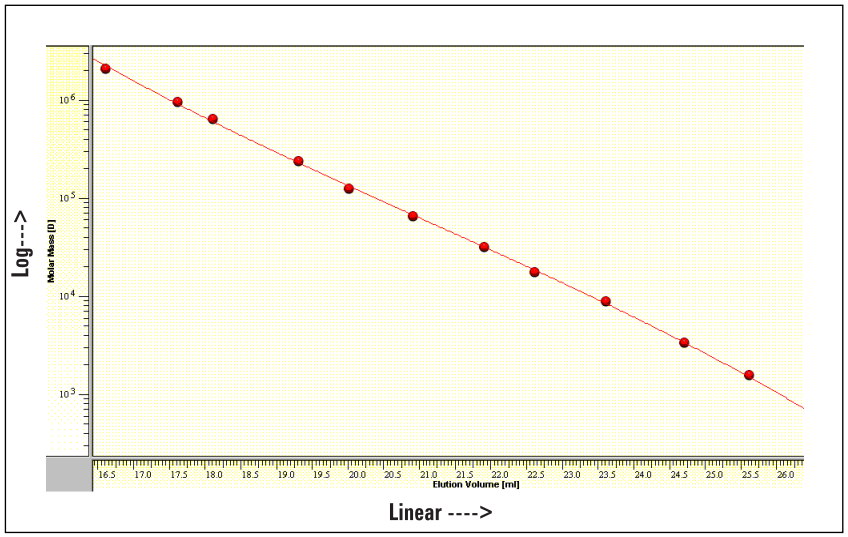

Figure 2
Typical GPC-SEC calibration plot

Molecular weight averages and molar mass distributions

Simple transfer of the sample elution volume into the peak apex molecular weight M_p is not sufficient because it characterizes the sample only in a single point. For better characterization the eluted peak is divided into several equidistant volume slices and the molecular weight averages are calculated, as shown in the equations on the right, where h(M) is the slice height at a molecular weight M. The most important averages are M_n and M_w. M_n provides information on the flexibility and M_w on the strength of the material. The molecular weight averages describe the polymer at different points of the peak. This can also be achieved using traditional techniques such as membrane osmometry or light scattering. GPC-SEC, however, is the only technique which in addition yields the molecular weight distribution. This is a plot of the statistical frequency of molecular weights versus the log of the molecular weight. The molecular weight or molar mass distribution is most important to characterize polymers. The molecular weight averages describe only average properties of the sample. Figure 3 shows the molar mass distributions of three polymers with identical molecular weight averages. The completely different molar mass distributions indicate clearly that they have different properties.

Number average molecular weight: $$M_n = \frac{\sum h(M) \cdot M}{\sum h(M)} = \frac{\sum w(M)}{\sum w(M)/M}$$

Weight average molecular weight: $$M_w = \frac{\sum h(M) \cdot M^2}{\sum h(M) \cdot M} = \frac{\sum w(M) \cdot M}{\sum w(M)}$$

z- average molecular weight: $$M_z = \frac{\sum h(M) \cdot M^3}{\sum h(M) \cdot M^2} = \frac{\sum w(M) \cdot M^2}{\sum w(M) \cdot M}$$

Viscosity average molecular weight: $$M_v = \left(\frac{\sum w(M) \cdot M^a}{\sum w(M)} \right)^{1/a}$$

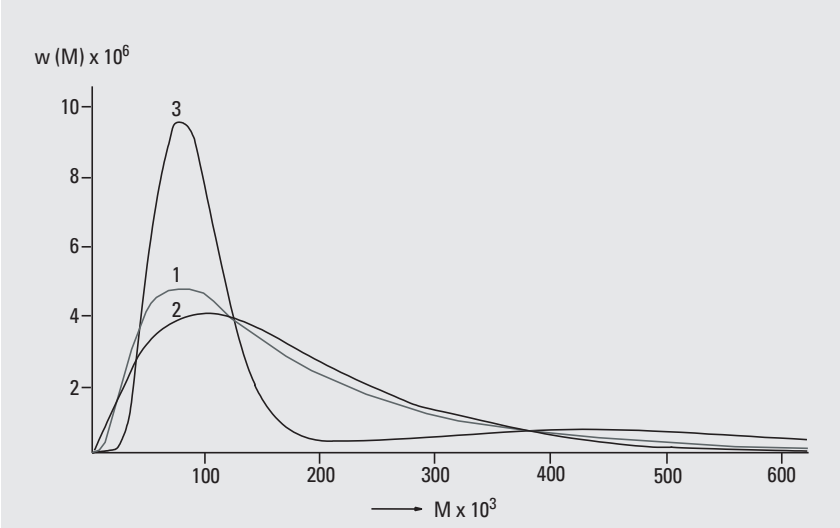

Figure 3
Molar mass distributions of three polymers with the same molecular weight averages

Mobile phase selection

In theory, the mobile phase serves only to dissolve the sample and carry it through the column. In other modes of HPLC, such as partition, adsorption and ion-exchange, there is interaction between the mobile phase and the stationary phase on the column packing and retention can be varied by changing the strength of the mobile phase. In GPC-SEC a change in mobile phase may cause a relatively small change in retention due to a change in hydrodynamic volume of the sample in different mobile phases. Also, a change in mobile phase may cause a change in pore size of the gel packing due to swelling or shrinking of the gel. These changes in retention are very small compared to the changes seen in the other HPLC modes. In GPC-SEC the mobile phase serves only to dissolve the sample and carry it through the column and a change in solvent produces a relatively small change in retention. Therefore, gradient elution is not used.

The mobile phases can be roughly devided into organic and aqueous mobile phases. Tetrahydrofuran(THF) is the most frequently used organic solvent. It is used for a wide range of polymers as polystyrene, poly(methyl metacrylate), epoxy resins, polycarbonate, polyvinylchloride, and polystyreneacrylonitrile. Other solvents include toluene, dimethylacetamide and dimethylformamide. For more information on mobile phases and columns recommended for a wide selection of polymer, see reference 1.

Column packings

Two general types of column packings are available: polymeric gels and silica gels. There are advantages and limitations to both types of packings. Polymeric gels are widely used. Adsorption effects are negligible, however, there are restrictions on solvents that can be used with these gels. Also, the gels can be damaged by pressure "shocks" since they are compressible. The silica packings are more stable physically and are compatible with a wide range of mobile phases. However, adsorption can be a problem with the silica packings unless the surface is deactivated. Highly-crosslinked polystyrene/divinylbenzene particles as packed in the Agilent PLgel columns are among the most widely used columns for polymer separations with organic mobile phases. They are available with different particle and pore sizes to cover a wide range of polymer molecular weight distributions (figure 4). For the analysis of broad distributed polymers one column alone is not sufficient. Such wide ranges usually require sets of several columns, typically between two to three (up to six). For more information on mobile phases and columns recommended for a wide selection of polymers, see reference 1.

An alternative to polystyrene/divinylbenzene based stationary phases are the ZORBAX PSM phases, which are available as small (5 µm) porous microspheres (PSM) in a deactivated and an untreated version. The deactivated version has been silanized for use with non-polar to relatively polar polymers in non-aqueous or partially aqueous solvents. The untreated version is for use with both non-aqueous and aqueous

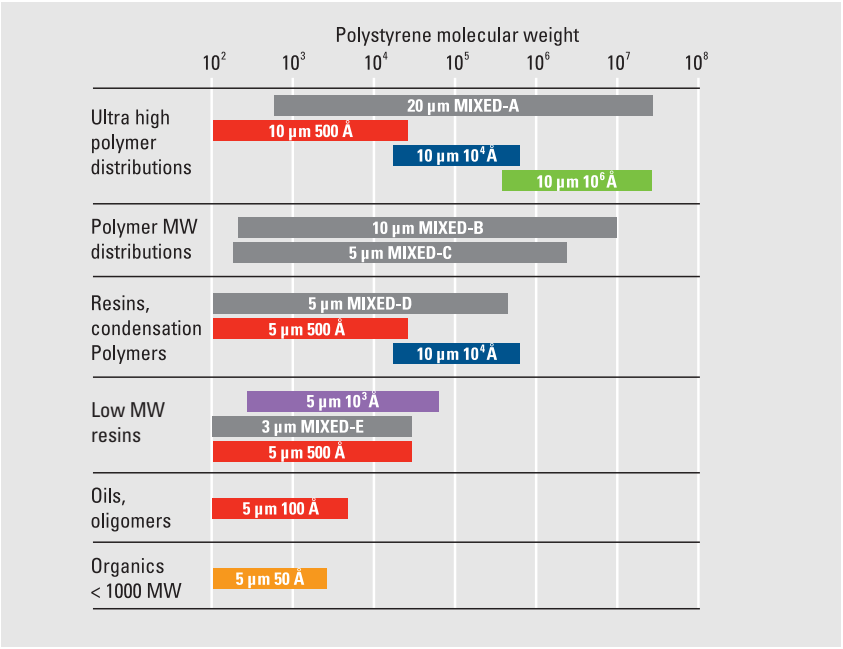

Figure 4
Molecular weight application range of PLgel columns

mobile phases. Dedicated to analyses with aqueous mobile phases are the Agilent PL aquagel-OH columns with their extremely hydrophilic polyhydroxyl surface. They can handle most neutral hydrophilic polymers, and the capability extends to the analysis of high molecular weight polymers (figure 5) including polyacrylamides and polyethylene oxides.

Instrument requirements

Due to the special calibration procedure using a linear elution volume (retention time) on the x-axis versus a logarithmic molecular weight on the y-axis the requirements on hardware and software are very demanding. Accuracy and precision of molecular weight data depends on several hard- and software parameters as listed in table 1.

One of the most important parameters is flow precision. Table 2 shows the strong influence of flow deviations on the weight average molecular weight M_w measured for a polystyrene sample.
The system was calibrated at a flow rate of 1.0 ml/min. When analyzed at exactly this flow rate the M_w value is 35400. Table 2 shows that for a flow deviation of only +0.60 % or +1.30 % errors of 11.0 and even 23.6 % occur.

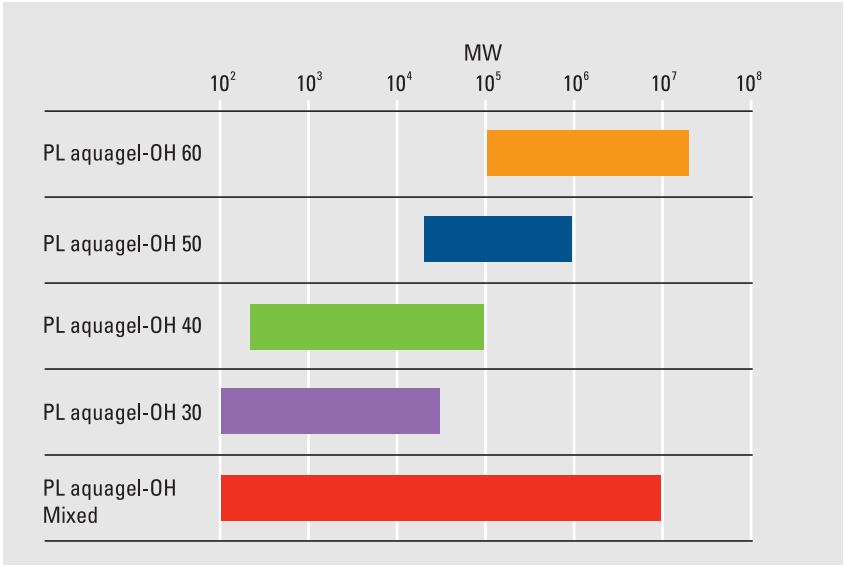

Figure 5
Molecular weight application range of PL aquagel-OH columns

Hardware Parameters	Software Parameters
• Column stability • Precise pump flow with retention time precision < 0.1 % • Column temperature precision ± 0.15 °C • Lowest short-term and long-term noise • Autosampler with low maintenance	• Precision of calculation procedures • Precision of baseline setting • Precision of setting the calculation start and end marks • Number of data points user selectable • Various calibration routines • Automated and interactive data analysis and reporting • Possibility to use an internal standard correction for flow rate changes

Table 1
Hardware and software parameters influencing accuracy and precision of molecular weight data

Flow [ml/min]	Flow deviation [%]	M_w	M_w deviation [%]
1.013	+1.30	43400	+23.6
1.006	+0.60	39300	+11.0
1.0	0	35400	0
0.992	-0.80	31100	-12.2
0.985	-1.50	27700	-21.80

Table 2
Influence of flow variations on molecular weight

Column temperature stability between calibration and sample run is also important. A 4 °C change, as it can easily occur if the column compartment is not thermostated, will create an error of 2.6 %.

On the software side it is important that the software is correctly installed and calculates correctly. State-of-the art GPC-SEC software therefore offers installation verification and system verification routines. The installation verification routine should be performed after installation and later on periodically to prove that all parts are correctly installed. System verification is used to prove that the software is calculating properly. A data file and a calibration file–provided as a protected part of the program–will be processed and a report will be generated as a printout. The GPC raw data from the known sample is processed in exactly the same way as data acquired by the Agilent ChemStation. This ensures that not only the final calculations are verified but also the complete data processing path. The results are then compared to the theoretical results and the system verification test is only passed if results differ less than a specified percentage. Hardware and software parameter effects on accuracy and precision of molecular weight data are discussed further in references 2 and 3.

Refractive index detection is most frequently used for polymer characterization by GPC-SEC. Some polymers, such as polyethyleneoxides, dextrans, celluloses, do not absorb in the UV-visible range.

On the other hand there are several polymers, that can be analyzed with UV-visible detection provided the eluent is transparent and the correct detection wavelength is selected. Examples are aromatic groups containing polymers as polystyrenes or poly(styreneacrylonitrile)s but also polymers without aromatic groups such as poly(methyl methacrylate)s, polybutadienes, polycarbonates, polyamides and polyacrylic acids. Figure 6 shows an overlay of a poly(methyl methacrylate) (PMMA) analysis obtained with refractive index and UV detection. One advantage of dual detection is that the operator receives more information about the sample. The PMMA chromatograms are very similar in the polymer region but show distinct differences in the oligomer region due to the better sensitivity of the UV detector.

If the UV detector is a diode array detector spectra can be acquired during the analysis and used for peak identification and peak purity control. For an example refer to reference 5. A further advantage of UV-visible detection is lower baseline noise and drift. This should have an influence on the accuracy and the precision of the molecular weight data.

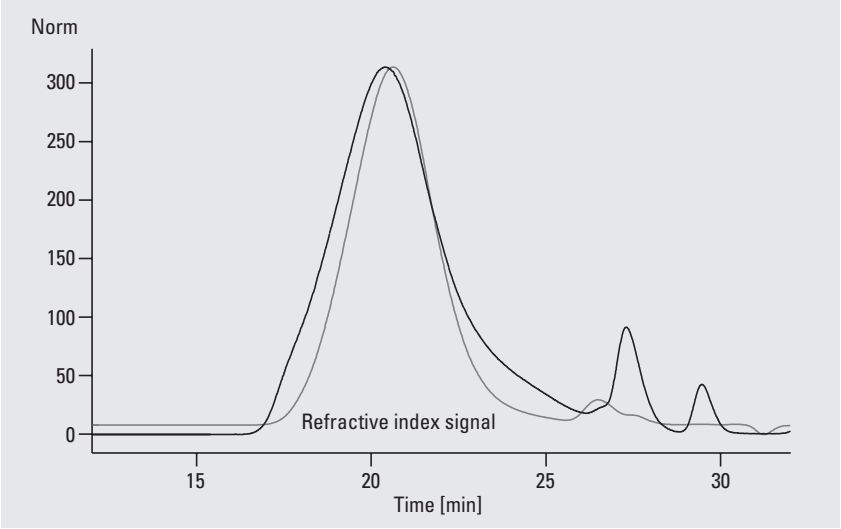

Figure 6
Overlay of poly(methyl metacrylate) chromatograms obtained with UV and refractive index detection

To study this we analyzed a technical polystyrene sample with UV-DAD-and refractive index detection in series. Table 3 shows the average M_n and M_w values and the respective relative standard deviations calculated from 10 automatic analyses.

State-of-the-art refractive detection has significantly improved in terms of baseline noise, wander, drift and automation capabilities. Therefore, the data in table 3 is very similar for the two detectors with some difference in the precision data. The precision data for UV-visible detection is typically better than the refractive index detection data by a factor of approximately two.

	Average value		Precision	
	M_n	M_w	M_n	M_w
Reference value	86000 (GPC)	246000 (light scattering)	-	-
UV-DAD	90700	265000	0.69	0.33
RID	91530	265000	1.24	0.36

Table 3
Comparison of accuracy and precision obtained with UV-DAD and refractive index detection

Conclusion

GPC-SEC is the most widely used technique for the analysis of polymers. It can be used for samples soluble in organic and aqueous eluents and molecular weights from approximately 100 to several million Dalton. In contrast to traditional techniques it yields all molecular weight averages and the molecular weight distribution. To obtain accurate and reliable results the demands on hardware and software are more stringent than for other HPLC modes.

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Printed 10/2000
Publication Number 5988-0110EN



Agilent Technologies
Innovating the HP Way

LC-ICP-MS Connection Kit for Agilent 7500 Series

Technology



- **Combining Agilent's expertise in LC and ICP-MS**
- **Easy switching between coupled and standalone configurations**
- **Optimized sample introduction compatible with standard and capillary LC applications**
- **Robust plasma capable of handling any organic mobile phase or gradient, including acetonitrile**
- **High ion transmission delivers high sensitivity even at capillary LC flow rates (20 mL/min)**
- **High stability electronics and mass analyzer for excellent long-term reproducibility**
- **Fully compatible with Agilent Plasma Chromatographic software for real-time data analysis**
- **Allows routine and overnight running of integrated LC-ICP-MS analyses**
- **Routine detection and quantification of elemental species**
- **Isotope analysis capability of ICP-MS enables isotope dilution and isotope tracer studies to be performed**

Why LC-ICP-MS?

The measurement capability of existing liquid chromatography (LC) detectors may be limited in terms of sensitivity or specificity. Current and future applications are likely to require the analysis of inorganic species and organometallic compounds at ever lower concentrations, so alternative detection systems are necessary. Inductively coupled plasma mass spectrometry (ICP-MS) provides good selectivity (element specific analysis and even isotopic information) and ultra-trace detection limits for most elements.

Samples are introduced into a high-temperature argon plasma where they are decomposed, atomized, and ionized. Ions are introduced into the mass spectrometer for detection and identification. ICP-MS provides information regarding the total metal concentrations in a sample. When used in combination with a front-end separation technique such as LC, ICP-MS becomes a highly sensitive detector that can be used for a variety of speciation applications. LC-ICP-MS allows for the simultaneous separation and measurement of a variety of species/compounds in a single analytical run.

The LC-ICP-MS Connection Kit

The Agilent LC-ICP-MS Connection Kit contains all the components required to easily combine the Agilent 1100 LC with the Agilent 7500 ICP-MS. It includes all the necessary fittings, tubing, and cables for complete synchronization of the LC and ICP-MS. An internal standard can be added post-column via the 7500 on-board pump. This provides additional flexibility and allows continuous point-by-point correction to provide the ultimate in data quality.

Fully Integrated LC-ICP-MS Analysis

The Agilent 7500 Series sample introduction, robust plasma system, and interface can be configured to handle organic samples on a routine basis, without high plasma or interface loading. Typical mobile phases, such as methanol or 65% acetonitrile, can be introduced to the ICP-MS over extended periods. The column eluent is directed into the nebulizer/spray chamber via a connecting block. Using this configuration, the ICP-MS becomes a very sensitive elemental detector for the LC.

See Figure 1 for a schematic of the LC-ICP-MS system.

In a typical analysis, an ICP-MS sequence is created containing information on calibration standards, unknown samples, and any QC samples, such as those used for recalibration of retention times. The LC sequence controls sample injection and then sends a "start" signal to the ICP-MS. After data collection, the Plasma Chromatographic Software (Plasma Chrom) automatically locates and integrates the peaks and generates quantitative results for each compound identified in the sample, based upon a response curve generated from the standards analyzed. A quantitative analysis report can be printed automatically in real-time during the sequence, or the data can be manipulated (for example, using different integration parameters) and quantified offline at a later date.

Ease of Use

Chromatographic data analysis is conducted via the optional Plasma Chrom module of the Agilent 7500 ChemStation software suite. The software enables, for the first time, the analysis of chromatographic data in real-time. Based on Agilent's renowned ChemStation chromatographic software, Plasma Chrom incorporates all of the

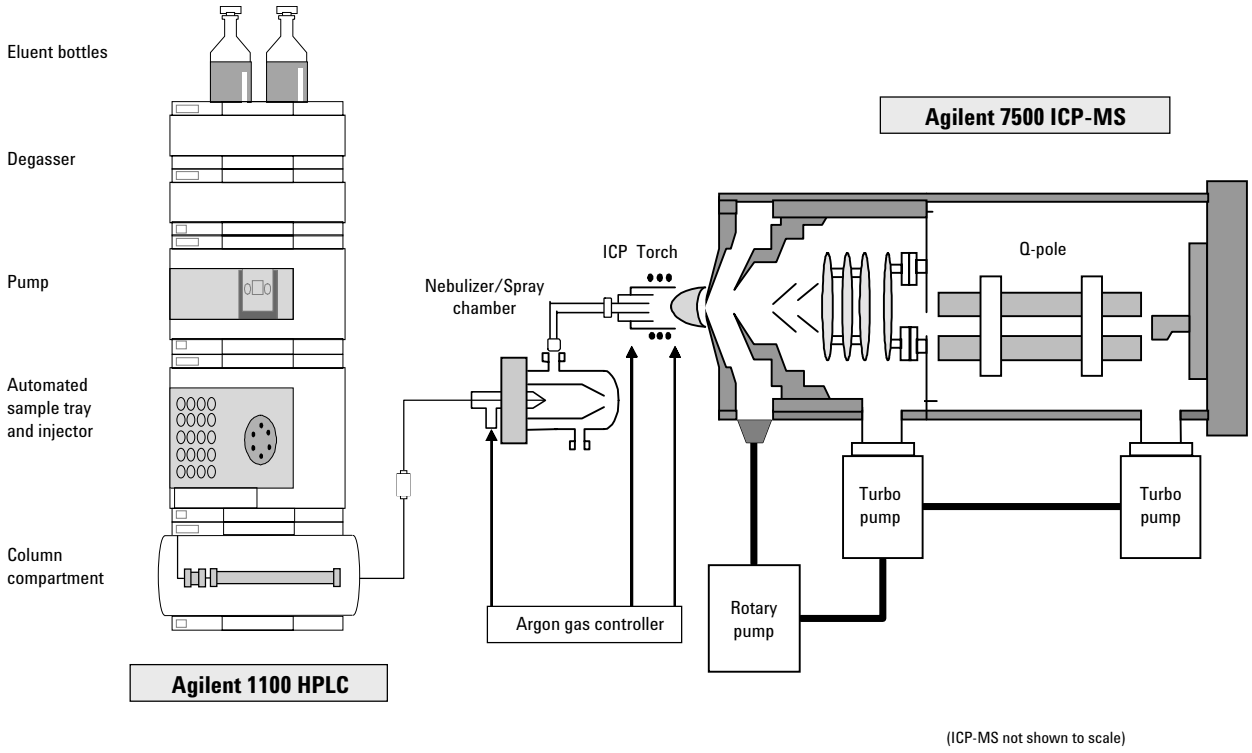

Figure 1. Schematic of Agilent 1100 HPLC coupled to Agilent 7500 ICP-MS.

features that chromatographers expect, such as real-time quality control (QC), advanced peak integration routines and confirmation of target analytes. Moreover, operation of the fully integrated Agilent LC-ICP-MS system is easy, making it suitable for both an R&D setting and for routine use.

Applications

Agilent has developed the fully automated LC-ICP-MS interface in response to the demand for routine and overnight running of analyses in the environmental, clinical, nutritional, bio/pharmaceutical research, and quality control areas. LC-ICP-MS is applicable wherever the quantification of different species, forms, oxidation states, or biomolecules associated with trace elements is required. The ICP-MS adds the capacity to measure isotopic composition, so isotope ratio measurements, isotope dilution analysis, isotopic spike recovery, and tracer studies can be carried out.

Figure 2 displays a series of chromatograms obtained from a 12-hour long-term stability study (20 overlaid chromatograms, obtained from separate visits to sample vials during the 12 hours). The sample was a mixed organotin solution containing diphenyltin (DPhT), dibutyltin (DBT), triphenyltin (TPhT), and tributyltin (TBT), each at 2 ppb, running running with an acetonitrile (65%), acetic acid (10%) mobile phase (minor contaminants can be observed just before the tributyltin (TBT) peak). No reoptimization or retention time calibration was performed. The excellent stability and reproducibility of the system is clearly demonstrated.

Conclusions

Agilent's LC-ICP-MS is opening up new possibilities for speciation measurement. The long-term reproducibility of the Agilent system will enable, for the first time, the study of species interconversion and equilibria within a given sample matrix. This has far reaching implications in terms of the development of new speciation standards and the validation of speciation measurement.

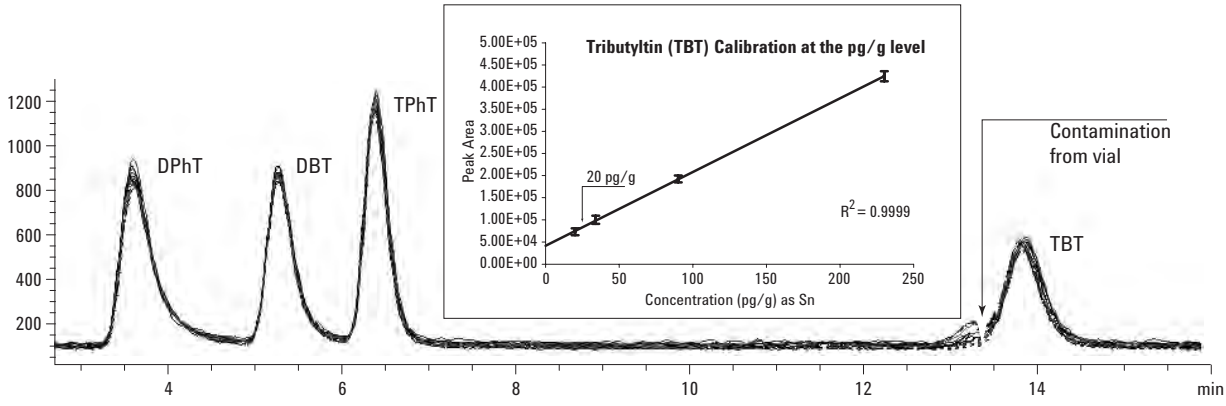

Figure 2. Twenty overlaid chromatograms showing 12-hour long-term stability for a mixed organotin standard running acetonitrile/acetic acid mobile phase. Included is a low-level calibration for tributyltin. Data courtesy of LGC (Teddington) Ltd, UK.

Ordering Information for the Agilent LC-ICP-MS Connection Kit

Description	Part no.
LC-ICP-MS Connection Kit	G1833-65200
Kit contents:	
Sample tubing	
Union joint	
Tee joint	
Connectors	
Ferrules	
APG remote cable	

Required 1100 Series LC Configuration for:

Automated analysis

Description	Order code
Agilent 1100 Series HPLC	
Iso pump	G1310A
Autosampler	G1313A
Control module	G1323B

Manual analysis

Description	Order code
Agilent 1100 Series HPLC	
Iso pump	G1310A
Manual injector assembly	G1328B
Control module	G1323B

Note 1: If the 1100 LC is controlled by a standalone ChemStation PC, then it is not necessary to order the G1323B Control Module.

Note 2: Additional optional items, not included in the LC connection kit, are required for the analysis of organic mobile phases.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

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Printed in the USA
November 21, 2001
5988-4393EN



Agilent Technologies

ZORBAX Eclipse XDB HPLC Columns

The "Perfect Fit" for Developing Better HPLC Methods

Technical Overview

- Excellent peak shape for basic, acidic or neutral compounds
- High performance over a wide pH range
- Rugged, reproducible chromatography from column-to-column and lot-to-lot
- More selectivity options for method development

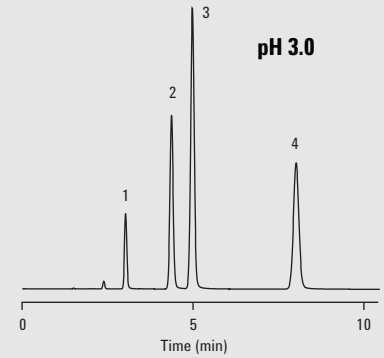
More and more chromatographers are developing their analytical and LC/MS separations on ZORBAX Eclipse XDB HPLC columns. Why? Because ZORBAX Eclipse XDB columns solve many of their separation challenges. In fact, Agilent Technologies includes a 4.6 x 150 mm, 5 µm ZORBAX Eclipse XDB-C8 column with every Agilent 1100 HPLC instrument it delivers. As Figure 1 shows, ZORBAX Eclipse XDB columns, specifically designed to extend column life and provide excellent peak shape for basic compounds in the pH range of 6 - 9, also deliver outstanding performance at low pH, as well.



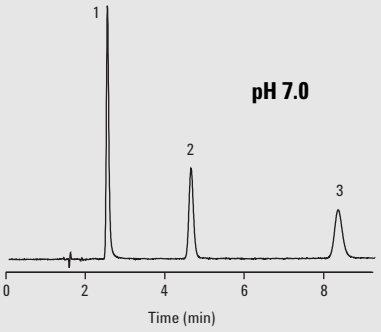
More detailed information about the benefits of the Eclipse column family can be found in this brochure.

Figure 1
Good Peak Shape for Acids, Bases and Neutrals at Low and Intermediate pH

ZORBAX Eclipse XDB-C18, 4.6 x 150 mm, 5 µm
(Agilent Part No. 993967-902)



Mobile Phase:	80% 25 mM Na_2HPO_4, pH 3.0 20% Methanol
Flow Rate:	1.0 mL/min
Temperature:	35°C
Sample:	1. Theobromine 2. Theophylline 3. 1,7-dimethylxanthine 4. Caffeine



Mobile Phase:	70% 25 mM Na_2HPO_4, pH 7.0 30% Methanol
Flow Rate:	1 mL/min
Temperature:	RT
Detection:	UV 254 nm
Sample:	1. Procainamide 2. N-acetylprocainamide 3. N-propionylprocainamide

Eclipse XDB HPLC Column Technology Provides:

Excellent Peak Shape



eXtra Dense Bonding + High Purity Silica = Excellent Peak Shape

eXtra Dense Bonding is key to the exceptional performance of ZORBAX Eclipse XDB columns at intermediate pH (Figure 2). This dense bonding is accomplished by adding an extra-dense monolayer of C18, C8 or Phenyl silane to the ultra-pure, fully-hydroxylated, ZORBAX Rx-silica surface. The packing is then endcapped not once, but twice, using two different and unique endcapping reagents. This combination of extra-dense surface coverage by the bonded phase and double endcapping produces a highly, deactivated stationary phase that virtually eliminates undesirable interactions between polar solutes and the silica surface. As a result, superior peak shape, high efficiency, and long-term chromatographic reproducibility are assured when using Eclipse XDB HPLC columns at both intermediate and low pH.

The exceptional and reproducible performance at low pH that you can expect from the Eclipse XDB is demonstrated in Figure 3. In this example, acetylsalicylic acid, and the base, dextromethorphan, are consistently separated with excellent peak shape at low pH on three different Eclipse XDB-C8 columns from three different lots of packing material.

Figure 2
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 7

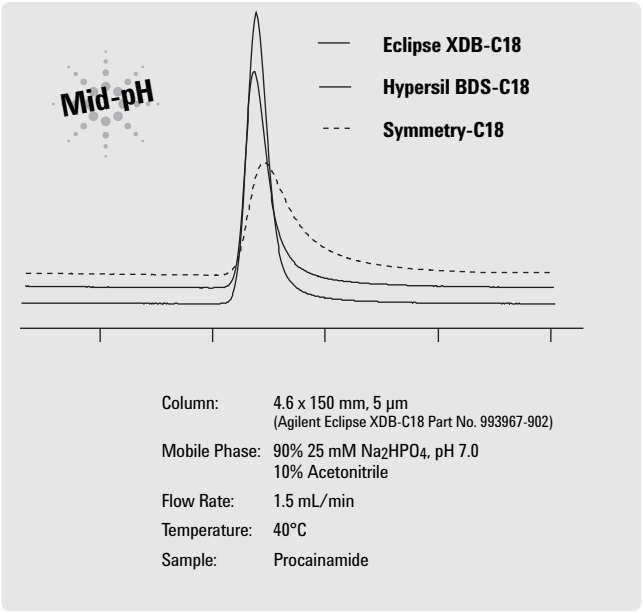
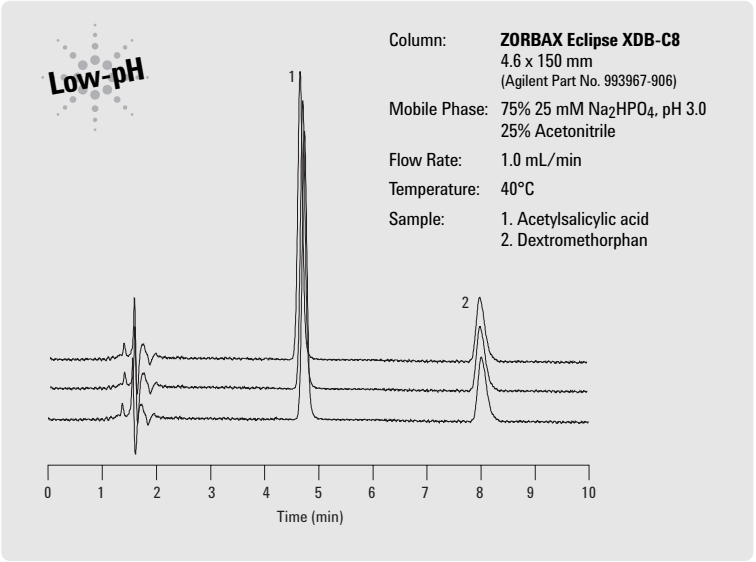


Figure 3
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 3



Eclipse XDB HPLC Column Technology Provides:

Long Column Life



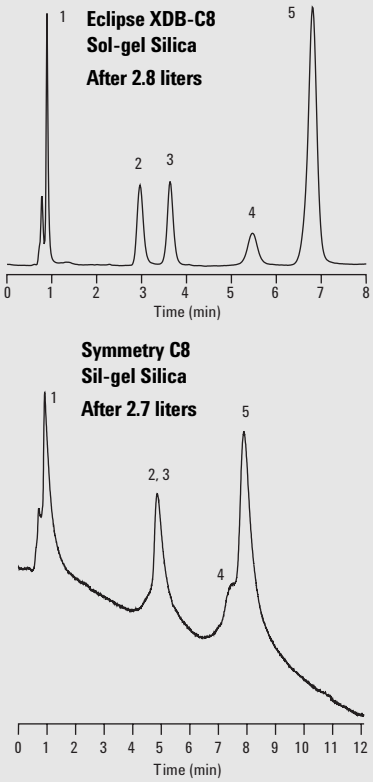
Eclipse XDB columns not only provide excellent peak shape but they are also exceptionally durable. In fact, the spherical ZORBAX Rx-SIL particles are the most durable, porous, 5 and 3.5 µm, silica particles commercially available. They are manufactured using a patented and proprietary process, forming thick, hard-walled silica, commonly referred to in the literature as "sol-gel" silica.

Because of the strength of the ZORBAX particle, all ZORBAX columns are consistently and reliably packed at pressures exceeding 8000 psi. The result is a durable column that can easily tolerate pressures up to 5000 psi in regular use without a loss in efficiency or a reduced lifetime.

Long Column Life at Intermediate pH

This thick, hard-walled "sol-gel" silica resists dissolution at intermediate pH and when densely bonded, the resultant Eclipse XDB column provides excellent column performance and increased column lifetime, even under the stressed intermediate pH conditions described in Figure 4.

Figure 4
Accelerated Column Aging Study Demonstrates the Durability of Eclipse XDB-C8 Over Waters' Symmetry C8



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase:
20% Acetonitrile
80% 250 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

Temperature: 60°C

Test Conditions:

Mobile Phase:
60% Acetonitrile
40% 10 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

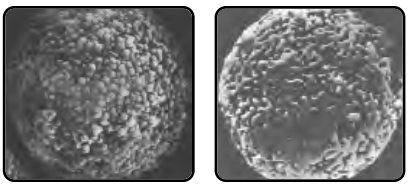
Temperature: 40°C

Sample: Tricyclic Antidepressants
1. Uracil 2. Nortriptyline 3. Doxepin
4. Amitriptyline 5. Trimipramine

Many commercial, base-deactivated, silica-based HPLC columns use manufacturing processes that produce a less robust "sil-gel" silica particle. The walls of these resulting high-surface area materials (typically 300 m^2/g for an 80-100Å pore material) are thinner and less uniform and can easily crush under high pressure conditions. Moreover, in many cases, they fail to withstand the high pressures of high flow rate LC/MS and High-Throughput methods.

The Eclipse XDB thick, hard-walled "sol-gel" is compared to the thin-walled "sil-gel" silica, used to make most of today's base-deactivated products, in electron micrographs shown in Figure 5.

Figure 5
Eclipse XDB is Based on Thick, Hard-Walled, Sol-gel Silica



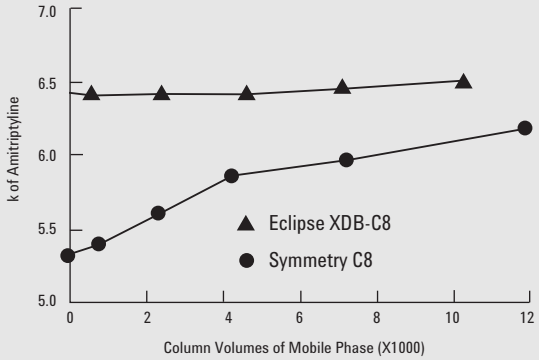
Sol-gel **Sil-gel**

Eclipse XDB columns are made with patented "hard wall" sol-gel silica particles. The thick walls of this silica are more resistant to dissolution than the "thin wall" sil-gel silica that is used to make most base-deactivated columns.

Long Column Life at Low pH

At low pH, Eclipse XDB columns provide better column life than most commercially available reversed-phase HPLC columns. Figure 6 summarizes the results from a low-pH accelerated aging study where loss in column performance is measured by a loss in bonded phase, indirectly measured by the change in retention of amitriptyline. The results show that less than 3% of the Eclipse XDB column performance is lost when exposed to 12,000 mL of a pH 3 mobile phase at 60°C. Under these same conditions more than 14% of column performance is lost on another popular "deactivated" reversed-phase HPLC column.

Figure 6
Accelerated Column Aging Study Demonstrates the Durability of ZORBAX Eclipse XDB-C8 Over Waters' Symmetry



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.5 mL/min

Temperature: 60°C

Retention Test Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.0 mL/min

Temperature: 60°C

Sample: Amitriptyline

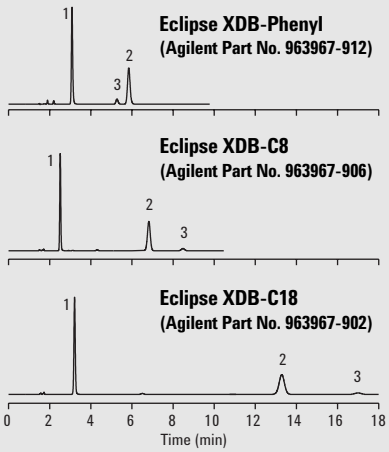
Eclipse XDB HPLC Column Technology Provides:

More Selectivity Options C18, C8 and Phenyl

Eclipse XDB HPLC columns are available as C18, C8 and Phenyl bonded phases. The Eclipse XDB-Phenyl phase complements both the most retentive Eclipse XDB-C18 and the moderately retentive Eclipse XDB-C8. The Eclipse XDB-Phenyl offers unique selectivity as well as reduced retention of non-polar and moderately polar compounds while maintaining retention of polar analytes.

These benefits are illustrated in Figure 7. Sunscreen components are well retained on the Eclipse XDB-C18, although the analysis time is long. Analysis time is reduced with more than acceptable resolution when using the Eclipse XDB-C8 or Eclipse XDB-Phenyl column, where analysis time is shortened by 50 or 61 percent, respectively.

Figure 7
Eclipse XDB-Phenyl Columns Offer Unique Selectivity and Short Analysis Times



Column: **ZORBAX Eclipse XDB**
4.6 x 150 mm, 3.5 µm

Mobile Phase: 85% Methanol
15% Water

Flow Rate: 1.0 mL/min

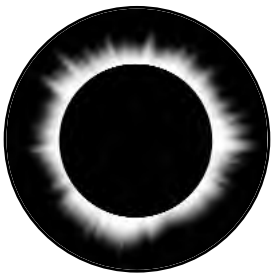
Temperature: 30°C

Detection: UV 310 nm

Sample: 1. Oxybenzone
2. Padimate O
3. Ethylhexyl Salicylate

Eclipse XDB HPLC Column Technology Provides:

Rapid Resolution

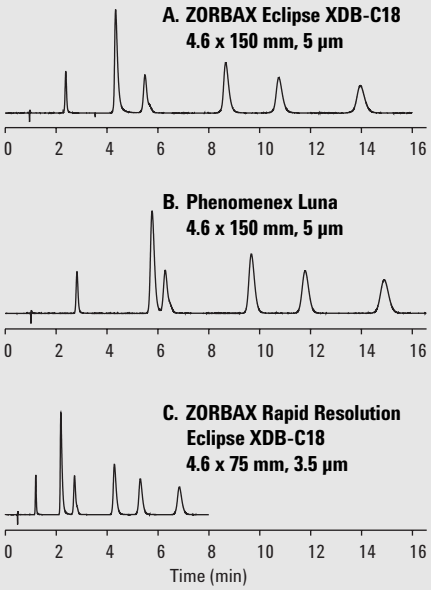


Eclipse XDB columns, available in 5 and 3.5 µm particle size packings, are highly efficient. In Figure 8A, six basic antidepressant compounds are well separated on a 5 µm, 4.6 x 150 mm, Eclipse XDB-C18 column using a methanol-phosphate mobile phase at pH 7. Peaks elute in sharp bands having average peak widths at half-height of 0.19 minutes. When the same sample was run on the Phenomenex Luna column, resolution between nortriptyline and doxepin ($R_{2,3}$) decreased significantly, with average peak widths at half-height being 47% wider, averaging 0.28 minutes for this assay.

For faster analysis, shorter Rapid Resolution Eclipse XDB columns, packed with 3.5 µm particles, provide equally efficient separations with reduced analysis times. This is demonstrated in Figure 8C, where analysis time is decreased by 50% — as retention decreases from 15.6 to 6.8 minutes — while resolution is maintained for this highly basic antidepressant sample.

If you have any questions about this or other applications in this bulletin, call 800-227-9770 select option 4 and ask for HPLC column technical support.

Figure 8
Eclipse XDB Provides High Efficiency and Rapid Resolution



Mobile Phase: 73% Methanol
27% 50 mM Phosphate, pH 7
Flow Rate: 1.5 mL/min.
Temperature: 40°C
Sample: Antidepressants
1. Imipramine
2. Nortriptyline
3. Doxepin
4. Doxylamine succinate
5. Amitriptyline
6. Cloripramine

Developing reliable reversed-phase methods for basic, acidic and neutral compounds just got easier . . .

Order Your ZORBAX Eclipse XDB HPLC Columns today!

ZORBAX Eclipse XDB Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse XDB-C18	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	10%
ZORBAX Eclipse XDB-C8	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.6%
ZORBAX Eclipse XDB-Phenyl	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.2%

Symmetry® is a registered trademark of Waters Corporation.
Luna® is a registered trademark of Phenomenex.

ZORBAX Eclipse XDB Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11
Standard Columns and Bulk Packings					
Semi-Prep	9.4 x 250	5	990967-202	990967-206	
Analytical	4.6 x 250	5	990967-902	990967-906	990967-912
Analytical	4.6 x 150	5	993967-902	993967-906	993967-912
Analytical	4.6 x 50	5	946975-902	946975-906	
Rapid Resolution	4.6 x 150	3.5	963967-902	963967-906	963967-912
Rapid Resolution	4.6 x 100	3.5	961967-902	961967-906	
Rapid Resolution	4.6 x 75	3.5	966967-902	966967-906	966967-912
Rapid Resolution	4.6 x 50	3.5	935967-902	935967-906	935967-912
Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312
Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312
Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312
Solvent Saver Plus	3.0 x 100	3.5	961967-302	961967-306	961967-312
Solvent Saver Plus	3.0 x 75	3.5	966954-302		
Narrow Bore	2.1 x 150	5	993700-902	993700-906	993700-912
Narrow Bore	2.1 x 50	5	960967-902	960967-906	960967-912
Narrow Bore RR*	2.1 x 150	3.5	930990-902	930990-906	
Narrow Bore RR	2.1 x 100	3.5	961753-902	961753-906	
Narrow Bore RR	2.1 x 75	3.5	966735-902		
Narrow Bore RR	2.1 x 50	3.5	971700-902	971700-906	
MicroBore RR	1.0 x 150	3.5	963600-902	963600-906	
MicroBore RR	1.0 x 50	3.5	965600-902	965600-906	
MicroBore RR	1.0 x 30	3.5	961600-902	961600-906	
Bulk Packing, 2 grams		5	920966-902		
Guard Cartridges, 4/pk	4.6 x12.5	5	820950-925	820950-926	820950-927
Guard Cartridges, 4/pk	2.1 x12.5	5	821125-926	821125-926	821125-926
Guard Hardware Kit			820777-901	820777-901	820777-901
Agilent Cartridge Columns					
Analytical	4.6 x 250	5	7995118-585	7995108-585	
Analytical	4.6 x 150	5	7995118-595	7995108-595	
Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344	
Solvent Saver	3.0 x 75	3.5	7995230-344		
Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504	
Cartridge Holder			5021-1845	5021-1845	
High Throughput Cartridge Columns (requires Hardware Kit 820222-901)					
Rapid Resolution Cartridge	4.6 x 30	3.5	933975-902	933975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	933975-932	933975-936	
Rapid Resolution Cartridge	4.6 x 15	3.5	931975-902	931975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	931975-932	931975-936	
Rapid Resolution Cartridge	2.1 x 30	3.5	973700-902	973700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	973700-932	973700-936	
Rapid Resolution Cartridge	2.1 x 15	3.5	975700-902	975700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	975700-932	975700-936	
Hardware Kit for High Throughput Columns			820222-901	820222-901	
CombiHT Columns (end fittings required)					
CombiHT	21.2 x 150	5	970150-902	970150-906	
CombiHT	21.2 x 100	5	970100-902	970100-906	
CombiHT	21.2 x 50	5	970050-902	970050-906	
CombiHT End Fittings (2) (required for use)			820400-901	820400-901	
Capillary Glass-lined Columns					
Capillary	0.5 x 250	5	5064-8286		
Capillary	0.5 x 150	5	5064-8287		
Capillary RR	0.5 x 150	3.5	5064-8288		
Capillary RR	0.5 x 35	3.5	5064-8298		
Capillary	0.3 x 250	5	5064-8269		
Capillary	0.3 x 150	5	5064-8291		
Capillary RR	0.3 x 150	3.5	5064-8271		
Guard Cartridges	0.5 x 35	5	5064-8296		
Guard Cartridges	0.3 x 35	5	5064-8297		

*RR: Rapid Resolution 3.5 µm columns.

For more information on these and other columns consult the Agilent web site at **www.agilent.com.**

Configurations not shown are available upon request.



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For the latest information on the complete line of Agilent Technologies columns and supplies for analytical instruments, see our online catalog at www.agilent.com/chem on the World Wide Web, or contact your local Agilent sales office. For all other areas contact Agilent or your local authorized distributor.

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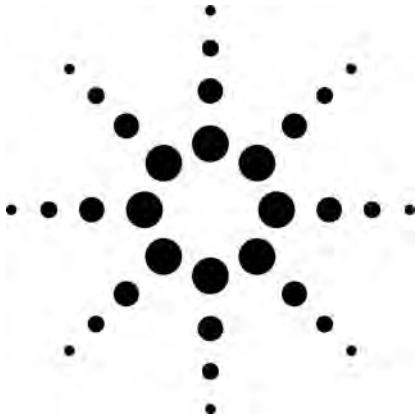
Printed in the USA
December 3, 2002
5988-8367EN

GS-OxyPLOT: A PLOT Column for the GC Analysis of Oxygenated Hydrocarbons

Technical Overview



Allen Vickers

Introduction

GS-OxyPLOT is a porous layer open tubular (PLOT) column. The stationary phase is a proprietary, salt deactivated adsorbent with a high chromatographic selectivity for low molecular weight oxygenated hydrocarbons. It is designed for and ideally suited for application in the ASTM methods listed in Table 1. It is an appropriate replacement for Varian's CP-LowOx column, usually with little to no changes in analytical parameters. This column is particularly useful for the trace analysis of oxygenates such as those listed in Table 2. Other oxygenated hydrocarbons are also suitable for analysis with this column subject to limitations given below.

The column can be used as a single, primary analytical separation column for oxygenated compounds. In complex sample matrices that have high molecular weight species (ca. 300 mol. wt. and higher) and/or species with insufficiently high vapor pressure to migrate through the GS-OxyPLOT, this column can be used in multidimensional GC systems with other columns that have vastly different polarity and lower selectivity toward oxygenated hydrocarbons. For example, a nonpolar DB-1 column can be used as an injection precolumn to retain low volatility solutes, allowing the less retained, polar oxygenated solutes to move into the GS-OxyPLOT. Since the stationary phase of GS-OxyPLOT is an oxygenate adsorbent phase, the oxygenates that enter the column are trapped. As the GC oven temperature is increased, the oxygenates will begin to migrate and are separated in the column prior to detection.

When first installed, the GS-OxyPLOT should be conditioned at 300 °C for at least 3 hours. Experience has shown that this column has an infinite shelf life, but when the column has not been in use for extended periods of time, longer conditioning times of 8 hours or more may be required to obtain retention time stability. The column can be stored with septa placed over the ends of the column, returned to the original column box, and stored at normal ambient temperatures for future use.

GS-OxyPLOT has a minimum temperature limit of 0 °C, an isothermal maximum temperature limit of 300 °C, and an oven program maximum temperature of 350 °C. Because the stationary phase is a strong adsorbent for polar compounds, especially water, it is recommended that when the column is installed in a GC, but idle, that the GC oven be set to an isothermal temperature of 220 °C with normal carrier gas flow, so that the instrument can be brought back into operation quickly when samples are ready to be analyzed. Otherwise, if the column is left at low oven temperatures, it may require reconditioning at 300 °C for several hours to obtain stable retention times.

Saturated hydrocarbon solutes have virtually no interaction with the GS-OxyPLOT and elute from the column so long as the column temperature is hot enough to induce a high enough vapor pressure for the solute to move in the carrier gas. Normal alkanes up to C_{18} will elute from GS-OxyPLOT within the program temperature maximum limit of the column. Because of the highly polar character of the GS-OxyPLOT phase, as would be

expected for oxygenate-selective PLOT column, the column has a relatively low sample load capacity for these nonpolar solutes. The low sample loading capacity is manifested chromatographically as a tailing peaking, indicative of phase overload in GS-OxyPLOT columns. Unsaturated hydrocarbons and aromatic hydrocarbons have relatively high retention. Injection of these organic compound classes should be limited to organic compounds with 11 carbons or less to prevent the column from fouling. As with the normal alkanes, the alkyl benzenes will show phase overloading at relatively low concentrations.

While GS-OxyPLOT is an ideal analytical solution for low molecular weight, oxygenated hydrocarbons, like all other similar oxygenate-selective PLOT columns,it is not recommended for higher molecular weight alkenals (e.g., 1-hexenal and 1-ocetenal). The combined interaction of the unsaturated and carbonyl functional groups can instigate tailing due to strong interactions and in some cases reaction between the phase and solutes.

Table 1. ASTM Standardized Methods for Which GS-OxyPLOT Is Specifically Designed

ASTM Method D7059	Determination of Methanol in Crude Oils by Gas Chromatography with Flame Ionization Detection
Proposed ASTM Method	Determination of C_1 to C_5 Oxygenates at Trace Levels in High Ethanol Content Gasoline Streams by Multidimensional Chromatography with Flame Ionization Detection*
Proposed ASTM Method	Determination of Oxygenates in Ethene, Propene, and C_4 and C_5 Hydrocarbon Matrices by Gas Chromatography and Flame Ionization Detection*

*These are "proposed methods" (i.e., do not have method designation numbers) that are destined for approval by ASTM Committee D2. These methods have already been accepted by, and are being implemented in, petrochemical refineries around the world.

Table 2. Examples of Oxygenated Compounds Suitable for GC Analysis Using the GS-OxyPLOT Column

1. Dimethyl Ether	13. Acetone
2. Diethyl Ether	14. Isovaleraldehyde
3. Acetaldehyde	15. Valeraldehyde
4. Ethyl t-Butyl Ether	16. Methyl Ethyl Ketone
5. Methyl t-Butyl Ether	17. Ethanol
6. Diisopropyl Ether	18. 1-Propanol
7. Propionaldehyde	19. Isopropyl Alcohol
8. tert-Amyl Methyl Ether	20. Allyl Alcohol
9. Propyl Ether	21. Isobutyl Alcohol
10. Isobutraldehyde	22. tert-Butyl Alcohol
11. Butylaldehyde	23. sec-Butyl Alcohol
12. Methanol	24. n-Butyl Alcohol
	25. 2-Methyl-2-Pentanol

Ordering Information for the GS-OxyPLOT Column

ID (mm)	Length (m)	Film Thickness (µm)	Temperature Limit (°C)	Cage Size	Part Number
0.53	10	10	350	7″	115-4912
0.53	10	10	350	5″	115-4912E

References

1. A. K. Vickers, “A ‘Solid’ Alternative for Analyzing Oxygenated Hydrocarbons—Agilent’s New Capillary GC PLOT Column,” Agilent Technologies publication 5989-6323EN, Feb 2006.
2. A New Megabore GC Column for the Adsorption and Chromatographic Separation of Oxygenates in Hydrocarbon Matrices, poster, Pittcon07-27.
3. Analysis and Chromatographic Separation of Oxygenates in Hydrocarbon Matrices, Power Point presentation, Pittcon07-20.

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Printed in the USA
March 9, 2007
5989-6447EN



Agilent Technologies

Successful Detection Using the Agilent 255 Nitrogen Chemiluminescence Detector (255 NCD)

Technical Overview

Introduction

The Agilent 255 Nitrogen Chemiluminescence Detector (255 NCD) can easily detect organic compounds containing nitrogen after conversion of the compounds to nitric oxide.

Frequently, chemists at Agilent are asked what nitrogen compounds are detected by the 255 NCD. Successful detection of nitrogen-containing compounds requires the conversion of these compounds to nitric oxide. The 255 NCD stainless steel burner converts nitrogen compounds to nitric oxide in a hydrogen and oxygen plasma at temperatures greater than 1800 °C.

The 255 NCD can easily detect organic compounds containing nitrogen after conversion of the compounds to nitric oxide. The stainless steel burner can also convert inorganic compounds such as ammonia and hydrazine to nitric oxide. The nitric oxide from the stainless steel burner reacts with ozone in the chemiluminescence reaction cell to produce a chemiluminescence reaction. A red optical filter allows transmission of the light from the chemiluminescence nitrogen reaction while suppressing chemiluminescence signals from other chemical species.

The selectivity of the 255 NCD results from the fact that not all compounds exhibit chemiluminescence when mixed with ozone. Also, the stainless burner cannot convert all compounds to nitric oxide.

Some compounds giving little or no response with the 255 NCD include carbon dioxide, water, nitrogen, oxygen, noble gases, and chlorinated hydrocarbons. These compounds represent the major constituents of many sample matrices. None of these compounds interfere significantly with the 255 NCD and determination of trace levels of the nitrogen-containing analytes.

Examples of Compounds Detected by the 255 NCD

- Amines
- Carbazoles
- Indoles
- Nitro-compounds
- Nitriles
- Nitrosamines
- Pyridines
- Quinolines
- Ammonia
- Hydrazine
- Hydrogen cyanide
- Nitric oxide, NO
- Nitrogen dioxide, NO_2
- NO_X

Compounds Not Detected by the 255 NCD

- Carbon dioxide
- Nitrogen gas
- Water
- Hydrocarbons

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Printed in the USA
May 17, 2007
5989-6770EN



Agilent Technologies

Agilent Model 255 Nitrogen Chemiluminescence Detector (NCD) Analysis of Adhesive Samples Using the NCD

Technical Overview

Introduction

The Agilent Model 255 NCD can easily replace a NPD for the analysis of 2-pyrrolidone and 1-vinyl-2-pyrrolidinone in adhesive samples.

A short-term evaluation was performed for the analysis of adhesive samples for two of the starting materials, 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. According to government regulations, the concentration of 1-vinyl-2-pyrrolidinone cannot exceed 800 ppm in the final product.

The primary objective for the analysis of adhesive was to demonstrate the repeatability and stability of the Model 255 NCD. The secondary objective of the study was to prove the NCD could replace the nitrogen-phosphorus detector (NPD) being used for the application. Table 1 compares the NCD and NPD.

The results from the evaluation demonstrated the Model 255 NCD could easily replace a NPD to monitor 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. The gas chromatograph and the Model 255 NCD were calibrated once and not recalibrated again during the 3-day demonstration. Representative precision data are shown in Tables 2 and 3. The gas chromatograph made over 160 injections of the adhesive samples during the 3-day evaluation with the percent relative standard deviation for the Model 255 NCD of less than 4%. The Model 255 was also linear from 20 to 3000 ppm. Figures 1 and 2 illustrate chromatographic response at high and low levels, respectively. Unlike an NPD, the sample matrix did not affect the detector performance.

For the 2-pyrrolidone and 1-vinyl-2-pyrrolidinone analysis, the NCD provides very good short-term and long-term precision. The NCD is unaffected by high levels of the sample matrix, and its use would require less day-to-day maintenance than an NPD. Use of the Model 255 NCD versus an NPD would result in more accurate and precise results and would reduce the level of instrument maintenance required.

Table 1. Comparison of Agilent NCD to NPD

	Agilent 255 NCD	NPD
Response	Equimolar	Non-equimolar
Quenching	No	Yes
Selectivity	> 107 gN/gC	105 gN/gC
Sensitivity	< 5 pg/sec	0.4 pg/sec
Ease of Use	Straightforward	Daily maintenance required

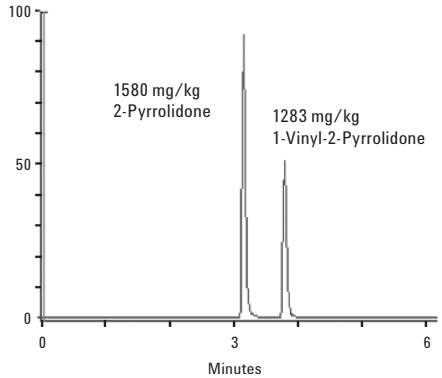

Figure 1. Agilent Model 255 analysis of adhesive standard.

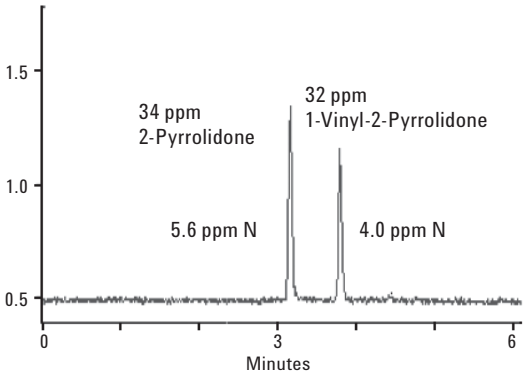

Figure 2. Agilent Model 255 NCD analysis of adhesive sample diluted in tetrahydrofuran.

GC Operating Conditions

(Agilent 6890 with EPC)

Temperature:	160 °C isothermal
Helium carrier:	2.2 mL/min
Split injection:	45.5:1 split
	200 °C
	1 µL injection volume

NCD Burner Conditions

Temperature:	800 °C
Hydrogen flow rate:	25 mL/min
Oxygen flow rate:	10 mL/min
Column:	20 Rtx-5, 0.32 mm id
	3 µm film thickness

Sample Preparation

Samples diluted in toluene
Dilution factors of 1:25 to 1:50

Table 2. Summary of 2-Pyrrolidone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	25.0	1094	2.2
Adhesive 2	43	1:41	28.1	1163	2.2
Adhesive 3	43	1:28	34.0	1405	2.2
Adhesive 4	43	1:45	20.3	838	1.7

Table 3. Summary of 1-Vinyl-2-Pyrrolidinone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	27.1	1188	2.2
Adhesive 2	43	1:41	15.1	624	2.2
Adhesive 3	43	1:28	32.5	1342	2.2
Adhesive 4	43	1:45	Not detected	Not detected	

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Printed in the USA
August 16, 2007
5989-6771EN

Agilent Technologies

Agilent Model 255 Nitrogen Chemiluminescence Detector (NCD) Simultaneous Hydrocarbon Analysis with the NCD and an FID

Technical Overview

Introduction

The nitrogen specificity of the Agilent Model 255 NCD and the universal detection of the flame ionization detector (FID) can provide a detailed analysis of a sample matrix. Chemiluminescence detection enables isolation of nitrogen-containing compounds, while the FID provides universal response for major components in many sample matrices.

Agilent has developed a detector interface to allow simultaneous universal and nitrogen specific detection for gas chromatography. An FID is used for universal detection and the Model 255 NCD is used for the specific detection of nitrogen compounds.

To perform simultaneous NCD and FID analysis, the capillary column is connected directly to the FID following the gas chromatograph manufacturer's installation instruction. The column effluent flows into the FID and then immediately continues through to the stainless steel burner of the NCD. The FID uses oxygen instead of air and the makeup gas is helium rather than nitrogen. Use of oxygen and helium avoids the formation of background nitric oxide in the flame of the detector.

The simultaneous mode is useful when the concentration of nitrogen in individual compounds is greater than 1 ppm. The detection scheme is also useful when the matrix of interest is unknown and there is a need for hydrocarbon data on the sample.

When necessary, the stainless steel burner is easily converted to Direct Analysis mode for better nitrogen sensitivity. The stainless steel burner was designed for use for either simultaneous NCD and FID analysis or specific nitrogen analysis only.

Figure 1 shows a chromatogram with nitrobenzene, 3-methylindole, and 9-methylcarbazole in toluene that demonstrates both the equimolar response and specificity of the NCD. The concentration of nitrogen is approximately 25 ppm for each compound. Also notice the lack of a solvent peak at the beginning of the NCD analysis. A benefit of the NCD is that non-nitrogen containing hydrocarbons in the sample are transparent to the NCD. The sample compounds flow from the column into the FID and the FID measures the hydrocarbon response. A portion of the FID effluent flows directly into the burner of the NCD.

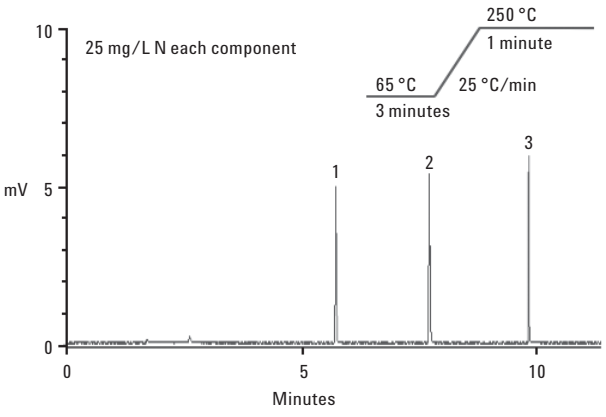

Figure 1. NCD analysis.

Table 1. Precision of Simultaneous NCD and FID Analysis

Number of runs	Compound concentration (ppm)	N concentration (ppm)	Model 255 NCD (% RSD)	FID (% RSD)
Nitrobenzene	218	25	2.3	1.5
3-methylindole	234	25	2.2	1.3
9-methylcarbazole	326	25	2.5	1.5

Notice the number of other small impurity peaks present in the FID chromatogram (Figure 2). The NCD did not detect these peaks since the NCD is specific only for nitrogen. If any of the small compounds contained nitrogen, the NCD would have detected them. Also, with the equimolar response of the NCD, it is possible to determine the concentration of nitrogen impurities in the sample.

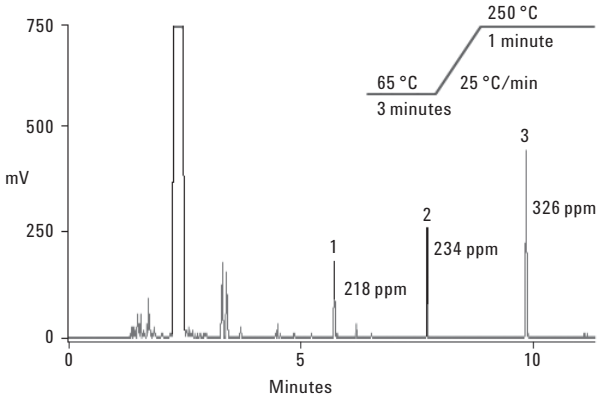

Figure 2. FID analysis.

GC Operating Conditions
(Agilent 6890 with EPC)

Initial temperature:	65 °C for 3 min
Temperature ramp:	25 °C/min
Final temperature:	250 °C for 1 min
Helium carrier:	2.2 mL/min
Split injection:	50:1 split
	250 °C
	2 µL injection volume

NCD Burner Condition

Temperature:	800 °C
Hydrogen flow rate:	25 mL/min
Oxygen flow rate:	10 mL/min
Column:	30 m HP-5, 0.32 mm id
	0.25 µm film thickness

Components

Peak 1:	Nitrobenzene
Peak 2:	Methylindole
Peak 3:	9-Methylcarbazole

The toluene sample with nitrobenzene, 3-methylindole, and 9-methylcarbazole was analyzed 143 times over a 3-day period to demonstrate the stability of the simultaneous NCD and FID analysis. The results of 2.5% relative standard deviation and less demonstrate the stability of the NCD when operated in tandem with the FID (see Table 1). The results also demonstrate that the tandem NCD/FID operation does not affect the performance of the FID. Furthermore, the results also demonstrate the consistency in transferring a fraction of the FID exhaust gases to the NCD.

The nitrogen specificity of the NCD and the universal detection of the FID can provide a detailed analysis. Chemiluminescence detection enables isolation of nitrogen-containing compounds in the sample, while the FID provides universal response for the major compounds of many sample matrices.

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Printed in the USA
May 17, 2007
5989-6772EN

Sulfur Compounds in Air – Agilent Model 355 SCD

Technical Overview

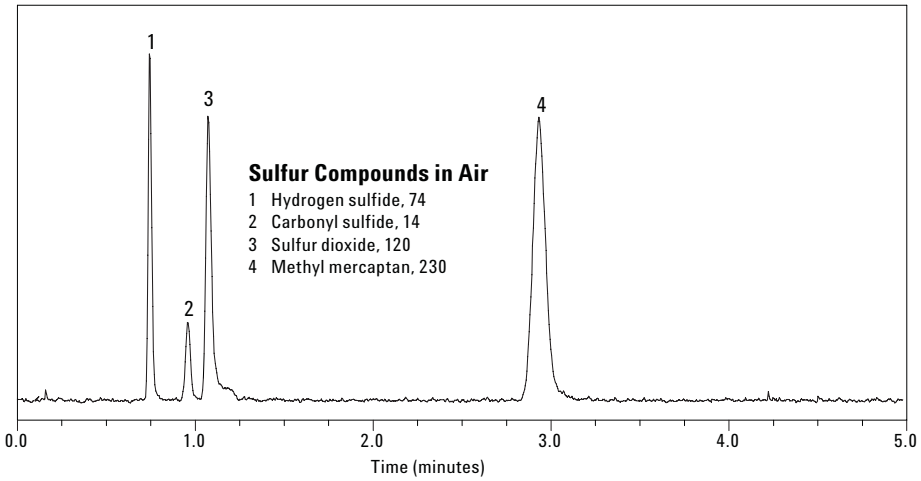
Introduction

The reliable measurement of sulfur gases in air is extremely important. Many sulfur compounds are toxic and notorious for their obnoxious odors even when present at only parts per billion levels. Gaseous sulfur compounds may be generated and emitted by various industrial processes, such as petroleum refining, ore smelting, and kraft paper pulping. Measurement of gaseous sulfur compounds aids in protection of the environment and human health. There are numerous natural sources of sulfur gases–vegetation, animals, soils, volcanoes, etc.–and measurement of sulfur gases is also of great importance in understanding atmospheric chemistry.

Gas chromatography with sulfur chemiluminescence detection (SCD) provides a rapid means to identify and quantify various sulfur compounds that may be present in air. Unlike other sulfur selective detectors, such as the flame photometric detector (FPD), the SCD produces a linear and equimolar response to sulfur compounds without significant hydrocarbon quenching or interferences. Furthermore, the Model 355 SCD is at least 10 times more sensitive and 100 times more selective than the FPD.

The following chromatogram illustrates the ability of the SCD to speciate and quantitate sulfur compounds at levels less than 1 ppm in an air sample without any sample preconcentration.

Conditions are as follows: Model 355 SCD operated according to standard conditions; 1 mL sample size; column: 30 m, 0.32 mm id, 4 µm methyl silicone WCOT fused silica; temperature program: –25 °C isothermal. The gas chromatograph was a Agilent Technologies Model 5890 Series II equipped with electronic pressure programming for compressing the initial bandwidth.



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Printed in the USA
May 22, 2007
5989-6778EN



Agilent Technologies

Agilent Model 355 Sulfur Chemiluminescence Detector (SCD): Thiophene in Benzene

Technical Overview

Introduction

This overview discusses the analysis of benzene for sulfur contamination. The sensitivity, selectivity, and linear response of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) in the analysis of trace levels of thiophene in benzene illustrates that the Model 355 is well-suited for low-level sulfur analysis.

Benzene is the basic unit of the aromatic class of compounds. The primary sources of benzene are from extraction of hydrocarbon crude distillates, refinery catalytic reforming, carbonization of coal, and the hydrodealkylation of a toluene charge stock. [1] The hydrodealkylation reaction results in the conversion of about 90% of the aromatics in the feed with a selectivity factor of about 95%. Thiophene, which also occurs in the light hydrocarbon fractions distilled from crude stocks and coal tar, is present in levels from 0.4 to 1.4 wt. %. [2] Traditionally, thiophene has been extracted by washing with H_2SO_4 to produce a sweeter product. However, this and other processes of thiophene removal still have difficulty reaching the level of purity required by many chemical markets. Consequently, it is often important to monitor trace levels of thiophene in benzene. The three main applications for benzene are production of ethylbenzene, cumene, and cyclohexane. These three products account for 80% of the benzene consumed as a chemical feedstock. [2]

These species can be classified as intermediates for a wide range of final products, including dyes, resins, solvents, and polymers, including nylon. The purity requirements for synthetic applications continue to become more confining, thereby increasing the need to monitor sulfur removal efficiency and to verify the purity of starting materials.

The data in Figure 1 illustrate the sensitivity of the 355 SCD for trace level analysis of sulfur in a hydrocarbon matrix without interference. Figure 2 displays the linear response of the SCD at trace levels. Correlation coefficients for five orders of magnitude were better than $R^2 = 0.999$. The selectivity of the 355 SCD for sulfur over hydrocarbon is shown in Figures 1 and 3, where sulfur chromatograms show no hydrocarbon interference from the eluting benzene.

The data in Figures 1 and 2 were collected on a Agilent 6890 gas chromatograph with a Agilent 355 SCD directly attached. The chromatograms in Figure 3 were collected simultaneously without column splitting, using the flame ionization detector attachment to the SCD. The chromatographic conditions for the trace analysis are summarized in Table 1.

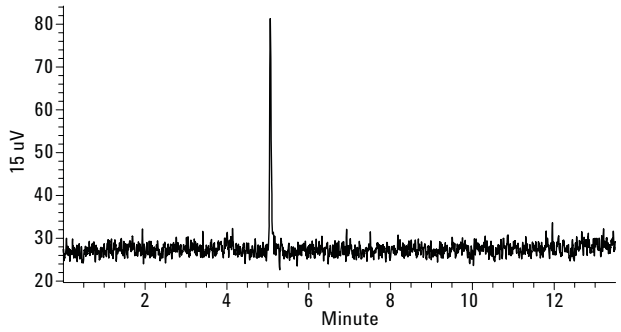

Figure 1. Chromatogram illustrating the analysis of 15 ppb thiophene (as sulfur, split 1:10).

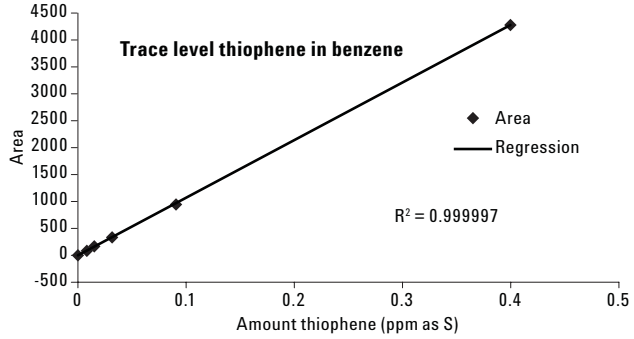

Figure 2. Linearity of trace level analysis of thiophene in benzene.

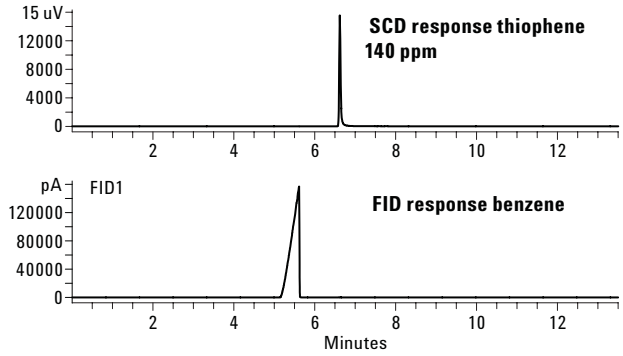

Figure 3. Simultaneous FID-SCD chromatogram on SUPELCOWAX-10.

Table 1. Chromatographic Conditions

Injection temperature	120 °C
Injection volume	1 µL
Initial temperature	30 °C
Initial time	2 min
Rate	10 °C/min
Final temperature	125 °C
Final time	2 min
Split ratio	1:10
Flow mode	Constant flow
Column flow	2 mL/min
Column type	SUPELCOWAX-10
Length	30 m
Internal diameter	0.32 mm
Film thickness	1 µm

References

1. James H. Gary and Glenn E. Handwerk, "Petroleum Refining Technology and Economics," Marcel Dekker Inc., New York, NY, 1984, Chapter 14.

2. Wolfgang Y. Gerhartz, Stephen Yamamoto, et. al. eds., "Ullmann's Encyclopedia of Industrial Chemistry," VCH Publisher, Deerfield Beach, FL, 1985, vol. A3.

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Printed in the USA
June 7, 2007
5989-6786EN

Agilent 355 Sulfur Chemiluminescence Detector (355 SCD): Sulfur Compounds in Ethylene and Propylene

Technical Overview

Introduction

This technical overview briefly describes the analysis of ethylene and propylene gases for trace amounts of hydrogen sulfide and carbonyl sulfide as well as other volatile sulfur compounds using gas chromatography and sulfur selective detection. The method provides for the determination of individual volatile sulfur-containing compounds, as well as the determination of total sulfur content in chemical feedstocks.

The measurement of trace amounts of volatile sulfur compounds in ethylene and proplyene is important because of the contaminant nature of these compounds in hydrocarbon feedstocks. Accurate gas chromatographic determinations of trace volatile sulfur compounds involve unique analytical difficulties due to the chemical nature of these compounds. Volatile sulfur compounds are particularly reactive and adsorptive in nature, making trace level analysis reliant on exceptionally good chromatographic technique, using inert sample handling systems and valving, and selective detection that is minimally affected by matrix interference. Because of their respective boiling point ranges, the measurement of hydrogen sulfide in ethylene and carbonyl sulfide in propylene is generally of great concern.

This analysis is especially difficult using detectors such as the flame photometric detectors, where coelution of the analyte and solvent contribute to hydrocarbon quenching and interference, which may result in erroneous results. The following chromatograms illustrate the ability of the SCD to selectively detect trace levels of volatile sulfur compounds in hydrocarbon gas samples without suffering from any quenching or interference from the hydrocarbon matrix.

The analyses presented here were performed on an Agilent 5890 Series II gas chromatograph equipped with a split/splitless injector. The Agilent Model 355 Sulfur Chemiluminescence Detector (SCD) was directly connected to an Astec Gaspro capillary column and operated according to standard conditions.

Figure 1 illustrates the power of the 355 SCD for the analysis of COS in propylene. A 1-mL propylene sample containing 60 ppb wt sulfur as COS was introduced to the GC with no pretreatment. The injector was operated with a split ratio of 1:6, and the linear velocity was approximately 38 cm sec^{-1}. Temperature programming for this analysis was as follows: 50 °C for 1 min to 100 °C at 10 °C/min. This chromatogram verifies the selectivity of the Agilent 355 SCD for sulfur over carbon, with no hydrocarbon response or anomalies visible in the baseline. Also evident is the sensitivity of the SCD to sulfur species, making it ideal for trace analysis.

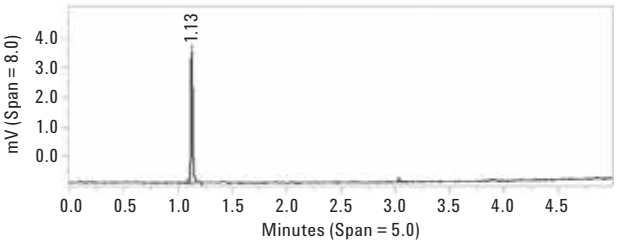

Figure 1. 60 ppb carbonyl sulfide in propylene.

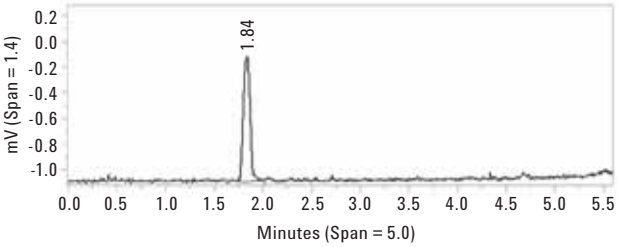

Figure 2. 100 ppb hydrogen sulfide in ethylene.

Figure 2 illustrates the analysis of 100 ppb hydrogen sulfide in ethylene using the GasPro column and SCD. As above, a 1-mL sample (gas) volume was introduced with a split 1:6. The oven temperature program started at 40 °C for 1 min and raised at 10 °C/min to the final temperature of 100 °C. In this case the temperature ramp for the analysis was deliberately set so that there would be a simultaneous elution of the ethylene and the hydrogen sulfide. As expected, this resulted in the exhibited band broadening; however, there was no evident quenching of the sulfur response and no hydrocarbon response or baseline anomalies. Although the SCD works well under these conditions, it is always recommended to separate the analyte from the matrix if at all possible to reduce solvent effects.

Recent developments in chromatographic column technology allow the ambient separation of hydrogen sulfide and carbonyl sulfide in hydrocarbon matrices. For ambient separation of H_2S and COS as well as light mercaptans and sulfides, capillary columns such as the Chrompack CP-SilicaPLOT (30 m 0.32 mm id) or the Astec Gaspro (15 m 0.32 mm id) are ideal. The retention characteristics of these columns are unique and seem to be best suited for light applications.

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Printed in the USA
June 14, 2007
5989-6791EN



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A Unified Gas Chromatography Method for Aromatic Solvent Analysis

Application

Gas Chromatography

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Abstract

A single, easy-to-use GC method for aromatic solvent purity analysis is described that meets the chromatographic requirements of ten separate ASTM methods. This method can be used to obtain identical results on both the Agilent 6890 and 6850 Series Gas Chromatographs designed for the method development lab and the routine production lab respectively. Reproducibility of results between instruments, between labs, and over time are further improved by applying the technique of retention time locking to this unified method.

Introduction

The producers and users of many aromatic hydrocarbons evaluate the product quality by measuring the purity of the material along with specific contaminants. For these types of measurements the most commonly used analysis technique is gas chromatography (GC). In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and published a number of GC methods specific to an aromatic compound or class of compounds.[1] These methods have evolved over time to meet the requirements of new materials specifications or to incorporate new GC technologies (i.e. capillary columns replacing packed columns). The result of this evolution is a large number of methods that are remarkably alike. In practice, many QA laboratories that support a variety of chemical processes typically devote one GC instrument to each ASTM method they must run.

Recently, there has been a move by many chemical companies to consolidate lab facilities, simplify measurements, and reduce the costs that chemical measurements add to production. Laboratory space is expensive and is becoming limited. Where three or four GCs were operating in the past, there is now only space and budget for one or two. Another part of this trend is to have non-traditional personnel such as plant operators; technicians and engineers perform chemical analyses. Since these personnel are not trained as analytical chemists, simpler methods are needed to perform the analyses without losing measurement performance.

Accommodating these changes in the lab environment makes it necessary to explore alternative approaches to performing GC analyses. One approach is to develop a method that combines the elements of several separate ASTM methods.

A single method has a number of advantages over multiple methods. Fewer GCs could be used in place of a larger number of instruments previously dedicated to individual methods; thus reducing required lab space. By running one method, any GC could also serve as a backup for instruments that are undergoing maintenance or repair. This would result in shorter down times and better utilization of lab space. A single method would also eliminate the need to stock multiple columns and supplies. Plant operators would also find it easier to use since they would only need to be trained once on a single procedure.

Another important advantage to a single aromatics method lies in the use of retention time locking (RTL). RTL is a technique that allows any Agilent 6890 or 6850 GC systems running the same method to obtain nearly identical retention times. Comparing data between instruments, between laboratories, or over time can be difficult due to variations in retention times. This is further complicated when using multiple methods since the different columns and operating conditions result in different retention times for the same compound. For instance, there are eight ASTM methods that measure p-xylene; however, p-xylene retention times range from 6 to 16 minutes depending on the method's operating conditions (column, flow, temperature). By using one method for all aromatic samples, retention time variations can be reduced to less than 0.5 minutes. Then by applying RTL to this method, system-to-system retention time variations can be further reduced to less than 0.03 minutes. Retention time precision on this order greatly simplifies comparison of data between systems, between laboratories, and over time.

This application note describes a GC method that is chromatographically suitable for a wide range of samples typically analyzed by ten different ASTM methods. Table 1 lists these ten methods along with the ASTM recommended columns and reporting specifications.

Table 1. Ten ASTM Methods for the GC Analysis of Aromatic Solvents

ASTM Method	Title	Liquid phase	Column type	Report specifications
D2306	Std Test for C8 Aromatic Hydrocarbons	0.25 µm Carbowax	Capillary 50 m × 0.25 mm	wt% of individual C8 HC
D2360	Std Test for Trace Impurities in Monocyclic Hydrocarbons	0.32 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual aromatic impurities, total impurities, purity
D3760	Std Test for Cumene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, cumene purity (wt%)
D3797	Std Test for o-Xylene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, o-xylene purity (wt%)
D3798	Std Test for p-Xylene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, total impurities, p-xylene purity (wt%)
D4492	Std Test for Benzene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, benzene purity(wt%)
D4534	Std Test for Benzene in Cyclic Products	10%TCEPE on Chromasorb P	Packed 3.7 m × 3.175 mm	wt% of benzene
D5060	Std Test for Impurities in Ethylbenzene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, ethylbenzene purity
D5135	Std Test for Styrene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, styrene purity
D5917	Std Test for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)	0.25 µm Carbowax	Capillary 60 m × 0.32 mm	wt% individual impurities, wt% total non-aromatics, wt% total C9 aromatics, purity of main component

Experimental

Two Agilent 6890 Plus Series gas chromatographs and four Agilent 6850 gas chromatographs were used for this work. Each GC was equipped with a split/splitless capillary inlet, a flame ionization detector (FID) and an Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with high-pressure Merlin Microseal Septa (Agilent Part no. 5182-3442) and spilt-optimized liners (Agilent Part no. 5183-4647). Injections were made using 10 µL gas-tight syringes (Agilent Part no. 5181-8809) designed for use with the Merlin Microseal. Table 2 lists the instrument conditions used for this method. An Agilent Chemstation was used for all instrument control, data acquisition and data analysis.

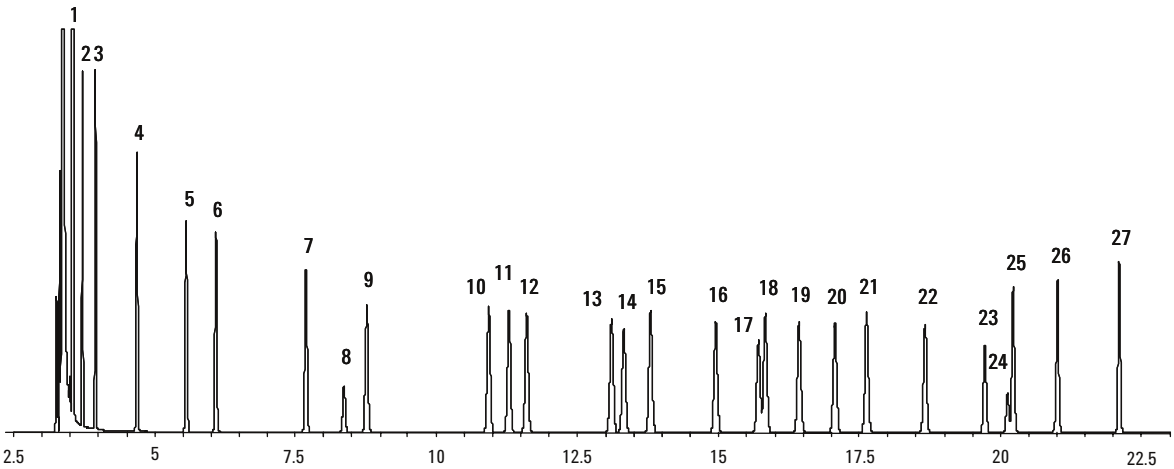
Table 2. Conditions for Unified Aromatic Solvents Method

Column	HP-Innowax, 60 m × 0.32 mm × 0.5 µm Agilent Part no.19091N-216
Carrier Gas	Helium @ 20.00 psi constant pressure mode
Inlet	Split/Splitless @ 250 °C 100:1 to 400:1 split ratio
Oven Temp	75 °C (10 min); 3 °C/min to 100 °C (0 min) 10 °C/min to 145 °C (0 min)
Detector	FID @ 250 °C Data acquisition rate @ 20 Hz
Injection Size	0.1 to 1.0 µL

An n-hexane solution was prepared containing 0.1 wt% of all the aromatic solvents and impurities specified for analysis by the ten ASTM methods listed in Table 1. This standard was used to develop the RTL calibration and to assess the separation of each compound. Final evaluation of this unified method was done by running the recommended standards specified in each of the ten ASTM methods.

Results and Discussion

Figure 1 shows a chromatogram of the hexane solution containing an aggregate of aromatic solvents and impurities. For most compounds, baseline resolution was achieved. There are two pairs that are only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, are also not resolved in the original ASTM method (D-5060 Impurities in ethylbenzene) and, along with o-ethyltoluene, are reported as total ethyltoluene. Therefore, the results presented here represent the same result obtained with the original ASTM method. A second pair, diethylbenzene and n-butylbenzene are also only partially resolved. Again, this does not present a problem since these two components are not typically found together in the same material. Diethylbenzene is sometimes found as a contaminant in ethylbenzene (ASTM D-5060) while n-butylbenzene is used as the internal standard for cumene analysis (ASTM D3760).



1	heptane	8	1,4-dioxane	15	o-xylene	22	tridecane
2	cycohexane	9	undecane	16	proplybenzene	23	diethylbenzene isomer
3	octane	10	ethylbenzene	17	p-ethyltoluene	24	diethylbenzene isomer
4	nonane	11	p-xylene	18	m-ethyltoluene	25	n-butylbenzene
5	benzene	12	m-xylene	19	t-butylbenzene	26	α-methylstyrene
6	decane	13	cumene	20	s-butylbenzene	27	phenylacetylene
7	toluene	14	dodecane	21	styrene		

Figure 1. Separation of the 27 compounds analyzed by the ten ASTM aromatics methods listed in Table 1.

Retention Time Locking (RTL)

Retention time locking calibration was performed using t-butylbenzene as the target peak. Figure 2 shows the five RTL calibration runs with the retention times of t-butylbenzene indicated and Figure 3 shows the RTL calibration. These calibration runs do not have to be repeated by anyone wishing to lock this method on their Agilent 6890 or 6850 GC systems. To use this RTL calibration, simply create a new method with conditions outlined in Table 2, then use the Chemstation RTL software to create a new RTL calibration and enter the data shown in Figure 3. The GC can then be locked by running a sample containing t-butylbenzene and using the RTL software to re-lock the method. The general theory and use of RTL is detailed in previous publications.[2]

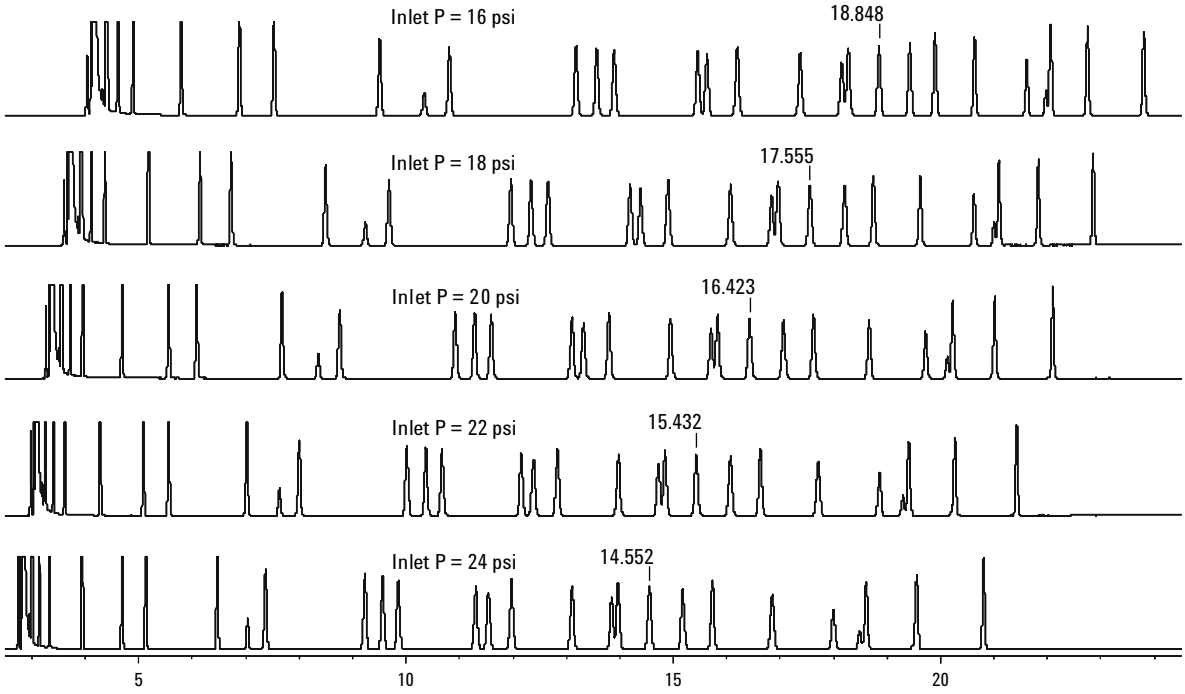


Figure 2. Retention time locking calibration runs using t-butylbenzene as the RTL target peak.

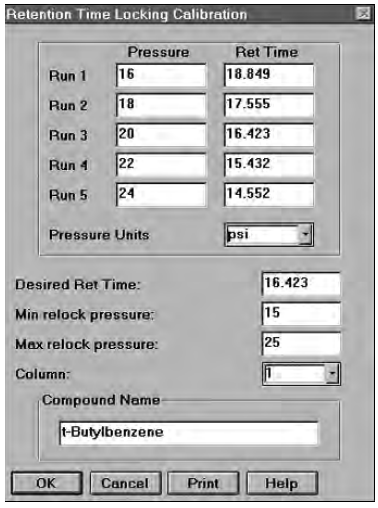


Figure 3. Retention time locking calibration using t-butylbenzene as the RTL target peak.

A total of six GC systems, two 6890s and four 6850s, were configured to run this unified method. Each GC was retention time locked using a t-butylbenzene target retention time of 16.423 minutes. Figure 4 shows an overlay of the locked chromatograms from each of the six GCs. Table 3 lists the retention times and precision of each compound in the standard mix. Excellent retention time precision was observed for the 6890 and 6850 instruments across the entire time range of the chromatographic run. Peaks falling within the initial 10-minute isothermal time had a standard deviation of about 0.02 minutes. Those peaks eluting during the 3 °C/min program ramp had a standard deviation of 0.01 minutes and those eluting in the 10 °C/min ramp showed a standard deviation of 0.03 minutes

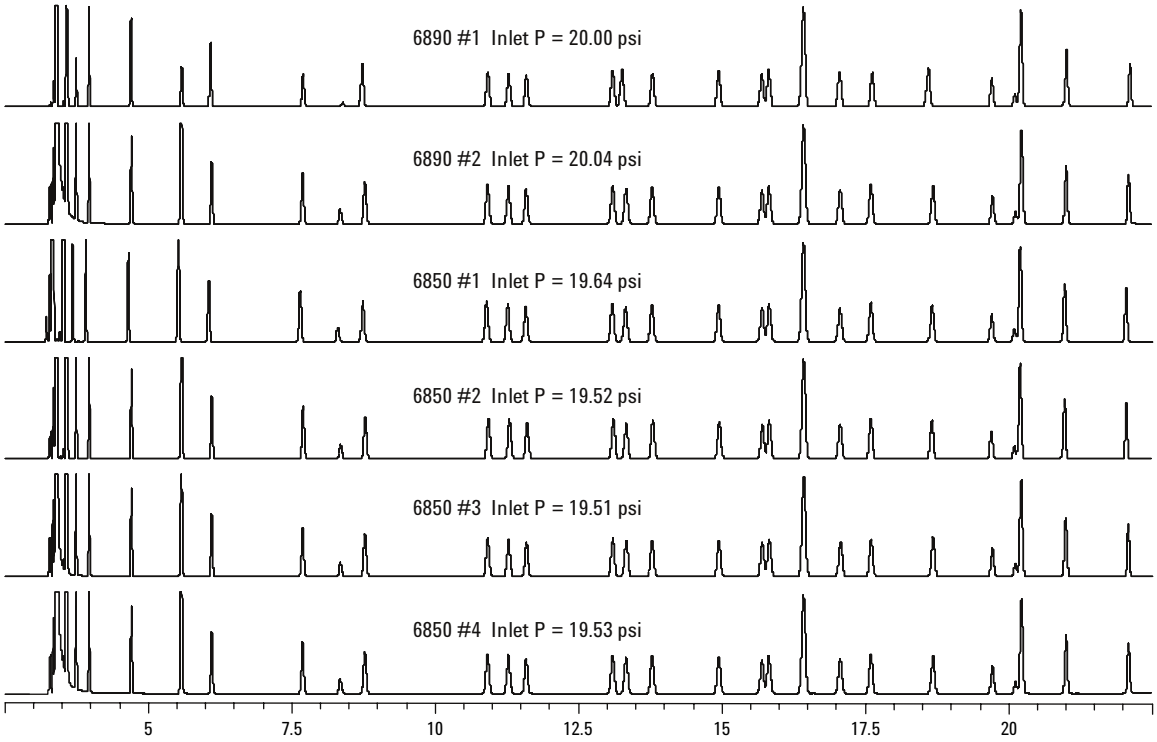


Figure 4. Using RTL, excellent retention time precision was observed for all 27 compounds analyzed using the unified aromatics method. Details of retention time precision are listed in Table 3.

Table 3. Retention Time Precision for Each Compound Analyzed by the Unified Method

	Retention time (min)							
Compound	**6890 #1**	**6890 #2**	**6850 #1**	**6850 #2**	**6850 #3**	**6850 #4**	**Std Dev**	**Range**
heptane	3.572	3.568	3.508	3.569	3.566	3.568	0.025	0.064
cyclohexane	3.745	3.742	3.682	3.743	3.741	3.742	0.025	0.063
octane	3.969	3.971	3.911	3.972	3.970	3.971	0.024	0.061
nonane	4.696	4.704	4.646	4.705	4.703	4.704	0.023	0.059
benzene	5.581	5.572	5.518	5.576	5.572	5.572	0.023	0.063
decane	6.084	6.105	6.053	6.106	6.104	6.105	0.021	0.053
toluene	7.694	7.686	7.646	7.695	7.687	7.686	0.018	0.049
1,4-dioxane	8.386	8.342	8.306	8.350	8.346	8.342	0.025	0.080
undecane	8.732	8.776	8.741	8.782	8.777	8.776	0.022	0.050
ethylbenzene	10.922	10.915	10.899	10.932	10.918	10.915	0.011	0.033
p-xylene	11.282	11.278	11.267	11.295	11.280	11.278	0.009	0.028
m-xylene	11.592	11.587	11.577	11.604	11.589	11.587	0.009	0.027
cumene	13.097	13.097	13.089	13.110	13.098	13.097	0.007	0.021
dodecane	13.264	13.334	13.323	13.337	13.333	13.334	0.028	0.073
o-xylene	13.790	13.781	13.778	13.795	13.782	13.781	0.007	0.017
propybenzene	14.940	14.943	14.939	14.951	14.945	14.943	0.004	0.012
p-ethyltoluene	15.696	15.699	15.699	15.706	15.702	15.699	0.003	0.010
m-ethyltoluene	15.819	15.820	15.820	15.827	15.823	15.820	0.003	0.008
t-butylbenzene	16.423	16.424	16.420	16.426	16.426	16.424	0.002	0.006
s-butylbenzene	17.049	17.060	17.053	17.059	17.063	17.060	0.005	0.014
styrene	17.623	17.600	17.600	17.600	17.603	17.600	0.009	0.023
tridecane	18.602	18.683	18.665	18.661	18.681	18.683	0.031	0.081
diethylbenzene	19.707	19.718	19.701	19.700	19.713	19.718	0.008	0.018
diethylbenzene	20.111	20.123	20.101	20.101	20.116	20.123	0.010	0.022
n-butylbenzene	20.217	20.225	20.201	20.203	20.219	20.225	0.011	0.024
α-methylstyrene	21.011	21.003	20.976	20.975	20.994	21.003	0.015	0.036
phenyacetylene	22.115	22.090	22.050	22.050	22.081	22.090	0.025	0.065
						Avg	***0.015***	***0.039***

For this method it is not always necessary to use t-butylbenzene to perform retention time locking. Analysts who want to use this method for samples not containing t-butylbenzene can select another compound as the RTL target peak. Compounds that do not elute near temperature program transitions can serve as RTL target peaks. Table 4 lists the other suitable RTL target compounds along with the retention time data for constructing alternate RTL calibrations for this method. For instance, if one were preparing the benzene standard prescribed by ASTM method D4492, the toluene in that standard could serve as the RTL target compound. It is not necessary to perform the five RTL calibration runs. Simply create a new RTL calibration using the inlet pressures and toluene retention times from Table 4. This example of an RTL calibration using toluene is shown in Figure 5.

Table 4. Retention Time Locking Calibration Data for Unified Aromatics Method

	Retention time (min) at each inlet pressure				
Compound	**16.00 psi**	**18.00 psi**	**20.00 psi**	**22.00 psi**	**24.00 psi**
nonane	5.794	5.174	4.682	4.279	3.943
benzene	6.880	6.143	5.558	5.080	4.681
toluene	9.507	8.489	7.680	7.018	6.468
cumene	15.460	14.188	13.100	12.148	11.305
o-xylene	16.189	14.897	13.791	12.825	11.969
propylbenzene	17.370	16.064	14.646	13.968	13.100
t-butylbenzene*	18.849	17.555	16.423	15.432	14.552
s-butylbenzene	19.424	18.201	17.061	16.063	15.176
n-butylbenzene	22.054	21.090	20.220	19.404	18.607
styrene	19.891	18.743	17.620	16.621	15.733
α-methylstyrene	22.745	21.824	21.010	20.261	19.552
phenylacetylene	23.795	22.852	22.097	21.421	20.800

*t-butylbenzene used as RTL target peak for this publication (target RT = 16.423 min).

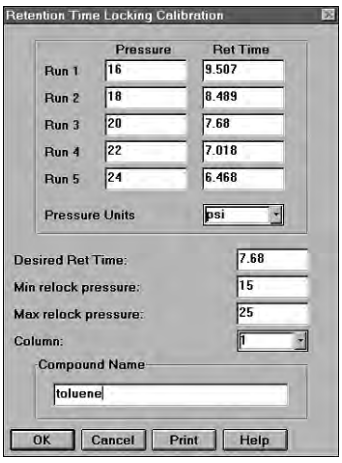


Figure 5. Alternate retention time locking calibration for the unified aromatics method that uses toluene as the locking target compound.

Evaluation of Calibration Standards

The calibration standards specified by each of the ten ASTM methods were prepared and run using this unified method. Each standard was run with Agilent 6890 and Agilent 6850 series gas chromatographs that were retention time locked using t-butylbenzene as the target peak (RT = 16.423 min.).

D2306 - Standard Test for C8 Aromatic Hydrocarbons

Figure 6 shows the chromatograms of the D2306 calibration standard run on Agilent 6890 and 6850 gas chromatographs. The injection size for both runs was 0.1 µL and the split ratio was 400:1.

D2360 - Standard Test for Trace Impurities in Monocyclic Hydrocarbons

The standard calibration mix specified by D2360 was prepared in p-xylene. Figure 7 shows the chromatograms of the D2360 calibration standard. Injection size was 1.0 µL and the split ratio was 100:1. The ethylbenzene peak (RT = 10.98 min) elutes just before p-xylene and was much broader than the other contaminants. This peak shape was due to a reverse solvent effect caused by the overloaded p-xylene along with an oven starting temperature (75 °C) that was much lower than the p-xylene boiling point (138 °C). A broad ethylbenzene peak was also observed in the original ASTM D2360 method.[3]

D3760 - Standard Test for Analysis of Isopropylbenzene (Cumene)

Figure 8 shows the chromatograms of the D3760 calibration standard. The injection size for both runs was 1.0 µL and the split ratio was 100:1. The xylene isomers' concentrations were not listed because they were not added to the standard, but were present as trace contaminants in the cumene used to prepare the standard. Since both GCs are retention time locked, the identification of each xylene isomer could be easily made.

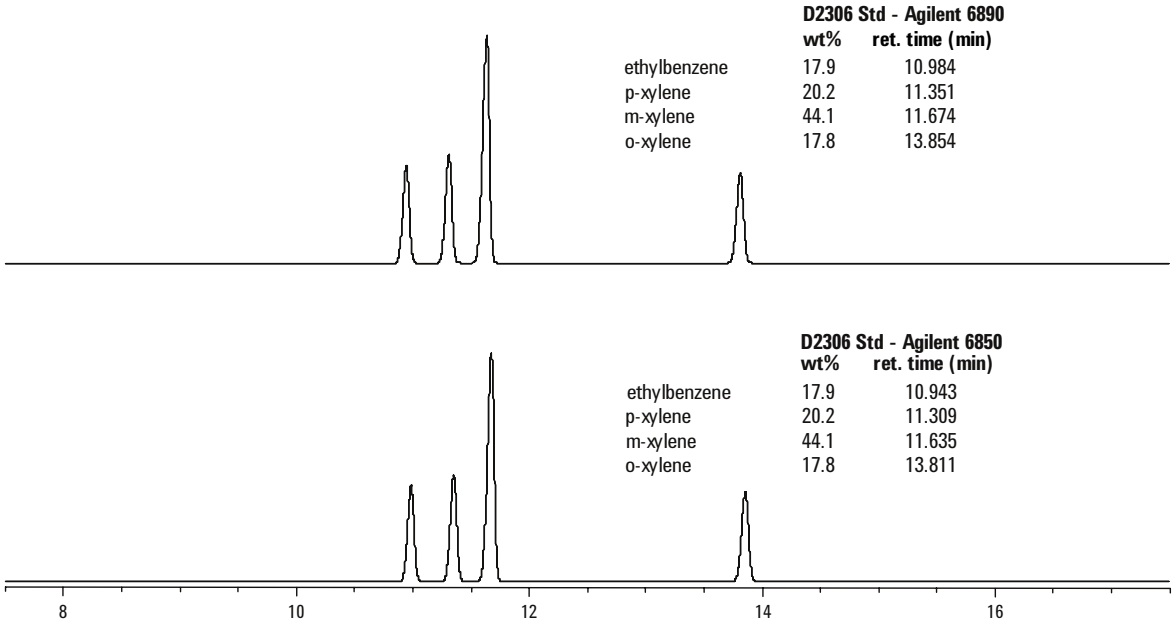


Figure 6. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

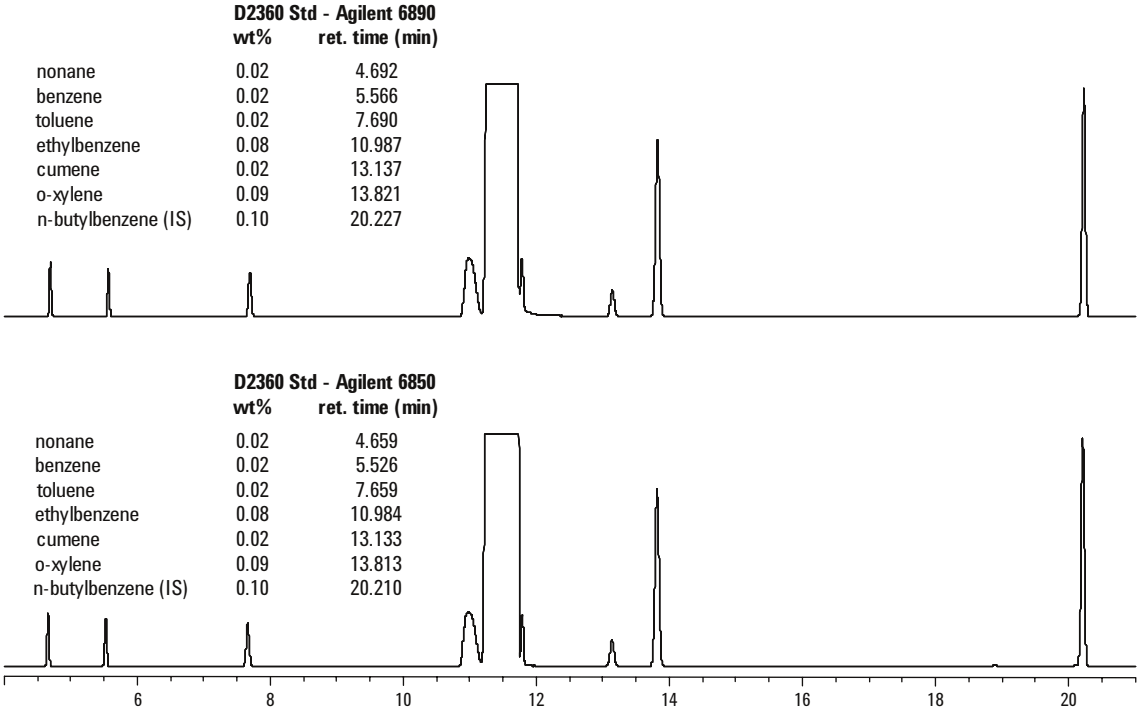


Figure 7. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

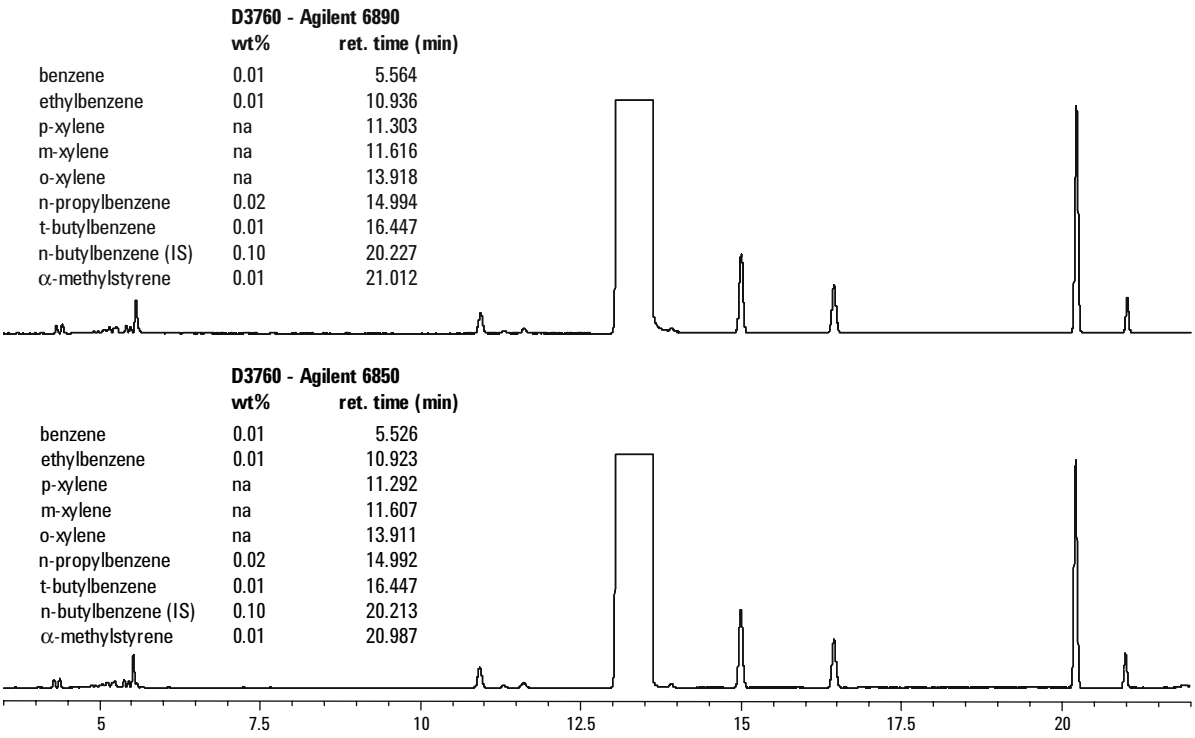


Figure 8. ASTM D3760 isopropylbenzene (cumene) quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D3797 - Standard Test Method for Analysis of o-Xylene

Figure 9 shows the chromatograms of the D3797 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1. The broadening of the cumene peak (RT = 13.28 min) was due to the reverse solvent effect of the overloaded o-xylene peak. This was also observed in the original ASTM D3797 method.

D3798 - Standard Test Method for Analysis of p-Xylene

Figure 10 shows the chromatograms of the D3798 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1. The ethylbenzene peak shows the same broadening observed in the D2360 standard. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Figure 11 shows the details of this separation using the unified method. For each GC this requirement was met for both the ethylbenzene and the m-xylene.

D4492 - Standard Test for Analysis of Benzene

Figure 12 shows the chromatograms of the D4492 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

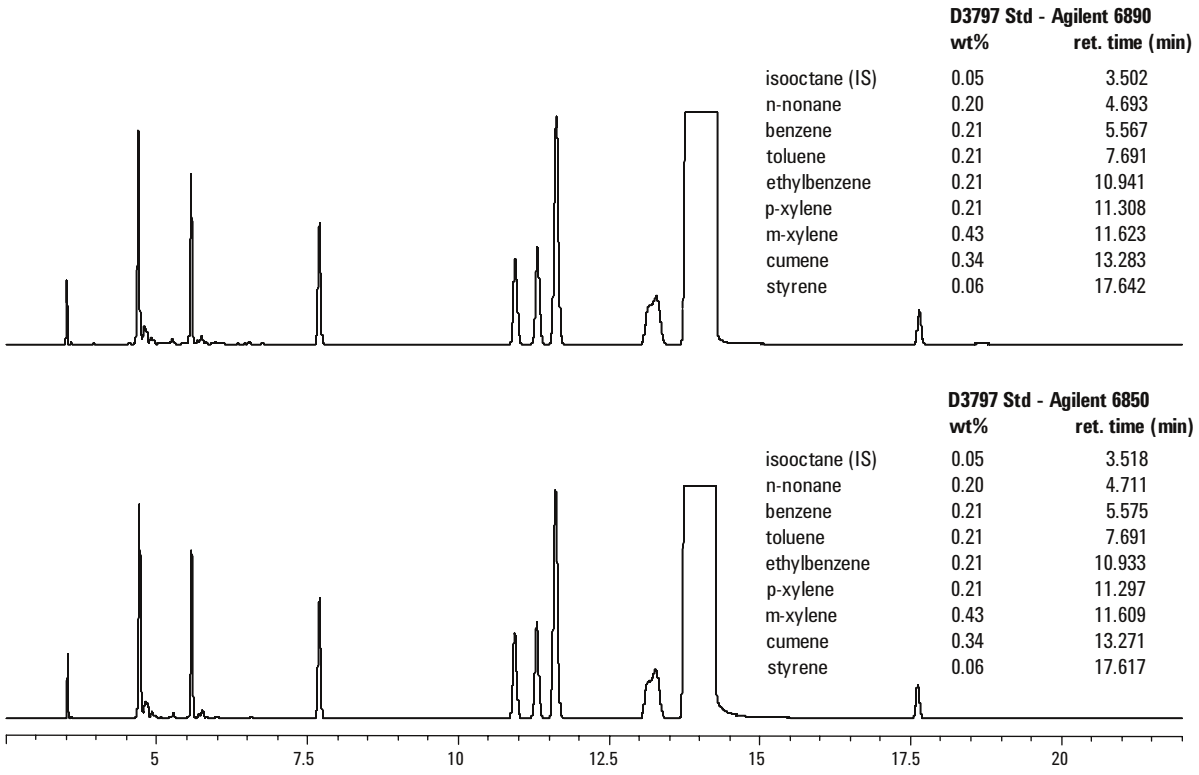


Figure 9. ASTM D3797 o-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

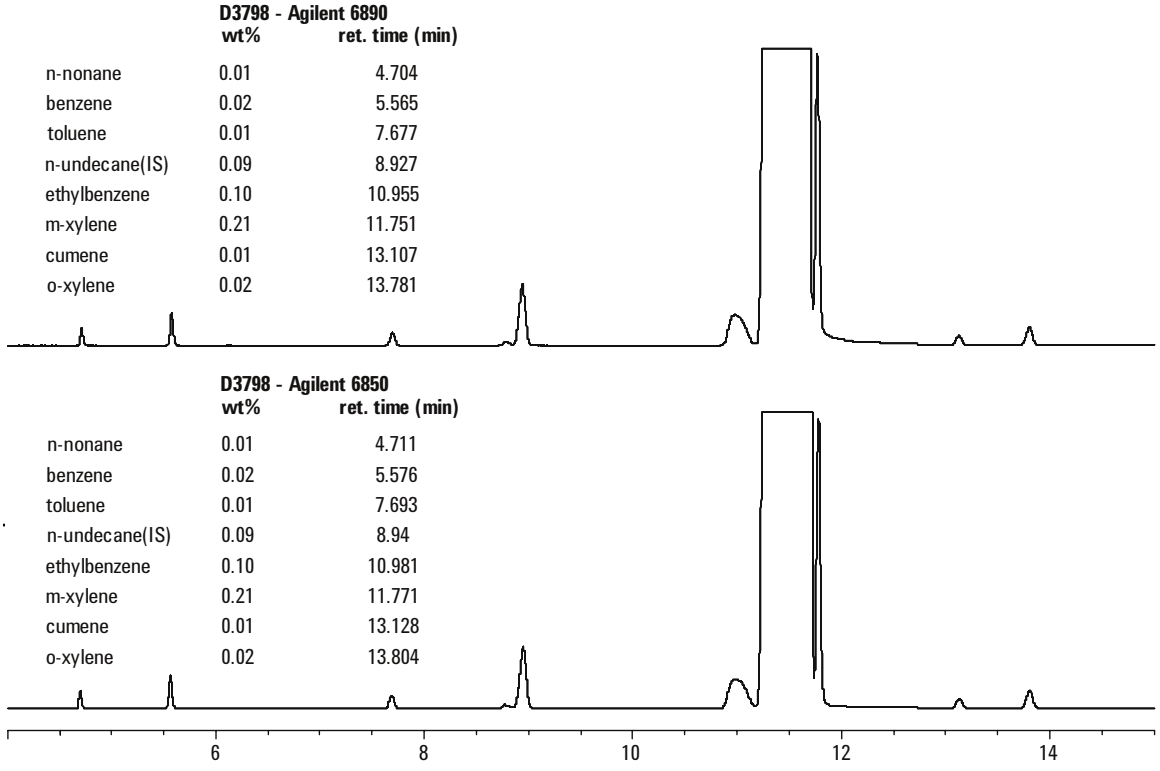


Figure 10. ASTM D3798 p-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

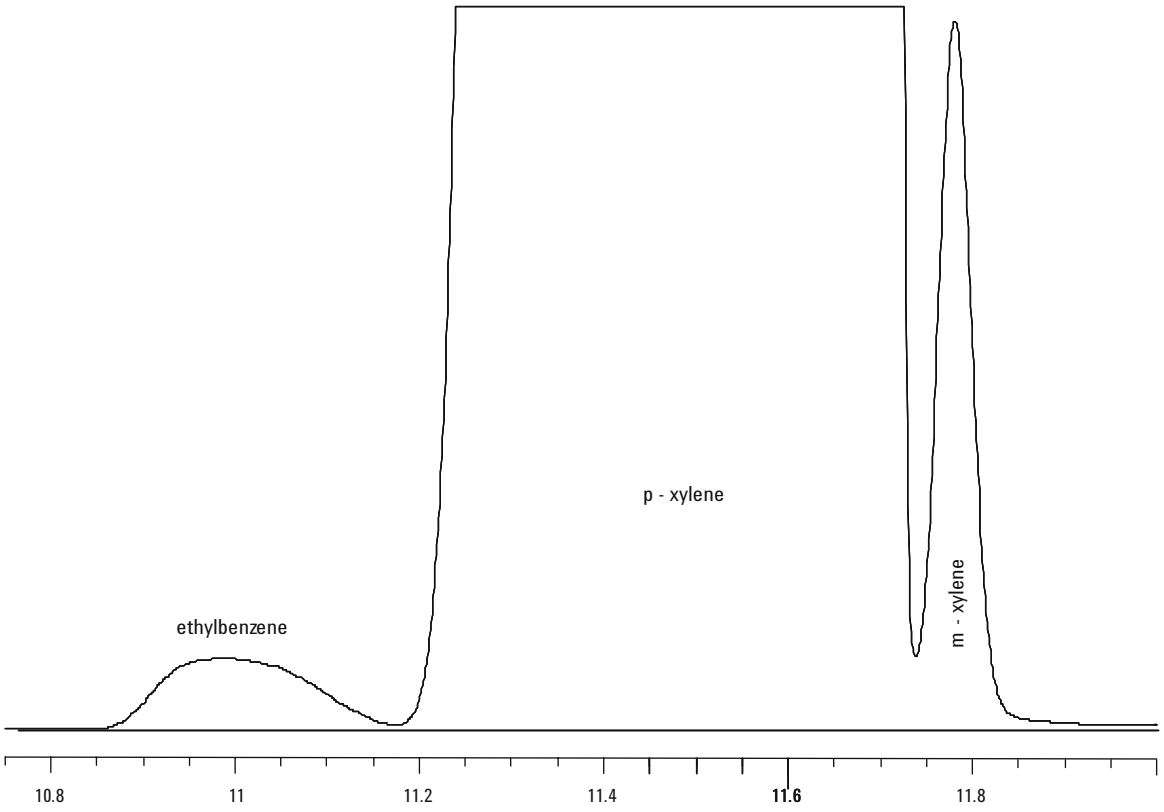


Figure 11. Expanded view from Figure 10 shows excellent separation of m-xylene from p-xylene peak using the unified aromatics method.

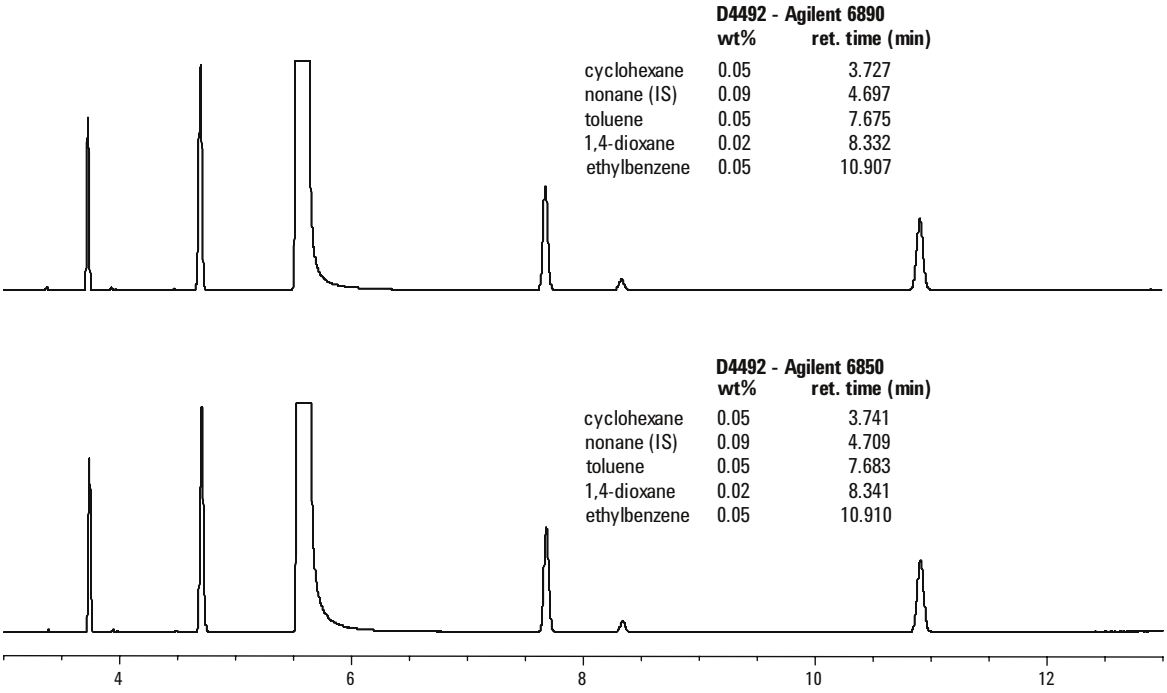


Figure 12. ASTM D4492 benzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cyclohexane

Figure 13 shows the chromatograms of the D4534 calibration standard containing 8 mg/kg (ppm) benzene in cyclohexane. The injection size was 1.0 µL and the split ratio was 100:1.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Toluene

Figure 14 shows the chromatograms of the D4534 calibration standard containing 9 mg/kg (ppm) benzene in toluene. The injection size was 1.0 µL and the split ratio was 100:1. Several contaminants were found in the toluene used to prepare this standard. Most of these contaminants were identified, but the peak at 15.3 minutes did not correspond to the retention times of those listed in Table 3. If the GC systems were not retention time locked, one might assume that this contaminant could be n-propylbenzene or p-ethyltoluene. However, given the retention time precision expected with RTL, it is clear that this contaminant is an unknown.

GC/MS is the best approach to identify this unknown, but under the same GC conditions, GC/MS retention times are often considerably faster than those obtained using atmospheric detectors. However, by combining retention time locking with a technique called method translation, one can obtain GC/MS retention times nearly identical to those found with conventional GC.[4] This makes identifying unknown peaks much easier. Figure 15 shows the D4534 toluene standard run on both the Agilent 6850 and the Agilent 5973 GC/MS. A mass spectral library search of the unknown peak at 15.320 minutes identifies this compound as chlorobenzene. The source of the chlorobenzene was found to be the toluene used to prepare the standard.

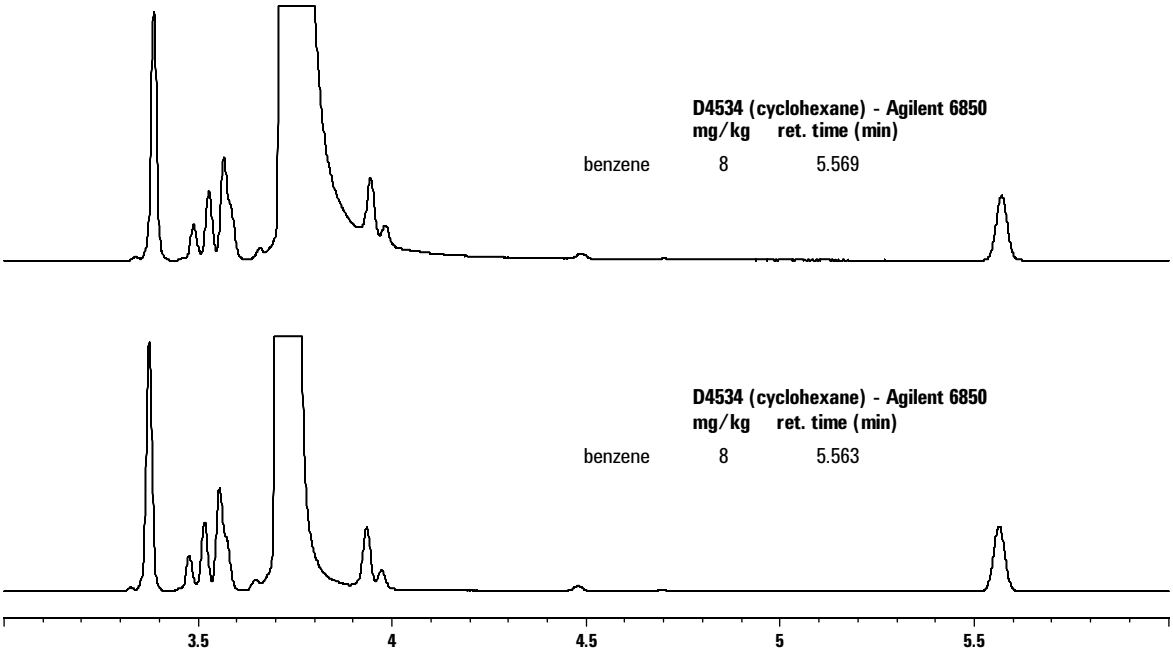


Figure 13. ASTM 4534 cyclohexane quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

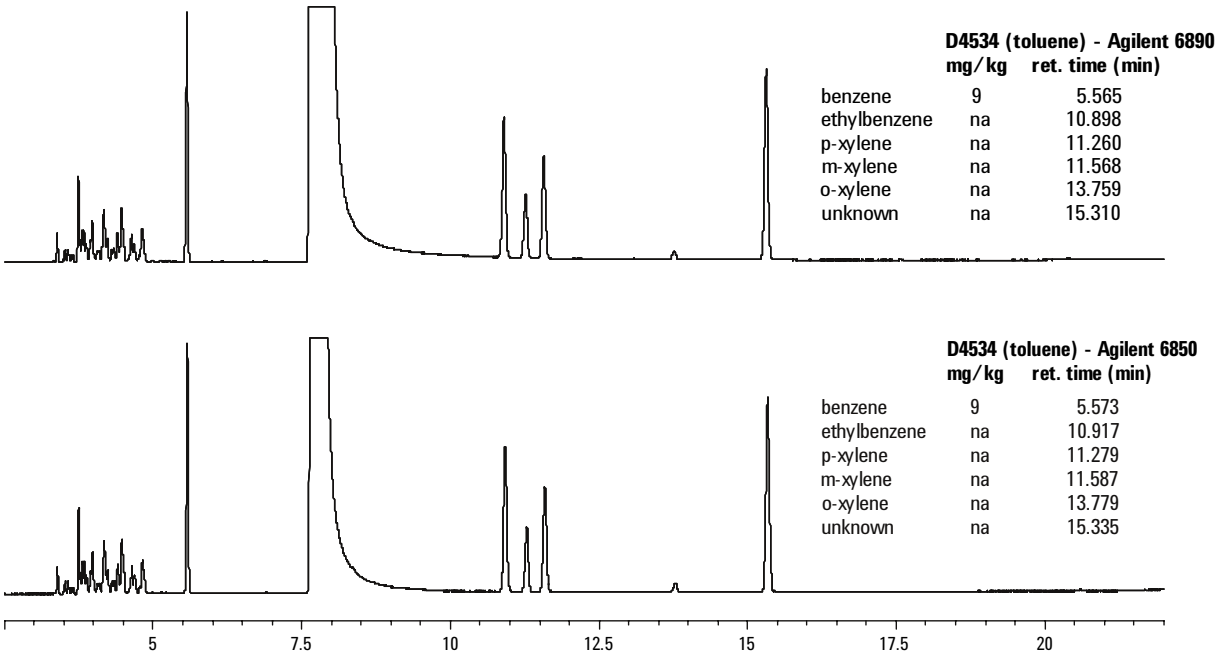


Figure 14. ASTM D4534 toluene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

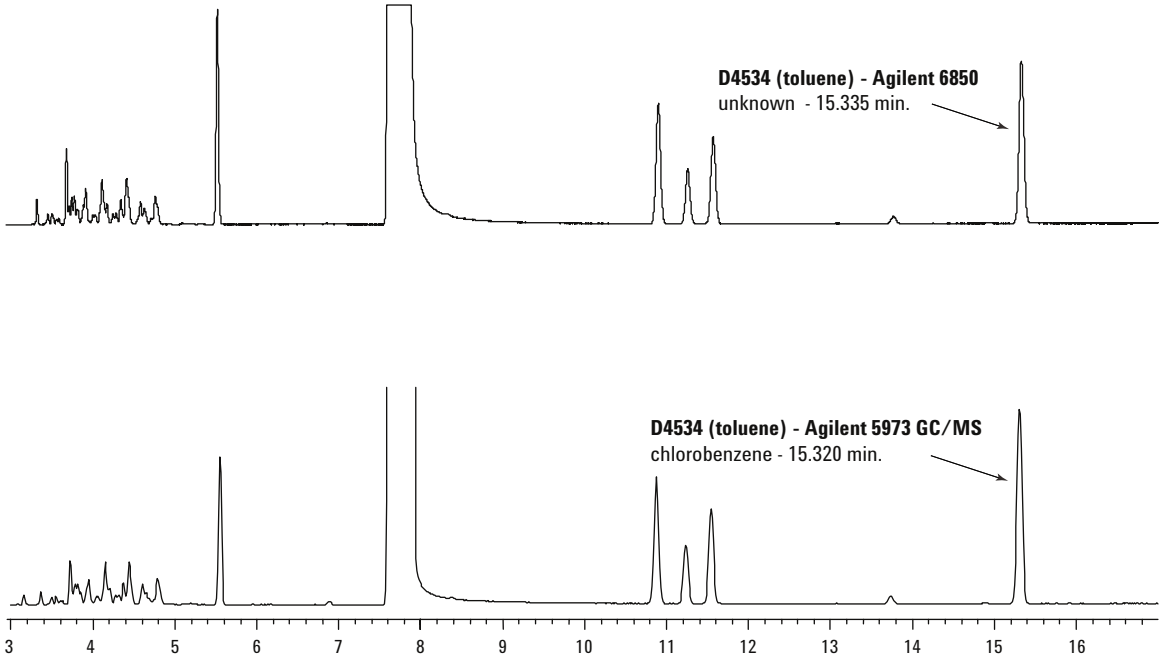


Figure 15. Unknown contaminant found in D4534 toluene standard (top) was identified as chlorobenzene using the retention time locked unified aromatics method run on the Agilent 5973 GC/MS (bottom).

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cumene

Figure 16 shows the chromatograms of the D4535 calibration standard containing 5 mg/kg (ppm) of benzene in cumene. The injection size was 1.0 µL and the split ratio was 100:1. Details of these chromatograms are shown in Figure 17. Although benzene is well resolved, there are still some C9 hydrocarbons that elute near benzene. These compounds represent a potential source of interference when measuring small amounts of benzene (less than 5 mg/kg) in cumene.

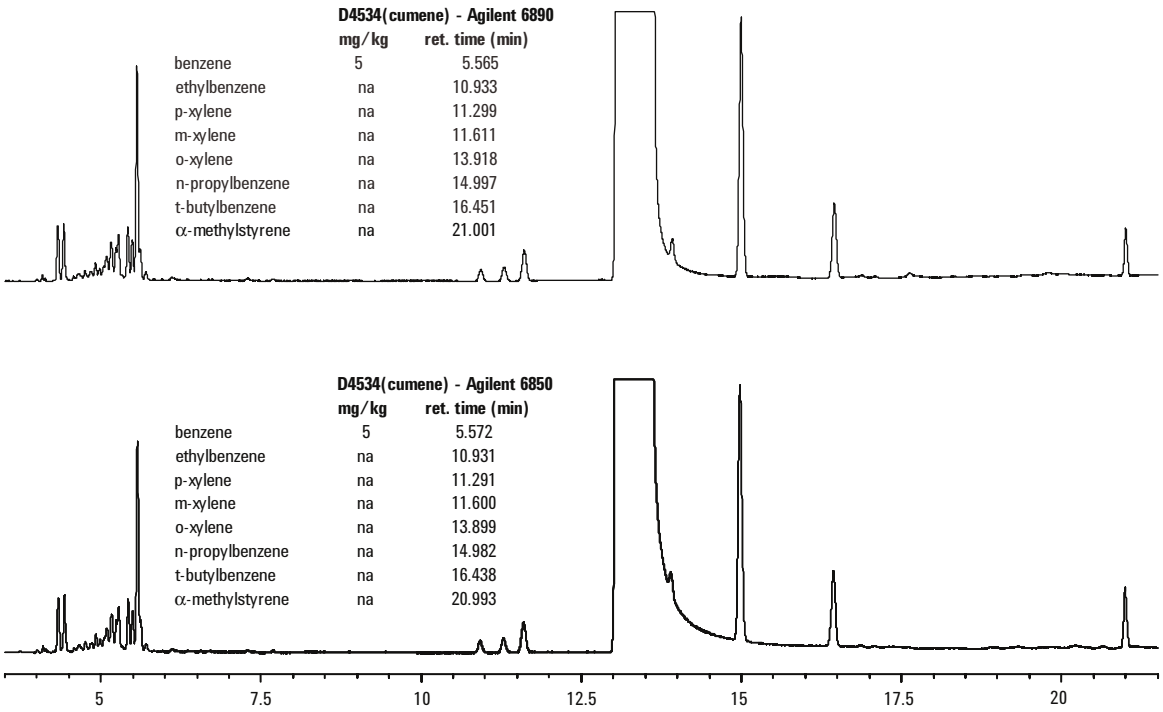


Figure 16. ASTM D4534 cumene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

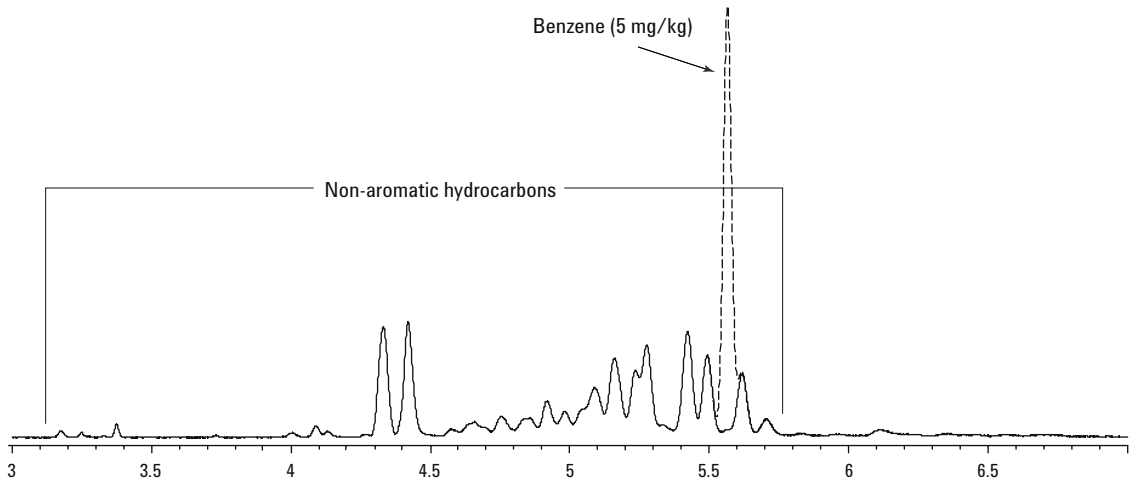


Figure 17. Details of the D4534 cumene standard showing the separation of 5 mg/kg of benzene from non-aromatic hydrocarbons typically found in cumene.

D5060 Standard Test Method for Determining Impurities in High-Purity Ethylbenzene

Figure 18 shows the chromatograms of the D5060 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

D5135 Standard Test Method for Analysis of Styrene by Capillary Gas Chromatography

Figure 19 shows the chromatograms of the D5135 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

D5917 Standard Test for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)

This method is identical to D2360 without the addition of the internal standard, n-butylbenzene, so that the chromatogram shown in Figure 7 is a good representation of an expected result. However, since n-butylbenzene is not included in the standard or samples for D5917, the run time of the unified method can be reduced to approximately 15 minutes.

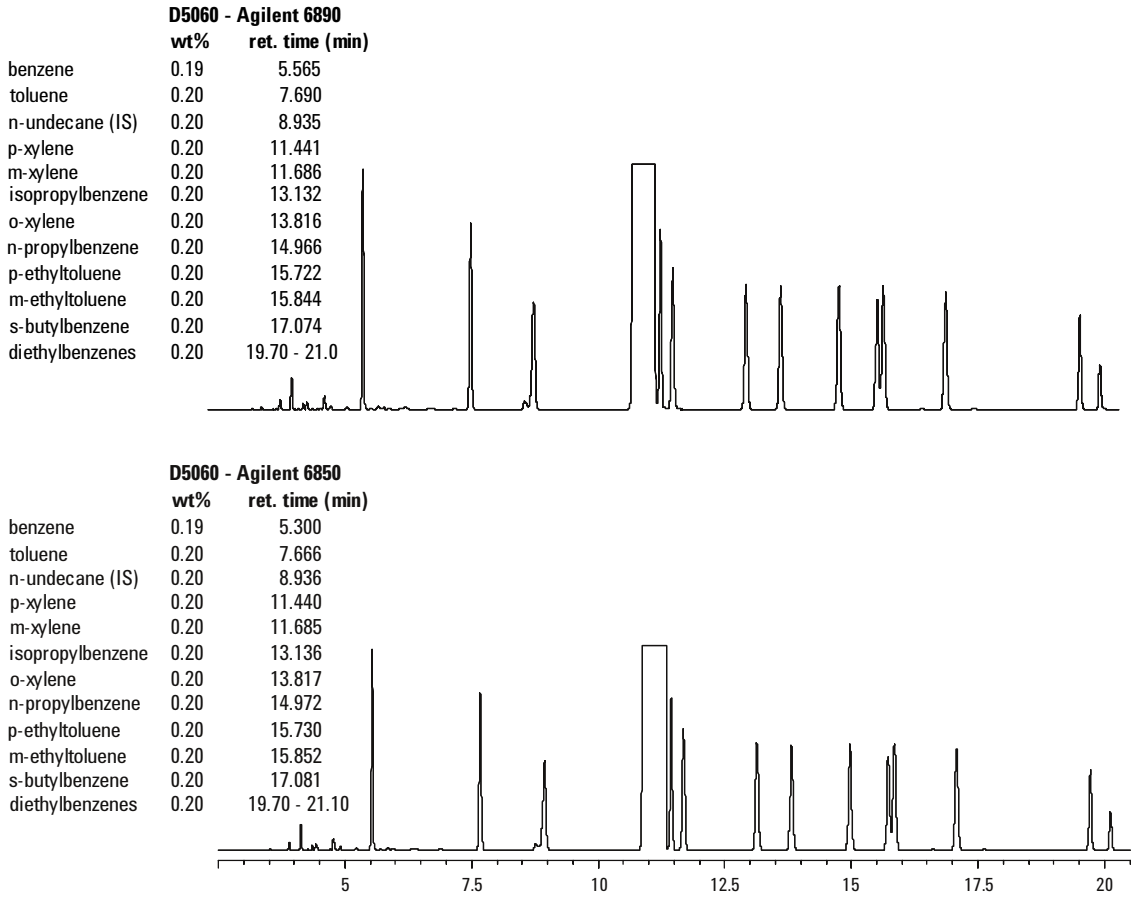


Figure 18. ASTM D5060 ethylbenzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

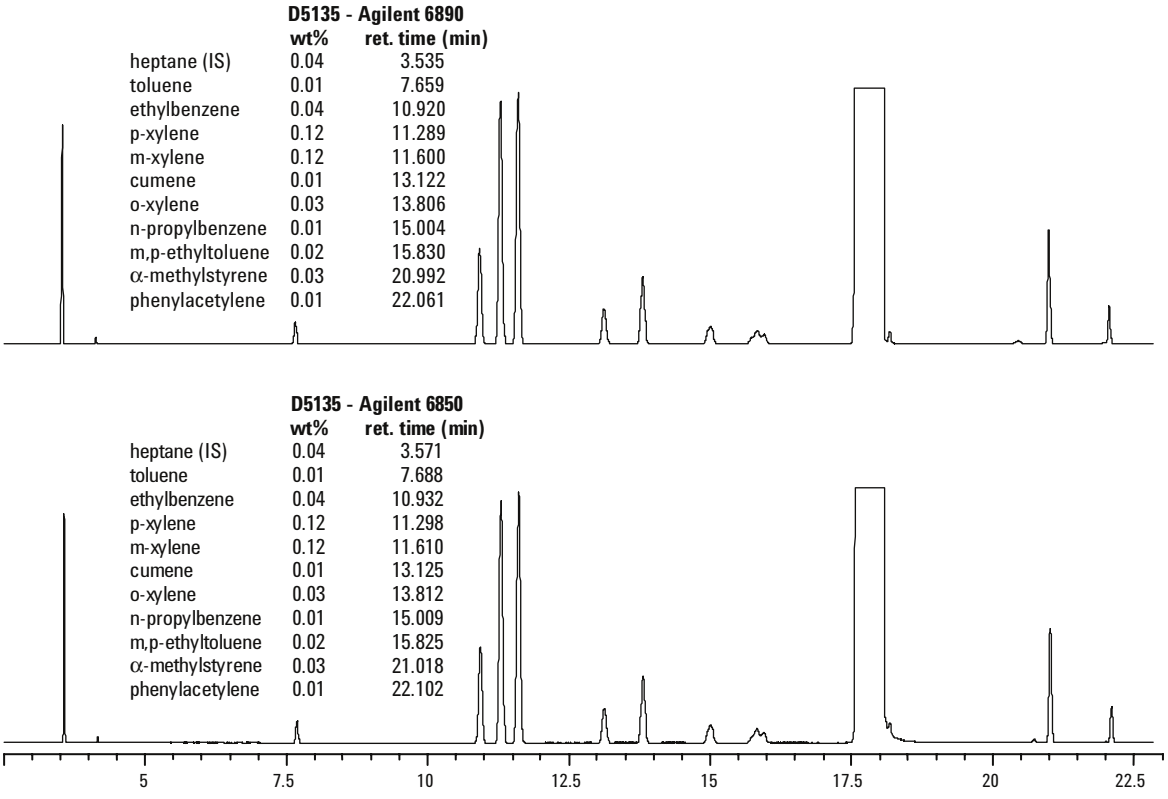


Figure 19. ASTM D5135 quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

Conclusions

The analysis of many different bulk aromatic solvents in the QA/QC laboratory presents the analyst with an array of ASTM methods specific to each material. In an effort to simplify these measurements for today's laboratory environment, the chromatographies of ten ASTM methods were consolidated into one method. This unified method can resolve the 27 compounds found in aromatic materials and can successfully run the calibration standards used by each ASTM method to determine solvent purity. This versatile method can be run on both the Agilent 6890 and 6850 GC to yield consistent results between the method development lab and the plant production lab. To further improve performance, retention time locking (RTL) was applied to the unified method so that retention time standard deviation for each compound in any sample is less than 0.03 minutes. This allows easy comparison of results between instruments, laboratories and over time. The retention time locked unified method meets the need for a fast, simple method that can be run in today's production laboratories.

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Printed in the USA
August 6, 2001
5988-3741EN



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A Unified Method for the Analysis of Aromatic Solvents Using the Agilent 6820 Gas Chromatography System

Application

Petrochemical

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Abstract

A single method for analyzing aromatic solvent purity is described. This Unified Aromatic Solvent application consolidates ten separated American Society of Testing and Materials methods into an easy-to-use gas chromatography method. An Agilent 6820 gas chromatography system configured with split/splitless inlet and flame ionization detector is used. The successful use of nitrogen as a carrier gas for this application is also demonstrated.

Introduction

Measuring and determining the purity of aromatic hydrocarbons is a critical measurement for many of the QA/QC labs in the chemical and petrochemical industry. In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and published a number of gas chromatography (GC) methods used to measure the purity of specific aromatic compounds or classes of compounds such as ethylbenzene, styrene, o-Xylene, p-Xylene, etc. These methods also measure key impurities in many of these materials [1].

Most of the China National Criteria in the petrochemical industry comply with these ASTM methods. Many labs need to run several different ASTM methods to ensure the quality of all products. This analysis can be difficult and expensive to perform. One solution to this problem is to develop a single GC method that is the chromatographic equivalent of the separate ASTM methods [2]. A single method would simplify the operation of the lab and offer a number of other advantages. Fewer GCs would replace a large number of instruments previously dedicated to individual methods, and lab space would be reduced. By running one method, any GC could serve as a backup for instruments undergoing maintenance or repair, resulting in shorter down times. A single method would also decrease the need to stock multiple columns and supplies. So, a simplified method with correct measurement performance and an easy-to-use, reliable GC system is necessary for this routine work.

This application describes a GC method that is chromatographically suitable for a wide range of aromatic hydrocarbon samples typically analyzed by 10 ASTM methods (Table 1). Considering that nitrogen (N_2) is the common carrier gas in China,

this application employs the method using N_2 as the carrier gas. The advantage for many Chinese customers is reduced cost without the loss of chromatographic performances such as resolution and response. However, there is a somewhat longer analysis time compared to Helium (He) as the carrier gas.

Table 1. Ten ASTM Methods for the GC Analysis of Aromatic Solvents

ASTM Method	Title	Capillary column type
D2306	Standard test for C8 aromatic hydrocarbons	Carbowax 50 m × 0.25 mm × 0.25 µm
D2360	Standard test for trace impurities in monocyclic hydrocarbons	Carbowax 60 m × 0.32 mm × 0.32 µm
D3760	Standard test for cumene	Carbowax 50 m × 0.32 mm × 0.25 µm
D3797	Standard test for o-Xylene	Carbowax 60 m × 0.32 mm × 0.50 µm
D3798	Standard test for p-Xylene	Carbowax 50 m × 0.32 mm × 0.25 µm
D4492	Standard test for benzene	Carbowax 50 m × 0.32 mm × 0.25 µm
D4534	Standard test for benzene in cyclic products	Packed 10% TCEPE on Chromasorb 3.7 m × 3.175 mm
D5060	Standard test for impurities in ethylbenzene	Carbowax 60 m × 0.32 mm × 0.50 µm
D5135	Standard test for styrene	Carbowax 60 m × 0.32 mm × 0.5 µm
D5917	Standard test for trace impurities in monocyclic hydrocarbons	Carbowax 60 m × 0.32 mm × 0.25 µm

Experimental

Experiments were performed on the Agilent 6820 GC equipped with a split/splitless inlet and flame ionization detector (FID). The conditions are shown in Table 2. An Agilent split liner (Agilent part number 19251-60540) and an Agilent advanced green septa (Agilent part number 5183-4759) were used. Manual injections used a 10 µL syringe with N_2 as the carrier gas. The Agilent Cerity Networked Data System for Chemical QA/QC was used for 6820 GC control, data acquisition, and data analysis.

Table 2. Conditions for the Unified Aromatic Solvents Method

GC	Agilent 6820 Gas Chromatograph
Data system	Agilent NDS Cerity for Chemical QA/QC
Inlet	Split/splitess; 250 °C, split mode, with 100:1 split ratio
Injection	1 µL
Carrier	Nitrogen, 7.6 psi at 75 °C
Column	HP-Innowax, 60 m × 0.32 mm × 0.5 µm column (Agilent p/n 19091N-216)
Oven	75 °C (23 min) with 1.3 °C /min to 100 °C (0 min) 4.4 °C /min to 145 °C (0 min) post run 220 °C (5 min)
Detector	FID, 250 °C

A 50 mL n-Hexane solution was prepared containing 0.1 wt% of 24 aromatic solvents as listed in Figure 1. The standard sample for p-Xylene, o-Xylene, ethylene, and styrene were used for injection.

Results and Discussion

Figure 1 shows a chromatogram of the hexane solution containing an aggregate of aromatic solvents and impurities. For most compounds, baseline resolution was achieved with the exception of partially resolved p-Ethyltoluene and m-Ethyltoluene. In the original ASTM method D5060, the components of this pair of ethyltoluene isomers are not resolved either [3]. Using N_2 as the carrier gas shows good resolution and response.

D3797-Standard Test Method for Analysis of o-Xylene

This test method covers the analysis of normally occurring impurities in o-Xylene and the measurement of o-Xylene purity by GC. It is suitable for setting specifications on o-Xylene and for use as an internal quality control tool where o-Xylene is used in a manufacturing process. Figure 2 shows the chromatograms of o-Xylene using N_2 as carrier gas. The injection size was 1 µL and the split ratio was 100:1. The broadening of the cumene peak was due to the reverse solvent effect of the overloaded o-Xylene, as the original ASTM D3797 method observed [4].

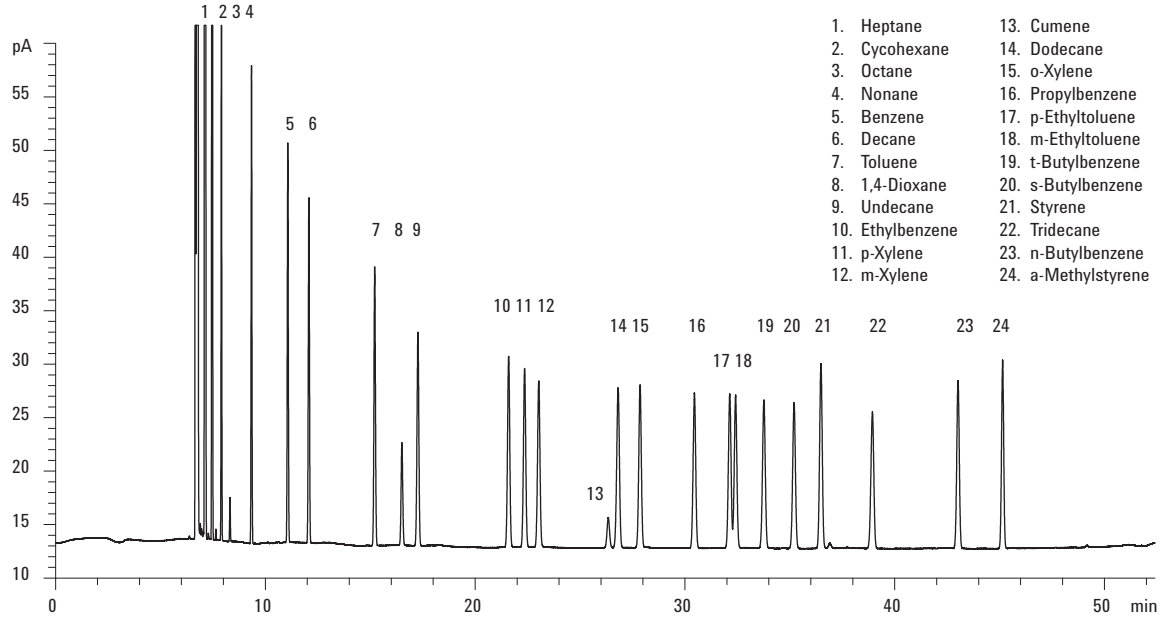

Figure 1. Chromatogram of a unified method to separate 24 compounds with N_2 as carrier gas.

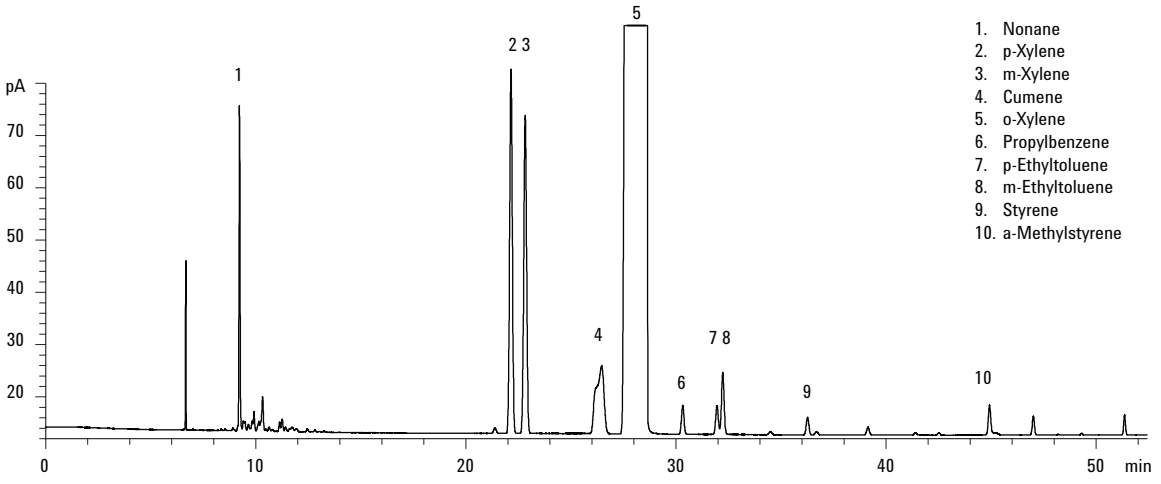

Figure 2. o-Xylene standard run on Agilent 6820 GC using N_2 as carrier gas. The split ratio was 100:1.

D3798-Standard Test Method for Analysis of p-Xylene

This test method covers the determination of known hydrocarbon impurities in p-Xylene and the measurement of p-Xylene purity by GC. It is generally used for the analysis of p-Xylene of 99% or greater purity. It is suitable for setting specifications as an internal quality control tool or development and research work. Figure 3 shows the chromatogram of the p-Xylene standard sample, using N_2 as carrier gas. The injection size was 1.0 µL and the split ratio was 100:1. The original ASTM D3798 method specifies that the valley points between the large p-Xylene peak and the ethylbenzene and m-Xylene contaminants should be less than 50% of the contaminants' peak height [5].

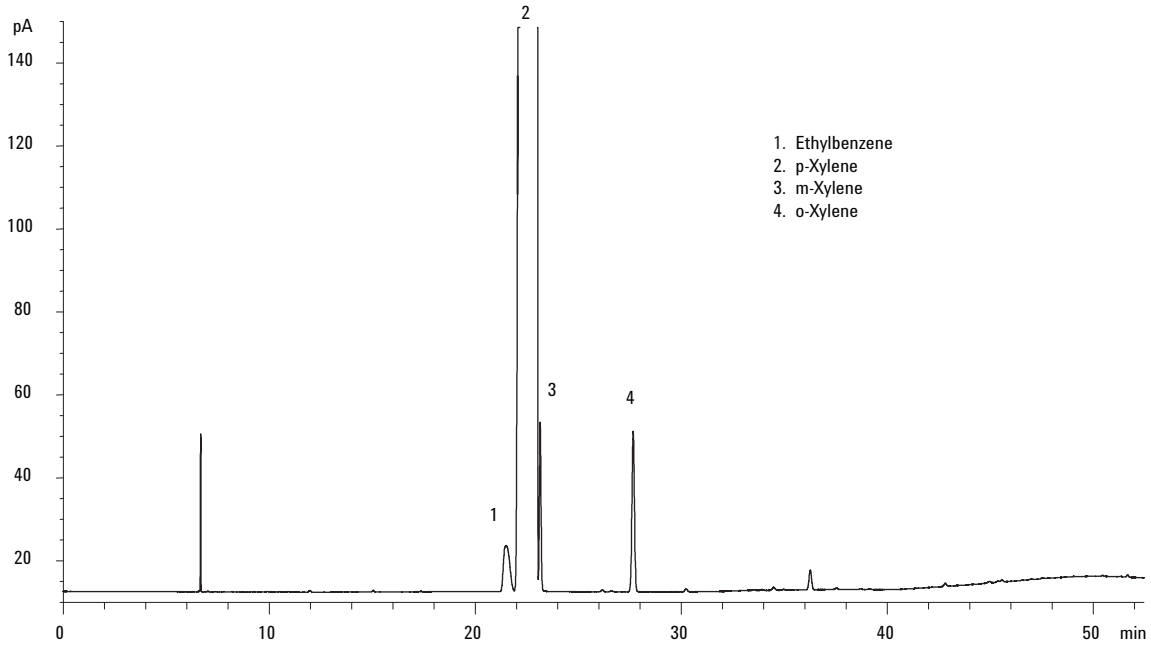

Figure 3. p-Xylene standard run on Agilent 6820 GC using N_2 as carrier gas, split ratio was 100:1.

D5060 Standard Test Method for Determining Impurities in High-Purity Ethylbenzene

The test is suitable for setting specifications on ethylbenzene and for use as an internal quality control tool where ethylbenzene is used in the manufacturing process. It may be used in development or research work involving ethylbenzene. Figure 4 shows the chromatogram of the ethylbenzene standard sample. The injection size was 1.0 µL and the split ratio was 100:1.

D5135 Standard Test Method for Analysis of Styrene

This test method covers the determination of the impurities in styrene and and the measurement of styrene by GC. It is designed to obtain styrene purity on the basis of impurities normally present in styrene and may be used for final product inspections and process control. Figure 5 shows the chromatogram of the styrene standard sample. The injection size was 1.0 µL and the split ratio was 100:1.

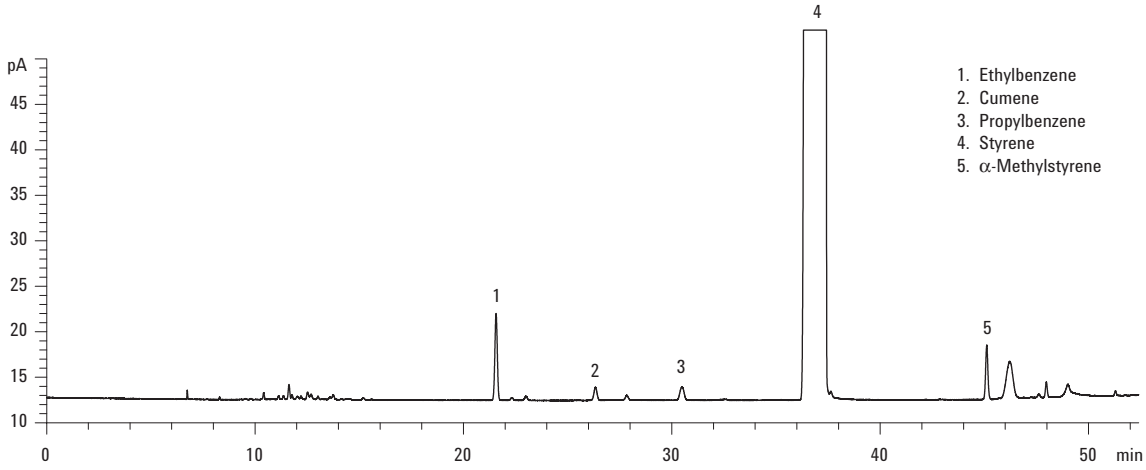

Figure 4. Ethylbenzene standard sample run on Agilent 6820 GC using N_2 as carrier gas. The split ratio was 100:1.

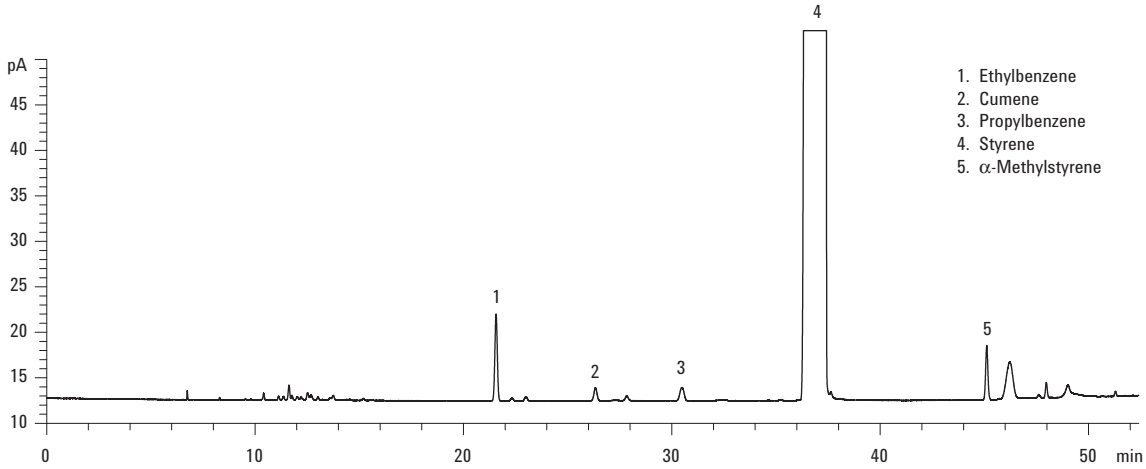

Figure 5. Styrene standard sample run on Agilent 6820 GC using N_2 as carrier gas. The split ratio was 100:1.

Conclusions

A simplified method is described for aromatic solvent purity analysis. The unified method can resolve a number of compounds found in aromatic materials and can successfully run the calibration standards used by each ASTM method to determine solvent purity. The Agilent 6820 GC system shows good applicability by using N_2 as the carrier gas. The single method with the Agilent 6820 GC system is easy to set up for determination of the aromatic solvent purity in the routine lab; it also decreases the need to stock multiple columns and supplies.

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Printed in the USA
May 7, 2003
5988-9278EN



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Analysis of Trace (mg/kg) Thiophene in Benzene Using Two-Dimensional Gas Chromatography and Flame Ionization Detection

Application

Petrochemical

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USA

Abstract

A two-dimensional gas chromatography system using a simplified Deans switch design is used for the analysis of trace (mg/kg) thiophene in benzene. This system uses two GC columns of different selectivity, INNOWax and PLOT Q, to completely separate the thiophene from the interfering sample matrix. Because of this high resolution, standard flame ionization detectors can be used in place of more expensive and complex sulfur selective detectors. The system provides precise qualitative and quantitative analyses results that are in agreement with those using sulfur selective detectors. This versatile system can also perform a standard American Society for Testing and Materials benzene purity analysis at the same time the thiophene measurement is made.

Introduction

Benzene is an important feedstock for the production of a number of key commodity chemicals. Many of these processes use expensive catalysts that can be easily poisoned by small amounts of sulfur containing impurities. In an effort to reduce costs, improve efficiency, and increase product quality, the sulfur specifications in benzene have been continuously lowered. Thiophene is a common sulfur impurity found in benzene and is difficult to analyze by gas chromatography (GC) at trace levels (mg/kg). This is because thiophene is an aromatic compound with a boiling point and molecular weight that is similar to benzene, therefore it has nearly the same chromatographic behavior as benzene. Successful GC analysis of thiophene has been performed using non-quenching selective detectors such as sulfur chemiluminescence (SCD) and atomic emission detectors (AED) [1]. However, these detectors are expensive and much more complex than a flame ionization detector (FID).

Two-dimensional gas chromatography (2-D GC) offers another approach to this analysis. Two columns of different selectivity can be combined to completely separate thiophene from benzene and other chromatographic interferences. Once the thiophene is completely separated from the large benzene peak, it can be easily detected using an FID. The keys to successful 2-D GC analysis are the column selection and the device that couples together the two columns. A 2-D GC system developed for the analysis of oxygenates and aromatics in gasoline uses a simplified Deans switch as the column-coupling device [2]. This application note describes a 2-D GC application using a similar Deans switch system for the analysis of trace thiophene in pure benzene.

Experimental

An Agilent 6890N gas chromatograph was equipped with a split/splitless injector, a pneumatics control module (PCM), two FIDs, and an automatic liquid sampler (ALS). An HP-INNOWax column was used as the primary column and an HP-PLOT Q was used as the secondary column. The two columns were linked using a simplified fluidic Deans switch. Figure 1 shows the instrument configuration using this hardware and Table 1 lists the details of the hardware configuration. The instrument operating conditions are outlined in Table 2 for this analysis. For all standards and samples, the injection volume was 4 µL.

Determination of electronic pressure control (EPC) pressures, flow rates, and the fixed restrictor dimensions were performed using a Deans switch calculator software program that is specially designed for this system. This calculator program is included with the Deans switch hardware option

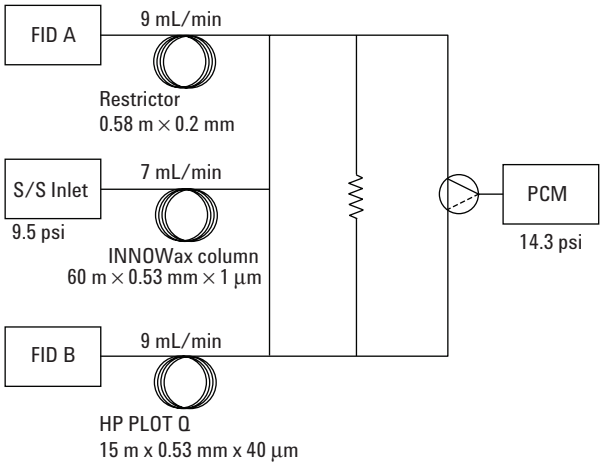

Figure 1. Deans switch configuration for the analysis of trace thiophene in benzene with an FID. A preliminary separation is performed using the primary INNOWax column. The thiophene peak is heart-cut from the INNOWax column to the secondary HP-PLOT Q column. Complete separation of the thiophene from benzene is then performed on the PLOT Q column.

Table 1. Agilent 6890 Hardware Configuration for 2-D GC

Standard 6890N GC hardware	
G1540N	Agilent 6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210 (2 of each)	FID with EPC control
Option 309	Pneumatics control module with EPC control
SP1 2310-0129	General purpose Deans switch kit, factory installed (may be ordered as an add-on kit for existing 6890 GC Agilent part no. G2855A)
G2613A	Agilent 7683 Autoinjector
Columns	
Primary column	INNOWax column, 1.00 µm film, 60 m × 0.53 mm id (Agilent part no. 19095N-126)
Secondary column*	HP PLOT Q column, 40 µm film, 15 m × 0.53 mm id (Agilent part no. 19095P-Q03)
Fixed restrictor*	Deactivated fused silica tubing, 0.58 m × 0.2 mm id (Agilent part no. 160-2205-10)
Data system	
G2070A	Agilent Multitechnique ChemStation
Other consumables	
Agilent part no. 5181-1267	10-µL Fixed tapered needle autoinjector syringe
Agilent part no. 5183-4647	Inlet liner optimized for splitless operation
Agilent part no. 5183-4759	Advanced green septa

*The Deans switch calculator was used to determine the correct EPC pressures for column flows and the dimensions of the fixed restrictor. Since the film thickness of the PLOT Q column is large (40 µm), the effective id of this column was reduced to 0.45 µm for the purpose of determining the column pressures and flows.

Table 2. Instrument Conditions

Injection port	Splitless mode
Temperature	200 °C
Pressure*	14.33 psi helium, constant pressure mode
Purge time	0.75 min.
Purge flow	100 mL/min
Injection size	2 µL–4 µL, splitless
INNOWax column flow	7 mL/min, constant pressure mode
Pneumatics control module (PCM)*	9.46 psi helium, constant pressure mode
HP PLOT Q column flow	9 mL/min
FID temperatures	250 °C
Oven temperature program:	
Initial temp	60 °C for 1 min
Ramp #1	25 °C/min to 80 °C for 9 min
Ramp #2	50 °C/min to 140 °C for 9 min
Ramp #3	35 °C/min to 225 °C for 0 min

*These pressures were calculated using a custom Deans switch software program to achieve the necessary column flow rates.

for the Agilent 6890N GC. All instrument set points required for any successful 6890N based Deans switch application can be quickly and easily determined using this software.

For the preparation of calibration standards, HPLC grade 99.9+% benzene (Sigma-Aldrich part no. 27,070-9) and 99+% thiophene (Sigma-Aldrich part no. T3,180-1) were used. The HPLC grade benzene was selected because it contained less than 0.003 mg/kg of thiophene contamination. This benzene was further purified by a three-step recrystallization. Calibration standards were prepared in the purified benzene that contained 0.02, 0.05, 0.1, 0.2, 0.5, 1, and 2 mg/kg of thiophene in benzene.

Heart-cut times for this analysis were determined by injecting the 2 mg/kg calibration standard into the 2-D GC system while keeping the fluidic switch in the “off” position. This allowed the standard to be separated only with the primary INNOWax column and detected using FID A (Figure 1). From this chromatogram, the cut times for thiophene were calculated using the peak start and end times.

A second gas chromatograph with an atomic emission detector (GC-AED) was used to perform a crosscheck to the 2-D GC system for the analysis of thiophene. The GC-AED was equipped with the same INNOWax capillary column listed in Table 1 and the AED was configured for sulfur selective detection. The GC-AED was also used to evaluate the thiophene content in the benzene used as a blank and for the calibration standards.

Results and Discussion

Heart-cut time setting – The 2 mg/kg calibration standard was run 12 times over a 24-hour period with no heart cutting. This established the retention time (RT) precision of the thiophene peak on the INNOWax column so that the heart-cut time could be determined. Figure 2 shows an overlay of these 12 chromatograms. The average RT for thiophene was 8.055 minutes with a relative standard deviation of 0.1%. The average peak start time was 7.995 minutes (%RSD = 0.1) and the average peak end time was 8.137 minutes (%RSD = 0.1). With this high degree of RT precision, a narrow heart-cut between 7.95 and 8.18 minutes was used

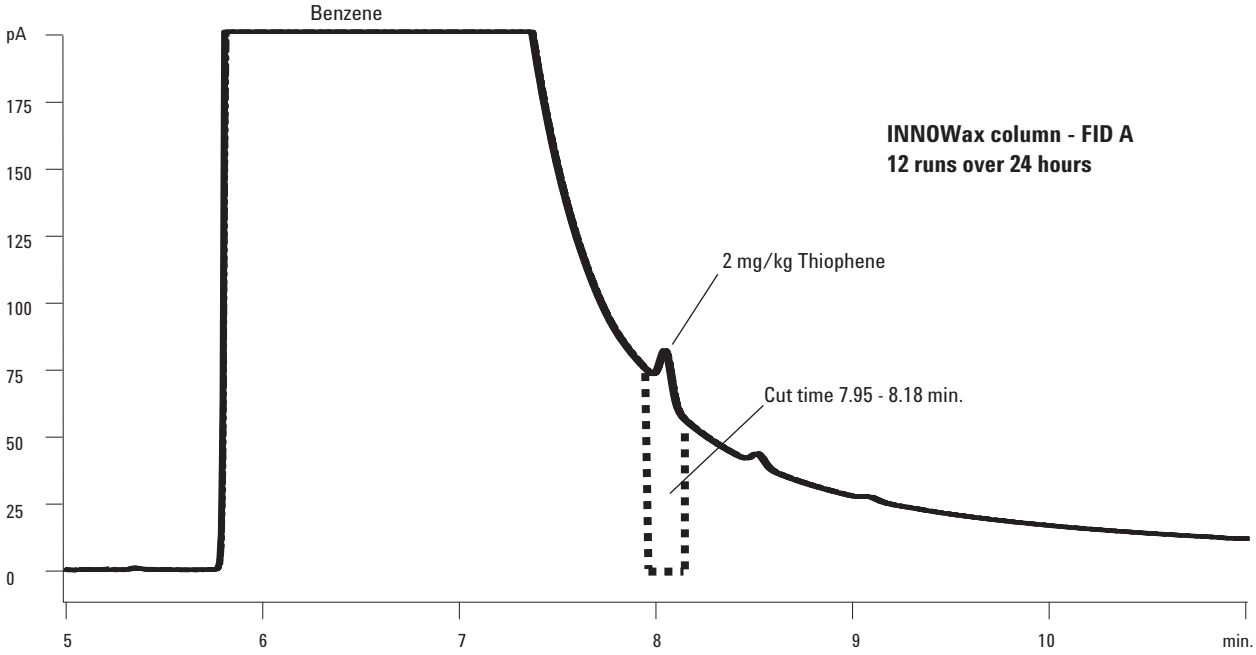

Figure 2. Twelve separate runs of the 2 mg/kg thiophene standard were made over a 24-hour period. The excellent RT precision allows a narrow heart-cut time from 7.95 to 8.18 min for the thiophene peak.

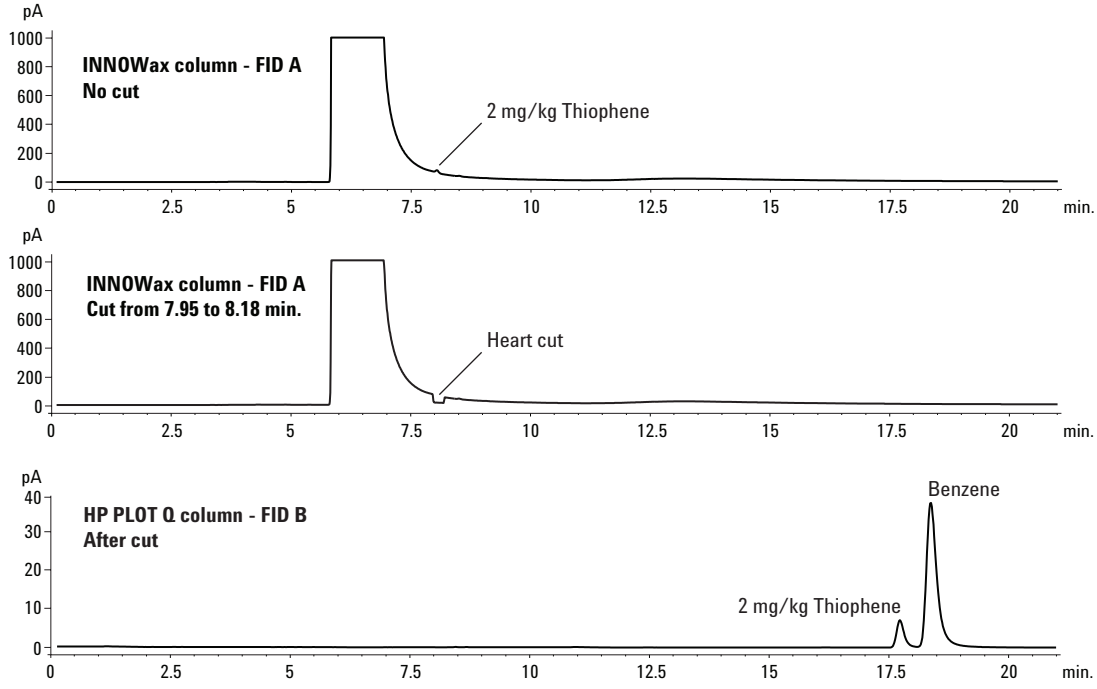

Figure 3. The upper chromatogram shows the 2 mg/kg standard separated on the INNOWax column with no heart cutting. In the middle chromatogram, the Deans switch transfers the INNOWax column eluent to the HP-PLOT Q column. The lower chromatogram shows the separation of this heart cut on the PLOT Q column. Note that the thiophene is well separated from the benzene matrix and elutes before the benzene on the PLOT Q column.

to transfer the thiophene peak from the INNOWax column to the PLOT Q column. The dimensions of the fixed restrictor were determined using the Deans switch calculator software to give a negligible hold-up time (0.002 minutes) from the INNOWax column to FID A. Therefore, the measured peak start and end times can be directly used to determine the heart-cut window.

Using this cut window, the 2 mg/kg standard was run on the 2-D GC system so that the thiophene peak was automatically cut from the INNOWax column to the HP-PLOT Q column (Figure 3). Since thiophene is not completely separated from the large benzene peak on the INNOWax column, a portion of the benzene peak was also cut to the HP-PLOT Q column. After transfer to the PLOT Q column, the thiophene was completely separated from the residual benzene (Figure 3). The selectivity of the PLOT Q column was quite different from the INNOWax column. This is shown in Figure 3 where the thiophene peak was observed to elute before the benzene peak on the PLOT Q column. Having the thiophene elute first also made peak integration easier since the small thiophene peak was not eluting in benzene tail.

Calibration – A seven-point calibration was made using standards from 0.02 mg/kg to 2 mg/kg. Figure 4 shows the linear regression results of this calibration. The use of an FID greatly simplifies the detection and quantitation of sulfur compounds since it has a linear response over a large dynamic range instead of the quadratic response of some sulfur selective detectors (that is, flame photometric detector [FPD], and pulsed flame photometric detector [PFPF]).

Sample analysis – Two benzene samples were run using the 2-D GC system for the analysis of thiophene. The first sample was commercially purchased and described as “thiophene-free.” The second sample was industrial benzene used as a styrene feedstock. Each sample was analyzed a total of 15 times over a 5-day period using the same GC conditions and heart-cut window used for the calibration standards. Figures 5 and 6 show the chromatographic results for these samples obtained with this 2-D GC system. The precisions for RT, thiophene peak area, and quantitative results are shown in Table 3 for both samples. The high precision for the RT and quantitative results obtained with the 2-D GC system shows the stability of the Deans switch design.

Table 3. 2-D GC Analysis Results for Two Benzene Samples

	"Thiophene free"	Styrene feed
RT (min)	17.718	17.718
(% RSD)	(0.03)	(0.03)
Amount (mg/kg)	0.2	0.06
(% RSD)	(2.2)	(4.8)

A GC-AED system was also used to analyze thiophene in these two benzene samples. Ten runs per sample were made over a 3-day period. For the “thiophene-free” benzene, 0.2 mg/kg of thiophene (% RSD = 1.0) was measured and for the styrene feed sample, 0.05 mg/kg of thiophene (%RSD = 1.8). These results compare favorably with those obtained on the 2-D GC system (Table 3).

The standard benzene analysis described by ASTM method D4492 uses a 60-meter INNOWax column to measure overall benzene purity and key impurities such as hydrocarbons, toluene, and 1,4-dioxane [1]. Since this 2-D GC system also uses INNOWax as the primary column, this system can also be used to perform this standard benzene analysis at the same time as the thiophene determination is made.

On an INNOWax column, C_8 and C_9 hydrocarbons are known to co-elute with benzene. These hydrocarbons represent a potential interference

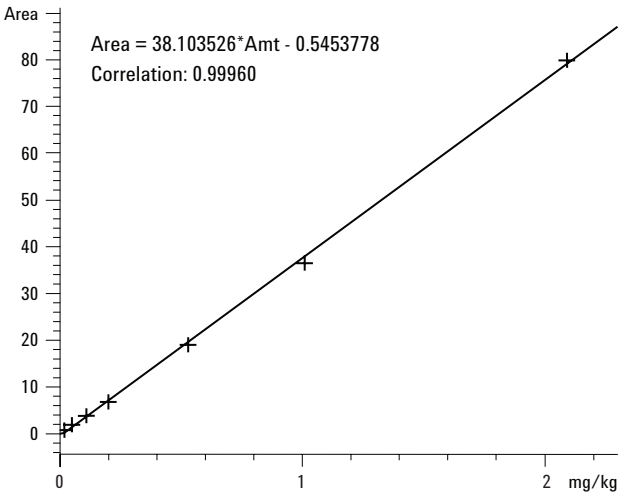

Figure 4. Seven-point calibration using 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 mg/kg thiophene in benzene standards.

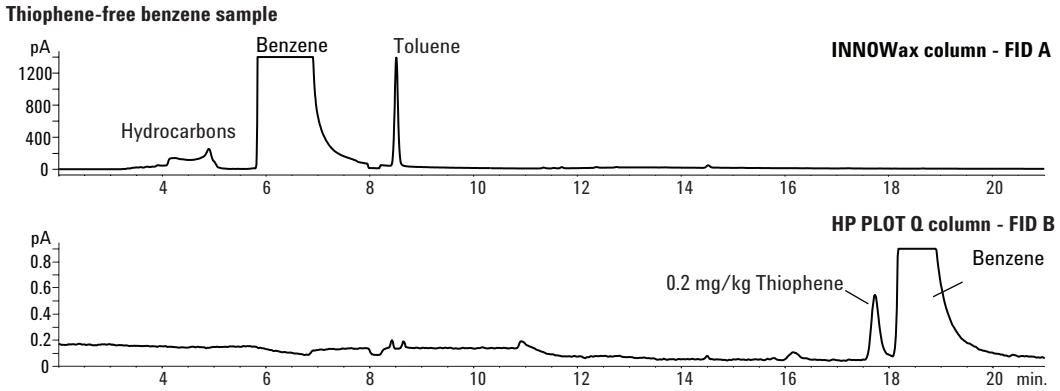

Figure 5. Analysis of thiophene for thiophene-free benzene using the 2-D GC system.

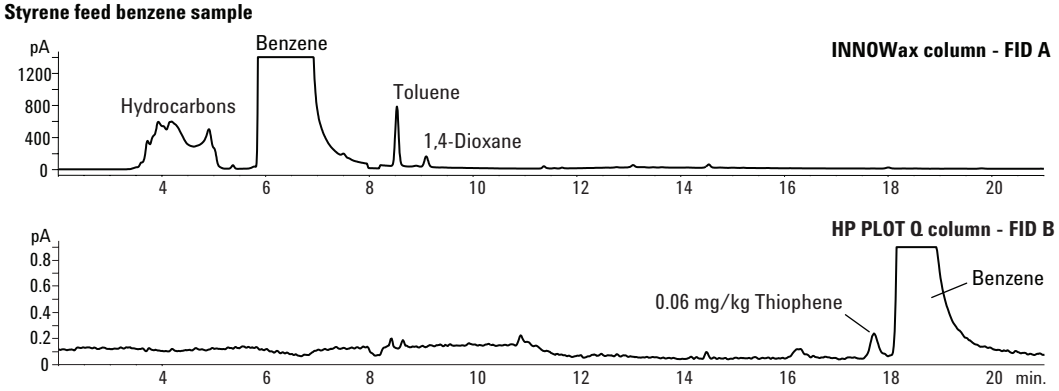

Figure 6. Analysis of thiophene for styrene feed benzene using the 2-D GC system.

with thiophene if they are in the heart-cut and cannot be resolved by the PLOT Q column. A benzene blank sample was spiked with 0.2 wt% of petroleum naphtha containing a number of C_8 and C_9 hydrocarbons. This sample was analyzed using the 2-D GC system under the same conditions as for the thiophene analysis. No hydrocarbon peaks were observed on the PLOT Q column at the RT where thiophene was expected (Figure 7). Therefore, any hydrocarbons contained in the heart cut were successfully separated from thiophene by the PLOT Q column.

Since the PLOT Q column was shown to completely separate thiophene from benzene, one would be tempted to use this column alone without the 2-D GC. However, to achieve the desired sensitivity, a 2 to 4 µL splitless injection is required. Since PLOT columns have very low capacities, an injection of this size would overload the column with benzene with a resultant loss of resolution. The 2-D approach described in this application note uses the INNOWax column to greatly reduce the amount of benzene that is loaded on the PLOT Q column. This assures that the thiophene can be separated and detected in a pure benzene matrix. Additionally, 2-D GC eliminated any other potential chromatographic interferences, such as hydrocarbons, so that nonselective GC detectors can be used for trace thiophene determination.

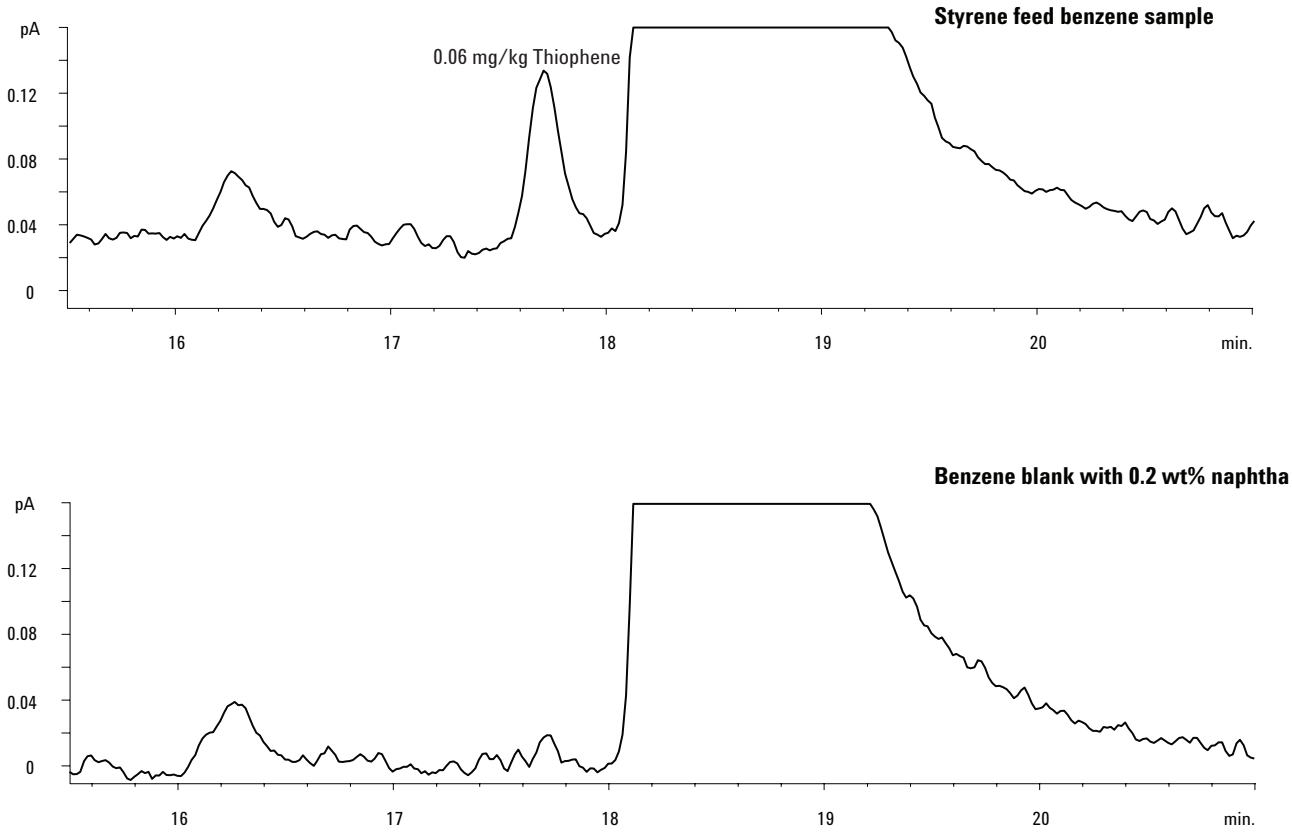

Figure 7. The upper chromatogram shows a the elution of thiophene on the PLOT Q column after heart-cutting. In the lower chromatogram, a benzene blank was spiked with 0.2 wt% of petroleum naphtha and analyzed by the 2-D GC system. No hydrocarbon interferences were observed to elute at the same time as the thiophene peak.

Conclusion

The 2-D GC system described in this application note was used to measure trace (mg/kg) quantities of thiophene in pure benzene using standard flame ionization detection. The use of two columns with different selectivity completely separates the thiophene peak from the benzene and other interfering matrix compounds. This system was shown to be precise and stable over extended periods of time. The quantitative results for thiophene that can be obtained with this system are nearly identical with those obtained using more expensive and complex GC systems with sulfur selective detectors. Additionally, the system was also shown to be versatile and cost effective since it can perform both the thiophene determination and overall benzene purity analysis in one run, on one system.

References

1. Annual Book of ASTM Standards, Vol. 06.04 “Paint - Solvents; Aromatic Hydrocarbons,” ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA.
2. McCurry, J.D. and Quimby, B.D., “Two-dimensional Gas Chromatographic Analysis of Oxygenates and Aromatics in Gasoline Using a Heart-Cutting Technique,” Agilent Technologies, publication 5988-6696EN www.agilent.com/chem

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Printed in the USA
April 24, 2003
5988-9455EN



Agilent Technologies

Two-Dimensional Gas Chromatographic Analysis of Trace Benzene in Styrene

Application

HPI

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Abstract

A two-dimensional gas chromatographic method for the analysis of trace benzene in styrene is described. This method employs the Agilent 6890N GC system equipped with a Deans switch device. The method can be used in a prefractionator mode to analyze trace benzene in styrene according to the proposed ASTM method [1]. It can also be used in a heart-cutting mode to transfer more precisely just the benzene peak from the primary column to the secondary column. The Agilent 6890N electronic pneumatics control and flow calculation software are used for setting up the precise flows and pressures required. Excellent calibration and sensitivity of this system is demonstrated in both prefractionator mode and heart-cutting mode, and the interference can be greatly reduced by the heart-cutting.

Introduction

The American Society of Testing and Materials (ASTM) method D5135 uses a single column GC to measure the overall purity of styrene, including benzene down to 1000 ppm [2]. However, industry now desires to lower the benzene content in styrene to 10 ppm. Styrene also contains trace amounts of C_8 and C_9 hydrocarbons that cannot be separated from trace benzene with a single GC column. A fast analytical method for trace benzene in styrene is needed for final product inspections, process control, establishing specifications, and research work.

ASTM has proposed a two-dimensional gas chromatography (2-D GC) method to completely separate trace levels of benzene from other contaminants in styrene. This method operates in a prefractionator mode where the primary column retains the high boiling styrene components while the low boiling contaminants (including benzene) are transferred to a second column. After the transfer is complete, the benzene is easily separated from the interfering hydrocarbons on the second column. This method uses two columns of different polarity coupled together with a device that allows one or more discrete effluent segments to be transferred from the first to the second column. There are two different switching devices that can be used with this method. The first device uses a rotary valve to transfer the benzene from column one to column two. The second uses a fluidic switch to transfer the benzene. The fluidic switch is similar in design

to the Agilent Deans switch for the 6890N GC system. While the Agilent Deans switch works well as a prefractionator, it can also be used in a heart-cutting mode to offer more precise transfer of benzene from the first column to the second column, thus further improving chromatographic resolution.

Two-dimensional GC is an old idea that was first demonstrated by Deans over 30 years ago. Although the Deans switch approach was useful, it did not gain widespread use because of problems associated with unreliable column connections, column flow drift, oven temperature imprecision, and column variability [3]. These problems caused poor overall retention time (RT) precision, thus forcing the analyst to use very wide cut times to ensure transfer of the target analyst from column one to column two. These wider cut times resulted in the transfer of more interference peaks and a loss in overall resolution.

Advanced technology of the Agilent 6890N GC improves the performance of 2-D GC. It makes the construction of 2-D GC systems much simpler and easier to use. Modern column connections have low dead volume and are inert and reliable. The Agilent 6890N electronic pneumatics control (EPC) and oven controls provide better RT precision that allows narrower cut times for better resolution and quantitative precision. New Deans Switch Software used for calculating pressures makes the 2-D GC method development faster and easier.

Experimental

Deans Switch System Design

Heart-Cutting Mode

Figure 1 shows the Agilent 6890N GC system configured with the Deans switch hardware. The sample is injected into the split/splitless (S/S) inlet and is initially separated on the non-polar methyl silicone (HP-1) primary column. With the fluidic switch in the "off" position, the pneumatics control module (PCM) delivers 8.54 mL/min of helium through the lower flow path. This supplies 6.54 mL/min of helium to the secondary column coated with a polar polyethylene glycol (INNOWax) phase. The remaining 2 mL/min is used to divert to the HP-1 column eluent to flame ionization detector (FID) A.

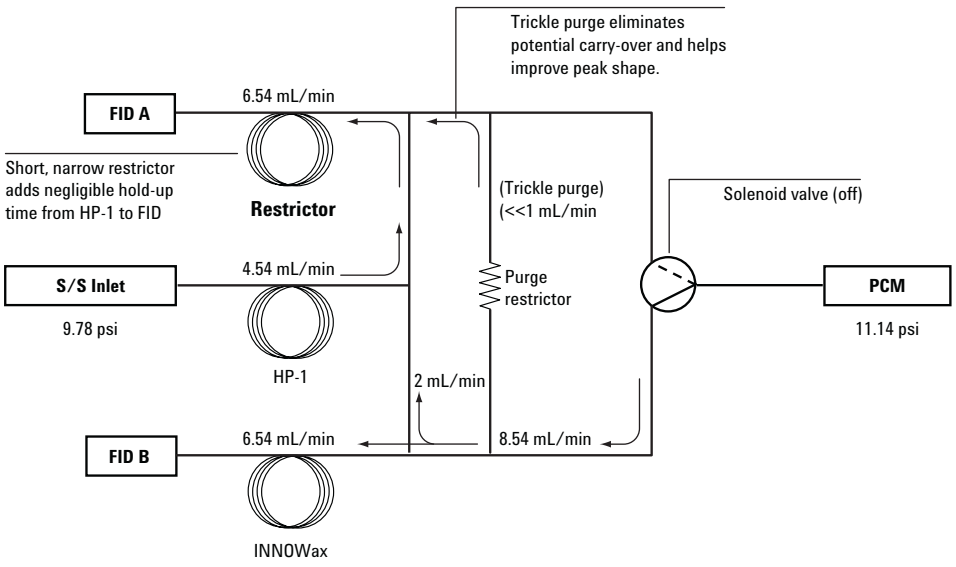

Figure 1. Solenoid valve off - no heart-cutting.

Figure 2 shows the flow path when heart-cutting with the Deans switch. Just before the benzene and any unresolved hydrocarbons elute from the HP-1 column, the fluidic switch is set to the "on" position. Helium from the PCM is now directed through the upper flow path so that 6.54 mL/min goes through the restrictor and 2 mL/min diverts the benzene and the hydrocarbons from the HP-1 column to the INNOWax column. The benzene is then separated from the hydrocarbons by the INNOWax column and detected on FID B. After the peaks are loaded onto the INNOWax column, the fluidic switch is returned to the "off" position shown in Figure 1, so that any peaks still on the HP-1 column are eluted to FID A.

Prefractional Mode

The Agilent 6890N Deans switch can also be used as a prefractionator. With the solenoid valve in the "on" position (Figure 2), the sample is injected into the S/S inlet and a partial separation is made on the nonpolar HP-1 column. The PCM delivers 8.54 mL/min of helium through the upper flow path. This supplies 6.54 mL/min of helium to the restrictor and 2 mL/min transfers fractions that are boiling point equal or less than benzene to the INNOWax secondary column.

When the benzene and lighter compounds are transferred to the INNOWax column, the fluidic switch is set to the "off" position, shown in Figure 1. The PCM delivers 8.54 mL/min of helium through the lower flow path. This supplies 6.54 mL/min of helium to the INNOWax column to separate the benzene from the hydrocarbons with detection on FID B.

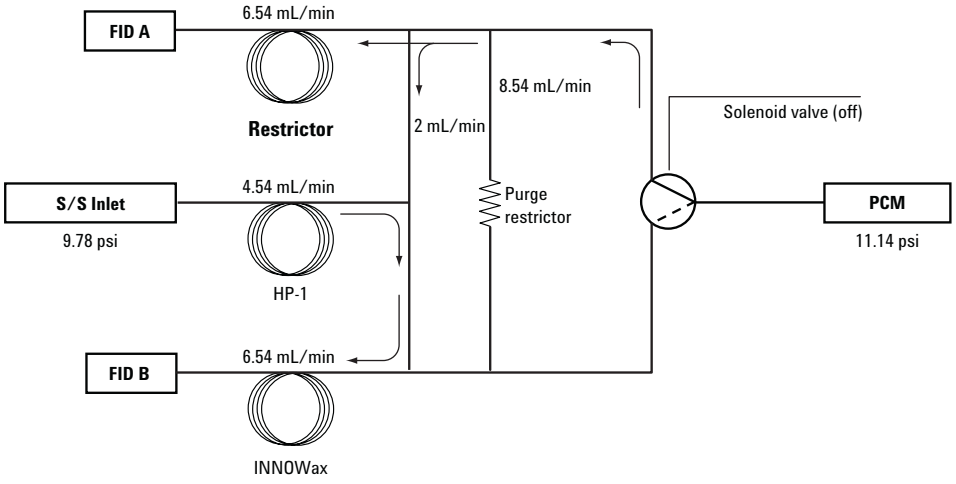

Figure 2. Solenoid valve on heart-cutting from HP-1 to INNOWax column.

Method Development Tool - Deans Switch Calculator

A powerful method development tool, Deans Switch Calculator, is used to easily set the correct column flows, inlet pressure, PCM pressure, and restrictor size. After the method parameters are decided, they are entered into designated fields in the software (shown in Figure 3). The Deans Switch Calculator will then determine the pressure needed to obtain the desired flow rates from the primary column inlet and the PCM. The software will also calculate the dimension for the fixed restrictor.

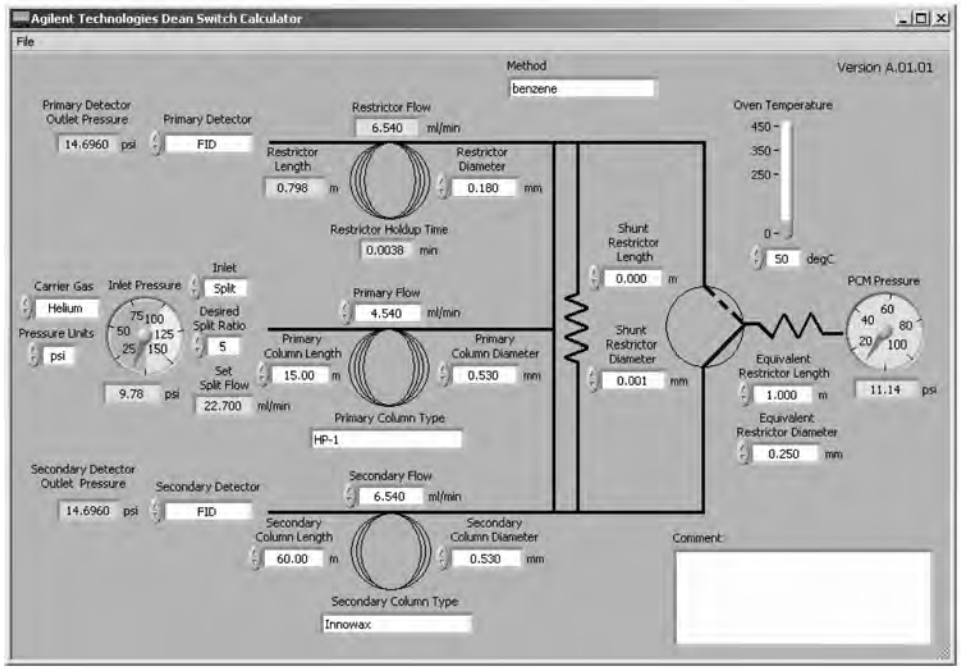

Figure 3. Deans Switch Calculator.

Hardware Configuration

The details of the system hardware for this analysis are listed in Table 1.

Table 1. Agilent 6890 Hardware Configuration

Standard 6890GC hardware		
G1540N	Agilent 6890N series GC	1
112	Capillary split/splitless inlet with EPC control	1
210	FID with EPC control	2
309	PCM with EPC control	1
G2613A	Agilent 7683 autoinjector	1
2310-0129	Deans switch kit	1
Columns		
Primary column	HP-1 15 m, 0.53 mm, 3.0 µm (p/n 19095Z-421)	1
Secondary column	INNOWax 60 m, 0.53 mm, 1.0 µm (p/n 9095N-126)	1
Data system		
G2070A	Agilent multitechnique ChemStation rev. A.08.01	
Optional consumables		
5812-3442	Merlin microseal high-pressure septum	
5182-0875	5-µL fixed straight needle autoinjector syringe for Merlin Microseal	
5183-4647	Inlet liner optimized for split operation	
Standards		
	Benzene, 99.8% minimum purity	
	Styrene, the highest purity available, but not less than 99.6%	

Instrument Conditions

Instrument conditions are listed in Table 2.

Table 2. Instrument Conditions

Split/Splitless inlet	Split mode, with 5:1 split ratio
Temperature	250 °C
Pressure	9.78 psi helium
Split ratio	5:1
HP-1 Column flow	4.54 mL/min, constant pressure mode
INNOWax column flow	6.54 mL/min, constant pressure mode
PCM	11.1psi
FID Temperatures	250 °C
Oven temperature program	50 °C for 4 min, 20 °C/min to 220 °C, for 2 min
FID H_2 flows	40 mL/min
FID Air flows	450 mL/min
FID Makeup gas (He) flows	45 mL/min

Results and Discussion

Heart-Cutting Mode

Agilent Deans switch design can be used in a heart-cutting mode, the results show very good sensitivity, precision, and linearity. The heart-cutting mode can precisely transfer just the benzene peak from the primary column to the secondary column.

Sensitivity

Figure 4 shows a chromatogram of 1-ppm benzene in styrene. It demonstrates the very good performance of the Agilent Deans switch system for sensitive and quantitative detection of 1-ppm benzene in styrene.

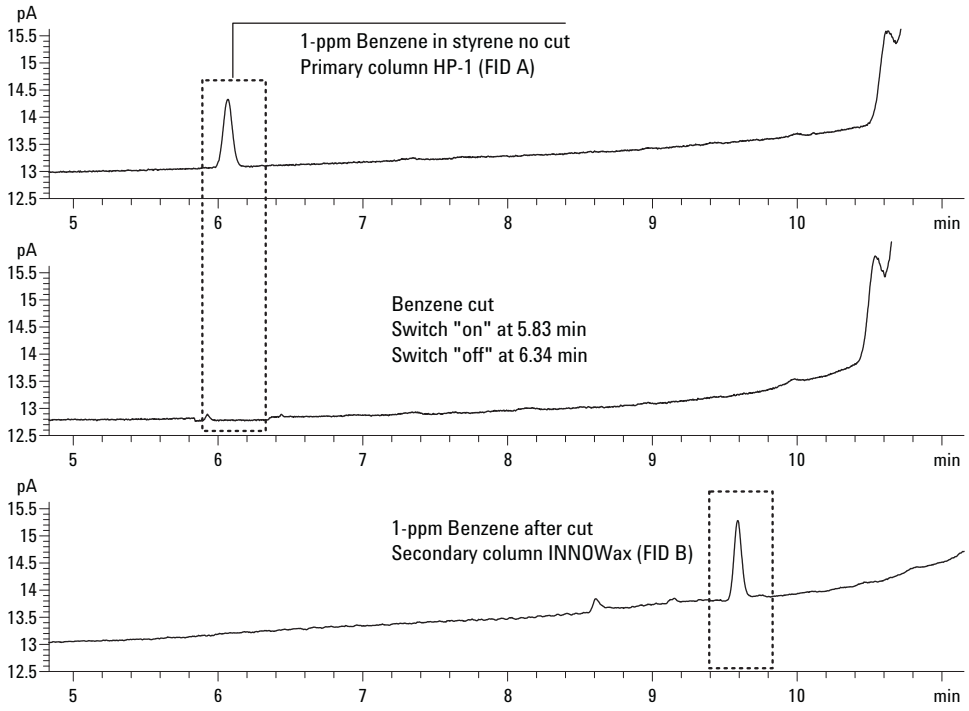

Figure 4. Benzene 1-ppm in styrene can be detected at good signal to noise (S/N) with heart-cutting mode.

Precision

Table 3 shows the excellent RT and detector response repeatability when heart-cutting with the Agilent Deans switch for this method.

Table 3. Repeatability of 2-D GC Method with Heart-Cutting Model

	1-ppm Benzene in styrene		10-ppm Benzene in styrene		100-ppm Benzene in styrene		1000-ppm Benzene in styrene	
	Area	RT	Area	RT	Area	RT	Area	RT
Run 1	5.4	9.589	55.2	9.555	569.6	9.557	5848.8	9.556
Run 2	5.4	9.566	55.7	9.553	572.5	9.556	5898.8	9.555
Run 3	5.4	9.560	55.2	9.555	567.5	9.556	5920.5	9.555
Run 4	5.4	9.561	55.5	9.557	569.5	9.556	5886.1	9.555
Run 5	5.4	9.560	55.4	9.557	570.9	9.554	5821.2	9.556
Average	**5.4**	**9.567**	**55.4**	**9.5554**	**570**	**9.561**	**5875.08**	**9.5554**
SD	**0.00**	**0.01**	**0.190**	**0.0015**	**1.6565**	**0.0023**	**35.6111**	**0.0005**
%RSD	**0.00**	**0.12**	**0.34**	**0.02**	**0.29**	**0.02**	**0.61**	**0.01**

Linearity

Regression statistics of four-point linear calibration curve for benzene containing 1-ppm to 1000-ppm in styrene is shown in Figure 5.

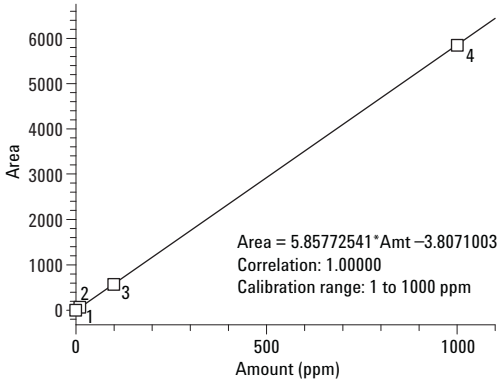

Figure 5. Calibration for styrene containing 1- to 1000-ppm benzene with heart-cutting mode.

Prefractionator Mode

Agilent Deans switch design can be used in a prefractionator mode to analyze trace benzene in styrene according to the proposed ASTM method. The result demonstrates excellent sensitivity and precision.

Sensitivity

Figure 6 shows a chromatogram of 1-ppm benzene in styrene. The 1 ppm of benzene is easily detected at good S/N.

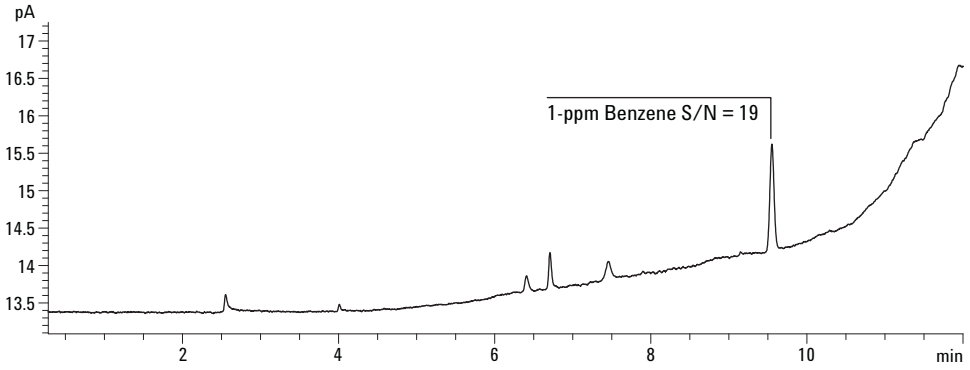

Figure 6. Benzene 1-ppm in styrene

Precision

Table 4 shows a very good repeatability for calibration standards from 1-ppm to 1000-ppm benzene when using the Agilent Deans switch as a prefractionator.

Table 4. Repeatability of 2-D Method with Prefractionator Model

	1-ppm Benzene in styrene		**10-ppm Benzene in styrene**		**100-ppm Benzene in styrene**		**1000-ppm Benzene in styrene**	
	Area	RT	Area	RT	Area	RT	Area	RT
Run 1	5.3	9.552	54.1	9.540	543.4	9.547	5492.1	9.498
Run 2	5.3	9.550	54.0	9.540	543.8	9.547	5492.8	9.500
Run 3	5.3	9.542	53.8	9.541	538.3	9.544	5489.7	9.504
Run 4	5.3	9.544	53.4	9.545	539.1	9.545	5507.7	9.522
Run 5	5.2	9.540	53.9	9.548	538.5	9.544	5525.9	9.544
Average	**5.3**	**9.546**	**53.8**	**9.543**	**540.6**	**9.545**	**5501.6**	**9.514**
SD	**0.04**	**0.005**	**0.2**	**0.003**	**2.4**	**0.001**	**13.7**	**0.017**
%RSD	**0.76**	**0.05**	**0.45**	**0.03**	**0.45**	**0.01**	**0.25**	**0.18**

Linearity

Figure 7 shows a calibration for styrene containing 1 to 1000-ppm benzene with prefractionator mode.

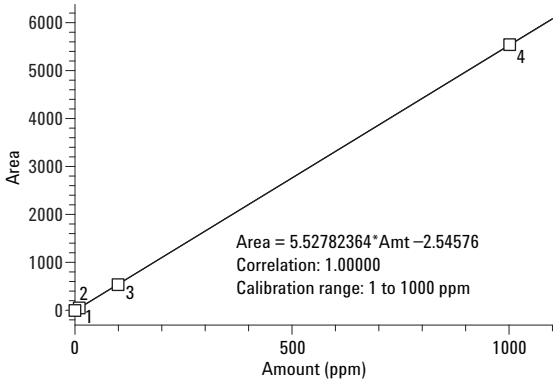

Figure 7. Calibration for styrene containing 1- to 1000-ppm benzene with prefractionator mode.

The Comparison Between Heart-Cutting Mode and the Prefractionator Mode

Comparing heart-cutting technique to the prefractionator technique, the former one can greatly reduce interference, because the heart-cutting can precisely transfer just the benzene and any co-eluting compounds from the primary column to the secondary column. While in the prefractionator mode, all compounds that elute before benzene on the HP-1 column are also transferred to the INNOWax column. These extra compounds are a potential source of interference on the INNOWax column. To show that heart-cutting can reduce potential interference, a styrene sample was spiked with 1000 ppm of natural gasoline to increase the amount and number of hydrocarbons. This sample was run using both the heart-cutting mode and the prefractionator mode. Figures 8 and 9 show the results of this experiment.

On the nonpolar primary column (HP-1), the components elute in boiling point order and the benzene peak co-elutes with hydrocarbons that have boiling points similar to benzene. After heart-cutting to the polar INNOWax column, the benzene is easily separated from the less polar hydrocarbons as shown in Figure 8. In the prefractionator mode, the benzene and all lighter compounds are transferred to the polar INNOWax column, on which there are hydrocarbons peaks including benzene peaks as shown in Figure 9. This demonstrates that heart-cutting greatly reduces the number of potential interferences for this method.

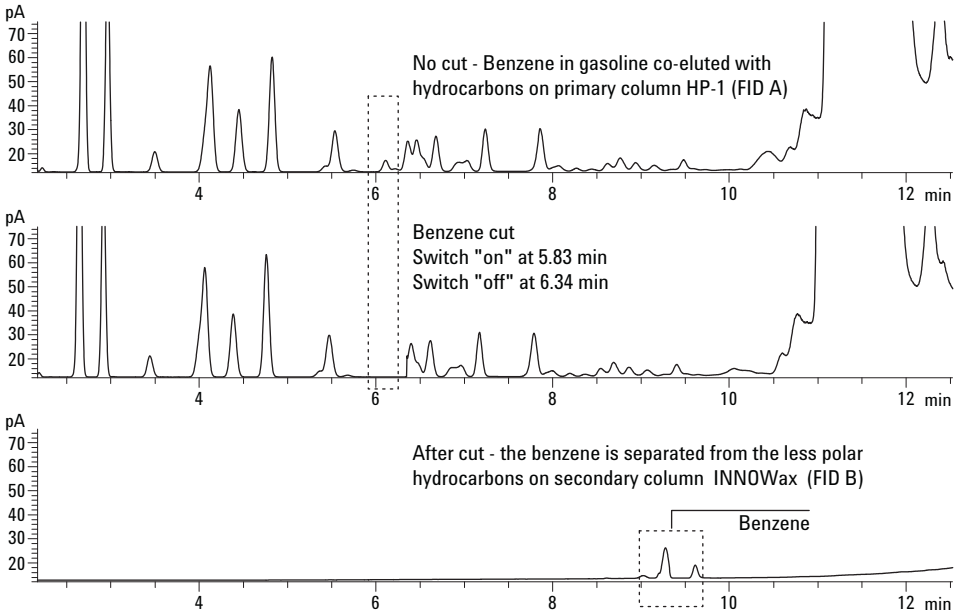

Figure 8. One thousand ppm gasoline in styrene with heart-cutting mode with reduced hydrocarbon peaks in the lower chromatogram.

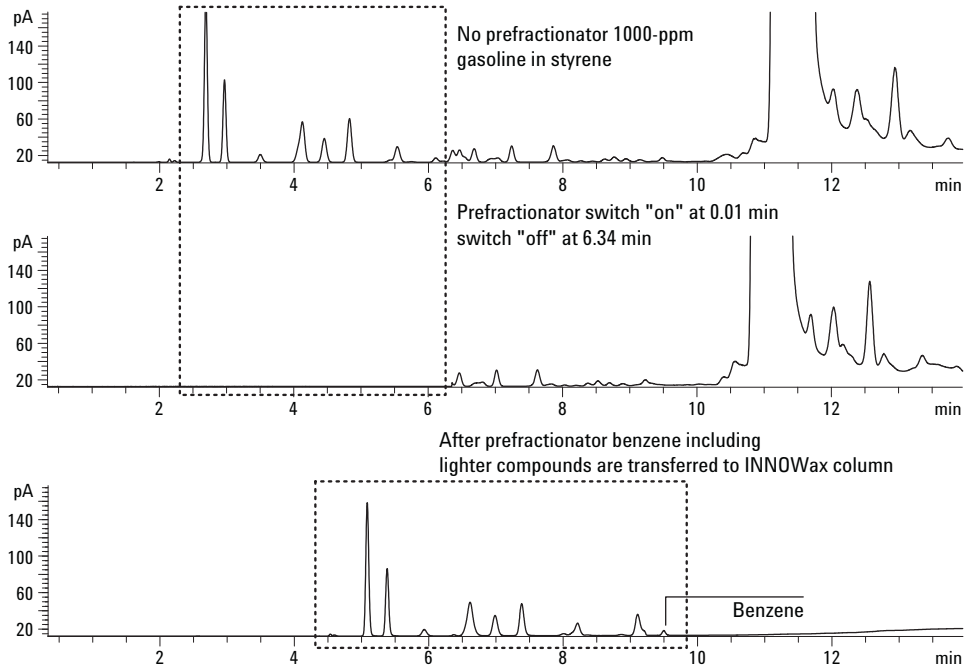

Figure 9. One thousand ppm gasoline in styrene with prefractionator mode. A larger number of peaks are observed in the lower chromatogram.

Conclusions

An easy and reliable 2-D GC system with a simplified Deans switch can be used in a prefractionator mode and in a heart-cutting mode to analyze trace benzene in styrene. In this system the Agilent 6890 EPC provides better RT precision that allows narrower cut times for better resolution and quantitative precision. Deans Switch Calculator makes the method development faster and easier. As the results have demonstrated, prefractionator and heart-cutting modes have an excellent calibration, linearity, and repeatability. The heart-cutting can greatly reduce the interference and benzene co-eluted with hydrocarbons on the nonpolar primary column and can be completely separated after heart-cutting to the polar secondary column. The system was able to detect 1-ppm benzene in styrene with excellent signal to noise.

References

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Printed in the USA
March 2, 2004
5989-0594EN

Fast Analysis of Aromatic Solvent with 0.18 mm ID GC column

Application

Gas Chromatography

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Abstract

Fast GC is one possible way to improve productivity. By reducing the internal diameter of the capillary column, a higher efficiency per unit of column length is obtained in capillary GC. Combined with shorter column length, the application of high-efficiency 0.18-mm-id GC column results in faster analyses compared to conventional 0.25-mm- or 0.32-mm-id columns without losing measurement performance. A single, rapid GC method for aromatic solvent purity analysis is described.

Introduction

Determination of the purity of aromatic hydrocarbons is critical for many QA and QC laboratories in the chemical and petrochemical industry. In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and published a number of GC methods specifically for an aromatic compound or a class of aromatic compounds such as styrene, o-xylene, p-xylene, and ethylbenzene. Table 1 lists 10 ASTM methods along with the recommended columns and specifications [1].

Many QA/QC labs need to run these different ASTM methods to ensure the quality of all products. These analyses can be difficult and expensive to perform. Because many of these ASTM methods are remarkably alike, it is highly desirable to develop a single method that is the chromatographic equivalent of the individual methods. Detailed discussions on an unified aromatic solvent method are available in the literature [2, 3].

Due to demands for increased productivity, many QC/QA laboratories need to analyze large numbers of samples every day. Faster analysis is highly desirable for increased sample throughput and therefore lower cost per sample.

Table 1. Ten ASTM Methods for the GC Analysis of Aromatic Solvents

ASTM Method	Title	Liquid phase	Column type	Report specifications
D2306	Std test for C8 aromatic hydrocarbons	0.25 µm Carbowax	Capillary 50 m × 0.25 mm	wt% of individual C8 HC
D2360	Std test for trace impurities in monocyclic hydrocarbons	0.32 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual aromatic impurities, total impurities, purity
D3760	Std test for cumene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, cumene purity (wt%)
D3797	Std test for o-xylene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, o-xylene purity (wt%)
D3798	Std test for p-xylene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, total impurities, p-xylene purity (wt %)
D4492	Std test for benzene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, benzene purity (wt%)
D4534	Std test for benzene in cyclic products	10% TCEPE on Chromasorb P	Packed 3.7 m × 3.175 mm	wt% of benzene
D5060	Std test for impurities in ethylbenzene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, ethylbenzene purity
D5135	Std test for styrene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, styrene purity
D5917	Std test for trace impurities in monocyclic hydrocarbons (ESTD Cal)	0.25 µm Carbowax	Capillary 60 m × 0.32 mm	wt% individual impurities, wt% total nonaromatics, wt% total C9 aromatics, purity of main component

Experimental

High-Efficiency Capillary GC Columns

Efficiency is often related to the number of theoretical plates, which increases linearly with decreasing column internal diameter (id). For instance, 0.18 mm id columns typically produce 5,800 to 6,600 theoretical plates per meter, whereas columns with 0.25 to 0.32 mm id typically produce 3,600 to 4,600 plates per meter. The efficiency improvement for the 0.18 mm id columns allows for better signal-to-noise ratios. Since decreasing the internal diameter results in an increase of the column efficiency per meter, the column length can be reduced while keeping the resolution constant. Therefore, the use of 0.18 mm id columns, also known as the high-efficiency GC columns, can help gas chromatographers substantially reduce their sample analysis time.

While it is true that an even smaller id column, such as 0.1 mm id, could lead to higher efficiency per meter, routine analysis with such a column imposes high demands on instrumentation. It requires higher inlet pressures, better split control, and faster oven temperature heating rates. On the contrary, 0.18 mm id columns are conveniently compatible with existing standard GC equipment without the need for system modifications. Smaller id, shorter length columns require less carrier flow to achieve separations, thus reduce carrier gas usage. Therefore, high-efficiency 0.18 mm id columns can provide an easy and inexpensive way to speed up GC analysis without compromising resolution.

One note of caution when going to smaller id columns is lower sample capacity. With some special samples, it is important to find a balance among speed, sensitivity, and resolution to meet the laboratory goals. For most applications in the chemical, petrochemical, food, or flavor/fragrance industries, however, the use of HE GC columns can offer an important reduction in analysis time and, consequently, a higher sample throughput.

The purpose of this application is to demonstrate in depth the use of high-efficiency 0.18 mm id columns for faster analysis of aromatic solvents with the unified aromatic solvent analysis method.

Results and Discussion

One Agilent 6890N Series gas chromatograph and two Agilent 7890 gas chromatographs were used for this work. Each GC was equipped with a split/splitless capillary inlet, a flame ionization detector (FID), and an Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with a long-lifetime septa (Agilent part no. 5183-4761) and split-optimized liners (Agilent part no. 5183-4647). Injections were made using 10-µL syringes (Agilent part no. 5181-3354). Agilent ChemStation was used for all instrument control, data acquisition, and data analysis.

A 50-mL n-Hexane solution was prepared containing 0.1 wt% of 27 compounds; that is, all the aromatic solvents and impurities specified for analysis by the 10 ASTM methods.

Table 2 lists the experimental conditions for Method 1 where the unified aromatic solvent analysis was performed using a conventional 60 m × 0.32 mm × 0.5 µm HP-INNOWax column (Agilent part no. 19091N-213). The GC chromatogram is shown in Figure 1.

Table 2. Conditions for Unified Aromatic Solvents Method Using a Conventional Column (Method 1)

Column	HP-INNOWax, 60 m × 0.32 mm × 0.50 µm
Carrier gas	Helium at 20.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	75 °C (10 min); 3 °C/min to 100 °C (0 min) 10 °C/min to 145 °C (0 min)
Detector	FID at 250 °C
Data acquisition rate	At 20 Hz
Injection size	1 µL

The experiment was then repeated with a high-efficiency 20 m × 0.18 mm × 0.18 µm HP-INNOWax column (Agilent part no. 19091N-577) (Method 2). Agilent GC Method Translation Software (http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm) was used to translate Method 1 to Method 2. Three translation modes, namely the "translate only," "best efficiency," and "fast analysis," were attempted with the new column dimensions. However, co-elution of dodecane and o-xylene was observed for all three translated methods. According to ASTM methods, some modifications of the temperature programs were therefore necessary to achieve a similar resolution to Method 1. The resulting experimental conditions are provided in Table 3 along with the chromatogram in Figure 2.

Table 3. Conditions for Aromatic Solvents Separations on a High-Efficiency Column (Method 2)

Column	HP-Innowax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 25.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	50 °C (2 min); 15 °C/min to 90 °C (0 min); 20 °C/min to 145 °C (1min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 µL

In order to achieve even faster separation while balancing speed and resolution, Agilent GC Method Translation Software was used to translate Method 1 to Method 3 while selecting "fast analysis" mode and using the same high-efficiency GC column. But dodecane and o-xylene could not achieve baseline separation with the obtained method as stated previously. According to ASTM methods, the obtained method conditions were used with minor adjustments of the initial temperature from 75 °C to 70 °C and the initial hold of 2 minutes to 3 minutes. Then baseline separation was obtained for dodecane and o-xylene (Rs = 2.78). Detailed experimental conditions are provided in Table 4 with the GC chromatogram in Figure 3.

Table 4. Conditions for Fast Aromatic Solvents Analysis (Method 3)

Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 33.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 to 600:1 split ratio
Oven temp	70 °C (3 min); 45 °C/min to 145 °C (1 min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 to 1.0 µL

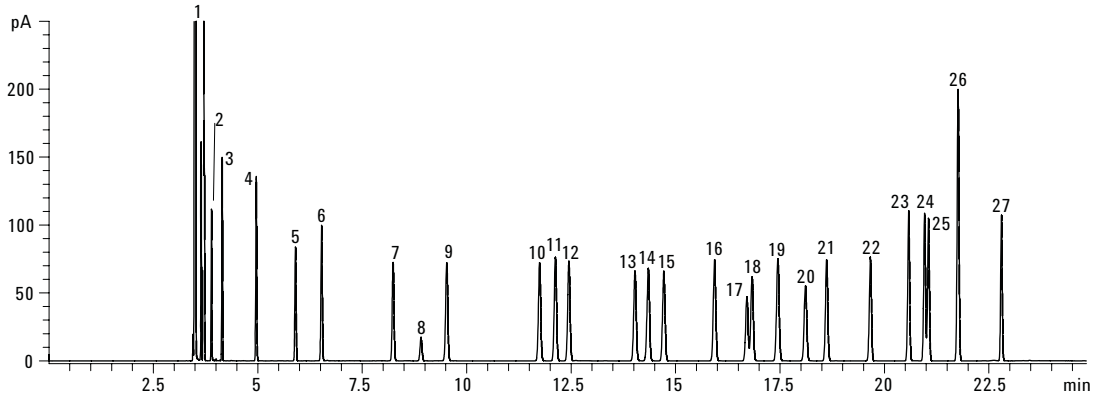


Figure 1. Unified aromatic solvent method with a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column.

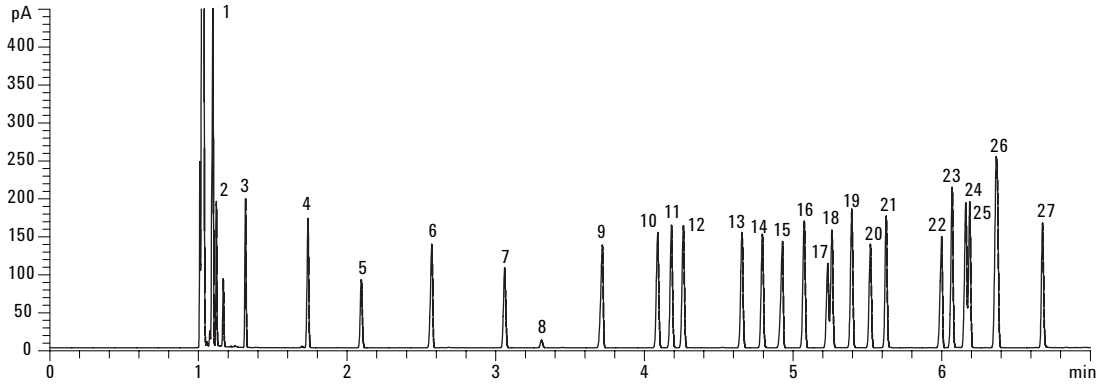


Figure 2. Separation of the same aromatic solvent with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column.

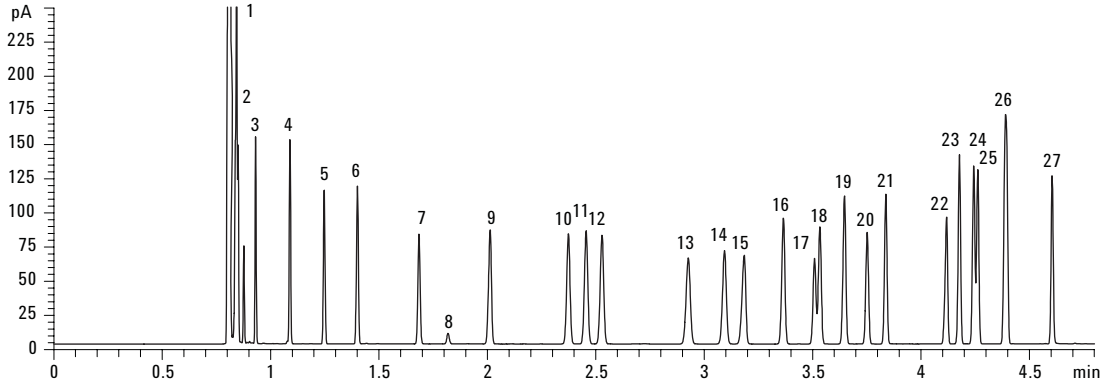


Figure 3. Optimized unified aromatic solvent method with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column.

1	Heptane	8	1,4-Dioxan	15	o-Xylene	22	Tridecan
2	Cyclohexane	9	Undecane	16	Propylbenzene	23	1,3-Diethylbenzene
3	Octane	10	Ethylbenzene	17	p-Ethyltoluene	24	1,2-Diethylbenzene
4	Nonane	11	p-Xylene	18	m-Ethyltoluene	25	n-Butylbenzene
5	Benzene	12	m-Xylene	19	t-Butylbenzene	26	a-Methylstyrene
6	Decane	13	Cumene	20	s-Butylbenzene	27	Phenylacetylene
7	Toluene	14	Dodecane	21	Styrene		

Figures 1, 2, and 3 show the chromatograms of the hexane solution containing an aggregate of aromatic solvents and impurities for Method 1, Method 2, and Method 3, respectively. As indicated in the three chromatograms, baseline resolution was achieved for most of the compounds of interest except for two compound pairs, which were only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, was also not resolved in the original ASTM method (D-5060, impurities in ethylbenzene) and, along with o-ethyltoluene, was reported as total ethyltoluene. A second pair, diethylbenzene and n-butylbenzene, was also partially resolved. However, this should not present a

problem since they are not typically found together within the same material. Diethylbenzene is sometimes found as a contaminant in ethyl benzene (ASTM Method D-5060) while n-butyl benzene is used as the internal standard for cumene analysis (ASTM Method D-3760).

The sample run time for Method 1 was 23 minutes (Figure 1), whereas it was 7 minutes for Method 2 (Figure 2). The 3x speedup was achieved by using a shorter and narrower bore high-efficiency column. The optimized Method 3 allowed for even faster analysis time at 5 minutes (Figure 3), resulting in 4.6x speedup as compared to Method 1. As shown in Table 5, similar resolution was obtained in spite of significant acceleration, indicating that fast sample throughput can be achieved with the high-efficiency columns without compromise on resolution.

Influence of Carrier Gas on Analysis Time

The type of carrier gas and its velocity highly impact resolution and retention time. Too high or too low of a carrier gas velocity results in loss of resolution. It is therefore important to set a correct gas velocity to achieve a right balance of resolution and analysis time.

Hydrogen, helium, and nitrogen are the most common carrier gases used. The use of hydrogen as a carrier gas provides a faster analysis with almost equivalent resolution because the optimum linear carrier gas velocity is higher due to the higher diffusivity of hydrogen. At the optimal flow rates of 12, 20, and 35 cm/s for nitrogen, helium, and hydrogen, respectively, the analysis times would be 35/12 to 35/20 to 1 for nitrogen, helium, and hydrogen, respectively.

Nitrogen vs. Helium Carrier Gas

To investigate the effect of carrier gas on sample analysis time, Agilent GC method translation software was used where “translate only” mode was chosen so that all experimental conditions were held constant except for the carrier gas. Method 1 was translated to Method 4 where a nitrogen carrier was used (see Figure 4 and Table 6). As shown in Figure 5, the run time for a nitrogen carrier was about 60 minutes compared to 23 minutes with a helium carrier when using a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column.

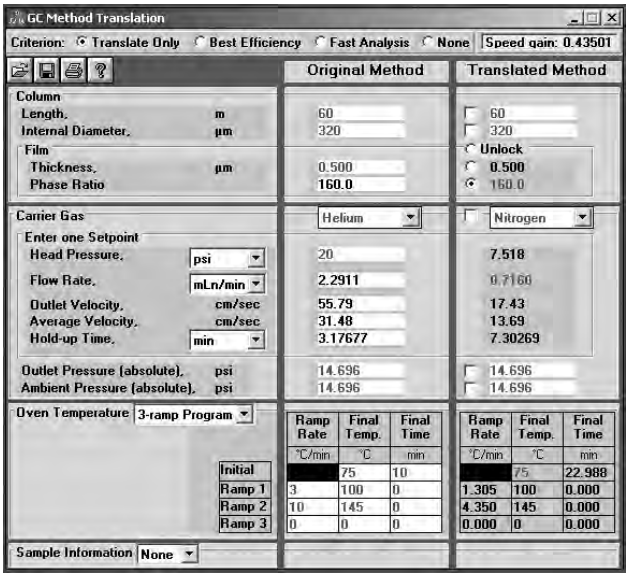


Figure 4. Method translation software input screen for a nitrogen carrier.

Table 6. Experimental Conditions for Unified Aromatic Solvents Method Using Nitrogen Carrier Gas (Method 4)

Column	HP-INNOWax, 60 m × 0.32 mm × 0.50 µm
Carrier gas	Nitrogen at 7.60 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	75 °C (23 min); 1.3 °C/min to 100 °C (0 min) 4.4 °C/min to 145 °C (0 min)
Detector	FID at 250 °C
Data acquisition rate	At 20 Hz
Injection size	0.2 µL

Table 5. Comparison of Resolution of Difficult-to-Separate Compound Pairs Under Different Experimental Conditions

Compound	Ethylbenzene/p-xylene	p-Xylene/m-xylene	p-Ethyltoluene/m-ethyltoluene	Diethylbenzene/n-butylbenzene
Method 1	3.25	3.10	1.10	1.11
Method 2	3.14	2.72	1.00	0.97
Method 3	2.84	2.47	0.94	0.88

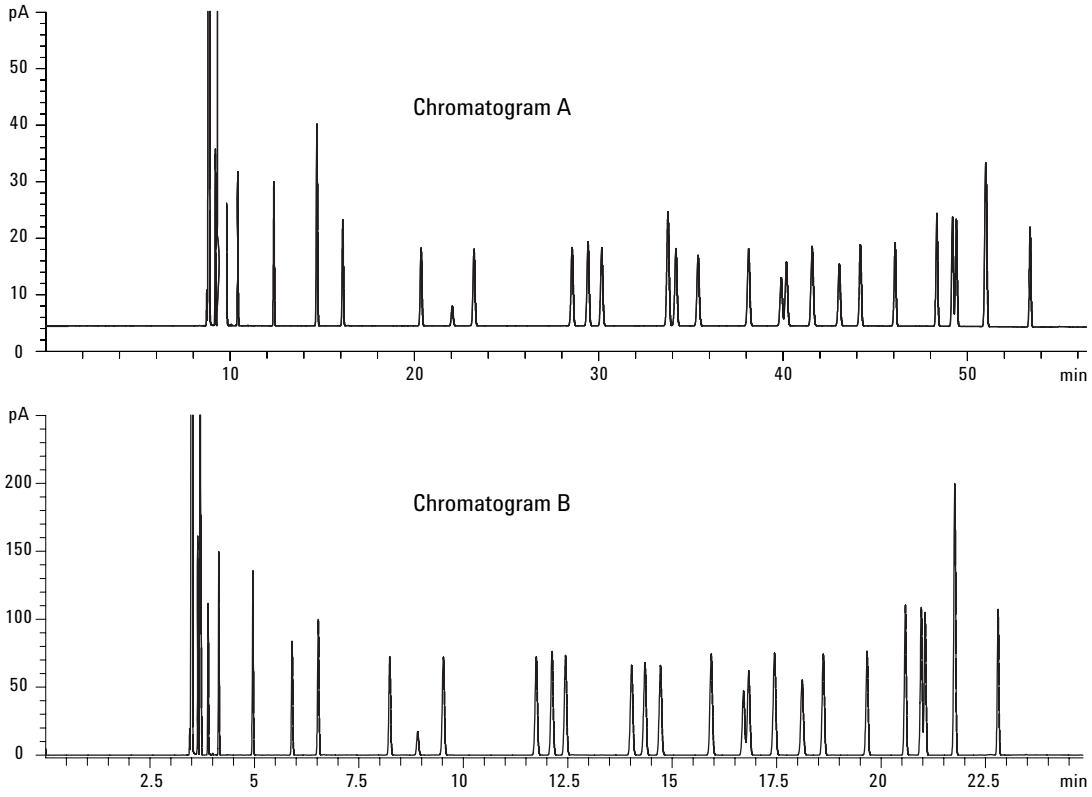


Figure 5. Comparison of unified aromatic solvent analysis using nitrogen and helium carrier gases with a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column. 5a. Nitrogen carrier gas (Method 4). 5b. Helium carrier gas (Method 1).

Hydrogen vs. Helium Carrier Gas

A faster analysis can be achieved by switching the carrier gas from helium to hydrogen on the same coumn. Method 3 was translated to Method 5 using the method translation software (see Figure 6); the detailed experimental condition is provided in Table 7. As shown in Figure 7, the total run time was decreased from 5 to 3 minutes by changing the carrier gas from helium to hydrogen while keeping the peaks well separated.

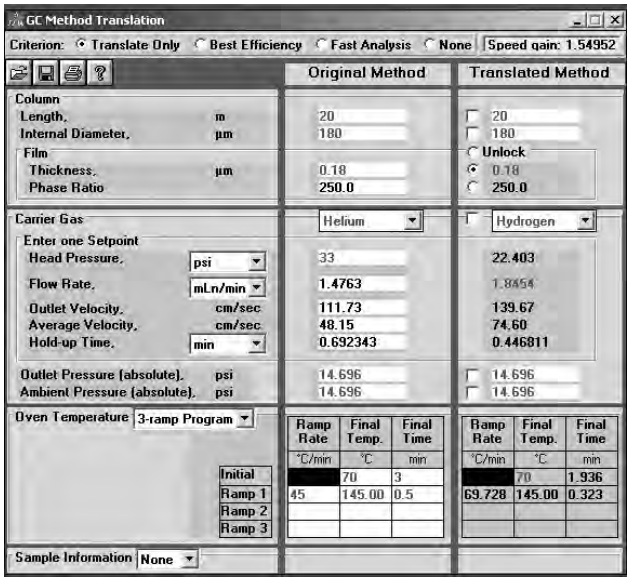


Figure 6. Method translation software input screen for a hydrogen carrier.

Table 7. Experimental Conditions for Unified Aromatic Solvents Method Using Hydrogen Carrier Gas (Method 5)

Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Hydrogen at 22.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	70 °C (2 min); 70 °C/min to 145 °C (0.5 min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 µL

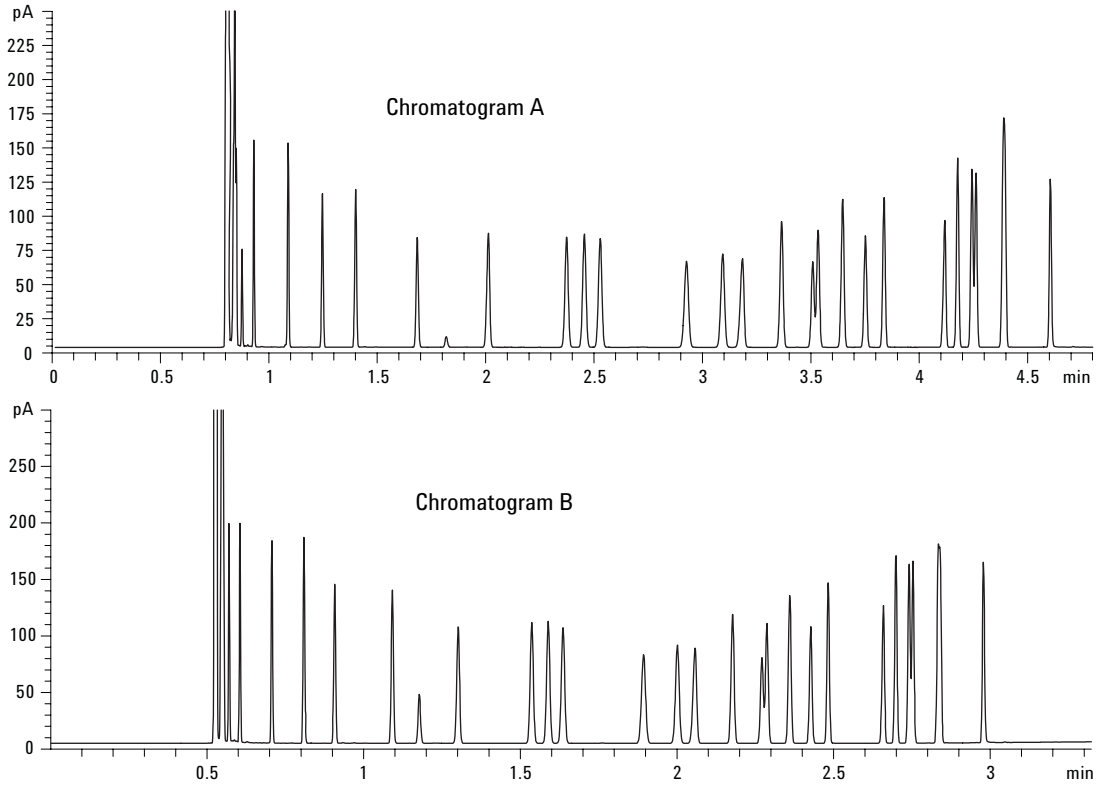


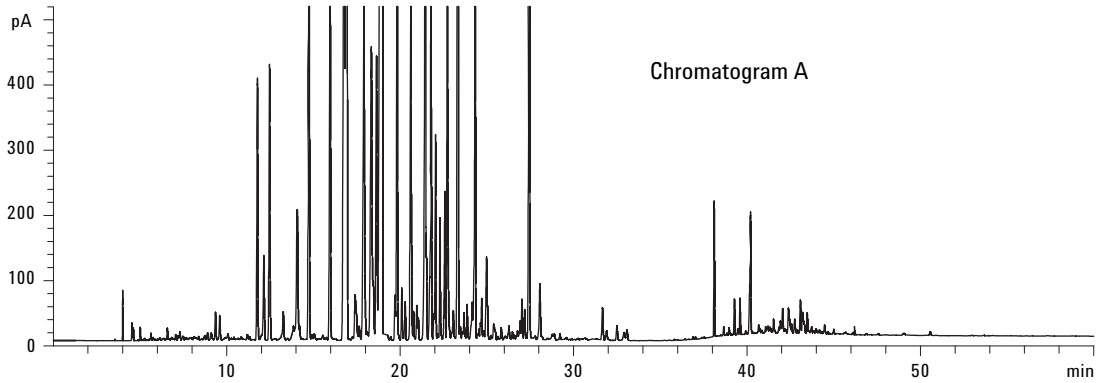
Figure 7. Comparison of unified aromatic solvent analysis using helium and hydrogen carrier gases with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column. 7a. Helium carrier gas (Method 5). 7b. Hydrogen carrier gas (Method 3).

Complex Matrix Sample

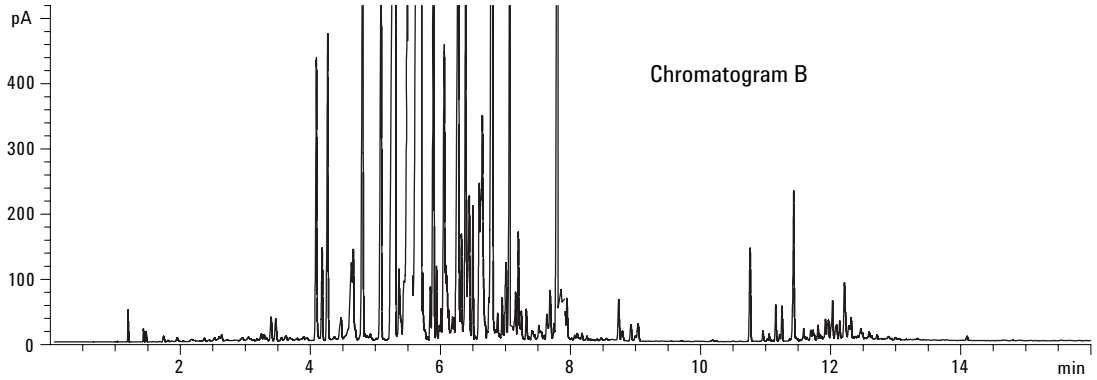
To validate the practicality of fast GC application using high-efficiency GC columns, a real aromatic solvent sample offered by a large-scale integrated petrochemical company was analyzed using the same experimental conditions as those for the standards (Methods 1, 2, and 3); the chromatograms are provided in Figures 8a, 8b, and 8c. A detailed comparison of the center sections is also provided in Figures 8d and 8e.

Although the analysis time is a bit longer with Method 2 compared to Method 3, the overall resolution obtained is slightly better for Method 2 (see Figures 8e and 8f). On the other hand, all the key compounds, including benzene, toluene, ethylbenzene, m-xylene, p-xylene, o-xylene, propylbenzene, and a-methylstyrene, were well separated with all three methods.

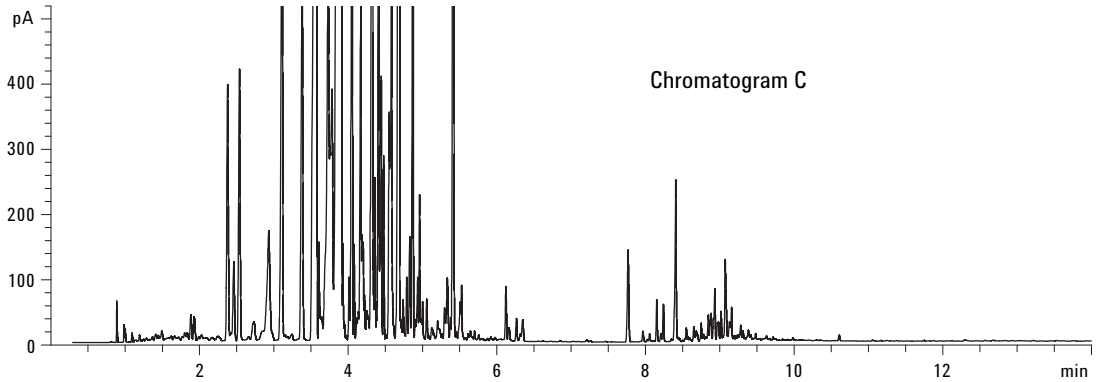
For complex matrix samples, a balance between speed and resolution must be selected according to the laboratory goals. In this case, it demonstrates that a complex matrix sample can be separated well on a high-efficiency 0.18 mm id GC column, where a more than 3x improvement in run time was accomplished compared to a 0.32 mm id column using a helium carrier.



Column	HP-INNOWax, 60 m × 0.32 mm × 0.50 µm
Carrier gas	Helium at 20.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C; 50:1 split ratio
Oven temp	75 °C (10 min); 3 °C/min to 100 °C (0 min); 10 °C/min to 145 °C (12.17 min), 25 °C/min to 220 °C (22 min)
Detector	FID at 250 °C
Injection size	0.2 µL



Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 25.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C; 150:1 split ratio
Oven temp	50 °C (2 min); 15 °C/min to 90 °C (0 min); 20 °C/min to 145 °C (3 min), 80°C/min to 220 °C (8 min)
Detector	FID at 250 °C
Injection size	0.2 µL



Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 33.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C; 150:1 split ratio
Oven temp	70 °C (3 min); 45 °C/min to 145 °C (3 min), 80 °C/min to 220 °C (8 min)
Detector	FID at 250 °C
Injection size	0.2 µL

Figure 8. Comparison of real aromatic solvent sample separations (a) and (d) Method 1, (b) and (e) Method 2, and (c) and (f) Method 3.

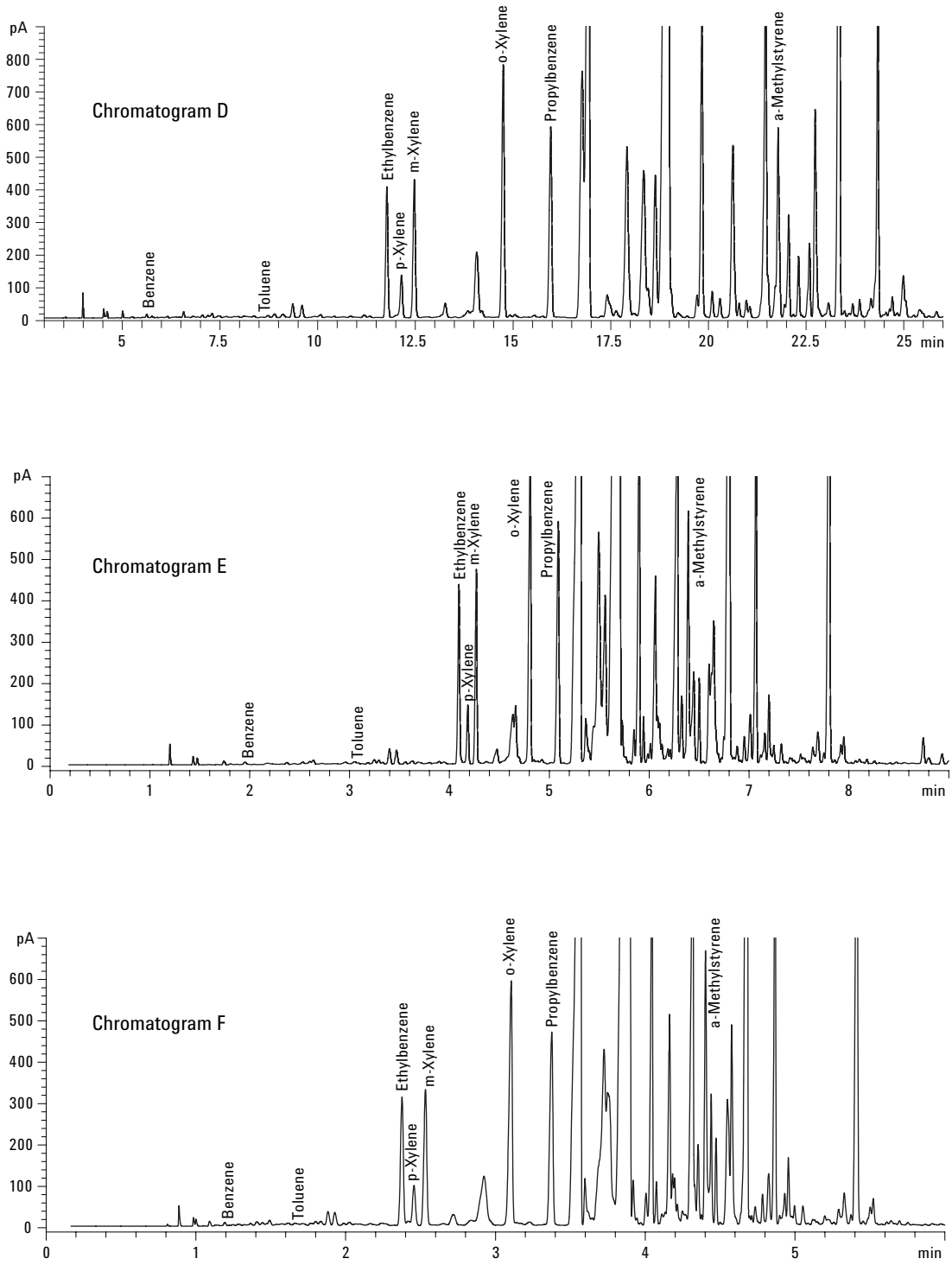


Figure 8. Comparison of real aromatic solvent sample separations (a) and (d) Method 1, (b) and (e) Method 2, and (c) and (f) Method 3. (continued)

Evaluation of Individual ASTM Calibration Standards

To evaluate the applicability of high-efficiency GC columns on individual ASTM calibration standards, experiments were carried out with Methods 1 and 3, respectively, on a 7890 gas chromatography system. All standards were prepared as outlined by the ASTM methods.

D2306 – Standard Test for C8 Aromatic Hydrocarbons

Concentration of ASTM D2306 standard calibration mix is quite high. It is therefore a challenge regarding the capacity of the high-efficiency 0.18 mm id column. The workaround is to inject a small volume with a high split ratio. In this experiment, the injection size was 0.2 µL and the split ratio was 600:1. As shown in Figure 9, the run time for the high-efficiency GC column was about 4.5 times shorter than that of the traditional one. The resolution is acceptable in spite of the high concentration of the calibration standard (see Table 8).

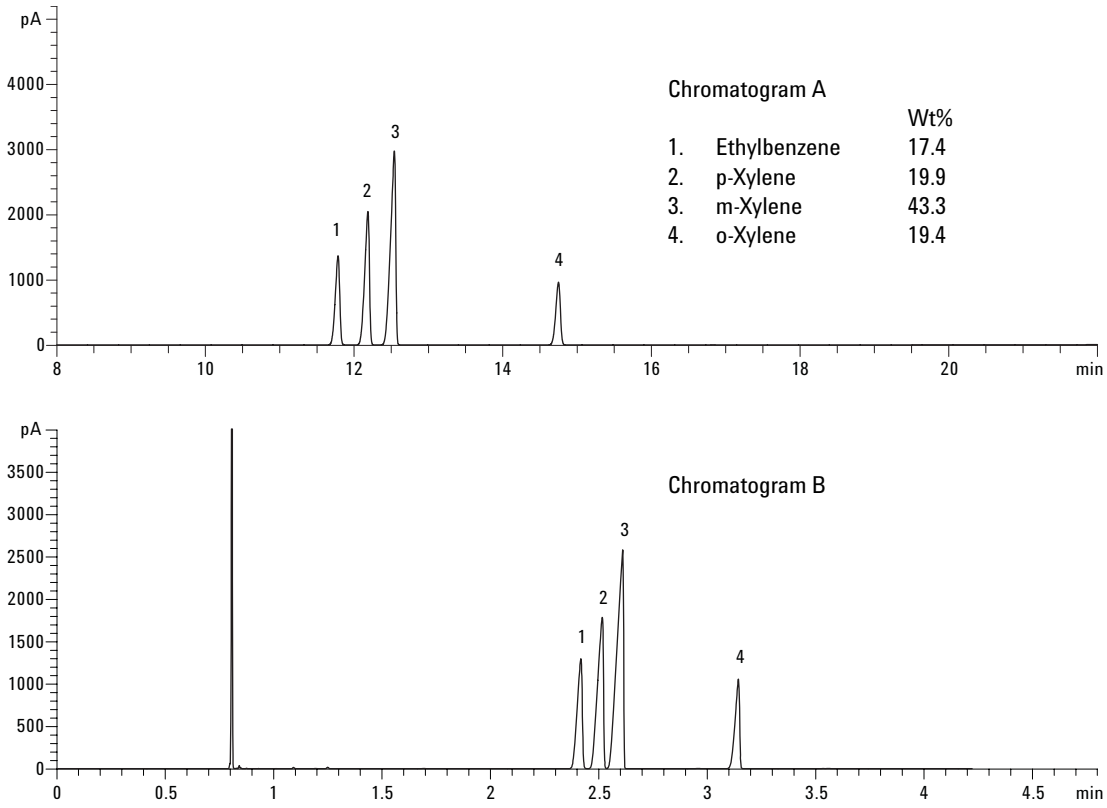


Figure 9. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standards (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (method 3).

Table 8. Comparison of Resolution Under Different Experimental Condition

Compound	Ethylbenzene/p-xylene	p-Xylene/m-xylene	m-Xylene/o-xylene
Method 1	3.52	2.86	18.11
Method 3	2.10	1.73	11.20

D2360 – Standard Test for Trace Impurities in Monocyclic Hydrocarbons

The standard calibration mix specified by D2360 was prepared in p-xylene. Injection size for this run was 0.2 µL and the split ratio was 200:1.

Similar resolution was obtained for the compounds of interest (Figure 10), except for the sample run time being decreased from 21.05 minutes (Method 1) to 4.28 minutes (Method 3).

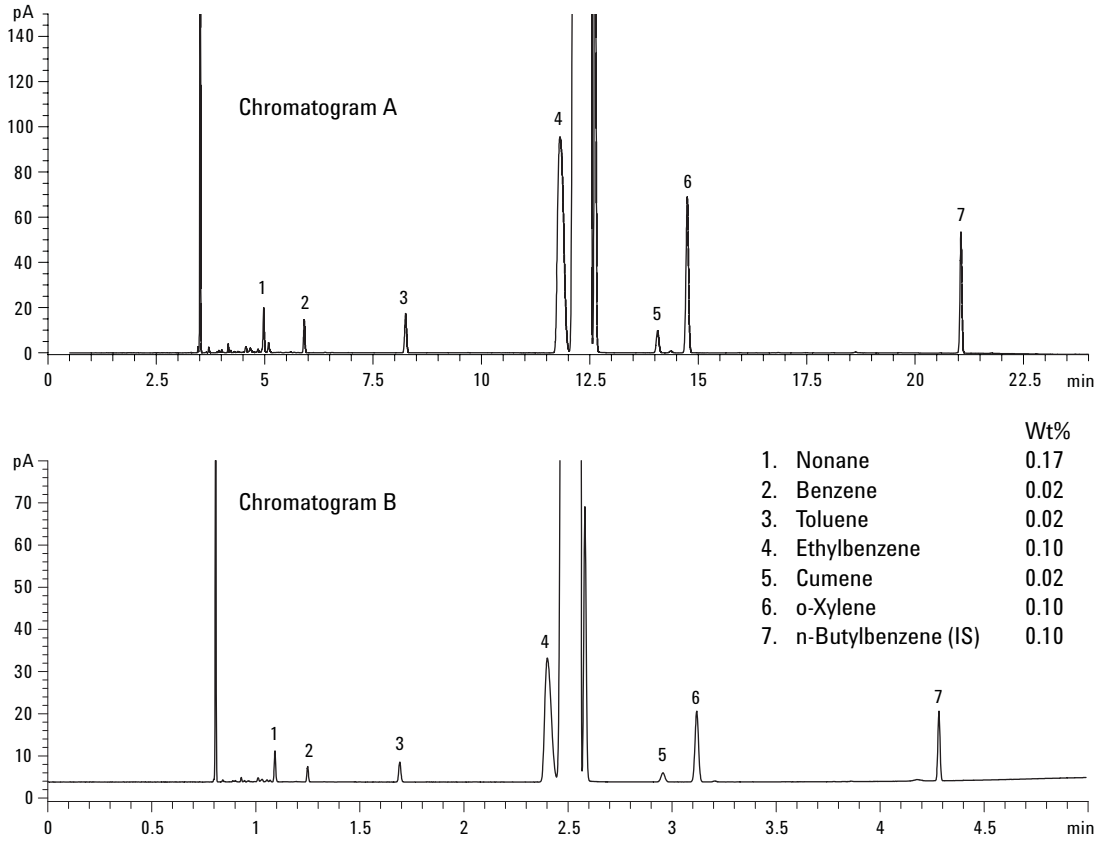
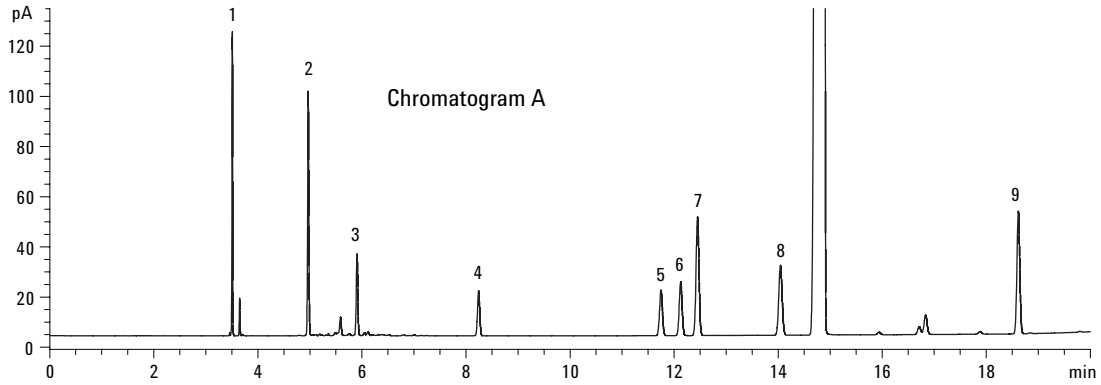


Figure 10. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

Figure 11 shows the chromatograms of the D3797 calibration standard. Injection size for this run was 0.2 µL and the split ratio was 100:1.

The broadening of the cumene peak was due to the reverse solvent effect of the overloaded o-xylene peak. This was also observed in the original ASTM D3797 method [4]. Comparison of the chromatograms in Figure 11 indicates that the D3797 calibration standard can be separated well on a high-efficiency 0.18 mm id GC column without loss of resolution.



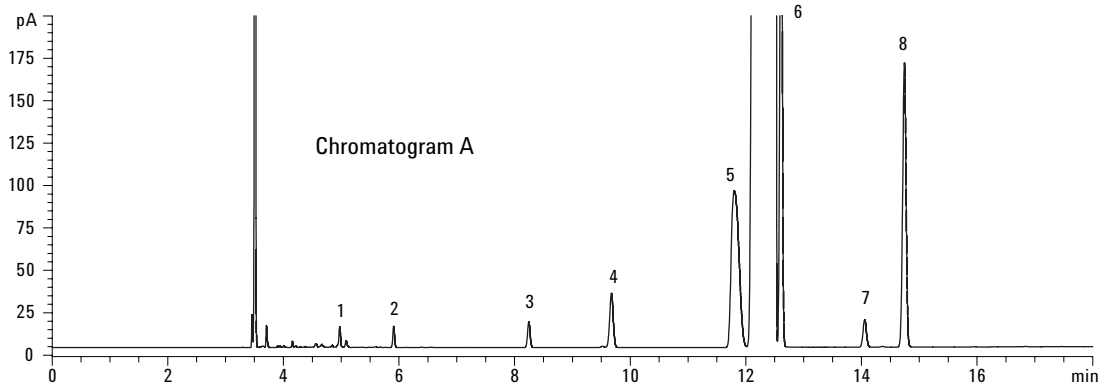
		Wt%			Wt%
1.	Isooctane (IS)	0.05	6.	p-Xylene	0.21
2.	Nonane	0.21	7.	m-Xylene	0.42
3.	Benzene	0.20	8.	Cumene	0.31
4.	Toluene	0.21	9.	Styrene	0.12
5.	Ethylbenzene	0.21			

Figure 11. o-Xylene standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

D3798 – Standard Test Method for Analysis of p-Xylene

This test method covers the determination of known hydrocarbon impurities in p-xylene and the measurement of p-xylene purity by GC. It is generally used for the analysis of p-xylene of 99% or greater purity.

Figure 12 shows the chromatograms of the D3798 calibration standard. Injection size for this run was 0.2 µL and the split ratio was 100:1. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Excellent separation was obtained for the critical compounds (Figure 13) with great reproducibility (Figure 14) when using a high-efficiency GC column.



		Wt%			Wt%
1.	Nonane	0.01	5.	Ethylbenzene	0.10
2.	Benzene	0.02	6.	m-Xylene	0.20
3.	Toluene	0.01	7.	Cumene	0.01
4.	Undecane (IS)	0.08	8.	o-Xylene	0.10

Figure 12. p-Xylene standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

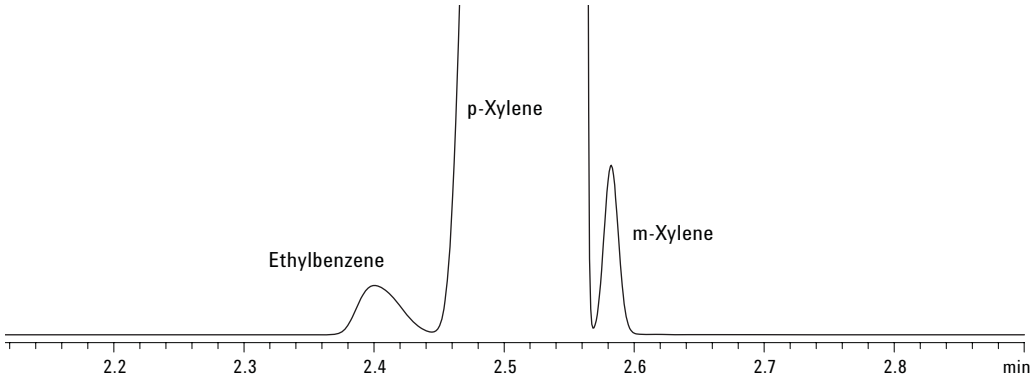


Figure 13. Expanded view from Figure 7 shows excellent separation of m-Xylene peak from p-Xylene peak using the fast GC method.

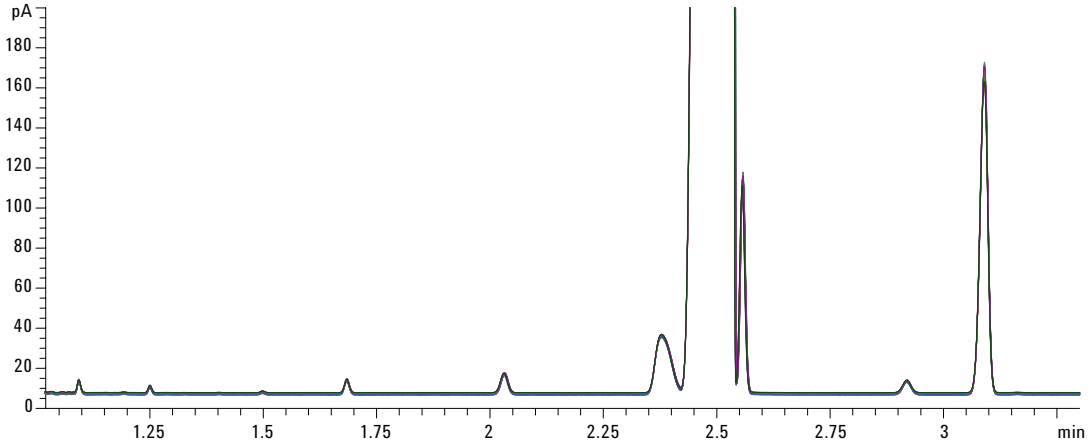


Figure 14. D3798 standard 30th run overlaid using a high-efficiency GC column.

D4492 – Standard Test for Analysis of Benzene

This test method determines the normally occurring trace impurities in, and the purity of, finished benzene. It is applicable for aromatic impurities from 0.001 to 0.010 weight % in benzene. Injection size for this run was 0.2 µL and the split ratio was 50:1.

Figure 15 compares the chromatograms of the D4492 calibration standard with Methods 1 and 3, where good separation of the D4492 calibration standards can be achieved with a high-efficiency column but with 80% saving on analysis time.

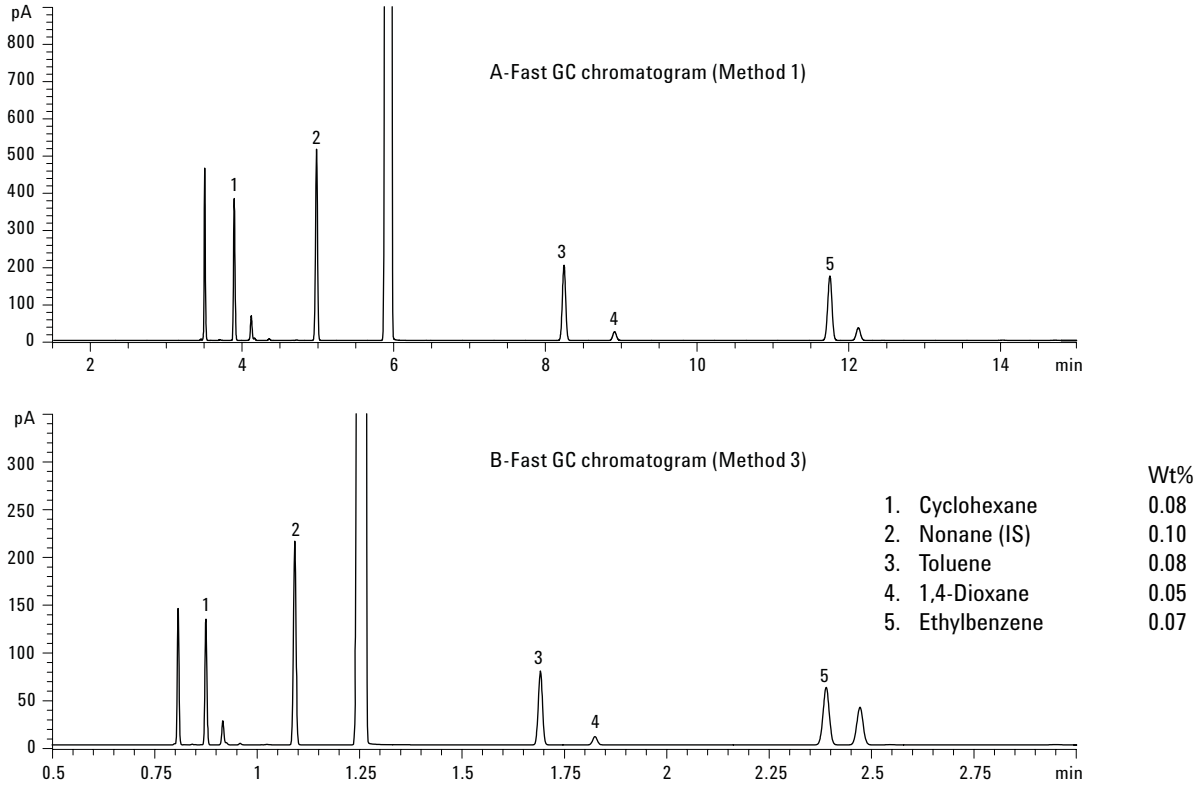


Figure 15. ASTM D4492 benzene quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

In summary, the analysis time for Method 3 is on average 5x shorter than that for Method 1 when working with the calibration standard samples.

Conclusions

Fast GC applications can significantly improve laboratory productivity by decreasing analysis time. This application showcases the practicality of high-efficiency GC columns in daily aromatic solvent analysis and the associated time savings achieved with these columns. By using high-efficiency GC columns with smaller inner diameters and shorter column lengths as well as an appropriate carrier gas (for example, helium or hydrogen), higher sample throughput and lower cost per sample is achievable [5] for chemical and petrochemical laboratories.

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5. Ken Lynam and Yun Zou, "A Faster Solution for Unified Volatile Organic Analysis with 0.18 mm ID GC Columns," *Separation Times*, Vol 20, Number 5, 2007

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Printed in the USA
November 28, 2007
5989-7623EN



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Analysis of Trace Hydrocarbon Impurities in Benzene by Agilent 7820A Gas Chromatograph

Chunxiao Wang and Wenmin Liu

Application Brief

HPI

Knowledge of impurities in benzene provides critical quality control information where benzene is either produced or used in a manufacturing process. ASTM D4492 [1] was used for analyzing these impurities, including nonaromatics containing up to nine carbon atoms, toluene, C8 aromatics, and 1,4-dioxane. The Agilent 7820A gas chromatograph offers an efficient and easy-to-use platform for the analysis of benzene and may other aromatic solvents. For this application, an Agilent 7820A GC is configured with a split/splitless capillary inlet and a flame ionization detector (FID). Agilent EZChrom Elite Compact software is used to control the 7820A GC and provide data acquisition/data analysis. The Agilent 7820A GC supports an automatic liquid sampler (ALS), allowing fully unattended operation – from injection all the way through final reporting.

Experimental

Table 1. Typical GC Conditions

Inlet settings	250 °C, Split ratio: 100:1 to 30:1
Injection volume	0.5 µL
Column	HP-INNOWax 60 m × 0.32 µm × 0.5 µm
Column flow (He)	2.6 mL/min (21.8 at 75 °C), constant flow mode
Oven temperature program	For impurities in benzene: 75 °C (10 min); 3 °C/min to 100 °C For aromatic solvent: 75 °C (10 min); 3 °C/min to 100 °C 10 °C/min to 145 °C
FID setting	
Temperature	250 °C
H2 flow	40 mL/min
Air flow	400 mL/min
Make up (N2)	25 mL/min
Data acquisition rate:	20 Hz

Highlights

- An easy-to-use, single-column method for benzene as well as a wide range of aromatic solvent purity analyses meets the chromatographic requirements of 10 separate ASTM methods. Therefore fewer GCs, stock columns, and supplies are required to analyze many different types of samples.
- EPC control and automatic injection ensures excellent repeatability for both retention time and peak area.
- The wide dynamic response range of the FID enables a quantitative analysis of samples containing both very high and very low concentrations in a single run.

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Discussion

The Agilent 7820A GC with full electronic pneumatics control (EPC) on all inlets and detectors ensures good repeatability and also makes it fast and easy to set and to save the pressures and flows. Figure 1 shows the chromatograms of the D4492 calibration standard. Excellent repeatability for retention time with RSD of approximately 0.03 to 0.01% and peak area with RSD of about 1.6% are shown in Table 2.

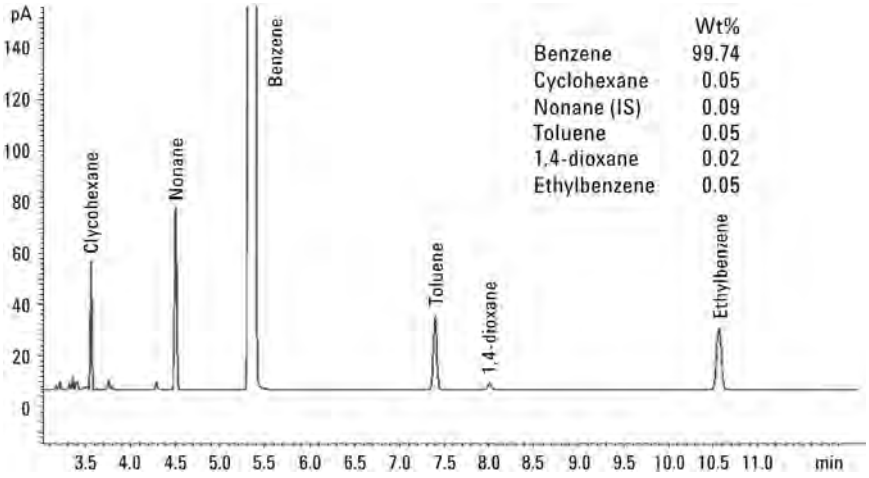


Figure 1. ASTM D4492 benzene calibration standard. *Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 100:1.*

Table 2. Repeatability–ASTM D4492 Benzene Calibration Standard (11 runs) with First Run Included

	Cyclohexane	Nonane	Bezene	Toluene	1,4-dioxane	Ethylbenzene
			Peak Area			
1	430130	861450	900088289	590385	56288	689141
2	425791	848159	888131170	581775	55693	677502
3	437496	874885	915251703	599534	57071	698269
4	439204	879141	918796665	601857	57355	701225
5	438646	876346	917995860	601138	57056	700462
6	436941	876809	914994185	599823	57743	699919
7	423567	844923	885230656	580241	55487	675473
8	420259	843030	878870585	577475	55392	673593
9	422665	844761	883243038	579572	55419	675665
10	430741	865226	901189833	591633	56211	691217
11	431032	865007	901921807	592037	56118	691200
Mean:	430588	861794	900519436	590497	56348	688515
Std Dev:	6852	14298	14909746	9406	837	11061
%RSD:	1.59	1.66	1.66	1.59	1.49	1.61

Table 2. ***Repeatability–ASTM D4492 Benzene Calibration Standard (11 runs) with First Run Included*** *(Continued)*

	Cyclohexane	Nonane	Bezene	Toluene	1,4-dioxane	Ethylbenzene
			Retention Time			
1	3.562	4.503	5.369	7.397	8.003	10.561
2	3.562	4.504	5.371	7.398	8.005	10.563
3	3.562	4.504	5.371	7.398	8.007	10.565
4	3.561	4.503	5.370	7.398	8.006	10.563
5	3.561	4.503	5.370	7.398	8.006	10.563
6	3.561	4.503	5.369	7.398	8.007	10.563
7	3.561	4.503	5.369	7.398	8.006	10.563
8	3.561	4.503	5.369	7.398	8.006	10.563
9	3.561	4.504	5.370	7.398	8.006	10.563
10	3.563	4.506	5.372	7.400	8.007	10.567
11	3.563	4.506	5.372	7.400	8.009	10.565
Mean:	3.562	4.504	5.370	7.398	8.006	10.564
Std Dev:	0.0008	0.0012	0.0012	0.0009	0.0015	0.0016
%RSD:	0.02	0.03	0.02	0.01	0.02	0.01

The FID has a very wide dynamic response range due to its full digital path. This enables a quantitative analysis of samples containing very high and very low concentrations in a single run. Figure 2 shows that trace impurities spiked in benzene, trace level (10 ppm) ethyl benzene, and > 99% benzene can be quantitative analyzed in a single run.

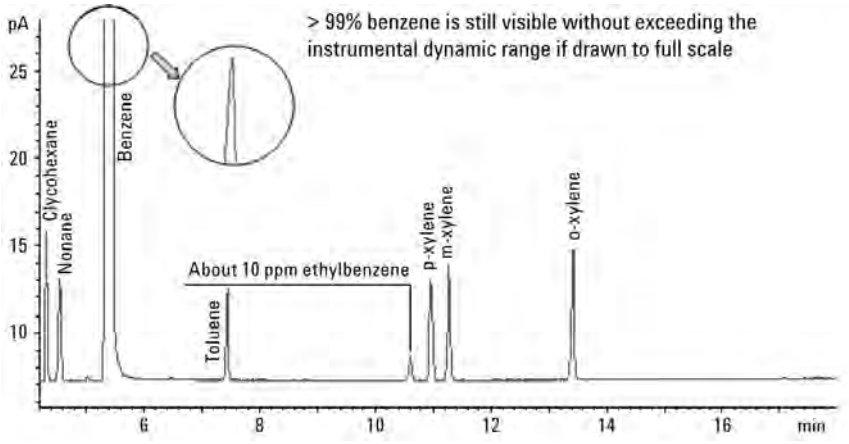
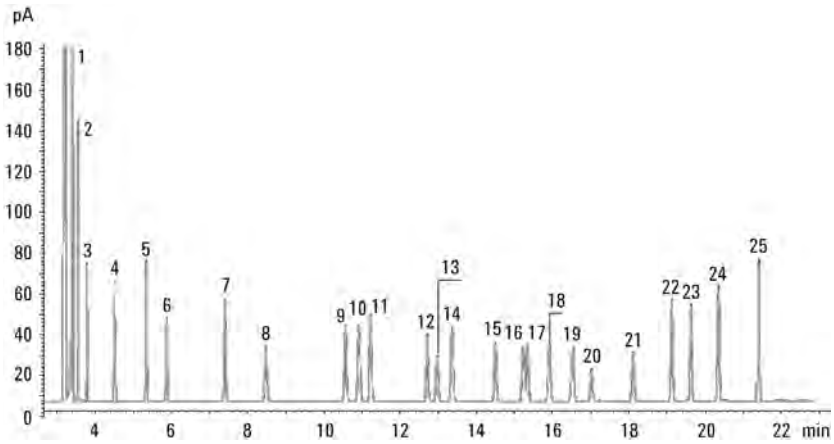


Figure 2. ***Analysis of trace impurities spiked in benzene.*** *Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 30:1.*

This system is also chromatographically suitable for a wide range of aromatic solvent samples according to 10 different ASTM aromatics methods as mentioned in reference 2. An n-hexane solution was prepared containing 0.1 wt% of aromatic solvents and impurities specified by the 10 ASTM methods for the analysis; the chromatographic overlay of 11 runs demonstrates outstanding repeatability as shown in Figure 3.



1. Heptane
2. Cyclohexane
3. Octane
4. Nonane
5. Benzene
6. Decane
7. Toluene
8. Undecane
9. Ethylbenzene
10. p-xylene
11. m-xylene
12. Cumene
13. Dodecane
14. o-xylene
15. Propylbenzene
16. p-ethyltoluene
17. m-ethyltoluene
18. t-butylbenzene
19. s-butylbenzene
20. Styrene
21. Tridecane
22. Diethylbenzene
23. n-butylbenzene
24. a-methylstyrene
25. Phenyacetylene

Figure 3. Chromatographic overlay of 11 runs of aromatic solvent specified by 10 ASTM methods.
Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C, 10 °C/min to 145 °C. Sample size: 0.5 µL, Split ratio: 100:1.

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2. James D. McCurry, “A Unified Gas Chromatography Method for Aromatic Solvent Analysis,” Agilent Technologies publication 5988-3741EN

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Printed in the USA
February 9, 2009
5990-3548EN

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Two-Dimensional Gas Chromatographic Analysis of Trace Benzene in Styrene

Application

HPI

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Abstract

A two-dimensional gas chromatographic method for the analysis of trace benzene in styrene is described. This method employs the Agilent 6890N GC system equipped with a Deans switch device. The method can be used in a prefractionator mode to analyze trace benzene in styrene according to the proposed ASTM method [1]. It can also be used in a heart-cutting mode to transfer more precisely just the benzene peak from the primary column to the secondary column. The Agilent 6890N electronic pneumatics control and flow calculation software are used for setting up the precise flows and pressures required. Excellent calibration and sensitivity of this system is demonstrated in both prefractionator mode and heart-cutting mode, and the interference can be greatly reduced by the heart-cutting.

Introduction

The American Society of Testing and Materials (ASTM) method D5135 uses a single column GC to measure the overall purity of styrene, including benzene down to 1000 ppm [2]. However, industry now desires to lower the benzene content in styrene to 10 ppm. Styrene also contains trace amounts of C_8 and C_9 hydrocarbons that cannot be separated from trace benzene with a single GC column. A fast analytical method for trace benzene in styrene is needed for final product inspections, process control, establishing specifications, and research work.

ASTM has proposed a two-dimensional gas chromatography (2-D GC) method to completely separate trace levels of benzene from other contaminants in styrene. This method operates in a prefractionator mode where the primary column retains the high boiling styrene components while the low boiling contaminants (including benzene) are transferred to a second column. After the transfer is complete, the benzene is easily separated from the interfering hydrocarbons on the second column. This method uses two columns of different polarity coupled together with a device that allows one or more discrete effluent segments to be transferred from the first to the second column. There are two different switching devices that can be used with this method. The first device uses a rotary valve to transfer the benzene from column one to column two. The second uses a fluidic switch to transfer the benzene. The fluidic switch is similar in design

to the Agilent Deans switch for the 6890N GC system. While the Agilent Deans switch works well as a prefractionator, it can also be used in a heart-cutting mode to offer more precise transfer of benzene from the first column to the second column, thus further improving chromatographic resolution.

Two-dimensional GC is an old idea that was first demonstrated by Deans over 30 years ago. Although the Deans switch approach was useful, it did not gain widespread use because of problems associated with unreliable column connections, column flow drift, oven temperature imprecision, and column variability [3]. These problems caused poor overall retention time (RT) precision, thus forcing the analyst to use very wide cut times to ensure transfer of the target analyst from column one to column two. These wider cut times resulted in the transfer of more interference peaks and a loss in overall resolution.

Advanced technology of the Agilent 6890N GC improves the performance of 2-D GC. It makes the construction of 2-D GC systems much simpler and easier to use. Modern column connections have low dead volume and are inert and reliable. The Agilent 6890N electronic pneumatics control (EPC) and oven controls provide better RT precision that allows narrower cut times for better resolution and quantitative precision. New Deans Switch Software used for calculating pressures makes the 2-D GC method development faster and easier.

Experimental

Deans Switch System Design

Heart-Cutting Mode

Figure 1 shows the Agilent 6890N GC system configured with the Deans switch hardware. The sample is injected into the split/splitless (S/S) inlet and is initially separated on the non-polar methyl silicone (HP-1) primary column. With the fluidic switch in the "off" position, the pneumatics control module (PCM) delivers 8.54 mL/min of helium through the lower flow path. This supplies 6.54 mL/min of helium to the secondary column coated with a polar polyethylene glycol (INNOWax) phase. The remaining 2 mL/min is used to divert to the HP-1 column eluent to flame ionization detector (FID) A.

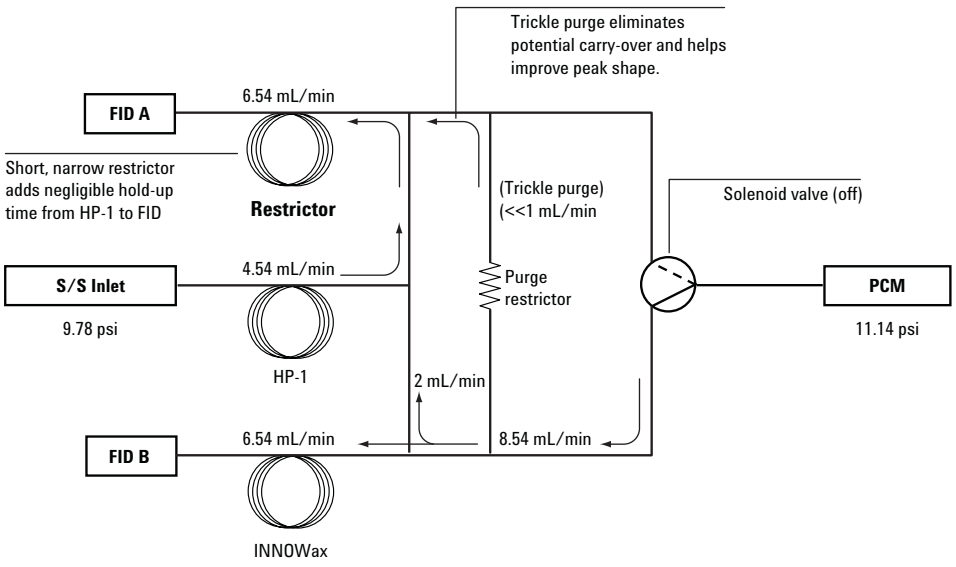

Figure 1. Solenoid valve off - no heart-cutting.

Figure 2 shows the flow path when heart-cutting with the Deans switch. Just before the benzene and any unresolved hydrocarbons elute from the HP-1 column, the fluidic switch is set to the "on" position. Helium from the PCM is now directed through the upper flow path so that 6.54 mL/min goes through the restrictor and 2 mL/min diverts the benzene and the hydrocarbons from the HP-1 column to the INNOWax column. The benzene is then separated from the hydrocarbons by the INNOWax column and detected on FID B. After the peaks are loaded onto the INNOWax column, the fluidic switch is returned to the "off" position shown in Figure 1, so that any peaks still on the HP-1 column are eluted to FID A.

Prefractional Mode

The Agilent 6890N Deans switch can also be used as a prefractionator. With the solenoid valve in the "on" position (Figure 2), the sample is injected into the S/S inlet and a partial separation is made on the nonpolar HP-1 column. The PCM delivers 8.54 mL/min of helium through the upper flow path. This supplies 6.54 mL/min of helium to the restrictor and 2 mL/min transfers fractions that are boiling point equal or less than benzene to the INNOWax secondary column.

When the benzene and lighter compounds are transferred to the INNOWax column, the fluidic switch is set to the "off" position, shown in Figure 1. The PCM delivers 8.54 mL/min of helium through the lower flow path. This supplies 6.54 mL/min of helium to the INNOWax column to separate the benzene from the hydrocarbons with detection on FID B.

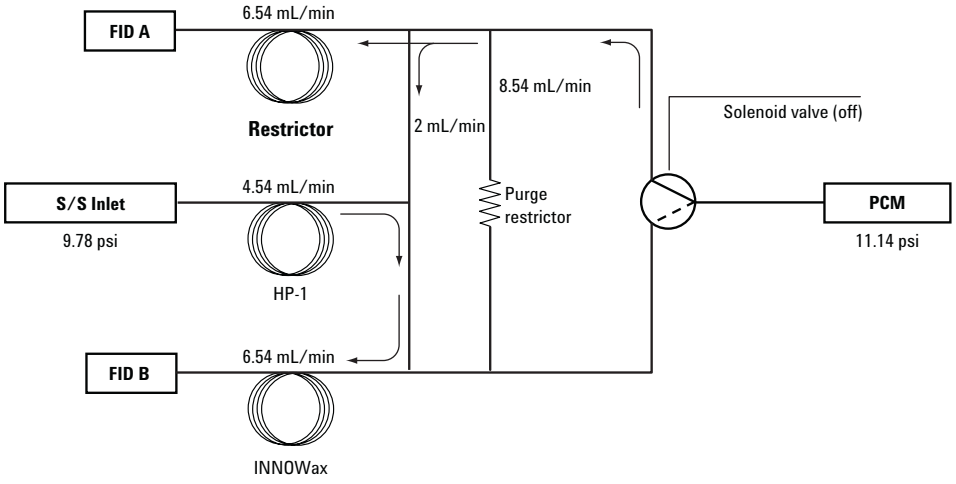

Figure 2. Solenoid valve on heart-cutting from HP-1 to INNOWax column.

Method Development Tool - Deans Switch Calculator

A powerful method development tool, Deans Switch Calculator, is used to easily set the correct column flows, inlet pressure, PCM pressure, and restrictor size. After the method parameters are decided, they are entered into designated fields in the software (shown in Figure 3). The Deans Switch Calculator will then determine the pressure needed to obtain the desired flow rates from the primary column inlet and the PCM. The software will also calculate the dimension for the fixed restrictor.

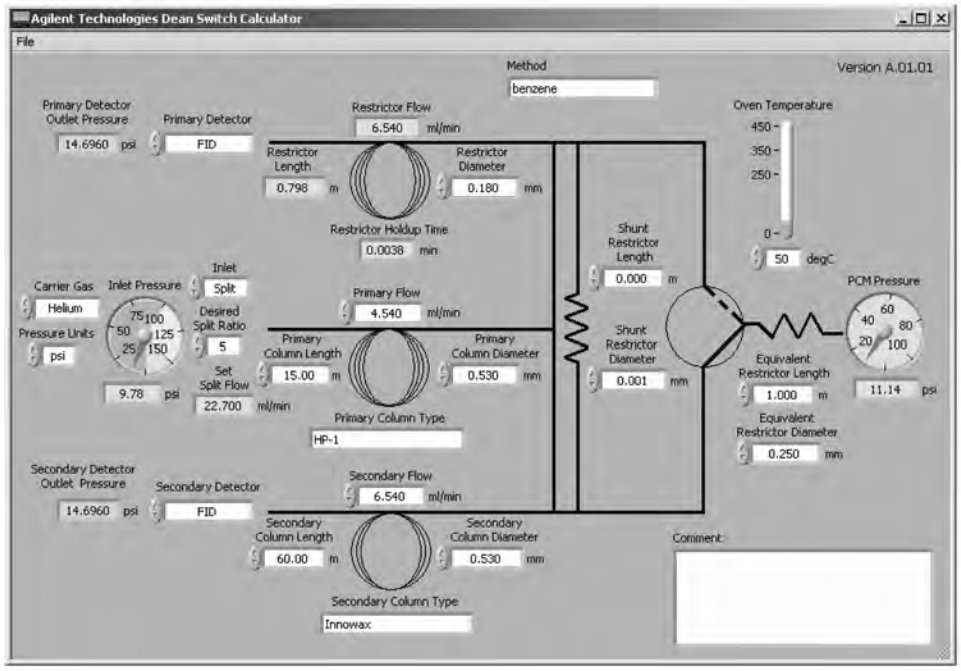

Figure 3. Deans Switch Calculator.

Hardware Configuration

The details of the system hardware for this analysis are listed in Table 1.

Table 1. Agilent 6890 Hardware Configuration

Standard 6890GC hardware		
G1540N	Agilent 6890N series GC	1
112	Capillary split/splitless inlet with EPC control	1
210	FID with EPC control	2
309	PCM with EPC control	1
G2613A	Agilent 7683 autoinjector	1
2310-0129	Deans switch kit	1
Columns		
Primary column	HP-1 15 m, 0.53 mm, 3.0 µm (p/n 19095Z-421)	1
Secondary column	INNOWax 60 m, 0.53 mm, 1.0 µm (p/n 9095N-126)	1
Data system		
G2070A	Agilent multitechnique ChemStation rev. A.08.01	
Optional consumables		
5812-3442	Merlin microseal high-pressure septum	
5182-0875	5-µL fixed straight needle autoinjector syringe for Merlin Microseal	
5183-4647	Inlet liner optimized for split operation	
Standards		
	Benzene, 99.8% minimum purity	
	Styrene, the highest purity available, but not less than 99.6%	

Instrument Conditions

Instrument conditions are listed in Table 2.

Table 2. Instrument Conditions

Split/Splitless inlet	Split mode, with 5:1 split ratio
Temperature	250 °C
Pressure	9.78 psi helium
Split ratio	5:1
HP-1 Column flow	4.54 mL/min, constant pressure mode
INNOWax column flow	6.54 mL/min, constant pressure mode
PCM	11.1psi
FID Temperatures	250 °C
Oven temperature program	50 °C for 4 min, 20 °C/min to 220 °C, for 2 min
FID H_2 flows	40 mL/min
FID Air flows	450 mL/min
FID Makeup gas (He) flows	45 mL/min

Results and Discussion

Heart-Cutting Mode

Agilent Deans switch design can be used in a heart-cutting mode, the results show very good sensitivity, precision, and linearity. The heart-cutting mode can precisely transfer just the benzene peak from the primary column to the secondary column.

Sensitivity

Figure 4 shows a chromatogram of 1-ppm benzene in styrene. It demonstrates the very good performance of the Agilent Deans switch system for sensitive and quantitative detection of 1-ppm benzene in styrene.

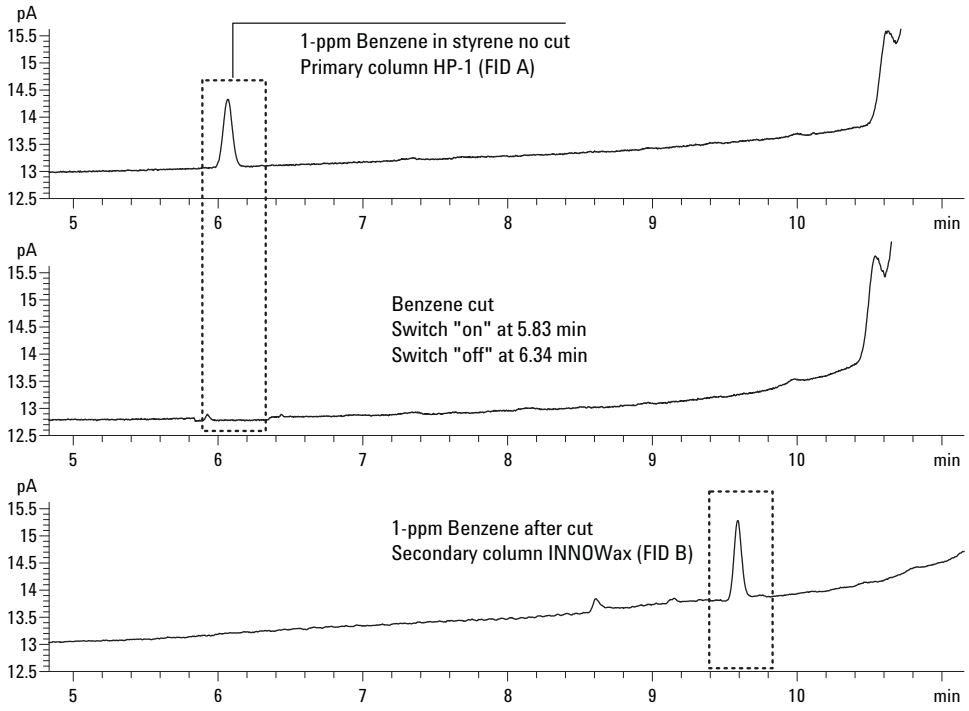

Figure 4. Benzene 1-ppm in styrene can be detected at good signal to noise (S/N) with heart-cutting mode.

Precision

Table 3 shows the excellent RT and detector response repeatability when heart-cutting with the Agilent Deans switch for this method.

Table 3. Repeatability of 2-D GC Method with Heart-Cutting Model

	1-ppm Benzene in styrene		10-ppm Benzene in styrene		100-ppm Benzene in styrene		1000-ppm Benzene in styrene	
	Area	RT	Area	RT	Area	RT	Area	RT
Run 1	5.4	9.589	55.2	9.555	569.6	9.557	5848.8	9.556
Run 2	5.4	9.566	55.7	9.553	572.5	9.556	5898.8	9.555
Run 3	5.4	9.560	55.2	9.555	567.5	9.556	5920.5	9.555
Run 4	5.4	9.561	55.5	9.557	569.5	9.556	5886.1	9.555
Run 5	5.4	9.560	55.4	9.557	570.9	9.554	5821.2	9.556
Average	**5.4**	**9.567**	**55.4**	**9.5554**	**570**	**9.561**	**5875.08**	**9.5554**
SD	**0.00**	**0.01**	**0.190**	**0.0015**	**1.6565**	**0.0023**	**35.6111**	**0.0005**
%RSD	**0.00**	**0.12**	**0.34**	**0.02**	**0.29**	**0.02**	**0.61**	**0.01**

Linearity

Regression statistics of four-point linear calibration curve for benzene containing 1-ppm to 1000-ppm in styrene is shown in Figure 5.

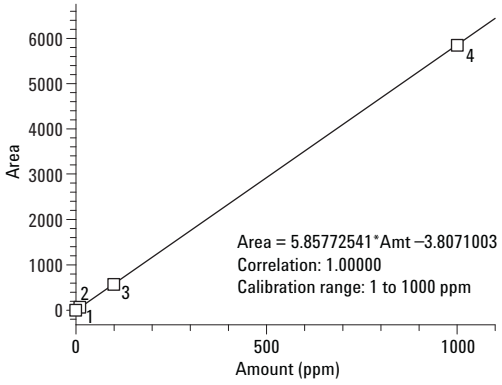

Figure 5. Calibration for styrene containing 1- to 1000-ppm benzene with heart-cutting mode.

Prefractionator Mode

Agilent Deans switch design can be used in a prefractionator mode to analyze trace benzene in styrene according to the proposed ASTM method. The result demonstrates excellent sensitivity and precision.

Sensitivity

Figure 6 shows a chromatogram of 1-ppm benzene in styrene. The 1 ppm of benzene is easily detected at good S/N.

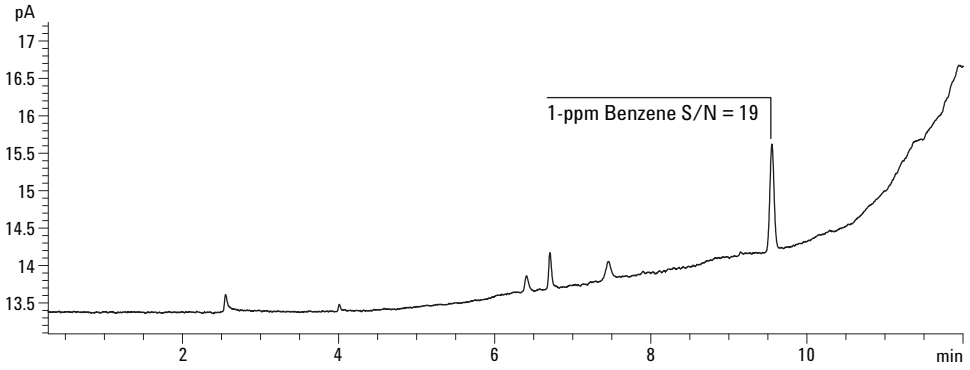

Figure 6. Benzene 1-ppm in styrene

Precision

Table 4 shows a very good repeatability for calibration standards from 1-ppm to 1000-ppm benzene when using the Agilent Deans switch as a prefractionator.

Table 4. Repeatability of 2-D Method with Prefractionator Model

	1-ppm Benzene in styrene		**10-ppm Benzene in styrene**		**100-ppm Benzene in styrene**		**1000-ppm Benzene in styrene**	
	Area	RT	Area	RT	Area	RT	Area	RT
Run 1	5.3	9.552	54.1	9.540	543.4	9.547	5492.1	9.498
Run 2	5.3	9.550	54.0	9.540	543.8	9.547	5492.8	9.500
Run 3	5.3	9.542	53.8	9.541	538.3	9.544	5489.7	9.504
Run 4	5.3	9.544	53.4	9.545	539.1	9.545	5507.7	9.522
Run 5	5.2	9.540	53.9	9.548	538.5	9.544	5525.9	9.544
Average	**5.3**	**9.546**	**53.8**	**9.543**	**540.6**	**9.545**	**5501.6**	**9.514**
SD	**0.04**	**0.005**	**0.2**	**0.003**	**2.4**	**0.001**	**13.7**	**0.017**
%RSD	**0.76**	**0.05**	**0.45**	**0.03**	**0.45**	**0.01**	**0.25**	**0.18**

Linearity

Figure 7 shows a calibration for styrene containing 1 to 1000-ppm benzene with prefractionator mode.

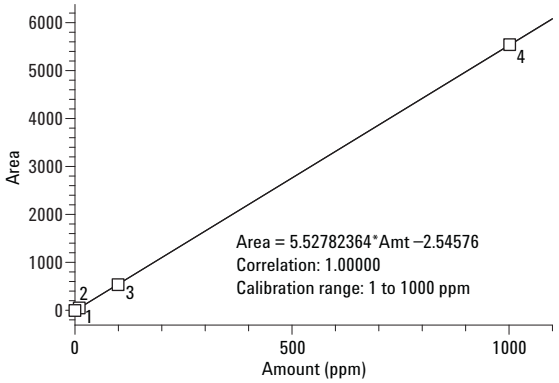

Figure 7. Calibration for styrene containing 1- to 1000-ppm benzene with prefractionator mode.

The Comparison Between Heart-Cutting Mode and the Prefractionator Mode

Comparing heart-cutting technique to the prefractionator technique, the former one can greatly reduce interference, because the heart-cutting can precisely transfer just the benzene and any co-eluting compounds from the primary column to the secondary column. While in the prefractionator mode, all compounds that elute before benzene on the HP-1 column are also transferred to the INNOWax column. These extra compounds are a potential source of interference on the INNOWax column. To show that heart-cutting can reduce potential interference, a styrene sample was spiked with 1000 ppm of natural gasoline to increase the amount and number of hydrocarbons. This sample was run using both the heart-cutting mode and the prefractionator mode. Figures 8 and 9 show the results of this experiment.

On the nonpolar primary column (HP-1), the components elute in boiling point order and the benzene peak co-elutes with hydrocarbons that have boiling points similar to benzene. After heart-cutting to the polar INNOWax column, the benzene is easily separated from the less polar hydrocarbons as shown in Figure 8. In the prefractionator mode, the benzene and all lighter compounds are transferred to the polar INNOWax column, on which there are hydrocarbons peaks including benzene peaks as shown in Figure 9. This demonstrates that heart-cutting greatly reduces the number of potential interferences for this method.

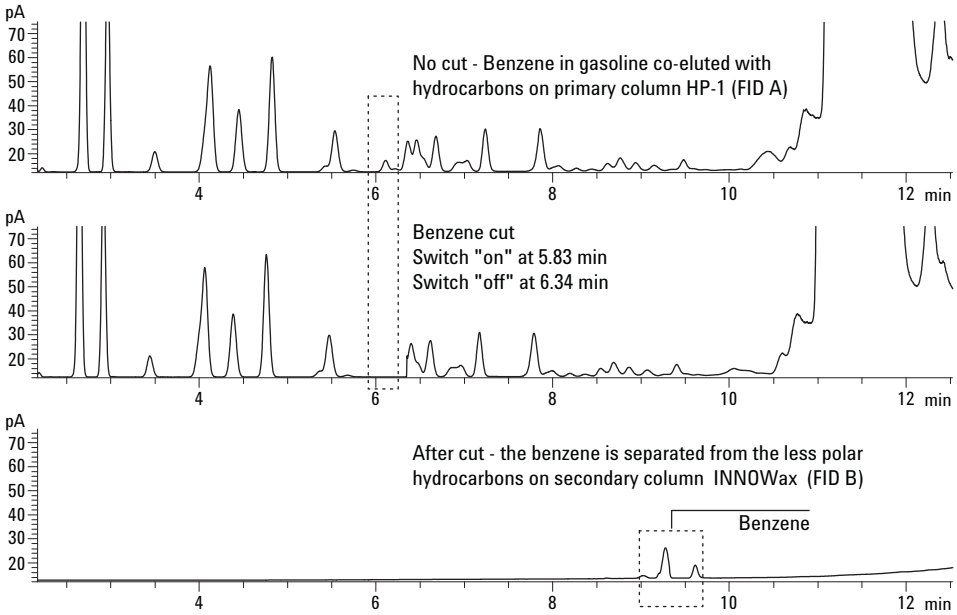

Figure 8. One thousand ppm gasoline in styrene with heart-cutting mode with reduced hydrocarbon peaks in the lower chromatogram.

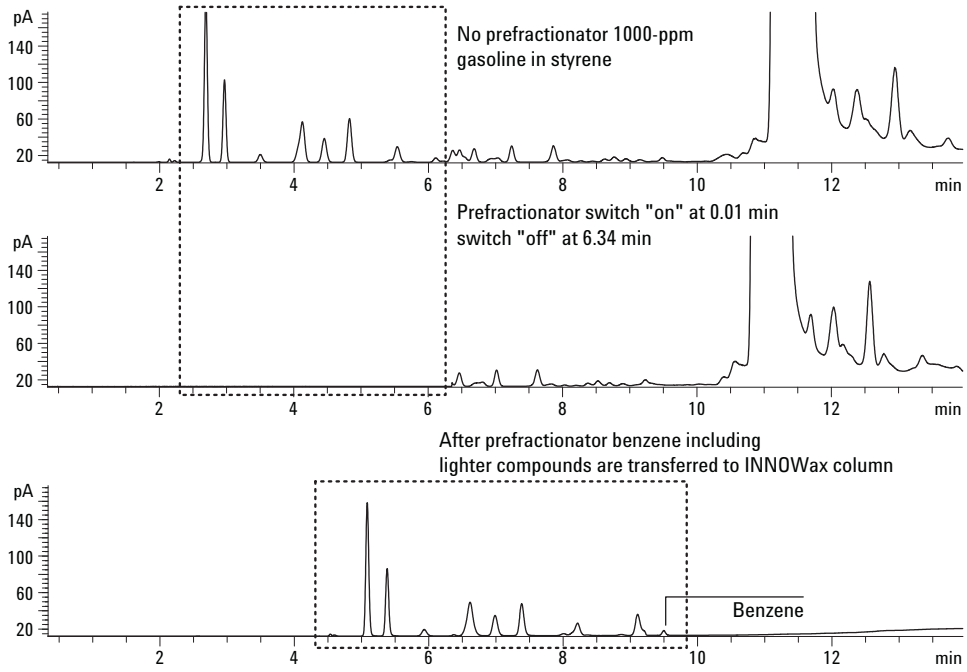

Figure 9. One thousand ppm gasoline in styrene with prefractionator mode. A larger number of peaks are observed in the lower chromatogram.

Conclusions

An easy and reliable 2-D GC system with a simplified Deans switch can be used in a prefractionator mode and in a heart-cutting mode to analyze trace benzene in styrene. In this system the Agilent 6890 EPC provides better RT precision that allows narrower cut times for better resolution and quantitative precision. Deans Switch Calculator makes the method development faster and easier. As the results have demonstrated, prefractionator and heart-cutting modes have an excellent calibration, linearity, and repeatability. The heart-cutting can greatly reduce the interference and benzene co-eluted with hydrocarbons on the nonpolar primary column and can be completely separated after heart-cutting to the polar secondary column. The system was able to detect 1-ppm benzene in styrene with excellent signal to noise.

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Printed in the USA
March 2, 2004
5989-0594EN



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Analysis of Essential Oils by Fast Capillary GC Using The Agilent 6890 Series GC

Application

Gas Chromatography

November 1995

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Abstract

By reducing the internal diameter of the capillary column, a higher efficiency per unit of column length is obtained in capillary GC (CGC). The application of narrow bore columns, therefore, results in faster analyses compared to conventional columns while maintaining the resolution.

The use of narrow bore columns in routine analysis, however, make high demands on instrumentation. The 6890 Series GC offers electronic control of column flow and split flow, faster oven heating and faster electronics, making fast capillary GC accessible for routine applications. Method translation software is an additional tool to translate an existing operating procedure into a method using a narrow bore column. The performance of the 6890 Series GC in fast CGC is demonstrated by the analysis of some essential oils.

Introduction

The ultimate goal of a gas chromatographic method is to obtain complete resolution of a given mixture in the shortest possible analysis time. For trace analysis, sensitivity and sample loadability are additional requirements. In order to meet these goals, capillary columns with a length of 25 to 30 m and an internal diameter of 0.25 to 0.32 mm are most frequently used in routine applications. These capillary columns offer high efficiency (100,000 plates) and sufficient sample capacity and loadability. Decreasing the internal diameter results in an increase of the column efficiency and, therefore, the column length can be reduced while keeping the resolution constant[1-4]. A 10 m x 0.1 mm i.d. column, for instance, offers the same resolution as a 25 m x 0.25 mm i.d. column. Because the column is 2.5 times shorter, the analysis time is reduced drastically. Moreover, because the van Deemter curves are flatter for narrow bore columns and the optimum gas velocities are higher, the speed of analysis can further be increased without reducing the resolution. The price to pay for the gain in speed of analysis is the lower sample capacity, resulting in the practical consequence that only small volumes can be injected. In practice, split injection with a high split ratio is used. Many applications in chemical, petrochemical, food and, flavor and fragrance analysis, however, do not need ultimate sensitivity and for these applications the use of narrow bore columns can offer an important reduction in analysis time and, consequently a higher sample throughput, while maintaining resolution.

Until now, the major obstacle for the implementation of narrow bore columns in routine CGC were instrumental requirements. The use of narrow bore columns requires high inlet pressures, better split flow control, fast oven temperature heating rates and fast electronics. With the introduction of the 6890 Series GC a major step was made in meeting these GC requirements.

The 6890 Series GC indeed offers:

High inlet pressures: The maximum inlet pressure is 150 psi (1030 kPa).

This pressure is high enough for 0.1 mm i.d. columns up to 50 m long and for 0.05 mm i.d. columns up to 15 m long when using hydrogen carrier gas.

Electronic pneumatic control of carrier and detector gases: Both the column head pressure and the split flow are controlled electronically from the keyboard or the ChemStation. This results in excellent repeatibility of flow settings.

Gas saver mode: Due to the high split ratio used, a very high flow of carrier gas exits the split vent. Because this high split vent flow is only needed during injection, the gas saver mode can be activated after injection to reduce the split flow for reducing gas consumption and for safety reasons when hydrogen is used as the carrier gas.

Fast oven heating: The 6890 Series GC allows oven heating rates up to 120°C/min. Heating rates of 50°C/min or more are sometimes necessary in order to fully exploit the speed gain that can be obtained using narrow bore columns.

Fast electronics: A data sampling rate up to 200 Hz makes signal acquisition of very sharp and fast eluting peaks possible.

Using narrow bore columns, different operational conditions are used. Because little information is yet available on the use of narrow bore columns, the transfer of standard operating procedures for conventional capillary columns into operating procedures for narrow bore columns might be difficult. In this respect, the development of method translation software[5] is very helpful for translating a standard operating procedure developed for a standard column to an operating procedure for a narrow bore column. After performing the analyses on the standard column, the optimized conditions are introduced in the program and all operational conditions for the narrow bore column are calculated in order to obtain the same resolution. The gain in analysis time is also predicted.

In this application note, the performance of the 6890 Series GC for fast CGC of essential oils is illustrated.

Experimental

Samples

Two samples were analyzed. Sample A is an artificial mixture of flavor compounds used for perfuming soaps and detergents. The sample was diluted to 1% in dichloromethane. Sample B is a natural lavender oil, also diluted to 1% in dichloromethane.

Analytical Conditions

The analyses were performed on a 6890 Series GC. Automated split injection was done using a 7673 autosampler. The instrumental configuration and analytical conditions are summarized in Table 1. First the analyses were performed on a 30 m x 0.25 mm i.d. x 0.25 µm HP-5 standard capillary column.

The conditions applied with this column are listed in experimental conditions A. The same analyses were then performed on a 15 m x 0.1 mm i.d. x 0.1 µm HP-5 narrow bore column. The conditions applied with this column were calculated by the method translation software and are listed in experimental conditions B. From the method translation software, a speed gain by a factor 2.4 was predicted.

Table 1. Instrumentation

Chromatographic System	
Gas chromatograph	6890 Series
Inlet	Split/splitless
Detector	FID
Automatic sampler	7673
Liner	Split/splitless liner, 4 mm i.d. with glass wool plug, (part no. 19251-60540)
Data handling	ChemStation (DOS Series)
Column A	30 m x 0.32 mm i.d. x 0.25 µm H-5
Column B	15 m x 0.10 mm i.d. x 0.1 µm H-5
Experimental Conditions A	
Inlet temperature	250°C
Injection volume	1 µL
Split ratio	1/50
Carrier gas	Hydrogen
Head pressure	6 psi
Flow/velocity	1 mL/min-35 cm/s
Split flow	49 mL/min
Gas saver	OFF
Oven temperature	40°C, 5°C/min to 190°C
Detector temperature	250°C
Detector gases.	Hydrogen: 35 mL/min; Air: 400 mL/min; Helium: 30 mL/min
Experimental Conditions B	
Inlet temperature	250°C
Injection volume	1 µL
Split ratio	1/530
Carrier gas	Hydrogen
Head pressure	29 psi
Flow/velocity	1.8 mL/min-88 cm/s
Split flow	192 mL/min
Gas saver	ON at 1 min
Oven temperature	40°C, 12°C/min to 190°C
Detector temperature	250°C
Detector gases	Hydrogen: 35 mL/min; Air: 400 mL/min; Helium: 30 mL/min

Results and Conditions

The analysis of the artificial flavor sample is shown in Figure 1. A good separation is obtained in an analysis time of 30 minutes.

The same analysis was then performed on the narrow bore column, applying the operation conditions calculated by the method translation software. The resulting chromatogram is shown in Figure 2. By comparing both figures, it is obvious that practically the same resolution is obtained. Using the narrow bore column, the analysis time is only 12.5 minutes, perfectly corresponding with the predicted speed gain by a factor 2.4.

Figure 3. Analysis of lavender oil on a 15 m x 0.10 mm i.d. x 0.1 µm HP-5 column.

Using the same analytical conditions, a solution of a natural lavender oil was analyzed. The chromatogram is shown in Figure 3. The main peaks in this essential oil correspond to linalool, camphor, linalyl acetate and geranyl acetate. For these four compounds, the retention time and peak area reproducibility was calculated for 10 runs. The results are given in Tables 2 and 3. The standard deviation on the retention times was smaller than 0.002 minutes (RSD < 0.03) The relative standard deviation on peak areas was better than 1 %.

Conclusion

The Agilent 6890 Series GC offers excellent potentials for fast capillary GC. The electronic pneumatic control of the column head pressure and the split flow, the gas saving option, the fast and reproducible oven heating and the fast electronics, result in high reproducibility of retention times and peak areas. Using the method translation software, an existing operating procedure applying a standard capillary column can be translated into an operating procedure for a narrow bore column, resulting in a faster analysis while maintaining resolution.

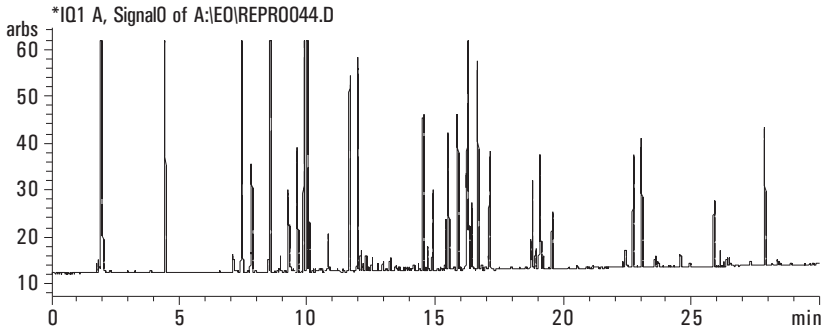

Figure 1. Analysis of an artificial flavor sample on a 30 m x 0.25 mm i.d. x 0.25 µm HP-5 column.

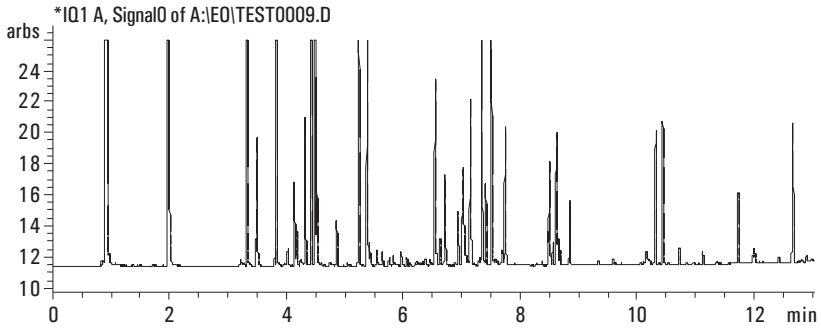

Figure 2. Analysis of an artificial flavor sample on a 15 m x 0.10 mm i.d. x 0.1 µm HP-5 column.

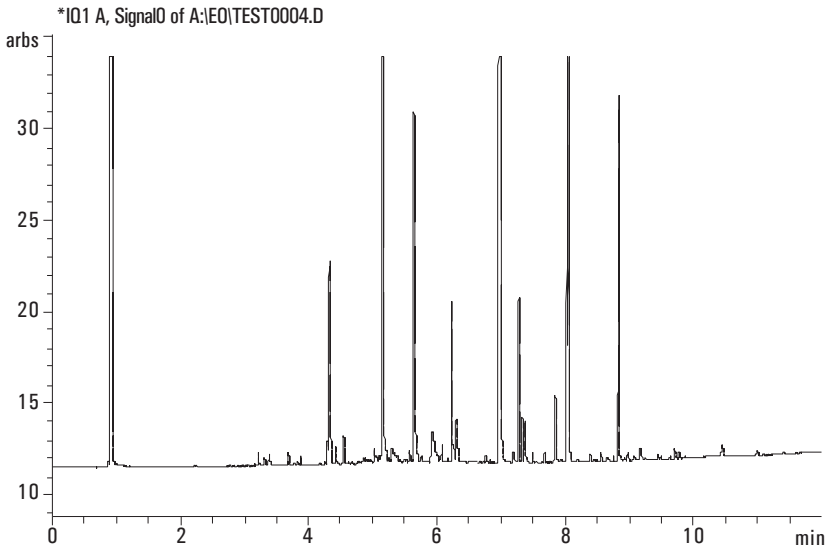

Figure 3. Analysis of lavender oil on a 15 m x 0.10 mm i.d. x 0.1 µm HP-5 column.

Table 2. Peak Area Precision for the Analysis of Lavender Oil Using a 15 m x 0.10 mm i.d. x 0.1 µm HP-5 Column

Run	Peak Linalool	Camphor	Linalylacetate	Geranylacetate
1		23.80851	103.47838	87.62983
2	115.93026	23.98765	103.49316	87.55368
3	117.01113	24.19413	104.4487	88.41127
4	116.82664	24.10895	104.15176	88.263
5	117.13489	23.91479	104.39146	88.3009
6	117.0449	24.00706	104.41306	88.30447
7	118.30569	24.23741	105.43989	89.23877
8	117.31309	24.34547	104.68652	88.69226
9	118.18525	24.18469	105.25503	89.21329
10	118.06036	24.06571	105.05595	89.24325
MEAN	117.16897	24.085437	104.481391	88.485072
S	0.849920602	0.161532422	0.666212109	0.617913328
RSD (%)	0.725	0.671	0.638	0.698

Table 3. Retention Time Precision for the Analysis of Lavender Oil Using a 15 m x 0.10 mm i.d. x 0.1 µm HP-5 Column

Run	Peak Linalool	Camphor	Linalylacetate	Geranylacetate
1	5.112	5.592	6.925	7.96
2	5.113	5.592	6.924	7.959
3	5.114	5.591	6.925	7.96
4	5.114	5.593	6.924	7.96
5	5.114	5.593	6.924	7.959
6	5.112	5.591	6.924	7.959
7	5.114	5.593	6.925	7.96
8	5.114	5.593	6.924	7.961
9	5.114	5.593	6.927	7.963
10	5.114	5.593	6.927	7.965
MEAN	5.1135	5.5924	6.9249	7.9606
S	0.000849837	0.000843274	0.001197219	0.00195505
RSD (%)	0.0166	0.0151	0.0173	0.0246

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Printed in the USA 3/2000
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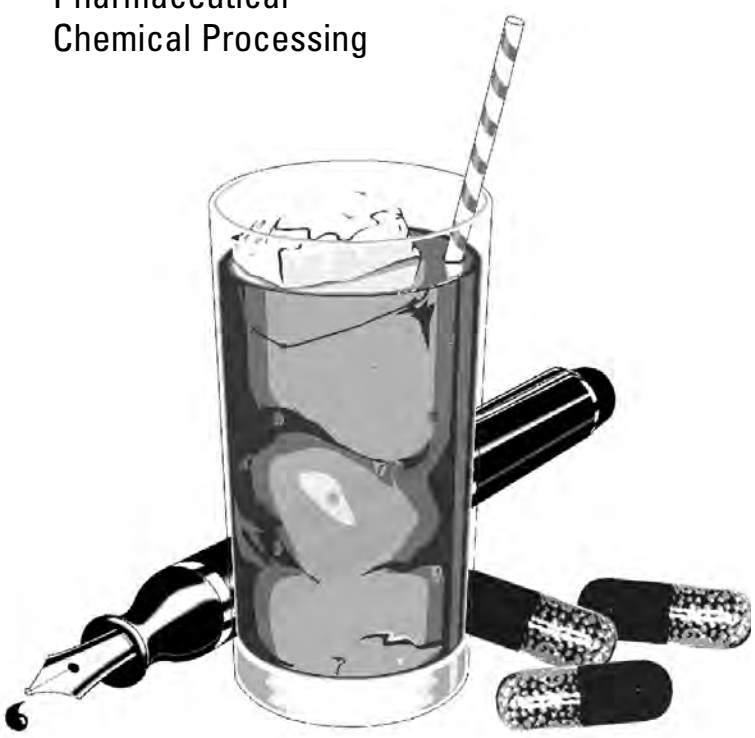
Sensitive Analysis of Synthetic Colors using HPLC and Diode- Array Detection at 190–950 nm

Application Note

Food and Flavors
Pharmaceutical
Chemical Processing

Angelika Gratzfeld-Hüsgen
Rainer Schuster



In this study synthetic dyes were analyzed using ion-pair, reversed phase chromatography on a special base-deactivated HPLC column. This separation mechanism was chosen to reduce tailing effects of highly polar colors. Detection was performed using a new design of diode-array detector based on two lamps — a deuterium lamp and a tungsten lamp. This ensured highest light output at 190 – 950 nm, which resulted in lowest detection limits over the entire wavelength range. It was possible to analyze blue, black or green colors in the low ng range at their absorption maxima of 600 – 700 nm. Complete spectra, feasible from 190 – 950 nm, facilitated identification with an automated library search.

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Introduction

Synthetic colors are widely used in the food, pharmaceutical and chemical industries.

The regulation of colors and the need for quality control relating to traces of starting products and by-products have forced the development of analytical methods. Nowadays various HPLC methods are in use, based either in ion-pair reversed phase chromatography or ion-exchange chromatography. UV absorption is the detection method of choice. The UV absorption maxima of colors are very characteristic, starting with maxima at about 400 nm for yellow colors, 500 nm for red colors and at 600 – 700 nm for green, blue and black colors. To analyze all different colors at maximum sensitivity and selectivity, the light output from the detector lamp should be high across the complete wavelength range. For black, green and blue colors, which show absorption maxima at or above 600 nm, this is not possible with conventional UV-Visible detectors based on a one-lamp design. For example, deuterium lamps have their maximum output in the UV range, whereas the visible range shows low light output.

A further analytical problem is the tailing-free separation of dyes. Figure 1 shows that some colors are of more polar nature, which sometimes causes problems even on reversed phases depending on the strength of polarity tailing. This results in worse detection limits and integration problems.

In the following study we evaluated the influence of:

- special deactivated columns on the separation of colors using different mobile phases,
- a newly designed diode-array detection system on limit of detection for colors which absorb at above 500 nm, and
- low noise and complete spectra on identification of sample compounds using automated library search in the low mAU range.

Experimental

For the experiments the Agilent 1100 Series HPLC system was used. The system comprised a low-pressure quaternary gradient pump with vacuum degasser, autosampler, Peltier-regulated column compartment, and diode-array detector with a wavelength range of 190 – 950nm. System control and data evaluation was done through an Agilent ChemStation for HPLC.

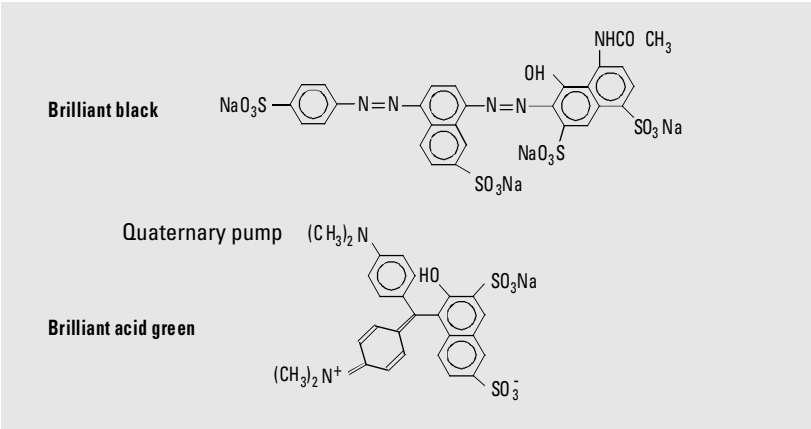

Figure 1
Chemical structure of brilliant black and brilliant green BS

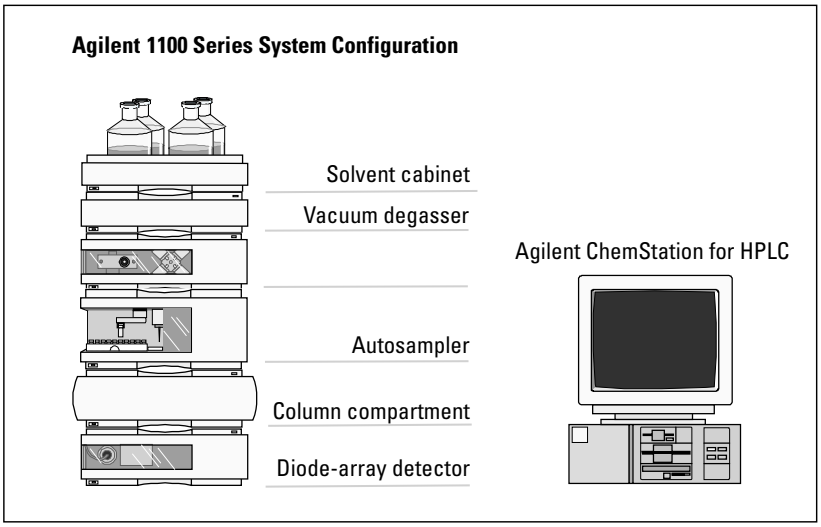

Figure 2
HPLC system used

Results and discussion

Separation of synthetic colors
During method development a special base-deactivated column and four different mobile phases were evaluated. Two mobile phases were simple buffer systems and the other two phases contained ion-pairing reagents (see figure 3). A 125 x 3 mm Hypersil BDS, 3-µm column (Agilent part number 79926BD-363) was used. The use of 3-mm id. and 3-µm material allowed optimum flow rates below 1 ml/min. This saved purchasing disposal costs of solvents. Mobile phases C and D showed tailing for compounds with sulfonic groups. Using ion-pairing chromatography (see figure 3, examples A and B), the separation of colors with different functional groups and different chemical structure was achieved with minimum or no tailing for all different color classes. As a conclusion, we recommend using mobile phases from A.

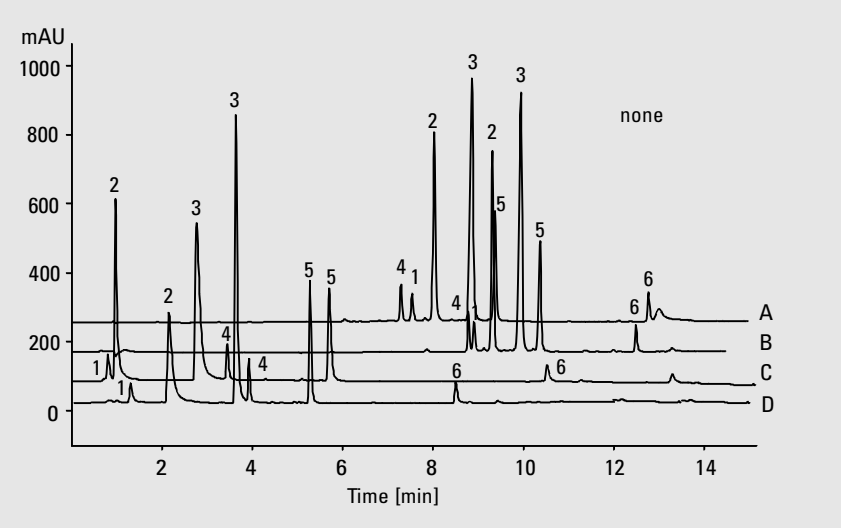

Figure 3
Separation of synthetic colors on BDS column using different mobile phases

Peak	Compound name	Number of sulfonic group
1	E102 Tartrazine	2
2	E123 Amaranth	3
3	E124 Ponceau 4R	3
4	E110 Sunset yellow	2
5	E125 Scarlet red	none
6	E127 Erythrosine	

Table 1
Content of sulfonic groups for colors shown in figure 3

Chromatographic conditions

For the application examples that follow we used mobile phases from A.

Column : 125 × 3 mm Hypersil BDS, 3 µm (HP part number 79926BD-363)

Mobile phase A: A = 0.01 M NaH_2PO_4 + 0.001 M Tetrabutylammonium-dihydrogenphosphate pH 4.2
B = acetonitrile (ACN)

Gradient for A: start with 15 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 15 % ACN

Mobile phase B A = 0.01 M NaH_2PO_4 + 0.001 M Tetrabutyl ammoniumhydrogen sulfate pH 4.8
B = acetonitrile (ACN)

Gradient for B: start with 15 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 15 % ACN

Mobile phase C A = 0.01 M ammoniumacetate pH = 4.9, B = ACN

Gradient for C: start with 7 %, in 10 min to 40 %, in 14 min to 90 %, until 19 min at 90 %, in 20 min to 7 % ACN

Mobile phase D A = 0.01 M NaH2P04, pH = 4.3, B = acetronitrile (ACN)

Gradient for D: start with 5 %, in 10 min to 60 %, in 14 min to 90 %, until 19 min at 90 % in 20 min to 5 % ACN

Stop time 20 min
Post time 4 min
Flow rate 0.8 ml/min
Col. temp. 40 °C
Inject. vol. 1 µl
Detector signal A: 254 nm/50 nm (for optimization of separation)
B: 350 nm/20nm,
C: 465 nm/30 nm,
D: 600 nm/40 nm
E: 750/40

Detection of synthetic colors using diode-array UV-visible absorption detector

The diode-array detector used here was equipped with two lamps, a deuterium lamp and a tungsten lamp. This ensured highest light output from 190 to 950 nm and therefore lowest detection limits over the entire wavelength range. The use of 1024 diodes and a programmable slit ensured highest spectral resolution. This gave the following advantages for the analysis of colors:

- acquisition of five signals simulataneously,
- highest sensitivity and selectivity even for blue, green and black colors with absorption maxima above 500 nm,
- complete spectral data up to 950 nm, and
- optimization of signal to noise ratio using different slit width without the need to exchange optical slits mechanically.

Figure 4 shows the complete spectra of a yellow, red and two blue colors.

For each of the analyzed colors characteristic absorption maxima were obtained. The yellow color tartrazine had its maxima at around 400 nm, the red color amaranth absorbed best at about 500 nm, the blue color patent blue had its maxima around 600 nm whereas the darker blue color brilliant blue showed its maxima at 740 nm. This clearly demonstrated that several signals had to be acquired for optimum sensitivity and selectivity for all colors.

The spectra of pure compounds can be stored in spectral libraries and used for later identification of colors in food, paints or pharmaceutical preparations.

That sensitivity is of utmost importance is shown in figure 8. The quality of inks is often determined by the content of traces of other colors, which may be unwanted by-products from production process or which are added to influence the nuance of a color. Therefore the quantitation and identification of trace compounds is as important as the determination of main compounds.

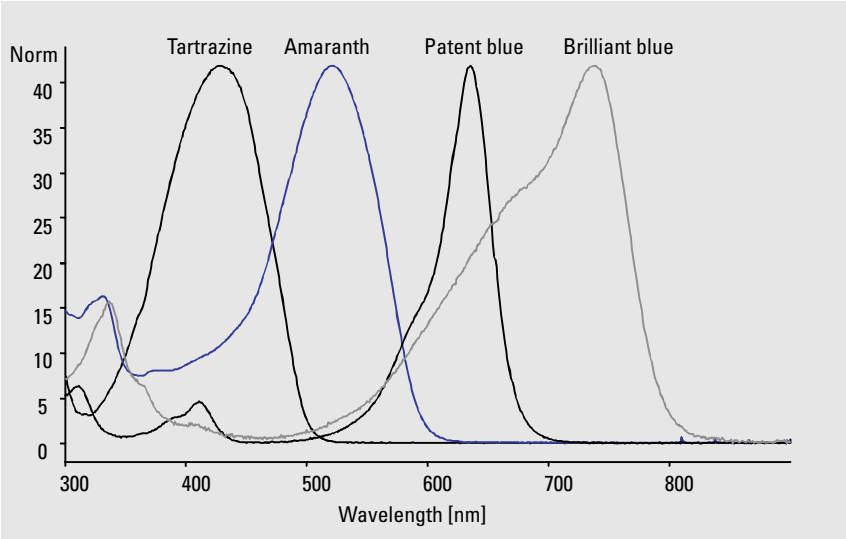

Figure 4
Spectra of different colors

As already demonstrated colors have very characteristic spectra which can be used to identify peaks not only by retention times but also by spectral data. The detector used here allowed identification using spectral data, see figure 5, even in the low mAU absorption range.

The *detection limit* for the evaluated colors, measured at their specific absorption maximum, was in the low ng range.

The *repeatability* of the HPLC method used here was measured using the standard mixture of figure 3. The relative standard deviation for retention times measured over 10 runs was below 0.2 %. For the areas the relative standard deviation was below 1 %.

The *linearity* was evaluated using blue ink color measured at 600 nm. Linearity was given from the low ng up to the low µg range.

Application Examples

Food colors

Colors are vital constituents of foods and probably the first characteristic perceived by the human senses. Today synthetic dyes have widely replaced natural colors. Table 2 lists some of the most frequently used food colors.

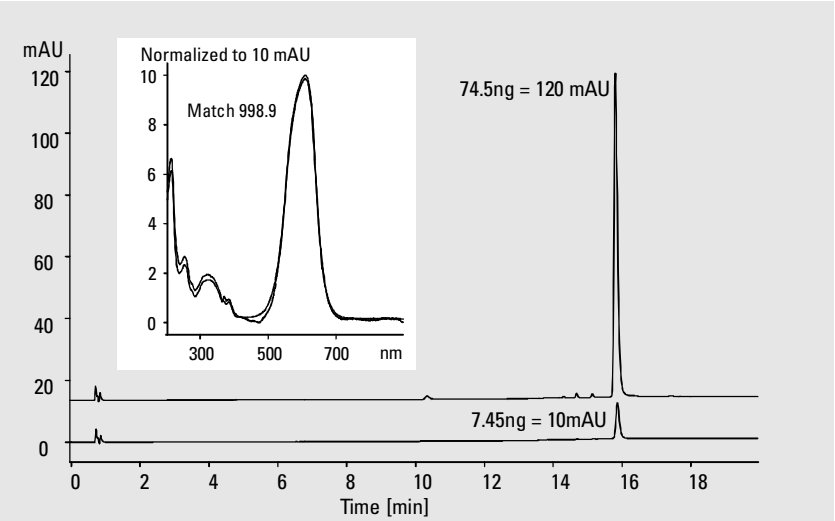

Figure 5
Identification of spectra in the low mAU absorption range for a blue ink color through overlay of trace level sample spectrum with library spectrum

EC	Name	Food, Drug & Cosmetics	CI
E102	Tartrazine	FD&C Yellow No. 5	19140
E103	Chryosine		14270
E104	Quinoline yellow	FD&C Yellow No. 10	47005
E105	Yellow		13015
E110	Sunset yellow	FCF FD&C Yellow No. 6	15985
E111	Sunset yellow	FD&C Orange No. 2	15980
E122	Azorubine	Carmoisine	14720
E123	Amaranth FD&C	Red No. 2	16185
E124	Ponceau 4R	Ponceau 4R	16255
E125	Scarlet red	Scarlet red	14815
E126	Ponceau 6R		16290
E127	Erythrosine	FD&C Red No. 3	45430
E131	Patent blue V	FD&C Violet No. 1	42051
E132	Indigo carmine	FD&C Blue No. 2	73015
E142	Acid brilliant green	FD&C Green	No. 3
E151	Black PN		28440
	Ponceau SX	FD&C Red No. 4	

Table 2
List of commonly-used colorings in EC and US classifications with color index numbers (CI

These dyes are used to supplement and enhance the natural colors destroyed during processing or storage, and substantially increase the appeal and acceptability of foodstuffs where no natural colors exist, for example, soft drinks or ice cream.

The usage of synthetic colors is well regulated worldwide, but the regulations differ from one country to the next. To ensure compliance with regulatory requirements, the used colors have to be identified and qualified according to national directives.

As an example of the determination of colors in foodstuffs we analyzed the synthetic colors used for a green carbonated drink — *a woodruff-ade*. The sample was injected directly and the compounds were identified using a library search (figure 6).

The green color was produced by a mixture of quinoline yellow and patent blue. The yellow color quinoline yellow split into four peaks showing an absorption maximum at 410 nm. The blue color patent blue had its maxima at 600 nm.

Colors for pharmaceutical preparations

The pharmaceutical industry uses colors, for example, for tablets, capsules and syrups. Here the intention is not only to improve the optical appearance but also to give more safety to the consumer. For example, different colors may help to avoid errors for a patient who has to take several medicaments.

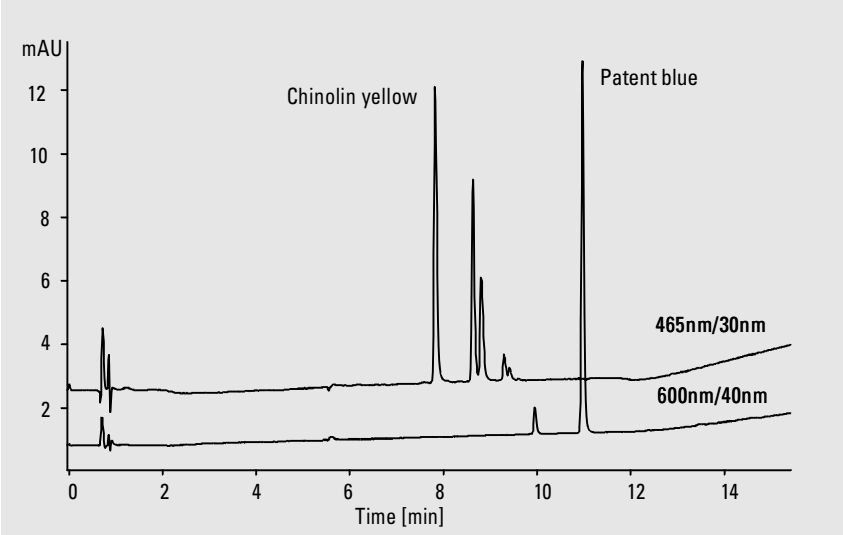

Figure 6
Chromatogram from the analysis of woodruff lemondade

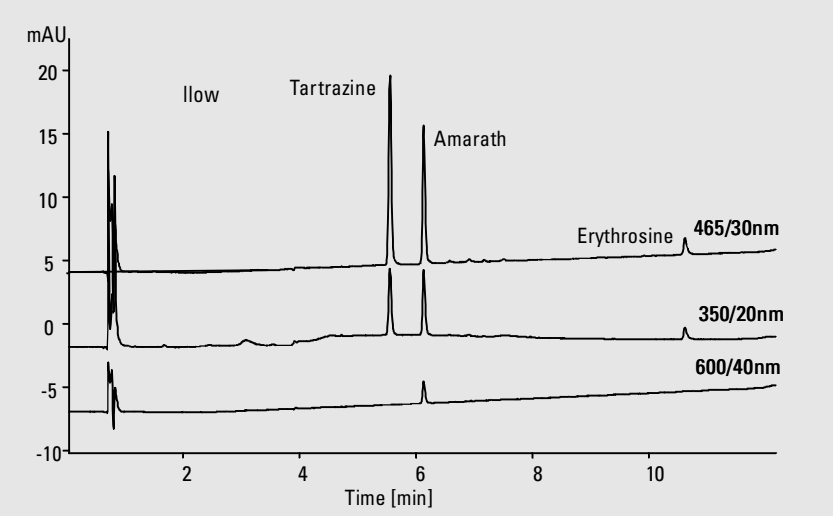

Figure 7
Chromatogram from the analysis of a red capsule

Figure 7 shows the analysis of tablet capsules which were dissolved in water, filtered and injected directly.

The red coloring comprised two red colors erythrosine and amaranth and a yellow color tartrazine.

Ink colors

The quality of ink colors relating to color brightness, stability against light and reproducibility of the same colors nuance for years, is determined by the accurate composition of different dyes in different concentrations. Here the right concentration of color traces is as important as the concentration of the main color compounds.

In figure 8 the chromatograms of a blue and a black ink color are overlaid showing that both inks do not only differ in one main compound but also in some trace compounds.

The *blue* color compound in the black ink is producing the main difference between black and blue.

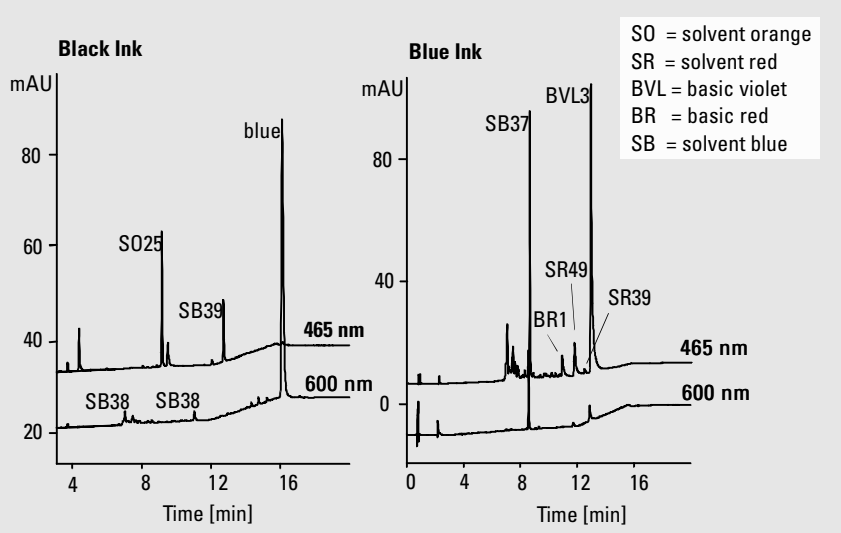

Figure 8
Comparison between a black and a blue ink

Conclusion

In this study we have demonstrated that the separation and detection of synthetic colors can be improved.

Ion-pairing reversed phase chromatography on a special deactivated column allowed the separation of dyes of different polarity with no or only slight tailing.

The UV-visible detection, especially from 400 to 950 nm, gained sensitivity by increasing the light output with a tungsten lamp in addition to a deuterium lamp.

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Publication Number 5964-3559E



Agilent Technologies
Innovating the HP Way

Predictable Translation of Capillary Gas Chromatography Methods for Fast GC

Application

Gas Chromatography

March 1997

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Abstract

The time required for capillary gas chromatography (GC) runs is often the major factor in sample turnaround time. Fast GC can significantly improve laboratory productivity by decreasing analysis time. However, converting established capillary GC methods to fast GC can be a daunting task.

The Agilent GC method translation software is a freeware program that logically and predictably translates traditional capillary GC methods. The translation program preserves the elution order for each compound, so that identification of each peak does not have to be repeated. Validation for the new method is made easier. The method translation software will instantly calculate temperature and flow conditions to meet various requirements including retention time locking, best theoretical efficiency, and decreased run time.

This application note discusses considerations involved in choosing fast GC over conventional GC, describes the Agilent GC method translation software, and demonstrates translating methods for faster analysis of solvents, styrene monomers, reformate gasoline, hydrocarbon emissions, semivolatile mixtures, and a chemical process intermediate.

Key Words

Capillary GC, fast GC, gas chromatography, laboratory productivity, method translation, solvent analysis, styrene monomer analysis, reformate gasoline analysis, hydrocarbon emission analysis, semivolatile analysis.

Introduction

A goal of many laboratories is to decrease the turnaround time for each sample. Shorter turnaround times mean quicker analytical results, lower operating costs, increased laboratory productivity, and higher revenues. Fast gas chromatography (GC) promises faster sample analysis, and is thus appealing as a component of increased productivity.

Fast GC is only one possible way to improve productivity. Many of the contributions to the total time required for sample analysis are not affected by the GC run time.

A complete analysis involves sample preparation, sample introduction, the GC cycle time (including run time and oven cool-down), data analysis, report generation, and the time required to document and track each sample. If the GC run time is short compared to the other steps, changing to fast GC may not result in concomitant productivity improvement. Fast GC is most appropriate when GC run times are a major contribution to total sample turnaround time. The laboratory should streamline as many operations as possible as part of a total productivity improvement program.

Once the GC run time is targeted as an area to improve, there are a

Table 1. Benefits and Drawbacks of Changing to Fast GC Methods

Parameter	Benefit	Detriment
Increase speed of injection process	Faster, more reproducible injections; automation	Cost of purchasing an automatic injector
Decrease column id (50 mm = current practical limit)	Faster optimum carrier gas velocity; shorter columns (higher plates/m); fastest possible separations	Lower column capacity; requires high inlet pressure; requires high split ratios or low injection volume
Increase carrier gas velocity	No new equipment/supplies required	Lower resolution; may degrade detector performance
Shorten column	Standard id columns (capacity); low pressure drops; standard hardware	Low separation power (resolution)
Perform isothermal analysis	No cool down cycle; short time until elution of early peaks of interest	No focusing possible; no splitless or cool on-column possible; applicable to narrow volatility range of solutes unless using multiple columns; contamination by late eluters
Increase oven program rate	Elutes full volatility range in minimum time	Lower resolution; limited by instrument capabilities; peak reversals possible
Change carrier gas (H_2 > He > N_2)	Same efficiency in shorter time with H_2; cost savings	Safety issues with H_2

number of ways to speed up the GC analysis time. However, there are tradeoffs in any attempt to decrease analysis time. A balance between speed, sensitivity, and resolution must be selected for each analysis to meet the laboratory goals. Table 1 lists the major benefits and potential disadvantages of optimizing each run parameter for speed. Adapting methods for fast GC can be complicated because peak reversals are common, and some fast GC methods decrease separation efficiency. This is illustrated in figure 1.

Figure 1, chromatogram A, shows a standard GC chromatogram for a semivolatile mixture using splitless injection. The run time is 45 minutes. Figure 1, chromatogram B, shows the same sample using fast GC. The run time has been decreased from 45 minutes to 12.5 minutes, but the oven temperature program was changed with little regard for possible peak shifting or reversals.

Although the chromatograms are similar and most peaks are adequately separated, a closer examination reveals problems typical of method adaptation. Figure 1, chromatograms C and D, compare the center sections of the two chromatograms A and B, respectively.

The three peaks labeled “1” in chromatogram C are reversed in chromatogram D. The peak pair at “2” is reversed in the fast GC run, and the two peaks at “3” in chromatogram C co-elute in chromatogram D.

These changes mean that validating the new method will be a time-consuming process. The Agilent GC method translation software avoids these problems by making the change from traditional capillary GC methods to fast GC methods predictable.

It locks the elution order for each compound, so peaks do not have to be painstakingly identified. This decreases the time necessary to validate the new method. The method translation software instantly calculates the correct oven temperature program and column head pressure as a function of the new column dimensions, phase ratio, and carrier gas type.

When using the method translation software to calculate conditions for a different column, the new column should have a stationary phase identical to the original column. Columns of 100 percent methyl and 5 percent phenyl/95 percent methyl can often be used interchangeably from different manufacturers, but more polar columns can vary significantly between manufacturers. Method translation may not preserve the elution order for phases that are chemically different. As a column ages, the stationary phase may decompose or become contaminated. This can also affect peak elution order initially and over time.

Method Translation Software

The Agilent GC method translation software, version 2.0, is a freeware program that simplifies fast GC method development. (For the web site address to download the freeware, refer to the last page of this publication.) The main data screen of the software is shown in figure 2.

The tool offers selection of several modes of method translation:

- **Translate Only**. Translates the current method to a new one based on a change of column dimensions, carrier gas type,

outlet pressure, and/or phase ratio. The relative retention times of the peaks are locked, so the order of elution is preserved. In "Translate Only" mode, the relative efficiency of the current method is maintained. This is useful if you need to convert a method from the literature, to convert an established method to use a column with different dimensions or a different phase ratio, or to change to a detector with a different outlet pressure.

- **Best Efficiency**. Calculates new conditions (using your current column) that correspond to the theoretical optimum gas flow rate for the gratest separation efficiency for most compounds. Simply enter your current conditions, and the program adjusts the temperatures and flow rate to match the theoretical optimum. The elution order of the peaks will stay the same, but the retention time will probably change.
- **Fast Analysis**. Calculates the temperature and pressure for your current column and carrier gas for a run that has an outlet carrier gas flow that is twice as fast as the "Best Efficiency" mode. Depending on the pressure drop across the column, run time will decrease 1.5 to 2 times. As always with method translation, the elution order of your current method will hold constant.

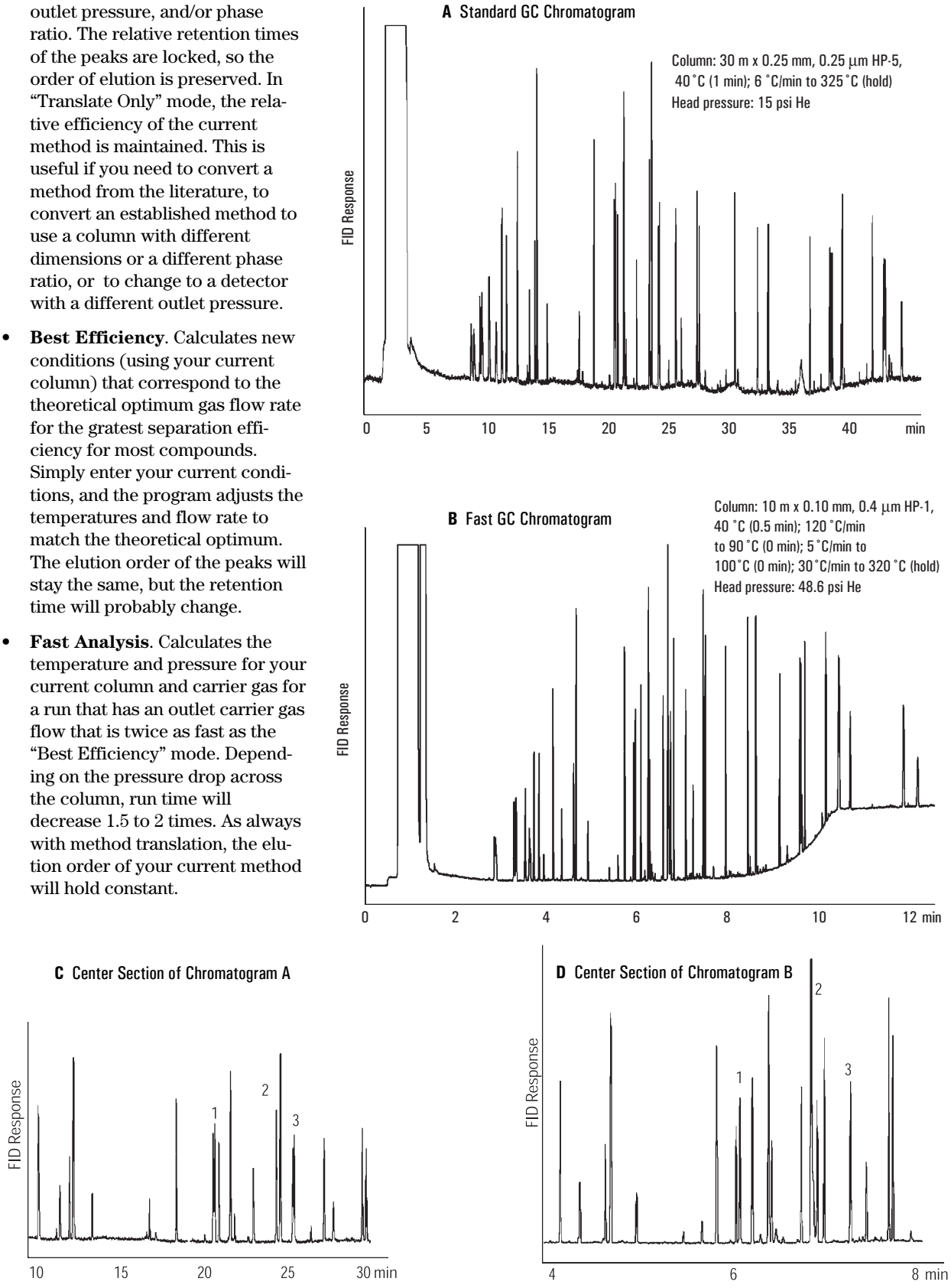


Figure 1. Comparison of traditional GC and fast GC for a splitless injection of a semivolatile mixture. (Concentration: 1 ppm; injection size: 0.5 mL)

- **None**. Allows you to change any run parameter of interest. You can decrease method development time by examining the effects of various parameters on the speed, head pressure, and oven temperature ramp of your method before you do any chromatography. This mode is also useful to try out changes to your current method. For instance, if your current method is already at maximum efficiency and you need to maintain separation power, you can enter a smaller column id to see the exact impact on run time. If you have excess separation power, you can calculate conditions for a shorter column or faster gas flow rate. The method translation software allows you to rapidly develop a feel for the effects f each parameter on speed of analysis, pressure, and oven temperature program rate.

Separation power (resolution) is a function of column dimensions, flow, and oven temperature. When a new column is selected to maintain separation efficiency, the GC method translation software will make sure that the flow and oven temperature ramp rates are scaled appropriately.

Experimental

All experiments were performed using an Agilent 6890 Series gas chromatograph (GC) with the 240-volt option. The 6890 Series GC has many features specifically designed for the successful migration to faster GC methods. These include:

- 100- and 150-psi EPC split/splitless inlet
- Automated split ratios to 7500:1
- Fast detector sampling rates (0.1–200 Hz) for flame ionization detection (FID) and nitrogen-phosphorus detection (NPD) with a ChemStation

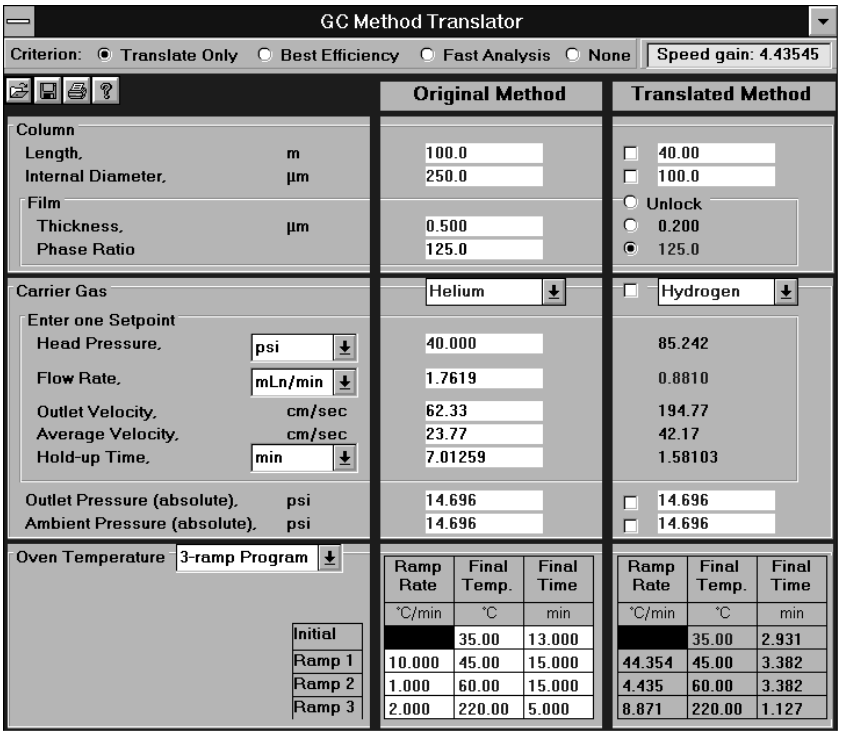


Figure 2. The Agilent GC Method Translation Software, Version 2.0a.

- Fast oven program rates (up to 120 °C/min, depending on the temperature range)

A G1916A automatic liquid sampler (ALS) was used for sample introduction, and an Agilent ChemStation was used for instrument control and data acquisition. Refer to the individual chromatograms for details about columns, temperatures, split ratios, carrier gas, gas flows, and oven ramps.

Injection Liner

To maximize sensitivity and resolution with split injections, it is important to obtain good peak shape. Using a different column size may require a different size liner to maintain good peak shape. Figure 3 demonstrates the difference in peak shape obtained with a 2-mm liner versus a 4-mm liner with glass wool plugs. The smaller diameter liner improves the peak shape for the 0.05-mm id column in this example.

Results and Discussion

Examples of fast GC for analysis of hydrocarbons in gasoline and simulated distillation are discussed in other Agilent publications.[1,2] This application note demonstrates the time savings achieved for various other analyses.

Chemical Process Intermediate

Figure 4 shows the development of a fast GC analysis for a chemical process intermediate. Figure 4, chromatogram A, shows the original method; figure 4, chromatogram B, shows the translated method with the column dimensions decreased by a factor of three and the relative retention times of the peaks constant. For even faster analysis, the column was shortened, as shown in figure 4, chromatogram C.

The relative retention times are still correct, and the run is almost eight

times as fast. In figure 4, chromatogram C, some resolution is lost due to the shorter column. Scaling the column to 10 m × 0.05 mm (0.2 mm) would have provided the identical resolution because of the higher efficiency per unit length of the smaller id column.

Solvent Analysis

A traditional capillary GC method for analysis of solvent from a commercial paint thinner is shown in figure 5, chromatogram A. Using the method translation software with the criterion of "fast analysis" selected, the carrier gas outlet flow rate was doubled. The tool calculated the oven temperature ramp that would maintain relative peak retention times at the faster flow rate. The resulting chromatogram is shown in figure 5, chromatogram B. The run time has decreased from 9 minutes to 6 minutes, and the peaks are still well separated.

Figure 6 shows the effect of changing carrier gas from helium to hydrogen in a similar analysis using smaller id columns. Because the theoretical optimum flow rate is faster for hydrogen than for helium, changing to hydrogen can significantly decrease the run time while not requiring high head pressures.

In figure 6, the flow rate of hydrogen was increased beyond that used for helium to reduce analysis time even

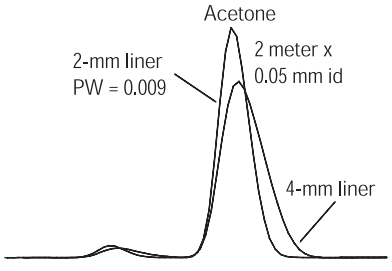


Figure 3. Peak shape vs liner diameter.

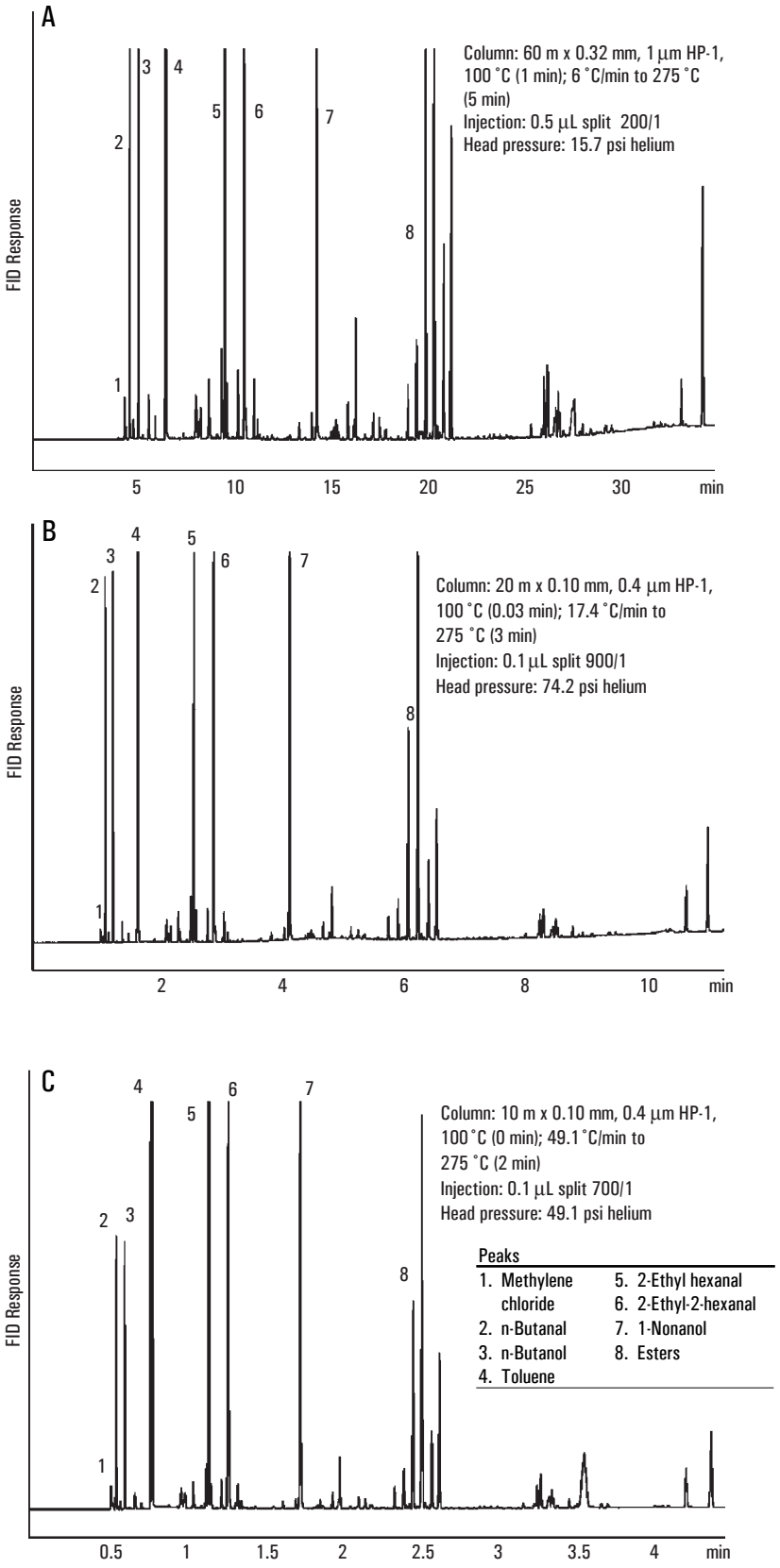


Figure 4. A chemical process intermediate on an HP-1 column at three different column sizes and phase ratios.

more. The conditions were not "translated." In the analyses shown in figures 5 and 6 (less than 10 highly resolved peaks), the use of method translation is optional. This is not the case with more complex samples.

Styrene Monomer Impurities

A standard GC analysis for impurities in styrene monomer takes about 12 minutes, as shown in figure 7. Using a smaller column with a faster oven program rate and slower carrier gas flow rate, an equivalent analysis was obtained in 3.6 minutes.

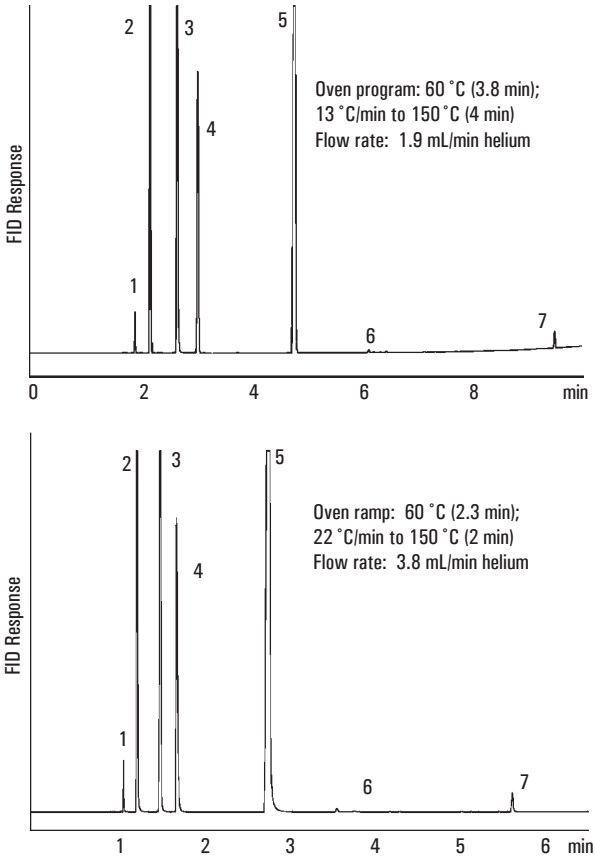
Reformate Gasoline Analysis

The development of a fast GC method for reformate gasoline analysis is shown in figure 8, chromatogram A, which shows a standard GC analysis that takes about 20 minutes. In figure 8, chromatogram B, a smaller column decreased the run time to 6 minutes. In figure 8, chromatogram C, the carrier gas was changed from helium to hydrogen, and the run time decreased to less than 4 minutes. The peaks are still well resolved and the order of elution is maintained. Table 2 shows the conditions used for the chromatograms in figure 8.

Conclusions

Despite the improvements in instrumentation, fast chromatography will always involve tradeoffs among speed, sensitivity, and resolution. The discussion here details considerations involved in choosing fast chromatography, discusses Agilent method translation software, and gives specific examples of some types of mixtures that are amenable to fast GC.

Use of Agilent method translation software eases the migration to faster methods by providing the conditions that will maintain the current order of elution. It can also highlight the potential instrument limitations (head

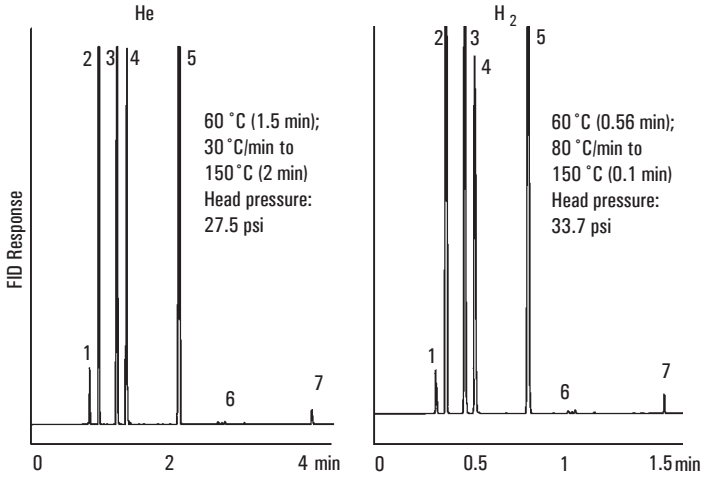


Peaks

1. Propylene oxide
2. Acetone
3. Methanol
4. Methylene chloride
5. Toluene
6. C_8 aromatics
7. 4-Hydroxy-4-methyl-2-pentanone

Column: 30 m x 0.32 mm, 0.5-mm HP-INNOWax
Injection: 0.5 µL
Split ratio: 200:1

Figure 5. Comparison of solvent analysis from a commercial paint remover at two flow rates



Column: 10 m x 0.10 µm HP-Wax
Injection size: 0.1 µL
Split ratio: 1000:1

Figure 6. Comparison of using helium and hydrogen carrier gas for analysis of a commercial paint remover

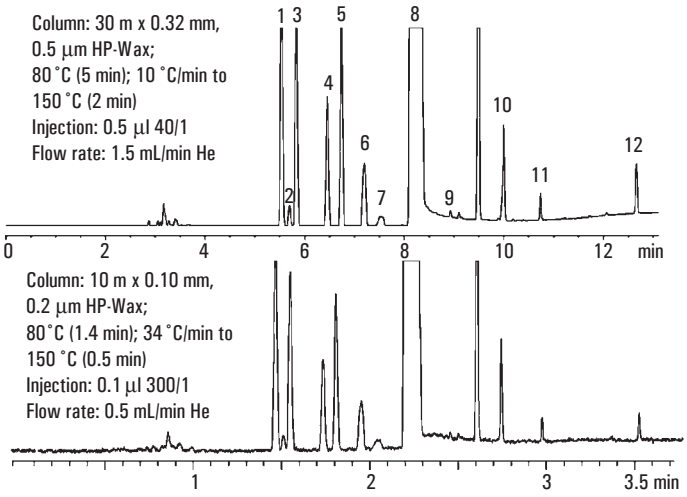
pressure or oven temperature program rate) that would be associated with translating a current method for use on a smaller id column.

To Obtain Agilent Method Translation Freeware

The Agilent method translation software can be downloaded from the Chemical Analysis Group (CAG) section of the Agilent home page on the World Wide Web at http://www.chem.agilent.com/cag/servsup/usersoft/main.html.

Table 2. Conditions for Fast GC of Reformate Gasoline

	A	B	C
Column	30 m x 0.32 mm	10 m x 0.32 mm	10 m x 0.10 mm
df	0.5 µm	0.2 µm	0.2 µm
Phase	HP-Wax	HP-Wax	HP-Wax
Carrier	Helium	Helium	Hydrogen
Pressure	6.4 psi	37.3 psi	21.9 psi
Flow	1.2 mL/min	0.37 mL/min	0.33 mL/min
Temperature	60 °C (hold 4 min)	60 °C (hold 1 min)	60 °C (hold 0.7 min)
Rate 1	10 °C/min	36.7 °C/min	55.1 °C/min
Final Temp	140 °C	140 °C	140 °C
Rate 2	15 °C/min	55.1 °C/min	70 °C/min
Final Temp	200 °C (hold 4 min)	200 °C (hold 2 min)	200 °C (hold 1 min)
Injection Volume	0.5 µL; split 200:1	0.1 µL; split 800:1	0.1 µL; split 800:1



Peaks

1. Ethylbenzene	7. p/m-Ethyl toluene
2. p-Xylene	8. Styrene
3. m-Xylene	9. a-Methylstyrene
4. Isopropyl benzene	10. Phenylacetylene
5. o-Xylene	11. b-Methylstyrene
6. n-Propylbenxene	12. Benzaldehyde

Figure 7. Comparison of traditional capillary GC and fast GC for the analysis of impurities in styrene monomer.

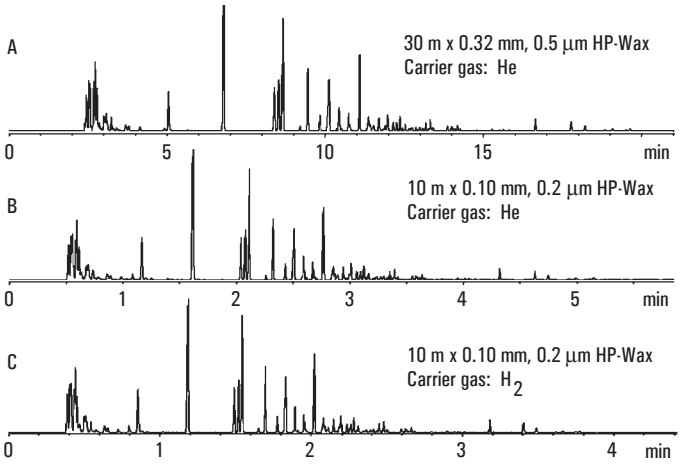


Figure 8. Development of fast GC for reformate gasoline.

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Printed in the USA 3/2000
5965-7673E



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Analysis of Nitroaromatics and Nitro-Polycyclic Aromatic Hydrocarbons by Capillary Gas Chromatography with the Agilent 6890 Micro-ECD

Application

Gas Chromatography

March 1997

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Abstract

A new electron capture detector (ECD) for the Agilent 6890 Series gas chromatograph (GC) allows very sensitive detection of nitroaromatic compounds at low picogram levels with a linear response over three orders of magnitude.

This application note describes the performance of the new 6890 Series Micro-ECD when analyzing two types of nitro-aromatic compounds—explosives and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs).

Introduction

Electron capture detection is most often used for the sensitive and selective detection of halogenated compounds. However, other compound classes also have electron capturing properties and can, therefore, be detected at low levels using an electron capture detector (ECD). Compounds containing a nitro-function—particularly nitroaromatics—are strong electron-capturing molecules. The ECD provides a very sensitive tool for trace analysis of these solutes.

This application note demonstrates that the 6890 Series Micro-ECD provides an extremely sensitive alternative to the typical NPD or MS detection[1, 2] for nitro-PAHs and explosives.

Experimental

The analyses were performed on an 6890 Series GC. Injection was automated splitless using an Agilent 7673 automatic sampler. The instrument configuration and analytical conditions used for the analysis of the nitro-PAHs and explosives are summarized in table 1.

Results and Discussion

The sensitivity of the ECD depends on the makeup flow rate. The 6890 Micro-ECD optimized the argon/5% methane (Ar/CH_4) makeup gas flow rate for the analysis of nitro-PAHs. Nitropyrene was used as test solute. The makeup flow rate was varied from 10 to 80 mL/min; at each setting, five runs were made.

Figure 1 shows the mean peak areas plotted versus the makeup flow rate. The optimum flow rate was obtained between 20 to 30 mL/min. At lower flow rates, the peak area decreased and the detector became less stable, shown in the increasing standard deviation on peak area. At higher flow

Table 1. Instrumental Configuration and Analytical Conditions

Chromatographic System	
Gas chromatograph	6890 Series
Inlet	Split/splitless
Detector	Micro-ECD
Automatic sampler	7673 Series
Liner	Single taper deactivated (part number 5181-3316)
Data handling	ChemStation (DOS Series)
Column	30 m x 0.25 mm id x 0.25 μm HP-5 MS (part number 19091S-433)
Experimental Conditions	
Inlet temperature	250 °C
Injection volume	1 μL
Injection mode	Splitless
Purge time	0.75 min
Purge flow	50 mL/min
Carrier gas	Hydrogen
Head pressure	58 kPa at 50 °C
Carrier gas mode	Constant flow
Flow, velocity	1.4 mL/min, 40 cm/s
Oven temperature	50 °C, 1 min initial, 20 °C/min to 320 °C, 0.5 min hold
Detector temperature	320 °C
Detector gases	Argon/5% methane: 20 mL/min

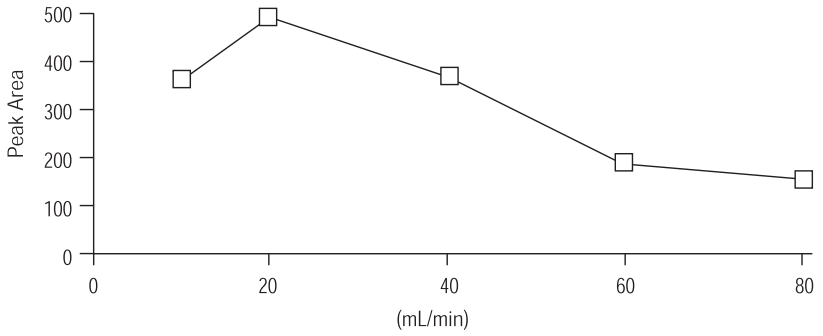

Figure 1. Peak area of 1-nitropyrene versus argon/5% methane makeup gas flow rate.

rates, the detector was stable (exhibiting a small standard deviation), but sensitivity drastically decreased.

Nitrogen is an alternative makeup gas for electron capture detection. It can usually be used interchangeably with Ar/CH_4; similar results for the effect of makeup gas flow rate are expected.

Next, the linearity of the detector response was measured. Using nitropyrene as the test solute, standard solutions of 1, 10, 50, 100 and 1,000 ppb were analyzed. The calibration curve for this compound, as shown in figure 2, exhibits a very good correlation coefficient (r = 0.99996).

Nitrated Polycyclic Aromatic Hydrocarbons

Nitro-PAHs are an important class of environmental pollutants.[3] Polycyclic aromatic compounds are formed during incomplete combustion of organic material. In the presence of nitrogen oxides (NO_x), the neutral PAHs (such as naphthalene or pyrene) are converted into nitro-PAHs.[3–5]

The nitro-PAHs have much higher mutagenic and carcinogenic activity than the neutral PAHs, but their extremely low concentration (measured as pg/m^3) in environmental samples, particularly air particulates, makes them difficult to monitor. Very sensitive detection is needed.

Using the optimized GC conditions, a mixture of 11 nitro-PAHs, each having a concentration of 40 pg/mL (40 ppb), was analyzed. The chromatogram for this analysis is shown in figure 3. Good peak shapes were obtained for all compounds. The detection limit, which varied from 0.1 to 1 pg for the different PAHs, is at least one order of magnitude lower than that obtained by nitrogen-phosphorus detection (NPD), mass spectrometry (MS), or MS-MS.[2] It can, therefore, be concluded that the 6890 Micro-ECD offers greater sensitivity for the detection of these nitro-PAHs than other methods.

Explosives

Explosives can be present as residues at chemical waste sites or on materials close to an explosion. Sensitive and fast methods are needed for analyzing and monitoring these compounds for environmental remediation or forensic evidence.

Although explosives are often analyzed by high pressure liquid chromatography (EPA method 8330), capillary gas chromatography (CGC) can provide a good alternative for most solutes using NPD or MS. Some of the nitro-aromatics are included in the target compound lists of EPA methods 8090 and 8270 (CGC-MS).

Explosives such as TNT (2,4,6-trinitrotoluene) contain one or more nitro-functions. CGC-ECD can provide a very sensitive and fast screening method for detecting these compounds.

The chromatogram in figure 4 shows the results of a standard mixture of explosives using the analytical conditions in table 1. The concentration of the test solutes was 100 pg/mL

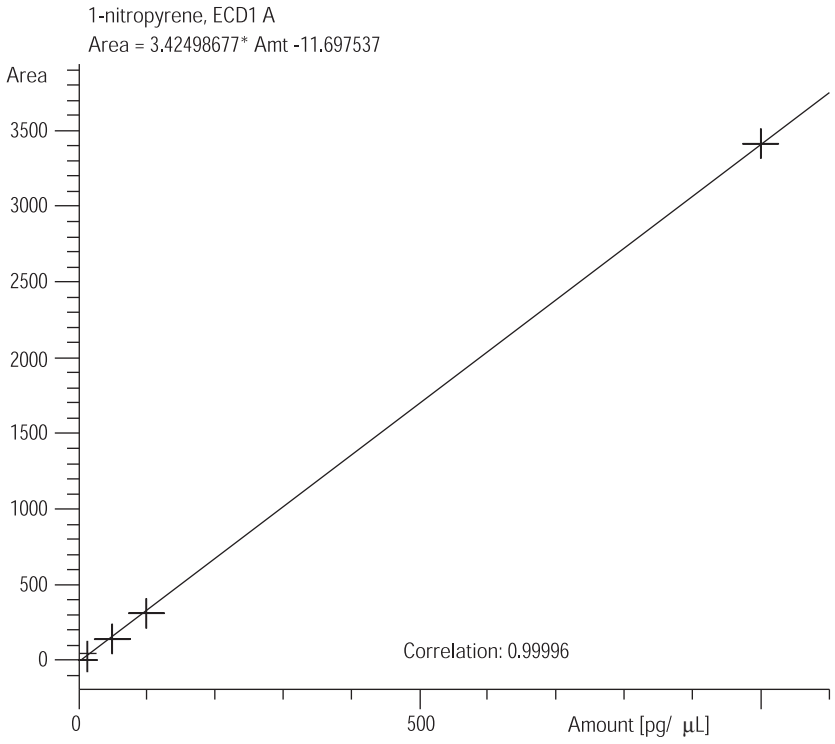

Figure 2. Calibration curve for 1-nitropyrene from 1 to 1,000 ppb

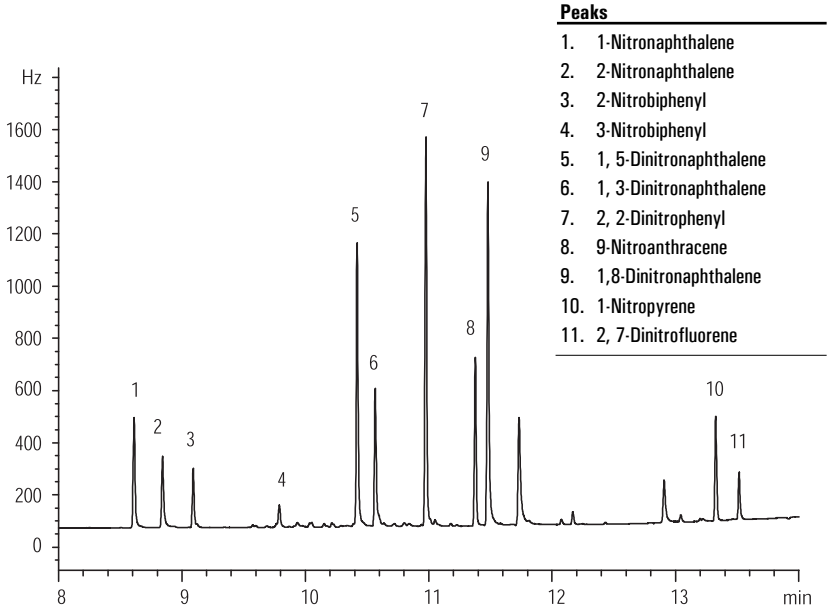

Figure 3. CGC-ECD analysis of nitrated polycyclic aromatic hydrocarbons (solute concentration: 40 ppb)

(100 ppb), except for 1,2-dinitrobenzene, which was present as an impurity. As the chromatogram shows, the different nitro-, dinitro-, trinitro-, and amino-nitro-compounds are well separated and elute with good peak shape.

The ECD response is dependent on the number of nitro-groups. For the mono-nitroaromatics, the detection limit is around 10 pg, while for the di- and tri-nitroaromatics the detection limit is below 1 pg. This example confirms that CGC-ECD can be used as a fast screening method for the analysis of this category of explosives.

Conclusion

The Agilent 6890 Series Micro-ECD allows very sensitive detection of nitroaromatic compounds. The detector was successfully used for the analysis of nitrated polycyclic aromatic hydrocarbons and explosives. Detection limits below 1 pg were obtained, and the detector was found to give a linear response over three orders of magnitude.

Peaks	
1. Nitrobenzene	7. 1,2-Dinitrobenzene (impurity)
2. 2-Nitrotoluene	8. 2,4-Dinitrotoluene
3. 3-Nitrotoluene	9. 1,3,5-Trinitrobenzene
4. 4-Nitrotoluene	10. 2,4,6-Trinitrotoluene
5. 1,3-Dinitrobenzene	11. 4-amino-2,6-Dinitrotoluene
6. 2,6-Dinitrotoluene	12. 2-amino-4,6-Dinitrotoluene

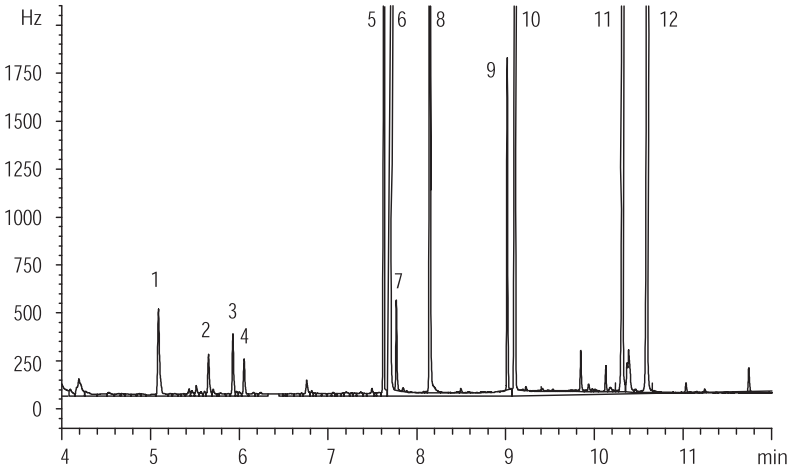

Figure 4. CGC-ECD analysis of explosives (solute concentration: 100 ppb)

References

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Printed in the USA 3/2000
5965-8015E

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Analysis of Parabenes in Body Lotion by Capillary Electrochromatography

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Chemical

Abstract

Cosmetics may contain a number of additives in addition to the active ingredients which convey color or other properties of the product. They also contain preservatives so that the shelf life may be extended. Parabenes are used in many cosmetics because of their ability to retard microbial growth and prolong the shelf-life of the cosmetic products. Subsequently the levels of preservatives in cosmetics need to be monitored.

Capillary Electrochromatography (CEC) is a fusion of liquid chromatography and capillary electrophoresis, which preserves the best aspects of both techniques. This application brief describes the application of CEC to the quantitative analysis of parabenes in body lotion.

The method needed minimal sample preparation and provided quantitative data.

Experimental

All experiments were performed using the Agilent Capillary Electrophoresis system equipped with diode array detector and computer controlled via Agilent ChemStation software. CEC capillary columns were Agilent CEC C18. The Agilent CE system is uniquely designed for operating CEC in that it can apply up to 12 bar pressure simultaneously to both vials in order to suppress bubble formation and maximize reliability.

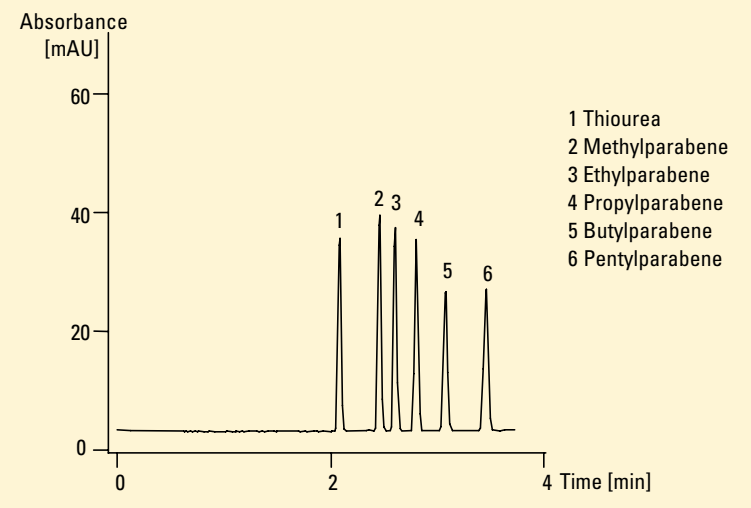

Figure 1
CEC separation of a standard mixture of 5 parabenes at a concentration of 5 mg/ml each

Conditions

Column
CEC C18 3 µm, 250 (335) × 0.1 mm i.d.
Mobile Phase
80/20 ACN/TRIS 25 mM pH 8.0
Detection 254/10 nm
(All spectra in peak were recorded)
Injection 10 s at 5 k
Run 25 kV, 20 °C
Pressure 8 bar both sides

Sample preparation
Body lotion diluted with mobile phase and filtered through 0.45 µm

Results

Figure 1 shows the CEC separation of a standard mixture of 5 parabenes at a concentration of 5 mg/ml each. The first eluting peak is thiourea, and as a non-retained marker can be used to indicate the linear velocity of the mobile phase through the column. Such a marker for is necessary since the flow is physico-chemically dependent. Reproducibility of migration time was sufficient to identify the 4 peaks apparent in the body lotion analysis as parabenes (figure 2). This was confirmed by spectral analysis which also indicated that the peak for ethylparabene contained an unknown component. Response was linear over the range 2 to 20 mg/ml and methyl-, propyl- and butyl parabene were calculated at concentrations of 276 mg/ml, 19 mg/ml and 49 mg/ml respectively.

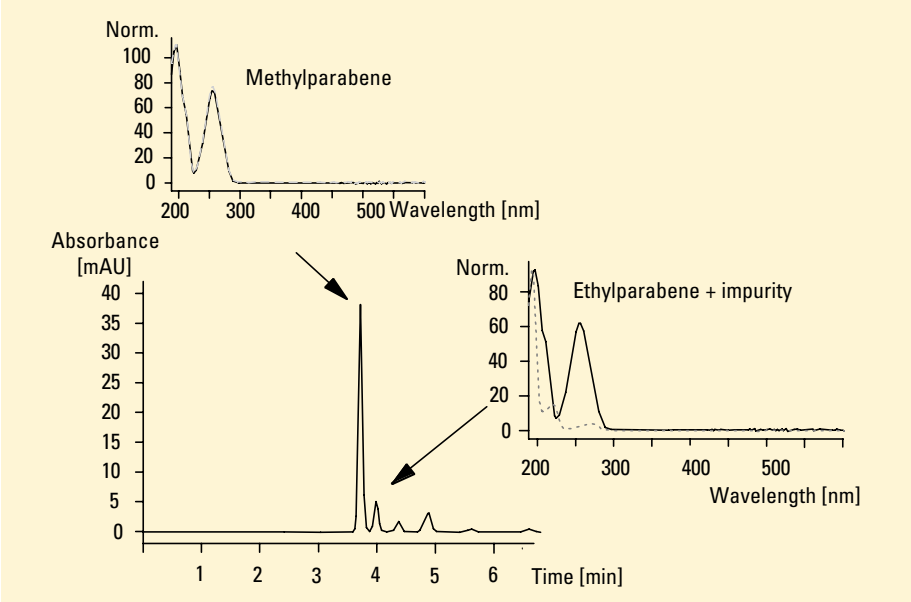

Figure 2
Spectral identification of parabenes in body lotion

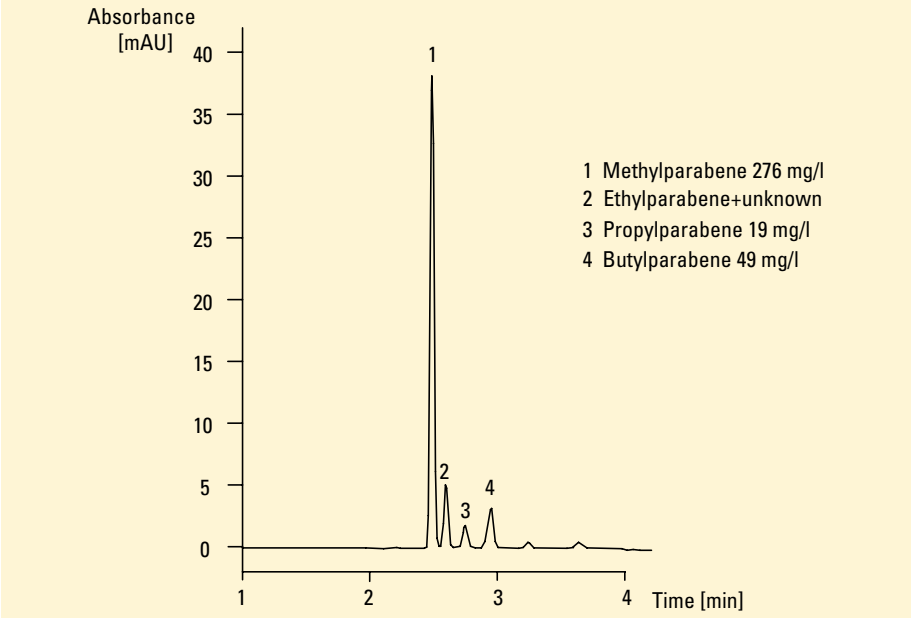

Figure 3
Quantitation of parabenes in body lotion by CEC

Conclusions

CEC can be used for the facile determination of parabenes in body lotion. The method is quick and simple and can provide quantitative determination of parabene levels in the cosmetic sample.

Equipment

- Agilent Capillary Electrophoresis System
- Agilent ChemStation + software

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Transfer of HPLC methods to Capillary Electrochromatography

Gordon Ross,
Monika Dittmann
and Gerard Rozing

Chemical/
pharmaceutical

Abstract

Capillary Electrochromatography combines the separation principle of HPLC (partitioning between mobile and stationary phases) with the high efficiency of capillary electroseparation methods in CEC. The electroosmotic flow (EOF) inherent in capillary electrophoretic separations is used to transport solute and mobile phase through a packed capillary column. The properties of the EOF allow much higher efficiencies than can be realized with LC. CEC can also be characterized by its low solvent consumption and reduced costs. Therefore, there are a number of advantages which may be gained by transferring a method from LC to CEC. This application brief aims to identify the parameters which must be appreciated in order to successfully transfer a method from LC to CEC.

Experimental

All CEC experiments were performed using the Agilent CE system, equipped for CEC operation and with a built in diode array detector. The system includes an Agilent ChemStation for system control, data collection and data analysis. LC separations were performed on an HP 1090 liquid chromatography system. CEC columns were supplied by Agilent Technologies. Buffer salts were of the highest purity available and organic solvents were HPLC grade. All buffers were filtered and degassed prior to use. Buffers/mobile phase were adjusted to pH prior to addition of organic modifiers.

The main point of difference between LC and CEC is in the motive force which transports the mobile phase through the column. In LC this is achieved through pressure drive flow, whereas in CEC this is achieved via the EOF. The flow velocity of the EOF is dependent upon properties of the mobile phase (viscosity, dielectric constant, buffer ionic strength), as well as those of the stationary phase (surface charge density). This application brief intends to outline some of the effects on linear velocity of varying stationary or mobile phases.

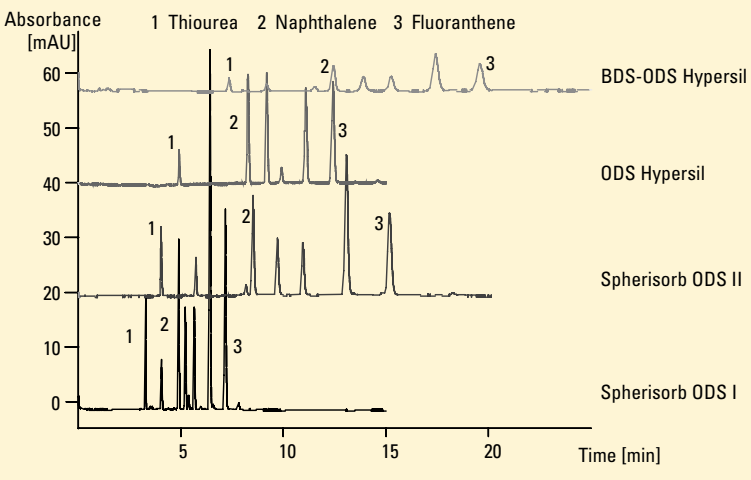

Figure 1
Separation of PAHs on different C18 phases

Conditions

Capillary CEC Hypersil C18, 3 µm
250 mm (350 mm) × 0.1 mm i.d.
Mobile phase
80/20 ACN/TRIS HCl.50 mM; pH 8.0
Voltage 20 kV
Temperature 20 °C
Injection 3 s at 5 kV
Pressure 10 bar both sides
Detection Sig. 254/10 nm, reference off

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The EOF in a packed column is dependent upon the packing materials. Figure 1 shows the separation of a mixture of polyaromatic hydrocarbons (PAHs) on different C18 stationary phases. The samples were not identical but thiourea (flow marker), naphthalene and fuoranthrene were included in all samples. The fastest EOF is observed on the two non-endcapped materials CEC Hypersil C18 and Spherisorb ODS 1. With the encapped ODS Hypersil the flow is reduced by a factor of approximately 2 and with the base deactivated phase (BDS Hypersil) it is reduced by a factor of 3.

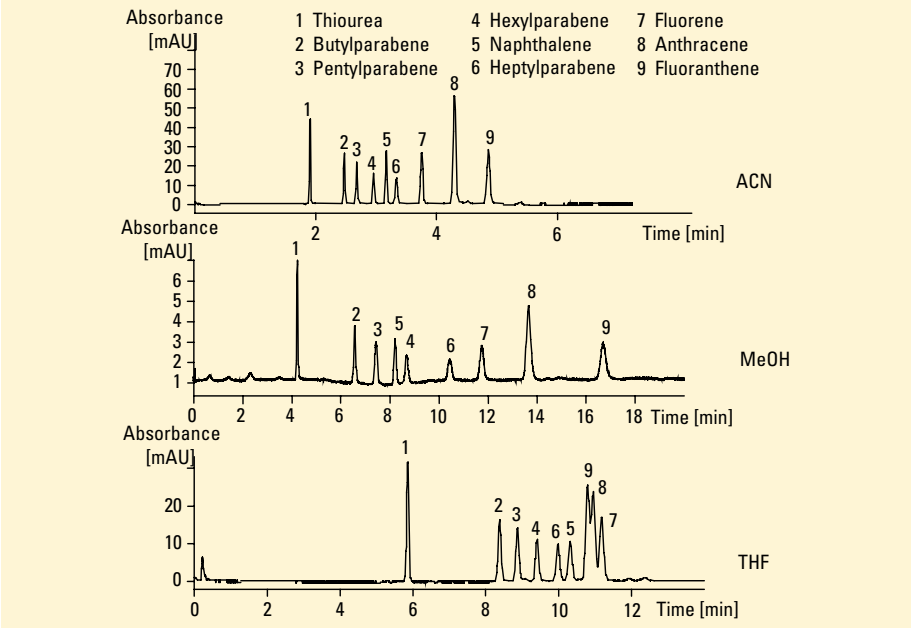

Figure 2
Effect of organic modifier of EOF, retention and selectivity in CEC

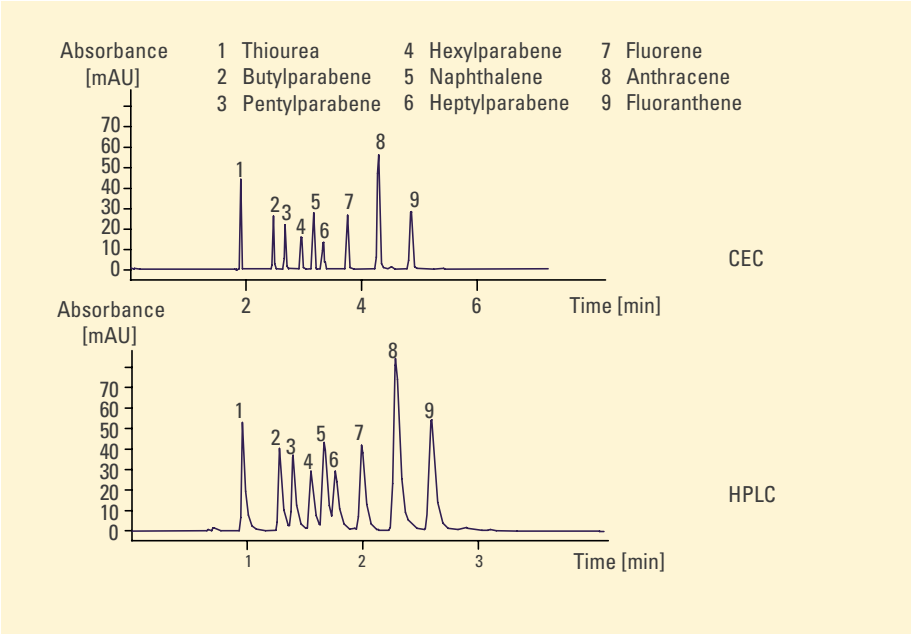

Figure 3
Analysis of test mixture by HPLC and by CEC

Figure 2 shows the effect of different organic modifiers on the CEC separation of a standard mixture. Compared to acetonitrile, the flow observed is approximately 2 times slower with methanol and 3 times slower with THF. This is due to differences in viscosity and dielectric constant of the different organic modifiers. Figure 3 shows the separation of a neural test mix by CEC and by LC on the same column. The selectives are the same in both cases.

Conclusions

LC separations can be successfully transferred to CEC operation. Just as in LC, variation of the stationary and mobile phase is a useful tool for method optimization. Modulation of the mobile and stationary phase can change the EOF and thus the speed of separation.

Equipment

- Agilent Capillary Electrophoresis system
- HP 1090 LC system
- Agilent ChemStation + software

Monika Dittmann and Gerard Rozing are R&D chemists, and Gordon Ross is application chemist at Agilent Technologies, Waldbronn, Germany.

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Gradient LC analysis of herbicides and polyaromatic hydrocarbons by isocratic Capillary Electrochromatography

Gordon Ross,
Thomas Adam
and Monika Dittmann

Environmental/chemical

Abstract
Capillary Electrochromatography (CEC) combines the separation principle of HPLC (partitioning between mobile and stationary phases) with the high efficiency of capillary electroseparation methods. In CEC the electroosmotic flow (EOF) inherent in capillary electrophoretic separations is used to transport solute and mobile phase through a packed capillary column. The properties of the EOF provides higher efficiencies than can be realized with LC. This can be sufficient to allow the transfer of methods conventionally performed by gradient LC to be performed by isocratic CEC.

Experimental
All CEC experiments were performed using the Agilent CE system, equipped for CEC operation and with a built in diode array detector. The system includes an Agilent ChemStation for system control, data collection and data analysis. CEC columns were supplied by Agilent Technologies. Buffer salts were of the highest purity available and organic solvents were HPLC grade. All buffers were filtered and degassed prior to use. Buffers/mobile phase were adjusted to pH prior to the addition of organic modifiers.

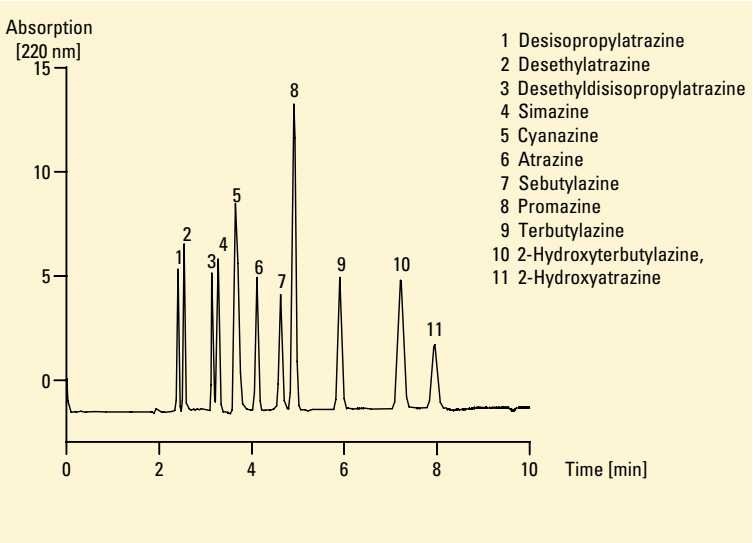

Figure 1
Isocratic CEC alternative to gradient HPLC separation of herbicides

Figure 1 shows the separation of a series of herbicides by CEC. The separation is normally achieved using gradient elution LC. The same is true for figure 2. Here the analysis is of polyaromatic hydrocarbons

Conditions

Column
250 mm × 100 µm; Sperisorb ODS1
Mobile Phase
60 % acetonitrile/40 % 25 mM TRIS pH 8
Voltage
30 kV
Temperature
15 °C

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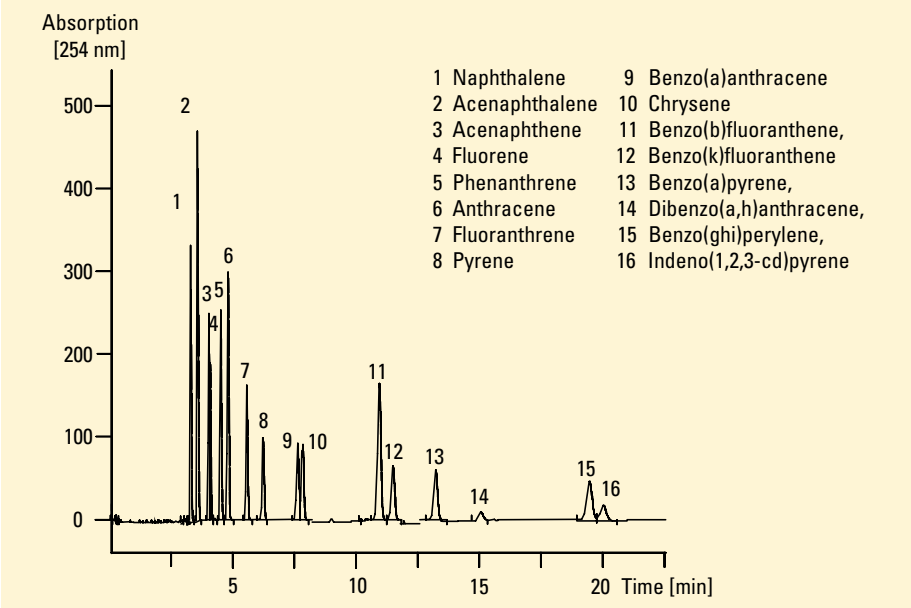


Figure 2
Fast CEC separation of EPA 16 PAH standard on CEC hypersil C18

which are of environmental significance and interest. Conventional analysis of these compounds can be achieved in a similar time however with isocratic CEC operation there is no inter-analysis time required for re-generation of the LC column.

Conclusions

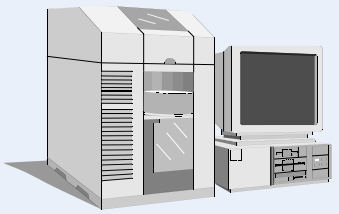
Some gradient LC separations can be succesfully performed using isocratic CEC. Very similar separations can be achieved in the same time frame. Time for re-equilibration of the LC column is not needed and therefore the overall analysis time is reduced.

Conditions

Column
CEC Hypersil C18, 250 mm (350 mm) × 0.1 mm i.d., 2.5 µm
Cell Standard
Eluent
90 % TRIS-HCl 50 mM, pH 8
Voltage 30 kV
Temperature 20 °C
Pressure 10 bar both sides

Equipment

- Agilent Capillary Electrophoresis System
- Agilent ChemStation + software



Monika Dittmann is an R&D chemist and Gordon Ross is an application chemists at Agilent Technologies, Waldbronn, Germany. Thomas Adam is at the University of Mainz, Germany.

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Publication Number 5965-9032E

Sensitive Analysis of Bisphenol A in Polycarbonates using HPLC and Fluorescence Detection

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Bisphenol A can be used as an antioxidant for softeners, fungicide and as an intermediate during the production process of polycarbonates, epoxids phenol resins and dyes.

Method performance and results

Bisphenol A was analyzed using reversed phase HPLC. A diode array detector and a fluorescence detector were used as the detection systems. With diode array detection a spectrum can be taken and used as an identification tool in addition to retention times. However, using state-of-the-art equipment fluorescence detection is about 10 times more sensitive than diode array detection.

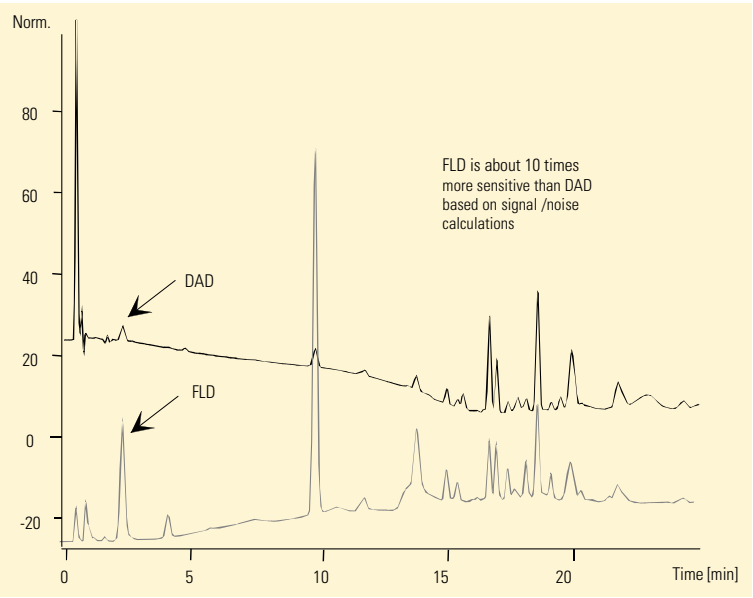
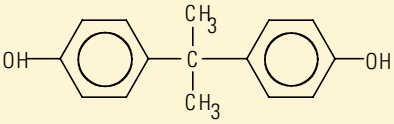


Figure 1
EIC traces from amine standards



Bisphenol A

Conditions

Column
100 x 2.1 mm Hypersil ODS, 5 µm
Mobile Phase
A = Water, B = Acetonitrile
Gradient
at start 40%B, at 15 min 95% B,
Post Time 4 min
Flow Rate 0.4 ml/min
Oven Temp 25 °C
Injection Vol 1 µl
Diode array detector
230/20 nm Reference 440/80 nm
Fluorescence detector
Ex = 225 nm, Em = 310 nm

Sample preparation
40 ml of a 10 % solution of PC in methylenechloride are extracted with 20 ml 0.1n NaOH. 10 ml of NaOH phase are filled with methanol to 50 ml.

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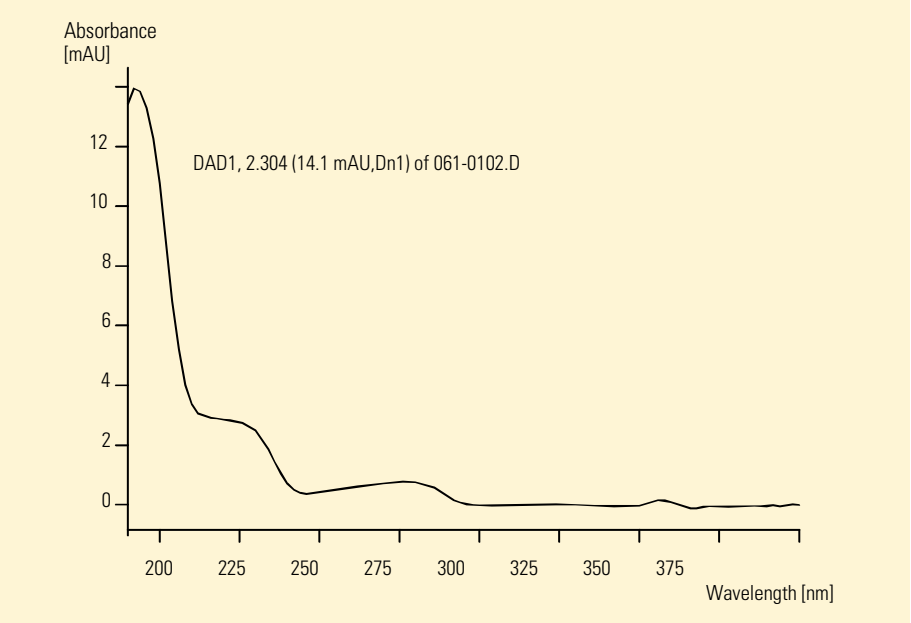


Figure 2
Spectrum of Bisphenol A

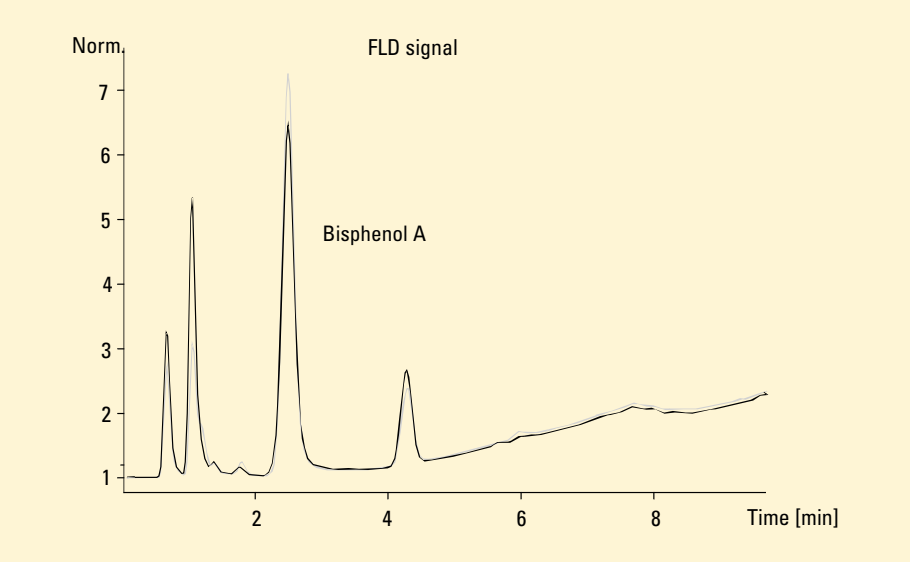


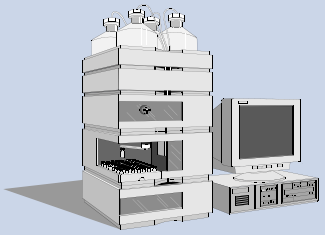
Figure 3
Content of Bisphenol A in two different polycarbonates

Equipment

Agilent 1100 Series

- degasser
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector and/or FLD

Agilent ChemStation + software



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Analysis of Polymethylmethacrylate (PMMA) using Gel Permeation Chromatography

Angelika Gratzfeld-Huesgen

Polymer /chemical industry

Abstract

Polymethylmethacrylates are used as homo and co-polymers for the production of safety glasses, Plexiglas and glasses for optics, cars and dishes. The mol masses vary from 120000 to 180000 g/mol. In 1988 1.5 Mio.t. were used worldwide.

The performance of PMMA depends on the molecular weight of the polymer. To ensure quality, molecular weight (MW) data has to be evaluated for each batch of polymer that is produced. Gel Permeation Chromatography is an analytical tool for the characterization of polymers which are soluble in organic solvents. The separation is based on the differences in size of the polymer molecules, and provides primary result molecular weight distribution curves. This means that molecular weight data and quantitative data are calculated after calibration with standards of known molecular weight.

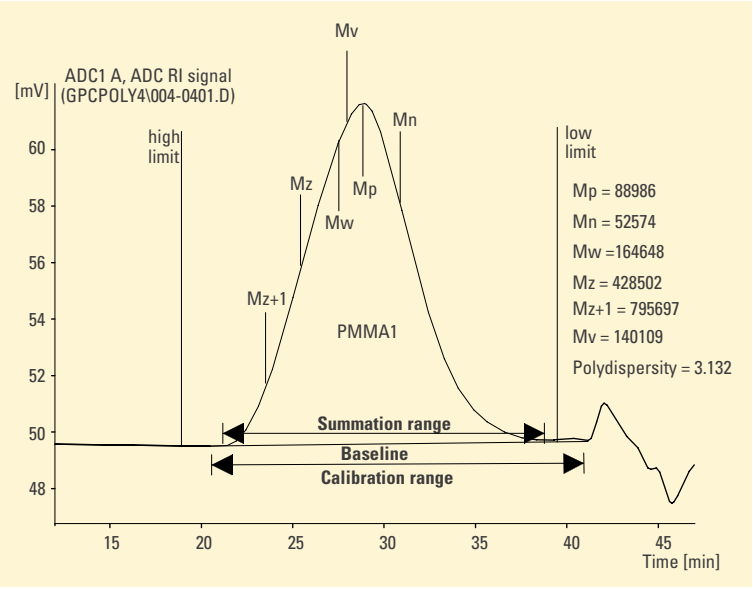

Figure 1
Standard Chromatogram

Conditions

Column
3 ˘ PSS GPC 8 ˘ 300 mm, 5 µm, 10^6, 10^5, 10^3 A
Mobile phase
Tetrahydrofurane (THF)
Flow rate
0.8 ml/min
Oven Temp
20 ºC
Injection vol 10 µl
Refractive index detector

Sample preparation
26 mg sample dissolved in 1 ml THF
Polystyrene standards from PSS were used for narrow standard calibration

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Method Performance

Having set up the chromatographic and GPC calculation procedures including the calibration, the polymer can be analyzed and MW and MWD (molecular weight distribution) data can be calculated. After analysis of the polymer, the baseline and summation range have to be defined. The baseline points should be selected within a flat part of the graph before and after the polymer peak. The summation range should be within the calibrated range and marked either side with lines indicating the high and low limits. The start and end points of the peak need to be carefully selected.

Method performance

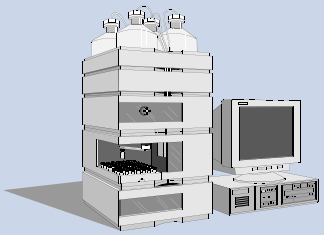
Precision of weight average molecular weight (rsd of Mw) = < 1 %
Precision of number weight average molecular weight (rsd of Mn) = < 2 %

Equipment

Agilent 1100 Series:

- isocratic pump
- degasser (recommended)
- autosampler
- thermostatted column compartment
- diode array detector and/or HP 1047A refractive index detector

Agilent ChemStation + software + polymer labs GPC software



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Released 06/97
Publication Number 5965-9045E

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Retention Time Locking: Concepts and Applications

Application

Gas Chromatography

December 1997

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Abstract

The concepts and applications of retention time locking (RTL) are described. RTL simplifies the process of transferring methods from chromatographic instrument to chromatographic instrument, column to column, and detector to detector. The analysis of impurities in styrene according to ASTM D 5135 is used to demonstrate the efficacy of the approach. Using RTL, the retention times matched within an average of 0.16% (0.02–0.03 minute) in constant pressure modes.

Key Words

Retention time locking, method validation, styrene analysis, ASTM D 5135, capillary gas chromatography, laboratory productivity

Introduction

Retention time is the fundamental qualitative measurement of chromatography. Most peak identification is performed by comparing the retention time of the unknown peak with that of a standard. It is much easier to identify peaks and validate methods if there is no variation in the retention time of each analyte.

However, shifts in retention time occur frequently. Routine maintenance procedures such as column trimming alter retention times. In a multi-instrument laboratory running duplicate methods, the retention times for each instrument will differ from each other, even when run under nominally identical conditions. These differences in retention times mean that each instrument must have a separate calibration and integration event table, making it time-consuming to transfer methods from one instrument to another. Differences in retention time also complicate comparison of data between instruments and over time.

Retention time locking (RTL) is the ability to very closely match chromatographic retention times in any Agilent 6890 gas chromatograph (GC) system to those in another 6890 GC system with the same nominal column.

There are several subtle effects that combine to cause retention time differences between similarly configured GC systems. Columns of the same part number can vary slightly in length, diameter, and film thickness.

GC pneumatics can have small variations in the actual inlet pressure applied at a given setpoint. The actual temperature of the GC oven also has minute but real deviations from the indicated value. The sum of these and other effects result in the observed retention time differences between similarly configured GC systems.

The pneumatics and oven temperature control of the 6890 GC have advanced the state of the art in GC hardware accuracy and precision. Agilent's advances in fused silica capillary column technology have resulted in highly reproducible column-to-column retention characteristics. With these advances, retention time precision for a given peak in a single GC setup is usually better than 0.01 minute. However, even with these advances in columns and instrument hardware, the sum of the effects mentioned above can cause retention time differences between identically configured GC systems of as much as 0.4 minute.

It would be impractical to control all of the instrument and column variables to a degree where retention time differences between similarly configured GC systems are removed. There is, however, a means of greatly reducing these differences. By making an adjustment in the inlet pressure, the retention times on a given GC setup can be closely matched to those of a similarly configured GC system. RTL is based on this principle. The process of RTL is to determine what adjustment in inlet pressure is necessary to achieve the desired match in retention times. Agilent RTL software (G2080AA), which integrates into the Agilent GC ChemStation (version A.05.02 or later), provides the tool required to determine the correct inlet pressure quickly and simply.

There are several advantages gained by using RTL in the laboratory. Peak identification becomes easier and more reliable. It is easier to compare data both between instruments and over time. Comparison of data when using different detectors for analyte identification is simplified. Transferring methods from instrument to instrument or laboratory to laboratory is easier because calibration time windows normally will not require readjustment. Validation of system performance is easier. With "locked" GC methods, the development and use of retention time data bases for unknown identification is much more straightforward.

To maintain a locked method, RTL should be performed whenever:

- The column is changed or trimmed
- The method is installed on a new instrument
- A detector of different outlet pressure is used
- System performance is validated
- Troubleshooting chromatographic problems

To lock a given method for the first-time or for the reasons below, one must first develop a retention time versus pressure (RT vs. P) calibration.

Even when using columns with the same part number (same id, stationary phase type, phase ratio, and same nominal length), separate/different locking calibration curves are needed when using:

- Systems with different column outlet pressures (FID/atmospheric, MSD/vacuum, AED/elevated)
- Columns differing from the "nominal" length by more than 15% (e.g., due to trimming)
- Systems where the predicted locking pressure falls outside the range of the current calibration

A specific solute (usually one found in the normal method calibration standard) must be chosen and then used for both developing the locking calibration and locking all future systems. The solute, or target peak, should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Solutes that are very polar or subject to degradation should be avoided.

Once the target solute has been chosen and all other chromatographic parameters of the method have been determined, five calibration runs are performed. The runs are made at conditions identical to the nominal method except that four of the runs are made at different pressures. The pressures used are typically:

- Target pressure – 20%
- Target pressure – 10%
- Target pressure (nominal method pressure)
- Target pressure + 10%
- Target pressure + 20%

The retention time of the target compound is determined for each run. The resulting five pairs of inlet pressures and corresponding retention times are entered into the ChemStation software to generate an RTL calibration file.

Figure 1 shows the dialog box used to enter the calibration data. After the data is entered, a plot is displayed, as shown in figure 2. The maximum departure of the fitted curve from the data is given for both time and pressure. If the fit is acceptable, the retention time versus pressure calibration is stored and becomes part of the GC

method. This calibration need only be generated once. Subsequent users of the method can use this calibration when running the method on a similar instrument setup, regardless of location.

To relock a system or lock a new one:

1. Set up the method conditions and run a standard containing the target compound.
2. Enter the actual retention time of the target compound into the "(Re)Lock current method" dialog box (see figure 3).
3. Update the 6890 method with the new calculated pressure, and save the method.
4. Validate the retention time lock by injecting the standard at the new pressure, and compare the retention time obtained to the desired retention time.
5. Repeat steps 2 to 4, if necessary.

A Note on Constant Flow versus Constant Pressure Modes of EPC Operation

Many GC chromatographers prefer to use the "constant flow mode" of EPC operation. In this mode, inlet pressure increases automatically to maintain constant outlet flow rate as the oven temperature increases during the run. Constant flow mode reduces run time and ensures that flow-sensitive detectors see a constant column effluent flow.

The "constant pressure" mode of EPC operation is also popular. In this mode, the pressure remains constant during the run (outlet flow will decrease as temperature increases). For those wishing to reduce run time in constant pressure mode, a higher pressure can be chosen. For

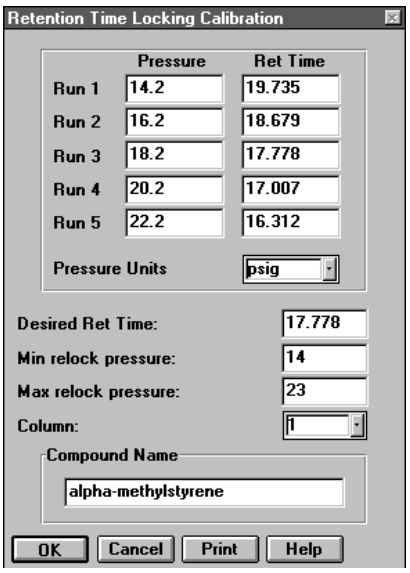

Figure 1. Dialog box used for entering retention time locking calibration data

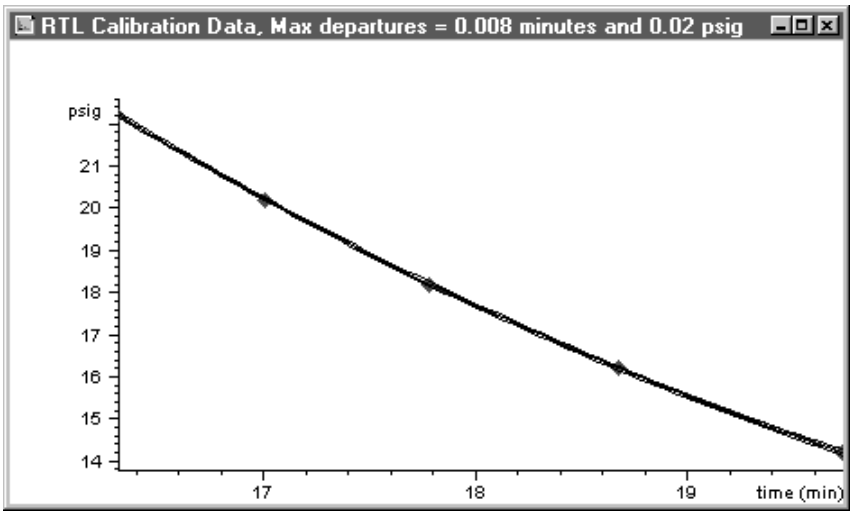

Figure 2. Plot of calibration data as displayed by RTL software

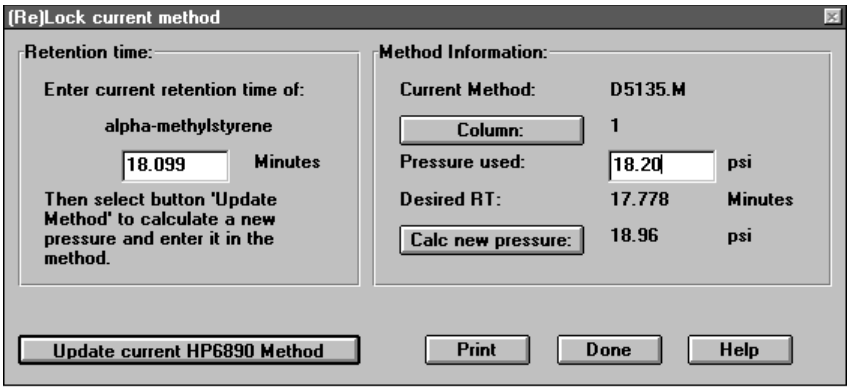

Figure 3. Dialog box used to calculate locking pressure and update the 6890 method

flow-sensitive detectors, one can set "constant column flow + makeup" via the 6890 keyboard or ChemStation. In this mode, the makeup flow is increased as the column flow decreases to keep the sum of the two constant.

The underlying theory of RTL predicts that constant pressure mode of EPC provides the closest matching of retention times. If one desires to compare data from systems with very different configurations, such as GC/FID to GC/MSD, it is best to use constant pressure mode. As can be seen from the styrene analysis data herein, retention time matching between systems of the same configuration (GC/FID, in this case) is still quite good in the constant flow mode.

This application note shows the use of RTL to lock retention times between multiple chromatographic instruments, columns, and detector types and demonstrates RTL in both constant flow and constant pressure modes.

Experimental

Two 6890 Series GC systems were used. Each system was equipped with:

- Electronic pneumatics control (EPC)
- Split/splitless inlet (250 °C, He carrier gas, split 80:1)
- Automatic liquid sampler
- GC ChemStation (version A.05.02)
- Flame ionization detector (FID)
- 60 m × 0.32 mm, 0.5 mm HP-INNOWax column (part no. 19091N-216)
- Temperature program: 80 °C (9 min), 5 °C/min to 150 °C

The inlet pressures/flows used are indicated with each chromatogram.

A third 6890 Series GC was also used. This system was equipped with an Agilent 5973 mass selective detector (MSD) and was used for peak identification. The GC-MSD chromatographic parameters used were the same as the GC systems noted above except for the inlet pressures as indicated.

Results and Discussion

GC-FID to GC-FID Locking

Figure 4 shows the original chromatogram (GC system 1) obtained from running a styrene sample under the conditions specified in ASTM D 5135.[1] Many of the typical impurities found in styrene are found here. The phenylacetylene peak represents about 60 ppm. The peaks are identified in table 1.

The sample was then run at four other pressures to collect the five data pairs for RTL calibration. Because this method was run in constant flow mode, the pressures entered into the RTL software were the initial pressures. The α-methylstyrene peak (peak 10) was chosen as the target compound. The calibration data are shown in figure 1.

The method conditions and RTL calibration were then moved to GC system 2, a different GC and column. The sample was run at the original method inlet pressure of 18.2 psi. The chromatogram obtained using this scouting run is overlaid on the original chromatogram in figure 5. The retention times shifted about 0.3 minute on the second GC. This is a typical result obtained when trying to replicate an analysis on a second instrument or with a second column.

The retention time of α-methylstyrene was entered into the RTL software

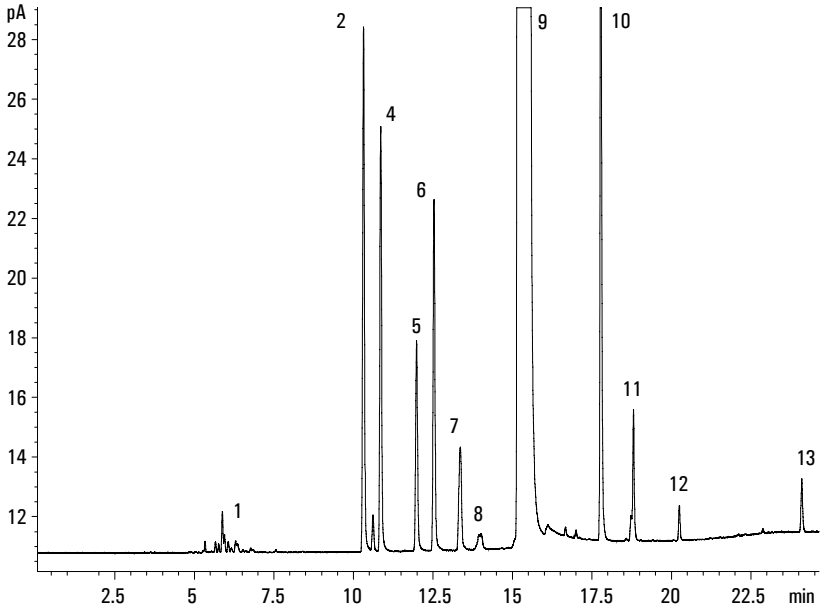

Figure 4. Styrene sample run on GC system 1 at 18.2 psi initial pressure, constant flow mode

dialog box on GC system 2, as shown in figure 3. The RTL software indicated the initial pressure should be modified from 18.2 psi to 18.96 psi. The new initial pressure was entered into the method and saved.

Figure 6 compares the chromatograms obtained from the original run and after locking retention times using the α-methylstyrene. Table 2 compares the retention times before and after using this approach. The retention times are now closely matched.

GC-FID to GC-MSD Locking

A second experiment was conducted to lock the original method from GC system 1 to the GC-MSD. This is useful for identification of unknown impurities that show up in the FID chromatogram. For example, there is a shoulder evident on the front side of the phenylacetylene peak in figure 4. It would simplify locating the impurity in the GC-MSD data if the retention times closely matched that of the GC-FID.

Because constant pressure mode is preferred when comparing data from FID and MSD systems, constant pressure mode was chosen, and the styrene sample was re-run on GC system 1 at 18.2 psi for reference.

The next step was to determine the chromatographic conditions to be used on the GC-MSD. The Agilent method translation software tool was used to calculate the conditions necessary to have the peaks elute in the identical order on the two systems.[2,3] Because the retention times need to match, the dead time and temperature program used for running the GC-MSD must be the same as the GC

Table 1. Peak Identities for Figure 4

Peak #	Name	Peak #	Name
1	Nonaromatics	8	p/m-Ethyltoluene
2	Ethylbenzene	9	Styrene
3	p-Xylene	10	α-Methylstyrene
4	m-Xylene	11	Phenylacetylene
5	i-Propylbenzene	12	β-Methylstyrene
6	o-Xylene	13	Benzaldehyde
7	n-Propylbenzene		

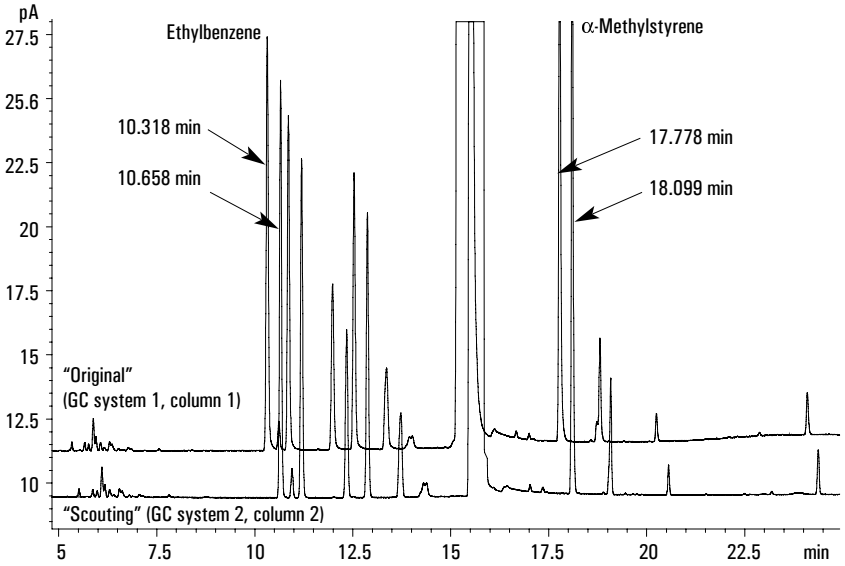

Figure 5. Comparison of original chromatogram on GC system 1 with GC system 2 before retention time locking

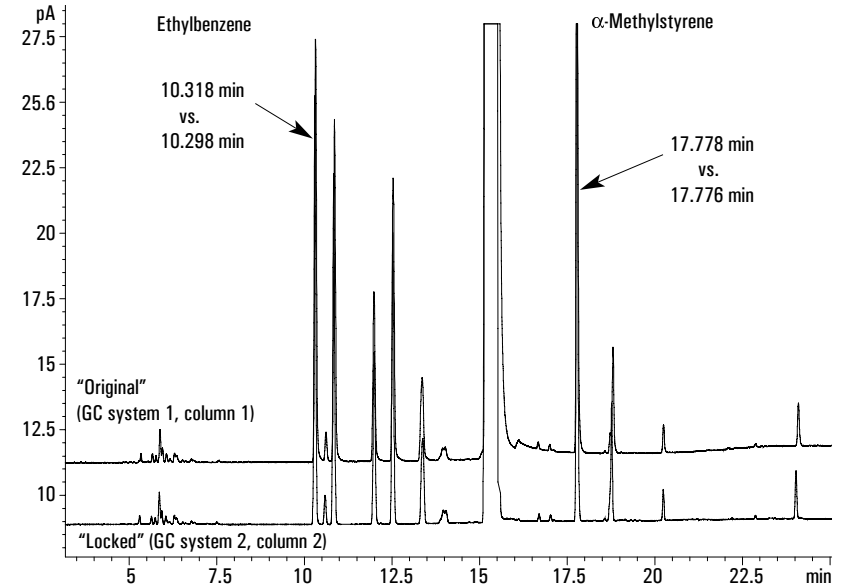

Figure 6. Comparison of original chromatogram on GC system 1 with GC System 2 after retention time locking

method. The pressure used, however, will be different due to the difference in column outlet pressure. The GC-MSD inlet pressure is calculated using the "none" mode of the method translation software (figure 7). In this mode, the holdup time between the two columns was forced to be identical to the GC-FID. This gives a speed gain of 1. The pressure calculated for use on the GC-MSD was 8.44 psi. Note that this calculated pressure is only the nominal pressure required to get similar retention times, not the exact locking pressure.

A different RTL calibration is required for GC-MSD because the outlet pressure is vacuum, and that of the FID is atmospheric pressure. Five runs were made on the GC-MSD system bracketing the 8.44 psi nominal method pressure. Because the GC-MSD used in this study was not equipped with RTL software, a dummy method was created in GC system 1 and the GC-MSD RTL calibration data was entered into it. A scouting run of the Styrene sample was made on the GC-MSD, and the α-methylstyrene retention time was used for locking. The locking inlet pressure calculated with the dummy method was 7.9 psi and was entered into the GC-MSD.

Figure 8 shows the resulting matched chromatograms from the GC-FID and GC-MSD. As seen in table 3, the retention times are now closely matched within 0.02 minute.

Figure 9 shows the MSD first choice of library search result of the impurity that created the shoulder on the front side of the Phenylacetylene peak. RTL ensured that this shoulder remained separated on the MSD system and eluted at the same time for easy comparison to the FID results.

Table 2. GC-FID Retention Times Before and After Locking for Styrene Impurities (Constant Flow Conditions). Chromatograms Shown in Figures 4, 5, and 6.

Component	Original Run GC 1/Column 1 18.2 psi	GC2–GC1 Before RTL	Scouting Run GC 2/Column 2 18.2 psi	GC2–GC1 After RTL	Locking Run GC 2/Column 2 19.0 psi
Ethylbenzene	10.318	0.340	10.658	–0.020	10.298
p-Xylene	10.616	0.333	10.949	–0.026	10.590
m-Xylene	10.858	0.337	11.195	–0.022	10.836
i-Propylbenzene	11.985	0.359	12.344	+0.005	11.990
o-Xylene	12.533	0.345	12.878	–0.012	12.521
n-Propylbenzene	13..360	0.364	13.724	–0.016	13.376
α-Methylstyrene*	17.778	0.321	18.099	–0.002	17.776
Phenylacetylene	18.806	0.275	19.081	–0.040	18.766
β-Methylstyrene	20.248	0.310	20.558	–0.006	20.242
Benzaldehyde	24.097	0.279	24.376	–0.069	24.028
Average Δ		0.326		0.028	

* Used in locking calculation

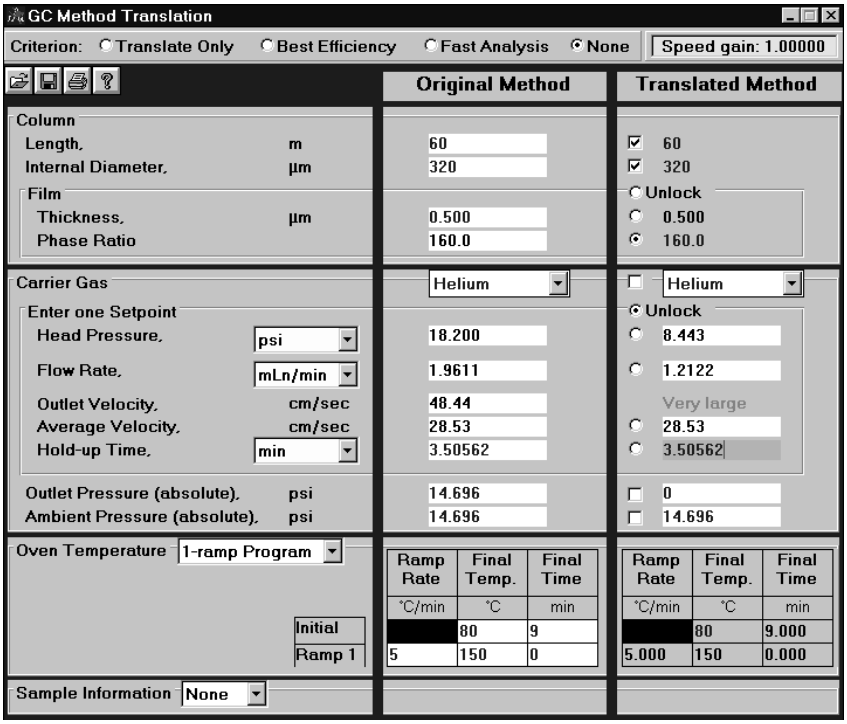

Figure 7. Method translation software provides scaled conditions for GC systems with different configurations

Conclusions

Retention time locking facilitates replicating results from instrument to instrument, from column to column, and from detector to detector by locking retention times. The retention times of a styrene sample analyzed according to ASTM D 5135 matched to within 0.06 minute after locking.

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3. GC Pressure/Flow Calculator for Windows, Version 2.0 and Method Translation Tool Version 2.0. Available at http://www.chem.agilent.com/servsup/usersoft/main.html.

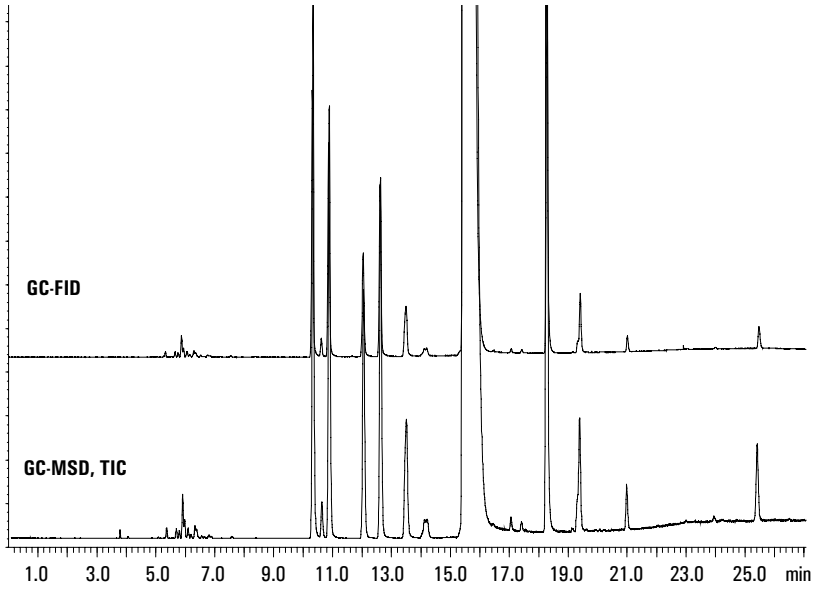

Figure 8. Comparison of chromatogram on GC system 1 with GC-MSD system after retention time locking, Constant Pressure Mode

Table 3. GC-FID vs. GC-MSD, Method Translated then Locked—Retention Times (Constant Pressure Conditions)

Component	GC-FID Original 18.2 psi	GC-MSD 7.9 psi	RT Difference min
Ethylbenzene	10.315	10.338	0.023
p-Xylene	10.620	10.642	0.022
m-Xylene	10.869	10.890	0.021
i-Propylbenzene	12.038	12.053	0.015
o-Xylene	12.613	12.630	0.017
n-Propylbenzene	13.492	13.508	0.016
a-Methylstyrene*	18.276	18.267	–0.009
Phenylacetylene	19.406	19.389	–0.017
b-Methylstyrene	21.008	20.987	–0.011
Benzaldehyde	25.475	25.415	–0.060
		Average	0.021

* Used in locking calculation

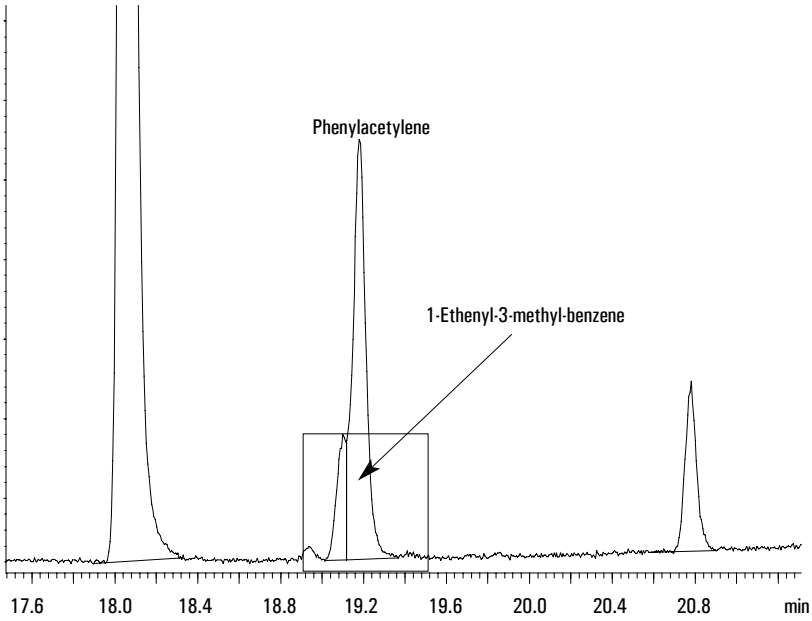

Figure 9. GC-MSD identification of impurity in shoulder of phenylacetylene peak

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Printed in the USA 3/2000
5966-2469E



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Analysis of Sulfur Anions in Kraft Liquors using Capillary Electrophoresis

Maria Serwe

Inorganics

Abstract

The proper function of Kraft recovery furnaces is a very important part of the chemical recovery process at a Kraft pulp mill. In the recovery furnace sulfur anions, such as sulfate in the black liquor are converted to sulfide anions in the green liquor. Sulfide anions in the pulping reactions produce stronger paper and if its concentration is too low, the paper produced loses its strength. If the sulfide concentration is very high, polluting emissions from the Kraft recovery furnaces are increased. Wet chemical tests and Ion Chromatography (IC) have traditionally been used to analyze process liquors. CE is a worthy replacement for IC due to less buffer consumption, less maintenance, and considerably shorter run times.

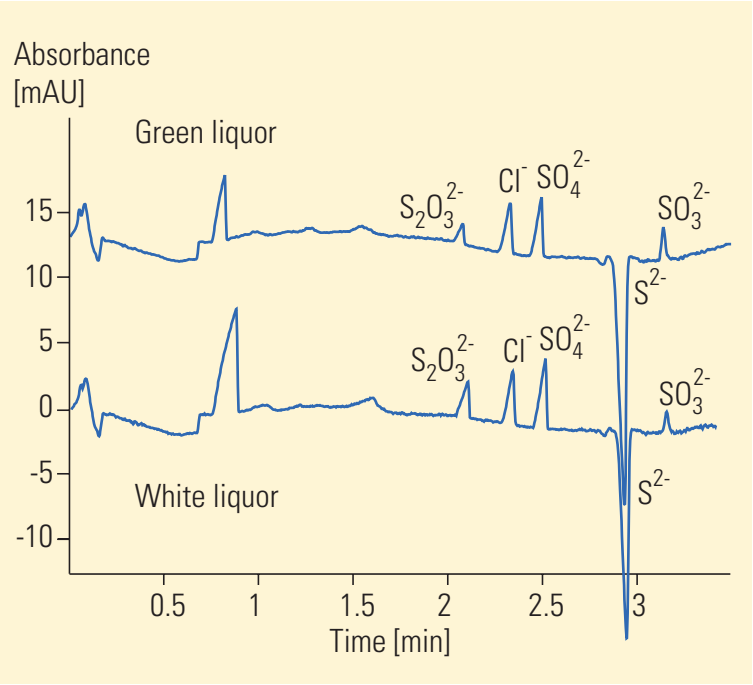

Figure 1
Analysis of green and white Kraft liquors

Conditions

Sample
green and white Kraft liquor
Injection
1 sec @ 50 mbar sample,
dip inlet in water,
2 sec @ 50 mbar buffer
Capillary
effective length 24.5 cm
total length 33 cm
internal diameter 50 µm
Buffer
2.25 mM pyromellitic acid
6.5 mM sodium hydroxide
0.75 mM hexamethonium hydroxide
1.6 mM triethanolamine, pH 11.2
Voltage
-18 kV
Temperature
35 °C
Detection
signal 350/50 nm
reference 235/10 nm
Preconditioning
2 min flush with 0.1 N NaOH
4.2 min flush with buffer,
at 1 bar each

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Experimental and Results

In the pulp and paper industry Kraft liquors must be monitored for the anion content both to monitor the quality of the liquors and for environmental reasons. CE is used to measure sulfur anions (sulfide, sulfate, thiosulfate and sulfite), chloride anions and oxalate anions. Wet chemical tests are used for hydroxide, carbonate, sulfide and sulfate anions. The sulfate and sulfide anions are used to calculate the percent reduction of the green and white liquor, which is one measure of the efficiency of the recovery furnace. Control personnel uses the results of the tests to adjust process control parameters that influence the degree of conversion of sulfur containing anions to sulfide anions.

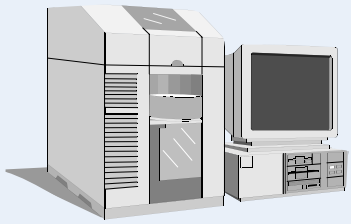
CE is also used to measure the amount of chloride ions in Kraft recovery process liquors. Chloride concentrations tend to build up in the recovery process to an equilibrium level depending on inputs to the system. High chloride concentration can be detrimental because it increases the rate of corrosion for some type of metals.

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and computer control via the Agilent Chemstation. The chemical method is based on the Agilent Inorganic Anion Kit. The pH of the buffer is raised to make sulfur species stable (for details see conditions next to figure). Migration times were stabilized by flushing the capillary with sodium hydroxide and buffer and replenishing buffer vials prior to each run. Available modification to the Agilent CE system provides for blanketing the replenishment system with helium to prevent oxidation of sulfide and sulfite anions. All standards (10,000 ppm sulfide, sulfate, Cl) were prepared in 0.1 N NaOH. Sample preparation consisted simply of diluting the sample 1:200 with water.

The assay was linear over the range 10 to 100 ppm with an $R^2 = 0.99997$. The detection limit in the Kraft liquors was approximately 1 ppm. Samples containing 20g/l sulfide and 1 g/l of the other components could be routinely analyzed.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation + 3D software
- Agilent Inorganic Anion Kit (part number 5063-6511)



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Released 12/98
Publication Number 5968-3306E

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application

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Use of Liquid Reagents for Positive Chemical Ionization on the 5973 MSD

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Introduction

Two powerful features of the technique of positive chemical ionization (PCI) mass spectrometry are selectivity and control over the extent of fragmentation of the analytes of interest. This is made possible by selecting reagent gases that have different proton affinities (PAs). The potential advantages in using liquids as PCI reagents are their availability and purity, ease of handling and introduction into the mass spectrometer, low consumption, and low cost. For example, the common organic solvents—methanol, acetone, and acetonitrile—have high vapor pressures and a range of PAs that make them attractive as potential PCI reagents (Table 1). Intense adducts of the molecular ion (M^+) are possible for additional confidence in molecular identity. This is helpful in situations in which dehydration or abstraction mechanisms may convolute establishing molecular identity. Also these additional intense confirming adduct ions can improve quantitative confidence. For example, methane, the most common PCI reagent, usually produces a protonated molecular ion $(M+H)^+$ as the most abundant ion for an analyte in PCI spectra with the $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$ adducts being less than approximately 10% and 5%, respectively. These ratios are very stable and are reproducible on the Agilent Technologies 5973 MSD and, by adjusting the source parameters, can be easily controlled in such a way as to be favorable for confirmation work.

The situation in PCI is very compound-specific; however, to generally explore liquid reagents and adduct formation, benzophenone (BZP) was chosen as a model compound (Figure 1).

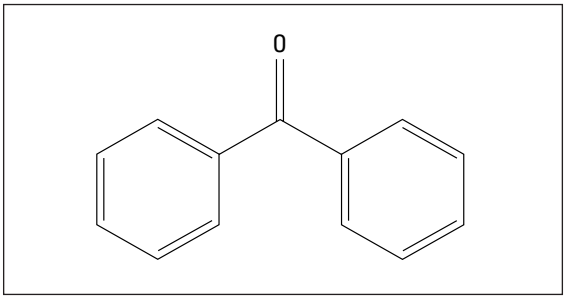

Figure 1. Benzophenone: $C_{13}H_{10}O$, molecular weight 182 g/mole.

Table 1. Compound Physical and Thermochemical Properties.

Compound	Molecular Weight	Vapor Pressure (at °C)	Proton Affinity (kcal/mole)[1]
Methane	16	–	131.6, 162.6
Methanol	32	125 torr (25°C)	181.9
Acetonitrile	41	89 torr (25°C)	188.2
Acetone	58	184 torr (20°C)	196.7
Ammonia	17	–	204.0

Experimental

The Agilent Technologies 6890 GC Plus with the 5973 Mass Selective Detector with CI option was used for all experiments. A PCI autotune with methane reagent gas was used for the initial source tuning parameters and lens voltages.

Liquids were introduced into the CI manifold through a short length of Teflon tubing (0.317 cm o.d. tubing with 0.159 cm wall) with a 1/8-inch Swagelok connector. Liquids were contained in either a standard 1-liter HPLC bottle (Figure 2) or in a custom glass tube of 100 ml capacity (Figure 3) and positioned below the manifold to avoid siphoning of liquid into the instrument. The end of the Teflon tubing was suspended just above the surface of the liquid, and the vessel was purged with argon or helium prior to use. The liquid's vapor displaces air or purge gas in the vessels and generates an atmosphere that consists primarily of the chemical of interest. Placing the vessel in an ultrasonic cleaner assists in generating vapor.

The flow into the source, which is directly proportional to source pressure, was controlled by the standard 5973 CI manifold. Although this reagent gas flow is continuously adjustable, experiments with each liquid were conducted at four flow increments: 5%, 10%, 15% and 20% of the total flow of 5 ml/min. In each experiment, benzophenone was injected and spectra were recorded in full scan with a 200-amu mass range. The mass range was adjusted to avoid collecting reagent ions.

Results

The most critical parameters affecting the intensities of reagent ions in the source and the abundances of the $[BZP+H]^+$ and $[BZP+Adduct]^+$ products were source temperature and pressure. As expected, the lower the source temperature, the higher the abundance of the adducts. A source temperature of 150°C was selected. The results for each liquid are presented as the intensities of the reagent ions in the source versus flow setting. These reagent ion intensities are normalized to the most abundant reagent monomer ion ($[Reagent+H]^+$) intensity. Similarly, abundances of the $[BZP+H]^+$ and the $[BZP+Reagent+H]^+$ products versus source pressure are presented relative to the most abundant $[BZP+H]^+$ produced at any flow setting.



Figure 2. HPLC Bottle as liquid CI reagent container.



Figure 3. 100 ml liquid CI reagent tube.

Methanol Results

Methanol (MeOH) easily generated dimers and trimers in the source (Figure 4). A flow setting of 10% generated the greatest abundance of the $[MeOH+H]^+$ reagent ion. As flow into the source increased, the degree of "solvation" of the proton increased $[H^+ + (MeOH)_n]$, and at the highest setting used there was 4 times more dimer and 3 times more trimer than monomer.

Despite the high abundances of dimers and trimers, relatively small amounts of adduct were formed with benzophenone (Figure 5). The highest flow setting applied generated the highest amounts of total reagent ions, as well as product $[BZP+H]^+$ and $[BZP+MeOH+H]^+$ ions.

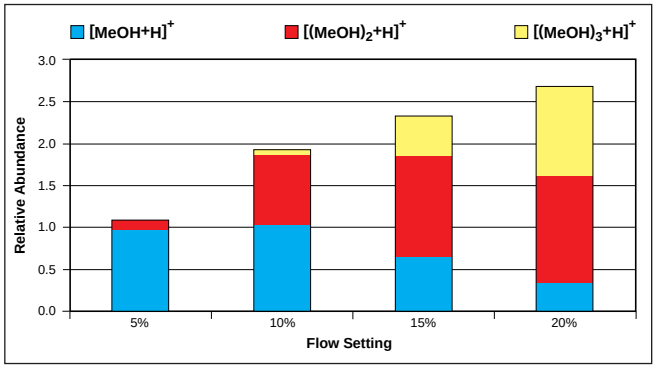

Figure 4. Abundance of methanol PCI reagent ions as a function of flow setting.

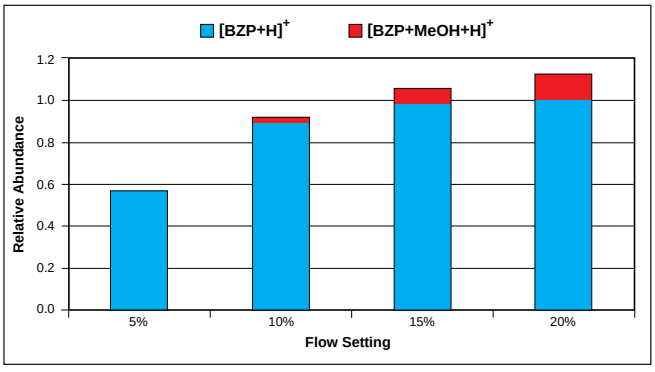

Figure 5. Benzophenone product on abundances as a function of methanol reagent gas flow setting.

Acetonitrile Results

Acetonitrile (ACN) readily formed dimers in the source (Figure 6). The most abundant monomer was formed at the lowest source pressure studied, the 5% flow setting, and the abundance of dimers was approximately 50% that of monomers. The next higher increment in flow to the source, the 10% flow setting, equivalent to doubling the source pressure of ACN, generated the largest total ion intensity in the source (sum of monomers and dimers) and the largest amount of $[BZP+H]^+$ product ion (Figure 7). As the source pressure was further increased from 10% to 20%, the abundance of $[(ACN)_2+H]^+$ reagent ion remained relatively constant, but the intensity of $[ACN+H]^+$ reagent decreased. Simultaneously the percentage of adduct ion increased until at the highest source pressure studied, the $[BZP+ACN+H]^+$ adduct ion intensity was nearly 75% that of the $[BZP+H]^+$ ion. However, the product ion signal reached a maximum near the 10% flow setting.

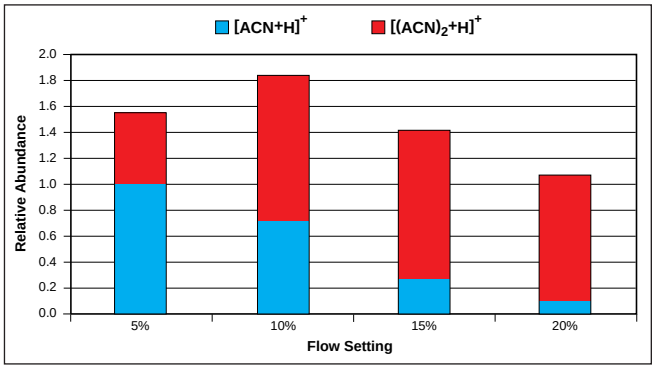

Figure 6. Acetonitrile PCI reagent ions as a function of flow setting.

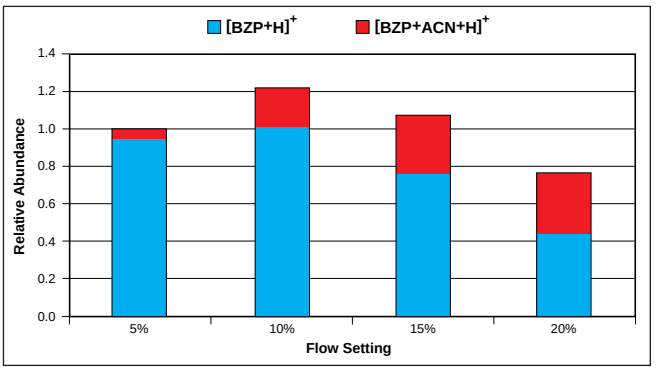

Figure 7. Benzophenone product ion abundances as a function of acetonitrile reagent gas flow setting.

Acetone Results

The trends for acetone were similar to those for acetonitrile. Protonated acetone reagent dimers dominated monomers in the source at every flow setting studied, most likely because of the large dipole moment and molecular size (Figure 8). The absolute dimer abundance was essentially constant at the four flow settings, but monomer abundance decreased as the source pressure increased, similar to the findings for acetonitrile. At the 20% flow setting, dimer abundance was more than 40 times that of the monomers.

Product ions were similarly dominated by adduct formation over protonated BZP at all settings, and $[BZP+H]^+$ decreased as flow to the source increased (Figure 9). The 5% and 10% flow settings produced the maximum total signal and relative adduct intensities of 65% and 190% of $[BZP+H]^+$. This implies that a situation that is optimum for quantitation may be produced where the intensity of the quantitation ion and confirming ion are of equal intensity; that is, when $[BZP+H]^+ = [BZP+Acetone+H]^+$.

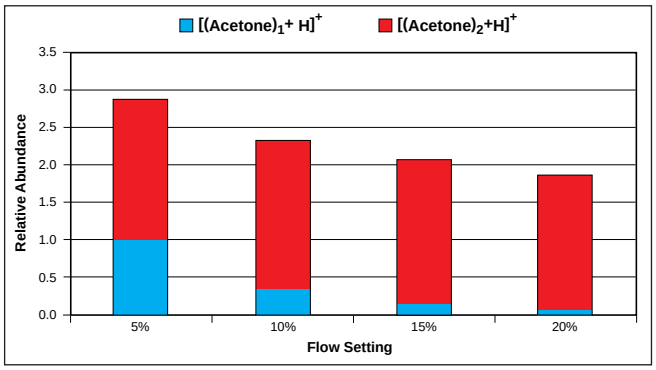

Figure 8. Acetone PCI reagent ions as a function of flow setting.

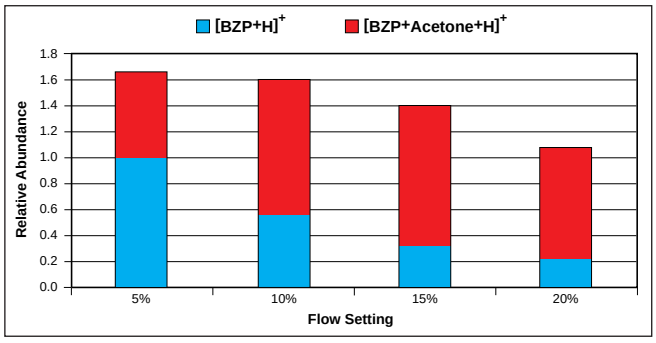

Figure 9. Benzophenone product ion abundances as function of acetone reagent gas flow setting.

Deuterated Liquid Reagents: CD_3CN

Deuterated organic solvents are readily available and inexpensive. In PCI they can produce $[M+D]^+$, as well as identifying adducts. A mixture of 50:50 (w/w) of CH_3CN and CD_3CN was used as the CI reagent at a flow setting of 20%. Using this reagent, a variety of species is possible in the source. Table 2 shows the source ion composition and relative intensity in the source at this setting.

The resulting PCI mass spectrum for benzophenone shows intense BZP+D and BZP+H ions, as well as a variety of adducts (Figure 10). Table 3 lists the product ion compositions, mass-to-charge ratios, and relative abundances.

Table 2. Reagent Ion Composition, Mass-To-Charge Ratio and Relative Abundance generated by a 50:50 mixture of CH_3CN and CD_3CN.

Reagent Ion Formula	m/z (Relative Abundance)
$[CH_3CN+CD_3CN+H]^+$	86 (100%)
$[CH_3CN+CD_3CN+D]^+$	87 (80%)
$[(CH_3CN)_2+H]^+$	83 (62%)
$[(CH_3CN)_2+D]^+$	84 (62%)
$[(CD_3CN)_2+H]^+$	89 (44%)
$[(CD_3CN)_2+D]^+$	90 (40%)
$[(CH_3CN)+H]^+$	42 (5%)
$[(CH_3CN)+D]^+$	43 (4%)

Table 3. Product Ion Composition, Mass-To-Charge Ratio and Relative Abundance generated by a 50:50 mixture of CH_3CN and CD_3CN.

Product Ion	m/z (Relative Abundance)
$[BZP+D]^+$	184 (100%)
$[BZP+H]^+$	183 (88%)
$[BZP+CH_3CN+H]^+$	224 (35%)
$[BZP+CH_3CN+D]^+$	225 (31%)
$[BZP+CD_3CN+H]^+$	227 (32%)
$[BZP+CD_3CN+D]^+$	228 (30%)

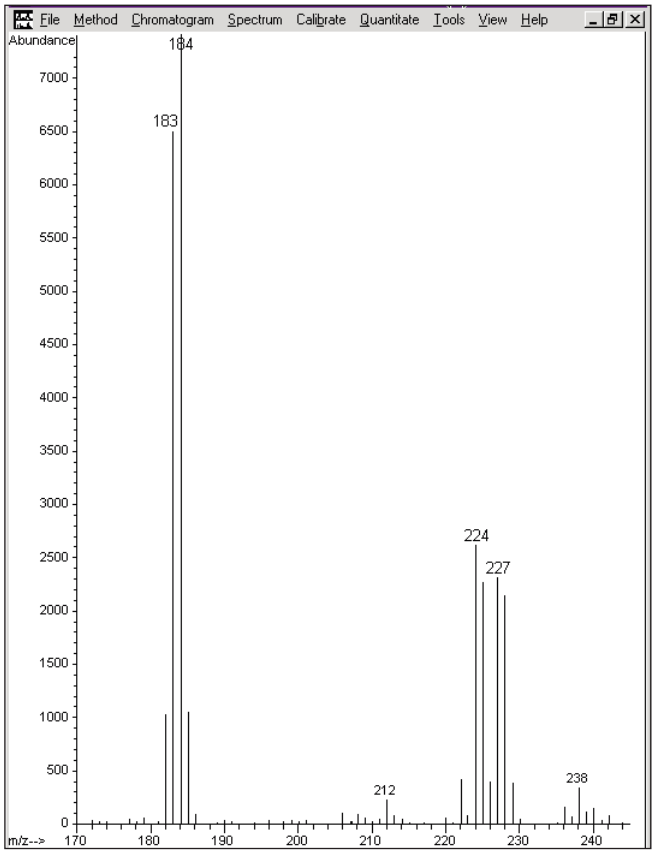

Figure 10. Benzophenone PCI spectra with acetonitrile/acetonitrile-d_3 (50:50 w/w) PCI reagent gas.

Conclusions

Liquid reagents are *easily* implemented on the 5973 MSD-CI for use in PCI. The instrument parameters provide a great deal of flexibility, which allows the analyst to optimize PCI performance for the compounds of interest. The most important parameters for any specific reagent gas or analyte are source temperature and reagent pressure. By adjusting the source parameters of temperature and reagent flow setting, the intensity of the protonated molecular ion can be optimized or the abundance of adducts or both. Just as with the traditional CI gases (methane, ammonia, etc.), these adducts can be utilized to confirm the analyte molecular weight since they will result in a known m/z value and relative abundance of M+Adduct ion. For example, the results for PCI of benzophenone with acetone show that the highest intensity of protonated benzophenone occurs at the lowest setting (Figure 9). However, increasing the flow slightly allows the adduct to increase with little loss in total intensity; a situation useful in quantitation where an intense confirming ion is useful. The results for acetonitrile show that equal intensities of the protonated benzophenone and benzophenone-adduct are not achieved until the flow setting has been raised to 20% (Figure 7).

Similarly, mixtures of native and deuterated liquid reagents can generate a variety of confirming adduct ions in addition to M+1 and M+2. This feature may be helpful in distinguishing ions formed by abstraction $(M–1)^+$ or dehydration $(M–H_2O)^+$ from those molecular ions expected via molecular protonation which can confound the determination of molecular weights. In other words, if one expects to see M+2 or M+Adduct in the mass spectrum of a particular compound but they are not observed, it suggests that another mechanism of ionization is taking place such as loss of hydrogen, $[M–H]^+$, or water, $[M–H_2O]^+$. This in turn provides some information about chemical structure and functional groups. The ability of the 5973 MSD-CI to conveniently manipulate the reagent source pressure allows the analyst to manipulate the ratios of adduct formation and protonation to aid in confirmation or quantitation.

However, it is *highly* recommended that the classic gaseous reagents be explored before resorting to liquid reagents. Methane and ammonia span a greater range of proton affinities than the liquid reagents and are well characterized in their behavior and operation. All the aforementioned conclusions about source parameters and adduct formation, etc., are equally valid for the gaseous PCI reagents. The most efficient and straightforward approach for confirming identity, studying functional groups, and arranging favorable quantitation ratios and one that is extremely easy to implement on the 5973 MSD-CI, is to first apply methane and then ammonia. Further, perdeuterated methane and ammonia are both readily available and relatively inexpensive and, since CI gas use is only of the order of 0.5–1 ml/min, their use is economically feasible.

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Method Precautions

NEVER HEAT flammable solvents such as those used in this study! Use of halogenated solvents and carbon disulfide should be avoided as these may affect the electron capture negative ionization sensitivity.

Acknowledgments

The authors are grateful to the Agilent Technologies Gas Phase Application Chemists Council for their technical review of the manuscript.

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Printed in the U.S.A. December 1999
(23) 5968-5707E

application

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An approach to the determination of N-nitrosodimethylamine at part-per-quadrillion levels using Positive Chemical Ionization and Large-Volume Injection

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Introduction

N-nitrosodimethylamine (NDMA, Figure 1) is one of a series of nitroso compounds known to be carcinogenic. NDMA is found in nitrate-cured or smoked meats,[1] cheeses,[2] tobacco smoke,[3] cooked foods and in beverages such as beer[4] (both foreign and domestic[5–7]). The presence of NDMA in surface waters designated for use drinking water use is of particular concern and the U.S. Environmental Protection Agency (EPA) has promulgated a regulatory standard for these waters of 0.7 ng/l (700 ppq). When in 1998 NDMA was detected in California drinking water, the source was associated with the production and use of a rocket fuel component, unsymmetrical dimethylhydrazine. In response, the California Department of Health Services (DHS) announced an action level in drinking water of 2 ng/l (2 ppt). However, the best available methods in the literature provide *detection* limits on the order of 1–3 ng/l. EPA methods 625 and 1625 specify a detection limit for NDMA of 50 ppb—25,000 times the California DHS action level and 70,000 times the EPA regulatory standard. It follows that using existing methodologies, *any* detection of NDMA represents a violation.

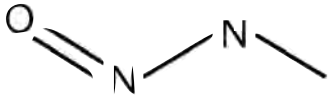

Figure 1. N-nitrosodimethylamine, $(CH_3)_2N_2O$, 74 g/mole, CAS Registry No. 62-75-9

Determining NDMA at ppt or ppq concentrations in water is an analytical challenge. The extraction methods that have been applied, such as liquid-liquid or solid-phase extraction,[8–10] produce concentration factors of 500 to 1000, but overall recoveries are generally low. The high polarity and volatility of NDMA contribute to lowered recoveries and extensive extract concentration by evaporation can lead to high losses.

To increase sensitivity and specificity, one prevalent detection scheme involves use of the chemiluminescent nitrogen detector. Electron impact mass spectrometry has also been used but the fragmentation pattern is not very favorable (Figure 2). While the molecular ion at 74 m/z may be a reliable quantitation ion, the confirming ions at 42 and 43 m/z are hardly unique and are easily compromised by fragments from interferences.

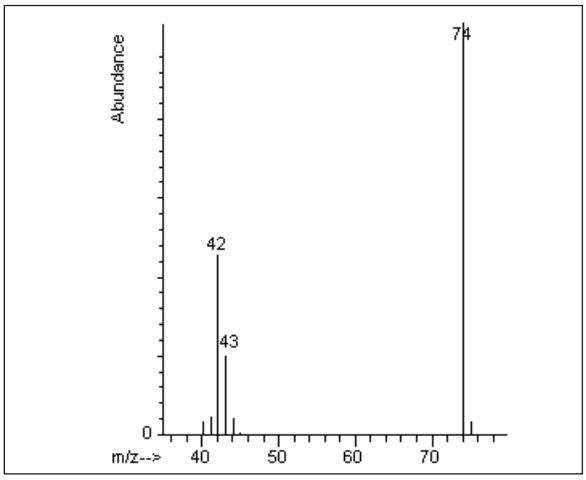

Figure 2. Electron impact ionization mass spectrum of NDMA

One approach to overcoming the unfavorable electron impact (EI) ionization mass spectrum of NDMA is to apply positive chemical ionization (PCI). PCI can provide enhanced analyte selectivity and sensitivity. Utilizing large-volume injection (LVI) should lower the concentration of NDMA that can be detected in an extracted sample. This note describes the combined application of these two techniques as a possible approach to determining NDMA at ppt and ppq concentrations.

Experimental

NDMA standards were made by serial dilution in 1-ml of dichloromethane from a 100 ng/µl standard (Ultra Scientific, Kingstown, RI; part number NS-100). Dichloromethane was chosen as the solvent, because this solvent is used in both liquid-liquid and solid-phase extraction techniques.

Instrumental Section

The 6980 Plus GC / 5973 MSD with chemical ionization option was operated in the selected-ion-monitoring mode (SIM) with ammonia reagent gas. An HP-210 GC column 50%-trifluoropropyl-50%-methyl-siloxane (30-m, .25 mm i.d., 0.5 µm film thickness, Part Number 19091C-733) was used with a 5-m, 0.32 mm i.d. uncoated retention gap (Part Number 19091-60600) joined by a press-fit connector (Part Number 5062-3555). A 100-µl syringe was used in the integrated automated liquid sampler 7683 injector for the 50-µl injections. GC oven conditions and mass selective detector settings are given in Tables 1 and 2.

Table 1. GC and Injector parameters

Oven Temperature Program	Temp	Time
Initial Temperature	45°C	3.00 min
Ramp 50°C / min	180°C	0.50 min
GC Oven Equilibrium Time		3.00 min
MSD Transfer Line		225°C
Inlet Mode		Split
Split Flow		50 ml / min
Gas Saver		Off
Column Flow (Helium carrier gas)		2.0 ml / min
Mode		Constant Flow
Outlet Pressure		Vacuum
Injection Volume		50 µl
Syringe Size		100 µl
Plunger Speed		Slow
Solvent Washes A, B	Methanol*	Dichloromethane

* A solvent that "wets" the glass bore improves syringe life.

Table 2. MSD parameters

Tune File *	PCINH3.U
Ammonia Reagent Gas Flow	10 %
EM Voltage	PCI CH_4 AutoTune + 400V
MS Quadrupole Temp	106°C
MS Source Temp	250°C
Acquisition Mode	SIM
Solvent Delay	5.25 min
SIM Ions	Dwell
75.1 amu	80 msec
92.1 amu	80 msec

* PCI Autotune parameters were used for these experiments. Autotune provides high sensitivity over a large mass range, but even greater sensitivity for these low molecular weight ions can be achieved by manual adjustment of the tuning parameters.

Large-Volume Injections

The APEX ProSep™ 800 Series XT Plus Preseparation System Inlet (APEX Technologies, Cincinnati, OH) was used as the inlet for large-volume injections.[11, 12] Injections were made into a fused-silica preseparation column packed with deactivated fused-silica wool in the top 3 to 7 cm of the column (available from APEX). The ProSep Precolumn Temperature Module and Flow Module parameters that were successful for this particular preseparation column are given in Tables 3 and 4. This is a very flexible device, and the parameters given can be further optimized to provide better performance for particular extracted matrices. For example, a higher final precolumn temperature than 180°C can be applied to remove high-boiling contaminants.

Table 3. ProSep Precolumn Temperature Program

	Target	Duration
Initial	45°C	0.05 min
250°C / min	180°C #	6.00 min

Higher bake-out temperatures are recommended for extracted samples.

Table 4. ProSep Precolumn Mode Program

	Mode	Duration
Initial	Split	0.05 min
1	Splitless	0.07 min*
2	GC Split **	2.50 min*

*These times should be appropriately optimized.

** It is recommended that ProSep Split be implemented instead of simply GC Split due to superior venting.

Results

The application of PCI with ammonia reagent gas to NMDA produces a simplified mass spectrum consisting only of protonated NDMA, $[NDMA+H]^+$, and the ammonium adduct, $[NDMA+NH_4]^+$, which correspond to 75 m/z and 92 m/z, respectively. PCI provides a three-fold advantage over the EI approach. First, the relatively non-unique 74, 43, 42 m/z ions of the EI have been replaced by higher-mass ions. Second, PCI provides increased sensitivity for NDMA and a reduction in low-mass, "background" ions which enhances the signal-to-noise ratio. Third, by manipulating the ammonia gas flow, the abundances of the 92 m/z and 75 m/z ions can be controlled. As the ammonia flow into the source is increased, the abundance of the $[NDMA+NH_4]^+$ adduct also increases, allowing the ratio of 92 m/z to 75 m/z to be controlled by the analyst. For example, at 0.4 ml/min of ammonia—a relatively low flow setting of the reagent gas mass flow controller (8% of the total 5-ml/min provided by the controller)—the ratio of the protonated form to adduct is biased toward the protonated form: $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 4$ **:** 3. At higher flows, the situation reverses and $[NDMA+NH_4]^+$ predominates, e.g., at 0.9 ml/min ammonia (18% flow setting) $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 1$ **:** 5. It is therefore possible to produce an intense confirming ion for quantitative applications. A good compromise between signal intensities and ion abundancies was achieved at a 0.5 ml/min ammonia flow setting. Figure 3 shows the 75 m/z and 92 m/z SIM signals for a 40-fg/µL standard for this flow. Under these conditions, $[NDMA+H]^+$ is 79% of $[NDMA+NH_4]^+$ according to the integrated signal areas.

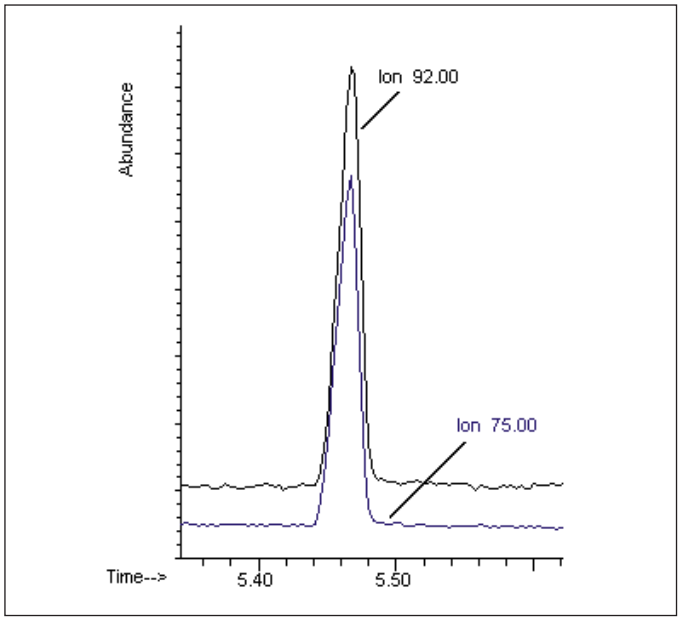

Figure 3. Extracted ion chromatogram for NDMA at 40-fg/µl using PCI-SIM with NH_3 reagent gas.

Figure 4 shows the results of a linear regression of the response of the 92 m/z ion for 50-µl injections of NDMA standards from 20-fg/µl to 4000-fg/µl. The regression fit was very good, $r^2 = 0.999$, considering the propagation of error in the dilutions. The relative standard deviation in the response factors was less than 6% and could be improved by using a perdeuterated or ^{15}N-labeled NDMA surrogate.

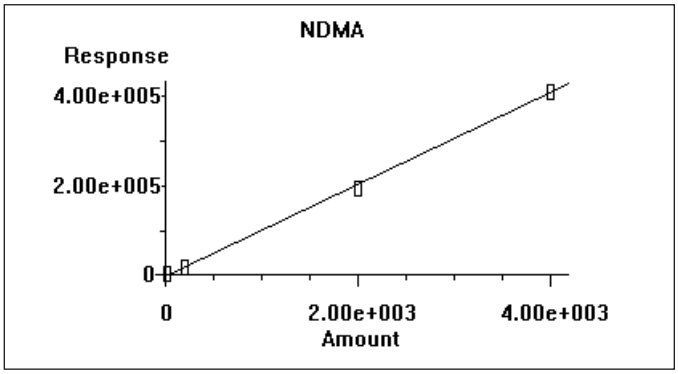

Figure 4. Linear regression of response of the 92 m/z ion versus NDMA concentration from 20-fg/µl to 4000 fg/µl, $r^2 = 0.999$.

Table 5 shows the excellent degree of reproducibility in the ratio of 75 m/z confirming ion to 92 m/z target ion over a wide range of concentrations. The absolute value of the ratio was 0.79, with a relative standard deviation of < 3%. This high precision is important to the degree of confidence in confirming and quantitating NDMA.

Table 5. Reproducibilities of the ratio of the integrated areas of 75 m/z : 92 m/z and the response of the 92 m/z target ion for 5 injections at 5 concentrations.

Concentration as fg NDMA / µl	RSD Ratio 75 mz / 92 m/z	RSD Response by 92 m/z area
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

Table 5 also shows the excellent reproducibility of the response of the 92 m/z ion for replicate 50-µl injections. Even at the 20-fg/µl concentration, precision is better than 3%.

Conclusions

Concentration and recovery factors for NDMA using present published methodologies suggest effective preconcentration of NDMA in samples to be on the order of 500, e.g., 60–70% recovery of NDMA in extraction of a 1-liter water sample. This implies that the low 20-fg/µl NDMA standard corresponds to a sample concentration of 40 pg/l, or 40 parts-per-quadrillion. Alternatively, to quantitate NDMA at 0.5 ppt in water, which is 4 times lower than the California DHS limit and slightly lower than the EPA regulated limit, quantitating at 20 fg/µl is equivalent to requiring the extraction of *only* 80 ml of water even if recoveries are still only 50%. Extracting small volumes presents a significant simplification of the process and offers savings in solvent and related materials, and in processing time.

With NDMA eluting in about 5 1/2 minutes, the analysis is fast, and the run-to-run cycle time is short—less than 13 minutes between injections. The method may be further optimized for even more rapid analysis.

The 5973 MSD provides very stable ratios for the confirming ion that can be optimized for quantitative purposes as described. In contrast to EI, in which many possible interfering fragment ions are possible that may distort the ratio of the target and confirming ion(s), PCI with ammonia is unlikely to cause fragmentation-induced interferences because of the relatively "gentle" nature of ammonia reagent gas. Interferences could occur involving compounds with molecular weights of 74 or 91 g/mole eluting at the same retention time but that is unlikely scenario.

The high degree of reproducibility in the injections, even at very low NDMA concentrations, demonstrates the robustness of large-volume injections using the APEX ProSep with the 6890/5973 MSD. It should be emphasized that the reproducibility of 2.4% for the replicate 50-µl injections of the 20-fg/µl standard reported here was for the *absolute* response. Use of an internal standard should further lower the deviation in response and improve quantitation.

Using this approach it should now be possible to satisfy the 2 ppt action level for NDMA set by the State of California and the 700 ppq regulatory standard promulgated by the U.S. EPA.

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Acknowledgments

Harry Prest is very grateful to Dr. William Steeber of the California Department of Health Services, Los Angeles, CA and John M. Hughes of the Gas Phase Chemists Council for their review of the manuscript.

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Printed in the U.S.A. September 1999
(23) 5968-7799E

Revision 1.0 - December 1999

Quality control of paint resins

Application

Heinz Goetz

Resins such as alkyd or acrylic resins are essential ingredients of paints. The rapid determination of the resin quality is of particular interest. The capability to respond quickly to quality control requirements increases productivity and therefore profit. This example shows the quality control analysis of two resins used for high quality paints in the car industry. One resin showed good adhesion properties while the other one failed. The poor quality resin failed because the high molecular weight fraction was not present (figure 1, hatched area).

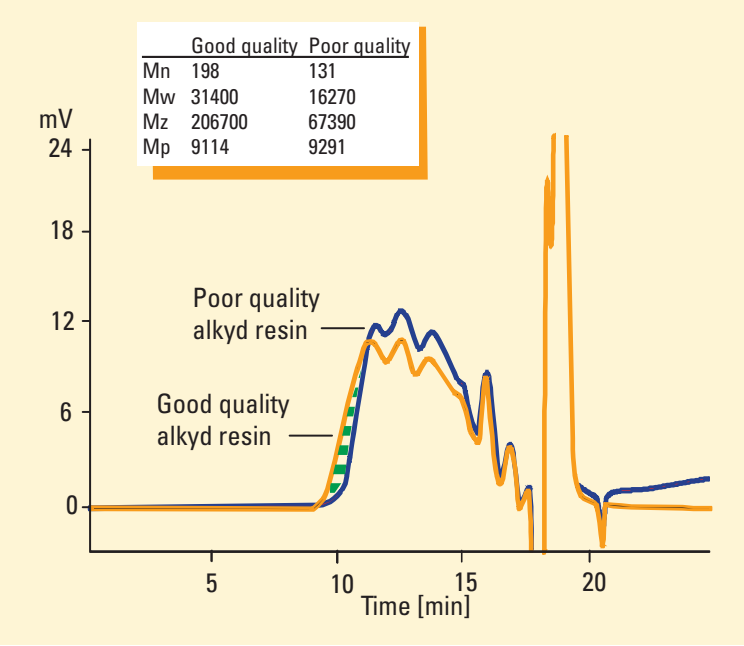

Figure 1
Quality control of two resins

Conditions

Sample preparation
Resins were dissolved in THF. Polystyrene EasyCal vial standards (Agilent p/n 5064-8281) were used for narrow standard calibration.

Column
PLgel 10^2 Å, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-501) in series with a PLgel 5 x 10^3, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-502) and a PLgel 10^4 Å, 7.5 x 300 mm, 5 µm (Agilent p/n 79911GP-504)

Mobile phase
Tetrahydrofuran

Flow rate
1.5 mL/min

Column compartment temperature
20 °C

Injection volume
100 µL

Detector
Refractive index detector

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The ChemStation GPC data analysis software provides the conventional graphical information of the chromatograms with the additional rapid numeric data in form of molecular weight averages and the report subsets. With the report subsets we could easily determine that the high molecular weight fraction (between the arrows) was 22 % for the high quality polymer but only 14 % for the low quality polymer.

HPLC performance

RSD of M_w < 1%
RSD of M_n < 2%

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

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Released 09/2000
Publication Number 5988-0114EN

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Analysis of polyvinyl alcohol

Application

Heinz Goetz

Polyvinyl alcohols (PVA) are industrially synthesized by the catalytic reaction of polyvinyl acetates with alcohols, typically methanol. Due to properties such as excellent biological degradeability, water solubility , toxilogical harmlessness they are widely used as emulgators, binding agents in adhesives, salves and haircream. The properties can be varied with the molecular weight distribution and the molecular weight which ranges from 20000 to 100000 g/mol. Both parameters can be fast and reliably monitored by aqueous SEC. This is a convenient method for quality control analyis, and is more informative in production control and end-use performance evaluation than single-point viscosity measurements.

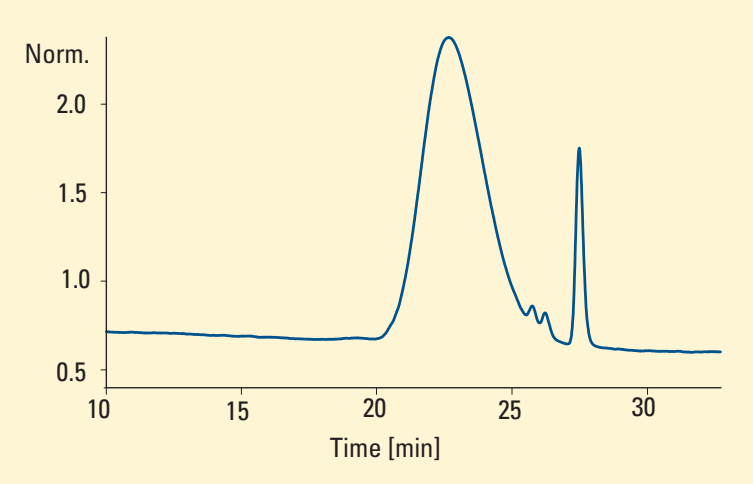

Figure 1
SEC chromatogram of polyvinyl alcohol

Conditions

Sample preparation
PVA was dissolved in the mobile phase (concentration 0.1 %)

Column
3 × PL aquagel-OH 30 in series, 7.5 × 300 mm, 8 µm (Agilent p/n 79911GF-MXA) in series with PL aquagel-OH 30, 7.5 × 300 mm, 8 µm (Agilent p/n 79911GF-083)

Mobile phase
0.2 M $NaNOH_3$, NaH_2PO_4, pH 7

Flow rate
1 mL/min

Column compartment temperature
25 ° C

Injection volume
100 µl

Detector
Refractive index detector

Polymer standards
Polyethylene oxide EasyCal standards in vials for calibration (Agilent p/n 5064-8280)

HPLC performance

RSD of M_w	< 1.5 %
RSD of M_n	< 3 %

Equipment

Agilent 1100 Series GPC-SEC system
consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

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Publication Number 5988-0123EN

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Creating and Using a Performance-Based API-LC/MSD Mass Spectral Library with NIST MS Search Software

Application

Liquid Chromatography/Mass Spectrometry

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Abstract

Currently, due to the different tune requirements, instrument parameters, and hardware differences, universal liquid chromatography/mass spectrometry libraries do not exist as they do for gas chromatography/mass spectrometry electron impact systems. However, using performance-based tune criteria for instruments, consistent and reproducible spectra can be obtained. This is the concept for creating universal searchable liquid chromatography/mass spectrometry libraries.

This application note shows step-by-step procedures for tuning the liquid chromatography/mass selective detector (LC/MSD) to performance-based criteria, for creating a spectral library, and for searching the library. A mass spectral library developed under the performance-based conditions can be used on any LC/MSD tuned to performance-based criteria.

Introduction

As liquid chromatography/mass spectrometry (LC/MS) becomes easier to use and readily available in analytical labs in all industries, the need for searchable mass spectral libraries for data interpretation becomes more apparent. Currently, due to differences in tune requirements, instrument parameters, and hardware, universal LC/MS libraries do not exist as they do for gas chromatography/mass spectrometry (GC/MS) electron impact (EI) systems. LC/MS libraries created from different vendor systems are not transferable. Libraries created from the same system may not be usable after system service.

Using performance-based conditions for different liquid chromatography/mass selective detector (LC/MSD) systems, consistent and reproducible spectra can be obtained with or without collision-induced-dissociation (CID). These spectra can be searched against a library created under the same performance-based criteria but perhaps from a different LC/MSD system [1].

The performance-based conditions are standard conditions that are defined by instrument performance (ion ratios of the tune compounds) rather than just specific instrument settings from Autotune. The instrument is first tuned to fixed ion

optic voltages and then set to the fragmentor voltage to meet the standardized ion-ratio criteria of the tune compounds. Such performance-based results should be comparable from one instrument to another, whether of the same type or from different manufacturers.

To search universal mass spectra libraries with high confirmation confidence, the performance-based conditions include low-, mid-, and high-fragmentation energies. The instrument must be set to certain ion ratios using the tune mixture for each energy level. The three energy levels yield varying degrees of fragmentation of the tune compounds or analytes. These fragmentor voltages are also the CID energies. Three CID libraries (low-, mid-, and high-energy) are created from analyzing standards at three different energy levels. The actual sample analysis thus involves the low-, mid-, and high-fragmentation energies (that is, three separate injections) to obtain the molecular weight and the structural information of the peaks of interest. The analyst can then search the low-, mid-, and high-energy libraries respectively to identify the peaks with confirmation from different energy-level libraries. It is not necessary to make three libraries. All the library entries can be put together in one library.

This application note talks about using the National Institute of Science and Technology (NIST) MS search software to create and use a performance-based mass spectral library.

Experimental

The Agilent ES Tune Mix (part no. G2421A) was chosen as the tune mixture. This mix includes a series of fluorinated compounds and is designed to fragment across a wide range of fragmentation voltages, providing a variety of masses to use at different energy levels. The tune mix was diluted 1:1 using a mixture of 50% acetonitrile and 50% water for flow injection analysis (FIA). FIA is an established process for fast, multiple analyses that bypasses the chromatographic column and injects the sample directly into the detector. The time between injections is usually 0.5–1 minute.

Procedure for tuning the 1100 LC/MSD to performance-based criteria

1. Autotune the 1100 LC/MSD (see Table 1).

Table 1. The Conditions Used to Tune 1100 LC/MSD to Designated Tune Criteria

1100 LC/MSD	G1946D (SL)
Mode	ESI Positive
FIA volume	5 µL
Flow rate	200 µL/min
Mobile phase	50%:50% water:acetonitrile
Scan range	50–800 Da
Gain	1.0
Threshold	150
Stepsize	0.1
Peak width	0.12 min
Drying gas temperature	350 °C
Drying gas flow	10 L/min
Nebulizer pressure	30 psi
Capillary voltage	3500 V
Fragmentor voltage	Varied

2. Prepare the tuning mix in a 2-mL sample vial by diluting the ES tuning mix 1:1 in mobile phase (1:1 water:acetonitrile) for FIA.
3. Run FIA series (from the Method menu) across all fragmentor ranges for the desired CID energy levels (see Figures 1 and 2). The voltage is stepped at 5- or 10-V increments over the range shown below. A portion of a typical FIA analysis while varying the fragmentor voltage from 50 to 400 V appears in Figure 3. The mass spectra should be stored in “Condensed” mode as noted in Figure 2.

1100 LC/MSD	G1946A and G1946B MSD	G1946C and G1946D MSD
Low energy	70–110 V	80–180 V
Mid energy	130–170 V	210–310 V
High energy	200–240 V	320–400 V

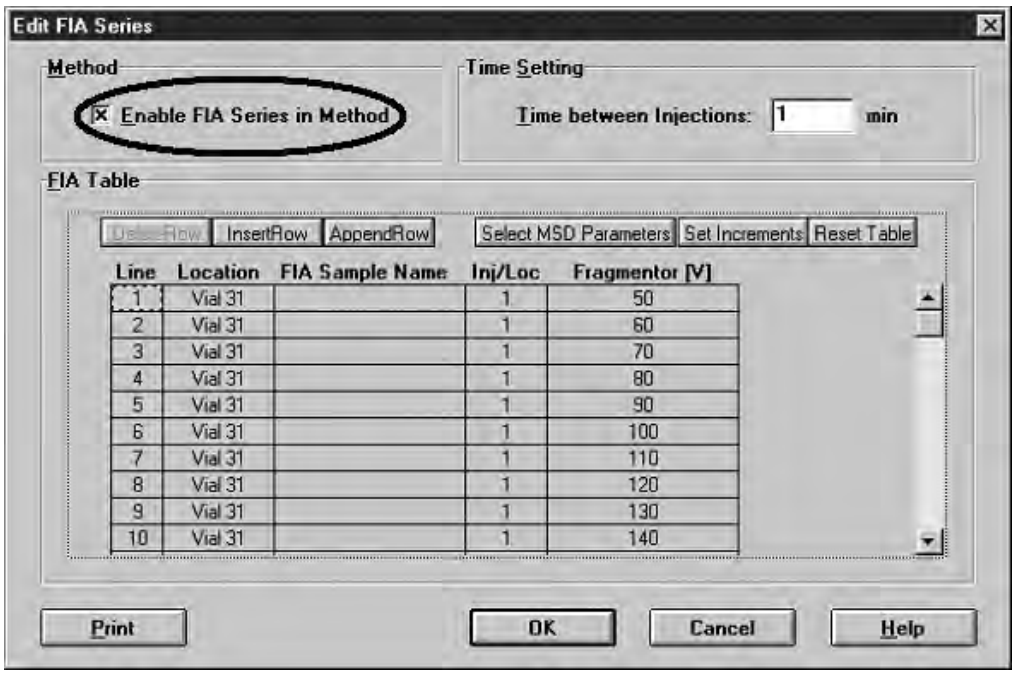

Figure 1. Creating an FIA series in a method.

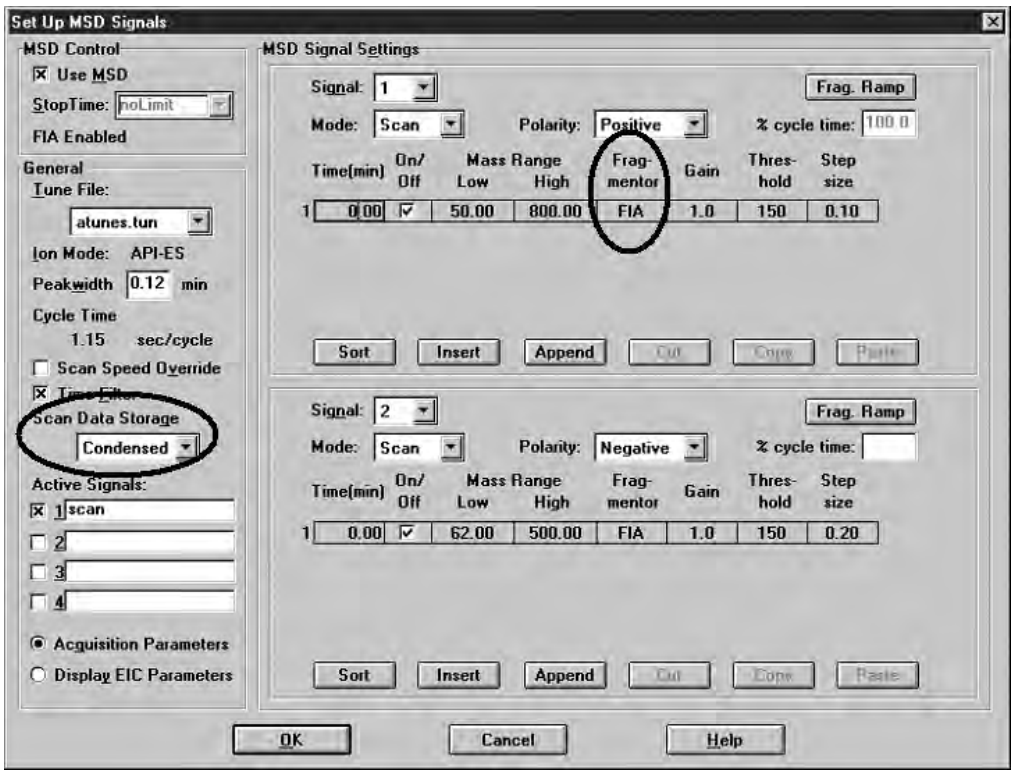

Figure 2. Set up MSD data acquisition parameters with the Fragmentor set to "FIA" and the data storage set to "Condensed".

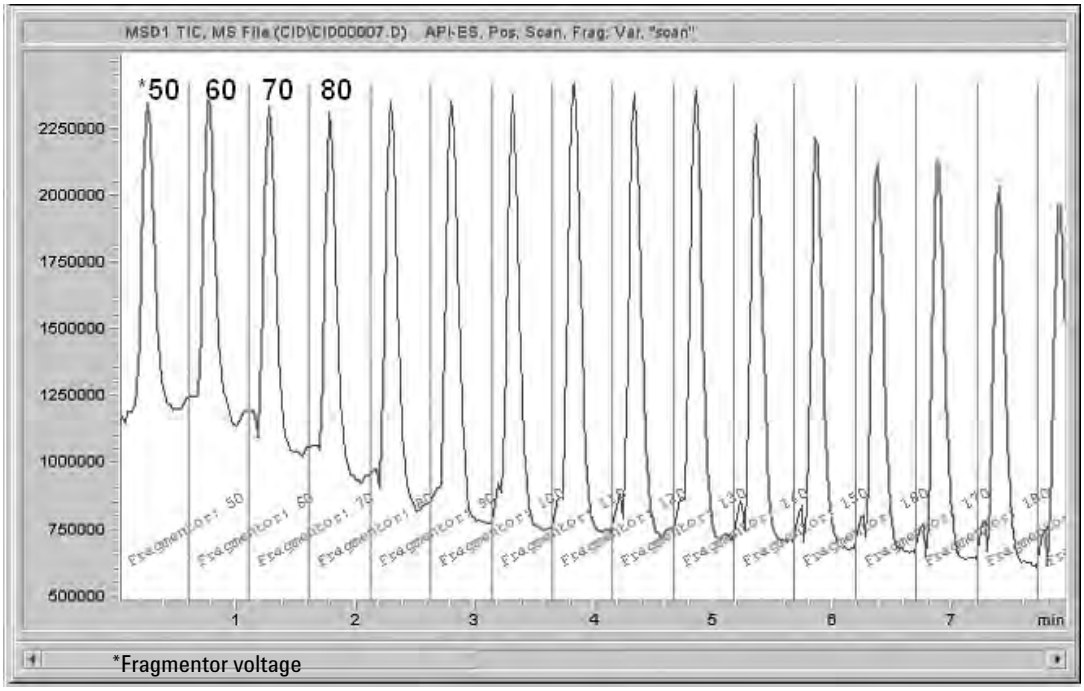

Figure 3. A portion of a typical FIA when varying the fragmentor voltage from 50 to 400 V.

4. Select a fragmentor voltage that best satisfies the criteria listed in Table 2 for each CID energy (low, mid, or high). Display the FIA in "Extract Ions" mode to help the voltage selection process.

Table 2 Performance-based Criteria for Generating CID Mass Spectra Library

Low-energy fragmentor range 70–110 V (or 80–180 V)	
Ion ratio	**Criterion**
290/586	50%–200%
322/118	<100%
622/118	100%–300%
Mid-energy fragmentor range 130–170 V (or 210–310 V)	
Ion ratio	**Criterion**
540/290	50%–200%
540/260	50%–200%
322/228	<100%
High-energy fragmentor range 200–240 V (or 320–400 V)	
Ion ratio	**Criterion**
790/622	50%–200%
678/622	<150%
540/678	<100%

5. Store the three fragmentor voltages that best meet the low-, mid-, and high-fragmentation criteria in three individual method files. These methods will be used to generate libraries or generate data to be searched in a previously generated library. Typically, variations in fragmentor voltages of 10 V result in little change in the match or search factor. These fragmentor voltages can also be used when the 1100 MSD is switched to electrospray ionization (ESI) negative ion detection.

Procedure for adding LC/MSD spectra into the NIST Mass Spectral Search Program [2, 3]

1. Make sure the NIST search program (G1043A) is installed on the LC/MSD ChemStation PC. If not, load the program onto the PC.
2. Start the LC/MSD ChemStation and Load an ultraviolet (UV) library (Spectra → Library → Open library...).

3. Figure 4 shows that two additional commands and icons are created to link the LC/MSD ChemStation to NIST MS search software.

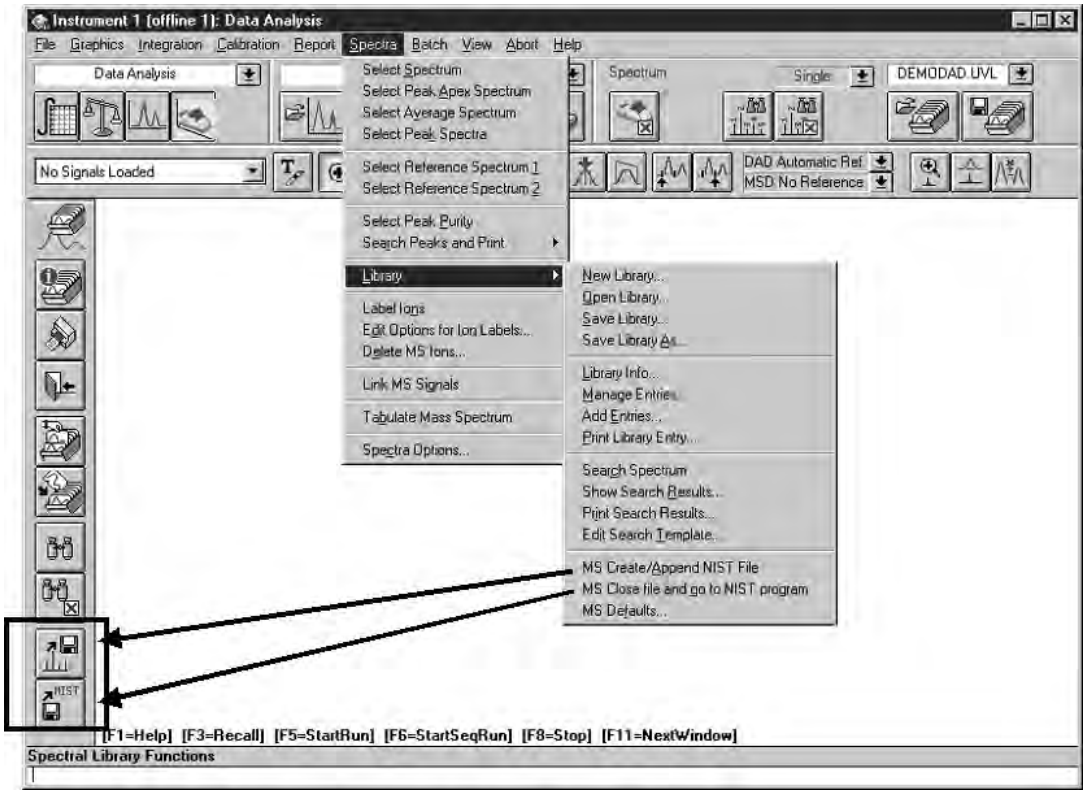

Figure 4. Two additional commands and icons are created to link the LC/MSD ChemStation to the NIST MS search software.

4. Select Spectra → Library → MS Default... to customize. Select "Append" to append (not overwrite) selected spectrum from the ChemStation to the clipboard (see Figure 5).

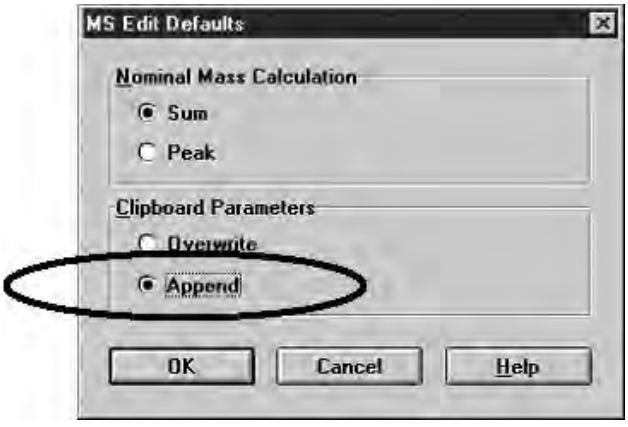

Figure 5. Select "Append" to append (not overwrite) selected spectrum from the ChemStation to the clipboard.

5. Analyze target compounds (standards) by LC/MSD using the three fragmentor voltages determined by the performance-based tune. Perform a quick check with a few standards to make sure the different CID energies can generate both molecular weight and fragmentation information.
6. Generate the apex spectrum of a peak and do a background subtraction to eliminate matrix interference, if any.
7. Verify there is no co-elution by comparing the spectra at the front, the apex, and the tail of the peak.

8. Press “Create/Append MS/NIST file” to convert the MS spectrum (of peak 1) into NIST format (see Figure 6).

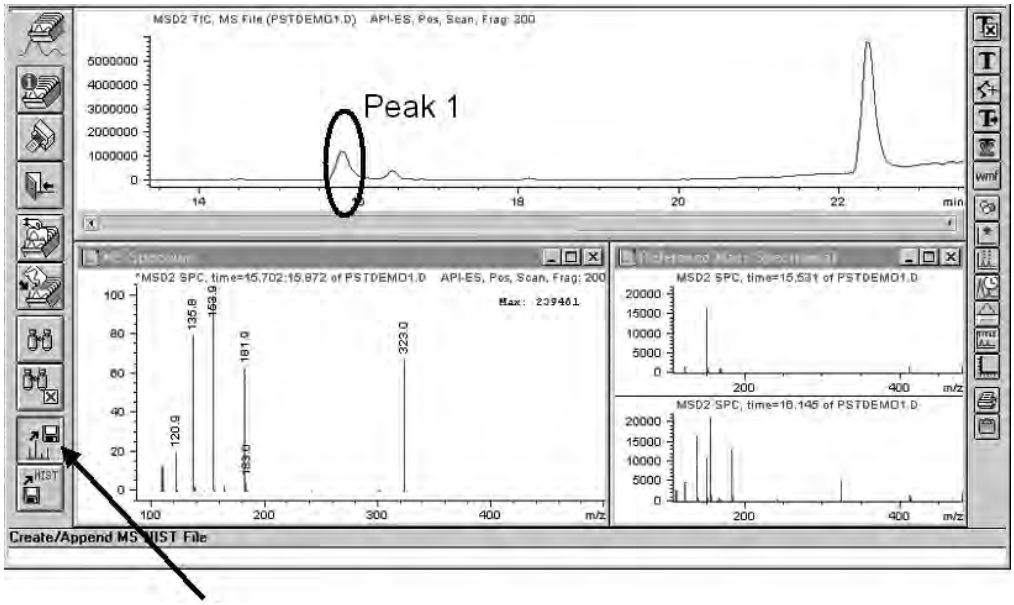

Figure 6. Press "Create/Append MS/NIST file" to convert the MS spectrum (of peak 1) into NIST format.

9. Continue steps 6, 7, and 8 to append other spectra into the clipboard.
10. When all the spectra (in this case, three spectra) are appended to the clipboard, press “Close MS NIST file and go to NIST program” to bring up the NIST program with the three spectra accumulated in the clipboard (see Figure 7).

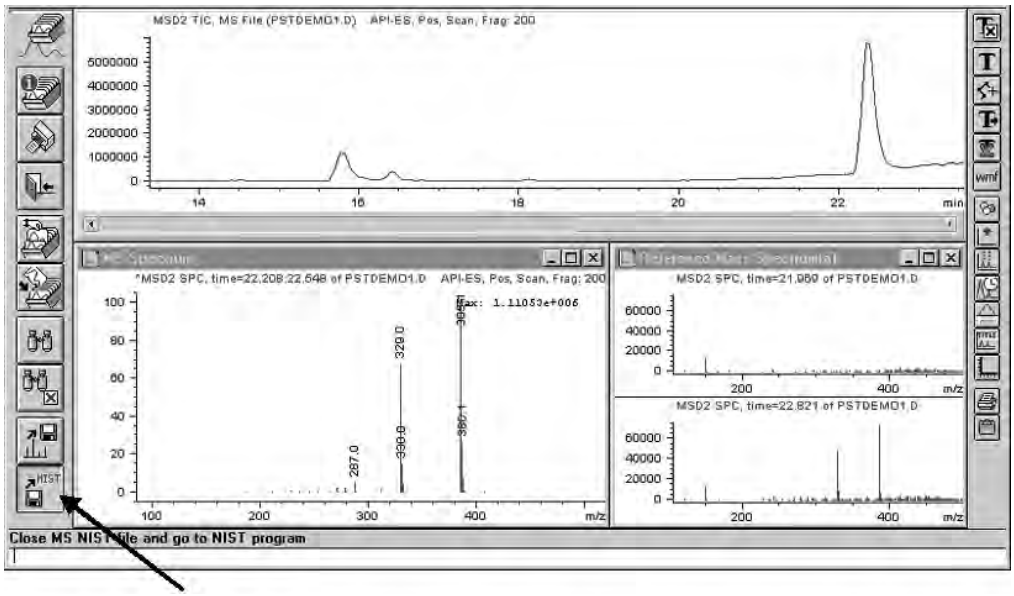

Figure 7. Press "Close MS NIST file and go to NIST program" to bring up the NIST program.

11. Figure 8 is the screen capture of the NIST MS search program with the three spectra from the LC/MSD ChemStation. Your screen may not look exactly the same as the figure.

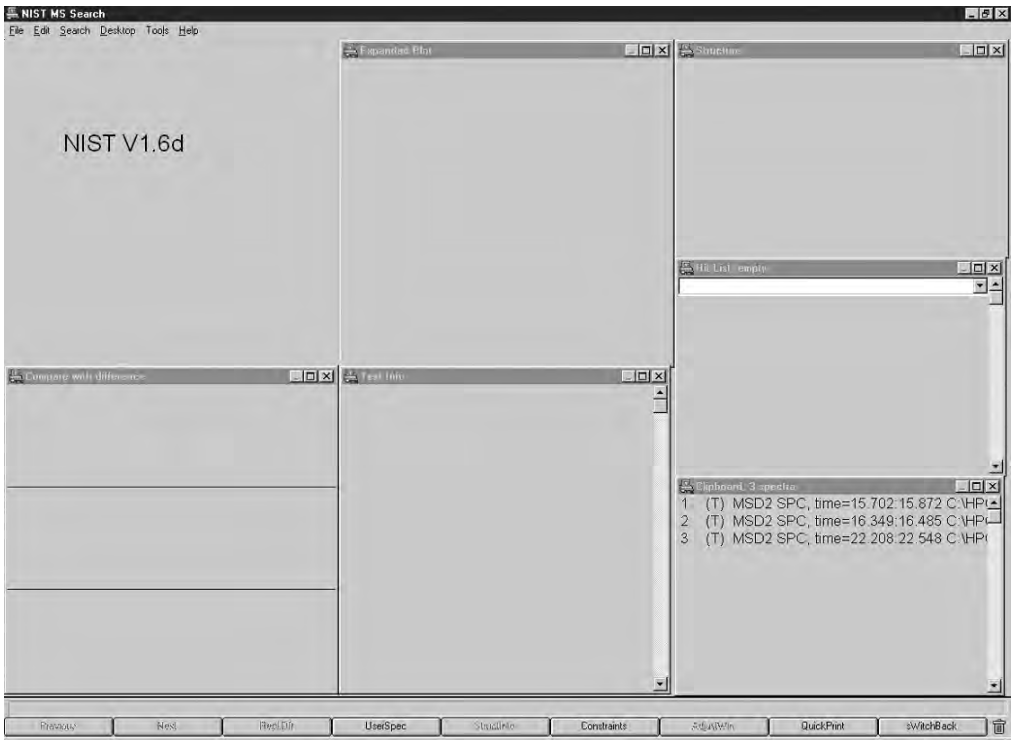

Figure 8. The screen capture of the NIST MS search program with the three spectra from the LC/MSD ChemStation.

Procedure for generating mass spectral library entries using the NIST Mass Spectral Search Program

1. (Continue from Figure 8) Select "Librarian..." command from the Tools menu.
2. As seen in Figure 9, all three entries from the clipboard appear in the “User Library Manager” window. Highlight the first entry, and press “Edit”.



Figure 9. All three entries in the clipboard are shown in the "User Library Manager" screen. Highlight a TextFile entry and press "Edit".

3. Enter the available information for each entry: Name, Chem. Formula, Mol. Weight, Comments, etc. Press “Structure” to attach a structure to the entry, if there is one (see Figure 10). After all the information and structure are entered, press “Save” to complete the edit of the first entry in the library.
4. Repeat the process for the other entries in the library manager.

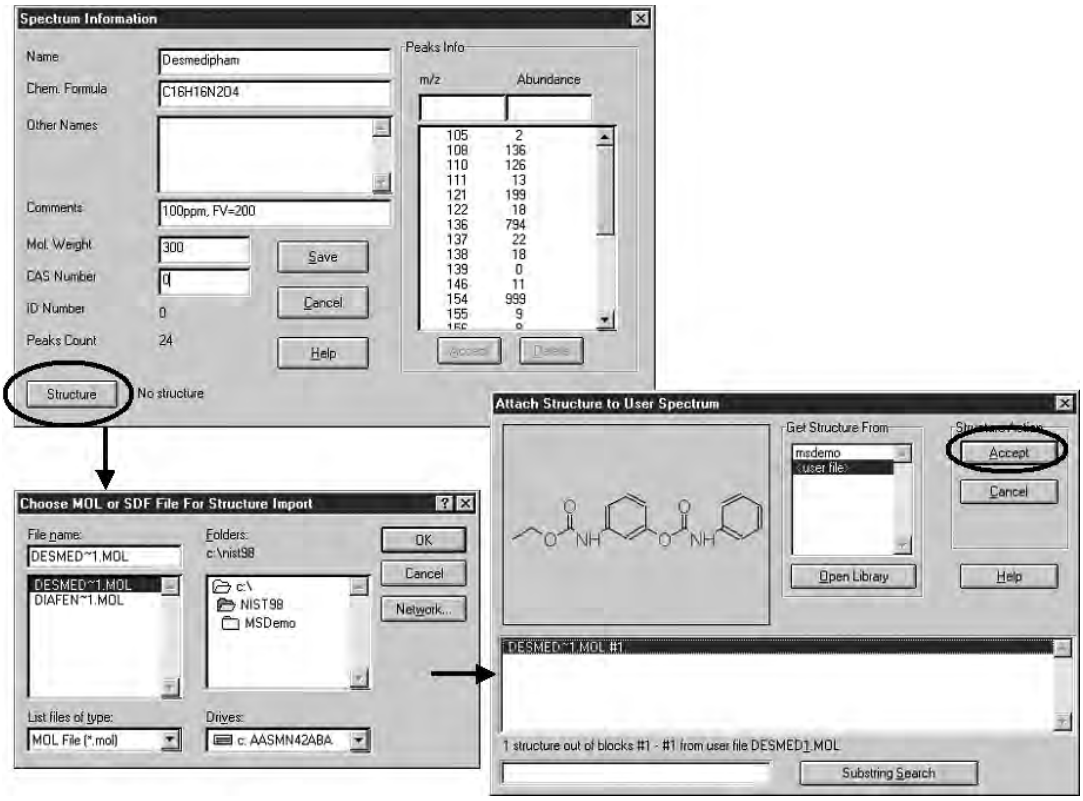

Figure 10. Enter the text and add a structure for the entry.

5. When all entries are updated with the compound name, chemical formula, molecular weight, molecular structure, and additional comments, press “SelAll” to highlight all the entries (see Figure 11).

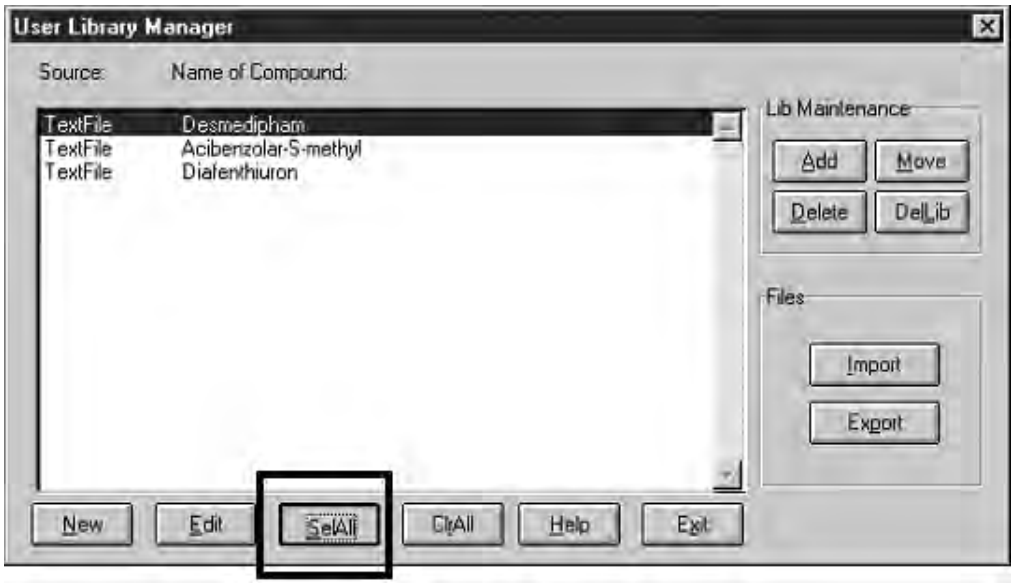

Figure 11. When all the entries are updated, press "SelAll" to highlight all the entries.

6. Press “Add” to add all the entries to an existing library or to a new library. In this example, all the entries were saved to a new library called pest200 (see Figure 12).

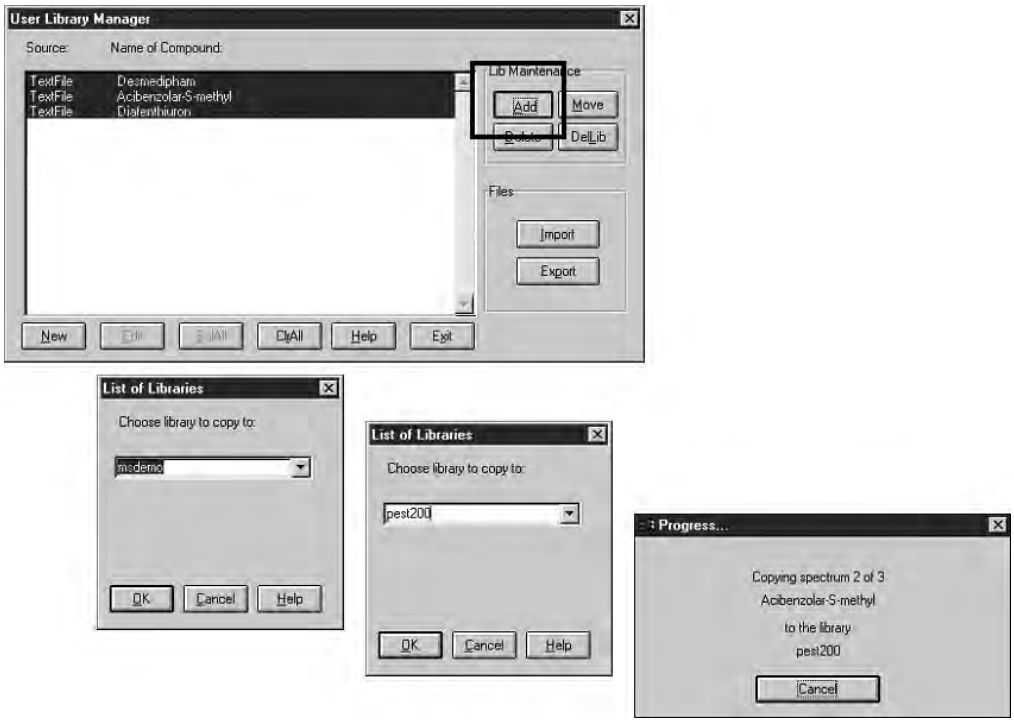

Figure 12. Press "Add" to add all the entries to an existing library or to a new library. In this example, all the entries were saved to a new library called pest200.

In general, store CID spectra in a CID library corresponding to each CID energy. For example Pest_low and Pest_mid are libraries for the low- and mid-CID energies. Storing the spectra in separate libraries for low-, mid-, and high-energies allows the relevant libraries to be searched in a sequential fashion. For example you can choose to search only the mid-energy library if you analyze the unknown using only the mid-CID energy. Of course, all the spectra from the different CID energies can be stored in one library for searching.

Procedure for Searching CID Mass Spectral Libraries Using the NIST Mass Spectral Search Program

1. Analyze unknown(s) using the three performance-based CID energies that you used to build the library. Best results are obtained if all three energies are used (three separate injections) and searched against a library (or three libraries) that contains mass spectra for the three energies.
2. Generate the apex spectrum of a peak and do a background subtraction to eliminate matrix interference, if any.

3. Verify there is no coelution by comparing the spectra at the front, the apex, and the tail of the peak.
4. Press “Create/Append MS/NIST file” to convert the MS spectrum into NIST format (see Figure 6).
5. Continue steps 2–4 to append other spectra to the clipboard.
6. When all the spectra are appended to the clipboard, press “Close MS NIST file and go to NIST program” to bring up the NIST MS search program with the spectra accumulated in the clipboard (see Figure 7).
7. In the NIST MS search program, press “UserSpec” to specify a library (see Figure 13).

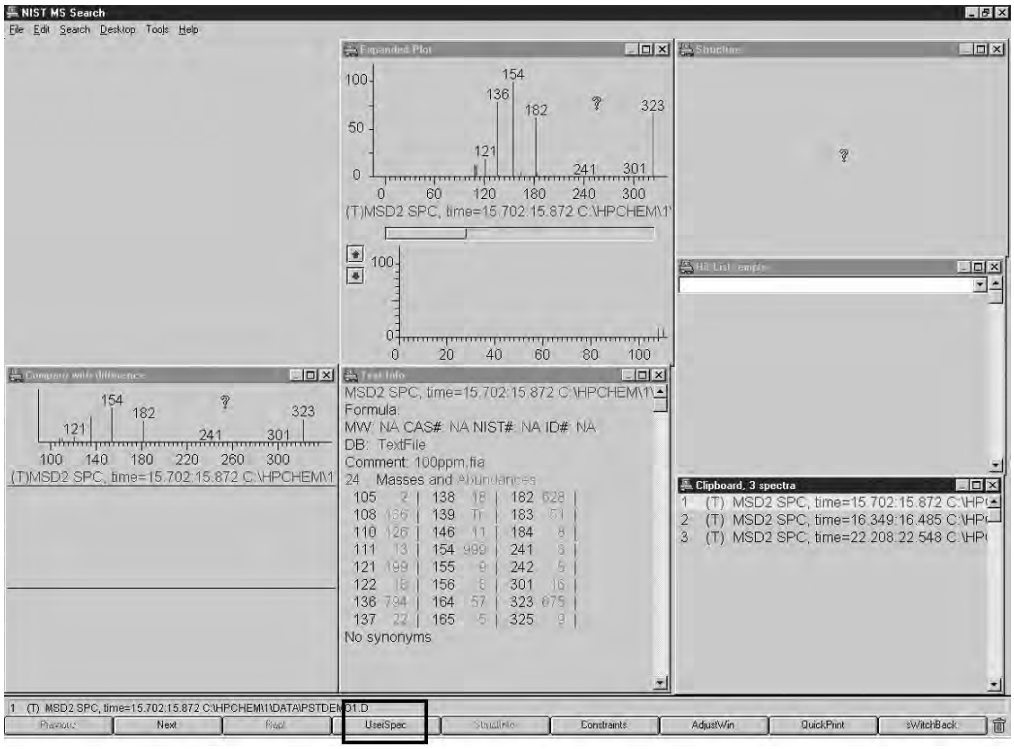

Figure 13. In the NIST MS search program, press "UserSpec" to specify a library.

8. Press “Libs” to add or remove libraries for searching. In this example, pest200 was added to the search list (see Figure 14). If there are more libraries to be searched, add them to the right side of the panel. Press “OK”.

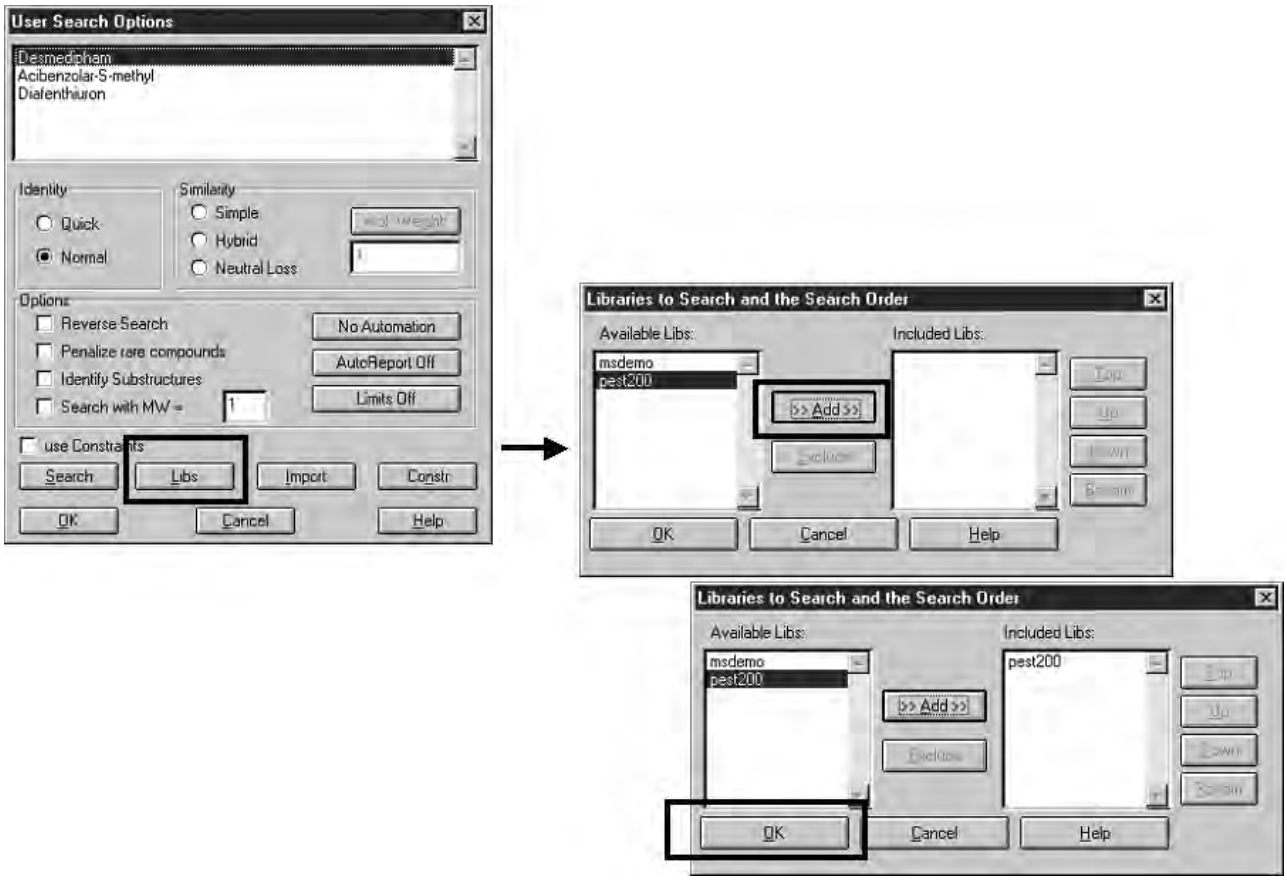

Figure 14. Press "Libs" to add or remove libraries for searching. In this example, pest200 was added to the search list.

9. Double-click on a clipboard entry to search against the pest200 library (see Figure 15). In general, a search factor (in the InLib window of the NIST program) greater than 800 is considered acceptable for identification.

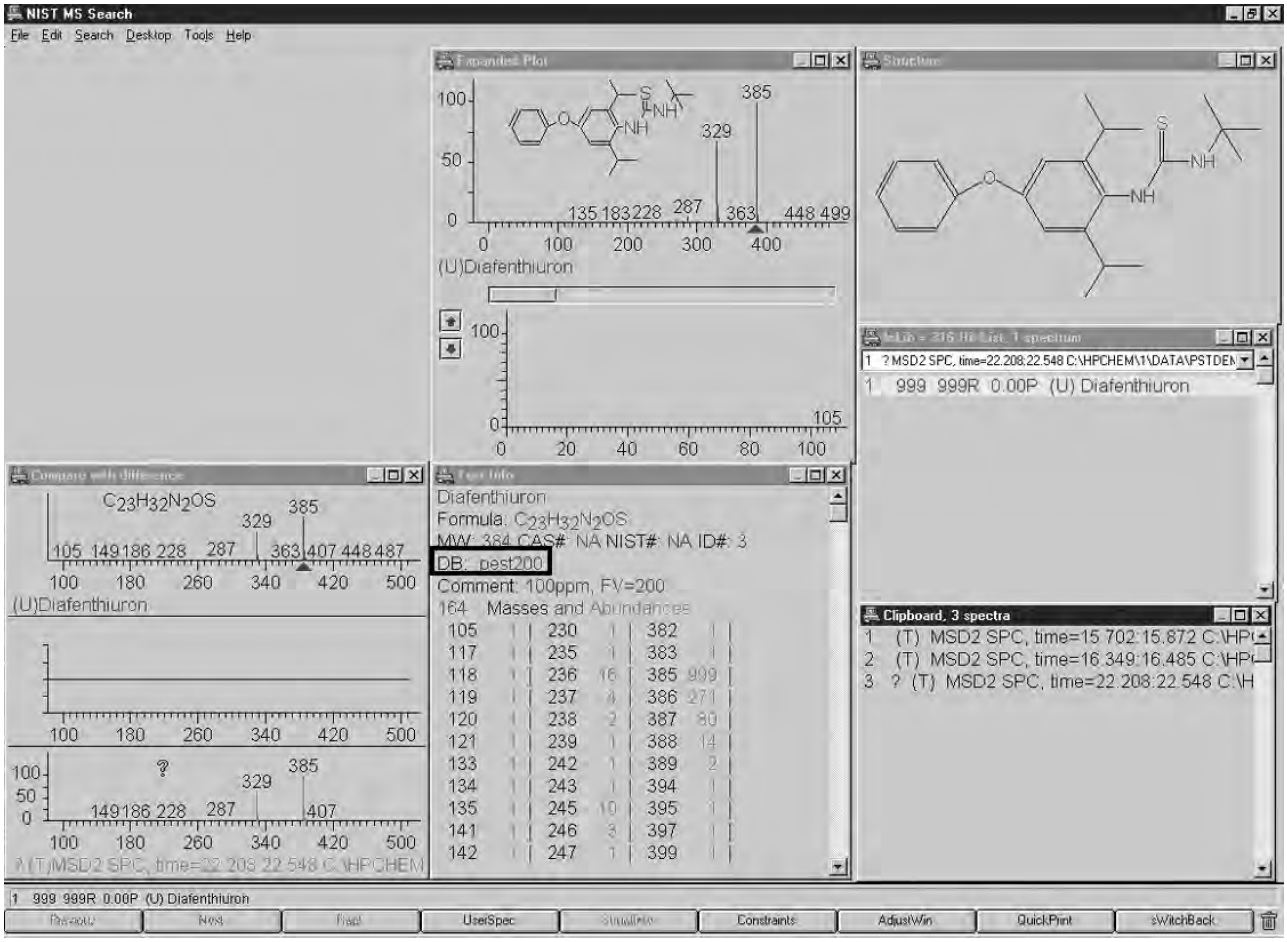

Figure 15. Double-click on a clipboard entry to search against the pest200 library. A reverse search factor greater than 800 is considered acceptable for identification.

Discussion

The solubility of the compounds in the ES Tune Mix solution may change over time. Therefore, a relatively fresh solution should be used for the performance-based tuning to achieve the most consistent results.

Sodiation will change the fragmentation pattern. Therefore, it is important to minimize and monitor the different sodium adducts in the solution and to analyze the samples using all three CID energies.

A pesticide library with over 200 compounds was built on a G1946A LC/MSD Quad system. The library was then copied to a G1946D LC/MSD Quad system. An analysis of some of the target compounds done on the G1946D LC/MSD was successfully searched against the library.

Conclusion

This application note gives step-by-step procedures to create and use a performance-based LC/MSD mass spectral library. The performance-based conditions are standard conditions defined by achieving a set of ion ratios of the tune mixture by adjusting the fragmentor voltage. The instrument is first tuned to fixed ion optic voltages and then set to the fragmentor voltage to meet the standardized ion-ratio criteria of the tune compounds. Therefore, the performance-based results should be comparable from one instrument to another, whether of the same type or from different manufacturers, as is currently true for GC/MS. A mass spectral library developed under the performance-based conditions can be used on any LC/MSD system tuned to performance-based criteria.

References

1. J. M. Hough, C. A. Haney, R. D. Voyksner, and R. Bereman, “Evaluation of Electrospray Transport CID for the Generation of Searchable Libraries,” Analytical Chemistry, 72, 2265-2270, 2000.
2. Sparkman and Agnew, “A New Dimension In Mass Spectral Library Searching,” Agilent Application Note, publication 5965-8424E, December 1999.
3. http://www.nist.gov/srd/nist1a.htm

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Printed in the U.S.A.
April 4, 2002
5988-5445EN



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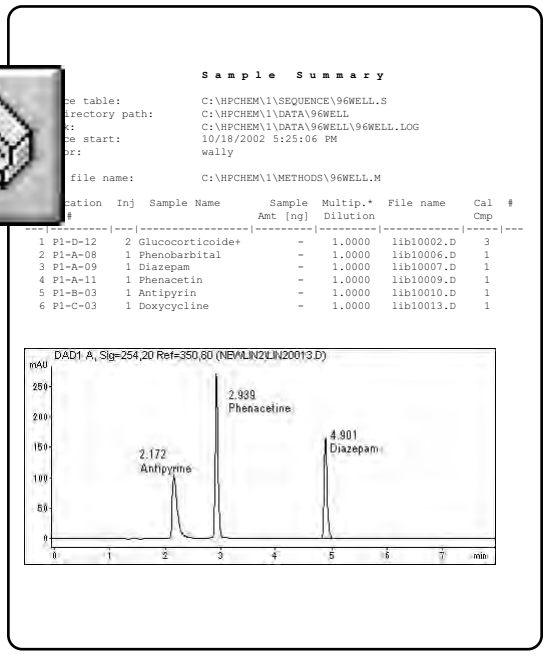
Using Agilent ChemStation to generate summary reports for a single analysis or a sequence of analyses

Application

Angelika Gratzfeld-Huesgen





Sample Summary

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t:	C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
ce start:	10/18/2002 5:25:06 PM
or:	wally
file name:	C:\HPCHEM\1\METHODS\96WELL.M

cation #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal # Cmp
1 P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3
2 P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1
3 P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1
4 P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1
5 P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1
6 P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1

Introduction

The Agilent ChemStation base software includes a wide range of built-in report styles and types. For example, it provides standard reports such as area percent (AREA%), external standard (ESTD), internal standard (ISTD), and normalized (NORM) reports as well as system suitability reports and sequence summary reports with statistical evaluation of retention times, areas, heights and more.

For each type of report the user can determine the amount of information that is included in the report. The ChemStation base software also provides a report editor for customizing reports — a topic that is beyond the scope of this note.

This Application Note describes how to set up the different report types, explaining the software screens and giving example reports. The main objective is to give guidelines and to provide strategies on how to use the different built-in reports in the ChemStation base software.

Agilent Technologies

Equipment

The data for the report examples was generated using an Agilent 1100 Series HPLC system comprising the following modules.

- high pressure gradient pump
- micro-vacuum degasser
- well plate sampler
- thermostatted column compartment
- diode array detector

The Agilent ChemStation base software including the 3D data evaluation module, revision A.08.04, was used for instrument control, data acquisition, data handling, sample tracking, and reporting.

Report setup on ChemStation

The standard reporting function in the ChemStation base software provides for single run reports or sample-set reports for a full sequence of runs, whereby these so-called sequence summary reports can only be generated after completion of the sequence. The content of the sequence summary reports is defined by the acquisition sequence.

Further, the ChemStation base software includes a wide range of built-in standard reports that allow users to define the content and amount of printed information. Whereas this functionality meets the requirements of most standard applications to a large extent, it does not have the flexibility to create additional table elements for non-chromatographic information, charts or custom calculations.

If such extended reporting capabilities are required, it is recommended to use the ChemStation Plus data system including the ChemStore data organization module.

The ChemStation base software offers four types of report.

- Individual run reports, which can be generated automatically after each run or sequence, provide quick and easy printouts of results.

- Sequence summary reports provide comprehensive infomation for a full set of samples, including full GLP/GMP details. They are generated automatically at the end of a sequence and may include individual reports as well as statistical summary reports.

- Batch reports provide direct printouts of first-pass review modifications and results. They are generated during reprocessing of data from a complete sequence or of a subset of one sequence using ChemStation batch review.

- Advanced custom reports for requirements that go beyond the scope of the previous types. These include customized reports for individual runs or complete sequences and can also be obtained automatically after each run or sequence.

The following sections focus on the individual-run and sequence-summary report types, which are built-in as standard in the ChemStation base software, and explain in detail how to use and set up these report types.

Qualitative reports for individual runs

Qualitative reports are used mainly during the development of a separation or when a quick decision is needed as to whether a compound is present or not. Here the separation of peaks is of primary interest and a short AREA% report is sufficient. Particularly during method development it does not make sense to obtain reports with quantitative results.

Setup

To obtain an automated printout of an individual report such as a short AREA% report, the item *Standard Data Analysis* must be selected in the *Run Time Checklist*, which is part of the overall method for acquisition, data analysis and reporting, see figure 1. This screen is part of the *Edit Entire Method* dialog or can be accessed directly from the *Method* menu of the *Method and Run Control* view.

The item shown in figure 1 must be selected when the calculation of results is required, such as for printing reports, including sequence summary reports, with or without individual run reports.

Configuration

To obtain qualitative reports the item *Calculate* in the group *Quantitative Results* must be set to *Percent* as shown in figure 2.

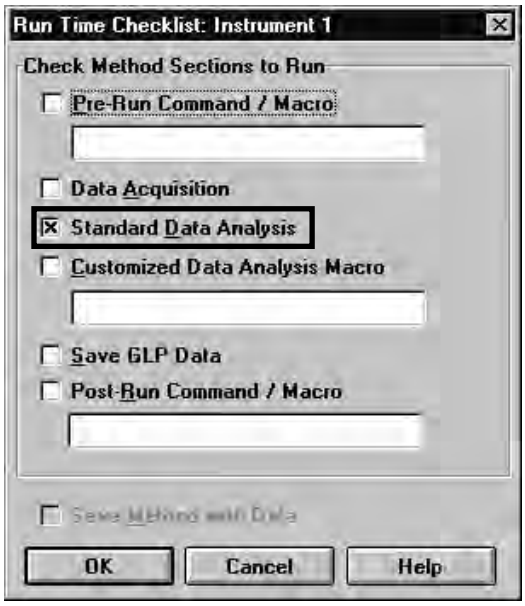


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

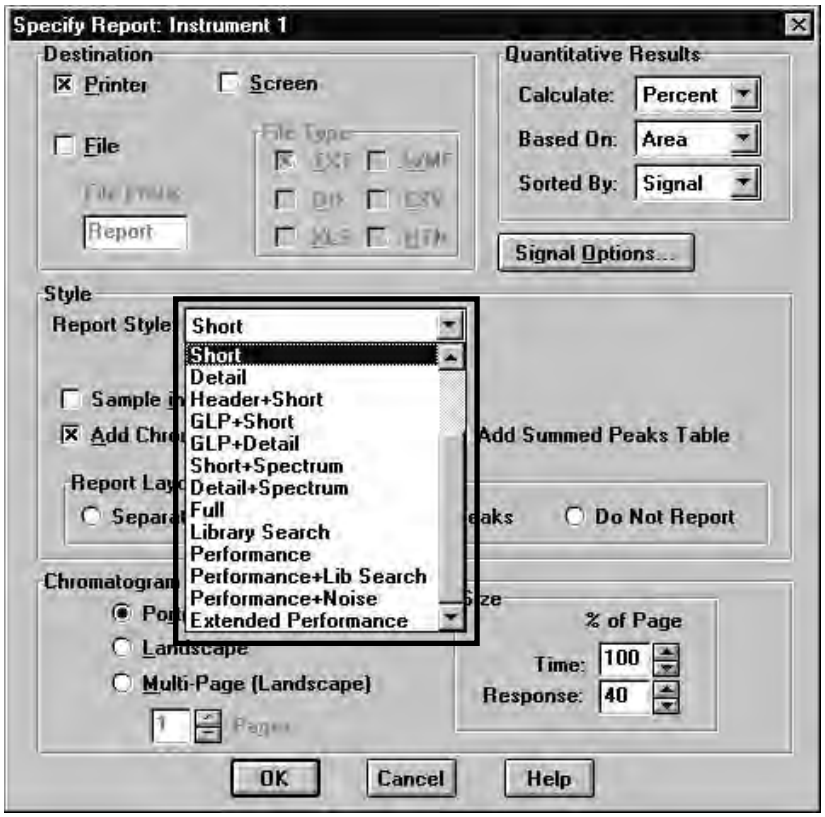


Figure 2
Specifying individual run reports

There are three ways to set up reports for individual runs.

1 Using the report smart icon in the *Method and Run Control* view.
2 Using part of the *Edit Entire Method* wizard
3 Using the *Data Analysis* view by selecting *Report* and then *Specify Report*.

Figure 2 shows the setup screen for run reports. Several report styles are available, covering a broad spectrum of report types. The report output can be sent to a printer, displayed on the screen or saved to a file. Multiple report destinations can be selected at a time. Other report parameters allow to include chromatograms, in landscape or portrait format or even distributed over several pages, and to define the way unknown compounds are reported.

An example of an AREA% report is given on page 12, containing information about the used method, data filename, time of injection, chromatogram and report.

The report styles that are available depend on the installed software modules. For example, the report styles Short+Spectrum, Detail+Spectrum and Library Serach are only available when the 3D data evaluation module is installed.

During method development the combination of *Percent* and *Performance* in reporting can be a valid tool to find out about k', resolution, selectivity, peak width and, for isocratic runs, the number of plates. An example is given on page 19.

Calculation procedures such as **Percent** (for others such as ESTD and ISTD, see section "Quantitative reports for individual runs") can be combined with any of the available standard reports shown in figure 2.

Qualitative reports can not use calculations based on standards such as ESTD and ISTD.

Quantitative reports for individual runs

Quantitative reports offer compound identification and compound quantification. They are mainly used with known samples or reference results in method optimization and quality control areas.

Setup

Before a quantitative report can be generated, standard samples with known compound concentrations have to be run and a calibration table has to be set up.

Peak integration should always be optimized before a peak is used as a reference in the calibration table and before the calibration tasks are done. To optimize integration, load a sample file with known sample concentration and then use the *Integration* tool set in the *Data Analysis* screen. When integration is optimized and saved, the calibration table can be created.

The calibration table is set up in *Data Analysis* from the *Calibration* menu, see figure 3.

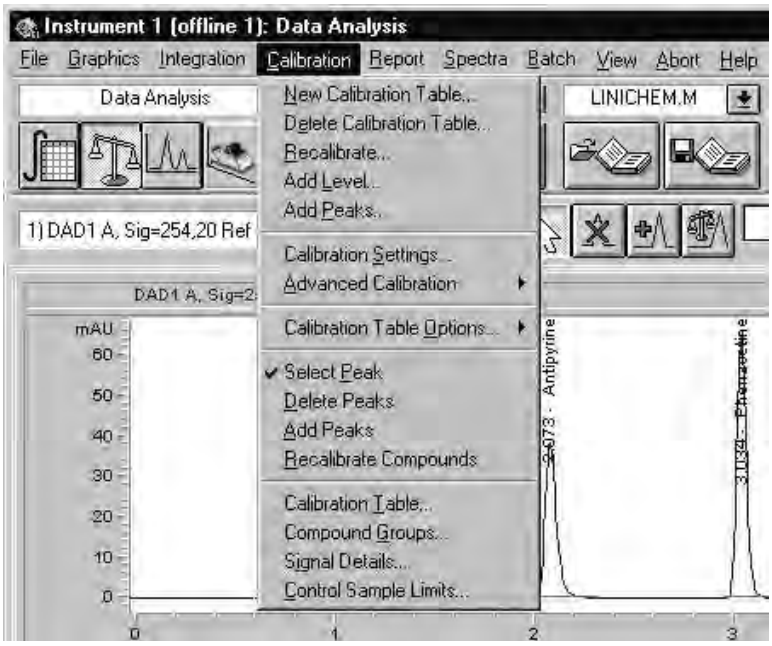


Figure 3
Calibration setup menu

In the following example we set up a multilevel calibration with four calibration levels. Multilevel calibrations use multiple files to complete the calibration. One file defines one level—completion of a four-level calibration thus requires four files. The steps involved are as follows.

1 Load the first file and click on *New Calibration Table*.

2 Calibrate each peak by selecting the peak (left mouse click), and filling in compound name and compound amount.

3 Repeat step 2 for all peaks.

4 When all peaks in the file are calibrated, load the next file with the next concentration. Use the *Add Level* tool to fill in the amounts for the next concentration level (level two).

5 Repeat step 4 for level three and four.

The calibration is stored as part of the ChemStation method. It is saved by simply saving the method. Every calibration update is easily accessible by loading the method, modifying (for example, updating) the calibration files and saving the new method revision.

Setup

When the calibration is complete all prerequisites for generating a quantitative report are met. The first step in generating a report is to specify the report style as described in the section "Qualitative reports for individual runs." The calibration of the method now offers access to all predefined report styles such as standards reports or normalized reports or, when running a sequence, to sequence summary reports (see separate section later.)

The calculation of results can be a normalized (NORM) area determination or based on an external standard (ESTD) or internal standard (ISTD). Result calculations can be based on area or height. Figure 4 shows selection of *External Standard Method* as calculation procedure and *Short* as *Report Style*. An example is given on page 13.

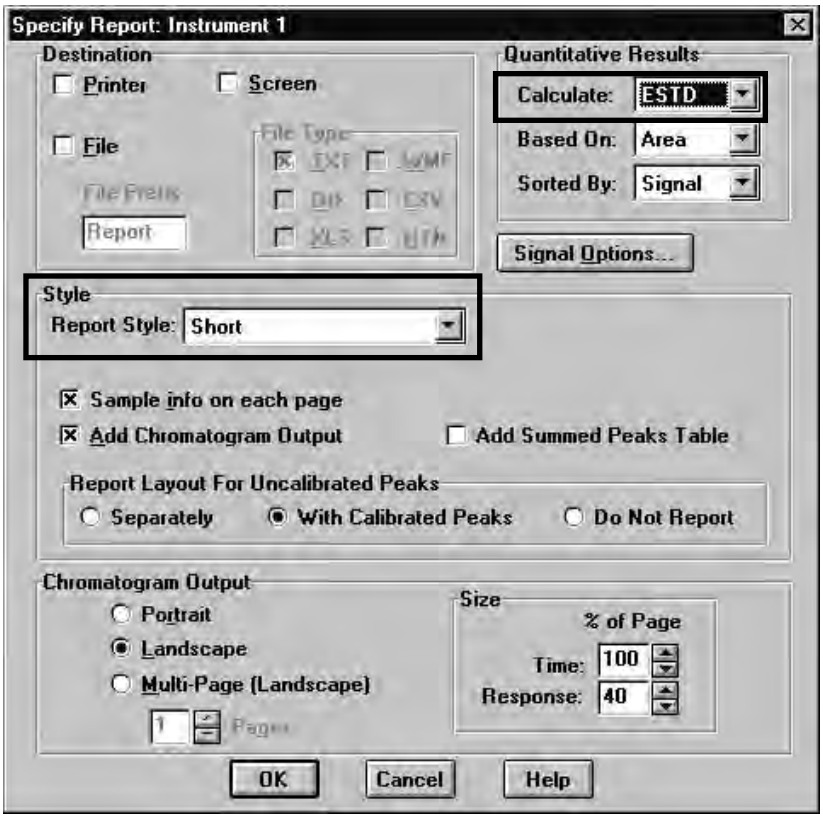


Figure 4
Selection of external standard report and short report style

Configuration

Additional report features can be specified such as output format for the chromatogram (including multipage outputs), picture size and the documentation of uncalibrated (which means unknown) peaks in the *Specify Report* screen as shown in figure 4. Any report style (see figure 2) can also be combined with any calculation procedure. Examples are given on pages 13 through 21.

- ESTD combined with report style *Short* (p 13)
- ESTD combined with report style *Library Search* (p 14)
- ESTD combined with report style *GLP+Short* (p 16)
- ESTD combined with report style *Performance* (p 19)
- ESTD combined with report style *Detail* (p 20)

Similar to the calibration, the report configuration is saved with the ChemStation method. Thus all data analysis steps for integration, calibration, result calculation and reporting are saved under one "umbrella" tool. Once setup, reuse of all steps is automated by simply reapplying the method to any sample under investigation.

The method that has been set up for data acquisition, integration, calibration and reporting has to be saved under a unique name to ensure that samples are analyzed and evaluated using the correct conditions.

Final report output

Final report outputs are quick and easy to obtain with ChemStation. Both qualitative and quantitative reports offer the same options and use identical tools to generate the final report.

Reports can be
- sent to a printer
- displayed on the screen for a quick review or preview when setting up report options
- saved to a file in HTML, CSV, XML, TXT, WMF, or DIF format

It is possible to combine all output types, for example, to get a printed copy on paper, an online report display on the screen and a file copy on the local hard disk.

The user can choose either
- automated report output at the end of each sample analysis (or reanalysis), or
- interactive report output at user request



Figure 5
Run method for automated method execution and result output

Automated report output

An automated report is output whenever the ChemStation method is executed and at least one report destination is selected in the *Specify Report* screen, see figure 4. If no report output is desired, simply leave all report destination check boxes blank.

Method execution typically is used to analyze a sample or to reapply changes in calculations or calibration during data analysis. To execute a method, simply press *F5* or select *Run method* from the ChemStation *Run control* menu as shown in figure 5.

If the user wants to re-analyze data without data acquisition, *Data Acquisition* must be disabled in the *Run Time Checklist*, see figure 1.

Interactive report printout

Manual report output is available from the ChemStation *Data Analysis* view. It is designed to preview report outputs on the screen during report configuration or to get an individual sample report during interactive result analysis or result review.

The *Data Analysis* view is designed to set up advanced reports such as library searches, detailed spectrum reports and others. It has a separate report menu and additional smart icons for report setup, preview and output to a printer as shown in figure 6.

When the user wants a report during their data review session, they simply press the preview or print button and immediately get the report on the screen or on paper.



Figure 6
Report menu and smart icons (far right) in ChemStation Data Analysis view

Sequence summary reports

In contrast to individual run reports, sequence summary reports can only be generated for a complete set of samples that have been analyzed in one continuos sequence. The sequence summary report (also referred to as a system suitability report) is designed to meet the specific needs of GLP and GMP regulations in the pharmaceutical industry as well as comparable ISO and DIN regulations in other industries.

In addition to result calculation and result documentation, all regulations require additional documentation on how the results have been obtained and how "well" the analytical system behaved during analysis. The sequence summary report is a single all-inclusive report style, combining the analytical result with full documentation of how the result was obtained and the system suitability information, thereby providing a comprehensive report that addresses all regulatory requirements.

Sequence summary reports are frequently used in quality control work. These reports include the analytical results along with documented evidence of the system's suitability for the analytical purpose. System suitability is defined in the various Pharmacopoeia guidelines and it typically includes system performance information based on parameters such as peak width, theoretical plate number, resolution and others.

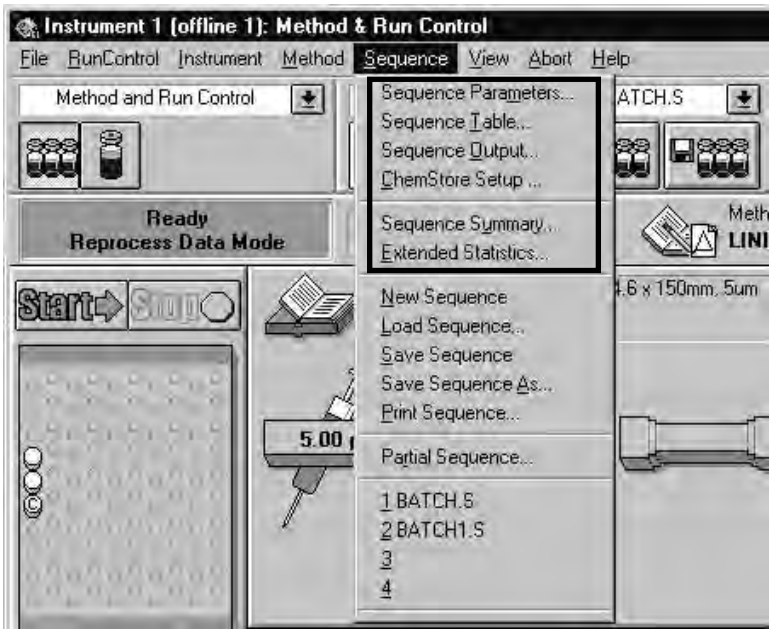


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

All these parameters are available in the report style, but the user must configure the report to suit their own specific needs. The following section describes setup and configuration of a sequence summary report in ChemStation.

Setup and configuration

After each sequence of runs a sequence summary report can be printed. Typically this is done to obtain statistical results and determine system suitability. In addition to the entries in the sequence table and before the report can be calculated and printed, several data inputs for sequence parameter and sequence output are required, see figure 7.

In the *Sequence Parameters* screen (figure 8) the item *Parts of Method to Run* must be set to *According to Runtime Checklist*. This entry determines which part of a method is executed during a sequence and *According to Runtime Checklist* refers to the run-time checklist configuration that was previously edited as part of the method in order to obtain integration and quantitative results.

If data acquisition is completed and the user wants to reanalyze a sequence of samples without data acquisition, the option *Reprocessing Only* allows to recalculate the sequence summary report easily.

In the *Sequence Output* screen the report destination and the content of a sequence summary report are defined by selecting the appropriate check boxes, see figure 9.

The content of the sequence summary report is defined by the items on the right side of the scrreen shown in figure 9. Selecting *Setup* in the *Sequence Output* dialog box accesses this configuration screen. The sequence summary report allows a variety of informations to be printed in one continuously enumerated report.

In addition to a wide selection of statistical results from sample and/or calibration runs, other items can be selected such as sample summary reports that list all acquired samples, com-



Figure 8
Sequence parameters screen

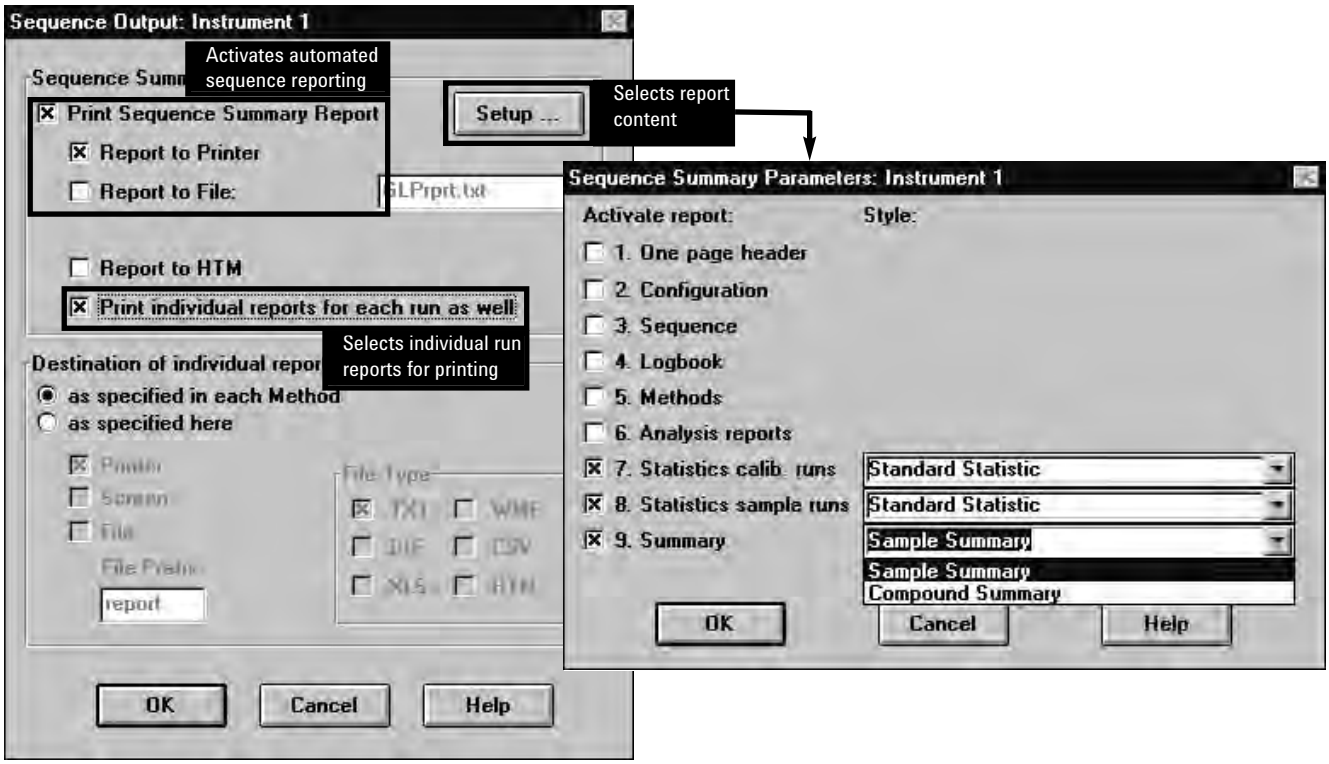


Figure 9
Selection of report destination and content of a sequence summary report

plete printouts of all parameters in the methods that were used, printouts of sequence logbooks and so on.

It is also possible to include the individual result reports for each run as part of the summary report instead of individual printouts after the end of each run.

The statistical evaluation of sequence runs is defined in the *Extended Statistic Parameter* screen, see figure 10. Statistical results can be obtained for all parameter shown in this dialog box. Either standard deviation or relative standard deviation or 95% confidence interval can be applied and upper/lower limits for each parameter can be specified.

A calibrated method is necessary to be able obtain statistical results.

Figure 11 shows the *Sequence Table* screen, in which it is important to ensure that the sample type is correctly set to *Sample*, *Calibration* or *Control Sample*, because statistical calculations can be selected based on sample type.

Figure 12 shows an example of a sequence summary report. It contains information about the analyzed samples such as location, sample name, filename, and so on. The header includes information such as operator name, the used chromatographic method, and date of acquisition.

Further report examples can be found on pages 11 through 35.

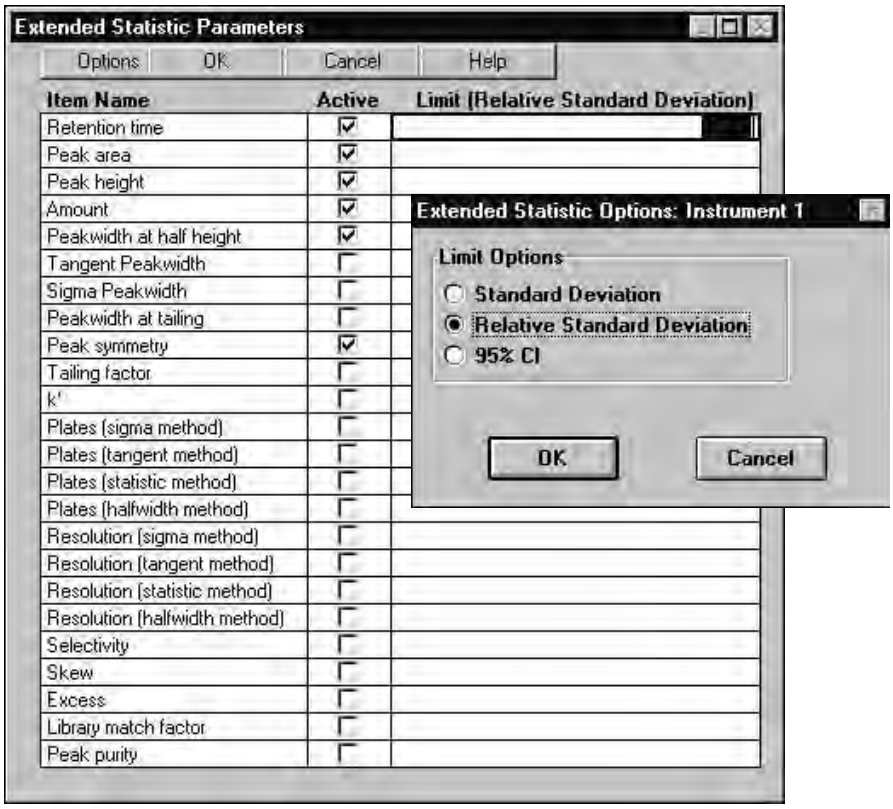


Figure 10
Setup of statistical calculations for sequence runs

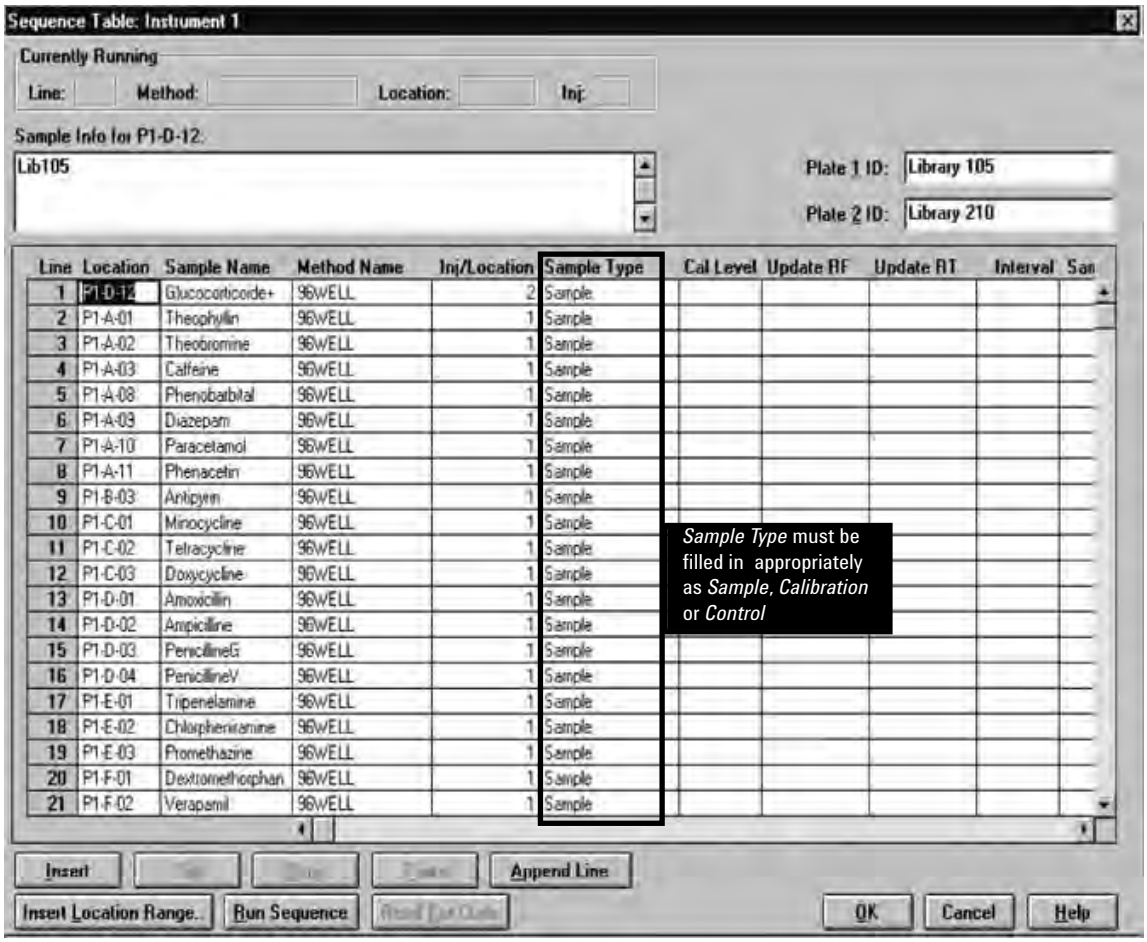


Figure 11
The Sequence Table screen

```
S a m p l e   S u m m a r y

Sequence table:          C:\HPCHEM\1\SEQUENCE\96WELL.S
Data directory path:     C:\HPCHEM\1\DATA\96WELL
Logbook:                 C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
Sequence start:          10/18/2002 5:25:06 PM
Operator:                agratz

Method file name:        C:\HPCHEM\1\METHODS\96WELL.M

Run Location Inj Sample Name       Sample    Multip.* File name    Cal #
 #     #                              Amt [ng]  Dilution               Cmp
---|---------|---|----------------|---------|---------|------------|-----|---
  1 P1-D-12    2 Glucocorticoide+     -      1.0000   lib10002.D    3
  2 P1-A-08    1 Phenobarbital        -      1.0000   lib10006.D    1
  3 P1-A-09    1 Diazepam             -      1.0000   lib10007.D    1
  4 P1-A-11    1 Phenacetin           -      1.0000   lib10009.D    1
  5 P1-B-03    1 Antipyrin            -      1.0000   lib10010.D    1
  6 P1-C-03    1 Doxycycline          -      1.0000   lib10013.D    1
```

Figure 12
Example of a sequence sample summary report

Conclusion

The built-in single-run and sequences summary reports that are available in the ChemStation base software offer a wide range of reporting capabilities. The various reports give access to all important sample-related information quickly and easily. For all report types the user can select the amount of information to be included, from a simple qualitative report on one page through detailed quantitative reports to comprehensive and powerful sequence summary reports. Knowledge of a report editor is not required to be able to set up the ChemStation reports.

Reports can be obtained after each run or at the end of a sequence. With the ChemStation Method concept users starting from scratch can have a printed result copy of any type in less than 10 minutes – once set up the report is available within seconds after run completion. ChemStation reports are easy to configure, fast to obtain and quickly stored and managed.

Appendix

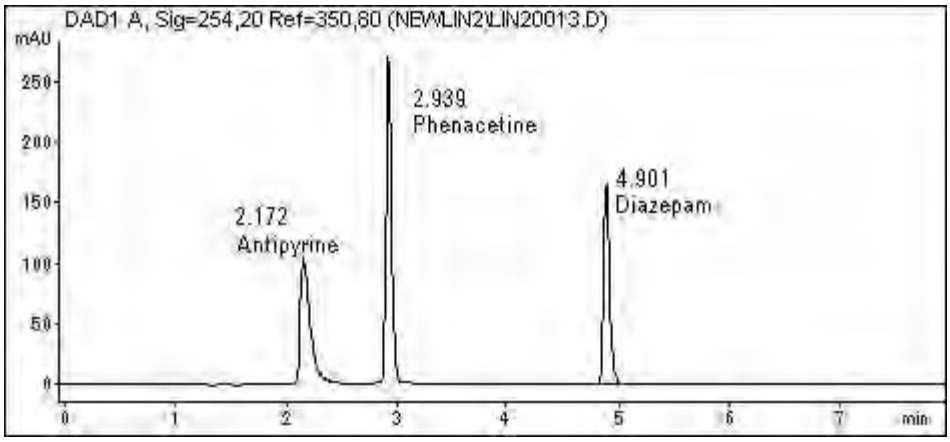
The following pages show examples of summary reports that can be generated with the ChemStation base software. The examples were generated using the print-to-file function and may have different pagination than a report printed directly from the ChemStation. Reports shown include:

- Short Area Percent Report
- Short ESTD Report
- Spectral Library Search Report
- Short GLP Report
- Performance Report
- Detail Report
- Extended Performance Report
- Sequence Summary Report – Compound Summary
- Sequence Summary Report – Standard Statistics for Sample Runs

Short Area Percent Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 8:54:14 AM agratz

================================================================
Injection Date  : 10/25/00 8:47:20 AM            Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                             Inj :   1
                                                 Inj Volume :  1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 8:53:08 AM by agratz
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
================================================================
```




```
                          Area Percent Report
================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 8:52:20 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

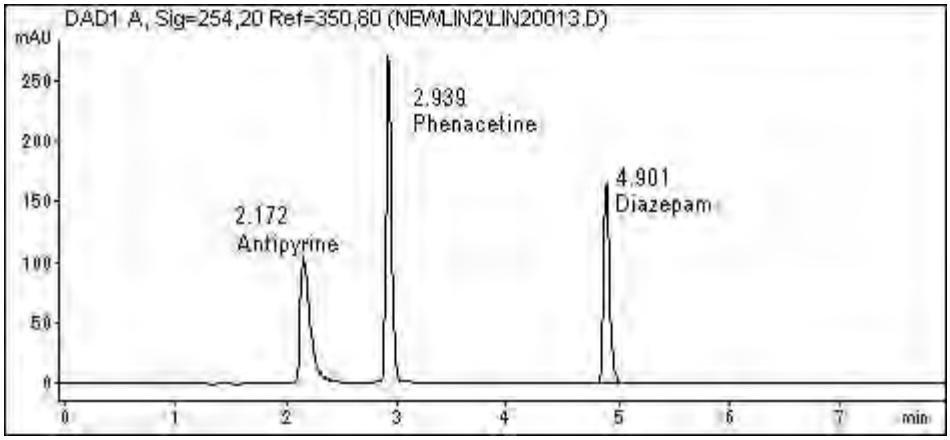
Peak RetTime  Type    Width     Area      Area     Name
  #    [min]           [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
  1  1.424    BV    0.0829    10.51506   0.4743 ?
  2  2.172    BB    0.0933   661.70422  29.8443 Antipyrine
  3  2.939    BB    0.0535   934.32690  42.1402 Phenacetine
  4  4.901    BB    0.0566   610.64050  27.5412 Diazepam
Totals :                   2217.18669

================================================================
                          *** End of Report ***
```

Short ESTD Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:09:23 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                          Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !       Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:09:14 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                        External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

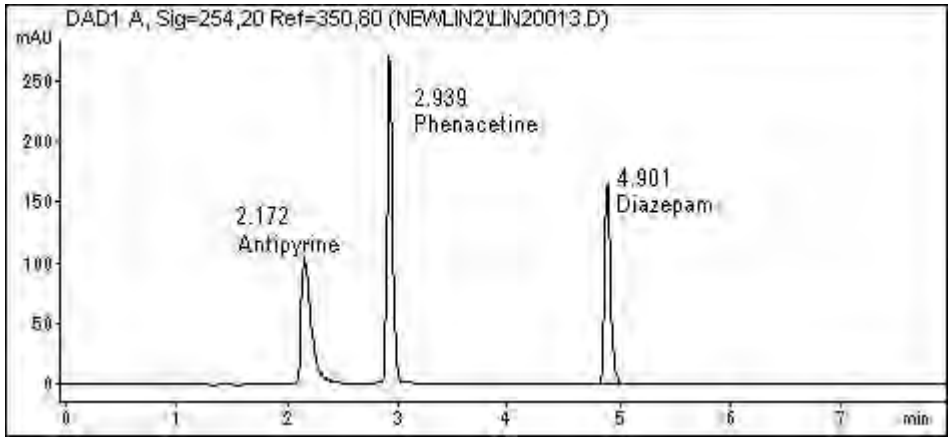
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB      661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB      934.32690    1.00317   937.28787     Phenacetine
  4.901 BB      610.64050 9.81915e-1   599.59734     Diazepam
Totals :                             1975.58590

=====================================================================
                            *** End of Report ***
```

Spectral Library Search Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:28:46 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:28:26 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                    External Standard Report
=====================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Library search mode:     Automatic library search

Library file No.       : 1
Library file name      : D:\HPCHEM\1\METHODS\LINICHEM.M\PHARMA.UVL
Match threshold        :     950        Purity threshold: Calculated
Time window  left [%] :    5.00         Case sensitive : No
Time window right [%] :    5.00             Whole word : No
Wavelength shift       :     0.0       Compare spectrum : Yes
Absorbance threshold   :     0.0           Search logic : OR
Search range           :     All
```

Spectral Library Search Report (continued)

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!
Calibrated compounds:

Meas. RetTime [min]	Library RetTime [min]	CalTbl RetTime [min]	Sig	Amount [ng]	Purity Factor	#	Library Match	Name
2.172	2.177	2.071	1	438.70069	1000	1	1000	Antipyrine
2.939	2.944	3.038	1	937.28787	1000	1	1000	Phenacetine
4.901	4.904	5.090	1	599.59734	1000	1	1000	Diazepam

Note(s):
u: compound identified at upslope. Purity factor exceeds threshold.
d: compound identified at downslope. Purity factor exceeds threshold.

==========

*** End of Report ***

Short GLP Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:31:21 AM agratz

This is a special file, named RPTHEAD.TXT, in the directory of a method
which allows you to customize the report header page.
It can be used to identify the laboratory which uses the method.

This file is printed on the first page with the report styles:

   Header+Short, GLP+Short, GLP+Detail, Short+Spec, Detail+Spec, Full


                  XXXX    XXX
                 XX  XX    XX
                XX         XX        XXXXX    XXX XX
                XX         XX XXX   XX     X   XX X XX
                XX     X    XXX XX   XXXXXXX   XX X XX
                 XX  XX    XX  XX   XX         XX    XX
                  XXXX    XXX  XXX   XXXXX    XXX   XXX


    XXXXXX      X                   X          XX
   XX     X     XX                  XX
   XX          XXXXX     XXXXX     XXXXX      XXX       XXXX     XX XXX
    XXXXX      XX            X      XX          XX      XX  XX    XXX XX
        XX     XX       XXXXXX      XX          XX      XX  XX    XX  XX
   X    XX     XX XX   X    XX      XX XX       XX      XX  XX    XX  XX
   XXXXXX       XXX    XXXXX X      XXX       XXXX      XXXX     XX  XX


                                                              X
         XX XXX     XXXXX    XX XXX      XXXX    XX XXX     XXXXX
          XXX XX   XX     X    XX  XX    XX  XX    XXX XX     XX
          XX       XXXXXXX    XX  XX    XX  XX    XX         XX
          XX       XX          XXXXX     XX  XX    XX         XX XX
         XXXX       XXXXX     XX          XXXX    XXXX         XXX
                              XXXX


         XXX                                XXX
          XX                                 XX
          XX         XXXXX     XXXXX         XX     XXXXX    XX XXX
          XX XXX   XX     X         X      XXXXX    XX     X    XXX XX
          XXX XX   XXXXXXX   XXXXXX    XX  XX    XXXXXXX    XX
          XX  XX   XX         X    XX    XX  XX    XX          XX
         XXX  XXX   XXXXX    XXXXX X    XXXX X    XXXXX    XXXX
```

Short GLP Report (continued)

```
==================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                               Inj :   1
                                                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:31:10 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
Module                                Firmware revision  Serial number
-----------------------------------|------------------|---------------
1100 Wellplate Autosampler            A.04.08            DE02700294
1100 Column Thermostat                A.04.06            DE53400174
1100 Diode Array Detector             S.03.91            DE00900051
1100 Binary Pump                      A.04.06            DE53500104
1100 Sample Thermostat                n/a                DE82203241

Software Revisions for:
- Acquisition:   Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies
==================================================================

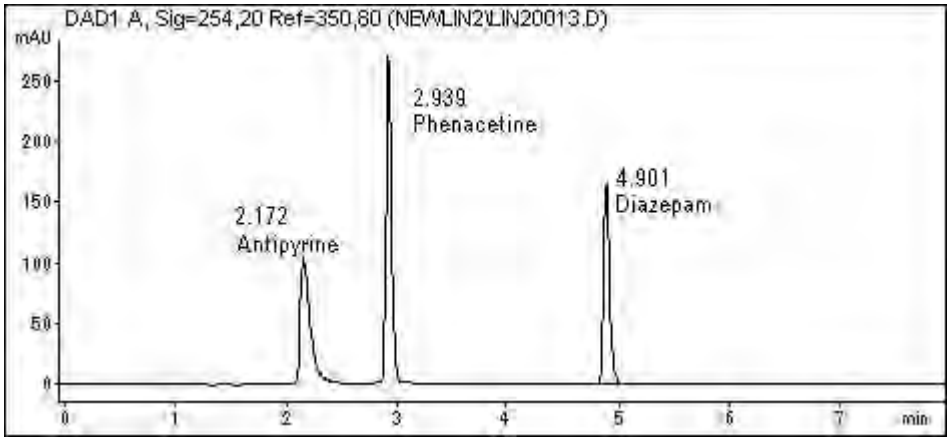
Instrument Conditions   :    At Start            At Stop
Air Temperature (Tray)  :      20.1   °C
Column Temp. (left)     :      40.0                40.0   °C
Column Temp. (right)    :      40.0                40.0   °C
Pressure                :      69.8                75.7   bar
Flow                    :       1.200               1.200 ml/min

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp          :       2.44              454.9   h
DAD 1, Visible Lamp     :       2.44              424.1   h

Solvent Description     :
PMP1, Solvent A         : Water
PMP1, Solvent B         : acn
==================================================================
```

Short GLP Report (continued)

```
                                Run Logbook
=====================================================================
Method        Method started:  line# 7 vial# 2 inj# 1    10:46:18 10/25/00
Method        Instrument running sample Vial 2           10:46:18 10/25/00
1100 ALS    1 Air temperature (tray) = 20.1 °C           10:47:21 10/25/00
1100 PMP    1 Pressure = 69.8 bar                        10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:55:21 10/25/00
1100 PMP    1 Pressure = 75.7 bar                        10:55:21 10/25/00
Method        Instrument run completed                  10:55:23 10/25/00
Method        Method completed                           10:55:23 10/25/00
=====================================================================
=====================================================================
```




```
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

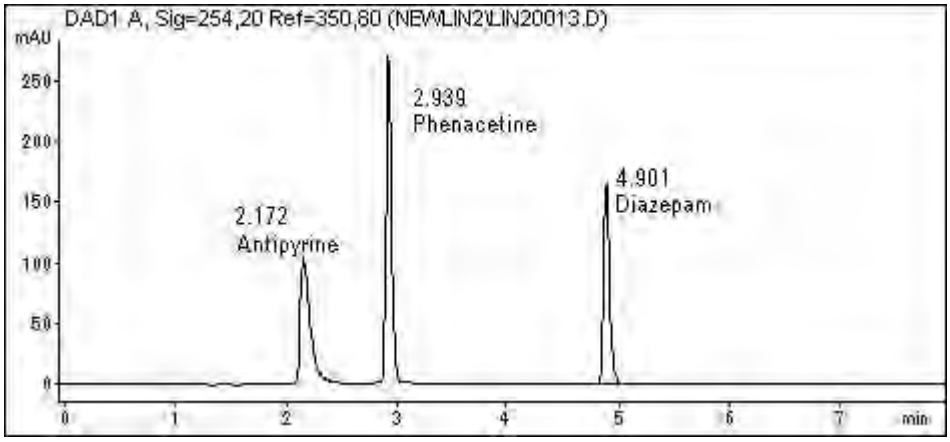
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1  438.70069     Antipyrine
  2.939 BB       934.32690    1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1  599.59734     Diazepam
Totals :                               1975.58590

=====================================================================
                           *** End of Report ***
```

Performance report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:36:38 AM agratz

======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                   Inj Volume : 1 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:36:32 AM by agratz (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150mm, 5µm
======================================================================
```




```
     External Standard Report with Performance
======================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!

RetTime     k'  Sig    Amount     Symm.  Width    Plates Resol  Name
 [min]                  [ng]             [min]           ution
-------|------|---|----------||-----|-------|-------|-----|----------
 2.172    0.81  1    438.70069    0.44  0.0883     3351  4.47 Antipyrine
 2.939    1.45  1    937.28787    0.83  0.0524    17435  6.40 Phenacetine
 4.901    3.08  1    599.59734    0.80  0.0550    43990 21.47 Diazepam

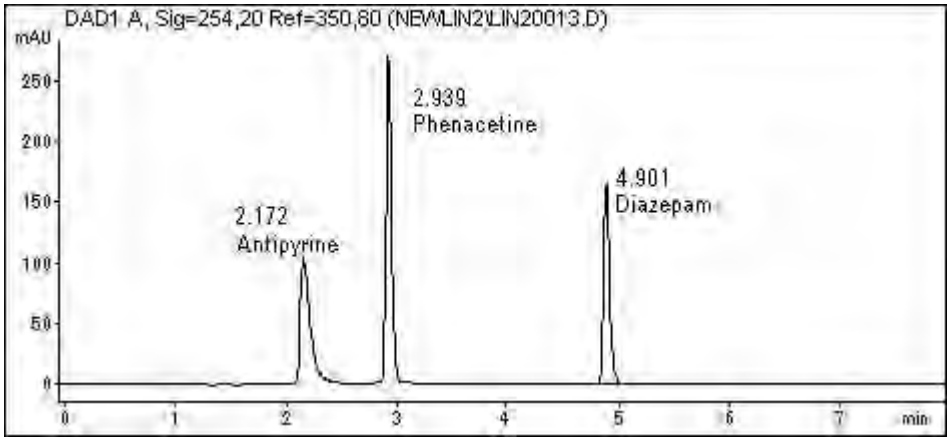
======================================================================
                          *** End of Report ***
```

Page 1 of 1

Detail report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:51:47 AM agratz

======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================
```




```
                  External Standard Report
======================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

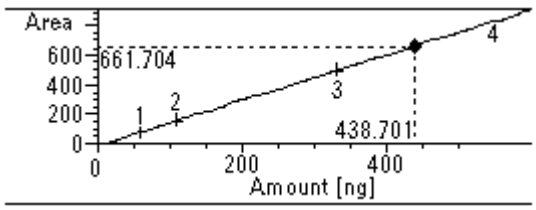
Signal 1: DAD1 A, Sig=254,20 Ref=350,80

RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1  438.70069     Antipyrine
  2.939 BB       934.32690    1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1  599.59734     Diazepam
Totals :                               1975.58590
======================================================================
```

Detail report (continued)

```
======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================

======================================================================
----------------------------------------------------------------------
                        Calibration Curves
======================================================================
```




```
Antipyrine at exp. RT: 2.071
DAD1 A, Sig=254,20 Ref=350,80
Correlation:          1.00000
Residual Std. Dev.:   0.00000
Formula: y = ax^3 + bx^2 + cx + d
      a:     1.00818e-7
      b:     9.51014e-5
      c:     1.57593
      d:   -19.85331
      x: Amount (ng)
      y: Area
```

:
:
:

The header information and calibration curve is repeated for each peak

```
======================================================================
                        *** End of Report ***
```

Extended Performance Report

```
Data File D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D

                  Extended Performance Report

Instrument: Instrument 1

Module                          Firmware revision         Serial number
-------------------------------------------------------------------------
1100 Quaternary Pump            A.04.11                   DEl 1116042
1100 Wellplate Autosampler      A.04.13                   DE02700294
1100 Column Thermostat          A.04.11                   DE53400174
1100 Diode Array Detector       A.04.11                   DEO0900051
1100 Sample Thermostat          n/a                       DE82203241

Specials:
micro column switching valve installed in oven

Software Revisions for:
-Acquisition: Rev. A.08.04 [982] Copyright @ Agilent Technologies
-Data Analysis: Rev. A.08.04 [1008] Copyright @ Agilent Technologies

Column Description: XDB-C8
Product# Zorbax Batch#: b99024
Serial# USLLO00162
Diameter 2.1 mm Length: 30.0 mm
Particle size 3.5 mm Void volume 0.08 ml
Maximum Pressure 350 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment: system suitability

Analysis method: D:\HPCHEM\l\METHODS\SYSSUIP.M

Sample information for vial#: 21

  Sample Name:           calanti+     Multiplier:  1.00
  Injection#:            5            Dilution:    1.00
  Injection volume:      3 µl

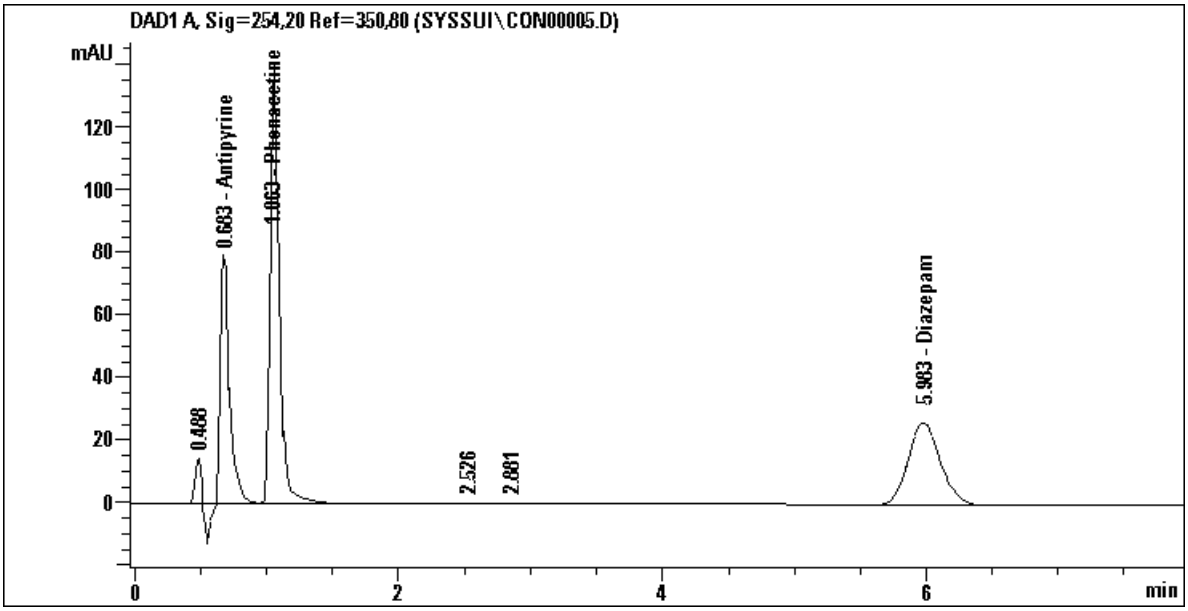
Acquisition information:
  Operator:              agratz
  Date/Time:             2/11/029:06:34 AM
  Data file name:        D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D
  Method file name:      D:\HPCHEM\l\METHODS\SYSSUIP.M

  Flow:                  0.200 ml/min
  Pressure at start:     85 bar              Pressure at end:    88 bar
  Temperature at start:  25.1°C Temperature  at end:            25.0°C
```

Extended Performance Report (continued)

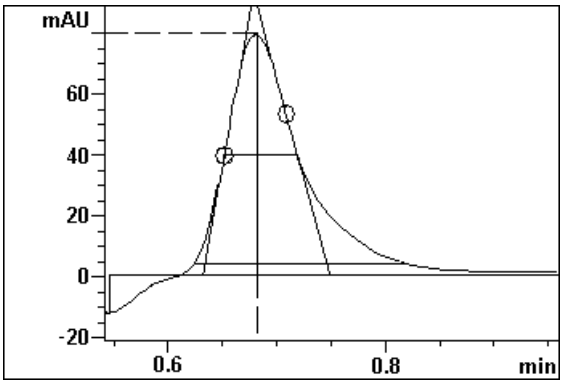
```
Solvents:              PMP1, Solvent A     water
                       PMP1, Solvent B     ACN
                       PMP1, Solvent C
                       PMP1, Solvent D

Signal description: DAD1 A, Sig=254,20 Ref=350,80
```




```
Compound# 2    :  Antipyrine
Amount [ng]:         51.1385

Peak description [min]:
      Signal: DAD1 A, Sig=254,20 Ref=350,80
      RetTime: 0.583      K':    0.706
      Height:  79.78  Area:      371.2
      Start:      0.546   End:   0.956
      Skew:    0.898   Excess:   1.643
      Width at half height:      0.067
            5 sigma:             0.196
            tangent:             0.117
            tailing:             0.190
      Symmetry:                  0.483
      USP Tailing:               1.657
      Integration type:            HV
      Time increment [macc]:     400.0
      Data points:                  66
```



Extended Performance Report (continued)

```
Statistical moments (BB peak detection):    Efficiency:  Plates per ...
     M0:  514.1                                      column       meter
     M1:  0.699                Tangent method         541        18020
     M2:  0.00341              Halfwidth method       581        19360
     M3:  0.000179             5 sigma method         385        10153
     M4: 0.000054              Statistical            143        4782

Relationship to preceeding peak:             Selectivity: 3.217
Resolution Tangent method: 2.015             5 sigma method 1.700
Halfwidth method 2.034                       Statistical method 1.067



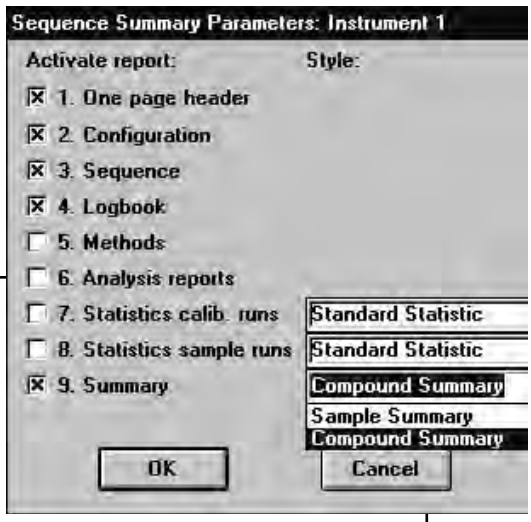
                                        :
                                        :
                                        :
```

The peak description and statistical moments are repeated for each compound

```
=====================================================================
                            *** End of Report ***
```

Page 3 of 3

Sequence Summary Report – Compound Summary




```
  XXXXXX      XX        XXXXXX
 XX    XX     XX        XX    XX
 XX           XX        XX    XX
 XX           XX        XXXXXX
 XX    XX     XX        XX
 XX    XX     XX        XX
 XX    XX     XX        XX
  XXXXXX      XXXXXX    XX
```

S E Q U E N C E

S U M M A R Y

R E P O R T

A.G Huesgen

.....
Date/Signature

I n s t r u m e n t C o n f i g u r a t i o n

Instrument: Instrument 1

Module	Firmware revision	Serial number
1100 Wellplate Autosampler	A.04.08	DE02700294
1100 Column Thermostat	A.04.06	DE53400174
1100 Diode Array Detector	S.03.91	DE00900051
1100 Binary Pump	A.04.06	DE53500104
1100 Sample Thermostat	n/a	DE82203241

Software Revisions for:
- Acquisition: Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies

Sequence Summary Report – Compound Summary (continued)

S e q u e n c e

Sequence Parameters:

Operator:	agratz
Data File Naming:	Prefix/Counter
Signal 1 Prefix:	Lin2
Counter:	0001
Data Directory:	D:\HPCHEM\1\DATA\
Data Subdirectory:	NEWLIN2
Part of Methods to run:	Reprocessing only
	Use SAMPLE.MAC
Wait Time after loading Method:	0 min
Barcode Reader:	not used
Sequence Timeout:	0 min
Shutdown Cmd/Macro:	none
Sequence Comment:	Linearity Test

Sequence Table:

Sample Information Part:

Line	Location	Sample Information
1	Vial 1	1:10 diluted stock solution
2	Vial 1	1:10 diluted stock solution
3	Vial 1	1:10 diluted stock solution
4	Vial 1	1:10 diluted stock solution
5	Vial 1	1:10 diluted stock solution
6	Vial 1	1:10 diluted stock solution
7	Vial 2	1:100 diluted stock solution
8	Vial 2	1:100 diluted stock solution
9	Vial 2	1:100 diluted stock solution
10	Vial 2	1:100 diluted stock solution
11	Vial 2	1:100 diluted stock solution

Sequence Summary Report – Compound Summary (continued)

```
     Method and Injection Info Part:

     Line Location SampleName        Method   Inj SampleType InjVolume DataFile
     ==== ======== ================ ======== === ========== ========= ==========

     1    Vial 1   1:10dil.         LINICHEM 2   Sample        0.1
     2    Vial 1   1:10dil.         LINICHEM 2   Sample        0.5
     3    Vial 1   1:10dil.         LINICHEM 2   Sample        1
     4    Vial 1   1:10dil.         LINICHEM 2   Sample        3
     5    Vial 1   1:10dil.         LINICHEM 2   Sample        5
     6    Vial 1   1:10dil.         LINICHEM 2   Sample        10
     7    Vial 2   1:100dil.        LINICHEM 2   Sample        25
     8    Vial 2   1:100dil.        LINICHEM 2   Sample        50
     9    Vial 2   1:100dil.        LINICHEM 2   Sample        75
     10   Vial 2   1:100dil.        LINICHEM 2   Sample        100
     11   Vial 2   1:100dil.        LINICHEM 2   Sample        0.1


     Calibration Part:

     Line Location SampleName        Method   CalLev Update RF Update RT Interval
     ==== ======== ================ ======== ====== ========= ========= =========



     Quantification Part:

     Line Location SampleName        SampleAmount ISTDAmt   Multiplier Dilution
     ==== ======== ================ ============ =======   ========== =========

     1    Vial 1   1:10dil.
     2    Vial 1   1:10dil.
     3    Vial 1   1:10dil.
     4    Vial 1   1:10dil.
     5    Vial 1   1:10dil.
     6    Vial 1   1:10dil.
     7    Vial 2   1:100dil.
     8    Vial 2   1:100dil.
     9    Vial 2   1:100dil.
     10   Vial 2   1:100dil.
     11   Vial 2   1:100dil.


Sequence Output Parameters:

      Print Sequence Summary Report (SSR):              Yes
           SSR to Printer:                              Yes
           SSR to File:                                 Yes
           SSR File Name:                               GLPrprt.txt
           SSR to HTML:                                 No
           Print individual reports for each run:       No
```

Sequence Summary Report – Compound Summary (continued)

```
Sequence Summary Parameters:

One page header:                        Yes
Print Configuration:                    Yes
Print Sequence:                         Yes
Print Logbook:                          Yes
Print Method(s):                        No
Print Analysis reports:                 No
Print Statistics for Calib. runs:       No
Statistic Sample runs style:            No
Summary style:                          Compound Summary


                              L o g b o o k
24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                          Time      Date
-------------------------------------------------------------------------------------
Sequence      LIN2.S started                                         10:47:06 01/24/02
Method        Loading Method LINICHEM.M                              10:47:07 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 1                10:47:08 01/24/02
CP Macro      Analyzing rawdata Lin20001.D                           10:47:08 01/24/02
Method        Method completed                                       10:47:10 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 2                10:47:11 01/24/02
CP Macro      Analyzing rawdata Lin20002.D                           10:47:11 01/24/02
Method        Method completed                                       10:47:13 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 1                10:47:14 01/24/02
CP Macro      Analyzing rawdata Lin20003.D                           10:47:14 01/24/02
Method        Method completed                                       10:47:16 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 2                10:47:17 01/24/02
CP Macro      Analyzing rawdata Lin20004.D                           10:47:18 01/24/02
Method        Method completed                                       10:47:19 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 1                10:47:21 01/24/02
CP Macro      Analyzing rawdata Lin20005.D                           10:47:21 01/24/02
Method        Method completed                                       10:47:22 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 2                10:47:24 01/24/02
CP Macro      Analyzing rawdata Lin20006.D                           10:47:24 01/24/02
Method        Method completed                                       10:47:26 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 1                10:47:27 01/24/02
CP Macro      Analyzing rawdata Lin20007.D                           10:47:27 01/24/02
Method        Method completed                                       10:47:29 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 2                10:47:30 01/24/02
CP Macro      Analyzing rawdata Lin20008.D                           10:47:30 01/24/02
Method        Method completed                                       10:47:32 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 1                10:47:33 01/24/02
CP Macro      Analyzing rawdata Lin20009.D                           10:47:34 01/24/02
Method        Method completed                                       10:47:35 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 2                10:47:37 01/24/02
CP Macro      Analyzing rawdata Lin20010.D                           10:47:37 01/24/02
Method        Method completed                                       10:47:39 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 1                10:47:40 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

```
CP Macro      Analyzing rawdata Lin20011.D                          10:47:40 01/24/02
Method        Method completed                                      10:47:42 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 2               10:47:43 01/24/02
CP Macro      Analyzing rawdata Lin20012.D                          10:47:43 01/24/02
Method        Method completed                                      10:47:45 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 1               10:47:46 01/24/02
CP Macro      Analyzing rawdata Lin20013.D                          10:47:47 01/24/02
Method        Method completed                                      10:47:48 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 2               10:47:50 01/24/02
CP Macro      Analyzing rawdata Lin20014.D                          10:47:50 01/24/02

24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                         Time      Date
-------------------------------------------------------------------------------------
Method        Method completed                                      10:47:51 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 1               10:47:53 01/24/02
CP Macro      Analyzing rawdata Lin20015.D                          10:47:53 01/24/02
Method        Method completed                                      10:47:55 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 2               10:47:56 01/24/02
CP Macro      Analyzing rawdata Lin20016.D                          10:47:56 01/24/02
Method        Method completed                                      10:47:58 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 1               10:47:59 01/24/02
CP Macro      Analyzing rawdata Lin20017.D                          10:47:59 01/24/02
Method        Method completed                                      10:48:01 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 2               10:48:02 01/24/02
CP Macro      Analyzing rawdata Lin20018.D                          10:48:03 01/24/02
Method        Method completed                                      10:48:04 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 1              10:48:06 01/24/02
CP Macro      Analyzing rawdata Lin20019.D                          10:48:06 01/24/02
Method        Method completed                                      10:48:08 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 2              10:48:09 01/24/02
CP Macro      Analyzing rawdata Lin20020.D                          10:48:09 01/24/02
Method        Method completed                                      10:48:11 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 1              10:48:12 01/24/02
CP Macro      Analyzing rawdata Lin20021.D                          10:48:13 01/24/02
Method        Method completed                                      10:48:14 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 2              10:48:16 01/24/02
CP Macro      Analyzing rawdata Lin20022.D                          10:48:16 01/24/02
Method        Method completed                                      10:48:18 01/24/02
Sequence      LIN2.S completed                                      10:48:19 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

C o m p o u n d S u m m a r y

Sequence table: D:\HPCHEM\CORE\LIN2.S
Data directory path: D:\HPCHEM\1\DATA\NEWLIN2
Logbook: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG
Sequence start: 10/25/00 6:58:26 AM
Operator: agratz

Method file name: D:\HPCHEM\1\METHODS\LINICHEM.M

Sample Name	Sample Amt [ng]	Multip.* Dilution	FileName .D	RetTime [min]	Amount [ng]	Compound
sample1	0.00000	1.0000	Lin20001	2.071	-	-
				3.005	41.80740	Phenacetine
				5.061	27.57288	Diazepam
sample2	0.00000	1.0000	Lin20002	2.071	-	-
				2.927	37.71584	Phenacetine
				4.931	24.68503	Diazepam
sample3	0.00000	1.0000	Lin20003	2.159	113.94044	Antipyrine
				2.921	249.65462	Phenacetine
				4.927	162.09926	Diazepam
sample4	0.00000	1.0000	Lin20004	2.138	115.89423	Antipyrine
				2.888	254.19389	Phenacetine
				4.893	167.32050	Diazepam
sample5	0.00000	1.0000	Lin20005	2.071	-	-
				2.967	533.16102	Phenacetine
				4.977	350.64724	Diazepam
sample6	0.00000	1.0000	Lin20006	2.071	-	-
				2.935	555.34634	Phenacetine
				4.885	359.02135	Diazepam
sample7	0.00000	1.0000	Lin20007	2.120	770.88338	Antipyrine
				2.932	1659.61614	Phenacetine
				4.939	1090.77773	Diazepam
sample8	0.00000	1.0000	Lin20008	2.156	766.86882	Antipyrine
				2.978	1658.25754	Phenacetine
				4.990	1088.46781	Diazepam
sample9	0.00000	1.0000	Lin20009	2.112	1298.20959	Antipyrine
				2.956	2780.26621	Phenacetine
				4.874	1801.76061	Diazepam
sample10	0.00000	1.0000	Lin20010	2.125	1265.65752	Antipyrine
				2.931	2753.00356	Phenacetine
				4.917	1784.44912	Diazepam
sample11	0.00000	1.0000	Lin20011	2.070	2206.34622	Antipyrine
				2.928	4737.72659	Phenacetine
				4.931	3055.52966	Diazepam
sample12	0.00000	1.0000	Lin20012	2.157	2219.77978	Antipyrine
				2.959	4771.25573	Phenacetine
				4.905	3043.14819	Diazepam
sample13	0.00000	1.0000	Lin20013	2.172	438.70069	Antipyrine
				2.939	937.28787	Phenacetine
				4.901	599.59734	Diazepam

Sequence Summary Report – Compound Summary (continued)

sample14	0.00000	1.0000	Lin20014	2.137	431.19756	Antipyrine
				2.920	922.41613	Phenacetine
				4.914	598.82718	Diazepam
sample15	0.00000	1.0000	Lin20015	2.130	1050.21043	Antipyrine
				2.956	2257.23577	Phenacetine
				4.946	1454.09021	Diazepam
sample16	0.00000	1.0000	Lin20016	2.071	-	-
				3.062	2266.63554	Phenacetine
				4.914	1450.54300	Diazepam
sample17	0.00000	1.0000	Lin20017	2.112	1860.82017	Antipyrine
				2.958	4083.57167	Phenacetine
				4.943	2601.71134	Diazepam
sample18	0.00000	1.0000	Lin20018	2.114	1846.79895	Antipyrine
				2.970	4045.19575	Phenacetine
				4.970	2576.86650	Diazepam
sample19	0.00000	1.0000	Lin20019	2.152	2485.47770	Antipyrine
				3.019	5268.86688	Phenacetine
				4.973	3410.01754	Diazepam
sample20	0.00000	1.0000	Lin20020	2.135	2489.66113	Antipyrine
				2.975	5298.02094	Phenacetine
				4.943	3415.39103	Diazepam
sample21	0.00000	1.0000	Lin20021	2.155	2961.16799	Antipyrine
				3.010	6013.24563	Phenacetine
				5.003	4037.60722	Diazepam
sample22	0.00000	1.0000	Lin20022	2.156	2983.41614	Antipyrine
				3.042	6012.35737	Phenacetine
				4.988	4010.73532	Diazepam

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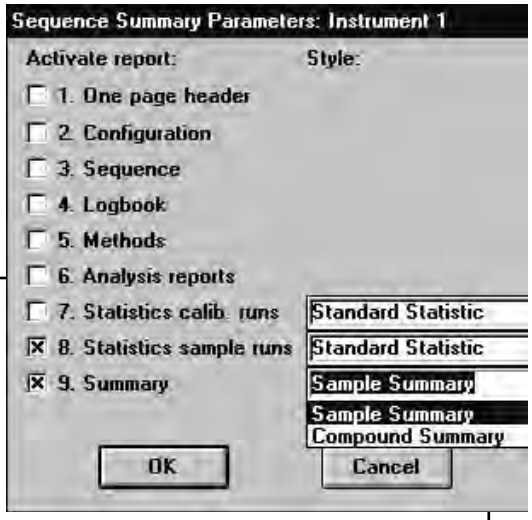
*** End of Report ***

Sequence Summary Report – Standard Statistics for Sample Runs




```
                    S t a t i s t i c    R e p o r t

Sequence table:          D:\HPCHEM\1\SEQUENCE\NEWLIN.S
Data directory path:     D:\HPCHEM\1\DATA\NEWLIN
Operator:                agratz

Method file name:        D:\HPCHEM\1\METHODS\LINI2.M
```

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	Vial 2	1	8/24/00 12:42:04 AM	new00061.D	sample1
2	Vial 2	2	8/24/00 12:51:09 AM	new00062.D	sample2
3	Vial 2	3	8/24/00 1:00:14 AM	new00063.D	sample3
4	Vial 2	4	8/24/00 1:09:18 AM	new00064.D	sample4
5	Vial 2	5	8/24/00 1:18:21 AM	new00065.D	sample5
6	Vial 2	6	8/24/00 1:27:25 AM	new00066.D	sample6
7	Vial 2	7	8/24/00 1:36:30 AM	new00067.D	sample7
8	Vial 2	8	8/24/00 1:45:34 AM	new00068.D	sample8
9	Vial 2	9	8/24/00 1:54:38 AM	new00069.D	sample9
10	Vial 2	10	8/24/00 2:03:42 AM	new00070.D	sample10

Compound: Antipyrine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BV	2.071	26.23064	834.52417	215.75279	0.0594	0.74
2	BV	2.071	26.28149	836.14185	216.26503	0.0594	0.74
3	BV	2.070	26.22879	834.46539	215.85945	0.0594	0.74
4	BV	2.070	26.27553	835.95233	216.52124	0.0594	0.74
5	BV	2.070	26.21720	834.09644	215.51944	0.0594	0.74
6	BV	2.070	26.19317	833.33203	216.02470	0.0593	0.74
7	BV	2.070	26.27779	836.02423	216.93185	0.0592	0.74
8	BV	2.072	26.29524	836.57941	216.89178	0.0593	0.74
9	BV	2.072	26.22549	834.36017	216.09763	0.0593	0.74
10	BV	2.071	26.21184	833.92590	216.06882	0.0593	0.74
Mean:		2.071	26.24372	834.94019	216.19327	0.0594	0.74
S.D.:		6.81e-4	3.53636e-2	1.12509	4.66512e-1	6.63e-5	1e-3
RSD :		0.033	1.34751e-1	1.34751e-1	2.15784e-1	0.1117	0.20
95% CI:		4.87e-4	2.52976e-2	8.04838e-1	3.33722e-1	4.74e-5	1e-3

Sequence Summary Report – Standard Statistics for Sample Runs

Compound: Phenacetine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	3.035	12.05932	1203.01074	357.49438	0.0528	0.88
2	BB	3.035	12.07862	1204.93591	357.76285	0.0527	0.87
3	BB	3.035	12.05487	1202.56653	357.16501	0.0527	0.88
4	BB	3.035	12.07567	1204.64221	357.80615	0.0527	0.88
5	BB	3.036	12.05951	1203.02979	356.62448	0.0528	0.87
6	BB	3.036	12.02965	1200.05090	356.52957	0.0528	0.88
7	BB	3.037	12.08083	1205.15625	357.92139	0.0527	0.88
8	BB	3.037	12.06433	1203.51099	357.60211	0.0527	0.88
9	BB	3.039	12.05340	1202.42065	356.89868	0.0527	0.87
10	BB	3.038	12.04430	1201.51282	356.41678	0.0528	0.88
Mean:		3.036	12.06005	1203.08368	357.22214	0.0527	0.88
S.D.:		1.35e-3	1.59266e-2	1.58880	5.70986e-1	3.70e-5	6e-3
RSD :		0.045	1.32061e-1	1.32061e-1	1.59840e-1	0.0702	0.68
95% CI:		9.69e-4	1.13932e-2	1.13656	4.08458e-1	2.65e-5	4e-3

Compound: Diazepam (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	5.085	17.51478	820.56067	228.97469	0.0556	0.84
2	BB	5.086	17.54309	821.88702	229.58243	0.0557	0.84
3	BB	5.085	17.51162	820.41229	229.04759	0.0557	0.84
4	BB	5.084	17.54478	821.96600	229.60602	0.0557	0.84
5	BB	5.086	17.51105	820.38562	229.37668	0.0556	0.84
6	BB	5.087	17.47411	818.65503	228.69946	0.0556	0.85
7	BB	5.088	17.54951	822.18774	229.63567	0.0556	0.84
8	BB	5.088	17.51423	820.53491	229.10289	0.0556	0.84
9	BB	5.090	17.51381	820.51508	229.17131	0.0557	0.84
10	BB	5.090	17.50570	820.13525	228.79688	0.0556	0.84
Mean:		5.087	17.51827	820.72396	229.19936	0.0556	0.84
S.D.:		2.12e-3	2.24801e-2	1.05318	3.38200e-1	3.77e-5	2e-3
RSD :		0.042	1.28324e-1	1.28324e-1	1.47557e-1	0.0678	0.29
95% CI:		1.52e-3	1.60813e-2	7.53401e-1	2.41934e-1	2.70e-5	2e-3

Sequence Summary Report – Standard Statistics for Sample Runs

```
                  S a m p l e    S u m m a r y


Sequence table:                          D:\HPCHEM\1\SEQUENCE\NEWLIN.S
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Logbook:                                 D:\HPCHEM\1\DATA\NEWLIN\NEWLIN.LOG
Sequence start:                          8/24/00 12:42:04 AM
Statistic report on calibration runs:  1
Operator:                                agratz

Method file name:                        D:\HPCHEM\1\METHODS\LINI2.M

Run Location Inj Sample Name Sample Amt Multip.* File name Cal # Page
 #             #                    [ng]    Dilution               Cmp  #
---|--------|---|----------------|----------|--------|----------|-|---|---
  1 Vial 2    1  sample1               -    1.0000  new00061.D *   3  -
  2 Vial 2    2  sample2               -    1.0000  new00062.D *   3  -
  3 Vial 2    3  sample3               -    1.0000  new00063.D *   3  -
  4 Vial 2    4  sample4               -    1.0000  new00064.D *   3  -
  5 Vial 2    5  sample5               -    1.0000  new00065.D *   3  -
  6 Vial 2    6  sample6               -    1.0000  new00066.D *   3  -
  7 Vial 2    7  sample7               -    1.0000  new00067.D *   3  -
  8 Vial 2    8  sample8               -    1.0000  new00068.D *   3  -
  9 Vial 2    9  sample9               -    1.0000  new00069.D *   3  -
 10 Vial 2   10  sample10              -    1.0000  new00070.D *   3  -


================================================================================
                            *** End of Report ***
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www.agilent.com/chem/nds

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Published March 1, 2003
Publication Number 5988-9011EN



Agilent Technologies

Optimizing the Agilent Technologies 6890 Series GC for High Performance MS Analysis

Technical Overview

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Abstract

Trace level GC/MS analysis requires a system that is performing at its best. Without a properly optimized GC, the mass spectrometer may not give the sensitivity expected. In other words, when more of the sample gets from the injection port to the ion source, the more likely a detector will produce a signal. Also, if the chemical noise from the GC is too high, the signal-to-noise ratio and ability to detect small analyte concentrations will be reduced. This note is a "how-to" guide for improving the GC performance. This will, in many instances, improve the overall performance of a GC/MS system. This guide is specific for the Agilent Technologies 6890 Series GC used with the Agilent Technologies 5973 MSD.

Supplies

In order to improve the system performance, some supplies should be on hand. These may not give significant improvements by themselves, but when installed together, they will give the best results. Many of the referenced supplies have changed over the past few years and will continue to be improved. It is important to stay informed and purchase the most recently updated versions of the consumables. Refer to www.agilent.com/chem for the latest comsumables available.

The Carrier Gas Line

The GC carrier gas should be at least 99.999% helium. Lower grades of helium are available and may contain impurities that can damage the GC column (for example, oxygen) and contribute to the chemical noise background. Even with a high purity gas there may be trace water, oxygen, and hydrocarbons. Putting a trap in the carrier line will eliminate these contaminants (see Figure 1). The mass spectrometer (MS) gas purifier trap is recommended and shipped with all new mass selective detectors (MSDs). It must be installed diagonally or vertically for optimum performance. Do not install it horizontally. High-purity helium also increases the effective lifetime of traps.

Precleaned, refrigeration grade 1/8-inch copper tubing should be used with high quality carrier gas. Other tubing can be cleaned by running solvents (methanol, ethyl acetate, hexane) through it in a water aspirator vacuum setup. The use of chlorinated solvents is not recommended due to

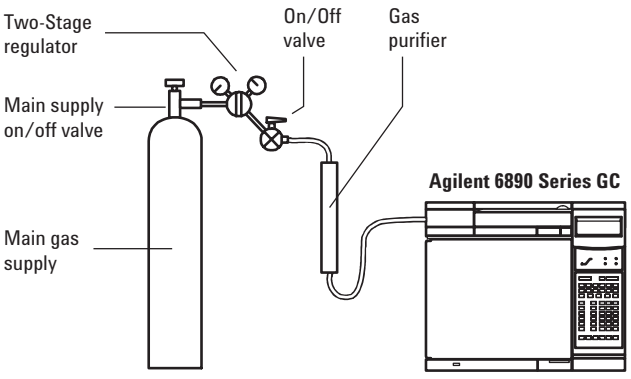

Figure 1. General gas plumbing assembly. The MS gas purifier must be installed diagonally or vertically.

possible long term contamination of flow lines and controllers. Another commonly used cleaning technique is to heat the copper tubing with a Bunsen burner, propane torch, or heat gun, while helium is flowing through the tubing. This is done after connecting the tubing to the helium supply but before connecting it to the GC. This process bakes off all the volatile contaminants. Take proper safety precautions while heating the tubing. Laboratory manifold systems, especially when new, tend to have hydrocarbon contaminants. Purging the new lines, before connecting the clean tubing to analytical instruments, is essential. The supplies needed for the carrier gas line are:

- ≥99.999% He: Gas supplier
- Clean copper tubing (50 ft): p/n 5180-4196
- Mass Spectrometer gas purifier: p/n RMSH-2
- Bracket to mount the gas purifier: p/n UMC-5-2

Splitless Inlet Consumables

The capillary inlet (Figure 2) has many consumable parts that should be kept on hand. Many of these consumables, such as liners (5), come in a variety of designs (Appendix A). The proper liner to use is dependent on the application. For trace level analysis, the single tapered, deactivated liner is recommended. The Viton O-ring (4) holding the liner in place should be replaced periodically to reduce the chance of leaking. The seal and the washer in the bottom of the injection port (10, 11) should be replaced whenever the reducing nut (12) is removed. The recommended seal is gold plated to reduce metal catalyzed thermal degradation of analytes. Septa (2) should be replaced quite frequently: for example, every 100 injections. The low bleed, precored, red septa should be used. Keeping a beaker of septa in an oven at 250 °C at all times will eliminate the need to condition the septum once it is in place. A Merlin Microseal™ is highly recommended over a conventional septum nut and septum (1, 2). The Microseal eliminates the need for septa and lasts for tens of thousands of injections without leaking. It is most appropriate with the Automatic Liquid Sampler (ALS) injection tower and only works with untapered, blunt tip syringe needles. The Microseal has been improved this past year. Older seals have a maximum pressure rating of 30 psi. The new seals are rated to 100 psi. The new seal and nut are recommended. The gold nuts (stamped “303C”) are not compatible with the new seals. A gray nut, stamped “221B,” should be installed.

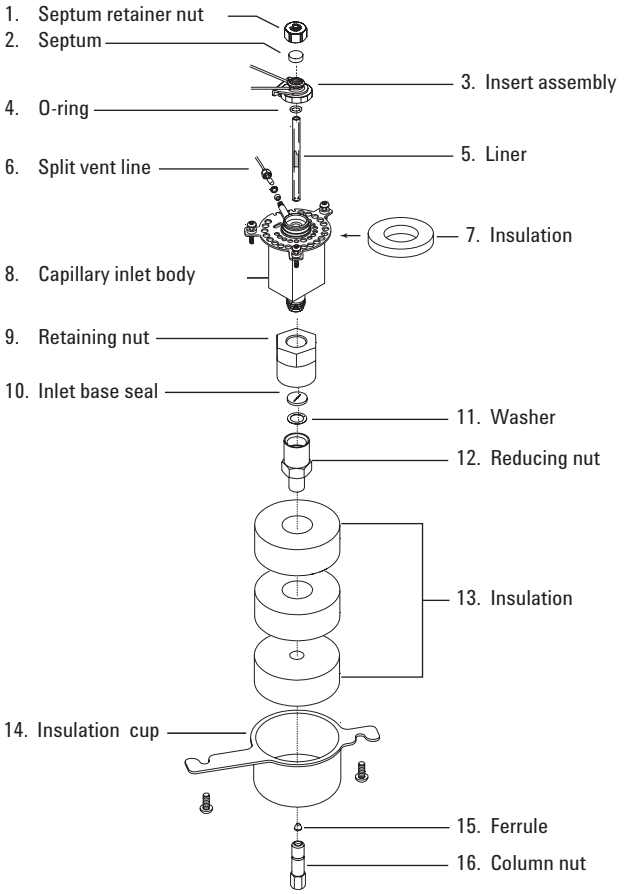

Figure 2. Capillary inlet assembly.

Electronic pneumatic control (EPC) is an integral part of the Agilent 6890 Series GC. The EPC version of the Agilent 6890 is required when using an Agilent 5973 MSD. The manual version of the Agilent 6890 will not work with the Agilent 5973 MSD. Electronic control gives the best repeatability in retention time and area counts. Using the electronic pulsed splitless mode allows for complete transfer of larger volume injections (up to 5 µL) onto the column. Even larger volume injections (>5 µL) may result in more inlet maintenance, especially with dirty samples.

The Agilent 6890 does have an inlet that accommodates injections up to 250 µL. It is called the programmable temperature vaporizer (PTV). It works with the ALS to deliver large volume injections (LVI). The inlet works by venting the solvent before analysis. The analyte is trapped and concentrated. It is then delivered to the column as a single plug.

The list of splitless inlet consumables is:

- Molded Septa (11 mm, red, 25/pk): p/n 5181-3383

Or

- High Pressure Merlin Microseal starter kit: p/n 5182-3442
- Merlin Microseal septum: p/n 5182-3444
- Merlin Microseal septum nut: p/n 5182-3445
- Liner, single taper, deactivated, no glass wool: p/n 5181-3316
- Viton O-ring (12/pk): p/n 5180-4182
- Gold plated seal: p/n 18740-20885
- Washer (to go with seal): p/n 5061-5869
- 10-µL Blunt needle syringe: p/n 9301-0713

Column Consumables

The optimal choice of column is once again dependent on the application. For trace-level, high-sensitivity applications, a column with a thin film and low bleed is best. A 30 m, 0.25-mm id, 0.25-µm film, 5% phenyl–95% methyl silicone column is a versatile column used for many applications. Special low-bleed MS columns cost more but give better results.

The proper column nut and ferrule combination are critical for a leaktight seal. Newer column nuts may not be compatible with all ferrules. The proper ferrule will be dependent on column outer diameter (od) and is specified below. The ferrule should only be slightly larger than the column od. The use of 100% graphite ferrules is not recommended, as they are easily over-tightened causing graphite to extrude into the injection port. This will be apparent when disassembling the injection port. If there are pieces of graphite in the bottom of the injection port, the ferrule(s) was/were overtightened. The presence of graphite in a hot injection port can cause thermally labile compounds to degrade. It can also affect the chromatography and cause tailing. Thus, 10% graphite, 90% Vespel ferrules are highly recommended. Vespel® ferrules will shrink as they are heated. Conditioning them for 4 hours in a 250 °C oven will preshrink them before use. Alternatively, the column nuts (Figure 2, number 16) can be retightened after the column oven cycles a few times.

The column, column nut, and ferrules supplies include:

- 30-m column, 0.25-mm id, 0.25 µm, low bleed (HP-5MS): p/n 19091S-433
- Column nuts (wrench-tighten only) 2/pk: p/n 5181-8830
- Ferrules for 0.2-mm id columns, 10/pk: p/n 5062-3516
- Ferrules for 0.25-mm id columns, 10/pk: p/n 5181-3323
- Ferrules for 0.32-mm id columns, 10/pk: p/n 5062-3514
- Ceramic scoring wafer (column cutter), 4/pk: p/n 5181-8836

A sharp column-cutting tool is needed for making clean cuts. The ceramic scoring wafers or sapphire square edge pens are desirable. The diamond point pens are harder to use. Ceramic scoring wafers are extremely sharp. They should be used with care. An X-ACTO® or Swiss Army knife is not a column cutting tool.

Use a 10× magnifier to assure that the cut is clean and no column shards are lodged inside the column.

Interfacing the Column to the MS

The column is connected to the MS through an interface that is sealed with a column nut and ferrule. The specific ferrule used depends on the column diameter. Never use a 100% graphite ferrule. Similar to the injection port, pieces of graphite may extrude into the interface and contaminate the MS. The ferrules required are 15% graphite, 85% Vespel. The column nut listed is brass; stainless steel nuts should never be substituted. Stainless nuts may damage the threads on the interface. Damaged threads cause air leaks and replacement of the entire interface.

The MS interface supplies include:

- Brass column nut: p/n 05988-20066
- Ferrules for 0.2 and 0.25-mm id columns, 10/pk: p/n 5062-3508
- Ferrules for 0.32-mm id columns, 10/pk: p/n 5062-3506

Installation of Consumables

This section assumes that you are going to perform preventative maintenance (PM) on your Agilent 6890 Series GC. If this is a new gas chromatography/mass selective detector (GC/MSD) system, many of these steps will be completed by a Customer Engineer during installation. Before beginning the PM, please read this section carefully. The necessary manuals will be referenced frequently. These manuals can be downloaded from our Web site at www.agilent.com/chem.

The manuals necessary include:

- Agilent 6890N GC User Information Manual: G1530-90210
- Agilent 6890 Series GC Service Manual: G1530-90220
- Agilent 5973 Series MSD Hardware Installation Manual: G2589-90006
- Agilent 5973 inert MSD Hardware Manual: G2589-90071
- Agilent 5973 Series MSD Site Preparation Guide: G2589-90070

With all of the consumable supplies previously mentioned at hand, a proper PM can be completed. To begin a PM, it is necessary to cool the GC zones (oven, inlet, MS interface). Vent the Agilent 6890 Series MSD. Please refer to the MS hardware manuals for venting instructions.

Installation of Gas Supplies

Follow the directions in the GC site preparation/installation manual and install the gas line supplies. Take care in making the Swagelok connections. The trap can break if too much stress is placed on the connection during tightening. Leak check all connections with a helium leak detector. (No Snoop please!) Make sure to purge all lines with helium before connecting them to the GC.

Installation of Capillary Inlet Supplies

Before handling any of the injection port supplies, wash hands and/or wear lint-free gloves. Oils on the hands will be transferred to these parts and become background in the system, requiring extra bakeout time. Following the instructions in the GC operating manual, remove the septum nut, septum, and liner. Discard the septum, liner, and liner O-ring. Open the oven door, loosen the 1/16-inch column nut, and remove the column and nut.

Remove the insulation cup and any necessary insulation (Figure 2, number 14) to provide access to the reducing nut (Figure 2, number 12). If the lower insulation cup was not in place, find it, because this piece improves the inlet temperature profile.

With a 1/2-inch wrench, remove the reducing nut (Figure 2, number 12). Due to heat cycling of the GC, the reducing nut will be very tight. Remove the seal and the washer (Figure 2, number 10, 11) and discard. Place a new washer in the reducing nut and a new seal (flat side with groove up). Hand-tighten the reducing nut back into place within the retaining nut and then wrench-tighten until very tight. Replace the insulation cup. Insert a new liner and O-ring. The single taper liners are installed with the taper down, toward the column end of the inlet. Hand-tighten the insert assembly (Figure 2, number 3). Add the Merlin Microseal or proper preconditioned septum and septum nut. The molded septum is installed with the hole up. Follow the directions supplied with the Merlin Microseal to insure proper installation. If the green septum nut is used, wrench-tighten the weldment and septum nut with the septum nut wrench until the C-ring lifts off the top of the green septum nut.

At this point, the inlet should be leak checked. Follow the directions in the GC maintenance and troubleshooting section of the manual.

Column Installation

Working with fused silica columns may be dangerous. Wear proper eye protection. Inspect the column for damage or breakage. Unweave 0.5–1 coil of the column from its basket to make it easier to install. Push a septum onto the inlet end of the column about 10 cm. Put the column nut and appropriate ferrule on the column. Cut 5–10 cm off the inlet end of the column. Check the cut with a 10× magnifier; the cut should be straight, not jagged, with no column shards within the column. If the cut is jagged or shards are inside, try again. After a clean cut is obtained, mark the proper column position with the septum (Figure 3). The septum will hold the column nut and ferrule in place. Place the column on the column hanger. Insert the column nut into the inlet reducing nut and finger-tighten. Wrench-tighten the column nut. The column should be stationary in the ferrule. Carefully slide the septum down away from the nut without disturbing the column positioning. The septum can be left in place if desired. Using the GC keypad or the MS ChemStation software, input the

Table 1. Head Pressures and Calculated Flowrates for a Splitless Inlet at an Oven Temperature of 25 °C with the Outlet Pressure set to Vacuum

Column id (mm)	Length (meters)	Head pressure (psi)	Linear velocity (cm/s)	Column flow (mL/min)
0.20	12	6.0	57	1.0
0.20	25	15.0	39	1.0
0.20	50	28.0	28	1.0
0.25	30	6.2	36	1.0
0.32	30	3.4	50	2.0
0.32	50	5.5	34	1.5

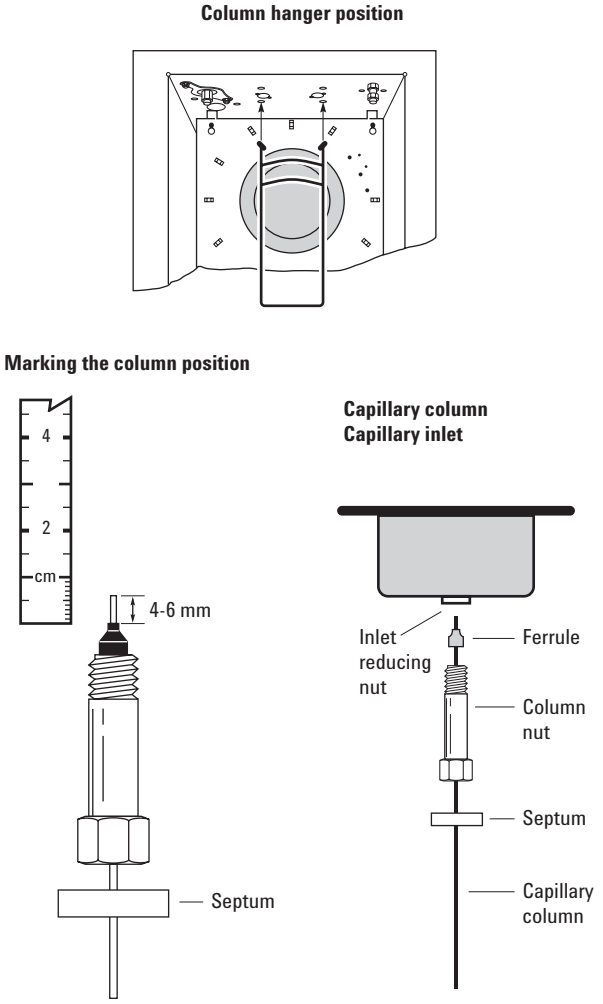

Figure 3. Proper installation of capillary columns in a capillary inlet.

Column Dimensions, set Outlet Pressure to Vacuum and Column Flow between 1 and 1.5 mL/min helium (Table 1). The Split Vent Flow should be approximately 50 mL/min. These parameters are accessed through the inlet and column screens. Place the detector end of the column into a beaker of water and check for bubbles to show helium flow. Heat the injection port. When the injection port reaches the setpoint temperature, retighten the column nut in case it loosened. Check once again for column flow. Remove the end of the column from the beaker and close the oven door. Condition the column by slowly (5 °C/min) ramping it to its maximum operating temperature. Leave it at that temperature for least 2 hours; overnight is preferable. The maximum operating temperature for an HP-5MS column is 325 °C. Cool the oven to ambient and insert the interface nut and ferrule onto the column. Properly cut off 5–10 cm of the column. Properly place the column into the interface by following the directions in the MS hardware manual for the Agilent 5973 and for the Agilent 6890 series MSD. Hand-tighten the interface nut and then wrench-tighten the nut. The nut should be tightened only until you hear two squeaks. This is a firm seal. Pump down the detector as directed by the MSD manual (for the Agilent 5973 and for the Agilent 6890 Series MSD). Keep the oven at ambient temperature until the source is hot. Check the interface connection after the interface is heated. The interface nut may need additional tightening.

Tips for Better Method Performance

Numerous splitless parameters need to be optimized for the best splitless injection. Those parameters include:

- Injection port temperature
- Column flow
- Liner design
- Solvent
- Sample volume
- Sample volatility
- Splitless valve time
- Injection speed

The proper inlet temperature is needed to evaporate high boiling point compounds without thermally degrading other compounds. Normally, the inlet temperature is a compromise between these two factors. A good starting point is 250 °C.

Liner design is one of the most difficult choices simply because of the variety of liners available. The features that are most important in a liner are the volume, whether it is deactivated or not, and whether or not it contains deactivated glass wool. As a general choice for high sensitivity work, a 4-mm single tapered, deactivated liner with no glass wool is recommended. For large volume injections (≥2 µL) and for the highest repeatability (especially with small volume injections of ≤0.5 µL [1]), deactivated glass wool is necessary. For dirty samples, deactivated glass wool helps to keep the nonvolatiles from getting to the column, but too much deactivated glass wool can greatly decrease sensitivity. Often, the most appropriate liner must be determined through experimentation. Please note: Removing and/or breaking deactivated glass wool creates active sites.

Splitless valve timing is critical. The ON time (splitless mode) should to be long enough to assure that all of the injected sample reaches the column.

A textbook splitless injection has the liner volume swept at least two times (during the "ON" time). A 4-mm liner has an approximate volume of 1 mL. With a GC/MS flow rate of 1 mL/min, a 2-min splitless injection would be necessary.

However, this long splitless time has not been common. There are two reasons for this:

- Conventional (manually controlled) capillary inlets were pressure regulated (constant pressure, regardless of oven temperature) and not flow regulated (changing pressure with oven temperature), so a higher-than-optimal flow was set initially so that the flow did not go to zero at high oven temperatures. Thus, a typical splitless or "OFF" time has been between 0.5 to 1.5 min.
- Liner volumes smaller than the textbook examples have typically been used. Since a 2-mm liner (250 µL volume) was more commonly employed, the splitless time was proportionally shorter.

Finally, with the programmable control afforded by EPC, flows can be reliably pulsed during the injection process. With pulsed splitless injections, flows during the splitless time can be 2-6 mL/min, resulting in splitless times less than 2 min for a 4-mm id liner. The pulsed splitless injection mode on the Agilent 6890 is recommended for GC/MS work. After the injection pulse, the system returns to analytical flow rates of 1–4 mL/min He. The highest flow allowable depends on the MSD. Refer to the appropriate MS hardware manual for your detector's limit.

Unless all analytes have high boiling points, the initial oven temperature should be set to take advantage of the solvent effect. The solvent effect focuses the analytes on the head of the column. The oven temperature should typically be set to ≥10 °C below the boiling point of the solvent used (Table 2). On the other hand, the MS interface temperature should be hot enough to avoid loss of analytes on cold spots. The interface should be set to the maximum oven temperature for the analysis or 10 °C–15 °C higher if the upper temperature limit for the column is not exceeded. The default method temperature is 280 °C; the interface temperature should be optimized as part of method development.

Finally, the rate of auto-injection of a sample has been studied for splitless injections. It has been found that fast injections, such as with the ALS, tend to give the most repeatable and non-discriminating results.

Table 2. Boling and Initial Oven Temperatures for Common Solvents

Solvent	Boiling point (°C)	Initial oven temperature (°C)
Diethyl ether	36	10 to 25
n-Pentane	36	10 to 25
Methylene chloride	40	10 to 35
Carbon disulfide	46	10 to 35
Acetone	56	25 to 45
Chloroform	61	25 to 50
Methanol	65	35 to 55
n-Hexane	69	40 to 60
Ethyl acetate	77	45 to 65
Acetonitrile	82	50 to 70
n-Heptane	98	70 to 90
Isooctane	99	70 to 90
Toluene	111	80 to 100

Using Pulsed Splitless Injections

Pulsed splitless injections are the best way to do splitless injections. EPC of the splitless inlet allows for high flow rates initially, followed by more typical GC/MS flow rates. A pulsed splitless injection transfers more of the sample onto the column and allows for increased injection volumes up to 5 µL. When the injected volume is flash vaporized, the required expansion volume for the solvent is greatly increased (solvent choice also effects expansion volume). The use of higher initial inlet pressures reduces the volume ($P_1V_1 = P_2V_2$) so the entire injected volume can move to the column. The higher pressure also decreases the likelihood that highly volatile compounds will escape out the top of the injection port through the septum purge vent (Figures 4 and 5). In the case of thermally labile compounds, the faster they leave the hot injection port the less likely they are to degrade [2, 3]. The flow rate is then reduced to the value desired for the chromatographic separation. This flow is held constant by increasing the pressure as the oven temperature increases. Figure 6 is a graphical representation of the pulsed splitless technique with constant flow. Pulsed splitless injections should always be used when sensitivity and/or repeatability are critical. Refer to the GC operating manual for how to set up a pulsed splitless injection.

Electronic pressure and flow control of carrier gases not only help with larger volumes, they also help to decrease run times and maintain stable MS sensitivity by keeping the carrier gas flow constant. These lead to shorter analyses, higher sensitivity, and higher reproducibility. The benefits for the analyst are more chromatographic analyses per shift, better data, and higher revenues per instrument.

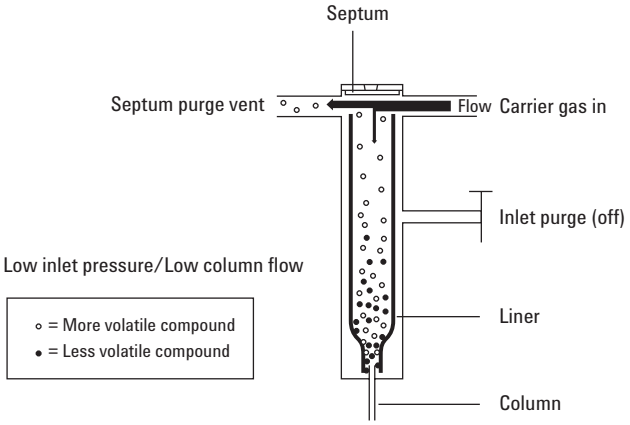

Figure 4. A low initial inlet pressure causes loss of volatile compounds.

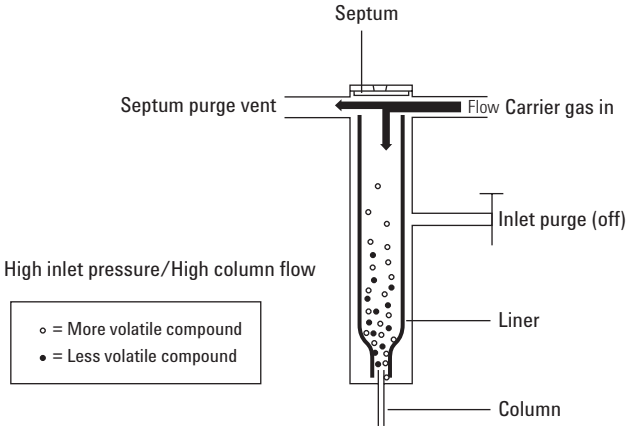

Figure 5. With the correct inlet pressure there is no loss of volatile compounds.

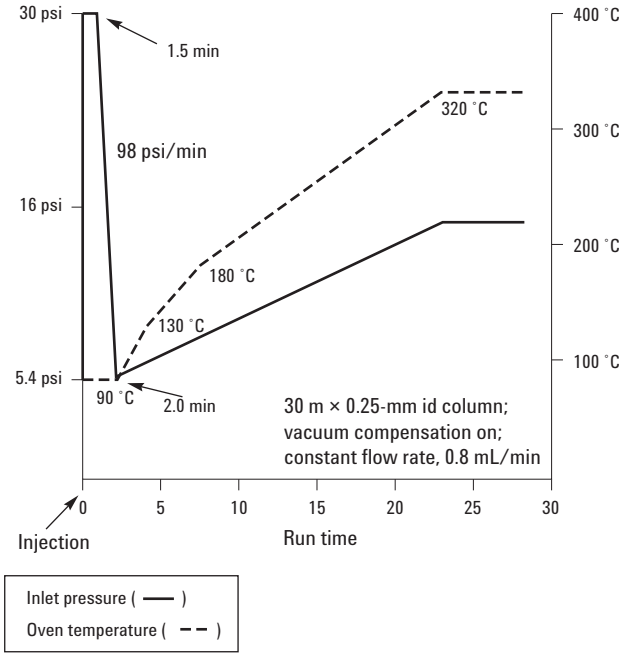

Figure 6. Pulsed splitless injection technique employing constant flow with EPC. This technique allows larger injection volumes and inhibits the loss of volatile compounds out of the septum purge vent.

Summary

Following the instructions in this guide will improve analytical results with your GC/MSD system. Contamination interferring with the determination of your analytes will be minimized and sample transfer optimized, both improving sensitivity.

A final note: the choice of vials and septa will affect your results. Screw cap vials are not recommended for GC/MS analyses. Application note "Effects of Vial Septa Used in GC/ECD Analysis of Trace Organics" Agilent Technologies publication 5091-8980E www.agilent.com/chem will help you make the right choice.

References

1. "Analyzing aromatics in reformulated gasoline by GC/MS", Agilent Technologies, publication (23) 5964-0116E.
2. Philip L. Wylie et al. Improving the Analysis of Pesticides by Optimizing Splitless Injections. 1995 Pittsburgh Conference paper number 347.
3. Philip L. Wylie and Katsura Uchiyama. Improved Gas Chromatogaphic Analysis of Organophoshorous Pesticides with Pulsed Splitless Injection. (1996) *JAOAC* **79**: 571-577.

Appendix A

Capillary Inlet System Liners

Liner	Part no.	Price	Configuration: ID volume	Glass type	Deactivated	Glass wool packing**	Applications: Fast injection (7673 ALS) Split	Fast injection (7673 ALS) Split-less	Slow & manual injection Split	Slow & manual injection Split-less	Linear seals: Fluoro-carbon (Max 350 °C)	Linear seals: Graphite (350 °C and above)
Single-Taper liner	5062-3587	34	4 mm (0.8-mm end) 900 L	Borosilicate	YES	YES**	C	●	C	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Single-Taper liner	5181-3316	31	4 mm (0.8-mm end) 900 L	Borosilicate	YES	NO	—	A	—	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Double-Taper liner	5181-3315	34	4 mm (0.8-mm end) 800 L	Borosilicate	YES	NO	—	A	—	●	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Capillary liner	19251-60540	26	4 mm 990 L	Borosilicate	NO	YES**	●	D	●	D	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Split liner	18740-60840	46	4 mm with cup	Borosilicate	NO	YES + column packing	—	—	E	—	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Split liner	18740-80190	41	4 mm with cup	Borosilicate	NO	NO	—	—	●	—	5180-4182 (12/pk) 12	5180-4168 (12/pk) 48
Splitless liner	18740-80220	27	2 mm 250 L	Quartz (Purity 8)	NO	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Splitless liner	5181-8818	30	2 mm 250 L	Quartz (Purity 8)	YES	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48
Direct liner	18740-80200	17	1.5 mm 140 L	Borosilicate	NO	NO	—	A,B	—	B	5180-4182 (12/pk) 12	5180-4173 (12/pk) 48

● General recommendation

○ See notes at left regarding use

* Quality discounts available; please inquire.

** Silanized glass wool, 10 gm, (pesticide grade) (Agilent p/n 5181-3317).

A. Can be used without the glass wool in some applications, but liners with glass wool are generally recommended for best reproducibility in fast injections.

B. Recommended only for small (< 0.5 µL) volumes, depending on solvent and conditions.

C. The glass wool packing as supplied may not provide adequate mixing for good precision in split injections. Liners can be packed with silanized glass wool positioned as in the straight 4-mm capillary liner, part number 19251-60540.

D. Taper liners are recommended for best performance in this application, particularly with labile samples and wide-boilingrange mixtures.

E. Not recommended for use with EPC.

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Printed in the USA
November 14, 2003
5988-9944EN



Agilent Technologies

Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns

Application Note

Michael Frank



Abstract

The need to increase the daily throughputs of LC systems is a constant desire. Now, with the Agilent 1200 Series Rapid Resolution LC system highest throughputs are possible, and in combination with the Agilent ZORBAX RRHT columns and the increased pressure and temperature range of the LC system, excellent chromatographic resolution can be achieved even at run times below one minute.

This Application Note describes the correct set-up of the instrument which is the key for optimal results with narrow bore columns, such as a 2.1 mm x 50 mm column packed with sub two micron particles. Peak capacities in the range of fifty in analysis times as short as 24 seconds and peak widths as narrow as 200 milliseconds are shown. The well-balanced use of all possible module options to achieve shortest cycle times with throughputs far beyond 1500 samples per day is described.

Agilent Technologies

Introduction

Particularly analytical service laboratories in the pharmaceutical industry, responsible for analyzing chemical libraries[1] or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates[2] are faced with the challenge to increase their throughput, but also to maintain a high chromatographic resolution. In 2003 Agilent Technologies introduced sub two micron particles in their RRHT column series. Because of the small particle size, the chromatographic resolution obtainable with these columns is superior to standard particle sizes such as 3.5 µm or even 5 µm. Due to a unique silica manufacturing process, Agilent ZORBAX RRHT columns show a significantly reduced backpressure, if compared to similar column dimensions of other manufacturers. Excellent chromatographic results are achieved in a very short analysis time with the Agilent 1200 Series Rapid Resolution LC system, which facilitates an increased pressure range and flow rates from 0.05 up to 5 mL/min using column diameters ranging from 2.1-mm id up to 4.6-mm id. This Application Note will focus on 2.1-mm id columns only.
Not only are the run times of the analyses important for high throughput, but also the overhead time. The Agilent 1200 Series Rapid Resolution LC system can be optimized to achieve highest throughputs with exceptionally good overall system performance.

Experimental

An important issue when dealing with narrow bore columns, especially in gradient mode where smallest peak widths can be achieved, is to have small extra column volumes. This also includes any volumes in front of the sampling device, because any volume after the solvent mixing point will increase the time for the gradient composition to reach the column. This results in an increased run time. The Agilent 1200 Series Rapid Resolution LC system can be reconfigured within a few minutes to provide appropriate system volumes for different column ids. Here, the pumps are set-up in the low delay volume configuration with an internal volume of approximately 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744). Consequently, only capillaries of 0.12 mm id are used beyond the injection valve. In the Agilent 1200 Series thermostatted column compartment SL the newly introduced low dispersion heat exchangers with 1.6 µL internal volume were used. In some experiments, the Agilent 1200 Series Rapid Resolution LC is set up for alternating column regeneration to achieve highest throughput using the ACR-capillary kit (G1316-68721) and 2.1-mm id columns[3]. The high pressure rated 2-position/10-port valve in the thermostatted column compartment was only placed into the flow path if alternating column regeneration was used indeed.

The instrument set-up is as follows (figure 1):

- Agilent 1200 Series binary pump SL with the new Agilent 1200 Series micro vacuum degasser
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration
- Agilent 1200 Series diode-array detector SL with a 2-µL/3-mm cell
- ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm

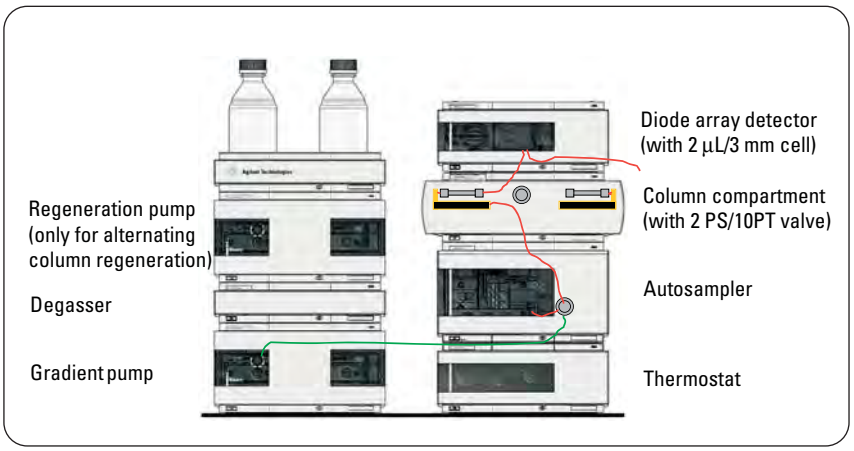

Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and lowest pump ripple. The pump hardware is significantly different from the standard binary pump. In the Agilent 1200 Series binary pump SL the pressure transducer is separate from the damper which has been modified to have a lower delay volume (pressure dependent ranging from 80-280 µL). In this study the pumps were used in the low delay volume configuration without the mixer and damper in the flow path. In contrast to the standard binary pump the pump heads of the binary pump SL have an additional damping coil (500 µL volume each) to allow damping in the low delay volume configuration. This does not add to the gradient delay volume because it is before the mixing point. Anyhow, pressure ripples are also strongly suppressed by the Electronic Damping Control (EDC). The pressure range of the pump and all other modules is increased to 600 bar.

Only one sample, the so-called "phenone-mix", was used in the course of this study to keep variations low. The sample consists of nine compounds: acetanilid, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. Unless otherwise stated, the concentration was 0.1 µg/µL for each compound except butyrophenone which was 0.2 µg/µL. The solvent was water-acetonitril 2:1.

Results and discussion

The most frequently sold particle size in chromatographic columns today is 5 µm. Of course, fast and ultra fast LC is also possible with columns packed with particles of these larger diameters – the reduced back pressure is even beneficial to allow higher flow rates. However, resolution will be sacrificed because conditions are usually far on the right side of the van-Deemter-optimum. Here, the big advantage of the RRHT columns with particles of less than 2 µm diameter is proven. The van Deemter optimum is shifted further to the right and the curve is much flatter at the onset because the "resistance of mass transfer" term is diminished (figure 2). In figure 3 the analysis on a 2.1-mm id column with 1.8-µm particles is compared to the linear scaled analysis on the same stationary phase but on 5 µm particles packed in a 4.6-mm id-column. The gain in resolution is obvious – from Rs = 2.1 up to Rs = 3.5 for the critical pair which matches the theoretically expected value of a 1.66 fold increase in resolution. Also note that there is a saving in solvent consumption of 8.6 mL in the "standard" HPLC analysis and only 1.8 mL in the ultra fast HPLC analysis.

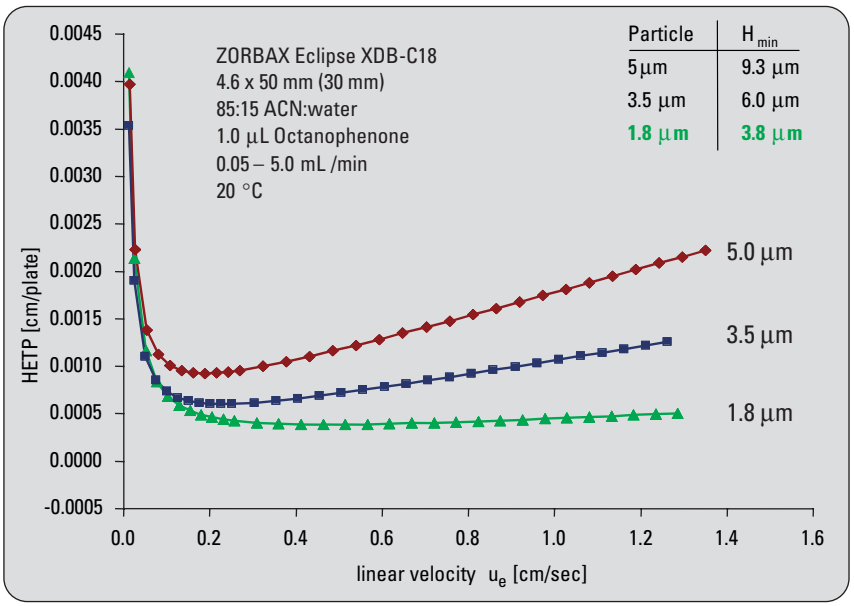

Figure 2
Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.

For gradient separation the dependencies of the capacity factor can be expressed as:

$$k^* = 0.87 \cdot tg \cdot \frac{F}{Vm \cdot \Delta\%B \cdot S}$$

(tg = gradient time, F = flow rate, Vm = column void volume, △%B = gradient steepness, S = solvent and solute dependent factor)

If the product of the gradient time and flow rate, the so-called gradient volume, is kept constant together with all other parameters, the gradient time might be decreased while the flow rate is increased. Thus, the capacity factors of two compounds will stay constant and if no large alteration of the plate height occurs, the resolution will not change significantly, either. The final point is the big advantage of the sub two micron particles – the van-Deemter curve is nearly flat on the right side of the minimum (figure 2) and flow rates can be increased with only little increase in plate heights. However, the equation is an empirical one and deviations may occur especially under extreme conditions.

With a two-step approach, highest gradient speeds with virtually no loss or only little loss in resolution can be achieved. In the first step, start from a medium temperature and begin to increase the flow rate up to the pressure maximum. Subsequently the temperature should be increased to lower the viscosity of the solvent and then the flow rate is increased again. It may be worthwhile to check the resolution with two identical gradients but with different temperatures to see the influence of the temperature change on the resolution which may be very compound dependent. In figure 4 the result of this approach is shown. A nearly 7-fold increase in separation speed could be achieved with still baseline separation of the critical pair before meeting the pressure and temperature limit (the maximum temperature is a function of flow, temperature, number of controlled Peltier elements and of the heat capacity of the solvent used).

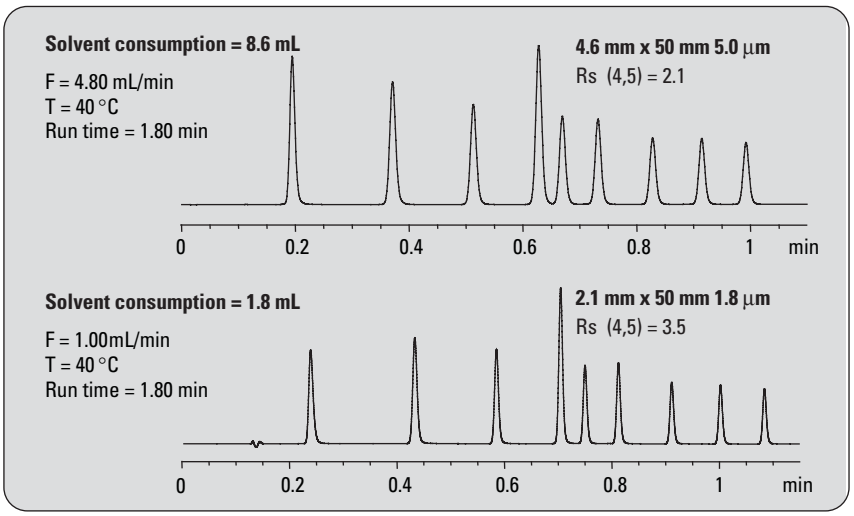

Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

Conditions:	4.6-mm id column used on standard Agilent 1200 system	
Solvent:	A = Water, B = ACN	
Temperature:	40 °C	
Column:	**2.1 mm x 50 mm, 1.8 µm**	**4.6 mm x 50 mm, 5.0 µm**
Flow:	1.0 mL/min	4.8 mL/min (scaled from 2.1 mm col.)
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 % B	1.11 min 35 % B
Stoptime:	1.15 min	1.15 min
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	245 nm (8), ref. 450 nm (80)
Peakwidth:	>0.0025 min (0.05 s res.time), 80 Hz	>0.01 min (>0.2 s), 20 Hz
Injection volume:	1 µL	5 µL (not scaled)

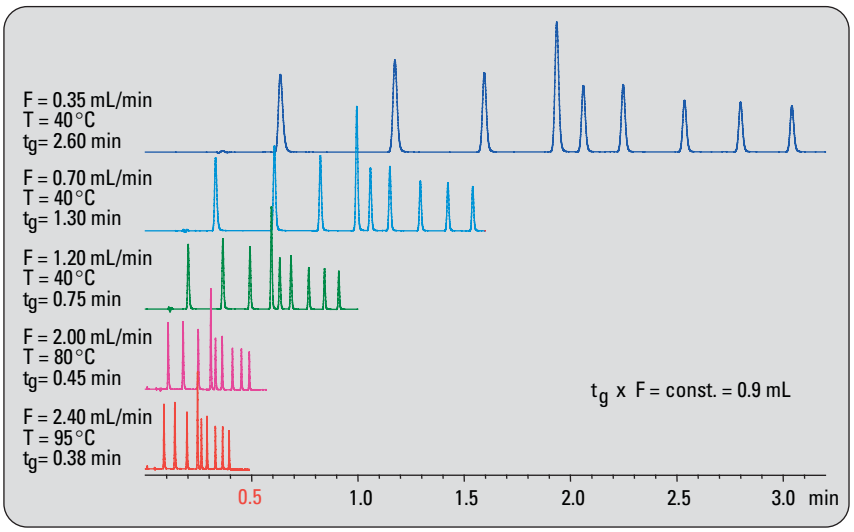

Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

Conditions:
Solvent: A = water, B = ACN
Temp.: 40 °C, 80 °C, 95 °C
Flow: 0.35, 0.70, 1.20, 2.00, 2.40 mL/min
Gradient: 0.00 min 35 %B
2.60 min 95 %B
3.20 min 95 %B
3.21 min 35 %B
Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL
Stop time: 3.20 min
Post time: 2.00 min
Wavelength: 245 nm (8), Ref. 450 nm (100)
Peak width: >0.0025 min (0.05 s response time), 80 Hz

The last chromatogram is enlarged in figure 5 and reveals the details of this separation. The first peak is eluted after only five seconds and peaks with a width at half height of less than 200 ms are achievable. Within twenty-four seconds nine compounds are separated with a peak capacity in the range of fifty.

Retention time precision at highest analysis speed

High analysis speed is meaningless without precision. One basic performance criteria for HPLC pumps is the precision of gradient formation measured by the precision of retention times of repeated gradients. However, the stability of the column temperature must also be taken into consideration, because temperature fluctuations will also influence the retention times of a given sample. In table 1 and figure 6 the results from the 10-fold repeated analysis of a standard sample are listed and since the deviation between individual runs is so small, the octanophenone peak is enlarged in a separate window. This sample contains compounds that are both not retained and refer to isocratically eluted compounds found at the starting conditions of the gradient, as well as highly unpolar and strongly retained compounds. The analyses

Conditions:

Solvent:	A = Water, B = ACN
Temp.:	40 °C, 80 °C
Flow:	0.35 mL/min, 1.20 mL/min, 2.0 mL/min
Gradient:	0.00 min 35%B
	2.60 min 95%B
	3.20 min 95%B
	3.21 min 35%B
	Time values for F = 0.35 mL/min.
	For all other flow rates times are
	scaled so that (time x flow) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Injection vol.:	1.0 µL

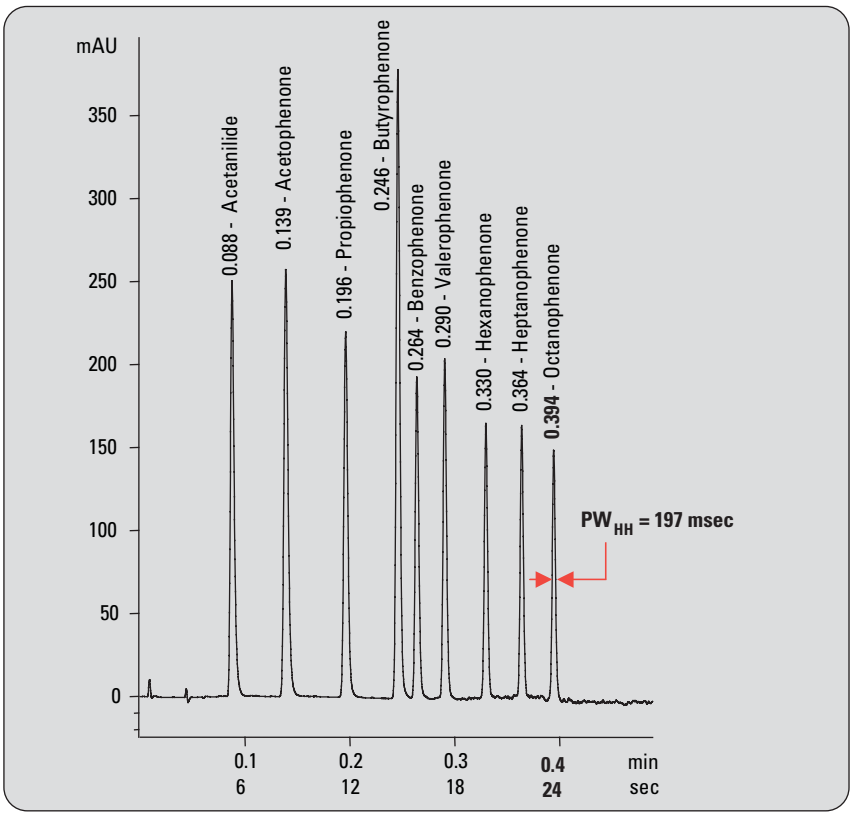

Figure 5
Separation of a nine compound mixture under ultra fast conditions.

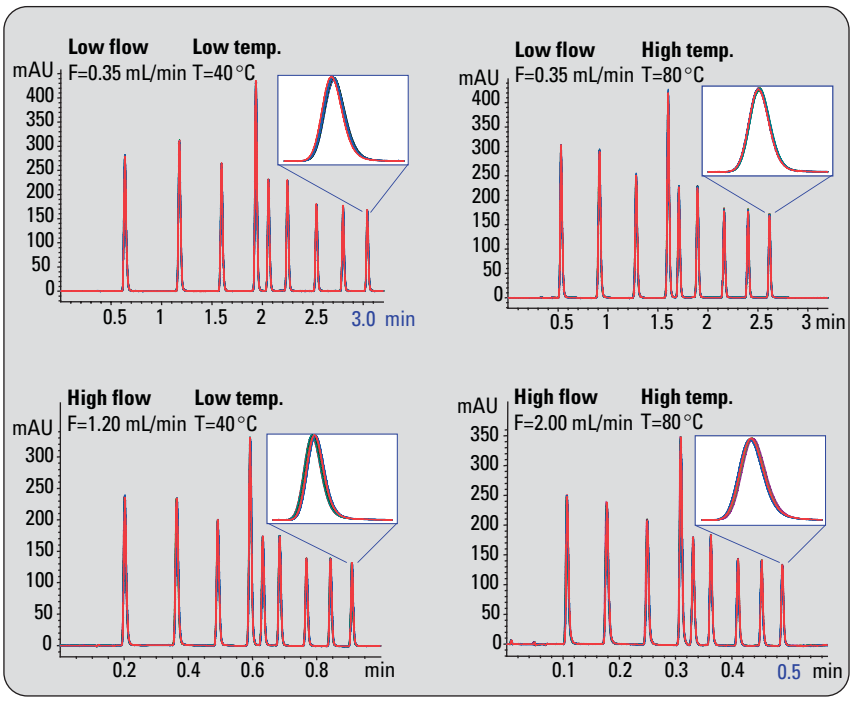

Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

were done at high and low flow rates as well as with high and low temperatures as in the examples shown earlier. In all cases the mean retention time precision is below 0.3 % RSD, which was the specification of the Agilent 1100 Series LC system. Of course, the results are also in line with the specifications for the new Agilent 1200 Series Rapid Resolution LC system which is < 0.07 % RSD or < 0.02 min SD, whichever is met first. At these high gradient speeds, the SD criteria are always met. The RSD criteria are also met for both fast-LC gradients of 2.6 min duration (0.35 mL/min flow rate). Even at ultra-fast gradient speeds, the retention time precisions are still below or only slightly higher than 0.1% RSD (table 1).

Improving the cycle-time

Not only is the gradient speed important when dealing with high-throughput analysis but furthermore the over all cycle time of the entire system, which is the time between two consecutive analyses. A good method to measure the cycle time is by using the time stamp the data file is assigned by the operating system of the computer. Clearly, optimizing the cycle time has some drawbacks. For example, extensive needle cleaning procedures are in contradiction with a high sampling speed. Table 2 gives an overview of important parameters influencing the cycle time. Using 1.8-µm particle size columns together with an optimized HPLC system very short run times can be achieved without sacrificing chromatographic resolution. Combining short run times together with low overhead times will result in a high daily throughput. In figure 7 the cycle time and daily throughput is shown for two

	0.35 mL/min, 40°C		0.35 mL/min, 80°C		1.20 mL/min, 40°C		2.00 mL/min, 80°C	
	SD	% RSD	SD	% RSD	SD	% RSD	SD	% RSD
Average	0.00107	0.067	0.00084	0.070	0.00048	0.098	0.00031	0.134

Table 1
Standard deviations (mAU) and %RSD (n=10) of the retention times under different chromatographic conditions in temperature and flow.

Module	Parameter	Effect on cycle time	Other effects
Pump	Low delay volume setting	Reduced retention times, run time can be shortened, reduced cycle time	Increased pressure ripple, slightly increased mixing noise if modifiers such as TFA are used.
Autosampler	Automatic Delay Volume Reduction (ADVR) – activated	Reduced delay volume, reduced retention times, run time can be shortened, reduced cycle time	Increased carry-over
	ADVR activated and Overlapped Injection (OI)	Enables parallel sampling, thus reduces the cycle time independently of the below listed settings (as long as the overall sampling speed does not exceed the gradient and post time)	Increased carry-over
	no OI – Needle Wash	Increased sampling time with increasing wash time	Reduced carry-over with longer needle wash time
	no OI – Equilibration time	Increased sampling time with increased equilibration time	Better injection precision with longer equilibration time
	no OI – Draw/Eject speed	Low speed causes increased sampling time	Low speed results in better injection precision
Column compartment	Alternating column regeneration	Saves column wash-out and equilibration time, reduces cycle time enormously	Additional hardware required, slightly increased extra column volume, slightly different retention times between columns possible
Detector	Pre-run and/or post-run balance	Increased cycle time	Baseline drifts possible if not applied
	Spectral data acquisition with high data rate, small band width and broad wavelength range large data files	Depending on computer power and additional processes running might increase cycle time because of writing speed	Reduced information content if no spectral data acquired or with lower resolution
Software	Data analysis with acquisition	Increased cycle time, depending on computer power and number of peaks	Data analysis has to be done offline is no set
	Save method with data	Slightly increased cycle time	Information is missing if method is not saved
	Execution of pre-run or post-run macros	Increased cycle time, depending on macro	Depending on macro
System	LC controlled over local network between computer and LC (and MS) only	Faster data and method transfer between computer and LC because of reduced net work traffic reduced cycle time	Additional hardware might be necessary (use independent acquisition computer)
	Number of detectors	More detectors produce a higher data amount and lower the data transfer speed, resulting in higher cycle times	More detectors higher information content

Table 2
Influence of various parameters on the overall cycle time.

different methods – both giving virtually the same resolution. The first method (0.45 min gradient) utilizes alternating column regeneration and high temperatures to allow high flow rates and speed optimized settings. A cycle time of 49 s could be achieved, resulting in a theoretical daily throughput of more than 1700 samples per day. The second method (0.90 min gradient) does not use high temperatures or alternating column regeneration and the time saving of some simple and often forgotten method options are shown. By optimizing these parameters the real cycle time gets as close to 8 s to the run time (stop time plus post time) and allows a daily throughput of more than 700 samples per day. By sub-optimal method set up this can easily drop to below 500 samples per day if options like automatic delay volume reduction, overlapped injection or offline data-analysis are not used.

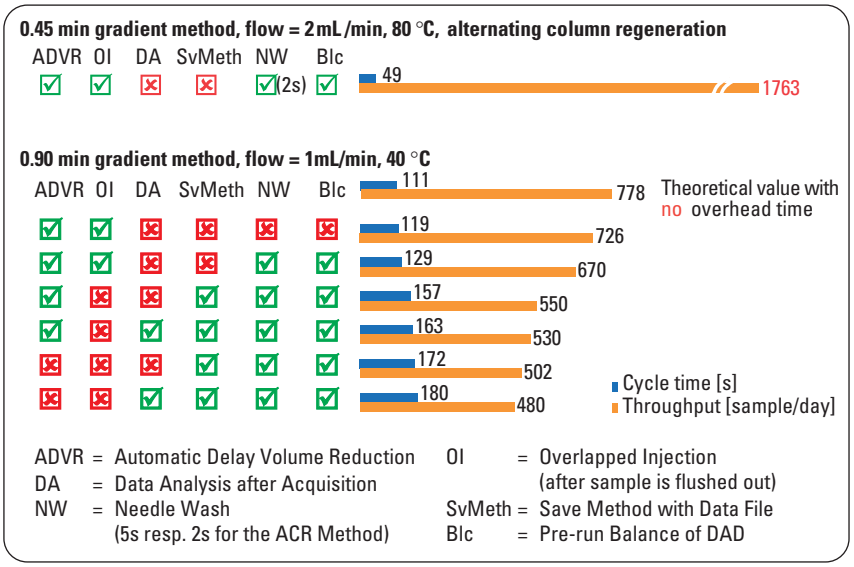

Figure 7
Cycle time and daily throughput optimization.

Chromatographic conditions:

Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	80 °C	
Flow:	2.0 mL/min	
ADVR:	Yes	
Gradient:	**Gradient-Pump**	**Regeneration-Pump**
	0.00 min 35 %B	0.00 min 35 %B
	0.45 min 95 %B	0.01 min 95 %B
	0.46 min 35 %B	0.11 min 95 %B
	0.57 min 35 %B	0.12 min 35 %B
Stoptime:	0.57 min	no limit
Posttime:	off	off
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	none	
Injection volume:	1.0 µL	
Injector:	Overlapped injection, 2 s needle wash, sample flush-out factor = 10, draw/eject speed = 100 µL/min	
Valve:	next position	

No Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	40 °C	
Flow:	1.0 mL/min	
ADVR:	Yes	No
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 %B	1.11 min 35 %B
Stoptime:	1.15 min	*1.40 min (add. 300 µL extra column volume, increased retention times)*
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	all, 190-500 nm, BW = 1 nm	
Injection volume:	1.0 µL	
Injector:	See figure 7, 2 s equilibration time	

Conclusion

The Agilent 1200 Series Rapid Resolution LC system is a powerful tool to achieve highest chromatographic resolutions and also highest throughputs. The extended pressure range allows the usage of columns packed with stationary phases with particles sizes below 2 µm, for example, Agilent RRHT columns with particle sizes of 1.8 µm. These columns not only allow an increase in linear flow rates with virtually no loss in resolution but also have an inherently higher resolution compared to 3.5 µm or even 5.0 µm particle sizes. The possibility to switch the pump into its low delay volume configuration allows the use of the entire bandwidth of today's widely used column ids – from 4.6 mm down to 2.1 mm and even 1.0 mm. As illustrated above, the system has uncompromised performance characteristics even at highest gradient speeds.

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www.agilent.com/chem/1200rr

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Published March 1, 2006
Publication Number 5989-4502EN



Agilent Technologies

Process Monitoring of Bisphenol-A in Industrial Feedstock using High Throughput HPLC

Application

Process Control

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Abstract

The chromatographic method used to monitor the Bisphenol-A manufacturing process was improved using Agilent RRHT Eclipse XDB-C18 columns. These columns use 1.8-µm particles versus conventional 3.5-µm or 5-µm particles. The improved method allowed seven times faster analyses, improved resolution, and higher sensitivity.

Introduction

Bisphenol-A (Figure 1) is a highly versatile material used to manufacture many modern products. It is also known as 4,4"-Isopropylidenediphenol, 4,4"-(1-Methylethylidene) bisphenol, or simply BPA.

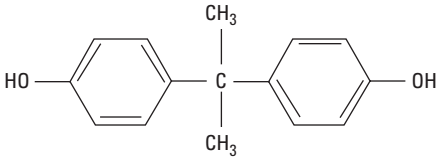

Figure 1. Bisphenol A

Every year, 2.8 million tons of BPA are produced. BPA is a building block for polycarbonate plastic and epoxy resins. Polycarbonate plastic is prized for its scratch resistance, optical clarity, and heat and electrical resistance. Because of these attributes, it is used for eyewear, CD/DVD disks, electronics, and food and drink containers. Epoxy resins are used for protective coatings because of their combination of inertness, chemical resistance, adhesion, and formability. For example, metal food cans are lined to protect taste. Epoxy resins are also used as a component in dental sealants and as a component in dental composites providing an alternative to mercury amalgam in veneers and fillings. Other uses include fungicides, polymer antioxidants, and components in automobiles and appliances.

BPA is produced through an acid-catalyzed condensation reaction of phenol with acetone. During condensation, a number of phenol-based byproducts are also formed. HPLC is used to determine the composition of many of the process streams in a commercial BPA plant.

Here we describe the use of new HPLC column technology for the possible improvement to one of the HPLC methods used in a commercial BPA facility.

Method Optimization and Scalability

The existing HPLC method was proven and robust; however, it was complicated. We sought a similar chromatogram, based on the original method, but using simpler method parameters. Because of the challenge of changing many chromatographic parameters, essentially redeveloping the method, we chose a 4.6 × 50 mm, 1.8-µm Eclipse XDB-C18 column for experiments to reduce the time required. Smaller particles packed in shorter columns increase the speed of analysis and still provide enough efficiency to maintain resolution equivalent to longer columns packed with larger particles. After several trials, we developed a method that produced a chromatogram similar to the original. The short analysis time is a major advantage of Rapid Resolution High Throughput (RRHT) technology. Whereas a handful of experimental runs would take an entire work day using a typical analytical-sized column (50 min/run), the series of runs took about an hour (7.5 min/run), using an RRHT column.

We incrementally scaled up to a 4.6 × 250 mm column. Figure 2 shows an overlay of the sample analyzed by three 4.6-mm id columns of different lengths and particle sizes. Injection volume was also changed proportionally to length. The smaller ZORBAX particles speed up the analysis while maintaining resolution. In fact, resolution increased when using the RRHT columns despite their shorter length.

One reason this method can be easily scaled (up or down) is the uniform spherical Eclipse XDB-C18 packing. It has a proprietary engineered particle size distribution, based on ZORBAX silica with a controlled surface area and pore size. The robust proprietary packing material and proven column manufacturing techniques consistently yield reproducible columns with similar chromatographic performance, independent of the column dimensions.

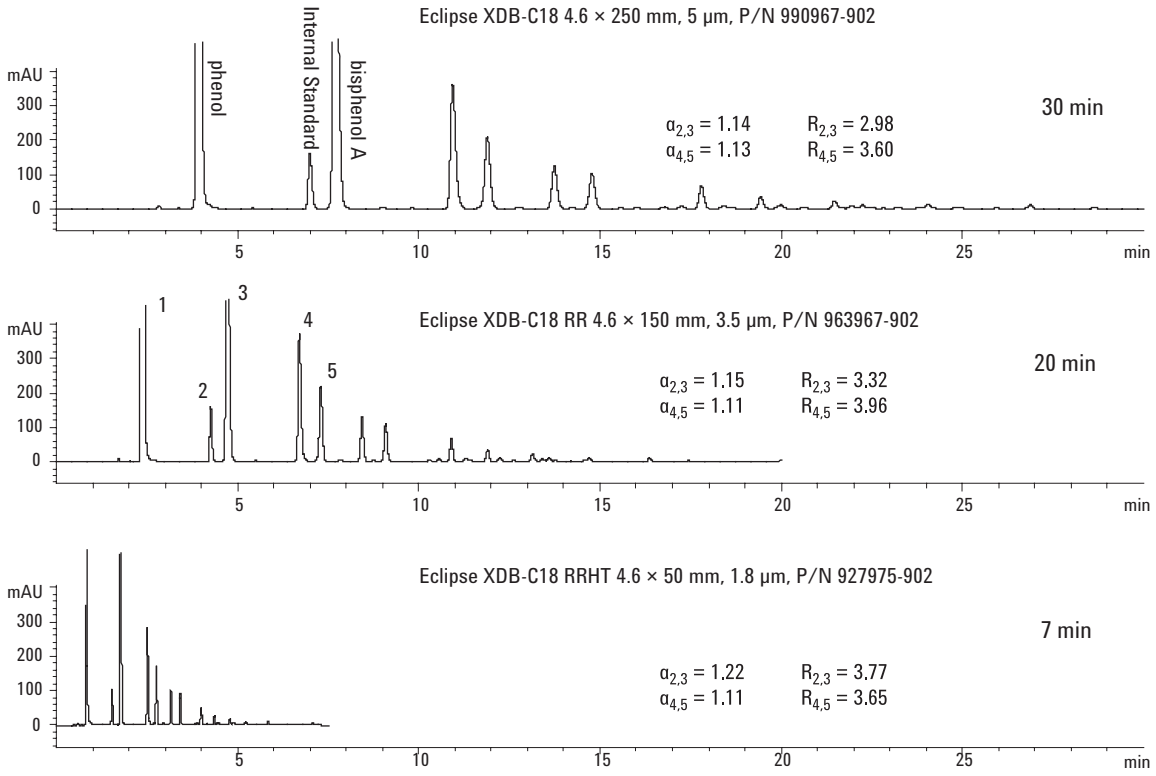

Figure 2. RRHT column configuration increased both speed and resolution.

Particle size does influence resolution. The influence can be noticed when comparing columns of identical dimensions, packed with three different particle sizes. Figure 3 shows the shortened Bisphenol-A analysis using different particle-sized Eclipse XDB-C18 columns. Resolution (Rs) is related to selectivity (α), efficiency (N) and retention (k´):

$$Rs = (1/4)(\alpha-1)\sqrt{N}\,[k'/(1+k')]$$

Factors affecting the selectivity term (stationary phase, mobile phase) and retention term (mobile phase, temperature) are constant for the three chromatograms. The efficiency term is influenced by column length, linear velocity of the mobile phase (both constant), and particle size (varied in Figure 3). N increases as particle size decreases. In Figure 3 the selectivity factors (α) and retention remain about the same, but resolution actually increases. The increase in resolution due to the decrease in particle size highlights the advantage of using smaller particles. The similar selectivity and retention highlight the suitability of ZORBAX Eclipse XDB-C18 columns for scaling methods, especially to more rapid, high-throughput methods.

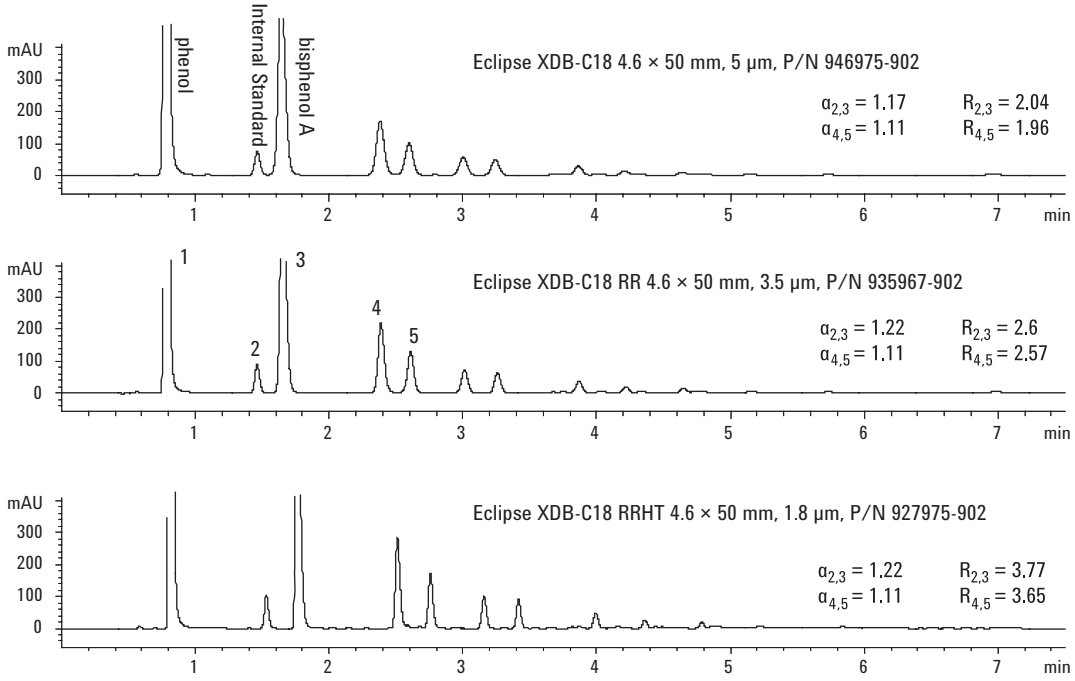

Figure 3. Effect of particle size on resolution and selectivity.

Comparing the Existing Method to the RRHT Method

Figure 4 compares the original BPA separation to the RRHT separation. The top chromatogram is an example of the analysis using the original commercial method, and the bottom is an example of the process sample analyzed with the RRHT method. The method developed with the new column technology clearly increases productivity.

Analysis time is reduced at least six-fold; solvent consumption is reduced about 12.5 times, from 100 mL/analysis to only 7.5 mL/analysis. Interestingly, the peak shape of Bisphenol-A is more symmetrical using Eclipse XDB-C18 as compared to the current C18 column used in the original analysis. The more Gaussian peak shape eluted by the Eclipse XDB-C18 column is important for accurate quantification. Other method improvements such as a simplified gradient and a binary mobile phase are listed in Table 1.

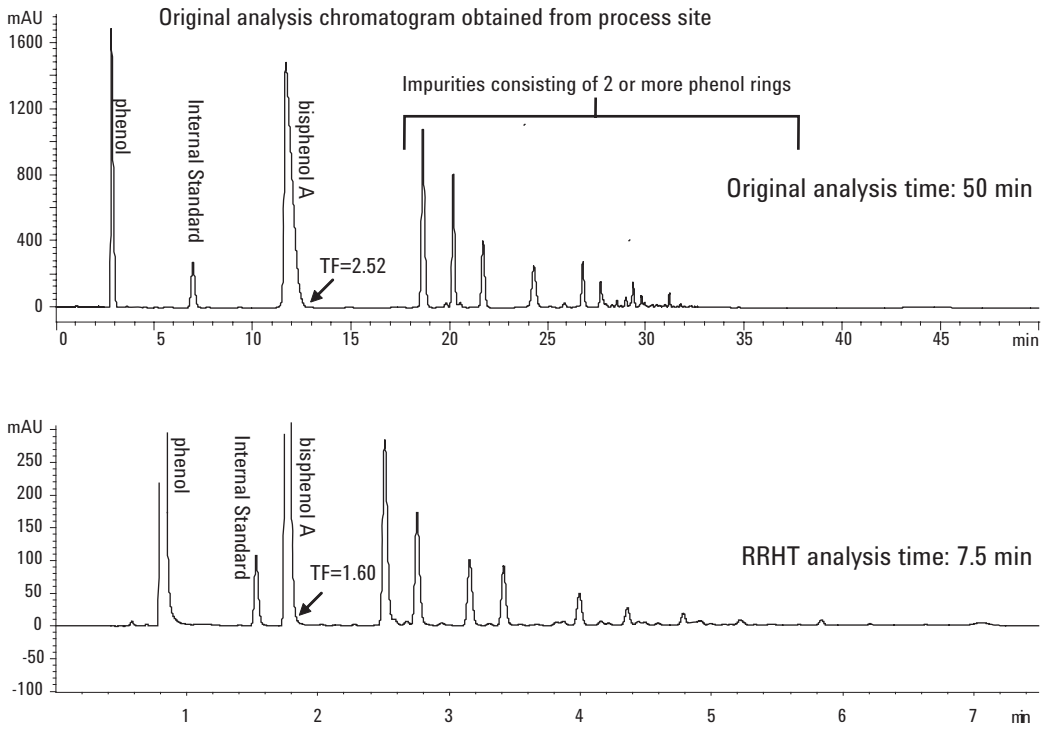

Figure 4. Comparison of methods; original to RRHT.

Table 1. Current and Improved Method Parameters

Original

- Column: Supelco LC-18, 4.6 × 250 mm, 5 µm
- Mobile phase: A: 0.025% H_3PO_4, B: ACN, C: MeOH
- Flow: 2 mL/min
- Temperature: 35 °C
- Sample size: 20 µL
- Gradient: segmented, has isocratic holds

Time	% A:B:C
0	65:25:10
13	65:25:10
18	50:40:10
23	50:40:10
27	30:50:20
32	0:70:30
35	0:70:30
36	0:60:40
40	0:50:50
43	0:20:80
48	65:25:10

RRHT

- Column: ZORBAX XDB-C18, 4.6 × 50 mm, 1.8 µm
- Mobile phase: A: 0.1% formic acid, B: ACN: MeOH (200:800)
- Flow: 1 mL/min
- Temperature: 25 °C
- Sample size: 2 µL
- Gradient: linear, no isocratic holds

Time	% B
0	60
6	95
6.01	60
8	60

Conclusion

Converting an existing method to a high-throughput method is one way to improve lab productivity. Using RRHT columns initially for method development also improves productivity. Eclipse XDB-C18 RRHT columns are a good choice for converting existing C18 methods into high-throughput methods. Smaller particles packed into shorter columns provide comparable resolution to larger particles packed into longer columns in a fraction of the time. RRHT columns are advantageous for gradient method development because gradient re-equilibration is time-consuming and often overlooked in the total analysis time. Methods developed on Agilent RRHT columns can be scaled easily because of the highly uniform particles, bonded phase chemistry, and column manufacturing techniques. An existing method developed on a "traditional analytical-sized" column was easily converted to a high throughput method using an Eclipse XDB-C18 RRHT column. The method was incrementally scaled up to an analytical-sized column, and it performed with predictable results on various column dimensions and particle sizes. The predictability of the results supports Eclipse XDB-C18 RRHT columns' ability to easily improve applications and transfer them into high-throughput and high-resolution applications.

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Printed in the USA
July 26, 2006
5989-5231EN



Agilent Technologies

Improving Productivity and Extending Column Life with Backflush

Application Brief

Chin-Kai Meng

All Industries

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, μECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

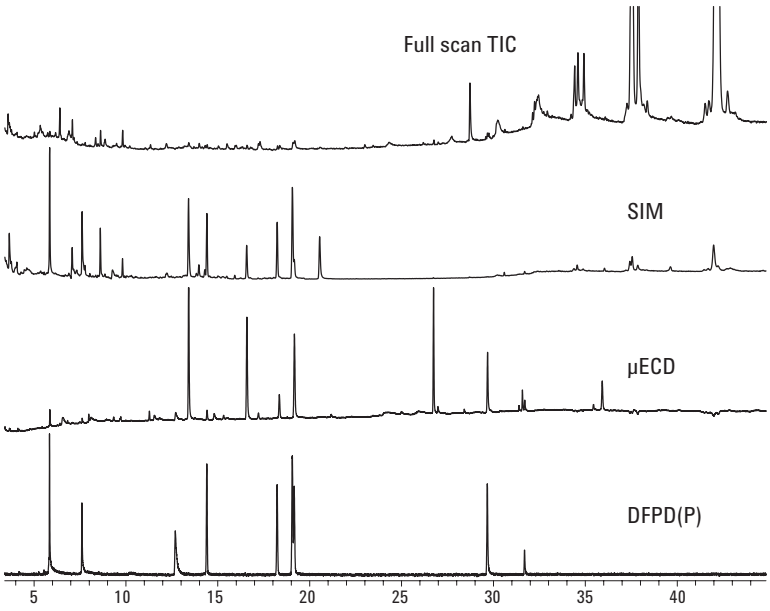

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

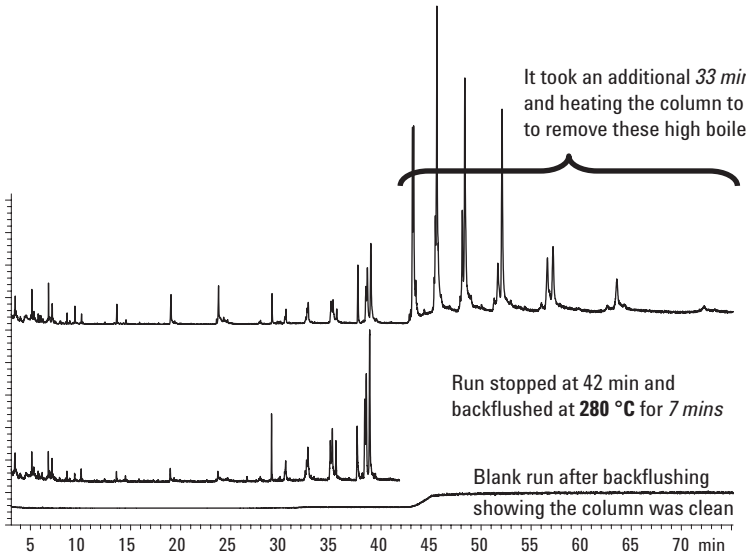

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

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2. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Application Note, 5989-5111EN, June 2006.

Acknowledgement

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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Printed in the USA
December 26, 2006
5989-6018EN

Comprehensive GC System Based on Flow Modulation for the 7890A GC

Application Brief

Introduction

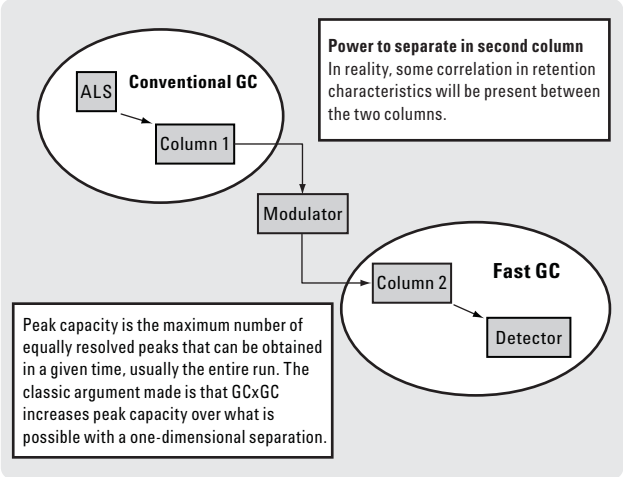
A hardware solution is available on the 7890A for the practice of comprehensive GC. The system uses a capillary flow modulator controlled by the 7890A GC. The system is offered with factory checkout using an FID detector. Other detectors, preferably those operating at 50 Hz or greater, can be used.

Comprehensive two-dimensional (2D) GC, or GCxGC, is a powerful technique that can be used to separate very complex mixtures, such as those found in the hydrocarbon processing, environmental, and food/fragrance industries.

The method uses two columns, typically of very different polarities, installed in series with a modulator in between. The second column is much shorter than the first column to effect a fast separation. The entire assembly is located inside the GC oven.

The modulator performs three functions:

1. It collects effluent from the first column for a fraction of the time equal to peak width. For example, if a peak from column one is six seconds wide, the modulator will accumulate material every two to three seconds, thereby dividing the peak from the first column into two or three "cuts."
2. It focuses the material collected from each cut into a very narrow band through flow compression.
3. It introduces the bands sequentially onto the second column, resulting in additional separation for each band injected onto the second column.



Comprehensive 2D GC uses a primary column (conventional separation), a flow modulator, a second column (very fast separation), and a fast detector.

This technique provides a second dimension of information that can increase the peak resolution and capacity.

A number of different modulator designs have been described in the literature, most relying on thermal cycling to focus the bands from the first column and release them into the second column. Some disadvantages to this approach are:

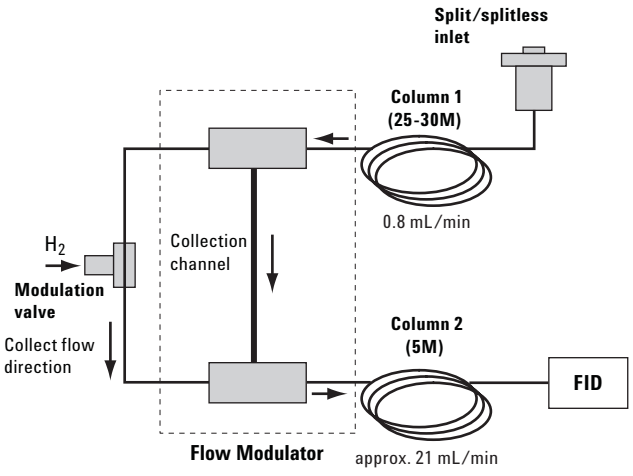
- Large usage of expensive cryogenic gases leading to a high cost of analysis
- Complexity of the hardware
- Longer analysis times

Agilent's proprietary Capillary Flow Technology and fourth-generation Electronic Pneumatics Control (EPC) enable the use of a differential flow modulator to conduct comprehensive 2D-GC without the use of cryogenic gases or complex hardware.

The key to operation is the flow differential (typically 20 to 1) between the second and first columns, respectively. This compresses and focuses the analytes present in any given modulation "inject" pulse into the second column. Precise timing of the modulator is made possible by installing a driver board in the Aux det 2 detector slot of the 7890A mainframe.

The Capillary Flow Technology modulator uses a deactivated, stainless steel structure with all flow splitters and the collector channel incorporated internally in the device. It has low thermal mass so it can track the oven temperature very closely, and its GC oven location allows precise temperature control without lag during programmed runs. All external connections are made using Agilent's Ultimate Union technology for leak-free operation and extremely small, well-swept volumes. A micro three-way solenoid valve, installed on the side of the gas chromatograph, connects to a pneumatics control module (PCM) to accurately and precisely control the flows through the modulator.

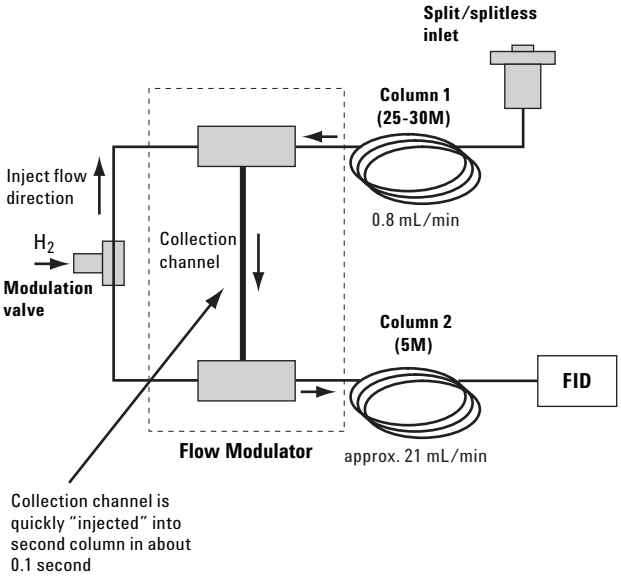
The figures below illustrate the modulator. A three-way solenoid valve receives a controlled supply of hydrogen gas from a PCM. The periodic switching of this three-way valve drives the modulator. The precisely timed and synchronized switching between the collect and inject states directs discrete sample pulses continuously to the second column for additional fast separation throughout the chromatographic run. Both columns are run in constant flow mode. For optimal performance, injection size and split ratio should be carefully adjusted to avoid overloading, which can lead to excessive peak tailing.



Flow rates and flow directions during the load or collect portion of the modulation cycle

Load or collect state (above): At the beginning of this state, the collection channel is filled with hydrogen gas from a previous injection cycle flush.

The primary column effluent enters the modulator's top tee connection and flows into the collection channel. The analytes from this column enter one end of the collection channel. Hydrogen flow from the PCM/three-way micro valve exits the modulator at the bottom tee and is sent to the second column.



Flow rates and flow directions during the transfer or inject portion of the modulation cycle

Inject or flush state (above): Hydrogen gas flow from the three-way solenoid valve is directed to the top tee. A high flow of typically 20 mL/min for about 0.1 second rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes collected in the channel undergo rapid separation.

What is required:

- Agilent 7890A GC with firmware version A.04.06 or higher
- FID with 200 Hz data collection rate or other fast detector
- Split/splitless inlet
- Capillary Flow Technology modulator option or accessory
- Capillary Flow Technology modulator checkout kit
- Pneumatics control module (PCM)
- Agilent GC ChemStation B.03.02 or other data collection and analysis system that can control the flow modulator cycle
- 30-m × 0.25-mm × 0.25-µm DB-5ms column (included with option or accessory)
- 5-m × 0.25-mm × 0.15-µm INNOWax column (included with option or accessory)
- 2D data analysis software, GC Image recommended (not provided by Agilent)
- Internal column nuts and SilTite ferrules

Ordering Information

Description	Part number
7890A GC with Capillary Flow Technology Modulator (requires checkout kit)	G3440A Option 887 or accessory G3486A
7890A GC with 200 Hz FID	G3440A Option 211 or accessory G3462A
7890A GC with split/splitless inlet	G3440A Option 112 or accessory G3452A
Capillary Flow Technology modulator checkout kit	G3487A
PCM for 7890A GC	G3440A Option 309 or accessory G3471A
SilTite metal ferrules, 1/16-in × 0.4-mm id, 10/pk, includes 2 column nuts	5184-3569
Agilent 32-bit ChemStation for 1 GC	G2070BA
Agilent 32-bit ChemStation Bundle for 1 GC includes: – G2070BA 32-bit ChemStation software – Computer with monitor and Windows operating system – Printer	G1875BA
2D GC software Recommend GC Image software, which can be purchased from Zoex Corporation	www.zoex.com

Application Examples

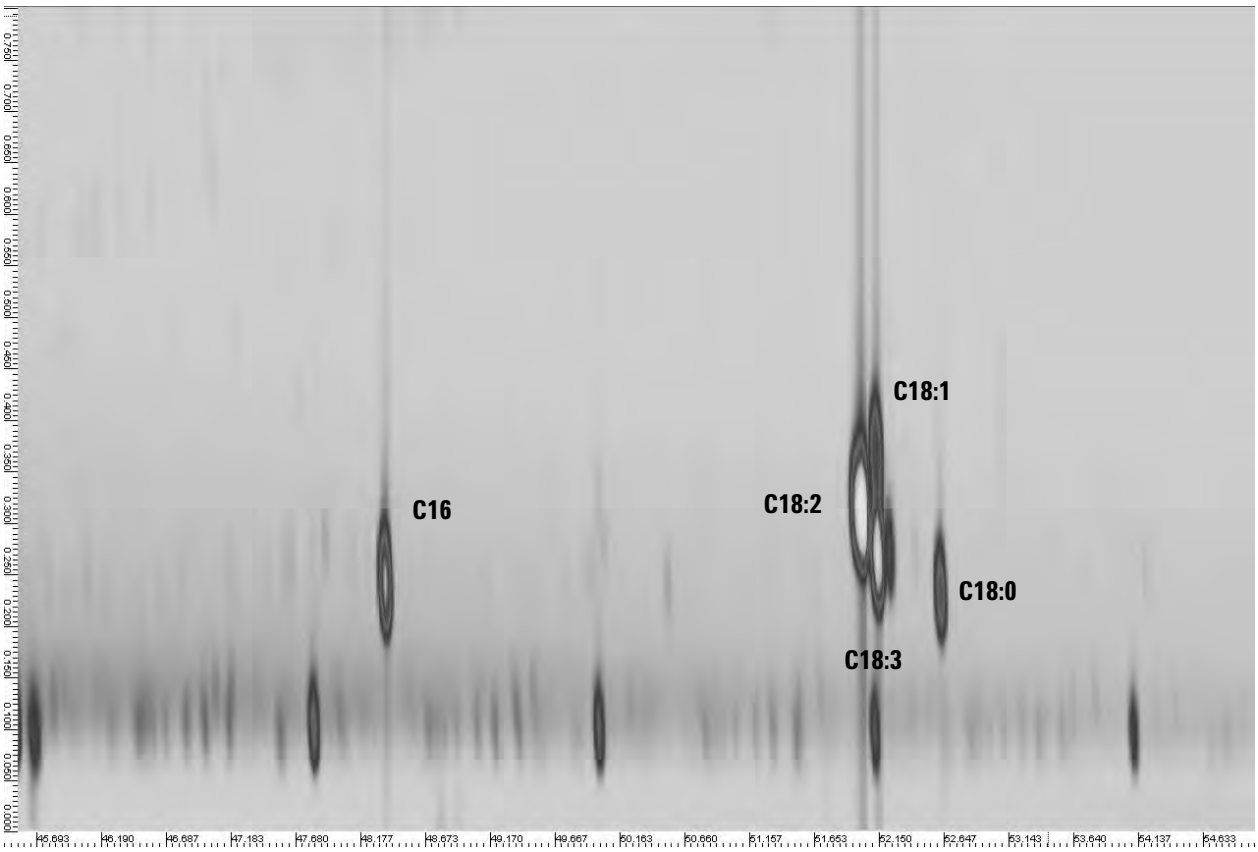
Several applications are shown. Note that primary column lengths have been chosen to give optimal results. While the 30M column that is shipped with the system is an excellent choice for a wide range of applications, other lengths can be used to optimize a given separation. Various columns have been used in these examples to illustrate some of the possibilities. The GC Image software package was used for processing the ChemStation data.

1. B20 biodiesel based on soy FAMES. Section of the 2D image showing the C16 and C18 FAMES is shown.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

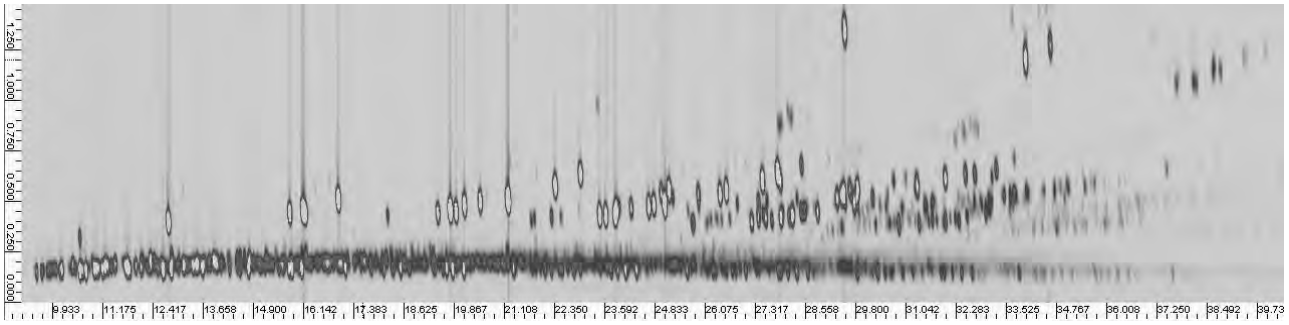


2. Complete 2D image of a sample of heavy gasoline. Each series of substituted 1-ring aromatics is well separated, making hydrocarbon class grouping possible.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

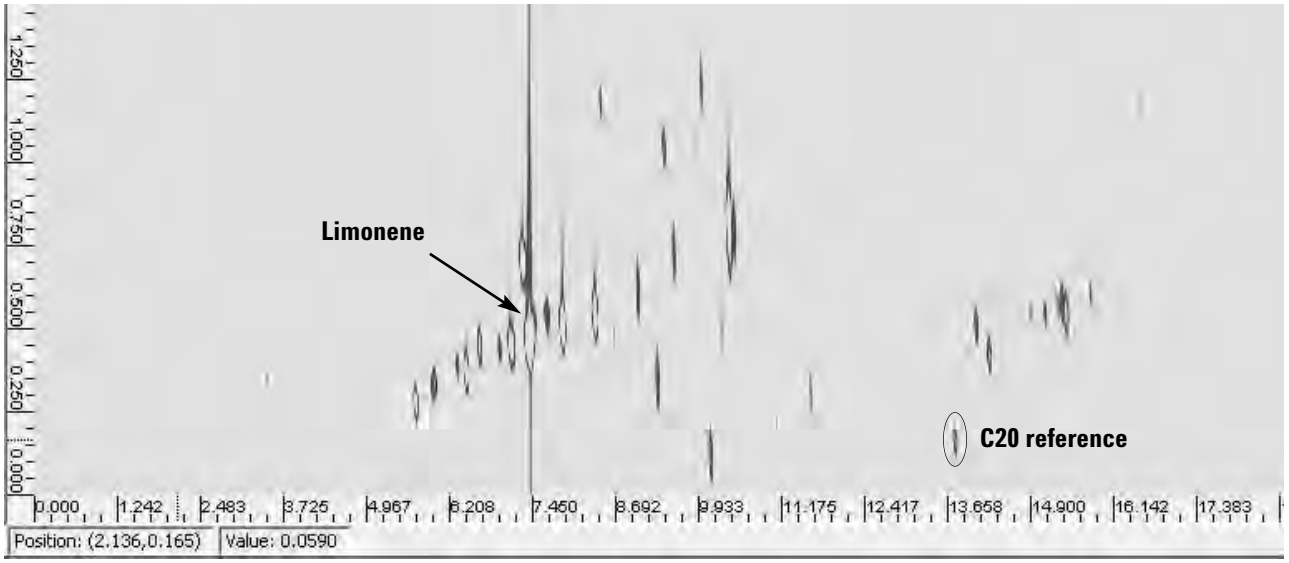


3. Lime oil 2D image.

   Column 1: 15 m × 0.25 m × 0.25 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm DB-17HT

   Modulation: 1.40 s load, 0.10 s inject



Thermal vs. Flow Modulation

Since competitors offer only systems based on thermal modulation, the following table summarizes the key points about the respective approaches of thermal vs. flow modulation.

Thermal modulation	Differential Flow modulation
Cryo-focusing provides potentially narrower peaks in second dimension	Peak widths comparable to thermal. Usually no more than 20% wider. Many users want to sum regions of peaks where peak width is not as critical
Lower flows – Can be used with high-vacuum detectors (TOF)	MSD can be used with a splitter over limited scan range
Large consumption of cryogen	No cryogen required
Complex hardware design, set-up, and maintenance	Simple, reliable Capillary Flow Technology based hardware; small thermal foot print
Long chromatographic runs required for best performance	Run times comparable to a 1D separation
System price (estimate) $60 to $70K	Agilent system approximately $60K (list)

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Printed in the USA
April 1, 2008
5989-8060EN



Agilent Technologies

Ultra-low level impurity analysis by capillary zone electrophoresis

Application Note

Pharmaceutical

Author

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Abstract

Capillary zone electrophoresis (CZE) is an inherently high efficiency liquid phase separation technique. This makes it very suitable for the separation of closely related compounds and can be of particular use in the analysis of drug impurities. Regulatory requirements demand the demonstration of the purity of any drug substance and the acceptable criteria for presence of impurities are around 0.1 %. This means that the analysis not only has to have an adequate sensitivity, but also that the linear range is such that the minor component may be quantitatively reported as a corrected (area/area) percent of the main component. By using the Agilent CE high sensitivity detection cell, sensitivity can be increased by an order of magnitude, with linearity increased over 3-fold compared to conventional capillaries. This enables the determination of impurities in drug substances below the 0.1 % area/area level. Figure 1 shows the linear range achievable using the high-sensitivity cell, illustrating its linearity over 2.2 AU.

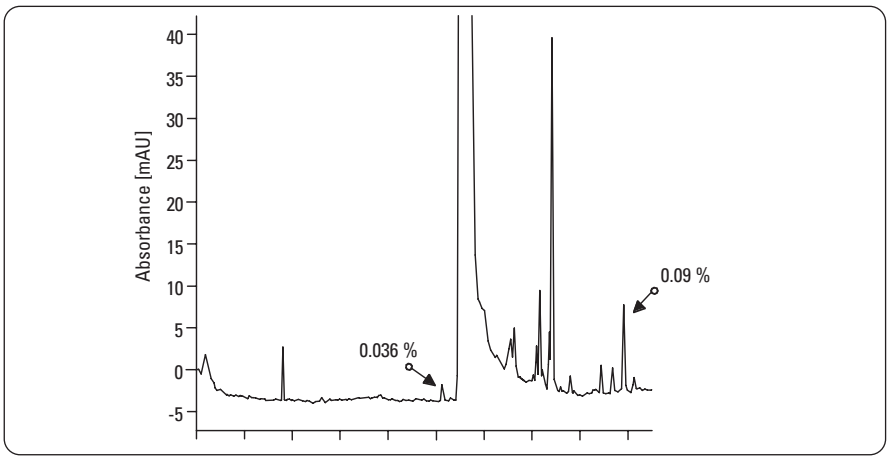

Figure 1
Linear range of high sensitivity cell.

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Experimental

All experiments were performed on the Agilent Capillary Electrophoresis system which is computer controlled via Agilent ChemStation software. High sensitivity detection was achieved using the Agilent High-Sensitivity Detection Cell (part number G1600-68713) and capillaries.

During a shelf life study of ranitidine, the anti-ulcer drug was analyzed for the presence of impurities after 12 months exposure to light and room temperature. Figure 2 shows the analysis of the main peak indicating that this is at the upper levels of the linear range of detection. An expanded view of the base of the peak shows the number of impurities present at very low levels. Given the linear range of the detection cell, it is possible to calculate the impurity level of these peaks at less than 0.1 % area/area of the main peak.

Conclusion

CZE in conjunction with the high-sensitivity detection cell, may be used to determine impurity levels in drugs at less than 0.1 % area/area. This level is appropriate to that required for regulatory submissions.

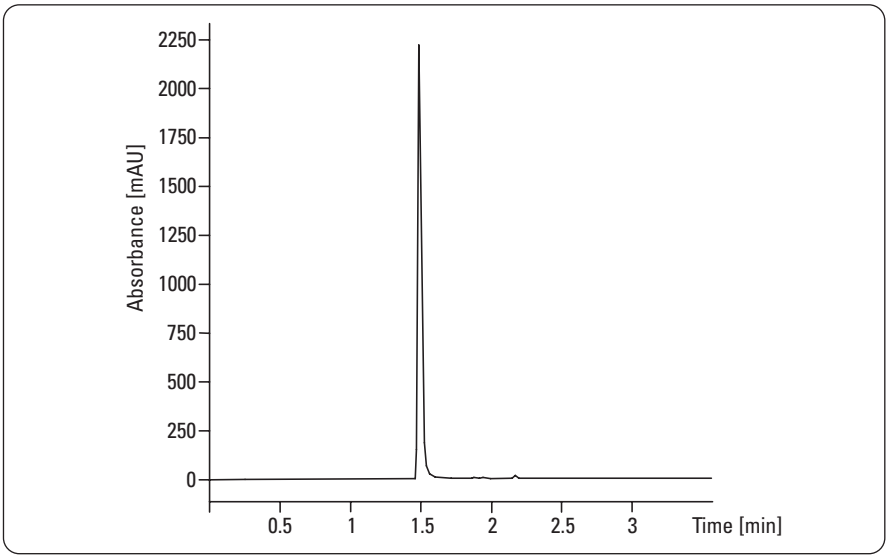

Figure 2
CZE analysis of ranitidine.

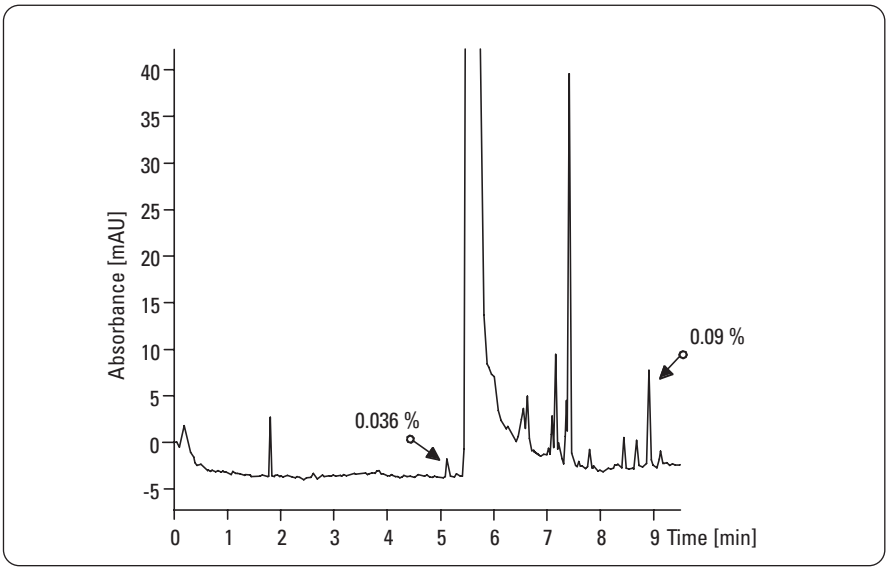

Figure 3
Low level determination of ranitidine impurities.

Chromatographic conditions (figures 2, 3)

Buffer:	20 mM borate pH 9.3
Capillary:	56 cm eff (64.5) x 75 µm id
Injection:	200 mbars
Run:	20 °C, 30 kV
Detection:	225/20 nm (high sensitivity cell)

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Published March 1, 2009
Publication Number 5990-3381EN

Agilent Technologies

Analysis of anions in power plant waters using capillary electrophoresis

Application Note

Power Generating Industry

Authors

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Abstract

Power plant circuit waters must be ultrapure and treated to minimize corrosion. Additives are ammonia and/or morpholine (pH control), and hydrazine (powerful reducing agent). Corrosion products, consisting mainly of insoluble metal species, build up deposits in the tubings where trace ionic contaminants from the feedwater can concentrate resulting in a highly corrosive local environment. Low levels of small anions and cations must therefore be monitored in order to minimize operational costs and contribute to the safe operation of the plant. Low ppb levels of small inorganic and organic anions are analyzed fast and reliably by capillary electrophoresis (CE) using indirect UV detection.

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Experimental and results

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with DAD detection and computer control via Agilent ChemStation. The method is based on the Agilent Inorganic Anion Solutions Kit (part number 5063-6511). A bubble cell capillary was used to increase detection limits. Additionally, the capillary effective length was reduced to 50 cm to shorten analysis times without sacrifying resolution between nitrate and oxalate. All vials (glass vials for buffer, polypropylene vials for samples) and clear olefine vial caps were washed overnight with deionized water (Milli-Q Plus or comparable quality) to eliminate contamination. Deionized water was also used to prepare the anion standards daily. Gluconic acid [10 mM] was added to all standards and samples to decrease carbonation.

New capillaries were subjected to a conditioning procedure: 10 minutes flush 1 N NaOH, 3 minutes flush 0.1 N NaOH, dip inlet/outlet in water, 15/30 minutes flush from two different buffer vials. Each flush step used its own waste vial. Afterwards, 10 standard runs injecting hydrodynamically from a 100 ppb standard solution in water were performed thereby equilibrating the capillary.

Prior to each analytical run a preconditioning procedure together with automatic buffer vial replenishment was performed to keep migration times stable. This procedure and more details are found in the caption of figure 1, which shows the analysis of anions in reconstituted secondary circuit water matrix.

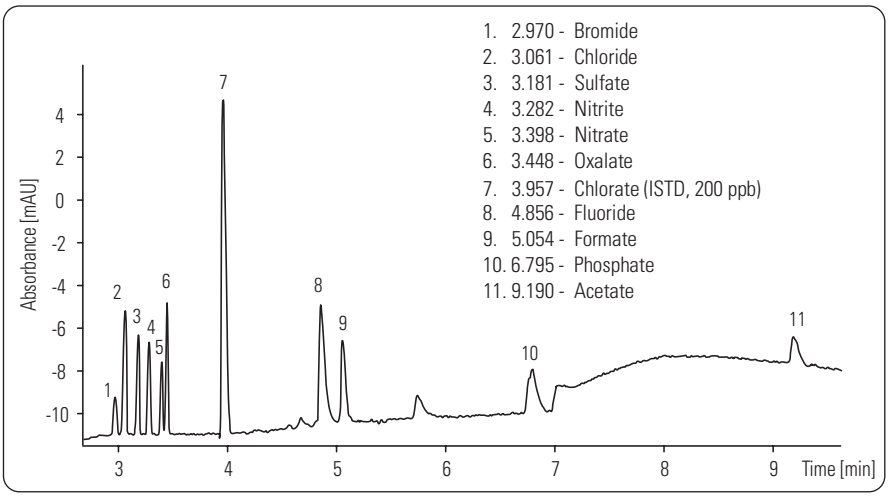

Figure 1
The analysis of anions in reconstituted secondary circuit water matrix.

Chromatographic conditions

Sample:	20 ppb anions in matrix, (5 ppm morpholine, 50 ppb hydrazine, 300 ppb ammonia, 300 ppb methylamine, 5 ppm borate)
Injection:	-5 kV x 20 sec
Capillary:	length = 58.5 cm, effective length = 50 cm; 50 µgm id, BF3
Buffer:	inorganic anion buffer
Temperature:	30 °C
Voltage:	30 k, negative polarity
Detection:	signal 350/50, reference 245/10
Precondition:	flush 2 min 0.1 N NaOH, dip both ends in water, flush 2 x 2 min with electrolyte

The assay was linear over the range 10–30 ppb ($r^2 > 0.98$) with a detection limit of 1-5 ppb. This was sufficient for reliable determination of ion concentrations normally found in circuit water samples. Repeatability (n = 6, with internal standard) was 0.08 - 0.19 % RSD for migration times and 2.68–5.63 % RSD for corrected peak areas (except for acetate, 10.44 % RSD). This excellent migration time repeatability was achieved even in the presence of a complex sample matrix. The repeatability of peak areas was sufficient for reliable quantitation.

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Published March 1, 2009
Publication Number 5990-3385EN

Agilent Technologies

Screening Impurities in Fine Chemicals Using the Agilent 1290 Infinity LC System

Application Note

Fine Chemical

Authors

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Abstract

The Agilent 1290 Infinity LC System with ultra violet/visible (UV/VIS) Diode Array detection (DAD) is used to analyze octyl-dimethyl-p-aminobenzoic acid for the presence of impurities. The system is used for the chromatographic separation of the compound from its impurities on 3.0 and 2.1 mm id C18 columns, of various lengths, with 1.8-um packing materials prepared in 600-bar (9000 psi) or special 1200-bar (18,000 psi) configurations. The ability of the 1290 Infinity LC System to operate with long, high resolution columns under conditions of rapid analysis is demonstrated with low viscosity acetonitrile (ACN) and higher viscosity methanol (MeOH) solvent conditions.

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Introduction

The analysis of impurities in starting materials, intermediates and finished products intended for a wide range of final uses is essential for ensuring product quality, performance, and consumer safety. The general conditions for successful analysis of impurities by high-performance liquid chromatography (HPLC) include gradient elution and multi-wavelength monitoring of the overall separation and may benefit from other detectors including evaporative light scattering (ELSD) and mass spectrometers (MS). Because impurity determination is the primary goal, one needs to ensure that mobile phase, vials, and HPLC components are free of minor impurities that might lead to confusing results during the analysis. Careful preparation of diluent blanks and blanks that might represent contamination sources due to additional sample preparation, such as filtration, are also appropriate. The analysis sequence is likely to include runs of the production material, solvent or diluent blank runs. It is also typical to include limit standards prepared by diluting the primary component to the lowest level where detection of impurities might be required. Finally, it is generally essential to include an authentic high purity reference standard.

Para-aminobenzoic acid (PABA) has historically been used as an ultra-violet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer a derivative in the form of octyl-dimethyl-p-aminobenzoic acid (OD-PABA), is currently and more commonly used. However, PABA may be formed as a degradate of OD-PABA, so it is important to monitor its potential presence in samples of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, for the purposes of safety and economics. In this work we investigate the capability of the Agilent 1290 Infinity LC system (UHPLC system with 1200 bar pressure limit) to detect impurities in OD-PABA samples with UV/VIS Diode Array detection.

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

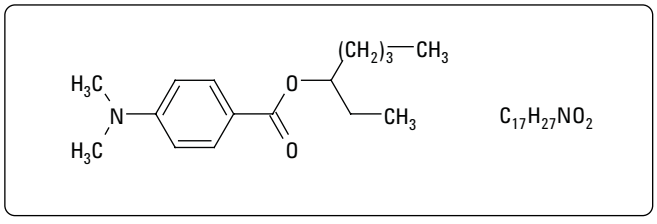

Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Experimental

Sample Preparation

The primary OD-PABA solution was prepared at a concentration of 1 mg/mL in 2-propanol and subsequently diluted to lower concentrations as needed. Injection volumes of 0.2–2 µL were made into the LC/DAD system.

LC Method Details

LC Conditions

Agilent 1290 Infinity LC system binary pump G4220A,
Agilent 1290 Infinity LC system autosampler G4226A
Agilent Thermostatted Column Compartment G1316C with switching valve
Agilent 1290 Infinity system diode array UV/VIS detector G4212A with 10 mm path fiber optic flow cell

Columns:	(See individual figures for specific usage) Agilent ZORBAX SB-C18 RRHT, 3 mm × 50 mm, 1.8 µm 600 bar p/n 827975-302 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 100 mm, 1.8 µm 1200 bar, p/n 858700-902 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 150 mm, 1.8 µm 1200 bar, p/n 859700-902
Column temp:	40 °C
Mobile phase:	A = HPLC grade water B = Acetonitrile (ACN) or methanol (MeOH) (See individual figures)
Flow rate:	See individual figures
Gradient:	Gradient: the gradient conditions were either 40% to 90% ACN or 50% to 100% MeOH. The gradient slope was maintained at 3.5% organic phase increase per column volume, altering gradient time and flow rate accordingly. This is based on calculations using a modification of the Agilent Method Translator. [1]

UV Conditions

Monitoring 210, 254, 280 and 320 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

The UV response of OD-PABA, with four wavelengths monitored, is shown at a retention time of 2 min in Figure 2. Multi-wavelength monitoring of the separation provides a simple way to account for multiple impurities and assist in the selection of a final wavelength condition that can maximize sensitivity for all detected analytes.

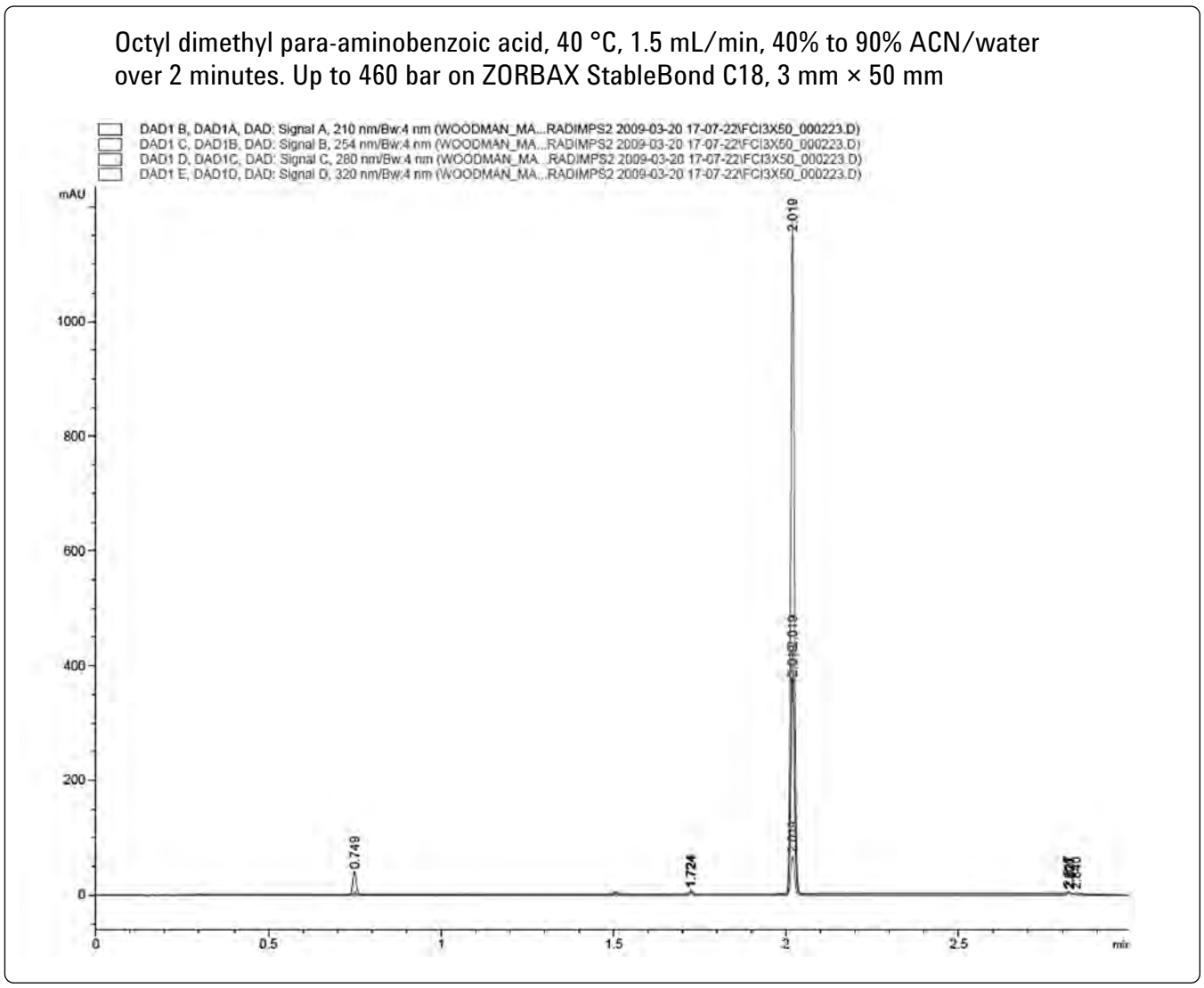

Figure 2. Multi-wavelength UV chromatogram of OD-PABA production material on a 3 mm × 50 mm ZORBAX Rapid Resolution High Throughput (RRHT) column. The chromatogram demonstrates the typical difficulties encountered with this type of separation, which are a need for wide dynamic range detection and sensitive impurity measurement. The peak at 0.75 minutes is confirmed by retention time matching and UV spectra to be PABA, the primary impurity in the mixture.

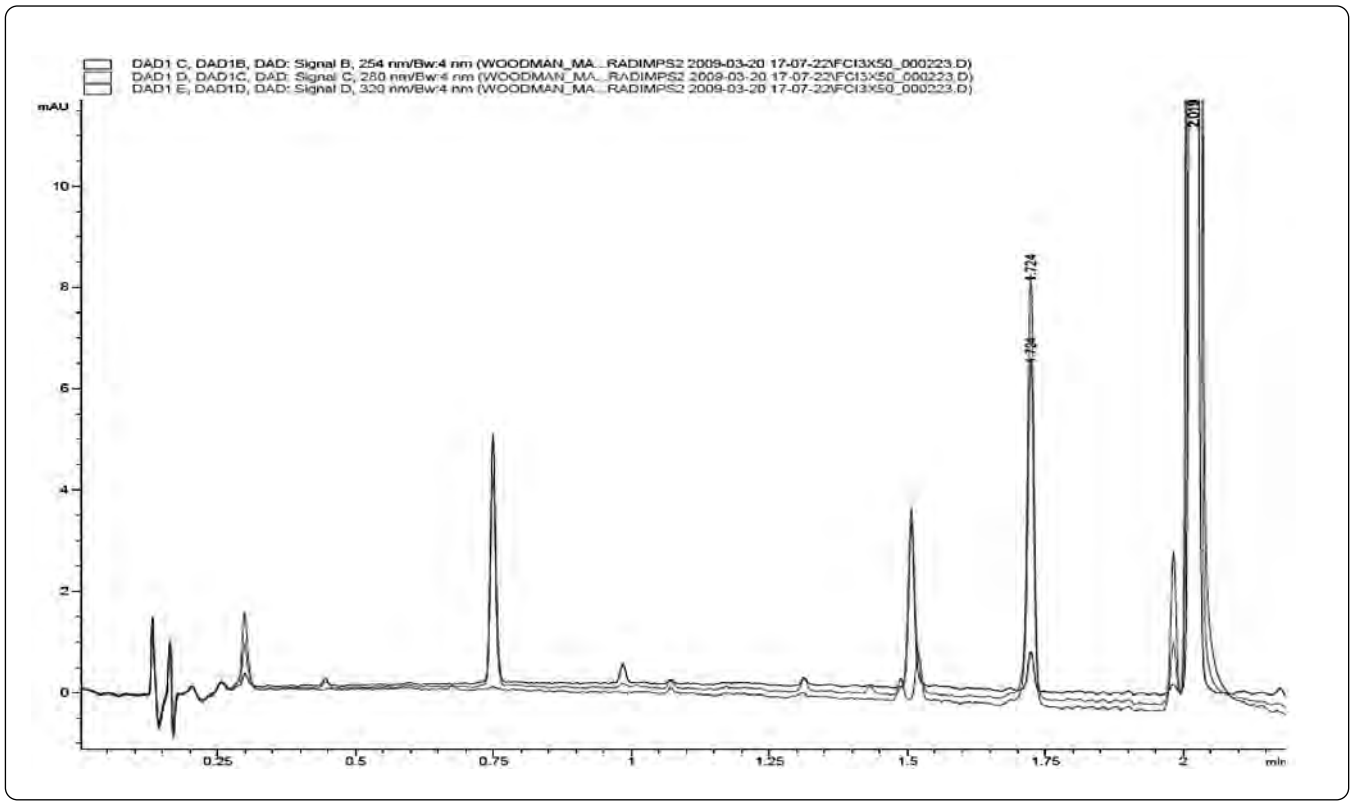

Figure 3. An expanded presentation of the chromatogram shown in Figure 2 based on the 3 mm × 50 mm gradient separation.

In Figure 3 the expanded multi-wavelength chromatogram allows us to see close detail and shows the number of impurities, as well as several areas where chromatographic resolution is clearly inadequate for individual component measurement. Despite the small particle size used in this column, the relatively short length limits the total resolution. As we move to longer column dimensions we will often reduce column diameter to reduce overall solvent consumption at the same time.

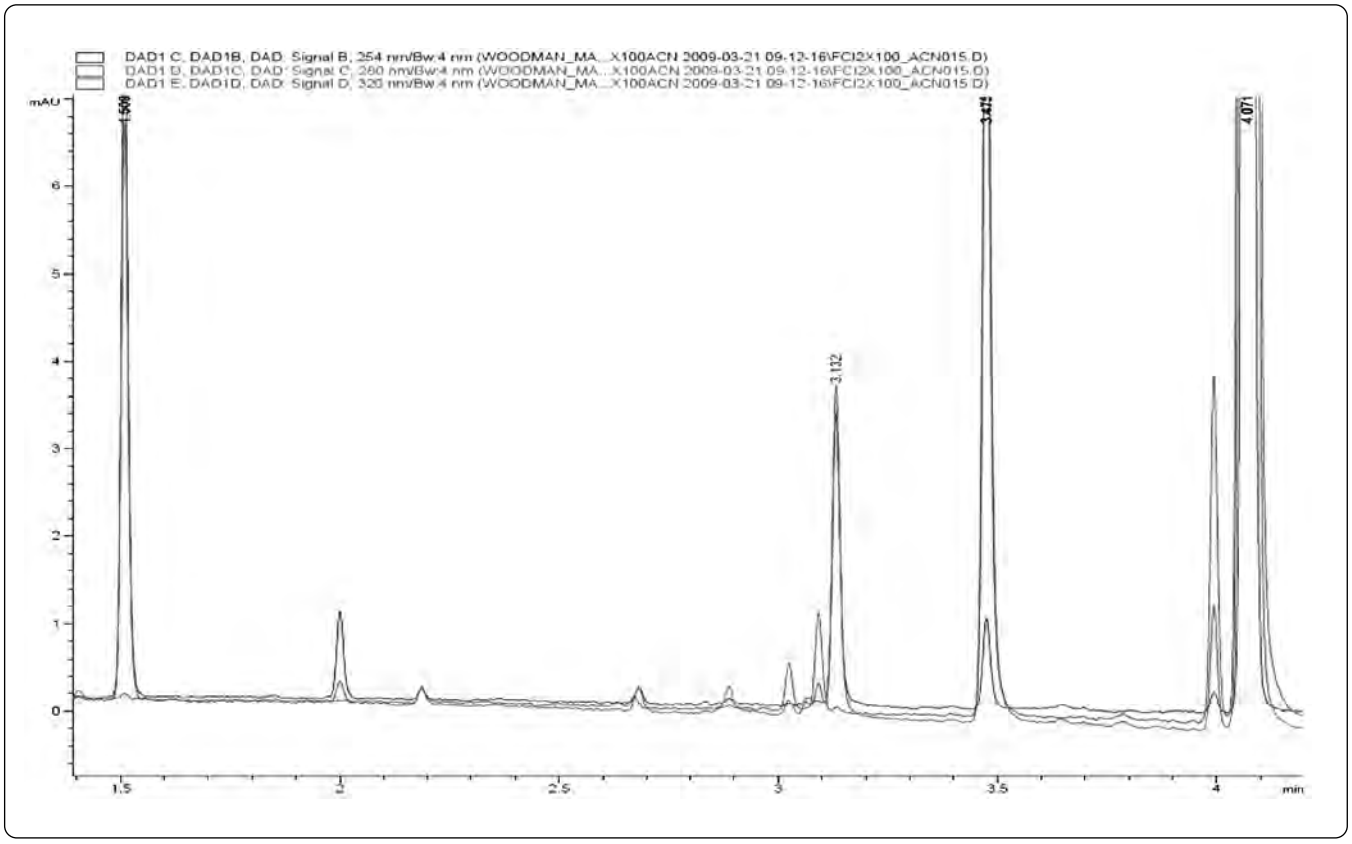

Figure 4. Analysis of the standard material on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18 column prepared for operation at 1200 bar pressure limit. Acetonitrile water gradient, 0.74 mL per minute, gradient time 4.0 minutes.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution it would be practical to explore longer columns or explore alternative mobile phase or column chemistries.

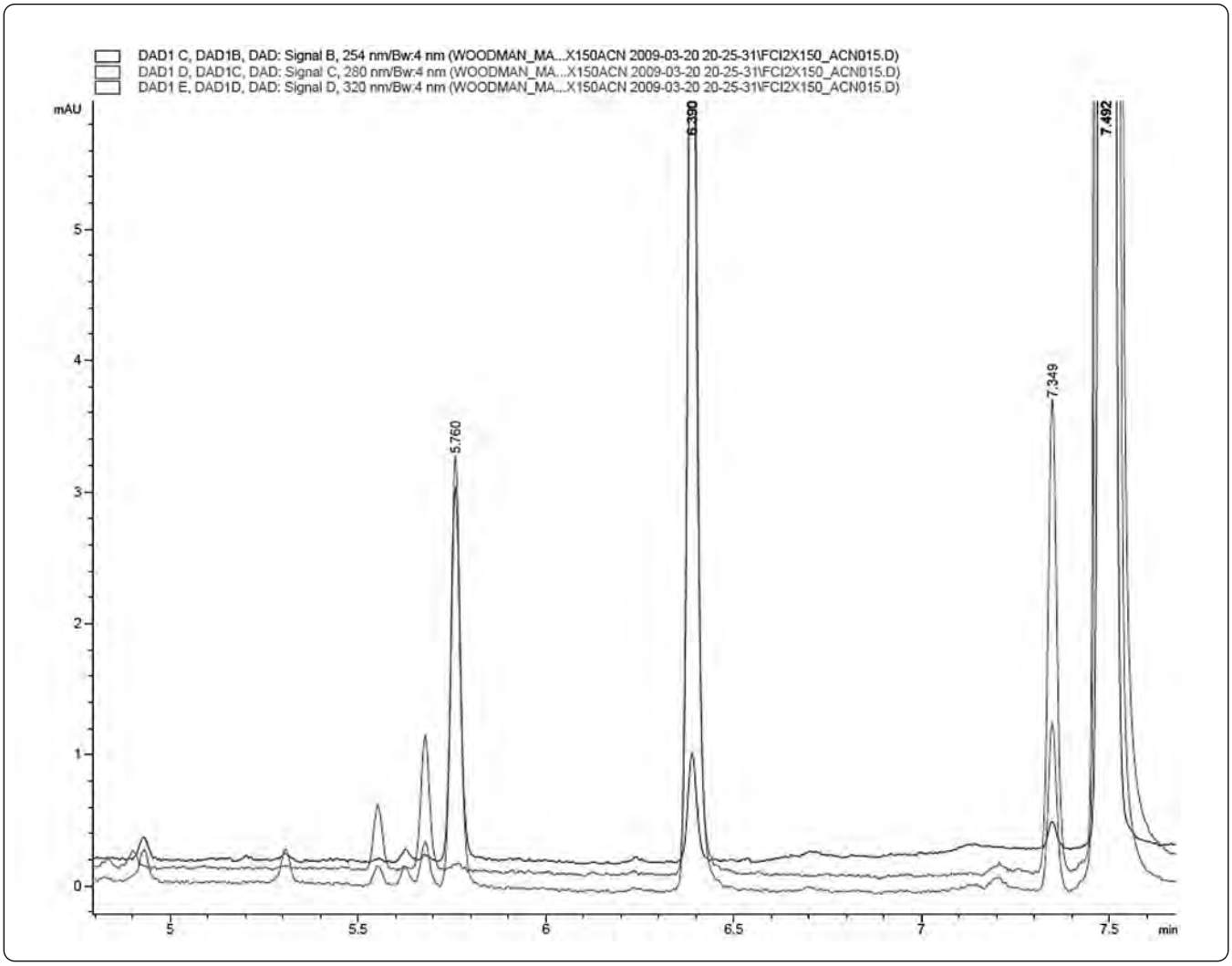

Figure 5. An expanded view of the acetonitrile separation using the same gradient slope on a 2.1 mm × 150 mm column rated for 1200 bar operating pressure. Agilent ZORBAX StableBond C18, 1.8 µm.

The increased column length clearly gives more resolution, however the increased back pressure also limits the flow rate if one is to operate in a conservative range of operating pressure. The Agilent 1290 Infinity LC system and associated ZORBAX Rapid Resolution High Definition (RRHD) chemistries are capable of operating pressures up to 1200 bar, approximately 18,000 psi. To ensure robust and rugged system operation many users typically specify the upper pressure limit for a method at a value less than 80% of the rated operating pressure.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm.

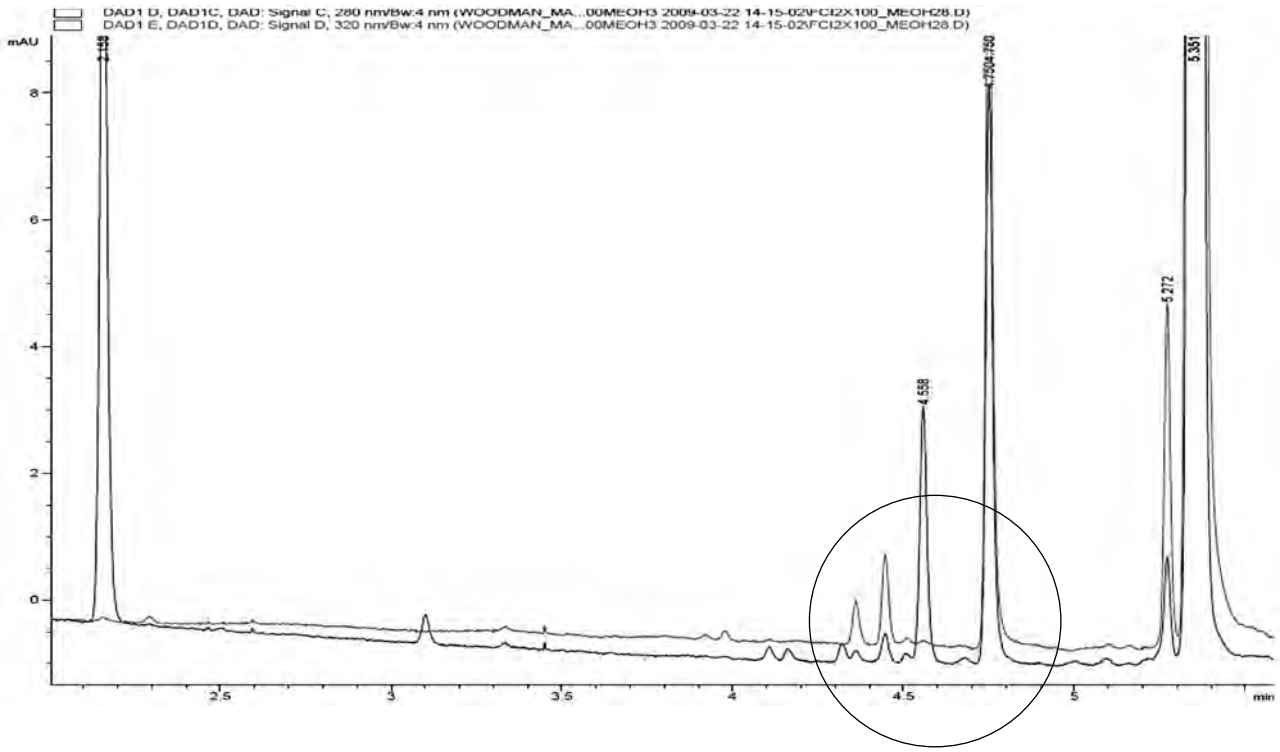

Figure 6. Separation of the sample mixture on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18, using methanol as the organic phase. Flow rate 0.52 mL/min gradient time 5.7 min, for a gradient of 5% to 100% methanol

When considering the fundamental components of the resolution equation we are all quite familiar with the concepts of capacity, selectivity, and efficiency. Increasing the column length, like decreasing the particle size of the packing material, will increase the efficiency of the overall separation. Because the increase in efficiency yields a relatively low return in terms of resolution, users often need to ensure that the capacity factor is optimized by exploring alternative chemical variables that could promote increased selectivity in the separation.

In Figure 6 we see the dramatic results achieved by changing the separation conditions from using acetonitrile as the organic phase to methanol. If this separation was highly dependent on monitoring the separation at very low wavelengths one might find the UV cutoff of the methanol, 205 nm, to be problematic. In this example, however, the highly conjugated structures of the parents and related impurity structures allow sensitive detection at wavelengths well above the UV cutoff of common organic solvents used in reversed phase chromatography. In about the same amount of analysis time as the example in Figure 5, we achieve significantly higher selectivity leading to more resolved impurities while reducing overall solvent consumption and eliminating the need for expensive acetonitrile as the organic phase.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm, 40 °C.

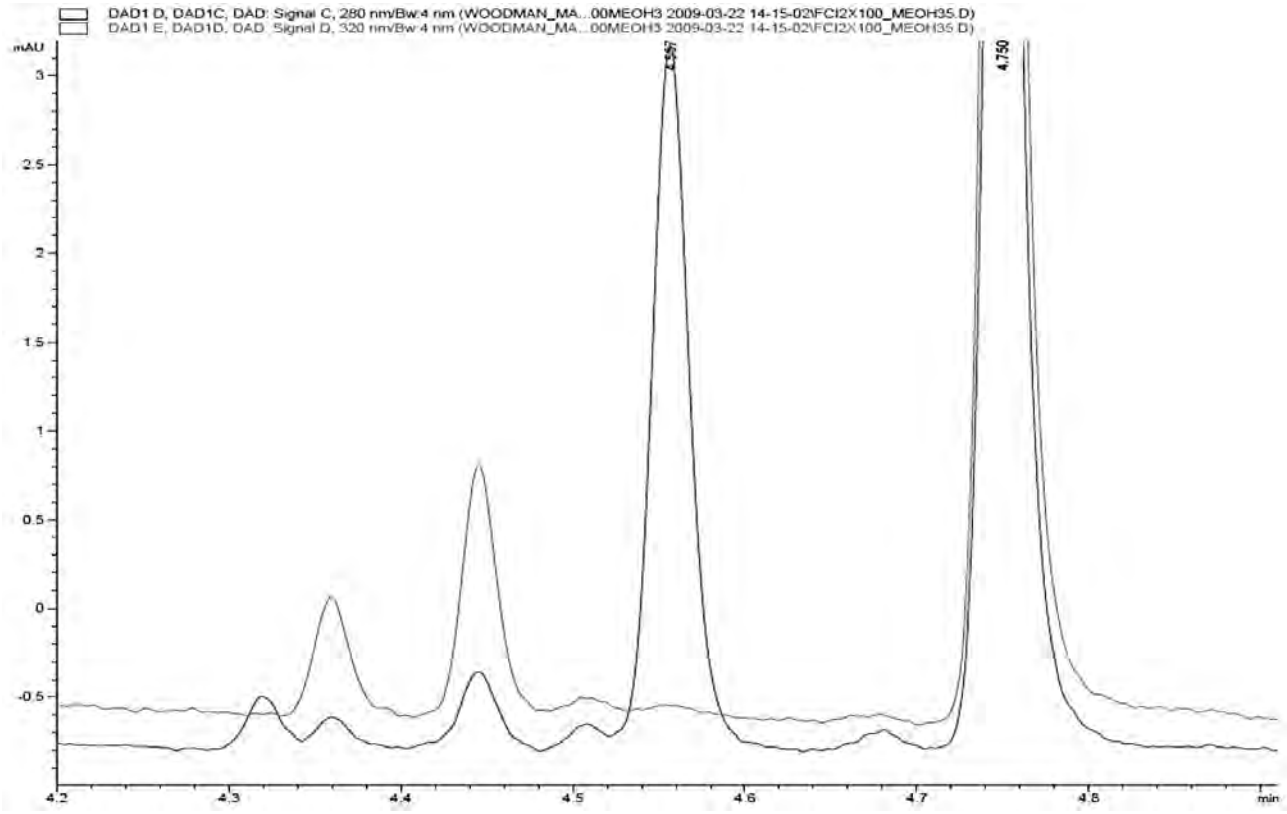

Figure 7. An expanded view of the small region of the chromatogram in Figure 6 showing a significant number of low concentration impurities. Conditions as in Figure 6. Estimated impurity concentrations for the smallest peaks in this figure are less than 0.02%.

Conclusions

The detection of low-level impurities in synthetic materials and highly refined natural products is of critical importance to the ultimate utility of these substances. Rapid analysis by HPLC using high-resolution columns and appropriately chosen organic phases ensures consistent results with rapid analysis turnaround time. Using the Agilent 1290 Infinity LC system, we were able to easily demonstrate UHPLC capabilities well within the operating range of the designed system. Higher throughput could still be achieved with this system by increasing flow rate and simultaneously reducing the gradient segment time to reproduce the gradient slope in a shorter total analysis time.

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References

1. http://www.chem.agilent.com/en-US/products/instruments/lc/pages/gp60931.aspx)

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Printed in the USA
August 3, 2009
5990-4293EN



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Determination of Formic Acid in Acetic Acid for Industrial Use by Agilent 7820A GC

Application Brief

Wenmin Liu, Chunxiao Wang

HPI

With rising prices of crude oil and a future shortage of oil and gas resources, people are relying on the development of the coal chemical industry.

Acetic acid is an important intermediate in coal chemical synthesis. It is used in the production of polyethylene, cellulose acetate, and polyvinyl, as well as synthetic fibres and fabrics. The production of acetic acid will remain high over the next three years. In China, it is estimated that the production capacity of alcohol-to-acetic acid would be 730,000 tons per year in 2010.

The purity of acetic acid determinates the quality of the final synthetic products. Formic acid is one of the main impurities in acetic acid. Many analytical methods for the analysis of formic acid in acetic acid have been developed using gas chromatography. For example, in the GB/T 1628.5-2000 method, packed column and manual sample injection is used with poor separation and repeatability which impacts the quantification of formic acid.

In this application brief, a new analytical method was developed on a new Agilent GC platform, the Agilent 7820A GC System. The GC was configured with a micro thermal conductivity detector (μTCD) which provides an easy to use method for the determination of formic acid in acetic acid. To achieve a better separation, an Agilent J&W DB-FFAP (30 m × 320 μm, 0.25 μm) capillary column was used as the analytical column.

Highlights

- The Agilent 7820A GC coupled with a μTCD provides a simple method for analysis of formic acid in acetic acid.
- ALS and EPC ensure good repeatabiltiy and ease of use which makes the 7820GC appropriate for routine analysis in QA/QC labs.
- Using a capillary column as the analytical column ensures better separation of formic acid in acetic acid compared to the China GB method.

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Experimental

Analytical conditions

Inlet	150 °C, Split ratio: 10:1
Injection volume	1 µL
Column	Agilent J&W DB-FFAP, 30 m × 320 µm, 0.25 µm (p/n 123-3232)
Carrier gas	He, Constant flow: 1.5 mL/min
Oven	80 °C (3 min) 8 °C/min 150 °C (5 min)
Detector	µTCD: 200 °C, Reference gas: 15 mL/min, Makeup gas: 6.5 mL/min FID: 300 °C, H_2: 30 mL/min; Air: 350 mL/min; Makeup flow (N_2): 60 mL/min
Autosampler	Agilent 7693A automatic liquid sampler
Data analysis system	EZChrom Elite Compact

Results

Figure 1 shows the chromatogram of the analysis of formic acid in acetic acid at 1% (weight to weight) on the Agilent 7820A GC. From the chromatogram it could be seen that formic acid elutes after acetic acid on the Agilent J&W DB-FFAP column, which is confirmed by the Agilent 6890 GC and the Agilent 5975C series GC/MSD. In this experiment, a flame ionization detector (FID) was also used to confirm the detection of formic acid. The results are shown in Figure 2.

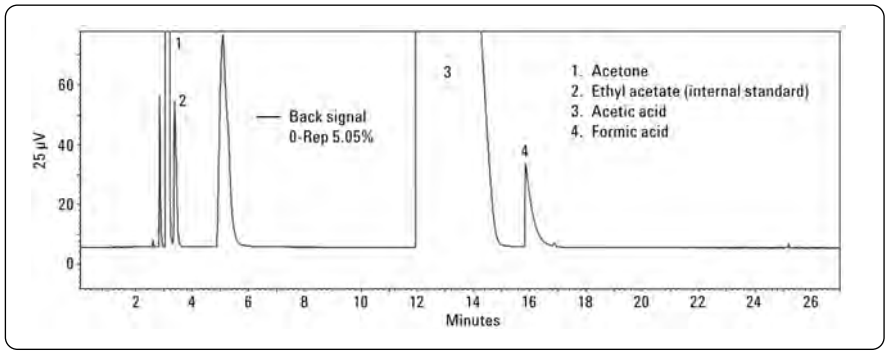

Figure 1. Chromatogram of formic acid analysis in acetic acid on the TCD channel.

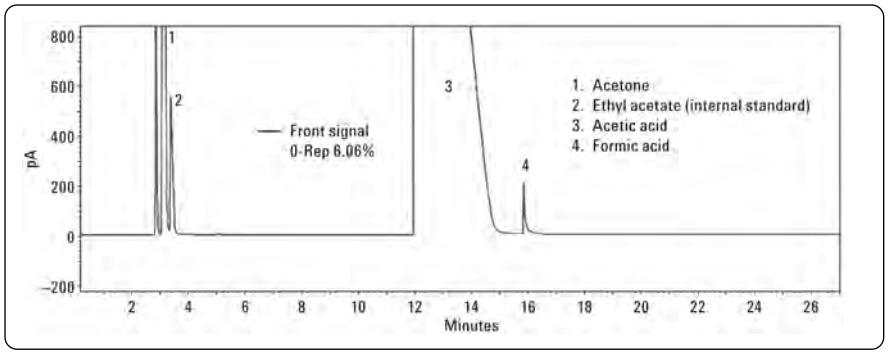

Figure 2. Chromatogram of formic acid analysis in acetic acid on the FID channel.

Agilent μTCD is a proprietary designed single-filament flow switching detector. This design eliminates the need for a reference column, by exposing the filament to column effluent and reference flows at a frequency of 5 Hz. There is no other reference column that assures a stable baseline even with a ramped temperature program. Compared to the typical TCD, the smaller volume cell of μTCD provides higher sensitivity.

The detection limits of formic acid on μTCD was tested using a series of diluted formic acid samples. The method has a signal-to-noise of 6.8 for a formic acid concentration of 0.05 wt%. A method precision of 1.75% RSD for five injections was also calculated for the 0.05 wt% concentration. These excellent results were attributed to the Electronic Pressure Control (EPC) and precision auto sampler that are the key features of the Agilent 7820A GC.

In this method, ethyl acetate was used as the internal standard according to the GB/T 1628.5-2000. Concentrations of 0.1%, 0.5%, 1%, 10% (weight to weight) formic acid in acetic acid standard solution were made with ethyl acetate as an internal standard. The results show that from 0.1% to 10% (weight to weight), formic acid response to concentration was linear with an $r^2 = 0.9917$.

Conclusions

The Agilent 7820A GC coupled with a μTCD provides a simple method for analysis of formic acid in acetic acid. Use of a capillary column ensures good separation of impurities and acetic acid in both high concentrations and low concentrations. The stable and sensitive μTCD is a good choice for formic acid analysis compared to an FID which has a relatively low response. The 7693A autosampler with a capacity of 16 sample vials and 7820A GC EPC control ensure good repeatability and ease of operation, which is suitable for the fast growing coal to chemical industry and routine analysis labs where feedstock and intermediate quality control is important. The data processing system, EZChrom Elite Compact software specially designed for Agilent 7820 GC, is easy to use and provides commonly used report templates.

References

1. GB/T 1628.5-2000. Determination of formic acid in acetic acid by gas chromatography.

Wenmin Liu, Chunxiao Wang are application chemist based at Agilent Technologies, Shanghai, China.

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August 25, 2009
5990-4456EN



Agilent Technologies

Analysis of impurities in fine chemical octyl-dimethyl-4-aminobenzoate using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Fine Chemicals

Author

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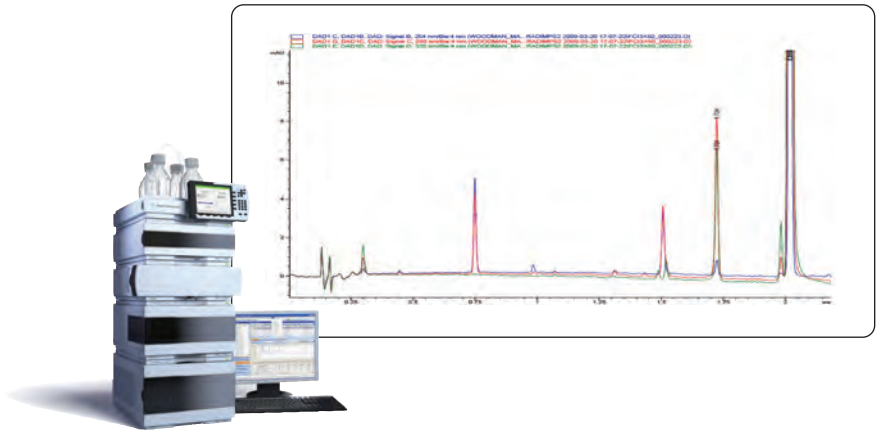
Wilmington, DE 19808
USA



Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system and has the flexibility to operate with a wide range of column dimensions and particle sizes. Additionally, advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of small impurities in fine chemicals.

Introduction

The combined benefits are demonstrated by a separation of impurities found in a sample of octyl-dimethyl-4-aminobenzoate (Figure 1). The high pressure capability of the system allows the use of methanol, as well as acetonitrile, to explore the selectivity of the two solvents. At 1.5 mL/min, using a simple 2-min gradient and a 3.0 mm × 50 mm 1.8 µm column, the analysis time is only 3 min. The separation of the main components is shown in Figure 2.

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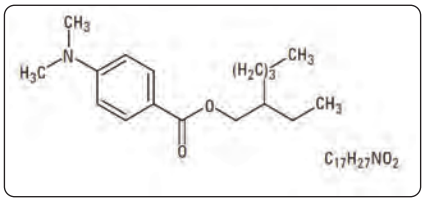

Figure 1
Structure of the cited compound.

The speed, resolution and flexibility of the system are further demonstrated by a separation of the sample using methanol or acetonitrile with low solvent consumption 2.1 mm id, 1.8 µm columns. The flow rate and gradient conditions are optimized for each solvent, to produce a gradient separation with maximum pressure of approximately 850 bar, a conservative setting for the 1200-bar capability of the Agilent 1290 Infinity LC. The separation of the main components, with the two organic solvents, is shown in Figure 3a (acetonitrile, top panel) and 3b (methanol, lower panel), where the chromatograms are zoomed to the region of peaks shown from approximately 1.2-2.5 min in Figure 2.

Configuration

- G4220A 1290 Infinity Binary Pump with Integrated Vacuum Degasser
- G4226A 1290 Infinity Autosampler
- G1316C 1290 Infinity Thermostatted Column Compartment
- G4212A 1290 Infinity Diode Array Detector

Conclusion

The combined high flow and high pressure capability of the system allows one to use high efficiency columns, producing rapid separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns. Impurity detection, due to high detector sensitivity and stability, is estimated to be < 0.01%.

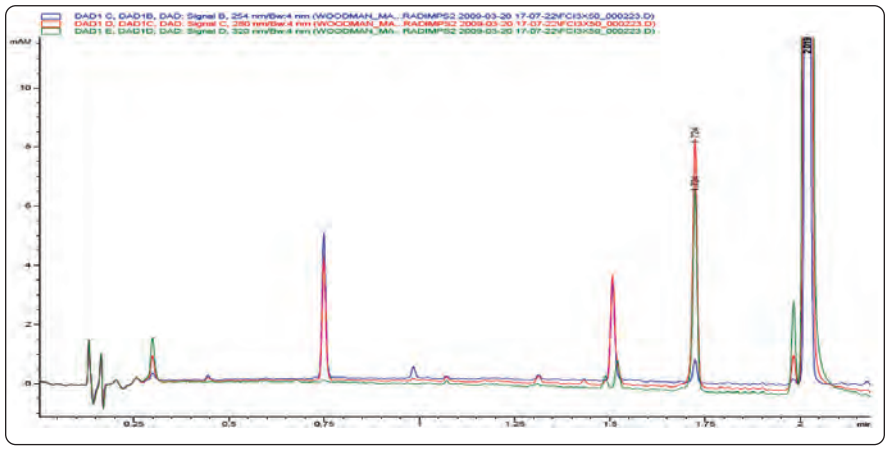

Figure 2
Initial separation conditions showing a need for greater resolution and selectivity. Sample: Octyl dimethyl para-aminobenzoate, 1 mg/mL. Gradient: 1.5 mL/min, 40% to 90% ACN/water over 2 minutes. Up to 460 bar on ZORBAX StableBond RRHT C18, 3 mm × 50 mm, 1.8 µm, 40 °C. 0.75 minute retention: 4-amino-benzoic acid; 2.1 minute retention: Octyl dimethyl para-aminobenzoate.

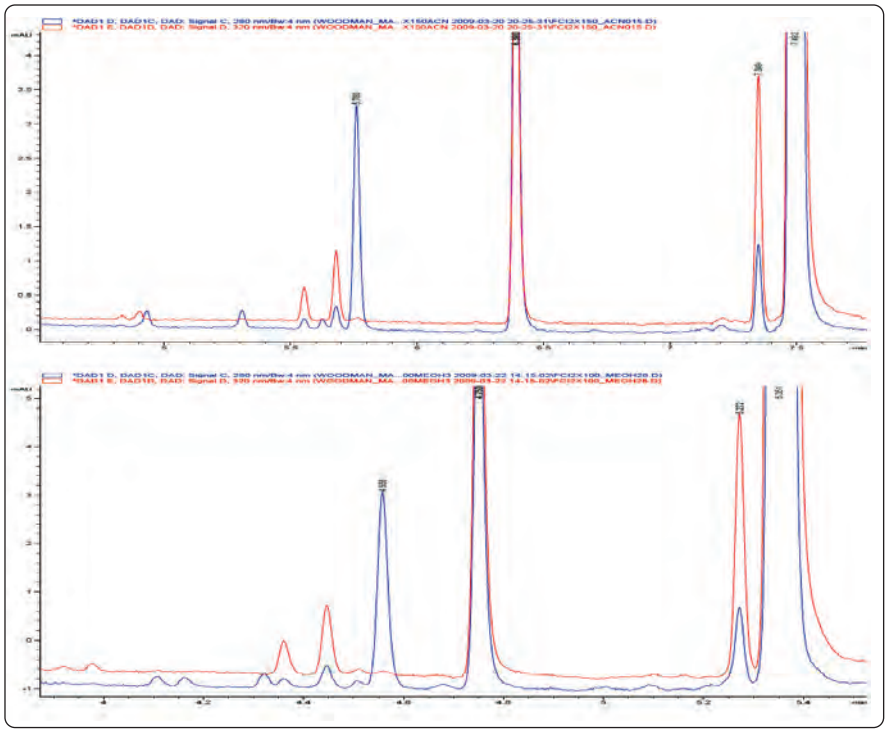

Figure 3
Results using ACN vs. MeOH with the same gradient slope on the 1290 Infinity LC. Sample: ODPABA working standard, 1 mg/mL. Conditions: ACN gradient 0.6 mL/min, 40% to 90% ACN/water over 7.4 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm × 150 mm, 1.8 µm, 40 °C. Methanol gradient 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm × 100 mm 1.8 µm, 40 °C. The increased selectivity of methanol allowed a shorter column to be used, decreasing run time and avoiding the use of more expensive acetonitrile mobile phase.

www.agilent.com/chem/1290

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October 1, 2009
Publication Number 5990-4880EN

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Specialty Chemical

Technical Overviews

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ZORBAX Eclipse XDB HPLC Columns

The "Perfect Fit" for Developing Better HPLC Methods

Technical Overview

- Excellent peak shape for basic, acidic or neutral compounds
- High performance over a wide pH range
- Rugged, reproducible chromatography from column-to-column and lot-to-lot
- More selectivity options for method development

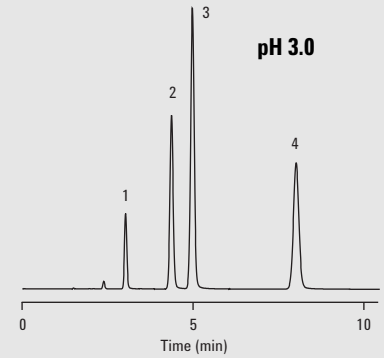
More and more chromatographers are developing their analytical and LC/MS separations on ZORBAX Eclipse XDB HPLC columns. Why? Because ZORBAX Eclipse XDB columns solve many of their separation challenges. In fact, Agilent Technologies includes a 4.6 x 150 mm, 5 µm ZORBAX Eclipse XDB-C8 column with every Agilent 1100 HPLC instrument it delivers. As Figure 1 shows, ZORBAX Eclipse XDB columns, specifically designed to extend column life and provide excellent peak shape for basic compounds in the pH range of 6 - 9, also deliver outstanding performance at low pH, as well.



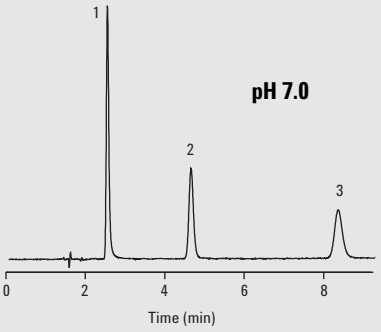
More detailed information about the benefits of the Eclipse column family can be found in this brochure.

Figure 1
Good Peak Shape for Acids, Bases and Neutrals at Low and Intermediate pH

ZORBAX Eclipse XDB-C18, 4.6 x 150 mm, 5 µm
(Agilent Part No. 993967-902)



Mobile Phase:	80% 25 mM Na_2HPO_4, pH 3.0 20% Methanol
Flow Rate:	1.0 mL/min
Temperature:	35°C
Sample:	1. Theobromine 2. Theophylline 3. 1,7-dimethylxanthine 4. Caffeine



Mobile Phase:	70% 25 mM Na_2HPO_4, pH 7.0 30% Methanol
Flow Rate:	1 mL/min
Temperature:	RT
Detection:	UV 254 nm
Sample:	1. Procainamide 2. N-acetylprocainamide 3. N-propionylprocainamide

Eclipse XDB HPLC Column Technology Provides:

Excellent Peak Shape



eXtra Dense Bonding + High Purity Silica = Excellent Peak Shape

eXtra Dense Bonding is key to the exceptional performance of ZORBAX Eclipse XDB columns at intermediate pH (Figure 2). This dense bonding is accomplished by adding an extra-dense monolayer of C18, C8 or Phenyl silane to the ultra-pure, fully-hydroxylated, ZORBAX Rx-silica surface. The packing is then endcapped not once, but twice, using two different and unique endcapping reagents. This combination of extra-dense surface coverage by the bonded phase and double endcapping produces a highly, deactivated stationary phase that virtually eliminates undesirable interactions between polar solutes and the silica surface. As a result, superior peak shape, high efficiency, and long-term chromatographic reproducibility are assured when using Eclipse XDB HPLC columns at both intermediate and low pH.

The exceptional and reproducible performance at low pH that you can expect from the Eclipse XDB is demonstrated in Figure 3. In this example, acetylsalicylic acid, and the base, dextromethorphan, are consistently separated with excellent peak shape at low pH on three different Eclipse XDB-C8 columns from three different lots of packing material.

Figure 2
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 7

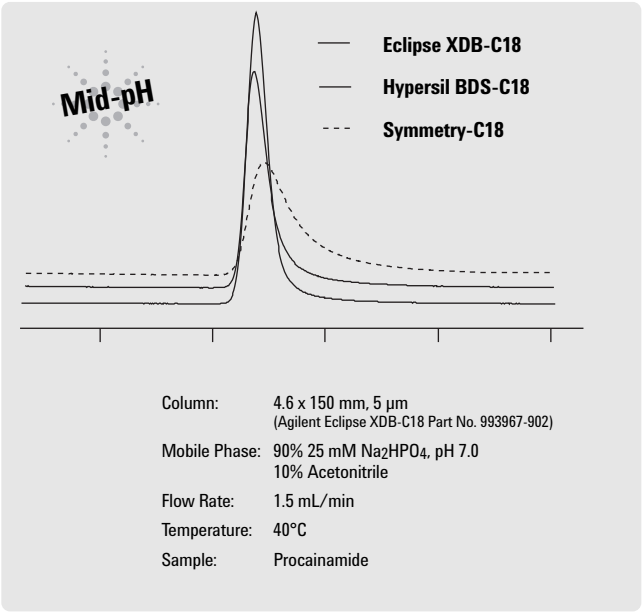
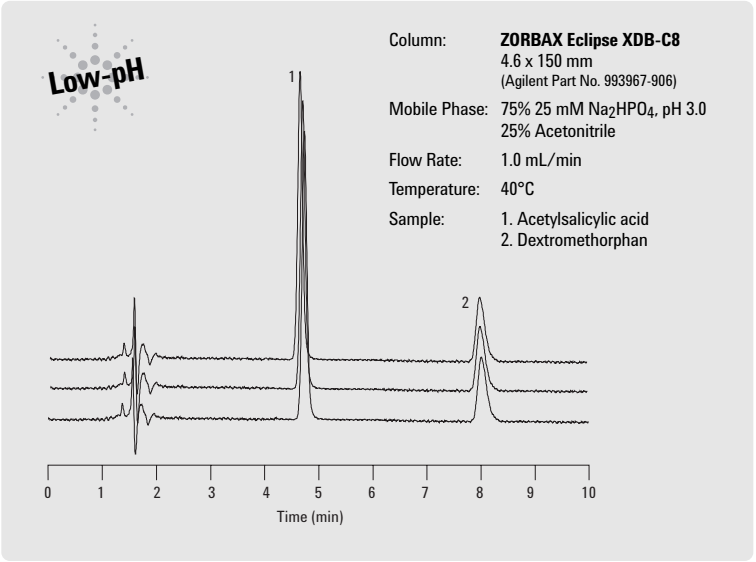


Figure 3
eXtra Dense Bonding (XDB) and Double Endcapping Improves Peak Shape for Polar Compounds at pH 3



Eclipse XDB HPLC Column Technology Provides:

Long Column Life



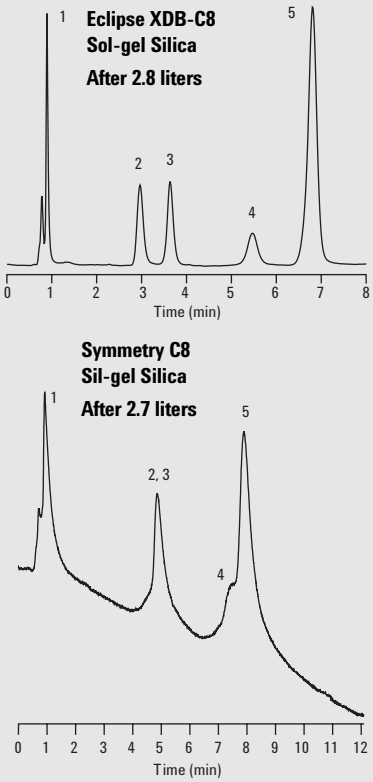
Eclipse XDB columns not only provide excellent peak shape but they are also exceptionally durable. In fact, the spherical ZORBAX Rx-SIL particles are the most durable, porous, 5 and 3.5 µm, silica particles commercially available. They are manufactured using a patented and proprietary process, forming thick, hard-walled silica, commonly referred to in the literature as "sol-gel" silica.

Because of the strength of the ZORBAX particle, all ZORBAX columns are consistently and reliably packed at pressures exceeding 8000 psi. The result is a durable column that can easily tolerate pressures up to 5000 psi in regular use without a loss in efficiency or a reduced lifetime.

Long Column Life at Intermediate pH

This thick, hard-walled "sol-gel" silica resists dissolution at intermediate pH and when densely bonded, the resultant Eclipse XDB column provides excellent column performance and increased column lifetime, even under the stressed intermediate pH conditions described in Figure 4.

Figure 4
Accelerated Column Aging Study Demonstrates the Durability of Eclipse XDB-C8 Over Waters' Symmetry C8



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase:
20% Acetonitrile
80% 250 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

Temperature: 60°C

Test Conditions:

Mobile Phase:
60% Acetonitrile
40% 10 mM phosphate buffer, pH 7

Flow Rate: 1.5 mL/min

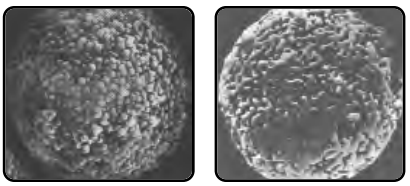
Temperature: 40°C

Sample: Tricyclic Antidepressants
1. Uracil 2. Nortriptyline 3. Doxepin
4. Amitriptyline 5. Trimipramine

Many commercial, base-deactivated, silica-based HPLC columns use manufacturing processes that produce a less robust "sil-gel" silica particle. The walls of these resulting high-surface area materials (typically 300 m^2/g for an 80-100Å pore material) are thinner and less uniform and can easily crush under high pressure conditions. Moreover, in many cases, they fail to withstand the high pressures of high flow rate LC/MS and High-Throughput methods.

The Eclipse XDB thick, hard-walled "sol-gel" is compared to the thin-walled "sil-gel" silica, used to make most of today's base-deactivated products, in electron micrographs shown in Figure 5.

Figure 5
Eclipse XDB is Based on Thick, Hard-Walled, Sol-gel Silica



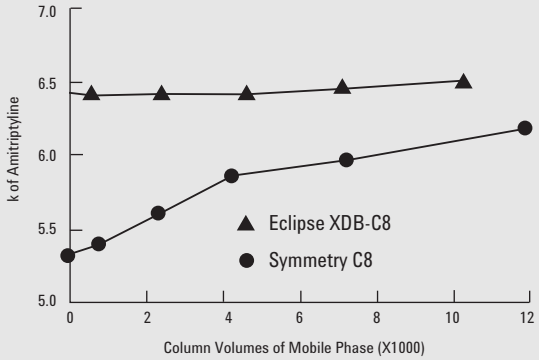
Sol-gel **Sil-gel**

Eclipse XDB columns are made with patented "hard wall" sol-gel silica particles. The thick walls of this silica are more resistant to dissolution than the "thin wall" sil-gel silica that is used to make most base-deactivated columns.

Long Column Life at Low pH

At low pH, Eclipse XDB columns provide better column life than most commercially available reversed-phase HPLC columns. Figure 6 summarizes the results from a low-pH accelerated aging study where loss in column performance is measured by a loss in bonded phase, indirectly measured by the change in retention of amitriptyline. The results show that less than 3% of the Eclipse XDB column performance is lost when exposed to 12,000 mL of a pH 3 mobile phase at 60°C. Under these same conditions more than 14% of column performance is lost on another popular "deactivated" reversed-phase HPLC column.

Figure 6
Accelerated Column Aging Study Demonstrates the Durability of ZORBAX Eclipse XDB-C8 Over Waters' Symmetry



Column: 4.6 x 150 mm

Purge Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.5 mL/min

Temperature: 60°C

Retention Test Conditions:

Mobile Phase: 30% Acetonitrile
70% 50 mM NaOAc-HCl, pH 3

Flow Rate: 1.0 mL/min

Temperature: 60°C

Sample: Amitriptyline

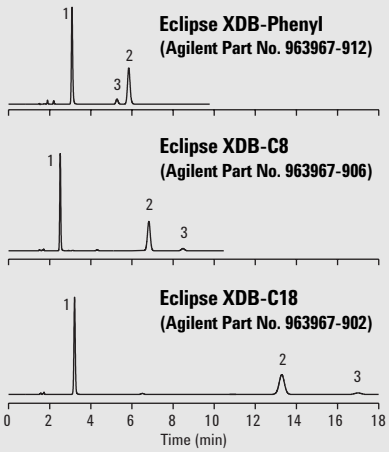
Eclipse XDB HPLC Column Technology Provides:

More Selectivity Options C18, C8 and Phenyl

Eclipse XDB HPLC columns are available as C18, C8 and Phenyl bonded phases. The Eclipse XDB-Phenyl phase complements both the most retentive Eclipse XDB-C18 and the moderately retentive Eclipse XDB-C8. The Eclipse XDB-Phenyl offers unique selectivity as well as reduced retention of non-polar and moderately polar compounds while maintaining retention of polar analytes.

These benefits are illustrated in Figure 7. Sunscreen components are well retained on the Eclipse XDB-C18, although the analysis time is long. Analysis time is reduced with more than acceptable resolution when using the Eclipse XDB-C8 or Eclipse XDB-Phenyl column, where analysis time is shortened by 50 or 61 percent, respectively.

Figure 7
Eclipse XDB-Phenyl Columns Offer Unique Selectivity and Short Analysis Times



Column: **ZORBAX Eclipse XDB**
4.6 x 150 mm, 3.5 µm

Mobile Phase: 85% Methanol
15% Water

Flow Rate: 1.0 mL/min

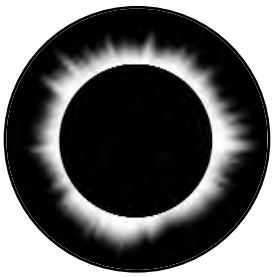
Temperature: 30°C

Detection: UV 310 nm

Sample: 1. Oxybenzone
2. Padimate O
3. Ethylhexyl Salicylate

Eclipse XDB HPLC Column Technology Provides:

Rapid Resolution

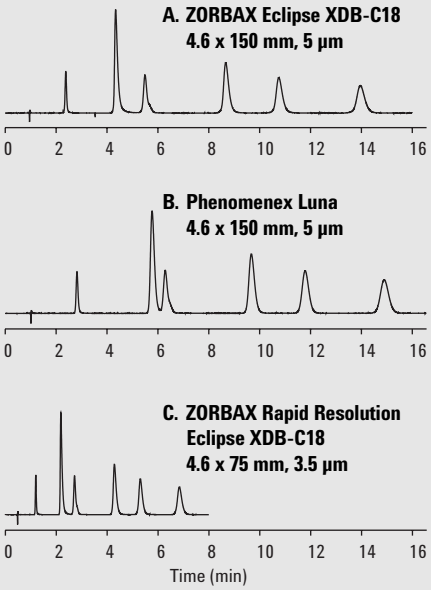


Eclipse XDB columns, available in 5 and 3.5 µm particle size packings, are highly efficient. In Figure 8A, six basic antidepressant compounds are well separated on a 5 µm, 4.6 x 150 mm, Eclipse XDB-C18 column using a methanol-phosphate mobile phase at pH 7. Peaks elute in sharp bands having average peak widths at half-height of 0.19 minutes. When the same sample was run on the Phenomenex Luna column, resolution between nortriptyline and doxepin ($R_{2,3}$) decreased significantly, with average peak widths at half-height being 47% wider, averaging 0.28 minutes for this assay.

For faster analysis, shorter Rapid Resolution Eclipse XDB columns, packed with 3.5 µm particles, provide equally efficient separations with reduced analysis times. This is demonstrated in Figure 8C, where analysis time is decreased by 50% — as retention decreases from 15.6 to 6.8 minutes — while resolution is maintained for this highly basic antidepressant sample.

If you have any questions about this or other applications in this bulletin, call 800-227-9770 select option 4 and ask for HPLC column technical support.

Figure 8
Eclipse XDB Provides High Efficiency and Rapid Resolution



Mobile Phase: 73% Methanol
27% 50 mM Phosphate, pH 7
Flow Rate: 1.5 mL/min.
Temperature: 40°C
Sample: Antidepressants
1. Imipramine
2. Nortriptyline
3. Doxepin
4. Doxylamine succinate
5. Amitriptyline
6. Cloripramine

Developing reliable reversed-phase methods for basic, acidic and neutral compounds just got easier . . .

Order Your ZORBAX Eclipse XDB HPLC Columns today!

ZORBAX Eclipse XDB Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse XDB-C18	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	10%
ZORBAX Eclipse XDB-C8	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.6%
ZORBAX Eclipse XDB-Phenyl	80Å	180 m^2/g	60°C	2.0 - 9.0	Double	7.2%

Symmetry® is a registered trademark of Waters Corporation.
Luna® is a registered trademark of Phenomenex.

ZORBAX Eclipse XDB Column Ordering Information

Column Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11
Standard Columns and Bulk Packings					
Semi-Prep	9.4 x 250	5	990967-202	990967-206	
Analytical	4.6 x 250	5	990967-902	990967-906	990967-912
Analytical	4.6 x 150	5	993967-902	993967-906	993967-912
Analytical	4.6 x 50	5	946975-902	946975-906	
Rapid Resolution	4.6 x 150	3.5	963967-902	963967-906	963967-912
Rapid Resolution	4.6 x 100	3.5	961967-902	961967-906	
Rapid Resolution	4.6 x 75	3.5	966967-902	966967-906	966967-912
Rapid Resolution	4.6 x 50	3.5	935967-902	935967-906	935967-912
Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312
Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312
Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312
Solvent Saver Plus	3.0 x 100	3.5	961967-302	961967-306	961967-312
Solvent Saver Plus	3.0 x 75	3.5	966954-302		
Narrow Bore	2.1 x 150	5	993700-902	993700-906	993700-912
Narrow Bore	2.1 x 50	5	960967-902	960967-906	960967-912
Narrow Bore RR*	2.1 x 150	3.5	930990-902	930990-906	
Narrow Bore RR	2.1 x 100	3.5	961753-902	961753-906	
Narrow Bore RR	2.1 x 75	3.5	966735-902		
Narrow Bore RR	2.1 x 50	3.5	971700-902	971700-906	
MicroBore RR	1.0 x 150	3.5	963600-902	963600-906	
MicroBore RR	1.0 x 50	3.5	965600-902	965600-906	
MicroBore RR	1.0 x 30	3.5	961600-902	961600-906	
Bulk Packing, 2 grams		5	920966-902		
Guard Cartridges, 4/pk	4.6 x12.5	5	820950-925	820950-926	820950-927
Guard Cartridges, 4/pk	2.1 x12.5	5	821125-926	821125-926	821125-926
Guard Hardware Kit			820777-901	820777-901	820777-901
Agilent Cartridge Columns					
Analytical	4.6 x 250	5	7995118-585	7995108-585	
Analytical	4.6 x 150	5	7995118-595	7995108-595	
Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344	
Solvent Saver	3.0 x 75	3.5	7995230-344		
Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504	
Cartridge Holder			5021-1845	5021-1845	
High Throughput Cartridge Columns (requires Hardware Kit 820222-901)					
Rapid Resolution Cartridge	4.6 x 30	3.5	933975-902	933975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	933975-932	933975-936	
Rapid Resolution Cartridge	4.6 x 15	3.5	931975-902	931975-906	
Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	931975-932	931975-936	
Rapid Resolution Cartridge	2.1 x 30	3.5	973700-902	973700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	973700-932	973700-936	
Rapid Resolution Cartridge	2.1 x 15	3.5	975700-902	975700-906	
Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	975700-932	975700-936	
Hardware Kit for High Throughput Columns			820222-901	820222-901	
CombiHT Columns (end fittings required)					
CombiHT	21.2 x 150	5	970150-902	970150-906	
CombiHT	21.2 x 100	5	970100-902	970100-906	
CombiHT	21.2 x 50	5	970050-902	970050-906	
CombiHT End Fittings (2) (required for use)			820400-901	820400-901	
Capillary Glass-lined Columns					
Capillary	0.5 x 250	5	5064-8286		
Capillary	0.5 x 150	5	5064-8287		
Capillary RR	0.5 x 150	3.5	5064-8288		
Capillary RR	0.5 x 35	3.5	5064-8298		
Capillary	0.3 x 250	5	5064-8269		
Capillary	0.3 x 150	5	5064-8291		
Capillary RR	0.3 x 150	3.5	5064-8271		
Guard Cartridges	0.5 x 35	5	5064-8296		
Guard Cartridges	0.3 x 35	5	5064-8297		

*RR: Rapid Resolution 3.5 µm columns.

For more information on these and other columns consult the Agilent web site at **www.agilent.com.**

Configurations not shown are available upon request.



Agilent Technologies

For the latest information on the complete line of Agilent Technologies columns and supplies for analytical instruments, see our online catalog at www.agilent.com/chem on the World Wide Web, or contact your local Agilent sales office. For all other areas contact Agilent or your local authorized distributor.

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Printed in the USA
December 3, 2002
5988-8367EN

Agilent Model 255 Nitrogen Chemiluminescence Detector (NCD) Analysis of Adhesive Samples Using the NCD

Technical Overview

Introduction

The Agilent Model 255 NCD can easily replace a NPD for the analysis of 2-pyrrolidone and 1-vinyl-2-pyrrolidinone in adhesive samples.

A short-term evaluation was performed for the analysis of adhesive samples for two of the starting materials, 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. According to government regulations, the concentration of 1-vinyl-2-pyrrolidinone cannot exceed 800 ppm in the final product.

The primary objective for the analysis of adhesive was to demonstrate the repeatability and stability of the Model 255 NCD. The secondary objective of the study was to prove the NCD could replace the nitrogen-phosphorus detector (NPD) being used for the application. Table 1 compares the NCD and NPD.

The results from the evaluation demonstrated the Model 255 NCD could easily replace a NPD to monitor 2-pyrrolidone and 1-vinyl-2-pyrrolidinone. The gas chromatograph and the Model 255 NCD were calibrated once and not recalibrated again during the 3-day demonstration. Representative precision data are shown in Tables 2 and 3. The gas chromatograph made over 160 injections of the adhesive samples during the 3-day evaluation with the percent relative standard deviation for the Model 255 NCD of less than 4%. The Model 255 was also linear from 20 to 3000 ppm. Figures 1 and 2 illustrate chromatographic response at high and low levels, respectively. Unlike an NPD, the sample matrix did not affect the detector performance.

For the 2-pyrrolidone and 1-vinyl-2-pyrrolidinone analysis, the NCD provides very good short-term and long-term precision. The NCD is unaffected by high levels of the sample matrix, and its use would require less day-to-day maintenance than an NPD. Use of the Model 255 NCD versus an NPD would result in more accurate and precise results and would reduce the level of instrument maintenance required.

Table 1. Comparison of Agilent NCD to NPD

	Agilent 255 NCD	NPD
Response	Equimolar	Non-equimolar
Quenching	No	Yes
Selectivity	> 107 gN/gC	105 gN/gC
Sensitivity	< 5 pg/sec	0.4 pg/sec
Ease of Use	Straightforward	Daily maintenance required

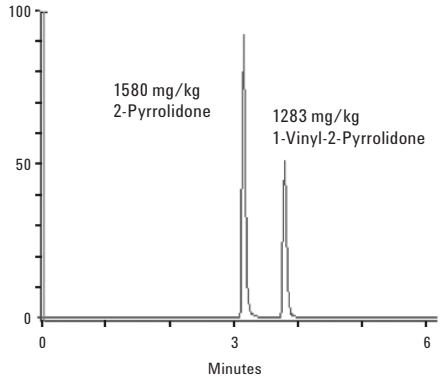

Figure 1. Agilent Model 255 analysis of adhesive standard.

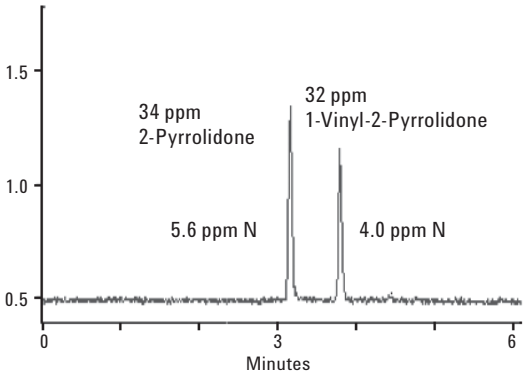

Figure 2. Agilent Model 255 NCD analysis of adhesive sample diluted in tetrahydrofuran.

GC Operating Conditions

(Agilent 6890 with EPC)

Temperature:	160 °C isothermal
Helium carrier:	2.2 mL/min
Split injection:	45.5:1 split
	200 °C
	1 µL injection volume

NCD Burner Conditions

Temperature:	800 °C
Hydrogen flow rate:	25 mL/min
Oxygen flow rate:	10 mL/min
Column:	20 Rtx-5, 0.32 mm id
	3 µm film thickness

Sample Preparation

Samples diluted in toluene
Dilution factors of 1:25 to 1:50

Table 2. Summary of 2-Pyrrolidone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	25.0	1094	2.2
Adhesive 2	43	1:41	28.1	1163	2.2
Adhesive 3	43	1:28	34.0	1405	2.2
Adhesive 4	43	1:45	20.3	838	1.7

Table 3. Summary of 1-Vinyl-2-Pyrrolidinone in Adhesive Results

Sample number	Number of runs	Dilution factor	Diluted concentration (ppm)	Sample concentration (ppm)	RSD (%)
Adhesive 1	43	1:44	27.1	1188	2.2
Adhesive 2	43	1:41	15.1	624	2.2
Adhesive 3	43	1:28	32.5	1342	2.2
Adhesive 4	43	1:45	Not detected	Not detected	

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Printed in the USA
August 16, 2007
5989-6771EN

Agilent Technologies

Agilent Model 355 Sulfur Chemiluminescence Detector (SCD): Thiophene in Benzene

Technical Overview

Introduction

This overview discusses the analysis of benzene for sulfur contamination. The sensitivity, selectivity, and linear response of the Agilent 355 Sulfur Chemiluminescence Detector (SCD) in the analysis of trace levels of thiophene in benzene illustrates that the Model 355 is well-suited for low-level sulfur analysis.

Benzene is the basic unit of the aromatic class of compounds. The primary sources of benzene are from extraction of hydrocarbon crude distillates, refinery catalytic reforming, carbonization of coal, and the hydrodealkylation of a toluene charge stock. [1] The hydrodealkylation reaction results in the conversion of about 90% of the aromatics in the feed with a selectivity factor of about 95%. Thiophene, which also occurs in the light hydrocarbon fractions distilled from crude stocks and coal tar, is present in levels from 0.4 to 1.4 wt. %. [2] Traditionally, thiophene has been extracted by washing with H_2SO_4 to produce a sweeter product. However, this and other processes of thiophene removal still have difficulty reaching the level of purity required by many chemical markets. Consequently, it is often important to monitor trace levels of thiophene in benzene. The three main applications for benzene are production of ethylbenzene, cumene, and cyclohexane. These three products account for 80% of the benzene consumed as a chemical feedstock. [2]

These species can be classified as intermediates for a wide range of final products, including dyes, resins, solvents, and polymers, including nylon. The purity requirements for synthetic applications continue to become more confining, thereby increasing the need to monitor sulfur removal efficiency and to verify the purity of starting materials.

The data in Figure 1 illustrate the sensitivity of the 355 SCD for trace level analysis of sulfur in a hydrocarbon matrix without interference. Figure 2 displays the linear response of the SCD at trace levels. Correlation coefficients for five orders of magnitude were better than $R^2 = 0.999$. The selectivity of the 355 SCD for sulfur over hydrocarbon is shown in Figures 1 and 3, where sulfur chromatograms show no hydrocarbon interference from the eluting benzene.

The data in Figures 1 and 2 were collected on a Agilent 6890 gas chromatograph with a Agilent 355 SCD directly attached. The chromatograms in Figure 3 were collected simultaneously without column splitting, using the flame ionization detector attachment to the SCD. The chromatographic conditions for the trace analysis are summarized in Table 1.

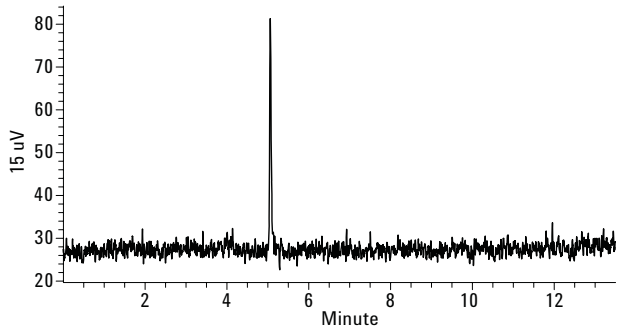

Figure 1. Chromatogram illustrating the analysis of 15 ppb thiophene (as sulfur, split 1:10).

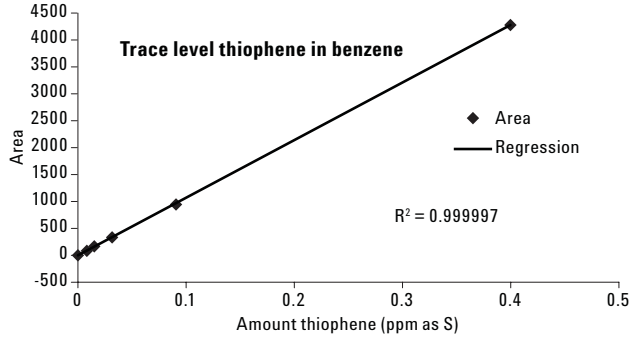

Figure 2. Linearity of trace level analysis of thiophene in benzene.

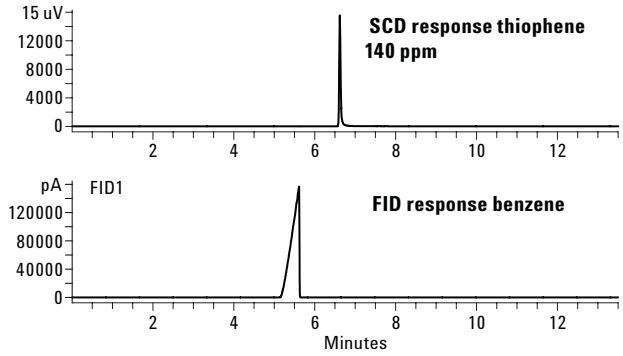

Figure 3. Simultaneous FID-SCD chromatogram on SUPELCOWAX-10.

Table 1. Chromatographic Conditions

Injection temperature	120 °C
Injection volume	1 µL
Initial temperature	30 °C
Initial time	2 min
Rate	10 °C/min
Final temperature	125 °C
Final time	2 min
Split ratio	1:10
Flow mode	Constant flow
Column flow	2 mL/min
Column type	SUPELCOWAX-10
Length	30 m
Internal diameter	0.32 mm
Film thickness	1 µm

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Printed in the USA
June 7, 2007
5989-6786EN



Alternative Fuels

Applications

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Fast Determination of Denatured Fuel Ethanol Purity by Two-Dimensional Gas Chromatography

Application

Petrochemical

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Abstract

Two-dimensional gas chromatography not only provides improved resolution, it can also provide much faster analysis times. A two-dimensional gas chromatography system based on a simplified Deans switch design is used to analyze the alcohol content in denatured fuel grade ethanol. By using two short columns of different selectivity, the two-dimensional gas chromatography application completes this analysis 10 times faster than the standard American Society for Testing and Materials D5501 method [1] without the use of cryogenic oven cooling. This easy-to-use system also provides high qualitative and quantitative precision for this measurement.

Introduction

Oxygenated compounds are added to gasoline to improve octane rating and reduce emissions of smog-producing combustion products. Until recently, methyl-tert-butyl ether (MtBE) has been the most widely used oxygenated additive in reformulated gasoline (RFG). However, problems with groundwater contamination by MtBE have led to the designation of ethanol as the preferred gasoline additive.

Fermentation and distillation of biomass is used to produce a fuel grade ethanol that contains 85%–98% ethanol and natural impurities such as water and methanol. Before it can be used as a gasoline additive, fuel ethanol is denatured with approximately 2 to 5 vol% of natural gasoline to make it unsuitable for beverage use. This final product is known as denatured fuel ethanol. ASTM Specification D4806 outlines the performance requirements for denatured fuel ethanol [2].

The overall purity of denatured fuel ethanol must be measured by gas chromatography (GC) to meet the ASTM specifications and to determine the amount of methanol impurity present in the material. Since methanol is also an oxygen containing species, its concentration must be determined since it can affect the resulting properties of the RFG. However the natural gasoline denaturant complicates these measurements because it is difficult to separate methanol and ethanol from the hydrocarbons present in the natural gasoline. ASTM method D5501 uses GC with a 150-meter methyl silicone capillary column and subambient oven temperatures to separate the methanol and ethanol from the low-boiling hydrocarbons [1]. While this approach is effective, the run times are in excess of 40-minutes and the method requires the use of a cryogenic coolant.

Two-dimensional gas chromatography (2-D GC) offers another solution to this analysis that is also faster. By combining two short columns of different selectivity, complete separation of the

polar alcohol from the nonpolar hydrocarbons can be made in much less time than is needed with a single, long column. The primary column can make a fast, yet incomplete separation of the target compounds from the majority of compounds in the matrix. As the target compounds and any interference elute from the primary column, they can be selectively transferred to the secondary column. Complete separation of the target compound from interferences is achieved on this column. This technique has been successfully applied to the analysis of oxygenates and aromatics in bulk gasoline samples [3].

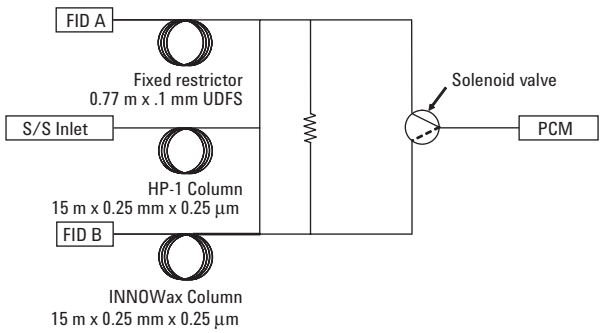

Figure 1. Deans switch configuration for the fast analysis of denatured fuel ethanol with an FID.

Experimental

An Agilent 6890N GC was equipped with a split/splitless injector, a pneumatics control module (PCM), two flame ionization detectors (FIDs) and an automatic liquid sampler. A short, nonpolar HP-1 column was used as the primary column and a polar INNOWax was used as the secondary column. The two columns were linked using a fluidic Deans switch. Figure 1 shows the instrument configuration using this hardware and Table 1 list the details of the hardware configuration.

Determination of electronic pressure control (EPC) pressures, flow rates, and the fixed restrictor dimensions were performed using a Deans switch calculator software program that is specially designed for this system. This calculator program is included with the Deans switch hardware option for the Agilent 6890N GC. All instrument set points required for any successful 6890N based Deans switch application can be quickly and easily determined using this software.

A sample of denatured fuel ethanol containing 0.3 vol% methanol, 95.2 vol% ethanol and 4.0 vol% natural gasoline was prepared using absolute ethanol. The water content of this sample was not measured. A denaturant blank sample was prepared containing 4.0 vol% natural gasoline in isooctane. These samples were used to determine the heart-cut times for the 2-D GC method.

Instrument conditions for this analysis are listed in Table 2. The sample is injected into the split/splitless inlet and is separated on the 15-meter HP-1 primary column. With the solenoid switch in the “off” position, the HP-1 column effuent is directed to FID A. Just before the methanol and ethanol peaks elute from the HP-1 column, the solenoid is automatically set to the “on” position and the HP-1 column effuent is redirected to the INNOWax column. After the ethanol peak has been cut to the INNOWax column, the solenoid valve is set back to the “off” position. The separation of the alcohol peaks from the hydrocarbons is then completed on the INNOWax column and detected on FID B.

Table 1. Agilent 6890 Hardware Configuration for 2-D GC

Standard 6890N GC hardware	
G1540N	Agilent 6890N Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 210 (2 of each)	FID with EPC control
Option 309	Pneumatics control module with EPC control
SP1 2310-0129	General purpose Deans switch kit, factory installed (may be ordered as an add-on kit for existing 6890 GC Agilent part no. G2855A)
G2613A	Agilent 7683 Autoinjector
Columns	
Primary column	HP-1 column, 0.25 µm film, 15 m × 0.25 mm id (Agilent part no. 19091S-931)
Secondary column	INNOWax column, 0.25 µm film, 15 m × 0.25 mm id (Agilent part no. 19091N-131)
Fixed restrictor*	Deactivated fused silica tubing, 0.38 m × 0.1 mm id (Agilent part no. 160-2635-10)
Data system	
G2070A	Agilent Multitechnique ChemStation
Other consumables	
Agilent part no. 5181-1267	10 µL Fixed tapered needle autoinjector syringe
Agilent part no. 5183-4647	Inlet liner optimized for splitless operation
Agilent part no. 5183-4759	Advanced green septa

*The Deans switch calculator was used to determine the correct EPC pressures for column flows and the dimensions of the fixed restrictor.

Table 2. Instrument Conditions

Split/Splitless injection port	
Temperature	225 °C
Pressure*	25 psi helium
Split ratio	600:1
Injection size	0.2 µL
HP-1 column flow	2.0 mL/min, constant pressure mode
Pneumatics control module (PCM)*	19 psi helium, constant pressure mode
INNOWax column flow	3.0 mL/min
FID temperatures	250 °C
Oven temperature program	70 °C for 3 min, 25 °C/min to 225 °C

*These pressures were calculated using a custom Deans switch software program to achieve the necessary column flow rates.

Results and Discussion

ASTM method D5501 can easily separate methanol and ethanol from the hydrocarbons in the gasoline denaturant. This method achieves the required chromatographic resolution by using a large number of chromatographic plates provided by the very long capillary column and the subambient oven temperatures. Figure 2 shows a typical denatured fuel ethanol analysis using this method. Although the methanol and ethanol separation is completed in 12 minutes, the time required to elute all of the gasoline components can extend to 60 minutes.

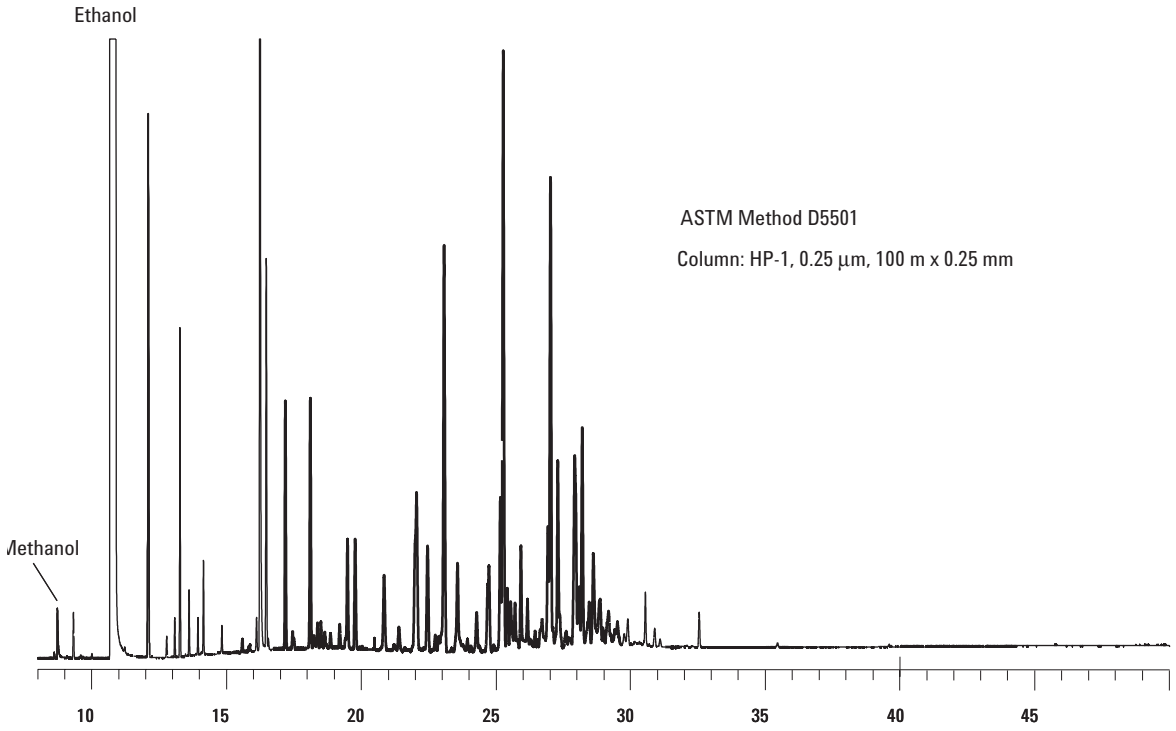

Figure 2. A typical analysis of denatured fuel ethanol using ASTM method D5501. The long column (100 m) and subambient starting temperature (15 °C) contributes to an overall analysis time of 40 minutes or longer.

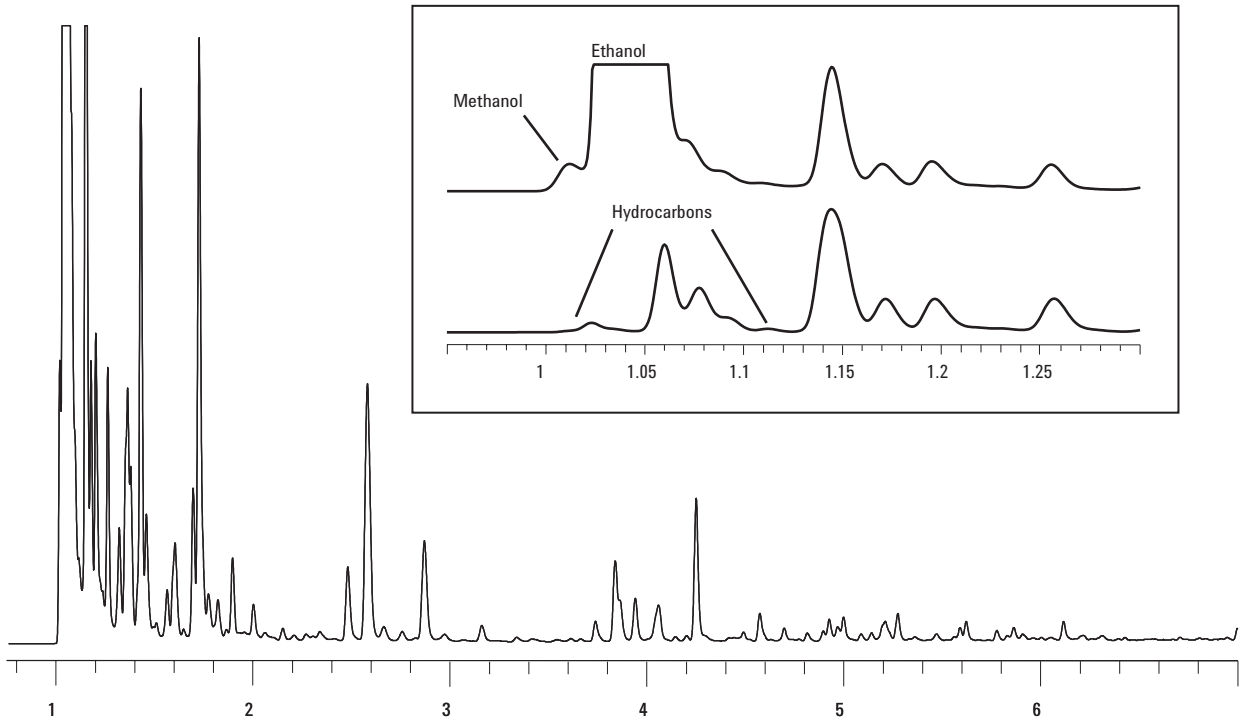

Figure 3. Analysis of denatured fuel ethanol using a much shorter (15 m) HP-1 column. The analysis time is much faster, but the methanol is not separated from the ethanol and there is significant co-elution of the alcohol peaks with hydrocarbons from the natural gasoline. The inset chromatogram shows a comparison of the denatured fuel ethanol sample with a natural gasoline blank.

An easy way to get faster chromatography is to use shorter columns, but one must pay a resolution penalty for the speed gain [4]. See Figure 3. A 15-meter methyl silicone column was used to analyze the fuel ethanol sample in less than 7 minutes. However, the methanol peak is not completely resolved from the ethanol. It also appears that several other compounds are not resolved from the two alcohol peaks. The inset chromatogram in Figure 3 shows a comparison of the denatured fuel ethanol sample with the natural gasoline blank. There are a number of hydrocarbons in the natural gasoline that elute at the same time as the methanol and ethanol with this short capillary column.

Two-dimensional GC can solve this problem by combining two shorter GC columns with different selectivity. For this analysis, a 15-meter HP-1 column was used to perform a preliminary boiling point separation of the alcohols from the bulk of the natural gasoline components. The Deans switch selectively transferred the alcohol peaks and any interferences to a polar INNOWax

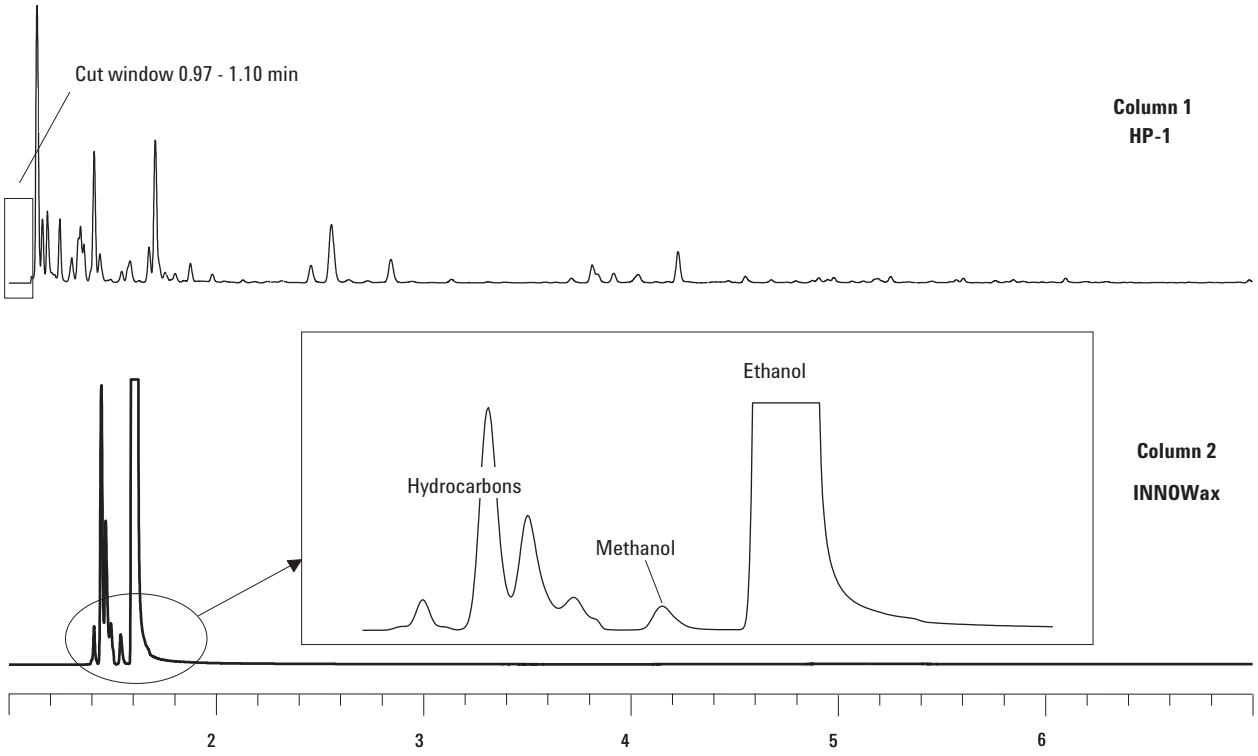

Figure 4. Analysis of denatured fuel ethanol by 2-D GC using a Deans switch to heart cut the alcohol peaks from a 15-m HP-1 column to a 15-m INNOWax column. The heart-cut window is from 0.97 to 1.10 minutes. The chromatographic run is completed in less than 7 minutes using this technique.

column, where the nonpolar hydrocarbons were quickly eluted and the polar alcohols retained and separated. Figure 4 shows the results of this separation using the 2-D GC system. On the HP-1 column, the methanol and ethanol elution time was between 0.97 and 1.10 minutes. This time was use used to heart-cut the alcohols from the HP-1 column to the INNOWax column. The inset chromatogram in Figure 4 shows that the interfering hydrocarbons are separated from the alcohols and the methanol and ethanol are completely resolved.

A sample of the natural gasoline was also run by 2-D GC under the same conditions used for the denatured fuel ethanol analysis. This was done to assure that no other hydrocarbons co-eluted with the alcohol peaks after heart cutting to the INNOWax column. Figure 5 shows that the 2-D GC system using the HP-1 column and the INNOWax column completely eliminated any interference from the gasoline denaturant with the alcohol peaks.

The overall analysis time of this 2-D GC approach is also much faster than the ASTM method. The choice of the INNOWax column was made so that the starting oven temperature could be set to 70 °C, instead of 15 °C used in the original ASTM method. This higher oven temperature allowed the GC recycle time to be approximately 10 minutes shorter, thus reducing the overall run time of the method. The higher temperature also avoids the expense and complication of using cryogens.

The precision of the 2-D GC method was evaluated by running a denatured fuel ethanol sample 30 times over a 5-day period. Table 3 shows the retention time (RT), detector response and volume percent precisions for methanol and ethanol analysis. The high degree of both qualitative (RT) and quantitative precision indicates that the 2-D GC system used for this analysis was stable over extended periods of time.

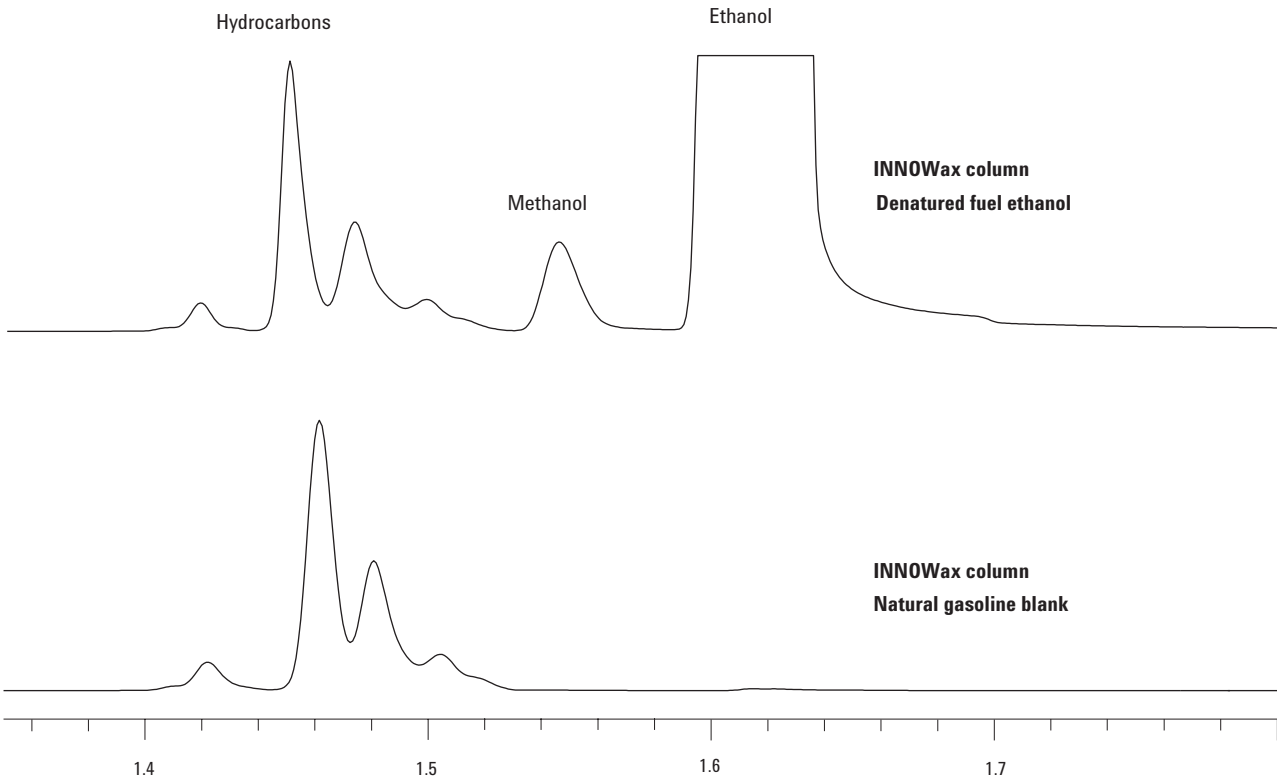

Figure 5. The upper chromatogram shows the analysis of denatured fuel ethanol using the 2-D GC system. The lower chromatograms shows a sample of the natural gasoline denaturant run on the same 2-D GC system. No hydrocarbons in the gasoline blank elute on the INNOWax column at the same time as methanol and ethanol.

Table 3. Measurement Precision for the Analysis of Denatured Fuel Ethanol by 2-D GC

	Methanol			Ethanol		
	RT (min)	**Area counts**	**Vol%**	**RT (min)**	**Area counts**	**Vol%**
Average	1.552	47.92	0.3	1.622	20410.55	95.4
SD	0.0004	0.35	0.002	0.0004	118.93	0.6
%RSD	0.025	0.74	0.7	0.023	0.58	0.6

Conclusion

Two-dimensional GC offers both high resolution of target compounds in complex matrices and a significant improvement in analysis speed when compared to high resolution GC methods that use a single, long capillary column. This is shown for the analysis of ethanol and methanol content in denatured fuel grade ethanol. A 2-D GC system with a simplified Deans switch used two short columns of different selectivity to quickly separate the alcohols in fuel ethanol from the natural gasoline hydrocarbons used as a denaturant. The 7-minute GC analysis time with this system was shown to be nearly 10-times faster than the standard ASTM methodology. The overall run time was also faster since the 2-D method used an initial oven temperature that was well above ambient and did not require cryogenic coolants. This 2-D GC approach also gave precise results over an extended period of time.

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Printed in the USA
April 24, 2003
5988-9460EN



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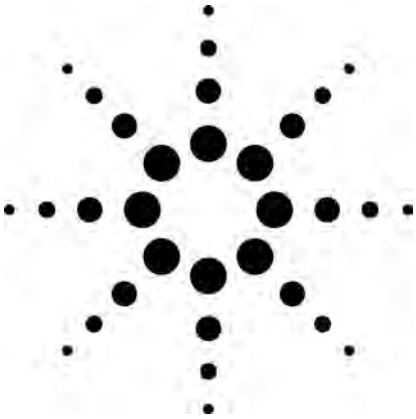
Determining the Ester and Linoleic Acid Methyl Ester Content to Comply with EN14103

Application



HPI

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Abstract

Gas chromatography with a split/splitless inlet and FID is used to determine the ester and linoleic acid methyl ester content of fatty acid methyl esters (FAME) intended for use as pure biofuel or as a blending component for heating and diesel fuels. The method is suitable for FAME containing methyl esters between C14 and C24. This application used the Agilent 6850 System and HP-INNOWax column; calibration was achieved with internal standards of methyl heptadecanoate. After analyzing several different types of biodiesel, excellent precision was obtained, exceeding the EN14103 method specifications.

Introduction

Biodiesel fuel is produced when a vegetable oil or an animal fat reacts with methanol in the presence of a catalyst to yield fatty acid methyl esters (FAME) and glycerin, which is removed. FAME is a pure biodiesel fuel called B100. A "green" fuel, biodiesel is biodegradable, nontoxic, and is essentially free of sulfur and aromatics. It is rapidly gaining momentum worldwide as an alternative fuel source for diesel engines.

Only biodiesel fuel meeting the specifications of ASTM D6751 or EN14214 is acceptable for use as a motor fuel. Several GC methods have been developed to determine if a biodiesel meets the specification. For example, EN14103 determines the ester and linoleic acid methyl ester content; EN14105 and ASTM D6584 determine free and total glycerin and mono-, di-, and triglyceride content; and EN14110 is for methanol. EN14106, which determines free glycerol, is not commonly used since 14105/ASTM D6584 provides more complete results.

Three major GC biodiesel solutions—EN14103, EN14105/ASTM D6584, and EN14110—were developed for the Agilent GC platform. This application describes the performance of EN14103 on the Agilent 6850 GC.

Experimental

The application was conducted with the Agilent 6850 GC with FID, split/splitless inlet, and

HP-INNOWax column (30 m × 320 µm id × 0.25 µm film of polyethylene glycol). A solution of methyl heptadecanoate in heptane (10 mg/mL or 5 mg/mL) was used as a calibration for quantification.

Gas Chromatographic Conditions

Inlet Temperature:	250°C
Split ratio:	80:1
Injection volume:	1µL
Column flow (He):	1.5 mL/min, constant flow mode
FID temperatures:	300°C
H_2 flow:	40 mL/min
Air flow:	400 mL/min
Make up (N_2):	40 mL/min
Oven program:	210 °C hold 9 min, to 230 °C at 20°C/min, hold 10 min
Column:	30m x 320mm x 0.25 µm HP-INNOWax (Part no. 19091N-113)
Calibration standard:	Solution of methyl heptadecancate in heptane (5 mg/mL)

Sample Preparation

Accurately weigh approximately 250mg of sample in 10-mL vial, and then add 5 mL of methyl heptadecanoate solution using a pipette.

Results and Discussion

Several samples of B100 biodiesel made from vegetable oils and animal oils were analyzed. Figure 1 and Figure 2 are the chromatograms of rapeseed oil and pork oil, respectively.

The HP-INNOWax column exhibits excellent separation for the methyl esters between C14 and C24, which obtain baseline separation. To achieve a satisfactory compromise between resolution and analysis time, esters between C14 and C20 were separated isothermally at 200 °C; esters between C22 and C24 were separated at 230 °C.

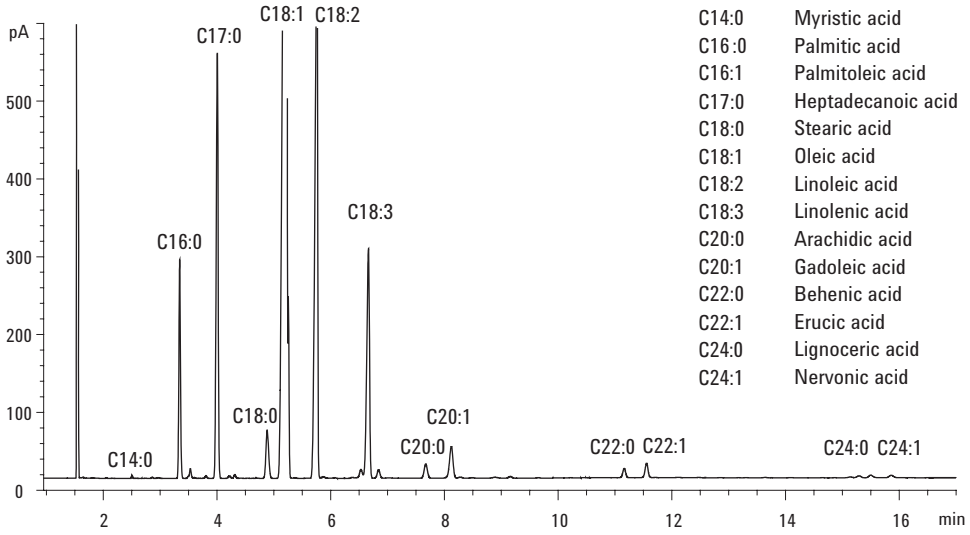

Figure 1. Chromatogram of rapeseed methyl esters.

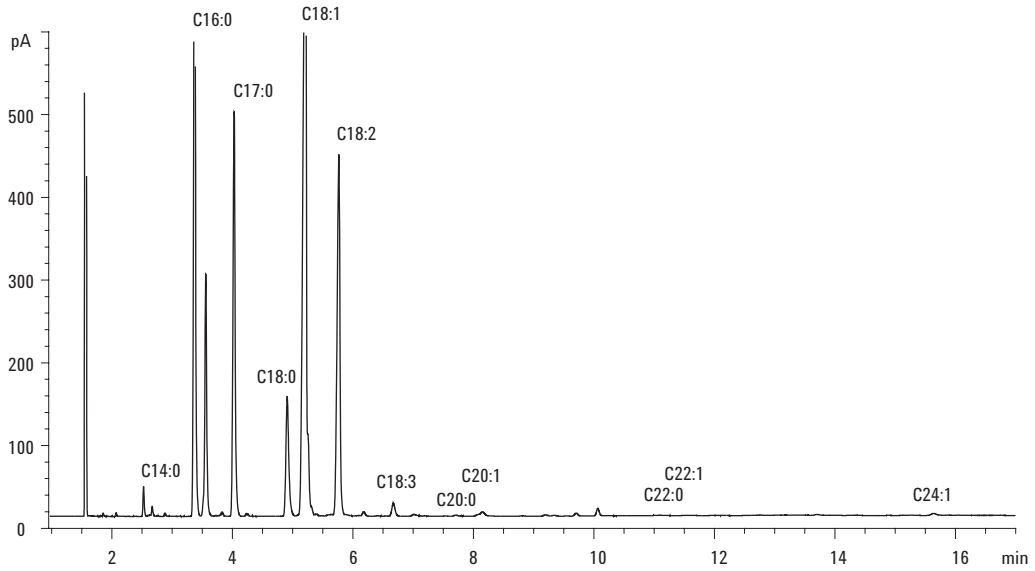

Figure 2. Chromatogram of pork methyl esters.

Quantitative results for different types of biodiesel fuel, such as rapeseed oil, soybean oil, chicken oil and pork oil are shown in Table 1. It can be seen that rapeseed oil contains a higher concentration of C18:1 (55.68% m/m) and soybean oil contains a higher concentration of C18:2 (48.66% m/m). The animal oils (chicken and pork) contain a higher concentration of C18:1; however, compared with vegetable oil, animal oil contains a higher concentration of C16:0.

Table 2 shows excellent repeatability, exceeding the specification of EN14103. The data in Table 3 demonstrate that most RSD% is within 1%.

Table 1. Observed FAME Composition in % (m/m) of Different Type Oil

		Average, % (m/m)			
Component FAME		**Rapeseed oil**	**Soybean oil**	**Chicken oil**	**Pork oil**
Myristic acid	C14:0	0.04	0.07	1.12	0.43
Palmitic acid	C16:0	4.12	9.90	17.63	15.62
Palmitoleic acid	C16:1	0.05	0.02	2.15	5.28
Stearic acid	C18:0	1.57	4.27	9.91	3.93
Oleic acid	C18:1	55.68	22.54	34.32	28.48
Linoleic acid	C18:2	17.82	48.66	7.38	11.87
Linolenic acid	C18:3	7.61	7.27	0.37	0.48
Arachidic acid	C20:0	0.56	0.32	0.14	0.05
Gadoleic acid	C20:1	1.31	0.18	0.73	0.29
Behenic acid	C22:0	0.32	0.32		
Erucic acid	C22:1	0.51			
Lignoceric acid	C24:0	0.15		0.08	
Nervonic acid	C24:1	0.16	0.16	0.15	0.15

Table 2. Repeatability* for Different Type Biodiesel

		Observed			
	EN14103 Spec (m/m)	Soybean (m/m)	Rapeseed (m/m)	Chicken (m/m)	Pork (m/m)
Ester content	1.6%(m/m)	0.065%(m/m)	0.254%(m/m)	0.021%(m/m)	0.098%(m/m)
C18:3 content	0.1%(m/m)	0.005%(m/m)	0.018%(m/m)	0.002%(m/m)	0.012%(m/m)

*The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment with a short time interval.

Table 3. Relative Standard Deviation Data (RSD%) for FAME Analysis

		RSD%, %(m/m) (Average=5)			
Component FAME		Rapeseed oil	Soybean oil	Chicken oil	Pork oil
Myristic acid	C14:0	0.38	0.34	0.17	0.16
Palmitic acid	C16:0	0.05	0.01	0.05	0.03
Palmitoleic acid	C16:1	0.17	1.02	0.16	0.11
Stearic acid	C18:0	0.16	0.49	0.06	0.23
Oleic acid	C18:1	0.14	0.04	0.03	0.10
Linoleic acid	C18:2	0.14	0.02	0.03	0.47
Linolenic acid	C18:3	0.11	0.03	0.28	0.95
Arachidic acid	C20:0	0.35	0.16	0.32	1.55
Gadoleic acid	C20:1	0.46	1.05	0.20	0.63
Behenic acid	C22:0	0.34	0.49		
Erucic acid	C22:1	0.23			
Lignoceric acid	C24:0	1.31		1.14	
Nervonic acid	C24:1	1.15	1.31	1.49	0.89

Conclusions

The ester and linoleic acid methyl ester content present in the different types of biodiesel fuel produced from rapeseed, soybean, chicken, and pork were quantitatively analyzed using the Agilent 6850 System equipped with a split/splitless inlet, FID, and HP-INNOWax column. Calibration was achieved with internal standards, methyl heptadecanoate. The results show excellent repeatability, exceeding the specification of EN14103. The relative standard deviation is less than 1% for almost all methyl esters.

References

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Printed in the USA
December 7, 2006
5989-5924EN

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Comprehensive Flow Modulated Two-Dimensional Gas Chromatography System

Application Brief

Roger L. Firor

Two-dimensional GC (GC x GC), a relatively new gas chromatographic technique, is being recognized for its powerful separation capabilities for the analysis of complex mixtures. The methodology involves the use of two capillary columns of usually very different polarities installed in series. Between the two columns a device known simply as a flow modulator is installed and interfaced to an auxillary programmable control module (PCM) on an Agilent 7890A gas chromatograph through a three-way solenoid valve. In the flow modulator, analyte bands from the first column are collected in a fixed volume channel and successively injected very quickly into the short second column in very narrow bands.[1] Any separation that occurs on the first column is preserved during transfer to the second column. In summary, GC x GC can greatly increase peak resolution and peak capacity.

The unique flow modulator used in this system is based on Agilent's Capillary Flow Technology hardware and does not require the use of cryogenics for focusing. The modulator consists of a planar structure where flow splitters and collector channel are all incorporated internally to the device. All external connections are made through Agilent CPM fittings (ultimate union technology) incorporated into the plate for zero unswept volumes and leak proof seals. A three-way solenoid valve is installed on top of the GC oven and is interfaced to a PCM module. Experimental conditions used are shown below.

Experimental

GC:	Agilent 7890A, FID at 200 Hz data collection rate, split/splitless inlet
Carrier:	Hydrogen
Column 1:	30 m x 0.25 mm x 0.25 µm HP-5ms, 19091S-433
Column 1 Pressure:	21.5 psig at 50 °C, constant flow mode
Column 2:	5 m x 0.25 mm x 0.15 µm HP-INNOWax
Column 2 Flow:	20 mL/min, constant flow mode
Oven Program:	50 °C (1.0 min) to 260 °C (4 min) @ 8 °C/min.
Modulator Period:	1.4 seconds collect, 0.12 seconds flush typical

GC x GC analysis software: GC Image[2]

An illustration of the modulator is given in Figure 1. The precisely timed and synchronized periodic switching between collect and flush states directs sample pulses continuously to the second column for additional separation during the

Highlights

- Flow modulation offers a viable alternative to thermal modulation without the burdens imposed by cryo requirements for comprehensive GC x GC.
- Modulation, timing (collect and inject), and synchronization are all integrated into the 7890A GC system for easy setup and operation.
- Agilent's fifth-generation electronic pneumatic control with setting to three decimal points combined with Capillary Flow Technology hardware forms the basis for an easy-to-use GC x GC system.

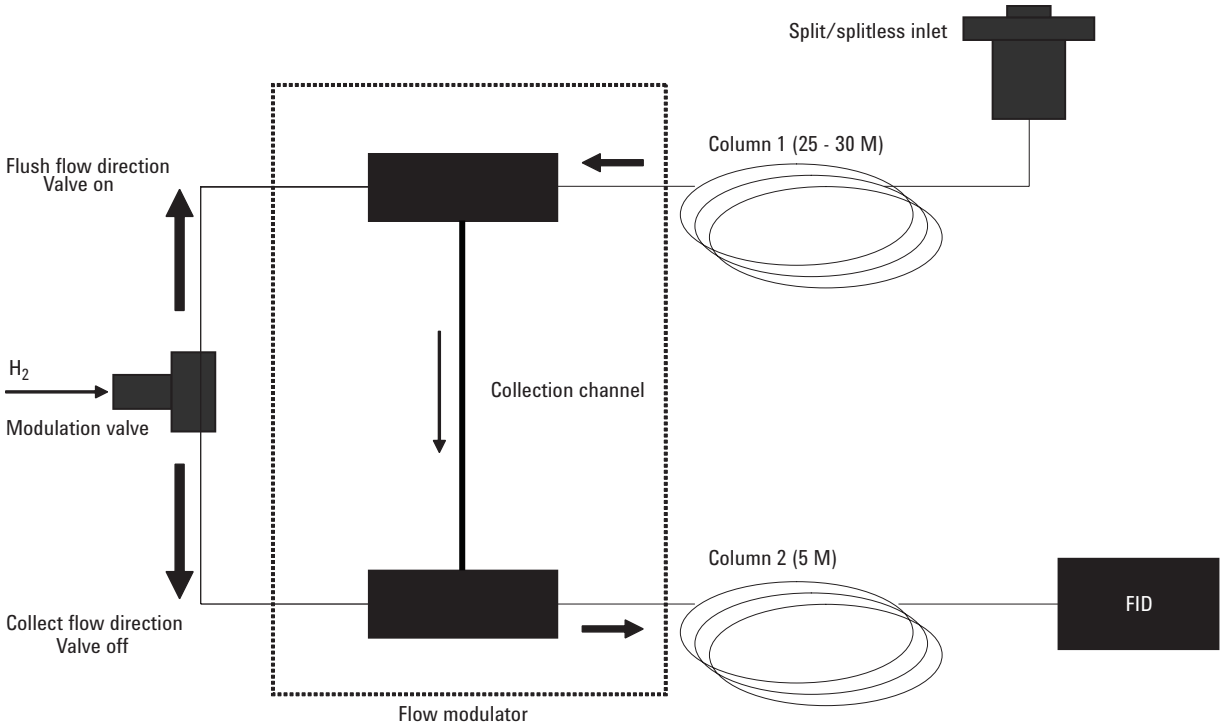

Figure 1. Flow modulator design – differential flow system.

length of the chromatographic run.

Discussion

Figures 2a and 2b show unmodulated and modulated peaks, respectively, of a pure analyte. In this example, n-butylbenzene is shown with approximately three modulations across the first-column peak. The areas of the modulated peaks should ideally equal the area of the un-modulated peak. In other words, no material should be lost in the transfer to the second column. Area agreement was within 3% for this test. Peak widths at half height for modulated butylbenzene are approximately 100 ms. Very narrow peaks as required for the technique are

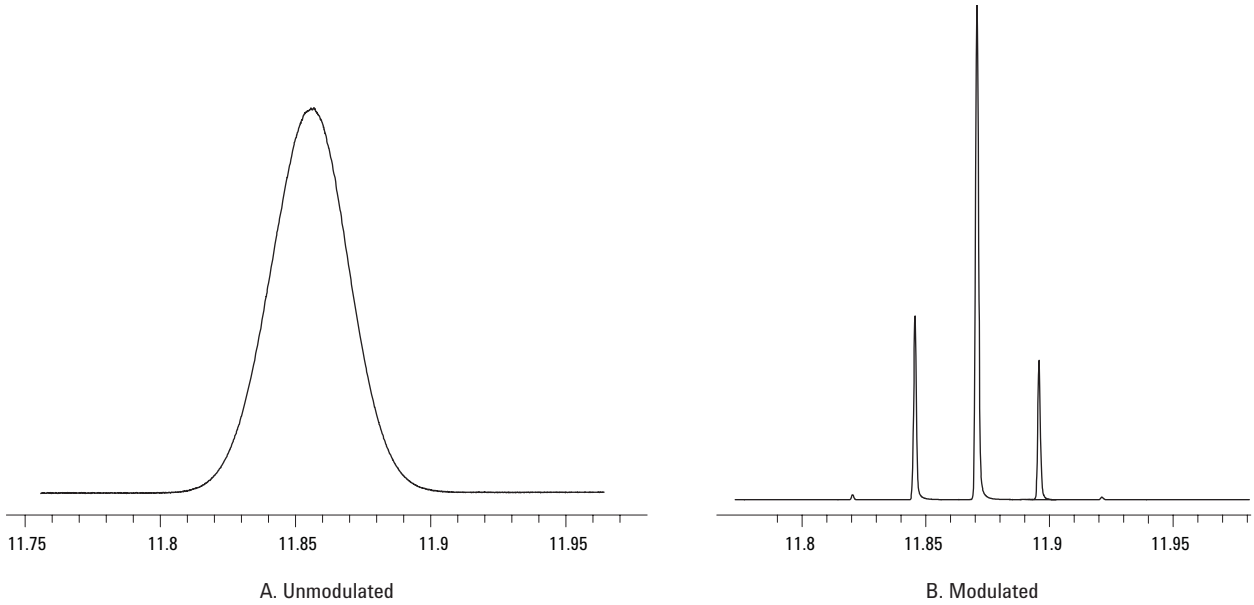

Figure 2. Modulated and unmodulated n-butylbenzene (not to same scale).

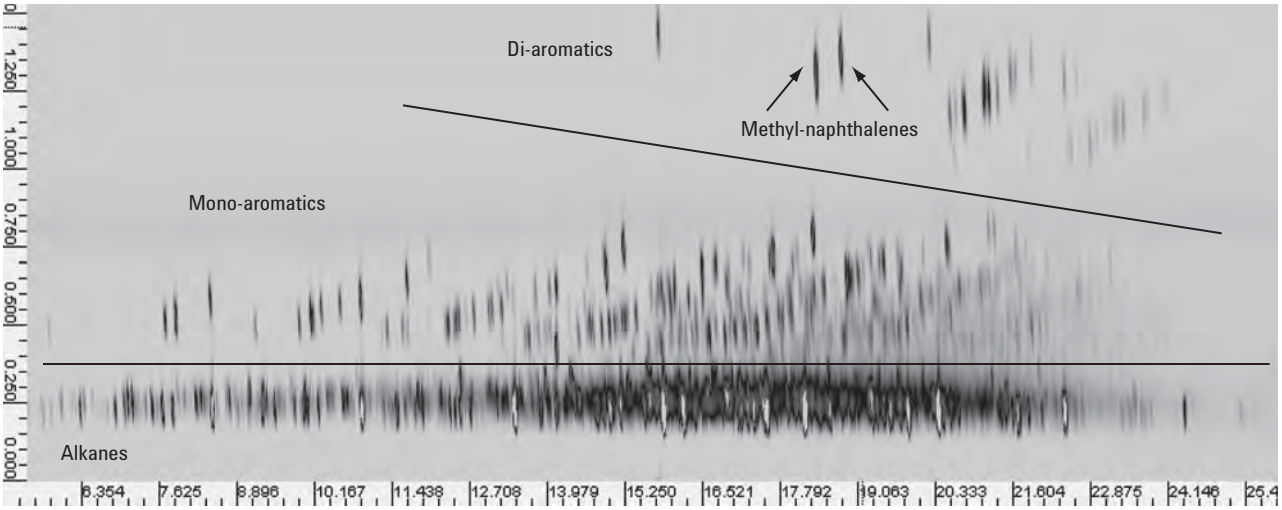

Figure 3. A 2D image of No. 2 kerosene.

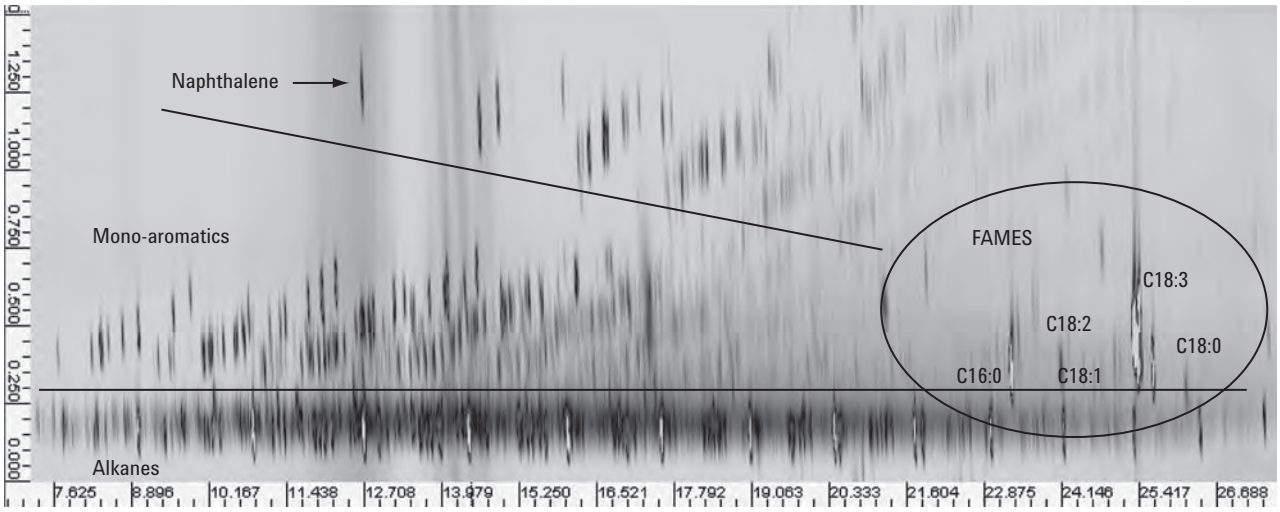

Figure 4. A B20 soy-based biodiesel (20% methyl ester, 80% diesel).

seen that approach the peak widths obtained by thermal modulation systems. Flow modulation has the distinct advantage of not requiring cryo fluid for focusing.

Use of a nonpolar column followed by a polar column produces hydrocarbon-type retention in the following order: alkanes, cyclic alkanes, olefins, single-ring aromatics, and multi-ring aromatics. An example of a 2D image of No. 2 kerosene is shown in Figure 3. Chemical classes are clearly discernable, with good resolution seen for the aromatics. Another example, B20 soy-based biodiesel (20% methyl ester, 80% diesel) is shown in Figure 4. Here the C16 and C18 fatty acid methyl esters are indicated. Data processing for all samples was performed using GC Image[2].

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Printed in the USA
January 17, 2007
5989-6078EN

Agilent Technologies

Analysis of Glycerin and Glycerides in Biodiesel (B100) Using ASTM D6584 and EN14105

Application

HPI/Petrochemicals/Polymers

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Abstract

The analysis of free glycerin (glycerol) and total glycerides (mono-, di-, and triglycerides) in B100 biodiesel was performed according to ASTM method D6584 and CEN method EN14105. Method improvements were demonstrated through the use of a 530-µm id high-temperature fused-silica retention gap coupled to the analytical column. This was made possible with an Agilent Capillary Flow Technology Ultimate Union designed for inert, high-temperature GC oven operation. This configuration on the Agilent 7890A GC System showed calibration and precision performance that exceeded both D6584 and EN14105 specifications. This application provides complete system configuration as well as guidelines for successful analysis of free glycerin and total glycerides in biodiesel.

Introduction

Biodiesel is a motor or heating fuel produced from renewable vegetable oils or animal fats. With the high cost and limited availability of crude oil, renewable fuels like biodiesel are seen as a way to replace, supplement, or extend traditional petroleum fuels. Biodiesel is produced by a process called transesterification. The vegetable oil is reacted with methanol in the presence of a catalyst to produce a mixture of fatty acid methyl esters (FAME) and glycerin. After removal of the glycerin and other contaminants, the remaining FAME mixture is pure biodiesel. Depending on the oil source, a typical biodiesel contains FAME mixtures having both saturated and unsaturated carbon chains from C_8 to C_{24}. Table 1 shows the distribution and relative amounts of FAME found in biodiesel made from common plant oils.[1]

Pure biodiesel is generally not used as a fuel, but instead it is blended with petroleum diesel. Biodiesel is defined by the notation Bxx, where xx indicates the volume percent of FAME content in the liquid. Using this nomenclature, B100 is pure FAME, B50 contains 50 volume % FAME, B5 contains 5 volume % FAME, etc. Common commercial biodiesel blends are B2, B5, and B20.

Before biodiesel can be sold as a fuel or blending stock, it must first meet a defined standard. ASTM standard D6751 and European Committee of Standardization (CEN) standard EN14214 set similar specifications for biodiesel blending and motor fuels.[2,3] In each standard, an important specification is a limit on the amounts of free glycerin and glycerides in biodiesel. Free glycerin is a byproduct of biodiesel production. Mono-glycerides, diglycerides, and triglycerides are partially reacted oils that may be contaminants in the finished biodiesel. High amounts of free glycerin can cause problems due to separation. High amounts of glycerides and glycerin can result in increased engine deposits. Table 2 shows the limits set by each standard.

Table 1. Distribution and Relative Amounts of FAMEs Derived from Vegetable Oils

	Weight Percent FAMEs											
Oil type	**C8:0**	**C10:0**	**C12:0**	**C14:0**	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C20:0 C18:3**	**C20:1 C22:0**	**C22:1**
Rapeseed					2–5	0.2	1–2	10–15	10–20	5–10	0.9	50–60
Soybean				0.3	7–11	0–1	3–6	22–34	50–60	2–10	5–10	
Palm				1–6	32–47		1–6	40–52	2–11			
Coconut	5–9	4–10	45–52	13–18	7–10		1–4	5–8	1–3			
Palm kernel	2–4	3–7	44–51	14–19	6–9	0–1	1–3	10–18	1–2		1–2	

Table 2. Free and Total Glycerin Specifications for Biodiesel

	EN14214		ASTM D6571	
	Limit (% m/m)	**Test method**	**Limit (% m/m)**	**Test method**
Free glycerin	0.02 max	EN14105	0.020 max	D6584
Monoglycerides	0.80 max	EN14105	NA	D6584
Diglycerides	0.20 max	EN14105	NA	D6584
Triglycerides	0.20 max	EN14105	NA	D6584
Total glycerin	0.25 max	EN14105	0.240 max	D6584

ASTM and CEN have defined several physical and chemical test methods to meet the standard specifications. An important chemical test measures the free glycerin and glyceride content in B100. Two gas chromatographic methods, EN14105 and D6584, were developed to make this measurement.[4,5] Both are nearly identical in sample preparation, instrument configuration, operating conditions, and reporting. Since glycerin and glycerides are polar and high boiling, they must first be derivatized to improve volatility and reduce activity before injection into the GC. A cool-on-column inlet (COC) and high-temperature capillary column are used to make the analysis of these compounds easier. Another important consideration when using these methods is the source of the biodiesel. Both methods were developed for B100 derived from vegetable oils such as rapeseed, soybean, sunflower, and palm. It is known that these methods are not suitable for B100 derived from lauric acid oils, such as coconut and palm kernel oils.

Experimental

Instrument Configuration

Table 3 lists the details of the GC configuration used for this work. A 530-µm id high-temperature retention gap was used between the on-column inlet and the analytical capillary column to improve sample vaporization and provide easy sample injection using a standard tapered needle syringe. An Agilent Capillary Flow Technology Ultimate Union was used to join the retention gap and the analytical column. Table 4 shows the GC operating conditions used for this analysis.

Standard and Sample Preparation

Agilent Technologies biodiesel standards were used containing glycerin, monoolein, diolein, triolein, butanetriol (internal standard #1), and tricaprin (internal standard #2) at concentrations specified in the ASTM and CEN methods. A list of these standards and other chemical reagents used for this analysis are shown in Table 3.

Five GC calibration standards were prepared by mixing aliquots of the individual stock standards in proportions specified by the ASTM and CEN methods. After mixing, 100 µL of the derivatization agent, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added to each calibration standard. After 20 minutes, 8 mL of reagent grade n-heptane was added to each calibration standard. These final reaction mixtures were directly injected into the gas chromatograph.

Sample preparation followed the procedure in the ASTM and CEN methods. Two samples of B100, from soybean oil and rapeseed oil, were used for this application. Each sample was run two times over four consecutive days with fresh calibration standards prepared and run for each analysis.

Table 3. System Configuration (SP1 7890-0294)

Standard 7890A GC hardware	
G3440A	Agilent 7890A Series GC
Option 122	Cool-on-column inlet with electronic pneumatics control (EPC)
Option 211	Capillary flame ionization detector (FID) with EPC control
G2613A	Agilent 7683 Autoinjector
Columns	
Analytical column	DB-5ht, 15 m x 0.32 mm id x 0.1-µm film (part no. 123-5711)
High-temperature retention gap	Deactivated fused-silica tubing, 1 m x 0.53 mm id (part no.160-2865-5 comes in 5-m lengths)
Union	Capillary Flow Technology Ultimate Union Kit (part no. G3182-61580)
Union ferrules	0.32-mm column Siltite ferrules (part no. 5188-5362) 0.53-mm column Siltite ferrules (part no. 5188-5363)
Data system	
	Agilent Multitechnique ChemStation
Consumables	
5181-1267	10-µL Teflon fixed autoinjector syringe
Standards and reagents	
5190-1408	Biodiesel D6584 kit, 5 calibration standard solutions and 2 internal standard solutions
5190-1407	Biodiesel MSTFA derivatization kit, 10 x 1 mL ampoule

Table 4. Instrument Conditions

Cool-on-column inlet	
Mode	Ramped
Initial temperature	oven track, approx 50 °C
Pressure	7.6 psi helium
Injection amount	1 µL
Initial column flow	3.0 mL/min, constant pressure mode
FID temperature	380 °C
Oven temperature program	50 °C for 1 min, 15 °C/min to 180 °C, hold 0 min 7 °C/min to 230, hold 0 min 30 °C/min to 380, hold 10 min

Results and Discussion

After running the standards, Agilent ChemStation was used to calculate linear calibration curves for glycerin, monoolein, diolein, and triolein. The curves for each compound showed excellent linearity and y-intercepts near zero. These curves are shown in Figure 1. The correlation coefficients (r^2) for each compound exceeded the specification of 0.99 set forth in the ASTM and CEN methods.

Figure 2 shows the typical chromatograms obtained for samples of soybean B100 and rapeseed B100. The large peaks observed in each chromatogram are the FAMEs present in the samples. Figure 3 shows the selected regions of the rapeseed chromatogram where glycerin, monoglycerides, diglycerides, and triglycerides elute. Peak identification for each compound is made using the relative retention times published in the ASTM method (Table 5). The retention time of the first internal standard, 1,2,4-butanetriol, was used to identify glycerin. The retention time of the second internal standard, tricaprin, was used to identify the monoglycerides, diglycerides, and triglycerides.

Using the approach detailed in the ASTM and CEN methods, the amount of glycerin in each sample was calculated with the calibration functions derived from the glycerin calibration curve. Likewise, the amount of monoglycerides, diglycerides, and triglycerides was determined from the monoolein, diolein, and triolein calibration functions, respectively. Table 6 list the amounts of glycerin and glycerides found in each sample.

Precision of the analysis was measured using repeatability, which is the difference between two successive analyses of the same sample run on the same day by a single operator on the same instrument. This repeatability measurement was made for each sample over four consecutive days. Table 7 shows the results of the daily precision measurements compared to the specifications from the ASTM D6584 method. These results show excellent single-day precision as determined by repeatability.

ASTM D6584 and EN14105 are not easy methods to run for a number of reasons: the sample preparation is lengthy and difficult; the sample injection

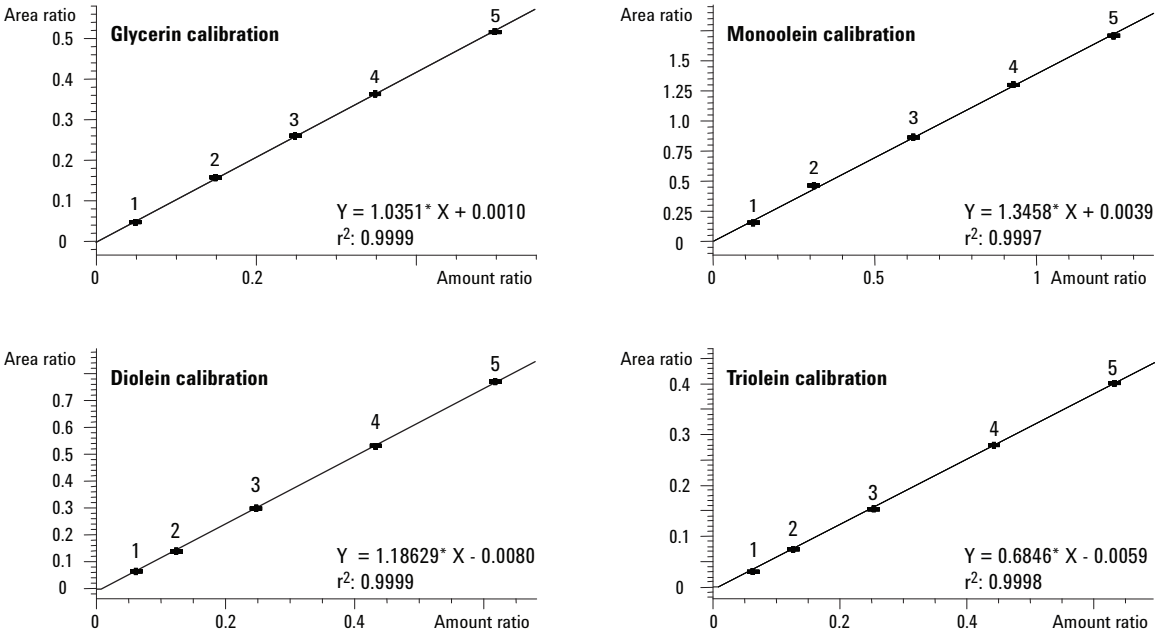

Figure 1. Calibration curves for glycerin, monoolein, diolein, and triolein.

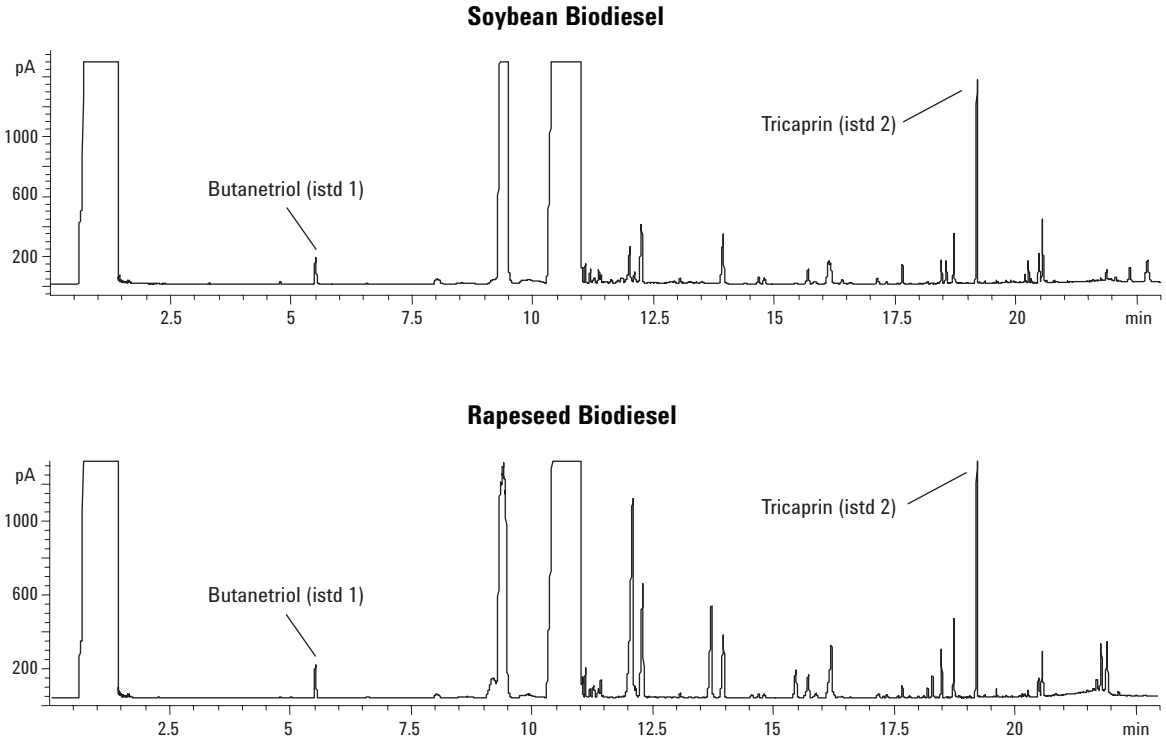

Figure 2. Chromatograms showing typical analysis of free and total glycerins in two B100 biodiesel samples.

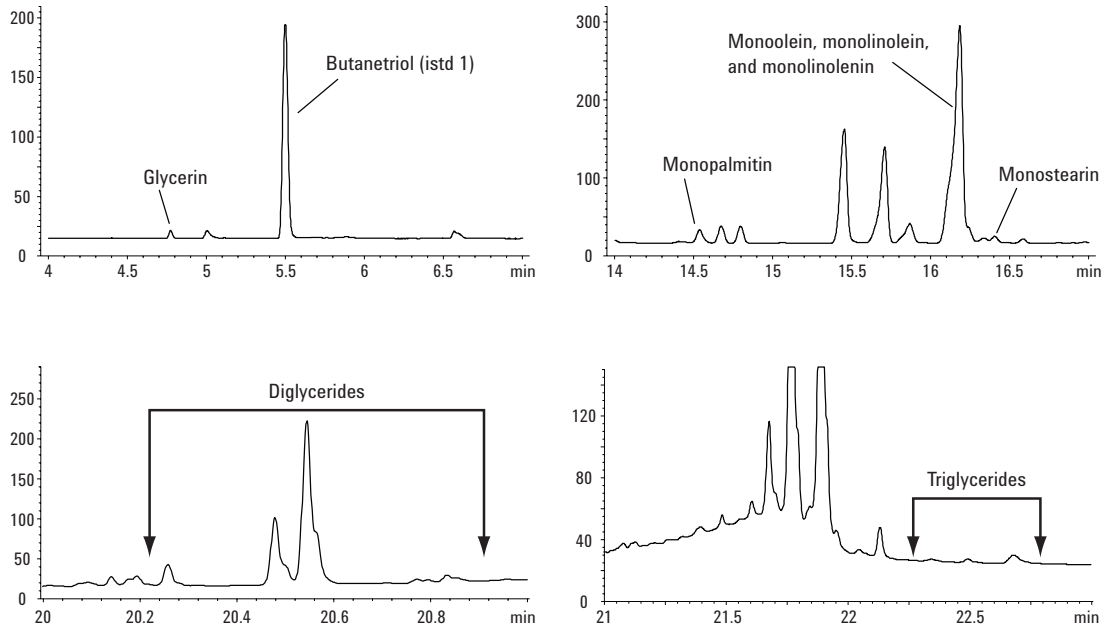

Figure 3. Details of glycerin, monoglycerides, diglycerides, and triglycerides found in a sample of rapeseed B100 biodiesel.

onto a 0.32-mm id column is not easily automated; and calibration can be difficult. However, there are a number of guidelines and procedures that can be followed to obtain good, precise results.

Sample and Standard Preparation

1. Prepare fresh calibration standards every day. Once the standards are prepared they should not be stored for more than several hours.
2. Use commercially prepared stock or final calibration standards packaged in sealed, glass ampoules. If all of the standard solutions are not used in a single day, do not save for later use. Water can accumulate in the solutions and this will inhibit derivatization.
3. Only use derivatization-grade MSTFA. Lesser grades contain solvents that can reduce the effectiveness of the reagent. It is best to purchase MSTFA in small quantities packaged in sealed, glass ampoules. As with the standards, discard any unused MSTFA.
4. Use only clean, dry glassware and pipettes.
5. Only analyze finished product B100. This method should not be used for process samples since high methanol content or water content will inhibit derivatization.
6. Run all samples immediately after preparation. Do not store prepared sample for more than several hours, especially in humid environments.

Table 5. Relative Retention Times Used for Peak Identification

	RRT (int std 1)	RRT (int std 2)
Glycerin	0.85	
1,2,3-Butanetriol (int std 1)	1.00	
Monopalmitin		0.76
Monoolein, monolinolein, monolinolenin, monostearin		0.83 – 0.86
Tricaprin (int std 2)		1.00
Diglycerides		1.05 – 1.09
Triglycerides		1.16 – 1.31

GC Analysis

It is recommended that a retention gap be used between the GC inlet and the column. The retention gap will improve peak shape and sample vaporization, as well as maintain column efficiency. Figure 4 shows the improvement in peak shape for glycerin and 1,2,3-butanetriol when using a 0.53-mm id retention gap. A retention gap will also prolong the column life since it traps any nonvolatile compound contained in the sample. A 0.53-mm id retention gap will also make sample injection easier since it can easily accommodate the standard single tapered syringe needle.

Table 6. Weight Percent of Free and Total Glycerin

	%(m/m) in Soybean B100 Biodiesel			
	Day 1 (avg)*	Day 2 (avg)*	Day 3 (avg)*	Day 4 (avg)*
Free glycerin	0.004	0.004	0.004	0.004
Monoglycerides	0.287	0.280	0.285	0.290
Diglycerides	0.533	0.527	0.533	0.546
Triglycerides	0.387	0.371	0.340	0.304
	%(m/m) in Rapeseed B100 Biodiesel			
	Day 1 (avg)*	Day 2 (avg)*	Day 3 (avg)*	Day 4 (avg)*
Free glycerin	0.002	0.002	0.002	0.002
Monoglycerides	0.365	0.375	0.370	0.371
Diglycerides	0.256	0.262	0.256	0.256
Triglycerides	0.021	0.019	0.018	0.016

*Average of 2 runs per day for each sample.

Table 7. Repeatability Results for Two B100 Biodiesel Samples Over Four Days

	Soybean B100 Biodiesel				
	ASTM D6584 Specification (% m/m)	Observed repeatability (%m/m) Day 1	Day 2	Day 3	Day 4
Glycerin	0.001	0.000	0.000	0.000	0.000
Monoglycerides	0.021	0.005	0.007	0.007	0.000
Diglycerides	0.021	0.008	0.008	0.014	0.000
Triglycerides	0.032	0.008	0.004	0.005	0.000

	Rapeseed B100 Biodiesel				
	ASTM D6584 Specification (% m/m)	Observed repeatability (%m/m) Day 1	Day 2	Day 3	Day 4
Glycerin	0.001	0.000	0.000	0.000	0.000
Monoglycerides	0.021	0.007	0.000	0.006	0.000
Diglycerides	0.021	0.003	0.002	0.000	0.000
Triglycerides	0.032	0.002	0.000	0.001	0.000

One problem with using a retention gap is the high oven temperature (380 °C) required for triglyceride elution. Most fused-silica tubing cannot be used above 350 °C. Also, traditional column unions can leak above that temperature. The Agilent Capillary Flow Technology Ultimate Union combined with special high-temperature fused-silica tubing can solve this problem. The Ultimate Union is made with deactivated stainless steel that can be taken to 400 °C without losing inertness. The high-temperature polyimide coating on the retention gap has extended lifetime up to 380 °C.

Successfully using this Union first requires that the retention gap and column be correctly installed using the metal ferrules designed for the Union. Next, the Union must be completely supported so that no weight is placed on the column connections. A bracket is supplied with the Ultimate Union Kit to support the union fitting to the GC oven wall. Failure to do this will result in a large leak after only a few runs above 350 °C, resulting in column damage. Figure 5 shows a correct installation with the Union supported on its bracket in the GC oven. From this photo it can be seen there is no stress on the column or retention gap. Additionally, to extend the lifetime of this connection, the oven temperature should be kept at 50 °C between analyses. It is also recommended that the Union be checked for leaks before running samples. If a leak is detected, make a new connection to the Union with a new ferrule, and evaluate the column performance before running samples.

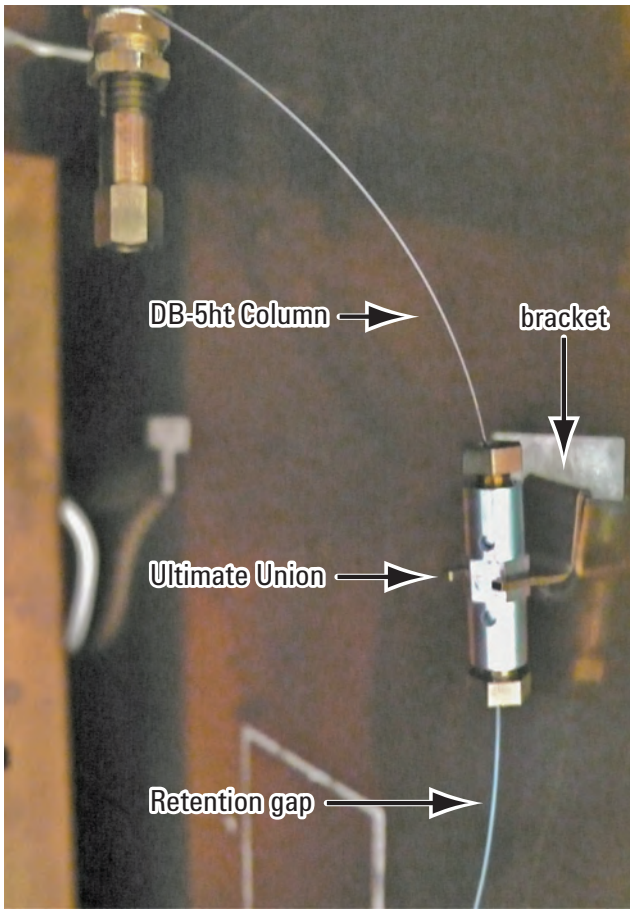

Figure 5. Details of the retention gap and analytical column joined with a Capillary Flow Technology Ultimate Union.

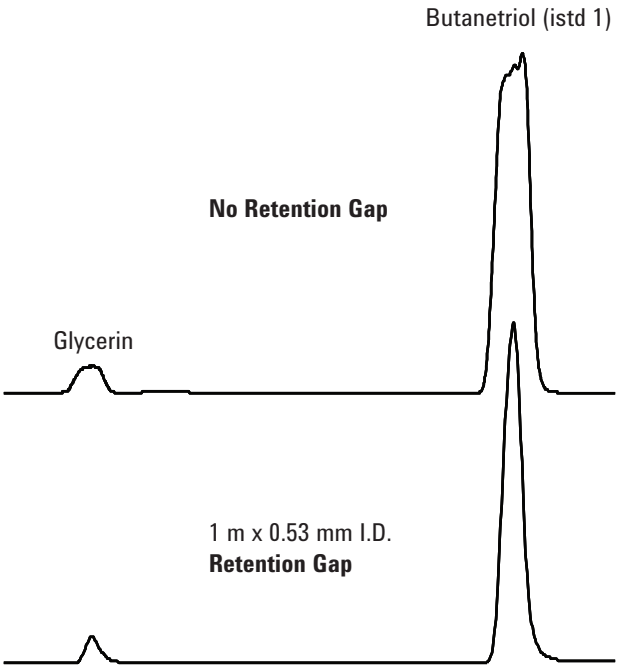

Figure 4. Improved peak shape for glycerin and 1,2,3-butanetriol when using a retention gap and the Capillary Flow Technology Ultimate Union.

Conclusions

The analysis of free and total glycerins can be done using ASTM D6584 or EN14105. Both methods are nearly identical in sample preparation and analysis. This application described the configuration of an Agilent 7890A gas chromatograph for these methods. By combining careful and deliberate sample preparation with a high-temperature retention gap and a Capillary Flow Technology Ultimate Union, this system can obtain results that meet or exceed the methods' calibration and precision specifications.

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Printed in the USA
November 9, 2007
5989-7269EN



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Direct Elemental Analysis of Biodiesel by 7500ce ICP-MS with ORS

Application Note

Petrochemical

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Abstract

A simple and sensitive method is described for the measurement of multiple trace elements in a range of biofuels using an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) featuring the Octopole Reaction System (ORS). Each sample was analyzed directly following a simple dilution in kerosene. The ORS effectively removes matrix and plasma-based spectral interferences to allow measurement of all important analytes, including sodium, potassium, lead, vanadium, and sulfur, at levels lower than those possible by ICP-optical emission spectroscopy (OES). The detection limits (DLs) and background equivalent concentrations (BECs) were in the µg/kg (ppb) range for all elements except sulfur (mg/kg). Good spike recovery is presented for a rapeseed biodiesel sample and petrochemical diesel (between 90 and 120%, with the majority of elements within 5% of the target value). A 10-hour stability test using a spiked (approx 10 µg/kg) rapeseed material yielded excellent precision for almost all elements (between 6.4% for boron and 1.3% for arsenic). The results show that the 7500ce methodology can be used for the routine measurement of trace metals in biofuels.

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Introduction

Biodiesel is a renewable fuel derived from natural oils that can be used in diesel-engine vehicles with little or no modification. Biodiesel refers to a collection of alkyl esters derived from renewable or "bio" sources such as cooking oils, animal fats, or plant oils. In its pure form it is termed fatty acid methyl ester (FAME). Commercial biodiesel is usually a blend of the pure FAME and fossil-fuel petrochemical diesel. The blend ratio is given as its B number, for example, B5 (5% FAME 95% petro); B100 (100% FAME).

Elements of interest are catalyst residues (usually Na and K), sulfur due to emission legislation and the low sulfur requirements of modern diesel engines, and catalyst poison elements such as lead, vanadium, and mercury. Although lead, vanadium, and mercury tend to be naturally low in the raw oil stock, they can still be found in the final product from contamination during oil processing or final FAME production. Common fuel additives include silicon (anti-foaming agent), manganese (burn improver), and other specialist materials such as additives for marine applications (chromium, iron, and nickel products). These elements need to be monitored to ensure correct dosage. Traditional analytical techniques for analysis of biofuels are ICP-optical emission spectroscopy (OES) and atomic absorption spectroscopy (AAS/FAAS). However, these techniques suffer from intense interferences due to an increased background continuum from the carbon matrix, so they are limited to a few elements or have limited detection limits.

Experimental

Standards and internal standards were prepared from ~1,000 mg/kg metallo-organic oils (Spex Certiprep, Metuchen, New Jersey, USA, and Conostan, Conoco-Phillips, Bartlesville, OK, USA). Internal standards were added to all samples and standards prior to analysis to compensate for viscosity differences. Samples and standards were prepared by simple 3x dilution in kerosene (Purum, Fluka Sigma-Aldrich, St. Louis, USA) using weight/weight preparation. Five samples were analyzed: processed soybean and rapeseed oils, pork and poultry tallow (both B100), and petrochemical diesel taken from a local gasoline station.

An Agilent 7500ce ICP-MS fitted with an Octopole Reaction System (ORS) for removal of polyatomic interferences was used for this study. The instrument was operated using hydrogen, helium, xenon, and no-gas cell modes. All modes were acquired sequentially during a single visit to the sample vial. Instrumental conditions are given in Table 1.

Table 1. 7500ce ICP-MS Operating Conditions

Forward power	1550W
Plasma gas	15 L/min
Auxillary gas	1 L/min
Sampling depth	8 mm
Carrier gas	0.9 L/min
Oxygen (50% O_2 in Ar)	0.12 L/min
ORS Cell Gas Flow Rates	
Helium (He)	5.7 mL/min
Hydrogen (H_2)	6.5 mL/min
Xenon (Xe)	0.54 mL/min

The ORS operating in helium collision mode removes polyatomic interferences using a process known as kinetic energy discrimination (KED). The larger interfering species experience more collisions with He atoms and lose energy as they pass through the cell. An energy differential is applied to prevent the lower energy polyatomic ions from entering the mass filter. Reaction mode using hydrogen gas was used to remove the intense plasma-based species such as $^{14}N_2$ on ^{28}Si, $^{38}Ar^1H$ on ^{39}K, and ^{40}Ar on ^{40}Ca. The interference is neutralized or converted to another species by reaction. Finally, sulfur can be measured most effectively by removing the O_2 interference by reaction with Xe cell gas using a third cell gas controller (Agilent product number G3264A). For interference-free analytes, the cell can be operated in no-gas mode, that is, with no cell gas added to the ORS cell. Oxygen was added to the plasma by an additional mass flow controller (Agilent product number G3145B) to remove excess carbon from the plasma and to prevent it from condensing on the interface and ion lenses; this was used in conjunction with the organic solvent introduction kit (Agilent product number G1833-65038). Note that, while a 50% O_2 in argon mix was used for this application, a 20% O_2 in argon mix is the preferred option, as this provides more precise control of the O_2 flow rate, which may be critical in certain applications, such as low-level S and P analysis.

Results and Discussion

Detection limit (DL) and background equivalent concentration (BEC) for the kerosene diluent and a diluted soy B100 sample are given in Table 2. The DLs and BECs were in the µg/g (ppb) range for all elements except sulfur (mg/kg). The calibration profiles for vanadium (Figure 1) were obtained with and without cell gas. The large offset for the calibration graph in no-gas mode (due to matrix interference) is removed by the ORS in helium mode. Figure 2 shows a calibration plot for ^{32}S in Xe gas mode, demonstrating the effective removal of the O_2 interference by the ORS.

Tables 3 and 4 show the analytical results, including spike recoveries for rapeseed FAME and petrochemical diesel. Recoveries for all elements were between 90 and 120%, but the majority fell within 5% of the target value. This indicates reliable interference removal for the spiked matrices and the applicability of the technique for trace and ultratrace determination of metallic impurities. The data for sodium is particularly high for the B100 rape oil and pork tallow samples; this is an indication of inadequate cleanup procedures of the final FAME product.

Table 2. Detection Limits (3s) and Background Equivalent Concentrations (BEC) for Kerosene Solvent and 3x Diluted B100 Soy FAME Data Reported as µg/kg (S as mg/kg)

			Kerosene		B100 Soy					Kerosene		B100 Soy	
Element	**Mass**	**Mode**	**DL**	**BEC**	**DL**	**BEC**	**Element**	**Mass**	**Mode**	**DL**	**BEC**	**DL**	**BEC**
Be	9	NoGas	0.0153	0.0234	0.0109	0.0423	Ni	60	He	0.0250	0.0367	0.126	0.165
B	10	NoGas	0.344	4.63	6.57	30.1	Cu	63	He	0.0525	0.681	0.0264	0.832
Na	23	NoGas	2.99	18.8	1.19	62.5	Cu	65	He	0.0723	0.675	0.101	0.834
Mg	24	H_2	1.37	8.90	8.65	9.77	Zn	66	He	0.0393	0.0685	0.211	0.963
Si	28	H_2	5.17	53.3	7.44	48.7	As	75	He	0.0896	0.192	0.0660	0.547
P	31	He	10.0	52.0	22.7	811	Sr	88	NoGas	0.0335	0.121	0.0631	0.215
S	32	Xe	0.124	0.569	0.0293	0.980	Mo	95	NoGas	0.500	0.411	0.371	0.332
K	39	H_2	0.649	1.34	2.10	7.25	Ag	107	NoGas	0.0374	0.0832	0.149	0.155
Ca	40	H_2	0.568	2.95	6.40	9.21	Cd	111	NoGas	0.121	0.138	0.108	0.0913
Ti	47	He	0.125	0.0396	0.706	0.680	Sn	118	NoGas	0.0173	0.0901	0.411	84.8
V	51	He	0.0198	0.0435	0.0409	0.134	Sb	121	NoGas	0.0261	0.0827	0.0395	0.0895
Cr	52	H_2	0.0935	0.0772	0.0224	0.402	Ba	137	NoGas	0.0472	0.145	0.0990	1.87
Mn	55	He	0.0249	0.0527	0.0563	0.101	W	182	NoGas	0.0111	0.0231	0.0177	0.0261
Fe	56	He	0.0447	0.129	0.0869	4.21	Hg	201	NoGas	0.0147	0.107	0.123	0.403
Co	59	He	0.0113	0.0245	0.0337	0.0400	Pb	208	NoGas	0.00724	0.0595	0.0226	0.0666

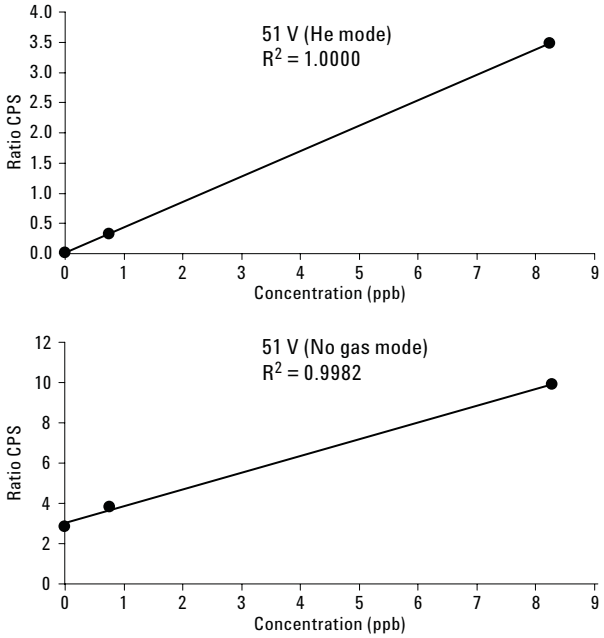

Figure 1. Calibration plots for ^{51}V in helium gas mode (top) and no-gas mode (bottom) demonstrating effective interference removal by the ORS.

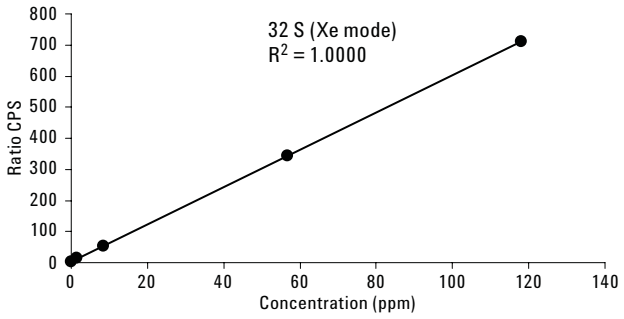

Figure 2. Calibration plot for ^{32}S in Xe gas mode.

Table 3. Analytical Data B100 FAME and a Petrochemical Diesel Sample (Corrected for Dilution Factor)

Element	Mass	Mode	Units	B100 rape	B100 soy	B100 poultry	B100 pork	Petrodiesel
Na	23	He	µg/kg	1,040	127	1,430	614	20.0
K	39	H_2	µg/kg	15.4	19.1	50.6	22.4	6.28
S	32	Xe	mg/kg	3.20	1.29	15.9	18.9	9.85
Be	9	NoGas	µg/kg	0.0609	0.0681	0.0241	0.0202	N/D
B	10	H_2	µg/kg	40.3	81.2	334	54.6	5.08
Mg	24	H_2	µg/kg	8.57	6.16	12.1	7.69	6.80
Si	28	H_2	µg/kg	39.8	6.02	8,220	159	372
P	31	He	µg/kg	21.4	2,120	171	85.5	28.5
Ca	40	H_2	µg/kg	131	20.8	135	26.8	2.57
Ti	47	He	µg/kg	0.342	2.01	8.08	2.45	0.0798
V	51	He	µg/kg	0.186	0.302	1.36	0.310	N/D
Cr	52	H_2	µg/kg	1.36	1.04	0.718	0.394	0.446
Cr	53	H_2	µg/kg	1.09	0.939	0.765	0.376	0.390
Mn	55	He	µg/kg	0.450	0.170	0.364	0.114	0.190
Fe	56	He	µg/kg	4.61	12.3	50.8	5.21	30.5
Co	59	He	µg/kg	0.0449	0.0578	0.124	0.0856	N/D
Ni	60	He	µg/kg	3.64	0.416	0.816	0.397	0.239
Cu	63	He	µg/kg	3.28	0.730	11.5	5.49	2.09
Cu	65	He	µg/kg	3.23	0.752	11.5	5.64	2.12
Zn	66	He	µg/kg	17.9	2.80	27.4	7.74	47.0
As	75	He	µg/kg	1.29	1.18	1.08	1.02	0.145
Sr	88	NoGas	µg/kg	4.59	0.339	3.47	0.583	N/D
Mo	95	NoGas	µg/kg	0.263	N/D	N/D	N/D	N/D
Ag	107	NoGas	µg/kg	0.563	0.257	3.15	0.617	0.0527
Cd	111	NoGas	µg/kg	0.304	N/D	0.589	N/D	N/D
Sn	118	NoGas	µg/kg	N/D	131	1.71	0.138	N/D
Sb	121	NoGas	µg/kg	0.0920	0.0528	0.399	0.109	N/D
Ba	137	NoGas	µg/kg	14.6	5.35	55.8	4.64	N/D
W	182	NoGas	µg/kg	0.121	0.0181	0.112	0.0252	N/D
Hg	201	NoGas	µg/kg	0.582	0.404	0.396	0.791	N/D
Pb	208	NoGas	µg/kg	0.372	0.0450	0.385	0.0693	0.191

Table 4. Spike Recovery Data for a Rape FAME and Petrochemical Sample – Spiked with a Multielement Mixed Concentration Solution – Recoveries Are Presented as %

Sample	Mass	Mode	Units	B100 rape	Rape spike	% recovery	Diesel spike	% recovery
Na	23	He	µg/kg	1,040	Spike too low	197	105	
K	39	H_2	µg/kg	15.4	185	98.2	172	98.5
S	32	Xe	mg/kg	3.20	13.1	101	18.3	108
Be	9	NoGas	µg/kg	0.0609	164	94.9	164	97.3
B	10	H_2	µg/kg	40.3	207	96.8	170	103
Mg	24	H_2	µg/kg	8.57	170	93.6	154	87.1
Si	28	H_2	µg/kg	39.8	202	94.0	Spike too low	–
P	31	He	µg/kg	21.4	211	110	234	122
Ca	40	H_2	µg/kg	131	285	89.2	169	98.7
Ti	47	He	µg/kg	0.342	165	95.5	164	97.1
V	51	He	µg/kg	0.186	170	98.4	172	102
Cr	52	H_2	µg/kg	1.36	171	98.2	169	100
Cr	53	H_2	µg/kg	1.09	167	96.0	163	96.4
Mn	55	He	µg/kg	0.450	167	96.7	168	99.7
Fe	56	He	µg/kg	4.61	182	103	184	90.9
Co	59	He	µg/kg	0.0449	184	101	185	110
Ni	60	He	µg/kg	3.64	193	110	191	113
Cu	63	He	µg/kg	3.28	198	113	200	117
Cu	65	He	µg/kg	3.23	198	113	201	118
Zn	66	He	µg/kg	17.9	225	120	250	121
As	75	He	µg/kg	1.29	230	133	227	108
Sr	88	NoGas	µg/kg	4.59	175	98.9	172	102
Mo	95	NoGas	µg/kg	0.263	161	99.9	164	97.1
Ag	107	NoGas	µg/kg	0.563	183	106	197	117
Cd	111	NoGas	µg/kg	0.304	198	115	200	118
Sn	118	NoGas	µg/kg	N/D	165	95.8	168	99.6
Sb	121	NoGas	µg/kg	0.0920	169	97.9	173	102
Ba	137	NoGas	µg/kg	14.6	172	91.2	156	92.7
W	182	NoGas	µg/kg	0.121	159	97.5	173	102
Hg	201	NoGas	µg/kg	0.582	Not in spike mix	–	–	–
Pb	208	NoGas	µg/kg	0.372	157	90.5	170	100

Instrumental stability was tested for 10 hours using a spiked (approximately 10 µg/kg) rapeseed material. Repeatability over the 10-hour run varied between 6.4% for boron to 1.3% for arsenic, indicating good reproducibility for an extended sample set; the normalized stability plot is displayed in Figure 3.

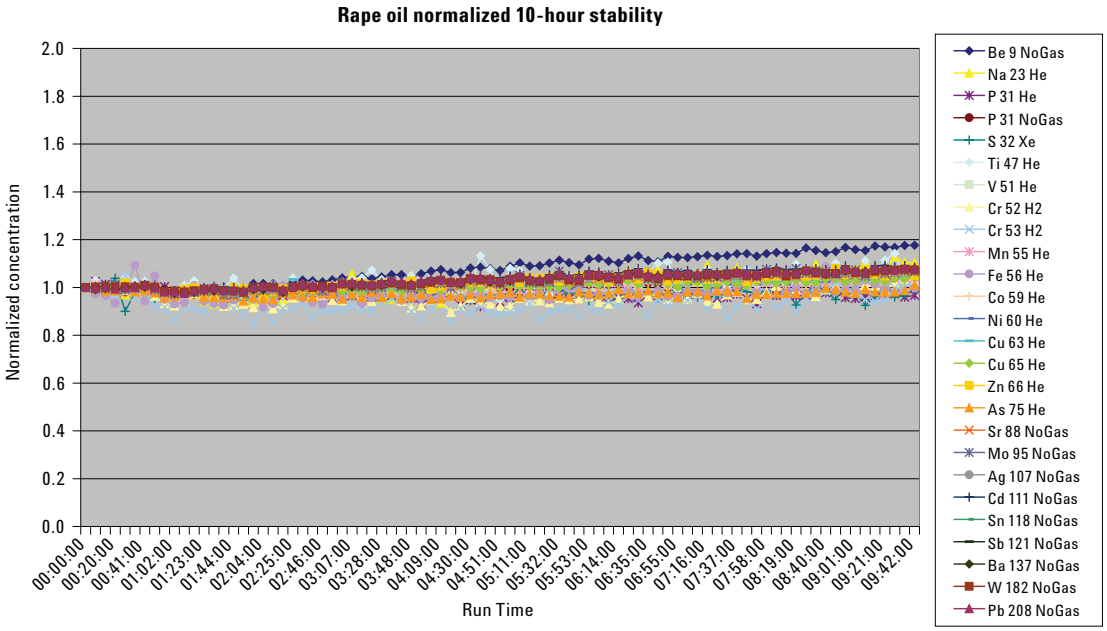

Figure 3. Normalized stability plot for 3x diluted rapeseed B100 biodiesel. Sample diluted in kerosene and run repeatedly over a 10-hour period.

Conclusions

The methodology outlined shows that the 7500ce ICP-MS is suitable for the routine analysis of biofuels following a simple dilution step in kerosene. The use of the ORS in the appropriate gas mode efficiently removes plasma-based and matrix-based interferences, improving detection limits for all key elements compared to ICP-OES. (BECs were generally limited by background contaminant levels). The 7500ce also offers a wider elemental coverage compared to ICP-OES and AAS, which are typically used for determining a limited elemental suite in FAME. The lower backgrounds and greater dynamic range and predictability of interferences simplify the analysis significantly.

NOTE: The 7500ce has now been superseded by the 7500cx. For this application, the 7500cx should be fitted with the optional H_2 cell gas line (and low flow third cell gas line if sulfur is to be measured).

References

The full paper of this work was originally published in *Analytical and Bioanalytical Chemistry* (2007) 389:753-761. The publication is also available online at www.springerlink.com: "Direct elemental analysis of biodiesel by inductively coupled plasma-mass spectrometry," (DOI 10.1007/s00216-007-1439-0).

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Published in the USA
November 19, 2008
5989-7649EN

Agilent Technologies

Comprehensive GC System Based on Flow Modulation for the 7890A GC

Application Brief

Introduction

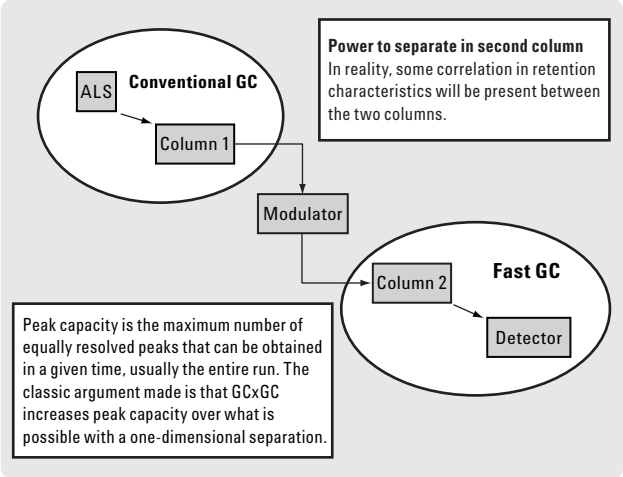
A hardware solution is available on the 7890A for the practice of comprehensive GC. The system uses a capillary flow modulator controlled by the 7890A GC. The system is offered with factory checkout using an FID detector. Other detectors, preferably those operating at 50 Hz or greater, can be used.

Comprehensive two-dimensional (2D) GC, or GCxGC, is a powerful technique that can be used to separate very complex mixtures, such as those found in the hydrocarbon processing, environmental, and food/fragrance industries.

The method uses two columns, typically of very different polarities, installed in series with a modulator in between. The second column is much shorter than the first column to effect a fast separation. The entire assembly is located inside the GC oven.

The modulator performs three functions:

1. It collects effluent from the first column for a fraction of the time equal to peak width. For example, if a peak from column one is six seconds wide, the modulator will accumulate material every two to three seconds, thereby dividing the peak from the first column into two or three "cuts."
2. It focuses the material collected from each cut into a very narrow band through flow compression.
3. It introduces the bands sequentially onto the second column, resulting in additional separation for each band injected onto the second column.



Comprehensive 2D GC uses a primary column (conventional separation), a flow modulator, a second column (very fast separation), and a fast detector.

This technique provides a second dimension of information that can increase the peak resolution and capacity.

A number of different modulator designs have been described in the literature, most relying on thermal cycling to focus the bands from the first column and release them into the second column. Some disadvantages to this approach are:

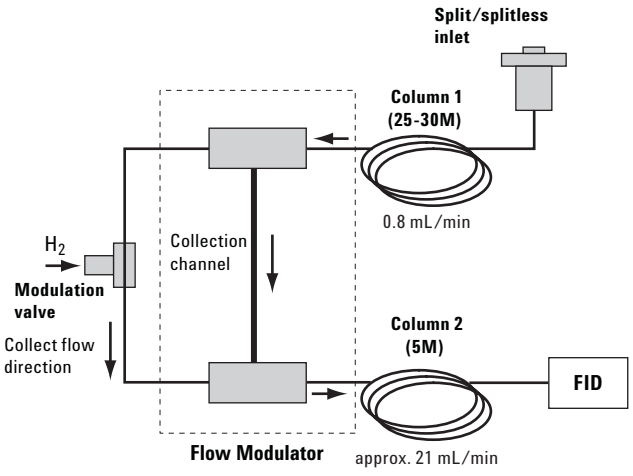
- Large usage of expensive cryogenic gases leading to a high cost of analysis
- Complexity of the hardware
- Longer analysis times

Agilent's proprietary Capillary Flow Technology and fourth-generation Electronic Pneumatics Control (EPC) enable the use of a differential flow modulator to conduct comprehensive 2D-GC without the use of cryogenic gases or complex hardware.

The key to operation is the flow differential (typically 20 to 1) between the second and first columns, respectively. This compresses and focuses the analytes present in any given modulation "inject" pulse into the second column. Precise timing of the modulator is made possible by installing a driver board in the Aux det 2 detector slot of the 7890A mainframe.

The Capillary Flow Technology modulator uses a deactivated, stainless steel structure with all flow splitters and the collector channel incorporated internally in the device. It has low thermal mass so it can track the oven temperature very closely, and its GC oven location allows precise temperature control without lag during programmed runs. All external connections are made using Agilent's Ultimate Union technology for leak-free operation and extremely small, well-swept volumes. A micro three-way solenoid valve, installed on the side of the gas chromatograph, connects to a pneumatics control module (PCM) to accurately and precisely control the flows through the modulator.

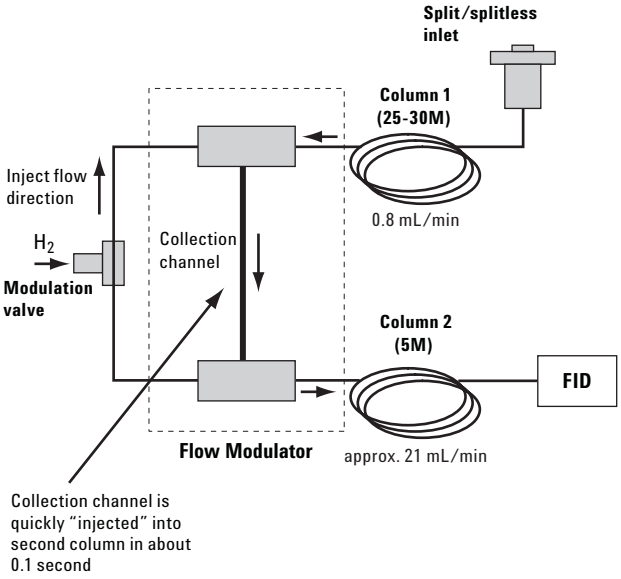
The figures below illustrate the modulator. A three-way solenoid valve receives a controlled supply of hydrogen gas from a PCM. The periodic switching of this three-way valve drives the modulator. The precisely timed and synchronized switching between the collect and inject states directs discrete sample pulses continuously to the second column for additional fast separation throughout the chromatographic run. Both columns are run in constant flow mode. For optimal performance, injection size and split ratio should be carefully adjusted to avoid overloading, which can lead to excessive peak tailing.



Flow rates and flow directions during the load or collect portion of the modulation cycle

Load or collect state (above): At the beginning of this state, the collection channel is filled with hydrogen gas from a previous injection cycle flush.

The primary column effluent enters the modulator's top tee connection and flows into the collection channel. The analytes from this column enter one end of the collection channel. Hydrogen flow from the PCM/three-way micro valve exits the modulator at the bottom tee and is sent to the second column.



Flow rates and flow directions during the transfer or inject portion of the modulation cycle

Inject or flush state (above): Hydrogen gas flow from the three-way solenoid valve is directed to the top tee. A high flow of typically 20 mL/min for about 0.1 second rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes collected in the channel undergo rapid separation.

What is required:

- Agilent 7890A GC with firmware version A.04.06 or higher
- FID with 200 Hz data collection rate or other fast detector
- Split/splitless inlet
- Capillary Flow Technology modulator option or accessory
- Capillary Flow Technology modulator checkout kit
- Pneumatics control module (PCM)
- Agilent GC ChemStation B.03.02 or other data collection and analysis system that can control the flow modulator cycle
- 30-m × 0.25-mm × 0.25-µm DB-5ms column (included with option or accessory)
- 5-m × 0.25-mm × 0.15-µm INNOWax column (included with option or accessory)
- 2D data analysis software, GC Image recommended (not provided by Agilent)
- Internal column nuts and SilTite ferrules

Ordering Information

Description	Part number
7890A GC with Capillary Flow Technology Modulator (requires checkout kit)	G3440A Option 887 or accessory G3486A
7890A GC with 200 Hz FID	G3440A Option 211 or accessory G3462A
7890A GC with split/splitless inlet	G3440A Option 112 or accessory G3452A
Capillary Flow Technology modulator checkout kit	G3487A
PCM for 7890A GC	G3440A Option 309 or accessory G3471A
SilTite metal ferrules, 1/16-in × 0.4-mm id, 10/pk, includes 2 column nuts	5184-3569
Agilent 32-bit ChemStation for 1 GC	G2070BA
Agilent 32-bit ChemStation Bundle for 1 GC includes: – G2070BA 32-bit ChemStation software – Computer with monitor and Windows operating system – Printer	G1875BA
2D GC software Recommend GC Image software, which can be purchased from Zoex Corporation	www.zoex.com

Application Examples

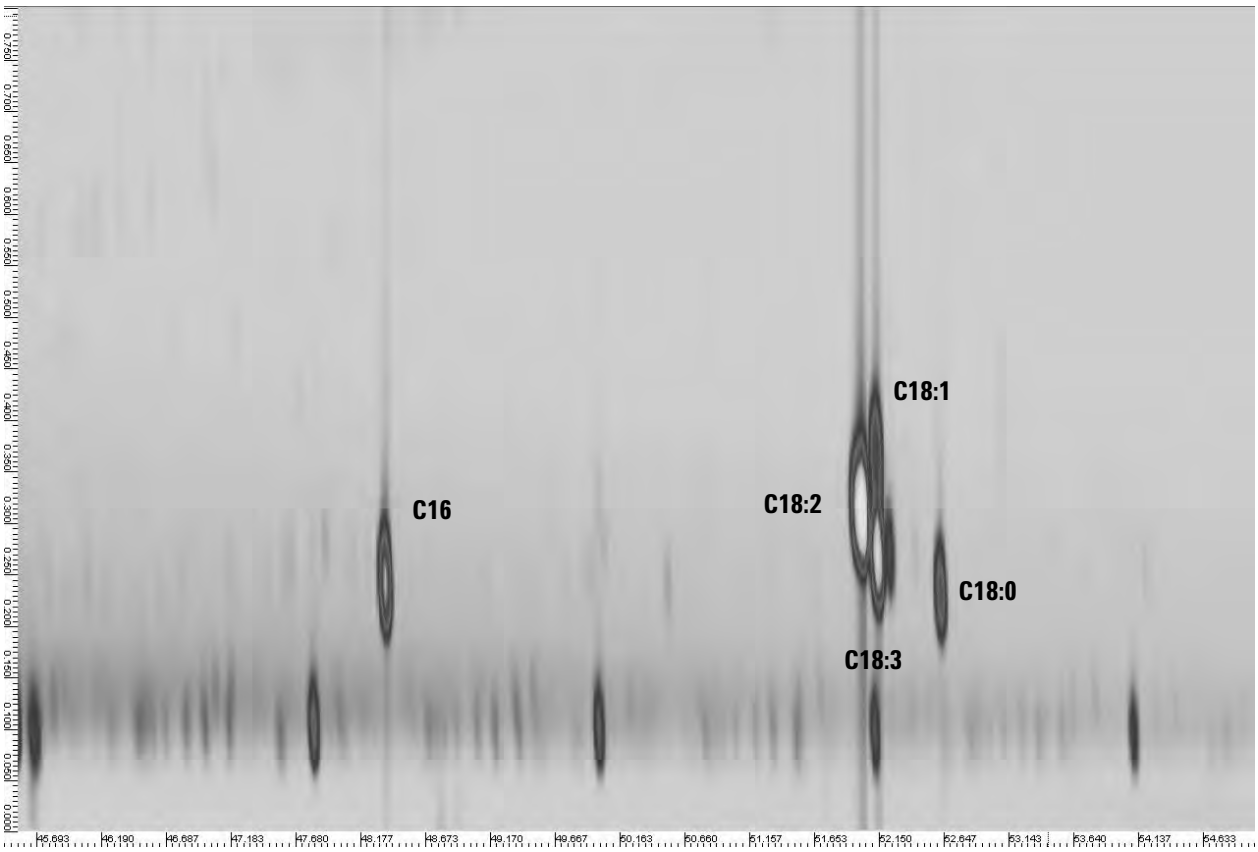
Several applications are shown. Note that primary column lengths have been chosen to give optimal results. While the 30M column that is shipped with the system is an excellent choice for a wide range of applications, other lengths can be used to optimize a given separation. Various columns have been used in these examples to illustrate some of the possibilities. The GC Image software package was used for processing the ChemStation data.

1. B20 biodiesel based on soy FAMES. Section of the 2D image showing the C16 and C18 FAMES is shown.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

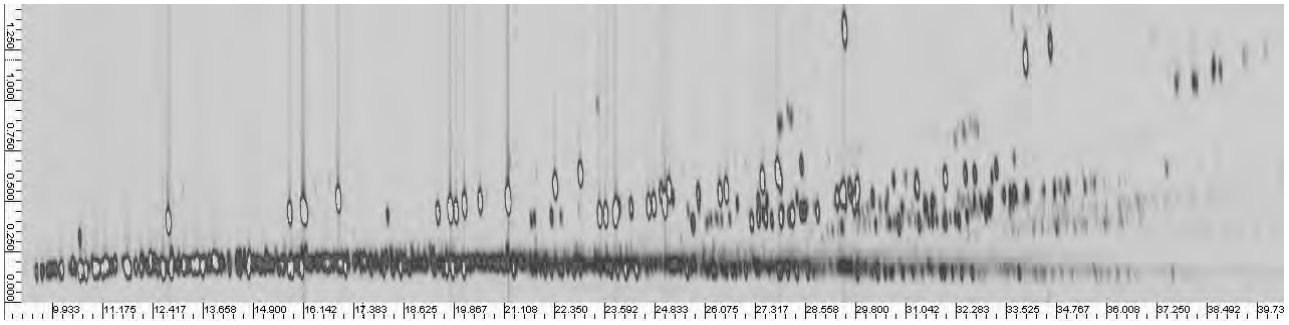


2. Complete 2D image of a sample of heavy gasoline. Each series of substituted 1-ring aromatics is well separated, making hydrocarbon class grouping possible.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

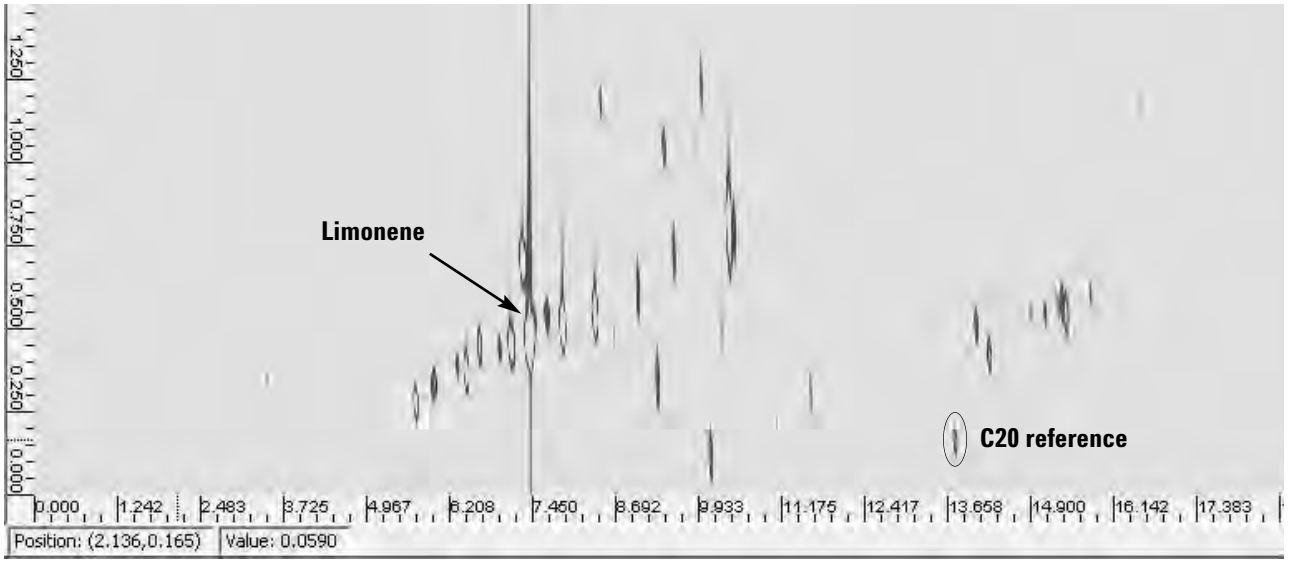


3. Lime oil 2D image.

   Column 1: 15 m × 0.25 m × 0.25 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm DB-17HT

   Modulation: 1.40 s load, 0.10 s inject



Thermal vs. Flow Modulation

Since competitors offer only systems based on thermal modulation, the following table summarizes the key points about the respective approaches of thermal vs. flow modulation.

Thermal modulation	Differential Flow modulation
Cryo-focusing provides potentially narrower peaks in second dimension	Peak widths comparable to thermal. Usually no more than 20% wider. Many users want to sum regions of peaks where peak width is not as critical
Lower flows – Can be used with high-vacuum detectors (TOF)	MSD can be used with a splitter over limited scan range
Large consumption of cryogen	No cryogen required
Complex hardware design, set-up, and maintenance	Simple, reliable Capillary Flow Technology based hardware; small thermal foot print
Long chromatographic runs required for best performance	Run times comparable to a 1D separation
System price (estimate) $60 to $70K	Agilent system approximately $60K (list)

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Printed in the USA
April 1, 2008
5989-8060EN



Agilent Technologies

Analysis of Fatty Acid Methyl Ester (FAME) Content and Distribution in Biodiesel Blends Using Heart-Cutting 2D Gas Chromatography

Application

HPI/Biofuel

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Abstract

The analysis of the fatty acid methyl ester (FAME) content in blended biodiesel samples is described using a heart-cutting two-dimensional (2D) gas chromatographic (GC) system. A Capillary Flow Technology Deans switch is used to interface a primary nonpolar capillary column to a secondary polar capillary column. The primary column separates most of the petroleum hydrocarbons from the FAMEs. The FAMEs are selectively transferred to the secondary column, where they are completely resolved from the remaining hydrocarbon matrix. The instrument is calibrated using the total response of all separated FAME peaks over a range of 1 to 25 volume percent. After calibration, a sample of commercially blended B20 biodiesel is analyzed; the results show excellent quantitative precision. The distribution of individual FAMEs is also determined and the results show that the commercial sample contains biodiesel made from soybean oil. The separation of palm oil and coconut oil FAMEs in biodiesel blends is also demonstrated using the heart-cutting 2D GC approach.

Introduction

High crude oil prices combined with disruptions in supply and refining capacity have driven the price of motor fuels to new highs and created spot shortages throughout the world. This has given new urgency to the development of locally produced alternative renewable fuels. This effort offers the potential to reduce reliance on crude oil as well as lower emissions of airborne pollutants and greenhouse gases.

Biodiesel is a motor or heating fuel produced from renewable vegetable oils derived from crops such as sunflower, soybean, rapeseed, and palm. Biodiesel is made by transesterification of vegetable oil or animal fats to produce a mixture of fatty acid methyl esters (FAMEs). Pure biodiesel is called B100 and must meet industry standard specifications before it can be used as a fuel or blending stock. The distribution of FAMEs in a B100 mixture depends on the feedstock source as shown in Table 1.[1] The relative amounts of FAMEs in biodiesel can vary widely and have different effects on both the fuel and handling properties.[2]

Table 1. Fatty Acid Distribution of Common Biodiesel Feedstocks

	Fatty Acid Distribution											
Oil Type	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0 C22:0	C20:1 C22:1
Soybean				0.3	7–11	0–1	3–6	22–34	50–60	2–10	5–10	
Rapeseed					2–5	0.2	1–2	10–15	10–20	5–10	0.9	50–60
Palm				1–6	32–47		1–6	40–52	2–11			
Coconut	5–9	4–10	44–51	13–18	7–10		1–4	5–8	1–3			

Pure biodiesel is a relatively simple mixture and gas chromatography (GC) is routinely used to test product quality. Commercially, pure biodiesel is blended with no. 2 petroleum diesel to create a motor fuel with 1 to 20 volume percent (vol%) of total FAME content. These blends are designated B1 to B20, respectively. As a blend, it is difficult to quantify the FAME content in the presence of the petroleum hydrocarbons using conventional capillary GC. EN14331 is the only industry standard GC method for measuring the FAME content in biodiesel blends.[3] This method requires atmospheric pressure silica-column liquid chromatography (LC) to physically separate the petroleum diesel from the FAMEs in the sample. The FAME fractions from the silica column are then analyzed using GC. This method is time-consuming and is only scoped for 5 vol% (B5) or lower biodiesel blends.

Two-dimensional (2D) GC offers a higher resolution solution to the analysis of very complex mixtures. The most widely practiced 2D GC technique is called heart-cutting. Selected, unresolved peaks are transferred from one column to another column of different selectivity where a second separation takes place. By carefully choosing the columns and instrument conditions, it is possible to obtain higher resolution for several compounds in a complex mixture. A device commonly used to transfer peaks from one column to the next is a Deans switch. Due to improvements in GC hardware, there has been renewed interest in heart-cutting methods for the analysis of petroleum and petrochemical products.[4–7] Recently a new type of Deans switch has been developed using Capillary Flow Technology to further improve the precision and performance of heart-cutting 2D GC.[7, 8] This application describes a new method using a Capillary Flow Technology Deans switch to separate the FAME compounds in biodiesel blends.

Experimental

An Agilent 7890A GC was equipped according to the details outlined in Table 2. After column installation, the GC conditions were set according to the data in Table 3. Instrument pressures, flow rates, and the fixed restrictor dimensions were determined using a Deans switch calculator software program designed for this system. This calculator program is included with the Deans switch hardware option for the Agilent 7890A GC.

Determination of Heart-Cut Times

A low erucic rapeseed oil reference standard was used to determine retention times and cut times on the HP-5ms column. This standard was dissolved in 5 mL of hexane containing 10 mg/mL of methyl heneicosanoate (C21:0) as the internal standard. The standard was injected with the Deans switch set in the off position during the entire run. This same standard was then run using these cut times to determine the retention time of each FAME peak on the HP-INNOWax column. Alternatively, a sample of the biodiesel blending stock could be used as a standard for determining heart-cut times.

Once the retention times and cut times for each FAME group were determined, a matrix blank was run using these cut times. This will determine if there is any potential interference from the matrix that is not resolved by the secondary column. For this work, a no. 2 diesel fuel containing no biodiesel was used as the matrix.

System Calibration and Sample Analysis

Calibration standards were prepared by mixing no. 2 diesel fuel with a commercially available B100 soybean biodiesel in 12-mL vials equipped with Teflon-lined caps. Standards were made to

Table 2. System Configuration

Standard 7890A GC hardware	
G3440A	Agilent 7890A Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 211 (2 of each)	Capillary FID with EPC control
Option 309	Pneumatics control module with EPC control
Option 888	Factory installed Capillary Flow Technology Deans Switch
G2613A	Agilent 7683 autoinjector
Columns	
Primary column	HP-5ms, 15 m × 0.25 mm id × 0.1 µm (part no. 19091S-331)
Secondary column	HP-INNOWax, 30 m × 0.25 mm id × 0.5 µm (part no. 19091N-233)
Deans restrictor	Deactivated fused silica tubing, 0.77 m × 0.1 mm id (part no. 160-2635-5)
Data system	
G2070	Agilent multi-technique ChemStation
Optional consumables	
5181-1267	10 µL Teflon fixed autoinjector syringe
5183-4647	Inlet liner optimized for split operation
Standards	
H3265-100MG*	Methyl heneicosanoate (C21:0)
O7756-1AMP*	Low erucic rapeseed oil reference standard, 100 mg

*Available from Sigma-Aldrich, PO Box 14508, St. Louis, MO 63178, USA

Table 3. Instrument Conditions

Injection port	Split mode, 200:1 split ratio
Temperature	250 °C
EPC pressure	33.86 psi helium, constant pressure mode
Injection size	0.2 µL
HP-5ms column flow	1.5 mL/min
Pneumatics control module	30.70 psi helium, constant pressure mode
HP-INNOWax column flow	3.5 mL/min
FID temperatures	275 °C
Oven temperature program	
Initial temperature	50 °C for 0 min
Ramp number 1	20 °C/min to 210 °C for 18 min
Ramp number 2	20 °C/min to 230 °C for 13 min

represent 1, 2, 5, 10, and 25 vol% biodiesel blends. A commercially blended soybean B20 fuel was obtained from Uncle Willie's Deli & Fuel (Woodside, DE, USA) for use as a test sample. A 10 mg/mL solution of methyl heneicosanoate (C21:0) in chromatographic-grade hexane was prepared for use as an internal standard. Each calibration standard and sample was prepared for GC analysis by weighing a 250-mg aliquot and adding 1 mL of the internal standard solution. After calibration, the B20 biodiesel sample was analyzed as a performance check of the system.

Results and Discussion

The HP-5ms primary column separation of the FAMEs in the rapeseed oil reference sample is shown in Figure 1. The HP-5ms column does not completely separate the individual FAMEs; however, they generally are separated by groups according to the number of carbons in the fatty acid chain. Figure 1 also shows a chromatogram of pure no. 2 diesel fuel on the HP-5ms column. Most of the hydrocarbons elute before the first FAME peak, methyl myristate (C14:0). Therefore, co-elution of FAMEs and hydrocarbons primarily occurs between 9 and 15 minutes on the HP-5ms column.

The heart-cut times of each FAME group were determined from the data shown in Figure 1. The HP-5ms retention times, the heart-cut times, and the HP-INNOWax retention times are summarized in Table 4. Due to slight variations in columns and hardware, the retention times and cut times listed in Table 4 cannot be used for every system. Instead, each analyst must determine the correct heart-cut times and secondary column retention times for their system.

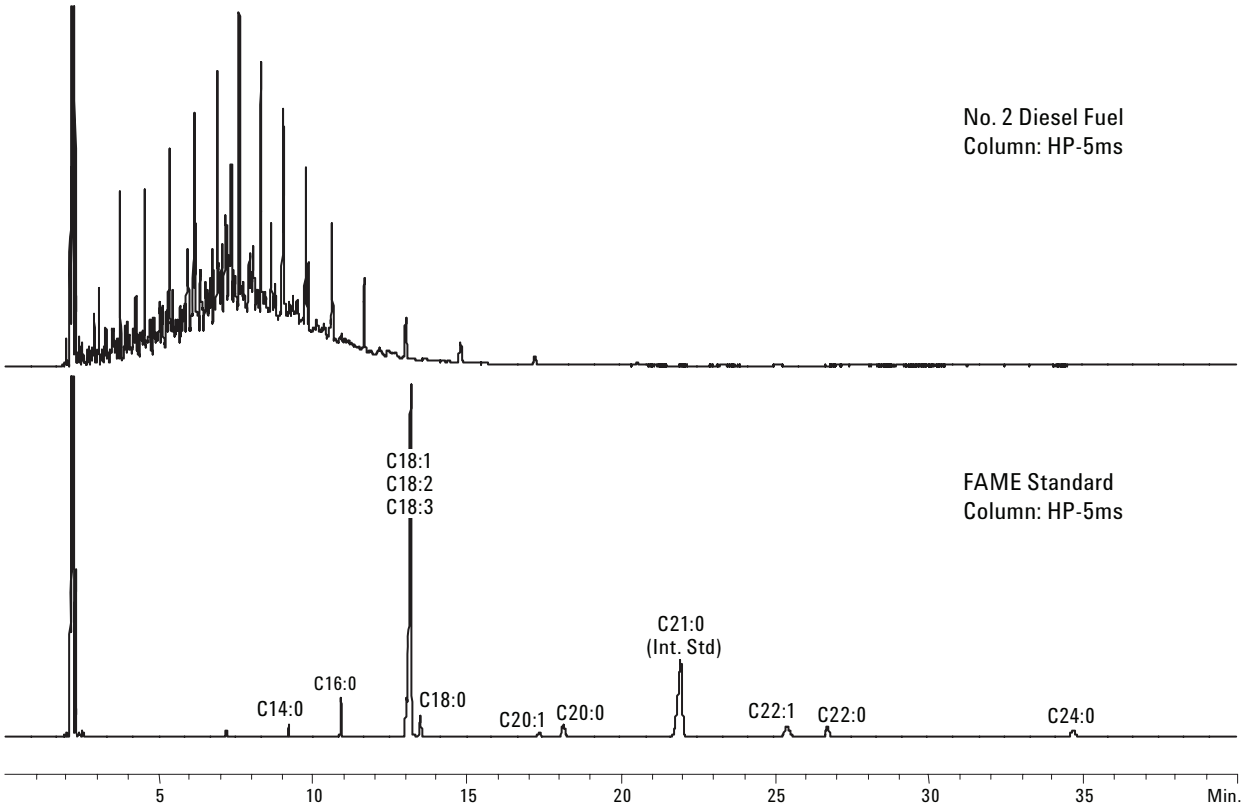

Figure 1. The lower chromatogram shows the separation of FAMEs on the primary HP-5ms column. The upper chromatogram shows the separation of pure no. 2 diesel fuel on the same column. A blended biodiesel fuel containing FAMEs and no. 2 diesel fuel would have unresolved compounds between 2 and 15 minutes on this column.

Table 4. Cut Times for C14 to C22 FAMES as Shown in Figures 1 and 2

FAME	Carbon number	HP-5ms RT (min.)	Cut time (min)	HP-INNOWax RT (min)
Methyl-myristate	C14:0	9.21	9.10 – 9.28	12.46
Methyl-palmitate	C16:0	10.90	10.77 – 11.02	15.99
Methyl linolenate	C18:3	13.03	12.85 – 13.60	25.63
Methyl-oleate	C18.1	13.16	12.85 – 13.60	22.26
Methyl-linoelate	C18.2	13.16	12.85 – 13.60	23.41
Methyl stearate	C18:0	13.49	12.85 – 13.60	22.00
Methyl-eicosanoate	C20:1	17.35	17.14 – 17.50	30.18
Methyl-arachidate	C20:0	18.13	17.90 – 18.31	30.10
Methyl-heneicosanoate (Int. Std.)	C21:0	21.94	21.53 – 22.25	34.49
Methyl-erucate	C22:1	25.40	25.06 – 26.43	40.18
Methyl-behenate	C22:0	26.70	26.43 – 26.91	40.18

After heart-cutting, the FAME peaks are transferred from the HP-5ms column to the secondary HP-INNOWax column, where most are further resolved into their individual components as shown in Figure 2. However, for the C20 group and C22 group, resolution was lost within each group after heart-cutting to the HP-INNOWax column. For analysts who prefer to maintain the separation within these two groups, it is not necessary to use heart-cutting since these FAMEs elute after the petroleum hydrocarbons on the primary HP-5ms column.

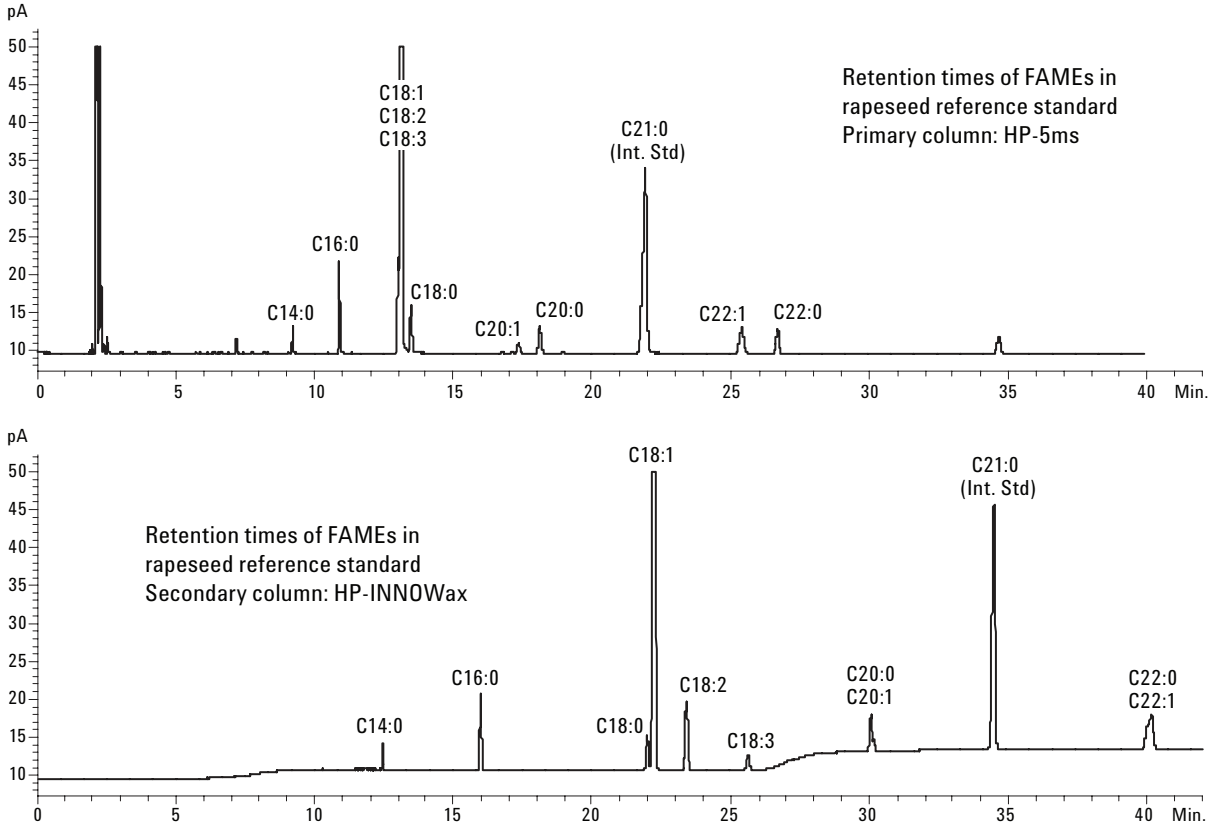

Figure 2. The upper chromatogram shows the separation of FAMEs on the primary HP-5ms column before heart-cutting. After heart-cutting, the lower chromatogram shows the separation of the FAMEs on the HP-INNOWax secondary column.

Using the heart-cut times obtained from the previous experiment, a sample of pure no. 2 diesel was run to observe any potential matrix interference with the FAMEs on the secondary column. Figure 3 shows a comparison of the FAME separation and the matrix hydrocarbons on the HP-INNOWax column. For the major FAME components found in biodiesel, there are no significant co-elutions with petroleum hydrocarbons on the INNOWax column after heart-cutting. Due to variations in composition of different types of petroleum diesel fuel, practitioners of this method should perform this experiment using the no. 2 diesel fuel found in their blends.

The system was calibrated for quantitative analysis by running the standards described in the experimental section. Since the total amount of biodiesel in the blends is distributed among several FAME peaks, it is not possible to use any single peak for quantification. Instead, the area responses of all FAME peaks are summed to represent the total amount of biodiesel in the blend for calibration. This operation is accomplished using the peak grouping calibration functions in the Agilent ChemStation.

A least-squares linear calibration curve was prepared using the summed area response of all FAME peaks relative to the area response of the C21:0 internal standard. The calibration curve shows good linearity for biodiesel blends containing total FAME concentrations from 1 vol% to 25 vol% (Figure 4). The commercially blended biodiesel sample was run five times after calibration and one of the chromatograms is shown in Figure 5. The results summarized in Table 5 show the commercial sample contained a total FAME content of 20.5%, which confirms the sample as a B20 biodiesel blend.

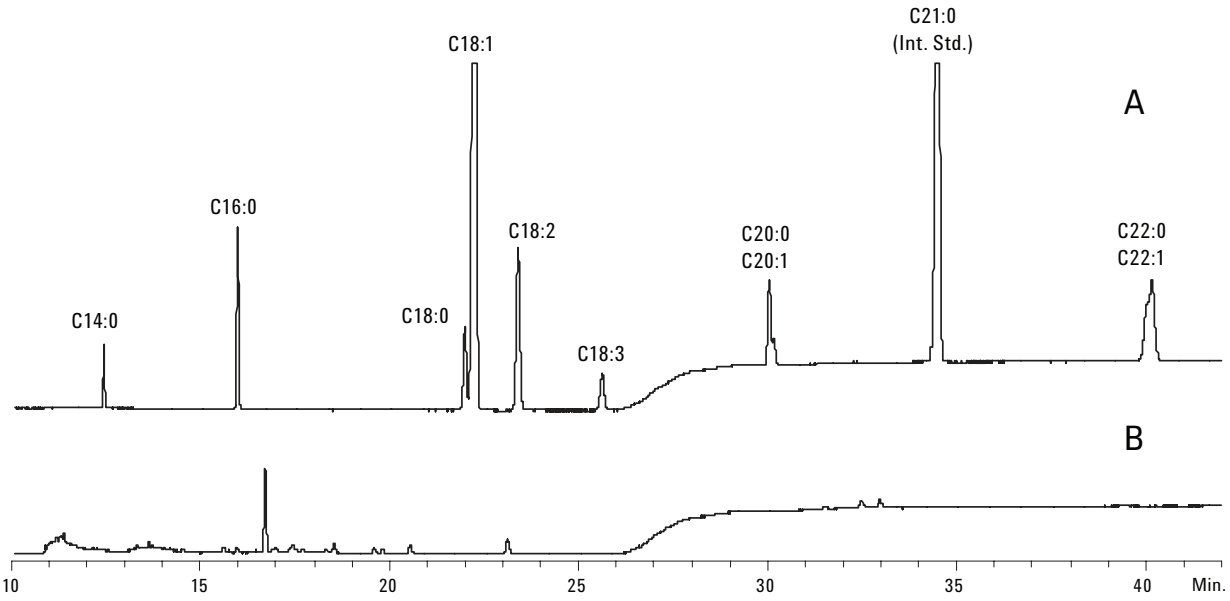

Figure 3. The upper chromatogram (A) shows the retention times of FAMEs on the secondary HP-INNOWax column after heart-cutting. The lower chromatogram (B) shows the hydrocarbon matrix of no. 2 diesel fuels after heart-cutting. No large peaks from the hydrocarbon matrix were found to co-elute with the FAME peaks after heart-cutting to the HP-INNOWax column.

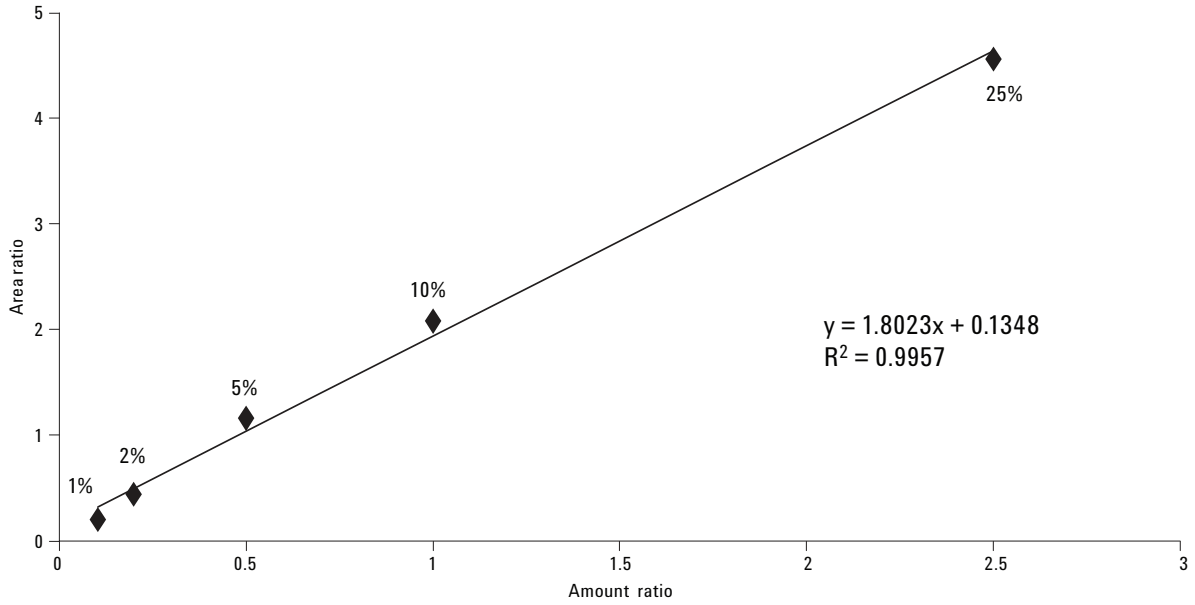

Figure 4. A calibration curve for biodiesel blends containing soybean biodiesel between 1 vol% (B1) and 25 vol% (B25). This calibration was prepared using the total peak areas of all FAMEs found in the blends.

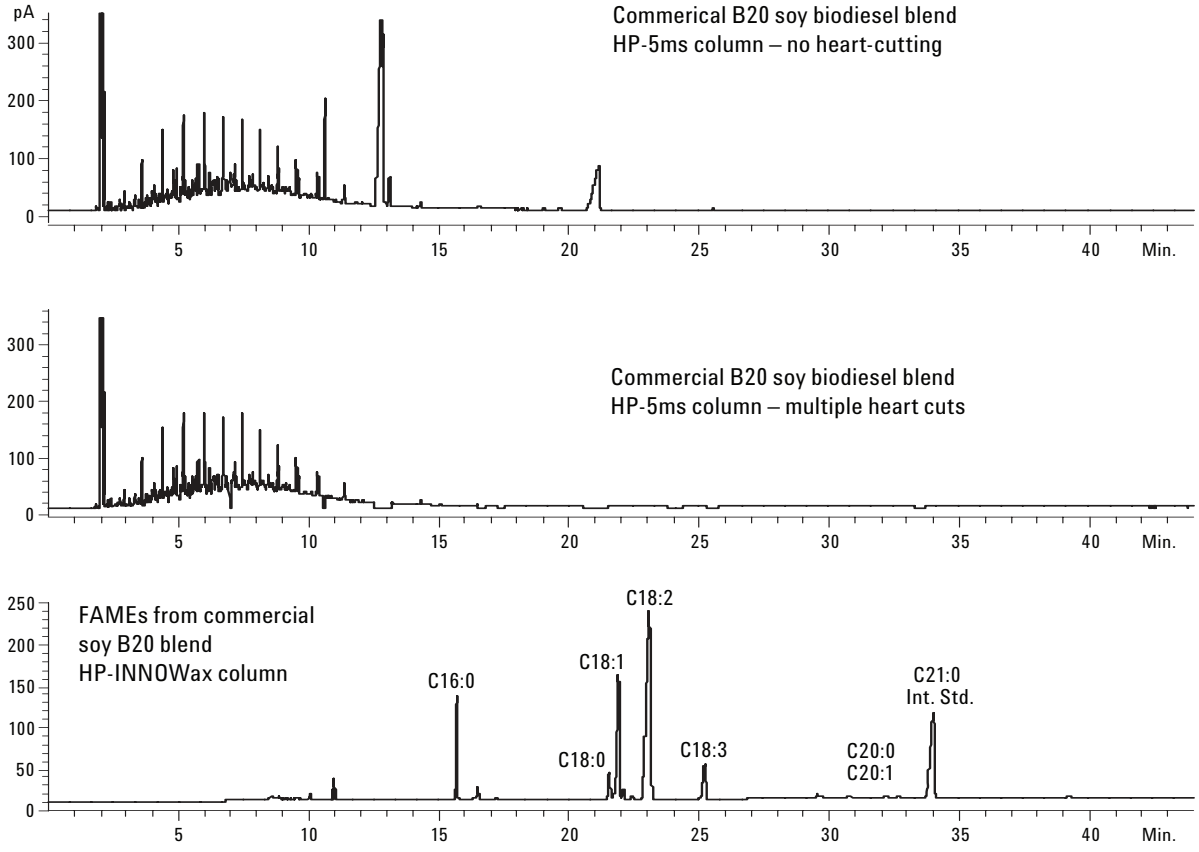

Figure 5. A commercially prepared B20 sample containing soybean biodiesel was analyzed using heart-cutting 2D GC. The upper chromatogram shows the primary column separation before heart-cutting. The middle and lower chromatograms show the sample after heart-cutting the FAMEs from the HP5ms column to the HP-INNOWax column.

Table 5. Analysis of a B20 (20 vol%) Soybean Biodiesel Commercial Blend

Run	Volume %
1	20.4
2	20.5
3	20.5
4	20.5
5	20.5
Average	20.5
RSD (%)	0.1

Since this method can identify individual FAMEs in a biodiesel blend, it is possible to determine the relative distribution of the esters in the fuel. This data can be useful in determining the type of feedstock used to make the B100 blending stock. The identity and distribution of FAMEs found in the B20 biodiesel blend sample indicates that soybean oil was the biodiesel feedstock (Table 6).

This heart-cutting 2D GC technique can also be used for measuring FAMEs in other types of biodiesel blends. In many regions throughout the world, tropical vegetable oils such as palm and coconut are used to make B100 biodiesel. Biodiesel blends made from these tropical oils can also be analyzed using this method. This is demonstrated in Figures 6 and 7, where B20 blends containing palm biodiesel and coconut biodiesel are measured using this method. The FAMEs derived from palm oil are somewhat less complicated than those derived from soybean. Methyl palmitate is the major peak. No methyl linolenate (C18:3) or C20 FAMEs were found in the sample.

Table 6. Distribution of FAMEs Found in B20 Bodiesel Blend

	Mass Fraction of FAME in Biodiesel					
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0, C20:1
% found in B20 sample	11	4	22	53	8	2
% expected in soy	7–11	3–6	22–34	50–60	2–10	5–10

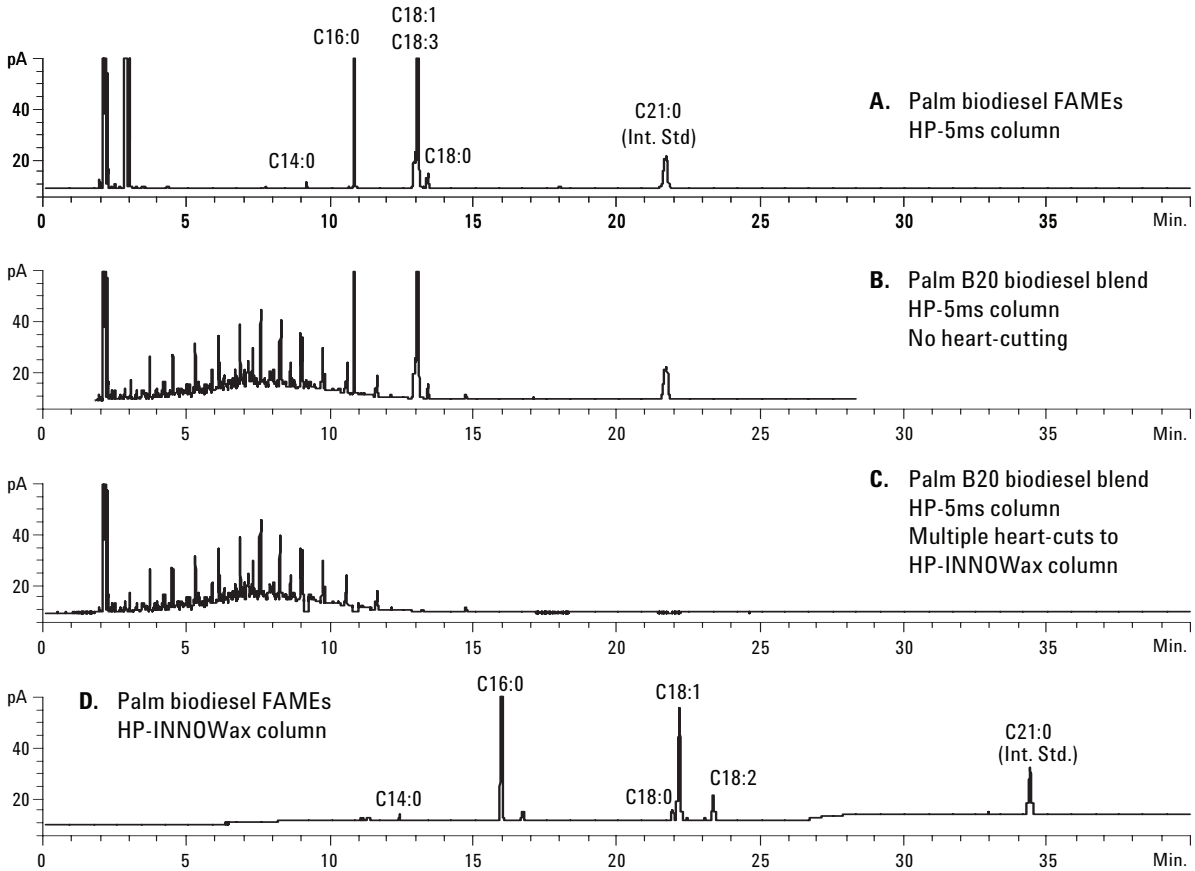

Figure 6. (A) Palm oil FAMEs on the primary HP-5ms column. (B) Palm B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of palm FAMEs in B20 biodiesel blend using heart-cutting 2D GC.

Coconut oil contains a wider and lighter range of fatty acids as shown in Table 1. The complexity of coconut biodiesel results in more co-elution of FAMEs with hydrocarbons in blended fuels.

However, the heart-cutting 2D GC technique used in this method can successfully separate coconut methyl ester from these hydrocarbons, as shown in Figure 7.

Table 7. Cut Times for C8 to C18 Coconut Oil FAMES as Shown in Figures 7

FAME	Carbon number	HP-5ms RT (min)	Cut-time (min)	HP-INNOWax RT (min)
Methyl-caprylate	C8:0	4.72	4.68 – 4.77	6.62
Methyl-decanoate	C10:0	6.30	6.26 – 6.35	8.27
Methyl-laurate	C12:0	7.77	7.71 – 7.85	10.05
Methyl-myristate	C14:0	9.18	9.11 – 9.25	12.44
Methyl-palmitate	C16:0	10.85	10.78 – 10.92	15.96
Methyl-linoleate	C18:2	13.03	12.85 – 13.16	23.36
Methyl-oleate	C18:1	13.03	12.85 – 13.16	22.15
Methyl stearate	C18:0	13.74	13.34 – 13.49	21.95
Methyl-heneicosanoate (Int. Std.)	C21:0	21.73	21.47 – 21.91	34.43

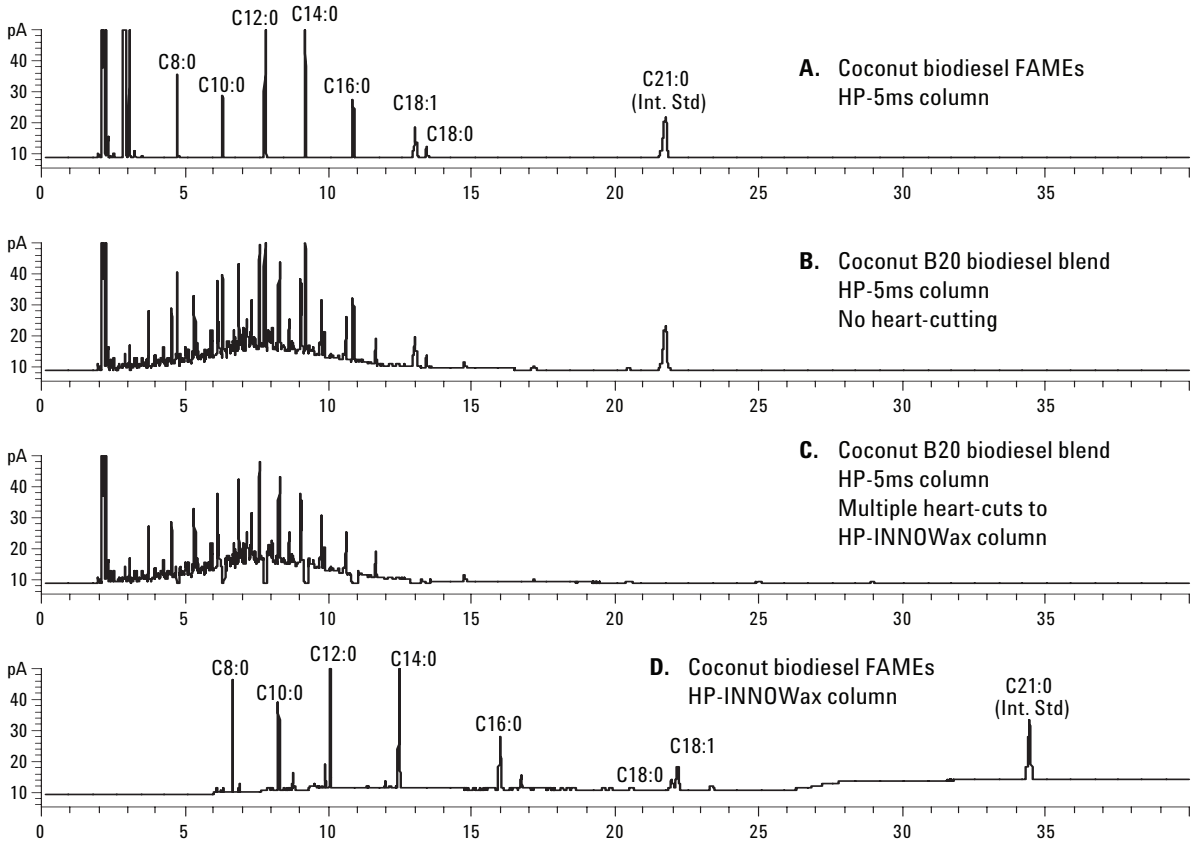

Figure 7. (A) Coconut oil FAMEs on the primary HP-5ms column. (B) Coconut B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of coconut FAMEs in B20 biodiesel blend using heart-cutting 2D GC.

Conclusions

The analysis of biodiesel content in biodiesel blends is a unique challenge due to the complexity of the sample. Conventional single capillary column GC cannot provide sufficient resolution to quantify the biodiesel using a universal detector such as an FID. Multidimensional GC provides a powerful tool for measuring both the total amount of biodiesel in the blend as well as the distribution of biodiesel FAMEs. A Capillary Flow Technology Deans switch can precisely heart-cut biodiesel FAMEs from a nonpolar column to a polar column. The first-dimension column separates the bulk of the hydrocarbons from the FAMEs. The secondary column resolves the FAMEs from co-eluting hydrocarbons and further separates individual FAME peaks. The improved chromatographic resolution of this technique allows the quantitative analysis of the total biodiesel content in a blend as well as the distribution of FAMEs contained in the sample.

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Printed in the USA
March 21, 2008
5989-8107EN



Agilent Technologies

Automated Standard and Sample Preparation for Multiple Gas Chromatographic Analyses of Biodiesel

Application Note

HPI/Petrochemical/Polymer

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Abstract

An approach to automating the biodiesel standard and sample preparation prior to GC analysis is presented using the enhanced capabilities of the Agilent 7693A Automated Liquid Sampler (ALS). A derivatization protocol for the silyation of glycerol, mono-, and di-glycerides was developed for the 7693A ALS using a procedure described in the ASTM D6584 and EN14105 methods [1,2]. A second automated protocol was developed for adding an internal standard to biodiesel samples prior to the fatty acid methyl ester (FAME) analysis according to the EN14103 method [3]. The resulting calibrations and GC sample runs of these methods shows excellent comparison to manual preparation methods.

Agilent Technologies

Introduction

Several industry standard gas chromatographic methods are used to determine the product quality of pure biodiesel, also known as B100. Two of these methods, ASTM D6584 and EN14105, require very complex and time consuming procedures for preparing calibration standards and finished B100 samples. These methods also use expensive and toxic reagents adding to the cost of analysis. While modern instruments possess many automation capabilities with regards to the gas chromatographic process, manual sample and standard preparation remains a tedious chore that can significantly contribute to errors and lost precision in GC analyses.

The new Agilent 7693A ALS offers new capabilities for automatic standard and sample preparation at the GC instrument. This paper describes these capabilities for improving the productivity of three biodiesel GC methods. Both ASTM D6584 and EN14105 require the derivatization of non-volatile glycerides in B100 prior to GC analyses. Additionally, the calibration standards for these methods must also undergo the same derivatization procedure. Careful configuration of the Agilent 7890A GC with the 7693A ALS can eliminate most manual preparation steps for the D6584 and EN14105 methods. A third biodiesel method, EN14103, is used to measure the fatty acid methyl ester (FAME) and linolenic acid methyl ester content in B100 samples. This method requires the addition of an internal standard solution to each B100 sample. This sample preparation step can also be performed using the 7693A ALS.

Experimental

An Agilent 7890A GC was configured according to Table 1. In addition to this configuration, an optional isothermal, external column oven was placed on the top of the GC to accommodate an HP-INNOWax column for the EN14103 analysis. This was necessary to avoid damage to this column when the high temperature D6584 or EN14105 methods were run. GC conditions for these methods are listed in Tables 2 and 3. This hardware configuration allows up to five different GC methods for biodiesel analysis to be run on a single instrument:

- **ASTM D6584** - Determination of Free and Total Glycerin and Mono-, Di-, Triglyceride Content
- **EN14105** - Determination of Free and Total Glycerol and Mono-, Di-, Triglyceride Content
- **EN14103** - Determination of Ester and Linolenic Acid Methyl Ester Content
- **EN14110** - Determination of Methanol Content
- **EN14106** - Determination of Free Glycerol

Table 1. 7890A GC Configuration for 5-in-1 Biodiesel Analyses

Front inlet	Split/splitless with split optimized liner (5188-6576)
Rear inlet	Cool-on-column
Column 1	HP-INNOWax, 30 m × 0.32 mm id × 0.25 µm film (19091N-113V) - special configuration for external column oven
Column 2	DB-5ht, 15 m × 0.32 mm id × 0.1 µm film (123-BD11) with 2 m × 0.53 mm id retention gap (160-2865-5)
Detectors	Dual flame ionization (FID)
Firmware version	A.01.10 or greater
Data system	Chemstation rev. B.04.01 or greater

Table 2. GC Conditions for Analysis of Free and Total Glycerin in B100 Biodiesel (ASTM D6584 or EN14105)

Cool-on-column inlet	
Initial temperature	50 deg °C
Temperature program	Oven track mode
Column 2 (DB-5ht) flow	Helium at 3 mL/min measured at 50 deg °C
Column temperature	
Initial	50 °C for 1 min
Rate 1	15 °C/min to 180 °C, hold 0 min
Rate 2	7 °C/min to 230 °C, hold 0 min
Rate 3	30 °C/min to 380 °C, hold 10 min
Flame ionization detector	380 °C

Table 3. GC Conditions for Determination of Ester and Linoleic Acid Methyl Ester Content (EN14103)

Split/splitless inlet	
Temperature	250 °C
Split flow	100 mL/min
Column 2 (HP-INNOWax) flow	Helium at 1 mL/min
Column temperature	210 °C for 30 min.
Flame ionization detector	250 °C

Combining automated sample preparation and automated sample injection with GC analysis was accomplished using two 7693A towers and the 7693A sample tray. One tower was used for sample preparation while the second tower was used to inject the prepared sample onto the GC column. Special accessories on the sample tray were used to mix and heat samples as required by the preparation protocols. Table 4 lists the individual 7693A ALS components used for this paper. Control of the towers and sample tray was accomplished using control software built into the Agilent Chromatography Data systems.

Table 4 - 7693A ALS Configuration

Front tower	7693A Autoinjector (G4513A)
Rear tower	7693A Autoinjector (G4513A)
Sample tray	7693A with heater, mixed, bar code reader (G4520A)
Sample preparation syringe	100 µL, PTFE plunger (5183-2042)
GC Inlet injection syringe	10 µL PTFE plunger (5181-3354)

Table 5 lists the standards and reagents necessary for ASTM D6584 and EN14105. The preparation procedures described by these methods result in a final volume of 15 mL for each sample. The maximum vial size used by the 7693A ALS is 2 mL. Therefore the standard and sample preparation procedures must be scaled to 10% for the final volume to fit into the 2 mL vials. Shown below is the 10% scaled protocol used by the 7693A ALS for the D6584 standard preparation. The same protocol was used for the EN14105 standard preparation.

1. Move an empty 2 mL vial from the sample tray to the front tower.
2. Add 100 µL calibration standard mixture #1 to vial (100 uL syringe).
3. Add 10 µL ISTD1 solution (butanetriol) to vial using front tower.
4. Add 10 µL ISTD2 solution (tricaprin) to vial using front tower.
5. Add 10 µL derivatization reagent (MSTFA) to vial using front tower.
6. Transfer vial to mixer and mix for 1 minute.
7. Transfer vial to heater and react for 30 minutes at room temperature.
8. Transfer vial to front tower.
9. Add 800 µL n-heptane to vial using front tower.
10. Transfer vial to mixer and mix for 1 minute.
11. Transfer vial to rear tower (10 µL syringe).
12. Inject 1 µL on-column using rear tower.
13. Repeat for calibration standard mixes, numbers 2 - 5.

Table 5. Standards and Reagents used for Analysis of Free and Total Glycerin in B100 Biodiesel

ASTM D6584 standard kit (5190-1408)	Contains 2 pre-mixed internal standards and 5 pre-mixed calibration standards
EN14105 standard kit (5190-1409)	Contains 2 pre-mixed internal standards and 4 pre-mixed calibration standards
MSTFA derivatization reagent (5190-1407)	Contains 10 × 1 mL ampoules of N-methyl-N-(trimethylsilyl) trifluoro-acetamide
Monoglyceride retention time standard (5190-1410)	Contains 3 × 1 mL ampoules of pre-mixed monoglycerides for retention time identification

The B100 sample preparation protocol was slightly different. First, it was necessary to weigh 10 mg of each soybean B100 sample into separate 2 mL ALS vials. After the samples were weighed into the vials, they were placed in the 7693A sample tray for automated preparation using this protocol:

1. Move vial containing 10 mg B100 sample from sample tray to front tower.
2. Add 10 µL ISTD1 solution (butanetriol) to vial using front tower.
3. Add 10 µL ISTD2 solution (tricaprin) to vial using front tower.
4. Add 10 µL derivatization reagent (MSTFA) to vial using front tower.
5. Transfer to vial mixer and mix for 1 minute.
6. Transfer to vial heater and react for 30 minutes at room temperature.
7. Transfer vial to front tower.
8. Add 800 µL n-heptane to vial using front tower.
9. Transfer vial to mixer and mix for 1 minute.
10. Transfer vial to rear tower (10 µL syringe).
11. Inject 1 µL on-column using rear tower.
12. Repeat for other B100 samples.

The sample preparation required by EN14103 is much simpler. Three B100 samples sourced from rape seed oil, palm oil, and coconut oil were used for this study. After 10 mg of each B100 sample was weighed into 2 mL ALS vials, the following 7693A protocol was used for sample preparation:

1. Move vial containing 10 mg B100 sample from sample tray to rear tower (100 µL syringe).
2. Add 500 µL ISTD Solution (10 mg/mL C17:0 in n-heptane) to vial using rear tower (100 µL Syringe).
3. Transfer vial to mixer and mix for 1 minute.
4. Transfer vial to front tower (10 µL syringe).
5. Inject 1 µL onto split inlet using front tower (10 µL syringe).
6. Repeat for other B100 Samples.

Results

ASTM D6584 - Determination of Free and Total Glycerin and Mono-, Di-, Triglyceride Content

The automated standard preparation resulted in a 5-level calibration for glycerol, monoolein, diolein and triolein with two internal standards. Figure 1 shows the chromatograms generated for each standard. Visually, it was difficult to determine if the standards were prepared correctly using the 7693A ALS. A better representation of properly prepared standards was the individual linear calibration curves shown in Figure 2. This data shows excellent linearity for each compound that exceeds the requirements of ASTM D6584 (r^2 = 0.99 or greater). Figure 3 shows a comparison of a soybean B100 sample prepared manually and automatically. Details of the four different glyceride elution regions are shown in Figure 4. This data shows that the 10% scaled automated sample preparation yields the same chromatographic result as the full manual sample preparation.

EN14103 - Determination of Ester and Linolenic Acid Methyl Ester Content

Figures 5, 6 and 7 show three different biodiesel samples analyzed using the EN14103 method. For each sample, the automated sample preparation using the 7693A ALS yielded the same chromatographic result as the manual preparation.

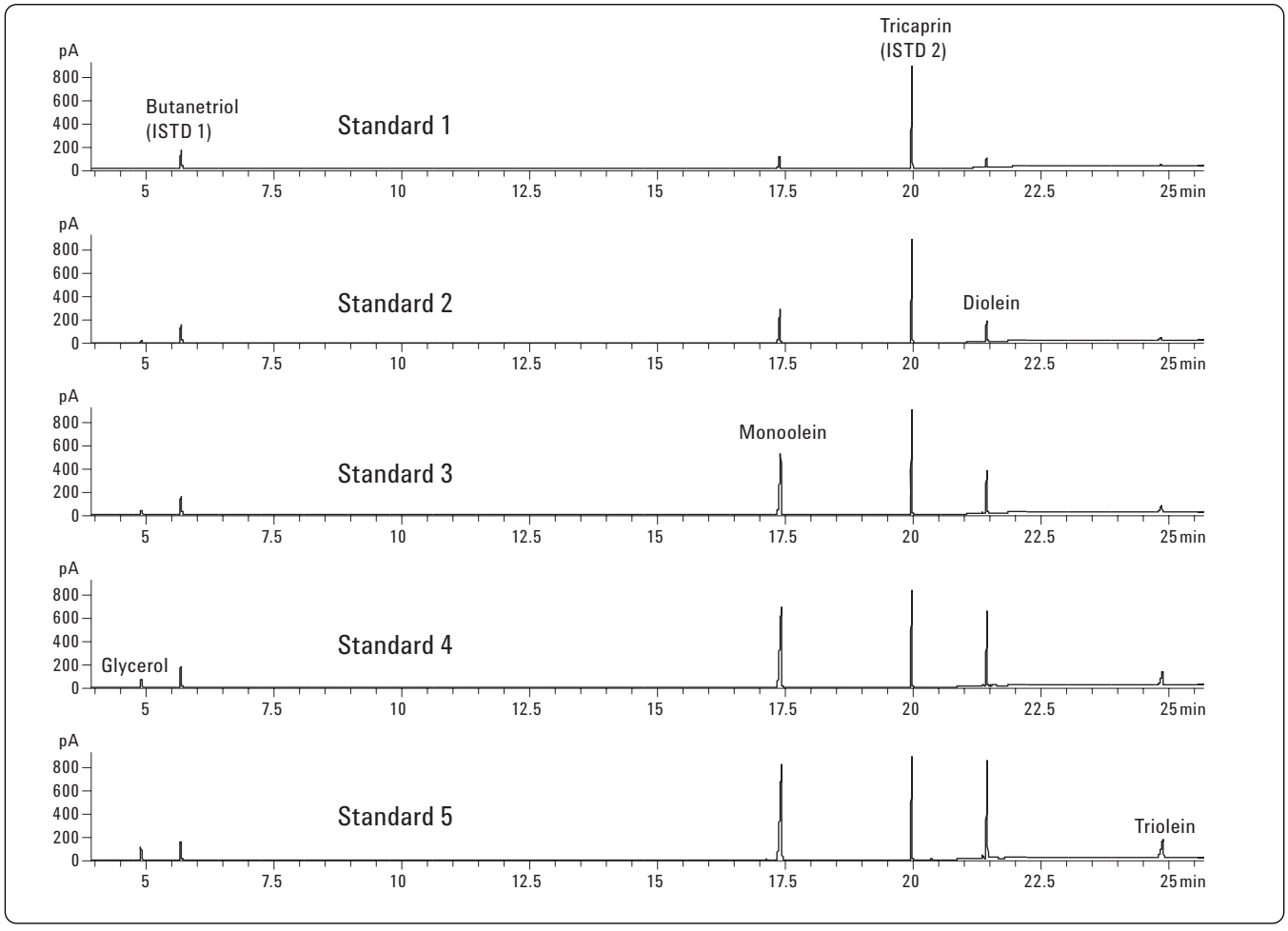

Figure 1. A five level ASTM D6584 calibration was automatically prepared using the Agilent 7693A ALS and run on the Agilent 7890A Biodiesel Solution.

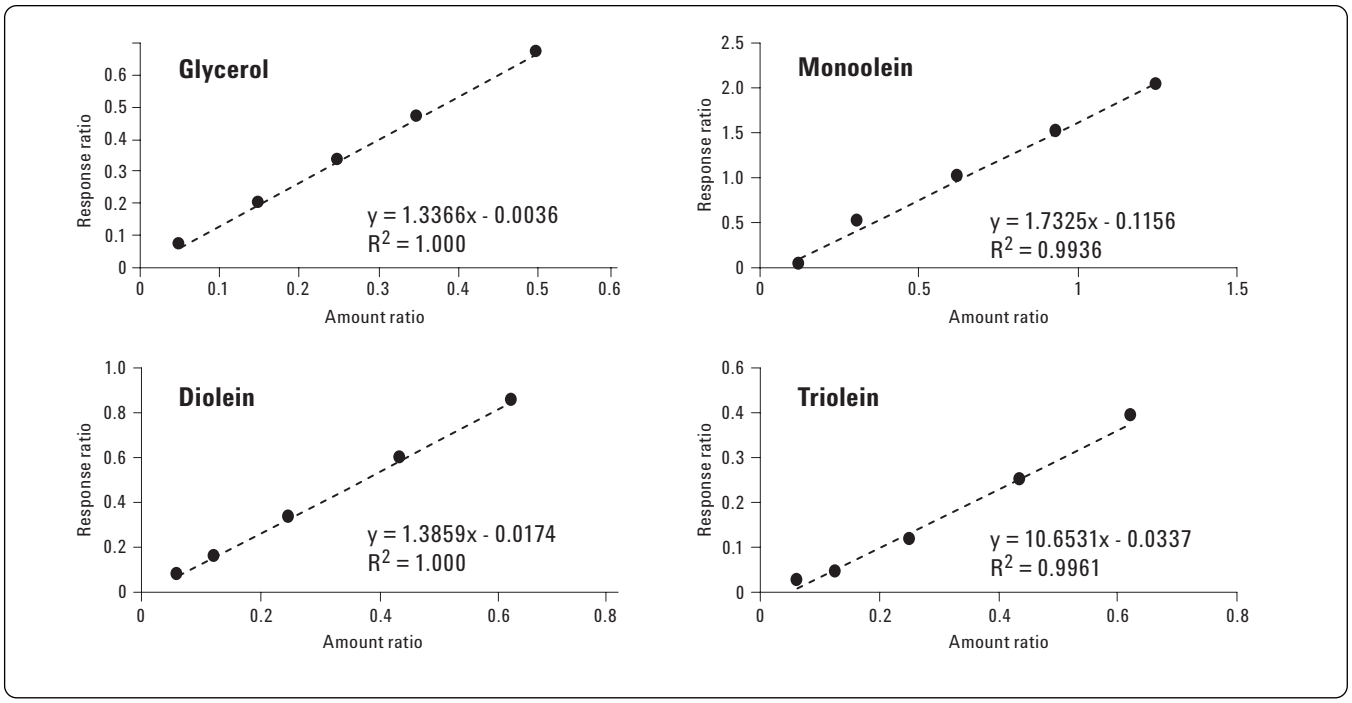

Figure 2. Five point linear calibration curves resulting from automated standard preparation with the Agilent 7693A ALS.

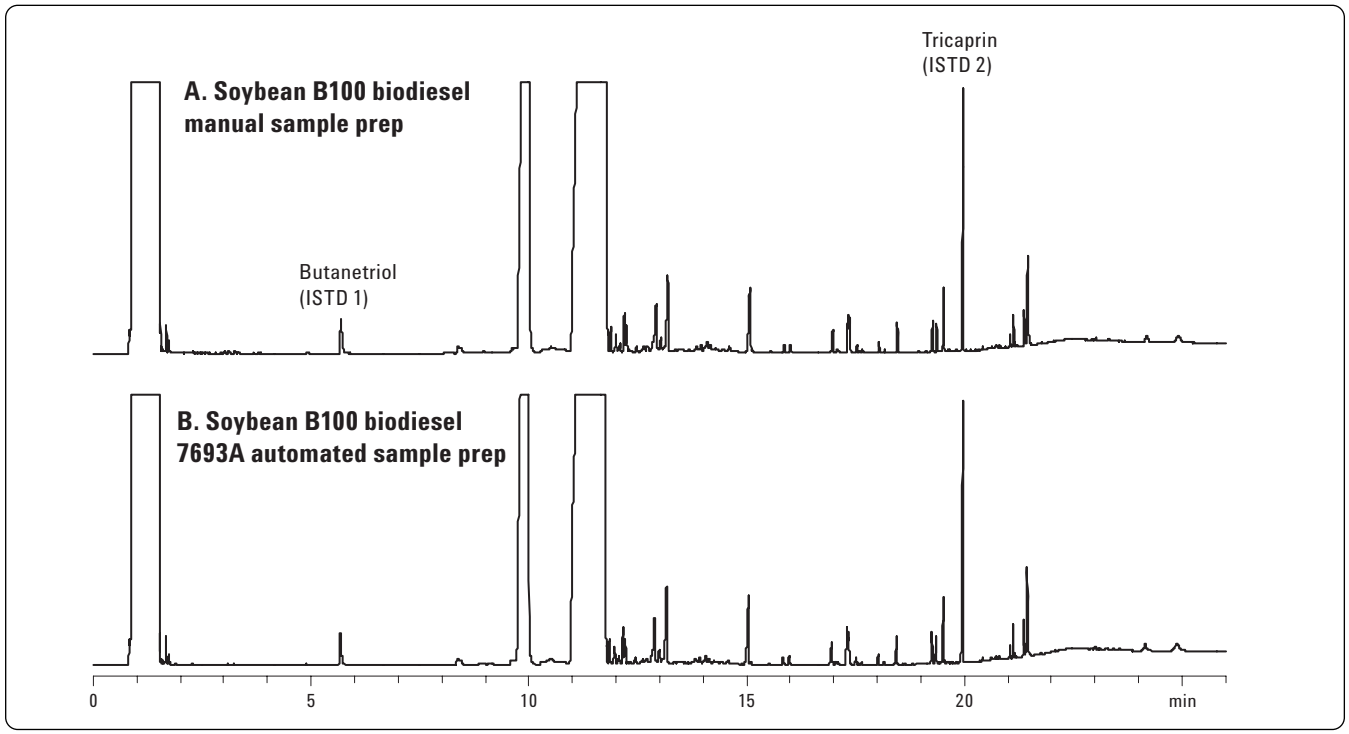

Figure 3. Comparison of a soybean B100 sample prepared (A) manually according to the ASTM D6584 protocol and (B) the automatic 10% scaled protocol using the Agilent 7693A ALS and Agilent 7890A Biodiesel Solution.

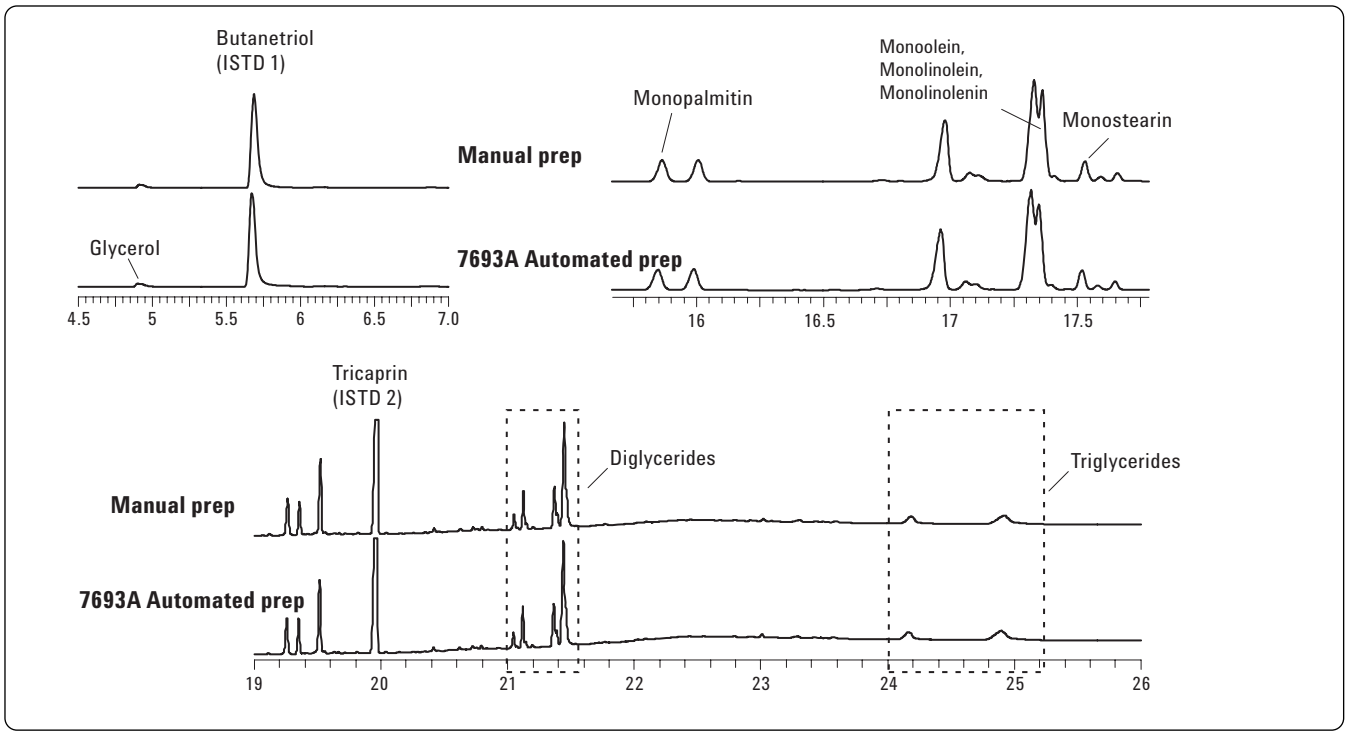

Figure 4. Details of the glycerol, mono-, di-, and tri-glycerides contained in a soybean B100 sample. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

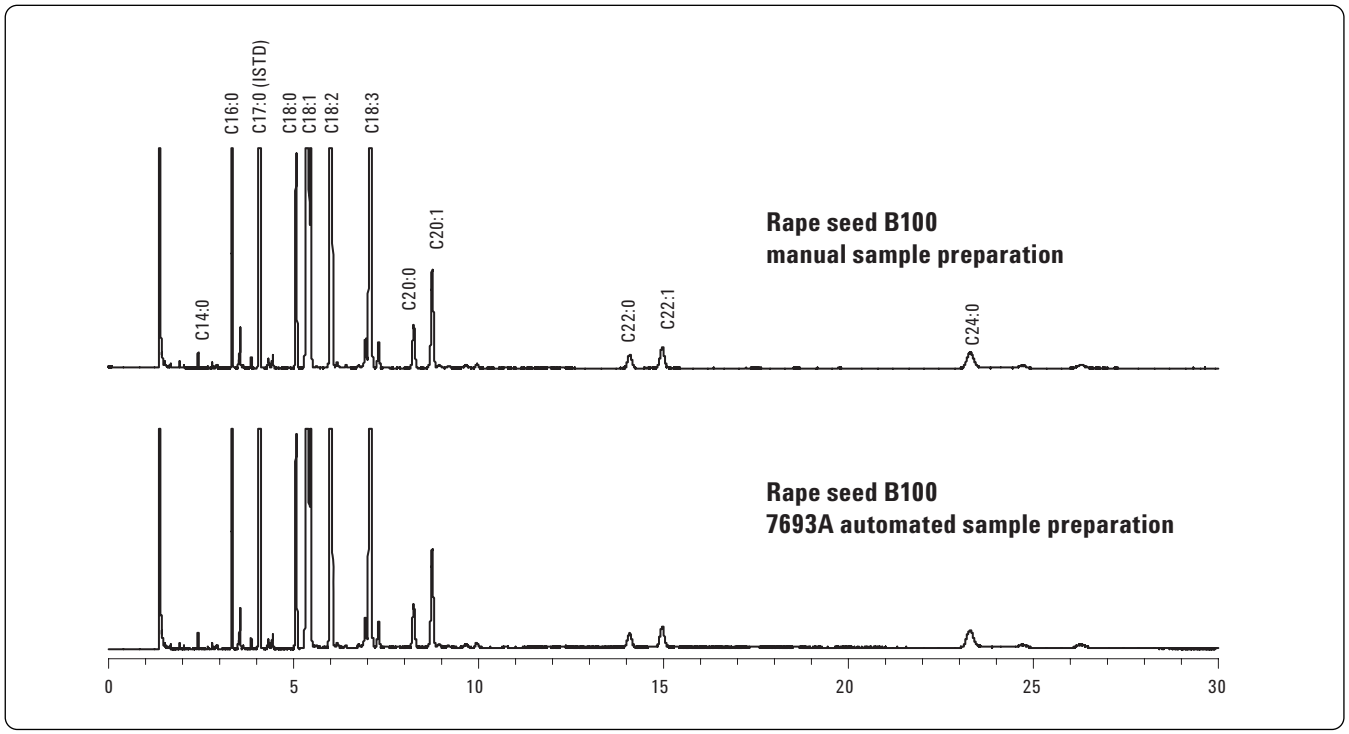

Figure 5. Analysis FAMEs in rape seed B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

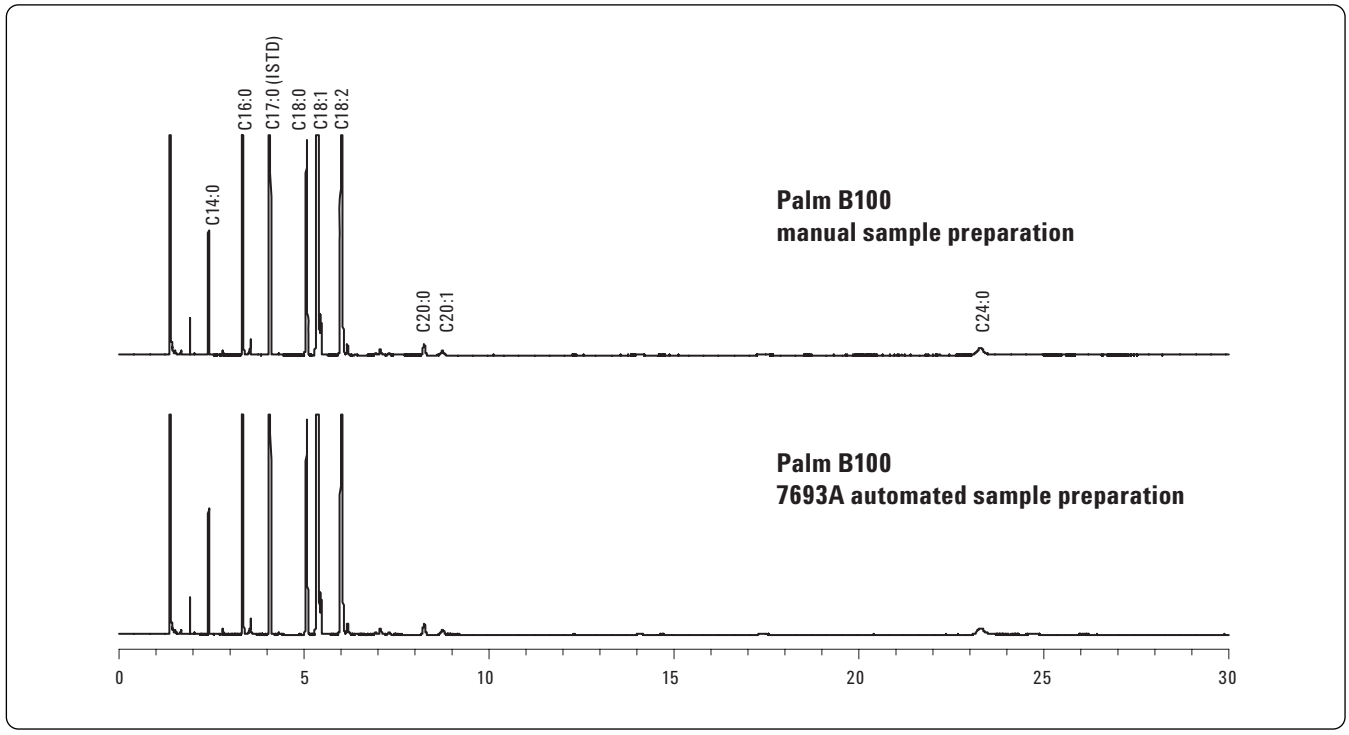

Figure 6. Analysis FAMEs in palm oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

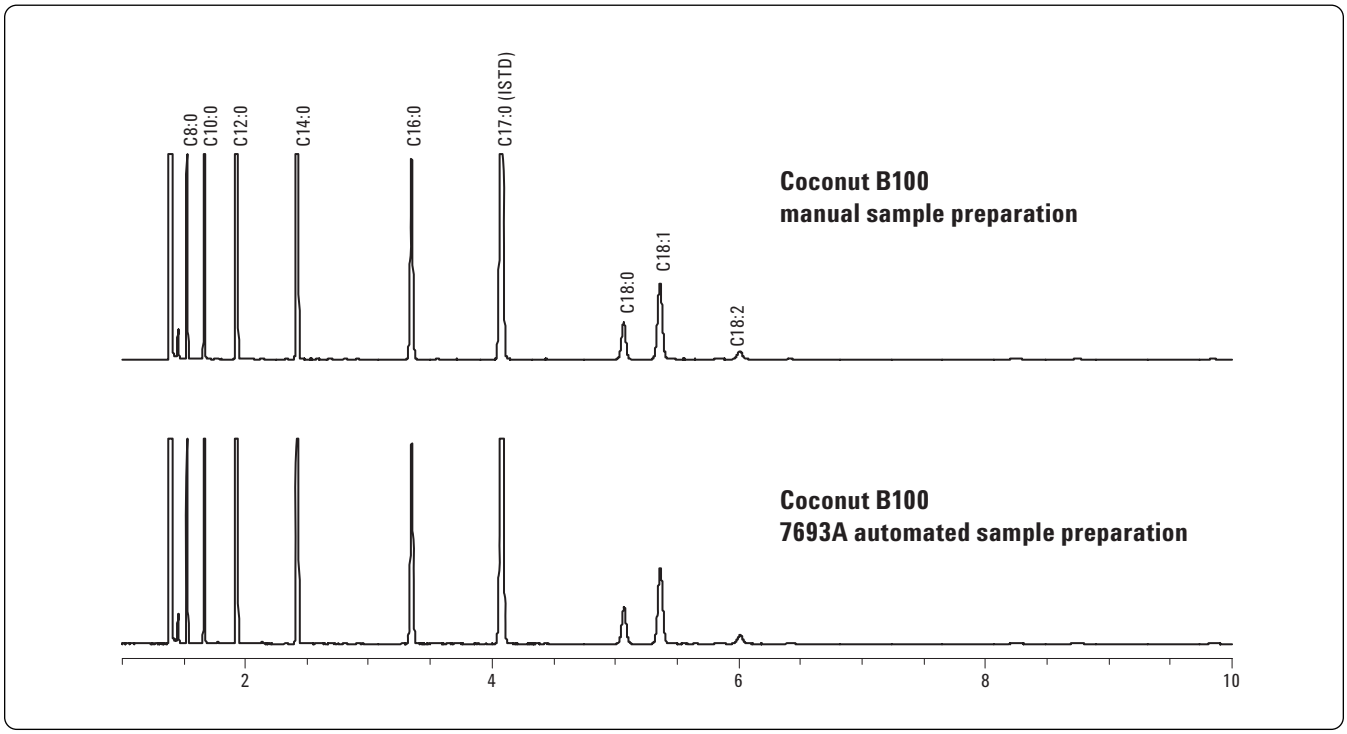

Figure 7. Analysis FAMEs in coconut oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

Conclusions

In the past, measuring biodiesel quality was divided into two parts: sample preparation and GC analysis. Sample preparation was complex, becoming a major contributor to errors and the need to repeat sample analyses. This paper demonstrates the automated sample and standard preparation capabilities of the Agilent 7693A ALS applied to the analysis of B100 biodiesel. A complex manual derivatization procedure used by the ASTM D6584 and EN14105 methods for measuring free and total glycerine was successfully converted to a completely automated protocol with the 7693A ALS. This sample preparation protocol was integrated with the GC analysis conditions on the Agilent 7890A Biodiesel Analysis Solution creating a totally automated system. An automated sample preparation and GC analysis was also developed for the analysis of FAMEs and methyl linolenate using method EN14103. Automated sample preparation combined with automated GC analysis offers improved reliability and productivity for laboratories concerned with obtaining the highest quality results for biodiesel analyses. Additionally, automated sample preparation can reduce the usage of expensive reagents and standards and the exposure of laboratory personnel to toxic materials.

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2. "EN14105 Fat and Oil Derivatives - Fatty Acid Methyl Esters (FAME) - Determination of Free and Total Glycerol and Mono-, Di- and Triglyceride Content"; European Committee for Standardization: Management Centre, rue de Stassart 36, B-1050 Brussels, 2003.
3. "EN14103 Fat and Oil Derivatives - Fatty Acid Methyl Esters (FAME) - Determination of Ester and Linolenic Acid Methyl Ester Content"; European Committee for Standardization: Management Centre, rue de Stassart 36, B-1050 Brussels, 2003.

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Printed in the USA
April 13, 2009
5990-3781EN



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Boiling Point Range of Fatty Acid Methyl Esters Using the 7890A Gas Chromatograph, Low Thermal Mass (LTM) System, and 7693A Tower and Tray

Application Note

Hydrocarbon Processing

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Abstract

Two Agilent 7890A Series GC systems were used to determine the boiling point distribution of Biodiesel (B100 and B20). First, the standard oven was used to produce runs of about 16 minutes. This was followed by a 7890A equipped with an LTM system and 1 five-inch module which does an 8 minute run. Both systems used the Multimode inlet in temperature programmed split mode for sample introduction and a dual tower Agilent 7693A Automatic Liquid Sampler configuration with a 150-vial sample tray for sample prep and injection.

Agilent Technologies

Introduction

ASTM method D7398 describes procedures for determining the boiling point range distribution of pure biodiesel (B100) and biodiesel blends of B1 and higher. To ensure that unreacted triglycerides are detected, the gas chromatograph is temperature programmed to 400 °C. Only the procedures involving calibration and running of pure biodiesel and biodiesel blends will be demonstrated in this work. Some sample preparation is normally involved which includes dissolution of a Polywax 500 standard that involves heating, mixing, and sample dilution of the biodiesel. These sample preps can be largely automated using the Agilent 7693A Series injector and tray system. Simulated distillation software is then used to compute the boiling range distribution. A standard 7890A GC and a 7890A/Low Thermal Mass (LTM) was used to analyze the prepared samples.

Experimental

Standard 7890A System

Inlet:	Multimode, G3510, 325 °C (0 min) to 400 °C at 200 °C/min
Liner:	Single taper liner with glass wool, 5183-4647
7890A oven:	40 °C (0 min) to 400 °C at 15 °C/min
Column:	5M × 0.53 mm × 0.15 µm DB-HT SimDis, 145-1001
Flow:	Constant flow mode at 14 mL/min He
Injection:	0.1 µL split 4:1, PW500 standard, 1 µL

7890A/LTM System

Inlet:	Multimode, G3510, 220 °C (0 min) to 400 °C (2 min) at 300 °C/min
LTM column module:	5 m × 0.53 mm × 0.15 µm DB-HT SimDis
Module connections:	0.7 m deactivated ProSteel on inlet and outlet
7890A oven:	325 °C isothermal
LTM module program:	40 °C (0 min) to 400 °C at 50 °C/min
FID:	400 °C
Inlet pressure ramp:	2.5 psi (0 min) to 9.5 psi (1 min) at 1 psi/min
Injection:	0.1 µL, split 10 to 1, PW500 standard, 1 µL

Deactivated Ultimate Unions, part no. 3182-60580, are used with the LTM module for connection of the ProSteel retention gaps to the column ends.

The 7693A injectors are installed with the 150-vial sample tray which includes a mixer/barcode reader and heater compartment for the purpose of sample prep and injection. The front tower uses a 5-µL syringe and the rear tower uses a 250-µL syringe which requires the large syringe carriage G4521A.

Data is processed using ChemStation 4.01 and the Agilent SimDis software, part number G2887BA. Example sample preparation programs from the ChemStation are shown below for system calibration and biodiesel samples.

Sample Prep Programs Using the 7693A

Table 1. Sample Program for the Preparation of PW500 with C5-C18 Mix Added

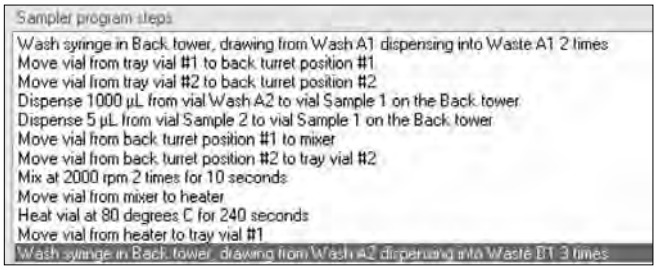
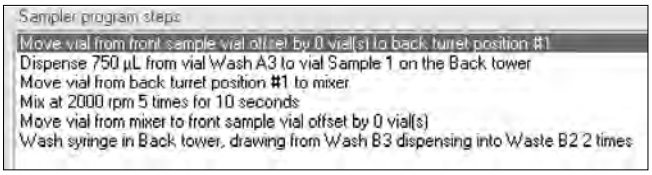


Table 2. Sample Program for the Dilution of a Biodiesel Sample Starting with 0.5 mL Biodiesel in a 2 mL Vial



Discussion

The calibration setup pane from the SimDis software is shown in Figure 1 for the LTM system. The mix of C5-C18 plus Polywax 500 gives a calibration from C_8 to C_{78}, covering the boiling point range of B100 (including unreacted components) and biodiesel blends. In Figures 1 and 2, calibration plot panes from the SimDis software with assigned carbon numbers are shown for the LTM and standard 7890A systems, respectively. Typical elution times for C_{70} are 7.5 minutes and 22 minutes for LTM and standard systems, respectively. Both show symmetric distributions indicating good inlet sample transfer with minimal discrimination. Figure 3 shows the chromatogram of a B20 soy-based biodiesel run on the LTM system. C_{16} and C_{18} fatty acid methyl esters can be seen above the diesel background.

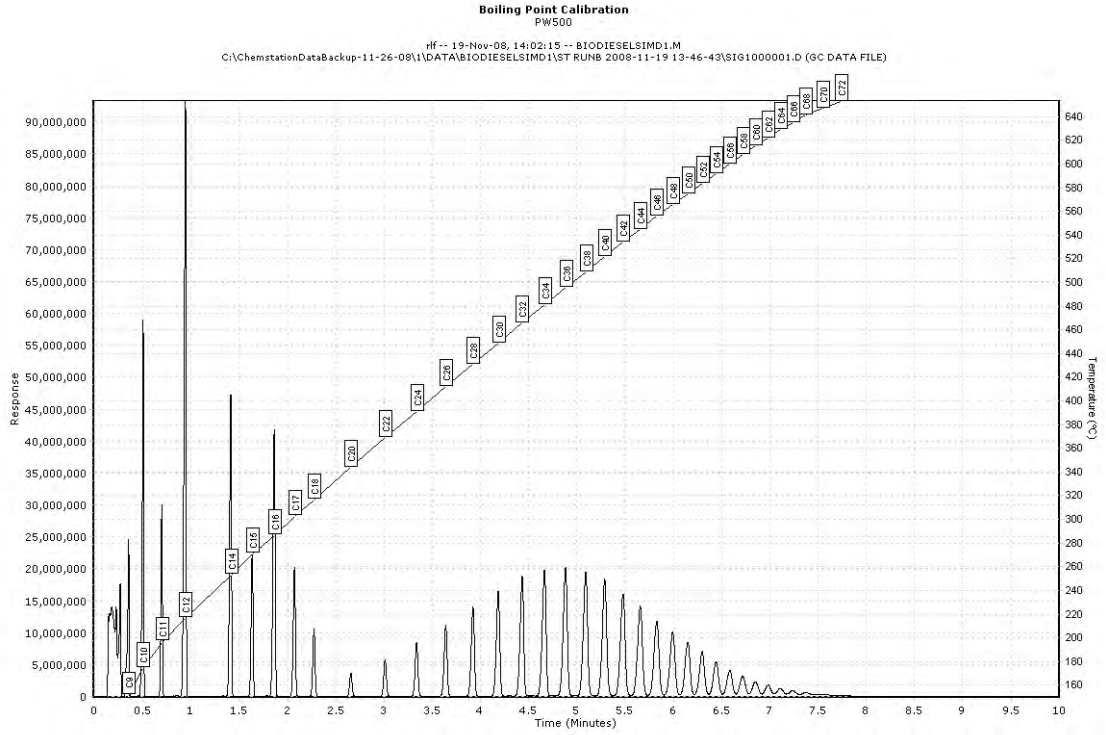


Figure 1. Calibration curve on LTM system from C9 to C72 prepared from PW500 and C5-C18 mix.

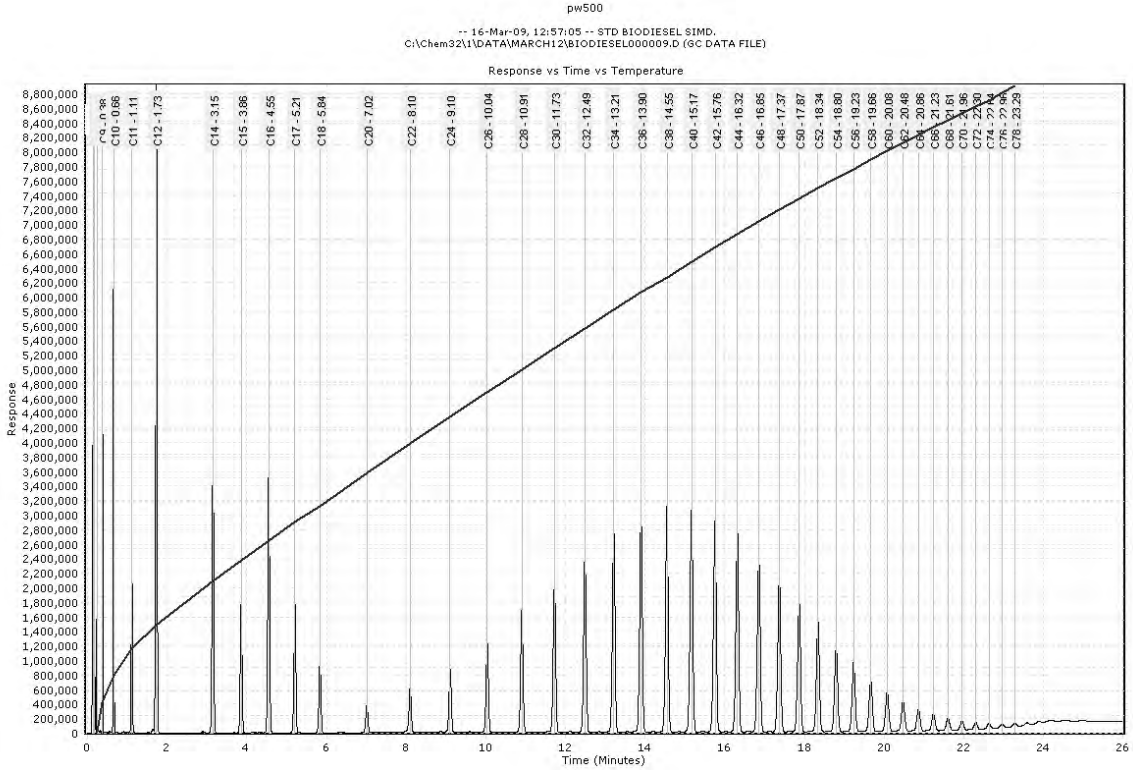


Figure 2. Calibration curve on standard 7890A GC.

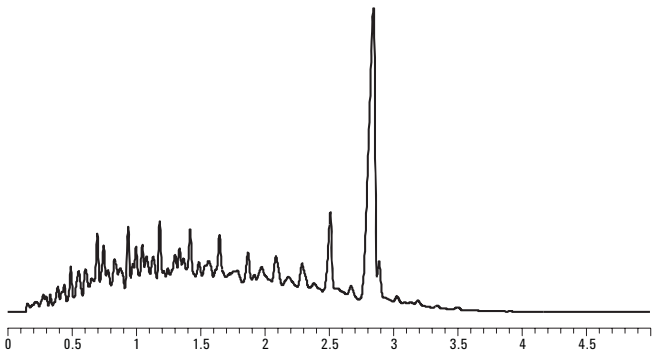


Figure 3. Chromatogram of B20 Soy based biodiesel using the LTM system.

A boiling point distribution of B100 sourced from rapeseed is shown in Figure 4. In Figure 5, two chromatograms are shown in an overlay. These are both B100 production biodiesel from two different plants. Note the different ratios of the C_{16} group (6.6 min.) to the C_{18} group (7.5 min.) in these samples. Lastly, calculated boiling point distributions for both samples are shown in Figures 6 and 7.

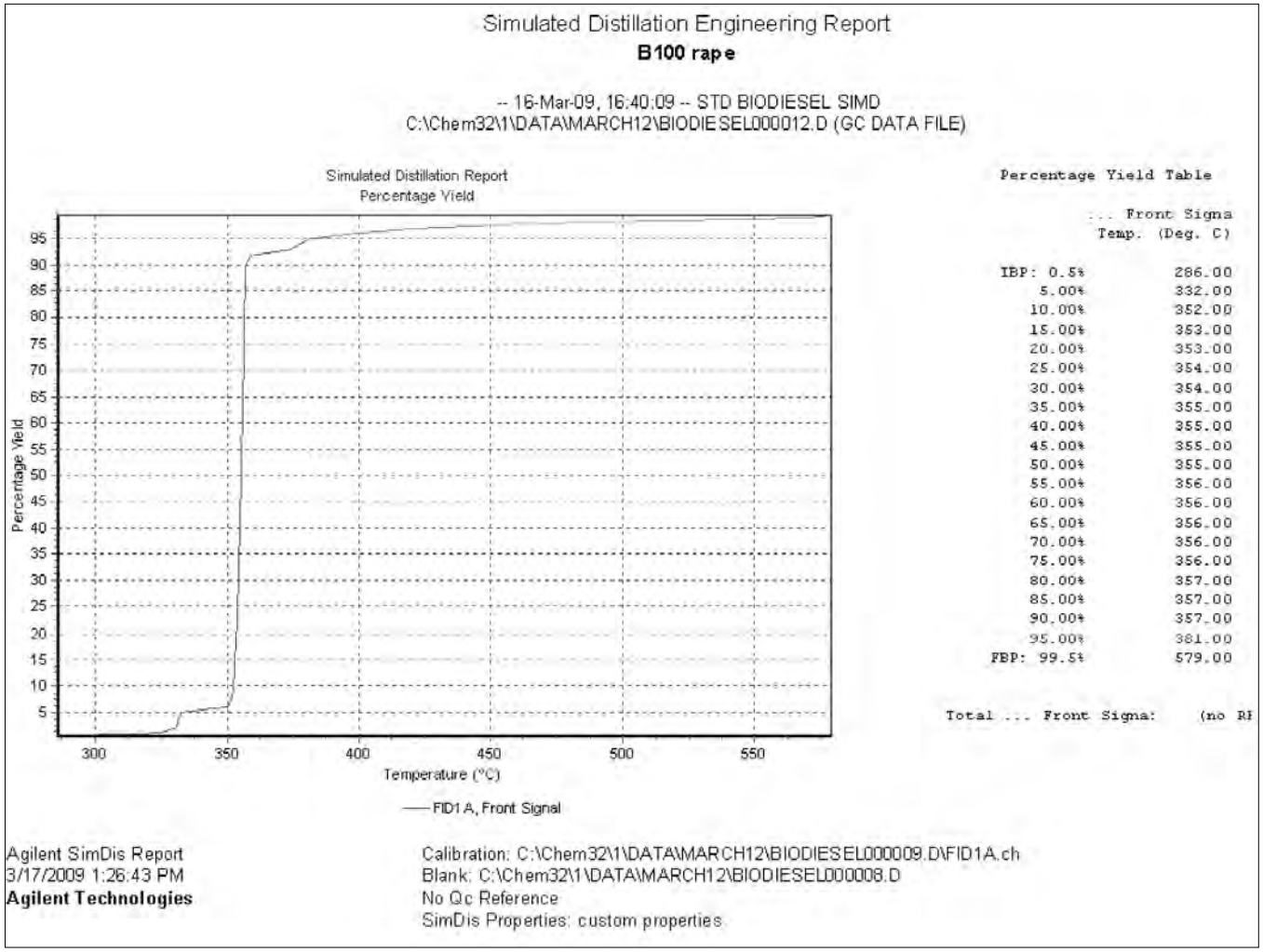


Figure 4. Boiling point distribution for rapeseed B100.

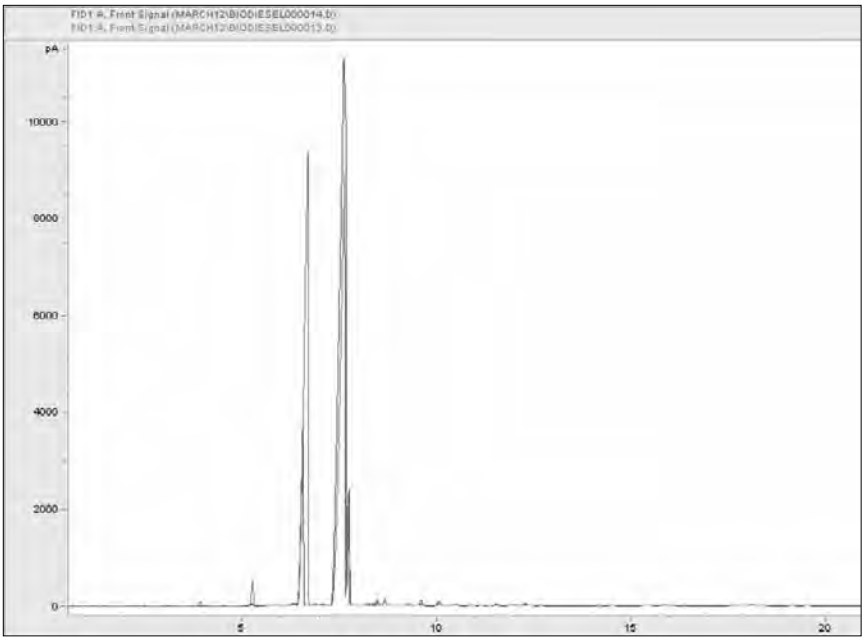


Figure 5. Overlay of two B100 samples from different producers. Both are soy based biodiesel. Producer A: signal 14, Producer B: signal 13.

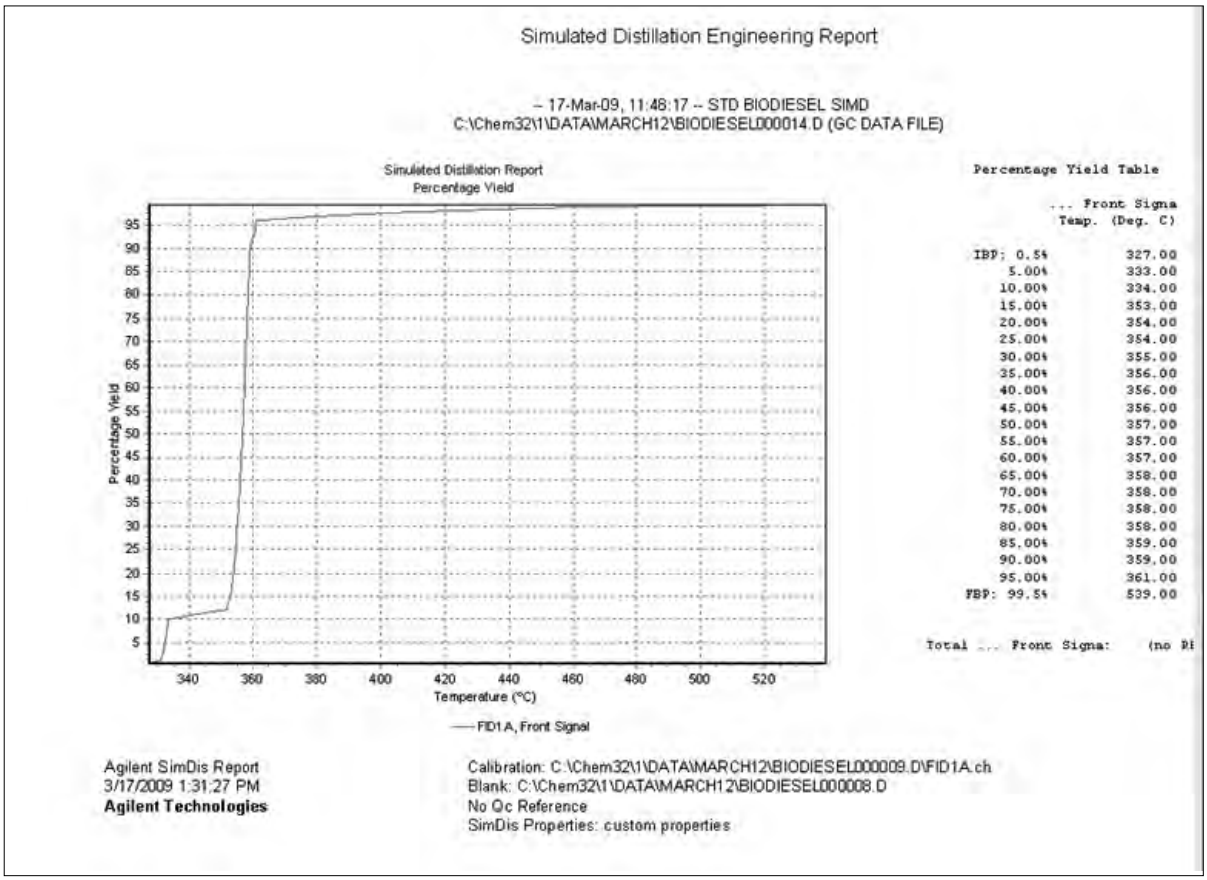


Figure 6. Boiling point distribution of B100, producer A.

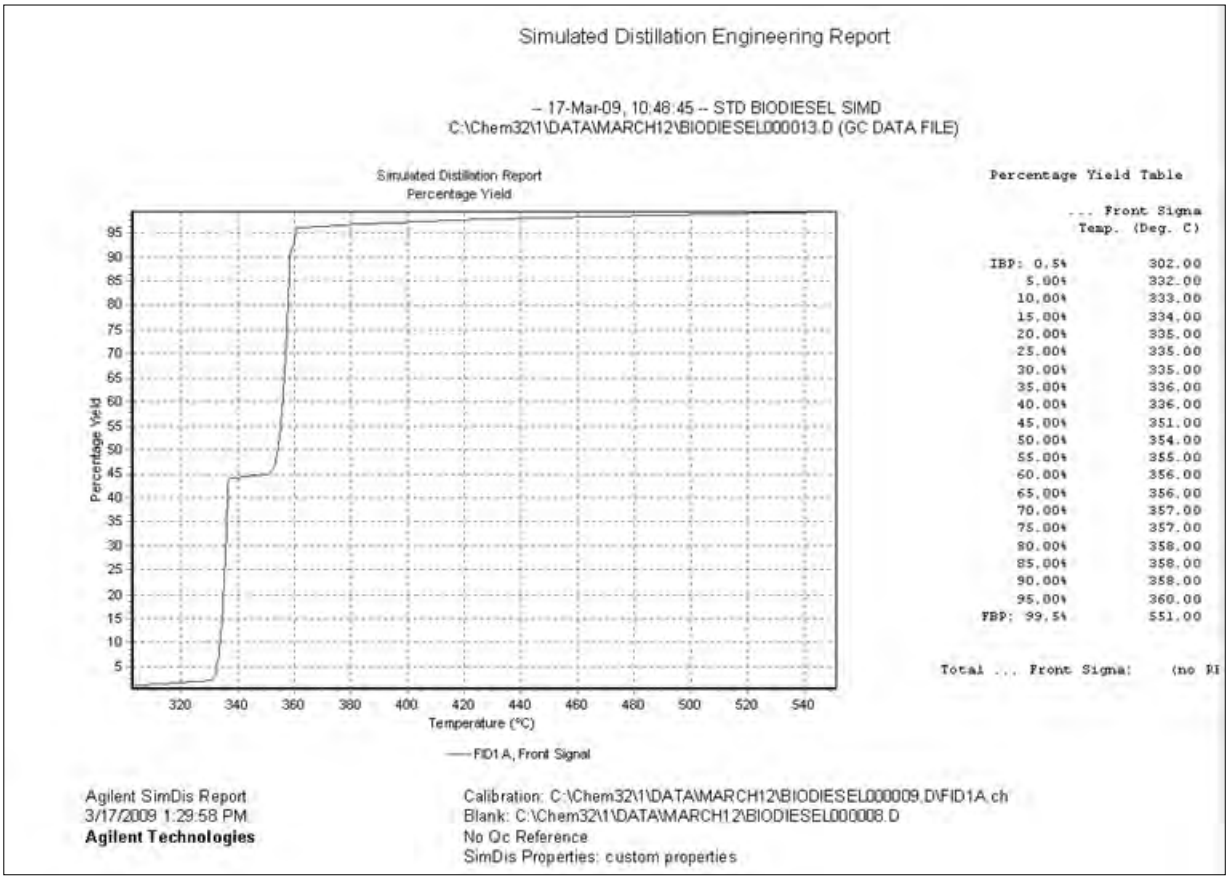


Figure 7. Boiling point distribution of B100 from producer B.

Summary

Simulated distillation is a powerful tool for characterization of biodiesel and biodiesel blends for a variety of starting oils. Besides determining the fatty acid methyl ester boiling point distribution, some information on the amount of un-reacted oil can be ascertained. The technique is also useful to determine authenticity and product consistency for quality control. The Agilent 7890A Series GC with the Agilent 7693A Automatic Liquid Sampler tower/tray system forms a complete analysis system from sample prep to boiling point distribution reporting using SimDis software integrated in the GC ChemStation.

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Printed in the USA
April 20, 2009
5990-3868EN

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High-Resolution Analysis of Intact Triglycerides by Reversed Phase HPLC Using the Agilent 1290 Infinity LC UHPLC System

Application Note

Food, Hydrocarbon Processing

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Abstract

The Agilent 1290 Infinity LC System with ultraviolet/visible (UV/VIS) Diode Array detection (DAD) is used to analyze triglycerides in soybean oil under non-aqueous reversed phase gradient conditions. The Agilent 1290 Infinity LC System was used for the chromatographic separation of the sample on 3.0 and 2.1 mm id C18 columns, of various lengths, with 1.8-µm packing materials prepared in 600 bar (9000 psi) or special 1200 bar (18,000 psi) configurations. The ability of the Agilent 1290 Infinity LC System to operate with long, high resolution columns is demonstrated with isopropanol (IPA) or methyl tert butyl ether (MTBE) as the strong solvent and acetonitrile as the weak component of the mobile phase mixture.

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Introduction

The analysis of intact triglycerides from animal or vegetable sources has many practical uses including understanding the chemical composition of the triglyceride, assessing fuel potential, and understanding lipid metabolism and behavior in living systems. The general conditions for successful analysis of these components by high-performance liquid chromatograph (HPLC) include gradient elution and low-wavelength monitoring of the overall separation. Because triglycerides have relatively few chromophores it is also beneficial to use evaporative light scattering detectors (ELSD) or mass spectrometers to facilitate other views of the separation.

During the development of this application, we analyzed a number of vegetable oils from various sources including soy, corn, rice bran, safflower, grape seed, olive, and palm oil. Because of the wide abundance of soybean oil in the United States and its growing significance in the production of biofuels, most of this work was standardized on maximizing the resolution of soybean oil triglycerides. These general conditions, however, are also suitable for a wide variety of samples including samples from animal lipid sources.

Intact triglycerides generally have very low water solubility and as such are commonly separated by normal phase chromatography, which separates species largely based on differences in polar functional groups, or by reversed phase chromatography operating in a non-aqueous mode of separation, which has more selectivity for small differences on carbon character such as chain length or unsaturation.

According to information published by Perkins [1] the predominant fatty acids, which are the building blocks of triglycerides on a glycerol backbone, found in soybean oil are myristic (14:0), palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). Many other minor fatty acids are also present and because all of the fatty acids are randomly constructed into triglycerides, an extensive permutation of fatty acid substructure is obviously possible. Because the predominant difference between fatty acids consists of carbon chain length and number of double bonds, most of the diversity in triglycerides is found in the rather non-polar organic structural features. As a result, reversed phase chromatography is most useful for this application. Triglycerides have extremely poor solubility in water so one normally chooses either a high organic starting position, with respect to the aqueous content or, as in this work, a completely non-aqueous separation environment.

The typical structure of a triglyceride is shown in Figure 1. [2] In this example, from top to bottom, palmitic acid (C16:0), oleic acid (C18:1), alpha-linolenic acid (C18:3) are shown with respect to chain length and degree of unsaturation. The chemical formula is $C_{55}H_{98}O_6$.

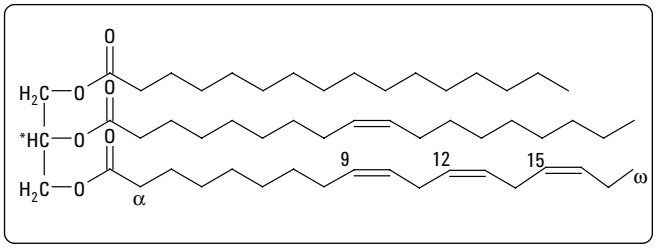

Figure 1. Typical triglyceride structure.

Experimental

Sample Preparation

The primary solution was prepared at a concentration of 10 mg/mL, in 2-propanol or 2:1 volume to volume MeOH/MTBE, and subsequently diluted to lower concentrations as needed. Injection volumes of 0.2-2 µL were made into the LC/DAD system.

LC Method Details

LC Conditions

Agilent 1290 Infinity LC System binary pump G4220A,
Agilent 1290 Infinity LC System autosampler G4226A
Agilent Thermostatted Column Compartment G1316C with switching valve
Agilent 1290 Infinity LC System diode array UV/VIS detector G4212A with 10 mm path fiber optic flow cell

Columns:	(See individual figures for specific usage) Agilent ZORBAX SB-C18 RRHT, 3 mm × 150 mm, 1.8 µm 600 bar, p/n 829975-302 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 100 mm, 1.8 µm 1200 bar, p/n 858700-902 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 150 mm, 1.8 µm 1200 bar, p/n 859700-902 In some cases, columns were coupled to extend the length and resolution.
Column temp:	20 °C or 30 °C
Mobile phase:	A = acetonitrile B = isopropanol (IPA) or tert butyl methyl ether (MTBE) (See individual figures)
Flow rate:	See individual figures
Gradient:	The gradient conditions were either 20% to 60% IPA or 10% to 40% MTBE, based on the strong eluting strength of MTBE when compared to IPA. The gradient slope was maintained at 2.6% organic phase increase per column volume for IPA gradients and 2.0% with MTBE, altering gradient time and flow rate accordingly. This was determined by calculations using a modification of the Agilent Method Translator. [3]

UV Conditions

Monitoring 210, 220 and 230 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

A typical gradient separation of triglycerides using acetonitrile IPA gradient is shown in Figure 2.

Some general comments are appropriate about the conditions and chromatographic profile shown in Figure 2. While it would be ideal to consider less expensive methanol as the weak eluent, introduction of methanol or denatured ethanol containing methanol has consistently shown a dramatic reduction in the overall resolution of the triglycerides. The significant increase in operating pressure, when running the gradient from acetonitrile to IPA, is clearly limiting and undesirable. Increasing the operating temperature of the column as a means of reducing solvent viscosity has proven to be undesirable because the chromatographic resolution tends to collapse as temperature increases.

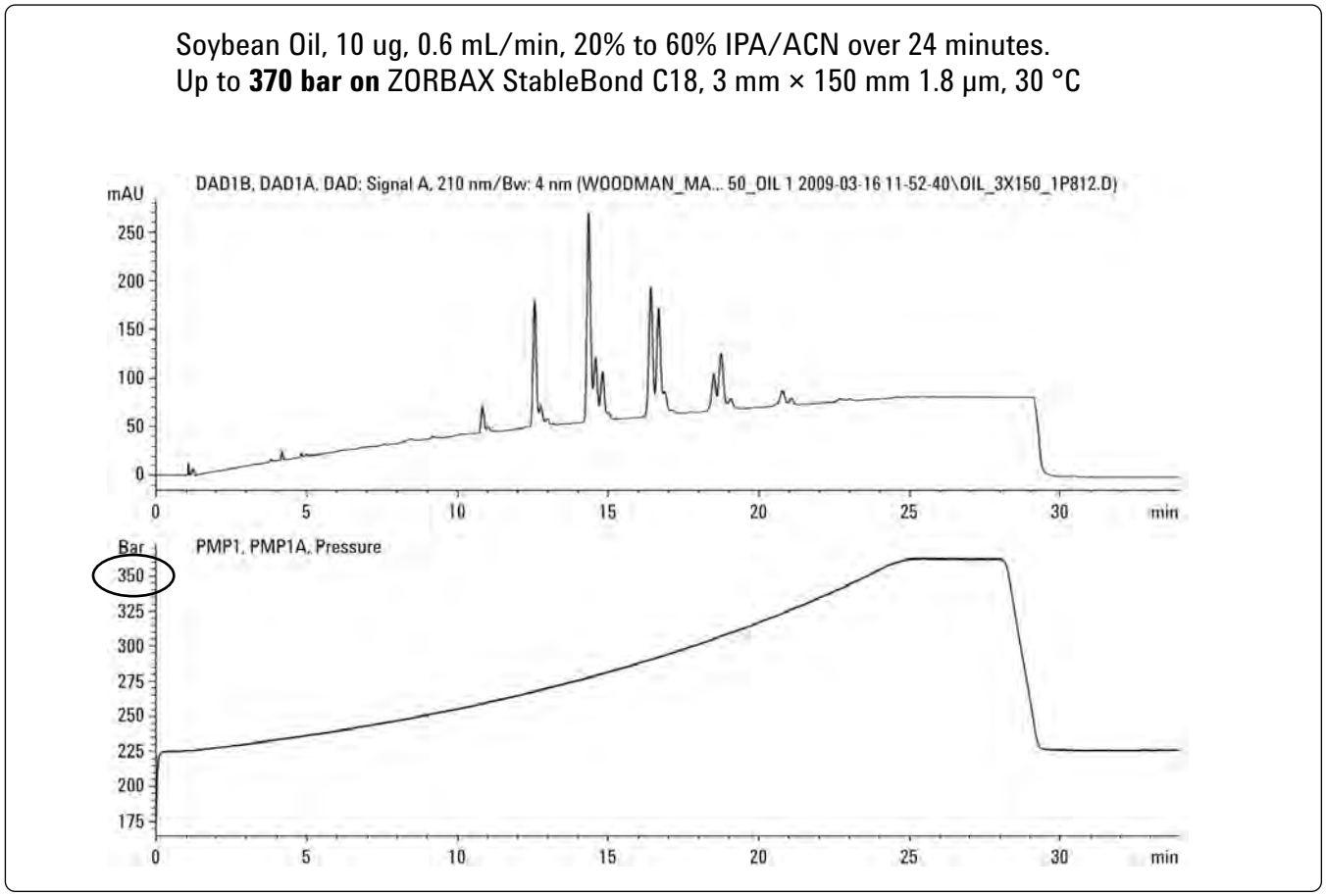

Figure 2. A 210-nm UV chromatogram of soybean oil sample on a 3 mm × 150 mm ZORBAX Rapid Resolution High Throughput (RRHT), 1.8 µm column, upper panel. System pressure trace showing the general progress of the gradient elution, lower panel. Flow rate 0.6 mL/min, gradient time 24 min. Strong solvent, isopropanol. The chromatogram demonstrates the typical difficulty encountered with this type of separation, which is small clusters of chromatographically similar triglycerides. These clusters are not positional isomers of the same carbon number and degree of unsaturation, rather a mixture of various chain lengths and number of double bonds as shown by mass spectrometric evaluation.

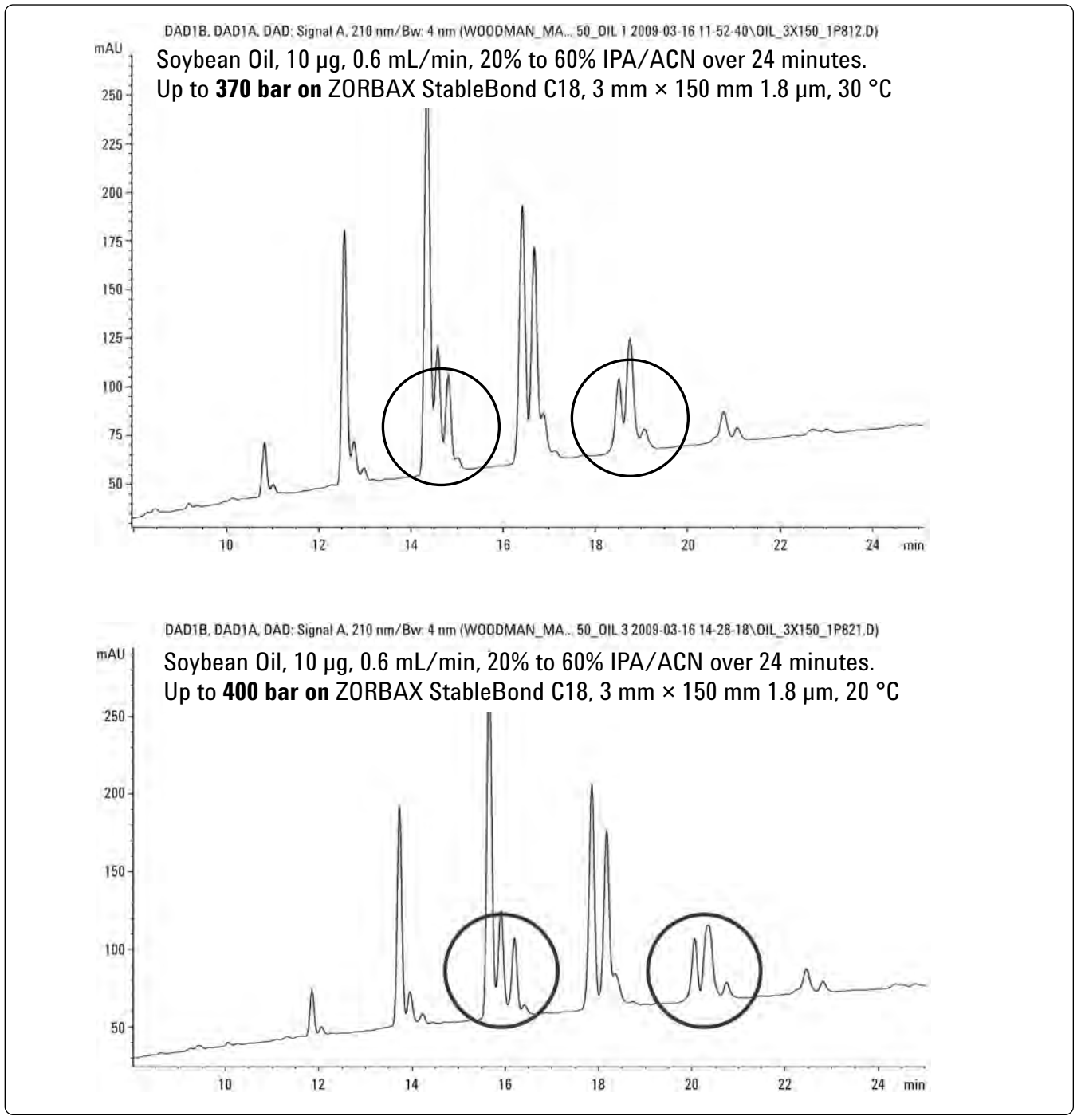

Figure 3. An expanded presentation of the chromatogram shown in Figure 2 at 30 °C, upper panel, compared with the same conditions in Figure 2 operating the column at 20 °C.

In Figure 3 we see the improvement achieved by operating the separation at 20 °C rather than 30 °C. The operating pressure increase is approximately 10% at the lower temperature. While many of our separations have been performed at 30 °C as a compromise between separation and operating pressure, the availability of the Agilent 1290 Infinity LC System with increased operating pressure capability has allowed us to reduce the temperature to 20 °C and demonstrate a usable improvement in separation.

Soybean Oil, 10 µg, 0.29 mL/min, 20% to 60% IPA/ACN over 27 minutes.
Up to **760 bar on** ZORBAX StableBond C18, 2.1 mm × 250 mm 1.8 µm, 20 °C

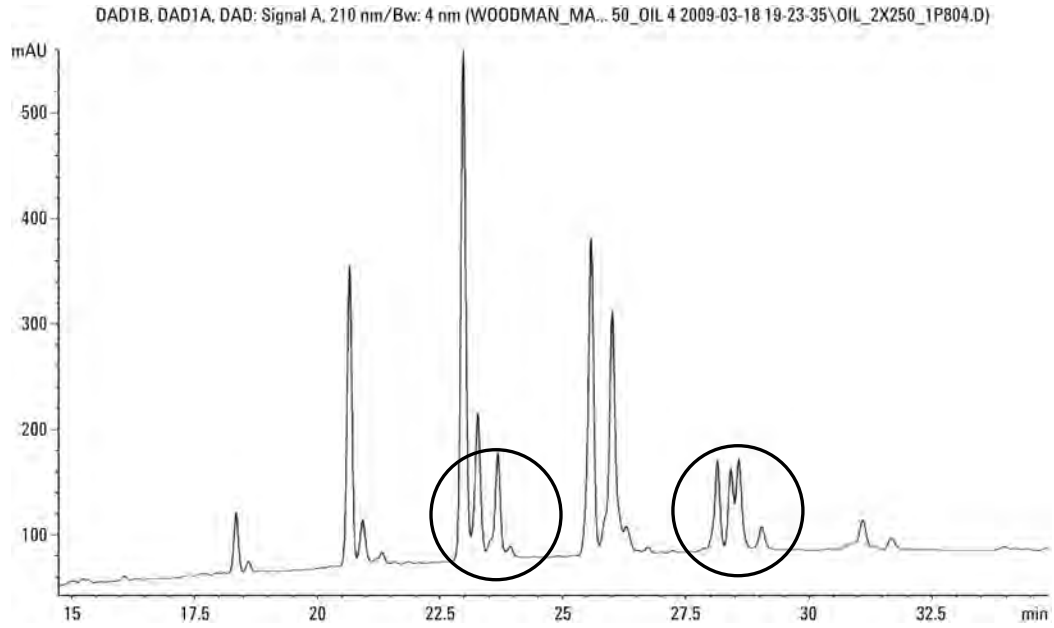

Figure 4. Analysis of the soybean oil sample on an Agilent ZORBAX StableBond C18 column, 2.1 mm × 250 mm, 1.8 µm, (150 mm in series with 100 mm) prepared for operation at 1200 bar pressure limit. Flow rate 0.29 mL/min, gradient time 27 min. Maximum observed pressure 760 bar.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution, it would be practical to explore longer columns or explore alternative mobile phase or column chemistries. As with most very high performance separations, rate-limiting features tend to include operating pressure, operating temperature, and maximum flow rate. The triglyceride separations evaluated thus far have not been receptive to operation at higher column temperatures or higher flow rates, presumably because of their relatively high molecular weight and flexible organic structure. Even when gradient slope translations are carefully made to ensure organic strength consistency from method to method, operating at higher flow rates has consistently shown degradation of the overall separation. Because the isopropanol has significantly high viscosity and high pressure, it seemed appropriate to consider other non-polar solvents that are miscible with acetonitrile and friendly to low UV detection, as a substitute for isopropanol.

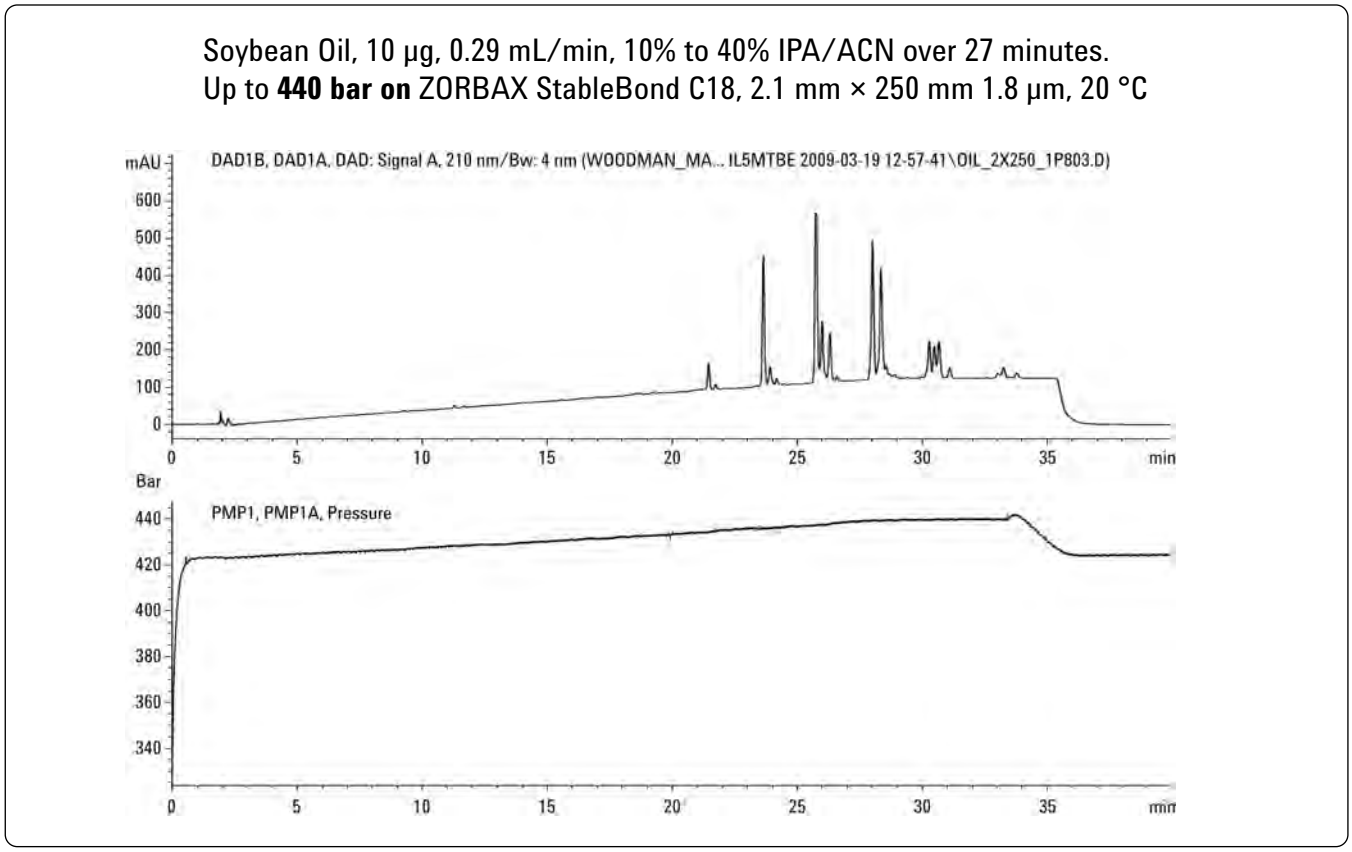

Figure 5. By substituting MTBE for isopropanol with otherwise the same conditions as Figure 4, and then re-optimizing the gradient for the significant increase in eluting strength of MTBE, we arrive at a new set of operating conditions where there is only a small difference in operating pressure over the gradient run. Flow rate 0.29 mL/min, gradient 27 min for 10% to 40% MTBE, maximum observed pressure 440 bar.

In Figure 5, the change to MTBE and subsequent readjustment of the gradient resulted in a separation that was very comparable to the original isopropanol separation, however at a much lower maximum operating pressure. In view of the prior evidence and comments regarding increased temperature or flow rate resulting in degraded separation, it seemed that the most appropriate way to take advantage of the new operating pressure capability of the Agilent 1290 Infinity LC System was to continue to increase the column length. The Agilent 1290 Infinity LC System and associated ZORBAX chemistries are capable of operating pressures up to 1200 bar, or approximately 18,000 psi. To ensure robust and rugged system operation many users typically specify the upper pressure limit for a method at a value less than 80% of the rated operating pressure.

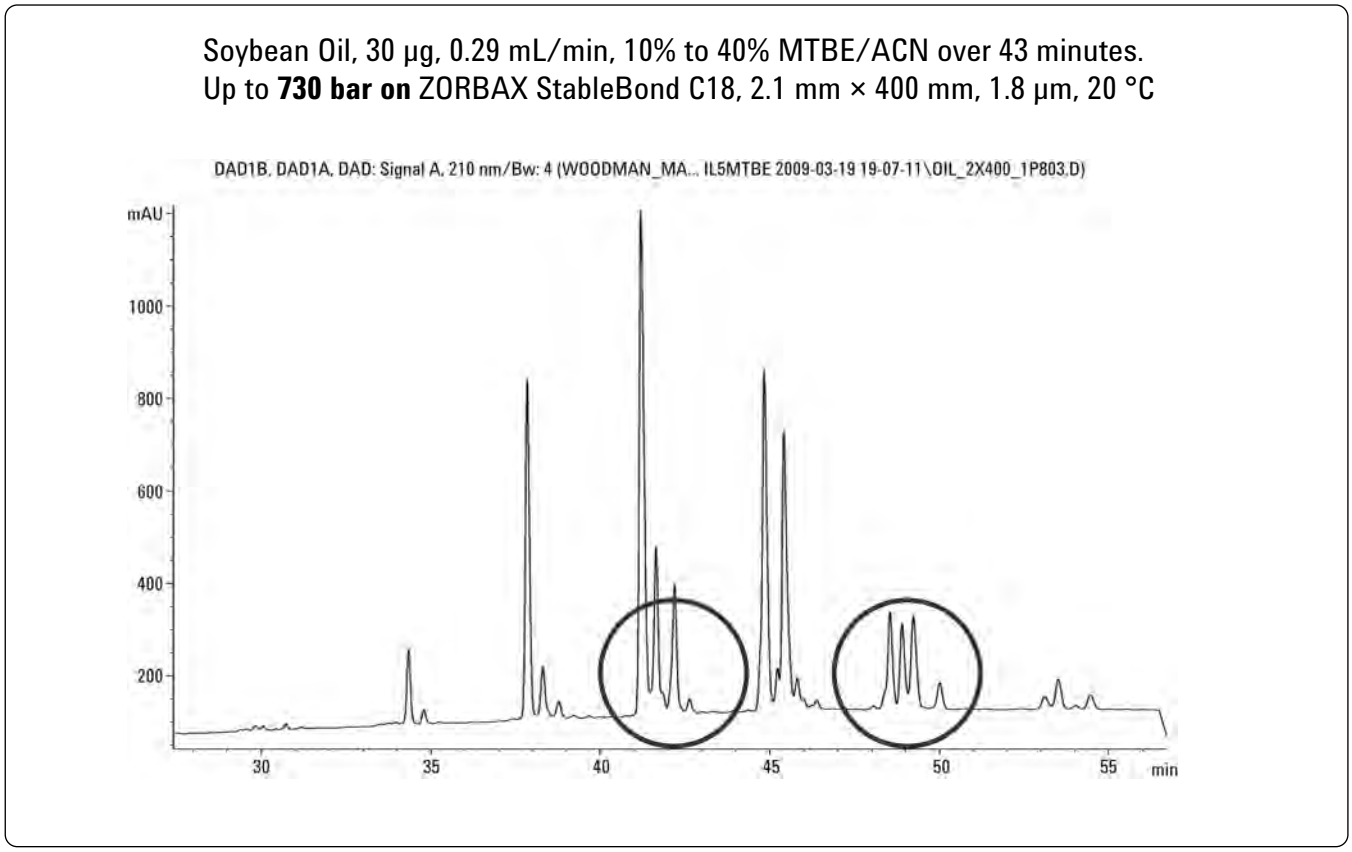

Figure 6. Separation of the soybean oil sample on a 2.1 mm × 400 mm ZORBAX StableBond C18, 1.8 µm 1200 bar columns (150 mm + 150 mm + 100 mm in series). Flow rate 0.29 mL/min gradient time 43 min, for a gradient of 10% to 40% MTBE. Maximum operating pressure 730 bar at 20 °C.

As shown in Figure 6, having previously optimized the column temperature, operating flow rate and gradient slope for the best possible balance between resolution and analysis time, and after investigating a variety of solvents as candidates for both the weak solvent and strong solvent choice, we are left with an ultimate opportunity to operate on a very long column set of 1.8 µm particle size columns under conditions ideal for the separation of this group of triglycerides. With an operating pressure of only 730 bar, which is about 60% of the rated capability of the Agilent 1290 Infinity LC System, it is clearly possible to consider even longer column lengths or a further reduction in the operating temperature as both of these seem promising in terms of delivering even higher resolution out of the mixture.

The separation with MTBE or isopropanol can be adapted for use with a mass spectrometer as one of the detectors. In previous studies (see www.Agilent.com/chem ASMS 2009 for a poster on this subject) we have been able to demonstrate the capability of quickly and confidently identifying the composition of many of the triglycerides found in this and other samples. For optimum electrospray performance in the non-aqueous, non-buffered environment it was useful to do post UV detector addition of a mixture of methanol and water with ammonium formate buffer to enhance ionization and to ensure a consistent ability to preserve the molecular ion into the mass spectrometer. It has been shown by McIntyre [4] that the presence of ammonium formate in the mobile phase significantly improves the probability that a molecular ion will be formed and preserved in the mass analyzer portion of a mass spectrometer.

Conclusions

Using the Agilent 1290 Infinity LC System, we were able to easily demonstrate UHPLC capabilities well within the operating range of the instrument. The significantly enhanced resolution afforded by long sub-2 micron particle size columns in the sub-ambient column compartment environment will contribute significantly to our understanding of the major and minor composition of this sample and other similar materials. This should significantly enhance the contribution of liquid chromatography to the understanding of seed oil composition, the role of triglycerides in metabolism, and the area of lipidomics where great interest has been directed on the LC separation coupled to time-of-flight high-resolution mass spectrometry (LC/TOF).

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Printed in the USA
August 3, 2009
5990-4292EN

Agilent Technologies

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