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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%

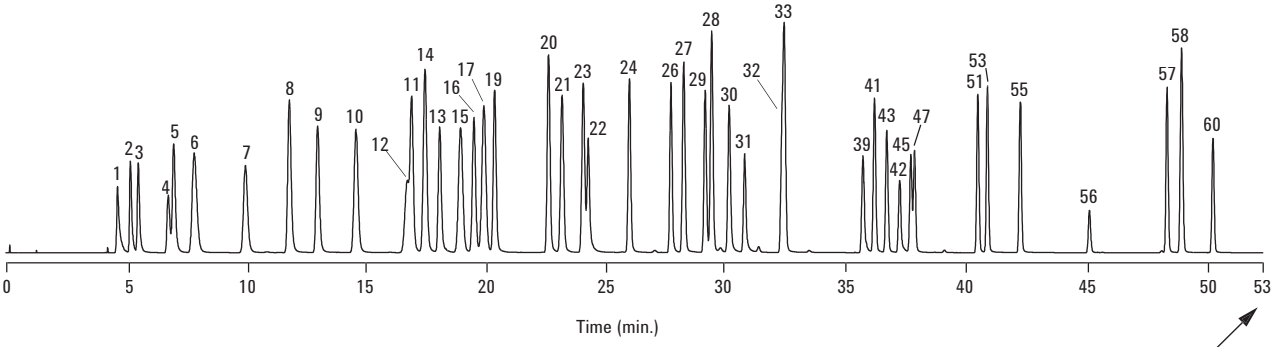
Compound List for all Chromatograms

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!

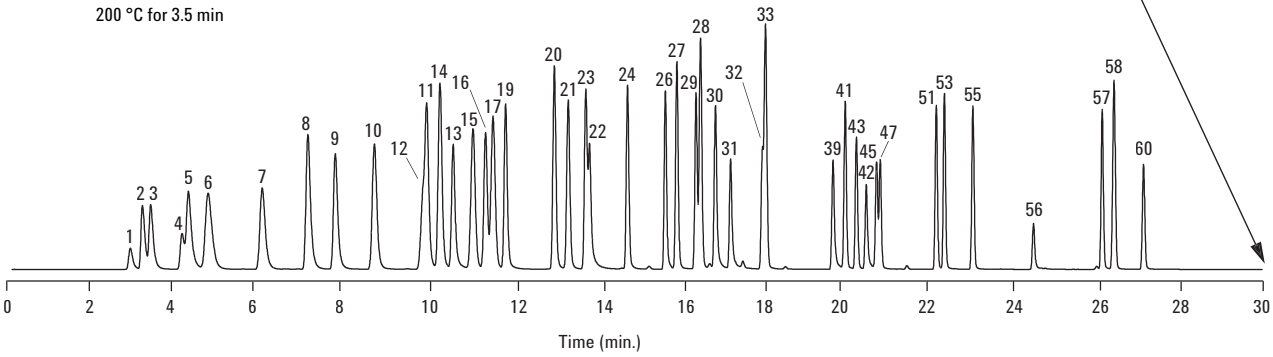


Figure 1. Analysis time comparison

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

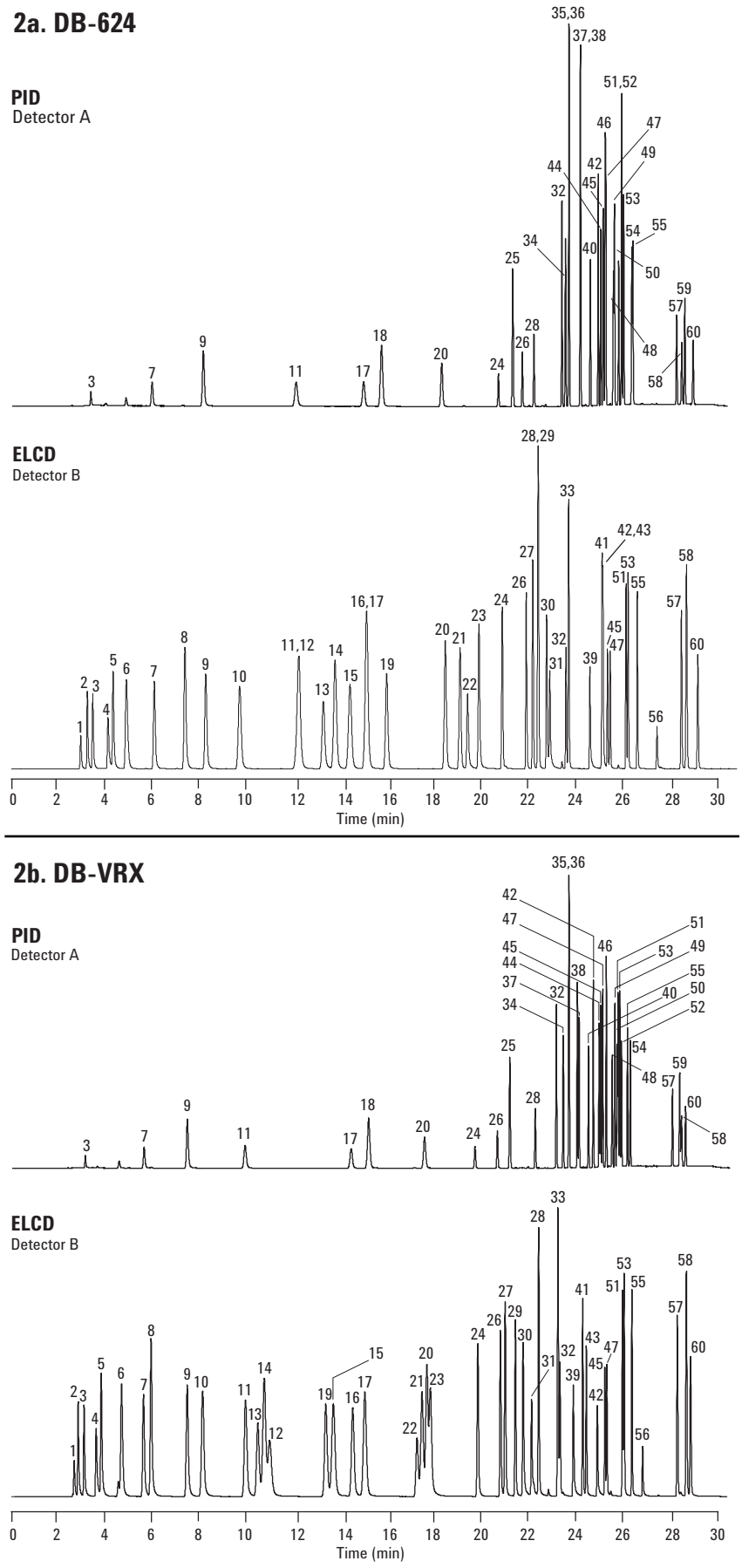


Figure 2a and 2b. High-Speed Megabore dual column applications.

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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Agilent Technologies

DuraGuard Columns: GC Columns with Built-In Protection

Application

Guard columns/retention gaps without the use of unions

- **Minimize front-end contamination of the column and increase column lifetime**
- **Aid in focusing sample onto the front end of the column for excellent peak shape**
- **Minimize the amount of mass selective detector (MSD) source contamination originating from the column**

All this with no leaks, no added activity, and no hassle

Deactivated fused silica tubing is commonly added to the front of an analytical column to act as a guard column or retention gap. It can also be added to the back of the analytical column as a transfer line into the MSD to minimize the amount of source contamination originating from the column.

Historically, deactivated tubing has been connected to the analytical column by using a union. These are difficult to install requiring a great deal of care and skill to ensure they will work properly. With incorrect installation unions can cause leaks resulting in column degradation, dead volume resulting in peak shape problems, or activity problems resulting in peak shape problems and/or response loss. Leaks are especially a problem when the union is located close to the MSD when using deactivated fused silica for the transfer line.

DuraGuard columns, with a built in length of deactivated fused silica tubing, avoid these potential problems. The deactivated fused silica and the analytical column are made with a single, continuous piece of fused silica tubing, thus eliminating the need for the union. Installation hassles, peak shape problems and leaks associated with unions are history. Samples containing difficult analytes such as pesticides or drugs can be chromatographed without any undesirable contributions from the union.

Guard Columns

DuraGuard columns are especially beneficial as guard columns when analyzing samples containing low levels of chemically active compounds. Unions can be active towards these analytes and can cause peak-shape problems, which in turn result in poor detection limits. DuraGuard columns eliminate the potentially active union by using a single piece of fused silica tubing. Agilent Technologies' special deactivation process results in extremely inert columns and tubing for a broad range of analyte types.

Guard columns are used when samples contain nonvolatile residues that contaminate the column. The nonvolatile residues deposit in the guard column and not in the analytical column. This greatly reduces the interaction between the residues and the sample since the guard column does not retain the solutes (because it contains no stationary phase). Also, the residues do not coat the stationary phase which often results in poor peak shapes. Periodic cutting or trimming of the guard column is usually required upon a build-up of residues. Guard columns 5–10 meters in length allow for substantial trimming before the entire guard column requires replacement. The onset of peak shape problems is the usual indicator that the guard column needs trimming or changing.

Retention Gaps

DuraGuard columns offer the user the benefits of a retention gap without the hassle of making critical clean column cuts and installing the fused silica tubing to the front of their analytical column with a union. By avoiding the union there are no additional sources of leaks or activity. The only difference is the improved peak shape of the analytes.

Retention gaps are used to improve peak shape for some types of samples, columns and GC conditions. Use of 3–5 meters of tubing is required to obtain the benefits of a retention gap. The situations that benefit the most from retention gaps are large volume injections (>2 µL) and solvent-stationary phase polarity mismatches for splitless, Megabore direct and on-column injections. Peak shapes are sometimes distorted when using combinations of these conditions. Polarity mismatches occur when the sample solvent and column stationary phase are very different in polarity. The greatest improvement is seen for the peaks eluting closest to the solvent front or solutes very similar to the solvent in polarity. The benefits of a retention gap are often unintentionally obtained when using a guard column.

MSD Transfer Line

DuraGuard columns help minimize source contamination without the potential for leaks. The vacuum system of the MSD makes it especially difficult to maintain a leak free system - particularly the closer the connection is to the MSD. The use of unions with Mass Spec Detectors has always been tricky and prone to leakage. By using a single piece of fused silica, there are no additional connections to cause leaks.

Using a piece of deactivated fused silica as the transfer line to an MSD can reduce the frequency of source cleaning. Often the MSD transfer line temperature is at or above the columns upper temperature limit and thermal degradation of the stationary phase occurs. Volatile polymer breakdown products are carried into the MSD and can deposit in the MSD ion source. Using deactivated fused silica tubing as the MSD transfer line eliminates the presence of polymer in the heated zone and decreases the amount of material that can contaminate the MSD source thus decreasing the frequency of required source cleanings.

Results

Figure 1 is an FID chromatogram of a complex test mixture separated using a combination DuraGuard column. Note the peak shape quality for notoriously difficult to analyze compounds.

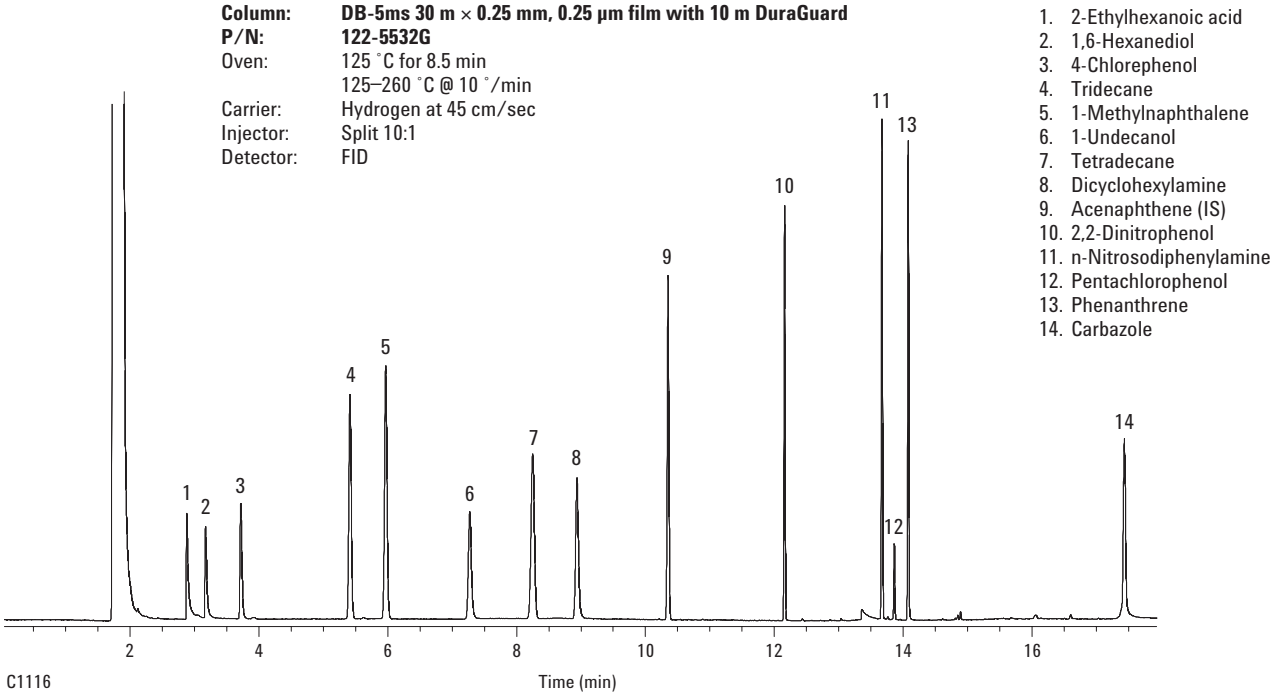

Figure 1. Chromatogram of test mixture using combination guard and analytical columns.

Want a Guard Column or Retention Gap of a Different Internal Diameter?

If you would prefer a guard column with a different diameter than your analytical column, save yourself the hassle of assembling union connections and let us do it for you! Agilent Technologies offers the dependable Leak-free connection service to meet your analytical needs: short guard columns, long guard columns, different diameters, or dual columns. Whatever you need, Agilent Technologies can provide through our Custom Column shop.

Our Leak-free connection service results in a dependable, long lasting leak-free connection. We use high quality glass press fit unions with polyimide sealing resin to ensure the connection will last. See Figure 2. At Agilent Technologies our technicians have years of experience in creating leak-free connections and in using special techniques to keep the polyimide sealing resin out of the flow path. Once the connection is carefully made, the resin is cured and the product is tested for leaks.

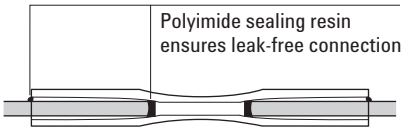

Figure 2. Detail of glass press fit union with polyimide sealing resin.

DuraGuard Column Order Guide

Part number	Phase	Inner diameter (mm)	Length (m)	Film thickness (µm)	DRGD Length (m)
122-1032G	DB-1	0.25	30	0.25	10
122-5532G	DB-5ms	0.25	30	0.25	10
122-5536G	DB-5ms	0.25	30	0.5	10
122-5533G	DB-5ms	0.25	30	1	10
122-5562G	DB-5ms	0.25	60	0.25	10
125-5537G	DB-5ms	0.53	30	0.5	10
122-1232G	DB-XLB	0.25	30	0.25	10
125-0732G	DB-1701	0.53	30	1	10
125-1334G5	DB-624	0.53	30	3	5

DuraGuard columns of different phases and dimensions are available through Agilent Technologies custom column shop. Any DB polysiloxane or low bleed phase can be made as a DuraGuard column with 0.18 mm id or larger fused silica tubing. Ask for a custom column quote (part number 100-2000 and specify the phase, id, length, and film thickness of analytical column, and desired length of DuraGuard).

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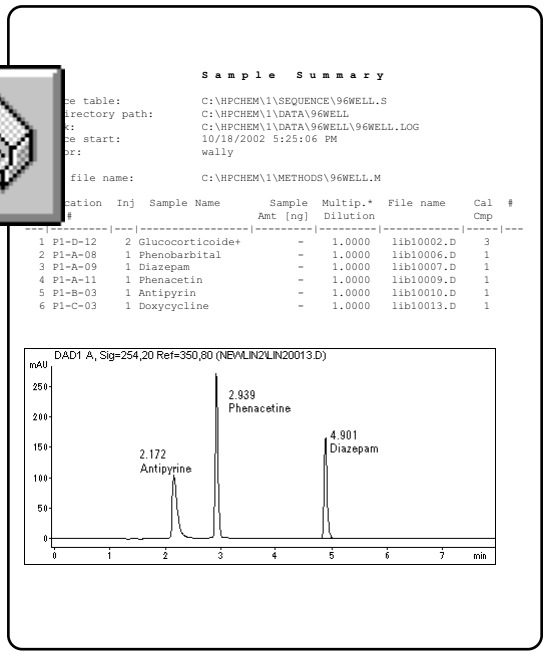
Agilent Technologies

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.

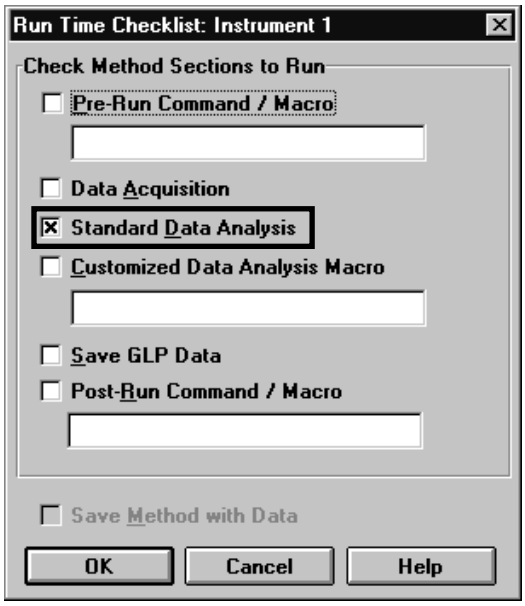


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

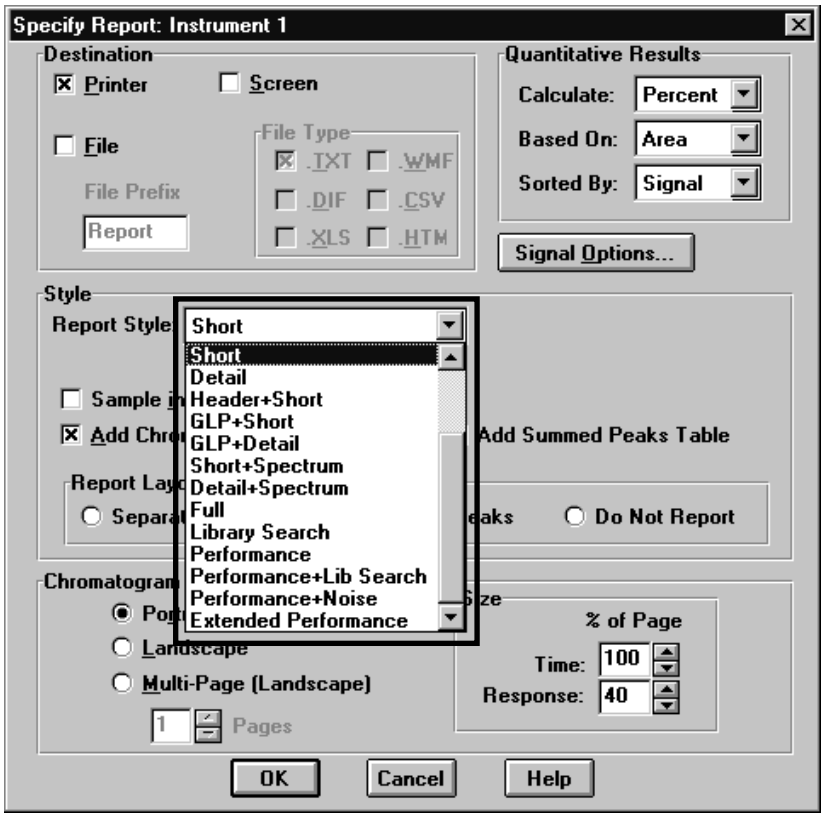


Figure 2
Specifying individual run reports

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.

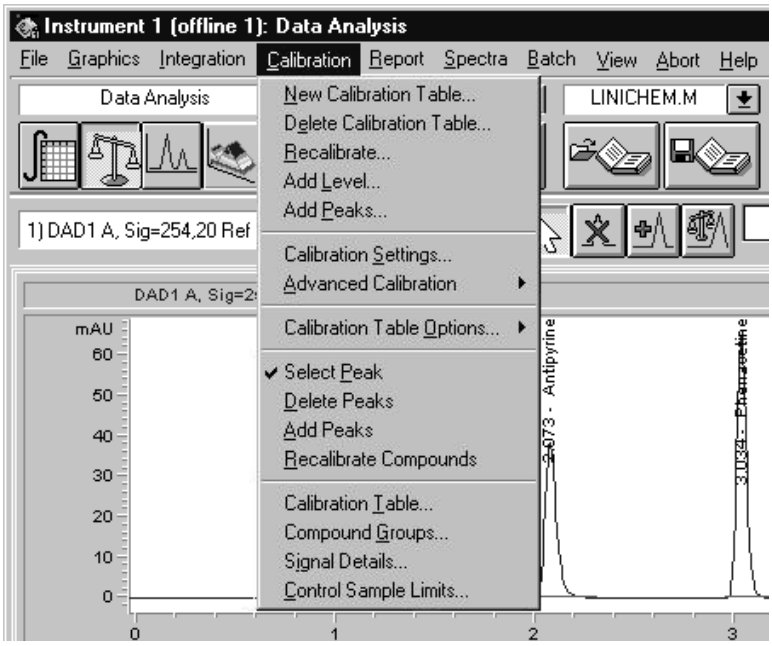


Figure 3
Calibration setup menu

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.

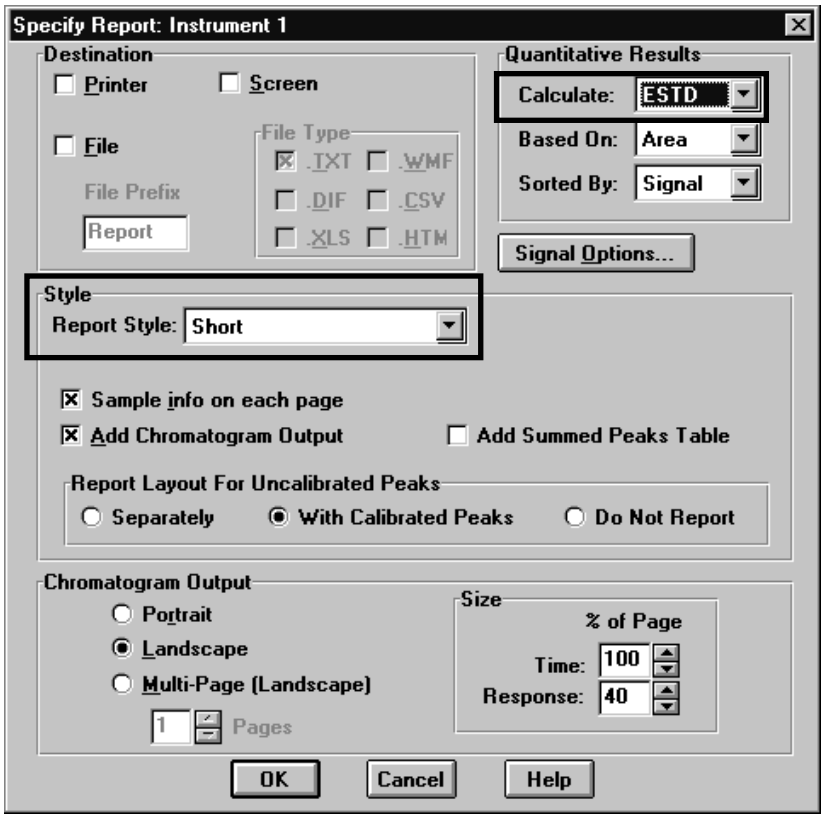


Figure 4
Selection of external standard report and short report style

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

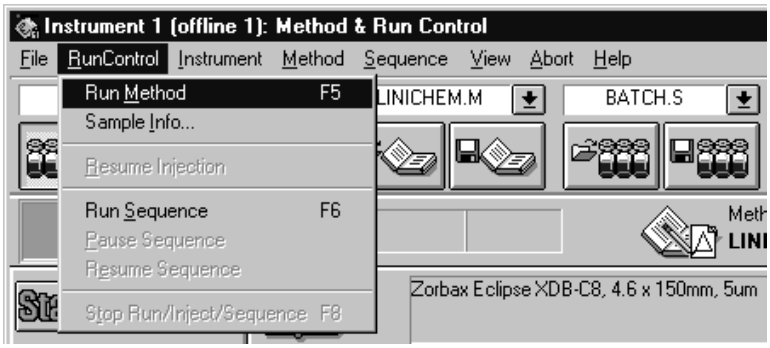


Figure 5
Run method for automated method execution and result output

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.

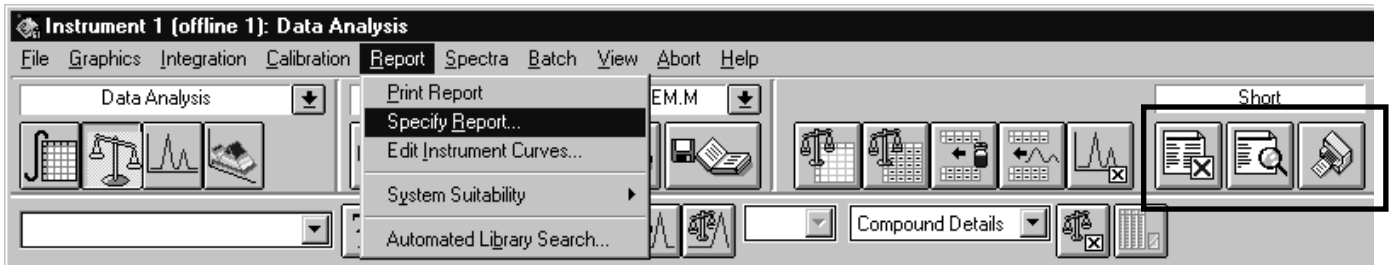


Figure 6
Report menu and smart icons (far right) in ChemStation Data Analysis view

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.

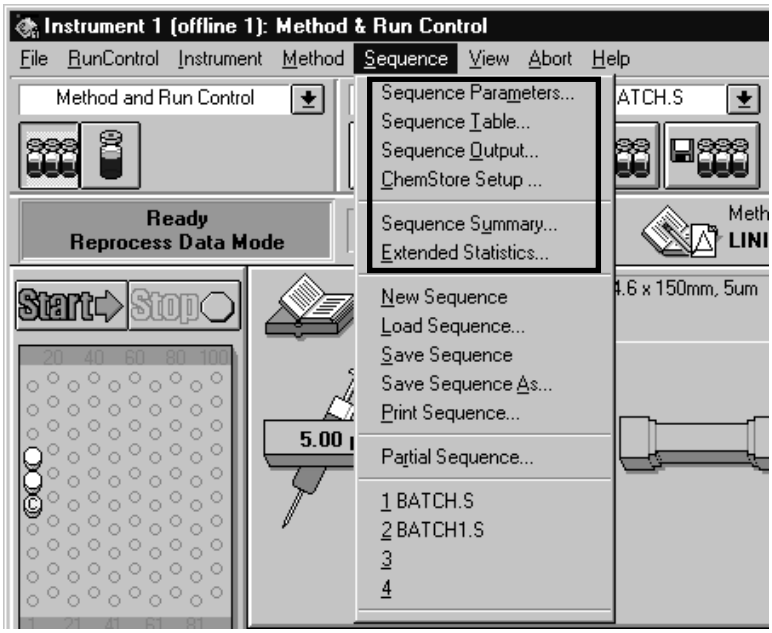


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

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-

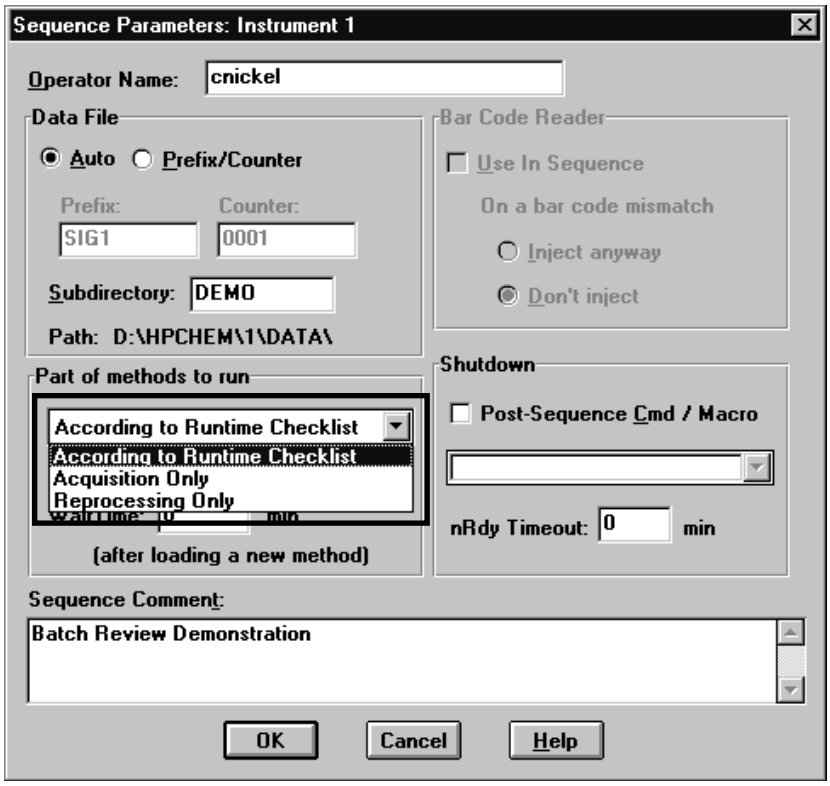


Figure 8
Sequence parameters screen

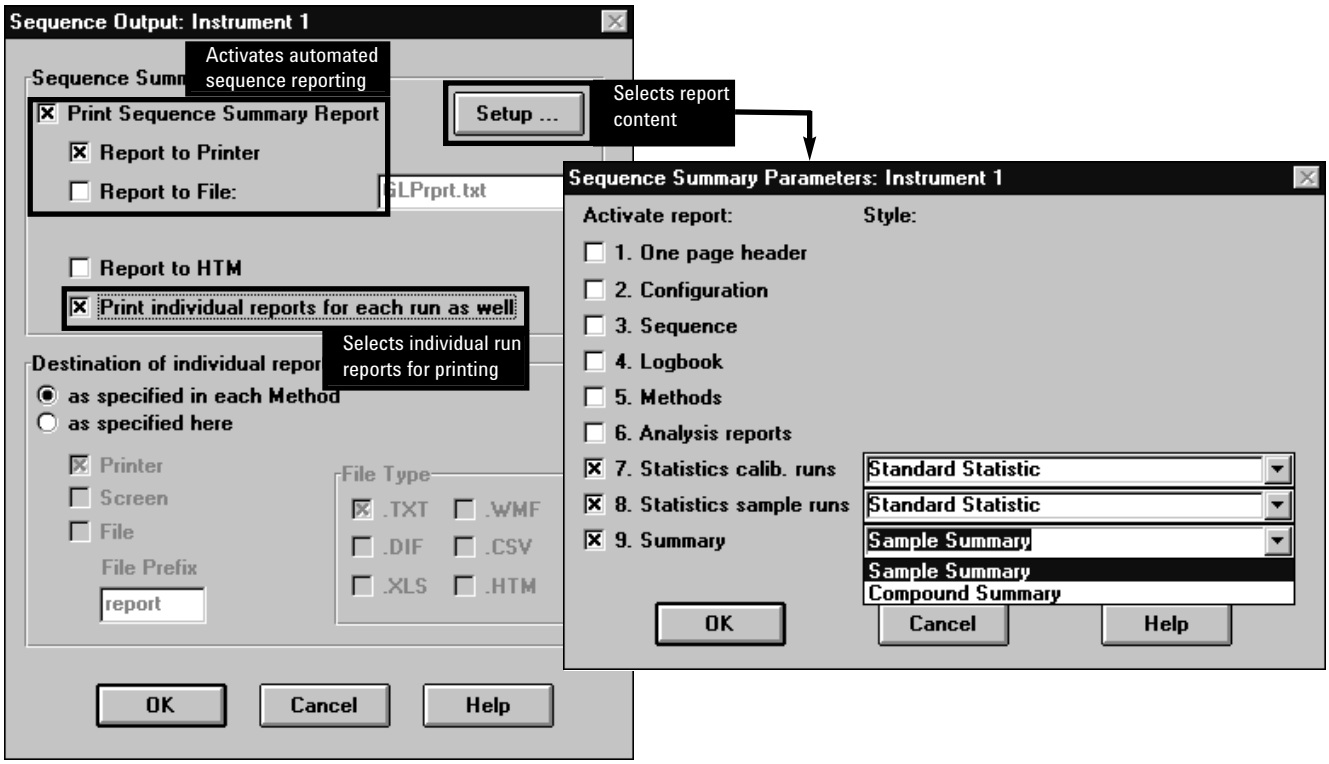


Figure 9
Selection of report destination and content of a sequence summary report

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.

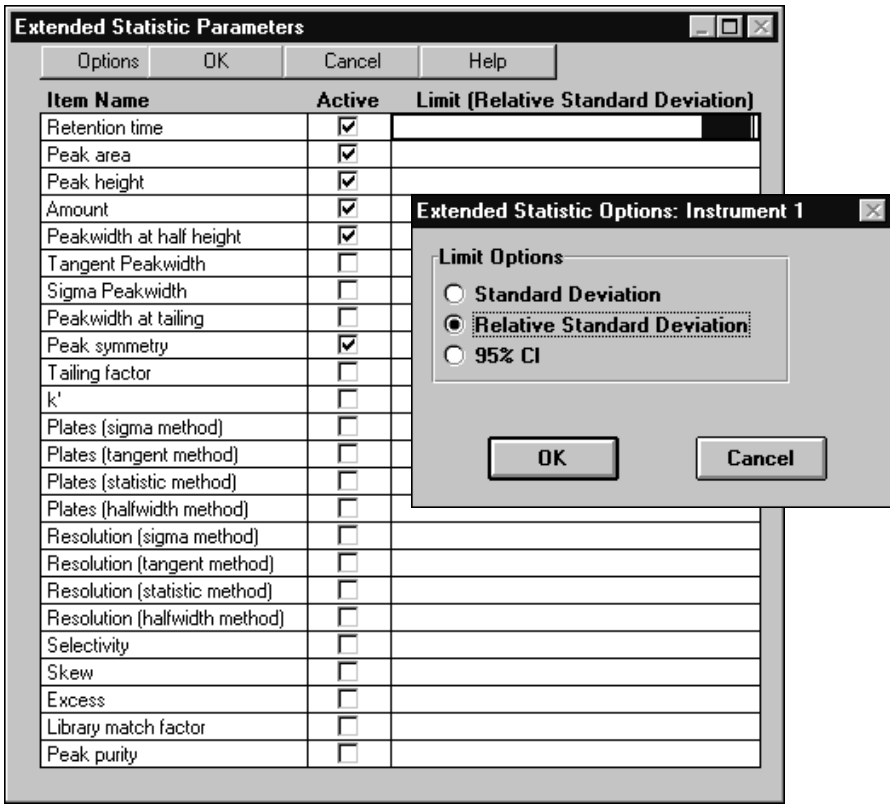


Figure 10
Setup of statistical calculations for sequence runs

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.

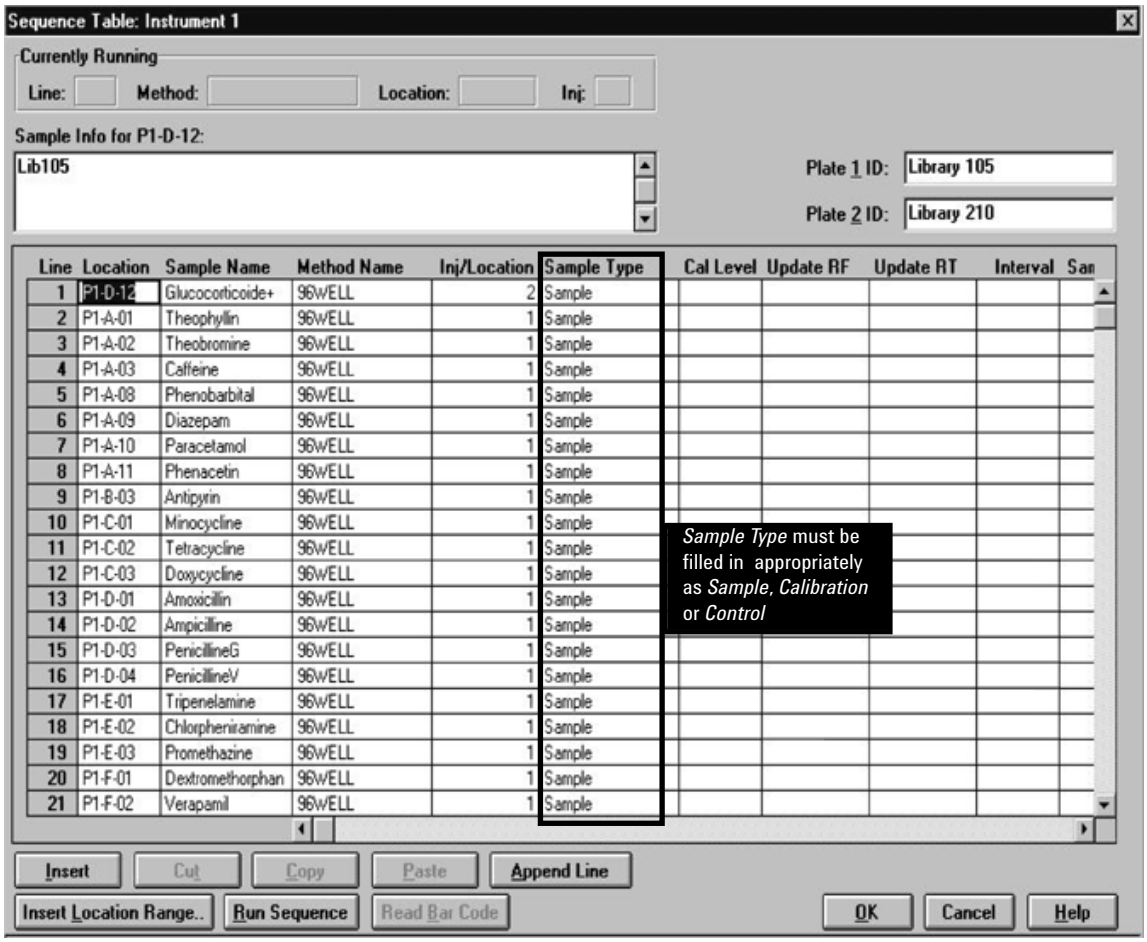


Figure 11
The Sequence Table screen

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 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	

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.

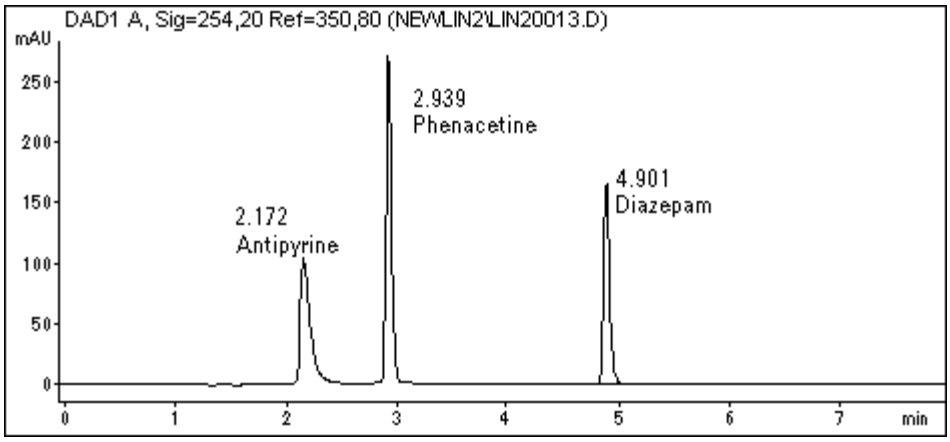
Appendix

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

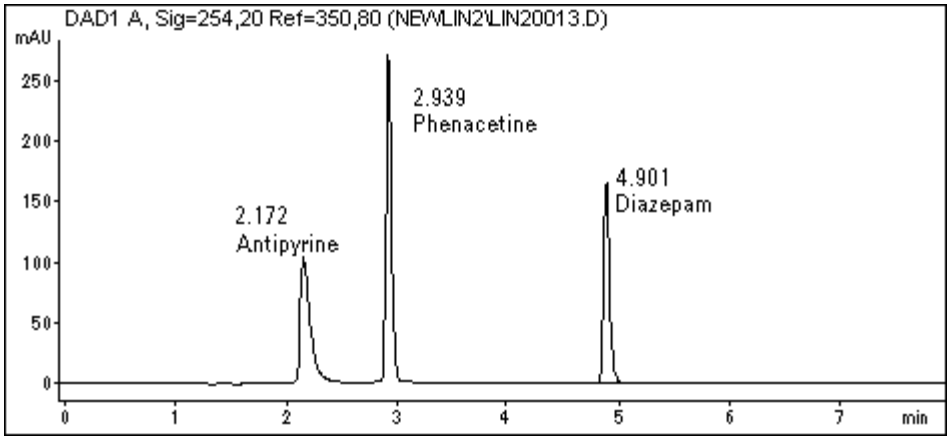
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 8:54:14 AM agratz

==================================================================
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

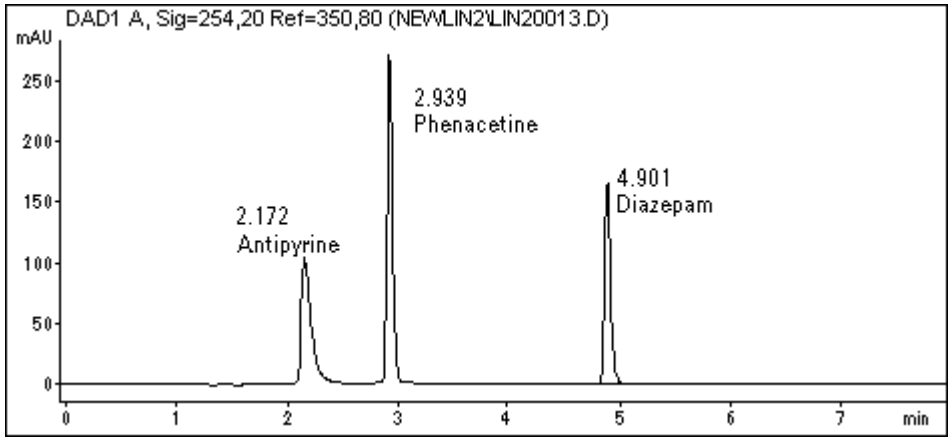
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:09:23 AM agratz

=====================================================================
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

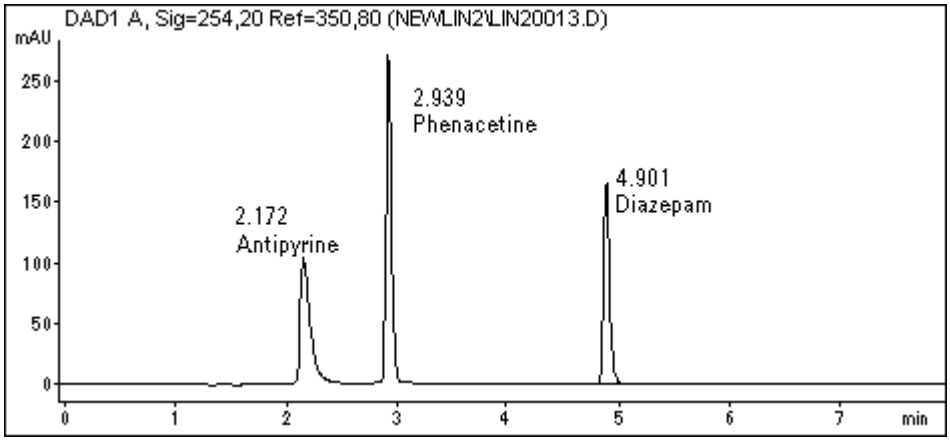
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:31:21 AM agratz

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)

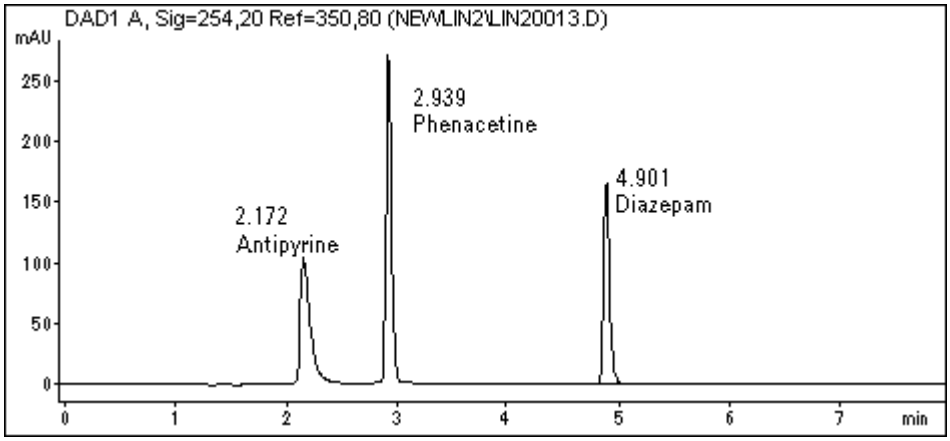
```
                                Run Logbook
=====================================================================
Method        Method started:  line# 7 vial# 2 inj# 1    10:46:18 10/25/00
Method        Instrument running sample Vial 2           10:46:18 10/25/00
1100 ALS    1 Air temperature (tray) = 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

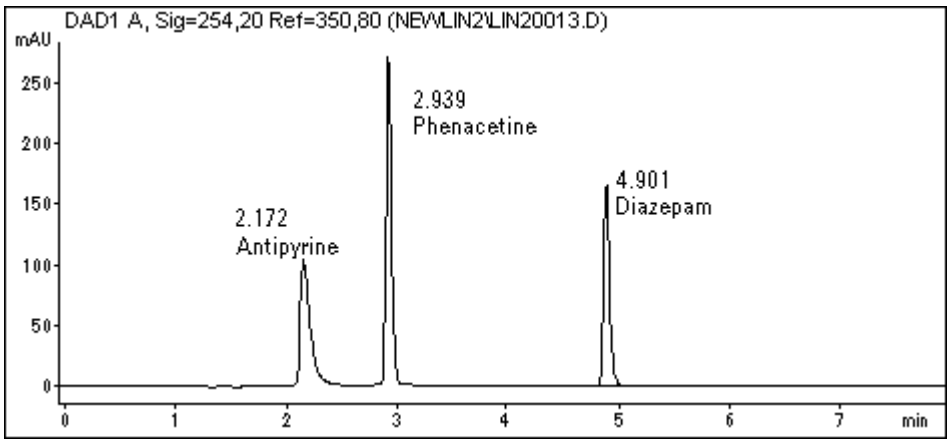
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:36:38 AM agratz

================================================================
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 ***
```

Detail report

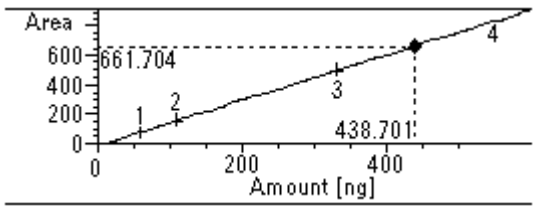
```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:51:47 AM agratz

================================================================
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

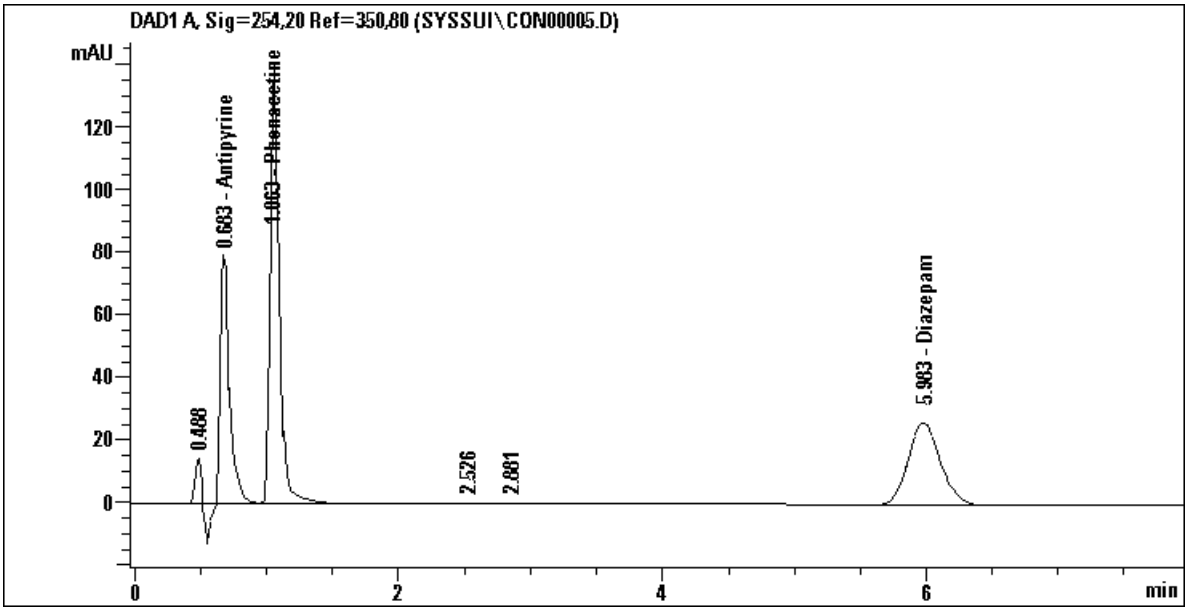
```
======================================================================
                      *** End of Report ***
```

Extended Performance Report

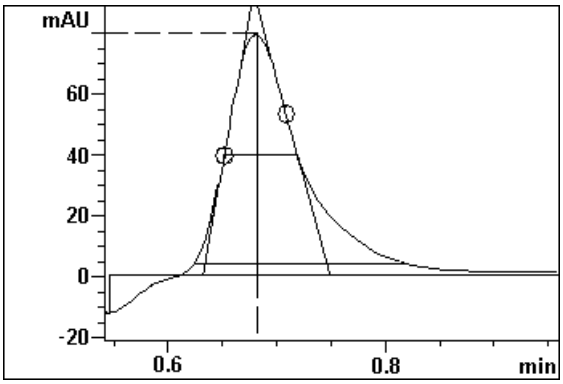
```
Data File D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D

                  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
```

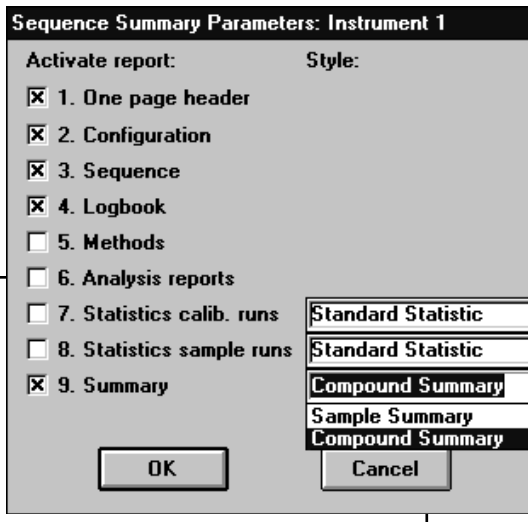


Extended Performance Report (continued)

```
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

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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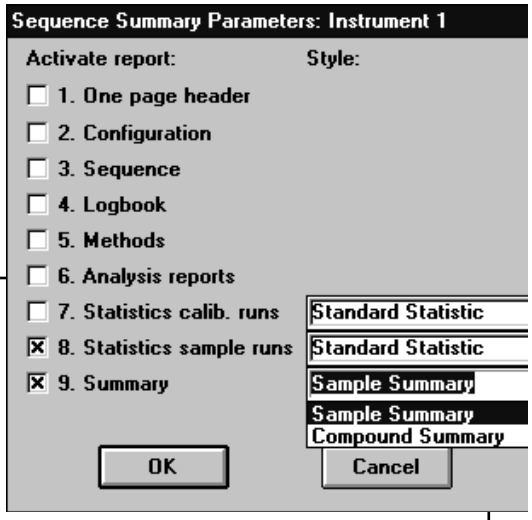
*** End of Report ***

Sequence Summary Report – Standard Statistics for Sample Runs

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                    S t a t i s t i c    R e p o r t

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Operator:                agratz

Method file name:        D:\HPCHEM\1\METHODS\LINI2.M
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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

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                  S a m p l e    S u m m a r y


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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  #
---|--------|---|----------------|----------|--------|----------|-|---|---
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  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  -


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                           *** End of Report ***
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www.agilent.com/chem/nds

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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

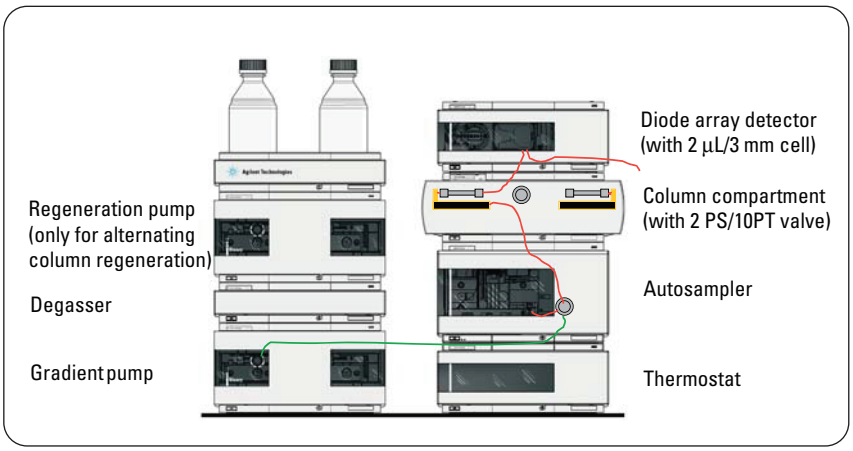

Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

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.

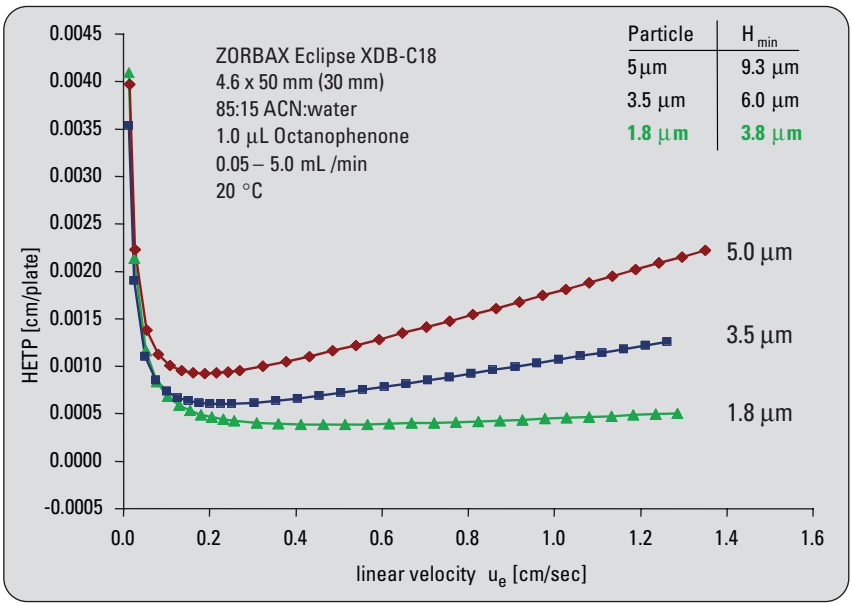

Figure 2
Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.

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).

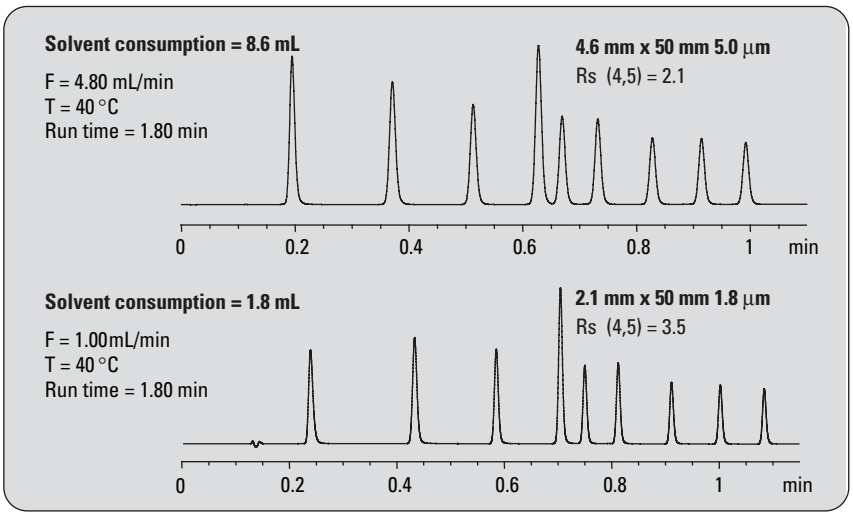

Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

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)

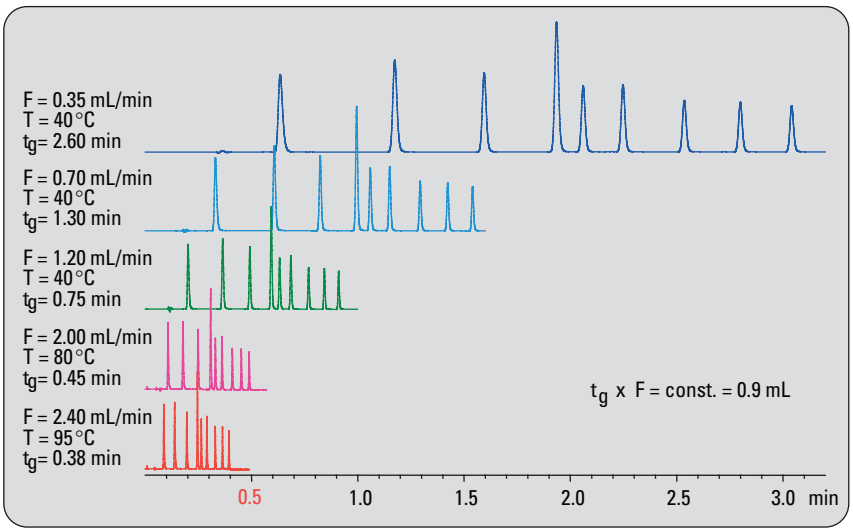

Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

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

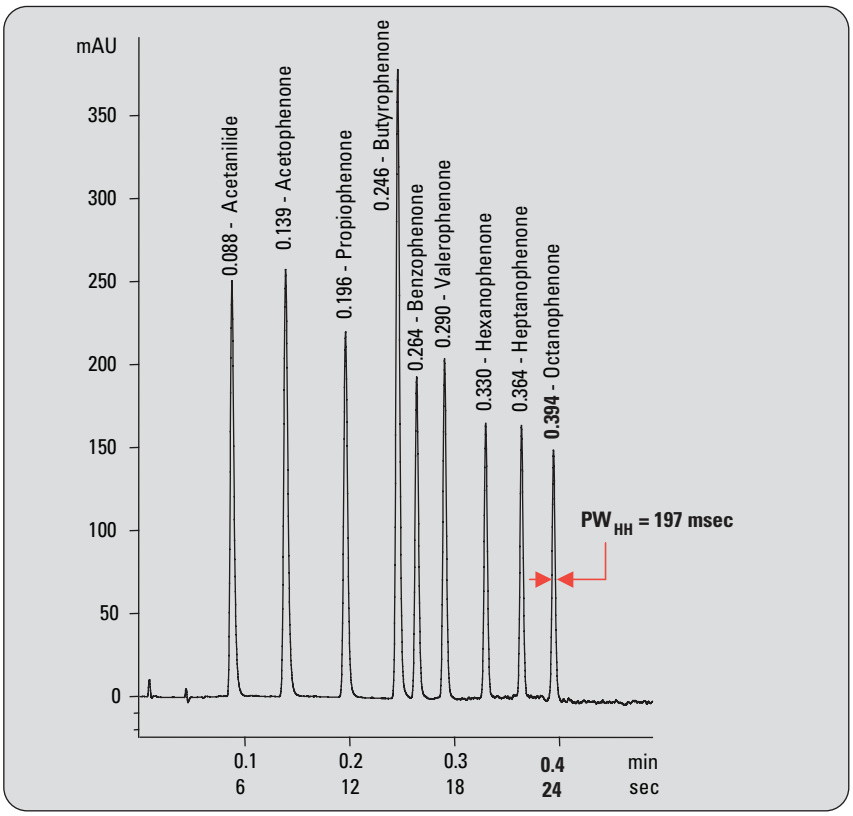

Figure 5
Separation of a nine compound mixture under ultra fast conditions.

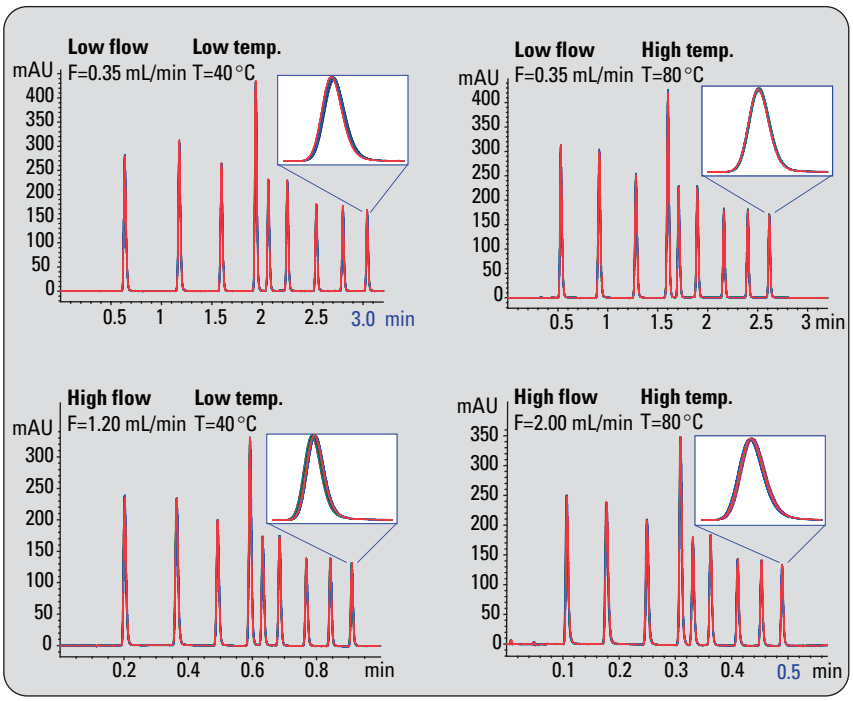

Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

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.

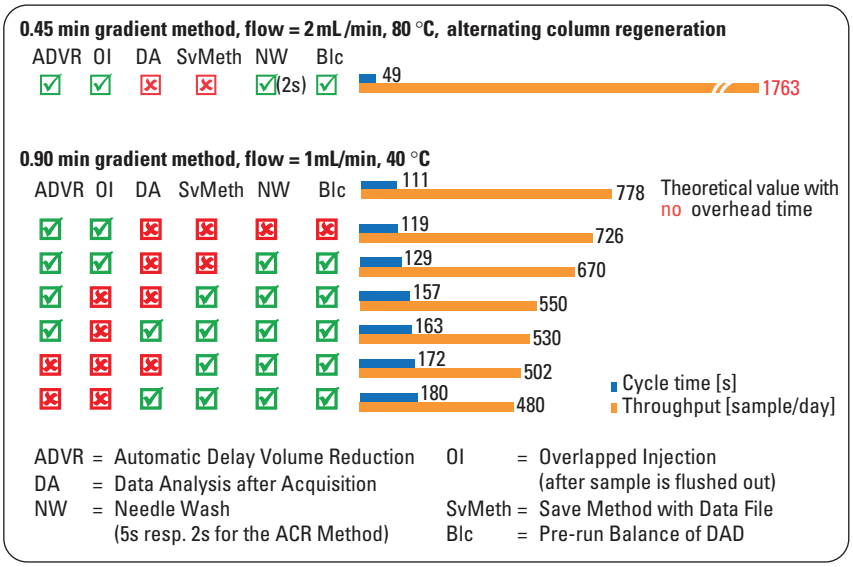

Figure 7
Cycle time and daily throughput optimization.

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.

References

1.

Jeremy R. Kenseth, Shelly J. Coldiron, "High-throughput characterization and quality control of small-molecule combinatorial libraries", *Curr. Opin. Chem. Biol. 8; 418-423*; **2004.**

Jill Hochlowski, Xueheng Cheng, "Current Application of Mass Spectrometry to Combinatorial Chemistry", *Anal. Chem. 74, 2679-2690;* **2002.**

2.

R. Kostiainen, et al., "Liquid chromatography/atmospheric pressure ionization-mass spectrometry in drug metabolism studies", J. *Mass Spectrom.*, *38*, *357-372*; **2003.**

Garry Siuzdak, et al., "The application of mass spectrometry in pharmacokinetics studies", *Spectroscopy 17 681-691;* **2003.**

3.

Udo Huber, „High throughput HPLC – Alternating column regeneration with the Agilent 1100 Series valve solutions" A*gilent Application Note*, *Publication number 5988-7831EN;* **2002.**

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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.

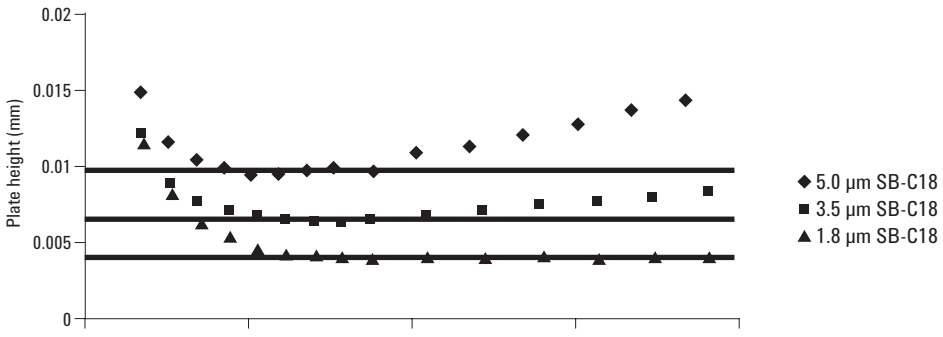
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

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

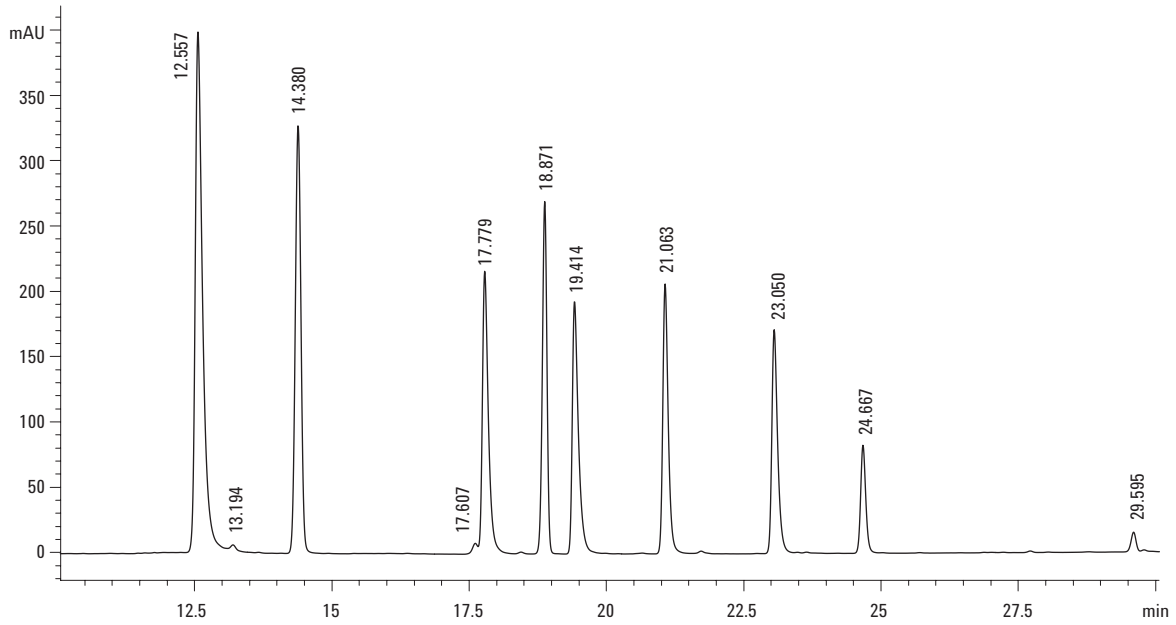
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

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

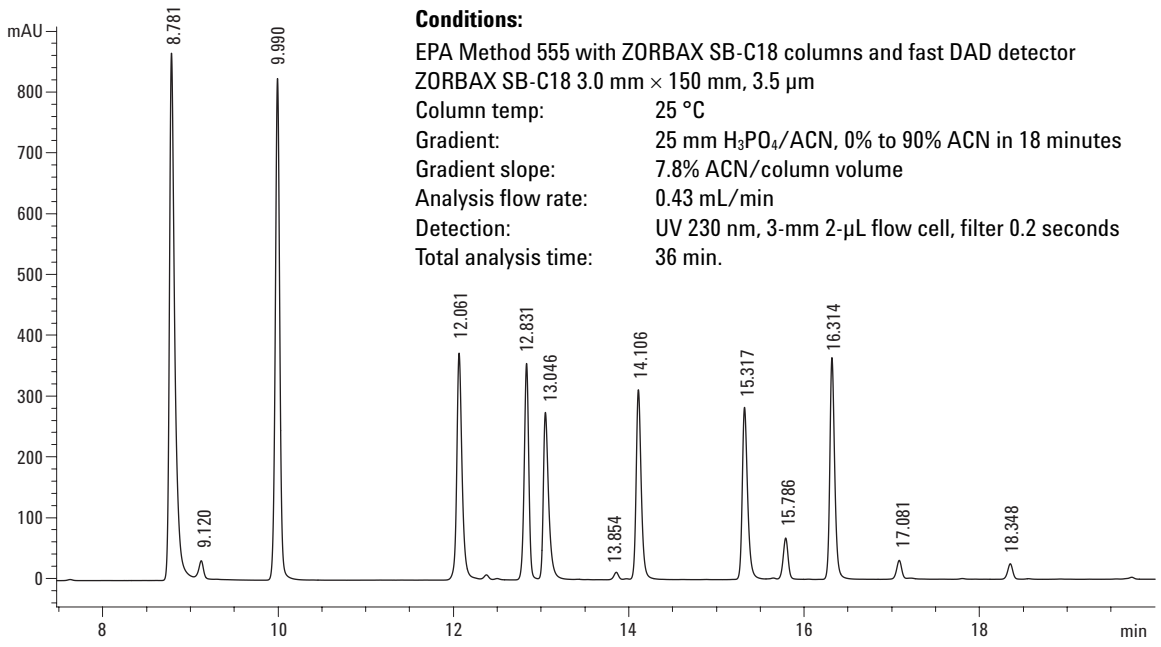


Conditions

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.

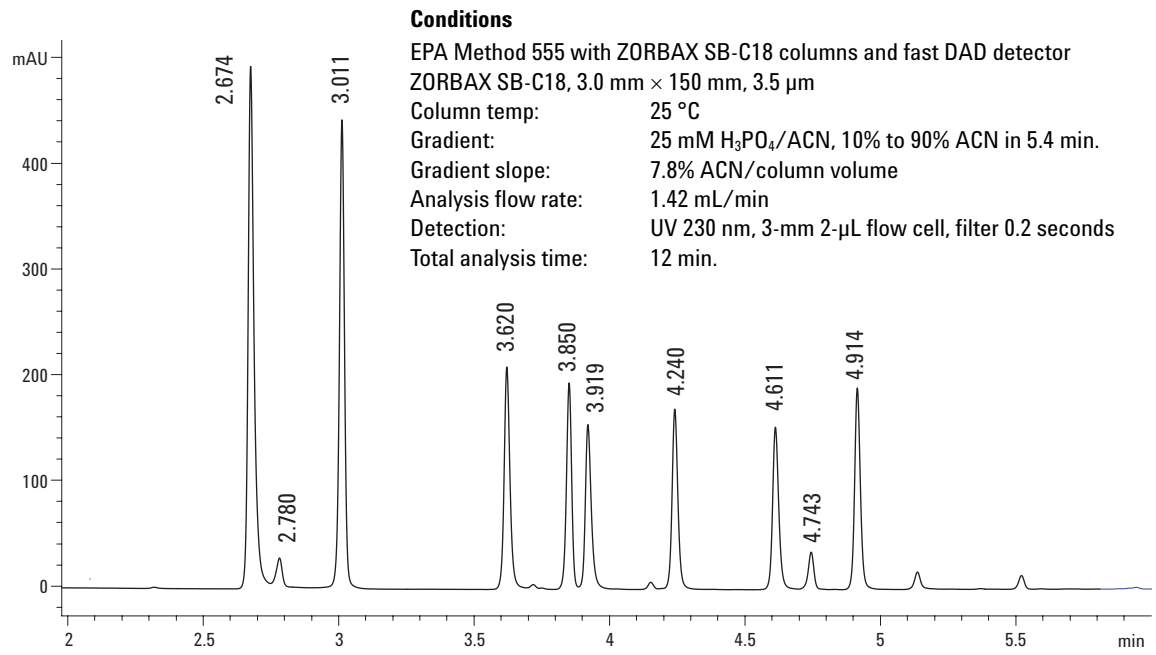


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

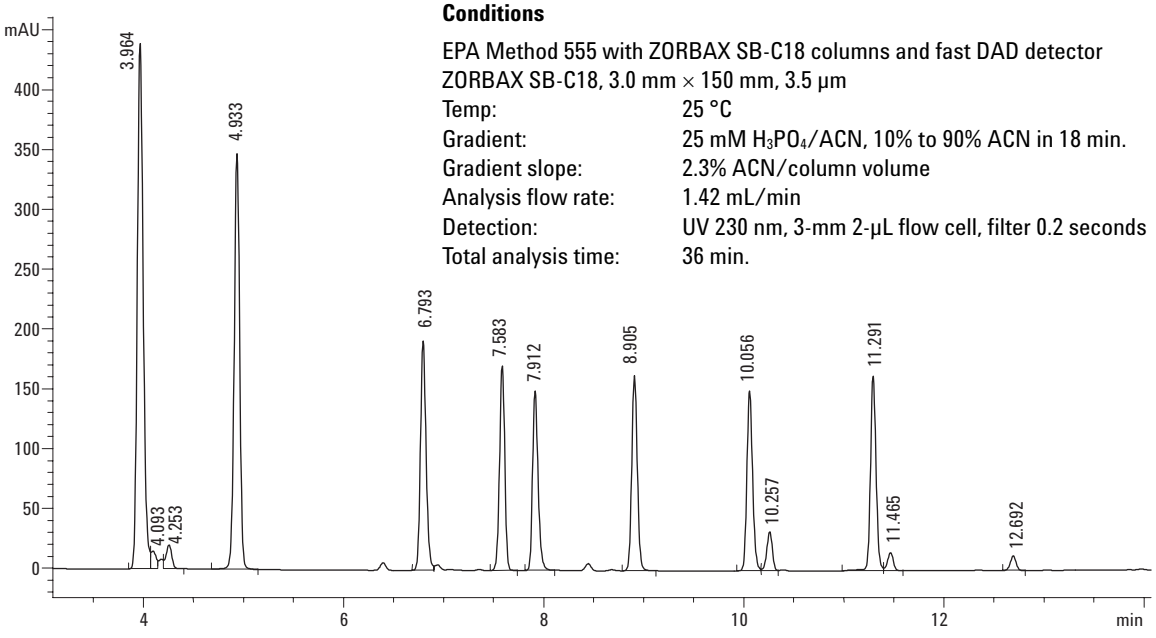


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

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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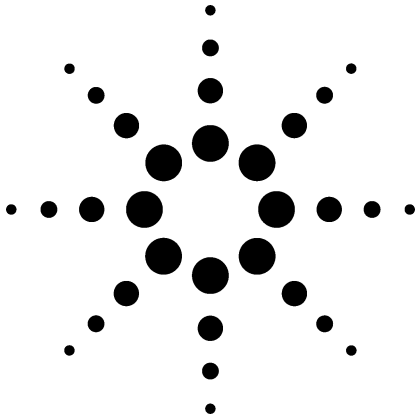
Determining the Ester and Linoleic Acid Methyl Ester Content to Comply with EN14103

Application



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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.

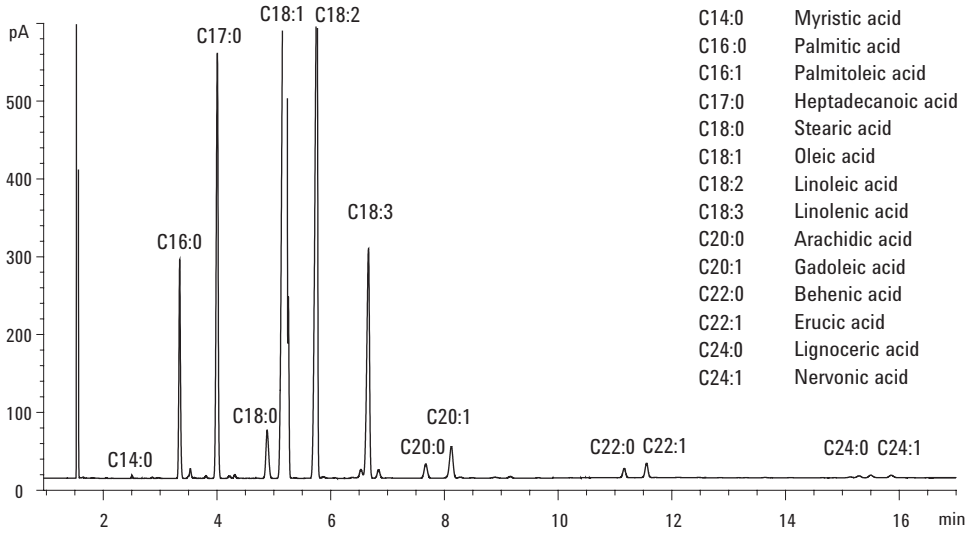

Figure 1. Chromatogram of rapeseed methyl esters.

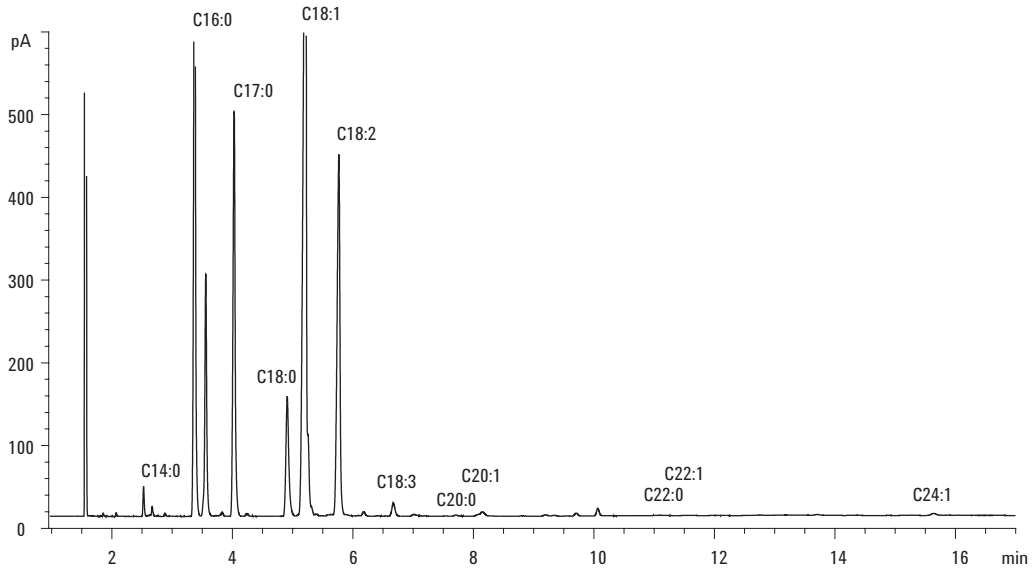

Figure 2. Chromatogram of pork methyl esters.

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

1. EN14103, "Fat and oil derivatives. Fatty acid methyl esters (FAME) Determination of ester and linolenic acid methyl ester contents."
2. ASTM D6751-03, "Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels."

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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

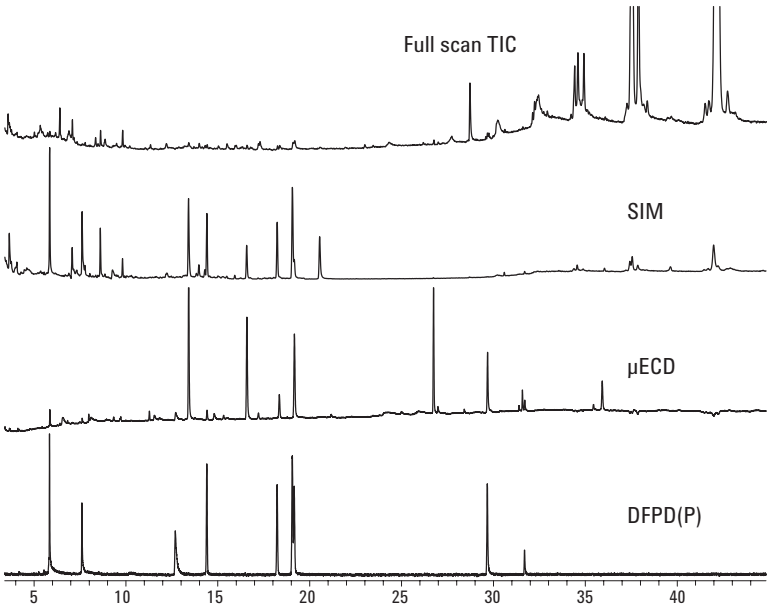

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

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.

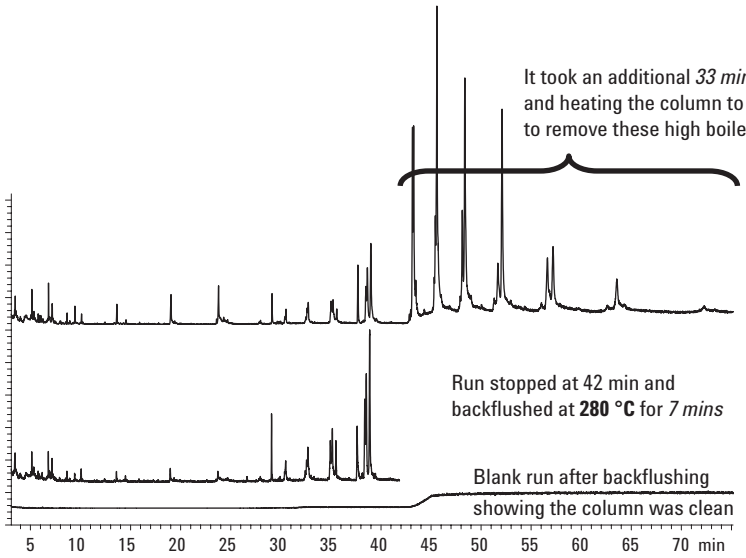

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Agilent Application Note, 5989-3299EN, July 2005.
2. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Application Note, 5989-5111EN, June 2006.

Acknowledgement

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A Column-Flow Independent Configuration for QuickSwap

Application

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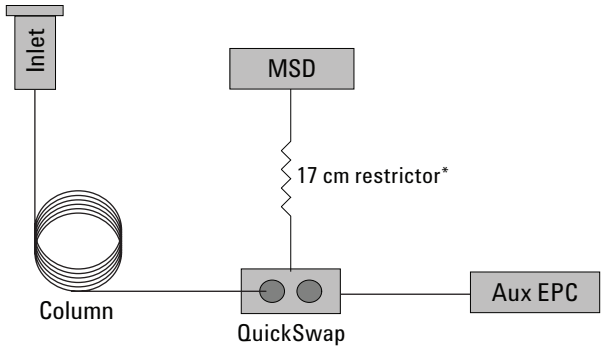
Abstract

A flexible configuration of QuickSwap is presented that allows use of larger id columns, pressure pulse injections, and variable column flow rates without having to change the restrictor or QuickSwap pressure. The split configuration can be set up such that the MSD is run at optimal flow rate. Examples are presented for several different columns and experimental conditions.

Introduction

QuickSwap is a recently introduced Capillary Flow Technology device designed to improve the usability of GC/MSD systems. It allows you to change columns and do inlet maintenance without venting the mass spectrometer. It also facilitates use of the backflush technique. The basic concepts, benefits, and use of QuickSwap are described in several Agilent Technologies publications [1-4] and are illustrated in Figures 1 and 2.

As can be seen from Figure 1, if the column is disconnected from QuickSwap, a flow of inert gas from the Aux EPC will prevent air from entering the MSD.



*QuickSwap restrictor, P, and T are selected for desired flow to MSD, usually the maximum flow that the current application requires.

Figure 1. General concept of QuickSwap.

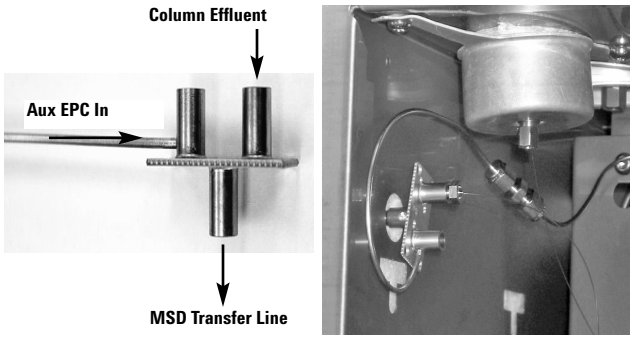


Figure 2. QuickSwap is pictured on the left showing permanent (Aux EPC In) and temporary connections. A picture of a normal QuickSwap installation is shown on the right.

In the standard configuration of QuickSwap, you must determine before installation what the maximum expected flow will be from the analytical capillary column being used. This value is in turn used to select the proper restrictor size (the four available sizes are 92 µm, 100 µm, 110 µm, and 120 µm id), the transfer line temperature, and QuickSwap pressure.

If the flow from the analytical column exceeds that originally planned for, then the pressure at QuickSwap will exceed its setpoint and the GC will go "not ready." This can happen if you do any of the following:

- Do pressure pulse injections, wherein the flow during injection is typically two to three times that during the run
- Increase column flow rate, as you might do when doing a method speed-up with method translation
- Do a retention time locking calibration, where inlet pressure is increased 20% over the nominal pressure
- Change to larger-dimension columns

In these examples, you would need to increase QuickSwap pressure and/or lower restrictor temperature or cool the system and install a new restrictor in order to accommodate the higher flows.

On the other hand, if you were to use a restrictor that allowed excess flow to the MSD, method performance (for example, detection limit and linear dynamic range) might be worse. So, it is important to plan carefully when using the normal QuickSwap configuration to get the right balance in performance and usability.

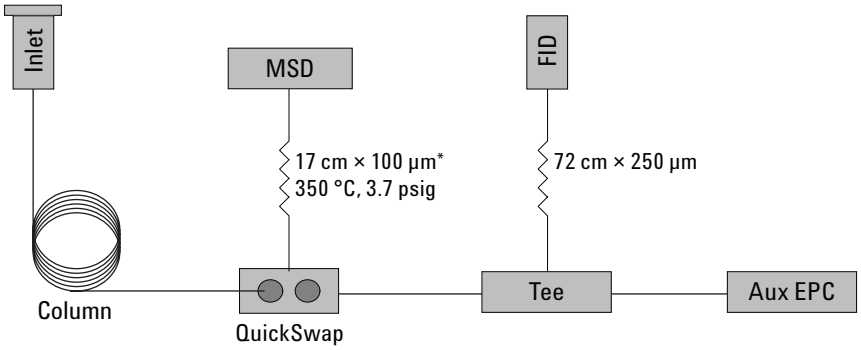
In general, when flow to the MSD changes,

- Tune parameters can change
- Response can change
- S/N and limit of detection can change

An alternate configuration was conceived of that allows the MSD to be run at optimal flow rate and improves flexibility and usability of QuickSwap [QS] in a wider range of potentially useful situations. This configuration incorporates a split between the Aux EPC module and QS and is illustrated in Figure 3.

This configuration has several advantages over the standard configuration. It:

- Simplifies initial setup (restrictor choices)
- Simplifies changes to existing methods



*In this example, the restrictor, transfer line temperature, and QuickSwap pressure were chosen to allow approximately 1 mL/min flow to the MSD—corresponding to its optimal performance regime.

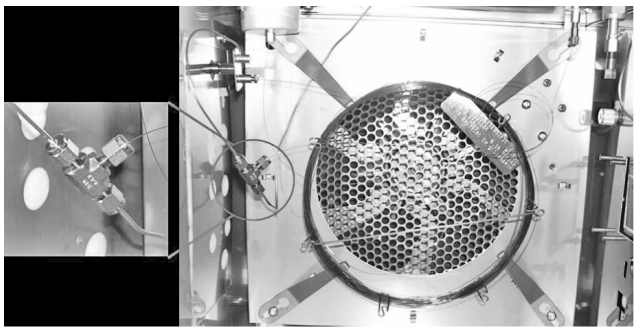
Figure 3. Flexible configuration includes addition of a split vent path on the Aux EPC line leading to QuickSwap.

- Simplifies retention time locking applications with QS
- Allows pressure pulse injections without having to change QS restrictor
- Allows more aggressive backflush conditions than if larger restrictors were used
- Allows method translation and speed up without having to change QS restrictor
- Allows use of medium- and large-bore columns with MSD

In some applications, there are some valid reasons why you might consider larger-bore capillary columns. These include:

- Higher sample capacity (solvent peaks don't tail as much, polar solutes don't front as much)
- Better robustness (better able to handle dirty samples)
- More amenable to large-volume injections—especially the solvent vapor exit version
- Less problematic cool on-column injections (more rugged larger id needles can be used)

However, the problem of higher flow rates associated with larger id columns has limited applications in GC/MS. MSD users are probably aware that there is an optimum flow above which MSD performance degrades. For most MSDs with electron impact sources and standard drawout lenses, optimal performance coincides with a flow rate range of 1 to 1.5 mL/min. Above that, signal and S/N fall approximately linearly with respect to flow rate increases.



Experimental

An 80-ppm mixture of semivolatiles and surrogates was selected based on a validated "fast" USEPA 8270 method [5]. A reference chromatogram is shown in Figure 4.

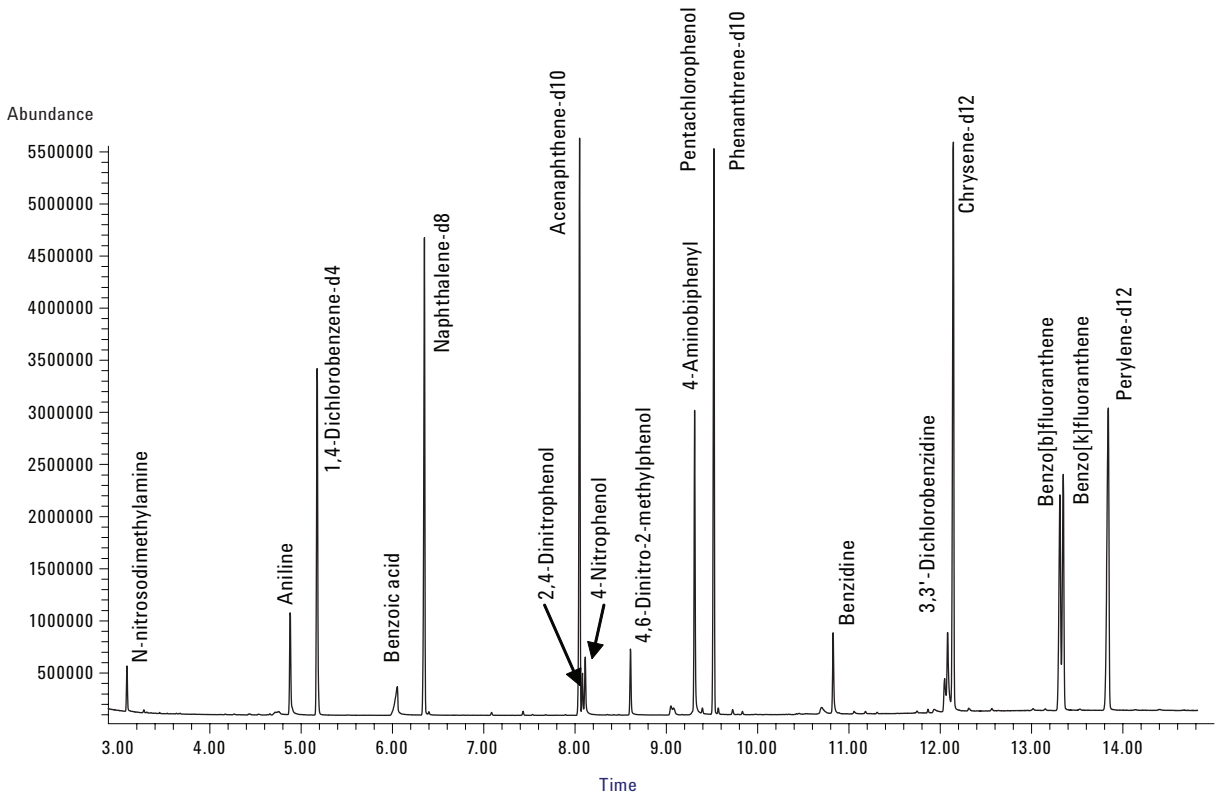


Figure 4. Reference chromatogram for Fast 8270 method.

Restrictor and setpoints were chosen for the flexible split configuration such that approximately 1 mL/min would go to the MSD. Several different combinations of QuickSwap restrictor and setpoints could be used to yield a flow rate in the optimal range for MSD with EI source. These are listed in Table 1.

Table 1. Restrictor and Setpoint Combinations Corresonding to the Optimal Flow Rate Range of the MSD

QuickSwap restrictor id (µm)	QuickSwap pressure (psig)	Transfer line temperature (°C)	Flow to MSD (mL/min)
92 (G3185-60361)	4.0	250	1.0
92	4.0	195	1.2
100 (G3185-60362)	3.7	350	1.0
100	2.7	250	1.2
110 (G3185-60363)	0.5	350	1.0
110	1.4	325	1.2

Referring back to Figure 3, now let's examine the flexible QuickSwap configuration in more detail. In this study, the 1/16-inch Swagelok union connecting the line from QuickSwap to that coming from the Aux EPC was replaced with a stainless steel tee (refer to the parts list). To the third leg of the tee, a restrictor was added leading to a flame ionization detector (FID) to allow monitoring of vented material. In an alternate configuration, one can put the tee outside the oven by cutting the Aux EPC tubing on the top of the GC, and then plumb the restrictor to a separate split vent trap (such as that used to trap vented sample on the split/splitless inlet; refer to the parts list). This configuration is recommended to capture potentially noxious sample components that are vented if an FID is not being used to combust them. The split vent trap cartridge is also easily replaced with a fresh one if and when it is necessary.

The dimensions of the vent restrictor is not as critical as the one used for QuickSwap. The vent flow rate needs to be more than that reasonably expected for the analytical column used and experiments to be conducted. However, there is little downside to using a restrictor with "moderately excessive flow," except that one is wasting clean purge gas from the Aux EPC. In this example, the restrictor was chosen to yield approximately 10 mL/min at the initial oven temp (50 °C) and QuickSwap pressure (3.7 psig).

For experiments where the column flow is less than the 1 mL/min nominal flow to the MSD, makeup gas would be supplied by the Aux EPC to make up the difference and pure purge gas would vent through the FID. In those cases where the column flow exceeds 1 mL/min, the excess would back up the Aux EPC line to the tee, where it will mix with the purge gas and be vented to the FID and detected. In effect, any flow > 1 mL/min is vented while the flow to the MSD remains constant at its optimum.

To test the flexibility of this configuration, several different sizes of columns and several different flow rates were examined using the same semivolatiles sample used earlier. The columns and conditions are listed in Table 2. Again, constant pressure mode conditions were chosen to yield approximately the same void times for the three different columns so that solute retention times would be similar. Later, other flows were tried as were constant flow modes.

Table 2. Conditions for Constant Pressure Mode Experiments (Void times nominally matched at 1.239 min. Conditions: Oven program: 50 °C (1 min) → 350 °C (3 min) @ 20 °C/min; QuickSwap restrictor = 17 cm x 100 µm id at 3.7 psig and 350 °C, yielding 1.0 mL/min flow to MSD; 0.5 µL splitless injection with a 2-min purge delay, inlet at 275 °C)

Dimensions	Head pressure	Initial flow (@ 50 °C)	Ending flow (350 °C)	Relative capacity
20 m x 180 µm	20.5 psig	0.70 mL/min	0.23 mL/min	1 X
30 m x 250 µm	23.4 psig	2.18 mL/min	0.72 mL/min	2.2 X
30 m x 530 µm	7.93 psig	6.85 mL/min	2.26 mL/min	18 X

The results of the comparison are shown in Figure 5. Several points are worth stating.

1. Columns were quickly switched without venting the MSD (a key benefit of QuickSwap).
2. No pump down, retuning, or equilibration time were required prior to applying new pressure setpoints and acquiring data for the different columns.
3. The retention times are approximately the same on each column—a result of determining the setpoints that would yield the same void time.
4. Peak widths, shapes and heights reflect a composite of chromatographic phenomena such as relative stationary phase capacities, column efficiencies, deviation of actual flow from optimal flow, and the amount of post-column split to vent. For example, one might think that the 180-µm id column should have the narrowest peaks (highest efficiency); however, one can see from Table 2 that the flow rate decreases from the optimal flow rate of 0.7 mL/min at the start of the run to well below that at the end. This will cause peaks to be wider than they would be at optimal flow. In contrast, the flow rate of the 250-µm id column starts higher than the 1 mL/min optimal flow but remains at an optimal or faster-than-optimal rate for most of the run. This will cause the peak widths for the 250-µm id column to be narrower than that of the 180-µm id column.
5. The benzoic acid peak (#4) is less distorted on the 530-µm id column as a consequence of the larger column capacity. This is one of the benefits of using larger id columns.
6. The relative elution order is the same for the three columns. This is a consequence of matching void times and using constant pressure mode. This would not be the case when using constant flow mode (see Figure 7).

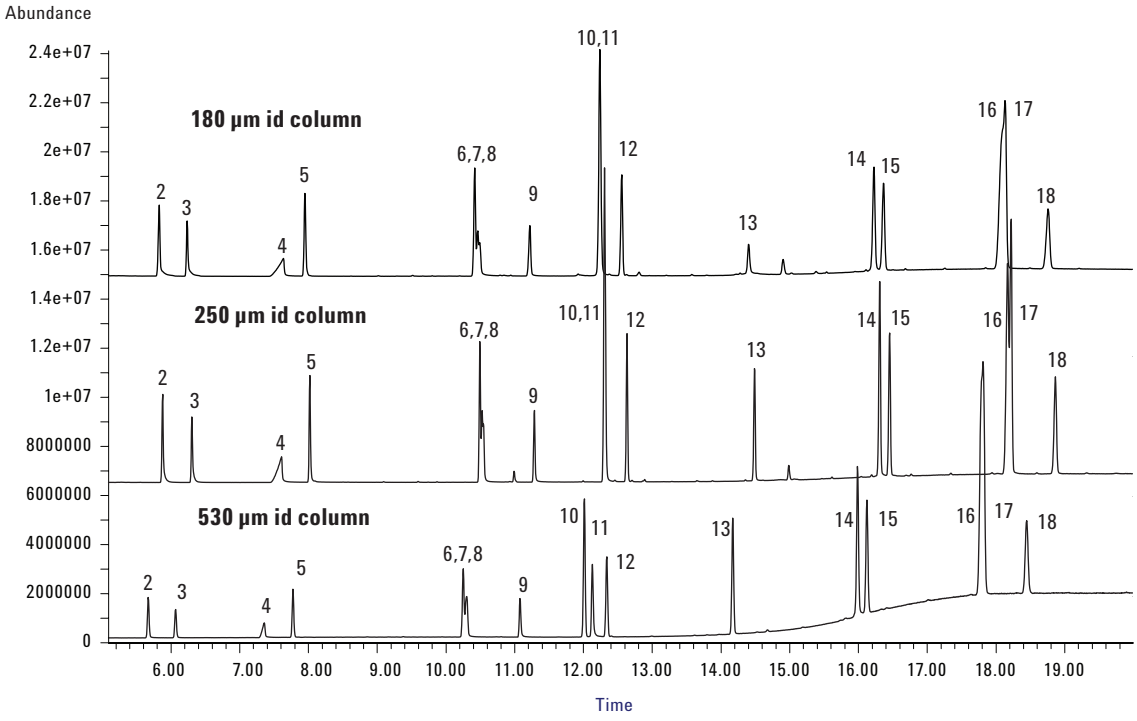


Figure 5. Constant pressure mode analysis with three different column dimensions; 0.5-µL splitless injections of 80-ppm semivolatiles test sample, with flow conditions from Table 2.

As can be seen in Figure 6, the FID signal indicates what was split to the FID when column flow exceeded the 1 mL/min flow to the MSD. At no time does the 180-µm id column flow exceed 1 mL/min, so there is nothing vented and no FID signal. For the 250-µm id column, the flow at initial conditions is > 1 mL/min, and the excess flow is split to the FID, as indicated by a solvent peak. Yet as flow decreases during the run (a normal consequence of constant pressure mode conditions), column effluent all goes to the MSD and FID signal remains flat. For the 530-µm id column, flow is always > 1 mL/min, so some flow is always being vented through the FID. This is easily seen in the inset of Figure 6, where the scale is expanded and peaks can be seen throughout the FID chromatogram.

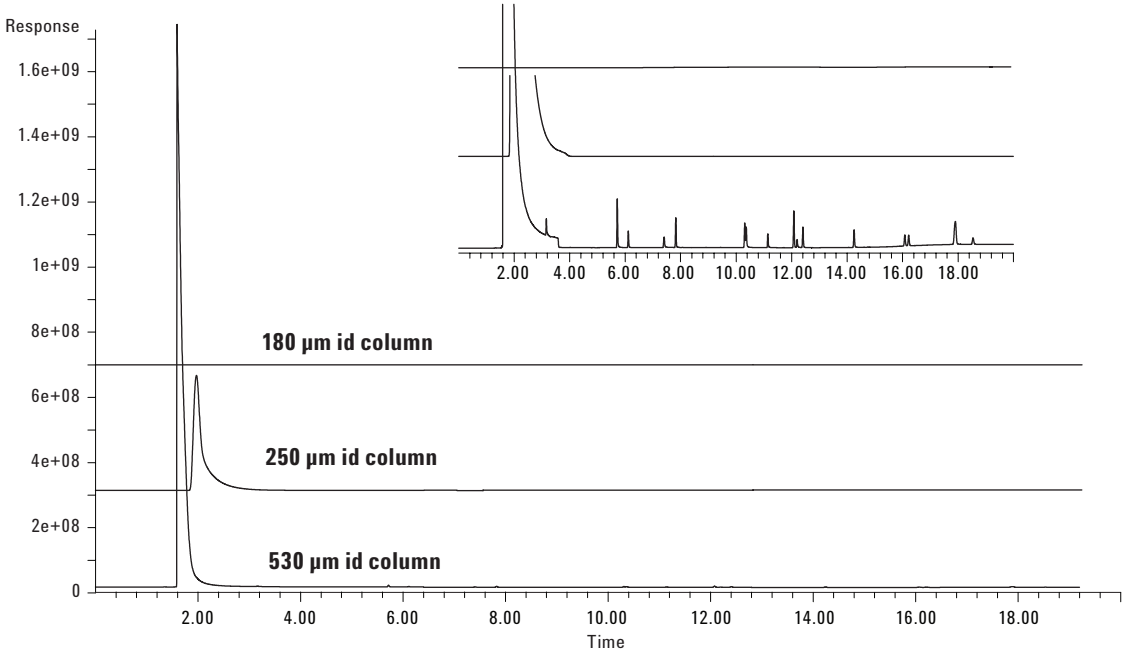


Figure 6. FID signal of vent stream shows what is vented when column flow exceeds flow to MSD.

Table 3. Constant Flow Mode Conditions (Lower flow for each column is its optimal flow, the higher is 2X optimum. Other instrumental paramters were the same as those used for constant pressure mode experiments.)

Dimensions	Outlet flow
20 m X 180 µm	0.72 mL/min
20 m X 180 µm	1.44 mL/min
30 m X 250 µm	2.5 mL/min
30 m X 250 µm	1.0 mL/min
30 m X 530 µm	2.1 mL/min
30 m X 530 µm	7.0 mL/min

Constant flow mode was also evaluated. Conditions for constant flow modes are given in Table 3. Two flow rates were chosen for each column: optimal flow rates (the lower of the two) and 2X optimum.

The MSD TIC for each column at optimal flow rates is shown in Figure 7, with the corresponding FID vent signal in Figure 8. It can clearly be seen that for the 250-µm and 180-mm id columns, no column effluent is split to the FID. Since the flow rate of the 530-µm id column is approximately 2X the flow the MSD, half of the column effluent is split to the FID.

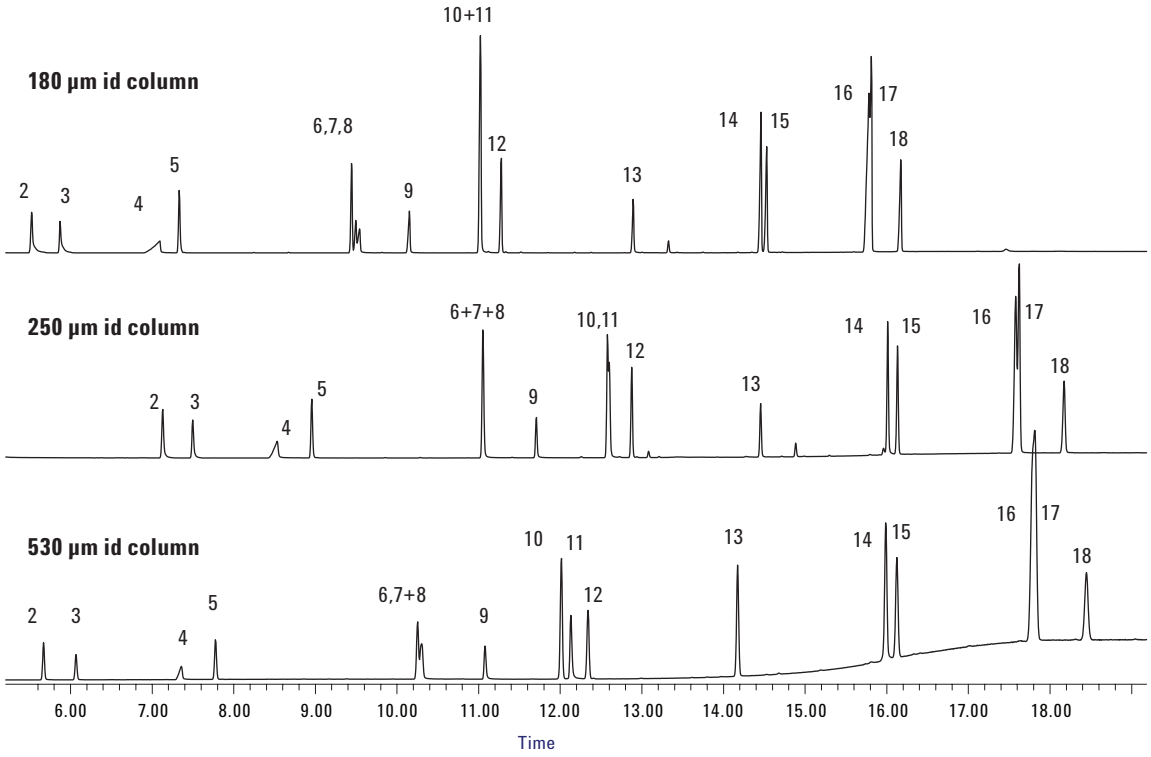


Figure 7. TIC chromatograms for the three columns under optimal constant flow mode conditions.

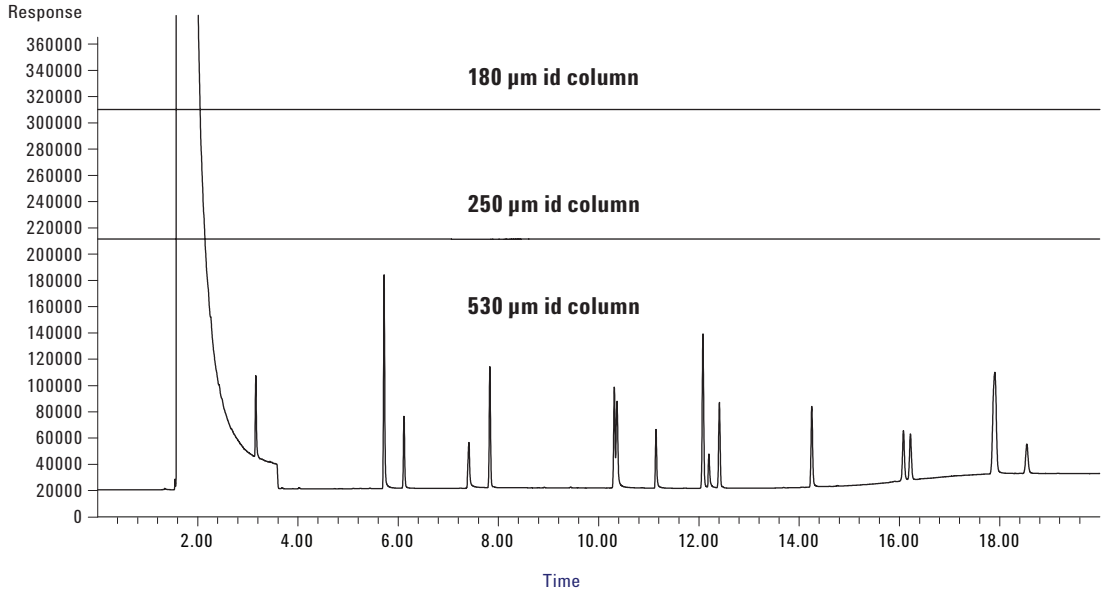


Figure 8. FID vent signal for three columns under optimal flow conditions. Only the 530-µm id column has a flow that exceeds the 1 mL/min flow to the MSD.

Results for the 2X optimal flow conditions are shown in Figures 9 and 10. The flexibility of the QuickSwap split configuration is highlighted here in that no adjustments were made to QuickSwap restrictor size, transfer line temperature, or Aux EPC pressure in order to accommodate all of the flow changes. Only the columns and their individual flow conditions were changed. The QuickSwap split passively accommodated all excess flow.

Notice in Figure 9 that the higher the excess column flow, the less of the sample goes to the MSD (more is split to vent, as seen in Figure 10). The fact that less sample is getting to the MSD might be considered a serious disadvantage for some analyses, but this is tempered by the fact that the larger column has higher sample capacity, so larger sample volumes could be injected without suffering overload (peak distortion). In addition, the larger diameter columns usually generate wider peaks, so a larger value can be selected for MSD sampling (for example, samples = 2^3 or 2^4 instead of 2^2). This will result in higher S/N. So, if one seeks the benefits of larger id columns for MS analysis, one can easily accommodate them with this QuickSwap configuration with only a small compromise.

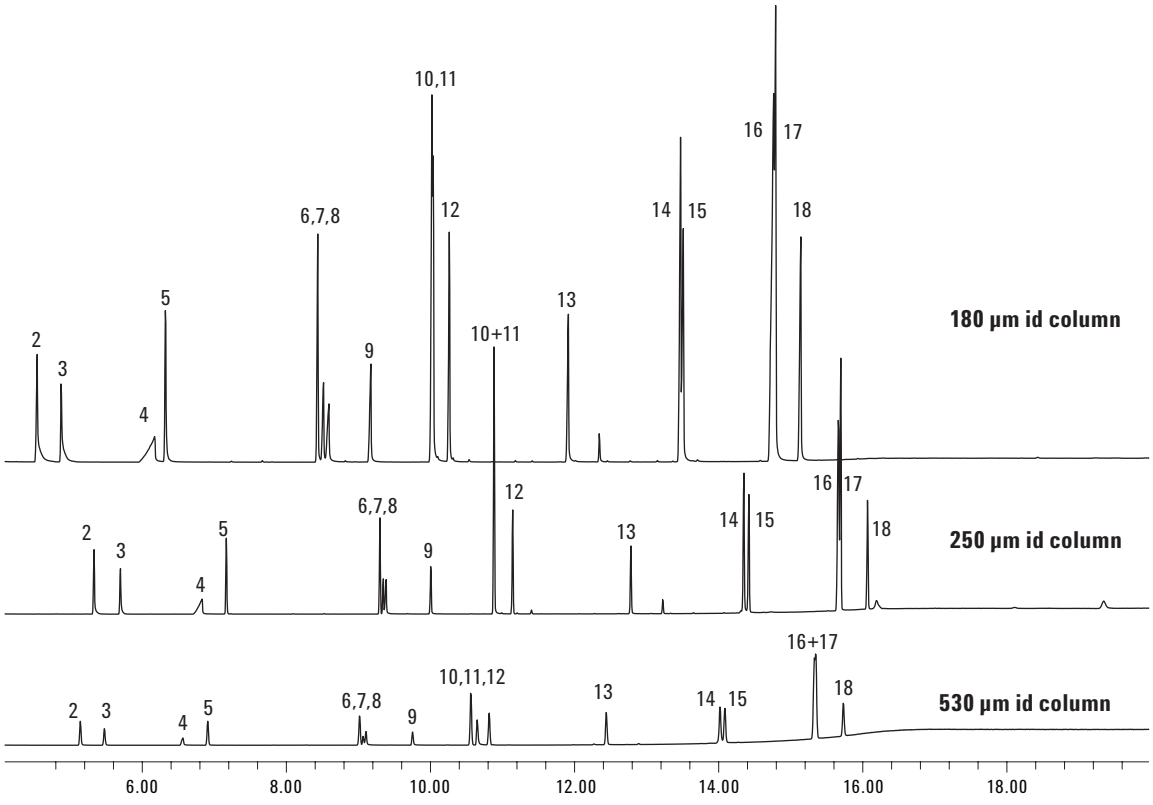


Figure 9. Comparison of MSD TIC chromatograms for three columns run at 2X optimal constant flow mode. Scale is constant for the three, showing the absolute amount of sample reaching the MSD.

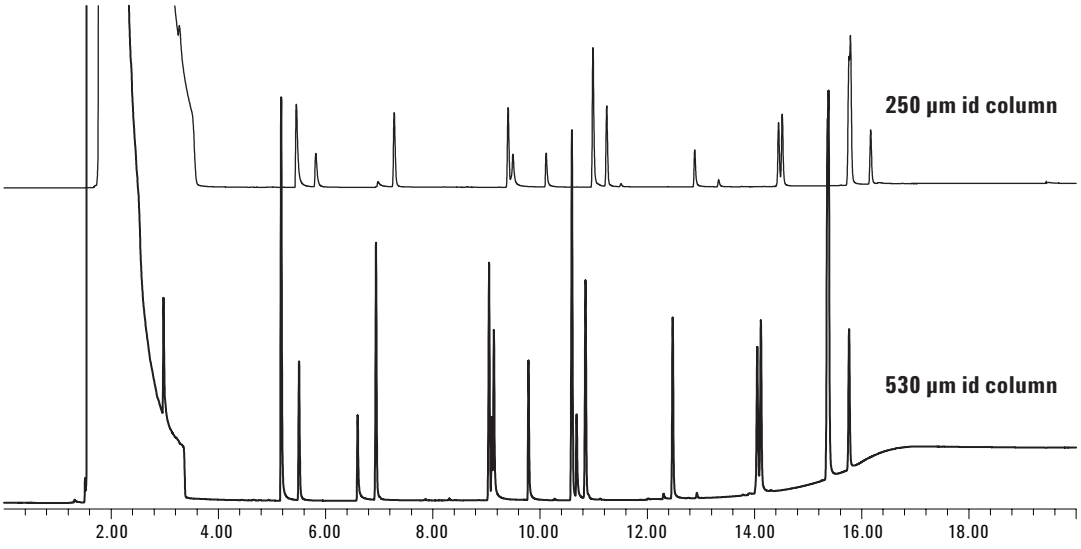


Figure 10. FID vent signals for the two largest columns operated at 2X optimal constant flow rate conditions.

Pressure-pulse injection is often used to minimize the time labile samples stay in the inlet and to avoid inlet overload when large volume sample injections. With this technique, pressures are typically two to three times the starting pressure of the standard analysis. As such, the flow through the column is increased significantly. In the standard QuickSwap configuration, this higher flow can exceed the ability of the chosen QuickSwap restrictor to handle at the selected QuickSwap (Aux EPC) pressure. When this happens, pressure exceeds the setpoint, the GC goes "not ready," and automated injection does not proceed. With the flexible split configuration for QuickSwap described herein, the extra flow during pressure pulse injection is vented, so there is no issue with maintaining setpoint.

A pressure pulse injection was done with the 250-µm id column to verify that the split configuration would accommodate the extra flow. The pulse pressure was 50 psi (approximately two times the standard pressure) for 1 min, after which the pressure returned to 23.41 psig for the remainder of the run. For the standard run, the pressure was 23.41 psig for the whole time. No other changes were made to experimental conditions.

Figure 11 compares MSD TIC chromatograms for the standard and pulsed-pressure experiments. One can see a slightly earlier retention time for the first couple of peaks in the pressure pulse experiment (this is typical due to the higher initial column flows). Other than that, the chromatograms are indistinguishable.

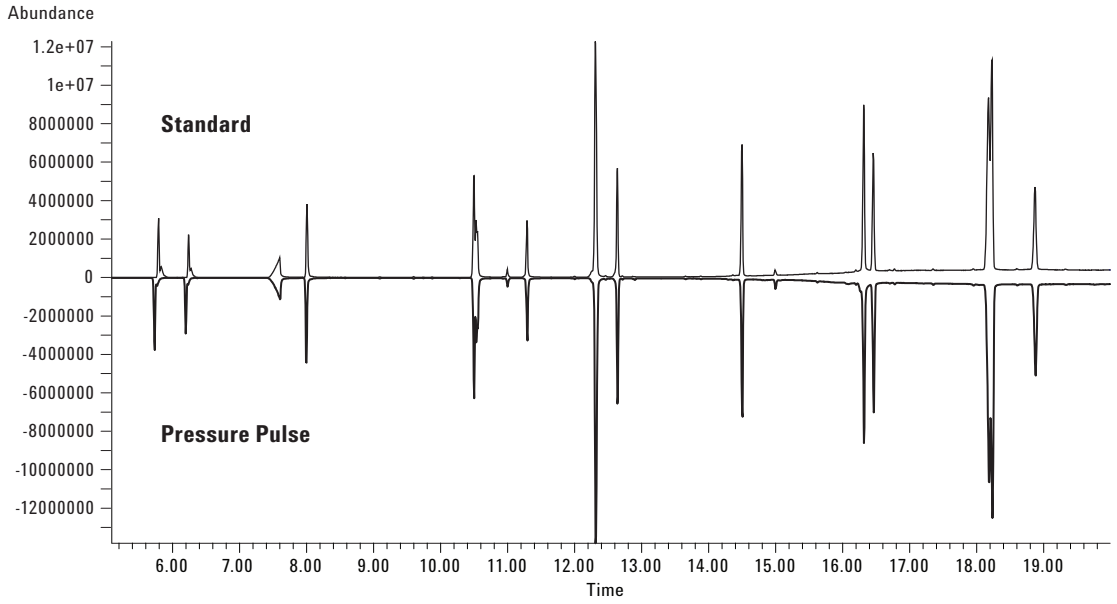


Figure 11. Comparison of standard and pressure-pulse injection modes. No adjustment of QuickSwap pressure was required for the pressure-pulse mode—a benefit of using QuickSwap split configuration.

As can be seen from the FID vent signal, (Figure 12), more solvent is vented in the pressure-pulse injection than in the standard because of the higher initial flow. Yet for the analytical portion of the run after completion of the pressure pulse period (1 min), the column flows are the same in the two cases and decrease to near or below 1 mL/min. As a result, there is no excess column flow to split to the FID and the FID baseline is flat.

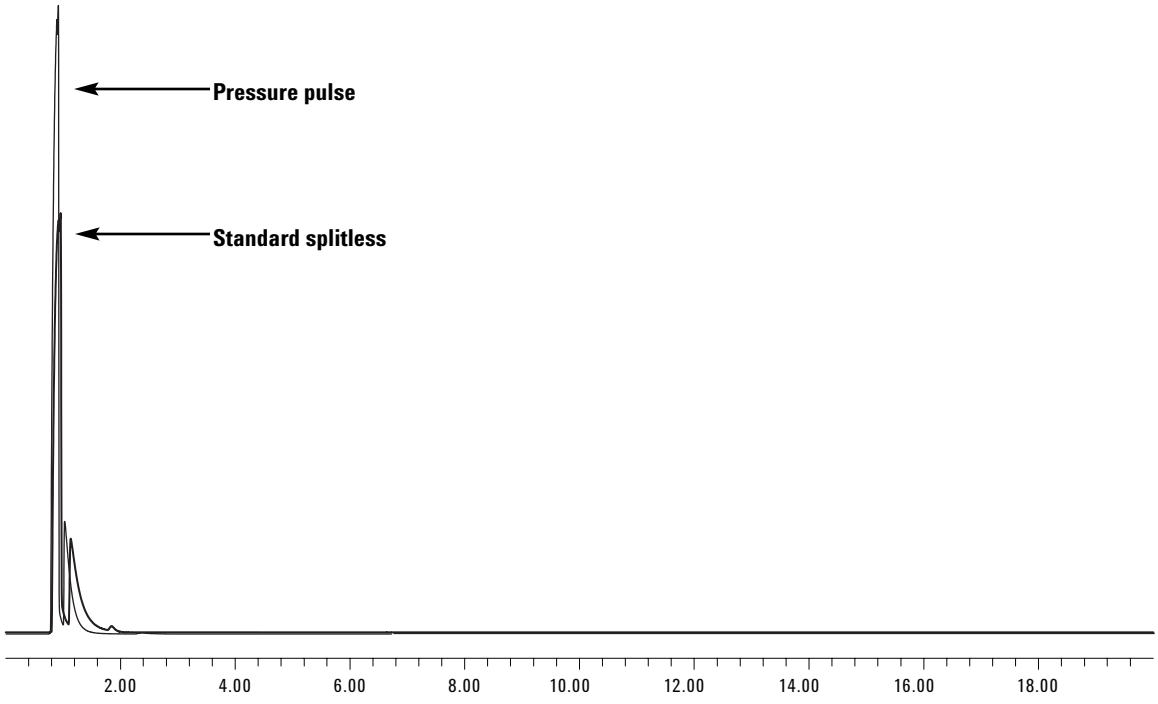


Figure 12. FID vent signal for pressure-pulse injection versus standard splitless injection.

Conclusions

The QuickSwap split configuration provides a flexible and simple alternative to the standard configuration. The split configuration can benefit MSD users who change columns frequently, seek the benefits of using larger id columns, and/or use pressure pulse injection. The configuration allows the MSD to run at optimal flow conditions while accommodating a wide range of column flows.

References

1. "How QuickSwap Works," f03002.pdf.
2. "Agilent G3185B QuickSwap Accessory Installation and Setup," Agilent publication number G3185-90100.
3. "Agilent G3185B QuickSwap Accessory Reference Manual," Agilent publication number G3185-90101.
4. "Simplified Backflush Using Agilent 6890 GC," Agilent publication number 5989-5111EN.
5. "Fast USEPA 8270 Semivolatiles Analysis Using the 6890N/5975 Inert GC/MSD," Agilent publication number 5989-2981EN.

Parts List

Part	Description	Part number
QuickSwap	Kit	G3185B
QuickSwap restrictors	92 µm	G3185-60361
	100 µm	G3185-60362
	110 µm	G3185-60363
1/16" tee	Regular	0100-0782
	ZDV	0100-0969
SilTite 1/16" ferrules	For connecting 1/16" SS lines	G2855-2055
Deactivated FS	250-µm id FID vent restrictor	160-2255-5
Split vent trap	Kit—vent alternative to FID	G1544-0124
1/16" straight union		0100-0124
SilTite ferrules for capillary	250 µm	5188-5361
column connections	320 µm	5188-5362
	530 µm	5188-5363
20 m X 180 mm X 0.36 mm	DB-5.625	121-5622
30 m X 250 mm X 0.5 mm	DB-5MS	122-5536
30 m X 530 mm X 1 mm	DB-5	125-503J

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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

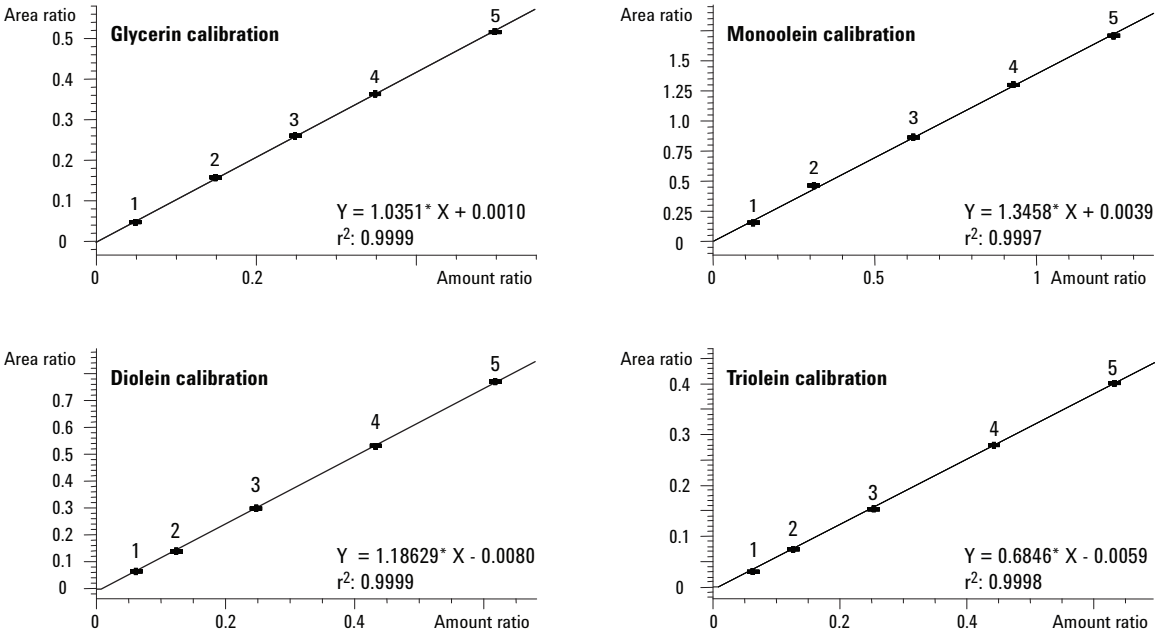

Figure 1. Calibration curves for glycerin, monoolein, diolein, and triolein.

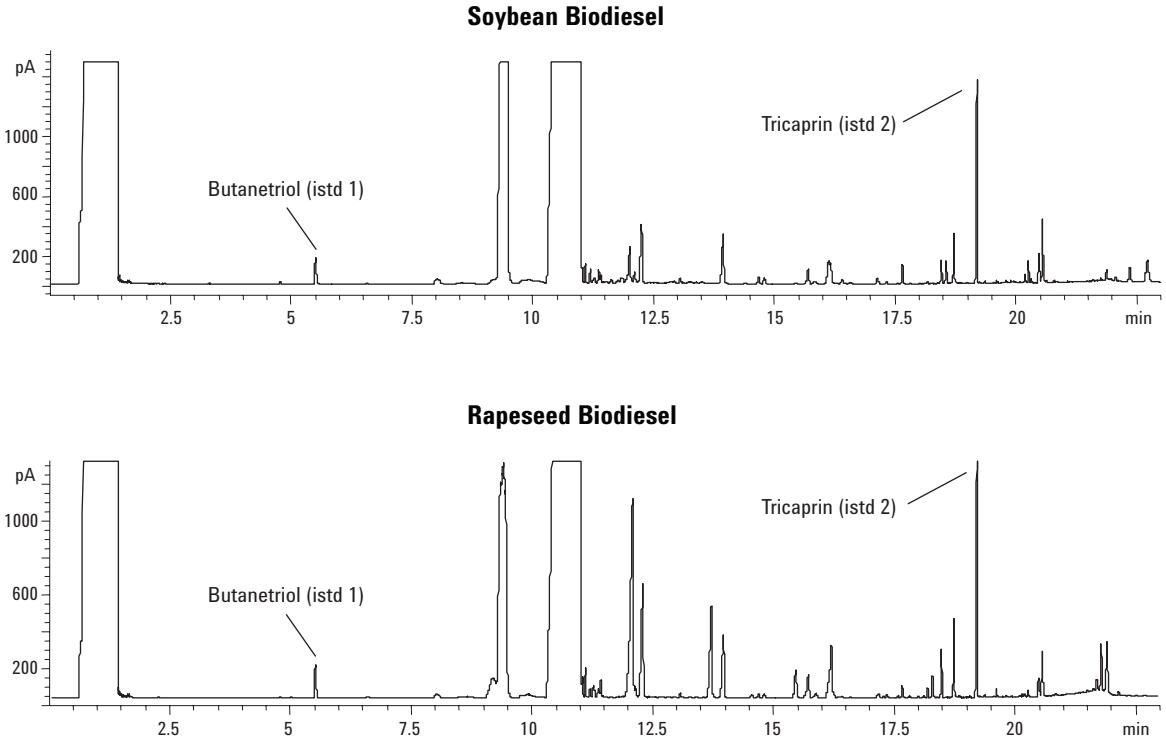

Figure 2. Chromatograms showing typical analysis of free and total glycerins in two B100 biodiesel samples.

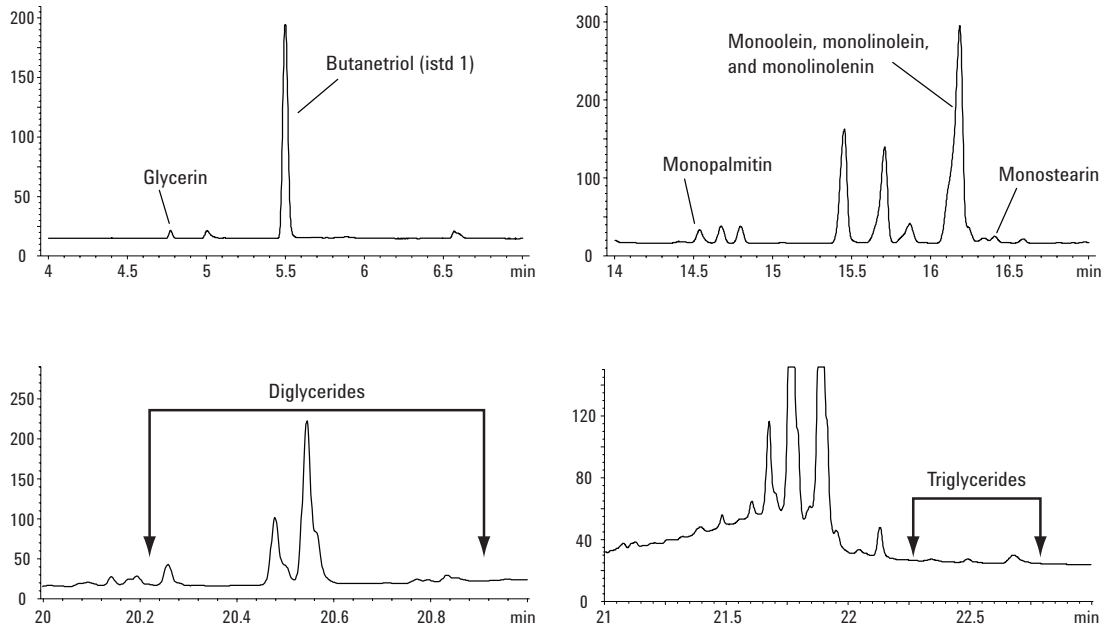

Figure 3. Details of glycerin, monoglycerides, diglycerides, and triglycerides found in a sample of rapeseed B100 biodiesel.

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.

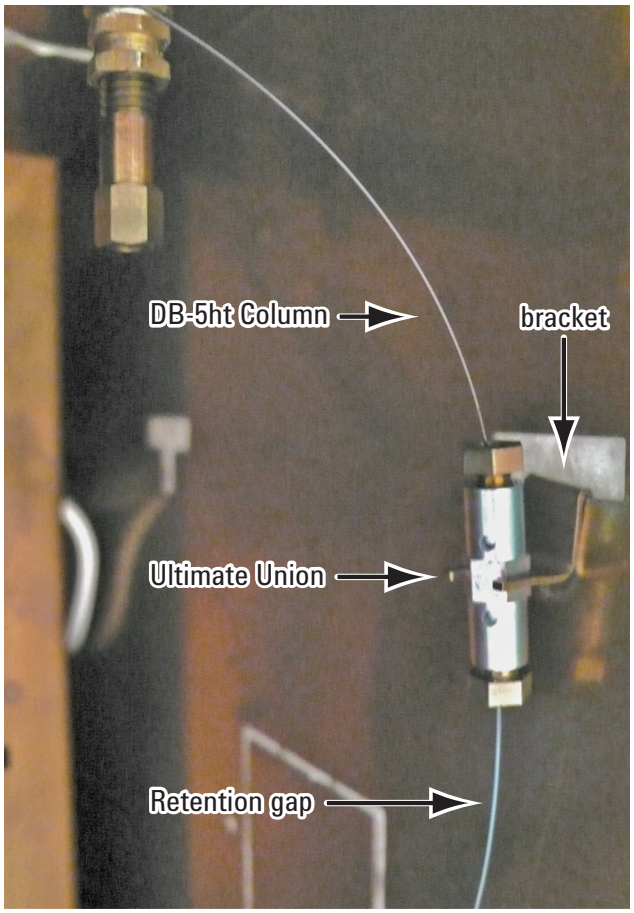

Figure 5. Details of the retention gap and analytical column joined with a Capillary Flow Technology Ultimate Union.

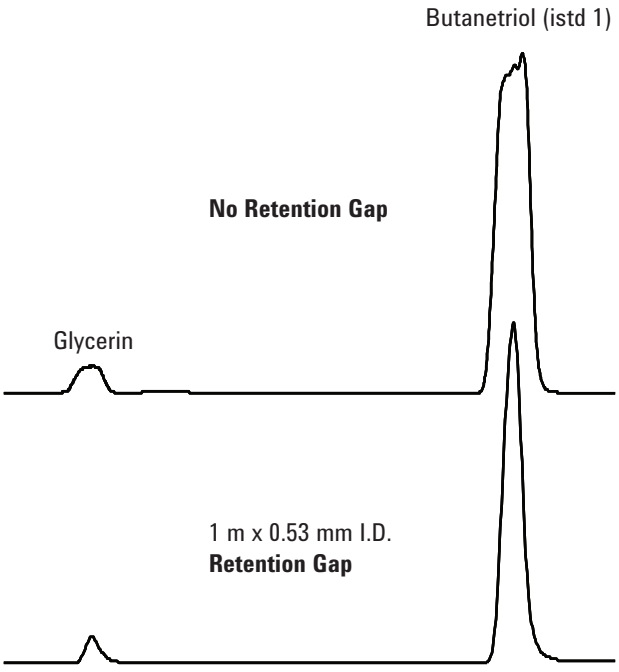

Figure 4. Improved peak shape for glycerin and 1,2,3-butanetriol when using a retention gap and the Capillary Flow Technology Ultimate Union.

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.

References

1. K. Shaine Tyson, “Biodiesel Handling and Use Guidelines,” National Renewable Energy Laboratory, NREL/TP-580-30004, September 2001
2. “D6751 Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuel,” ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA
3. “EN14214 Fatty Acid Methyl Esters (FAME) for Diesel Engines, Requirements and Test Methods,” European Committee for Standardization: Management Centre, rue de Stassart 36, B-1050 Brussels, 2003

www.agilent.com/chem

4. “D6585 Test Method for Determination of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography,” ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA, USA, 2003
5. “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

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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.

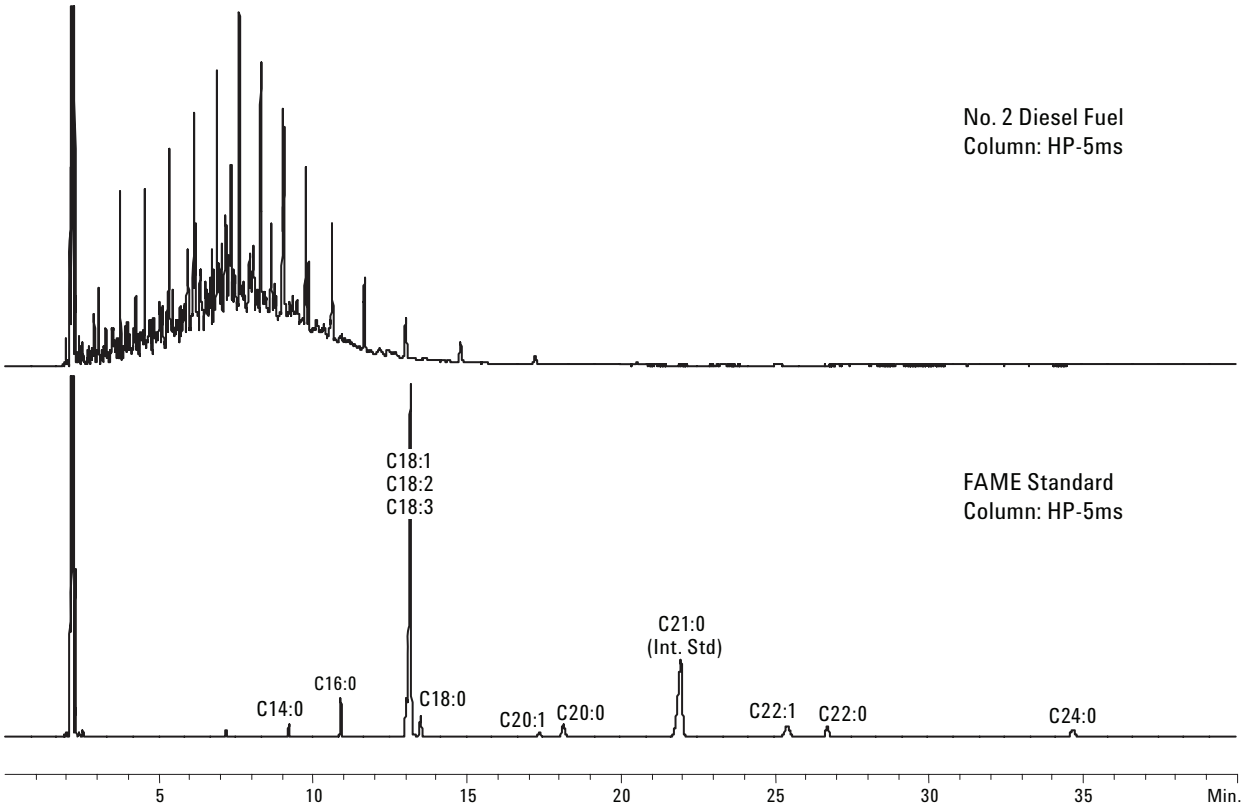

Figure 1. The lower chromatogram shows the separation of FAMEs on the primary HP-5ms column. The upper chromatogram shows the separation of pure no. 2 diesel fuel on the same column. A blended biodiesel fuel containing FAMEs and no. 2 diesel fuel would have unresolved compounds between 2 and 15 minutes 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.

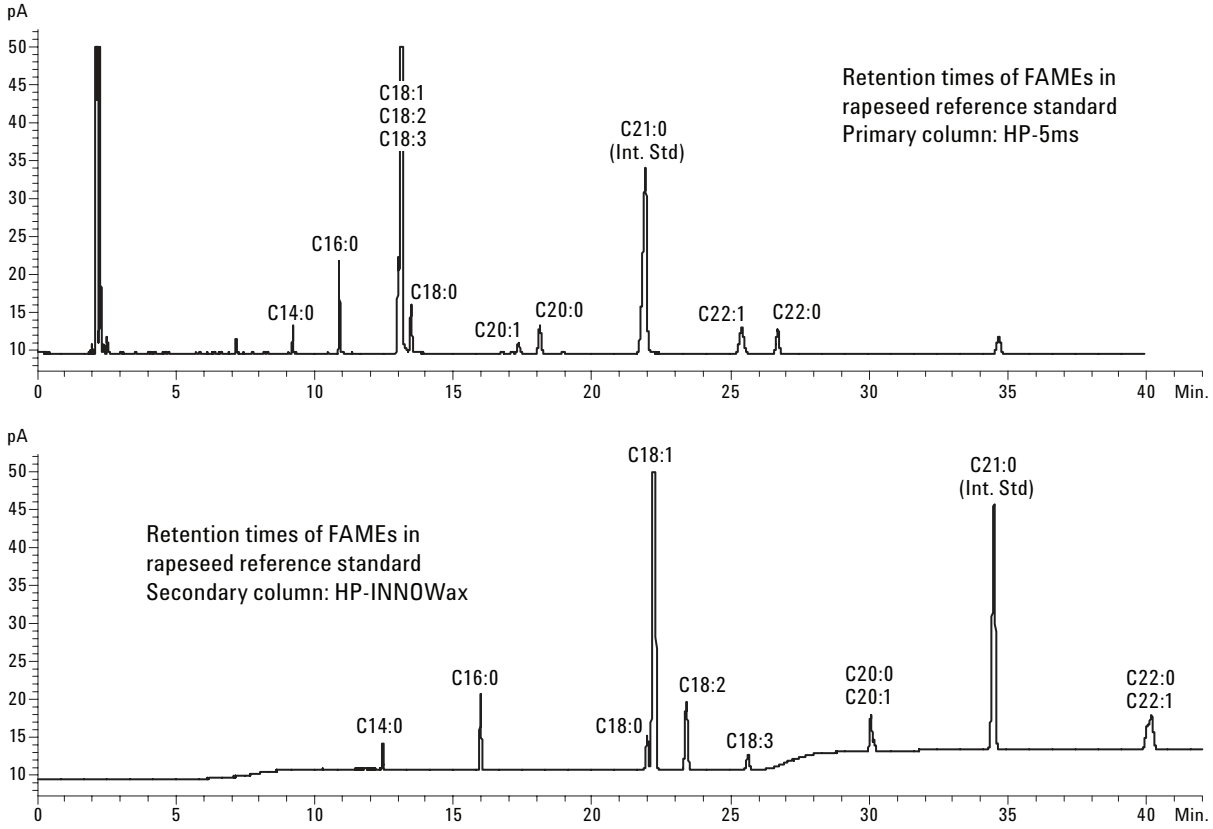

Figure 2. The upper chromatogram shows the separation of FAMEs on the primary HP-5ms column before heart-cutting. After heart-cutting, the lower chromatogram shows the separation of the FAMEs on the HP-INNOWax secondary column.

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.

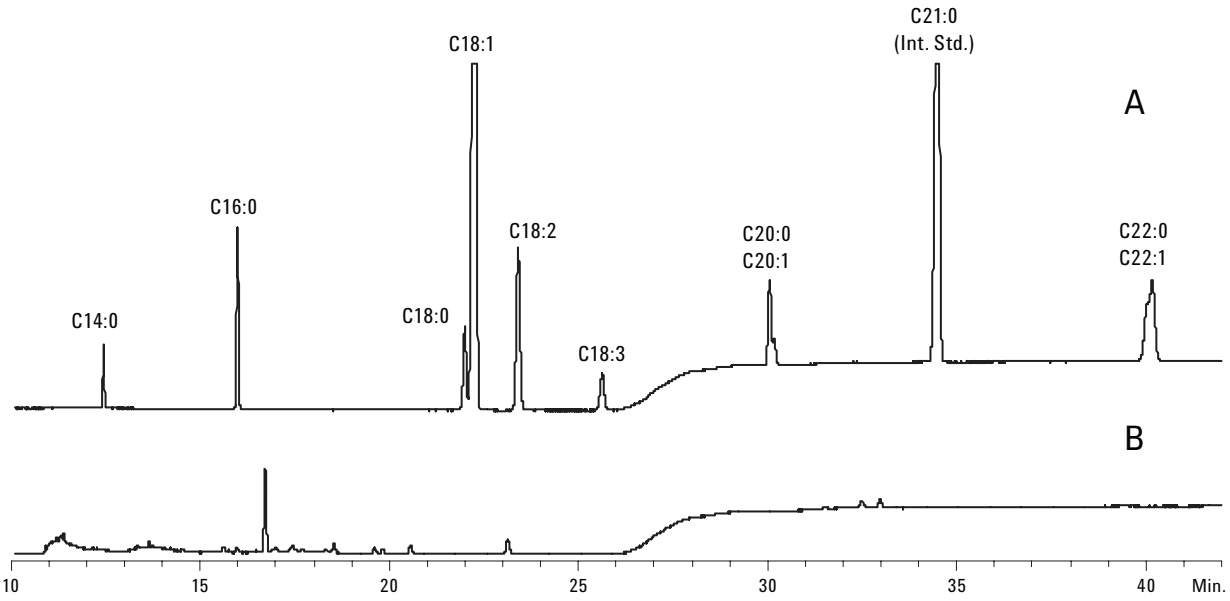

Figure 3. The upper chromatogram (A) shows the retention times of FAMEs on the secondary HP-INNOWax column after heart-cutting. The lower chromatogram (B) shows the hydrocarbon matrix of no. 2 diesel fuels after heart-cutting. No large peaks from the hydrocarbon matrix were found to co-elute with the FAME peaks after heart-cutting to the HP-INNOWax column.

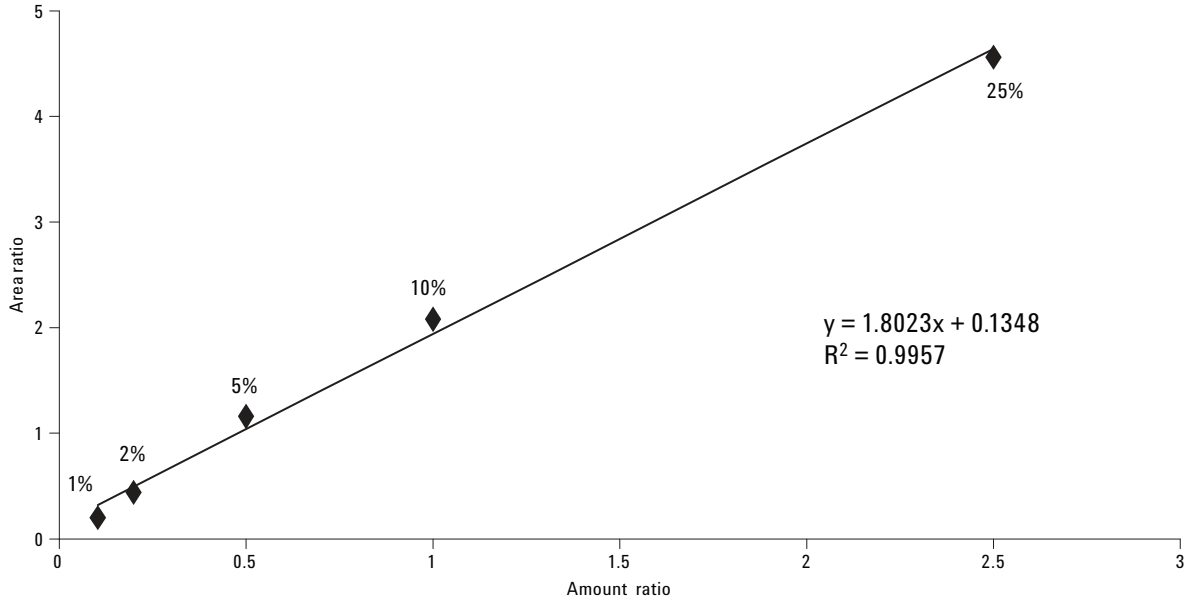

Figure 4. A calibration curve for biodiesel blends containing soybean biodiesel between 1 vol% (B1) and 25 vol% (B25). This calibration was prepared using the total peak areas of all FAMEs found in the blends.

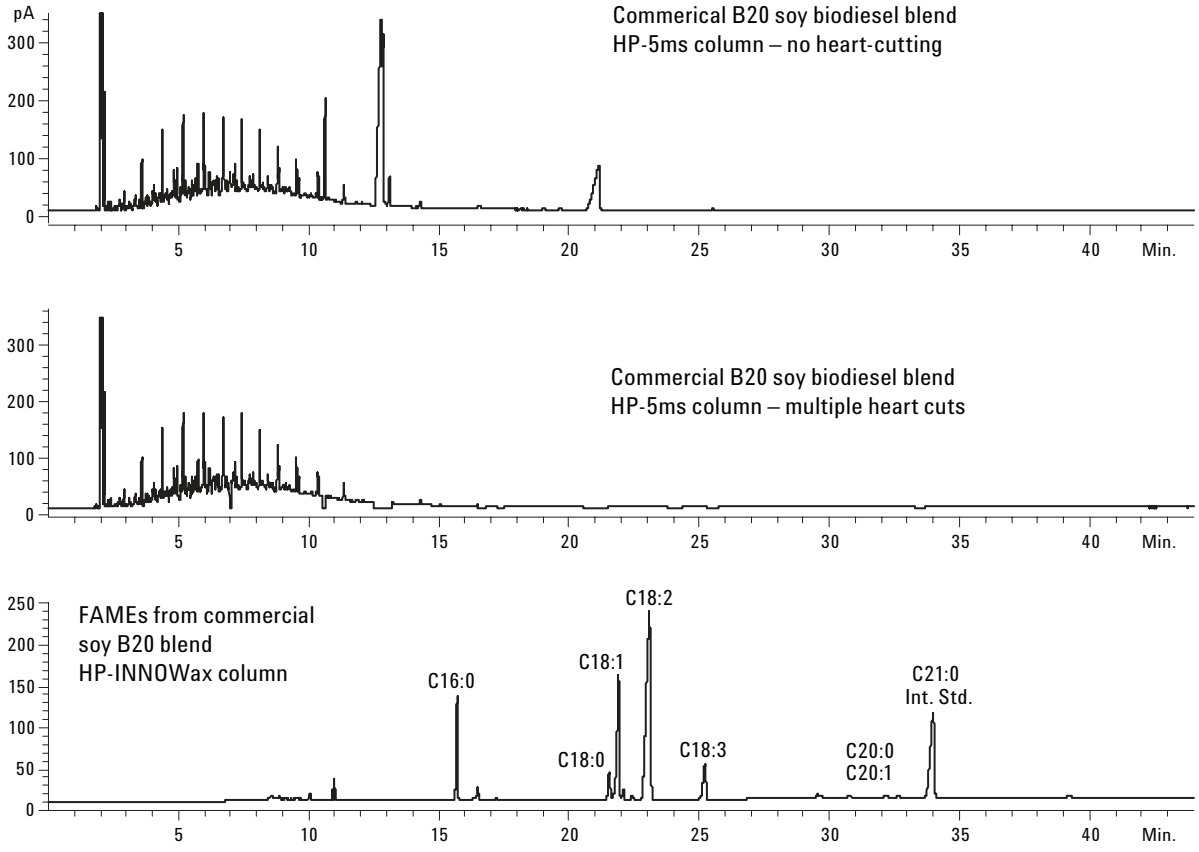

Figure 5. A commercially prepared B20 sample containing soybean biodiesel was analyzed using heart-cutting 2D GC. The upper chromatogram shows the primary column separation before heart-cutting. The middle and lower chromatograms show the sample after heart-cutting the FAMEs from the HP5ms column to 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

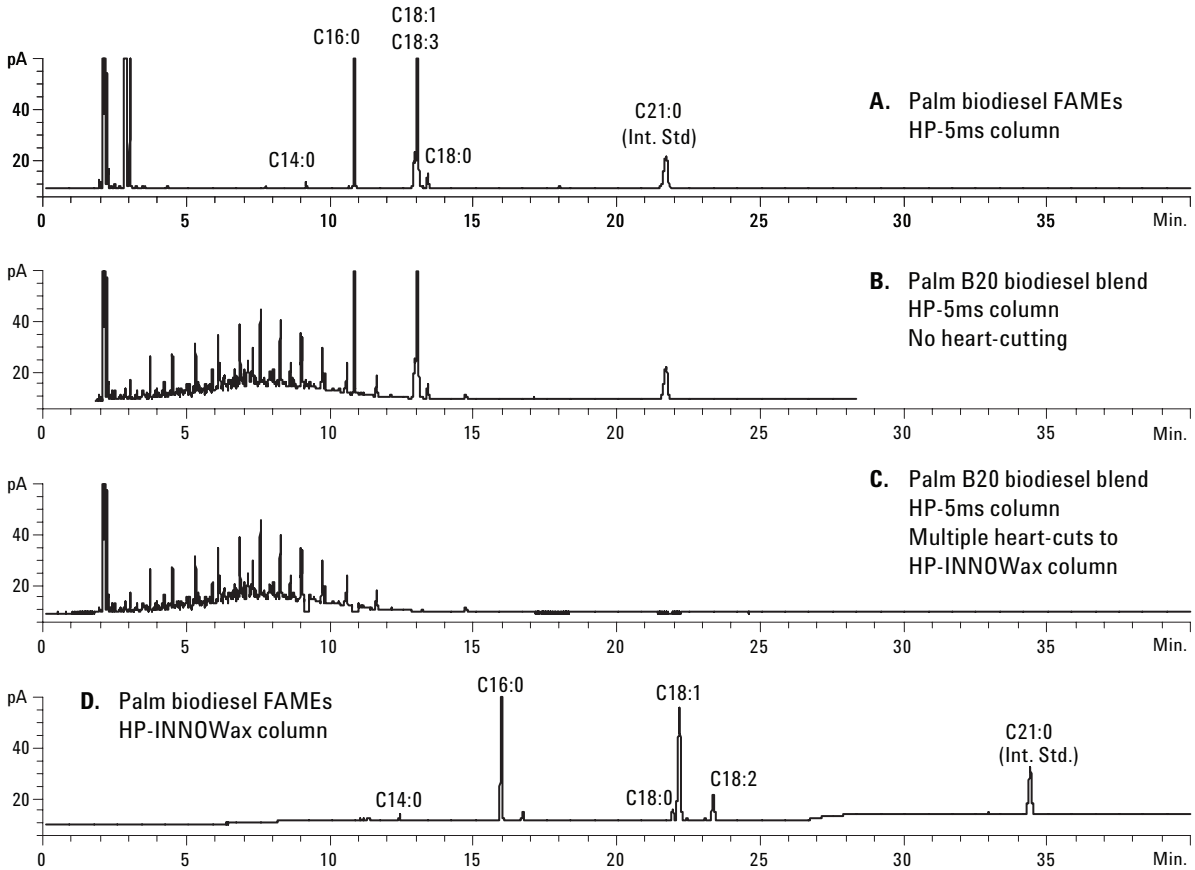

Figure 6. (A) Palm oil FAMEs on the primary HP-5ms column. (B) Palm B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of palm FAMEs in B20 biodiesel blend using heart-cutting 2D GC.

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

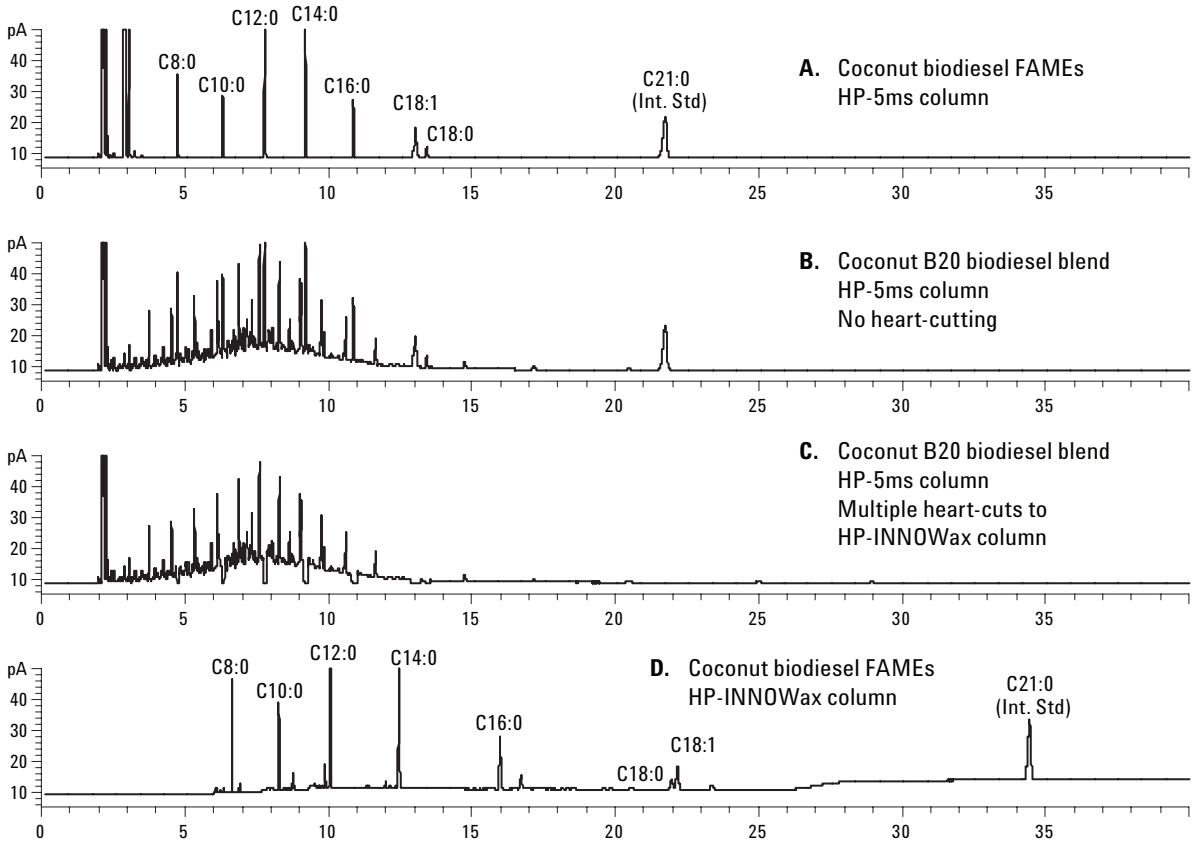

Figure 7. (A) Coconut oil FAMEs on the primary HP-5ms column. (B) Coconut B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of coconut FAMEs in B20 biodiesel blend using heart-cutting 2D GC.

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.

References

1. K. Shaine Tyson, "Biodiesel Handling and Use Guidelines," National Renewable Energy Laboratory, NREL/TP-580-30004, September 2001.
2. K. S. Tyson, and R. L. McCormick, "2006 Biodiesel Handling Use and Guidelines," Third Ed., National Renewable Energy Laboratory, DOE/GO-102006-2358, September 2006.
3. "EN14331 Liquid Petroleum Products – Separation and Characterisation of Fatty Acid Methyl Esters (FAMEs) from Middle Distillates," European Committee for Standardization: Management Centre, rue de Stassart 36, B-1050 Brussels, 2004.

www.agilent.com/chem

4. J. D. McCurry and B. D. Quimby, "Two-Dimensional Gas Chromatography Analysis of Components in Fuel and Fuel Additives Using a Simplified Heart-Cutting GC System," *J Chromatogr Sci*, 41 (10): 524–527 Nov – Dec 2003.
5. J. McCurry and B. Qumby, "Two-dimensional Gas Chromatographic Analysis of Oxygenates and Aromatics in Gasoline Using a Heart-Cutting Technique," Agilent Technologies publication 5988-6696EN, May 2002.
6. J. McCurry, "Fast Determination of Denatured Fuel Ethanol Purity by Two-Dimensional Gas Chromatography," Agilent Technologies publication 5988-9460EN, April 2003.
7. J. McCurry, "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," Agilent Technologies publication 5989-1840EN, November 2004.
8. B. D. Quimby, J. D. McCurry, and W. M. Norman, "Capillary Flow Technology for Gas Chromatography: Reinvigorating a Mature Analytical Technique," LCGC The Peak, Advanstar Communication, April 30, 2007 (p7–15).

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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.

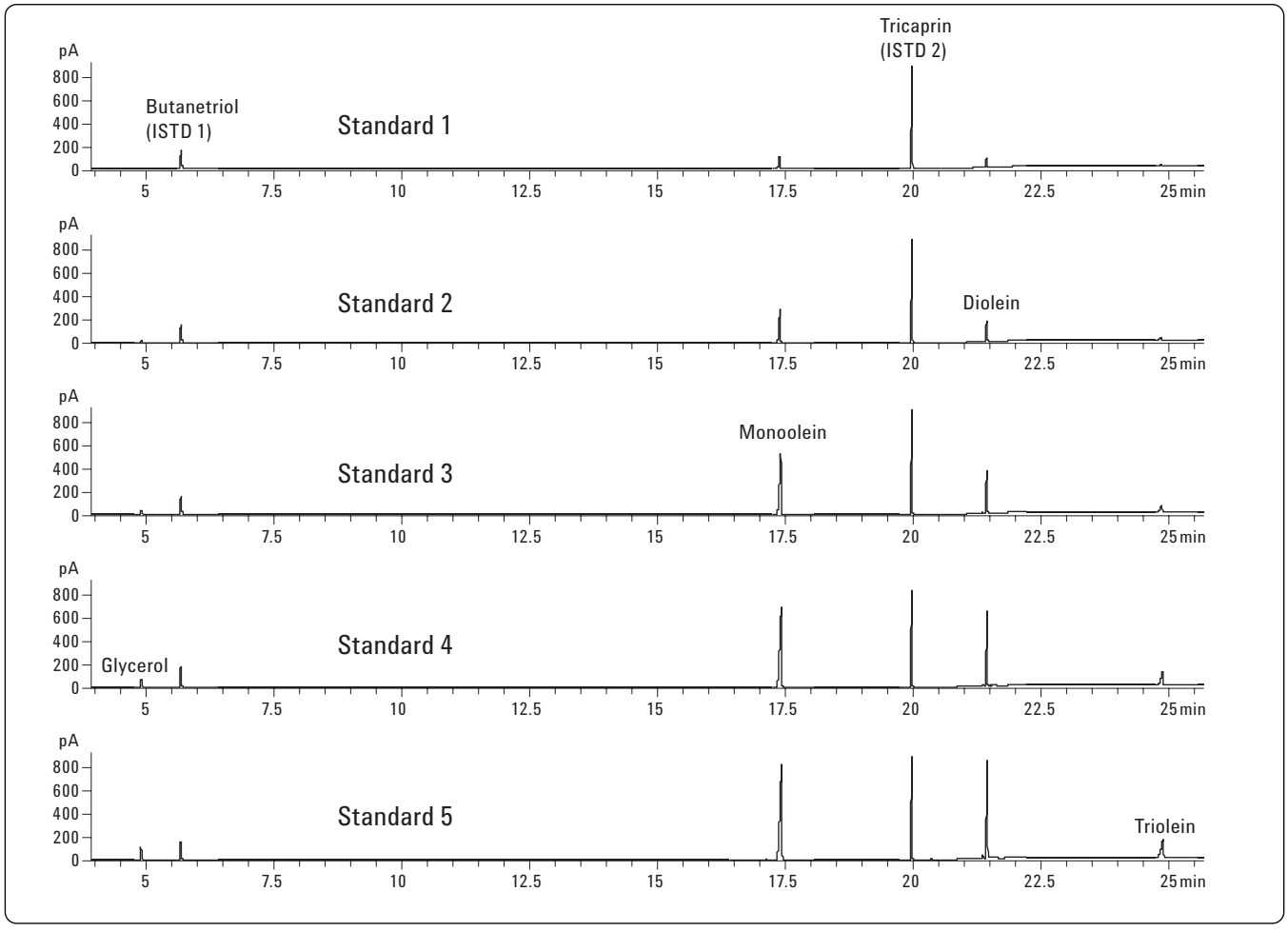

Figure 1. A five level ASTM D6584 calibration was automatically prepared using the Agilent 7693A ALS and run on the Agilent 7890A Biodiesel Solution.

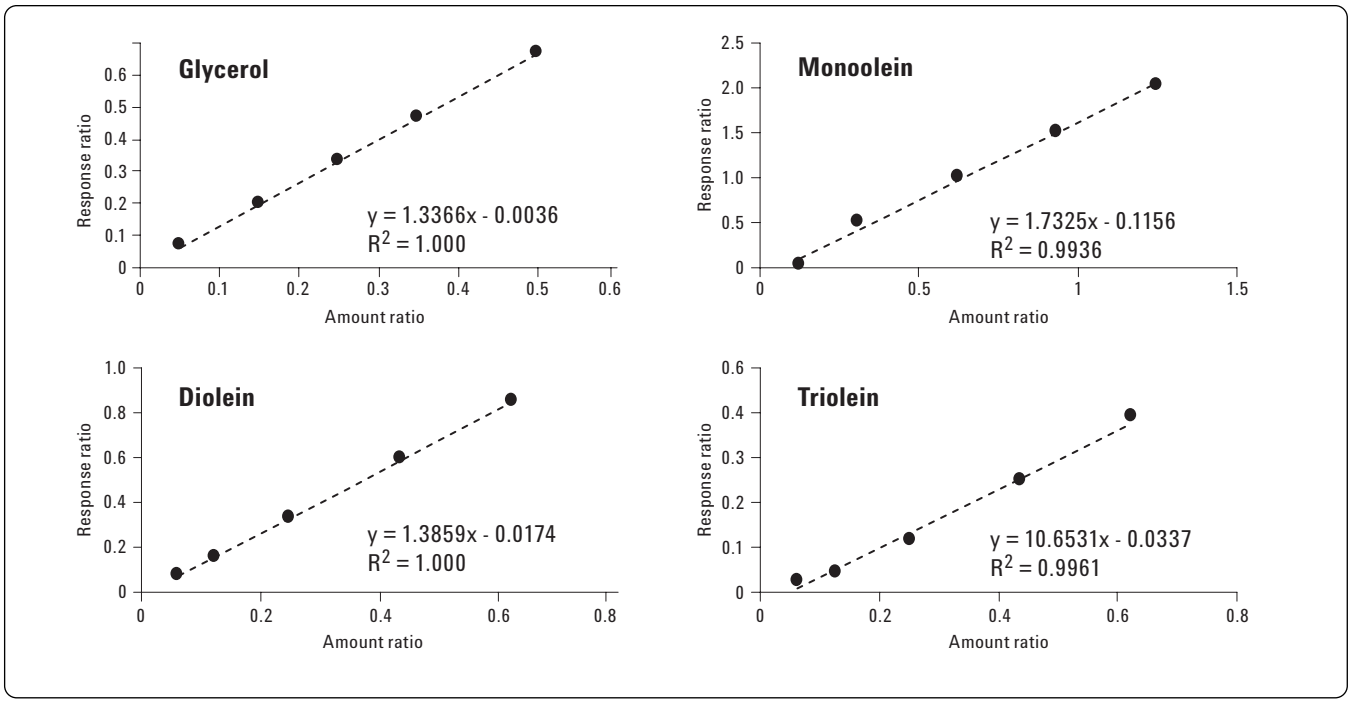

Figure 2. Five point linear calibration curves resulting from automated standard preparation with the Agilent 7693A ALS.

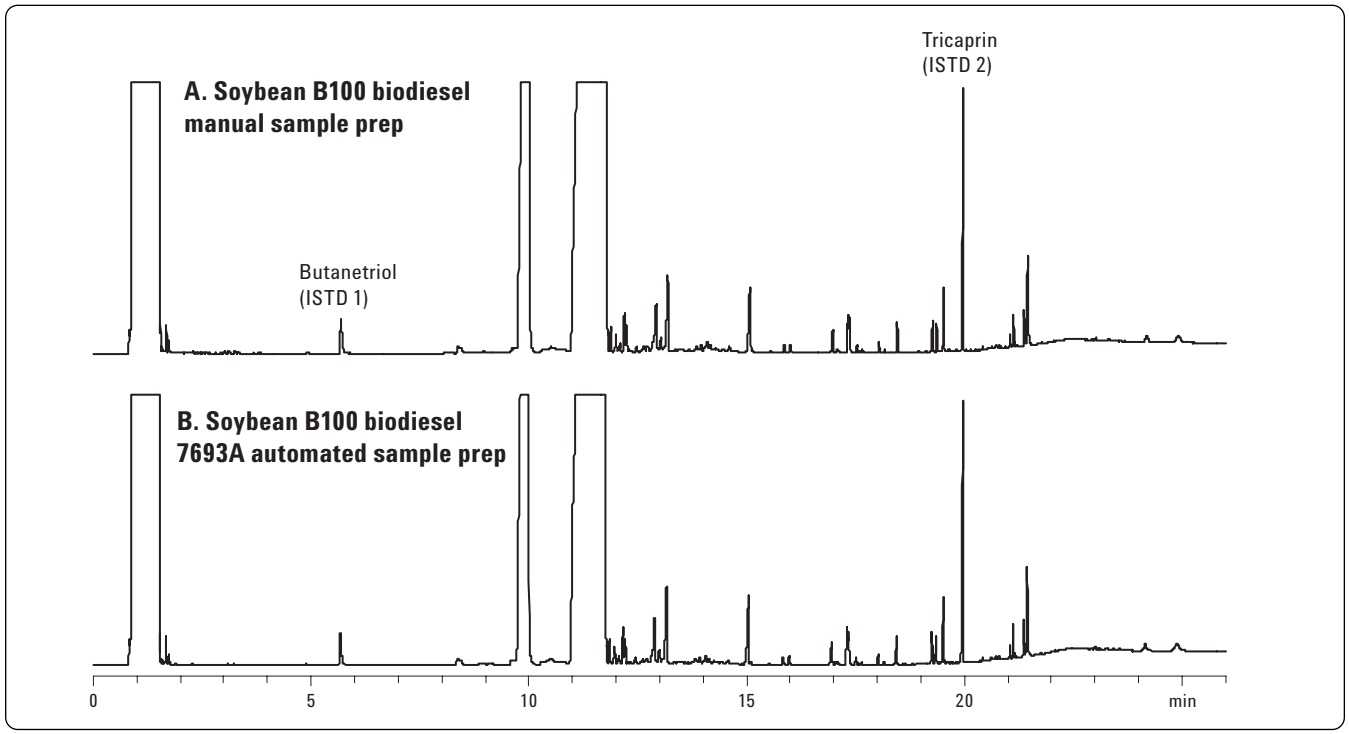

Figure 3. Comparison of a soybean B100 sample prepared (A) manually according to the ASTM D6584 protocol and (B) the automatic 10% scaled protocol using the Agilent 7693A ALS and Agilent 7890A Biodiesel Solution.

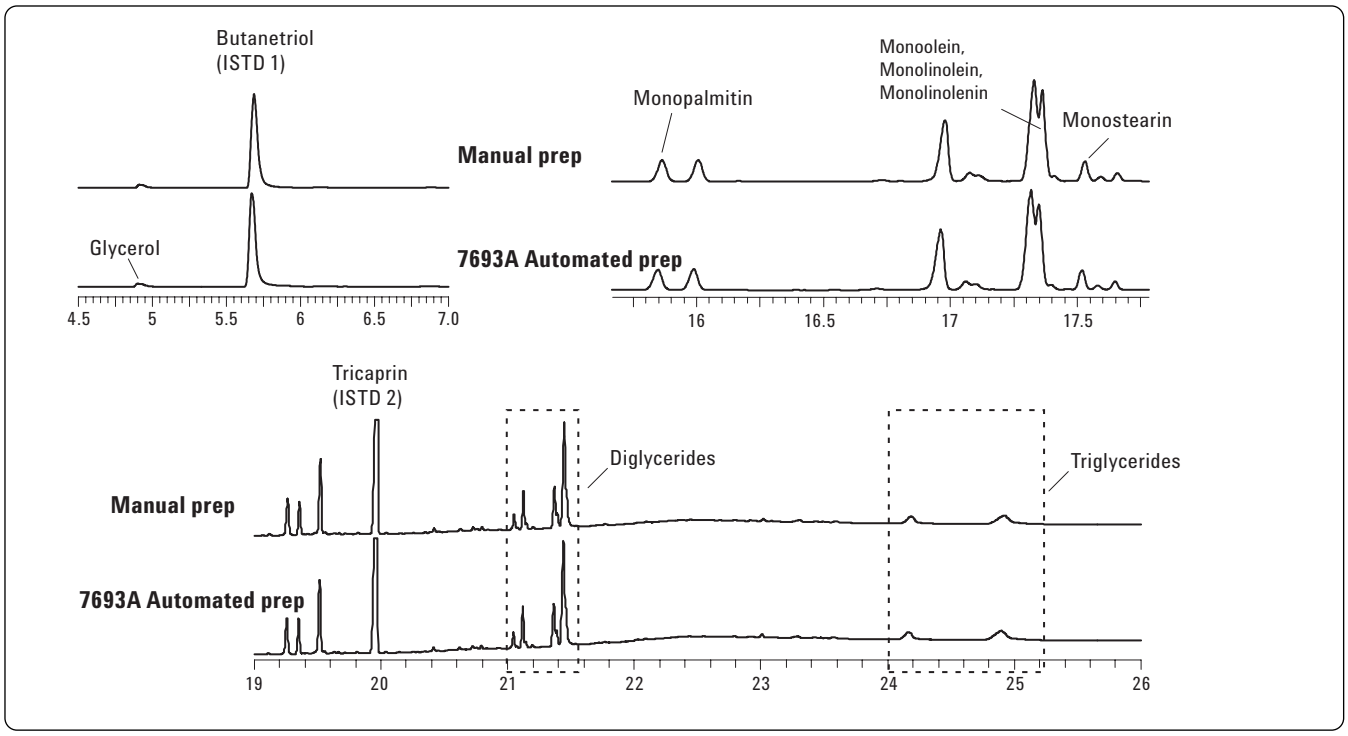

Figure 4. Details of the glycerol, mono-, di-, and tri-glycerides contained in a soybean B100 sample. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

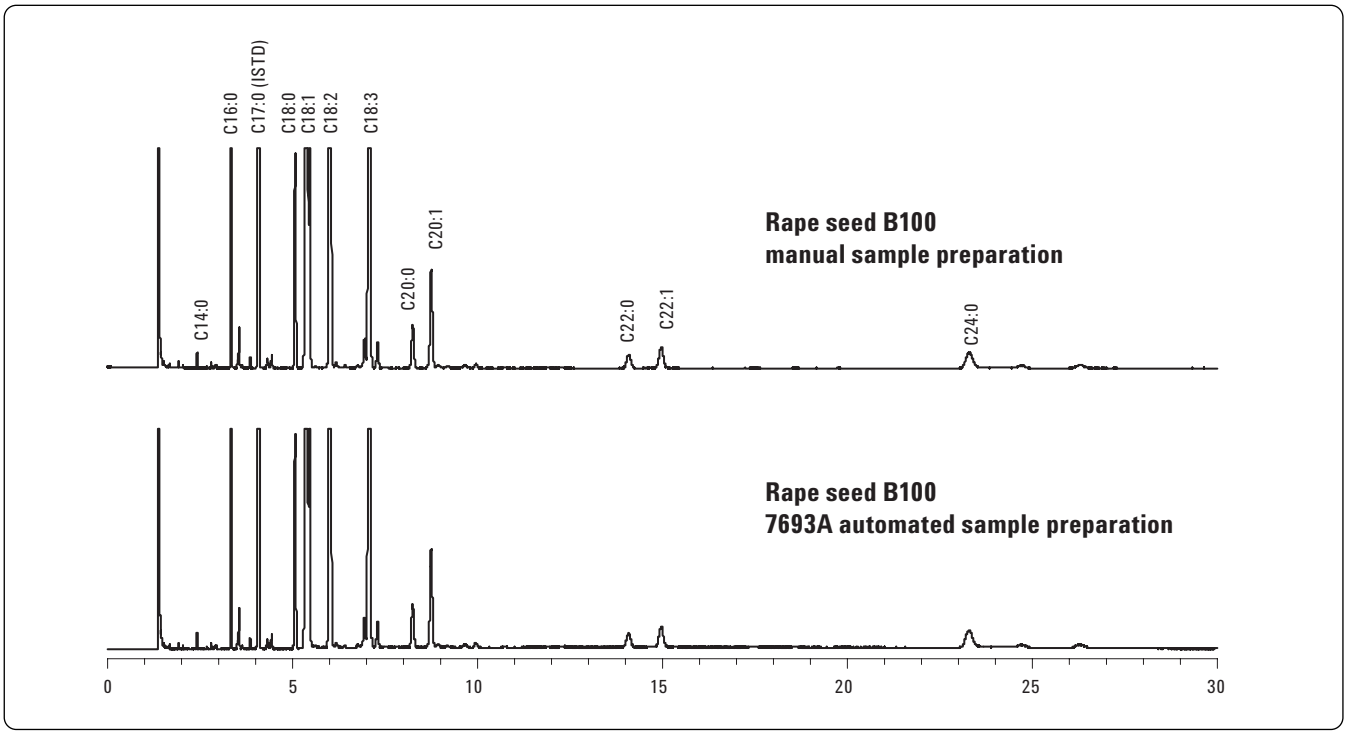

Figure 5. Analysis FAMEs in rape seed B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

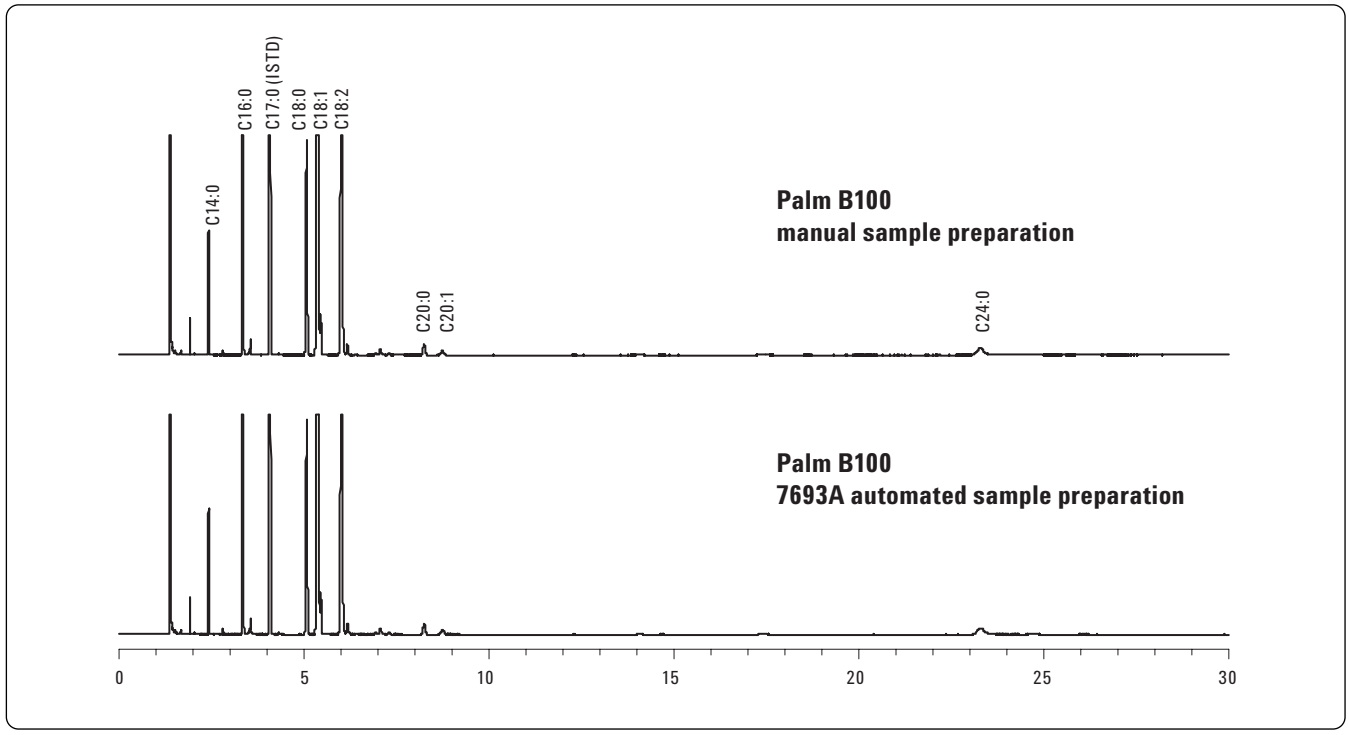

Figure 6. Analysis FAMEs in palm oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

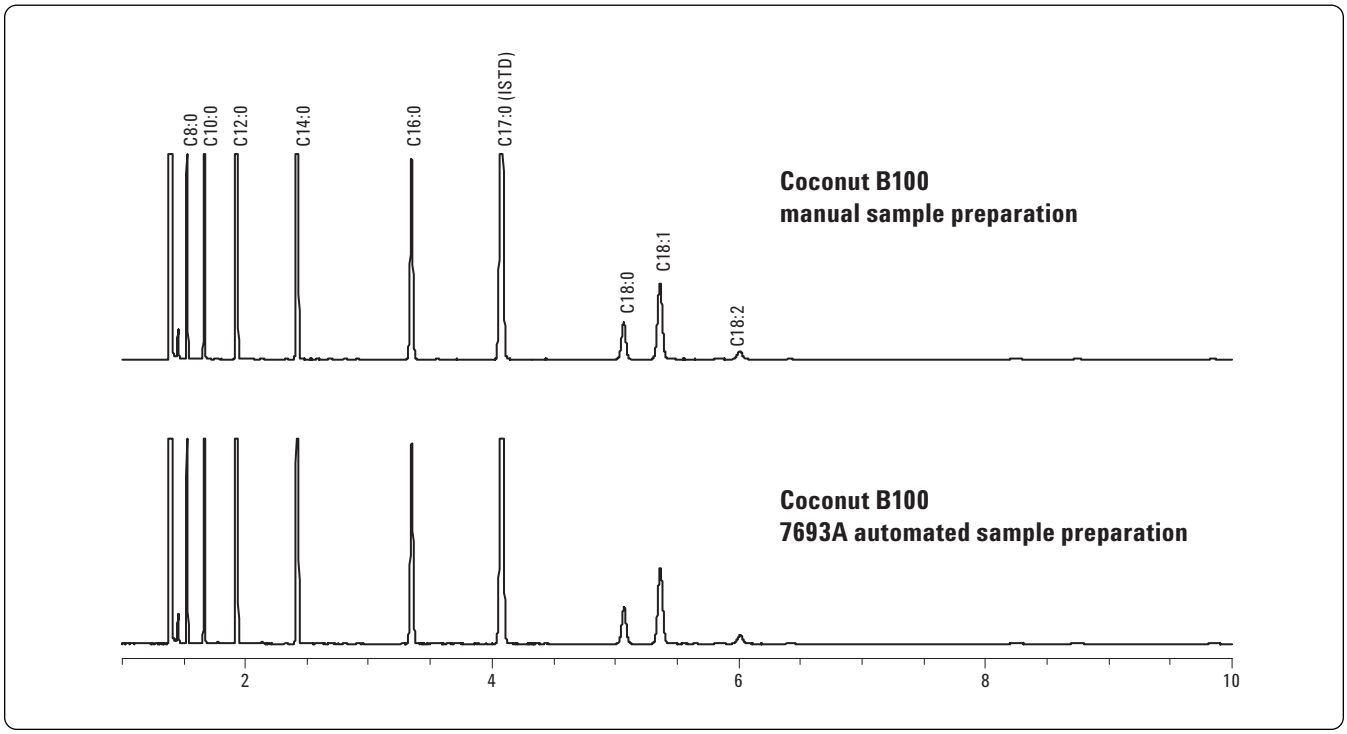

Figure 7. Analysis FAMEs in coconut oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

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.

References

1. "D6585 Test Method for Determination of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography"; ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2007.
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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Agilent Technologies

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.

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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

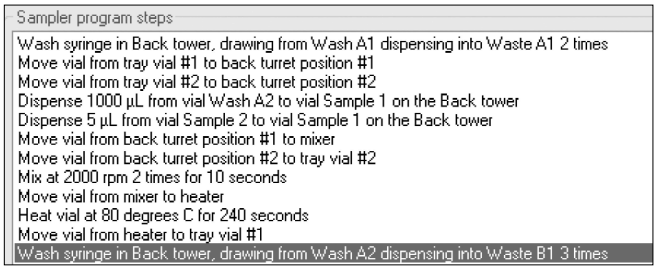
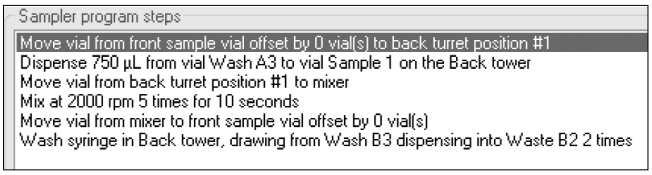


Table 2. Sample Program for the Dilution of a Biodiesel Sample Starting with 0.5 mL Biodiesel in a 2 mL Vial



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.

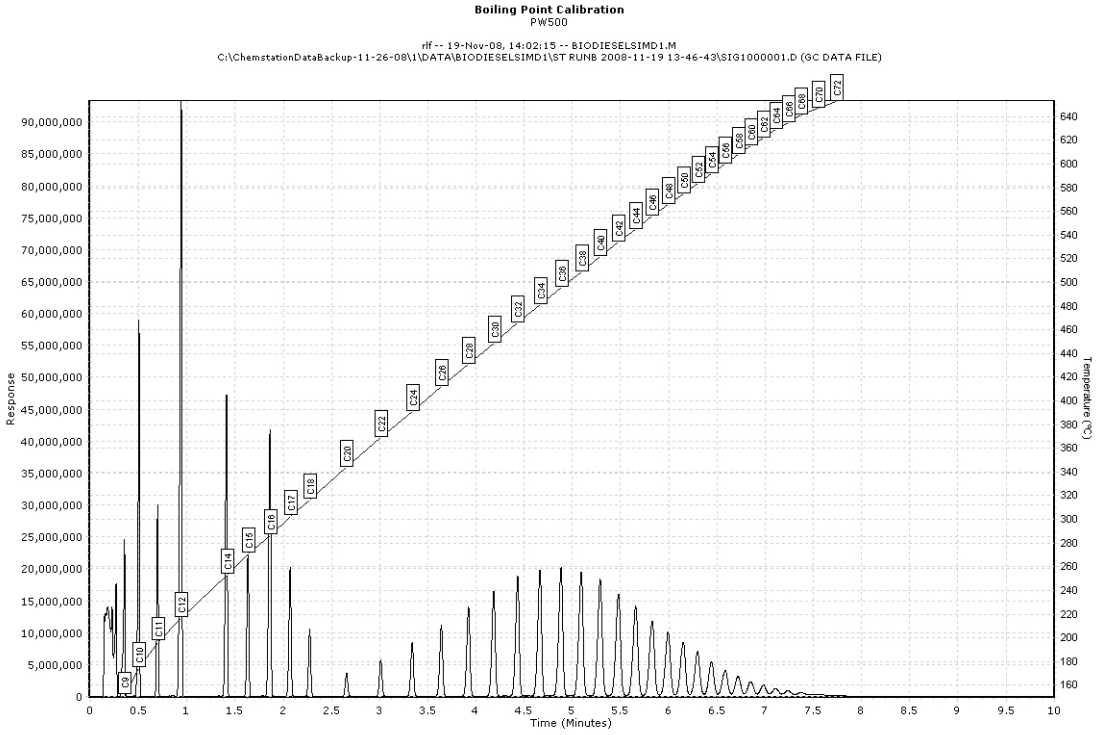


Figure 1. Calibration curve on LTM system from C9 to C72 prepared from PW500 and C5-C18 mix.

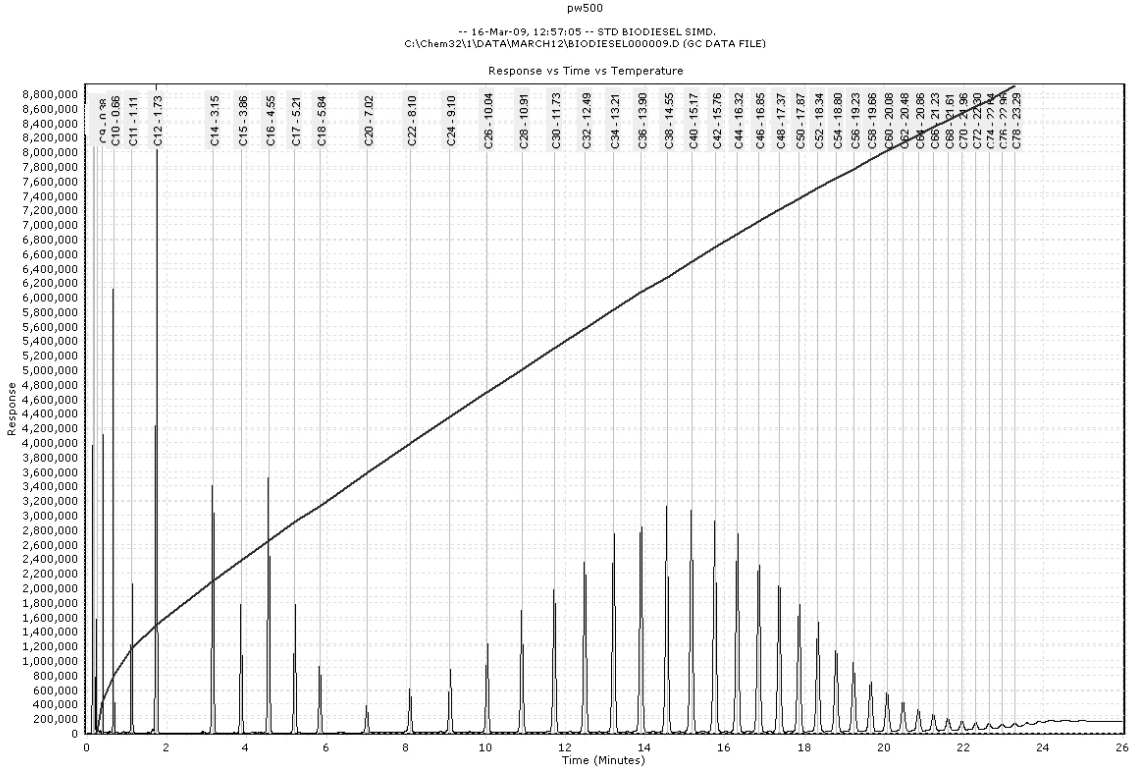


Figure 2. Calibration curve on standard 7890A GC.

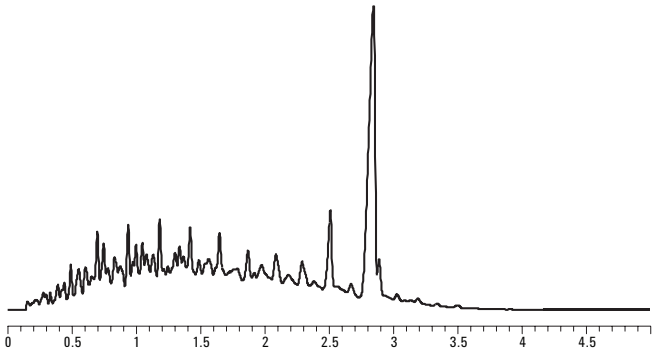


Figure 3. Chromatogram of B20 Soy based biodiesel using the LTM system.

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.

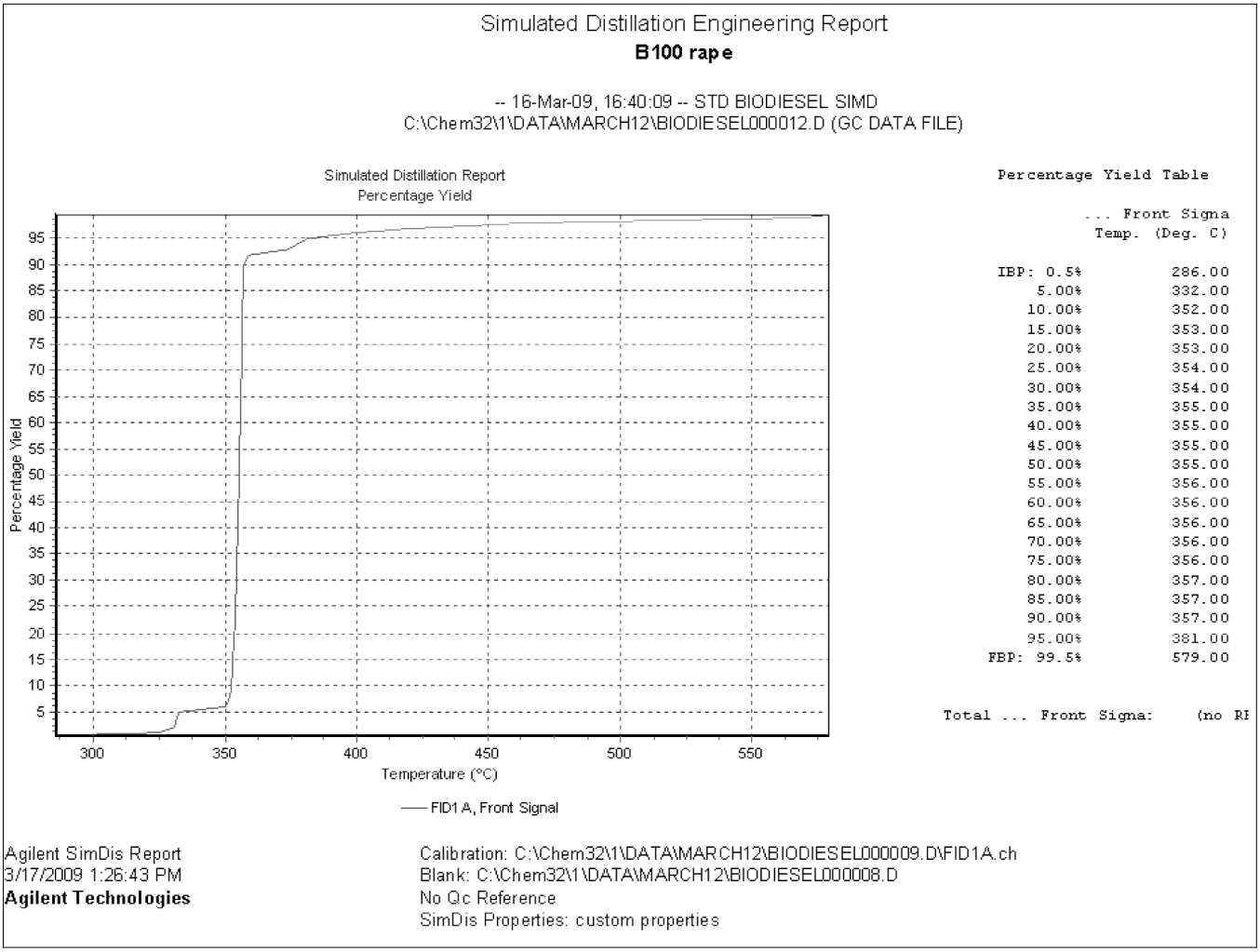


Figure 4. Boiling point distribution for rapeseed B100.

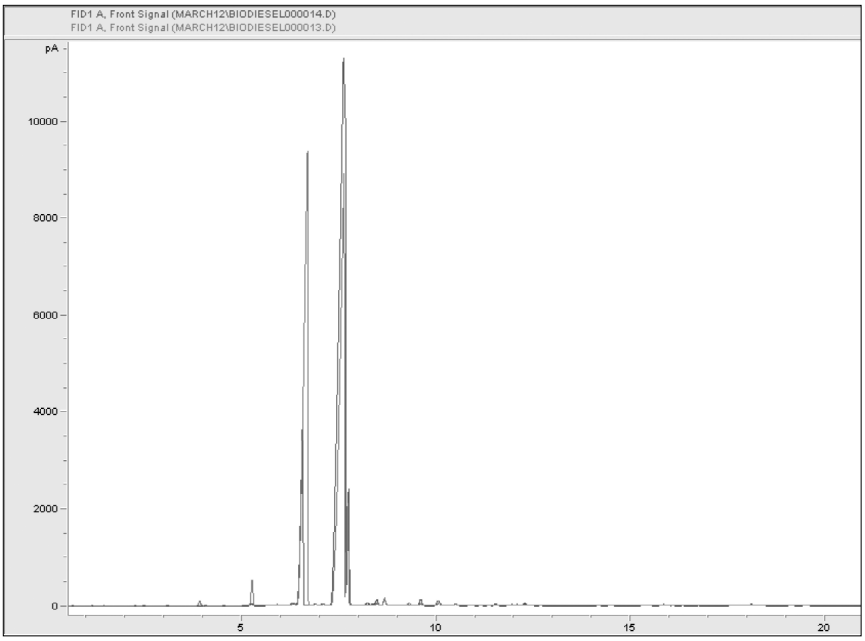


Figure 5. Overlay of two B100 samples from different producers. Both are soy based biodiesel. Producer A: signal 14, Producer B: signal 13.

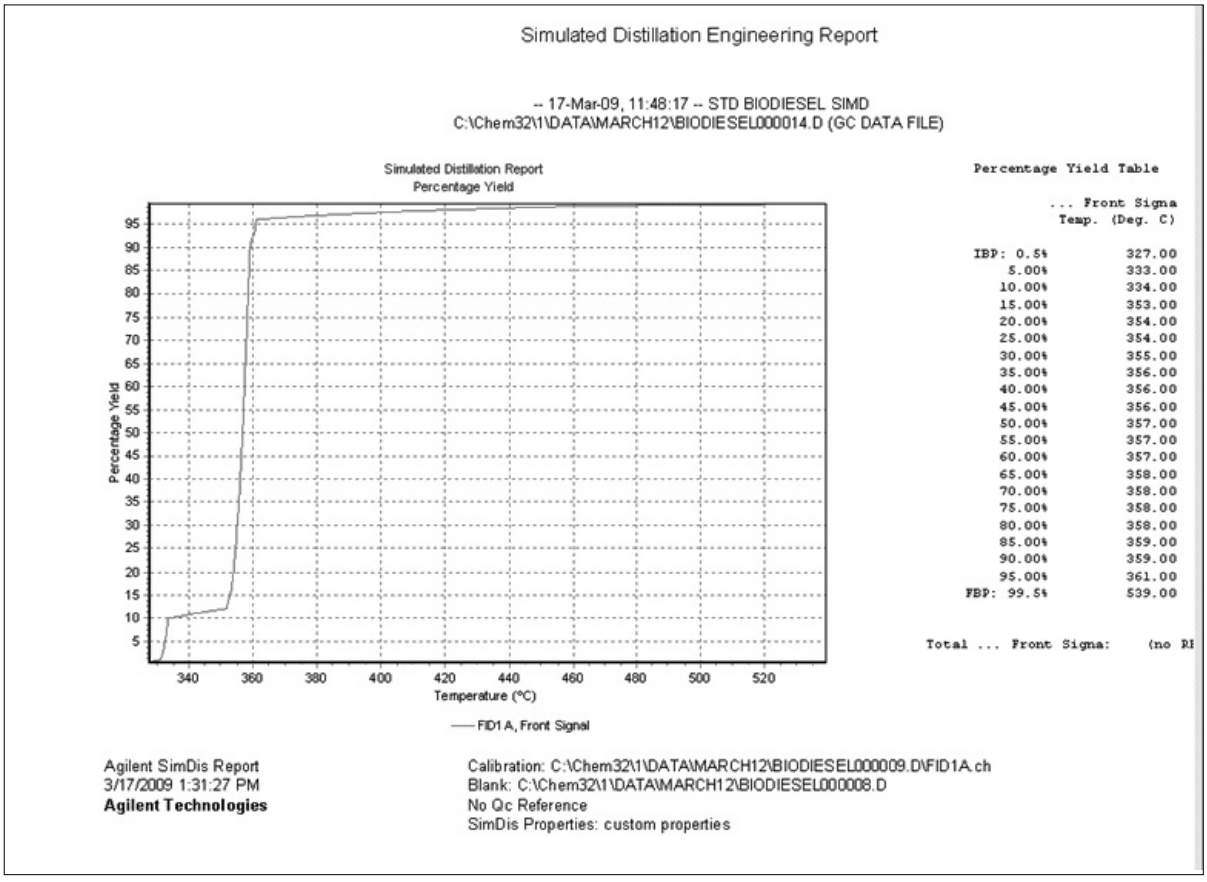


Figure 6. Boiling point distribution of B100, producer A.

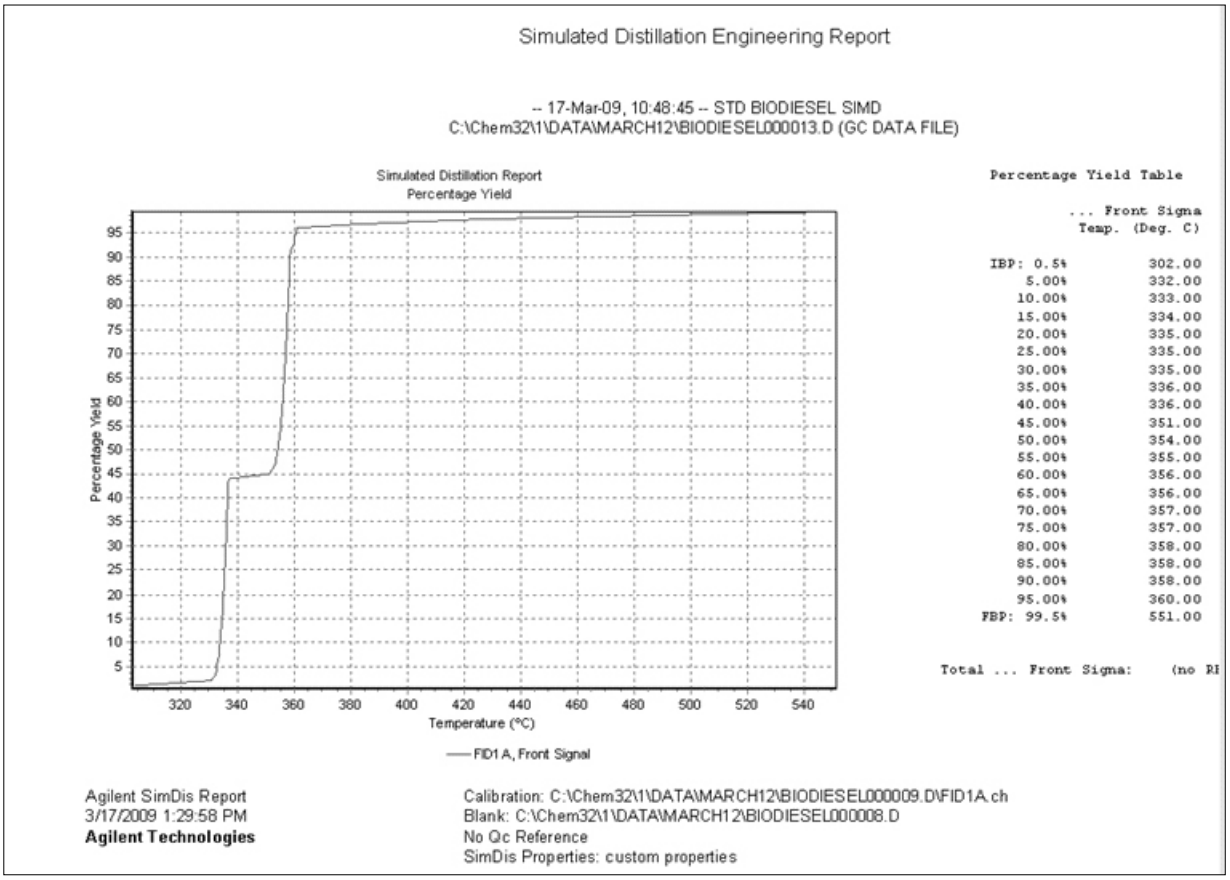


Figure 7. Boiling point distribution of B100 from producer B.

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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Agilent Technologies

Achieving Lower Detection Limits Easily with the Agilent Multimode Inlet (MMI)

Application Note

All Industries

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Abstract

This application note discusses three injection techniques: hot splitless, cold splitless, and solvent vent mode available on the Multimode Inlet. The cold splitless and solvent vent mode injections allow analysts to achieve a lower detection limit by making large volume injections (LVI). A total ion chromatogram overlay of 40-ppb pesticide standards from 2-µL hot splitless, 10-µL cold splitless and 25-µL solvent vent illustrates the improvement in signal-to-noise ratios using LVI.

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Introduction

A growing number of analysts are exploring large volume injection (LVI) techniques to improve existing analyses. With traditional liquid injection techniques in capillary gas chromatography, most inlets and columns can only handle 1 – 2 µL at a time. Attempts to increase the injection volume can lead to broadened and distorted analyte peaks, large and long solvent peak tails, and saturated or damaged detectors.

The purpose of increasing the injection volume is normally to improve detection limits in trace analysis. By introducing more of the sample to the system, the mass of analyte reaching the detector will be proportionally increased, resulting in larger peak areas and peak heights. If the baseline noise is constant, larger peak heights mean greater signal to noise ratios and lower system detection limits. An additional benefit of LVI is the ability to reduce the amount of sample originally processed. By injecting 10 – 100 times more volume of processed sample and concentrating it in the inlet, the sample preparation can start with 10 – 100 times smaller sample volume and still achieve the same mass of analyte on column. Another advantage of using LVI (solvent vent) is the decrease in solvent that actually reaches the detector. Usually, only 10 – 30% of the injection solvent actually enters the column and makes it to the detector.

LVI can be applied to injection volumes ranging from a few microliters up to 1 mL or more. In most LVI approaches, the sample solvent is evaporated and removed from the inlet system before the analytes are transferred to the separation column. In this way, LVI is similar to nitrogen evaporation or rotary evaporation of the solvent, with the added benefit of being performed in the GC inlet rather than in a fume hood. Analytes that would be lost during nitrogen evaporation may be retained in the inlet and successfully analyzed via LVI. Furthermore, the LVI process can be automated and is reproducible. As in the other evaporation techniques, the LVI approach is a function of the solvent type, the inlet temperature, the vent flow of evaporation gas, and the analyte boiling point. In addition, the inlet pressure during evaporation and the inlet liner have an impact on the rate of solvent removal and analyte recovery. These parameters will be discussed in this application note.

Experimental

MMI Operational Modes

The Agilent Multimode Inlet (MMI) uses the same liners and consumables as a standard split/splitless inlet, making it compatible with existing hot split and splitless methods. Its operational modes include: Hot Split/Splitless (also in pulsed mode), Cold Split/Splitless (also in pulsed mode), Solvent Vent and Direct mode.

Hot Splitless (for 1 – 3 µL injections)

For most analysts considering LVI, their current methods are using hot splitless injection. This proven and reliable sample introduction technique has worked well for almost 40 years; however, it does present some challenges to the sample integrity and to the method developer. First, the inlet must be hot enough to flash vaporize the solvent and analytes so that the resulting vapor cloud can be transferred to the column. The inlet liner volume must be sufficiently large to contain this vapor cloud. If the liner volume is too small, the vaporized sample can overflow the liner and reach reactive surfaces, leading to analyte loss. In addition, the pressure wave generated by the vaporized sample can push back against the incoming carrier gas and enter sensitive pressure and flow control systems. Using the Agilent pressure/flow calculator [1], a 1-µL injection of acetone into an inlet at 240 °C and 14.5 psig expands to 288 µL of gas. Most inlet liners for standard split/splitless inlets have a nominal volume of 1 mL. An increase of injection volume to only 3.5 µL under these conditions creates a vapor cloud of 1 mL which could easily overflow the inlet liner.

Hot splitless injection also creates a challenging environment for thermally unstable or labile analytes. Compounds such as the organochlorine pesticides DDT and endrin can rearrange to form breakdown compounds. This process is accelerated with the inlet temperatures normally used to analyze them. Effective chemical deactivation of the liner can minimize analyte breakdown. However, high inlet temperatures can decrease the lifetime of deactivated liners.

Another challenge created by hot splitless injection is the opportunity for needle fractionation or analyte discrimination. The needle temperature increases as the sample is being transferred from the syringe to the inlet because the needle is in contact with the septum. The rise in needle temperature can cause the solvent to "boil" away and deposit high boiling analytes inside the needle. To avoid this fractionation problem, some analysts load a solvent plug into the syringe first and then draw up the desired sample volume (available in 7693A Automatic Liquid Sampler). The thought is that the solvent plug will wash any deposits into the inlet. An effective way to address this problem is to make a high speed injection. This minimizes the time the needle is in contact with the septum and the time the sample touches the needle. Even with these issues, hot splitless injection is a well-accepted technique. An alternative technique, such as cold splitless can address these concerns and improve the analysis results.

Cold Splitless (for 1 – 10 µL injections)

MMI's versatile temperature programmability allows it to perform cold split and splitless analyses. In cold splitless mode, the MMI is cooled to a temperature below the normal boiling point of the sample solvent so that when the sample is injected, no vaporization takes place. The injection is simply a liquid transfer from the syringe to the inlet. Once the syringe is removed from the inlet, the inlet is heated to vaporize the sample and transfer it to the column. The solvent vaporizes first and moves to column, allowing analyte focusing to take place as in normal hot splitless injections. The analytes subsequently vaporize and move to the column. The main advantage is that the analytes vaporize at the lowest possible inlet temperature, rather than at a constant high temperature. This minimizes thermal degradation while still allowing a wide range of analytes to vaporize. Cold splitless operations also do not thermally stress the liner as harshly as hot splitless does, prolonging its usable life. Cold splitless can also extend the amount of sample that can be injected in some cases. If a slow inlet temperature program is used, the solvent can be vaporized slowly and will not overflow the liner volume. As long as the analytes can be refocused on the column, slow inlet temperature programs cause no detrimental effects to the chromatography.

Solvent Vent (for 5 – 1000 µL injections)

The solvent vent mode is the method which enables MMI to do LVI of more than 5 µL. In solvent vent mode, the inlet is kept at a low initial temperature during sample injection. Pneumatically, the inlet is in split mode with a low inlet pressure. The flow of gas through the inlet liner and out to vent removes the evaporating solvent. The sample is injected slowly so that the incoming liquid is deposited on the liner wall and the solvent evaporates at a similar rate. Once the entire sample has been injected, the inlet switches to a splitless mode for analyte transfer. The inlet is then heated to vaporize the concentrated sample and any remaining solvent and the vapor is transferred to the column. After a sufficient period to ensure the sample transfer, the inlet is then switched to a purge mode to allow any remaining material in the inlet liner to be vented. During the sample injection and solvent venting period, the GC oven has been held at an appropriate temperature to allow the solvent to refocus the analytes on the column. When this refocusing is complete, the oven is then programmed to perform the separation.

LVI Method Development

An effective procedure for developing an LVI method on a MMI is to run the existing method first to determine peak areas for a small volume injection. Such results serve as a baseline for evaluating the LVI method performance. The next step is to switch to the solvent vent mode with a slightly larger injection volume (for example, 2 to 5 times larger). By comparing the resulting peak areas and accounting for the increased injection volume, the analyte recovery can be calculated and conditions can be further optimized.

Backflush

A traditional bakeout step for removing late eluters can be very time consuming for samples with complicated matrices, even as long as the analysis time. Capillary flow devices (in this case, a purged ultimate union) provide backflush [2, 3] capability. "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. By reversing column flow immediately after the last compound of interest has eluted, the long bake-out time for highly retained components can be eliminated. Therefore, the column bleed and ghost peaks are minimized, the column will last longer, and the MS ion source will require less frequent cleaning. The split vent trap may require replacement more frequently than usual.

Instrument Parameters

GC	Agilent 7890A
MS	Agilent 5975C MSD
Column	HP-5MS UI, 15 m × 0.25 mm × 0.25 µm (19091S-431UI), from inlet to purged union
MMI	Constant pressure (~18 psi), chlorpyrifos-methyl RT locked to 8.297 min, 2 psi at post run for backflush
MMI liner	Double taper deactivated, Helix (5188-5398)
Septum purge	3 mL/min
Purged Union	4 psi; 70 psi at post run for backflush
Restrictor	0.7 m × 0.15 mm deactivated fused silica tubing (from purged union to MSD)
Syringes	10 µL, for splitless injections (5181-3354) 50 µL, for solvent vent mode (5183-0318)
ALS	Agilent 7693A
MS parameters	
Solvent delay	2.5 min
Gain factor	1
Mass range	44–550
Threshold	0
Samples	2
Tune file	atune.u

Oven

Initial temperature	70 °C
Initial hold time	1 min
Rate 1	50 °C/min
Temperature 1	150 °C
Hold time	0 min
Rate 2	6 °C/min
Temperature 2	200 °C
Hold time	0 min
Rate 3	16 °C/min
Temperature 3	280 °C
Hold time	5 min
Total runtime	20.933 min
Post run	5 min (for backflush)
Oven post run temp	280 °C

Sample: 40-ppb pesticide standards in acetone (for a list of compounds, see Figure 5).

Multimode Inlet (MMI)

Parameter	Hot Splitless	Cold Splitless	Solvent Vent
Initial temperature	280 °C	30 °C	35 °C
Initial time	–	0.01 min	0.35 min
Rate 1	–	700 C/min	700 °C/min
Final temperature	–	320 °C	320 °C
Vent flow	–	–	150 mL/min
Vent pressure	–	–	5 psig
Vent time	–	–	0.33 min (from
	–	–	calculator, Figure 3)
Purge time	0.75 min	1.25 min	1.5 min
Purge flow	50 mL/min	50 mL/min	50 mL/min
Injection volume	2 µL	10 µL	25 µL
Injection speed	Fast	Fast	75 µL/min (from
	–	–	calculator, Figure 3)
Cryo	–	On (liquid CO_2)	On (liquid CO_2)
Cryo fault detection	–	On	On
Cryo use temperature	–	125 °C	125 °C
Time out detection	–	On (15 min)	On (15 min)

The parameters for the 25-µL Solvent Vent injection were determined with the Solvent Elimination Calculator integrated in the ChemStation. This calculator was designed to help determine reasonable starting conditions for LVI methods. When the MMI is put into the PTV Solvent Vent mode, an additional button appears in the inlet screen, shown in Figure 1.

In the first screen of the Solvent Elimination Calculator (Figure 2), the sample solvent and desired injection volume are selected and entered. The calculator "knows" the syringe currently installed and will only allow 50% of that volume to be injected at once. Larger injection volumes can be entered into the calculator but the injection volume will not be downloadable. The calculator also requests the boiling point of the earliest eluting analyte, as this allows the initial inlet temperature to be selected. If the boiling point is unknown, the temperature should be left at 150 °C as this will work for a wide range of analytes.

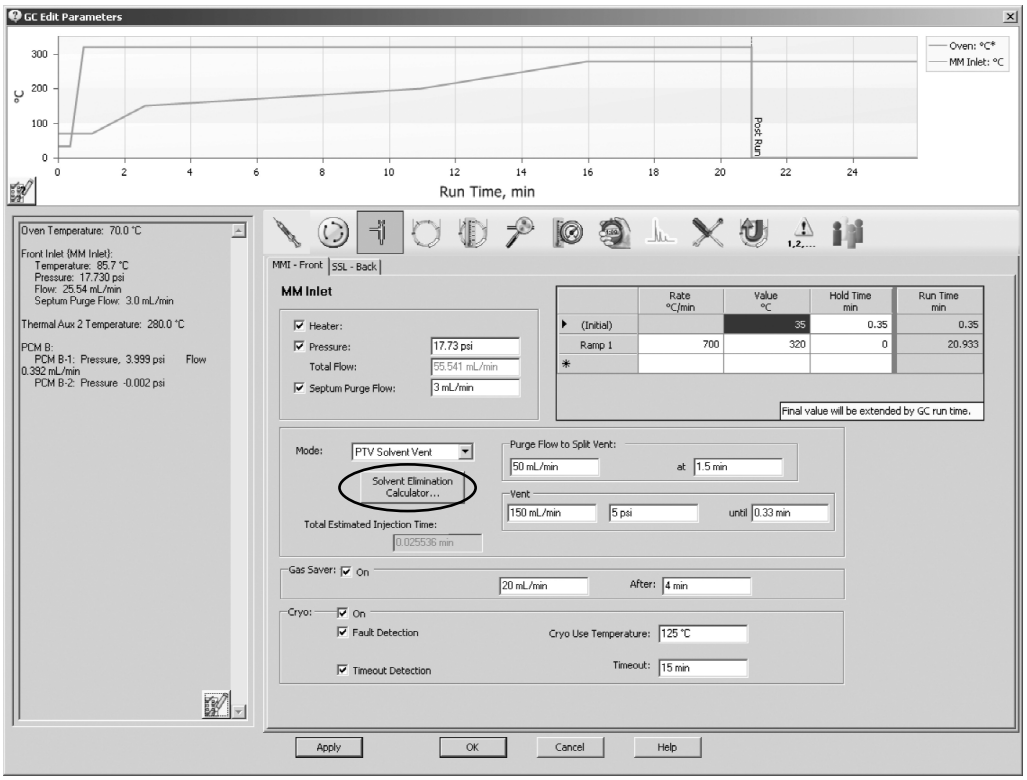

Figure 1. Multimode Inlet "Solvent Elimination Calculator" imbedded in ChemStation for easy method development.

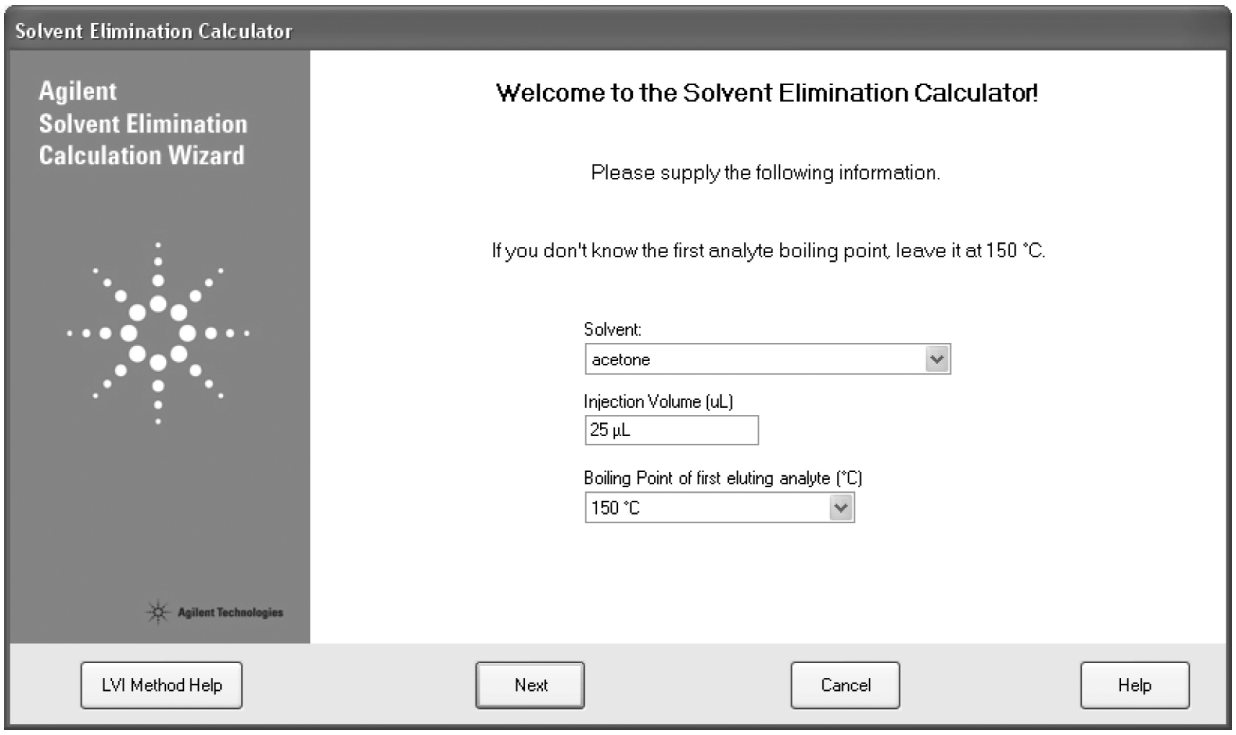

Figure 2. Select solvent of choice and enter the injection volume to start the calculation.

Figure 3 shows the calculation screen. The calculator uses an initial set of inlet conditions to determine the solvent elimination rate according to fundamental theory [4]. This "Elimination Rate" does not account for other factors (for example, local cooling due to solvent evaporation) specific to LVI and is normally faster than that determined from practical experience. The "Suggested Injection Rate" does consider these factors and is designed to leave a small amount of solvent in the liner at the end of the venting period. This solvent serves as a liquid "trap" for the more volatile analytes and promotes their recovery. The "Suggested Vent Time" is determined by dividing the injection volume by the "Suggested Injection Rate."

Several variables for determining elimination rate can be set by the user in the lower portion of the window. A small change in inlet temperature has a significant impact on elimination rate. Vent flow has a linear effect such that a decrease by a factor of two in vent flow gives an equal decrease in elimination rate. As the vent pressure decreases, the elimination rate increases. Bear in mind that the vent pressure also impacts the amount of solvent that reaches the column during venting. As the vent pressure is increased, more solvent is loaded onto the column before the analytes are transferred. Finally, the type of solvent, specifically its normal boiling point, has a substantial impact on the elimination rate.

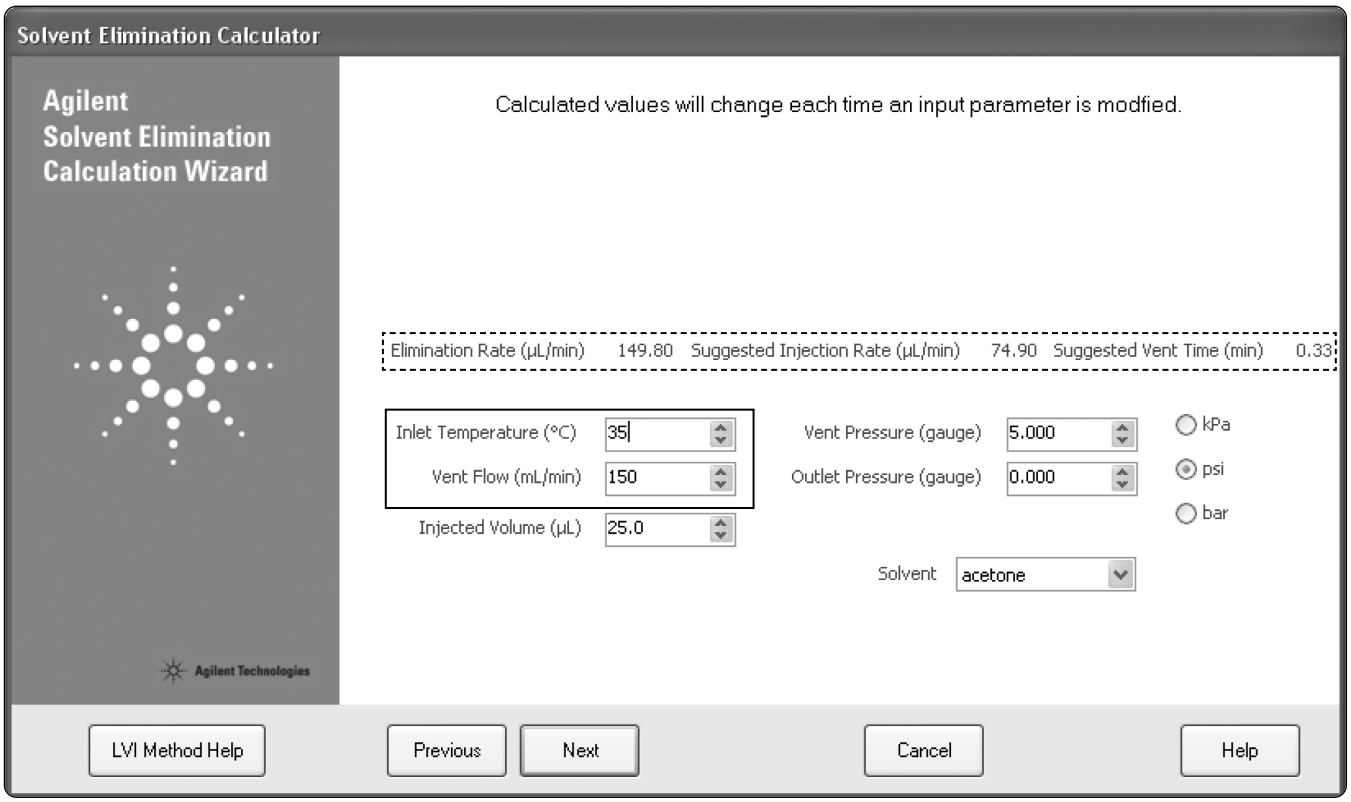

Figure 3. The calculator calculates the injection rate and vent time according to the selected inlet temperature and vent flow.

The download screen in Figure 4 shows all of the method changes that are downloaded to the edit parameters screen. The check boxes allow the user to accept (by checking) or reject any of these parameters. The oven initial temperature and hold times are not automatically checked in case the current method requires these values to be unchanged (for example, a Retention Time Locked method).

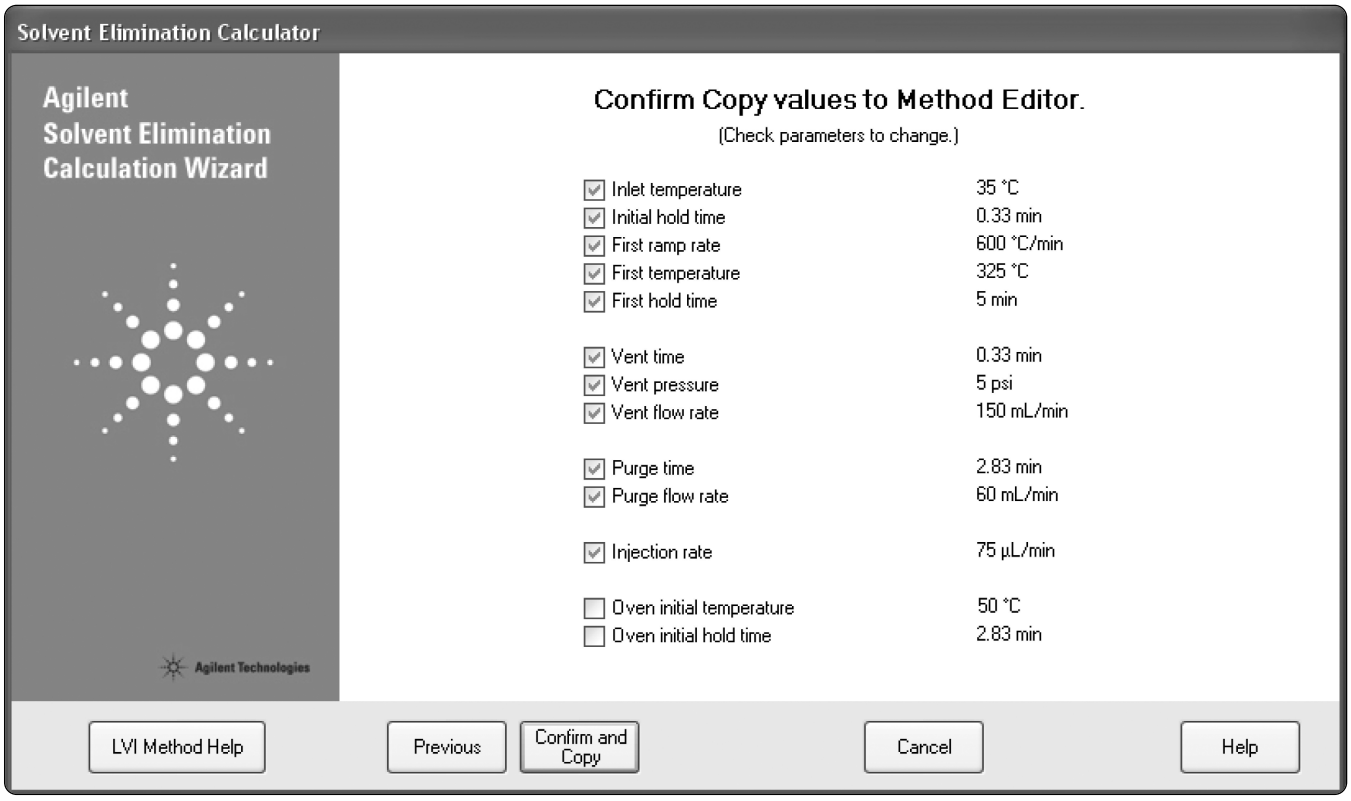

Figure 4. Confirm values suggested by the Calculator and download to ChemStation.

Results and Discussion

Figure 5 compares the responses of a 40-ppb standard solution from three injection modes.

The bottom total ion chromatogram (TIC) is a typical 2-µL hot splitless injection. Some of the 40-ppb pesticides are barely visible (80 pg each on column). The middle TIC is from a 10-µL cold splitless injection. The MMI starting temperature was 30 °C. In this TIC, the on column amount for each analyte is 400 pg. Lastly, the top TIC is from a 25-µL solvent vent injection with MMI starting temperature at 35 °C. In this TIC, the signal-to-noise ratio is significantly better than the TIC from hot splitless injection (bottom TIC), as noted in the Introduction section. The peak shape and resolution are maintained, even with the 25-µL injection volume. This implies that the solvent was mostly eliminated during the injection.

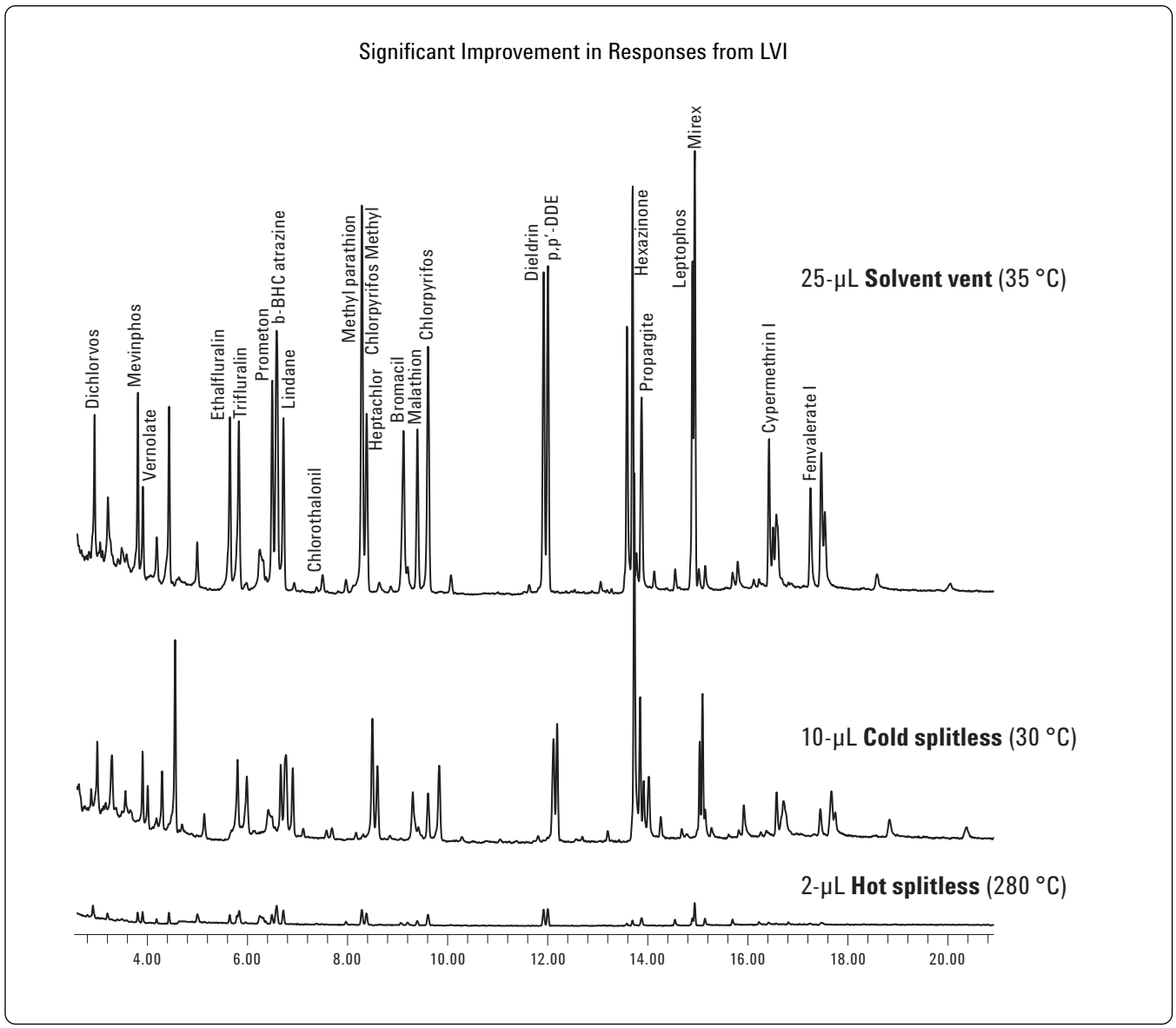

Figure 5. Overlay of total ion chromatograms (TICs) from three injection modes, plotted on the same scale.

Conclusion

The new Agilent Multimode Inlet (MMI) has the same form factor and uses the same consumables (for example, liners, o-rings and septa) as the existing split/splitless inlet, allowing existing hot splitless methods to be replicated. In addition, the temperature programmability permits both cold splitless and large volume injection (LVI) methods for improved detection limits. An integrated Solvent Elimination Calculator provides a complete set of initial conditions for easy LVI method development. The application results show a significant signal-to-noise improvement (lower detection limits) comparing the 25-µL solvent vent injection to the 2-µL hot splitless injection.

References

1. Agilent Pressure/Flow Calculator Included in the Instrument Utility DVD, available with each gas chromatograph and MMI accessory kit.
2. Chin-Kai Meng, "Improving Productivity and Extending Column Life with Backflush, "Agilent Technologies publication, 5989-6018EN, December 2006.
3. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Technologies publication, 5989-5111EN, June 2006.
4. J. Stanieski and J. Rijks, *Journal of Chromatography* 623 (1992) 105-113.

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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 $C_{55}H_{98}O_6$.

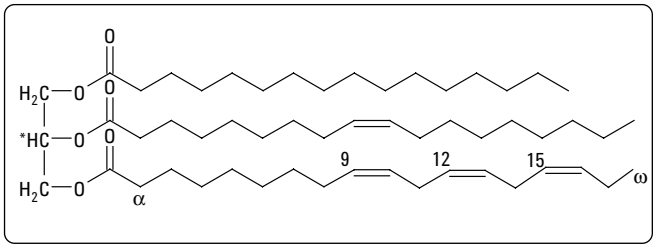

Figure 1. Typical triglyceride structure.

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.

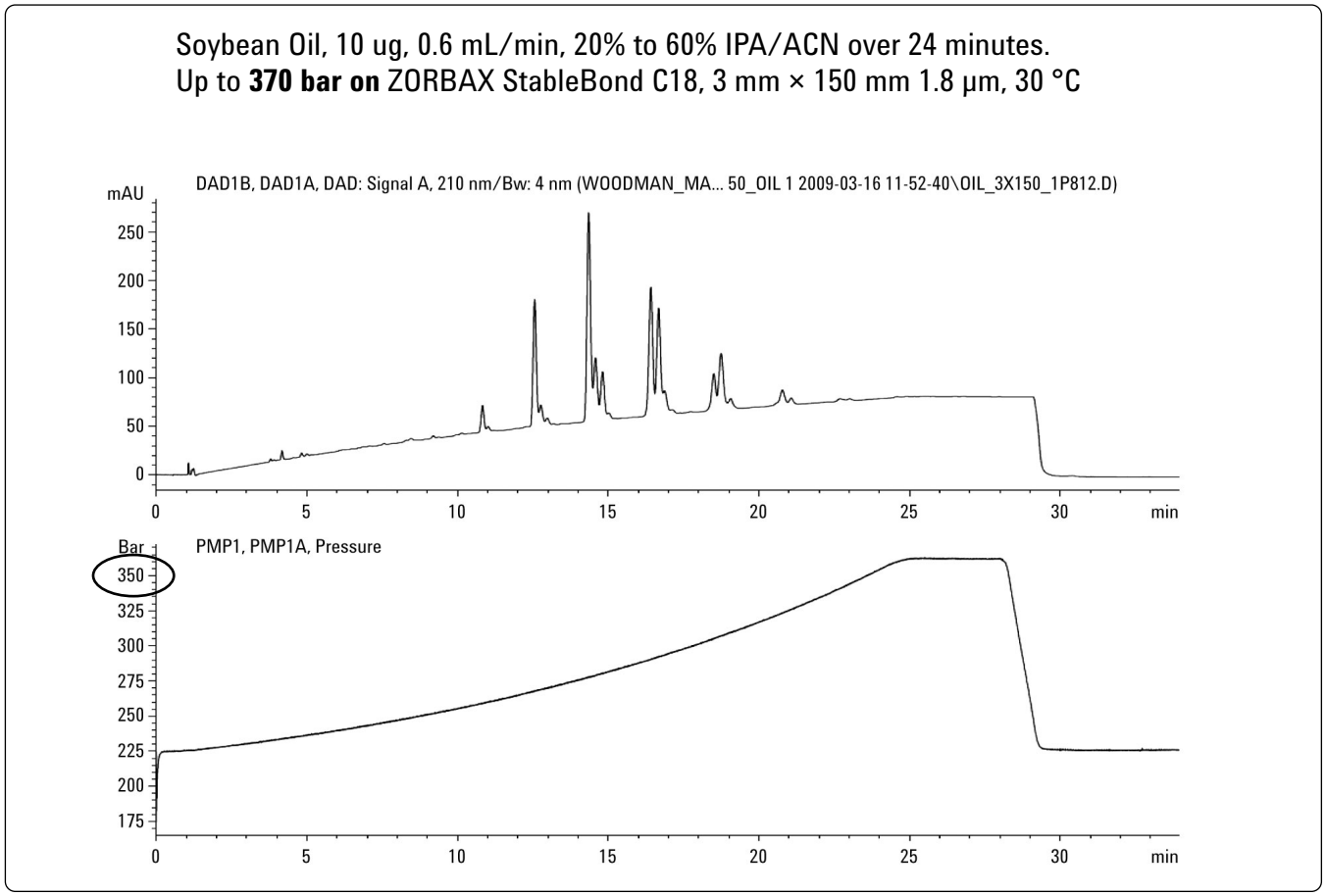

Figure 2. A 210-nm UV chromatogram of soybean oil sample on a 3 mm × 150 mm ZORBAX Rapid Resolution High Throughput (RRHT), 1.8 µm column, upper panel. System pressure trace showing the general progress of the gradient elution, lower panel. Flow rate 0.6 mL/min, gradient time 24 min. Strong solvent, 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.

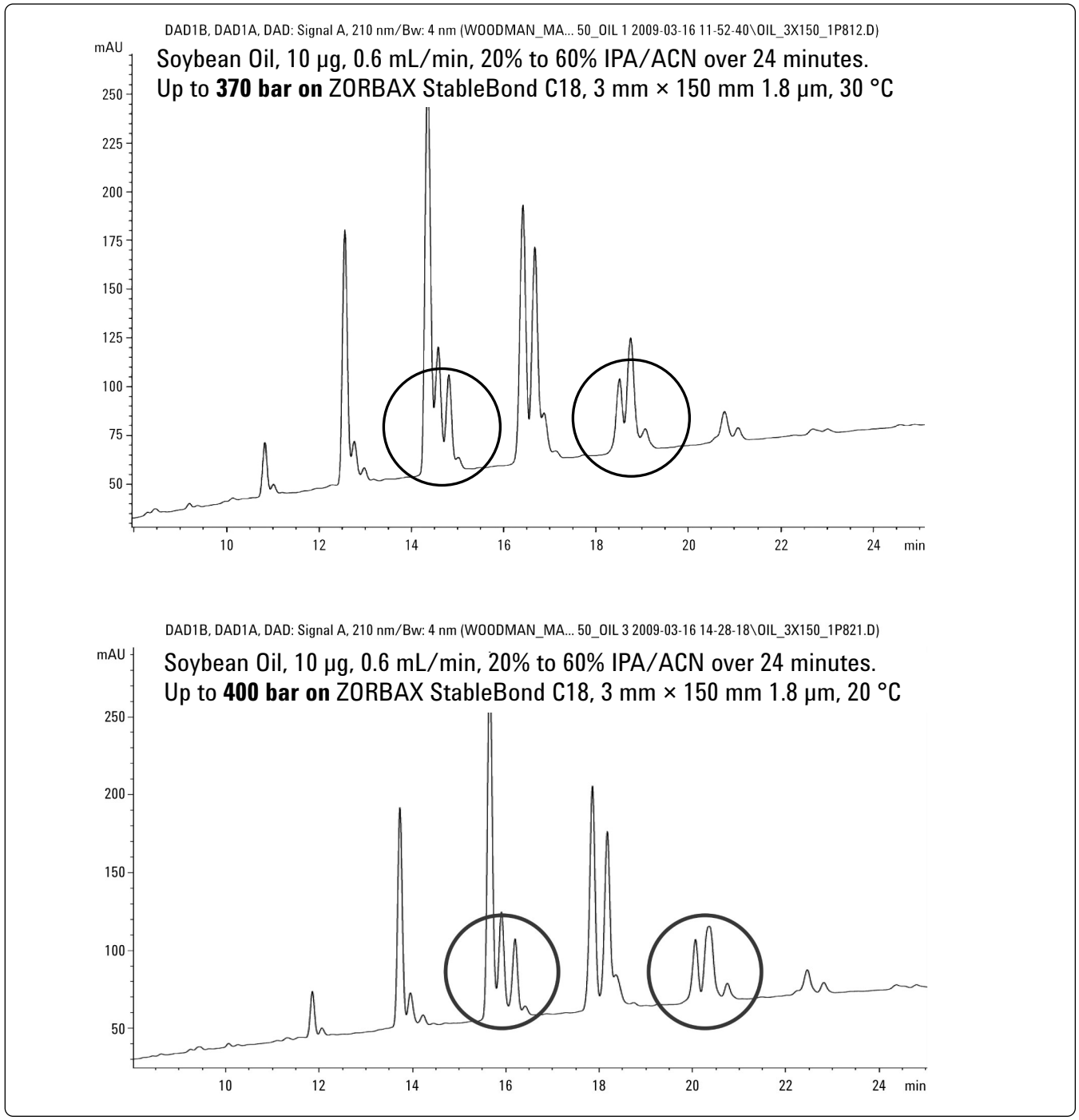

Figure 3. An expanded presentation of the chromatogram shown in Figure 2 at 30 °C, upper panel, compared with the same conditions in Figure 2 operating the column at 20 °C.

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.

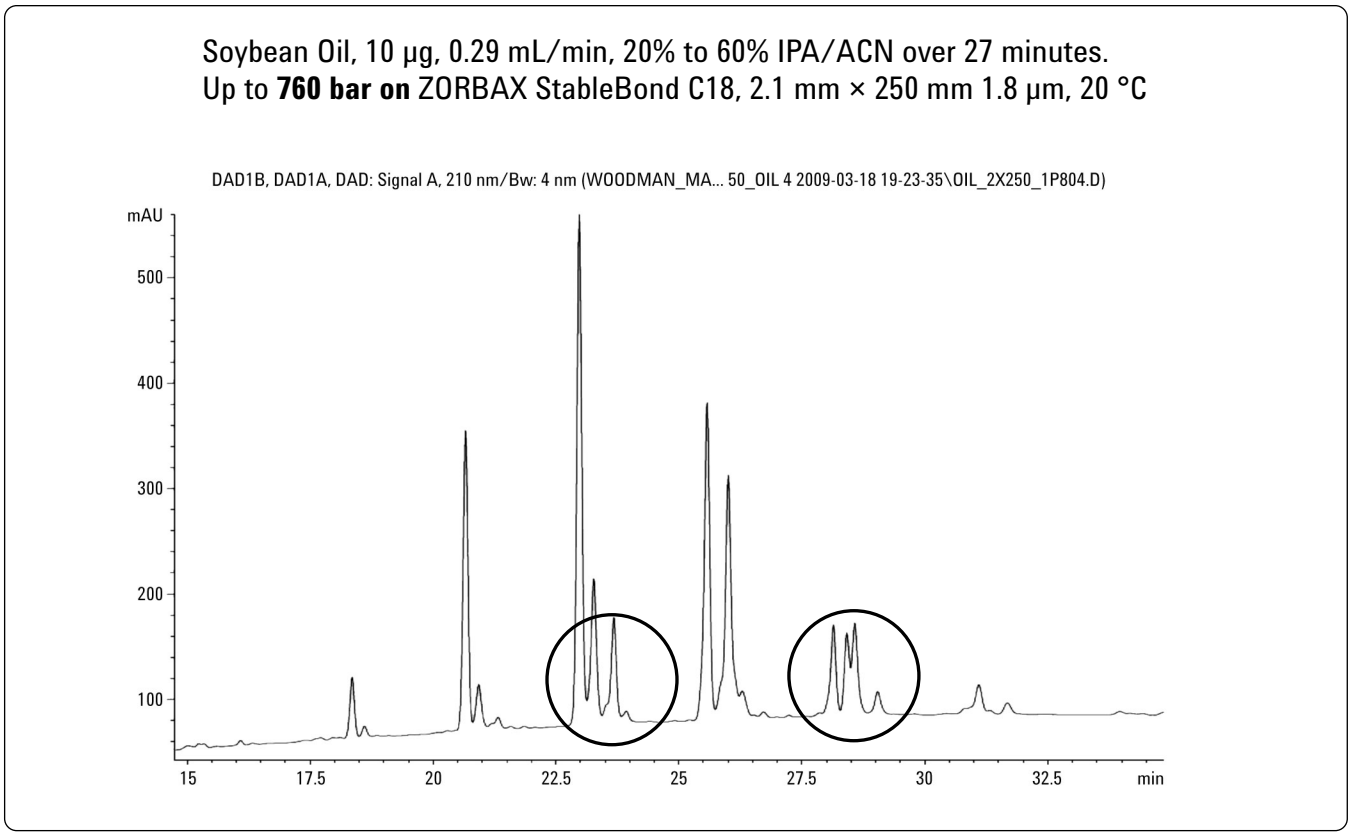

Figure 4. Analysis of the soybean oil sample on an Agilent ZORBAX StableBond C18 column, 2.1 mm × 250 mm, 1.8 µm, (150 mm in series with 100 mm) prepared for operation at 1200 bar pressure limit. Flow rate 0.29 mL/min, gradient time 27 min. Maximum observed pressure 760 bar.

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.

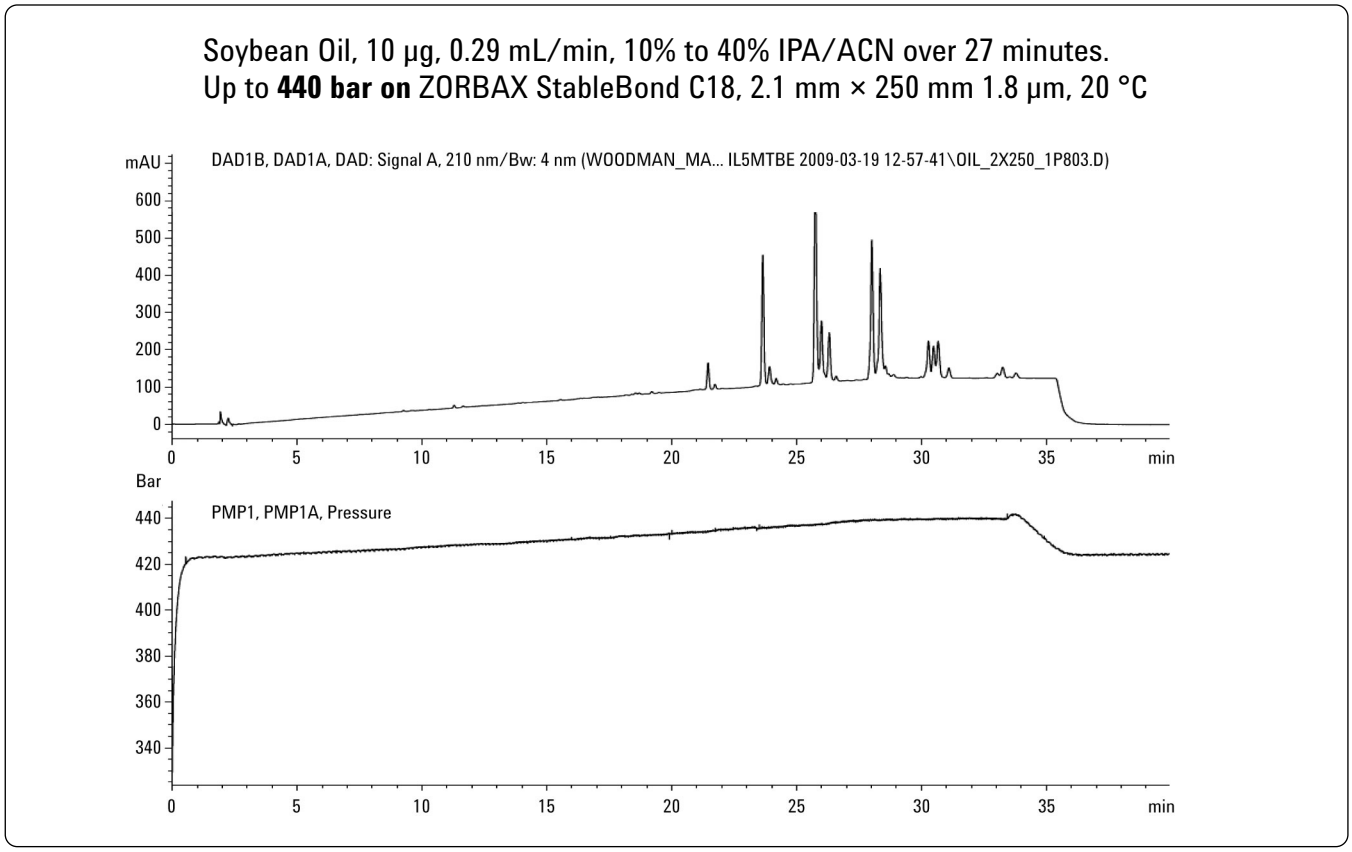

Figure 5. By substituting MTBE for isopropanol with otherwise the same conditions as Figure 4, and then re-optimizing the gradient for the significant increase in eluting strength of MTBE, we arrive at a new set of operating conditions where there is only a small difference in operating pressure over 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.

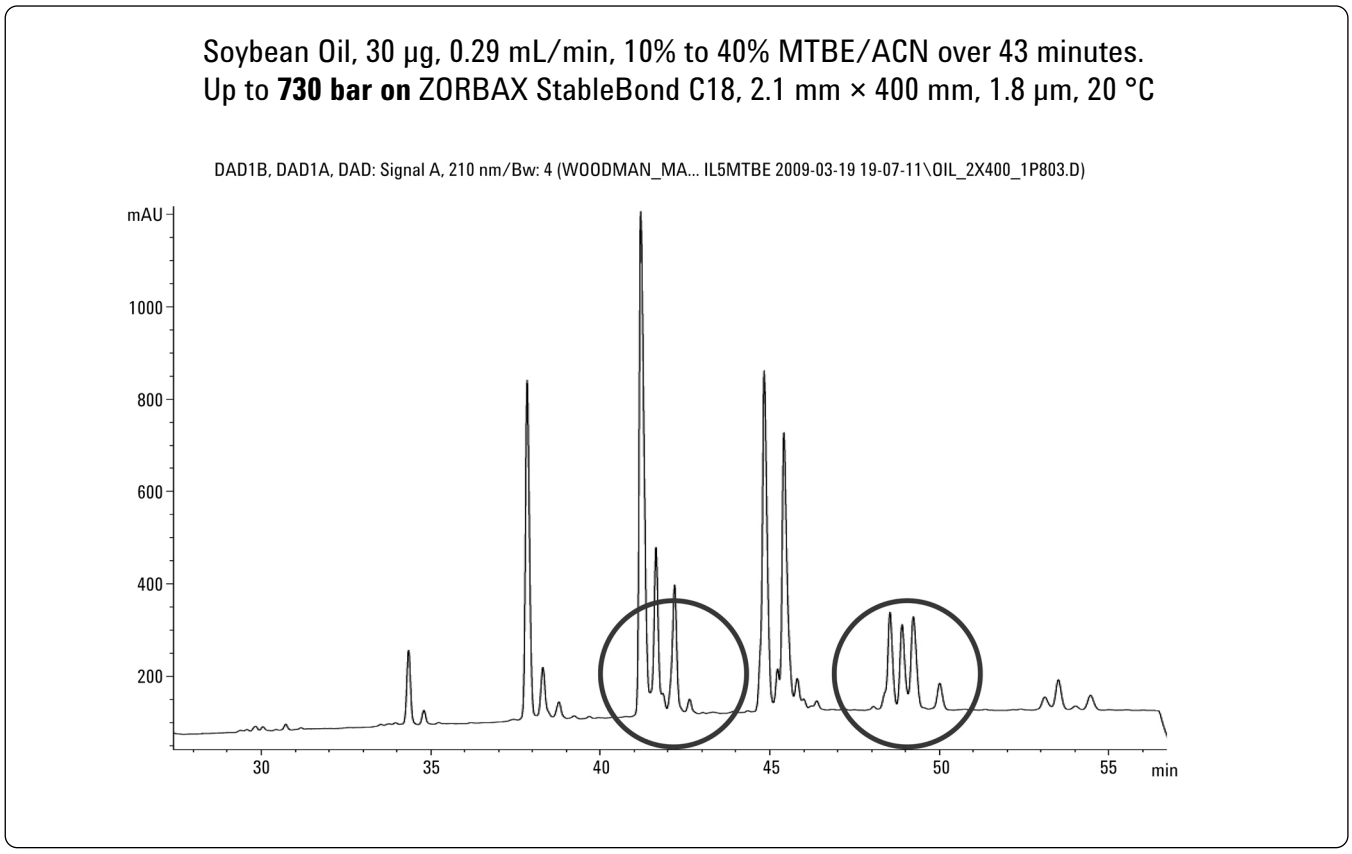

Figure 6. Separation of the soybean oil sample on a 2.1 mm × 400 mm ZORBAX StableBond C18, 1.8 µm 1200 bar columns (150 mm + 150 mm + 100 mm in series). Flow rate 0.29 mL/min gradient time 43 min, for a gradient of 10% to 40% MTBE. Maximum operating pressure 730 bar at 20 °C.

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).

References

1. E. G. Perkins, "Analyses of Fats, Oils, and Derivatives," AOCS Press, 1993
2. http://en.wikipedia.org/wiki/File:Fat_triglyceride_shorthand_formula.PNG
3. (http://www.chem.agilent.com/en-US/products/instruments/lc/pages/gp60931.aspx)
4. D. McIntyre, “The Analysis of Triglycerides in Edible Oils by APCI LC/MS”, May, 2000 Agilent Technologies publication 5968-0878E

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High resolution of complex lipids (triglycerides) using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Lipid Analysis

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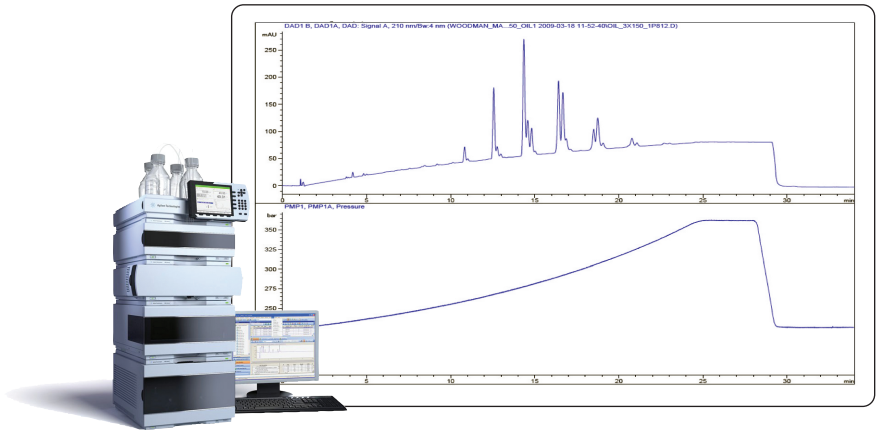

Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. With a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system, and the flexibility to operate a wide range of column dimensions and particle sizes, it is extremely useful for method transfer from any HPLC or UHPLC to the 1290 Infinity system. It allows the user to access capabilities not otherwise available.

Introduction

The typical HPLC resolution is shown by a separation of complex triglycerides in vegetable oil. Using a 24-min gradient and a 3.0 mm × 150 mm, 1.8 µm column, the analysis time of 35 min is typical; however, resolution is insufficient for good compositional investigation of the mixture. The separation of the main components is shown in Figure 1.

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The high resolution of the system is further demonstrated by separation on a much longer column, using more of the power range of the system. At 0.29 mL/min, incorporating a shallow gradient condition and an RRHD, 2.1 mm × 400 mm, 1.8 µm column, the separation is dramatically improved. The separation of the main components is shown in Figure 2. Subambient column temperature control, a standard feature of the Agilent Thermostatted Column Compartment, has significant advantages for many difficult isomer separations, including enantiomeric separations, and for shape-selective separations such as polycyclic aromatic hydrocarbons.

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 high resolution and pressure capability of the system allows one to use high efficiency 2.1 mm id columns, generating approximately 97,000 theoretical plates and having approximately 400% lower solvent consumption compared to 4.6 mm id columns. With nearly 3 times higher efficiency, run time was increased by only about 80%. The low flow rate and high resolution facilitate the interfacing of the separation to high resolution TOF and QTOF mass spectrometers to produce high confidence peak identification and compositional information.

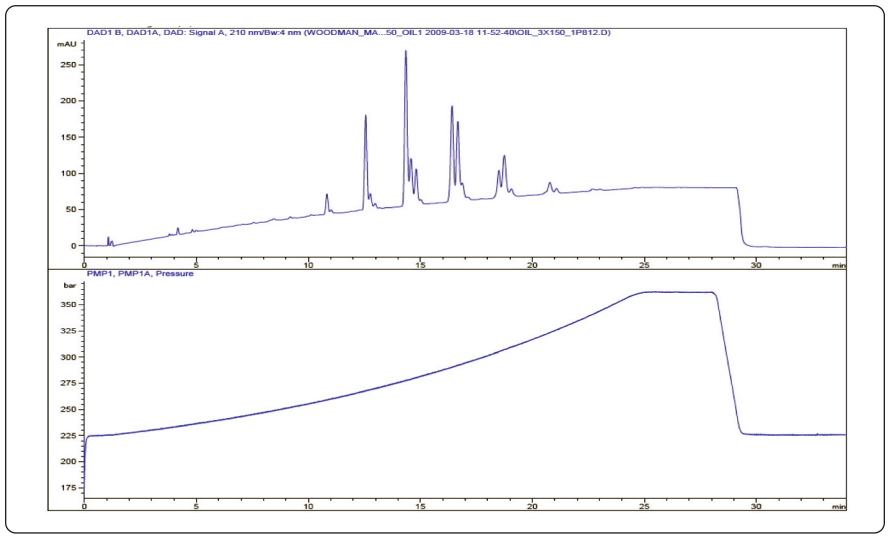

Figure 1
Analysis of vegetable oil components on the 1290 Infinity LC. Sample: soybean oil, 10 mg/mL, 10 µg on column. Conditions: 0.6 mL/min, 20% to 60% IPA vs. ACN at 24 min, hold to 30, run 35 min, ZORBAX RRHT StableBond C18, 3 mm × 150 mm 1.8, µm, 30 °C. Maximum operating pressure, 370 bar.

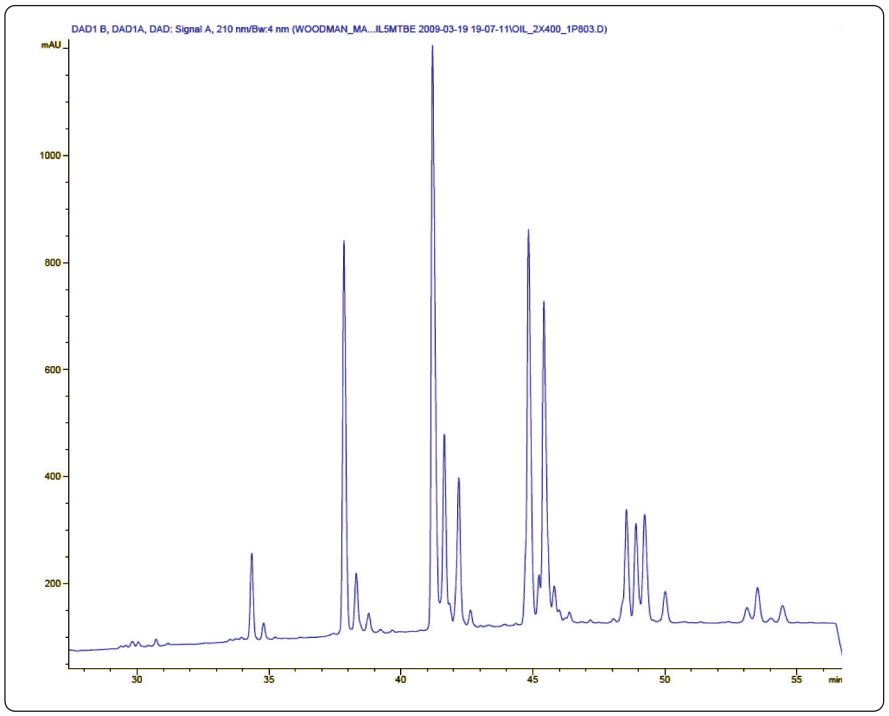

Figure 2
Analysis of soybean triglycerides on the 1290 Infinity LC. Sample: soybean oil, 10 mg/mL, 30 µg on column. Conditions: 0.29 mL/min, 10% to 40% MTBE vs. ACN at 42 minutes, hold to 55 minutes, run 60 minutes, 210 nm UV. ZORBAX RRHD StableBond C18, 2.1 mm × 400 mm (2–150 and 1–100 mm length in series), 1.8 µm, 20 °C. Operating pressure 730 bar.

www.agilent.com/chem/1290

October 1, 2009
Publication Number 5990-4881EN

Agilent Technologies

Analysis of Trace 2-Ethylhexyl Nitrate in Diesel Using Chemiluminescence Detector (NCD)

Application Brief

ChunXiao Wang and Roger Firor

HPI

An increase in the use of fleet diesel vehicles has helped define requirements for diesel fuel for light duty engines. One of these requirements is to recognize the influence of the cetane number on cold start properties, exhaust emissions and combustion noise. Several types of chemicals such as alkyl nitrates, ether nitrates or nitroso compounds have been identified as effective in increasing the cetane number. The most commonly used cetane enhancer is 2-ethylhexyl nitrate (2-EHN). ASTM D4046 standard test method is used for determining the amount of alkyl nitrate added to diesel fuel to judge compliance with specifications covering any alkyl nitrate. This method uses spectrophotometry with a detection range of 0.03 to 0.30 volume percent. The Agilent 7890A GC system configured with a chemiluminescence detector (NCD) provides an alternative method to ASTM D4046 with excellent results. Although the detection of 2-EHN is very difficult because of its low concentration in diesel fuel, a NCD can deliver both the required sensitivity and selectivity as shown in this analysis report.

Experiment

Table 1. Typical GC Conditions

Inlet:	250 °C, Split: 10:1
Column:	HP-5MS, 15 m × 0.32 mm, 0.32 µm, 3.9 mL/min, constant flow:
Oven:	60 °C (2 min), to 280 °C (8 min) at 20 °C/min
NCD	
Temperature:	200 °C
Detector pressure (Torr):	7.7
Dual plasma controller pressure (Torr):	110
Burner temperature:	905 (°C)
Hydrogen flow rate (sccm):	5
Oxidant flow rate (sccm):	10 (oxygen)

Highlights

- High sensitivity in analyzing nitrogen at low ppm levels. The results demonstrate a good signal-to-noise ratio for 2-EHN as nitrogen in diesel at the 1.87 ppm level.
- High selectivity for nitrogen over carbon. Analyzes nitrogen in diesel without suffering from any hydrocarbon interference.
- Linear response simplifies calibration. The results illustrate a linear response to nitrogen (2-EHN) over the concentration range of interest.
- An equimolar response simplifies quantification of unknowns, eliminating the need for determining separate response factors for individual nitrogen compounds.

Results

The Agilent 255 NCD delivers the sensitivity required for analysis of 2-EHN in diesel without hydrocarbon interference.

Figure 1 shows a good signal-to-noise for 2-EHN as nitrogen in diesel at 1.87 ppm. The result also demonstrates the selectivity of the detector showing no response from the diesel hydrocarbon background.

Trace 2-EHN added to diesel fuel can be found in pump diesel and B20 biodiesel. Figures 2 and 3 show chromatograms with nitrogen species in pump diesel and B20 biodiesel, respectively. The concentration determined for 2-EHN as nitrogen is 1.18 ppm and 18.7 ppm respectively. Also, other higher boiling nitrogen species are observed in both pump diesel and B20 biodiesel.

Figure 3 illustrates linear response to nitrogen (2-EHN) over the concentration range of the interest.

The precision for analysis of 2-EHN in pump diesel with an RSD of 1.15% is shown in Table 2.

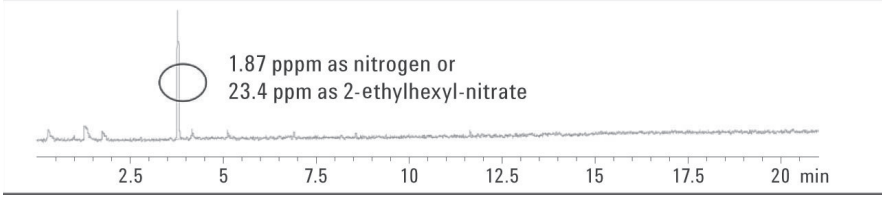

Figure1. Standard sample: 2-EHN at a concentration of 23.4 ppm in diesel (nitrogen free).

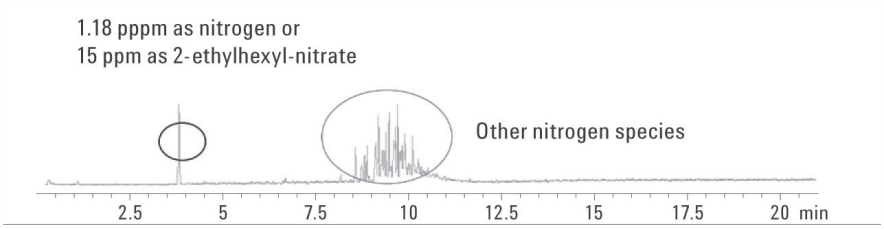

Figure 2. Nitrogen species in pump diesel fuel.

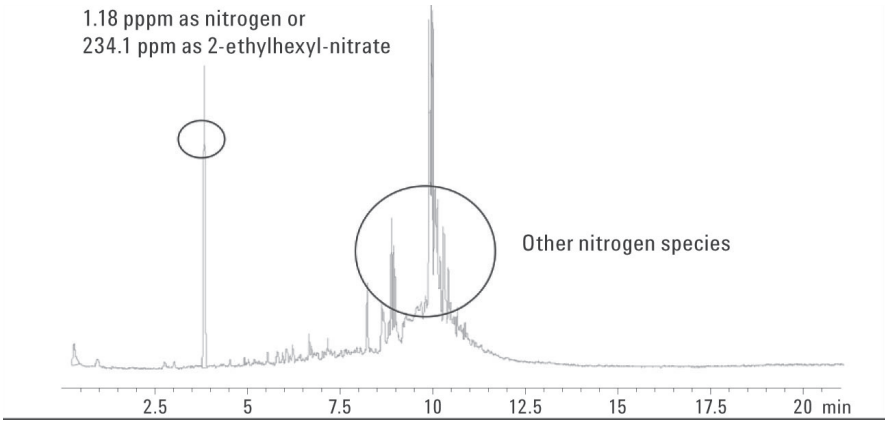

Figure 3. Nitrogen species in B20 biodiesel.

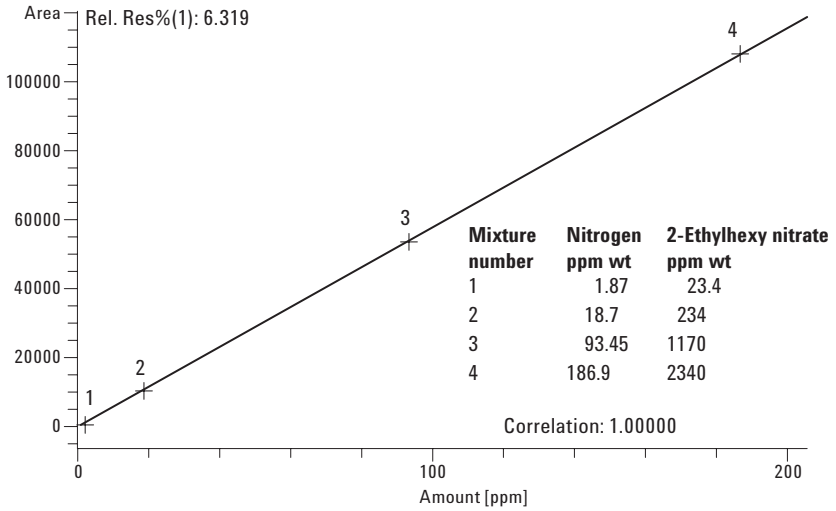

Figure 4. 2-ethylhexyl nitrate calibration.

Table 2. Method precision for analysis of 2-ethylhexyl nitrate in pump diesel.

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average	RSD%
Nitrogen, mg/kg	1.18	1.21	1.19	1.17	1.19	1.18	1.19	1.15

Sample run 6 times

References

1. ASTM D 4046-91(2005), "Standard Test Method for Alkyl Nitrate in Diesel Fuels by Spectrophotometry", Annual Book of Standards, Volume 05.04, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA.
2. Agilent Technical Overview, "Analysis of Trance Nitrogen Species in Benzene," Agilent Technologies publication. 5989-6774EN.

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Agilent Technologies

Analysis of Denatured Fuel Ethanol using ASTM Method D5501-09

Application Note

HPI/Energy/Renewable Fuels

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Abstract

Denatured fuel ethanol is the feedstock used to make different types of high ethanol content motor fuels. Before it can be used, the amount of ethanol and methanol must be measured to assure product quality. ASTM method D5501-09 uses high resolution gas chromatography to perform this analysis. In this paper, the Agilent 7890A GC system was configured to run D5501-09. Excellent system performance and precision were demonstrated using the 7890A GC. Combined with the Agilent MultiTechnique ChemStation, this system offers a complete, automated solution for denatured fuel ethanol analysis.

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Introduction

Ethanol is a key additive in gasoline, serving both as a smog reducer as well as a fuel supplement to reduce the overall use of petroleum. It is relatively easy to produce by fermenting sugars obtained from food crops such as corn and sugar cane. However, the future of ethanol fuel cannot rely on food. To solve this problem, researchers are investigating ways to convert polymeric biomass carbohydrates, such as cellulose, to fermentable sugars. These sugars can then be used as an ethanol fermentation feedstock into the existing production infrastructure.

Whether ethanol comes from food sugars or converted biomass, it is first denatured before use as a motor fuel. Hydrocarbons are common denaturants and ASTM Standard D4806 specifies the types of hydrocarbons that can be used as denaturants [1]. Once the hydrocarbons are added, the product is called denatured fuel ethanol. Commercial fuels are then made by blending denatured fuel ethanol with gasoline. To assure product quality, ASTM has published method D5501-09, which uses gas chromatography to measure the ethanol and methanol content in ethanol fuels [2]. This paper describes the configuration and performance of the Agilent 7890A GC System when running ASTM D5501-09 for the analysis of denatured fuel ethanol.

Experimental

An Agilent 7890A GC System was configured according to D5501-09 and is shown in Table 1. The operating conditions for this method are shown in Table 2. Prior to sample analysis, the GC inlet splitter linearity was checked to assure there was no sample discrimination. A splitter linearity mix was prepared using the procedure described in ASTM Practice D4307 [3]. Ten hydrocarbons ranging from C_5 to C_{11} were gravimetrically blended and the final weight percent of each hydrocarbon in the mix was recorded. This mix was run using the GC conditions shown in Table 2. Calibrations for ethanol, methanol and hydrocarbons were performed using standards obtained from Spectrum Quality Standards, Sugarland, TX USA. After calibration, a commercial denatured fuel ethanol sample was analyzed to determine the ethanol and methanol content.

Table 1. The Agilent 7890A GC System Instrument Configuration for ASTM Method D5501

Standard Agilent 7890A GC System Hardware	
G3440A	Agilent 7890A Series GC System
Option 113	150 psi Split/Splitless Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693 Automatic Liquid Sampler
GC Capillary Column	
Analytical Column	PDMS, 150 m × 0.25 mm id × 1.0 µm film
Data System	
G2070BA	Agilent MultiTechnique ChemStation rev B.04.01
Consumables	
5181-1273	5 µL autoinjector syringe
5183-4647	Single taper split liner with glass wool
5183-4759	Advanced green inlet septa
Calibration Standards	
ETOH5501CAL	D5501 Calibration Set
Spectrum Quality Standards PO Box 2346 Sugarland, TX 77487-2346 USA	

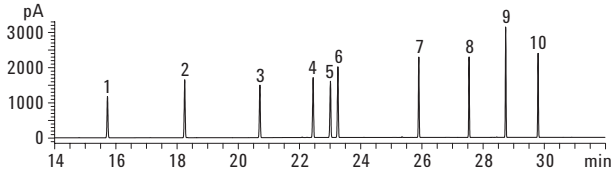
Table 2. GC Operating Conditions for ASTM Method D5501

Split/Splitless Inlet	
Temperature	300 °C
Pressure	Helium at 66 psi
Split ratio	200:1
Septum Purge	3 mL/min
Sample Size	0.5 µL injection
Initial column flow	2.34 mL/min, constant flow mode (24 cm/sec average linear velocity)
FID temperature	300 °C
Oven temperature program	60 °C for 15 min 30 °C/min to 250 °C, hold for 23 min

Results

The splitter linearity test was performed to assure quantitative transfer of all compounds from the inlet to the column without any boiling point discrimination. The test sample contained saturated hydrocarbons between C_5 and C_{11}, which covers the boiling range typically found in denatured fuel ethanol. Using a relative mass response factor of 1, each hydrocarbon in the splitter linearity mix was quantified using a normalized percent calculation. The D5501-09 method specifies that the measured mass percent of each hydrocarbon must match the known mass percent within ±3% relative difference. Figure 1 shows the chromatogram of the splitter linearity mix and the results that meet the ASTM D5501-09 specification. This shows that optimal split injection, with no discrimination, can be easily achieved using the Agilent 7693A ALS fast injection and the Agilent split optimized inlet liner.

System calibration for methanol, ethanol and hydrocarbons was done by running seven calibration standards using the GC conditions listed in Table 2. Methanol was calibrated between 0.05 and 0.6 wt% while ethanol was calibrated between 93 and 98 wt%. The calibration for the hydrocarbon



	Compound	Known wt%	Calc wt%	Relative difference (%)
1	n-pentane	6.9	7.0	**2**
2	2,4-dimethylbutane	9.5	9.6	**1**
3	2,4-dimethylpentane	8.5	8.6	**1**
4	3-methylhexane	10.1	10.1	**1**
5	2,2,4-trimethylpentane	9.5	9.7	**1**
6	n-heptane	11.4	11.4	**0**
7	n-octane	10.9	10.8	**1**
8	Nonane	9.6	9.6	**1**
9	Decane	13.3	13.3	**1**
10	Undecane	10.3	10.2	**1**

Figure 1. Analysis of the splitter linearity test mix containing saturated hydrocarbons from C_5 to C_{11}. These results meet the D5501-09 criteria for splitter linearity.

response was done using n-heptane between 1.95 and 7.4 wt%. After the calibration data was collected and the peak integration optimized, the individual response factors (R) for methanol, ethanol and n-heptane were calculated at each calibration level. Using the response factor of n-heptane, the relative response factors (RR) for methanol and ethanol were then determined at each level using the formulas described in ASTM Practice D4626 [4].

The D5501-09 method allows a single level calibration using a standard containing methanol and ethanol amounts expected in the users' samples in order to save time and resources. For this paper, the amount of alcohols in the sample was not known, therefore average RRs were calculated from all seven calibration standards and are shown in Table 3. These average RRs were then used to quantify the alcohols found in the sample of denatured fuel ethanol.

Table 3. Calibration Data for Denatured Fuel Ethanol Analysis

n-Heptane	Methanol	Ethanol
Average RR (1.95 – 7.4 wt%)	**Average RR (0.05 – 0.6 wt%)**	**Average RR (93 – 98 wt%)**
1.00	2.97	2.06

A sample of commercial denatured fuel ethanol was obtained from a producer and analyzed using the Agilent 7890A GC System running ASTM method D5501-09. Five aliquots of the sample were each measured two times for a total of ten runs. An example chromatogram is shown in Figure 2. It is important to optimize the peak integration in order to correctly measure the methanol peak area. Failure to do so could add peak response from nearby C_4 hydrocarbons to the methanol peak resulting in results that are too high. An example of optimized methanol peak integration is shown in Figure 3.

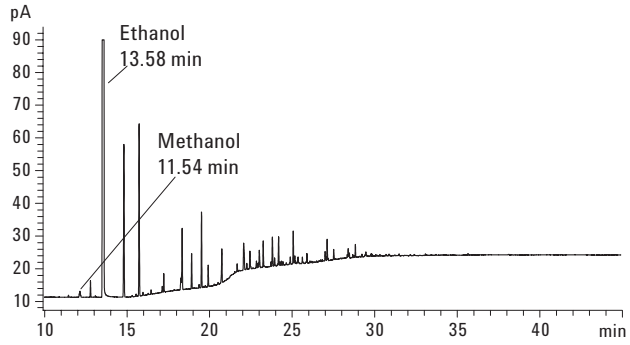


Figure 2. Analysis of a commercial denatured fuel ethanol sample using ASTM method D5501-09.

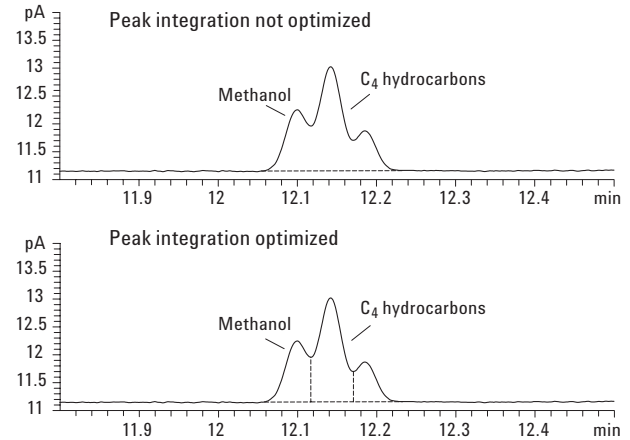


Figure 3. Optimizing the methanol peak integration is important for obtaining correct results.

Quantification of the alcohols in this sample was done using the average RRs calculated in Table 3. For all other peaks in the chromatogram, the n-heptane RR of 1 was used to measure the mass percent. Final reporting of all components was done using a normalized percent calculation as described in the D5501-09 method. The Agilent MultiTechnique ChemStation software can automatically perform both the average response factor calibration as well as the required normalized percent reporting. These results are shown in Table 4. Excellent system measurement precision was obtained for both the low level ethanol content as well as the very high level ethanol content.

Table 4. Results and Precision for the Analysis of Methanol and Ethanol in Denatured Fuel Ethanol.

Run	Methanol	Ethanol
1	0.02	97.81
2	0.02	97.83
3	0.02	97.81
4	0.02	97.82
5	0.02	97.79
6	0.02	97.81
7	0.02	97.78
8	0.02	97.76
9	0.02	97.77
10	0.02	97.74
Avg	**0.02**	**97.79**
Std Dev	**2.18e-4**	**0.03**
RSD	**1.16%**	**0.03%**

Conclusion

The measurement of methanol and ethanol in denatured fuel ethanol can be quite challenging due to the complexity of the hydrocarbon denaturant and the need to quantify near 100% ethanol as well as low level components in the sample. ASTM method D5501-09 uses high resolution gas chromatography to perform this measurement. In this paper, the Agilent 7890A GC Service was configured to run method D5501-09. The system showed no inlet discrimination so that quantitative sample transfer to the column could be made for the wide boiling range components found in denatured fuel ethanol. This was a key factor in the excellent precision shown in this paper. Calibration of a large ethanol concentration as well as a low-level methanol and hydrocarbon concentrations were done using the Agilent MultiTechnique ChemStation. The ChemStation was also able to automate the final calculations and reporting.

References

1. "D4806 - Standard Specification for Denatured Fuel Ethanol for Blending with Gasolines for Use as Automotive Spark-Ignition Engine Fuel"; ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.
2. "D5501-09 Standard Test Method for Determination of Ethanol Content of Denatured Fuel Ethanol by Gas Chromatography"; ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.
3. "D4307 - Standard Practice for Preparation of Liquid Blends for Use as Analytical Standards"; ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.
4. "D4626 – Standard Practice for Calculation of Gas Chromatographic Response Factors"; ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.

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Evaporation from 2-mL Vials on the Agilent 7696A Sample Prep WorkBench: Septa Unpierced, Septa Pierced with a Syringe Needle, Septa with an Open Hole

Application Note

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Introduction

In the course of sample analysis by gas chromatography, the vial septum may be pierced multiple times before each injection, often with multiple injections. Once the septum is pierced, solvent evaporation from the vial occurs. This usually does not create a reproducibility problem for GC analysis, even with multiple injections, unless the time between runs is an hour or longer. With the Agilent 7696A Sample Prep WorkBench, the number of times a septum is pierced may be greater, and the time before the final sample is analyzed may be much longer than is typical in GC.

Another problem that arises with the Agilent 7696A Sample Prep WorkBench is the need to withdraw large volumes from 2 mL vials. For example, transferring 0.5 mL solvent or sample from one vial to another can create a partial vacuum in the source vial. This results in poor reproducibility because the degree of vacuum varies from vial to vial and the amount of liquid actually transferred also varies. One way to eliminate this problem is to prepierce the septum with a small off-center hole so that no vacuum is created and the syringe needle is still wiped by the septum when withdrawn from the vial.

The evaporation rates of hexane (bp = 70 °C) and isooctane (bp = 100 °C) were measured at ambient temperature for three different septum scenarios to determine the magnitude of the problem. The three scenarios are as follows: a new unpierced septum, a septum prepierced approximately nine times, and a septum cored to prevent vacuum formation. Evaporation from the new, unpierced screw cap vial septa was considered negligible. Evaporation was greater with the septa pierced with a syringe needle and much greater with the cored septa.

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Experimental

Hardware

Vials: 2 mL glass screw cap (5182-0714)

Septum caps: With PTFE/red silicone rubber (5185-5820)

Septum types:

A = new, unpierced
B = pierced approximately 9 times with syringe needle
C = new, cored off-center with a 0.5 mm hole

The type B septa were prepierced with GC injections. The type C septa were cored with a miniature "cork borer" made from a brass tube (1/16" od × 0.035" id). One end was filed to create a sharp inner edge. The holes created were about 0.5 mm id.

Fifteen empty vials plus caps were weighed. Five contained type A septa, five contained type B and five contained type C. Vials were filled with about 1 mL of solvent each, reweighed, and placed in a Agilent 7696 sample tray. Vials were weighed again after 24 and 96 hr at room temperature (23 °C).

Results

The %loss/hr for the different septum types for hexane is:

A = 0
B = 0.3
C = 0.9

The %loss/hr for the different septum types for isooctane is:

A = 0
B = 0.1
C = 0.3

Table 1 lists average evaporation rates from vials with the different septa.

Conclusions

This data provides a rough idea of the effect solvent evaporation has on our preparation results. It is up to the user to determine what level of evaporation can be tolerated based on the specific method and length of time between initial and final samples in the preparation. When a method requires vacuum relief holes in the septa, the transfers should be performed early in the method if possible, and even perhaps as a separate method so that vials can be recapped before significant evaporation occurs.

Table 1. Average Evaporation Rates from Vials with the Different Septa

Solvent: hexane, bp = 70 °C

	Septum:	A		B		C	
	After:	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**
	24hr	0.00	0.00	7.27	0.30	21.06	0.88
	96hr	0.03	0.00	29.21	0.30	84.55	0.88

Solvent: isooctane, bp = 100 °C

	Septum:	A		B		C	
	After:	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**
	24hr	0.12	0.01	2.74	0.11	6.84	0.29
	96hr	0.65	0.01	11.38	0.12	28.26	0.29

A New, unpierced septa
B Septa prepierced about nine times
C Septa cored to prevent vacuum formation

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Agilent Technologies

Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance

Application Note

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Introduction

A challenge that arises more often than the analyst might like, is the need to prepare a set of samples by serial dilution. Serial dilution starts with a single sample of known concentration. It is then used to prepare a set of dilutions, each usually differing from the previous one, by a constant factor. Each sample is made from the previous one in the series. This task may be driven by the need to calibrate an instrument with specific analytes or measure such things as detector performance: linearity, sensitivity and minimum detectable level (MDL). If the samples are not stable over time, they may need to be prepared weekly or even daily. To minimize errors in manual preparations or reduce the frequency of tiresome dilutions, the user will often prepare larger volumes of sample than needed, which leads to unnecessary waste and expense.

The Agilent 7696A Sample Prep WorkBench provides a solution to this problem by automating the serial dilution process precisely so that small volumes of sample can be routinely prepared when needed over as large a concentration range as desired. The preparative method for serial dilution starts with a measured volume of solvent in an empty vial followed by a measured volume of sample. After mixing, this step is repeated using a new vial of solvent and an aliquot from the last dilution. For example, measuring the performance of a flame ionization detector (FID) requires a set of samples, each diluted by a factor of ten from the previous sample. The starting sample is a normal hydrocarbon such as n-tridecane (C_{13}). Each dilution consists of 90% solvent and 10% previous sample (v:v). A set of seven or eight samples, as prepared in this application, are required to demonstrate the normal seven orders of magnitude of FID linearity. As described below, eight sets of test samples were prepared over a two week period. Three were prepared manually and five with the Agilent 7696 Sample Prep Workbench at a total volume per sample of either 1 mL or 0.5 mL. Repeatability over all sets was excellent whether measured by sample weight in each set or by FID performance.

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Experimental

The Agilent 7696A Sample Prep WorkBench was used to prepare a set of eight samples, each diluted by a factor of ten from the previous sample. Two sequences were used so that samples could be weighed after each addition. The first used a method that added a fixed amount of solvent to each vial. The second started with a manually-prepared 10% solution of C_{13} in solvent, then added enough solution to the next vial to make a tenfold less concentrated solution. After mixing, an aliquot of the freshly made sample was used to make the next dilution in the series until the eight sample set was complete. The empty vials were tared, and then weighed after each sequence to measure reproducibility of transfers across the series. The same preparations were also done manually for comparison.

Hardware Configuration

The Agilent 7696A Sample Prep WorkBench was equipped with two Agilent 7693A Automated Liquid Samplers. The back injector contained an enhanced syringe carriage containing a 500-µL syringe (p/n G4513-60561). The front injector used a standard syringe carriage containing a 100-µL syringe (p/n 5183-2042). The back injector was used for solvent delivery to each of the empty vials (first sequence) and the front injector was used for sample transfer from one sample to the next (second sequence).

Sample Preparation

Two protocols were used that differed only in the volume of the prepared dilution. The first used 900 µL solvent + 100 µL sample and the second used half these amounts: 450 µL solvent + 50 µL sample.

A single Agilent 7696A Sample Prep WorkBench resource layout was used for both sequences:

Resource Layout:

Vial Range	Name	Type	Usage
2-9	MT vial	Empty container	1 use/vial
12-19	Solvent	Chemical resource	1 use/vial

The single sample required was a solution of 10% C_{13} in isooctane. It was prepared by adding 100 µL C_{13} to a 1 mL volumetric and diluting to mark.*

The first sequence prepared the 1 mL sample (900 µL + 100 µL) by adding 900 µL solvent to an empty vial (see Appendix for syringe parameters). The sequence specified vials 2 through 9.

* I started with the 10% C_{13} instead of 100% C_{13} to avoid any volume shrinkage that might occur when mixing two neat compounds by volume.

The second sequence specified sample dilutions according to the following steps. (see Appendix for syringe parameters):

Step	Function
1	Add 100 µL of Sample (Front) to vial #2
2	Mix vial #2 at 1500 RPM for 0 min 5 sec
3	Add 100 µL of vial #2 to vial #3
4	Mix vial #3 at 1500 RPM for 0 min 5 sec
5	Add 100 µL of vial #3 to vial #4
6	Mix vial #4 at 1500 RPM for 0 min 5 sec
7	Add 100 µL of vial #4 to vial #5
8	Mix vial #5 at 1500 RPM for 0 min 5 sec
9	Add 100 µL of vial #5 to vial #6
10	Mix vial #6 at 1500 RPM for 0 min 5 sec
11	Add 100 µL of vial #6 to vial #7
12	Mix vial #7 at 1500 RPM for 0 min 5 sec
13	Add 100 µL of vial #7 to vial #8
14	Mix vial #8 at 1500 RPM for 0 min 5 sec
15	Add 100 µL of vial #8 to vial #9
16	Mix vial #9 at 1500 RPM for 0 min 5 sec

Results

Over a period of two weeks, eight serial dilution runs were made: Three manual (two at 1 mL and one at 0.5 mL); five with the Agilent 7696A Sample Prep WorkBench (three at 1 mL and two at 0.5 mL).

Table 1. Reproducibility for Solvent Delivery (Average of Eight Samples)

Type	Manual	Manual	Manual	7696A	7696A	7696A	7696A	7696A
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Average weight (g)	*	0.6165	0.6151	0.3089	0.6176	0.6195	0.6180	0.3088
%SD	*	0.17	0.26	0.11	0.16	0.09	0.06	0.17

* Not measured.

Reproducibility for the second step was ±1 µL, for all but the last sample. Each sample except the last was used to prepare the next. The weight should not change because the same volume is added to and then removed from each sample. The average weight change regardless of whether a 1 mL or 0.5 mL preparation was involved was equivalent to ±1 µL. The volume increase of the last sample was 100 µL or 50 µL for the 1 mL and 0.5 mL volumes, respectively.

The total Agilent 7696A Sample Prep WorkBench runtime was 49 min for the 1 mL set of samples and 41 min for the 0.5 mL set. The time for the manual preparations was not measured.

Reproducibility of FID performance

The protocol used for FID linearity, sensitivity and MDL followed the ASTM protocol closely [1]. The major difference was the use of liquid samples rather than gas samples as specified by ASTM. All preparations were tested on the same FID. The linearity results (Figure 1) are essentially indistinguishable whether the samples were prepared by the Agilent 7696A Sample Prep WorkBench or manually. The average sensitivity and % SD were 26.3 and 2.4, respectively. This is very good performance for repeat runs on a single FID. The large spread in the MDL (Table 2) is caused by day-to-day variability in average detector noise in the region where C_{13} elutes. MDL is a sensitive function of noise. Table 2 and Figure 1 summarizes the results.

Table 2. FID MDL

Prep Type	Manual	Manual	Manual	7696	7696	7696	7696	7696
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Sensitivity (ma-s/gC)	27.2	25.7	25.8	26.8	26.8	25.5	26.6	25.5
MDL (pgC/s)	0.96	1.14	1.66	0.92	0.68	1.31	1.23	1.15

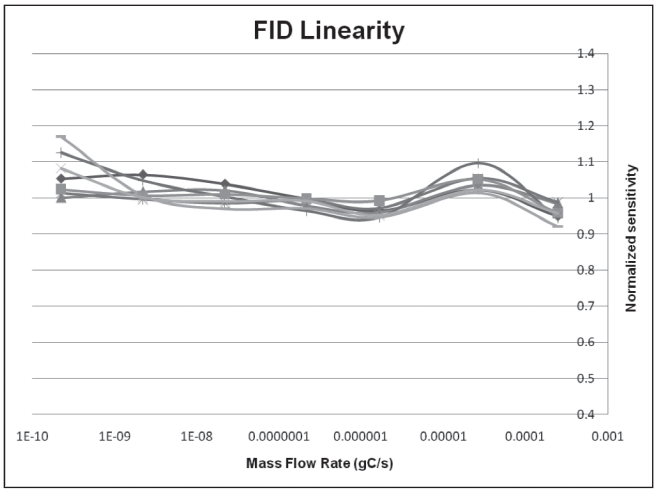

Figure 1. Linearity Plots for all eight runs overlaid.

Conclusion

The Agilent 7696A Sample Prep WorkBench simplifies the preparation of a set of samples by serial dilution. The user can prepare fresh samples only when needed at volumes no larger than necessary to satisfy the analytical requirements. The result is less boredom, less chance for operator error, less consumption of reagents, less waste disposal expense and better repeatability.

Appendix

500 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes					3			
Wash volume (µL)					50			
Draw speed (µL/min)					1250	1250		
Dispense speed (µL/min)					3000	3000		
Needle depth offset (mm)					0	0		
Viscosity delay(s)					2	2		
Turret solvent								
Air gap (% syr.vol.)						0		

100 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes		1		1	2			
Wash volume (µL)		10		20	10			
Draw speed (µL/min)		300		300	300	300		
Dispense speed (µL/min)		6000		6000	6000	6000		
Needle depth offset (mm)		0		0	0	0		
Viscosity delay(s)		2		2	2	2		
Turret solvent		A						
Air gap (% syr.vol.)						0		

Reference

1. ASTM E594-96 (2006) Standard Practice for Testing Flame Ionization Detectors used in Gas or supercritical Fluid Chromatography

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Improving the Analysis of Fatty Acid Methyl Esters Using Automated Sample Preparation Techniques

Application Note

Food Testing and Agriculture

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Abstract

An automated method for esterifying fatty acids in canola oil samples is presented. Using the Agilent 7696A Sample Prep WorkBench, a side-by-side comparison was undertaken comparing a manual method employing automatic pipettors to a method developed for this automated system. Using the Agilent 7696A Sample Prep WorkBench, preparation of 10 samples resulted in 3% RSD for both an acid-catalyzed and base-catalyzed reaction. When comparing the automated acid-catalyzed method to the manual preparation, the RSD improved by a factor of two. Furthermore, by automating the fatty acid preparation the amount of reagents consumed was reduced up to 50-fold. Overall, the automated method resulted in better precision and accuracy with smaller amounts of reagents used and less time required from the operator to complete the task.

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Introduction

The analysis of fatty acids (FAs) is commonly performed in many industries. The food industry routinely performs FA analysis since lipids are a major component in oils, meats, seeds, and other products [1-5]. Furthermore, with the increased importance on fat as part of dietary health and its role in maintaining a healthy disposition, the determination of FA composition has become increasingly common [1-2]. Biomedical applications use FA profiles as a diagnostic tool since FA composition effects biological membranes [3-4,6-7]. Fatty acids are also found in many household products and are used industrially in cosmetics and surfactants, among other things [2,8].

Gas chromatography has been the predominant technique used for analyzing FAs since the 1950's across these industries [3-4,9]. While FAs can be separated and analyzed with the appropriate analytical conditions, they present a number of challenges due to their polar nature and high boiling points. This generally results in long retention times and poor peak shape. For that reason, most methods use derivatization reactions to convert FAs to fatty acid methyl esters (FAMEs), which are easier to separate and exhibit better peak shape.

Converting FAs to FAMEs, regardless of the matrix or application, can be achieved in a number of ways, often involving a two step process of saponification followed by methylation. Lipids can also be esterified in one step through a process known as alcoholysis [4], although many applications, specifically food applications, still use a two-step procedure [1,2,5]. Whether multi-step or single-step, the process of converting FAs to FAMEs can be achieved in a number of ways and different applications require different derivatization reagents [10]. A majority of the reactions can be categorized as using acid, base, or silylating reagents or diazomethane, each with their own advantages and disadvantages.

When performing acid-catalyzed reactions, the most common reagents are boron trifluoride (BF_3) in methanol, hydrochloric acid (HCl), and sulfuric acid (H_2SO_4). Procedures using HCl or H_2SO_4 often call for refluxing the acid for up to an hour, depending on the acid concentration and the sample, for example, free fatty acids, phosphoglycerides, or triglycerides, to achieve complete methylation [3-4,10]. This method is very effective, but it can be costly and requires specific glassware and training. Using BF_3 as the methylating reagent provides the fastest results, because it can be complete within two minutes when boiling. However, this can lead to degradation of labile fatty acids and has a limited shelf life at room temperature [3].

Base-catalyzed reactions use sodium hydroxide (NaOH) or potassium hydroxide (KOH) in methanol. This method has many advantages. It is quick, a simple one-step process, occurs at room temperature, and avoids the degredation of labile FAs. However, base-catalyzed reactions do not work on free fatty acids, and therefore can be limiting in their applicability [3-4,10].

Two additional reagents can be used, but are rarely employed. Diazomethane provides a rapid derivatization technique, but it can produce byproducts that interfere with the compounds of interest [3,10]. Its toxicity and potential for explosion make it a rarely used reagent in recent years. Silylating reagents are also rarely used because of their sensitivity to water although they react fairly quickly and at moderate temperatures [10].

Automation of these methods can be advantageous in many ways and recently there have been more automated and microscale methods for converting FAs to FAMEs [11-15]. Generally, automated methods use smaller amounts of reagents, reduce an operator's potential exposure to hazardous chemicals, can reduce the time required to complete a task, and provide intervention-free operation for hours. Automating the preparation of FAMEs is possible with the Agilent 7696A Sample Prep WorkBench (Figure 1). It features a 150-vial tray, two liquid dispensing modules, a single vial heater, mixer, and barcode reader. In addition, the individual vial racks can be heated or cooled. The liquid dispensing modules can be configured with either a standard syringe or a large volume (250 µL or 500 µL) syringe. Most applications use a standard syringe (10 µL or 25 µL) in one module for fine manipulations of liquids and a large volume syringe in the other module for dispensing larger volumes. With the two dispensing modules, mixer, and heater, the Agilent 7696A Sample Prep WorkBench is capable of sample dilutions, internal standard additions, derivatizations, liquid/liquid extraction, as well as many other tasks.

Figure 1. Agilent 7696A Sample Prep WorkBench.

Using the Agilent 7696A Sample Prep WorkBench, two methods of extracting and methylating FAs in canola oil were examined: a base-catalyzed reaction and an acid-catalyzed reaction. Both methods were adapted from a previously published manual method using 20-mL test tubes [5]. Recoveries between 93% and 107% with RSDs < 5% were achieved. In addition, when modifying the manual method for use on the Agilent 7696A Sample Prep WorkBench, the reaction time was reduced from 2 hours to 20 minutes with up to a 50-fold decrease in reagent and solvent usage.

Experimental

Materials

Hexane (reagent grade), isooctane, and methanol (HPLC grade) were purchased from Burdick and Jackson (Muskegon, Michigan). Boron trifluoride (BF_3) in methanol (14% w/v) was obtained from Aldrich (St. Louis, MO). A solution of sodium hydroxide (reagent grade, Sigma Aldrich, St. Louis, MO) in methanol (NaOH) was made to yield a 2N solution. Sodium chloride (certified ACS, Fisher Scientific, Atlanta, GA) was used to make a 1 M solution in Milipure water (H)O/NaCl). While some reports suggest using a saturated solution [2,16], such a concentrated solution was found to precipitate in the system and cause syringe errors.

Individual FAs were obtained from Alltech (Waukegan, IL) and consisted of caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid. These were used to generate a 1 mg/mL solution in hexane. FAME standards were made from a custom mix obtained from ChemService (West Chester, PA) consisting of methyl pentanoate, methyl hexanoate, methyl heptanoate, methyl octanoate, methyl decanoate, methyl laurate, methyl myristate, methyl palmitate, methyl stearate, methyl eicosanoate, and methyl behenate at 1 mg/mL. A 1 mg/mL solution of lauric acid in hexane was used as a surrogate standard. A 1 mg/mL solution of decane (99+% Sigma, St. Louis, MO), dodecane (99+% Aldrich, St. Louis, MO), tetradecane (99+% Fluka, St. Louis, MO), and hexadecane (99+% Aldrich, St. Louis, MO) in isooctane was used as the internal standard. The canola oil was obtained from the local supermarket.

Two wash solvents were used in the Agilent 7696A Sample Prep WorkBench: hexane and acetone (Laboratory grade, Fisher Scientific, Atlanta, GA). Acetone was used for a majority of wash steps since it provided a solvent in which all the reagents used were miscible.

Instrumentation

The Agilent 7696A Sample Prep WorkBench (Agilent Technologies, Santa Clara, CA) was used to prepare calibration curve standards, free FA samples, and canola oil samples. For this application, the liquid dispensing modules were configured with a 25-µL syringe in the back module and a 500-µL syringe in the front module for larger volumes.

All analyses were performed on an Agilent 7890A gas chromatography (GC) System (Agilent Technologies, Santa Clara CA) equipped with a split/splitless inlet, operated in split mode (10:1) and a flame ionization detector. The inlet was held at 300 °C with a constant column flow rate of 3 mL/min. An Agilent HP5-MS column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies, Santa Clara) was used. A temperature program of 100 °C (5 min), 7 °C/min to 225 °C (5 min) was used to achieve separation of the FAME standards. The same temperature program was used for the canola oil samples as well, although baseline separation of all the compounds was not achieved. The detector was held at 300 °C and data collection was performed with Multi-Technique ChemStation.

The sample preparation method programming was performed with Easy SamplePrep, a drag and drop method editor developed for the Agilent 7696A Sample Prep WorkBench. Easy SamplePrep features a Resource Manager that allows users to allocate vials as chemical resources or empty vials. This way the user inputs all the solvents, reagents, standards, and empty vials needed for the sample preparation and the

Resource Manager keeps track of the vials as they are used throughout the program and sequence (Figure 2). With the resources configured in the Resource Manager, the sample preparation program is built. The Easy SamplePrep method editor allows the user to add steps in a manner similar to following a protocol or laboratory notebook and gives a textual display of the steps and the resources available (Figure 3).

Each sample prep step has a set of advanced parameters for a fully customizable program. In the Add Step, the Advanced Parameters allows the user to set parameters like wash volumes, draw and dispense speeds, and needle depths (Figure 4). The Mix Step can be customized with regard to speed and time, while the Heat Step allows the user to specify both time and temperature setpoints. The Flag as Result Step allows the user to select the vial that contains the finished sample for reporting purposes.

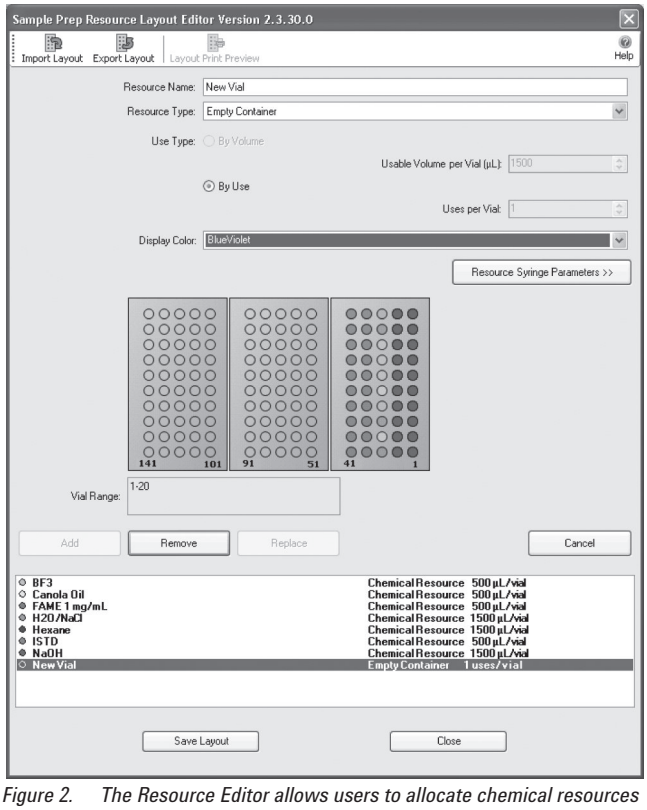

Figure 2. The Resource Editor allows users to allocate chemical resources and empty vials to be used during the sample preparation.

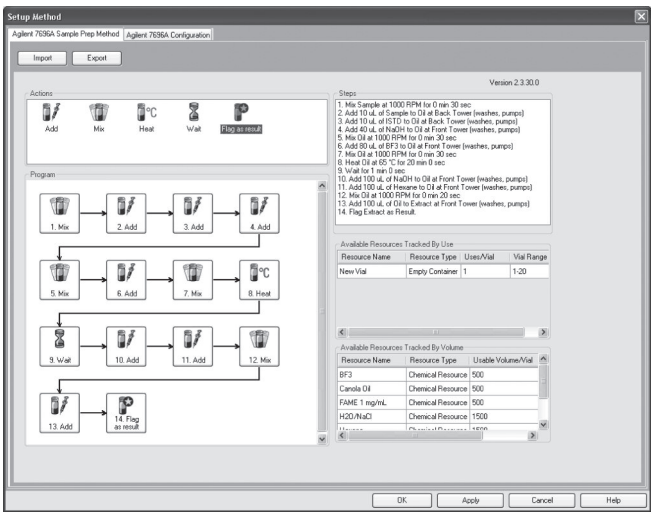

Figure3. SamplePrep Method Editor features drag and drop icons for easy step-wise programming. The steps used for the acid-catalyzed reaction are shown here.

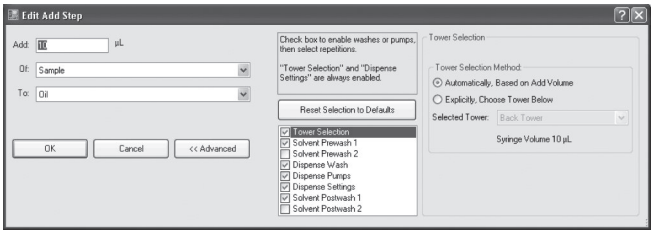

Figure4. Each sample prep step has advanced parameters, like the Add Step shown here, that allows the user to fully customize the operation.

Calibration Curve Generation

Prior to preparing samples with the Agilent 7696A Sample Prep WorkBench, a calibration curve was generated from the FAME standard with the instrument. An eight-level calibration curve consisting of the 11 FAMEs was generated spanning 1–500 ppm in approximately 100 µL.

Sample Preparation: Acid-Catalyzed Reaction

The original manual method followed the Association of Official Analytical Chemists Official Methods of Analysis and started with a 50-mg sample of canola oil in 20-mL test tubes and included two heating steps at 80 °C for 60 minutes each [5,17].

When converting the AOAC method to an automated one, the scale of the reaction was necessarily reduced since the Agilent 7696A Sample Prep WorkBench accepts only 2-mL autosampler vials. The manual method was reduced approximately 50-fold and applied to both the oil sample and a free FA sample.

Initially the oil, surrogate standard (lauric acid), and internal standard (alkanes) were added in separate steps. However, because of difficulty in achieving acceptable reproducibility when dispensing the oil, a solution consisting of 0.4 mL of the oil sample and 0.4 mL of the surrogate standard was made, greatly improving the reproducibility of the method.

An empty, 2-mL autosampler vial was capped and 10 µL of sample (either the free FA sample in hexane or oil/surrogate standard solution) and 10 µL of the internal standard were added. To the sample, 40 µL of NaOH was added and the mixture was vortexed at 1000 rpm for 30 sec. After saponification, 80 µL of BF_3 was added and the mixture was again vortexed at 1000 rpm for 30 sec. The mixture was then heated for 20 minutes at 65 °C to facilitate the reaction. After heating, the mixture was allowed to sit at room temperature for 2 minutes to let it cool slightly. To the cooled mixture, 100 µL of H_2O/NaCl and 100 µL of hexane was added to extract the newly formed FAMEs into the organic layer. The sample was mixed a final time for 20 sec at 1000 rpm and the top layer (100 µL) was transferred to a new, empty, capped 2-mL autosampler vial and taken to the GC for analysis.

Sample Preparation: Base-Catalyzed Reaction

As with the acid-catalyzed reaction, the manual preparation for the base-catalyzed reaction was too large to be prepared in a 2-mL autosampler vial since it started with a 100-mg oil sample in a 20-mL test tube [5]. For this reaction to work on the Agilent 7696A Sample Prep WorkBench, it was reduced approximately 10-fold.

Since the base-catalyzed reaction does not convert free fatty acids, the surrogate standard was omitted. A solution of 0.4 mL of oil and 0.4 mL of internal standard was again used to improve the reproducibility of the method. To an empty, capped 2-mL autosampler vial, 10 µL of sample (oil/internal standard solution) was added. To methylate the FAs and extract the newly formed FAMEs 100 µL of NaOH and 500 µL of hexane was added and the mixture was vortexed at 1000 rpm for 30 sec. After waiting 2 minutes, the top layer (100 µL) was transferred to a new, empty, capped 2-mL autosampler vial and taken to the GC for analysis. Unlike the acid-catalyzed reaction, this base-catalyzed reaction occurs in a single step and is complete within minutes.

Validation of the Acid-Catalyzed Reaction

Because the acid-catalyzed reaction works as well on lipid bound fatty acids as it does on free fatty acids, the method was performed on the free FA sample in hexane. Five samples were prepared on three different days to determine repeatability between samples as well as day-to-day reproducibility.

The same procedure was followed when performing the reaction manually. Volumes were added using adjustable pipettors and the reaction took place in a heated block for comparison. The manual procedure was performed alongside the automated procedure to give an accurate comparison between the manual and automated preparations.

Results and Discussion

Calibration

Excellent linearity was achieved for the eight standards made with the Agilent 7696A Sample Prep WorkBench. The calibration data are given in Table 1. Because the standards were made with a selection of saturated FAMEs, those were the only compounds that were identified and quantified in the oil and FA standard samples.

Table 1. Instrument Calibration Data for FAME Standards Prepared with the Agilent 7696A Sample Prep WorkBench

Analyte	R2	Linear regression
Methyl pentanoate	0.9997	y = 1.33x + 4.1175
Methyl hexanoate	0.9998	y = 1.4876x + 8.9684
Methyl heptanoate	0.9998	y = 1.5671x + 7.7412
Methyl octanoate	0.9998	y = 1.6669x + 8.2446
Methyl decanoate	0.9998	y = 1.7825x + 9.0499
Methyl laurate	0.9998	y = 1.8786x + 9.7365
Methyl myristate	0.9998	y = 1.9727x + 10.264
Methyl palmitate	0.9998	y = 1.9623x + 10.369
Methyl stearate	0.9998	y = 1.9828x + 10.64
Methyl eicosanoate	0.9998	y = 2.0155x + 10.826
Methyl behenate	0.9998	y = 2.087x + 11.266

Method Validation

Before the oil samples were examined, a free FA sample was prepared with the automated method and the manual method described above to validate the use of the Agilent 7696A Sample Prep WorkBench.

For the automated method, five samples prepared on any day resulted in an average RSD of < 2%. When comparing the five samples made on three different days with the Agilent 7696A Sample Prep WorkBench, good reproducibility was again achieved for all the compounds with retention times greater than methyl octanoate. The mean, standard deviation, relative

standard deviation, and recoveries are given in Table 2. Methyl octanoate is significanly lower than the other analytes due to its volatility and proximity to the solvent peak [3,4]. This data comprises 15 samples prepared over three days with triplicate injections.

Table 2. Results from the Free Fatty Acid Sample Acid-Catalyzed Reaction using an External Standard to Determine Concentration and Recovery

Analyte	Amount (ppm)	Standard Deviation (ppm)	Relative Standard Deviation (%)	Recovery (%)
Decane	81.8	0.968	1.18	97.3
Dodecane	84.6	0.517	0.611	102.4
Tetradecane	88.0	0.869	0.967	105.7
Hexadecane	92.2	1.02	1.11	111.4
Methyl octanoate	63.5	0.570	0.898	75.1
Methyl decanoate	101.2	0.175	0.173	97.5
Methyl laurate	110.1	0.681	0.619	104.8
Methyl myristate	97.0	0.713	0.735	102.6
Methyl palmitate	115.2	0.688	0.597	106.6
Methyl stearate	98.0	0.266	0.271	106.9
Methyl eicosanoate	80.6	0.394	0.489	104.0
Methyl behenate	90.0	1.12	1.25	99.9

The results from the automated method were then compared to those obtained using a manual method. The reproducibility was much worse for the manual method on any of the three days tested. Treating the manual data in the same manner as the automated data, the mean, standard deviation, relative standard deviation, and recoveries are given in Table 3. Recoveries are routinely higher for the manually prepared samples than those achieved with the automated preparation

Table 3. Results from the Free Fatty Acid Sample Prepared Manually with the Acid-Catalyzed Preparation using an External Standard to Determine Concentration and Recovery

Analyte	Amount (ppm)	Standard Deviation (ppm)	Relative Standard Deviation (%)	Recovery (%)
Decane	88.2	13.2	14.9	104.9
Dodecane	98.1	7.92	8.08	118.8
Tetradecane	104.2	6.81	6.53	125.0
Hexadecane	109.8	6.66	6.06	132.8
Methyl octanoate	94.6	14.1	14.9	111.9
Methyl decanoate	126.4	13.8	10.9	121.8
Methyl laurate	130.0	12.1	9.33	123.8
Methyl myristate	113.1	10.0	8.84	119.6
Methyl palmitate	134.4	12.0	8.93	124.3
Methyl stearate	114.0	9.89	8.67	124.3
Methyl eicosanoate	93.6	8.41	8.98	120.8
Methyl behenate	104.7	9.83	9.39	116.3

due to either a greater amount of standard added or slightly less hexane added, the later being more likely the case.

While the results shown in Table 2 and 3 were determined using the external calibration, the data was also examined using an internal standard. The peak areas were normalized to methyl laurate which produced better overall results than the absolute peak areas. Normalizing to methyl laurate, used here as the internal standard, provides results indifferent to the dilution. In doing so, RSDs generally improved. Using normalized peak areas, the relative standard deviation for the samples made both manually and with the automated method across the three days are presented in Table 4. Comparing the manual and automated results, it was clear that the automated method provided a viable solution for derivatizing fatty acids and could improve the reproducibility and recovery.

Table 4. Results from the Free Fatty Acid Sample Normalized to Methyl Laurate Prepared Both Manually and with the Automated Acid-Catalyzed Reaction

Analyte	Relative Standard Deviation-automated (%)	Relative Standard Deviation-manual (%)
Methyl octanoate	1.31	7.65
Methyl decanoate	0.452	2.63
Methyl laurate	–	–
Methyl myristate	0.425	1.05
Methyl palmitate	0.779	1.80
Methyl stearate	1.10	1.93
Methyl eicosanoate	1.59	1.72
Methyl behenate	2.62	1.77

Canola Oil analysis

After validating the automated method using the free FA sample, the Agilent 7696A Sample Prep WorkBench was used to prepare oil samples. As stated above, dispensing canola oil proved to be more difficult than originally thought due to its high viscosity. However, by mixing the oil sample with the surrogate standard, the viscosity of the solution was much closer to that of hexane and therefore easier to dispense reproducibly. The results are given in Table 5 with a representative chromatogram presented in Figure 5.

Eleven oil samples were prepared across two days. Because a fresh solution of oil and lauric acid was made each day, an average RSD for all 11 samples cannot be given. However, good reproducibility was still found. The average RSD for the six samples prepared on day one was 3.6%. The average RSD for the five samples prepared on day two was slightly lower at 2.5%. Using methyl laurate as an internal standard to normalize the FAMEs, the average RSD for all eleven samples decreases, as seen in Table 5. The average recovery for these samples was 101%.

Table 5. Results Using Both an External Standard (ES) and an Internal Standard (IS) for the Canola Oil Samples Prepared with the Agilent 7696A Sample Prep WorkBench and the Acid-Catalyzed Reaction

Analyte	Amount (ppm)	Standard Deviation (%)	Relative Standard Deviation-ES (%)	Relative Standard Deviation-IS (%)	Recovery (%)
Methyl laurate	51.0	1.91	3.74	–	97.1
Methyl palmitate	1499.6	57.8	3.85	0.778	–
Methyl stearate	306.8	12.9	4.20	0.928	–
Methyl eicosanoate	226.8	9.44	4.16	1.10	–
Methyl behenate	111.6	4.73	4.24	0.861	–

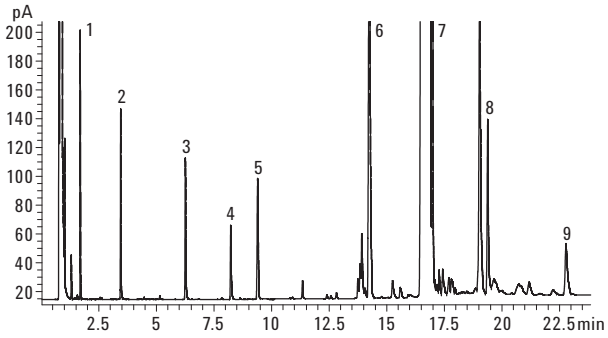

Figure 5. A typical chromatogram of a canola oil sample after automated acid-catalyzed sample preparation and analysis by GC. Compounds identified and quantified were 1) decane, 2) dodecane 3) tetrade cane, 4) methyl laurate, 5) hexadecane, 6) methyl palmitate, 7) methyl stearate, 8) methyl eicosanoate, and 9) methyl behenate. Other unidentified, uncalibrated peaks are various unsaturated FAMEs.

The base-catalyzed reaction provided excellent results as well. A total of 10 samples were prepared in one day and yielded similar reproducibility (Table 6). The average RSD for the 10 samples was 3.2%. Using hexadecane as the internal standard to normalize the peak areas did not sufficiently lower the RSDs as it did with the acid catalyzed reaction. The average recovery was found to be 94%.

Table 6. Results Using Both an External Standard (ES) and an Internal Standard (IS) for the Canola Oil Sample Prepared with the Agilent 7696A Sample Prep WorkBench and the Base-Catalyzed Reaction

Analyte	Amount (ppm)	Standard Deviation	Relative Standard Deviation-ES	Relative Standard Deviation-IS (%)	Recovery (%)
Hexadecane	9.66	0.215	2.23	–	99.2
Methyl palmitate	312.5	13.01	4.16	2.69	–
Methyl stearate	49.95	3.39	6.80	4.89	–
Methyl eicosanoate	40.98	1.66	4.04	2.15	–
Methyl behenate	18.07	0.945	5.23	2.83	–

Benefits of automated sample preparation

Automating the sample preparation procedure proves to be advantageous in many ways. By adapting this method to an automated one, the scale of the reaction was reduced. In doing so, the level of chemical exposure is reduced as well as the amount of solvent and reagent used. This increases the safety of the method and reduces the cost of the analysis.

More importantly, automating this method resulted in better recoveries and reproducibility. Automating this method resulted in reproducibilities at least twice as good as the compared manual method.

Conclusions

Two automated methods for derivatizing fatty acids to fatty acid methyl esters were described in this Application Note. Using the Agilent 7696A Sample Prep WorkBench, derivatization reactions were easily converted to automated methods with an increase in reproducibility. Furthermore, smaller volumes of solvents and reagents were used, which significantly reduced the cost per analysis. Excellent reproducibility and recovery were achieved for most compounds in both a fatty acid standard and a canola oil sample. These results show that methods such as these can be easily be adapted for use on the Agilent 7696A Sample Prep WorkBench with many advantages.

References

1 M. Petrovic, N. Kezic, and V. Bolanca, *Food Chemistry*, **122**, 285-291(2010).

2 K. M. Giffin and W. H. Wilson, "Preparation and Analysis of FAMEs by Automated Esterification/Capillary GC," Application Note 288-357, Hewlett-Packard No. (23)5965-1110E (1996).

3 K. Eder, *J. Chromatogr.*, B, **671**, 113-131 (1995).

4 G. Gutnikov, *J. Chromatogr.*, B, **671**, 71-89 (1995).

5 F. David, P. Sandra, P. Wylie, "Improving the Analysis of Fatty Acid Methyl Esters Using Retention Time Locked Method and Retention Time Databases," Application Note 5990-4822EN, Agilent Technologies publication 5988-5871EN (2003).

6. W. Welz, W. Sattler, H.-J. Leis, and E. Malle, *J. Chromatogr.*, B, **526**, 319-329 (1990).

7. N. Sanchez-Avila, J. M. Mata-Granados, J. Ruiz-Jimenez, and M. D. Luque de Castro, J. Chromatogr., A, **1216**, 6864-6874 (2009).

8. R. W. Johnson in Fatty Acids, E. Pryde (editor), AOCS Press, Champaign, IL, p. 608 and Part VIII (1979).
9. T. Seppanen-Laakso, I. Laakso, and R. Hiltunen, Anal. *Chim. Acta*, **465**, 39-62 (2002).
10. W. W. Christie in *Advances in Lipid Methodology – Two*, W. W. Christie (editor), Oily Press, Dundee, MI, pp. 69-111 (1993).
11. L. Mondello, P. Quinto Tranchida, P. Dugo, and G. Dugo, *J. Pharm. Biomed. Anal.*, **41**, 1566-1570 (2006).
12. R. Perkins, K. Summerhill, and J. Angove, *Chromatography Today*, Sept/Oct, 17-19 (2008).
13. M. Athar Masood and N. Salem Jr., *Lipids*, **43**, 171-180 (2008).
14. E. Ballesteros, M. Gallego, and M. Valcarcel, *Anal. Chim. Acta*, **282**, 581-588 (1993).
15. P. W. Park and R. E. Goins, *J. Food Sci.*, **59**, 1262-1266 (1994).
16. L.-E. Dayhuff and M. J. M. Wells, *J Chromatogr.*, A, **1098**, 144-149 (2005).
17. AOAC Official Methods of Analysis (1990), method 969.33.

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Improved Data Quality Through Automated Sample Preparation

Application Note

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Abstract

Sample preparation tasks can be extremely time-consuming and are often prone to errors, leading to poor reproducibility and accuracy. Many of these tasks, such as calibration curve generation, sample dilution, internal standard addition, or sample derivatization are performed daily, requiring significant resources as well. The Agilent 7696 Sample Prep WorkBench can perform many common sample prep tasks with better accuracy and precision than most manual methods, while using significantly fewer reagents and requiring less time from the operator. To demonstrate this, three sample preparation tasks were adapted for use on the Agilent 7696 Sample Prep WorkBench and yielded the same, if not better, results than the manual methods for accuracy and precision.

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Introduction

The Agilent 7696 Sample Prep WorkBench can perform many sample preparation tasks for either gas chromatographic (GC) or liquid chromatographic (LC) analyses. The Agilent 7696 Sample Prep WorkBench consists of two liquid dispensing modules, a single vial heater capable of reaching 80 °C, a single vial mixer, and barcode reader (Figure 1). This enables dilutions/aliquoting, liquid addition, heating for derivatization or digestion, liquid/liquid extractions, and sample mixing. Individual racks can also be heated and/or cooled. This sample preparation instrument can perform tasks with the same accuracy and precision as the Agilent 7693A Automatic Liquid Sampler only in an offline setting instead of on top of a GC [1]. Many sample preparation tasks such as sample dilution, calibration curve standard generation, and sample derivatization within both fields can be time consuming and resource intensive. Automating these procedures with the Agilent 7696 Sample Prep WorkBench therefore is beneficial in many ways.

Figure 1. The Agilent 7696 Sample Prep WorkBench.

A side-by-side comparison of manual and automated methods was performed for three common sample prep applications to demonstrate the improved data quality achieved through automated sample preparation. Sample dilution, calibration curve standard generation, and derivatizations were performed with success on the Agilent 7696 Sample Prep WorkBench.

Experimental

Three common sample preparation tasks were performed with the Agilent 7696 Sample Prep WorkBench. First, sample dilutions and internal standard additions were performed for analysis by both GC and LC. For the GC samples, 50 µL each of isooctane and a standard solution containing four analytes were added to an empty 2-mL autosampler vial. Additionally 0.5 µL of an internal standard solution (ISTD) containing three analytes was added to the vial. The solution was mixed using the onboard mixer before transferring the vials to a GC for analysis. The samples for LC followed a similar procedure. To an empty 2-mL autosampler vial, 187.5 µL of acetonitrile, 62.5 µL of a pesticide standard, and 125 µL of an ISTD were added. The sample was mixed before being transferred to an LC for analysis. For both of these sample dilutions, n=10.

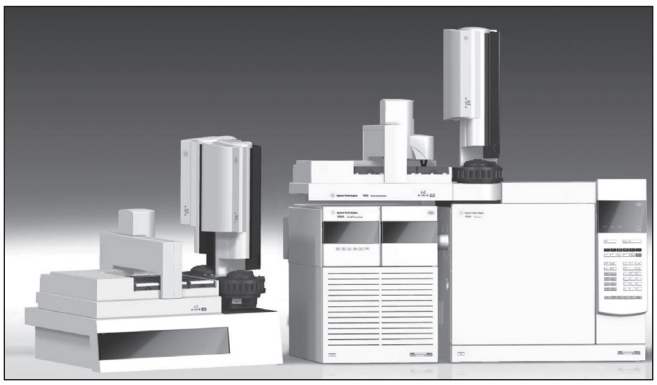

Figure 2. The Agilent 7696 Sample Prep WorkBench with a gas chromatograph and mass spectrometer.

Second, generic calibration curves for the GC were made in triplicate via linear dilution both manually in 10-mL volumetric flasks and with the Agilent 7696 Sample Prep WorkBench. To make the standards manually, small amounts of hexane was added to six clean, dry 10-mL volumetric flasks. Varying amounts of a stock solution containing five analytes at 5 mg/mL, ranging from 0.1 to 1 mL, were added using serological pipets. The flasks were diluted to the mark with hexane to yield concentrations of 50, 100, 200, 300, 400, and 500 ppm. For the automated method, 100 µL of hexane was added to six empty 2-mL autosampler vials. Again, varying amounts of the stock solution, ranging from 1 to 10 µL, was added to the vials yielding approximately the same concentrations.

Figure 3. The Agilent 7696 Sample Prep WorkBench with a liquid chromatograph.

Third, derivatization of fatty acids via silylation reaction was performed. For the manual prep, 100 µL of a silylating reagent was added to approximately 0.5 mL of a free fatty acid solution using an automatic pipettor. The solutions were heated to 70 °C using a heated block. The same derivatization was performed with the Agilent 7696 Sample Prep WorkBench using the single vial heater.

Results and Discussion

GC and LC Sample Dilution

For the 10 samples diluted for GC and LC analysis, the dispensed solvent, standard solution, and ISTD, was measured gravimetrically to determine the reproducibility of the dispensing action. Dispensing 50 µL with a 250 µL syringe results in a 0.5% relative standard deviation (RSD) for the 10 samples measured by weight. The samples were diluted within 1% accuracy, determined from the peak areas. The ISTD exhibited a slightly higher RSD. Dispensing 0.5 µL with a 25 µL syringe resulted in an RSD of 2% for the 10 samples. If a smaller syringe had been used to dispense the ISTD, a lower RSD, closer to that obtained when dispensing the solvent and standard, would have resulted. The added ISTD did not affect the accuracy of the diluted sample (Figure 4).

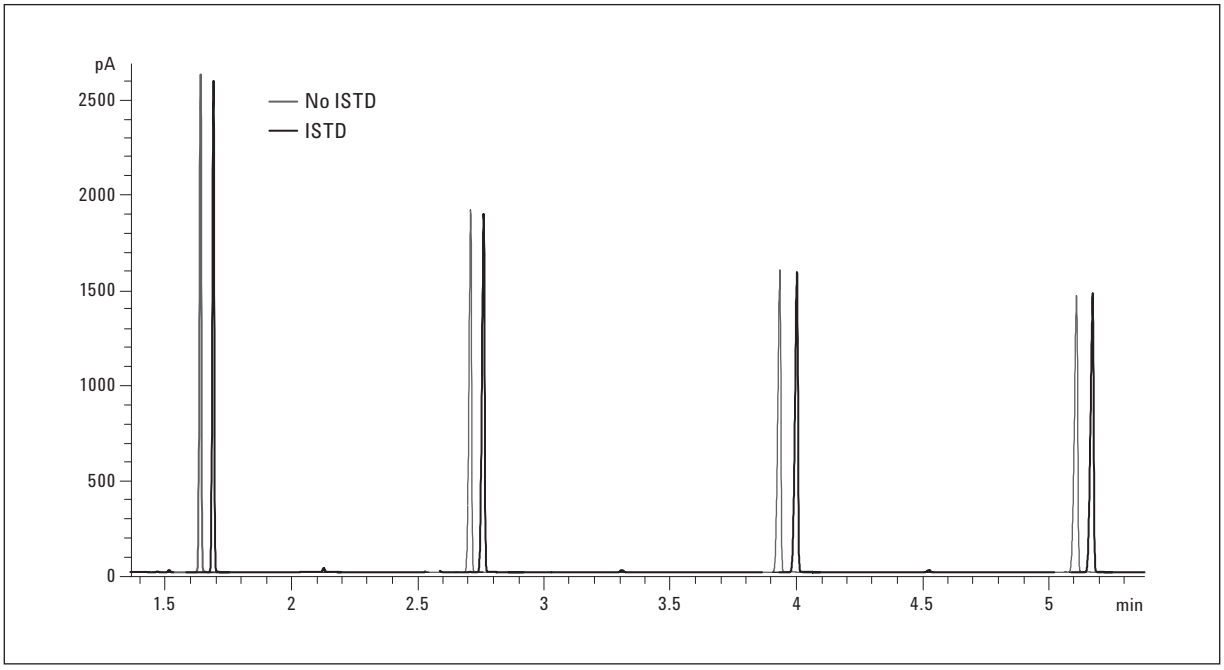

Figure 4. GC chromatograms (slightly offset) are shown for a standard solution dispensed and diluted with and without an ISTD added. No difference in peak areas are observed.

For the 10 samples diluted for LC analysis, similar results were obtained. Dispensing all three volumes with a 250 µL syringe resulted in a RSD of <0.5%, determined gravimetrically. By examining the peak areas after analysis, the dilutions were found to be accurate within 2% (Figure 5).

Calibration Curve Standard Preparation

Three sets of standards were made both manually and with the Agilent 7696 Sample Prep WorkBench. Comparing the three standard sets on the same plot highlighted the increased reproducibility of the Agilent 7696 Sample Prep WorkBench (Figure 6). While each individual curve yielded R^2 values of 0.999, when plotted together the R^2 value was reduced to 0.934 for the manually prepared standards. In contrast, the three curves prepared by the Agilent 7696 Sample prep WorkBench also yielded R^2 values of 0.999 for the individual curves, but when plotted together, the R^2 value was only reduced to 0.997.

Additionally, the relative response factor (RRF) was calculated for each set of standards. Calculating the RSD of the RRFs provides a measure of linearity and reproducibility. The individual calibration curves yielded good RSDs (<5%), demonstrating linear relationships. However, when comparing the three calibration curves together the superiority of the 7696 Sample Prep WorkBench made standards is evident. The average RSD of the RRFs for the three curves made manually was 16%; the three calibration curves made with the 7696 Sample Prep WorkBench gave an average RRF RSD of 4%.

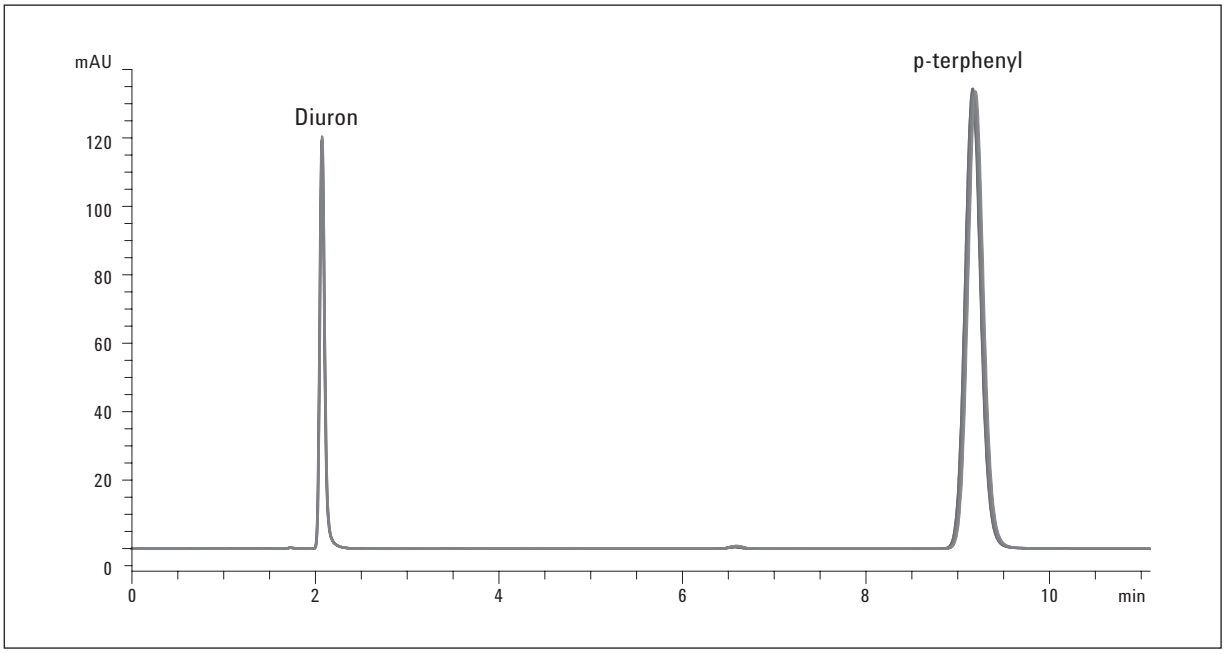

Figure 5. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

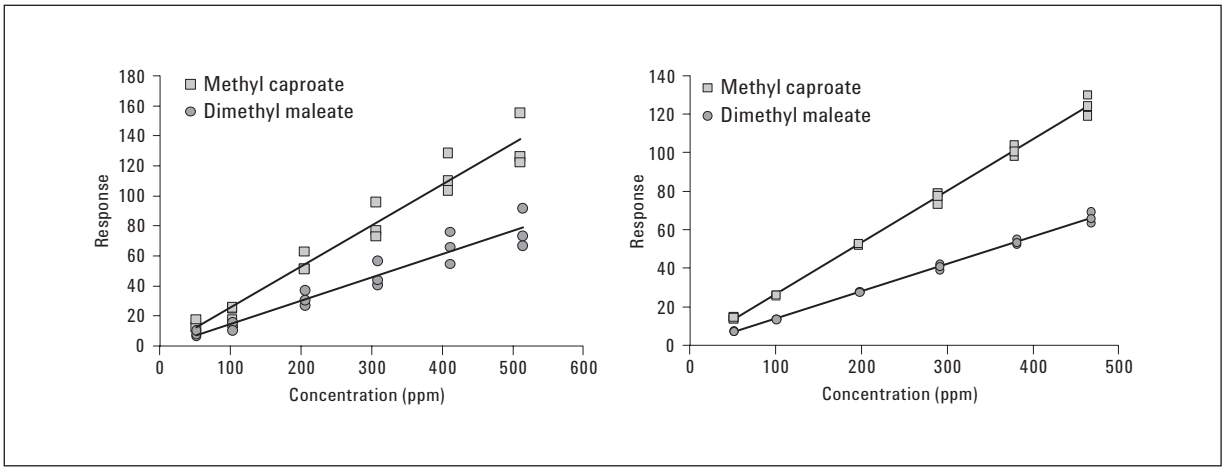

Figure 6. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with the Agilent 7696 Sample Prep WorkBench, are visibly more reproducible than the curves made manually on the left.

Fatty Acid Derivatization

For sample derivatization, identical results were obtained whether the sample was derivatized manually or with the Agilent 7696 Sample Prep WorkBench. For a set of four fatty acids, no discrimination was observed in either method when derivatizing with a silylating reagent (Table 1). However, as seen with other sample preparation tasks, the Agilent 7696 Sample Prep WorkBench is more reproducible in its liquid delivery. The RSD from the peak areas for the three samples prepared manually 0.9%. The RSD for the three samples prepared with the Agilent 7696 Sample Prep WorkBench was 0.7%.

Table 1. After normalizing the fatty acid peak areas to myristic acid, no discrimination was observed from automating the derivatization

Analyte	Ratio-manual	Ratio-automated
Capric acid	0.92	0.92
Capric acid	1.2	1.2
Myristic acid	1.0	1.0
Palmitic acid	1.1	1.1

Conclusions

The three sample preparation tasks presented in this application note highlight the increased reproducibility achieved by automation with the Agilent 7696 Sample Prep WorkBench. Sample dilutions are accurate and reproducible, calibration curve standards are more linear with fewer errors, and sample derivatizations can be performed without analyte discrimination. However, additional benefits can be reaped through sample prep automation with the Agilent 7696 Sample Prep WorkBench.

By automating calibration curve standard preparation, solvent and reagent usage is significantly reduced. Instead of using >60 mL of solvent to make up standards in 10-mL flasks, only 600 µL of solvent was used, excluding the wash vials. This can result in substantial cost savings for laboratories. Additionally, calibrations curve standards required approximately half the time to complete with the Agilent 7696 Sample Prep WorkBench, compared to making up the standards manually. While the other automated sample prep tasks require the same amount of time to complete as the manual methods, the Agilent 7696 Sample Prep WorkBench frees the operator to perform other tasks, such as experiment design or data analysis.

Overall there are many benefits to sample prep automation with the Agilent 7696 Sample Prep WorkBench. While freeing personnel to perform other tasks and reduced solvent usage are important, the largest benefit comes from the reproducibility and accuracy achieved with this system. The automated methods showed better reproducibility and accuracy with fewer errors, thereby improving the quality of the data.

Reference

1. Susanne Moyer, Dale Synder, Rebecca Veeneman, and Bill Wilson, "Typical Injection Performance for the Agilent 7693A Autoinjector," Agilent Technologies Publication 5990-4606EN.

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5990-6874EN

Agilent Technologies

Analysis of Sugars in Glycosylated Woody Biomass with the Agilent 1200 Series LC System

Application Note

Biofuels and Alternative Energy

Author

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Agilent Technologies Japan
Hachioji, Tokyo

Abstract

Although bioethanol is currently produced mainly from edible plants, research is underway into production methods based on non-edible plants, for example, wood. When wood is used as a raw material, glycosylation and fermentation are carried out by turning cellulose, hemicellulose, and lignin into small molecule compounds and subjecting the cellulose and hemicellulose to the action of enzymes. This is an example of analysis of sugars in wood sugar solutions obtained by low environmental impact hydrothermal treatment and mechanochemical treatment followed by enzyme-based glycosylation. The samples were kindly provided by Mr. Shigeki Sawayma, Head of the Research Team, and Mr. Katsuji Murakami, Chief Researcher, from the Biomass Research Center of the National Institute of Advanced Industrial Science and Technology.

Agilent Technologies

Configuration

Agilent 1200 Series LC System

- Agilent 1200 Series Quaternary Pump (G1354A)
- Agilent 1200 Series Standard Autosampler (G1329A)
- Agilent 1200 Series Thermostatted Column Compartment (G1316A)
- Agilent 1200 Series Evaporative Light Scattering Detector (G4218A)

Analytical Conditions

Column:	Shodex Asahipak NH2P-50 4E
Mobile phase:	Water/acetonitrile = 20/80
Flow rate:	1.0 mL/min
Column temperature:	30 °C
Injection volume:	20 µL
Sample concentration:	1000 ng/µL

A chromatogram of the reference solutions of sugars typically detected in wood sugar solutions is shown in Figure 1. Figures 2–6 show analytical results for wood sugar solutions obtained using different pre-treatment methods and raw materials. The samples were obtained by diluting wood sugar solutions with a mixture of water and acetonitrile (1:1) and passing the diluted solutions through a 0.22-µm filter.

The amount of the produced sugars and their ratios varied greatly depending on whether hydrothermal treatment or ball mill treatment was used. In addition, the amount of the produced sugars varied depending on the raw materials.

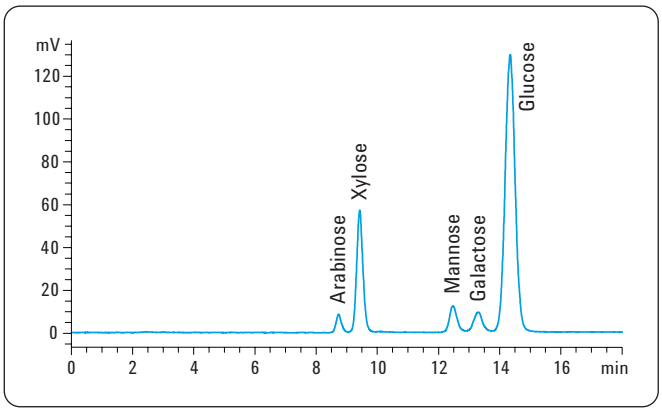

Figure 1
Chromatogram of reference solutions (1000 ng/µL each).

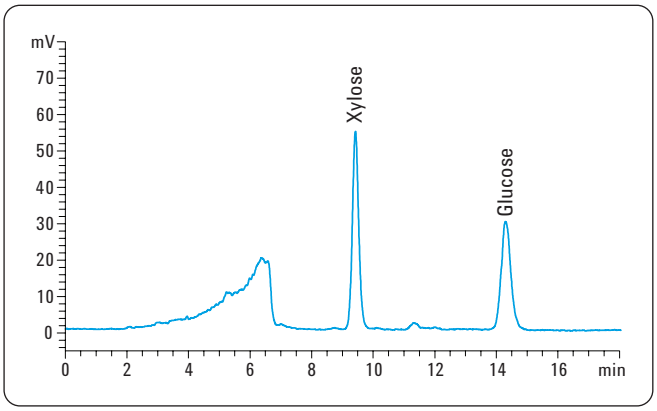

Figure 2
Bagasse, hydrothermal 180 °C 5 min.

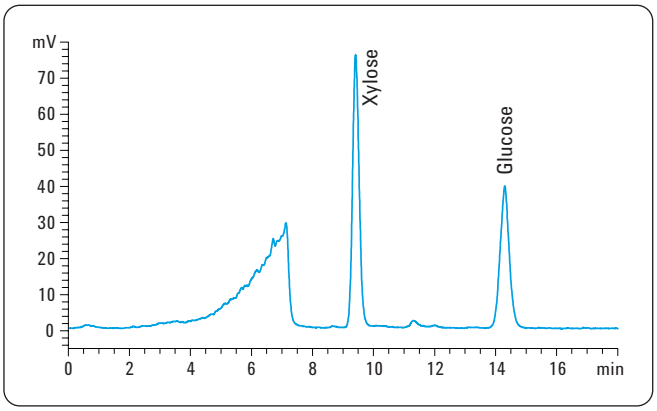

Figure 3
Bagasse, hydrothermal 160 °C 15 min.

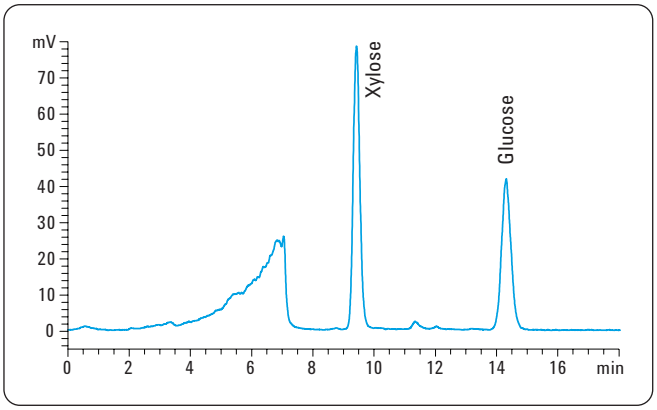

Figure 4
Bagasse, hydrothermal 160 °C 30 min, w/phosphoric acid.

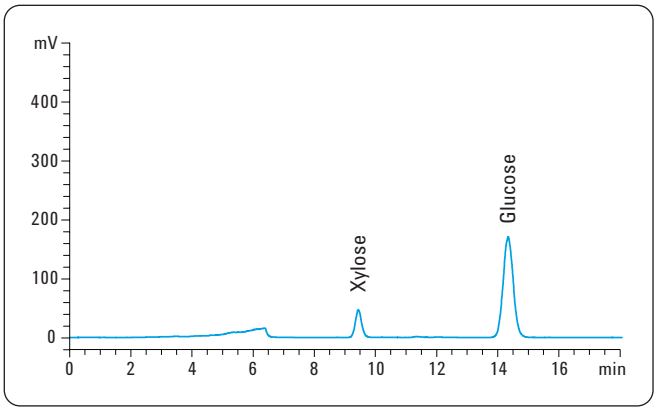

Figure 5
Bagasse, ball mill.

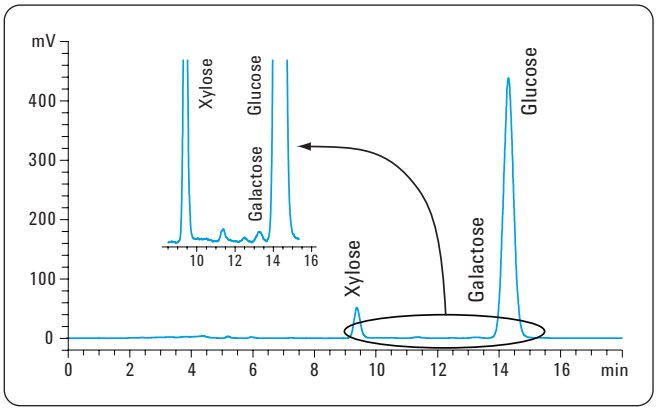

Figure 6
Eucalyptus, ball mill.

Figures 7–10 show analytical results for 2-fold dilution. A mannose peak was detected and several peaks believed to belong to oligosugars were observed subsequent to glucose elution. In addition, an unknown peak was detected prior to the mannose peak. It was found that there were few peaks believed to belong to oligosugars when the hydrothermal treatment was used and there were many peaks when the ball mill treatment was used.

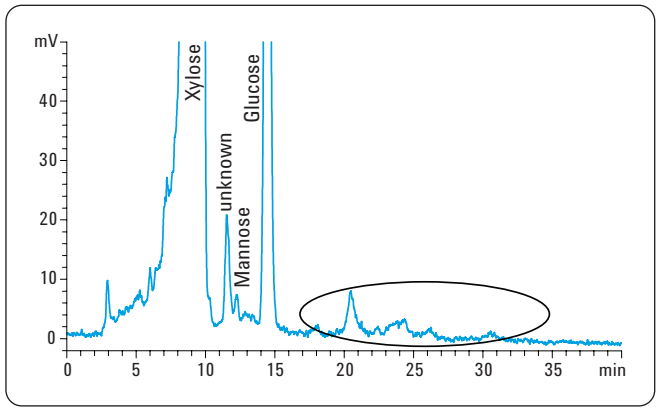

Figure 7
Bagasse, hydrothermal 180 °C 5 min (2-fold dilution).

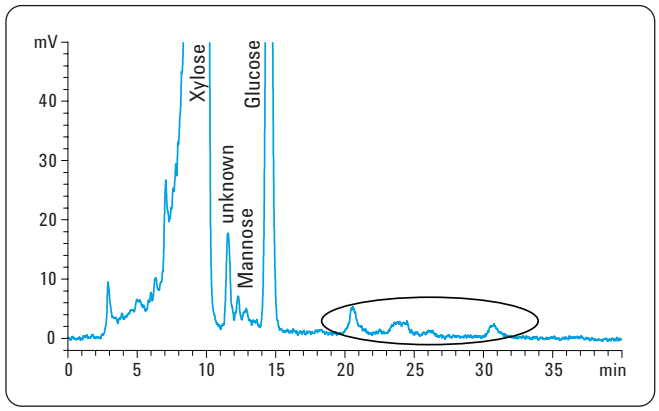

Figure 8
Bagasse, hydrothermal 160 °C 15 min (2-fold dilution).

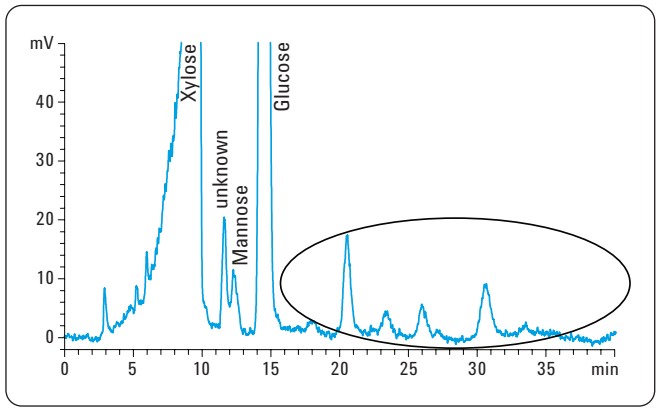

Figure 9
Bagasse, ball mill (2-fold dilution).

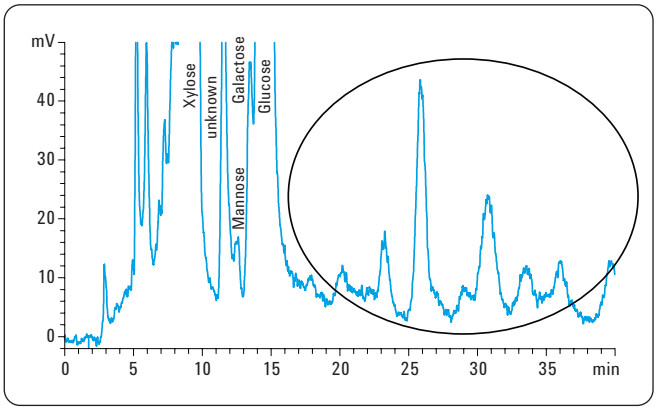

Figure 10
Eucalyptus, ball mill (2-fold dilution).

Conclusion

Sugars in glycosylated woody biomass are mainly xylose and glucose, but the concentration depends on the pre-treatment process. The Agilent 1200 Series LC system with the evaporative light scattering detector is suitable for sugar analysis in glycosylated woody biomass due to good sensitivity and good usability.

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Published February 1, 2011
Publication Number 5990-7318EN

Agilent Technologies

Analysis of Furfurals in Glycosylated Woody Biomass with the Agilent 1260 Infinity LC system

Application Note

Biofuels and Alternative Energy

Author

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Hachioji, Tokyo

Abstract

Although bioethanol is currently produced mainly from edible plants, research is underway into production methods based on non-edible plants, for example, wood. When wood is used as a raw material, furfural and 5-hydroxymethyl furfural (5-HMF) are produced as over-decomposition products of sugars during its glycosylation and fermentation. This is an example of analysis of furfurals in wood sugar solutions obtained by low environmental impact hydrothermal treatment and mechanochemical treatment followed by enzyme-based glycosylation. The samples were kindly provided by Mr. Shigeki Sawayama, Head of the Research Team, and Mr. Katsuji Murakami, Chief Researcher, from the Biomass Research Center of the National Institute of Advanced Industrial Science and Technology.

Agilent Technologies

The chromatograms of the reference solutions are shown in Figure 1.

Configuration

Agilent 1260 Infinity LC System

- Agilent 1260 Infinity Series Binary Pump SL (G1312B)
- Agilent 1260 Infinity Series High Performance Autosampler (G1367C)
- Agilent 1260 Infinity Series Thermostatted Column Compartment (G1316B)
- Agilent 1260 Infinity Series Diode Array Detector VL Plus (G1315C)

Analytical Conditions

Column:	Agilent ZORBAX RRHT SB-C18 3.0 mm × 50 mm,1.8 µm
Mobile phase:	0.1% formic acid/acetonitrile 3/97 (0–3 min)→ 10/90 (3–5 min)
Flow rate:	0.5 mL/min
Column temperature:	40 °C
Sample quantity:	2 µL
Detection:	Signal = 280 nm, bandwidth 4 nm, reference = 380 nm, bandwidth 80 nm

Figures 2–7 show analytical assay results of wood sugar solutions obtained using different pre-treatment methods and raw materials. The wood sugar solutions were diluted 10-fold with ultrapurified water and centrifuged, the supernatant was used for samples.

Conclusion

The amount of furfural and 5-HMF, which interfere with glycosylation and fermentation of biomass, depends on hydrothermal treatment process. The Agilent 1260 Infinity Series is suitable for the analysis in glycosylated woody biomass because of good sensitivity and good usability.

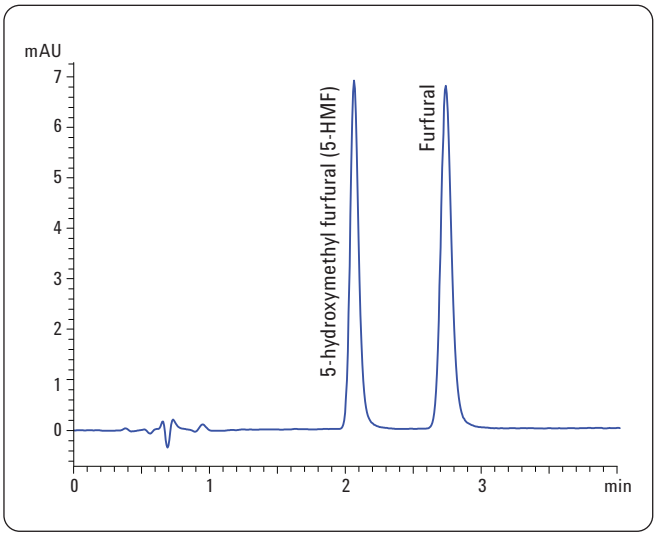

Figure 1
Chromatogram of Reference Solutions (1 mg/mL each).

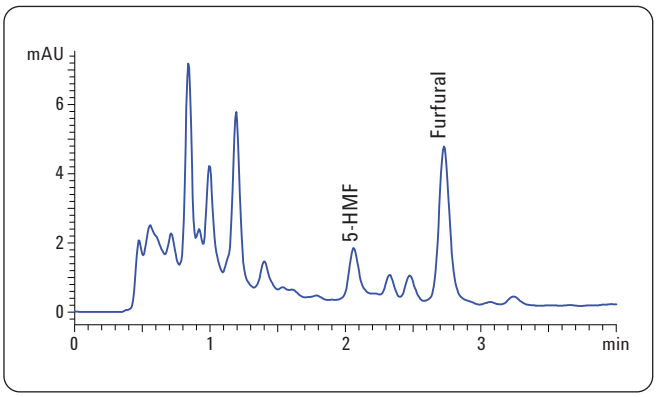

Figure 2
Eucalyptus, ball mill.

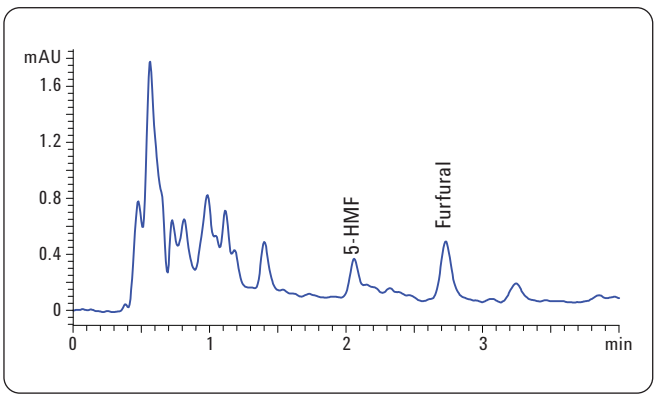

Figure 3
Bagasse, ball mill.

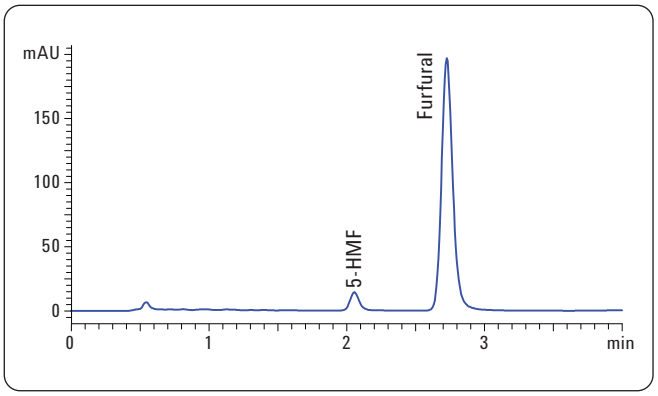

Figure 4
Bagasse, hydrothermal, 180 °C 5 min.

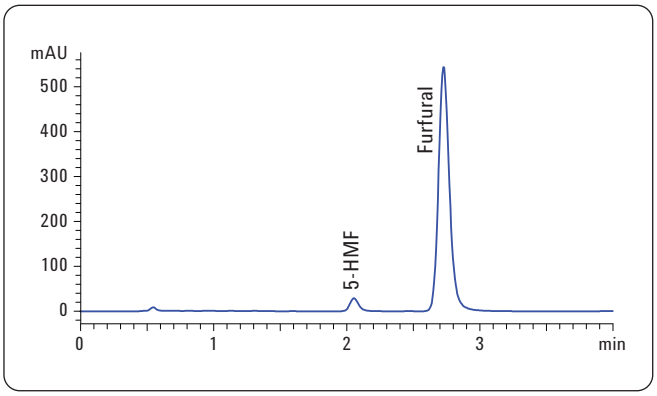

Figure 6
Bagasse, hydrothermal, 180 °C 30 min.

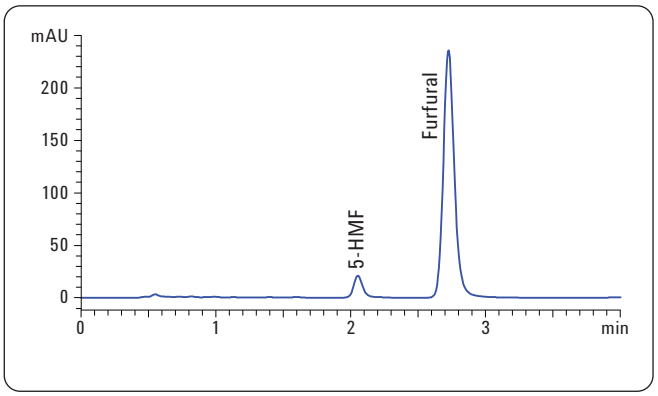

Figure 5
Bagasse, hydrothermal, 160 °C 15 min.

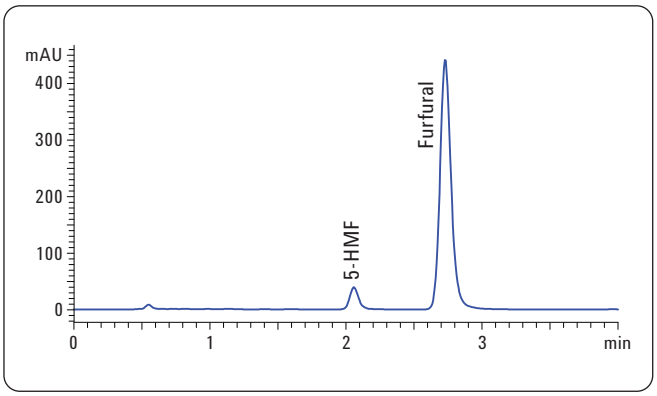

Figure 7
Bagasse, hydrothermal, 160 °C 30 min w/phosphoric acid.

Sample		5-HMF assay results [mg/L]	Furfural assay results [mg/L]
Eucalyptus	Ball mill	2.3	6.7
Bagasse	Ball mill	14.8	62.7
Bagasse	Hydrothermal treatment, 180 °C 5 min	22.6	298
Bagasse	Hydrothermal treatment, 160 °C 15 min	32.2	357
Bagasse	Hydrothermal treatment, 180 °C 30 min	45.5	826
Bagasse	Hydrothermal treatment, 160 °C 30 min w/phosphoric acid added	60.0	669

Table 1
Furfural assay results for samples (wood sugar solutions).

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Agilent Technologies

Analysis of FAME and TG in biodiesel fuel with the Agilent 1200 Series HPLC system

Application Note

Biofuels and Alternative Energy

Author

Keiko Nakamura
Agilent Technologies Japan
Hachioji, Tokyo

Abstract

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}.

In this Application Note an exemplary analysis of the concentration of FAME and triglycerides (TG) in biodiesel fuel (diesel oil) is shown. To establish calibration curves, methyl stearate was used as a reference compound for FAME concentration and trilinolein for TG concentration.

Agilent 1260 Infinity LC Verified

Agilent Technologies

Configuration

Agilent 1200 Series HPLC system

- Agilent 1200 Series Isocratic Pump (G1310A)
- Agilent 1200 Series Standard Autosampler (G1329A)
- Agilent 1200 Series Thermostatted Column Compartment (G1316A)
- Agilent 1200 Series Refractive Index Detector (RID) (G1362A)

A chromatogram of the reference solutions is shown in Figure 1. Figure 2 is an enlargement of the chromatogram shown in Figure 1. The retention time of methyl stearate (3.845 min) and the resolution of methyl stearate and trilinolein (10.54) met column performance criteria. Each of the solutions used for the calibration curves (Table 1) was analyzed twice. The calibration curves were obtained by plotting the concentration (1µg/10 µL) values (x) and the average surface area values (y) for each component (Figure 3 and Figure 4).

Analytical Conditions

Column:	Agilent ZORBAX Rx-SIL 4.6 mm × 250 mm, 5 µm
Mobile phase:	Hexane/2-propanol = 99.6:0.4
Flow rate:	1.0 mL/min
Column temperature:	40 °C
Injection volume:	5 µL

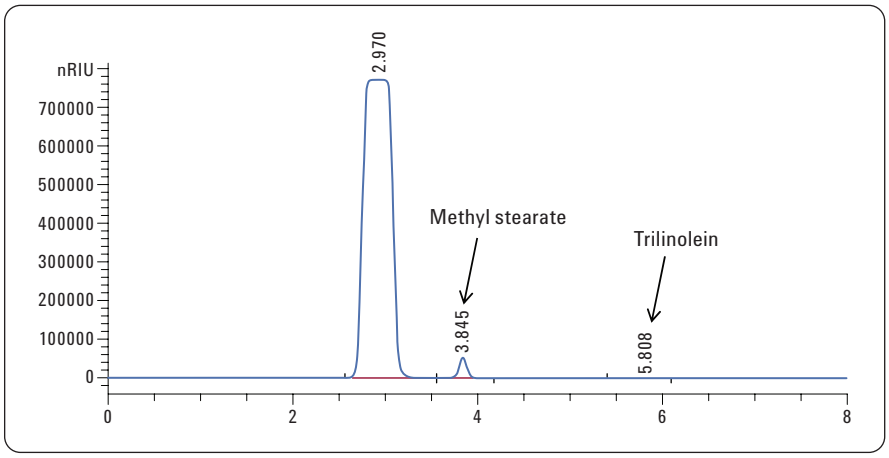

Figure 1
Exemplary analysis of reference compounds (methyl stearate: 100 µg/10 µL; trilinolein: 1 µg/10 µL).

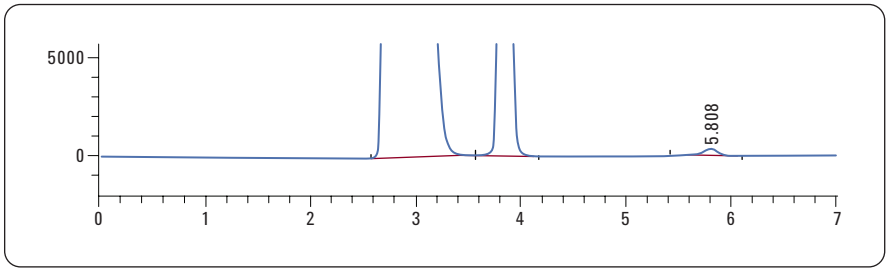

Figure 2
Enlarged Figure 1

Column performance		Figure 1 Results
Methyl stearate retention time	3.5 or more	3.845
Methyl stearate and trilinolein resolution	3 or more	10.54

Calibration curve solutions	Methyl stearat	Trilinolein
1	10	–
2	100	1
3	200	2
4	500	5
5 (undiluted)	1000	10

Table 1
Concentration of calibration curve solutions.

Conclusion

Since triglyceride in biodiesel fuel should be trace level, high sensitive analysis is required.

The Agilent 1200 Series LC system with a refractive index detector can detect small amount of Triglyceride (Quantification limit is 1 µg/10 µL) because of good baseline stability and low baseline noise.

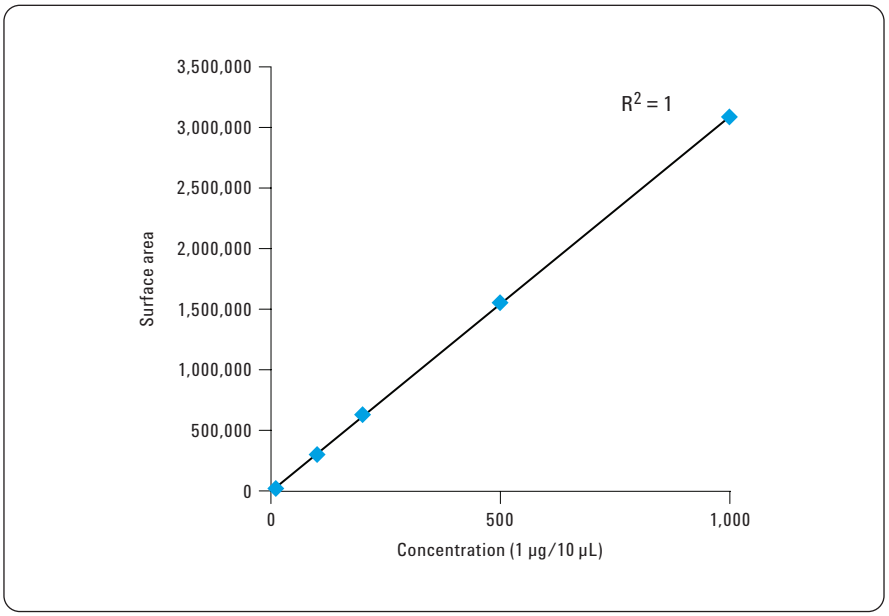

Figure 3
Methyl stearate calibration curve.

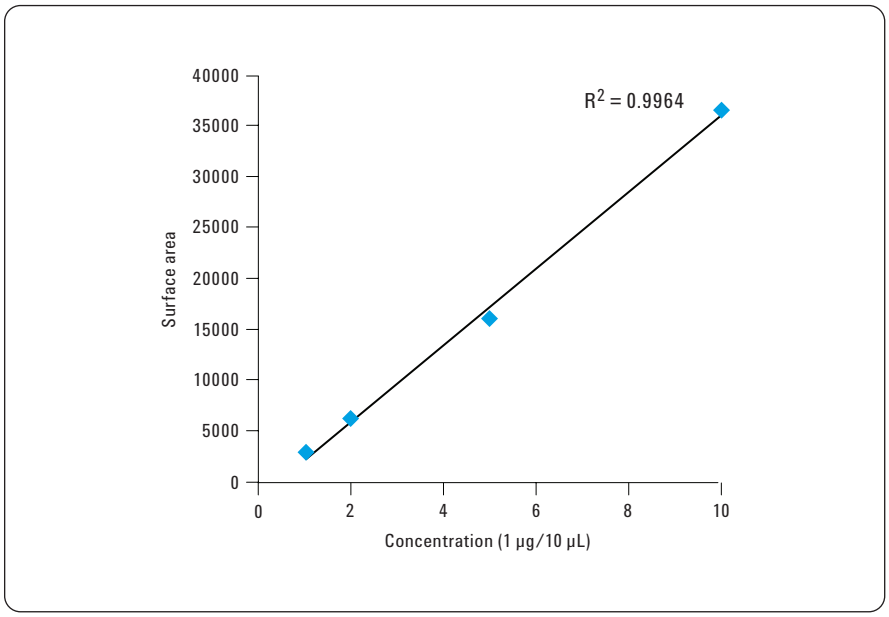

Figure 4
Trilinolein calibration curve.

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Agilent Technologies

Automation of a Complex, Multi-Step Sample Preparation Using the Standalone Agilent 7696A WorkBench

Application Note

Biofuels and Alternative Energy

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Abstract

The Agilent 7696A Sample Prep Workbench was used to automate a multi-step sample preparation. We chose ASTM method D6584 as a test case to demonstrate the capabilities of the WorkBench. This method requires a complex derivatization of non-volatile contaminants before analysis by gas chromatography. The WorkBench was used to prepare several different types of biodiesel and the calibration standard used to quantify the target contaminants. The results with the WorkBench prepared samples were nearly identical to those prepared manually. Analysis precision was very high and well within industry specifications for the WorkBench prepared samples. To further test the WorkBench, multiple groups of chemists developed an automated sample preparation for a biodiesel sample. The analysis results obtained between each group were also nearly identical with very high analysis precision.

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Introduction

In analytical chemistry, sample preparation can be as simple as adding a solvent or as complex as performing chemical reactions to improve the instrumental measurements that follow. While sample preparation is a critical component to any chemical measurement, chemists rarely look forward to performing this job, especially if it is complex, boring and involves handling unpleasant chemicals. As a result, manual sample preparation can be the source of many errors and poor precision. To help reduce errors and improve precision, many manual sample preparations are done using large amounts of chemicals and expensive volumetric glassware to make handling, dispensing, and measuring easier.

A good example of a difficult manual preparation is ASTM method D6584. This method measures the free and total glycerin content in B100 biodiesel to assure good product quality [1]. Since the various glycerins found in biodiesel are not volatile, they cannot be measured using gas chromatography (GC). Method D6584 describes a sample preparation protocol to derivatize these compounds with a trimethylsilation reagent so they can be analyzed with GC. The steps for this sample preparation are complex, time consuming, and use pyridine, a toxic solvent with a distinctly unpleasant odor. This explains the unpopularity of this sample prep among chemists working with biodiesel.

The Agilent 7696A Sample Prep WorkBench is a standalone instrument specifically designed to perform automated sample preparation [2,3]. It uses two 7693A injection towers to volumetrically transfer liquids between 2-mL vials. The vials containing various chemical resources, standards and samples are housed in three 50-position trays. The sample tray compartment houses a robotic arm to move vials, a vortex mixing station and a sample heating station.

Designing the 7896A WorkBench Procedure

The ASTM D6584 preparation procedure can be completely described in six individual steps as shown in Table 1. When done manually, this prep consumes large amounts of standards, reagents, solvents and disposable glassware. Since the Agilent WorkBench uses smaller 2-mL vials, this procedure can be scaled down by a factor of 10. The WorkBench also uses two pipetting syringes to transfer liquids, thus eliminating the expense of disposable glassware. Table 1 also shows how each step was scaled to accommodate the 2-mL vials used by the WorkBench.

Before building a WorkBench sample prep, we first defined the chemical resources needed to prepare the biodiesel samples and the position of those resources in the WorkBench trays. Table 2 shows each resource, their tray positions and the pipetting syringe parameters used to dispense each resource. The WorkBench software also provides a graphic, overhead view of the resources in the sample trays as shown in Figure 1. In this example, we show 10 samples in tray positions 1 to 10 and 10 n-heptane resource vials that will be used with each sample. The n-heptane vials are stored in tray positions 101 to 110.

Table 1. ASTM Method D6584 uses a six step derivatization of Glycerins in Biodiesel to prepare the samples for analysis by high temperature GC. Since the Agilent 7696A Sample Prep WorkBench uses 2-mL vials, the manual sample must be scaled down 10:1

Steps	Manual Sample Prep in 15-mL Vials	10:1 Scaling →	WorkBench Sample Prep using 2-mL Vials
1	Add 100 mg B100 to a 15-mL vial with Teflon screw cap		Add 10 mg B100 to a 2-mL vial with Teflon screw cap
2	Add 100 µL ISTD1 solution (butanetriol) to the vial		Add 10 µL ISTD1 solution (butanetriol) to the vial
3	Add 100 µL ISTD2 solution (tricaprin) to vial		Add 10 µL ISTD2 solution (tricaprin) to vial
4	Add 100 µL derivatization reagent (MSTFA) to vial and mix		Add 10 µL derivatization reagent (MSTFA) to vial and mix
5	React at room temperature for 15 minutes		React at room temperature for 15 minutes
6	Add 8 mL n-heptane to vial and mix		Add 800 µL n-heptane to vial and mix

Table 2. Four chemical resources are needed to completely derivatize Glycerins in Biodiesel. The resources, tray positions and syringe parameters are set in the Workbench Software. The syringe draw speeds are used to load each resource into the syringe. The syringe dispense speeds are used to transfer the resource into the 2-mL sample vials

Chemical resource	Tray position	Syringe size (µL)	Syringe draw speed (µL/min)	Syringe dispense speed (µL/min)
ISTD1 (1000 µg/mL butanetriol in pyridine)	51	100	250	500
ISTD2 (8000 µg/mL tricaprin in pyridine	52	100	250	500
MSTFA derivatization reagent	53	100	250	500
n-Heptane	101–110	250	500	2000

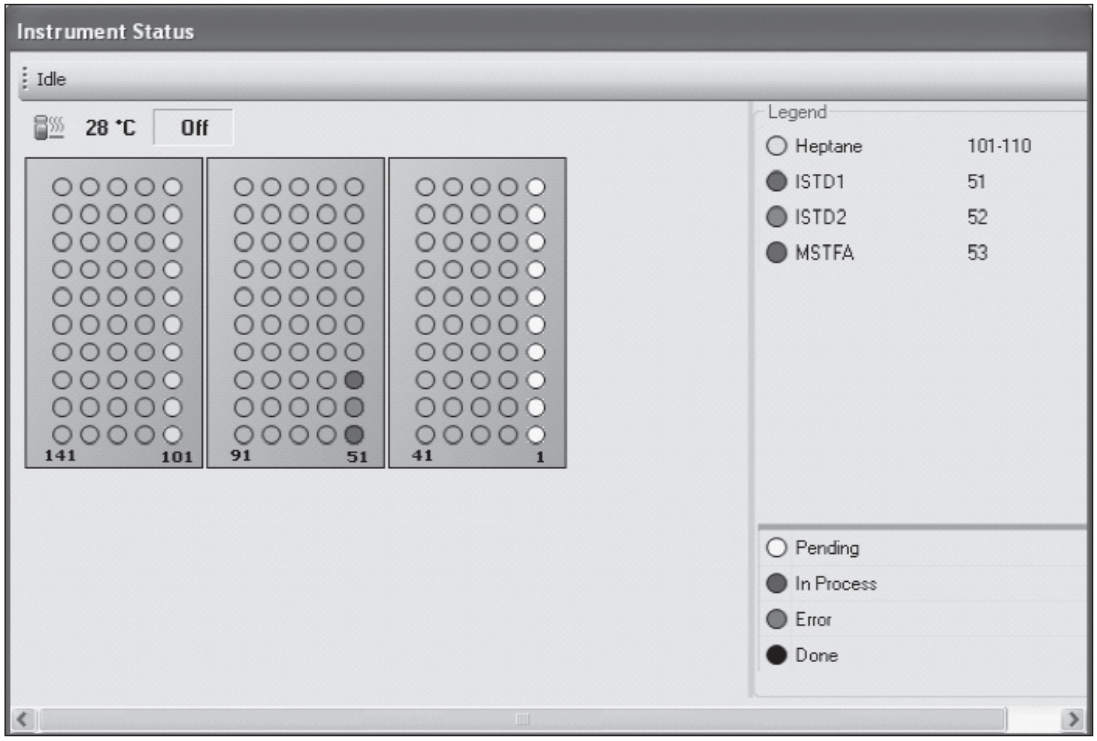

Figure 1. The WorkBench software provides an overhead view of each chemical resource in the sample trays. For this example, in addition to the chemical resources, 10 samples were placed in tray positions 1 to 10.

Sample weighing cannot be performed using the WorkBench because there is no analytical balance. Since weighing 10 mg of biodiesel can be very challenging, an Eppendorf Reference Adjustable-Volume Pipettor (10–100 µL) was used to transfer the sample. Weighing 10 mg of biodiesel was done by manually pipetting 11.4 µL of biodiesel into tared 2-mL vials and recording the weight to the nearest 0.1 mg.

To mimic the manual sample prep workflow, individual WorkBench methods were created for each step outlined in Table 2. For instance, we created a method called ADD_ISTD1.M to add the first internal standard solution (ISTD1) to every sample before adding the second internal standard (ISTD2) using method ADD_ISTD2.M. With this approach, we only needed to wash the syringe with solvent after switching to a different resource. This greatly reduces the amount of wash solvent needed and allows more samples to be prepared before refilling the wash solvent reservoirs. The final "script" for the WorkBench sample prep, including the syringe wash steps, is shown in Table 3. To run the complete sample prep, each method is run by the WorkBench sequence queue as shown in Figure 2.

Table 3. A final "Script" showing each step in the sample prep protocol and the corresponding Workbench Methods needed to perform each action

Steps	Biodiesel preparation protocol	Method name	Comments
1	Add 10 µL ISTD1 solution to every sample vial	ADD_ISTD1.M	Uses 100-µL syringe in rear tower
2	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
3	Add 10 µL ISTD2 solution to every sample vial	ADD_ISTD2.M	Uses 100-µL syringe in rear tower
4	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
5	Add 10 µL MSTFA reagent to every sample vial and mix	ADD_MSTFA.M	Uses 100-µL syringe in rear tower
6	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
7	React at room temperature for 15 minutes	Reaction.M	One 15 minute wait time is used for all samples
8	Add 800 µL n-heptane to every sample vial and mix	ADD_Heptane.M	Uses 250-µL syringe in front tower

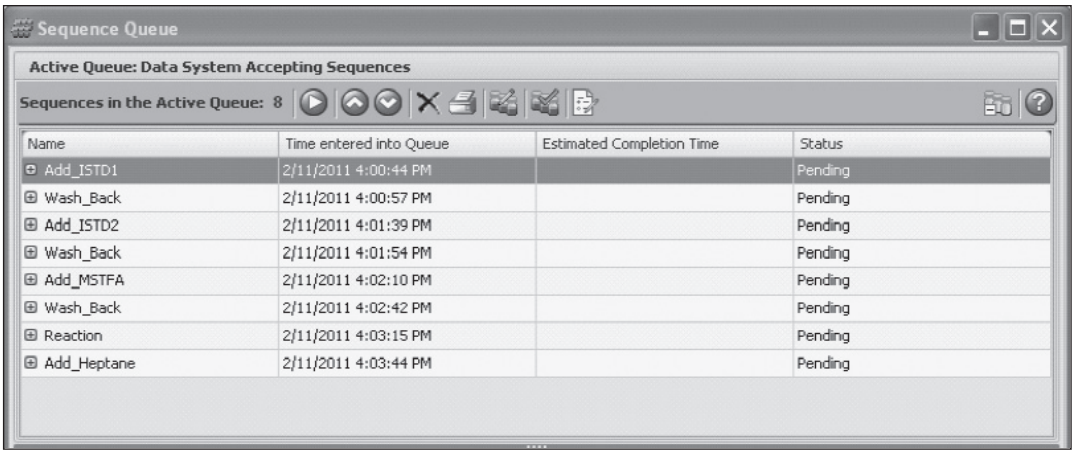

Figure 2. The WorkBench Sequence Queue is used to run the WorkBench methods described in Table 3.

Experimental

An Agilent 7890A GC was configured to run ASTM D6584. This configuration is outlined in Table 4. The GC conditions used to analyze the biodiesel samples and standards are shown in Table 5.

Preparation of GC calibration standards

ASTM D6584 also requires the derivatization of five calibration standards with the same preparation used for the samples. After running the standards by GC, the resulting calibration curves were evaluated for linearity before running any samples. The calibration standards were prepared both manually and with the WorkBench with the same protocol used for the samples. The calibration curves resulting from the manual prep were used to quantify the manually prepared biodiesel samples. The calibrations resulting from the WorkBench prepared standards were used to quantify the WorkBench prepared samples.

Comparison of manual sample prep and WorkBench sample prep

The first question many users will ask is "does a scaled WorkBench sample prep produce the same results as the manual sample prep?". To help answer that question, two different types of biodiesel samples were prepared using the manual ASTM protocol and the WorkBench. The first biodiesel sample came from a small local producer using canola oil as the feedstock. The second sample was supplied by a national producer using a soybean oil feedstock. For both the manual and WorkBench protocols, each biodiesel sample was prepared and analyzed in duplicate to evaluate the repeatability (single user precision) according to the ASTM method.

Multiuser precision - reproducibility

In order to evaluate multi-user precision, four different chemists were provided with a soybean biodiesel sample, calibration standards and a WorkBench with the chemical resources shown in Table 2. Each chemist was given the list of sample preparation steps outlined in Table 3 and asked to develop and use a WorkBench protocol. Duplicates of a soybean biodiesel sample were prepared using their WorkBench followed by GC analysis.

Table 4. Gas Chromatographic Instrument configuration used to analyze samples using ASTM Method

Standard Agilent 7890A GC	
Hardware	
G3440A	Agilent 7890A Series GC
Option 122	Cool-On-Column Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693A ALS
Columns	
Analytical Column	Select Biodiesel for Glycerides 15 m x 0.32mm id x 0.1 µm film (p/n cp9078)
Data System	Agilent Multi-Technique Chemstation
Consumables	
5181-1267	10 µL Teflon fixed autoinjector syringe
Standards and Reagents	
5190-1408	Biodiesel D6584 Calibration Standards Kit
5190-1407	Biodiesel MSTFA Kit
	Reagent grade n-heptane

Table 5. GC Instrument Conditions for ASTM Method D6584

Cool-on-column inlet	
Initial temperature	50 °C
Temperature program	Oven track mode
Column flow	Helium at 3 mL/min constant flow mode
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

Results

Preparation of GC calibration standards

The 5-level calibration curves for glycerin, monoolein, diolein and triolein are shown in Figure 3. The five standards used to create these curves were prepared with the Agilent WorkBench. The glycerin curve was used to quantify free glycerin in the biodiesel samples. The monoolein curve was used for the monoglycerides, the diolein curve for all diglycerides and the triolein curves for all triglycerides found in the samples. The same calibration standards were also prepared manually and used to construct calibration curves. In Table 6, we compared the calibration models for all four compounds from the manually prepared standards and the WorkBench prepared standards. The manually prepared standards and the WorkBench prepared standards yielded nearly identical calibration curves and the correlation coefficients (r^2) from the WorkBench prepared standards exceeded the ASTM specification of at least 0.99 or greater.

Table 6. The calibrations curves resulting from manual and WorkBench Preparation Protocols were very similar as shown by the respective slopes and intercepts for each compound. Both preparation Methods met the ASTM requirement for Correlation Coefficient Values (r^2) of 0.99 or greater

	Manual Prep			WorkBench		
Compound	**Slope**	**y-int**	**r^2**	**Slope**	**y-int**	**r^2**
Glycerin	1.0433	0.0028	0.9997	1.1027	0.0049	0.9995
Monoolein	1.3446	–0.0171	0.9997	1.3786	0.0044	1.0000
Diolein	1.2176	–0.0010	0.9999	1.2086	–0.0014	0.9999
Triolen	0.8303	–0.0018	0.9965	0.8703	0.0030	1.0000

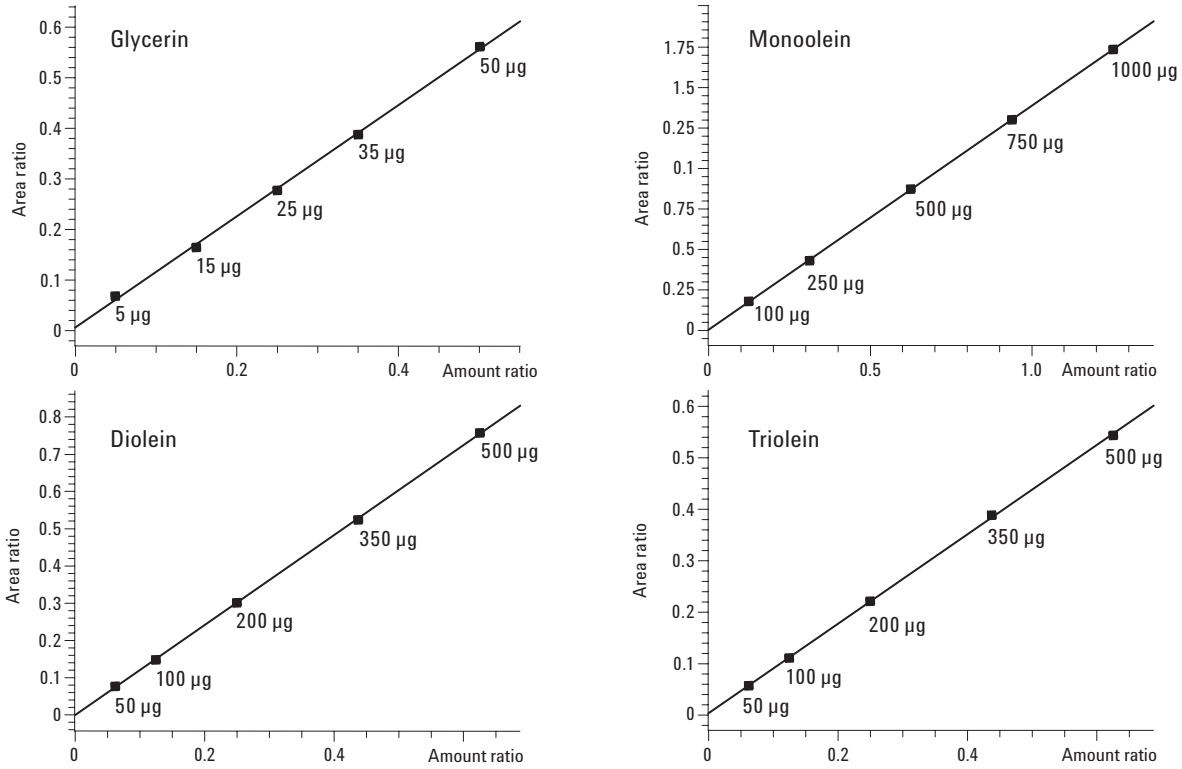

Figure 3. Calibration curves from standards prepared using the WorkBench.

Comparison of manual sample prep and WorkBench sample prep

The biodiesel samples prepared manually and with the WorkBench were analyzed according to ASTM method D6584. Figure 4 shows a comparison of biodiesel sample 1 (canola) chromatograms resulting from the manual prep and the WorkBench prep. In the regions where the various glycerins elute, both chromatograms look identical. For all samples, the free and total glycerins were quantified and the results are listed in Table 7. The WorkBench sample prep yielded results that were identical to those prepared manually. Both samples were prepared and analyzed in duplicate to determine the repeatability of the sample preparations. Repeatability (r) is used to measure the precision for a single operator by taking the difference between duplicate analyses of each sample. As seen in Table 7, the samples prepared using the WorkBench exceeded minimum repeatability specification set by ASTM for this analysis. This shows that after a 10-fold reduced scale, samples prepared with WorkBench can easily provide the same precise results as manually prepared samples using much larger amounts of chemicals, reagents and solvents.

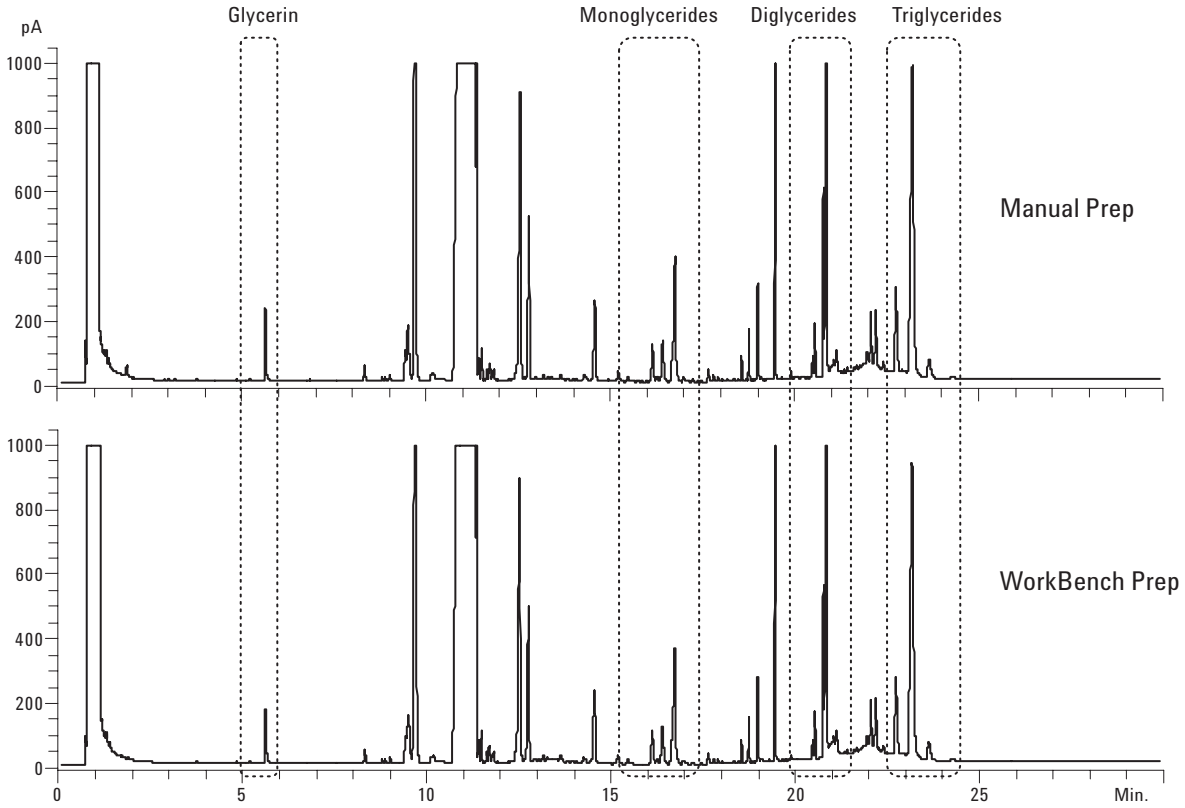

Figure 4. A comparison of data from a canola biodiesel sample prepared manually and using the Agilent WorkBench. These chromatograms show remarkable similarity in the four regions where glycerin, monoglycerides, diglycerides and triglycerides are separated.

Table 7. For two different types of Biodiesels, the WorkBench sample results were nearly identical to the samples prepared manually. The precision (repeatability) observed for the WorkBench samples were well within ASTM Specifications

Biodiesel Sample 1 (canola)

	Manual Prep			WorkBench			Reproducibility (r)
	Run 1	**Run 2**	**r**	**Run 1**	**Run 2**	**r**	**Specification**
Free Glycerin	0.000	0.000	0.000	0.000	0.000	0.000	2.58E-04
Monoglycerides	0.169	0.169		0.168	0.163		
Diglycerides	0.282	0.286		0.291	0.286		
Triglycerides	0.533	0.536		0.565	0.554		
Total Glycerin	0.984	0.991	0.007	1.023	1.003	0.020	0.083

Biodiesel Sample 2 (soybean)

	Manual Prep			WorkBench			Reproducibility (r)
	Run 1	**Run 2**	**r**	**Run 1**	**Run 2**	**r**	**Specification**
Free Glycerin	0.008	0.008	0.000	0.008	0.008	0.000	0.002
Monoglycerides	0.138	0.144		0.141	0.140		
Diglycerides	0.022	0.023		0.022	0.021		
Triglycerides	0.009	0.009		0.006	0.005		
Total Glycerin	0.177	0.184	0.007	0.176	0.174	0.002	0.046

Multiuser precision - reproducibility

Figure 5 shows the same soybean biodiesel sample independently prepared by four different chemists on four different days. The chromatography between each chemists is nearly identical. The quantitative results obtained by each chemist are shown in Table 8 along with an evaluation of the precision between groups (reproducibility). These results show a very high level of precision when several chemists develop an automated WorkBench protocol for preparing the same sample.

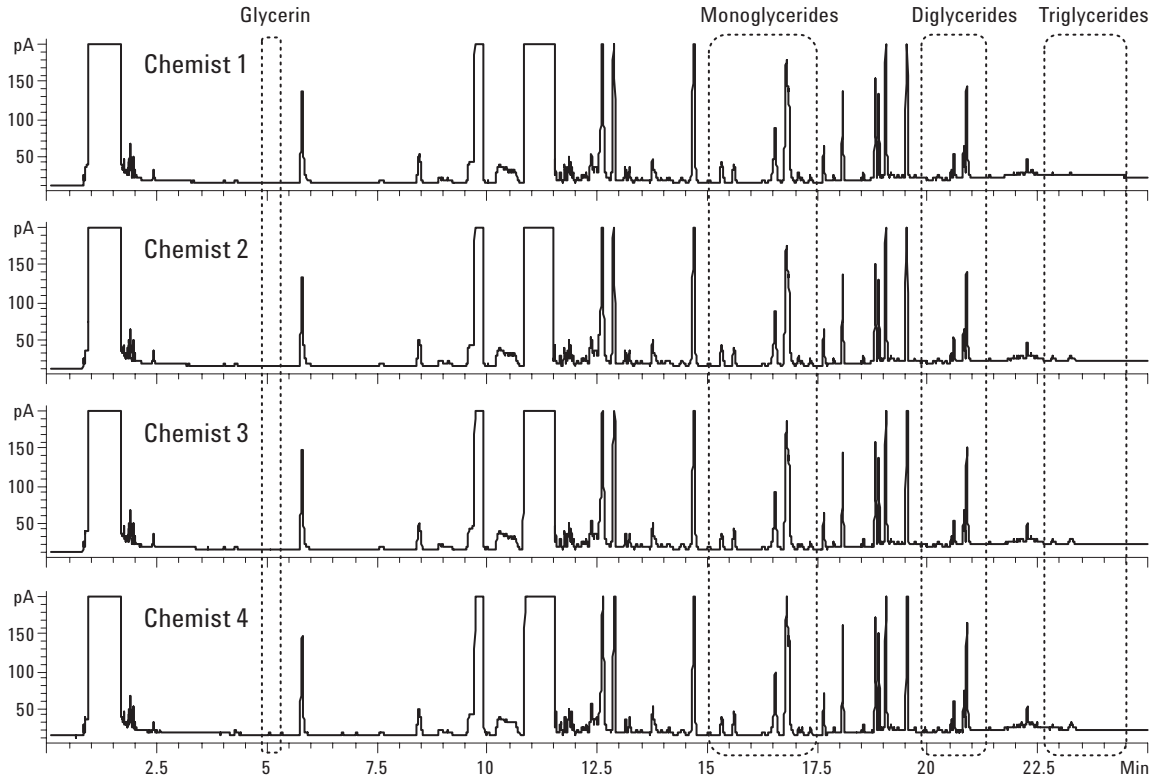

Figure 5. A comparison of data from a soybean biodiesel sample prepared by four different chemists working on four different days. Each chemist developed a WorkBench sample preparation protocol and then analyzed the samples using ASTM method D6584. The results are nearly identical.

Table 8. Each chemist obtained nearly the same results when using the Agilent WorkBench for Automated Sample Preparation. The precision (reproducibility) was well within the ASTM Specification for multiple operators

	Chemist 1			Chemist 2			Reproducibility	ASTM R
	Run 1	Run 2	Average	Run 1	Run 2	Average	(r)	Specification
Free Glycerin	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.007
Monoglycerides	0.107	0.114	0.111	0.109	0.118	0.113		
Diglycerides	0.032	0.034	0.033	0.033	0.036	0.034		
Triglycerides	0.009	0.009	0.009	0.008	0.009	0.008		
Total Glycerin	0.152	0.161	0.156	0.154	0.166	0.160	0.005	0.094

	Chemist 3			Chemist 4			Reproducibility	ASTM R
	Run 1	Run 2	Average	Run 1	Run 2	Average	(r)	Specification
Free Glycerin	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.007
Monoglycerides	0.116	0.114	0.115	0.113	0.114	0.113		
Diglycerides	0.033	0.033	0.033	0.032	0.033	0.032		
Triglycerides	0.007	0.007	0.007	0.006	0.006	0.006		
Total Glycerin	0.160	0.157	0.159	0.155	0.157	0.156	0.004	0.091

Conclusion

This paper demonstrates that a complex, multi-step sample preparation protocol can be automated with the Agilent 7696A WorkBench. Analytical results obtained with WorkBench prepared samples were the same as those obtained using a traditional manual sample preparation. Even after scaling the preparation steps for the 2-mL vials, the quantitative precision was very high with WorkBench prepared samples. Reducing the sample prep scale with the WorkBench also used 10 times less solvents, reagents, and calibration standards. Additionally, there was no need to use disposable glassware and expensive volumetric glassware.

References

1. "D6584 Test Method for Determination of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography", ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.
2. "Agilent 7696A Sample Prep WorkBench", Agilent Technologies, Publication Number 5990-6908EN, January 28, 2011.
3. Rebecca Veeneman and Dale Snyder, "Improved Data Quality through Automated Sample Preparation", Agilent Technologies, Publication Number 5990-6974EN, December 10, 2010.

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Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy

Fast, easy antioxidant measurement

Application Note

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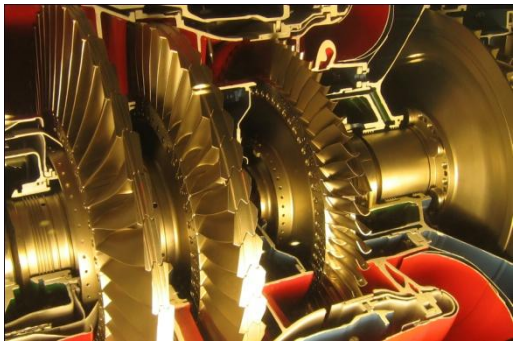

Abstract

Agilent 5500t FTIR spectrometers can independently measure phenolic and aminic antioxidants in turbine oil and provide the time sensitive results necessary to assist in preventing a non-scheduled shutdown by ensuring reliable operation of the turbine equipment. The 5500t FTIR system alerts, at pre-set warning levels, when the phenolic and aminic antioxidants are at or approaching minimal concentration milestones, and thus helps prevent turbine oils from reaching the critical point in the oxidation cycle of oil. Measurement is quick, easy and can be performed at-site. It requires no sample preparation, calibration, or electrode maintenance involved with voltammetric systems.

Agilent Technologies

Introduction

The Agilent 5500t FTIR (Fourier transform infrared) spectrometer, a compact, easy-to-use and affordable system, provides the ability to perform real-time, onsite analysis of high value assets such as turbines. With 5500t FTIR spectrometers, the lubrication specialist has the ability to simultaneously monitor key parameters such as oxidation, additive concentrations and levels of water in lubricants. This application note will demonstrate the ability to monitor the depletion of key additives using the 5500t FTIR spectrometer.

Antioxidants in turbine oil

The phenolic and aminic antioxidants in turbine oils function as preservatives, which prevent the oil from oxidizing and forming harmful varnish deposits. Oxidation causes turbine oils to quickly lose viscosity and wetting characteristics, which protect metal contact surfaces and prevent wear. Oxidation arises from a combination of sources including elevated temperatures, extreme pressures, high shear conditions, the presence of water and metal particles, and is accelerated by electrostatic sparking, particularly in certain gas turbine systems. Antioxidants inhibit the formation of these decomposition products, however once the antioxidants are consumed, the process accelerates exponentially and at a certain critical point, corrective action has negligible benefit. The 5500t FTIR system measures both the antioxidant levels and the amount of oxidation present, to ensure that corrective action is taken before this critical point is reached.

Measuring antioxidants in turbine oil with the Agilent 5500t FTIR

The primary and most abundant antioxidant is the phenolic antioxidant, which works synergistically with the aminic antioxidant. It is postulated that the phenolic antioxidant protects the workhorse aminic antioxidant, which has the ability to recharge itself over and over during the cycles of oxidation. This is consistent with data we have obtained, as will be demonstrated later in this application note.

The phenolic and aminic antioxidants in turbine oil have prominent absorbance bands in select regions of the infrared spectrum, thus enabling FTIR spectroscopy to be an ASTM preferred means of measurement. Figure 1 shows one of the major infrared bands of the phenolic antioxidant in turbine oil and the change in the band, as a function of time, as the antioxidant is depleted. Similarly, Figure 2 illustrates the incremental diminishment of the aminic antioxidant as the turbine oil ages. These bands are so characteristic of these two species that they are often called 'fingerprint bands' and they are the functional groups that are automatically tracked by the 5500t FTIR spectrometer software.

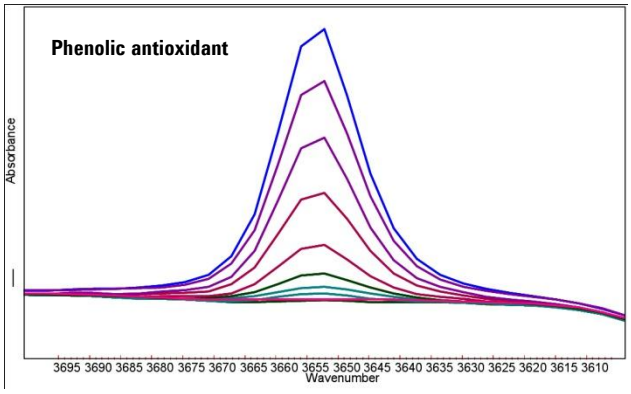

Figure 1. FTIR spectral overlay of the phenolic antioxidant functional group bands depleting as a function of time. The strongest band (light blue) is that of new ISO 32 turbine oil and the weakest absorbance (light green) is from turbine oil that has started to show some oxidation.

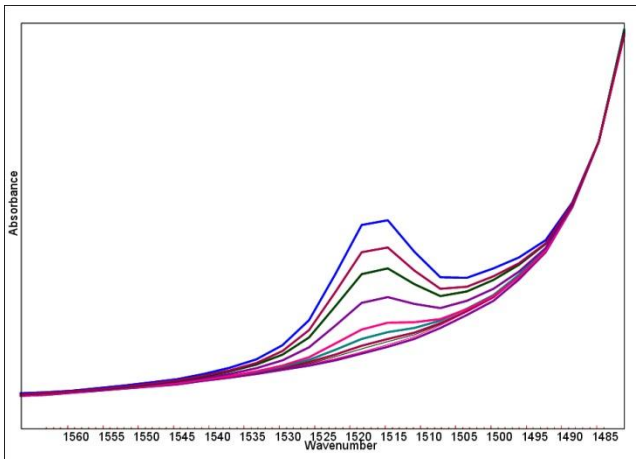

Figure 2. FTIR spectral overlay of the aminic antioxidant functional group depleting as a function of time. The strongest absorbance (red) is aminic antioxidant in new ISO 32 turbine oil and the weakest bands (blue and green) are from turbine oil with spent antioxidant.

The 5500t FTIR software (Figure 3) stores the FTIR spectrum of the initial new or reference oil. When in service used oil is measured, its spectrum is overlaid and compared to the reference oil. The user is provided a weight % for each phenolic and aminic antioxidant as well as a visual overlay of the spectral regions associated with each additive. The turbine oil methods also provide oxidation and nitration as a percentage of an upper limit, which is set from oxidation tests. The 5500t FTIR software is also programmed to inform the user via a yellow 'Monitor Frequently' warning when each additive is nearing the critical depletion points. Likewise, a red 'Change Immediately' warning is displayed on any additive, or other component such as water or oxidation, which has reached a critical threshold. Therefore, if both the phenolic and aminic antioxidants are in the red zone the critical saturation point for oxidation is imminent. The oxidation and ppm water are also provided with visual comparisons to the reference oil.

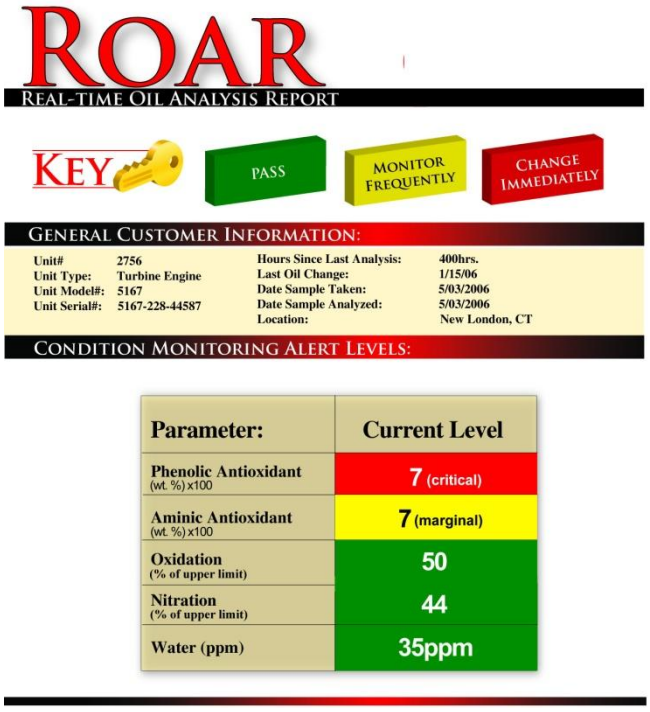

Figure 3. Agilent 5500t FTIR software presents the user with the specific concentration of phenolic and aminic antioxidants as well as crucial information about oxidation by-products and level of water contamination

The relationship between antioxidant depletion and oxidation

We will demonstrate the relationship of antioxidants and oxidation formation as well as the ability of the 5500t FTIR system to both predict and detect oxidation formation before the critical point is reached. Metallic iron and copper, known oxidation catalysts were added to used Chevron ISO 32 turbine oil that was in service 4 months in a steam turbine system. The iron and copper catalysts accelerate the inherent thermal oxidation mechanism, and are used in most oxidation potential tests such as RPVOT (D2272), Universal Oxidation Test (D6514 and D5846), and TOST (D943).

This mixture was heated at 135 °C for 26 days at atmospheric pressure in air, and small samples of the oil were removed every 2 to 3 days. The samples were analyzed using a 5500t FTIR spectrometer and the peak area measurements for phenolic antioxidant, aminic antioxidant, and oxidation products were recorded and plotted as a function of time as shown in Figure 4. As shown, the phenolic antioxidant diminishes to about 40% of the original amount in a relatively short time, however, the aminic antioxidant is observed to stay above 80% for almost the whole life span of the oil. Some of the initial drop in the phenolic antioxidant is due to evaporation which is a known problem with certain more simple phenolic antioxidants. The aminic antioxidant is observed to have three stages:

- Stage 1: The aminic antioxidant level is fairly constant and remains at this level approximately halfway thru the useful life of the oil. The initial slight increase in aminic may be due to volatiles in the oil, which can evaporate from the new oil during high temperature operation, thus slightly increasing the concentration of the aminic antioxidant.
- Stage 2: The aminic antioxidant depletes rapidly by about 25% at the mid-way point in the useful life of the oil.
- Stage 3: After the phenolic drops below 30% of the original concentration (70% depletion) the aminic begins a rapid descent from 80 to 40%. At this

critical point, the oxidation process accelerates exponentially. Corrective action would need to be taken prior to this stage in order to extend the useful lifespan of the oil.

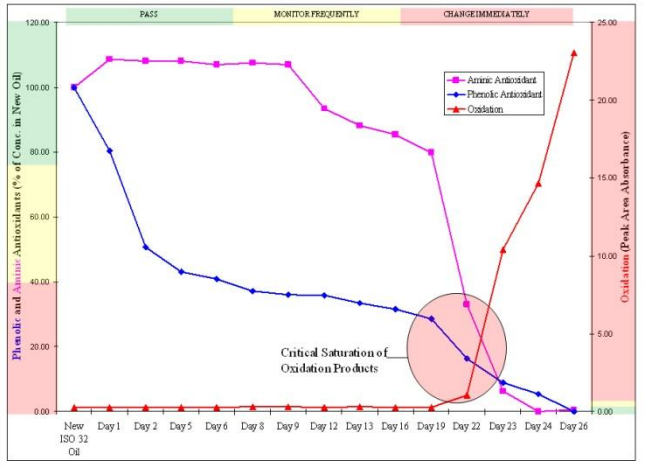

Figure 4. The additive depletion (% relative to new oil concentrations, left scale) and oxidation formation (right scale) trend analysis in thermally stressed ISO 32 turbine oil generated using the Agilent 5500t FTIR spectrometers

Lube 'useful life' measurements – Agilent 5500t FTIR versus voltammetric methods

As we have demonstrated in this application note, the 5500t FTIR system measures each antioxidant species individually, as well as providing a direct measurement of the degree of oxidation in the oil.

Cyclic voltammetric methods rely on mixing an exact amount of an oil sample with exact amounts of an electrolyte solution, the solution is shaken, at which point the antioxidants are extracted into the electrolyte solution. The results require a sample of the new oil for comparison and the used oil results are given in % depletion instead of exact concentrations such as weight %. This also causes inaccurate results if the used oil has been mixed with slightly different brands of oils. Another potential drawback to this technique is the antioxidant extraction from oil is never 100% efficient (typical extraction efficiencies are 75 to 95%), so not all of the active antioxidants are being measured. The pipetting required for voltammetric methods is not as accurate for higher viscosity oils, especially with gear oils or greases. Separate electrolyte solutions are needed for measuring oxidation and additional different solutions are needed to analyze crankcase or polyol ester based oils. The voltammetric method doesn't measure water or nitration, and contaminants in the oil such as EHC hydraulic fluid may cause inaccurate results. However, the 5500t FTIR spectrometer can detect the presence of contaminants such as EHC hydraulic fluid in turbine oils or gear oil in turbine oil.

The 5500t FTIR system requires only a drop of neat oil for its measurements and no sample preparation, whereas, voltammetric systems require careful pipetting techniques and an extraction step using an electrolyte solution. The FTIR system comes fully calibrated for weight % antioxidant functional groups in turbine, gear, hydraulic, and crankcase oils. Metal particles, water, or organic salts (that is, ionized carboxyls such as copper carboxylates) will not interfere with the antioxidant measurements using the 5500t FTIR system. The 5500t FTIR system has virtually no learning curve, requires no maintenance nor special chemicals or reagents for antioxidant measurement. Since the antioxidants can be monitored independently using the 5500t FTIR, re-additization can be carefully controlled and monitored. The effectiveness of top-offs, bleed and feed, filtration, and dehydration can be monitored as well. Mixing oil brands is not recommended, but the weight % phenolic and aminic antioxidants are still accurate measurements no matter what mineral oil basestocks are mixed together.

Conclusions

Agilent 5500t FTIR spectrometers are capable of independently measuring phenolic and aminic antioxidants in turbine oil and provide the time sensitive results necessary to assist personnel in preventing a non-scheduled shutdown by ensuring reliable operation of the turbine equipment. The 5500t FTIR system is designed to alert, at pre-set warning levels, when the phenolic and aminic antioxidants are at or approaching minimal concentration milestones, and thus help prevent turbine oils from reaching the critical point in the oxidation cycle of oil.

The capability of measuring additives in turbine oil by FTIR spectroscopy eliminates the issues associated with other measurements, including the need for sample preparation, calibrating, and maintaining electrodes based on voltammetric systems. The measurements are more rapid than electrode based antioxidant monitoring equipment, and minimize the dependency on the skill of the operator and the operating condition of the equipment. As importantly, the ability to measure antioxidant levels at-site via FTIR means that the results will be more convenient, more frequent, and obtained far more rapidly than samples that are sent for offsite analysis to a traditional oil analysis lab.

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Agilent Technologies

Biodiesel in diesel fuel using the Agilent 5500t FTIR by EN14078 method

Application Note

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Objective

Determine concentration of biodiesel in diesel fuel from 1% to 6% (v/v) per EN14078 procedure.

Samples

Two stock solutions in the concentrations of 20% (v/v) and 4% (v/v) of biodiesel in standard U.S. automotive diesel were made. These solutions were diluted to yield solutions of 0.8, 1.2, 3, 4, 6, 8 and 10% (v/v) biodiesel in diesel.

Experiment

Each of the above concentrations of biodiesel in diesel was measured using an Agilent 5500t FTIR spectrometer with a 100 µm pathlength Tumbler transmission cell; 32 scans were collected at 4 cm^{-1} resolution yielding a 15 second sample measurement time. Measurements were made in triplicate. A calibration curve was made according to the EN14078 procedure "Liquid petroleum products — Determination of fatty acid methyl esters (FAME) in middle distillates — Infrared spectroscopy method". The maximum absorbance at 1745 cm^{-1} was plotted versus volume percent of biodiesel.

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Results

The average absorbance measured from the lowest concentration (0.8%) was 0.15 Abs. The highest concentration (10%) produced an absorbance of 1.6 Abs. The FAME absorbances at 1745 cm^{-1} for all concentrations are shown in Figure 1. Note that all three replicates are shown in that figure.

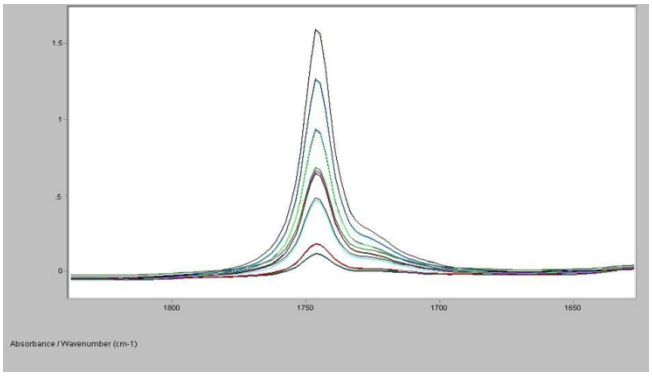

Figure 1. Absorbance at 1745 cm^{-1} of biodiesel in diesel fuel at 0.8, 1.2, 3, 4, 6, 8 and 10% (v/v)

The absorbance at 1745 cm^{-1} was measured on each of the samples using a two-point baseline at 1820 cm^{-1} and 1670 cm^{-1}. A calibration plot was drawn using two measurements at each concentration; it is shown in Figure 2. A simple linear regression yielded a correlation of 0.999.

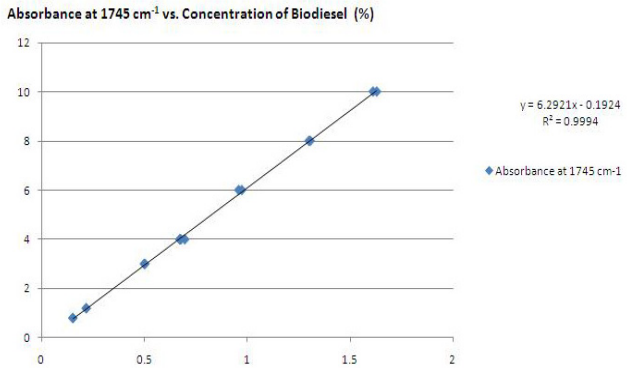

Figure 2. Calibration plot of biodiesel in diesel fuel showing linear fit of absorbance from 0.8 to 10% (v/v)

The data from the calibration was used to generate a method in the MicroLab software. Note that the concentrations are formatted at % x 10 in order to display the calculated value to 0.1%. The method is shown in Figure 3.

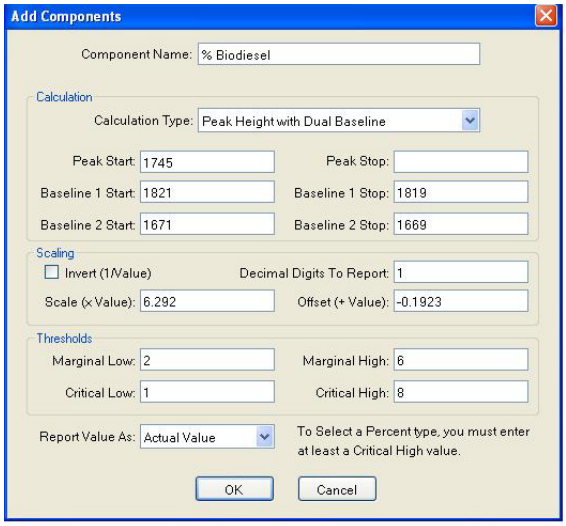

Figure 3. Biodiesel method in MicroLab software

This method was used in the MicroLab software to predict the concentration of the remaining samples. The average error was 0.129% (v/v) with a maximum error of 0.2% (v/v). The results are shown in Table 1, and an example of the MicroLab software results screen is shown in Figure 4.

Table 1. Results from samples measured with the biodiesel method in the MicroLab software

Actual %	Abs at 1745 cm^{-1}	Predicted %	Error (%)
0.8	0.154	0.8	0
1.2	0.219	1.1	0.1
3	0.504	2.90	0.1
4	0.696	3.9	0.1
6	0.971	5.8	0.2
8	1.3	7.8	0.2
10	1.631	9.8	0.2
		Average error:	0.13
		Maximum error:	0.20

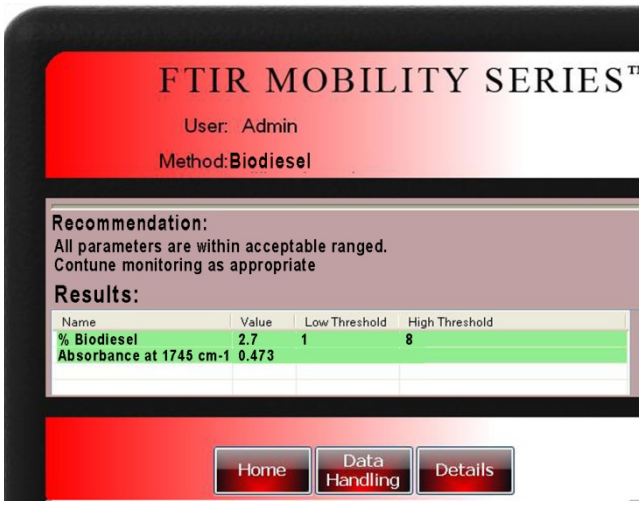

Figure 4. MicroLab results screen for a 3.0% sample of biodiesel in diesel

Conclusion

This experiment shows the ability of the Agilent 5500t FTIR spectrometer with the Tumbler transmission cell to quantify the amount of biodiesel in diesel fuel per the European Standard EN14078. The system using a 100 µm liquid cell produced ideal absorbances in the concentration range of interest (1.0 to 6.0% (v/v)). The MicroLab software can be easily configured to calculate the percent biodiesel in diesel fuel and presents the data in an easily understandable format.

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Low level detection of biodiesel in diesel fuel using the Agilent 5500t FTIR spectrometer

Application Note

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Background

Recent increases in production of biodiesel along with the high cost of crude oil have encouraged some producers to mix biodiesel with regular diesel fuel. Although biodiesel provides some environmental advantages, problems have been reported in the use of mixed fuels in engines designed for petroleum based diesel. Additionally, biodiesel can promote biological growth in the diesel fuel when stored for a period of time. In response to these issues there is a need to determine if biodiesel is present in regular diesel fuel, especially for industries which store large amounts of diesel fuel. The European Union has recently released regulations requiring the measurement of biodiesel in diesel and has issued an analytical test method, EN 14078, for testing.

In the United States, a recent ASTM ruling (D-975) allows shipments of up to 5% biodiesel in fuel without notification to the customer. This notification requirement does not meet the needs of all industries. As an example, the U.S. Nuclear Regulatory Commission (NRC) suggests lower limits for biodiesel in fuel blend for stationary standby diesel engines at nuclear plants because of the potential for instability of the higher percent biodiesel blends resulting from the buildup of oxidation products. These conflicting rulings make it incumbent on the user to verify the level of biodiesel before being placed in long-term storage.

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The Agilent 5500t FTIR spectrometer provides an easy to use means of measuring biodiesel in diesel. The EN 14078 method comes pre-programmed on the 5500t FTIR spectrometer; this method can determine the amount of biodiesel in the range between 1 % and 10 %. The design is easy to use and provides nearly instant answers. In some cases, however, even lower levels of detection are required. To meet these needs, Agilent Technologies has modified the EN 14078 method to provide detection down to 0.025 % biodiesel in diesel. The Low Level Biodiesel in Diesel method can quantitatively determine the amount of biodiesel in the range from 0.025 % to 5 % with the same easy to use system.

Experiment

Six standards of biodiesel in diesel were made by successive dilution in the range from 0.0 to 1.5 %. Each concentration was measured using an Agilent 5500t FTIR spectrometer with a 100 µm path length Tumbler transmission cell; 32 scans were collected at 4 cm^{-1} resolution yielding a 15 second sample measurement time. Measurements were made in triplicate on two separate instruments. A calibration curve was made using the 1745 cm^{-1} carbonyl band specified in the EN 14078 method. The EN method specifies peak height but to achieve lower limits of detection the peak area was used in this method.

Results

Figure 1 shows the carbonyl region of the spectrum of the 6 samples tested plus a blank. The lowest concentration of 0.025 % is clearly visible with an absorbance which can be discerned over the blank. The absorbance increases linearly all the way to the highest concentration at 1.5 % biodiesel.

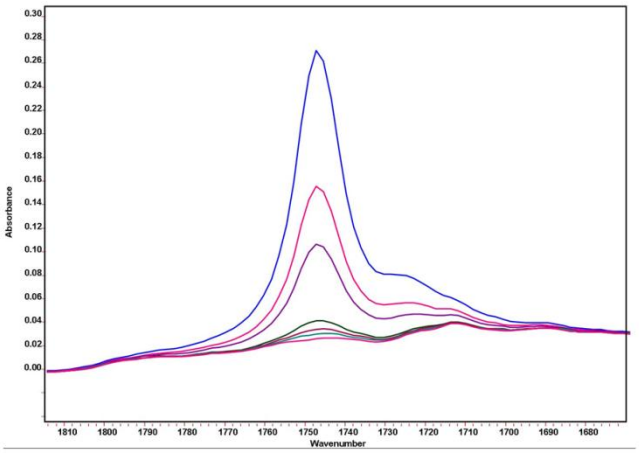

Figure 1. Absorbance at 1745 cm-1 of biodiesel in diesel fuel at 0.0, 0.025, 0.05, 0.1, 0.5, 0.8 and 1.5 % (v/v)

The calibration plot of the peak area of the 1745 cm^{-1} band is shown in figure 2. The plot shows an excellent correlation of R^2 = 0.9998.

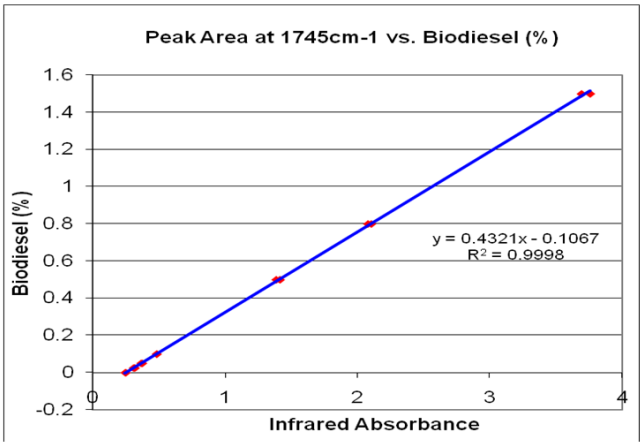

Figure 2. Calibration plot of biodiesel in diesel fuel showing linear fit of absorbance from 0 to 1.5 % (v/v)

The data from the calibration was used to generate a method in the MicroLab software. The method is shown in Figure 3.

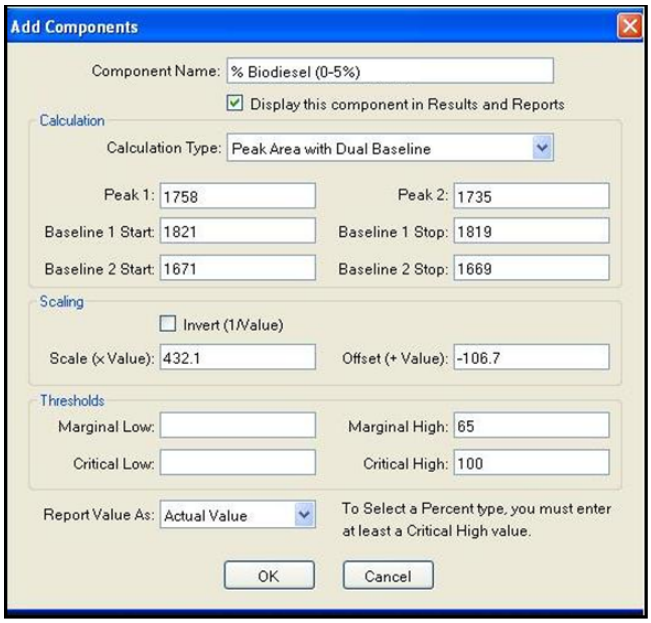

Figure 3. Biodiesel method in MicroLab software

This method was used in the MicroLab software to predict the concentration of a separate validation set. The validation set ranged from 0 to 5% biodiesel in diesel. The average relative error was 1% with a maximum relative error of 2%. These results indicate that the same method can be used to predict concentrations at least as high as 5%. The results are shown in Table 1, and an example of the MicroLab software results screen is shown in Figure 4.

Table 1. Results from samples measured with the biodiesel method in the MicroLab software

Actual %	Peak Area Abs at 1745	Predicted %	Error (%)
0	0.245	0	0.0
0.025	0.307	0.025	0.0
0.050	0.365	0.049	2.0
0.100	0.482	0.101	1.0
0.5	1.382	0.491	1.8
0.8	2.078	0.790	1.3
1.5	3.691	1.488	0.8
3.0	7.122	2.971	1.0
5.0	11.674	4.938	1.2
		Average error:	1.0
		Maximum error:	2.0

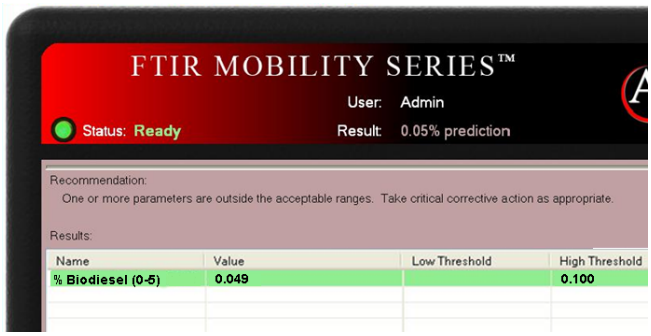

Figure 4. MicroLab results screen for a 0.05 % sample of biodiesel in diesel

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Agilent Technologies

Test method for low level detection of biodiesel in diesel using the Agilent 5500t FTIR spectrometer

Application Note

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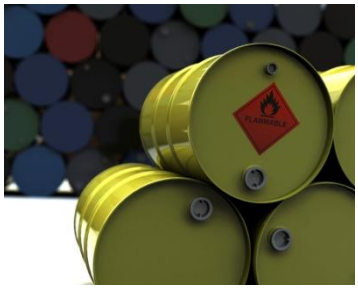

Introduction

Agilent Technologies 4500t and 5500t FTIR spectrometers are gaining rapid acceptance for measuring biodiesel (%FAME) in diesel fuel for applications where low level contamination of diesel fuel by FAME is problematic. Diesel fuel containing up to 5 % biodiesel meets the ASTM D975 standard, which does not require disclosure of the biodiesel level, and this can be a significant issue for certain diesel fuel users. Agilent has now developed an enhanced method for determining contamination levels of FAME in diesel. This method combines the more sensitive transmission IR sampling interface specified in EN 14078 with the universal algorithm and sample set specified in ASTM D7371 to produce the most sensitive and accurate method available. This enables the 5500t FTIR systems to quickly and accurately predict the percentage of biodiesel in diesel fuel in the range from 0.025 % to 20 %. In round robin testing, the accuracy of this method has been found to be superior to the other methods, especially for measuring low levels of biodiesel.

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Instrumentation

The Agilent biodiesel test method was designed around the 5500t FTIR series of portable spectrometers, equipped with the innovative, patented sampling interface. This sampling system has been engineered to provide a highly reproducible 100 micron transmission pathlength, as called for in the EN 14078 method. The sample interface is one area where the ASTM method differs from the EN method. The ASTM method specifies an attenuated total reflectance (ATR) sample interface; the EN method specifies a transmission sample interface. The ASTM ATR method is easy to use, but does not provide the level of detection required for measuring biodiesel contamination; the EN transmission cell method provides the sensitivity required, but traditional IR transmission cells are not easy to use with respect to both filling and cleaning, particularly for viscous liquids like diesel fuel.

Agilent FTIR transmission sampling interface is unique in that it provides the sensitivity and limit of detection as required in EN14078, but at the same time is as easy to use as the ATR cell employed in ASTM D7371. In the sampling system, the upper window of the transmission cell is mounted in a precision rotating assembly. This opens by rotating this window into the upward position. Then, a single drop of fuel is placed on the bottom transmission window, the upper window is then rotated back into the closed position creating a path length of 100 micrometers. Clean-up is equally straightforward, since the sample is simply wiped from the windows when the FTIR instrument is in the open position. This patented sample interface gives the ease of use of the ATR measurement with the path length and sensitivity of a transmission measurement. Furthermore, the design provides a path length reproducibility of better than 0.2 micrometers. Representative spectra measured on the 5500t FTIR spectrometer of biodiesel in diesel are shown in Figure 1.

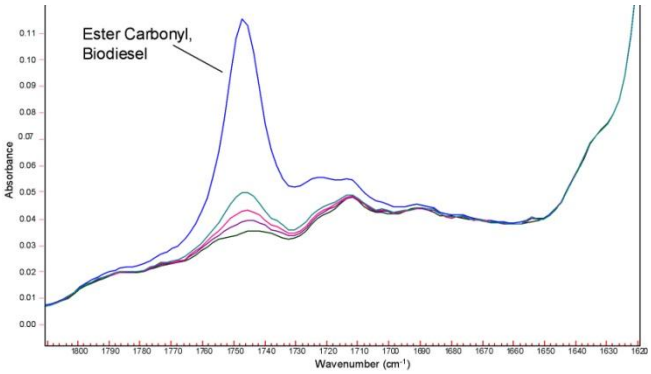

Figure 1. The overlaid IR spectra of diesel fuels with various ultra low concentrations of biodiesel, at 0.50 % (Blue), 0.10 % (Lt. Green), 0.05 % (Red), 0.025 % (Maroon), and 0.00 % (Dk. Green)

Calibration

In order to produce a quantitative measurement, the spectra generated from an infrared spectrometer must be calibrated with quantitative samples. The ASTM and EN methods specify different methods of quantitation. Both methods measure the carbonyl absorbance of the fatty acid methyl ester molecule; the EN method uses a simple linear fit to the band height while the ASTM method uses a multivariate, partial least squared (PLS) method. The univariate method specified in the EN method directly follows a Beers law calibration. As specified in the method, the absorbance of the carbonyl stretching frequency at 1745 cm^{-1} is measured with local baseline points at 1820 cm^{-1} and 1770 cm^{-1}. The absorbance intensity is then plotted against the concentration of 10 standards. A linear fit is used for the calibration curve.

ASTM D7371 specifies a more complicated multivariate PLS method. The method is still based on Beers Law; however, the full spectrum technique better accounts for baseline effects and interferents. In addition to the different algorithm, the ASTM method specifies a large collection of samples. The samples cover the entire calibration range and are made in three different diesel formulations: low, high, and ultra high Diesel Cetane Check Fuel (DCCF-Low, DCCF-High, and DCCF-Ultra High). The DCCF basestock fuels and biodiesel B100 used to create the biodiesel calibration and qualification

standards are in compliance with specifications described in Annex 2 (A2.1, A2.2.1, A2.2.2, and A2.2.3). Varying the aromatic content of the diesel fuel used in the calibration and qualification sets creates a more robust and accurate PLS model.

Agilent's transmission IR based method incorporates 3 calibration models similar to the ASTM 7371 method; the Microlab software automatically selects the result from the correct calibration to display without any user input. The calibration ranges are 0.025-1 %, 1-10 %, 10-25 % biodiesel in petroleum diesel. The PLS model for the low biodiesel range (0.025-1 %) consisted of 70 spectra preprocessed with mean centering, baseline correction, and thickness correction and uses a portion of ester carbonyl region of the mid IR spectrum (1950-1720 cm^{-1}) similar to the ASTM 7371 method.

The calibration for the second range (1-10% biodiesel) consists of 46 spectra preprocessed with mean centering and baseline correction. The model uses a portion of ester carbonyl region of the mid IR spectrum (1800-1720 cm^{-1}) similar to the ASTM 7371 method. The third calibration (10-25 % biodiesel) uses 40 spectra preprocessed with mean centering and baseline correction preprocessing. Three spectral regions are used : the ester carbonyl at 1846-1758 cm^{-1} and 1738-1719 cm^{-1}, and the ester C-O stretch at 1327-1119 cm^{-1}

Method Performance

Each calibration model was tested with both a cross validation (leave one out) and a separate validation set. The cross validation data was used to calculate the standard error of cross validation (SECV) and to prepare an actual versus predicted plot. The correlation of the actual versus predicted plot was also calculated. The results of each model are listed in Table 2. All models produced a correlation greater than R_2= 0.999 and an average relative error for the separate validation set of less than 1.5%.

The Agilent method was compared to the ASTM 7371 method by two other analytical labs in a blind round robin experiment initiated and conducted by a third party. Twenty samples were received with no identification of their composition and run with the 5500t FTIR. The Agilent method performed the best of all six biodiesel methods, including the ASTM 7371 methods. The total average relative error was only 2.1% (all samples, 2-20% range), the low level accuracy was much better than any other method at only 1.1% relative error.

Range	SECV	R^2	#Validation Samples	Avg. Relative Error
0.025 - 1 %	0.0016 %	0.9999	29	1.37 %
1% - 10 %	0.0164 %	0.9999	12	0.06 %
10% - 20%	0.04 %	0.9999	8	0.57 %

Conclusion

Two established standard techniques exist for measurement of biodiesel in fuel by infrared spectroscopy: ASTM D7371 and EN 14078. Unfortunately, both of those methods are focused on measurement of levels consistent with blended fuels; they do not address the needs of users who need to minimize the amount of biodiesel in their fuel supply. Agilent Technologies, employing its 5500t FTIR system, combines the transmission sample interface specified in the EN 14078 method with the algorithm and standards specified in ASTM E7371, yielding a method that accurately predicts the percentage of biodiesel in diesel fuel in the range from 0.025 % to 20 %. The accuracy of this method has been tested and found to be superior to other methods, especially for low levels of biodiesel. Thus, users who must quickly and accurately detect low level biodiesel contamination in their diesel fuel supply will find this new technology and methodology of great value.

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Agilent Technologies

Onsite quantitative FTIR analysis of water in turbine oil

Application Note

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Introduction

The availability of the Agilent 5500t FTIR spectrometers, which are compact, easy-to-use and affordable systems, provides new capabilities for real-time, on-site analysis of high value assets such as turbines. With the 5500t FTIR spectrometers, the lubrication specialist now has the ability to monitor key parameters such as oxidation, additive depletion and levels of water in lubricants. In this application brief, we will demonstrate that the Agilent 5500t FTIR spectrometer has the sensitivity, accuracy and reproducibility to determine the level of water in turbine oils without the difficulties associated with the conventional Karl Fischer technique.

Agilent Technologies

Water in turbine oil

An important parameter to measure

The amount of water in turbine oil is critical to the performance and longevity of the equipment. Excessive amounts of entrained water in the turbine oil can cause premature failure of the turbine unit, typically due to changes in the physical properties induced by the presence of water. Physical properties of oil affected by the presence of water include viscosity (measure of the oil's resistance to flow), specific gravity (density of the oil relative to that of water), and the surface tension (a measure of the stickiness between surface molecules of a liquid). All of these properties are important for the ability of the oil to coat, lubricate, and protect the critical mechanical clearances. In addition, water in turbine oil can accelerate additive depletion and contribute to chemical degradation mechanisms such as oxidation, nitration, and varnish formation.

On-site analysis is highly desirable

The ability to measure water on-site, as soon as possible after drawing the sample, is a substantial benefit in obtaining accurate water level results. Off-site analysis for trace water in oil may be compromised due to variability of water concentration introduced by storage, transportation, or shipment of a sample. Furthermore, turbine oils contain demulsifying additives that cause microscopic water droplets to separate from the oil and concentrate in layers at the bottom and sides of containers. This demulsifying action takes time to occur, and can cause large variations in analytical measurements. Also, oil samples can sometimes pick up or lose water simply depending on the type of sample container used.

Measuring water in turbine oil

Karl Fischer (KF) coulometric titration is typically used to determine the amount of water in turbine oils. Karl Fischer has some practical draw backs for on-site analysis including complicated sample preparation, the use of hazardous and expensive chemical reagents, and length of time required to perform the analysis. However, KF analysis is considered the "gold standard" method for analyzing water in oil because it provides accurate and precise answers.

FTIR spectroscopic analysis eliminates many of the concerns associated with measuring water via Karl Fischer titration. The spectroscopic method, can be performed in far less time than KF measurement, does not require reagents and when a rugged and easy-to-use FTIR system such as the 5500t instrument is used, FTIR is ideal for on-site analysis. Karl Fischer titrations require about 10-15 minutes to perform, with the instrument properly conditioned and equilibrated overnight. For KF analysis the oil must be carefully weighed on a high precision balance before and after injecting into the titration vessel. Following each analysis the KF instrument takes another 5-10 minutes to re-equilibrate. The FTIR analysis takes about 2 minutes to perform and is immediately ready for the next sample analysis after a simple cleaning with a tissue.

This application brief will demonstrate that FTIR spectroscopic analysis using the 5500t FTIR is as accurate and precise as the Karl Fischer method within the analytical range necessary for measuring water in turbine oil. Using the 5500t, we have developed two FTIR methods for water in turbine oil and have calibrated and evaluated them against the Gold Standard Karl Fischer procedure.

Water in turbine oil - the FTIR method

Used turbine oil (C&C Oil Co.) was homogenized with water and aged overnight at 70 °C to make a very high water standard. This standard was then diluted with various amounts of a used turbine oil mix, which contains oil in-service four months and another more degraded oil with a dark amber color. These dilutions had various amounts of water based on how much "as is" oil was added. The samples were mixed well and allowed to equilibrate for about an hour before they were analyzed by coulometric Karl Fischer titration (Metrohm 756 KF Coulometer) to determine the concentration of water. The samples were run in

duplicate by KF before the infrared spectra were acquired using the 5500t FTIR spectrometer. The water concentrations for the prepared standards ranged from 22-3720 ppm (parts per million). The water IR absorbance measurement for each standard sample was plotted versus the corresponding KF water data to obtain a residual least squares linear regression. The IR spectra were also analyzed using a partial least squares method to develop a regression model for the quantitative predictions of water in oil.

Calibration results

The IR analysis and calibration models indicate a very good correlation between the 5500t FTIR measurements and the Karl Fischer water data. Two different methods were developed for the quantitative measurement of water in oil using the 5500t spectrometer. The first is a relatively simple conventional IR absorbance model following Beer's Law that uses the region of the IR spectrum in which water strongly absorbs, known as the O-H stretch region. The second method uses multiple regions of the IR spectrum with partial least squares (PLS) chemometric modeling to reduce the effects of noise, baseline variance, and other interfering factors.

Beer's law model

In the first method, a peak area absorbance measurement provides a detection limit of about 30 ppm water in oil (Figure 1). The IR spectra of 15 samples with KF water values ranging from 7-270 ppm were used to build a linear calibration curve that follows Beer's Law (Figure 2). The weakest water absorbance in Figure 1 is new turbine oil with 30 ppm of water (Red) and the strongest water absorbance is shown in blue with a KF water value of 1460 ppm. The calibration plot is shown in Figure 2 with a correlation coefficient of R2=0.977 and a standard error of validation (SEV) of ~40 ppm (20-270 ppm range). The addition of higher water concentration standards to the calibration improves the correlation coefficient to R2=0.996.

Therefore, this calibration is optimized for the low levels of water (<500 ppm), but is still quite accurate for predications of higher water levels above 500 ppm if necessary.

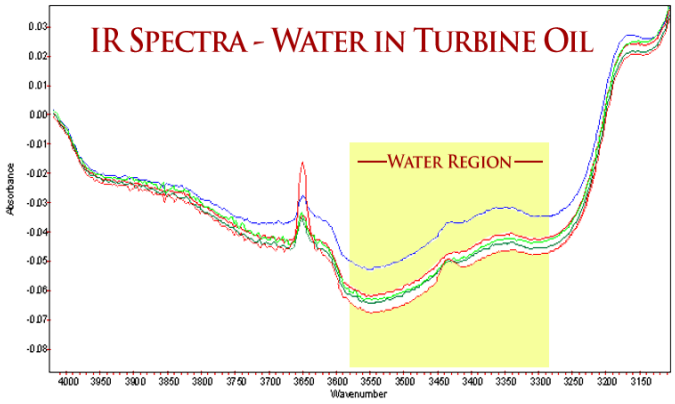

Figure 1. The overlaid IR spectra of turbine oil with the water absorbance region expanded, water values from bottom to top are 30 ppm (red), 80 ppm (dark green), 217 ppm (light green), 533 ppm (red), and 1460 ppm (blue)

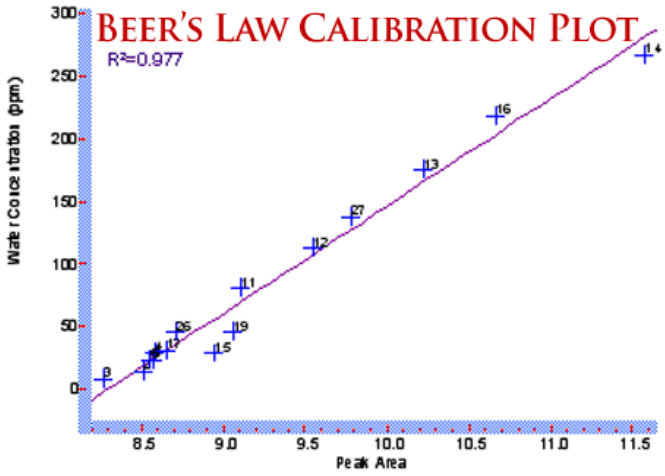

Figure 2. The calibration plot of KF water values (ppm) versus peak absorbance area for water in turbine oil using a Beer's Law peak area method

Pls model

The PLS chemometric model uses more sophisticated mathmatics to develop models that are typically more robust and accurate than the conventional Beer's Law IR absorbance method demonstrated above. Whereas both the PLS and the Beer's law quantitative methods for water in oil are sufficient for classification into 100 ppm ranges (i.e. <100 ppm, 100-200 ppm, 200-300 ppm, etc.), the PLS method provides the most accurate

KF water prediction values over the whole range of 30-1500 ppm.

In order to develop the PLS method for water in oil, we used 23 standards covering a range from 7-1460 ppm water. We then recorded the IR spectrum and measured the water level by the KF method. The two sets of results were correlated with partial least squares and the predicted versus actual KF values are plotted in Figure 3 and indicate a correlation coefficient of R^2=0.990.

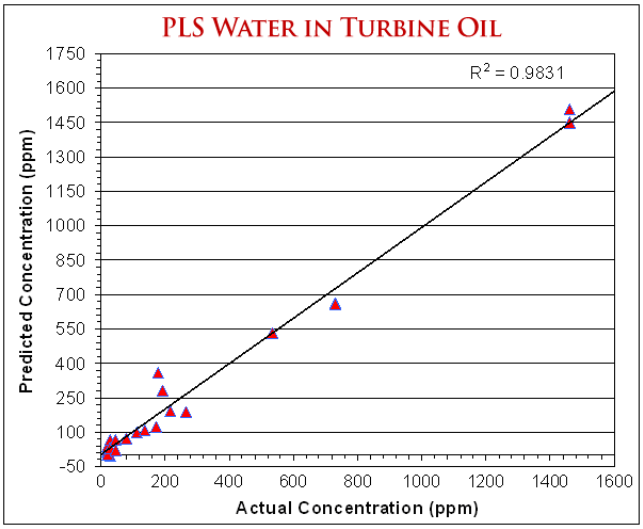

Figure 3. The PLS predicted versus actual plot of KF values using Agilent 4500 Series FTIR spectrometer

Predictions

To validate each FTIR method, 15 unknown mixtures were made by mixing used turbine oils with hydrated turbine oils, and running them by KF (in duplicate) and by FTIR (in triplicate). The coulometric KF performance was verified using 100 ppm and 1000 ppm NIST reference standards. It was found that thorough mixing was important to obtain quality data, due to the heterogeneous nature of water in turbine oil. Environmental and experimental factors caused the KF duplicate measurements to typically vary by 30-60 ppm, measured consecutively in the 100-1000 ppm range. The FTIR water predictions indicated similar variations in replicate measurements of the same sample. The averages of the replicate measurements by KF and FTIR are compared in Table 1. Good agreement with the KF measurements is observed for both FTIR methods, however, the PLS predictions are statistically better in the 100-1500 ppm range. The standard deviation between the averaged PLS predictions and the averaged KF data (0-700 ppm range) are all below 30 ppm, except for one sample (#11). The Beer's Law method predictions are better in the 0-100 ppm range, and are sufficient to classify the water concentrations into ranges as follows: <100 ppm, 100-200 ppm, 200-500 ppm, and 500+ ppm.

Validation Sample	Beer's Law (ppm water)	PLS (ppm water*)	KF (ppm water)
Turbine Oil 1	26.5	-	27.5
Turbine Oil 2	160	194.6	199.7
Turbine Oil 3	125.2	139	145.1
Turbine Oil 4	15.1	-	12.4
Turbine Oil 5	21	-	19.8
Turbine Oil 6	63	64.5	40.8
Turbine Oil 7	251.8	219.3	215.3
Turbine Oil 8	117.9	70.3	111.1
Turbine Oil 9	539.3	685.4	663.3
Turbine Oil 10	350	300	246
Turbine Oil 11	340.7	367.3	285.7
Turbine Oil 12	251.8	244.4	206.5
Turbine Oil 13	2979.3	3780.5	367.4
Turbine Oil 14	1100.3	1375	1027.5
Turbine Oil 15	1219.2	1541.9	1362.4

Conclusions

We have shown that the Agilent 5500t FTIR Spectrometer is capable of measuring water in oil at the levels that are critical to the reliable operation of the turbine equipment. The capability of measuring water in turbine oil by FTIR spectroscopy eliminates the issues associated with Karl Fischer measurements including the need for expensive and hazardous consumables, the time required for the KF measurement as well as the dependency on the skill of the operator and the operating condition of the KF equipment.

As importantly, the ability to measure water levels at-site via FTIR means that the results will be more accurate, more reproducible and obtained far more rapidly than samples that are sent for off-site analysis to a traditional oil analysis lab. We have observed that low ppm levels of water are observed to change on an hourly basis if left open to air - a sample that initially was 200 ppm can have less than 100 ppm if left in an open sample container overnight. This is also true if the sample container is not filled to the top, and water can evaporate into the head space (air) of the jar. One can only imagine the level of error that is introduced when half filled jars are sent to off site labs.

The Agilent 5500t FTIR spectrometer can detect water at the necessary warning levels. The system can alert when water reaches 100 ppm and then issue a critical warning if the water reaches 200 ppm. In addition to the analysis of water, Agilent's Mobility spectrometers can measure the depletion of additives and determine the levels of oxidation and nitration by-products in turbine oils.

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Portable measurement of biodiesel in diesel fuels by ASTM D7371-07 (FTIR-ATR-PLS method) with the Agilent 5500t FTIR spectrometer

Application Note

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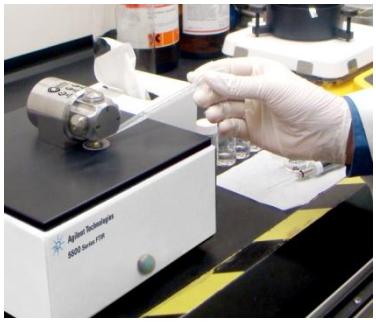

Background

Biodiesel blending with current ultra low sulfur diesel (ULSD) fuels is increasing in popularity for both large scale fleet use and individual small scale consumers. The test method detailed in this application brief can be used for quality control purposes in the production and distribution of diesel fuel and biodiesel blends. The ASTM D7371 method is applicable to 1-100 volume % biodiesel (FAME) concentrations in diesel fuel oils; it applies to all common 5 % (B5), 10 % (B10), and 20 % (B20) biodiesel blends. The ASTM D7371 method coupled with the Agilent 5500t FTIR spectrometer provides an easy, accurate, and portable means for measuring the biodiesel content of a blended fuel with petroleum diesel fuel.

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Experiment

Following the ASTM D7371 procedures, three different diesel fuels are used to create the calibration standards. The cetane index in diesel fuels is varied by changing the relative percentage of aromatic to aliphatic hydrocarbons; higher cetane index fuels have less aromatic compounds. Cetane index is typically lower during cold months. The ASTM D7371 is designed to account for these seasonal differences in the diesel fuels. The ASTM certified B100 Biodiesel was mixed with diesel fuel blended at three different cetane indexes, referred to in the D7371 as diesel cetane check fuel low, high and ultra high. As specified in the method, a total of 70 standards were produced with biodiesel concentrations ranging from 0-100%. In addition to the calibration standards, 21 qualification standards were created with different concentrations than the calibration standards. The qualification standards were used to determine the method's accuracy and robustness.

All standards were measured using the Agilent 5500 Series FTIR spectrometers with an integrated 9 reflection diamond attenuated total reflectance (ATR) sample interface. The spectra were collected using 64 scans at 4cm-1 resolution yielding a 30 second sample measurement time. A partial least squares (PLS) model was developed using Thermo Galactic PLS/IQ software. The model concentrates on the ester carbonyl and other absorbance bands specific to fatty acid methyl esters (FAME). The PLS models were incorporated into Microlab software for an easy end-user biodiesel in diesel fuel application.

Results

A series of spectra from the calibration set are shown in Figure 1. Bands due to biodiesel can be seen both at 1741cm^{-1} and between 1170-1245cm^{-1}; these areas are correlated to the concentration of biodiesel in the D7371 method. The absorbance increases linearly with the concentration throughout the whole range from 0-100 %.

This provides a very accurate and precise measurement using the 5500 Series FTIR spectrometers.

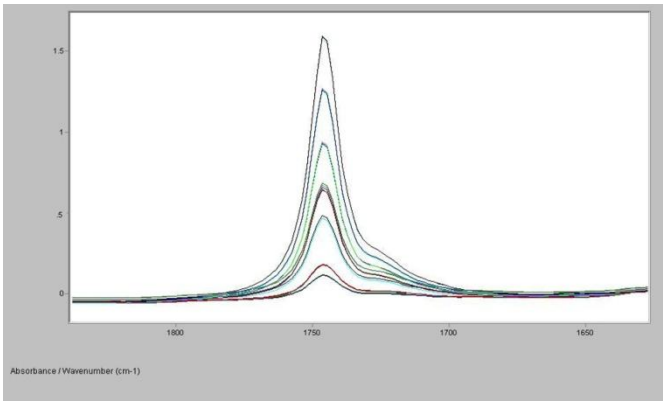

Figure 1. FTIR spectra overlaid of ASTM D7371 standards with biodiesel in diesel at 0, 2.5, 5, 10, 15, 20, 30, 50, 70, and 100 % biodiesel (v/v)

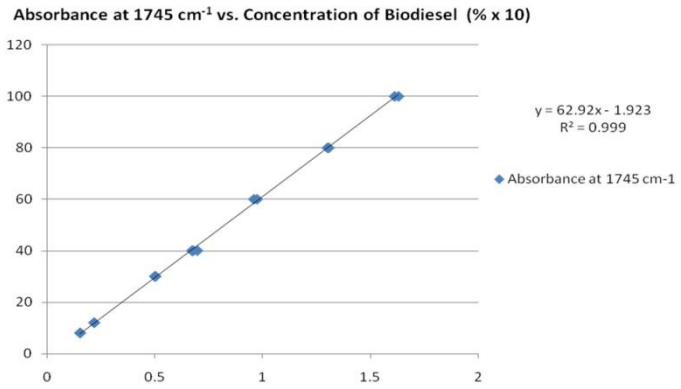

Figure 2. The PLS model's actual vs. predicted plot of biodiesel in diesel, low calibration set (0-10 % biodiesel)

ASTM D7371 specifies individual calibration models for the concentration ranges 0 -10 %, 10 - 30 % and 30 -100 %; each calibration model contains standards from each of the three cetane index diesel fuel stocks (ultra high, high and low). The 0-10 % calibration model results are plotted in Figure 2 as the actual (x-axis) vs. predicted (y-axis) biodiesel concentrations. The correlation coefficient for this model is R_2= 0.999. Results for the 10 - 30 % and 30 - 100 % models were similar. Each model uses 3 - 4 factors on mean centered data.

The three models based on the ASTM D7371 method were incorporated into a single method within the Microlab software. A screen shot showing one of the calibration definitions definition is shown in Figure 3.

The Microlab software also contains logic to report only the result from the correct model.

Using the "Component Reporting" feature, shown in Figure 4, which result will be shown to the user based on the predicted result. Using this feature, a single, correct result is present to the user even though results from three methods are calculated. This reduces confusion and allows samples to be measured by untrained users.

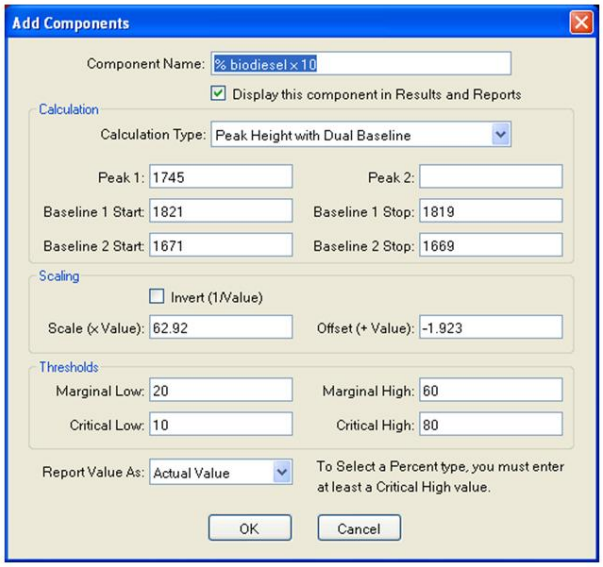

Figure 3. The Microlab methods editing feature where the 1-10 % biodiesel model is assigned

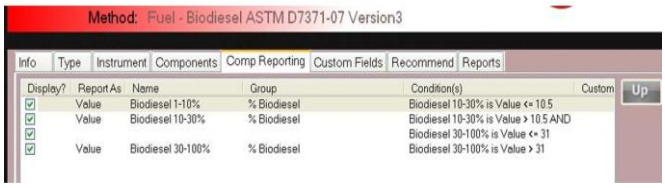

Figure 4. The conditional reporting setup window from the Microlab PC software, which determines the model results to be displayed when running a sample

The Microlab ASTM D7371 method was used to predict the concentrations of a separate qualification set. The qualification set covers the entire 0-100 % range of biodiesel in diesel, and the different cetane index diesel fuels were also used to make the qualification samples. The average relative error (1-100 % range) is 0.47 % and the maximum relative error is 1.56 %. The results of the separate validation are shown in Table 1. It should be noted that the standard error of qualification calculated for these tests is less than half the acceptable standard error of qualification listed in the ASTM method. A screen shot showing the software display for a 2.5 % biodiesel validation sample is shown in Figure 5.

Figure 5. Microlab results screen for a 2.50 vol % sample of biodiesel in diesel

Table 1. The results from the qualification set samples measured with the ASTM 7371 method in the Microlab software

Qualification Sample	Predicted Biodiesel (Vol %)	Actual Biodiesel (Vol %)	Error (%)
Q1	0.77	0.71	8.61
Q2	5.98	5.95	0.55
Q3	13.14	13.14	0.01
Q4	26.50	26.44	0.24
Q5	59.05	58.73	0.54
Q6	92.12	92.07	0.05
Q7	97.73	97.77	0.04
Q8	0.36	0.36	0.77
Q9	1.64	1.66	1.56
Q10	5.91	5.94	0.49
Q11	38.51	38.69	0.47
Q12	84.16	84.39	0.27
Q13	95.74	95.88	0.14
Q14	99.11	99.30	0.20
Q15	0.35	0.36	1.09
Q16	3.60	3.55	1.28
Q17	8.35	8.31	0.43
Q18	13.15	13.10	0.39
Q19	21.17	21.49	1.50
Q20	73.70	73.65	0.06
Q21	95.66	95.49	0.18
		Average Error Total (%)*:	0.47
		Maximum error (%*):	1.56
		Standard Error of Qualification (SEQ):**	0.08
		ASTM D7371 SEQ Limit (PSEQ):	0.21

Conclusions

This set of experiments show the ability of Agilent 5500 Series FTIR spectrometers with 9 reflection diamond ATR sample interface to meet the ASTM D7371 method. The method file which calculates the concentration in all ranges from 1 % to 100 % biodiesel and selectively reports the correct concentration is standard with all 5500 FTIR and 4500 FTIR systems. The results from a separate validation show that the instrument and method are very accurate while being very simple to use.

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Returning to Fixed Pathlength Infrared Spectroscopy: Gaining Detail and Removing the Obstacles

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Introduction

This article discusses the benefits of making infrared (IR) transmission measurements from liquids with a fixed pathlength. The pros and cons, mainly cons, of traditional fixed pathlength cells are reviewed first, with the main "cons" being difficulties with filling and cleaning, and the need to protect the IR windows from moisture. ATR has become a practical alternative method for a liquid, however, the technique, by nature, is a surface-based measurement and there are significant limitations in regard to physical pathlength, which is very short.

A new system that provides a fixed pathlength IR transmission measurement for liquid sample handling and analysis is reviewed. The system features and integrated FTIR and provides three user-selectable pathlengths that are factory fixed at the time of purchase; nominally set to 30, 50 and 100/150 microns that can be used without the customary drawbacks of a fixed pathlength cell. A special sampling point, called a DialPath head (Figure 1A/B), is used to locate the sample between a pair of specially designed zinc selenide (ZnSe) windows. These are constructed not to generate any optical interference pattern in the recorded spectrum. The sampling point is easily accessible and sample preparation is reduced to applying a drop of liquid on the lower "window" and after the measurement the window is cleaned by a wipe with a tissue, Q-tip or paper towel.

Fixed pathlength measurements have the ability to provide fine detail in the measured spectrum. This is an important fact for quality-based measurements where subtleties or small variations differentiate "good" from "bad" materials. Some example applications are reviewed that illustrate the benefits of fixed path measurements. Comparisons are made with a standard laboratory-based FTIR equipped with fixed pathlength transmission cells to confirm equivalency. The featured applications include measurements of dilute solutions, alternative fuels and food products (dairy products and edible oils).

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Background and the use of fixed pathlength cells

Originally, infrared spectroscopy was developed as a quantitative technique for liquid petroleum products (fuels and lubes) and polymers. It was later that it became the universal tool for material identification, as we know of today. The combination of material identification and quantitative response has made infrared spectroscopy unquestionably the most versatile instrumental method for chemical and physical analysis, covering a wide range of applications. As with any measurement, maintaining quantitative integrity by reproducible and accurate sampling is essential. In the infrared, maintaining a measureable pathlength, which is not trivial, is required for the accurate analysis of liquids. There are at least five critical factors to be considered and addressed:

- The need for a pathlength compatible with the absorption characteristics of the liquid in the mid-infrared (5000 cm^{-1} to 400 cm^{-1}/2.0 µm to 25 µm)
- Mechanical design issues of an accurate and reproducible short pathlength
- The filling, emptying and cleaning of the cells and the influence of the sample
- Window material selection based on the properties of the sample, and the optical characteristics of the window
- Alternative methods of sampling that reduce or overcome the difficulties associated with the sample...are they good substitutes?

It is obvious that there are important issues related to making infrared spectral measurements that become practical challenges. The first is the high infrared absorption cross section of most materials. Unlike other spectral regions, where cells or cuvettes are used with pathlengths measured in millimeters or centimeters, infrared measurements require pathlengths measured in microns. Generating a reproducible film of a sample this thin is a challenge. For years practical infrared spectral analysis has been performed with different methods of handling of liquid samples whereby the pathlength is controlled to the accuracy required for the analysis.

The standard, for 40 years, is the fixed pathlength cell, where the optical pathlength is generated by the use of thin spacers sandwiched between a pair of infrared transmitting windows. Two versions of these cells are used; demountable cells and sealed cells. Demountable cells are dismantled to simplify “filling”, “emptying” and cleaning. The windows are separated, and the sample is dropped into the void in the spacer, and then the top window is carefully replaced to form a sandwich with the liquid; taking care not to trap air. The problem with this approach is that assembly can be difficult and there is uncertainty in the pathlength formed. At best, it is a semi-quantitative approach to sample handling.

Sealed cells are required for accurate sampling. In a sealed cell the sample holder, the windows and the spacers have to be permanently fixed together. Such a cell is filled via special sample ports where the liquid is injected from a syringe into the cell. While this sounds simple, in practice it has significant practical drawbacks. Filling, where the liquid is “squeezed” into the confined space, which is at most 100 microns thick, is the first challenge. This can require the application of pressure from a syringe. This step requires extreme caution because the hydraulic pressure generated can damage the cell and can cause leaks. Originally, cells were sealed with special lead spacers treated with mercury to form an amalgam seal. Today, the use of these materials are not permitted, and non-toxic alternatives such as tin, steel or aluminum foils are used, sometimes in combination with an adhesive. Teflon sheet spacers are used in demountable cells and occasionally in sealed cells. However, the sealing integrity of Teflon-based spacers is questionable.

The next practical issue is emptying and cleaning the cell. As indicated above, a sealed, fixed pathlength cell is filled via filling ports. These are implemented by the use of a special drilled window, which is sealed against the metal front plate of the cell. This front plate has input tubes with female Luer fittings that couple to the

male Luer tip of a syringe. The entire assembly, mounting plates, seals, windows and the selected spacer form the sealed, fixed pathlength infrared cell. This is a fragile, complex component that requires skilled assembly, and careful use, maintenance and storage.

These cells have been the mainstay of liquid sample handling of liquids for nearly fifty years. They are not ideal, they are expensive, and they are difficult to fill, empty and clean. If handled correctly, they are usually filled and emptied by a pair of syringes connected to the filling ports of the cell. This action takes skill and dexterity, and if not carried out carefully it will lead to the formation of bubbles; a serious interference in the measurement. Incorrect use can lead to cell damage, with resultant leakage of fluid. Also, short pathlengths (less than 50 µm thick) are especially difficult to use with samples of medium to high viscosity. Emptying and cleaning are equally difficult, and again a syringe is used to draw out the sample, and then to flush solvent through the cell until the cell is clean. Careful selection of the solvent is important to ensure dissolution of the sample, ease of removal and to ensure inertness towards the windows.

The best windows for good infrared transparency are sodium chloride and potassium bromide. While these are good optically speaking, they are water soluble and are readily attacked (etched) by moisture in the sample or by humidity in ambient air. Calcium fluoride and barium fluoride are water insoluble and moisture resistant they have a restricted range of infrared transparency (optical cut-offs at 1100 cm^{-1} for CaF_2 and 870 cm^{-1} for BaF_2). A practical alternative is to use windows made from zinc selenide (ZnSe). This material provides transparency similar to NaCl, and can be used to 650 cm^{-1}. The material is very durable and is not attacked by water. Unfortunately, it is not in common use as a cell window because ZnSe has a high index of refraction (Index = 2.4) and it introduces an interference pattern (sine wave) into the spectrum of most liquids. This interference is above an acceptable level and in most cases is impossible to remove from a final spectrum.

In summary, practical issues interfere with the ability to obtain fixed pathlength infrared measurements of liquids in traditional cells:

- The pathlength is required to be between a few micrometers (µm) and a few hundred micrometers (<200 µm, <0.2 mm)
- The pathlength must be accurately defined and reproducible
- Fixed pathlength cells are difficult to fill, empty and clean
- Window materials need to be carefully selected; materials such as ZnSe, which appear to be ideal, are unsuitable because of optical interference caused by a high index of refraction

Practical alternatives for fixed pathlength infrared measurements

In the 1980s the application of ATR was extended to include liquids. Commercial accessories based on cylindrical internal reflectance elements (IREs) or horizontally mounted IREs provided a practical solution. Zinc selenide turns out to be a good match for this application because of its optical range, hardness, high index and water insolubility. Consequently, ATR has become a de facto standard for the handling of liquids. ATR is a surface phenomenon and the physical optical pathlength is only a few microns deep. The effective pathlength can be extended by multiple internal reflections, where the liquid sample has multiple interactions with the internal reflections. Optical geometries with nine or ten reflections produce an "effective pathlength" in the range of 10 µm to 25 µm, dependent on the analytical wavelength.

There are downsides to the ATR measurement linked to the mechanism of the internal reflection. First, the physical pathlength, per reflection is short and is wavelength and index dependent. Consequently, the actual, physical pathlength is not absolute and is effectively unknown and variable.

Also, zinc selenide, a popular IRE substrate, is ionic and its surface is chemically reactive. Practical alternatives to zinc selenide exist, with diamond being a candidate. Commercial accessories exist based on diamond with configurations that provide from single to nine reflections for liquid handling. Diamond is an ideal substrate; it is very hard and is chemically inert. Optically it is limited in size and optical transmission with a loss in throughput performance for configurations with multiple reflections (3x and 9x).

The success of horizontal ATR accessories and diamond tipped ATR sampling systems must not be underestimated. Most laboratories have implemented these systems for liquid sample measurements. However, the approach is a compromise for many measurements. Non-reproducibility is an issue, but this can be improved by integration of the ATR into a dedicated instrument with rigid, permanent mounting. Although some non-reproducibility (linked to the index of refraction) may still exist, the permanent mounting of the IRE provides a fixed sampling point and is a popular method for routine sample handling.

The benefits offered by an integrated ATR measurement can be improved by the combination of the ATR with an optimized FTIR spectral engine. In such systems the sample can be applied to the sampling point from a dropping pipette, and the analysis completed in a few seconds. Cleaning is reduced to simply wiping material off the ATR sampling surface with a soft tissue, possibly followed by the use of a small amount of solvent. Moving forward, a similar easy-to-use interface would provide the ideal scenario for a fixed pathlength measurement. Such a system would offer the benefits of real extended pathlength, with the simplicity of a "drop-it-on"/"wipe it off" sampling point, and a measurement that is not compromised by the sample.

An integrated measurement system from Agilent Technologies, the 5500 Series FTIR and sample handling system, has been developed and introduced, fulfills this "idealized" concept for fixed pathlength sample handling. The implementation covered in this article uses a three-position version of the company's 5500 DialPath FTIR rotary head, providing pathlengths of 30, 50 and 100 µm for the fixed path transmission measurements. This head, shown in Figure 1, is equipped with a slightly curved (bowed) zinc selenide window, which rotates to form a rigidly defined pathlength with the sample. Figure 1B shows the head located at position 1, which provides a nominal 30 µm optical path; the other two locations provide nominal 50 µm and 100 µm paths, respectively.

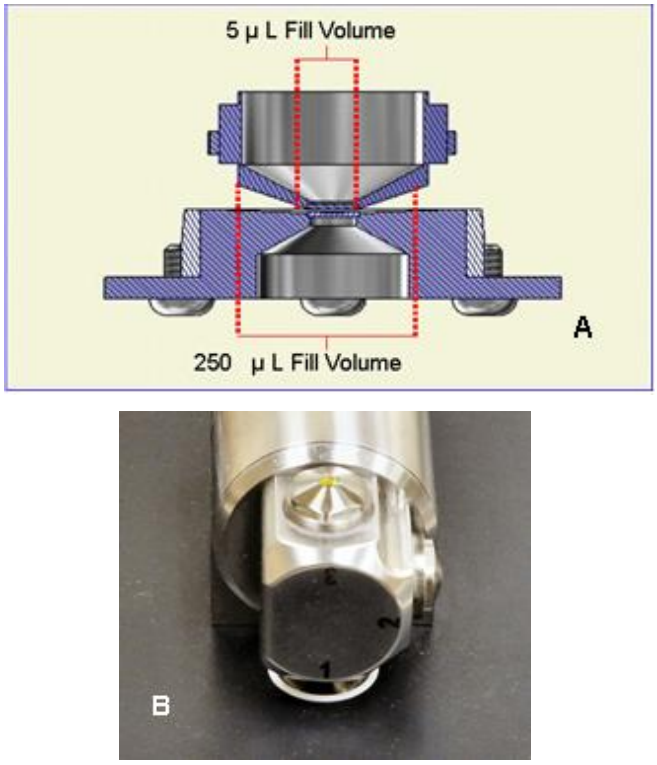


Figure 1. The 5500 DialPath FTIR sampling point concept (A); provides a user selectable pathlength, with one of three fixed/calibrated optical paths, designated 1, 2 and 3 (B)

This configuration provides the simplicity of the ATR sampling concept where the sample is dropped on to the small circular window, the sampling head is rotated in place, and the measurement made, in a few seconds. The liquid forms a uniform capillary film between the lower window and the window in the rotary head. The sweeping action of the rotary head produces a uniform film without any bubble interference. The slight curvature of the optical surface eliminates the opportunity to form an optical interference situation

between the two zinc selenide windows. The optical, mechanical and water insolubility benefits of the zinc selenide windows are realized without the negative impact of optical interference. The lack of optical interference can be appreciated by Figure 2, where the three baselines (100% lines) for the empty window cavities are presented. These spectra, recorded in approximately 13 seconds have a nominal 8000:1 SNR across the analytical range of 2100 cm^{-1} to 1100 cm^{-1}.

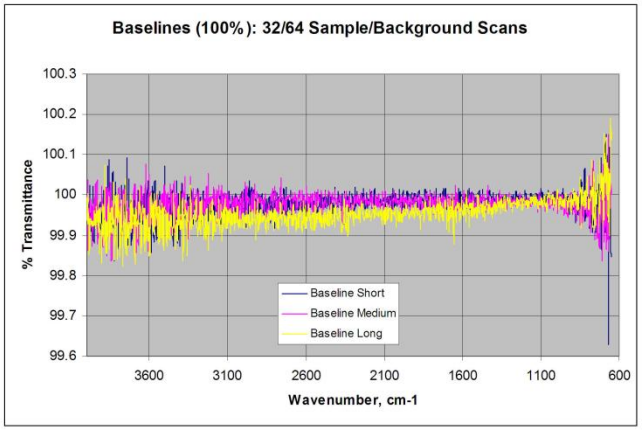


Figure 2. 100% Baseline performance; spectra from long, medium and short pathlengths presented with an average SNR of 8000:1 (2100 cm^{-1} to 1100 cm^{-1})

The SNR represented in Figure 2 is a significant result because it shows a flat 100% line without any artifacts caused by optical interference. The spectrum from a fixed pathlength cell constructed from zinc selenide windows would be dominated by a large sinusoidal pattern. This occurs with or without the sample in place. The lack of any interference pattern is further substantiated by the adherence to the square root law, where the SNR of the system is proportional to the square root of the number of scans (Figure 3). An excellent linear correlation is observed for the practical measurement timeframes; the presence of interference would result in significant deviation and curvature to this line.

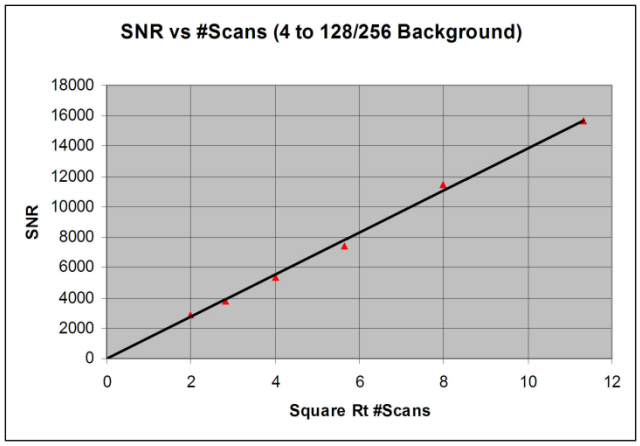


Figure 3. The adherence of the measurement system to the square root law of measured SNR

It is appropriate to compare the spectral data from a standard diamond ATR system with the fixed pathlength (5500 DialPath) measurement (Figure 4).

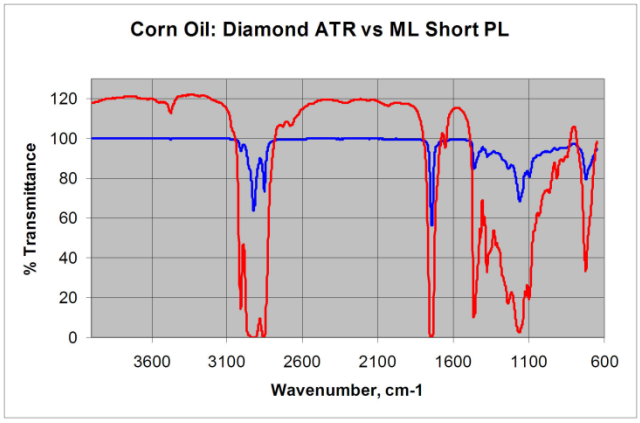
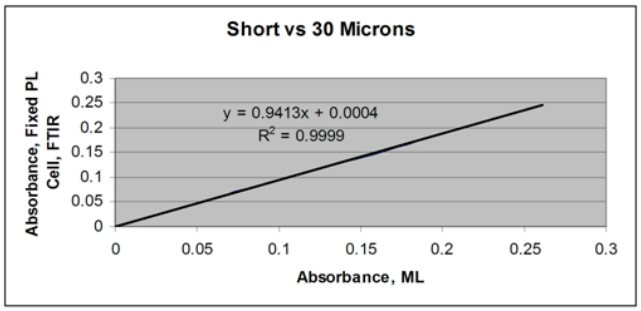


Figure 4. Comparison of the effective pathlength of a diamond ATR integrated system with the short fixed pathlength (~30 µm) transmission spectrum for corn oil

Both systems provide good quality spectral data, however, if one is looking for characteristic details in the spectrum for property measurements, such as the degree and type of unsaturation of an edible oil, then a long, fixed path measurement is required. One minor optical issue is that the high index of the ZnSe windows can be detected by the shift in the baseline of the corn oil above 100%. This result is the difference between the low index of the air (used for background), versus the higher index of the corn oil.

Analytically this is not a problem because the shift can be compensated from the absorbance form of the spectrum.

The reproducibility of the pathlength and the ability to dial in a longer pathlength are important attributes. The pathlength is defined by the height of the head from the measurement surface; a mechanical adjustment fixed at manufacture. The actual pathlength can be calibrated from the spectral response of fixed calibrated pathlengths in a standard lab instrument. The unit used for the data here was not pre-calibrated to exact values. The data shown in Figure 5 is taken from a series of standard xylene solutions prepared in carbon tetrachloride and recorded on the 5500a FTIR system. A parallel set of spectra were obtained on a commercial FTIR (PerkinElmer Spectrum 100) with a set of calibrated fixed pathlength, KBr cells (30µm, 50µm and 100µm). The results (Figure 5) indicate a high level of correlation between the two different sets of fixed pathlength spectra, providing calibration equations for the three 5500a system pathlengths; short = 31.9 µm, medium = 52.6 µm, and long = 114.7 µm.



	ML Pathlengths	PL Equation	Correlation
Short	31.9	y = 0.9413x + 0.0004	R2 = 0.9999
Medium	52.6	y = 0.9497x - 0.0013	R2 = 0.9998
Long	114.7	y = 0.8721x + 0.0018	R2 = 0.9992

Figure 5. Example calibration for the short pathlength (No 1) of the Agilent 5500 DialPath FTIR system based on comparisons with a calibrated fixed pathlength cell for a series of xylene solutions

These experiments have demonstrated that the fixed pathlengths of the 5500 DialPath system are highly reproducible, and once calibrated provide an accurate duplication of the fixed pathlength performance of the standard, calibrated fixed pathlength cells.

Practical applications of a fixed pathlength measurement system

The ability to measure with known fixed pathlengths is important for a wide range of applications. An obvious application is for the analysis of very dilute solutions where a pathlength of 100 µm or more is required. The application shown in Figure 6, are spectra of dilute solutions (<1% solute) of methanol are measured in a non-polar solvent (carbon tetrachloride).

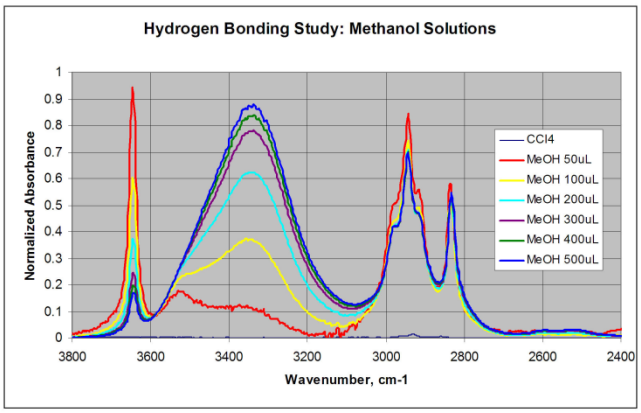


Figure 6: Dilute solutions of methanol in carbon tetrachloride; a study of the effects of hydrogen bonding in non-polar solvents

This is a classical measurement where changes in intermolecular hydrogen bonding are demonstrated. The normal condensed phase spectrum of methanol exhibits a broad absorption centered at 3450 cm^{-1} assigned to polymeric hydrogen bonding. Upon dilution with the non-polar solvent, this hydrogen bond profile changes as indicated in the red and yellow band profiles of Figure 6. These spectra correspond to the transition, through oligomeric forms to the non-bonded form with the narrow absorption at 3630 cm^{-1}. This experiment is only practical with a long path measurement (100+ µm in this case). The ATR method is impractical for this type of application.

The largest benefit of the open architecture of the 5500 DialPath system is the ability to handle medium to high viscosity liquids. Typical applications that are constrained by viscosity are measurements on vegetable oils (including cooking and edible oils), dairy products (such as milk, cream and butter products) and automotive products, including fuels, lubricating oils

and greases. While an ATR liquid measurement system might be used for some of these applications, the increased spectral detail of a longer pathlength is preferred for product quality and performance-related measurements. Figure 7 is important for both edible and cooking oils and products derived from these materials, such as biodiesel fuels. Recent regulations on food quality and safety have focused on the need to eliminate trans unsaturated fats from food preparation. The total level of unsaturates and the type of unsaturates, including the trans configuration can be determined from the spectral region from 1000 cm^{-1} to 650 cm^{-1}. In the case of biodiesel, many quality parameters are linked to components formed in the esterification process. These components, such as free acid, free glycerol and glyceride fragments can be determined from the spectrum. These include the OH stretching region featured in Figure 7A where residual water (from esterification) and free glyceride components can be detected and measured. These measurements require the extended pathlengths used in the spectra shown in Figure 7A/B (100+ µm pathlength).

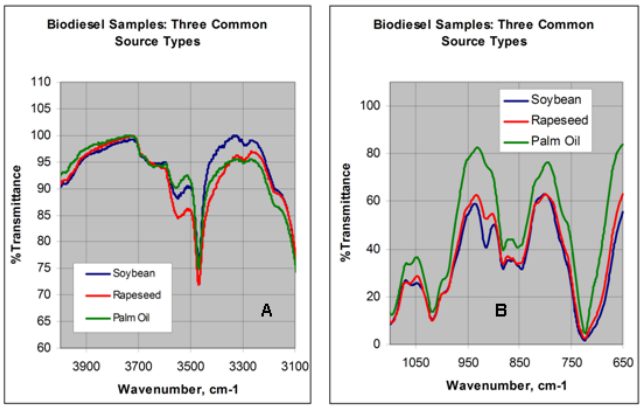


Figure 7. Detailed information from the base ester components used in the production of biodiesel methyl esters; hydroxyl (A) and unsaturation (B) functionalities

Another important issue for biodiesel is the level and type of unsaturation; a parameter linked to the chemical reactivity of unburned fuel residues in the engine oil. Three common types of biodiesel are illustrated in Figure 7B, ranging from the rapeseed derivatives (common in Europe), the soy based product (USA), and the palm oil based product often used in Latin America and the Caribbean. These differences correlate with unsaturation and chain length. These considerations equally apply to edible oils, where unsaturation, molecular weight and reactivity are relevant to use at high temperatures.

Another important application of fixed path infrared measurements to biodiesel fuel is in the qualification of biodiesel blends. While biodiesel may be used as 100% of the methyl ester fuel, it is seldom used or distributed in that form. 100% biodiesel has a negative impact on vehicle emissions and it can attack materials used in the fuel system of a vehicle (tubing, seals and gaskets) Many vehicle/engine manufacturers, do not recommend its use; its use may violate and even void the vehicle powertrain warranty. Typically the fuel is used diluted with hydrocarbon diesel fuel to give 5 % to 20 % in blends designated B5 to B20. Figure 8 illustrates the measurement of biodiesel blends covering the full range from B0 to B100. Good calibrations for this series are obtained as indicated in Figure 9.

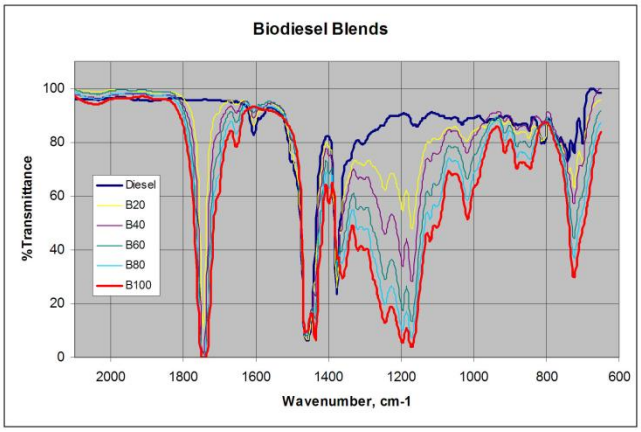


Figure 8. Measurement of biodiesel blends, experimental data from B0 (diesel fuel) to B100 (biodiesel) and intermediate biodiesel/diesel blends

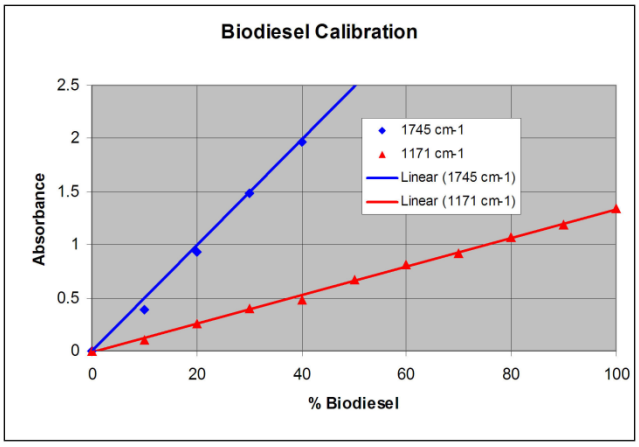


Figure 9. Quantitative measurement of biodiesel blends, B0, B10 to B90 and B10

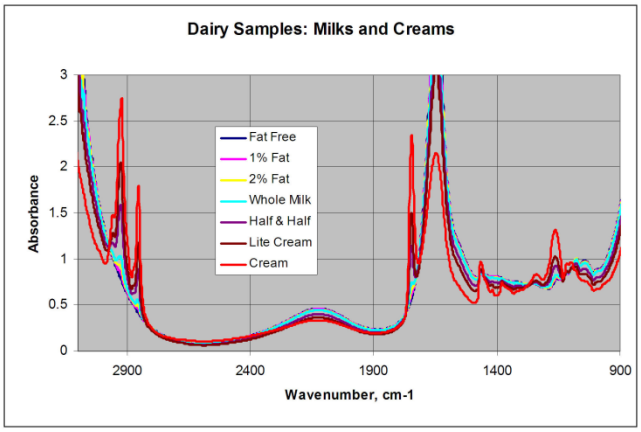


Figure 10. Dairy product spectra; short fixed pathlength (~30 mm), from fat free skim milk to standard heavy cream

The role of mid-infrared in the commercial analysis of milk and dairy products is well established. The measurement of raw milk in a fixed pathlength cell is used by regulatory agencies to control and standardize milk and dairy products. Standard methods exist for fat and protein content, which is used for the payment of the farmer. The performance and health of the dairy herd is also controlled, in pseudo real-time by monitoring fat/protein content. The results are used to control diet and medications. All of the relevant components in dairy products are derived from measurements of the infrared spectral data between 1800 cm^{-1} and 1000 cm^{-1}, a region that includes fat (ester), protein (amide bands) and sugars/lactose
(C-O-C, ether bands). Attempts to make these measurements in a standard sealed cell are fraught with difficulties. The accuracy of a fixed pathlength measurement is required, and the ease of handling high fat content materials, such as cream products, with the ease of cleaning, make the 5500a FTIR approach ideal for dairy product analysis.

Summary of the role and benefits of a “fixed” dial-a-pathlength system

This article has reintroduced the concept of making fixed pathlength mid-infrared transmission measurements without the complexity or the difficulties of the traditional sample handling method. A two-step approach summarized as “drop it on” and “wipe it off” is proposed, where the sample is put in place from a dropping pipette and is removed with the wipe of a paper towel. Anyone who has faced the challenges of working with the traditional fixed pathlength sealed cells can appreciate the ease of use and the simplicity of the system described. Traditional short path cells are impossible to fill with most liquids with average viscosity, and once filled, the cell can take five minutes or more to clean. The system described dramatically improves productivity and provides a platform for rapid, accurate quantitative analysis for all types of liquids.

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Agilent Technologies

Using the Agilent 490 Micro GC for the Monitoring of a Circulating Fluidized Bed Biomass Gasifier

Application Note

Micro Gas Chromatography, Reaction/Production Monitoring, Renewable Energy

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Abstract

Biomass has been recognized as a potential renewable and sustainable energy source. The Delft University of Technology researches the gasification of woody and agricultural biomass in a Circulating Fluidized Bed Reactor. The Agilent 490 Micro GC is used to characterize the product gas using a COX column for the permanent gases and a CP-Sil 5CB for the BTX compounds.

Introduction

There is a growing interest in sustainable heat and power generation using biomass. A possible way to use the biomass is through thermal conversion processes; combustion and gasification are the most well-known examples. The Process and Energy Department of the Delft University of Technology researched the gasification of woody and agricultural biomass in a Circulating Fluidized Bed. The product gas consists roughly of 5–15% Carbon monoxide, 10–15% Hydrogen, 3–5% Methane, 10–20% Carbon dioxide, 5–10% Nitrogen, and 40–70% Water, also (poly)aromatic compounds, minor inorganic species, and particles are present in the gas.

This product gas can be subsequently upgraded to Syngas (a mixture of Hydrogen, Carbon monoxide, Carbon dioxide and eventually water vapor). After applying the water-gas shift reaction ($CO + H_2O \rightarrow CO_2 + H_2$), Syngas could be used as a hydrogen-rich fuel gas for Fuel Cells. Other applications of Syngas are Fisher Tropsch processes (Gas to Liquid fuels), platform chemicals (like furfural), or the combustion in a gas turbine to generate heat and power. For the characterization of the product gas, the Agilent 490 Micro GC was used.

Experimental

Fluidization media and woody or agricultural biomass are fed into the Circulating Fluidized Bed Reactor, where the biomass is gasified at around 850 °C. The sample is taken from the product gas stream using a heated probe. Particles present in the sample are removed by the dust filter. Water vapor is stripped from the sample using two condensers. Figure 1 gives an overview of the sampling and sample conditioning setup. An external gas pump provides a continuous sample gas flow to the Agilent Micro GC. Every 3 min, the Micro GC starts an analytical run and analyses the gas sample on both column channels.

The Agilent 490 Micro GC used for the analysis of the product gas is equipped with a 1 m COX column channel for permanent gas analysis and a 4 m CP-Sil 5 CB column channel for the analysis of Benzene, Toluene and the Xylenes. The Micro GC conditions for both channels are displayed in Table 1.

Table 1. Agilent 490 Micro GC Instrument Conditions

	1 m COX	4 m CP-Sil 5 CB
Column temperature	100 °C	100 °C
Carrier gas	Argon, 200 kPa	Argon, 150 kPa
Injector temperature	110 °C	110 °C
Injection time	20 ms	40 ms
Detector sensitivity	Auto	High
Sample line temperature	110 °C	
Sampling mode	Continuous flow	
Sampling time	10 s	

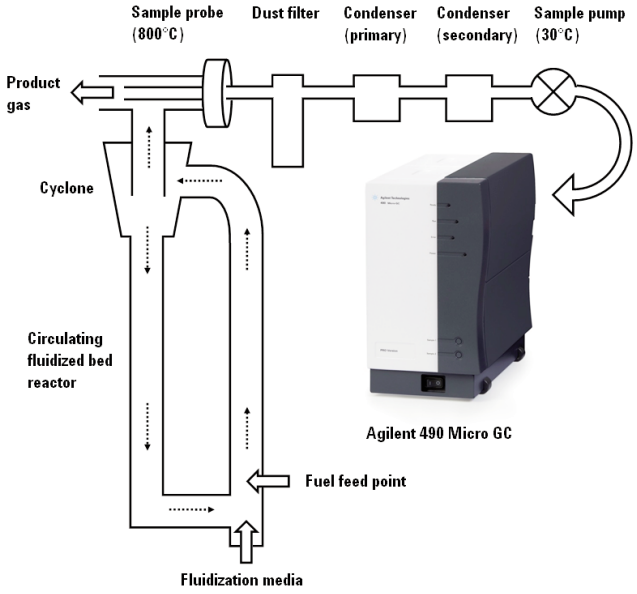

Figure 1. Reactor, sampling and sample conditioning setup.

Results and Discussion

The COX column shows an excellent separation for the permanent gases, as shown in Figure 2.

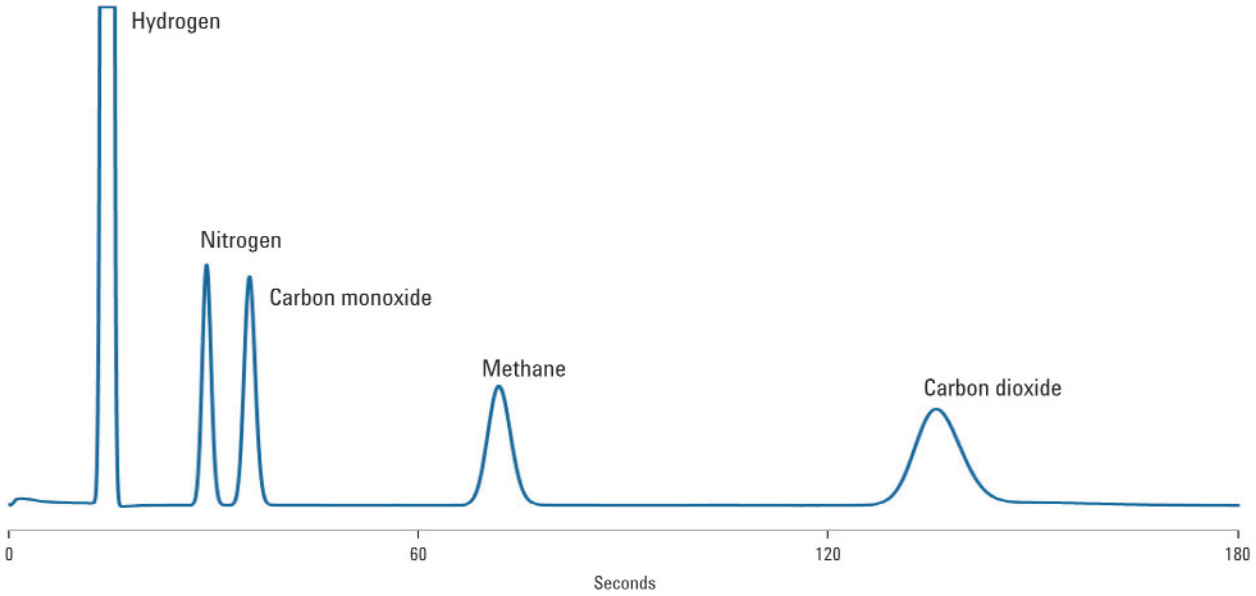

Figure 2. Permanent gases on the COX column.

Although the COX column does not separate oxygen and nitrogen, it is very suitable for the analysis of permanent gases including carbon dioxide. In the case of gasification, the product gas sample does not contain oxygen. When the sample contains both oxygen and nitrogen, and these gases need to be quantified separately, the use of a MolSieve5A column channel instead of the COX column channel is required. The COX column can be equipped with a back flush to vent. This option makes it possible to back flush later eluting compounds to reduce analysis time and to prolong column lifetime.

For each component a multi-level calibration (4 levels) is performed. Figures 3 and 4 show an excellent calibration curve for Methane and Carbon monoxide. For a linear regression, the R-Squared for these compounds is nearly perfect.

The BTX compounds are analyzed on a CP-Sil 5 CB column channel. The chromatogram in Figure 5 shows that all compounds are eluted in less than 90 sec. On the CP-Sil 5 CB column type it is not possible to separate meta- and para-Xylene. These compounds are reported in a single result. For all BTX compounds, a 4-level calibration is performed. Figure 6 shows an example of Benzene. R-squared (linear regression) for Benzene is 0.9969.

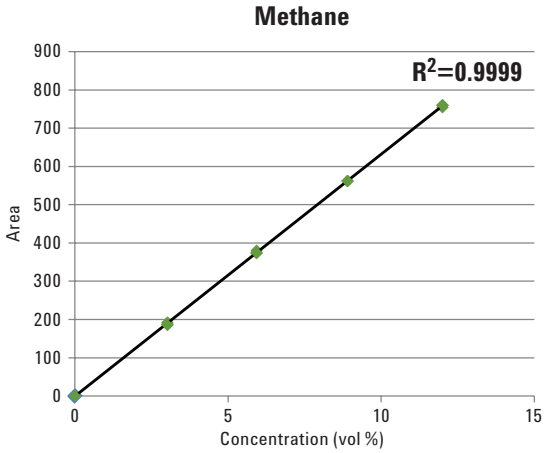

Figure 3. Calibration curve for methane.

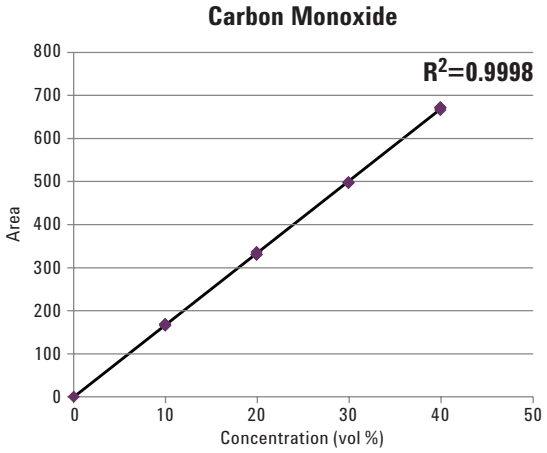

Figure 4. Calibration curve for Carbon monoxide.

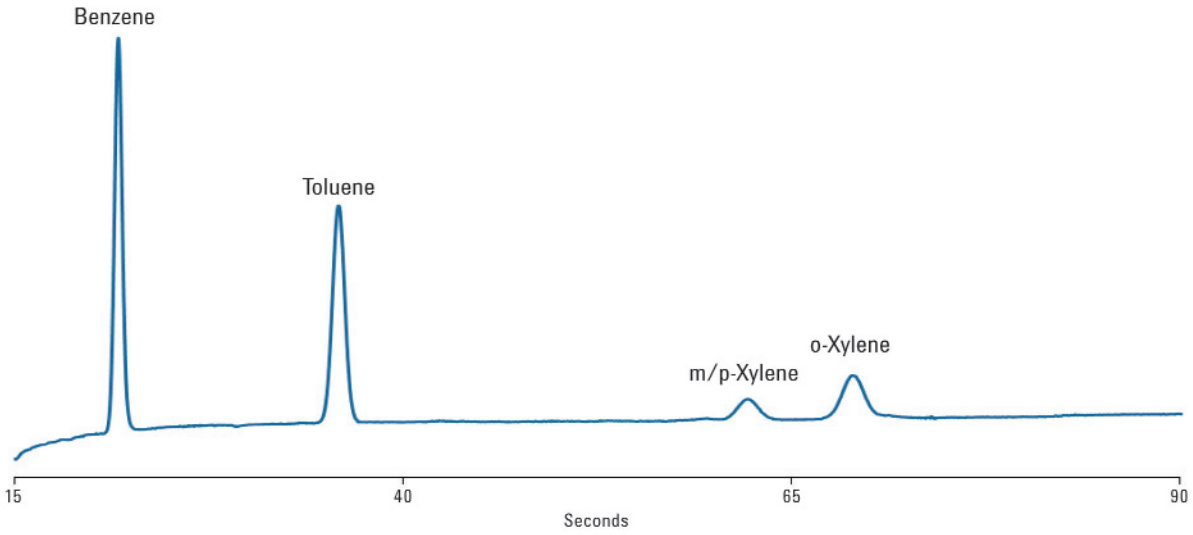

Figure 5. BTX analysis on the CP-Sil 5 CB column.

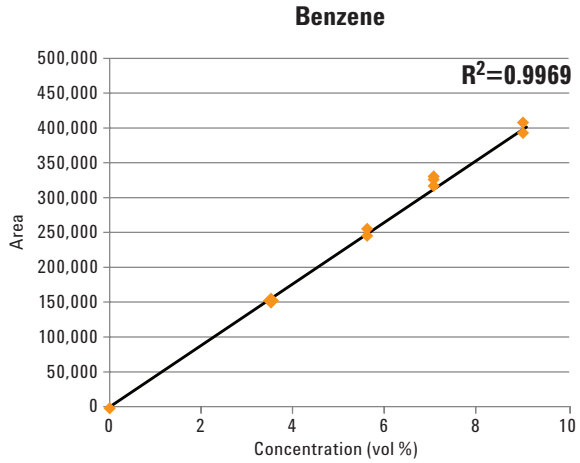

Figure 6. Calibration curve for Benzene.

Conclusion

The data presented in this application note clearly shows that the Agilent 490 Micro GC equipped with two column channels was capable of monitoring the product gas from the Circulating Fluidized Bed biomass gasifier. Within 180 sec the permanent gases were analyzed using a COX column channel. The BTX analysis was performed on a CP-Sil 5CB column channel with an analysis time of less than 90 sec.

The Agilent 490 Micro GC is considered a key apparatus for the quantification of the main product gas components in the gasification test rig at the Process & Energy Laboratory at Delft University of Technology. The main advantages of the 490 Micro GC analyzer are its reliability, short analysis times, ease of use (both hardware and software), and a certain degree of flexibility. The modular setup of the 490 Micro GC makes it possible to exchange the column modules if other gas components need to be analyzed.

The Agilent 490 Micro GC is a rugged, compact and portable lab-quality gas analysis platform. When the composition of gas mixtures is critical, count on this fifth generation micro gas chromatograph.

References

1. M.Siedlecki, R. Nieuwstraten, E. Simone, W. de Jong and A.H.M. Verkooijen; Delft University of Technology; 'Effect of Magnesite as Bed Material in a 100 kWth Steam-Oxygen Blown Circulating Fluidized-Bed Biomass Gasifier on Gas Composition and Tar Formation'; 2009.

2. Application note 5990-7054EN; Simone Darphorn-Hooijschuur and Marijn van Harmelen, Avantium Technologies; Remko van Loon and Coen Duvekot, Agilent Technologies; 'Permanent Gases on a COX Module Using an Agilent 490 Micro GC'; 2010.

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5990-8069EN

Agilent Technologies

Reversed Phase HPLC of Fatty Acids

Application Note

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Introduction

The analysis of lipids is of particular importance to the food industry; a variety of lipid compounds are used commercially, particularly in processed foods. Meat and cereals contain naturally-occuring lipids ranging from polar phospholipids, fatty acids, diglycerides and cholesterol to non-polar fats and oils (triglycerides). Processed foods may also contain additional spray dried or encapsulated fats and emulsifiers. Health issues related to excessive dietary fat intake include obesity, increased risk of some forms of cancer and cholesterol deposition in cardiovascular diseases such as atherosclerosis (hardening of the arteries). Identification and quantification of the different types of lipid and their fatty acid composition is therefore required, but is complicated by the difficulty in detection due to the absence of a strong UV chromophore.

Fatty acids are long hydrocarbon chains with terminal carboxylate groups, and form a major component of triacylglycerides, phospholipids and sphingolipids. More than 1000 naturally-occuring fatty acids have been identified, but most common lipids contain only a few of this extensive group. Biological systems usually contain fatty acids with an even number of carbon atoms, between 14 and 24, the most common between 16 and 18 carbon atoms. In animals, these chains are invariably unbranched. The hydrocarbon chain can contain one or more *cis* configuration double bonds. These double bonds dramatically affect the physical properties of the fatty acids. Stearic and oleic acid are both 18 carbon atoms long, but oleic acid has one double bond and a melting point of 13.4 °C, in comparison to stearic acid which is saturated and has a melting point of 69.6 °C.

Fatty acids that contain no double bonds, when analyzed by reversed phase chromatography, are separated by chain length, the shortest eluting first.

Agilent Technologies

A PLRP-S column can be used to separate fatty acids in a variety of media. These columns are robust enough to be stable at pH 1-14 and cope with vigorous clean up procedures and aggressive eluents. The Agilent evaporative light scattering detector is an ideal detector for the analysis of fatty acids. Although these acids can be detected by UV at 210 nm, the tetrahydrofuran itself will absorb. However, the changing composition of the eluent does not present a problem for the Agilent ELSD, as it is evaporated before reaching the light scattering cell. This method of detection produces a flat, stable baseline, as illustrated in the following examples using the same experimental conditions.

Conditions

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5800)
Eluent A: 60 mM Acetic acid
Eluent B: ACN
Eluent C: THF
Gradient: 35:60:5 to 0:90:10 in 20 min
Flow Rate: 0.5 mL/min
Detection: Agilent ELSD (neb=80 °C, evap=70 °C, gas=1.0 SLM)

Results and Discussion

Figure 1 shows good separation of seven fatty acids and Figure 2 shows two of the fatty acids in evening primrose oil. Good baseline resolution was achieved through the use of PLRP-S reversed phase material.

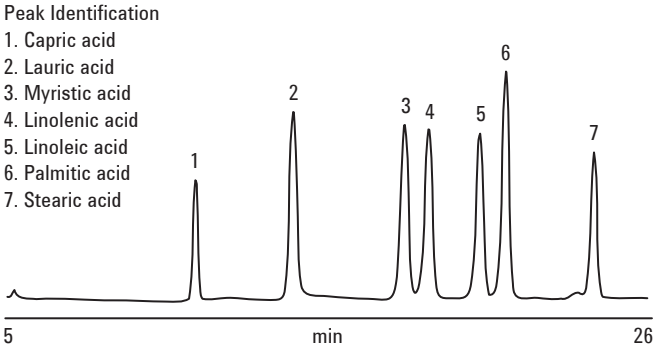

Figure 1. Separation of seven fatty acids using PLRP-S media.

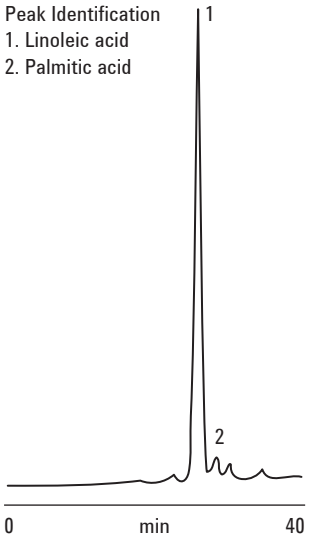

Figure 2. Fatty acid composition of evening primrose oil revealed by PLRP-S.

Conclusion

Coupling a PLRP-S column with the Agilent ELSD provides an ideal system for the quantitation of fatty acids, essential for their structural analysis and solute identification. As a single column, PLRP-S operates across the entire range of HPLC eluents. It is chemically stable and physically robust and so it is possible to switch between organic modifiers, such as ACN and tetrahydrofuran, and eluent pH 0 to 14.

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Agilent Technologies

Alkyl Glycerides from Frying Fat on Agilent PLgel 3 µm with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Alkyl glycerides are discrete molecules commonly found in frying fats and oils. Agilent's PLgel 3 µm 100Å columns for gel permeation chromatography have been specifically designed for the analysis of low molecular weight molecules from complex mixtures such as these. In this example, the columns are used for the analysis of alkyl glycerides, and for resolving individual molecules from complex alkyl glyceride mixtures such as occur in frying fats.

Analysis of Alkyl Glycerides

The samples were made up at 0.2% (w/v) in tetrahydrofuran and injected without further treatment. Figure 1 shows two overlaid chromatograms of lauryl and stearyl mono-, di-, and triglycerides, illustrating the base line resolution possible with these high efficiency columns.

Figure 2 shows the separation of a complex mixture of alkyl glycerides. Although base line resolution is not possible with two columns, the two-column set has resolved the individual components from the mixture, allowing identification of the molecules to be made after appropriate calibration.

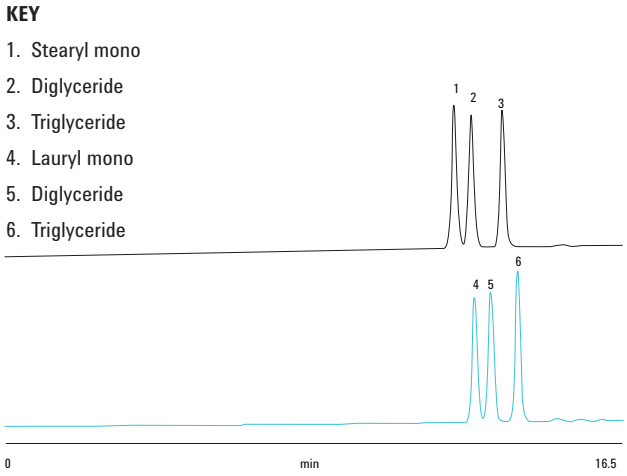

Figure 1. Overlaid chromatograms of lauryl and stearyl mono-, di-, and triglycerides, showing the base line resolution possible with high efficiency Agilent PLgel 3 µm columns.

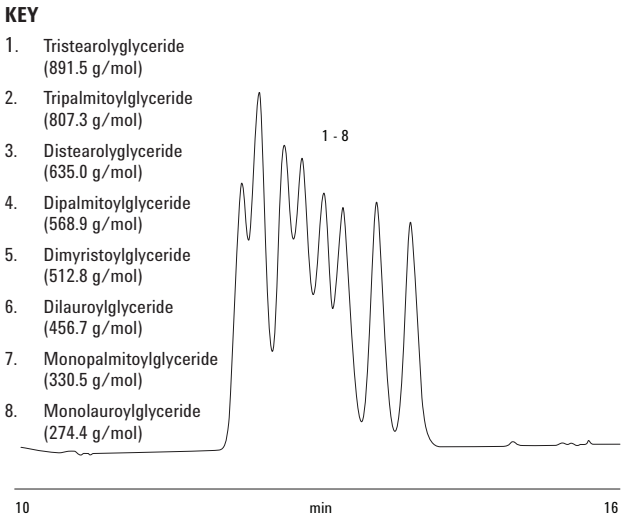

Figure 2. An Agilent PLgel 3 µm two-column set resolves individual components from an alkyl glyceride mixture.

Conditions

Samples	Alkyl glycerides
Columns	2 × Agilent PLgel 3 µm 100Å, 300 × 7.5 mm (p/n PL1110-6320)
Concentration	0.2% (w/v)
Eluent	THF
Flow rate	1.0 mL/min
Injection volume	20 µL
Detector	RI
System	Agilent PL-GPC 50

Further resolution could be obtained by adding additional columns, according to the equation:

$$Rsp = \frac{0.25}{\sigma D}$$

where Rsp is the specific resolution, σ is the peak variance (related to the peak width) and D is the slope of the calibration curve. Increasing the number of columns in the analysis reduces the slope of the calibration curve.

Conclusions

A sample of frying fat containing a complex mixture of alkyl glycerides was successfully separated using Agilent PLgel 3 µm 100Å columns to allow for further analysis and identification of the individual components. These low pore size columns are ideal for high resolution separations by gel permeation chromatography of low molecular weight compounds such as alkyl glycerides.

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Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer

Application note

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Abstract

The OneNeb nebulizer for inductively coupled plasma optical emission spectrometry (ICP-OES) features unique Flow Blurring technology. Compared to previous nebulizers, this universal nebulizer provides improved sensitivity, greater tolerance to dissolved salts and strong acids such as HF, resistance to most common organic solvents and efficient operation over a much wider flow rate range.

This application note demonstrates the superior performance of the OneNeb nebulizer compared to commercially available glass concentric nebulizers usually provided with ICP-OES instruments. Detection limits and reproducibility were better in a range of analytes and liquids.

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Introduction

The OneNeb nebulizer for use with an inductively coupled plasma optical emission spectrometer (ICP-OES) is a novel nebulizer that uses Flow Blurring technology. It is designed as a universal nebulizer offering a unique alternative to a variety of nebulizers by providing improved sensitivity, greater tolerance to dissolved salts and strong acids such as HF, resistance to most common organic solvents and efficient operation over a much wider flow rate range than existing nebulizers.

In this application note we will compare the performance of the OneNeb nebulizer to the commercially available glass concentric nebulizer normally fitted, using a range of performance criteria such as limits of detection and reproducibility using a range of analytes and liquids.

Description

The OneNeb nebulizer (Agilent part number 2010126900, Figure 1) is made completely from inert polymeric materials. It is physically robust and can withstand physical shocks that usually damage a glass concentric nebulizer.

Figure 1. OneNeb nebulizer

The capillary tubing extends nearly to the tip. The geometry at the tip, is carefully dimensioned to allow the carrier gas (in this case, argon) to mix with the sample liquid.

The OneNeb nebulizer uses Flow Blurring technology to mix argon with the sample to efficiently create an aerosol of smaller droplets with a narrower size distribution than conventional concentric nebulizers. Smaller droplets with narrow size distribution are more efficiently desolvated and excited in the plasma, ensuring better analytical precision and improved sensitivity.

By using Flow Blurring principles instead of the venturi effect for nebulization, the OneNeb is ideal for samples with high dissolved salts.

Other nebulizer designs

Concentric glass nebulizers (Figure 2) are the most common nebulizer type used in ICP-OES. The design features two concentric glass tubes with liquid pumped through the narrow inner capillary and argon forced through the gap between the inner sample capillary and outer quartz tube. A venturi effect creates an aerosol of relatively narrow droplet distribution, resulting in a nebulizer that provides good analytical RSD and detection limits. However, the narrow sample capillary is prone to blockages and precipitates forming on the end of the capillary that can affect nebulizer efficiency over time. Nebulizers using the venturi effect are not well suited for use with high dissolved salts because of this tendency to block.

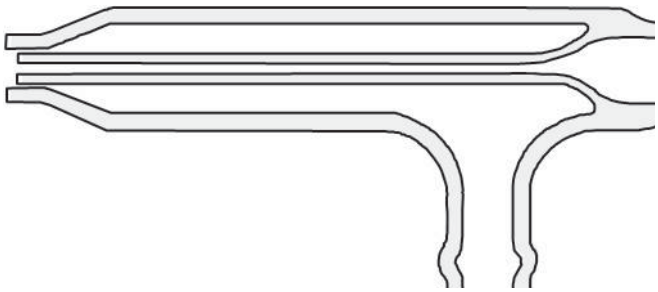

Figure 2. Concentric glass nebulizer

Nebulizers designed for samples with high total dissolved solids (TDS) such as the V-Groove nebulizer and cross-flow nebulizer do not rely on the venturi effect of the concentric glass nebulizer and are therefore more tolerant to dissolved salts. However, typically these nebulizers generate an aerosol with a wide range of droplet sizes resulting in higher analytical relative standard deviation and poorer detection limits.

Experimental

Instrumentation

An Agilent 725 ICP-OES with radially-viewed plasma and SPS 3 Sample Preparation System was used for this work.

The 725 ICP-OES features a custom-designed CCD detector, which provides true simultaneous measurement and full wavelength coverage from 167 to 785 nm. The CCD detector contains continuous angled arrays that are matched exactly to the two-dimensional image from the echelle optics. The thermally-stabilized optical system contains no moving parts, ensuring excellent long-term stability.

Operating parameters

- RF power: 1.3 kW
- Plasma gas flow: 15 L/min
- Auxiliary gas flow: 2.25 L/min
- Spray chamber: Single-pass and double-pass glass cyclonic
- Torch: Standard demountable with 0.38 mm quartz injection tube.
- Nebulizer flow: 0.7 L/min
- Replicate read time (for determining limits of detection): 30 s
- Number of replicates (for limits of detection): 10
- Stabilization time (for limits of detection): 30 s
- Replicate read time (for stability): 10 s
- Number of replicates (for stability): 6

Pump tubing

Two cases of pump tubing were used:

- Instrument: Orange-green (0.38 mm ID), of materials matched to the solvent being studied.
- Waste: Orange-orange (0.89 mm ID) Marprene for organic solutions.
- Instrument: Black-black (0.76 mm ID) for aqueous only.
- Waste: Blue-blue (1.65 mm ID) for aqueous only.

Results and discussion

The transport efficiency of the OneNeb at conventional flows is equivalent to a high-efficiency concentric glass nebulizer (Table1). As shown in Table 2, the OneNeb is capable of operating with even higher transport efficiency at very low sample flow rates, which a conventional concentric glass nebulizer is not capable of. Typically, for operation with low sample uptake rates, a specialized low flow nebulizer is required. The very high transport efficiency of the OneNeb at low flow rates makes it an ideal nebulizer for precious samples or samples with limited volumes, such as biological fluids.

Table 1. Transport efficiency at conventional ICP-OES uptake rates

Nebulizer	Solvent	Spray chamber	TE (%)
Glass concentric	Water	Double-pass	6.1
OneNeb	Water	Double-pass	6.6
OneNeb	Water	Single-pass	3.8–12.8

Table 2. Transport efficiency of OneNeb at very low uptake rates

Solvent	Spray chamber	TE (%)
Water (2–6% HNO_3)	Double-pass	12.5–18.79
Water (2–6% HNO_3)	Single-pass	17.7–31.4
ShellSol	Single-pass	44.0–48.7
Diisobutyl ketone	Single-pass	49.0

With organic solvents commonly used in ICP-OES analysis such as diisobutyl ketone and ShellSol, the OneNeb nebulizer provided excellent stability (Figures 3 and 4) over long-term runs, demonstrating excellent chemical resistance.

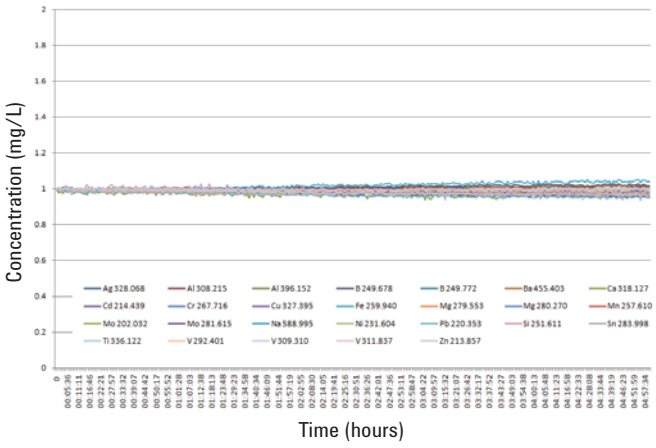

Figure 3. Long-term stability of the OneNeb nebulizer with diisobutyl ketone

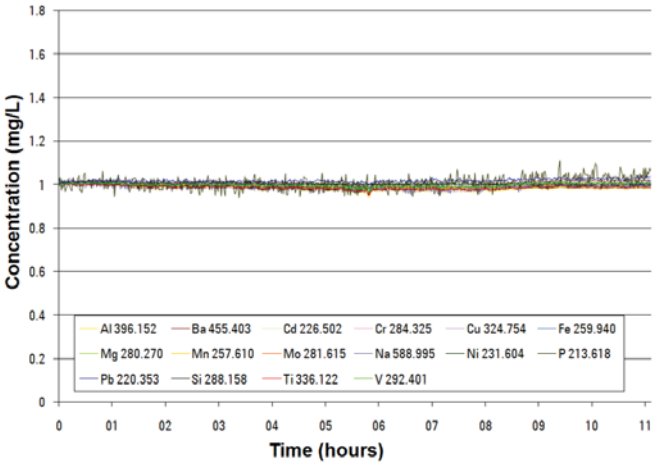

Figure 4. Long-term stability of the OneNeb nebulizer with ShellSol

The OneNeb nebulizer provided superior (>100% ratio) detection limits compared to the high performance concentric glass nebulizer for all elements analyzed, except for silver and zinc, which exhibited equivalent detection limits (Table 3).

Table 3. Comparison of 30 second detection limits (DLs) between concentric glass nebulizer (CGN) and OneNeb nebulizer

Element	CGN DL	OneNeb DL	DL ratio (%)
Ag 328.068	0.61	0.61	100
Al 167.019	1.94	1.53	127
As 188.980	12	9.84	122
Ba 455.403	0.07	0.05	162
Be 313.042	0.01	0.01	193
Ca 396.847	0.09	0.07	121
Cd 214.439	1.27	0.91	139
Co 238.892	1.9	1.7	110
Cr 267.716	0.86	0.70	123
Cu 327.395	1.76	0.96	183
Fe 238.204	0.90	0.68	132
K 766.491	59	38	154
Mg 279.553	0.05	0.05	107
Mn 257.610	0.19	0.15	131
Na 589.592	2	1.04	197
Ni 231.604	5	5	108
Pb 220.353	12	10	113
Se 196.026	17	13	133
Tl 190.794	15	12	129
V 292.401	1.24	0.96	129
Zn 213.857	0.50	0.49	101

Conclusion

The OneNeb nebulizer with Flow Blurring technology demonstrated excellent tolerance to samples with high TDS. Over weeks of extended testing of these high TDS samples, the OneNeb nebulizer proved virtually unblockable. This was in stark contrast to the regular failure of the glass concentric nebulizer due to blocking.

In terms of detection limits and tolerance to organic solvents, the OneNeb nebulizer proved superior to a high performance concentric glass nebulizer. Its resistance to strong acids such as HF proved similar to inert polymeric nebulizers. Tolerance to high TDS samples by the OneNeb nebulizer ranked it equal to nebulizers dedicated to handling high TDS such as V-groove nebulizers, without the deterioration in precision or detection limits in aqueous solutions.

The OneNeb nebulizer proved to be a genuinely universal nebulizer that is mechanically rugged and durable. It is competitive in price with a high performance concentric glass nebulizer. The OneNeb is capable of replacing many different types of nebulizers typically required to analyze the range of samples an ICP-OES is called upon to measure, without compromising performance. A universal nebulizer also simplifies method development and day-to-day operation by eliminating the need to decide which nebulizer is best for which sample, and reducing the need for many different nebulizers. It operates with very high nebulization efficiency at sample uptake rates from 40 µL/min, potentially allowing the analysis of volume limited samples.

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Using a Dual LTM Series II System with Flow Modulated Comprehensive GCxGC

Application Note

Application Area Identifier

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Abstract

A comprehensive GCxGC system based on differential flow modulation is described that uses three independent programmable ovens. The first dimension separation occurs in the 7890A air bath oven while two simultaneous second dimension separations occur on 5 inch LTM Series II modules. All columns operate in constant flow mode. Oven temperature programs can be customized independently for each column. Typically the two LTM columns will be of different polarities and phase ratios to maximize the information that can be gathered from the sample. A typical column configuration consists of a 20 m x 0.18 mm x 0.25 µm DB5ms for the first dimension, a 7 m × 0.25 mm × 0.2 µm HP-INNOWax for LTM module 1 and a 5 m × 0.25 mm × 0.15 µm DB17HT for LTM module 2. Many other column combinations are possible.

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Introduction

Conventional flow modulated GCxGC usually consists of one first dimension column and one second dimension column where both are subjected to the same temperature program. The basic one-oven system has been described previously [1,2]. Flow modulation also has the distinct advantage of not requiring cryo fluids for operation, rather it relies on a high flow differential between 1st and 2nd dimensions for operation.

Careful matching of the retention factors (k) between the first and second column is necessary in a one-oven system in order to produce meaningful 2D data and avoid the wrap around effect. The wrap around effect occurs when analytes injected onto the second column do not elute in one modulation cycle. However, the single oven system is in widespread use for a variety of applications and works well if k's are matched appropriately.

Flow modulated GCxGC works best when all columns are operated in constant flow mode. The Low Thermal Mass (LTM) Series II system is fully integrated into the GC and MSD ChemStations and Agilent 7890A firmware allowing control of all parameters. Since this integration enables LTM to operate in constant flow, the system can be easily interfaced to a flow modulated GCxGC 7890 system.

Experimental

A diagram of the system is shown in Figure 1. A Capillary Flow Technology (CFT) splitter is used to direct the out flow from the CTF modulator to two LTM column modules for a simultaneous dual channel GCxGC analysis. Each column operates with its own independent temperature program.

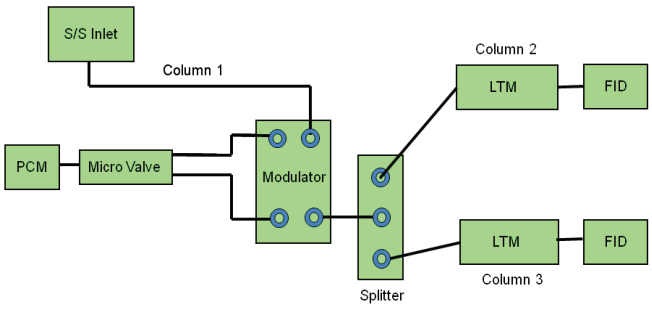

Figure 1. Diagram of the dual LTM GCxGC system.

The operation scheme of the flow modulator showing both the load and inject states is shown in Figure 2. Effluent for the first column fills the collection channel, and before significant diffusion or overfill occurs the three way valve is switched and a high flow (21 mL/min) controlled by the PCM injects the channel contents into the two second dimension columns. The modulation cycle then repeats based on the user set collect and inject times.

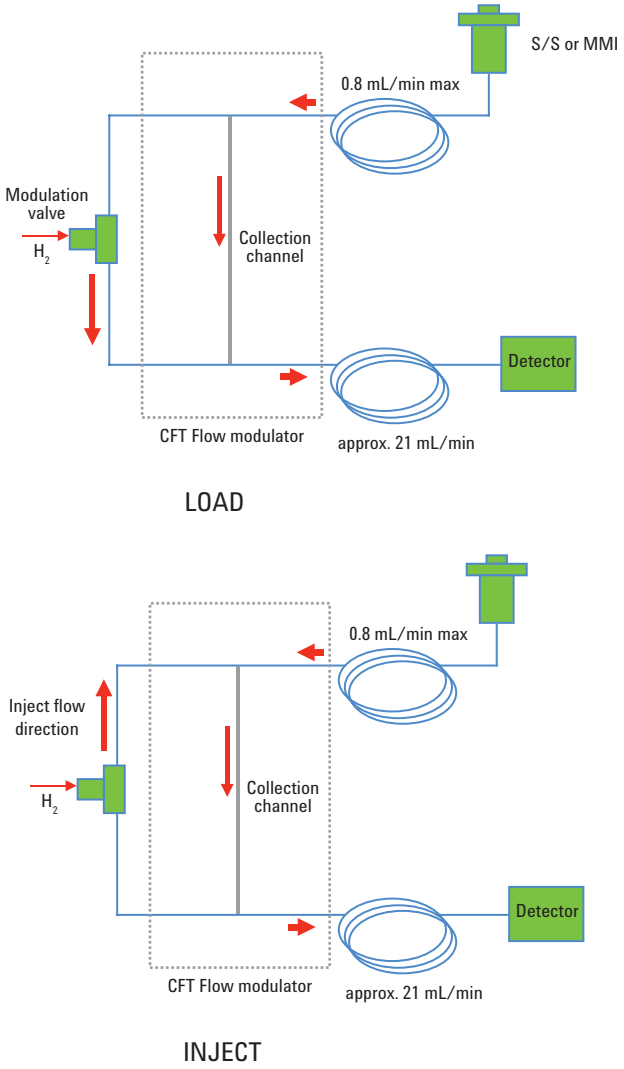

Figure 2. Operational detail of the flow modulator showing load and inject states.

Column 1 flow rate depends on column dimensions, but cannot exceed 0.8 mL/min. Figure 3 shows the relationship between modulation period and Column 1 flow rate.

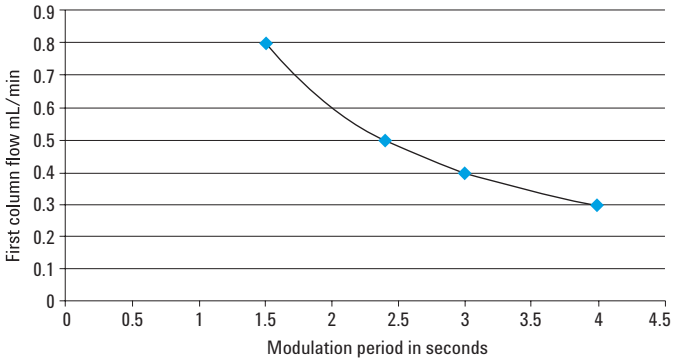

Figure 3. Relationship between modulation period and first dimension column flow rate.

Referring again to Figure 1, since LTM column flow rate is controlled by a single PCM, column flow will be the same in both modules provided they are of the same dimension. If this is not the case, the column configuration (in Chemstation) should set the PCM to control the longer or more restrictive column at 21 mL/min or greater. The second LTM column will then operate at a higher flow. Therefore, it is advisable that the two LTM columns do not differ greatly in length. Also, it is best to keep the second dimension columns at 0.25 mm ID. For this work, LTM column pairs were either both 5 meter or 5 and 7 meter. An example column configuration Chemstation pane for the system is shown in Figure 4.

	Column	Calibration Results	Inlet	Outlet	Heated By
1	Agilent 19091J-413: 400 °C: 7 m x 250 µm x 0.25 µm Additional Segments: inSeg Heated By Oven: 0.5 m x 250 µm x 0 µm outSeg Heated By Oven: 0.5 m x 250 µm x 0 µm HP-5 5% Phenyl Methyl Siloxan: <Not Inventoried>	Uncalibrated	PCM A-1	Front Detector	LTM-II
2	J&W Custom LTM 5M: 320 °C: 5 m x 250 µm x 0.15 µm Additional Segments: inSeg Heated By Oven: 0.3 m x 250 µm x 0 µm outSeg Heated By Oven: 0.6 m x 250 µm x 0 µm LTM 5M x 0.25 x 0.25: <Not Inventoried>	Uncalibrated	PCM A-1	Back Detector	LTM-II
3	450 °C: 20 m x 180 µm x 0.18 µm restrictor: <Not Inventoried>	Uncalibrated	Front Inlet	PCM A-1	Oven

Figure 4. Column configuration pane from the GC Chemstation showing set up of all three columns.

Hardware

Agilent 7890A GC with S/S inlet and dual FID's

Flow modulator	G3440A option887, and G3487A
If adding to existing GC	G3486A
CFT un-purged splitter	Kit: G3181-64010
LTM Series II	G6680A, 2-channel, 5-inch system, two power supplies

Firmware and Chemstation

Agilent 7890A firmware	A.01.12.1 or greater
ChemStation	B.04.03 DSP1, includes LTM II software

Typical Parameters

Carrier gas	Hydrogen
Primary column	20 m × 0.18 mm × 0.18 µm HP-1
LTM Module 1	7m × 0.25 mm × 0.25 µm HP- INNOWax, or 5 m × 0.25 mm × 0.15 µm HP- INNOWax
LTM Module 2	5 m × 0.25 mm × 0.15 µm DB17HT
Primary column flow	0.35 mL/min, 27.6 psi starting pressure
LTM 1	20 mL/min, 25.6 psi starting pressure (7 m column)
LTM 2	29 mL/min
Inlet	Split/splitless, 280 °C, 200-600 to 1 split
Primary oven program	35 °C (2 min) to 280 °C @ 3 °C/min
LTM 1 program	55 °C (3 min) to 270 °C @ 5 °C/min
LTM 2 program	60 °C (5 min) to 300 °C @ 3 °C/min
LTM InSeq retention gaps	0.5 m × 0.25 mm
LTM OutSeg retention gaps	0.5 m × 0.25 mm
Detectors	dual FID's at 300 °C

GCxGC Parameters

Load time	2.700 sec
Inject time	0.090 sec
Modulation period	2.799 sec

GCxGC Data Processing Software

GC Image, Version 2.1b4

Results and Discussion

In flow modulated GCxGC, greater flexibility in optimizing methods may be achieved by use of independent ovens for the first and second dimension columns. Correct matching of the retention factors between the 1st and 2nd dimension columns is critical for achieving the best performance with flow modulated GCxGC. If retention on the 2nd D column is too high, analytes injected during one modulation cycle may not elute completely before the next modulation begins.

When a second independent oven is available for the 2nd dimension column, more column choices are available in terms of phase ratio and length. Using a temperature offset, (2nd column starts at higher temp compared to 1st) may allow more retentive columns to be used. Then fine tuning the temperature ramp rate becomes an additional tool to help achieve a difficult separation throughout a 2D chromatographic run or in a particular section of a run. Employing an LTM module for the second dimension makes this possible.

The system can be further enhanced by inserting a CFT unpurged splitter between the modulator and the 2nd dimension. This allows two completely independent 2nd dimension LTM modules (with different stationary phase polarities) to be used which will yield two sets of 2D data for each run.

In figure 5a, a lower phase ratio 7 m INNOWax column is used for the analysis of a jet fuel. When both 1st and 2nd dimension columns are in the air bath oven, the standard 5 m × 0.25 mm × 0.15 µm column must be used to avoid wrap around at low oven ramp rates. With the second column configured as an LTM, longer, thicker film columns can be used to achieve better group separation while ensuring that all compounds will elute from the 2nd column in one modulation cycle. Figure 5b shows the same jet fuel analyzed simultaneously on a less polar 5 m × 0.25 mm × 0.15 µm DB17HT. Both offer useful information and allow different levels of compound group determination when using GC Image.

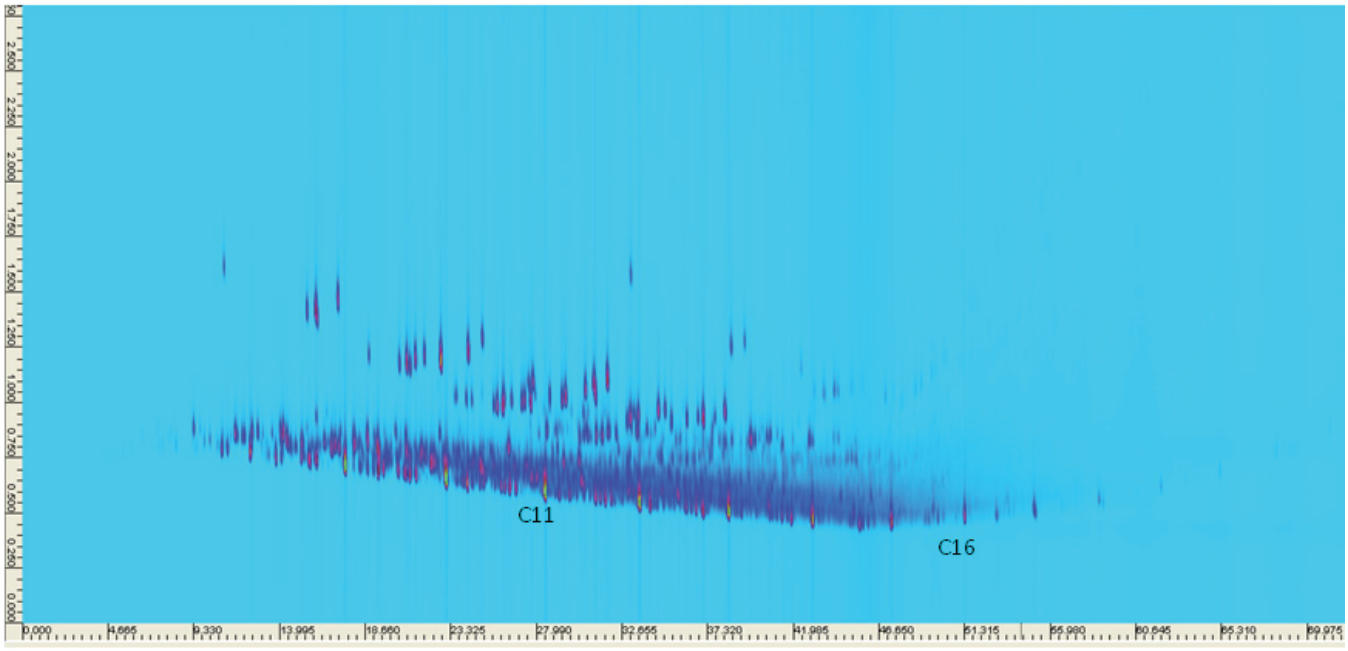

Figure 5a. Jet fuel 2D image. 7 m × 0.25 mm × 0.24 µm HP-INNOWax, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

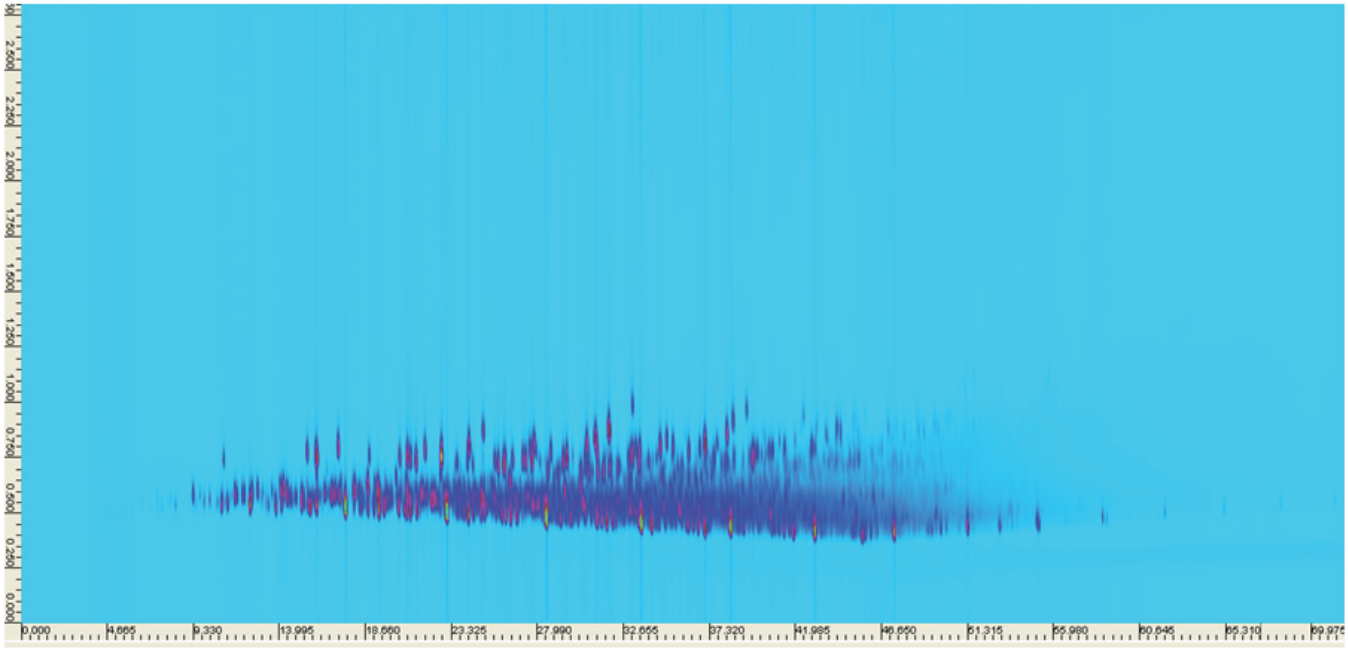

Figure 5b. Jet fuel on 5 m × 0.25 mm × 0.15 µm DB17HT, LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

2D images of a fragrance additive used in detergents is shown in figures 6a and 6b, on the 7 m INNOWax and DB17HT LTM columns, respectively. Peak 3, 4-tert-butyl-cyuclohexyl acetate, shown on the wax column eluted on a second modulation cycle. However, it remains well separated from other components and does not complicate interpretation of the 2D image. Labeled compounds determined by a GC × GC - 5975C MSD system.

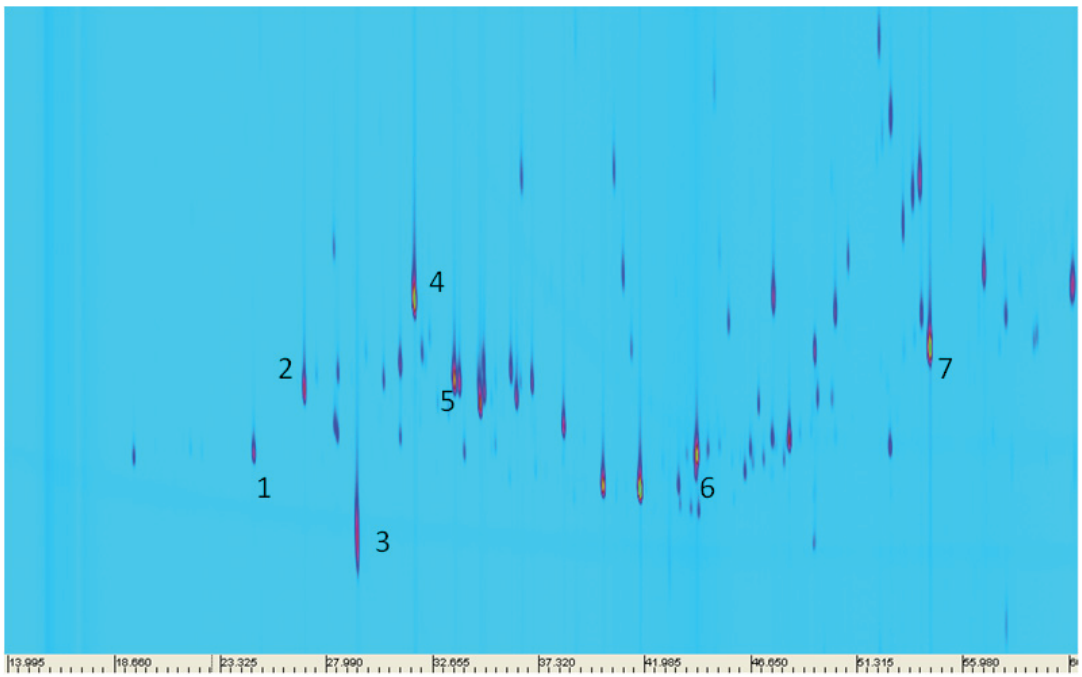

Figure 6a. Fragrance additive using 7 m INNOWax for 2nd dimension, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate.

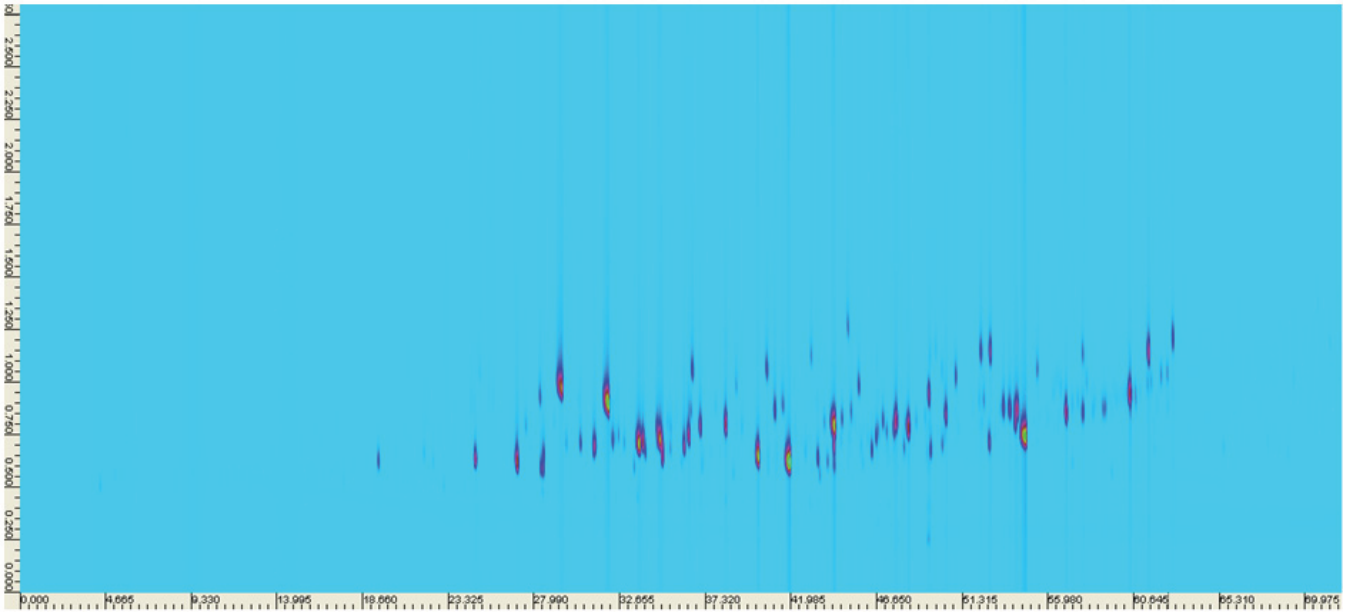

Figure 6b. Fragrance additive using 5m DB17HT for 2nd dimension separation. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

Lime oil images are shown in figures 7a and 7b. Only the regions around limonene are shown to highlight the separation differences on INNOWax and DB17HT. The 7M thicker film wax column separates minor components from dominate limonene. Compounds identified using a GC × GC - 5975C MSD system.

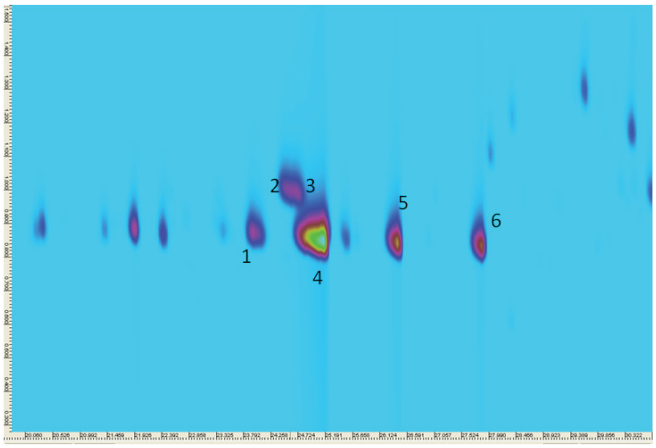

Figure 7a. Lime oil on the 7 m INNOWax. LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate 1.beta pinene, 2. 1,4 Cineol, 3. m-cymene, 4. Limonene, 5. Terpinen, 6. Terpinolen

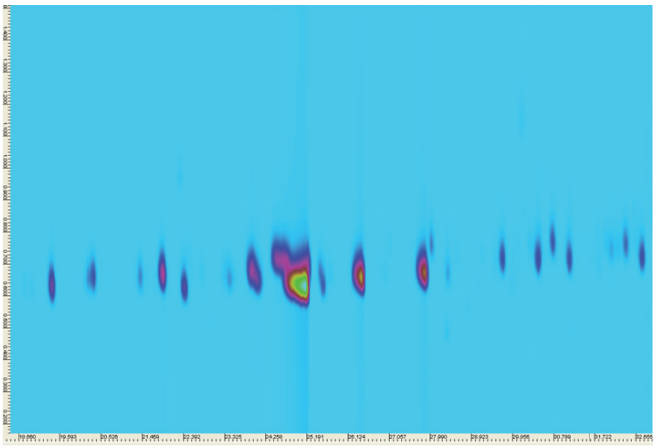

Figure 7b. Lime oil on the 5 m DB17HT. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

Finally, a 2D analysis of B20 (20% soy) biodiesel is shown in figure 8 using a 5 m × 0.25 mm × 0.15 µm INNOWax. Here, the LTM module and 7890 air oven are programmed at 3 °C/min. However the starting temperature of LTM is offset by minus 5 °C.

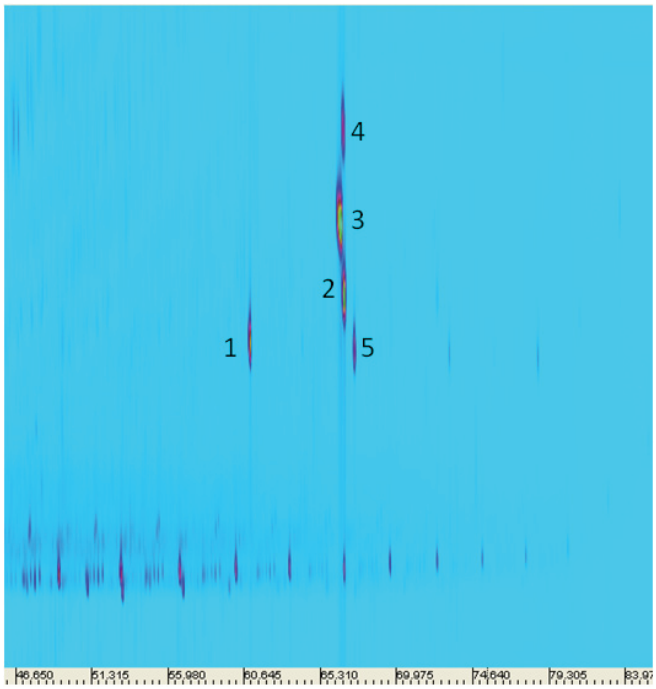

Figure 8. Separation of C16 and C18 fatty acid methyl esters in B20 biodiesel on a 5 m × 0.25 mm × 0.15 µm LTM INNOWax column in the 2nd dimension. LTM program: 30 °C (0 min) to 270 °C (5 min) @ 3 °C/min. 1. C16:0, 2. C18:1, 3. C18:3, 4. C18:3, 5. C18:0.

Conclusions

Comprehensive GCxGC is normally used when faced with a very difficult separation in a complex sample, perhaps a specific analyte determination. It is also a powerful tool for group determination, especially in fuels, and as a classification tool when used with chemometrics. The LTM series II system gives the analyst additional separation power and is easily interfaced to a flow modulated GCxGC system. Depending on how the system is configured, two or three independent temperatures programs can be used. This allows a wider range of column retention in the second dimension to be used.

This work is intended to illustrate some of the possibilities where comprehensive GC and LTM technology can be put to work. Only one combination of column stationary phases was tested (DB5ms-INNOWax-DB17HT). Many other combinations are possible. For example, some useful combinations to consider with the dual LTM system where different polarities are used include (INNOWax-DB1-DC200), and (DB1-DB200-DB35). Reversing polarities (most polar as 1st dimension) can be useful, i.e. (DB210-DB1-DB17) for problems where a few polar compounds must be separated from a complex non-polar matrix. When using LTM with GCxGC, appropriate matching of the retention factors of the 1st to 2nd dimension columns is still important; however LTM offers some additional flexibility to use lower phase ratio columns through temperature offsets and temperature ramps.

References

1. Comprehensive Flow Modulated Two-Dimensional Gas Chromatography, Roger L. Firor, Application Note 5989-6078EN, 2008
2. Comprehensive GC System Based on Flow Modulation for the 7890 GC, Roger L. Firor, Application Note 5989-8060EN, 2009

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Oligosaccharide Analysis on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Oligosaccharides are naturally occurring saccharides, typically with three to 10 component sugars. They are usually linked to lipids or amino acid side chains in proteins, and have many functions, such as cell-to-cell recognition in animals.

These compounds are soluble in water and polar organic solvents such as N-methylpyrrolidone (NMP), which may be required for more polar oligosaccharides such as starches, as this application note demonstrates. Gel permeation chromatography of oligosaccharides with NMP is straightforward using Agilent PLgel 5 µm columns.

Analysis of an Oligosaccharide

The viscosity of NMP is extremely high and operation at elevated temperature is recommended to reduce pressure and enhance resolution.

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Conditions

Column	Agilent PLgel 5 µm 500Å, 7.5 × 300 mm (p/n PL1110-6525)
Eluent	NMP
Flow rate	1.0 mL/min
Temp	80 °C
Detector	RI

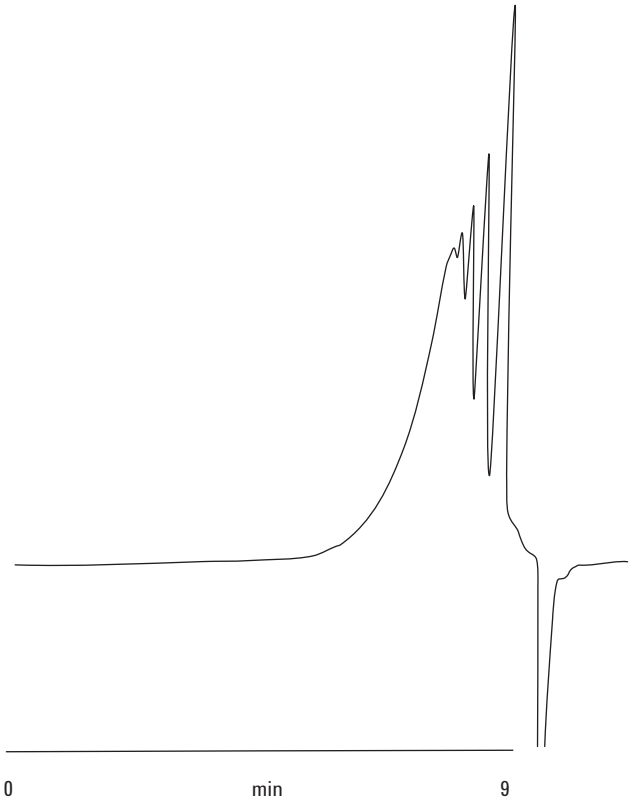

Figure 1. Separation of oligosaccharide on an Agilent PLgel 5 µm column.

Conclusions

Agilent PLgel columns in a polar solvent such as N-methylpyrrolidone can be used to analyze oligosaccharides by gel permeation chromatography.

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Agilent Technologies

Analysis of Lipids with the Agilent 385-ELSD and the Agilent 1260 Infinity LC

Sensitive detection through sub-ambient evaporation and nebulization

Application Note

Food Testing

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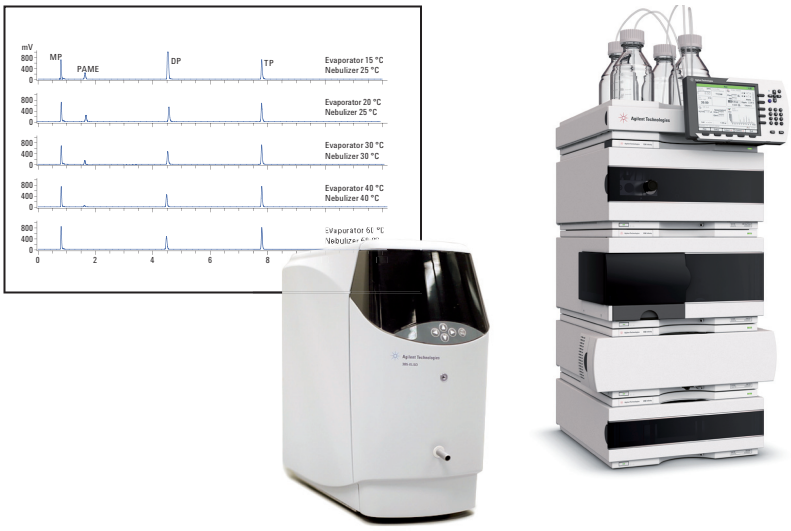

Abstract

This Application Note discusses how the Agilent 385-ELSD Evaporative Light Scattering Detector operates at various temperatures for a selection of lipid standards. The results clearly illustrate the benefit of sub-ambient evaporation and nebulization for certain (semi-)volatile compounds.

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Introduction

The Agilent 385-ELSD Evaporative Light Scattering Detector is equipped with a Peltier cooled evaporation tube which enables solvent removal at sub-ambient temperatures. This is an interesting feature for the detection of (semi-)volatile compounds. Evaporation at temperatures above ambient can lead to a loss of sensitivity, or even no detection at all for these compounds. This Application Note describes the analysis of lipid standard compounds with reversed-phase HPLC and ELSD. The detector temperature has a remarkable effect on the sensitivity for certain compounds.

Experimental

A standard mixture composed of 500 µg/mL 1-monopalmitin (MP), 1,2-dipalmitin (DP), tripalmitin (TP), and palmitic acid methyl ester (PAME) was made in mobile phase component B. This standard solution was injected under different detector temperature conditions. Five injections per condition were performed to evaluate the repeatability of injection and to obtain averaged/reliable results. The detectability, signal-to-noise ratio of PAME, and peak area of all compounds were compared for the different setpoints.

Results and discussion

Figure 1 shows the result for an injection of the standards at various temperatures. At sub-ambient conditions, that is evaporator temperature at 15 °C and nebulizer at 25 °C, all peaks are detected, and the signal for dipalmitin is outside the detector range.

Chromatographic conditions

An Agilent 1260 Infinity LC system with the following configuration was used:

- Agilent 1260 Infinity Quaternary Pump with integrated vacuum degasser (G1311B)
- Agilent 1260 Infinity Standard Autosampler (G4226A)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 385-ELSD Evaporative Light Scattering Detector (G4261A)

Method parameters

Column:	Agilent ZORBAX Eclipse XDB C18 RRHT, 2.1 mm L × 50 mm id, 1.8 µm d_p (p/n 927700-902)	
Mobile phase:	A = 0.1% acetic acid in methanol	
	B = isopropanol/hexane 50/40 v/v	
Flow rate:	1 mL/min	
Gradient:	0–1 min	0% B
	1–11 min	0–70% B
	11–12 min	70% B
	12–14 min	0% B (post-time)
Temperature:	25 °C	
Injection:	2.5 µL, needle wash (4 s, flushport, mobile phase B)	
Detection ELSD:	Nebulizer temperature	Varied (25–60 °C)
	Evaporator temperature	Varied (15–60 °C)
	Evaporator gas	1.6 SLM
	Detector rate	40 Hz
	Smoothing	3.0 s
	Gain	1

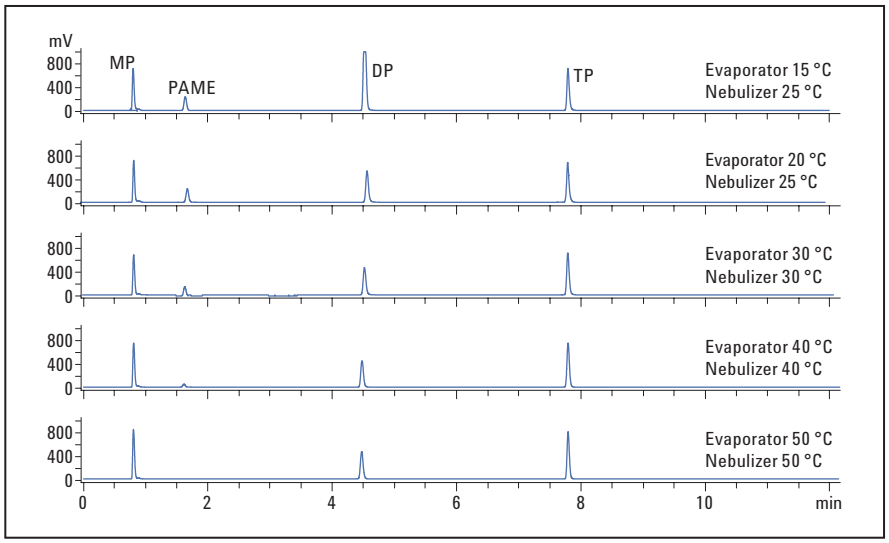

Figure 1
Analysis of the test mixture at various ELSD temperature settings.

When the evaporator temperature is slightly increased to 20 °C, the response for dipalmitin is within the detector range and remains relatively stable up to 60 °C (Table 1). The response for mono- and tripalmitin is significantly less dependent on the detector temperature.

The most striking influence of the evaporation/nebulization temperature is observed for the fatty acid methyl ester. Palmitic acid methyl ester (PAME) is easily detected at low temperatures, but contrary to the other compounds, the signal rapidly decreases when the ELSD is operated at temperatures above ambient. The signal-to-noise ratios (S/N) with the tested temperatures for this compound are shown in Table 1. Although the highest area is obtained at the lowest investigated temperature (15 °C), the signal-to-noise ratio, and consequently sensitivity, is maximal at ca. 25 °C (Figure 2). This is due to the slightly increased background noise observed with the low temperature settings. An overlay of a detail of the baseline for some representative temperatures are shown in Figure 3. This figure also illustrates the loss in signal intensity for PAME at higher temperatures. The compound is no longer detected at temperatures above 50 °C.

Conclusion

The advantage of low temperature nebulization and evaporation is demonstrated for a mixture of lipid standard compounds. The influence of the detector temperature on the detection of (semi-)volatile molecules can be impressive, therefore the option of a broad temperature range with sub-ambient capabilities is very useful for the analysis of such analytes.

Temperature		MP		TP		TP		PAME		
Evaporator	Nebulizer	Area	RSD%	Area	RSD%	Area	RSD%	Area	RSD%	S/N
15	25	1416	3.49	3931[1]	2.14	2032	3.38	735	1.26	398
20	25	1434	1.73	1637	3.30	1824	2.59	708	2.38	429
25	25	1362	1.74	1448	0.84	1869	0.49	607	1.41	530
30	30	1387	1.50	1403	0.97	1931	1.57	445	1.95	410
35	35	1473	1.13	1386	1.34	1882	1.57	270	3.41	267
40	40	1529	0.53	1408	2.84	1983	1.93	126	7.46	157
45	45	1602	1.68	1463	1.79	2031	1.08	50	2.82	48
50	50	1706	1.00	1527	0.90	2142	1.66	15	8.74	17
55	55	1759	1.65	1538	1.81	2196	1.58	N.D.	N.D.	< 3
60	60	1832	1.41	1574	1.46	2237	0.29	N.D.	N.D.	N.D.

(1) Out of range of the ELSD)

Table 1
Peak area and repeatability for the selected compounds and signal-to-noise ratio for PAME at various ELSD settings.

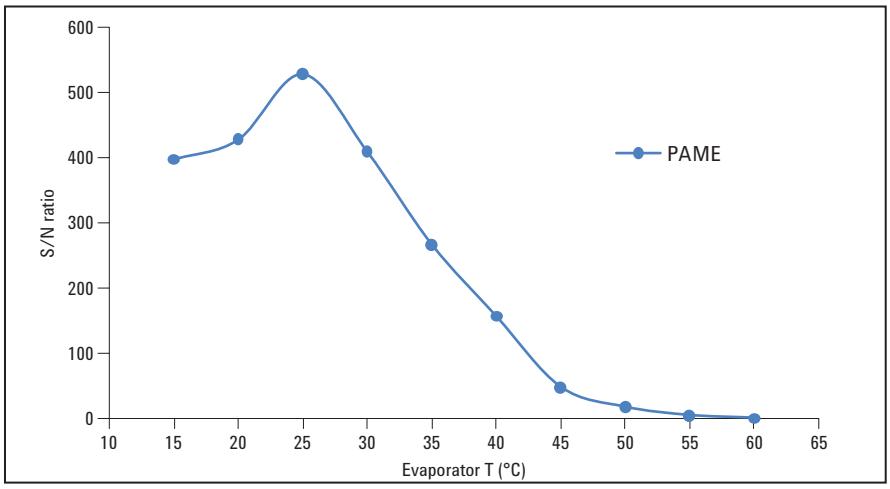

Figure 2
Influence of ELSD temperature on signal-to-noise ratio for PAME.

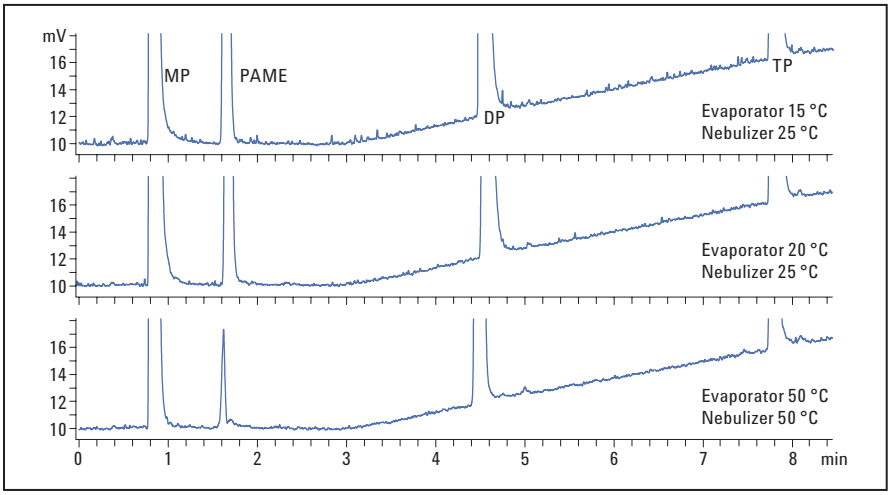

Figure 3
Detail on the baseline for the analysis of the test mixture at various ELSD temperature settings.

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Agilent Technologies

EN 15779 - Gas Chromatographic Analysis of Polyunsaturated FAME in Biodiesel Made From Algae and Marine Oils

Application Note

Fuels

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Abstract

The GC analysis of four common polyunsaturated fatty acid methyl esters (PUFA FAMEs) in algal biodiesel is described using method EN 15779. An Agilent 7890A GC system was configured and calibrated according to the procedure outlined in the method. Two samples of B100 biodiesel made from algae oil were each prepared in duplicate and analyzed according to the conditions set forth in the method. In each sample, the four PUFA FAMEs were chromatographically separated and quantified. The analysis precision was calculated and shown to exceed the specifications of the EN 15779 methods.

Introduction

Currently, most worldwide stocks of biodiesel are made from vegetable oils or animal fats. While these sources are cheap and convenient, they compete with food production resources. Current research involves finding nonfood sources of triglycerides harvested from plants that do not compete with food production. A promising source is algae cultivated in contained bioreactors, where both growth rates and oil yields are greater when compared to land-based crops. One potential problem with algae and marine oils is the high concentrations of polyunsaturated fatty acids (PUFA). After conversion to biodiesel fuel, PUFA FAMEs exhibit lower oxidation stability and higher rates of self-polymerization. These properties can cause engine fouling and fuel line or filter plugging if the PUFA FAME content is too high.

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To assure good algal biodiesel quality, the European Committee for Standardization (CEN) has developed a GC method to measure the amount of four predominant PUFA FAMEs found in these biodiesels (Table 1). The method is designated as EN 15779 [1]. This application note describes the configuration and performance of the Agilent 7890A GC system when using this method for the analysis of B100 biodiesel derived from algae oil.

Table 1. Polyunsaturated FAMEs Measured Using Method EN 15779

CAS number	Chemical name	Abbreviation
2566-89-4	Methyl eicosatetraenoate	C20:4 (n-6)
2734-47-6	Methyl eicosapentaenoate	C20:5 (n-3)
108698-02-8	Methyl docosapentaenoate	C22:5 (n-3)
28061-46-3	Methyl docosahexaenoate	C22:6 (n-3)

Experimental

An Agilent 7890A GC was configured and the instrument conditions were set according to the EN 15779 method. These details are shown in Tables 2 and 3. A 1.0 mg/mL solution of methyl tricosanoate (C23:0) in n-heptane was prepared for use as an internal standard. A 0.1 mg/mL solution of the four PUFA FAMEs (Table 1) was prepared in n-heptane containing 1.0 mg/mL of the internal standard (C23:0). This standard was used to determine the retention times for each PUFA FAME and the C23:0 internal standard. Two samples of algal B100 biodiesel were obtained for testing. Each sample was prepared by weighing 100 mg into a 2-mL autosampler vial and adding 1.0 mL of the C23:0 internal standard solution followed by mixing. The samples were prepared and run in duplicate to determine the repeatability of the analysis.

Table 2. 7890A GC Configuration for EN 15779

Standard Agilent 7890A GC system hardware	
Agilent 7890A Series GC (G3440A)	
Option 112	100 psi split/splitless Inlet with EPC control
Option 211	Capillary FID with EPC control
Agilent 7693 Autoinjector (G4513A)	
123-7032	DB-Wax Column, 0.32 mm × 30 m id × 0.25 µm

Table 3. Instrument Conditions for EN 15779 Method

Column oven conditions	
Initial oven temperature	150 °C for 1 min
Oven ramp 1	15 °C/min to 200 °C
Oven ramp 2	2 °C/min to 250 °C
Inlet and sampling conditions	
Column flow	Hydrogen at 1 mL/min constant flow
Inlet temperature	220 °C
Inlet mode	Split at 50:1 split ratio
Injection size	1 µL
Flame ionization detector conditions	
Detector temperature	250 °C

Results and Discussion

Figure 1 shows a chromatogram of the PUFA FAME reference standard run under the EN 15779 GC conditions. The retention times of each peak were noted on the chromatogram. These retention times were used to identify each of the four PUFA FAMEs found in the biodiesel samples.

The GC analysis of the two algal biodiesel samples is shown in Figure 2. The FAME profiles of the two samples are very similar, but the PUFA FAME content appears higher in sample 1. Quantification of the PUFA FAMEs was done using the theoretical response factors for each PUFA FAME published in the EN 15779 method. These response factors were corrected using the detector response of the C23:0 FAME internal standard added to each sample. This procedure helps to improve the accuracy of the final results. The weight percent of each PUFA FAME was calculated, and the total PUFA FAME content in the samples was reported by summing the individual FAMEs. Table 4 shows the results for the duplicate analyses of both algal biodiesel samples.

The analysis precision for each sample was determined by calculating the repeatability (r) for the duplicate runs. Repeatability is defined as the difference between duplicate sample results analyzed by a single operator on the same equipment in a short period of time, usually the same day. For the EN 15779 method, a repeatability specification was only determined for the total PUFA FAME result. Table 4 shows that this specification was exceeded for both samples when using the Agilent 7890A GC system.

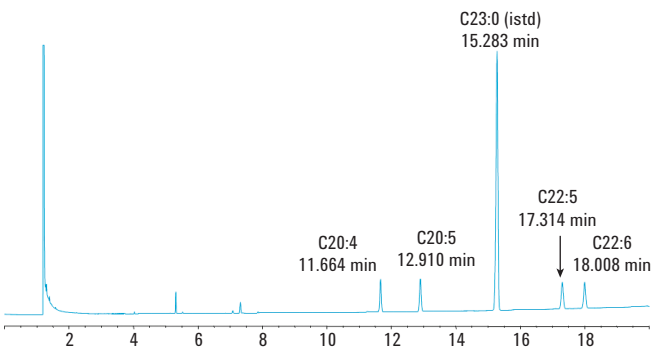

Figure 1. Chromatogram of the retention time standard containing the four PUFA FAMEs and the internal standard, methyl tricosonate (C23:0).

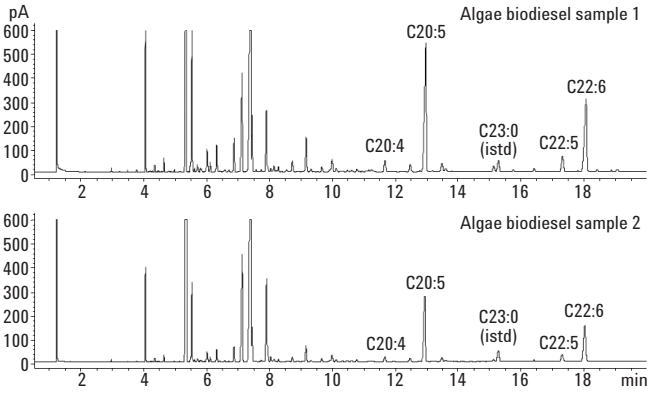

Figure 2. These chromatograms show the analysis of PUFA FAMEs in biodiesel samples made from two different algae oils.

Table 4. Reproducibility of Biodiesel Sample Runs

Run	C20:4 wt%	C20:5 wt%	C22:4 wt%	C22:6 wt%	Total PUFA
Algae biodiesel sample 1					
1	0.39	5.96	0.72	4.01	11.08
2	0.39	5.98	0.72	4.02	11.11
			Measured repeatability (r)		0.03
			EN 15779 Specification (r)		0.07
Algae biodiesel sample 2					
1	0.17	2.65	0.32	1.79	4.93
2	0.18	2.67	0.32	1.81	4.98
			Measured repeatability (r)		0.05
			EN 15779 Specification (r)		0.07

Excellent precision was observed for duplicate runs of each algal biodiesel sample. The reproducibility (r) measured for each sample exceeded the specification published in the EN 15779 method.

Conclusion

The analysis of PUFA FAMEs in biodiesel made from algal or marine oils can be easily done using EN method 15779 on an Agilent 7890A GC system. Calibration and reporting of the PUFA FAME content can be done according to the method's protocol using the standard tools within the Agilent Chemstation. After analyzing two algal oil biodiesel samples, the 7890A GC system provided results whose precision met the requirement of the EN 15779 method.

References

1. "EN15779 Petroleum products and fat and oil derivatives – Fatty acid methyl esters (FAME) for diesel engines – Determination of polyunsaturated fatty acid methyl esters (PUFA) by gas chromatography"; European Committee for Standardization: Management Centre, Avenue Matrix 17, B-1000 Brussels, 2009.

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Agilent Technologies

Determination of Total FAME and Linolenic Acid Methyl Esters in Biodiesel According to EN-14103

Application Note

Energy and Fuels

Author

Coen Duvekot

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Introduction

For biodiesel to be used as a motor fuel or blended with petroleum diesel, it must conform to standard specifications (ASTM D 6751 or EN-14214). There are standard GC methods in to determine if biodiesel conforms to the standard specifications, one of which is EN-14103, used to determine the ester and linoleic acid methyl ester content. Other methods include EN-14105 / ASTM D 6584 (free and total glycerine and mono, di, and triglyceride content), and EN-14110 (residual methanol). Agilent has designed GC solutions for each of these standard methods.

EN-14103 is used to verify that the ester content of fatty acid methyl esters (FAMEs) is greater than 90% (m/m) and that the linolenic acid content is between 1% (m/m) and 15% (m/m) consistent with the EN-14214 specifications.

This method is suitable for FAME that contains methyl esters between C_{14} and C_{24}.

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Materials and Methods

To prepare the sample, accurately weigh approximately 250 mg of sample in a 10 mL vial, then add 5 mL of methyl heptadecanoate solution (10 mg/mL) using a pipette.

Conditions

Column	Agilent Select Biodiesel for FAME, 0.32 mm × 30 m, 0.25 µm (p/n CP9080)
Instrument	Agilent GC
Software	Agilent Chromatography Data Station
Injection volume	1 µL
Injector	Split/splitless 1177, full EFC control, 250 °C, split 100 mL /min
Carrier gas	Helium, 12 psi (83 kPa)
Oven	210 °C isothermal
Detector	250 °C, FID, full EFC control

Results and Discussion

Figure 1 shows a separation of biodiesel, using the conditions outlined above.

The ester content (C), expressed as a mass fraction in percent, is calculated using Equation 1.

$$C = \frac{(\Sigma A) - AEI}{AEI} \times \frac{CEI \times VEI}{m} \times 100\ \%$$

where:

ΣA = total peak area from the FAME $C_{14:0}$ to $C_{24:1}$

AEI = peak area of methylheptadecanoate

CEI = concentration, in mg/mL, of the methylheptadecanoate solution

VEI = volume, in mL, of the methylheptadecanoate solution

m = mass, in mg, of the sample

Equation 1.

The linolenic acid methyl ester content (L), expressed as a mass fraction in percent, is calculated using Equation 2.

$$L = \frac{AL}{(\Sigma A) - AEI} \times 100\ \%$$

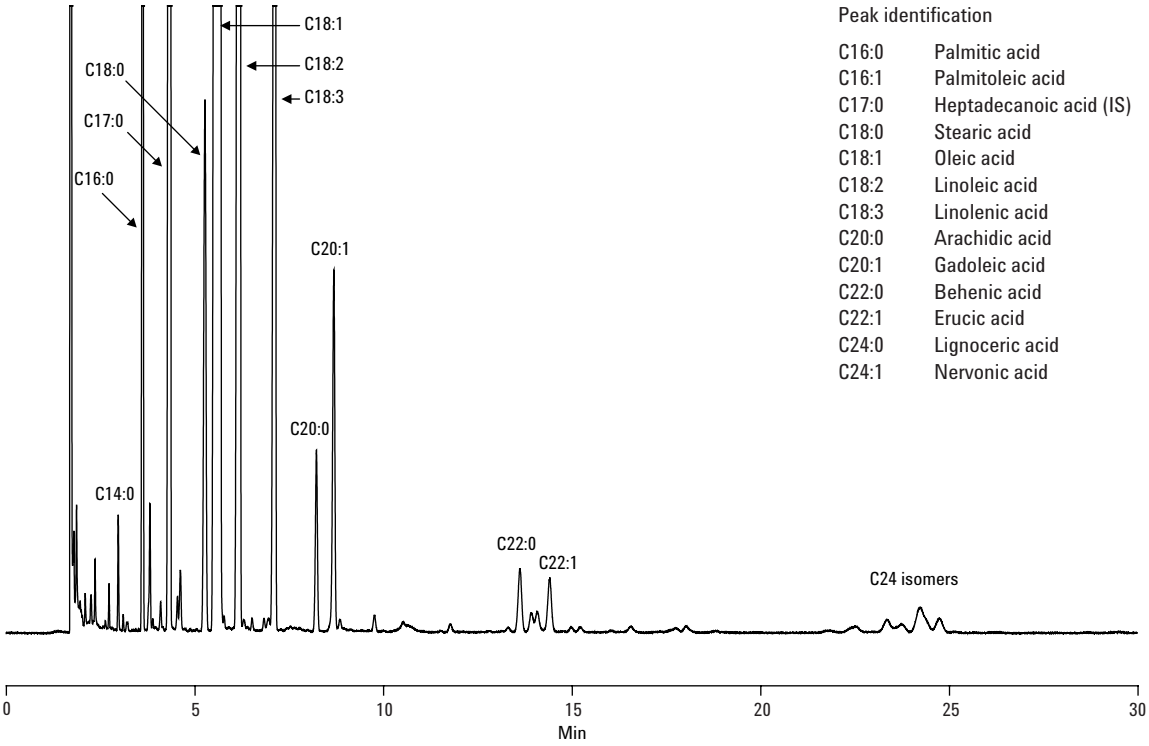
where:

ΣA = total peak area from the FAME $C_{14:0}$ to $C_{24:1}$

AEI = peak area of methylheptadecanoate

AL = peak area of linolenic acid methyl ester

Equation 2.



Peak identification

C16:0	Palmitic acid
C16:1	Palmitoleic acid
C17:0	Heptadecanoic acid (IS)
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:0	Arachidic acid
C20:1	Gadoleic acid
C22:0	Behenic acid
C22:1	Erucic acid
C24:0	Lignoceric acid
C24:1	Nervonic acid

Figure 1. Separation of biodiesel by gas chromatography using an Agilent Select Biodiesel for FAME column.

The results of the biodiesel assay are shown in Table 1.

Table 1. Analysis Results of Biodiesel

	Area (µV.min)	Quantity(mass %)
FAME content	103139	96.6
Linolenic acid	7599.2	7.1

The biodiesel sample was in accordance with the requirements stated in EN-14214, that is. FAME content > 96.5 % (m/m) and linolenic acid content < 12 % (m/m). To verify the integrity of the system, repeatability was determined. One sample was analyzed 15 times (Table 2 and Figure 2).

Table 2. Repeatability Results

Parameter	FAME (mass %)	Linolenic acid (mass %)
Average	96.4	7.1
Standard deviation	0.20	0.015
RSD (%)	0.21	0.21

A relative standard deviation of 0.21 % was achieved. Figure 2 shows the mass % results of the subsequent injections and the absolute difference obtained, compared to the specification limits.

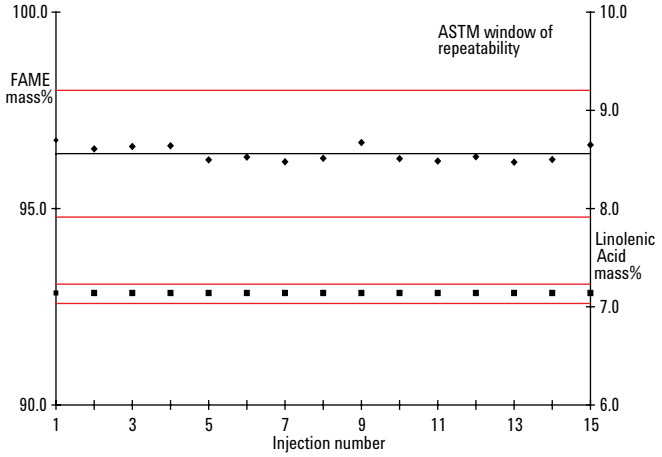


Figure 2. Repeatability data obtained during the analysis of biodiesel using an Agilent Select Biodiesel for FAME column. Red lines indicate the maximum and minimum variation limits specified in the method.

In the method, the absolute difference between two test results must be FAME > 1.6% (m/m) and linolenic acid > 0.1% (m/m). All results obtained are within the limits specified in the method.

Conclusion

The data clearly show the applicability of the Agilent GC system with Select Biodiesel for FAME column for the analysis of biodiesel according to the EN-14103 standard method, with good repeatability. The biodiesel sample fulfilled the requirements stated in EN-14214.

References

1. EN-14103. Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – determination of ester and linolenic acid methyl ester contents.
2. EN-14214:2003. Automotive fuels – Fatty Acid Methyl Esters (FAME) for diesel engines – requirements and test methods.

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Agilent Technologies

Determination of Methanol Content in Biodiesel Using Agilent Select Biodiesel for Methanol with Headspace Sampling to EN-14110

Application Note

Energy and Fuels

Author

Coen Duvekot

Agilent Technologies, Inc.

Introduction

The popularity and interest in biodiesel is growing significantly in many areas of the world and has become a commonly sought after alternative fuel source for use with diesel engines. Biodiesel is produced from vegetable oils or animal fats through transesterification using methanol to yield fatty acid methyl esters (FAMEs) and glycerine. The yield, pure FAME (once the glycerine and the residual methanol have been recovered/removed), is called B-100.

For biodiesel to be used as a motor fuel or blended with petroleum diesel, it must conform to standard specifications (ASTM D 6751 or EN-14214). GC methods determine whether biodiesel conforms to the standard specifications. One of these methods, EN-14110, is used to determine the methanol content. EN-14110 is applicable for a concentration range from 0.01 to 0.5% (m/m) methanol[a].

a The method is not applicable to mixtures of FAME that contain other low boiling components.

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Materials and Methods

Calibration solutions

Solution A	0.5 % (m/m) methanol in FAME
Solution B	0.1 % (m/m) methanol in FAME
Solution C	0.01 % (m/m) methanol in FAME

A 1 mL aliquot was accurately weighed, transferred into a 20 mL vial and then immediately capped.

Conditions

Sample	FAME mix with methanol content <0.001%
Column	Agilent Select Biodiesel for Methanol, 0.32 mm × 30 m, 3.0 µm (p/n CP9083)
Instrument	Agilent GC
Software	Agilent Chromatography Data Station
Headspace sampler	QHSS-40, sample loop mode (QUMA Elektronik & Analytik GmbH)
Sample loop	1 mL
Vial/heating	80 °C
Equilibrium time	45 min
Injection volume	1 µL
Injector	Split/splitless 1177, full EFC control, 250 °C, split 100 mL/min
Split rate	50:1
Detector	275 °C, FID
Oven	80 °C (0.5 min isothermal) at 20 °C/min to 160 °C (2 min)
Carrier gas	2.0 mL/min constant flow, helium

Results and Discussion

All three calibration solutions were analyzed twice and a calibration curve was obtained. Figure 1 is an overlay of the methanol peaks of the different calibration solutions. The calibration curve (Figure 2) shows excellent correlation with the method.

The correlation coefficient should be >0.95. In this case, the correlation coefficient was determined as 0.9998. A typical chromatogram of a biodiesel sample is shown in Figure 3.

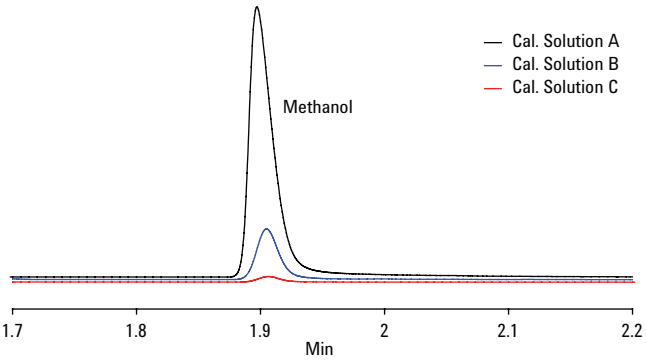

Figure 1. Overlay traces of calibration solutions obtained by gas chromatography using an Agilent Select Biodiesel for Methanol column.

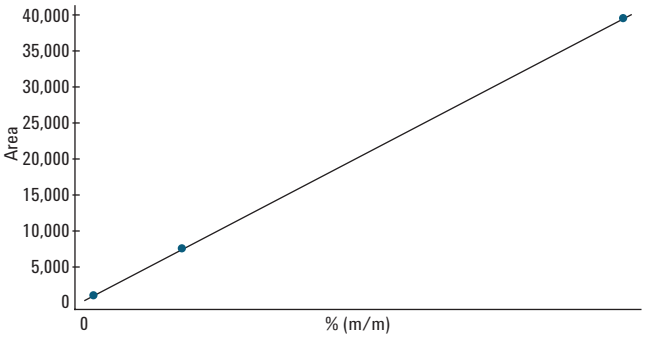

Figure 2 Calibration curve produced by an Agilent Select Biodiesel for Methanol column.

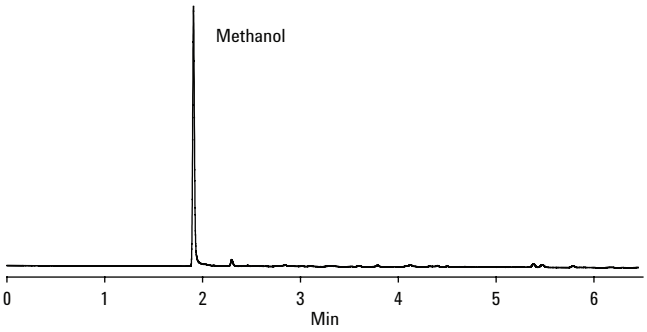

Figure 3. Typical headspace chromatogram for biodiesel produced by an Agilent Select Biodiesel for Methanol column.

Since biodiesel does not generally contain volatile components, other than methanol, identification and quantification is quite straightforward. Repeatability data are shown in Table 1 and Figure 4.

The methanol content of the biodiesel was 0.038% (m/m), thus meeting the specifications set in EN-14214, (methanol content <0.2 %). Furthermore, the repeatability figures indicated that the system was properly optimized for the analysis, as seen in Figure 4, where the analyses trend line is well within the repeatability window according to the EN-14110 method. In Figure 4, this is visualized by adding the average line and the window of repeatability set by the EN-14110 method.

Table 1. Repeatability Results

Parameter	Methanol (mass %)
N	15
Average	0.038
Standard deviation	0.0007
RSD (%)	1.96

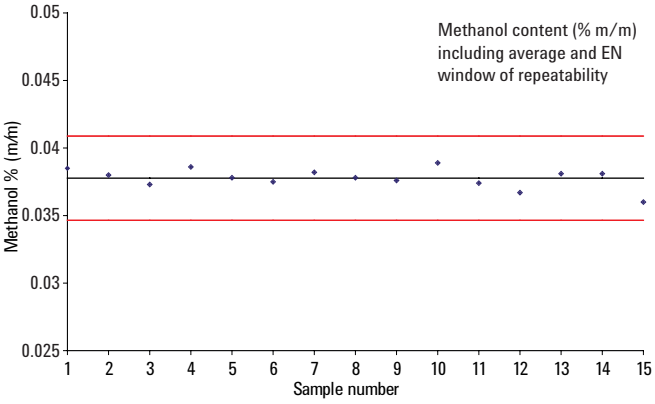

Figure 4. Repeatability values are within the specification boundaries established in EN-14214, as indicated by the red lines in the chart.

Conclusion

The GC headspace system (Agilent GC, QUMA Headspace Sampler and Agilent Select Biodiesel for Methanol column) was well suited for the determination of methanol content in biodiesel according to EN-14110, and the biodiesel tested met the specifications on methanol content according to EN-14214.

References

1. EN-14110 Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – determination of methanol content.
2. EN-14214 Automotive fuels – Fatty Acids Methyl Esters (FAME) for diesel engines – requirements and test methods

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Determination of Methanol Content in Biodiesel Using Agilent Select Biodiesel for Methanol

Application Note

Energy and Fuels

Author

John Oostdijk

Agilent Technologies, Inc.

Introduction

The purpose of the European Standard EN-14110 is to determine the methanol content of fatty acid methyl esters (FAMEs) intended for use as pure biodiesel, or as a blending component for domestic heating fuels and diesel fuels. The method is applicable for a concentration range from 0.01 % (m/m) to 0.5 % (m/m) methanol. Requirements stated in EN-14214:2003 are <0.2 % (m/m) methanol (MeOH).

The EN-14110 method is not applicable to mixtures of FAME that contain other low boiling components. However, by using the Agilent Select Biodiesel for Methanol column, a good separation from most of these minor compounds can be achieved, resulting in a reliable analysis. In this example, the determination was conducted using the internal standard method, which was appropriate for manual headspace analysis.

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Materials and Methods

Conditions

Sample	Reference FAME with methanol content less than 0.001 % (m/m)
Column	Select Biodiesel for Methanol 0.32 mm × 30 m, 3 µm (p/n CP9083)
Injector	1177, split/splitless
Injector temperature	275 °C, split 150 mL/min, cup liner
Oven	40 °C, isothermal
Carrier gas	Helium, 80 kPa (11.6 psi)
Detector	FID, 300 °C

Reference FAME

A reference sample of FAME with a low methanol content was prepared by extracting with water (taking 30 mL biodiesel and extracted four times with 10 mL water). Next, the extracted biodiesel layer was dried with $MgSO_4$ for 15 minutes. After filtration, the clear biodiesel layer was collected and analyzed. It was found to contain less than 0.001 % (m/m) methanol and no isopropanol[a].

Calibration curve

Using reference FAME, three calibration solutions (A, B, and C) were prepared, yielding concentrations of 0.462, 0.0919, and 0.0092 % (m/m) methanol, respectively.

Samples

Different biodiesel samples were obtained from several sources, including in-house prepared biodiesel. This sample was made from rapeseed oil, but not at optimized conditions, to ensure against a biodiesel sample of suspect quality.

Headspace conditions

From homogenized samples and standards, a 1 mL aliquot was accurately weighed into a 20 mL vial and 5 µL isopropanol (internal standard, IS) was added to each. Each vial was tightly capped to prevent leaking. Next, each vial was shaken and heated at 80 °C for 45 minutes and 100–200 µL headspace was injected into the GC using a syringe preheated to 60 °C.

Results and Discussion

Figure 1 is the chromatogram obtained from the separation of a biodiesel sample. The calibration curve was then recorded and calibration factors were calculated. See Table 1, for detailed results of the calibration, and Figure 2, for the recorded calibration curve. The average response factor was 1.18, with a coefficient of variation of 13.5%. The coefficient of variation was <15%, thus meeting the specifications.

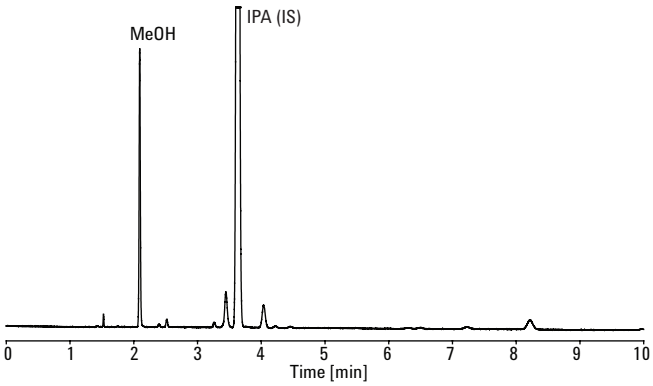

Figure 1. Chromatogram of a summer biodiesel (sample code 1) including some unknown volatiles.

Table 1. Calibration Data with Three Standards Using the Internal Standard Method

Code	Mass % MeOH	Mass % IPA	Mass % MeoH/IPA	µL gas sample	Mrea MeOH	Area IPA	Area MeOH/IPA	RF (F)
Reference FAME	0	0.393	0	200	4815	509865	0.0094	
CAL A	0.462	0.393	1.18	200	1261017	974438	1.2941	1.10
CAL A	0.462	0.393	1.18	100	765768	581293	1.3174	1.12
CAL B	0.0919	0.393	0.23	200	323222	1319992	0.2449	1.05
CAL C	0.00918	0.393	0.023	200	43372	1279566	0.0339	1.45
Average								**1.18**

a Reference FAME can also be obtained from commercial sources.

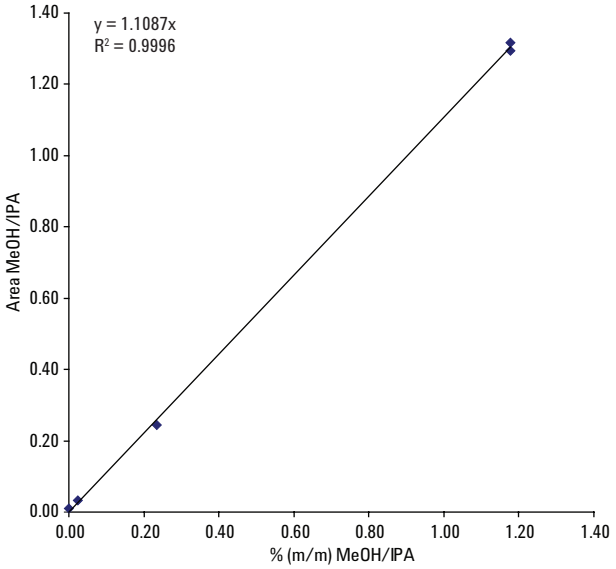

Figure 2. Calibration curve as obtained with the manual headspace method with isopropanol as internal standard.

As some trace level MeOH was present in the reference FAME, the response factor of the lowest calibration standard (C) was slightly higher. This, however, did not have a significant effect on the average response factor. Although use of a regression line was not described in the method, one was calculated and is shown in Figure 2. This resulted in a comparable (and probably better) value.

During this procedure, blank headspace samples were prepared by filling the vials with nitrogen and analyzed as before. These blank samples showed no carryover of MeOH and IPA, as well as no interfering peaks from vials, septa, or from the syringe. Seven different biodiesel (B-100) samples were analyzed (Table 2).

Table 2. Results of the Methanol Headspace Analysis of Different Biodiesel Samples

Code	Description*	Sample mass (g)	µL gas sample	Area MeOH	Area IPA	Area MeOH/IPA	Mass % MeOH
1	Summer biodiesel (2004)	0.910	200	73982	868326	0.0852	0.04
2	Winter biodiesel (3S)	0.884	200	138444	1083819	0.1277	0.06
3	Winter biodiesel (2S)	0.886	200	108693	1252320	0.0868	0.04
4	Winter biodiesel (1N)	0.878	200	146444	1232739	0.1188	0.06
5	FAME mix TOFA	0.890	200	155232	1052582	0.1475	0.07
6	Biodiesel ASTM round robin	0.880	200	206244	1096931	0.1880	0.09
7	In-house biodiesel	0.893	100	76234	107138	0.7115	0.32

**Descriptions of the biodiesel (B-100) samples are based on the original lab code, time period (Germany, summer or winter biodiesel as sold on the pump) or origin of sample; TOFA: Tall Oil fatty acid methyl ester (mainly C18:1 and C18:2 FAMEs).*

The sample weights actually used in the method were slightly modified due to the availability of limited sample. This did not change the principle of analysis and a good result was still achieved, even with a manual headspace method. Optimal sensitivity was obtained by varying the headspace temperature, sampling time, inlet split flow and sample amount injected. All samples analyzed were within standard specifications as stated in EN-14214 (max. 0.2 % (m/m) methanol), except the in-house prepared biodiesel of low quality, which was as expected.

Conclusion

These results clearly demonstrate that the Agilent Select Biodiesel for Methanol column achieved a better resolution than specified in the method (Rs > 1.5). The column provided good separation of other low boiling compounds that may be present and did not interfere with the methanol peak or isopropanol internal standard peak.

References

1. EN-14110 (June 2003) Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – determination of methanol content.
2. EN-14214:2003. Automotive fuels – Fatty Acid Methyl Esters (FAME) for diesel engines – requirements and test methods.

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Analysis of Free and Total Glycerol in B-100 Biodiesel Methyl Esters Using Agilent Select Biodiesel for Glycerides

Application Note

Energy and Fuels

Author

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Introduction

The American Standard, ASTM D 6584 [1], is the standard test method used to determine the free and total glycerol contents in fatty acid methyl esters (FAMEs), typically intended for pure biodiesel or as a blending component for domestic and diesel fuels. Total glycerol content is calculated from the results. The method is suitable for FAME from rapeseed, sunflower, and soybean oils. It is not suitable for FAME produced from or containing lauric oils, such as coconut and palm kernel oils, due to the problem of peak overlap.

The high performance Agilent Select Biodiesel for Glycerides metal capillary (UltiMetal) GC column was specifically developed for high temperature methods. This column will not break during extreme oven conditions and is produced with a pre-installed retention gap, which provides the performance and robustness required to run this application for an extended period of time.

Biodiesel is produced by transesterifying the parent oil or fat with an alcohol, usually methanol, in the presence of a catalyst, usually a strong base such as sodium or potassium hydroxide, or preferably and increasingly more commonly, alkoxides. The resulting product can contain not only the desired alkyl ester product but also unreacted starting material (TAG, triacylglycerides), residual alcohol, and residual catalysts. Glycerol is formed as a by-product and is separated from biodiesel in the production process. However, traces of glycerol can be found in the final biodiesel product. Since transesterification is a stepwise process, MAG (monoacylglycerides) and DAG (diacylglycerides) formed as intermediates can also be found in biodiesel [2].

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Experimental

Calculation of free and total glycerol

First, the free glycerol (G) and residual mono- (M), di- (D), and triglyceride (T) contents in FAME is determined. The total and bound glycerol content is calculated from the results using the following equation.

Total glycerol (GT) = free glycerol (G) + bound glycerol (BG)

Bound glycerol is calculated using the following equation.

Bound glycerol = 0.2591M + 0.1488D + 0.1044T

The detection range for free glycerol is 0.005 to 0.05 mass %. The detection range for total glycerol is 0.05 to 0.5 mass %. In the standard test method ASTM D 6751 [3], requirements for glycerol used as a blend component with diesel fuel oils are < 0.02 mass % for glycerol and < 0.240 mass % total glycerol.

ASTM 6751 and EN-14105 are two of the most commonly used standardized analytical methods for the analysis of biodiesel.

Materials and methods

Reagents

1,2,4-butanetriol, Internal Standard Solution 1, 1 mg/mL pyridine (IS1)

1,2,3-tricaproylglycerol (tricaprin), Internal Standard Solution 2, 8 mg/mL pyridine (IS2)

Reference materials: glycerol, 1-monooleoylglycerol (monoolein), 1,3-dioleolglycerol (diolein), 1,2,3-trioleoylglycerol (triolein) (GLC standard grade)

Monoglyceride mix (monopalmitin, monostearin, and monoolein), 10 mg/mL pyridine

Conditions

Column	Select Biodiesel for Glycerides UltiMetal 0.32 mm x 10 m, 0.1 µm, with retention gap (p/n CP9076)
Injection	Cold on-column (1093), full EFC control, 1 µL, reversed liner
Temperature	100 °C (1 min) to 370 °C at 15 °C/min
Oven	50 °C (1 min) to 180 °C at 15 °C/min to 230 °C at 7 °C/min to 380 °C (10 min) at 30 °C/min
Carrier gas	Helium, constant flow rate 3 mL/min
Detector	FID, full EFC control, 380 °C

Sample preparation

Different biodiesel samples were obtained from several sources, including in-house prepared biodiesel. This sample was made from rapeseed oil but not at optimized conditions to ensure against a biodiesel sample of suspect quality.

Standard mixtures and internal standard solutions were prepared according to the method. Approximately 100 µL of homogenized biodiesel sample was accurately weighed (± 0.1 mg) in a 20-mL vial, then 100 µL of Internal Standard 1, 100 µL of Internal Standard 2, and 100 µL of MSTFA were added to the sample vials. Care was exercised to ensure there was no contact with any moisture. The vials were hermetically sealed and shaken vigorously. After storing the vials at room temperature for 15 – 20 minutes, approximately 8 mL of heptane was added to each, then 1 µL of the reaction mixture was automatically injected into the gas chromatograph.

Results and Discussion

The method describes the transformation of the glycerol, mono-, and diglycerides into more volatile silylated derivatives in the presence of pyridine and N-methyl-N-trimethylsilyltrifl uoroacetamide (MSTFA). Figure 1 is a chromatogram of a typical B-100 biodiesel sample.

Calibration curves were obtained for glycerol, monoolein, diolein, and triolein. These calibration curves indicate the performance of the system. A typical calibration curve for triolein is shown in Figure 2. Regression coefficients are listed in Table 1.

Based on the calibration curves obtained for glycerol,

Table 1. Regression Coefficients as Calculated by Chromatography Data Station (r^2 must be ≥ 0.99)

Curve y = a x + b	a	b	r^2
Glycerol	1.15585	0.01729	0.9996
Monoolein	1.38085	–0.01468	0.9994
Diolein	1.13841	–0.00772	0.9994
Triolein	0.86610	–0.00720	0.9998

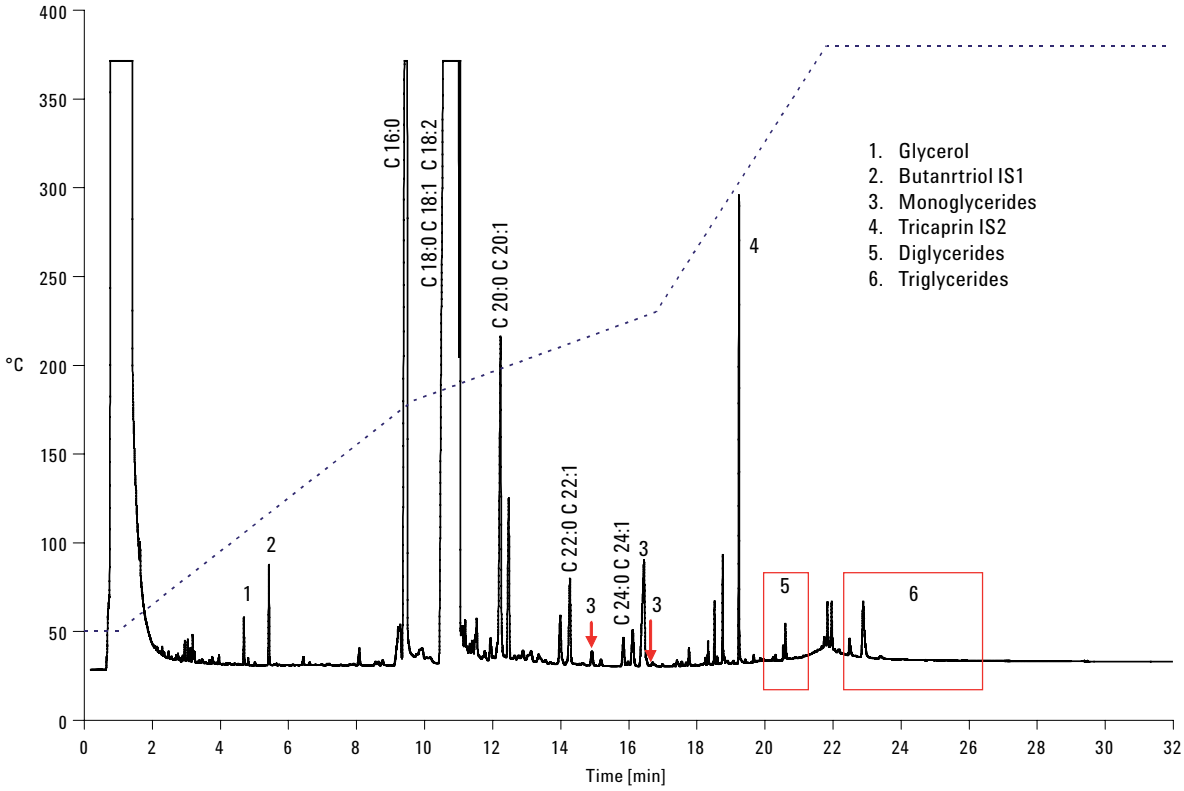

Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which consists mainly of C18 and C16 FAMEs and minor compounds, such as sterols.

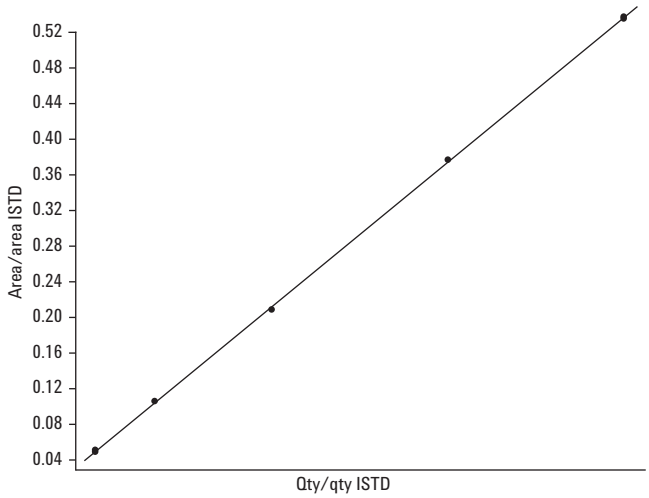

Figure 2. Calibration curve for triolein.

monolein, diolein, and triolein, biodiesel samples were analyzed and quantified: seven regular samples and one spiked sample (code 2, 3S) with extra glycerol (± 0.030 mg) and triglycerides (± 0.48 mg rapeseed oil) per 100 µL. Tables 2 and 3 show typical results for the actual biodiesel samples. Figure 3 depicts the repeatability of the analysis of the spiked sample. The column performance was constant during the analysis of standards and samples (plate number, peak symmetry, and peak width). The variation (expressed as standard deviation) in retention time was typically 0.022 minutes.

A challenging aspect of the method is to accurately integrate the correct peaks using optimized integration parameters. In this example, peak identification was based on a comparison with known standard components. Monoglycerides were integrated from 14.8–15.1 and 16.25–16.8 min, diglycerides from 20.1–20.9 min, and triglycerides from 22.3–26.5 min. The Chromatography Data Station calculates all the peak areas in a specified retention time window and then compares these areas to a calibration curve generated for a single component. See Figures 4, 5 and 6 for details.

Table 2. Typical Analysis Results of a Biodiesel Sample (Winter Biodiesel, Code 1N, 87.9 mg, Duplicate Analysis, Values not Rounded)

Name	Area 1 (µV.min)	Area 2 (µV.min)	Qty average % (m/m)	St. dev.	RSD %
Glycerol	401.6	444.4	0.0109	0.0003	3.08
Butanetriol (IS1 0.08 mg)	10802.7	11319.7	–	–	–
Monoglycerides	2575.4	2482.0	0.6192	0.0046	0.75
Tricaprin (IS2 0.8 mg)	470.3	410.2	–	–	–
Diglycerides	3257.7	3472.2	0.1753	0.0087	4.97
Triglycerides	11743.8	12173.9	0.0463	0.0047	10.2
Bound glycerol	–	–	0.1914	0.0006	0.31
Total glycerides	–	–	0.2023	0.0003	0.12

Table 3. Results of the Free and Total Glycerol Analysis of Biodiesel Samples from Different Origin (Average Mass %, RSD, n = 2)

Code	Description*	Sample mass (mg)	Glycerol	Monoglycerides	Diglycerides	Triglycerides	Bound glycerol	Total glycerides	Meet spec. D6751?
1	Summer biodiesel (2004)	88.8	0.0011^{v} (27%)	0.474 (2.9%)	0.315 (10%)	0.0179 (6.0%)	0.172 (0.7%)	0.173 (0.6%)	Passed
2	Winter biodiesel (3S)	88.6	0.0076 (2.4%)	0.640 (2.1%)	0.139 (2.7%)	0.0657 (7.4%)	0.193 (1.7%)	0.201 (1.7%)	Passed
3	Winter biodiesel (2S)	88.1	0.0014^{v} (31%)	0.644 (3.4%)	0.156 (1.3%)	0.0641 (6.4%)	0.197 (2.5%)	0.198 (2.7%)	Passed
4	Winter biodiesel (1N)	87.9	0.011 (3.1%)	0.619 (0.8%)	0.175 (5.0%)	0.046 (10.2%)	0.191 (0.3%)	0.202 (0.1%)	Passed
5	FAME mix TOFA	89.2	0.0007^{v} (94%)	0.013 (18.3%)	0.049 (4.3%)	0.0075 (0.0%)	0.012 (8.2%)	0.012^{v} (13.1%)	Passed
6	Biodiesel ASTM round robin	87.9	0.0005^{v} (76%)	0.490 (2.3%)	0.111 (2.5%)	0.239 (15.5%)	0.168 (0.8%)	0.169 (0.6%)	Passed
7	In-house prepared biodiesel	89.1	0.0037^{v} (0.6%)	1.743 (1.6%)	8.90 (0.2%)	25.1 (0.6%)	4.39 (1.6%)	4.40^ (1.6%)	Failed

*Descriptions of the biodiesel (B-100) samples are based on the original lab code, time period (Germany, summer or winter biodiesel as sold on the pump) or origin of sample; TOFA: Tall Oil fatty acid methyl ester (mainly C18:1 and C18:2 FAMEs). Sample 2 was analysed with n = 4 (see also Table 2). v = below detection range; ^ = above detection range.

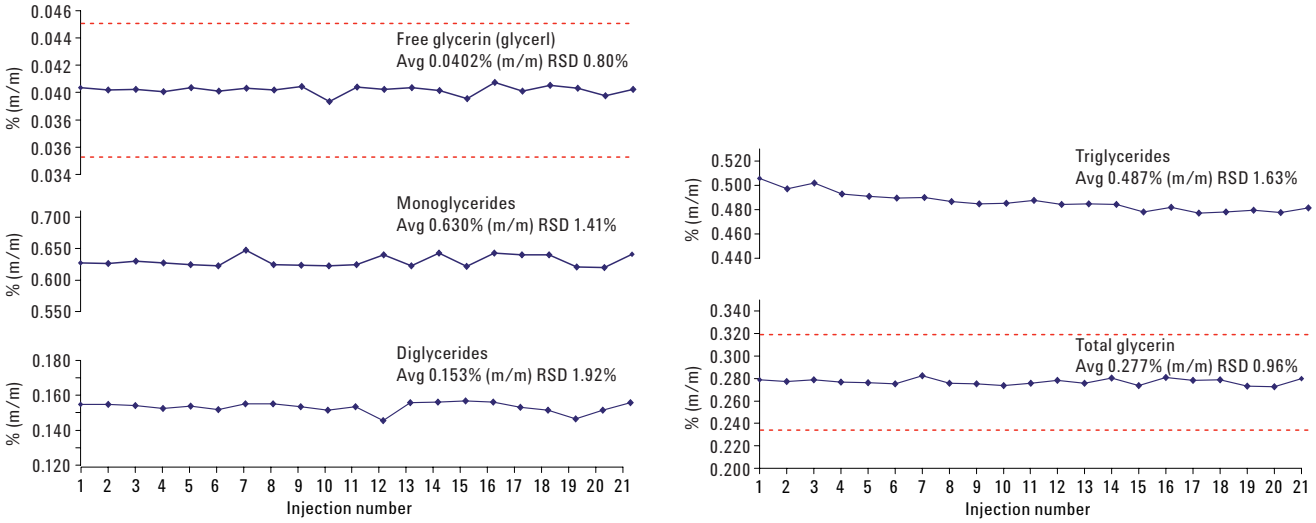

Figure 3. Typical repeatability of 21 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the ASTM D 6584 – 07.

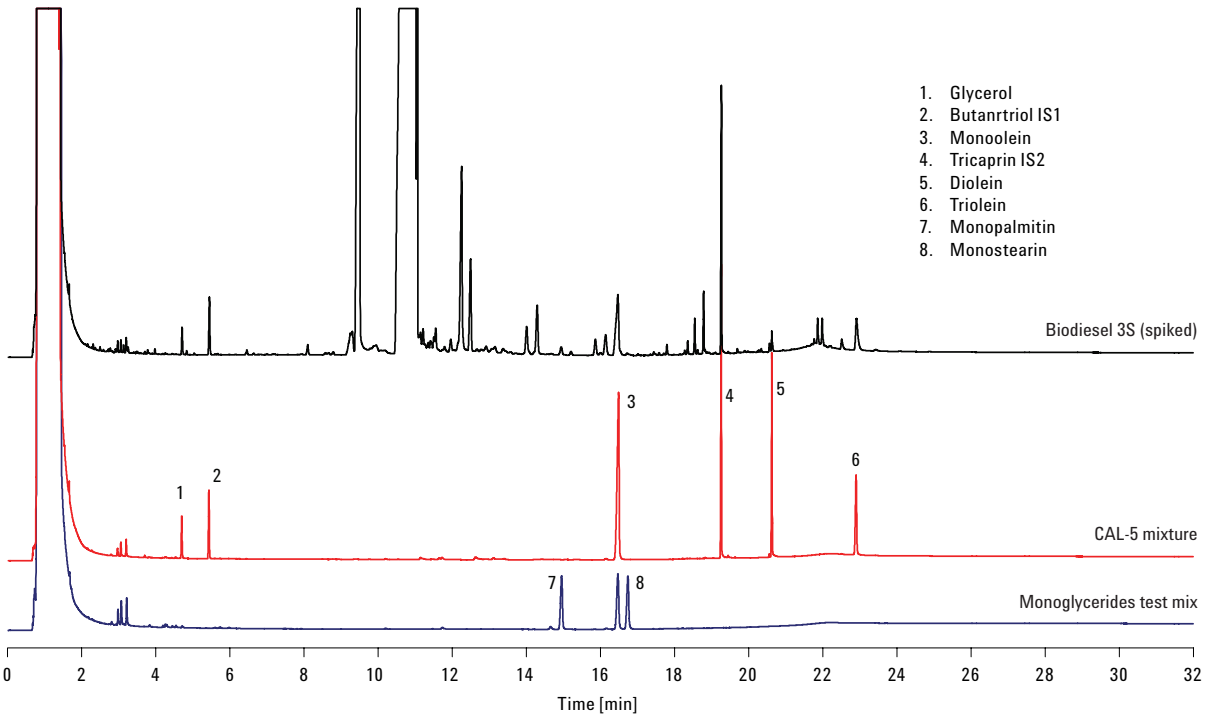

Figure 4. Details of identification of unknown peaks in a biodiesel sample.

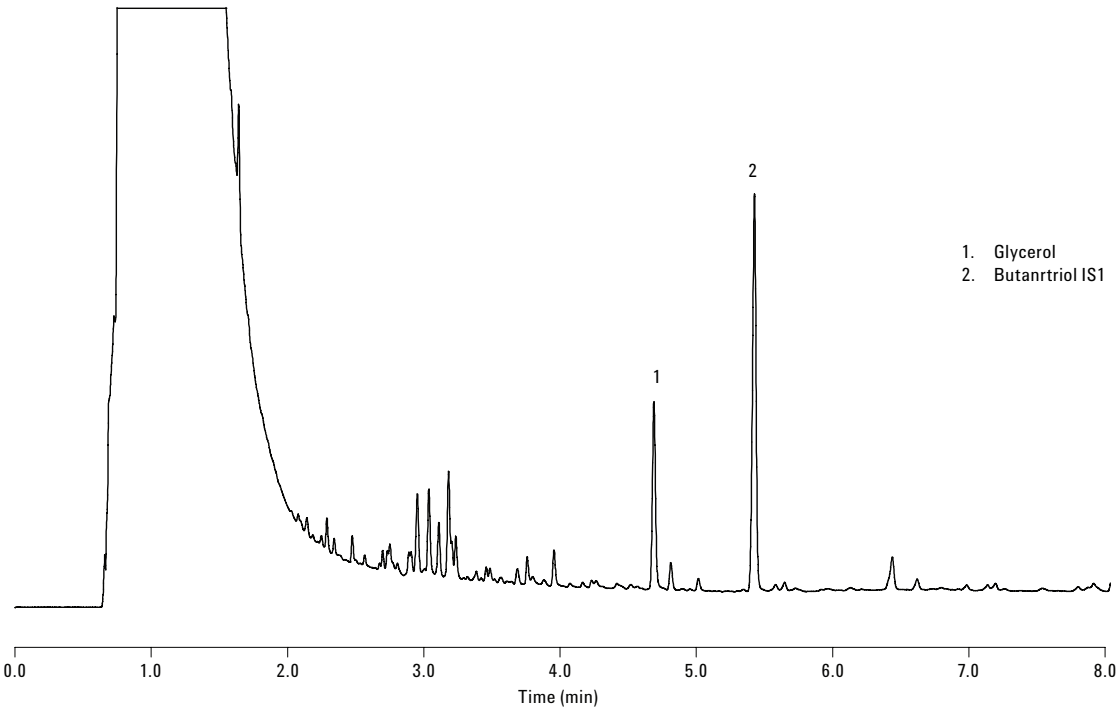

Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

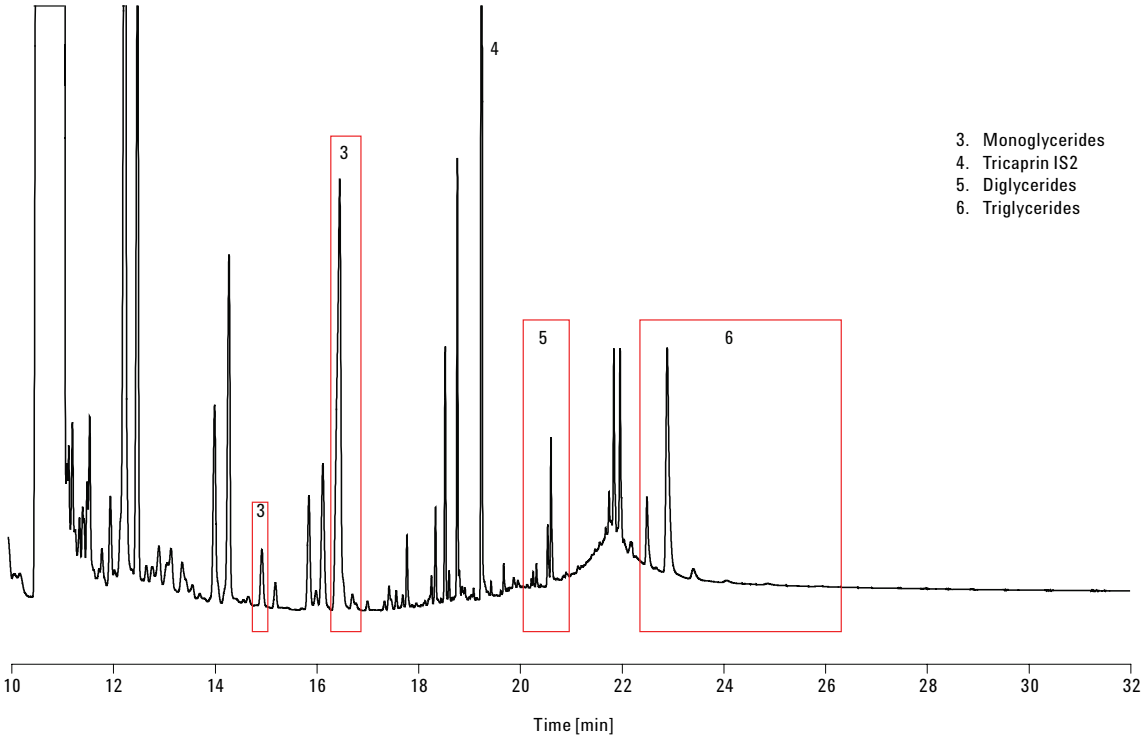

Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

It is evident in Figure 6 that there is excellent separation of the triglycerides and very low column bleed at 380 °C. Due to the oven ramp step of 30 °C/min, a broad background peak eluted from the biodiesel matrix at 22 minutes. This had minor effects on the analysis of the di- and triglycerides and is also shown in the ASTM method.

Conclusion

This application note demonstrates the suitability of an on-column injector and the Agilent Select Biodiesel for Glycerides UltiMetal column for the analysis of biodiesel by gas chromatography. The calibration curves and repeatability data demonstrate excellent system integrity, which makes the system ideally suited for the analysis of free, bound, and total glycerol, as well as mono-, di-, and triglyceride content in biodiesel in accordance with ASTM D 6584 [1].

All samples analyzed were within standard specifications as stated in ASTM D 6751 [2] with respect to the maximum level of free glycerol and total glycerol, except for the in-house prepared biodiesel of low quality, which was as expected. However, because of the noted low levels of both glycerol and triglycerides, one sample (3S) was spiked with glycerol and triglycerides so an assessment could be made on the column's separation performance for those components.

The Select Biodiesel for Glycerides column achieves good resolution of biodiesel samples and is a robust solution for this high temperature application. By using an already coupled and tested column from Agilent, problems associated with making a coupling are avoided. In addition, UltiMetal technology removes risks of degradation of the outer coating, and breakage, which makes this column a very robust solution with a long lifetime.

References

1. ASTM D 6584–07. Test Method of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography.
2. Knothe, G. (2006), Analyzing Biodiesel: Standards and other Methods. JAOCS 83, 823–833.
3. ASTM D 6751–06a. Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.

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Analysis of Free and Total Glycerol and Triglyceride Content in B-100 Biodiesel Methyl Esters Using Agilent Select Biodiesel for Glycerides

Application Note

Energy and Fuels

Author

John Oostdijk

Agilent Technologies, Inc.

Introduction

The European Standard, EN-14105 [1], is the standard test method used to determine the free glycerol and residual mono-, di-, and triglyceride contents in fatty acid methyl esters (FAMEs), typically intended for pure biodiesel or as a blending component for domestic and diesel fuels. Total glycerol content is calculated from the results. The method is suitable for FAME from rapeseed, sunflower, and soybean oils. It is not suitable for FAME produced from or containing lauric oils, such as coconut and palm kernel oils, due to the problem of peak overlap.

The high performance Agilent Select Biodiesel for Glycerides metal capillary (UltiMetal) GC column was specifically developed for high temperature methods. This column will not break during extreme oven conditions and is produced with a preinstalled retention gap, which provides the performance and robustness required to run this application for an extended period of time.

Biodiesel is produced by transesterifying the parent oil or fat with an alcohol, usually methanol, in the presence of a catalyst, usually a strong base such as sodium or potassium hydroxide, or preferably and increasingly more commonly, alkoxides. The resulting product can contain not only the desired alkyl ester product but also unreacted starting material (TAG, triacylglycerides), residual alcohol, and residual catalysts. Glycerol is formed as a by-product and is separated from biodiesel in the production process. However, traces of glycerol can be found in the final biodiesel product. Since transesterification is a stepwise process, MAG (monoacylglycerides) and DAG (diacylglycerides) formed as intermediates can also be found in biodiesel [2].

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Experimental

Calculation of free and total glycerol

The method is applicable for a concentration range from 0.005 – 0.05% (m / m) for glycerol (G), 0.25 – 1.25% (m/m) for monoglycerides (M), 0.05 – 0.5% (m/m) for diglycerides (D), and 0.05 – 0.4% (m/m) for triglycerides (T). The total glycerol (GT) content is calculated using the following equation.

$$GT = G + 0.255M + 0.146D + 0.103T$$

In the European standard method EN-14214:2003 [3], requirements for glycerol are 0.02, 0.8, 0.2, 0.2, and 0.25% (m/m). This method and ASTM D 6584 are two of the most commonly used standardized analytical methods for the analysis of biodiesel.

Materials and methods

Reagents

1,2,4-butanetriol, Internal Standard Solution 1, 1 mg/mL pyridine (IS1)

1,2,3-tricaproylglycerol (tricaprin), Internal Standard Solution 2, 8 mg/mL pyridine (IS2)

Reference materials: glycerol, 1-monooleoylglycerol (monoolein), 1,3-dioleolglycerol (diolein), 1,2,3-trioleoylglycerol (triolein) (GLC standard grade)

Monoglyceride mix (monopalmitin, monostearin, and monoolein), 10 mg/mL pyridine

Conditions

Column	Select Biodiesel for Glycerides UltiMetal 0.32 mm × 10 m, 0.1 µm, with retention gap (p/n CP9076)
Injection	Cold on-column (1093), full EFC control, 1 µL, reversed liner
Temperature	100 °C (1 min) to 370 °C at 15 °C/min
Oven	50 °C (1 min) to 180 °C at 15 °C/min to 230 °C at 7 °C/min to 370 °C (5 min) at 10 °C/min
Carrier gas	Helium, constant flow rate 4 mL/min
Detector	FID, full EFC control, 380 °C

Sample preparation

Standard mixtures and internal standard solutions were prepared according to the method. Approximately 100 µL of homogenized biodiesel sample was accurately weighed (± 0.1 mg) in a 20-mL vial, then 100 µL of Internal Standard 1, 100 µL of Internal Standard 2, and 100 µL of MSTFA were added to the sample vials. Care was exercised to ensure there was no contact with any moisture. The vials were hermetically sealed and shaken vigorously. After storing the vials at room temperature for 15–20 minutes, approximately 8 mL of heptane was added to each, then 1 µL of the reaction mixture was automatically injected into the gas chromatograph.

Results and Discussion

The method describes the transformation of the glycerol, mono-, and diglycerides into more volatile silylated derivatives in the presence of pyridine and N-methyl-N-trimethylsilyltrifl uoroacetamide (MSTFA). Figure 1 is a chromatogram of a typical B-100 biodiesel sample.

Calibration curves were obtained for glycerol, monoolein, diolein, and triolein. These calibration curves indicate the performance of the system. A typical calibration curve for diolein is shown in Figure 2. Regression coefficients are listed in Table 1.

Table 1. Regression Coefficients as Calculated by Chromatography Data Station (r^2 must be ≥ 0.95)

Curve y = a x + b	a	b	r^2
Glycerol	1.08243	0.00813	0.9994
Monoolein	1.35878	0.00105	1.0000
Diolein	1.13201	–0.00727	0.9999
Triolein	0.96579	–0.01490	0.9992

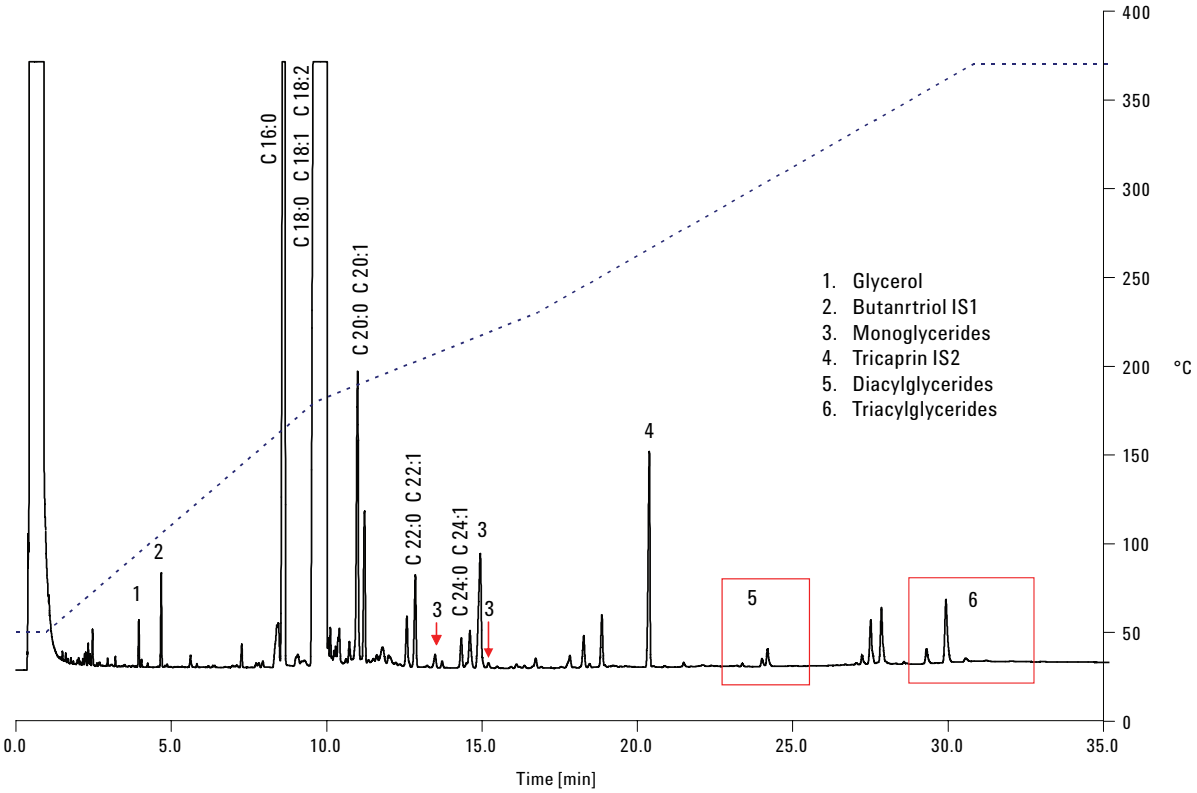

Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which consists mainly of C18 and C16 FAMEs and minor compounds, such as sterols.

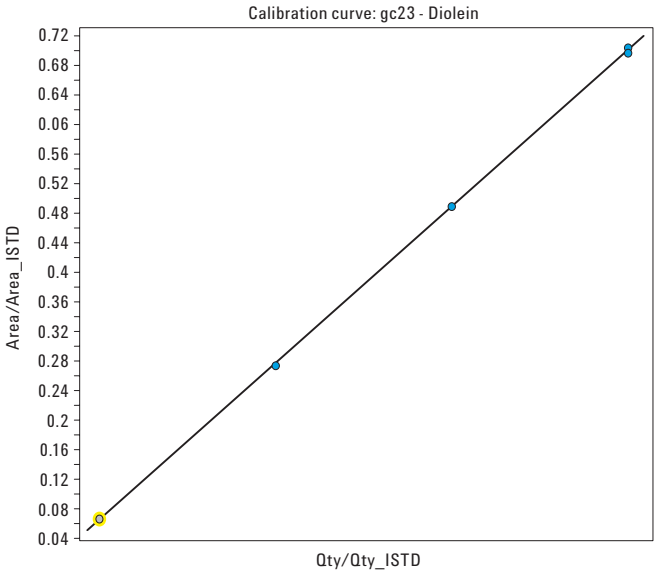

Figure 2. Calibration curve for diolein.

Based on the calibration curves obtained for glycerol, monoolein, diolein, and triolein, two biodiesel samples were analyzed and quantified: a sample with code 3S and the same sample spiked with extra glycerol (± 0.028 mg) and triglycerides (± 0.48 mg rapeseed oil) per 100 µL. Table 2 shows typical results for the actual biodiesel sample. Figure 3 depicts the repeatability of the analysis of the spiked sample. The column performance was constant during the analysis of standards and samples (plate number, peak symmetry and peak width). The variation (expressed as standard deviation) in retention time was typically 0.005 minutes.

A challenging aspect of the method is to accurately integrate the correct peaks using optimized integration parameters. In this example, peak identification was based on a comparison with known standard components. Monoglycerides were integrated from 13.15–13.60 and 14.50–15.40 minutes, diglycerides from 22.90–25.50 minutes, and triglycerides from 29–34 minutes. The Chromatography Data Station calculates all the peak areas in a specified retention time window and then compares these areas to a calibration curve generated for a single component. See Figures 4, 5, and 6 for details.

Table 2. Typical Analysis Results of a Biodiesel Sample (Winter Biodiesel, Code 1N, 89.5 mg, Duplicate Analysis, Values not Rounded)

Name	Area 1 (µV.min)	Area 2 (µV.min)	Qty average % (m/m)	St. dev.	RSD %
Glycerol	311.5	307.3	0.0072	0.00010	1.46
Butanetriol (IS1 0.08 mg)	3255.5	3272.7	–	–	–
Monoglycerides	17514.8	17656.2	0.7844	0.00003	0.003
Tricaprin (IS2 0.8 mg)	14675.8	14793.6	–	–	–
Diglycerides	2553.6	2573.2	0.1431	0.00003	0.02
Triglycerides	942.8	976.8	0.0741	0.0012	1.58
Total glycerol	–	–	0.2357	0.00002	0.01

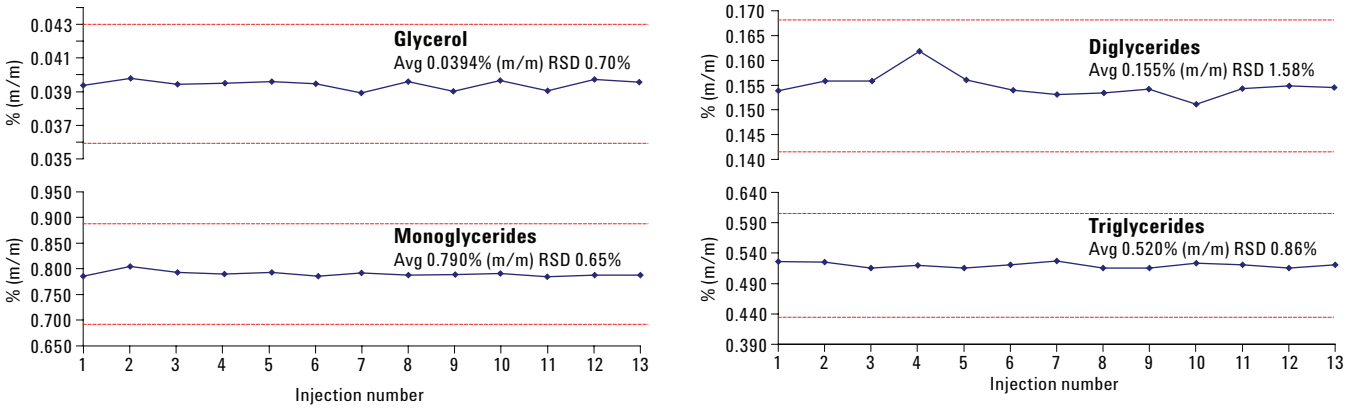

Figure 3. Typical repeatability of 13 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the EN-14105 method.

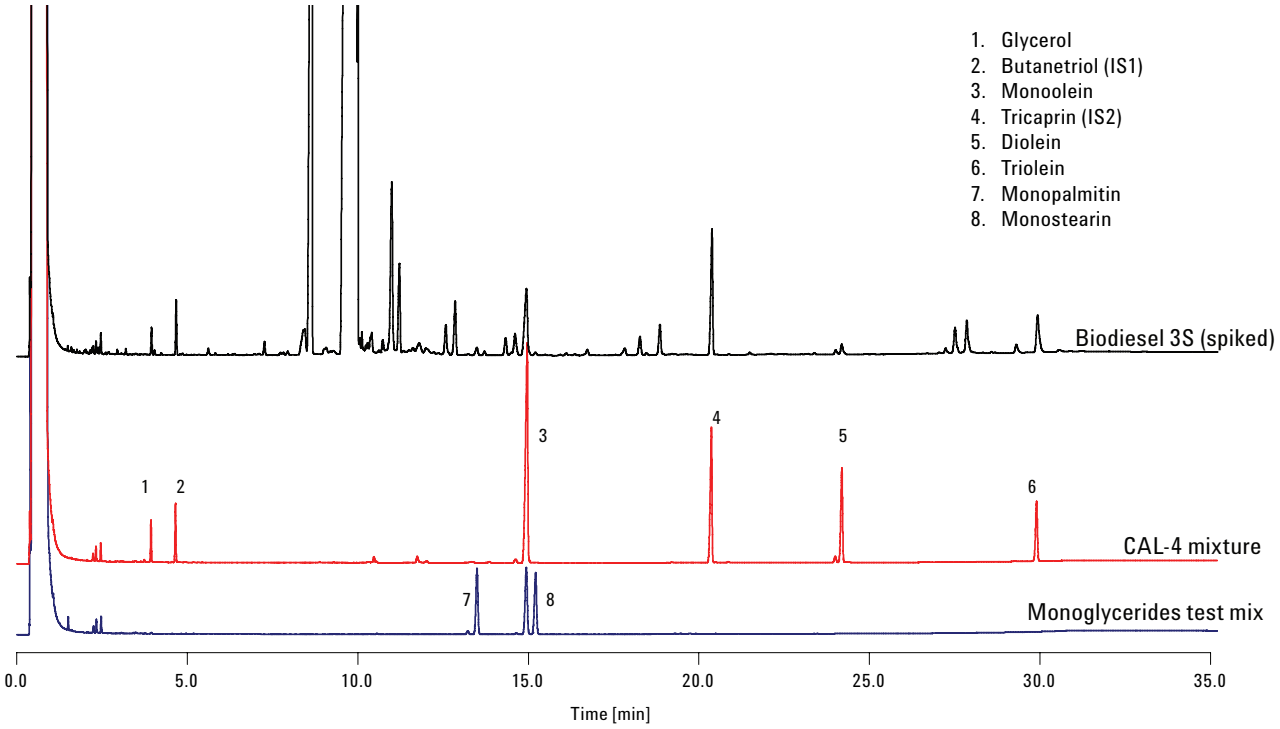

Figure 4. Details of identification of unknown peaks in a biodiesel sample .

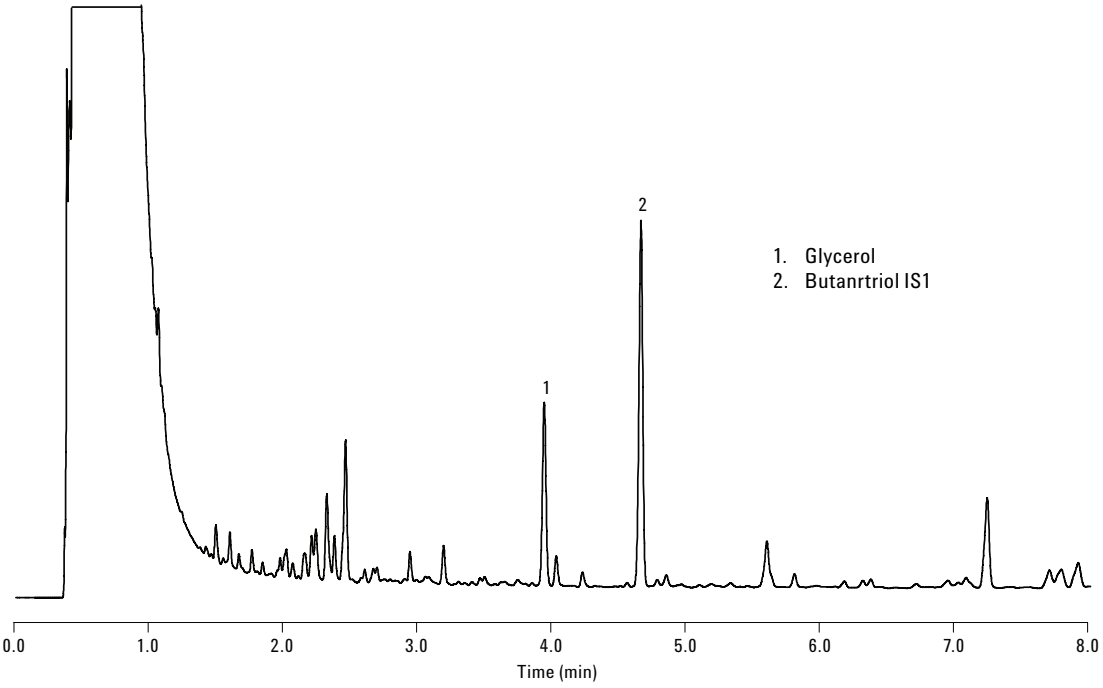

Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

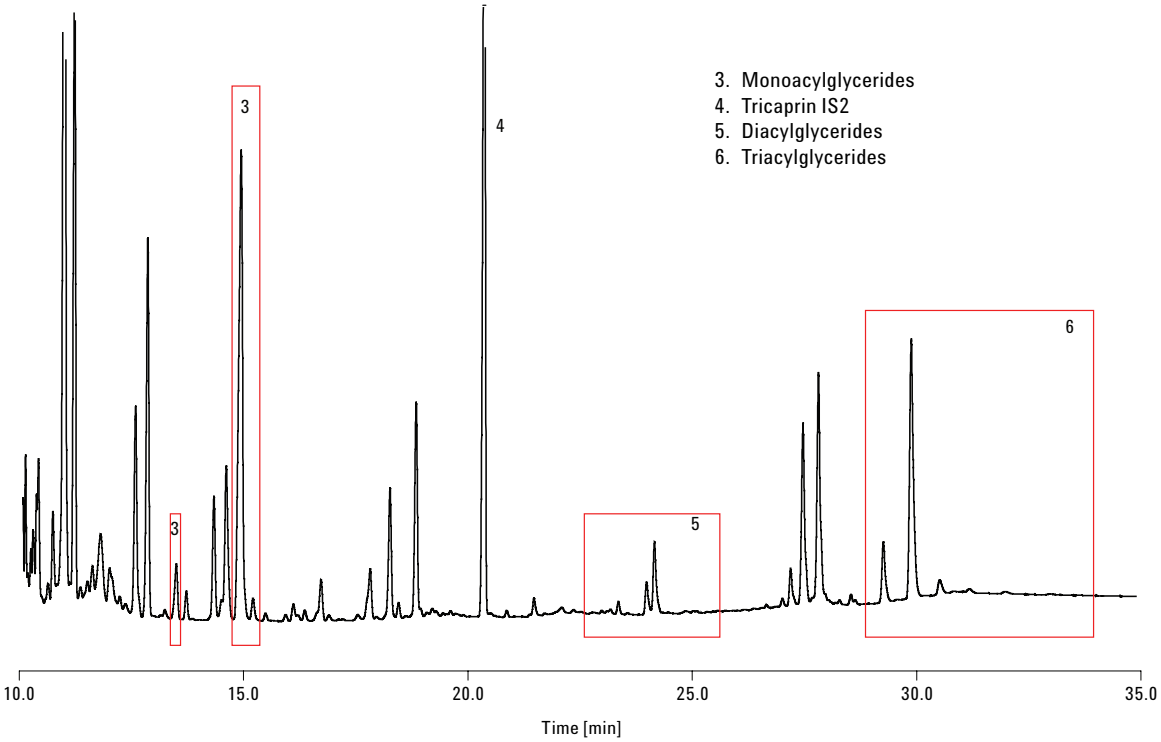

Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

The biodiesel sample analyzed was within the EN-14214 specification. However, because of the low levels of both glycerol and triglycerides, the sample was spiked with glycerol and triglycerides so an assessment could be made of the column's separation performance for those components. It is evident in Figure 6 that there is excellent separation of the triglycerides and very low column bleed at 370 °C.

Conclusion

This application note demonstrates the suitability of an on-column injector and the Agilent Select Biodiesel for Glycerides UltiMetal column for the analysis of biodiesel by gas chromatography. The calibration curves and repeatability data demonstrate excellent system integrity, which makes the system ideally suited for the analysis of free glycerol and total glycerol, as well as, mono-, di-, and triglyceride content in biodiesel in accordance with EN-14105 [1].

References

1. EN-14105. Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, triglyceride content.
2. Knothe, G. (2006), Analyzing Biodiesel: Standards and other Methods. JAOCS 83, 823–833.
3. EN-14214:2003. Automotive fuels – Fatty Acid Methyl Esters (FAME) for diesel engines – requirements and test methods.

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Agilent Technologies

Obtaining Optimum Performance When Using the SIPS Accessory

Application Note

Atomic Absorption

Introduction

The SIPS accessory, which was introduced in December 1994, was the first practical dilution system for flame AA to provide calibration from a single standard and fast, on-line dilution of over range samples. A few simple procedures, outlined in this information sheet, ensure reliable and productive operation of this accessory.

The Agilent SIPS pump tubing is manufactured from a composite material known as Santoprene. The pump tubing commonly used on VGA and ICP pumps is a single-mix polymer. All types of pump tubing, but especially composite tube materials, can sometimes show signs of "spalling" under normal operation. This is a variable effect in which very small particles of the tubing material break away. If severe spalling occurs, these particles can stick together and cause blockage of the nebulizer.

Spalling occurs in various degrees with all peristaltic pump tubing manufactured from composite materials. It is not unique to SIPS.

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The Effect of Spalling

The symptom of severe spalling is an initial increase in the absorbance followed by a decrease as the nebulizer capillary becomes increasingly blocked. A totally blocked nebulizer will cause the sample to be pumped into the diluent bottle thus contaminating the diluent. Sometimes the blockage may clear without intervention.

The extent of the blockage can depend on the nature of the solutions being pumped. It has been found that very dilute solutions are more likely to induce spalling and block the nebulizer than are concentrated solutions.

Why Use Composite Materials?

Composite materials produce long-wearing tubes that have consistent performance. Spalling usually has no noticeable effect. Some formulations, however, display a higher level of spalling. Naturally these are not recommended for use with SIPS.

Achieving Reliable SIPS Operation

There are four easy steps required to minimize spalling effects and to achieve reliable operation. These are:

1. Use only Agilent-supplied SIPS pump tubing
2. Determine, and use the correct arm pressure for each unit
3. Condition new pump tubes, and re-condition (used) tubes before a run
4. Add a detergent to the diluent

A brief summary of these procedures follow. The complete procedures are outlined in publication no. 85-101710-00, which is supplied with all batches of pump tubes.

Use Only Agilent-Supplied SIPS Pump Tubing

It is recommended that SIPS users obtain their pump tubing from Agilent only. Agilent supplied pump tubing is guaranteed to achieve our specified performance and this minimizes batch to batch variations. As with graphite tubes, individual batches of pump tubes are tested to ensure satisfactory operation. Only those batches passing our tests are accepted. Stretching and other problems have been noted with tube batches sampled from a range of vendors.

Determine the Correct Arm Pressure

When the SIPS is first installed, the user must determine the optimum arm pressure setting for that particular unit. This setting does vary from one SIPS unit to another. By optimizing the arm pressure setting, tube life is maximized and the optimum pumping efficiency is achieved.

In practice, this calibration does not have to be repeated when new tubes are installed as there is little variation from one batch of tubes to another.

The procedure need only be repeated if the SIPS unit is repaired or changed (for example, if a SIPS-10 is upgraded to a dual pump SIPS-20).

Condition the Pump Tubing

Before each use of a new pump tube, the pump tubing should be cleaned and conditioned, using the following procedure. Briefly, a dilute detergent solution (such as a 1% solution (mass/volume) of Triton X-100) is pumped through the tube for 15 minutes. Then distilled water is pumped for 30 minutes to rinse it. Once this time has elapsed, the SIPS unit is ready for regular operation.

If the pump tubing has been used previously, it is recommended that before use of the SIPS, the pump tubing is reconditioned. This is achieved by pumping a solution of 0.01 % Triton X-100 (mass/volume) through the tube for 15 minutes. This procedure can be completed while waiting for the hollow cathode lamp and the burner to warm-up and stabilize. Once this time has elapsed, the SIPS unit is ready for regular operation.

Add a Detergent

To minimize nebulizer blockage from spalling, it is recommended that all SIPS users add Triton X-100 (a readily available laboratory detergent) at a concentration of 0.01% (mass/volume) to the Rinse and Make-up (Diluent) solutions. The Triton X-100 evidently alters the surface of the particles so that the particles do not stick together, but pass through the nebulizer and disappear in the flame.

Summary

The SIPS accessory offers real time-saving and cost-saving benefits to users. Completing the simple procedures described above ensures users can achieve the best performance and the maximum benefit from their SIPS.

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Routine Maintenance for Atomic Absorption Spectrophotometers

Application Note

Atomic Absorption

Author

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Introduction

Instruments in good operating condition are a necessity in any analytical laboratory. This level of integrity can be achieved by a regular maintenance schedule with minimal work. The four main areas of such a program for atomic absorption spectrophotometers include:

- General instrument maintenance
- Gas supply maintenance
- Flame component maintenance
- Furnace component maintenance

The benefits of routine maintenance include:

- Increased instrument lifetime
- Reduced downtime
- Overall improvement in instrument performance; giving the operator greater confidence in the validity of his analytical results

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General Instrument Maintenance

Dust and condensed vapors can accumulate on the instrument case, and corrosive liquids can be spilled on the instrument. To minimize damage, wipe off the instrument with a damp, soft cloth using water or a mild detergent solution. DO NOT USE ORGANIC SOLVENTS. The sample compartment windows and the lamp windows can accumulate dust or fingerprints. In such cases, clean the windows with a soft tissue moistened with a methanol or ethanol and water solution. If the windows are not clean, the operator will observe noisy lamp signals and non-reproducible analytical results.

The remaining optical components are sealed, but they should not be exposed to corrosive vapors or a dusty atmosphere. In laboratories where high concentrations of dust or vapors are unavoidable, schedule a yearly check by a service engineer to maintain the efficiency of optical light transmission in the instrument. There is no need for an operator to clean the sealed optical components.

Gas Supply Maintenance

Three gases are suitable for flame M. Air and nitrous oxide are used as combustion support gases (oxidants). Acetylene is used as the fuel gas. Each gas is supplied to the instrument through piped supply systems and rubber hoses. Copper or copper alloy tubing may be used for the oxidant gases. Acetylene should only be supplied through stainless steel or black iron pipe. Check connections regularly between the supply and instrument for leaks, especially when tanks are changed using a soap solution or commercial leak detector. Check the rubber hoses connected to the instrument for fraying and cracking. In addition, each time a tank is changed, check the regulators and valves for proper operation.

Because potentially toxic gases are used or produced in the flame, it is necessary to use a suitable exhaust system with a minimum capacity of 6 m^3/min (200 cfm). A simple smoke test will indicate if it is functioning properly.

Compressed Air Supply

Air may be supplied to the instrument from cylinders, a house air system, or small compressor. Cylinders are the most expensive source of air, particularly where large amounts are consumed and cylinders must be changed frequently. If compressed air from an in-house supply is used, a filter/regulator assembly must be installed in the input line to the instrument. An acceptable “Air Service Unit” (Part No. 01 102093 00) may be ordered from any Agilent sales office.

Whatever source is used, the supply must be continuous and have a delivery pressure of 420 kPa (60 psi). The air must be clean, dry and oil free. Approximately 50% of all gas unit failures are caused by moisture or other impurities inthe air supply.

Excessive noise in the readout has also been attributed to contaminated air. An air filter assembly is therefore an essential component of the atomic absorption spectrophotometer, and its inclusion in the air supply installation is mandatory. Weekly, check the air filter for particle and moisture accumulation. When necessary, dismantle the air filter assembly and clean the filter element, bowl, and drain valve components. Use the following procedure for dismantling and cleaning the air filters supplied with the instrument.

1. Shut off the air supply and allow the system pressure to bleed off.
2. Unscrew the filter bowl, complete with automatic drain valve.
3. Unscrew the retaining ring and push the drain valve back into the bowl.
4. Unscrew the baffle carefully, and remove the filter and filter shield.
5. Clean the filter bowl, drain valve components, baffle, and filter shield by washing in a solution of soap and water. DO NOT USE ORGANIC SOLVENTS AS THEY WILL DESTROY THE BOWL AND VALVE COMPONENTS. Rinse thoroughly in fresh water.
6. Clean the filter element by washing in ethyl alcohol or similar solvent.
7. Ensure that all components are properly dried before reassembly.

Nitrous Oxide Supply

The nitrous oxide used for atomic absorption spectrophotometry must be oil free. If a heated regulator is not used, loss of regulation can occur due to the expansion cooling effect encountered when nitrous oxide is drawn from a cylinder. This can lead to erratic results and create a potential flashback situation with manual gas control units: An acceptable heated regulator may be ordered from any Agilent sales office. The consumption rate is dependent on the application, but is usually 10–20 liters per minute.

Acetylene Supply

Acetylene is the only combustible gas which is normally used in MS. The gas must be supplied packed in acetone. Some companies supply acetylene packed in proprietary solvents, but unfortunately the disadvantages outweigh the advantages. The major disadvantage is that the solvent may be carried over into the instrument and corrode the internal tubing, causing a potential explosion hazard. Ensure that the acetylene is at least 99.6% pure "M Grade" and packed in acetone.

The delivery pressure must be regulated and never exceed 105 kPa (15 psi). Check the instrument operation manual for the correct delivery pressure for the particular instrument being used. In addition, check the acetylene cylinder pressure daily, and maintain in excess of 700 kPa (100 psi) to prevent acetone from entering the gas line and degrading analytical results or causing damage to the instrument.

Flame Component Maintenance

The flame component section of the instrument can be divided into three areas; the nebulizer, spray chamber and burner. Each requires routine maintenance to assure optimum performance.

Nebulizer

The nebulizer area of the flame component consists of the capillary tubing and the nebulizer body. Always ensure that the plastic capillary tubing used for aspirating solutions is correctly fitted to the nebulizer capillary. Any leakage of air, tight bends, or kinks will cause unsteady, non-reproducible readings.

At times the plastic capillary tubing can become clogged and it will be necessary to cut off the clogged section or fit a new piece of capillary tubing (about 15 cm long). in any event, make sure the plastic capillary tubing fits tightly on the nebulizer capillary. The nebulizer capillary can also become clogged. If this occurs, proceed as follows:

1. TURN THE FLAME OFF.
2. Remove the plastic capillary tubing from the nebulizer.
3. Remove the nebulizer from the bung.
4. Dismantle the nebulizer as described in the instrument operation manual or the instruction manual supplied with the nebulizer.
5. Place the nebulizer in an ultrasonic cleaner containing 0.5% liquid soap solution such as Triton X-100 for 5 to 10 minutes. If the ultrasonic bath fails to clear the blockage, pass a burr-free nebulizer wire CAREFULLY through the nebulizer and then repeat the ultrasonic cleaning procedure.
6. Re-assemble the nebulizer in accordance with the instructions.
7. Install the cleaned nebulizer.

   Replace the plastic capillary tubing.

   If blockages are allowed to build up and are not removed, the analytical signal will steadily drop until no absorbance is observed.
8. Check the nebulizer body, capillary, and venturi occasionally for corrosion. Nebulizer problems can be minimized by taking care to always aspirate 50–500 mL of distilled water at the end of each working day.

Spray Chamber

As the sample leaves the nebulizer it strikes the glass bead and breaks into an aerosol of fine droplets. The efficiency of the glass bead can be degraded by surface cracks, pitting and the accumulation of solid material. The reduction in bead efficiency can cause lower absorbance readings and noisy signals. When removing the nebulizer for inspection, always check the glass bead. Look for pitting, cracks, breakage, ensure that the adjusting mechanism operates properly and that the bead is correctly positioned over the nebulizer outlet (venturi).

While the nebulizer and glass bead are removed from the instrument for inspection, the spray chamber and liquid trap should be removed, dismantled, and cleaned. Discard the liquid in the liquid trap and wash both the spray chamber and liquid trap thoroughly with laboratory detergent and warm water. Rinse completely with distilled water and dry all components. Refill the liquid trap and reassemble the spray chamber, checking for any distortion of O-rings or blockages in the gas inlets. Reconnect the drain hose. If a bottle or jug is used to collect the waste solutions, check that the hose is not below the level of the waste. If the hose is below that level, absorbance readings will steadily decrease with occasional abrupt increases as intermittent drainage of the spray chamber occurs. Therefore, it is necessary to daily check the level of the waste and to dispose of it frequently. This is imperative when using organic solvents because of the potential hazards introduced by flammable liquids. Only wide necked, plastic containers can safely be used to collect the waste solutions.

Burner

The final area of concern in the flame component is the burner. During aspiration of certain solutions, carbon and/or salt deposits can build up on the burner causing changes in

the fuel/oxidant ratio and flame profile, potential clipping of the optical beam, and degradation of the analytical signal. To minimize the accumulation of salts, a dilute solution of acid (HNO_3) may be aspirated between samples. However, if salts continue to build up, turn off the flame and use the brass cleaning strip supplied with the instrument. Insert the strip in the burner slot and move it back and forth through the slot. This should dislodge any particles which will then be carried away once the flame is lit and water aspirated.

DO NOT USE SHARP OBJECTS such as razors to clean the burner as they can nick the slot and form areas where salt and carbon can accumulate at an accelerated rate.

If this type of cleaning is inadequate, remove the burner, invert, and soak it in warm soapy water. A scrub brush will facilitate cleaning. Soaking may also be done in dilute acid (0.5% HNO_3). Ultrasonic cleaners containing dilute non-ionic detergent only are another alternative for cleaning. After cleaning, thoroughly rinse the burner with distilled water and dry before installing in the instrument. NEVER DISASSEMBLE THE BURNER FOR CLEANING. IMPROPERLY RE-ASSEMBLED BURNERS WILL LEAK COMBUSTIBLE GAS MIXTURES, POTENTIALLY CAUSING EXPLOSIONS.

Each day after all analyses are completed, 50–100 mL of distilled water should be aspirated to clean the nebulizer, spray chamber, and burner. This is even more important after aspirating solutions containing high concentrations of Cu, Ag, and Hg, since these elements can form explosive acetylides. The entire burner/nebulizer assembly should be disassembled and thoroughly cleaned after analyzing these types of solutions. The burner should be removed weekly, scrubbed with a laboratory detergent, and rinsed with distilled water.

Furnace Component Maintenance

The graphite furnace accessory maintenance can be divided into three major areas; the gas and water supplies, the workhead, and the autosampler. Each plays an important role in obtaining valid analytical results. The following general maintenance program refers to the GTA-95.

Gas and Water Supplies

Normally the gases used in FAAS are inert gases such as N_2 and Ar. Either one may be used, but must be clean, dry, and of high purity. The regulated pressure should be 100–340 kPa (15–50 psi). At times the incorporation of air may be useful to fully ash a sample. However, air should not be used at ash temperatures higher than 500 °C because of the accelerated rate of graphite component deterioration at elevated temperatures.

The water supply, used to cool the furnace, may be supplied either from a laboratory tap or a cooling-recirculating pump. If a recirculating pump is used the water must be kept below 40 °C. The water used must be clean and free of corrosive contamination. The flow should be 1.5–2 liters/minute. Maximum permissible pressure is 200 kPa (30 psi).

Workhead

The workhead is a closed assembly with quartz windows on either end. Before starting an analysis, check the windows for dust or fingerprints. If needed, clean both sides of the quartz windows with a soft tissue moistened with an alcohol/water solution. Never use coarse cloths or abrasive cleaning agents. While the windows are removed, inspect the gas inlets on the window mountings. If the graphite components have deteriorated extensively, graphite particulates may have dropped into the gas inlets, blocking the proper flow of gas. This will cause further graphite deterioration at an accelerated rate and lead to poor analytical performance. To clean, carefully blow out the particulates with a supply of air. Inspect the inside of the window mountings and clean off any sample residue which may have deposited over time.

In the center of the workhead are the graphite components. At frequent, regular intervals, remove the graphite tube atomizer and inspect the inside of the graphite shield. Ensure that the bore and the injector hole area are free of loose carbon or sample residue. Check the electrodes on either end of the graphite shield for proper tapering. If the tapering is worn or burnt, the electrodes will not make the correct contact with the graphite tubing, causing fluctuations in applied power resulting in irreproducibility. The electrodes also have a series of gas inlets which must be free of loose carbon or sample residue.

Above the graphite shield is the titanium chimney. Injected sample or sample residue from the ash/atomize cycles may deposit in this area. A cotton swab soaked with alcohol can be used to clean both the inside and outside of the chimney. Alternatively, the titanium chimney may be soaked in dilute acid to remove deposits.

Autosampler

The components of the autosampler requiring routine maintenance are the rinse bottle, syringe, and capillary tubing, the proper care of which will minimize contamination and improve reproducibility of analytical results.

Regularly remove the rinse bottle for cleaning. This involves soaking the bottle in 20% HNO_3 followed by rinsing with distilled-deionized water. Refill the bottle with a solution of 0.01–0.05% HNO_3 in distilled-deionized water. The solution

may also include 0.005% v/v Triton X-100 R. The Triton helps maintain the sample capillary in clean condition and assists in obtaining good precision.

At times, graphite particulates may accumulate on the capillary tip and should be carefully removed with a tissue. If these particulates are not removed, the dispensing characteristics of the capillary may change. Contamination of the capillary may become a problem when using some matrix modifiers. In such cases, direct the capillary to a vial containing 20% HNO_3, draw up 70 µL, and stop the autosampler while the capillary is in the vial. After a period of a few minutes, the autosampler RESET should be utilized to rinse out the acid solution. This will clean the internal and external areas of the capillary. Similarly, organic residues can be removed by directing the capillary to a vial of acetone and repeating the above procedure. The PTFE capillary should be treated carefully during cleaning and operation. If bends or kinks appear, it can take time to reshape, and while doing so the repeatability of injection may be degraded. If the capillary tip is damaged, the damaged portion should be cut off at a 90° angle with a sharp scalpel or razor blade.

The final area of the autosampler maintenance schedule is the syringe. Daily, check for bubbles in both the capillary and syringe. Any bubbles in the system can cause dispensing errors and lead to erroneous results. Follow the instructions in the operating manual to free the system of bubbles. If the bubbles continue to cling to the syringe, it may need cleaning. The syringe can be washed with a mild detergent solution and thoroughly rinsed with deionized water. Ensure that contamination is not introduced through the syringe. Be particularly careful not to bend the plunger while washing the syringe.

Conclusion

Attached is a routine maintenance schedule for atomic absorption spectrophotometers (Figure 1). By adhering to this program, the overall integrity of the atomic absorption spectrophotometer can be maintained and the laboratory analyst will reap the benefits of increased instrument lifetime, reduced downtime, and gain greater confidence in the analytical results.

Maintenance Schedule (Flame AA)	
Daily	**Completed**
1. Check Gas	
2. Check Exhaust system with smoke test	
3. Empty the drain receptacle	
4. Clean lamp and sample compartment windows	
5. Rinse spray chamber with 50-100 mL of distilled water	
Weekly	
1. Disassemble spray chamber	
(a) Check glassbead	
(b) Check nebulizer components	
(c) Wash the spray chamber and liquid trap	
(d) Scrub the burner	
(e) Change the liquid in the liquid trap	
(f) Check the O-rings	
2. Check air filter assembly	
3. Wipe off instrument	
4. At Time of Gas Tank Change	
5. Check for leaks	
6. Check for operation of the regulators	
7. Check for operation of the shut off valves	
8. Check the gas supply hoses	
Yearly	
1. Schedule an Agilent service engineer to perform Preventive Maintenance	

Figure 1. Routine maintenance schedule for atomic absorption spectrophotometers.

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Guidelines for Using Non-Aqueous Solvents in Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Author

Jonathan Moffett

Introduction

Much of our environment consists of water. Therefore the bulk of AA methodology deals with water as a solvent. The use of water also has advantages:

- Restricted density range
- Relatively constant viscosity
- Constant specific heat
- Nonflammable
- Transparent in UV and visible region

The relatively constant physical properties allow optimized design of nebulizers, spray-chamber and burner. Background correction is not necessary for many applications.

Some disadvantages of water as a solvent include:

- Potentially corrosive action towards metal
- Dissolved solids levels can be very high
- Flame characteristics affected by cooling

The first can be controlled by careful selection of instrument construction materials. Correct instrument setup (such as glass bead adjustment) can substantially minimize flame perturbation caused by the last two.

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The use of non-aqueous (mainly organic) solvents for AA is necessary for certain applications. These include:

- Solvent extraction of metal chelates
- Direct analysis of petroleum products like oil
- Direct analysis of edible oil products
- Direct analysis of pharmaceuticals

The use of organic solvents introduces many complicating aspects including:

- Wide range of densities
- Differing viscosities
- Flammability
- Major effect on flame stoichiometry
- Relatively low flashpoints
- Effect on plastics
- Irritating and noxious fumes
- Increased care required for safe disposal

This wide range of physical and chemical properties (Table 1) makes it difficult to anticipate all the requirements of a particular application. An instrument used with organic solvents must be more flexible than one used for aqueous solvents. The operator also requires more training, especially with the safety aspects. Materials used to protect an instrument from corrosive aqueous solutions are often attacked by organic solvents. Sometimes expensive alternative materials must be used in instrument construction.

Safety Aspects

Organic solvents generally used in AA include the following:

- Hydrocarbon (kerosene, white spirit, xylene)
- Ketone (MIBK, DIBK)
- Alcohol (butanol)
- Ester (isobutylacetate)

The most widely used solvents are usually either a hydrocarbon or a ketone. Further information may be found in Table 1.

Table 1. Physical Properties of Some Organic Solvents

Solvent	Flash point °C	Boiling point °C	Specific gravity
4-Methylpentan-2-one (MIBK)	22	118	0.79
2-Methylpropan-2-ol	23	148	0.83
m-Xylene	29	139	0.86
Cyclohexanone	34	155	0.95
Kerosene (Jet-A1)	39-74	175-325	0.78
3-Heptanone	46	148	0.82
Shellsol T	50	186-214	0.75
White spirit (Pegasol)	55	179-194	0.76
2,6-Dimethylheptan-4-one (DIBK)	60	166	0.81
Cyclohexanol	68	161	0.96
Tetrahydronapthalene (Tetralin)	71	207	0.76

Note: The flash point is the lowest temperature at which the liquid gives sufficient vapor to form an ignitable mixture with air and to produce a flame when an ignition source is brought near the surface of the liquid.

To varying degrees, all organic solvents are both flammable and toxic. The use of organic solvents requires great care.

Organic solvents should be kept in glass bottles. The bottles should be stored in a metal cabinet or in a separate storage area well away from flames and other ignition sources. When using solvents only a relatively small quantity (less than 2 L) should be open to the atmosphere at any one time. In addition most countries have legislation which applies to the storage and handling of flammable liquids. These legal aspects must also be considered.

Prolonged exposure to organic solvent fumes is a health risk. All work with them should be carried out in a fume cupboard which has adequate venting. Samples not being analyzed should be covered. If a sampler is used, it should be placed in an venting system which removes the vapors from the area.

There is always a risk of fire from fumes reaching the flame and adequate ventilation must be provided for the instrument itself. These vapors also absorb ultraviolet radiation and if present in the sample beam light path, can cause a significant background signal.

The plastics materials and paints used in the instrument and its accessories should be protected from direct contact with any solvents. Nearly all plastics except fluorinated plastics are affected to some degree by organic solvents and will swell and distort. Instrument parts are made to close tolerances and such changes may cause malfunctions. Generally if allowed to dry thoroughly these parts will return to their original shape.

A plastic waste container must be used for the instrument wastes. A flashback may shatter a glass waste container with potentially dangerous results. The waste container must be emptied often. All wastes including those from the instrument must be stored in approved containers. Legislation should be consulted for proper disposal of all waste liquids.

The following should never be used as solvents for AA (especially flame):

- Halogenated hydrocarbons (chloroform, Freon)
- Very low boiling point hydrocarbons (petroleum spirit)
- Ethers and acetone
- Tetramethylfuran (TMF)
- Dimethylsulphoxide (DMSO)

Halogenated hydrocarbons are toxic. If aspirated into a flame, even more dangerous gases (phosgene is the most common) are produced.

The other solvents in the list are extremely hazardous in the vicinity of a naked flame because they are volatile. Some are so flammable that they could support a spectrometer flame without acetylene.

Standards

Atomic absorption spectrometric measurement and calibration is based on comparison. Care is needed in preparing standards to obtain accurate results. The amount of care and time needed depends on how accurate the results must be.

Aqueous standard solutions are not generally suitable to calibrate an instrument for organic work. Hydrated metal cations in water have different physical and chemical properties to metallo-organic compounds in an organic solvent.

Metal compounds soluble in organic solvents are commercially available. These can either be dry powders or else dissolved in a matrix oil.

The oil-based standards are easy to use. Single element standards can be weighed out and blended together. This multi-element standard can then be weighed into a clean base matrix. If it is not known whether the base matrix is free of the analyte of interest, then the calibration should be treated as a standard additions calibration. This prepared standard is then diluted by an organic solvent to give a working standard to calibrate the instrument. This approach allows the matrix and concentration range to be adapted to specific requirements. Companies such as Conostan (Ponca City, OK USA) and National Spectrographic Laboratories (Cleveland, OH USA) offer a range of single and multi-element standards that only need dilution to the required levels. Most countries have agents who represent these companies.

The dry standards are typically the cyclobutyrate salts of most metals. The powders are stable and can be stored for long periods. Dissolving the powders can be time consuming and may require two or three liquids. Once dissolved, they may be used in the same way as the oil-based standards. Chemical companies supplying atomic absorption standards also offer the dry powder standards.

Some ways of checking standards accuracy and instrument calibration are:

- Recovery studies
- Measure reference materials
- Inter-laboratory studies

A recovery study is done by spiking a sample with a known amount of standard. The absorption of the sample and spiked sample are measured and the respective concentration calibrated. Percent recovery is calculated by the following equation (US EPA abbreviations are used):

$$\% \text{ Recovery} = (SSR - SR)/SA \times 100$$

where:
SSR = spiked sample result
SR = sample result
SA = spike added

Reference materials are check samples which have accurately known compositions. There are organizations which supply reference materials. A list of these is given in later in this document. Consult their catalogs for further information. Reference materials should be treated in the same way as the other samples. A measured result should be within experimental error of the certified result. These materials could also be used as calibration standards. This is not recommended for two reasons:

- Cost is very high
- Calibration standards and quality control (QC) samples should have different sources to reduce systematic errors

Inter-laboratory studies require the cooperation of laboratories doing the same type of analyses. A sample is divided among the laboratories and measured. The results are all collated and compared. When done as a long term project, this method can monitor a laboratory's performance and allows any necessary remedial action to be taken.

Calculations

Units

Concentration of oil standards are generally expressed as µg/g or ppm (mass).

For solutions presented to the instrument for aspiration, the range is generally in mg/L or ppm (volume).

The term ppm (parts per million) in particular must be very carefully defined. An oil standard may contain 500 µg/g of the element of interest. If diluted 1:10, the solution contains 50 mg/L. To allow direct comparison of oil samples, the concentration of the standard can be entered as 500 in the instrument software. However, when comparing absorbances with other studies, it must be remembered that the solution concentration is 50 mg/L. The unit part per million (ppm) is therefore somewhat ambiguous and will not be used in this discussion.

Dilution

Very often organic samples cannot be presented directly to an instrument's nebulizer. For example an oil sample is too viscous to be aspirated directly without dilution. A gasoline sample is too flammable to be used with a flame instrument. These must be diluted in a suitable miscible liquid. Dilution must be done to allow meaningful measurement of the analyte in question. A 1:5 or 1:10 dilution is usually appropriate for the determination of copper or iron in used oil analysis. The determination of zinc or sodium may require a greater dilution and/or selection of a suitably sensitive resonance line. Burner rotation may also be necessary to reduce sensitivity.

Remember that when the sample has been diluted, the analyte concentration must be carefully defined. It must be very clearly stated whether the concentration refers to the analyte in the original sample or in the diluted solution.

Some examples of typical dilutions are given below.

Case 1: Preparation of oil standards using an oil-soluble metallo-organic salt.

Mass (in grams) of salt to be weighed out, m, can be calculated by equation 1.

$$\text{mass salt} = \frac{MC}{10{,}000\ P}\ \text{grams} \qquad (1)$$

where M is mass of oil standard required (g)
C is concentration of analyte in oil (µg/g))
P is percent analyte in salt

Example 1: Prepare a 500 µg/g Si standard in 100 g oil. The silicon was assayed at 14.29% in the salt. Using equation 1,

$$\text{mass salt} = \frac{100 \times 500}{10{,}000 \times 14.29} = 0.3499\ \text{g}$$

Method: Weigh out 0.3499 g salt. Dissolve in xylene and organic solubilizers (refer to the instructions provided by the chemical supplier) with warming. Add 80–90 g warm base oil with stirring. Cool. Make up to 100.00 g.

Case 2: Preparation of an oil standard using an oil dissolved standard and clean base oil.

Mass of oil standard (in grams) to be weighed out, m, can be calculated by equation 2.

$$\text{mass oil standard} = \frac{M\ C}{S}\ \text{grams} \qquad (2)$$

where M = mass of standard to be prepared
C = concentration of analyte required
S = stock oil concentration

Example 2: Prepare 10 g of multi-element oil containing 120 µg/g Cu and 300 µg/g Al starting with 5000 µg/g standards.

Using equation 2,

$$\text{Cu}\quad m = \frac{10 \times 120}{5000} = 0.2400\ \text{g} \qquad \text{Al}\quad m = \frac{10 \times 300}{5000} = 0.6000\ \text{g}$$

Method: Weigh out 0.2400 g of the copper standard and 0.6000 g of the aluminium standard. Dissolve in about 8–9 g of warm base oil. Cool. Make up to 10.000 g.

Case 3: Prepare 20 g of a standard to analyze an oil sample with less than or equal to 1.5% Zn.

In this case, there are two possible methods. One method is to make up a standard from the cyclobutyrate salt (assayed at 16.18% Zn) as shown in Case 1.

Method 1: 1.5% Zn = 1.5 × 10,000 µg/g Zn
From equation 1: $m = \frac{20 \times 1.5 \times 10\ 000}{10{,}000 \times 16.18} = 1.854$ g

Dissolve the salt in xylene and organic solubilizer as recommended by the chemical supplier. Add about 18 g warmed clean base oil with stirring. Make up to 20.000 g.

To reduce the amount of diluent required, the 307.6 nm resonance line could be used in this analysis. A 1:5 or 1:10 dilution

would be sufficient. Note that the signal to noise ratio for the 307.6 line is not as good as the 213.9 line, but would still give acceptable results.

Another method is to use a variation of Case 2 and make up a standard from a more easily handled oil-based standard. However the sample (15 000 µg/g) is more concentrated than the standard (usually 5 000 µg/ g). So this method uses a different dilution for the sample compared to that for the standard. If the very sensitive 213.9 nm zinc line is used, then a 1:10 000 dilution of sample is necessary to obtain about 1.5 mg/L. Such a large dilution would mean that the sample solution would have almost the same physical properties as the solvent.

If a 5000 µg/g standard is used, a 150 µg/g working standard can be made which only has to be diluted 1:100. At a 1:100 dilution the physical properties of the standard solution would also be similar to the solvent.

Method 2:

From equation 2 $m = \frac{20 \times 150}{5000} = 0.600$ g

Weigh out the oil standard. Add about 12 g warm clean base oil with stirring. Cool. Make up to 20.000 g.

Dilute the sample by weighing out 1.000 g and dissolving in 100 mL solvent solution. Pipette out 1 mL of the solution and make up to 100 mL. This is the solution to be analyzed.

Dilute the standard by weighing out 1.000 g and dissolve in 100 mL solvent solution. This standard is equivalent to 1.5% Zn in the original oil sample.

Ionic Suppression

A nitrous oxide-acetylene flame is recommended for the measurement of the Group II elements (magnesium, calcium, strontium, barium). Under these conditions, the analytes are partially ionized and require the use of an ionization suppressant for their accurate measurement. An organic soluble potassium or sodium salt is added to the standards and samples to give a final concentration of 2000–5000 ppm. The salts are either napthenates, sulphonates or cyclobutyrates.

A branched capillary to aspirate an ionization suppressant and sample simultaneously has been described [1] and it has been claimed to work with organic samples. This has not yet seen wide application.

Hardware

Spraychamber: Check that the components are resistant to solvent attack and do not distort. Removable components should be checked to ensure they are not binding or tight.

O-Rings: Inspect these frequently. KALREZ O-rings are resistant to solvent attack and are available as sets.

Liquid Trap: This should be filled with the liquid being aspirated or a liquid miscible with the solvent being aspirated.

It is recommended that the spraychamber and liquid trap be dismantled and cleaned at the end of each working day. Wash with hot water and detergent or acetone and allow to dry. Reassemble while checking the O-Rings.

Nebulizer: An adjustable nebulizer which allows control of the uptake rate is necessary. The uptake can be continuously varied from zero up to about 10 mL/min.

An adjustable nebulizer does not have a thimble like the standard preset nebulizer. Instead it has a housing with an uptake control. Refer to the instructions on initial setup.

Setting the correct uptake rate should be done using an air-acetylene flame and the selected solvent:

1. Check nebulizer is set for zero uptake rate
2. Light flame and adjust gas flows to give a very lean flame
3. Place capillary in solvent
4. Slowly rotate uptake control clockwise until flame is beginning to become fuel-rich (some yellow may be seen)
5. Measure and record uptake

   Generally, MIBK, DIBK and xylene - 2 mL/min white spirit, kerosene - 4 mL/min. The nitrous oxide-acetylene flame can tolerate higher uptake rates (MIBK - 6 mL/min).

   A high uptake rate is not desirable for a number of reasons: the flame may be extinguished between samples because of insufficient fuel; the risk of background and inter-element interferences is increased; the gains in signal are usually not significant enough.

Burner: An air-acetylene burner should only require periodic cleaning. The use of organic solvents however increases the possibility of carbon buildup with the nitrous oxide-acetylene flame. More frequent cleaning of the nitrous oxide-acetylene burner may be needed.

A carefully cleaned burner gives the best performance and

reduces salt blocking and carbon build-up. The use of a brass strip is no longer recommended. Studies revealed that a metal strip does not clean sufficiently well and that it does not polish the jaws [2]. For optimum performance, any burner should be cleaned as follows:

1. Use a card (for example, business card) and a brass polish (for example, "Brasso")
2. Wet card on both sides with polish
3. Slide card into slot
4. Move card up and down to polish inside of burner jaws
5. Rub card along top of slot
6. Scrub with a soft nylon brush (for example, toothbrush) using hot water and detergent
7. Use ultrasonic bath if available
8. Rinse with hot running water
9. Rinse with distilled water
10. Allow to dry or use a card to remove water from inside slot

Background correction: The organic nature of the matrix means that UV absorption is significant. Background correction is more likely to be required for most elements. Background studies are recommended to determine if correction is needed.

Programmable Gas Box: The sample uptake rate affects the flow of oxidant through the nebulizer into the spraychamber. At low sample uptake rates in the air-acetylene flame, the oxidant flow must be set somewhat higher than the default 13.0 L/min. It is suggested the flow should be about 19 L/min.

Graphite Furnace Operation

Many of the practical precautions of flame are not needed for graphite furnace operation. For example the fire potential is greatly reduced because there is no naked flame and the volumes involved are very small. However some precautions are still necessary. Guidelines for handling, storing and disposing organic solvents must still be observed.

The chemical nature of the metallo-organic compounds means that organic standards may still be required for calibration.

The solvent used for dilution should not be too volatile. A furnace run can take a long time. The solution concentrations could be affected because of evaporation. The ketones (MIBK and DIBK) are probably the most suitable general purpose solvents for furnace work. They are miscible with many organic compounds and solvents. DIBK is also immiscible with water.

The organic phase is very mobile. When injected into a furnace, this mobility may cause more spreading than is desirable. To control droplet spreading in the furnace, a partition graphite tube should be used. Some analyzes of volatile elements like lead and cadmium may require the use of a platform [3]. The platform controls droplet spreading provided no more than about 20 mL is injected. For both types of atomization (wall and platform), the hot injection facility can also be used to control spreading. For example, using DIBK as a solvent the inject temperature on the sampler page can be set to 130 °C and the injection rate slowed down to 5. This facility also helps shorten the time needed to dry the injected solution and allows faster furnace cycles [4].

The solution in the rinse bottle of the sampler does not have to be organic. The rinse solution can be distilled water with 0.01% nitric acid and 0.1% Triton X-100 (a non-ionic detergent)3. If the samples are such that the dispenser tip is not being cleaned, a slightly higher concentration of Triton X-100 may be tried. A small amount (0.5 - 1%) of propan-2-ol in the rinse solution as well can assist with keeping the tip free of grease and oil.

Safety Checkpoints

Choose a Suitable Solvent Which Has the Following Properties

- Miscible with sample
- Uitably high flashpoint
- Density greater than 0.75
- No toxic by-products formed

Handling Solvents

- Use small volumes near instrument
- Keep solutions covered when not in use
- Do not inhale vapors
- Empty waste vessel often
- Use fume cupboard for solution preparation
- Dispose of all wastes carefully and responsibly
- Do not mix with nitric or perchloric acids or wastes

Instrument

- Fill liquid trap with suitable solvent before starting
- Attach tube to spraychamber vent and allow other end to vent safely away from flame
- Install an efficient exhaust system above instrument
- Keep burner clean
- Do not clean burner while flame is on
- Drain liquid trap at the end of each day
- Wash spraychamber and allow to dry overnight; check condition of O-rings often

References

1. R. J. Watling, L. O'Neill and J. Haines, Spectrochim. Acta, Part B, **1990**, 45B, 955.
2. J. B. Willis, B. J. Sturman and B. D. Frary, J. Anal. At. Spectrom., **1990**, 5(5), 399.
3. J. H. Moffett, Varian Instruments At Work, November 1985, AA-55 M. B. Knowles, J. Anal. At. Spectrom., **1989**, 4(3), 257.

Company Addresses

Conostan Division Continental Oil Co. PO Drawer 1267 Ponca City OK 74601 U.S.A.

National Spectrographic Laboratories Inc. 19500 South Miles Road Cleveland OH 44128 U.S.A.

Bureau of Analyzed Samples Ltd Newham Hall Newby, Middlesbrough, TS8 9EA England

U.S. Department of Commerce National Institute of Science and Technology Gaithersburg, MD, 20899 U.S.A.

Commission of European Communities Community Bureau of Reference (BCR) 200 Rue de la Roi B-1049 Brussels Belgium

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Agilent Technologies

Agilent Oil Analyzer: customizing analysis methods

Application Note

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Introduction

Traditionally, the analysis of used oils has been conducted by physical and wet chemical methods. FTIR spectroscopy has become a routinely used technique to analyze used oils, providing the following major advantages[1]:

- Ability to simultaneously determine several parameters from a single experiment
- Increase in speed of analysis
- More cost effective than traditional techniques
- Mobility and portability allowing remote on-site analysis

The Agilent FTIR Oil Analyzer is designed to meet the requirements of the US Department of Defense Joint Oil Analysis Program (JOAP)[2] for use in their condition monitoring program as well as commercial applications. It is optimized for monitoring relative changes in various indicators of oil conditions (oil failure symptoms) using a standardized protocol developed by the Joint Oil Analysis Program Technical Support Center (JOAP-TSC). This protocol sets the data extraction algorithm for several types of petroleum and synthetic-based lubricants and hydraulic fluids, and eliminates the need for reference samples as spectral subtraction is no longer required.

The Agilent Oil Analyzer software allows users to readily customize existing methods as well as create new methods to measure other parameters and properties of lubricants defined by the user. The methods can be easily adjusted for performing analysis of samples where spectral subtraction is required.

Agilent Technologies

This application note describes the tools available with the Agilent FTIR Oil Analyzer and procedures that a user should follow to customize analysis methods, while reinforcing the importance of reliable calibration in quantitative spectral analysis.

Analysis methods

The sampling and analyzing procedures available in the Agilent FTIR Oil Analyzer conform to the ASTM E 2412–04 "Standard practice for condition monitoring of used lubricants by trending analysis using Fourier Transform Infrared (FTIR) Spectrometry"[3]. These methods provide a generalized protocol for condition monitoring of contaminants and breakdown products in used lubricants including water, ethylene glycol, fuels, incorrect oil, soot, oxidation, nitration and sulfonation. The methods are based on calculating trends and distributions from mid-IR absorption measurements, and encompass both direct and differential (spectral subtraction) trend analysis approaches.

The Agilent Oil Analyzer software is configured to run twelve predefined analysis methods that correspond to different classes of lubricating oils or hydraulic fluids, and their applications with differing limits. The methods are:

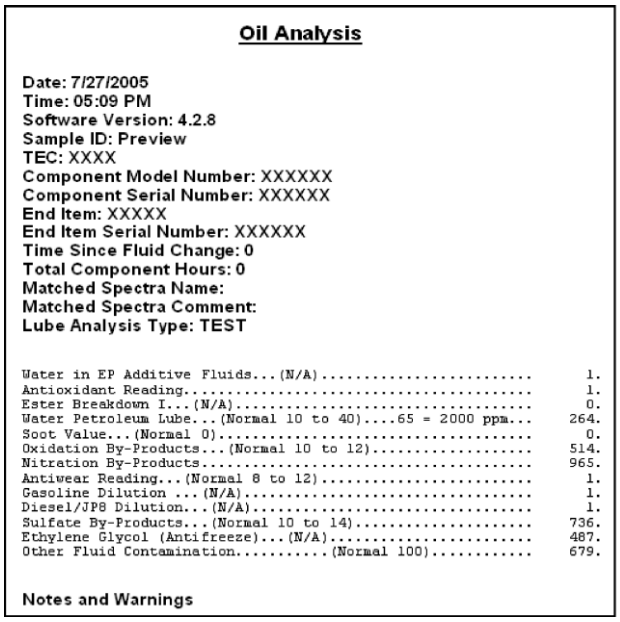
- Aircraft hydraulic (Mil-H-83282)
- Aircraft hydraulic (Mil-H-83282_350 ppm limit for water)
- Dextron transmission fluid
- Engine crankcase (Diesel_gasoline_natural gas)
- Fire retardant hydraulic (Mil-H-46170)
- Gas turbine or Helo Gbx (Mil-L-23699)
- Ground equipment hydraulic (Mil-L-2104_10W)
- Ground equipment synthetic hydraulic (Mil-H-5606)
- Marine diesel crankcase (Mil-L-9000)
- Conostan IR OTS fluid
- Steam turbine (Mil-L-17331)
- Generic or undetermined (Unknown lubricant type)

Each of the methods measures numerical indicators (parameters) that are related to the oil's condition. The software then generates a report that contains thirteen measurement parameters, as listed below:

- Water in EP fluids
- Antioxidant reading
- Ester breakdown
- Water in petroleum
- Soot value
- Oxidation by-products
- Nitration by-products
- Antiwear reading
- Gasoline dilution
- Diesel/JP8 dilution
- Sulfate by-products
- Ethylene glycol
- Other fluid contamination

Additionally, a separate procedure for predicting Total Base Number (TBN) is available and can be integrated into existing methods.

The parameters are reported in the units of spectral absorbance (peak areas or heights) rather than in physical concentrations, such as ppm, wt.% or mg of KOH. Figure 1 shows an example of a typical standard Oil Analysis report.



Oil Analysis

Date: 7/27/2005
Time: 05:09 PM
Software Version: 4.2.8
Sample ID: Preview
TEC: XXXX
Component Model Number: XXXXXX
Component Serial Number: XXXXXX
End Item: XXXXX
End Item Serial Number: XXXXXX
Time Since Fluid Change: 0
Total Component Hours: 0
Matched Spectra Name:
Matched Spectra Comment:
Lube Analysis Type: TEST

Water in EP Additive Fluids...(N/A)	1.
Antioxidant Reading	1.
Ester Breakdown I...(N/A)	0.
Water Petroleum Lube...(Normal 10 to 40)....65 = 2000 ppm...	264.
Soot Value...(Normal 0)	0.
Oxidation By-Products...(Normal 10 to 12)	514.
Nitration By-Products	965.
Antiwear Reading...(Normal 8 to 12)	1.
Gasoline Dilution ...(N/A)	1.
Diesel/JP8 Dilution...(N/A)	1.
Sulfate By-Products...(Normal 10 to 14)	736.
Ethylene Glycol (Antifreeze)...(N/A)	487.
Other Fluid Contamination.....(Normal 100)	679.

Notes and Warnings

Figure 1. Typical standard Oil Analysis report

Calibration

All analysis methods in the Agilent FTIR Oil Analyzer consist of a set of calibration models (procedures) in the form of corresponding files with an indication of the calibration model's type (univariate, or multivariate, or a combination). The analysis method may be composed of one or several calibration files.

The construction of calibration models in quantitative spectral analysis is a two-step procedure: calibration and validation. In the calibration step, indirect instrumental measurements (spectra) are obtained from standard samples in which the value of the parameter of interest has been determined by a standard reference method (an accurate direct measurement method). The set of spectra and results from the reference method, referred to as the calibration set or training set, is used to construct a model that relates parameter values to the spectra. Before the calibration model is accepted and used for prediction, it should be validated by a set of independent (not used in the calibration set) samples of known parameter concentrations (validation set). If parameters from the validation set fall within acceptable accuracy limits using the model derived from the calibration set, an acceptable model has been constructed that can be used to predict for new "unknown" samples.

To build a univariate calibration model, it is necessary to specify a single measurement from a spectrum, such as peak area or height that demonstrates the most distinctive spectral response for the parameter of interest. The univariate calibration and prediction procedures are available as a standard part of Resolutions/Resolutions Pro software and are defined as a simple quantitative analysis. The analysis is described in detail in the Resolutions online help and the corresponding system reference manuals for previous software versions (Win-IR Pro and Merlin). The user must generate a quantitative calibration document and save it as *.BSQ file using Resolutions/Resolutions Pro (Win-IR Pro or Merlin) software.

Where spectral responses attributed to different parameters overlap and the selective spectral measurements for the parameter of interest is very difficult, univariate models may not be reliable. Multivariate methods such as Principal Component Regression (PCR) and Partial Least Squares (PLS) allow multiple responses at the selected wavenumbers to be used. These methods are better suited to extracting spectral information where bands overlap and it is difficult to discern the relevant spectral regions attributable to a particular parameter. The main advantage of multivariate methods is the ability to calibrate for a parameter of interest when it correlates in a complicated (non-specific) way with multiple spectral regions, while minimizing background matrix interferences in the lubricants.

The Agilent Oil Analysis software allows multivariate calibration models created with the use of third party software to be incorporated in analysis methods. The PLSplus IQ package available as an additional application in the Galactic GRAMS/AI (GRAMS/32) software suite must be used. The "PLSplus IQ User's guide" gives step-by-step instructions on how to construct and validate a multivariate calibration model

as well as theory of advanced statistical analysis in spectroscopic quantitative analysis. The user must build an accurate calibration model and save it into a *.CAL file using PLSplus IQ.

The validity of empirically-built calibration models depends heavily on how well the standard samples (calibration set) represents the unknown samples to be analyzed (prediction set). In all cases, the selection of standard samples to be used for calibration must adequately cover the expected range of measurement parameters in the prediction set. This means that the expected extreme values for each parameter of interest in unknown samples must be included in the calibration set, as extrapolation outside the calibrated value range can be unreliable. It is important to ensure that any phenomena that influence the spectral measurements (e.g., not only the total amount of soot but its particle size distribution) also vary in the calibration set over ranges that span the levels of the phenomena occurring in the prediction set. It is also very important to minimize the errors in the standard sample parameters that are used to construct the empirical calibration model, as any calibration model can only be as accurate as the reference measurements from which it was constructed.

Many conditions can affect the results obtained from FTIR lubricant monitoring such as lubricant type, engine type, operational conditions, environmental conditions, etc. When the conditions are changed significantly, new calibration models and methods may be required to ensure accurate prediction of oil properties. For instance, new calibrations may be required when a new oil type with a different base stock and additive chemistries comes for the analysis.

Care must be taken when measuring overall oil quality parameters such as Total Acid Number (TAN) and Total Base Number (TBN) using FTIR spectroscopy. The secondary formation of acidic products in lubricants is characterized by TAN or indirectly by TBN, which assesses the consumption of basic reserve additives in the oil. While the various acids or bases present in a lubricant could, in principle, be individually quantified based on their characteristic absorption bands, no unique absorption bands can be directly related to TAN or TBN. Thus, only indirect FTIR spectroscopic methods for TAN and TBN have been standardized to date. In addition, there is a large discrepancy in new lubricant TAN values, from less than 0.1 mg KOH/g for R&O type oils to 9 or higher for some synthetic oils in industrial applications. On the other hand, the incremental decrease in TBN used to indicate that a product is failing, varies in broad ranges: some oils may have a new TBN value of 12, but rapidly decrease to a value of 3, whereas other synthetic oils may have the beginning TBN of 40.

A calibration model for TBN is currently available in Agilent Oil Analyzer. The calibration is intended for prediction of the values in gasoline and diesel engine oils having typical baseline numbers not higher than 12 mg KOH/g.

Note that in many individual cases, in order to estimate TAN and TBN satisfactorily the user needs to construct a multivariate calibration model that would cover the higher range of values as well as take into account any other factors that could influence the accuracy and the reproducibility of spectral measurements.

Method editor

Once the univariate or multivariate calibration models are built, the corresponding *.BSQ or *CAL files must be moved or copied into the directory C [Local Disk]:\ Program Files\Varian\Resolutions\Oil Analyzer\Methods. This is the storage location for the available calibration and method files. Then, log in as Administrator to the Agilent Oil Analysis software and enter the Method editor. Follow the Chapter 11 “Method Editor” in “Agilent Oil Analyzer operational manual” to incorporate the calibrations to an existing method or to develop a new method.

Note that spectral subtraction is available in the Agilent Oil Analyzer but was not utilized in JOAP protocol. It is not considered to be practical in view of the deployability aspect of many JOAP laboratories and that the required sample volume would increase because of the necessity of new oil samples to act as references. In order to apply the spectral subtraction procedure, the user needs to select "Use spectral subtraction" option in the Sampling method group in the General option dialog and edit the relevant analysis method, by clearing the "Zero less than Zero" check box in all the associated calibration models. Refer to Chapter 4 "General Options–Setup" and Chapter 11 "Method Editor" of "Agilent Oil Analyzer operational manual" for more information.

Conclusion

FTIR spectroscopy has been gaining increased acceptance as a method of choice for used oil analysis. Designed and optimized as a complete system for predictive maintenance programs, according to JOAP standards, the Agilent FTIR Oil Analyzer combines specific capabilities with the flexibility to be successfully used in any oil analysis laboratory.

The Oil Analyzer software allows new and improved analysis methods to be built and ensures that new types of lubricating oils and fluids used in a variety of different machinery are timely and reliably monitored and tested.

The software allows the user to include PCR/PLS methods to measure oil parameters and convert the units of spectral absorbance into physical results (ppm, wt.%, cSt, mg KOH/g oil, etc.) applying spectral subtraction if needed.

References

[1] Larry A. Toms, "Machinery Oil Analysis. Methods, Automation & Benefits", 2nd ed., Coastal Skills Training, Virginia Beach, VA, 1998.

[2] Allison M. Toms, "FTIR for the Joint Oil Analysis Program", in Proc. 1994 Joint Oil Analysis Program International Condition Monitoring Conference, Squalls, M., ed., JOAP-TSC, Pensacola, FL (1994), pp.387-419.

[3] Available from www.astm.org

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AA or ICP – Which Do You Choose?

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

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Introduction

For many analysts Atomic Absorption Spectrometry (AAS) is a well established and understood technique. However, even though Inductively Coupled Plasma Emission Spectrometry (ICP-ES) instrumentation has been commercially available for over a decade, the technique has proven to be more complex. This article discusses the main differences between the two techniques.

AAS Versus ICP

The basic difference between the two techniques is that one relies upon an atomic absorption process while the other is an atomic/ionic emission spectroscopic technique. The next essential difference is the means by which the atomic or ionic species are generated. A combustion flame or graphite furnace is typically used for AA while ICP-ES uses a plasma.

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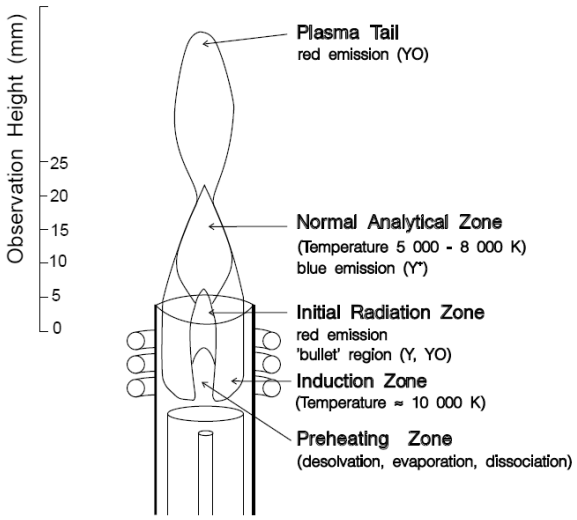

Figure 1. A plasma used for emission spectrometry. The regions refer to those seen when a Yttrium solution is introduced.

The typical maximum temperature for an air/acetylene flame is 2300 °C while for nitrous oxide acetylene, it is 2900 °C. Temperatures as high as 10,000 K can be reached in an argon plasma.

Detection Limits

The comparison of detection limits in Table 1 highlights the following differences:

- Furnace AA detection limits are generally better in all cases where the element can be atomized.
- Detection limits for Group I elements (for example, Na, K) are generally better by flame AAS than by ICP.
- Detection limits for refractory elements (for example, B, Ti, V, Al) are better by ICP than by flame AAS.
- Non metals such as sulfur, nitrogen, carbon, and the halogens (for example, I, Cl, Br) can only be determined by ICP.

While it is possible to determine phosphorous by AAS, its detection limit by ICP is more than three orders of magnitude better.

Optimum detection of non metals such as S, N and halogens by ICP-ES can only be achieved if a vacuum monochromator, with purged transfer optics, is used. The optics must be purged to exclude atmospheric oxygen and eliminating its absorption.

Sulfur can be measured at 180.73 nm by purging the monochromator. To detect the primary aluminium wavelength at 167.08 nm, the monochromator must first be evacuated, then purged with the inert plasma gas.

Note that a continuous flow vapor generation accessory can be used with either ICP-ES or AAS for improved detection limits for As, Se, Hg, Sb, Bi and Ge.

Sample Throughput

In ICP-ES, the rate at which samples may be determined depends on the type of instrument: both simultaneous and sequential ICP spectrometers are available. Most ICP spectrometers purchased are the sequential type, providing maximum flexibility of choice of element and analytical wavelength. Surveys have shown that most analysts are interested in 6–15 elements per sample and choose to pump the sample (which increases washout times) to improve precision and accuracy by minimizing viscosity effects. Simultaneous ICP spectrometers demonstrate an advantage in analytical speed over sequential ICP spectrometers when more than 6 elements/sample are measured.

If a "one off" sample is presented for a few elements, flame AAS is faster. However, with flame equilibration time, program recall and monochromator condition changes, the cross over point where sequential ICP becomes faster than AAS is approximately 6 elements/sample for routine analysis.

Unattended Operation

Flame AAS cannot be left completely unattended for safety reasons. An ICP-ES instrument or graphite furnace AA can be left to run overnight as no combustible gases are involved, effectively increasing the working day from 8 hours to 24 hours.

Linear Dynamic Range

The inductively coupled plasma is doughnut shaped (with a “hollow” center). The sample aerosol enters the base of the plasma via the injector tube. The “optical thinness” of the ICP results in little self absorption and is the main reason for the large linear dynamic range of about 10^5. For example, copper can be measured at the 324.75 nm wavelength from its detection limit of about 0.002 ppm to over 200 ppm. In ICP, extrapolation of two point calibrations can be accurately used to achieve orders of magnitude above the top standard. This compares to a linear dynamic range of typically 10^3 for AAS.

Interferences

Chemical
Chemical interferences are relatively common in AA, especially with graphite furnace AA, but may be minimized with chemical modifiers.

ICP-ES is almost free from chemical interferences. The chemical bonds that still exist at below 3000 °C are completely ruptured at above 6000 °C. The high temperatures reached in a plasma eliminate chemical interferences, which accounts (for the most part) for the better detection limits achieved for refractory elements.

Ionization
The ICP contains a large number of free electrons, so ionization interferences for most applications are virtually nonexistent. Ionization interferences can be encountered when determining elements in matrices that contain very high concentrations of Group I elements (for example, Na & K). However, these effects can be minimized by optimizing the plasma viewing height.

Ionization interferences may also be found in AAS, such as, when measuring certain Group II elements in a nitrous oxide flame. An ionization buffer such as Cs, Li or K can be added to both samples and standards to minimize this effect.

Spectral
The optical requirements of AAS are fairly simple. The monochromator only needs to distinguish a spectral line emitted from the hollow cathode lamp from other nearby lines. The lamp itself only emits a few spectral lines. Most elements require 0.5 nm resolution with only iron, nickel and cobalt of the common elements requiring 0.2 nm or better.

In ICP-ES, the rich spectra present in the plasma means that there is a greater possibility of spectral interference. Spectral resolutions of 0.010 nm or better are required to resolve nearby interfering lines from the atomic and ionic analytical emission signals of interest.

Spectral interference in sequential ICP spectrometers can, in most cases, be overcome by selecting a different elemental wavelength with similar detection limits. With simultaneous ICP spectrometers, the elements and the wavelengths which may be determined are fixed at the time of purchase, and an alternative line may not be available. In this case, inter-element correction may be used to minimize the spectral interference.

Physical
These interferences relate to the different properties of various samples and can affect sample transport and droplet formation. ICP tends to be more susceptible to such interference because of the smaller droplet size required and lower transport efficiency.

Precision
Precision can be termed short term (or within-run) and long term (over a period of one day). For AAS a precision of 0.1–1% is typical for the short term, but recalibration is required over a longer period. With ICP-ES the short term precision is typically 0.3–2%, but precisions of 2–5% are not uncommon over an 8 hour period without recalibration.

One technique used to eliminate backlash in the grating drive mechanism of ICP spectrometers is by scanning and measuring at the same time. This method of measurement can be termed as “measurement on the move” and effectively results in poor short term precision. A more recent method drives the grating to a wavelength near the analytical peak. A refractor scan is then performed over a smaller wavelength region in order to identify and locate the peak position. Finally the refractor plate is repositioned “at the peak” where the replicate measurements are then performed. This method offers better precision.

AAS v ICP – A quick guide

	ICP-OES	Flame AAS	Furnace AAS
Detection limits	Best for : Refractories Non metals P, S, B, Al V, Ba, Ti	Best for : Group I metals Na, K Volatile elements Pb, Zn Rare Earths	Best for : All elements except : B,W,U, Refractories, for example P, S Halogens
Sample throughput	Best if more than 6 elements/sample	Best if less than 6 elements/sample	Slow (typically 4 mins/element)
Linear dynamic range	10^5	10^3	10^2
Precision Short term Long term (over 8 hrs)	0.3 – 2% Less than 5%	0.1 – 1%	0.5 – 5%
Interferences			
Spectral	Many	Virtually none	Minimal
Chemical	Virtually none	Some	Many
Ionization	Minimal	Some	Minimal
Operating costs	High	Low	Relatively high
Combustible gases	No	Yes	No

Table 1. Guide to ICP/AAS Analytical Values

				ICP	Flame AA			Zeeman Furnace AA			
Element		AA λ (nm)	ICP λ (nm)	Detection limit µg/L	Characteristic conc µg/L	Detection limit µg/L	Flame type	Characteristic** conc µg/L	Mass pg	MSR %	EI
Silver	Ag	328.1	328.068	3	30	2	Air	0.035	0.7	97	Ag
Aluminium	Al	309.3	167.081	1.5	800	30	N_2O	0.25	5	100	Al
Arsenic	As	193.7	188.985	12	500	300	N_2O	0.5	10*	86	As
Gold	Au	242.8	267.595	5.5	100	10	Air	0.22	4.4	94	Au
Boron	B	249.8	249.773	1.5	8000	500	N_2O	43	855*	70	B
Barium	Ba	553.6	455.403	0.07	200	20	N_2O	0.85	17	100	Ba
Beryllium	Be	234.9	313.042	0.2	15	1	N_2O	0.025	0.5	64	Be
Bismuth	Bi	223.1	223.061	12	200	50	Air	0.45	9	88	Bi
Bromine	Br		163.340	6000							Br
Carbon	C		247.856	65						–	C
Calcium	Ca	422.7	393.366	0.03	10	1	N_2O	0.03	0.6	94	Ca
Cadmium	Cd	228.8	228.802	1.5	10	2	Air	0.01	0.2*	87	Cd
Cerium	Ce	520.0	418.660	7.5	100000	100000	N_2O			–	Ce
Chlorine	Cl		725.665	200000						–	Cl
Cobalt	Co	240.7	228.616	5	50	5	Air	0.21	4.2	98	Co
Chromium	Cr	357.9	267.716	4	50	6	N_2O	0.075	1.5	100	Cr
Cesium	Cs	852.1	455.531	3200	20	4	Air	0.55	11	58	Cs
Copper	Cu	324.7	324.754	2	30	3	Air	0.3	6	84	Cu
Dysprosium	Dy	421.2	353.170	0.3	600	30	N_2O	2.3	45	100	Dy
Erbium	Er	400.8	337.271	0.7	500	50	N_2O	5	100	100	Er
Europium	Eu	459.4	381.967	0.3	300	1.5	N_2O	1.3	25	100	Eu
Iron	Fe	248.3	259.940	1.5	50	6	Air	0.06	1.2	97	Fe
Gallium	Ga	294.4	417.206	6.5	800	100	Air	0.23	4.5*	80	Ga
Gadolinium	Gd	368.4	342.247	2.5	20000	2000	N_2O			–	Gd
Germanium	Ge	265.1	265.118	13	1000	200	N_2O	0.45	9*	100	Ge
Hafnium	Hf	307.3	264.141	4	10000	2000	N_2O			–	Hf
Mercury	Hg	253.7	184.950	8.5	1500	200	Air	7.5	150*	69	Hg

*Modifier used to obtain these results.

**20 µL injection

***The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Table 1. Guide to ICP/AAS Analytical Values (continued)

Element		AA λ (nm)	ICP λ (nm)	ICP Detection limit µg/L	Flame AA Characteristic conc µg/L	Flame AA Detection limit µg/L	Flame type	Zeeman Furnace AA Characteristic** conc µg/L	Zeeman Furnace AA Characteristic*** Mass pg	MSR %	EI
Holmium	Ho	410.4	345.600	0.5	700	40	N_2O			–	Ho
Iodine	I		178.276	60							I
Indium	In	303.9	325.609	18	150	40	Air	0.35	7.0*	100	In
Iridium	Ir	208.9	224.268	3.5	800	500	Air	6.8	135	97	Ir
Potassium	K	766.5	766.490	10	7	3	Air	0.02	0.4	90	K
Lanthanum	La	550.1	379.478	0.02	40000	2000	N_2O			–	La
Lithium	Li	670.8	670.784	0.6	20	2	Air	0.2	4	49	Li
Lutetium	Lu	336.0	261.542	0.05	7000	300	N_2O			–	Lu
Magnesium	Mg	285.2	279.553	0.1	3	0.3	Air	0.01	0.2	75	Mg
Manganese	Mn	279.5	257.610	0.3	20	2	Air	0.03	0.6	92	Mn
Molybdenum	Mo	313.3	202.030	4	300	20	N_2O	0.35	7	96	Mo
Nitrogen	N		174.272	50 000							N
Sodium	Na	589.0	588.995	1	3	0.2	Air	0.005	0.1	92	Na
Niobium	Nb	334.9	309.418	4	20000	2000	N_2O			–	Nb
Neodymium	Nd	492.5	401.225	2	6000	1000	N_2O			–	Nd
Nickel	Ni	232.0	231.604	5.5	70	10	Air	0.24	4.8	98	Ni
Osmium	Os	290.9	225.585	5	1000	100	N_2O			–	Os
Phosphorous	P	213.6	177.499	18	120000	40000	N_2O	110	2200*	69	P
Lead	Pb	217.0	220.353	14	100	10	Air	0.28	5.5	92	Pb
Palladium	Pd	244.8	340.458	7	50	10	Air	0.43	8.6	100	Pd
Praseodymium	Pr	495.1	417.939	0.8	20000	10000	N_2O			–	Pr
Platinum	Pt	265.9	265.945	20	1000	100	Air	3.5	70	82	Pt
Rubidium	Rb	780.0	780.023	35	50	10	Air	0.05	1	90	Rb
Rhenium	Re	346.1	227.525	11	8000	1000	N_2O			–	Re
Rhodium	Rh	343.5	343.489	5	100	5	Air	0.4	8	95	Rh
Ruthenium	Ru	349.9	267.876	5.5	400	100	Air	0.75	15	100	Ru
Sulphur	S		180.734	20						–	S
Antimony	Sb	217.6	217.581	18	300	40	Air	0.5	10	96	Sb
Scandium	Sc	391.2	361.384	0.4	300	50	N_2O			–	Sc
Selenium	Se	196.0	196.026	37	1000	500	N_2O	0.7	14*	92	Se
Silicon	Si	251.6	251.611	5	1500	300	N_2O	0.75	15	100	Si
Samarium	Sm	429.7	442.434	7	6000	1000	N_2O			–	Sm
Tin	Sn	235.5	242.949	15	700	100	N_2O	0.5	10*	93	Sn
Strontium	Sr	460.7	407.771	0.02	40	2	N_2O	0.1	2	94	Sr
Tantalum	Ta	271.5	268.517	9	10000	2000	N_2O			–	Ta
Terbium	Tb	432.7	350.917	5	7000	700	N_2O	0.18	3.5	90	Tb
Tellurium	Te	214.3	214.281	27	200	30	Air	0.45	9*	93	Te
Thorium	Th		274.716	17						–	Th
Titanium	Ti	364.3	334.941	0.6	1000	100	N_2O	2.5	50	100	Ti
Thallium	Tl	276.8	351.924	16	200	20	Air	0.75	15	63	Tl
Thulium	Tm	371.8	346.220	1.5	300	20	N_2O			–	Tm
Uranium	U	358.5	385.958	18	100000	40000	N_2O			–	U
Vanadium	V	318.5	309.311	2	700	100	N_2O	1.1	22	79	V
Tungsten	W	255.1	239.709	17	5000	1000	N_2O			–	W
Yttrium	Y	410.2	371.030	0.2	2000	200	N_2O			–	Y
Ytterbium	Yb	398.8	328.937	0.3	60	4	N_2O	0.15	3	97	Yb
Zinc	Zn	213.9	213.856	0.9	8	1.0	Air	0.0075	0.15	92	Zn
Zirconium	Zr	360.1	339.198	1.5	9000	1000	N_2O			–	Zr

*Modifier used to obtain these results.

** 20 µL injection

*** The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Analytical Requirements

Before deciding which technique is appropriate, the chemist must define both present and future analytical requirements. That is:

- Number of samples/week?
- What matrices need to be analyzed? For example, steels, bronzes, effluents, soils.
- How many elements need to be determined for each sample type?
- What are the typical sample volumes?
- What elements need to be determined?
- What concentration ranges are present in the matrices?
- Would an Internal Standard be useful? For example, where the samples may change in viscosity from sample to sample, for example, battery acid analysis.
- What expertise do the operators have?
- How much money is available to purchase or lease costs/month?
- Cost of ownership and running costs. Can the user afford an automated AAS or ICP-ES, or is a simple AAS sufficient?

The answers to these questions will help you to decide which is the preferred technique. Sometimes the answer is further complicated by the fact that neither flame AAS nor ICP-ES will satisfy all requirements. You may find, as many do, that both an ICP-ES and a furnace AAS will be necessary to meet the analytical requirements.

For Deuterium Furnace systems, the equivalent Characteristic Concentration and Characteristic Mass is easily calculated using the following conversion:

CMn = CMz X MSR (%)/100 CCn = CCz X MSR (%)/100

where:

CMn = Characteristic Mass for Deuterium Furnace Systems

CMz = Characteristic Mass for Zeeman Furnace Systems (from Table 1)

MSR = Magnetic Sensitivity Ratio (as % from Table 1)

CCn = Characteristic Concentration for Deuterium Furnace Systems

CCz = Characteristic Concentration for Zeeman Furnace Systems (from Table 1).

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Improving Throughput for Oils Analysis by ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

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Introduction

Trend analysis of wear metals in lubricating oils is a proven, cost-effective predictive maintenance technique. The presence and levels of various metal elements in lubricating oils gives an indication of the type of wear occurring in an engine. For example, an increase in the level of copper may indicate increased wear of bushings. Non-metals such as silicon, boron and phosphorus elements can also be determined. Monitoring the levels of wear metals and other elements in lubricating oils provides many benefits apart from predicting engine failure. For example, machinery can be kept up and running until maintenance becomes necessary, avoiding premature maintenance. Potential problems can be associated with specific components, eliminating complete teardowns.

The inductively coupled plasma optical emission spectroscopy (ICP-OES) technique for monitoring wear metals is the method of choice for trend analysis because it is fast and accurate. For the busy laboratory, not only is accuracy and long-term stability important; sample throughput is often a vital factor. The most significant contributor to the time taken for an analysis is the sample introduction system; the actual measurement time is most often less than one tenth of the total analysis time. This work shows that the use of a novel pump tubing arrangement can improve the speed of analysis. Using an improved sample introduction system, it was possible to accurately determine key wear metals and other elements in less than 50 seconds per sample using one simple method.

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Experimental

Instrumental

A Vista-PRO simultaneous ICP-OES with a radially viewed plasma was used. The radial plasma configuration is the accepted standard for the oils industry. The radial plasma orientation allows direct venting of combustion products, thereby reducing carbon build-up on the torch. The highly efficient 40 MHz free-running RF generator is easily able to cope with solvents to produce a stable, robust plasma with excellent long term stability. The instrument was fitted with a 3 channel peristaltic pump to allow a modified pump tubing configuration for faster sample uptake and washout. A glass concentric nebulizer with wide internal bore size was used to better handle particulates, and a glass double-pass spraychamber was used to prevent overloading the plasma with sample. Optimized instrument operating conditions are set out in Table 1.

Table 1. Instrument Operating Conditions

Parameter	Setting	Part number (where applicable)
Power	1.35 kW	
Plasma gas flow	15.0 L/min	
Auxilliary gas flow	2.25 L/min	
Nebulizer pressure or flow	110 kPa or 0.60 L/min	
Viewing height	10 mm	
Pump speed	12 rpm	
Sample uptake delay	15 s	
Stabilization time	5 s	
Rinse time	10 s	
Replicate read time	1 s	
Replicates	2	
Nebulizer type	Slurry glass concentric	20-100976-00
Torch type	Radial fully demountable torch kit (includes bracket and clamp)	99-101064-00
Spraychamber	Twister double pass	79-100437-00
Sample tubing to nebulizer	Grey/grey solvent flex	37-100352-00
Sample tubing to waste	Black/black solvent flex	37-100348-00
Tubing to waste from spraychamber	Solvent flex waste tubing	37-100354-00
Transfer tubing	Solvent flex transfer tubing ¼"internal diameter	37-100378-00
Drain tubing	Purple/black solvent flex	37-100470-00
Autosampler	AIM 1250*	

* Manufactured by A.I. Scientific, Scarborough, Qld, Australia

Standards and Reagents

Calibration solutions of 5, 10, 25, 50, 100, and 250 mg/L were prepared from Conostan S-21 certified standard, which contains 21 elements (Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Ti, V, Zn) at 500 mg/kg in oil. These calibration solutions were viscosity matched using Conostan base oil 75. Single element standards of Ca, Fe, Pb, P, and Zn were prepared from certified 5000 mg/kg Conostan standards (Conostan Division, Conoco Specialty Products Inc., Ponca City, OK, USA). The single element standard concentrations prepared were 10, 25, 50, 100, 250, 500, 1000 and 2500 mg/L. Jet-A1 kerosene (Mobil, Melbourne, Australia) was used as diluent.

Results

Detection Limits

In general, sensitive emission line wavelengths have lower detection limits than less sensitive emission line wavelengths for any given element. This is because sensitive emission lines produce a larger signal for a given concentration than less sensitive emission lines. Thus, low concentrations can be better detected using a sensitive emission line wavelength than an insensitive one. Frequently, detection limits improve with increasing read time because readout noise is reduced. The detection limits of various elements in kerosene are shown in Table 2. All detection limits in the table are below 1 mg/L, which easily allows trace levels of wear metals to be detected and a trend to be observed, even at low levels.

Table 2. Detection Limits of Elements in Kerosene at 2, 5 and 10 Seconds Integration Time

Element and emission line wavelength	3 σ Detection limits (mg/L) 1 s	2 s	3 s
Ag 328.068	0.006	0.003	0.002
Al 308.215	0.05	0.02	0.02
Al 396.152	0.05	0.02	0.01
B 249.772	0.021	0.007	0.005
Ba 455.403	0.003	0.002	0.001
Ba 493.408	0.0010	0.0007	0.0005
Ca 317.933	0.02	0.01	0.01
Ca 396.847	0.002	0.002	0.002
Cd 226.502	0.023	0.003	0.002
Cr 284.325	0.012	0.005	0.003
Cu 327.395	0.011	0.004	0.003
Fe 259.940	0.014	0.006	0.005
Fe 274.932	0.06	0.02	0.02
Mg 280.270	0.001	0.001	0.001
Mn 257.610	0.002	0.001	0.000
Mo 202.032	0.072	0.009	0.005
Na 589.592	0.004	0.002	0.002
Ni 230.299	0.08	0.02	0.01
P 213.618	0.26	0.03	0.02
Pb 220.353	0.39	0.05	0.03
Si 251.608	0.05	0.02	0.02
Sn 283.998	0.11	0.04	0.02
Ti 336.122	0.003	0.002	0.001
V 311.837	0.012	0.004	0.003
Z n 206.200	0.063	0.007	0.005
Zn 213.857	0.017	0.002	0.002

Linear Range

In general, the maximum accurately measurable concentration of an element is obtained by using a less sensitive emission line wavelength for that element. Although sensitive emission line wavelengths have lower detection limits than insensitive ones, insensitive emission line wavelengths can measure higher maximum concentrations. Some elements, such as calcium and phosphorus, may be present at high concentrations in oils, so a high maximum measurable concentration is desirable. The wavelengths chosen for analysis reflect a compromize between best detection limits and desired concentration range.

Table 3. Maximum Measurable Concentration of Selected Elements at Specified Emission Line Wavelengths

Element and emission line wavelength	Maximum concentration (mg/L)
Ag 328.068	250+
Al 308.215	250+
Al 396.192	100
B 249.772	250+
Ba 455.403	100
Ba 493.408	250+
Ca 317.933	2500
Ca 396.847	100
Cd 226.502	250+
Cr 284.325	250+
Cu 327.395	250+
Fe 259.940	250+
Fe 274.932	1000
Mg 280.270	100
Mn 257.610	250+
Mo 202.032	250+
Na 589.592	250+
Ni 230.299	250+
P 213.618	2500
Pb 220.353	1500
Si 251.608	250+
Sn 283.998	250+
Ti 336.122	250+
V 311.837	250+
Zn 206.200	2500
Zn 213.857	250

* Note that 250+ designates an accurately measurable concentration that may surpass 250 mg/L.

Modified Pump Tubing Setup

To speed up sample delivery to the plasma, the flow rate of sample through the autosampler probe was increased based on the "rapid flow" concept conceived by Shane Elliott and investigated as applied to organic solutions by Ross Ashdown (both from Agilent). The idea is to increase the flow rate of sample from the autosampler to the peristaltic pump. To increase the sample flow rate, a wider internal diameter peristaltic pump tubing could have been used, but this would overload the nebulizer, adversely affecting nebulization. Instead, an additional sample peristaltic pump tube was introduced to the system via a T-piece inserted between the end of the autosampler line and the start of the sample peristaltic pump tubing so that sample would flow through two sample perstaltic pump tubings instead of one. One of the peristaltic pump tubes was directed to the nebulizer, and the other to waste, which avoided overloading the nebulizer with sample. By having sample flow through two pump tubings, the sample flow rate through the autosampler probe up to the point where the T-piece was inserted was increased, thus reducing sample uptake time.

To measure sample uptake time, kerosene was introduced to the autosampler probe manually after aspirating air, and the time taken for the plasma to turn bright green (which indicates that organic solution is being aspirated into the plasma) was measured by stopwatch. Table 4 shows that using the modified pump tubing setup, the sample uptake time was decreased by approximately 10 seconds. An added benefit of decreasing sample uptake time is that the time taken to achieve a fixed degree of washout is also reduced.

Table 4. Time Saved Using Modified Pump Tubing Setup

Pump tubing configuration	Acutal sample uptake time (s)	Sample uptake time in method (s)
Standard	24	25
Modified	15	15

Washout

To determine the washout achieved in an autosampler run, an analysis was performed where a blank kerosene solution was measured immediately following a solution containing 1000 mg/L of Fe. These two solutions were then measured in pairs six times each. Table 5 shows that three orders of reduction in sample concentration was achieved in an autosampler run with a rinse time of 10 seconds. If a more thorough rinse was required, then SmartRinse could have been used. The SmartRinse feature of the ICP Expert software optimizes the rinse time for each sample, ensuring that the rinse time is only as long as required to return the signal to that of a blank for each wavelength in the analysis [1]. This means that high concentration samples will take longer to analyze than low concentration samples. For this work, a washout of three orders was acceptable, so a short, fixed rinse time was used.

Table 5. Blank Results After Measuring 1000 mg/L Iron. This Demonstrates that Three Orders of Washout is Achieved with a Rinse Time of 10 Seconds.

Kerosene blank measurement number	Measured Fe conc. (mg/L)
2	0.66
4	0.77
6	0.79
8	0.79
10	0.80
12	0.64

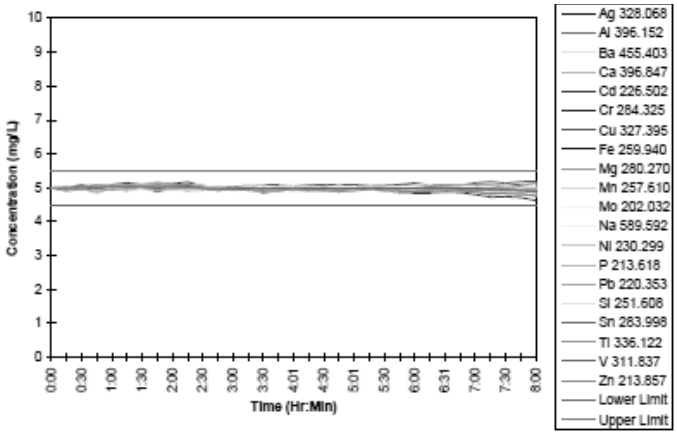

Figure 1. Stability of the Vista-PRO radial instrument over 8 hours. Results remained within ±10% for all elements in the 5 mg/L S21 kerosene solution without internal standardization or recalibration.

Long-Term Stability

A 5 mg/L solution of S21 elements in Jet-A1 kerosene was analysed continuously over an eight hour period. No recalibrations were performed, and no internal standard was used. Figure 1 shows that results remained within 10% of the true value over the entire 8 hours. Precision was typically better than 2 %RSD.

Conclusion

The Vista-PRO radial ICP-OES provides excellent throughput at 47 seconds per sample using a simple optimized sample introduction system. The detection limits and maximum measurable concentration of selected wavelengths allows typical oil samples to be analysed, while the excellent stability allows continuous running without recalibration, providing a saving on costs by reducing analysis time and the amount of standard solution used.

Acknowledgements

The author would like to thank Shane Elliott (Varian Australia) for the initial concept and his advice with alternative sample pump tubing configurations, Ross Ashdown (Varian U.K.) for his early work with fast throughput for organics, Barry Sturman, Alan Wiseman and Kate Pearson-Santiago (Varian Australia) for editing, and Glyn Russell (Varian Australia) for his input, encouragement and review of this work.

Reference

1. I. Szikla, SmartRinse - the latest advance in maximizing

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Agilent Technologies

Agilent Hi-Plex Columns for Sugar Separation: Effects of Temperature and Mobile Phase

Application Note

Food

Author

Stephen Ball

Agilent Technologies, Inc.

Introduction

The Agilent Hi-Plex H is a high-performance ligand-exchange chromatography column. The column is based on polystyrene/divinylbenzene with an 8% crosslinking and hydrogen counter ion. Typically used for the analysis of sugars, sugar alcohols, and organic acids, its monodisperse sulfonated packing gives improved column efficiency, lower column pressure, and assured batch-to-batch reproducibility.

This application note investigates the effect of temperature and mobile-phase acid concentration on the separation of sugars and organic acids in wine.

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Materials and Reagents

Sample Preparation

The seven compounds listed in Table 1 were weighed into the same vial in the quantities described and dissolved in 10 mL of 0.01 M H_2SO_4. Injection volume was 20 µL.

Table 1. Compound Quantities

Constituent	Amount (g)
1. Citric acid	0.1010
2. Tartaric acid	0.1032
3. Glucose	0.1011
4. Malic acid	0.1018
5. Fructose	0.1011
6. Lactic acid	0.1015
7. Glycerol	0.1131

Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.01 M H_2SO_4
Flow rate	0.4 mL/min
Temperature	> 75 °C
Detector	RI

Results and Discussion

This mixture of sugars and organic acids is particularly difficult to analyze as several of the compounds elute very closely together and often simultaneously. However, these results show that as the temperature is increased, the minimum resolution of the separation gradually increases until all seven compounds are nearly separated.

At 35 °C, there are two pairs of co-eluted peaks, but by increasing the temperature to 55 °C, they have started to split into pairs of peaks. This increase in temperature, however, causes fructose and malic acid to become co-eluted. By increasing the temperature further to 75 °C, these two components begin to separate in the reverse order (Figure 1).

To gain complete separation of all seven compounds, this analysis would need to be run above 75 °C.

By varying the concentration of the sulfuric acid in the mobile phase, the selectivity of the column can be altered (Figure 2). At high concentrations, fructose and malic acid co-elute. Therefore, by reducing the mobile phase acid concentration, the minimum resolution of the separation can be improved. However, this reduction in mobile-phase acid strength does result in slightly fronted peak shapes for the organic acids. The best separation obtained consisted of a 0.003 M H_2SO_4, where malic acid elutes almost halfway between glucose and fructose.

By comparing these two sets of results, it can be seen that temperature is a more powerful tool in gaining complete separation of all seven compounds in this particular mixture, as resolution between them can be achieved while maintaining good peak shape.

Peak identification

1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Lactic acid
7. Glycerol

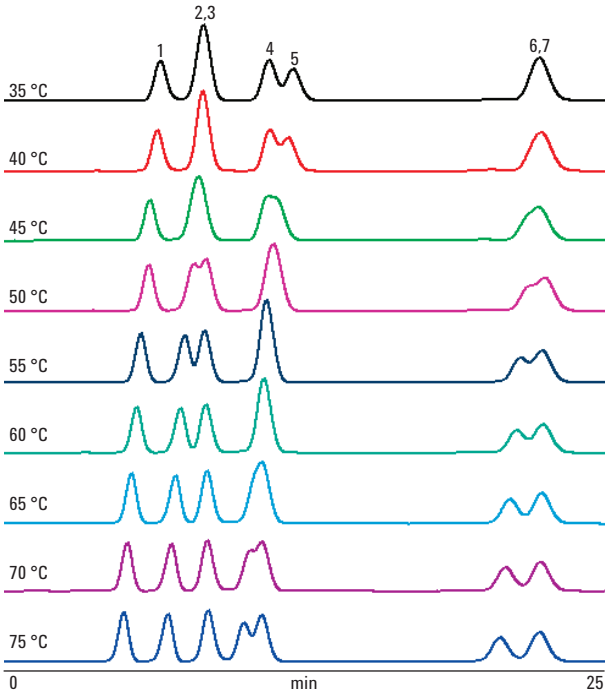

Figure 1. Effect of temperature on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

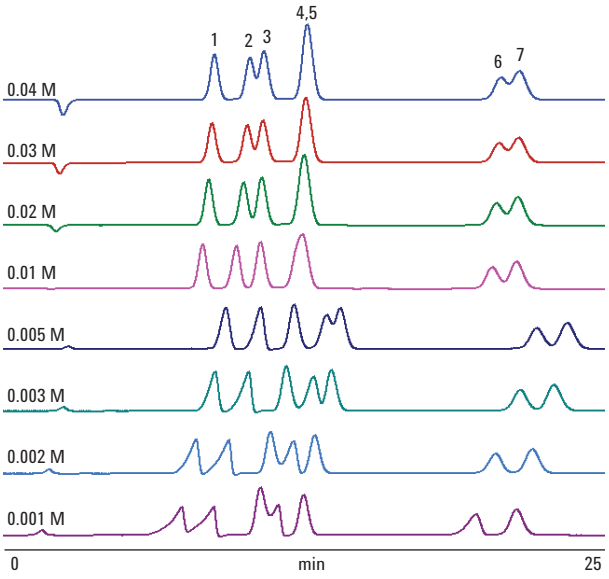

Figure 2. Effect of acid concentration on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

Conclusion

The analysis of wines demonstrates how Agilent Hi-Plex H columns provide optimum resolution of closely eluting compounds, enabling quantitation of each. These columns are ideal for the analysis of sugar alcohols and sugar molecules, using sulfuric acid as the mobile phase. The Hi-Plex H is also the column of choice for the analysis of organic acids, using dilute mineral acid as eluent. By using the columns at higher operating temperatures, closely eluting compounds can be resolved.

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Agilent Technologies

Simple Analysis of Carbohydrates by HPLC Using Evaporative Light Scattering Detection

Application Note

Food

Author

Stephen Bullock

Agilent Technologies, Inc.

Introduction

The separation, identification, and quantification of simple sugars can be readily achieved using chromatography. High-performance liquid chromatography (HPLC) is perhaps the simplest technique, often requiring little in the way of sample preparation, particularly with liquids.

Sugars may be detected with the Agilent evaporative light scattering detector (ELSD) and an Agilent Hi-Plex column that has strong cation-exchange resins available in differing ionic forms. The sulfonated column resin gives a fundamental improvement in performance and overcomes the problems of low efficiencies and high backpressures encountered with soft gels. The separation mechanism is achieved initially by size exclusion, with larger oligosaccharides eluting before smaller monosaccharides, and then by ligand-exchange interaction of the numerous hydroxyl groups on the sugar molecules with the metal ion associated with the resin. Hi-Plex columns are used at elevated temperature with isocratic eluents.

As neutral carbohydrates have limited UV activity, the most commonly used detector with these columns is refractive index (RI). However, there are a number of issues related to the use of RI detectors, including baseline stability and sensitivity. A better method of detection is provided by evaporative light scattering detection. The Agilent ELSD does not require the solutes of interest to have any optical properties. The principle of operation is a three-stage process. The first stage involves the nebulization of the eluent; the second, the evaporation of the solvent to leave solute particles; and the third, the detection of the light scattered by the solid solute particles as they pass through the light beam. The only requirement for using the Agilent ELSD is that the eluent be more volatile than the solutes.

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When using Agilent Hi-Plex columns for the analysis of carbohydrates, water (with no buffer or added salt) is used as the eluent, making this an ideal application for the Agilent ELSD because neutral carbohydrates have little UV activity.

Hi-Plex resins are available in 8% crosslinked calcium and lead forms for the analysis of mono- and disaccharides and in hydrogen (acid) forms for the analysis of sugar alcohols and organic acids. Also available is a 4% crosslinked sodium form for the separation of high molecular weight oligosaccharides, such as corn syrups, to Dp 9.

Instrumentation

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Detector	Agilent ELSD

Materials and Reagents

Mobile phase 100% DI H_2O

Results and Discussion

A separation of standard sugars – raffinose, lactose, glucose, galactose, and fructose – was obtained using the detection system (Figure 1). Calibration curves were produced for the six solutes in the test mixtures, as shown in Figure 2.

Conclusion

The separation and detection of raffinose, lactose, glucose, galactose, and fructose are readily achieved using water as the mobile phase with an Agilent Hi-Plex Ca column and the Agilent ELSD. This system avoids the use, high cost, and disposal implications of toxic acetonitrile when separations are performed on amino silica columns. In addition, Hi-Plex stays active in the presence of sugar molecules. Together with fast dissolution, this benefit results in long lifetimes compared to amino silica columns.

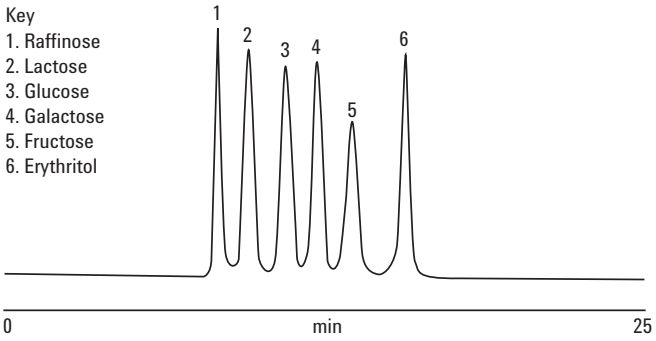

Figure 1. Good separation of six simple sugars using the Agilent ELSD and an Agilent Hi-Plex Ca column.

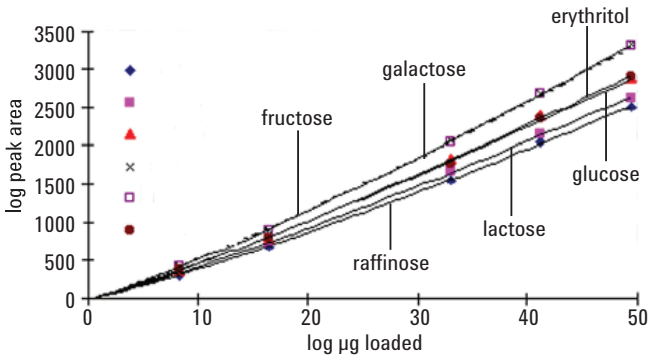

Figure 2. Calibration curves of six sugars using the Agilent ELSD and an Agilent Hi-Plex Ca system.

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Agilent Technologies

Rapid Profiling of Saccharides Using HPLC with ELSD for Improved Precision

Application Note

Food

Author

Stephen Bullock

Agilent Technologies, Inc.

Introduction

Saccharides are of great importance in nature, and chemists and biochemists require sensitive and robust analytical methods for their identification and quantification.

These compounds do not possess a UV chromophore and are therefore not suited to UV detection. Normally, the nonchromophoric sugar separations would be performed using a refractive index (RI) detector. However, RI commonly suffers from baseline instability and poor sensitivity. Due to the nonvolatile nature of saccharides, evaporative light scattering detection (ELSD) using the Agilent ELSD, is better for this type of analysis, offering excellent baseline stability. There are a number of HPLC methods to quantify saccharides, with one of the most simple being the use of a calcium ligand-exchange column, Agilent Hi-Plex Ca, with water as the eluent.

The Hi-Plex Ca column contains a monodisperse sulphonated polystyrene incorporating 8% divinylbenzene with a calcium counter ion and provides a separation based on a combination of both size exclusion and ligand-exchange chromatography. These soft gel columns are operated at elevated temperature in order to reduce operating pressure and permit regular flow rates to be employed. Sensitivity in saccharide detection is achieved by using the Agilent ELSD. In addition to an increase in sensitivity, this detector also gives a more stable, drift-free baseline, improving the precision of the quantitation.

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Experimental

Instrumentation

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Detector	Agilent ELSD

Materials and Reagents

Mobile phase 100% DI H_2O

Sample Preparation

Saccharides were dissolved in water at 1.0 mg/mL.

Results and Discussion

Figure 1 shows that the five saccharide standards are well-resolved and the baseline is extremely stable.

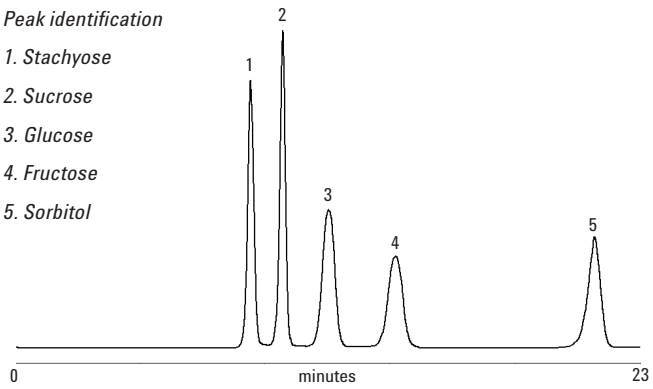

Figure 1. Excellent separation to baseline of five saccharides by Agilent Hi-Plex Ca columns with the Agilent ELSD.

Conclusion

Combining the Agilent Hi-Plex Ca column with the Agilent ELSD provides an excellent solution for resolving saccharides. The sulfonated resin in Hi-Plex Ca offers a fundamental improvement in performance. Its monodisperse sulfonated packing overcomes problems of low efficiencies and high backpressures encountered with soft gels. The Agilent ELSD surpasses other ELSDs for low-temperature HPLC applications with semivolatile compounds. The Agilent ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100% water at a flow rate of 5 mL/min can be removed at 30 °C.

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Agilent Technologies

HPLC Analysis of Sugars and Glycoproteins in Biological Systems

Application Note

Bioanalysis

Author

Linda Lloyd

Agilent Technologies, Inc.

Introduction

Free sugars, other than glucose and fructose, rarely occur in nature. However, other sugars such as the pentoses (arabinose, xylose, and ribose) and the hexoses (mannose and galactose) are found as integral parts of biological macromolecules. The function of the carbohydrate components of these macromolecules is diverse; they can be important either biochemically, for example targeting sequences in glycoproteins, or structurally, as in DNA and RNA. The sequences of monosaccharides are only obtained as breakdown products during fermentation.

Levels of lactate and pyruvate found in blood serum are important indicators of health. Elevated blood lactate levels are indicative of diabetes, and pyruvate of possible heavy metal poisoning. It is important to reduce sample handling to a minimum during analysis of complex biological fluids. Because samples can often be analyzed directly using Agilent Hi-Plex H columns, the potential for errors is reduced. Hi-Plex columns are well suited to the fast analysis of sugars and glycoproteins in plant and animal tissues.

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Experimental

Instrumentation

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Detector	RI

Materials and Reagents

Mobile phase	0.005 M H_2SO_4 (sugars), 0.0005 M H_2SO_4 (compounds of physiological significance)

Conditions

Flow rate	0.6 mL/min
Temperatures	60 °C (sugars), 55 °C (compounds of physiological significance)

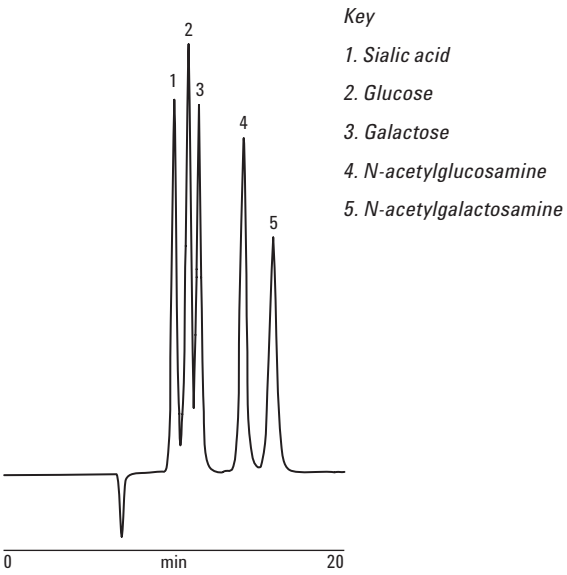

Figure 1. Good separation of glucose and galactose from their derivatives achieved by HPLC with Agilent Hi-Plex H columns.

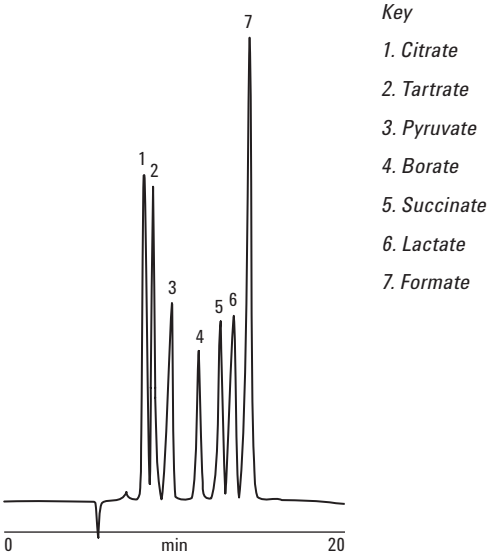

Figure 2. The presence of lactate and pyruvate revealed by HPLC with Agilent Hi-Plex H columns.

Results and Discussion

Figure 1 shows how Agilent Hi-Plex H columns separate glucose- and galactose-free sugars from their derivatives N-acetylglucosamine and N-acetylgalactosamine. The latter are found in the cell membranes of higher organisms as carbohydrate residues, linking the amino acid chain to the carbohydrate component of membrane glycoproteins. Figure 2 shows good resolution of lactate and pyruvate from other salts found in animal tissues.

Conclusion

Agilent Hi-Plex columns are packed with sulfonated resin, giving a fundamental improvement in performance to overcome the problems of low efficiencies and high back-pressures encountered with soft gels. The columns are available in hydrogen form for fast analysis of glycoproteins and sugars in biological systems. Accurate determination of composition and content is ensured.

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Agilent Technologies

Oligosaccharide Analysis on Agilent Hi-Plex Phases

Application Note

Food

Authors

Stephen Ball, Linda Lloyd

Agilent Technologies, Inc.

Introduction

Oligosaccharides are saccharide polymers containing usually three to ten component sugars. Some common oligosaccharides include fructooligosaccharides, found in many vegetables, and galactooligosaccharides, which also occur naturally. These compounds are only partially digestible by humans. However, glucose oligosaccharides, produced by the hydrolysis of starch, are a major energy source.

Oligosaccharides can be analyzed on two Agilent Hi-Plex phases, in order to determine the quantities of each different chain length in the sample. The Agilent Hi-Plex Na, with a crosslinking of 4% and a particle size of 10 µm, can separate oligosaccharides up to Dp 8. Alternatively, the Agilent Hi-Plex Ca (Duo), with an 8% crosslinking, can be used for the faster separation of oligosaccharides up to Dp 5.

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Because the Agilent Hi-Plex Na material has a crosslinking of 4%, it has the largest pore size of the entire range. This, in turn, allows the Hi-Plex Na to resolve the higher oligomers and gives definition in excess of Dp 8 for the oligomers of glucose, as shown in Figure 1.

Conditions

Column	Agilent Hi-Plex Na, 7.7 × 300 mm, 10 µm (p/n PL1171-6140)
Mobile phase	100% DI H_2O
Flow rate	0.4 mL/min
Temperature	85 °C
Detector	RI

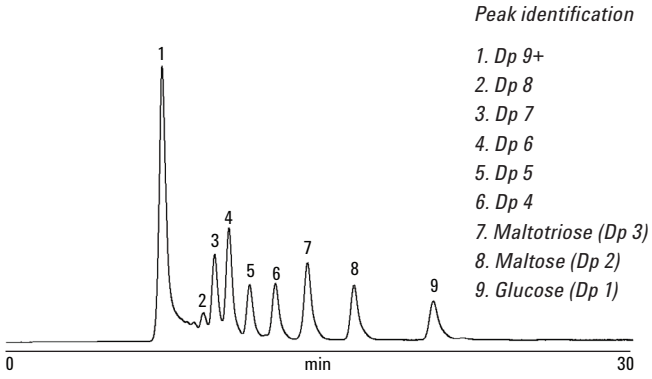

Figure 1. Oligosaccharide separation up to Dp 8 using an Agilent Hi-Plex Na column.

As the Hi-Plex Ca (Duo) is an 8% crosslinked material, its separation mechanism is predominantly size, but the higher crosslinked density reduces the number of oligomers that can be resolved, typically Dp 5 and below.

This material has improved mechanical strength relative to the softer Hi-Plex Na, and its calcium counter ion gives improved ligand-exchange capabilities, per Figure 2.

Conditions

Column	Agilent Hi-Plex Ca (Duo), 6.5 × 300 mm, 8 µm (p/n PL1F70-6850)
Mobile phase	100% DI H_2O
Flow rate	0.5 mL/min
Temperature	90 °C
Detector	RI

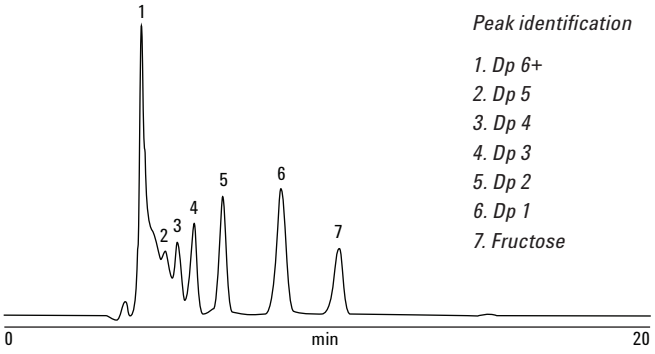

Figure 2. Oligosaccharide separation up to Dp 5 using an Agilent Hi-Plex Ca (Duo) column.

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Agilent Technologies

Analysis of a Sugar, Organic Acid, and Ethanol Reference Sample

Application Note

Food

Authors

Stephen Ball, Linda Lloyd

Agilent Technologies, Inc.

Introduction

Food products commonly contain a mixture of sugars (of varying chain lengths), sugar alcohols, and organic acids. This application note shows how an Agilent Hi-Plex H ligand-exchange chromatography column and refractive index (RI) detector can be used to quantify levels of these components in a sample of food.

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Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Sample	Sugars + acid mixture
Sample size	10 mg/mL each
Mobile phase	0.05 M H_2SO_4
Flow rate	0.5 mL/min
Injection volume	20 µL
Temperature	Ambient
Detector	RI

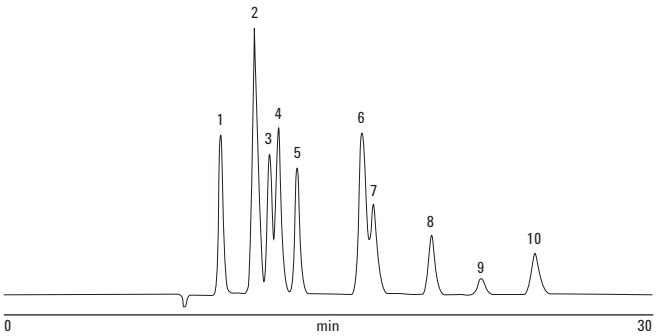

Figure 1. Separation of a sugars and acids mixture using an Agilent Hi-Plex H 8 µm column. See Table 1 for peak identification.

Table 1. Peak Identification for Figure 1

Peak	Name	Time (min)	Height (µV)	Area (%)	Width 50% (min)	As. USP	10% Asymmetry	Res. HW	Plate counts	Plates/m
1	Sucrose	10.04	193813.4	9.998	0.23	1.20	1.15	0.00	10870	36233
2	Citric acid, glucose	11.60	323837.5	21.448	0.30	1.24	1.18	3.48	8312	27708
3	Tartaric acid	12.28	170815.1	9.038	0.26	0.87	0.91	1.46	12812	42705
4	Fructose	12.70	203381.4	12.313	0.28	1.29	1.15	0.91	11430	38099
5	Malic acid	13.55	154736.4	9.548	0.27	1.14	1.12	1.85	14375	47918
6	Lactic acid, glycerol	16.53	196730.6	16.474	0.41	0.98	1.05	5.22	9104	30346
7	Succinic acid	17.07	110886.3	8.846	0.40	1.66	1.47	0.78	10298	34325
8	Acetic acid	19.72	73828.0	5.480	0.33	1.17	1.16	4.28	19217	64056
9	Methanol	22.00	19729.7	1.724	0.36	1.25	1.14	3.88	20778	69259
10	Ethanol	24.47	50557.3	5.131	0.42	1.25	1.20	3.75	19042	63475
Total			**1498315.5**	**100.000**						

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Agilent Technologies

Cellulose Hydrolysate Analysis by HPLC

Application Note

Biofuels

Authors

Stephen Ball, Linda Lloyd,
Keeley Mapp
Agilent Technologies, Inc.

Introduction

Cellulose is a polysaccharide consisting of a linear chain of several hundred to over ten thousand linked D-glucose units. It provides the structure of the cell wall in green plants. Cellulose can be hydrolyzed into its glucose units by treating it with concentrated acids at high temperature. Alternatively, enzymes such as the endo-acting cellulase break cellulose down into individual glucose units.

HPLC using an Agilent Hi-Plex Ca column analyzes the breakdown products of an enzymic digestion of cellulose.

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Experimental

An isocratic HPLC system was set up with a column block heater and an RI detector.

Conditions

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Mobile phase	100% DI H_2O
Flow rate	0.6 mL/min
Temperature	85 °C

Sample Preparation

A 10 mg/mL solution of cellulase (CAS 9012-54-8) in water was adjusted to an approximate pH of 4.5 with 0.01 M HCl. Ten milliliters of this solution were then added to 0.1 g of chromatography-grade cellulose (CAS 9004-34-6) in a 25 mL conical flask.

The contents of the flask were left in a water bath and heated to 40 °C for 24 hours, during which time 1 mL aliquots were extracted for analysis. Each sample and the liquid remaining after 24 hours were passed through a 0.45 µm syringe filter to remove any remaining cellulose from the sample (effectively preventing any further hydrolysis). All samples were stored in the freezer before analysis.

Twenty microliter injections were made of each sample to analyze for breakdown products.

Results

The following chromatograms track the levels of the sugars resulting from the enzymic hydrolysis of cellulose over time.

Aliquots were collected at 2 hours through the process, 19 hours (after being left overnight), 21 hours, and finally 24 hours.

Discussion

From an early stage in the process, two different sugar molecules begin to form in the solution: glucose and cellobiose.

Cellobiose is a disaccharide derived from the condensation of two glucose molecules linked in a ß (1 ⇒ 4) bond. This is a byproduct of the enzyme-catalyzed hydrolysis of cellulose.

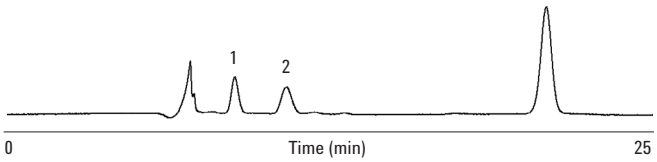
As the elapsed time increases, so does the concentration of cellobiose and glucose, indicating that increased numbers of cellulose chains are breaking down into smaller sugar units.

The additional peaks at the beginning and end of the chromatograms are likely to be additional side-products of the hydrolysis reaction or cellulase itself present in solution.

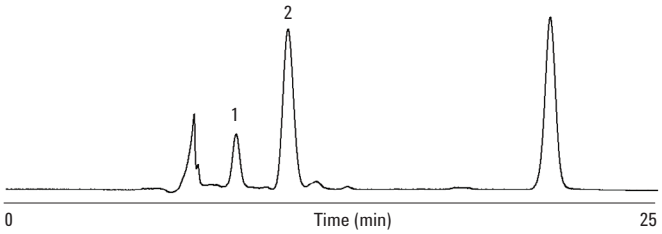
Peak identification (for all figures)

1. Glucose
2. Cellobiose

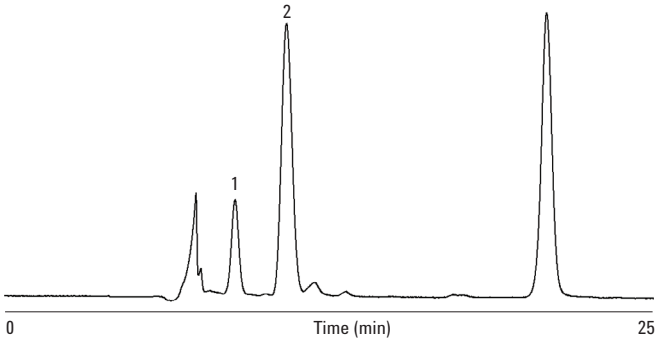
Reaction time: 2 hours



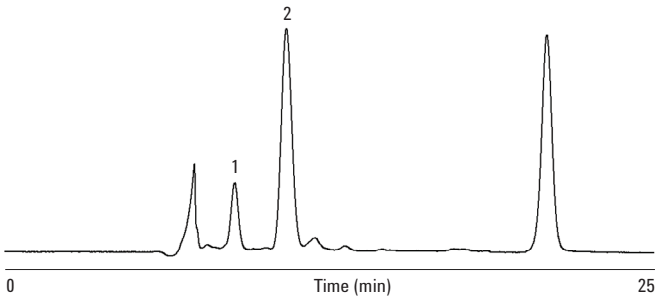
Reaction time: 19 hours



Reaction time: 21 hours



Reaction time: 24 hours



Conclusion

The Agilent Hi-Plex Ca column can be used to quantify the levels of the sugars in solution that result from the hydrolysis of cellulose.

A potentially useful application of this HPLC procedure is in the quality control of a glucose manufacture process or in the biofuels industry, where enzymes are often used to break down cellulose and hemicelluloses.

For More Information

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www.agilent.com/chem

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SI-01921

Agilent Technologies

Analysis of Bioethanol Fermentation Products Using an Agilent Hi-Plex H Column

Application Note

Biofuels

Author

A. Ewen

Prüf- und Forschungsinstitut

Pirmasens

Germany

Introduction

The fermentation of biomass to ethanol is an economically important process. For the control of the fermentation, HPLC is the method of choice because it can separate and detect carbohydrates (starting material), acids (potential inhibitors), fusel alcohols (byproducts), and ethanol (product) in one step without prior derivatization. This application note describes the analysis of a sample of a batch bioethanol fermentation product using an Agilent Hi-Plex H column.

Agilent Technologies

Materials and Methods

The sample (a suspension of biomass and yeast in water) was pretreated by filtration through a 0.45 µm membrane.

Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.005 M H_2SO_4
Gradient	Isocratic
Flow rate	0.7 mL/min
Injection volume	20 µL
Sample concentration	Glucose ~ 37 g/L
	Xylose ~ 3.5 g/L
	Succinic acid ~ 1 g/L
	Lactic acid ~ 200 mg/L
	Glycerol ~ 10 g/L
	Acetic acid ~ 700 mg/L
	Methanol ~ 1.5 g/L
	Acetaldehyde ~ 300 mg/L
	Ethanol ~ 87 g/L
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

Results

The chromatogram clearly indicates that the biomass sample contains large amounts of starting material, organic acids, byproducts, and final product mixed together.

Conclusion

A sample from a batch fermentation was resolved with good separation using an Agilent Hi-Plex H column. Hi-Plex H columns are ideal for the analysis of sugar alcohols and sugar molecules using water as the mobile phase. Hi-Plex H is also the column of choice for the analysis of organic acids, using dilute acid as eluent. The use of a ligand-exchange chromatography column such as Hi-Plex H significantly reduces the need for complicated sample preparation (typically involving elution through an ion-exchange resin bed), as retention is brought about not only by ion exchange, but also by ion exclusion and partitioning on this type of column.

For More Information

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Peak identification

1 - 4 di- and oligosaccharides
5 Glucose
6 Xylose
7 Succinic acid
8 Lactic acid
9 Glycerol
10 Acetic acid
11 Acetaldehyde
12 Methanol
13 Ethanol

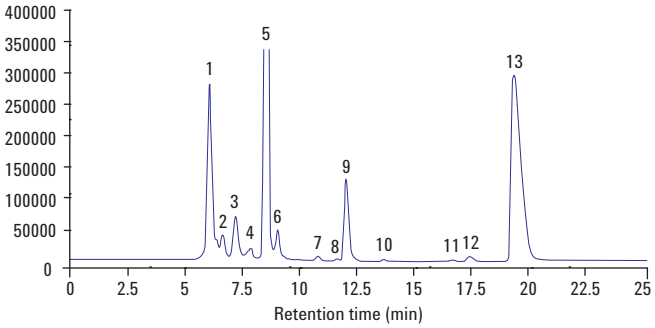

Figure 1. HPLC chromatogram of biomass and yeast in water, using an Agilent Hi-Plex H column.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-1941

Agilent Technologies

Analysis of Byproducts in Fermentation Liquids Using an Agilent Hi-Plex H Column

Application Note

Food and Beverage

Author

A. Ewen

Prüf- und Forschungsinstitut

Pirmasens

Germany

Introduction

Biomass fermentation has grown in importance because diverse products such as fuel, lubricants, and chemicals can be derived. One option for this use of biomass is the fermentation of xylose from hemicelluloses, to xylitol, a sugar substitute. For the HPLC analysis of fermentation liquids, the US NREL Biomass Program method *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples* can be applied.

Agilent Technologies

Materials and Methods

Two fermentation samples were analyzed. The first was obtained by a hydrothermal digestion of straw (as an example of biomass) that destroys the hemicelluloses and frees the xylose. Following partial evaporation of water, the second sample was obtained after fermentation of xylose to xylitol.

Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.005 M H_2SO_4
Gradient	Isocratic
Flow rate	0.7 mL/min
Injection volume	20 µL
Sample concentration	Xylose ~ 8 g/L
	Glucose ~ 1.5 g/L
	Xylitol ~ 13 g/L
	Furfural 10 ~ 500 mg/L
	Hydroxymethylfurfural ~ 100 mg/L
	Acetic acid ~ 1000 mg/L
	Ethanol ~ 2000 mg/L
	Lactic acid ~ 2500 mg/L
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

Results

After hydrothermal digestion, a large quantity of xylose is present in solution, as expected (Figure 1). Figure 2 shows that further fermentation of the sample converts a large quantity of this xylose into xylitol and gives a very large RI response for this sugar alcohol.

Conclusion

The Agilent Hi-Plex H column is specially suited for the analysis of byproducts and degradation products (acids, alcohols, furfural, hydroxymethylfurfural), such as those produced by biomass fermentation. The column is recommended for use with samples that contain high levels of organic acids or for simultaneous analysis of these acids and sugars, using sulfuric acid as the mobile phase.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

Peak identification for Figures 1 and 2

1 Glucose
2 Xylose
3 Arabinose
4 Xylitol
5 Lactic acid
6 Glycerol
7 Acetic acid
8 Ethanol
9 Hydroxymethylfurfural (HMF)
10 Furfural

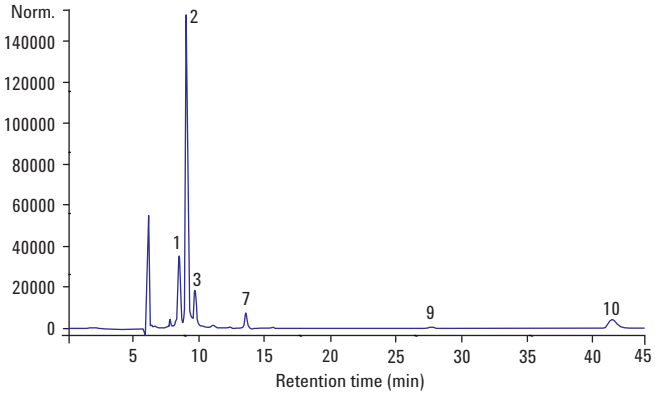

Figure 1. Analysis of a sample of straw after hydrothermal digestion using an Agilent Hi-Plex H column.

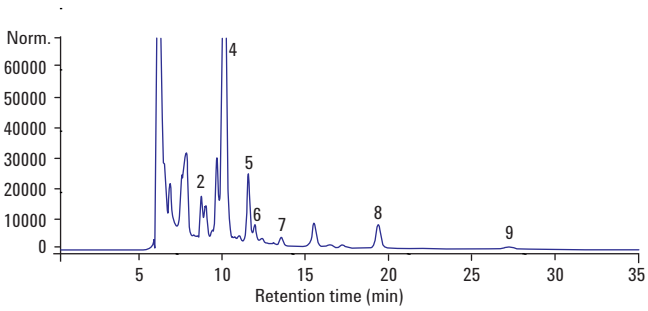

Figure 2. Components of a straw sample after fermentation of xylose to xylitol.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-1942

Agilent Technologies

Analyzing Liquid Fractions of Biogas Processes by HPLC

Application Note

Biofuels

Author

A. Ewen

Prüf- und Forschungsinstitut

Pirmasens

Germany

Introduction

For process control of the ever-growing number of biogas plants, knowledge of acetic and propionic acid concentration is crucial, since high levels of propionic acid can indicate biological problems. Analysis of free fatty acids can be done by GC or by HPLC. This application note shows the analysis of a specimen of a biogas plant liquor using HPLC with an Agilent Hi-Plex H column.

Agilent Technologies

Materials and Methods

The sample was steam distilled according to *German standard methods for the examination of water, waste water and sludge — Sludge and sediments (group S) — Part 19: Determination of the steam-volatile organic acids (S 19)* and pretreated by filtration through a 0.45 µm membrane before analysis. Since caproic acid is very seldom found in biogas plant liquors, the method can be halted after the elution of isovaleric acid.

Conditions

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.005 M H_2SO_4
Gradient	Isocratic
Flow rate	0.7 mL/min
Injection volume	20 µL
Sample concentration	200 mg/mL for each acid
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

Results and Discussion

Figure 1 shows a separation of a standard mixture of free fatty acids.

Figure 2 shows the main constituents of the biogas liquor, which includes some of the fatty acids in the standard mix.

Conclusion

A sample of biogas plant liquor was successfully separated using HPLC with an Agilent Hi-Plex H column. Hi-Plex H is the column of choice for the analysis of organic acids, using dilute mineral acid as eluent. The use of a ligand-exchange chromatography column such as Hi-Plex H significantly reduces the need for complicated sample preparation (typically involving elution through an ion-exchange resin bed). This is because retention is brought about not only by ion exchange, but also by ion exclusion and partitioning on this type of column.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

Peak identification (for both figures)

1. Formic acid
2. Acetic acid
3. Propionic acid
4. Isobutyric acid
5. Butyric acid
6. Isovaleric acid
7. 3,3-Dimethyl butyric acid (Internal Standard)
8. Valeric acid
9. Isocaproic acid
10. Caproic acid

x. Ethanol

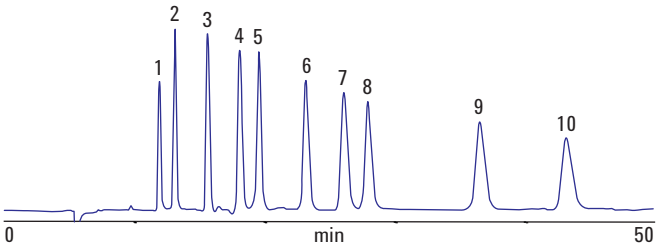

Figure 1. Analysis of a standard mixture of free fatty acids on an Agilent Hi-Plex H column.

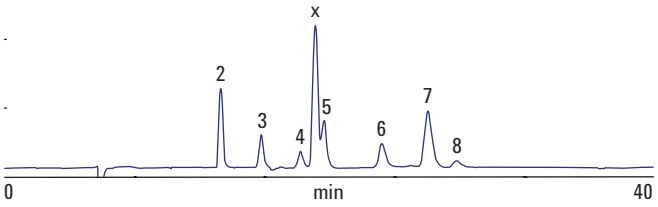

Figure 2. Separation of a biogas plant liquor by an Agilent Hi-Plex H column.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-01943

Agilent Technologies

Analysis of Sugars from Biomass Fermentation

Application Note

Biofuels

Author

A. Ewen

Prüf- und Forschungsinstitut

Pirmasens

Germany

Introduction

Biomass fermentation has grown in importance because diverse products such as fuel, lubricants, and chemicals can be derived. One option for this use of biomass is the fermentation of xylose (from hemicelluloses) to xylitol, a sugar substitute. For the HPLC analysis of fermentation liquids, the US NREL Biomass Program method *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples* can be applied.

This application note shows the analysis of the main hemicellulose-forming carbohydrates in a sample obtained by a hydrothermal digestion of straw. The hemicelluloses are hydrolyzed, and, in addition to other monosaccharides, free xylose can be obtained. While the Agilent Hi-Plex H column is especially suited for the analysis of byproducts and degradation products (Application Note SI-1942), the sugars are best analyzed with an Agilent Hi-Plex Pb column.

Agilent Technologies

Materials and Methods

Conditions

Column	Agilent Hi-Plex Pb, 7.7 × 300 mm, 8 µm (p/n PL1170-6820)
Mobile phase	100% DI H_2O
Gradient	Isocratic
Flow rate	0.5 mL/min
Injection volume	20 µL
Sample concentraion	1 g/L for each component
Temperature	70 °C
Pressure	2.5 MPa (25 bar, 360 psi)
Detector	RI (55 °C)

Results

Figures 1 and 2 highlight the main constituents of hemicellulose and straw after hydrothermal digestion. Clearly, this digestion process yields large quantities of xylose that elute after 29 minutes, as well as glucose and arabinose to a lesser extent.

Conclusion

The sugar composition of straw after hydrothermal digestion is readily determined using water as the mobile phase with an Agilent Hi-Plex Pb column. Analyses with these columns avoid the use, high cost, and disposal implications of toxic acetonitrile when separations are performed on amino silica columns. In addition, Hi-Plex stays active in the presence of sugar molecules. Together with fast dissolution, this benefit results in long lifetimes compared to amino silica columns.

For More Information

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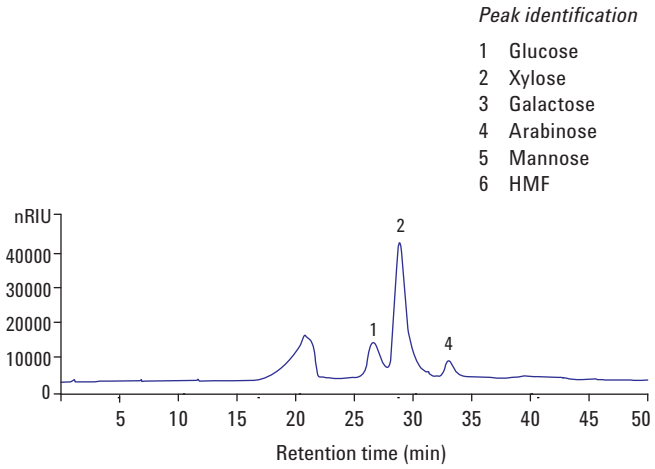

Figure 1. Analysis of a sample of straw after hydrothermal digestion using an Agilent Hi-Plex Pb column.

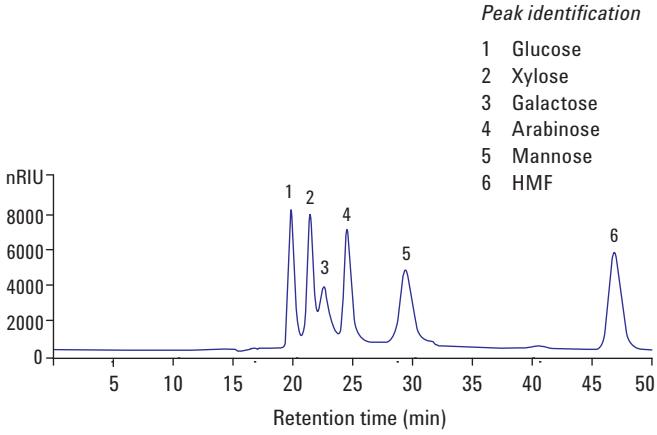

Figure 2. Standard curve of the main hemicellulose-forming monosaccharides and hydroxymethylfurfural (HMF), an oxidation product of sugars.

www.agilent.com/chem

Published in USA, August 3, 2011
SI-1944

Agilent Technologies

Organic Acids in Silage

Application Note

Food and Environmental

Author

A. Ewen

Prüf- und Forschungsinstitut

Pirmasens

Germany

Introduction

In addition to other factors, the concentration of the three fermentation acids lactic acid, acetic acid, and butyric acid is a criterion for the quality of silages. HPLC is the choice for this analysis, since volatile and nonvolatile acids can be determined together without prior derivatization. This application note shows the analysis of several specimens of silages (grass, whole plant, and corn) using an Agilent Hi-Plex H column.

Agilent Technologies

Materials and Methods

The extraction of the acids is done according to EN 13037 (soil improvers and growing media determination of pH) by adding 1.25 L of water to 250 mL of silage and agitating for one hour. The sample was pretreated by filtration through a 0.45 µm membrane.

Conditions

Column	Agilent Hi-Plex H, 7.7 x 300 mm, 8 µm (p/n PL1170-6830)	
Mobile phase	0.005 M H_2SO_4	
Gradient	Isocratic	
Flow rate	0.7 mL/min	
Injection volume	20 µL	
Sample concentration	Glucose	50 – 1500 mg/L
	Succinic acid	50 – 125 mg/L
	Lactic acid	750 – 1000 mg/L
	Acetic acid	200 – 450 mg/L
	Ethanol	80 – 700 mg/L
Temperature	60 °C	
Pressure	4.6 MPa (46 bar, 670 psi)	
Detector	RI (55 °C)	

Results and Discussion

Figure 1 shows the analysis of grass silage, which has undergone a homofermentative process leading mostly to lactic acid and a small amount of ethanol. An example for a heterofermentative process is shown in Figure 2. Here the silage of corn yielded not only lactic acid, but also acetic acid and ethanol. Figure 3 shows the analysis of whole plant silage, which has undergone an untypical process, leaving a large amount of free sugars.

Conclusion

Samples of silage from different crops were successfully separated by HPLC with an Agilent Hi-Plex H column.

Hi-Plex H is the column of choice for the analysis of organic acids in complex matrices, using dilute mineral acid as eluent. Hi-Plex columns are packed with sulfonated resin, giving a fundamental improvement in performance. They contain monodisperse sulfonated packing to overcome the problems of low efficiencies and high backpressures encountered with soft gels.

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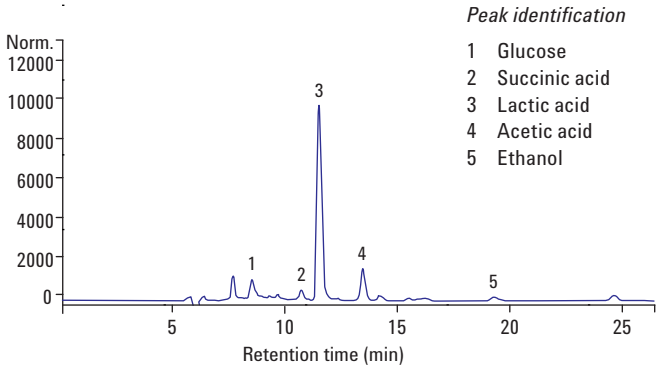


Figure 1. Analysis of grass silage using an Agilent Hi-Plex H column.

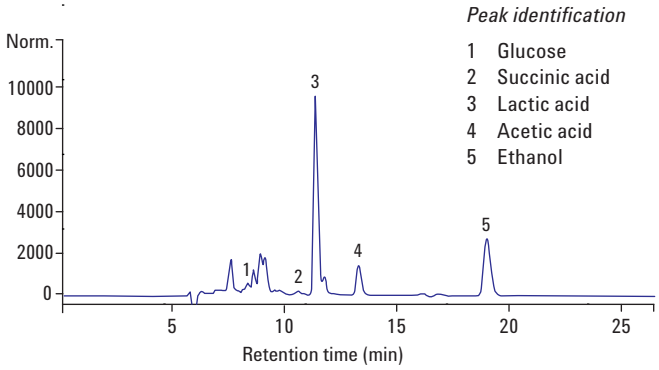


Figure 2. Separation of corn silage using an Agilent Hi-Plex H column.

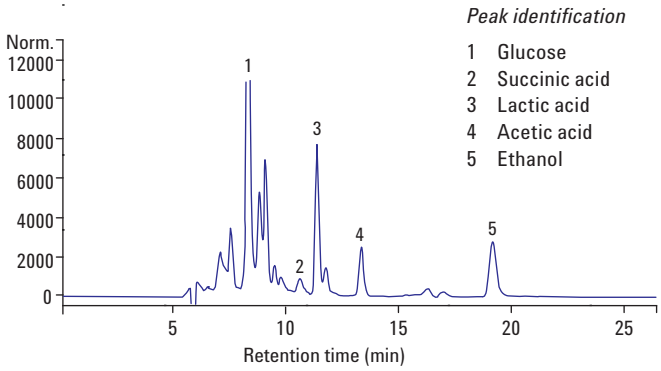


Figure 3. Analysis of whole plant silage using an Agilent Hi-Plex H column.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-1945

Agilent Technologies

High Temperature GC Analysis of Fischer-Tropsch Reaction Products using the Agilent J&W FactorFour VF-5ht Ultimetal Column

Application Note

Authors

Johan Kuipers
Agilent Technologies, Inc.

Susanne Buchwaldt
Sasol Wax GmbH.

Introduction

Alternative fuels that are not derived from petroleum, such as biodiesel, biomass to liquid (BTL) or gas to liquid (GTL) diesel, are increasingly being developed and adopted. Although the Fischer-Tropsch process is an established technology for the production of GTL or coal to liquid synthetic petroleum products, its popularity is hampered by high capital costs and the uncertain and volatile price of crude oil.

Fischer-Tropsch reaction products mainly consist of straight and branched alkanes. Long chain hydrocarbons up to C100 may be formed in this process. The GC analyses of these high boiling hydrocarbons products require oven temperatures up to 440 °C in order to elute the C90 – C100 HC fraction. The UltiMetal column, coated with the highly temperature stable and durable VF-5ms arylene stabilized liquid phase, is successfully applied for this HT-GC analysis.

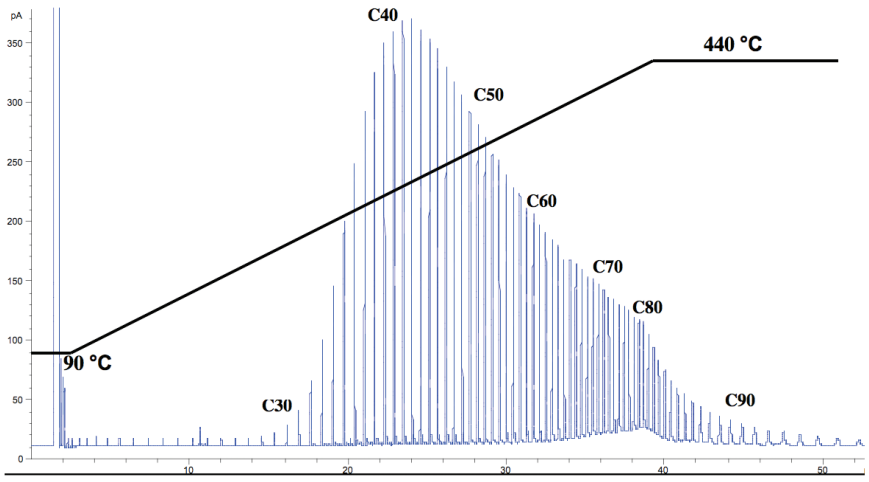


Figure 1. High temperature GC analysis of Fischer-Tropsch synthetic paraffins on VF-5ht UltiMetal column

Agilent Technologies

Results and Discussion

The VF-5ht UltiMetal column shows an excellent durability over many months and multiple analysis cycles as illustrated by the elution profile in figure 2. The retention times of the C35 –C55 middle fraction have shortened somewhat compared to the original first analysis on a new column due to a minor loss of stationary phase. This illustrates the robustness of the column considering the longer term exposure of the column to an oven temperature of 440 °C. Even after 500 analyses cycles equaling a 125 hour exposure to 440 °C, the resolution and peak profiles are still excellent, indicating the high thermal stability of the VF-5ms liquid phase.

Obviously, regular fused silica tubing with its polyimide outercoating will not be able to withstand these high operating temperatures. For this reason, the VF-5ms liquid phase has been coated on UltiMetal-treated SS tubing, which provides a virtually unbreakable metal column material with excellent inertness properties similar to fused silica tubing. The Agilent UltiMetal tubing is manufactured using proprietary deactivation technologies.

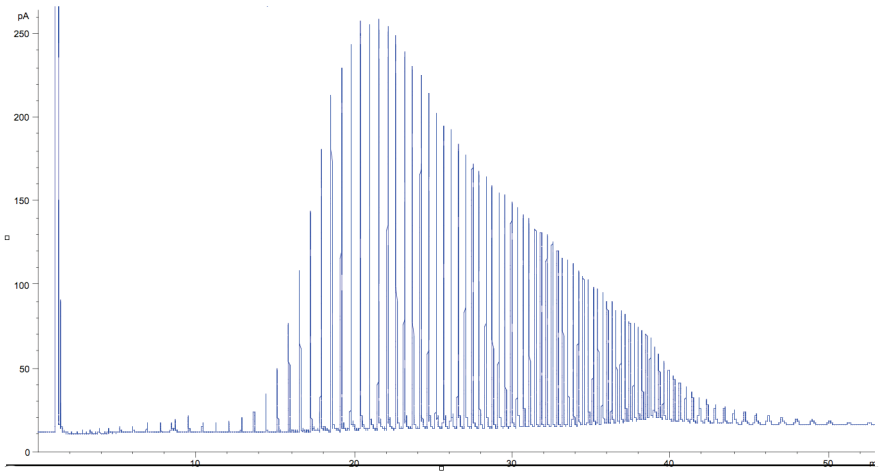


Figure 2. High temperature GC analysis of Fischer-Tropsch synthetic paraffins on VF-5ht UltiMetal column (500 analyses cycles)

Data - courtesy of Susanne Buchwaldt, Sasol Wax GmbH

Conditions

Column:	VF-5ht UltiMetal, 30 m x 0.32 mm x 0.10 µm (part number CP9096)
Sample volume:	1 µl
Carrier gas:	2.5 ml/min Hydrogen, constant flow
Injector:	Cool on-column
Temperature:	90 °C, 25 °C/min, 150 °C, 8 °C/min, 440 °C (15 min)
Detector:	FID, 440 °C

www.agilent.com/chem

Published in UK, October 08, 2010
SI-2105

Agilent Technologies

Separation of Permanent Gases on a Liquid Phase

Separation of 5 permanent gases on a WCOT column with a liquid phase with high retention

Application Note

Authors

Rick Hamerlinck and Norbert Reuter
Agilent Technologies, Inc.

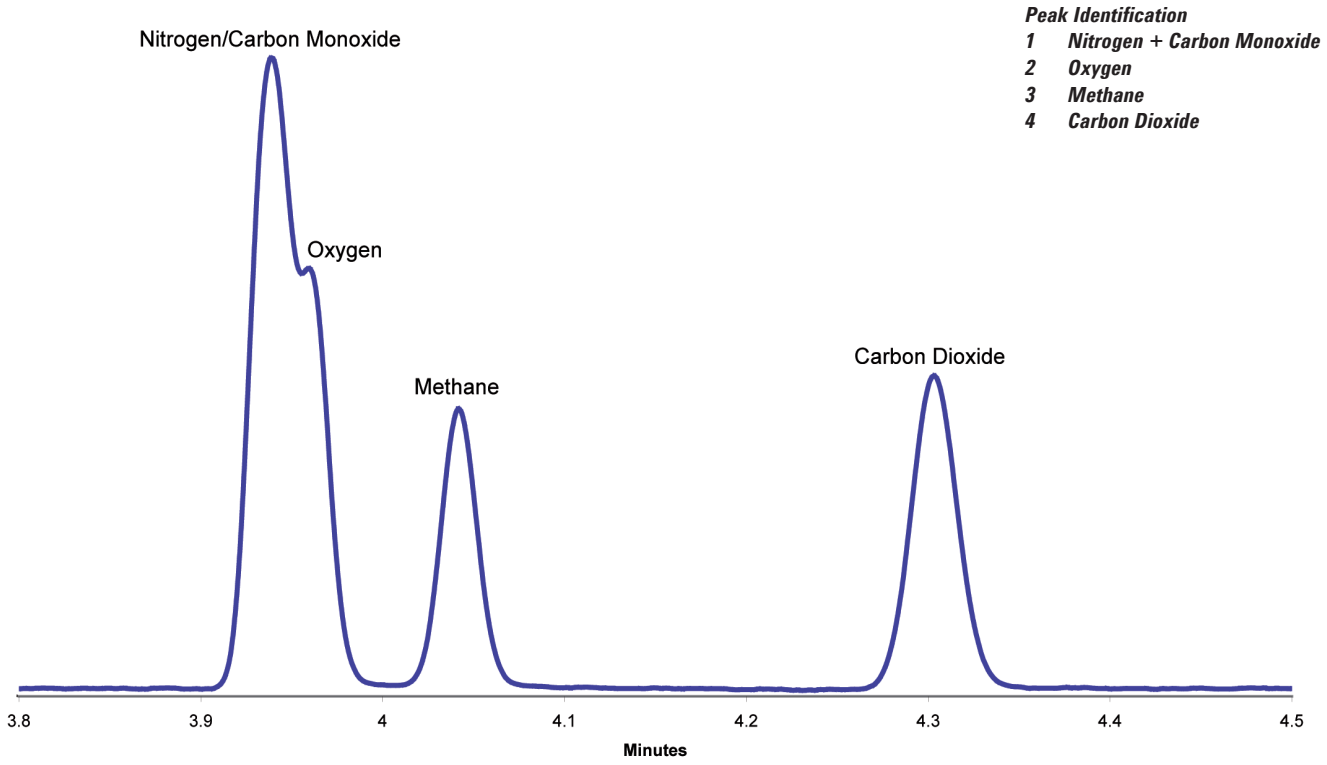
Introduction

Normally permanent gases are separated by PLOT (porous layer open tubular) columns with their high retentive phases. With WCOT (wall coated open tubular) columns sub-ambient temperatures are normally necessary. Thick films, like the 8 µm film thickness of the Agilent J&W Select CP-Sil 5CB for Formaldehyde, allow the use of high-inert liquid phases for the (pre-) separation of the standard permanent gases from carbon dioxide for possible column switching at normal ambient temperatures.

Agilent Technologies

Materials and Methods

Technique:	GC-Capillary Medium Bore	Carrier Gas:	Helium at 25 psi (170 kPa)	Detector:	Thermal Conductivity Detector at 220 °C (Filament Temp. 280 °C)
Instrument:	GC Gas Chromatograph	Temp Program:	35 °C isothermal	Sample:	All Gases 1% in Helium
Column:	CP-Sil 5 CB for Formaldehyde, 0.32 mm x 60 m, df=8 µm (part number CP7475)	Injector:	Split/Splitless-Injector (1177) at 250 °C		
		Inj Volume:	500 µL (split ratio 1:20)		



Peak Identification

1. ***Nitrogen + Carbon Monoxide***
2. ***Oxygen***
3. ***Methane***
4. ***Carbon Dioxide***

Analysis of permanent gases at 35°C

www.agilent.com/chem

Published in UK, October 08, 2010
SI-02166

Agilent Technologies

Separation of Fatty Acid Methyl Esters (FAME) on an Agilent J&W Select CP-Sil 88 for FAME GC Column

Application Note

Authors

Frau Thomae and
Frau Dr. Schwendig-Radke
Office for Consumer Protection,
Mettmann

Introduction

The routine, detailed separation of fatty acid methyl esters (FAME) requires high polarity liquid phases, which will differentiate between the multiple FAME isomers. The CP-Sil 88 is among the GC columns frequently used for the profiling of complex FAME mixtures. It is based on a stabilized, highly subsituted cyanopropyl siloxane phase and due to its highly polar properties is able to effectively separate on small structural differences of many positional FAME isomers. This application note shows the routine separation of fatty acid methyl esters (FAME) using a Select CP-Sil 88 column and GC-FID resulting in excellent selectivity; all 37 components were resolved in one run.

Agilent Technologies

Conditions

Technique:	GC-FID
Column:	Select CP-Sil 88 for FAME, 100 m x 0.25 mm df=0.2 µm (part number CP7489)
Oven:	80 °C, 4 °C/min to 220 °C, 5 min, 4 °C/min to 240 °C, 10 min
Carrier Gas:	Helium, constant flow 1.0 mL/min
Injection:	Splitflow 20 mL/min, Temperature = 250 °C
Injection Volume:	0.5 µL
Detection:	FID, 270 °C
Sample:	37 component FAME mix
Sample Solvent:	Dichloromethane
Sample Conc:	2-4% (w/w)

Peak Identification

1. C4:0
2. C6:0
3. C8:0
4. C10:0
5. C11:0
6. C12:0
7. C13:0
8. C14:0
9. C14:1
10. C15:0
11. C15:1
12. C16:0
13. C16:1
14. C17:0
15. C17:1
16. C18:0
17. C18:1 trans 9
18. C18:1 cis 9
19. C18:2, trans 9, 12
20. C18:2 cis 9,12
21. C20:0
22. C18:3 cis 6,9,12 gamma
23. C20:1 cis 11
24. C18:3 cis 9,12,15 alpha
25. C21:0
26. C20:2 cis 11,14
27. C22:0
28. C20:3 cis 8,11,14
29. C22:1 cis 13
30. C20:3 cis 11,14,17
31. C20:4 cis 5,8,11,14
32. C23:0
33. C22:2 cis 13,16
34. C24:0
35. C20:5 cis 5,8,11,14,17 EPA
36. C24:1 cis 15
37. C22:6 cis 4,7,10,13,16,19 DHA

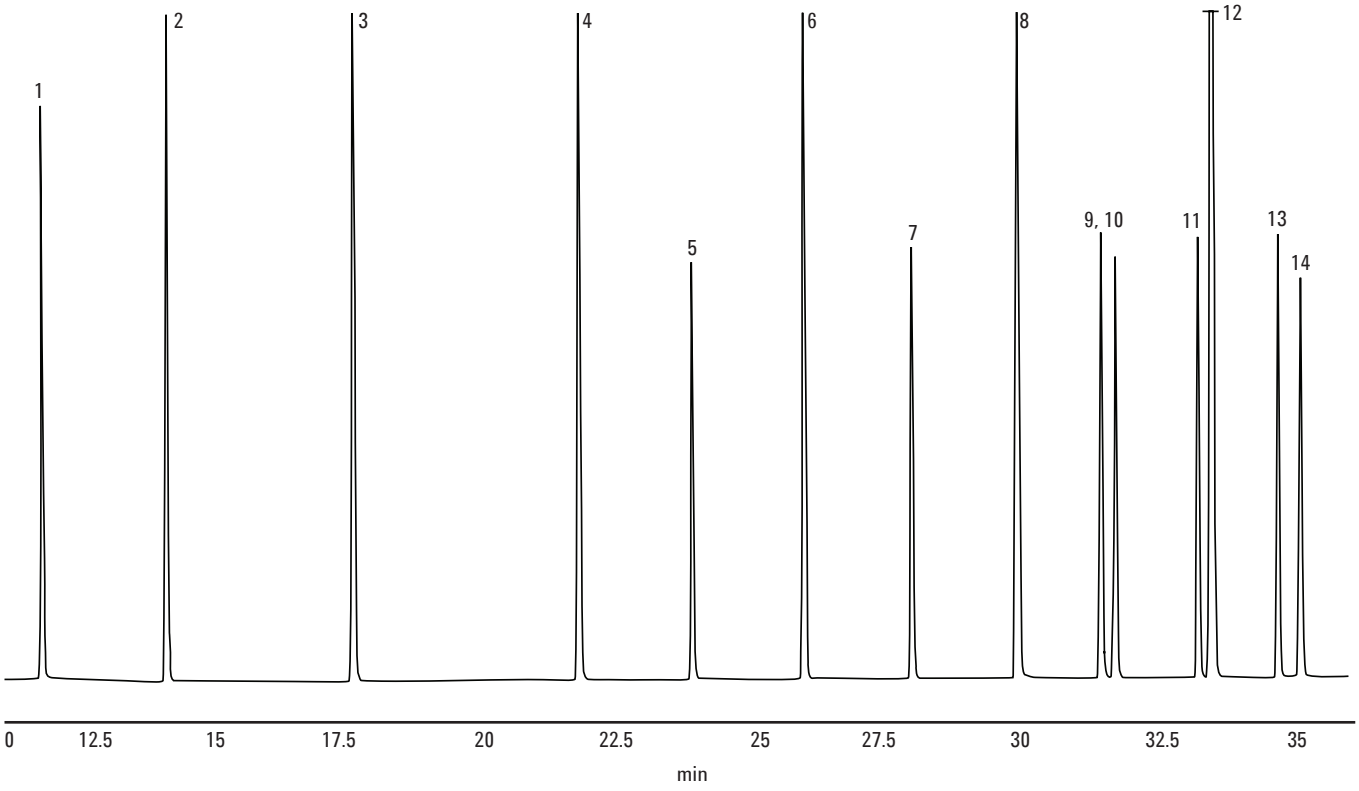


Figure 1a. Separation of a 37 component FAME mix (peaks 1-14)

Peak Identification

1.	*C4:0*
2.	*C6:0*
3.	*C8:0*
4.	*C10:0*
5.	*C11:0*
6.	*C12:0*
7.	*C13:0*
8.	*C14:0*
9.	*C14:1*
10.	*C15:0*
11.	*C15:1*
12.	*C16:0*
13.	*C16:1*
14.	*C17:0*
15.	*C17:1*
16.	*C18:0*
17.	*C18:1 trans 9*
18.	*C18:1 cis 9*
19.	*C18:2, trans 9, 12*
20.	*C18:2 cis 9,12*
21.	*C20:0*
22.	*C18:3 cis 6,9,12 gamma*
23.	*C20:1 cis 11*
24.	*C18:3 cis 9,12,15 alpha*
25.	*C21:0*
26.	*C20:2 cis 11,14*
27.	*C22:0*
28.	*C20:3 cis 8,11,14*
29.	*C22:1 cis 13*
30.	*C20:3 cis 11,14,17*
31.	*C20:4 cis 5,8,11,14*
32.	*C23:0*
33.	*C22:2 cis 13,16*
34.	*C24:0*
35.	*C20:5 cis 5,8,11,14,17 EPA*
36.	*C24:1 cis 15*
37.	*C22:6 cis 4,7,10,13,16,19 DHA*

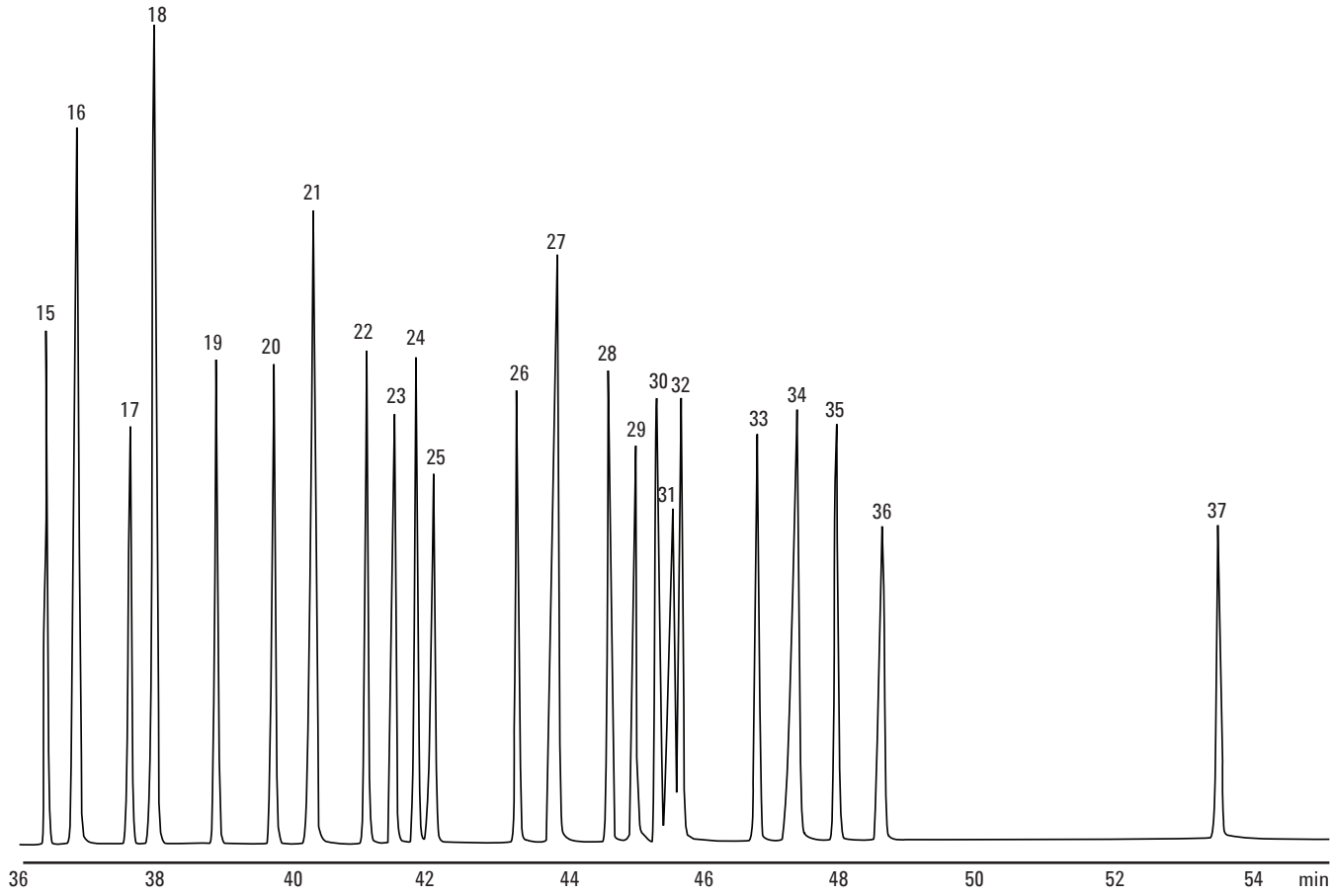


Figure 1b. Separation of a 37 component FAME mix (peaks 15-37)

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Agilent Technologies

Analysis of Biogas with the 490 Micro GC Gas Chromatograph

Application Note

Authors

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Introduction

Biogas is a gas mixture produced by biological processes, from the anaerobic fermentation of organic material such as biomass, manure or sewage, municipal waste, green waste and energy crops. Swamp gas is a naturally produced biogas.

The main components of biogas are methane and carbon dioxide, with some carbon monoxide and hydrogen. Biogas can be used as biofuel, as a low-cost fuel for any heating purpose. It also has a role in modern waste management to run any type of heat engine, to generate either mechanical or electrical power. To increase caloric values it might be necessary to remove some of the carbon dioxide. Biogas can be compressed, much like liquified natural gas, and used to power motor vehicles. For this purpose it is necessary to remove hydrogen sulfide. Biogas is a renewable fuel, and so it qualifies for renewable energy subsidies in some parts of the world. Due to the increasing interest in biogas, there is a growing demand for fast and efficient analysis technology. That is where the new generation micro GC from Agilent, the 490 Micro GC, can play a significant role.

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Instrumentation

Depending on the type of biogas to be analyzed, two configurations are available. If the sample contains only permanent gases and the hydrocarbons methane, ethane and propane, a dual channel GC is ideal. If higher hydrocarbons are also present in the sample, a third channel is needed and therefore the quad version of the micro-GC is recommended.

490 Micro GC Gas Chromatograph

Dual channel:

- Channel 1, CP-Molsieve column
- Channel 2, CP-PoraPLOT U column

Quad equipped with three channels:

- Channel 1, CP-Molsieve column
- Channel 2, CP-PoraPLOT U column
- Channel 3, CP-Sil 5 CB column

GC control and data handling software: Galaxie Chromatography Data System

Conditions

Table 1. GC conditions

	Inj Time (ms)	Inj Temp (° C)	Column Temp (° C)	Carrier Gas	Pressure (kPa)	Back Flush (sec)
Ch1	40	80	80	Ar	150	9
Ch2	100	80	100	He	100	10
Ch3	100	80	60	He	150	-

Results and Discussion

The sample can be introduced to the 490 Micro GC either pressurized (reduced to max 1 bar) via a Tedlar sampling bag, or by using continuous flow. In this case the sample was pressurized, see Figure 1.

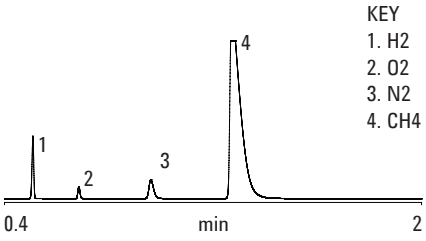

Figure 1. Permanent gases on the CP-Molsieve channel

As biogas and related samples may contain large amounts of CO2, water and higher hydrocarbons it was necessary to back flush these components. Water and CO2, in particular, adsorb to the stationary phase and change chromatographic properties. Higher hydrocarbons eventually elute but cause higher noise and thus reduced sensitivity.

An indication of changed chromatographic properties is a drift in retention time. Table 2 shows repeatability figures on the CP-Molsieve channel. Repeatability figures of retention time and quantity are presented.

Table 2. Repeatability figures for the CP-Molsieve channel

Run #	Tr (min) Hydrogen	Tr (min) Oxygen	Tr (min) Nitrogen	Tr (min) Methane	QTY (%) Hydrogen	QTY (%) Oxygen	QTY (%) Nitrogen	QTY (%) Methane
1	0.5095	0.6858	0.9618	1.2745	1.0253	2.0183	8.0511	84.5107
2	0.5097	0.6858	0.962	1.2748	1.0222	2.012	8.057	82.945
3	0.509	0.6852	0.961	1.2727	1.0375	2.0272	8.0874	88.3207
4	0.5095	0.6857	0.9617	1.2743	1.0239	2.0155	8.0307	83.2869
5	0.5095	0.6858	0.9622	1.2748	1.0292	2.0197	8.0516	85.4475
6	0.5095	0.6858	0.962	1.2743	1.0329	2.0258	8.0664	86.787
7	0.5097	0.686	0.9622	1.2748	1.0306	2.0254	8.0589	85.2073
8	0.5092	0.6853	0.9617	1.2735	1.0365	2.0303	8.0875	88.3182
9	0.5095	0.6858	0.962	1.2745	1.0278	2.0188	8.0446	85.3981
10	0.5098	0.6862	0.9623	1.2753	1.0252	2.0165	8.0182	83.0202
11	0.5093	0.6855	0.9617	1.2736	1.0347	2.0277	8.0754	87.3976
12	0.5092	0.6855	0.9615	1.2735	1.0398	2.0358	8.099	88.1668
13	0.5092	0.6855	0.9617	1.2737	1.0368	2.032	8.0797	87.1916
14	0.5097	0.686	0.9622	1.2752	1.0264	2.0143	8.0082	82.8409
15	0.5092	0.6853	0.9615	1.2735	1.0361	2.0294	8.0688	87.3486
Average	0.5094	0.6857	0.9618	1.2742	1.0310	2.0232	8.0569	85.7458
Std Dev	0.0002	0.0003	0.0003	0.0007	0.0057	0.0073	0.0274	2.0592
RSD %	0.05%	0.04%	0.04%	0.06%	0.55%	0.36%	0.34%	2.40%

The very low relative standard deviation (RSD%) figures in Table 2 clearly show that the CP-Molsieve channel was working with very good repeatability. There was no drift in retention time and the analysis results for quantity were also very repeatable.

Figure 2 shows the chromatogram of the CP-PoraPLOT U channel. Separation of carbon dioxide, ethane, hydrogen sulfide and propane was achieved.

Baseline separation of all components of interest was obtained. Higher hydrocarbons were back flushed to vent, which prevented later eluting components from disturbing the next analysis. The results presented in Table 3 show very good repeatability figures for the CP-PoraPLOT U channel. RSD% below 0.1% for retention times and quantification illustrate the system's suitability for this type of analysis.

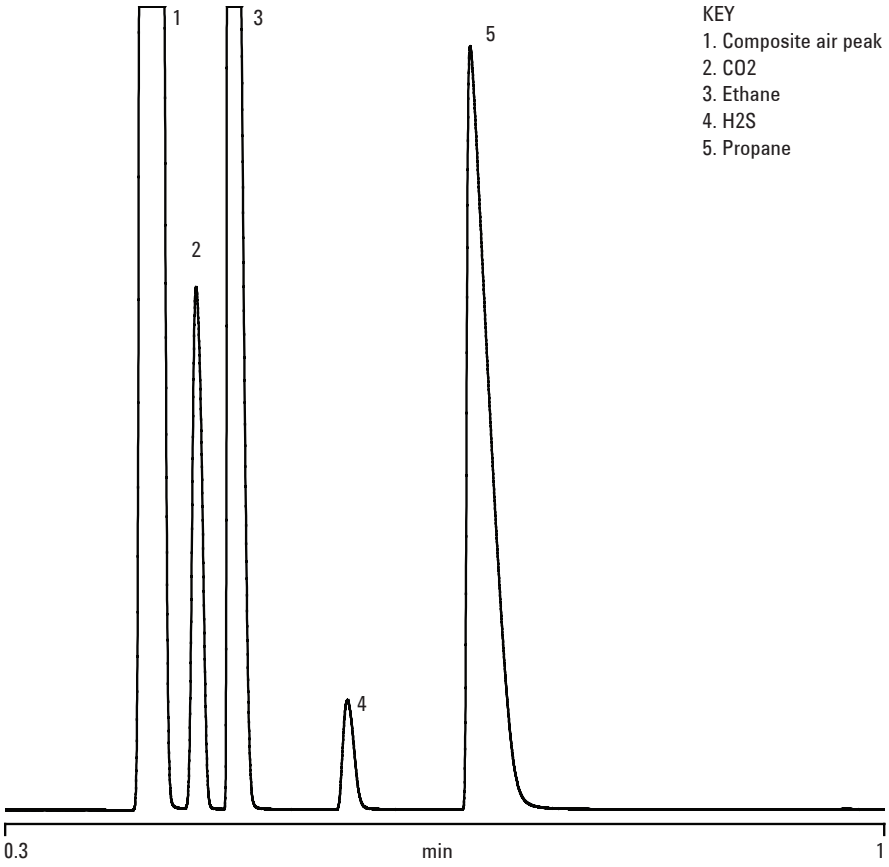

Figure 2. CO2, H2S, ethane and propane on the CP-PoraPLOT U channel

Table 3. Repeatability figures of the CP-PoraPLOT U channel

Run #	Tr (min) Air Peak	Tr (min) CO2	Tr (min) Ethane	Tr (min) Propane	QTY (%) CO2	QTY (%) Ethane	QTY (%) Propane
1	0.4115	0.4522	0.4833	0.6808	1.9866	4.0032	2.9955
2	0.4113	0.452	0.4832	0.6807	1.988	4.0048	2.9967
3	0.4117	0.4525	0.4837	0.6815	1.9921	4.0121	3.0015
4	0.4117	0.4525	0.4837	0.6813	1.99	4.0073	2.9985
5	0.4115	0.4522	0.4833	0.6808	1.9921	4.011	3.0014
6	0.4115	0.4523	0.4835	0.681	1.991	4.0089	2.9992
7	0.4115	0.4522	0.4835	0.6808	1.9896	4.0059	2.9973
8	0.4113	0.4522	0.4833	0.6808	1.9908	4.0073	2.9986
9	0.4115	0.4522	0.4833	0.6808	1.9927	4.011	3.0027
10	0.4115	0.4522	0.4833	0.6808	1.9912	4.0069	2.9984
11	0.4115	0.4522	0.4833	0.6808	1.9933	4.0113	3.0018
12	0.4115	0.4522	0.4833	0.6807	1.9927	4.0103	3.0008
13	0.4113	0.4522	0.4833	0.6807	1.9908	4.0062	2.9978
14	0.4113	0.452	0.4832	0.6807	1.9928	4.0096	2.9994
15	0.4118	0.4525	0.4837	0.6815	1.9919	4.0076	2.9992

Finally, Figure 3 is a chromatogram of the separation and determination of the (higher) hydrocarbons. The column used was a CP-Sil 5 CB. This extra channel expanded the application range of biogas analysis to blends with C3 and or C4 LPGs.

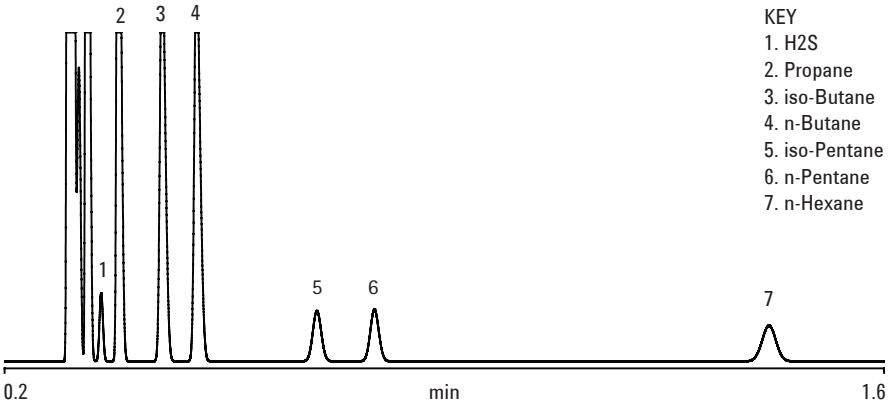

Figure 3. Higher hydrocarbons on the CP-Sil 5 CB channel

Table 4 shows the repeatability figures of the CP-Sil 5 CB channel. Again, very good repeatability figures were obtained. Relative standard deviation was well below 0.05% for retention times and below 0.15% for quantitative measurements.

Table 4. Repeatability figures of the CP-Sil 5 CB channel

Run #	Tr (min) Air Peak	Tr (min) Ethane	Tr (min) Propane	Tr (min) iso-Butane	Tr (min) n-Butane	QTY (%) Ethane	QTY (%) Propane	QTY (%) iso-Butane	QTY (%) n-Butane
1	0.3025	0.3333	0.3833	0.455	0.5107	4.0108	3.0222	0.501	0.5005
2	0.3023	0.3333	0.3833	0.455	0.5105	4.0092	3.0179	0.501	0.5004
3	0.3023	0.3333	0.3832	0.455	0.5105	4.0139	3.0171	0.5007	0.5002
4	0.3023	0.3332	0.3832	0.4548	0.5103	4.0116	3.0136	0.5009	0.5003
5	0.3022	0.3332	0.383	0.4547	0.5102	4.0129	3.0131	0.5007	0.5004
6	0.3023	0.3332	0.3832	0.4547	0.5102	4.0096	3.0111	0.5007	0.5003
7	0.3023	0.3332	0.3832	0.4548	0.5103	4.0102	3.0095	0.5006	0.5002
8	0.3022	0.3332	0.383	0.4547	0.5102	4.0126	3.0104	0.5009	0.5004
9	0.3023	0.3332	0.3832	0.4548	0.5103	4.0119	3.009	0.5007	0.5003
10	0.3023	0.3332	0.3832	0.4548	0.5103	4.0098	3.009	0.5009	0.5003
11	0.3023	0.3332	0.3832	0.4548	0.5103	4.0112	3.0092	0.5011	0.5005
12	0.3023	0.3332	0.3832	0.4548	0.5103	4.0091	3.0078	0.5008	0.5
13	0.3022	0.333	0.383	0.4547	0.5102	4.0128	3.0101	0.5014	0.5007
14	0.3022	0.333	0.383	0.4547	0.5102	4.0099	3.0083	0.501	0.5003
15	0.3022	0.333	0.383	0.4547	0.5102	4.0098	3.0083	0.5009	0.5002
Average	0.3023	0.3332	0.3831	0.4548	0.5103	4.0110	3.0118	0.5009	0.5003
Std Dev	0.0001	0.0001	0.0001	0.0001	0.0001	0.0015	0.0042	0.0002	0.0002
RSD %	0.03%	0.03%	0.03%	0.02%	0.03%	0.04%	0.14%	0.04%	0.03%

490 Micro GC Configuration for Biogas depends on Sample Type

Regular biogas contains methane, oxygen, nitrogen, carbon dioxide, hydrogen sulfide, and sometimes some hydrogen and carbon monoxide. For this type of sample a two channel 490 Micro GC is perfectly suited. Channel 1, configured with a CP-Molsieve column, will separate and analyze hydrogen, oxygen, nitrogen, methane and carbon monoxide. Channel 2, equipped with a CP-PoraPLOT U column, will analyze carbon dioxide and hydrogen sulfide. This configuration can even be used if ethane and propane are present in the sample. If, however, C4+ hydrocarbons also have to be analyzed, a third CP-Sil 5 CB channel is required, together with the 490 Micro GC QUAD.

Conclusion

All results clearly showed that the system configuration was perfectly capable of analyzing biogas.

The CP-Molsieve channel separated and analyzed permanent gases such as hydrogen, oxygen, nitrogen and methane. With some changes in chromatographic parameters even carbon monoxide can be analyzed on this channel. Higher hydrocarbons, as well as moisture and carbon dioxide, were back flushed to vent ensuring trouble free operation, perfect repeatability and a long column lifetime.

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Agilent Technologies

Sensitivity Enhancement for Flame Atomic Absorption Spectrometry Using an Atom Concentrator Tube, the ACT 80

Application Note

Atomic Absorption

Author

Jonathan Moffett

Abstract

A simple attachment to enhance the sensitivity of flame atomic absorption spectrometry (FAAS) is described along with some performance results and practical applications. An historical review is also presented.

Introduction

In theory, atomic absorption spectrometry (AAS), is very simple: introduce ground state (metal) atoms into the appropriate instrument's optical path and measure the absorption of light at an appropriate wavelength [1]. The device that generates the atoms is called an atomizer and there are several types:

- Flame
- Vapor generation (cold and heated)
- Graphite furnace
- Cathodic discharge [2,3]

The flame atomization system offers several advantages:

- Relative freedom from interference
- Low capital cost
- Low running cost
- Rapid and simple operation

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Flame atomic absorption spectrometry (FAAS) is routinely used to measure solutions at the parts per million level–equivalent to one gram of element per 1000 kg of solution–which is suitable for a wide range of analyses. The other atomizers offer such benefits as greater sensitivity or minimal sample preparation. However the initial outlay and running expenses can be higher. Much closer attention to the chemistry of the samples is also required. Consequently various schemes have been devised to enhance the sensitivity of FAAS without incurring the expense associated with the other techniques. Some of the more commonly used methods as well as some speculative ideas will be outlined.

Enhancements in FAAS

All methods to improve the sensitivity of FAAS must involve at least one of the following stages:

- Sample preparation/preconcentration
- Nebulization
- Atomization

Each of these techniques is discussed in turn.

Sample

The simplest and cheapest methods for improving sensitivity rely on increasing the concentration of the sample solution. After sample dissolution, one of the following methods of sample preconcentration may be applied:

- Solvent evaporation
- Solvent extraction (for example, APDC/MIBK)
- Ion-exchange (for example, Chelex-100)
- Co-precipitation

While all are used [4], the method of solvent extraction (chelating the analyte and extracting with an organic solvent) is probably the most common. All of the methods are slow, increase the possibility of contamination and need a sample volume of at least 10 to 100 mL. The ion-exchange technique is the only one which could be developed into an automated online system and may overcome the speed and contamination problems.

Nebulization

Nebulization is the physical process of changing the bulk solution into a spray of fine droplets and mixing the droplets with the combustion gases.

The premix (laminar flow) burner assembly is invariably used in commercial FAAS instruments (Figure 1). A venturi is used to create a low pressure zone which draws up and causes nebulization of the solution. An impact bead breaks up the droplets even further. Mixing paddles or baffles may also be used to improve gas mixing and to remove larger droplets. The gas mixture is then passed into the burner and the combustion zone.

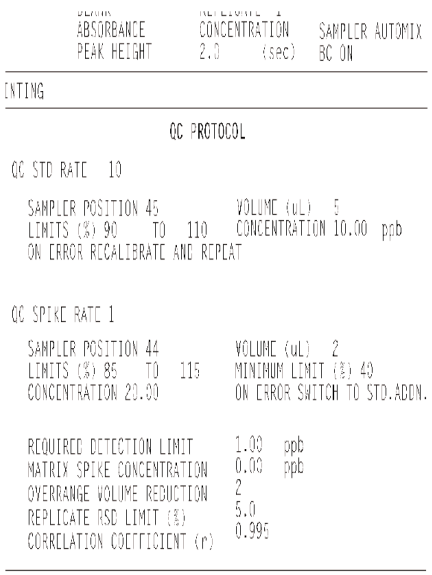

Figure 1. The Agilent Mark-VI spraychamber: (1) nebulizer, (2) ceramic faceplate, (3) adjustable glass bead, (4) drainage tube, (5) dual-head mixing paddle, (6) enhanced slope floor.

The main advantage of the premix burner assembly is its low noise and reproducibility. Agilent Technologies has introduced a new nebulizer [5], spraychamber [6], and a burner [7] to enhance further these benefits. However these improvements were not intended to improve the sensitivity significantly.

The difficulty of improving sensitivity can be demonstrated by using some typical numbers from this process. The nebulization process is only about 10% efficient so an uptake rate of 5 mL/min implies 0.5 mL/min passes through the burner. In most instruments 15–20 L/min of gas also flows through the burner. The effective dilution of the sample is therefore approximately 0.5/15000 or 1/30000.

The spraychamber would appear to be the obvious area to look for improvements in sensitivity. However even after decades of research and experimentation further significant improvements have yet to be made.

A heated spraychamber has been described which improves sensitivity for dilute, low solid solutions [8,9]. It appears likely that the premix spraychamber has been refined to its optimum

performance.

Logically the next potential area for improvement would be the nebulizer. Indeed it is possible to adjust the standard Agilent nebulizer to improve substantially the sensitivity for aqueous copper solutions. However the penalty of this mode of operation is an increased uptake rate and larger droplets in the flame. This would be perfectly acceptable if all samples behaved like aqueous copper solutions. In practice, under these conditions most solutions are known to cause unacceptable problems such as inter-element interferences, signal noise and blocking of the burner or nebulizer. Therefore obtaining sensitivity by increasing uptake rate is not recommended. Other nebulization schemes have been proposed. For example, it is quite feasible to use ultrasonic vibrations for improved nebulization. A different approach is to use electrostatic precipitation of the solid solutes in the aerosol [10-12]. However both techniques have yet to find wide acceptance in FAAS.

Atomization

The physical changes occurring to the solution aerosol in a flame are summarized in Reference 1. Work has been done on trying to understand the process better [8,13,14] but knowledge is still somewhat empirical, even without considering the chemical aspects or interferences. The number of analyte atoms present should in principle depend only on the volume of liquid reaching the combustion zone and the efficiency of atom formation. The flame sensitivity is determined by the number of ground state analyte atoms present in the optical path.

If the removal rate of the atoms from the optical path could be reduced, then an improvement in sensitivity should be observed. Such an approach was pioneered by Robinson [15] on a total combustion burner. Watling [16,17] experimented using a laminar flow burner with a slotted tube above the flame and Brown *et al* [18–20] have done additional work. (It should be mentioned that the Delves cup technique [21] also uses a tube.) This scheme is discussed in more detail in the following section.

A closely related approach pioneered by Lau [22] and investigated by several others [23–31] is to trap the atoms physically on the surface of a narrow diameter water-cooled silica tube placed just above the cone of the flame. After a suitable collecting period, the atom-trap tube is allowed to heat up (by stopping the flow and removing the water) and atoms are released to give an enhanced transient signal. Enhancements of 10 to 30 times have been reported. Practical difficulties have limited the application of this technique.

Atom Concentrator Tube, ACT 80

Watling, in 1977, described a slotted quartz tube which he placed over a conventional AA-6 air-acetylene burner and observed an improvement in analytical sensitivity [16,17].

The commercially available ACT 80 is a quartz tube 150 mm long with two lengthwise cuts. The longer slot is 100 mm × 2 mm, the shorter 80 mm × 2 mm. These cuts are angled at 120 degrees to each other relative to the tube's axis. The ACT 80 is installed in a standard Agilent Vapor Generation Accessory (VGA 76) cell holder and fits on a burner as does the VGA 76 cell. The longer slot is aligned over the burner slot; the shorter faces towards the rear of the instrument away from the holder. As with the VGA 76 cell, only the air-acetylene flame can be used as a hotter flame would destroy the tube. Figure 2 shows the tube in its holder.

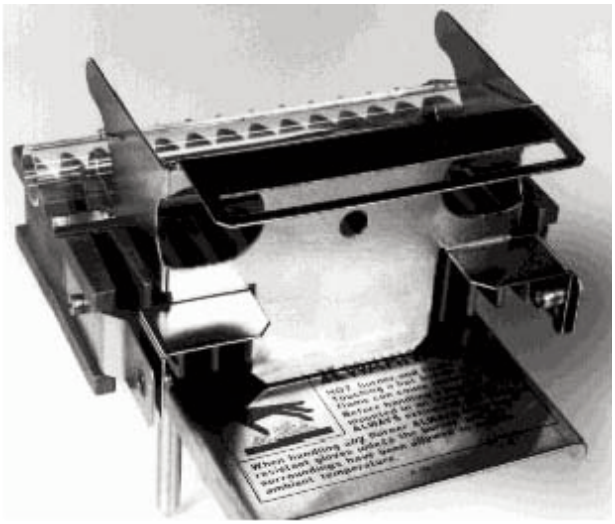

Figure 2. The ACT 80 Atom Concentrator Tube.

The ACT 80 tube must also be optically aligned so that the long axis of the tube coincides with the light beam. It was found in practice that the burner and ACT 80 needed to be lowered about 7 mm (equivalent to the radius of the tube).

Experimental

The performance of the ACT 80 was evaluated using SpectrAA-300/400 spectrometers fitted with a Mark VI spray-chamber and a Mark VA or a Mark VI air-acetylene burner. A VGA cell holder clamp was attached to the burner. Instrument default conditions were used for all measured elements. Where nitrous oxide-acetylene was the default flame, air-acetylene was used instead. Oxidant flow was 13.5 L/min and

acetylene flow 2.0 L/min. Delay time was 20 s and the read time period was 10 s integrated. All measurements were made after the system had been operated at least ten minutes to reach equilibrium.

Results and signal graphics were sent out to a printer. In addition, sample absorbances were sent to an ASCII file for further data manipulation.

Standard solutions were made from BDH (Poole, England) Spectrosol 1000 mg/L standards. Solutions and blanks were acidified with Analar grade concentrated nitric acid to give 0.5% v/v in final volume. Water was distilled from a Pyrex still and deionized with a Waters Milli-Q system to 18 MOhms conductivity.

Practical Points

The ACT 80 must be tilted back out of the way when lighting the flame. Otherwise for tongue-of-flame igniters a significant amount of acetylene builds up inside the ACT 80 with subsequent noisy ignition. Mechanical igniters would physically damage the ACT 80.

Flame composition is also an important factor. It was found that a lean to stoichiometric flame was needed. A rich flame causes soot formation and the signal noise becomes unacceptably high. Elements requiring a rich flame such as arsenic, chromium or molybdenum are therefore not usefully measured using the ACT 80. It was noted with arsenic that each blank signal increased and the blank and solution absorbances tended to give the same value. While this observation is not strong evidence for a memory effect, it cannot yet be eliminated. Alkali and alkaline earth (Group I and II) metals which etch heated silica [22] are also not usefully measured with this technique.

Devitrification of the tube inevitably occurs and starts initially around the inlet slot. The presence of Group I and II metals tends to accelerate this process. However it is possible to aspirate strong solutions (1000 mg/L or greater) of aluminium or lanthanum which provide a protective coating [23] and so retard the devitrification process. This should be done each time the tube is used and must be repeated on a regular basis. Tube lifetimes for samples with simple acidified matrices for example, water or dilute solutions of solids should typically be several hours of continuous operation. At a rate of approximately 200 samples/hour many samples may be determined using one tube.

Lifetime is maximized by continuous operation because cooling and reheating stresses the quartz.

Results and Discussion

Performance

As a guide to performance, improvements in characteristic concentration and detection limit were measured for selected air-acetylene elements. For both values the absorbance of a dilute solution of the analyte must be measured. The absorbance must be determined on a linear portion of the calibration graph and so concentrations were selected to be approximately equal to the characteristic to determine the characteristic concentration (determined using values previously published by Agilent). In practice ten measurements of the solution were made interspersed by measurement of the blank solution. Measurements of each series were done without the ACT-80 and repeated with the ACT-80 fitted (the burner height was reoptimized as needed).

Each element required a large number of readings and to avoid transcription errors the measurements were also printed to an ASCII file. This file was subsequently read by a BASIC program written to extract the absorbance values and perform the necessary calculations. Each solution absorbance was corrected by subtracting the mean of the two adjacent blank readings. The mean and standard deviation of the ten corrected absorbances were used to determine the characteristic concentration and detection limit values. These values were then loaded into a a LOTUS1-2-3 spreadsheet to generate Table 1.

Table 1 also lists, for reference only, Agilent data on detection limit and characteristic concentration values. The values found from this study were obtained using fixed air-acetylene flows and should not be directly compared with values obtained by optimizing conditions for each element.

The following points are drawn from Table 1:

1. All the elements listed showed some improvement in sensitivity. These tended to be consistent as indicated by duplicate runs. Copper was repeated on different systems.
2. All improvements appear to be about 2X to 3X, which reflects the findings of Watling [16,17] and Brown [18–20].
3. Generally there was a corresponding improvement in detection limit. The statistical nature of detection limit means direct comparisons should be interpreted cautiously but since the improvement factor is almost always greater than unity it is inferred that the ACT-80 does improve detection limits. Gold, cadmium and lead appear to show the best improvements.
4. Iron and platinum showed no significant improvements in characteristic concentration or detection limit.

Table 1. Comparison of Detection Limits and Characteristic Concentrations for Selected Air-Acetylene Flame Elements

	Characteristic concentration				Detection limit			
Element	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor
Ag	0.030	0.0134	0.0049	2.7	0.002	0.0019	0.0020	1.0
Au	0.100	0.1226	0.0451	2.7	0.010	0.0148	0.0036	4.1
Bi	0.200	0.2647	0.0919	2.9	0.050	0.0766	0.0177	4.3
Bi		0.2498	0.0903	2.8		0.0414	0.0211	2.0
Cd	0.010	0.0123	0.0054	2.3	0.002	0.0047	0.0011	4.3
Cu	0.030	0.0422	0.0214	2.0	0.003	0.0055	0.0056	1.0
Cu		0.0496	0.0212	2.3		0.0047	0.0034	1.4
Cu *		0.0448	0.0189	2.4		0.0066	0.0065	1.0
Fe	0.050	0.0538	0.0362	1.5	0.006	0.0110	0.0102	1.1
Hg	1.500	2.4278	0.8581	2.8	0.150	0.3094	0.1121	2.8
Mn	0.029	0.0291	0.0141	2.1	0.002	0.0025	0.0019	1.3
Pb	0.100	0.1182	0.0404	2.9	0.010	0.0301	0.0090	3.3
Pt	1.000	2.0064	1.9328	1.0	0.100	0.1220	0.0967	1.3
Sb	0.300	0.3866	0.1244	3.1	0.040	0.0678	0.0462	1.5
Se	1.000	0.3356	0.1010	3.3	0.500	0.1381	0.0927	1.5
Te	0.200	0.2476	0.0903	2.7	0.030	0.0760	0.0492	1.5
Tl	0.200	0.1509	0.0588	2.6	0.020	0.0112	0.0052	2.2

Notes:
-Ten readings were taken and the mean calculated for each value.
-Uptake rate was fixed at 6 mL/min.
-All conditions constant except for burner height ("Ht").
-"Ht" is burner position as shown on the instrument's burner vertical scale.
-Concentrations are about 10 times detection limit (except for Cu which was 100 times).*
-Quoted results for Se used nitrous oxide-acetylene flame. This study used an air-acetylene flame.
-Some elements show replicate results. With Cu, results were from different burners.

The following definitions apply:

$$\text{Detection limit} = \frac{2 \times \text{Standard Deviation} \times \text{Concentration}}{\text{Mean Absorbance}}$$

(IUPAC now recommend detection limit to be 3 times standard deviation, for comparison with literature values 2 times is used here.)

$$\text{Characteristic concentration} = \frac{0.0044 \times \text{Concentration}}{\text{Mean Absorbance}}$$

As an illustration, signal graphics for a standard lead solution measured with and without the ACT-80 tube in place are shown in Figure 3.

Variation in tube dimensions were not investigated, however Brown used a tube 8 mm id (Watling did not specify dimensions). The similarity between the results of this study and the published data indicates that the enhancement is not influenced greatly by the tube dimensions.

Watling suggested the flame characteristics are being affected in a way to encourage atom residence time in the optical path. Whether the flame has less entrained air or the reducing interconal zone is broadened or the diffusion of atoms is slowed down requires more work to elucidate. However, it appears that atoms are not trapped but merely delayed.

The sensitivity of the nitrous oxide-acetylene flame would perhaps also benefit from this technique but its higher temperature (2600 °C) means that the tube would need to be very refractory. The Delves cup method has been applied to the nitrous oxide-acetylene flame [32] so a refractory atom concentrator tube may be feasible.

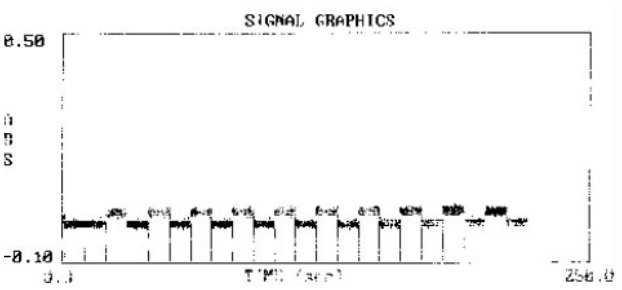

Figure 3(a). Pb signal compared to blank without ACT-80 tube.

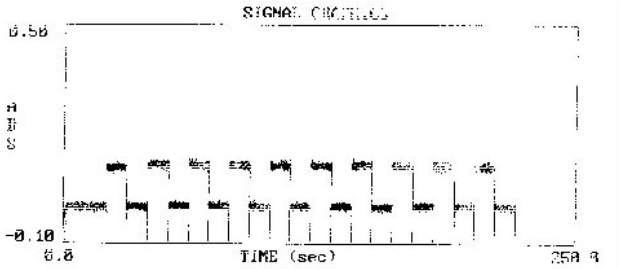

Figure 3(b). Pb signal compared to blank with ACT-80 tube.

Calibration Graphs

Calibration graphs were generated for four selected elements. The highest standard was selected to give about 0.3 Abs without the ACT-80 tube. As shown in Figure 4 the slope is clearly increased as would be anticipated from the improvements seen for the characteristic concentration. The graph for selenium shows that curvature is apparently more pronounced with the ACT-80 in place. However the same curvature is seen with higher solution concentrations without the tube in place. To corroborate this, the highest standard concentration used with the ACT-80 gave an absorbance equivalent to a standard three times the concentration without the tube.

Practical Applications

To illustrate the use of the tube in practical applications, quality control samples supplied by the United States Environmental Protection Agency (US EPA) were measured against aqueous standards. The levels of cadmium, copper and lead in EPA samples #4 and #5 are at or below the quoted detection limits for normal flame operation. A limited amount of National Bureau of Standards SRM 1643b water was also available and used for cadmium determinations.

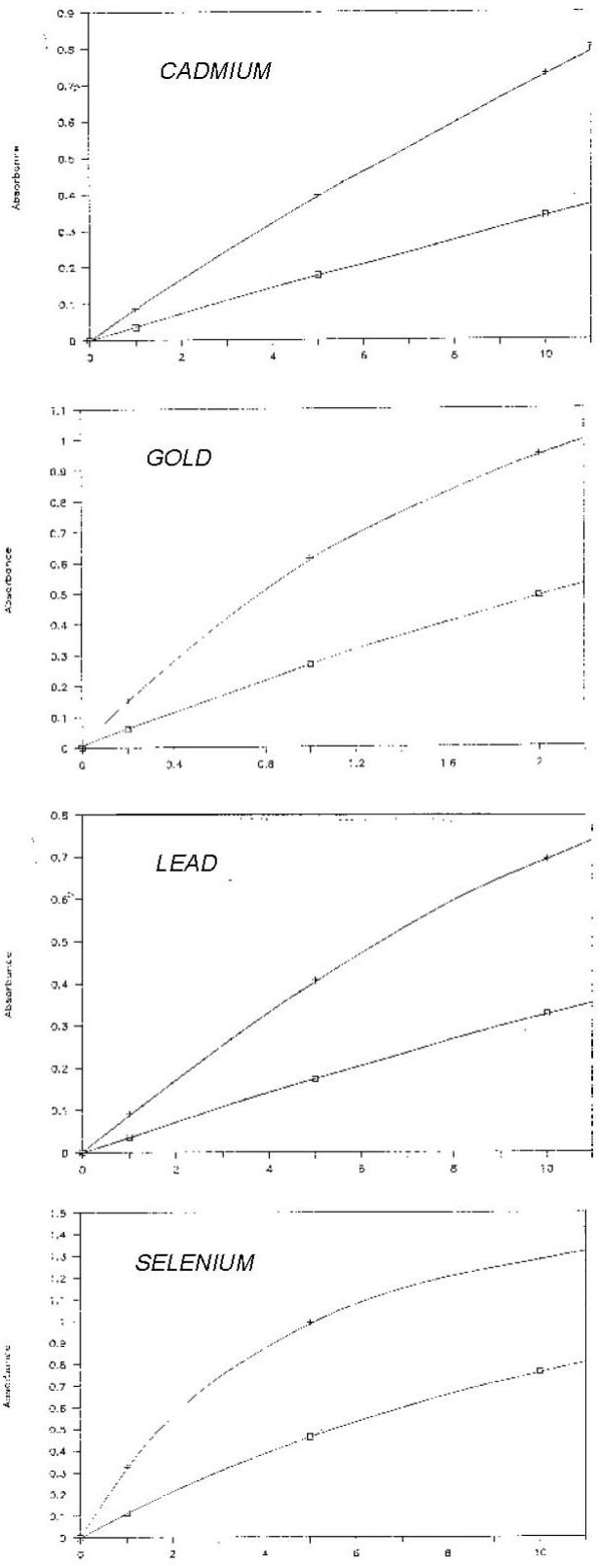

Figure 4. Calibration graphs of selected elements showing improvement in sensitivity. (+ = ACT-80, □ = normal FAAS)

The recommended instrument settings were used for each element. A delay time of five seconds and a read time of three seconds with three replicates were used. With these conditions about 200 solutions could be measured per hour. At least ten readings were taken for each sample to calculate standard deviations. The calibration graphs obtained are shown in Figure 5. A summary of the measured means and standard deviations are listed in Table 2. It can be seen that the measured results agree closely with the certified values even when working at the quoted detection limit for normal flame operation.

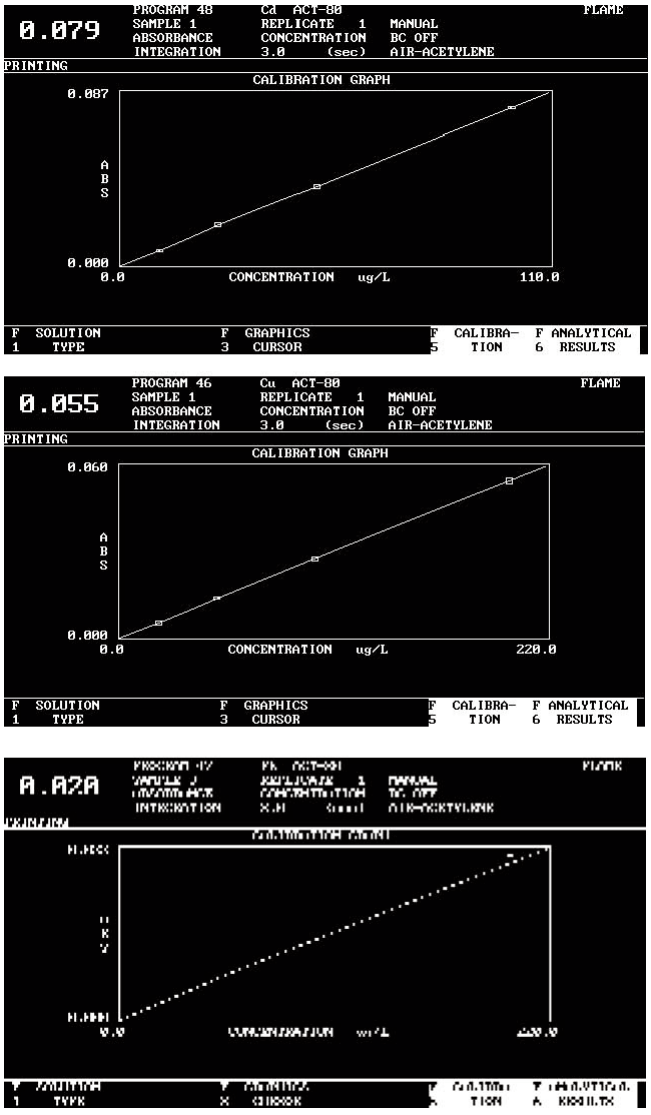

Figure 5. Calibration graphs used to measure quality control samples.

Table 2 Results for Quality Control Samples

Material	Mean ng/g	SD	Mean abs	Comments
Results for Cd using ACT-80				
US EPA sample 4	2.38	0.17		
Found	1.5	0.3	0.001	At quoted detection limit
US EPA sample 5	12.3	1.4		
Found	12.1	0.2	0.009	
NBS SRM 1643b	20	1		
Found	20.6	1.0	0.017	
Results for Cu using ACT-80				
US EPA sample 4	11.3	2.6		
Found	11.7	0.2	0.003	
US EPA sample 5	49.4	3.5		
Found	49.6	0.5	0.014	
Results for Pb using ACT-80				
US EPA sample 4	24.7	3.7		
Found	23.8	2.8	0.002	Twice quoted detection limit
US EPA sample 5	122	14.8		
Found	127.6	2.2	0.013	

Notes: Ten or more readings were taken for each solution.
SD is the standard deviation.

Conclusion

There is a measurable improvement in signal using the ACT-80. The improvements seen are comparable with those previously published. This study shows that there is an improvement in characteristic concentration between two and three times that of the normal FAAS. Detection limits generally show somewhat similar improvements. The ACT-80 is simple, cost effective and offers benefits in low level analyses.

References

1 P. A. Bennett, E. Rothery, Introducing Atomic Absorption Analysis, Varian Techtron, Australia., 1983.

2 A. E. Bernhard, Spectroscopy, **2**(6), 24, (1987).

3 K. R. Hess, R. K. Marcus, Spectroscopy, **2**(9), 27, (1987).

4 See for example "Annual Reports on Analytical Atomic Spectroscopy", Vols **1-14**, Royal Society of Chemistry; and the reviews in Journal of Analytical Atomic Spectrometry.

5. B. T. Sturman, Journal of Analytical Atomic Spectrometry, **1**, 55, (1986).

6. M. Knowles, Varian Instruments At Work, April, AA-80, (1988).

7. J. B. Willis, B. D. Frary, B. T. Sturman, in press.
8. A. Hell, "Advanced Laminar Flow Burner for Atomic Absorption," 5th Australian Spectroscopy Conference, Perth, June (1965).
9. A. Hell, W. F. Ulrich, N. Shifrin, J. Ramirez-Munez, Applied Optics, **7**, 1317-23 (1968).
10. P. A. Michalik, R. Stephens, Talanta, **28**, 37-41, (1981).
11. P. A. Michalik, R. Stephens, Talanta, **28**, 43-7, (1981).
12. P. A. Michalik, R. Stephens, Talanta, **29**, 443-6 (1982).
13. J. B. Willis, Spectrochimica Acta, **25B**, 487-512 (1970).
14. B. V L'vov, D. A. Katskov, L. P. Kruglikova, L. K. Polzik, Spectrochimica Acta, **31B**, 49-80, (1976).
15. J. W. Robinson, Analytica Chimica Acta, **27**, 465 (1962).
16. R. J. Watling, Analytica Chimica Acta, **94**, 181-6 (1977).
17. R. J. Watling, Analytica Chimica Acta, **97**, 395-8 (1978).
18. A. Taylor, A. A. Brown, Analyst, **108**, 1159-61 (1983).
19. A. A. Brown, A. Taylor, Analyst, **109**, 1455-9 (1984).
20. A. A. Brown, B. A. Milner, A. Taylor, Analyst, **110**, 501-5 (1985).
21. D. T. Delves, Analyst (London), **95**, 431 (1970).
22. C. Lau, A. Held, R. Stephens, Canadian Journal of Spectroscopy, **21**, 100-4 (1976).
23. J. Khalighie, A. M. Ure, T. S. West, Analytica Chimica Acta, **107**, 191-200 (1979).
24. J. Khalighie, A. M. Ure, T. S. West, Analytica Chimica Acta, **117**, 257-66 (1980).
25. J. Khalighie, A. M. Ure, T. S. West, Analytica Chimica Acta, **131**, 27-36 (1981).
26. J. Khalighie, A. M. Ure, T. S. West, Analytica Chimica Acta, **134**, 271-81 (1982).
27. J. Khalighie, A. M. Ure, T. S. West, Analytica Chimica Acta, **141**, 213-24 (1982).
28. C. M. Lau, A. M. Ure, T. S. West, Analytica Chimica Acta, **146**, 171-9 (1983).
29. C. M. Lau, A. M. Ure, T. S. West, Analytical Proceedings, **20**, 114-7 (1983).
30. C. Hallam, K. C. Thompson, Analyst, **110**, 497¬500 (1985).
31. S. Bradshaw, A. J. Gascoigne, J. B. Headridge, J. H. Moffett, Analytica Chimica Acta, **197**, 323-5 (1987).
32. M. Kahl, D. G. Mitchell, G. L. Kaufman, K. M. Aldous, Analytica Chimica Acta, **87**, 215 (1976).

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Sensitivity Enhancement for Flame AAS Using an Atom Concentrator Tube for Elements Dissolved in Organic Solvents

Application Note

Atomic Absorption

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Introduction

The application of a slotted tube placed on an ordinary atomic absorption burner head in order to increase the sensitivity and detection limit for a number of elements in flame-atomic absorption spectrometry (FAAS) was first demonstrated by Watling [1,2]. A very similar technique had been used before in combination with either a nickel "cup" [3] or a tantalum "boat"[4] for the same purpose. The enhancement effect using the combination of a slotted tube and an ordinary acetylene/air flame was later confirmed by several authors who demonstrated that the sensitivity and the detection limit could typically be improved by a factor of 2–5 for easily atomized elements [5–11].

Extraction of aqueous samples into a small volume of an organic solvent after addition of a complexing agent in order to enhance the detection limit is a well established method [12–14]. A concentration factor of at least 20 times can easily be achieved.

Moreover, it is also well known that atomizing organic solutions (especially those rich in oxygen, for example, ketones) can result in 3–5 times better sensitivity for many elements [15] and references therein. Thus the improvement in sensitivity for flame-AAS after extraction should be about 20 x (3–5) = 60 – 100 times.

A combination of extraction into an organic solvent and the atom concentrator tube should thus theoretically result in a total improvement in sensitivity and detection limit of (60 to 100) × (2 to 3) = 120 to 300 times.

Surprisingly, the possibility of combining these techniques has not been investigated. The present paper therefore reports results from a number of experiments using the atom concentrator tube for organic solutions of some metals. For comparison the same solutions have been analyzed without the concentrator tube.

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Experimental

Apparatus

An Agilent SpectrAA-10BQ Atomic Absorption Spectrometer equipped with a Mark VI burner head was used together with an Agilent Atom Concentrator Tube (ACT 80) including a special metal holder constructed to fit the quartz tube to this particular burner–the holder being identical with that used for the quartz tube of the Agilent Vapor Generation Accessory (VGA-77). The quartz tube was 150 mm long with two lengthwise cuts 2 mm wide by 100 and 80 mm long respectively, angled at 120 degrees relative to each other. New tubes were conditioned in the flame by nebulizing a 1% lanthanum nitrate solution for 10–15 min before use in order to prolong the tube life.

The built-in instrument graphics together with an Epson RX-80 printer were used for the recording of the signals and for construction of the calibration graphs.

Gas flow-rates of acetylene for the organic and aqueous solutions were 1.2 and 1.8 L/min respectively. The air flow-rate was 12 L/min in both cases.

The instrument parameters were as follows:

Measurement time	4 sec
Delay time	4 sec
Replicates	3
Recommended SBW and wavelength for each element	Background correction was not used

Experiments

Test solutions containing mixtures of Ag, Cu, Fe Ni and Pb made by appropriate dilutions of a metallo-organic standard mixture of the elements (Conostan S-12 100 ppm (Wt)) with methyl isobutyl ketone (MIBK) were used. A corresponding series of aqueous metal standards were made by diluting a stock solution made from the appropriate amounts of the respective metal nitrates (of A.R. grade) dissolved in water.

The following concentrations were measured: 0, 2, 4, 6, 8 and 10 mg/L of each metal.

The instrument calculated and displayed the calibration graph for each element. From the four graphs: for example, water, MIBK, water + ACT and MIBK + ACT the relative enhancement factors were calculated for each element using the absorbance values for 6 mg/L. The factors are given in Table 1.

Results and Discussion

Both the aqueous and the MIBK-solutions were measured with and without the ACT tube. The No.1 value in the table should be compared with those obtained for No. 4. Both series demonstrated the enhancement factors that can be expected when the ACT is used and that the tube indeed has almost the same effect for organic solutions. Comparison of No. 2 and No. 6 confirms this.

Experiment No. 3 illustrates the total enhancement obtained using an organic solution combined with the concentrator tube relative to aqueous solutions without the tube.

No. 5 shows that atomizing MIBK-solutions without the tube is always more effective than atomizing aqueous solutions with the tube.

The results in Table 1 also confirm that the enhancement effect using the tube is best for the easily atomized elements.

Conclusion

The results show that using a quartz atom concentrator tube for metal compounds in methyl isobutyl ketone solutions will result in the same enhancement of the sensitivity as for aqueous solutions multiplied with a factor of 3–4 due to the beneficial (exothermal) atomizing conditions for organic solvents (see above). This can be utilized in the application of extraction methods for the determination of ions present in water samples thus achieving a much better detection limit relative to that obtained for aqueous samples without extraction.

It is evident that the enhancement effect is caused mostly by the prolonged residence time of the atoms in the light path and is most pronounced for the easily atomized elements. Thus for iron (and nickel) the tube does not seem to offer any advantage at all. This can be explained by the lower temperature inside the quartz tube, this being too low for an effective atomization of the more refractive elements. For such elements it is better to atomize an organic solution without tube.

In many cases, the combination of extraction of metal complexes into organic solvents using an atom concentrator tube for flame-AAS could be an alternative to the graphite furnace technique, for instance for sea-water samples. This approach can be even more attractive if using the extraction equipment recently described for a fast, non-manual extraction of large volumes which can solve the problems associated with the use of the conventional and inconvenient separatory funnels [15].

Alternatively, programmable probe height of the SPS-5 Flame Sampler may be used to advantage in the extraction procedure.

The SPS-5 probe operates through a range of 160 mm. When two immiscible liquids are in a test tube, the probe may be programmed to descend into the upper liquid layer. Thus, the extraction procedure could be as follows:

- Pipette a volume of sample into a stopped test tube, and add a known volume of extractant
- Then pipette a volume of organic solvent into the tube, stopper and shake it
- Remove the stopper, start the SPS-5 Flame Sampler
- The probe will then descend into the upper organic layer. This eliminates the use of separatory funnels.

Table 1. Enhancement Factors for Pb, Cu, Ag, Fe and Ni

	Pb	Cu	Ag	Fe	Ni
MIBK/ACT / MIBK	2.4	1.6	2.8	0.6	1.1
MIBK/ACT / AQ/ACT	3.3	4.0	3.8	2.1	n.d.
MIBK/ACT / aq	8.6	6.0	10.9	2.2	n.d.
AQ/ACT / aq	2.7	1.5	2.8	1.0	n.d.
MIBK / aq/ACT	1.3	2.5	1.3	3.5	n.d.
MIBK / aq	3.6	3.8	3.6	3.6	n.d.

n.d. = Not determined

References

1. R. Watling J Anal Chim Acta, **1977**, 94, 181.
2. R. Watling J Anal Chim Acta, **1978**, 97, 395.
3. H. T. Delves Analyst, **1970**, 95, 431.
4. R. Bye, Fresnius' Z. Anal Chem, **1981**, 306, 30.
5. A. Taylor and A. A. Brown. Analyst, **1983**, 108, 1159.
6. A. A. Brown and A. Taylor Analyst,**1984**, 109, 1455.
7. A. A. Brown, B. A. Milner and A. Taylor Analyst, **1985**, 110, 501.
8. M. Harriot, D. Thorburn Nurns and N. Chimpales Anal Proc, **1991**, 28, 193.
9. S. Xu, L. Sun and Z. Fang Talanta, **1992**, 39, 581.
10. J. Moffet Spectroscopy, **1990**, 5, 41.
11. J. Moffet AA-91 Varian Instruments At Work, **1989**.
12. K. Kramling and H. Peterson Anal Chim Acta, **1974**, 70 35.
13. L. G. Danielsson B. Magnusson and S. Wasterlund Anal Chim Acta, **1978**, 98, 47.
14. J. D. Kinrade and J. G. Van Loon Anal Chem, 1974.
15. J. G. Welz B Atomic Absorption Spectrometry Verlag Chemie Weinheim, **1976**.
16. R. Bye, T Agasuster and A Asheim Fresnius' J Anal Chem, **1993,** 345, 111.

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