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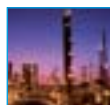
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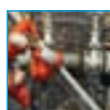
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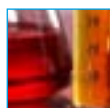
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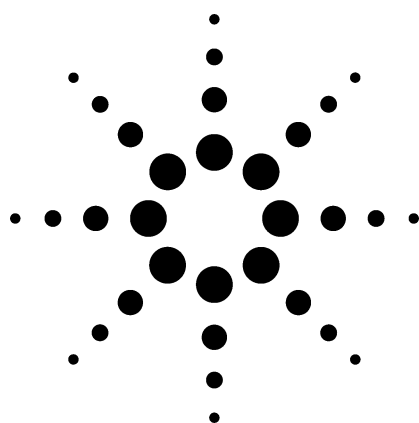
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A Column-Flow Independent Configuration for QuickSwap



Application

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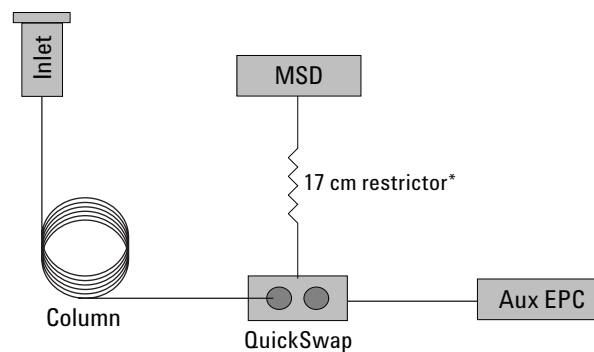
Abstract

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.



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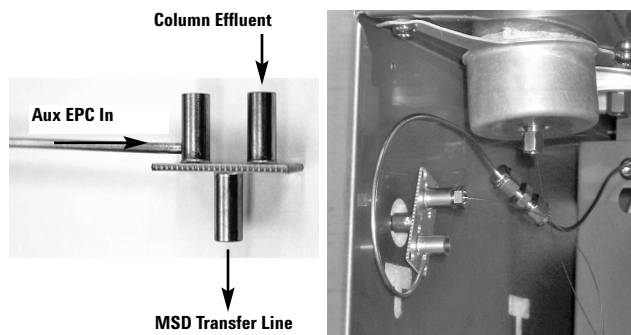


Figure 2. QuickSwap is pictured on the left showing permanent (Aux EPC In) and temporary connections. A picture of a normal QuickSwap installation is shown on the right.

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.

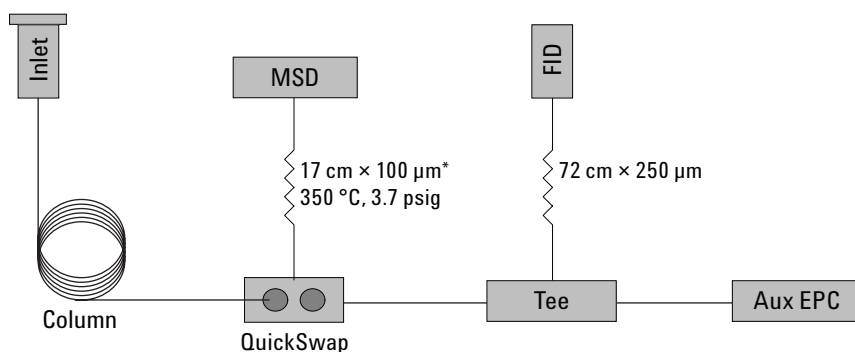
In general, when flow to the MSD changes,

- 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.

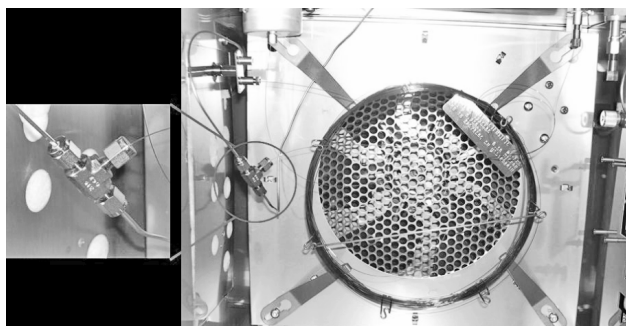
- Simplifies retention time locking applications with QS
- Allows pressure pulse injections without having to change QS restrictor
- Allows more aggressive backflush conditions than if larger restrictors were used
- Allows method translation and speed up without having to change QS restrictor
- Allows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

tions 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.

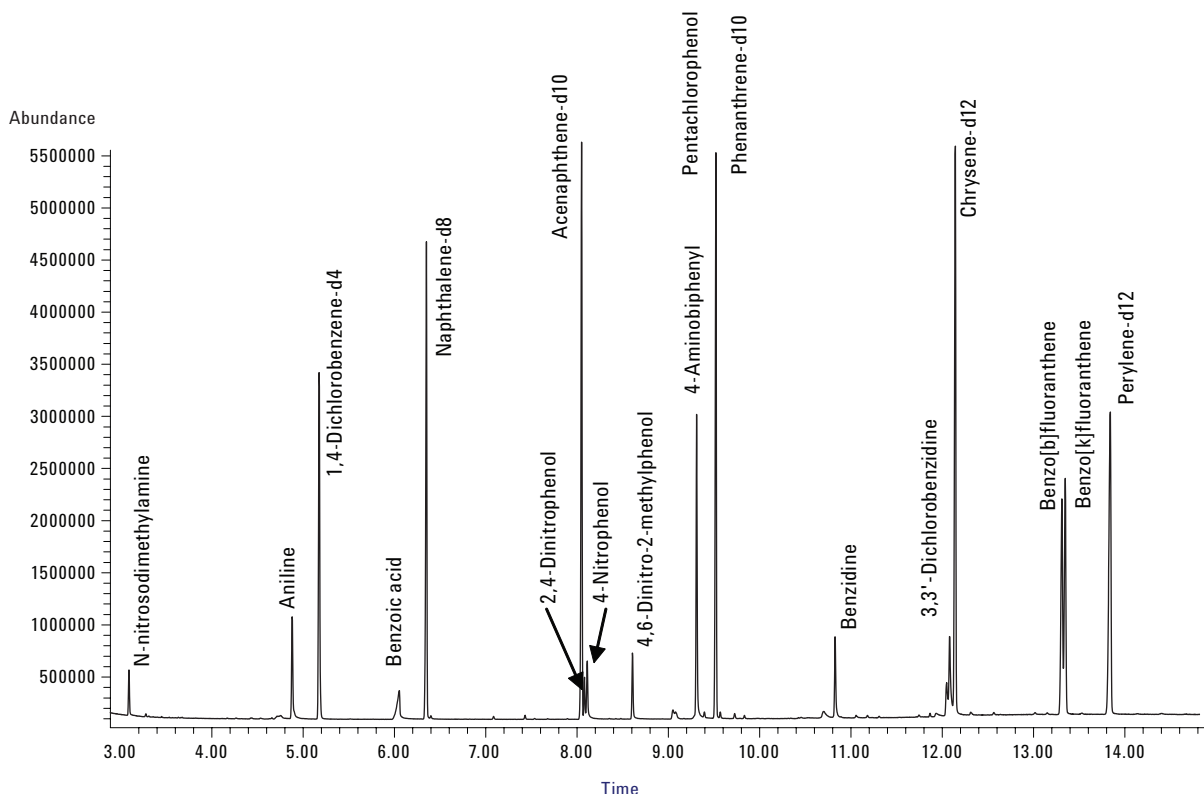


Figure 4. Reference chromatogram for Fast 8270 method.

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 Corresponding to the Optimal Flow Rate Range of the MSD

QuickSwap restrictor id (μm)	QuickSwap pressure (psig)	Transfer line temperature ($^{\circ}\text{C}$)	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 semi-volatiles 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) $\rightarrow$ 350°C (3 min) @ $20^{\circ}\text{C}/\text{min}$; 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
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).

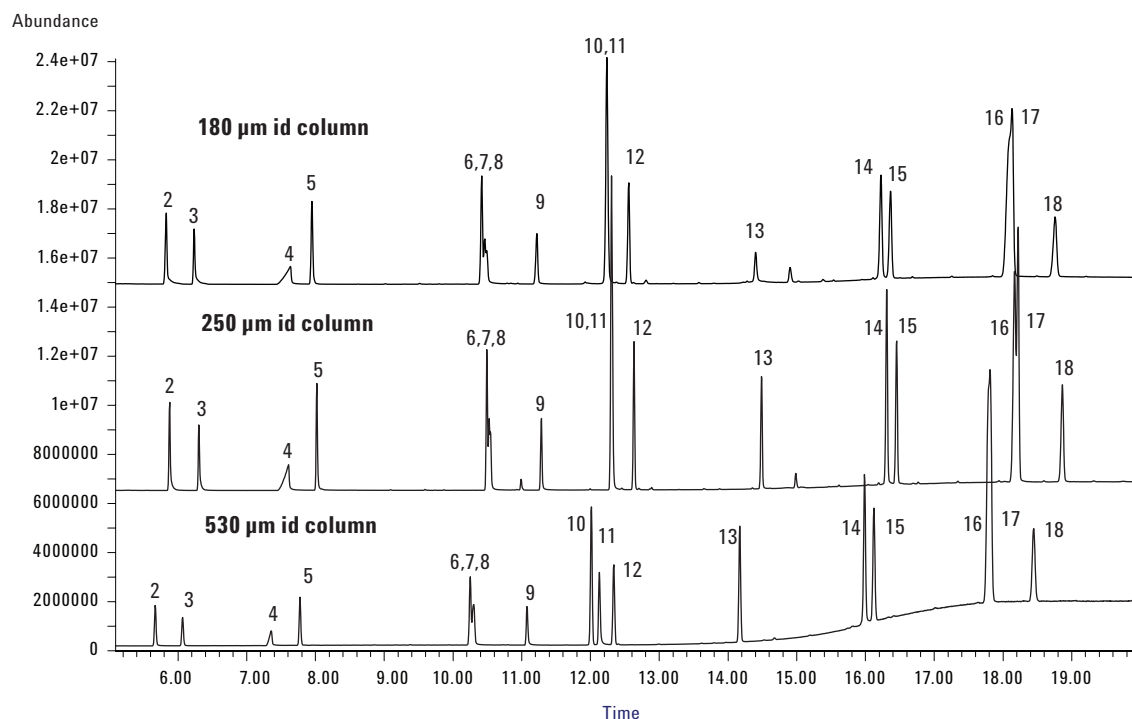


Figure 5. Constant pressure mode analysis with three different column dimensions; 0.5- μL splitless injections of 80-ppm semi-volatiles test sample, with flow conditions from Table 2.

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- μ m id column flow exceed 1 mL/min, so there is nothing vented and no FID signal. For the 250- μ m 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- μ m 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.

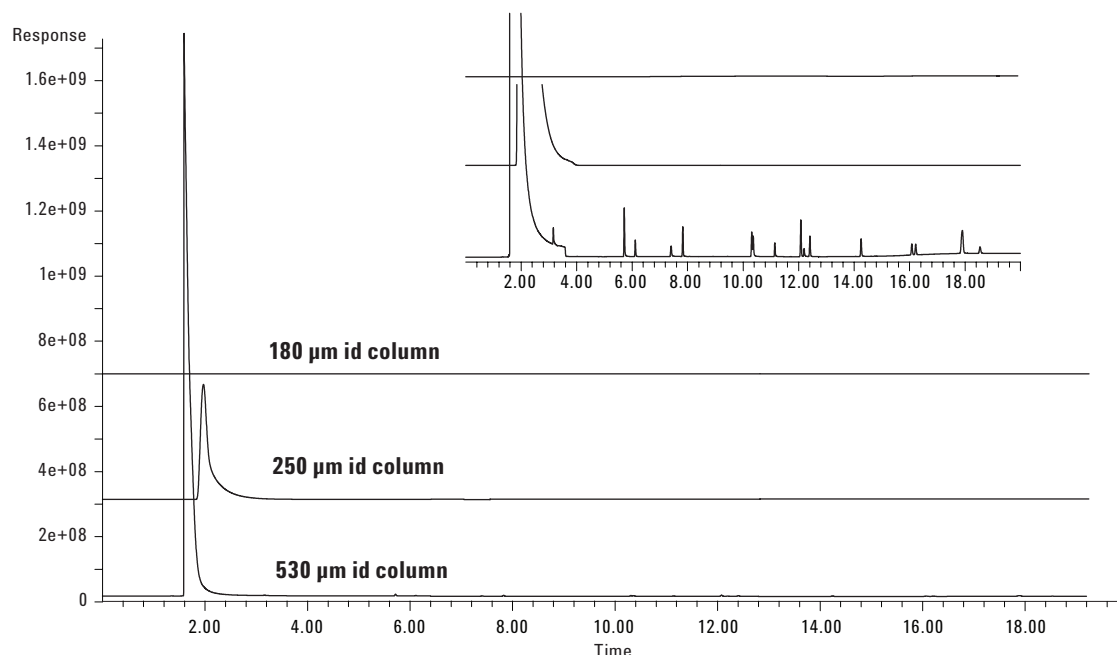


Figure 6. FID signal of vent stream shows what is vented when column flow exceeds flow to MSD.

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

Dimensions	Outlet flow
20 m X 180 μ m	0.72 mL/min
20 m X 180 μ m	1.44 mL/min
30 m X 250 μ m	2.5 mL/min
30 m X 250 μ m	1.0 mL/min
30 m X 530 μ m	2.1 mL/min
30 m X 530 μ m	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- μ m and 180- μ m id columns, no column effluent is split to the FID. Since the flow rate of the 530- μ m id column is approximately 2X the flow the MSD, half of the column effluent is split to the FID.

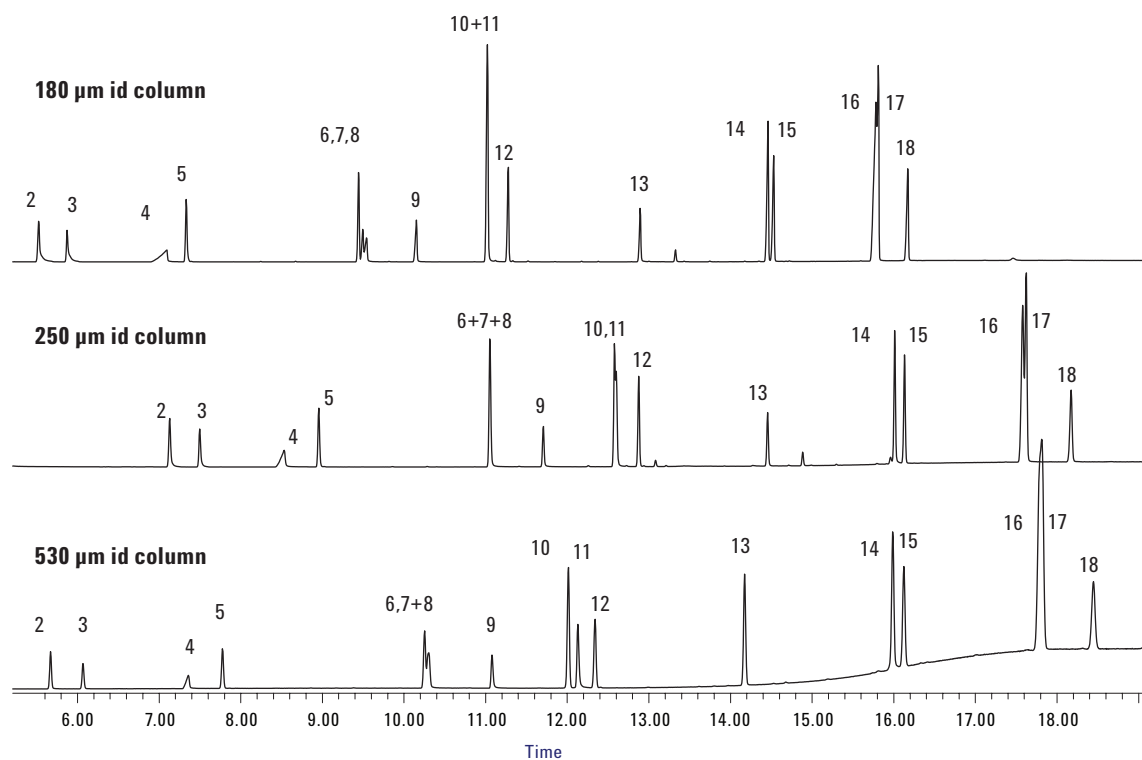


Figure 7. TIC chromatograms for the three columns under optimal constant flow mode conditions.

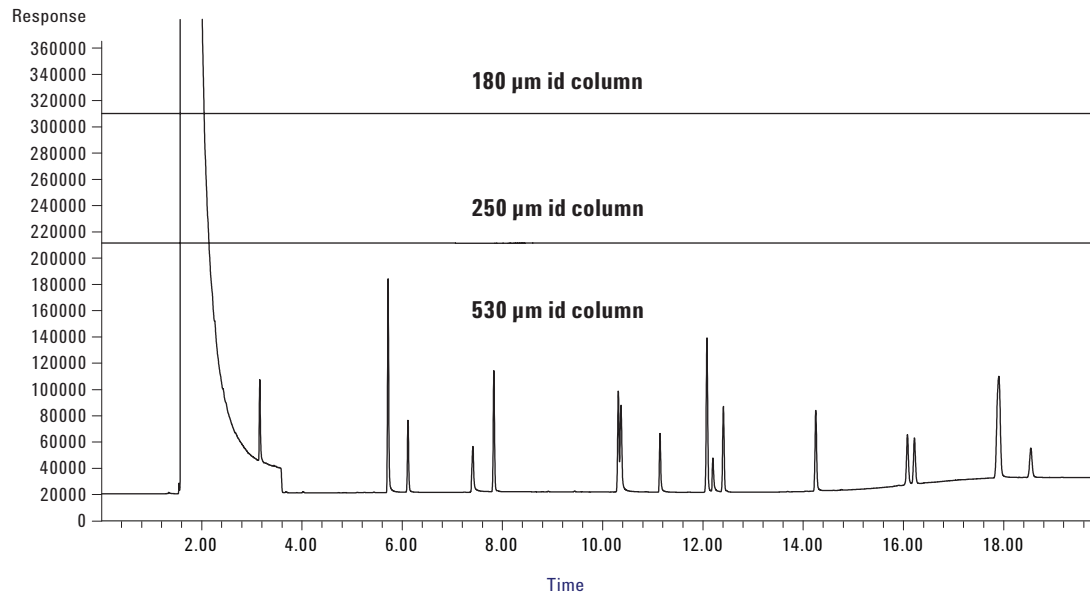


Figure 8. FID vent signal for three columns under optimal flow conditions. Only the 530-µm id column has a flow that exceeds the 1 mL/min flow to the MSD.

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.

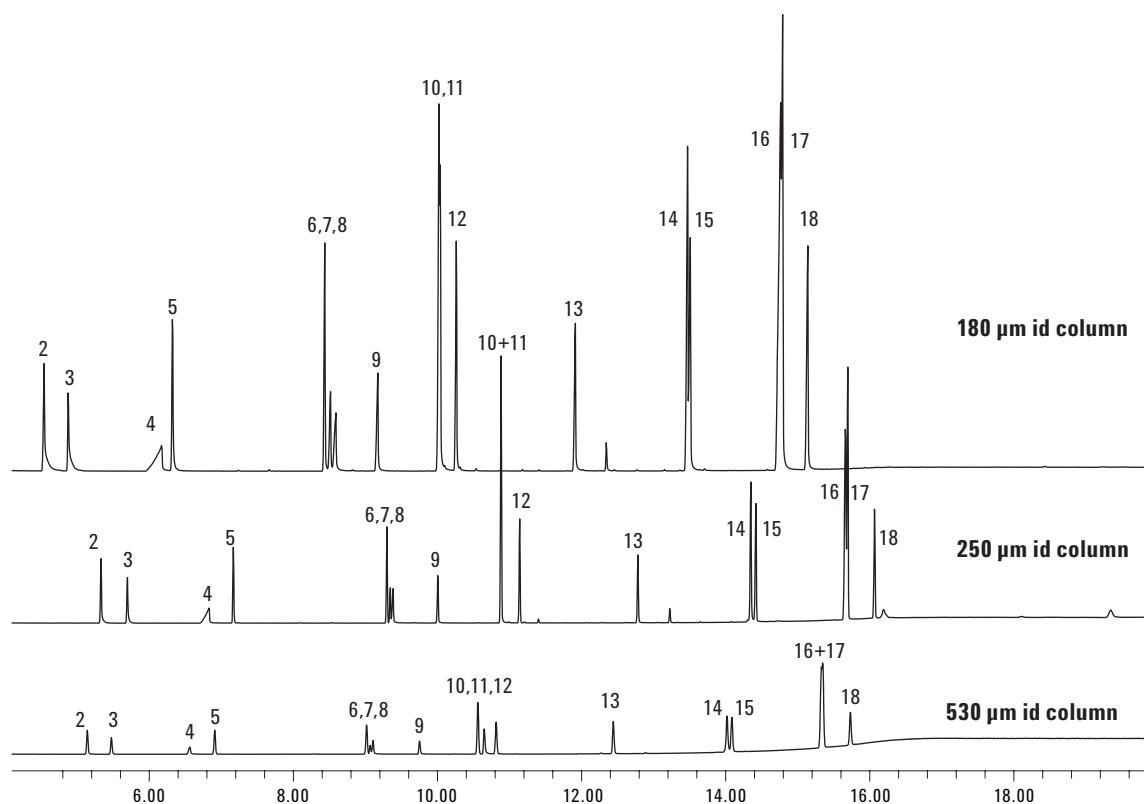


Figure 9. Comparison of MSD TIC chromatograms for three columns run at 2X optimal constant flow mode. Scale is constant for the three, showing the absolute amount of sample reaching the MSD.

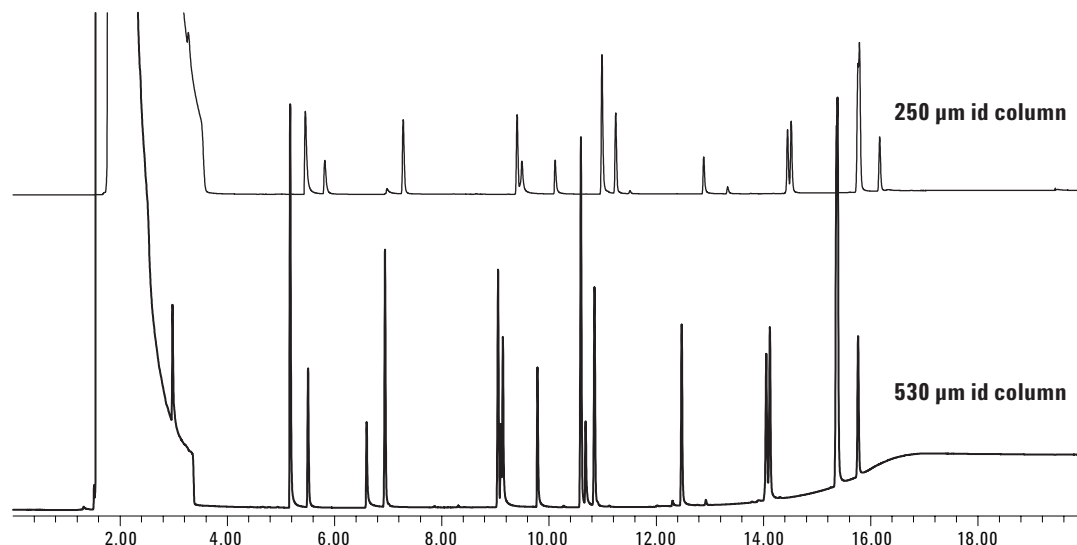


Figure 10. FID vent signals for the two largest columns operated at 2X optimal constant flow rate conditions.

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.

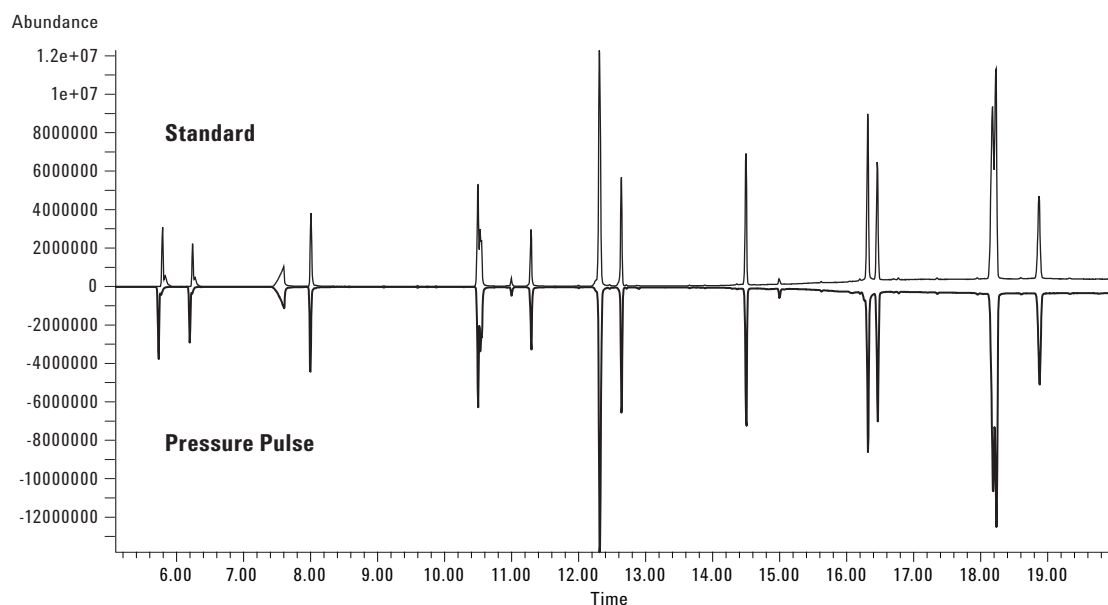


Figure 11. Comparison of standard and pressure-pulse injection modes. No adjustment of QuickSwap pressure was required for the pressure-pulse mode—a benefit of using QuickSwap split configuration.

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.

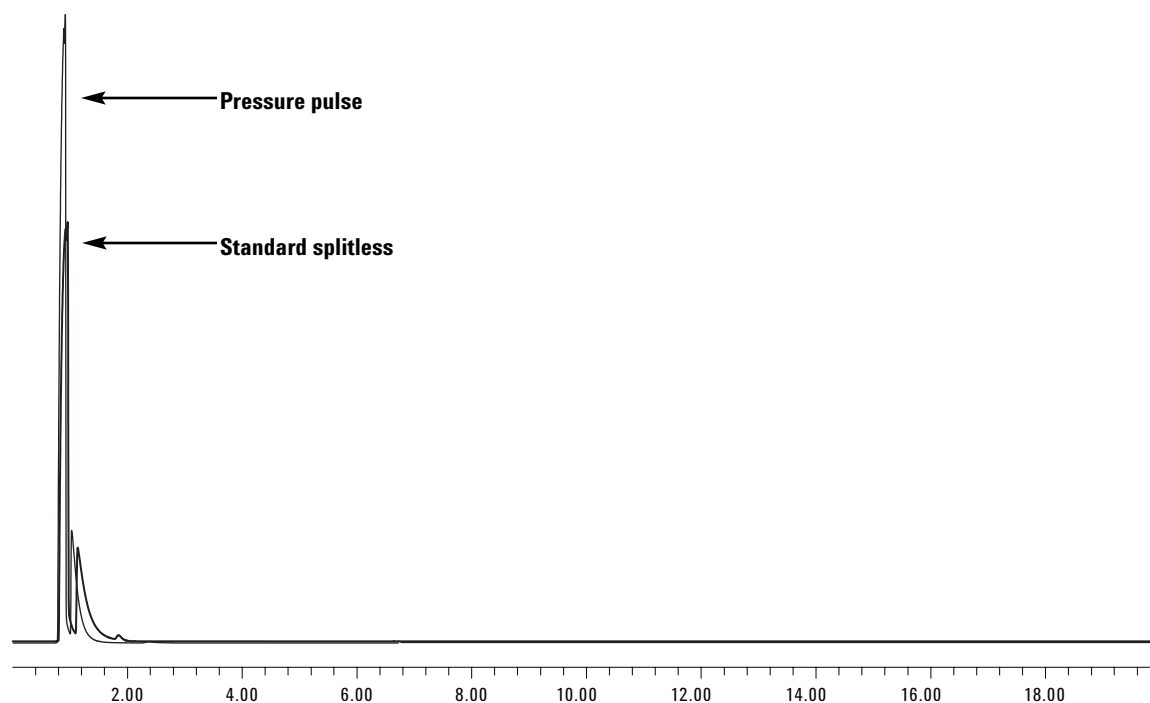


Figure 12. FID vent signal for pressure-pulse injection versus standard splitless injection.

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 column connections	250 µm	5188-5361
	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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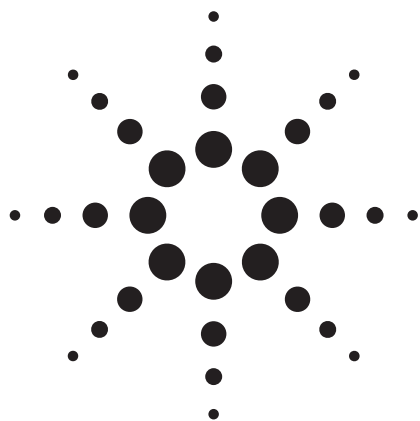
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AA or ICP – Which Do You Choose?

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Geoff Tyler

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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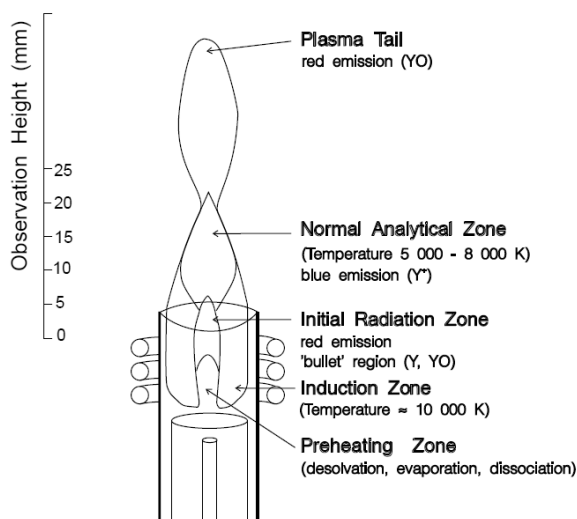


Figure 1. A plasma used for emission spectrometry. The regions refer to those seen when a Yttrium solution is introduced.

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	0.3 – 2%	0.1 – 1%	0.5 – 5%
Short term	Less than 5%		
Long term (over 8 hrs)			
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

Element		AA λ (nm)	ICP λ (nm)	ICP Detection limit µg/L	Flame AA Characteristic conc µg/L	Detection limit µg/L	Flame type	Zeeman Furnace AA 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 ₂ O	0.25	5	100	Al
Arsenic	As	193.7	188.985	12	500	300	N ₂ O	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 ₂ O	43	855*	70	B
Barium	Ba	553.6	455.403	0.07	200	20	N ₂ O	0.85	17	100	Ba
Beryllium	Be	234.9	313.042	0.2	15	1	N ₂ O	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 ₂ O	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 ₂ O			–	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 ₂ O	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 ₂ O	2.3	45	100	Dy
Erbium	Er	400.8	337.271	0.7	500	50	N ₂ O	5	100	100	Er
Europium	Eu	459.4	381.967	0.3	300	1.5	N ₂ O	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 ₂ O			–	Gd
Germanium	Ge	265.1	265.118	13	1000	200	N ₂ O	0.45	9*	100	Ge
Hafnium	Hf	307.3	264.141	4	10000	2000	N ₂ O			–	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	Detection limit µg/L	Flame type	Zeeman Furnace AA Characteristic*** conc** µg/L	Mass pg	MSR %	EI
Holmium	Ho	410.4	345.600	0.5	700	40	N ₂ O			—	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 ₂ O			—	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 ₂ O			—	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 ₂ O	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 ₂ O			—	Nb
Neodymium	Nd	492.5	401.225	2	6000	1000	N ₂ O			—	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 ₂ O			—	Os
Phosphorous	P	213.6	177.499	18	120000	40000	N ₂ O	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 ₂ O			—	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 ₂ O			—	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 ₂ O			—	Sc
Selenium	Se	196.0	196.026	37	1000	500	N ₂ O	0.7	14*	92	Se
Silicon	Si	251.6	251.611	5	1500	300	N ₂ O	0.75	15	100	Si
Samarium	Sm	429.7	442.434	7	6000	1000	N ₂ O			—	Sm
Tin	Sn	235.5	242.949	15	700	100	N ₂ O	0.5	10*	93	Sn
Strontium	Sr	460.7	407.771	0.02	40	2	N ₂ O	0.1	2	94	Sr
Tantalum	Ta	271.5	268.517	9	10000	2000	N ₂ O			—	Ta
Terbium	Tb	432.7	350.917	5	7000	700	N ₂ O	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 ₂ O	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 ₂ O			—	Tm
Uranium	U	358.5	385.958	18	100000	40000	N ₂ O			—	U
Vanadium	V	318.5	309.311	2	700	100	N ₂ O	1.1	22	79	V
Tungsten	W	255.1	239.709	17	5000	1000	N ₂ O			—	W
Yttrium	Y	410.2	371.030	0.2	2000	200	N ₂ O			—	Y
Ytterbium	Yb	398.8	328.937	0.3	60	4	N ₂ O	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 ₂ O			—	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:

$$CM_n = CM_z \times MSR (\%)/100 \quad CC_n = CC_z \times MSR (\%)/100$$

where:

CM_n = Characteristic Mass for Deuterium Furnace Systems

CM_z = Characteristic Mass for Zeeman Furnace Systems (from Table 1)

MSR = Magnetic Sensitivity Ratio (as % from Table 1)

CC_n = Characteristic Concentration for Deuterium Furnace Systems

CC_z = Characteristic Concentration for Zeeman Furnace Systems (from Table 1).

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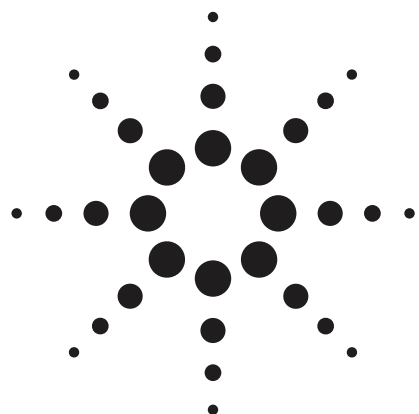
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Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns

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 libraries¹ or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates² 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 columns³. 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

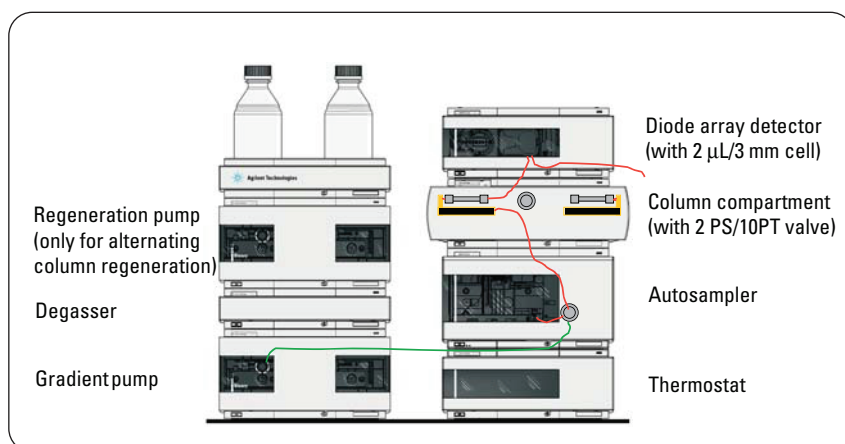


Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and low-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 $\mu\text{g}/\mu\text{L}$ for each compound except butyrophenone which was 0.2 $\mu\text{g}/\mu\text{L}$. The solvent was water-acetonitrile 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

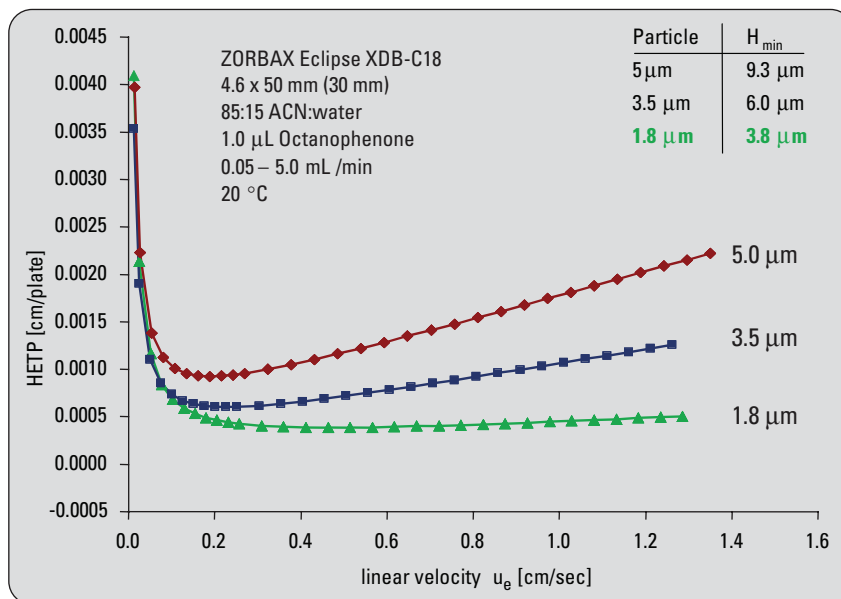


Figure 2
Van Deemter curves of columns packed with 1.8 μm , 3.5 μm and 5.0 μm particles.

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 $R_s = 2.1$ up to $R_s = 3.5$ 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.

For gradient separation the dependencies of the capacity factor can be expressed as:

$$k^* = 0.87 \cdot tg \cdot \frac{F}{V_m \cdot \Delta\%B \cdot S}$$

(*tg* = gradient time, *F* = flow rate, *V_m* = column void volume, $\Delta\%B$ = 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 base-line 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).

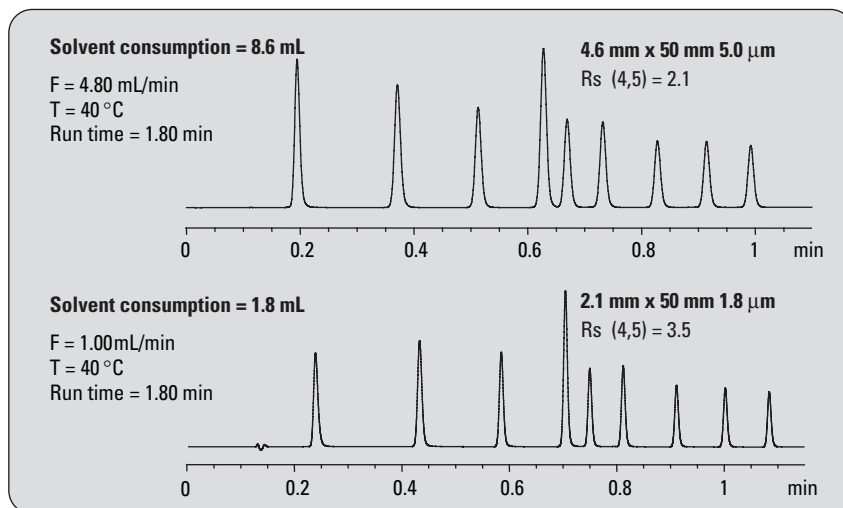


Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

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 0.90 min 95 %B 1.10 min 95 %B 1.11 min 35 %B 1.15 min	0.00 min 35 %B 0.90 min 95 %B 1.10 min 95 %B 1.11 min 35 %B 1.15 min
Stoptime:	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)

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
<i>Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL</i>	
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

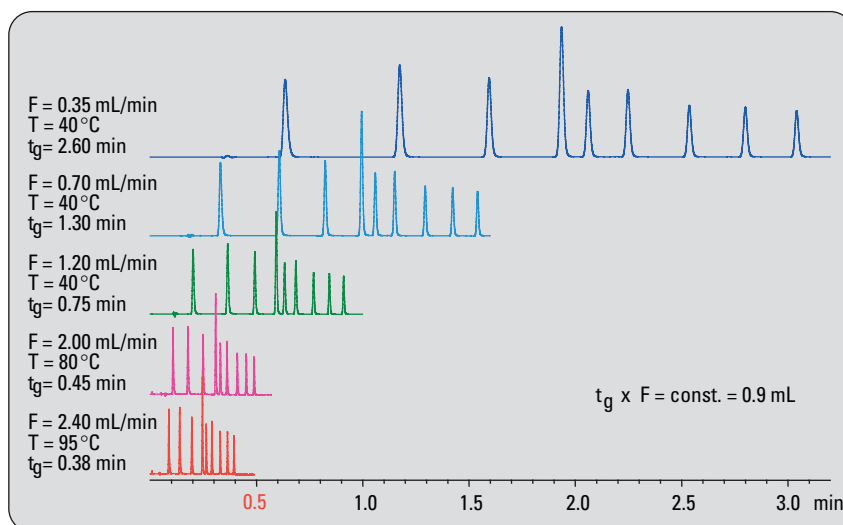


Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

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

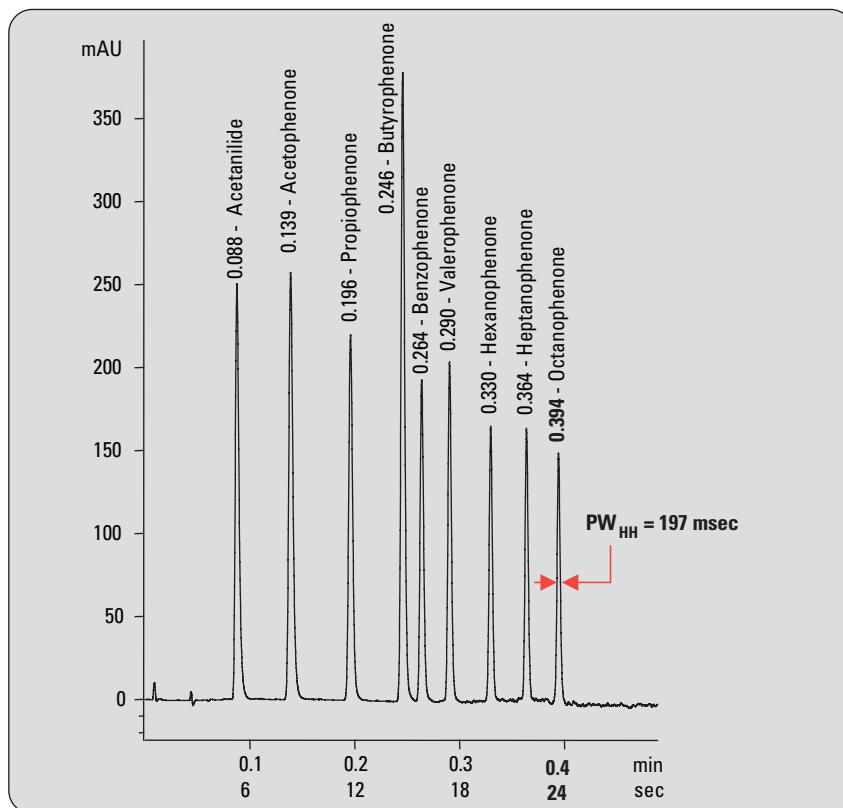


Figure 5
Separation of a nine compound mixture under ultra fast conditions.

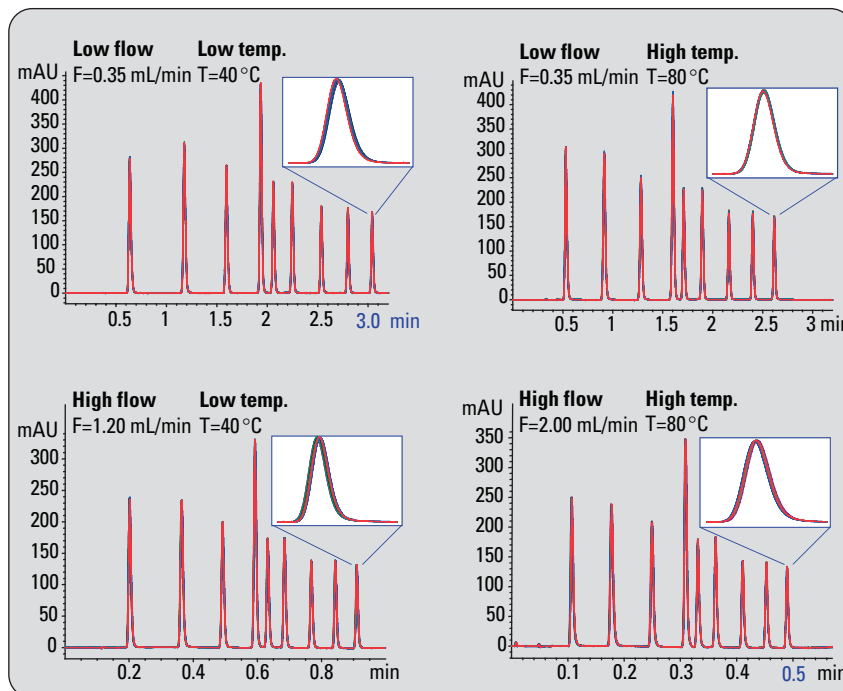


Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

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- μ m 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.
	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
Column compartment	no OI – Draw/Eject speed	Low speed causes increased sampling time	Low speed results in better injection precision
	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.

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

0.45 min gradient method, flow = 2 mL/min, 80 °C, alternating column regeneration

ADVR	OI	DA	SvMeth	NW	Blc	Cycle time [s]	Throughput [sample/day]
✓	✓	✗	✗	✓(2s)	✓	49	1763

0.90 min gradient method, flow = 1 mL/min, 40 °C

ADVR	OI	DA	SvMeth	NW	Blc	Cycle time [s]	Throughput [sample/day]	
✓	✓	✗	✗	✗	✗	111	778	Theoretical value with no overhead time
✓	✓	✗	✗	✗	✗	119	726	
✓	✓	✗	✗	✓	✓	129	670	
✓	✗	✗	✓	✓	✓	157	550	
✓	✗	✓	✓	✓	✓	163	530	
✗	✗	✗	✓	✓	✓	172	502	
✗	✗	✓	✓	✓	✓	180	480	

ADVR = Automatic Delay Volume Reduction
 DA = Data Analysis after Acquisition
 NW = Needle Wash
 (5s resp. 2s for the ACR Method)

OI = Overlapped Injection
 (after sample is flushed out)
 SvMeth = Save Method with Data File
 Blc = Pre-run Balance of DAD

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

0.00 min 35 %B
 0.45 min 95 %B
 0.46 min 35 %B
 0.57 min 35 %B

Regeneration-Pump

0.00 min 35 %B
 0.01 min 95 %B
 0.11 min 95 %B
 0.12 min 35 %B

Stoptime: 0.57 min
 Posttime: 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
 Gradient:

0.00 min 35 %B
 0.90 min 95 %B
 1.10 min 95 %B
 1.11 min 35 %B
 1.15 min

No

0.00 min 35 %B
 0.90 min 95 %B
 1.10 min 95 %B
 1.11 min 35 %B

Stoptime: 1.15 min
 Posttime: 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

1.40 min (add. 300 µL extra column volume, increased retention times)
 0.70 min

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. Kostianen, 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" *Agilent Application Note, Publication number 5988-7831EN*; **2002**.

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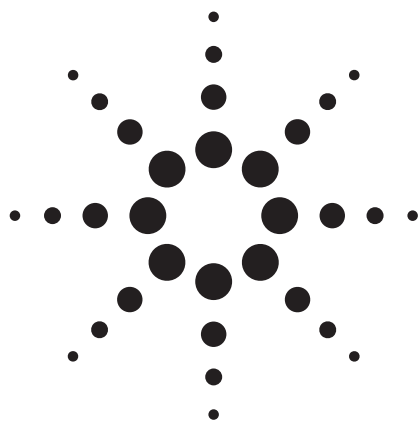
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Agilent Technologies



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- μ L hot splitless, 10- μ L cold splitless and 25- μ L 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.

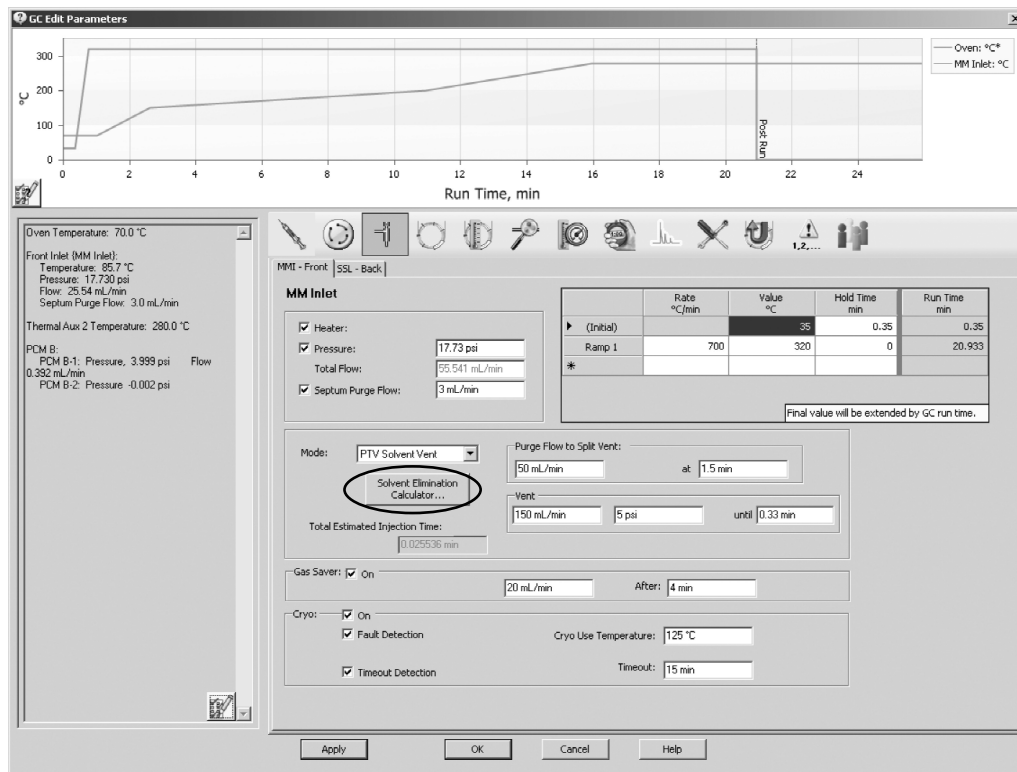


Figure 1. Multimode Inlet "Solvent Elimination Calculator" imbedded in ChemStation for easy method development.

Agilent Solvent Elimination Calculation Wizard

Welcome to the Solvent Elimination Calculator!

Please supply the following information.

If you don't know the first analyte boiling point, leave it at 150 °C.

Solvent: acetone

Injection Volume (µL): 25 µL

Boiling Point of first eluting analyte (°C): 150 °C

LVI Method Help Next Cancel Help

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.

Solvent Elimination Calculator

Agilent
Solvent Elimination
Calculation Wizard

Calculated values will change each time an input parameter is modified.

Elimination Rate (µL/min) 149.80 Suggested Injection Rate (µL/min) 74.90 Suggested Vent Time (min) 0.33

Inlet Temperature (°C) 35 Vent Pressure (gauge) 5.000 ☐ kPa
 Vent Flow (mL/min) 150 Outlet Pressure (gauge) 0.000 ☒ psi
 Injected Volume (µL) 25.0 ☐ bar

Solvent acetone

LVI Method Help Previous Next Cancel Help

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).

Confirm Copy values to Method Editor.	
(Check parameters to change.)	
☒ Inlet temperature	35 °C
☒ Initial hold time	0.33 min
☒ First ramp rate	600 °C/min
☒ First temperature	325 °C
☒ First hold time	5 min
☒ Vent time	0.33 min
☒ Vent pressure	5 psi
☒ Vent flow rate	150 mL/min
☒ Purge time	2.83 min
☒ Purge flow rate	60 mL/min
☒ Injection rate	75 µL/min
☐ Oven initial temperature	50 °C
☐ Oven initial hold time	2.83 min

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- μ L hot splitless injection. Some of the 40-ppb pesticides are barely visible (80 pg each on column). The middle TIC is from a 10- μ L 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- μ L 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- μ L injection volume. This implies that the solvent was mostly eliminated during the injection.

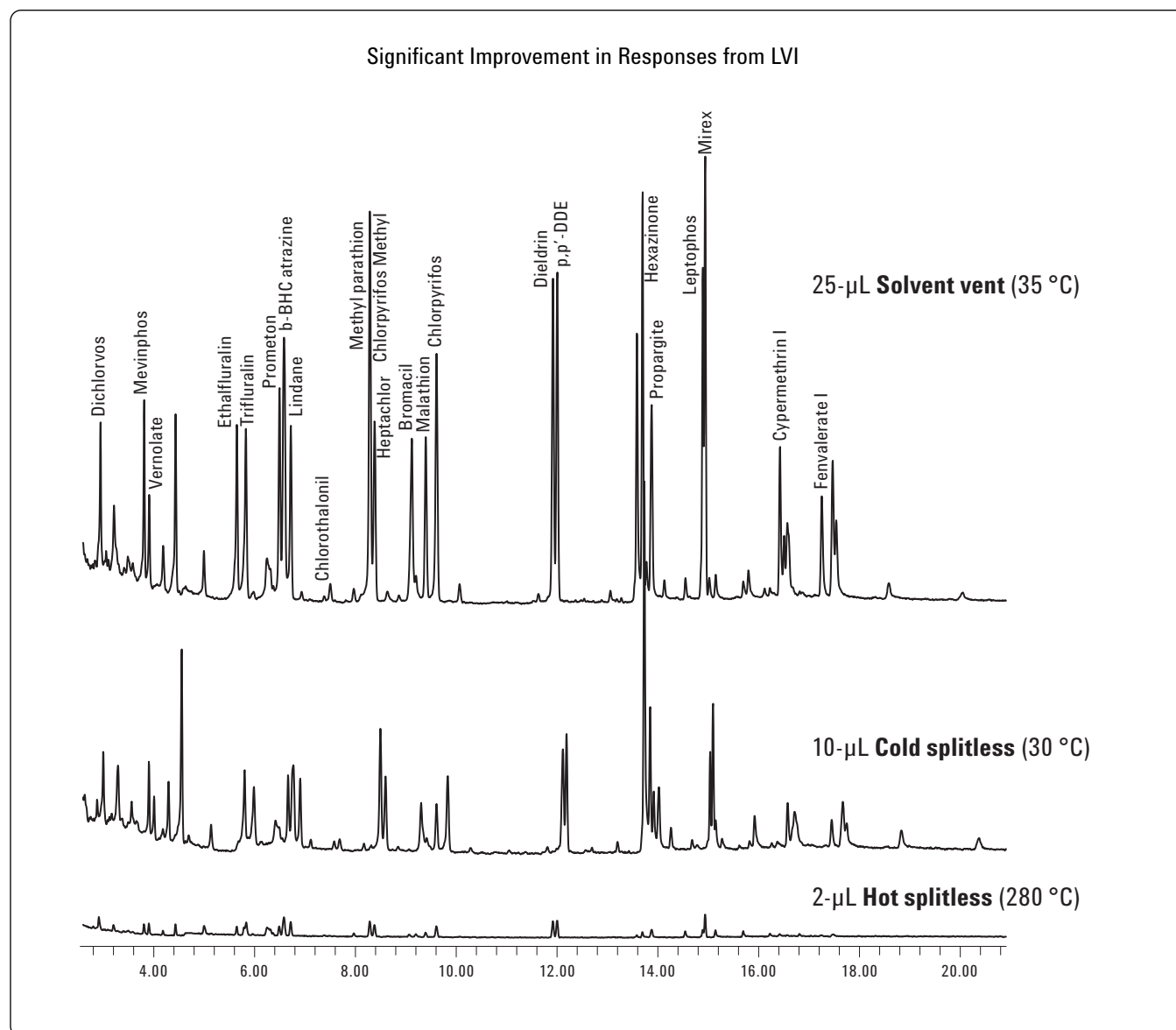


Figure 5. Overlay of total ion chromatograms (TICs) from three injection modes, plotted on the same scale.

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- μ L solvent vent injection to the 2- μ L 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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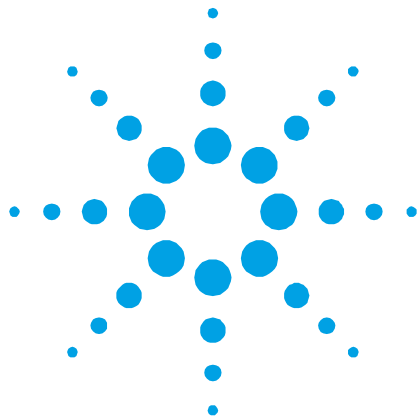
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Agilent Technologies



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 advantages¹:

- 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.



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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/JPS 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

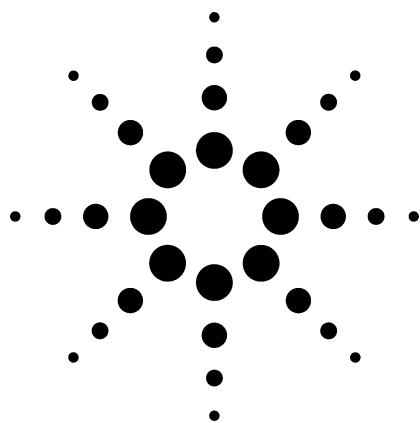
- ¹ Larry A. Toms, "Machinery Oil Analysis. Methods, Automation & Benefits", 2nd ed., Coastal Skills Training, Virginia Beach, VA, 1998.
- ² Allison M. Toms, "FTIR for the Joint Oil Analysis Program", in Proc. 1994 Joint Oil Analysis Program International Condition Monitoring Conference, Squalls, M., ed., JOAP-TSC, Pensacola, FL (1994), pp.387-419.
- ³ Available from www.astm.org

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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.



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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.

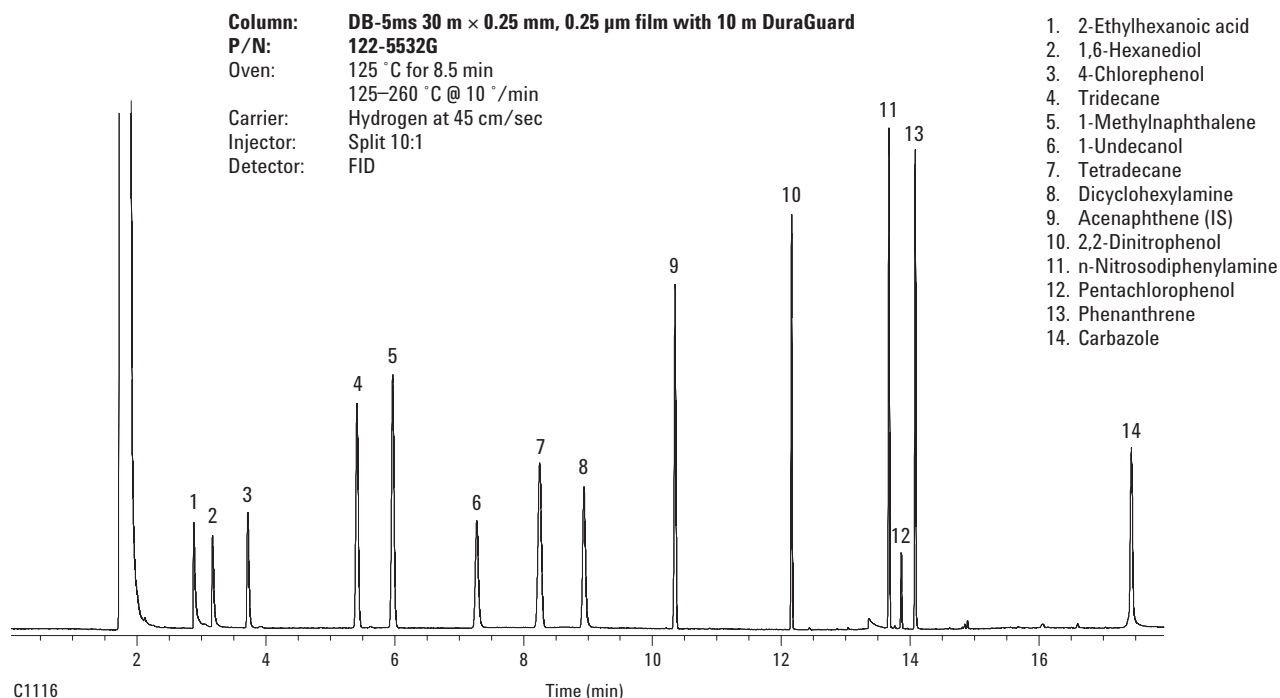


Figure 1. Chromatogram of test mixture using combination guard and analytical columns.

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.

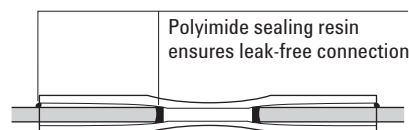


Figure 2. Detail of glass press fit union with polyimide sealing resin.

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).

For More Information

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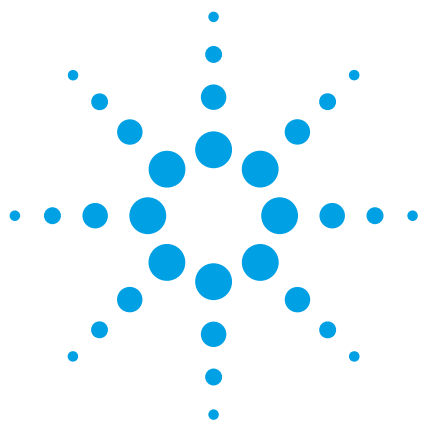
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Agilent Technologies



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.

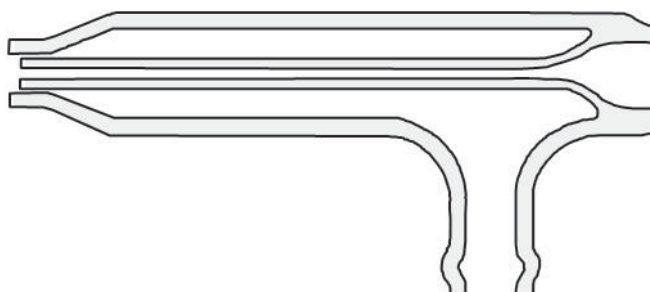


Figure 2. Concentric glass nebulizer

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 (Table 1). 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.

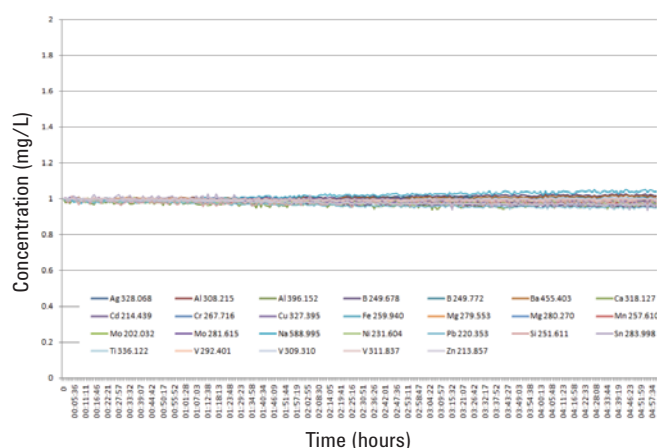


Figure 3. Long-term stability of the OneNeb nebulizer with diisobutyl ketone

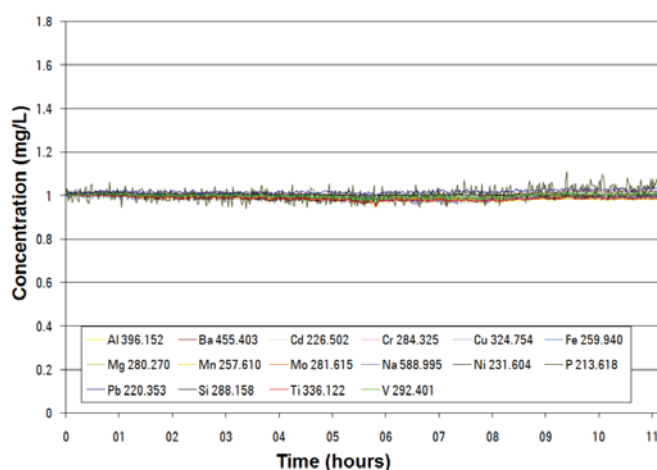


Figure 4. Long-term stability of the OneNeb nebulizer with ShellSol

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
Ti 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 $\mu\text{L}/\text{min}$, potentially allowing the analysis of volume limited samples.

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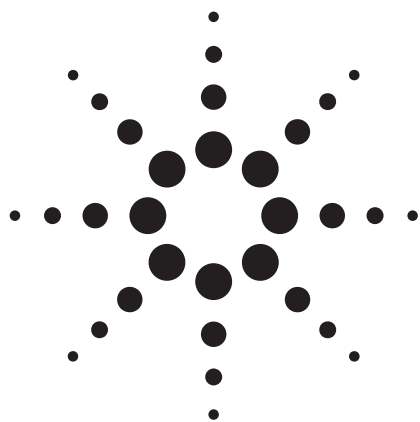
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Agilent Technologies



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 x 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).

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

Solvent: hexane, bp = 70 °C

	Septum:	A		B		C	
		%loss	%loss/hr	%loss	%loss/hr	%loss	%loss/hr
After:							
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	
		%loss	%loss/hr	%loss	%loss/hr	%loss	%loss/hr
After:							
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

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.

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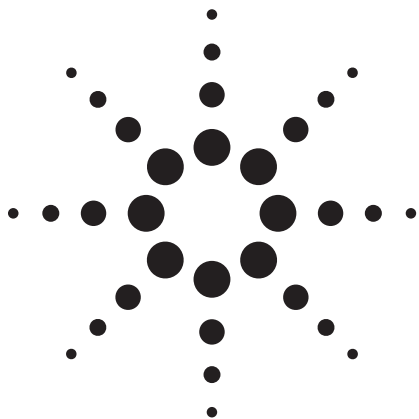
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Guidelines for Using Non-Aqueous Solvents in Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Author

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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
Tetrahydronaphthalene (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):

$$\% \text{ Recovery} = (\text{SSR} - \text{SR}) / \text{SA} \times 100$$

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 $\mu\text{g/g}$ 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 $500 \mu\text{g/g}$ 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.

$$\text{mass salt} = \frac{MC}{10,000 P} \text{ grams} \quad (1)$$

where M is mass of oil standard required (g)
 C is concentration of analyte in oil ($\mu\text{g/g}$)
 P is percent analyte in salt

Example 1: Prepare a $500 \mu\text{g/g}$ 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.

$$\text{mass oil standard} = \frac{MC}{S} \text{ grams} \quad (2)$$

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 $120 \mu\text{g/g}$ Cu and $300 \mu\text{g/g}$ Al starting with $5000 \mu\text{g/g}$ standards.

Using equation 2,

$$\begin{array}{ll} \text{Cu} & \text{Al} \\ m = \frac{10 \times 120}{5000} & m = \frac{10 \times 300}{5000} \\ = 0.2400 \text{ g} & = 0.6000 \text{ g} \end{array}$$

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.

$$\begin{array}{ll} \text{Method 1:} & 1.5\% \text{ Zn} = 1.5 \times 10,000 \mu\text{g/g Zn} \\ \text{From equation 1:} & m = \frac{20 \times 1.5 \times 10,000}{10,000 \times 16.18} = 1.854 \text{ g} \end{array}$$

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:

$$\text{From equation 2} \quad m = \frac{20 \times 150}{5000} = 0.600 \text{ g}$$

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 naphthenates, sulphonates or cyclobutyrate.

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 analytes 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
- Suitably 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

National Physical Laboratory Office of Reference Materials Teddington, Middlesex, TW1 0LW England

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Improving Productivity and Extending Column Life with Backflush

Application Brief

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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, μ ECD, 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

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.

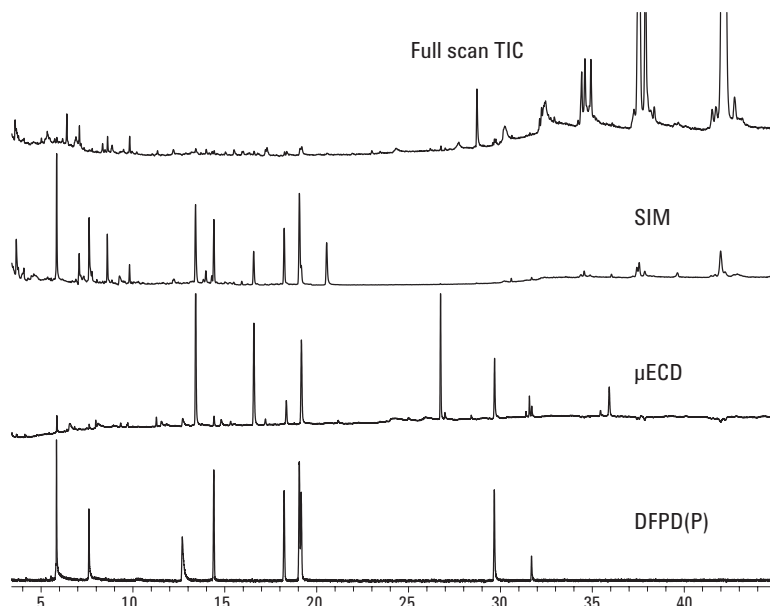


Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.



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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

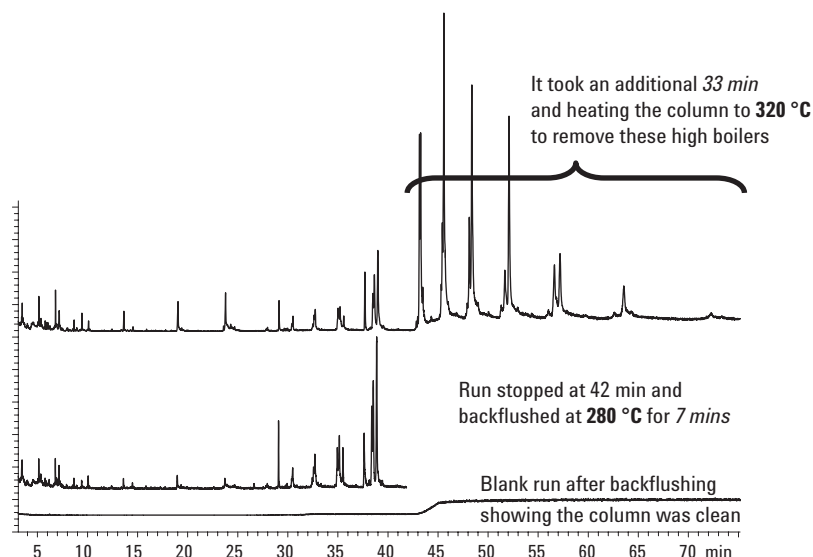


Figure 2. Three total ion chromatograms comparing the results with and without backflush.

of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μ ECD, 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

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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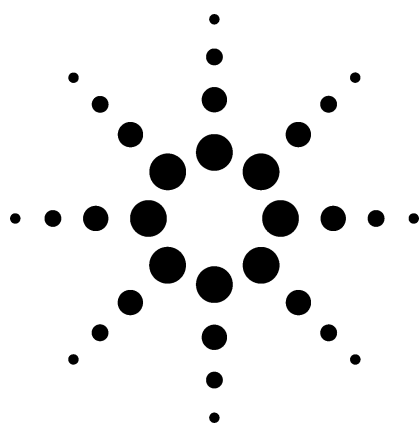
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Improving the Effectiveness of Method Translation for Fast and High Resolution Separations

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 L_1/L_2 , 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.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam. column 2}}{\text{Diam. column 1}} \right]^2 = \text{Flow}_{\text{col. 2}} \quad (\text{eq. 1})$$

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,



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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 opti-

um 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

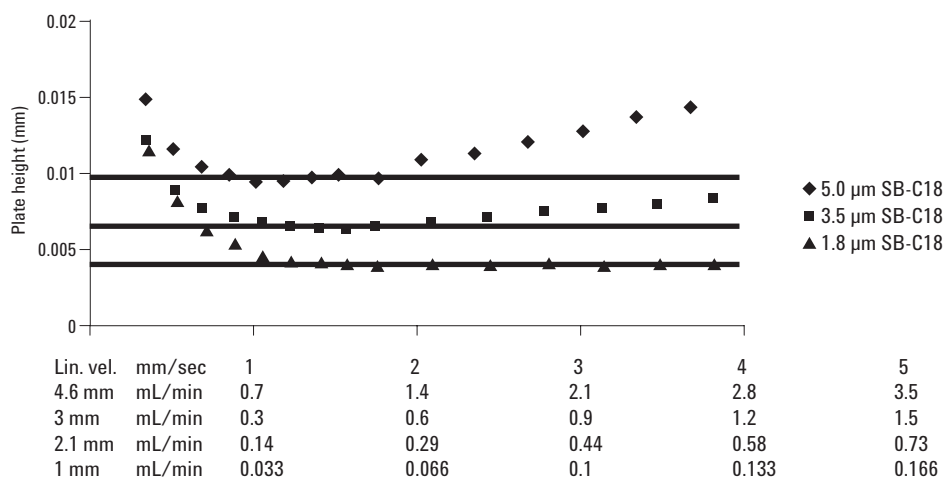


Figure 1. van Deemter plot with various flow rates and particle sizes.

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

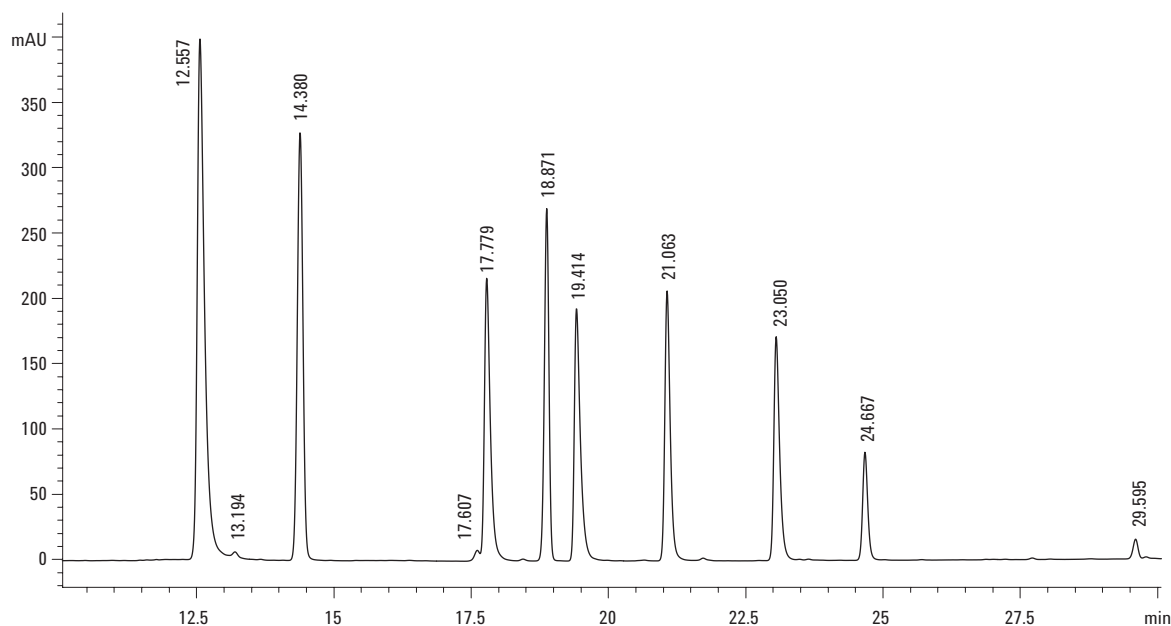
Standard mixture of chlorinated phenoxy acid herbicides,
 100 μg/mL in methanol

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 H₃PO₄

Gradient slope: 7.8% ACN/column volume

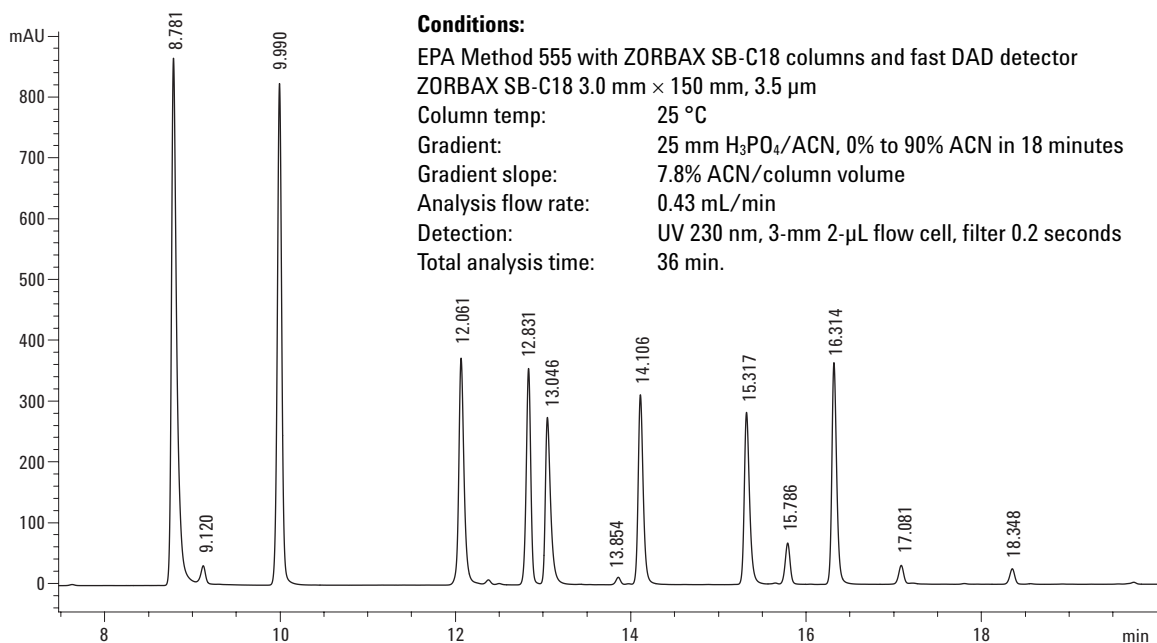
Analysis flow rate: 1 mL/min

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 H₃PO₄/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.

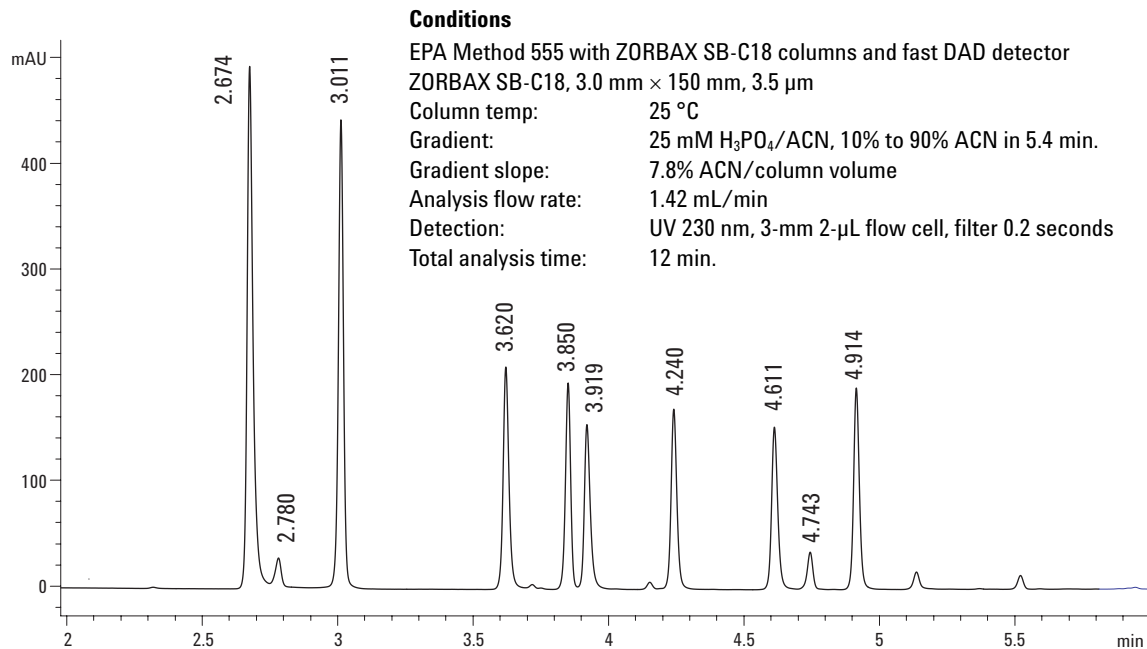


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-μm ZORBAX SB-C18.

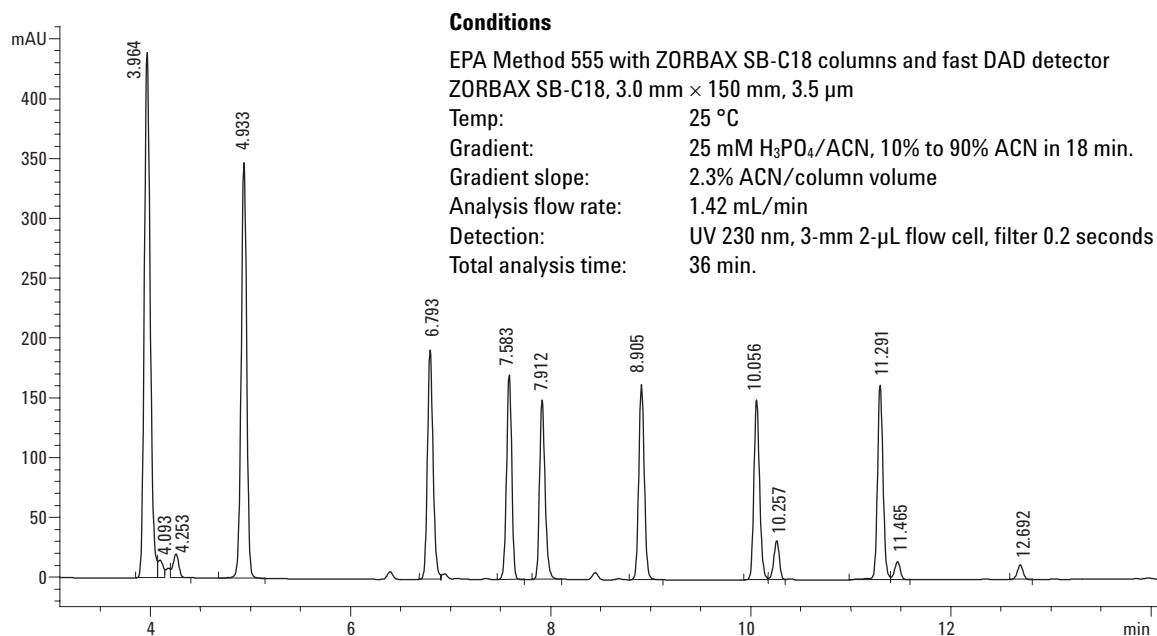


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-μm ZORBAX SB-C18.

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- μ L injection of dilute actone

Table 1. Volumetric Measurements of Various Flow Cells

Flow cell	Elution volume (μ L)	Half height width (μ L)	5 Sigma width (μ L)
New SL 2 μ L 3 mm	11	5	12
Micro 6 mm 1.7 μ L (n = 2)	14	6	18
Semi-micro 6 mm 5 μ L (n = 2)	13	6.5	18.5
Standard 10 mm 13 μ L	26	11	26
New SL 10 mm 13 μ L	27	11	25

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- μ L to the 13- μ L flow cells is 11 μ L, we observe an increase of 15 to 16 μ L because of the larger diameter inlet tubing integral to the larger volume flow cells.

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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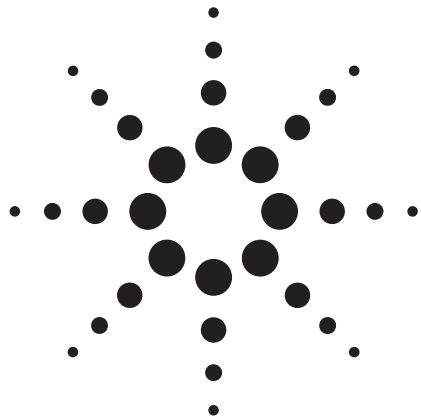
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Improving Throughput for Oils Analysis by ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Ingrid Szikla

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 spray-chamber 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	
Auxiliary 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
Zn 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 compromise 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 peristaltic 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	Actual 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

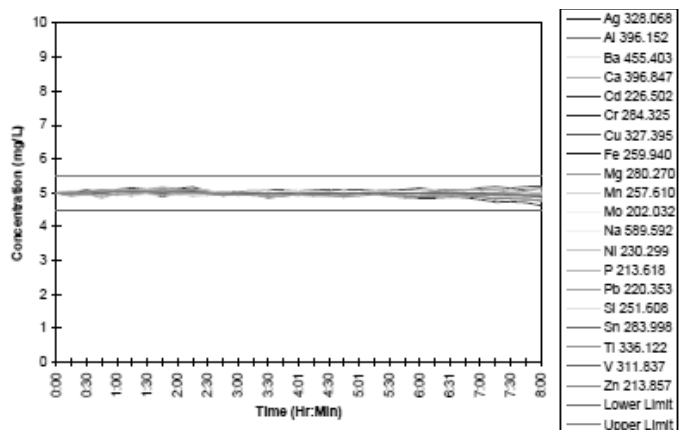


Figure 1. Stability of the Vista-PRO radial instrument over 8 hours. Results remained within $\pm 10\%$ for all elements in the 5 mg/L S21 kerosene solution without internal standardization or recalibration.

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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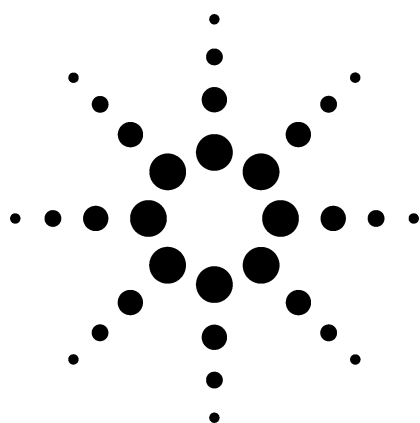
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Increasing Sample Throughput with High-Speed Megabore Columns

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 (B) 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 μ m	1997 (28)
	75 meters	0.540 mm	3.00 μ m	1447
DB-624	75 meters	0.446 mm	2.55 μ m	1402 (22)
	75 meters	0.546 mm	3.00 μ m	1090
DB-502.2	75 meters	0.453 mm	2.55 μ m	1526 (20)
	105 meters	0.544 mm	3.00 μ m	873
DB-WAX	30 meters	0.447 mm	0.85 μ m	1656 (25)
	30 meters	0.544 mm	1.00 μ m	1357
DB-1	30 meters	0.455 mm	1.30 μ m	1477 (27)
	30 meters	0.551 mm	1.50 μ m	1357
DB-5	30 meters	0.446 mm	1.30 μ m	1895 (23)
	30 meters	0.540 mm	1.50 μ m	1454
DB-608	30 meters	0.450 mm	0.71 μ m	1477 (23)
	30 meters	0.535 mm	0.83 μ m	1134

[1] Phase ratio (B) held constant for all columns

[2] Average 24%



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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-Dichloroethene
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

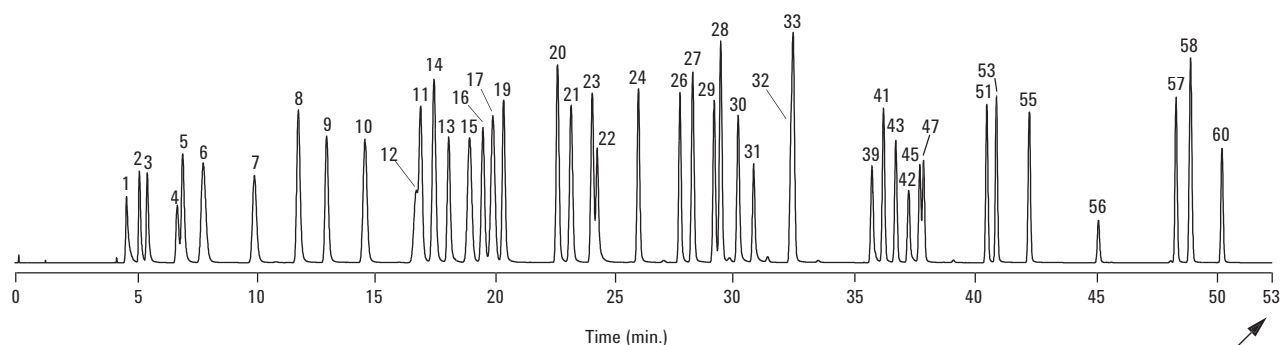
41. 1,1,2,2-Tetrachloroethane
42. Bromobenzene
43. 1,2,3-Trichloropropane
44. n-Propylbenzene
45. 2-Chlorotoluene
46. 1,2,3-Trimethylbenzene
47. 4-Chlorotoluene
48. tert-Butylbenzene
49. 1,2,4-Trimethylbenzene
50. sec-Butylbenzene
51. 1,3-Dichlorobenzene
52. para-Isopropyltoluene
53. 1,4-Dichlorobenzene
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 μ m
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 μ m
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

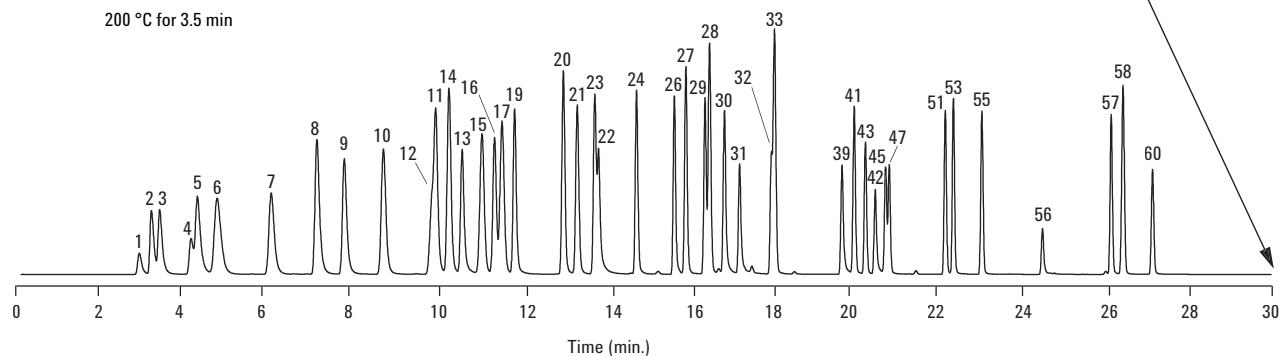


Figure 1. Analysis time comparison

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

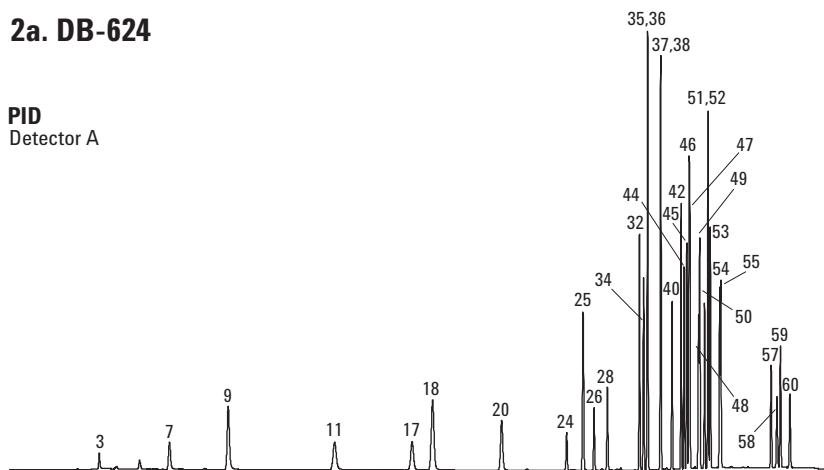
200 °C for 4 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water

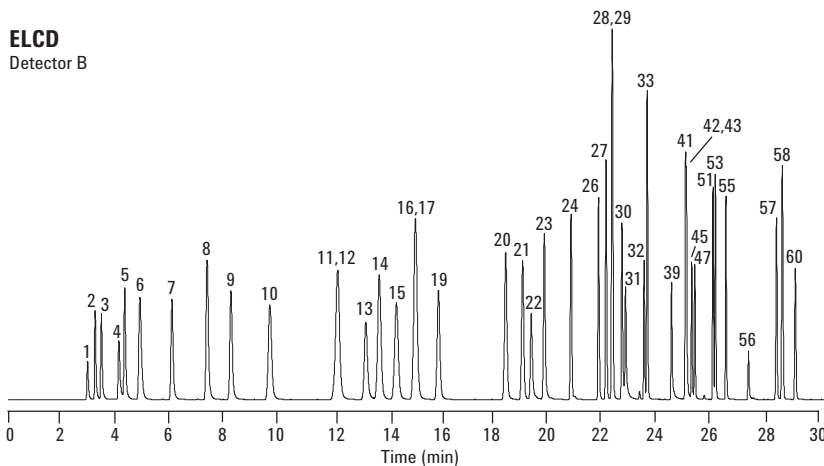
Trap: Tenax/Silica gel/Charcoal

Detector A: Photoionization detector (PID)
(OIA 4430) at 220 °CDetector B: Electrolytic conductivity detector
(ELCD) (OIA 4420) with NiCat
reaction tube in the halogen
mode**2a. DB-624****PID**

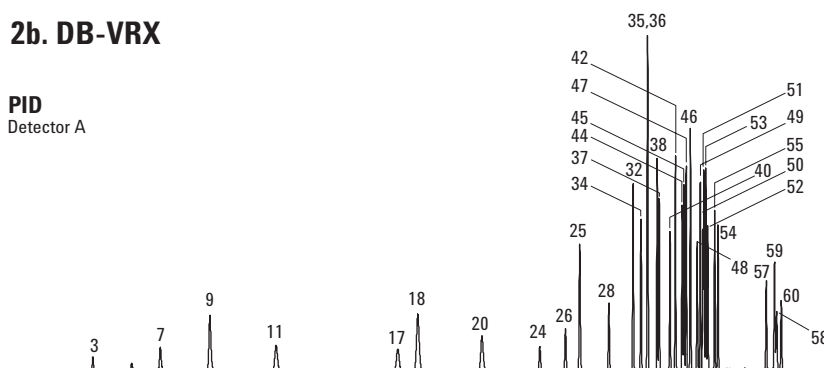
Detector A

**ELCD**

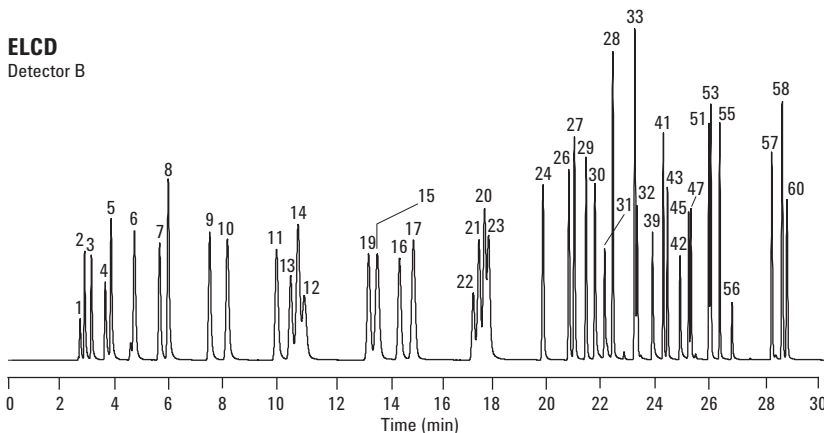
Detector B

**2b. DB-VRX****PID**

Detector A

**ELCD**

Detector B

**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

¹Additional 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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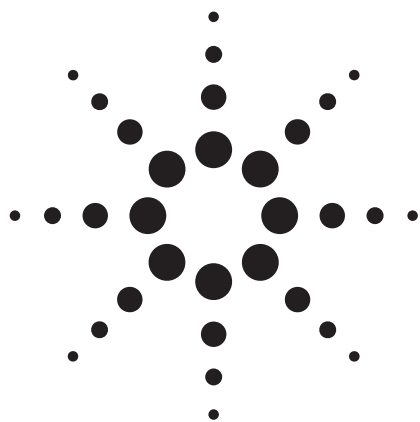
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Obtaining Optimum Performance When Using the SIPS Accessory

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 re-conditioned. 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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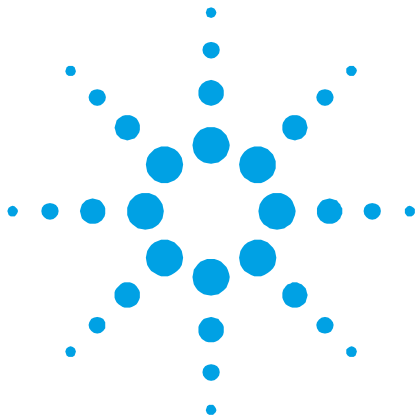
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Returning to Fixed Pathlength Infrared Spectroscopy: Gaining Detail and Removing the Obstacles

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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 an 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} / $2.0\text{ }\mu\text{m}$ to $25\text{ }\mu\text{m}$)
- 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 ($<200\text{ }\mu\text{m}$, $<0.2\text{ mm}$)
- 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 $10\text{ }\mu\text{m}$ to $25\text{ }\mu\text{m}$, 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, fulfilling 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.

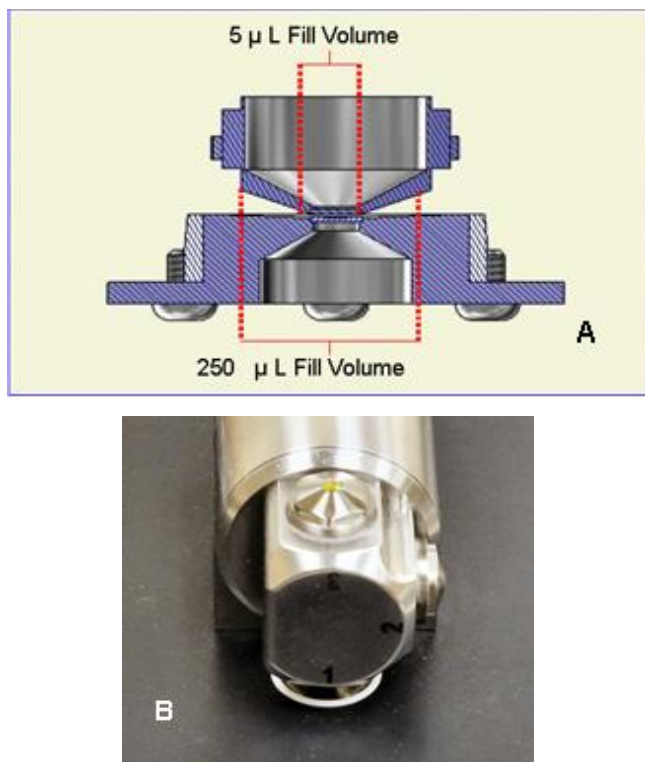


Figure 1. The 5500 DialPath FTIR sampling point concept (A); provides a user selectable pathlength, with one of three fixed/calibrated optical paths, designated 1, 2 and 3 (B)

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} .

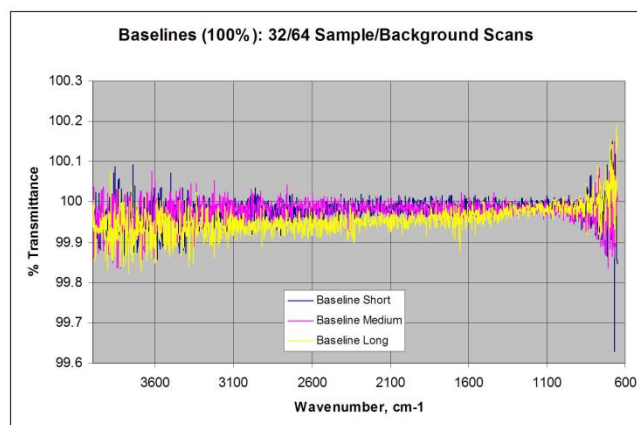


Figure 2. 100% Baseline performance; spectra from long, medium and short pathlengths presented with an average SNR of 8000:1 (2100 cm^{-1} to 1100 cm^{-1})

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.

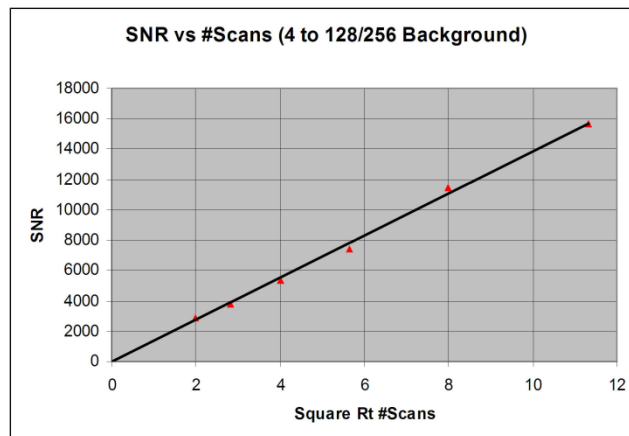


Figure 3. The adherence of the measurement system to the square root law of measured SNR

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

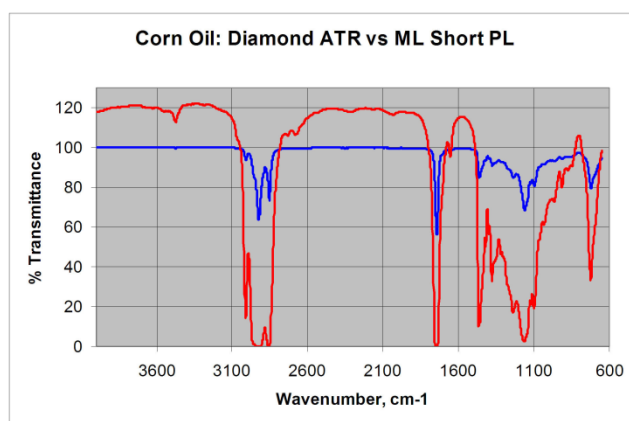


Figure 4. Comparison of the effective pathlength of a diamond ATR integrated system with the short fixed pathlength ($\sim 30 \mu\text{m}$) transmission spectrum for corn oil

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 (30 μ m, 50 μ m and 100 μ m). 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 μ m, medium = 52.6 μ m, and long = 114.7 μ m.

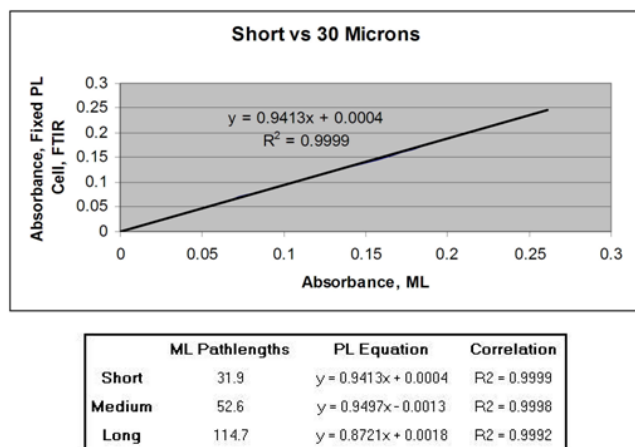


Figure 5. Example calibration for the short pathlength (No 1) of the Agilent 5500 DialPath FTIR system based on comparisons with a calibrated fixed pathlength cell for a series of xylene solutions

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 μ m 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).

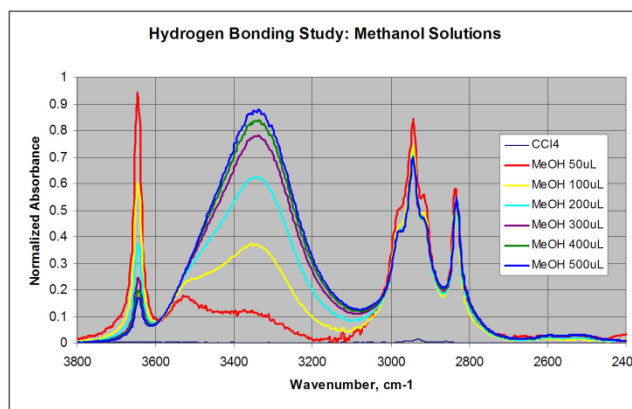


Figure 6: Dilute solutions of methanol in carbon tetrachloride; a study of the effects of hydrogen bonding in non-polar solvents

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+ μ m 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 ($100+\text{ }\mu\text{m}$ pathlength).

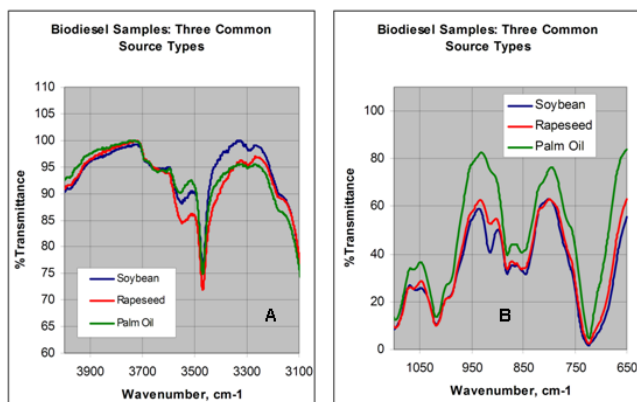


Figure 7. Detailed information from the base ester components used in the production of biodiesel methyl esters; hydroxyl (A) and unsaturation (B) functionalities

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.

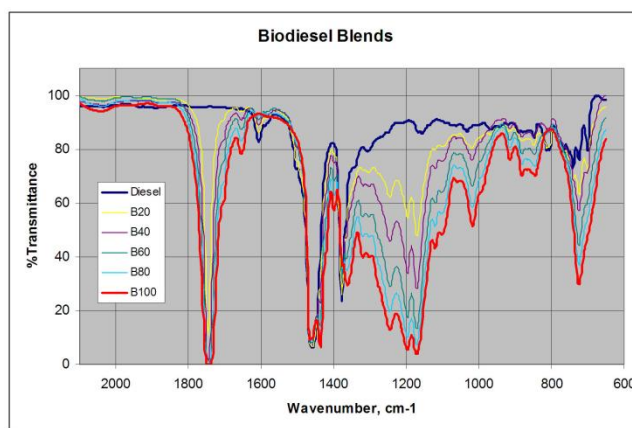


Figure 8. Measurement of biodiesel blends, experimental data from B0 (diesel fuel) to B100 (biodiesel) and intermediate biodiesel/diesel blends

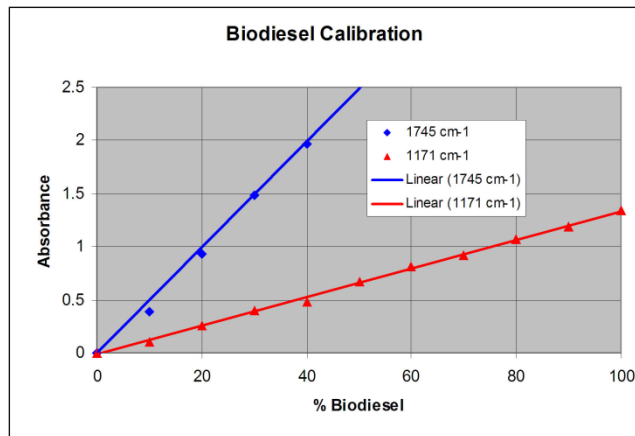


Figure 9. Quantitative measurement of biodiesel blends, B0, B10 to B90 and B100

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.

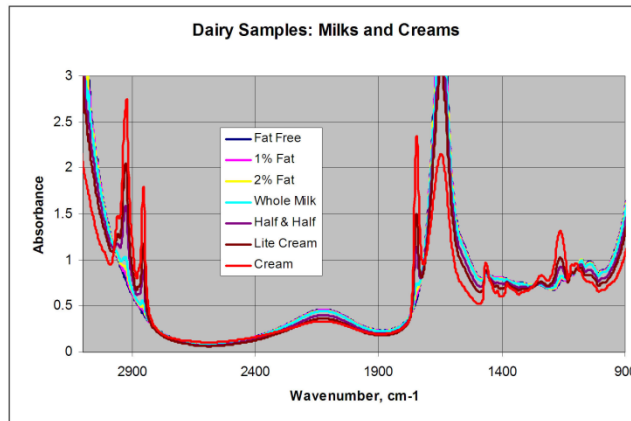


Figure 10. Dairy product spectra; short fixed pathlength (~30 mm), from fat free skim milk to standard heavy cream

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

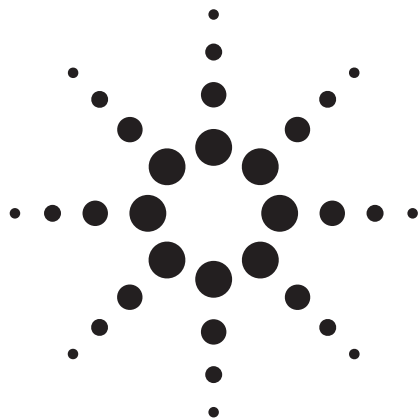
This article has reintroduced the concept of making fixed pathlength mid-infrared transmission measurements without the complexity or the difficulties of the traditional sample handling method. A two-step approach summarized as “drop it on” and “wipe it off” is proposed, where the sample is put in place 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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Routine Maintenance for Atomic Absorption Spectrophotometers

Application Note

Atomic Absorption

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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 m³/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 in the 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 block-

age, 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% HNO₃, 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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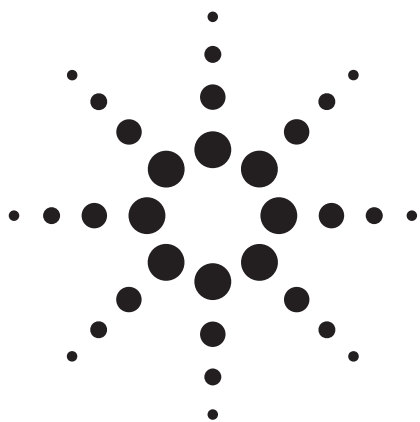
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Sensitivity Enhancement for Flame AAS Using an Atom Concentrator Tube for Elements Dissolved in Organic Solvents

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 $20 \times (3-5) = 60 - 100$ 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 $(60 \text{ to } 100) \times (2 \text{ to } 3) = 120 \text{ to } 300$ 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 length-wise 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
<u>MIBK/ACT</u> MIBK	2.4	1.6	2.8	0.6	1.1
<u>MIBK/ACT</u> AQ/ACT	3.3	4.0	3.8	2.1	n.d.
<u>MIBK/ACT</u> aq	8.6	6.0	10.9	2.2	n.d.
<u>AQ/ACT</u> aq	2.7	1.5	2.8	1.0	n.d.
<u>MIBK</u> aq/ACT	1.3	2.5	1.3	3.5	n.d.
<u>MIBK</u> 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, Fresenius' 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 Fresenius' J Anal Chem, **1993**, 345, 111.

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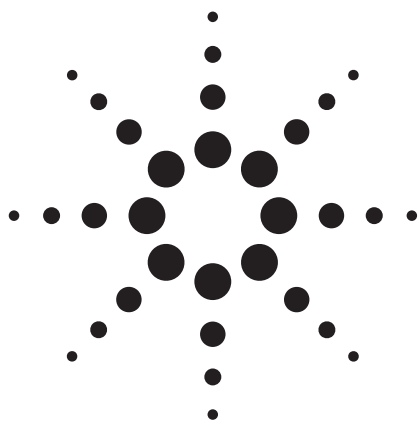
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Sensitivity Enhancement for Flame Atomic Absorption Spectrometry Using an Atom Concentrator Tube, the ACT 80

Application Note

Atomic Absorption

Author

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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.

PEAK ABSORBANCE PEAK HEIGHT	NEBULIZER CONCENTRATION (%)	SAMPLER AUTOMIX BC ON
INTING		
QC PROTOCOL		
QC STD RATE 10		
SAMPLER POSITION 45	VOLUME (uL) 5	
LIMITS (%) 90 TO 110	CONCENTRATION 10.00 ppb	
ON ERROR RECALIBRATE AND REPEAT		
QC SPIKE RATE 1		
SAMPLER POSITION 44	VOLUME (uL) 2	
LIMITS (%) 85 TO 115	MINIMUM LIMIT (%) 40	
CONCENTRATION 20.00	ON ERROR SWITCH TO STD. ADDN.	
REQUIRED DETECTION LIMIT	1.00 ppb	
MATRIX SPIKE CONCENTRATION	0.00 ppb	
OVERRANGE VOLUME REDUCTION	2	
REPLICATE RSD LIMIT (%)	5.0	
CORRELATION COEFFICIENT (r)	0.999	

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.

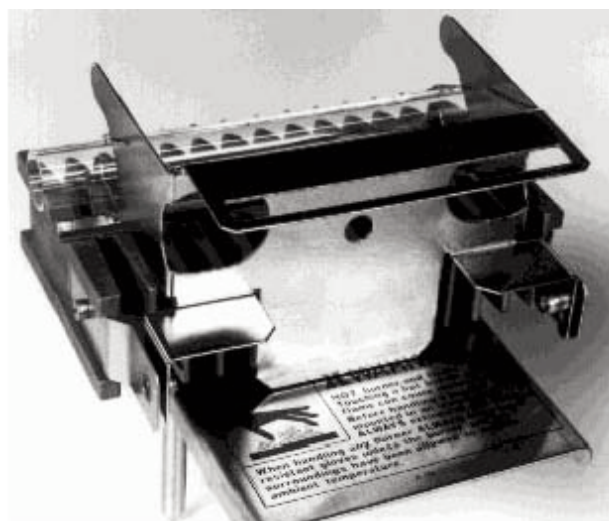


Figure 2. The ACT 80 Atom Concentrator Tube.

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 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

Element	Literature FAAS	Characteristic concentration			Literature FAAS	Detection limit		
		Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor		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.

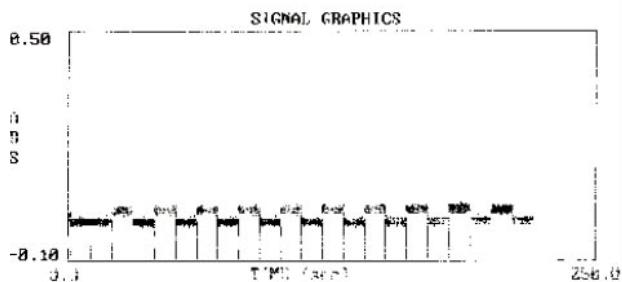


Figure 3(a). Pb signal compared to blank without ACT-80 tube.

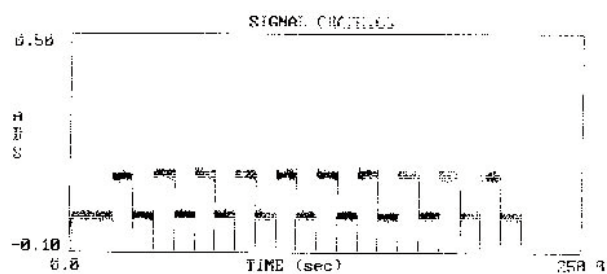


Figure 3(b). Pb signal compared to blank with ACT-80 tube.

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.

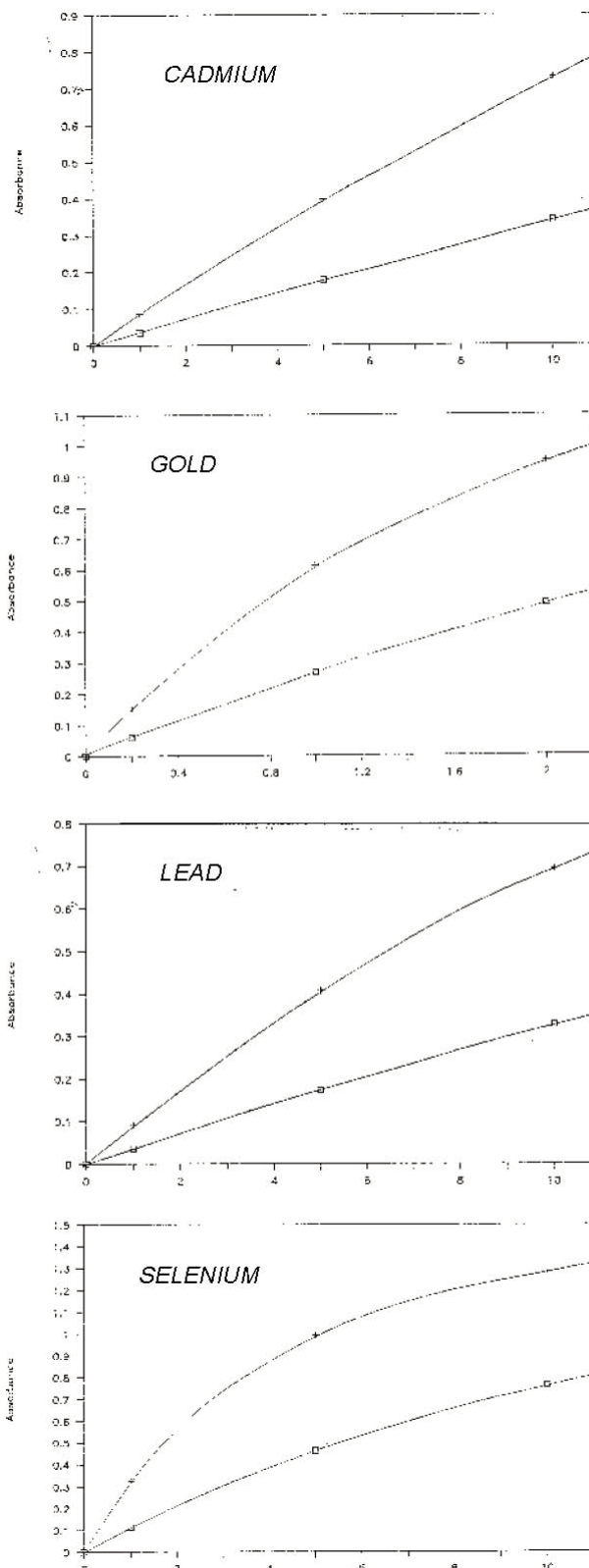


Figure 4. Calibration graphs of selected elements showing improvement in sensitivity. (+ = ACT-80, □ = normal FAAS)

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.

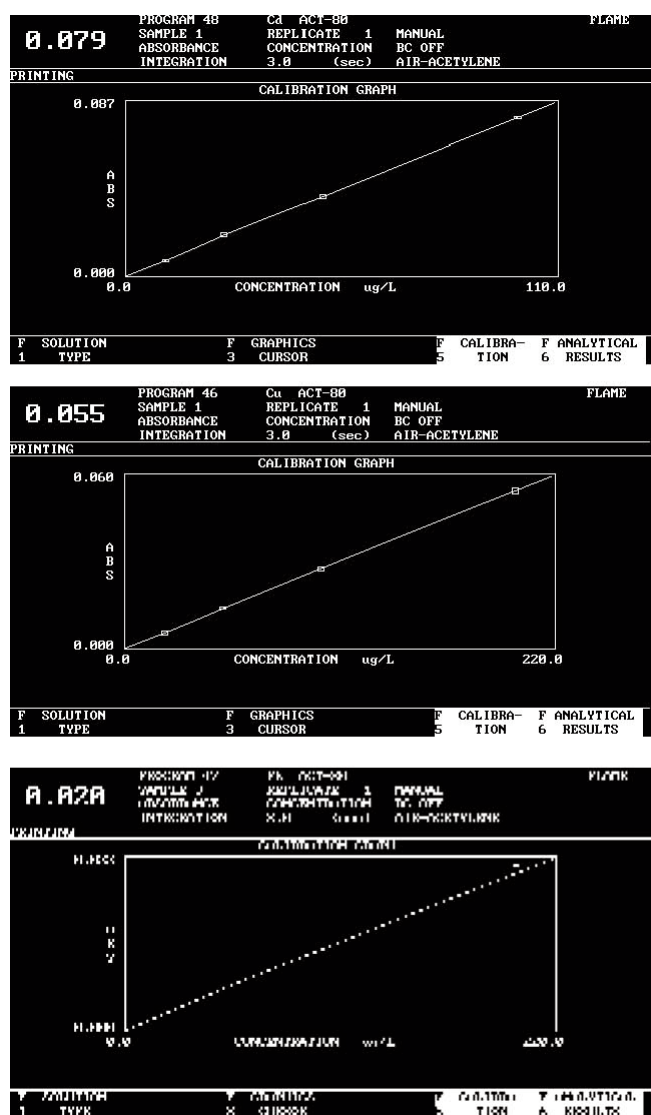


Figure 5. Calibration graphs used to measure quality control samples.

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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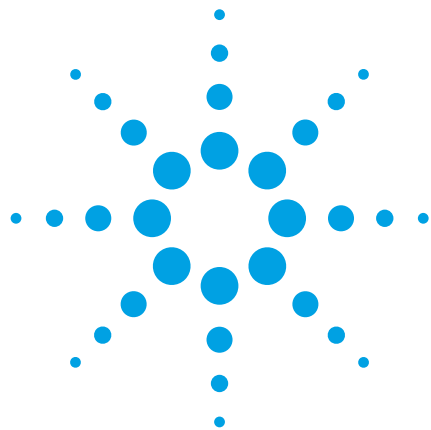
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Separation of Permanent Gases on a Liquid Phase

Separation of 5 permanent gases on a WCOT column with a liquid phase with high retention

Application Note

Authors

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Introduction

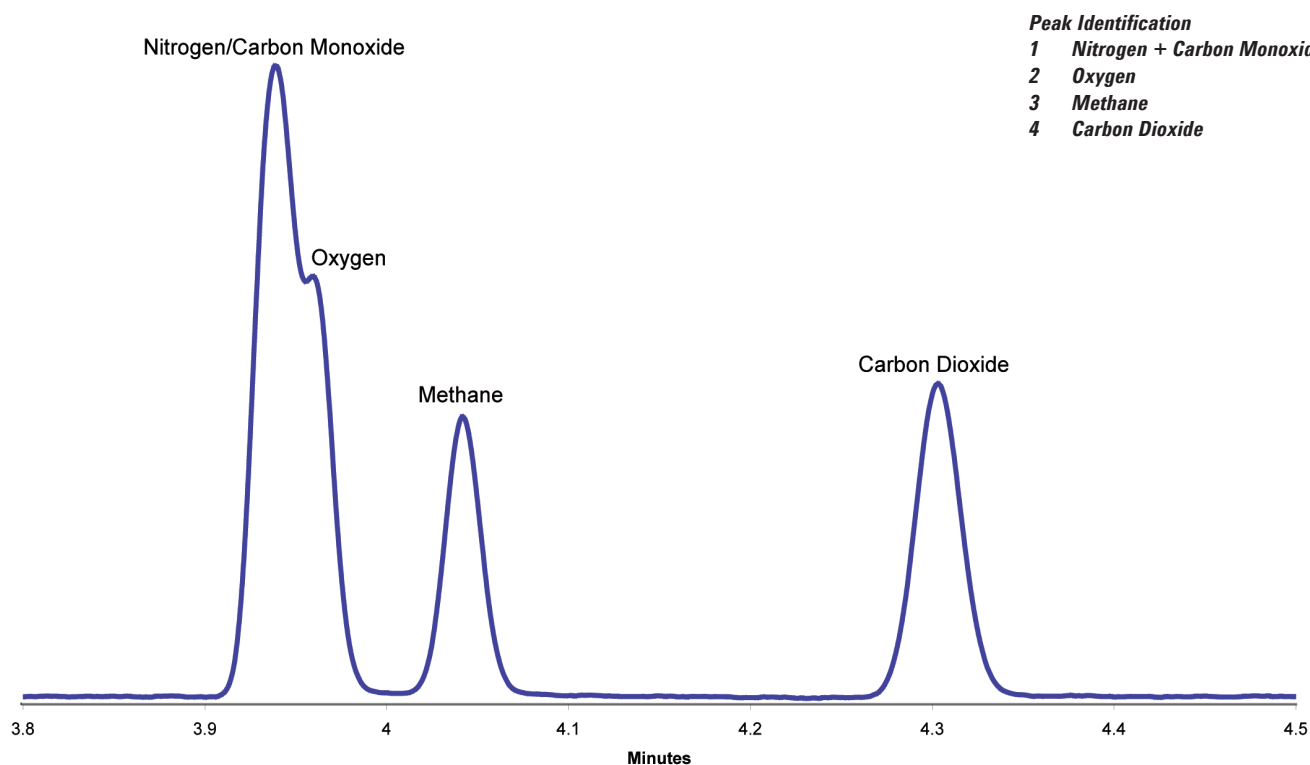
Normally permanent gases are separated by PLOT (porous layer open tubular) columns with their high retentive phases. With WCOT (wall coated open tubular) columns sub-ambient temperatures are normally necessary. Thick films, like the 8 μm film thickness of the Agilent J&W Select CP-Sil 5CB for Formaldehyde, 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.



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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)		



Analysis of permanent gases at 35°C

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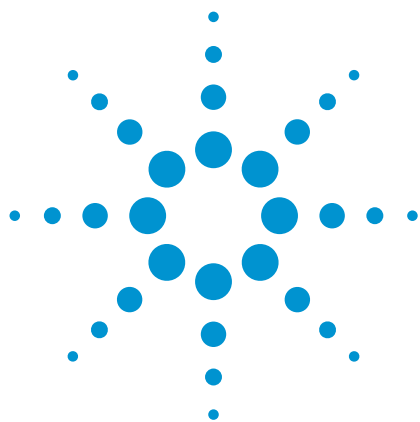
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Using a Dual LTM Series II System with Flow Modulated Comprehensive GCxGC

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 $\times$ 0.18 mm $\times$ 0.25 μ m DB5ms for the first dimension, a 7 m $\times$ 0.25 mm $\times$ 0.2 μ m HP-INNOWax for LTM module 1 and a 5 m $\times$ 0.25 mm $\times$ 0.15 μ m 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.

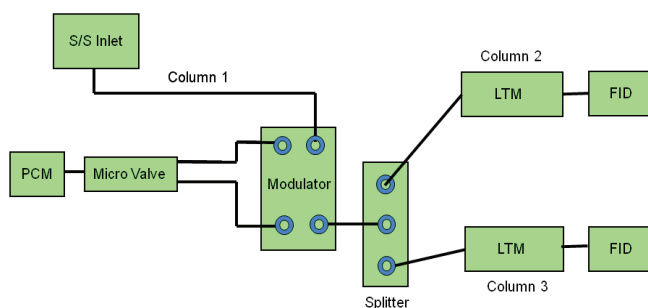


Figure 1. Diagram of the dual LTM GCxGC system.

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 overflow 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.

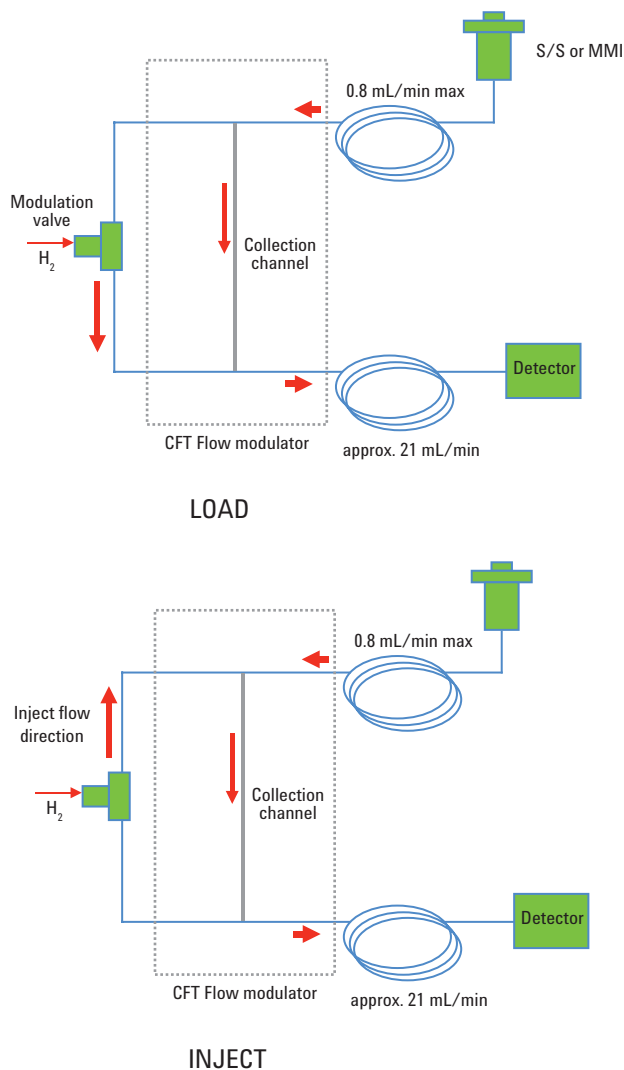


Figure 2. Operational detail of the flow modulator showing load and inject states.

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.

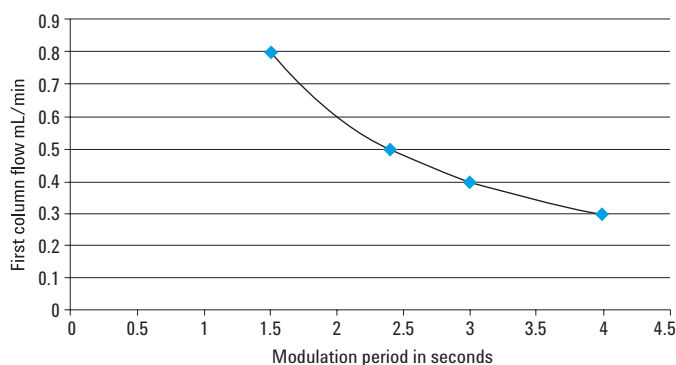


Figure 3. Relationship between modulation period and first dimension column flow rate.

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	7 m × 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 OutSeq 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.

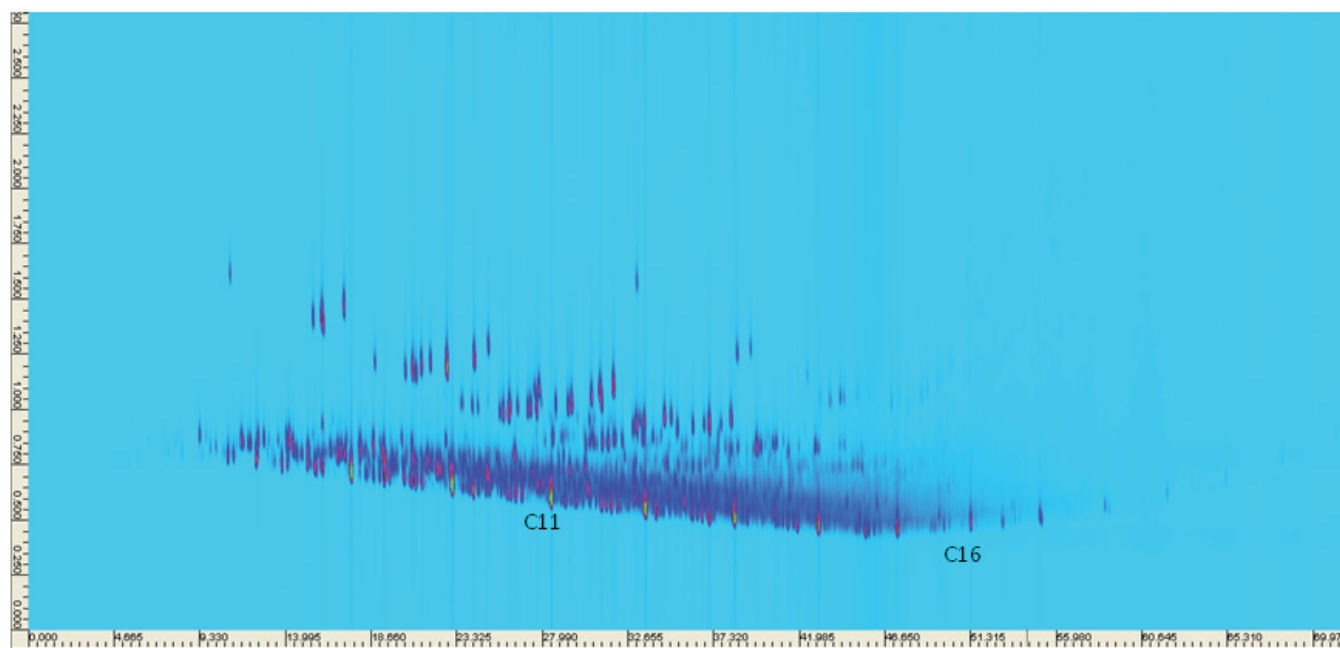


Figure 5a. Jet fuel 2D image. 7 m $\times$ 0.25 mm $\times$ 0.24 μ m HP-INNOWax, LTM program: 55 $^{\circ}$ C (3 min) to 270 $^{\circ}$ C @ 5 $^{\circ}$ C/min. 7890A program: 35 $^{\circ}$ C (2 min) to 280 $^{\circ}$ C @ 3 $^{\circ}$ C/min.

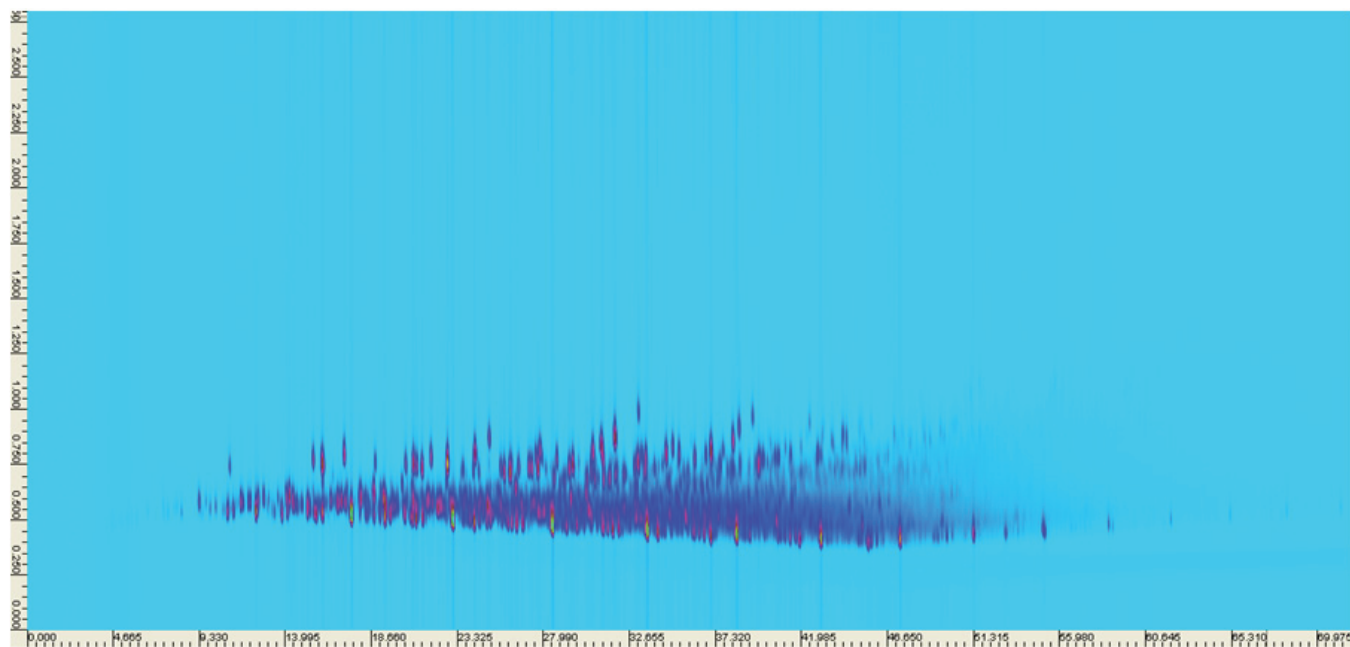


Figure 5b. Jet fuel on 5 m $\times$ 0.25 mm $\times$ 0.15 μ m DB17HT, LTM program: 60 $^{\circ}$ C (5 min) to 300 $^{\circ}$ C @ 3 $^{\circ}$ C/min. 7890A program: 35 $^{\circ}$ C (2 min) to 280 $^{\circ}$ C @ 3 $^{\circ}$ C/min.

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-cyclohexyl 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 $\times$ GC - 5975C MSD system.

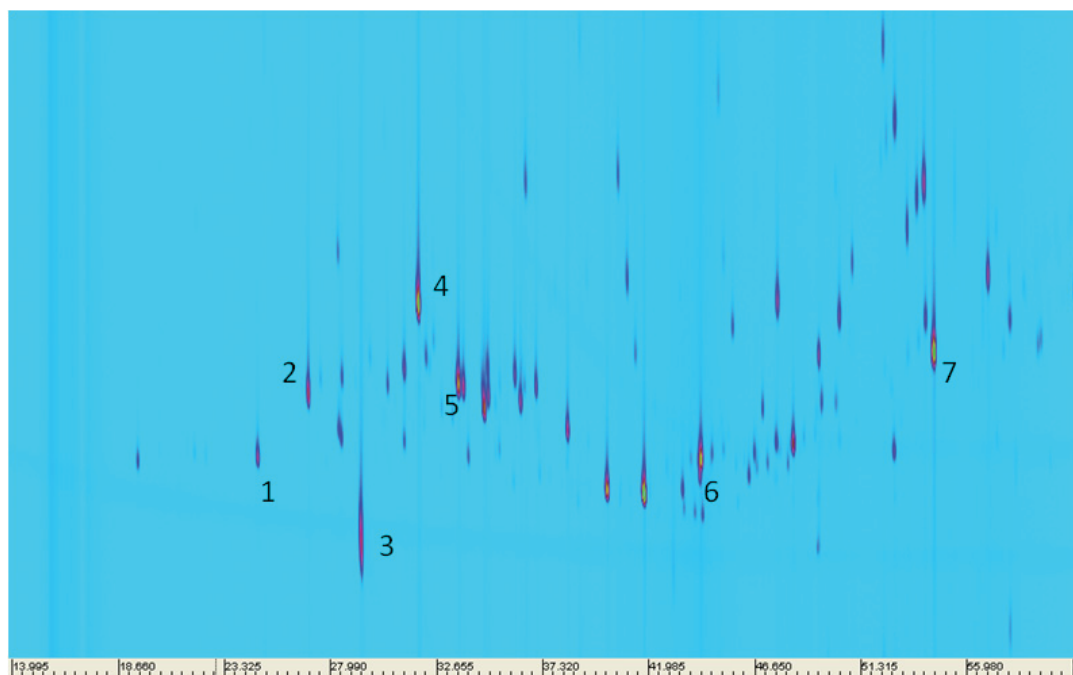


Figure 6a. Fragrance additive using 7 m INNOWax for 2nd dimension, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl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

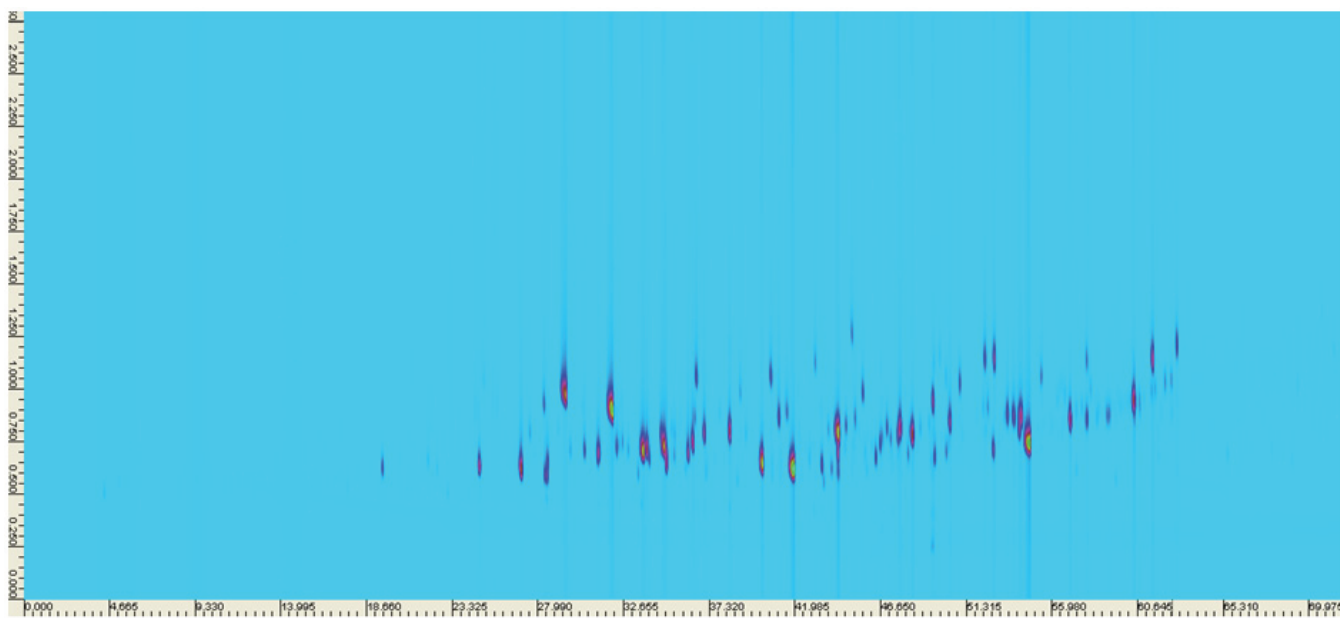


Figure 6b. Fragrance additive using 5m DB17HT for 2nd dimension separation. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

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.

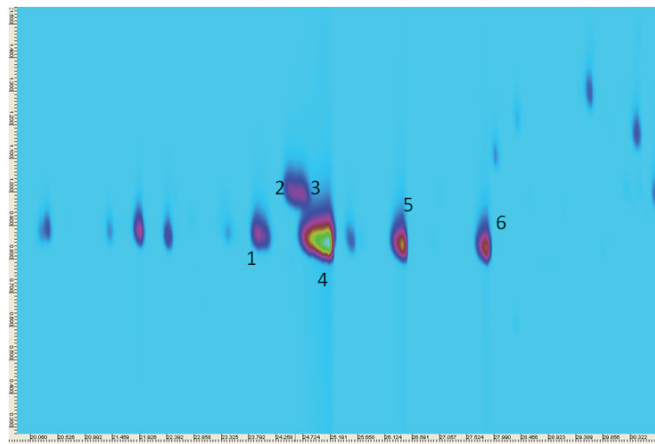


Figure 7a. Lime oil on the 7 m INNOWax. LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate 1.beta pinene,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

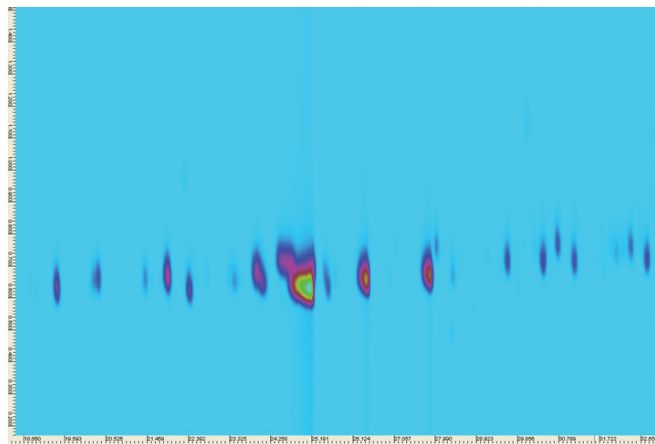


Figure 7b. Lime oil on the 5 m DB17HT. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

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.

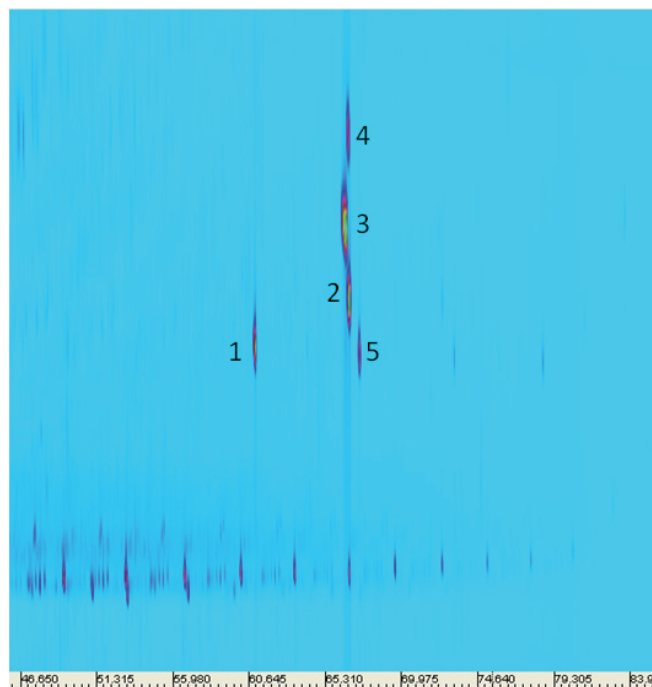


Figure 8. Separation of C16 and C18 fatty acid methyl esters in B20 biodiesel on a 5 m × 0.25 mm × 0.15 μm LTM INNOWax column in the 2nd dimension. LTM program: 30 °C (0 min) to 270 °C (5 min) @ 3 °C/min. 1. C16:0, 2. C18:1, 3. C18:3, 4. C18:3, 5. C18:0.

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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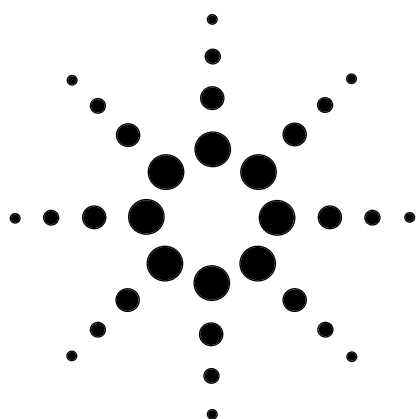
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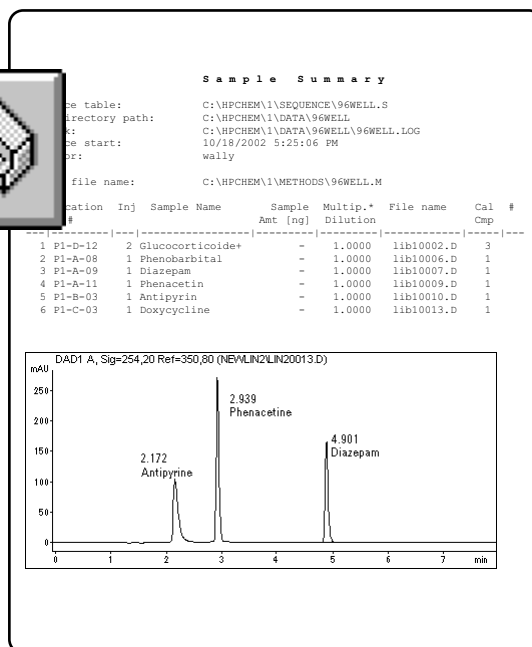
Agilent Technologies



Using Agilent ChemStation to generate summary reports for a single analysis or a sequence of analyses

Application

Angelika Gratzfeld-Huesgen



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 information 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.

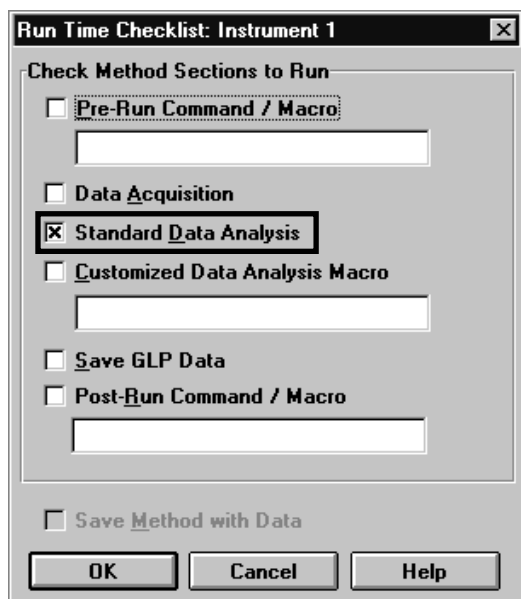


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

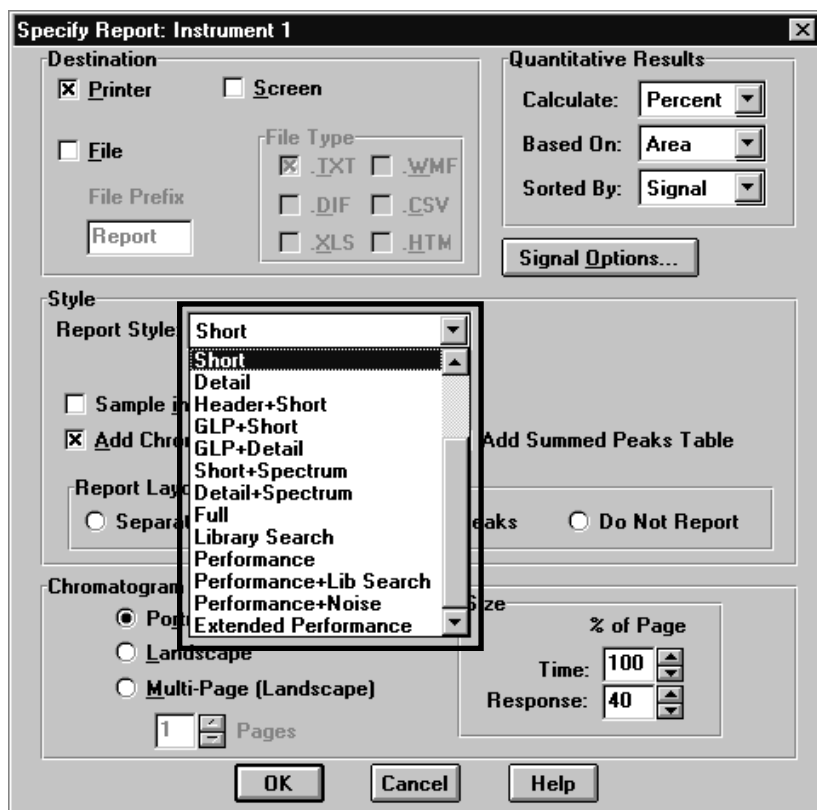


Figure 2
Specifying individual run reports

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 Search 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.

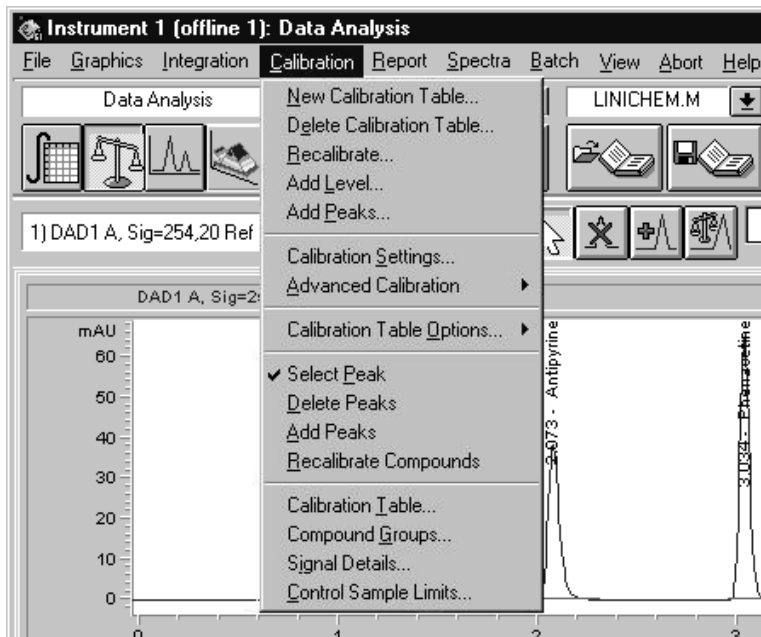


Figure 3
Calibration setup menu

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.

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)

Figure 4
Selection of external standard report and short report style

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

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.



Figure 5
Run method for automated method execution and result output

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 continuous 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.

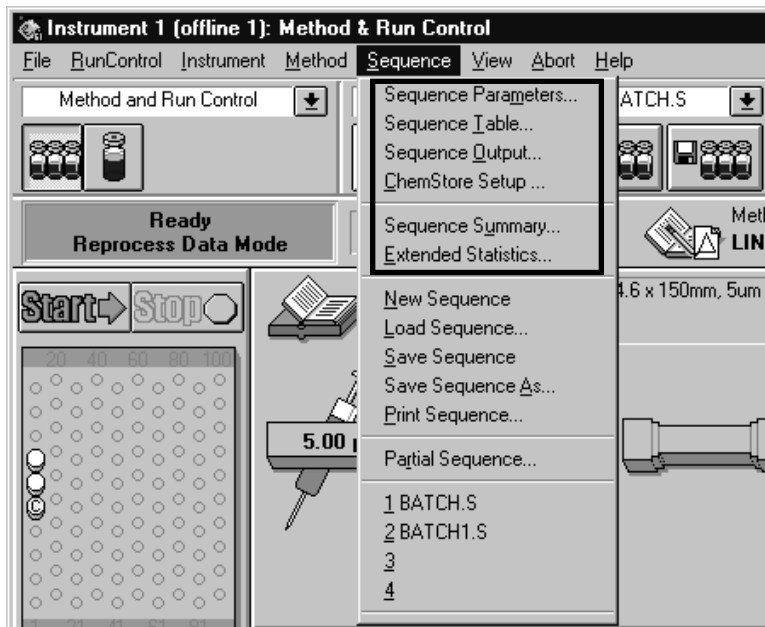


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

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 screen shown in figure 9. Selecting *Setup* in the *Sequence Output* dialog box accesses this configuration screen. The sequence summary report allows a variety of informations to be printed in one continuously enumerated report.

In addition to a wide selection of statistical results from sample and/or calibration runs, other items can be selected such as sample summary reports that list all acquired samples, com-

Figure 8
Sequence parameters screen

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

plete printouts of all parameters in the methods that were used, printouts of sequence logbooks and so on.

It is also possible to include the individual result reports for each run as part of the summary report instead of individual printouts after the end of each run.

The statistical evaluation of sequence runs is defined in the *Extended Statistic Parameter* screen, see figure 10. Statistical results can be obtained for all parameter shown in this dialog box. Either standard deviation or relative standard deviation or 95% confidence interval can be applied and upper/lower limits for each parameter can be specified.

A calibrated method is necessary to be able obtain statistical results.

Figure 11 shows the *Sequence Table* screen, in which it is important to ensure that the sample type is correctly set to *Sample*, *Calibration* or *Control Sample*, because statistical calculations can be selected based on sample type.

Figure 12 shows an example of a sequence summary report. It contains information about the analyzed samples such as location, sample name, filename, and so on. The header includes information such as operator name, the used chromatographic method, and date of acquisition.

Further report examples can be found on pages 11 through 35.

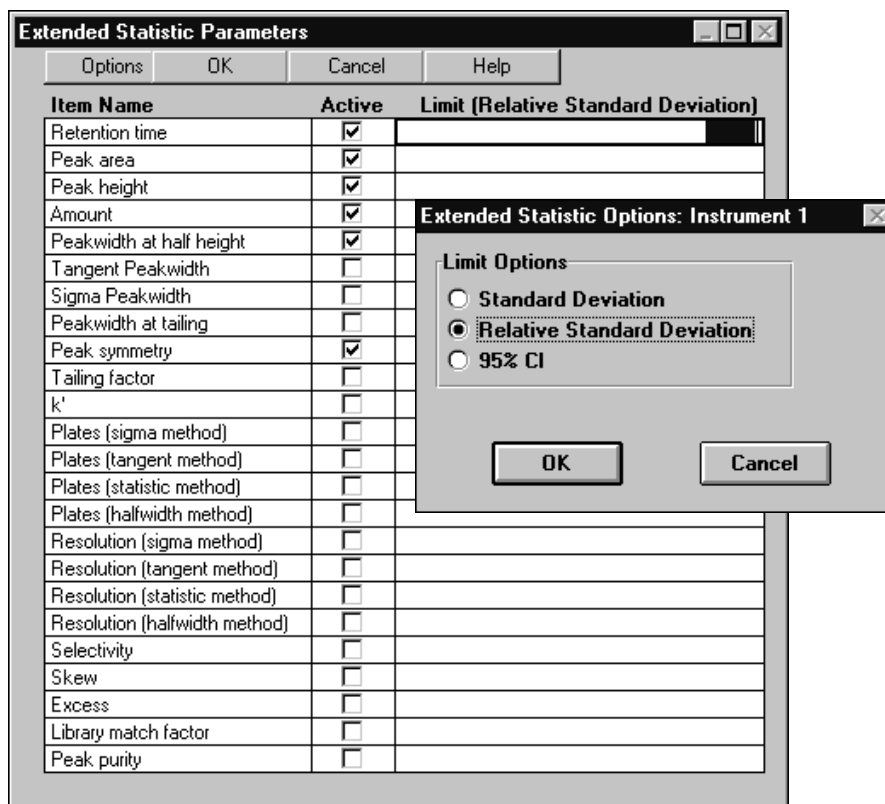


Figure 10
Setup of statistical calculations for sequence runs

Sequence Table: Instrument 1

Currently Running

Line: Method: Location: Inj:

Sample Info for P1-D-12:

Lib105

Plate 1 ID:

Plate 2 ID:

Line	Location	Sample Name	Method Name	Inj/Location	Sample Type	Cal Level	Update RF	Update RT	Interval	San
1	P1-D-12	Glucocorticoide+	96WELL	2	Sample					
2	P1-A-01	Theophyllin	96WELL	1	Sample					
3	P1-A-02	Theobromine	96WELL	1	Sample					
4	P1-A-03	Caffeine	96WELL	1	Sample					
5	P1-A-08	Phenobarbital	96WELL	1	Sample					
6	P1-A-09	Diazepam	96WELL	1	Sample					
7	P1-A-10	Paracetamol	96WELL	1	Sample					
8	P1-A-11	Phenacetin	96WELL	1	Sample					
9	P1-B-03	Antipyrin	96WELL	1	Sample					
10	P1-C-01	Minocycline	96WELL	1	Sample					
11	P1-C-02	Tetracycline	96WELL	1	Sample					
12	P1-C-03	Doxycycline	96WELL	1	Sample					
13	P1-D-01	Amoxicillin	96WELL	1	Sample					
14	P1-D-02	Ampicilline	96WELL	1	Sample					
15	P1-D-03	PenicillineG	96WELL	1	Sample					
16	P1-D-04	PenicillineV	96WELL	1	Sample					
17	P1-E-01	Triphenelamine	96WELL	1	Sample					
18	P1-E-02	Chlorpheniramine	96WELL	1	Sample					
19	P1-E-03	Promethazine	96WELL	1	Sample					
20	P1-F-01	Dextromethorphan	96WELL	1	Sample					
21	P1-F-02	Verapamil	96WELL	1	Sample					

Sample Type must be filled in appropriately as Sample, Calibration or Control

Insert Cut Copy Paste Append Line

Insert Location Range.. Run Sequence Read Bar Code

OK Cancel Help

Figure 11
The Sequence Table screen

Sample Summary

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	Multip.* [ng]	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

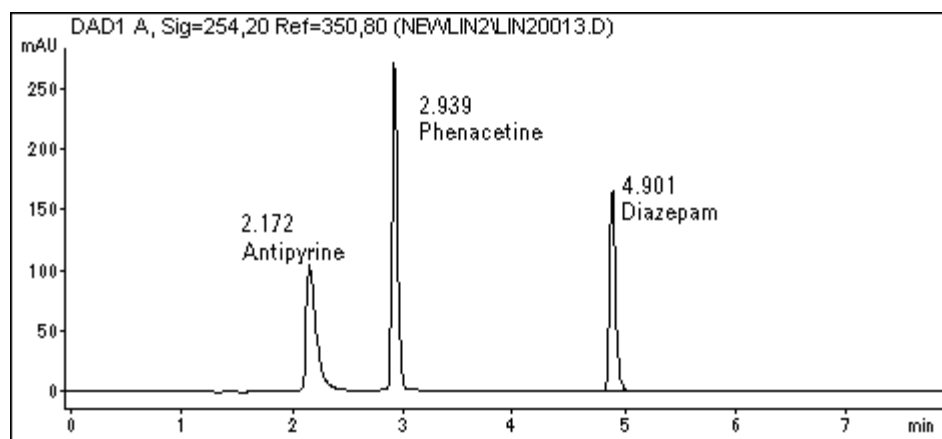
The following pages show examples of summary reports that can be generated with the ChemStation base software. The examples were generated using the print-to-file function and may have different pagination than a report printed directly from the ChemStation. Reports shown include:

- 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 [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
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		

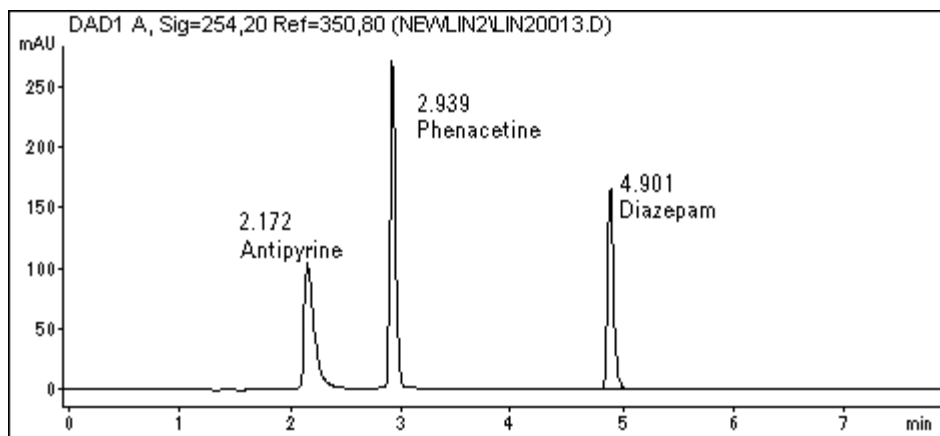
*** End of Report ***

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 [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng]	Grp	Name
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		

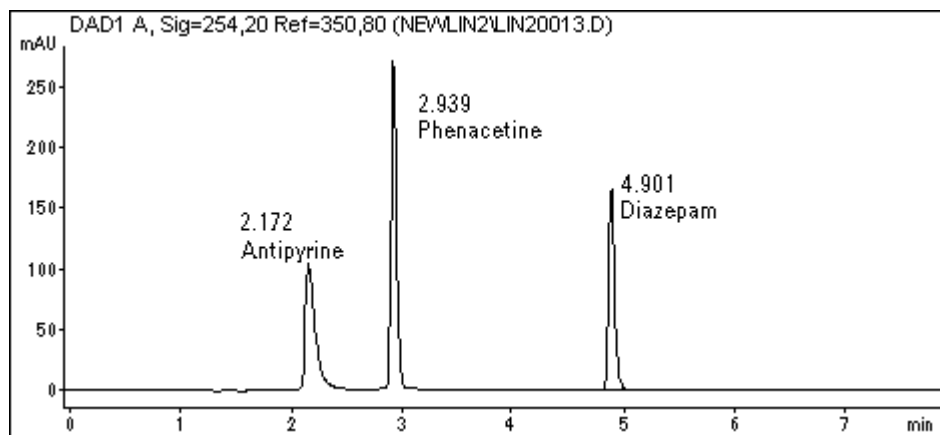
*** End of Report ***

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.	Library	CalTbl						
RetTime	RetTime	RetTime	Sig	Amount	Purity	Library	Name	
[min]	[min]	[min]		[ng]	Factor	# Match		
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      XXXXXX  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      XXXXXX  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
Column Temp. (right)  :      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   :
PMPL, Solvent A       : Water
PMPL, Solvent B       : acn
=====
```

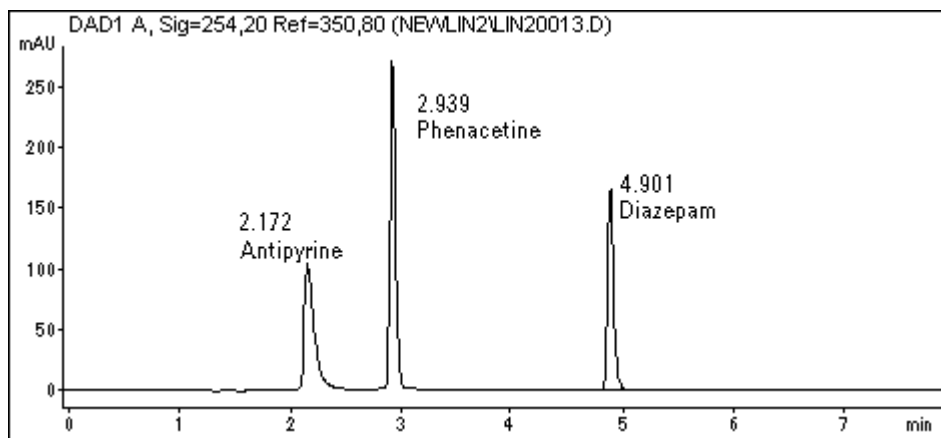
Short GLP Report (continued)

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 [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng]	Grp	Name
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 ***

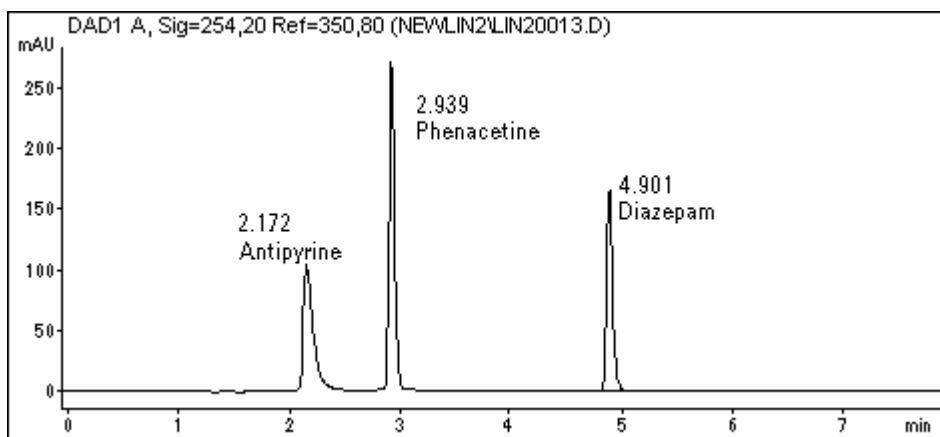
Performance report

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!

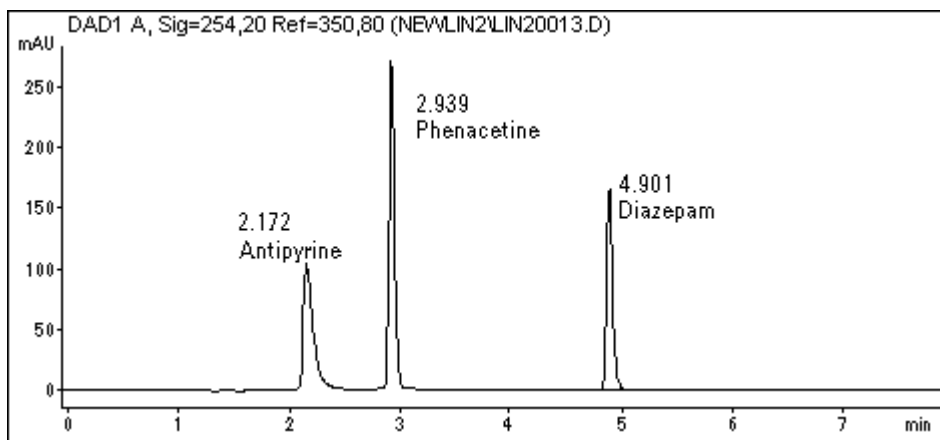
RetTime [min]	k'	Sig	Amount [ng]	Symm.	Width [min]	Plates	Resol	Name
2.172	0.81	1	438.70069	0.44	0.0883	3351	4.47	Antipyrine
2.939	1.45	1	937.28787	0.83	0.0524	17435	6.40	Phenacetine
4.901	3.08	1	599.59734	0.80	0.0550	43990	21.47	Diazepam

*** 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

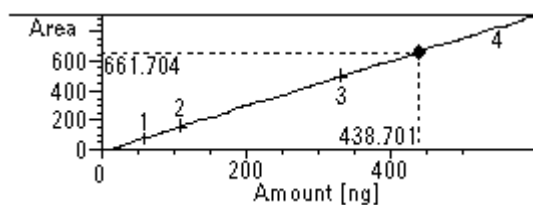
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng]	Grp	Name
2.172	BB	661.70422	6.62986e-1	438.70069		Antipyrine
2.939	BB	934.32690	1.00317	937.28787		Phenacetine
4.901	BB	610.64050	9.81915e-1	599.59734		Diazepam
Totals :				1975.58590		

=====

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 = ax3 + bx2 + 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\CON00005.D

Extended Performance Report

Instrument: Instrument 1

Module	Firmware revision	Serial number
1100 Quaternary Pump	A.04.11	DE1 1116042
1100 Wellplate Autosampler	A.04.13	DE02700294
1100 Column Thermostat	A.04.11	DE53400174
1100 Diode Array Detector	A.04.11	DE00900051
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\1\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:

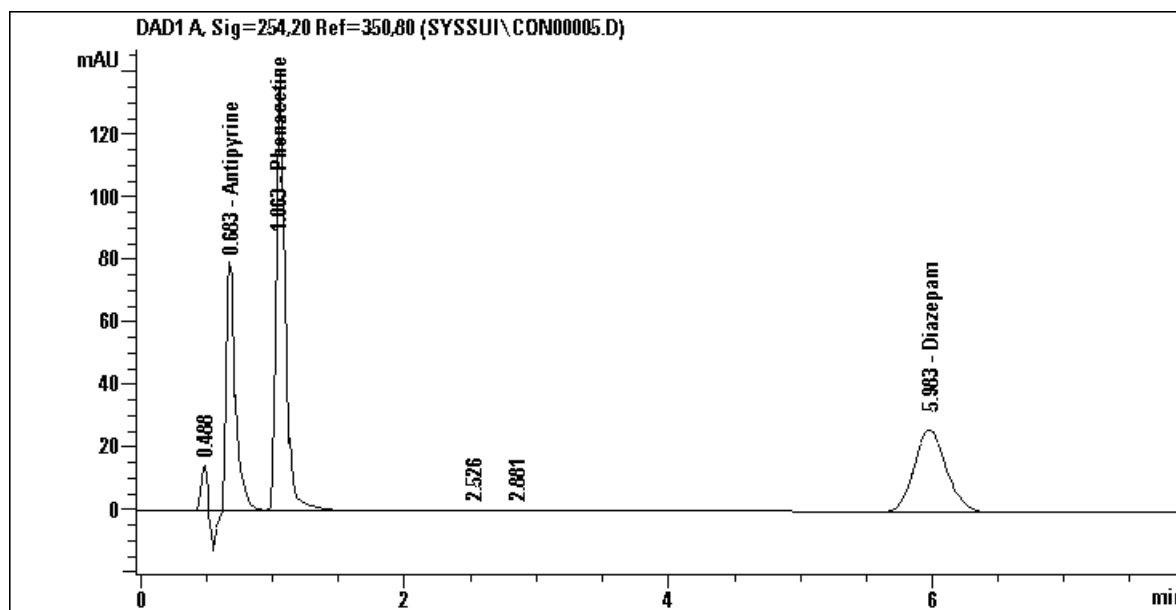
Operator:	agratz
Date/Time:	2/11/029:06:34 AM
Data file name:	D:\HPCHEM\1\DATA\SYSSUI\CON00005.D
Method file name:	D:\HPCHEM\1\METHODS\SYSSUIP.M

Flow:	0.200 ml/min		
Pressure at start:	85 bar	Pressure at end:	88 bar
Temperature at start:	25.1°C	Temperature at end:	25.0°C

Extended Performance Report (continued)

Solvents: PMP1, Solvent A water
PMP1, Solvent B ACN
PMP1, Solvent C
PMP1, Solvent D

Signal description: DAD1 A, Sig=254,20 Ref=350,80



Compound# 2 : Antipyrine
Amount [ng]: 51.1385

Peak description [min]:

Signal: DAD1 A, Sig=254,20 Ref=350,80

RetTime: 0.583 K': 0.706

Height: 79.78 Area: 371.2

Start: 0.546 End: 0.956

Skew: 0.898 Excess: 1.643

Width at half height: 0.067

5 sigma: 0.196

tangent: 0.117

tailing: 0.190

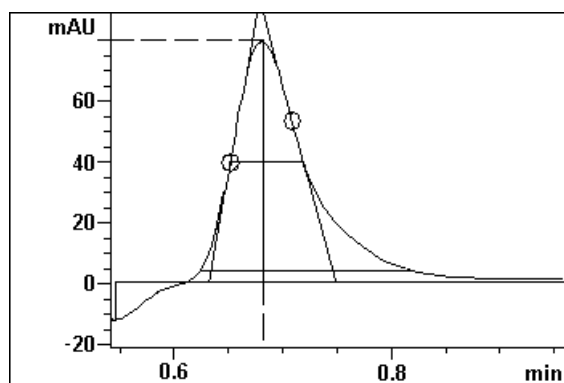
Symmetry: 0.483

USP Tailing: 1.657

Integration type: HV

Time increment [macc]: 400.0

Data points: 66



Extended Performance Report (continued)

Statistical moments (BB peak detection):		Efficiency:	Plates per ...
M0:	514.1		column meter
M1:	0.699	Tangent method	541 18020
M2:	0.00341	Halfwidth method	581 19360
M3:	0.000179	5 sigma method	385 10153
M4:	0.000054	Statistical	143 4782

Relationship to preceeding peak:	Selectivity: 3.217
Resolution Tangent method: 2.015	5 sigma method 1.700
Halfwidth method 2.034	Statistical method 1.067

:
:
:

The peak description
and statistical moments
are repeated for each
compound

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

Sequence Summary Report – Compound Summary

```
XXXXXX  XX  XXXXXX
XX  XX  XX  XX  XX
XX  XX  XX  XX  XX
XX  XX  XX  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 Parameters: Instrument 1

Activate report:

Style:

- ☒ 1. One page header
- ☒ 2. Configuration
- ☒ 3. Sequence
- ☒ 4. Logbook
- ☐ 5. Methods
- ☐ 6. Analysis reports
- ☐ 7. Statistics calib. runs
- ☐ 8. Statistics sample runs
- ☒ 9. Summary

Standard Statistic

Standard Statistic

Compound Summary

Sample Summary

Compound Summary

OK

Cancel

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)

Compound Summary

Sequence table: D:\HPCHEM\CORE\LIN2.S
 Data directory path: D:\HPCHEM\1\DATA\NEWLIN2
 Logbook: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG
 Sequence start: 10/25/00 6:58:26 AM
 Operator: agratz

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

Sample Name	Sample Amt [ng]	Multip.* Dilution	FileName .D	RetTime [min]	Amount [ng]	Compound
sample1	0.00000	1.0000	Lin20001	2.071	-	-
				3.005	41.80740	Phenacetine
				5.061	27.57288	Diazepam
sample2	0.00000	1.0000	Lin20002	2.071	-	-
				2.927	37.71584	Phenacetine
				4.931	24.68503	Diazepam
sample3	0.00000	1.0000	Lin20003	2.159	113.94044	Antipyrine
				2.921	249.65462	Phenacetine
				4.927	162.09926	Diazepam
sample4	0.00000	1.0000	Lin20004	2.138	115.89423	Antipyrine
				2.888	254.19389	Phenacetine
				4.893	167.32050	Diazepam
sample5	0.00000	1.0000	Lin20005	2.071	-	-
				2.967	533.16102	Phenacetine
				4.977	350.64724	Diazepam
sample6	0.00000	1.0000	Lin20006	2.071	-	-
				2.935	555.34634	Phenacetine
				4.885	359.02135	Diazepam
sample7	0.00000	1.0000	Lin20007	2.120	770.88338	Antipyrine
				2.932	1659.61614	Phenacetine
				4.939	1090.77773	Diazepam
sample8	0.00000	1.0000	Lin20008	2.156	766.86882	Antipyrine
				2.978	1658.25754	Phenacetine
				4.990	1088.46781	Diazepam
sample9	0.00000	1.0000	Lin20009	2.112	1298.20959	Antipyrine
				2.956	2780.26621	Phenacetine
				4.874	1801.76061	Diazepam
sample10	0.00000	1.0000	Lin20010	2.125	1265.65752	Antipyrine
				2.931	2753.00356	Phenacetine
				4.917	1784.44912	Diazepam
sample11	0.00000	1.0000	Lin20011	2.070	2206.34622	Antipyrine
				2.928	4737.72659	Phenacetine
				4.931	3055.52966	Diazepam
sample12	0.00000	1.0000	Lin20012	2.157	2219.77978	Antipyrine
				2.959	4771.25573	Phenacetine
				4.905	3043.14819	Diazepam
sample13	0.00000	1.0000	Lin20013	2.172	438.70069	Antipyrine
				2.939	937.28787	Phenacetine
				4.901	599.59734	Diazepam

Sequence Summary Report – Compound Summary (continued)

sample14	0.00000	1.0000	Lin20014	2.137	431.19756	Antipyrine
				2.920	922.41613	Phenacetine
				4.914	598.82718	Diazepam
sample15	0.00000	1.0000	Lin20015	2.130	1050.21043	Antipyrine
				2.956	2257.23577	Phenacetine
				4.946	1454.09021	Diazepam
sample16	0.00000	1.0000	Lin20016	2.071	-	-
				3.062	2266.63554	Phenacetine
				4.914	1450.54300	Diazepam
sample17	0.00000	1.0000	Lin20017	2.112	1860.82017	Antipyrine
				2.958	4083.57167	Phenacetine
				4.943	2601.71134	Diazepam
sample18	0.00000	1.0000	Lin20018	2.114	1846.79895	Antipyrine
				2.970	4045.19575	Phenacetine
				4.970	2576.86650	Diazepam
sample19	0.00000	1.0000	Lin20019	2.152	2485.47770	Antipyrine
				3.019	5268.86688	Phenacetine
				4.973	3410.01754	Diazepam
sample20	0.00000	1.0000	Lin20020	2.135	2489.66113	Antipyrine
				2.975	5298.02094	Phenacetine
				4.943	3415.39103	Diazepam
sample21	0.00000	1.0000	Lin20021	2.155	2961.16799	Antipyrine
				3.010	6013.24563	Phenacetine
				5.003	4037.60722	Diazepam
sample22	0.00000	1.0000	Lin20022	2.156	2983.41614	Antipyrine
				3.042	6012.35737	Phenacetine
				4.988	4010.73532	Diazepam

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

Sequence Summary Report – Standard Statistics for Sample Runs

Sequence Summary Parameters: Instrument 1

Activate report: Style:

☐ 1. One page header

☐ 2. Configuration

☐ 3. Sequence

☐ 4. Logbook

☐ 5. Methods

☐ 6. Analysis reports

☐ 7. Statistics calib. runs

☒ 8. Statistics sample runs

☒ 9. Summary

Standard Statistic

Standard Statistic

Sample Summary

Sample Summary

Compound Summary

OK Cancel

Statistic Report

Sequence table: D:\HPCHEM\1\SEQUENCE\NEWLIN.S
 Data directory path: D:\HPCHEM\1\DATA\NEWLIN
 Operator: agratz

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

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	Vial 2	1	8/24/00 12:42:04 AM	new00061.D	sample1
2	Vial 2	2	8/24/00 12:51:09 AM	new00062.D	sample2
3	Vial 2	3	8/24/00 1:00:14 AM	new00063.D	sample3
4	Vial 2	4	8/24/00 1:09:18 AM	new00064.D	sample4
5	Vial 2	5	8/24/00 1:18:21 AM	new00065.D	sample5
6	Vial 2	6	8/24/00 1:27:25 AM	new00066.D	sample6
7	Vial 2	7	8/24/00 1:36:30 AM	new00067.D	sample7
8	Vial 2	8	8/24/00 1:45:34 AM	new00068.D	sample8
9	Vial 2	9	8/24/00 1:54:38 AM	new00069.D	sample9
10	Vial 2	10	8/24/00 2:03:42 AM	new00070.D	sample10

Compound: Antipyrine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BV	2.071	26.23064	834.52417	215.75279	0.0594	0.74
2	BV	2.071	26.28149	836.14185	216.26503	0.0594	0.74
3	BV	2.070	26.22879	834.46539	215.85945	0.0594	0.74
4	BV	2.070	26.27553	835.95233	216.52124	0.0594	0.74
5	BV	2.070	26.21720	834.09644	215.51944	0.0594	0.74
6	BV	2.070	26.19317	833.33203	216.02470	0.0593	0.74
7	BV	2.070	26.27779	836.02423	216.93185	0.0592	0.74
8	BV	2.072	26.29524	836.57941	216.89178	0.0593	0.74
9	BV	2.072	26.22549	834.36017	216.09763	0.0593	0.74
10	BV	2.071	26.21184	833.92590	216.06882	0.0593	0.74

Mean:		2.071	26.24372	834.94019	216.19327	0.0594	0.74
S.D.:		6.81e-4	3.53636e-2	1.12509	4.66512e-1	6.63e-5	1e-3
RSD :		0.033	1.34751e-1	1.34751e-1	2.15784e-1	0.1117	0.20
95% CI:		4.87e-4	2.52976e-2	8.04838e-1	3.33722e-1	4.74e-5	1e-3

Sequence Summary Report – Standard Statistics for Sample Runs

Compound: Phenacetine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	3.035	12.05932	1203.01074	357.49438	0.0528	0.88
2	BB	3.035	12.07862	1204.93591	357.76285	0.0527	0.87
3	BB	3.035	12.05487	1202.56653	357.16501	0.0527	0.88
4	BB	3.035	12.07567	1204.64221	357.80615	0.0527	0.88
5	BB	3.036	12.05951	1203.02979	356.62448	0.0528	0.87
6	BB	3.036	12.02965	1200.05090	356.52957	0.0528	0.88
7	BB	3.037	12.08083	1205.15625	357.92139	0.0527	0.88
8	BB	3.037	12.06433	1203.51099	357.60211	0.0527	0.88
9	BB	3.039	12.05340	1202.42065	356.89868	0.0527	0.87
10	BB	3.038	12.04430	1201.51282	356.41678	0.0528	0.88
----- ----- ----- ----- ----- ----- ----- -----							
Mean:		3.036	12.06005	1203.08368	357.22214	0.0527	0.88
S.D.:		1.35e-3	1.59266e-2	1.58880	5.70986e-1	3.70e-5	6e-3
RSD :		0.045	1.32061e-1	1.32061e-1	1.59840e-1	0.0702	0.68
95% CI:		9.69e-4	1.13932e-2	1.13656	4.08458e-1	2.65e-5	4e-3

Compound: Diazepam (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	5.085	17.51478	820.56067	228.97469	0.0556	0.84
2	BB	5.086	17.54309	821.88702	229.58243	0.0557	0.84
3	BB	5.085	17.51162	820.41229	229.04759	0.0557	0.84
4	BB	5.084	17.54478	821.96600	229.60602	0.0557	0.84
5	BB	5.086	17.51105	820.38562	229.37668	0.0556	0.84
6	BB	5.087	17.47411	818.65503	228.69946	0.0556	0.85
7	BB	5.088	17.54951	822.18774	229.63567	0.0556	0.84
8	BB	5.088	17.51423	820.53491	229.10289	0.0556	0.84
9	BB	5.090	17.51381	820.51508	229.17131	0.0557	0.84
10	BB	5.090	17.50570	820.13525	228.79688	0.0556	0.84
----- ----- ----- ----- ----- ----- ----- -----							
Mean:		5.087	17.51827	820.72396	229.19936	0.0556	0.84
S.D.:		2.12e-3	2.24801e-2	1.05318	3.38200e-1	3.77e-5	2e-3
RSD :		0.042	1.28324e-1	1.28324e-1	1.47557e-1	0.0678	0.29
95% CI:		1.52e-3	1.60813e-2	7.53401e-1	2.41934e-1	2.70e-5	2e-3

Sequence Summary Report – Standard Statistics for Sample Runs

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

Sequence table: D:\HPCHEM\1\SEQUENCE\NEWLIN.S
Data directory path: D:\HPCHEM\1\DATA\NEWLIN
Logbook: D:\HPCHEM\1\DATA\NEWLIN\NEWLIN.LOG
Sequence start: 8/24/00 12:42:04 AM
Statistic report on calibration runs: 1
Operator: agratz

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

Run #	Location	Inj #	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal #	Page	#
1	Vial 2	1	sample1	-	1.0000	new00061.D	*	3	-
2	Vial 2	2	sample2	-	1.0000	new00062.D	*	3	-
3	Vial 2	3	sample3	-	1.0000	new00063.D	*	3	-
4	Vial 2	4	sample4	-	1.0000	new00064.D	*	3	-
5	Vial 2	5	sample5	-	1.0000	new00065.D	*	3	-
6	Vial 2	6	sample6	-	1.0000	new00066.D	*	3	-
7	Vial 2	7	sample7	-	1.0000	new00067.D	*	3	-
8	Vial 2	8	sample8	-	1.0000	new00068.D	*	3	-
9	Vial 2	9	sample9	-	1.0000	new00069.D	*	3	-
10	Vial 2	10	sample10	-	1.0000	new00070.D	*	3	-

*** End of Report ***

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www.agilent.com/chem/nds

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BIOFUELS & ALTERNATIVE ENERGY

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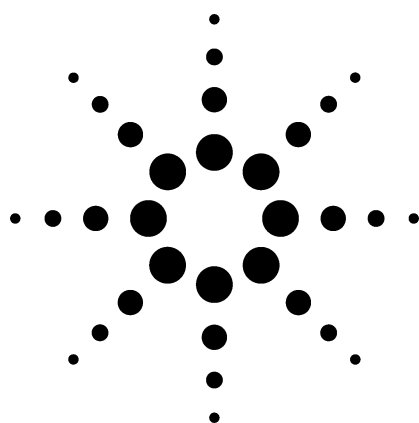
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Determining the Ester and Linoleic Acid Methyl Ester Content to Comply with EN14103

Application



HPI

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Abstract

Gas chromatography with a split/splitless inlet and FID is used to determine the ester and linoleic acid methyl ester content of fatty acid methyl esters (FAME) intended for use as pure biofuel or as a blending component for heating and diesel fuels. The method is suitable for FAME containing methyl esters between C14 and C24. This application used the Agilent 6850 System and HP-INNOWax column; calibration was achieved with internal standards of methyl heptadecanoate. After analyzing several different types of biodiesel, excellent precision was obtained, exceeding the EN14103 method specifications.

Introduction

Biodiesel fuel is produced when a vegetable oil or an animal fat reacts with methanol in the presence of a catalyst to yield fatty acid methyl esters (FAME) and glycerin, which is removed. FAME is a pure biodiesel fuel called B100. A "green" fuel, biodiesel is biodegradable, nontoxic, and is essentially free of sulfur and aromatics. It is rapidly gaining momentum worldwide as an alternative fuel source for diesel engines.

Only biodiesel fuel meeting the specifications of ASTM D6751 or EN14214 is acceptable for use as a motor fuel. Several GC methods have been developed to determine if a biodiesel meets the specification. For example, EN14103 determines the ester and linoleic acid methyl ester content; EN14105 and ASTM D6584 determine free and total glycerin and mono-, di-, and triglyceride content; and EN14110 is for methanol. EN14106, which determines free glycerol, is not commonly used since 14105/ASTM D6584 provides more complete results.

Three major GC biodiesel solutions—EN14103, EN14105/ASTM D6584, and EN14110—were developed for the Agilent GC platform. This application describes the performance of EN14103 on the Agilent 6850 GC.

Experimental

The application was conducted with the Agilent 6850 GC with FID, split/splitless inlet, and



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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: 1 µL
 Column flow (He): 1.5 mL/min, constant flow mode
 FID temperatures: 300°C
 H₂ flow: 40 mL/min
 Air flow: 400 mL/min
 Make up (N₂): 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 heptadecanoate 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.

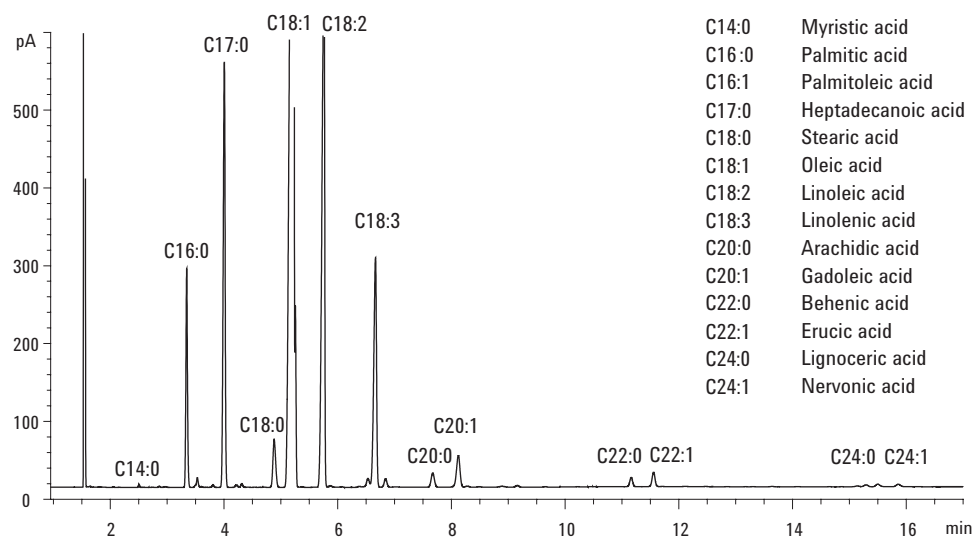


Figure 1. Chromatogram of rapeseed methyl esters.

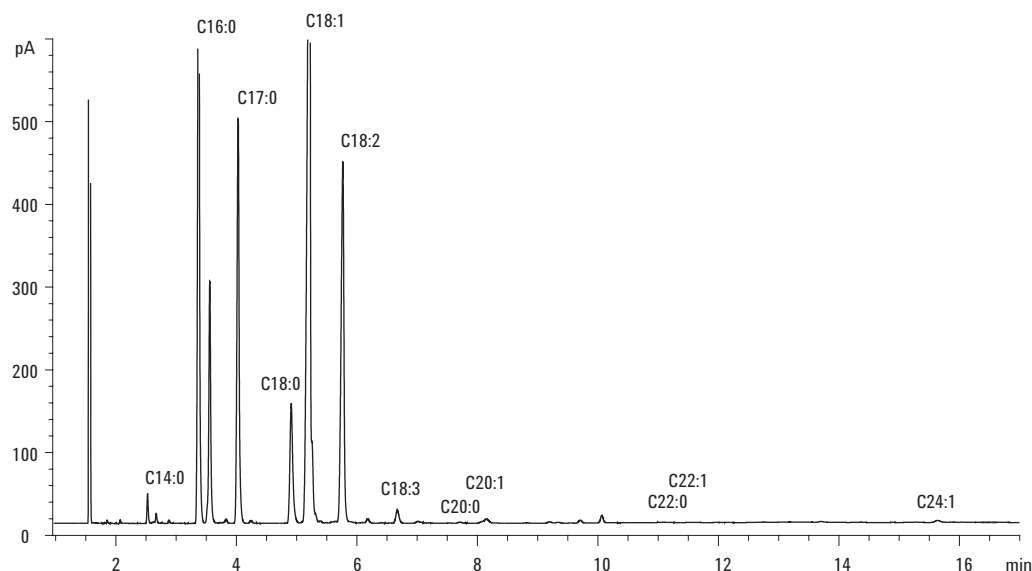


Figure 2. Chromatogram of pork methyl esters.

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

Component FAME		Average, % (m/m)			
		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

Component FAME		RSD%, %(m/m) (Average=5)			
		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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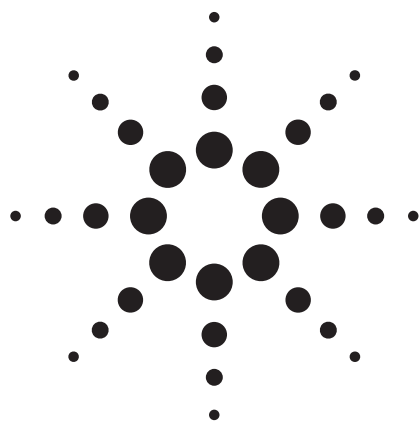
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Analysis of Glycerin and Glycerides in Biodiesel (B100) Using ASTM D6584 and EN14105



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- μ m 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 C₈ to C₂₄. 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.



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Table 1. Distribution and Relative Amounts of FAMES Derived from Vegetable Oils

	Weight Percent FAMES											
										C20:0	C20:1	
Oil type	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	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- μ m 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 tri-caprin (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 μ L 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- μ m 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 Silitite ferrules (part no. 5188-5362) 0.53-mm column Silitite ferrules (part no. 5188-5363)

Data system

Agilent Multitechnique ChemStation

Consumables

5181-1267	10- μ L 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 μ L
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, tricaprins, 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

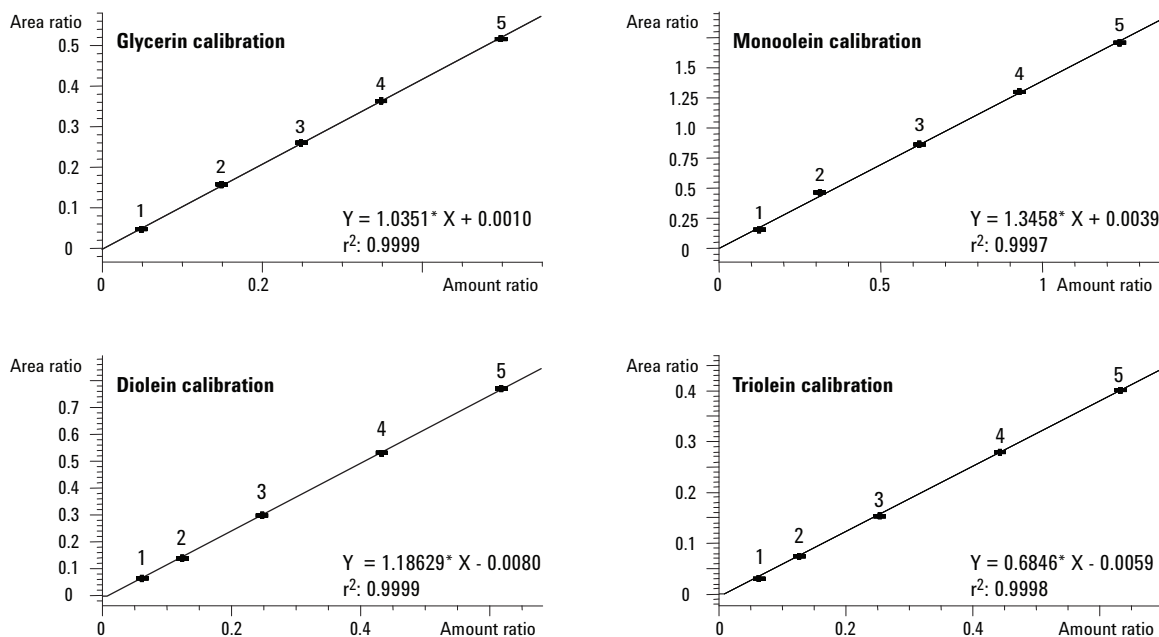


Figure 1. Calibration curves for glycerin, monoolein, diolein, and triolein.

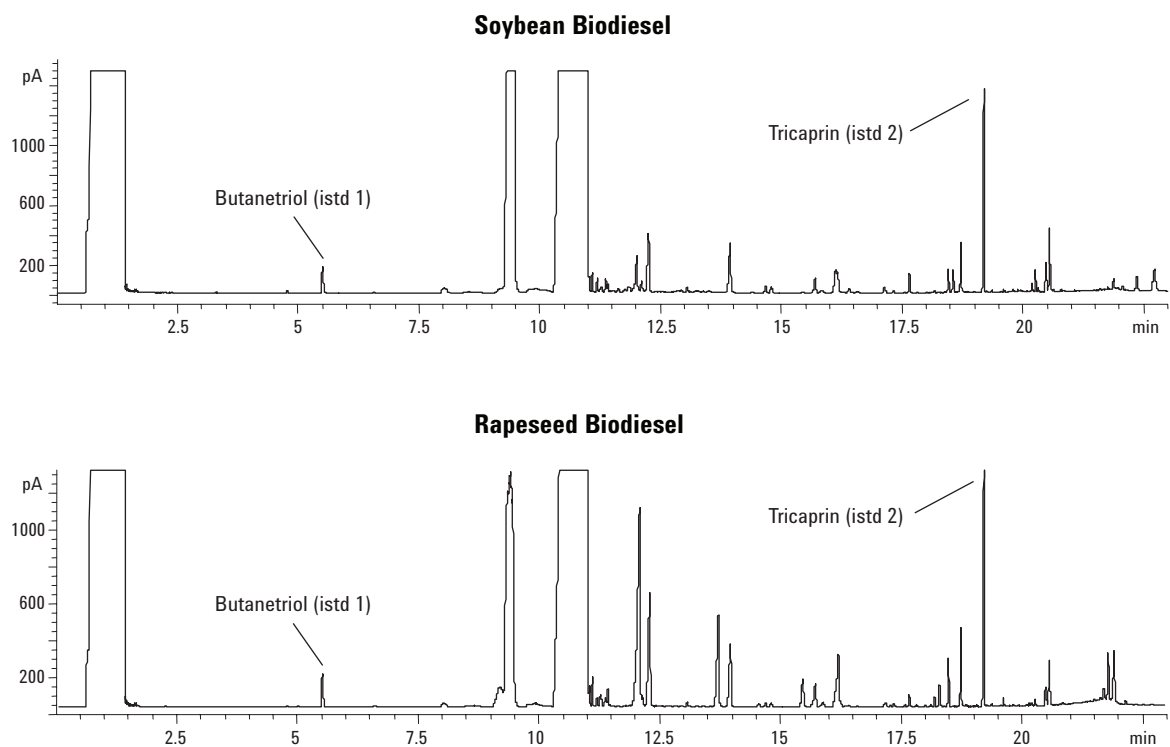


Figure 2. Chromatograms showing typical analysis of free and total glycerins in two B100 biodiesel samples.

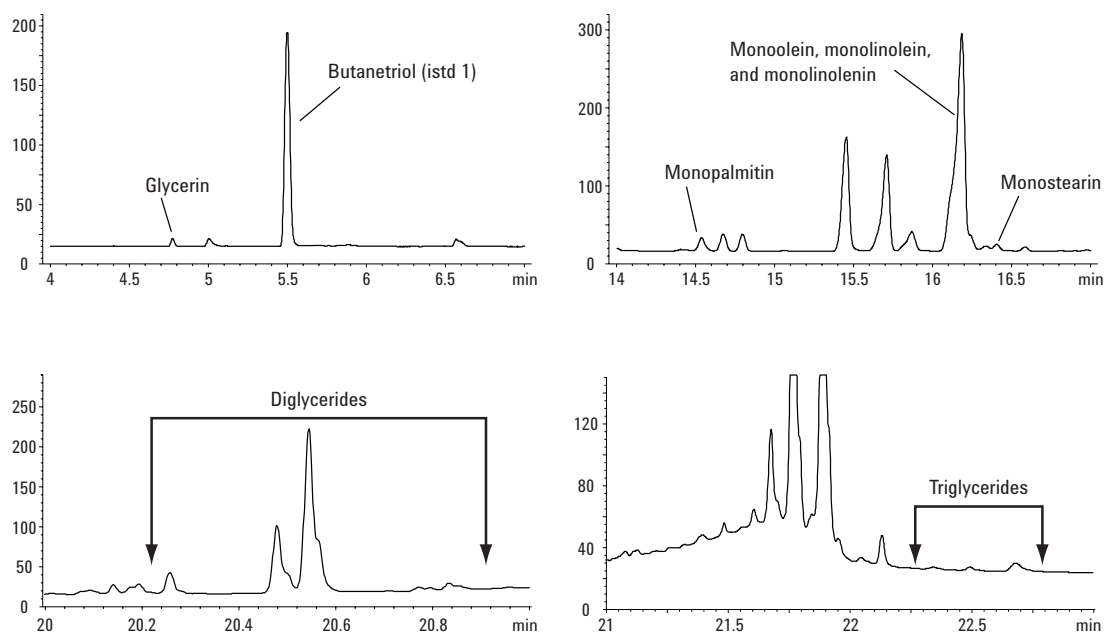


Figure 3. Details of glycerol, monoglycerides, diglycerides, and triglycerides found in a sample of rapeseed B100 biodiesel.

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

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
Monolein, monolinolein, monolinolenin, monostearin		0.83 – 0.86
Tricaprin (int std 2)		1.00
Diglycerides		1.05 – 1.09
Triglycerides		1.16 – 1.31

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.

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

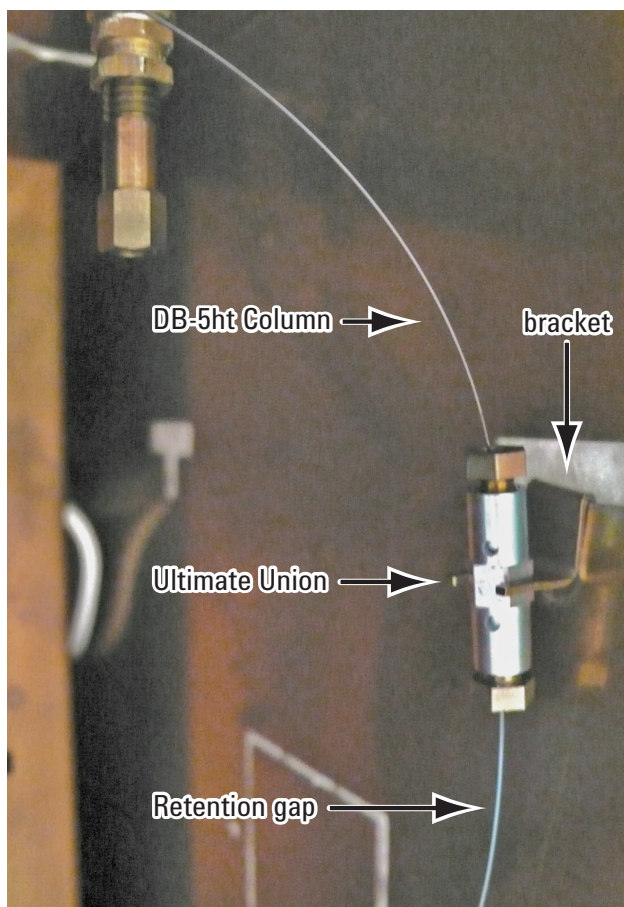


Figure 5. Details of the retention gap and analytical column joined with a Capillary Flow Technology Ultimate Union.

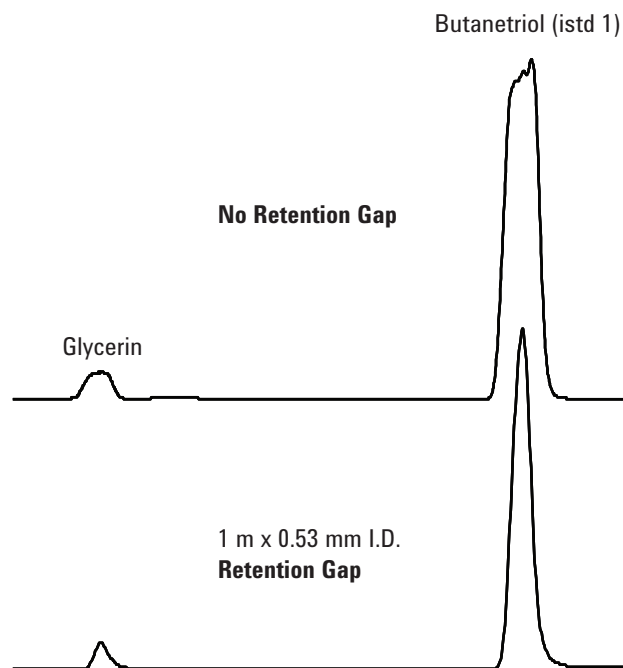


Figure 4. Improved peak shape for glycerin and 1,2,3-butanetriol when using a retention gap and the Capillary Flow Technology Ultimate Union.

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.

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
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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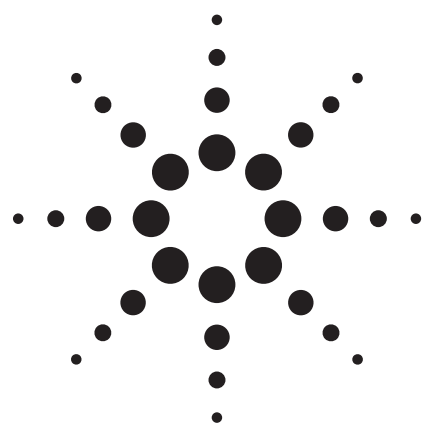
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Analysis of Fatty Acid Methyl Ester (FAME) Content and Distribution in Biodiesel Blends Using Heart-Cutting 2D Gas Chromatography

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 preci-

sion. 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]



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Table 1. Fatty Acid Distribution of Common Biodiesel Feedstocks

Oil Type	Fatty Acid Distribution										C20:0	C20:1
	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C22:0	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
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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)
07756-1AMP*	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 pre-

pared 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.

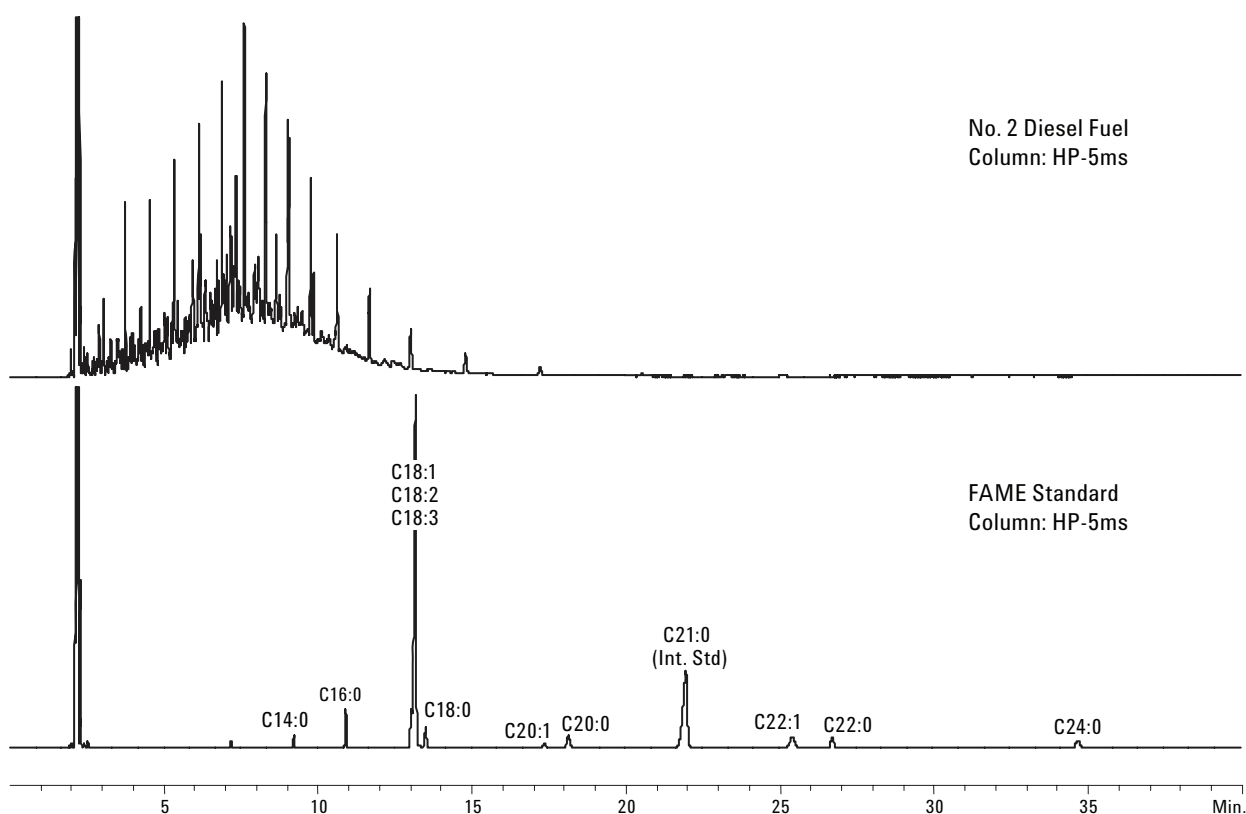


Figure 1. The lower chromatogram shows the separation of FAMES on the primary HP-5ms column. The upper chromatogram shows the separation of pure no. 2 diesel fuel on the same column. A blended biodiesel fuel containing FAMES and no. 2 diesel fuel would have unresolved compounds between 2 and 15 minutes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

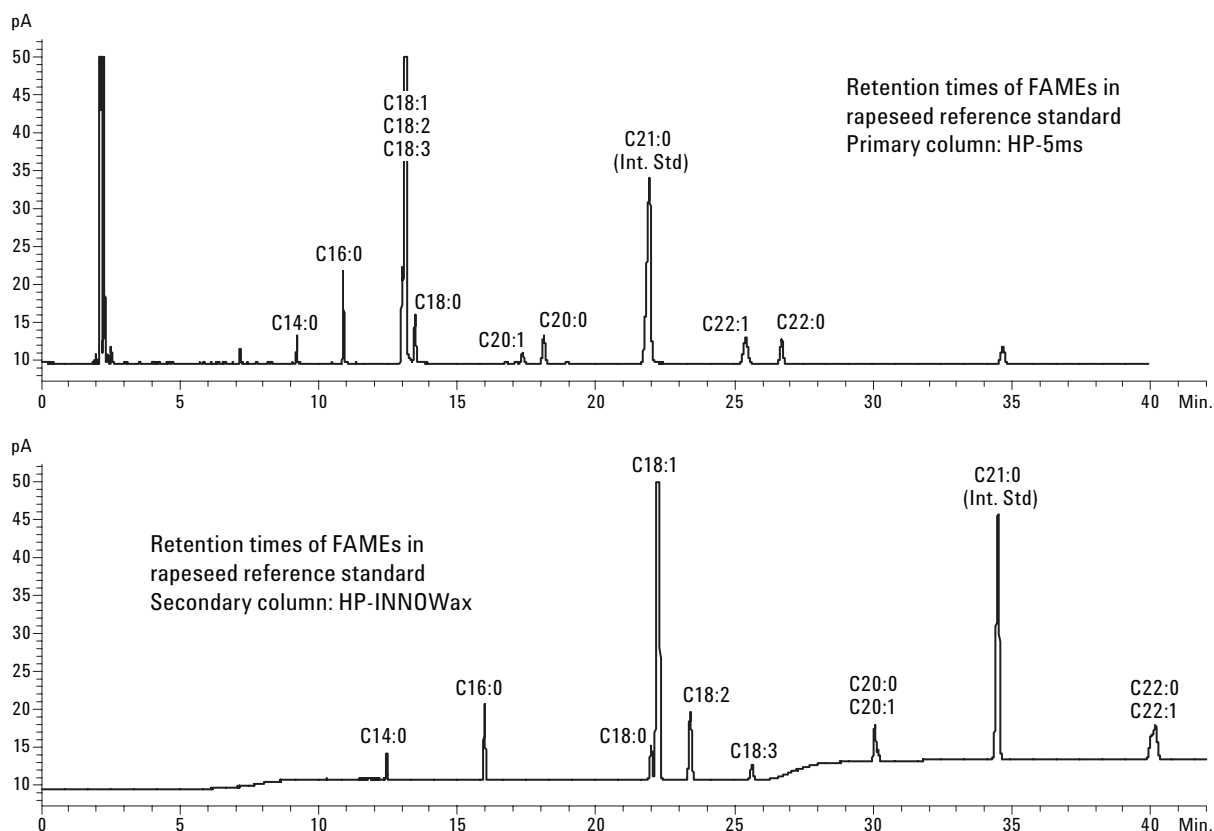


Figure 2. The upper chromatogram shows the separation of FAMES on the primary HP-5ms column before heart-cutting. After heart-cutting, the lower chromatogram shows the separation of the FAMES on the HP-INNOWax secondary column.

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.

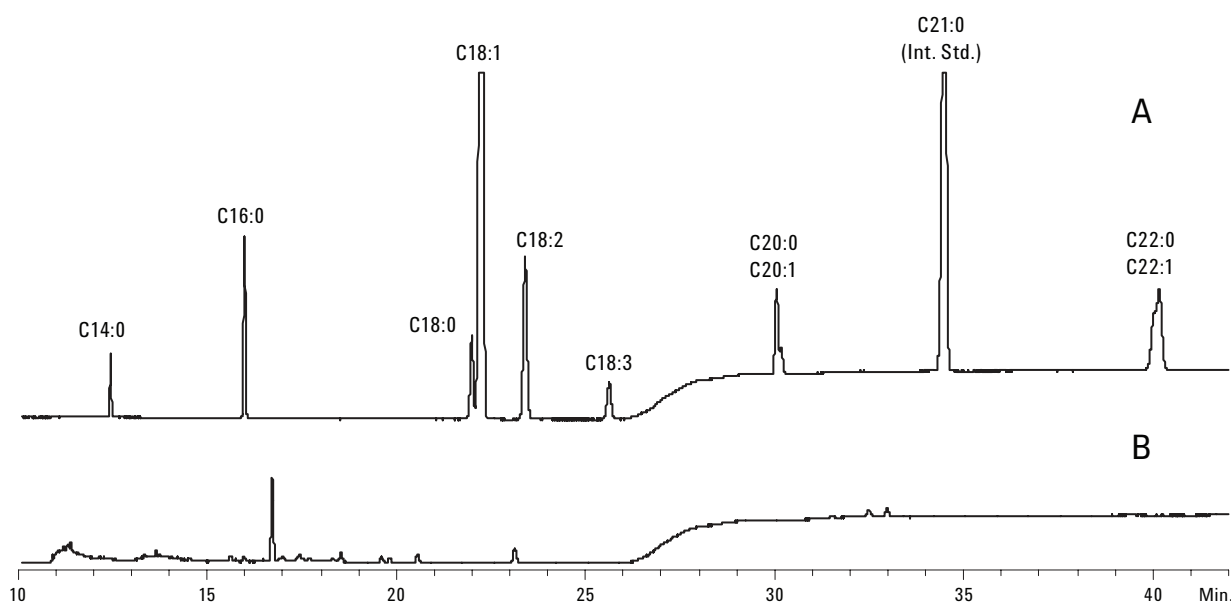


Figure 3. The upper chromatogram (A) shows the retention times of FAMES on the secondary HP-INNOWax column after heart-cutting. The lower chromatogram (B) shows the hydrocarbon matrix of no. 2 diesel fuels after heart-cutting. No large peaks from the hydrocarbon matrix were found to co-elute with the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

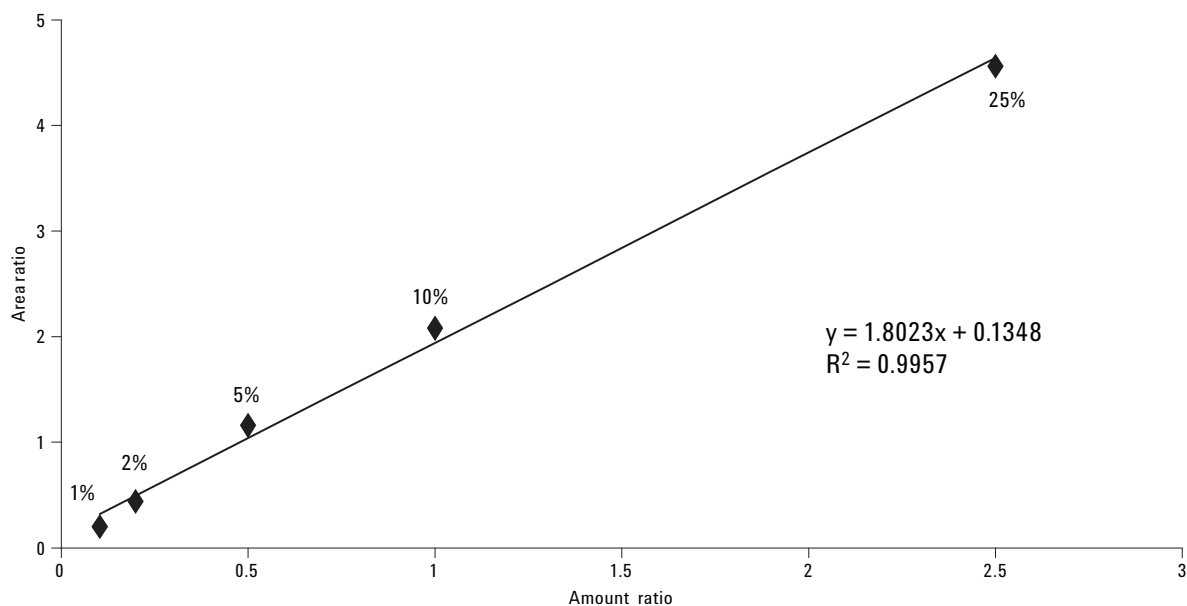


Figure 4. A calibration curve for biodiesel blends containing soybean biodiesel between 1 vol% (B1) and 25 vol% (B25). This calibration was prepared using the total peak areas of all FAMES found in the blends.

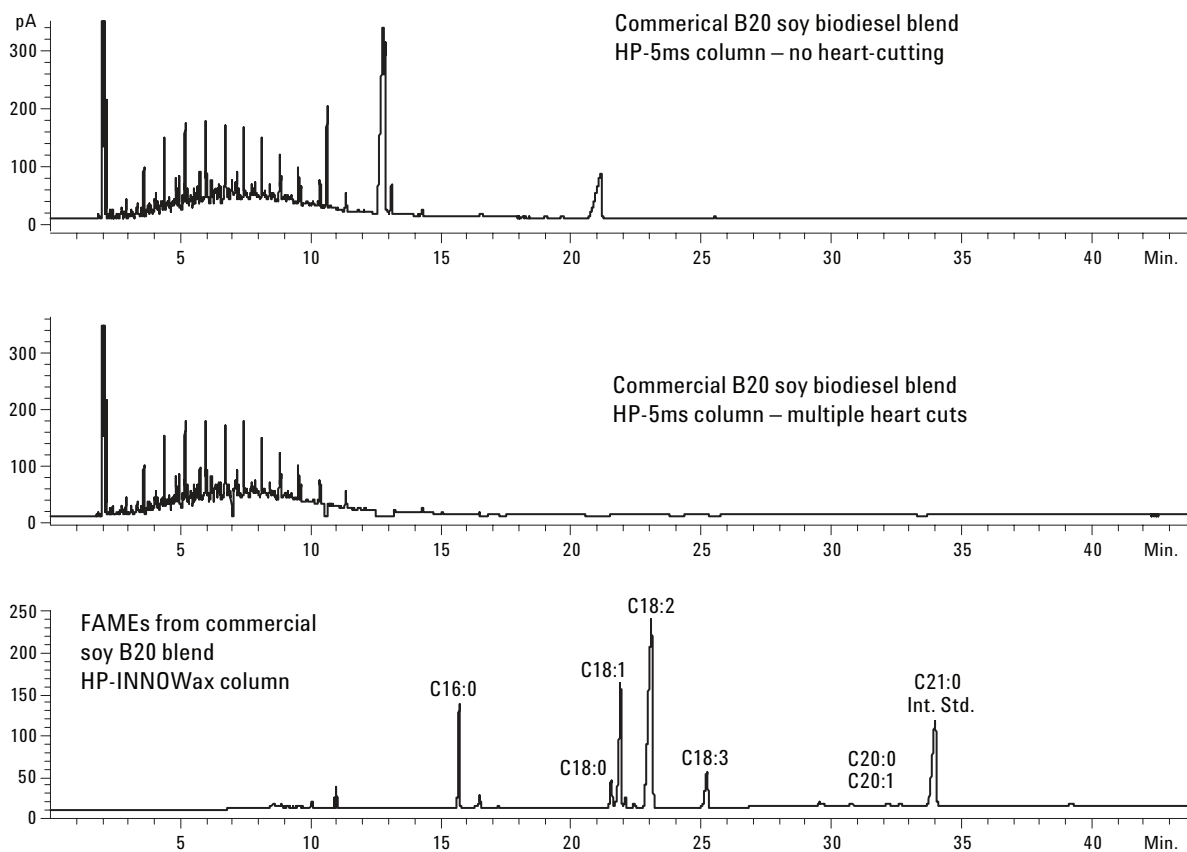


Figure 5. A commercially prepared B20 sample containing soybean biodiesel was analyzed using heart-cutting 2D GC. The upper chromatogram shows the primary column separation before heart-cutting. The middle and lower chromatograms show the sample after heart-cutting the FAMES from the HP5ms column to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

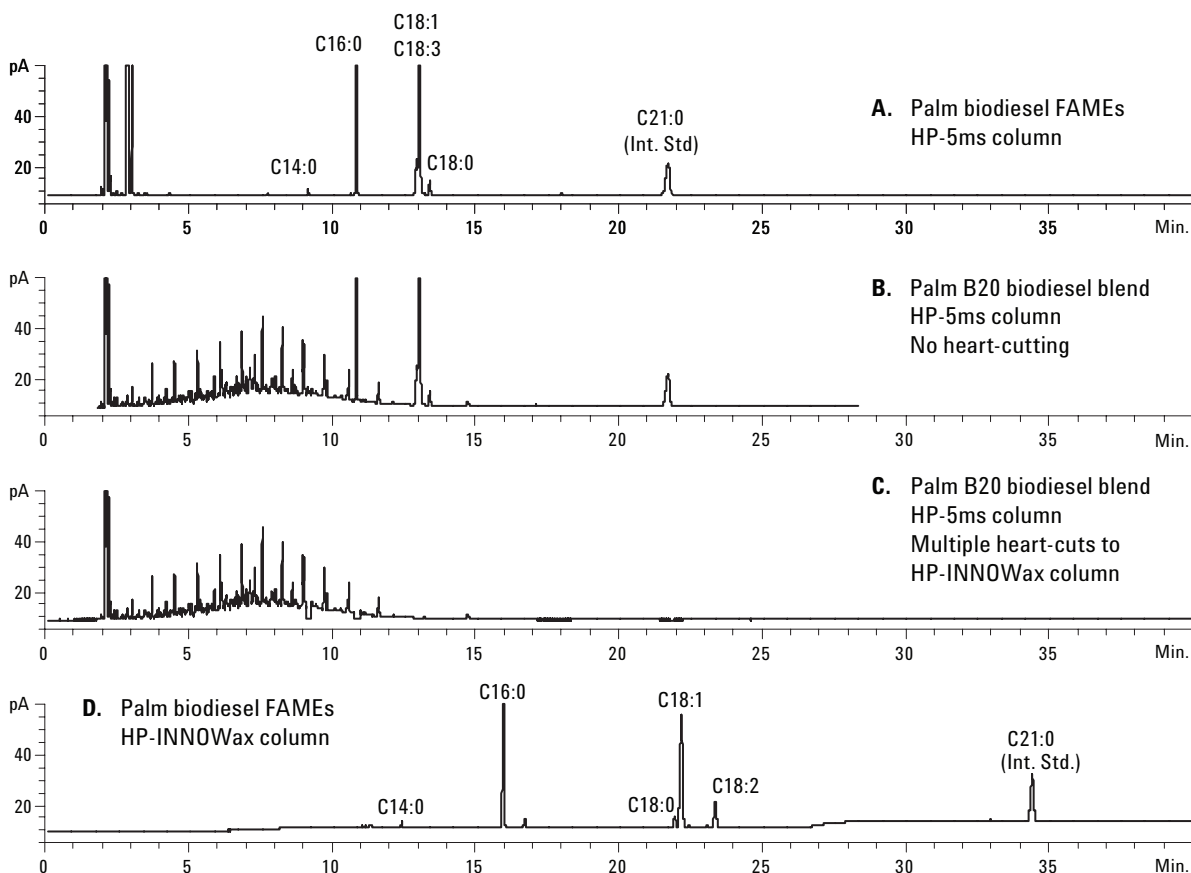


Figure 6. (A) Palm oil FAMES on the primary HP-5ms column. (B) Palm B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of palm FAMES in B20 biodiesel blend using heart-cutting 2D GC.

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

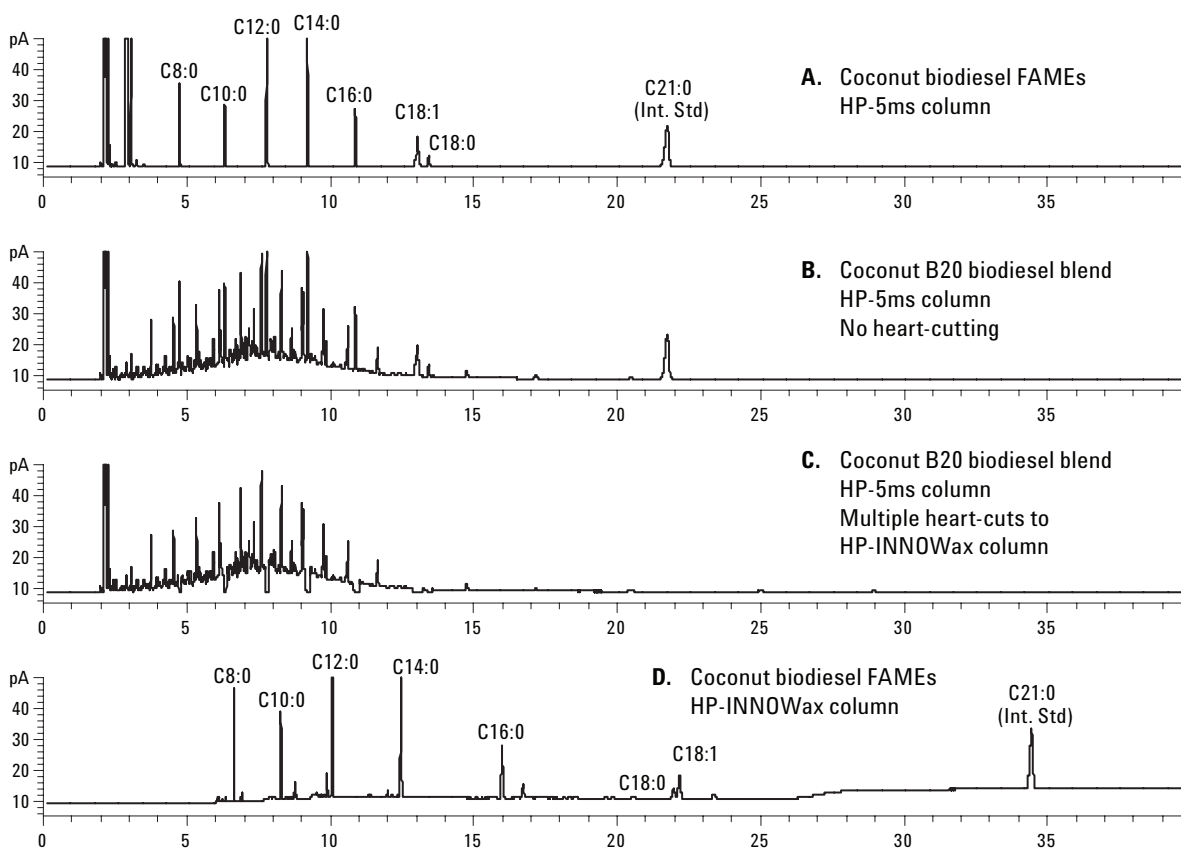


Figure 7. (A) Coconut oil FAMES on the primary HP-5ms column. (B) Coconut B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of coconut FAMES in B20 biodiesel blend using heart-cutting 2D GC.

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.
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. Quimby, "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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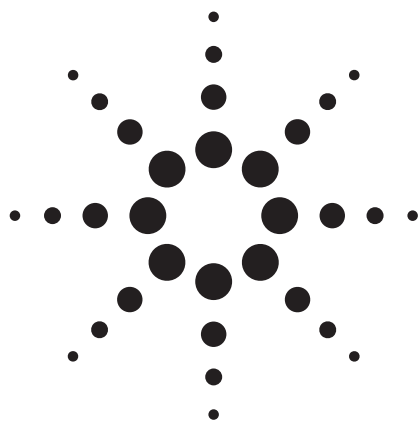
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Automated Standard and Sample Preparation for Multiple Gas Chromatographic Analyses of Biodiesel

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 silylation 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.



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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 μ L calibration standard mixture #1 to vial (100 μ L syringe).
3. Add 10 μ L ISTD1 solution (butanetriol) to vial using front tower.
4. Add 10 μ L ISTD2 solution (tricaprin) to vial using front tower.
5. Add 10 μ L 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 μ L n-heptane to vial using front tower.
10. Transfer vial to mixer and mix for 1 minute.
11. Transfer vial to rear tower (10 μ L syringe).
12. Inject 1 μ L 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 $\times$ 1 mL ampoules of N-methyl-N-(trimethylsilyl) trifluoro-acetamide
Monoglyceride retention time standard (5190-1410)	Contains 3 $\times$ 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 μ L ISTD1 solution (butanetriol) to vial using front tower.
3. Add 10 μ L ISTD2 solution (tricaprin) to vial using front tower.
4. Add 10 μ L 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 μ L n-heptane to vial using front tower.
9. Transfer vial to mixer and mix for 1 minute.
10. Transfer vial to rear tower (10 μ L syringe).
11. Inject 1 μ L 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 μ L syringe).
2. Add 500 μ L ISTD Solution (10 mg/mL C17:0 in n-heptane) to vial using rear tower (100 μ L Syringe).
3. Transfer vial to mixer and mix for 1 minute.
4. Transfer vial to front tower (10 μ L syringe).
5. Inject 1 μ L onto split inlet using front tower (10 μ L 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.

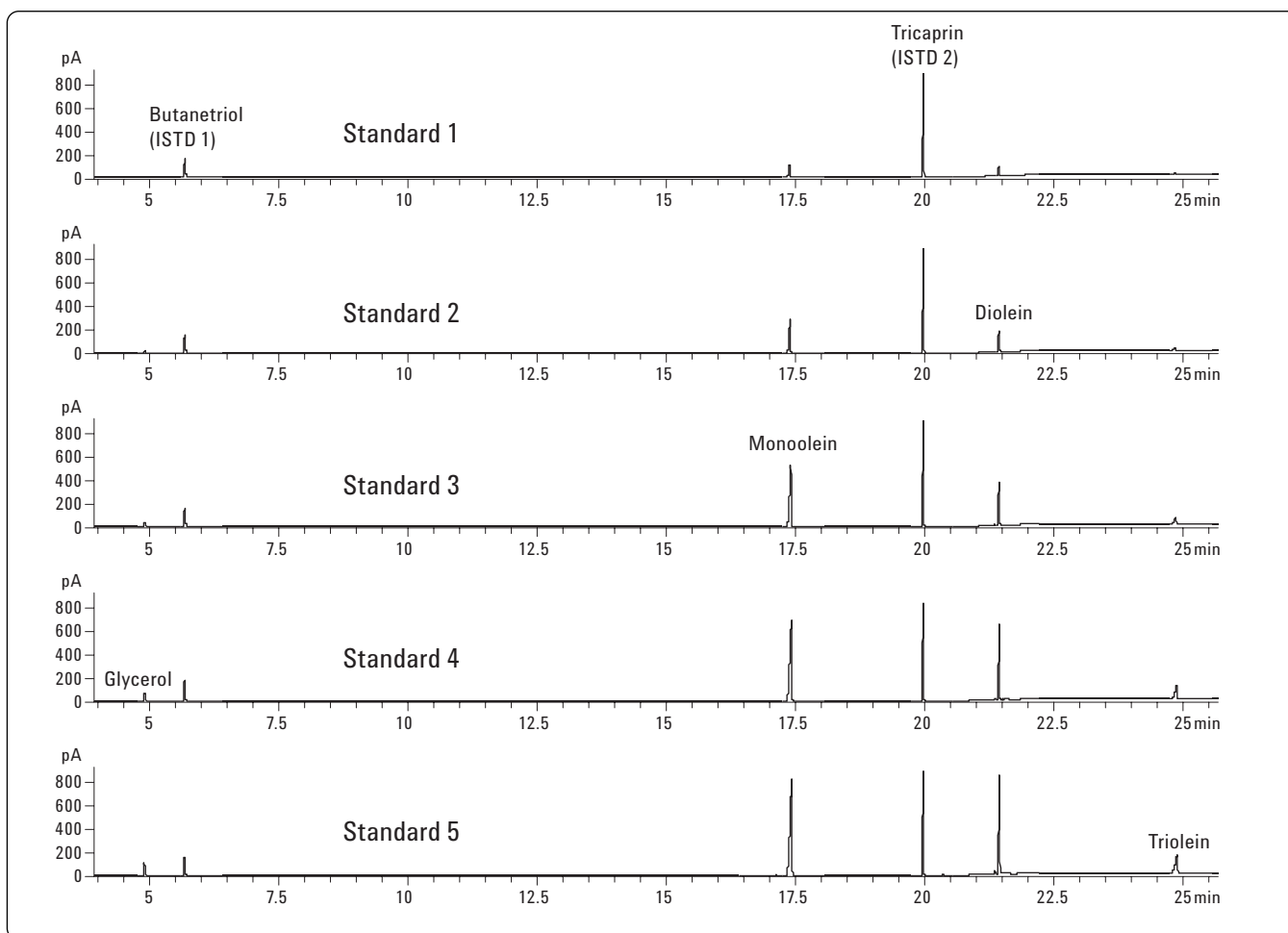


Figure 1. A five level ASTM D6584 calibration was automatically prepared using the Agilent 7693A ALS and run on the Agilent 7890A Biodiesel Solution.

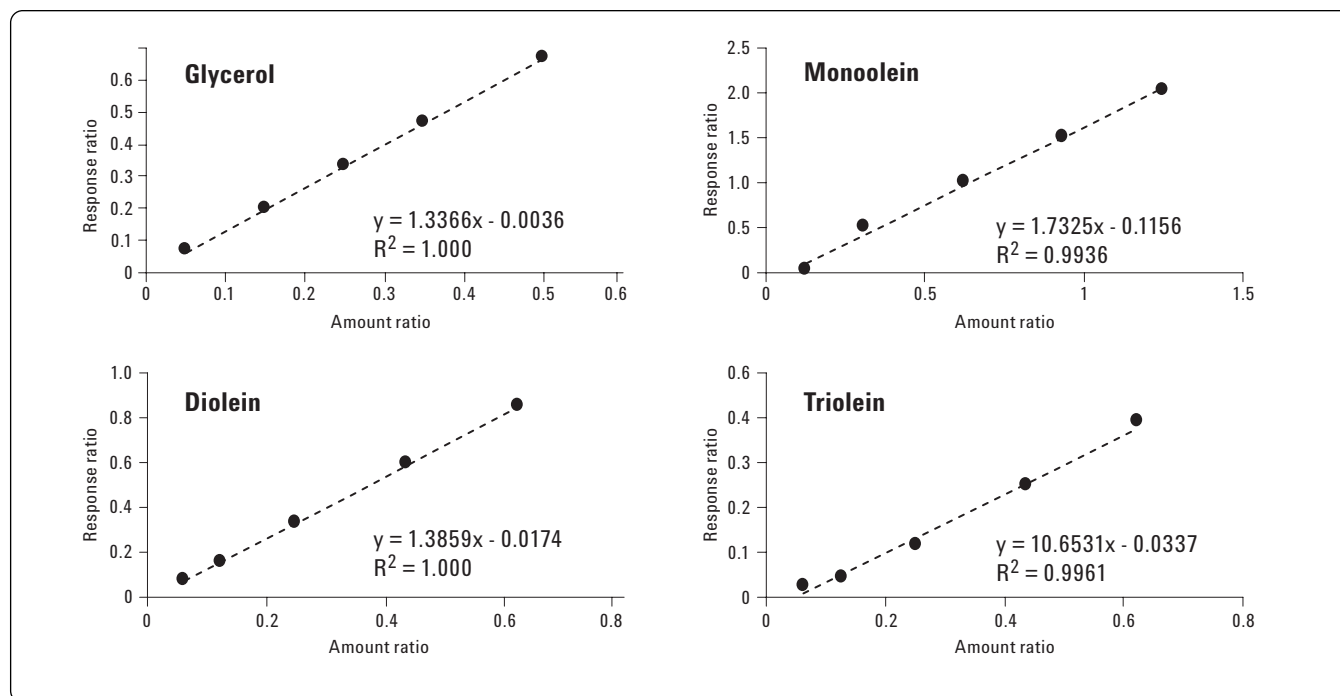


Figure 2. Five point linear calibration curves resulting from automated standard preparation with the Agilent 7693A ALS.

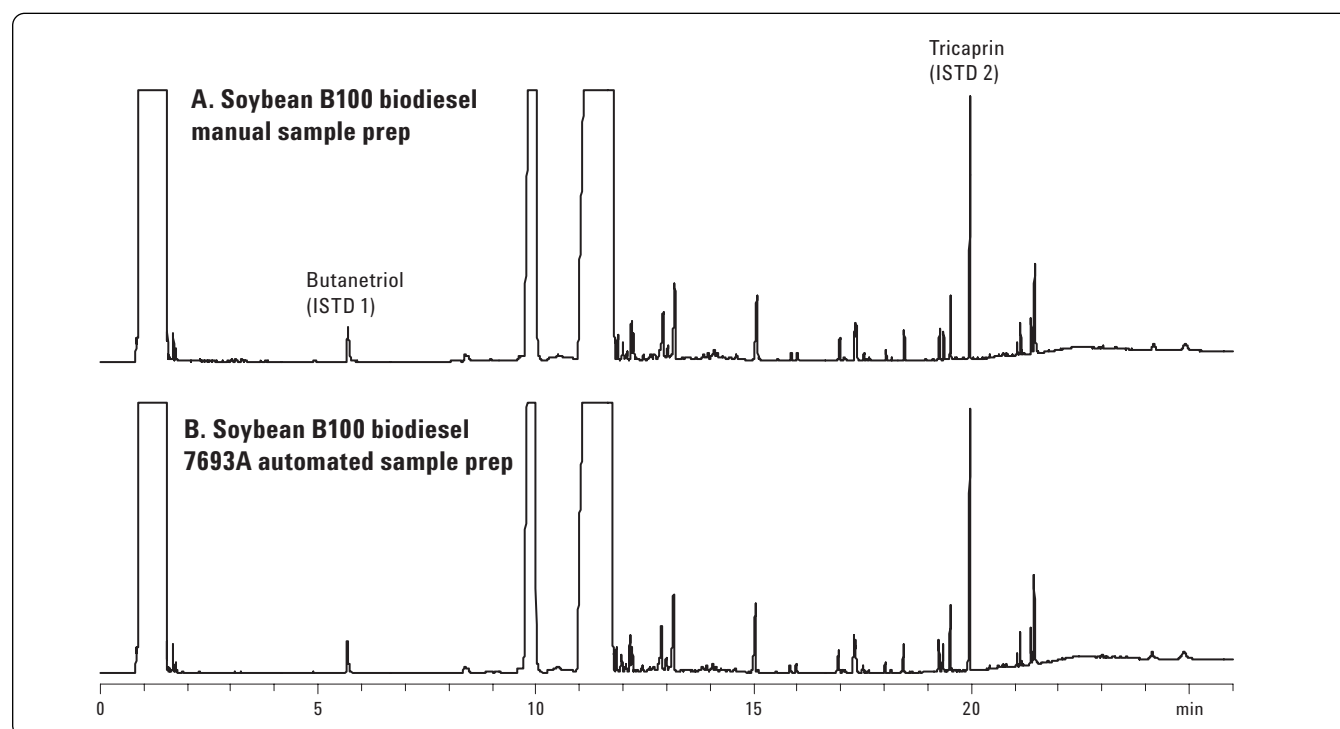


Figure 3. Comparison of a soybean B100 sample prepared (A) manually according to the ASTM D6584 protocol and (B) the automatic 10% scaled protocol using the Agilent 7693A ALS and Agilent 7890A Biodiesel Solution.

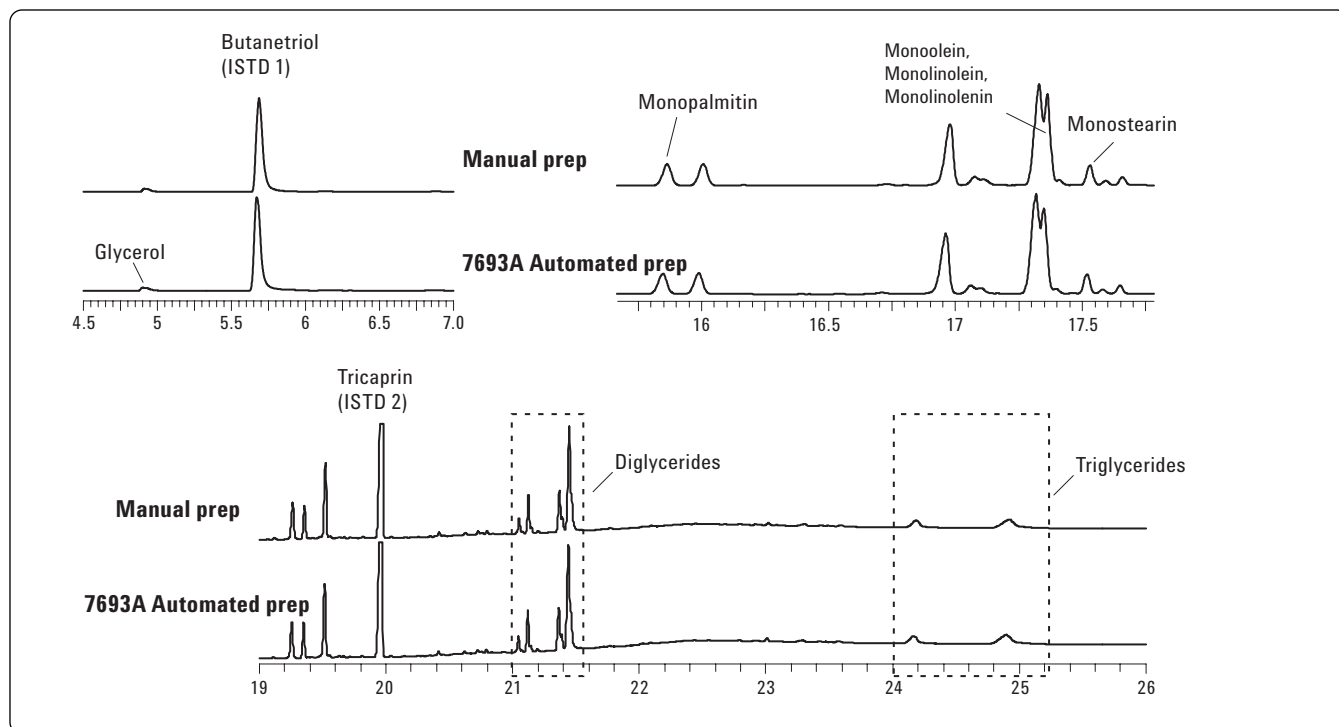


Figure 4. Details of the glycerol, mono-, di-, and tri-glycerides contained in a soybean B100 sample. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

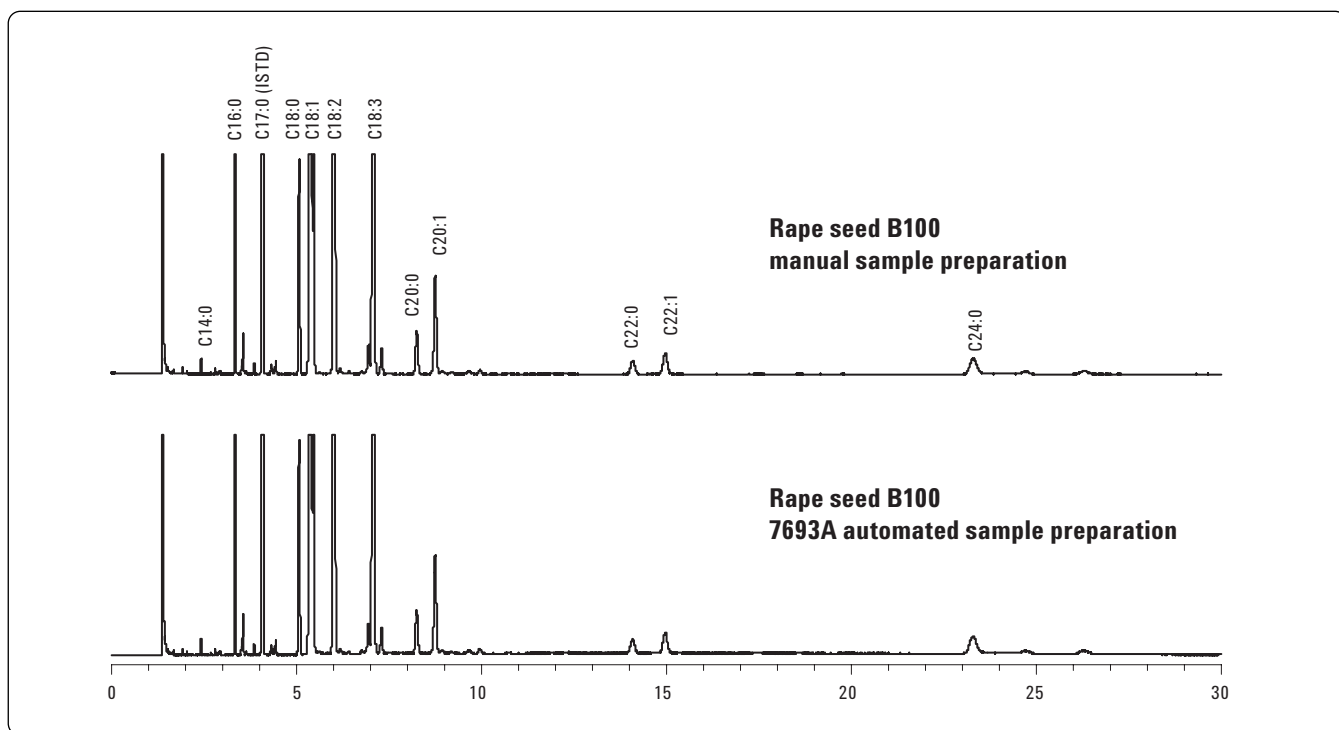


Figure 5. Analysis FAMES in rape seed B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

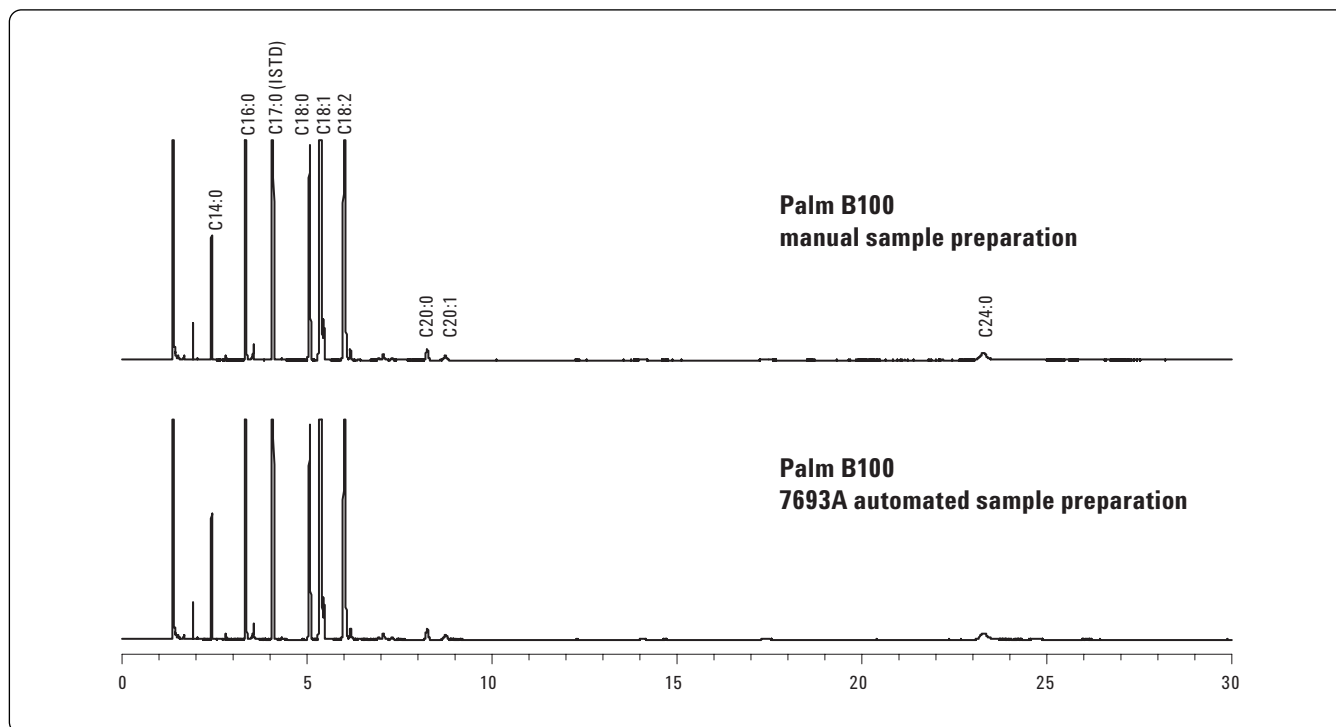


Figure 6. Analysis FAMEs in palm oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

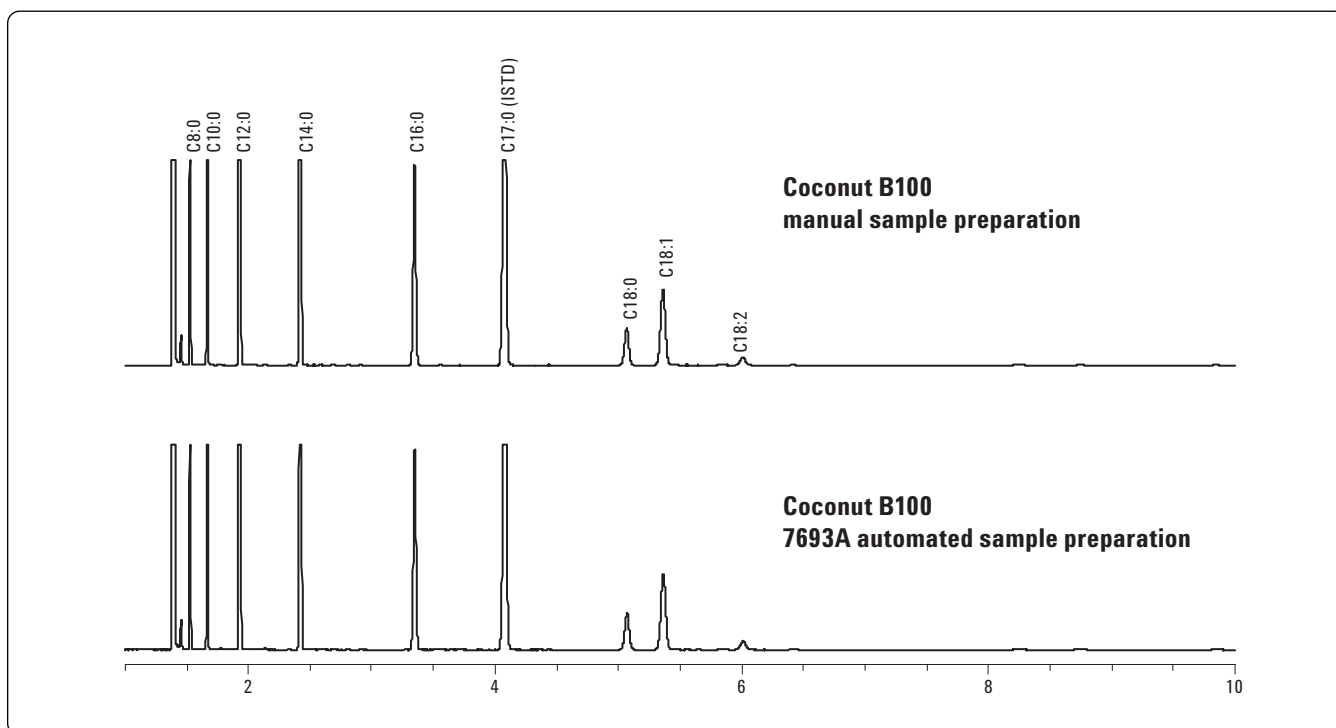


Figure 7. Analysis FAMEs in coconut oil B100 biodiesel after manual and automated addition of internal standard. The automated sample preparation yields the same chromatographic result as the manual sample preparation.

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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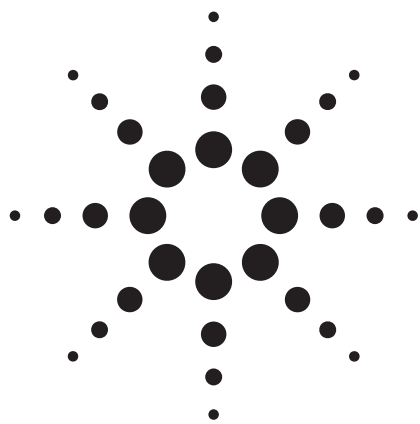
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Boiling Point Range of Fatty Acid Methyl Esters Using the 7890A Gas Chromatograph, Low Thermal Mass (LTM) System, and 7693A Tower and Tray

Application Note

Hydrocarbon Processing

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Abstract

Two Agilent 7890A Series GC systems were used to determine the boiling point distribution of Biodiesel (B100 and B20). First, the standard oven was used to produce runs of about 16 minutes. This was followed by a 7890A equipped with an LTM system and 1 five-inch module which does an 8 minute run. Both systems used the Multimode inlet in temperature programmed split mode for sample introduction and a dual tower Agilent 7693A Automatic Liquid Sampler configuration with a 150-vial sample tray for sample prep and injection.



Agilent Technologies

Introduction

ASTM method D7398 describes procedures for determining the boiling point range distribution of pure biodiesel (B100) and biodiesel blends of B1 and higher. To ensure that unreacted triglycerides are detected, the gas chromatograph is temperature programmed to 400 °C. Only the procedures involving calibration and running of pure biodiesel and biodiesel blends will be demonstrated in this work. Some sample preparation is normally involved which includes dissolution of a Polywax 500 standard that involves heating, mixing, and sample dilution of the biodiesel. These sample preps can be largely automated using the Agilent 7693A Series injector and tray system. Simulated distillation software is then used to compute the boiling range distribution. A standard 7890A GC and a 7890A/Low Thermal Mass (LTM) was used to analyze the prepared samples.

Experimental

Standard 7890A System

Inlet:	Multimode, G3510, 325 °C (0 min) to 400 °C at 200 °C/min
Liner:	Single taper liner with glass wool, 5183-4647
7890A oven:	40 °C (0 min) to 400 °C at 15 °C/min
Column:	5M × 0.53 mm × 0.15 µm DB-HT SimDis, 145-1001
Flow:	Constant flow mode at 14 mL/min He
Injection:	0.1 µL split 4:1, PW500 standard, 1 µL

7890A/LTM System

Inlet:	Multimode, G3510, 220 °C (0 min) to 400 °C (2 min) at 300 °C/min
LTM column module:	5 m × 0.53 mm × 0.15 µm DB-HT SimDis
Module connections:	0.7 m deactivated ProSteel on inlet and outlet
7890A oven:	325 °C isothermal
LTM module program:	40 °C (0 min) to 400 °C at 50 °C/min
FID:	400 °C
Inlet pressure ramp:	2.5 psi (0 min) to 9.5 psi (1 min) at 1 psi/min
Injection:	0.1 µL, split 10 to 1, PW500 standard, 1 µL

Deactivated Ultimate Unions, part no. 3182-60580, are used with the LTM module for connection of the ProSteel retention gaps to the column ends.

The 7693A injectors are installed with the 150-vial sample tray which includes a mixer/barcode reader and heater compartment for the purpose of sample prep and injection. The front tower uses a 5-µL syringe and the rear tower uses a 250-µL syringe which requires the large syringe carriage G4521A.

Data is processed using ChemStation 4.01 and the Agilent SimDis software, part number G2887BA. Example sample preparation programs from the ChemStation are shown below for system calibration and biodiesel samples.

Sample Prep Programs Using the 7693A

Table 1. Sample Program for the Preparation of PW500 with C5-C18 Mix Added

Sampler program steps
Wash syringe in Back tower, drawing from Wash A1 dispensing into Waste A1 2 times
Move vial from tray vial #1 to back turret position #1
Move vial from tray vial #2 to back turret position #2
Dispense 1000 µL from vial Wash A2 to vial Sample 1 on the Back tower
Dispense 5 µL from vial Sample 2 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to mixer
Move vial from back turret position #2 to tray vial #2
Mix at 2000 rpm 2 times for 10 seconds
Move vial from mixer to heater
Heat vial at 80 degrees C for 240 seconds
Move vial from heater to tray vial #1
Wash syringe in Back tower, drawing from Wash A2 dispensing into Waste B1 3 times

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

Sampler program steps
Move vial from front sample vial offset by 0 vial(s) to back turret position #1
Dispense 750 µL from vial Wash A3 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to mixer
Mix at 2000 rpm 5 times for 10 seconds
Move vial from mixer to front sample vial offset by 0 vial(s)
Wash syringe in Back tower, drawing from Wash B3 dispensing into Waste B2 2 times

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 C₈ to C₇₈, 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 C₇₀ 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. C₁₆ and C₁₈ fatty acid methyl esters can be seen above the diesel background.

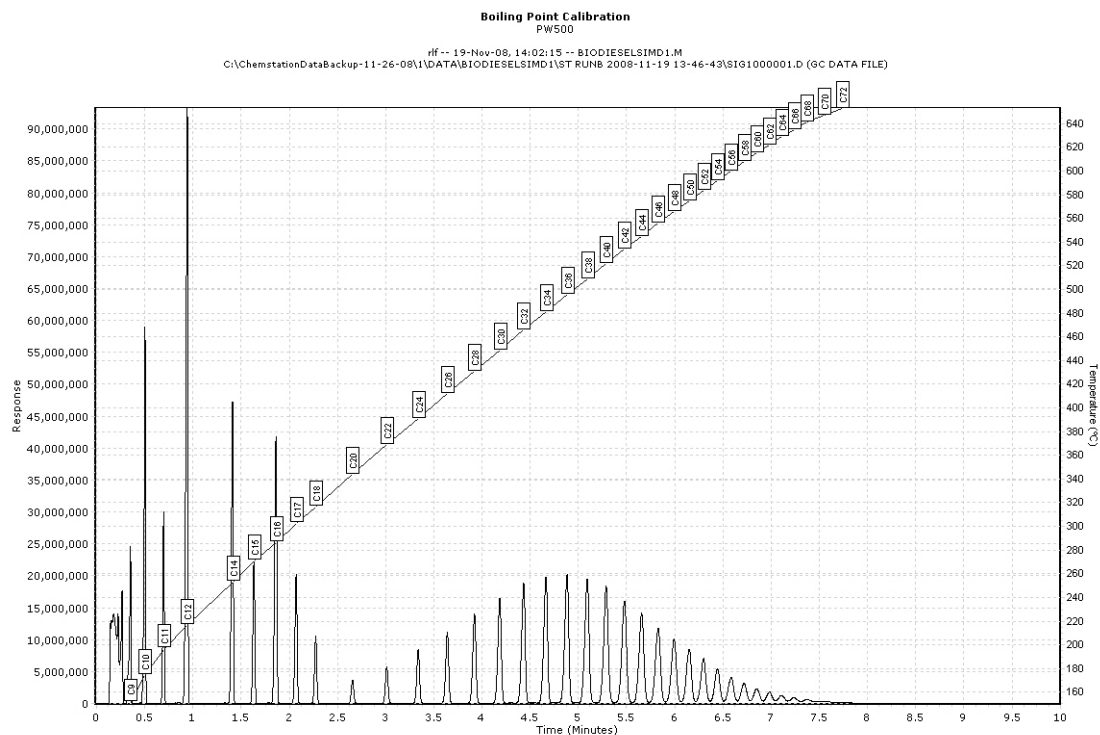


Figure 1. Calibration curve on LTM system from C9 to C72 prepared from PW500 and C5-C18 mix.

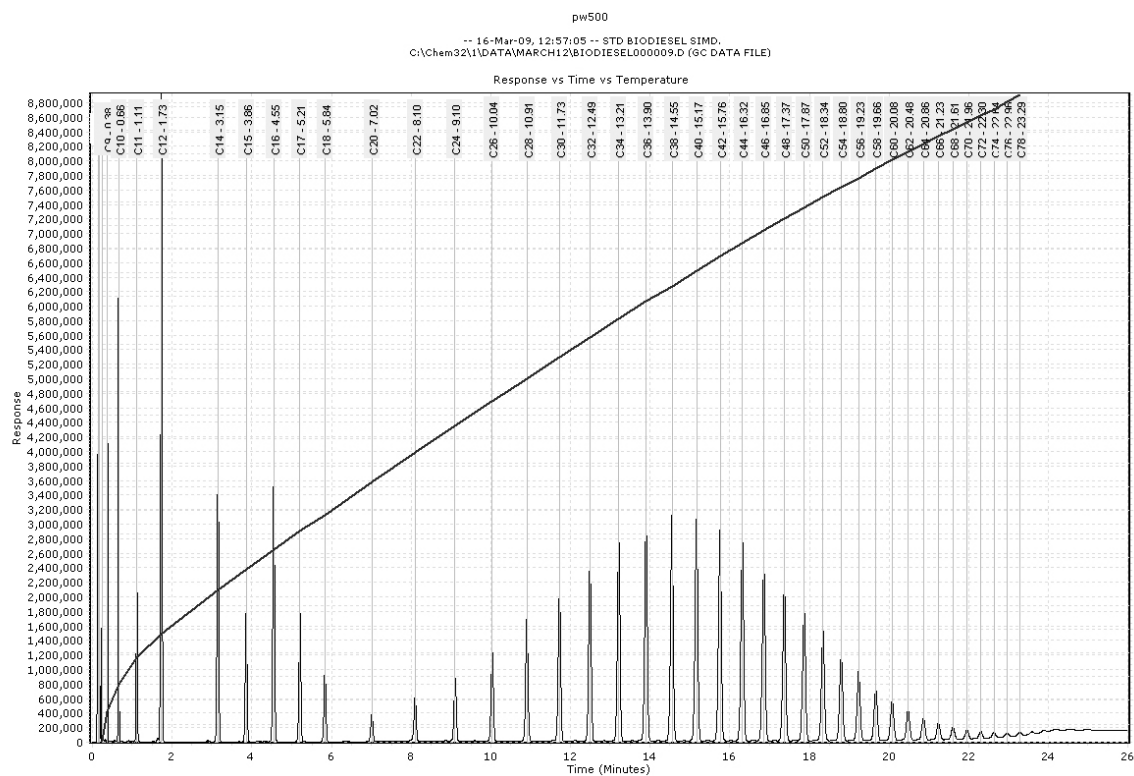


Figure 2. Calibration curve on standard 7890A GC.

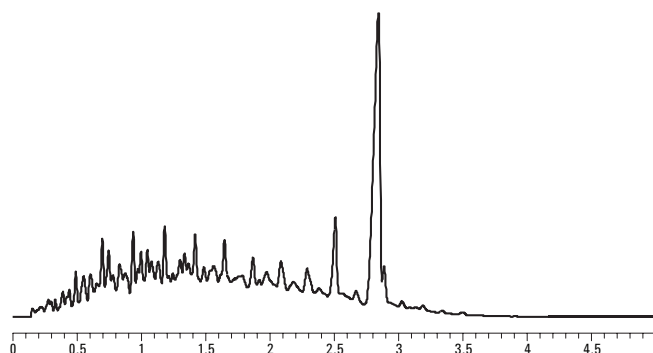


Figure 3. Chromatogram of B20 Soy based biodiesel using the LTM system.

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 C₁₆ group (6.6 min.) to the C₁₈ group (7.5 min.) in these samples. Lastly, calculated boiling point distributions for both samples are shown in Figures 6 and 7.

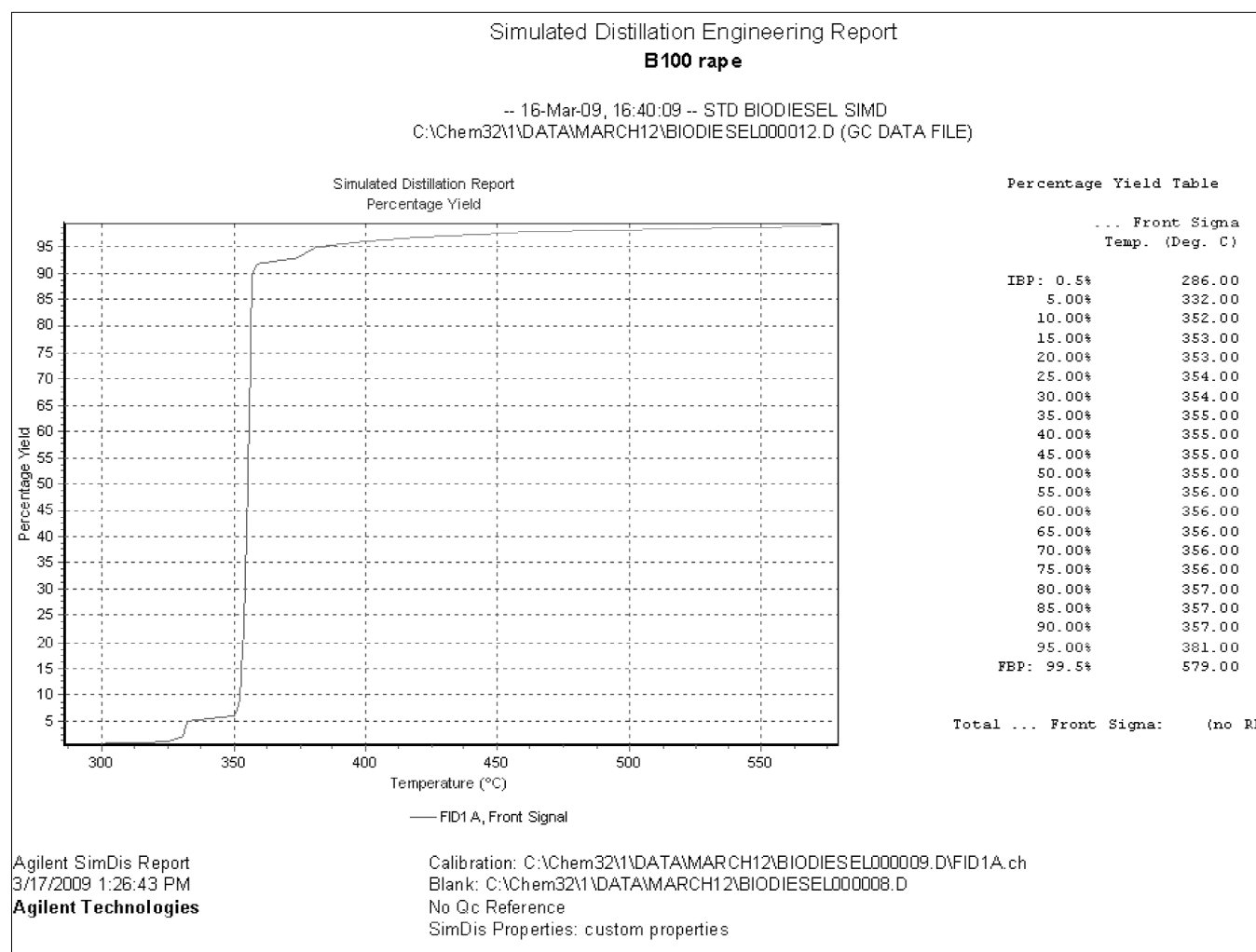


Figure 4. Boiling point distribution for rapeseed B100.

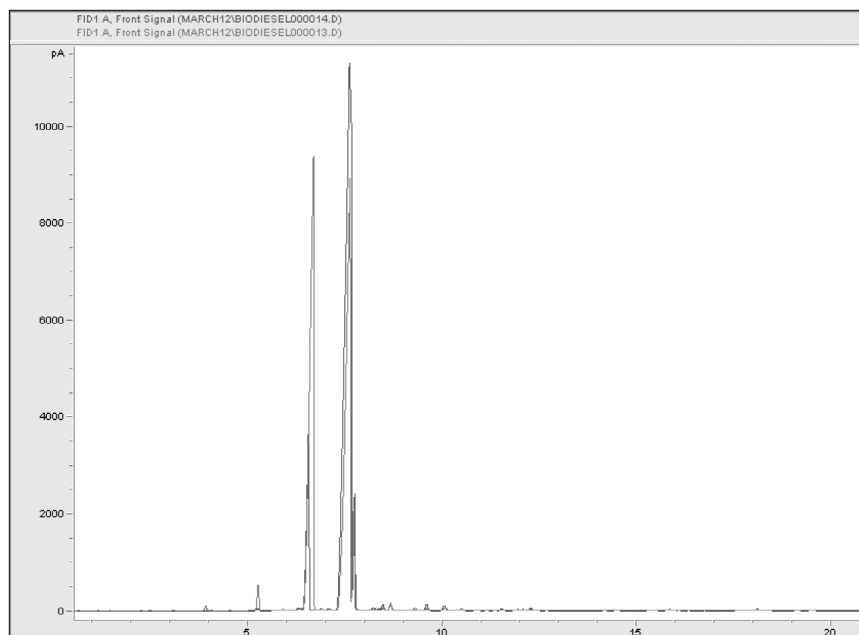


Figure 5. Overlay of two B100 samples from different producers. Both are soy based biodiesel. Producer A: signal 14, Producer B: signal 13.

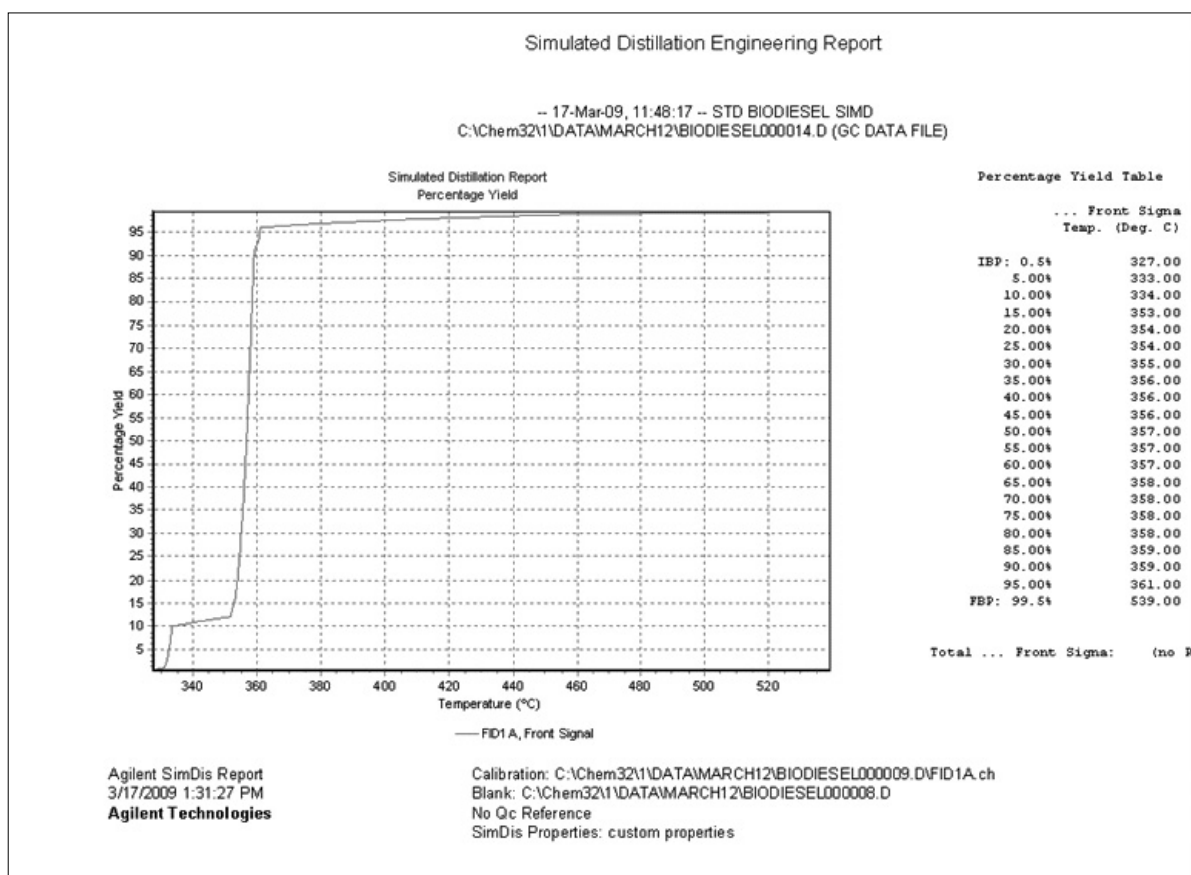


Figure 6. Boiling point distribution of B100, producer A.

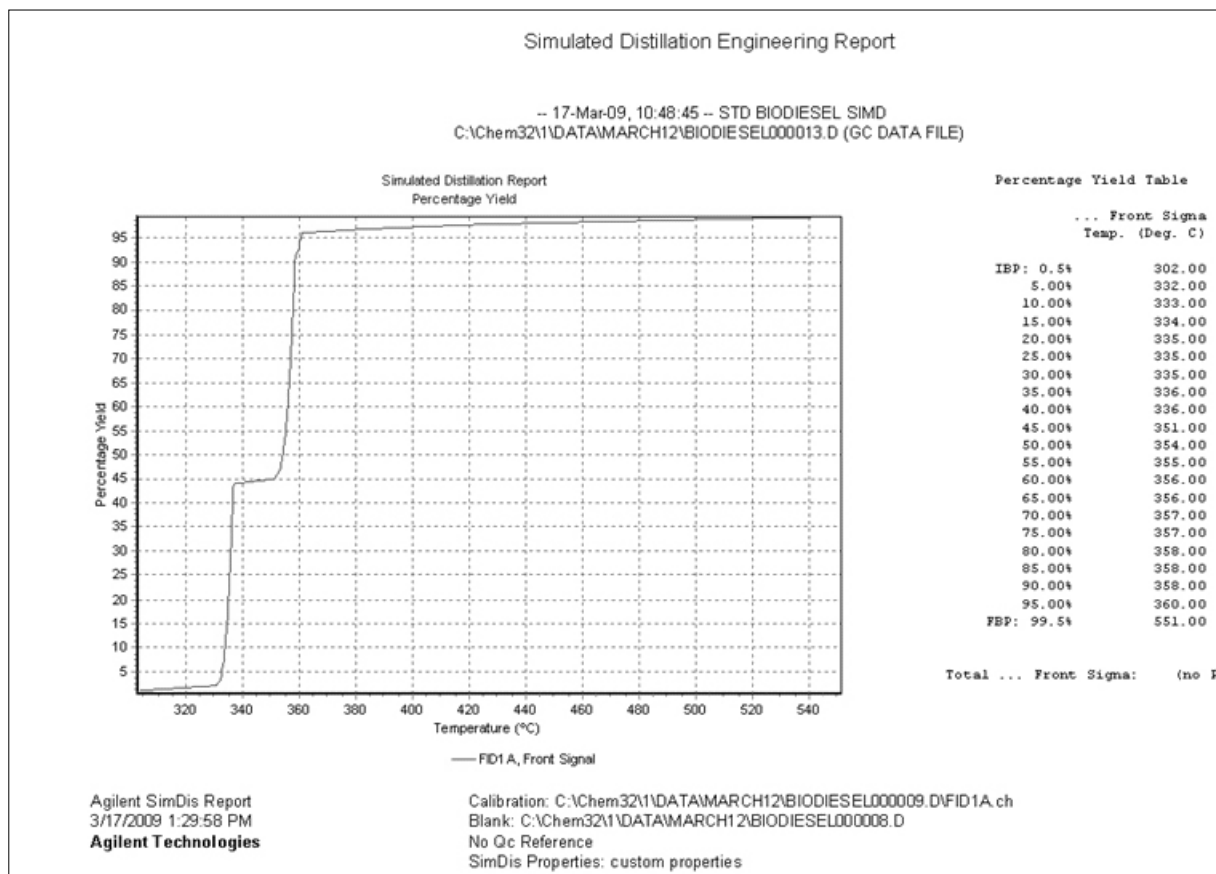


Figure 7. Boiling point distribution of B100 from producer B.

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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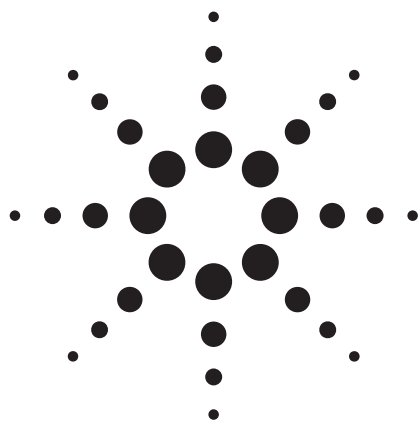
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High-Resolution Analysis of Intact Triglycerides by Reversed Phase HPLC Using the Agilent 1290 Infinity LC UHPLC System

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- μ m 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.



Agilent Technologies

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 bio-fuels, 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 $C_{55}H_{98}O_6$.

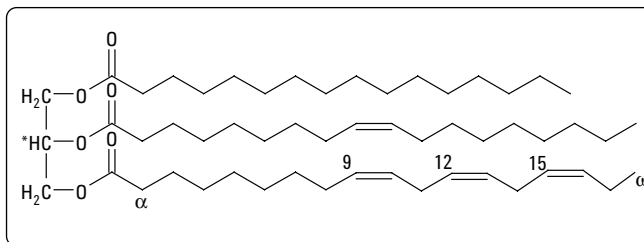


Figure 1. Typical triglyceride structure.

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 μ L 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 $\times$ 150 mm, 1.8 μ m 600 bar, p/n 829975-302
Agilent ZORBAX SB-C18 RRHD, 2.1 mm $\times$ 100 mm, 1.8 μ m 1200 bar, p/n 858700-902
Agilent ZORBAX SB-C18 RRHD, 2.1 mm $\times$ 150 mm, 1.8 μ m 1200 bar, p/n 859700-902
In some cases, columns were coupled to extend the length and resolution.

Column temp: 20 $^{\circ}$ C or 30 $^{\circ}$ C

Mobile phase: A = acetonitrile
B = isopropanol (IPA) or tert butyl methyl ether (MTBE) (See individual figures)

Flow rate: See individual figures

Gradient: The gradient conditions were either 20% to 60% IPA or 10% to 40% MTBE, based on the strong eluting strength of MTBE when compared to IPA. The gradient slope was maintained at 2.6% organic phase increase per column volume for IPA gradients and 2.0% with MTBE, altering gradient time and flow rate accordingly. This was determined by calculations using a modification of the Agilent Method Translator. [3]

UV Conditions

Monitoring 210, 220 and 230 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

A typical gradient separation of triglycerides using acetonitrile IPA gradient is shown in Figure 2.

Some general comments are appropriate about the conditions and chromatographic profile shown in Figure 2. While it would be ideal to consider less expensive methanol as the weak eluent, introduction of methanol or denatured ethanol

containing methanol has consistently shown a dramatic reduction in the overall resolution of the triglycerides. The significant increase in operating pressure, when running the gradient from acetonitrile to IPA, is clearly limiting and undesirable. Increasing the operating temperature of the column as a means of reducing solvent viscosity has proven to be undesirable because the chromatographic resolution tends to collapse as temperature increases.

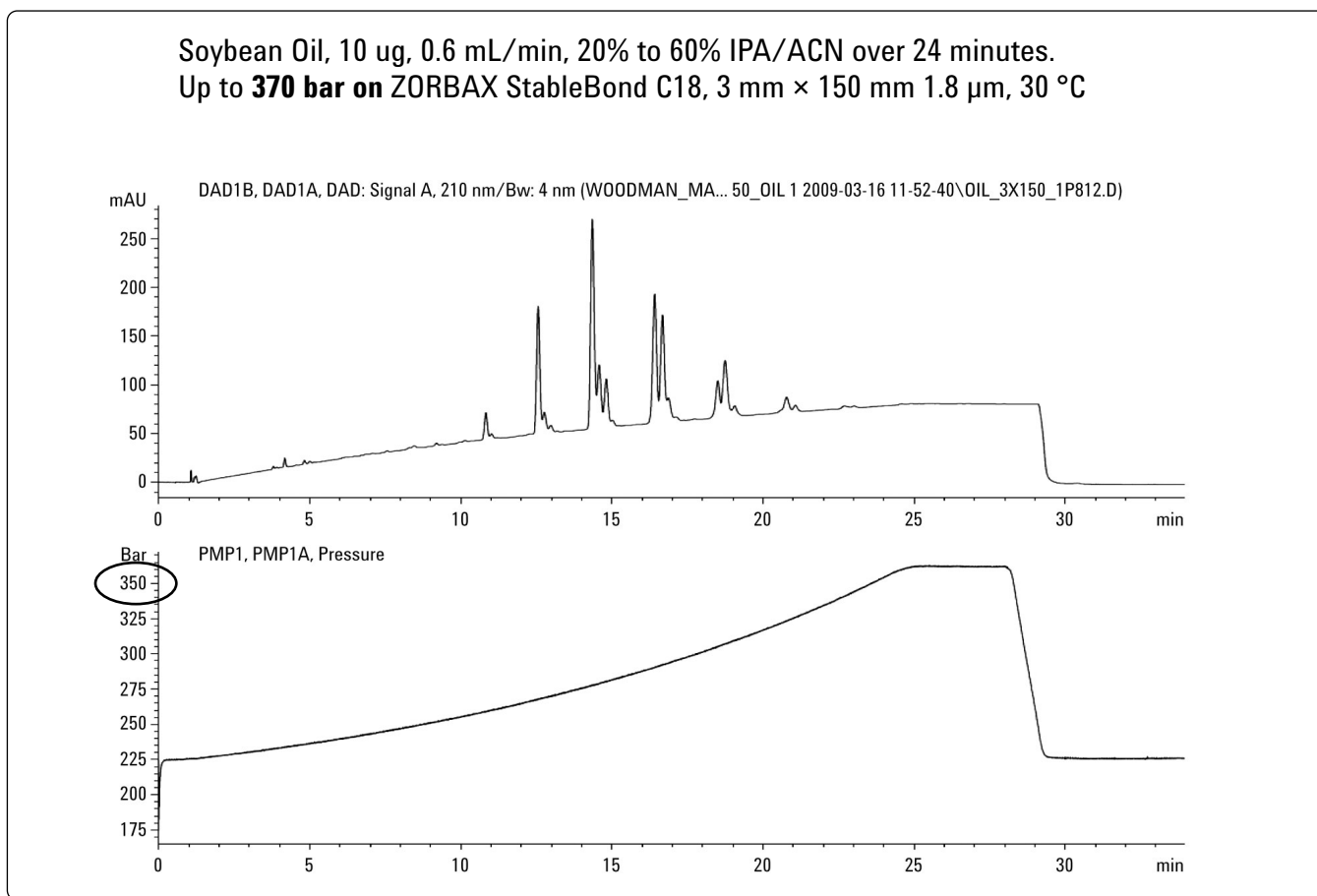


Figure 2. A 210-nm UV chromatogram of soybean oil sample on a 3 mm × 150 mm ZORBAX Rapid Resolution High Throughput (RRHT), 1.8 µm column, upper panel. System pressure trace showing the general progress of the gradient elution, lower panel. Flow rate 0.6 mL/min, gradient time 24 min. Strong solvent, isopropanol. The chromatogram demonstrates the typical difficulty encountered with this type of separation, which is small clusters of chromatographically similar triglycerides. These clusters are not positional isomers of the same carbon number and degree of unsaturation, rather a mixture of various chain lengths and number of double bonds as shown by mass spectrometric evaluation.

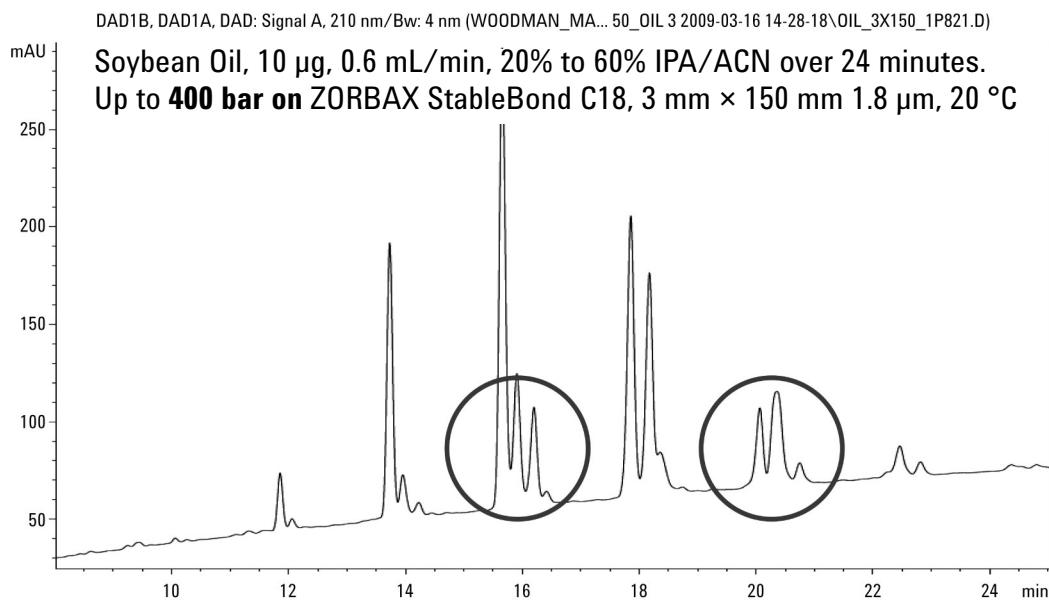
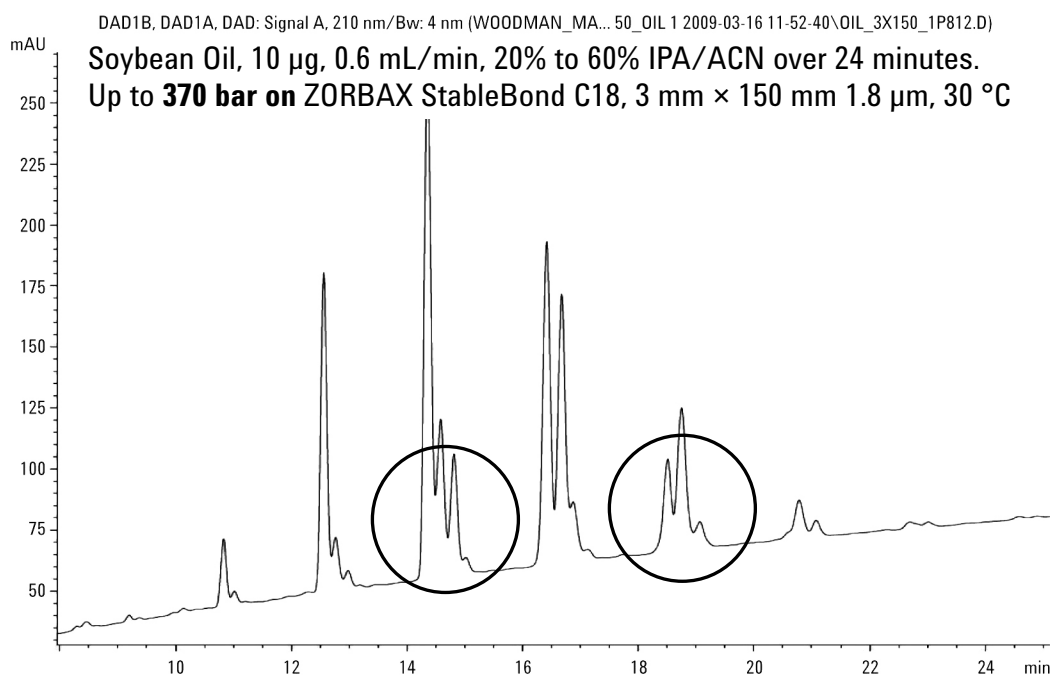


Figure 3. An expanded presentation of the chromatogram shown in Figure 2 at 30 °C, upper panel, compared with the same conditions in Figure 2 operating the column at 20 °C.

In Figure 3 we see the improvement achieved by operating the separation at 20 °C rather than 30 °C. The operating pressure increase is approximately 10% at the lower temperature. While many of our separations have been performed at 30 °C

as a compromise between separation and operating pressure, the availability of the Agilent 1290 Infinity LC System with increased operating pressure capability has allowed us to reduce the temperature to 20 °C and demonstrate a usable improvement in separation.

Soybean Oil, 10 µg, 0.29 mL/min, 20% to 60% IPA/ACN over 27 minutes.
Up to **760 bar** on ZORBAX StableBond C18, 2.1 mm × 250 mm 1.8 µm, 20 °C

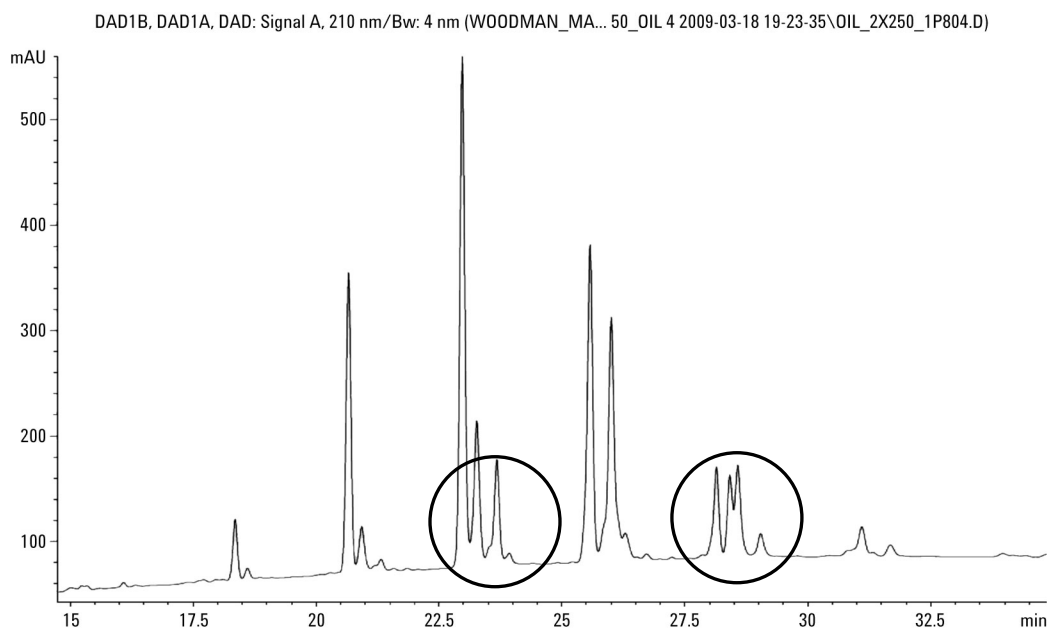


Figure 4. Analysis of the soybean oil sample on an Agilent ZORBAX StableBond C18 column, 2.1 mm × 250 mm, 1.8 µm, (150 mm in series with 100 mm) prepared for operation at 1200 bar pressure limit. Flow rate 0.29 mL/min, gradient time 27 min. Maximum observed pressure 760 bar.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution, it would be practical to explore longer columns or explore alternative mobile phase or column chemistries. As with most very high performance separations, rate-limiting features tend to include operating pressure, operating temperature, and maximum flow rate. The triglyceride separations evaluated thus far have not been receptive to operation at higher column temperatures or higher flow rates, presumably because of their

relatively high molecular weight and flexible organic structure. Even when gradient slope translations are carefully made to ensure organic strength consistency from method to method, operating at higher flow rates has consistently shown degradation of the overall separation. Because the isopropanol has significantly high viscosity and high pressure, it seemed appropriate to consider other non-polar solvents that are miscible with acetonitrile and friendly to low UV detection, as a substitute for isopropanol.

Soybean Oil, 10 µg, 0.29 mL/min, 10% to 40% IPA/ACN over 27 minutes.
Up to **440 bar** on ZORBAX StableBond C18, 2.1 mm × 250 mm 1.8 µm, 20 °C

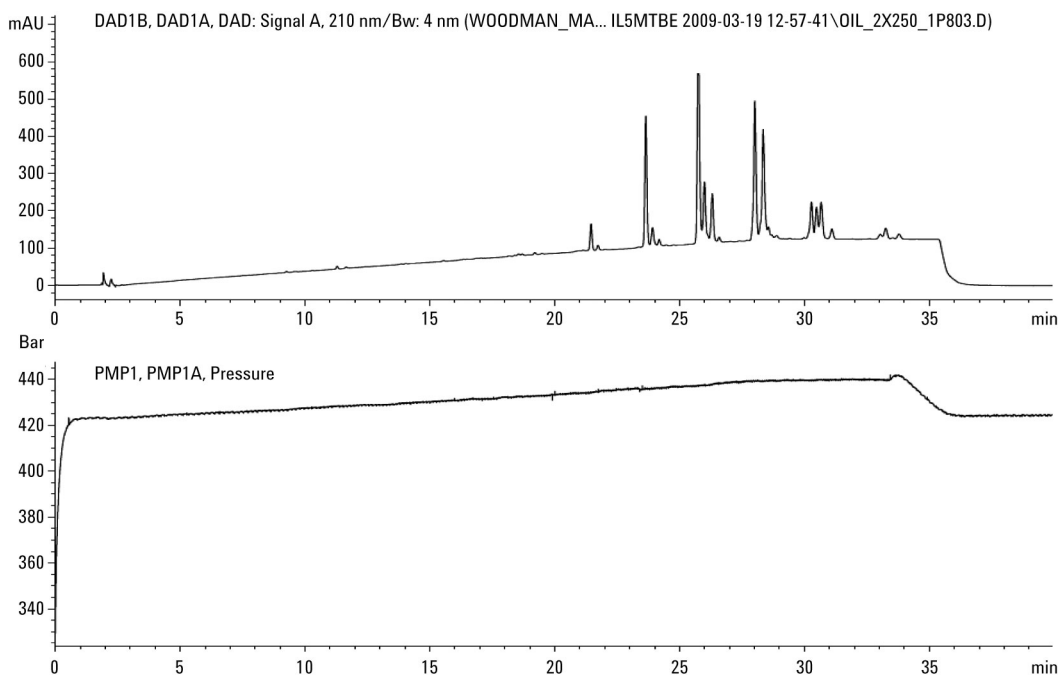


Figure 5. By substituting MTBE for isopropanol with otherwise the same conditions as Figure 4, and then re-optimizing the gradient for the significant increase in eluting strength of MTBE, we arrive at a new set of operating conditions where there is only a small difference in operating pressure over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

Soybean Oil, 30 µg, 0.29 mL/min, 10% to 40% MTBE/ACN over 43 minutes.
Up to **730 bar** on ZORBAX StableBond C18, 2.1 mm × 400 mm, 1.8 µm, 20 °C

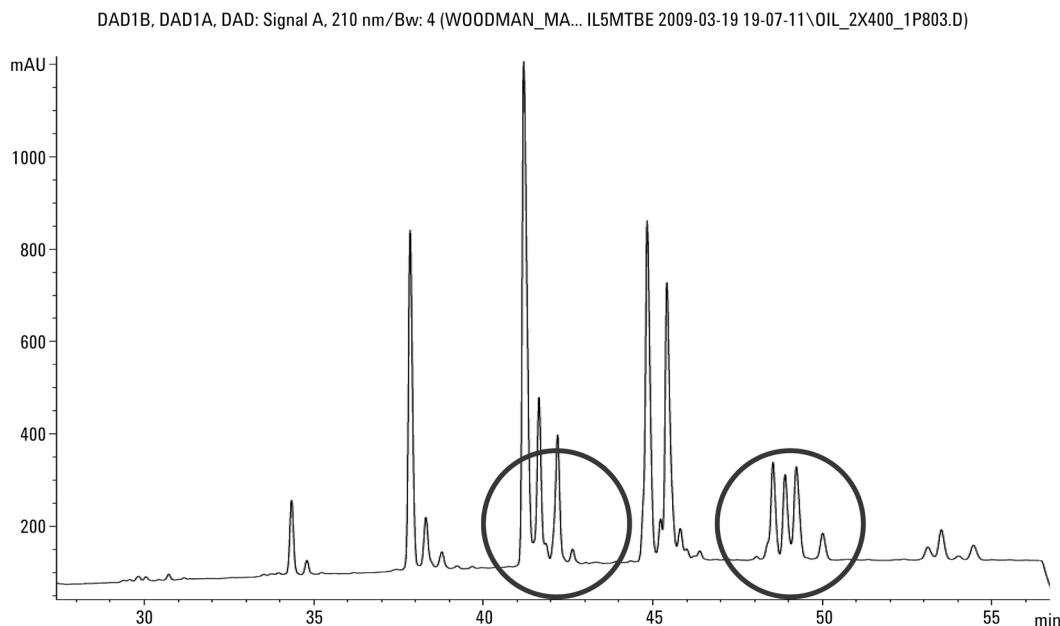


Figure 6. Separation of the soybean oil sample on a 2.1 mm × 400 mm ZORBAX StableBond C18, 1.8 µm 1200 bar columns (150 mm + 150 mm + 100 mm in series). Flow rate 0.29 mL/min gradient time 43 min, for a gradient of 10% to 40% MTBE. Maximum operating pressure 730 bar at 20 °C.

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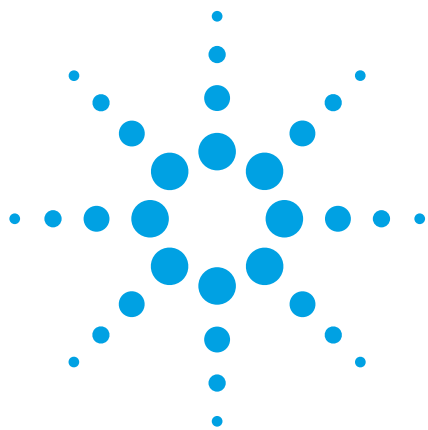
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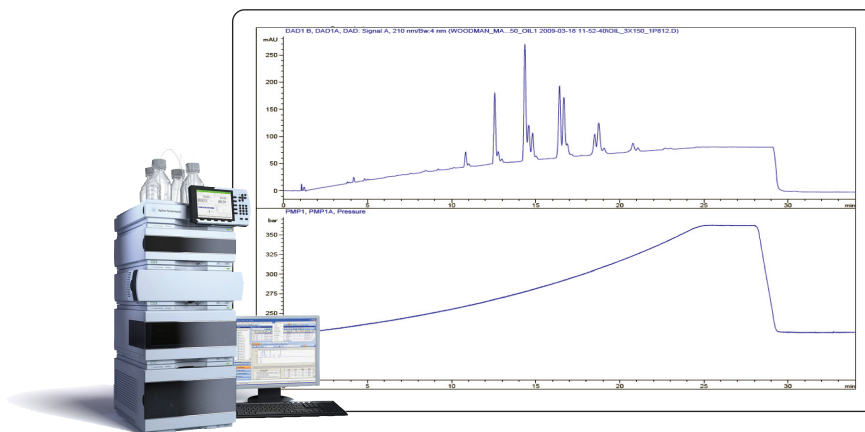
High resolution of complex lipids (triglycerides) using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 μm columns

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 $\times$ 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.

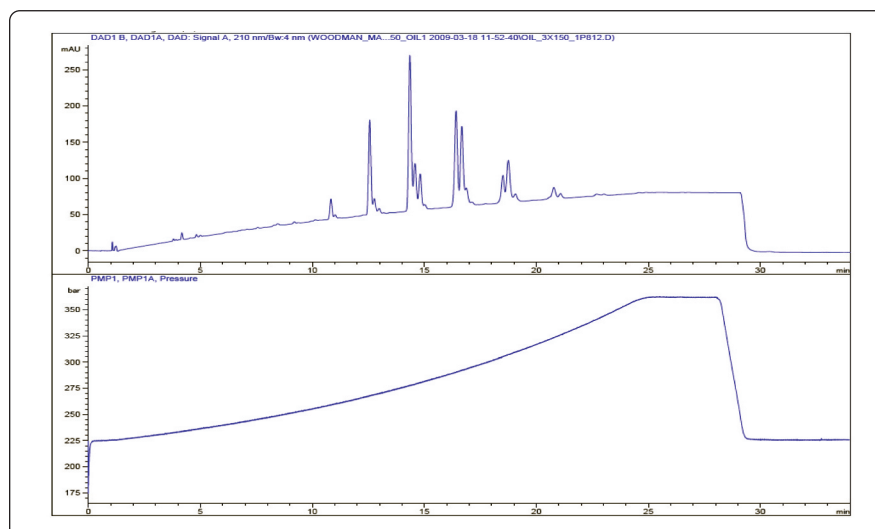


Figure 1

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.

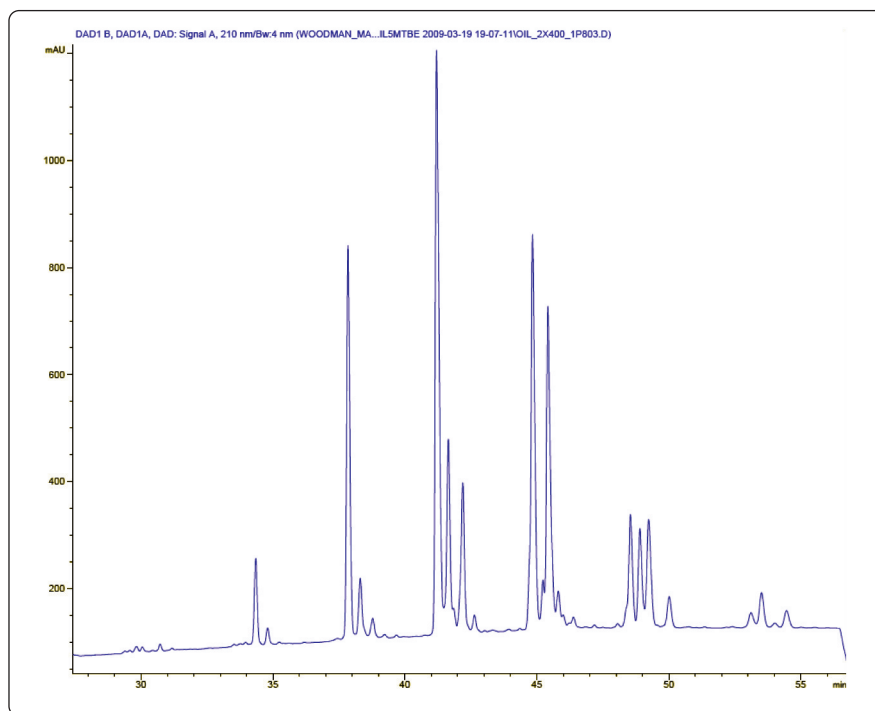


Figure 2

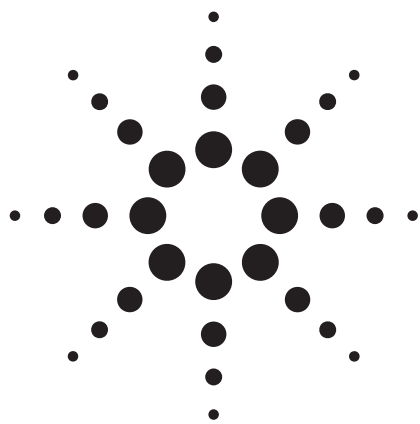
Analysis of soybean triglycerides on the 1290 Infinity LC. Sample: soybean oil, 10 mg/mL, 30 µg on column. Conditions: 0.29 mL/min, 10% to 40% MTBE vs. ACN at 42 minutes, hold to 55 minutes, run 60 minutes, 210 nm UV. ZORBAX RRHD StableBond C18, 2.1 mm × 400 mm (2–150 and 1–100 mm length in series),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

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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.



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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.

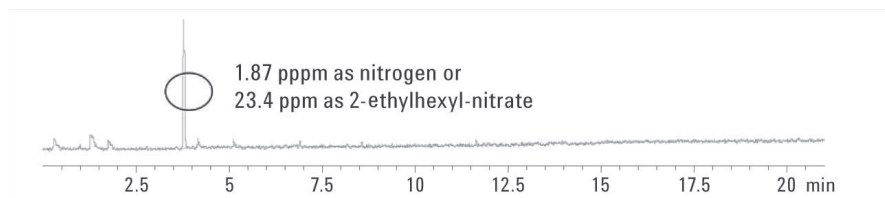


Figure1. Standard sample: 2-EHN at a concentration of 23.4 ppm in diesel (nitrogen free).

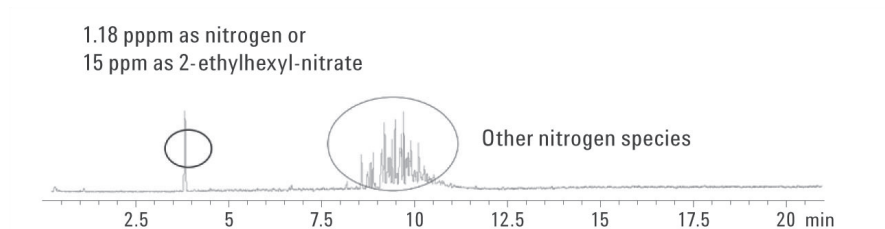


Figure 2. Nitrogen species in pump diesel fuel.

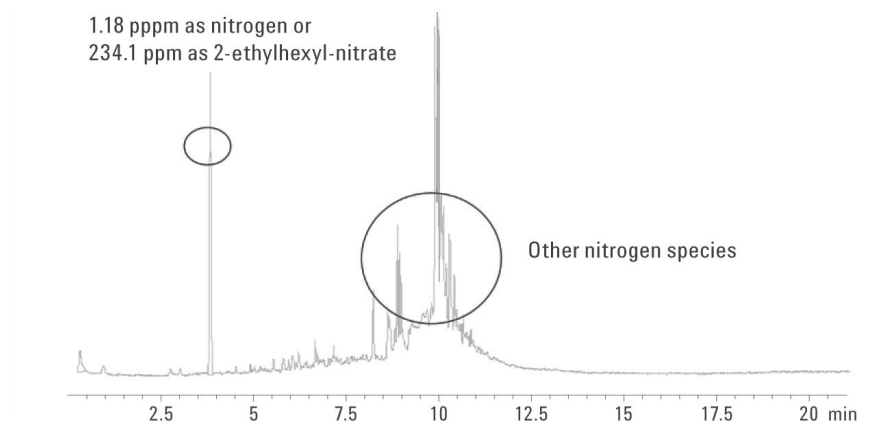


Figure 3. Nitrogen species in B20 biodiesel.

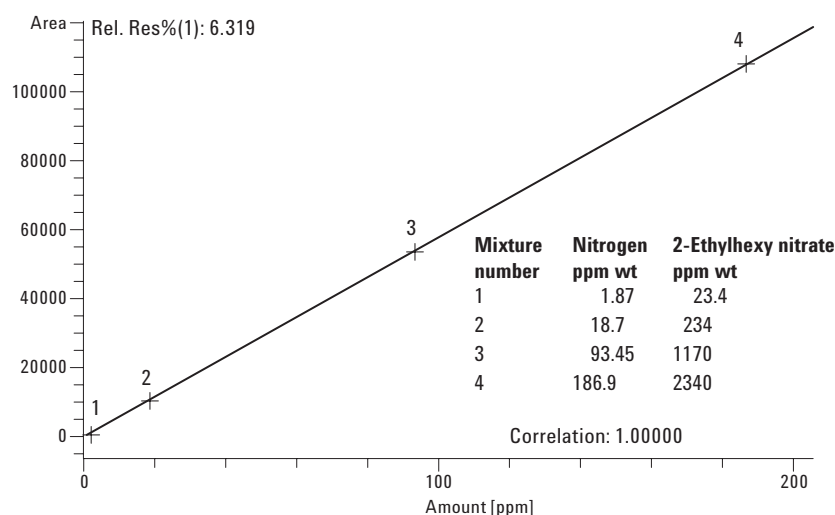


Figure 4. 2-ethylhexyl nitrate calibration.

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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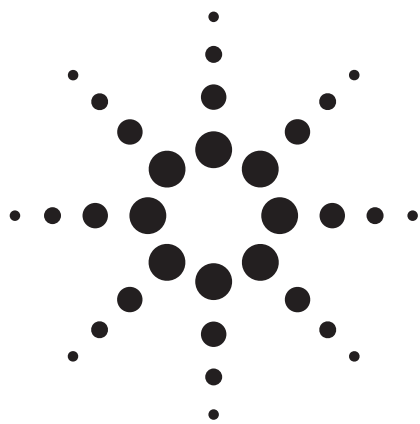
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Analysis of Denatured Fuel Ethanol using ASTM Method D5501-09

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 C₅ to C₁₁ 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.

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 C₅ and C₁₁, which

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

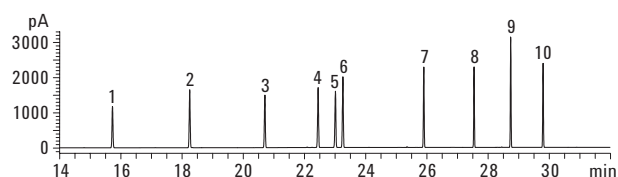
Standard Agilent 7890A GC System Hardware	
G3440A	Agilent 7890A Series GC System
Option 113	150 psi Split/Splitless Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693 Automatic Liquid Sampler
GC Capillary Column	
Analytical Column	PDMS, 150 m × 0.25 mm id × 1.0 µm film
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

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.

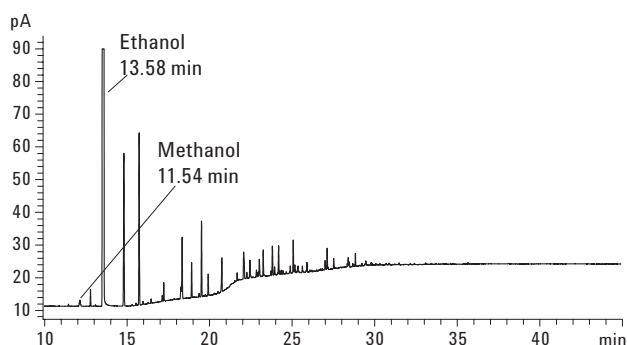


Figure 2. Analysis of a commercial denatured fuel ethanol sample using ASTM method D5501-09.

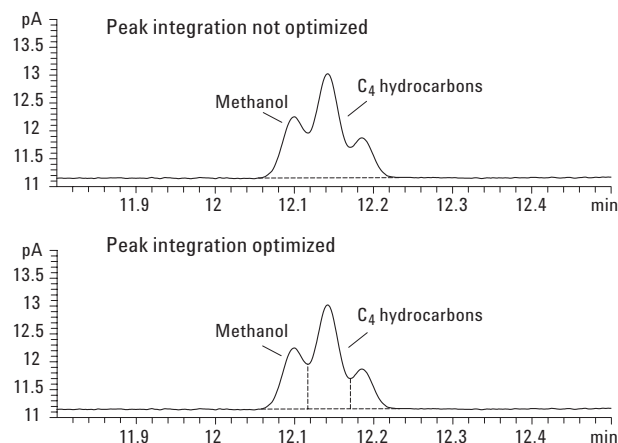


Figure 3. Optimizing the methanol peak integration is important for obtaining correct results.

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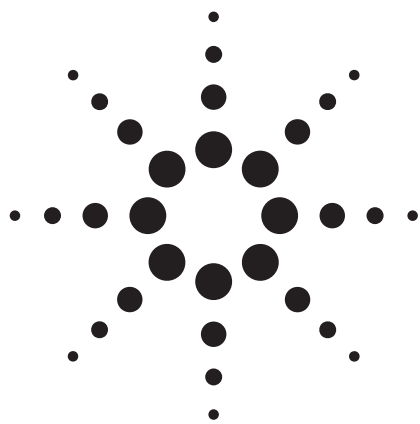
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Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance

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- μ L syringe (p/n G4513-60561). The front injector used a standard syringe carriage containing a 100- μ L 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 μ L solvent + 100 μ L sample and the second used half these amounts: 450 μ L solvent + 50 μ L 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 μ L C_{13} to a 1 mL volumetric and diluting to mark.*

The first sequence prepared the 1 mL sample (900 μ L + 100 μ L) by adding 900 μ L 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 μ L of Sample (Front) to vial #2
2	Mix vial #2 at 1500 RPM for 0 min 5 sec
3	Add 100 μ L of vial #2 to vial #3
4	Mix vial #3 at 1500 RPM for 0 min 5 sec
5	Add 100 μ L of vial #3 to vial #4
6	Mix vial #4 at 1500 RPM for 0 min 5 sec
7	Add 100 μ L of vial #4 to vial #5
8	Mix vial #5 at 1500 RPM for 0 min 5 sec
9	Add 100 μ L of vial #5 to vial #6
10	Mix vial #6 at 1500 RPM for 0 min 5 sec
11	Add 100 μ L of vial #6 to vial #7
12	Mix vial #7 at 1500 RPM for 0 min 5 sec
13	Add 100 μ L of vial #7 to vial #8
14	Mix vial #8 at 1500 RPM for 0 min 5 sec
15	Add 100 μ L 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 μ L, 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 μ L. The volume increase of the last sample was 100 μ L or 50 μ L 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

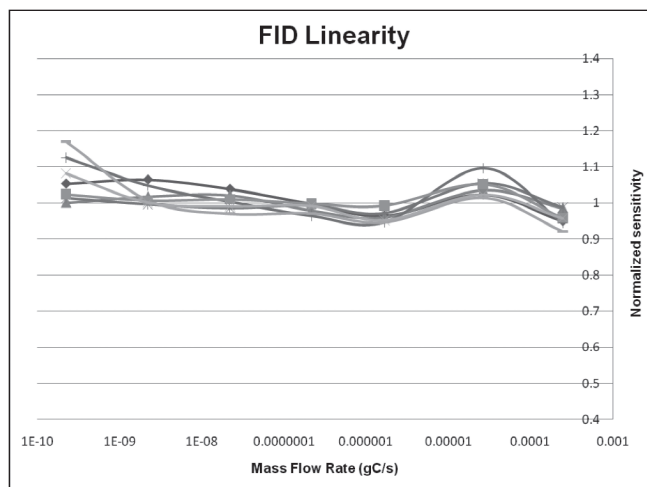


Figure 1. Linearity Plots for all eight runs overlaid.

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 μ L 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 (μ L)					50			
Draw speed (μ L/min)					1250	1250		
Dispense speed (μ L/min)					3000	3000		
Needle depth offset (mm)					0	0		
Viscosity delay(s)					2	2		
Turret solvent								
Air gap (% syr.vol.)						0		

100 μ L 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 (μ L)		10		20	10			
Draw speed (μ L/min)		300		300	300	300		
Dispense speed (μ L/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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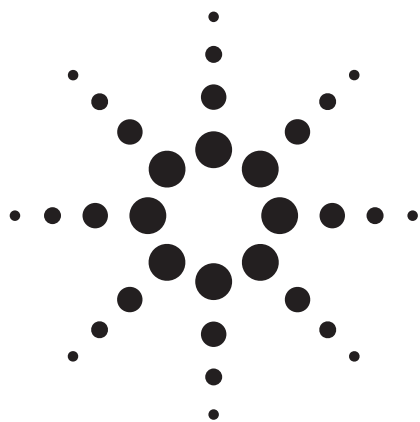
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Agilent Technologies



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.



Agilent Technologies

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 affects 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 degradation 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 Milipore water ($\text{H}_2\text{O}/\text{NaCl}$). 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 $\times$ 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.

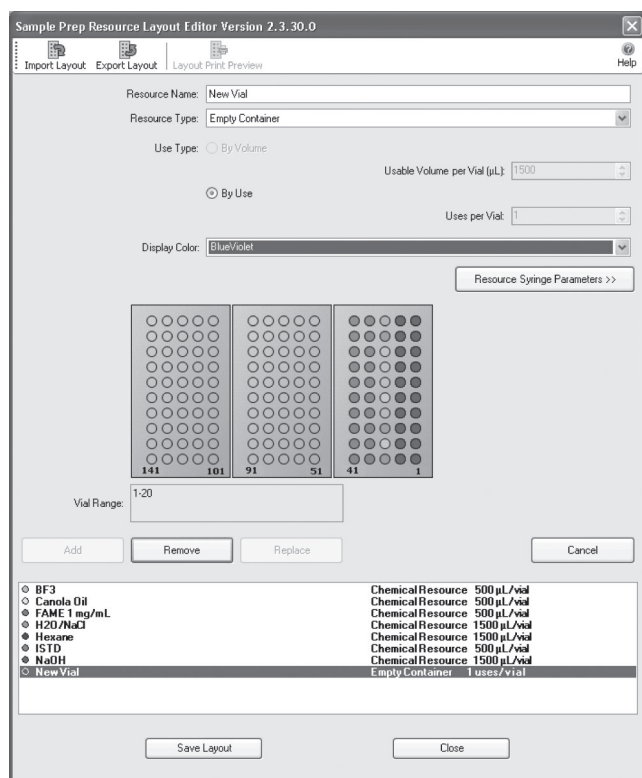


Figure 2. The Resource Editor allows users to allocate chemical resources and empty vials to be used during the sample preparation.

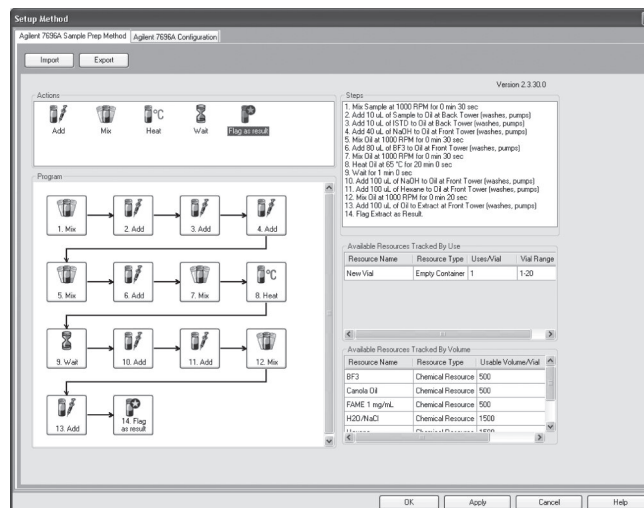


Figure 3. SamplePrep Method Editor features drag and drop icons for easy step-wise programming. The steps used for the acid-catalyzed reaction are shown here.

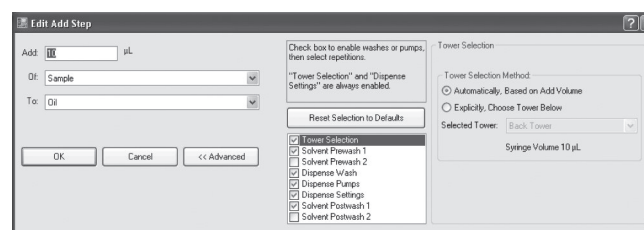


Figure 4. Each sample prep step has advanced parameters, like the Add Step shown here, that allows the user to fully customize the operation.

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 μ L of sample (either the free FA sample in hexane or oil/surrogate standard solution) and 10 μ L of the internal standard were added. To the sample, 40 μ L of NaOH was added and the mixture was vortexed at 1000 rpm for 30 sec. After saponification, 80 μ L of BF₃ 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 μ L of H₂O/NaCl and 100 μ L 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 μ L) 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 μ L of sample (oil/internal standard solution) was added. To methylate the FAs and extract the newly formed FAMES 100 μ L of NaOH and 500 μ L of hexane was added and the mixture was vortexed at 1000 rpm for 30 sec. After waiting 2 minutes, the top layer (100 μ L) 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 significantly 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	—

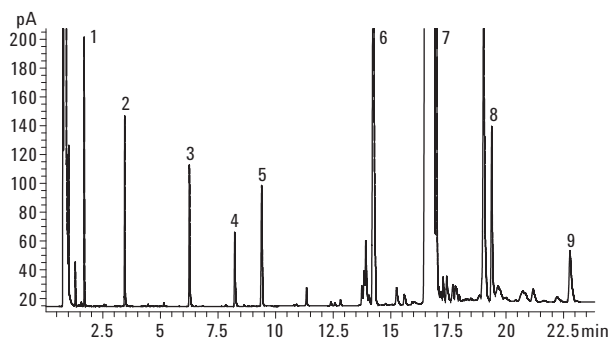


Figure 5. A typical chromatogram of a canola oil sample after automated acid-catalyzed sample preparation and analysis by GC. Compounds identified and quantified were 1) decane, 2) dodecane 3) tetradecane, 4) methyl laurate, 5) hexadecane, 6) methyl palmitate, 7) methyl stearate, 8) methyl eicosanoate, 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 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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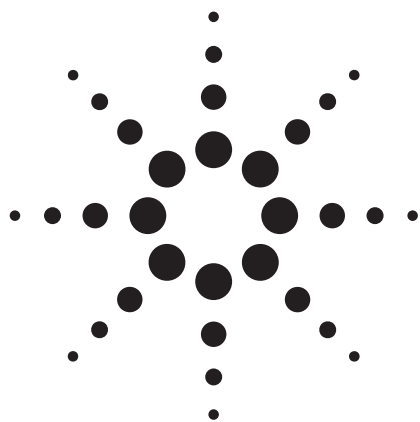
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Agilent Technologies



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 iso-octane 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.

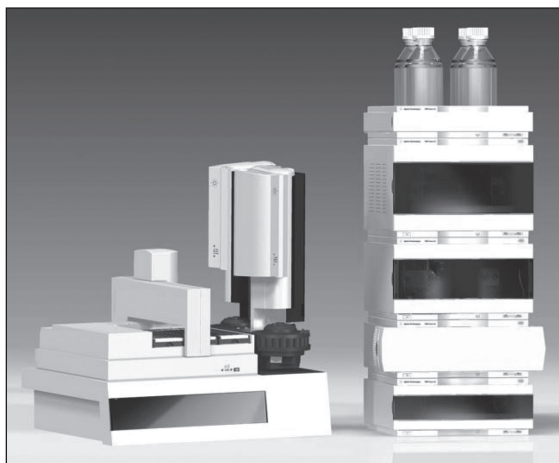


Figure 3. The Agilent 7696 Sample Prep WorkBench with a liquid chromatograph.

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 $^{\circ}\text{C}$ 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).

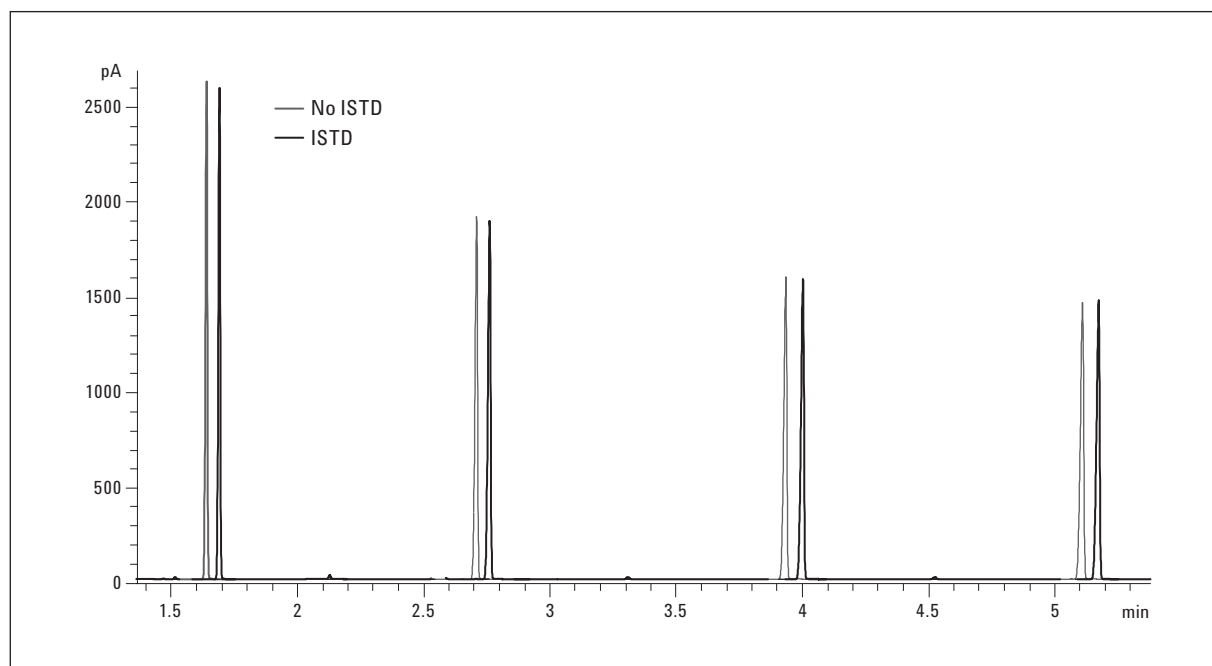


Figure 4. GC chromatograms (slightly offset) are shown for a standard solution dispensed and diluted with and without an ISTD added. No difference in peak areas are observed.

For the 10 samples diluted for LC analysis, similar results were obtained. Dispensing all three volumes with a 250 μ L 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 con-

trast, 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%.

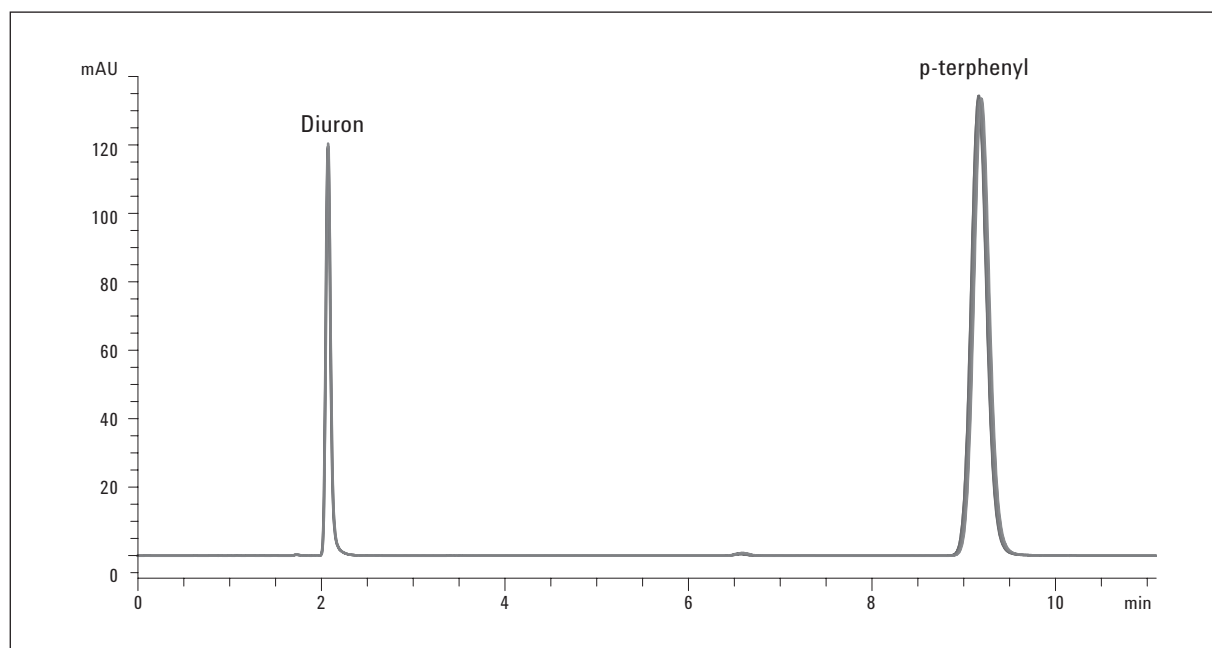


Figure 5. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

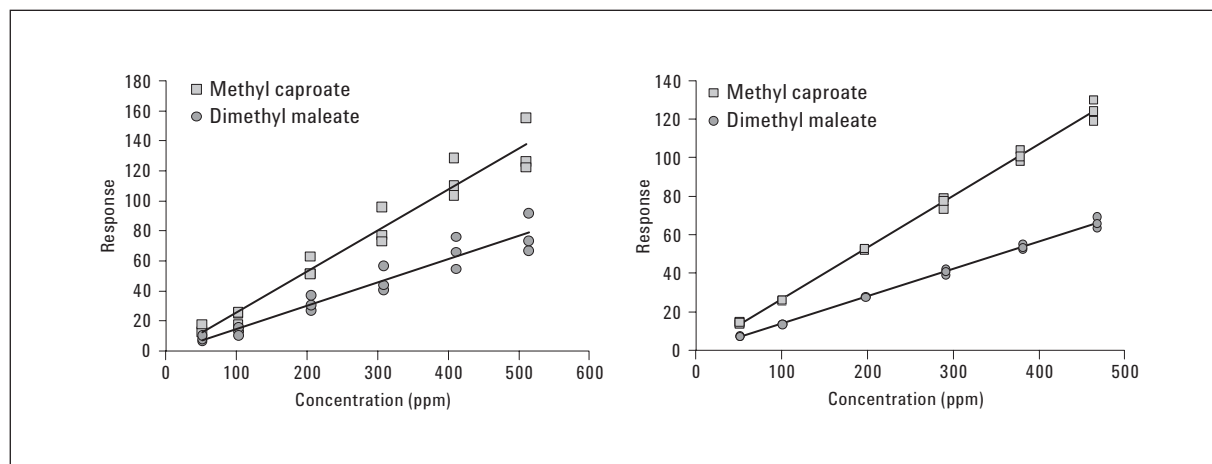


Figure 6. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with the Agilent 7696 Sample Prep WorkBench, are visibly more reproducible than the curves made manually on the left.

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 μ L of solvent was used, excluding the wash vials. This can result in substantial cost savings for laboratories. Additionally, calibration 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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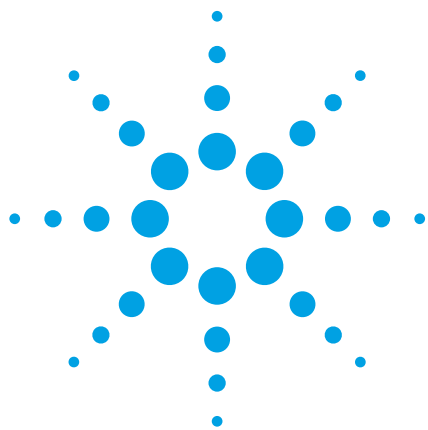
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Analysis of Sugars in Glycosylated Woody Biomass with the Agilent 1200 Series LC System

Application Note

Biofuels and Alternative Energy

Author

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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.



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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.

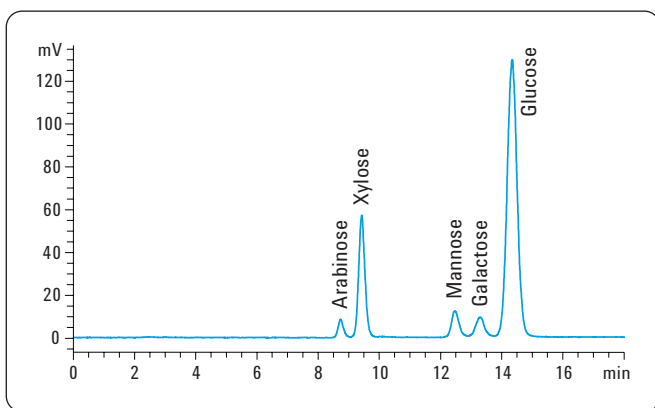


Figure 1
Chromatogram of reference solutions (1000 ng/µL each).

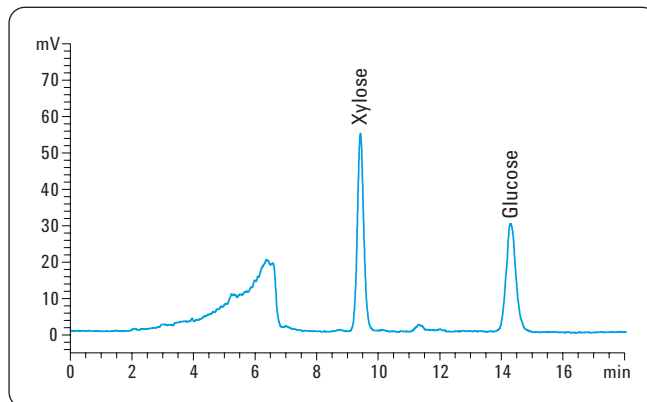


Figure 2
Bagasse, hydrothermal 180 °C 5 min.

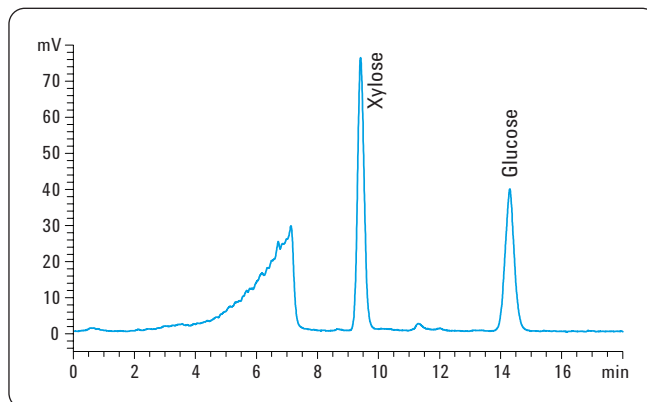


Figure 3
Bagasse, hydrothermal 160 °C 15 min.

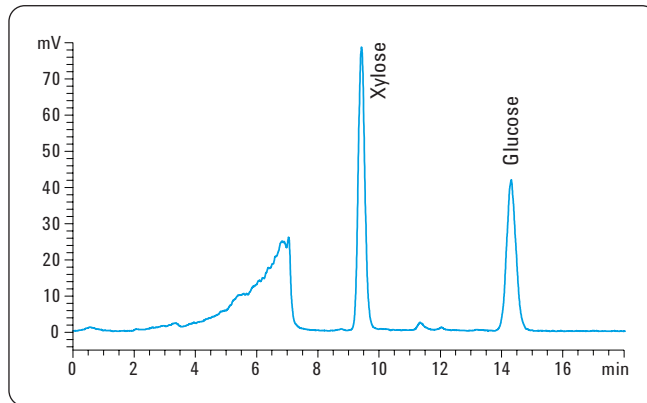


Figure 4
Bagasse, hydrothermal 160 °C 30 min, w/phosphoric acid.

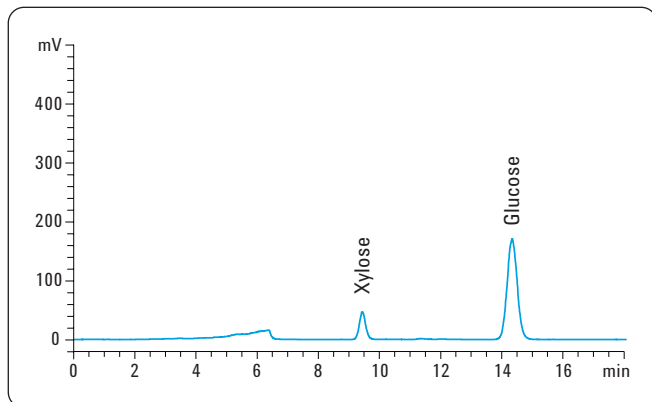


Figure 5
Bagasse, ball mill.

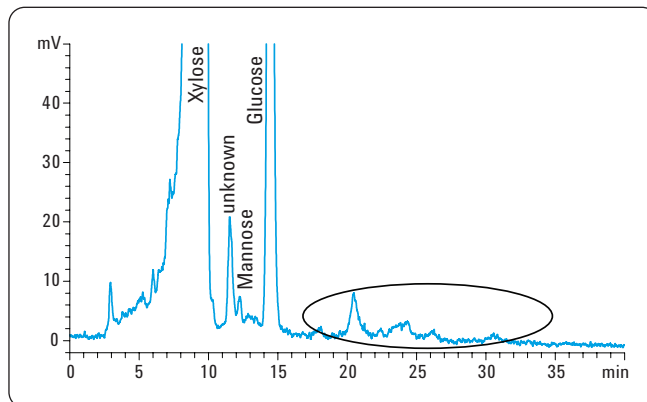


Figure 7
Bagasse, hydrothermal 180 °C 5 min (2-fold dilution).

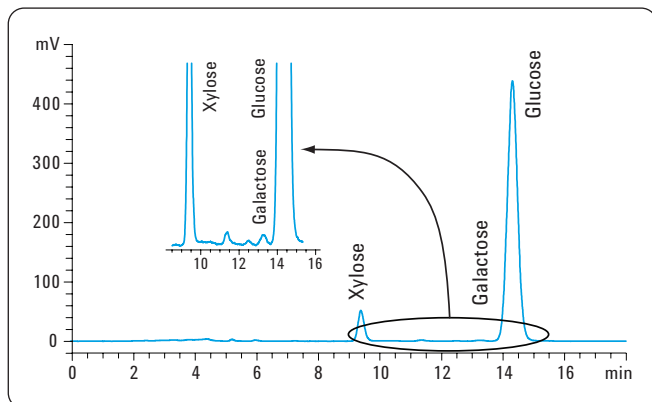


Figure 6
Eucalyptus, ball mill.

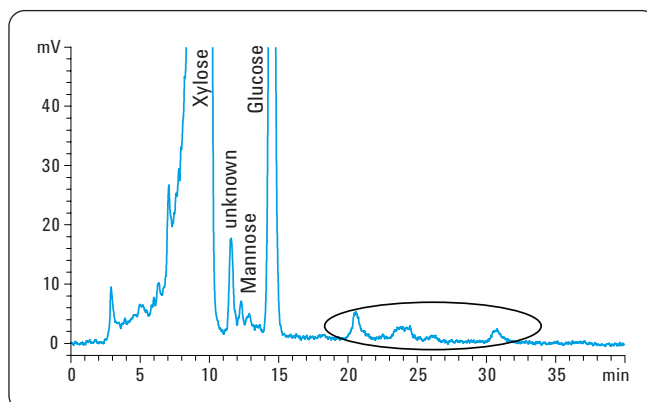


Figure 8
Bagasse, hydrothermal 160 °C 15 min (2-fold dilution).

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.

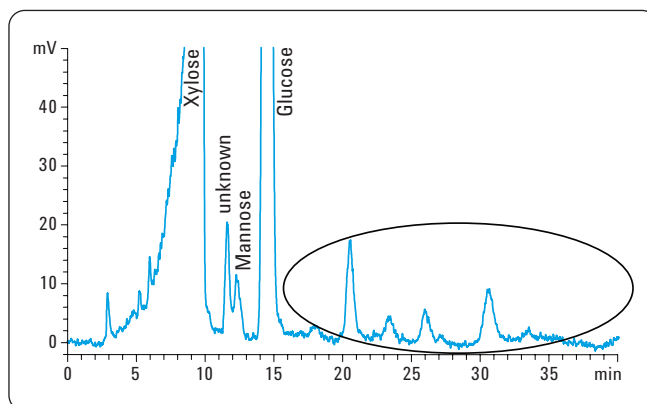


Figure 9
Bagasse, ball mill (2-fold dilution).

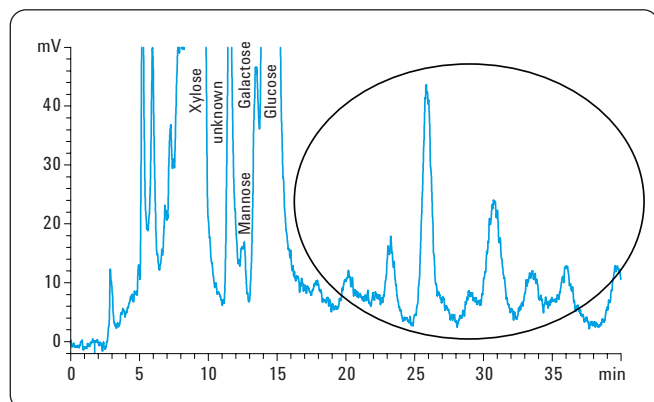


Figure 10
Eucalyptus, ball mill (2-fold dilution).

Conclusion

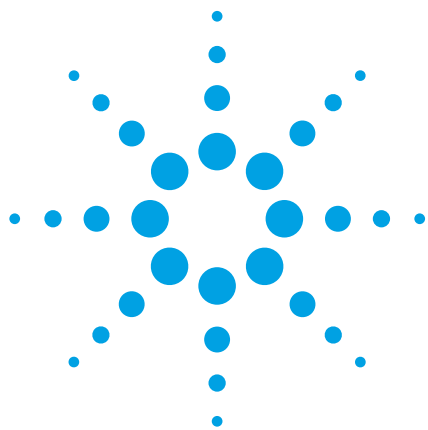
Sugars in glycosylated woody biomass are mainly xylose and glucose, but the concentration depends on the pre-treatment process. The Agilent 1200 Series LC system with the evaporative light scattering detector is suitable for sugar analysis in glycosylated woody biomass due to good sensitivity and good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

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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.



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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.

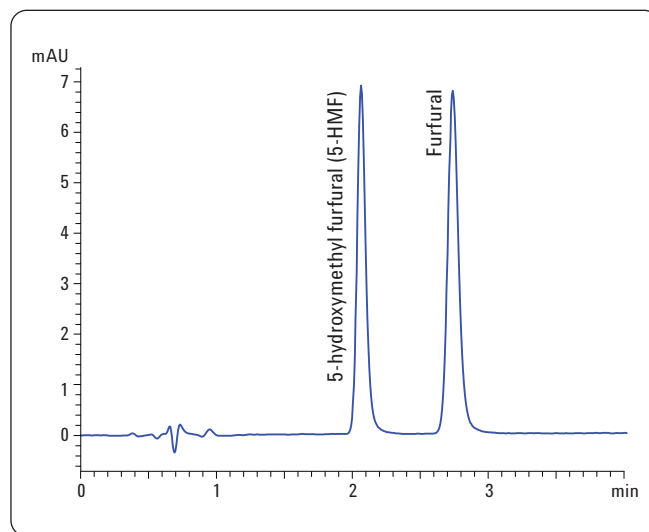


Figure 1
Chromatogram of Reference Solutions (1 mg/mL each).

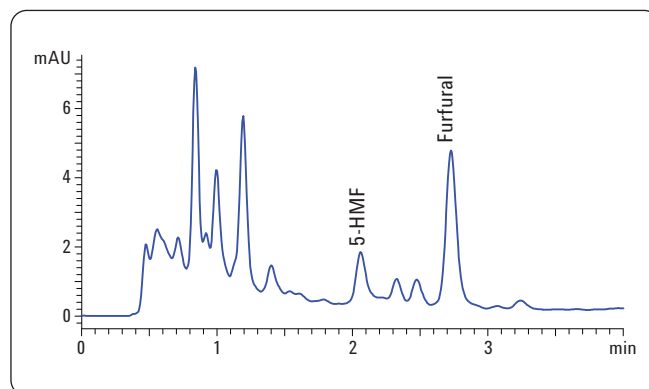


Figure 2
Eucalyptus, ball mill.

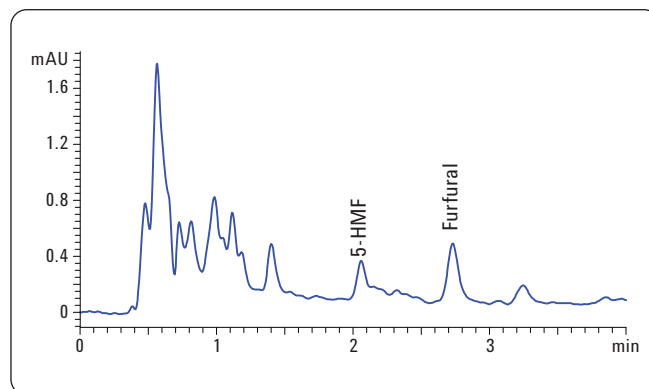


Figure 3
Bagasse, ball mill.

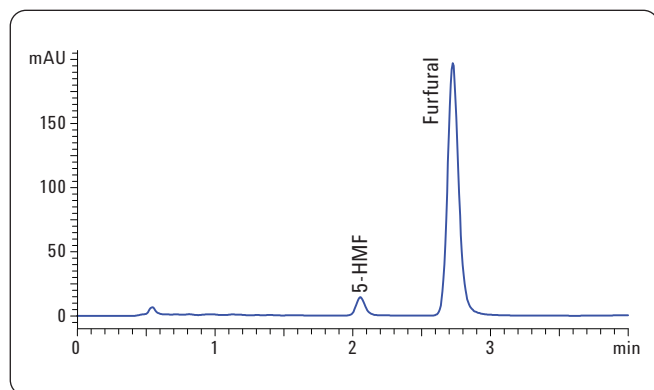


Figure 4
Bagasse, hydrothermal, 180 °C 5 min.

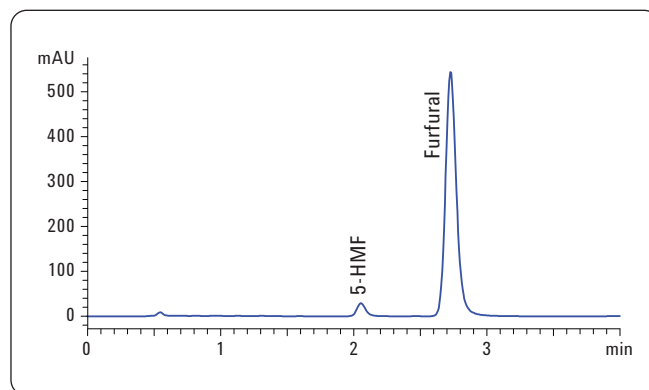


Figure 6
Bagasse, hydrothermal, 180 °C 30 min.

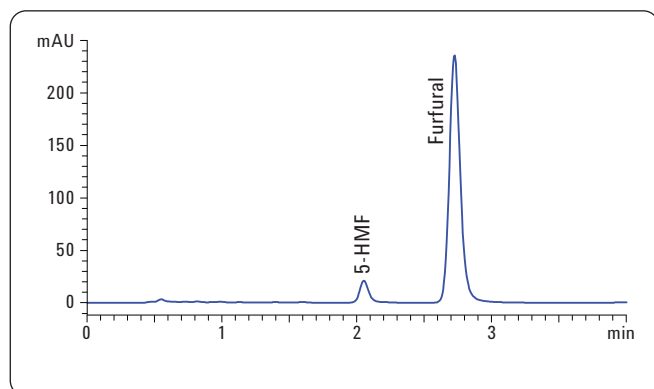


Figure 5
Bagasse, hydrothermal, 160 °C 15 min.

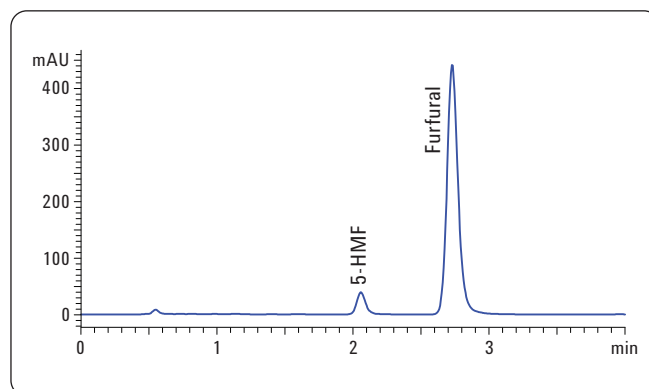


Figure 7
Bagasse, hydrothermal, 160 °C 30 min w/phosphoric acid.

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

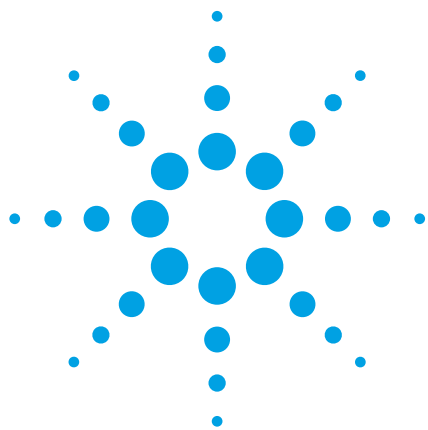
Table 1
Furfural assay results for samples (wood sugar solutions).

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

Application Note

Biofuels and Alternative Energy

Author

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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 triolein for TG concentration.



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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 (1 µg/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

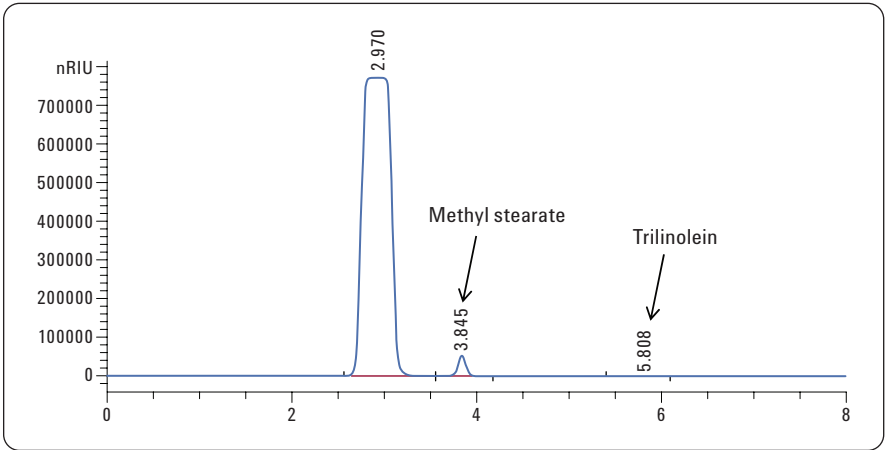


Figure 1
Exemplary analysis of reference compounds (methyl stearate: 100 µg/10 µL; trilinolein: 1 µg/10 µL).

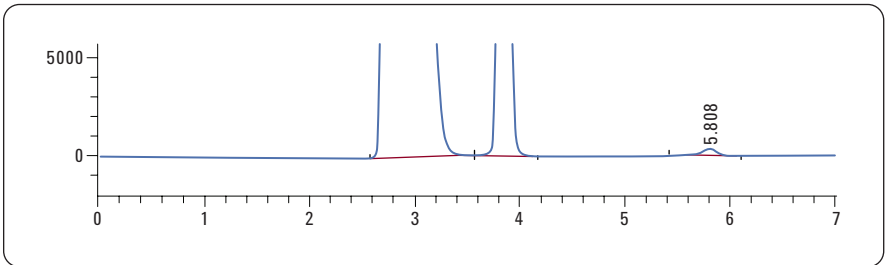


Figure 2
Enlarged Figure 1

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.

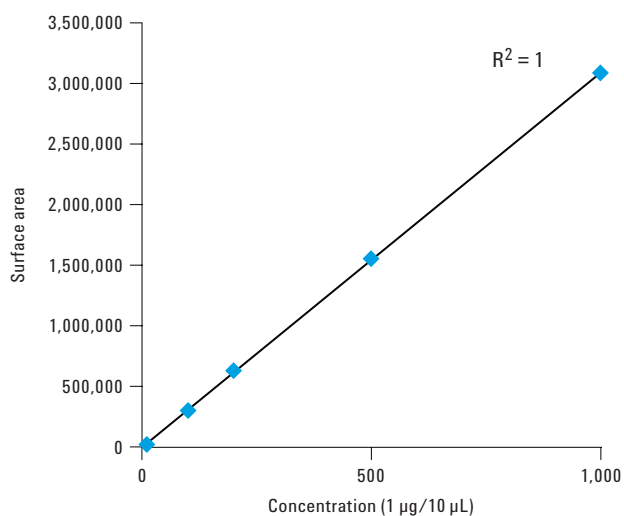


Figure 3
Methyl stearate calibration curve.

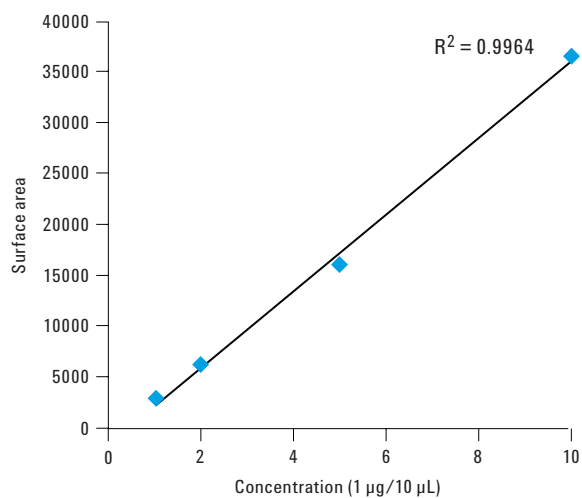


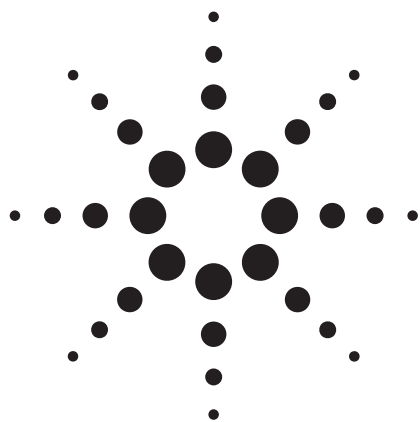
Figure 4
Trilinolein calibration curve.

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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 trimethylsilylation 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 volumet-

rically 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 tricaprln in pyridine)	52	100	250	500
MSTFA derivatization reagent	53	100	250	500
n-Heptane	101–110	250	500	2000

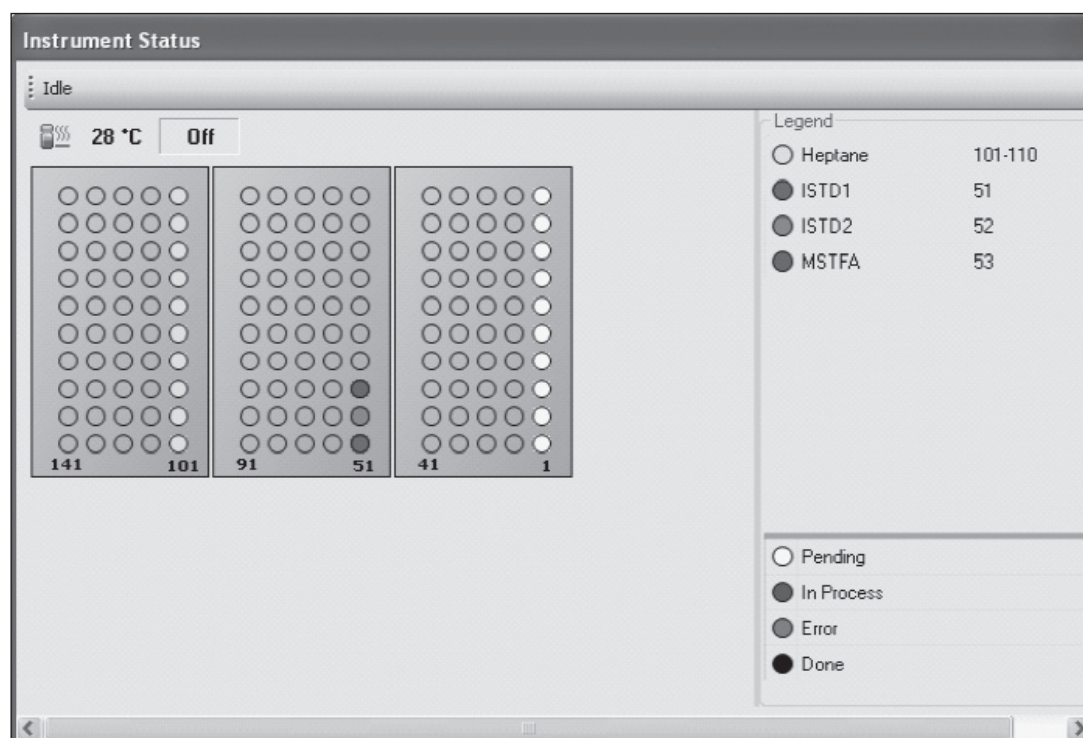


Figure 1. The WorkBench software provides an overhead view of each chemical resource in the sample trays. For this example, in addition to the chemical resources, 10 samples were placed in tray positions 1 to 10.

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

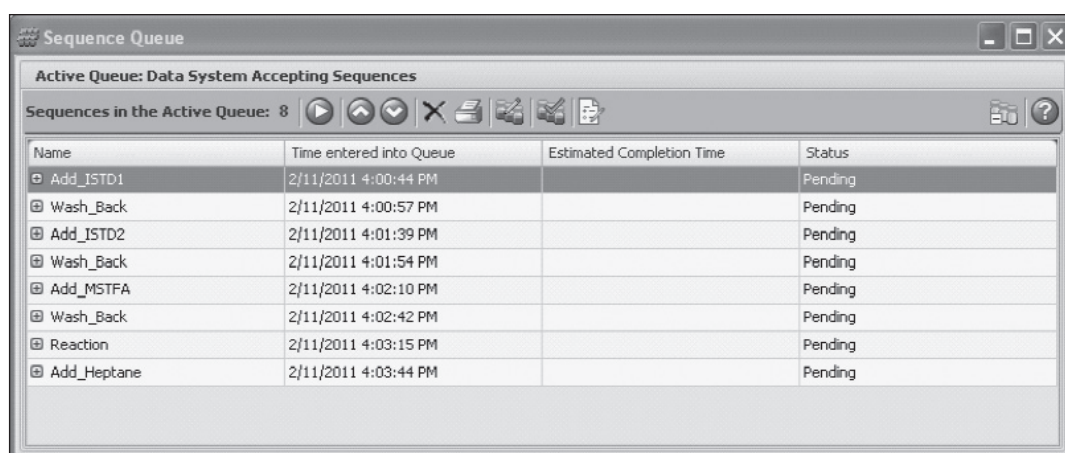


Figure 2. The WorkBench Sequence Queue is used to run the WorkBench methods described in Table 3.

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.

Multuser 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

Compound	Manual Prep			WorkBench		
	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
Triolein	0.8303	-0.0018	0.9965	0.8703	0.0030	1.0000

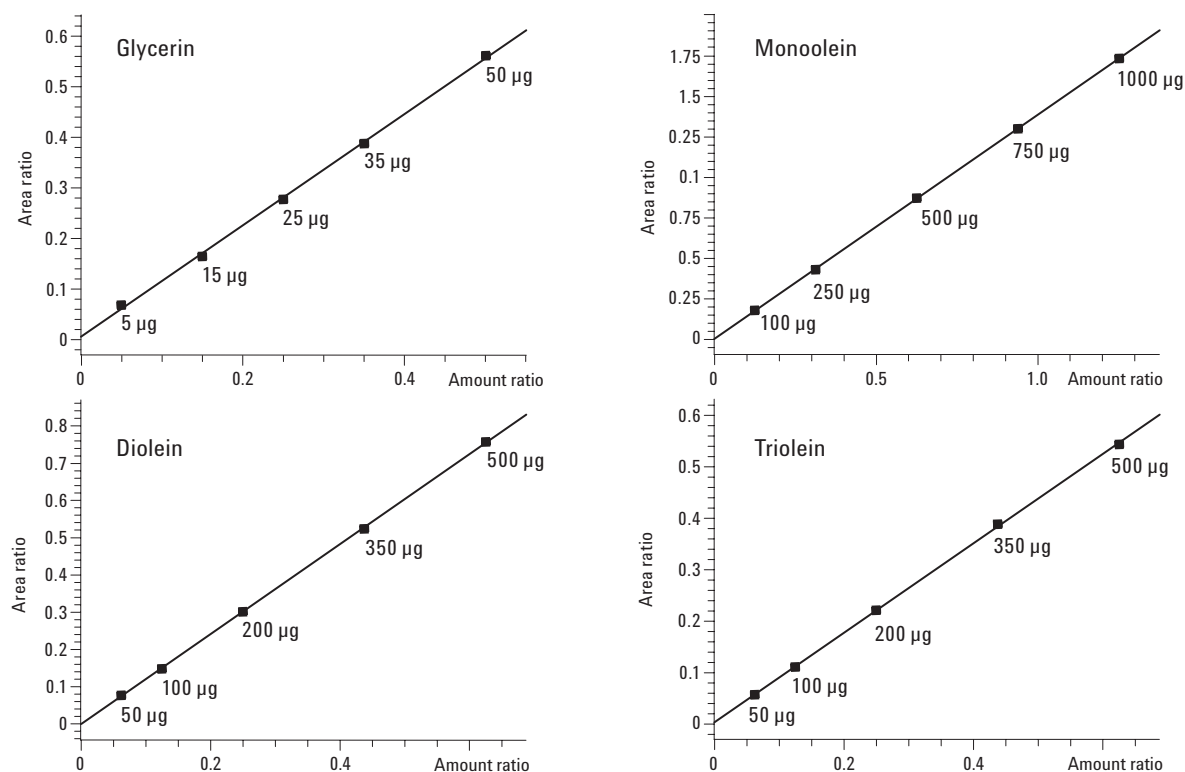


Figure 3. Calibration curves from standards prepared using the WorkBench.

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.

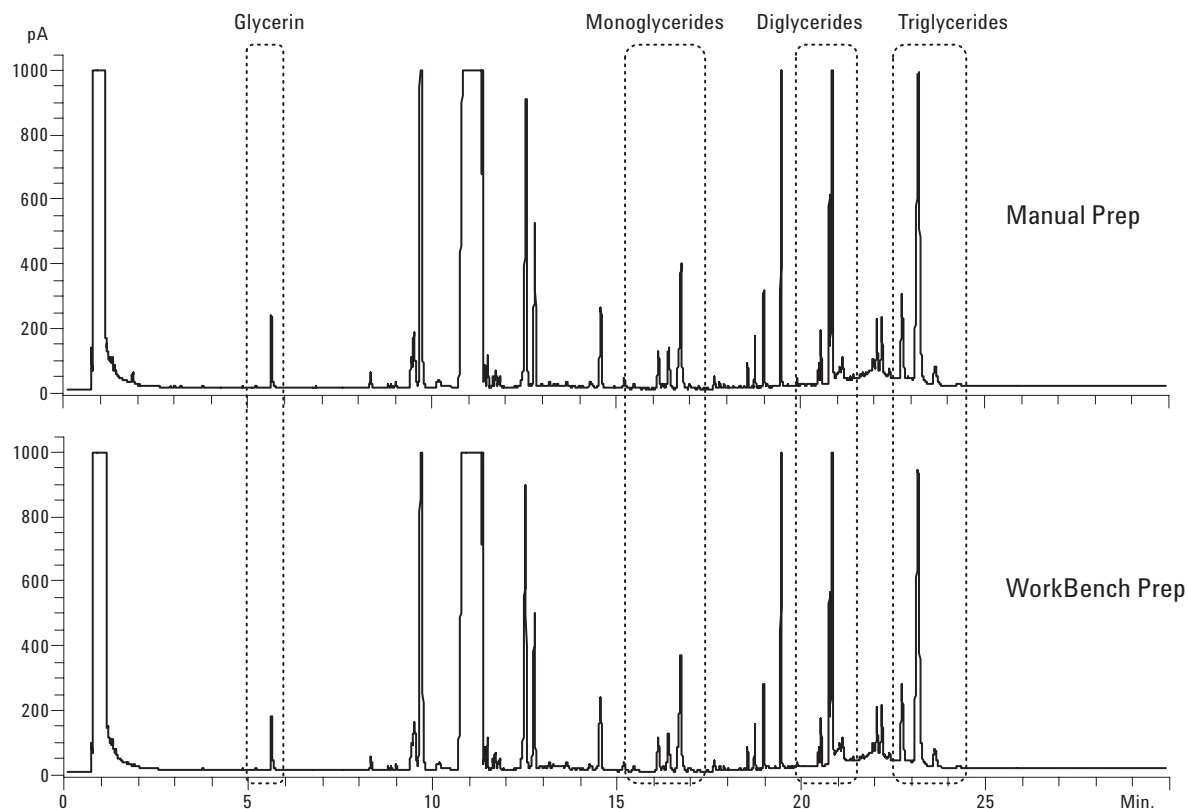


Figure 4. A comparison of data from a canola biodiesel sample prepared manually and using the Agilent WorkBench. These chromatograms show remarkable similarity in the four regions where glycerin, monoglycerides, diglycerides and triglycerides are separated.

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.

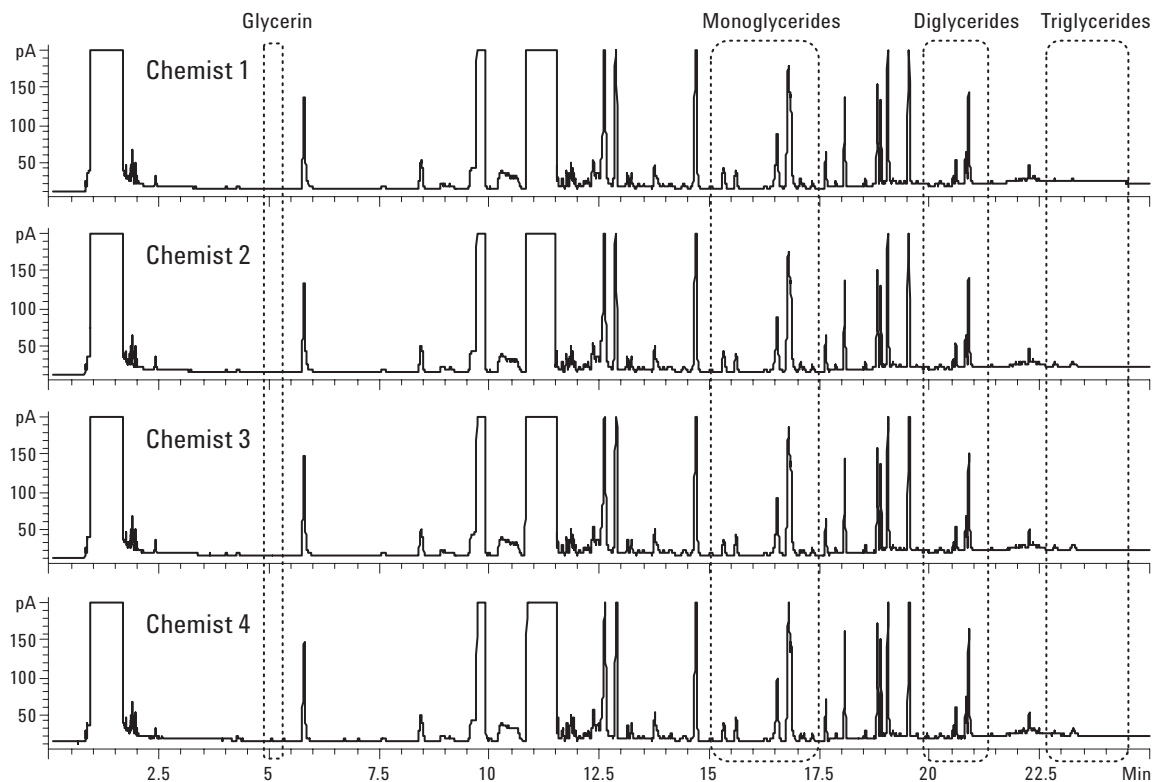


Figure 5. A comparison of data from a soybean biodiesel sample prepared by four different chemists working on four different days. Each chemist developed a WorkBench sample preparation protocol and then analyzed the samples using ASTM method D6584. The results are nearly identical.

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.

For More Information

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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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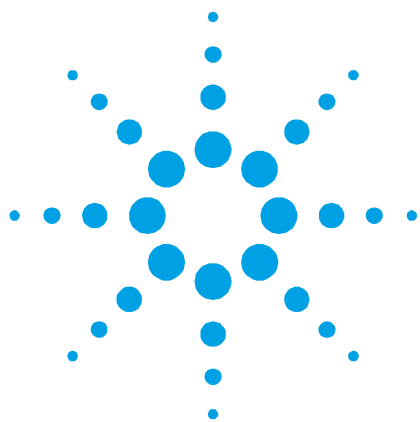
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Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy

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.



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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.

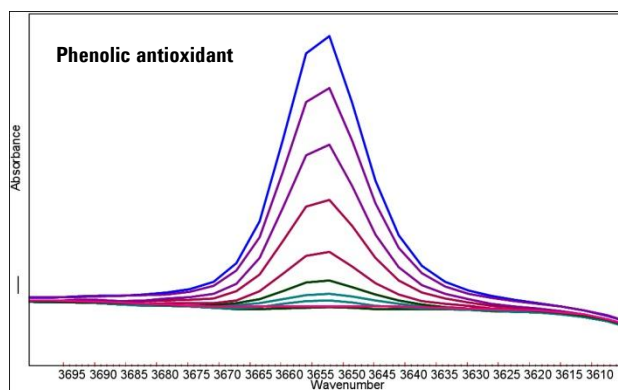


Figure 1. FTIR spectral overlay of the phenolic antioxidant functional group bands depleting as a function of time. The strongest band (light blue) is that of new ISO 32 turbine oil and the weakest absorbance (light green) is from turbine oil that has started to show some oxidation.

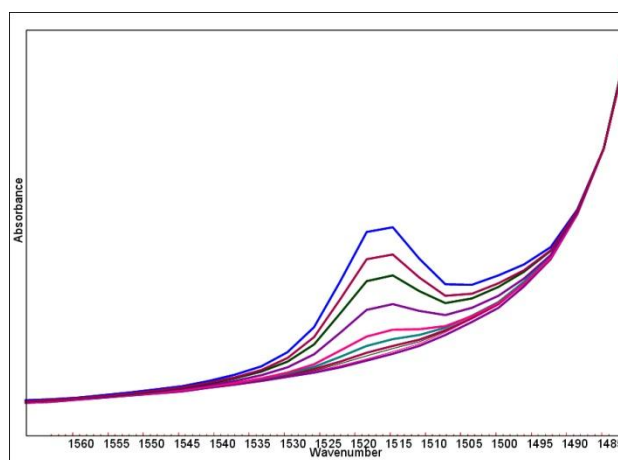


Figure 2. FTIR spectral overlay of the aminic antioxidant functional group depleting as a function of time. The strongest absorbance (red) is aminic antioxidant in new ISO 32 turbine oil and the weakest bands (blue and green) are from turbine oil with spent antioxidant.

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.

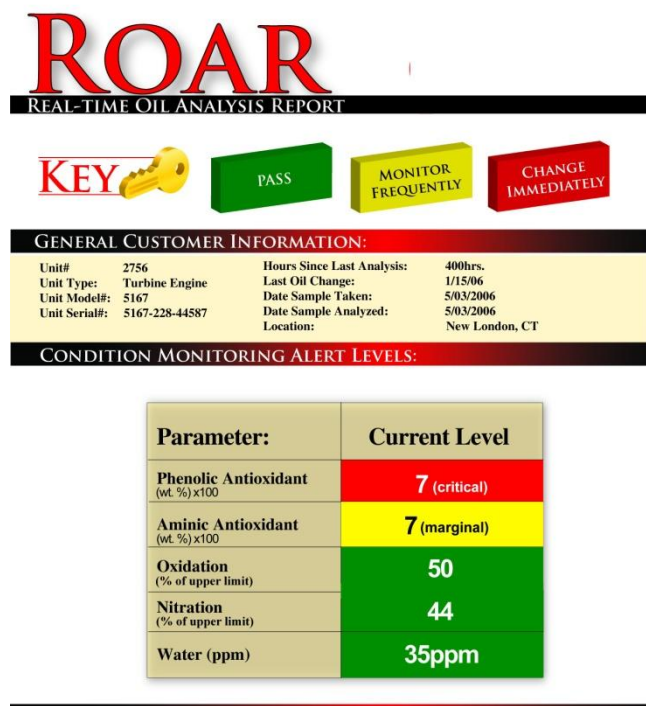


Figure 3. Agilent 5500t FTIR software presents the user with the specific concentration of phenolic and aminic antioxidants as well as crucial information about oxidation by-products and level of water contamination

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.

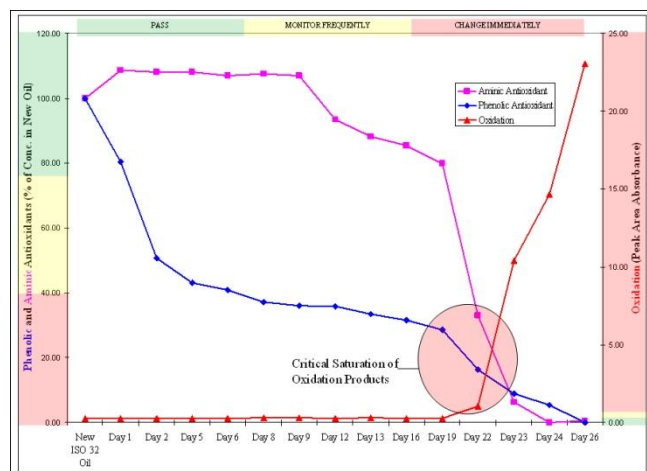


Figure 4. The additive depletion (% relative to new oil concentrations, left scale) and oxidation formation (right scale) trend analysis in thermally stressed ISO 32 turbine oil generated using the Agilent 5500t FTIR spectrometers

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.

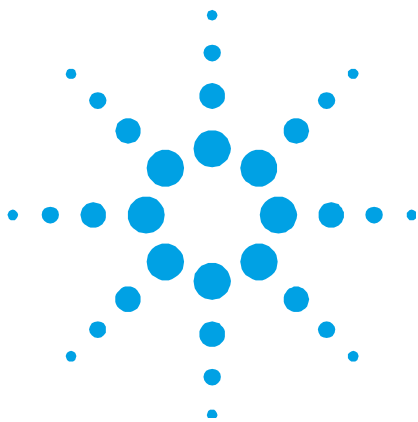
The capability of measuring additives in turbine oil by FTIR spectroscopy eliminates the issues associated with other measurements, including the need for sample preparation, calibrating, and maintaining electrodes based on voltammetric systems. The measurements are more rapid than electrode based antioxidant 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.

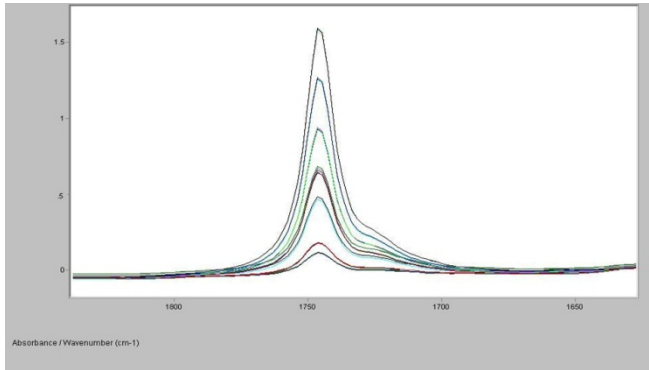


Figure 1. Absorbance at 1745 cm⁻¹ of biodiesel in diesel fuel at 0.8, 1.2, 3, 4, 6, 8 and 10% (v/v)

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.

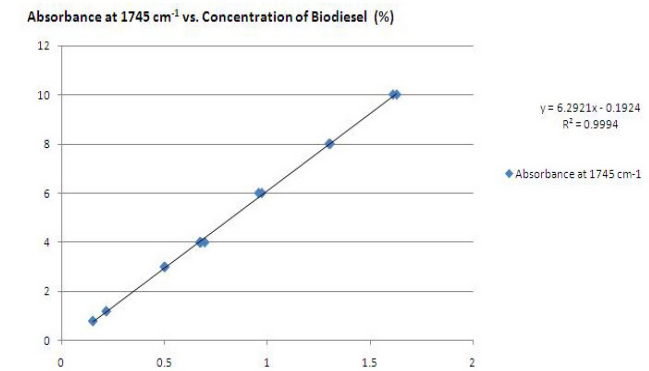


Figure 2. Calibration plot of biodiesel in diesel fuel showing linear fit of absorbance from 0.8 to 10% (v/v)

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.

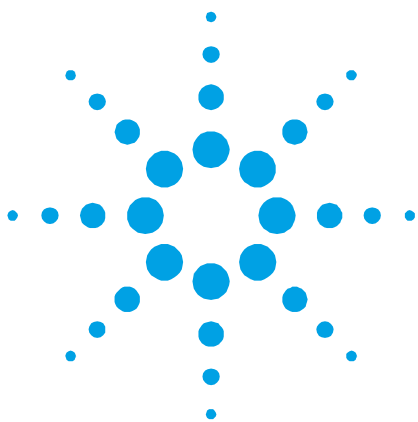


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Agilent Technologies



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.

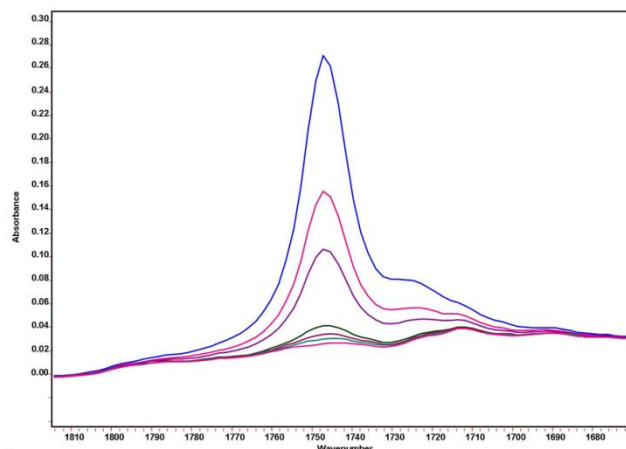


Figure 1. Absorbance at 1745 cm^{-1} of biodiesel in diesel fuel at 0.0, 0.025, 0.05, 0.1, 0.5, 0.8 and 1.5 % (v/v)

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 $R^2 = 0.9998$.

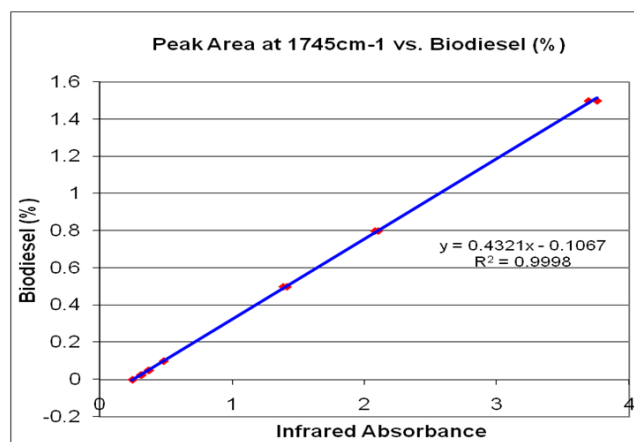


Figure 2. Calibration plot of biodiesel in diesel fuel showing linear fit of absorbance from 0 to 1.5 % (v/v)

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

Add Components

Component Name: % Biodiesel (0-5%)

☒ Display this component in Results and Reports

Calculation

Calculation Type: Peak Area with Dual Baseline

Peak 1: 1758 Peak 2: 1735

Baseline 1 Start: 1821 Baseline 1 Stop: 1819

Baseline 2 Start: 1671 Baseline 2 Stop: 1669

Scaling

☐ Invert (1/Value)

Scale (x Value): 432.1 Offset (+ Value): -106.7

Thresholds

Marginal Low: Marginal High: 65

Critical Low: Critical High: 100

Report Value As: Actual Value

To Select a Percent type, you must enter at least a Critical High value.

OK Cancel

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

FTIR MOBILITY SERIES™

User: Admin

Status: Ready

Result: 0.05% prediction

Recommendation:
One or more parameters are outside the acceptable ranges. Take critical corrective action as appropriate.

Results:

Name	Value	Low Threshold	High Threshold
% Biodiesel (0-5)	0.049		0.100

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

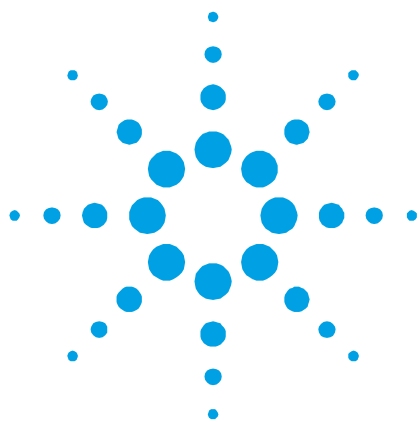


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Agilent Technologies



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.

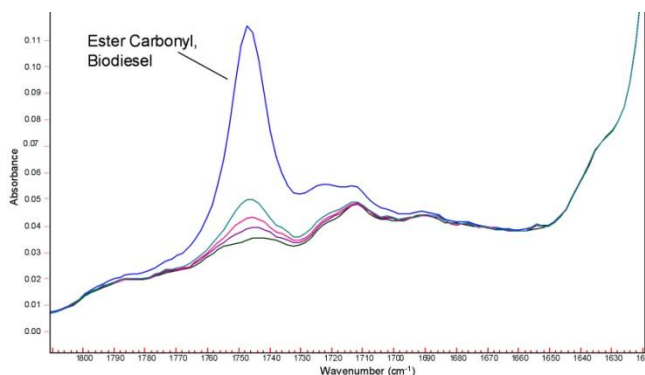


Figure 1. The overlaid IR spectra of diesel fuels with various ultra low concentrations of biodiesel, at 0.50 % (Blue), 0.10 % (Lt. Green), 0.05 % (Red), 0.025 % (Maroon), and 0.00 % (Dk. Green)

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 interferences. 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.

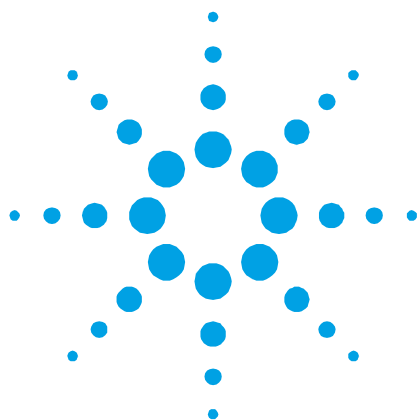


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Agilent Technologies



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.



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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 $R^2=0.977$ 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 $R^2=0.996$.

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

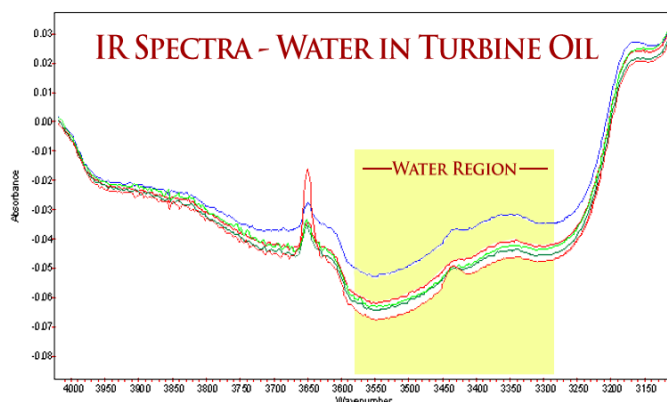


Figure 1. The overlaid IR spectra of turbine oil with the water absorbance region expanded, water values from bottom to top are 30 ppm (red), 80 ppm (dark green), 217 ppm (light green), 533 ppm (red), and 1460 ppm (blue)

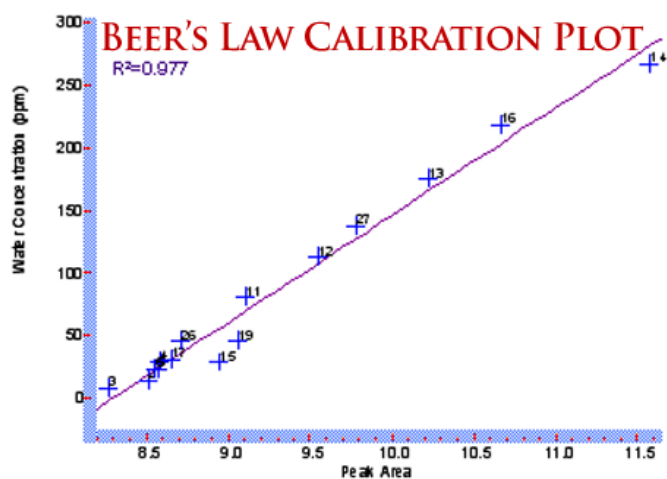


Figure 2. The calibration plot of KF water values (ppm) versus peak absorbance area for water in turbine oil using a Beer's Law peak area method

PLS model

The PLS chemometric model uses more sophisticated mathematics 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 $R^2=0.990$.

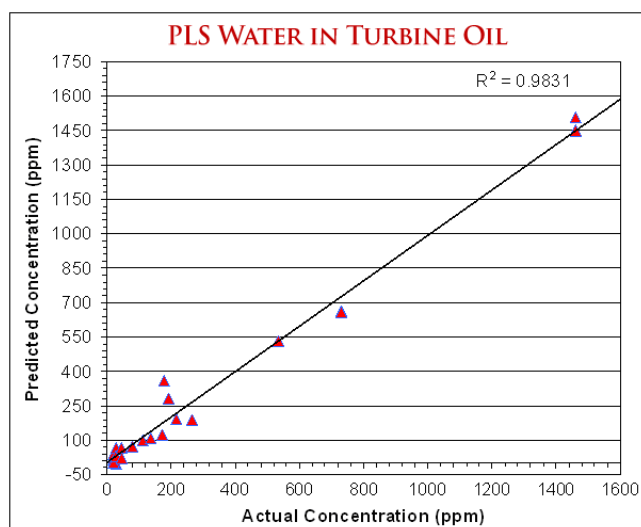


Figure 3. The PLS predicted versus actual plot of KF values using Agilent 4500 Series FTIR spectrometer

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.

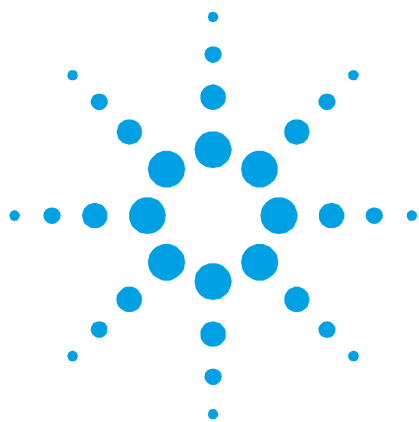


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Agilent Technologies



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.

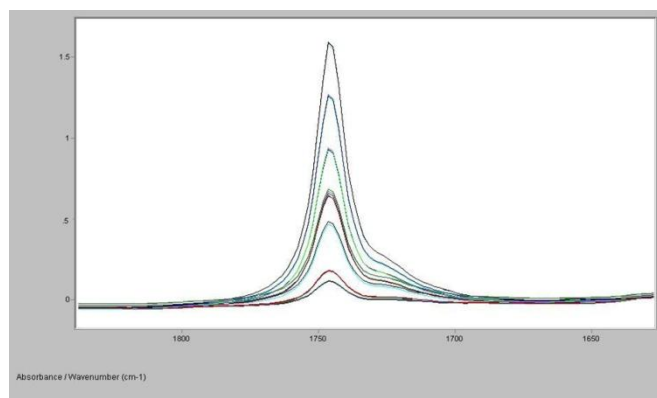


Figure 1. FTIR spectra overlaid of ASTM D7371 standards with biodiesel in diesel at 0, 2.5, 5, 10, 15, 20, 30, 50, 70, and 100 % biodiesel (v/v)

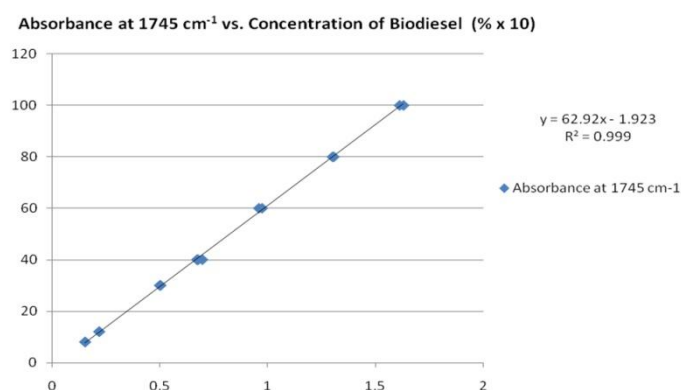


Figure 2. The PLS model's actual vs. predicted plot of biodiesel in diesel, low calibration set (0-10 % biodiesel)

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

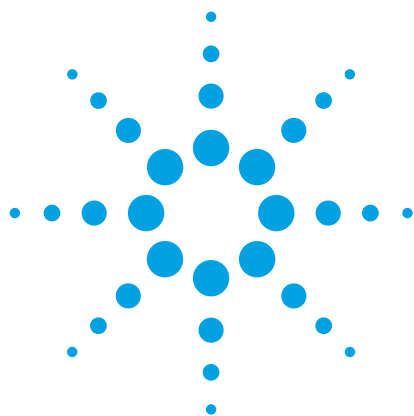
This set of experiments show the ability of Agilent 5500 Series FTIR spectrometers with 9 reflection diamond ATR sample interface to meet the ASTM D7371 method. The method file which calculates the concentration in all ranges from 1 % to 100 % biodiesel and selectively reports the correct concentration 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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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 ($\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$), 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.



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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	

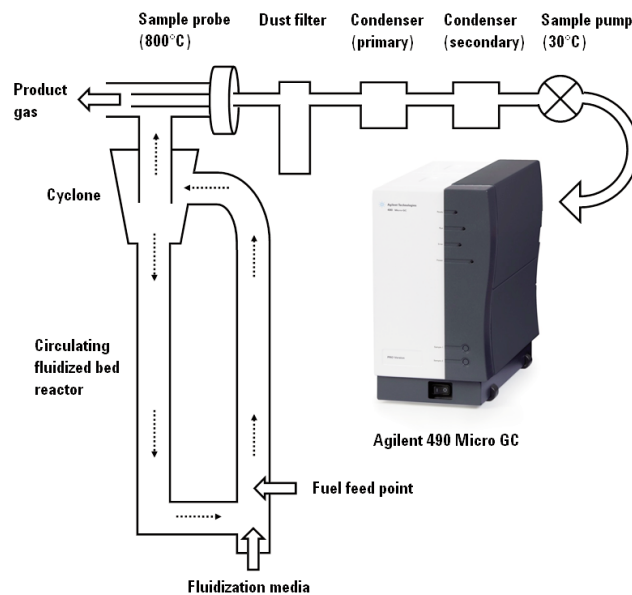


Figure 1. Reactor, sampling and sample conditioning setup.

Results and Discussion

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

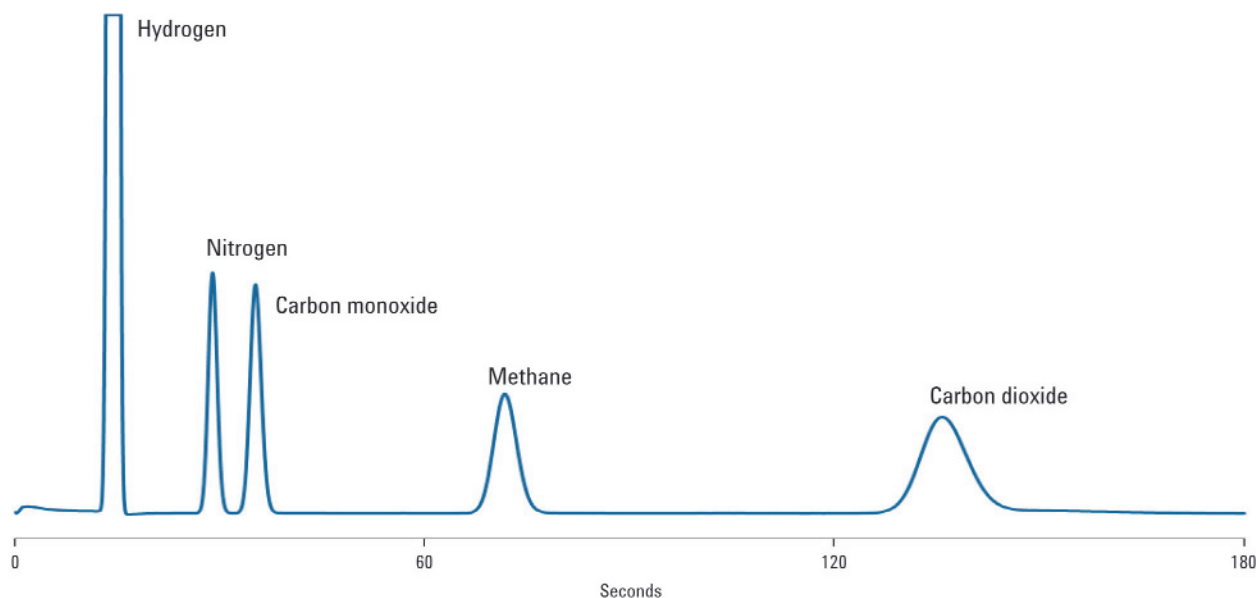


Figure 2. Permanent gases on the COX column.

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 per-

formed. 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.

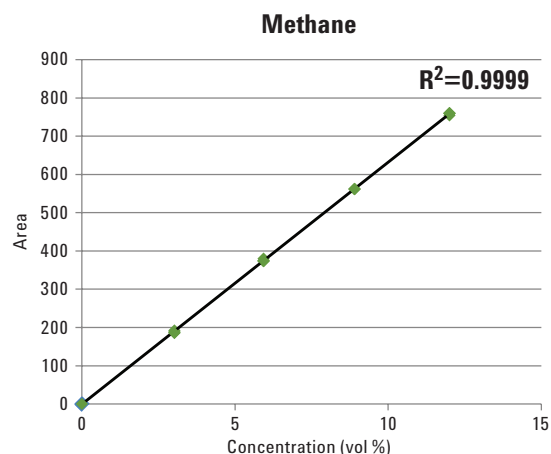


Figure 3. Calibration curve for methane.

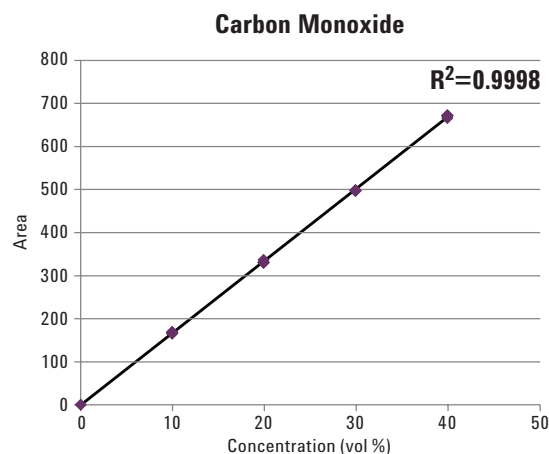


Figure 4. Calibration curve for Carbon monoxide.

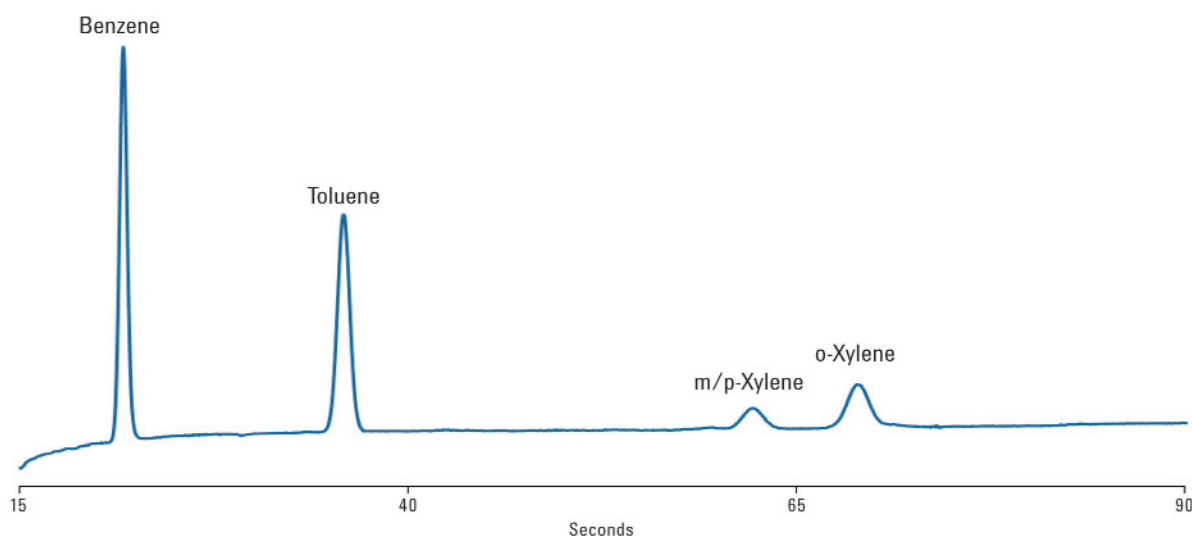


Figure 5. BTX analysis on the CP-Sil 5 CB column.

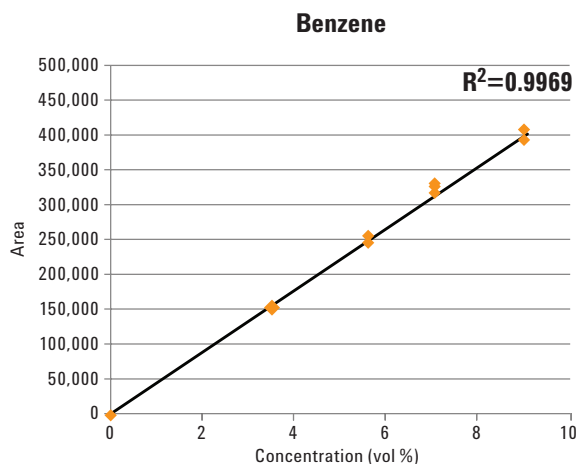


Figure 6. Calibration curve for Benzene.

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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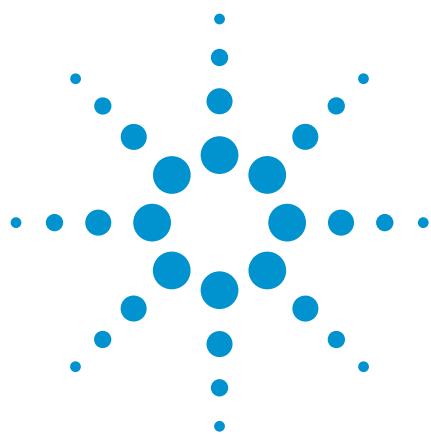
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Reversed Phase HPLC of Fatty Acids

Application Note

Author

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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-occurring 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-occurring 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.



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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.

Peak Identification

1. Capric acid
2. Lauric acid
3. Myristic acid
4. Linolenic acid
5. Linoleic acid
6. Palmitic acid
7. Stearic acid

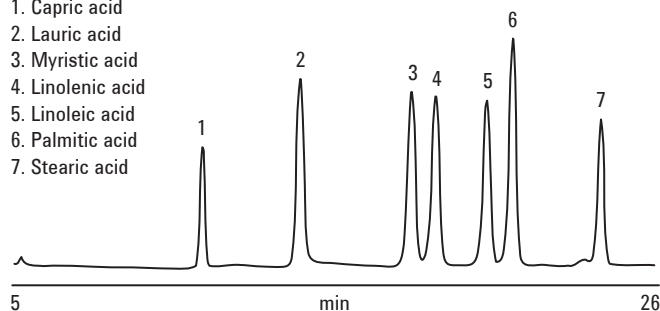


Figure 1. Separation of seven fatty acids using PLRP-S media.

Peak Identification

1. Linoleic acid
2. Palmitic acid

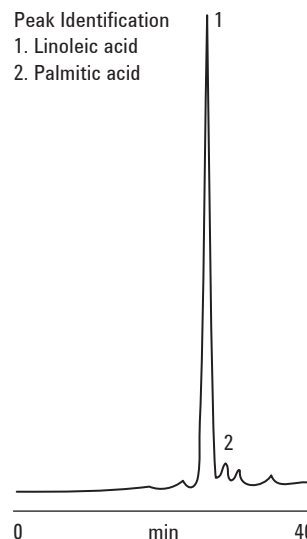


Figure 2. Fatty acid composition of evening primrose oil revealed by PLRP-S.

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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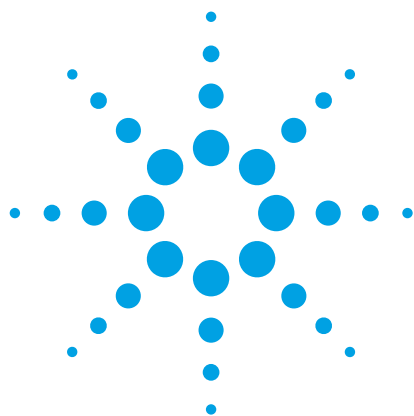
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5990-8158EN



Agilent Technologies



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

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Alkyl glycerides are discrete molecules commonly found in frying fats and oils. Agilent's PLgel 3 μ m 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.



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KEY

1. Stearyl mono
2. Diglyceride
3. Triglyceride
4. Lauryl mono
5. Diglyceride
6. Triglyceride

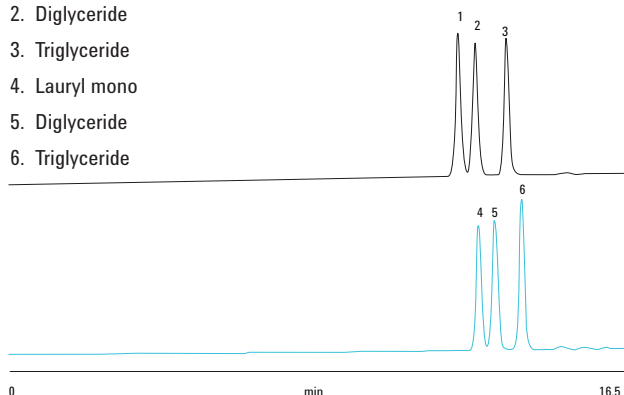


Figure 1. Overlaid chromatograms of lauryl and stearyl mono-, di-, and triglycerides, showing the base line resolution possible with high efficiency Agilent PLgel 3 μ m columns.

KEY

1. Tristearoylglyceride (891.5 g/mol)
2. Tripalmitoylglyceride (807.3 g/mol)
3. Distearoylglyceride (635.0 g/mol)
4. Dipalmitoylglyceride (568.9 g/mol)
5. Dimyristoylglyceride (512.8 g/mol)
6. Dilauroylglyceride (456.7 g/mol)
7. Monopalmitoylglyceride (330.5 g/mol)
8. Monolauroylglyceride (274.4 g/mol)

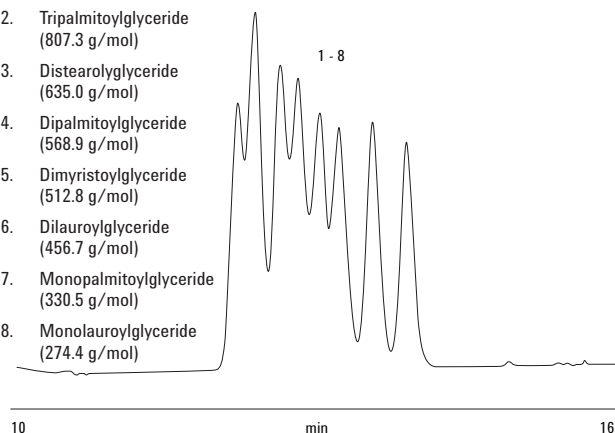


Figure 2. An Agilent PLgel 3 μ m two-column set resolves individual components from an alkyl glyceride mixture.

Conditions

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

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

$$R_{sp} = \frac{0.25}{\sigma D}$$

where R_{sp} 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 μ m 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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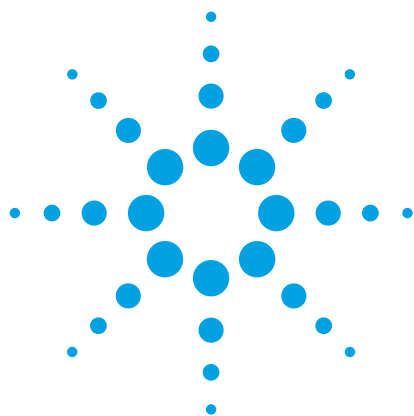
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Agilent Technologies



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 μ m 500Å, 7.5 $\times$ 300 mm (p/n PL1110-6525)
Eluent	NMP
Flow rate	1.0 mL/min
Temp	80 °C
Detector	RI

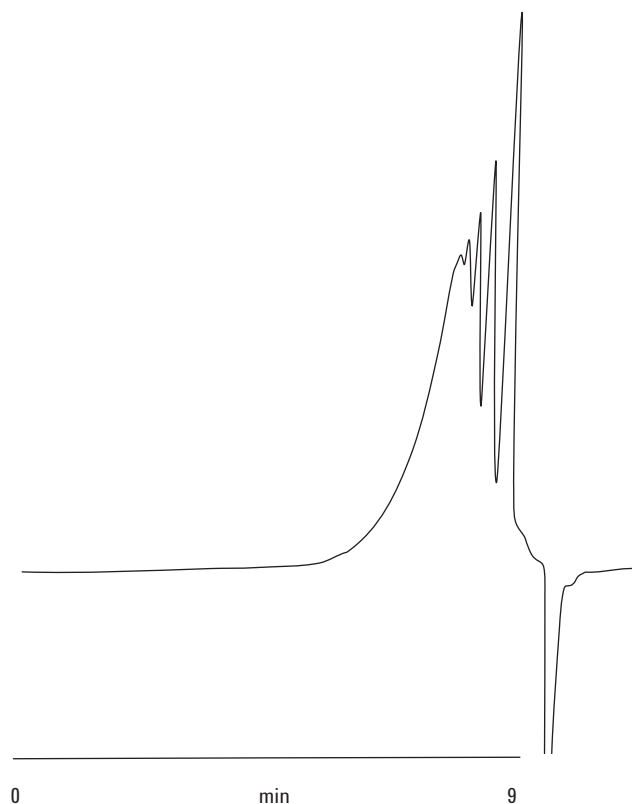


Figure 1. Separation of oligosaccharide on an Agilent PLgel 5 μ m column.

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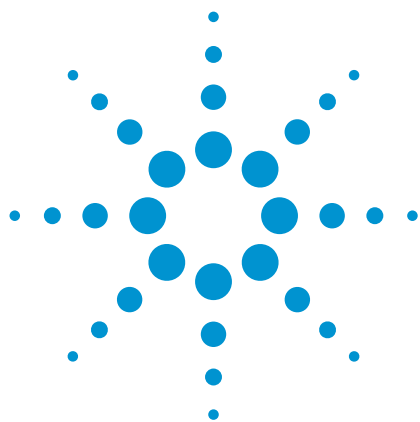
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Analysis of Lipids with the Agilent 385-ELSD and the Agilent 1260 Infinity LC

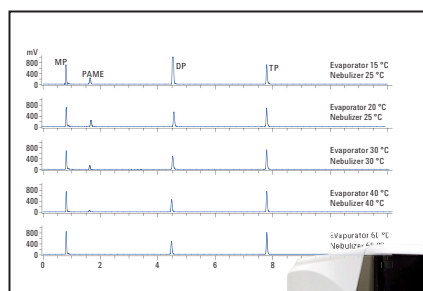
Sensitive detection through sub-ambient evaporation and nebulization

Application Note

Food Testing

Authors

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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

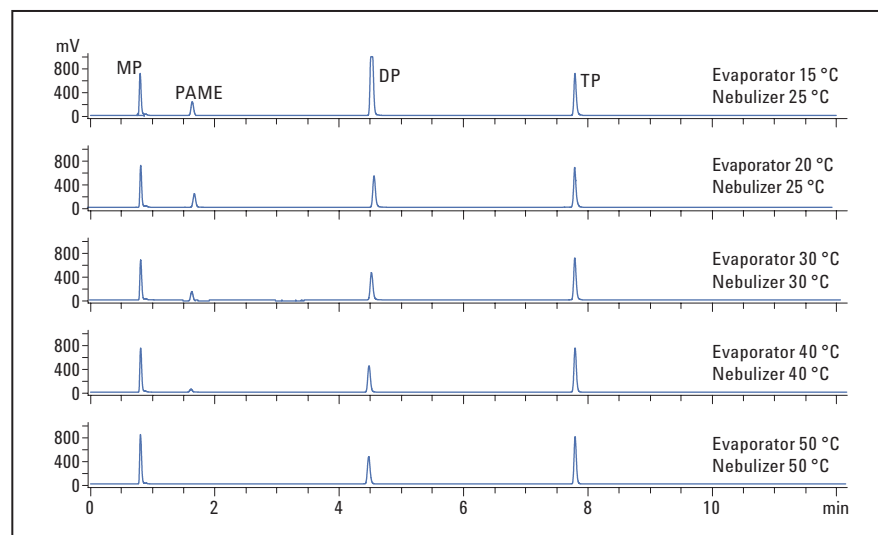


Figure 1
Analysis of the test mixture at various ELSD temperature settings.

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		S/N
Evaporator	Nebulizer	Area	RSD%	Area	RSD%	Area	RSD%	Area	RSD%	
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.

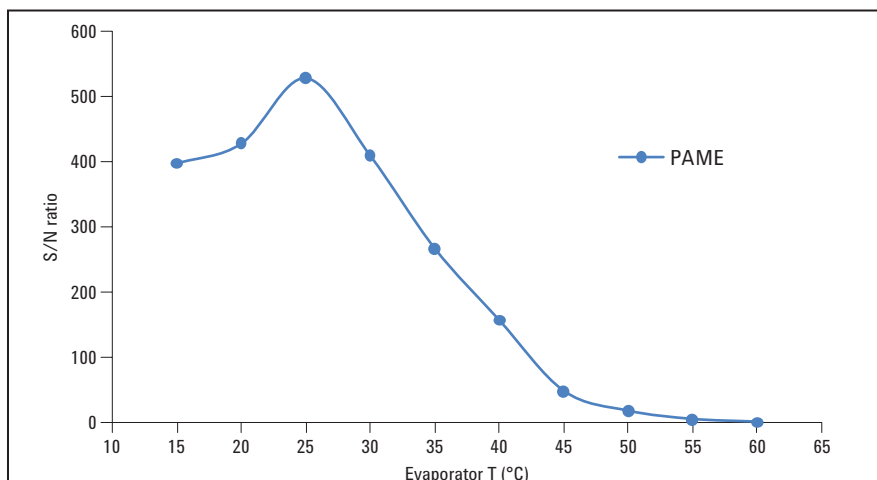


Figure 2

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

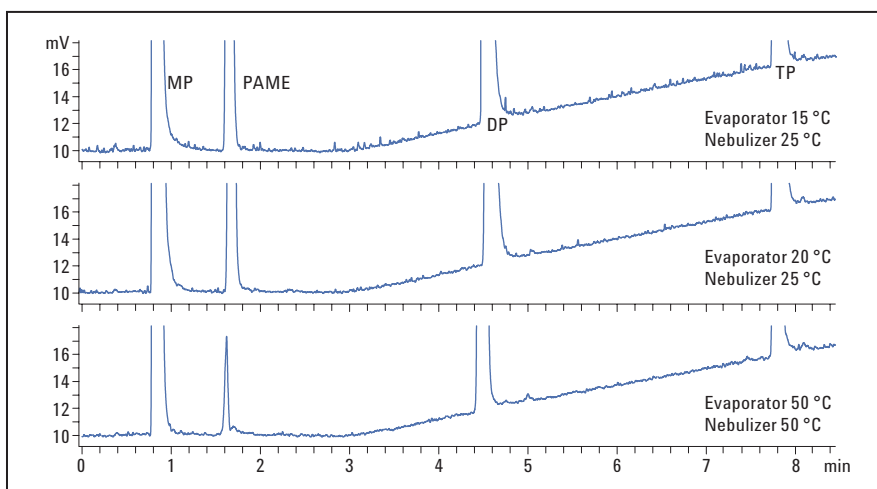


Figure 3

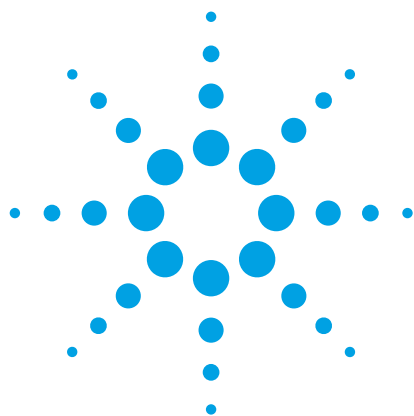
Detail on the baseline for the analysis of the test mixture at various ELSD temperature settings.

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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.

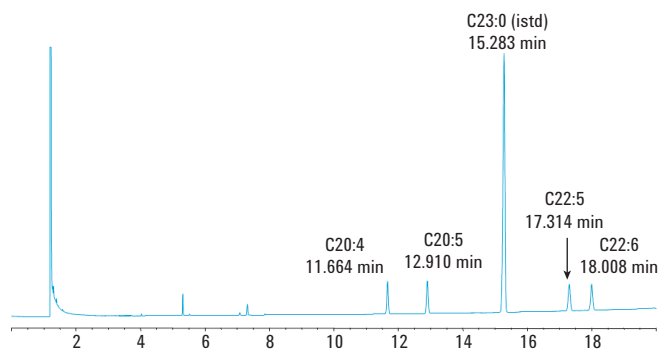


Figure 1. Chromatogram of the retention time standard containing the four PUFA FAMES and the internal standard, methyl tricosonate (C23:0).

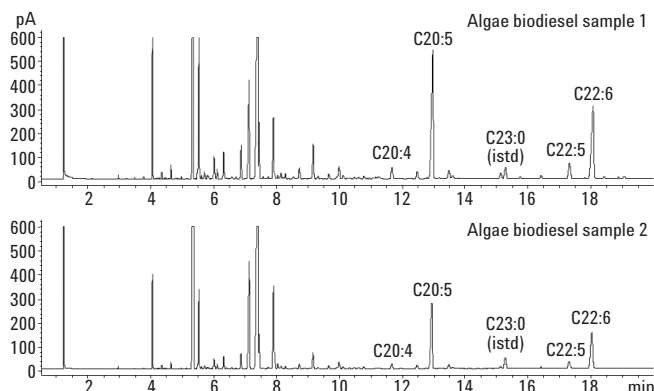


Figure 2. These chromatograms show the analysis of PUFA FAMES in biodiesel samples made from two different algae oils.

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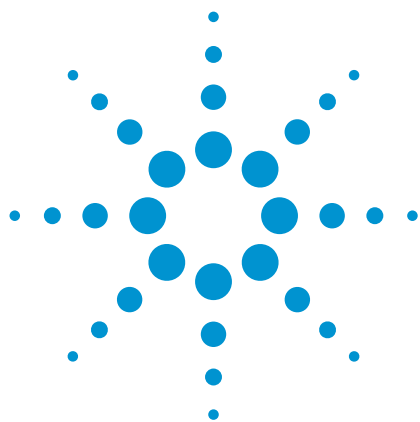
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Determination of Total FAME and Linolenic Acid Methyl Esters in Biodiesel According to EN-14103

Application Note

Energy and Fuels

Author

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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.

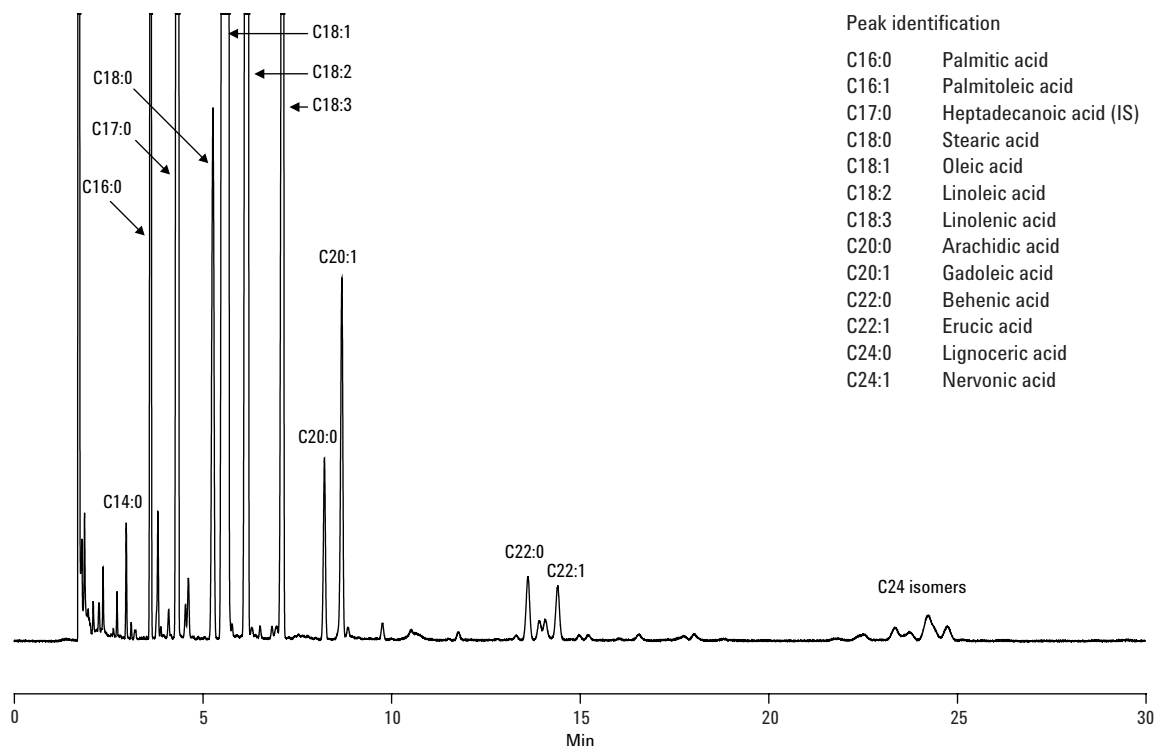


Figure 1. Separation of biodiesel by gas chromatography using an Agilent Select Biodiesel for FAME column.

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

Table 1. Analysis Results of Biodiesel

	Area ($\mu\text{V}\cdot\text{min}$)	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.

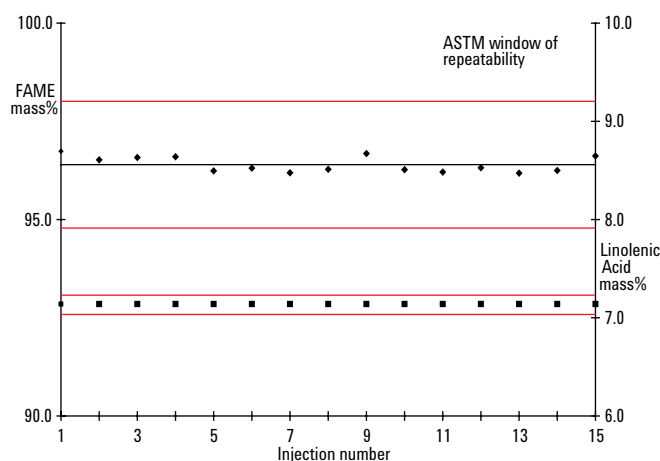


Figure 2. Repeatability data obtained during the analysis of biodiesel using an Agilent Select Biodiesel for FAME column. Red lines indicate the maximum and minimum variation limits specified in the method.

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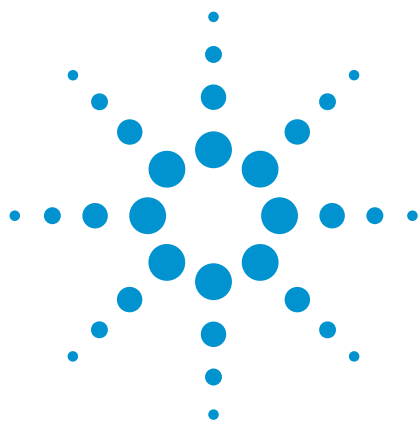
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Determination of Methanol Content in Biodiesel Using Agilent Select Biodiesel for Methanol with Headspace Sampling to EN-14110

Application Note

Energy and Fuels

Author

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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.

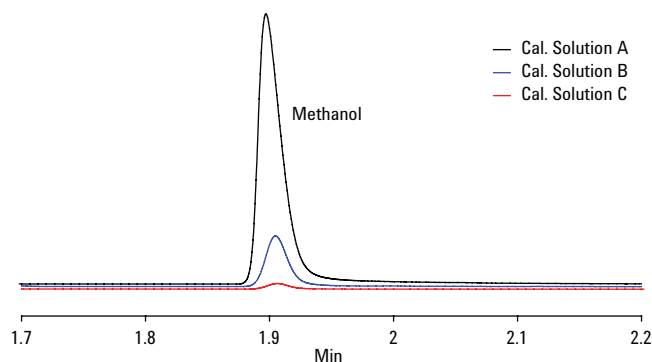


Figure 1. Overlay traces of calibration solutions obtained by gas chromatography using an Agilent Select Biodiesel for Methanol column.

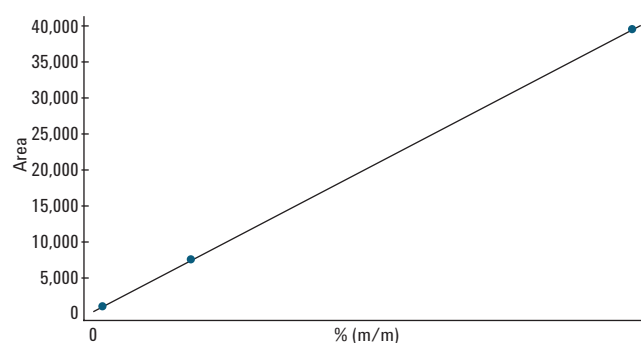


Figure 2. Calibration curve produced by an Agilent Select Biodiesel for Methanol column.

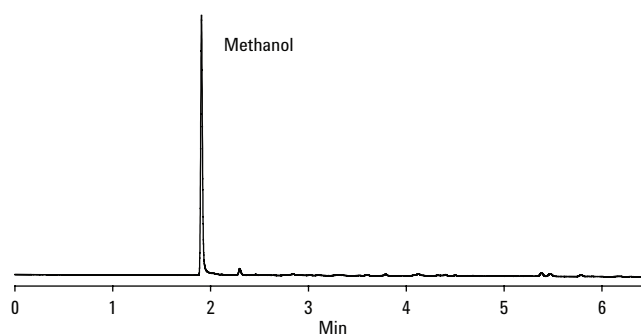


Figure 3. Typical headspace chromatogram for biodiesel produced by an Agilent Select Biodiesel for Methanol column.

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.

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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Table 1. Repeatability Results

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

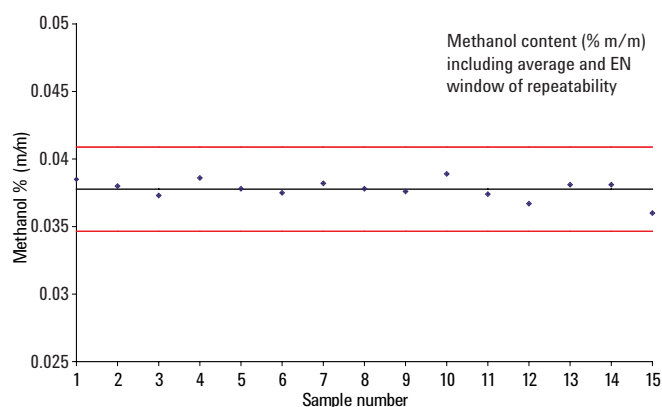


Figure 4. Repeatability values are within the specification boundaries established in EN-14214, as indicated by the red lines in the chart.

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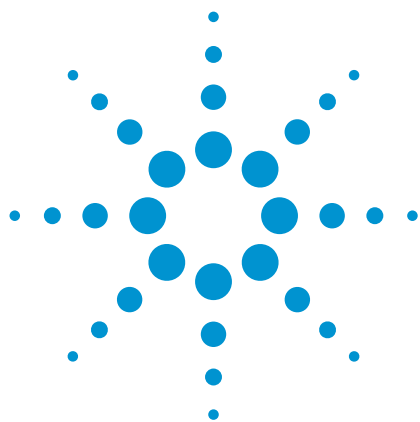
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Determination of Methanol Content in Biodiesel Using Agilent Select Biodiesel for Methanol

Application Note

Energy and Fuels

Author

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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.

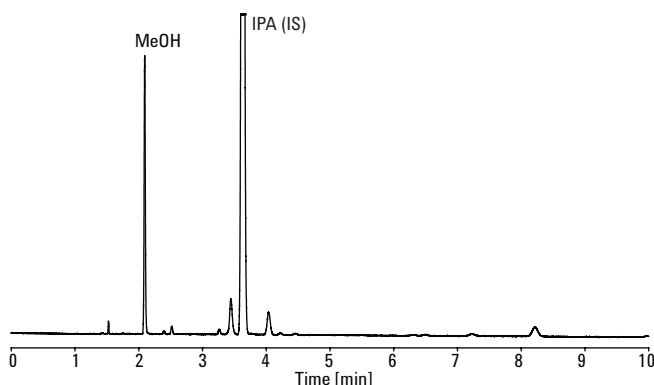


Figure 1. Chromatogram of a summer biodiesel (sample code 1) including some unknown volatiles.

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.

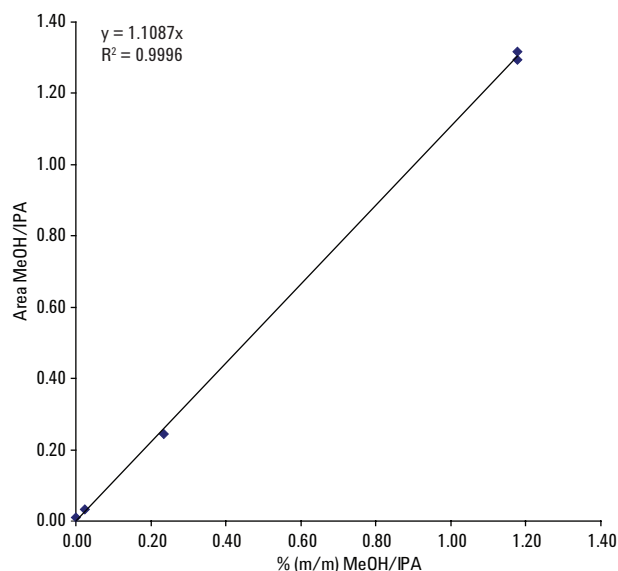


Figure 2. Calibration curve as obtained with the manual headspace method with isopropanol as internal standard.

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).

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 ($R_s > 1.5$). 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.

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

Code	Description*	Sample mass (g)	μ L 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).

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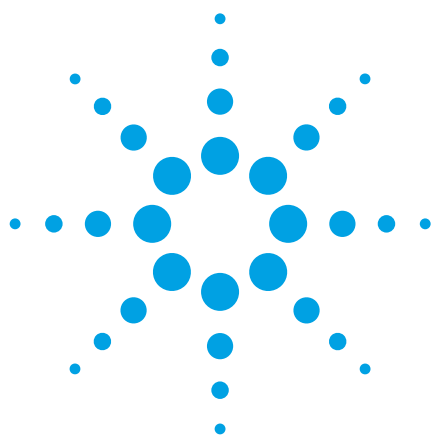
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Analysis of Free and Total Glycerol in B-100 Biodiesel Methyl Esters Using Agilent Select Biodiesel for Glycerides

Application Note

Energy and Fuels

Author

John Oostdijk
Agilent Technologies, Inc.

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-dioleoylglycerol (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 μ m, with retention gap (p/n CP9076)
Injection	Cold on-column (1093), full EFC control, 1 μ L, 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 μ L of homogenized biodiesel sample was accurately weighed ($\pm$ 0.1 mg) in a 20-mL vial, then 100 μ L of Internal Standard 1, 100 μ L of Internal Standard 2, and 100 μ L 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 μ L 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-trimethylsilyltrifluoroacetamide (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

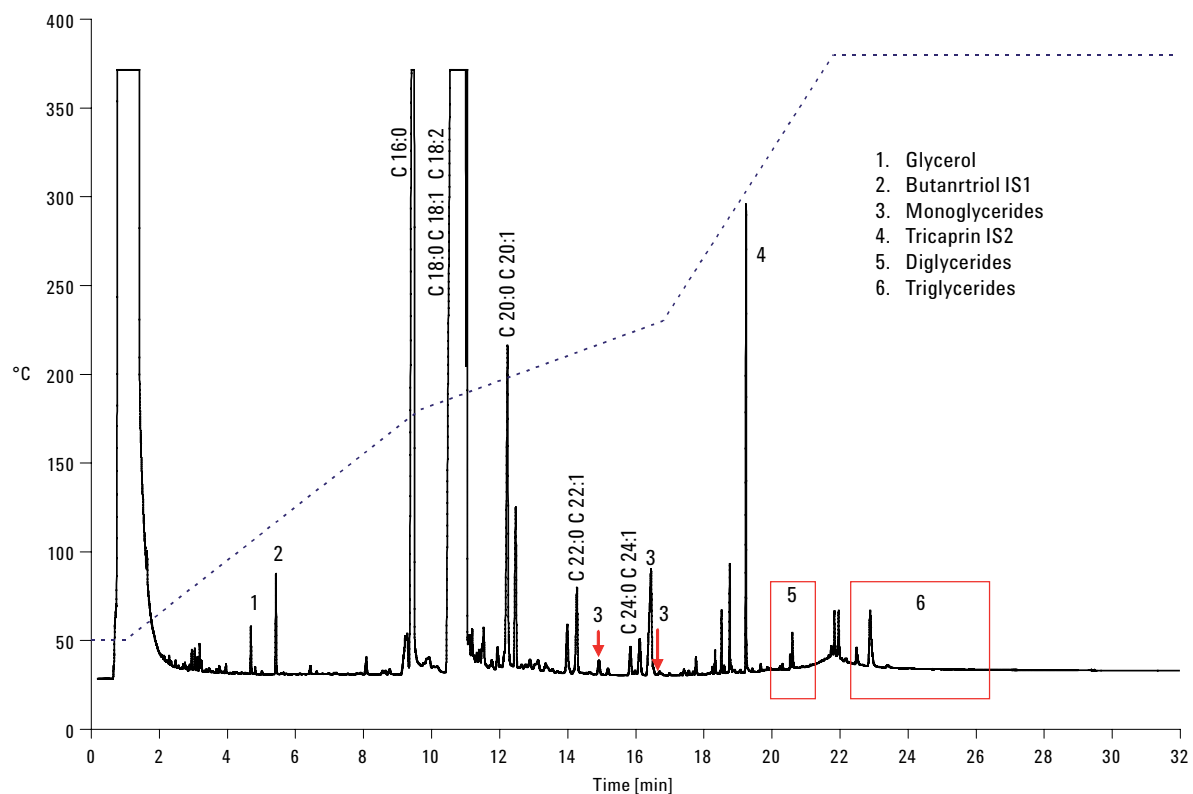


Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which consists mainly of C18 and C16 FAMES and minor compounds, such as sterols.

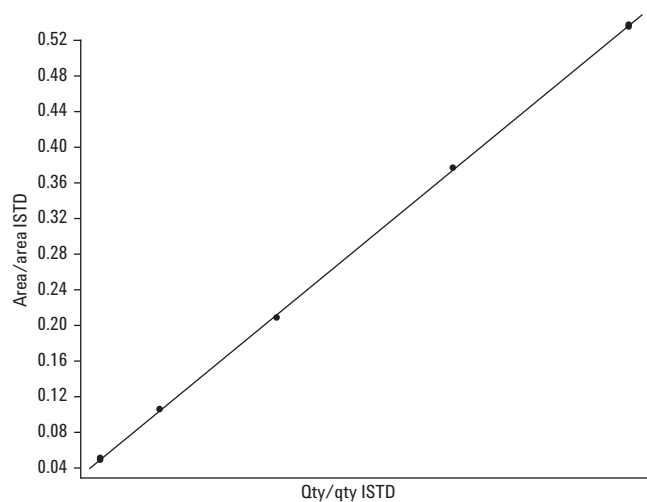


Figure 2. Calibration curve for triolein.

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 μ L. 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 (μ V.min)	Area 2 (μ V.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.

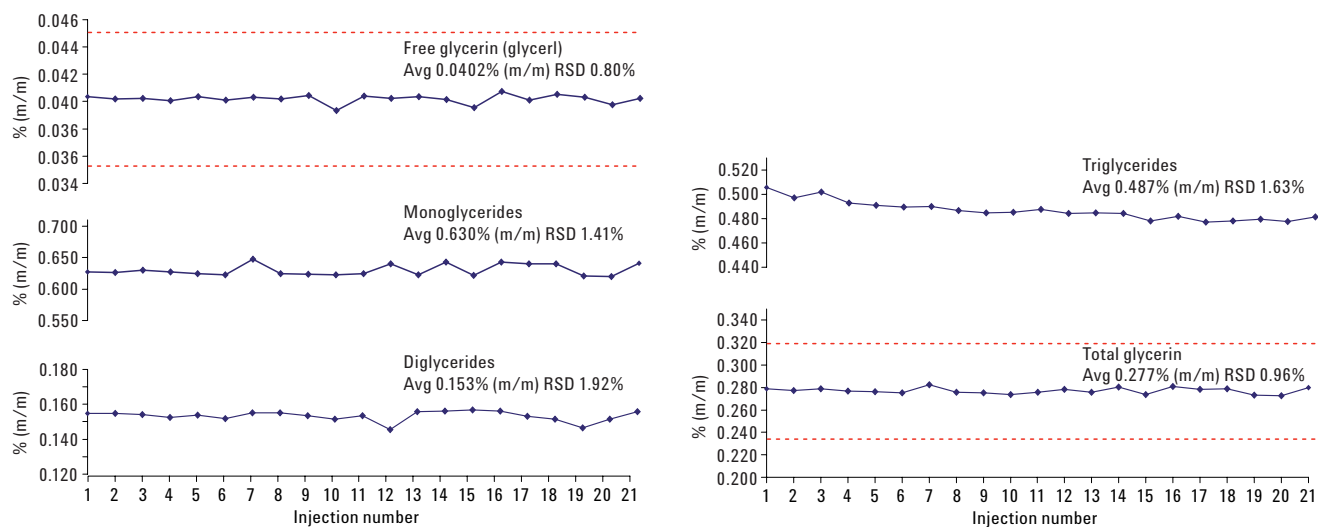


Figure 3. Typical repeatability of 21 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the ASTM D 6584 – 07.

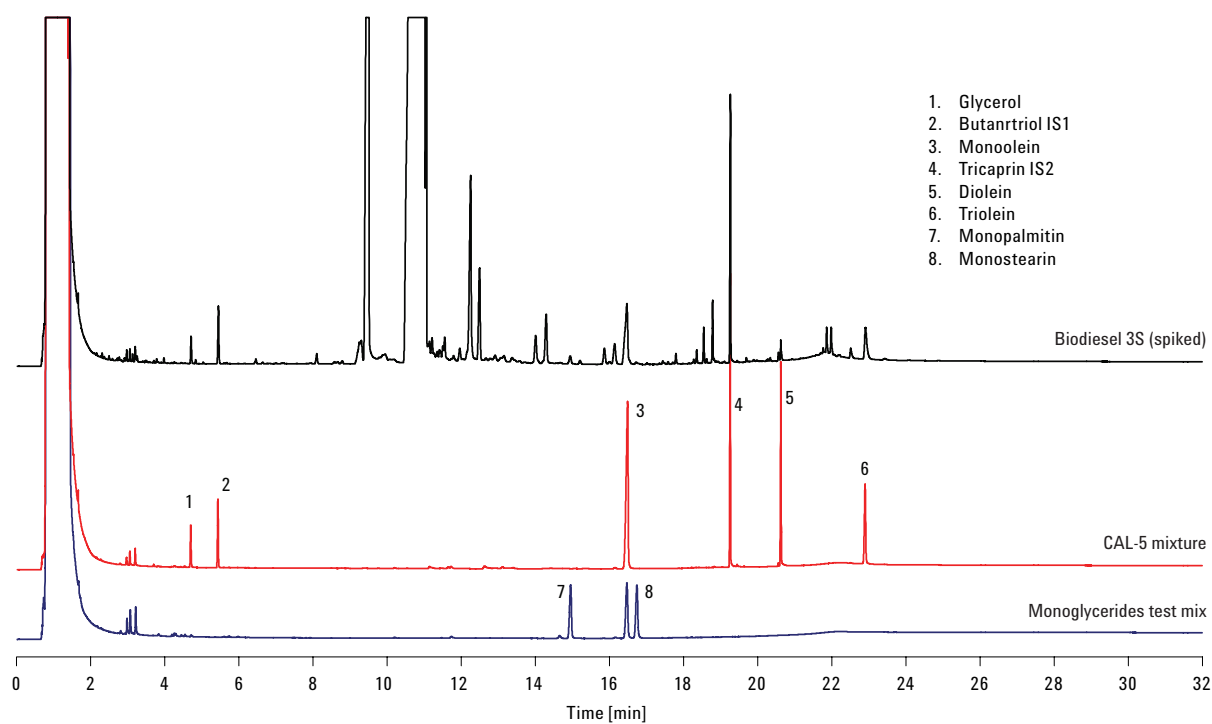


Figure 4. Details of identification of unknown peaks in a biodiesel sample.

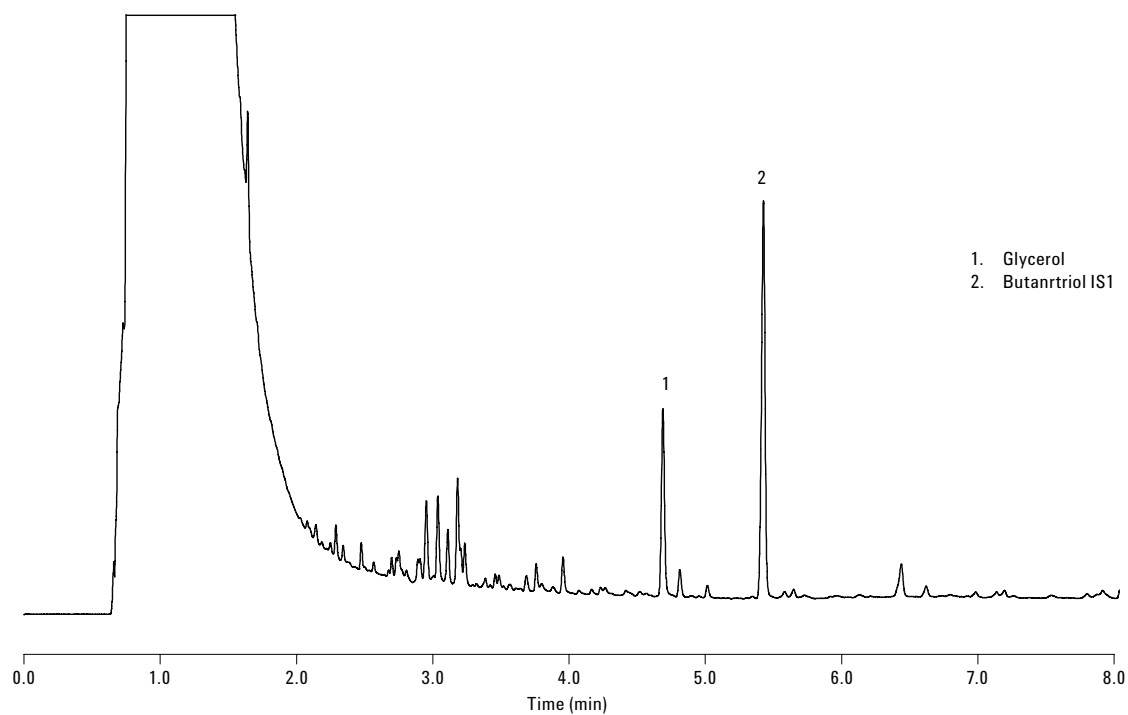


Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

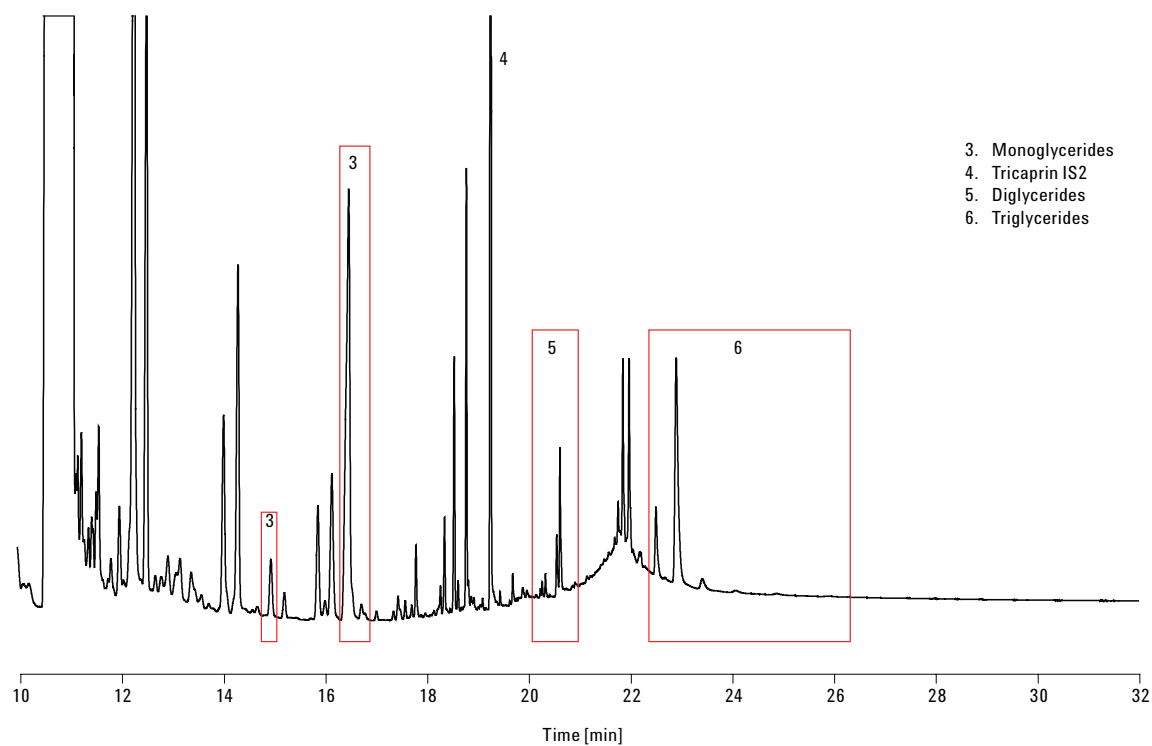


Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

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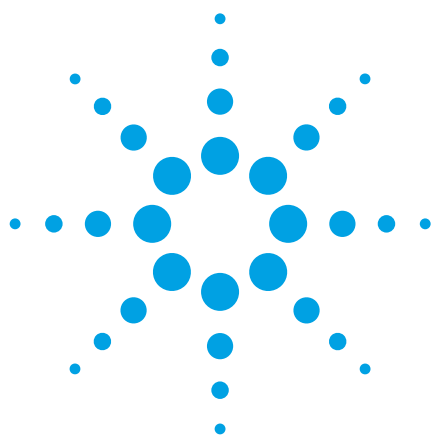
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Analysis of Free and Total Glycerol and Triglyceride Content in B-100 Biodiesel Methyl Esters Using Agilent Select Biodiesel for Glycerides

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 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

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-dioleoylglycerol (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-trimethylsilyltrifluoroacetamide (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

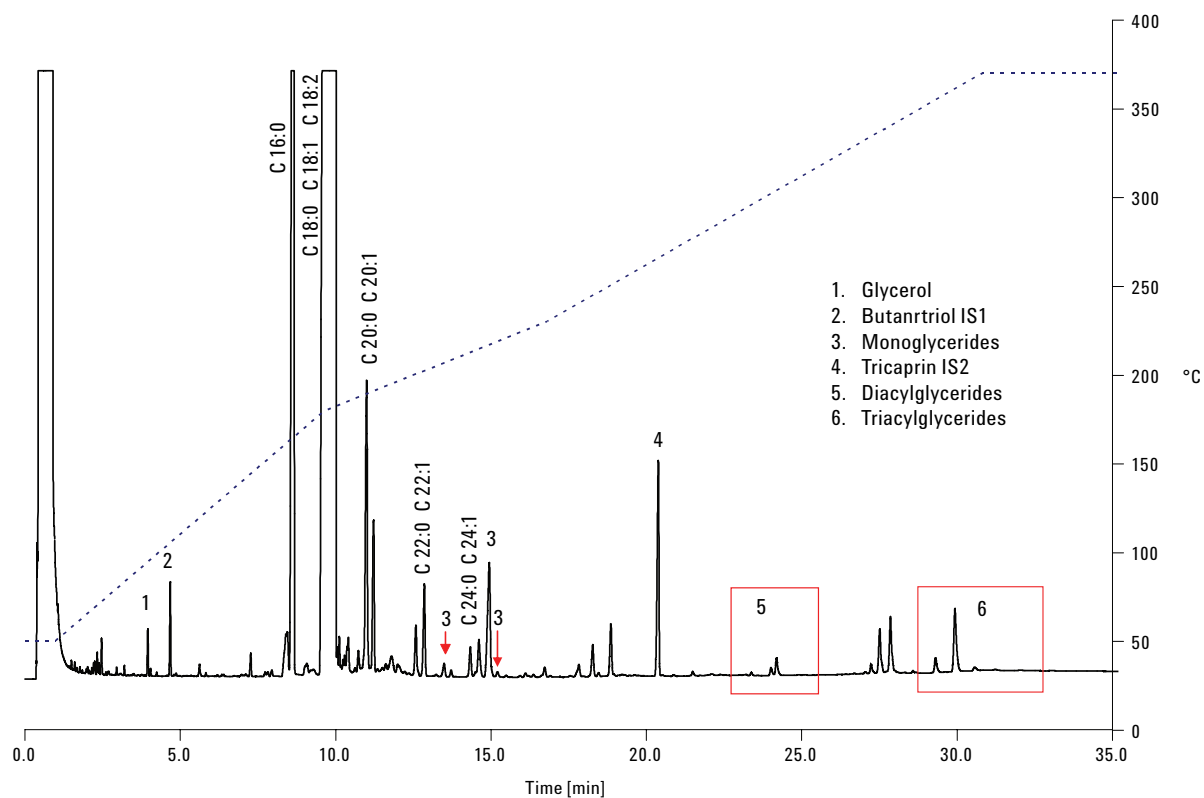


Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which consists mainly of C18 and C16 FAMES and minor compounds, such as sterols.

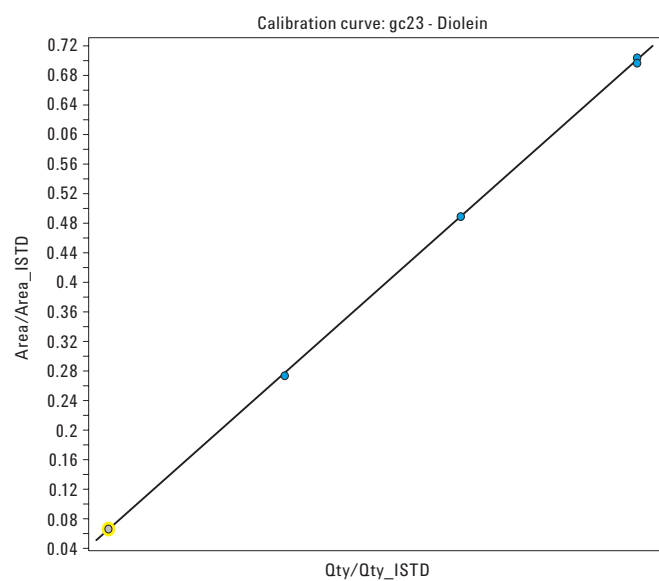


Figure 2. Calibration curve for diolein.

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 μ L. 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 (μ V.min)	Area 2 (μ V.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

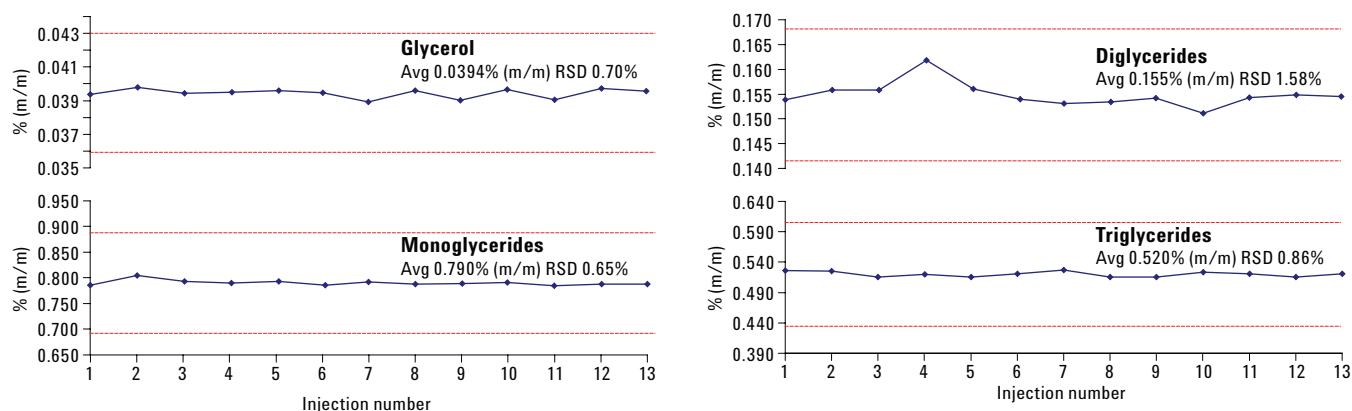


Figure 3. Typical repeatability of 13 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the EN-14105 method.

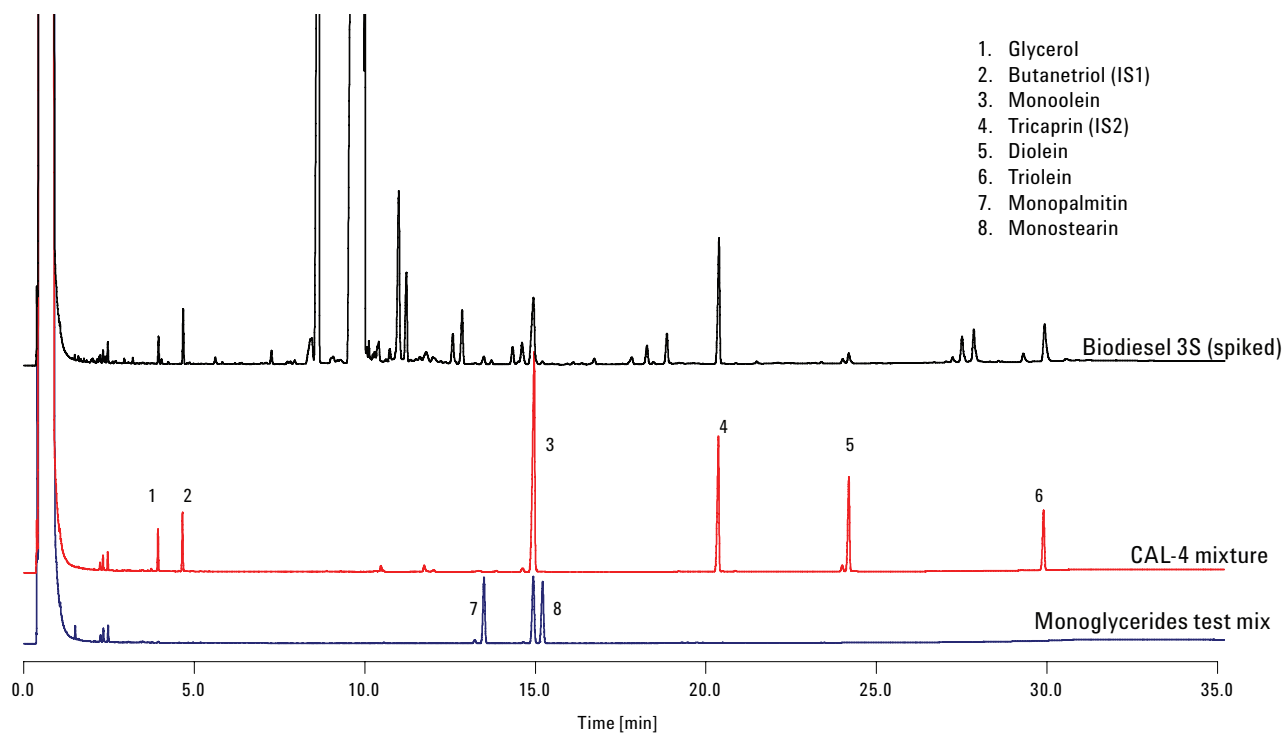


Figure 4. Details of identification of unknown peaks in a biodiesel sample .

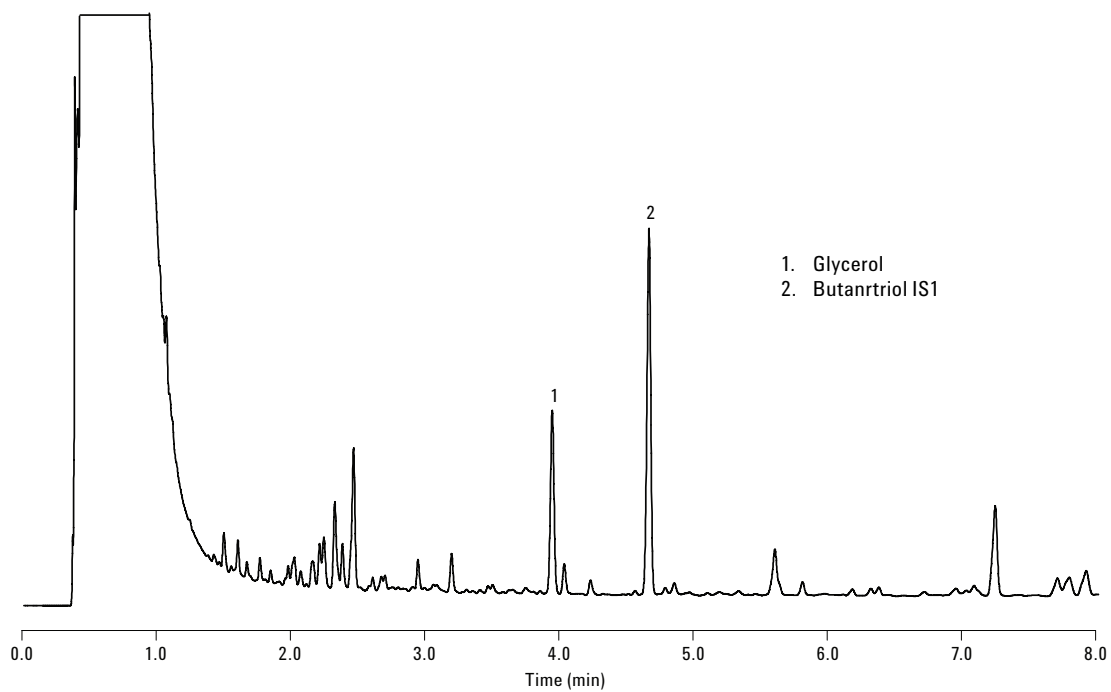


Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

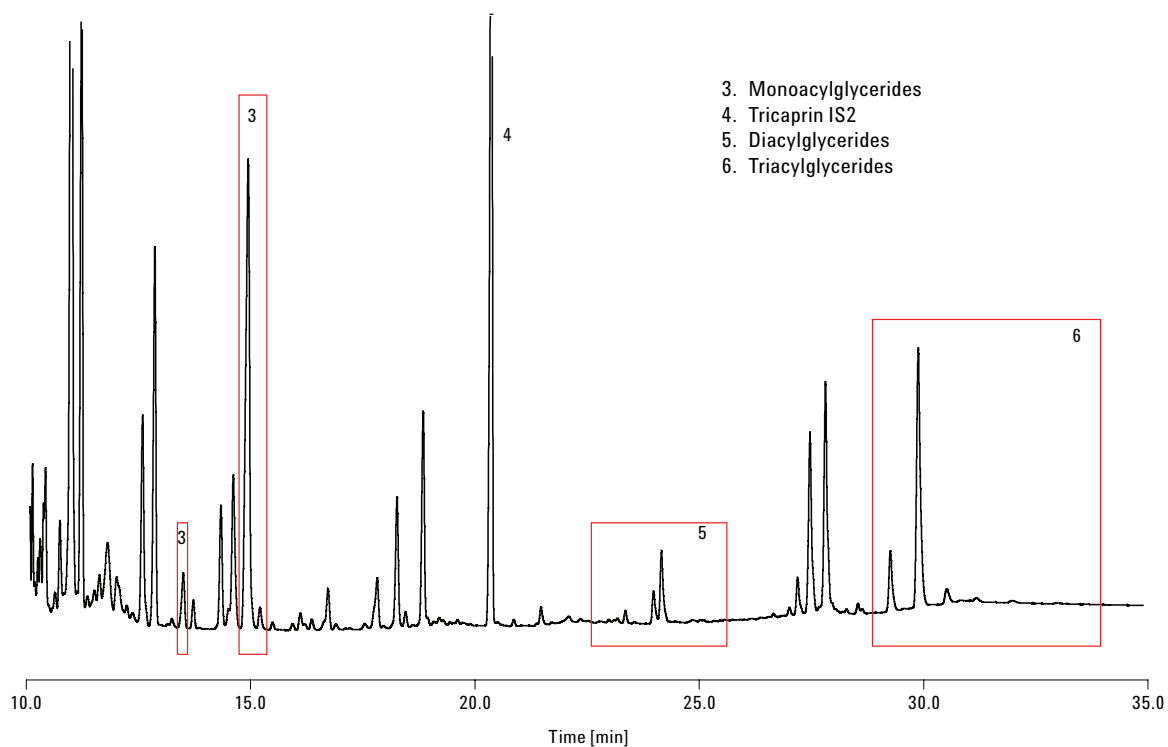


Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

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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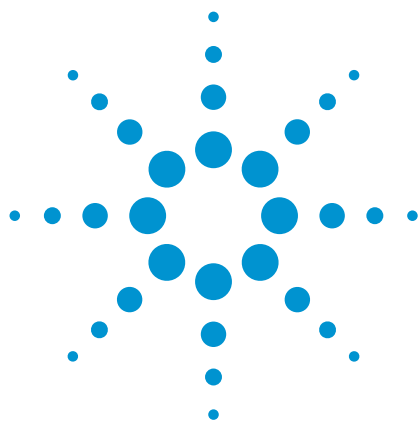
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Agilent Hi-Plex Columns for Sugar Separation: Effects of Temperature and Mobile Phase

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 $\times$ 300 mm, 8 μm (p/n PL1170-6830)
Mobile phase	0.01 M H_2SO_4
Flow rate	0.4 mL/min
Temperature	> 75 $^\circ\text{C}$
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 $^\circ\text{C}$, there are two pairs of co-eluted peaks, but by increasing the temperature to 55 $^\circ\text{C}$, 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 $^\circ\text{C}$, 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 $^\circ\text{C}$.

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

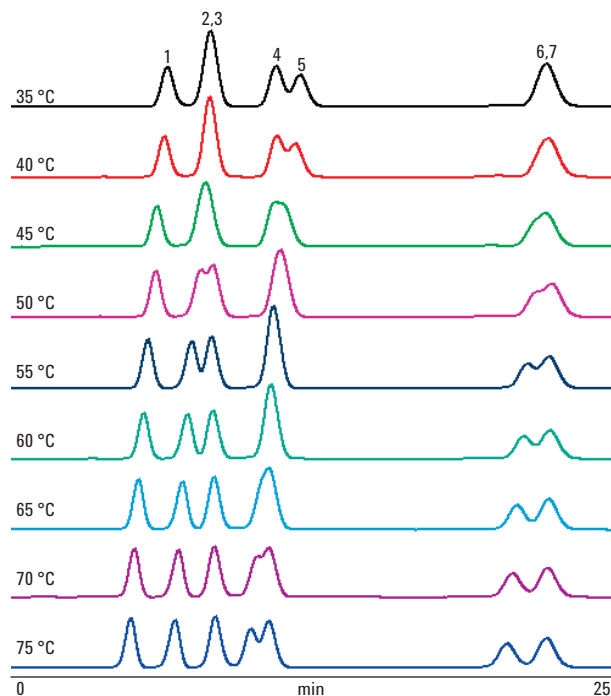


Figure 1. Effect of temperature on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

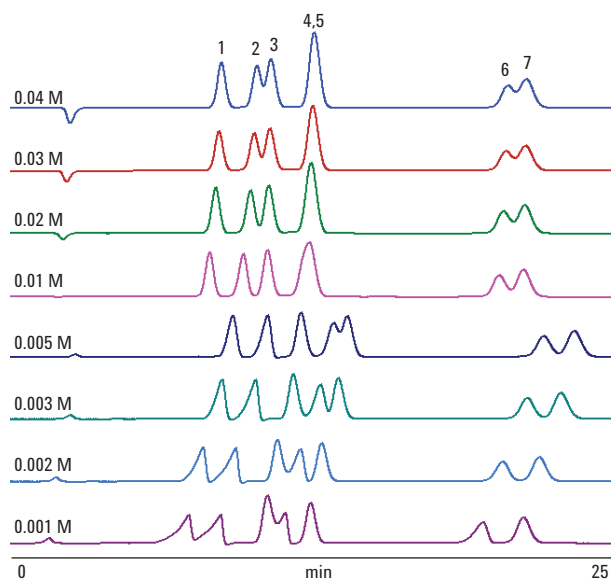


Figure 2. Effect of acid concentration on the separation of sugars and organic acids on an Agilent Hi-Plex H column.

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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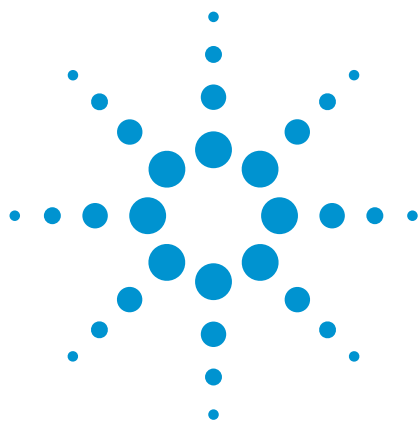
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Agilent Technologies



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 H₂O

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.

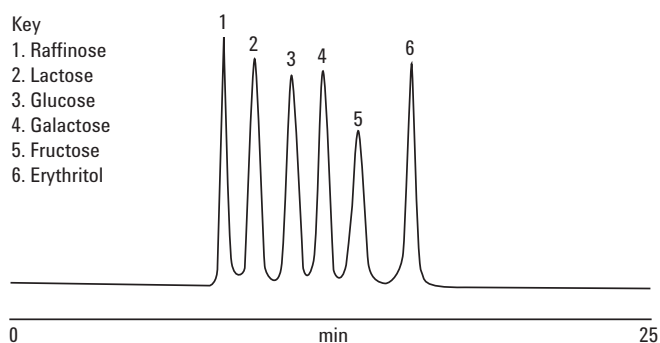


Figure 1. Good separation of six simple sugars using the Agilent ELSD and an Agilent Hi-Plex Ca column.

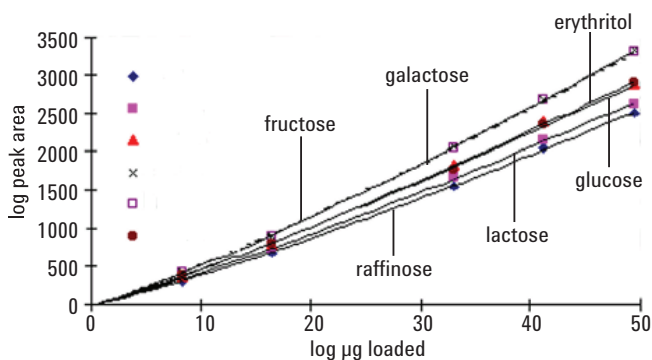


Figure 2. Calibration curves of six sugars using the Agilent ELSD and an Agilent Hi-Plex Ca system.

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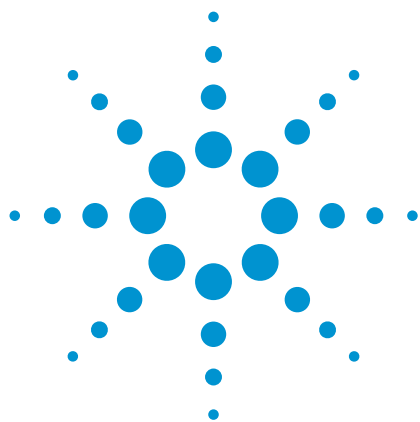
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Agilent Technologies



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 H₂O

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.

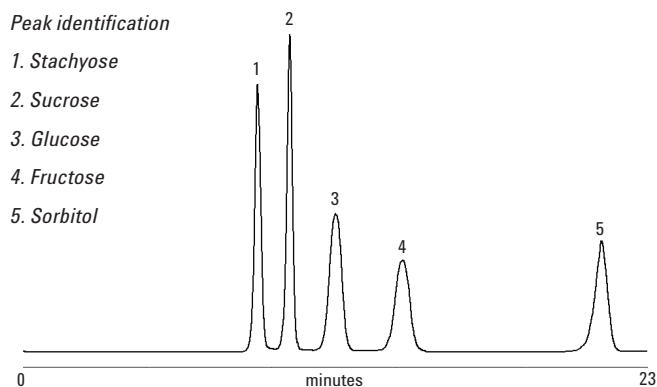


Figure 1. Excellent separation to baseline of five saccharides by Agilent Hi-Plex Ca columns with the Agilent ELSD.

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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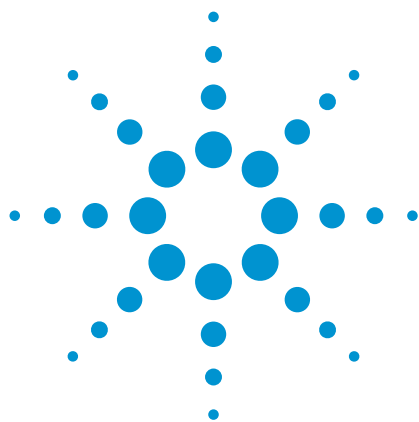
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Agilent Technologies



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 H₂SO₄ (sugars), 0.0005 M H₂SO₄
(compounds of physiological significance)

Conditions

Flow rate 0.6 mL/min
Temperatures 60 °C (sugars), 55 °C (compounds of physiological significance)

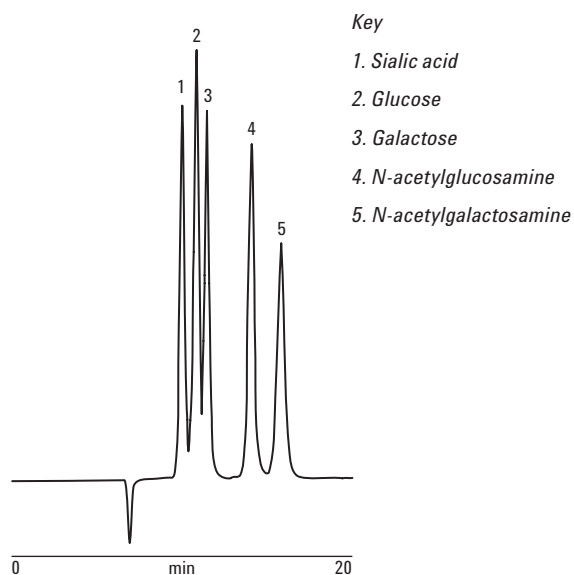


Figure 1. Good separation of glucose and galactose from their derivatives achieved by HPLC with Agilent Hi-Plex H columns.

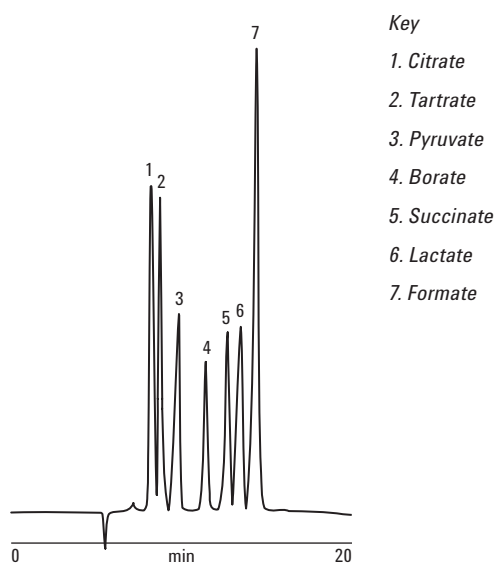


Figure 2. The presence of lactate and pyruvate revealed by HPLC with Agilent Hi-Plex H columns.

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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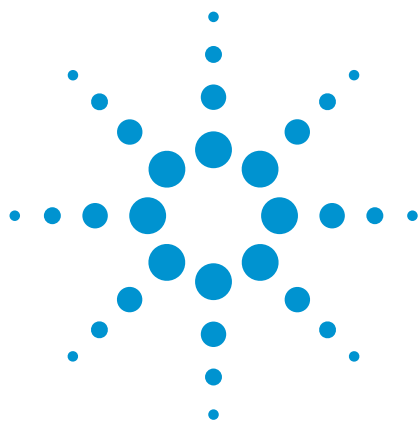
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Oligosaccharide Analysis on Agilent Hi-Plex Phases

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 ₂ O
Flow rate	0.4 mL/min
Temperature	85 °C
Detector	RI

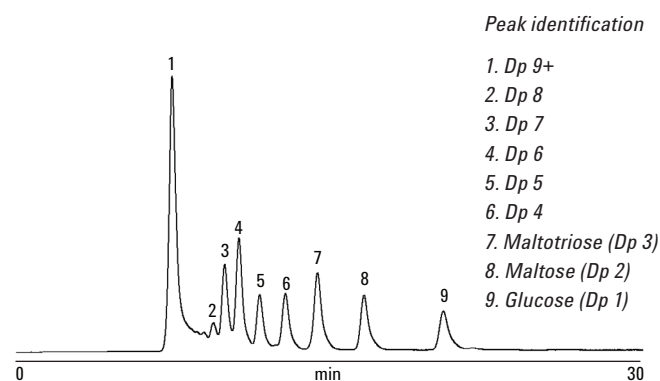


Figure 1. Oligosaccharide separation up to Dp 8 using an Agilent Hi-Plex Na column.

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 ₂ O
Flow rate	0.5 mL/min
Temperature	90 °C
Detector	RI

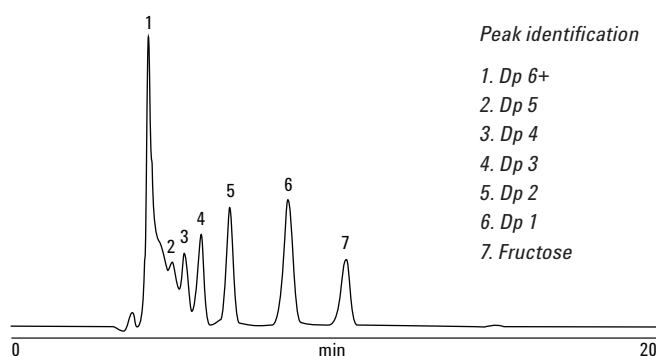


Figure 2. Oligosaccharide separation up to Dp 5 using an Agilent Hi-Plex Ca (Duo) column.

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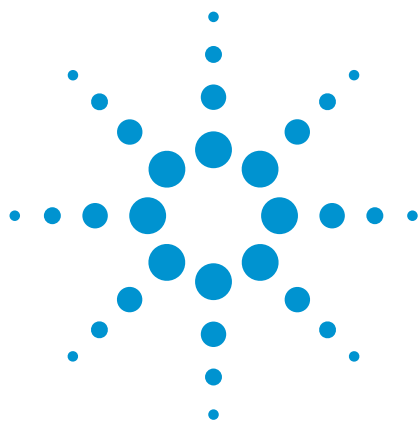
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Agilent Technologies



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 ₂ SO ₄
Flow rate	0.5 mL/min
Injection volume	20 µL
Temperature	Ambient
Detector	RI

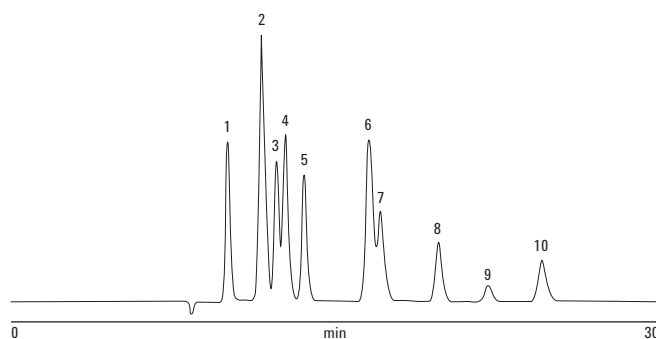


Figure 1. Separation of a sugars and acids mixture using an Agilent Hi-Plex H 8 µm column. See Table 1 for peak identification.

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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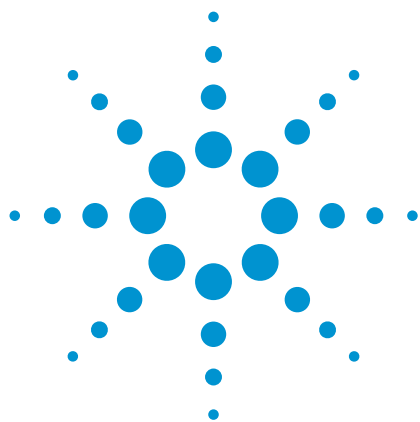
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Agilent Technologies



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 ₂ O
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.

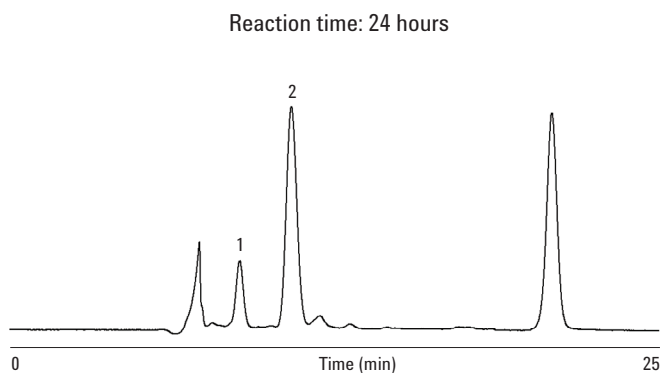
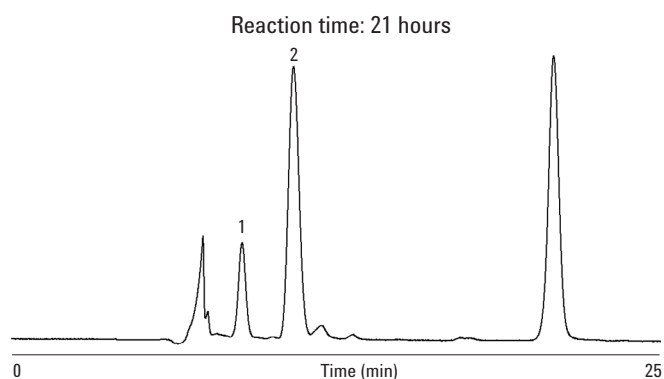
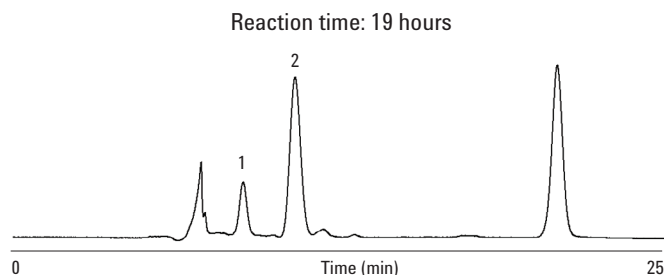
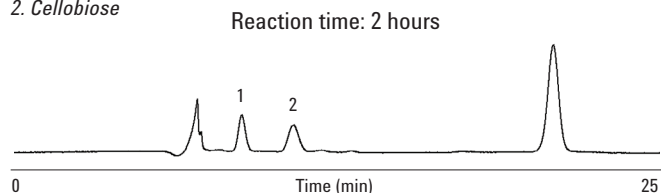
Cellobiose is a disaccharide derived from the condensation of two glucose molecules linked in a β (1 $\Rightarrow$ 4) bond. This is a byproduct of the enzyme-catalyzed hydrolysis of cellulose.

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



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.

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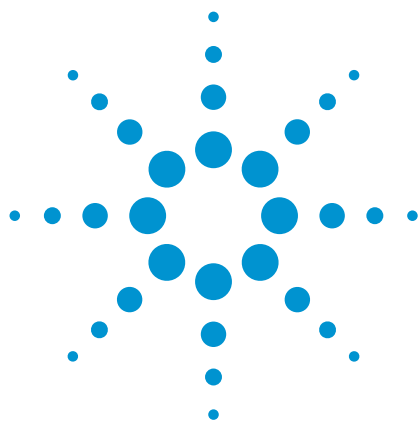
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Agilent Technologies



Analysis of Bioethanol Fermentation Products Using an Agilent Hi-Plex H Column

Application Note

Biofuels

Author

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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.



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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 ₂ SO 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.

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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

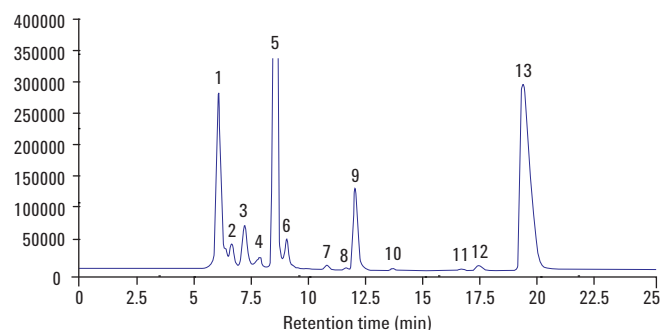


Figure 1. HPLC chromatogram of biomass and yeast in water, using an Agilent Hi-Plex H column.

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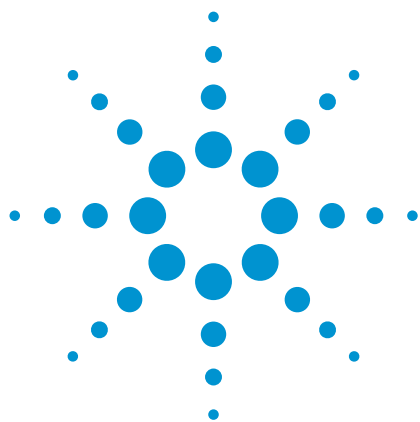
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Agilent Technologies



Analysis of Byproducts in Fermentation Liquids Using an Agilent Hi-Plex H Column

Application Note

Food and Beverage

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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.



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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 ₂ SO 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

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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

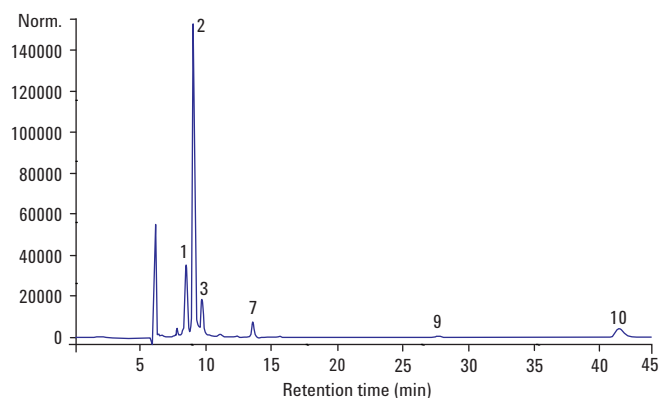


Figure 1. Analysis of a sample of straw after hydrothermal digestion using an Agilent Hi-Plex H column.

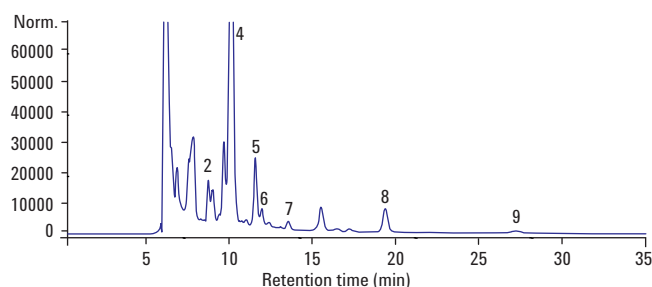


Figure 2. Components of a straw sample after fermentation of xylose to xylitol.

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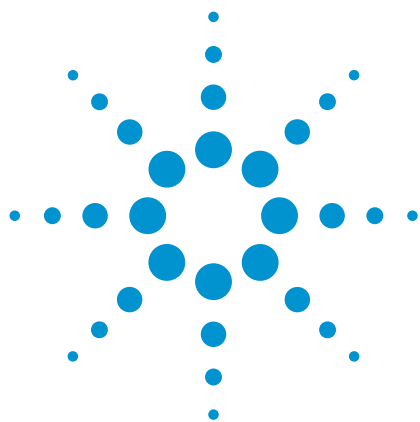
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Agilent Technologies



Analyzing Liquid Fractions of Biogas Processes by HPLC

Application Note

Biofuels

Author

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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.



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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 ₂ SO 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.

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Peak identification (for both figures)

- | | |
|--------------------|--|
| 1. Formic acid | 7. 3,3-Dimethyl butyric acid (Internal Standard) |
| 2. Acetic acid | 8. Valeric acid |
| 3. Propionic acid | 9. Isocaproic acid |
| 4. Isobutyric acid | 10. Caproic acid |
| 5. Butyric acid | x. Ethanol |
| 6. Isovaleric acid | |

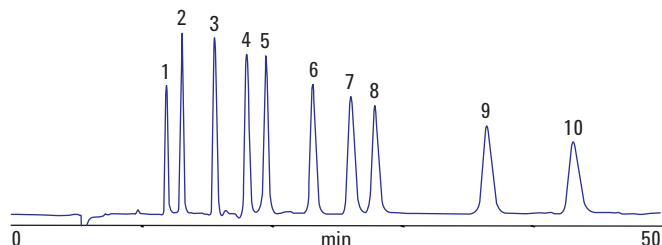


Figure 1. Analysis of a standard mixture of free fatty acids on an Agilent Hi-Plex H column.

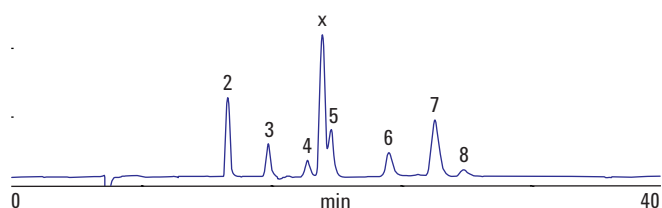


Figure 2. Separation of a biogas plant liquor by an Agilent Hi-Plex H column.

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Agilent Technologies



Analysis of Sugars from Biomass Fermentation

Application Note

Biofuels

Author

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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.



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Materials and Methods

Conditions

Column	Agilent Hi-Plex Pb, 7.7 × 300 mm, 8 µm (p/n PL1170-6820)
Mobile phase	100% DI H ₂ O
Gradient	Isocratic
Flow rate	0.5 mL/min
Injection volume	20 µL
Sample concentration	1 g/L for each component
Temperature	70 °C
Pressure	2.5 MPa (25 bar, 360 psi)
Detector	RI (55 °C)

Peak identification

- 1 Glucose
- 2 Xylose
- 3 Galactose
- 4 Arabinose
- 5 Mannose
- 6 HMF

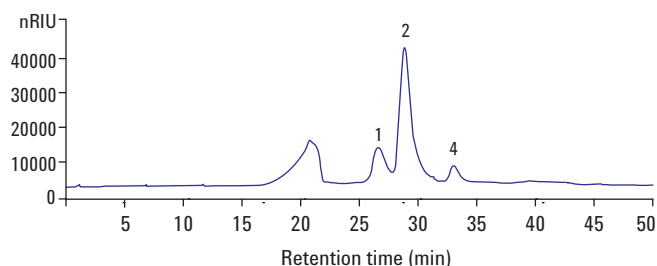


Figure 1. Analysis of a sample of straw after hydrothermal digestion using an Agilent Hi-Plex Pb column.

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.

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Peak identification

- 1 Glucose
- 2 Xylose
- 3 Galactose
- 4 Arabinose
- 5 Mannose
- 6 HMF

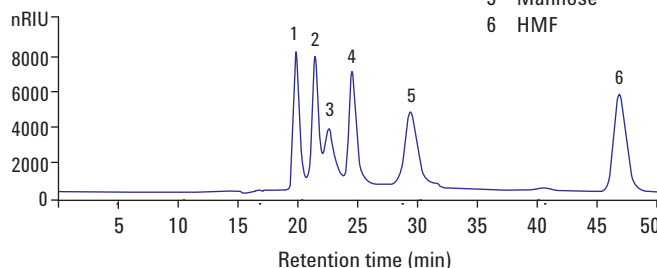


Figure 2. Standard curve of the main hemicellulose-forming monosaccharides and hydroxymethylfurfural (HMF), an oxidation product of sugars.

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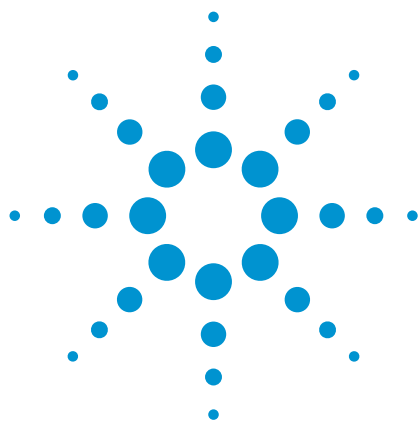
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Agilent Technologies



Organic Acids in Silage

Application Note

Food and Environmental

Author

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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.



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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 ₂ SO 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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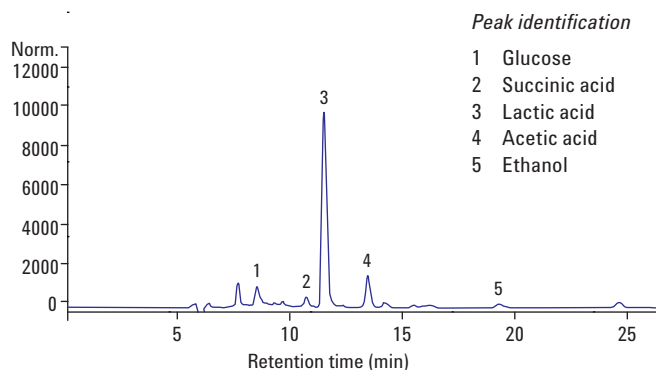


Figure 1. Analysis of grass silage using an Agilent Hi-Plex H column.

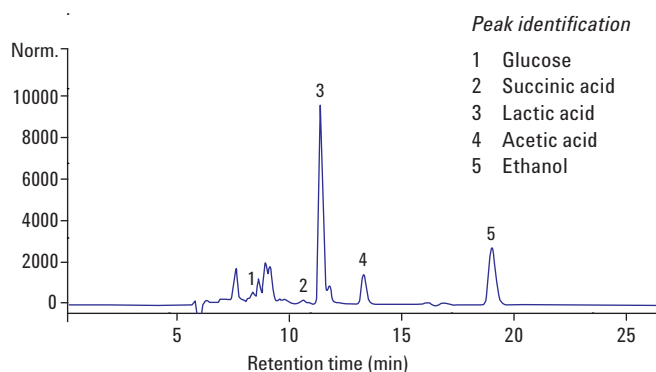


Figure 2. Separation of corn silage using an Agilent Hi-Plex H column.

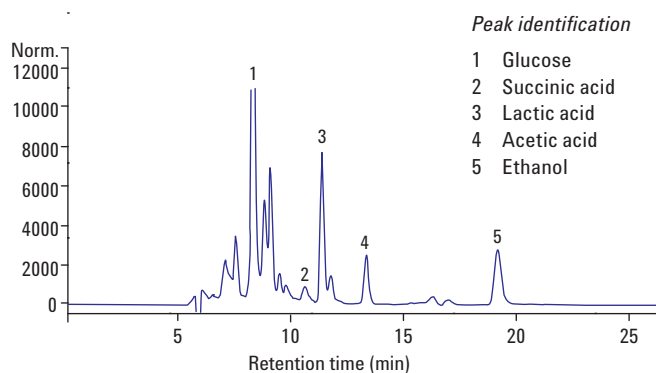


Figure 3. Analysis of whole plant silage using an Agilent Hi-Plex H column.

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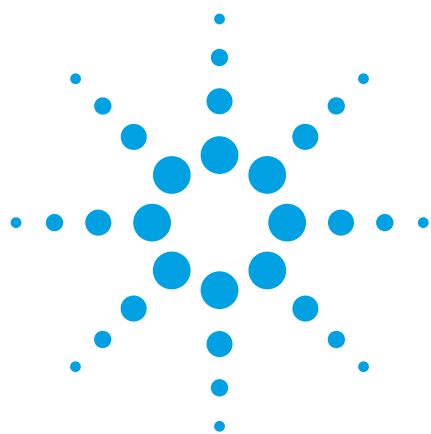
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Agilent Technologies



High Temperature GC Analysis of Fischer-Tropsch Reaction Products using the Agilent J&W FactorFour VF-5ht Ultimetall 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.

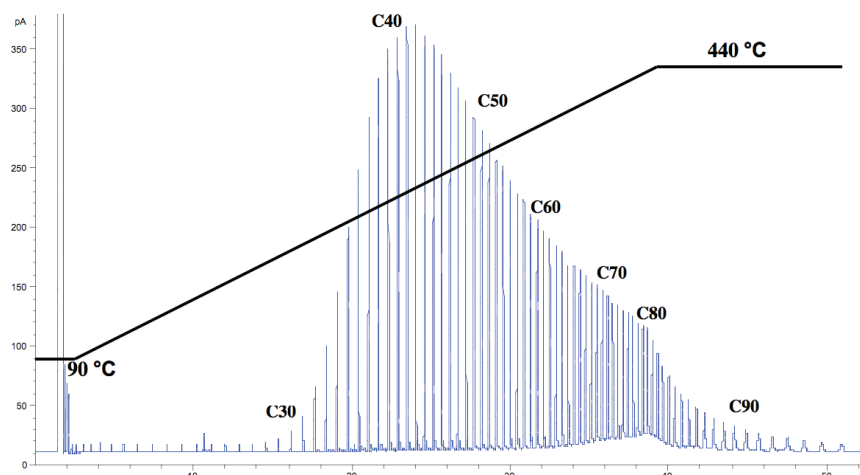


Figure 1. High temperature GC analysis of Fischer-Tropsch synthetic paraffins on VF-5ht UltiMetal column



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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.

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

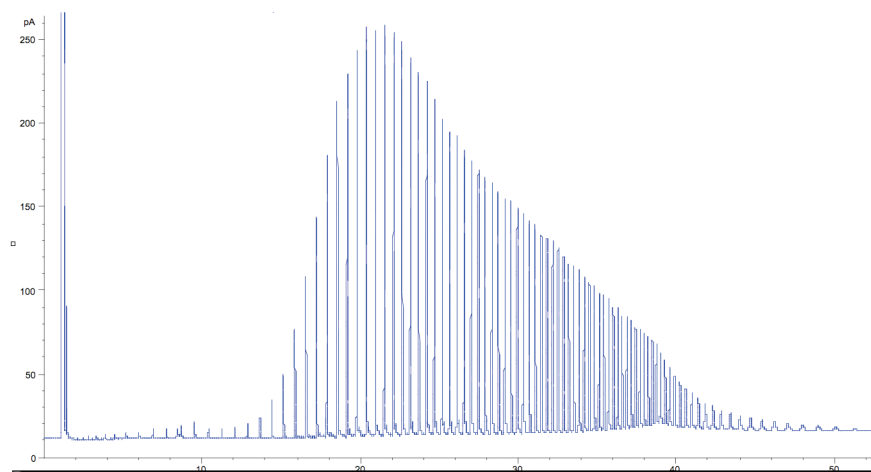


Figure 2. High temperature GC analysis of Fischer-Tropsch synthetic paraffins on VF-5ht UltiMetal column (500 analyses cycles)

Data - courtesy of Susanne Buchwaldt, Sasol Wax GmbH

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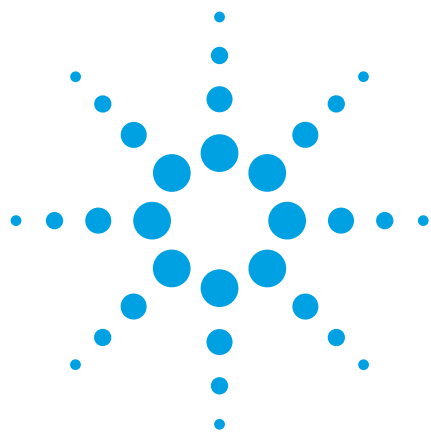
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SI-2105



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 substituted 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		Peak Identification	
Technique:	GC-FID	1.	C4:0
Column:	Select CP-Sil 88 for FAME, 100 m x 0.25 mm df=0.2 µm (part number CP7489)	2.	C6:0
Oven:	80 °C, 4 °C/min to 220 °C, 5 min, 4 °C/min to 240 °C, 10 min	3.	C8:0
Carrier Gas:	Helium, constant flow 1.0 mL/min	4.	C10:0
Injection:	Splitflow 20 mL/min, Temperature = 250 °C	5.	C11:0
Injection Volume:	0.5 µL	6.	C12:0
Detection:	FID, 270 °C	7.	C13:0
Sample:	37 component FAME mix	8.	C14:0
Sample Solvent:	Dichloromethane	9.	C14:1
Sample Conc:	2-4% (w/w)	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

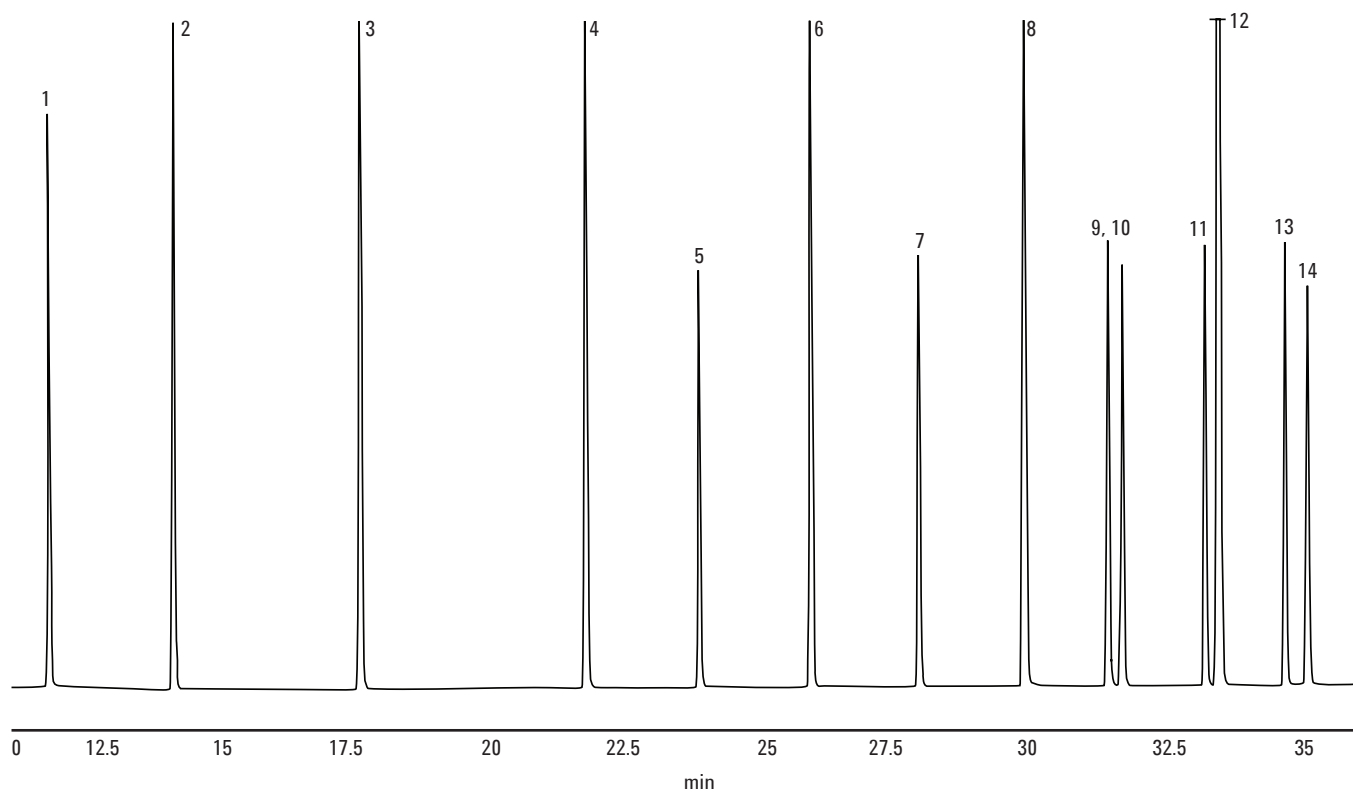


Figure 1a. Separation of a 37 component FAME mix (peaks 1-14)

Peak Identification

1. C4:0	14. C17:0	27. C22:0
2. C6:0	15. C17:1	28. C20:3 cis 8,11,14
3. C8:0	16. C18:0	29. C22:1 cis 13
4. C10:0	17. C18:1 trans 9	30. C20:3 cis 11,14,17
5. C11:0	18. C18:1 cis 9	31. C20:4 cis 5,8,11,14
6. C12:0	19. C18:2, trans 9, 12	32. C23:0
7. C13:0	20. C18:2 cis 9,12	33. C22:2 cis 13,16
8. C14:0	21. C20:0	34. C24:0
9. C14:1	22. C18:3 cis 6,9,12 gamma	35. C20:5 cis 5,8,11,14,17 EPA
10. C15:0	23. C20:1 cis 11	36. C24:1 cis 15
11. C15:1	24. C18:3 cis 9,12,15 alpha	37. C22:6 cis 4,7,10,13,16,19 DHA
12. C16:0	25. C21:0	
13. C16:1	26. C20:2 cis 11,14	

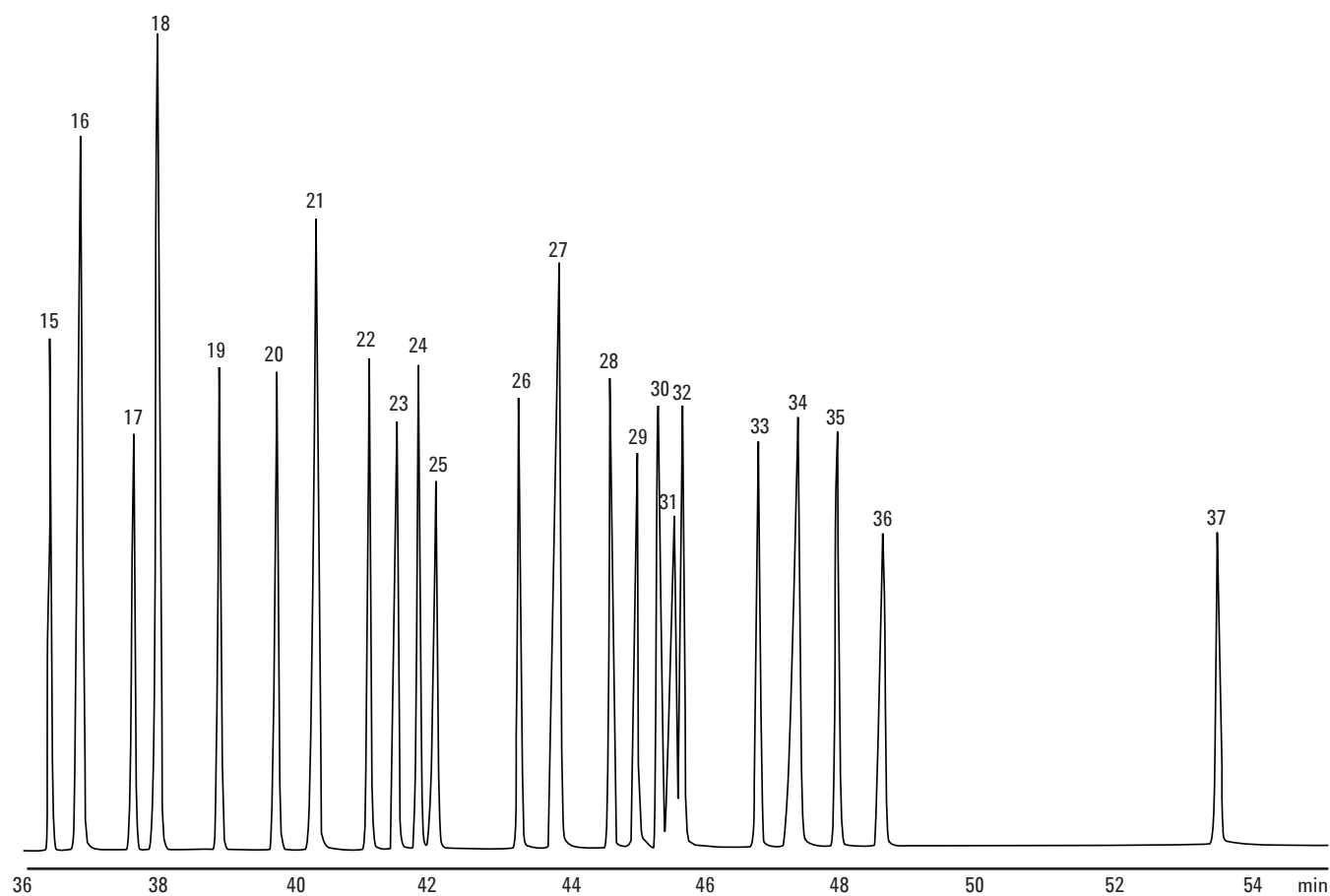


Figure 1b. Separation of a 37 component FAME mix (peaks 15-37)

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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

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.

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.

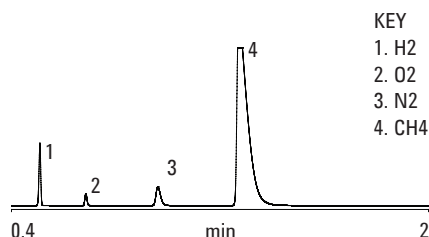


Figure 1. Permanent gases on the CP-Molsieve channel

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.

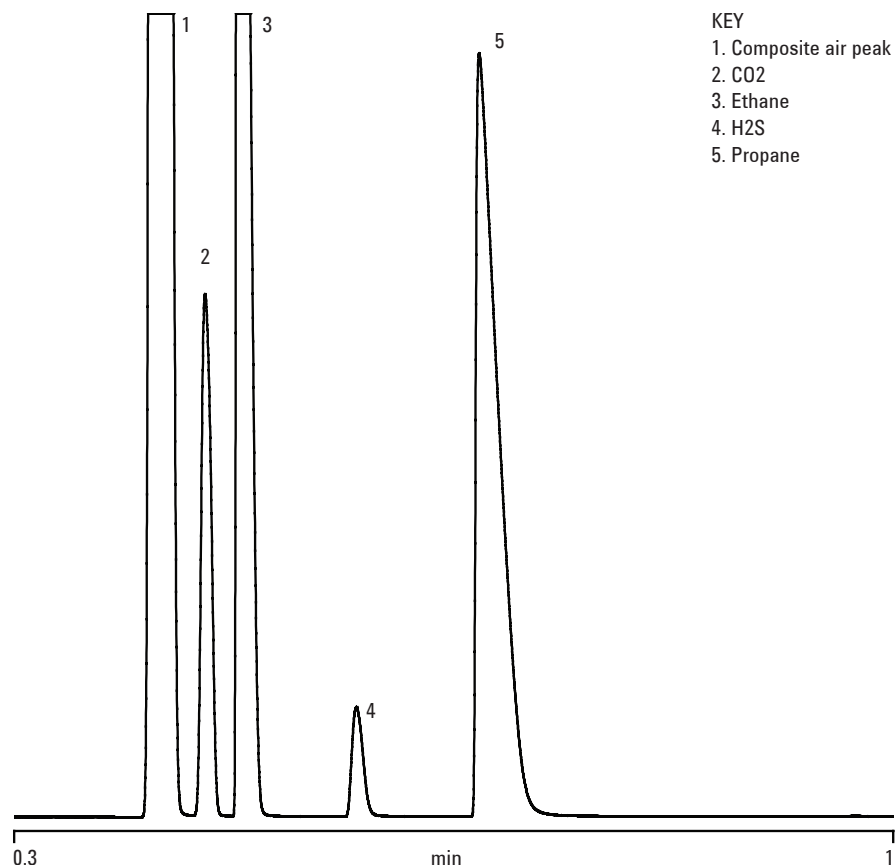


Figure 2. CO₂, H₂S, ethane and propane on the CP-PoraPLOT U channel

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.

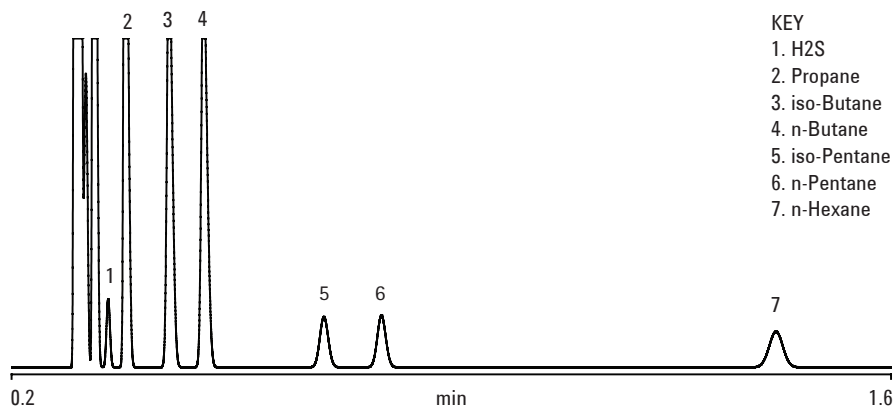


Figure 3. Higher hydrocarbons on the CP-Sil 5 CB channel

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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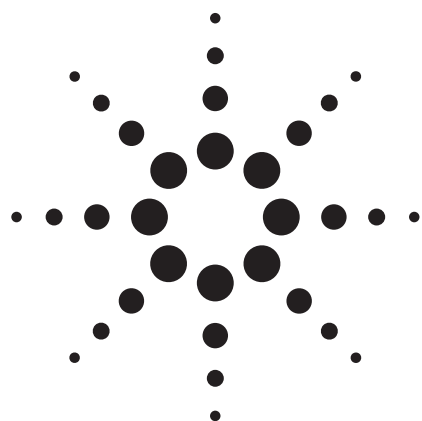
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The Measure of Confidence

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Parallel GC for Complete Refinery Gas Analysis



Application

Hydrocarbon Processing

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Abstract

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

Introduction

Refinery gas is a mixture of various gas streams produced in refinery processes. It can be used as a fuel gas, a final product, or a feedstock for further processing. An exact and fast analysis of the components is essential for optimizing refinery processes and controlling product quality. Refinery gas stream composition is very complex, typically containing hydrocarbons, permanent gases, sulfur compounds, and so on. Successful separation of such a complex gas mixture is often difficult using a single-channel GC system. Three parallel channel

analyses allow a separation problem to be divided into three sections. Each channel can optimize a particular part of the separation. TCD with helium carrier gas can be used for permanent gases analysis like O₂, N₂, CO, CO₂, H₂S, and COS. However, hydrogen has only a small difference in thermal conductivity compared to helium, making analysis by TCD using helium carrier gas difficult. To achieve full-range capability for hydrogen, an additional TCD with nitrogen or argon as a carrier is required. Light hydrocarbons are separated on an alumina PLOT column and detected on a FID.

The Agilent 7890A GC now supports an optional third detector (TCD), allowing simultaneous detection across three channels; this provides a complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and hydrocarbons to nC₅, C₆+ fraction within six minutes.

Experimental

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

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



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

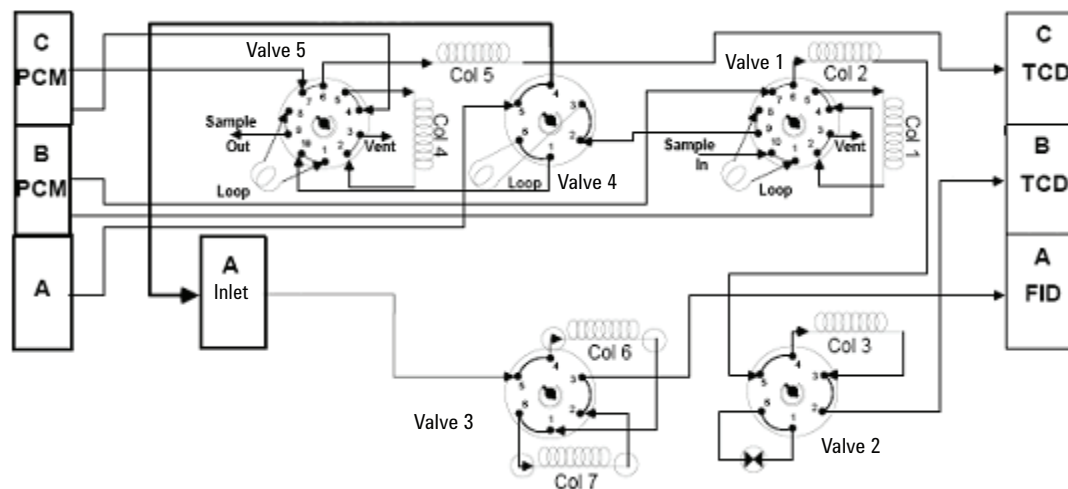
A new tube connector based on capillary flow technology is used to connect the valve to the capillary column to enhance the hydrocarbons analysis by improving the peak shape.

The second TCD channel (B TCD) employs three packed columns and two valves for the separation of permanent gases including O_2 , N_2 , CO , and CO_2 using helium as a carrier gas. Valve 1 is a 10-port valve used for gas sampling and backflushing heavier components; normally components heavier than ethylene are backflushed to vent when H_2S is not required to be analyzed. A six-port isolation

valve (valve 2) with adjustable restrictor is used to switch the molecular sieve 5A column in and out of the carrier stream. Initially, the isolated valve is in the OFF position so that unresolved components air, CO , and CH_4 pass quickly through the HayeSep Q (column 2) onto the molecular sieve (column 3). The valve is then switched to the ON position to trap them in column 3 and allow the CO_2 to bypass this column. When the CO_2 has eluted, valve 2 is switched back into the flow path to allow O_2 , N_2 , CH_4 , and CO to elute from the molecular sieve column.

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

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



Column 1 HayeSep Q 80/100 mesh
Column 2 HayeSep Q 80/100 mesh
Column 3 Molsieve 5A 60/80 mesh
Column 4 HayeSep Q 80/100 mesh

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

Figure 1. RGA valve system.

Table 1. Typical GC Conditions for Fast Refinery Gas Analysis

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

Table 2. RGA Calibration Gas Standards

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

Results and Discussion

Enhance Gas Analysis with Union Connector

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

Fast Refinery Gas Analysis (RGA)

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

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

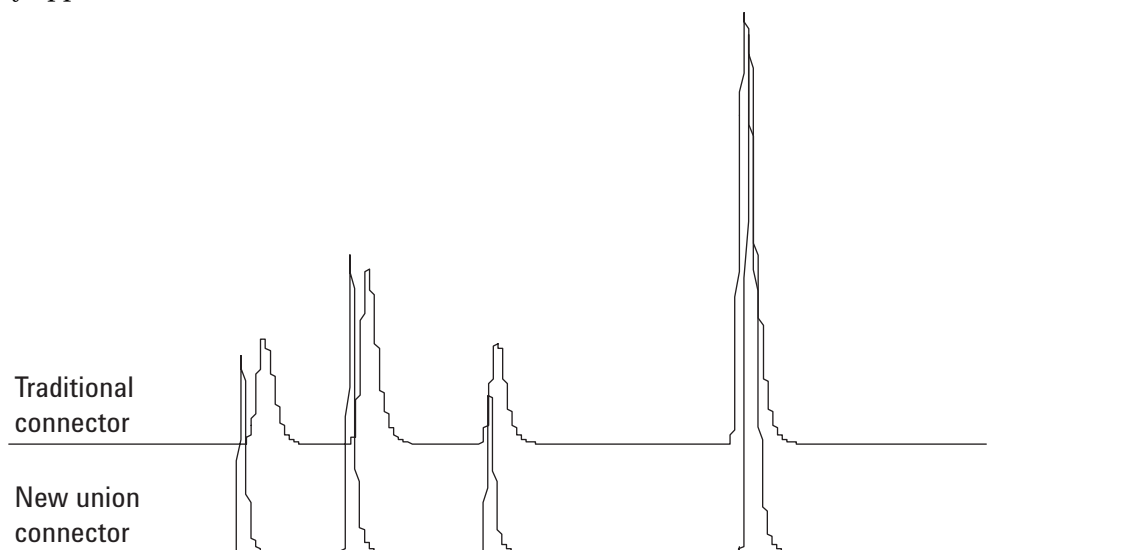


Figure 2. Hydrocarbon peaks obtained from traditional tube connector and new union connector.

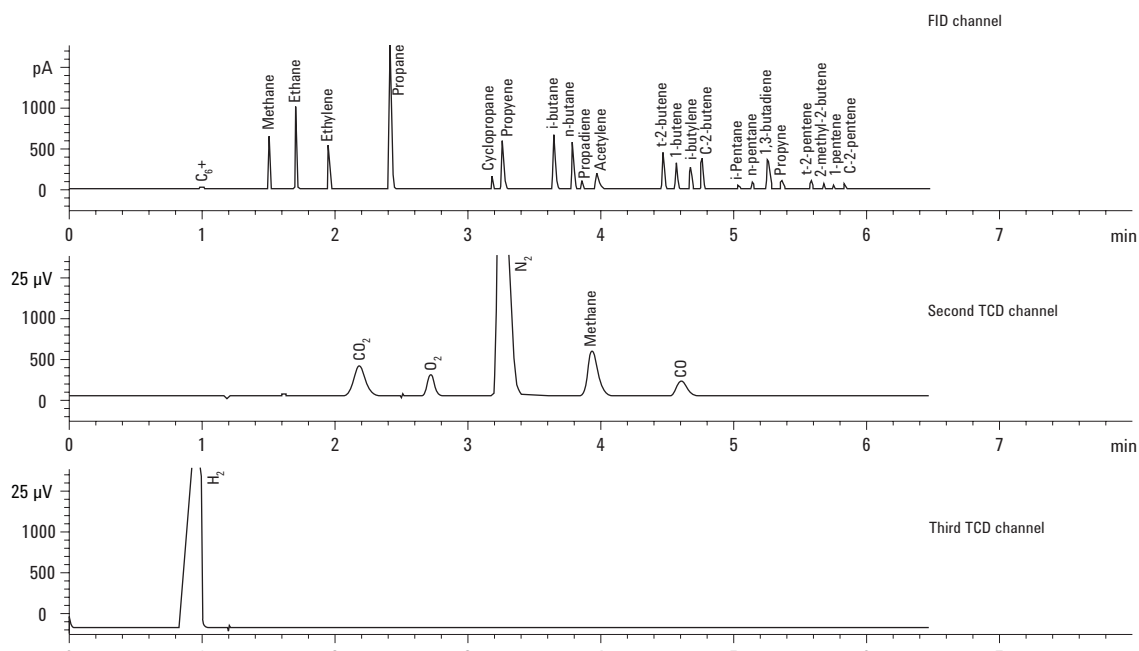


Figure 3. Refinery gas calibration standards analysis. The concentrations for each compound are shown in Table 2.

The top chromatogram (FID channel) is the hydrocarbon analysis. The PLOT alumina column provides excellent separation of hydrocarbons from C₁ to nC₅, including 22 isomers. Components heavier than nC₆ are backflushed early as a group (C₆+) through the precolumn. The middle chromatogram (second TCD channel) is the separation of permanent gases using helium as a carrier gas. The bottom chromatogram (third TCD channel) is the

separation of hydrogen, since hydrogen has only a little difference in thermal conductivity compared to helium. Use of an additional TCD with nitrogen (or argon) as a carrier gas improves the hydrogen detectability and linearity.

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

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

Compounds	Retention time			Average	Area	
	Average	Std. dev.	RSD%		Std. dev.	RSD%
C ₆ +	0.99648	0.00031	0.03	59.01	1.10	1.86
Methane	1.50780	0.00046	0.03	490.02	1.45	0.30
Ethane	1.70788	0.00052	0.03	807.40	2.35	0.29
Ethylene	1.95732	0.00071	0.04	472.31	1.31	0.28
Propane	2.41706	0.00075	0.03	1950.35	5.96	0.31
Cyclopropane	3.18506	0.00075	0.02	145.62	0.45	0.31
Propylene	3.26195	0.00072	0.02	732.90	2.01	0.27
i-butane	3.64883	0.00055	0.02	885.04	3.15	0.36
n-butane	3.79161	0.00070	0.02	682.13	2.59	0.38
Propadiene	3.86098	0.00095	0.02	109.08	0.65	0.60
Acetylene	3.96990	0.00120	0.03	348.17	2.39	0.69
t-2-butene	4.47301	0.00106	0.02	507.88	2.59	0.51
1-butene	4.57118	0.00110	0.02	332.39	2.03	0.61
i-butylene	4.67529	0.00121	0.03	260.95	1.95	0.75
c-2-butene	4.76367	0.00112	0.02	403.80	3.47	0.86
i-pentane	5.03923	0.00090	0.02	45.03	0.05	0.11
n-pentane	5.14583	0.00099	0.02	69.23	0.40	0.58
1,3-butadiene	5.25906	0.00122	0.02	485.49	3.66	0.75
Propyne	5.36385	0.00155	0.03	101.08	0.41	0.40
t-2-pentene	5.58664	0.00121	0.02	82.85	0.66	0.79
2-methyl-2-butene	5.68220	0.00117	0.02	62.54	0.61	0.98
1-pentene	5.75553	0.00126	0.02	39.57	0.38	0.96
c-2-pentene	5.83970	0.00131	0.02	59.08	0.50	0.85
CO ₂	2.18561	0.00221	0.10	2040.33	2.37	0.12
O ₂	2.72634	0.00060	0.02	930.68	6.53	0.70
N ₂	3.25170	0.00044	0.01	22500.18	68.87	0.31
CO	4.61692	0.00083	0.02	903.09	2.77	0.31
H ₂	0.9869	0.00099	0.10	16097.38	106.53	0.66

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

Flexibility for Hydrocarbon Analysis

The system is very flexible for hydrocarbon analysis. By setting up different valve (valve 3) switch times, the early backflush group can be C₆+ followed by individual C₁ to C₅ hydrocarbons as mentioned in fast RGA, or C₇+ followed by individual C₁ to C₆ hydrocarbons, or no backflush to separate C₁ to C₉ individual hydrocarbons. The top chromatogram in Figure 5 is the result with backflush group of C₆+, the middle one is that of C₇+, and the

bottom one is that of no backflush. With such flexibility, a wide range of refinery gas and natural gas compositions can be measured reliably without hardware or column changes.

H₂S and COS Analysis

H₂S and COS (methyl-mercaptan) can be analyzed on the rear TCD channel by adding an additional delay to the backflush time (valve 1) to allow H₂S and COS to elute onto column 2 (HayeSep Q). The analysis time is extended an additional 3 to 4 minutes, and requires a sample containing no water. Figure 6 shows the chromatogram of H₂S at approximately 500 ppm and COS 300 ppm with 1 mL sample size. The Nickel tubing packed columns and Hastelloy-C valves can be chosen for high concentration of H₂S analysis to minimize corrosion.

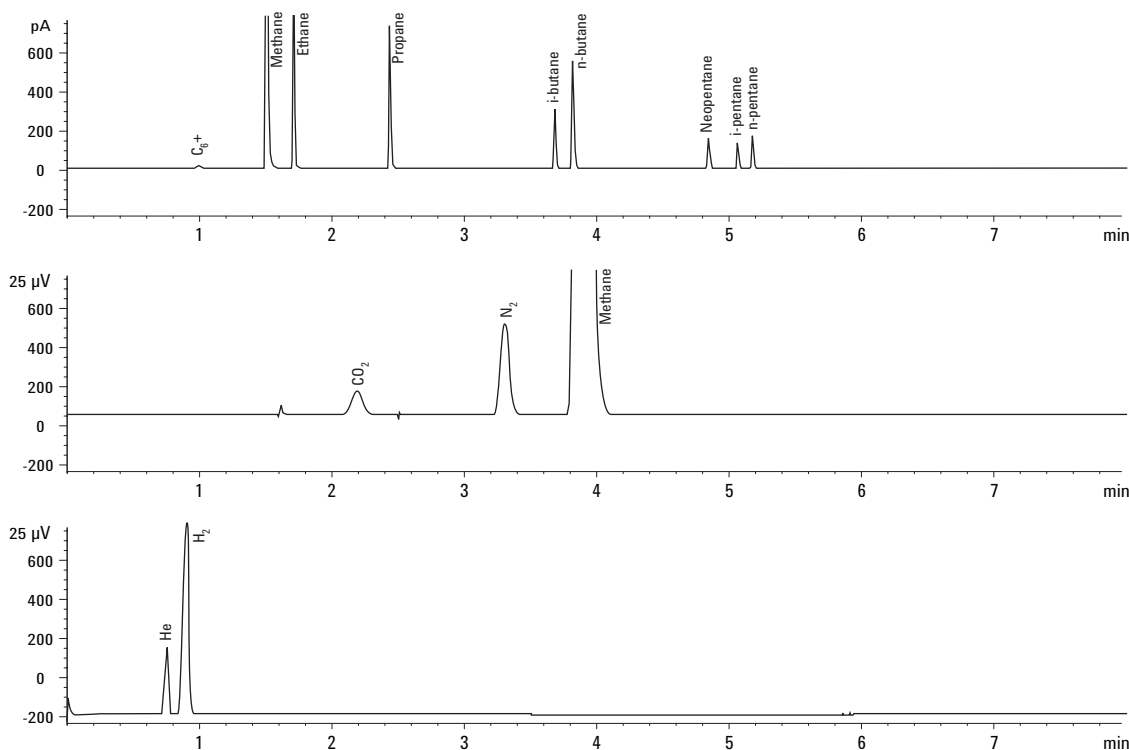


Figure 4. Natural gas analysis of a calibration gas.

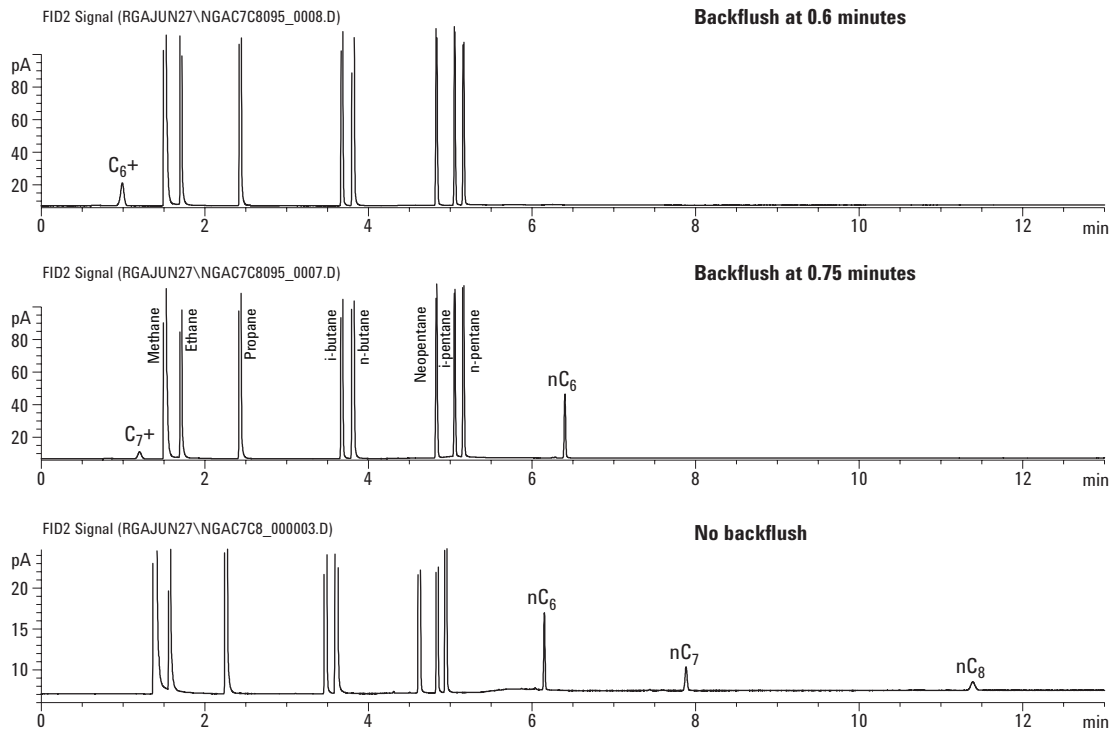


Figure 5. Chromatograms of light hydrocarbons on FID channel with different backflush times .

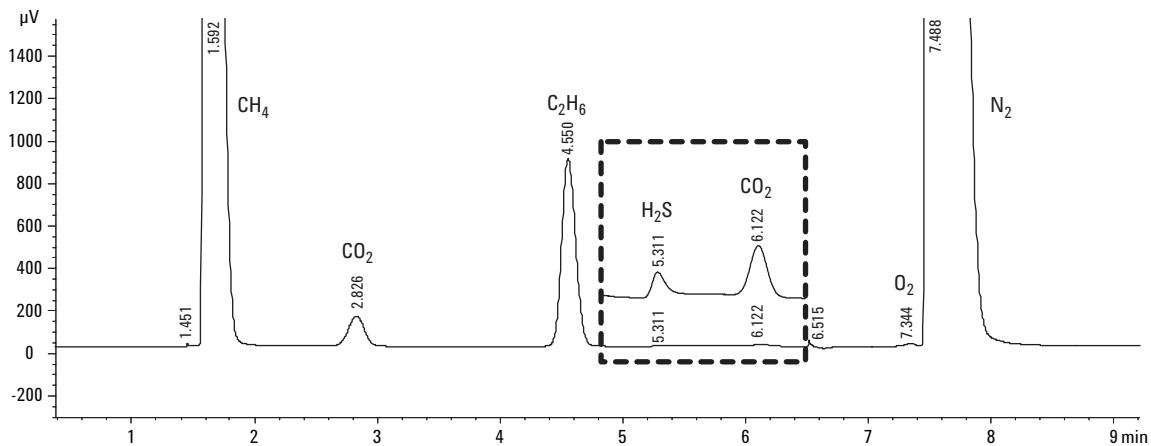


Figure 6. H₂S at approximately 500 ppm and COS 300 ppm on second TCD channel.

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

Reporting

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

Conclusions

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

One 7890A GC configured with three parallel channels with simultaneous operation provides complete analysis of permanent gases, including nitrogen, hydrogen, helium, oxygen, carbon monoxide, carbon dioxide, and all hydrocarbons to C₅ and C₆+ as a group within six minutes. A second TCD with nitrogen or argon as a carrier gas improves the hydrogen sensitivity and linearity.

The configuration is very flexible for hydrocarbon analysis, different backflush times may be set to obtain the early backflush group for C₆+ or C₇+, or no backflush to separate C₁ to C₁₀ individual hydrocarbons. In these cases, the analysis time is increased by 6 minutes. H₂S and COS can be analyzed on the same GC configuration; it requires 3 to 4 minutes of additional time.

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

References

1. ASTM D1945-03, "Standard Test Method for Analysis of Natural Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
2. ASTM D1946-90 (2006), "Standard Practice for Analysis of Reformed Gas by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
3. UOP Method 539, "Refinery Gas Analysis by Gas Chromatography," ASTM International, 100 Bar Harbor Drive, West Conshohocken, PA 19428, USA.

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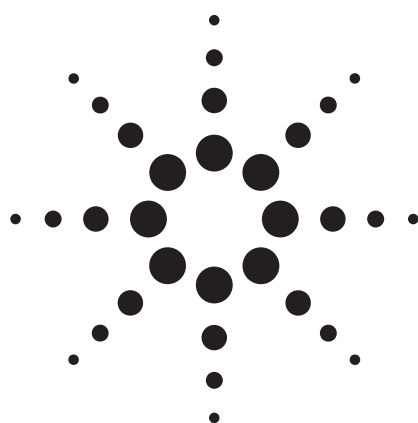
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Parallel GC for Complete RGA Analysis

Application Brief



Chunxiao Wang

A previous application brief [1] has shown that a 7890A GC configured with three parallel channels provides a complete refinery gas analysis (RGA) within six minutes. The configuration for fast RGA in the brief has been updated by adding a fifth valve, which can now be supported by the 7890A GC. The updated configuration is almost the same as the previous one except for the third channel (TCD) for H₂ analysis using N₂ or Ar as carrier gas to improve H₂ detectability and linearity. The updated configuration uses a 10-port valve with a pre-column for backflushing late-eluting components while H₂ is separating on the molsieve column instead of a three-way splitter plus split/splitless inlet.

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

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

Highlights

- One 7890A GC configured with three parallel channels with simultaneous detection provides a comprehensive, fast, and high-resolution analysis of refinery gas in 6 minutes.
- Use of optimized columns allows faster analysis of hydrocarbons and permanent gases using a single oven temperature program without the need for an additional column oven.
- A third TCD channel can be used for improving hydrogen detection and linearity by using nitrogen (or argon) as carrier gas.
- A new, easy-to-use union tubing connector based on capillary flow technology is used to connect valves and capillary columns to improve the chromatographic performance, including peak shape.
- Excellent results are achieved. The lowest detection limit is 50 ppm for all compounds, 500 ppm for hydrogen sulfide.
- ChemStation macro program is supplied for RGA reporting.
- The system can be obtained by ordering option SP1 7890-0322 for the standard fast RGA and 7890-0338 for the fast RGA with Hastelloy valves and nickel tubing for H₂S containing samples on the 7890A.

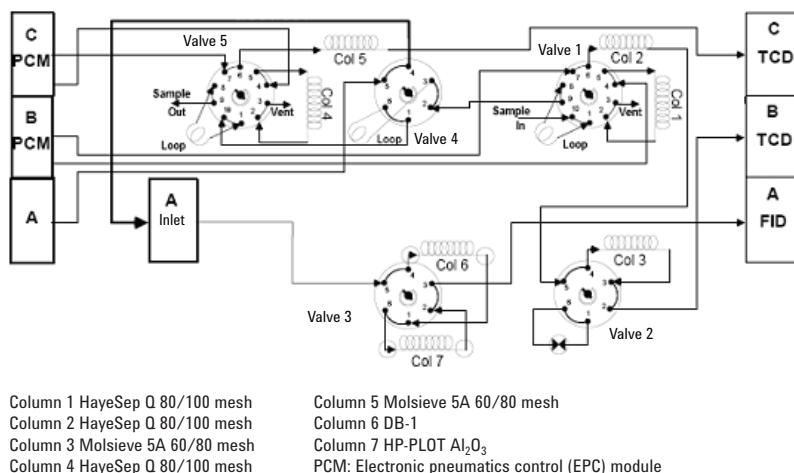


Figure1. RGA valve system.



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

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

Separation resulting from each channel is illustrated in Figure 2. The top chromatogram shows the hydrocarbon analysis. A PLOT AL203 column provides excellent separation of hydrocarbons from C1 to nC5 containing 22 isomers. Components heavier than nC6 are backflushed early in the run as a group (C6+) through a short DB-1 pre-column. The middle chromatogram shows the separation of permanent gases using helium as the carrier gas on the second TCD channel (B TCD). H₂S and COS can be analyzed on the second TCD channel as well, requiring 3 to 4 additional minutes. The bottom chromatogram shows the

separation of hydrogen. Because hydrogen has only a small difference in thermal conductivity compared to helium, it requires an additional TCD with nitrogen or argon as the carrier gas to improve the hydrogen detectability and linearity. All channels operate simultaneously to provide a comprehensive, fast analysis with high resolution of components. A macro program automatically provides the calculation of gas properties. Reports can be generated using formulas specified in the ASTM/GPA and/or ISO standards. Reports in mole%, weight%, volume%, or any combination of the three are available.

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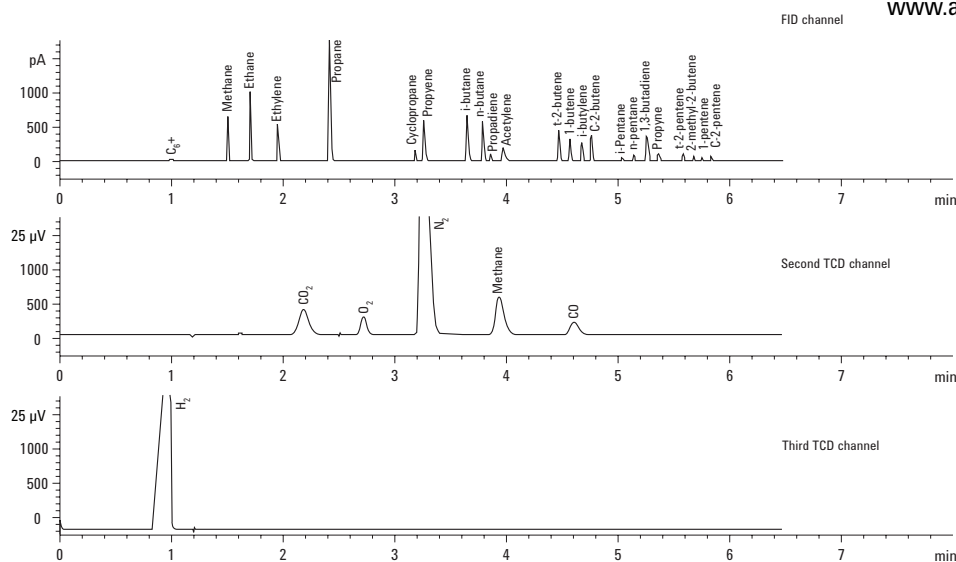


Figure 2. Refinery gas calibration standards analysis.

Reference

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

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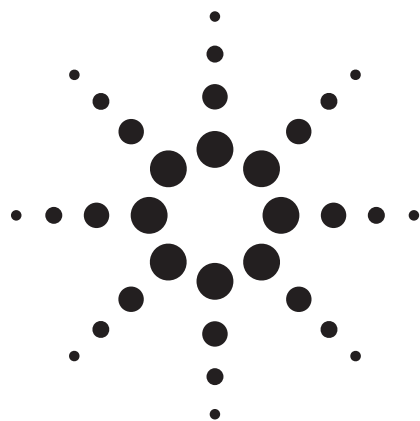
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Fast Analysis of Aromatic Solvent with 0.18 mm ID GC column



Application

Gas Chromatography

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Abstract

Fast GC is one possible way to improve productivity. By reducing the internal diameter of the capillary column, a higher efficiency per unit of column length is obtained in capillary GC. Combined with shorter column length, the application of high-efficiency 0.18-mm-id GC column results in faster analyses compared to conventional 0.25-mm- or 0.32-mm-id columns without losing measurement performance. A single, rapid GC method for aromatic solvent purity analysis is described.

Introduction

Determination of the purity of aromatic hydrocarbons is critical for many QA and QC laboratories in the chemical and petrochemical industry. In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and published a number of GC methods specifically for an aromatic compound or a class of aromatic compounds such as styrene, o-xylene, p-xylene, and ethylbenzene. Table 1 lists 10 ASTM methods along with the recommended columns and specifications [1].

Many QA/QC labs need to run these different ASTM methods to ensure the quality of all products. These analyses can be difficult and expensive to perform. Because many of these ASTM methods are remarkably alike, it is highly desirable to develop a single method that is the chromatographic equivalent of the individual methods. Detailed discussions on an unified aromatic solvent method are available in the literature [2, 3].

Due to demands for increased productivity, many QC/QA laboratories need to analyze large numbers of samples every day. Faster analysis is highly desirable for increased sample throughput and therefore lower cost per sample.



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Table 1. Ten ASTM Methods for the GC Analysis of Aromatic Solvents

ASTM Method	Title	Liquid phase	Column type	Report specifications
D2306	Std test for C8 aromatic hydrocarbons	0.25 μ m Carbowax	Capillary 50 m $\times$ 0.25 mm	wt% of individual C8 HC
D2360	Std test for trace impurities in monocyclic hydrocarbons	0.32 μ m Carbowax	Capillary 60 m $\times$ 0.32 mm	wt% of individual aromatic impurities, total impurities, purity
D3760	Std test for cumene	0.25 μ m Carbowax	Capillary 50 m $\times$ 0.32 mm	wt% of individual impurities, cumene purity (wt%)
D3797	Std test for o-xylene	0.5 μ m Carbowax	Capillary 60 m $\times$ 0.32 mm	wt% of individual impurities, o-xylene purity (wt%)
D3798	Std test for p-xylene	0.25 μ m Carbowax	Capillary 50 m $\times$ 0.32 mm	wt% of individual impurities, total impurities, p-xylene purity (wt %)
D4492	Std test for benzene	0.25 μ m Carbowax	Capillary 50 m $\times$ 0.32 mm	wt% of individual impurities, benzene purity (wt%)
D4534	Std test for benzene in cyclic products	10% TCEPE on Chromasorb P	Packed 3.7 m $\times$ 3.175 mm	wt% of benzene
D5060	Std test for impurities in ethylbenzene	0.5 μ m Carbowax	Capillary 60 m $\times$ 0.32 mm	wt% of individual impurities, ethylbenzene purity
D5135	Std test for styrene	0.5 μ m Carbowax	Capillary 60 m $\times$ 0.32 mm	wt% of individual impurities, styrene purity
D5917	Std test for trace impurities in monocyclic hydrocarbons (ESTD Cal)	0.25 μ m Carbowax	Capillary 60 m $\times$ 0.32 mm	wt% individual impurities, wt% total nonaromatics, wt% total C9 aromatics, purity of main component

Experimental

High-Efficiency Capillary GC Columns

Efficiency is often related to the number of theoretical plates, which increases linearly with decreasing column internal diameter (id). For instance, 0.18 mm id columns typically produce 5,800 to 6,600 theoretical plates per meter, whereas columns with 0.25 to 0.32 mm id typically produce 3,600 to 4,600 plates per meter. The efficiency improvement for the 0.18 mm id columns allows for better signal-to-noise ratios. Since decreasing the internal diameter results in an increase of the column efficiency per meter, the column length can be reduced while keeping the resolution constant. Therefore, the use of 0.18 mm id columns, also known as the high-efficiency GC columns, can help gas chromatographers substantially reduce their sample analysis time.

While it is true that an even smaller id column, such as 0.1 mm id, could lead to higher efficiency per meter, routine analysis with such a column imposes high demands on instrumentation. It requires higher inlet pressures, better split control,

and faster oven temperature heating rates. On the contrary, 0.18 mm id columns are conveniently compatible with existing standard GC equipment without the need for system modifications. Smaller id, shorter length columns require less carrier flow to achieve separations, thus reduce carrier gas usage. Therefore, high-efficiency 0.18 mm id columns can provide an easy and inexpensive way to speed up GC analysis without compromising resolution.

One note of caution when going to smaller id columns is lower sample capacity. With some special samples, it is important to find a balance among speed, sensitivity, and resolution to meet the laboratory goals. For most applications in the chemical, petrochemical, food, or flavor/fragrance industries, however, the use of HE GC columns can offer an important reduction in analysis time and, consequently, a higher sample throughput.

The purpose of this application is to demonstrate in depth the use of high-efficiency 0.18 mm id columns for faster analysis of aromatic solvents with the unified aromatic solvent analysis method.

Results and Discussion

One Agilent 6890N Series gas chromatograph and two Agilent 7890 gas chromatographs were used for this work. Each GC was equipped with a split/splitless capillary inlet, a flame ionization detector (FID), and an Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with a long-lifetime septa (Agilent part no. 5183-4761) and split-optimized liners (Agilent part no. 5183-4647). Injections were made using 10- μ L syringes (Agilent part no. 5181-3354). Agilent ChemStation was used for all instrument control, data acquisition, and data analysis.

A 50-mL n-Hexane solution was prepared containing 0.1 wt% of 27 compounds; that is, all the aromatic solvents and impurities specified for analysis by the 10 ASTM methods.

Table 2 lists the experimental conditions for Method 1 where the unified aromatic solvent analysis was performed using a conventional 60 m $\times$ 0.32 mm $\times$ 0.5 μ m HP-INNOWax column (Agilent part no. 19091N-213). The GC chromatogram is shown in Figure 1.

Table 2. Conditions for Unified Aromatic Solvents Method Using a Conventional Column (Method 1)

Column	HP-INNOWax, 60 m $\times$ 0.32 mm $\times$ 0.50 μ m
Carrier gas	Helium at 20.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	75 °C (10 min); 3 °C/min to 100 °C (0 min) 10 °C/min to 145 °C (0 min)
Detector	FID at 250 °C
Data acquisition rate	At 20 Hz
Injection size	1 μ L

The experiment was then repeated with a high-efficiency 20 m $\times$ 0.18 mm $\times$ 0.18 μ m HP-INNOWax column (Agilent part no. 19091N-577) (Method 2). Agilent GC Method Translation Software (<http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm>) was used to translate Method 1 to Method 2. Three translation modes, namely the “translate only,” “best efficiency,” and “fast analysis,” were attempted with the new column dimensions. However, co-elution of dodecane and o-xylene was observed for all three translated methods. According to ASTM methods, some modi-

fications of the temperature programs were therefore necessary to achieve a similar resolution to Method 1. The resulting experimental conditions are provided in Table 3 along with the chromatogram in Figure 2.

Table 3. Conditions for Aromatic Solvents Separations on a High-Efficiency Column (Method 2)

Column	HP-INNOWax, 20 m $\times$ 0.18 mm $\times$ 0.18 μ m
Carrier gas	Helium at 25.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	50 °C (2 min); 15 °C/min to 90 °C (0 min); 20 °C/min to 145 °C (1 min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 μ L

In order to achieve even faster separation while balancing speed and resolution, Agilent GC Method Translation Software was used to translate Method 1 to Method 3 while selecting “fast analysis” mode and using the same high-efficiency GC column. But dodecane and o-xylene could not achieve baseline separation with the obtained method as stated previously. According to ASTM methods, the obtained method conditions were used with minor adjustments of the initial temperature from 75 °C to 70 °C and the initial hold of 2 minutes to 3 minutes. Then baseline separation was obtained for dodecane and o-xylene (R_s = 2.78). Detailed experimental conditions are provided in Table 4 with the GC chromatogram in Figure 3.

Table 4. Conditions for Fast Aromatic Solvents Analysis (Method 3)

Column	HP-INNOWax, 20 m $\times$ 0.18 mm $\times$ 0.18 μ m
Carrier gas	Helium at 33.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 to 600:1 split ratio
Oven temp	70 °C (3 min); 45 °C/min to 145 °C (1 min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 to 1.0 μ L

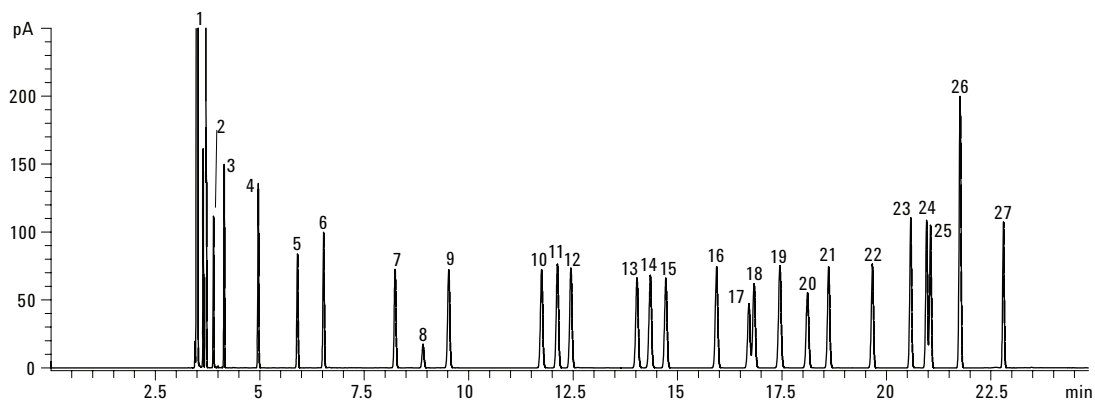


Figure 1. Unified aromatic solvent method with a 60 m × 0.32 mm × 0.5 μm HP-INNOWax column.

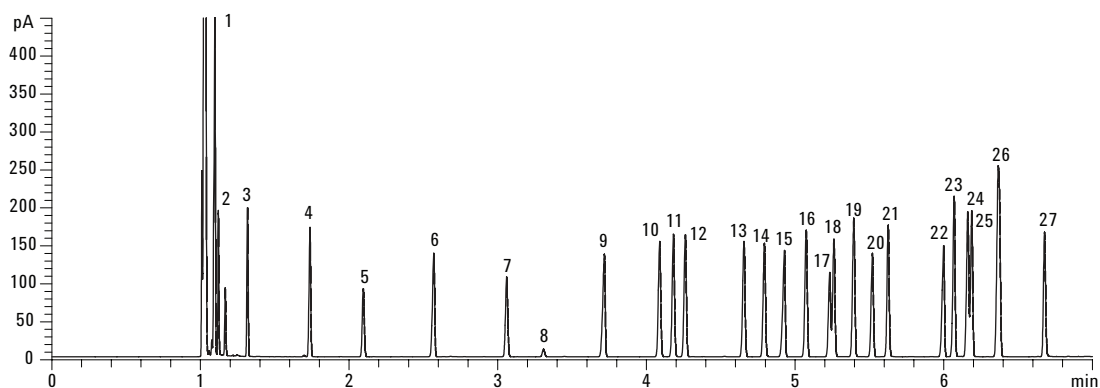


Figure 2. Separation of the same aromatic solvent with a 20 m × 0.18 mm × 0.18 μm HP-INNOWax column.

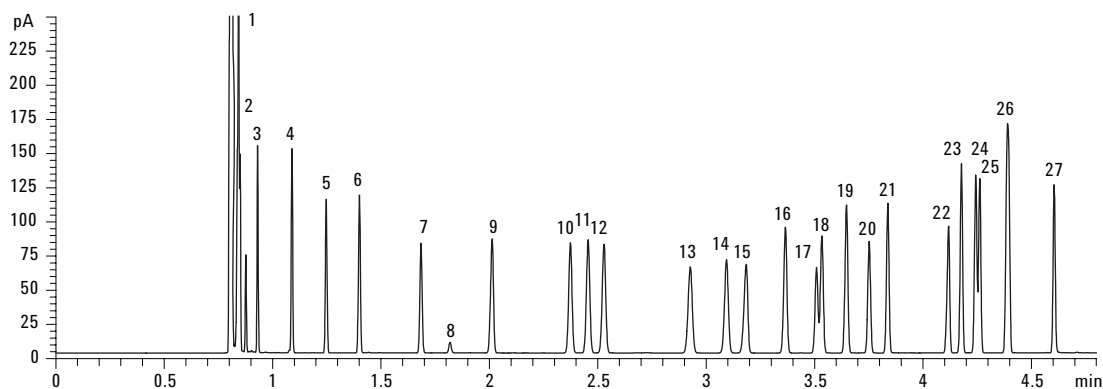


Figure 3. Optimized unified aromatic solvent method with a 20 m × 0.18 mm × 0.18 μm HP-INNOWax column.

1 Heptane	8 1,4-Dioxan	15 o-Xylene	22 Tridecan
2 Cyclohexane	9 Undecane	16 Propylbenzene	23 1,3-Diethylbenzene
3 Octane	10 Ethylbenzene	17 p-Ethyltoluene	24 1,2-Diethylbenzene
4 Nonane	11 p-Xylene	18 m-Ethyltoluene	25 n-Butylbenzene
5 Benzene	12 m-Xylene	19 t-Butylbenzene	26 a-Methylstyrene
6 Decane	13 Cumene	20 s-Butylbenzene	27 Phenylacetylene
7 Toluene	14 Dodecane	21 Styrene	

Figures 1, 2, and 3 show the chromatograms of the hexane solution containing an aggregate of aromatic solvents and impurities for Method 1, Method 2, and Method 3, respectively. As indicated in the three chromatograms, baseline resolution was achieved for most of the compounds of interest except for two compound pairs, which were

only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, was also not resolved in the original ASTM method (D-5060, impurities in ethylbenzene) and, along with o-ethyltoluene, was reported as total ethyltoluene. A second pair, diethylbenzene and n-butylbenzene, was also partially resolved. However, this should not present a

problem since they are not typically found together within the same material. Diethylbenzene is sometimes found as a contaminant in ethyl benzene (ASTM Method D-5060) while n-butyl benzene is used as the internal standard for cumene analysis (ASTM Method D-3760).

The sample run time for Method 1 was 23 minutes (Figure 1), whereas it was 7 minutes for Method 2 (Figure 2). The 3x speedup was achieved by using a shorter and narrower bore high-efficiency column. The optimized Method 3 allowed for even faster analysis time at 5 minutes (Figure 3), resulting in 4.6x speedup as compared to Method 1. As shown in Table 5, similar resolution was obtained in spite of significant acceleration, indicating that fast sample throughput can be achieved with the high-efficiency columns without compromise on resolution.

Influence of Carrier Gas on Analysis Time

The type of carrier gas and its velocity highly impact resolution and retention time. Too high or too low of a carrier gas velocity results in loss of resolution. It is therefore important to set a correct gas velocity to achieve a right balance of resolution and analysis time.

Hydrogen, helium, and nitrogen are the most common carrier gases used. The use of hydrogen as a carrier gas provides a faster analysis with almost equivalent resolution because the optimum linear carrier gas velocity is higher due to the higher diffusivity of hydrogen. At the optimal flow rates of 12, 20, and 35 cm/s for nitrogen, helium, and hydrogen, respectively, the analysis times would be 35/12 to 35/20 to 1 for nitrogen, helium, and hydrogen, respectively.

Nitrogen vs. Helium Carrier Gas

To investigate the effect of carrier gas on sample analysis time, Agilent GC method translation software was used where “translate only” mode was

chosen so that all experimental conditions were held constant except for the carrier gas. Method 1 was translated to Method 4 where a nitrogen carrier was used (see Figure 4 and Table 6). As shown in Figure 5, the run time for a nitrogen carrier was about 60 minutes compared to 23 minutes with a helium carrier when using a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column.

Figure 4. Method translation software input screen for a nitrogen carrier.

Table 6. Experimental Conditions for Unified Aromatic Solvents Method Using Nitrogen Carrier Gas (Method 4)

Column	HP-INNOWax, 60 m × 0.32 mm × 0.50 µm
Carrier gas	Nitrogen at 7.60 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	75 °C (23 min); 1.3 °C/min to 100 °C (0 min) 4.4 °C/min to 145 °C (0 min)
Detector	FID at 250 °C
Data acquisition rate	At 20 Hz
Injection size	0.2 µL

Table 5. Comparison of Resolution of Difficult-to-Separate Compound Pairs Under Different Experimental Conditions

Compound	Ethylbenzene/p-xylene	p-Xylene/m-xylene	p-Ethyltoluene/m-ethyltoluene	Diethylbenzene/n-butylbenzene
Method 1	3.25	3.10	1.10	1.11
Method 2	3.14	2.72	1.00	0.97
Method 3	2.84	2.47	0.94	0.88

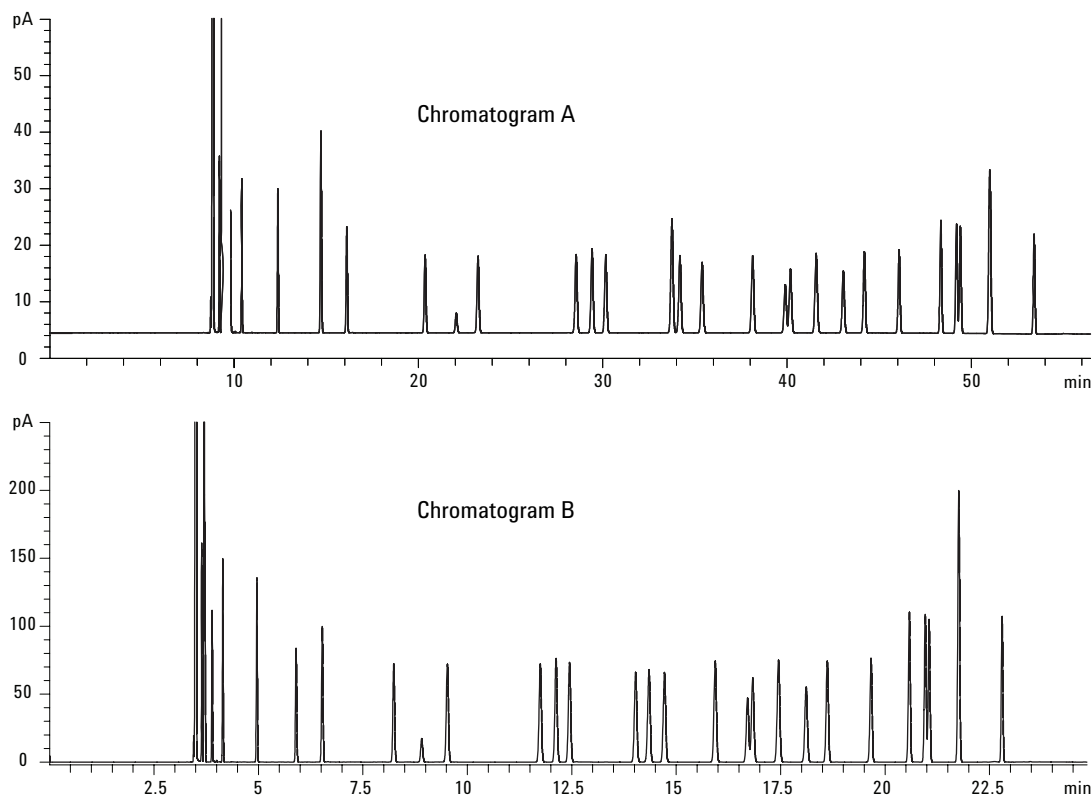


Figure 5. Comparison of unified aromatic solvent analysis using nitrogen and helium carrier gases with a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column. 5a. Nitrogen carrier gas (Method 4). 5b. Helium carrier gas (Method 1).

Hydrogen vs. Helium Carrier Gas

A faster analysis can be achieved by switching the carrier gas from helium to hydrogen on the same column. Method 3 was translated to Method 5 using the method translation software (see Figure 6); the

detailed experimental condition is provided in Table 7. As shown in Figure 7, the total run time was decreased from 5 to 3 minutes by changing the carrier gas from helium to hydrogen while keeping the peaks well separated.

GC Method Translation				
Criterion: ☒ Translate Only ☐ Best Efficiency ☐ Fast Analysis ☐ None Speed gain: 1.54952				
	Original Method		Translated Method	
Column Length, m	20		☐ 20	
Internal Diameter, µm	180		☐ 180	
Film Thickness, µm	0.18		☒ 0.18	
Phase Ratio	250.0		☐ 250.0	
Carrier Gas	Helium		☐ Hydrogen	
Enter one Setpoint				
Head Pressure, psi	33		22.403	
Flow Rate, mL/min	1.4763		1.8454	
Outlet Velocity, cm/sec	111.73		139.67	
Average Velocity, cm/sec	48.15		74.60	
Hold-up Time, min	0.692343		0.446811	
Outlet Pressure (absolute), psi	14.696		☐ 14.696	
Ambient Pressure (absolute), psi	14.696		☐ 14.696	
Oven Temperature	3-ramp Program			
	Ramp Rate	Final Temp.	Final Time	
	°C/min	°C	min	
Initial		70	3	
Ramp 1	45	145.00	0.5	
Ramp 2				
Ramp 3				
	Ramp Rate	Final Temp.	Final Time	
	°C/min	°C	min	
	69.728	145.00	0.323	
Sample Information	None			

Figure 6. Method translation software input screen for a hydrogen carrier.

Table 7. Experimental Conditions for Unified Aromatic Solvents Method Using Hydrogen Carrier Gas (Method 5)

Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Hydrogen at 22.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C 100:1 split ratio
Oven temp	70 °C (2 min); 70 °C/min to 145 °C (0.5 min)
Detector	FID at 250 °C
Data acquisition rate	At 50 Hz
Injection size	0.2 µL

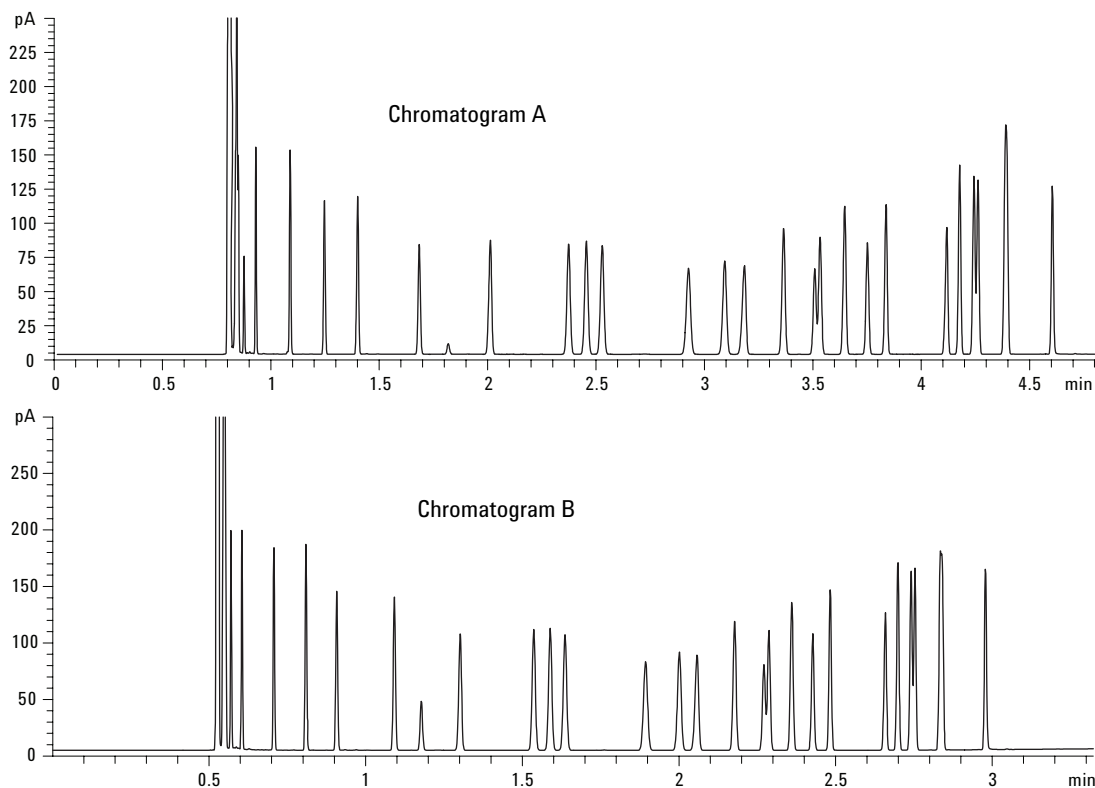


Figure 7. Comparison of unified aromatic solvent analysis using helium and hydrogen carrier gases with a $20\text{ m} \times 0.18\text{ mm} \times 0.18\text{ }\mu\text{m}$ HP-INNOWax column. 7a. Helium carrier gas (Method 5). 7b. Hydrogen carrier gas (Method 3).

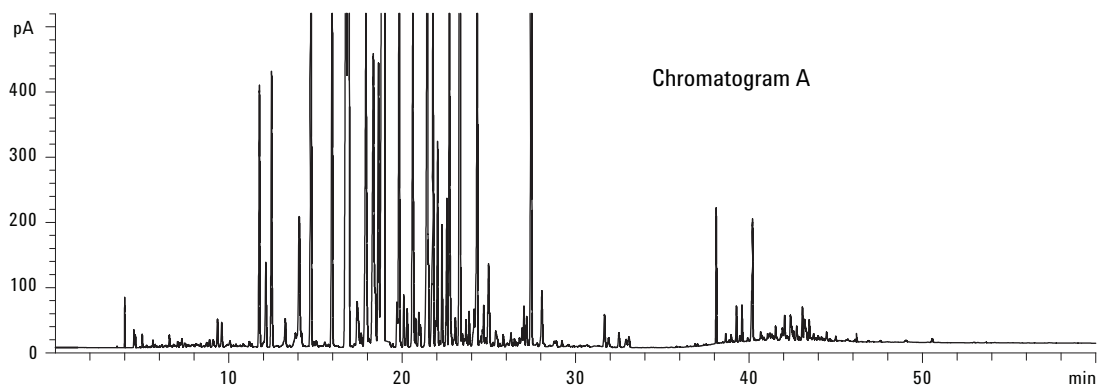
Complex Matrix Sample

To validate the practicality of fast GC application using high-efficiency GC columns, a real aromatic solvent sample offered by a large-scale integrated petrochemical company was analyzed using the same experimental conditions as those for the standards (Methods 1, 2, and 3); the chromatograms are provided in Figures 8a, 8b, and 8c. A detailed comparison of the center sections is also provided in Figures 8d and 8e.

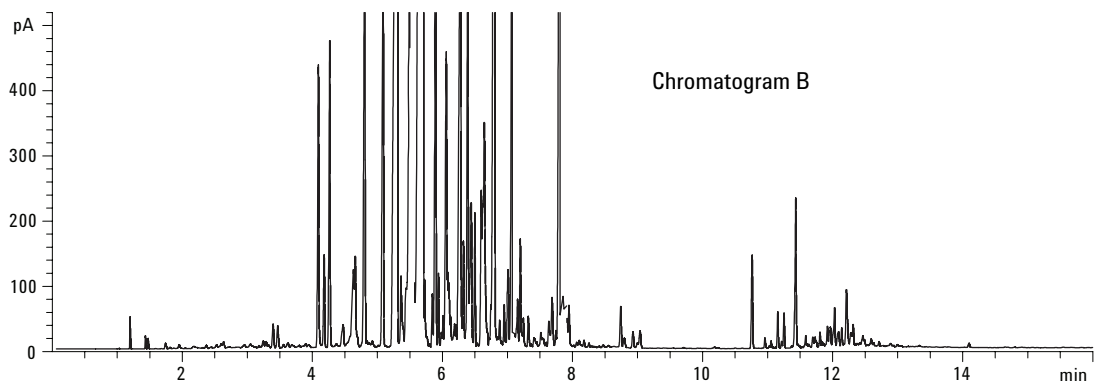
Although the analysis time is a bit longer with Method 2 compared to Method 3, the overall resolution obtained is slightly better for Method 2 (see

Figures 8e and 8f). On the other hand, all the key compounds, including benzene, toluene, ethylbenzene, m-xylene, p-xylene, o-xylene, propylbenzene, and a-methylstyrene, were well separated with all three methods.

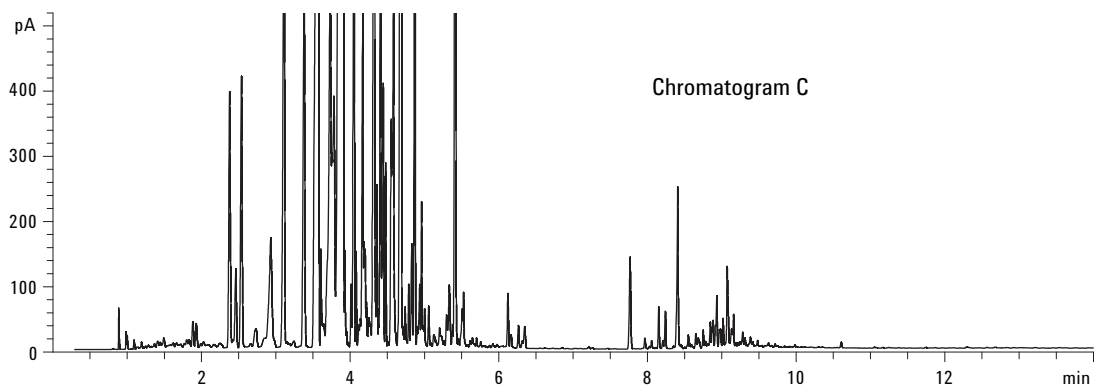
For complex matrix samples, a balance between speed and resolution must be selected according to the laboratory goals. In this case, it demonstrates that a complex matrix sample can be separated well on a high-efficiency 0.18 mm id GC column, where a more than 3x improvement in run time was accomplished compared to a 0.32 mm id column using a helium carrier.



Column HP-INNOWax, 60 m $\times$ 0.32 mm $\times$ 0.50 μ m
 Carrier gas Helium at 20.00 psi constant pressure mode
 Inlet Split/splitless at 250 $^{\circ}$ C; 50:1 split ratio
 Oven temp 75 $^{\circ}$ C (10 min); 3 $^{\circ}$ C/min to 100 $^{\circ}$ C (0 min); 10 $^{\circ}$ C/min to 145 $^{\circ}$ C (12.17 min), 25 $^{\circ}$ C/min to 220 $^{\circ}$ C (22 min)
 Detector FID at 250 $^{\circ}$ C
 Injection size 0.2 μ L



Column HP-INNOWax, 20 m $\times$ 0.18 mm $\times$ 0.18 μ m
 Carrier gas Helium at 25.00 psi constant pressure mode
 Inlet Split/splitless at 250 $^{\circ}$ C; 150:1 split ratio
 Oven temp 50 $^{\circ}$ C (2 min); 15 $^{\circ}$ C/min to 90 $^{\circ}$ C (0 min); 20 $^{\circ}$ C/min to 145 $^{\circ}$ C (3 min), 80 $^{\circ}$ C/min to 220 $^{\circ}$ C (8 min)
 Detector FID at 250 $^{\circ}$ C
 Injection size 0.2 μ L



Column HP-INNOWax, 20 m $\times$ 0.18 mm $\times$ 0.18 μ m
 Carrier gas Helium at 33.00 psi constant pressure mode
 Inlet Split/splitless at 250 $^{\circ}$ C; 150:1 split ratio
 Oven temp 70 $^{\circ}$ C (3 min); 45 $^{\circ}$ C/min to 145 $^{\circ}$ C (3 min), 80 $^{\circ}$ C/min to 220 $^{\circ}$ C (8 min)
 Detector FID at 250 $^{\circ}$ C
 Injection size 0.2 μ L

Figure 8. Comparison of real aromatic solvent sample separations (a) and (d) Method 1, (b) and (e) Method 2, and (c) and (f) Method 3.

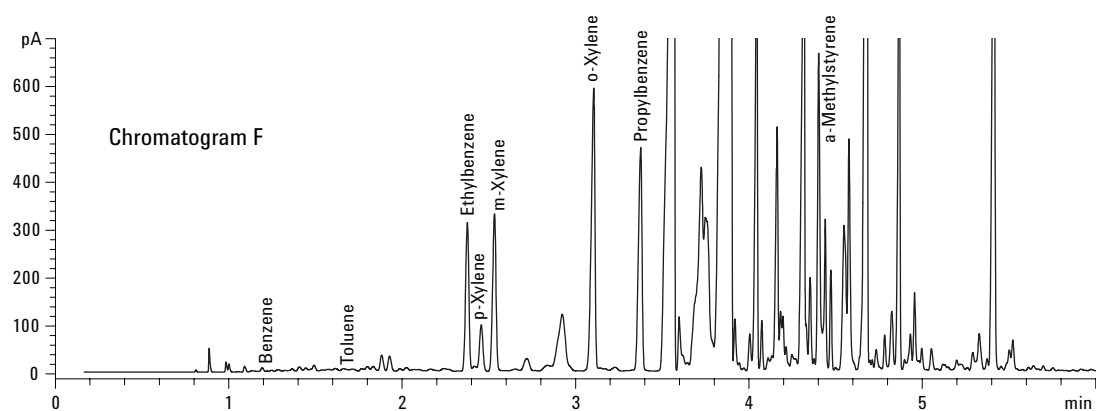
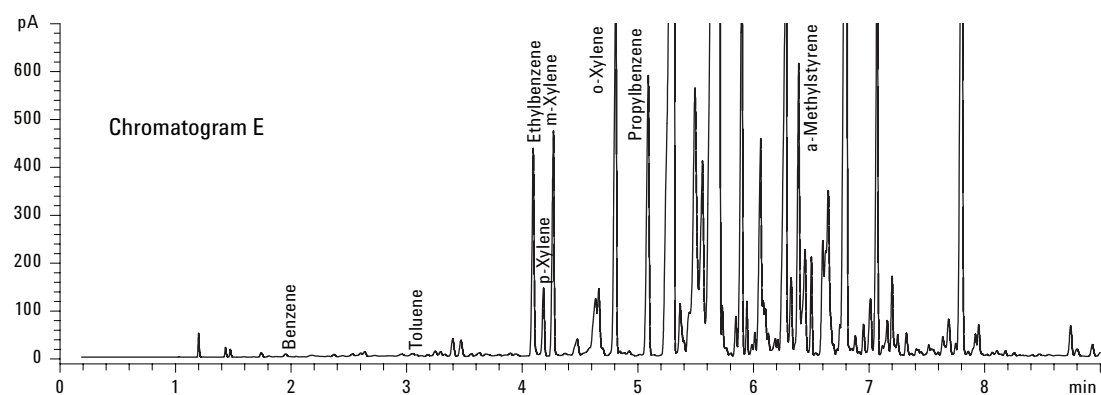
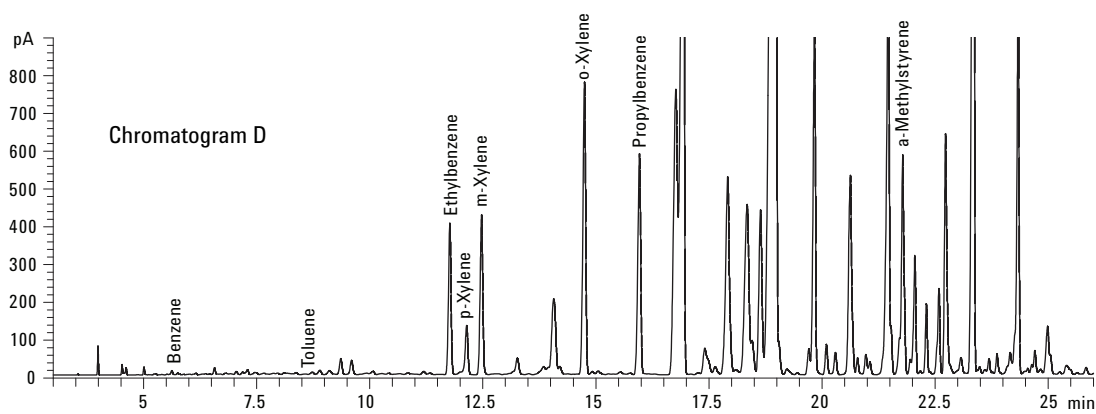


Figure 8. Comparison of real aromatic solvent sample separations (a) and (d) Method 1, (b) and (e) Method 2, and (c) and (f) Method 3. (continued)

Evaluation of Individual ASTM Calibration Standards

To evaluate the applicability of high-efficiency GC columns on individual ASTM calibration standards, experiments were carried out with Methods 1 and 3, respectively, on a 7890 gas chromatography system. All standards were prepared as outlined by the ASTM methods.

D2306 – Standard Test for C8 Aromatic Hydrocarbons

Concentration of ASTM D2306 standard calibration mix is quite high. It is therefore a challenge

regarding the capacity of the high-efficiency 0.18 mm id column. The workaround is to inject a small volume with a high split ratio. In this experiment, the injection size was 0.2 μ L and the split ratio was 600:1. As shown in Figure 9, the run time for the high-efficiency GC column was about 4.5 times shorter than that of the traditional one. The resolution is acceptable in spite of the high concentration of the calibration standard (see Table 8).

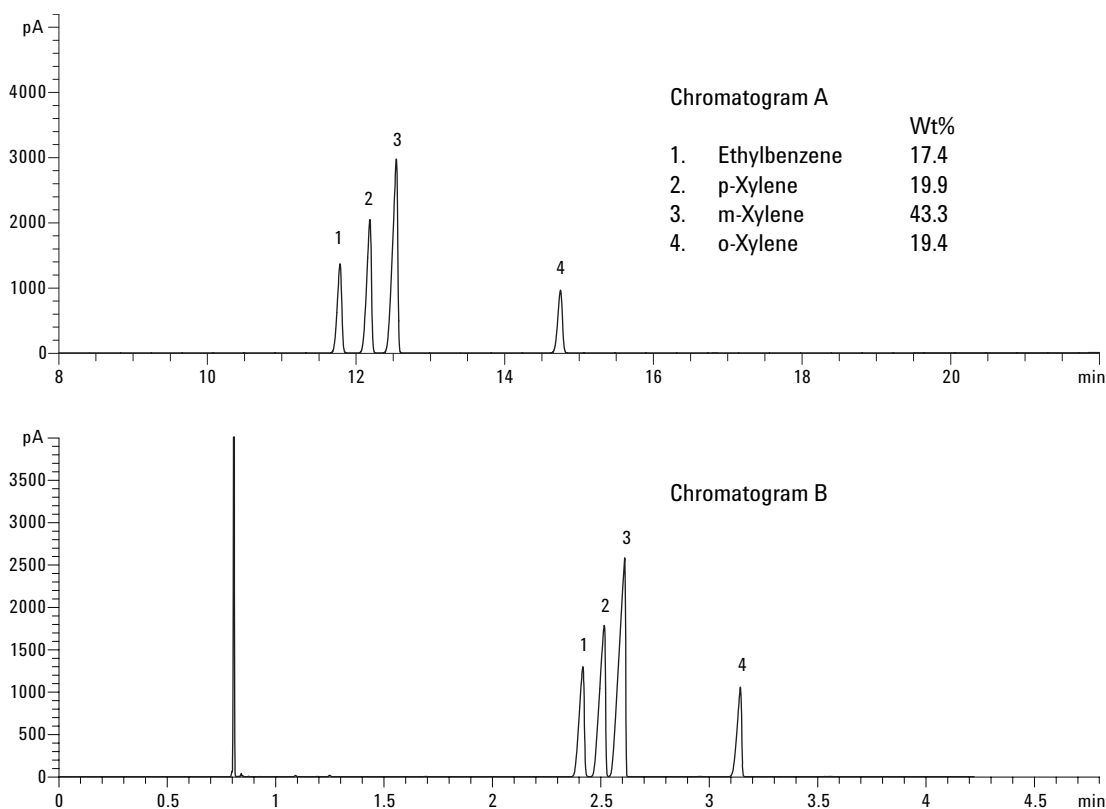


Figure 9. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standards (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (method 3).

Table 8. Comparison of Resolution Under Different Experimental Condition

Compound	Ethylbenzene/p-xylene	p-Xylene/m-xylene	m-Xylene/o-xylene
Method 1	3.52	2.86	18.11
Method 3	2.10	1.73	11.20

D2360 – Standard Test for Trace Impurities in Monocyclic Hydrocarbons

The standard calibration mix specified by D2360 was prepared in p-xylene. Injection size for this run was 0.2 μ L and the split ratio was 200:1.

Similar resolution was obtained for the compounds of interest (Figure 10), except for the sample run time being decreased from 21.05 minutes (Method 1) to 4.28 minutes (Method 3).

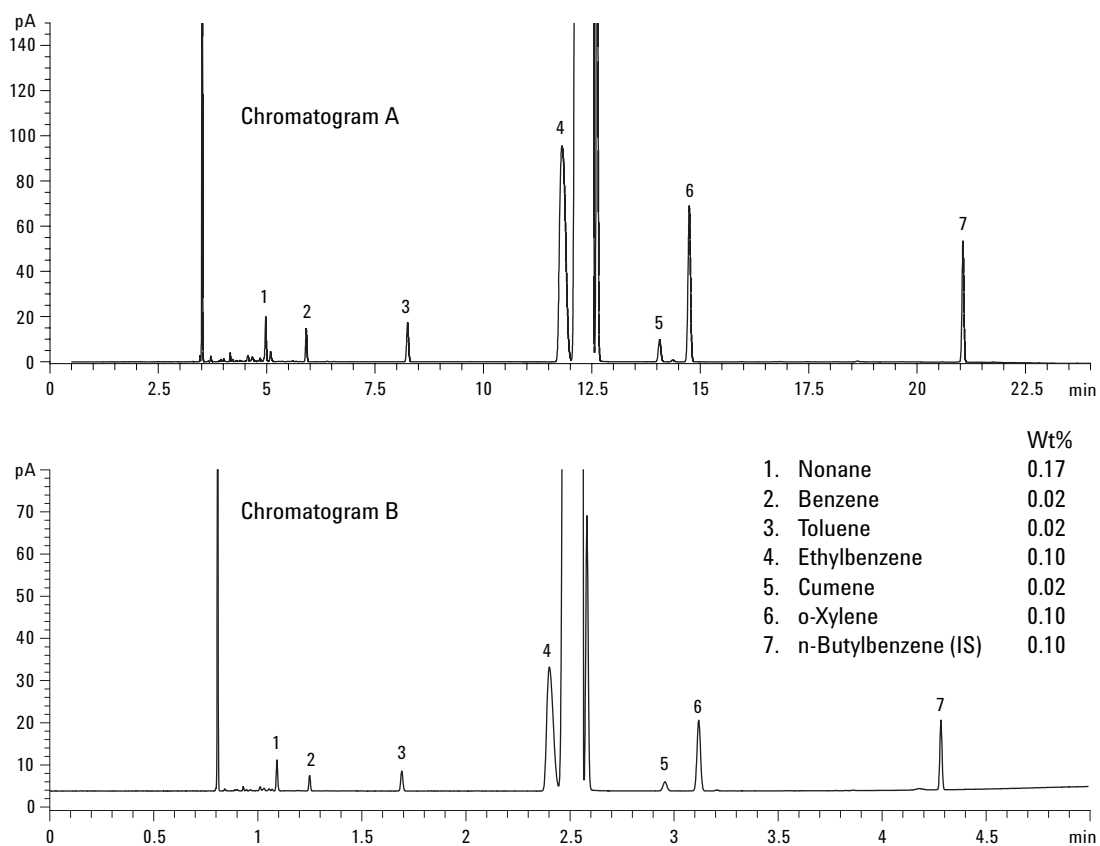


Figure 10. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

D3797 – Standard Test Method for Analysis of o-Xylene

Figure 11 shows the chromatograms of the D3797 calibration standard. Injection size for this run was 0.2 μ L and the split ratio was 100:1.

The broadening of the cumene peak was due to the reverse solvent effect of the overloaded o-xylene peak. This was also observed in the original ASTM D3797 method [4]. Comparison of the chromatograms in Figure 11 indicates that the D3797 calibration standard can be separated well on a high-efficiency 0.18 mm id GC column without loss of resolution.

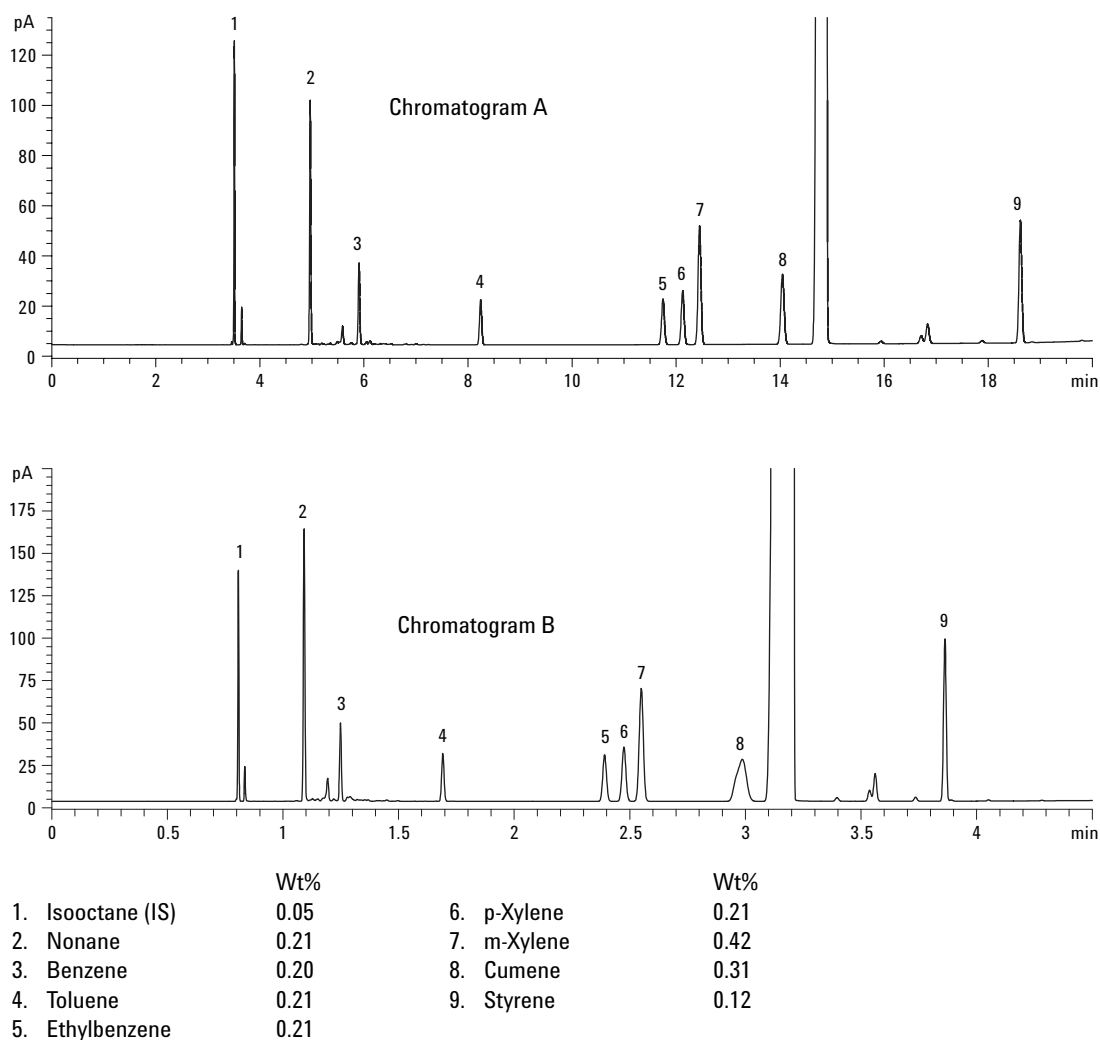


Figure 11. o-Xylene standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

D3798 – Standard Test Method for Analysis of p-Xylene

This test method covers the determination of known hydrocarbon impurities in p-xylene and the measurement of p-xylene purity by GC. It is generally used for the analysis of p-xylene of 99% or greater purity.

Figure 12 shows the chromatograms of the D3798 calibration standard. Injection size for this run

was 0.2 μ L and the split ratio was 100:1. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Excellent separation was obtained for the critical compounds (Figure 13) with great reproducibility (Figure 14) when using a high-efficiency GC column.

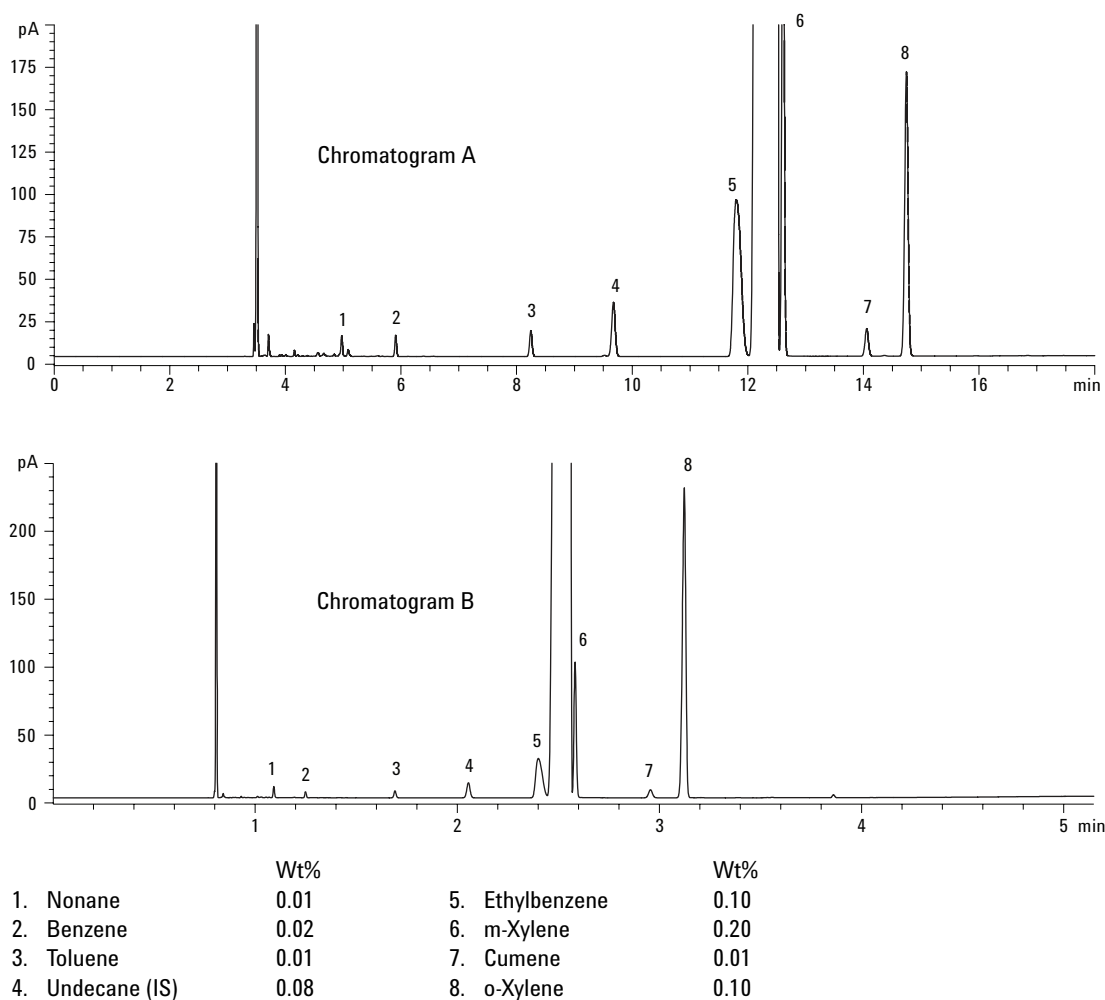


Figure 12. p-Xylene standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

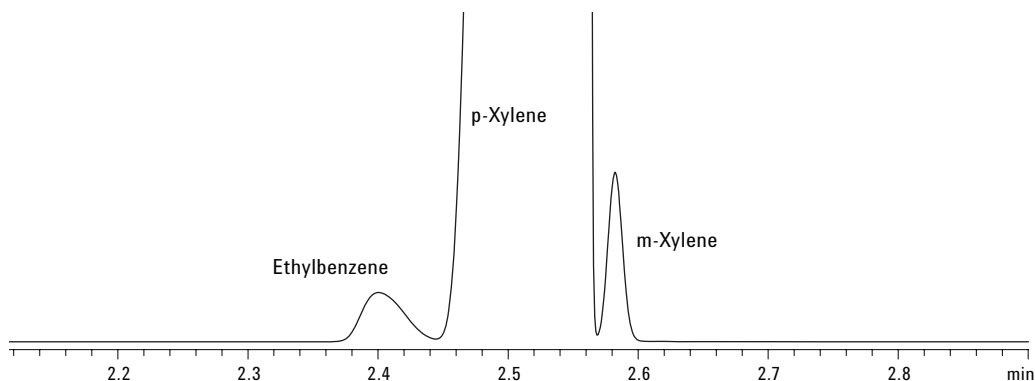


Figure 13. Expanded view from Figure 7 shows excellent separation of m-Xylene peak from p-Xylene peak using the fast GC method.

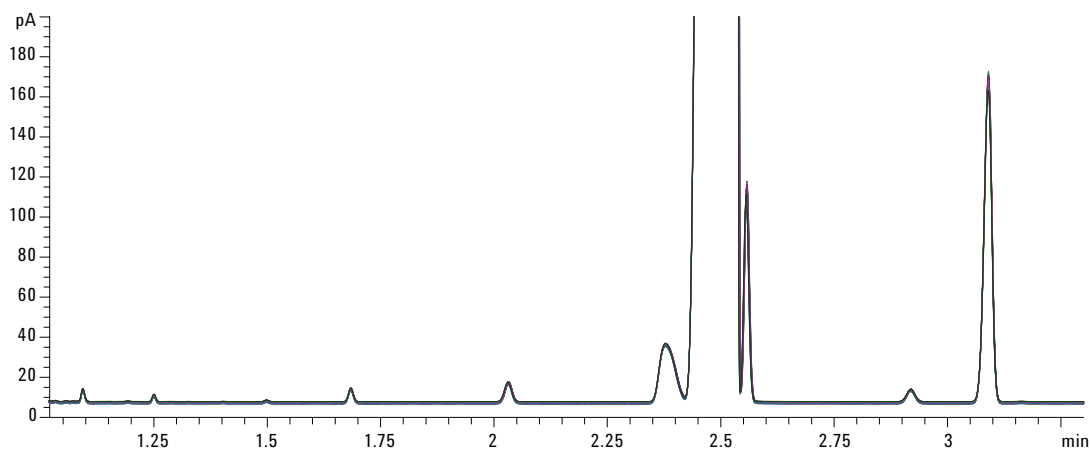


Figure 14. D3798 standard 30th run overlaid using a high-efficiency GC column.

D4492 – Standard Test for Analysis of Benzene

This test method determines the normally occurring trace impurities in, and the purity of, finished benzene. It is applicable for aromatic impurities from 0.001 to 0.010 weight % in benzene. Injection size for this run was 0.2 μ L and the split ratio was 50:1.

Figure 15 compares the chromatograms of the D4492 calibration standard with Methods 1 and 3, where good separation of the D4492 calibration standards can be achieved with a high-efficiency column but with 80% saving on analysis time.

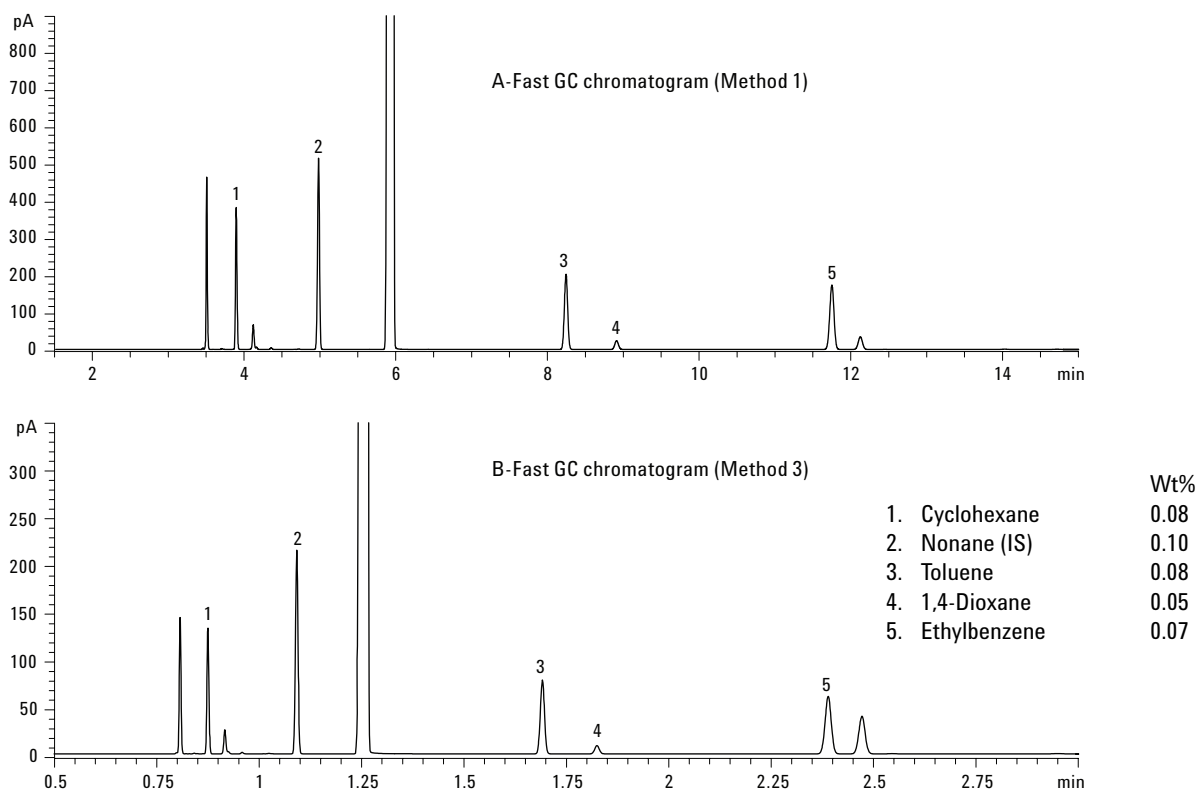


Figure 15. ASTM D4492 benzene quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

In summary, the analysis time for Method 3 is on average 5x shorter than that for Method 1 when working with the calibration standard samples.

Conclusions

Fast GC applications can significantly improve laboratory productivity by decreasing analysis time. This application showcases the practicality of high-efficiency GC columns in daily aromatic solvent analysis and the associated time savings achieved with these columns. By using high-efficiency GC columns with smaller inner diameters and shorter column lengths as well as an appropriate carrier gas (for example, helium or hydrogen), higher sample throughput and lower cost per sample is achievable [5] for chemical and petrochemical laboratories.

References

1. Annual Book of ASTM Standards, Vol. 6.04 "Paint – Solvents; Aromatic Hydrocarbons," ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA
2. James D. McCurry, "A Unified Gas Chromatography Method for Aromatic Solvent Analysis," Agilent Technologies, publication number 5988-3741EN
3. Yuehua Zhou, "A Unified Method for the Analysis of Aromatic Solvent Using the Agilent 6820 Gas Chromatography System," Agilent Technologies, publication number 5988-9278EN
4. ASTM Method D3797, Standard Test for Analysis of o-xylene, Annual Book of ASTM Standards, Vol. 6.04, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA
5. Ken Lynam and Yun Zou, "A Faster Solution for Unified Volatile Organic Analysis with 0.18 mm ID GC Columns," *Separation Times*, Vol 20, Number 5, 2007

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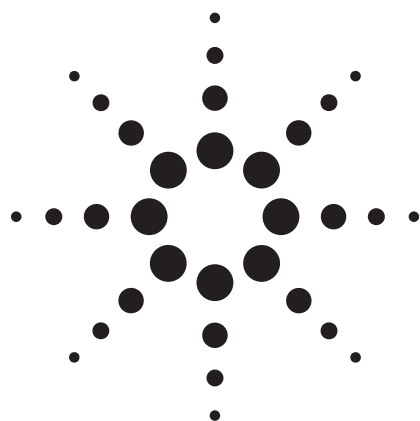
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Investigation of the Unique Selectivity and Stability of Agilent GS-OxyPLOT Columns



Application

Gas Chromatography

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Abstract

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

Introduction

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

Methanol is one of the oxygenates that often present in light hydrocarbon streams. For example, it is added to natural gas and production of crude oil to prevent hydration of hydrocarbons during transportation via pipelines. Therefore, it is important

to accurately measure the content of methanol from light hydrocarbons at different concentrations, including at trace levels.

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

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

Experimental

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



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

Results and Discussion

Analysis of Normal Hydrocarbons and Methanol

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

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

Table 1. Conditions for Normal Hydrocarbons and Methanol Analysis

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

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

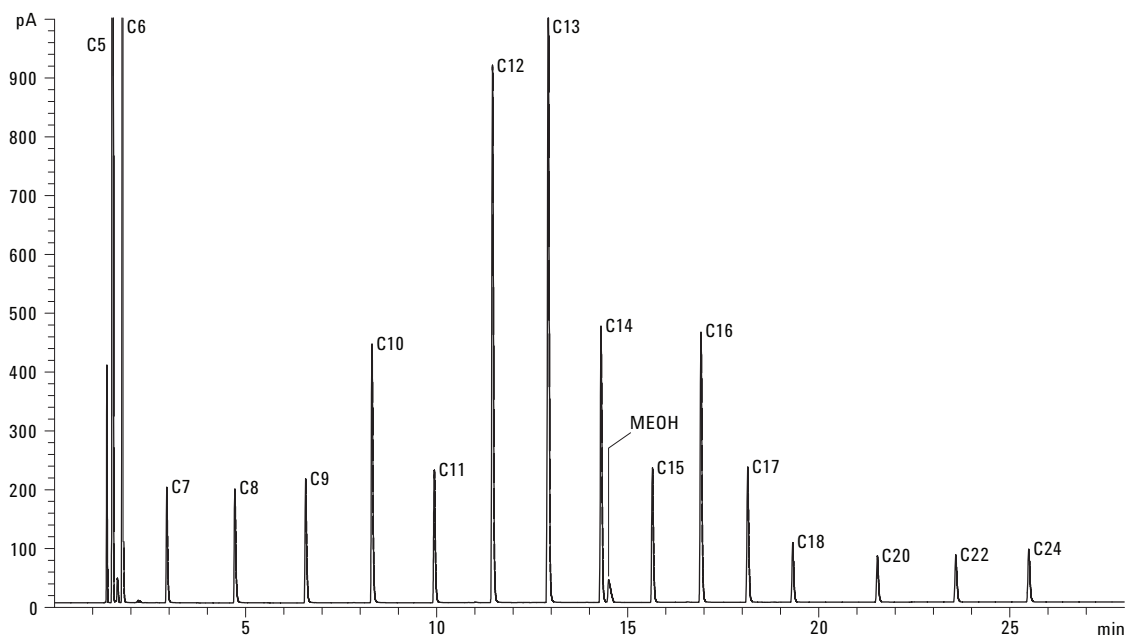


Figure 1. Analysis of methanol and normal hydrocarbons on a GS-OxyPLOT column, 10 m × 0.53 mm × 10 µm.

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

Analysis of Alcohols

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

Sample

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

As can be seen in Figure 2, all of the alcohols are separated and eluted with good peak shape within

Table 2. Conditions for Alcohols Analysis

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

an analysis time of 15 min. In this experiment, oven temperature was set up to 300 °C. Thanks to its advanced dynamic coating process, Agilent's GS-OxyPLOT stationary phase exhibits virtually no detector spiking due to particle generation from the phase coating [3].

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

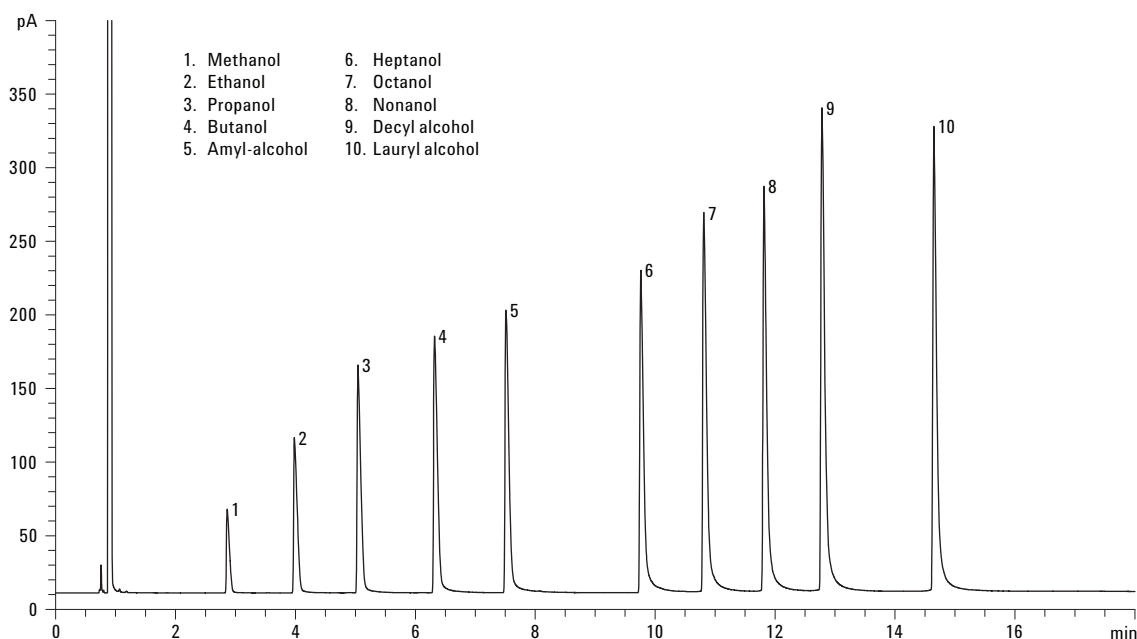


Figure 2. Separation of alcohols using GS-OxyPLOT, 10 m × 0.53 mm × 10 µm.

Influence of Temperature on the Selectivity of GS-OxyPLOT

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

Table 3. Conditions for Kovats Retention Indices Test

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

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

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

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

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

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

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

Influence of Moisture on GS-OxyPLOT

Some PLOT columns can adsorb water, which can lead to changes in retention times and selectivity

for analytes. Therefore, column performance will be influenced greatly in the presence of water. Although cumbersome solvent-extraction procedures can be performed before injection, injecting sample that contains water is, in some cases, unavoidable.

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

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

Table 5. Conditions for Moisture Test

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

As shown in Figure 3, the area of n-pentadecane remained the same before and after 100 injections of water. However, compared with the area before injecting water, the area of methanol (peak 6) decreased by 50%, and the area of acetone (peak 7) decreased by 14.4% after 100 injections of water (see Table 6). It demonstrated that water can affect the activity of GS-OxyPLOT, especially for the analysis of those relatively low molecular weight oxygenated compounds, such as methanol and acetone.

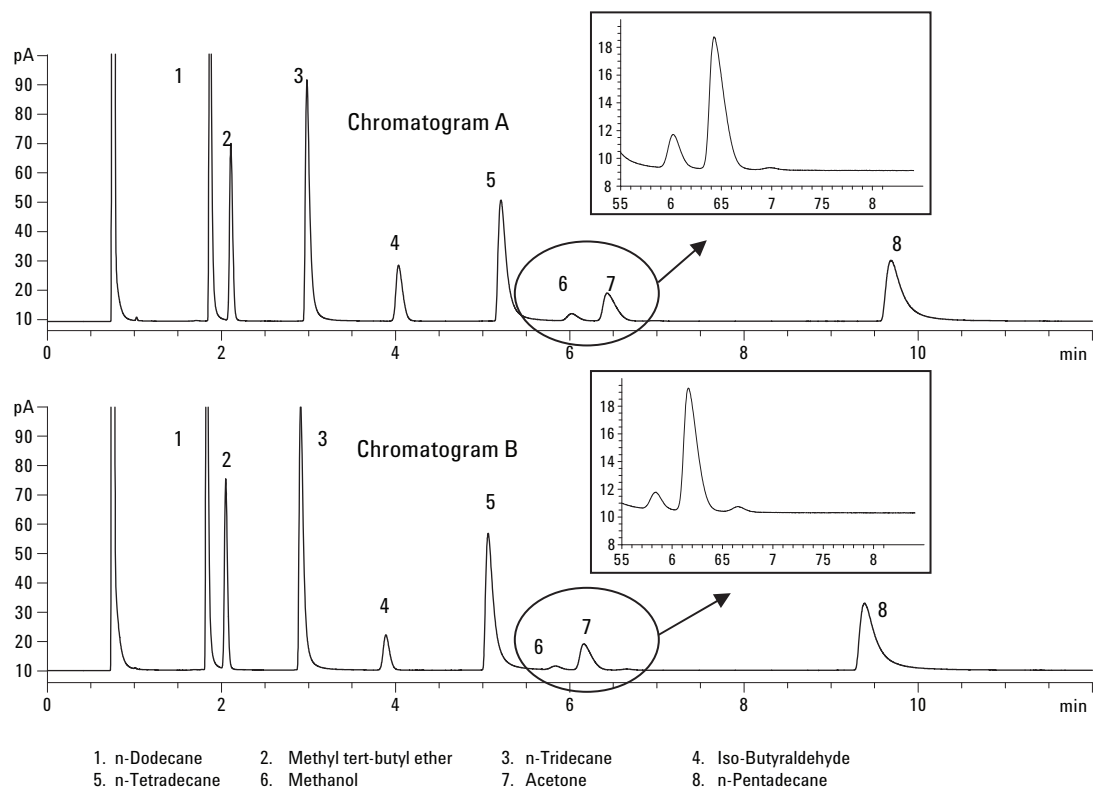


Figure 3. Comparison of test mixture separation before (A) and after (B) 100 injections of water.

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

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

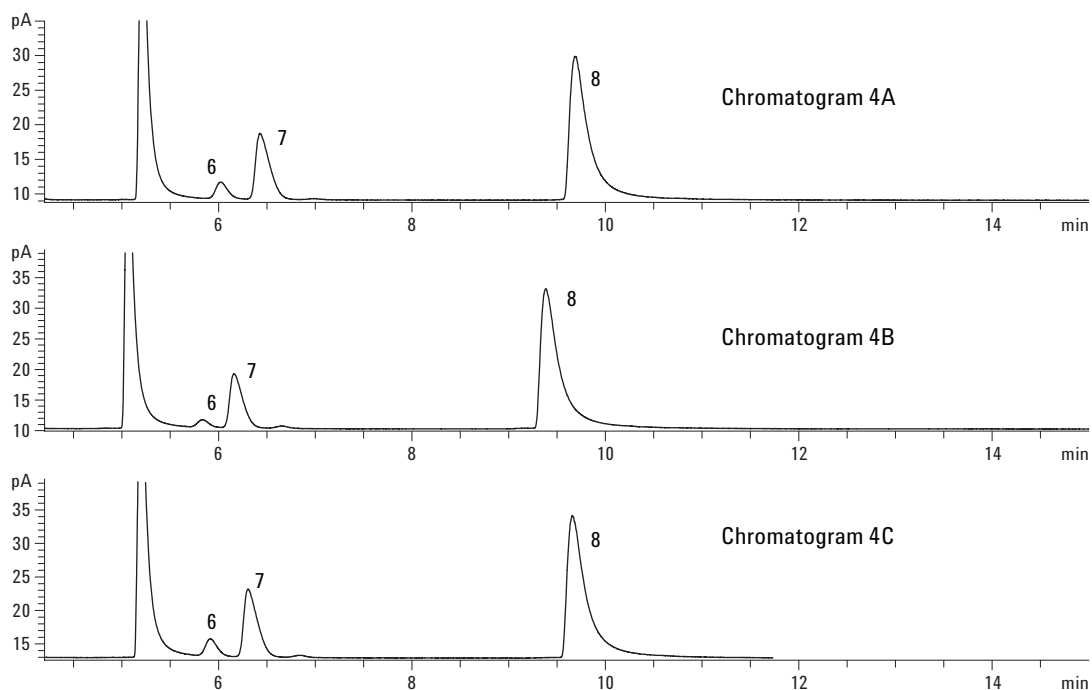


Figure 4. Expanded view shows comparison of test mixture separation on GS-OxyPLOT.
4A. Before injection of water. 4B. After 100 injections of water. 4C. After conditioning the column.

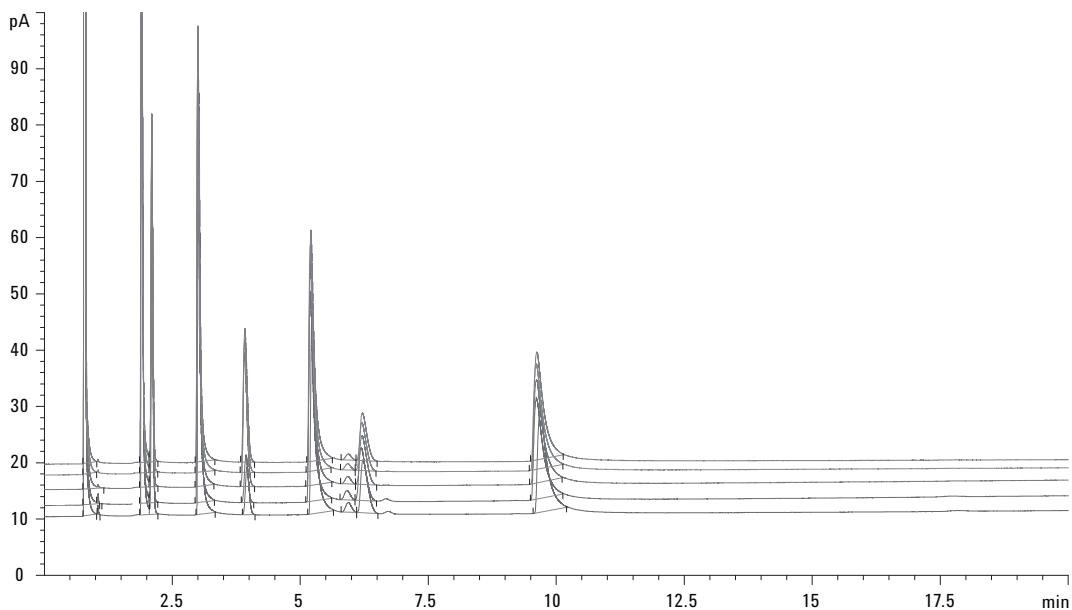
Table 6. Comparison of Test Mixture Separation

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

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

Determination of Methanol

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

**Figure 5. Fifth run overlaid using GS-OxyPLOT (after conditioning column).****Table 7. Peak Area Reproducibility and Retention Time Reproducibility on GS-OxyPLOT (after conditioning column)**

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

Reagents and Materials

Carrier gas, Helium, > 99.95% purity

Methanol, > 99.9% purity

1-propanol, > 99.9% purity, and containing
< 500 ppm methanol

Toluene, > 99.9% purity, and containing < 0.5 ppm
methanol

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

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

Linearity

Under the conditions listed in Table 8, the
methanol calibration standards were analyzed. The
linearity is shown by plotting the response ratio of
methanol and internal standard 1-propanol against

their amount ratio (see Figure 6). For methanol,
good linearity was gained ranging from 5 to
1,500 ppm. The correlation r^2 value for the calibra-
tion curve is higher than 0.999.

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

Table 8. System Settings for the Calibration Curve

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

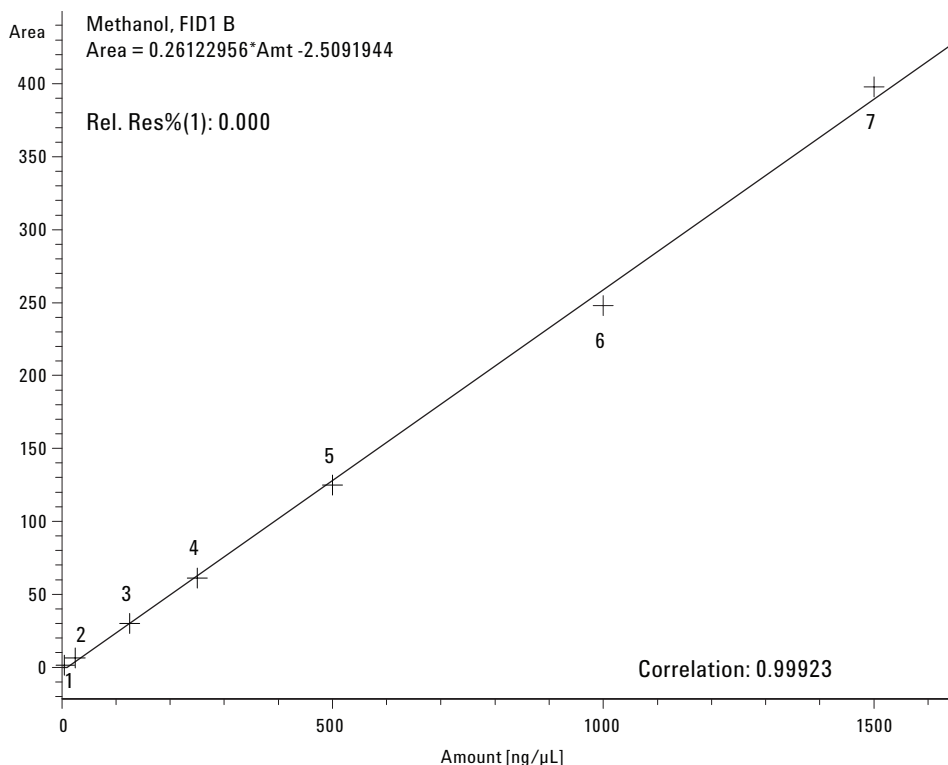


Figure 6. The calibration curve of methanol in toluene.

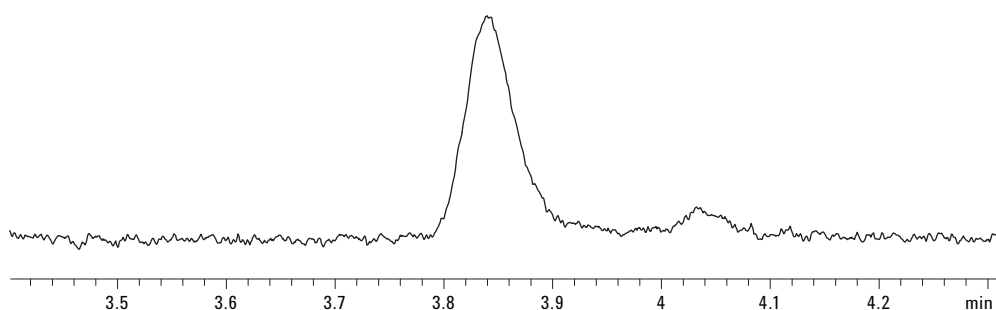


Figure 7. Test mixture of 5 ppm methanol in toluene.

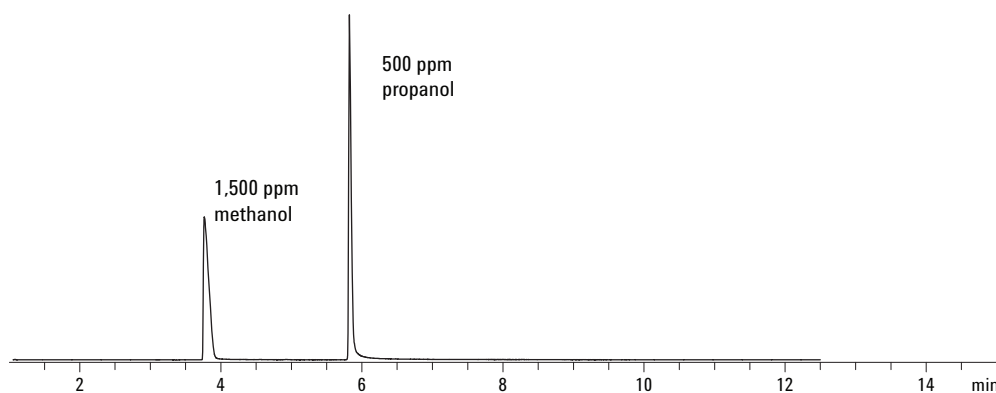


Figure 8. Test mixture of 1,500 ppm methanol in toluene.

Repeatability

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

Life Span

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

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

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

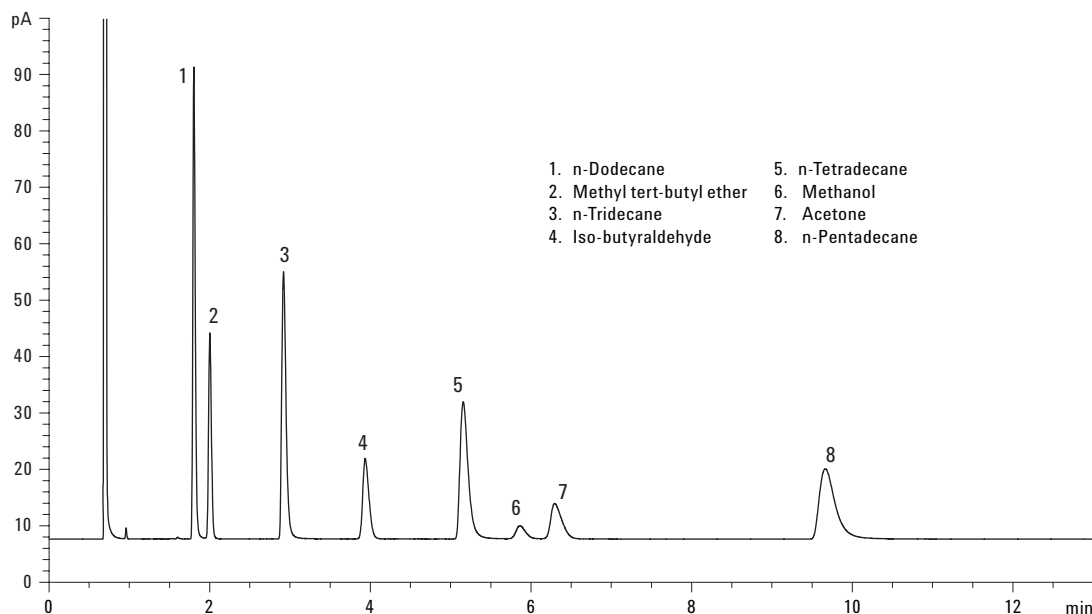


Figure 9. Chromatogram of performance mixture after 1,500 injections.

Conclusions

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

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

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

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

References

1. A. K. Vickers, "GS-OxyPLOT: A PLOT Column for the GC Analysis of Oxygenated Hydrocarbons," Agilent Technologies publication 5989-6447EN, March 2007
2. "Agilent J&W GS-OxyPLOT Capillary GC Columns," Agilent Technologies publication 5989-6489EN
3. A. K. Vickers, "A 'Solid' Alternative for Analyzing Oxygenated Hydrocarbons — Agilent's New Capillary GC PLOT Column," Agilent Technologies publication 5989-6323EN, February 2006
4. Standard test method for the determination of methanol in crude oils by multidimensional gas chromatography, ASTM D7059-04, July 2004

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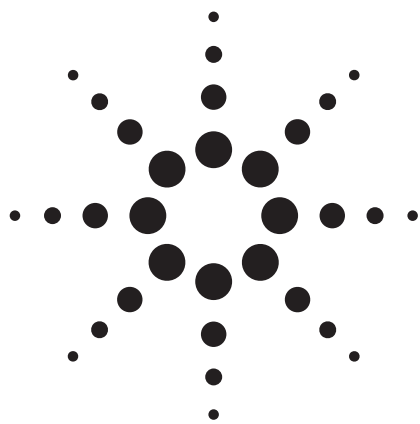
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Agilent Technologies



Fast Hydrocarbon and Sulfur Simulated Distillation Using the Agilent Low Thermal Mass (LTM) System on the 7890A GC and 355 Sulfur Chemiluminescence Detector

Application Note

Hydrocarbon Processing

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Abstract

A fast multielement simulated distillation method (SimDis) based on Low Thermal Mass (LTM) column technology is described. The LTM system technology using resistive heating allows rapid temperature programming with extremely fast column cool-down. Significantly shortened analytical cycle times as compared to conventional air bath GC ovens are achieved. The method combines hydrocarbon SimDis with an FID with sulfur SimDis using the Agilent 355 sulfur chemiluminescence detector (SCD) in a series configuration. The results show that the LTM method produces a run time for both hydrocarbon and sulfur boiling point distribution at least six times shorter than the conventional ASTM D2887 procedure [1]. The results agree with the specification in ASTM D2887 for the reference gas oil checkout sample.



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Introduction

Sulfur and carbon simulated distillation provide meaningful information to optimize refining processes and demonstrate compliance with petroleum product specifications. To meet the increasing needs for greater productivity, a fast sulfur and carbon SimDis method was developed using LTM technology. LTM technology was developed by RVM Scientific and acquired by Agilent Technologies in 2008 [2]. The Agilent 7890A system is designed to work with the LTM column module components to heat and cool the column very efficiently to significantly shorten analytical cycle times as compared to conventional air bath GC oven techniques involving much higher thermal mass. Aside from selectivity, temperature programming is an extremely useful feature of gas chromatography. When appropriate column dimensions and flow rates are chosen, high program rates (such as, 200 °C/min) can be used to great advantage. Agilent's method translation software can be useful in this regard [3]. For example, as temperature program rates increase, a corresponding increase in column flow rate should also be considered to achieve more optimal overall system performance. The system (except external power supply) is built into a replacement GC oven door, which is mounted as an add-on to an Agilent 7890A GC. The method described here combines hydrocarbon SimDis with sulfur selective SimDis using an FID and an SCD in series with simultaneous acquisition of both signals.

Experimental

This two-channel SimDis application uses the Agilent 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet and an SCD mounted in series with an FID by using a special adapter. A 5-inch format LTM column module is used for the analysis. Samples, such as boiling point (BP) calibration C5-C40, polywaxes, and resids, that are too viscous or waxy to sample with a syringe are heated to approximately 80 °C for a few minutes before injection.

The SimDis application software can process one or two channels of signal data (FID and SCD) from the GC ChemStation. The software is based on four modules: Browse, Setup, SimDis, and Report. Each module provides specific functions to rapidly perform multielement SimDis calculations. For example, the Setup module allows you to assign the files to use for BP Calibration, Blank, and QC reference. The detailed GC conditions used are listed in Table 1.

Table 1. Gas Chromatographic Conditions

HT-PTV inlet	
Temperature	350 °C
Split ratio	30:1
Injection volume	0.1 µL
7890/LTM	
Column (LTM)	DB-1 5 m × 320 mm × 1 µm
LTM temperature program	45 to 350 °C at 150 °C/min, hold 1 min (GC oven: 300 °C, held for duration)
Column flow (He)	Ramp pressure: 18 psi to 42 psi at 11.8 psi/min
FID temperatures	350 °C
H ₂ flow	40 mL/min
Air flow	400 mL/min
Make up (N ₂)	40 mL/min
Data rate	5 Hz
SCD	
Burner temperature	800 °C
Vacuum of burner	324 Torr
Vacuum of reaction cell	11.6 Torr
H ₂	40 SCCM
Air	8.3 SCCM
Data rate (AIB)	5 Hz
LTM system	G6578A, bundle for 1-channel 5-inch modules

Processing Two-Channel Data in the SimDis Software

The Agilent SimDis System enables the use of gas chromatography to determine the boiling point range distribution and percent recovery of petroleum fractions. The SimDis software allows for reports to be generated in two ways: automated and manual. Both require the user to first manually set a blank, calibration, and optional QC reference chromatogram. When working with dual channels, the SimDis software requires that each channel be labeled by the detector type rather than the defaults used by the GC ChemStation. Since the SCD operates off the analog input board (AIB), its signal begins with "AIB." For this reason, the Post-Run command macro SCDnamer.mac must be run to rename the signal file. The macro renames the AIB2B.ch channel as SCD1.ch. If the channel name is not corrected, the software will switch the FID and SCD channels during analysis, giving faulty results. The macro code to do this is shown below. It assumes that the AIB is in the rear position (B).

! SCDNamer call this as a post run command when an SCD is installed

! it renames the dual channel AIB2B.ch to SCD1.ch to allow simdis to properly calibrate

NAME SCDNamer

! This macro renames the SCD files named as AIB2B.ch to SCD1.ch

if filestat(mode,dadatapath\$+dadatfile\$+"\AIB2B.CH")=1

```
rename dadatapath$+dadatfile$+"\AIB2B.ch",dadat-  
path$+dadatfile$+"\SCD1.ch"
```

```
print "File Renamed"
```

```
else
```

```
print "No AIB2B File found"
```

```
endif
```

```
RETURN
```

```
ENDMACRO
```

Under the Setup tab of SimDis, select the default chromatograms to be used for the calculation. All desired solvent masking should be done here. The calibration run should have major carbon peaks identified; this can be done with an imported calibration table from ChemStation or manually in SimDis. The QC reference should have concentrations and dilution factors entered. Now enter the SimDis pane and set the parameters to be used. Do not include the solvent peak in the calculations and account for the baseline and noise using a zeroing method. In order to get proper percent recovery values make sure Normalize to 100% is not selected and a proper dilution factor is entered. Set the settings as default. For

automation, enter ChemStation and select SimDis > Setup > Use SimDis Defaults under Data Analysis and select a report to generate after each run. For manual operation, select the desired chromatogram under the Report pane in SimDis and enter the report pane to view and print results.

Results and Discussion

The LTM column module employs a unique direct resistive heating technology using ceramic-insulated heating wire where contact with the capillary column is maximized. This is packaged in a very low mass assembly. Accurate and precise temperature sensing is possible by incorporating the temperature sensor with the capillary GC column. This technology greatly reduces GC analytical cycle times, addressing demands for greater productivity. The result for the D2887 reference gas oil (RGO) shows that the run time using LTM is less than 2.5 minutes, or about six times faster compared to conventional ASTM D2887 procedures. The SimDis results agree with the specifications of ASTM D2887 with excellent repeatability (see Table 2). The upper chromatogram in Figure 1 shows the D2887 RGO analysis by conventional air bath GC oven and the lower chromatogram shows RGO by LTM GC. The run time is about 2.5 minutes and 15 minutes for LTM and conventional air bath GC, respectively.

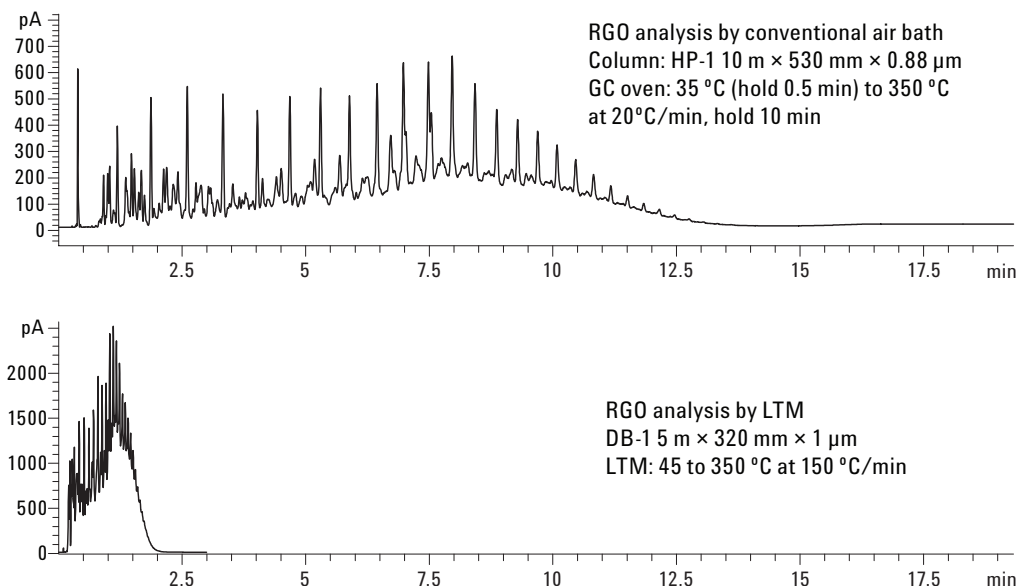


Figure 1. Comparison of RGO analysis by LTM and air bath GC ovens.

Table 2. RGO SimDis Results with the LTM System

ASTM D2887 Values			Observed Value												
	BP, °C	Allowable difference	1	2	3	4	5	6	7	8	9	10	Average	Difference	RSD%
IBP	115	7.6	112	113	112	113	112	113	112	113	112	113	112.5	2.5	0.47
10%	176	4.1	173	174	173	174	173	174	173	174	173	174	173.5	2.5	0.30
20%	224	4.9	220	220	220	220	220	220	220	220	220	220	220	4	0.00
30%	259	4.7	254	255	255	255	255	255	255	255	255	255	254.9	4.1	0.12
40%	289	4.3	285	286	285	287	285	286	285	286	286	286	285.7	3.3	0.24
50%	312	4.3	309	310	309	310	309	309	309	309	309	309	309.2	2.8	0.14
60%	332	4.3	329	330	329	330	329	330	329	329	329	329	329.3	2.7	0.15
70%	354	4.3	350	352	351	352	351	352	351	352	351	352	351.4	2.6	0.20
80%	378	4.3	375	376	375	377	375	376	375	376	375	376	375.6	2.4	0.19
90%	407	4.3	404	405	405	406	404	406	404	405	404	405	404.8	2.2	0.19
FBP	475	11.8	474	475	475	476	475	476	475	475	475	475	475.1	−0.1	0.12

A mixture of C5 through C40 n-alkanes with known boiling points which are used for establishing the correlation between RT (min) and BP (°C) can be separated in less than 2.5 minutes with repeatability in retention time better than 0.1 percent relative standard deviation (RSD). Table 3 shows

RT stability with RSD of approximately 0.02 to 0.15 percent (C40) using a rapid temperature program of 150 °C/min ramped from 45 to 350 °C. These results indicate a lack of cold spots and temperature nonuniformity in the LTM column module. Figure 2 is an overlay of five consecutive runs of the C5 to C40 calibration mixture.

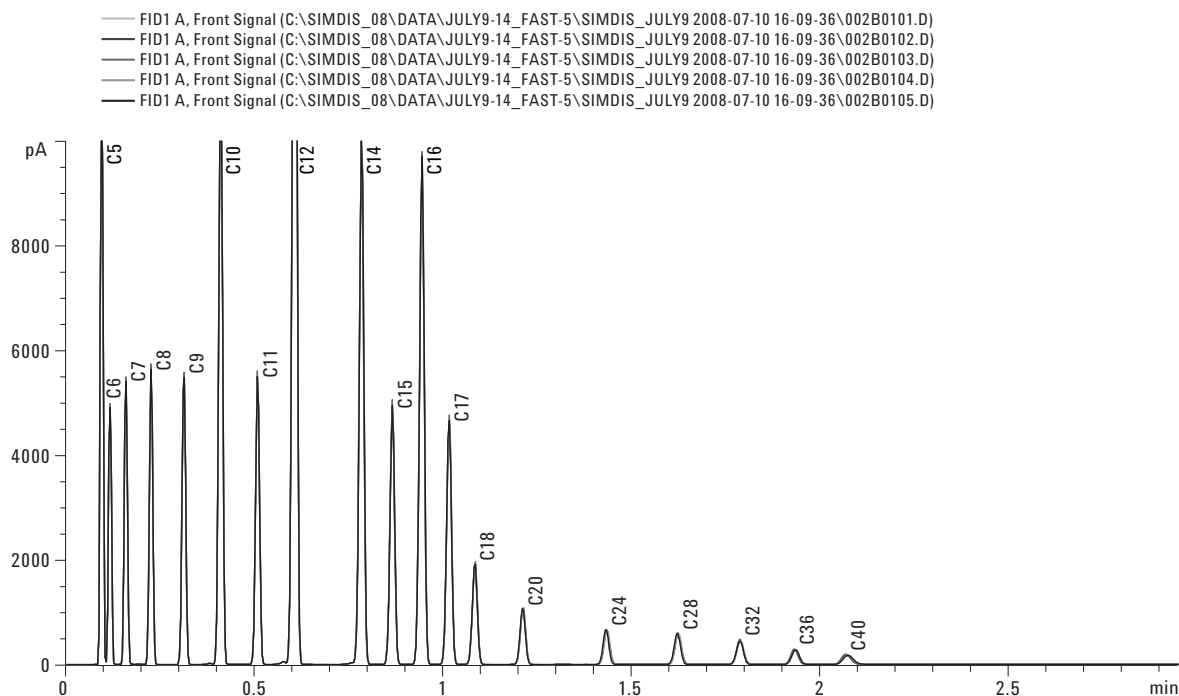


Figure 2. Overlays of five consecutive runs of the C5 to C40 calibration mixture. Chromatographic overlay shows outstanding repeatability. LTM: 45 to 350 °C at 150 °C/min.

Table3. Retention Time Repeatability of C5 to C40 Calibration Mixture (n = 5)

	1	2	3	4	5	Average	SD	RSD%
C5	0.09766	0.09760	0.09766	0.09763	0.09764	0.09764	2.33E-05	0.024
C6	0.11999	0.11992	0.11994	0.11989	0.11993	0.11994	3.67E-05	0.031
C7	0.16285	0.16274	0.16270	0.16263	0.16270	0.16272	8.07E-05	0.050
C8	0.23072	0.23051	0.23045	0.23036	0.23045	0.23050	0.000135	0.059
C9	0.31908	0.31883	0.31877	0.31867	0.31874	0.31882	0.000158	0.049
C10	0.41803	0.41775	0.41779	0.41777	0.41780	0.41783	0.000117	0.028
C11	0.51730	0.51707	0.51711	0.51716	0.51719	0.51717	8.61E-05	0.017
C12	0.61713	0.61688	0.61723	0.61716	0.61721	0.61712	0.000138	0.022
C14	0.79706	0.79676	0.79746	0.79707	0.79733	0.79714	0.000271	0.034
C15	0.87963	0.87964	0.88004	0.87985	0.88009	0.87985	0.000216	0.025
C16	0.95952	0.95940	0.95986	0.95941	0.95966	0.95957	0.000192	0.020
C17	1.03290	1.03263	1.03314	1.03279	1.03257	1.03281	0.000225	0.022
C18	1.10255	1.10190	1.10250	1.10236	1.10201	1.10226	0.00029	0.026
C20	1.23235	1.23131	1.23203	1.23151	1.23060	1.23156	0.000676	0.055
C24	1.45819	1.45721	1.45683	1.45772	1.45470	1.45693	0.001348	0.093
C28	1.65011	1.64884	1.64878	1.65001	1.64698	1.64895	0.001261	0.076
C32	1.81666	1.81587	1.81535	1.81739	1.81562	1.81618	0.000834	0.046
C36	1.96355	1.96259	1.96200	1.96611	1.96522	1.96389	0.001738	0.088
C40	2.10071	2.10110	2.10021	2.10619	2.10665	2.10297	0.003168	0.151

This work combines hydrocarbon SimDis with sulfur selective SimDis using an SCD mounted over the FID for simultaneous dual detector acquisition. In this configuration, about 20% of the FID exhaust gases enter the SCD burner, reducing sensitivity to 1/5 observed in an SCD-only configuration. This series configuration provides more than enough sensitivity for SimDis. A sample with known sulfur concentration is used as a QC reference (external reference sample) to calibrate

response factors (RFs) for the sulfur channel needed for calculation of total sulfur. Sulfur linearity of the LTM method was checked by injecting the standard mixture at different concentration levels, ranging from 100 to 1,500 ng/ μ L (ppm). The calibration curve is displayed in Figure 3, giving a correlation coefficient above 0.999.

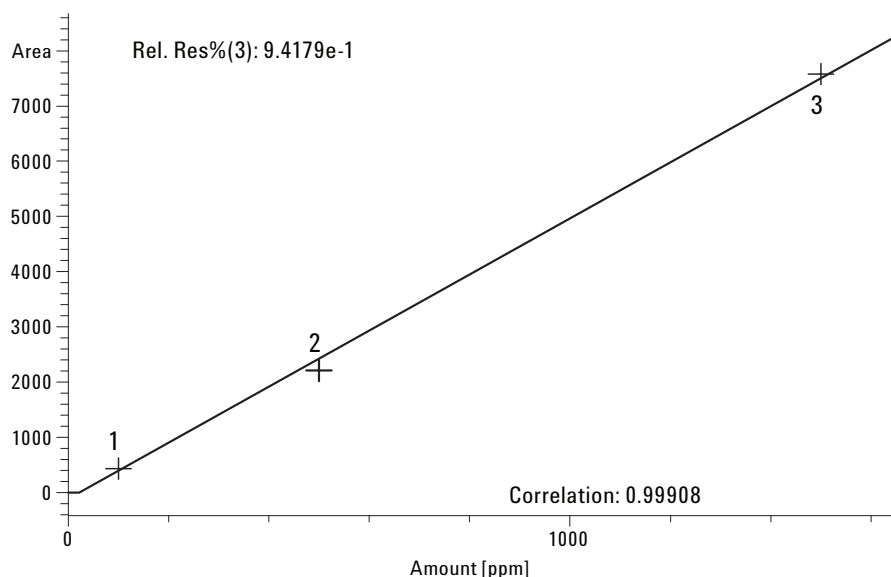


Figure 3. Linearity of sulfur with the SCD and LTM system.

A high-sulfur light cycle oil (LCO) sample was analyzed. Data were processed for hydrocarbon and sulfur simulated distillation, yielding excellent repeatability as shown in Table 4. The RSD is less than 0.4 percent for hydrocarbon and 0.5 percent

except that of FBP for sulfur. The average total sulfur is 260.8 ppm with an RSD of 4.83 percent. Figure 4 shows the chromatograms for sulfur (top) and carbon (bottom).

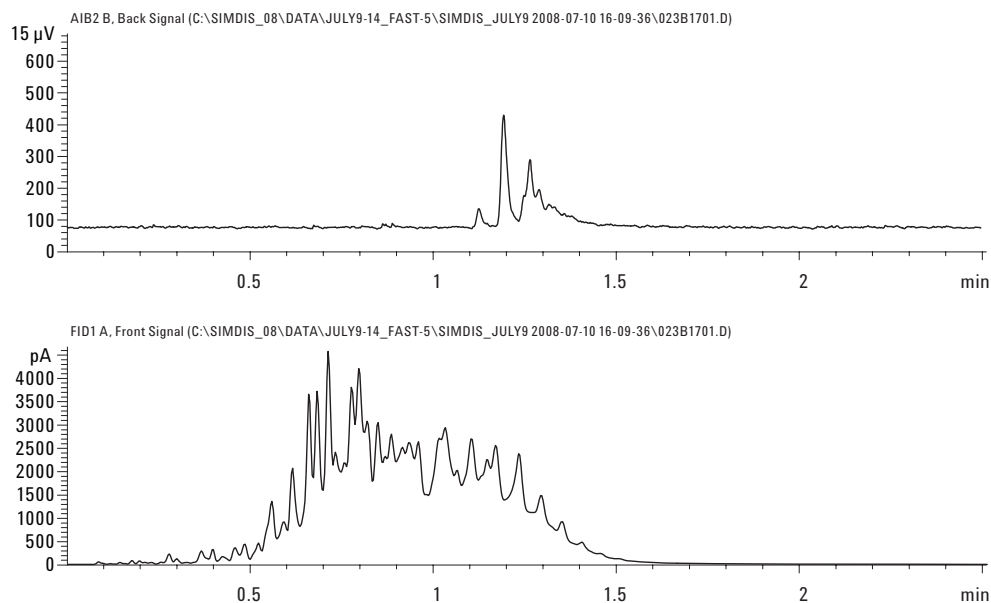


Figure 4. Chromatograms for sulfur and carbon. Sulfur top chromatogram.

Table 4. Hydrocarbon and Sulfur SimDis Results for Light Cycle Oil

Hydrocarbon SimDis												
	BP, °C										Average	RSD%
IBP	139	140	139	140	140	139	140	139	140	139	139.5	0.38
10%	222	223	222	223	223	222	223	222	224	223	222.7	0.30
20%	235	236	235	236	236	235	236	235	236	235	235.5	0.22
30%	249	250	249	250	250	249	250	249	250	249	249.5	0.21
40%	262	262	262	262	262	262	262	262	262	262	262.0	0.00
50%	276	277	277	277	277	277	277	276	277	277	276.8	0.15
60%	293	294	293	294	294	293	294	293	294	293	293.5	0.18
70%	308	309	308	309	309	308	309	308	309	308	308.5	0.17
80%	326	326	326	326	327	326	326	326	326	326	326.1	0.10
90%	347	347	347	347	347	347	347	347	347	347	347.0	0.00
FBP	411	411	411	412	413	412	412	412	412	412	411.8	0.15
Sulfur SimDis												
	BP, °C											
IBP	319	320	318	319	320	319	318	319	319	318	318.9	0.22
10%	334	334	334	334	334	334	334	334	334	334	334.0	0.00
20%	335	335	335	335	336	335	335	335	335	335	335.1	0.09
30%	337	337	337	337	337	337	337	337	337	337	337.0	0.00
40%	341	340	343	341	340	345	344	342	341	342	341.9	0.50
50%	349	349	349	349	349	350	349	349	349	349	349.1	0.09
60%	352	351	352	351	352	352	351	352	351	352	351.6	0.14
70%	355	354	355	355	355	355	355	355	355	355	354.9	0.08
80%	361	358	360	360	359	360	360	359	359	360	359.6	0.22
90%	368	365	367	367	366	369	368	367	367	367	367.1	0.30
FBP	399	384	385	386	383	407	392	384	395	395	391.0	2.05
Total sulfur (ppm)	260	237	255	262	252	281	273	254	263	273	260.8	4.83

Conclusions

A fast dual detector (FID and SCD in series) simulated distillation method using an LTM system on the 7890A GC provides a cycle time improvement over conventional systems of about 6X.

The SimDis result for ASTM D2887 RGO shows that the LTM system is equivalent to that of air-bath GCs. Results agree with the RGO specification of ASTM D2887 with RSDs of 0.12 to 0.47 percent across the reported percent off range. Wide boiling range hydrocarbons (C5 to C40 BP calibration mixtures) also show very good RT stability with RSD of 0.02 to 0.15 percent using a rapid column module temperature program of 150 °C /min ramped from 45 to 350 °C and sample injection using the HT-PTV. Sulfur linearity of the LTM/SCD system reveals an excellent correlation coefficient, above 0.999. SimDis of a high sulfur LCO sample shows an RSD of less than 0.4 percent for hydrocarbon and 0.5 percent except that of FBP for sulfur. The average total sulfur is 260.8 ppm, with an RSD of 4.83 percent.

References

1. ASTM D2887-06 a, "Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography," Annual Book of Standards, Volume 05.01, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA
2. "Agilent Low Thermal Mass (LTM) System for Gas Chromatography," Agilent Technologies publication 5989-8711EN, June 2008
3. Method Translation Software. Download from Agilent.com:
<http://www.agilent.com/chem/mts>

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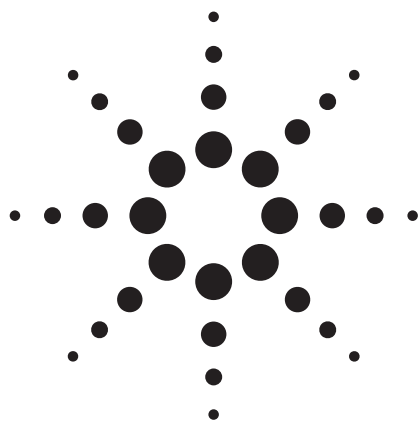
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Dual Channel Simulated Distillation of Carbon and Sulfur with the Agilent 7890A GC and 355 Sulfur Chemiluminescence Detector

Application Note

Hydrocarbon Processing

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Abstract

Two-channel simulated distillation by gas chromatography (GC) for both hydrocarbons and sulfur is described. The method utilizes a 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet and a sulfur chemiluminescence detector (SCD) mounted in series with a flame ionization detector (FID) by use of a special mounting adapter. A simulated distillation (SimDis) software program provides an easy-to-use solution for sulfur and hydrocarbon simulated distillation. The data show that observed boiling point (BP) values agree with the ASTM D2887 consensus BP values within the allowable differences. The system also demonstrates very good repeatability for both hydrocarbon and sulfur SimDis. An example of a light cycle oil (LCO) analyzed according to D2887 is also included.



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Introduction

Sulfur and hydrocarbon simulated distillation results provide meaningful information to optimize refining processes and ensure compliance with petroleum product specifications. A previous application note [1] describes a 6890 GC based system for hydrocarbon simulated distillation by ASTM D2887 [2]. Now with the highly selective Agilent Sulfur Chemiluminescence Detector (SCD), sulfur simulated distillation is possible. This 7890A GC based simulated distillation system consists of acquiring and analyzing simultaneously the specific detector data for hydrocarbon (FID) and sulfur (SCD).

Experimental

This two-channel SimDis application uses the Agilent 7890A GC configured with a high-temperature programmable temperature vaporizer (HT-PTV) inlet, and an SCD mounted onto an FID using a special adapter. Detailed GC conditions used are listed in Table 1.

Table 1. 7890A Gas Chromatographic Conditions (1) D2887, (2) D7213

HT-PTV inlet typical temperature programs	(1) 225 to 350 °C (hold 15 min) at 200 °C / min to 225 °C at 100 °C / min (2) 50 to 420 °C (hold 15 min) at 200 °C / min to 50 °C at 100 °C / min
Split ratio	(1) 4:1 for diluted sample, 20:1 for nondiluted sample (2) 1:1
Injection volume	(1) 0.1 µL (2) 0.5 to 1 µL
Column	(1) HP-1 10 m × 530 µm × 0.88 µm (19095z-021) (2) DB-HT-SimDis 5 m × 530 µm × 0.15 µm (145-1001)
Column flow (He)	(1) 13 mL/min, constant flow mode (2) 16 mL/min, constant flow mode
FID temperatures	(1) 350 °C (2) 400 °C
H ₂ flow	40 mL/min
Air flow	400 mL/min
Make up (N ₂)	40mL/min
SCD	
Burner temperature	800 °C
Vacuum of burner	324 torr
Vacuum of reaction cell	11.6 torr
H ₂	40 SCCM
Air	8.3 SCCM
Oven programs	(1) 35 °C (hold 0.5 min) to 350 °C at 20 °C/min , hold 10 min (2) 40 to 420 °C at 20 °C/min , hold 6 min
Data acquisition rate	5 Hz typical

SimDis Software

The processes of SimDis analysis include: blank analysis for baseline subtraction, calibration for establishing the relationship between boiling point and retention time (RT), validation for verifying both the chromatographic conditions and calculations in the method, and sample analysis. The Agilent SimDis software divides these functions under separate tabs that make navigation and data processing straightforward. The software is based on four modules: Browse, Setup, SimDis, and Report. For example, the Setup module allows you to configure the files to use for BP calibration, blank selection, and QC reference. Partial integration with the GC ChemStation sequence makes automated data analysis possible.

Processing Two Signals

The software can process one or two channels of signal data (FID and SCD for example) from GC ChemStation data files. When working with dual channels, the SimDis software requires that each channel be labeled by the detector type rather than the defaults used by the GC ChemStation. Since the SCD operates off the analog input board (AIB), its signal begins with "AIB." For this reason, the post-run command macro SCDnamer.mac must be run to rename the signal file. The macro renames the AIB2B.ch channel as SCD1.ch. If the channel name is not corrected, the software will switch the FID and SCD channels during analysis, giving faulty results. The macro code to do this is shown below. It assumes the AIB is in the rear position (B).

```
!=====
! SCDNamer call this as a post run command when an SCD is
! installed
! it renames the dual channel AIB2B.ch to SCD1.ch to allow
! simdis to
! properly calibrate
!=====
NAME SCDNamer
! This macro renames the SCD files named as AIB2B.ch to
SCD1.ch
if filestat(mode,dadatapath$+dadatfile$+"\AIB2B.CH")=1
rename dadatapath$+dadatfile$+"\AIB2B.ch",dadatapath$+dadatfile$+"\SCD1.ch"
print "File Renamed"
else
print "No AIB2B File found"
endif
RETURN
ENDMACRO
```

Results and Discussion

Calibration

A calibration mixture containing a series of known n-alkanes can be used for establishing the relationship between BP and RT. C5 to C40 is used for ASTM D2887, and Polywax 500 dissolved in toluene is used to calibrate ASTM D7213 [3]. Since both are too viscous or waxy at ambient temperature to sample with a syringe, they need to be heated manually to approximately 80 °C before injection. RT repeatability is key for consistent correlation of BP and RT. Figure 1 and Figure 2

show overlays of consecutive runs of C5 to C40 and Polywax 500, respectively. Tables 2 and 3 show repeatability for both RT and area.

Polywax 500 Sample Preparation

Place approximately 80 mg of Polywax 500 in a 2-mL vial. Add about 1.5 mL toluene followed by the addition of a suitable mixture of n-paraffins from C5 to C18 (Agilent SimDis calibration No.2). The final concentration should be approximately one part of (C5–C18) to 20 parts of toluene. Initially heat the solution to 80 °C to dissolve the Polywax 500.

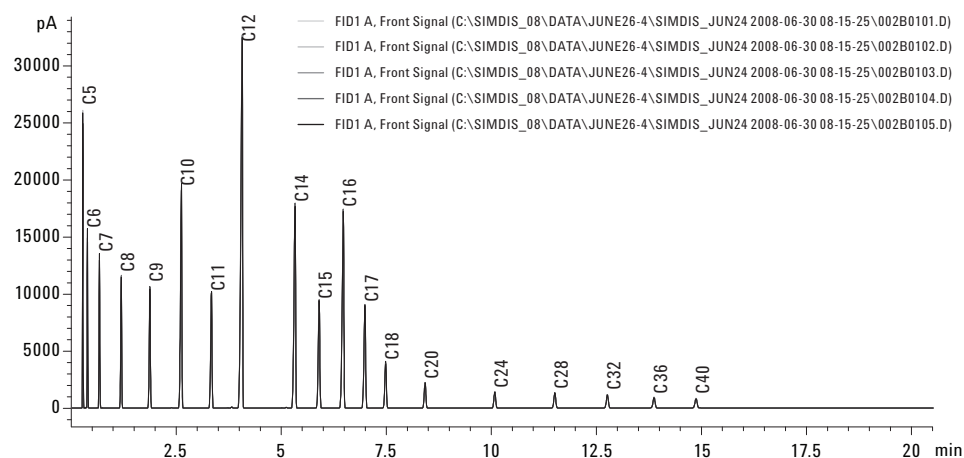


Figure 1. Overlay of five consecutive runs of C5 to C40 calibration mix, vial heated to 80 °C for 3 min prior to injection. GC conditions are listed in Table 1, items (1).

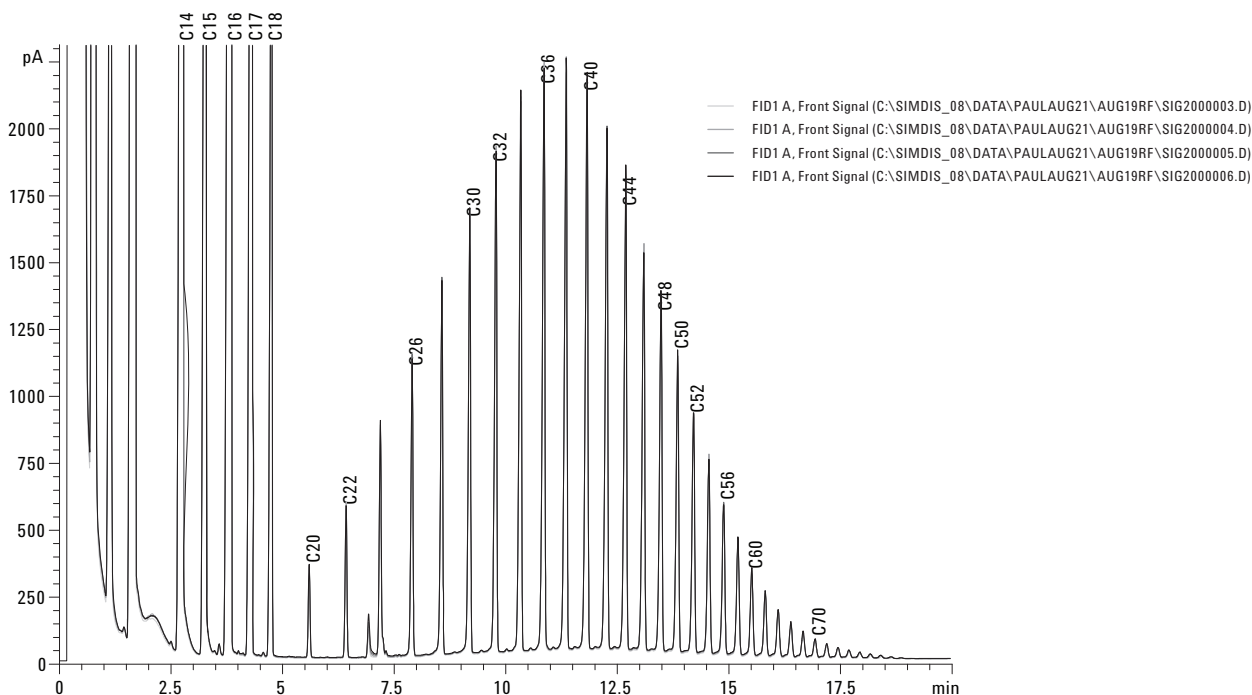


Figure 2. Overlay of four consecutive runs of Polywax 500 plus C5–C18. GC conditions are listed in Table 1, items (2).

Table 2. Repeatability for C5 to C40, n = 10

	Retention Time			Area		
	Average	STDEV	RSD%	Average	STDEV	RSD%
C5	0.275	0.000	0.06	19870	126	0.64
C6	0.388	0.000	0.09	14020	83	0.60
C7	0.673	0.001	0.17	16527	108	0.65
C8	1.192	0.002	0.16	18693	81	0.43
C9	1.874	0.002	0.12	20383	107	0.53
C10	2.622	0.003	0.10	43561	280	0.64
C11	3.338	0.002	0.07	22730	158	0.69
C12	4.068	0.002	0.05	94289	714	0.76
C14	5.327	0.002	0.03	48149	393	0.82
C15	5.902	0.002	0.03	24268	199	0.82
C16	6.477	0.001	0.02	49175	408	0.83
C17	6.991	0.001	0.02	24448	201	0.82
C18	7.485	0.000	0.00	10552	84	0.80
C20	8.424	0.001	0.01	6187	53	0.86
C24	10.083	0.000	0.00	4293	17	0.40
C28	11.512	0.001	0.01	4288	45	1.06
C32	12.762	0.002	0.01	3988	66	1.66
C36	13.874	0.001	0.01	3407	66	1.94
C40	14.874	0.002	0.01	3238	69	2.14

QC Reference

A QC reference sample is the basis for quantifying total sulfur and allows the direct entry of response factors for calculation based on total area and user-entered concentrations of sulfur. In this application, a diesel sample (SDF-1X-4, AccuStandard, Inc., New Haven, CT) with a sulfur concentration of 100 µg/g

Table3. Repeatability of Polywax 500 Plus C5 to C18, n = 10

	Retention Time			Area		
	Average	STDEV	RSD%	Average	STDEV	RSD%
C14	2.769	0.002	0.07	49126	953	1.94
C15	3.278	0.002	0.05	24337	469	1.93
C16	3.847	0.002	0.05	49304	948	1.92
C17	4.311	0.002	0.05	24597	470	1.91
C18	4.753	0.001	0.03	11374	218	1.92
C20	5.596	0.001	0.01	952	17	1.80
C22	6.424	0.001	0.01	1635	30	1.81
C26	7.904	0.001	0.01	3615	62	1.71
C32	9.783	0.001	0.01	6856	105	1.53
C36	10.858	0.001	0.01	8418	137	1.63
C40	11.823	0.001	0.01	8432	128	1.52
C44	12.690	0.002	0.01	7037	137	1.95
C48	13.480	0.001	0.01	5288	104	1.98
C52	14.208	0.001	0.01	3677	67	1.83
C60	15.512	0.001	0.01	1353	19	1.40
C70	16.931	0.002	0.01	273	5	1.92

is used as the QC external reference for calibration of response factors for the SCD channel. This is needed for calculation of total sulfur in the sample. Figure 3 shows the graphic pane from the SimDis software for of the QC reference.

Reference Gas Oil Analysis

To meet the requirements of ASTM D2887, the reference gas oil (RGO) sample analysis must be performed to verify both the chromatographic performance and the calculation algo-

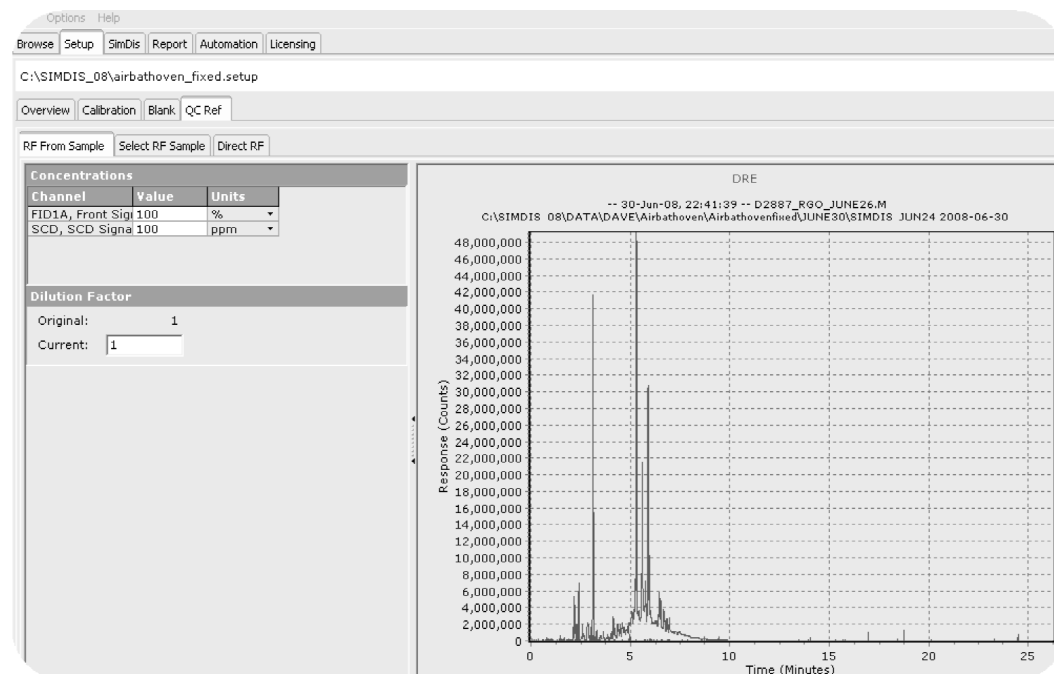


Figure 3. QC reference setup. GC conditions are listed in Table 1, items (1).

rithms involved in this test method. Figure 4 shows the chromatograms of RGO for both the hydrocarbon and sulfur channels. Tables 4 and 5 show the results for six runs of RGO

analysis. The data show that observed BP values agree with the ASTM D2887 consensus BP values within the allowable differences and with good repeatability.

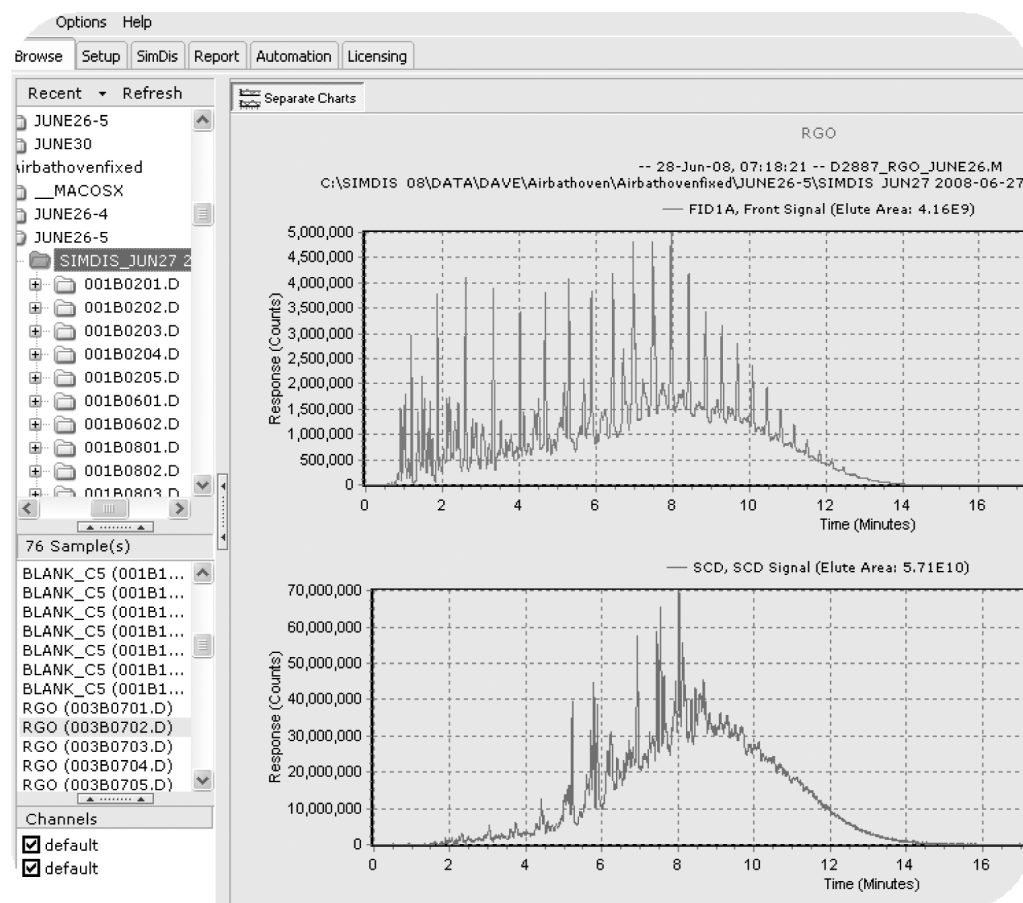


Figure 4. Chromatograms of RGO for hydrocarbon and sulfur channels. GC conditions are listed in Table 1, items (1).

Table 4. Hydrocarbon SimDis Results for Reference Gas Oil (Six runs shown.)

ASTM D2887 Values											
OFF %	BP, °C	Allowable Difference	1	2	3	4	5	6	Average	Difference	RSD%
IBP	115	7.6	114	114	114	114	114	114	114	1	0.00
10%	176	4.1	174	174	174	174	174	174	174	2	0.00
20%	224	4.9	223	223	223	223	223	223	223	1	0.00
30%	259	4.7	258	258	258	258	258	258	258	1	0.00
40%	289	4.3	287	287	287	287	287	287	287	2	0.00
50%	312	4.3	311	311	311	311	311	311	311	1	0.00
60%	332	4.3	330	330	330	330	330	330	330	2	0.00
70%	354	4.3	352	352	351	352	352	352	352	2	0.12
80%	378	4.3	376	376	376	376	376	376	376	2	0.00
90%	407	4.3	405	405	405	405	405	405	405	2	0.00
FBP	475	11.8	471	471	471	471	471	471	471	4	0.00

Table 5. Sulfur SimDis Results for Reference Gas Oil, BP in °C

OFF%	1	2	3	4	5	6	Average	STDEV	RSD%
IBP	168	169	169	167	165	169	168	1.60	0.95
10%	265	265	265	265	265	265	265	0.00	0.00
20%	293	293	293	293	293	293	293	0.00	0.00
30%	314	314	314	314	314	314	314	0.00	0.00
40%	329	330	330	330	330	330	330	0.41	0.12
50%	344	344	344	344	344	345	344	0.41	0.12
60%	359	359	359	360	360	360	360	0.55	0.15
70%	376	376	377	377	377	377	377	0.52	0.14
80%	396	396	396	397	397	398	397	0.82	0.21
85%	408	408	408	409	409	409	409	0.55	0.13
90%	422	422	423	423	424	424	423	0.89	0.21
FBP	495	495	495	499	499	501	497	2.66	0.53

Light Cycle Oil Analysis

To illustrate repeatability, chromatographic overlays are shown in Figures 5a and 5b for an LCO sample. Tables 6 and 7 list the results for hydrocarbon and sulfur SimDis, respectively. The average total sulfur content calculated is 248 ppm with 3.5% RSD.

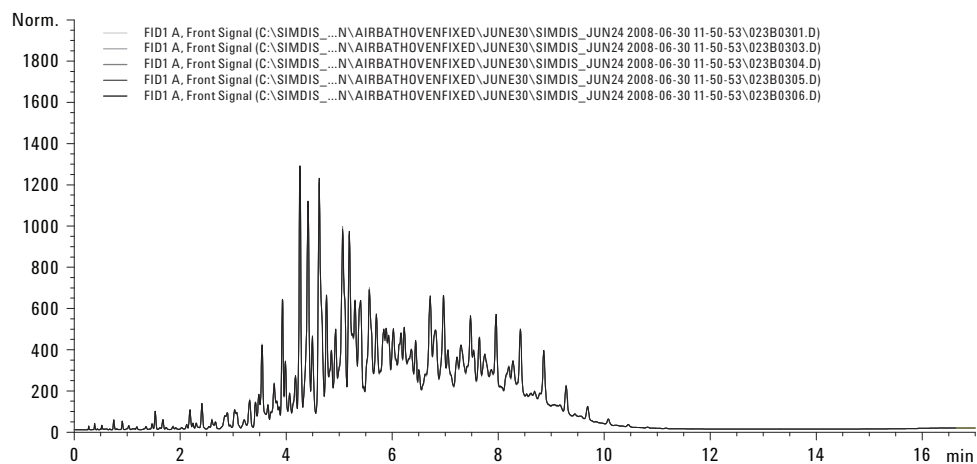


Figure 5a. Carbon SimDis of LCO. Five-run overlay.

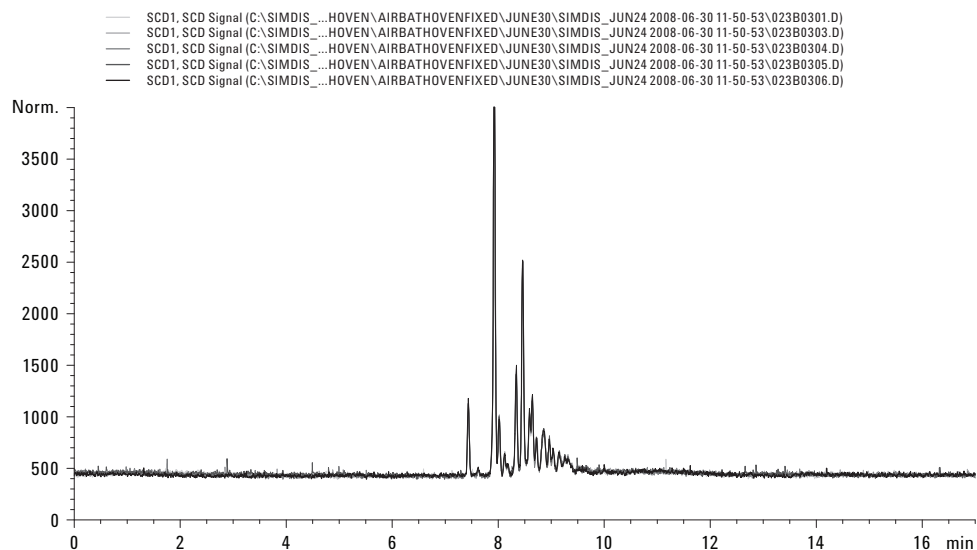


Figure 5b. Sulfur SimDis of LCO. Five-run overlay.

Table 6. Carbon SimDis Results for LCO, BP in °C

OFF%	1	2	3	4	5	Average	SD	RSD%
IBP	141	140	140	140	139	140	0.71	0.51
10%	221	221	221	221	221	221	0.00	0.00
20%	233	233	233	233	234	233	0.45	0.19
30%	247	247	247	247	247	247	0.00	0.00
40%	260	260	261	260	261	260	0.55	0.21
50%	274	275	275	275	275	275	0.45	0.16
60%	291	292	292	292	292	292	0.45	0.15
70%	306	307	307	307	307	307	0.45	0.15
80%	324	324	324	324	324	324	0.00	0.00
90%	344	344	344	344	344	344	0.00	0.00
FBP	391	391	391	391	392	391	0.45	0.11

Table 7. Sulfur SimDis Results for LCO, BP in °C

OFF%	1	2	3	4	5	Average	SD	RSD%
IBP	314	314	314	314	314	314	0.00	0.00
10%	328	329	328	328	328	328	0.45	0.14
20%	329	329	329	329	329	329	0.00	0.00
30%	329	329	329	329	329	329	0.00	0.00
40%	332	332	332	332	332	332	0.00	0.00
50%	342	342	342	342	342	342	0.00	0.00
60%	345	345	345	345	345	345	0.00	0.00
70%	347	347	346	346	347	347	0.55	0.16
80%	351	351	350	350	351	351	0.55	0.16
90%	359	359	357	359	358	358	0.89	0.25
FBP	375	375	371	374	371	373	2.05	0.55
Sulfur, ppm	254	250	240	238	258	248	8.62	3.48

Conclusions

This new SimDis procedure utilizes a 7890A GC configured with the HT-PTV inlet, and an SCD mounted in series with an FID. The Agilent SimDis software is capable of processing both FID and SCD data channels, providing a solution for hydrocarbon and sulfur simulated distillation.

Sulfur simulation distillation has been demonstrated using the Agilent 355 sulfur chemiluminescence detector. With a selectivity over carbon of approximately 10^6 , reliable boiling point distributions of sulfur in petroleum fractions can be obtained.

References

1. C. Wang and R. Firor, "Simulated Distillation System for ASTM D2887," Agilent Technologies, publication 5989-2726EN
2. ASTM D2887-06 a, "Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography," Annual Book of Standards, Volume 05.01, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA
3. ASTM D 7213-05, "Standard Test Method for Boiling Point Distribution of Petroleum Distillates from 100 °C to 615 °C by Gas Chromatography," Annual Book of Standards, Volume 05.04, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA

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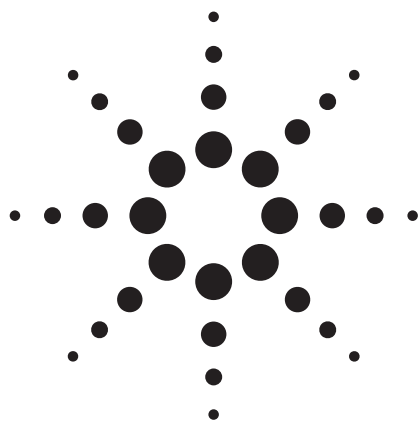
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Analysis of Trace Hydrocarbon Impurities in Benzene by Agilent 7820A Gas Chromatograph

Chunxiao Wang and Wenmin Liu

Application Brief

HPI

Knowledge of impurities in benzene provides critical quality control information where benzene is either produced or used in a manufacturing process. ASTM D4492 [1] was used for analyzing these impurities, including nonaromatics containing up to nine carbon atoms, toluene, C8 aromatics, and 1,4-dioxane. The Agilent 7820A gas chromatograph offers an efficient and easy-to-use platform for the analysis of benzene and many other aromatic solvents. For this application, an Agilent 7820A GC is configured with a split/splitless capillary inlet and a flame ionization detector (FID). Agilent EZChrom Elite Compact software is used to control the 7820A GC and provide data acquisition/data analysis. The Agilent 7820A GC supports an automatic liquid sampler (ALS), allowing fully unattended operation – from injection all the way through final reporting.

Experimental

Table 1. Typical GC Conditions

Inlet settings	250 °C, Split ratio: 100:1 to 30:1
Injection volume	0.5 µL
Column	HP-INNOWax 60 m × 0.32 µm × 0.5 µm
Column flow (He)	2.6 mL/min (21.8 at 75 °C), constant flow mode
Oven temperature program	For impurities in benzene: 75 °C (10 min); 3 °C/min to 100 °C For aromatic solvent: 75 °C (10 min); 3 °C/min to 100 °C 10 °C/min to 145 °C
FID setting	
Temperature	250 °C
H ₂ flow	40 mL/min
Air flow	400 mL/min
Make up (N ₂)	25 mL/min
Data acquisition rate:	20 Hz

Highlights

- An easy-to-use, single-column method for benzene as well as a wide range of aromatic solvent purity analyses meets the chromatographic requirements of 10 separate ASTM methods. Therefore fewer GCs, stock columns, and supplies are required to analyze many different types of samples.
- EPC control and automatic injection ensures excellent repeatability for both retention time and peak area.
- The wide dynamic response range of the FID enables a quantitative analysis of samples containing both very high and very low concentrations in a single run.



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Discussion

The Agilent 7820A GC with full electronic pneumatics control (EPC) on all inlets and detectors ensures good repeatability and also makes it fast and easy to set and to save the pressures and flows. Figure 1 shows the chromatograms of the D4492 calibration standard. Excellent repeatability for retention time with RSD of approximately 0.03 to 0.01% and peak area with RSD of about 1.6% are shown in Table 2.

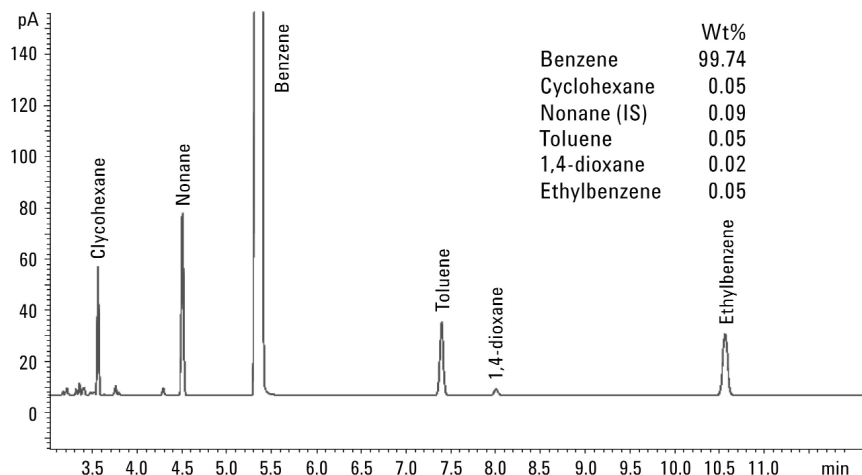


Figure 1. ASTM D4492 benzene calibration standard. Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 100:1.

Table 2. Repeatability–ASTM D4492 Benzene Calibration Standard (11 runs) with First Run Included

	Cyclohexane	Nonane	Bezene	Toluene	1,4-dioxane	Ethylbenzene
	Peak Area					
1	430130	861450	900088289	590385	56288	689141
2	425791	848159	888131170	581775	55693	677502
3	437496	874885	915251703	599534	57071	698269
4	439204	879141	918796665	601857	57355	701225
5	438646	876346	917995860	601138	57056	700462
6	436941	876809	914994185	599823	57743	699919
7	423567	844923	885230656	580241	55487	675473
8	420259	843030	878870585	577475	55392	673593
9	422665	844761	883243038	579572	55419	675665
10	430741	865226	901189833	591633	56211	691217
11	431032	865007	901921807	592037	56118	691200
Mean:	430588	861794	900519436	590497	56348	688515
Std Dev:	6852	14298	14909746	9406	837	11061
%RSD:	1.59	1.66	1.66	1.59	1.49	1.61

Table 2. Repeatability–ASTM D4492 Benzene Calibration Standard (11 runs) with First Run Included (Continued)

	Cyclohexane	Nonane	Bezene	Toluene	1,4-dioxane	Ethylbenzene
	Retention Time					
1	3.562	4.503	5.369	7.397	8.003	10.561
2	3.562	4.504	5.371	7.398	8.005	10.563
3	3.562	4.504	5.371	7.398	8.007	10.565
4	3.561	4.503	5.370	7.398	8.006	10.563
5	3.561	4.503	5.370	7.398	8.006	10.563
6	3.561	4.503	5.369	7.398	8.007	10.563
7	3.561	4.503	5.369	7.398	8.006	10.563
8	3.561	4.503	5.369	7.398	8.006	10.563
9	3.561	4.504	5.370	7.398	8.006	10.563
10	3.563	4.506	5.372	7.400	8.007	10.567
11	3.563	4.506	5.372	7.400	8.009	10.565
Mean:	3.562	4.504	5.370	7.398	8.006	10.564
Std Dev:	0.0008	0.0012	0.0012	0.0009	0.0015	0.0016
%RSD:	0.02	0.03	0.02	0.01	0.02	0.01

The FID has a very wide dynamic response range due to its full digital path. This enables a quantitative analysis of samples containing very high and very low concentrations in a single run. Figure 2 shows that trace impurities spiked in benzene, trace level (10 ppm) ethyl benzene, and > 99% benzene can be quantitative analyzed in a single run.

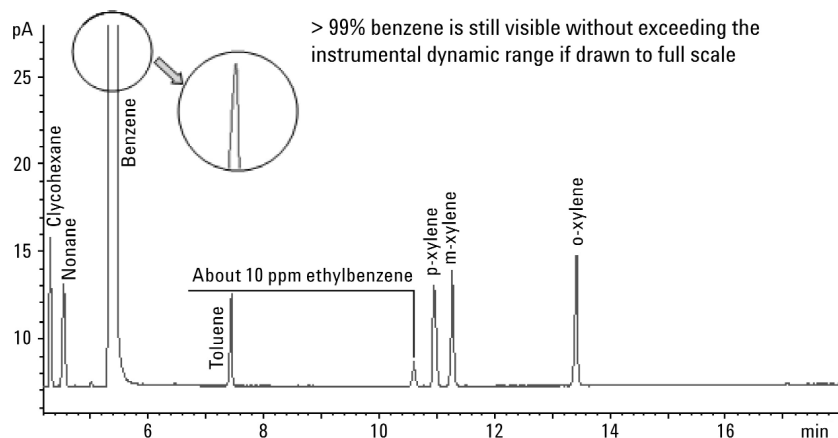
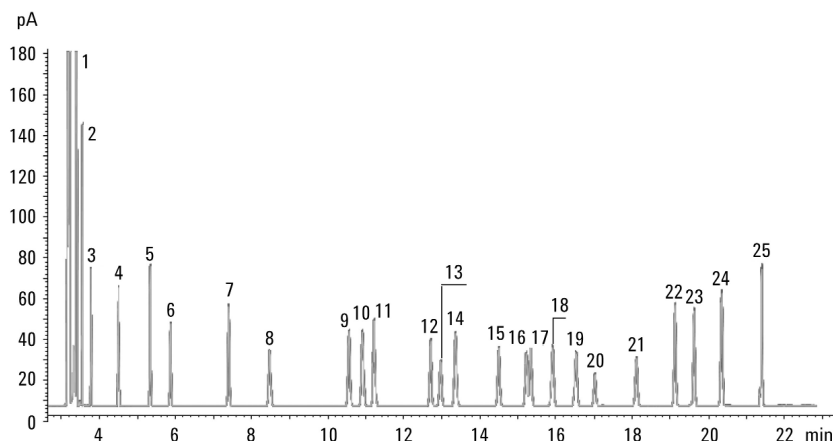


Figure 2. Analysis of trace impurities spiked in benzene. Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 30:1.

This system is also chromatographically suitable for a wide range of aromatic solvent samples according to 10 different ASTM aromatics methods as mentioned in reference 2. An n-hexane solution was prepared containing 0.1 wt% of aromatic solvents and impurities specified by the 10 ASTM methods for the analysis; the chromatographic overlay of 11 runs demonstrates outstanding repeatability as shown in Figure 3.



- | | | | |
|----------------|-----------------|--------------------|---------------------|
| 1. Heptane | 7. Toluene | 13. Dodecane | 19. s-butylbenzene |
| 2. Cyclohexane | 8. Undecane | 14. o-xylene | 20. Styrene |
| 3. Octane | 9. Ethylbenzene | 15. Propylbenzene | 21. Tridecane |
| 4. Nonane | 10. p-xylene | 16. p-ethyltoluene | 22. Diethylbenzene |
| 5. Benzene | 11. m-xylene | 17. m-ethyltoluene | 23. n-butylbenzene |
| 6. Decane | 12. Cumene | 18. t-butylbenzene | 24. a-methylstyrene |
| | | | 25. Phenylacetylene |

Figure 3. Chromatographic overlay of 11 runs of aromatic solvent specified by 10 ASTM methods.
 Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C, 10 °C/min to 145 °C.
 Sample size: 0.5 µL, Split ratio: 100:1.

References

1. ASTM D4492-98, "Standard Test Method for Analysis of Benzene by Gas Chromatography," Annual Book of Standards, Volume 06.04, ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA
2. James D. McCurry, "A Unified Gas Chromatography Method for Aromatic Solvent Analysis," Agilent Technologies publication 5988-3741EN

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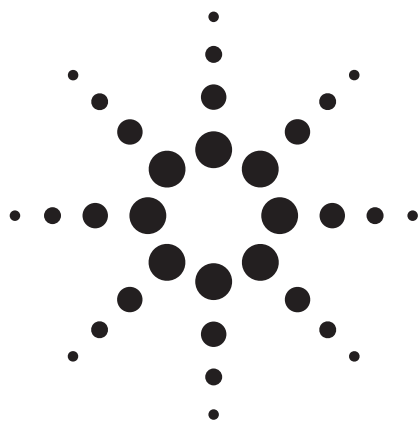
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Automated Preparation of Simulated Distillation Samples for ASTM Methods D2887, D7213, D7398 and D6352 using a Dual Tower 7693A and Tray System

Application Note

Hydrocarbon Processing

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Abstract

A dual tower 7693A and tray system installed on the 7890A Gas Chromatograph was used for preparation of hydrocarbon calibration standards, solvent blanks, and actual petroleum samples for the purpose of analysis by simulated distillation (SimDis). The front tower is equipped with a 5 or 10 μ L syringe while the back tower is equipped with a 250 or 500 μ L syringe. A 150 sample tray with heater and mixer/barcode reader is also used. Procedures are described for sample preparation for ASTM D2887, D7213, D7398 and D6352. The Multimode Inlet, G3510, operated in a temperature programmed split mode was used for all samples.



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Introduction

Sample and calibration standard preparation for various simulated distillation methods is normally a manual process requiring dilution, mixing, and heating. Many procedures use volatile toxic solvents such as carbon disulfide. ASTM method D2887 commonly uses CS₂ for sample dilution while D6352 may use CS₂ or toluene for polywax calibration standard preparation. Sample heating is required for many of these procedures. Using the automation capabilities of the 7693A tower and tray system improves lab safety as well when working with CS₂ and other solvents by avoiding manual handling and uncontrolled heating of mixtures.

Experimental

For all experiments, the 7890A GC was equipped with dual 7693A towers and tray. The front tower used a standard 5 or 10 µL syringe and the rear tower was equipped with the optional large syringe carriage with either a 250 or 500 µL syringe. Sample prep procedures were done on the rear tower and sample injection occurred on the front tower. The 7890A was configured with the multimode inlet operated in temperature programmed split mode. Detection was with FID. In addition, two 7890A oven systems were used. The first configuration used the conventional air bath oven and the second used the Low Thermal Mass (LTM) system. Instrumental parameters for various configurations are listed in Table 1.

Table 1. 7890A SimDis parameters

LTM System for D2887

LTM module	5M × 0.32 mm × 0.50 µm DB1, 5 inch format
7890A oven	300 °C isothermal
Inlet	Multimode, 270 °C (0 min) to 355 °C at 200 °C/min
Liner	Single taper with glass wool, 5183-4647
Split ratio	20:1
Pressure program (Inlet)	8 psi (0 min) – 42 psi (0.9 min) at 14 psi/min
LTM program	40 °C (0 sec) to 350 °C (30 sec) at 100 °C/min

Standard System for D2887

Column	10M × 0.53 mm × 3.0 µm D2887
Oven	40 °C (0 min) to 350 °C (5 min) at 15 °C/min
Inlet	Multimode, G3510, 50 °C (0 min) to 330 °C (4 min) at 200 °C/min
Liner	Single taper with glass wool, 5183-4647
Split	4 to 1
Flow	3.2 psig at 40 °C, constant flow mode

7890A system for D7213 and D7398 (Polywax 500 calibration)

LTM

Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis 5-inch LTM format
Oven	LTM configuration, 7890A oven 325 °C isothermal, module 40 °C (0 min) to 400 °C (30 sec) at 50 °C/min
Inlet	Multimode, 270 °C (0 min) to 400 °C (3 min) at 300 °C/min
Split ratio	4 to 1 and 10 to 1
Pressure program	2.5 psi (0 min) to 9.5 psi (1.0 min) at 1 psi/min

Standard Air Bath Oven

Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis
Oven program	40 °C (0 min) to 400 °C (5 min) at 15 °C/min
Inlet	Multimode, 210 °C (0 min) to 400 °C (10 min) at 200 °C/min
Split ratio	4 to 1
Flow	15 mL/min, constant flow mode

7890A system for D6352 (Polywax 655 calibration)

Column	5M × 0.53 mm × 0.15 µm DB-HT SimDis
Oven program	40 °C (0 min) to 430 °C (5 min) at 15 °C/min
Inlet	Multimode, 250 °C (0 min) to 430 °C (hold until end of run) at 200 °C/min
Split ratio	4 to 1
Flow	16 mL/min, constant flow mode

7693A System

Front tower	5 or 10 µL syringe, G4513A
Back tower	250 or 500 µL syringe, G4521A syringe carriage
Tray	150 sample capacity with heater and mixer/barcode reader, G4520A
Inlet	G3510 Multimode, CO ₂ cooled
ChemStation	B.04.01
7890A firmware	A.01.10 or greater

Discussion

A typical sample preparation program for D2887 setup is shown in Table 2. This illustrates just one way to program preparation of the calibration standard, reference gas oil (RGO), and blank that are necessary to set up a system for routine analyses. The commands can be assembled in other ways to produce the same end result. The following vials and tray locations are used with this program.

Tray position 1	Calibration mix, 0.5 µL of C5 to C40, Agilent part number 5080-8716
Tray position 2	1 mL RGO, Agilent part number 5060-9086
Tray position 3 to 5	Empty vials with 100 µL inserts, Agilent part number 5188-6592

When the procedure is complete, vial 3 will be the prepared RGO for injection, vial 4 will be the prepared calibration mix

for injection, and vial 5 will be a CS₂ blank. Next, a three-line sequence is set up that starts with vial 4 (calibration mix). Vial 4 is run with the ChemStation method set with this procedure active, then vial 3 (RGO) and vial 5 (CS₂ blank) are run using the same method but with the prep procedure inactive (unchecked in ChemStation's 7890A Injector Program pane under edit 7890A Parameters parameters menu because these samples are already prepared from the method in the first line of the sequence table). For all three samples, the core ChemStation method performs a sample preheat at 80 °C and a sample mix at 500 rpm for 20 seconds before injection. Lastly, the calibration, prepared RGO, and blank vials are fitted with 100 µL inserts so that the solvent amounts used for the procedure are minimized. Please note that when these inserts are used, mixing should be limited to speeds of approximately 500 rpm to avoid "spilling" liquid over the top of the insert into the bottom of the 2-mL vial.

Preparation of polywax standards for the higher temperature SimDis method is always challenging due to their low solubility. Solvents such as CS₂ and toluene are commonly used, and

Table 2. Sample prep procedure for D2887

Sampler program steps	
Move vial from front sample vial offset by -3 vial(s) to back turret position #1	
Dispense 750 µL from vial Wash A3 to vial Sample 1 on the Back tower	
Move vial from back turret position #1 to front sample vial offset by -3 vial(s)	
Move vial from front sample vial offset by -1 vial(s) to back turret position #3	
Move vial from front sample vial offset by 0 vial(s) to back turret position #2	
Load 150 µL from vial Wash A1 with 0 µL airgap	
Load 50 µL from vial Sample 3 with 0 µL airgap	
Load 0 µL from vial Waste A1 with 0 µL airgap	
Load 150 µL from vial Wash A1 with 0 µL airgap	
Load 0 µL from vial Sample 2 with 0 µL airgap	
Move vial from front sample vial offset by -3 vial(s) to heater	
Heat vial at 80 degrees C for 300 seconds	
Move vial from heater to back turret position #1	
Load 5 µL from vial Sample 1 with 0 µL airgap	
Load 0 µL from vial Sample 2 with 0 µL airgap	
Load 150 µL from vial Wash A2 with 0 µL airgap	
Wait for 1 minutes	
Load 0 µL from vial Waste A3 with 0 µL airgap	
Dispense 150 µL from vial Wash A3 to vial Waste A1 on the Back tower	
Wash syringe in Back tower, drawing from Wash A2 dispensing into Waste B1 3 times	
Move vial from back turret position #1 to front sample vial offset by -3 vial(s)	
Move vial from back turret position #2 to front sample vial offset by 0 vial(s)	
Move vial from front sample vial offset by -2 vial(s) to back turret position #1	
Dispense 20 µL from vial Sample 1 to vial Sample 3 on the Back tower	
Move vial from back turret position #3 to front sample vial offset by -1 vial(s)	
Move vial from back turret position #1 to front sample vial offset by -2 vial(s)	
Move vial from front sample vial offset by 1 vial(s) to back turret position #1	
Wash syringe in Back tower, drawing from Wash B3 dispensing into Waste B2 3 times	
Dispense 150 µL from vial Wash A3 to vial Sample 1 on the Back tower	
Move vial from back turret position #1 to front sample vial offset by 1 vial(s)	
Wash syringe in Back tower, drawing from Wash A1 dispensing into Waste A1 2 times	
Wash syringe in Front tower, drawing from Wash A1 dispensing into Waste A1 2 times	

heating of the solvent/polywax vial is required just prior to injection. This entire procedure can be automated with the 7693A tower and tray system. The basic procedure for Polywax 500 is as follows:

- Place approximately 80–100 mg of Polywax 500 in a 2-mL vial and seal
- Add 125 μ L of a C20/toluene solution to the polywax vial
- Add 1.25 mL of toluene to the polywax-C20 vial
- Mix the vial
- Heat the vial at 80 °C for 4 min
- Return to tray

- Heat one final time (3 min. typical) just prior to injection

Table 3 shows the basic prep procedure using a dual tower/tray system automating the steps shown above. The only manual step is adding the solid polywax to Vial 1. Vial 2 contains a C20/toluene mixture. Preparation of this sample could be automated as well. This procedure is applicable to D7213 SimDis and D7398 (Boiling Range Distribution of Fatty Acid Methyl Esters).

A resulting chromatogram from injection of the prepared Polywax 500 vial (vial 1) is shown in Figure 1. A symmetric distribution of the polywax fragments with good resolution to C80 can be seen.

Table 3. Preparation of Polywax 500

Sampler program steps
Wash syringe in Back tower, drawing from 'Wash A1' dispensing into 'Waste A1' 2 times
Move vial from tray vial #1 to back turret position #1
Move vial from tray vial #2 to back turret position #2
Dispense 1000 μ L from vial 'Wash A2' to vial Sample 1 on the Back tower
Dispense 5 μ L from vial Sample 2 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to mixer
Move vial from back turret position #2 to tray vial #2
Mix at 2000 rpm 2 times for 10 seconds
Move vial from mixer to heater
Heat vial at 80 degrees C for 240 seconds
Move vial from heater to tray vial #1
Wash syringe in Back tower, drawing from 'Wash A2' dispensing into 'Waste B1' 3 times

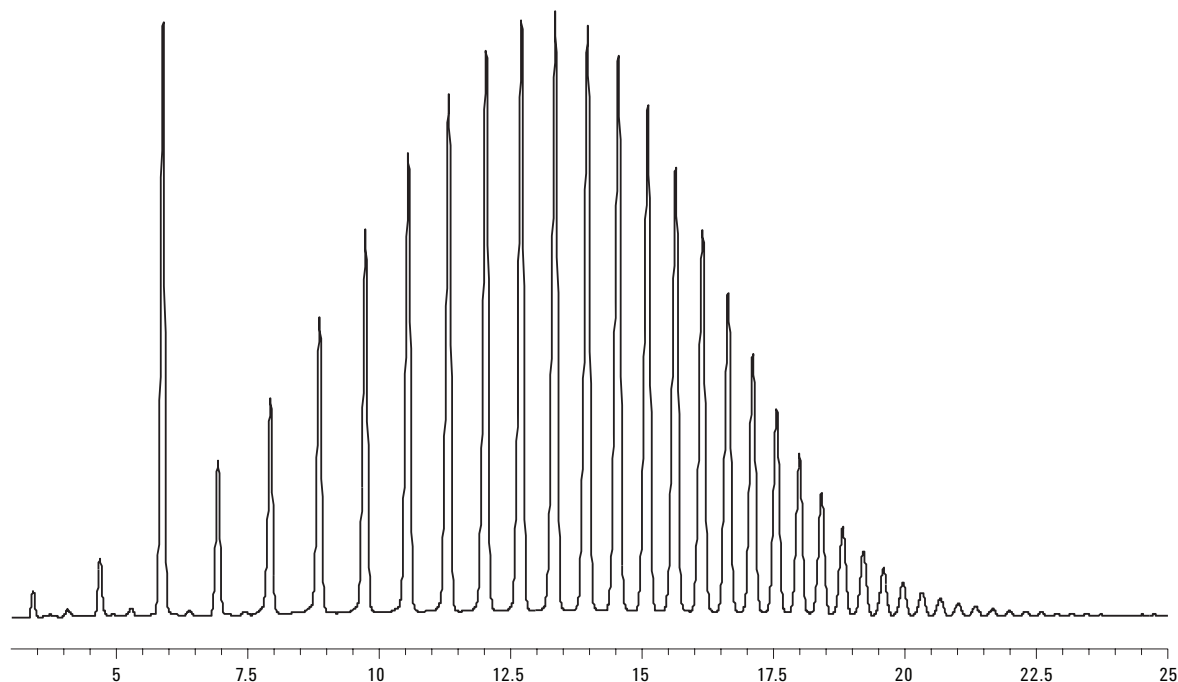


Figure 1. Polywax 500 with C20 marker. Multimode inlet with 7890A oven.

The preparation program for Polywax 655 is essentially the same as shown above for Polywax 500 except that heating is extended to 6 minutes, for better dissolution. Then just prior to injection, the prepared vial is heated for another 3 minutes. In the chromatogram shown below in Figure 2, a small amount (5 μ L) of C5-C18 mix was added to the Polywax 655/toluene solution as part of the automated procedure.

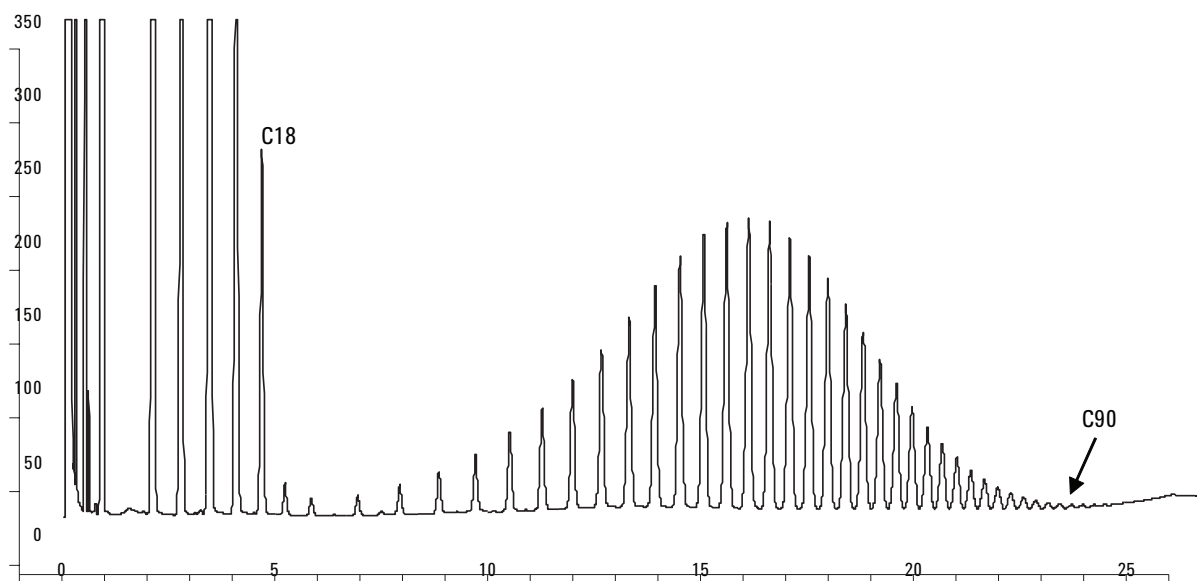


Figure 2. Chromatogram of Polywax 655.

The chromatogram was produced with the multimode inlet used in temperature-programmed split mode. Good definition of polyethylene fragmented to C110 is shown in Figure 3 where the last 5 minutes of the chromatogram are enlarged to show detail. Producing this detail out to C110 is extremely difficult for most chromatographic systems. The 7890A/7693A system produces excellent results with this sample.

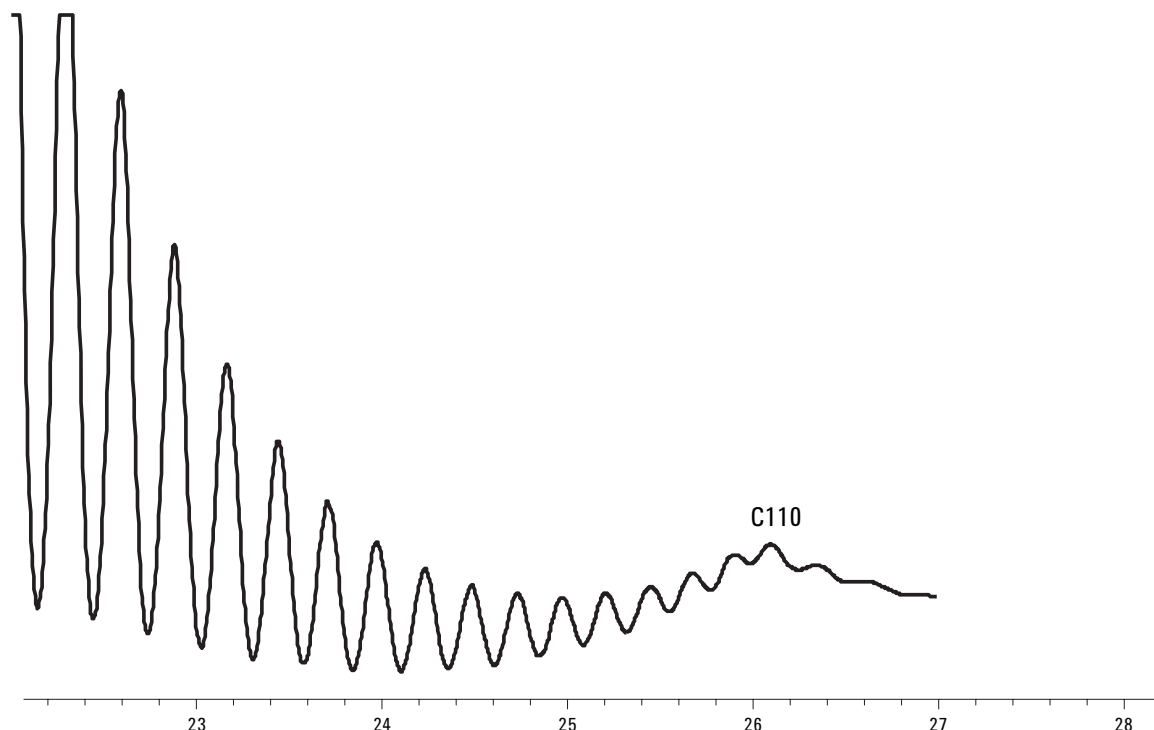


Figure 3. Polywax 655 to C110. Multimode inlet program: 150 °C (0 min) to 430 °C (hold until end of run) at 200 °C/min. 7890A oven: 40 °C (0 min) to 430 °C (5 min) at 15 °C/min. 3 µL injection. Solvent is toluene.

Reproducibility of the sample preparation steps is excellent as seen in Figure 4, for the dilution of a heavy vacuum gas oil sample (HVGO). The program steps that were followed to produce these chromatograms are given in Table 4. The back tower equipped with a 500-µL syringe, was used for sample preparation and the front tower with a 5-µL syringe was used

for sample injection. Carbon disulfide was used for sample dilution. This program assumes a sequence is run using vial 2. Vial 1 is the stock HVGO sample that is first prepared by adding 0.5 g of the oil to a 2-mL vial. This material is extremely viscous and cannot be drawn into a syringe. Therefore the program performs a fully automated two-stage dilution prior to injection.

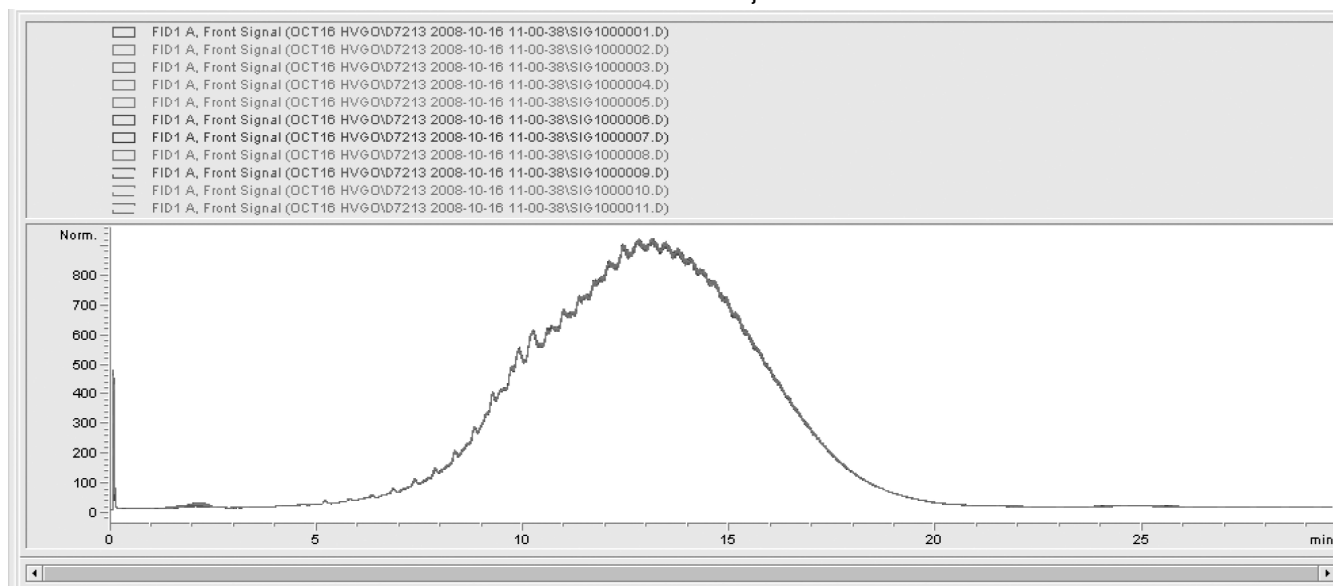


Figure 4. Overlay of 11 runs of HVGO, each prepared using 7693A towers and tray.

Table 4. Preparation of HVGO for injection. CS_2 is used as the solvent.

Sampler program steps
Move vial from front sample vial offset by -1 vial(s) to back turret position #1
Dispense 600 μ L from vial Wash A3 to vial Sample 1 on the Back tower
Move vial from back turret position #1 to heater
Move vial from front sample vial offset by 0 vial(s) to back turret position #1
Heat vial at 60 degrees C for 180 seconds
Mix at 3000 rpm 3 times for 20 seconds
Move vial from heater to back turret position #2
Dispense 250 μ L from vial Sample 2 to vial Sample 1 on the Back tower
Wash syringe in Back tower, drawing from Wash A3 dispensing into Waste A1 3 times
Dispense 1000 μ L from vial Wash A1 to vial Sample 1 on the Back tower
Move vial from back turret position #2 to front sample vial offset by -1 vial(s)
Move vial from back turret position #1 to mixer
Mix at 3000 rpm 4 times for 20 seconds
Move vial from mixer to front sample vial offset by 0 vial(s)

Conclusions

Difficult sample preparation procedures that are commonly used for petroleum and fuel samples can be easily automated with the 7693A tower and tray system for the 7890A and the 6890A. The system is particularly well suited for preparation of polywax calibration samples that are used for higher temperature methods. Tasks such as mixing, solid dissolution, dilution, heating, and internal standard addition are easily accomplished.

Chromatographic performance is enhanced through use of the multimode inlet. Using standard split injection liners, good sample capacity without carryover and with minimal discrimination of wide boiling samples is seen. The inlet was used in the temperature-programmed split mode for this work. Cryo cooling was not used, however, cryo can be used optionally to shorten inlet cool down between runs if desired.

For samples that fall within the boiling point range of D2887, D7213, and D7398, the Low Thermal Mass (LTM) system can be used to shorten typical analysis cycle times by 30 to 50% [1]. The high temperature method D6352 requires the standard 7890A oven.

The sample prep procedures listed here represent just one way of accomplishing a given task. Given the commands available with the system, there are many variants that will lead to the same end result.

Reference

1. C. Wang, R. Firor, and P. Tripp, "Fast Hydrocarbon and Sulfur Simulated Distillation Using the Agilent Low Thermal Mass (LTM) System on the 7890A GC and 355 Sulfur Chemiluminescence Detector," November 2008, Agilent Technologies publication 5990-3174EN.

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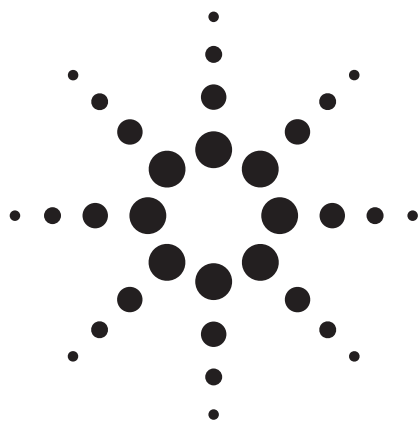
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Agilent Technologies



Implementation of the Pressure Controlled Tee for Backflushing for the 7000 Series Triple Quadrupole Mass Spectrometer: Implications for Sensitivity

Application Note

Environmental, Drug testing, Forensics and Metabolomics

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Abstract

The 7000 Series Triple Quadrupole Mass Spectrometer is designed to provide the most sensitive and specific analytical determinations in gas chromatography. The enhanced specificity of GC/MS/MS analysis encourages a more relaxed or minimized sample preparation strategy with the aim of higher sample throughput and sample turnaround time. However these more complex samples can rapidly degrade the gas chromatograph (GC) and mass spectrometer (MS) performance. Applying backflushing helps maintain the GC and the MS source by removing later eluting compounds from the column before reaching the MS, and prior to the next sample injection. This note cites the results of the Pressure Controlled Tee (PCT) configuration for rapid and universal GC/MS backflushing applied to selected reaction monitoring experiments on the Agilent 7890 GC used with the Agilent 7000 Series Triple Quadrupole Mass Spectrometer. The results show no loss in sensitivity and precision as measured for the peak area, height or signal-to-noise ratio in the 272→241 m/z for octafluoronaphthalene. This means that rapid backflushing and quick column and inlet maintenance without venting the MS system can be incorporated without a loss in detection limits using a simple PCT configuration.



Agilent Technologies

Introduction

Agilent's GC Capillary Flow Technologies (CFTs) have greatly expanded the capability and flexibility of gas chromatographic analysis. CFT can provide rapid column and inlet maintenance without MS venting as well as backflushing of the accumulated sample matrix preventing carryover which compromises the column performance. One such CFT arrangement, the Pressure Controlled Tee [1], has been shown to provide rapid backflushing and enhance analytical robustness in GC/MSD single quadrupole analysis in a very challenging sample matrix [2]. The expectation of reduced sample preparation steps for analyses using GC/MS/MS suggests that backflushing is essential to prevent source and column fouling and maintain sample throughput. In fact, MS/MS analysis of complex hydrocarbon samples has already demonstrated rising baselines and carryover in the absence of comprehensive backflushing [3]. However, concerns exist that implementing additional "plumbing" may compromise the signal and therefore the detection limits in MS/MS analysis. This note presents sensitivity results for the 7000A Triple Quadrupole GC/MS in multiple reaction monitoring (MRM) mode using the Pressure Controlled Tee (PCT) configuration.

Experimental

The target compound is the checkout compound, octafluoronaphthalene (OFN), which represents a model compound for demonstrating MRM detection thresholds. Studies were conducted at concentrations of 100 fg using the Pressure Controlled Tee (PCT) configuration.

The experimental arrangement is shown schematically in Figure 1. The typical 30-m analytical column is replaced by two 15-m columns joined by the Purged Ultimate Union. Makeup gas can be supplied by either an Auxiliary Electronic Pneumatic Control (Aux EPC) or Pressure Control module (PCM) but here an Aux EPC was used. The installation and use of the PCT are described in the G1472A kit and included documents [4]. It should be noted that the PCT is a very flexible configuration and many column combinations are possible. The one in this demonstration is the simplest to illustrate.

The same method MRM acquisition parameters were applied for the OFN as the standard instrument checkout method however the GC parameters were altered to include a backflushing period and to terminate the GC at 200 °C (Table 1). This shortened the runtime by about one third compared with the standard method. Data was also collected using a continuous 30-m column as in the checkout with the same tune file and standard checkout GC parameters.

Table 1. GC Parameters for OFN Testing with Backflush

Configuration:	(the actual arrangement is two – 15-m columns but the GC is configured as follows)
Column 1:	Front Inlet to MSD Outlet: HP-5ms 30 m × 0.25 mm, 0.25 µm film, helium carrier at 1.20 mL/min.
Column 2:	AUX EPC Channel 4 to MSD Outlet: HP-5ms 15 m × 0.25 mm, 0.25 µm film, helium carrier at 1.22 mL/min.
GC oven:	45 °C for 2.25 min, 40 °C/min to 195 °C (6 minutes runtime)
Post run:	200 °C for 2 minutes
Column 1:	0.25 mL/min (flow is actually reversed)
Column 2:	5 mL/min

Results

Replicate injections showed no statistically significant difference between the peak areas or heights for the 272 *m/z* → 241 *m/z* transition (Table 2). Further, retention time reproducibility was unaffected by addition of the PCT and a standard deviation of less than 0.1 secs was achieved both with and without the PCT. (The absolute retention times can shift slightly due to a slight difference in the absolute flows and lengths). Signal-to-noise was also indistinguishable between the two configurations although noise is uniformly low in selected reaction mode so variation in the signal-to-noise ratio can be high. The degree of correspondence is best illustrated in Figure 2.

Conclusions

Since part of the strategy for sample analysis by MRM is minimized sample cleanup to increase sample throughput, protecting the mass spectrometer source integrity and GC column phase through backflushing will help achieve system robustness. Introducing the Pressure Controlled Tee configuration into the 7890 GC combined with the 7000 Series MS maintains signal height and area without degrading compound retention time reproducibility or other performance metrics. This means that rapid backflushing and service of the injection port and column (without venting the mass spectrometer) can be introduced without compromising analytical integrity. In this way the PCT is a powerful addition to the analytical strategy for tandem mass spectrometry.

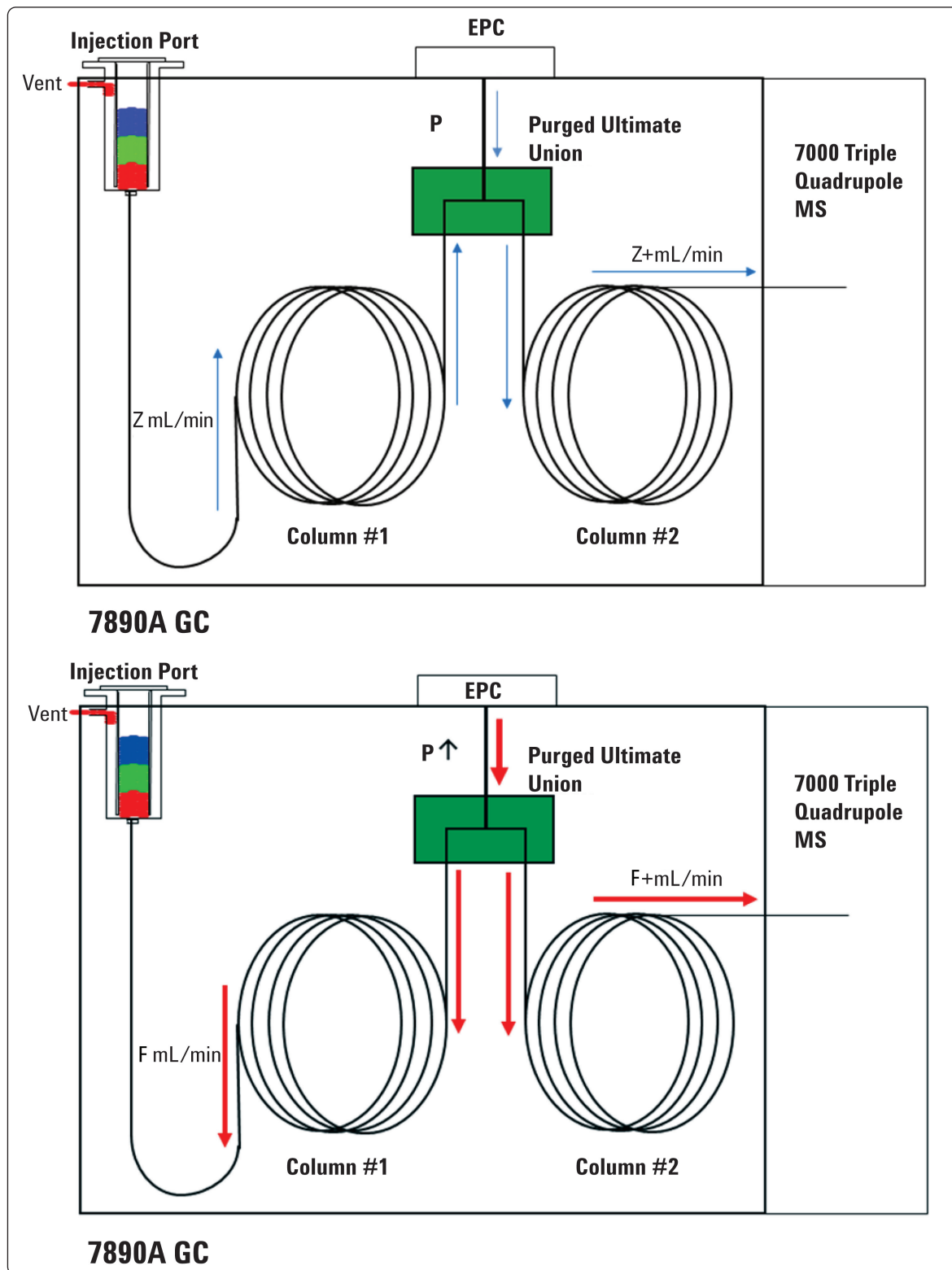


Figure 1 The Pressure Controlled Tee arrangement. Upper panel shows the forward flow during analysis and the lower panel shows the column flows during backflushing.

Table 2. Comparison between PCT and Continuous Column configurations for MRM 272→241 m/z transition for octafluoronaphthalene (OFN). To simplify the comparison, the results for the peak area, height and signal-to-noise (S/N) ratio have been normalized against the standard configuration.

GC configuration	RT variation (s)	Normalized MRM Signal area	Normalized MRM RSD (%)	Normalized MRM Signal height	Normalized MRM RSD (%)	Normalized MRM S/N
Continuous column without PCT	0.063	1	14	1	15	1
With PCT	0.098	1.1	15	1.0	16	1.2

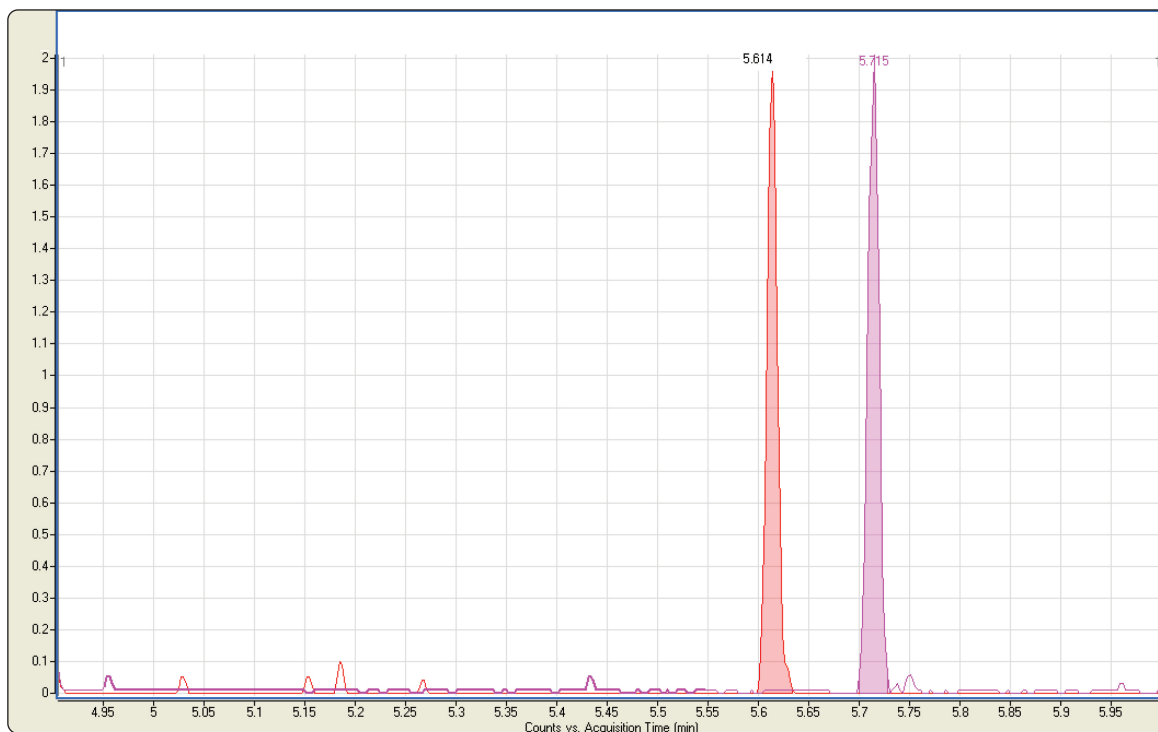


Figure 2. OFN 272 → 241 m/z transition without the PCT (5.614 min) and using the PCT (5.715 min). The same absolute abundance scale is applied to both.

References

- 1 "Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination," Agilent Technologies publication 5989-8664EN
- 2 "Capillary Flow Technology for GC/MS: Efficacy of the Simple Tee Configuration for Robust Analysis Using Rapid Backflushing for Matrix Elimination," Agilent Technologies publication 5989-9359EN
- 3 "Enhanced Sensitivity for Biomarker Characterization in Crude Oil by GC-SRM," in press
- 4 "The Rapid Universal GC/MS Backflushing Kit," part number G1472A and contains the manual (G1472-90001) which describes this configuration and operation for 5973 or 5975 or 7000 series Mass Selective Detectors.

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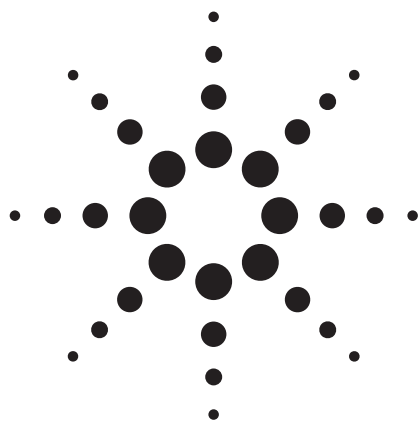
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Agilent Technologies



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

Application Note

HPI

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Highlights

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

Abstract

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



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Introduction

Analysis of permanent gases and light hydrocarbons has been widely employed in the petrochemical, chemical and energy industries. These permanent gases, such as O₂, N₂, CH₄, CO, and CO₂ are the common target compounds in natural gas, petroleum gas, synthesis gas, purified gas, water gas, blast furnace gas, stack gas, and so on. Understanding the concentrations of these components is important for petrochemical, chemical and energy industrial processes. The 7820A 3-valve system offers an easy-to-use and powerful platform for the analysis of these kinds of samples.

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

Experimental

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

Table 1. Gas Chromatographic Conditions

Sample loop size	0.25 mL
FID channel flow	5 mL/min
FID temp	300 °C
FID channel carrier	N ₂
Capillary splitter temp	200 °C
Split ratio	25:1
TCD channel flow	30 mL/min
TCD temp	250 °C
TCD channel carrier	He
Valve box temp	120 °C
Oven program	45 °C (6 min) >180 °C (2.25 min) at 20 °C/min

Table 2. Time Events

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

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

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

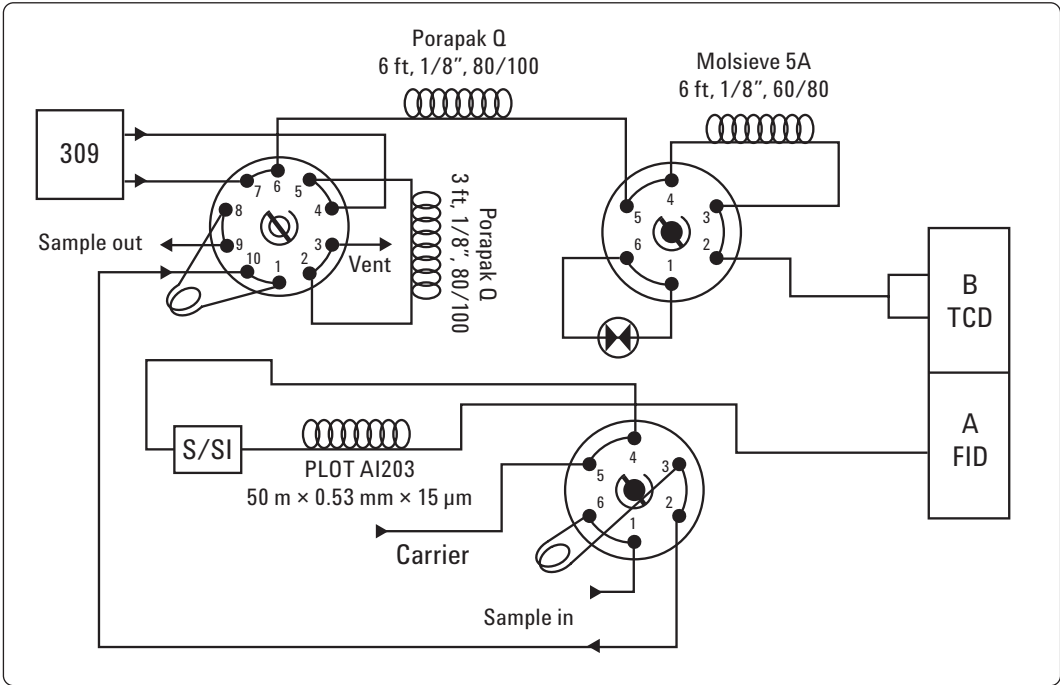


Figure 1. Valve diagram for dual-channel natural gas analysis.

Table 3. Concentrations of the Standard Gases

Components	H ₂	O ₂	N ₂	CO	CO ₂	CH ₄	C ₂ H ₆	C ₃ H ₈	iC ₄	nC ₄	iC ₅	nC ₅	nC ₆
Conc. (%)	6.09	3.00	9.97	1.99	3.48	71.92	2.00	0.99	0.11	0.10	0.12	0.12	0.11

Results

Chromatograms

Chromatograms for the FID and TCD channels of standard gas are shown in Figures 2 and 3. Hydrocarbons from C1 to C6 are separated by a PLOT Al₂O₃ column in approximately 15 minutes. For natural gas samples containing hydrocarbons higher than C6, the final temperatures of the oven program can be modified to 220 °C for the elution of hydrocarbons up to C11.

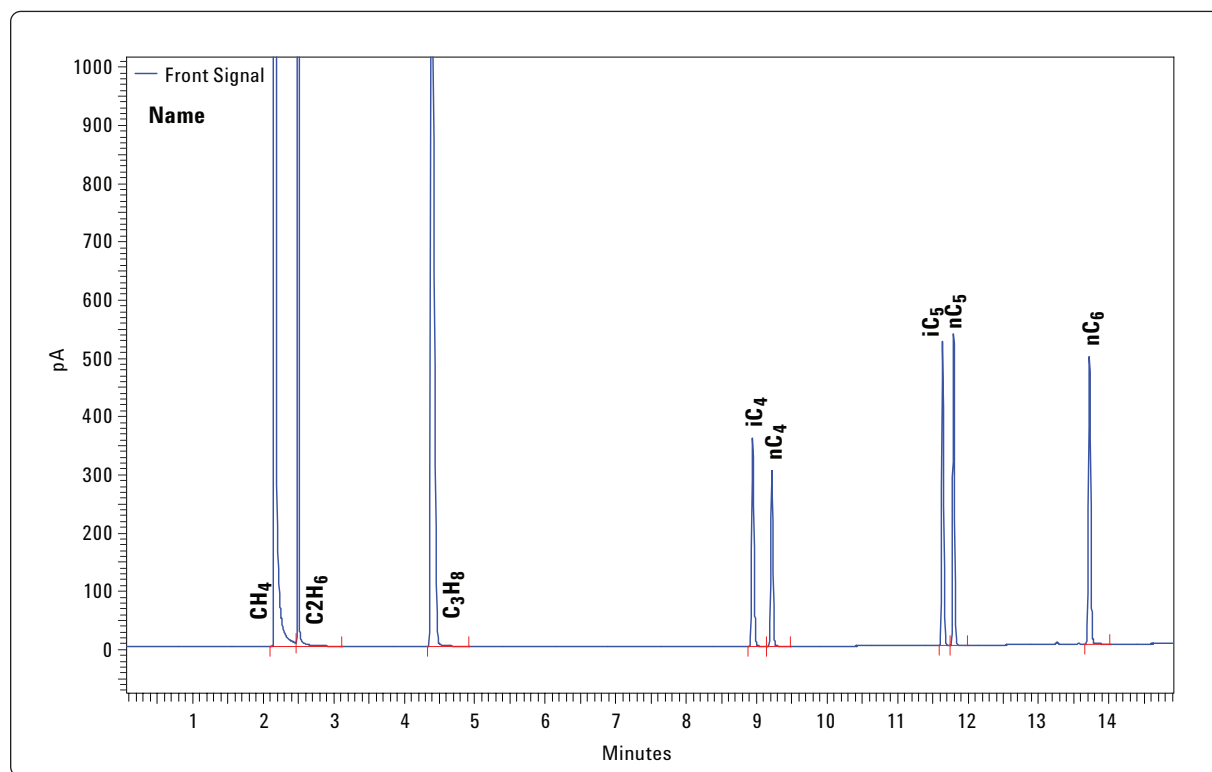


Figure 2. FID Channel chromatogram of CH₄, C₂H₆, C₃H₈, iC₄, nC₄, iC₅, nC₅, and nC₆.

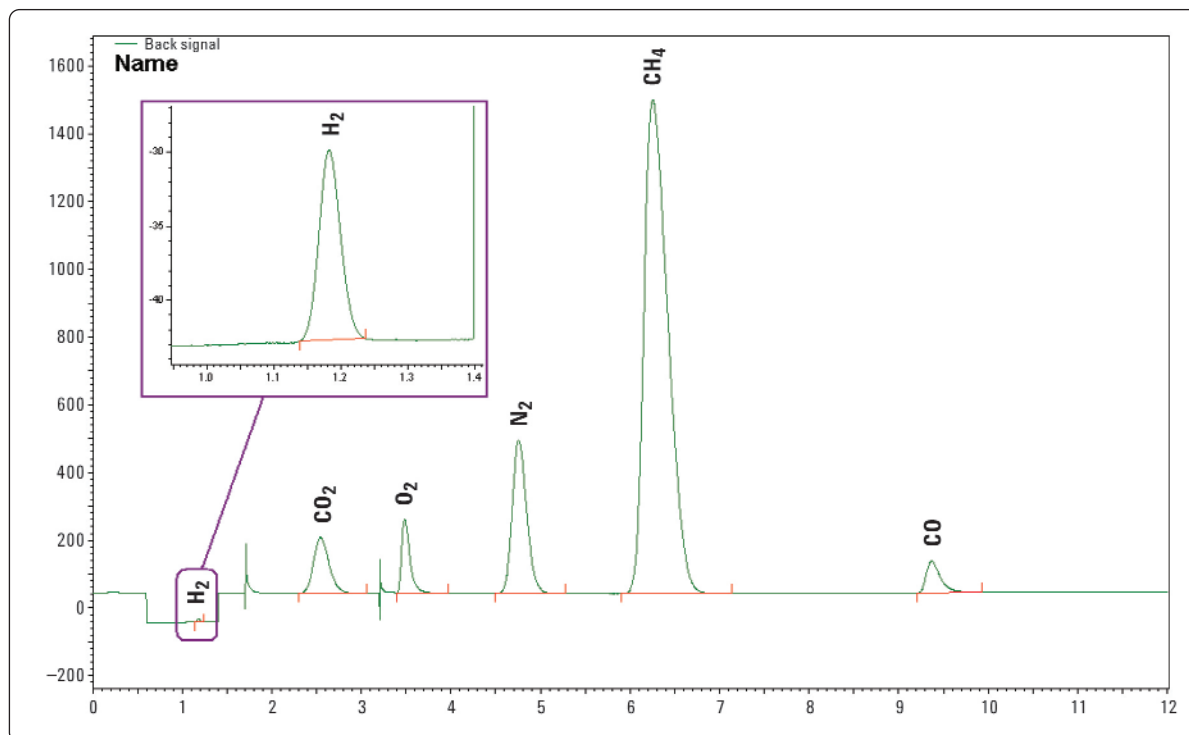


Figure 3. TCD Channel chromatogram of H₂, O₂, CO₂, N₂, CH₄, CO.

Linearity

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

Table 4. Linearity Results of TCD Channel

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

Repeatability

The relative standard deviations (RSD) for all hydrocarbon components were lower than 0.8% by using split injection on the FID channel. This was due to the full electronic pneumatics control (EPC) from injector to detector on 7820A. Results of the TCD channel also show excellent repeatability (Table 5). Component concentrations were 0.305%, 0.174%, 0.15%, 0.5%, 3.596%, and 0.1%, respectively for H₂, CO₂, O₂, N₂, CH₄, and CO.

Table 5. TCD Channel Repeatability

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

Low Level Permanent Gases

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

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

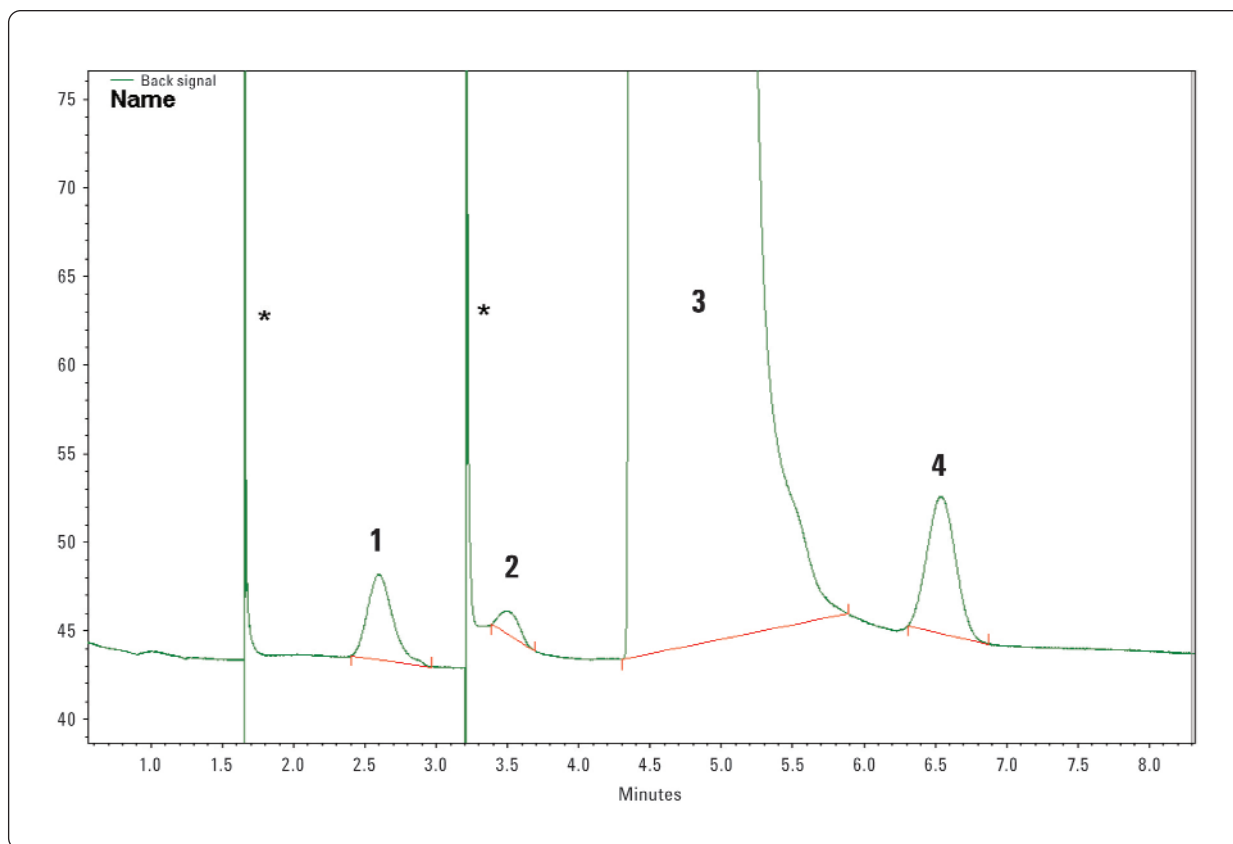


Figure 4. Chromatogram of low level permanent gas standard mix.

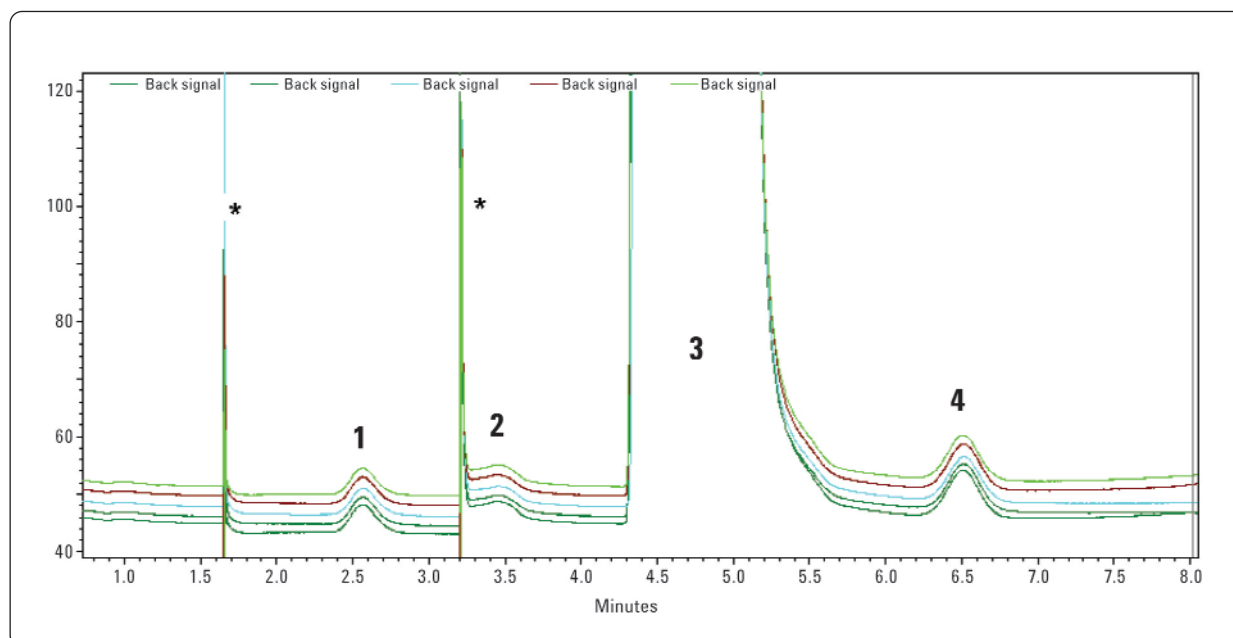


Figure 5. Overlapped chromatograms of five runs.

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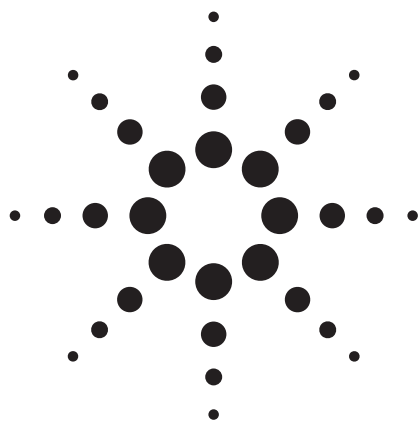
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Enhanced Sensitivity for Biomarker Characterization in Petroleum Using Triple Quadrupole GC/MS and Backflushing

Application Note

Environmental

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Abstract

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



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Introduction

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

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

Experimental

Standards and Samples

STANFORD-1 is a new external standard for quantitative biomarker analysis. It is a mixture of pure biomarker standards and paraffin-free saturate fractions from Paleozoic, Mesozoic,

Cenozoic, biodegraded, terrestrially-influenced, carbonate/evaporate-sourced, and open-marine sourced petroleum samples. It contains known quantities of most, if not all, commonly used biomarkers and two internal standards, BTI-6 and 5- β cholane, which are useful for quantifying hopane and sterane biomarkers across diverse GC/MS systems.

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

Instruments

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

MS SRM Parameters

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

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

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

Table 2. Analysis Parameters for Precision Experiments*

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

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

Results and Discussion

Backflushing with a Pressure Controlled Tee Configuration

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

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

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

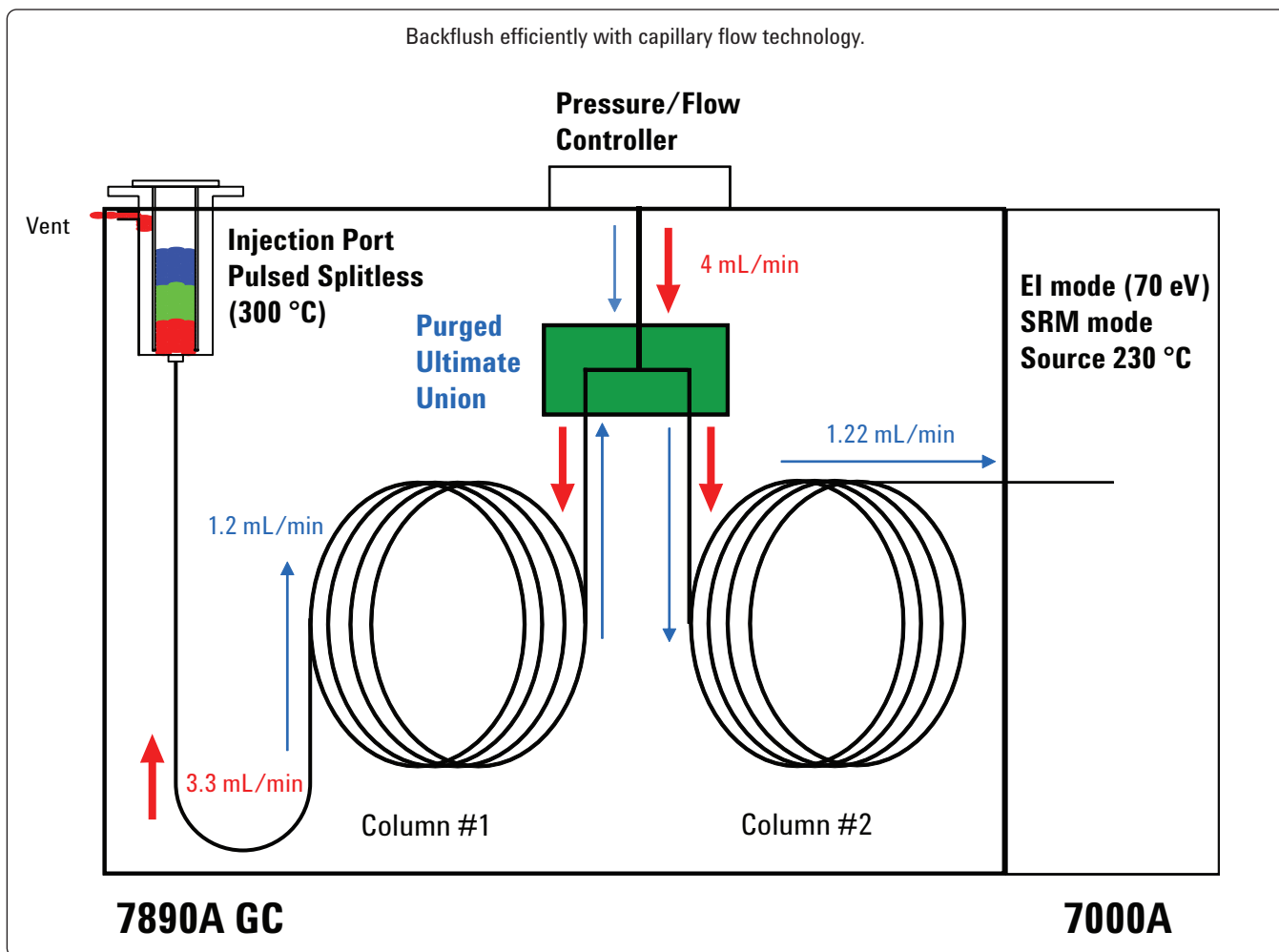


Figure 1. Schematic of the Pressure Controlled Tee GC/MS configuration. The narrower (blue) lines indicate the forward flow during analysis and the thicker (red) lines indicate the backflushing post-run state.

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

Faster Analysis of Biomarkers

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

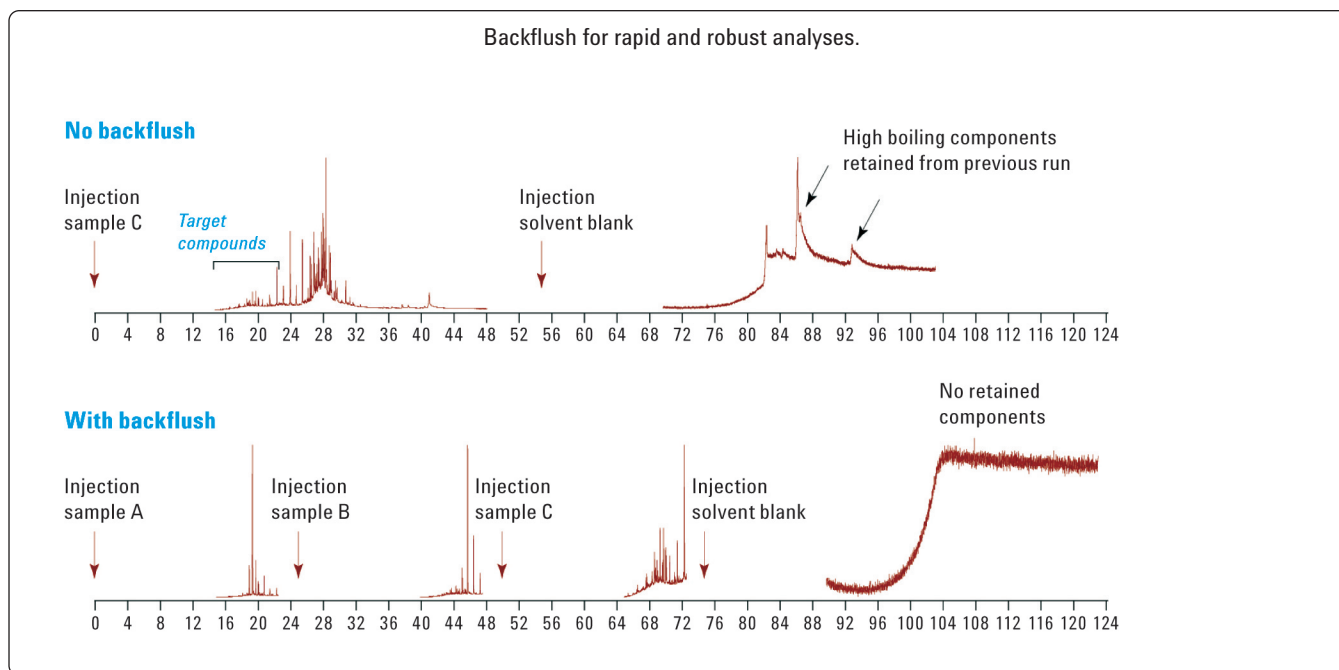


Figure 2. Petroleum samples, including one from Williston Basin source rocks (Sample C) which contains many late eluting, high molecular weight hydrocarbons, were analyzed without (top) and with (bottom) backflushing (40 m configuration). The target compounds comprise a subset of the total number of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

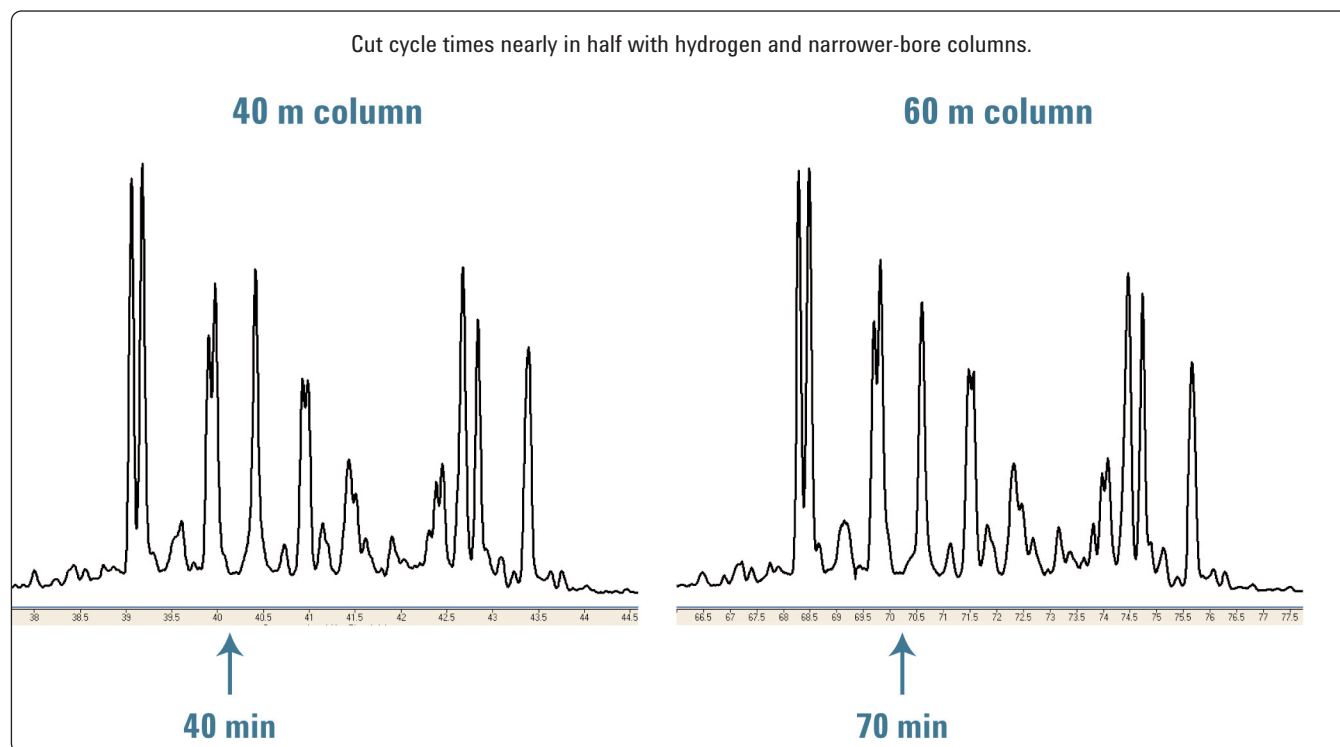


Figure 3. C28 steranes were analyzed using m/z transition 386→217 on either a 60 meter, 0.25 μm column and helium carrier gas, or a 40 meter, 0.18 μm column with hydrogen carrier gas. Employing hydrogen and the smaller bore column reduces analytical time significantly without loss in compound resolution.

Sensitivity, Selectivity and Precision

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

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

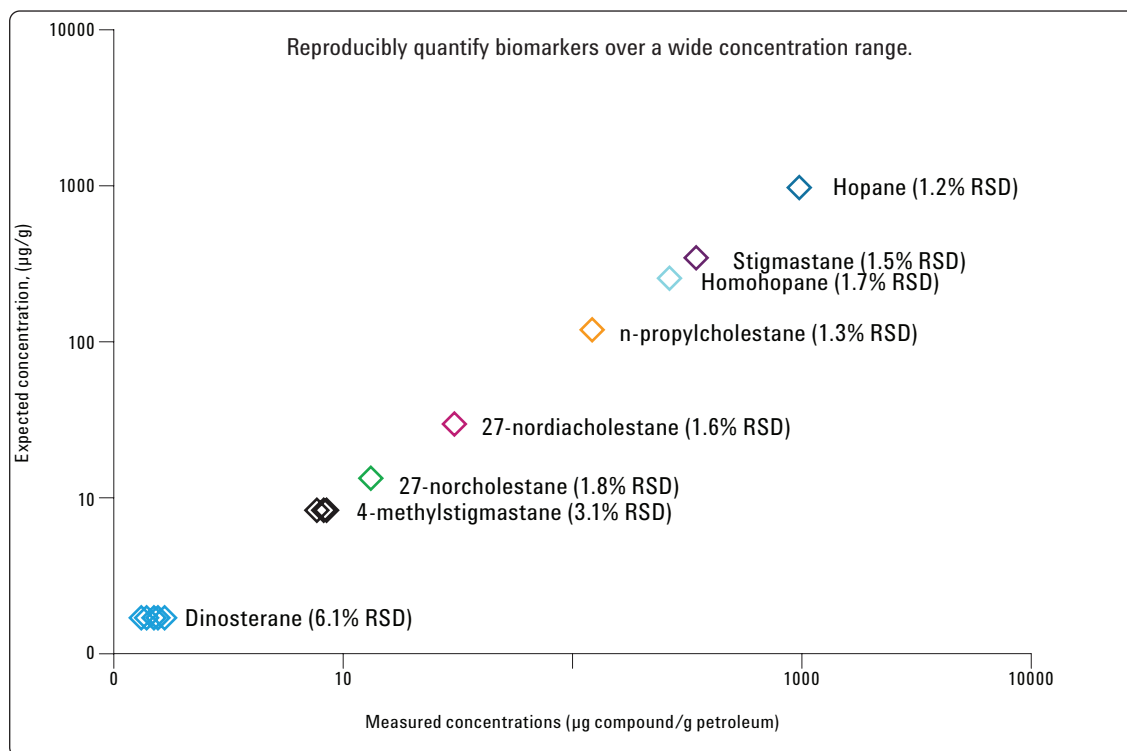


Figure 4a. Precision experiment results for eight biomarkers of widely varying concentrations contained within the STANFORD-1 standard. Ten sequential analyses were performed over a 15 hour period using the 60-m column PCT configuration. See Table 3 for transitions.

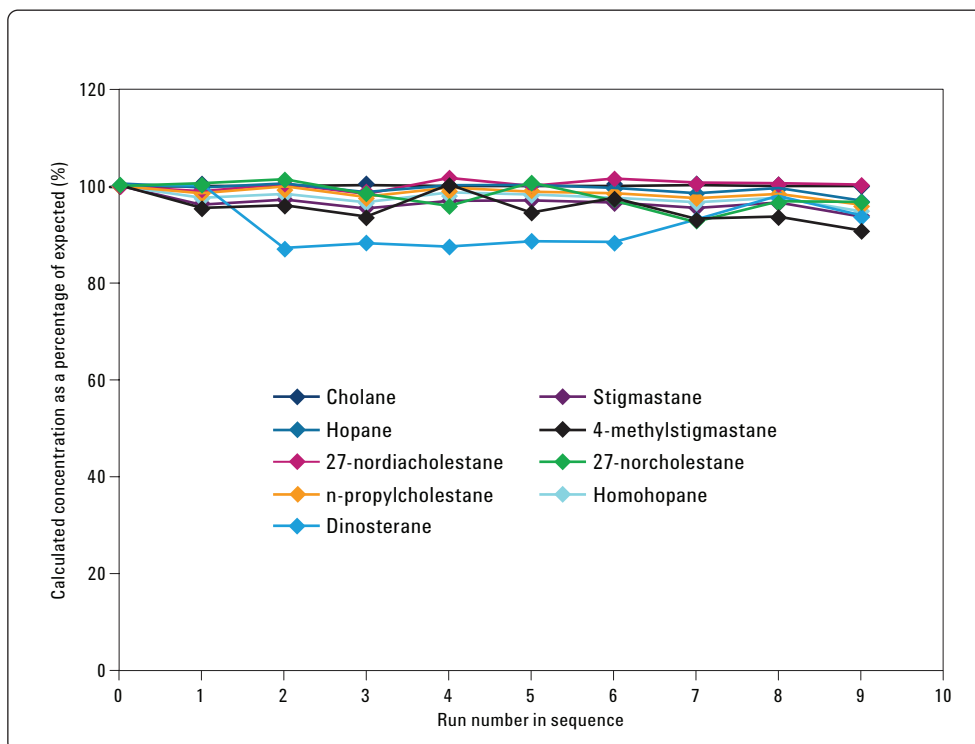


Figure 4b. The data from the analysis described in Figure 4a were plotted as calculated concentration of each biomarker versus the expected concentration over 10 analyses.

Deconvolving Oil Mixtures

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

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

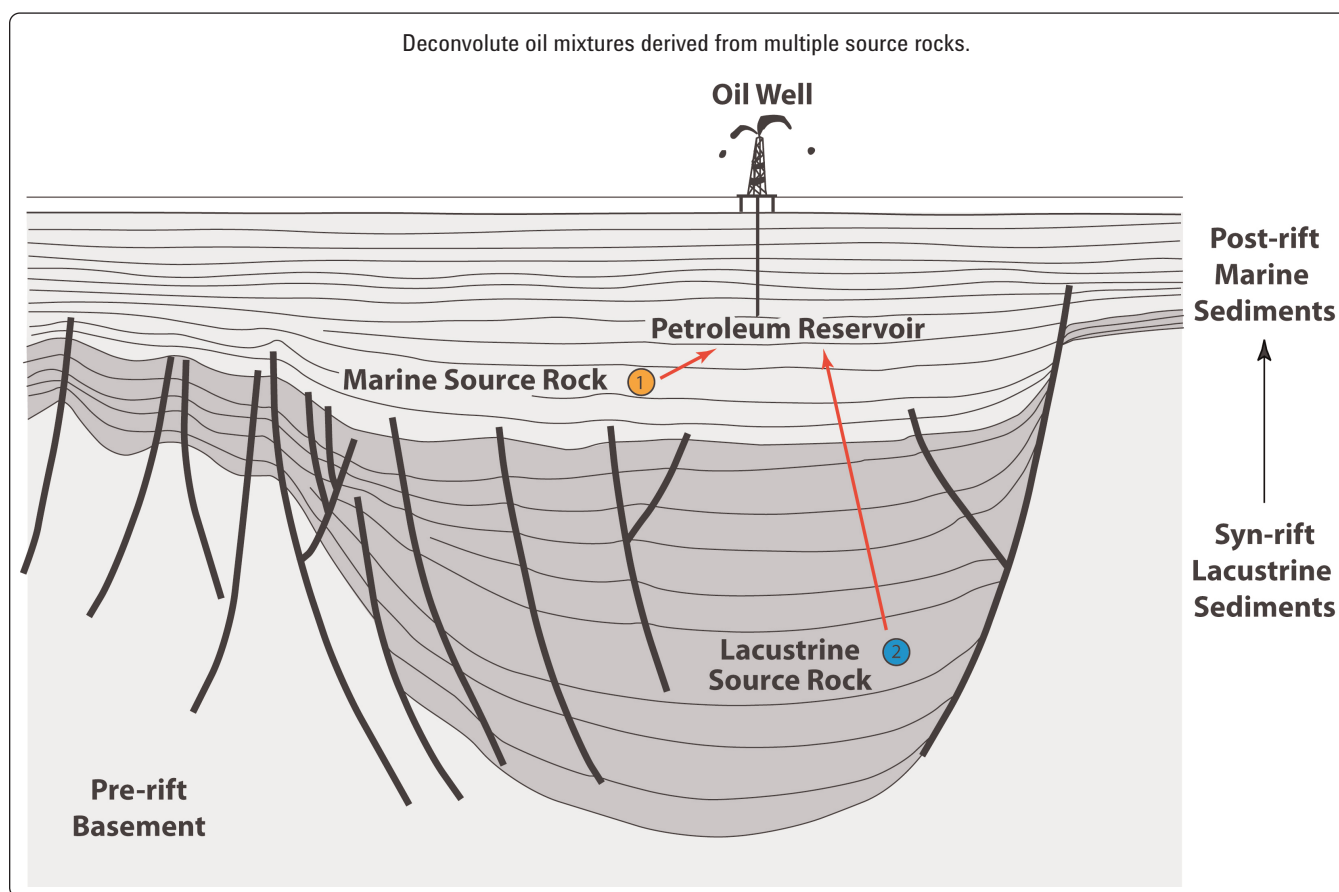


Figure 5. Diagram of an oil deposit containing source rocks from both marine (1) and lacustrine (2) sources.

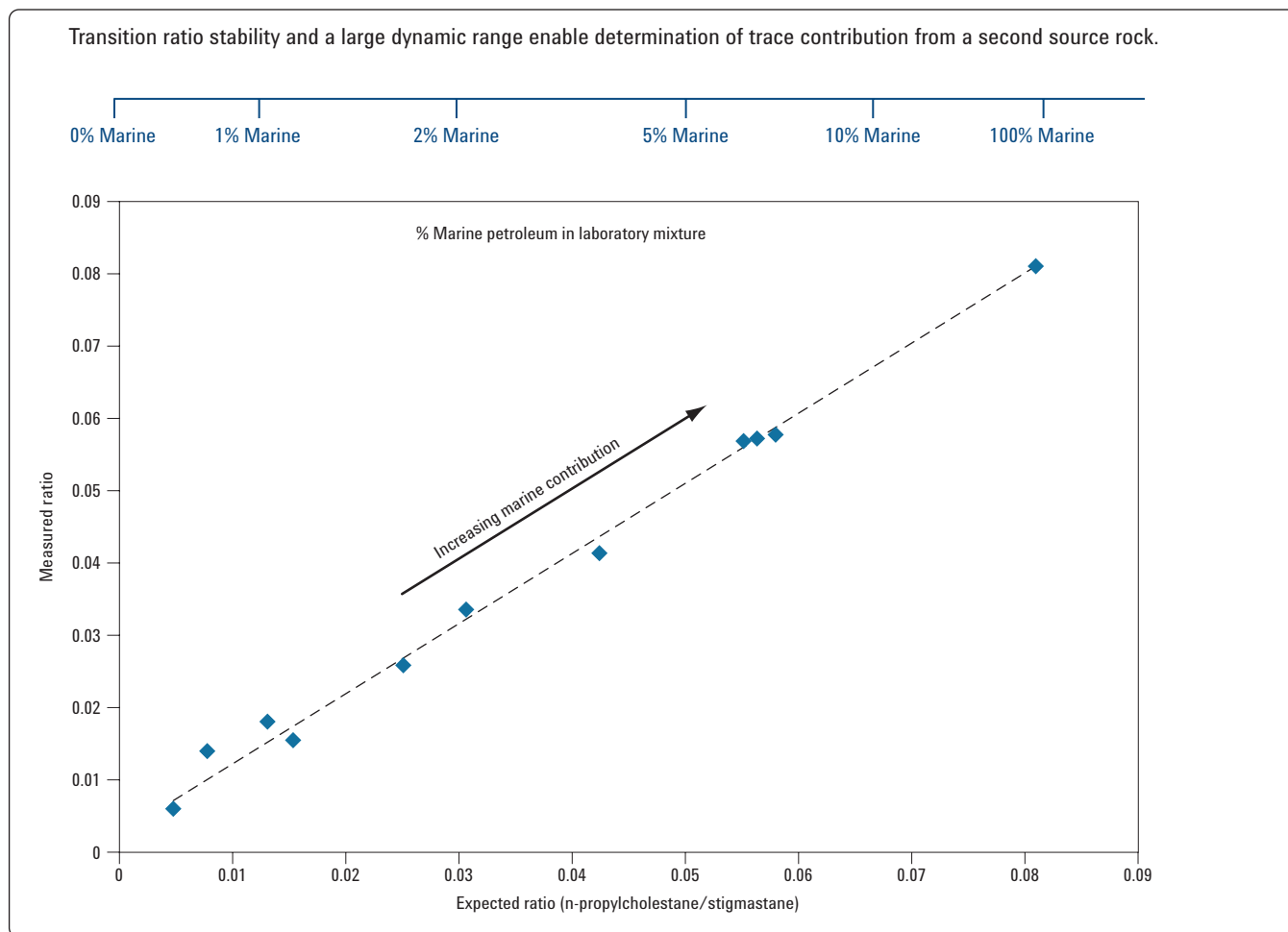


Figure 6. A series of laboratory mixtures consisting of various percentages of a marine petroleum sample in a lacustrine sample were analyzed for stigmastane, a ubiquitous component present in petroleum from both sources, and n-propylcholestane, a compound unique to oil from lacustrine source rock. The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

Conclusions

The Agilent 7000A Triple Quadrupole MS with 7890 GC using backflushing is a viable approach to the routine analysis of petroleum biomarkers, providing increased sensitivity, better selectivity and the potential to greatly reduce analysis time versus traditional GC/MS analysis. Column backflush provides higher sample throughput with lower carryover and source maintenance, and the use of hydrogen carrier gas and

narrower bore columns reduces run times nearly two-fold at no significant loss in chromatographic resolution. The SRM speed, linearity, dynamic range and transition ratio stability of the 7000A Triple Quadrupole mass spectrometer enable quantitative characterization for the fingerprinting of petroleum samples and the deconvolution of complex petroleum mixtures.

References

1. Peters, K. E., Walters, C. C. & Moldowan, J. M. 2005. The Biomarker Guide. Volume 2: Biomarkers and Isotopes in Petroleum Exploration and Earth History. Second Edition. (First edition published 1993 by Chevron Texaco.) 1132 pp. total. Cambridge, New York, Melbourne: Cambridge University Press. ISBN 0 521 83763 4.
2. H. Prest, C. Foucault and Y. Aubut, Capillary Flow Technology for GC/MS: Efficacy of the Simple Tee Configuration for Robust Analysis Using Rapid Backflushing for Matrix Elimination, Agilent Technologies Publication 5989-9359EN.
3. H. Prest, Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination, Agilent Technologies Publication 5989-8664EN.
4. H. Prest, The Pressure Controlled Tee (PCT): Configurations, Installation and Use, Agilent Technologies Technical Document G1472-90001.

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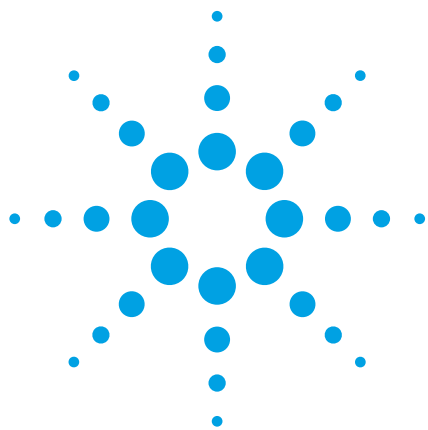
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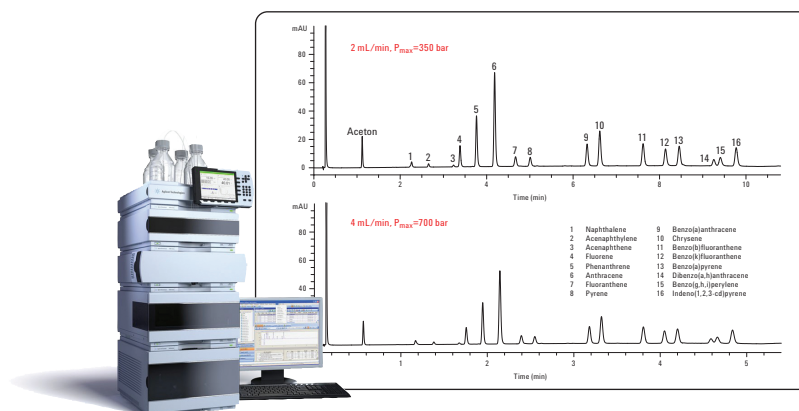
Fast analysis of polyaromatic hydrocarbons using the Agilent 1290 Infinity LC and Eclipse PAH columns

Application Note

Environmental

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The Agilent 1290 Infinity LC has a broader power range (the combination of pressure and flow capabilities) than any other commercially available system. This is extremely useful for method transfer from one (U)HPLC to the Agilent 1290 Infinity LC system and allows the analyst to develop methods that are impossible to run on these other systems.

The flow and pressure capabilities are illustrated by a separation of 16 polyaromatic hydrocarbons (PAHs) at high pressure and flow rate. At 2 mL/min, the analysis time is approximately 11 min. Doubling the flow rate and gradient speed allows the sample to be analyzed in 5.5 min with a maximum pressure of 700 bar. The combination of high flow (4 mL/min) and pressure is useful in this case to increase the sample throughput. The separation of the PAHs is shown in Figure 1.



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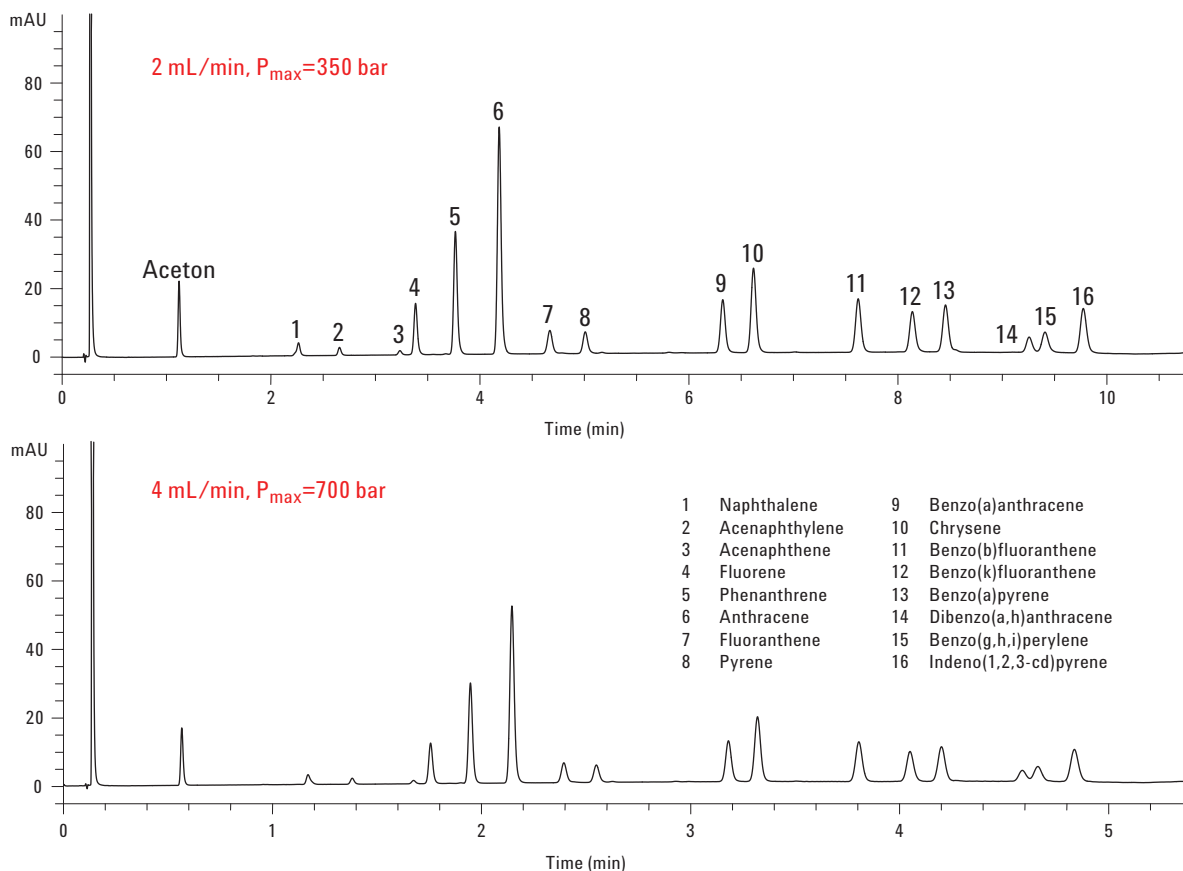


Figure 1

Analysis of 16 PAHs on the 1290 Infinity LC. Sample: standard solution of 16 PAHs, 50 µg/mL each.

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

Method:

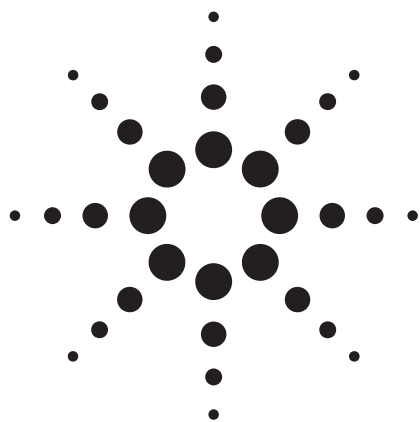
Column: ZORBAX Eclipse PAH 4.6 mm × 50 mm, 1.8 µm
 Mobile phase: A = water, B = acetonitrile
 Flow rate and gradient: 2 mL/min 0–0.33 min 40% B
 0.33–10 min 40–100% B
 4 mL/min 0–0.17 min 40% B
 0.17–5 min 40–100% B
 Injection volume: 0.2 µL
 Detector: Sig = 254/10 nm, Ref = off, 40 Hz
 Temperature: 25 °C

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Prefractionator for Reliable Analysis of the Light Ends of Crude Oil and other Petroleum Fractions

Application Note

Hydrocarbon Processing

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Abstract

A precolumn backflush system based on capillary columns using midpoint pressure control is described. Midpoint backflush is made possible with a Capillary Flow Technology (CFT) purged union controlled by an AUX EPC channel on the Agilent 7890A GC system. The key application discussed is prefractionation of crude oil that provides a high resolution separation of the C4 to C12 cut. A general backflush method using Polywax 500 is presented to illustrate the backflush concept.



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Introduction

The concept of backflushing in gas chromatography has been a mainstay of many petrochemical and gas analysis applications for over 40 years. Most use some implementation of a packed or micropacked precolumn connected to a mechanical valve. The analytical separation can then be done with either a packed or capillary column while the precolumn is backflushed to vent. Now precolumn backflush can be implemented in capillary only systems using either a standard split/splitless inlet or multimode inlet (MMI). Any application where sample components elute (or in some cases never elute) after the last compound of interest is a good candidate for a backflush implementation.

Process engineers and chemists working in the petroleum industry often have a need to analyze in detail the lighter fraction of a wide boiling raw material or feedstock. While GC is always the separation method of choice for petroleum and petrochemical samples, real limitations exist concerning the boiling point range or maximum carbon number that can be accommodated by a given capillary column. Many petroleum materials contain high boilers that can never elute. Analysis time can also be an issue even for compatible samples and columns because heavy material may require 60 minutes or longer to elute from the column. Now, the analysis of wide range petroleum material such as crude oils can be easily optimized, providing a high resolution time optimized separation for only the fraction required.

Crude oil analysis serves as an excellent example. A detailed analysis of the hydrocarbons in the C4 to C12 fraction is extremely valuable to the process engineer looking for the best method of refining the material. It is also valuable for determining the crude oil's value. Typically prefractionator or precolumn backflush GC configurations are based on packed precolumns and mechanical valves that can require specialized inlets. These systems require frequent maintenance, can suffer from poor thermal control, and are not optimized for high resolution separations. Agilent offers a unique solution based on a simple in-oven Capillary Flow Technology (CFT) device, the Purged Union (p/n G3186-60580). An MMI, AUX module, and FID complete the required hardware on the Agilent 7890A GC system. The configuration is compatible with all GC detectors including the MSD.

Experimental

A diagram of the basic system is shown below in Figure 1. The MMI is used in temperature programmed split mode to assist with cleaning out the liner during backflush while an

AUX channel controls analytical column flow. Injection is handled by the 7693A Tower and Tray system where basic sample prep (mixing, dilution, and heating) is used for automated sample prep.

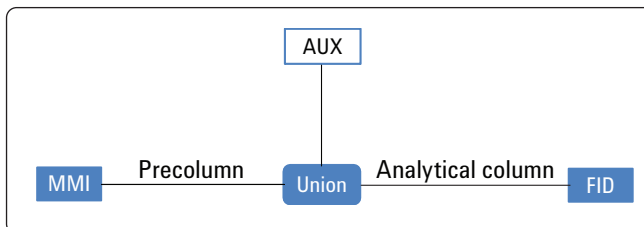


Figure 1. Basic precolumn backflush configuration with purged union.

Parameters for crude oil analysis of C4 to C12/C13

Sample:	Various crude oils
Inlet:	Multimode, 250:1 split
Inlet program:	250 °C (0.3 min) to 425 °C (60 min) at 200 °C/min
Oven program:	35 °C (10 min) to 160 °C (1 min) at 1 °C/min then 15 °C/min to 240 °C
Column 1:	2 m × 0.32 mm deactivated retention gap
Column 1 Flow:	0.9 mL/min in constant flow mode
Column 2:	100 m × 0.25 mm, 0.5 µm DB-Petro
Column 2 Flow:	1.2 mL/min in constant flow mode
Backflush after C12:	1.3 min approx.

Parameters for wide boiling range generic method

Sample:	Polywax 500
Inlet:	MMI, 10:1 split
Inlet program:	350 °C (0 min) to 425 °C (20 min)
Oven program:	50 °C (0 min) to 355 °C (5 min) at 15 °C/min
Column 1:	1 m × 0.53 mm deactivated retention gap
Column 1 Flow:	9 mL/min
Column 2:	5 m × 0.53 mm × 0.15 µm DB-HT
Column 2 Flow:	12 mL/min
Backflush times:	Various

The general procedure for precolumn backflush can be illustrated using a wide boiling range sample such as Polywax 500 (PW 500) where backflushing at specific carbon numbers can be easily accomplished. Setup panes for the PW500 analysis are shown in Figures 2A and 2B for precolumn and analytical column, respectively. Note that backflush is triggered by programming a rapid pressure drop at the inlet to the precolumn, which is the MMI in this example. First, defining the inlet and outlet sources for the columns is critical. The inlet to the precolumn is the MMI and the outlet an Aux channel. For the analytical column, the inlet is the Aux and FID the outlet.

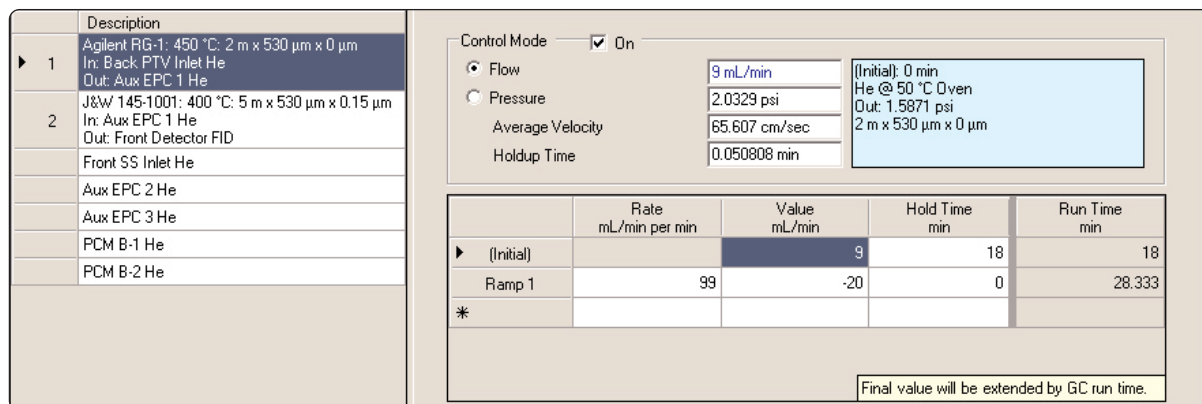


Figure 2A. Precolumn flows. Backflush starts at 18 min in this example.

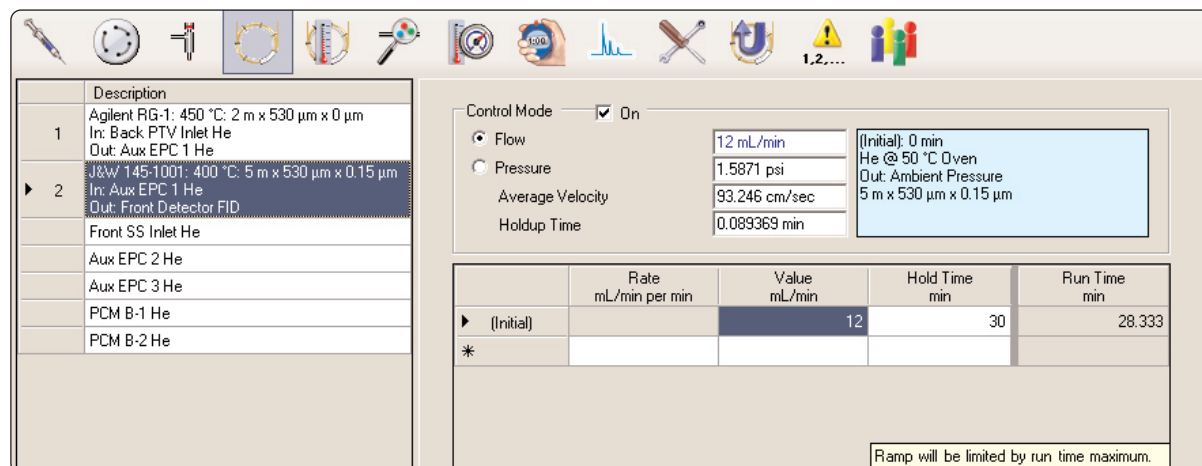


Figure 2B. Analytical column flow set at 12 ml/min for the entire run.

Note that at certain backflush times, only part of the last hydrocarbon is transferred to the analytical column. This occurs because individual compounds will be spread out and distorted on the precolumn. Backflush times can usually be fine tuned to make a clean cut with the polyethylene fragments that make up PW500 since they occur at even carbon numbers only (Figure 3).

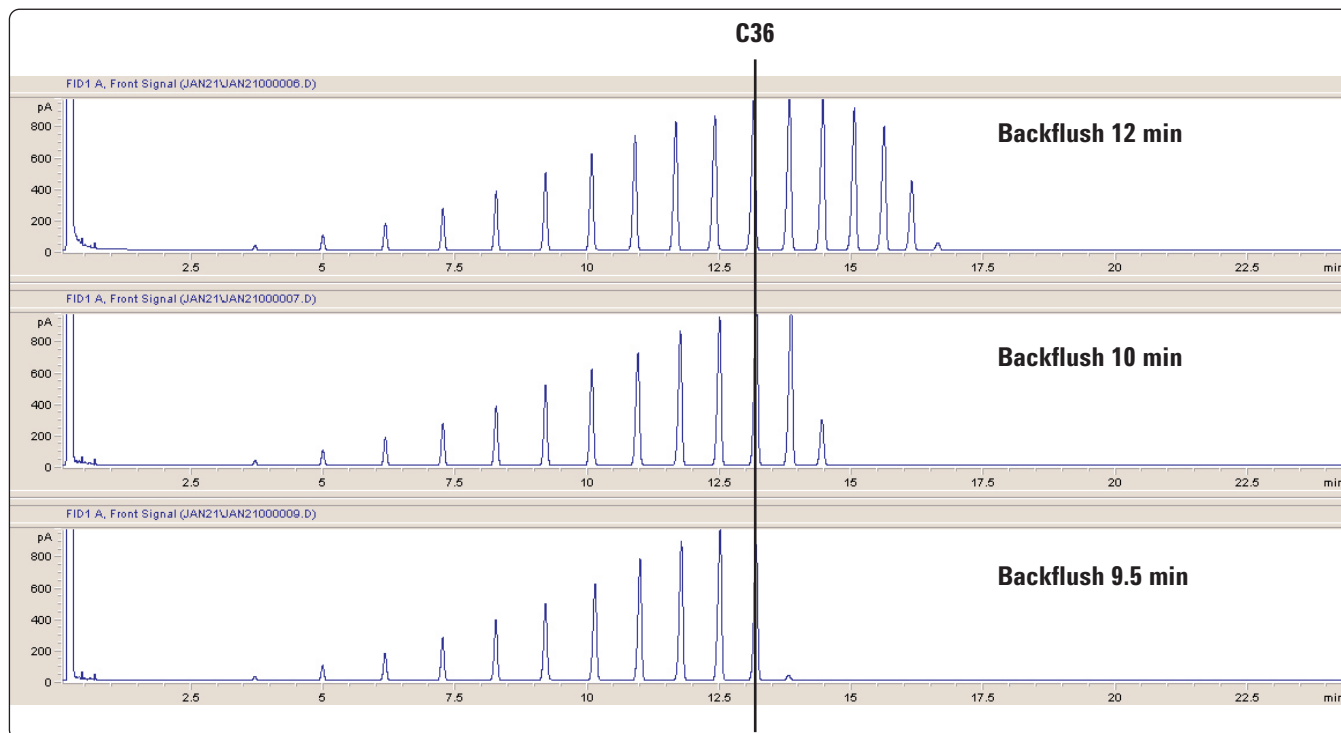


Figure 3. Polywax 500 chromatograms at three backflush times.

A plot of backflush time versus carbon number can be constructed as shown in Figures 4A and 4B. While a polynomial curve fit is best (Figure 4A), a linear regression will give a very good prediction of an appropriate backflush time at any desired carbon number (Figure 4B). The equation

$$\text{BF Time} = (\text{Carbon number} - 5.56)/3.68$$

can be used to give very close to ideal times for the columns and conditions stated here. Any change in the parameters would require a new equation. When developing a new application three to four points would be enough to establish the relationship between carbon number and backflush time using an appropriate test mixture. This is easily done using a ChemStation sequence for fast method optimization.

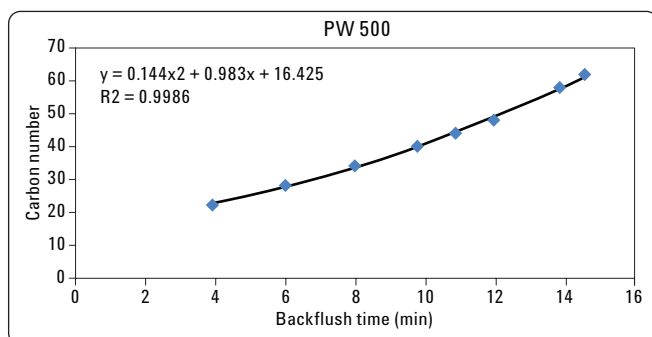


Figure 4A. Polynomial fit.

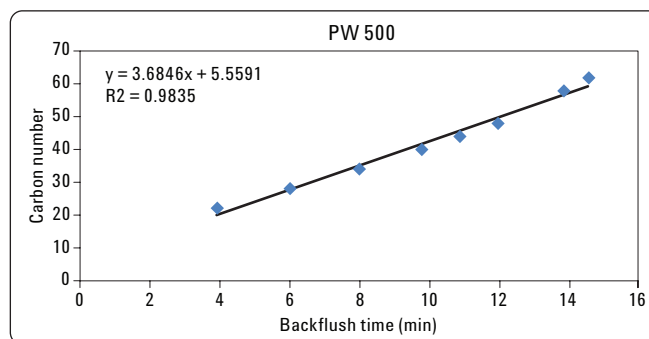


Figure 4B. Linear regression.

Discussion

Crude oil analysis is used as an example to show system setup and typical results. The precolumn usually consists of a short piece of deactivated fused silica, and the analytical column is chosen to provide sufficient separation power for the application. The columns used for crude oil analysis are 2 m × 0.32 mm deactivated retention gap, and 100 m × 0.25 mm × 0.50 μm DB-PETRO for the pre and analytical columns, respectively. Many possibilities exist for choice of pre and analytical columns for customizing the system for a particular application. Attention must be given to the pressure differential between

the inlet and aux to assure stable operation when choosing columns and conditions. Differences less than 0.1 psig must be avoided.

To begin system setup, the EPC channels must first be zeroed. This is necessary because the pressure difference between the MMI pressure and the Aux pressure may be as small as 0.1 psig. This can be seen in Figure 5 where the flow calculator is used to determine the flow settings for the crude oil prefractionation system. Flow calculator software can be downloaded from the Agilent web site. [1]

Next the "Quick swap" PID constants need to be uploaded to the Aux channel. This is done with the LMD Update Utility Tool for the 7890A. Flow or pressure is set first for the analytical column controlled by an Aux channel, then Flow or pressure is set for the precolumn controlled by the MMI. As a

general rule, the precolumn flow should be set between 70% and 85% of the analytical column flow.

Fine tuning the backflush time is easily done by running a sequence of several methods with a slightly different backflush time in each using a mix of hydrocarbons from C5 to C17 (p/n 5080-8769). A given hydrocarbon will elute from the uncoated precolumn at a lower temperature than it would from the analytical column. Exactly how much lower is highly dependent on the phase ratio of the analytical column. Therefore it is best to start with a relatively quick backflush and then adjust the time upwards to allow all of the desired boiling point range to pass into the analytical column for separation. As shown in Figure 6, the area of the C13 peak increases as the backflush time is lengthened. The final desired backflush time is reached once the area becomes constant (BF = 1.30 min).

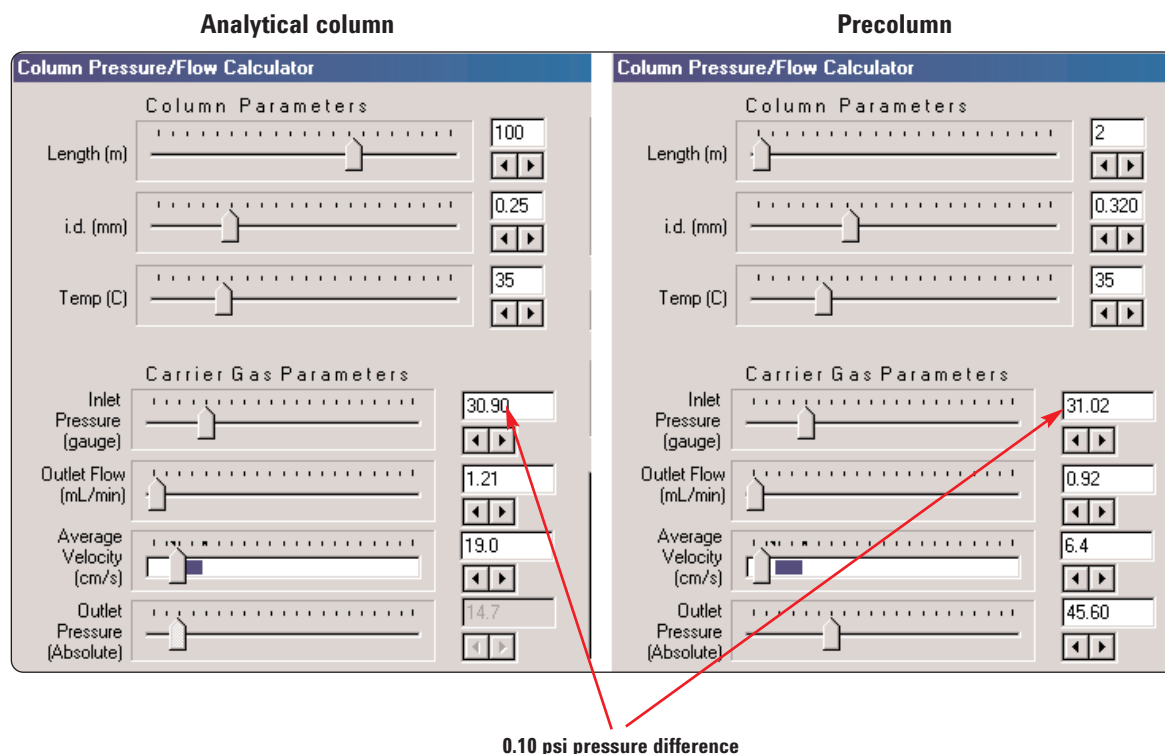


Figure 5. Pressure and flow setting for the analytical column (left pane) and precolumn (right pane).

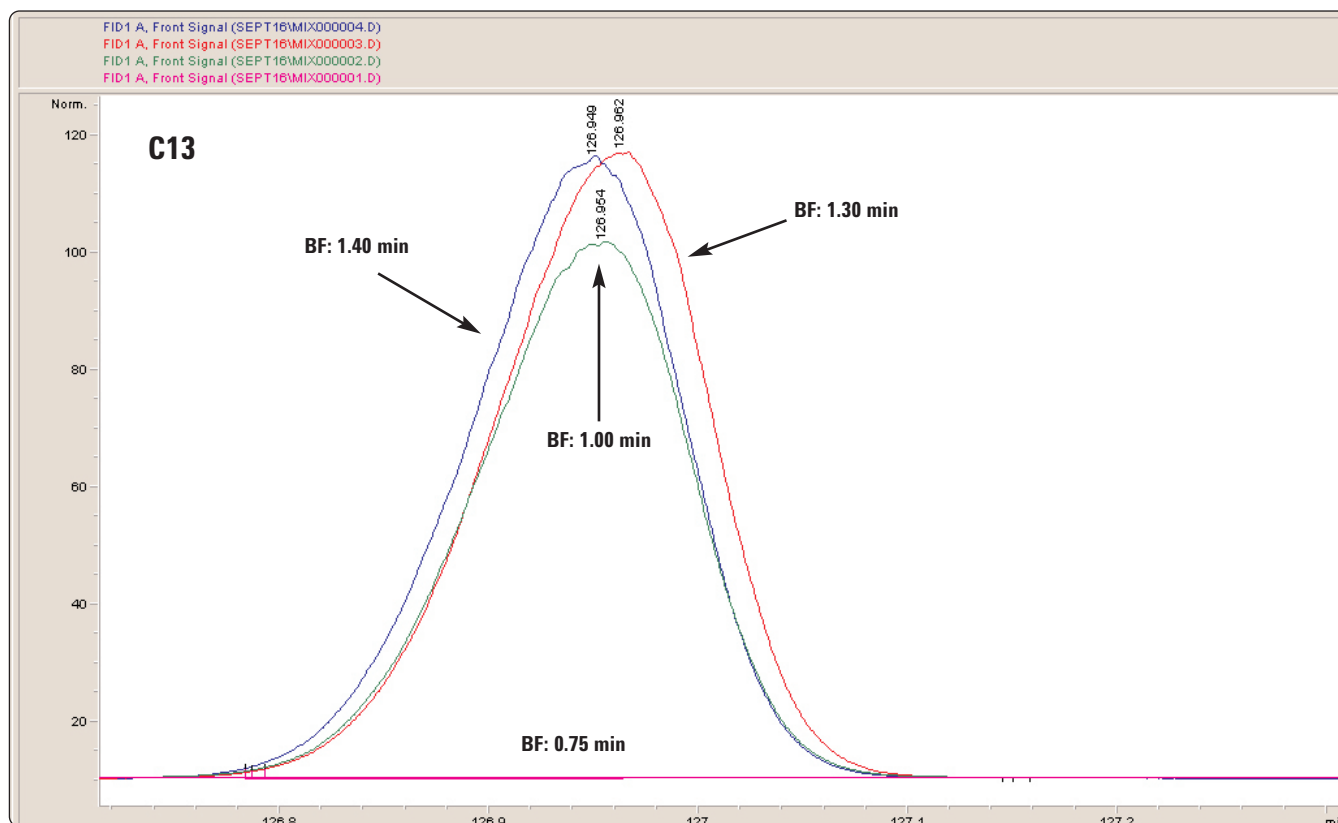


Figure 6. Fine tuning backflush time for ending transfer at C13. Trace at baseline: BF = 0.75 min, Peak at height of 100pa: BF = 1.00 min, Peaks at 117 pa: BF = 1.3 min and 1.4 min.

Easily Protect the Analytical Column with Backflush

Without backflush, a crude oil sample would contaminate and render the 100 m column useless. Setting the system to perform a backflush of the precolumn after approximately C12 has transferred to the 100 m column allows a high resolution separation to occur while the heavier fraction of the crude oil

is backflushed through the MMI's split vent. The MMI is also programmed to 425 °C to assist in cleaning the inlet liner during backflush. A single taper liner with glass wool is used (Agilent p/n 5183-4647). ChemStation screens showing setup conditions for the pre and analytical columns are shown in Figures 7A and 7B, respectively.

Description	
1	J&W 2.0RG: 450 °C: 2 m x 320 µm x 0 µm In: Back PTV Inlet He Out: Aux EPC 1 He
2	J&W 122-10A6: 325 °C: 100 m x 250 µm x 0.5 µm In: Aux EPC 1 He Out: Front Detector FID
	Front SS Inlet He
	Aux EPC 2 He
	Aux EPC 3 He
	PCM B-1 He
	PCM B-2 He

Control Mode ☒ On
☒ Flow
☐ Pressure

	Rate mL/min per min	Value mL/min	Hold Time min	Run Time min
(Initial)		0.9	1.3	1.3
▶ Ramp 1	99	-50	100	180
*				

Final value will be extended by GC run time.

Figure 7A. Precolumn set to backflush at 1.3 min.

Oven Temperature: 35.0 °C
Back Inlet (PTV Inlet):
Temperature: 250.0 °C
Pressure: 30.974 psi
Flow: 229.4 mL/min
Septum Purge Flow: 3.0 mL/min
APC 1,2,3:
Aux EPC 1: 30.858 psi
Aux EPC 2: -0.415 psi
Aux EPC 3: 0.002 psi
Column Flows:
Column 1: 0.903 mL/min
Column 2: 1.200 mL/min

Description	
1	J&W 2.0RG: 450 °C: 2 m x 320 µm x 0 µm In: Back PTV Inlet He Out: Aux EPC 1 He
2	J&W 122-10A6: 325 °C: 100 m x 250 µm x 0.5 µm In: Aux EPC 1 He Out: Front Detector FID
	Front SS Inlet He
	Aux EPC 2 He
	Aux EPC 3 He
	PCM B-1 He
	PCM B-2 He

Control Mode ☒ On
☒ Flow
☐ Pressure
Average Velocity
Holdup Time

1.2 mL/min
30.859 psi
19.043 cm/sec
8.7523 min

(Initial): 0 min
He @ 35 °C Oven
Out: Ambient Pressure
100 m x 250 µm x 0.5 µm

	Rate mL/min per min	Value mL/min	Hold Time min	Run Time min
▶ (Initial)		1.2	0	
*				

Final value will be extended by GC run time.

Figure 7B. ChemStation panes for configuring backflush and column flow.

Note that precolumn flow (0.9 mL/min) is set to approximately 80% of the analytical column flow. This is a good general rule to follow for method development. The same control mode should be set for both columns, either pressure or flow. Under the conditions used, setting the backflush time at 1.3 min allows up to C12 to pass into the analytical column. A 0.32 mm id precolumn is used instead of one with the same diameter as the analytical column simply because it has more sample capacity and therefore less peak distortion. Peak capacity will be largely dependent on surface area in uncoated retention gaps.

Four crude oils with prefractionation up to approximately C12 are shown in Figure 8. The resulting detailed C4-C12 hydrocarbon analysis provides valuable information to help the process chemist develop the best refining strategy. This system could be coupled with DHA software to provide comprehensive peak identification. The information could also be combined with crude oil simulated distillation for a complete GC sample characterization.

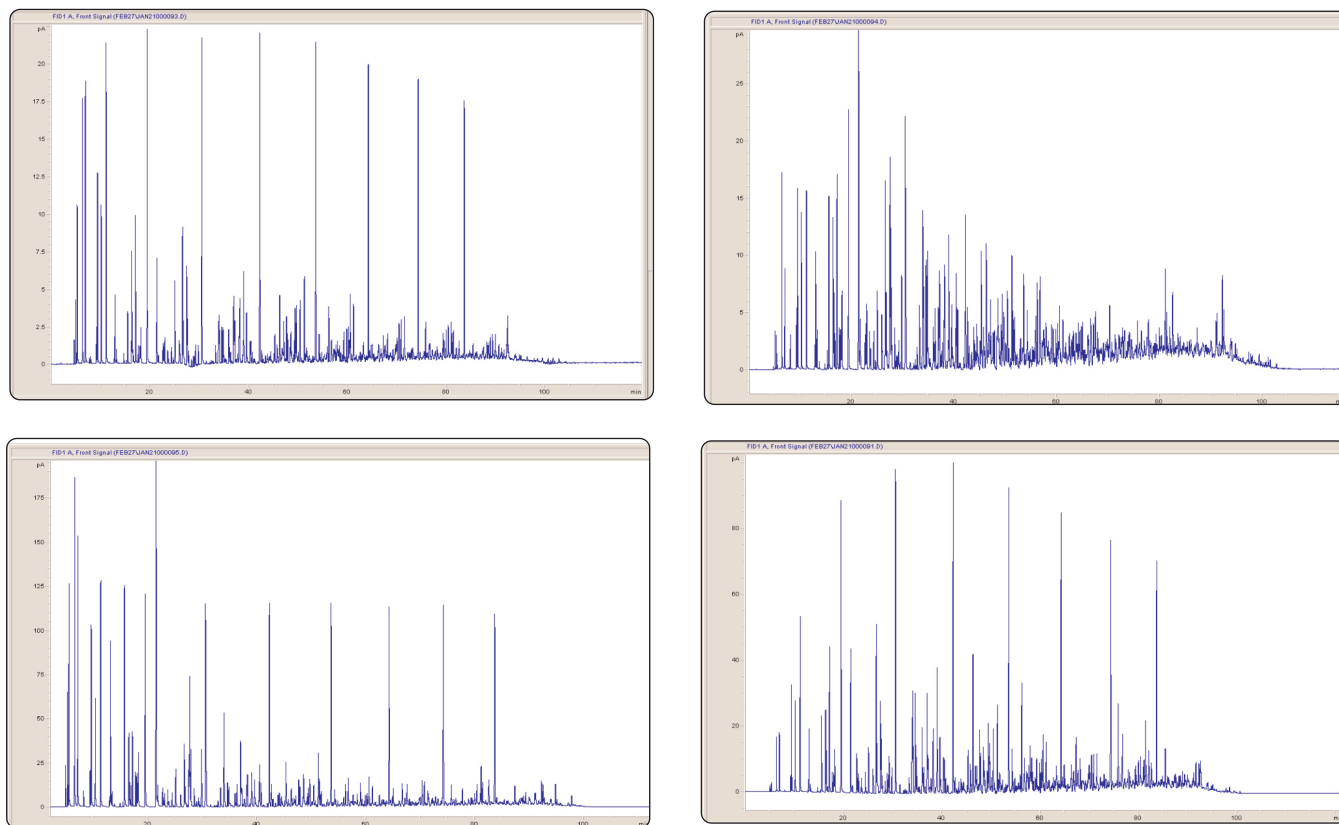


Figure 8. Four crude oils from different regions. Backflushed between C12 and C13.

Backflush With no Traces of High Molecular Weight Contamination

Figure 9 shows 12 consecutive injections of crude oil and analysis of the C4 to C12 fraction on the DB-Petro column. Retention time repeatability is better than 0.002 min and the

baselines show no signs of variability from residual material. This indicates a clean and complete backflush of each run. Typically a liner change should be made after approximately 50 to 75 crude oil injections to be conservative.

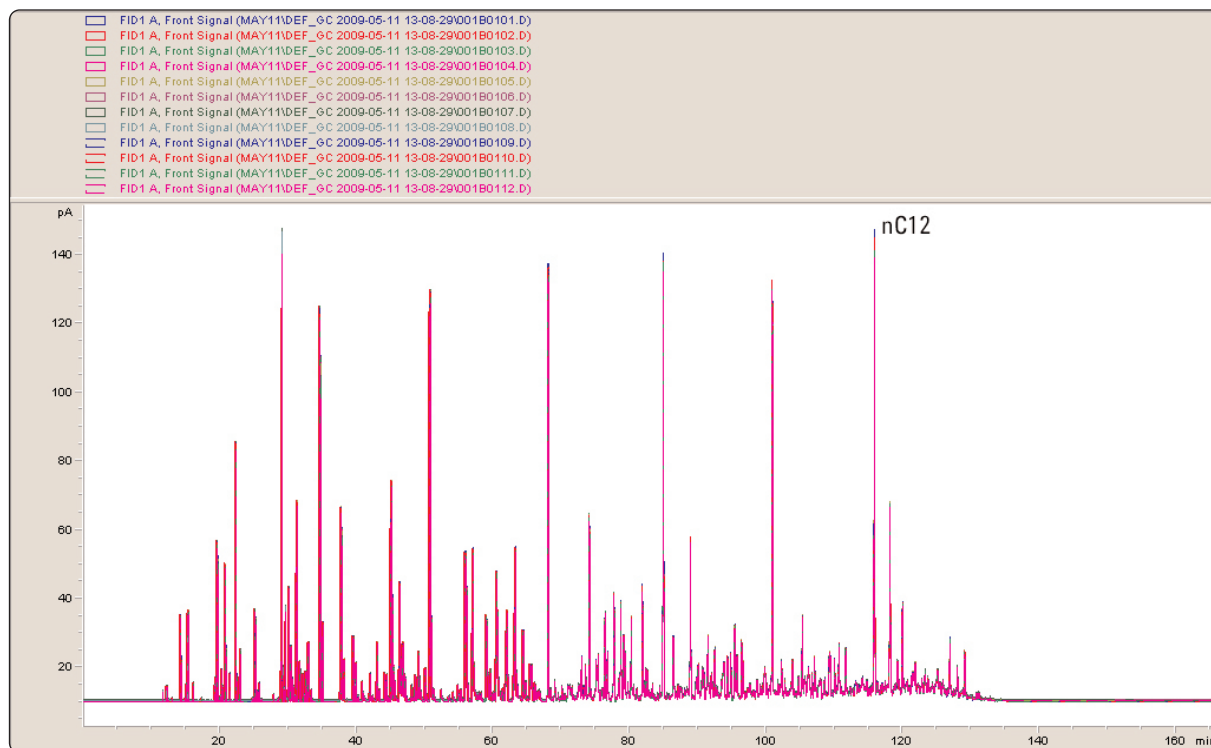


Figure 9. Overlay of twelve runs of crude oil backflushed between C12 and C13.

Conclusions

First and foremost, the system allows GC analysis of many wide molecular weight range samples that otherwise could not be injected without damaging the column or detector. Midpoint pressure control allows the analytical column to run at the desired flow while the precolumn is backflushed during the run. Further, the use of an uncoated precolumn transfers the desired compounds at a low temperature. This has the added benefit of faster backflushing of the heavier material. However, coated precolumns can also be used, and in some applications the use of a thin stationary phase will be advantageous. Columns will have longer lifetimes with improved retention time stability. Many combinations of pre and analytical columns can be used to address just about any GC application where light or early eluting material needs to be separated from heavier material that should not be introduced to an analytical column for either time savings or column protection. Example applications include additives in fuels and biodiesel analysis.

The configuration is compatible with the MSD as high carrier flows to the detector do not occur during backflush. In most cases, even a diffusion pump system can be used since the analytical column is usually of high resolution and the column flow during backflush will be low.

The Agilent 7890A GC system with precise and stable electronic pneumatic control enables midpoint backflush with a variety of column lengths, stationary phases and internal diameters. The CFT purged union designed for leak-free connections, superior inertness, and lack of unswept volumes yields chromatographic performance identical to single column systems.

References

1. Flow Calculator software: www.agilent.com/chem/flowcalculator

For More Information

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

www.agilent.com/chem

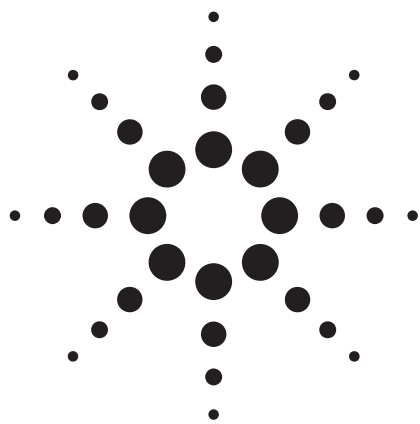
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Automated Preparation of Simulated Distillation Standards and Samples for ASTM Methods D2887, D7213, D7398 and D6352 using the 7693A System with Easy SamplePrep Software

Application Note

Hydrocarbon Processing

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Abstract

A dual tower Agilent 7693A and tray system installed on the Agilent 7890A GC system is used for the preparation of hydrocarbon calibration standards, solvent blanks, and petroleum samples for analysis by simulated distillation (SimDis). The front tower is equipped with a 5- μ L syringe while the back tower is equipped with a 250- μ L syringe. A 150 sample tray with heater and mixer/barcode reader is also used. Procedures are described for sample preparation for ASTM D2887, D7213, D7398 and D6352. The Multimode Inlet (MMI), G3510, operated in a temperature programmed split mode is used for all samples. On-line sample preparation programs are constructed using Easy SamplePrep software, an add-on software module for the multitechnique ChemStation.

Introduction

Sample and calibration standard preparation for various simulated distillation methods is normally a manual process requiring dilution, mixing, and heating. Many procedures use volatile toxic solvents such as carbon disulfide. ASTM method D2887 commonly uses CS₂ for sample dilution while D6352 may use CS₂ or toluene for polywax calibration standard preparation. Sample heating, mixing and solvent addition is available with the automation capabilities of the Agilent 7693A tower and tray system. Lab safety is improved by using small quantities of solvents with controlled heating, and mixing in sealed 2-mL vials.



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Experimental

The Agilent 7890A GC system was equipped with two Agilent 7693A towers and 150 sample tray. The front tower used a standard 5- μ L or 10- μ L syringe and the rear tower was equipped with the optional large syringe carriage with a 250- μ L syringe. Sample prep procedures were done by manipulating vials in the sample tray and in the tower turrets. Sample injection occurred on the front tower. The Agilent 7890A was configured with the multimode inlet (MMI) operating in temperature programmed split mode. Detection was with FID. Instrumental parameters for various configurations are listed in Table 1.

Discussion

A number of options or paths to construct sample prep programs using the drag and drop icon implementation of Easy SamplePrep software is possible. This discussion will in general illustrate just one possible solution for each procedure. Screen captures are used to detail the steps and advanced syringe settings.

Table 1. Agilent 7890A GC System SimDis Parameters

System for D2887	
Column	10 m $\times$ 0.53 mm, 3.0 μ m DB-2887
Oven	40 $^{\circ}$ C (0 min) to 350 $^{\circ}$ C (5 min) @ 15 $^{\circ}$ C/min
Inlet	Multimode (MMI), G3510, 100 $^{\circ}$ C (0 min) to 340 $^{\circ}$ C (to end of run) @ 250 $^{\circ}$ C/min
Liner	Single taper with glass wool, No. 5183-4711
Split	4 to 1
Flow	12 mL/min, constant flow mode
System for D7213 and D7398 (Polywax 500 calibration)	
Column	5 m $\times$ 0.53 mm, 0.15 μ m DB-HT SimDis
Oven Program	35 $^{\circ}$ C (0 min) to 400 $^{\circ}$ C (5 min) @ 10 $^{\circ}$ C/min
Inlet	Multimode (MMI), 100 $^{\circ}$ C (0 min) to 400 $^{\circ}$ C (20 min) @ 250 $^{\circ}$ C/min
Split ratio	5 to 1
Flow	14 mL/min, constant flow mode
Agilent 7890A GC system for D6352 (Polywax 655 calibration)	
Column	5 m $\times$ 0.53 mm, 0.15 μ m DB-HT SimDis
Oven Program	35 $^{\circ}$ C (0 min) to 435 $^{\circ}$ C (2 min) @ 10 $^{\circ}$ C/min
Inlet	Multimode (MMI), 100 $^{\circ}$ C (0 min) to 430 $^{\circ}$ C (hold to end of run) @ 250 $^{\circ}$ C/min
Split ratio	5 to 1
Flow	15 mL/min, constant flow mode
Agilent 7693A system	
Front tower	5 μ L syringe, G4513A
Back tower	250 μ L syringe, G4521A syringe carriage
Tray	150 sample capacity with Heater/Mixer/Bar Code Reader, G4520A
ChemStation	B.04.02 SP1
Sample Prep	G7300AA, Easy SamplePrep
Agilent 7890A GC system firmware	A.01.10.3 or greater
Standards and vials	
Calibration mix, C5-C40, No. 5080-8716	
Calibration mix, C5-C18, No. 5080-8768	
RGO, No. 5060-9086	
PW500, No. 5188-5316	
PW655, No. 5188-5317	
Empty vials with 100 μ L inserts, No. 5188-6592	
Simulated Distillation Software	
G2887BA	

Two-milliliter vial resources are assigned in user defined tray locations as shown in Figure 1. These are the resources needed for methods D2887, D7213, D6352, and D7398. The poly-wax standards are handled differently, usually as “Sample (front)” vials when the front tower is used for injection. Resource vials are specified for use by maximum volume extracted or by number of allowed uses. Ensure that appropriate syringe details such as draw and dispense speeds for the handling of a given chemical resource are set. An example of advanced settings for use of CS₂ is shown in Figure 2.

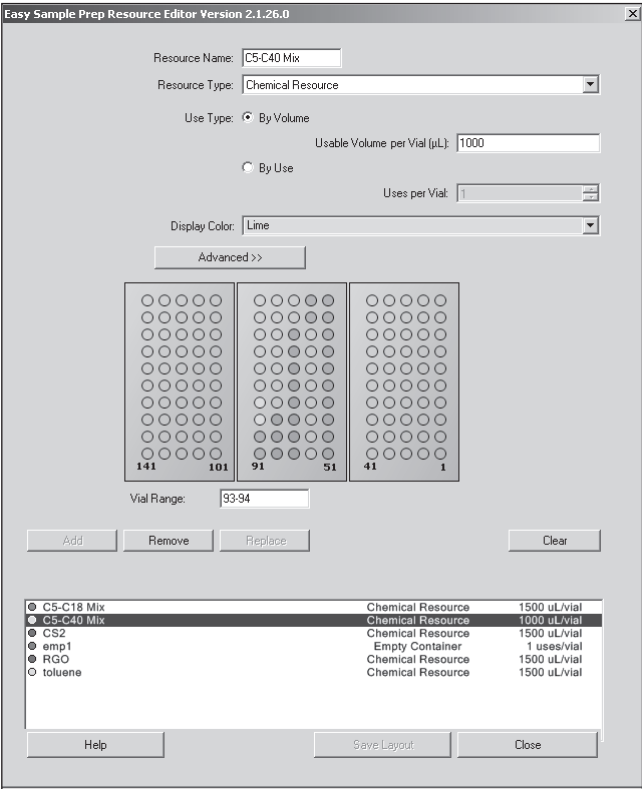


Figure 1. Example resource layout for various simulated distillation procedures. Each resource is assigned a unique color.

A typical sample preparation program for D2887 setup (blank, calibration, reference gas oil) may consist of a sequence of three methods, each for a specific sample prep and injection. An example sequence is shown in Figure 3. This illustrates preparation of the blank, calibration standard, and reference gas oil (RGO) samples necessary to set up and verify a system for routine analyses.

The Easy SamplePrep programs used for methods CS2 BLANK, C5C40 CAL 2887, and RGO 2887 are shown in Figures 4, 5, and 6, respectively. Using three methods in a sequence is convenient since each method has different integration parameters.

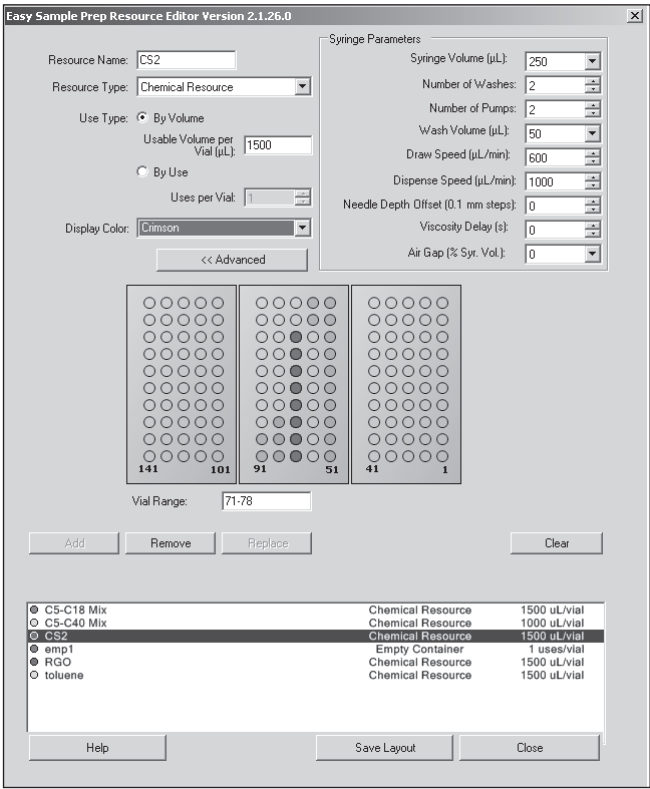


Figure 2. Advanced parameters shown (upper right box) for chemical resource CS₂.

Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type
1	1	blank	CS2 BLANK	2	Sample
2	1	C5-C40	C5C40 CAL 2887	1	Sample
3	1	RGO	RGO 2887	1	Sample

Figure 3. Sequence for setup of D2887. Each method contains the appropriate EasySample Prep procedure.

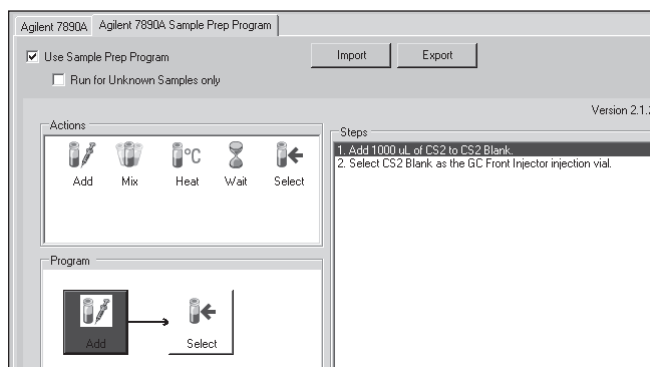


Figure 4. Easy Sample Prep program for the preparation of a CS_2 blank. An empty tray vial has been assigned the name "CS2 Blank". The select icon indicates that the prepared vial is to be injected.

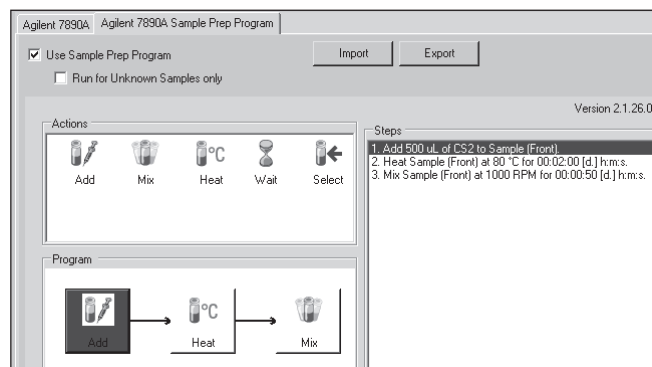


Figure 5. Easy Sample Prep program for preparation and injection of the C5 to C40 calibration mix. The "sample [front]" label defines sequence vials for the front tower.

A

Resource Name	Resource Type	Uses/Vial	Vial Ran
C5-C18 Mix	Chemical Resource	10	91-100
Empty	Empty Container	1	41-42

Resource Name	Resource Type	Usable Volume/Vial
CS2	Chemical Resource	1500
RGO	Chemical Resource	500
Toluene	Chemical Resource	1500

B

C

Figure 6a. Easy Sample Prep program for preparation and injection of RGO. An empty tray vial(empty) has been assigned the name "RGO Dilute" during the "Add" step and is selected for injection after prep. "Selected" vials override the vial number given in the sequence table.

Figure 6b. Add steps for RGO and vial naming.

Figure 6c. Adding carbon disulfide to the RGO vial.

Upon completion of the sequence, all three prepared vials will have been injected producing data files ready for analysis by simulated distillation software. Note that two blanks are run to ensure both are the same; otherwise, additional blanks should be run. As an alternate setup, the calibration, prepared RGO, and blank vials can be fitted with 100- μ L inserts to minimize solvent and resource amounts used for the procedure. Please note that when these inserts are used, limit mixing to speeds of approximately 500 rpm to avoid “spilling” liquid over the top of the insert into the bottom of the 2-mL vial.

Syringe washing is important to incorporate into these programs to avoid contamination or carryover for each vial addition. An example of settings is shown in Figure 7.

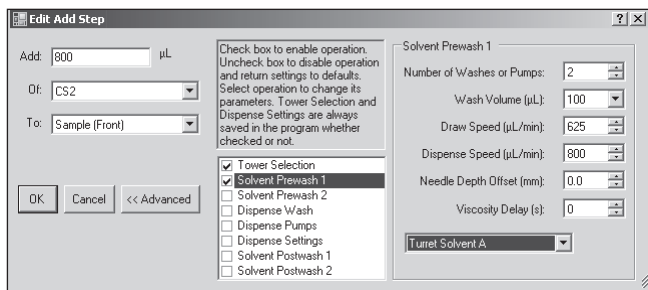


Figure 7. An example using solvent wash vial 1 (5 mL) in the turret of tower A.

Preparation of polywax standards for the higher temperature SimDis methods can be challenging due to their low solubility. Solvents such as CS₂ and toluene are commonly used. Heating of the solvent/polywax vial is required just prior to injection. This entire procedure can be automated with the Agilent 7693A tower and tray system. The basic procedure for Polywax 655 is as follows:

- Manually place approximately 80 – 100 mg of polywax 500 in a 2 mL vial and seal
- Add 1.5 mL of toluene to the polywax vial
- Add 10 μ L of C5-C18 to the polywax-toluene vial
- Mix the vial
- Heat the vial at 80 °C for 4 min.
- Return to tray
- Heat one final time just prior to injection by setting injection/tray parameters in the core ChemStation method

Figure 8 shows the basic prep procedure using a dual tower/tray system automating the steps shown above. The only manual step is adding the solid polywax 500 to Vial 1 (Sample front). This procedure is applicable to D7213 SimDis and D7398 (Boiling Range Distribution of Fatty Acid Methyl Esters).

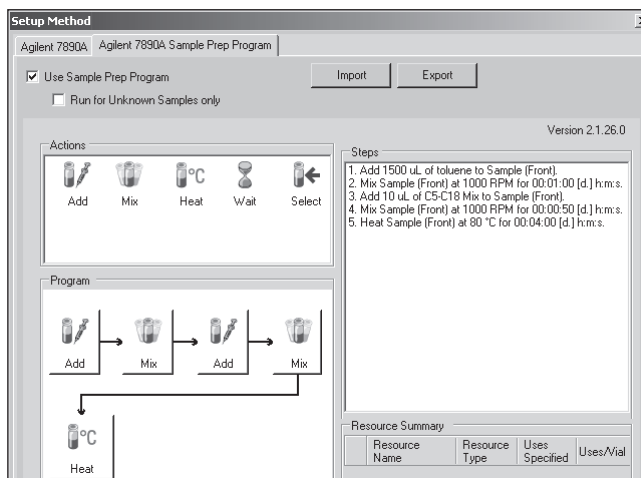


Figure 8. Polywax 500 prep procedure.

A resulting chromatogram for the injection of the prepared PW500 (vial 1) is shown in Figure 9. A symmetric distribution of the polyethylene fragments with good resolution to C80 is shown.

The preparation program for Polywax 655 is essentially the same as shown above for PW500 except that heating is extended for 6 minutes typically for dissolution. Prior to injection, the prepared vial is heated for another 3 minutes. Parameters for this second heating step are set under the core ChemStation injection parameter menu item. In the chromatogram shown in Figure 10, a small amount (5 μ L) of C₅-C₁₈ mix was added to the PW655/toluene solution as part of the automated procedure. This allows calibration starting at C12.

The chromatogram was produced with the multimode inlet set in temperature programmed split mode. Good definition of polyethylene fragments to over C110 are seen in Figure 11. The last 5 minutes of the chromatogram are zoomed to show detail. Producing this detail out to C110 is extremely difficult for any chromatographic system.

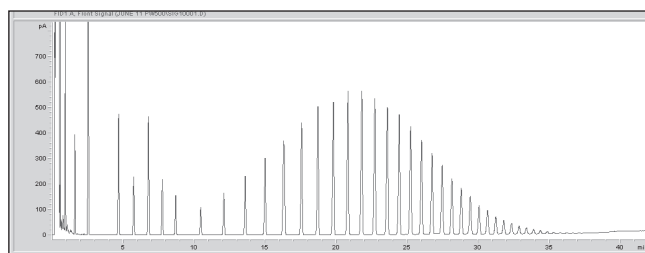


Figure 9. Polywax 500 with C₅-C₁₈ added. Multimode inlet, 2.5- μ L injection.

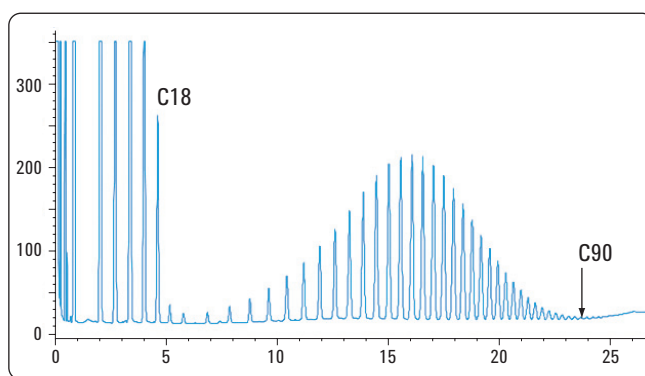


Figure 10. Chromatogram of PW 655.

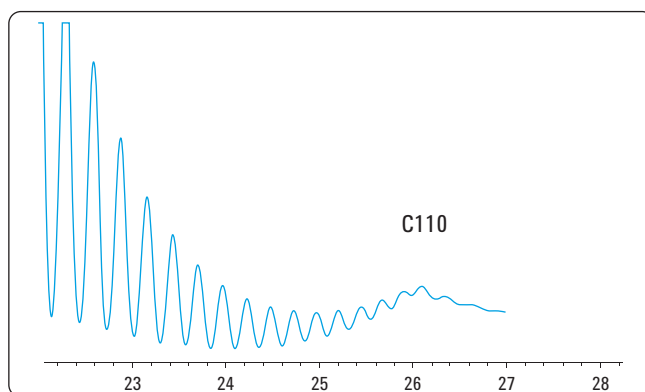


Figure 11. Polywax 655 to C110. Multimode inlet program: 150 $^{\circ}$ C (0 min) to 430 $^{\circ}$ C (hold to end of run) @ 200 $^{\circ}$ C/min. 7890A oven: 40 $^{\circ}$ C (0 min) to 430 $^{\circ}$ C (5 min) @ 15 $^{\circ}$ C/min. 3- μ L injection. Solvent is toluene.

Reproducibility of sample preparation steps for the dilution of a heavy vacuum gas oil sample (HVGO) is illustrated in Figure 12. Carbon disulfide was used for sample dilution. Tray vial 1 is the stock HVGO sample, prepared by manually adding 0.5 g of the oil to a 2-mL vial. This material is extremely viscous and cannot be drawn into a syringe without dilution. The program performs a fully automated dilution prior to injection. (Figure 13)

Summary

Difficult sample preparation procedures that are commonly used for petroleum and fuel samples can be easily automated on-line with the Agilent 7693A tower and tray system for the Agilent 7890A GC system and Agilent 6890N Network GC system, including A, and Plus models using the Easy SamplePrep add-on software for the multitechnique ChemStation. The system is particularly well suited for preparation of polywax calibration samples used for higher temperature methods. Tasks such as mixing, solid dissolution, dilution, heating, viscosity reduction, and internal standard addition are easily accomplished by assembling icon based instructions. User contact with toxic solvents such as CS_2 is greatly reduced. The software monitors used resources and moves to the next available resource vial as assigned in the resource table when needed.

Chromatographic performance is enhanced through use of the multimode inlet. Using standard split injection liners, good sample capacity without carryover and with minimal discrimination of wide boiling samples is achieved. The inlet was used in the temperature programmed split mode for this work. Cryo cooling was not used, however, carbon dioxide cryo can be used optionally to shorten inlet cool down if desired.

The sample prep procedures listed here represent just one way of accomplishing a given task. Using the icon based commands available with the system, there are many variants that lead to the same end result.

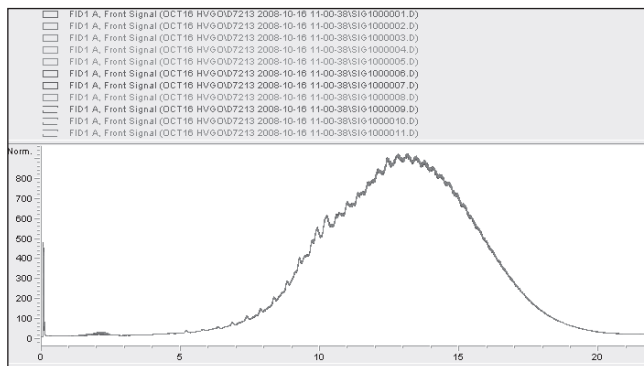


Figure 12. Overlay of 11 runs of HVGO, each prepared by using a Easy Sample Prep program.

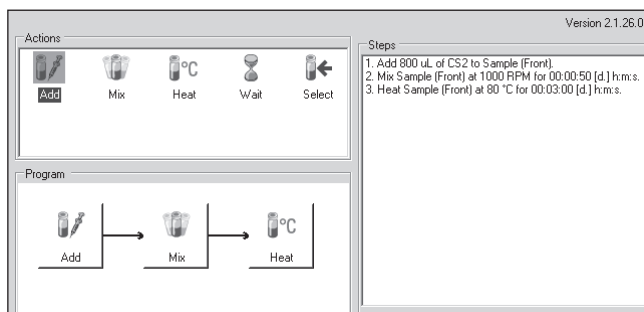


Figure 13. Preparation of HVGO for injection. CS_2 is used as the solvent.

Reference

1. Roger L. Firor, "Automated Preparation of Simulated Distillation Samples for ASTM Methods D2887, D7213, D7398, and D6352 using a Dual Tower 7693A and Tray System", April 2009, Agilent Technologies publication 5990-3778EN.

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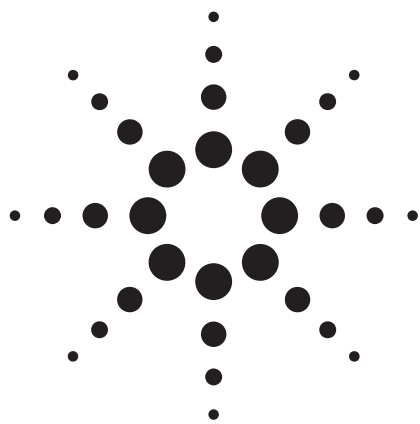
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GC Analysis of Sulfur Components in Propylene using a Pulsed Flame Photometric Detector

Application Note

Hydrocarbon Processing

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Abstract

The Agilent J&W Select Low Sulfur column measures trace levels of target components in C3 hydrocarbon streams without any matrix interference.

Introduction

Hydrogen sulfide (H_2S), carbonyl sulfide (COS) and methyl mercaptan (CH_3SH) are common components in light hydrocarbon streams. They have corrosive and toxic properties, causing damage to pipes and equipment. The emission of undesired odors caused by volatile sulfur compounds in intermediates and final products have serious economic and environmental impact. In addition, the presence of sulfur can affect the performance of industrial processes, causing chemical reactions, loss of catalyst activity (catalyst poisoning), and ultimately lower yield.

These sulfur components must be quantified at low ppb levels. They can be measured with sulfur specific detection devices like the Pulsed Flame Photometric Detector (PFPD) but large sample volumes are needed to reach the desired low parts per billion (ppb) detection limits. This creates matrix overload and quenching effects (decreased signal/sensitivity due to background interferences) on most sulfur specific detectors, limiting the detector's sensitivity and linearity and raising quantification limits. The capillary PLOT column, Agilent J&W Select Low Sulfur column, with a novel stationary phase was developed for the analysis of sulfur species such as H_2S , COS and CH_3SH in light hydrocarbon C3 matrices, with high loadability properties and unique selectivity giving baseline resolution for sulfur components and matrix components.



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Experimental

Technique:	GC-PFPD
Column:	Agilent J&W Select Low Sulfur, 60 m × 0.32 mm (p/n CP8575)
Oven:	65 °C isotherm
Carrier gas:	Helium, constant flow, 2.0 mL/min
Injector:	200 °C, split 1:20
Detector:	PFPD, 200 °C
Sample:	Propylene matrix containing ~500 ppb H ₂ S, COS, and CH ₃ SH
Injection volume:	1 mL
Injection:	Gas sampling valve

Results and Discussion

The stationary phase shows good selectivity between H₂S, COS and low mercaptans in various C3 hydrocarbon matrices. Therefore, co-elution of the sulfur components and the matrix, which causes “quenching”, is avoided.

The system was equipped with a gas sampling valve. The gas sampling valve event table is shown in Table 1. The detector settings are shown in Table 2.

Table 1. Gas Sampling Valve Event Table

Time (min)	Gas sampling valve
Initial	Fill
0.01	Inject
1.00	Fill

Table 2. Detector PFPD Settings

Combustion gases	
Air (1)	17 mL/min
H ₂	13 mL/min
Air (2)	10 mL/min
Trigger level	250 mV
Tube voltage	550 V
Sampling delay	6 ms
Sampling width	20 ms

Figure 1 shows the chromatogram of sulfur compounds H₂S, COS, and CH₃SH in a propylene matrix. Methyl mercaptan shows peak broadening from column overloading by the large amount of propylene. The propylene matrix elutes between COS and methyl mercaptan.

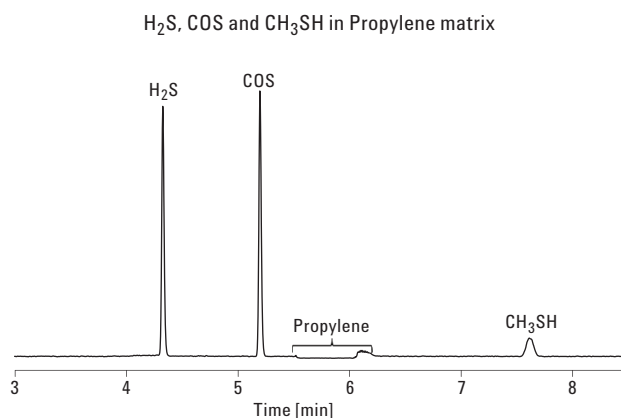


Figure 1. Chromatogram of sulfur compounds H₂S, COS and CH₃SH in a propylene matrix, using the Agilent J&W Select Low Sulfur with GC-PFPD.

Conclusion

The Agilent J&W Select Low Sulfur used in a GC with a sulfur specific detector, such as a PFPD, can detect H₂S, COS and CH₃SH at trace levels in a propylene matrix as a result of excellent separation of the sulfur compounds and the matrix. Separating the matrix from the sulfur components eliminates the “quenching” effects caused by the matrix. This provides a better response for the sulfur compounds. The column provides a good response for reactive sulfur compounds, such as H₂S, which makes detections of 20 ppb possible.

Although this is a PLOT column, no spikes will be observed because this column does not shed particles. It can therefore be used safely in combination with valves.

References

1. W. Wardencki (1998) Review “Problems with the determination of environmental sulphur compounds by gas chromatography.” J. Chromatog. A. 793: 1-19.
2. Roger L. Firor and Bruce D. Quimby, “Comparison of Sulfur Selective Detectors for Low-Level Analysis in Gaseous Streams,” Agilent Technologies publication 5988-2426EN.

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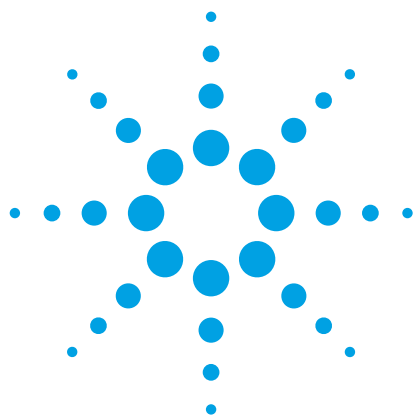
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Asphalt Analysis with Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Asphalt is the residual left when practically everything that can be recovered from crude oil by high vacuum, high temperature distillation has been vaporized. The result is a sticky, near solid material containing a vast array of compounds varying from paraffins to highly condensed aromatics. The choice of solvent for use as eluent in gel permeation chromatography (GPC) is very important. Polar materials in asphalt tend to associate and be adsorbed on the packing material. In addition, GPC results are affected by interactions with the solvent, which affect the apparent hydrodynamic volume.

Analysis of Asphalt

Many asphalt applications can be successfully carried out using tetrahydrofuran (THF) as eluent. Figure 1 shows such a comparison of two batches of asphalt, indicating differences in molecular weight distribution. Agilent PLgel GPC columns are compatible with solvents covering a wide range of polarity.



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Conditions for Figure 1

Columns	2 × Agilent PLgel 5 µm MIXED-D, 7.5 × 300 mm (p/n PL1110-6504)
Eluent	THF (stabilized)
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

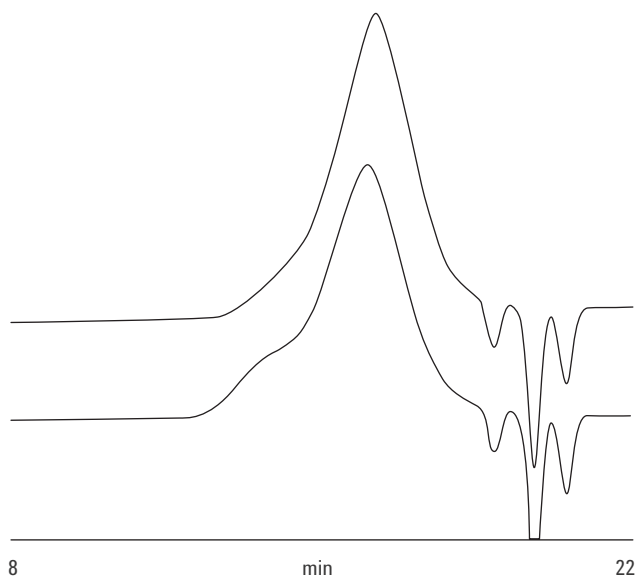


Figure 1. Comparing two batches of asphalt using an Agilent PLgel 5 µm MIXED-D two-column set with tetrahydrofuran as eluent.

Conditions for Figures 2 and 3

Columns	2 × Agilent PLgel 3 µm MIXED-E, 7.5 × 300 mm (p/n PL1110-6300)
Eluent	o-Xylene
Flow rate	0.5 mL/min
Detector	RI
System	Agilent PL-GPC 50

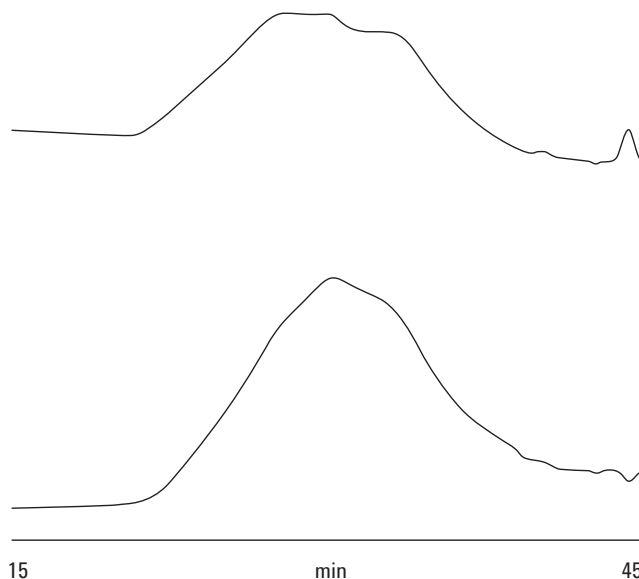


Figure 2. Comparing two batches of asphalt using an Agilent PLgel 5 µm MIXED-E two-column set with o-xylene as eluent.

Figure 2 shows another comparison of two polydisperse asphalt samples, this time analyzed using xylene as eluent. The higher viscosity of this solvent requires either a reduction in flow rate or elevation of temperature to reduce column operating pressure. The polystyrene standards separation for this application is illustrated in Figure 3. Other suitable solvents include benzene, toluene, and chloroform, all of which are compatible with PLgel columns.

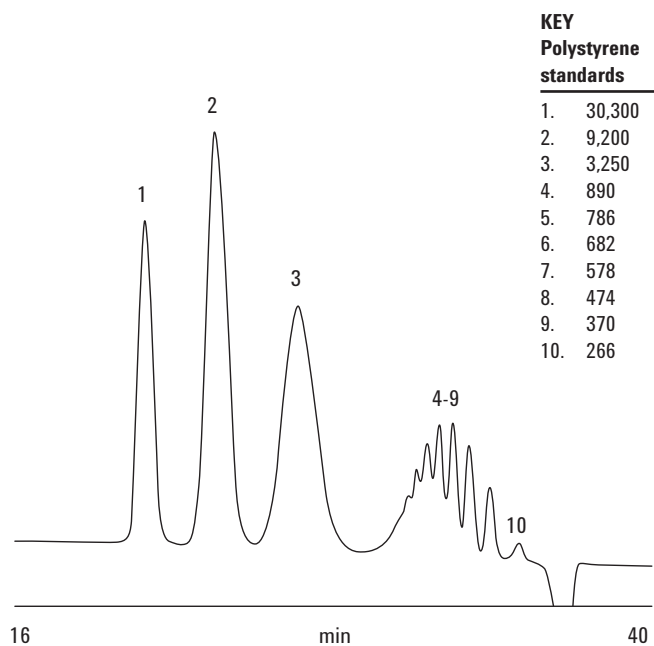


Figure 3. Separation of low molecular weight polystyrene standards on Agilent PLgel 3 μ m MIXED-E columns.

Conclusions

Agilent PLgel columns can be used in a variety of solvents to investigate the composition of complex materials such as asphalt by gel permeation chromatography.

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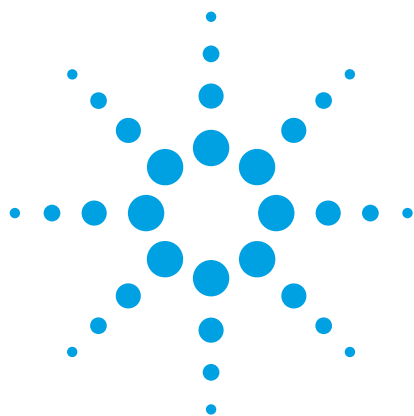
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Petroleum Analysis with Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Crude oil, or petroleum, is the main source of organic chemicals for industry. The major chemicals are derived from two constituents of oil, xylene, and naphtha. These raw materials are then broken down into more basic products, such as polyethylene, polypropylene, elastomers, asphalts, and liquid hydrocarbons. Characterization of such products is commonly achieved using gel permeation chromatography (GPC). This involves a liquid chromatographic separation from which a molecular weight distribution calculation can be made following calibration of the system with suitable polymer standards. The diversity of petroleum products demands a variety of Agilent PLgel GPC columns for optimized analysis.

Analysis of Asphalt

Low molecular weight liquid hydrocarbons require high resolution of individual components. This is illustrated in Figure 1, where three linear hydrocarbons are resolved easily to base line in a reasonably short analysis time.



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Conditions for Figure 1

Columns	2 × Agilent PLgel 5 µm 100Å, 7.5 × 300 mm (p/n PL1110-6520)
Eluent	1,2,4-Trichlorobenzene
Flow rate	1.0 mL/min
Temp	100 °C
Detector	RI
System	Agilent PL-GPC 220

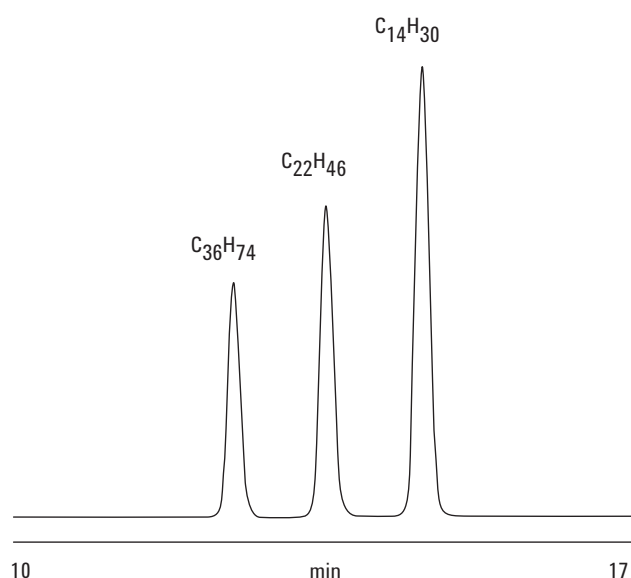


Figure 1. Low molecular weight linear hydrocarbons resolved to base line using an Agilent PLgel 5 µm two-column set.

The Agilent PLgel 5 µm 100Å columns have a GPC exclusion limit of 5,000 molecular weight (polystyrene equivalent), and efficiency is typically > 60,000 plates/m. Intermediate products can be analyzed using the Agilent PLgel 5 µm MIXED-D column, which has a linear molecular weight resolving range up to an exclusion limit of around 500,000 molecular weight. The 5-µm particle size maintains high column efficiency, therefore, fewer columns are required, and analysis time is relatively short.

Figure 2 shows the analysis of asphalt used in road surfacing. Subsequent information regarding the molecular weight distribution of such materials is invaluable in determining processability and final properties.

Conditions for Figure 2

Columns	2 × Agilent PLgel 5 µm MIXED-D, 7.5 × 300 mm (p/n PL1110-6504)
Eluent	THF
Flow rate	1.0 mL/min
Temp	50 °C
Detector	RI
System	Agilent PL-GPC 50

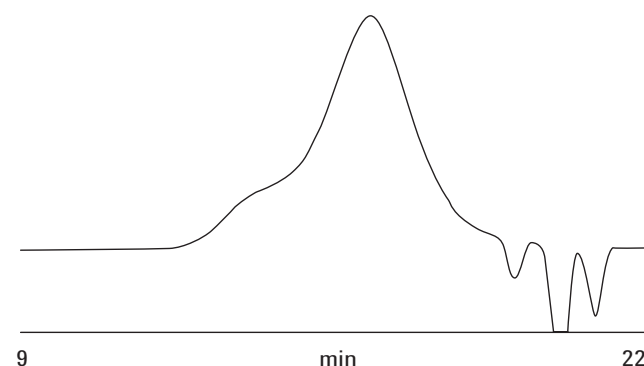


Figure 2. Asphalt separation on an Agilent PLgel 5 µm MIXED-D two-column set.

High molecular weight polyolefin polymers tend to exhibit very broad molecular weight distribution. In these applications, small particle size packings are not desirable since the incidence of polymer shear degradation is apparent. The Agilent PLgel 20 µm MIXED-A column is ideally suited, with a high exclusion limit (40,000,000 g/mol polystyrene equivalent). Its larger particle size, with subsequent lower efficiency, means that three or four columns are required in series. Figure 3 shows typical polyethylene and polypropylene analyses on Agilent PLgel 20 µm MIXED-A columns.

Conditions for Figure 3

Columns	3 × Agilent PLgel 20 µm MIXED-A, 300 × 7.5 mm (p/n PL1110-6200)
Eluent	1,2,4-Trichlorobenzene
Flow rate	1.0 mL/min
Temp	160 °C
Detector	RI
System	PL-GPC 220

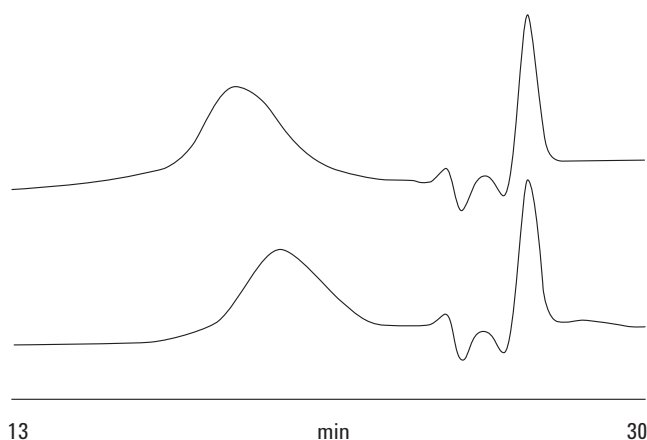


Figure 3. Polyethylene and polypropylene separated on an Agilent PLgel 20 µm MIXED-A three-column set.

Conclusions

For many polyolefin-based products, solubility is limited to solvents such as trichlorobenzene at temperatures in excess of the crystalline melting point. This implies that the GPC system must be carefully temperature-controlled throughout. In these examples, temperatures between 50 and 160 °C were used. As there is no UV chromophore, RI is the most common detection technique. However, RI detection is well known for its temperature instability, and in general, dedicated integrated high temperature GPC systems are preferred. The Agilent PL-GPC 220 system is ideal for these applications. Agilent PLgel 20 µm MIXED-A columns operate successfully at the elevated temperatures required for polyolefin analysis. However, for highly crystalline polyolefins of very high molecular weight (100,000,000 g/mol polystyrene equivalent), Agilent PLgel Olexis column are preferred.

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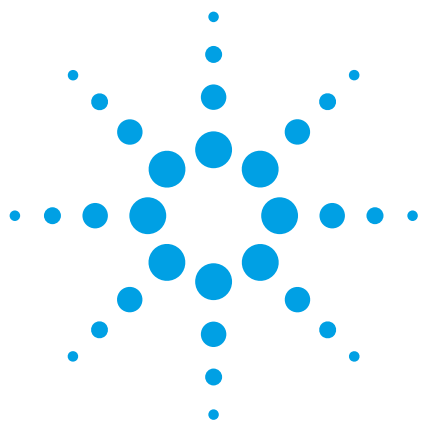
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Agilent Technologies



Determination of major and minor elements in geological samples using the 4100 MP-AES

Application note

Geochemistry, metals and mining

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Introduction

Establishing the elemental composition and grade of base metal ores is carried out at different stages of mining and processing. It is used in advanced exploration to confirm the feasibility of mining the ore, and in the assay of the ore concentrate. The determination of the concentration of base metals requires a technique with good accuracy and precision that is capable of generating and managing data from the analysis of large numbers of samples. Consequently, there are also very high expectations in terms of sample throughput and reliability.

In addition, ore-grade samples contain a range of concentrations. It would not be unusual to see high percentage level analytes present alongside low parts per million level analytes. A sample with 40% copper would not be unexpected, and as such, any analysis technique used needs to be able to cope with this range of concentrations.



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Typically, the determination of major and minor elements in geological samples is performed using flame atomic absorption spectroscopy (FAAS) and/or inductively coupled plasma optical emission spectroscopy (ICP-OES). Agilent has developed a new instrument, the 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES), that out-performs FAAS in terms of analytical performance, and offers lower operating costs and unattended overnight operation. The 4100 MP-AES is a highly automated multi-element system with high matrix tolerance and high sample throughput. This ensures that challenging samples can be analyzed quickly and reliably with little operator training and minimal method development.

Experimental

Instrumentation

The 4100 MP-AES is a fast sequential multi-element analytical technique that uses a microwave-induced plasma to provide analysis of liquid sample using a conventional sample introduction system. As MP-AES relies on the generation of a microwave plasma using nitrogen, no flammable gases such as acetylene are required. This reduces running costs and improves lab safety. Nitrogen can be supplied from bottled gas or the Agilent 4107 Nitrogen Generator. This alleviates the difficulty and costs in sourcing gases such as acetylene, specially in remote locations.

The potential of the Agilent 4100 MP-AES for the analysis of ore-grade base metal samples was investigated following preparation of the samples by a four-acid digestion procedure.

Samples and sample preparation

Ore-grade samples require different digestion techniques to geochemical exploration samples in order to dissolve the high metal concentrations and retain them in solution. As a result, dilution factors are greater than with geochemical exploration samples, resulting in higher detection limits, and also higher upper limits.

The preparation method used involves taking 0.4 g of sample and performing a four-acid (HNO_3 - HClO_4 - HF - HCl) digestion. The process is completed by further addition of hydrochloric acid and deionized water, followed by cooling to room temperature. The resulting solution

was diluted to 100 mL with de-ionized water resulting in a final matrix of 30% HCl. This represents a 250-fold sample dilution.

The 4100 MP-AES operating parameters were optimized as shown in Table 1.

Table 1. Agilent 4100 MP-AES operating parameters

Instrument parameter	Setting
Nebulizer pressure	80–240 kPa
Read time	3 s (10 s for Ag)
Stabilization time	15 s
Background correction	Auto

Results and discussion

Method detection limits (MDLs)

Method detection limits (MDLs) for silver, copper, molybdenum, nickel, lead, and zinc were determined. Table 2 shows that excellent MDLs can be obtained using the 4100 MP-AES.

Table 2. MDLs for digestions of ore-grade base metal samples

Analyte	Wavelength (nm)	MDL (ppm)
Ag	328.068	0.04
Cu	327.395	0.1
Mo	379.398	0.7
Ni	352.453	0.4
Pb	405.781	0.4
Zn	213.857	0.7

Quantitative analysis

A batch of base metal ore sample digests were prepared using the sample preparation method. These sample were analysed by the 4100 MP-AES using a blank and three matrix-matched standard solutions.

Accuracy

The results obtained by the 4100 MP-AES for Ag, Cu, Mo, Ni, Pb and Zn were plotted against the expected results — see Figures 1 to 7. The graphs show good correlation (accuracy) over the calibration range, up to ~4% for copper, indicating that MP-AES is a suitable technique for the application.

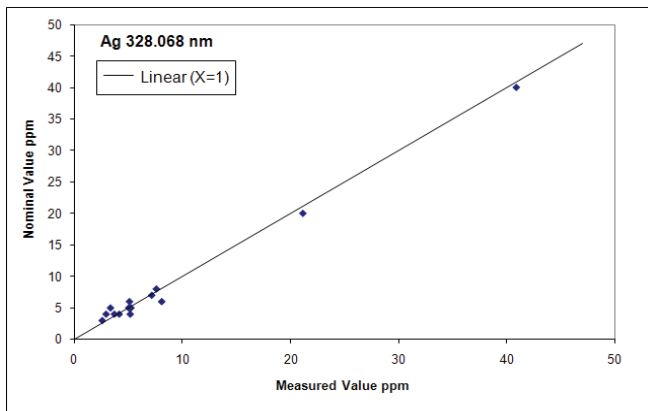


Figure 1. MP-AES measured results for Ag compared to nominal values

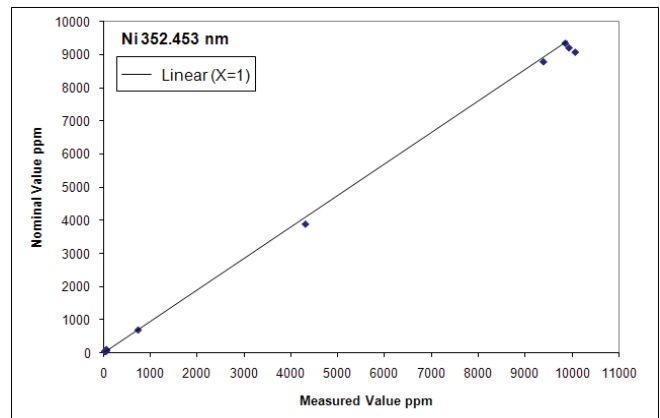


Figure 4. MP-AES measured results for Ni compared to nominal values

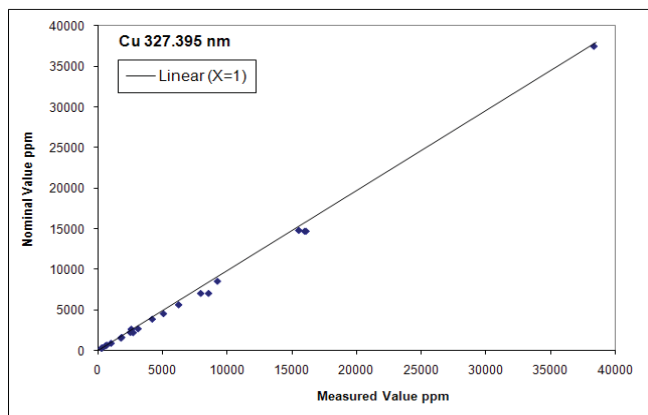


Figure 2. MP-AES measured results for Cu compared to nominal values

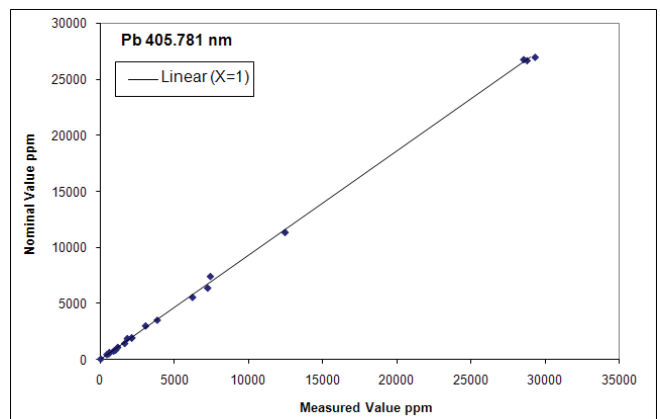


Figure 5. MP-AES measured results for Pb compared to nominal values

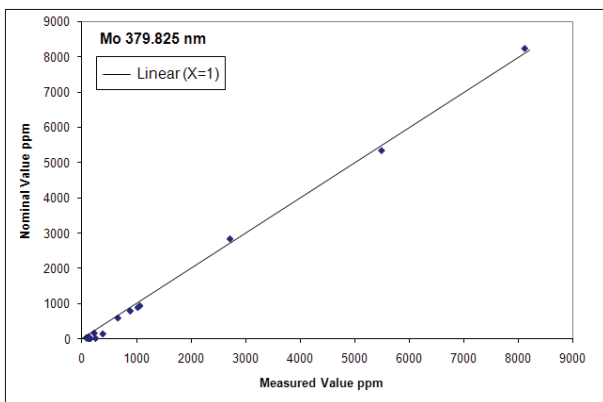


Figure 3. MP-AES measured results for Mo compared to nominal values

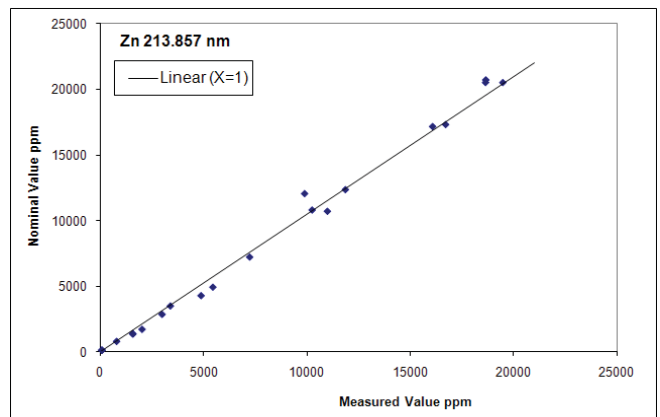


Figure 6. MP-AES measured results for Zn at the 213.857 nm wavelength compared to nominal values

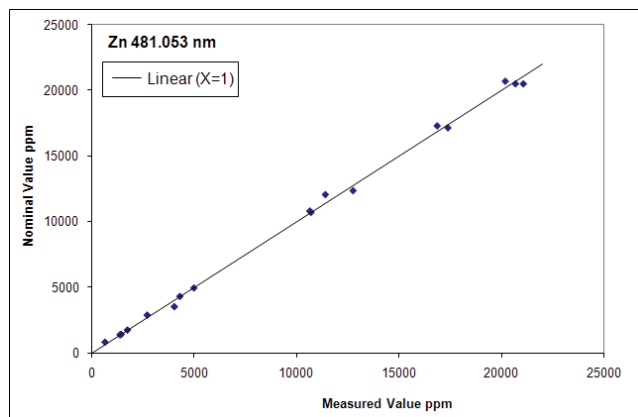


Figure 7. MP-AES measured results for Zn at the 481.053 nm wavelength compared to nominal values

Conclusions

A series of geological samples has been successfully analyzed using the Agilent 4100 MP-AES, following a sample preparation procedure that is commonly used to prepare base metal ores for analysis by geochemical laboratories. Quantitative results were obtained for 6 elements present in a batch of 23 base metal ore samples, with measured concentrations ranging from less than 1 ppm to 29%. The comparative results for Ag,

Cu, Mo, Ni, Pb and Zn obtained using MP-AES show that the 4100 MP-AES is well suited to this application. It also offers additional benefits over comparative techniques such as flame AAS, through lower operating costs, improved safety and the ability to perform the analysis using unattended overnight operation.

Operating costs can be further reduced by directly generating nitrogen from compressed air using an Agilent 4107 Nitrogen Generator. This is particularly advantageous in areas where gas supplies are expensive, difficult to obtain or pose logistics challenges in transporting bulk cylinders to site. The 4100 MP-AES also offers the possibility of installations in remote locations. This enables laboratories to analyze samples locally at the source rather than transporting the samples to a central laboratory for analysis, as is the current practice. Furthermore, the Agilent 4100 MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide.

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Fast Refinery Gas Analysis Using the 490 Micro GC QUAD

Application Note

Authors

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Introduction

There is a large variation in the composition and source of refinery gases. Therefore, the precise and accurate analysis of these gases is a significant challenge in today's refineries. Typical sources include fluid coking overheads, ethylene, propylene, fuel gas, stack gas, off gas, etc. The physical stream ranges from gas to highly pressurized gas or liquid.

Very fast refinery gas analysis (RGA) is possible with the portable 490 Micro GC QUAD. This note describes the use of the 490 Micro GC for RGA, with results obtained in about two minutes.



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Instrumentation

490 Micro GC QUAD

- Channel 1: Molsieve with back flush
- Channel 2: CP-PoraPlot U with back flush
- Channel 3: Aluminium oxide with back flush
- Channel 4: CP-Sil 5 CB

The Molsieve channel and the aluminium oxide channel are equipped with extra in-line filters between the manifold and the column module to ensure moisture and carbon-dioxide-free carrier gas. This enhances column lifetime and, most importantly, leads to stable retention times.

GC control and data handling software: Galaxie Chromatography Software.

Materials and Reagents

Channel 1, equipped with a Molsieve column, separates and analyzes the permanent gases except for carbon dioxide. Channel 2, with a CP-PoraPLOT U column, separates and analyzes the C2 gases and hydrogen sulfide. The C3 and C4 hydrocarbons are analyzed on the third channel with an Al2O3 column. Finally, the higher hydrocarbons are analyzed on the fourth channel, with a CP-Sil 5 CB column.

Table 1. Peak identification and composition of gas standards

Gas Standard		
Peak #	Component	Amt (%)
1	Hydrogen	Bal
3	Oxygen	
4	Nitrogen	
5	Methane	
6	Carbon monoxide	

Refinery Gas standard

Peak #	Component	Amt (%)	Peak #	Component	Amt (%)
2	Helium	Bal	15	Propadiene	0.62
4	Nitrogen	5.1	16	n-Butane	1.0
5	Methane	24.9	17	tr-2-Butylene	0.5
6	Carbon monoxide	1.0	18	1-Butylene	0.5
7	Carbon dioxide	0.5	19	iso-Butylene	1.01
8	Ethylene	24.9	20	cis-2-Butylene	0.5
9	Ethane	5.0	21	iso-Pentane	0.5
10	Acetylene	1.0	22	Methyl acetylene	1.0
11	Hydrogen sulfide	1.01	23	n-Pentane	0.2
12	Propane	5.0	24	1, 3-Butadiene	1.0
13	Propylene	5.0	25	n-Hexane	0.2
14	iso-Butane	0.5			

Conditions

Table 2. Chromatographic conditions

	Channel 1	Channel 2	Channel 3	Channel 4
	10 m Molsieve	10 m CP-PoraPLOT U	10 m Al2O3/KCL	8 m CP-Sil 5 CB
Injector Temp (°C)	110	110	110	110
Column Temp (°C)	80	100	100	80
Carrier Gas	Argon	Helium	Helium	Helium
Column Head Pressure (kPa)	150	205	70	205
Injection Time (ms)	40	10	10	100
Back Flush Time (s)	11	7.1	33	N/A

Results and Discussion

Figures 1 and 2 show chromatograms of the Molsieve channel 1.

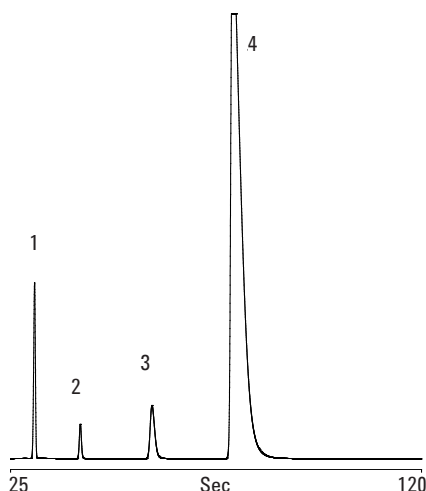


Figure 1. Standard gas on the Molsieve column, channel 1

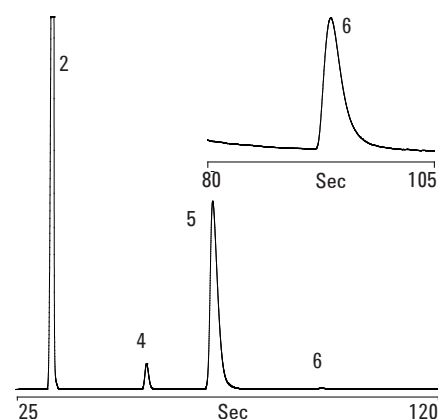


Figure 2. Refinery gas on the Molsieve column, channel 1

Hydrogen or helium, oxygen, nitrogen methane and carbon monoxide were separated and analyzed. Later eluting components were back flushed to vent.

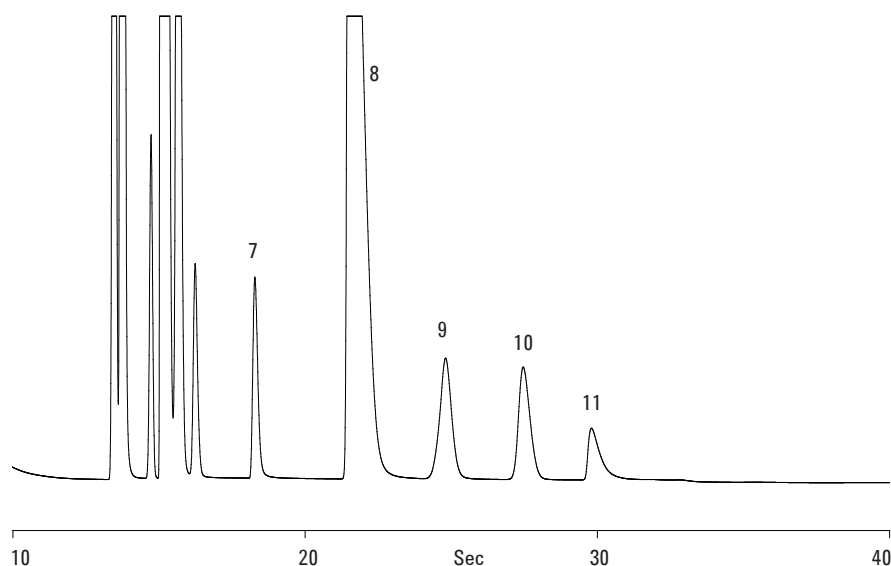


Figure 3. Refinery gas on the CP-PoraPLOT U column, channel 2

On the CP-PoraPLOT U channel (channel 2), the C2 hydrocarbons, hydrogen sulfide and carbon dioxide were separated and analyzed. The channel was equipped with a back flush later eluting components to vent.

On channel 2 the C3 and C4 saturated and unsaturated hydrocarbons were separated and analyzed. This channel was also equipped with back flush in order to prevent the later eluting hydrocarbons from entering the analytical column. This prevented the later eluting components from interfering with the next analysis causing “ghost” peaks and/or baseline drift and higher noise. Furthermore, this channel was equipped with extra filters in the carrier gas lines, effectively protecting the analytical column from traces of moisture and carbon dioxide that could influence the chromatographic properties of the stationary phase in the long term.

Stable retention times are key factors for good chromatographic results. Repeatability results derived from Table 3 and Figure 5 for retention times are superb with RSDs around 0.1% and no drift.

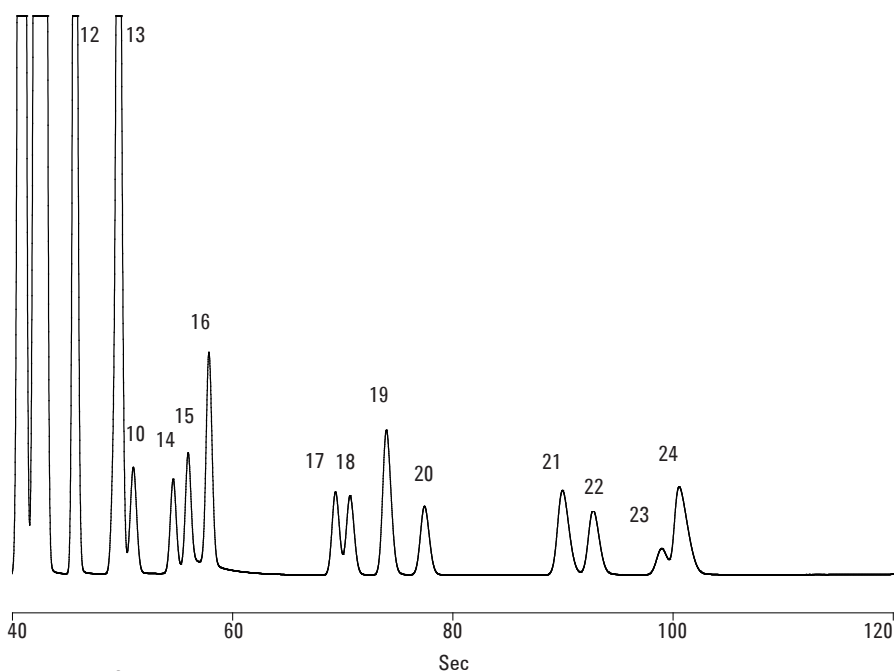


Figure 4. Refinery gas on the aluminium oxide column, channel 3

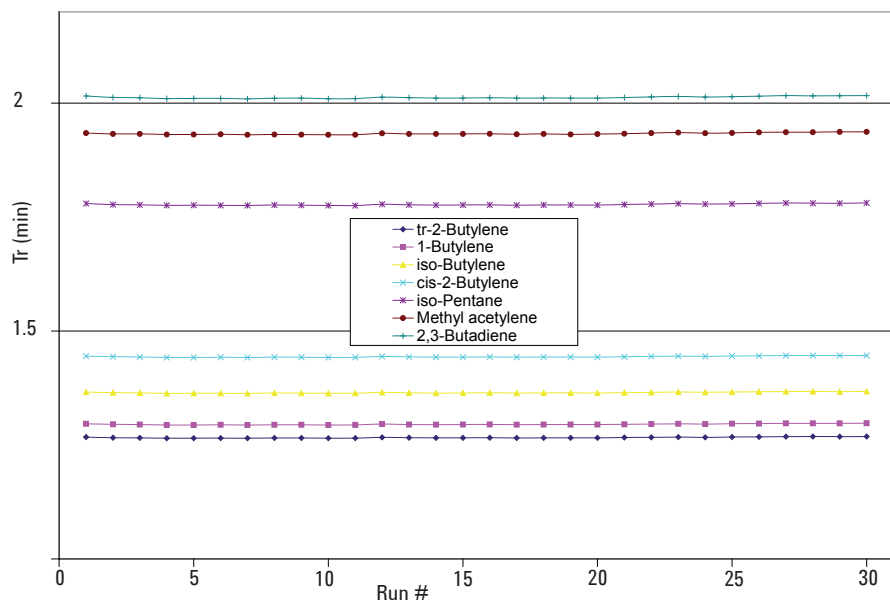


Figure 5. Repeatability figures for the aluminium oxide channel, channel 3

Table 3. Repeatability figures for the aluminium oxide channel

Run #	Tr (min) tr-2-Butylene	Tr (min) 1-Butylene	Tr (min) iso-Butylene	Tr (min) cis-2-Butylene	Tr (min) iso-Pentane	Tr (min) Methyl acetylene	Tr (min) 2, 3-Butadiene
1	1.2672	1.2963	1.366	1.4447	1.7797	1.934	2.0155
2	1.266	1.2952	1.3647	1.4437	1.7772	1.9322	2.0122
3	1.2657	1.2948	1.3643	1.443	1.7768	1.9323	2.0115
4	1.2647	1.2938	1.3632	1.442	1.7755	1.931	2.0097
5	1.2647	1.2937	1.3633	1.442	1.7758	1.931	2.0102
6	1.265	1.2942	1.3633	1.4423	1.7757	1.9315	2.0102
7	1.2648	1.2938	1.3632	1.442	1.7753	1.9303	2.0092
8	1.2653	1.2943	1.364	1.4427	1.7763	1.931	2.0105
9	1.2653	1.2943	1.3638	1.4423	1.776	1.9308	2.0108
10	1.2647	1.2938	1.3633	1.442	1.7753	1.9305	2.0095
11	1.265	1.294	1.3633	1.4422	1.7752	1.9303	2.0098
12	1.2667	1.2958	1.3653	1.444	1.778	1.9337	2.0128
13	1.2658	1.2948	1.3643	1.4432	1.7768	1.9322	2.0117
14	1.2655	1.2945	1.3638	1.4427	1.7762	1.9322	2.0108
15	1.2655	1.2947	1.364	1.4428	1.7763	1.9322	2.011
16	1.2658	1.295	1.3645	1.4432	1.7768	1.9325	2.0115
17	1.2653	1.2945	1.3638	1.4425	1.776	1.9315	2.0107
18	1.2657	1.2948	1.3642	1.443	1.7765	1.9322	2.011
19	1.2657	1.2947	1.3642	1.443	1.7765	1.9312	2.0108
20	1.2655	1.2947	1.364	1.4428	1.7762	1.932	2.0108
21	1.2663	1.2953	1.3648	1.4435	1.7775	1.9328	2.012
22	1.2667	1.2958	1.3653	1.4443	1.7782	1.934	2.0133
23	1.2672	1.2963	1.366	1.4448	1.7793	1.9353	2.0145
24	1.2667	1.2958	1.3655	1.4443	1.7782	1.9338	2.013
25	1.2675	1.2967	1.3662	1.445	1.7788	1.9343	2.0138
26	1.2678	1.2968	1.3667	1.4455	1.7798	1.9357	2.015
27	1.2683	1.2975	1.367	1.446	1.7807	1.936	2.0162
28	1.2685	1.2975	1.3673	1.4462	1.7803	1.936	2.0158
29	1.2682	1.2973	1.3668	1.446	1.7802	1.9367	2.016
30	1.2685	1.2977	1.3673	1.4462	1.781	1.9367	2.0163
Average	1.2662	1.2953	1.3648	1.4436	1.7774	1.9329	2.0122
Std Dev	0.0012	0.0012	0.0013	0.0014	0.0018	0.0020	0.0022
Rsd %	0.10%	0.09%	0.10%	0.10%	0.10%	0.10%	0.11%

Table 4. Reproducibility figures

Day	tr-2-Butylene	1-Butylene	iso-Butylene	cis-2-Butylene	iso-Pentane	Methyl acetylene	2, 3-Butadiene
1	1.2695	1.2988	1.3687	1.4481	1.7849	1.9406	2.0216
2	1.2678	1.2970	1.3668	1.4458	1.7815	1.9370	2.0173
3	1.2668	1.2958	1.3654	1.4443	1.7787	1.9339	2.0137
4	1.2665	1.2956	1.3652	1.4439	1.7781	1.9333	2.0130
8	1.2697	1.2989	1.3689	1.4483	1.7854	1.9405	2.0222
9	1.2681	1.2973	1.3671	1.4462	1.7821	1.9367	2.0180
10	1.2667	1.2957	1.3655	1.4443	1.7785	1.9345	2.0139
Average	1.2679	1.2970	1.3668	1.4458	1.7813	1.9366	2.0171
St. dev.	0.0013	0.0014	0.0015	0.0018	0.0031	0.0030	0.0037
RSD	0.10%	0.11%	0.11%	0.13%	0.17%	0.15%	0.19%

Table 4 and Figure 6 show the effects over several days. RSDs are only slightly higher when compared to the “results-per-day” which is to be expected. However, the results are very good, demonstrating the suitability of the Al₂O₃ channel for this type of analysis.

RSDs below 0.2% are shown in Table 4. During the ten day laboratory experiments no drift in retention times were observed, as can be seen in Figure 6.

Figure 6 shows no drift in retention time of components analyzed on the Al₂O₃ channel over ten days.

Figure 7 shows a chromatogram of refinery gas on the CP-Sil 5 CB channel. In this case the higher hydrocarbons C₅+ were analyzed.

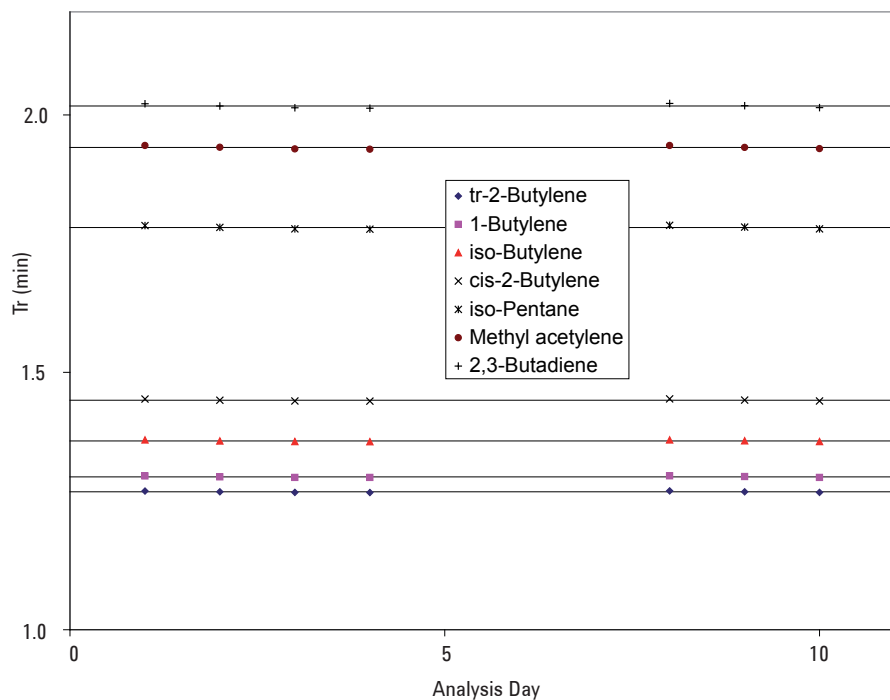


Figure 6. Reproducibility of the aluminium oxide channel, channel 3

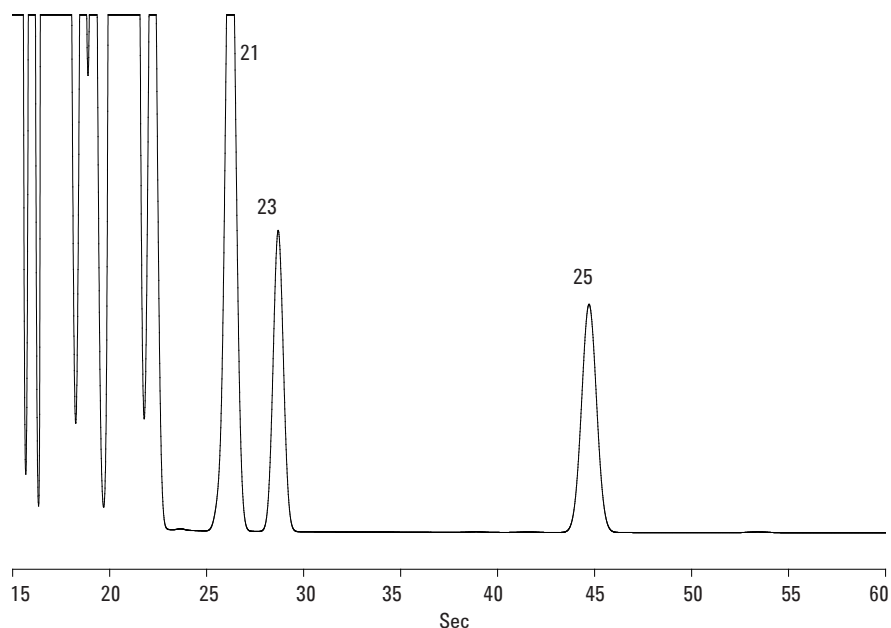


Figure 7. Refinery gas on the CP-Sil 5 CB column, channel 4

Conclusion

The 490 Micro GC QUAD was successfully used for the analysis of refinery gas. The permanent gases helium, hydrogen, oxygen, nitrogen, methane and carbon monoxide were analyzed on the Molsieve channel. The C2 hydrocarbons, carbon dioxide and hydrogen sulfide were analyzed on the second channel equipped with a CP-PoraPLOT U column. On the third channel, with an aluminium oxide column, the C3 and C4 hydrocarbons were analyzed. This channel was equipped with extra in-line filters to ensure moisture and carbon-dioxide-free carrier gas. This significantly enhanced column lifetime and ensured long-term stable retention times. Finally, the fourth channel, equipped with a CP-Sil 5 CB column, analyzed the C5+ hydrocarbons.

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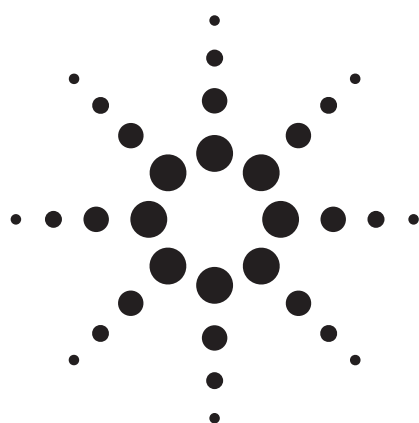
NATURAL GAS

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The Measure of Confidence

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

Application

Hydrocarbon Processing

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Abstract

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

Introduction

There are several known techniques for injecting volatile liquefied hydrocarbons in gas chromatographs. The simplest tools are high-pressure

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

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

Experimental

Injection Device

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



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

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

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

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or
G3470A Aux EPC module

6890GC

G1570A Aux EPC or
G2317A PCM module

The PCM is the preferred source for both GCs.

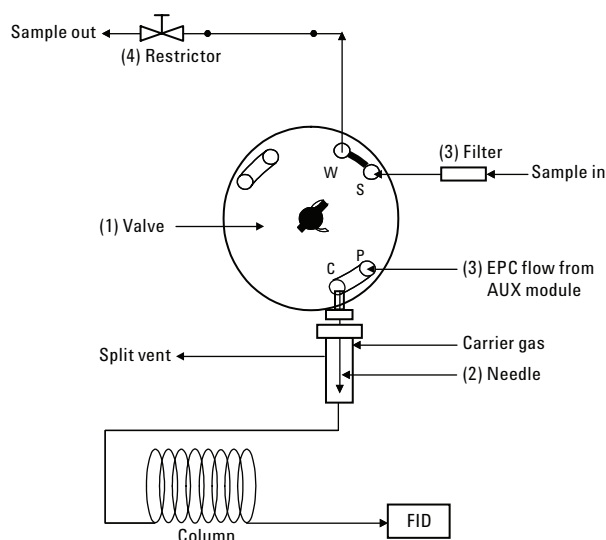


Figure 1. Flow diagram of the HPLI device.

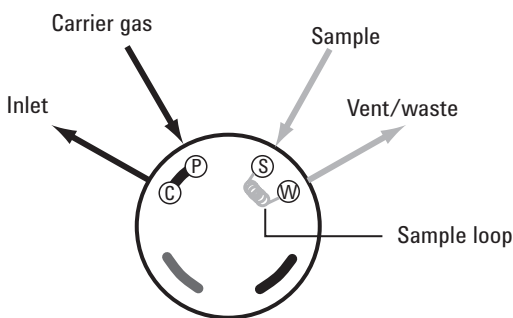
Samples for System Evaluation

- Liquefied natural gas: Calibration standard, 1,200 psi, with nC7-nC9 (0.102%–0.0503%)
- Liquefied ethylene: Purity 99.5, 1,200 psi
- Pressurized propylene: Grade C. P., purity 99.0%, 200 psi
- Pressurized propane + n-butane: 50.0%:50.0%, 200 psi
- Pressurized 1, 3-butadiene: Purity 99.5%, 180 psi
- n-Hexane + 1.0 % 2# BP standard (Agilent PN 5080-8768, nC5–nC18)
- nC5–nC40 D2887 1# BP standard (Agilent PN 5080-8716, diluted by CS₂)
- Glycols, including monoethylene glycol, diethylene glycol, and triethylene glycol
- C8 to C16 hydrocarbons at 100 ppm each

Operating Process

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

Load



Inject

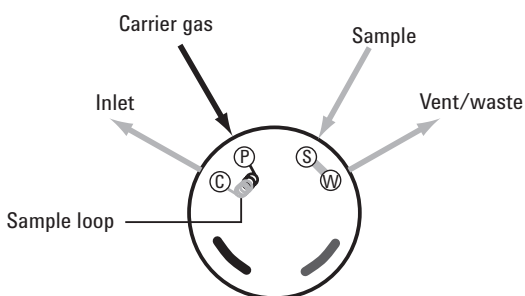


Figure 2. Pneumatic and sample paths in load and inject positions.

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

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

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



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

Table 1. Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	HPLI device at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.5- μ L (0.2 μ L for C5–C40) device supplied with 0.06- μ L rotor
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H ₂ , 35 mL/min Air, 400 mL/min

Table 2. Columns and Parameters

Samples	Columns	Column flow mL/min	Split ratio	Temperature program	Sample pressure psig
Natural gas	30 m $\times$ 0.53 mm $\times$ 0.5 μ m DB-1 #125-1037	8	40:1	35 °C, 1 min 20 °C/min to 180 °C, 1 min	1200
Ethylene	50 m $\times$ 0.53 mm $\times$ 15 μ m AL203 PLOT/KCL + 30 m $\times$ 0.53 mm $\times$ 5 μ m DB-1, #19095P-K25 and #125-1035	8	20:1	35 °C, 2 min 4 °C/min to 160 °C, 3.8 min	1100
Propylene	50 m $\times$ 0.53 mm HP AL203 PLOT + 30 m $\times$ 0.53 mm $\times$ 5 μ m DB-1	7	25:1	35 °C, 2 min 4 °C/min to 160 °C, 1.8 min	180
Propane + n-butane	30 m $\times$ 0.53 mm $\times$ 1.0 μ m DB-1, #125-103J	5	50:1	35 °C	150
1,3-Butadiene	50 m $\times$ 0.53 mm AL203 PLOT/KCL	10	15:1	35 °C, 2 min 10 °C/min to 195 °C, 15 min	180
n-Hexane	30 m $\times$ 0.53 mm $\times$ 1.0 μ m DB-1	5	50:1	45 °C	N/A
nC5-nC40	10 m $\times$ 0.53 mm $\times$ 0.88 μ m HP-1, #19095Z-021	10	15:1	35 °C, 1 min 15 °C/min to 350 °C, 5 min	N/A
Glycols	30 m $\times$ 0.25 mm $\times$ 1.0 μ m HP-1 ms	1.8	15:1	50 °C, 3 min 15 °C/min to 250 °C, 2 min	

Results and Discussion

Check for Carryover

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

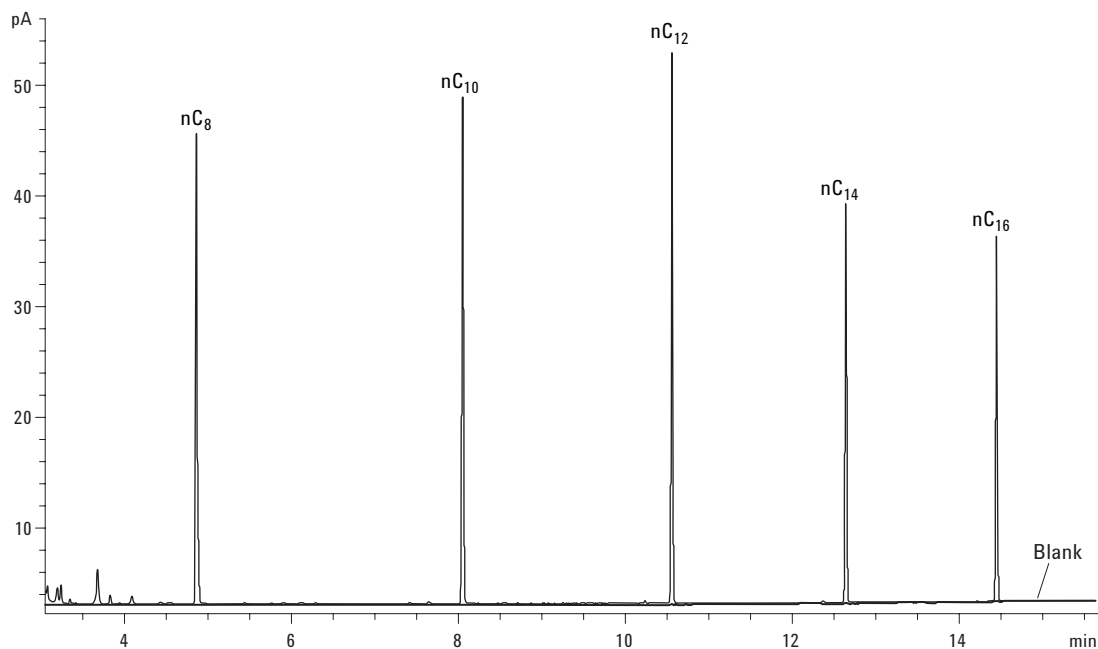


Figure 4. Overlay of standard versus blank (100 ppm each in cyclohexane).

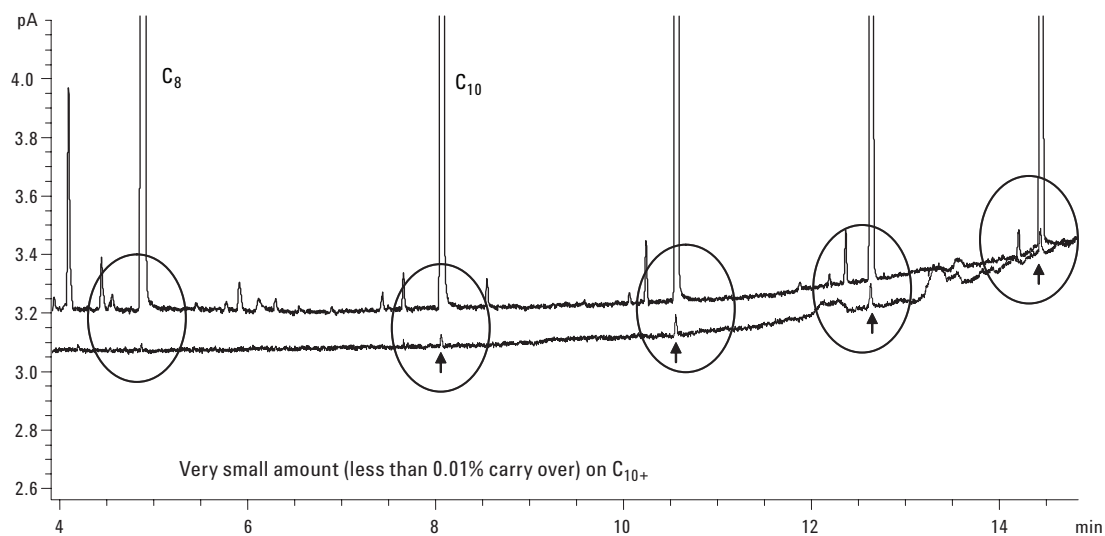


Figure 5. Carryover less than 0.01% on C₁₀₊.

Sample Analysis

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

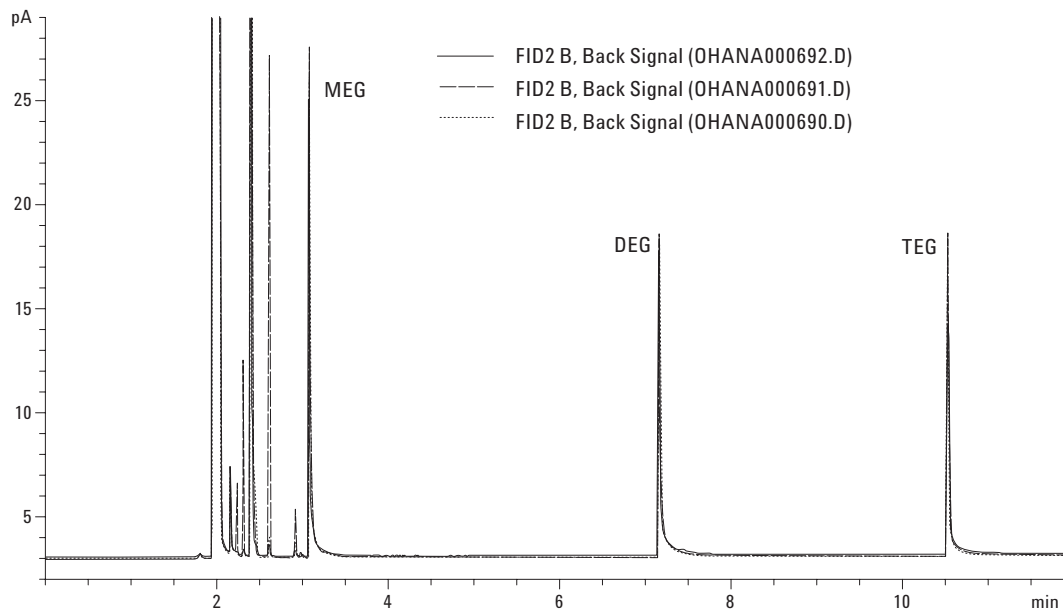


Figure 6. Triplicate run of 100 ppm each of MEG, DEG, and TEG in IPA.

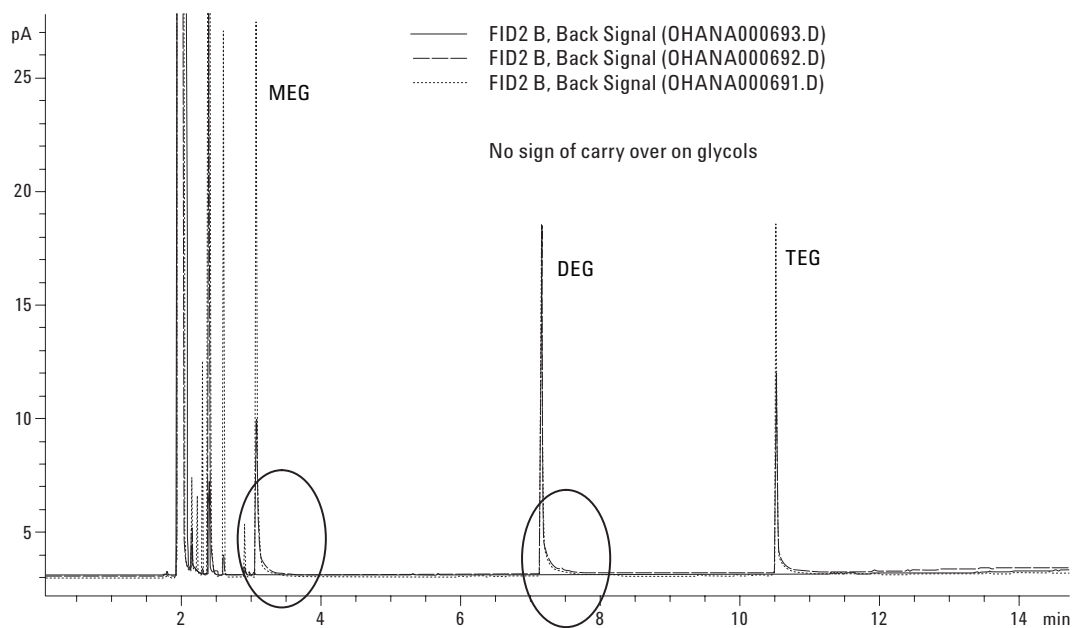


Figure 7. Glycols versus blank. Two standard duplicates, blank run immediately after injection of standard.

A. Liquefied Natural Gas

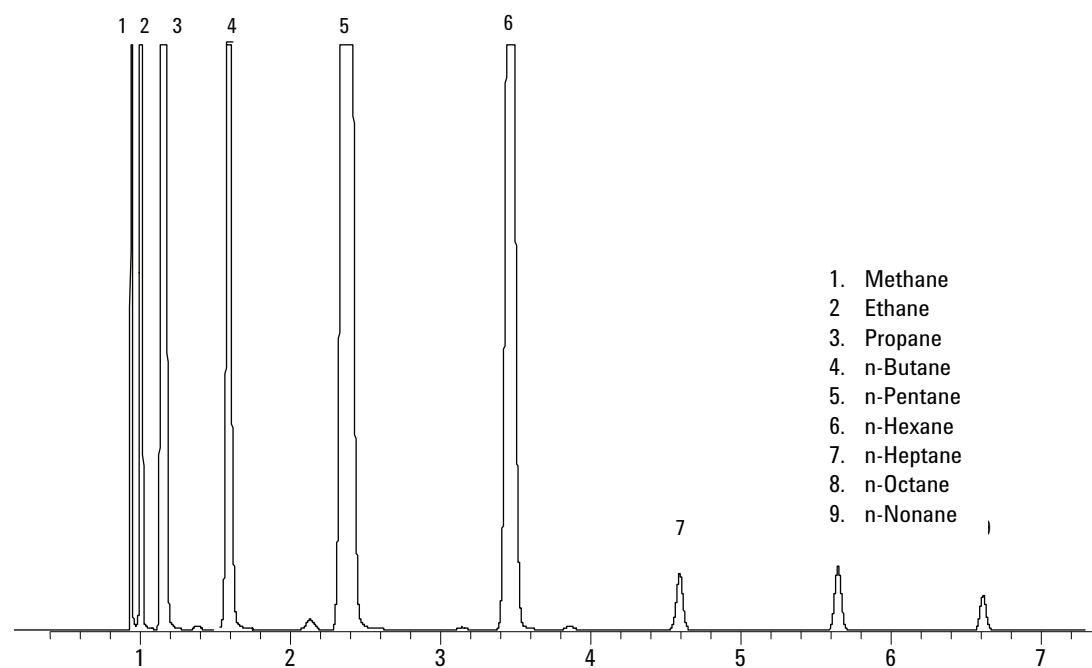


Figure 8. Chromatogram of liquefied natural gas (calibration standard).

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

B. Liquefied Ethylene

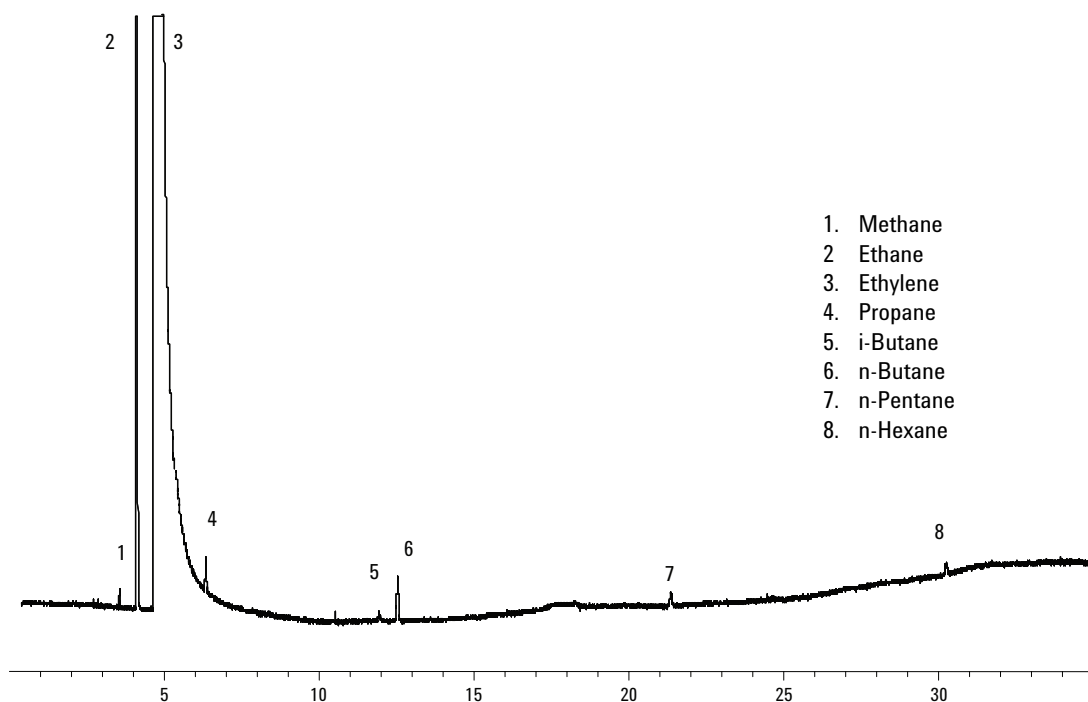


Figure 9. Chromatogram of liquefied ethylene.

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

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

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

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

C. Pressurized Propylene

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

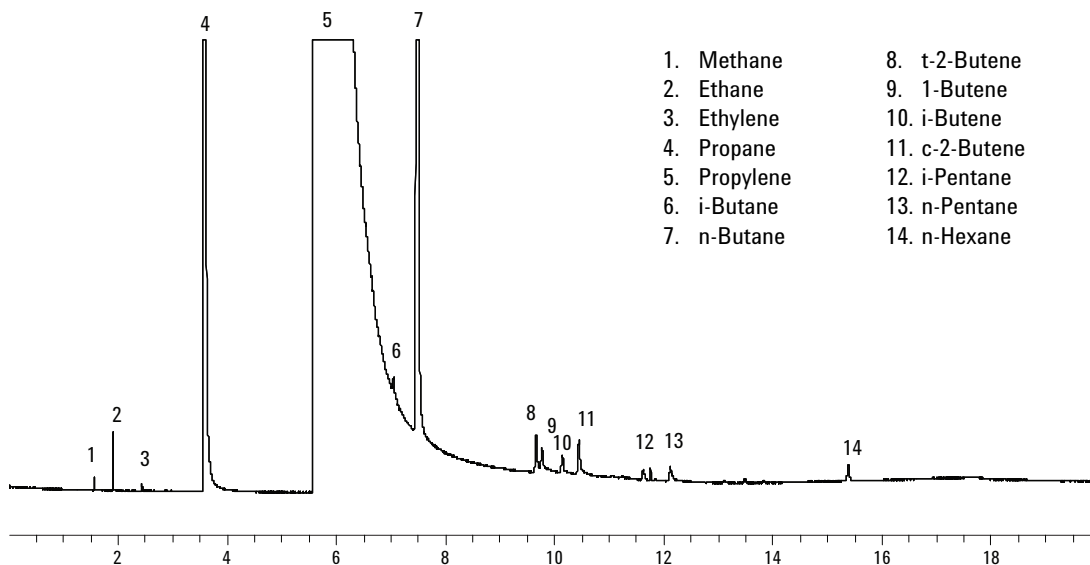


Figure 10. Chromatogram of pressurized propylene.

D. Pressurized 1,3-Butadiene

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

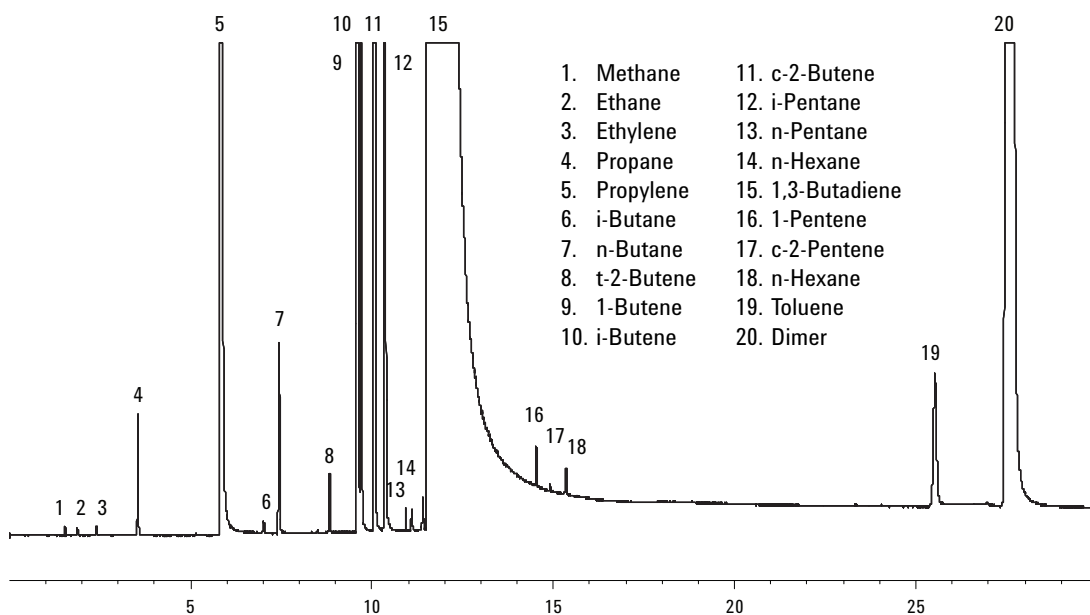


Figure 11. Chromatogram of pressurized 1,3-butadiene.

E. Pressurized Propane + n-Butane

This is a quantitative calibration sample:

Propane:n-Butane = 50%:50%.

The chromatogram is shown in Figure 12 with the results of a quantitative analysis shown in Table 4.

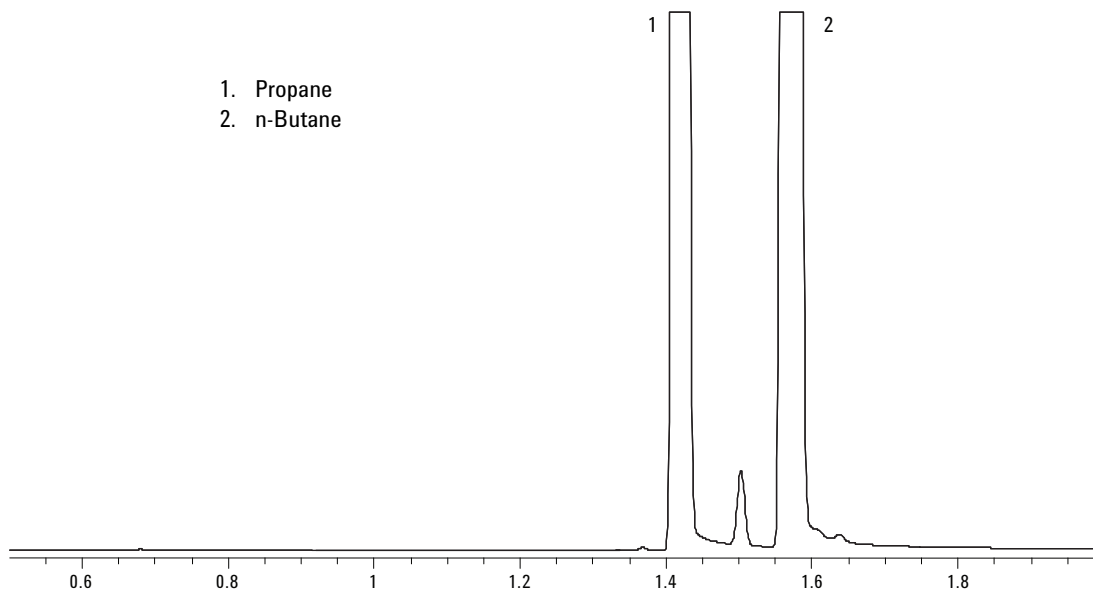


Figure 12. Chromatogram of pressurized propane + n-butane.

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

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

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

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

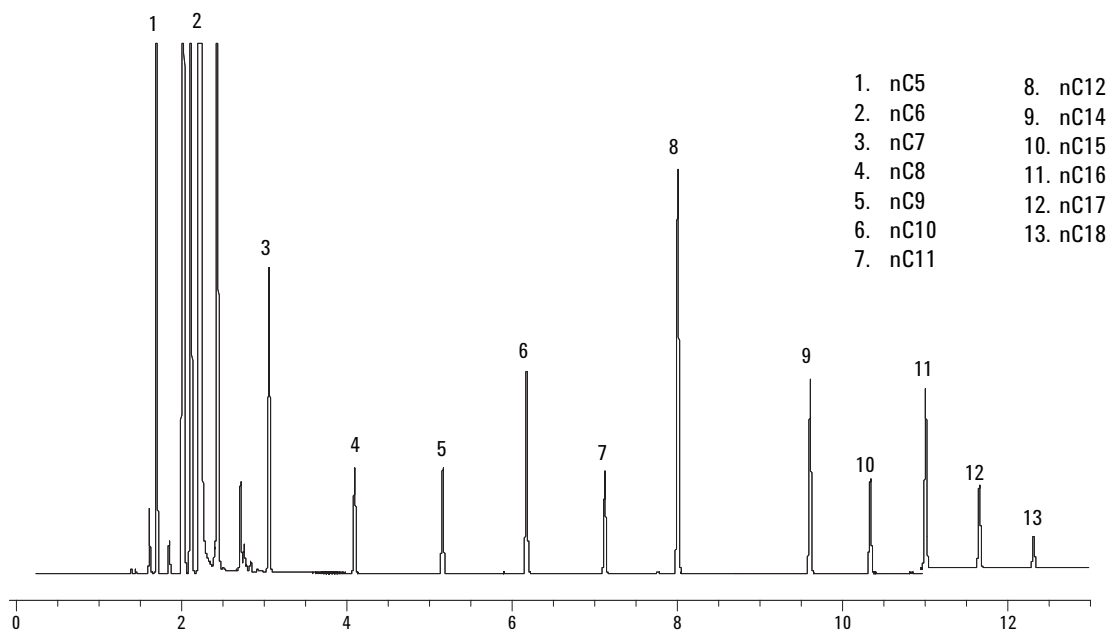


Figure 13. Chromatogram of n-hexane + 1.0% BP standard.

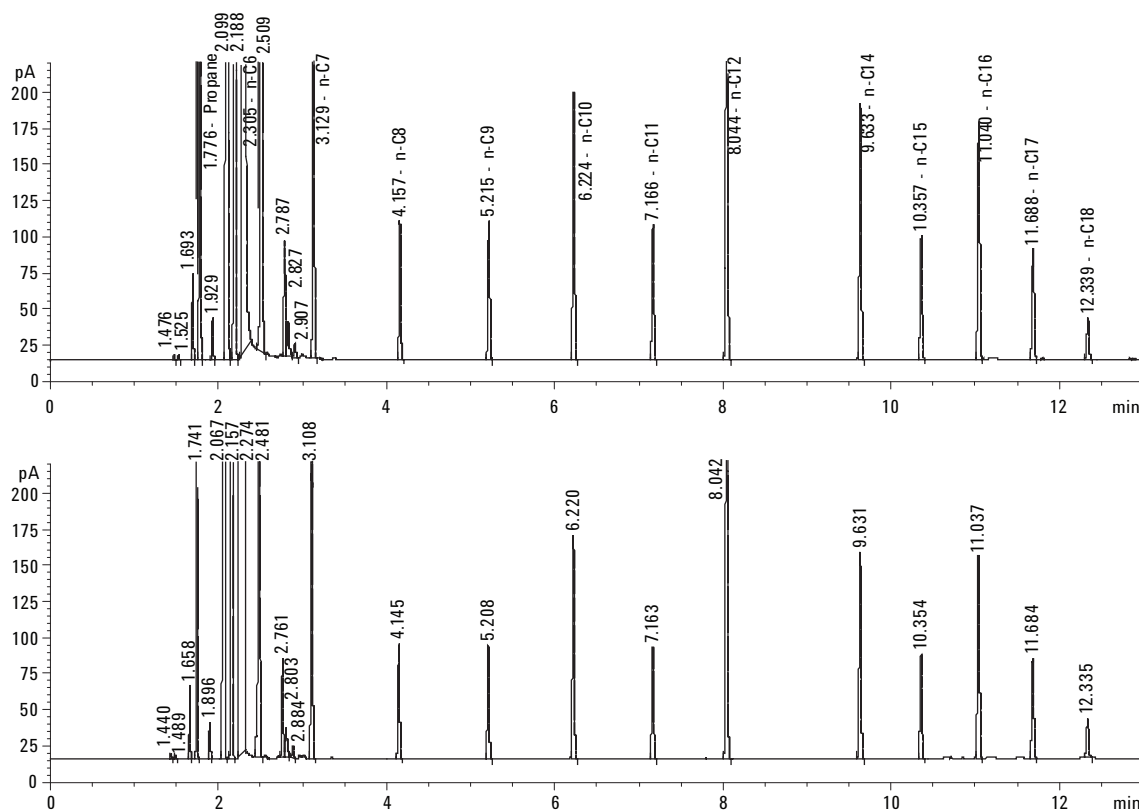


Figure 14. Chromatograms of n-hexane + 1.0% BP standard. Top: HPLI. Bottom: ALS (syringe).

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

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

The peak width of hexane at top: 0.0209 min

The peak width of hexane at bottom: 0.0195 min

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

G. nC5-nC40 (D2887 BP Standard Diluted by CS₂)

A sample with hydrocarbons (nC5-nC40 D2887 1# BP standard diluted by CS₂) is also run on HPLI. The chromatogram is shown in Figure 15.

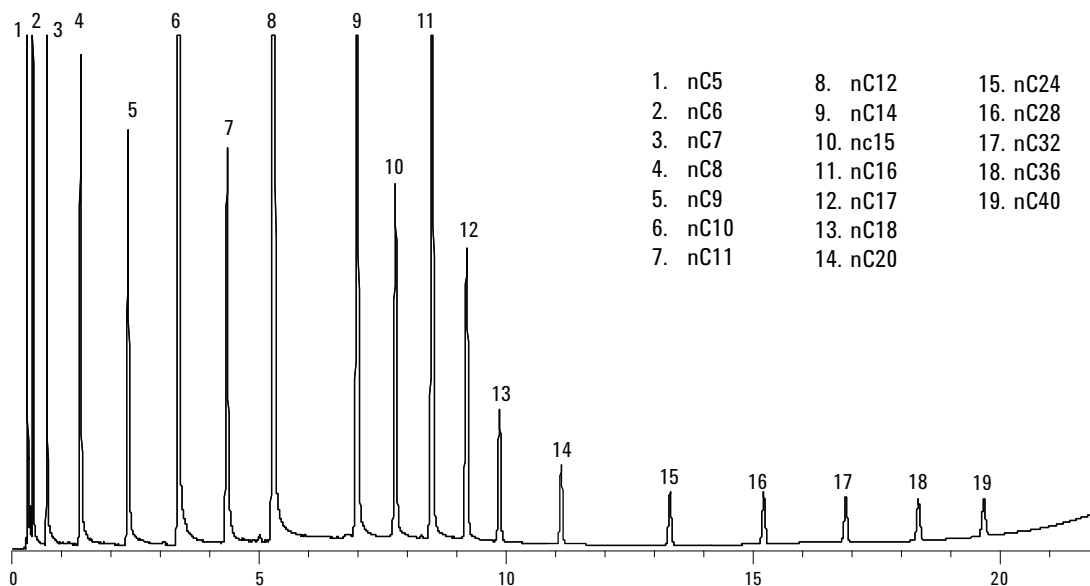


Figure 15. Chromatogram of nC5-nC40 (D2887 BP standard diluted by CS₂).

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

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

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

Conclusions

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

References

1. C. J. Cowper and A. J. DeRose, "The Analysis of Gases by Chromatography" (Pergamon Series in Analytical Chemistry, Vol. 7), Pergamon Press, Oxford, 1983, Ch. 6.
2. K. J. Rygle, G. P. Feulmer, and R. F. Scheideman, *J. Chromatogr. Sci.*, 22 (1984) 514–519.
3. Jim Luong, Ronda Gras, and Richard Tymko, *J. Chromatogr. Sci.*, 41 (2003) 550–5.

Acknowledgement

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

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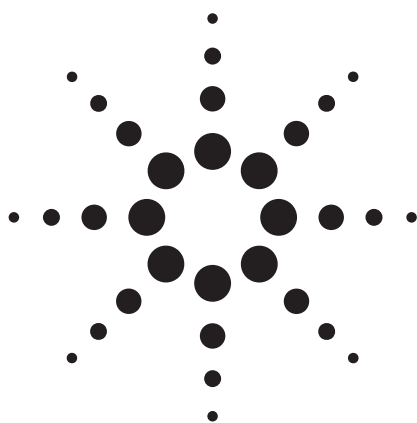
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High-Pressure Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs

Accessory G3505A

Introduction

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

Injection Device

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

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

Ordering Information

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

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

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

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or
G3470A Aux EPC module

6890GC

G1570A Aux EPC or
G2317A PCM module

The PCM is the preferred source for both GCs.



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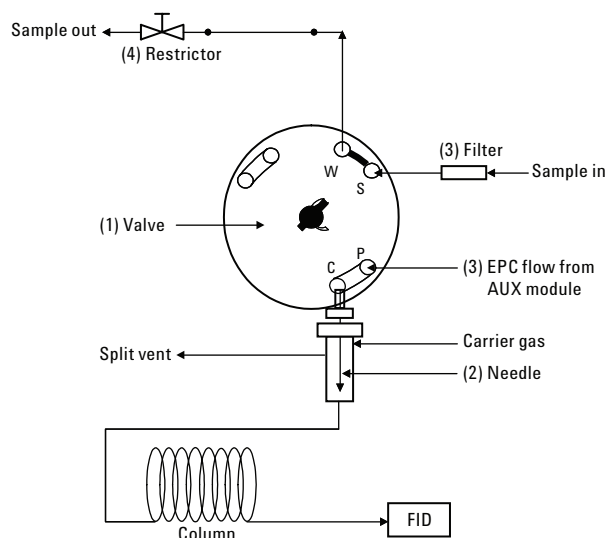


Figure 1. Flow diagram of the high-pressure injection device (HPLI).

Typical Instrumental Conditions

Gas chromatograph	Agilent 7890A
Injection source	High-pressure injection device (HPLI) at near ambient temperature
Injection port	Split/splitless, 250 °C (350 °C for C5–C40)
Sample size	0.06 µL
Carrier gas	Helium
Aux or PCM	150 mL/min (Helium)
FID	250 °C (350 °C for C5–C40) H ₂ , 35 mL/min Air, 400 mL/min



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

Sample Chromatograms

Pressurized Propylene

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

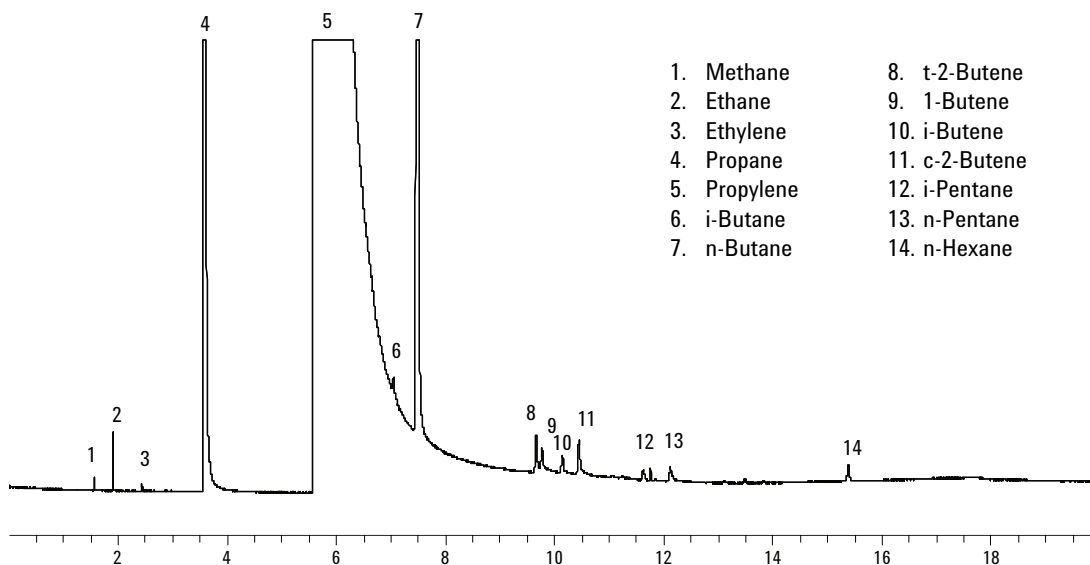


Figure 2. Chromatogram of pressurized propylene.

Pressurized 1,3-Butadiene

Figure 3 is an example of C4 hydrocarbons analysis showing 1,3-butadiene purity.

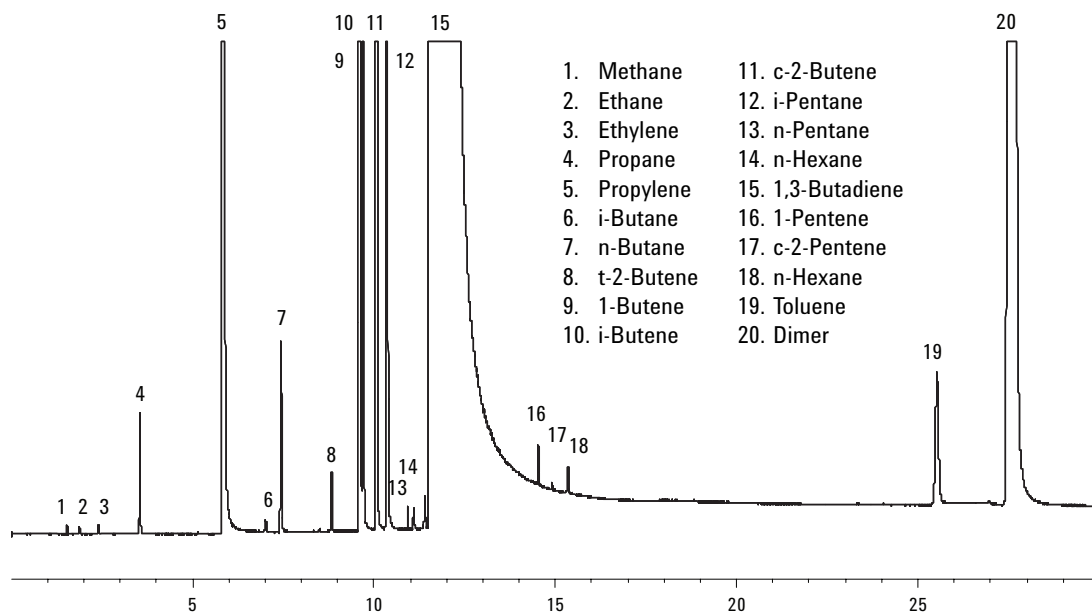


Figure 3. Chromatogram of pressurized 1,3-butadiene.

Summary

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

Competitive Advantages

The HPLI can be used with a wide variety of sample streams or pressurized vessels. Because the sampling valve is interfaced directly to the inlet with an inert needle, loss or adsorption of

analytes is minimized compared to conventional liquid sample valve systems. Compared to other gas chromatographic vaporizers for handling pressurized or nonpressurized samples, the Agilent HPLI has the following advantages:

- Better results with polar analytes such as glycols
- Superior inertness
- Low discrimination (no discrimination up to C₁₆)
- Flexibility: Install or uninstall in less than 10 minutes
- Good for trace impurity analysis with 0.5 µL rotor
- Excellent repeatability, typically RSDs below 1 %

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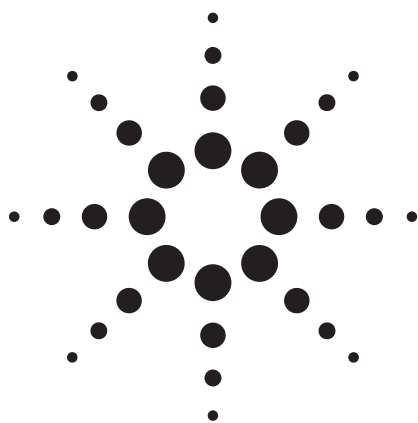
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Detection of Sulfur Compounds in Natural Gas According to ASTM D5504 with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) on the 7890A Gas Chromatograph

Application

Hydrocarbon Processing

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Abstract

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

Introduction

Many sources of natural gas and petroleum gases contain varying amounts and types of sulfur compounds. The analysis of gaseous sulfur compounds is difficult because they are polar, reactive, and present at trace levels. Sulfur compounds pose problems both in sampling and analysis. Analysis of sulfur compounds many times requires special treatment to sample pathways to ensure inertness

to the reactive sulfur species. Sampling must be done using containers proven to be nonreactive. Laboratory equipment must also be inert and well conditioned to ensure reliable results. Frequent calibration using stable standards is required in sulfur analysis [1].

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

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

Experimental

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



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

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

Table 1. Sulfur Standards in Helium

1.	Hydrogen sulfide	H ₂ S
2.	Carbonyl sulfide	COS
3.	Methyl mercaptan	CH ₃ SH
4.	Ethyl mercaptan	CH ₄ CH ₃ SH
5.	Dimethyl sulfide	CH ₃ SCH ₃
6.	Carbon disulfide	CS ₂
7.	2-propanethiol	CH ₃ SHC ₂ H ₅
8.	Tert-butyl mercaptan	(CH ₃) ₃ CSH
9.	1-propanethiol	CH ₃ (CH ₂) ₂ SH
10.	Thiophene	C ₄ H ₄ S
11.	n-butanethiol	CH ₃ (CH ₂) ₃ SH
12.	Diethyl sulfide	CH ₃ CH ₂ SCH ₂ CH ₃
13.	Methyl ethyl sulfide	CH ₃ SCH ₂ CH ₃
14.	2-methyl-1-propanethiol	(CH ₃) ₂ CHCH ₂ SH
15.	1-methyl-1-propanethiol	CH ₃ CH ₂ CHSHCH ₃

Experimental Conditions

GC Conditions

Front Inlet Split/splitless (Sulfinert-treated capillary inlet system)

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

SCD Conditions

Burner temperature	800 °C
Vacuum of burner	372 torr
Vacuum of reaction cell	5 torr
H ₂	40 mL/min
Air	53 mL/min

Results and Discussion

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

Figure 2 is the chromatogram of low-level sulfur compounds at 1.35 ppb (H₂S), which is prepared by the point-of-use gas blending system. Table 2 is the calculated signal to noise (S/N) of each compound, from the achieved data. It can be seen that DP SCD can detect low-level sulfur compounds.

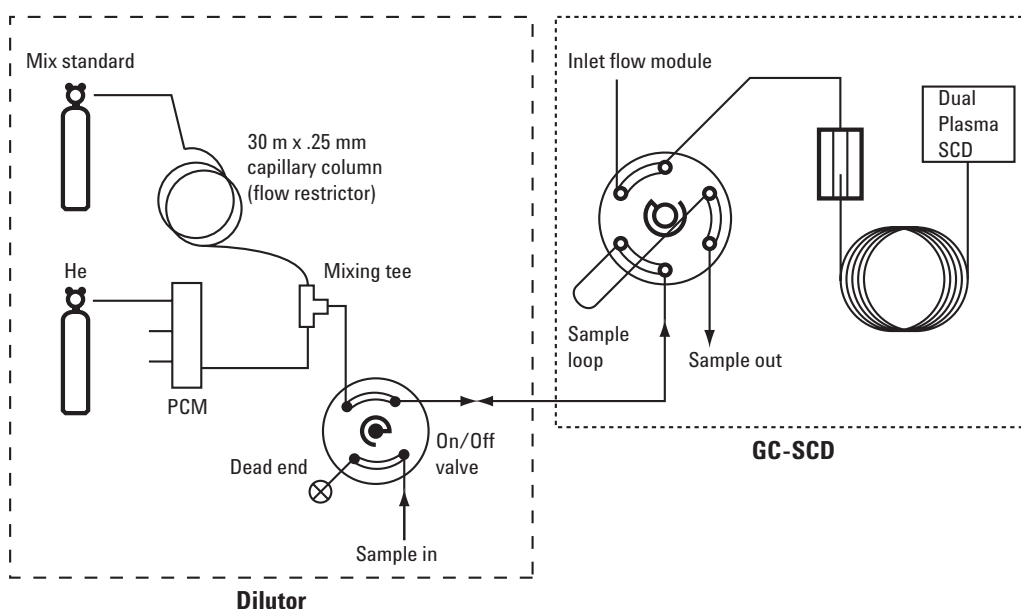
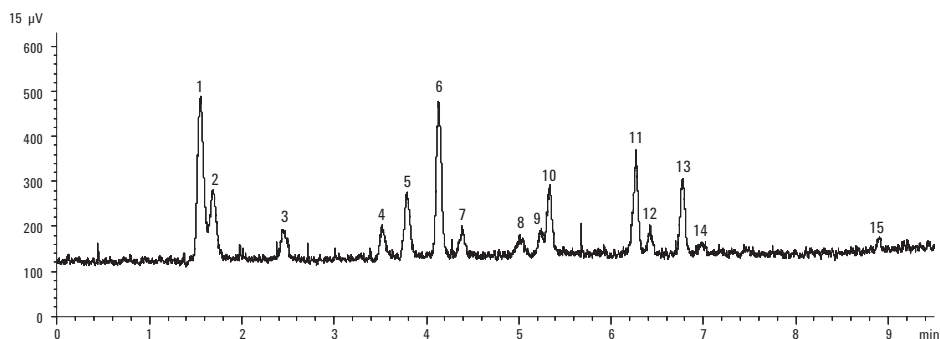


Figure 1. Diagram of online dilutor GC-DP SCD.

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

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

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

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

injection mode, the valve is switched to the off position, the pressure in the sample loop balances to ambient pressure, and then the sample is injected into the GC.

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

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

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

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

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

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

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

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

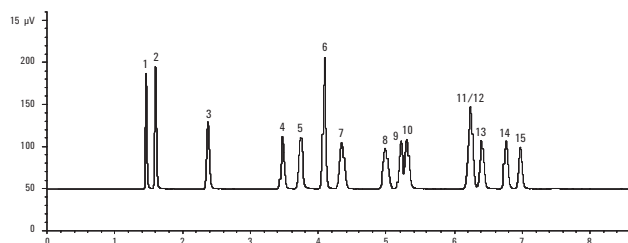


Figure 3. Chromatogram of sulfurs in natural gas. (Refer to Table 1 for peak identification.)

Natural Gas Sample Analysis

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

Table 5. Result of the Three Real Samples

Samples		H ₂ S	COS	Methyl Mercaptan
BLEND AL	Conc. (ppm, v/v)	2.3	2.0	2.0
	RSD (% , n = 5)	2.3	0.3	1.4
BLEND 6	Conc. (ppm, v/v)	27.1	21.9	17.3
	RSD (% , n = 5)	1.2	0.4	2.3
BLEND 12	Conc. (ppm, v/v)	15.0	9.2	10.1
	RSD (% , n = 5)	0.7	0.6	0.6
Standard natural gas	Conc. (ppm, v/v)	2.0	0.8	0.9
	RSD (% , n = 5)	1.7	2.5	1.7

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Conclusions

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

References

1. ASTM D5504-98: Standard test method for determination of sulfur compounds in natural gas and gaseous fuels by gas chromatography and chemiluminescence
2. Roger L. Firor, "Volatile Sulfur in Natural Gas, Refinery Gas, and Liquefied Petroleum Gas," Agilent Technologies publication 5988-2791EN
3. Roger L. Firor and Bruce D. Quimby, "A Comparison of Sulfur Selective Detectors for Low-Level Analysis in Gaseous Streams," Agilent Technologies publication 5988-2426EN

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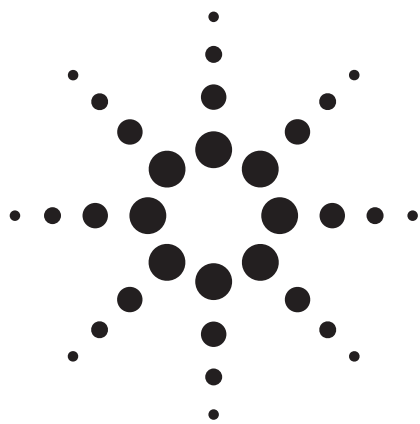
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Agilent Technologies



Permanent Gases on a COX Module Using a Agilent 490 Micro GC

Application Note

Micro Gas Chromatography

Authors

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Abstract

This application note demonstrates the capabilities of the COX column with the Agilent 490 Micro GC, including separation of permanent gases and backflush possibilities to ensure extended column lifetimes.

Introduction

Separation of permanent gases is usually performed on a Molsieve column. This column offers the best separation for all permanent gases but also has some severe drawbacks. Water and carbon dioxide do not elute from a Molsieve column under regular GC conditions. A bake out at high temperatures (250 – 300 °C) is needed to fully regenerate the column. Regeneration is very time consuming in a Micro GC usually taking overnight or longer because the maximum temperature is 180 °C. In addition, it is likely that regeneration from moisture does not occur at this temperature.

If there is no need to separate oxygen and nitrogen, the COX column is a better alternative. It delivers good separation of permanent gases, and carbon dioxide elutes from the column. COX is an ideal alternative for a Molsieve column, offering prolonged lifetime and instrument uptime.



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Experimental

Instrumentation

An Agilent 490 Micro GC system with a COX column module was used for these experiments. The COX column module was equipped with a heated injector and an optional precolumn with backflush.

Conditions

Column temperature	100 °C
Carrier gas	Argon, 100 kPa
Backflush to vent time	13 s
Injection time	80 ms
Injection temperature	110 °C
Sample line temperature	100 °C
Sampling time	30 s
Stabilization time	5 s
Run time	200 s

Sample Information

Standard gas samples were used. Concentrations were in % levels.

Table 1. Repeatability Figures Per Component on Peak Area

Run	He	H ₂	N ₂	CO	CH ₄	CO ₂
1	943213	16024030	20593423	1439534	1535598	1064007
2	947355	16092042	20685887	1444814	1538714	1062243
3	949818	16142635	20749728	1446996	1544418	1070193
4	949808	16167426	20781405	1449939	1542239	1066091
5	952725	16194789	20815739	1453498	1539162	1066940
6	952107	16206479	20826967	1456289	1543749	1063772
7	954648	16228802	20856620	1455219	1548126	1074325
8	954635	16249294	20879589	1456795	1547760	1079645
9	955454	16251565	20883920	1456611	1552320	1064839
10	955872	16250493	20901246	1473831	1547242	1065483
Average	951563.5	16180756	20797452	1453353	1543933	1067754
St. Dev	4053	75870	97930	9249	5122	5456
RSD%	0.43	0.47	0.47	0.64	0.33	0.51

Results and Discussion

The above settings produce the chromatogram shown in Figure 1, with repeatability data in Table 1.

The chromatogram shows a baseline separation of helium and hydrogen. Oxygen and nitrogen eluted as a single peak but separate from carbon monoxide and methane. Carbon dioxide eluted perfectly.

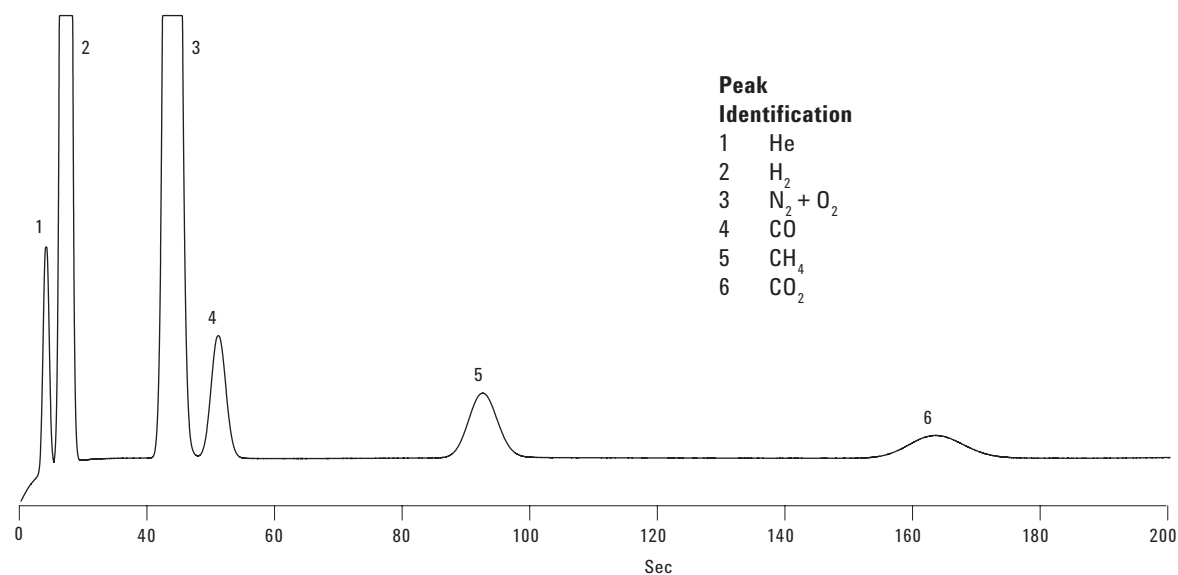


Figure 1. Excellent baseline separation of a gas sample on a COX column.

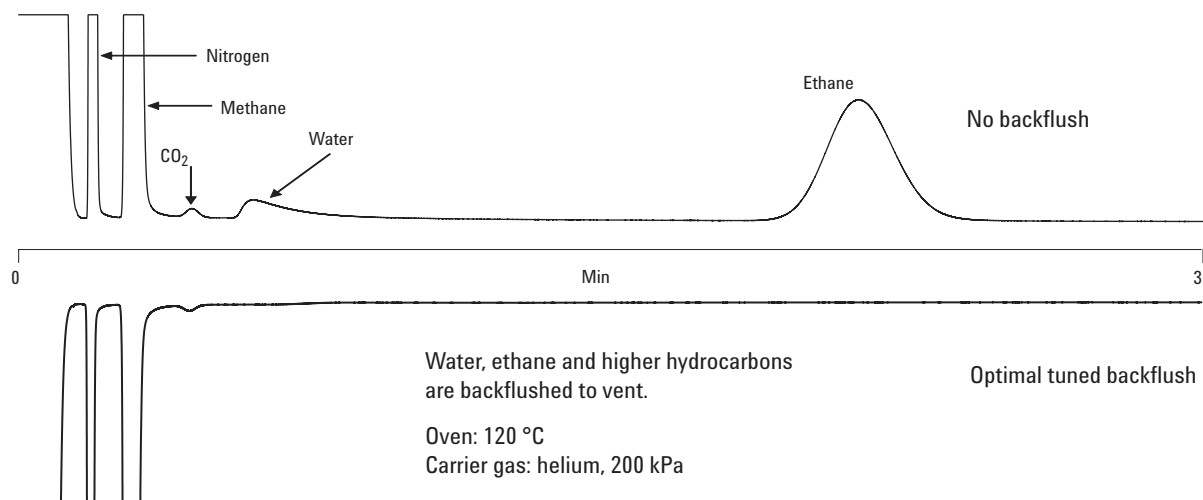


Figure 2. Backflush of water and ethane.

Other components such as water and higher hydrocarbons were backflushed to vent.

If the backflush time is set at a high value then virtually all the sample components enter the analytical column and eventually elute. However, if higher hydrocarbons are present the COX column is polluted because these components elute late and can influence the succeeding analysis.

Figure 2 shows the elution of water and ethane if no backflush is applied. If the backflush time is optimally tuned, water, ethane and higher hydrocarbons are backflushed to vent and does not enter the analytical column.

Conclusion

For the analysis of permanent gases the COX column is a good alternative to the commonly used Molsieve column.

Although the COX column does not separate oxygen and nitrogen, it does separate hydrogen and helium. In addition, carbon dioxide is analyzed and water elutes from the COX column. Repeatability figures are good, ensuring reliable analysis results.

The COX module can be equipped with a precolumn. This allows backflush of higher components and prolongs column lifetime.

The Agilent 490 Micro GC is a rugged, compact and portable “lab-quality” gas analysis platform. When the composition of gas mixtures is critical, this fifth generation Micro Gas Chromatograph generates more data in less time for faster and better performance.

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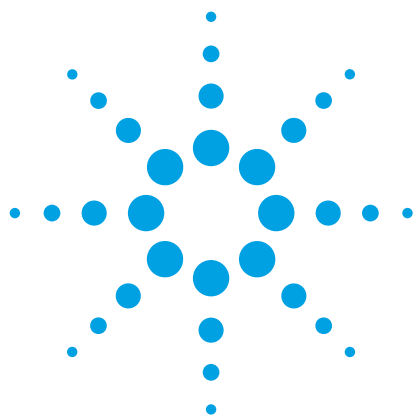
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Analysis of tert-Butyl Mercaptan in Natural Gas on a CP-Sil 13 CB Using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Hydrocarbon processing, Natural Gas Analysis

Author

Remko van Loon
Agilent Technologies Inc.
Middelburg, The Netherlands



Introduction

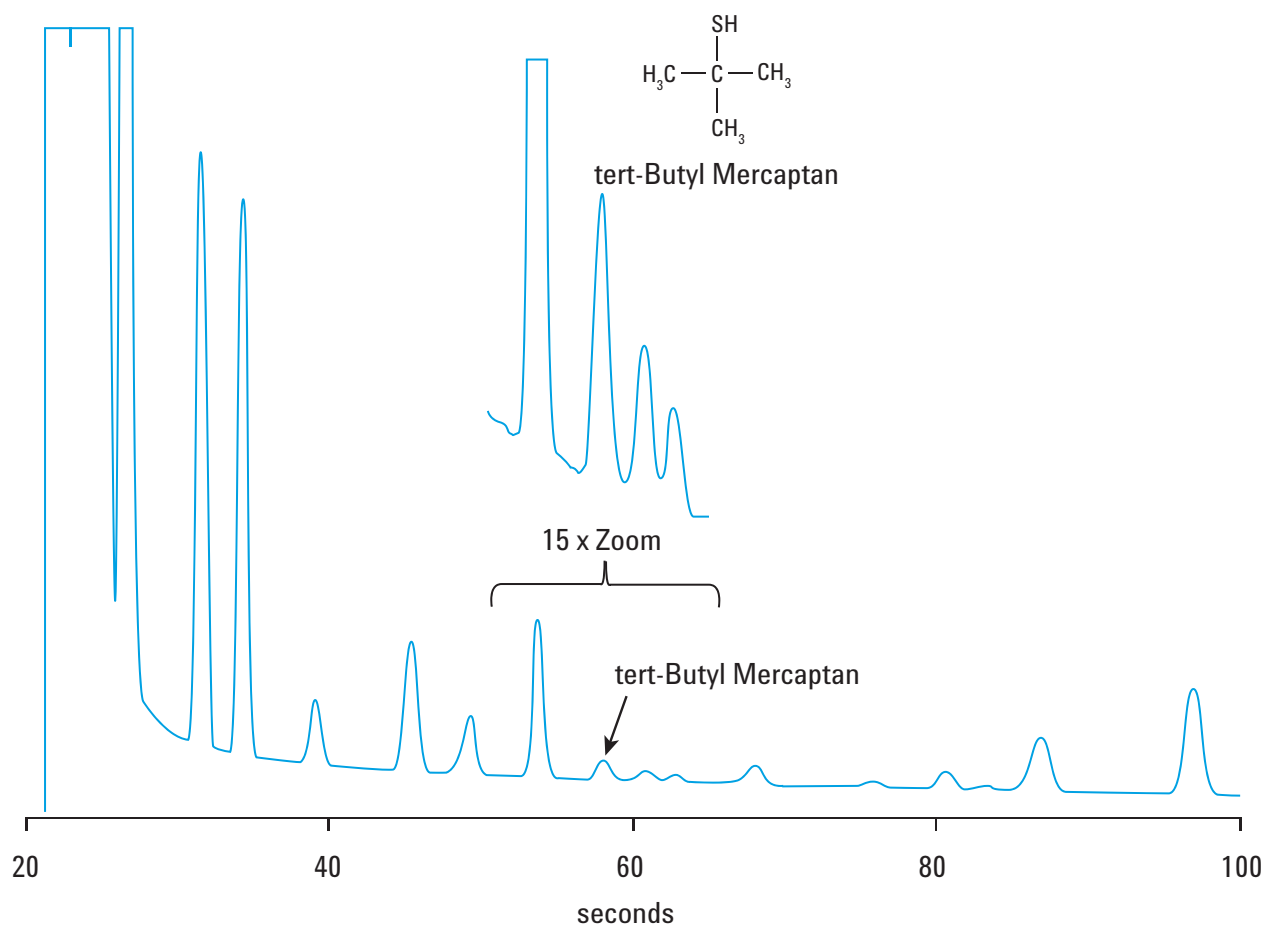
This application note shows the analysis of tertiary-butyl mercaptan (TBM) in a natural gas matrix using the Agilent 490 Micro GC. The dimensions and instrument conditions for the column channel used in this application note, a CP-Sil 13 CB, clearly shows the separation of TBM from the other compounds in the natural gas sample.

The advantage of the Agilent 490 Micro GC, in combination with the CP-Sil 13 CB column channel, is the ease of use and the speed of analysis. Tertiary-butyl mercaptan elutes just before 60 seconds and the total analysis time is only 100 seconds.

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 chromatography.



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Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	CP-Sil 13 CB for TBM
Column temperature	40°C
Carrier gas	Helium, 250 kPa
Injection time	255 msec

Sample information

Natural gas	Matrix
Tert-butyl mercaptan (TBM)	4 ppm

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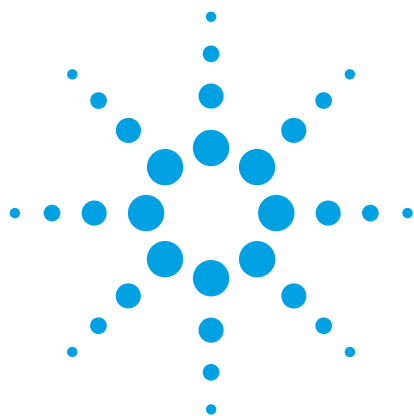
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Analysis of Tetrahydrothiophene (THT) in Natural Gas using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Natural Gas Analysis, Sulfur Compound Analysis

Author

Remko van Loon
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Middelburg
The Netherlands



Introduction

This application note shows the analysis of Tetrahydrothiophene (THT) in a Natural Gas matrix using the Agilent 490 Micro GC. THT consists of a five-membered ring containing a sulfur atom and four carbon atoms. THT is used as an odorant in Natural Gas, because of its smell.

The chromatogram clearly shows the separation of THT from the other compounds in the Natural Gas sample. The dimensions for the column channel used, a CP-Sil 19 CB for THT, are optimized for this application. Moreover, this column channel is factory tested to ensure the separation between THT and Nonane.

The advantage of the Agilent 490 Micro GC, in combination with the CP-Sil 19 CB column channel, is the ease-of-use and the speed of analysis. Tetrahydrothiophene elutes around 40 sec and the total analysis time is less than 90 sec.

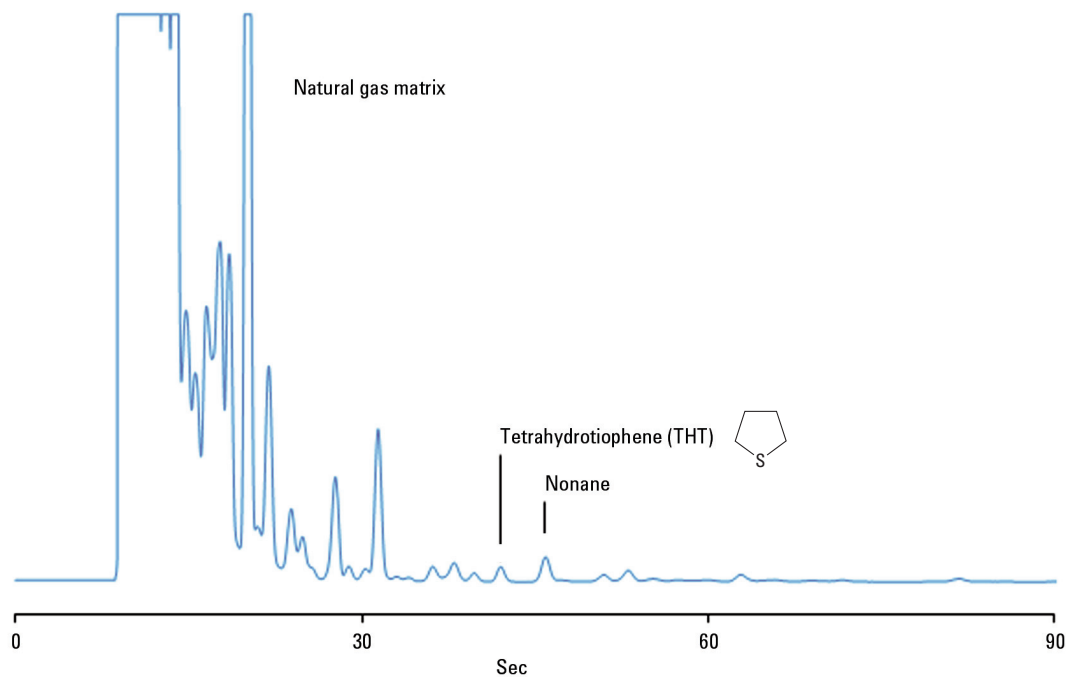
The Agilent 490 Micro GC is a rugged, compact, and portable lab-quality gas analysis platform. When the composition of gas mixtures is critical, count on this fifth generation micro gas chromatography.



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Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	CP-Sil 19 CB for THT
Column temperature	90 °C
Carrier gas	Helium, 200 kPa
Injection time	255 msec
Injector temperature	110 °C
Sampling time	30 sec



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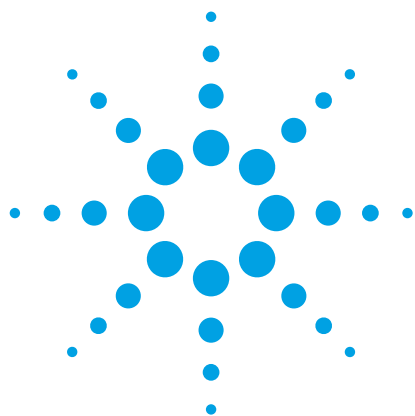
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Agilent Technologies



Analysis of Methyl Ethyl Sulfide (MES) in Natural Gas using the Agilent 490 Micro GC

Authors

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Application Note

Micro Gas Chromatography, Natural Gas Analysis, Sulphur Compound Analysis



Introduction

This application note shows the analysis of Methyl Ethyl Sulfide (MES) in natural gas using the Agilent 490 Micro GC. Methyl Ethyl Sulfide is an organosulfur compound with a characteristic odor, and therefore used in some countries as an odorant for natural gas.

This Micro GC column channel is equipped with a special dedicated column, MES in natural gas, for the separation of MES from the other compounds in natural gas. Moreover, this column channel is factory tested to ensure the separation between n-Decane and MES.

If you want the ability to measure anywhere and get the results you need in seconds, the Agilent 490 Micro GC is the ideal solution. With its rugged, compact, laboratory quality gas analysis platform, the 490 Micro GC generates more data in less time for faster, and better, business decisions.



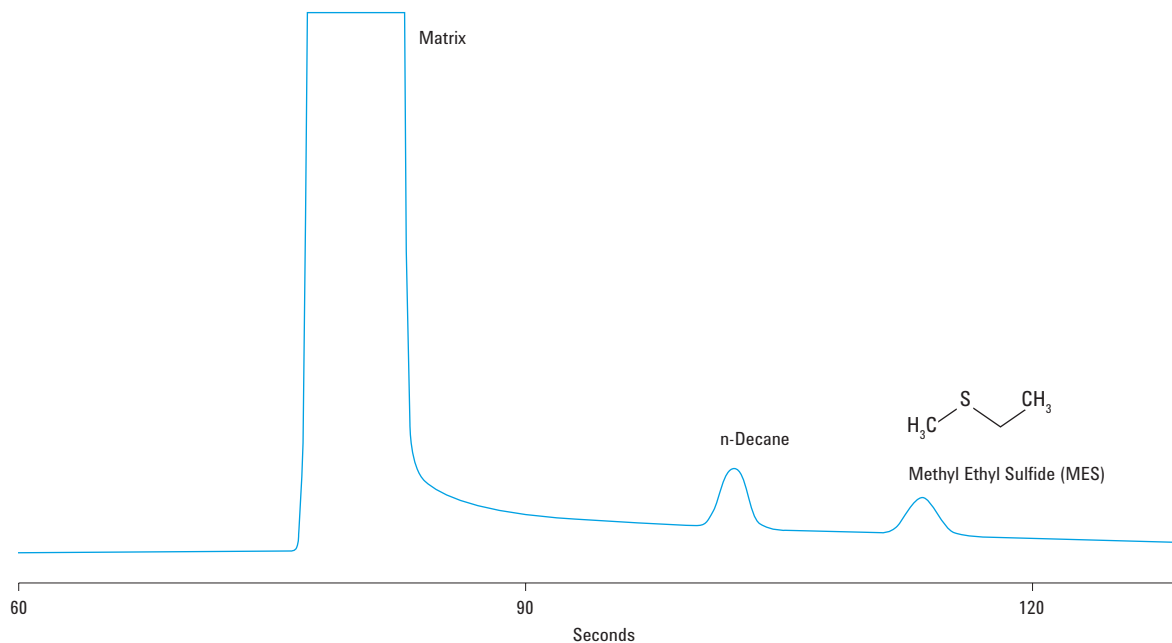
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Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	MES in natural gas
Column temperature	90 °C
Carrier gas	Helium, 70 kPa
Injection time	255 msec
Injector temperature	110 °C
Sampling time	30 sec

Sample information

n-Decane	11 ppm
Methyl Ethyl Sulfide (MES)	14 ppm



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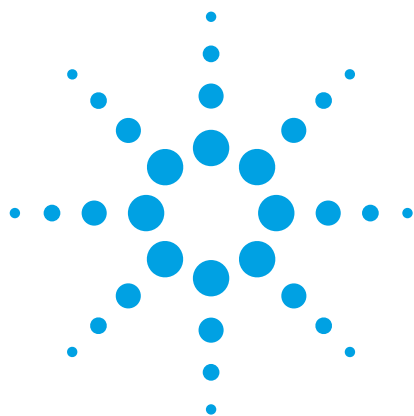
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C1 – C3 Hydrocarbon Analysis Using the Agilent 490 Micro GC – Separation Characteristics for PoraPLOT U and PoraPLOT Q Column Channels

Application Note

Micro Gas Chromatography, Hydrocarbon analysis

Author

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Introduction

This application note shows the possibilities and limitations in fast analysis of saturated and unsaturated C1 to C3 hydrocarbons using an Agilent 490 Micro GC. The chromatograms and results outline the similarities and differences when using a PoraPLOT U and a PoraPLOT Q column channels. Both the PoraPLOT U and the PoraPLOT Q are capable of resolving methane from the composite air peak and separate CO₂ from methane and the C2 hydrocarbons.

The PoraPLOT U column channel will have the following separation characteristics:

- Baseline separation for ethane, ethylene and acetylene
- Coelution of propane and propylene

The separation characteristics for the PoraPLOT Q column channel are:

- Coelution of ethylene and acetylene
- Baseline separation for propane and propylene



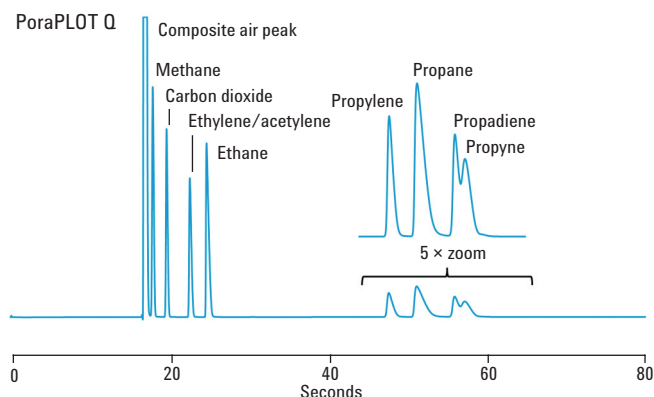
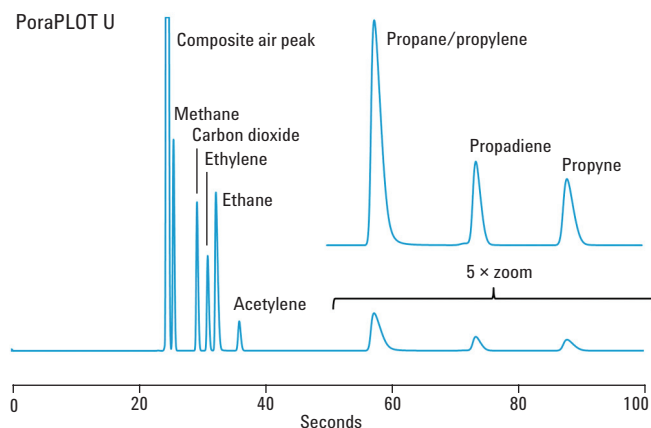
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If you want the ability to measure anywhere and get the results you need in seconds, the Agilent 490 Micro GC is the ideal solution. With its rugged, compact, laboratory quality gas analysis platform, the 490 Micro GC generates more data in less time for faster, and better, business decisions.

Instrumentation

For this application an Agilent 490 Micro GC (G3581A) equipped with a PoraPLOT U and a PoraPLOT Q was used. The setup parameters for the column is found in the table below.

	PoraPLOT U, 10 m	PoraPLOT Q, 10 m
Column temperature	80 °C	80 °C
Carrier gas	Helium, 200 kpa	Helium, 200 kpa
Injector temperature	110 °C	110 °C
Injection time	20 ms	20 ms



Sample information

Nitrogen	Balance
Methane	5.0 %
Carbon dioxide	3.0 %
Ethylene	2.0 %
Ethane	4.0 %
Acetylene	1.0 %
Propylene	1.0 %
Propane	2.0 %
1,2-Propadiene	0.97 %
Propyne	0.99 %

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Detailed Separation of C_1 - C_5 Light Hydrocarbons on CP- Al_2O_3 / Na_2SO_4 PLOT GC Column

Application Note

Author

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Introduction

Aluminum oxide PLOT columns are specifically designed for detailed C_1 - C_{10} hydrocarbon analysis in chemical and petrochemical industries. Due to the high selectivity of CP- Al_2O_3 PLOT columns, it is possible to analyze ppm to percent levels of any C_1 - C_5 impurities, including isomers, in main stream C_1 - C_5 products. CP- Al_2O_3 PLOT columns offer a higher level of analytical selectivity and efficiency compared to super-thick film non-polar liquid stationary phase columns.



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The aluminum oxide column carries a sodium sulfate (Na_2SO_4) deactivation layer. The deactivation provides a reproducible and stable selectivity up to 200 °C. Sodium sulfate deactivation results in a more polar surface than potassium chloride (KCl)-treated alumina, retaining unsaturated compounds such as ethylene, acetylene (ethyne) and methyl acetylene (propyne) more strongly than their unsaturated peers.

The analysis of light hydrocarbons is performed in refinery gas, liquefied petroleum gas (LPG) and natural gas. Refinery gas is a mixture of gases generated during refinery processes used to process crude oil into various petroleum products as intermediate products or high grade end-products. The composition of refinery gas may vary. Common components include butanes, butenes (butylenes), methane, ethane and ethene (ethylene). The aluminum PLOT column offers added value in separating all of the components.

Natural gas consists of methane, light hydrocarbons such as ethane, propane and butane, and small quantities of derivatives such as carbon dioxide and nitrogen. The precise composition of natural gas may differ from region to region. LPG is a mixture of light hydrocarbons. It occurs naturally in crude oil and natural gas production fields and is also produced in the oil refining process. The main component gases of LPG are propane and butane.

The $\text{CP-Al}_2\text{O}_3/\text{Na}_2\text{SO}_4$ GC column provides separation of the main components and gives detailed quantitative data on the impurities.

This application note shows the analysis of 18 light hydrocarbons on a $\text{CP-Al}_2\text{O}_3/\text{Na}_2\text{SO}_4$ GC column.

Materials and Methods

Technique: GC-FID
 Column: $\text{CP-Al}_2\text{O}_3/\text{Na}_2\text{SO}_4$, 50 m x 0.32 mm, df=5 µm (part number CP7565)
 Temperature: 70 °C, 3 °C/min, 170 °C
 Carrier Gas: Hydrogen, constant pressure, 100 kPa (1.0 bar, 14.5 psi)
 Injection: 250 °C, split 1:50
 Detection: FID, 275 °C
 Sample: Gas mixture, for concentrations see Table 1
 Injection Volume: 5 µL

Results and Discussion

Figure 1 shows the chromatogram of the detailed analysis of 18 hydrocarbons within 20 minutes. The $\text{CP-Al}_2\text{O}_3/\text{Na}_2\text{SO}_4$ column provided very good peak shape and baseline separation. The alkynes, acetylene and propyne, show some tailing. This tailing is typical for Na_2SO_4 deactivated Al_2O_3 PLOT and is caused by the higher interaction of the polar alkynes with the polar Na_2SO_4 deactivation layer.

Table 1. Peak Identification

Peak	Compound	Concentration % (moles in He)
1	Methane	24.9
2	Ethane	5.0
3	Ethene (ethylene)	24.9
4	Propane	5.0
5	Cyclopropane	0.50
6	Propene (propylene)	5.1
7	Isobutane	0.50
8	n-Butane	1.00
9	Propadiene	0.60
10	Ethyne (acetylene)	1.01
11	<i>trans</i> -2-Butene	0.50
12	1-Butene	0.50
13	Isobutene	1.00
14	<i>cis</i> -2-Butene	0.50
15	Isopentane	0.50
16	n-Pentane	0.199
17	1,3-Butadiene	1.00
18	Propyne (methyl acetylene)	1.01

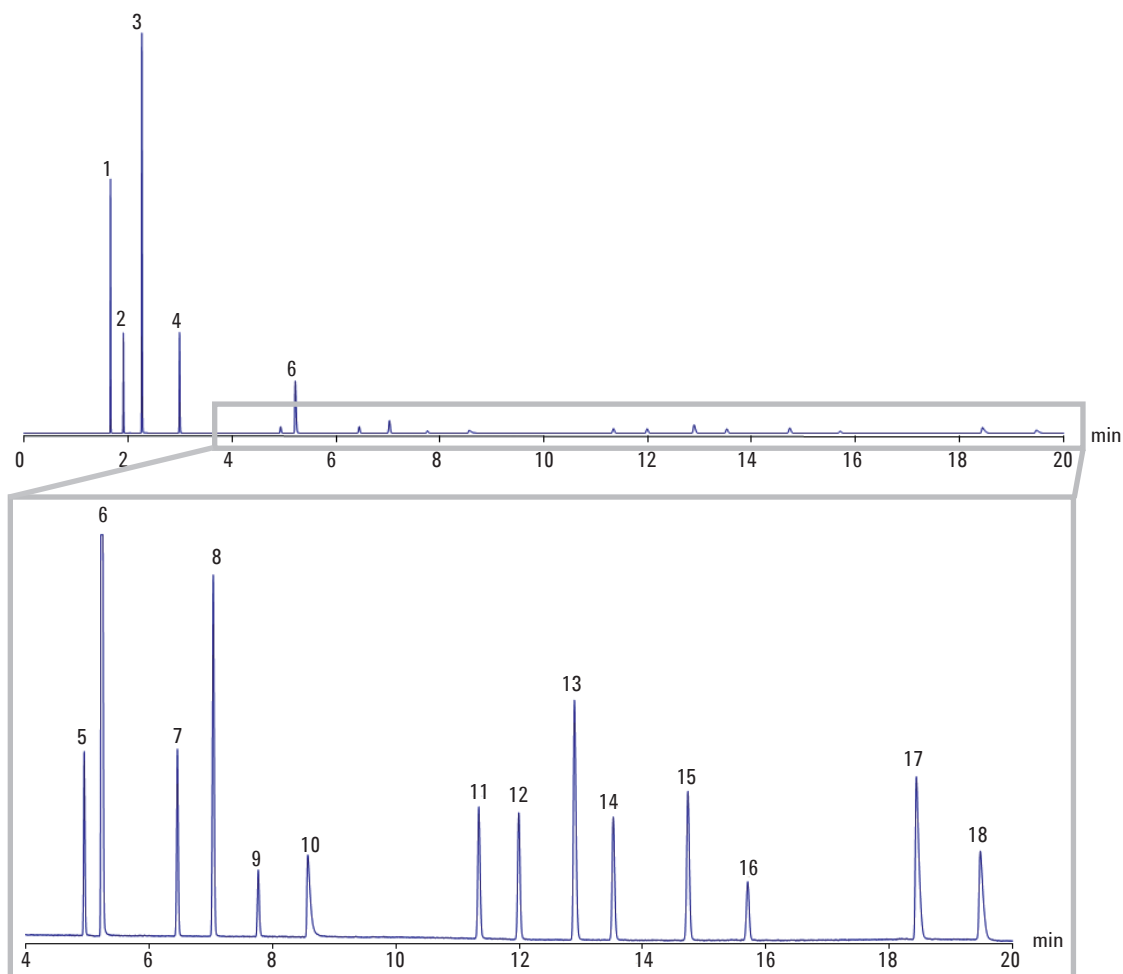


Figure 1. C_1 - C_5 hydrocarbons analyzed on a $CP-Al_2O_3/Na_2SO_4$ column

Conclusion

The $CP-Al_2O_3/Na_2SO_4$ column is very suitable for the analysis of light hydrocarbons. The sodium sulfate deactivation provides additional resolution for separating all C_4 isomers.

The robustness of the column allows temperatures up to 200 °C to be used, enabling bake-out of the column at the end of the analysis without changes in selectivity.

References

<http://www.gasterra.com/naturalgas/Pages/Whatisnaturalgas.aspx>
<http://www.wisegEEK.com/what-is-refinery-gas.htm>
<http://www.environment.gov.au/atmosphere/fuelquality/standards/lpg/faq.html#lpg>

www.agilent.com/chem

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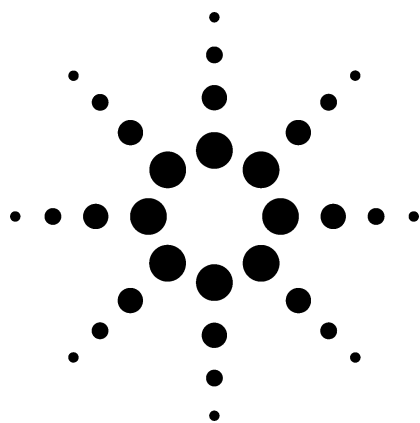
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A Unified Gas Chromatography Method for Aromatic Solvent Analysis

Application

Gas Chromatography

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Abstract

A single, easy-to-use GC method for aromatic solvent purity analysis is described that meets the chromatographic requirements of ten separate ASTM methods. This method can be used to obtain identical results on both the Agilent 6890 and 6850 Series Gas Chromatographs designed for the method development lab and the routine production lab respectively. Reproducibility of results between instruments, between labs, and over time are further improved by applying the technique of retention time locking to this unified method.

Introduction

The producers and users of many aromatic hydrocarbons evaluate the product quality by measuring the purity of the material along with specific contaminants. For these types of measurements the most commonly used analysis technique is gas chromatography (GC). In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and

published a number of GC methods specific to an aromatic compound or class of compounds.¹ These methods have evolved over time to meet the requirements of new materials specifications or to incorporate new GC technologies (i.e. capillary columns replacing packed columns). The result of this evolution is a large number of methods that are remarkably alike. In practice, many QA laboratories that support a variety of chemical processes typically devote one GC instrument to each ASTM method they must run.

Recently, there has been a move by many chemical companies to consolidate lab facilities, simplify measurements, and reduce the costs that chemical measurements add to production. Laboratory space is expensive and is becoming limited. Where three or four GCs were operating in the past, there is now only space and budget for one or two. Another part of this trend is to have non-traditional personnel such as plant operators; technicians and engineers perform chemical analyses. Since these personnel are not trained as analytical chemists, simpler methods are needed to perform the analyses without losing measurement performance.

Accommodating these changes in the lab environment makes it necessary to explore alternative approaches to performing GC analyses. One approach is to develop a method that combines the elements of several separate ASTM methods.



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A single method has a number of advantages over multiple methods. Fewer GCs could be used in place of a larger number of instruments previously dedicated to individual methods; thus reducing required lab space. By running one method, any GC could also serve as a backup for instruments that are undergoing maintenance or repair. This would result in shorter down times and better utilization of lab space. A single method would also eliminate the need to stock multiple columns and supplies. Plant operators would also find it easier to use since they would only need to be trained once on a single procedure.

Another important advantage to a single aromatics method lies in the use of retention time locking (RTL). RTL is a technique that allows any Agilent 6890 or 6850 GC systems running the same method to obtain nearly identical retention times. Comparing data between instruments, between laboratories, or over time can be difficult due to variations in retention times. This is further complicated when using multiple methods since the

different columns and operating conditions result in different retention times for the same compound. For instance, there are eight ASTM methods that measure p-xylene; however, p-xylene retention times range from 6 to 16 minutes depending on the method's operating conditions (column, flow, temperature). By using one method for all aromatic samples, retention time variations can be reduced to less than 0.5 minutes. Then by applying RTL to this method, system-to-system retention time variations can be further reduced to less than 0.03 minutes. Retention time precision on this order greatly simplifies comparison of data between systems, between laboratories, and over time.

This application note describes a GC method that is chromatographically suitable for a wide range of samples typically analyzed by ten different ASTM methods. Table 1 lists these ten methods along with the ASTM recommended columns and reporting specifications.

Table 1. Ten ASTM Methods for the GC Analysis of Aromatic Solvents

ASTM Method	Title	Liquid phase	Column type	Report specifications
D2306	Std Test for C8 Aromatic Hydrocarbons	0.25 µm Carbowax	Capillary 50 m × 0.25 mm	wt% of individual C8 HC
D2360	Std Test for Trace Impurities in Monocyclic Hydrocarbons	0.32 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual aromatic impurities, total impurities, purity
D3760	Std Test for Cumene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, cumene purity (wt%)
D3797	Std Test for o-Xylene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, o-xylene purity (wt%)
D3798	Std Test for p-Xylene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, total impurities, p-xylene purity (wt%)
D4492	Std Test for Benzene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, benzene purity(wt%)
D4534	Std Test for Benzene in Cyclic Products	10%TCEPE on Chromasorb P	Packed 3.7 m × 3.175 mm	wt% of benzene
D5060	Std Test for Impurities in Ethylbenzene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, ethylbenzene purity
D5135	Std Test for Styrene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, styrene purity
D5917	Std Test for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)	0.25 µm Carbowax	Capillary 60 m × 0.32 mm	wt% individual impurities, wt% total non-aromatics, wt% total C9 aromatics, purity of main component

Experimental

Two Agilent 6890 Plus Series gas chromatographs and four Agilent 6850 gas chromatographs were used for this work. Each GC was equipped with a split/splitless capillary inlet, a flame ionization detector (FID) and an Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with high-pressure Merlin Microseal Septa (Agilent Part no. 5182-3442) and split-optimized liners (Agilent Part no. 5183-4647). Injections were made using 10 μ L gas-tight syringes (Agilent Part no. 5181-8809) designed for use with the Merlin Microseal. Table 2 lists the instrument conditions used for this method. An Agilent Chemstation was used for all instrument control, data acquisition and data analysis.

Table 2. Conditions for Unified Aromatic Solvents Method

Column	HP-Innowax, 60 m $\times$ 0.32 mm $\times$ 0.5 μ m Agilent Part no.19091N-216
Carrier Gas	Helium @ 20.00 psi constant pressure mode
Inlet	Split/Splitless @ 250 $^{\circ}$ C 100:1 to 400:1 split ratio
Oven Temp	75 $^{\circ}$ C (10 min); 3 $^{\circ}$ C/min to 100 $^{\circ}$ C (0 min) 10 $^{\circ}$ C/min to 145 $^{\circ}$ C (0 min)
Detector	FID @ 250 $^{\circ}$ C Data acquisition rate @ 20 Hz
Injection Size	0.1 to 1.0 μ L

An n-hexane solution was prepared containing 0.1 wt% of all the aromatic solvents and impurities specified for analysis by the ten ASTM methods listed in Table 1. This standard was used to develop the RTL calibration and to assess the separation of each compound. Final evaluation of this unified method was done by running the recommended standards specified in each of the ten ASTM methods.

Results and Discussion

Figure 1 shows a chromatogram of the hexane solution containing an aggregate of aromatic solvents and impurities. For most compounds, baseline resolution was achieved. There are two pairs that are only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, are also not resolved in the original ASTM method (D-5060 Impurities in ethylbenzene) and, along with o-ethyltoluene, are reported as total ethyltoluene. Therefore, the results presented here represent the same result obtained with the original ASTM method. A second pair, diethylbenzene and n-butylbenzene are also only partially resolved. Again, this does not present a problem since these two components are not typically found together in the same material. Diethylbenzene is sometimes found as a contaminant in ethylbenzene (ASTM D-5060) while n-butylbenzene is used as the internal standard for cumene analysis (ASTM D3760).

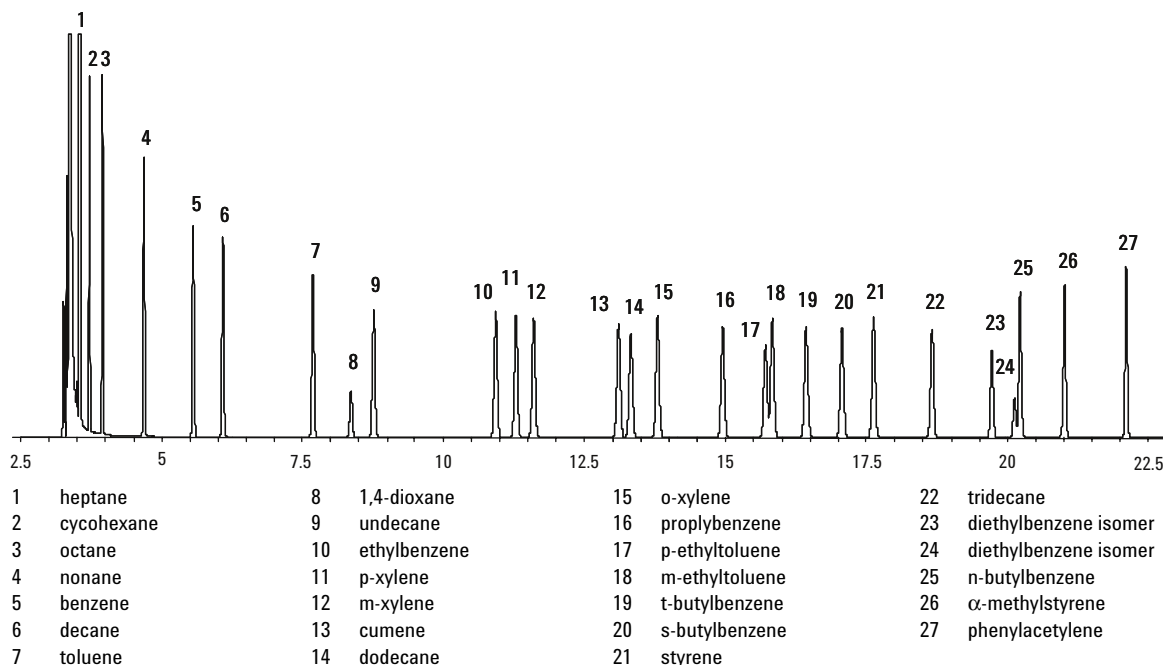


Figure 1. Separation of the 27 compounds analyzed by the ten ASTM aromatics methods listed in Table 1.

Retention Time Locking (RTL)

Retention time locking calibration was performed using t-butylbenzene as the target peak. Figure 2 shows the five RTL calibration runs with the retention times of t-butylbenzene indicated and Figure 3 shows the RTL calibration. These calibration runs do not have to be repeated by anyone wishing to lock this method on their Agilent 6890 or 6850 GC

systems. To use this RTL calibration, simply create a new method with conditions outlined in Table 2, then use the Chemstation RTL software to create a new RTL calibration and enter the data shown in Figure 3. The GC can then be locked by running a sample containing t-butylbenzene and using the RTL software to re-lock the method. The general theory and use of RTL is detailed in previous publications.²

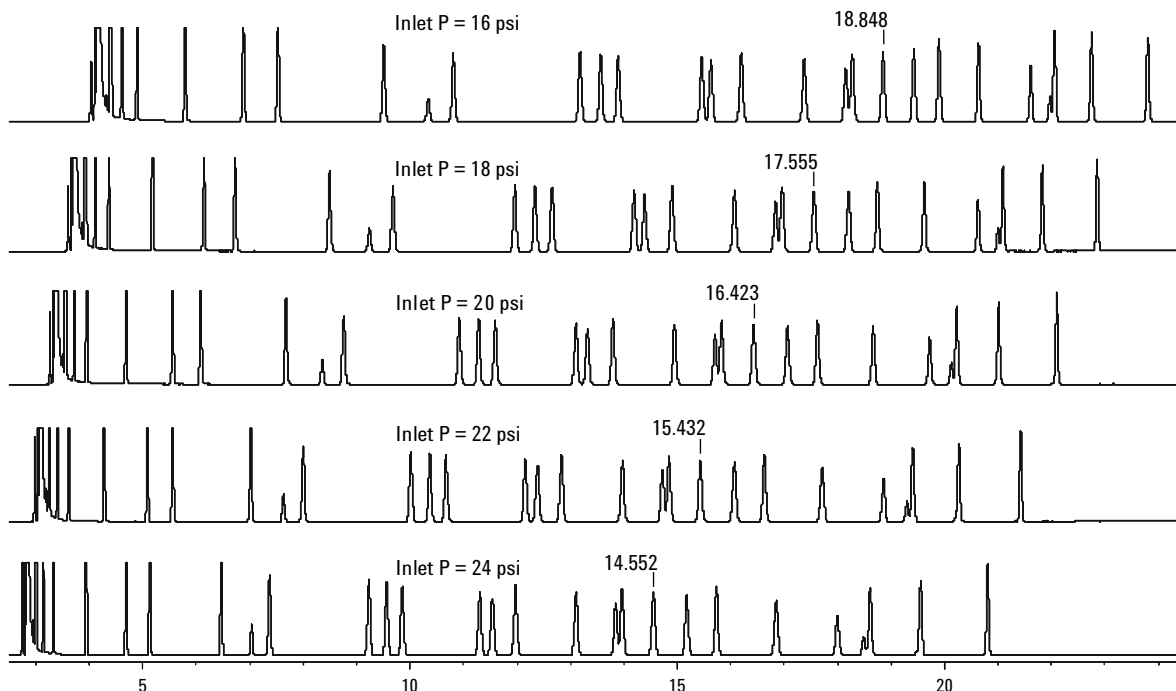


Figure 2. Retention time locking calibration runs using t-butylbenzene as the RTL target peak.

Run	Pressure	Ret Time
Run 1	16	18.849
Run 2	18	17.555
Run 3	20	16.423
Run 4	22	15.432
Run 5	24	14.552

Pressure Units: psi

Desired Ret Time: 16.423

Min relock pressure: 15

Max relock pressure: 25

Column: [dropdown]

Compound Name: t-Butylbenzene

Buttons: OK, Cancel, Print, Help

Figure 3. Retention time locking calibration using t-butylbenzene as the RTL target peak.

A total of six GC systems, two 6890s and four 6850s, were configured to run this unified method. Each GC was retention time locked using a t-butylbenzene target retention time of 16.423 minutes. Figure 4 shows an overlay of the locked chromatograms from each of the six GCs. Table 3 lists the retention times and precision of each compound in the standard mix. Excellent retention time precision was observed for the 6890 and 6850 instruments across the entire time range of the chromatographic run. Peaks falling within the initial 10-minute isothermal time had a standard deviation of about 0.02 minutes. Those peaks eluting during the 3 °C/min program ramp had a standard deviation of 0.01 minutes and those eluting in the 10 °C/min ramp showed a standard deviation of 0.03 minutes

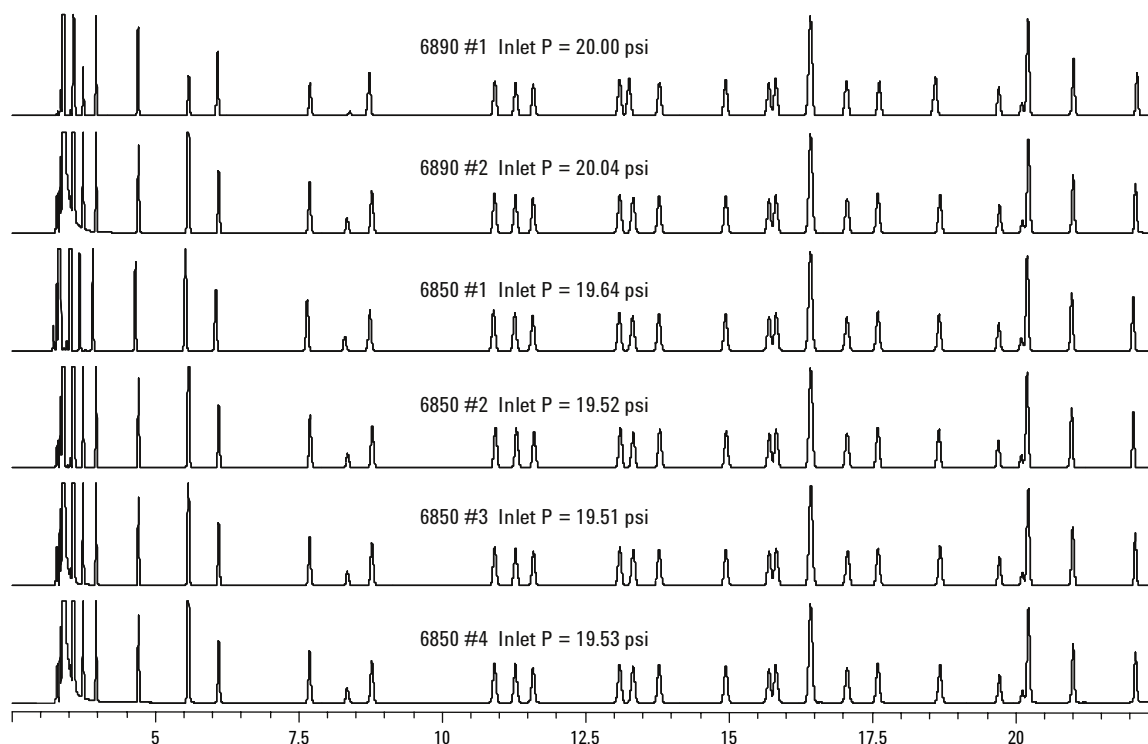


Figure 4. Using RTL, excellent retention time precision was observed for all 27 compounds analyzed using the unified aromatics method. Details of retention time precision are listed in Table 3.

Table 3. Retention Time Precision for Each Compound Analyzed by the Unified Method

Compound	Retention time (min)						Std Dev	Range
	6890 #1	6890 #2	6850 #1	6850 #2	6850 #3	6850 #4		
heptane	3.572	3.568	3.508	3.569	3.566	3.568	0.025	0.064
cyclohexane	3.745	3.742	3.682	3.743	3.741	3.742	0.025	0.063
octane	3.969	3.971	3.911	3.972	3.970	3.971	0.024	0.061
nonane	4.696	4.704	4.646	4.705	4.703	4.704	0.023	0.059
benzene	5.581	5.572	5.518	5.576	5.572	5.572	0.023	0.063
decane	6.084	6.105	6.053	6.106	6.104	6.105	0.021	0.053
toluene	7.694	7.686	7.646	7.695	7.687	7.686	0.018	0.049
1,4-dioxane	8.386	8.342	8.306	8.350	8.346	8.342	0.025	0.080
undecane	8.732	8.776	8.741	8.782	8.777	8.776	0.022	0.050
ethylbenzene	10.922	10.915	10.899	10.932	10.918	10.915	0.011	0.033
p-xylene	11.282	11.278	11.267	11.295	11.280	11.278	0.009	0.028
m-xylene	11.592	11.587	11.577	11.604	11.589	11.587	0.009	0.027
cumene	13.097	13.097	13.089	13.110	13.098	13.097	0.007	0.021
dodecane	13.264	13.334	13.323	13.337	13.333	13.334	0.028	0.073
o-xylene	13.790	13.781	13.778	13.795	13.782	13.781	0.007	0.017
propylbenzene	14.940	14.943	14.939	14.951	14.945	14.943	0.004	0.012
p-ethyltoluene	15.696	15.699	15.699	15.706	15.702	15.699	0.003	0.010
m-ethyltoluene	15.819	15.820	15.820	15.827	15.823	15.820	0.003	0.008
t-butylbenzene	16.423	16.424	16.420	16.426	16.426	16.424	0.002	0.006
s-butylbenzene	17.049	17.060	17.053	17.059	17.063	17.060	0.005	0.014
styrene	17.623	17.600	17.600	17.600	17.603	17.600	0.009	0.023
tridecane	18.602	18.683	18.665	18.661	18.681	18.683	0.031	0.081
diethylbenzene	19.707	19.718	19.701	19.700	19.713	19.718	0.008	0.018
diethylbenzene	20.111	20.123	20.101	20.101	20.116	20.123	0.010	0.022
n-butylbenzene	20.217	20.225	20.201	20.203	20.219	20.225	0.011	0.024
α -methylstyrene	21.011	21.003	20.976	20.975	20.994	21.003	0.015	0.036
phenylacetylene	22.115	22.090	22.050	22.050	22.081	22.090	0.025	0.065
Avg							0.015	0.039

For this method it is not always necessary to use t-butylbenzene to perform retention time locking. Analysts who want to use this method for samples not containing t-butylbenzene can select another compound as the RTL target peak. Compounds that do not elute near temperature program transitions can serve as RTL target peaks. Table 4 lists the other suitable RTL target compounds along with the retention time data for constructing alternate RTL calibrations for this method. For instance, if one were preparing the benzene standard prescribed by ASTM method D4492, the toluene in that standard could serve as the RTL target compound. It is not necessary to perform the five RTL calibration runs. Simply create a new RTL calibration using the inlet pressures and toluene retention times from Table 4. This example of an RTL calibration using toluene is shown in Figure 5.

Table 4. Retention Time Locking Calibration Data for Unified Aromatics Method

Compound	Retention time (min) at each inlet pressure				
	16.00 psi	18.00 psi	20.00 psi	22.00 psi	24.00 psi
nonane	5.794	5.174	4.682	4.279	3.943
benzene	6.880	6.143	5.558	5.080	4.681
toluene	9.507	8.489	7.680	7.018	6.468
cumene	15.460	14.188	13.100	12.148	11.305
o-xylene	16.189	14.897	13.791	12.825	11.969
propylbenzene	17.370	16.064	14.646	13.968	13.100
t-butylbenzene*	18.849	17.555	16.423	15.432	14.552
s-butylbenzene	19.424	18.201	17.061	16.063	15.176
n-butylbenzene	22.054	21.090	20.220	19.404	18.607
styrene	19.891	18.743	17.620	16.621	15.733
α -methylstyrene	22.745	21.824	21.010	20.261	19.552
phenylacetylene	23.795	22.852	22.097	21.421	20.800

*t-butylbenzene used as RTL target peak for this publication (target RT = 16.423 min).

	Pressure	Ret Time
Run 1	16	9.507
Run 2	18	8.489
Run 3	20	7.68
Run 4	22	7.018
Run 5	24	6.468

Pressure Units:

Desired Ret Time:

Min relock pressure:

Max relock pressure:

Column:

Compound Name:

OK Cancel Print Help

Figure 5. Alternate retention time locking calibration for the unified aromatics method that uses toluene as the locking target compound.

Evaluation of Calibration Standards

The calibration standards specified by each of the ten ASTM methods were prepared and run using this unified method. Each standard was run with Agilent 6890 and Agilent 6850 series gas chromatographs that were retention time locked using t-butylbenzene as the target peak (RT = 16.423 min.).

D2306 - Standard Test for C8 Aromatic Hydrocarbons

Figure 6 shows the chromatograms of the D2306 calibration standard run on Agilent 6890 and 6850 gas chromatographs. The injection size for both runs was 0.1 μ L and the split ratio was 400:1.

D2360 - Standard Test for Trace Impurities in Monocyclic Hydrocarbons

The standard calibration mix specified by D2360 was prepared in p-xylene. Figure 7 shows the chromatograms of the D2360 calibration standard. Injection size was 1.0 μ L and the split ratio was 100:1. The ethylbenzene peak (RT = 10.98 min) elutes just before p-xylene and was much broader than the other contaminants. This peak shape was due to a reverse solvent effect caused by the overloaded p-xylene along with an oven starting temperature (75 $^{\circ}$ C) that was much lower than the p-xylene boiling point (138 $^{\circ}$ C). A broad ethylbenzene peak was also observed in the original ASTM D2360 method.³

D3760 - Standard Test for Analysis of Isopropylbenzene (Cumene)

Figure 8 shows the chromatograms of the D3760 calibration standard. The injection size for both runs was 1.0 μ L and the split ratio was 100:1. The xylene isomers' concentrations were not listed because they were not added to the standard, but were present as trace contaminants in the cumene used to prepare the standard. Since both GCs are retention time locked, the identification of each xylene isomer could be easily made.

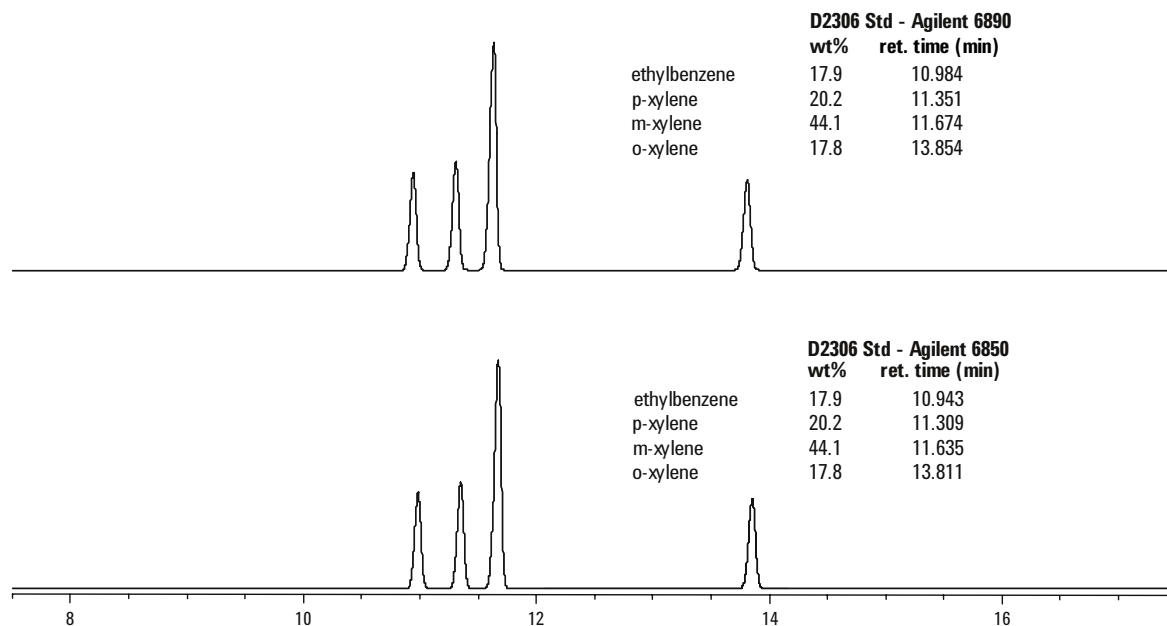


Figure 6. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

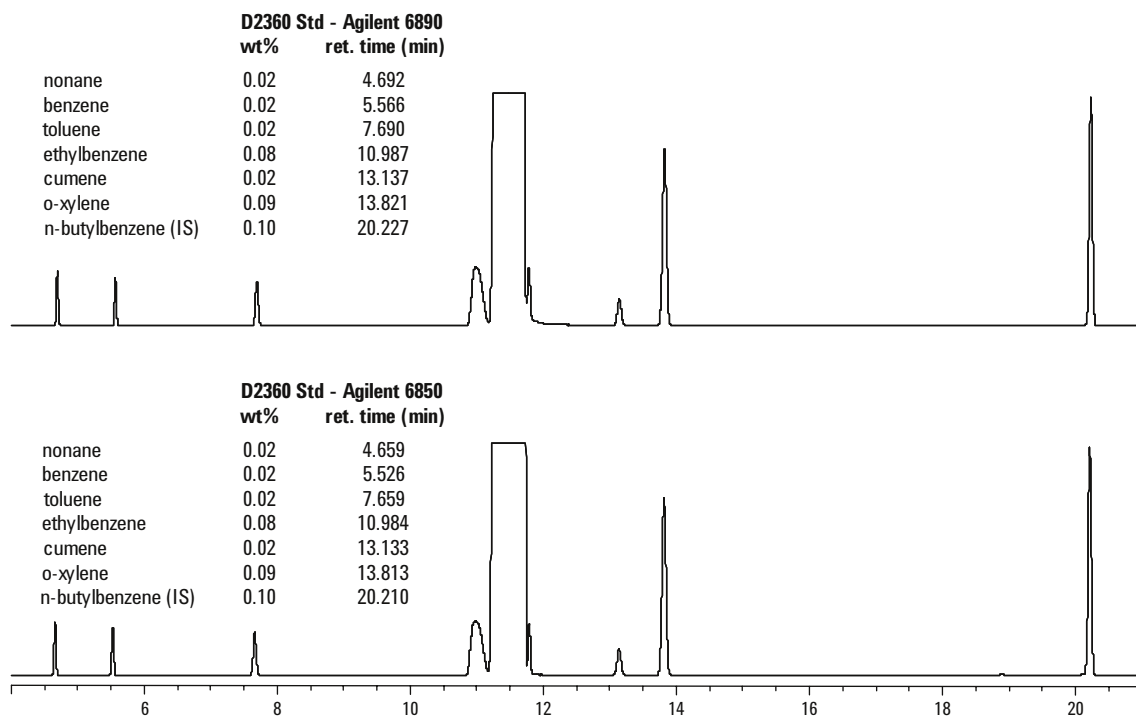


Figure 7. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

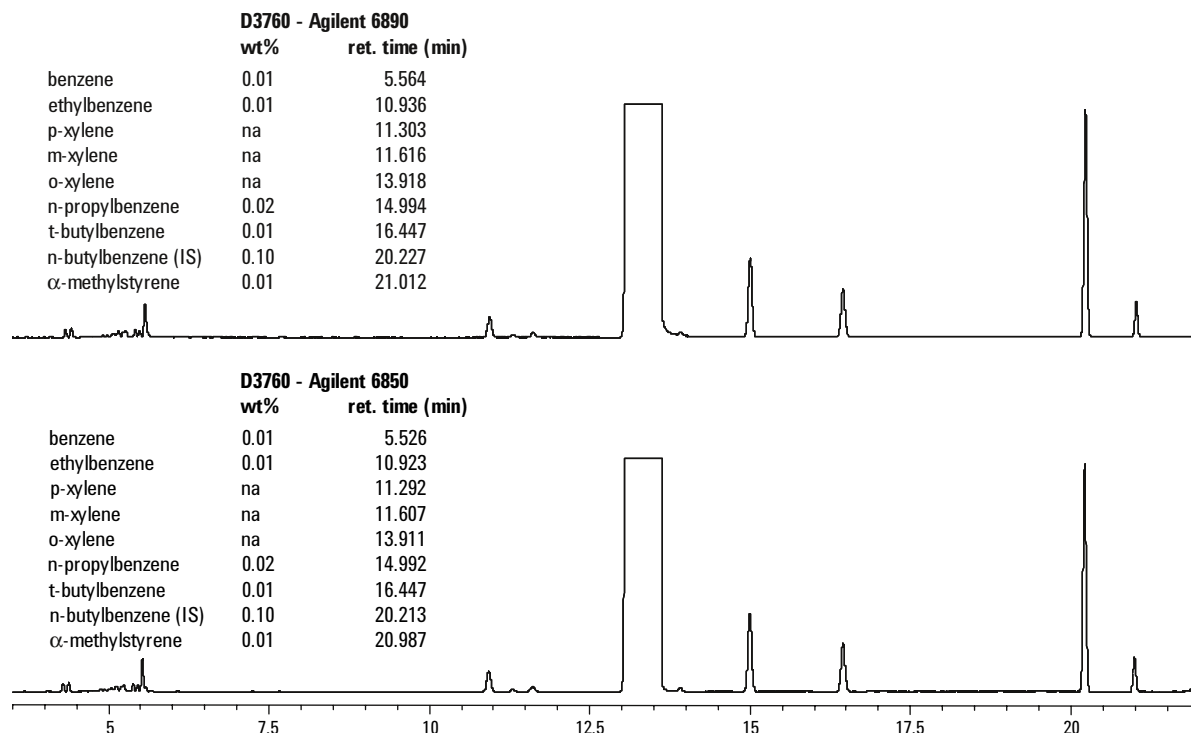


Figure 8. ASTM D3760 isopropylbenzene (cumene) quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D3797 - Standard Test Method for Analysis of o-Xylene

Figure 9 shows the chromatograms of the D3797 calibration standard. The injection size was 1.0 μ L and the split ratio was 100:1. The broadening of the cumene peak (RT = 13.28 min) was due to the reverse solvent effect of the overloaded o-xylene peak. This was also observed in the original ASTM D3797 method.

D3798 - Standard Test Method for Analysis of p-Xylene

Figure 10 shows the chromatograms of the D3798 calibration standard. The injection size was 1.0 μ L and the split ratio was 100:1. The ethylbenzene

peak shows the same broadening observed in the D2360 standard. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Figure 11 shows the details of this separation using the unified method. For each GC this requirement was met for both the ethylbenzene and the m-xylene.

D4492 - Standard Test for Analysis of Benzene

Figure 12 shows the chromatograms of the D4492 calibration standard. The injection size was 1.0 μ L and the split ratio was 100:1.

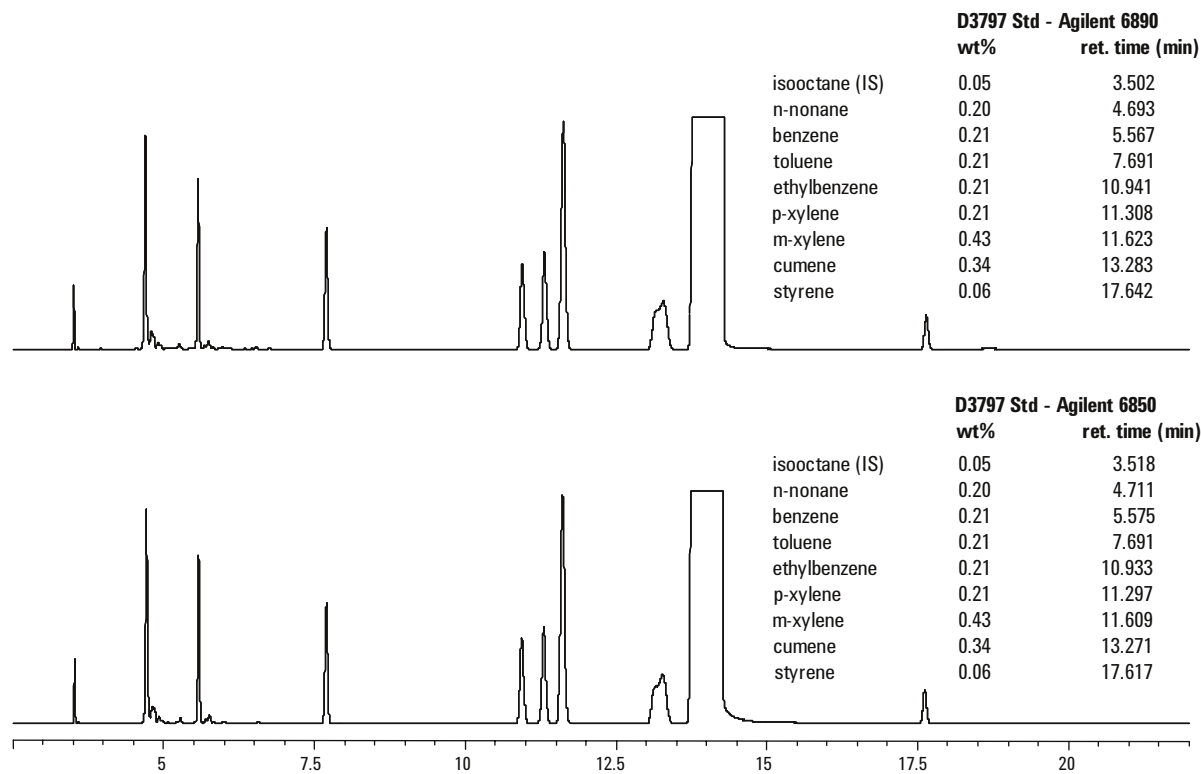


Figure 9. ASTM D3797 o-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

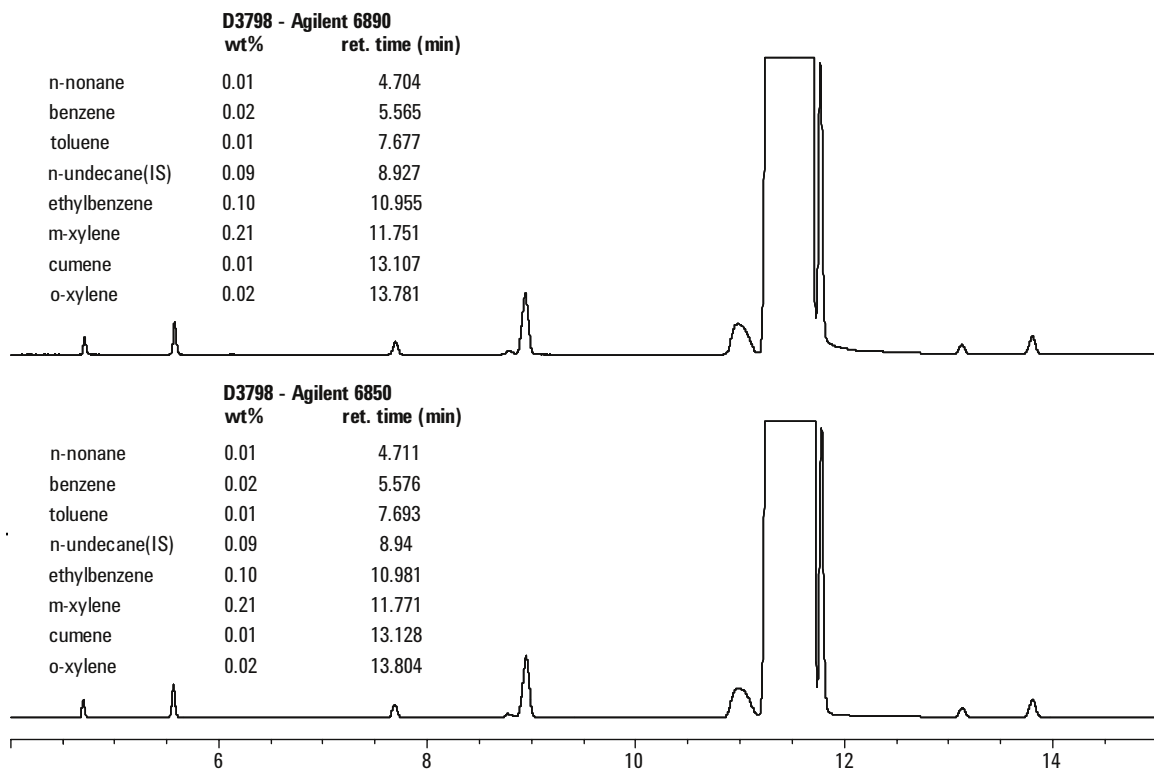


Figure 10. ASTM D3798 p-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

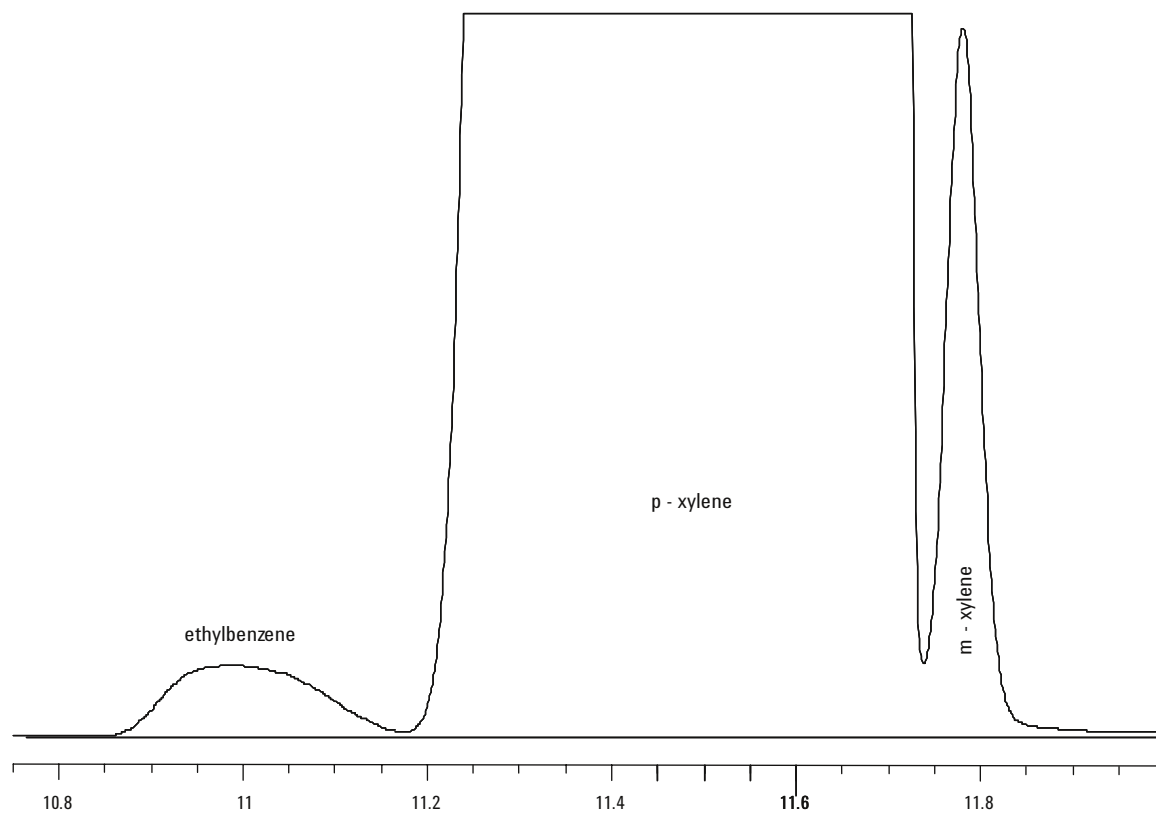


Figure 11. Expanded view from Figure 10 shows excellent separation of m-xylene from p-xylene peak using the unified aromatics method.

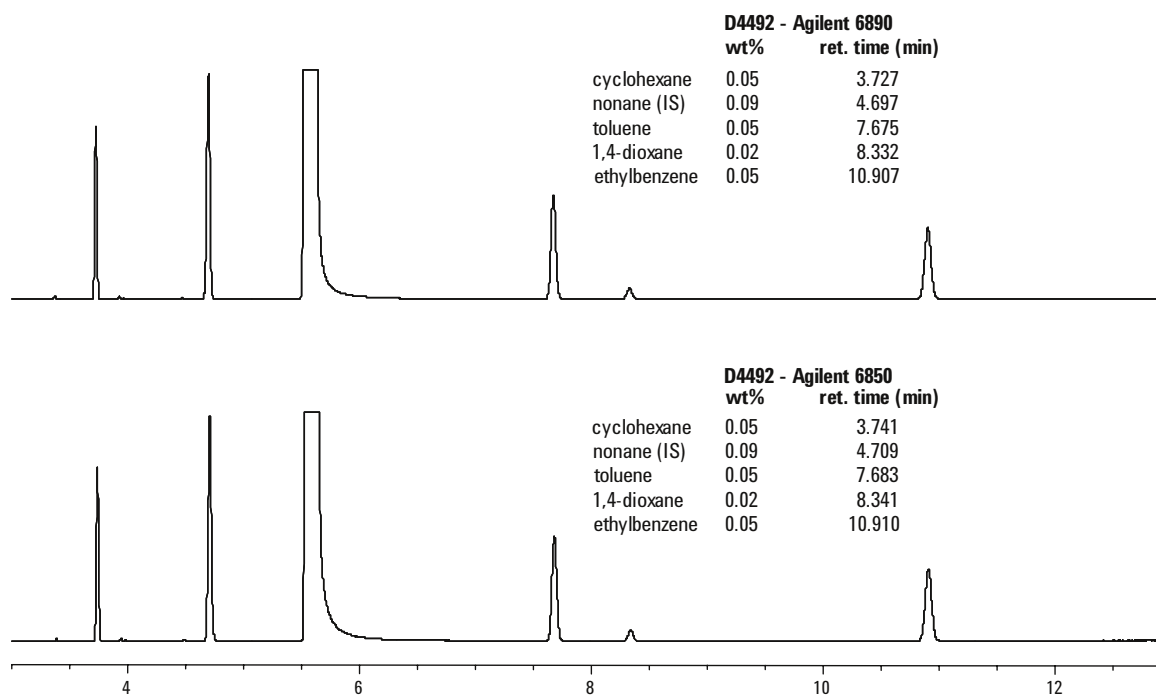


Figure 12. ASTM D4492 benzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cyclohexane

Figure 13 shows the chromatograms of the D4534 calibration standard containing 8 mg/kg (ppm) benzene in cyclohexane. The injection size was 1.0 μ L and the split ratio was 100:1.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Toluene

Figure 14 shows the chromatograms of the D4534 calibration standard containing 9 mg/kg (ppm) benzene in toluene. The injection size was 1.0 μ L and the split ratio was 100:1. Several contaminants were found in the toluene used to prepare this standard. Most of these contaminants were identified, but the peak at 15.3 minutes did not correspond to the retention times of those listed in Table 3. If the GC systems were not retention time

locked, one might assume that this contaminant could be n-propylbenzene or p-ethyltoluene. However, given the retention time precision expected with RTL, it is clear that this contaminant is an unknown.

GC/MS is the best approach to identify this unknown, but under the same GC conditions, GC/MS retention times are often considerably faster than those obtained using atmospheric detectors. However, by combining retention time locking with a technique called method translation, one can obtain GC/MS retention times nearly identical to those found with conventional GC.⁴ This makes identifying unknown peaks much easier. Figure 15 shows the D4534 toluene standard run on both the Agilent 6850 and the Agilent 5973 GC/MS. A mass spectral library search of the unknown peak at 15.320 minutes identifies this compound as chlorobenzene. The source of the chlorobenzene was found to be the toluene used to prepare the standard.

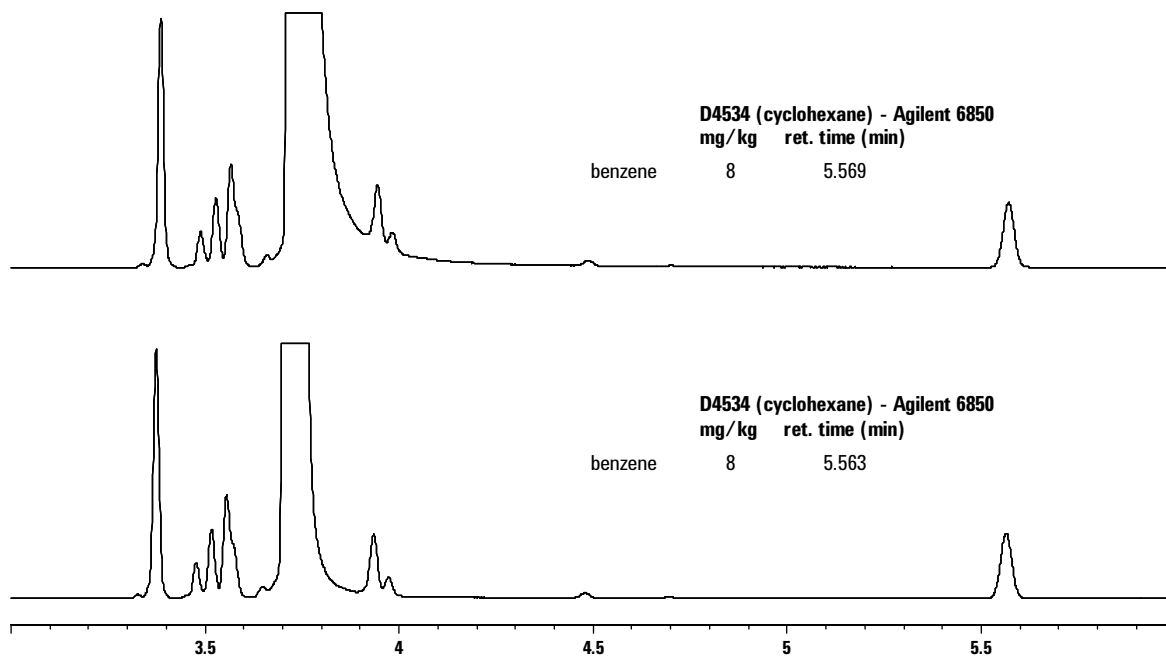


Figure 13. ASTM 4534 cyclohexane quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

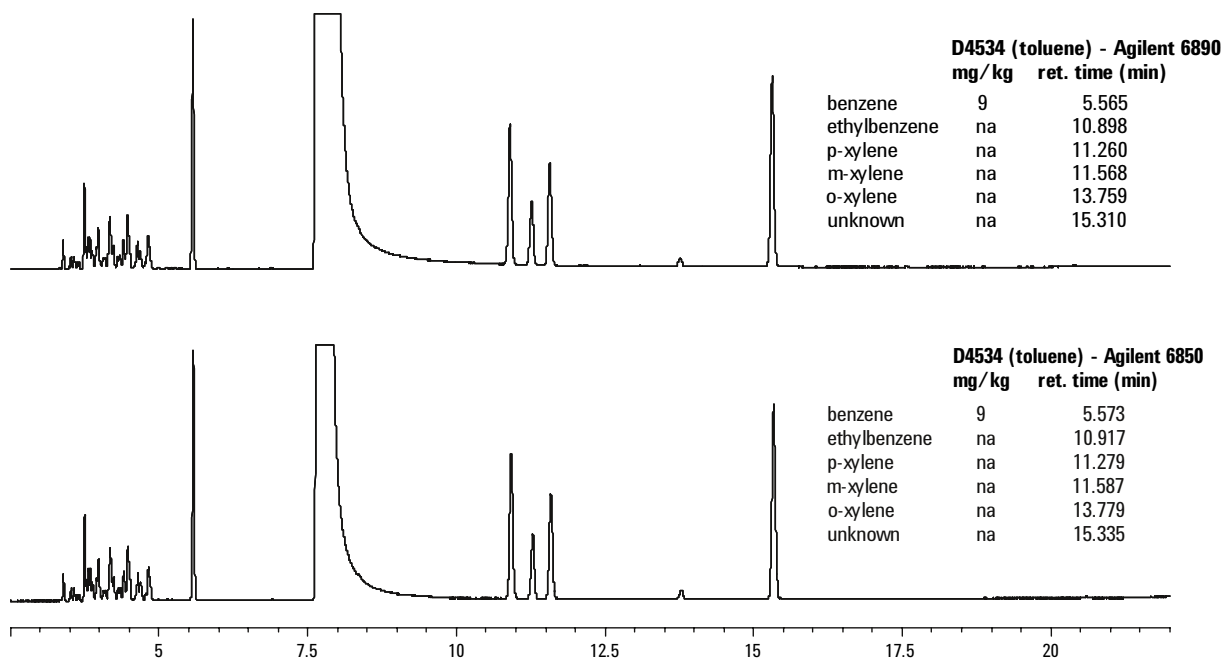


Figure 14. ASTM D4534 toluene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

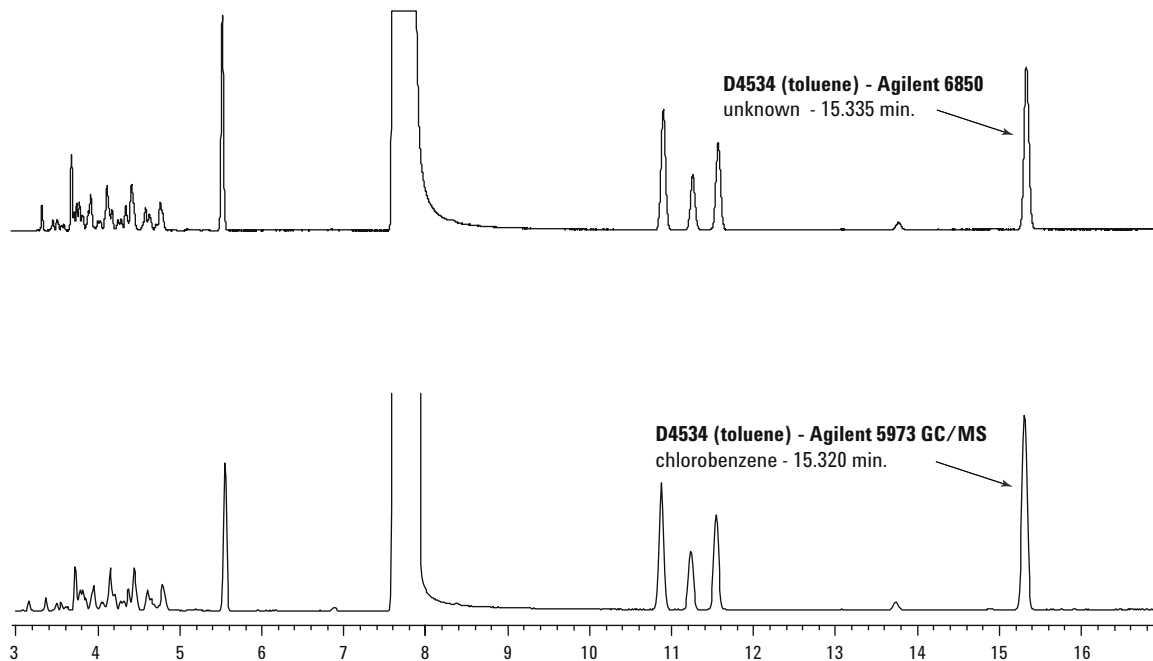


Figure 15. Unknown contaminant found in D4534 toluene standard (top) was identified as chlorobenzene using the retention time locked unified aromatics method run on the Agilent 5973 GC/MS (bottom).

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cumene

Figure 16 shows the chromatograms of the D4535 calibration standard containing 5 mg/kg (ppm) of benzene in cumene. The injection size was 1.0 μ L and the split ratio was 100:1. Details of these

chromatograms are shown in Figure 17. Although benzene is well resolved, there are still some C9 hydrocarbons that elute near benzene. These compounds represent a potential source of interference when measuring small amounts of benzene (less than 5 mg/kg) in cumene.

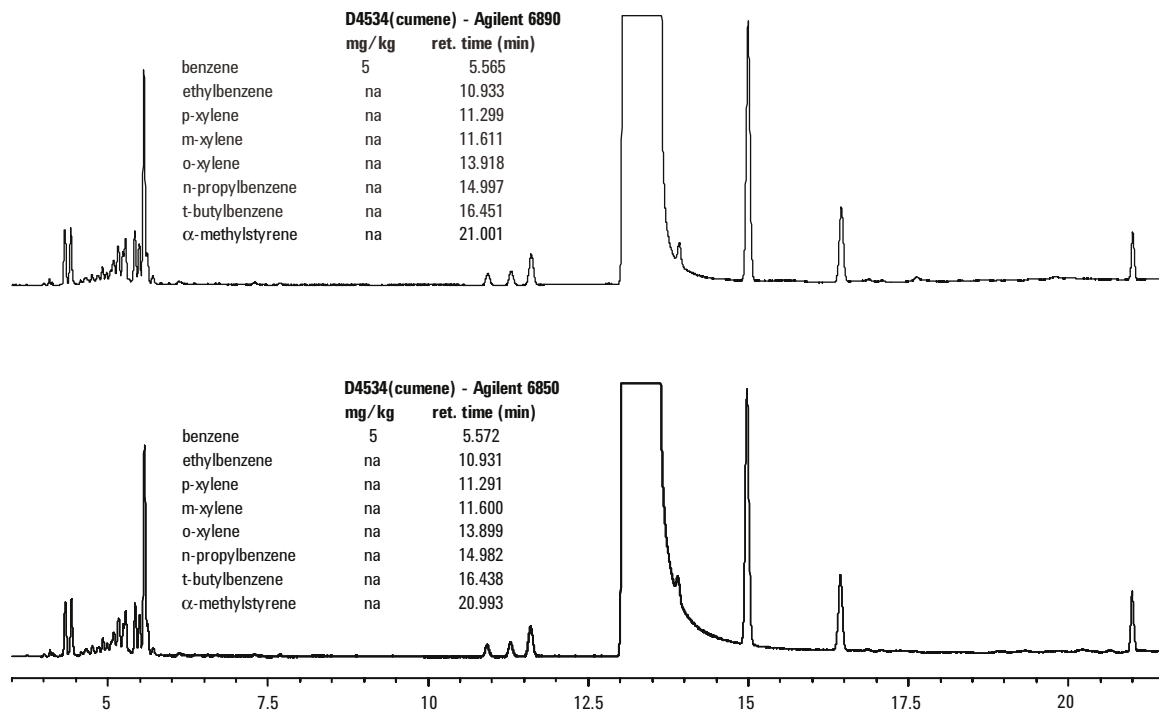


Figure 16. ASTM D4534 cumene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

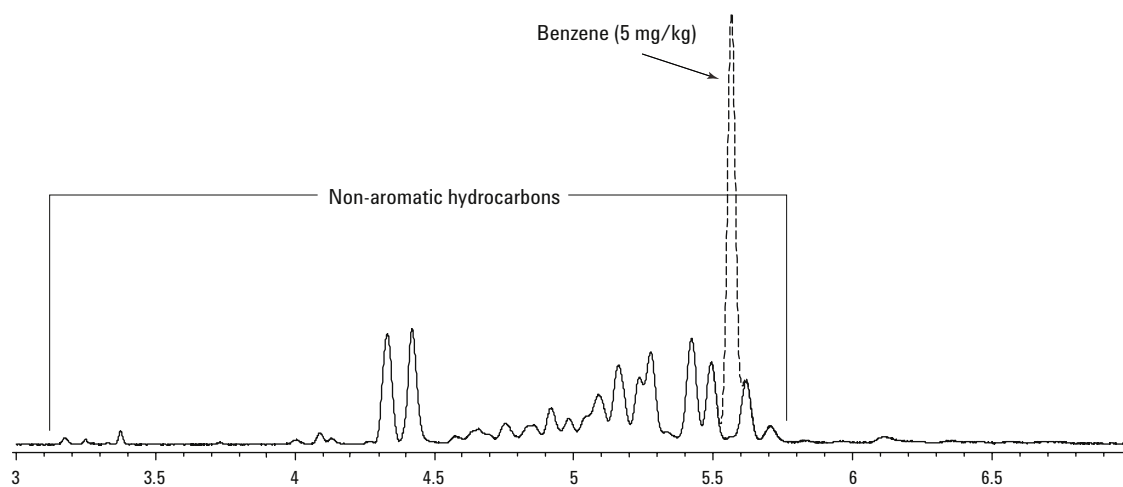


Figure 17. Details of the D4534 cumene standard showing the separation of 5 mg/kg of benzene from non-aromatic hydrocarbons typically found in cumene.

D5060 Standard Test Method for Determining Impurities in High-Purity Ethylbenzene

Figure 18 shows the chromatograms of the D5060 calibration standard. The injection size was 1.0 μ L and the split ratio was 100:1.

D5135 Standard Test Method for Analysis of Styrene by Capillary Gas Chromatography

Figure 19 shows the chromatograms of the D5135 calibration standard. The injection size was 1.0 μ L and the split ratio was 100:1.

D5917 Standard Test Method for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)

This method is identical to D2360 without the addition of the internal standard, n-butylbenzene, so that the chromatogram shown in Figure 7 is a good representation of an expected result. However, since n-butylbenzene is not included in the standard or samples for D5917, the run time of the unified method can be reduced to approximately 15 minutes.

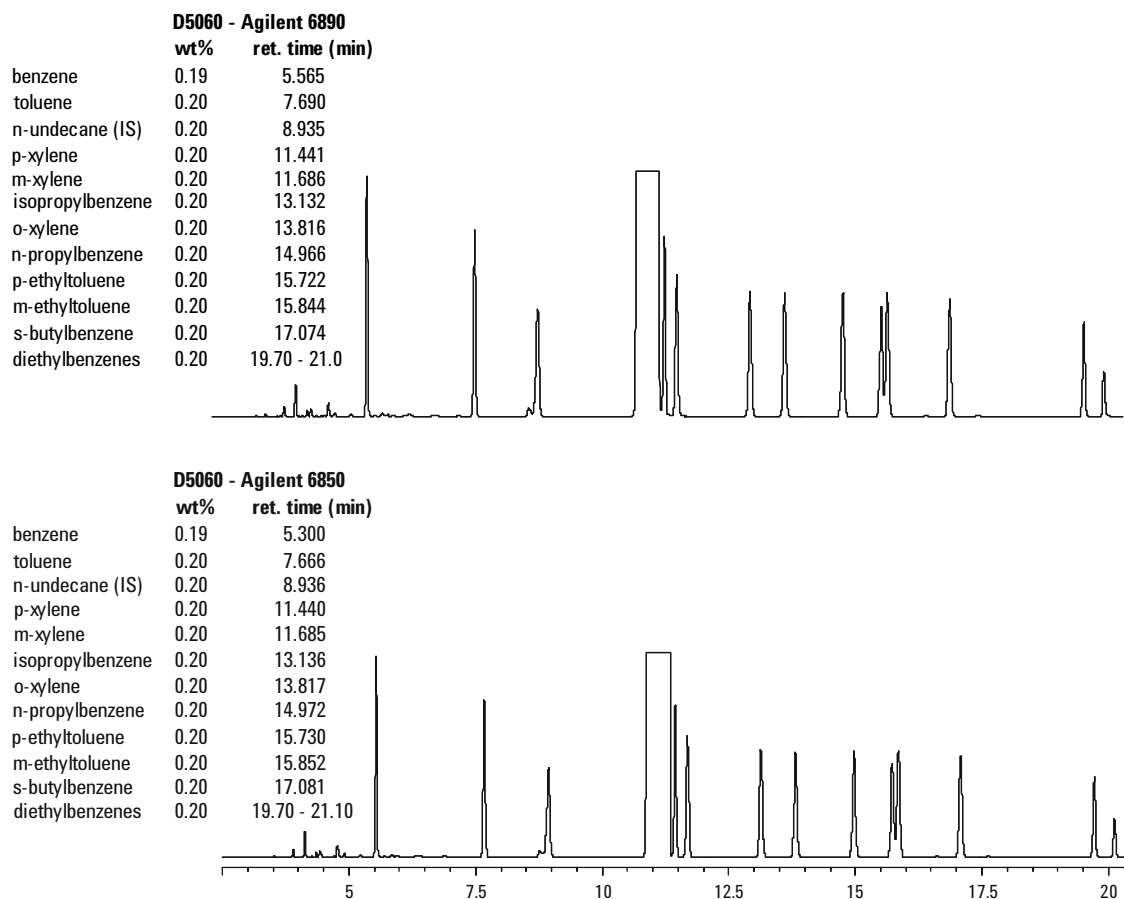


Figure 18. ASTM D5060 ethylbenzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

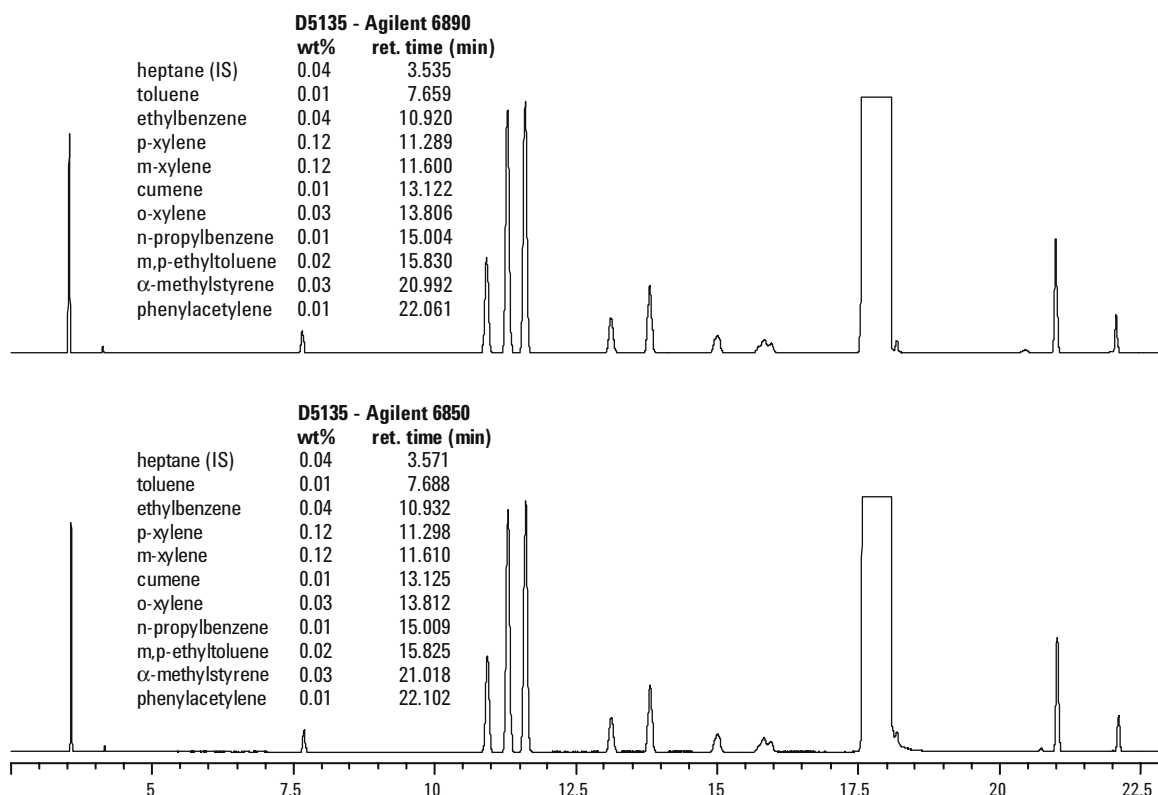


Figure 19. ASTM D5135 quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

Conclusions

The analysis of many different bulk aromatic solvents in the QA/QC laboratory presents the analyst with an array of ASTM methods specific to each material. In an effort to simplify these measurements for today's laboratory environment, the chromatographies of ten ASTM methods were consolidated into one method. This unified method can resolve the 27 compounds found in aromatic materials and can successfully run the calibration standards used by each ASTM method to determine solvent purity. This versatile method can be run on both the Agilent 6890 and 6850 GC to yield consistent results between the method development lab and the plant production lab. To further improve performance, retention time locking (RTL) was applied to the unified method so that retention time standard deviation for each compound in any sample is less than 0.03 minutes. This allows easy comparison of results between instruments, laboratories and over time. The retention time locked unified method meets the need for a fast, simple method that can be run in today's production laboratories.

References

1. Annual Book of ASTM Standards, Vol. 6.04 *Paint - Solvents; Aromatic Hydrocarbons*, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
2. V. Giarrocco, B.D. Quimby, and M.S. Klee, *Retention Time Locking: Concepts and Applications*, Agilent Technologies, Application Note 228-392, Publication number 5966-2469E, December 1997.
3. ASTM Method D2360, *Standard Test for Trace Impurities in Monocyclic Hydrocarbons*, Annual Book of ASTM Standards, Vol. 6.04, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
4. B.D. Quimby, L.M. Blumberg, M.S. Klee, and P.L. Wylie, *Precise Time-scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking*, Agilent Technologies, Publication number 5967-5820E, May 1998.

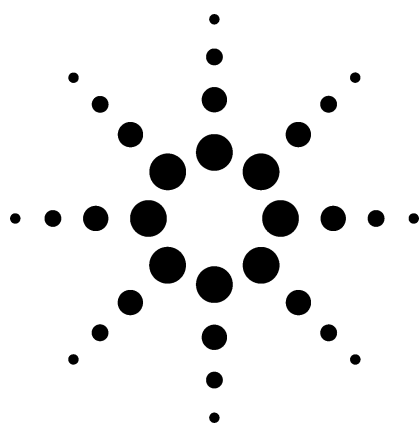
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Applying the 5975 inert MSD to the Higher Molecular Weight Polybrominated Diphenyl Ethers (PBDEs)

Application

Environmental, Component Testing

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This note presents the full scan spectra obtained for the PBDEs over the extended mass range of the 5975 inert MSD. The polymeric sample preparation and extraction protocols are cited elsewhere and supply two approaches to PBDE determinations [1].

Abstract

A previous application note presented results for analysis of the polybrominated diphenyl ethers (PBDEs) in polymers using the 5973N inert MSD [1]. Mass spectra were presented and interpreted for all of the important PBDEs. The new 5975 inert MSD provides many new features and improvements with expanded mass range to 1050 u being but one. This note presents the full spectra of the octa-, nona and decabrominated biphenyls ethers including ions that appear beyond the mass range of the previous 5973 MSD platform.

Introduction

PBDEs have become the “new PCBs” due to their widespread detection throughout the ecosystem. They have some structural and consequently mass spectral features in common with the polychlorinated biphenyls (PCBs) as well. The series of fragments formed by loss of chlorines ($M-nCl_2$) generates a number of intense ions useful in their determination. The PCBs also show relatively intense molecular ion clusters that assist in distinguishing the congeners. Similar attributes are expected and hoped for the PBDEs which show much more analytical difficulty than the PCBs.

Experimental

PBDE standards were acquired from Cambridge Isotope Laboratories (Andover, MA) and AccuStandard (New Haven, CT).

Instrumental Configuration and Conditions

The 6890 GC configuration and conditions are given in the previous application note [1]. The 5975 inert MSD system was operated in scan mode for acquisition of the PBDE spectra. The MSD scan operating parameters are cited in Table 1.

Table 1. 5975 inert MSD Configuration and Parameters

Mass spectrometer parameters	
Ionization mode	Electron impact
Ionization energy	70 eV
Tune parameters	Autotune
Electron multiplier voltage	Autotune + 400V
Scan mode	200–1000 u
Quadrupole temperature	150 °C
Inert source temperature	300 °C

Full conditions and parameters, as appropriate to the polymer analysis cited in reference 1, are available in the eMethod for this analysis (www.agilent.com/chem/emethods).



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Results

EI Spectra of the Higher Molecular Weight PBDEs

Figures 1, 2, and 3 present the full-scan spectra of an octa-, nona- and the decabromodiphenyl ether. Note that most intense ions in all cases are the $[M-Br_2]^+$ and the corresponding to $[M-Br_2]^{+2}$ ions. The relative abundance of the molecular ion clusters $[M]^+$ are under 30%. Figure 4 compares the

theoretical isotopic pattern to that experimentally obtained by the 5975 inert MSD. Agreement is good in both the abundance of the isotopes and the mass accuracy using the standard system Autotune. Mass accuracy agrees to within 0.2 m/z of the theoretical and experimental values. Table 2 presents the important ions for the PBDEs greater than the dibromodE. These ions are those most important to characterizing the technical mixtures used as additives to polymers.

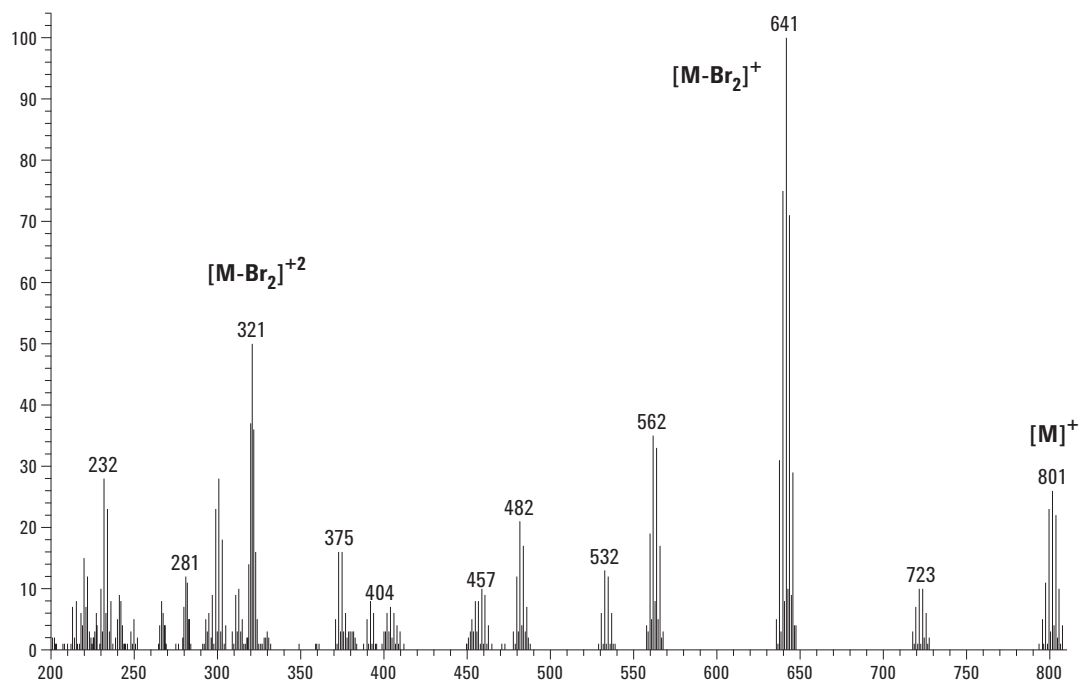


Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 m/z .

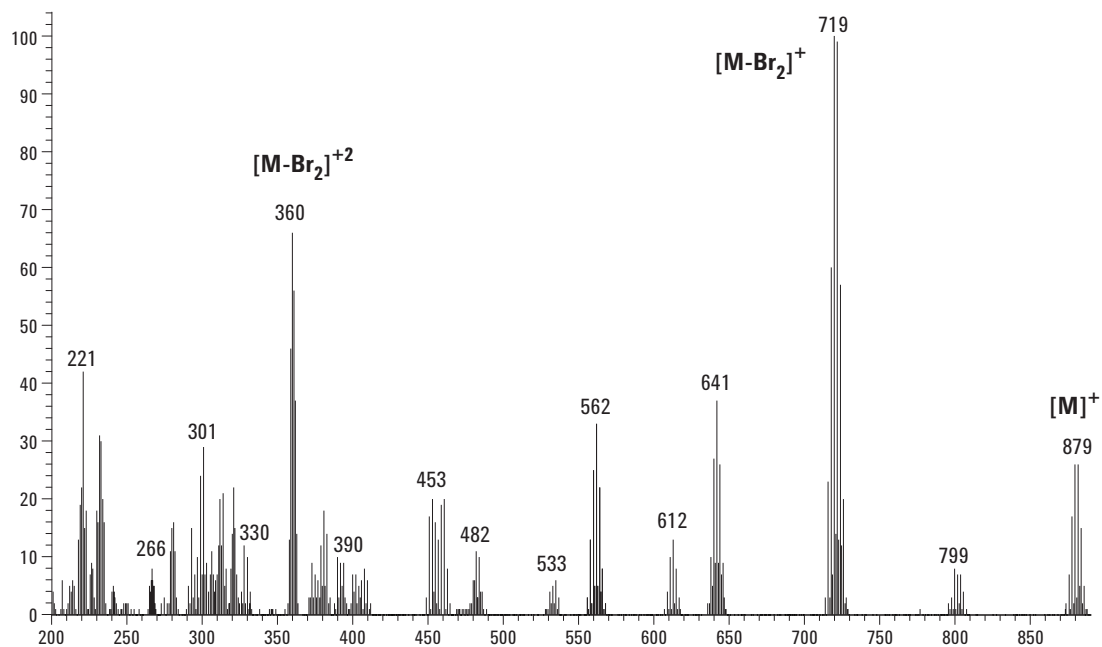


Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 m/z .

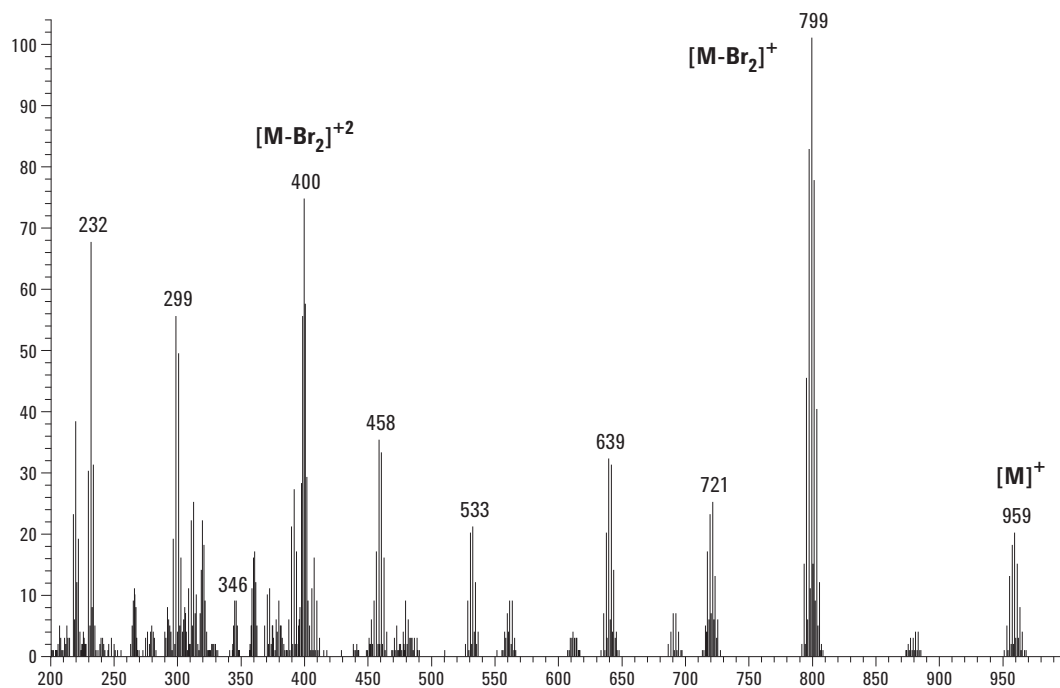


Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 m/z .

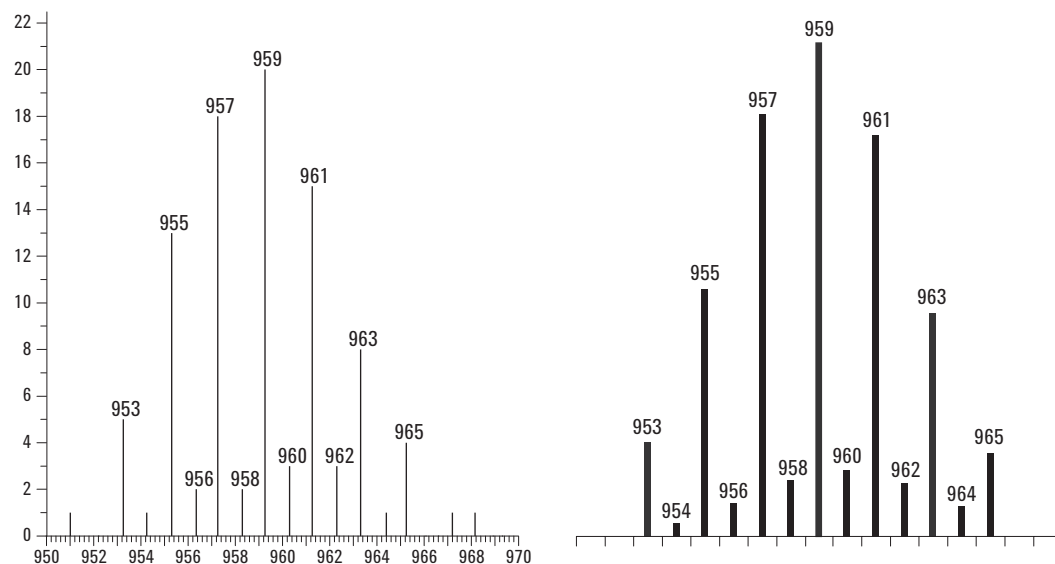


Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

Table 2. Important Ions for the PB_nDEs ($n > 2$)

PBDE bromination	$[M]^+$	$[M-Br_2]^+$	$[M-Br_2]^{+2}$
3	405.8	246.0	123.0
4	485.7	325.9	162.9
5	563.6	403.8	201.9
6	643.5	483.7	241.9
7	721.5	561.6	(280.8 **)
8	801.4	641.5	320.8
9	879.3	719.4	359.7
10	959.2	799.3	399.7

**The 280.8 and 281.8 m/z ions can be compromised by column bleed interferences so these have not been used in acquisition although they provide a useful diagnostic for column degradation.

The user should note the ion source and quadrupole temperature settings in Table 1. Figure 5 presents SIM acquisitions of several higher molecular weight PBDEs at source temperatures of 300 °C and 230 °C. Notice the signal height roughly doubles on average for the PBDEs at the higher ion source temperature. The insert in the figure shows the improvement in the peak shape for the hexabrominated diphenyl ether. This peak sharpening accounts for the increase in signal height. Since these compounds elute at higher temperatures

among other high boiling components that belong to the matrix, heating the quadrupole is important for robust and low maintenance operation in samples.

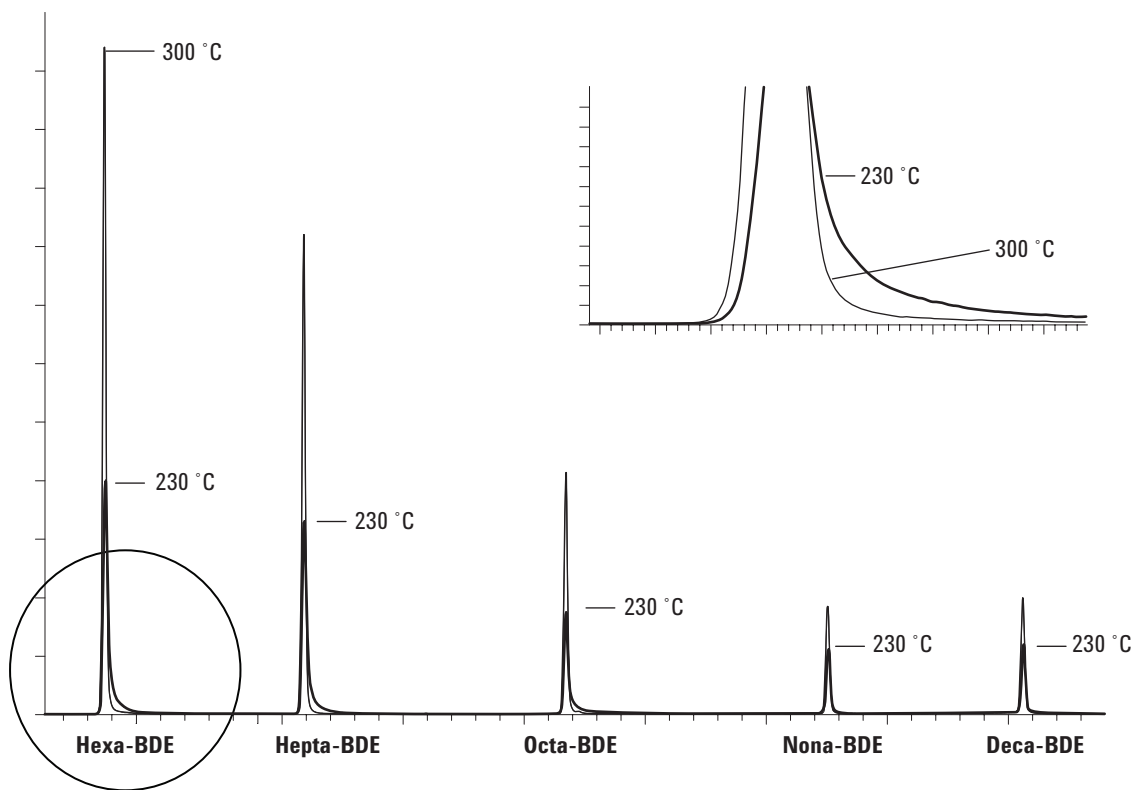


Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

Conclusions

The new 5975 inert MSD has an expanded set of features including mass range. High mass accuracy under standard autotuning is obtained even at the high masses typical of the brominated diphenyl ethers. As users survey higher mass compounds, the heated quadrupole and high temperature capabilities of the 5975 inert MSD will become even more important to rugged and robust analyses in complicated samples.

More details on the other relevant instrumental parameters are available in the eMethod (www.agilent.com/chem/emethods).

Reference

1. C. Tu, and H. Prest, Determination of polybrominated diphenyl ethers in polymeric materials using the 6890 GC/5973N Inert MSD

with electron impact ionization. Agilent Technologies, publication 5989-2850EN, www.agilent.com/chem

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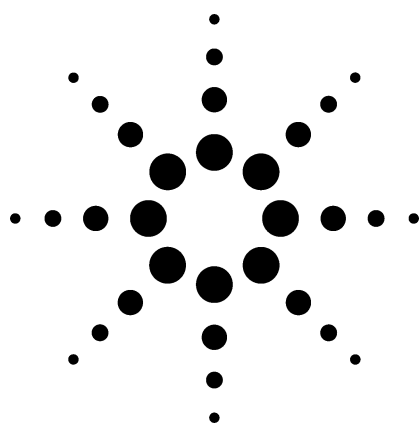
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Process Monitoring of Bisphenol-A in Industrial Feedstock using High Throughput HPLC

Application

Process Control

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Abstract

The chromatographic method used to monitor the Bisphenol-A manufacturing process was improved using Agilent RRHT Eclipse XDB-C18 columns. These columns use 1.8-µm particles versus conventional 3.5-µm or 5-µm particles. The improved method allowed seven times faster analyses, improved resolution, and higher sensitivity.

Introduction

Bisphenol-A (Figure 1) is a highly versatile material used to manufacture many modern products. It is also known as 4,4'-Isopropylidenediphenol, 4,4'-(1-Methylethylidene) bisphenol, or simply BPA.

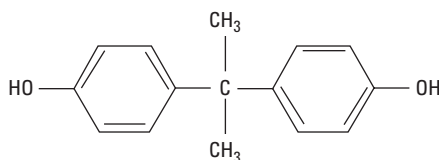


Figure 1. Bisphenol A

Every year, 2.8 million tons of BPA are produced. BPA is a building block for polycarbonate plastic and epoxy resins. Polycarbonate plastic is prized for its scratch resistance, optical clarity, and heat and electrical resistance. Because of these attributes, it is used for eyewear, CD/DVD disks, electronics, and food and drink containers. Epoxy resins are used for protective coatings because of their combination of inertness, chemical resistance, adhesion, and formability. For example, metal food cans are lined to protect taste. Epoxy resins are also used as a component in dental sealants and as a component in dental composites providing an alternative to mercury amalgam in veneers and fillings. Other uses include fungicides, polymer antioxidants, and components in automobiles and appliances.

BPA is produced through an acid-catalyzed condensation reaction of phenol with acetone. During condensation, a number of phenol-based byproducts are also formed. HPLC is used to determine the composition of many of the process streams in a commercial BPA plant.

Here we describe the use of new HPLC column technology for the possible improvement to one of the HPLC methods used in a commercial BPA facility.



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Method Optimization and Scalability

The existing HPLC method was proven and robust; however, it was complicated. We sought a similar chromatogram, based on the original method, but using simpler method parameters. Because of the challenge of changing many chromatographic parameters, essentially redeveloping the method, we chose a 4.6×50 mm, $1.8\text{-}\mu\text{m}$ Eclipse XDB-C18 column for experiments to reduce the time required. Smaller particles packed in shorter columns increase the speed of analysis and still provide enough efficiency to maintain resolution equivalent to longer columns packed with larger particles. After several trials, we developed a method that produced a chromatogram similar to the original. The short analysis time is a major advantage of Rapid Resolution High Throughput (RRHT) technology. Whereas a handful of experimental runs would take an entire work day using a typical analytical-sized column (50 min/run), the series of runs took about an hour (7.5 min/run), using an RRHT column.

We incrementally scaled up to a 4.6×250 mm column. Figure 2 shows an overlay of the sample analyzed by three 4.6-mm id columns of different lengths and particle sizes. Injection volume was also changed proportionally to length. The smaller ZORBAX particles speed up the analysis while maintaining resolution. In fact, resolution increased when using the RRHT columns despite their shorter length.

One reason this method can be easily scaled (up or down) is the uniform spherical Eclipse XDB-C18 packing. It has a proprietary engineered particle size distribution, based on ZORBAX silica with a controlled surface area and pore size. The robust proprietary packing material and proven column manufacturing techniques consistently yield reproducible columns with similar chromatographic performance, independent of the column dimensions.

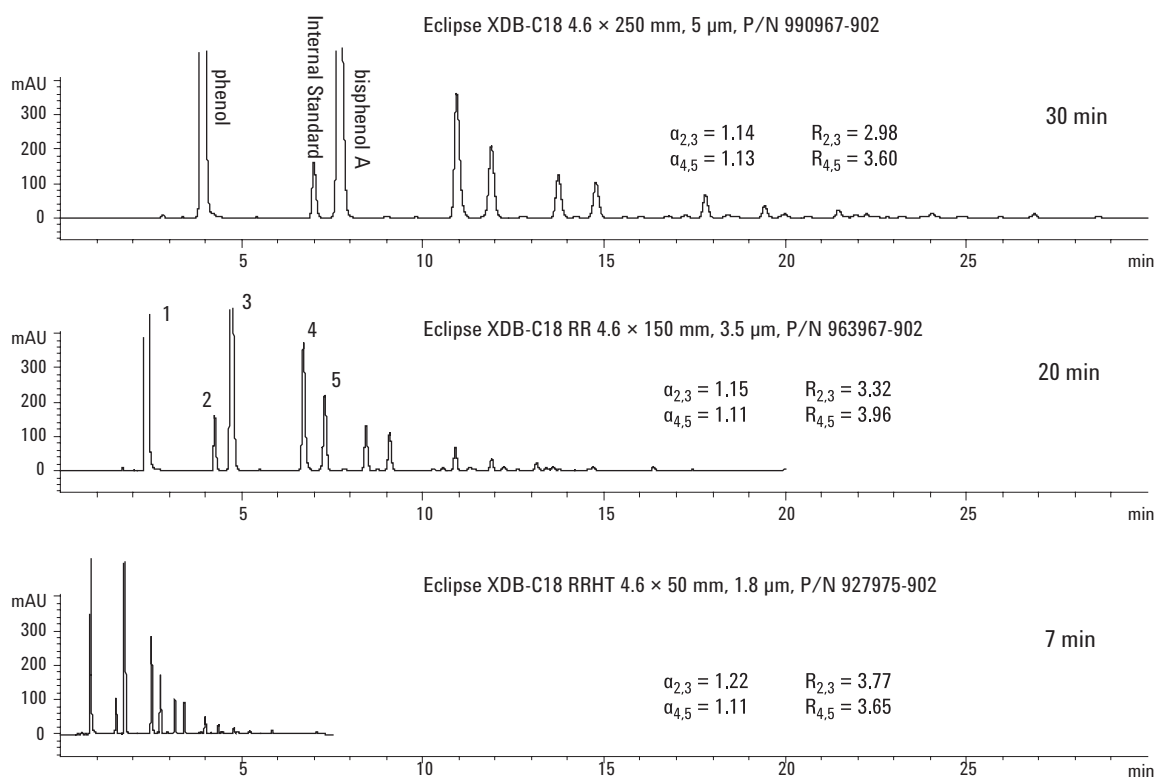


Figure 2. RRHT column configuration increased both speed and resolution.

Particle size does influence resolution. The influence can be noticed when comparing columns of identical dimensions, packed with three different particle sizes. Figure 3 shows the shortened Bisphenol-A analysis using different particle-sized Eclipse XDB-C18 columns. Resolution (R_s) is related to selectivity (α), efficiency (N) and retention (k'):

$$R_s = (1/4)(\alpha-1) \sqrt{N} [k'/(1+k')]$$

Factors affecting the selectivity term (stationary phase, mobile phase) and retention term (mobile phase, temperature) are constant for the three

chromatograms. The efficiency term is influenced by column length, linear velocity of the mobile phase (both constant), and particle size (varied in Figure 3). N increases as particle size decreases. In Figure 3 the selectivity factors (α) and retention remain about the same, but resolution actually increases. The increase in resolution due to the decrease in particle size highlights the advantage of using smaller particles. The similar selectivity and retention highlight the suitability of ZORBAX Eclipse XDB-C18 columns for scaling methods, especially to more rapid, high-throughput methods.

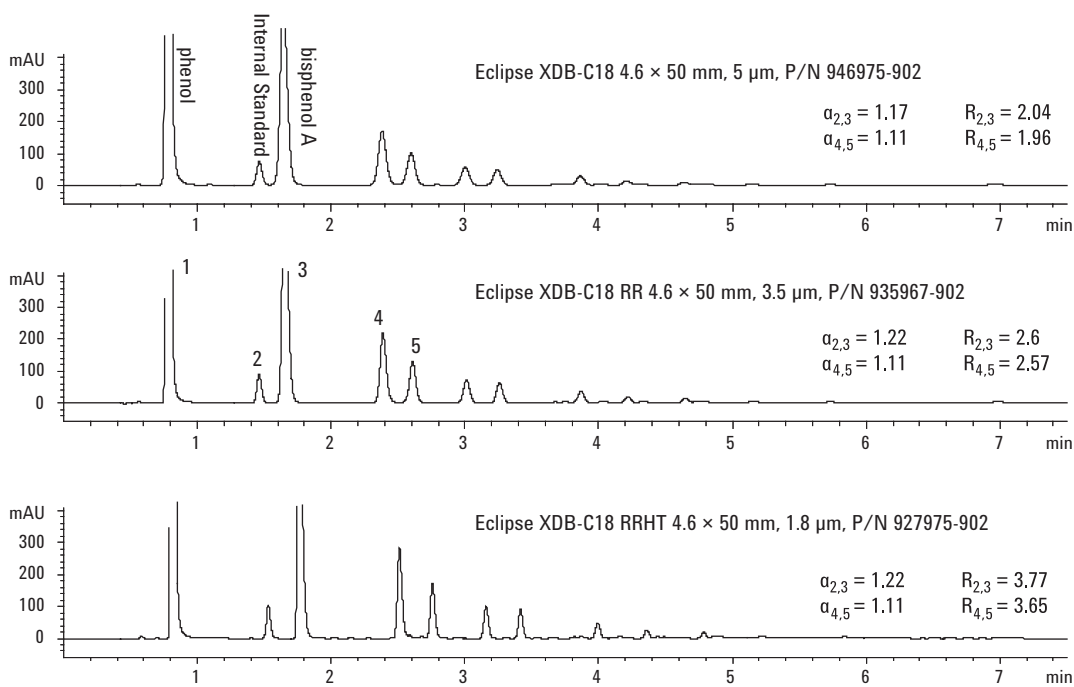


Figure 3. Effect of particle size on resolution and selectivity.

Comparing the Existing Method to the RRHT Method

Figure 4 compares the original BPA separation to the RRHT separation. The top chromatogram is an example of the analysis using the original commercial method, and the bottom is an example of the process sample analyzed with the RRHT method. The method developed with the new column technology clearly increases productivity.

Analysis time is reduced at least six-fold; solvent consumption is reduced about 12.5 times, from 100 mL/analysis to only 7.5 mL/analysis. Interestingly, the peak shape of Bisphenol-A is more symmetrical using Eclipse XDB-C18 as compared to the current C18 column used in the original analysis. The more Gaussian peak shape eluted by the Eclipse XDB-C18 column is important for accurate quantification. Other method improvements such as a simplified gradient and a binary mobile phase are listed in Table 1.

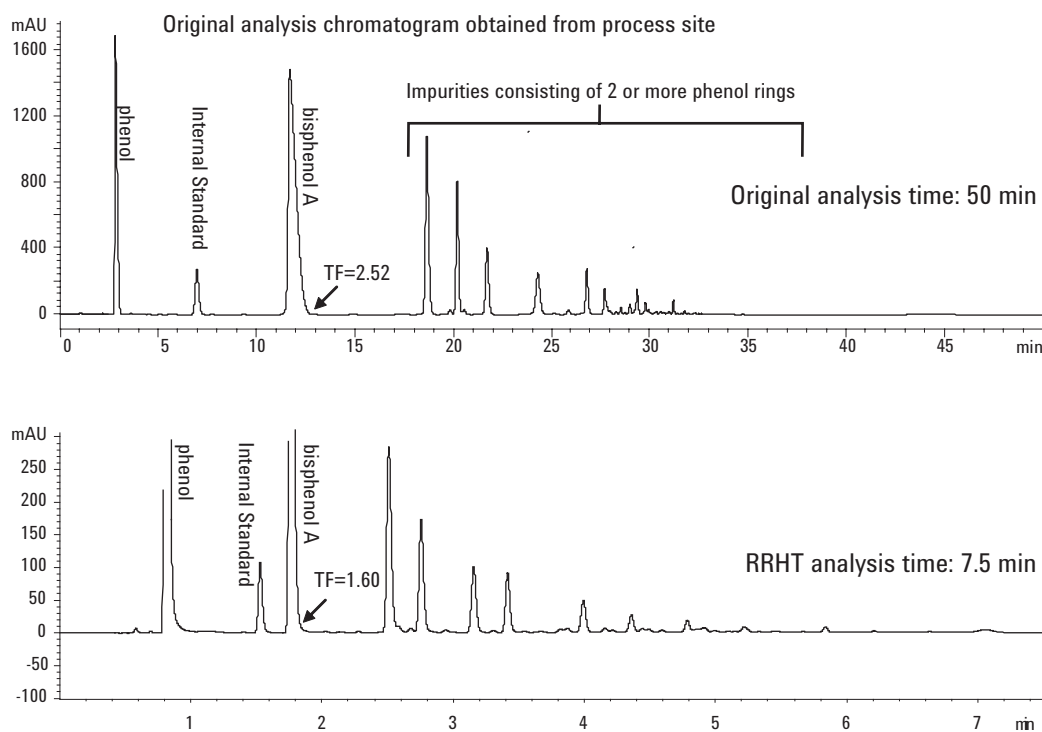


Figure 4. Comparison of methods; original to RRHT.

Table 1. Current and Improved Method Parameters

Original		RRHT	
• Column: Supelco LC-18, 4.6 × 250 mm, 5 µm • Mobile phase: A: 0.025% H₃PO₄, B: ACN, C: MeOH • Flow: 2 mL/min • Temperature: 35 °C • Sample size: 20 µL • Gradient: segmented, has isocratic holds		• Column: ZORBAX XDB-C18, 4.6 × 50 mm, 1.8 µm • Mobile phase: A: 0.1% formic acid, B: ACN: MeOH (200:800) • Flow: 1 mL/min • Temperature: 25 °C • Sample size: 2 µL • Gradient: linear, no isocratic holds	
Time	% A:B:C	Time	% B
0	65:25:10	0	60
13	65:25:10	6	95
18	50:40:10	6.01	60
23	50:40:10	8	60
27	30:50:20		
32	0:70:30		
35	0:70:30		
36	0:60:40		
40	0:50:50		
43	0:20:80		
48	65:25:10		

Conclusion

Converting an existing method to a high-throughput method is one way to improve lab productivity. Using RRHT columns initially for method development also improves productivity. Eclipse XDB-C18 RRHT columns are a good choice for converting existing C18 methods into high-throughput methods. Smaller particles packed into shorter columns provide comparable resolution to larger particles packed into longer columns in a fraction of the time. RRHT columns are advantageous for gradient method development because gradient re-equilibration is time-consuming and often overlooked in the total analysis time. Methods developed on Agilent RRHT columns can be scaled easily because of the highly uniform particles, bonded phase chemistry, and column manufacturing techniques. An existing method developed on a “traditional analytical-sized” column was easily converted to a high throughput method using an Eclipse XDB-C18 RRHT column. The method was incrementally scaled up to an analytical-sized column, and it performed with predictable results

on various column dimensions and particle sizes. The predictability of the results supports Eclipse XDB-C18 RRHT columns’ ability to easily improve applications and transfer them into high-throughput and high-resolution applications.

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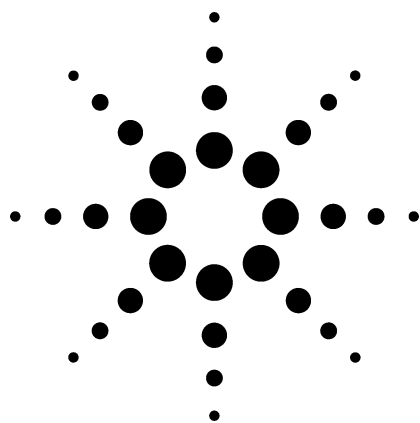
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Fast Analysis of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator

Application

Hydrocarbon Processing

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Abstract

Vitamin E (tocopherol), phenolic antioxidants and erucamide slip additives in polypropylene homopolymer formulations were resolved and detected using liquid chromatography with ultraviolet/visible detection, under guidelines suggested by ASTM Method D6042. Using the Agilent 1200 Rapid Resolution LC system with Agilent ZORBAX RRHT columns, the antioxidants could be rapidly separated with the same or improved resolution. The Agilent method translator was used to transfer the ASTM method into new methods based on the instrument parameters, column dimensions, and particle size in three modes: simple conversion and speed optimized and resolution optimized methods.

Introduction

Polymers are very popular all over the world owing to their unprecedented physical properties. Various additives are blended into polymeric materials to modify certain properties of the polymer formulation. Erucamide, Irganox 3114, Irganox 1010, Vitamin E (tocopherol), Irganox 1076, and Irgafos168 are often used as antioxidants to prevent the degradation of polypropylene homopolymer formulations by light, heat, and oxygen. In this work, with the goal to shorten the analysis time and reduce solvent consumption without losing separation quality, the existing ASTM method was recalculated for new operating conditions based on columns packed with smaller particle sizes. The chemical information of the antioxidants and Tinuvin P as internal standard is displayed in detail in Table 1.

Specific additives and their concentrations in polymer formulations are critical to the properties of polymer, and careful analysis is required to ensure that the additives and levels are appropriate for the intended use. This application will compare two different stationary phases according to analyte retention characteristics and peak shape, show the influence of different injection volume of real sample on the peak shape, and then will focus on showing how to use the method translator. The latter is used to transfer the conventional method to new methods using smaller size columns to perform simple conversion and to extend the method to greater speed and higher resolution.

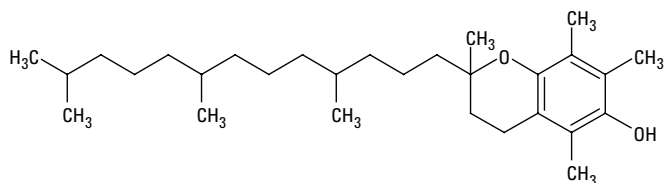


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Table 1. Chemical Information of Antioxidants and Tinuvin P

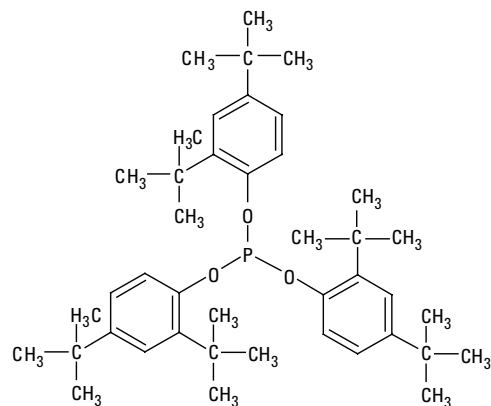
Name: Vitamin E
Formula: $C_{29}H_{50}O_2$
Molecular Weight: 430.71
CAS No.: 10191-41-0

DL-all-rac- α -Tocopherol



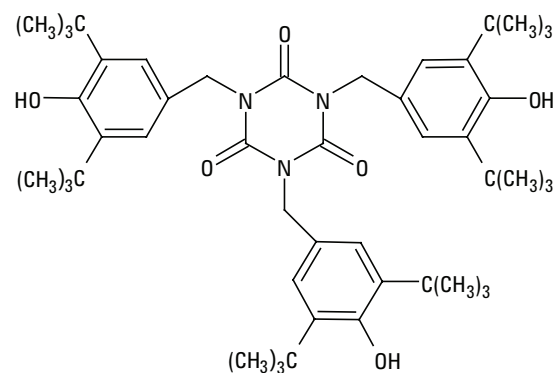
Name: Irgafos 168
Formula: $[(CH_3)_3C]_2C_6H_3O]_3P$
Molecular Weight: 646.92
CAS No.: 31570-04-4

Tris(2,4 di-tert-butylphenyl) phosphite



Name: Irganox 3114
Formula: $C_{48}H_{69}N_3O_6$
Molecular Weight: 784.08
CAS No.: 27676-62-6

Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate



Name: Erucamide
Formula: $CH_3(CH_2)_7CH=CH(CH_2)_{11}CONH_2$
Molecular Weight: 337.58
CAS No.: 112-84-5

cis-13-docosenamide

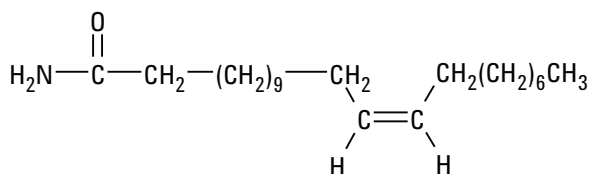
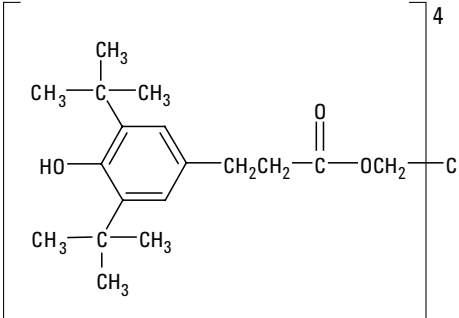
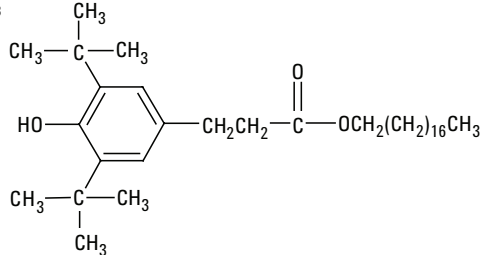
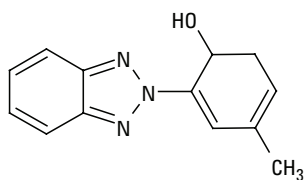


Table 1. Chemical Information of Antioxidants and Tinuvin P (Continued)

Name:	Irganox 1010	Pentaerythritol tetrakis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)
Formula:	$[\text{HOC}_6\text{H}_2[\text{C}(\text{CH}_3)_3]_2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_2]_4\text{C}$	
Molecular Weight:	1177.63	
CAS No.:	6683-19-8	
Name:	Irganox 1076	Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate
Formula:	$[(\text{CH}_3)_3\text{C}]_2\text{C}_6\text{H}_2(\text{OH})\text{CH}_2\text{CH}_2\text{CO}_2(\text{CH}_2)_{17}\text{CH}_3$	
Molecular Weight:	530.86	
CAS No.:	2082-79-3	
Name:	Tinuvin P	2-(2-hydroxy-5-methylphenyl)benzotriazole
Formula:	$\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}$	
Molecular Weight:	225.25	
CAS No.:	2440-22-4	

Experimental

System

Agilent 1200 Series Rapid Resolution LC (RRLC), consisting of:
G1379B micro vacuum degasser
G1312B binary pump SL
G1367C high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL with 3 mm, 2 µL flow cell
ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 50 mm, 1.8 µm

Mobile Phase

Gradients: A: water
B: acetonitrile (ACN)
Gradient conditions: See individual chromatograms
Column temperature: See individual chromatograms

Samples

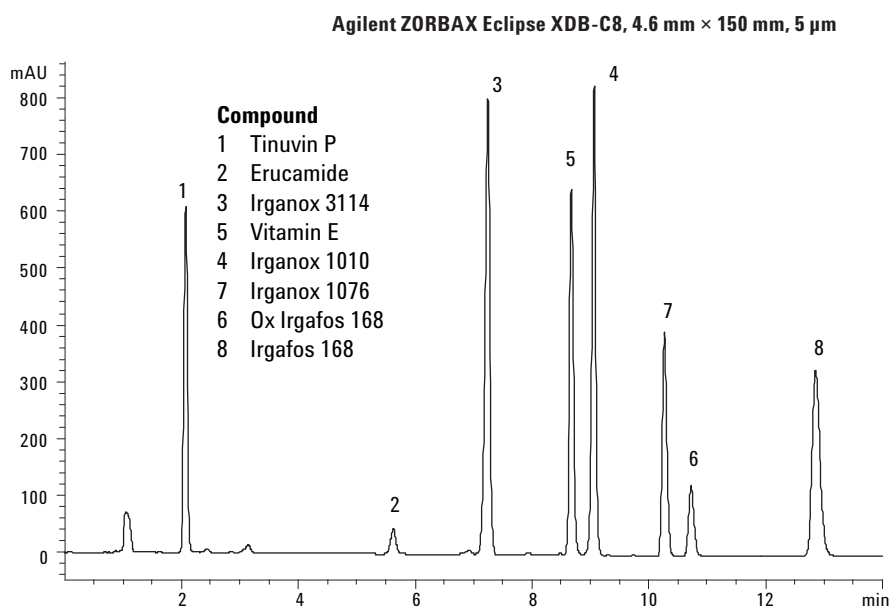
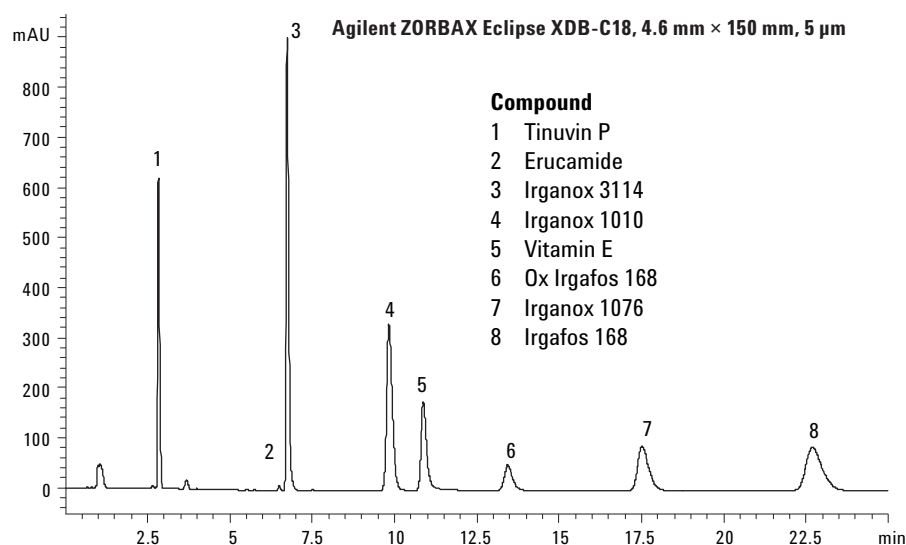
- Standard mixture of Tinuvin P, Erucamide, Irganox 3114, Irganox 1010, Vitamin E, Irganox 1076, and Irgafos168, all 200 µg/mL in isopropanol
- Polypropylene Homopolymer Formulation, from customer, extracted by ultrasonic according to the method ASTM D6042-04
- Polypropylene extract spiked with 20 µg/mL standard mixture

Results and Discussion

Selection of Stationary Phase for the Separation of Antioxidants

It is desirable during method development to select a column that will provide the optimal analyte separation and shortest analysis time. Reversed phase C18 columns are recommended by ASTM D6042-04; however, in our application we deter-

mined that the retention characteristics of ZORBAX XDB-C18 columns were too strong for the specified solvents, resulting in broad peak shape and quantitation difficulties for late-eluting peaks. Compared with ZORBAX XDB-C18 columns, ZORBAX XDB-C8 columns showed better retention capability and peak shape. Therefore, we chose the ZORBAX XDB-C8 column for further method development. The different separations with ZORBAX XDB-C18 and ZORBAX XDB-C8 columns are shown in the Figure 1.



Conditions

Mobile phase:	A: water; B: ACN	ZORBAX chemistry:	Eclipse XDB-C18	Eclipse XDB-C8
Flow rate:	1.5 mL/min	Gradient:	Min %B	Min %B
Wavelength:	200 nm		0.00 75	0.00 75
Injection volume:	10 μL		5.00 100	8.00 100
Column temperature:	50 °C		25.00 100	15.00 100
Column size:	4.6 mm × 150 mm, 5 μm		25.10 75	15.10 75
Sample:	Standard mixture, 200 μg/mL in isopropanol		30.00 75	20.00 75

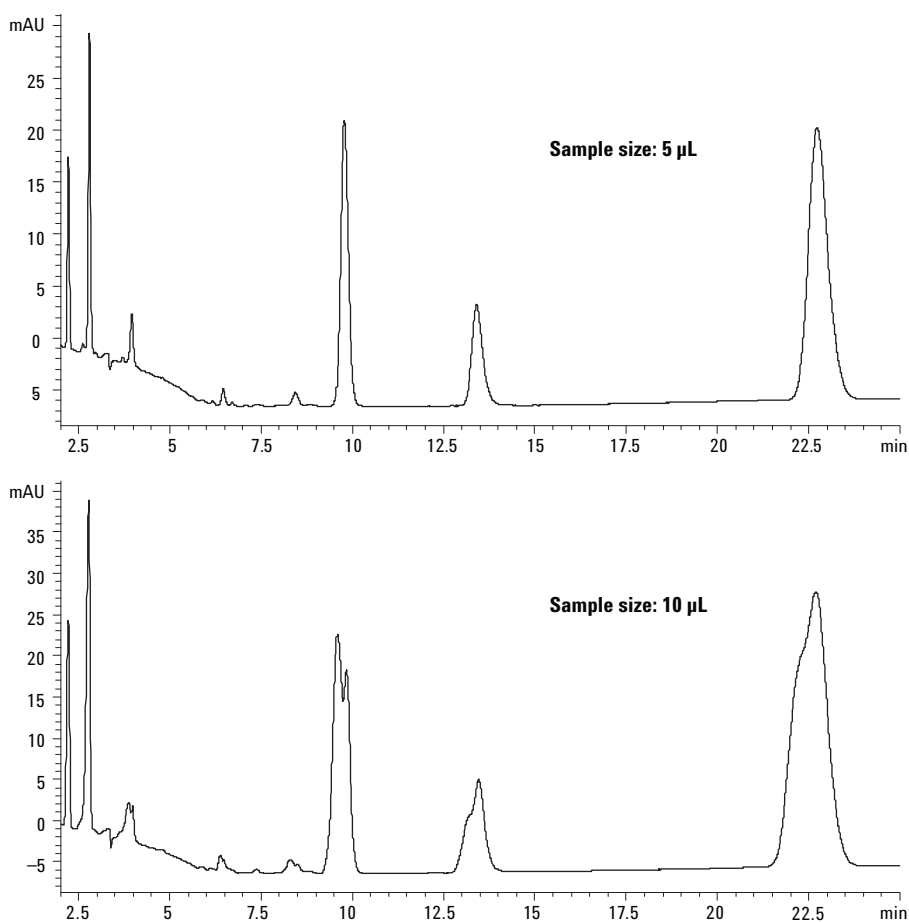
Figure 1. ZORBAX stationary phase comparison for antioxidants.

Injection Volume Influence of Real Sample Extraction Solution on the Peak Shape

According to ASTM D6042-04 [1], a solvent mixture of methylene chloride and cyclohexane (1/1 v/v) is used as the extraction solvent and, after filtration, the extracted solution is directly injected into the LC. Neither methylene chloride nor cyclohexane is miscible in the acetonitrile and water mobile phase. Peak splitting was observed when the injection volume was 10 μ L. We decreased the sample size of real sample and found that the volume of 5 μ L was suitable and free of solvent influence. The split and nonsplit peaks are shown in Figure 2. At the same time, the influence of injection volume was not found in the standard solution, which was dissolved in isopropanol per ASTM method guidance.

Fast Method Developed Based on New 1200 RRLLC with Method Translator

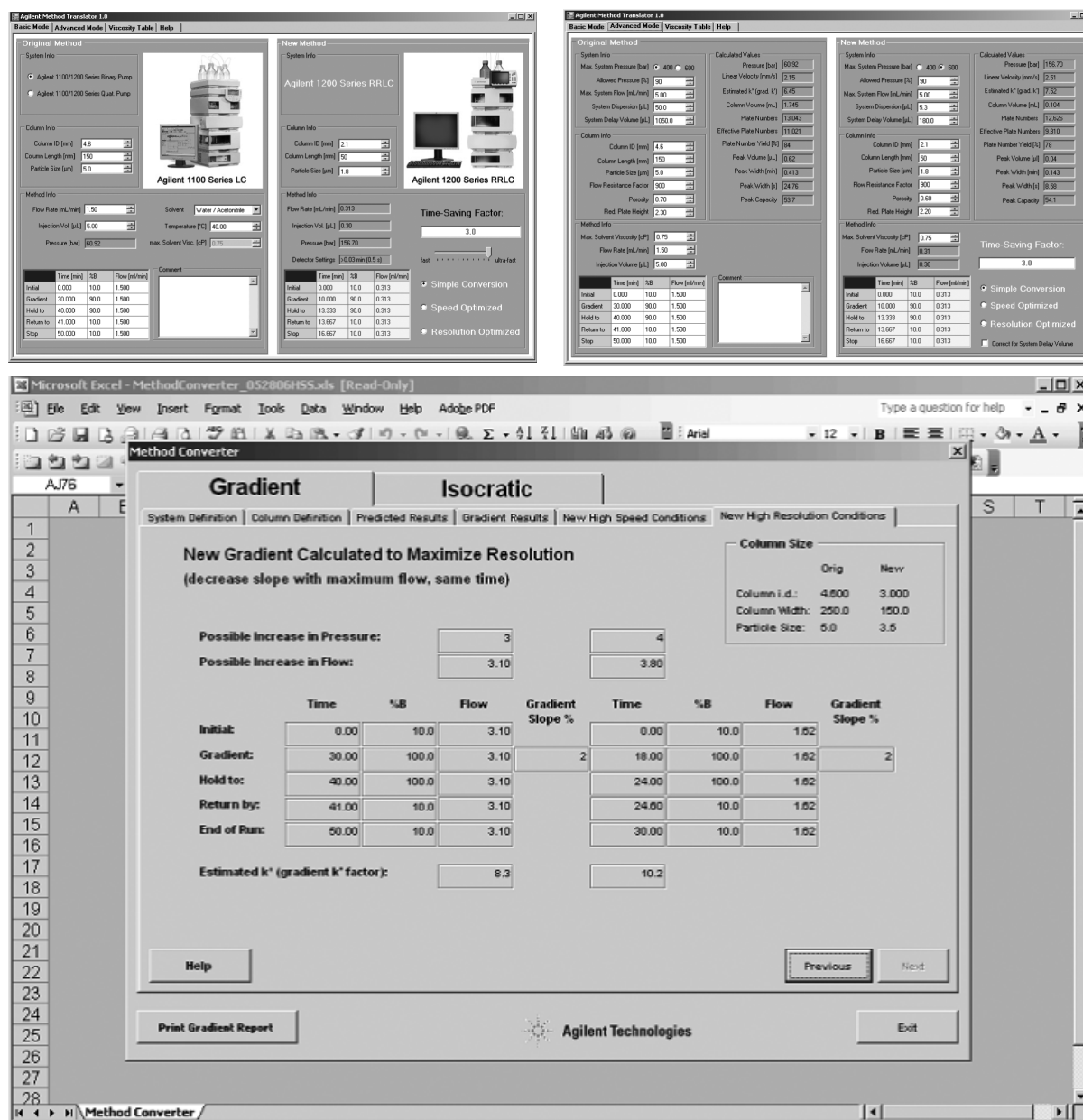
Due to the appearance of sub-two-micron columns and LC systems with higher pressure capabilities, the research of ultra-fast separation is more and more popular. Therefore, it is important to quickly and easily transfer conventional methods to fast or high-resolution methods. Agilent provides the users of RRLLC systems with two versions of method translators; one is a Microsoft.net version, which requires that Net-Framework 2.0 be resident on the computer, the other is a Microsoft Excel version, which requires that Excel be resident on the PC. The interface of the two translators is displayed in Figure 3.



Conditions

Mobile phase:	A: water; B: ACN	Gradient:	
Flow rate:	1.5 mL/min	Min	%B
Wavelength:	200 nm	0.00	75
Injection volume:	5 or 10 μ L	5.00	100
Column temperature:	50 $^{\circ}$ C	25.00	100
Column:	ZORBAX Eclipse XDB-C18	25.10	75
	4.6 mm $\times$ 150 mm, 5 μ m		
Sample:	Polypropylene extraction solution	30.00	75

Figure 2. Injection volume influence of real sample extraction solution on the peak shape.



The upper one is the Microsoft.net version, the lower one is the Microsoft Excel version.

Figure 3. Two different method translators.

Sample Preparation

The two versions of method translators provide three modes of method conversion; the first is the simple conversion, which has the same gradient slope as the conventional method, and changes the flow rate according to equation 1:

$$Flow_{Col.2} = \left[\frac{Diam_{Col.2}}{Diam_{Col.1}} \right]^2 \times Flow_{Col.1} \quad (\text{eq. 1})$$

The second is the speed optimized conversion, which has the same gradient slope as the conventional method and maximizes the flow rate according to the LC system pressure capability. The last is

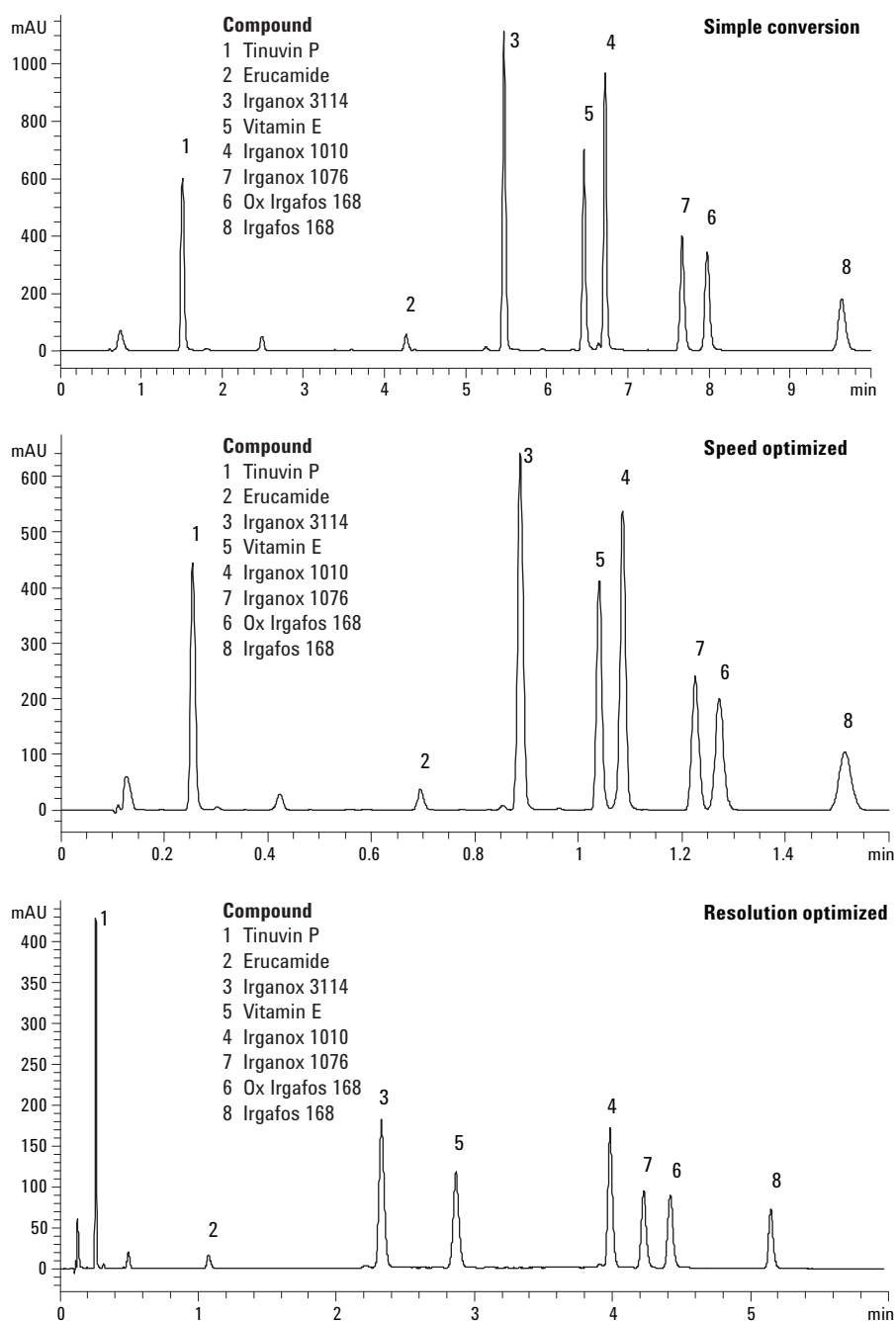
the resolution optimized conversion, which maximizes the flow rate according to the LC system pressure capability and has the same gradient time as the simple converted mode, resulting in a reduced gradient slope that normally yields higher peak resolution. For the different columns, the injection volumes should be changed according to the relationship displayed in equation 2.

$$Inj. vol._{Col.2} = \left[\frac{Volume_{Col.2}}{Volume_{Col.1}} \right] \times Inj. vol._{Col.1} \quad (\text{eq. 2})$$

As mentioned above, the method based on the ZORBAX Eclipse XDB-C8 4.6 mm x 150 mm, 5 µm, was selected as the initial method. Afterwards, the initial method was transferred with the method

translator into three modes on different column lengths (100, 50 mm) and particle sizes (3.5, 1.8 μm), respectively. Figures 4 and 5 show

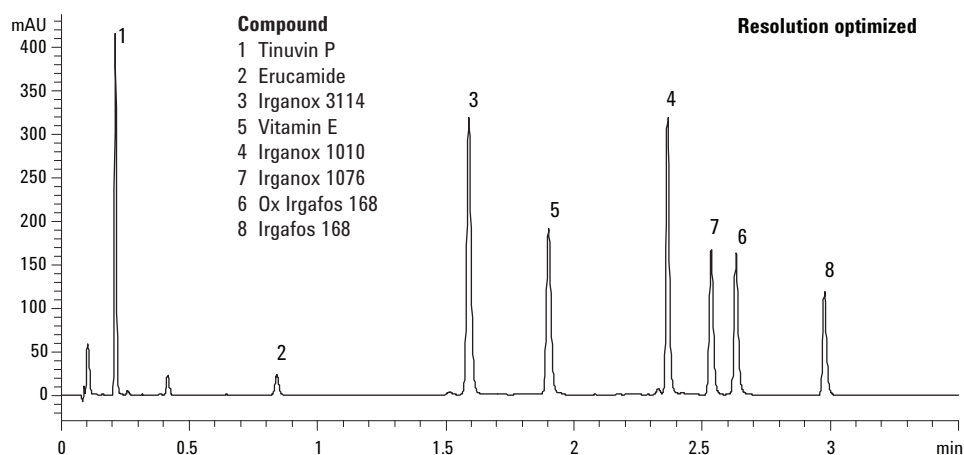
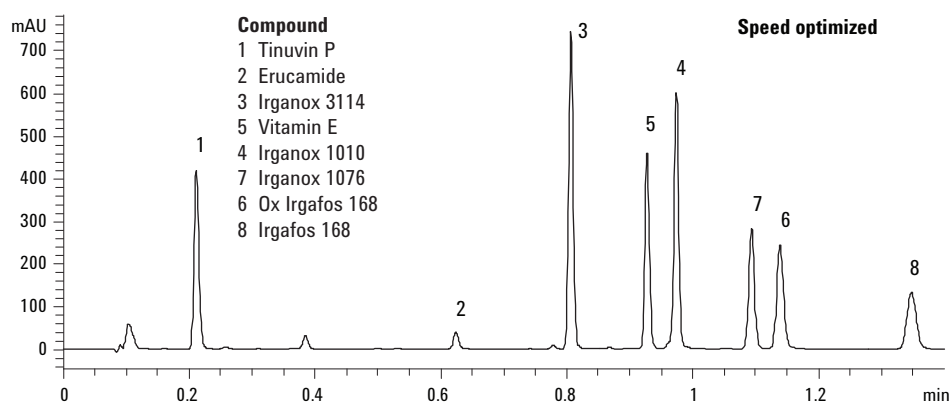
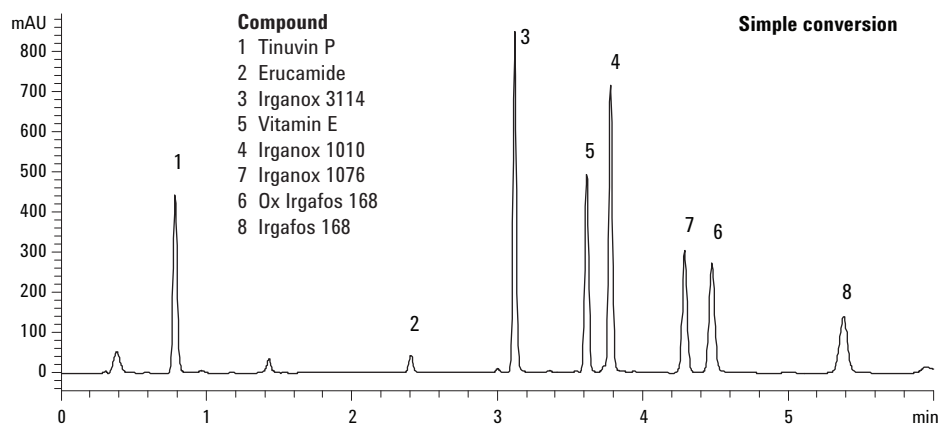
the separation of antioxidants in smaller particle size columns with the recalculated methods.



Conditions

Sample:	Standard mixture, 200 $\mu\text{g/mL}$ in isopropanol			
Mobile phase:	A: water; B: ACN			
Temperature:	50 $^{\circ}\text{C}$			
Wavelength:	200 nm			
Injection volume:	6.7 μL			
Column:	ZORBAX Eclipse XDB-C8 3.0 mm $\times$ 100 mm, 3.5 μm			
Mode:	Simple converted	Speed optimized	Resolution optimized	
Flow rate:	0.64 mL/min	4.00 mL/min	4.00 mL/min	
	Pressure:	110 bar	460 bar	460 bar
	Gradient slope:	3.1%	3.1%	0.5%
	Analysis time:	10 min	1.6 min	5.5 min

Figure 4. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm $\times$ 100 mm, 3.5 μm .



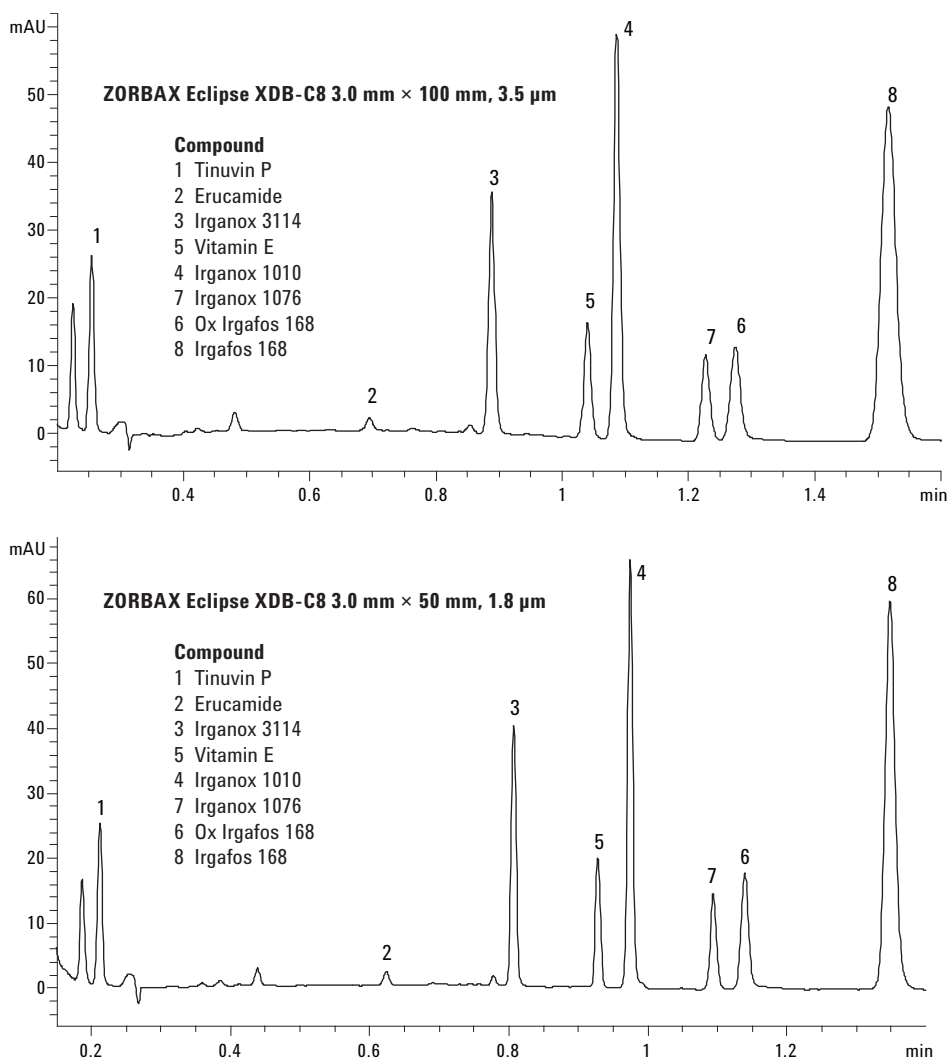
Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol		
Mobile phase:	A: water; B: ACN		
Temperature:	50 °C		
Wavelength:	200 nm		
Injection volume:	3.3 µL		
Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm		
Mode:	Simple converted	Speed optimized	Resolution optimized
Flow rate:	0.64 mL/min	2.50 mL/min	2.50 mL/min
Pressure:	160 bar	460 bar	460 bar
Gradient slope:	3.1%	3.1%	0.8%
Analysis time:	6 min	1.4 min	3 min

Figure 5. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm.

To identify the matrix influence on the separation, the polypropylene extract was spiked with 20 µg/mL standard mixture and injected into the LC system. Figure 6 depicts the separation of

spiked sample with the speed optimized method, which shows a sufficient separation of antioxidant in polymer matrix with about 10 times faster speed than the conventional method mentioned above.



Conditions

Sample:	Polypropylene extract spiked with 20 µg/mL standard mixture	
Mobile phase:	A: water; B: ACN	
Temperature:	50 °C	
Wavelength:	200 nm	
Stationary phase:	ZORBAX Eclipse XDB-C8	
Column size:	3.0 mm × 100 mm, 3.5 µm	3.0 mm × 50 mm, 1.8 µm
Injection volume:	3 µL	1 µL
Mode:	Speed optimized	Speed optimized
Flow rate:	4.00 mL/min	2.50 mL/min
Pressure:	460 bar	460 bar
Gradient slope:	3.1%	3.1%
Analysis time:	1.6 min	1.4 min

Figure 6. Separation of spiked polypropylene extract by the speed optimized method.

Conclusions

As an important innovation in the advancement of liquid chromatography, the Agilent 1200 Rapid Resolution LC system provides the customer not only a rapid separation with the same or similar resolution, but also includes a method translator to convert any initial conventional method to a fast or high-resolution method according to the requirements of the user. This note applies the method translation tool in the separation of polymer additives and demonstrates the ease-of-use and power of the method translator using separations of a standard mixture and spiked real sample.

References

1. ASTM D6042-04, "Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using Liquid Chromatography (LC)"
2. Michael Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations"
3. Michael Woodman, "Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection"

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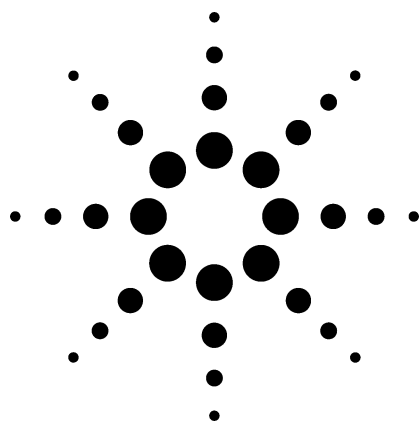
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Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC

Application

Hydrocarbon Processing

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Abstract

Liquid chromatography with ultraviolet/visible (UV/VIS) detection is a powerful approach for analyzing additives in polymer formulations. This application illustrates the use of the Agilent 1200 Series Rapid Resolution LC (RRLC) system for the separation of antioxidants and erucamide. The system can operate significantly faster than conventional HPLC without sacrificing resolution, precision, or sensitivity. The column chemistry and temperature influence on the separation and the sample preparation method are also discussed.

Introduction

Additives are incorporated into various polymeric materials to retard the degradation caused by ultraviolet light, heat, and oxygen or to modify processing characteristics. A rapid and accurate analytical method is required to ensure that the specified amount of an additive or combination of additives is incorporated into a polymer after the extrusion process. Conventional HPLC methods for additives [1,2] often require more than 30 minutes per analysis, while the application described here can achieve comparable results in as few as 3 minutes.

Agilent has developed an easy-to-use method conversion tool for transferring existing methods for higher speed and/or higher resolution. The tool was used for the method optimization in this application. [3]

This application examines additives mentioned in ASTM Methods D5815 and D1996. The chemical structures are shown in Table 1.



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Table 1. Polymer Additives in ASTM Methods D5815 and D1996

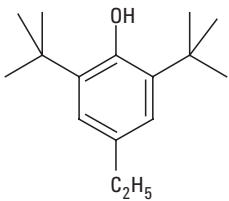
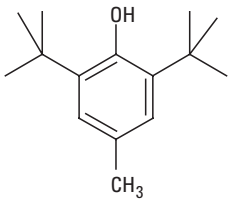
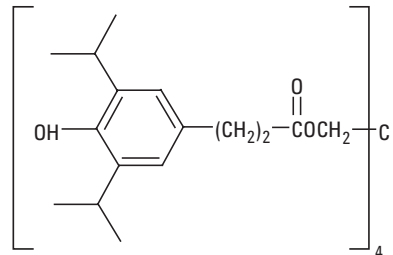
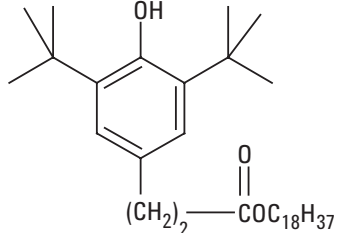
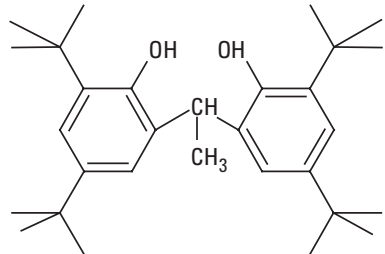
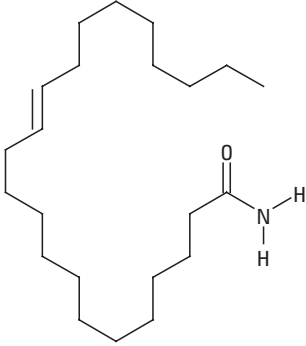
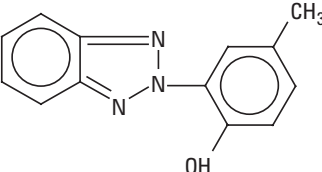
Registered trade name	CAS no.	Chemical name	Chemical structure
BHEB	4310-42-1	2,6-di-tert-butyl-4-ethyl-phenol or butylated hydroxyethyl benzene	
BHT	128-37-0	2,6-di-t-butyl-cresol or butylated hydroxy toluene	
Irganox 1010	6683-19-8	Tetrakis[methylene(3,5-di-t-butyl-4-hydroxy hydrocinnamate)] methane	
Irganox 1076	2082-79-3	Octadecyl-3,5-di-t-butyl-4-hydroxy hydrocinnamate	
Isonox 129	35958-30-6	2,2-ethylidene bis (4,6-di-t-butyl phenol)	

Table 1. Polymer Additives in ASTM Methods D5815 and D1996 (Continued)

Registered trade name	CAS no.	Chemical name	Chemical structure
Kemamide-E	112-84-5	Cis-13-docosenamide or Erucamide or Fatty acid amide (C ₂₂ H ₄₃ NO)	
Tinuvin P	2440-22-4	2(2'-hydroxy-5'-methyl phenyl) benzotriazole	

Experimental

System

Agilent 1200 Series rapid-resolution LC configured with
G1379B microvacuum degasser
G1312B binary pump SL
G1367B high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL
ChemStation 32-bit version B.02.01

Column

ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm
ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 μm
ZORBAX SB-C18, 4.6 mm × 150 mm, 5 μm
ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 μm

Mobile phase

Gradients: A: water
B: acetonitrile (ACN)

Gradient slope: See individual chromatograms for flow rate and gradient time

Column temperature: See individual chromatograms

Samples

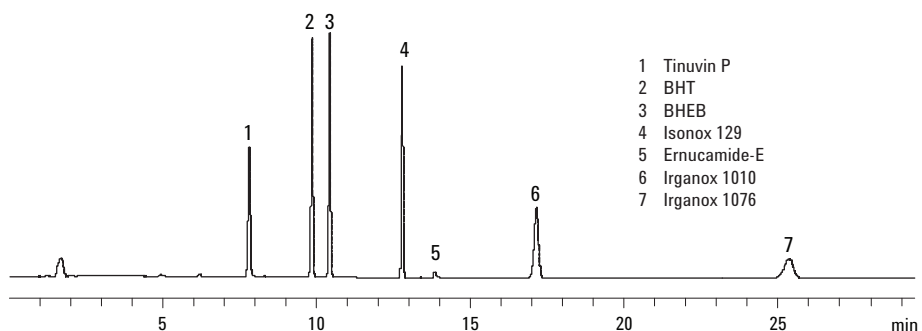
1. Standard mixture described in ASTM D5815 and D1996, 50 μg/mL, 200 μg/mL in isopropanol
2. Linear low-density polyethylene from customer, ground to 20 mesh, extracted by ultrasonic or reflux method

Results and Discussion

Fast Method Conversion

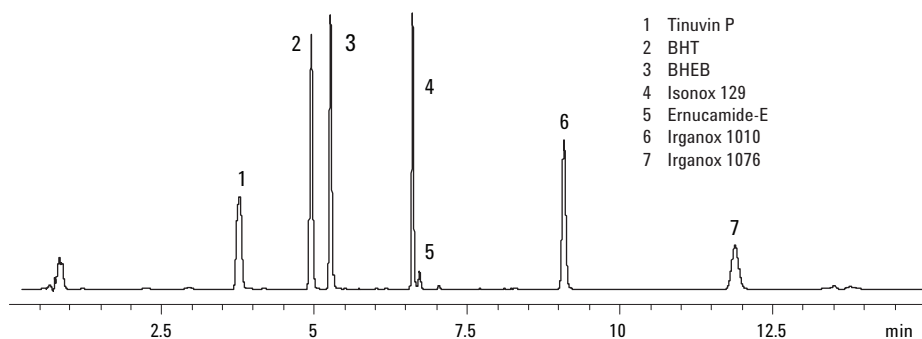
The separation was initially performed on a standard 4.6 mm × 150 mm, 5-μm ZORBAX Eclipse XDB-C18 column thermostatted to 60 °C (Figure 1) following the conditions in ASTM D5815 (or D1996). The method was then scaled in flow and time for exact translation to a 2.1 mm × 50 mm, 1.8-μm column (Figure 2). The analysis time was reduced from 25.5 to 12.5 minutes, and the solvent consumption was reduced from 25 to 2.5 mL.

The separation was then re-optimized for faster separation with the same gradient slope by increasing the flow rate from 0.21 to 0.9 mL/min and proportionately reducing the gradient time (Figure 3), achieving up to 10 times faster than conventional HPLC without sacrificing resolution, precision (showed in Table 2), or sensitivity. Figure 4 demonstrates that 1 ppm of additives can be determined with very good signal-to-noise response using the same condition in Figure 3, which exceeds the specification of 2 ppm of ASTM D5815 (or D1996). Peak 6, Irganox 1010, for example has a signal-to-noise response of 88 at 1 ppm.



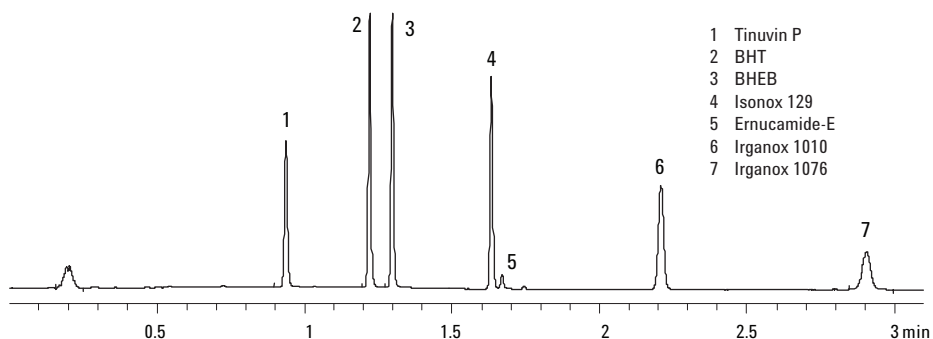
Conventional method:	Follow ASTM D5815 (or D1996) method with	Gradient	
	ZORBAX Eclipse XDB-C18,		%B
	4.6 mm × 150 mm, 5 μm	0	50
Sample:	Standard 50 μg/mL	11	100
Sample size:	10 μL	28	100
Detector:	UV 200 nm	28.1	50
Column temperature:	60 °C		
Mobile phase:	A: water		
	B: acetonitrile		
Flow rate:	1 mL/min		

Figure 1. Separation of additives standards on Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm.



Simple-converted:	Translate the conventional method to a	Gradient	
	ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 μm		%B
Sample:	Standard 50 μg/mL	0	50
Sample size:	2 μL	5.2	100
Detector:	UV 200 nm	12	100
Column temperature:	60 °C	12.1	50
Mobile phase:	A: water	15	50
	B: acetonitrile		
Flow rate:	0.21 mL/min (73 bar)		

Figure 2. Separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 μm.

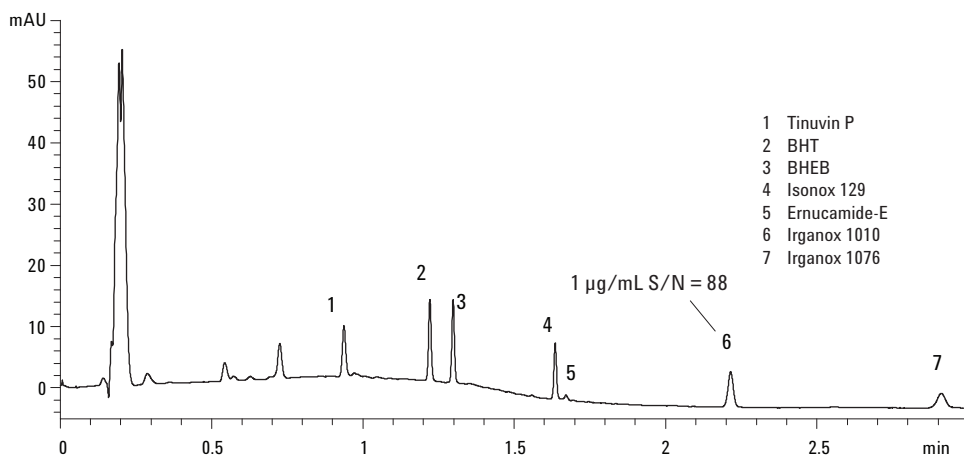


Speed-optimized:	Optimize the conventional method for speed with	Gradient	
	ZORBAX Eclipse XDB-C18,	%B	
	2.1 mm × 50 mm, 1.8 µm	0	50
Sample:	Standard 50 µg/mL	1.3	100
Sample size:	2 µL	3	100
Detector:	UV 200 nm	3.1	50
Column temperature:	60 °C	3.5	50
Mobile phase:	A: water		
	B: acetonitrile		
Flow rate:	0.9 mL/min (357 bar)		

Figure 3. Fast separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Table 2. Repeatability for the Methods of Conventional, Simple-Converted, and Speed-Optimized Methods (n = 5)

Compounds (50 ppm)	Conventional	Area, RSD%	
		Simple-converted	Speed-optimized
Tinuvin P	0.37	0.39	0.09
Erucamide	0.40	0.57	0.13
Irganox 3114	0.44	0.49	0.22
Irganox 1010	0.38	0.39	0.26
Vitamin E	0.58	0.80	0.68
Irganox 1076	0.58	1.49	0.17
Irgafos 168	0.53	0.77	0.32



Speed-optimized method for analysis of additives standards with concentration of 1 µg/mL LC conditions is identical to that in Figure 3

Figure 4. Fast separation of 1 µg/mL additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Optimized Column Temperature

Increasing column temperature can lower both solvent viscosity and nonspecific column/analyte interactions. The new ZORBAX StableBond RRHT columns can operate at temperatures up to 90 °C. We tested operating temperatures at 60, 75, 85, and 90 °C with a ZORBAX SB-C8 4.6 mm × 150 mm, 5-μm column. The results (Figure 5) show that the analysis time obtained from 60 °C to 85 °C is reduced from 23.5 minutes to 17 minutes; at 90 °C, only an additional 0.5 minute is saved. Based on the combined speed reduction and optimized resolution of peaks 4 and 5, 85 °C is chosen as a suitable column temperature.

The method was then scaled in flow and time for exact translation to a 4.6 mm × 50 mm, 1.8-μm column (Figure 6). Finally, the separation was optimized for faster separation by increasing the flow rate from 1 mL/min to 3.5 mL/min, with only a 1.7-minute analysis time (Figure 7). This is really an excellent procedure for high-throughput screening and quantitation of a large number of samples. Figure 8, the separation of an extract of linear low-density polyethylene (LLDPE) spiked with 20 μg/mL of standard solution, shows excellent separation with real sample matrix.

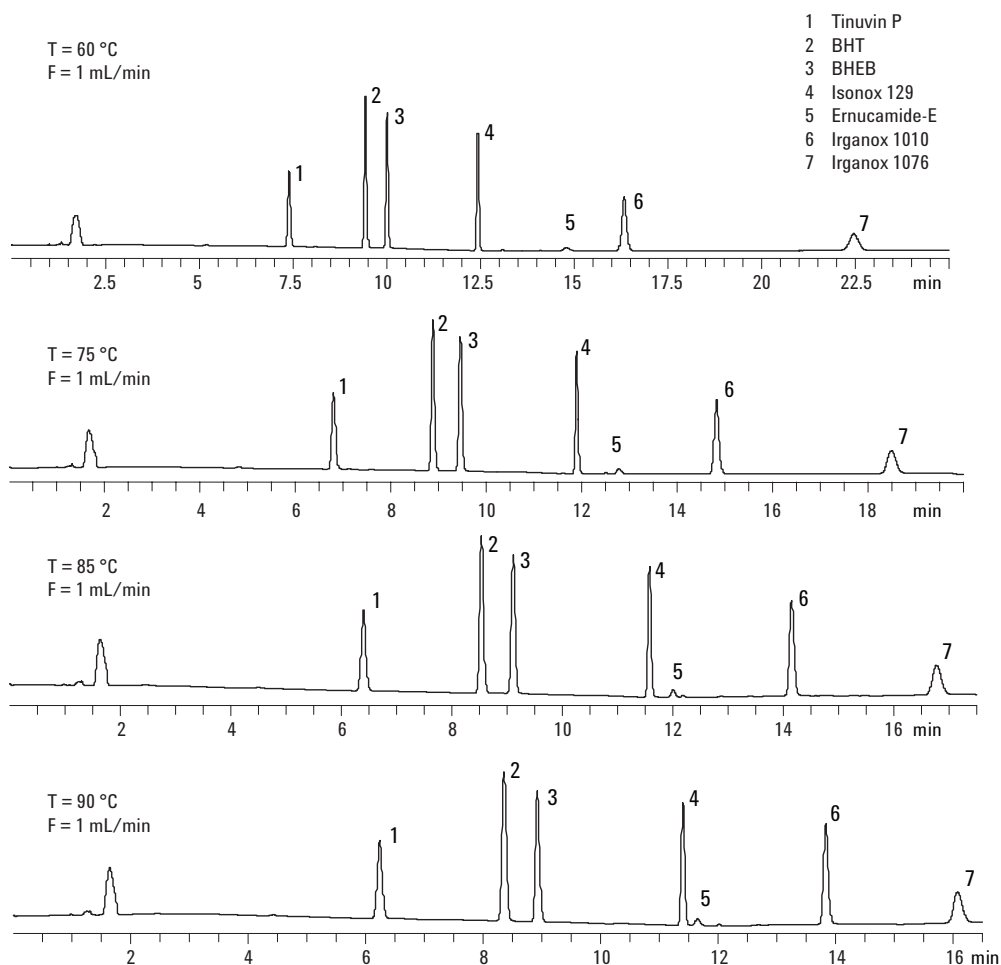
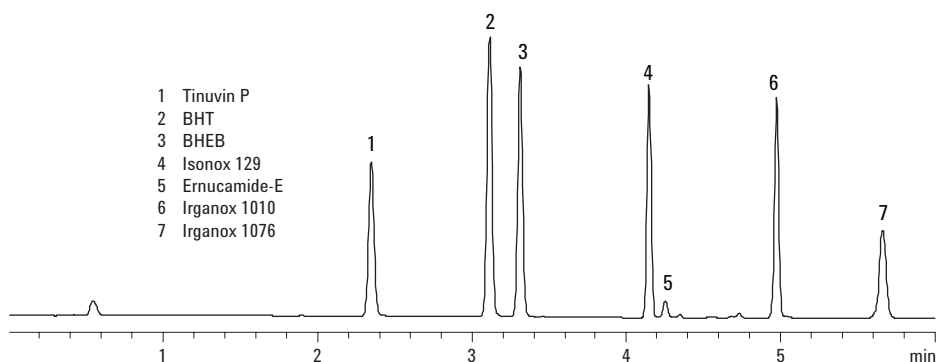
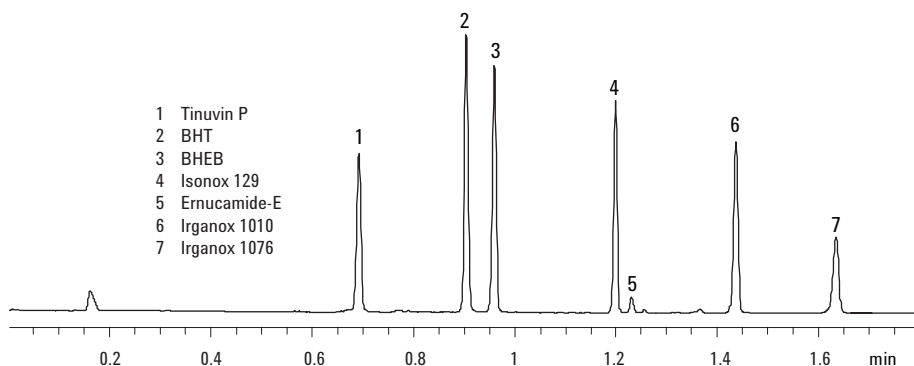


Figure 5. Separation of additives standards on ZORBAX StableBond RRHT SB-C18, 4.6 mm × 150 mm, 1.8 μm.



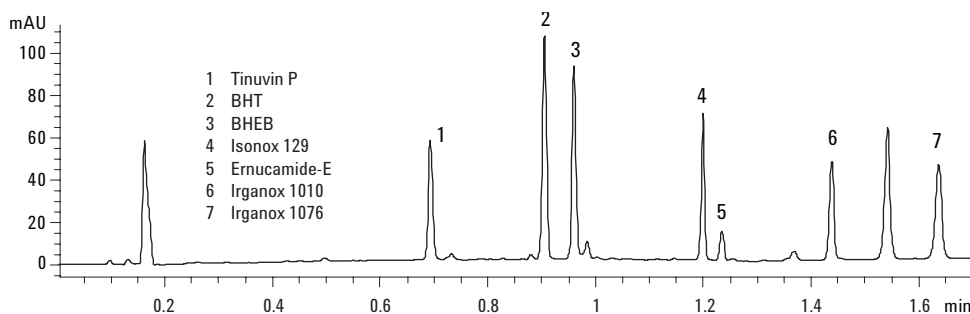
Sample: Standard 200 mg/mL
Sample size: 2 μ L
Detector: UV 200 nm
Mobile phase: A: water
B: acetonitrile
Gradient slope: 6.8%
Flow rate: 1 mL/min

Figure 6. Separation of additives standards on ZORBAX SB-C18, 4.6 mm $\times$ 50 mm, 1.8 μ m, at 85 $^{\circ}$ C.



Sample: Standard_200 mg/mL
Sample size: 2 μ L
Detector: UV 200 nm
Mobile phase: A: water
B: acetonitrile
Gradient slope: 6.8%
Flow rate: 3.5 mL/min

Figure 7. Fast separation of additives standards on ZORBAX SB-C18, 4.6 mm $\times$ 50 mm, 1.8 μ m, at 85 $^{\circ}$ C.



LC conditions are identical with those in Figure 7.

Figure 8. Fast separation of spiked real sample-LLDPE (20 μ g/mL) on ZORBAX SB-C18, 4.6 mm $\times$ 50 mm, 1.8 μ m, at 85 $^{\circ}$ C.

Sample Preparation

ASTM D5815 (or D1996) method recommends using a reflux apparatus for extracting additives in polymer. This requires periodic operator intervention over the 1.5-hour-long extraction period. To find a time-saving sample-preparation method, ultrasonic extraction was also tested, producing comparable results in 30 minutes. In terms of extraction efficiency, there is not much difference between these two methods. Figure 9 shows very good overlays of extractions by reflux and ultrasonic extraction methods for a LLDPE. Conditions are identical to those in Figure 1.

Conclusions

Liquid chromatography with ultraviolet/visible detection is an effective tool for analyzing additives in polymer formulations. The Agilent 1200 Series RRLC system equipped with RRHT 1.8- μ m columns was used to achieve up to 10 times faster than the conventional HPLC method. The ultrasonic extraction method allowed fast extraction without user intervention for a significant reduction in overall analysis time. Total time saved was more than 80 minutes per sample when compared

to the conventional analysis and extraction methods.

References

1. ASTM D5815-95, "Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Linear Low-Density Polyethylene Using Liquid Chromatography (LC)."
2. ASTM D1996-97, "Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Low-Density Polyethylene Using Liquid Chromatography (LC)."
3. Agilent Application Compendium CD, 5989-5130EN, June 2006.
4. Michael Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations," Agilent Technologies, publication 5989-5177EN.

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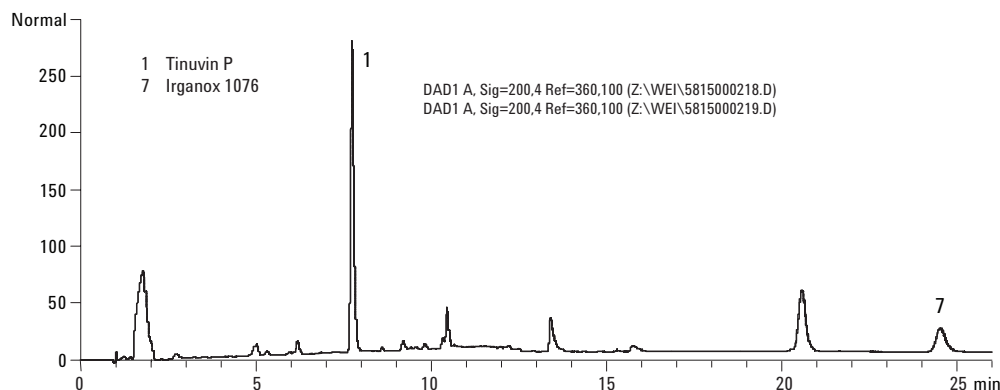


Figure 9. Chromatogram Overlays of extractions by reflux and ultrasonic extraction methods for LLDPE.

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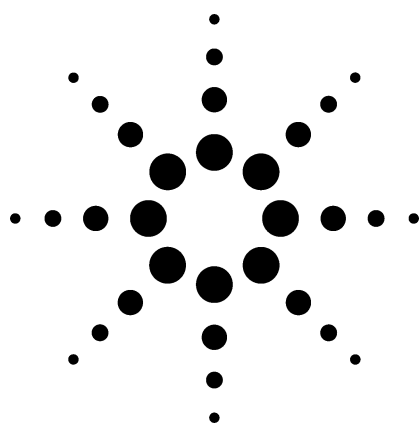
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Fast Analysis Method for Rubber Chemical Antidegradants Using 1200 Rapid Resolution Liquid Chromatography (RRLC) Systems with Rapid Resolution High Throughput (RRHT) Columns

Application

Hydrocarbon Processing

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Abstract

***p*-Phenylenediamine (PPD), a chemical antidegradant for rubber, and its analogs were found to be optimally determined using liquid chromatography with ultraviolet/visible detection according to the guidelines of ASTM Method D5666. Using the Agilent 1200 RRLC system with ZORBAX 1.8- μ m columns, the PPDs could be separated in one run with a total analysis time up to 6.4 times faster than the conventional method based on a 5- μ m column.**

Introduction

Various additives are artificially incorporated into polymeric materials to modify certain properties of the polymer. Therefore, the additives and their

concentration in the formulation are crucial to the properties of the end product. 77PD, DTPD, IPPD, PPD, and 6PPD (see Table 1) are often used as chemical antidegradants for rubber materials. The chemical information for five PPDs is displayed in detail in Table 1.

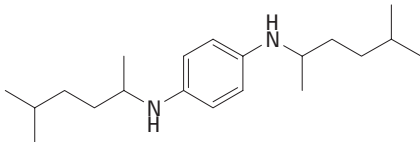
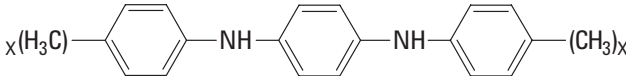
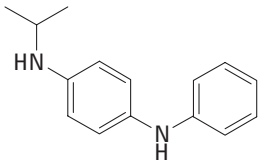
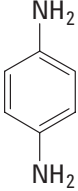
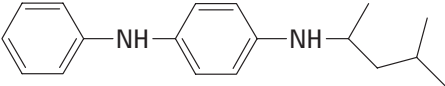
Liquid chromatography with ultraviolet/visible detection is a powerful approach to the qualitative and quantitative analysis of chemical antidegradants in rubber. The isocratic LC method for five PPDs is introduced by ASTM D5666. In this method, the five PPDs are divided into three groups and determined by three different methods (Table 1, ASTM Method D5666-95, 2004).

Agilent 1200 RRLC systems use conventional or sub-two-micron columns, in various lengths up to 300 mm, and can typically provide ultra-fast separations with the same or better resolution as the original method. This application will compare the retention capability and peak shape of the two different stationary phases and will focus on showing the separation of five PPDs in one run, within five minutes, using the 1200 RRLC system with Agilent RRHT reversed phase columns.



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Table 1. Chemical Information of Five PPDs

Trade Name	CAS Number	Chemical Structure and Chemical Name
77PD	3081-14-9	N,N'-bis-(1,4-dimethylpentyl)- <i>p</i> -phenylenediamine 
DTPD	27417-40-9	N,N'-ditolyl- <i>p</i> -phenylenediamine 
IPPD	101-72-4	N-isopropyl-N'-phenyl- <i>p</i> -phenylenediamine 
PPD	106-50-3	<i>p</i> -phenylenediamine 
6PPD	793-24-8	N-(1,3 dimethylbutyl)-N'-phenyl- <i>p</i> -phenylenediamine 

Experimental

System

Agilent 1200 Series Rapid Resolution LC, consisting of:
 G1379B micro vacuum degasser
 G1312B binary pump SL
 G1367C high-performance autosampler SL
 G1316B thermostatted column compartment SL
 G1315C UV/Vis diode array detector SL with 3-mm, 2-μL flow cell
 ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm
 Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 μm
 Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 μm
 Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 μm

Mobile Phase Conditions

A: Water with 0.1 g/L ethanolamine
 B: Acetonitrile (ACN) with 0.1 g/L ethanolamine

Samples

Mixture of 77PD, IPPD, PPD, DTPD, and 6PPD, all 50 μg/mL in acetonitrile. 77PD, IPPD, and PPD were standards from Sigma-Aldrich (St. Louis, Missouri, USA). DTPD and 6PPD were provided by a customer.

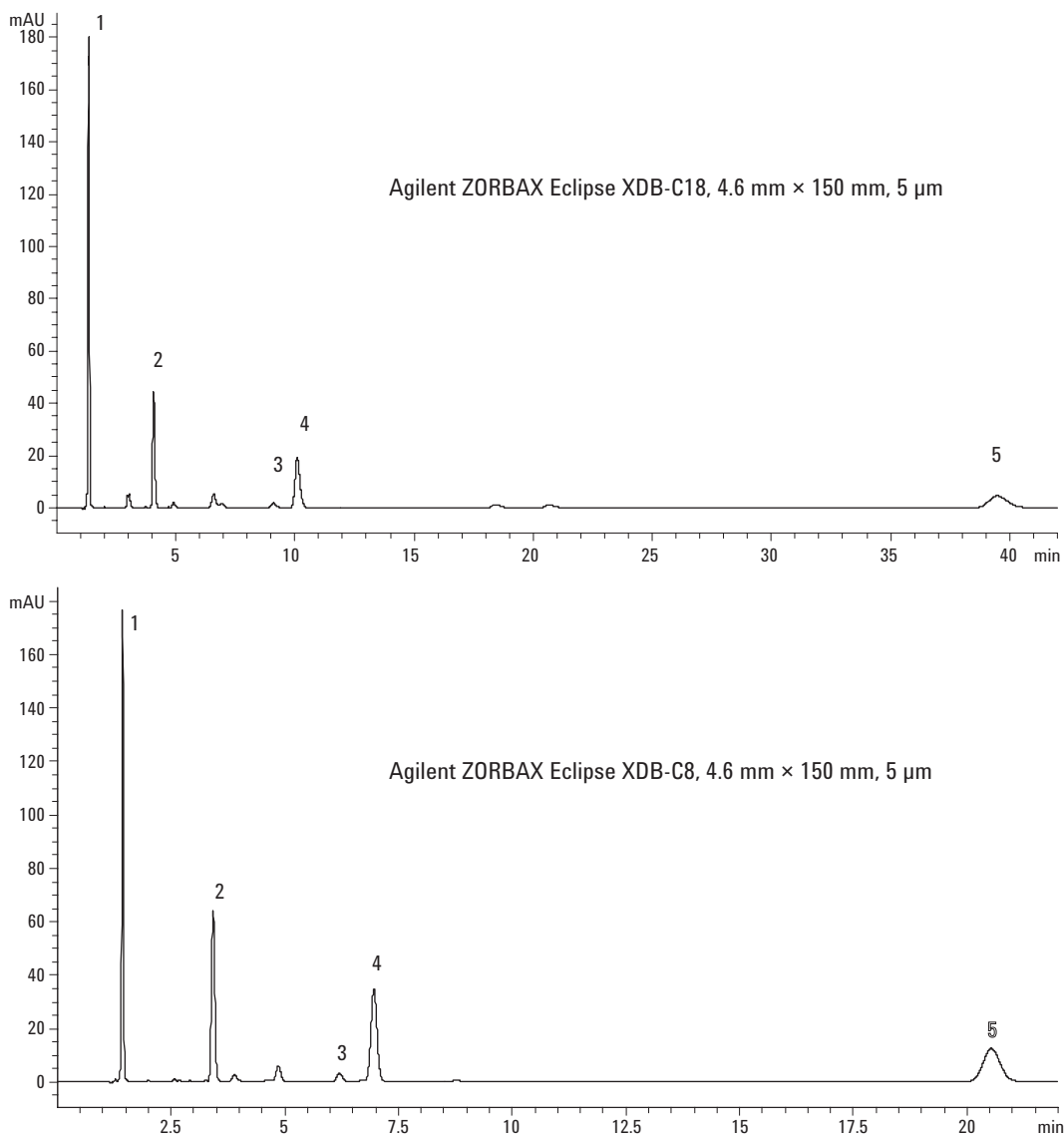
Results and Discussion

Selection of Stationary Phase for the Separation of Five PPDs

ASTM D5666-95 recommends a 10- to 15-cm long column packed with C18 grafted silica and 3- to 5-μm particle sizes. In our investigation, however, we observed that the retention characteristics of

ZORBAX Eclipse XDB-C18 were so strong that the total analysis time would be about 40 minutes or more. ZORBAX Eclipse XDB-C8 columns were found to have adequate resolution, and the reten-

tion time was only about half of the C18 column. Therefore, we chose the C8 column for further method development. The separations are shown in Figure 1.



Compound	Conditions	
1 PPD	Mobile phase:	A: water with 0.1 g/L ethanolamine B: ACN with 0.1 g/L ethanolamine
2 IPPD	Flow rate:	1.0 mL/min
3 DTPD	Wavelength:	260 nm
4 6PPD	Injection volume:	10 μL
5 77PD	Column temperature:	35 °C
	Sample:	Mixture, 50 μg/mL in ACN
	Isocratic composition:	30% A, 70% B (v/v)
	Column size:	4.6 mm × 150 mm, 5 μm
	Stationary phase:	Agilent ZORBAX Eclipse XDB-C18 Agilent ZORBAX Eclipse XDB-C8
	Analysis time:	42 min 22.5 min

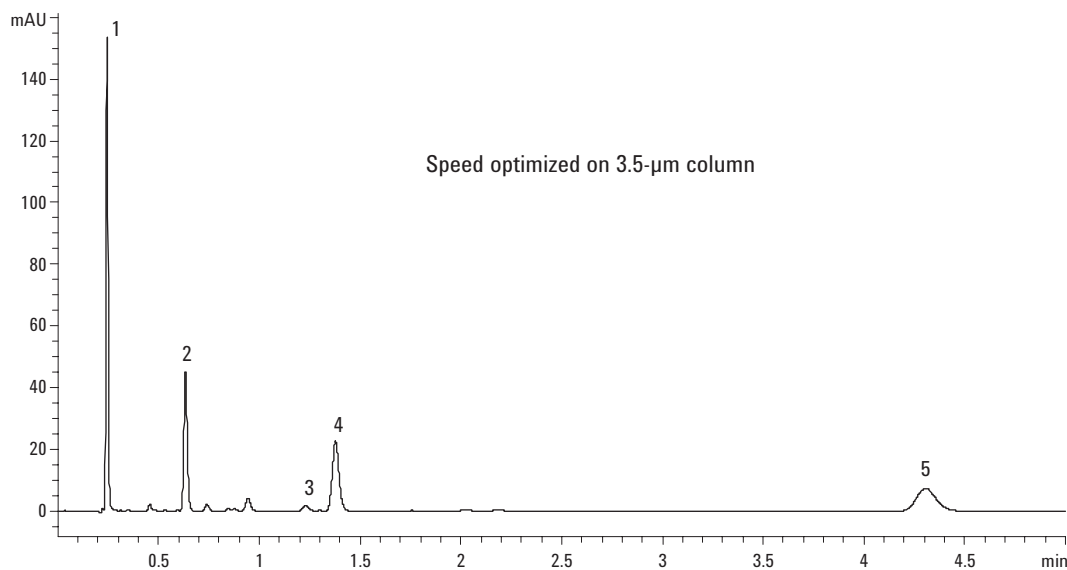
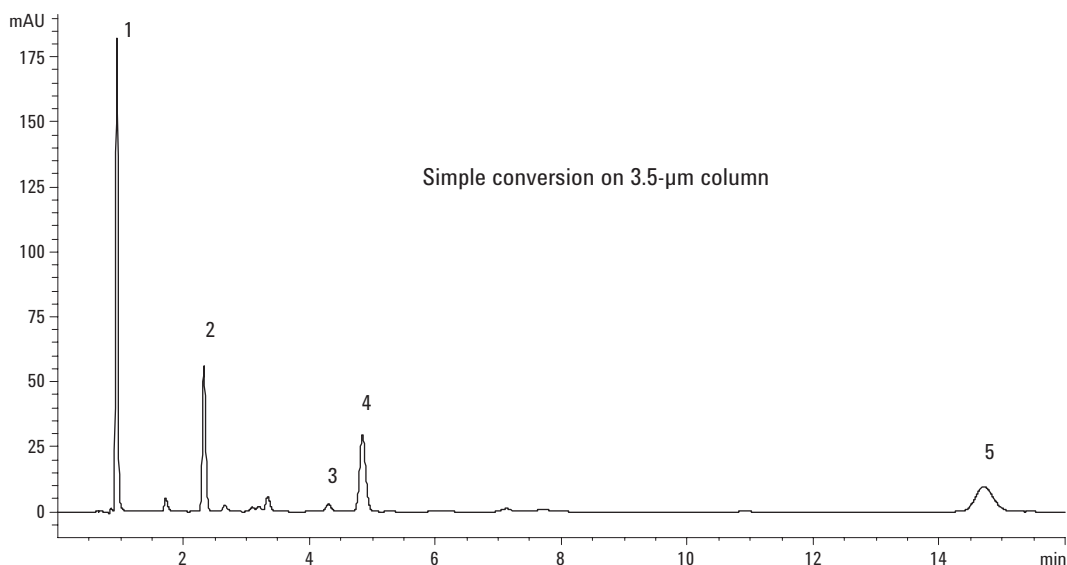
Figure 1. Column stationary phase comparison for five PPDs.

Fast Method Developed Based on New 1200 RRLC

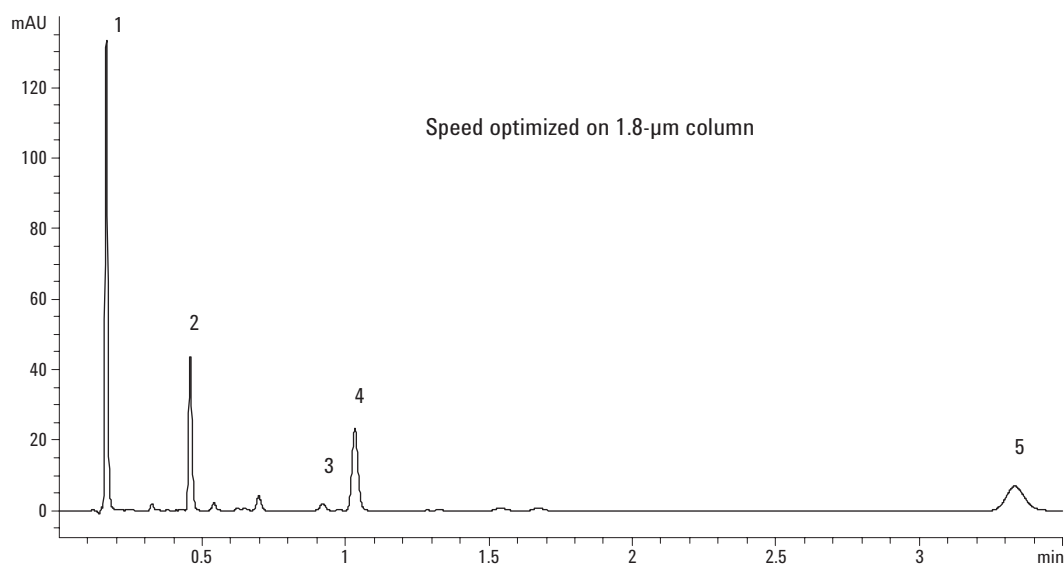
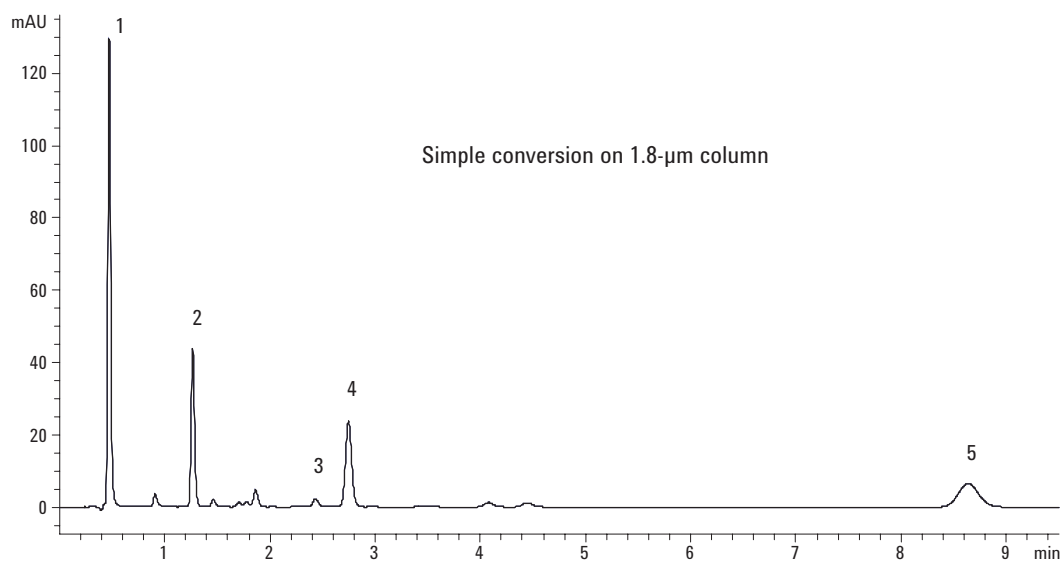
The popular desire of chromatographers is to decrease the analysis time and increase the daily throughput with the same or similar resolution. Nowadays, the Agilent 1200 RRLC system with higher pressure capability and a higher temperature range can provide excellent chromatographic resolution with much shorter run times. Furthermore, a constant concern is how to quickly and easily transfer conventional methods to fast methods. Agilent provides two versions of method translators: one is a Microsoft.net version requiring that Net-Framework 2.0 be resident on the computer, and the other is a Microsoft Excel version requiring that Excel be resident on the PC. When the ini-

tial method is an isocratic method, the method translator can provide two modes of faster methods. One is simple conversion, with the scaled flow rate according to the column diameter; the other is speed optimized conversion, with the maximum flow rate and pressure. In gradient mode, an additional option is a resolution optimized conversion.

This application uses a set of Agilent ZORBAX Eclipse XDB-C8 columns, including 4.6 mm × 150 mm (5 µm), 4.6 mm × 100 mm (3.5 µm), and 4.6 mm × 50 mm (1.8 µm). The method translator is used to transfer the initial method on a 5-µm column to two fast methods on 3.5-µm and 1.8-µm columns, respectively. The resulting separation of five PPDs is depicted in Figure 2.



Continued



Compound	Conditions			
1 PPD	Stationary phase:	Agilent ZORBAX Eclipse XDB-C8		
2 IPPD	Mobile phase:	A: water with 0.1 g/L ethanolamine		
3 DTPD		B: ACN with 0.1 g/L ethanolamine		
4 6PPD	Isocratic composition:	30% A, 70% B (v/v)		
5 77PD	Column temperature:	35 °C		
	Wavelength:	260 nm		
	Column size	4.6 mm × 100 mm, 3.5 μm	4.6 mm × 50 mm, 1.8 μm	
	Conversion mode:	Simple	Speed optimized	Simple Speed optimized
	Flow rate:	1.0 mL/min	4.0 mL/min	1.0 mL/min 3.0 mL/min
	Injection volume:	6.7 μL	6.7 μL	3.3 μL 3.3 μL
	Analysis time:	15 min	5 min	10 min 3.5 min

Figure 2. Separation of five PPDs on a smaller particle size column using the transferred methods.

Conclusions

As a powerful approach, liquid chromatography with ultraviolet\visible detection is often used to determine the chemical antidegradants in rubber. Agilent 1200 RRLC systems typically provide the customer with a rapid separation having the same or similar resolution. The method translator can convert any isocratic or gradient method to fast method according to customer requirements. This application details the selection of stationary phases for the separation of five PPDs, separates five PPDs with the RRLC system in one run, and applies the method translator to develop fast methods based on smaller particle size columns. With 1.8- μ m column, the total analysis time of five PPDs in one run is about 6.4 times faster than the original 5- μ m column method.

References

1. ASTM D5666-95 (Reapproved 2004)
"Standard Test Method for Rubber Chemical Antidegradants - Purity of *p*-Phenylenediamine (PPD) Antidegradants by High Performance Liquid Chromatography"
2. Michael Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations," Agilent Technologies publication 5989-5177EN, 2006.

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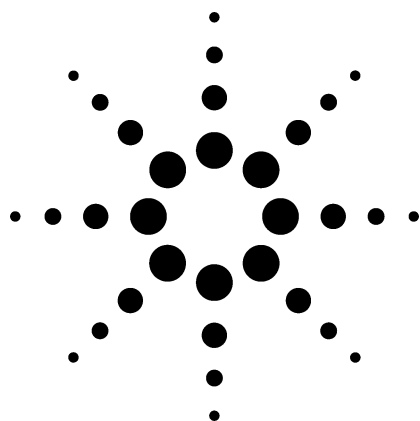
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Comprehensive Flow Modulated Two-Dimensional Gas Chromatography System



Application Brief

Roger L. Firor

Two-dimensional GC (GC x GC), a relatively new gas chromatographic technique, is being recognized for its powerful separation capabilities for the analysis of complex mixtures. The methodology involves the use of two capillary columns of usually very different polarities installed in series. Between the two columns a device known simply as a flow modulator is installed and interfaced to an auxiliary programmable control module (PCM) on an Agilent 7890A gas chromatograph through a three-way solenoid valve. In the flow modulator, analyte bands from the first column are collected in a fixed volume channel and successively injected very quickly into the short second column in very narrow bands.[1] Any separation that occurs on the first column is preserved during transfer to the second column. In summary, GC x GC can greatly increase peak resolution and peak capacity.

The unique flow modulator used in this system is based on Agilent's Capillary Flow Technology hardware and does not require the use of cryogenics for focusing. The modulator consists of a planar structure where flow splitters and collector channel are all incorporated internally to the device. All external connections are made through Agilent CPM fittings (ultimate union technology) incorporated into the plate for zero unswept volumes and leak proof seals. A three-way solenoid valve is installed on top of the GC oven and is interfaced to a PCM module. Experimental conditions used are shown below.

Experimental

GC:	Agilent 7890A, FID at 200 Hz data collection rate, split/splitless inlet
Carrier:	Hydrogen
Column 1:	30 m x 0.25 mm x 0.25 μ m HP-5ms, 19091S-433
Column 1 Pressure:	21.5 psig at 50 °C, constant flow mode
Column 2:	5 m x 0.25 mm x 0.15 μ m HP-INNOWax
Column 2 Flow:	20 mL/min, constant flow mode
Oven Program:	50 °C (1.0 min) to 260 °C (4 min) @ 8 °C/min.
Modulator Period:	1.4 seconds collect, 0.12 seconds flush typical
GC x GC analysis software:	GC Image[2]

An illustration of the modulator is given in Figure 1. The precisely timed and synchronized periodic switching between collect and flush states directs sample pulses continuously to the second column for additional separation during the

Highlights

- Flow modulation offers a viable alternative to thermal modulation without the burdens imposed by cryo requirements for comprehensive GC x GC.
- Modulation, timing (collect and inject), and synchronization are all integrated into the 7890A GC system for easy setup and operation.
- Agilent's fifth-generation electronic pneumatic control with setting to three decimal points combined with Capillary Flow Technology hardware forms the basis for an easy-to-use GC x GC system.



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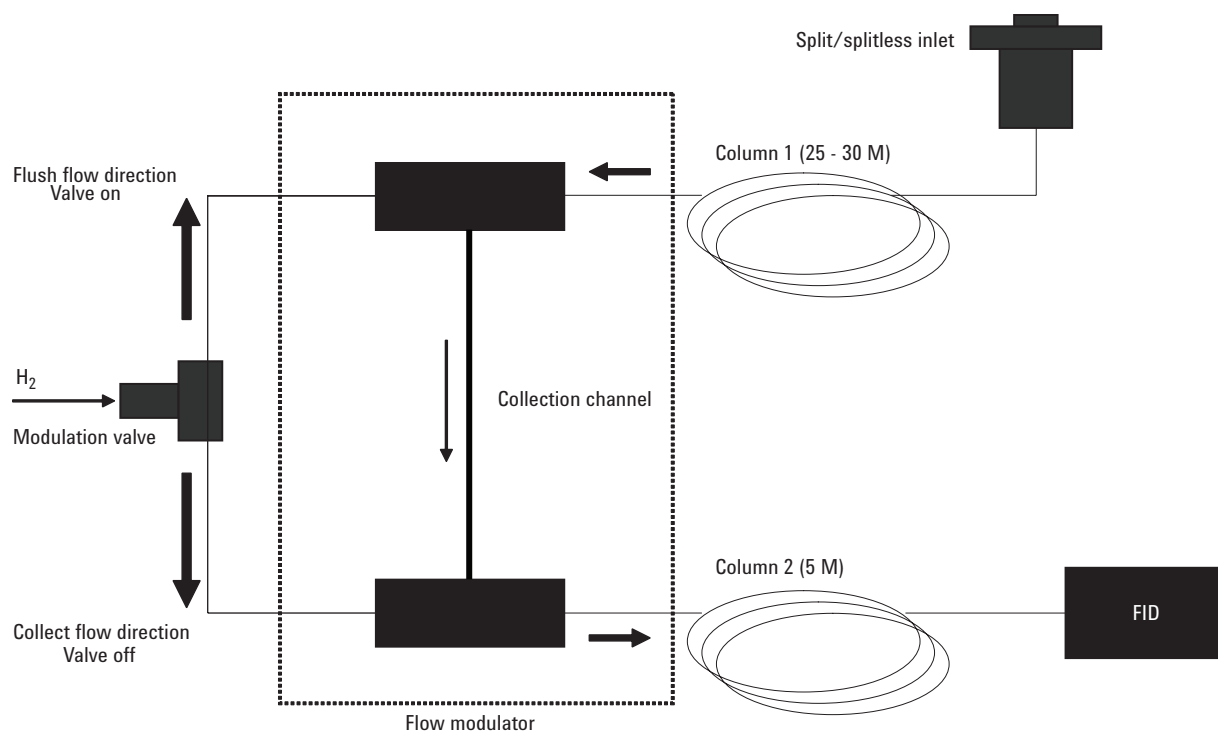


Figure 1. Flow modulator design – differential flow system.

length of the chromatographic run.

Discussion

Figures 2a and 2b show unmodulated and modulated peaks, respectively, of a pure analyte. In this example, n-butylbenzene is shown with approximately three modulations across the first-column peak. The areas of the modulated peaks should ideally equal the area of the un-modulated peak. In other words, no material should be lost in the transfer to the second column. Area agreement was within 3% for this test. Peak widths at half height for modulated butylbenzene are approximately 100 ms. Very narrow peaks as required for the technique are

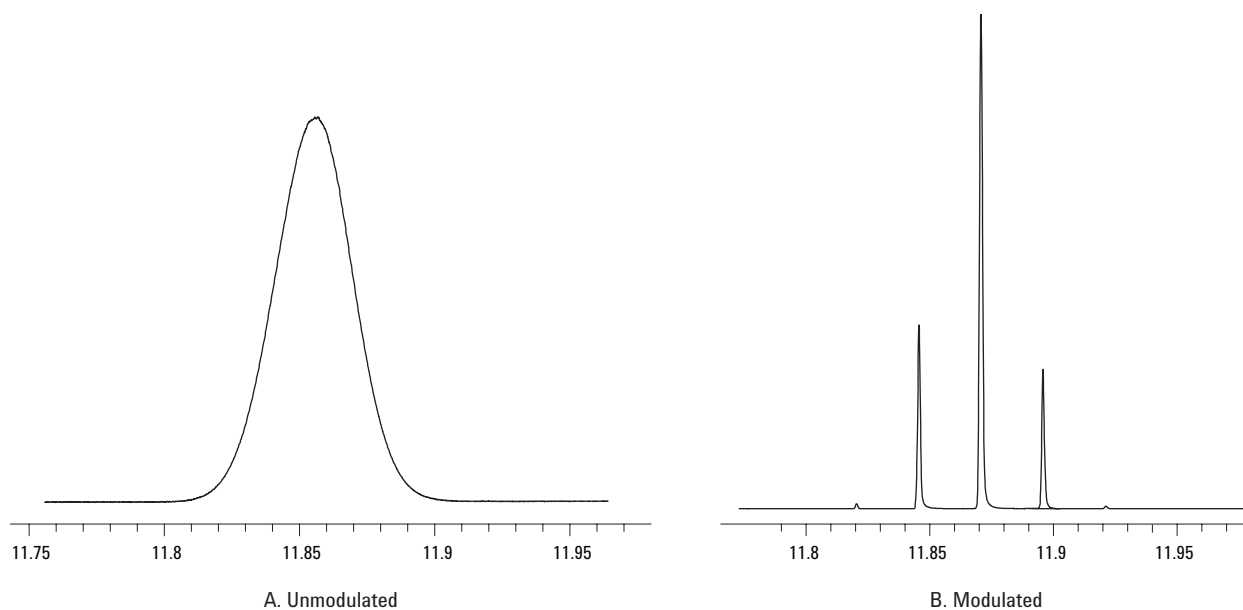


Figure 2. Modulated and unmodulated n-butylbenzene (not to same scale).

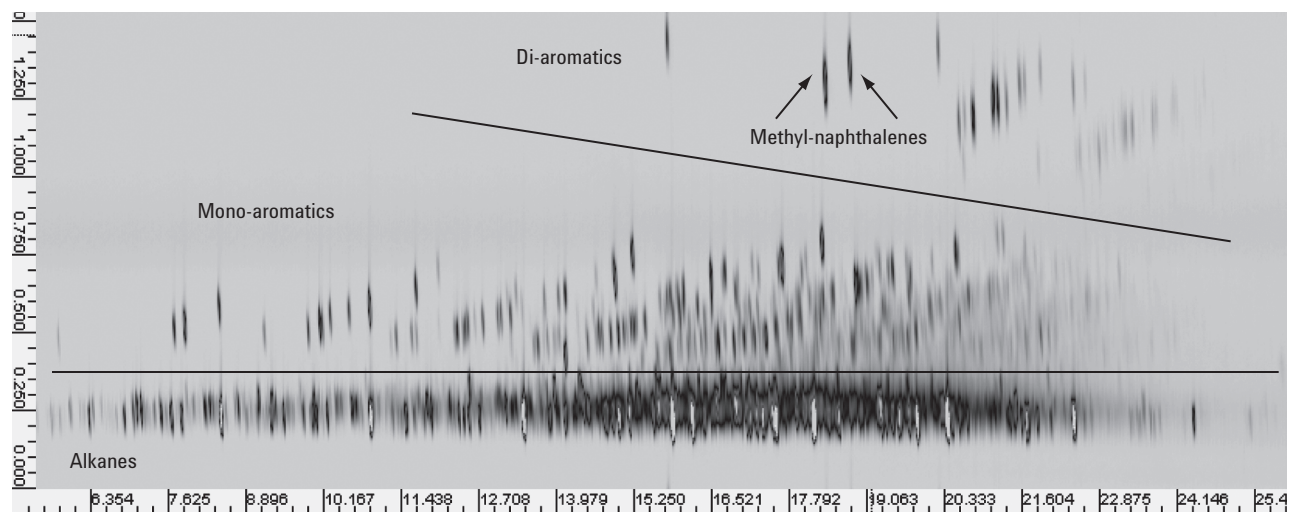


Figure 3. A 2D image of No. 2 kerosene.

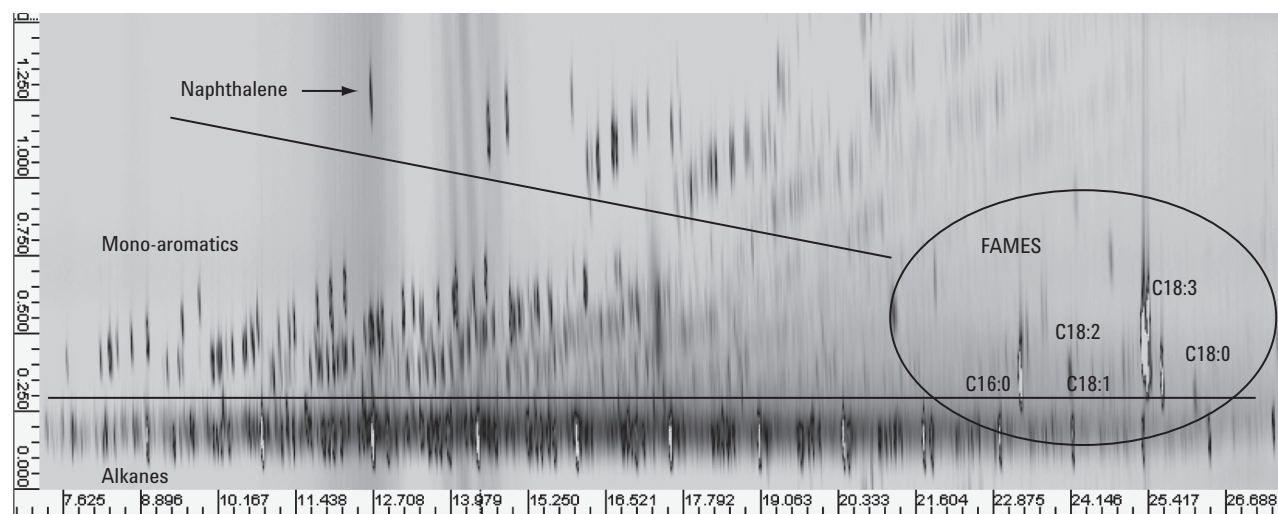


Figure 4. A B20 soy-based biodiesel (20% methyl ester, 80% diesel).

seen that approach the peak widths obtained by thermal modulation systems. Flow modulation has the distinct advantage of not requiring cryo fluid for focusing.

Use of a nonpolar column followed by a polar column produces hydrocarbon-type retention in the following order: alkanes, cyclic alkanes, olefins, single-ring aromatics, and multi-ring aromatics. An example of a 2D image of No. 2 kerosene is shown in Figure 3. Chemical classes are clearly discernable, with good resolution seen for the aromatics. Another example, B20 soy-based biodiesel (20% methyl ester, 80% diesel) is shown in Figure 4. Here the C16 and C18 fatty acid methyl esters are indicated. Data processing for all samples was performed using GC Image[2].

References

1. Pedro A. Bueno Jr., John V. Seeley, "Flow-switching device for comprehensive two-dimensional gas chromatography," *Journal of Chromatography A*, 1027 (2004) 3-10.
2. Zoex Corporation, 5010 Sea Oak Court, Pasadena, TX 77505, and GC Image, LLC, PO Box 57403, Lincoln, NE 68505.

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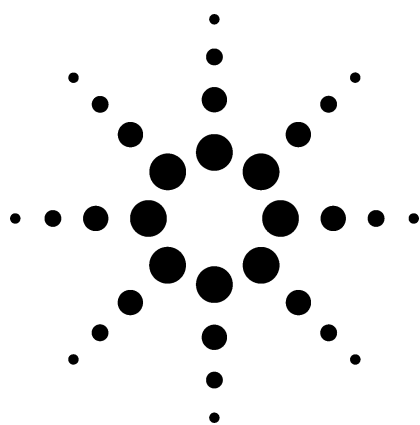
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Simultaneous Analysis of Trace Oxygenates and Hydrocarbons in Ethylene Feedstocks Using Agilent 7890A GC Capillary Flow Technology

Application Brief

James McCurry

The presence of trace hydrocarbons in ethylene can have damaging effects on both the process catalysts and the final polymer products. Test methods such as ASTM D6159 are used to ensure the quality of these feedstocks [1]. However, the analysis of other key contaminants, such as oxygenates, requires GC methods that run on separate instruments. This can be time consuming and expensive for the process analysis lab.

The Agilent 7890A GC serves as the ideal platform when analyzing different classes of trace compounds in ethylene. Maximum productivity can be realized by:

- Using Capillary Flow Technology to perform analysis of trace oxygenates and hydrocarbons in a single run through 2-D Deans switch chromatography.
- Automating the preparation of multilevel calibration standards using the new auxiliary electronic pneumatics control (EPC) modules.
- Protecting the sensitive and expensive alumina PLOT column by preventing polar oxygenates from entering the column.

Enhancing ASTM Method D6159 with Capillary Flow Technology 2-D GC

ASTM Method D6159 uses a methyl silicon column in series with an alumina PLOT column to resolve light hydrocarbons in ethylene. Polar oxygenated compounds cannot be analyzed on this column set because methyl silicon has insufficient selectivity and the alumina column will adsorb oxygenates, resulting in column damage. Wax-type liquid phases such as HP-INNOWax can easily separate polar compounds from light hydrocarbons using 2-D GC [2]. A wax column placed before an alumina column will retain polar compounds while the light hydrocarbons elute near the void volume. Therefore, if a Deans switch is placed between the columns, the hydrocarbons can be heart-cut from the wax to the alumina columns while oxygenates are held by the wax column. The optimized thermal and pneumatic performance of the Agilent 7890A Deans switch is a result of Capillary Flow Technology. This provides the high levels of retention time precision and narrow peak shape needed for optimal heart-cutting 2-D GC (Figure 1).

Highlights

- The Agilent 7890A GC Capillary Flow Technology combined with enhanced electronic pneumatics control (EPC) provide greater productivity and flexibility in the analysis of trace contaminants in ethylene.
- Multiple auxiliary EPC channels provide the ability to automatically generate gas calibration standards for trace level impurities.
- Enhancement of ASTM D6159 method with 2-D GC Deans switching measures trace oxygenates and hydrocarbons in a single run.



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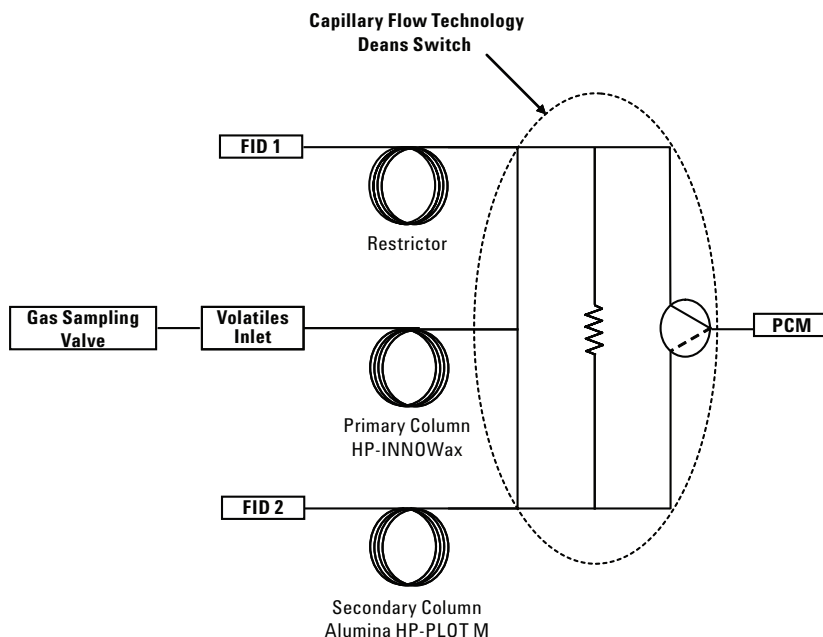


Figure 1. Configuration of Agilent 7890A for the 2-D GC analysis of trace oxygenates and hydrocarbons in ethylene.

Method Parameters for Enhanced ASTM D6159 Method

Primary column:	HP-INNOWax, 30 m × 0.32 mm id × 0.5 µm film (19091N-213)
Primary column flow:	Helium at 2.5 mL/min
Secondary column:	Alumina HP-PLOT M, 30 m × 0.53 mm id × 15 µm (19095P-M23)
Secondary column flow:	Helium at 6 mL/min
Oven temperature program:	40 °C for 6 min, 4 °C/min to 125 °C
Volatiles inlet conditions:	150 °C, 5:1 split
Sample loop:	250 µL at 65 °C
Detector temperature:	250 °C
Capillary Flow Technology:	2.3 to 4.5 min
Deans switch cut time	

Automating the Preparation of Trace-Level Calibration Standards

Another advantage of the Agilent 7890A GC is the expanded capabilities in EPC. These extra channels of auxiliary EPC are used with the dynamic blending system hardware to allow automated preparation of ppmV gas standards for calibration. This approach has been described for the automated preparation of trace sulfur compounds in various gas matrices [3].

Results

Figure 2 shows the 2-D GC analysis of methanol and C1 to C4 hydrocarbons in a sample of technical grade ethylene. The HP-INNOWax column first separates the polar methanol from the unresolved hydrocarbon peaks. The Deans switch transfers the hydrocarbons to the Agilent alumina HP-PLOT M column, where the C1 to C4 hydrocarbons are easily separated. This column is also shown to provide better separation of trace hydrocarbons from the large ethylene peaks, while maintaining excellent peak shape and intensity for the acetylene. The performance of this alumina column is maintained over many injections since the HP-INNOWax column prevents polar oxygenates (water, alcohols) from damaging the sensitive stationary phase. Table 1 shows very good precision using this method for a sample containing approximately 2 ppmV.

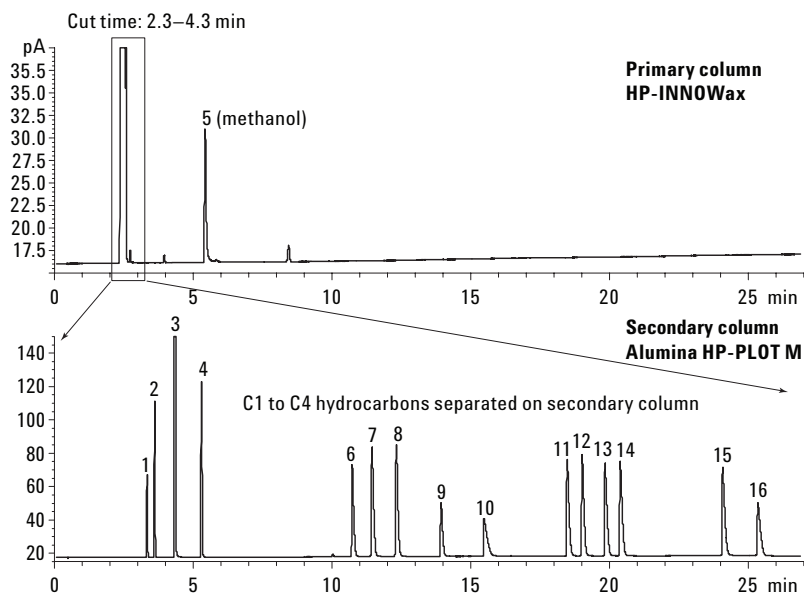


Figure 2. Capillary Flow Technology Deans switch used to separate 100 ppmV oxygenate and hydrocarbon impurities in ethylene.

Table 1. Method Precision for 2-D GC Analysis of Ethylene Impurities

Peak No.	Name	Avg. (ppmV)*	Std Dev*	%RSD*
1	Methane	2.1	0.011	0.5
2	Ethane	21.5	0.049	0.2
3	Ethylene	Balance	Balance	Balance
4	Propane	2.1	0.062	3.0
5	Methanol	2.1	0.081	3.8
6	Propylene	2.1	0.023	1.1
7	Isobutane	2.1	0.015	0.7
8	n-Butane	2.0	0.011	0.5
9	Propadiene	2.1	0.025	1.2
10	Acetylene	1.9	0.036	1.9
11	Tran-2-butene	2.1	0.011	0.5
12	1-Butene	2.0	0.013	0.7
13	Isobutylene	2.1	0.016	0.8
14	cis-2-butene	2.1	0.017	0.8
15	1,3-Butadiene	2.1	0.018	0.9
16	Methylacetylene	2.0	0.015	0.7

*Sample run 20 times

References

1. Annual Book of ASTM Standards, Vol. 05.03, "Petroleum Products and Lubricants (III), D5303 - D6553," ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428 USA.
2. J. D. McCurry, "Fast Determination of Denatured Fuel Ethanol Purity by Two-Dimensional Gas Chromatography," Agilent Technologies publication 5988-9460EN, April 2003.
3. R. L. Firor and B. D. Quimby, "Automated Dynamic Blending System for the Agilent 6890 Gas Chromatograph: Low Sulfur Detection," Agilent Technologies publication 5988-2465EN, April 2001.

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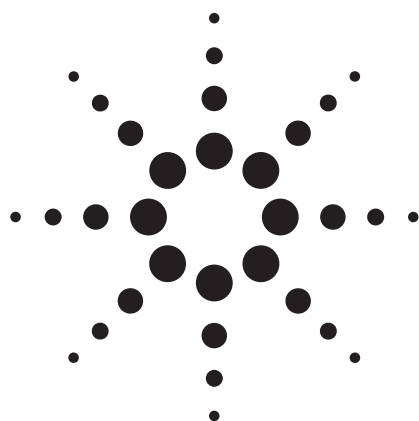
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GC/MS Analysis of PCBs in Waste Oil Using the Backflush Capability of the Agilent QuickSwap Accessory



Application

Environmental

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Abstract

Polychlorinated biphenyls (PCBs) in waste oil are typically analyzed by GC-ECD or GC/MS after solid phase extraction (SPE) cleanup. However, not all problematic matrix components are completely removed during cleanup and are injected into the analytical system, thereby contaminating the column and the detector.

In this application, a practical example of backflushing is presented using the Agilent QuickSwap accessory installed on a 7890 GC/5975 MSD system. Benefits of using QuickSwap instead of the traditional high-temperature bakeout procedure are demonstrated. Column and detector contamination were significantly reduced and sample throughput increased.

Introduction

The determination of polychlorinated biphenyls (PCBs) in mineral oils, including transformer oil,

waste oil, or solid waste in general, is a routine application in environmental laboratories. After dilution/dissolution of the oil sample, a solid-phase extraction sample cleanup is used to remove most of the matrix components. Several SPE methods are commonly applied, and some custom cartridges are available specifically for this purpose. According to EN 12766, for instance, a combination of silica and acidified silica/anion exchange ($\text{SiOH-H}_2\text{SO}_4/\text{SA}$) adsorbents is prescribed. The oil samples are diluted and applied to the cartridge in hexane solution and the PCB fraction is then immediately eluted with hexane rinse. The polar matrix compounds remain on the SPE cartridges [1].

In the PCB fraction, however, apolar matrix compounds elute from the cartridge with the PCBs. In one regard, this is not an immediate analytical problem, because when this sample fraction is analyzed by selective detectors like GC-ECD or GC/MS in selected ion monitoring (SIM) mode, the co-extracted solutes are not directly detected. However, their presence contaminates the inlet, column, and detector, causing continuously decreasing system performance. Symptoms such as drifting and increasingly noisy baseline, integration difficulties, decreasing chromatographic resolution, changing column selectivity, and decreasing detector S/N force more frequent inlet system, column, and MS source maintenance and potentially require re-running some samples.

Backflush is a technique that has recently become easier to implement with capillary GC separations due to the availability of Capillary Flow Technology devices [2-9]. One such device is the Agilent QuickSwap, whose primary function is to simplify



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changing columns and doing maintenance on GC/MS systems. QuickSwap provides a flow of clean carrier gas that excludes air from the mass spectrometer when columns are disconnected.

An auxiliary electronic pressure control (aux EPC) module or pressure control module (PCM) is typically used to supply the purge gas to QuickSwap and thereby offers the ability to program the pressure during the run. To backflush a capillary column, one need only raise the pressure of QuickSwap (the outlet of the column) higher than that of the inlet (the head of the column). The column flow reverses, eliminating remaining sample components from the head of the column and passing them out of the split vent of the inlet and onto the split vent trap.

Backflushing a column after elution of the compounds of interest is a very effective way of eliminating column contamination. Low-volatility contaminants from the most recently injected sample tend to remain at the head of the column until high oven temperatures are reached. So, by reversing the flow through the column, these contaminants need only flow a short distance to be removed from the column. In the traditional bake-out, they would need to travel through the full

length of the column to be removed. In addition to more effective removal of contaminants, cycle time is significantly reduced, columns are spared from exposure to the high temperatures typical of bake-outs, and detector contamination is reduced.

Sample Preparation

A typical procedure was used to prepare a BCR reference sample (BCR-449, waste mineral oil, high PCB level). A 10% dilution of the oil was made in hexane (1 g in 10 mL). From this solution, 250 μ L was applied to a series-combination of two cartridges: a 3 mL cartridge filled with 500 mg of silica treated with H₂SO₄ + 500 mg strong anion exchange resin and a 3-mL cartridge filled with 500 mg silica. The cartridges were preconditioned with hexane. The PCBs were eluted with 4 mL hexane. An aliquot of this solution was used for GC/MS analyses.

GC Conditions

All analyses were performed on an Agilent 7890A GC/5975 MSD system with QuickSwap option number 113 (with Aux EPC module). Injection was done using a 7683 ALS. The GC/MS conditions can be summarized in Table 1:

Table 1. GC/MS System Conditions

Column	30 m x 0.25 mm id x 0.25 μ m df	HP-5MS (Agilent P/N 19091-433)
Inlet	S/SI in splitless mode	280 °C, 0.75 min purge delay Purge flow rate: 50 mL/min
Carrier gas	Helium	
Run pressure	150 kPa constant pressure	2 mL/min initial flow rate
Backflush pressure	28 kPa	
QuickSwap	GC option number 113 or	Accessory kit G3185B
Restrictor	17 cm x 110 μ m id restrictor	Part number G3185-60363
Column outlet (QuickSwap)	Helium	
Run pressure	28 kPa He using AUX EPC	Through elution of PCBs
Backflush pressure	150 kPa	Held for 5 min after PCBs
Oven temperature program A (no backflush)	50 °C (1 min), 25 °C/min to 200 °C, 10 °C/min to 330 °C (10 min)	Total run time 30 min
Oven temperature program B (with backflush)	50 °C (1 min), 25 °C/min to 200 °C, 10 °C/min to 300 °C (5 min)	Total run time 22 min
MSD Setpoints	1.5 min solvent delay	260 °C MSD transfer line
SIM/scan settings (AutoTuned)	SIM ions 256, 258, 290, 292, 324, 326, 360, 362, 394, 396 (25 ms dwell time each)	Scan range 40–350 amu

Table 2. Sample Sequence

Run 1	Analysis of waste oil extract by GC/MS in scan/SIM mode – no backflush (oven program A)
Run 2	Blank run – no sample injection, no backflush (oven program A), same as run 1
Runs 3–5	Additional blank runs – same conditions as above (data not shown)
Run 6	Analysis of waste oil extract by GC/MS in scan/SIM mode – with backflush (oven program B)
Run 7	Blank run – no sample injection – same program as in runs 1–5

Results

Total ion chromatograms (TICs) obtained from GC/MS SIM mode are shown in Figure 1 (traditional bakeout with no backflush, A; with backflush, B). The PCBs of interest elute in the 9- to 16-min time range. The profiles obtained by both methods are very similar in PCB resolution and intensity. When these results are carefully scrutinized, little or no difference is noted. However, a clear baseline drop is observed in chromatogram B at 16.5 min, corresponding to the initiation of backflush. In usual backflush methods, the oven

temperature ramp and MS data acquisition are stopped when backflush is initiated. In Figure 1B, oven temperature was held at 300 °C, but acquisition was left on to show the drop in baseline when column flow reversed. In contrast to the backflush chromatogram (B), a “hump” is observed extending to 22 min in chromatogram A. This shows the presence of high-boiling matrix interferences in the sample extract and demonstrates the need for removal of these, either through a bakeout or backflush. By the end of the bakeout in 1B, the baseline appears to return to the initial level, indicating that the interferences had been removed.

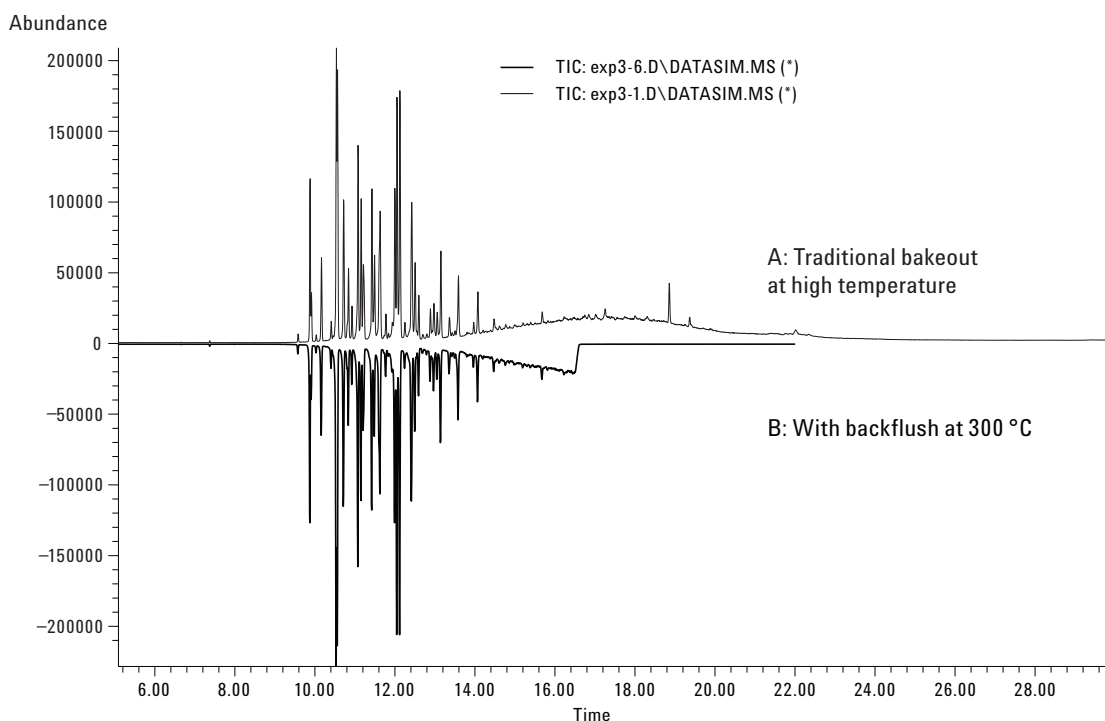


Figure 1. GC/MS total ion chromatograms (TICs) obtained for the analysis of PCBs in waste mineral oil without backflush (A) and with backflush (B)

A helpful recent enhancement of Agilent MSDs is the ability to acquire both SIM and scan data in the same run, termed “simultaneous SIM/Scan.” The advantage of simultaneous SIM/Scan is that the benefits of improved detection limits for target compounds with SIM acquisition can be coupled with the benefit of having full-scan data with which to identify unknowns using a library search or spectral interpretation. For the same analytical runs shown in Figure 1 (based on SIM data), total ion chromatograms from full scan data are shown in Figure 2A (no backflush) and Figure 2B (with backflush). The sample matrix interferents can be even more easily seen in these chromatograms. Since all ions in the 40 to 350 amu range are being monitored, the considerable amounts of material eluting after the PCBs of interest dominates the chromatogram. In fact, in the TICs shown in Figure 2, the low-level PCBs are not discernable due to the dominance of the hydrocarbon background. It appears that all the interferents were effectively removed by the bakeout, because the signal returns to baseline even though this was not the case.

To better demonstrate the inferiority of traditional bakeout to backflushing for removing residual

components, a blank run (no injection) was made after each of the analytical runs previously shown. The TIC scan-mode chromatogram after sample analysis with bakeout is shown in Figure 3A. To contrast the efficacy of backflush in removing contamination, the blank run done directly after sample analysis with backflush is shown in Figure 3B. In this chromatograph, only signal from normal column bleed was observed. In Figure 3A, the higher level of contamination was seen even after doing the 10 min bakeout at 330 °C and observing the apparent return of signal back to baseline. From this comparison, it is a clear that by relying on a typical bakeout, low-volatility material would continue to build up in the analytical system from run to run, ever increasing the level of background and interfering with subsequent analyses, requiring the column to be prematurely replaced. By backflushing, the low-volatility material was efficiently removed at lower temperatures in less time, while simultaneously lowering source contamination. Column lifetime would improve dramatically. In addition, the backflush method required less cooldown time after the run (from 300 °C instead of 330 °C). Total cycle time was thereby reduced by more than 25% by using backflush.

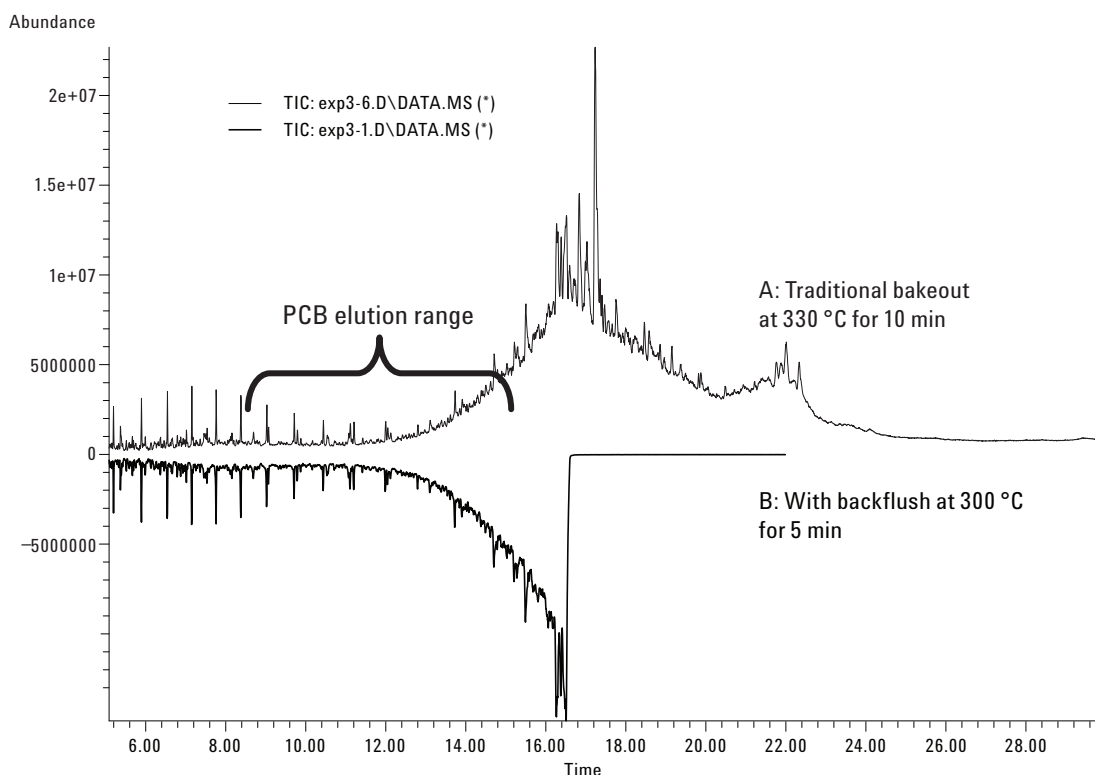


Figure 2. GC/MS TIC scan chromatograms obtained for the analysis of PCBs in waste mineral oil without backflush (A) and with backflush (B).

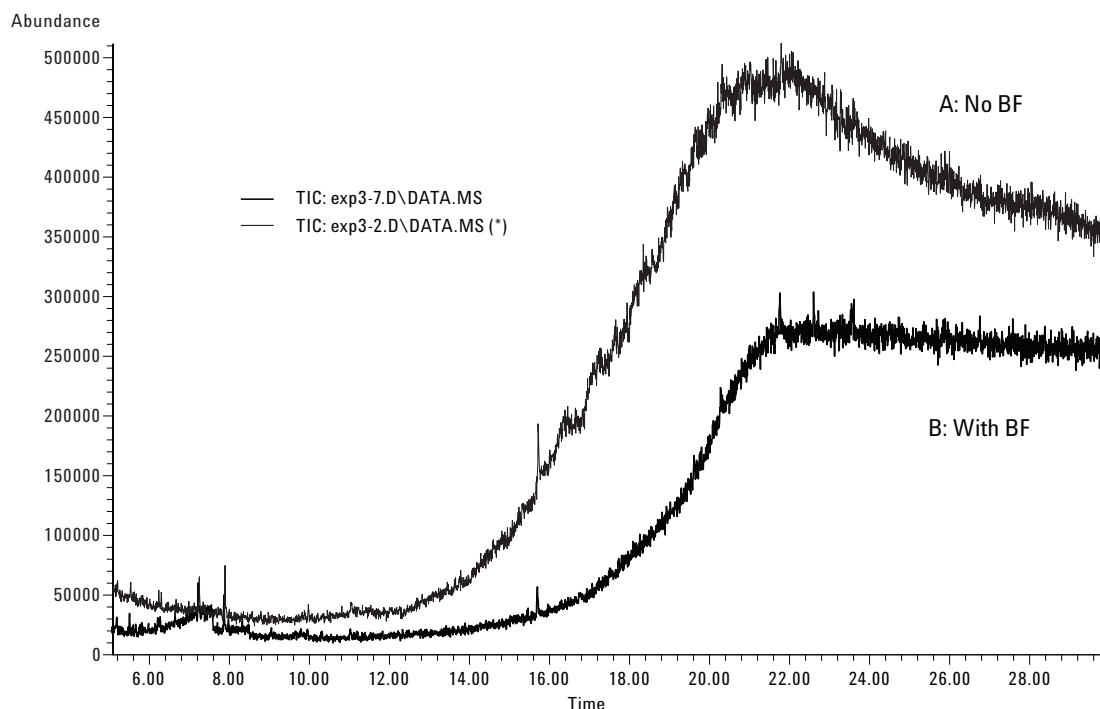


Figure 3. GC/MS TIC chromatograms obtained from scan data for a blank run after the analysis of a sampling with traditional bakeout (no backflush) (A) and with the use of backflush (B).

Conclusion

The benefits from using the backflush capability of QuickSwap on the 7890 GC/MSD were illustrated using an analysis of PCBs in waste mineral oil. The analytical portion of the analysis method was unchanged. There were no negative consequences from adding a backflush to the method. Several advantages were illustrated: improved cycle time, reduced column contamination, improved projected column lifetime, and reduced contamination of the MSD source. Adding backflush to current methods should be seriously considered to increase both laboratory productivity and quality of results.

References

1. Information on sample preparation and analysis of PCBs in waste oils can be found in reference methods DIN EN 12766 and DIN EN 61619
2. Chin-Kai Meng, "Improving Productivity and Extending Column Life with Backflush," 12/06, Agilent application note 5989-6018EN
3. Matthew S. Klee, "Simplified Backflush Using Agilent 6890 GC," 6/06, Agilent application note 5989-5111EN
4. Matthew S. Klee and Bruce Quimby, "A Column-Flow Independent Configuration for Quick-Swap," 5/07, Agilent application note 5989-6702EN
5. Frank David and Matthew S. Klee, "Analysis of Suspected Flavor and Fragrance Allergens in Cosmetics Using the 7890A GC and Capillary Column Backflush," 3/07, Agilent application note 5989-6460EN
6. Bruce Quimby, "Rapid Forensic Toxicology Screening Using an Agilent 7890A/NPD/5975/DRS GC/MSD System," 1/07, Agilent application note 5989-6066EN
7. Mike Szelewski, "Significant Cycle Time Reduction Using the Agilent 7890A/5975 GC/MSD for EPA Method 8270," 2/07 Agilent application note 5989-6026EN
8. Chunxiao Wang, "Parallel GC for Complete RGA Analysis," 1/07, Agilent publication 5989-6103EN
9. Philip Wylie, "Direct Injection of Fish Oil for the GC-ECD Analysis of PCBs: Results Using a Deans Switch With Backflushing," 1/07, Agilent application note 5989-6095EN

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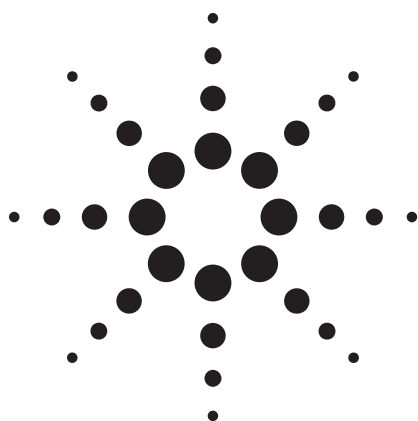
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Comprehensive GC System Based on Flow Modulation for the 7890A GC



Application Brief

Introduction

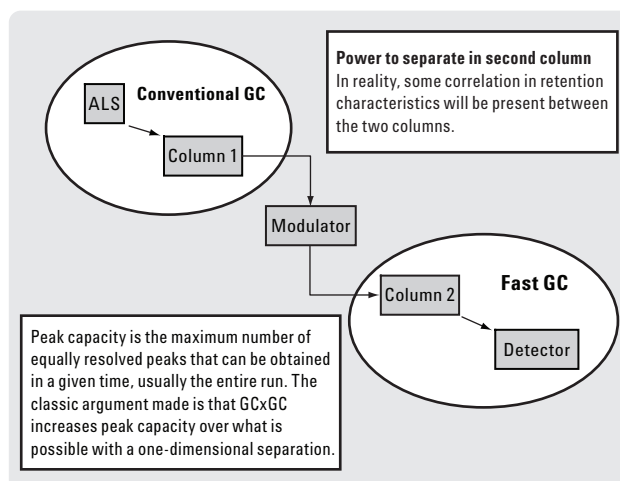
A hardware solution is available on the 7890A for the practice of comprehensive GC. The system uses a capillary flow modulator controlled by the 7890A GC. The system is offered with factory checkout using an FID detector. Other detectors, preferably those operating at 50 Hz or greater, can be used.

Comprehensive two-dimensional (2D) GC, or GCxGC, is a powerful technique that can be used to separate very complex mixtures, such as those found in the hydrocarbon processing, environmental, and food/fragrance industries.

The method uses two columns, typically of very different polarities, installed in series with a modulator in between. The second column is much shorter than the first column to effect a fast separation. The entire assembly is located inside the GC oven.

The modulator performs three functions:

1. It collects effluent from the first column for a fraction of the time equal to peak width. For example, if a peak from column one is six seconds wide, the modulator will accumulate material every two to three seconds, thereby dividing the peak from the first column into two or three “cuts.”
2. It focuses the material collected from each cut into a very narrow band through flow compression.
3. It introduces the bands sequentially onto the second column, resulting in additional separation for each band injected onto the second column.



Comprehensive 2D GC uses a primary column (conventional separation), a flow modulator, a second column (very fast separation), and a fast detector.

This technique provides a second dimension of information that can increase the peak resolution and capacity.

A number of different modulator designs have been described in the literature, most relying on thermal cycling to focus the bands from the first column and release them into the second column. Some disadvantages to this approach are:

- Large usage of expensive cryogenic gases leading to a high cost of analysis
- Complexity of the hardware
- Longer analysis times

Agilent's proprietary Capillary Flow Technology and fourth-generation Electronic Pneumatics Control (EPC) enable the use of a differential flow modulator to conduct comprehensive 2D-GC without the use of cryogenic gases or complex hardware.

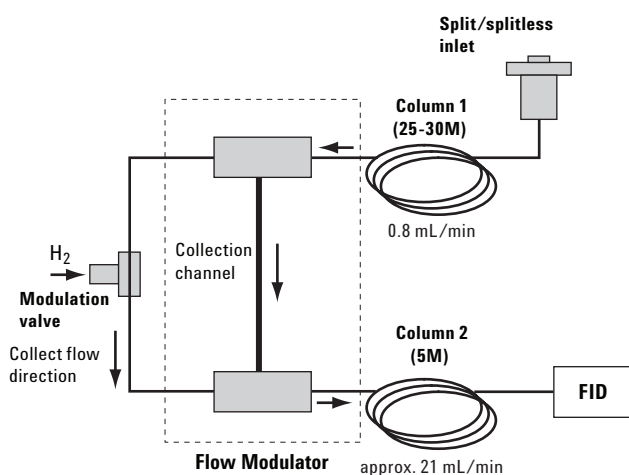


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The key to operation is the flow differential (typically 20 to 1) between the second and first columns, respectively. This compresses and focuses the analytes present in any given modulation “inject” pulse into the second column. Precise timing of the modulator is made possible by installing a driver board in the Aux det 2 detector slot of the 7890A main-frame.

The Capillary Flow Technology modulator uses a deactivated, stainless steel structure with all flow splitters and the collector channel incorporated internally in the device. It has low thermal mass so it can track the oven temperature very closely, and its GC oven location allows precise temperature control without lag during programmed runs. All external connections are made using Agilent’s Ultimate Union technology for leak-free operation and extremely small, well-swept volumes. A micro three-way solenoid valve, installed on the side of the gas chromatograph, connects to a pneumatics control module (PCM) to accurately and precisely control the flows through the modulator.

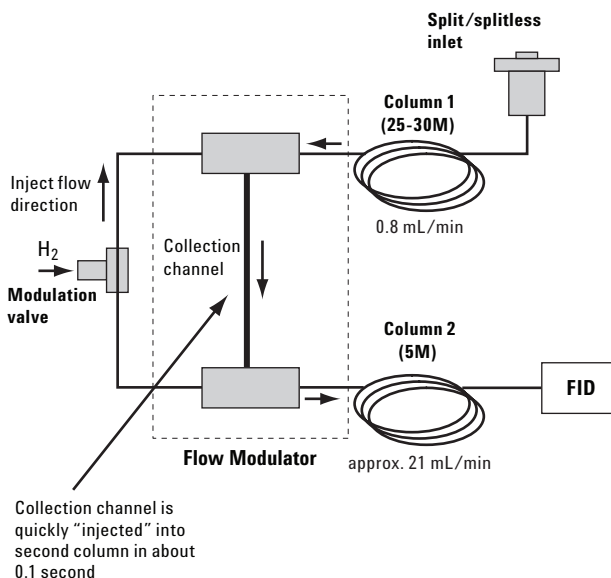
The figures below illustrate the modulator. A three-way solenoid valve receives a controlled supply of hydrogen gas from a PCM. The periodic switching of this three-way valve drives the modulator. The precisely timed and synchronized switching between the collect and inject states directs discrete sample pulses continuously to the second column for additional fast separation throughout the chromatographic run. Both columns are run in constant flow mode. For optimal performance, injection size and split ratio should be carefully adjusted to avoid overloading, which can lead to excessive peak tailing.



Flow rates and flow directions during the load or collect portion of the modulation cycle

Load or collect state (above): At the beginning of this state, the collection channel is filled with hydrogen gas from a previous injection cycle flush.

The primary column effluent enters the modulator’s top tee connection and flows into the collection channel. The analytes from this column enter one end of the collection channel. Hydrogen flow from the PCM/three-way micro valve exits the modulator at the bottom tee and is sent to the second column.



Flow rates and flow directions during the transfer or inject portion of the modulation cycle

Inject or flush state (above): Hydrogen gas flow from the three-way solenoid valve is directed to the top tee. A high flow of typically 20 mL/min for about 0.1 second rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes collected in the channel undergo rapid separation.

What is required:

- Agilent 7890A GC with firmware version A.04.06 or higher
- FID with 200 Hz data collection rate or other fast detector
- Split/splitless inlet
- Capillary Flow Technology modulator option or accessory
- Capillary Flow Technology modulator checkout kit
- Pneumatics control module (PCM)
- Agilent GC ChemStation B.03.02 or other data collection and analysis system that can control the flow modulator cycle
- 30-m × 0.25-mm × 0.25-μm DB-5ms column (included with option or accessory)
- 5-m × 0.25-mm × 0.15-μm INNOWax column (included with option or accessory)
- 2D data analysis software, GC Image recommended (not provided by Agilent)
- Internal column nuts and SiTite ferrules

Ordering Information

Description	Part number
7890A GC with Capillary Flow Technology Modulator (requires checkout kit)	G3440A Option 887 or accessory G3486A
7890A GC with 200 Hz FID	G3440A Option 211 or accessory G3462A
7890A GC with split/splitless inlet	G3440A Option 112 or accessory G3452A
Capillary Flow Technology modulator checkout kit	G3487A
PCM for 7890A GC	G3440A Option 309 or accessory G3471A
SilTite metal ferrules, 1/16-in × 0.4-mm id, 10/pk, includes 2 column nuts	5184-3569
Agilent 32-bit ChemStation for 1 GC	G2070BA
Agilent 32-bit ChemStation Bundle for 1 GC includes: – G2070BA 32-bit ChemStation software – Computer with monitor and Windows operating system – Printer	G1875BA
2D GC software	www.zoex.com
Recommend GC Image software, which can be purchased from Zoex Corporation	

Application Examples

Several applications are shown. Note that primary column lengths have been chosen to give optimal results. While the 30M column that is shipped with the system is an excellent choice for a wide range of applications, other lengths can be used to optimize a given separation. Various columns have been used in these examples to illustrate some of

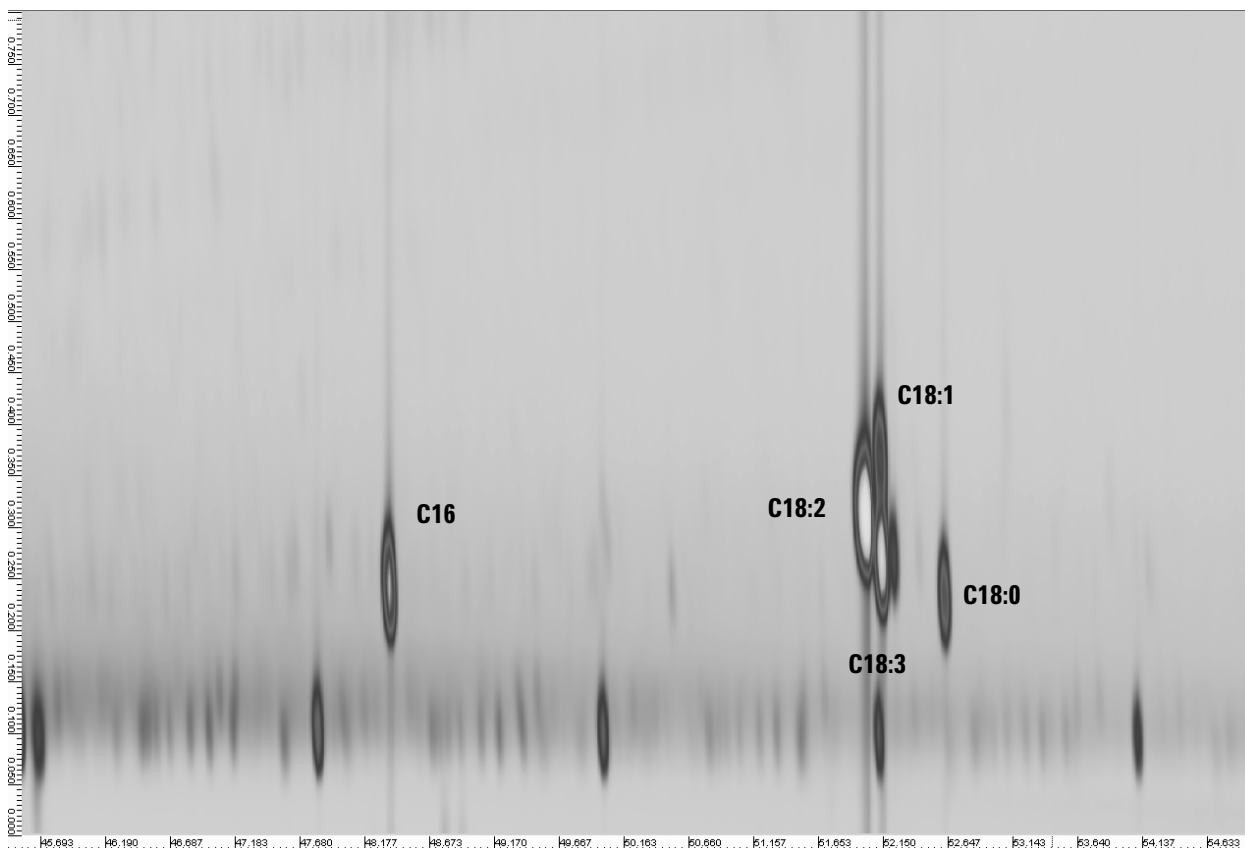
the possibilities. The GC Image software package was used for processing the ChemStation data.

1. B20 biodiesel based on soy FAMES. Section of the 2D image showing the C16 and C18 FAMES is shown.

Column 1: 60 m × 0.25 mm × 0.10 μm DB-5ms

Column 2: 5 m × 0.25 mm × 0.15 μm INNOWax

Modulation: 1.40 s load, 0.10 s inject

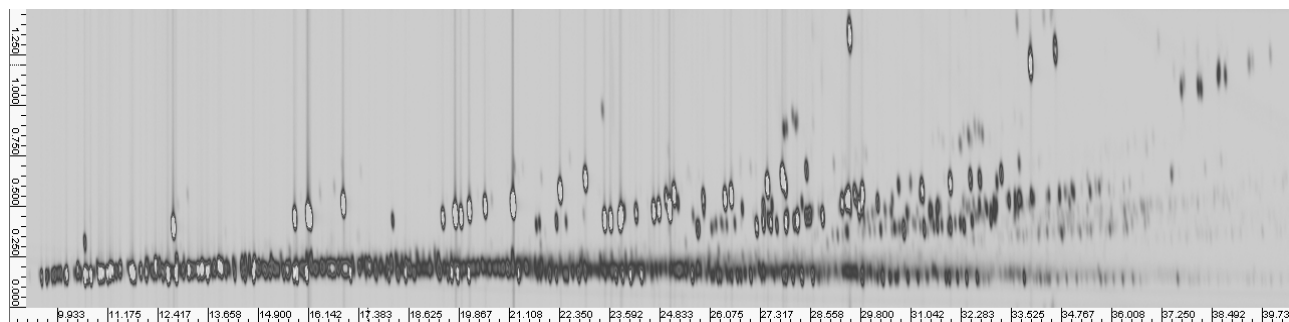


2. Complete 2D image of a sample of heavy gasoline. Each series of substituted 1-ring aromatics is well separated, making hydrocarbon class grouping possible.

Column 1: 60 m $\times$ 0.25 mm $\times$ 0.10 μ m DB-5ms

Column 2: 5 m $\times$ 0.25 mm $\times$ 0.15 μ m INNOWax

Modulation: 1.40 s load, 0.10 s inject

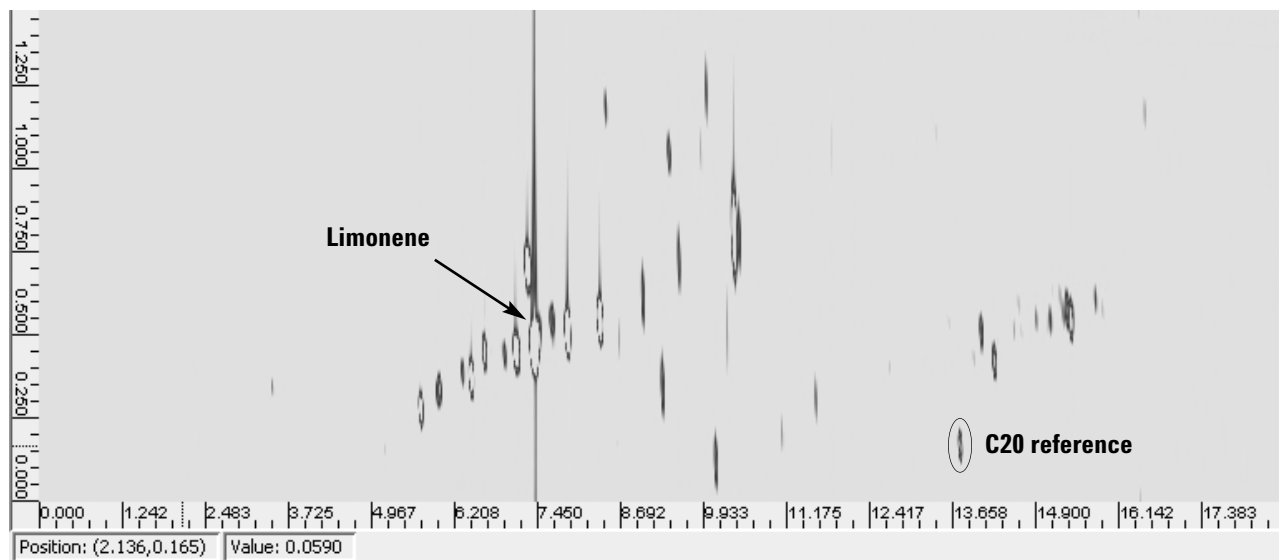


3. Lime oil 2D image.

Column 1: 15 m $\times$ 0.25 mm $\times$ 0.25 μ m DB-5ms

Column 2: 5 m $\times$ 0.25 mm $\times$ 0.15 μ m DB-17HT

Modulation: 1.40 s load, 0.10 s inject



Thermal vs. Flow Modulation

Since competitors offer only systems based on thermal modulation, the following table summarizes the key points about the respective approaches of thermal vs. flow modulation.

Thermal modulation	Differential Flow modulation
Cryo-focusing provides potentially narrower peaks in second dimension	Peak widths comparable to thermal. Usually no more than 20% wider. Many users want to sum regions of peaks where peak width is not as critical
Lower flows – Can be used with high-vacuum detectors (TOF)	MSD can be used with a splitter over limited scan range
Large consumption of cryogen	No cryogen required
Complex hardware design, set-up, and maintenance	Simple, reliable Capillary Flow Technology based hardware; small thermal foot print
Long chromatographic runs required for best performance	Run times comparable to a 1D separation
System price (estimate) \$60 to \$70K	Agilent system approximately \$60K (list)

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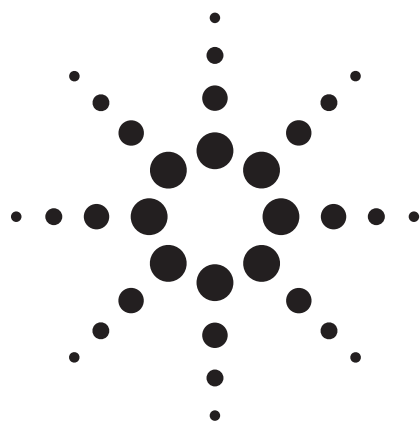
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The Use Of Automated Backflush on the 7890A/5975A GC-MS System



Setup Example Using Biomarkers

Application

Hydrocarbon Processing

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Abstract

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

Introduction

Until recently, the implementation of capillary column backflush has required a cumbersome conglomeration of parts and separate controllers. The nonintuitive combination of manual pressure regulators, timers, stand-alone valve controllers,

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

The benefits of backflush in capillary gas chromatography are myriad:

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

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



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

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

Experimental

Table 1 shows the analytical conditions used in a traditional GC-MS analysis of crude oil. The boiling point range of this oil sample is very wide (spanning C₄ to C₅₀), with the target components of interest eluting around 30 minutes in a 74-minute analysis (see Results and Discussion).

Table 1. Original Analytical Method Conditions

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

A 3-way purged splitter (Agilent part number G3183B) Capillary Flow Technology device was used for this application, in part to demonstrate its flexibility. The device has a purge and four

connections (Figure 1). As used herein, only two of the ports were used, one for the column outlet (port 3) and the other for the restrictor to the MSD (port 4). The other two ports (1 and 2) were plugged with solid wire instead of column connections. Very reliable connections are a feature of Capillary Flow Technology devices because of the use of soft metal ferrules. Care needs to be taken when making these connections, but the process is very straightforward and easily learned. The manuals provided with the various Capillary Flow Technology devices contain explicit instructions.

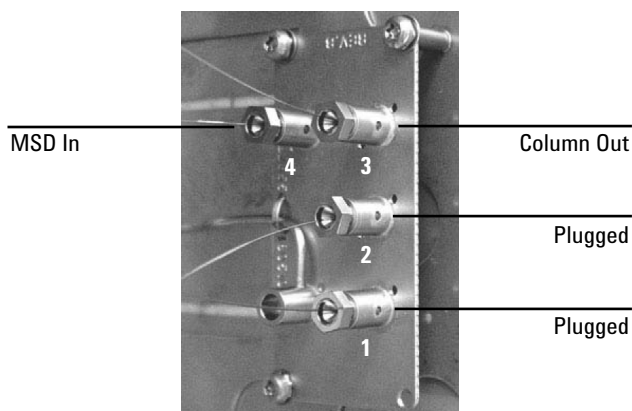


Figure 1. 3-way purged splitter. The column outlet was attached to port 3 and the MSD restrictor was attached to port 4. Ports 1 and 2 were plugged.

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

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

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

Table 2. Backflush Analytical Method Conditions

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

By setting up the required analytical column and restrictor with the correct inlet and outlet connections (Figures 2 to 4), the software automatically calculates the inlet pressure required to maintain analytical column flow. By selecting the “evaluate”

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

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

Description	
1	Agilent 190915-433; 325 °C; 30 m x 250 µm x 0.25 µm In: Front SS Inlet He Out: Aux Pressure 1 He
2	Agilent Restrictor; 360 °C; 1 m x 180 µm x 0 µm In: Aux Pressure 1 He Out: Vacuum
	Aux Pressure 2 N2
	Aux Pressure 3 N2

Control Mode ☒ On
☒ Flow
☐ Pressure

	Rate mL/min per min	Value mL/min	Hold Time min	Run Time min
▶		1.2	0	0
*				

Post Run: -5.6291 mL/min

Figure 2. Software setpoints for the analytical column.

Description	
1	Agilent 190915-433; 325 °C; 30 m x 250 µm x 0.25 µm In: Front SS Inlet He Out: Aux Pressure 1 He
2	Agilent Restrictor; 360 °C; 1 m x 180 µm x 0 µm In: Aux Pressure 1 He Out: Vacuum
	Aux Pressure 2 N2
	Aux Pressure 3 N2

Control Mode ☒ On
☐ Flow
☒ Pressure

	Rate psi/min	Value psi	Hold Time min	Run Time min
▶		1	0	0
*				

Post Run: 75 psi

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

	Description
▶ 1	Agilent 190915-433: 325 °C: 30 m x 250 µm x 0.25 µm In: Front SS Inlet He Out: Aux Pressure 1 He
2	Agilent Restrictor: 360 °C: 1 m x 180 µm x 0 µm In: Aux Pressure 1 He Out: Vacuum

Figure 4. Column inlet and outlet conditions.

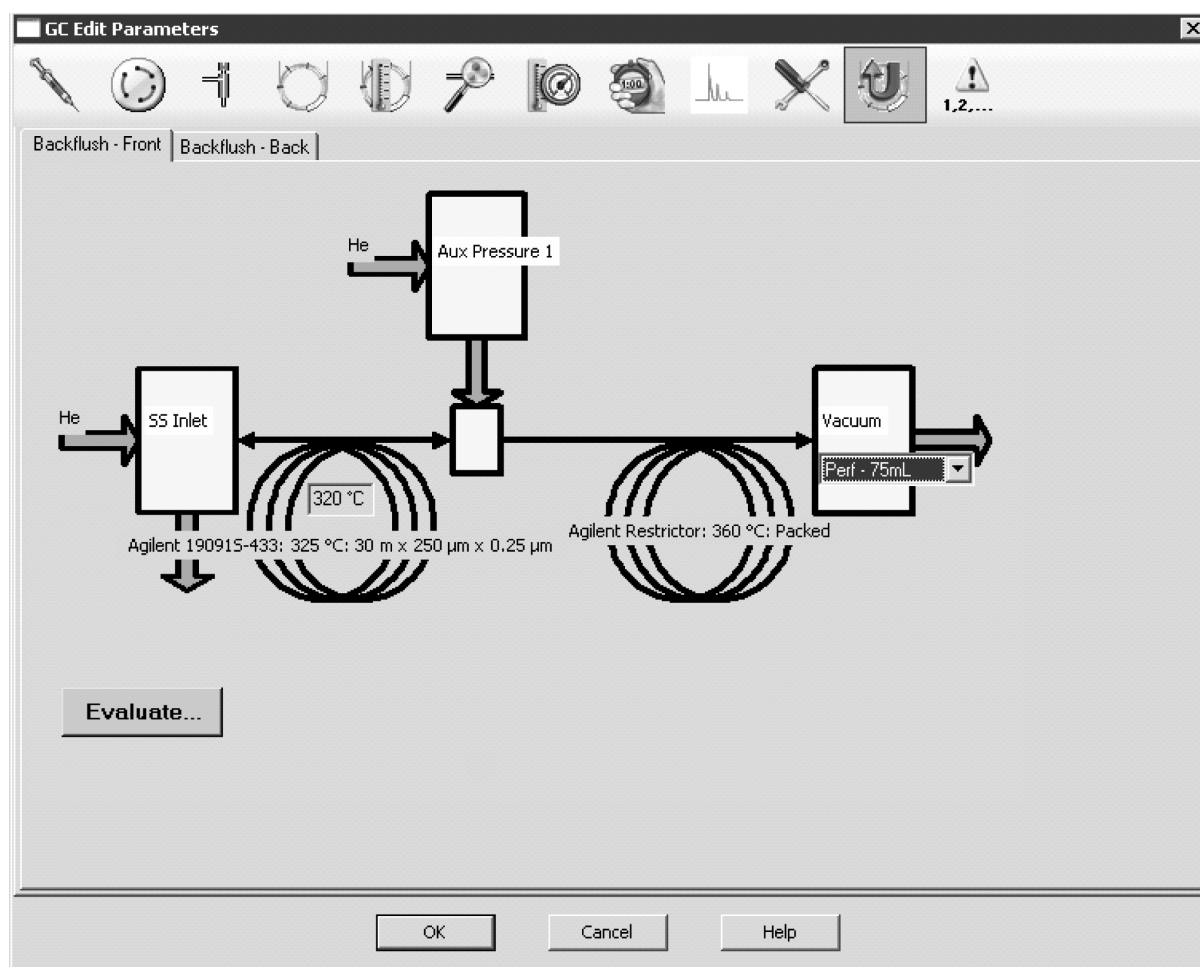


Figure 5. Interactive setup for backflush conditions in ChemStation.

Summary of Backflush Calculations

Oven Temperature
205 °C

Detector	Maximum Flow	Allowable Pressure	Flow at Chosen Pressure
Vacuum	75	79.654	67.783

Backflush Pressure
75 psi

Inlet Pressure during Backflush
1 psi

Backflush Flow to Inlet
8.0851 mL/min

Void Time
0.47227 min

Void Volumes
11.578

Backflush Time
5.4682 min

OK Cancel Help

Figure 6. Conditions uploaded to method setpoints.

Results and Discussion

The profile seen in Figure 9 is typical of many crude oils with complex distribution over a large boiling point range, with a large number of unresolved components. Another feature is the long tail

of high-boiling components that must be eluted after the compounds of interest. Figure 10 illustrates the three components of interest: a series of three methylbenzothiophenes through an extracted ion chromatogram (EIC) of m/z 198.

Review Backflush Settings [X]

The Backflush Calculations indicate setting the following Postrun Conditions:

Duration: 5.4682 min
Oven Temperature: 205 °C
Aux Pressure 1 Pressure: 75 psi
Front - SS Inlet Pressure: 1 psi

Choose 'OK' to make changes to method
Choose 'Cancel' to reconsider changes

OK Cancel

Figure 7. Conditions uploaded to method setpoints.

☒ Oven Temp On

Equilibration Time
0.5 min

Post Run Time:
5.4682 min

Cryo:
☐ On
☐ Quick Cool
 Cryo Use Temperature:
 0 °C
☐ Timeout Detection
 0 min
☐ Fault Detection

	Rate °C/min	Value °C	Hold Time min	Run Time min
▶		55	1	1
1	5	205	0	31
*				

Post Run: 205 °C

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

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

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

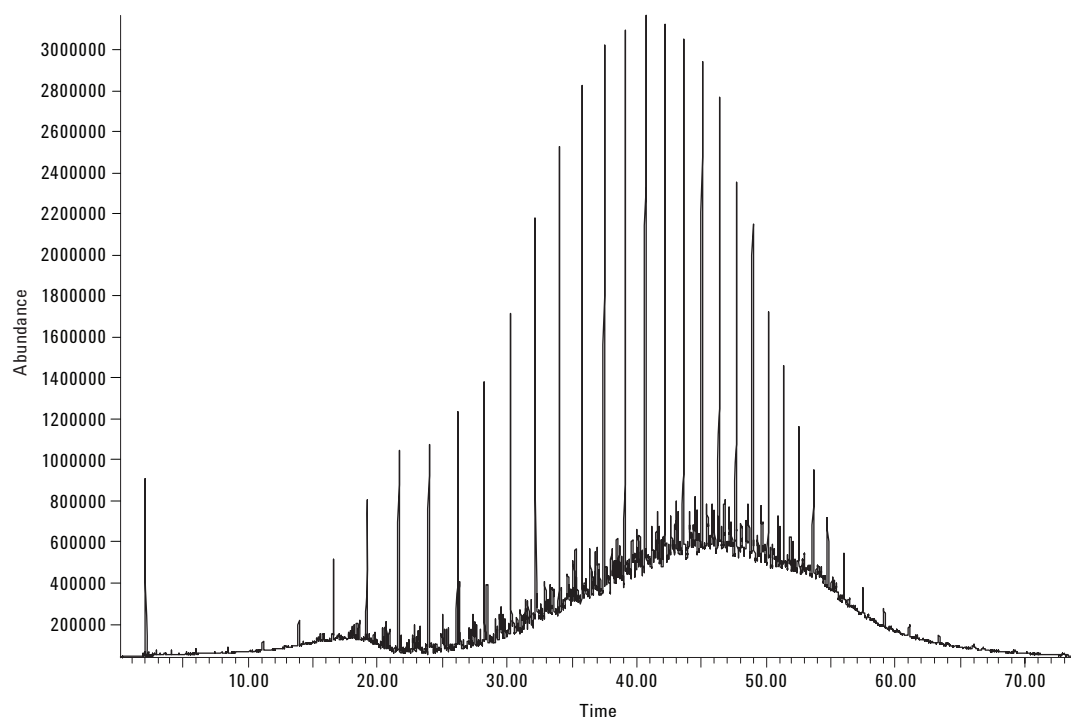


Figure 9. Total ion chromatogram (TIC) of normal analysis. Peaks of interest (benzothiophenes) are obscured by the high concentration of hydrocarbons.

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

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

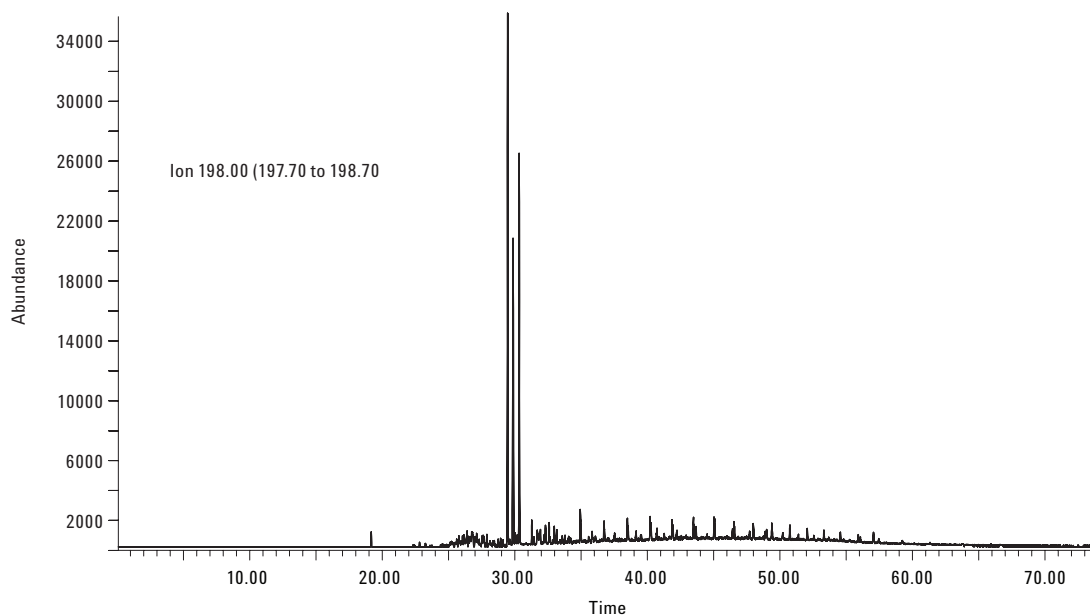


Figure 10. EIC of m/z 198 ion. The three methylbenzothiophene peaks of interest at approximately 30 minutes are easily visualized.

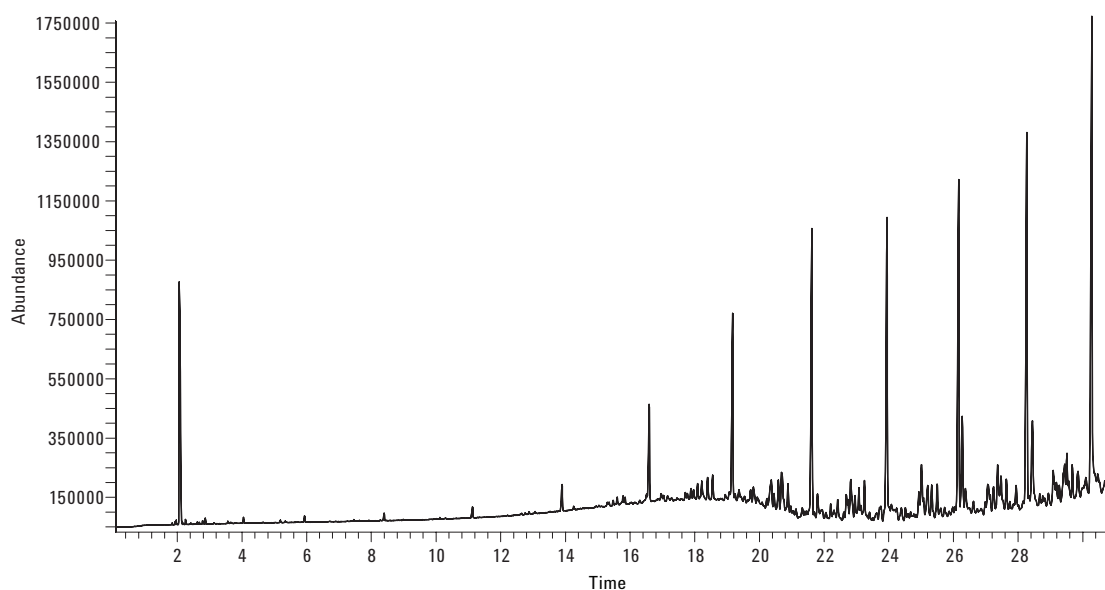


Figure 11. TIC of backflush run; run switched to backflush mode at 31 minutes.

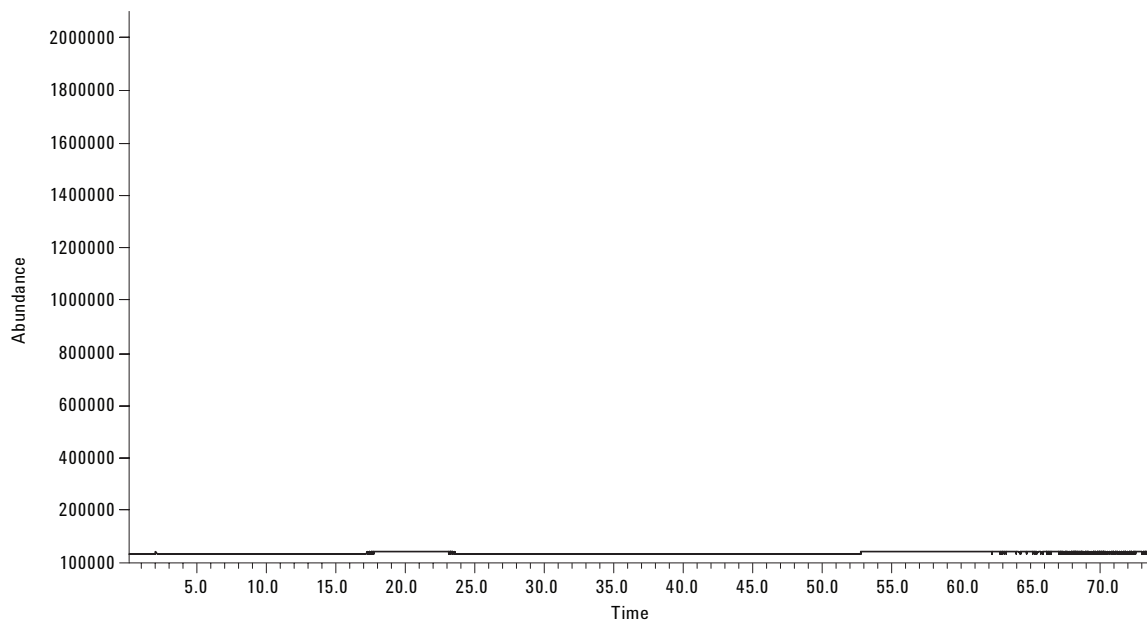


Figure 12. TIC of full run after the backflush with inset of the EIC of m/z 198.

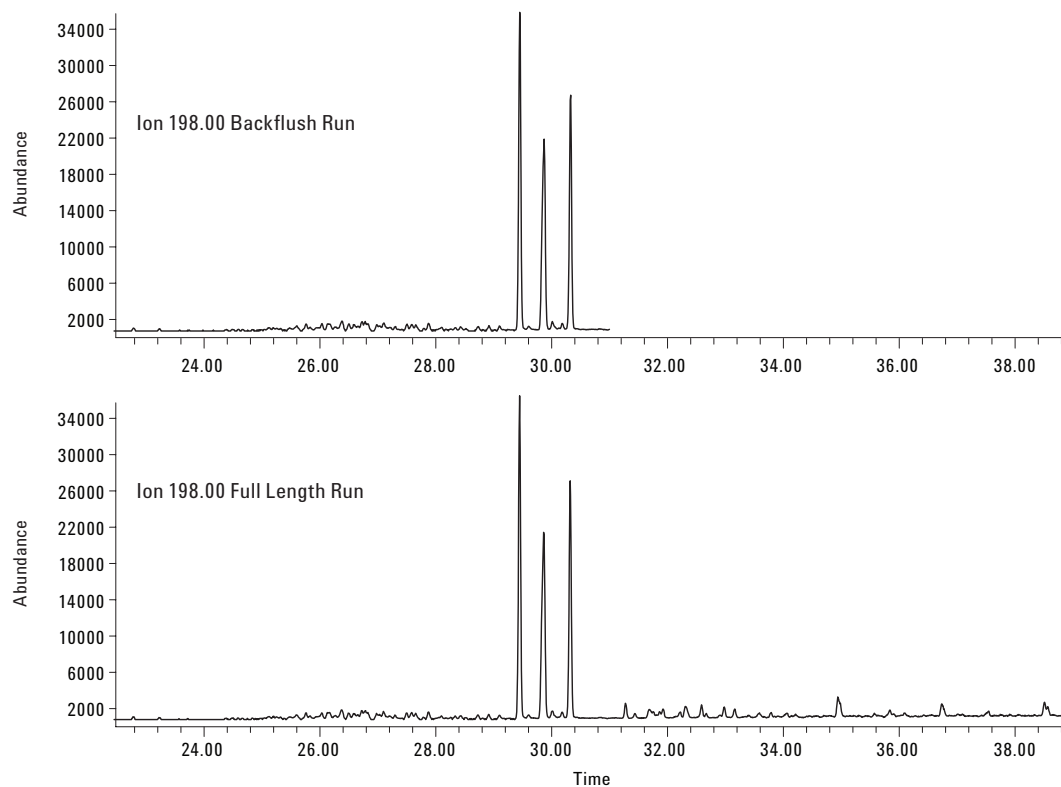


Figure 13. Overlay of EIC of m/z 198 from full run and backflush run, showing the exact matching of the analytical portion of each run for the three methylbenzothiophene biomarkers.

Conclusions

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

References

1. Russell Kinghorn, Courtney Milner, and Matthew S. Klee, "Simplified Backflush Using Agilent 6890 GC," Agilent Technologies publication 5989-5111EN
2. Chin Kai Meng, "Improving Productivity and Extending Column Life with Backflush," Agilent Technologies publication 5989-6018EN
3. Frank David and Matthew Klee, "GC/MS Analysis of PCBs in Waste Oil Using the Backflush Capability of the Agilent QuickSwap Accessory," Agilent Technologies publication 5989-7601EN
4. Frank David and Matthew Klee, "Analysis of Suspected Flavor and Fragrance Allergens in Cosmetics Using the 7890A GC and Capillary Column Backflush," Agilent Technologies publication 5989-6460EN
5. James McCurry, "Enhancements in the Operation and Precision of an ASTM D4815 Analyzer for the Determination of Oxygenates in Gasoline," Agilent Technologies publication 5988-4475EN
6. Mike Szelewski, "Significant Cycle Time Reduction Using the Agilent 7890A/5975A GC/MSD for EPA Method 8270," Agilent Technologies publication 5989-6026EN

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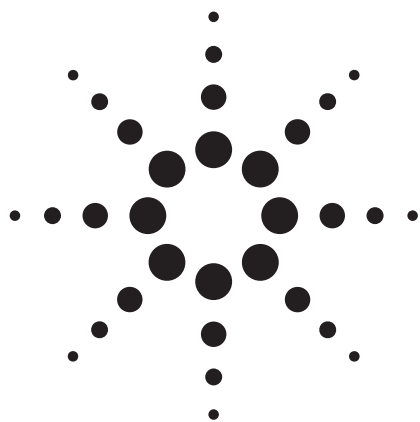
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Simultaneous Analysis of Greenhouse Gases by Gas Chromatography

Application

Environmental

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Abstract

Two analytical methods based on the Agilent 7890A GC system are developed for simultaneous analysis of methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O) in air samples. Each system has its own features to meet different requirements of greenhouse gases analyses. Both systems can easily be expanded to determine sulfur hexafluoride (SF_6). Results from both methods demonstrated high sensitivity and excellent repeatability for the required analyses.



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Introduction

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are considered the main greenhouse gases in the Earth's atmosphere. These gases trap heat in the atmosphere and affect the temperature of the Earth. Continuous measurement of these gases provides meaningful information to track greenhouse gas emission trends and help in the fight against climate change. On January 1, 2010, the U.S. Environmental Protection Agency will require large emitters of heat-trapping emissions to begin collecting greenhouse gas data under a new reporting system [1].

Two different configurations of Agilent 7890A GC systems have been developed for greenhouse gas analysis. These systems can also be used for other samples such as soil gases analysis or plant breathing studies where the analytes of interest contain gases such as CH₄, N₂O and CO₂ [2].

Method 1: SP1 7890-0468

An Agilent 7890A GC system is configured with a single channel using two detectors (FID and micro-ECD) for the analysis of CO₂, CH₄, N₂O, and SF₆ in air samples. Low concentrations of CO₂ can be analyzed by a methanizer with an FID.

Method 2: SP1 7890-0467

An Agilent 7890A GC is configured with two separate channels using three detectors (FID, TCD and micro-ECD) for the analysis of CO₂, CH₄, N₂O, and SF₆ in air samples. CO₂ can be analyzed at wide concentration levels. High levels of CO₂ can be analyzed by TCD and low concentrations can be analyzed by a methanizer with an FID.

A dynamic blending system is used to prepare the low level calibration standards using N₂ as a diluent.

Experimental and Results

Method 1: SP1 7890-0468

This system has three valves and two detectors using 1/8-in stainless steel packed columns (HayeSep Q 80/100). The methanizer/FID combination is used to measure low levels of CH₄ and CO₂, while the micro-ECD detects N₂O. The valve diagram is shown in Figure 1. The system can be modified to use a 6-port valve instead of a 10-port for automated headspace sampling. The typical GC conditions for Method 1 are listed in Table 1.

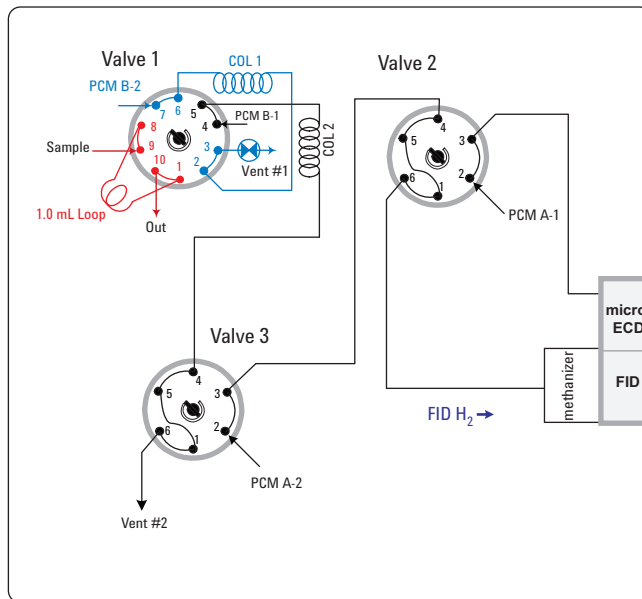


Figure 1. Configuration for SP1 7890-0468.

Table 1. Typical GC Conditions for Greenhouse Gas Analysis using Method 1

7890A GC

Valve temperature:	100 °C
Oven temperature:	60 °C
Post run at oven temperature of 110 °C for 2 minutes is recommended	
Methanizer Temperature:	375 °C
Sample loop:	1 mL
Column 1, 2 flow (N ₂):	21 mL/min (at 60 °C), constant pressure

FID

Temperature :	250 °C
H ₂ flow:	48 mL/min
Air flow:	500 mL/min
Make-up (N ₂):	2 mL/min

micro-ECD

Temperature :	350 °C
Make-up, 5% methane in Argon (Ar/5%CH ₄):	2 mL/min

Concentration of Gas Sample Standards

CH ₄ :	20.18 ppm v
CO ₂ :	376.4 ppm v
N ₂ O:	3.27 ppm v

Figure 2 illustrates a chromatogram of gas sample standards using Method 1. The sample is injected into a short HayeSep Q (column 1) which separates the components including air, CO₂ and CH₄ from water. All analytes after N₂O are back-flushed to vent 1. Air (O₂) should be directed away from the methanizer and micro-ECD and vented through vent 2. CO₂ is converted to CH₄ through the methanizer and measured by FID as shown in Figure 2B. After CO₂ elutes from column 2, the effluent is introduced to micro-ECD for measuring N₂O as shown in Figure 2A.

A repeatability study with 21 consecutive analyses was performed with results tabulated in Table 2. Excellent peak area repeatability for the analysis of CH₄, CO₂, and N₂O standards was observed with this configuration.

Table 2. Repeatability for Greenhouse Gas Standards (n=21, Excluding the First Run)

Name	Average (Area)	STDVE	RSD%
CH ₄	149.26	0.29	0.20
CO ₂	2779.04	17.16	0.62
N ₂ O	8253.96	11.06	0.13

To improve the sensitivity of micro-ECD, Ar-5% CH₄ is recommended as the make-up gas, which can lower the detection of N₂O to approximately 32 ppb with the good signal-to-noise (S/N) ratio as shown in Figure 3. The injected standard is prepared by dynamic blending with a 100-times dilution.

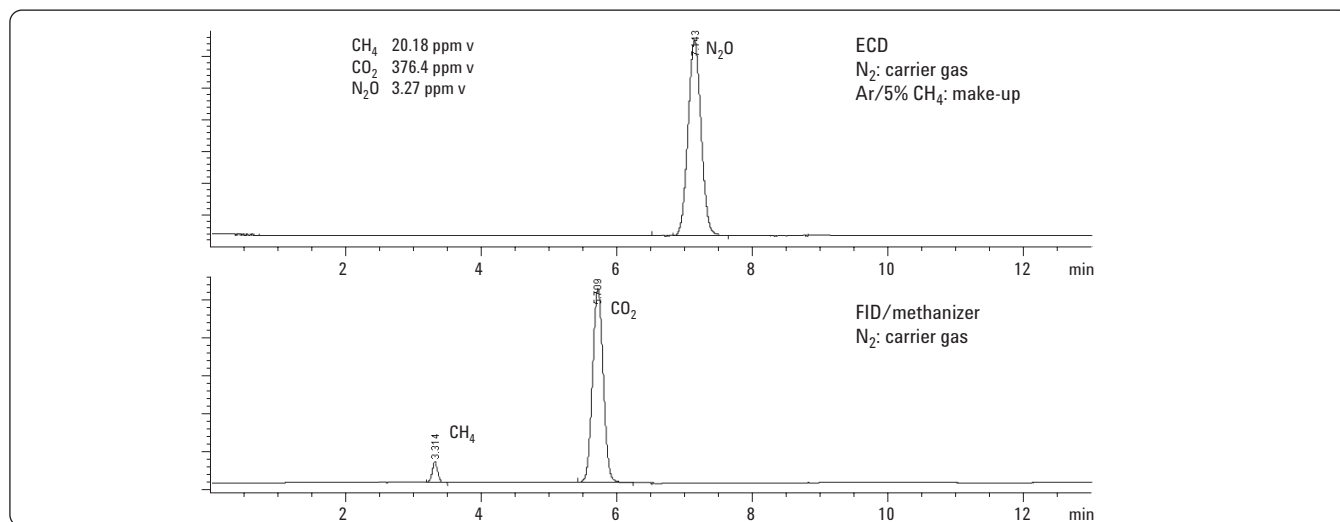


Figure 2. Analysis of greenhouse gases standards using Method 1.

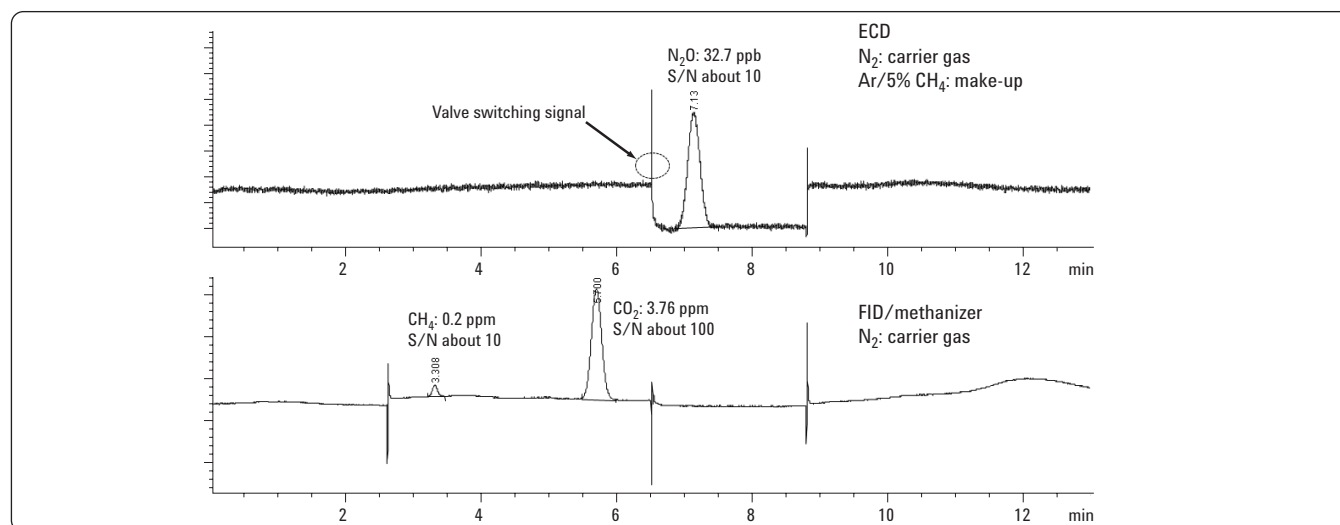


Figure 3. Chromatogram using Method 1 for CH₄, CO₂ and N₂O standards with a 100-times dilution.

The same configured system was used to analyze real samples. In this experiment, laboratory air is analyzed with Method 1. The chromatogram is shown in Figure 4. The measured concentrations of N_2O , CH_4 , and CO_2 are 473 ppb, 2.7 ppm, and 380 ppm respectively.

The system can easily include the analysis of SF_6 by delaying the backflush time (valve 1) to allow SF_6 to elute into column 1 (precursor). Figure 5 shows the chromatogram of SF_6 at approximately 0.5 ppb with a 1-mL sample size. The 0.5 ppb SF_6 standard is prepared by dynamic blending with 200 times dilution of the standard (original standard of SF_6 is 100 ppb).

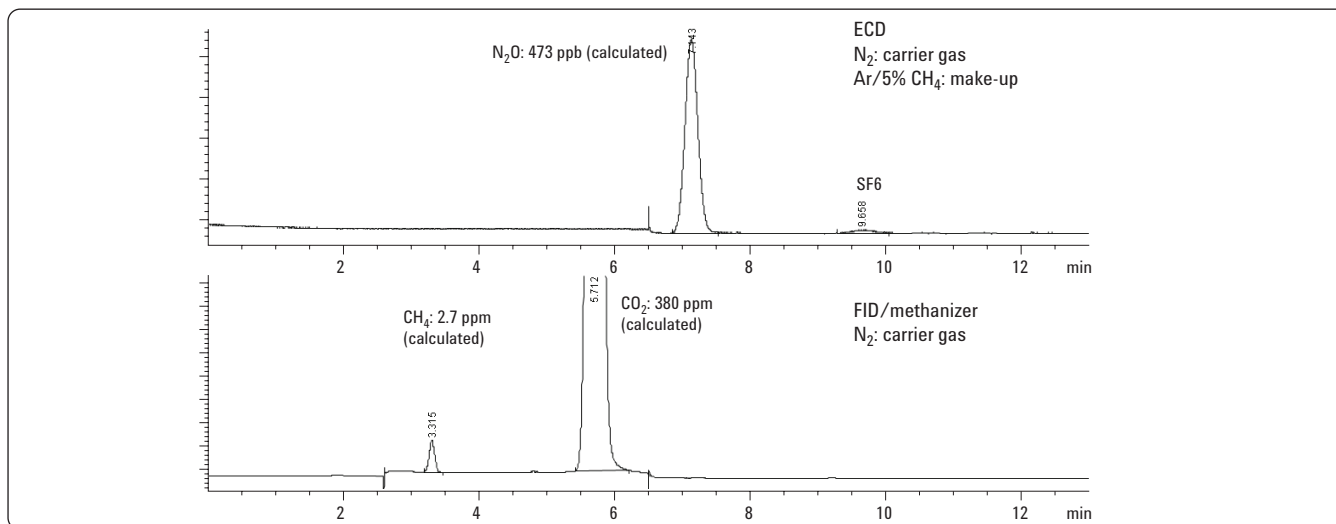


Figure 4. Chromatogram of real sample (laboratory air).

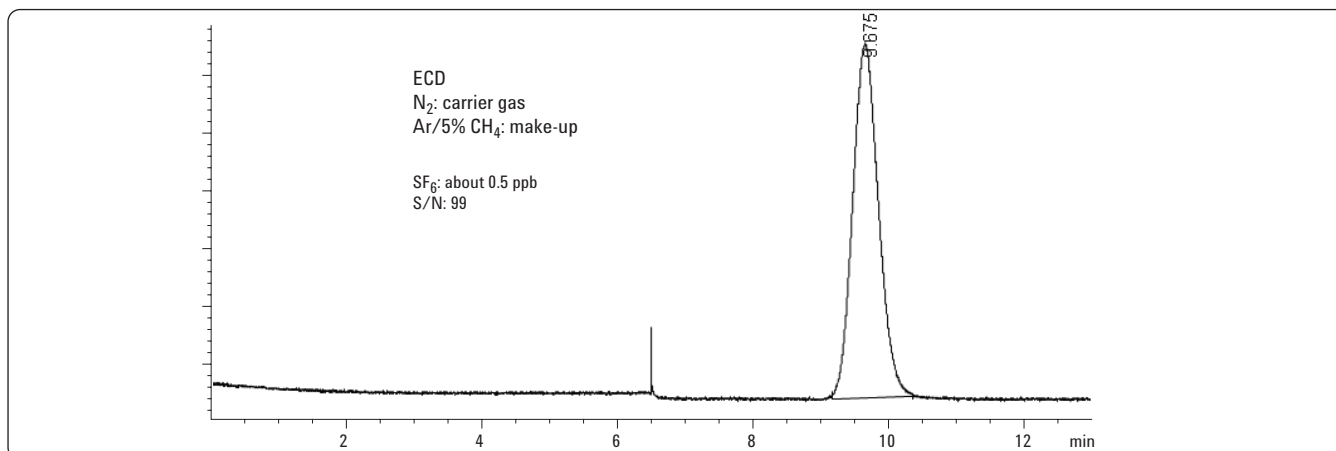


Figure 5. Chromatogram of SF_6 standard at approximately 0.5 ppb.

Method 2: SP1 7890-0467

This system consists of two separate channels with 1/8-in stainless steel packed columns (HayeSep Q 80/100). The first channel employs two valves with TCD and FID. The TCD and methanizer-FID are connected in series to measure CH₄ and CO₂. This channel provides the flexibility for CO₂ in varying levels. Low level CO₂ can be converted to CH₄ through the methanizer and measured by FID. The system is flexible depending on the requirements. The TCD can be used for high concentrations of CO₂. If only higher levels of CO₂ (higher than 50 ppm) analysis is required, the methanizer can be removed. This channel can be expanded to include O₂ and N₂ analysis by adding an additional Molsive column.

Another micro-ECD channel with two valves is dedicated to measuring N₂O and SF₆. Precolumns (column 1 and 2) direct heavier components (mainly water) to be backflushed to vent 1 and vent 4. O₂ should be excluded from the methanizer and micro-ECD and vented through vent 2 and vent 3. A typical plumbing diagram for this setup is shown in Figure 6. Typical GC conditions for Method 2 are listed in Table 3.

Table 3. Typical GC conditions for Greenhouse Gas Analysis Using Method 2

Valve temperature:	100 °C
Oven temperature:	60 °C
Post run at oven temperature of 110 °C for 2 min is recommended	
Sample loop:	1 mL
Column 1, 2 flow (He):	21 mL/min (at 60 °C), constant pressure
Column 3, 4 flow (N ₂):	21 mL/min (at 60 °C), constant pressure
FID	
Temperature:	250 °C
H ₂ flow:	48 mL/min
Air flow:	500 mL/min
Make-up (N ₂):	2 mL/min
TCD	
Temperature:	200 °C
Reference flow:	40 mL/min
Make-up:	2 mL/min
micro-ECD	
Temperature:	350 °C
Make-up, Ar/5% CH ₄ :	2 mL/min
Methanizer Temperature :	375 °C

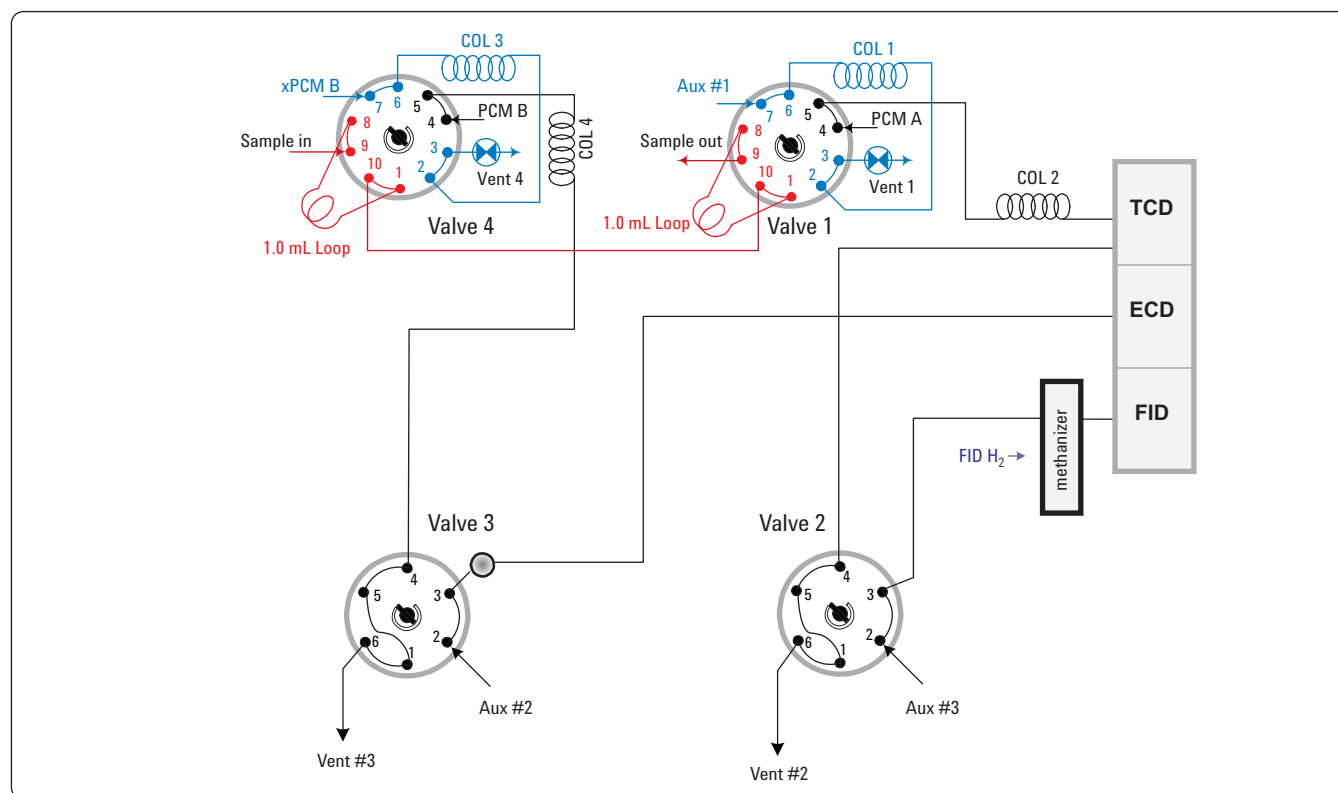


Figure 6. Valve configuration for Method 2.

Results obtained for greenhouse gases (N_2O , CH_4 , CO_2 and SF_6) by Method 2 are equivalent to those obtained by Method 1. In addition, with this setup, high levels of CO_2 can now be measured by the third detector, TCD. The dynamic blending system is also used for Method 2 to prepare the low level standards. Table 4 shows very good repeatability of peak areas for the analysis of the greenhouse gas standards.

Table 4. Repeatability for Greenhouse Gas Standards ($n=20$, Excluding the First Run)

Name	Average (Area)	STDVE	RSD%
CH_4	151.61	0.64	0.42
$\text{CO}_2(\text{FID})$	2788.51	14.72	0.53
N_2O	7467.92	13.91	0.19
$\text{CO}_2(\text{TCD})$	186.00	0.80	0.43

Real sample (laboratory air) is analyzed with Method 2. The chromatogram is shown in Figure 7. The concentrations of N_2O , CH_4 and CO_2 measured are 441 ppb, 2.2 ppm and 398 ppm respectively.

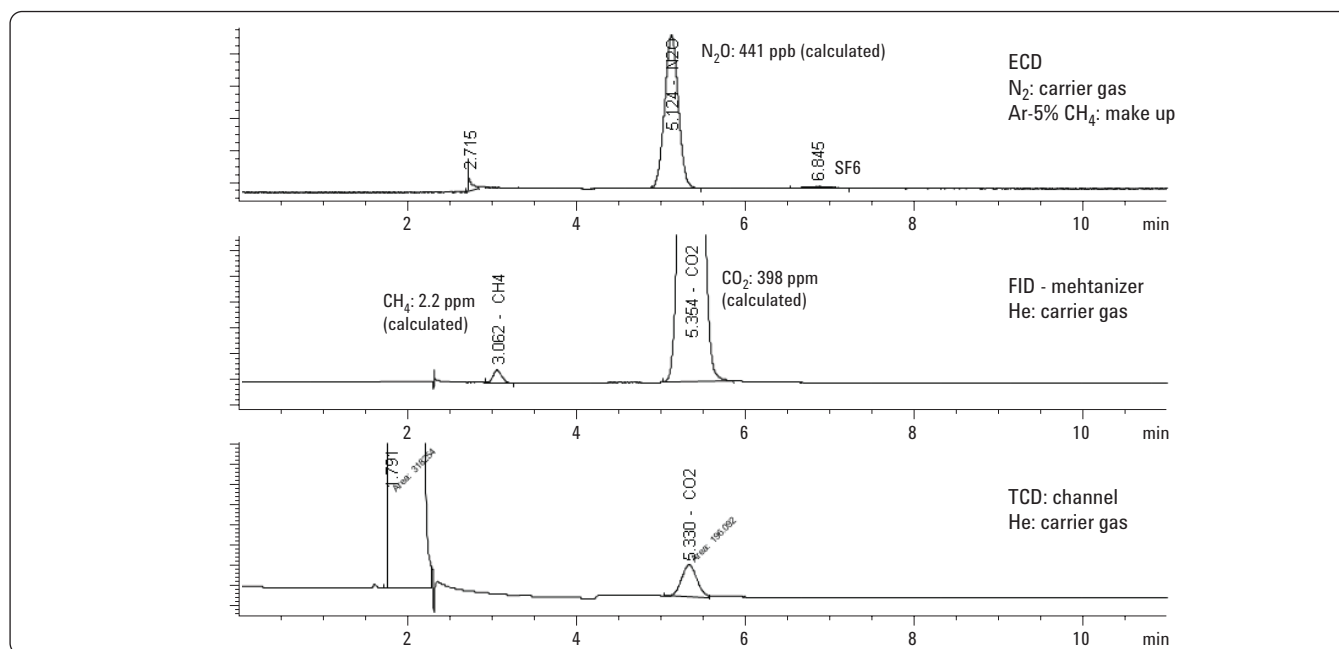


Figure 7. Chromatogram for real sample (laboratory air) using Method 2.

Conclusion

Two Agilent 7890A GC systems have been developed to meet the different requirements for simultaneous analyses of greenhouse gases including CH₄, CO₂, and N₂O in air samples.

Method 1 (SP1 7890-0468) has a simpler valve configuration and with minor modifications, accommodates autosampling by a headspace sampler.

Method 2 (SP1 7890-0467) has two separate channels with three detectors and can achieve even faster results. The separate channels increase flexibility to make the valve switching time less critical and the method easier to set up. The use of the third TCD allows measurement of a wide concentration range of CO₂ (0.2 ppm to 20%).

Results obtained on both analyzers are the same for greenhouse gases (N₂O, CH₄, CO₂ and SF₆).

References

1. Environmental Protection Agency (EPA), "40 CFR Parts 86, 87, 89 et al. Mandatory Reporting of Greenhouse Gases; Final Rule".
2. Teri Kanerva, Kristiina Regina, Kaisa Ramo, Katinka Ojanpera, Sirkku Manninen, "Fluxes of N₂O, CH₄ and CO₂ in a meadow ecosystem exposed to elevated ozone and carbon dioxide for three years", Environmental Pollution 145 (2007) 818-828.
3. European Environment Agency, Manual for the EEA greenhouse gases data viewer.

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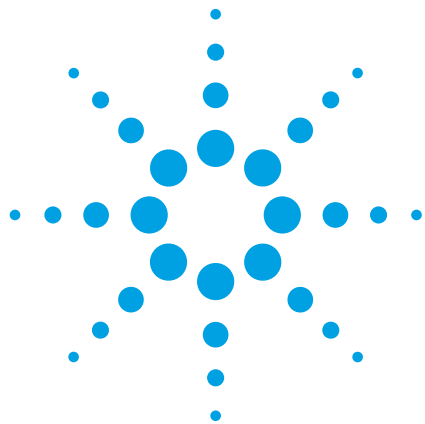
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Analysis of phenolic antioxidants and erucamide slip additives in polypropylene formulations

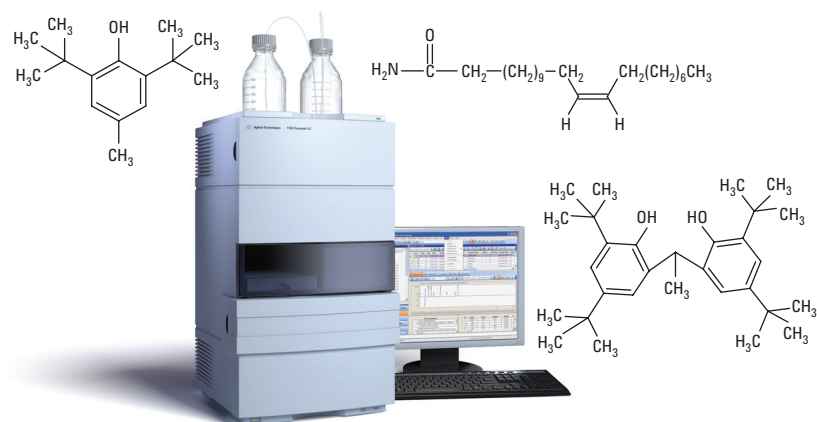
Excellent performance of the Agilent 1120 Compact LC and Agilent Method Translator to obtain ASTM-equivalent methods

Application Note

Food

Author

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Abstract

Phenolic antioxidants, such as vitamin E, and erucamide slip additives in polypropylene formulations were separated and detected using an Agilent 1120 Compact LC with UV/VIS detection. The method was executed under the guidelines of ASTM Method D6042-09 and adapted to various column dimensions with the help of Agilent Method Translator software (Version 2.0). Agilent ZORBAX Eclipse Plus columns were used for the separation. Linearity of the standards using the Agilent 1120 Compact LC was demonstrated. Tinuvin P was used as the internal standard for the experiment. The effects of reducing injection volume by changing column dimensions on peak shape and sensitivity are also discussed.



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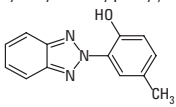
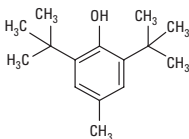
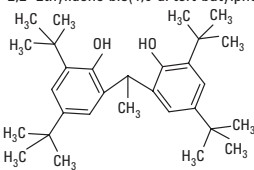
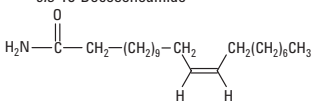
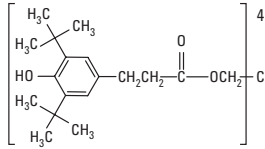
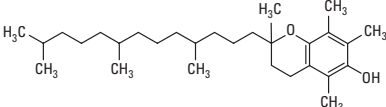
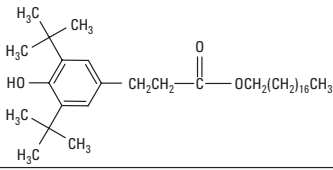
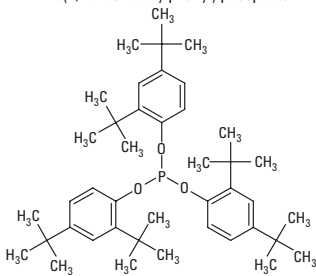
Name:	Tinuvin P	2-(2-Hydroxy-5-methylphenyl)benzotriazole
Formula:	C ₁₃ H ₁₁ N ₃ O	
Molecular weight:	225.25	
CAS No:	2440-22-4	
Name:	BHT	2,6-Di- <i>tert</i> -butyl- <i>p</i> -cresol
Formula:	C ₁₅ H ₂₄ O	
Molecular weight:	220.35	
CAS No:	128-37-0	
Name:	Isonox 129	2,2'-Ethylidene-bis(4,6-di- <i>tert</i> -butylphenol)
Formula:	C ₃₀ H ₄₆ O ₂	
Molecular weight:	438.69	
CAS No:	35958-30-6	
Name:	Erucamide	<i>cis</i> -13-Docosenoamide
Formula:	C ₂₂ H ₄₃ NO	
Molecular weight:	337.58	
CAS No:	112-84-5	
Name:	Irganox 1010	Pentaerythritol tetrakis(3,5-di- <i>tert</i> -butyl-4-hydroxyhydrocinnamate)
Formula:	C ₇₃ H ₁₀₈ O ₁₂	
Molecular weight:	1117.63	
CAS No:	6683-19-8	
Name:	Vitamin E	(±)- α -Tocopherol
Formula:	C ₂₈ H ₅₀ O ₂	
Molecular weight:	430.71	
CAS No:	10191-41-0	
Name:	Irganox 1076	Octadecyl 3-(3,5-di- <i>tert</i> -butyl-4-hydroxyphenyl)propionate
Formula:	C ₃₅ H ₆₂ O ₃	
Molecular weight:	530.86	
CAS No:	2082-79-3	
Name:	Irgafos 168	Tris(2,4-di- <i>tert</i> -butylphenyl) phosphate
Formula:	C ₄₂ H ₆₃ O ₃ P	
Molecular weight:	646.92	
CAS No:	31570-04-4	

Table 1
Chemical details of antioxidants and Tinuvin P

Introduction

Polymers are susceptible to degradation by ultraviolet light, heat and oxygen. To control this degradation process, various additives are blended into the polymeric material. These additives are also used to modify some of the physical properties of polymer formulations. Erucamide, Irgafos 168, Irganox 1010, Vitamin E (tocopherol), Irganox 1076, BHT, and Irgafos 168 are often used as antioxidants to prevent the degradation of polypropylene polymer formulations. HPLC methods are available for the quantitation of an additive or mix of additives in a polymeric material. ASTM D 6042-09¹ describes a conventional HPLC method for this purpose.

Accuracy of analysis is the most important requirement for ensuring that the additives and levels are suitable for the intended use. A liquid chromatography system with robust gradient pump, precise autosampler, and linear UV detector are required to execute this method. This Application Note evaluates the ability of the Agilent 1120 Compact LC to execute the ASTM D-6042 method for quantitation of the above mentioned additives. The ultraviolet (UV) absorbance for the compounds are measured at 200 nm and Tinuvin P is used as an internal standard according to the ASTM method. The samples for the analysis are prepared by an ultrasonic bath extraction procedure using a solvent mixture of 75:25 (v/v) dichloromethane:cyclohexane¹. Chemical details of antioxidants and Tinuvin P are tabulated in Table 1^{2,3}.

Experimental

Chemicals

All antioxidant standards and Tinuvin P were purchased from Sigma-Aldrich. HPLC grade water was freshly taken from a MilliQ water purification system. Super gradient grade acetonitrile (ACN), isopropyl alcohol (IPA) and dichloromethane (DCM) were purchased from Labscan (Bangkok Thailand). Cyclohexane for sample extraction was purchased from SD Fine Chemicals (India).

Sample/Solution preparation

- Individual stock solutions are prepared in IPA to 1000 µg/mL.
- Standard mixtures of BHT, Isonox 129, Erucamide, Irganox 1010, Vitamin E, Irganox 1076, Irgafos 168, all 125 µg/mL in IPA, were prepared by diluting individual standard stock solutions.

- Dichloromethane and cyclohexane were mixed in the ratio 75:25. A 7.5 mg amount of Tinuvin P was added to 150 mL of the above solvent mixture to prepare an extraction solvent mixture with an internal standard (Tinuvin P) concentration of 50 µg/mL.
- Samples: Three polypropylene formulations (self-prepared from polypropylene sample tubes, from polypropylene syringes, and packaged drinking water bottles) were extracted by ultrasonication according to Method ASTM-D6042-09. All three polypropylene formulation extracts were spiked with 25 µg/mL standard mix.
- Linearity levels of standard mixtures were prepared at the following concentrations: 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, 100 µg/mL. A concentration of approximately 50 µg/mL Tinuvin P was maintained in all linearity levels as an internal standard.

Columns

Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm (p/n 959993-902)

Agilent ZORBAX Eclipse Plus C18, 150 × 3.0 mm, 3.5 µm (p/n 959963-302)

Agilent ZORBAX Eclipse Plus C18, 100 × 3.0 mm, 3.5 µm (p/n 959961-302)

Agilent ZORBAX Eclipse Plus C18, 50 × 4.6 mm, 3.5 µm (p/n 959943-902)

LC system

An Agilent 1120 Compact LC, consisting of a gradient pump with integrated degasser, autosampler with vial tray, column oven, variable wavelength detector, and Agilent EZChrom Elite Compact software was used.

LC Parameters

The LC method used was ASTM-D6042-09. The run time was 30 min, with no post run time. The method details and the gradient used are tabulated in Table 2.

Parameter	Details
Column	Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm (p/n 959993-902)
Column Oven	50 °C
Mobile phase A	Water
Mobile phase B	Acetonitrile
Flow rate	1.5 mL/min
Needle wash	Using 100% acetonitrile
Injection volume	Variable, 10 µL and 5 µL
Detection	200/4 nm; no reference
Peak width	> 0.125 s
Data acquisition rate	20 Hz

Time	%B
0	75
5	100
25	100
25.1	75
30	75

Table 2
LC method details (ASTM method).

Procedure

A blank injection was performed in all trials to check the chromatographic interference in the resolution. Standard spike mix, extracted samples, and spiked extracted samples were also injected. The retention times of extraction solvents and individual standards were confirmed by individual standard injections.

Results and Discussion

Effect of injection volume on peak shape

According to method ASTM D 6042-09, DCM and cyclohexane in the ratio 75:25 are used for the extraction of antioxidants from polypropylene formulations. Both DCM and cyclohexane are immiscible with the acetonitrile and water mobile phases. Upon injection of the extract, a peak splitting is observed with an injection volume of 10 µL. When the injection volume was reduced from 10 µL to 5 µL, the peak shape improved (Figure 1). The effect of injection volume was not prominent with standards dissolved in IPA.

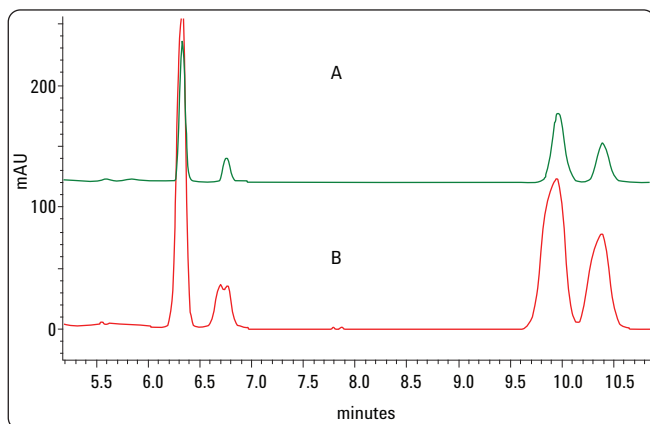


Figure 1
Effect of injection volume on peak shape. The lower trace (A) is with 10 µL injection volume and the upper trace (B) is with 5 µL.

Based on the observed injection volume effect, a 5-µm injection volume was maintained throughout this study. According to the ASTM method, the lowest level of phenolic antioxidant detectable under optimum conditions is approximately 2 µg/mL and the suggested injection volume is 10 µL. To maintain the same on-column concentration while checking the limit of detection, a 4 µg/mL standard solution was prepared and 5 µL were injected. The observed signal-to-noise (S/N) values for all individual peaks are included in Table 4.

Chromatographic representation of ASTM method

A standard spike mix of all additives at a concentration of 125 µg/mL in IPA was prepared and injected. The representative chromatogram of the standard spike solution as per ASTM method using an Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm column is shown in Figure 2.

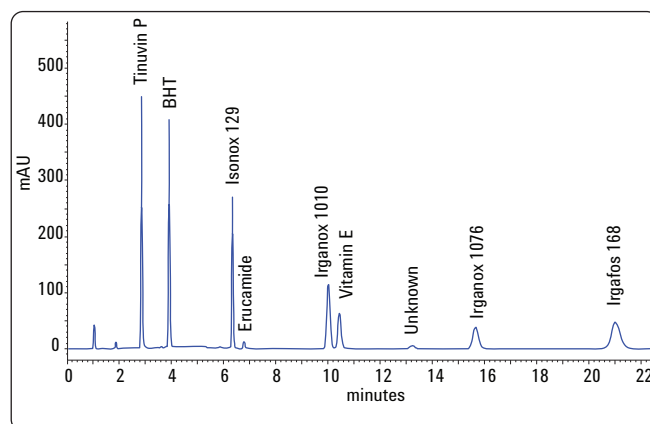


Figure 2
Representative chromatogram of a standard mix as per ASTM method (all at 125 µg/mL in IPA). (Column used: Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm).

An unknown peak was observed at approximately 13.2 minutes, which is an impurity from Irgafos 168.

The chromatograms of the three extracted samples overlaid with spiked standard mix is shown in Figure 3.

Erucamide was present in all three extracted samples. In Sample I, the unknown peak was very prominent along with trace amounts of Irganox 1010, Irgafos 168 and Irganox 1076. Sample II contained Irganox 1010, an unknown peak and Irgafos 168, while Isonox 129 and Irganox 1076 were present in trace levels. Sample III, contained Irganox 1010, an unknown impurity, Irganox 1076 and Irgafos 168 in trace levels only.

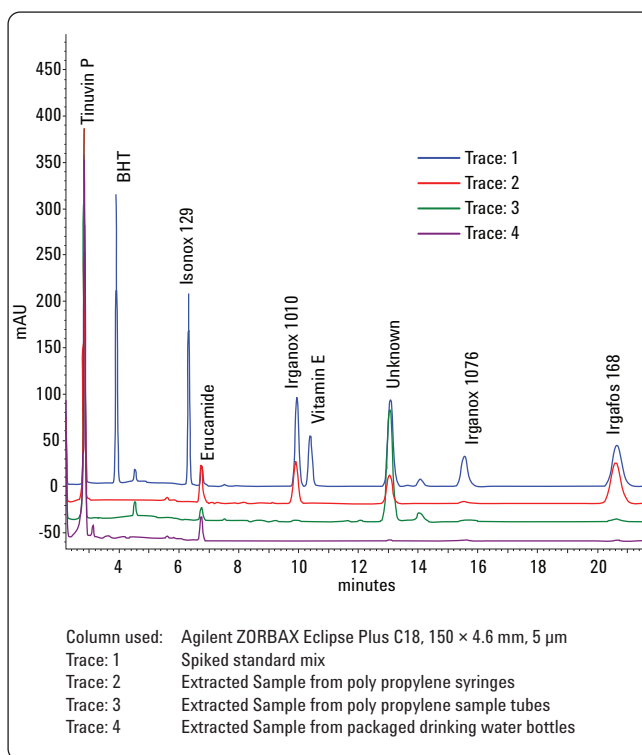


Figure 3
Chromatographic overlay of three extracted samples with spiked standard mixture.

Linearity and precision

Linearity tests were performed in the concentration range of 4 µg/mL to 100 µg/mL. The linearity levels were 4 ppm, 8 ppm, 16 ppm, 32 ppm, 64 ppm and 100 ppm. To demonstrate the precision of area and retention time, five replicate samples

for all linearity levels were injected and relative standard deviation (RSD) for retention time (RT) and area were calculated. The graphical representation of RSD for RT and area can be shown (Figure 4 a and b). The observed RSD values throughout the linearity levels for retention time (<0.18%) and for area (<0.92%) are well within the acceptance limit of 1.0% which confirms the excellent precision in area and retention time.

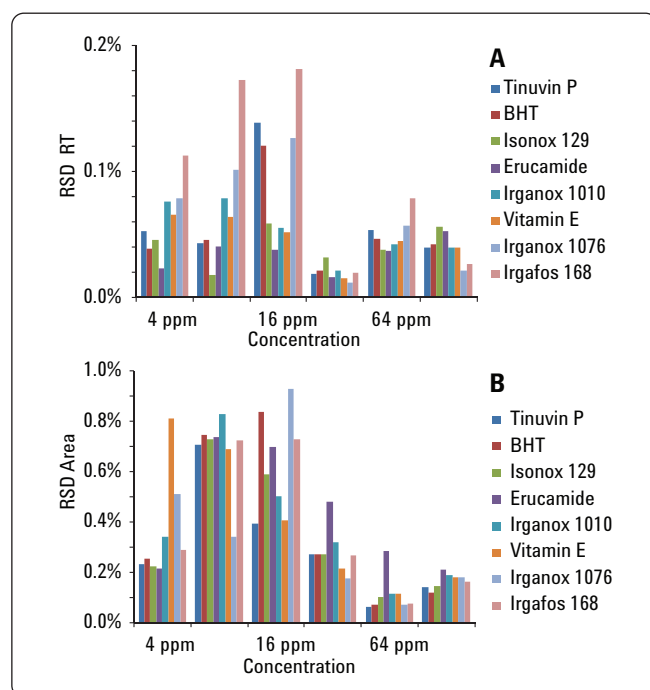


Figure 4
Graphical representation of RSD for (A) RT and (B) area.

A calibration graph was constructed by plotting the peak area of each standard against nominal concentrations (4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, and 100 µg/mL). The linearity of the relationship between peak area and concentration is demonstrated by the correlation coefficients obtained above $r^2 > 0.999$. The overlaid linearity curves for all standards are shown in Figure 5. ASTM signal-to-noise ratios for all standards at 20 ng on-column (4 ppm solution, 5 µL injection) concentration along with r^2 values are tabulated in Table 3. The RT RSD across the linearity levels is also included in Table 3.

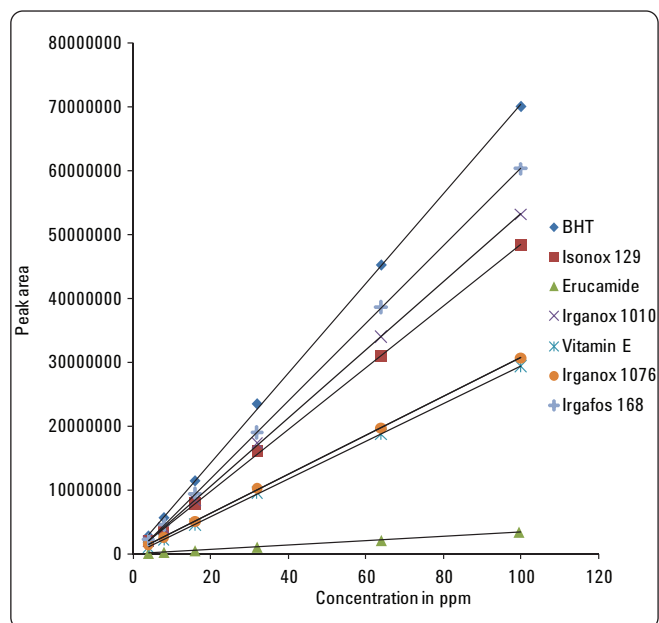


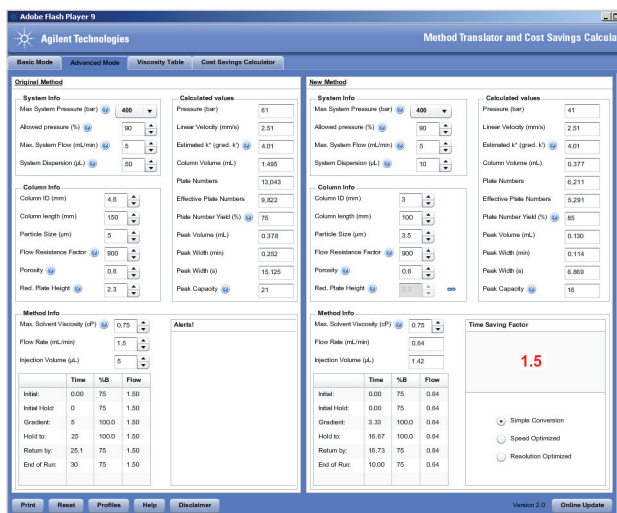
Figure 5
Linearity curves for all standards.

Compound	r^2 Values	S/N(ASTM) value at 4 ppm level, 5 µL	RT RSD across the linearity levels (6 levels)%
BHT	0.9998	650	0.19
Isonox 129	0.9999	234	0.05
Erucamide	0.9999	15	0.02
Irganox 1010	0.9999	245	0.12
Vitamin E	0.9999	131	0.10
Irganox 1076	0.9999	263	0.24
Irgafos 168	0.9999	244	0.38

Table 3
 r^2 values and S/N for each standard at 20 ng on-column concentration and RT RSD for each standard across linearity levels.

Trials with reducing column dimension and particle size

In order to demonstrate the improved sensitivity and reduced analysis time by changing the column dimensions and particle size, the initial ASTM method was recalculated using Agilent Method Translator software for three other column dimensions. Agilent ZORBAX Eclipse Plus C18 columns were used for this study. The screenshots of Method Translator are shown in Figure 6, and the corresponding chromatograms are shown in Figure 7.



Column 1

	Time	%B	Flow
Initial:	0.00	75	0.64
Initial Hold:	0.00	75	0.64
Gradient:	5.00	100.0	0.64
Hold to:	25.00	100.0	0.64
Return by:	25.10	75	0.64
End of Run:	30.00	75	0.64

- ☒ Simple Conversion
- ☐ Speed Optimized
- ☐ Resolution Optimized

Column 2

	Time	%B	Flow
Initial:	0.00	75	0.64
Initial Hold:	0.00	75	0.64
Gradient:	5.00	100.0	0.64
Hold to:	25.00	100.0	0.64
Return by:	25.10	75	0.64
End of Run:	30.00	75	0.64

- ☒ Simple Conversion
- ☐ Speed Optimized
- ☐ Resolution Optimized

Column 3

	Time	%B	Flow
Initial:	0.00	75	1.13
Initial Hold:	0.00	75	1.13
Gradient:	1.67	100.0	1.13
Hold to:	11.00	100.0	1.13
Return by:	8.37	75	1.13
End of Run:	10.00	75	1.13

- ☒ Simple Conversion
- ☐ Speed Optimized
- ☐ Resolution Optimized

Column 1: Agilent ZORBAX Eclipse Plus C18, 150 × 3.0 mm, 3.5 µm
 Column 2: Agilent ZORBAX Eclipse Plus C18, 100 × 3.0 mm, 3.5 µm
 Column 3: Agilent ZORBAX Eclipse Plus C18, 50 × 4.6 mm, 3.5 µm

Figure 6
 Agilent Method Translator screenshot.

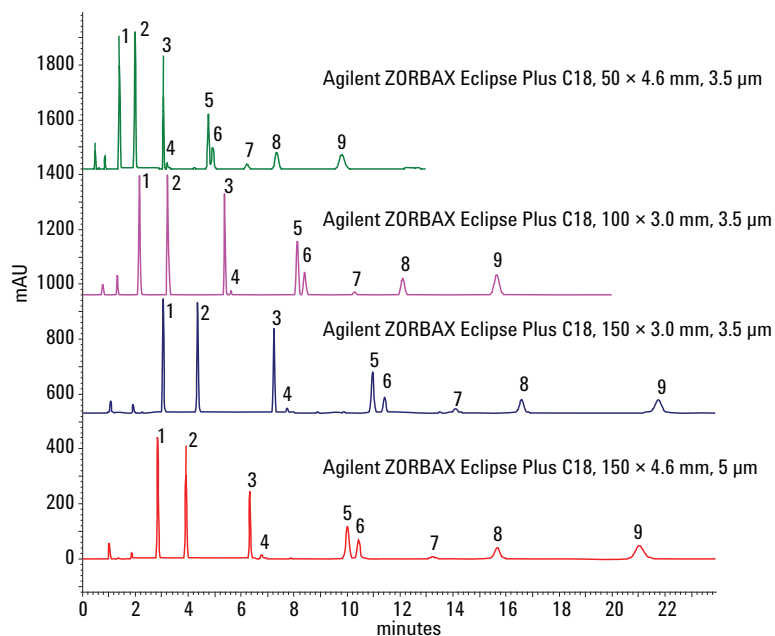


Figure 7
 Chromatographic separation of additives using various column dimensions.

Resolution values for two critical pairs (Isonox 129 and Erucamide and Irganox 1010 and Vitamin E) were evaluated to illustrate a comparison in chromatographic performance. (Table 4). The S/N value for Erucamide (20 ng on-column) is included in the same table. With 5 cm columns, the first solvent peak starts eluting at 0.4 minutes, affecting the ASTM S/N calculation. Therefore, S/N values were calculated manually by selecting 0 to 0.35 minutes as the noise region (ASTM uses a default noise region of 0 to 0.6 minutes for the calculation).

Critical pairs	150 × 4.6 mm 5 µm	150 × 3.0 mm	100 × 3.0 mm	50 × 4.6 mm
	Original method	3.5 µm	3.5 µm	3.5 µm
Isonox 129 Erucamide	4.25	5.71	3.17	2.47
Irganox 1010 Vitamin E	1.86	2.56	1.83	1.31
S/N value for 20 ng Erucamide on-column	48	168	225	108

Table 4
The resolution values for critical pairs along with observed S/N value for 20 ng on-column concentration of Erucamide using Agilent ZORBAX Eclipse Plus columns.

The Agilent ZORBAX Eclipse Plus 100 × 3.0 mm, 3.5 µm column gives a better peak height compared to other column dimensions used in this study. This improves S/N values above the conventional ASTM column dimension of 150 × 4.6 mm, 5 µm. The lowest resolution value observed with this column was 1.83, (between Irganox 1010 and Vitamin E) and is well within the Standard Pharmacopeia specification limit. Resolution provided by ZORBAX Eclipse Plus 50 × 4.6 mm, 3.5 µm column between Irganox 1010 and Vitamin E is usually not acceptable by Pharmacopeia (1.31), but included here to demonstrate the effect of resolution and sensitivity by reducing the column length.

Conclusions

Phenolic antioxidants, Vitamin E and Erucamide slip additives in polypropylene formulations are well resolved and detected using Agilent's 1120 Compact LC with variable wavelength detection at 200 nm UV/VIS detection. The excellent performance of Agilent 1120 Compact LC pump is established by demonstrating high precision for retention times and areas as well as excellent signal-to-noise ratios. Exceptional linearity of the Agilent 1120 Compact LC detector is demonstrated. The observed r^2 values for all standards are >0.999. This Application Note also demonstrates the applicability of Agilent Method Translator software to obtain an equivalent method from ASTM D6042-09 when using different column dimensions. Improvement in peak sensitivity and lowest detection limits are shown by reducing the column diameter and particle size.

References

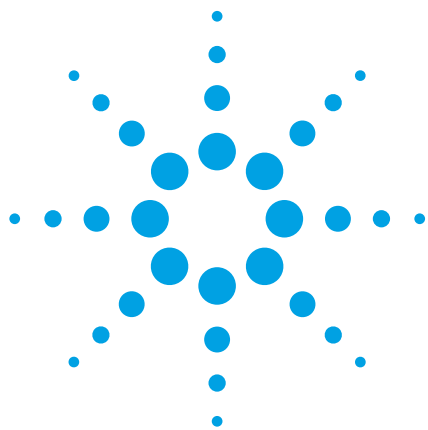
1. ASTM D6042-09, "Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Formulations Using Liquid Chromatography (LC)". Volume 08.03 Plastics (III): July 2009
2. Wei Luan, Chunxiao Wang (Shanghai-China) and Michael Woodman (Wilmington USA) "Fast Analysis of Tocopherol, Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator", Agilent Technologies Publication 5989-5849EN, 2009 .
3. Chun-Xiao Wang, Wei Luan (Shanghai-China) and Michael Woodman (Wilmington USA) "Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC", Agilent Technologies Publication 5989-5850EN 2009.

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High-throughput method development for aldehydes and ketones using an Agilent 1290 Infinity LC system and an Agilent ZORBAX StableBond HD column

Application Note

Environmental

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Abstract

This Application Note describes the development of a fast method for the determination of 13 aldehyde and ketone derivatives with the Agilent 1290 Infinity LC system. The method, which used acetone as organic co-solvent separates the analytes within 3.5 minutes.



Agilent Technologies

Introduction

Aldehydes and ketones are important compounds in the chemical industry. One of the most essential aldehydes is formaldehyde because it is used for the production of glued wood and synthetic resin. In addition, formaldehyde is one of the most used disinfectants and preservative agents worldwide. Another relevant aldehyde in the chemical industry is acetaldehyde. This chemical is frequently used as an organic solvent and is an important intermediate product in many industries. For example, acetaldehyde is principally used for the production of acetic acid. In general, aldehydes and ketones with middle carbon chain lengths are used as intermediate products during the production of gum, synthetic resin and plastic products. Therefore, many analytical methods exist for the determination of aldehydes and ketones in different matrices. The majority of these methods use the derivatization with 2,4-dinitrophenylhydrazine yielding the corresponding 2,4-dinitrophenylhydrazone. After that, an HPLC separation with UV detection at 360 nm is then performed.

The introduction of the Agilent 1290 Infinity LC system has improved LC-UV methods in several ways. The pressure of the Agilent 1290 Infinity LC system remains stable as high as 1200 bar at flow rates up to 2 mL/min. This is a significant enhancement in comparison to conventional HPLC systems. The most important advantage of the Agilent 1290 Infinity LC system is the small dwell volume of 125 µL (the volume from the point of mixing solvents A and B up to the column inlet including the autosampler). Because of this very small dwell volume, narrow bore columns can be used to shorten analysis time and reduce organic solvent consumption.

This Application Note focuses on LC method development for the determination of several aldehydes and ketones, as well as the advantages of the Agilent 1290 Infinity LC system.

A commercially available method development software package was used to determine the optimal method parameters. Four basic chromatographic runs were performed to determine the optimal column temperature and solvent gradient. These measurements comprised two linear solvent gradients from 5% to 100% B in 10 and 30 minutes at 20 °C and the same gradients at 40 °C. The measurements were performed on an Agilent ZORBAX StableBond RRHD C18 column (50 mm × 2.1 mm, 1.8 µm) by using acetone as an organic modifier. A method was then developed and experimentally confirmed with high agreement between prediction and experiment.

Experimental

All calculations were performed with Agilent ChemStation software version B.04.02 [65].

LC system

For method development, an Agilent 1290 Infinity LC system was used. The system consists of:

- Agilent 1290 Infinity Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment SL (G1316B)
- Agilent 1290 Infinity Diode Array Detector (G4212A)

Analyte mixture

The mixture of aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones is a certified reference material from Sigma-Aldrich (Catalog No. 47651-U) diluted in acetonitrile. In the mixture, each analyte has a concentration of 30 µg/mL of carbon.

The elution order for all analytes depicted in all figures is:

1. Formaldehyde-2,4-dinitrophenylhydrazone
2. Acetaldehyde-2,4-dinitrophenylhydrazone
3. Acrolein-2,4-dinitrophenylhydrazone
4. Acetone-2,4-dinitrophenylhydrazone
5. Propionaldehyde-2,4-dinitrophenylhydrazone
6. Crotonaldehyde-2,4-dinitrophenylhydrazone
7. Methacrolein-2,4-dinitrophenylhydrazone
8. 2-Butanone-2,4-dinitrophenylhydrazone
9. Butyraldehyde-2,4-dinitrophenylhydrazone
10. Benzaldehyde-2,4-dinitrophenylhydrazone
11. Valeraldehyde-2,4-dinitrophenylhydrazone
12. m-Tolualdehyde 2,4-dinitrophenylhydrazone
13. Hexaldehyde-2,4-dinitrophenylhydrazone

Results and discussion

Figure 1 shows the computer-optimized separation of 13 aldehyde 2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones on an Agilent ZORBAX StableBond RRHD C18 column within 3.5 minutes. Acetone was used as an organic co-solvent. All peaks are baseline separated with a critical resolution of 1.6 between peak pair 6 and 7. The critical resolution was calculated by the tangent method. The impurities, which are present in the reference material and highlighted by stars were not included in the method development. Figure 1 also shows a comparison of the programmed and effective solvent gradients. Due to a very small dwell volume, there is only a minor difference between the programmed and effective solvent gradients compared to a conventional HPLC system, which exhibits a dwell volume of approximately 1000 μL . This means that at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet with a delay of 0.83 minutes, so that the elution of the early-eluting analytes occurs under isocratic conditions. In other words, the elution of the early-eluting analytes cannot be affected by the solvent gradient. Using the Agilent 1290 Infinity LC system with a dwell volume of 125 μL at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet after 6.25 seconds and enables fast separations within a few minutes.

The chromatogram shown in Figure 1 is a high pressure application. Due to the applied flow rate of 1.2 mL/min and the 1.8 μm particle packed column, a pressure drop of 1100 bar during the solvent gradient can be observed. Figure 2 shows an overlay of ten consecutive chromatograms, demonstrating the robustness and reproducibility of the developed method.

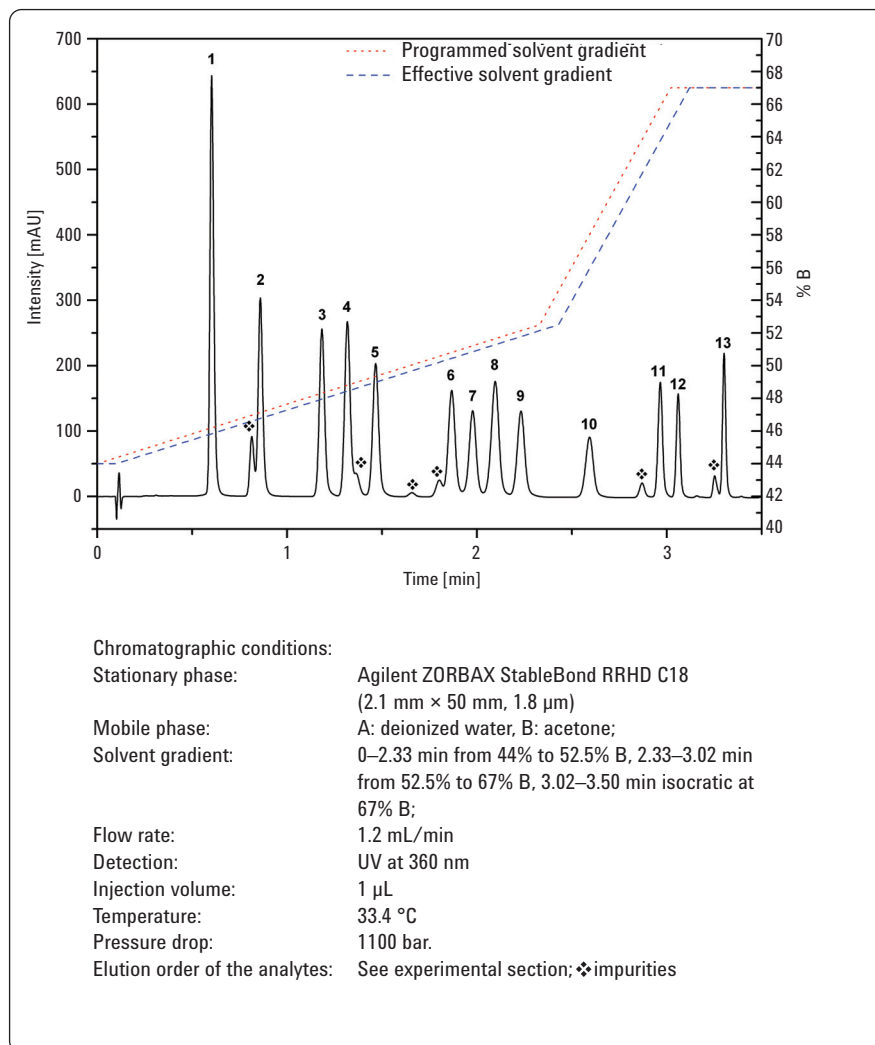


Figure 1
Separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

Figure 2 shows that there are virtually no differences among the ten chromatograms. This conclusion is confirmed by the relative standard deviation (RSD) of retention times of the analytes, which ranges between 0.03% and 0.09%.

Conclusion

The Agilent 1290 Infinity LC system is suitable for developing fast HPLC methods. The separation of 13 aldehyde and ketone derivatives was completed in around 3.5 minutes, using acetone as an organic modifier in the mobile phase. In addition, the method presented here illustrates that fast HPLC separations are only possible using HPLC systems with small dwell volumes. Finally, we have shown that the Agilent StableBond RRHD C18 column is suitable for separations where the pressure drop is greater than 1100 bar, without loss of separation efficiency.

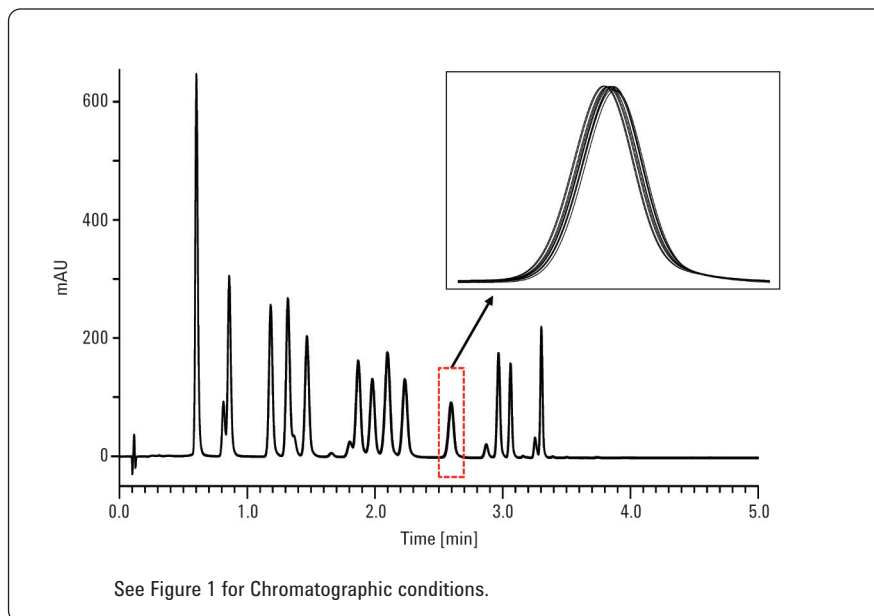


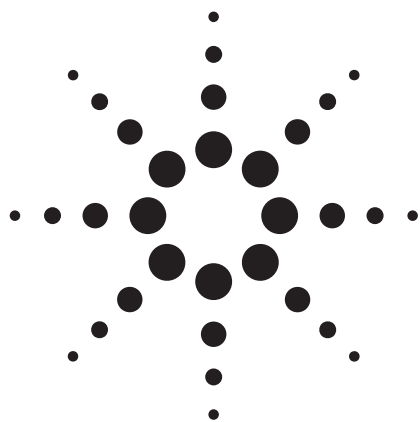
Figure 2
Overlay of 10 consecutive chromatograms of the separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

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Analysis of Low-level Polycyclic Aromatic Hydrocarbons (PAHs) in Rubber and Plastic Articles Using Agilent J&W DB-EUPAH GC column

Application Note

Gas Chromatography/Mass Spectrometry

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Abstract

Agilent J&W DB-EUPAH GC columns are designed for the analysis of EU-regulated priority PAHs. This application demonstrates a GC/MS method for the determination of 20 PAHs including 16 EPA-regulated priority PAHs and four commonly monitored PAHs including benzo(j)fluoranthene, benzo[e]pyrene, 2-methylnaphthalene and 1-methylnaphthalene in rubber and plastics using this type of column. To ensure the accuracy of results, the quantitation was performed with internal standardization using five isotopically-labeled PAHs including naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12 along with p-terphenyl-d14 as the surrogate standard. All 26 compounds were separated well with the DB-EUPAH column. The resulting good linearity and sample recovery demonstrate the high selectivity of the described method in this application note for trace-level detection and confirmation of the targeted PAHs in complex sample matrices.



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Introduction

Polycyclic aromatic hydrocarbons (PAHs), containing two to eight aromatic rings [1], are identified as some of the most persistent organic pollutants (POPs). Due to their well known carcinogenic and mutagenic properties, some PAHs are classified as priority pollutants by both the U.S. EPA and the European Commission. The U.S. EPA designated 16 PAH compounds as priority pollutants, including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The 16 EPA priority PAHs are often targeted for measurement in environmental samples.

PAHs may be present in oil, coal, rubber, and plastics. Some rubber products such as tires are produced using extender oils that may unintentionally contain various levels of PAHs. These extender oils along with the PAHs are incorporated into the rubber matrix and remain locked in the final products. In 2005, the European Commission adopted a Directive [2] restricting the marketing and use of certain PAHs in extender oils for tire production. All tires produced after 1 January 2010 are required to comply with the new Directive.

In the Directive, extender oils may not be used for the production of tires if they contain more than 1 mg/kg benzo(a)pyrene (BaP), or more than 10 mg/kg of the sum of the eight PAHs of concern including benzo(a)pyrene (BaP), benzo(e)pyrene (BeP), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), benzo(j)fluoranthene (BjFA), benzo(k)fluoranthene (BkFA) and dibenzo(a,h)anthracene (DBAaH). BeP and BjFA were not included in 16 EPA-regulated priority PAHs.

Five percent phenyl methylpolysiloxane stationary phase was the most commonly-cited GC column for the analysis of the 16 EPA-regulated PAHs, [3,4]. The resolution of the Benzo(b,k,j)fluoranthenes isomers were not easily obtained using this GC column.

In this application note, a DB-EUPAH column was chosen to provide the necessary separation for all 20 PAHs of interest in rubber and plastic articles. This includes the resolution of all the critical isomers such as the benzo(b,k,j)fluoranthenes. With its exceptional thermal stability, low column bleed at elevated temperatures, and consistent column inertness, the Agilent J&W DB-EUPAH column delivers fast, reliable results while meeting demanding regulatory requirements.

Experimental

The experiments were performed on one Agilent 7890 gas chromatograph equipped with an Agilent 5975C series GC/MSD, and an Agilent 7683 Automatic Liquid Sampler (ALS). The instrument conditions are listed in Table 1.

Chemicals and Standards

All standards in the experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA). The CAS numbers and nominal molecular mass are provided in Table 2.

Surrogate Spiking Solution

p-Terphenyl-d14 was used as a surrogate standard in this experiment. The surrogate spiking solution was prepared from aliquots of pure compound diluted with toluene to a concentration of 10 µg/mL, and served as a stock solution. Surrogate solution was added to all samples and all quality control samples prior to extraction.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Column:	Agilent J&W DB-EUPAH, 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627)
Inlet temperature:	290 °C
Carrier gas:	Helium, constant flow mode, 52 cm/s
Injection mode:	Splitless, purge flow 50 mL/min at 0.75 min
Injection volume:	1 µL
Oven:	120 °C (1 min), 8 °C/min to 200 °C (0.5 min), 11 °C/min to 270 °C, 2 °C/min to 300 °C, 320 °C (4 min)
Post run:	
MS Conditions	
Solvent delay:	2.8 min
MS temp:	250 °C (Source); 180 °C (Quad)
Transfer line:	290 °C
MS:	El, SIM/Scan
Scan mode:	Mass range (50-450 amu)
For other parameters, see Table 2	
Miscellaneous Parts	
Septa:	Long-lifetime septa (Agilent p/n 5183-4761)
Liner:	Splitless deactivated dual taper direct connect liner (Agilent p/n G1544-80700).
Syringe	5 µL syringe (Agilent p/n 5181-1273)

Table 2. Polycyclic Aromatic Hydrocarbons, CAS Number, Nominal Molecular Mass and Corresponding Ions

Compound	CAS No.	Nominal molecular mass	Corresponding ions
Naphthalene-d8*	1146-65-2	136	136 , 108, 68
Acenaphthene-d10*	15067-26-2	164	164 , 160
Phenanthrene-d10*	1719-06-8	188	188
Chrysene-d12*	1719-03-5	240	240 , 236, 120
p-Terphenyl-d14**	1718-51-0	244	244 , 122, 212
Perylene-d12*	1520-96-3	264	264 , 265, 260
Naphthalene	91-20-3	128	128 , 127, 129
2-methylnaphthalene	91-57-6	142	142 , 141, 115
1-methylnaphthalene	90-12-0	142	142 , 141, 115
Acenaphthylene	208-96-8	152	152 , 153, 151
Acenaphthene	83-32-9	154	153 , 154, 152
Fluorene	86-73-7	166	166 , 165, 167
Phenanthrene	85-01-8	178	178 , 176, 179
Anthracene	120-12-7	178	178 , 176, 179
Fluoranthene	206-44-0	202	202 , 200, 101
Pyrene	129-00-0	202	202 , 200, 101
Benzo(a)anthracene	56-55-3	228	228 , 226, 229
Chrysene	218-01-9	228	228 , 226, 229
Benzo(b)fluoranthene	505-99-2	252	252 , 253, 126
Benzo(j)fluoranthene	205-82-3	252	252 , 253, 126
Benzo(k)fluoranthene	207-08-9	252	252 , 253, 126
Benzo(e)pyrene	192-97-2	252	252 , 253, 126
Benzo(a)pyrene	50-32-8	252	252 , 253, 126
Indeno(1,2,3-cd)pyrene	193-39-5	276	276 , 138, 277
Dibenzo(a,h)anthracene	53-70-3	278	278 , 139, 279
Benzo(g,h,i)perylene	191-24-2	276	276 , 138, 277

1. * Internal standard

2. ** surrogate standard

3. Suggested quantitative ions are in bold.

Internal Standard Solution

The internal standard solution included naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12. The internal standard solution was made from aliquots of pure compounds, diluted with toluene to a concentration of 10 µg/mL, and served as a stock solution.

Calibration Solution

Calibrations solutions were prepared in five different concentrations ranging from 5 to 500 ppb by diluting commercially available certified solutions containing analytes of interest. Each standard solution contained 500 µg/L of internal standards (ISTDs).

Sample Preparation

According to ZLS standard ZEK 01.2-08 to prepare samples, rubber and plastic articles were cut into pieces no larger than 2–3 mm in size. Five hundred milligrams of cut pieces were extracted by 20 mL of toluene and mixed with internal standards for 1 hour in the ultrasonic bath at a temperature of 60 °C. After cooling to room temperature, an aliquot was taken from the extract for analysis.

One rubber sample and one plastic sample spiked with PAHs of interest at the 100 ppb level respectively were treated according to the procedure described above.

Results and Discussion

Figure 1 shows the GC/MS total ion chromatograms for the compounds of interest. Due to the presence of isomers, some compounds listed in Table 2 with the same quantitation ions could be eluting quite closely. Therefore good resolution is very important for these isomers to achieve accurate quantitative results. As shown in Figure 1, all 20 targeted PAH compounds were well-resolved with the DB-EUPAH column.

Figure 2 exhibits the baseline resolution of some critical PAH pairs of interest, including phenanthrene and anthracene (m/z 178), fluoranthene and pyrene (m/z 202), benzo[a]anthracene and chrysene (m/z 228), with excellent peak shapes using the DB-EUPAH column.

Benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(e)pyrene and benzo(a)pyrene are isomers with the same quantitation ion (m/z 252). According to 2005/ 69/ EC Directive, the content of benzo(a)pyrene was restricted to be less than 1 mg/kg. Figure 2 shows that benzo(a)pyrene can be separated well, and the resolution of benzo(a)pyrene and benzo(e)pyrene is 2.82 with the DB-EUPAH column. Benzo(b, k, j)fluoranthenes are difficult-to-chromatograph isomers. Benzo(b)fluoranthene (BbFA) and benzo(j)fluoranthene (BjFA) often show coelution on the commonly used 5% phenyl methylpolysiloxane stationary phase GC column. The resolution of benzo(b, j, k)fluoranthene is 1.41

and 1.63 respectively, meeting the requirement of analysis. The three benzo(b,k,j)fluoranthene isomers could be baseline separated, however, with the compromise of a extra 10-min run time. Therefore, it is a trade-off between speed and resolution. The described method in this application note was chosen to provide a reasonable sample run time when meeting the regulatory requirements.

Calibration curves were constructed from the data obtained by the 1- μ L injections of standards at 5, 50, 100, 250, 500 ppb. Each standard solution contained 500 ppb of internal standards (ISTDs). All the PAHs have excellent linearity with calibration coefficients greater than 0.998 as shown in Table 3.

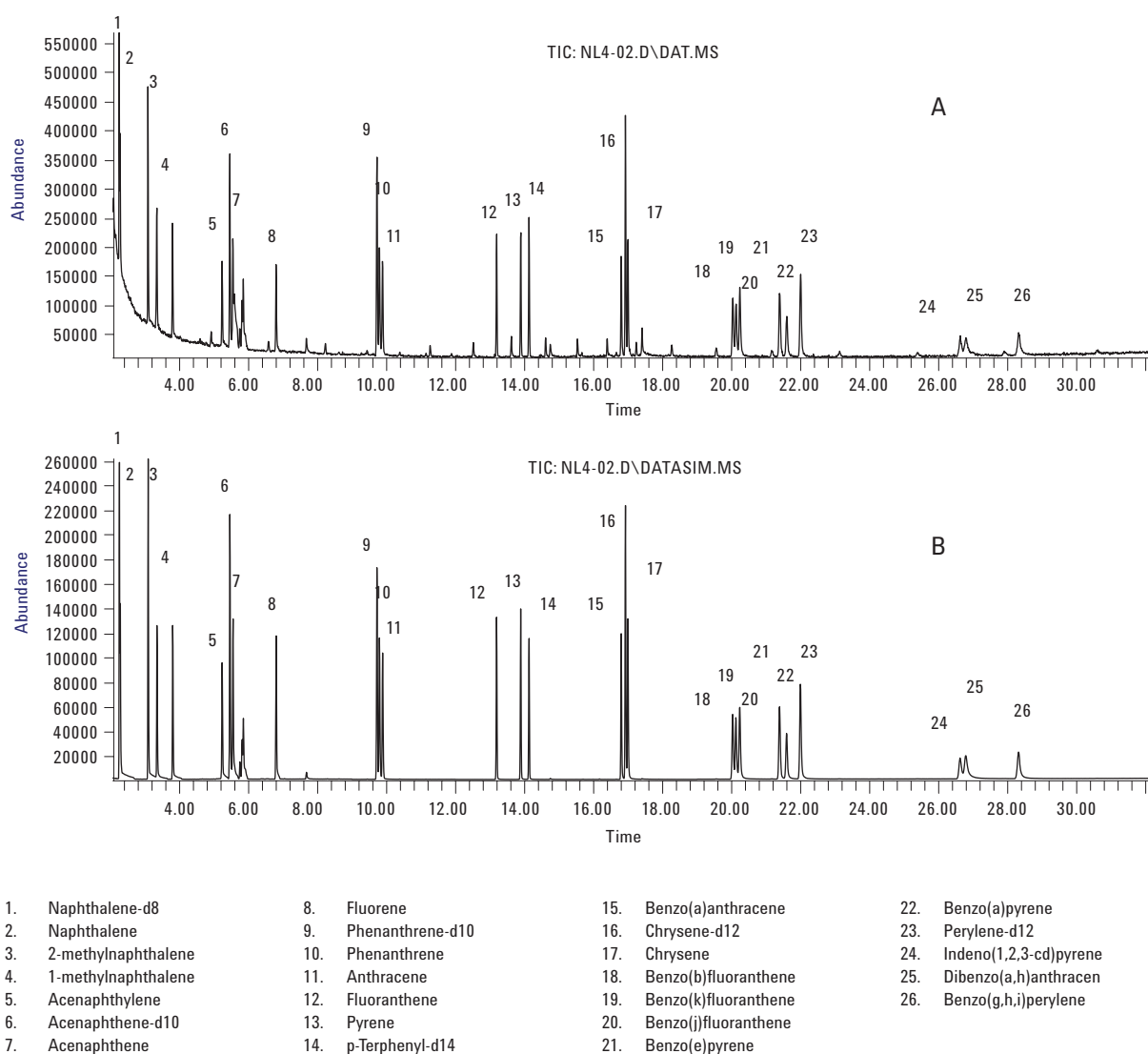


Figure 1. Twenty PAHs at 250 ppb each with ISTDs at 500 ppb each and surrogate at 250 ppb, using synchronous SIM/SCAN mode, A: SCAN mode B: SIM mode.

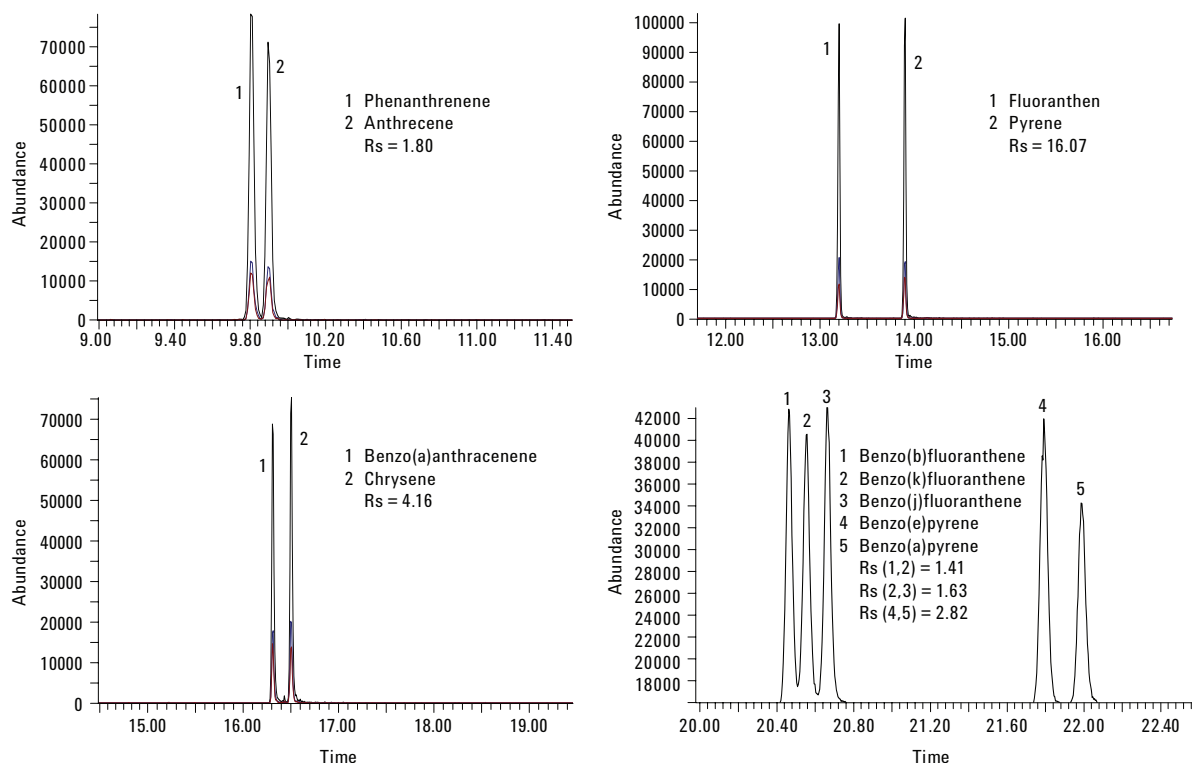


Figure 2. Resolution of Critical isomer Pairs with the DB-EUPAH column, 20 m $\times$ 0.18 mm $\times$ 0.14 μ m (Agilent p/n 121-9627).

The GC/MS TIC for matrix spiked extract is illustrated in Figure 3. The spiked samples were treated according to the procedure described in the sample preparation. The recovery data for the spiked samples are listed in Table 4. All data were based on three replicates of matrix spikes with the 20 targeted PAHs at the 100 ppb level. Good recoveries were achieved for all the compounds, ranging from 73.5% to 119.4%, satisfying both the US-EPA and EU regulatory requirements.

Conclusion

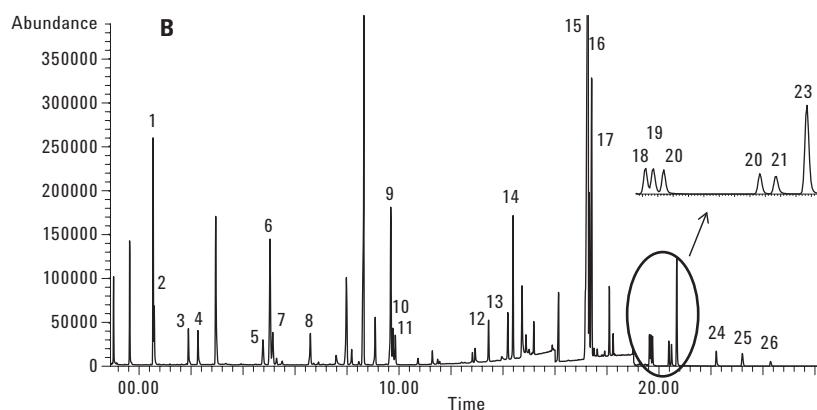
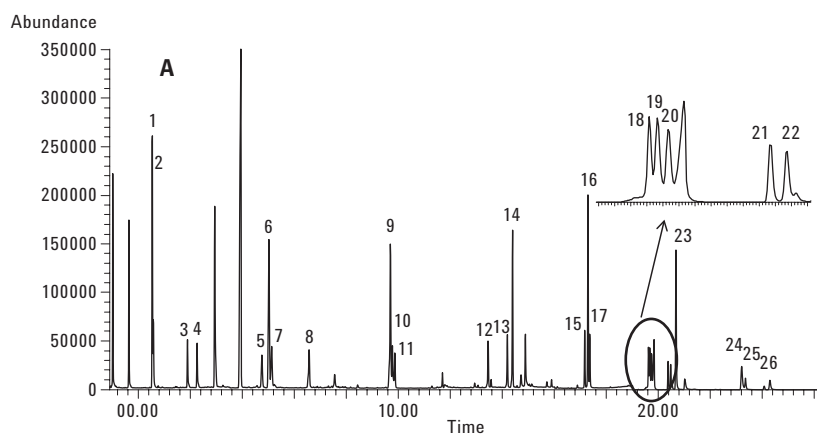
This application demonstrates a highly sensitive and selective GC/MS method for PAH analysis in rubber and plastic products using an Agilent J&W DB-EUPAH GC column. The DB-EUPAH column can effectively separate the 20 PAHs of interest, resolving all the critical, difficult-to-separate pairs. The system allows for trace-level detection of the PAHs in rubber and plastic articles. Good linearity and recoveries were achieved for all targeted compounds. The Agilent J&W DB-EUPAH column delivers fast, reliable results while meeting the requirements of both EPA and EU regulatory methods.

Table 3. The Regression Equations and Correlation Coefficient of PAHs

Compound	Range of linearity (ng)	Correlation coefficient (R ²)
Naphthalene	0.005-0.5	0.9997
2-Methylnaphthalene	0.005-0.5	0.9999
1-Methylnaphthalene	0.005-0.5	0.9998
Acenaphthylene	0.005-0.5	0.9996
Acenaphthene	0.005-0.5	0.9999
Fluorene	0.005-0.5	0.9994
Phenanthrene	0.005-0.5	0.9999
Anthracene	0.005-0.5	0.9992
Fluoranthene	0.005-0.5	0.9990
Pyrene	0.005-0.5	0.9996
Benzo[a]anthracene	0.005-0.5	0.9985
Chrysene	0.005-0.5	0.9998
Benzo[b]fluoranthene	0.005-0.5	0.9998
Benzo[j]fluoranthene	0.005-0.5	0.9983
Benzo[k]fluoranthene	0.005-0.5	0.9990
Benzo[e]pyrene	0.005-0.5	0.9992
Benzo[a]pyrene	0.005-0.5	0.9997
Indeno[1,2,3-c,d]pyrene	0.005-0.5	0.9989
Dibenzo[a,h]anthracene	0.005-0.5	0.9989
Benzo[g,h,i]perylene	0.005-0.5	0.9993

Table 4. Recoveries of PAHs in Substantial Plastic Sample and Rubble Sample

Compounds	Spiked (ppb)	Recovery (%) plastic sample	Recovery (%) rubber sample
Naphthalene	100	101.99	106.7
2-Methylnaphthalene	100	93.3	95.7
1-Methylnaphthalene	100	91.22	92.6
Acenaphthylene	100	111.32	120.8
Acenaphthene	100	98.45	109.0
Fluorene	100	107.66	114.6
Phenanthrene	100	92.54	109.1
Anthracene	100	106	110.8
Fluoranthene	100	110.52	119.4
Pyrene	100	111.62	104.0
Benzo[a]anthracene	100	102.61	118.5
Chrysene	100	107.67	95.9
Benzo[b]fluoranthene	100	103.18	114.6
Benzo[j]fluoranthene	100	103.02	118.9
Benzo[k]fluoranthene	100	91.13	88.8
Benzo[e]pyrene	100	90.8	92.3
Benzo[a]pyrene	100	106.58	119.2
Indeno(1,2,3-cd)pyrene	100	75.36	79.9
Dibenzo(a,h)anthracen	100	80.47	89.9
Benzo(g,h,i)perylene	100	76.45	73.5



1. Naphthalene-d8	8. Fluorene	15. Benzo(a)anthracene	22. Benzo(a)pyrene
2. Naphthalene	9. Phenanthrene-d10	16. Chrysene-d12	23. Perylene-d12
3. 2-methylnaphthalene	10. Phenanthrene	17. Chrysene	24. Indeno(1,2,3-cd)pyrene
4. 1-methylnaphthalene	11. Anthracene	18. Benzo(b)fluoranthene	25. Dibenzo(a,h)anthracene
5. Acenaphthylene	12. Fluoranthene	19. Benzo(k)fluoranthene	26. Benzo(g,h,i)perylene
6. Acenaphthene-d10	13. Pyrene	20. Benzo(j)fluoranthene	
7. Acenaphthene	14. p-Terphenyl-d14	21. Benzo(e)pyrene	

Figure 3. TIC of matrix spiked extract using Agilent GC/MS system and Agilent J&W DB-EUPAH 20 m $\times$ 0.18 mm, 0.14 μ m column (Agilent p/n 121-9627) A: plastic, B: rubber.

References

1. J. C. Fetzer, (2000). "The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons," Polycyclic Aromatic Compounds (New York: Wiley) 27: 143. doi:10.1080/10406630701268255. ISBN 0471363545.
2. European Union, Commission Recommendation, Directive 2005/69/EC, 15 November 2005.
3. Mike Szelewski, "Full Scan Low Level Polynuclear Aromatic Hydrocarbon Analysis using the Agilent Technologies 6890/5973 inert Gas Chromatograph/Mass Selective Detector," Agilent Technologies publication 5989-0264EN, www.agilent.com/chem.
4. Kenneth Lynam and Doris Smith, "Polycyclic Aromatic Hydrocarbon (PAH) Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column," Agilent Technologies publication 5989-9181EN, July 2008.
5. Doris Smith and Ken Lynam, "GC/MS Analysis of European Union (EU) Priority Polycyclic Aromatic Hydrocarbons (PAHs) using an Agilent J&W DB-EUPAH GC Column with a Column Performance Comparison" Agilent Technologies publication 5990-4883EN, 2009.
6. "How confident are you with your separations of critical PAH isomers?," Agilent Technologies publication 5990-5551EN.

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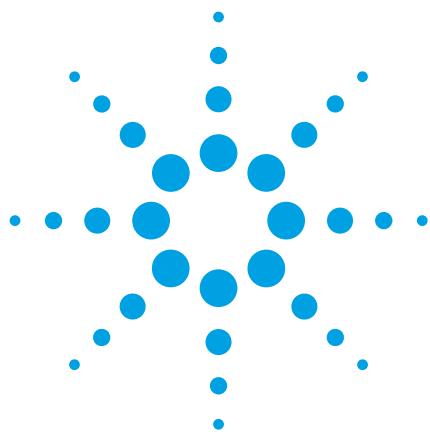
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Agilent Technologies



Low molecular weight resins - Analysis of low molecular weight resins and prepolymers by GPC/SEC

Application compendium

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The Measure of Confidence



Agilent Technologies

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Low molecular weight resins

Introduction

The term resin is used to describe materials manufactured by the addition polymerization of reactive monomers, often accompanied by the elimination of a small molecule. These synthetic methodologies result in materials with a relatively low molecular weight and a wide polydispersity, often containing oligomers and a considerable monomer content. A feature of these polymers is the presence of reactive groups at the end of the polymer and oligomer chains, and many such materials are used as prepolymers that can be further reacted to form new products.

Gel permeation chromatography/size exclusion chromatography (GPC/SEC) is a well-known technique for assessing the molecular weight distribution of polymers, a parameter that influences many physical properties. For example, the molecular weight of resin materials determines their physical state and their accessibility to further reactions. Characterizing and understanding the molecular weight distribution of resin materials is therefore key to their performance.

However, the polymers produced by condensation polymerizations tend to be highly functional and contain reactive groups, and many classes of material grouped by their reactive functionality, for example polyurethanes, have widely differing chemical structures. As a result, analysis of these materials by GPC/SEC can be challenging due to the possibility of interactions occurring between the packing material contained in the GPC/SEC column and structural elements of the material.

This application compendium discusses the analysis of low molecular weight resins and highlights those columns best suited for their analysis. A series of example analyses illustrate the quality of data that may be obtained.

Agilent produces GPC/SEC columns, standards and instruments that are ideally suited to the analysis of resins and low molecular weight prepolymers.

Agilent's GPC/SEC columns are ideal for applications that rely on extremely reproducible analysis, such as quality control environments in resin manufacturing. Agilent's columns include ranges suitable for use in organic and aqueous eluents, solvent mixtures and high polarity organic eluents, covering the requirements of the diversity of resins.

The column range includes products tailored to the analysis of low molecular weight materials, and includes high resolution columns, such as the OligoPore and PLgel 3 μm 100Å columns, that are designed for the analysis of oligomeric samples. With extensive options in particle size and pore size, Agilent's columns can be selected to match the molecular weight of the material under investigation simply, thereby ensuring that the best quality of data is obtained from the GPC/SEC experiment.

Agilent's range of integrated GPC/SEC instrumentation covers a temperature range from ambient to 220 °C. Agilent also produces a range of polymer standards to complement resin analysis.

These instruments allow all forms of the GPC/SEC experiment to be performed and can be used to analyze the complete range of resin materials, including those that require analysis in unusual solvents. Multiple detection options can be included in the instruments, such as light scattering and viscometry, and dedicated analysis software is available that allows the properties of resins to be analyzed in detail.

Characterization of low polarity resins

Low polarity resins may be analyzed on polystyrene/divinylbenzene columns in typical GPC solvents, such as THF.

The PlusPore series of columns has been specifically designed for *high resolution* GPC. These packing materials are based on the industry-standard, highly crosslinked polystyrene/divinylbenzene (PS/DVB) packing material, for the widest applicability and solvent compatability. Each is made using a novel polymerization process to produce particles which exhibit a specific, controlled pore structure for optimum GPC performance.

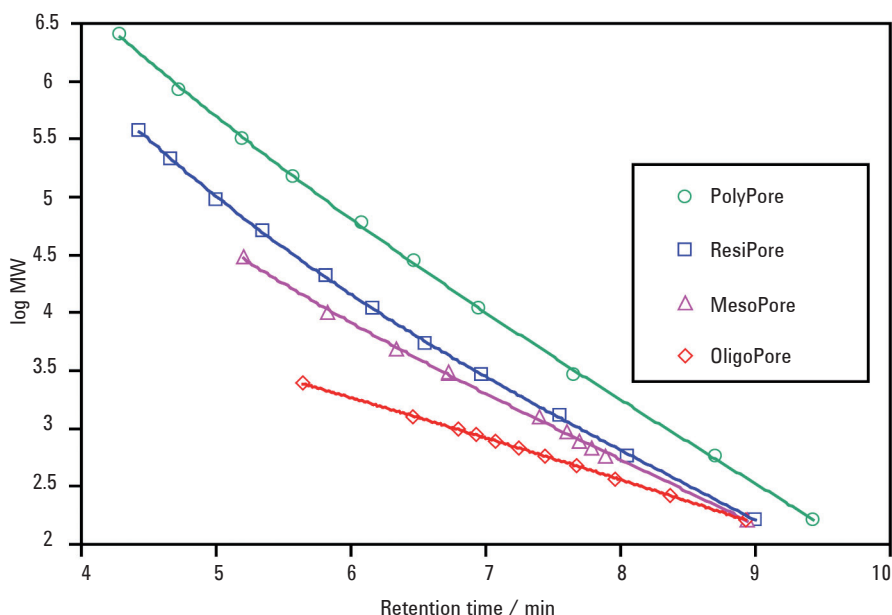


Figure 1. PlusPore calibration curves showing the resolving ranges and near linear calibrations of the columns

The ideal choice for polymer analysis

For high resolution polymer analysis, the PolyPore, ResiPore, MesoPore and OligoPore columns of the PlusPore product range exhibit a wide pore size distribution with near linear calibration curves covering an extended molecular weight range. These so-called 'multipore' structures have increased pore volume compared to regular PS/DVB packing materials. This results in very high resolution GPC columns designed for specific application areas. The highly crosslinked porous particles provide excellent chemical and physical stability and permit easy transfer across the full range of organic solvents with little change in the shape of the calibration curve or the efficiency of the columns. As this 'multipore' column technology does not require the combination of individual pore size packing materials, the result is high accuracy and precision *without* any artefacts in the shape of the molecular weight distribution (MWD).

Features and benefits of the PlusPore range

- High pore volume, high resolution - improved separations and efficiency
- Wide pore size distribution, optimized separation range - separates broad range, multiple usage
- Full solvent compatibility - easy transfer across range
- No MWD dislocations - maximized productivity

The composition of oligomers in resins is of great commercial importance, as is the determination of residual monomer in the quality control of polymers. These low molecular weight samples are routinely characterized by gel permeation chromatography (GPC). Ideally, separation of discrete components is required in order to identify and quantify specific components of interest. In order to achieve this, small particle size packings are used to produce high resolution separations. The MesoPore column, with an exclusion limit of 25,000 molecular weight, has a guaranteed minimum efficiency of 80,000 plates/meter. Typical application chromatograms are shown in Figures 2 to 6.

Sample: Isocyanate
Columns: 2 x MesoPore,
300 x 7.5 mm
(Part No. PL1113-6325)
Eluent: THF (stabilized)
Flow Rate: 1.0 mL/min
Detector: RI

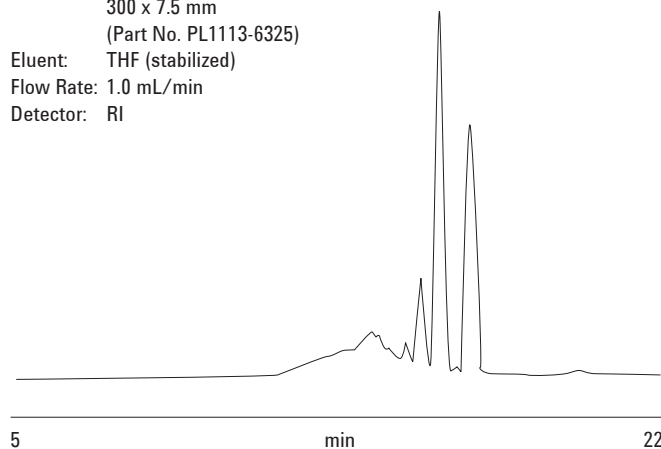


Figure 2. Chromatogram of an isocyanate sample showing polymer and oligomeric detail

Sample: Polyol
Columns: 2 x MesoPore,
300 x 7.5 mm
(Part No. PL1113-6325)
Eluent: THF (stabilized)
Flow Rate: 1.0 mL/min
Detector: UV, 240 nm

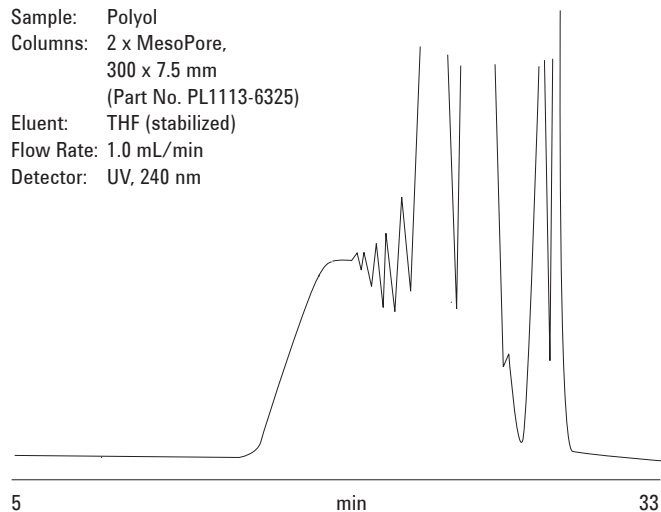


Figure 3. Chromatogram of a polyol sample showing the presence of large amounts of oligomers

Columns: 2 x MesoPore, 300 x 7.5 mm
(Part No. PL1113-6325)
Eluent: THF
Flow Rate: 1.0 mL/min
Inj Vol: 20 µl
Detector: RI

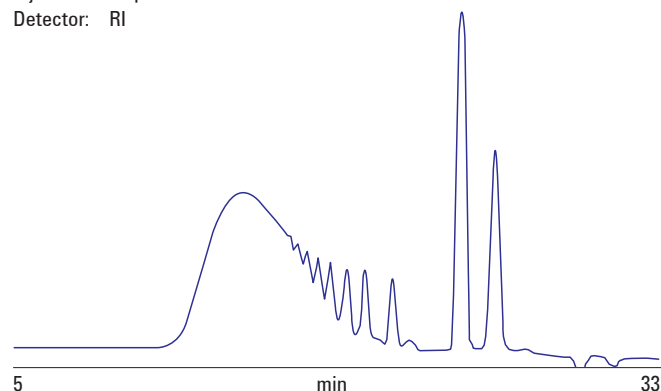


Figure 4. Chromatogram of a polyurethane sample with oligomers and residual monomers

Columns: 2 x MesoPore, 300 x 7.5 mm
(Part No. PL1113-6325)
Eluent: THF
Flow Rate: 1.0 mL/min
Inj Vol: 20 µl
Detector: RI

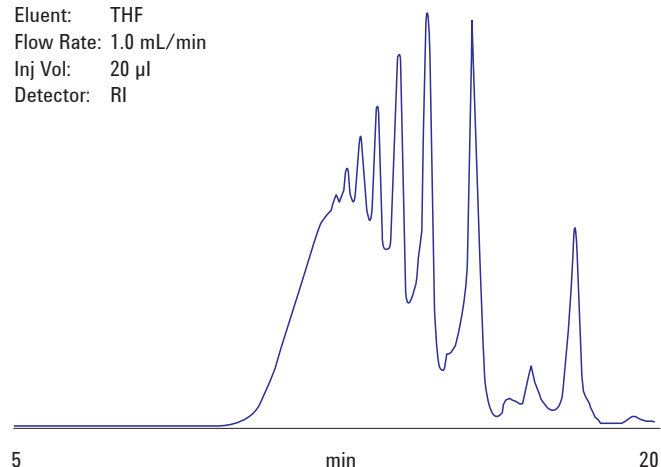


Figure 5. Chromatogram of an epoxy resin sample showing oligomers dominating the distribution

Columns: 2 x MesoPore, 300 x 7.5 mm
(Part No. PL1113-6325)

Eluent: THF

Flow Rate: 1.0 mL/min

Inj Vol: 20 µl

Detector: RI

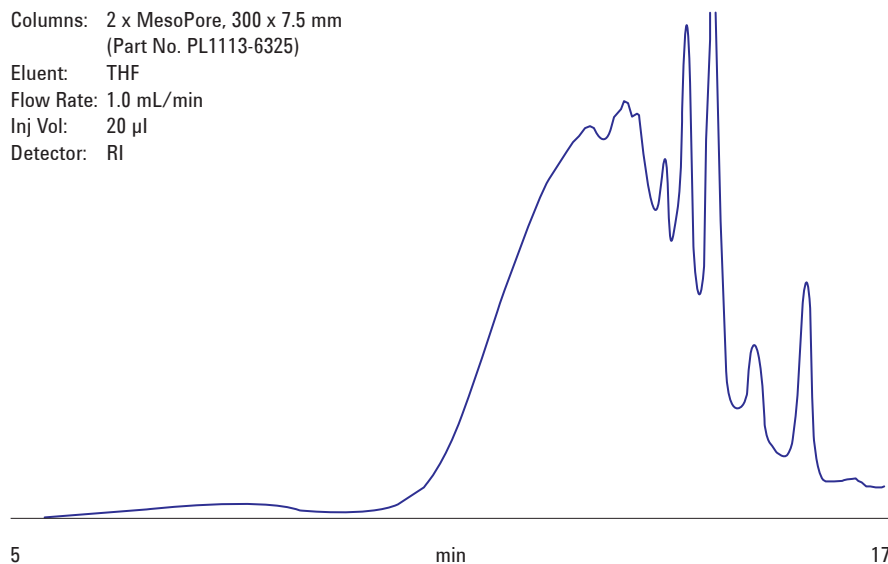


Figure 6. Chromatogram of a polyesterimide sample with a complex distribution of polymer and oligomer content

For higher molecular weight resins, the determination of molecular weight distribution is a primary objective in GPC analysis and columns with a broader resolving range are required. The ResiPore column has been specifically designed for such applications where material above 400,000 molecular weight is unlikely to be present. Figures 7 to 10 illustrate typical application chromatogram.

Sample: Paint resin

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)

Eluent: THF (stabilized)

Flow Rate: 1.0 mL/min

Detector: RI

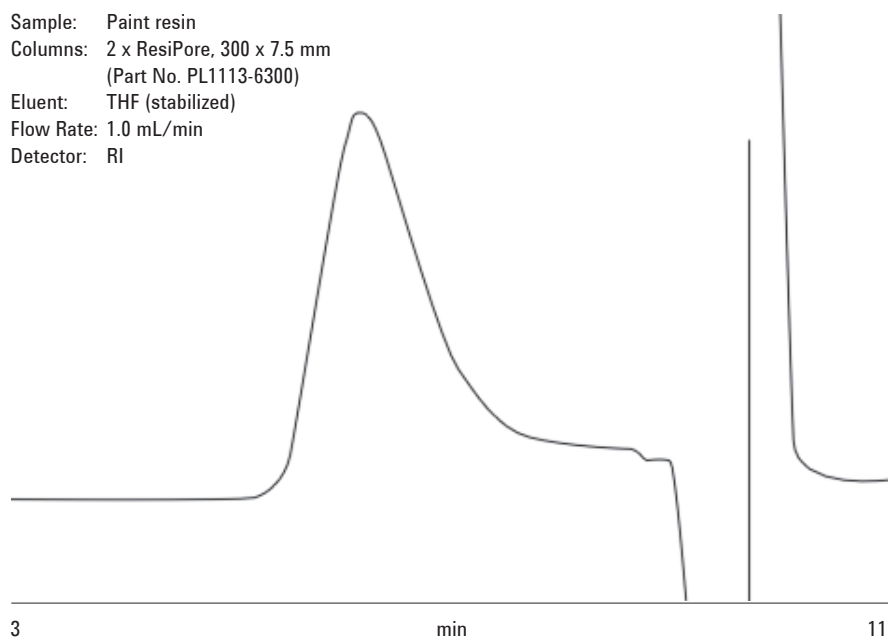


Figure 7. Chromatogram of a paint resin sample showing low molecular weight content with the polymer

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)

Eluent: THF

Flow Rate: 1.0 mL/min

Inj Vol: 20 μ l

Detector: RI

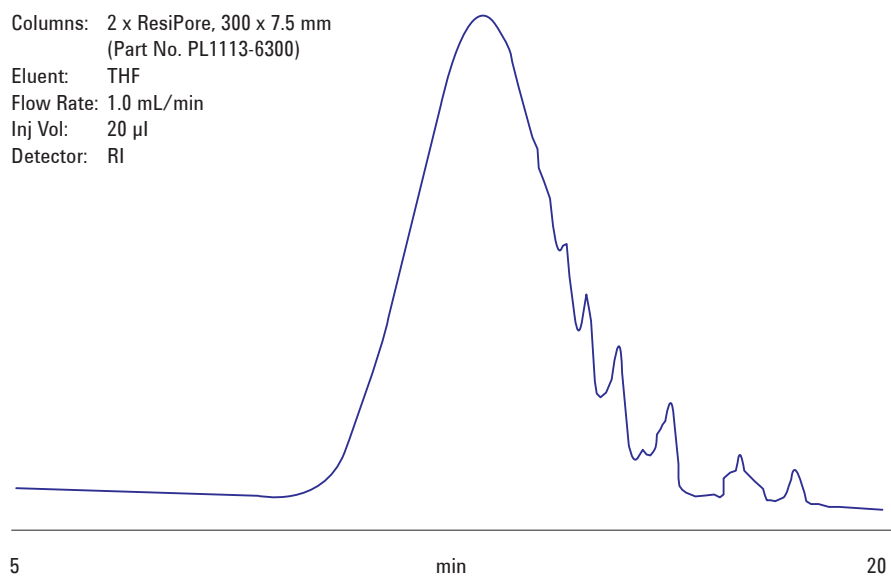


Figure 8. Chromatogram of higher molecular weight epoxy resin sample with some oligomer content

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)

Eluent: THF

Flow Rate: 1.0 mL/min

Inj Vol: 20 μ l

Detector: UV, 254 nm

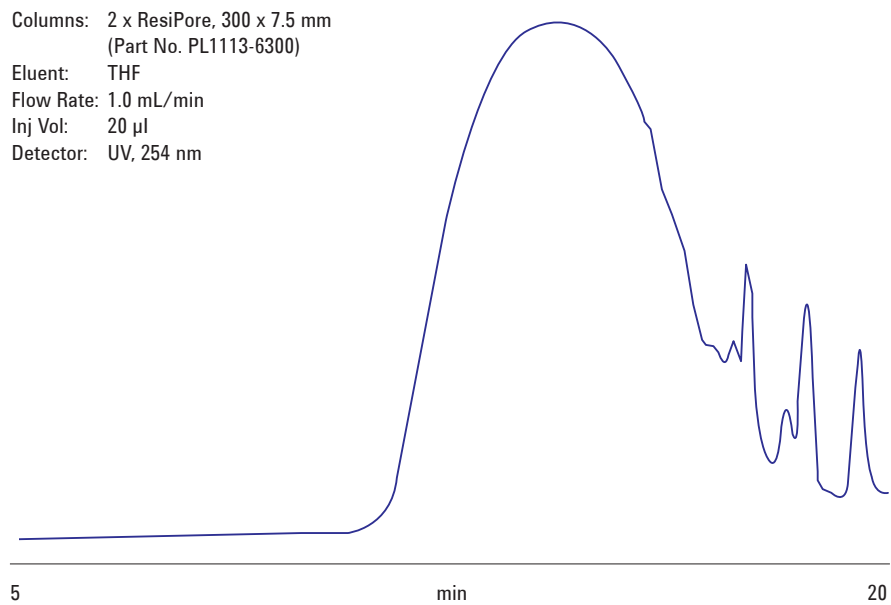


Figure 9. Chromatogram of an alkyd resin sample with a broad molecular weight distribution

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)

Eluent: THF

Flow Rate: 1.0 mL/min

Inj Vol: 20 μ l

Detector: UV, 254 nm

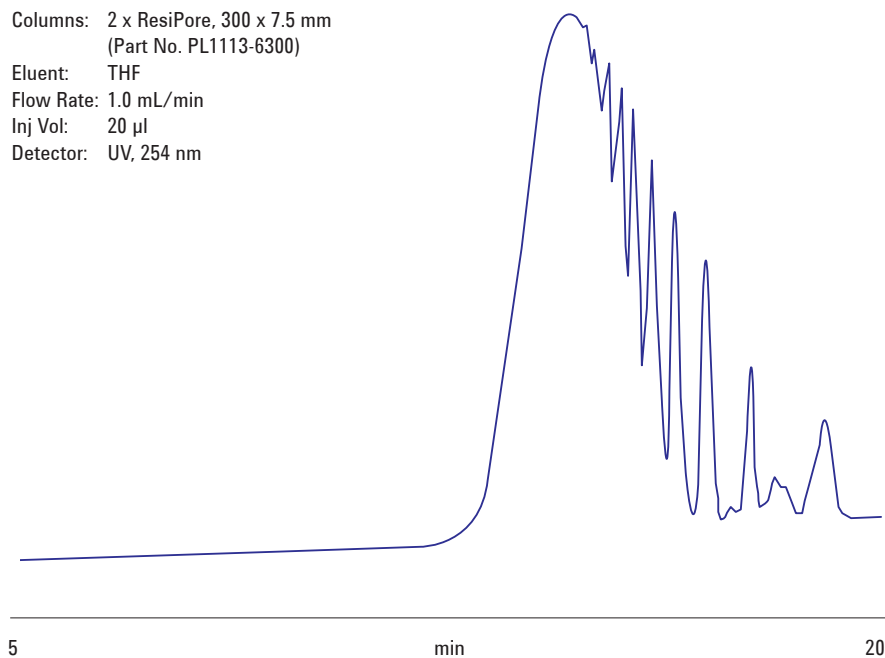


Figure 10. Chromatogram of a polyester sample showing oligomeric detail

Polyester polyol analysis

This separation demonstrates the resolution of the oligomeric species in a polyol sample prepared from adipic acid and butandiol using PLgel 3 μ m MIXED-E columns.

Columns: 2 x MesoPore, 300 x 7.5 mm
(Part No. PL1113-6325)

Eluent: THF

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm

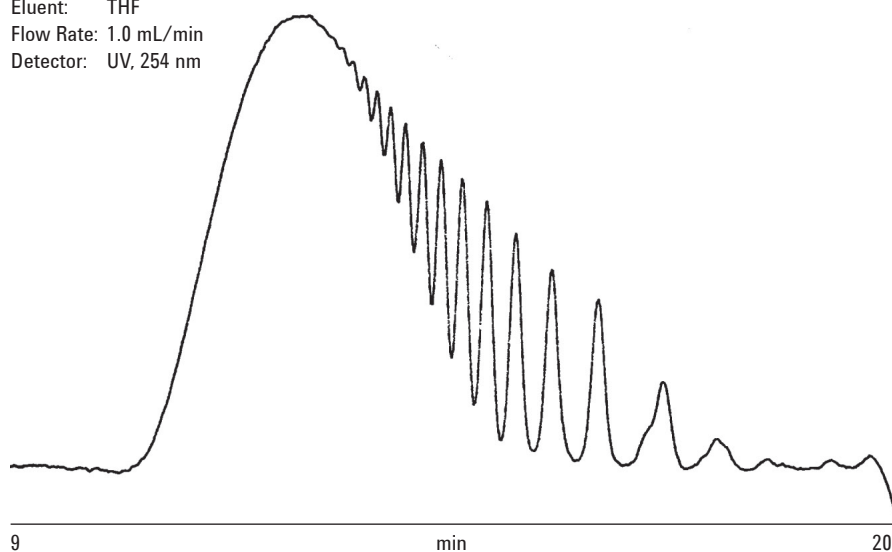


Figure 11. Chromatogram of a polyester polyol sample, a complex material with a UV chromophore

Phenolic resin analysis

Phenol-formaldehyde reactions produce two main products:

(a) Novolaks - under acidic conditions

(b) Resols - under basic conditions (excess aldehyde).

Chromatograms showing excellent oligomeric detail were produced for each product, when using high efficiency MesoPore columns.

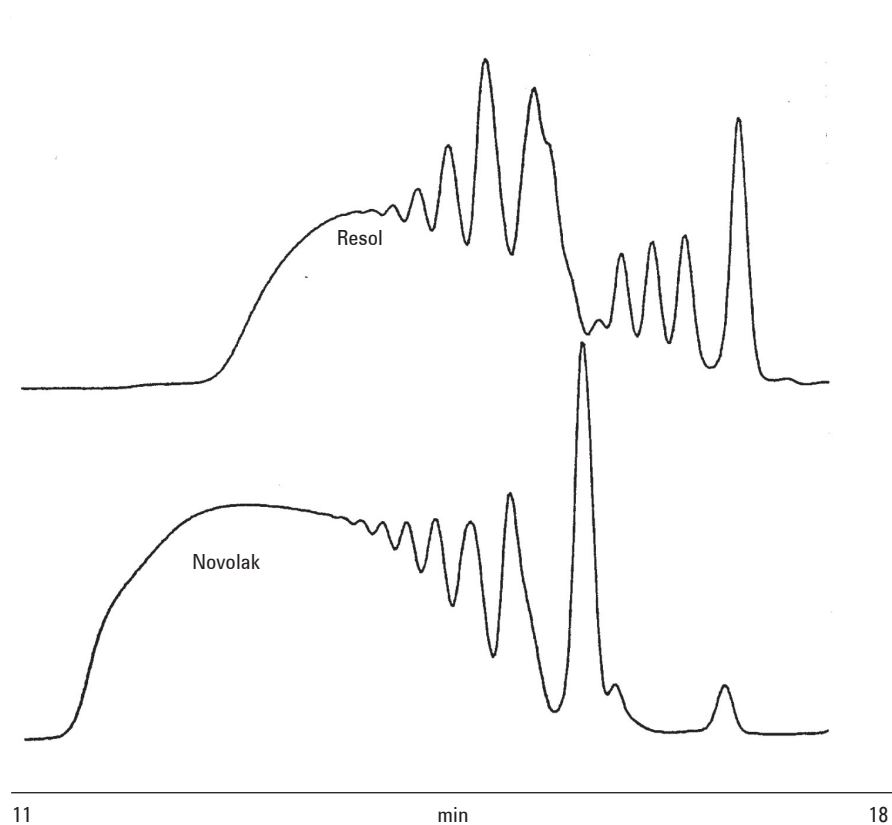


Figure 12. Overlaid chromatograms of two phenolic resin samples showing differences as a result of synthesis conditions

GPC analysis of adipate polyesters

Polyesters are produced from the condensation of a diacid with a dialcohol, eliminating water in the process. Depending on the acid and alcohol used, polyester can have a wide range of properties, including flexibility or hardness, stability to hydrolytic degradation and solvent, abrasion and shock resistance, properties which are useful for a wide range of applications. Adipate esters are produced by the condensation of a dialcohol with adipic acid.

Saturated adipate polyesters are used as cast elastomers; depending on the dialcohol used in the synthesis, linear or branched polyesters may be obtained. These polyesters are reacted with isocyanates to produce prepolymers with residual isocyanate groups that are precursors to mixed polyurethanes, a very important commercial class of material.

This application describes the analysis of two adipate polyesters by gel permeation chromatography (GPC) using two MesoPore columns in tetrahydrofuran with differential refractive index (RI) detection. Figure 13 shows two adipate polyester materials. The high resolution GPC columns are able to resolve the polymers into individual oligomers giving a characteristic peak shape that can be used to identify and 'fingerprint' different batches of polymers.

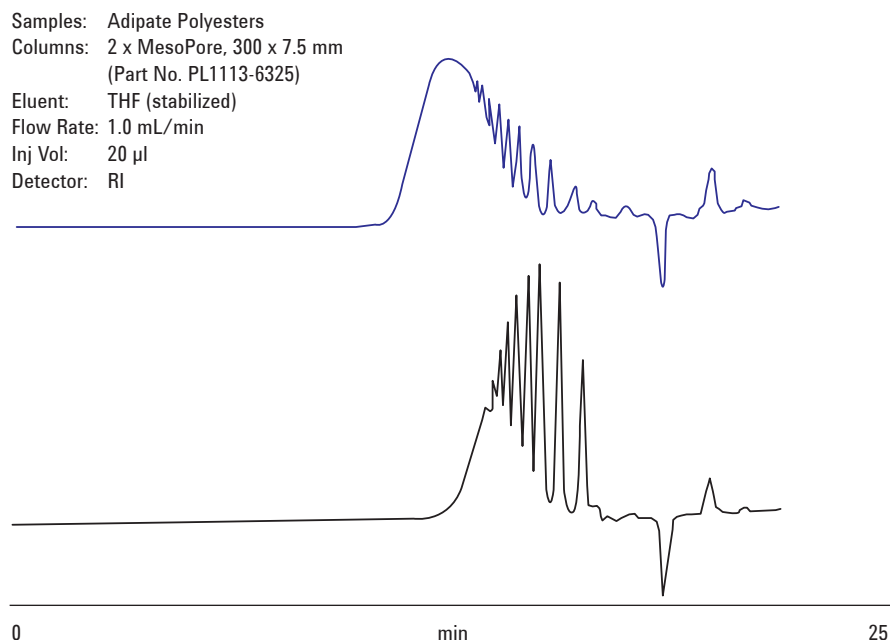


Figure 13. Overlaid chromatograms of adipate polyester samples showing clear differences between two batches

Higher molecular weight phenolic resins analysis

The term “phenolic resin” is used to describe a group of thermosetting resins produced through the reaction of phenol with an aldehyde. Phenolic resins were the earliest synthetic polymers to be developed (Bakelite, 1907), and possess useful mechanical and physical properties. Applications of phenolic resins include electrical insulation, molding, lamination and adhesives. Due to the relatively low cost and favorable properties of phenolic resins, they are produced in the greatest volume of all thermosetting polymers. Key characteristics of phenolic resins are their molecular weight distribution and oligomeric “fingerprint”, as these both have significant effect on the end use properties of the resin.

GPC is an ideal analytical tool for the examination of both of these characteristics. In this case, the use of high resolution GPC columns is advantageous, since these allow an optimized oligomeric separation and provide detailed information regarding the oligomeric sample composition.

These phenolic resins are non-polar so they can be analyzed in THF using PS/DVB columns.

In the GPC analysis detailed below, four distinct grades of phenolic resin have been analyzed by GPC using a ResiPore column set. Resulting from the small particle size (3 μm) and optimized pore size distribution of this column packing material, good resolution was obtained in the molecular weight range of interest.

The chromatography obtained from the GPC of each phenolic resin sample has been presented in Figure 14. Differential molecular weight distributions are given in Figure 15. This plot clearly shows significant differences in molecular weight distribution and the relative amounts of oligomeric material.

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)
(conditioned with 10 injections of a
typical sample solution at 10 mg/mL)
Eluent: THF (stabilized with 250 ppm BHT)
Flow Rate: 1.0 mL/min
Inj Vol: 20 μm
Detector: RI

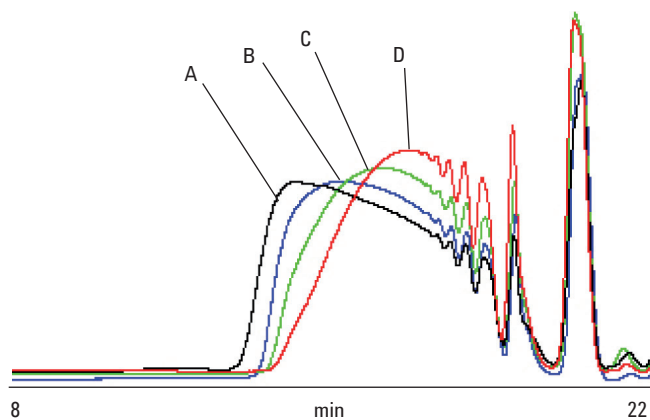


Figure 14. Overlaid GPC chromatograms obtained from four samples of phenolic resin showing differences in elution behavior

Columns: 2 x ResiPore, 300 x 7.5 mm
(Part No. PL1113-6300)
(conditioned with 10 injections of a
typical sample solution at 10 mg/mL)
Eluent: THF (stabilized with 250 ppm BHT)
Flow Rate: 1.0 mL/min
Inj Vol: 20 μm
Detector: RI

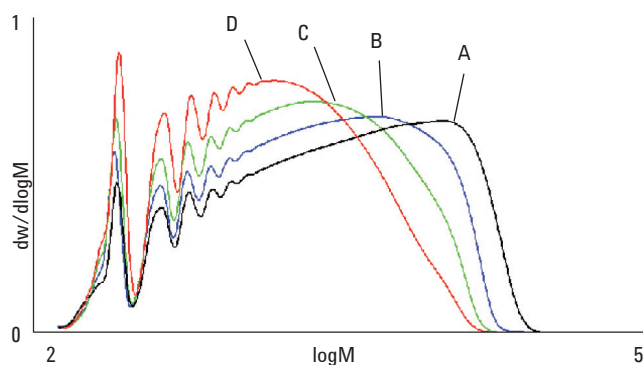


Figure 15. Overlaid molecular weight distributions from the GPC of four samples of phenolic resin - the same oligomers are present, but the overall molecular weights are very different

Preparative GPC separation of epoxy resin oligomers

Preparative GPC can be used to separate and isolate individual components of a sample based on size exclusion. By scaling up analytical separations, preparative GPC can be used to isolate practical quantities of individual components which can be used in further analysis. Agilent has developed the OligoPore preparative GPC column, which is ideally suited to the separation and isolation of individual oligomers from oligomer distributions and complex mixtures. This application illustrates the use of OligoPore preparative columns in the fractionation of epoxy oligomers. Figure 16 shows the general structure of an epoxy oligomer. A commercial epoxy resin, Epikote 828, is composed of two main epoxy oligomers where $n=0$ and $n=1$ and small amounts of the mono and di-epoxy water adducts.

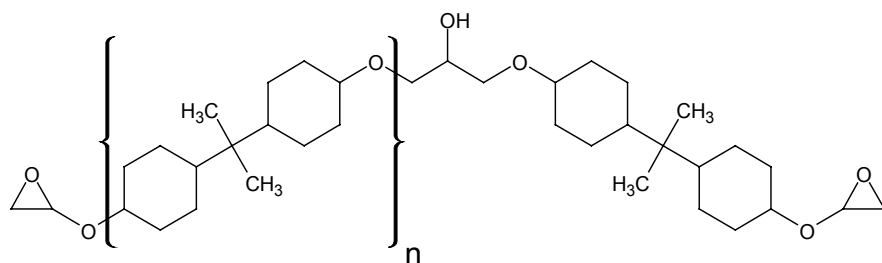


Figure 16. General structure of Epikote 828 epoxy resin oligomers

Analytical scale

Initially, the optimum loading of Epikote 828 on the OligoPore columns was analyzed on an analytical scale. Figure 17 shows analytical chromatograms at concentrations of 0.5% to 2.0% (w/v). The chromatograms show that Epikote 828 could be analyzed at a concentration of 2.0% (w/v) without serious loss of resolution.

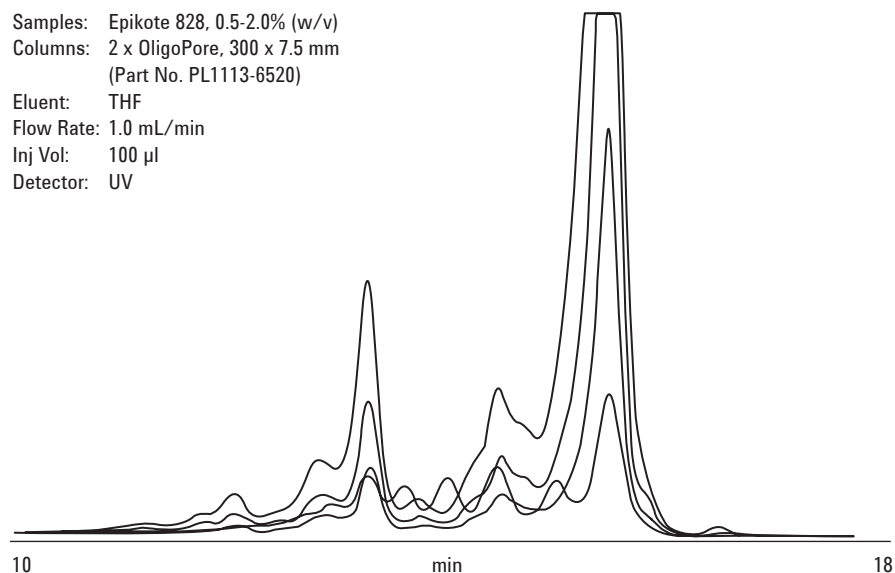


Figure 17. Overlaid chromatograms of epikote epoxy resin samples at different loadings

Preparative scale

OligoPore preparative columns were used to fractionate and purify the two oligomers from the resin. A preparative GPC system was set up with a 2 mL injection loop, two OligoPore 300 mm x 25 mm columns and a flow rate of 10.0 mL/min, an appropriate ten-fold scale-up over the analytical separation. The flow rate from the columns was split into two lines, ca 0.5 mL/min went to a UV detector, the remainder of the flow to a waste/fraction collector. The epoxy resin sample was injected at a concentration of 1.0% (w/v). Figure 18 shows a chromatogram of Epikote 828 obtained on the preparative columns indicating the resolution obtained. The sample was re-run and the two oligomers n=0 and n=1 were collected. The fractions were then analyzed on two OligoPore analytical columns.

Figure 19 shows the original analytical chromatogram of Epikote 828 run at a concentration of 2.0% (w/v) and an overlay of analytical chromatograms of the n=0 and n=1 oligomers collected from the OligoPore preparative GPC columns.

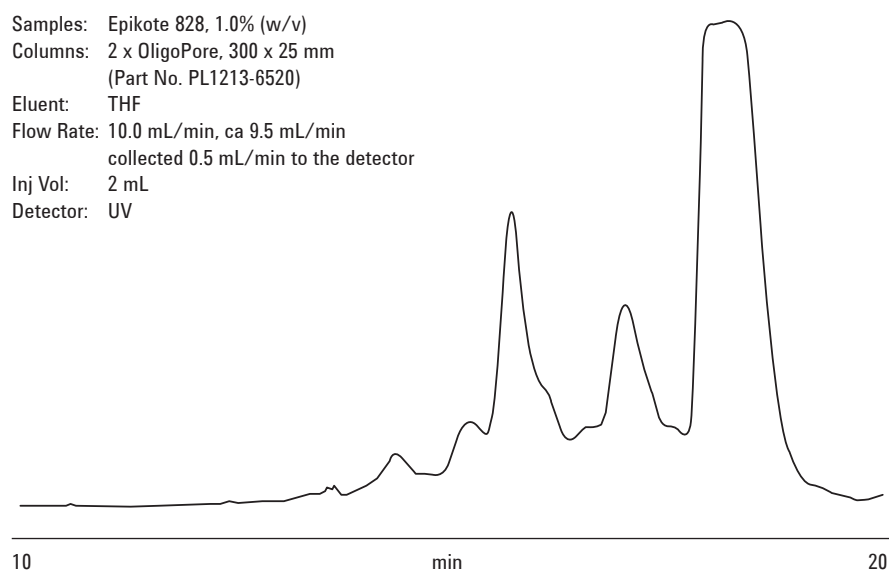


Figure 18. Chromatogram of an epikote 828 sample on a preparative scale column showing which oligomers could be isolated

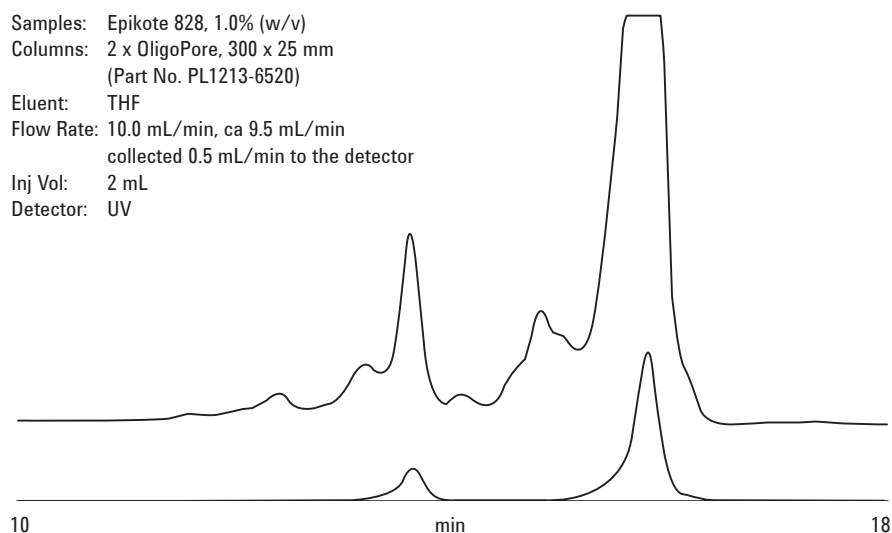


Figure 19. Overlaid chromatograms of an epikote 828 sample (top) and two separated fraction samples (bottom) showing the isolation of components through preparative GPC

Separation of epoxy resin oligomers

Epoxy resin prepolymers consist of oligomeric and polymeric diepoxides that are cured to form the finished product by the addition of a fixing or hardening agent. The formulation of the prepolymer is vital to controlling the physical properties of the final product. High resolution GPC can be used to investigate the oligomeric distributions of epoxy resin prepolymers for purposes of both formulation and quality control. When studying low molecular weight epoxy resins which elute close to total permeation, the Agilent evaporative light scattering detector (ELSD) is a good choice of detector due to the lack of system peaks, high sensitivity and excellent baseline stability obtained. This application outlines the analysis of four grades of epoxy resin using OligoPore columns and an ELS detector.

Figure 20 shows chromatograms of four different epoxy resins obtained on the OligoPore columns. The polymeric component of each of the samples was excluded on the OligoPore columns giving rise to a large peak at around 9 minutes, however, the oligomers were clearly resolved. The presence of peaks with identical retention times indicated that some of the same oligomers were present in each sample, however, clear differences in the oligomeric distributions of the four samples could be seen.

Samples: Epoxy resins
Columns: 2 x OligoPore, 300 x 7.5 mm
(Part No. PL1113-6520)
Eluent: THF + 250 ppm BHT
Flow Rate: 1.0 mL/min
Inj Vol: 100 µl
Detector: Agilent ELSD (neb = 40 °C,
evap = 80 °, gas = 1.0 SLM)

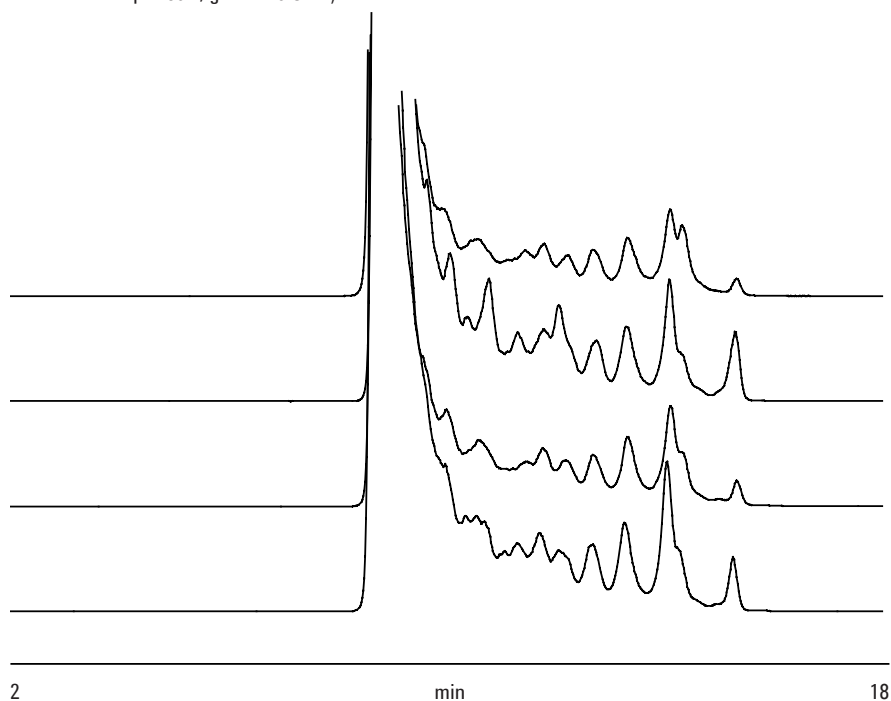


Figure 20. Overlaid chromatograms of epoxy resin samples, with excluded polymer components to focus on the oligomeric region

Characterization of high polarity resins

Increasingly, the choice of solvent for use as a GPC eluent is becoming more diverse since the polymers to be analyzed are more demanding in terms of solubility. Polar organic solvents are often the most suitable choice (see Table 1). However, such solvents usually exhibit relatively high viscosity, and demand the application of elevated temperature in order to improve the separation and reduce the column operating pressure.

The compatibility of GPC packing materials with this range of solvents assumes increasing importance in high performance separations of modern polymer systems. Column performance should be unaffected by solvent transfer, which demands a high degree of chemical and physical stability in the column bed.

Table 1. Solubility and eluent choices for different polymer types

Polymer Type	Solvent
ABS	DMF
Cellulose	DMSO/DMAC
Poly(acrylates)	DMF/DMAC
Poly(acrylonitrile)	DMF
Poly(ethylene oxide)	DMF
Poly(urethane)	DMF/DMAC
Poly(vinyl pyrrolidone)	DMF/DMAC

Intermediate polarity packing

PolarGel columns contain macroporous copolymer beads with a surface of balanced polarity, comprising hydrophobic and hydrophilic components. As the polarity of the bead surface is intermediate between the non-polar PLgel and the highly polar PL aquagel-OH materials, PolarGel is ideal for the analysis of high polarity polymers that are insoluble in water yet show interaction effects with styrene/divinyl benzene columns due to their high polarity.

PolarGel columns perform well in many applications that do not work well on typical organic GPC columns. The low swell of the PolarGel material in a range of solvents explains the stability of the packing, see Figure 21.

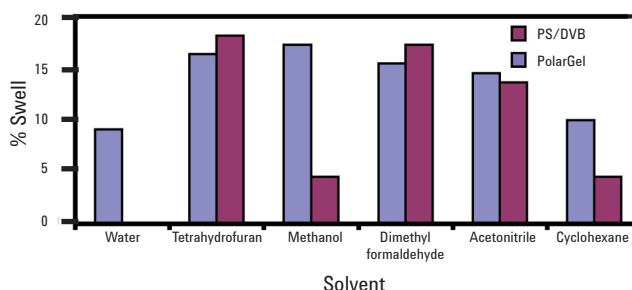
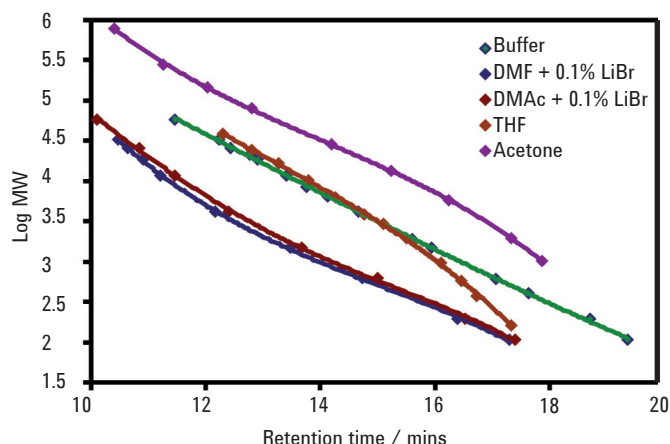


Figure 21. Swell data for PolarGel and a PS/DVB material showing the low swell of PolarGel

Broad solvent compatibility

As PolarGel columns combine intermediate surface polarity with low swell and high mechanical stability, they are used in a wide range of highly polar solvents, such as water, dimethyl formamide (DMF) and dimethyl acetamide (DMAc), and relatively low polarity solvents, such as tetrahydrofuran (THF), see figure 22. PolarGel-L columns are MIXED bed columns containing a number of constituents carefully blended to give a wide operating range focused at low molecular weight, making them suitable for a variety of applications up to ca. 30,000 g/mol (polyethylene glycol/oxide in water).



Samples: EasiVial PEG/PEO and PMMA
Standards (4 mL vials)
Columns: 2 x PolarGel-L, 300 x 7.5 mm
(Part No. PL1117-6830)
Eluent: Water, Acetone, DMF, DMAc, THF
Flow Rate: 1.0 mL/min
Inj Vol: 100 µl
Detector: Agilent 356-LC Refractive Index
Detector

Figure 22. Calibration curves of PolarGel-L in different solvents showing the near linear calibrations obtained in a solvent with a wide range of polarities

Analysis of phenol-formaldehyde rResins by GPC and PolarGel-M

Phenol-formaldehyde (P-F) resins are thermoplastic materials made with an excess of phenol in an acid catalyzed reaction with formaldehyde. P-F resins are commonly used as precursors to varnishes and other surface finish products.

PolarGel-M GPC columns are packed with low swell, macroporous copolymer beads that have a surface of balanced polarity, comprising hydrophobic and hydrophilic components. These allow PolarGel-M to be used in the analysis of high polarity polymers that are insoluble in water to give a more accurate representation of the molecular weight distribution of the polymer. If these polar polymers were to be analyzed with traditional styrene/divinylbenzene columns, interactions would cause artifacts in the peak shape and longer retention times, which would translate into apparently much lower molecular weight averages.

Two types of phenol-formaldehyde resin were analyzed to obtain an indication of differences in molecular weight, if any. The samples were made up in 0.2% (w/v) DMF, with 0.1% LiBr added to reduce sample aggregation, and injected without further treatment.

The results of the analyses are shown in the overlaid chromatograms and molecular weight distributions.

Columns: 2 x PolarGel-M, 300 x 7.5 mm
(Part No. PL1117-6800)
Eluent: DMF & 0.1% LiBr
Flow Rate: 1.0 mL/min
Inj Vol: 100 μ l
Temp: 50 $^{\circ}$ C
Detectors: Agilent PL-GPC 50 Plus
Integrated GPC/SEC System, RI

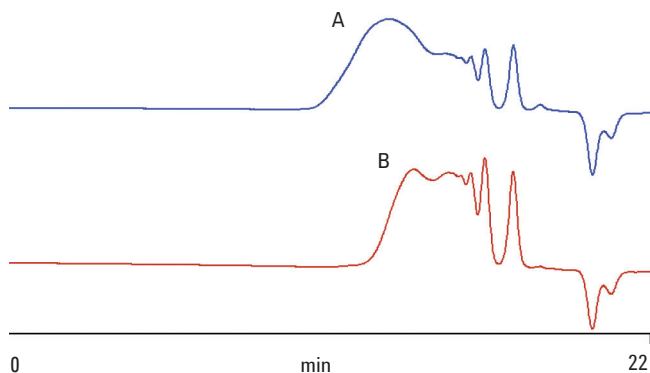


Figure 23. PolarGel-M reveals the composition of two phenol-formaldehyde resins, with clear differences between the materials

Columns: 2 x PolarGel-M, 300 x 7.5 mm
(Part No. PL1117-6800)
Eluent: DMF & 0.1% LiBr
Flow Rate: 1.0 mL/min
Inj Vol: 100 μ l
Temp: 50 $^{\circ}$ C
Detectors: Agilent PL-GPC 50 Plus
Integrated GPC/SEC System, RI

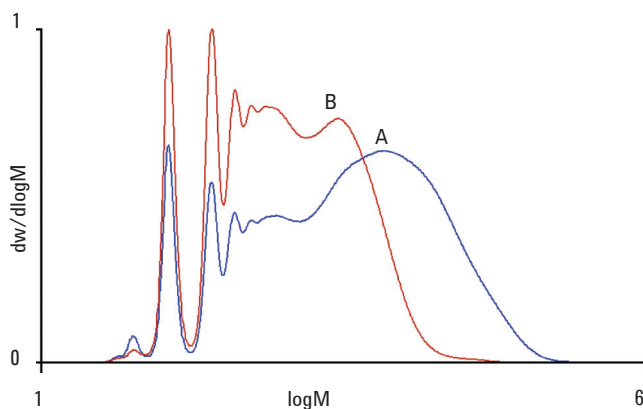


Figure 24. Overlaid molecular weight distributions of two phenol-formaldehyde resins show the same oligomers are present

GPC with PolarGel-M columns allows for the artifact-free calculation of the composition and molecular weight distributions of phenol-formaldehyde resins that are difficult to analyze on traditional, organic (PS/DVB) GPC columns.

GPC and PolarGel-M columns for the true representation of novolac resins

Novolac resins are thermoplastic materials made with an excess of phenol in an acid catalyzed reaction with formaldehyde. Novolacs are commonly employed as photoresists (light-sensitive materials used to form patterned surface coatings) and in varnishes. They have higher heat distortion temperatures and tend to be more expensive than regular epoxy resins.

PolarGel-M GPC columns are packed with low swell, macroporous copolymer beads that have a surface of balanced polarity, comprising hydrophobic and hydrophilic components. These allow PolarGel-M to be used in the analysis of high polarity polymers that are insoluble in water to give a more accurate representation of the molecular weight distribution of the polymer. If these polar polymers were to be analyzed with traditional styrene/divinylbenzene columns, interactions would cause artifacts in the peak shape and longer retention times, which would translate into apparently much lower molecular weight averages.

Two novolac resins were analyzed to obtain an indication of differences in molecular weight, if any. The samples were made up at 0.2% (w/v) in DMSO, with 0.1% LiBr added to reduce sample aggregation, and injected without further treatment.

Figure 25 shows the overlaid molecular weight distributions of two novolac resins.

GPC with PolarGel-M columns allows for the artifact, interaction free calculation of the composition and molecular weight distributions of novolac resins that are difficult to analyze on traditional, organic PS/DVB) GPC columns.

Columns: 2 x PolarGel-M, 300 x 7.5 mm
(Part No. PL1117-6800)
Eluent: DMSO & 0.1% LiBr
Flow Rate: 1.0 mL/min
Inj Vol: 50 $^{\circ}$ C
Detectors: Agilent PL-GPC 50 Plus Integrated
GPC/SEC System, RI

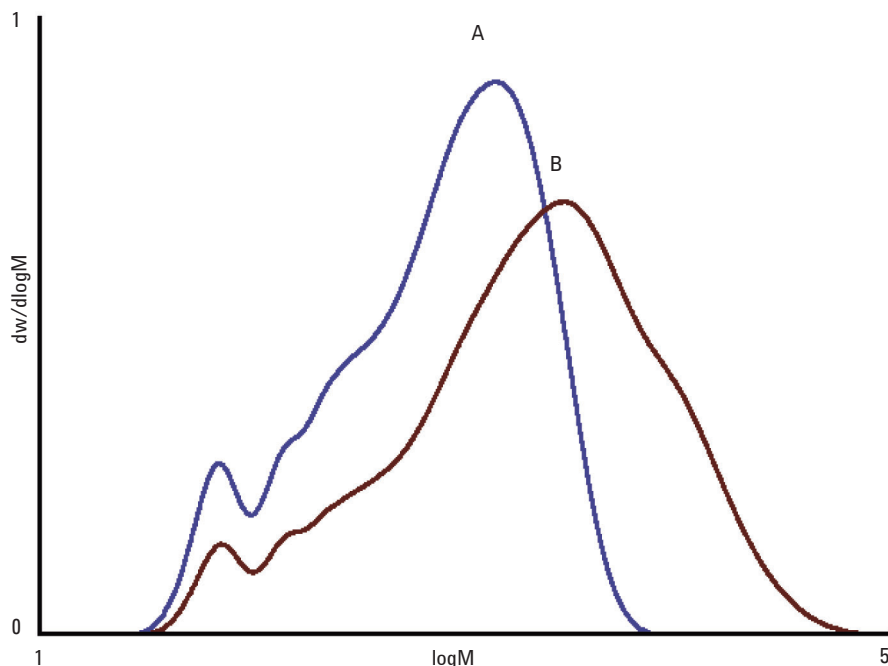


Figure 25. Overlaid molecular weight distributions of two novolac resins with very different molecular weights

Analysis of melamine resins by conventional Gel Permeation Chromatography using PolarGel-L columns on the PL-GPC 50 Plus

Melamine resins are durable thermosetting plastics formed by the condensation polymerization of melamine with formaldehyde. They are commonplace in the home as they are employed to laminate chipboard, creating inexpensive furniture, as well as being used in the manufacturing of kitchen tableware and food packaging. The molecular weight distribution of melamine resins determines many of the final properties of the polymer and therefore their application. Subtle differences in the molecular weight distribution of melamine resin samples is essential.

The molecular weight distributions of two different samples of melamine were investigated by conventional gel permeation chromatography using a set of two PolarGel-L (300 x 7.5 mm) columns and the PL-GPC 50 Plus Integrated GPC/SEC System. The samples were analyzed in the polar organic solvent dimethylacetamide (DMAc) which contained 0.1% LiBr.

Sample: Two samples of melamine resin
Column: 2 x PolarGel-L, 300 x 7.5 mm
(Part No. PL1117-6830)
Eluent: DMSO & 0.1% LiBr
Flow Rate: 1.0 mL/min
Inj Vol: 50 °C
Detectors: Agilent PL-GPC 50 Plus Integrated
GPC/SEC System, RI

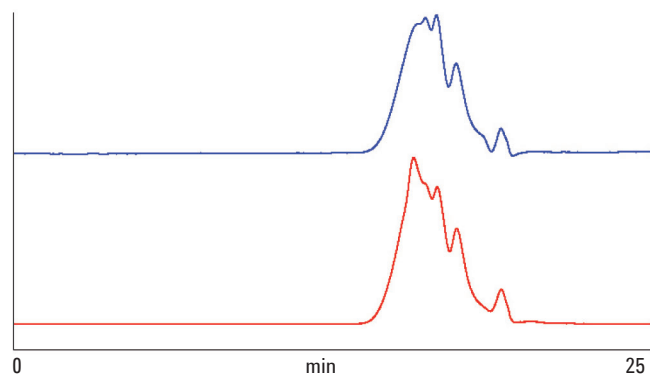


Figure 26. Chromatograms for two melamine samples showing obvious differences

Sample: Two samples of melamine resin
Column: 2 x PolarGel-L, 300 x 7.5 mm
(Part No. PL1117-6830)
Eluent: DMSO & 0.1% LiBr
Flow Rate: 1.0 mL/min
Inj Vol: 50 °C
Detectors: Agilent PL-GPC 50 Plus Integrated
GPC/SEC System, RI

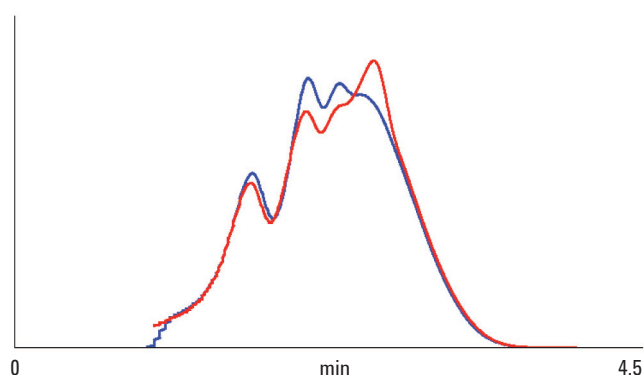


Figure 27. Overlaid molecular weight distributions for two melamine resin samples highlighting changes in the distributions

The two samples of melamine resins analyzed by conventional gel permeation chromatography on the PL-GPC 50 Plus clearly had quite different molecular weight distributions, with differing ratios of the various oligomers present.

The PL-GPC 50 Plus fitted with two PolarGel-L columns was used to successfully analyze two samples of melamine resin, indicating clear differences between the samples. The PolarGel-L columns are well suited to operation in highly polar solvents.

Analysis of resins

Resins comprise a complex group of natural and man-made, solid and semi-solid polymers for which there is no single, paramount analytical technique. To overcome this difficulty, Agilent makes a range of products in chromatography, NMR and spectroscopy that can be used for the investigation of this type of compound. Agilent's instruments and consumables elucidate the characteristics and composition of resins.

GC

For screening or fingerprinting of synthetic resins Agilent offers a range of high temperature GC columns, including DB-5ht, FactorFour VF-5ht, DB-2887, and CP-SimDist Ultimet. These columns are ideal for resin analysis using headspace sampling. To maximize column lifetime at high temperature, we recommend Agilent Gas Clean Filters to minimize oxygen and moisture content in carrier gas.

GC/MS

Threats to food safety through spoilage or adulteration have been of concern for many years. More recently, attention has focused on the perceived risk of harm from semi-volatile compounds given off by the plastic in which so much of our food is wrapped. These outgassed compounds, such as phthalates, can be detected by headspace GC/MS with the VF-5ms or VF-624ms columns and Agilent's ion trap instruments. Plastic wrapping of food can, however, have benefits when the atmosphere inside the pack is modified, by reducing the oxygen content to improve the keeping qualities of the food. Monitoring oxygen content is straightforward with CP-Molsieve columns. To assess CO₂ and moisture try Agilent's CP-PoraPLOT columns.

LC/MS

Additives are widely used to improve the performance characteristics of polymer resins. The analytical needs for additives analysis are qualitative identification, screening for potential contaminants (non-target analysis), and reliable, accurate quantitative determination of additive concentration in a complex matrix. It is a considerable analytical challenge to provide all of this information in a single analytical run. The Agilent 500-MS LC Ion Trap is ideal for this type of analysis, where sensitivity, reliability and productivity are essential. The 500-MS reliably detects and quantitates additives and non-target contaminants in complex matrices.

FT-IR

For most types of natural and synthetic resins, FT-IR from Agilent is a valuable tool for chemical identification of both known and unknown samples and also for QA/QC during manufacturing and post production. It can be used for the analysis of the liquid components (when they are in a viscous state) and of the final solidified samples. Depending on the type of resin, one can even study the process of resin-hardening (curing under different conditions) in 'real-time' using ATR-FT-IR. The fast scanning Agilent 670-IR FT-IR spectrometer is ideal for studying these kinetic processes. For most 'basic' resin analyses we recommend a 640-IR or 660-IR with a PIKE Diamond MIRacle ATR, for easy sample prep with a wide range of solvents and pH.

HPLC

PLRP-S 300Å columns provide oligomeric fingerprints of epoxy resins by reversed phase HPLC. Use these columns with any instrument in the Agilent 1200 Infinity Series, a suite of fully integrated, pre-configured and pre-tested HPLC instruments. Designed to get you quickly up and running, the Agilent 1200 Infinity Series is easy to customize, install and use.

NMR

If you work with compounds that are physically heterogeneous, such as non-filterable solid phase synthesis resins, then NMR with Agilent's NanoProbe is the best option. The NanoProbe combines the "high resolution" aspects of a liquids probe with the "MAS" aspects of a solids probe. Use NanoProbe with the new Agilent NMR System, engineered with a new technology designed for unsurpassed flexibility.

UV-Vis

UV-Vis spectroscopy is a valuable tool for the analysis of many polymers, including curable and composite dental resins. Agilent's Cary range of UV-Vis-NIR instruments has become the standard for researchers wanting to extend the boundaries of spectrophotometric measurement techniques. The range is equally at home in routine laboratories where reliability and ease of use are vital.

Find out how to take your resin analysis to the next level

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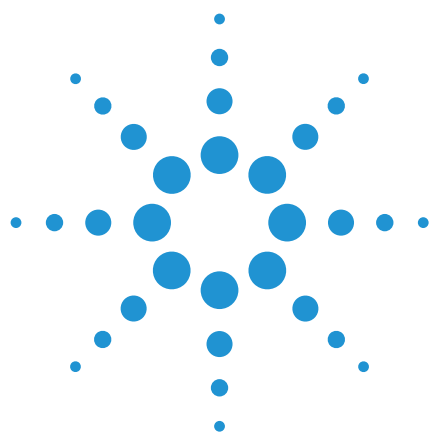


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Agilent Technologies



Biodegradable polymers - analysis of biodegradable polymers by GPC/SEC

Application compendium

Authors

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Agilent Technologies, Inc.



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Studying biodegradable polymers

Biodegradation is the degradation of a material by environmental factors such as sunlight, temperature changes or the action of microbes. In polymer science and engineering, the design of polymers susceptible to biodegradation is of increasing importance for two reasons – polymers that degrade naturally in the body to harmless products may be used in biological devices and in drug delivery, and polymers that break down in the environment are significantly ‘greener’ than traditional plastics.

Biodegradation is key to the suitability of materials for use in drug delivery devices or in temporary structures within the body, such as sutures. For these applications, the ability of the body to naturally break down the material used either as part of the application or post-event is very important, making the removal of the polymer simply a case of allowing the natural process of degradation to occur. Many materials are being investigated for these applications as medical science progresses.

The landfill crisis has made the production of non-polluting polymers for packaging and engineering uses a high priority. These materials need to be able to perform their function, but also break down in the environment with time, a difficult proposition.

For these materials, the rate of degradation and therefore the lifetime and performance of the polymer in the natural environment is related to the length of the polymer chains in the material, with degradation leading to scission of the polymer chains and a shortening of their length.

Gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC), a well-known technique for determining the molecular weight distribution of polymers, is therefore key to studying biodegradable materials by giving an insight into the rate at which a material might degrade, and revealing the presence of degraded polymer chains in a sample.

This application compendium shows examples of GPC applications involving different biodegradable polymers, derived from synthetic and natural sources.

Agilent Technologies produces the most extensive range of GPC/SEC columns, standards and instruments that are ideally suited to the analysis of biodegradable polymers.

Agilent’s columns are the most stable available, and include ranges suited for use in organic and aqueous eluents, solvent mixtures and high polarity organic eluents, covering the requirements of the diversity of biodegradable materials. With extensive options in particle and pore size, Agilent’s columns can be specifically selected to match the molecular weight of the material under investigation, thereby ensuring that the best quality data is obtained from the GPC/SEC experiment.

Agilent’s GPC/SEC columns are the most rugged and reliable on the market, making them ideal for applications that rely on extremely reproducible analysis such as in quality control environments.

Given that many biodegradable materials are destined for use in vivo, ensuring the quality of materials is of the upmost importance.

Agilent also manufactures narrow polydispersity standards with very highly characterized molecular weights that are used as calibration standards in the GPC/SEC analysis of biodegradable polymers.

Complementing Agilent’s column technology is the most extensive collection of integrated GPC/SEC instrumentation on the market covering the temperature range from ambient to 220 °C.

These instruments allow all forms of the GPC/SEC experiment to be performed and can be used to analyze the complete range of biodegradable materials. Multiple detection options can be included in the instruments, such as light scattering and viscometry, and dedicated analysis software is available that allows the biodegradation properties of the materials to be monitored.

Agilent’s complete range of columns and instrumentation offer a clear advantage in the analysis of biodegradable polymers.



Narrow dispersity polymer calibrants

Synthetic polymers

Poly(lactide-co-glycolide)

Application area: Drug delivery

Poly(lactide-co-glycolide) copolymers have found extensive applications in the pharmaceutical industry. The molecular weight distribution of the polymer can affect the properties of the end product, and is therefore of interest in both the areas of development and quality control.

The copolymer is quite polar in nature, but can be dissolved in several solvents suitable for gel permeation chromatography (GPC), notably tetrahydrofuran (THF) and chloroform.

Low boiling solvents like chloroform can suffer from outgassing effects. When employing refractive index detection, this can lead to chromatograms with noisy or drifting baselines. The Agilent 380-ELSD and Agilent 385-ELSD (cooled) evaporative light scattering detectors, on the other hand, always deliver baselines which are stable and drift-free. Furthermore, due to its evaporative nature, it provides chromatograms which are free from system peaks around total permeation which are commonly associated with refractive index detectors. Agilent's 380-ELSD and 385-ELSD also offer superior sensitivity compared to refractive index.

Poly(lactide-co-glycolide) copolymers are relatively low in molecular weight.

The Agilent PLgel 5 μ m MIXED-D columns with their high efficiency (>50,000 plates/meter) and broad resolving molecular weight range (up to 400,000 daltons relative to polystyrene), are the columns of choice for this application.

Figure 1 shows a typical raw data chromatogram for a poly(lactide-co-glycolide) sample.

Columns: 2 x PLgel 5 μ m MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent: Chloroform
Flow Rate: 1.0 mL/min
Detector: Agilent ELSD

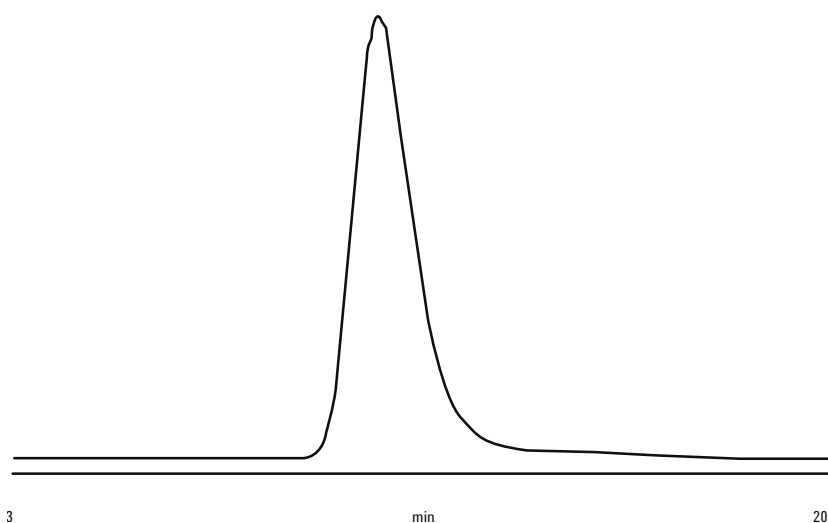


Figure 1. Typical raw data chromatogram for a poly(lactide-co-glycolide) sample showing a typical gaussian peak shape

Polycaprolactam

Application area: Drug delivery

Traditional drug delivery systems, such as the oral contraceptive pill, have a major disadvantage - the release of the active species is very non-linear, with typically a high dosage at the time of introduction followed by a steady decline as the drug is metabolized. A release profile of this kind is inefficient. Ideally, the dosage of the active compound into the body should remain at a constant level during treatment. The controlled delivery of drugs in vitro to produce linear dosing regimes is a major goal of therapeutic research. Polycaprolactam is a well-known polymer that biodegrades by enzymatic cleavage of ester bonds under conditions found within the human body. Introducing an active drug contained in a matrix of polycaprolactam into the body leads to the steady release of drug as the polymer matrix degrades. Appropriate inclusion of the drug into the matrix controls the rate of release

A critical parameter controlling the rate of degradation of biodegradable polymers is the molecular weight of the starting material. The higher the average molecular weight, the slower the rate of biological degradation. Measuring the molecular weight distributions of biodegradable polymers by gel permeation chromatography (GPC) is a critical part of research into controlled drug release with polymers. The chromatogram below shows polycaprolactam obtained in THF using two Agilent PLgel 5 μ m MIXED-C columns. The polymer eluted as a broad peak with an average molecular weight of 80,000 g/mol and a polydispersity of 2.5.

Sample: Polycaprolactam
Columns: 2 x PLgel 5 μ m MIXED-C, 300 x 7.5 mm (Part No. PL1110-6500)
Eluent: THF (stabilized)
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Detector: RI

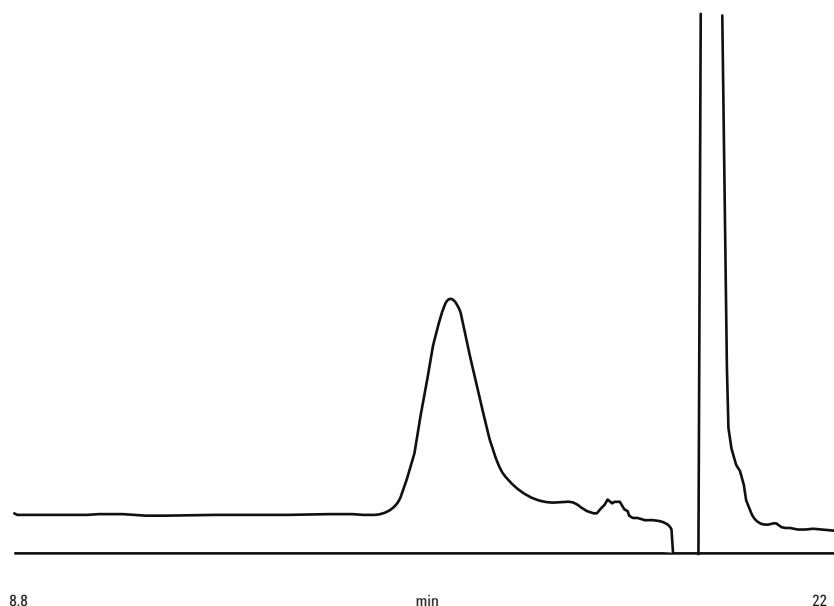


Figure 2. Typical raw data chromatogram for a sample of polycaprolactam containing low molecular weight components and showing a large system peak

Polyvinyl alcohol

Application areas: Adhesive, surfactant, surface properties

Fully or partially hydrolyzed grades of polyvinyl alcohol are normally specified according to their viscosity in solution. Aqueous SEC can be used to characterize these polymers in terms of molecular weight distribution. Three samples with the same degree of hydrolysis were compared by overlaying their molecular weight distributions. This is a convenient method of fingerprinting materials for quality control, and is more informative in production control and end-use performance evaluation than single point viscosity measurements.

Calibrants: Pullulan polysaccharides
Columns: 2 x Agilent PL aquagel-OH 40 μm , 300 x 7.5 mm (Part No. PL1149-6840)
Eluent: 0.25M NaNO_3 , 0.01M NaH_2PO_4 , pH 7
Flow Rate: 1.0 mL/min
Detector: RI

Table 1. Correlation of GPC results with polymer specification for PVA

Sample	Viscosity (mPa.s)	Mn	Mw
A	4.0	9771	29470
B	10.0	23339	80174
C	20.0	31210	102309

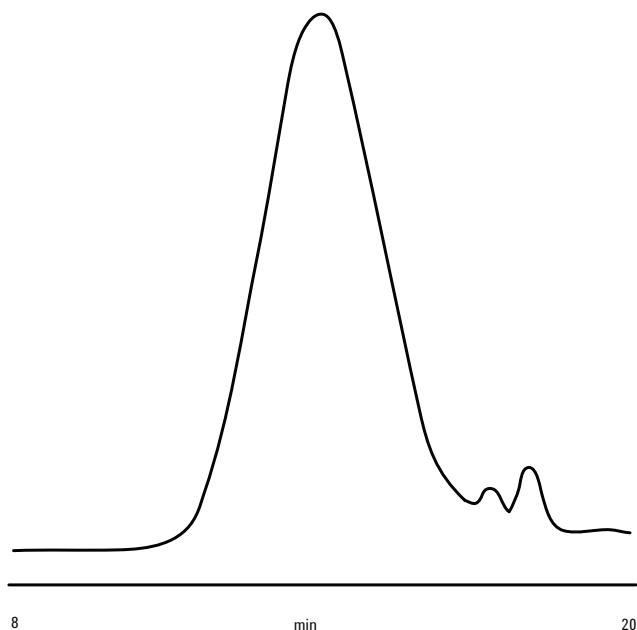


Figure 3. Raw data chromatogram (sample A) showing the presence of low molecular weight components along with the polymer peak

Polyethylene glycol (PEG)

Application areas: Excipient, dispersant, antifreeze

Polyethylene glycols are inert, water-soluble and biodegradable polymers used in a range of applications from medical formulations, protein conjugates to cosmetic products and antifreeze solutions. Manufactured by living polymerization processes, PEGs have narrow molecular weight distributions with physical properties controlled by their molecular weight.

Agilent PL aquagel-OH 30 8 μm high performance columns are ideal for relatively low molecular weight separations, combining low exclusion limit, high pore volume and high column efficiency ($>35,000$ plates/meter) for maximum resolution.

The separation below shows a range of PEG samples.

Columns: 2 x PL aquagel-OH 30 8 μm , 300 x 7.5 mm
(Part No. PL1120-6830)
Eluent: Water
Flow Rate: 1.0 mL/min
Detector: RI

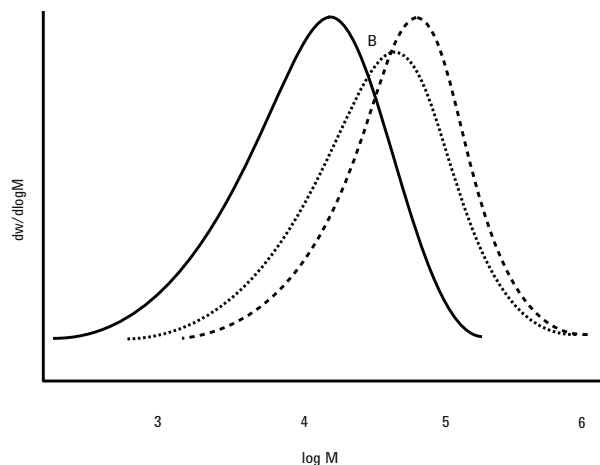


Figure 4. Example molecular weight distributions of the three polyvinyl alcohols with very different physical properties

Columns: 2 x PL aquagel-OH 30 8 μm , 300 x 7.5 mm
(Part No. PL1120-6830)
Eluent: Water
Flow Rate: 1.0 mL/min
Detector: RI

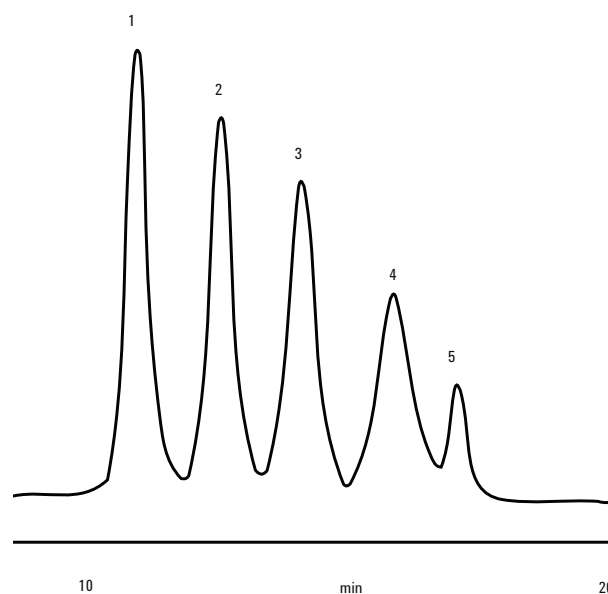


Figure 5. Resolution achieved in the separation of five PEG samples used for calibration

Naturally-occurring polymers

Natural rubber

Application area: Engineering material

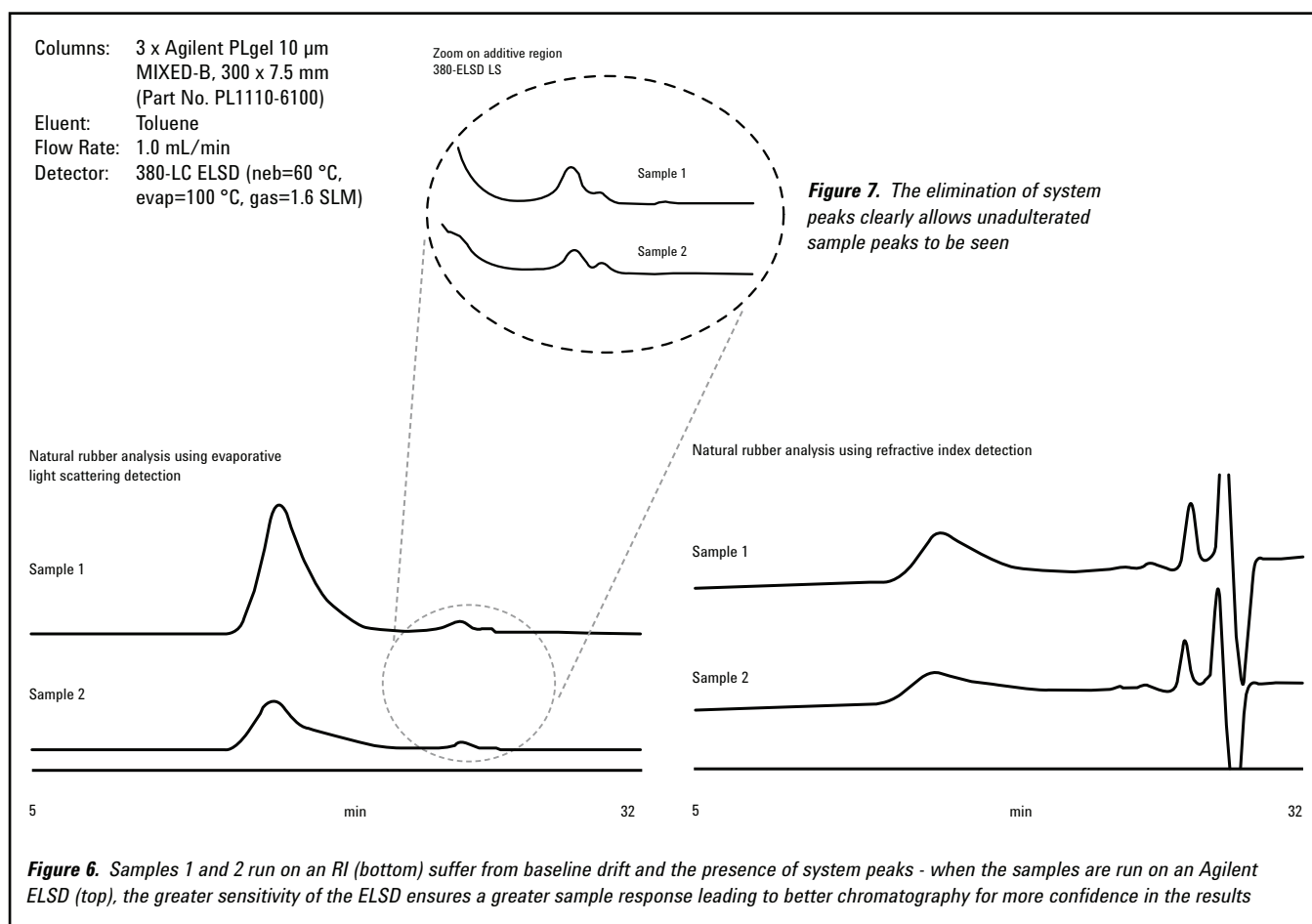
A biodegradable polymer produced from tree sap, natural rubber is an elastomer that has many uses in a wide range of industrial and household products. It is degraded slowly by bacterial action.

Solutions of natural rubber samples are generally very difficult to prepare for GPC due to the fact that the polymer contains relatively high levels of 'gel', which is partially crosslinked. An aliquot of the eluent is added to the weighed sample. It is allowed to swell and dissolve overnight, and then the gel material is filtered out (0.5 µm) prior to GPC analysis.

In this case, the actual polymer concentration can be significantly lower than the original concentration prepared depending on the gel content of the sample, and therefore detector response, usually RI, tends to be quite poor. ELSD exhibits significantly increased sensitivity compared to an RI and subsequently gives much greater response for this application. In addition, RI baseline drift, which commonly occurs, is very much emphasized when the actual peak response is so small.

ELSD always gives a flat baseline which, together with the improved response, makes baseline and peak setting much more reliable for GPC calculations.

A further problem with RI is sensitivity to system peaks around total permeation, which usually occurs even when samples are prepared in an aliquot of the eluent. These system peaks can interfere with low molecular weight components which are commonly found in natural rubber samples. This situation is very much improved when ELSD is employed, as system peaks are eliminated due to evaporation, leaving unadulterated sample peaks in the additives region.



Polyacrylic acid

Application areas: Adhesive, water treatment

Polyacrylic acid is a biodegradable water soluble polymer with numerous industrial applications, including as a super adsorbent (e.g. in disposable nappies), in water treatment as a metal ion scavenger and in the treatment of metal surfaces prior to coating.

The molecular weight distribution (MWD) of this material is an important parameter, as it strongly affects the end use properties of the polymer. Aqueous SEC is an ideal analytical tool for the measurement of the MWD of polyacrylic acid. Since polyacrylic acid is a polyelectrolyte, care must be taken in selecting the appropriate SEC conditions. In the SEC described below, a buffered mobile phase with a high electrolyte content was used to minimize non-size exclusion effects.

Agilent PL aquagel-OH MIXED-H columns were selected to provide good resolution over a wide molecular weight range. Column calibration was achieved using Agilent polyethylene oxide (PEO) EasiVial standards, see Figure 8.

Columns:	2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M NaNO ₃ + 0.01M NaH ₂ PO ₄ , adj to pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 μ L
Sample Conc:	PEO standards: 0.1-0.5 mg/mL Polyacrylic acid: approx 0.2% w/v
Detector:	RI

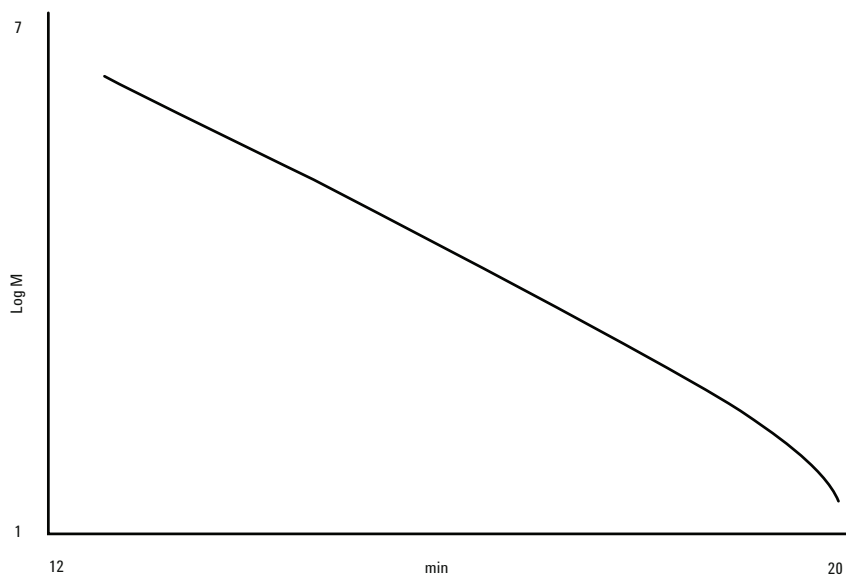


Figure 8. PL aquagel-OH MIXED-H 8 μ m column calibration using PEO EasiVial standards showing the relationship between retention time and the log of molecular weight

EasiVial standards

EasiVial standards provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). Each vial contains a mixture of four individual, highly characterized, narrow dispersity standards. The amount of each individual standard is carefully controlled during manufacture, allowing their use in SEC-viscometry which requires accurate concentrations.

Refractive index chromatograms obtained from each PEO EasiVial are presented in Figure 9.

Three polyacrylic acid samples (A, B and C) were chromatographed and their corresponding molecular weight distribution compared, see Figure 10.

Table 2. Comparison of molecular weight averages for the three samples of polyacrylic acid

Sample	Mn (g/mol)	Mw (g/mol)	PD
A	33,450	89,430	2.67
B	7,990	14,930	1.87
C	7,880	13,490	1.71

Sample A was found to possess a significantly higher molecular weight distribution compared to Samples B and C, which were found to be similar. Consequently, Sample A was expected to possess significantly different rheological properties compared to the remaining two samples. Closer examination of the samples showed Sample A to be significantly more viscous than B and C, which were similar. In addition, the MWD of Sample A was found to be bi-modal, which suggests that the sample may be a blend of more than one component. In conclusion, differences in the molecular weight distributions of the polyacrylic acids were identified. These differences were corroborated through visual examination of the samples' bulk viscosity.

Columns: 2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M NaNO₃ + 0.01M NaH₂PO₄, adj to pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Sample Conc: PEO standards: 0.1-0.5 mg/mL
Polyacrylic acid: approx 0.2% w/v
Detector: RI

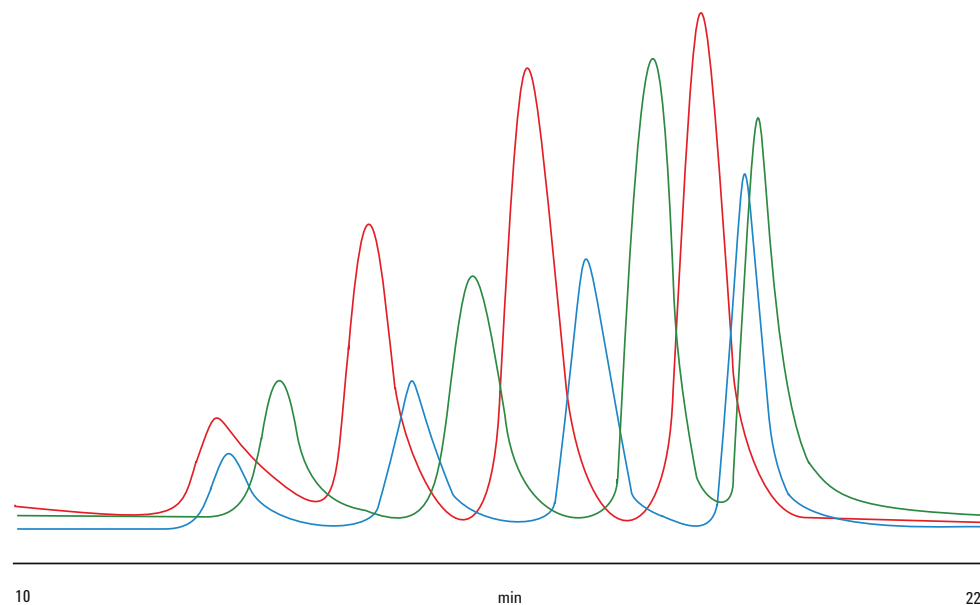


Figure 9. Example chromatograms of PEO EasiVial standards used to create the calibration

Columns: 2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M NaNO₃ + 0.01M NaH₂PO₄, adj to pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Sample Conc: PEO standards: 0.1-0.5 mg/mL
Polyacrylic acid: approx 0.2% w/v
Detector: RI

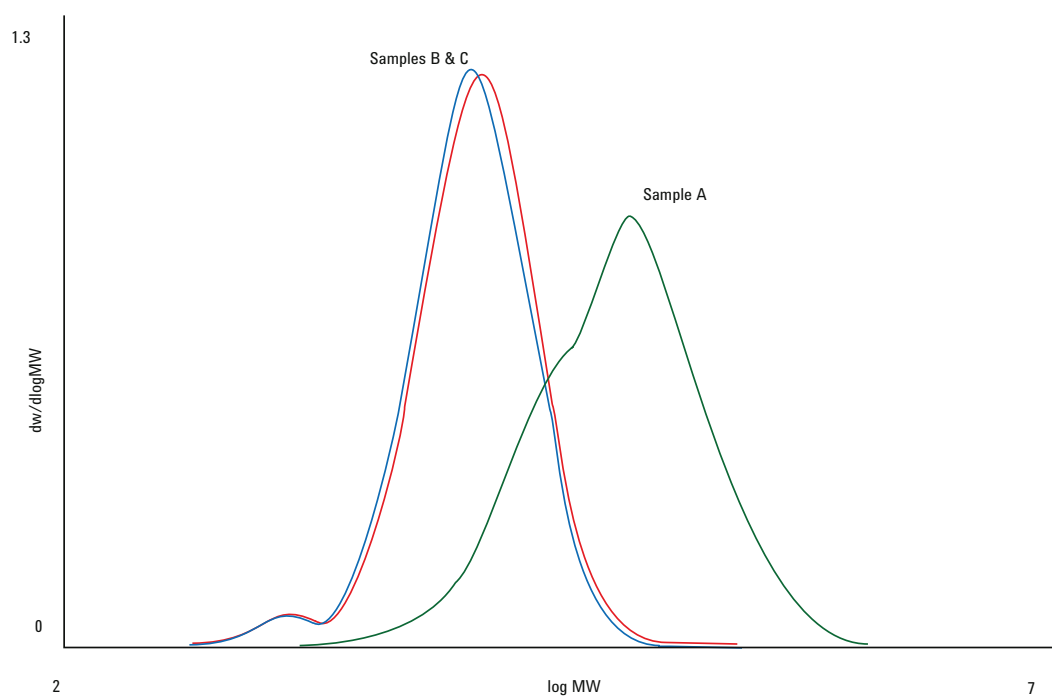


Figure 10. Molecular weight distribution of three polyacrylic acid samples showing a clear difference between one of the samples (A) and the other two (B and C)

Chitosan

Application areas: Drug delivery, paper production

Chitosan is a naturally-occurring polysaccharide made by alkaline N-deacetylation of chitin which is believed to be the second most abundant biomaterial after cellulose. The term chitosan does not refer to a uniquely-defined compound, but merely refers to a family of copolymers with various fractions of acetylated units containing both chitin and chitosan monomers.

The main interest in chitosan derives from its cationic nature in acidic solutions which provides unique properties relative to other polysaccharides, which are usually neutral or negatively charged. Application areas of chitosan include biomedical (e.g. wound healing, burn treatment and use as a hemostatic agent), paper production, textile finishes, photographic products, cements, heavy metal chelating agents and waste removal.

GPC/SEC can be used as a quality control tool for the determination of MW and MWD. Different molecular weights would be appropriate to particular applications.

Three grades of chitosan were analyzed using a column set comprising 2 x PL aquagel-OH MIXED 8 μ m columns. These columns offer resolution over a wide molecular weight range (up to 10,000,000 relative to PEO/PEG).

Due to the cationic nature of the samples, they were prepared in strong acid and were allowed to stand overnight to aid dissolution. They were analyzed in 0.5M sodium nitrate buffer and at low pH.

Raw data chromatograms and weight average molecular weight values (Mw) for the three chitosan samples are shown below.

The system was calibrated with narrow pullulan polysaccharide standards and the resulting calibration curve is illustrated below.

Columns: 2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm
(Part No. PL1149-6800)
Eluent: 0.5M NaNO₃, 0.01M NaH₂PO₄, pH 2
Flow Rate: 1.0 mL/min
Detector: RI

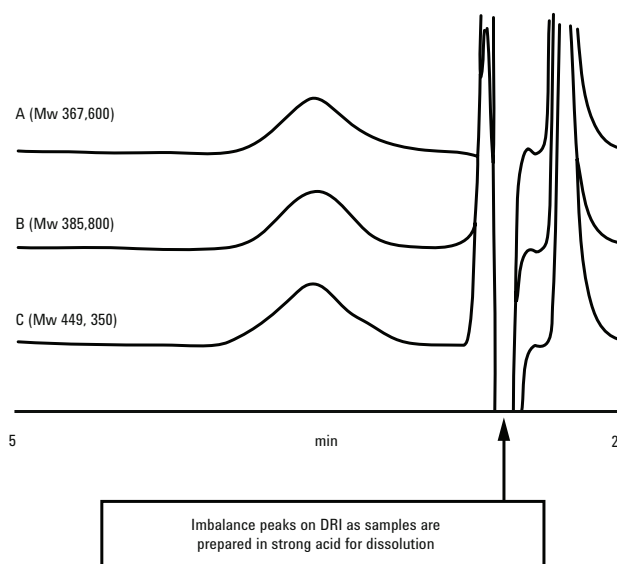


Figure 11. Raw data chromatograms of three chitosan samples showing typical peak shapes with strong imbalance peaks due to dissolution conditions

Columns: 2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm
(Part No. PL1149-6800)
Eluent: 0.5M NaNO₃, 0.01M NaH₂PO₄, pH 2
Flow Rate: 1.0 mL/min
Detector: RI

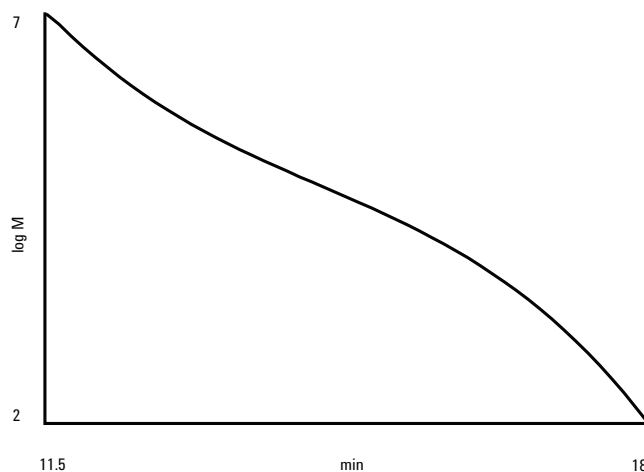


Figure 12. SEC calibration showing the resolving range of the PL aquagel-OH MIXED-H 8 μ m column set

Cellulosic polymer

Application areas: Thickening agent and viscosity modifier

The most abundant component of plant matter, cellulose, can be modified to produce a number of biodegradable materials with useful properties.

Carboxymethyl cellulose (CMC) is a cellulose derivative with some of the hydroxyl groups of the glucopyranose monomers of cellulose modified to contain carboxymethyl groups. CMC is a thickener used in the food industry where it has E number E466, and is also used to stabilize emulsions in ice cream. It is also a constituent of many non-food products, including toothpaste and water-based paints. Hydroxyethyl cellulose has some of the hydroxyl groups modified with ethyl chains, and is used as a gelling and thickening agent in cosmetics, cleaning solutions, and other household products.

Carboxymethyl cellulose

Calibrants: Pullulan polysaccharides
Columns: 2 x Agilent PL aquagel-OH 60 μ m, 300 x 7.5 mm (Part No. PL1149-6860)
1 x PL aquagel-OH 40 μ m, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent: 0.5M Na_2SO_4
Flow Rate: 1.0 mL/min
Detector: RI

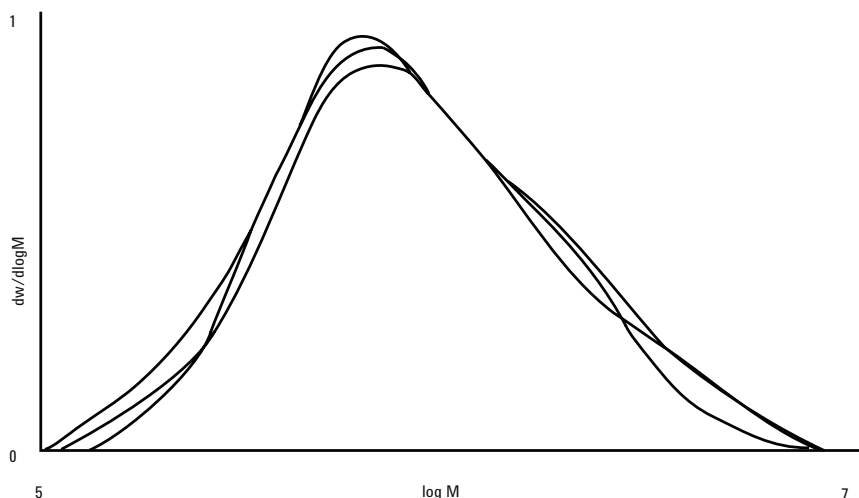


Figure 13. Example molecular weight distributions of three samples of carboxymethyl cellulose with subtle differences between samples

Hydroxyethyl cellulose

The correlation between the two measurements is good, showing that GPC is a viable measurement technique to viscosity when ensuring the quality of these samples.

Table 3. Correlation of viscosity data with GPC results for hydroxyethyl cellulose

	A	B	C
Viscosity Range (cps)	75-112	250-324	1500-2500
Mn	60,300	413,000	914,000
Mw	179,000	849,000	2,016,000
Mz	39,000	1,552,000	3,422,000

Calibrants: Pullulan polysaccharides
Columns: 2 x PL aquagel-OH 60 8 µm, 300 x 7.5 mm (Part No. PL1149-6860)
1 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent: 0.05M NaH₂PO₄, 0.25M NaCl, pH 7
Flow Rate: 1.0 mL/min
Detector: RI

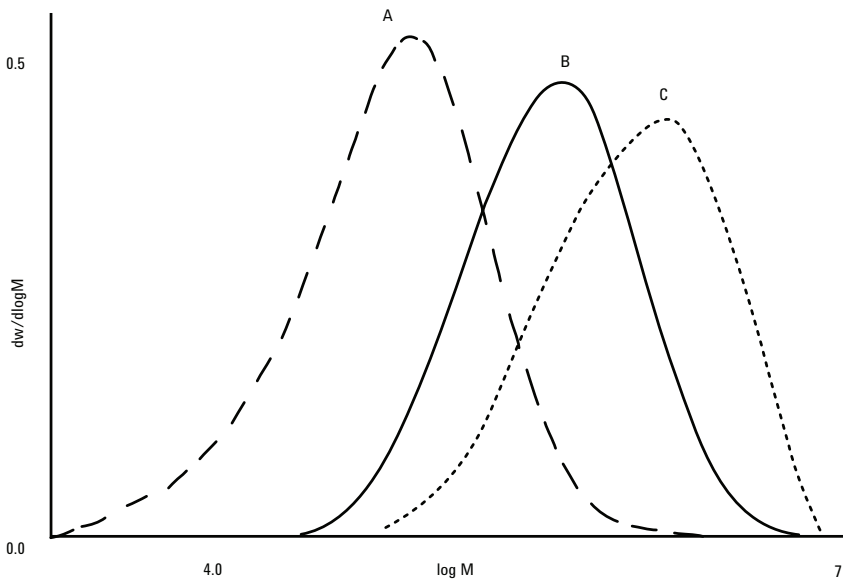


Figure 14. Example molecular weight distributions of three samples, A, B and C with very different properties

Methyl cellulose

Application areas: Emulsifier, treatment of constipation

Table 4. Correlation of viscosity data with GPC results for methyl cellulose

	A	B
Viscosity Range (cps)	85-115	4000-6000
Mn	131,000	484,000
Mw	369,000	1,023,000
Mz	691,000	1,884,000

There is good correlation between the viscosity data and molecular weight averages.

Calibrants: Pullulan polysaccharides
Columns: 2 x PL aquagel-OH 60 μ m, 300 x 7.5 mm (Part No. PL1149-6860)
1 x PL aquagel-OH 40 μ m, 300 x 7.5 mm (Part No. PL1149-6840)
Eluent: 0.05M NaH_2PO_4 , 0.25M NaCl, pH 7
Flow Rate: 1.0 mL/min
Detector: RI

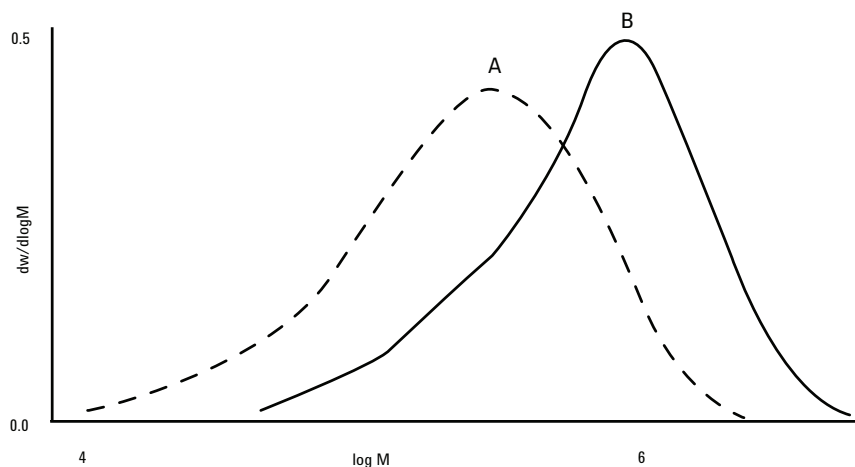


Figure 15. Example molecular weight distributions for two samples of methyl cellulose which behave very differently

Cellulose acetate analysis

Application areas: Photographic film base, adhesives, synthetic fibers

Used extensively in the photographic and packaging industries, cellulose acetate is soluble in a limited number of solvents. Here, dissolution was achieved in dimethylacetamide after gentle heating and stirring of the sample solution. Lithium chloride was added to the eluent to counter any polyelectrolyte effects.

Columns: 3 x PLgel 10 μ m MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Eluent: DMAc+0.5% LiCl
Flow Rate: 1.0 mL/min
Loading: 0.2% w/v, 100 μ L
Temp: 60 °C
Detector: GPC (RI)

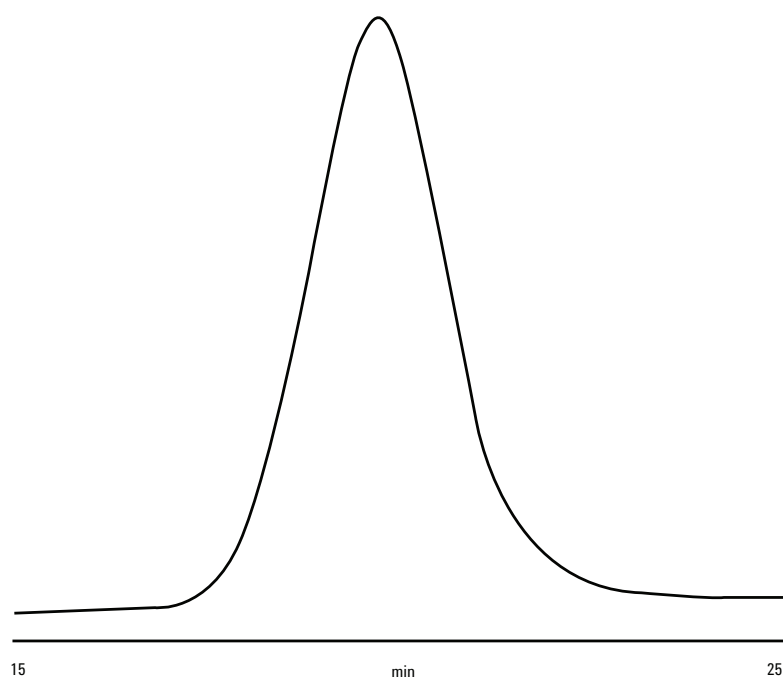


Figure 16. Example chromatogram for a sample of cellulose acetate analysis showing a typical polymer peak shape

Pectins

Application area: Gelling agent in food

Pectins are a class of polysaccharide gum found naturally in fruits such as apples, plums, grapes and cranberries. Structurally complex, pectins consist of 'smooth' and 'hairy' regions. The smooth regions are linear partially methylated poly(D-galacturonic) acid, the hairy regions alternating L-rhamnosyl and D-galacturonosyl residues containing L-arabinose and D-galactose branch points up to 20 residues long. As a result of this heterogeneous nature, pectins adopt complex structures in solution.

Applications of pectin are related to the formation of crosslinks through hydrogen bonding of the carboxylic acid groups, and include use as gelling agents, thickeners and water binders. Triple detection size exclusion chromatography employs a concentration detector, a viscometer and a light scattering detector to assess the molecular weight distribution and molecular structure of polymers without having to rely on column calibrations. This can be important when analyzing complex materials for which no structurally similar standards are available.

In this application, a sample of pectin was analyzed on the Agilent PL-GPC 50 integrated GPC system running at 30 °C fitted with a refractive index detector, an Agilent PL-BV 400 four capillary bridge viscometer and an Agilent PL-LS 15/90 dual angle light scattering detector (collecting scattered light at 15° and 90°). Two PL aquagel-OH MIXED 8 µm columns were used for the analysis with a 200 µL injection loop and a buffer solution of 0.2M NaNO₃, 0.01M NaH₂PO₄, adjusted to pH 7, as the eluent. The sample was prepared accurately at nominally 2 mg/mL in the eluent and filtered before injection through a 0.45 µm disposable filter. For the purpose of light scattering calculations, an average dn/dc value was used for the sample.

Figure 17 shows an overlay of the triple detector chromatograms for the pectin sample. The chromatograms obtained on the refractive index and light scattering detectors were clearly multimodal, as expected for a structurally heterogeneous material.

Columns: 2 x PL aquagel-OH MIXED 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M NaNO₃ + 0.01M NaH₂PO₄, adj pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Detectors: PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

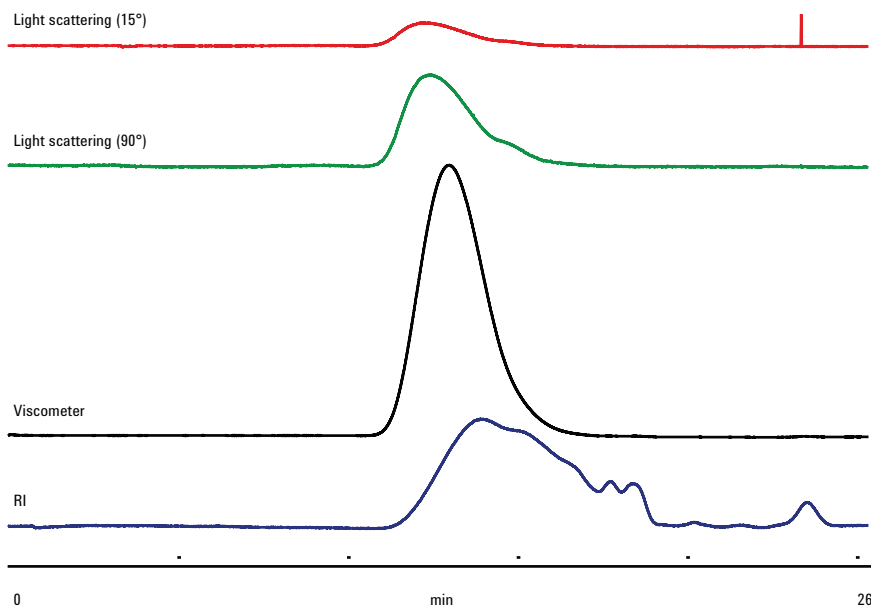
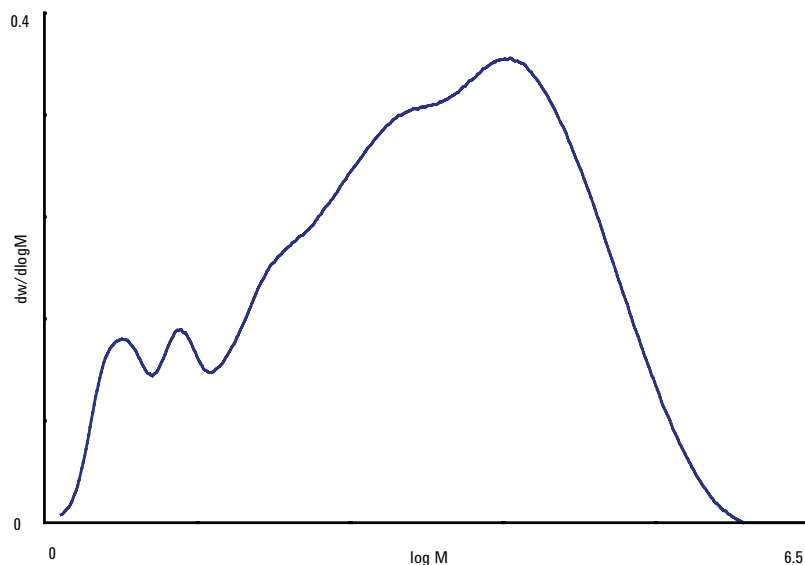


Figure 17. A typical multi detector overlay of chromatograms for a sample of pectin, showing the different responses of the detector

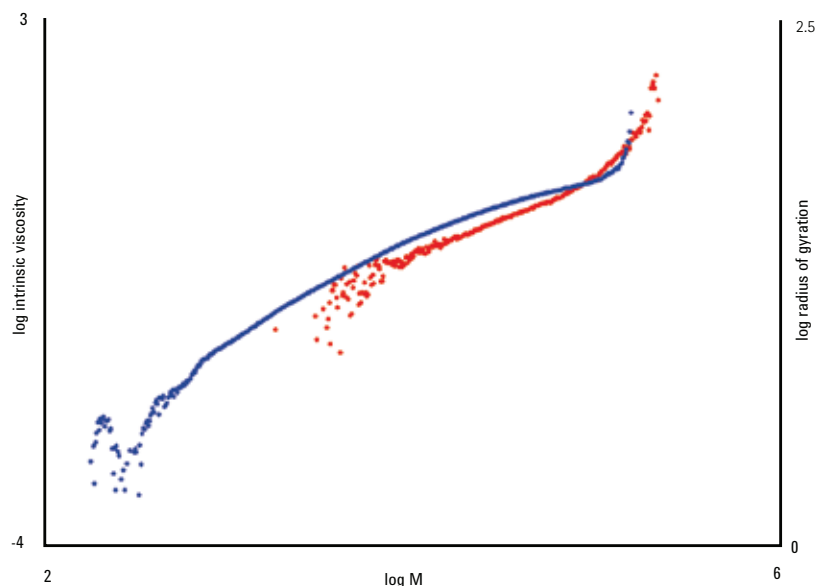
From the viscometry and light scattering data, Mark-Houwink (log intrinsic viscosity versus log M) and conformation (log radius of gyration versus log M) plots were generated for the pectin, shown overlaid in Figure 19.

The Mark-Houwink, and to some extent, the conformation plots show curvature over the entire molecular weight distribution, indicating a change in molecular density as a function of molecular weight, resulting from a variation in the relative amounts of 'smooth' and 'hairy' regions. This application demonstrates how the new PL-GPC 50 can be used for the analysis of structurally complex but commercially important materials by multi detector GPC.



Columns: 2 x PL aquagel-OH MIXED 8 μ m, 300 x 7.5 mm
(Part No. PL1149-6800)
Eluent: 0.2M NaNO₃ + 0.01M NaH₂PO₄, adj pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Detectors: PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

Figure 18. Molecular weight distribution calculated for the pectin showing a complex shape



Columns: 2 x PL aquagel-OH MIXED 8 μ m, 300 x 7.5 mm
(Part No. PL1149-6800)
Eluent: 0.2M NaNO₃ + 0.01M NaH₂PO₄, adj pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Detectors: PL-GPC 50, RI, PL-BV 400, PL-LS 15/90

Figure 19. Mark-Houwink and conformation data showing differences in structure between the two materials

More Agilent solutions for biodegradable polymers

UV-Vis-NIR spectroscopy

The Agilent Cary spectrophotometer series is the standard for researchers wanting to extend the boundaries of spectrophotometric measurement, and is equally at home in routine laboratories where reliability and ease of use are vital.

Fluorescence spectroscopy

The Cary Eclipse fluorescence spectrophotometer offers the high performance you've come to expect from a Cary, at a surprisingly low price.

FTIR spectroscopy

The compositional analysis of polymers is made easy with Agilent's FTIR spectrometers and microscopes which extract specific chemical information from extremely small sample areas.

Raman spectroscopy

Raman spectroscopy delivers qualitative and quantitative information on chemical species that make up biodegradable polymers.

X-Ray crystallography

X-ray crystallography was famously used to decipher the structure of the DNA polymeric protein in the early 1950s. These days, Rosalind Franklin would probably use the Agilent SuperNova system, the highest quality and most reliable diffractometer.

Nuclear magnetic resonance

The Agilent NMR System works in an integrated fashion, providing unsurpassed flexibility to implement a multitude of different experiments.

Dissolution release rate testing

Agilent's family of dissolution apparatus is the most comprehensive in the pharmaceutical industry for testing stents, patches, capsules and membranes.



The Agilent Cary 600 FTIR Series delivers the best analytical performance under real world conditions.



Agilent's SuperNova is the ideal diffractometer for both modern crystallographic research and leading analytical service laboratories.

Find out how to take your biodegradable polymers analysis to the next level

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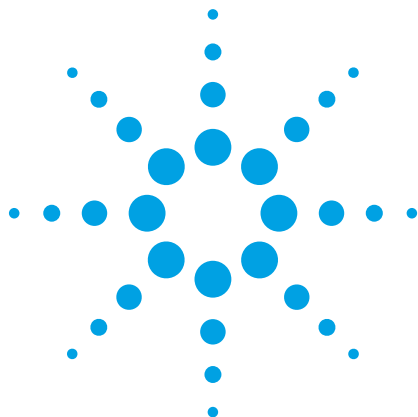
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Agilent Technologies



Analysis of engineering polymers by GPC/SEC

Application Compendium

Authors

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Agilent Technologies, Inc.



Polymer analysis by GPC/SEC

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Polymer analysis by GPC/SEC

Introduction

Increasingly, plastics are being utilized to perform structural and mechanical roles in the construction and engineering industries. The favorable properties of polymers, such as mechanical strength, durability, and resistance to chemical and physical degradation, coupled with their relative cheapness, means that polymers outperform many traditional materials, including wood and metals, in key applications. With the creation of new polymeric materials, this shift towards plastics is becoming even more pronounced as materials with new properties are designed and developed. An understanding of the behavior of polymers is key to designing new materials with appropriate performance characteristics for specific applications. Analysis of these materials is therefore a critical component of the development and manufacture of engineering polymers.

Gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many of their physical properties. Generally, increasing molecular weight leads to higher performance characteristics, while an increase in the width of the distribution (the polydispersity) leads to a loss of performance but an increase in the ease of processing.

Engineering polymers are particularly difficult to analyze – they are generally tough and difficult to dissolve, often requiring aggressive solvents and elevated temperatures. For these applications at high temperature, a high performance integrated GPC system, such as the Agilent PL-GPC 220 Integrated GPC/SEC System, is a necessity. The PL-GPC 220 has the highest temperature range of any system on the market. The following applications show the analysis of various types of engineering polymers and illustrate the conditions and equipment required.

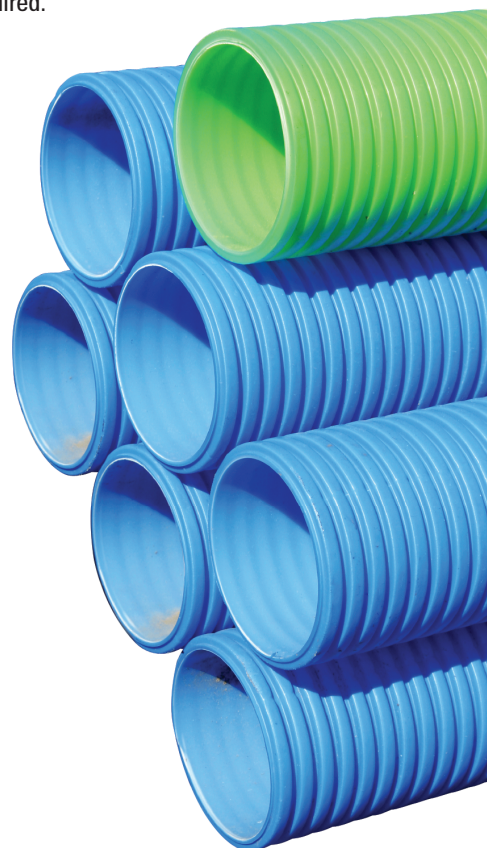


Table 1. Effects of molecular weight distribution on the properties of engineering polymers

	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	–
Decreasing distribution	+	+	–	+	+	+

Agilent's GPC/SEC technology

Agilent produces the most extensive range of GPC columns, standards, instruments and software, ideally suited to the analysis of engineering polymers.

GPC/SEC columns

Agilent's GPC columns are the most rugged and reliable on the market, making them ideal for applications performed in viscous solvents and at elevated temperatures where column lifetime can be an issue. The extensive column range includes products tailored to the analysis of engineering polymers that generally have high molecular weights and high viscosities, and includes specialist columns such as Agilent PLgel Olexis that are designed for the analysis of a specific material. With extensive options in particle and pore size, Agilent's columns can be selected to match the molecular weight of the material under investigation, thereby ensuring that the best quality of data is obtained from the GPC/SEC experiment.



Agilent offers a selection of GPC/SEC column dimensions

GPC/SEC instruments

Complementing Agilent's column technology is the most extensive collection of integrated GPC/SEC instrumentation on the market, covering the widest temperature range available, from ambient to 220 °C.

Agilent's PL-GPC 220 Integrated GPC/SEC System features unbeatable reproducibility for any GPC/SEC application, across the entire operating range. The PL-GPC 220 is an extremely flexible system, designed to run almost all polymer, solvent and temperature combinations, from 30 to 220 °C.

The PL-GPC 220 allows all forms of the GPC/SEC experiment to be performed and can be used to analyze the complete range of engineering materials, including those that require analysis at extremely high temperatures. Multiple detection options can be included in the instruments, such as light scattering and viscometry, and dedicated analysis software is available that allows the properties of engineering polymers to be analyzed in detail. Agilent's complete range of columns and instrumentation offer a clear advantage in the analysis of engineering polymers.



PL-GPC 220 Integrated GPC/SEC System

GPC/SEC standards

Narrow polydispersity polymer standards with very highly characterized molecular weights are used as calibration standards in the GPC analysis of engineering polymers. Polystyrene standards are the first choice for many organic solvents, either for conventional GPC column calibration or for calibrating light scattering and viscosity detectors.



Agilent's EasiVial calibration kit

For more information on Agilent's GPC/SEC products, visit www.agilent.com/chem/gpcsec

GPC/SEC of polymers in aggressive solvents

Aggressive solvents

Many polymers, especially those used in engineering applications, show only limited solubility in a small number of solvents. This is because high strength and toughness are usually a result of high molecular weight and/or high crystallinity. Increasing molecular weight requires untangling the molecular chains to dissolve the material, whereas increased crystallinity requires break-up of any inter-chain bonds that may be present.

The PL-GPC 220 Integrated GPC/SEC System is designed to allow the use of even the most aggressive solvents. The following applications illustrate the analysis of a range of engineering polymers that require aggressive solvents for solubility or as eluents during the analysis.

GPC analysis of polyether ether ketone (PEEK)

Application areas: High performance components, tubing in liquid chromatography

Polyether ether ketone (PEEK) was developed in 1977 by ICI and was one of the first of the new generation of engineering thermoplastics developed for chemical resistance, high mechanical strength and high thermal stability – the useful properties of the material are retained up to temperatures as high as 315 °C. A crystalline material with repeat units of two ethers and a ketone group in the polymer backbone, PEEK is a high cost material. For many applications, such as the manufacture of piston components in engines, the insulation of cables and the production of high performance aircraft parts, this cost is justified as there are no other plastics that can offer the same performance properties. The industrial performance of PEEK makes analysis of this material by GPC difficult. PEEK has excellent chemical resistance and is unaffected by many organic and inorganic chemicals, dissolving only in strong or concentrated anhydrous oxidizing agents. Previous methods for analyzing PEEK have involved mixtures of trichlorobenzene and phenol running at high temperatures.

For this analysis, the PEEK sample was dissolved in a small volume of dichloroacetic acid at 120 °C for two hours. After dissolution, the sample was diluted to the required concentration of 0.2% (w/v) with chloroform and injected into a system running at temperature after filtration to remove undissolved material.

The PEEK sample eluted as a broad polymer peak with an MW of 70,000 g/mol and a polydispersity of 2.2. The large system peak observed at the end of the run was due to the excess dichloroacetic acid used in the preparation of the sample.

Conditions

Sample: Polyether ether ketone (PEEK)
Columns: 2 x Agilent PLgel 10 µm MIXED-B,
300 x 7.5 mm (Part No. PL1110-6100)
Eluent: 80% chloroform
20% dichloroacetic acid
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Detector: PL-GPC 220

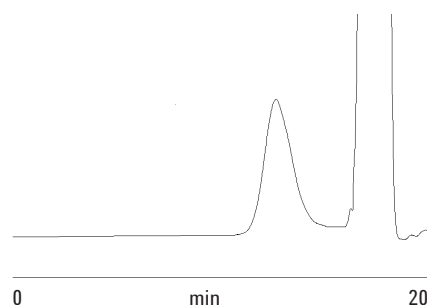


Figure 1. Chromatogram of a PEEK sample

GPC analysis of polybutylene terephthalate (PBT) resins in HFIP

Application areas: Machined parts

Polybutylene terephthalate (PBT) resins are used in a wide variety of applications in which toughness and resistance to damage are highly advantageous. However, mechanical and thermal stress during the production of molded parts can cause degradation, giving a reduction in desirable physical properties. The molecular weight distribution of the resin is a key measure of the onset of degradation and therefore of estimating the mechanical strength of the final product. PBT is soluble in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), a polar organic solvent, which is excellent for dissolving polar polymers such as polyamides and polyesters. The analysis was carried out in HFIP modified by the addition of 20 mM sodium trifluoroacetate to prevent aggregation. Two Agilent PL HFIPgel columns, designed specifically for HFIP applications, were employed for the analysis at a temperature of 40 °C. The PL-GPC 220 Integrated GPC/SEC System was used with differential refractive index and viscometry detection. GPC coupled with a molecular weight-sensitive viscometer allowed calculation of molecular weights based on hydrodynamic volume using the Universal Calibration approach, leading to molecular weights independent of the standards used to generate the column calibration. Agilent polymethylmethacrylate (PMMA) standards were employed to generate the Universal Calibration.

Table 2 shows the molecular weight averages and intrinsic viscosity for the sample before and after molding, as determined by GPC/viscometry. Clearly, the molecular weight distribution indicates that after molding, the material has suffered from degradation and is less robust than the virgin material.

Table 2. Molecular weight averages and intrinsic viscosity for the PBT resin sample

	Mn/g mol ⁻¹	Mw/g mol ⁻¹	Intrinsic viscosity/g ⁻¹
Virgin resin	24,400	48,600	0.535
Molded part	11,200	24,000	0.306

Conditions

Samples: PBT resin
Columns: 2 x PL HFIPgel,
300 x 7.5 mm (Part No. PL1114-6900HFIP)
Eluent: HFIP + 20 mM NaTFA
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Temp: 40 °C
Detectors: PL-GPC 220, viscometer

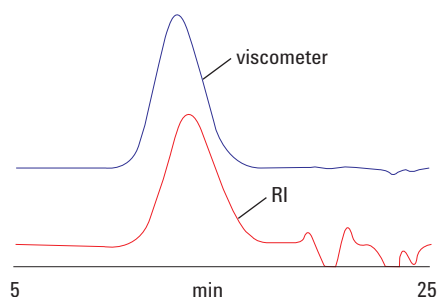


Figure 2. Example overlay of a dual-detector chromatogram of the virgin PBT resin before molding

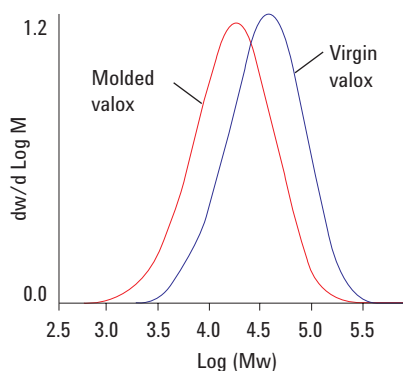


Figure 3. Molecular weight distributions of the two samples

Polyethylene terephthalate analysis in o-chlorophenol as an alternative solvent

As an alternative to the use of HFIP, PET can be analyzed in o-chlorophenol. This viscous solvent requires elevated temperatures and is a hazardous substance.

The samples were dissolved by heating to 110 °C for 30 minutes. The polymer remains in solution at room temperature but the high viscosity of the eluent means that high temperature GPC is necessary. Three grades of PET, with different intrinsic viscosities, were analyzed and compared, showing minor differences between the materials.

Conditions

Samples: PET resin
Columns: 2 x PLgel 10 μ m MIXED-B,
300 x 7.5 mm (Part No. PL1110-6100)
Eluent: o-chlorophenol
Flow Rate: 1.0 mL/min
Temp: 100 °C
Detection: PL-GPC 220

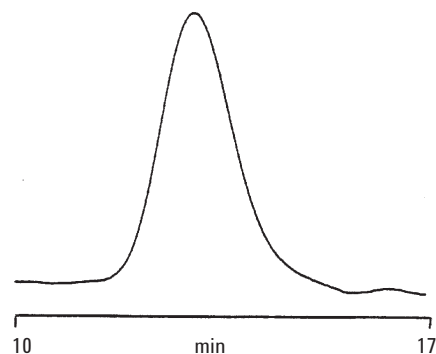


Figure 4. Chromatogram of a PET sample

Peak Identification

1. IV=0.72
2. IV=0.75
3. IV=0.84

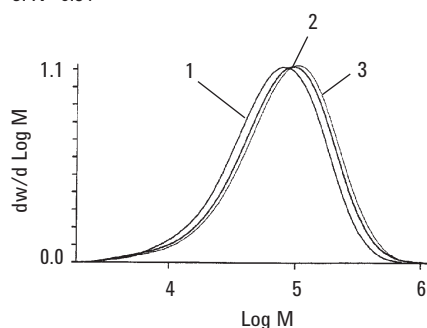


Figure 5. Molecular weight distributions of the PET samples

GPC/SEC of polymers at high temperature

High temperature analysis

Some highly crystalline polymers such as polyethylene show only limited solubility and only then at high temperatures. This is due to the fact that temperature is required to break down the ordered crystalline structure, and on cooling, the material will re-crystallize and precipitate from solution. For these applications, high temperature is required throughout the entire analysis to ensure that the samples remain in solution during the experiment. The PL-GPC 220 Integrated GPC/SEC System is capable of maintaining a constant temperature up to 200 °C between the point of injection, the columns and the detector cells, until the point of elution. The following applications illustrate the analysis of crystalline polymers at high temperatures using the PL-GPC 220.

Column selection for polyolefin analysis

Polyolefins range from low molecular weight hydrocarbon waxes to ultra high molecular weight rigid plastics. The molecular weight distribution of polyolefins is directly related to physical properties such as toughness, melt viscosity, and crystallinity. GPC/SEC is widely accepted as the preferred technique to fully characterize the molecular weight distribution of polyolefins.

The selection of a column set for the analysis of a polyolefin is dependent on the molecular weight range of the sample. Low molecular weight samples can be analyzed using high efficiency, relatively low pore size columns. Higher molecular weight materials require large particle size media to minimize shear effects, with a wide pore size distribution.

Figures 6 to 8 show typical data for four very different polyolefin samples, all obtained with the PL-GPC 220.

Conditions

Sample: Linear hydrocarbons
Columns: 2 x Agilent PLgel 3 μ m 100Å,
300 x 7.5 mm (Part No. PL1110-6320)
Eluent: TCB
Flow Rate: 0.8 mL/min
Inj Vol: 20 μ L
Temp: 145 °C
Detector: PL-GPC 220

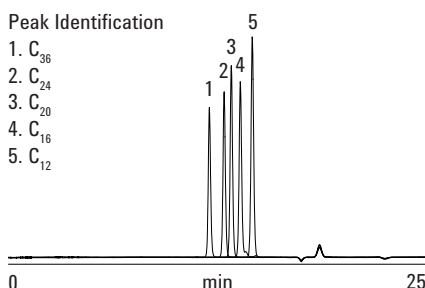


Figure 6. Separation of a selection of low molecular weight linear hydrocarbons analyzed using two PLgel 3 μ m 100Å columns

Conditions

Sample: Hydrocarbon wax
Columns: 2 x Agilent PLgel 5 μ m MIXED-D,
300 x 7.5 mm (Part No. PL1110-6504)
Eluent: TCB
Flow Rate: 1.0 mL/min
Inj Vol: 100 μ L
Temp: 160 °C
Detector: PL-GPC 220

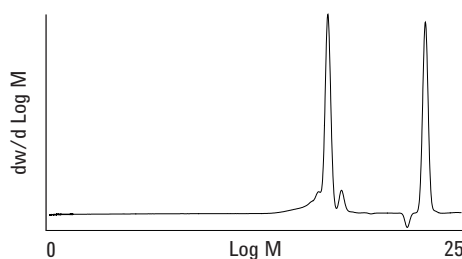


Figure 7. Chromatogram of a relatively low molecular weight hydrocarbon wax obtained on two PLgel 5 μ m MIXED-D columns

Conditions

Sample: Polyethylene
 Columns: 3 x PLgel Olexis, 300 x 7.5 mm
 (Part No. PL1110-6400)
 Eluent: TCB
 Flow Rate: 1.0 mL/min
 Inj Vol: 200 µL
 Temp: 160 °C
 Detector: PL-GPC 220

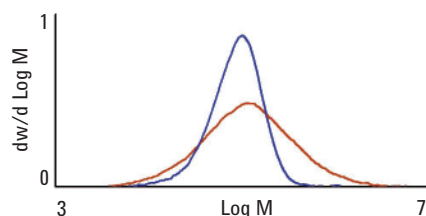


Figure 8. Overlaid molecular weight distributions of medium and high polydispersity polyethylene samples

High molecular weight materials require analysis on high pore size PLgel Olexis columns that minimize shear degradation, and are able to resolve up to 100,000,000 g/mol with a 13 µm particle size. These applications illustrate the diversity of polyolefin samples and indicate the flexibility of the PLgel series of columns in addressing the analysis of such samples.

High temperature GPC analysis of polypropylene on the PL-GPC 220 – repeatability study

Application areas: Plastic pipes, bottles and containers

The PL-GPC 220 Integrated GPC/SEC System is ideally suited to the analysis of polypropylene. A commercial sample of polypropylene (PP) was prepared at 1.5 mg/mL using the Agilent PL-SP 260VS Sample Preparation System with a dissolution temperature of 160 °C and a dissolution time of two hours. Six aliquots of the master batch solution were dispensed into the PL-GPC 220 autosampler vials and placed in the carousel where the hot zone temperature was 160 °C and the warm zone 80 °C.

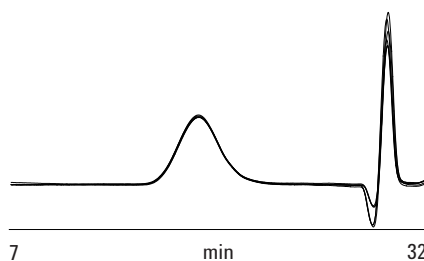


Figure 9. Overlay of the raw data chromatograms obtained for six consecutive injections of polypropylene

The data was analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene equivalent molecular weight averages that are shown in Table 3.

Polystyrene in TCB¹ $K=12.1 \times 10^{-5}$ $\alpha=0.707$

Polypropylene in TCB² $K=19.0 \times 10^{-5}$ $\alpha=0.725$

Table 3. Calculated molecular weights for six injections of polypropylene and calculated % variation

Injection Number	Mp	Mn	Mw
1	127,132	65,086	185,795
2	131,893	65,089	185,236
3	128,673	66,802	186,202
4	132,062	67,417	188,048
5	131,625	69,320	188,679
6	130,227	69,677	186,188
Mean	130,202	67,232	186,691
Std Dev	1,693	1,815	1,239
% Variation	0.13	2.70	0.66

The results illustrate the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 µm MIXED-B columns.

Conditions

Sample: Polypropylene
Columns: 3 x PLgel Olexis, 300 x 7.5 mm
(Part No. PL1110-6400)
Eluent: TCB + 0.0125% BHT
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Temp: 160 °C
Detector: PL-GPC 220

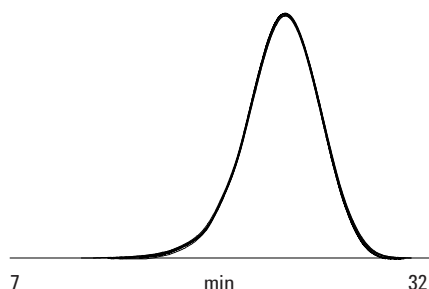


Figure 10. Overlay of the molecular weight distribution calculated for six consecutive injections of the polypropylene sample

References

- ¹ H. Col and D. K. Giddings, *J. Polym. Sci.*, (A2) **8** (1970) 89
² T.G. Scholte et al, *J. Appl. Polym. Sci.*, **29** (1984) 3763

Branching analysis of polyethylenes with Cirrus GPC Multi Detector Software

Application areas: Plastic bags and containers

The presence of long chain branching (over 6 carbons in length) in polyolefins strongly influences physical properties such as melt viscosity and mechanical strength. The distribution chain branches in polyolefins are determined by the polymerization mechanism and there is significant interest in the production of materials with well-defined and characterized molecular weight and branching distributions for specific applications.

Here we describe the analysis of three samples of polyethylene with the PL-GPC 220 by GPC/viscometry. Two of the samples had been synthesized by a mechanism to promote branching while the third was a standard linear reference material NBS 1475. The analysis was carried out at 160 °C with three PLgel Olexis columns in trichlorobenzene (TCB) with 0.015% butylated hydroxytoluene (BHT) as a stabilizer.

Refractive index and viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software using the Universal Calibration approach. Polystyrene standards were used to generate the Universal Calibration and the unbranched sample was used as a linear model in the determination of branching.

Figure 11 shows the molecular weight distributions for the three samples. The black plot is for the unbranched sample. Although there was some overlap, the samples clearly had significantly different molecular weights.

Figure 12 shows the Mark-Houwink plots for the three samples. The uppermost sample is the unbranched material. The other two samples have lower intrinsic viscosities at any given molecular weight, with the unbranched polymer indicating the presence of branching. This can be expressed in terms of g , the branching ratio, defined as follows, where ϵ is a constant:

$$g = \left[\frac{\text{Intrinsic viscosity (branched)}}{\text{Intrinsic viscosity (linear)}} \right]^{1/\epsilon}$$

Conditions

Samples: Polyethylenes
Columns: 3 x PLgel Olexis, 300 x 7.5 mm
(Part No. PL1110-6400)
Eluent: TCB + 0.015% BHT
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Temp: 160 °C
Detector: PL-GPC 220 + viscometer

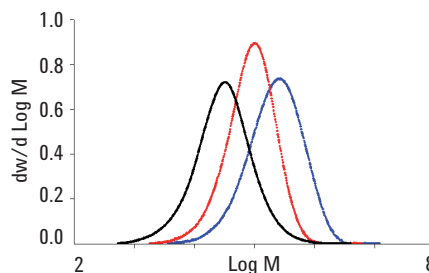


Figure 11. Molecular weight distribution plots for the three polyethylene samples – the black plot is for the unbranched sample

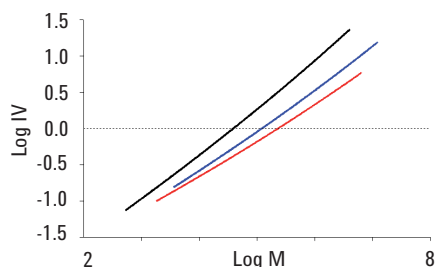


Figure 12. Mark-Houwink plots for three samples of polyethylene

The unbranched sample was used as the linear model and so gives a g value of unity (except at high molecular weight due to scatter in the data). The other two samples both exhibit a decrease in g as a function of molecular weight, indicating that as molecular weight increases, the number of branches increases. Based on these calculated g values, a branching number or number of branches per 1,000 carbon atoms can be generated. This is achieved by fitting the data into a model. The Cirrus GPC Multi Detector Software offers a selection of branching models that can be employed in this approach. In this case a model was used that calculates a number-average branching number, assuming a random distribution of branches on the polymer. Figures 13 and 14 show the g plots and branching-number plots obtained for the samples.

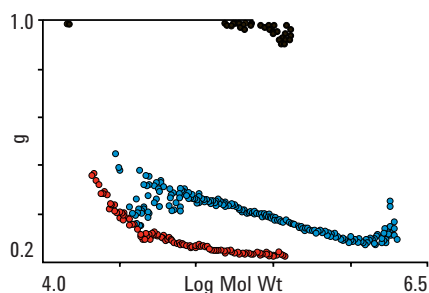


Figure 13. Branching ratio g plots for the three polyethylene samples – the black plot is the unbranched sample

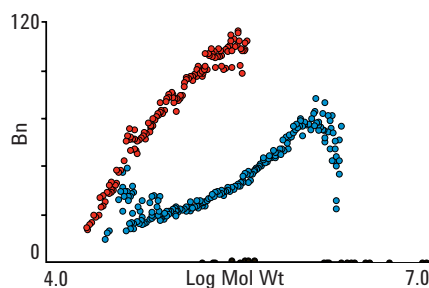


Figure 14. Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample

The results show that of the two branched samples, the trend in molecular weight distribution does not follow the trend in branching distribution. The sample showing the most branching at any given molecular weight has a lower molecular weight than the second sample. Clearly, understanding both the molecular weight and branching distributions will give an insight into the processability of the two materials.

Polyphenylene sulfide analysis

Application areas: High performance membranes, felts and insulators

Polyphenylene sulfide (PPS) is an engineering polymer with a rigid backbone of alternating aromatic rings linked by sulfur atoms. It is useful as a structural material due to its high resistance to both chemical and thermal attack, and the material is very stiff, even at high temperatures. PPS is used in a number of applications, including as a filter fabric for coal boilers, in felts used in paper making, in electrical insulation applications and in the manufacture of specialty membranes. PPS is naturally insulating, although the addition of a dopant can be used to make the material semi-conducting.

PPS is particularly difficult to analyze by GPC. The high chemical and thermal resistance of the material means that it is only soluble in specialist solvents such as ortho-chloronaphthalene at elevated temperatures of around 200 °C. The PL-GPC 220 is capable of operation at these temperatures, and the PLgel column material can perform the analysis of PPS.

Conditions

Columns: 3 x PLgel 10 µm MIXED-B,
300 x 7.5 mm (Part No. PL1110-6100)
Eluent: o-chloronaphthalene
Flow Rate: 1.0 mL/min
Temp: 210 °C
Detector: PL-GPC 220

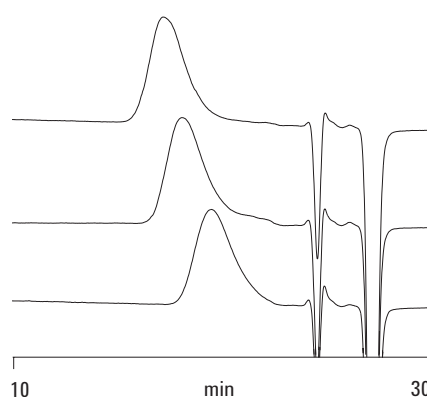


Figure 15. Overlaid chromatograms of three samples of polyphenylene sulfide

Ordering information

The following products are featured in this application compendium. For a full list of GPC/SEC part numbers, visit www.agilent.com/chem/store

Columns	
Description	Part No.
Agilent PLgel 3 µm 100Å, 300 x 7.5 mm	PL1110-6320
Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm	PL1110-6504
Agilent PLgel 5 µm MIXED-C, 300 x 7.5 mm	PL1110-6500
Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm	PL1110-6100
Agilent PLgel 20 µm MIXED-A, 300 x 7.5 mm	PL1110-6200
Agilent PLgel 20 µm MIXED-A LS, 300 x 7.5 mm	PL1110-6200LS*
Agilent PLgel Olexis, 300 x 7.5 mm	PL1110-6400
Agilent PL HFIPgel, 300 x 7.5 mm	PL1114-6900HFIP

Standards	
Description	Part No.
Agilent PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0301
Agilent PS-L EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0401
Agilent EasiCal PS-1 pre-prepared polystyrene kit	PL2010-0501
Agilent EasiCal PS-2 pre-prepared polystyrene kit	PL2010-0601
Agilent PM EasiVial 2 mL pre-weighed polymethylmethacrylate calibration kit	PL2020-0201
Agilent PM EasiVial 4 mL pre-weighed polymethylmethacrylate calibration kit	PL2020-0200

Instruments	
Description	Part No.
Agilent PL-GPC 220 Integrated GPC/SEC System	PL0820-0000
Agilent PL-BV 400HT Online Integrated Viscometer	PL0810-3050
Agilent PL-HTLS 15/90 Light Scattering Detector	PL0640-1200
Agilent PL-SP 260VS Sample Preparation System**	

Software	
Description	Part No.
Agilent Cirrus GPC Software	PL0570-2000
Agilent Cirrus GPC Multi Detector Software	PL0570-2020

* Low shedding for light scattering applications

** Contact your local sales office or distributor for different options

Companion products

The term engineering polymers includes a wide range of materials, and although gel permeation chromatography is the paramount technique in their analysis, there are other analytical techniques that may be employed. Agilent makes a range of instruments in molecular spectroscopy and X-ray crystallography that can be used for the investigation of these types of material. Agilent's advanced instruments elucidate their characteristics and composition, leading to a better understanding of the behavior and fitness for purpose of these increasingly valuable products.

UV-Vis-NIR spectroscopy

Agilent's Cary range of UV-Vis-NIR spectrophotometers has been synonymous with excellence and high performance for over 60 years. The Cary spectrophotometer series is the standard for researchers wanting to extend the boundaries of spectrophotometric measurement. The range is equally at home in routine laboratories where reliability and ease of use are vital for the quality control of polymers.



The Agilent 600-IR series provides the highest level of sensitivity combined with detailed structural and compositional information for information-rich detection

Fluorescence spectroscopy

Agilent's Cary Eclipse Fluorescence Spectrophotometer offers the high performance you've come to expect from a Cary, at a surprisingly low price. With xenon flash lamp technology, plug-and-identify electronics and feature-packed, intuitive software, the instrument embodies the Agilent and Cary names.

FTIR spectroscopy

The compositional analysis of polymers is made easy with Agilent's FTIR spectrometers and microscopes that provide the ability to extract specific chemical information from extremely small sample areas. Exclusive to Agilent and taking full advantage of our FTIR attenuated total reflection (ATR) imaging technology, the Specac Imaging Golden Gate Diamond ATR accessory also provides the highest quality chemical images that are distortion- and aberration-free with a preserved aspect ratio, while maintaining the Golden Gate's robustness and ease of use.

Raman spectroscopy

Raman spectroscopy delivers qualitative and quantitative information on chemical species that make up engineering polymers. Raman complements IR spectroscopy, particularly for the study of crystalline polymers. Agilent's Synergy FT-Raman module is the most compact FT-Raman accessory on the market, maintaining the versatility required by research spectroscopists.

X-ray crystallography

X-ray crystallography was famously used to decipher the structure of the DNA polymer in the early 1950s. These days, Rosalind Franklin would probably use Agilent's Xcalibur E, the expert diffractometer for the modern chemical crystallography laboratory and the most popular choice for single wavelength, small molecule crystallography.

Other GPC/SEC resources from Agilent

Agilent has published application compendia on biodegradable polymers, polyolefin analysis, elastomers, and low molecular weight resins. In addition, we also offer a comprehensive and informative range of literature for all aspects of GPC/SEC, including application notes, datasheets and technical overviews.

Publication	Publication number
Biodegradable polymers	5990-6920EN
Polyolefin analysis	5990-6971EN
Elastomers	5990-6866EN
Low molecular weight resins	5990-6845EN
Introduction to GPC/SEC	5990-6969EN
GPC/SEC reference poster	5990-6882EN
Column selection guide	5990-6868EN

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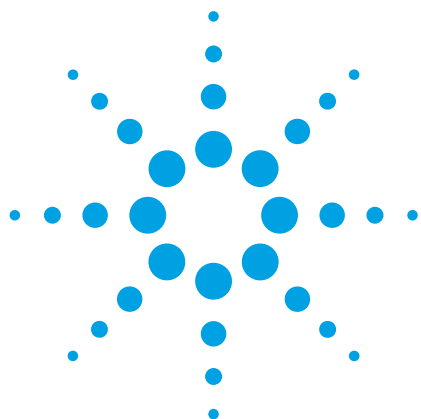
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Agilent Technologies



Analysis of polyolefins by GPC/SEC

Application Compendium

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Agilent Technologies, Inc.



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Polyolefin analysis by GPC/SEC

Introduction

Polyolefin is a general term describing polymers created from simple olefins or alkenes. Many different types of olefin exist, from the most simple, ethylene, to alpha-olefins of increasing complexity. Polyolefins are of great interest as two of them, polyethylene (polythene) and polypropylene, are among the highest tonnage polymers produced in the world. Interest in the analysis of polyolefins comes from the desire to create new materials with custom properties, from the development of new catalysts and from the need to perform quality control on polymer production.

Agilent has a long history of involvement in the analysis of polyolefins by gel permeation chromatography (GPC, also known as size exclusion chromatography, SEC). This application booklet describes Agilent's product portfolio for polyolefin analysis. Instrumentation, software, columns and standards are described, providing a complete package for the analysis of these important products. In addition, a wide range of applications are included that illustrate the performance of the complete solutions for polyolefin analysis offered by Agilent.

Gel permeation chromatography is a well-known technique for assessing the molecular weight distribution of polymers such as polyolefins. Molecular weight influences many of their physical characteristics, as shown in Table 1. In general, increasing molecular weight leads to higher performance, while an increase in the width of the distribution (the polydispersity) leads to a loss of performance but an increase in the ease of processing.

Many polyolefins, typically those containing over 10% ethylene and polypropylene monomers, are of limited solubility in a number of solvents. This is because the characteristic high strength and toughness of these materials results from their high crystallinity. Increased crystallinity requires break up of any inter-chain bonds in order to dissolve the material. Several solvents can be used, but in general the most effective is trichlorobenzene, a viscous solvent with a distinct odor. Ortho-dichlorobenzene is also used in some laboratories, but solubility in this solvent is less effective.

Table 1. Effects of molecular weight (Mw) and the impact of decreasing the width of distribution of Mw on polyolefins

	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

Polymer Laboratories was formed in 1976 to offer high quality columns, standards, instruments, and software for GPC/SEC. For over 30 years the company developed many market-leading products, including PLgel, PL aquagel-OH, PlusPore, PLgel Olexis, PolarGel columns, and EasiVial standards. Built on advanced in-house manufacturing technology, PL's products have the highest reputation for quality and performance, backed up by world-class technical and applications support.

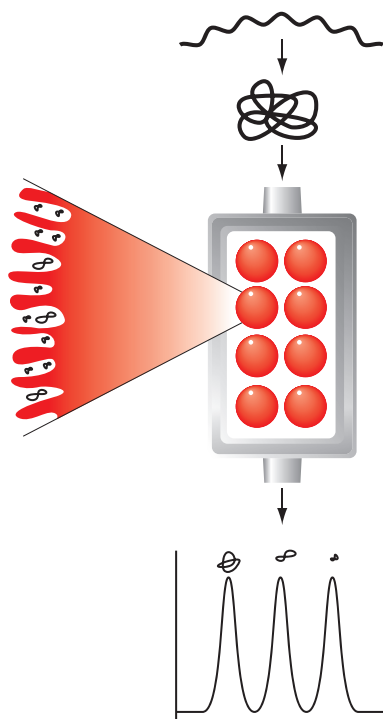
With the acquisition of PL, Agilent offers an even wider range of GPC and SEC solutions for all types of polymer characterization of synthetic and bio-molecular polymers, with options for conventional GPC all the way up to complex determinations using multi-column and multi-detection methods.

The GPC separation mechanism

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- Polymer coils introduced to eluent flowing through a column
- Column packed with insoluble porous beads with well-defined pore structure
- Size of pores similar to that of polymer coils
- Polymer coils diffuse in and out of the pores
- Result is elution based upon size – large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards

Highly crystalline polymers such as polyethylene are soluble only at high temperatures. This is because elevated temperatures are required to break down the ordered crystalline structure, and on cooling the material will re-crystallize and precipitate from solution. For these applications, high temperature is required throughout the entire analysis to ensure that the samples remain in solution. This places several requirements on the instrument for the successful analysis of polyolefins.

- Solvent choice is limited, mainly to 1,2,4-trichlorobenzene (TCB)
- Elevated temperature is required for dissolution, typically for 1 to 4 hours depending on molecular weight and crystallinity
- Column selection must be appropriate for the application in terms of molecular weight resolving range and efficiency of separation
- A high temperature GPC system is required to maintain all components at the analysis temperature, typically 135 to 170 °C, depending on molecular weight and crystallinity



Key

- Smaller coils can access many pores
- Larger coils can access few pores
- Very large coils access very few pores

GPC system requirements for polyolefin analysis

Autosampler, detectors, columns, injection valve and transfer tubing must all be capable of handling elevated temperatures during polyolefin analysis. A typical system schematic is shown in Figure 1.

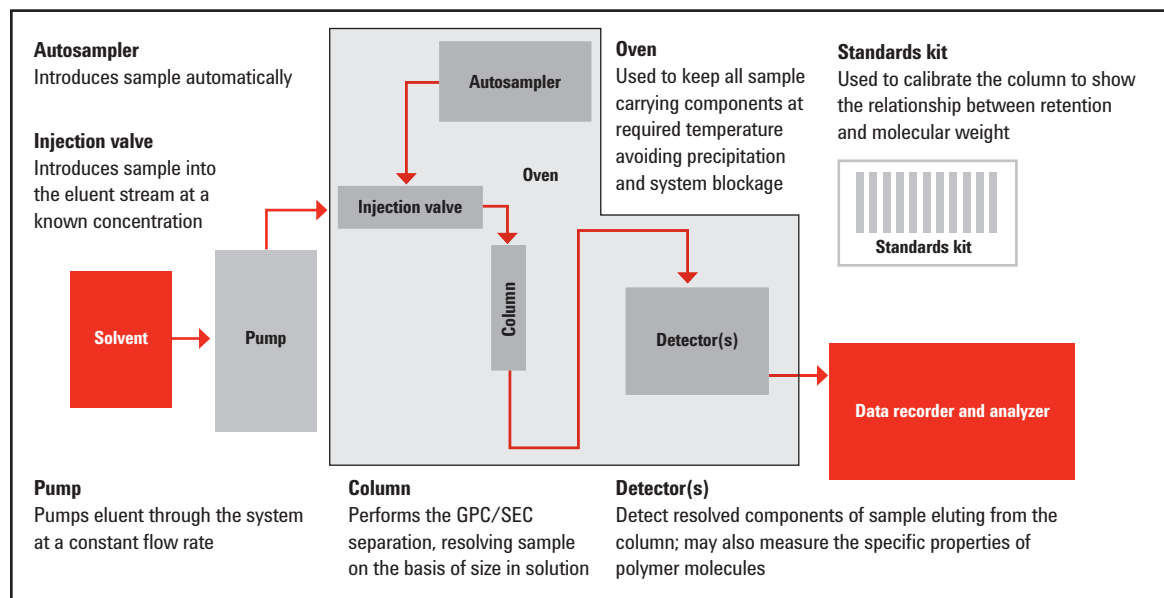


Figure 1. Schematic of a GPC system for polyolefin analysis

Sample preparation

Preparing polyolefin samples is time-consuming because high temperatures and long heating times are required to dissolve the sample (Table 2). Many polyolefins also display a lower density than common analytical solvents such as TCB, and so agitation of the sample is essential to ensure complete dissolution. Filtration may also be necessary to remove insoluble material such as fillers.

Table 2. Preparing a polyolefin sample for analysis

Material	Typical concentration (mg/mL)	Typical prep temp (°C)	Typical heating time (h)
Olefin wax	2 to 3	150	1
General PE or PP	2	150	4
Ultra-high-molecular-weight polyolefin	0.25 to 0.5	150	4 to 8

Agilent PL-SP 260VS Sample Preparation System

The PL-SP 260VS is designed for the manual dissolution and filtration of samples such as polyolefins prior to GPC analysis. The unit combines controlled heating across a temperature range of 30 to 260 °C (± 2 °C), with gentle agitation, user-selectable between 85 to 230 ($\pm 10\%$) rpm. With its temperature range and speed capabilities, the PL-SP 260VS is ideal for a wide range of polymer types, including even the most difficult of samples such as ultra-high-molecular-weight polyethylene.

Choice of vial types

The removable aluminium blocks for the heated compartment are available in several formats to accommodate a variety of vial types. The Standard Accessory Kit is used with standard sample preparation 20 mL vials (supplied) and either PL-GPC 220 2 mL autosampler vials or 4 mL autosampler vials from other vendors. The Custom Accessory Kits let you choose alternative vials, if necessary.



Agilent PL-SP 260VS Sample Preparation System

Efficient dispensing

A unique pipettor device efficiently dispenses filtered sample solution from the sample preparation vial directly into destination (autosampler) vials with minimal handling.

Choice of filtration media

Filtration of polyolefin samples is often required to remove insoluble fillers or gel content (Figure 2). Two filter media are available:

- Glass-fiber (nominal porosity 1 μm) – the preferred system for general applications (Figure 2)
- Porous stainless steel (nominal porosity 0.5, 5, and 10 μm)

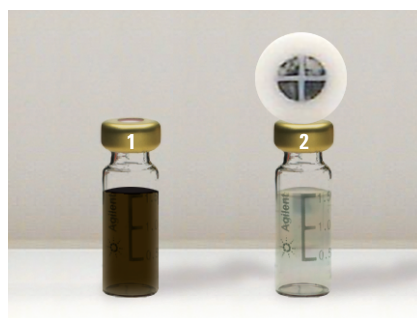


Figure 2. Filtering a carbon black polyethylene solution – 1. without filtration, 2. after filtration using a 1 μm glass-fiber filter

System, software and standards

The Agilent PL-GPC 220 Integrated GPC/SEC System for polyolefin analysis

The PL-GPC 220 is a leading system for the analysis of polyolefins at high temperature. Containing a number of features that have been specifically designed for polyolefin analysis, the PL-GPC 220 is the most versatile instrument for gel permeation chromatography.

Widest temperature range

The PL-GPC 220 features the widest operating range available: 30 to 220 °C, permitting analysis of virtually any polymer in any solvent. The multi-heater, forced-air oven is extremely stable, and accurately controls the temperature to within 0.05 °C. This minimizes detector baseline drift, ensuring the reproducible retention times so important in GPC.

High-precision isocratic pump – unrivalled reproducibility for precise results

The PL-GPC 220 incorporates a high-precision pump for the best pump performance available. Unbeatable flow reproducibility of 0.07% is achieved, not only in THF at near-ambient temperature, but also in TCB at temperatures above 140 °C.

Easy-access oven – changing columns and routine maintenance made simple

The column oven can comfortably hold six, 300 x 7.5 mm GPC columns. The oven operates at a convenient angle to allow for easy access for changing columns and the injector loop, providing comfortable and safe operation.



Agilent PL-GPC 220 Integrated GPC/SEC System

Enhanced RI sensitivity and stability

The improved refractive index (RI) detector includes a new photodiode and uses fiber optic technology to maximize sensitivity while minimizing baseline drift and noise, vital for good GPC/SEC. This RI detector delivers outstanding signal-to-noise ratios, even at 220 °C (Figure 3).

Conditions

Columns: 2 x Agilent PLgel 10 μ m MIXED-B,
300 x 7.5 mm (Part No. PL1110-6100)

Flow Rate: 1 mL/min

Inj Vol: 200 μ L

Detector: PL-GPC 220

Peak Identification

1. Mp = 1,460,000, conc. = 0.62 mg/mL

2. Mp = 9,860, conc. = 1.08 mg/mL

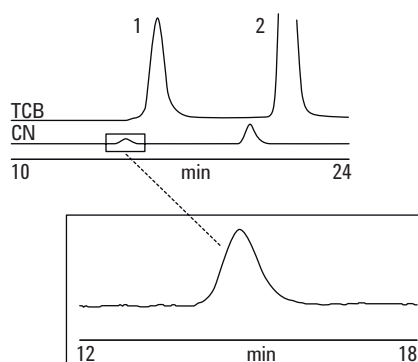


Figure 3. Excellent signal-to-noise demonstrated in the separation of polystyrene standards

Safety first – solvent leak detection and automated shutdown

Agilent's GPC/SEC systems incorporate integral sensors that constantly monitor the system. Vapor sensors are fitted in both the solvent module and column oven. The sensors can be programmed for sensitivity according to the solvent in use.

In the case of an unattended error, the system selects and activates the appropriate shutdown sequence depending on the nature of the error. Low solvent flow will be maintained, where possible, to avoid damage to valuable GPC columns.

An audit trail feature offers full status and error logging for system traceability.

Customized upgrade solutions

The oven easily handles multiple-detector upgrades such as light scattering and viscometry, and coupling to other techniques such as TREF (temperature rising elution fractionation), FTIR (fourier transform-infrared spectroscopy) and ELSD (evaporative light scattering detection). The oven holds up to four detectors in combination. For example, integrating RI, viscometry and light scattering would provide complete polymer characterization.

PC control – easy to program, easy to use

The PL-GPC 220 system for polymer characterization up to 220 °C features intuitive, comprehensive PC software control for full and flexible system management. With safety a pre-requisite, PC control uniquely permits remote use so that you do not need to be in the laboratory.

Interactive color-coded graphics provide ease-of-use. Simply click on the color-coded modules via the main screen to alter any run parameters. Flow rate, temperature and autosampler sequence are quickly and easily updated, and on-screen help is always available, if required (Figure 4).

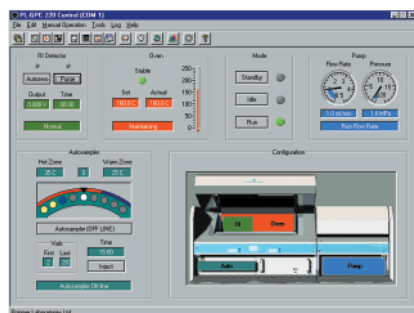


Figure 4. Software control of the PL-GPC 220

The time estimator calculates the amount of solvent you will need to run your samples. Input the day and time you want the system to start, then load your samples into the autosampler and let the PL-GPC 220 take care of the analysis for you.

The PL-GPC 220 is designed for true unattended operation. The system gradually heats to the analysis temperature, while the pump maintains a low flow of solvent through the column set. Once temperature is reached and stable, the pump ramps gradually to the flow rate required to run your sample. The PL-GPC 220 then automatically purges the RI detector and autozeros the baseline. Detector output is monitored and when stable, the autosampler loads and injects the first sample. Once the run sequence is complete, the flow rate automatically reduces to conserve solvent.

Integrated solvent delivery – safety by design

The solvent module in the PL-GPC 220 provides a safe, controlled environment in which solvent and waste are managed. Solvent handling is fully integrated and vented for operator safety, and the system does not need to be located in a fume hood.

The PL-GPC 220 includes an integral solvent degasser with a choice of solvent reservoir from 2 L bottles up to a 13 L stainless-steel tank. The solvent delivery module is thermostatically controlled to 30 °C, which ensures efficient, continuous and reproducible solvent delivery, even if the solvent is viscous or may be solid at near-ambient temperature (Figure 5).



Figure 5. Agilent PL-GPC 220 integrated solvent delivery system

Dual-zone-heated autosampler – no degradation of samples before injection

Agilent's innovative autosampler accommodates 39 samples in industry-standard 2 mL vials. Injection precision has been measured at better than 1% RSD with no cross contamination between samples, and without the need for rinse vials. The autosampler design features dual-zone heating to minimize thermal degradation. The warm and hot zones are independently programmable from ambient to 220 °C, and so the samples in the carousel waiting for injection are maintained at a lower holding temperature, then heated to analysis temperature prior to injection.

The vial is transferred to the column oven where the sample equilibrates before injection. This minimizes baseline disturbance and completely eliminates the risk of sample precipitation.

Agilent Cirrus GPC Software – the universal GPC solution

Cirrus is the powerful suite of GPC/multi-detector software from Agilent. Polymer Laboratories, now a part of Agilent, has been a supplier of industry-standard GPC software since the 1980s. Cirrus makes GPC calculations easy, whether in conventional GPC using a concentration detector or for multi-detector analysis with light scattering and viscosity.

Integration with existing LC software

Powerful, yet easy to use and learn, Cirrus is available for standalone GPC or for integrating GPC with LC. Cirrus utilizes the latest advances in software design to provide comprehensive calculation options, customized reporting, and high-resolution data capture with the Agilent PL DataStream.

Modular, flexible, and scalable

Cirrus is made to grow as your needs change. A suite of modules provides support for a variety of GPC techniques, such as multi-detector GPC, online FTIR detection and short-chain branching (SCB). Cirrus can be run on a standalone PC or provide a networked GPC solution.

Easy-to-use interface

Cirrus uses an intuitive graphical-user interface, so straightforward that new users can report results within an hour of installing the software. Cirrus is based on Agilent's Workbook concept to provide:

- A simple 'container' for data, parameters and results
- Automatic archiving of chromatograms, calibrations, and results
- Data traceability and data integrity
- Templates allowing predefinition of parameters and report content

Comprehensive calibration and calculation options

Cirrus offers a choice of calibration options.

- Conventional calibration using narrow standards
- Universal Calibration by viscometry or using Mark-Houwink coefficients
- Replicate entries of calibration points
- Three broad-standard calibration methods
- Averages and distributions can be calculated for any number of peaks in a chromatogram
- % of material can be reported for specific MW limits

A calibration overlay facility lets you view the effects of column performance over time.

Reviewing, collating, and condensing results

Cirrus meets the requirements of both QC/Routine and R&D environments, providing fully automated or interactive analysis. The software offers a number of powerful options to review, compare and extract information from archived data and results for inclusion into final reports.

Chromatograms and results can be reviewed both textually and graphically. This information can be exported in a variety of industry-standard formats. A powerful report designer provides total flexibility in report content and presentation. In Cirrus, all parameters relating to a chromatogram or results file are easily accessible via a comprehensive range of export options. Cirrus also ensures that data integrity and traceability are maintained throughout all operations.

Standards for column calibration in polyolefin analysis

Polymer standards from Agilent Technologies are the ideal reference materials for generating accurate, reliable GPC/SEC column calibrations, with the assurance of the ISO 9001:2000 quality standard. Additional applications for our highly characterized homopolymers exhibiting unique

characteristics are as model polymers for research and analytical method development. These quality polymer standards are supplied with extensive characterization that utilize a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign that all important peak molecular weight (Mp).

For polyolefin analysis, polyethylene and polystyrene standards are commonly employed. Agilent provides you with the widest choice of these materials to maximize your specific characterization needs. In addition, we supply other polymers as individual molecular weights, and broad distribution polymers for system validation or broad standard calibration procedures. A range of polymer standards available from Agilent are listed in Table 3.

Table 3. Standards selection guide

Polymer type	Individual Mw	Calibration kits	Agilent EasiCal	Agilent EasiVial	Type of GPC/SEC
Polystyrene	Yes	Yes	Yes	Yes	Organic
Polymethylmethacrylate	Yes	Yes		Yes	Organic
Polyethylene	Yes	Yes			Organic

Recommendations for setting up a GPC/SEC system for polyolefin analysis

The following questions will help you find the recommended columns and standards for any given application, as well as the system parameters such as injection volumes.

Choosing a column for GPC/SEC of polyolefins

Columns shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
1. What is the expected molecular weight?	High (up to several millions)	PLgel Olexis	PLgel Olexis is specifically designed for polyolefin analysis, offers optimal performance, also suitable for light scattering
<i>It may seem strange to ask this question, but in GPC/SEC the resolution of a column is related to the resolving range. Knowing something of the expected molecular weight of a sample helps to choose the best column that will give optimum results.</i>		PLgel 10 µm MIXED-B or PLgel 20 µm MIXED-A	The PLgel MIXED-A column resolves higher than the PLgel MIXED-B but at lower efficiency due to larger particle size
		PLgel MIXED-B LS or PLgel MIXED-A LS	Suitable for light scattering
	Intermediate (up to hundreds of thousands)	PLgel 5 µm MIXED-C or PLgel 5 µm MIXED-D	These PLgel columns are the most widely applicable for the majority of applications
	Low (up to tens of thousands)	PLgel 5 µm 500Å	The PLgel column provides high resolution and is designed for low-molecular-weight applications
	Very low (a few thousand)	PLgel 5 µm 100Å	The PLgel column gives high resolution at low Mw
	Unknown	PLgel Olexis	This PLgel column is designed for polyolefin analysis
2. How many columns to use?	Depends on the particle size of the columns	Particle size 20 µm, use 4 columns	Increased number of columns required for large particle sizes to make up for low efficiencies – PLgel Olexis is 13 µm
<i>The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution and the more columns are required to maintain the quality of the results. For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples during analysis.</i>		Particle size 13 µm, use 3 columns	
		Particle size 10 µm, use 3 columns	
		Particle size 5 µm, use 2 columns	
3. What standard is best?		Polystyrene (PS) or polyethylene (PE)	Polystyrene is the most commonly used standard in convenient EasiVial format, polyethylene is useful for generating PE based molecular weights
<i>Depending on analysis there are two options.</i>			

Columns for GPC analysis of polyolefins

Agilent produces a broad array of columns for the analysis of synthetic polymers and many of them are suitable for the analysis of polyolefins. However, the PLgel Olexis column is specifically designed for polyolefins with a wide range of molecular weights.

Agilent PLgel Olexis

PLgel Olexis is the optimum column choice for the analysis of very high-molecular-weight polymers such as polyolefins. Designed and manufactured specifically for these compounds, the column resolves up to 100,000,000 g/mol (polystyrene in THF). Packed with 13 μm particles for maximum resolution with minimal polymer shear, the columns also operate up to 220 $^{\circ}\text{C}$ for the analysis of highly crystalline materials. The column packing exhibits the excellent mechanical stability and robustness expected from the PLgel product range.

No shear degradation

The columns have a particle size of 13 μm , selected to give good efficiency in excess of 30,000 plates/m. In addition, the excellent size consistency of the particles (Figure 6) results in a very narrow particle size distribution that ensures no shear degradation.

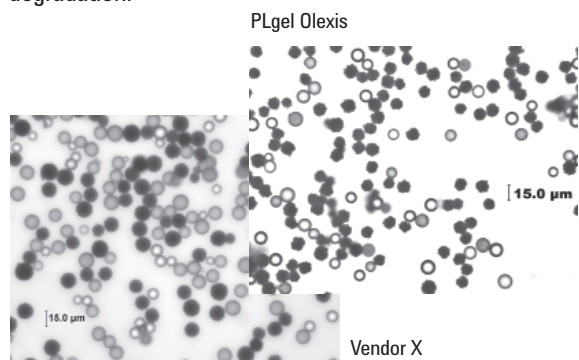


Figure 6. The superior size consistency of PLgel Olexis particles is clearly evident

High resolving range

Many new types of polyolefins have been developed recently with very high polydispersities. Determination of accurate polydispersities and modalities is critical in the research and development of these new polymers. PLgel Olexis completely satisfies this demand, for all polyolefin applications up to 100,000,000 g/mol.

Easy extrapolation

The large pore size of the particles makes them effective with many types of polyolefin. Linearity was introduced into the Agilent manufacturing process as a control criterion to ensure linear resolution across the operating range (Figure 7). The result is simplified extrapolation for calibrations.

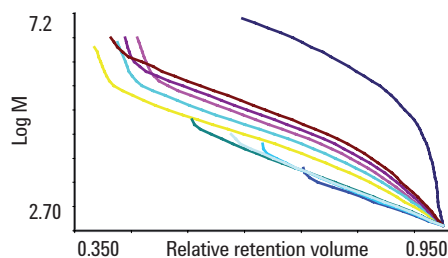


Figure 7. Some of the components of PLgel Olexis that contribute to its lack of artifacts

One column for all polyolefin applications

As the packing material in PLgel Olexis is an accurate blend of many components, smooth distributions are produced that truly reflect the sample composition (Figure 8). Dislocations are absent, so you can be sure that any unusual peak shapes represent the true nature of the sample and are not artifacts.

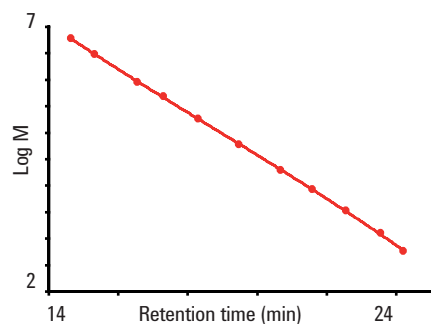


Figure 8. Careful blending delivers highly linear polystyrene calibrations with PLgel Olexis in TCB

The quality of the blending in PLgel Olexis columns means that polyolefins of very different polydispersity can be confidently analyzed on the same column set. Once again, PLgel Olexis provides trustworthy, clean and mono-modal peaks.



Polyolefin applications

The applications in this booklet illustrate the diversity of polyolefin samples, and reveal the flexibility of PLgel columns and the necessity for the PL-GPC 220 in addressing the analysis of such compounds.

Columns for high-molecular-weight polyolefins

Polyolefins range from low-molecular-weight hydrocarbon waxes to ultra-high-molecular-weight rigid plastics. The molecular weight distributions of polyolefins is directly related to physical properties such as toughness, melt viscosity and crystallinity. High-molecular-weight polyolefins tend to exhibit very broad molecular weight distribution (MWD). For such samples, small particles with small pore sizes are not desirable since shear degradation may occur, and so the high-pore-size particles of PLgel Olexis are recommended.

Conditions

Samples: Polyethylenes
Columns: 3 x PLgel Olexis,
300 x 7.5 mm (Part No. PL1110-6400)
Eluent: TCB + 0.015% BHT
Flow Rate: 1 mL/min
Inj Vol: 200 μ L
Temp: 160 $^{\circ}$ C
Detector: PL-GPC 220 (RI) + viscometer

Artifacts known as dislocations can arise in blended columns, resulting from a mismatch of the pore volume of components in the blend. Dislocations lead to false modalities and polydispersities. Avoiding dislocations was an integral part of the design brief for PLgel Olexis columns. Accurate blending of these components produces a column that gives a smooth molecular weight distribution, providing a true reflection of the shape of the MWD (Figure 9). PLgel Olexis is perfect for studies that require accurate polydispersity index and modality information.

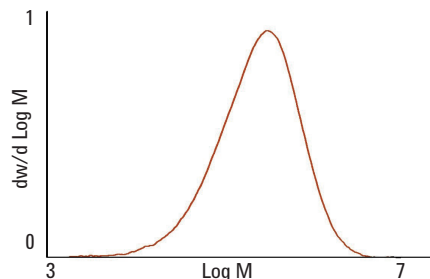


Figure 9. True representation of polyolefin molecular weight distribution with PLgel Olexis

Figure 10 shows a range of polyolefin samples analyzed on a PLgel Olexis column, covering the spread of molecular weights. There are no dislocations and the peak shape of the very broad samples shows true sample modality.

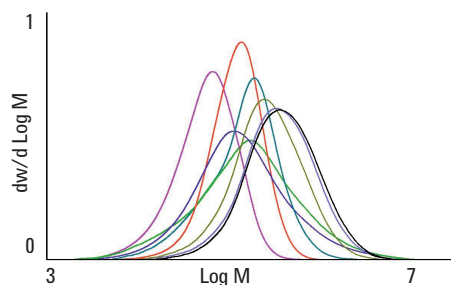


Figure 10. PLgel Olexis reveals true modalities across the range of polyolefins

Given the accurate resolving power of PLgel Olexis you can be sure that unusual peak shapes are real and not artifacts; unusual peak shapes of some samples will be true reflections of their modality. This is important for studies into reaction mechanisms and catalyst behavior (Figure 11).

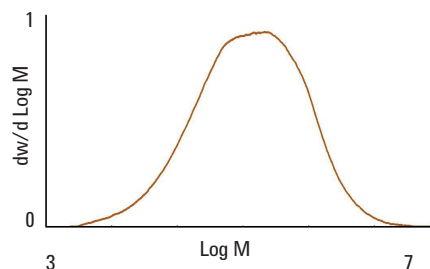


Figure 11. A true change in peak shape revealed by PLgel Olexis of a multi-modal material manufactured from a multi-site catalyst

Columns for lower-molecular-weight polyolefins

Crude oil, or petroleum, is the main source of organic chemicals for industry. The major chemicals are derived from two constituents of oil, xylene and naphtha. These raw materials are then broken down into more basic products, e.g. polyethylene, polypropylene, elastomers, asphalts and liquid hydrocarbons. Characterization of such products is commonly achieved using GPC. This involves a liquid chromatographic separation from which a molecular weight distribution calculation can be made following calibration of the system with suitable polymer standards. The diversity of petroleum products demands a variety of GPC column types for optimized analysis. Low-molecular-weight liquid hydrocarbons require high resolution of individual components. This is illustrated in Figure 12, where three linear hydrocarbons are resolved easily to base-line in a reasonably short analysis time.

Conditions

Samples: Linear hydrocarbons
Columns: 2 x Agilent PLgel 5 μ m 100Å,
300 x 7.5 mm (Part No. PL1110-6520)
Eluent: TCB
Flow Rate: 1 mL/min
Temp: 145 °C
Detector: PL-GPC 220

Peak Identification

1. C₃₆ H₆₄
2. C₂₂ H₄₆
3. C₁₄ H₃₀

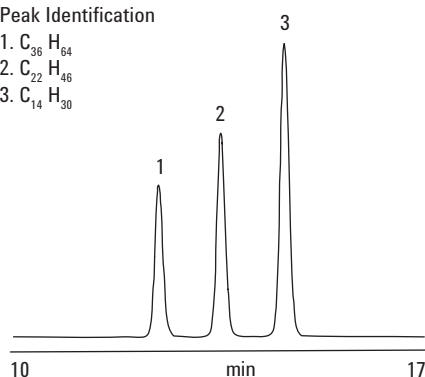


Figure 12. Linear hydrocarbons separated to base-line on a PLgel column set

Figure 13 shows the separation of a selection of low-molecular-weight linear hydrocarbons.

Conditions

Samples: Linear hydrocarbons
Columns: 2 x Agilent PLgel 3 μ m 100Å,
300 x 7.5 mm (Part No. PL1110-6320)
Eluent: TCB
Flow Rate: 0.8 mL/min
Inj Vol: 20 μ L
Temp: 145 °C
Detector: PL-GPC 220

Peak Identification

1. C₃₆
2. C₂₄
3. C₂₀
4. C₁₆
5. C₁₂

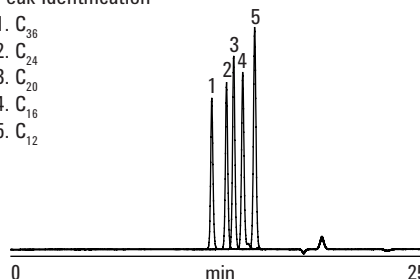


Figure 13. Separation of low-molecular-weight hydrocarbons

The PLgel 100Å columns have a GPC exclusion limit of 4,000 molecular weight (polystyrene equivalent). Intermediate products can be analyzed using the PLgel MIXED-D column that has a linear molecular weight resolving range up to an exclusion limit of around 400,000 molecular weight. The 5 μ m particle size maintains high column efficiency and thus fewer columns are required and analysis time is relatively short.

Figure 14 shows a chromatogram of a relatively low-molecular-weight hydrocarbon wax obtained on PLgel 5 µm MIXED-D columns.

Conditions

Samples: Linear hydrocarbons
 Columns: 2 x Agilent PLgel 5 µm MIXED-D,
 300 x 7.5 mm (Part No. PL1110-6504)
 Eluent: TCB
 Flow Rate: 1 mL/min
 Inj Vol: 200 µL
 Temp: 160 °C
 Detector: PL-GPC 220

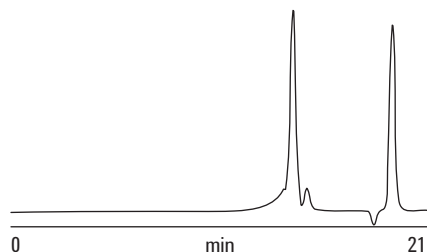


Figure 14. A low-molecular-weight wax

Figure 15 shows the analysis of an asphalt used in road surfacing. Subsequently derived information regarding the molecular weight distribution of such materials is invaluable in determining their processibility and final properties.

Conditions

Columns: 2 x PLgel 5 µm MIXED-D,
 300 x 7.5 mm (Part No. PL1110-6504)
 Eluent: THF
 Flow Rate: 1 mL/min
 Temp: 40 °C
 Detector: RI

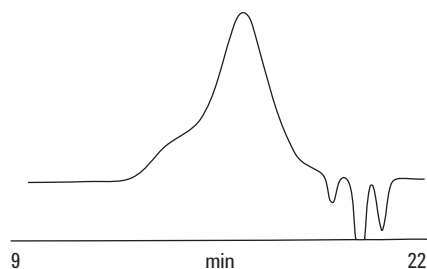


Figure 15. Fast analysis of asphalt on PLgel 5 µm MIXED-D columns

Repeatability study 1

A commercial sample of high-density polyethylene (HDPE) was prepared at 2 mg/mL using the PL-SP 260VS Sample Preparation System, with a dissolution temperature of 160 °C and a dissolution time of two hours. Eight aliquots of the master batch solution were dispensed into PL-GPC 220 autosampler vials and placed in the autosampler carousel of the PL-GPC 220 where the hot zone temperature was 160 °C and the warm zone 80 °C (Figure 16).

Conditions

Columns: 3 x PLgel 10 µm MIXED-B,
 300 x 7.5 mm (Part No. PL1110-6100)
 Eluent: TCB + 0.0125% BHT
 Flow Rate: 1 mL/min
 Inj Vol: 200 µL
 Temp: 160 °C
 Detector: PL-GPC 220

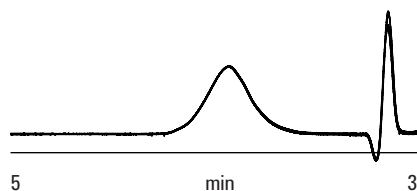


Figure 16. Overlay of the raw data chromatograms obtained for eight consecutive injections of HDPE

The data were analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene equivalent molecular weight averages that are shown in Table 4.

Polystyrene in TCB¹ $K = 12.1 \times 10^{-5}$ $a = 0.707$

Polyethylene in TCB² $K = 40.6 \times 10^{-5}$ $a = 0.725$

Table 4. Summary of results from eight injections of HDPE

Injection number	Mn	Mp	Mw
1	17,289	76,818	333,851
2	16,988	77,434	335,496
3	17,428	77,514	332,616
4	17,521	77,052	335,635
5	17,348	76,520	334,212
6	17,487	77,728	333,511
7	16,898	77,578	335,642
8	17,457	77,288	334,923
Mean	17,302	77,241	334,485
Std Dev	220	387	1,048
% Variation	1.3	0.5	0.3

Figure 17 shows an overlay of the molecular weight distribution calculated for the eight consecutive injections of the HDPE sample, and illustrates the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 μ m MIXED-B columns.

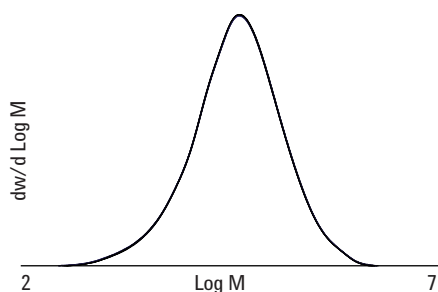


Figure 17. Molecular weight overlay of eight consecutive injections of HDPE

Repeatability study 2

A commercial sample of high-density polypropylene (HDPP) was prepared at 1.5 mg/mL using the PL-SP 260VS Sample Preparation System with a dissolution temperature of 160 °C and a dissolution time of two hours. Six aliquots of the master batch solution were dispensed into PL-GPC 220 autosampler vials and placed in the carousel where the hot zone temperature was 160 °C and the warm zone 80 °C.

Figure 18 shows an overlay of the raw data chromatograms obtained for six consecutive injections of the sample.

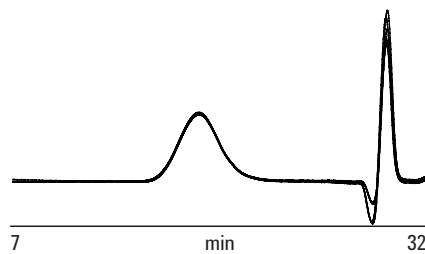


Figure 18. Overlay of the raw data chromatograms obtained for six consecutive injections of HDPP

The data were analyzed against a polystyrene standards calibration using the following Mark-Houwink parameters to obtain the polypropylene-equivalent molecular weight averages that are shown in Table 5.

Polystyrene in TCB¹ $K = 12.1 \times 10^{-5} \alpha = 0.707$

Polypropylene in TCB² $K = 19.0 \times 10^{-5} \alpha = 0.725$

Table 5. Overlay of the raw data chromatograms obtained for six consecutive injections of HDPP

Injection number	Mp	Mn	Mw
1	127,132	65,086	185,795
2	131,893	65,089	185,236
3	128,673	66,802	186,202
4	132,062	67,417	188,048
5	131,625	69,320	188,679
6	130,227	69,677	186,188
Mean	130,202	67,232	186,691
Std Dev	1,693	1,815	1,239
% Variation	0.13	2.70	0.66

Conditions

Columns: 3 x PLgel 10 μ m MIXED-B,
300 x 7.5 mm (Part No. PL1110-6100)
Eluent: TCB + 0.0125 BHT
Flow Rate: 1 mL/min
Inj Vol: 200 μ L
Temp: 160 $^{\circ}$ C
Detector: PL-GPC 220

Figure 19 shows an overlay of the molecular weight distribution calculated for the six consecutive injections of the HDPP sample that illustrates the excellent repeatability obtained with the PL-GPC 220 using PLgel 10 μ m MIXED-B columns.

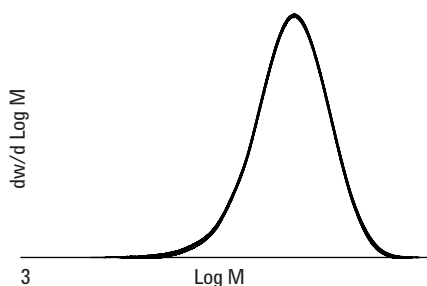


Figure 19. Molecular weight overlay of six consecutive injections of HDPP

References

- ¹ H. Coll and D. K. Gilding (1970) Universal calibration in GPC: a study of polystyrene, poly- α -methylstyrene, and polypropylene. *Journal of Polymer Science Part A-2: Polymer Physics*, 8, 89-103.
- ² T. G. Scholte, N. L. J. Meijerink, H. M. Schoffeleers and A.M.G. Brands (1984) Mark-Houwink equation and GPC calibration for linear short chain branched polyolefins, including polypropylene and ethylene-propylene copolymers. *Journal of Applied Polymer Science*, 29, 3763.

Specialist detectors

Multi-detector options for polyolefin analysis

Conventional GPC employs a refractive index or other concentration detector. However, polyolefins can be analyzed by multi-detector GPC that combines a concentration detector with a viscometer, a static light scattering detector, or both.

GPC viscometry – analysis using a concentration detector and viscometer

A viscometer may be housed inside the oven of the PL-GPC 220 to allow analysis of polyolefins by GPC viscometry. Using GPC viscometry, molecular weights are determined using the Universal Calibration method. A plot of molecular size as $\log(\text{molecular weight} \times \text{intrinsic viscosity})$ versus retention time is constructed for a series of narrow standards, based on the relationships in Equations 1 and 2.

Equation 1:

Hydrodynamic volume $\propto$ molecular weight $\times$ intrinsic viscosity

Equation 2:

$\log(\text{MW} \times \text{intrinsic viscosity}) \text{ versus retention time} \approx \log(\text{hydrodynamic volume}) \text{ versus retention time}$

PLgel Olexis columns are separated and calibrated in terms of size and so a Universal Calibration is obtained (Figure 20).

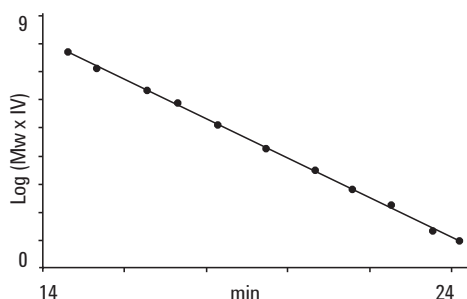


Figure 20. Multi-detector GPC Universal Calibration of a PLgel Olexis column

The Universal Calibration technique gives polyolefin molecular weights regardless of the calibrants used in the analysis. This allows cheaper calibrants such as polystyrene to be used while still providing accurate polyolefin results.

- Intrinsic viscosities are measured from the viscometer and concentration detector
- Accurate molecular weights are calculated assuming that the sample obeys the Universal Calibration (pure size exclusion is obtained)
- Radius of gyration is calculated using a model for the polymer behavior in solution

GPC light scattering – analysis employing a concentration detector and a light scattering detector

A dual-angle light scattering detector can be sited inside the oven of the PL-GPC 220 to allow analysis of polyolefins by GPC light scattering, employing the dissymmetry method. In GPC light scattering, accurate molecular weights are determined directly by using the response of the light scattering detector and the intensity of scattered light, as described in Equation 3.

Equation 3:

$$R_{\theta} = CM (dn/dc)^2 P_{\theta} K_{\theta}$$

R_{θ} is the detector response, CM is concentration $\times$ mass, dn/dc is the specific refractive index increment, P_{θ} is the particle scattering function and K_{θ} is the light scattering constant.

- Molecular weights are calculated directly from the light scattering response, calculating the particle scattering function from the ratio of intensities at 15° and 90°
- Radius of gyrations are determined from the particle scattering function by comparison of the two angles, but only if the molecule is over about 10 nm in size and the scattering intensity shows angular dependence
- Intrinsic viscosity is calculated using a model for the polymer behavior in solution

GPC triple detection – analysis using concentration, viscometry and light scattering data

In this technique, both a viscometer and a dual-angle light scattering detector are housed inside the PL-GPC 220. With GPC triple detection, molecular weights are determined directly using the response of the light scattering detector as described above.

- Molecular weights are calculated directly from the light scattering response, calculating the particle scattering function from the ratio of intensities at 15 ° and 90 °
- Radius of gyration is determined from the particle scattering function by comparison of the two angles but only if the molecule is over about 10 nm in size and the scattering intensity shows angular dependence
- Intrinsic viscosity is calculated from the viscometer trace

Comparisons between conventional GPC, GPC viscometry, GPC light scattering and GPC triple detection

Conventional GPC using only a concentration detector generates molecular weights on the basis of comparison to a series of calibration standards. However, unless the standards and samples are of the same chemistry and therefore same size in solution at any given molecular weight, the results are only relative as the GPC column separates on the basis of size not molecular weight. Conventional GPC only gives accurate results if standards of the same chemistry as the samples under investigation are used.

GPC viscometry and GPC light scattering, or GPC triple detection, can be used to determine 'absolute' molecular weights of samples, independent of the chemistry of standards used in the column calibration (GPC viscometry) or independent of column calibration entirely (GPC light scattering and GPC triple detection).

The values of molecular weight can vary between these techniques because the viscometer and light scattering detectors respond to different properties of the polymer, the viscometer to molecular density, and the light scattering detector to size in solution. Therefore, molecular weights calculated by these approaches will not necessarily have the same values.

Branching

Comparing long-chain branching in polyethylenes

Multi-detector GPC combined with branching calculations is an excellent way of comparing and identifying different kinds of polyethylene. These different materials, although of the same basic chemical structure, differ in their mode of manufacture and have very different physical properties.

LDPE – low-density polyethylene

Low-density polyethylene was the first grade of polyethylene manufactured in the 1930s. It exhibits relatively low crystallinity compared to other forms of polyethylene due to the presence of long branches on the polymer backbone (on about 2% of the carbon atoms). As a result, the tensile strength of the material is lower while resilience is higher. These long-chain branches are a result of ‘backbiting’ reactions in the synthetic processes used to manufacture the material. Multi-detector GPC can measure the level of branching in LDPE.

HDPE – high-density polyethylene

High-density polyethylene is manufactured using different catalysts than those used for LDPE, selected to give very low levels of branching from the backbone. HDPE therefore has higher density and crystallinity than LDPE, resulting in a tougher, more temperature-stable product. HDPE does not display long-chain branching.

LLDPE – linear low-density polyethylene

Linear low-density polyethylene is a newer material manufactured by incorporation of small quantities of alpha-olefins such as butane, hexane or octene into the polymer. LLDPE materials are more crystalline than LDPE, but are elastomeric and have a higher tensile strength and puncture resistance. Multi-detector GPC employing a viscometer and/or light scattering detector cannot be used to investigate the branching in LLDPE as changes in the density and size of the molecules compared to linear materials are very small and cannot be detected. GPC-FTIR is employed for short-chain branching analysis, as discussed on page 24.

Investigating branching in polyolefins

In multi-detector GPC, branching is assessed by investigating changes in molecular size or intrinsic viscosity as a function of increasing molecular weight. In all cases for polymers of the same chemistry, branched molecules always have lower Rg and IV values than linear analogs due to the presence of branch points.

In all methods, branching calculations can be performed on either the intrinsic viscosity (measured or calculated) or radius of gyration (measured or calculated) data. The quality of the branching results will depend on the quality of the source data (intrinsic viscosity or radius of gyration). Contraction factors are determined from the Mark-Houwink (log intrinsic viscosity versus log MW) or conformation (log radius of gyration versus log MW) plots using the relationships in Equation 4.

Equation 4:

Radius of gyration contraction factor

$$g = \left(\frac{Rg \text{ branched}}{Rg \text{ linear}} \right) MW$$

Intrinsic viscosity contraction factor

$$g' = \left(\frac{IV \text{ branched}}{IV \text{ linear}} \right) MW$$

where $g = g'^{(1/\epsilon)}$

ϵ (structure factor) = 0.5 to 1.5, typically 0.75

The value of g (directly or taken from the value of g' and an estimation of the structure factor, typically 0.75) is used along with the branching repeat unit (the molecular weight of the monomer multiplied by 1,000) to obtain branching numbers using a branching model. In the absence of structural data for the sample, a number-average ternary-branching model is used as shown in Equation 5.

Equation 5:

$$g = [(1 + B_n/7)^{1/2} + 4B_n/9\pi]^{-1/2}$$

where B_n = branches per 1,000 carbons

Branching numbers are expressed as number of branches per 1,000 carbons (from polyethylene investigations). If the polymer in question is not polyethylene then the actual branching number may not be directly meaningful. However, comparison between samples is still possible.

Analysis of branching in polyethylenes

Samples of LDPE, HDPE and LLDPE were analyzed with the PL-GPC 220 by triple detection.

Conditions

Columns: 3 x PLgel Olexis,
300 x 7.5 mm (Part No. PL1110-6400)
Eluent: TCB + 0.015% BHT
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Temp: 160 $^{\circ}$ C
Detector: PL-GPC 220 (RI) + viscometer + dual-angle light scattering

Refractive index, dual-angle light scattering and viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software. A polystyrene standard was used to generate the detector constants for the triple detection analysis.

Figure 21 shows the molecular weight distributions for the three samples. Although there was some overlap, the samples clearly had significantly different molecular weights.

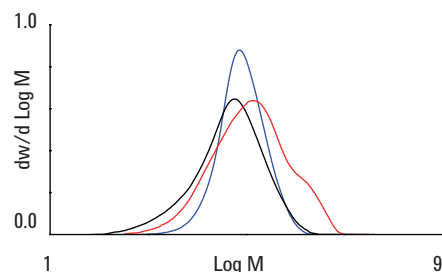


Figure 21. Overlaid molecular weight distributions for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red

Figure 22 shows the Mark-Houwink plots for the three samples using intrinsic viscosities generated from the viscometer and molecular weights from the light scattering detector.

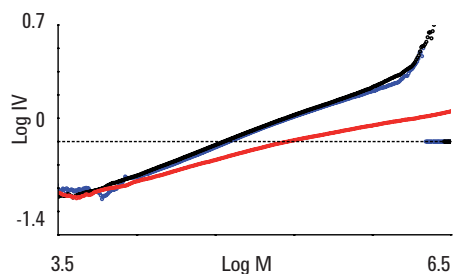


Figure 22. Overlaid Mark-Houwink plots for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red

The Mark-Houwink plot describes the change in the viscosity of the polymers as a function of increasing molecular weight. The HDPE and LLDPE samples overlay on the Mark-Houwink plot, indicating that the polymers have very similar structures. The Mark-Houwink parameters K (the intercept) and alpha (the slope) indicate that the materials contain no branching that can be detected by multi-detector GPC. However, the LDPE shows a clear deviation from the HDPE and LLDPE lines, with a decreasing slope as molecular weight increases. This is due to increased branching of the LDPE compared to the other materials as molecular weight increases lead to a reduction in viscosity.

Branching analysis of polyethylenes with Cirrus GPC Multi Detector Software

The presence of long-chain branching (over six carbons in length) in polyolefins strongly influences physical properties such as melt viscosity and mechanical strength. The distribution chain branches in polyolefins are determined by the polymerization mechanism and there is significant interest in the production of materials with well-defined and characterized molecular weight and branching distributions for specific applications.

Three samples of polyethylene, one HDPE and two LDPE, were analyzed using the PL-GPC 220 by GPC/viscometry. Two of the samples had been synthesized by a mechanism to promote branching, while the third was a standard linear reference material, NBS 1475.

Refractive index viscometry detectors were employed and the data was analyzed with Cirrus GPC Multi Detector Software using the Universal Calibration approach. Polystyrene standards were used to generate the Universal Calibration and the unbranched sample was used as a linear model in the determination of branching.

Figure 23 shows the molecular weight distributions for the three samples. The black plot is for the unbranched sample. Although there was some overlap, the samples clearly had significantly different molecular weights.

Figure 24 shows the Mark-Houwink plots for the three samples. The upper-most sample is the unbranched material. The other two samples have lower intrinsic viscosities at any given molecular weight, with the unbranched polymer indicating the presence of branching. This can be expressed in terms of g , the branching ratio, defined in Equation 6, where ϵ is a constant.

Equation 6:

$$g = \left(\frac{IV \text{ branched}}{IV \text{ linear}} \right)^{1/\epsilon}$$

Conditions

Samples: Polyethylenes
Columns: 3 x PLgel Olexis,
300 x 7.5 mm (Part No. PL1110-6400)
Eluent: TCB + 0.015% BHT
Flow Rate: 1.0 mL/min
Inj Vol: 200 μ L
Temp: 160 $^{\circ}$ C
Detector: PL-GPC 220 (RI) + viscometer

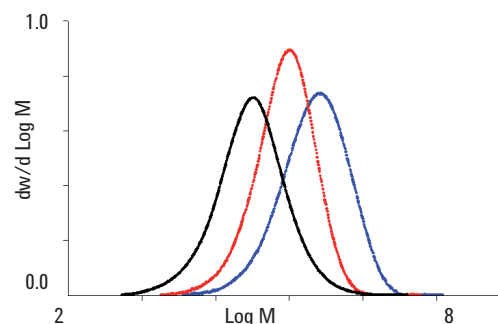


Figure 23. Molecular weight distribution plots for three polyethylene samples – the black plot is the unbranched sample

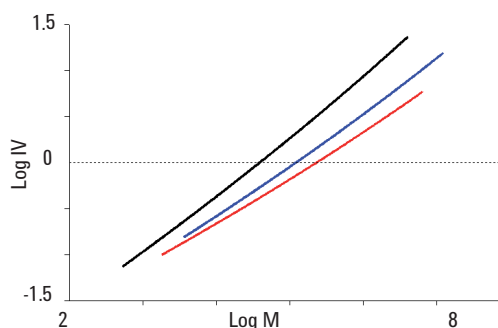


Figure 24. Mark-Houwink plots for three samples of polyethylene

The unbranched sample was used as the linear model and so gives a g value of unity (except at high molecular weight due to scatter in the data). The other two samples both exhibit a decrease in g as a function of molecular weight, indicating that as molecular weight increases the number of branches also increases. Based on these calculated g values, a branching number or number of branches per 1,000 carbon atoms can be generated. This is achieved by fitting the data into a model. The Cirrus GPC Multi Detector Software offers a selection of branching models that can be employed in this approach. In this case a model was used that calculates a number-average branching number assuming a random distribution of branches on the polymer. Figures 25 and 26 show the g plots and branching number plots obtained for the samples.

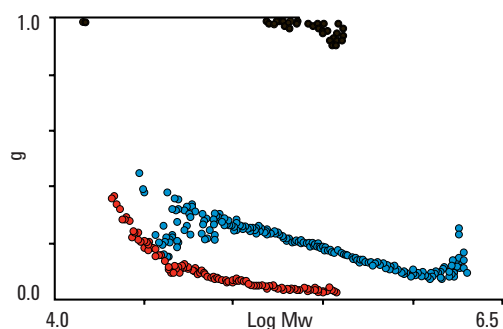


Figure 25. Branching ratio g plots for three polyethylene samples – the black plot is the unbranched sample

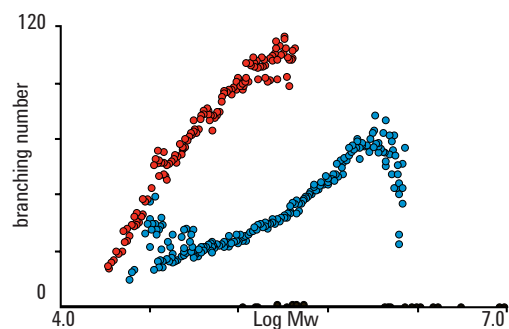


Figure 26. Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample

The results show that of the two, branched samples, the trend in molecular weight distribution does not follow the trend in branching distribution. The sample showing the most branching at any given molecular weight has a lower molecular weight than the second sample. Clearly, understanding both the molecular weight and branching distributions will give an insight into the processability of the two materials.

Analysis of branching in linear low-density polyethylene (LLDPE)

Fourier transform-infrared (FTIR) spectroscopy is a well-established technique used in compositional analysis of materials through the measurement of vibrational absorption bands. Polymers typically exhibit relatively simple absorption spectra, allowing them to be readily identified by comparison to library data and are therefore well suited to analysis by FTIR. Coupling FTIR detection with gel permeation chromatography is particularly advantageous as FTIR detection can be utilized as both concentration detector for molecular weight calculations and as a spectroscopic tool for compositional analysis, significantly enhancing the information available from a single GPC experiment.

Coupling a PL-GPC 220 system to one of the range of Agilent's FTIR spectrometers can be achieved using the PL-HTGPC-FTIR interface, which consists of a heated flow cell, a heated transfer line, and a temperature control box. The flow cell and transfer line can be heated up to 175 °C with an accuracy of ± 0.5 °C for polyolefin applications. To obtain good quality spectra, the FTIR spectrometer is fitted with a fast MCT (mercury-cadmium-telluride) detector. Data acquisition is performed through the spectrometer's time-resolved data-acquisition software.

GPC/FTIR analysis of polyethylene

Highly crystalline polyethylene is difficult to analyze by GPC due to its limited solubility in most organic solvents, and the high temperatures required for dissolution (typically over 135 °C). Trichlorobenzene (TCB) is the most commonly used solvent for these materials. TCB is also a suitable solvent for GPC analysis with FTIR detection as the solvent has a good absorption window between about 3,500 and 2,700 cm^{-1} , which corresponds to the $>\text{C-H}$ stretching region. CH vibrations dominate the solid-state spectra of polyethylene and so this absorption region is of key importance.

Focusing on the $>\text{C-H}$ stretching region, differences in the proportions of $>\text{CH}_2$ and $-\text{CH}_3$ groups in a sample can be seen in the relative intensities of the absorption bands. This dependence of the infrared spectra on the presence of $-\text{CH}_3$ and $>\text{CH}_2$ groups can be used to measure the level of short-chain branching (SCB) in polyethylene¹. These are branches less than six carbons long introduced by co-polymerization of ethylene with other alpha-olefins that cannot be detected by traditional multi-detector GPC experiments, as they do not affect the viscosity of the polymer. The level of SCB does, however, strongly influence crystallinity, density, and stress-crack resistance of polyethylene. By measuring the spectra of polyethylene containing SCB, the relative intensities of the stretching vibrations due to $-\text{CH}_3$ and $>\text{CH}_2$ groups can be measured and, providing that the monomers used to introduce SCB are known, the level of SCB can be estimated using chemometrics. Coupling the detector to a GPC system allows the SCB to be assessed (as a function of molecular weight).

Analysis of an ethylene-hexene copolymer by GPC/FTIR

A sample of ethylene co-polymerized with hexene was analyzed using the PL-GPC 220 coupled to an Agilent FTIR to assess the levels of short-chain branching.

Conditions

Column:	2 x PLgel Olexis, 300 x 7.5 mm (Part No. PL1110-6400)
Eluent:	Trichlorobenzene (with BHT)
Inj Vol:	200 μL
Flow Rate:	1.0 mL/min
Temp:	160 °C
Data Collection:	Time-resolved Agilent Resolutions Pro software collecting at 8.0 cm^{-1} resolution with 16 scan accumulations for 11 minutes, range 3,500 – 2,700 cm^{-1} with automatic solvent background subtraction
Detection:	Agilent PL-HTGPC-FTIR interfaced to an Agilent FTIR spectrometer fitted with an MCT detector

Cirrus GPC-FTIR SCB software was used to perform the experiments, calculating SCB based on a rigorous chemometrics approach. To determine molecular weight, the FTIR data was used as a concentration source for the generation of Figure 27, showing an overlay of the polymer weight and short-chain branching distribution obtained for a copolymer of ethylene and another alpha-olefin by FTIR. Clearly, in this case the level of co-monomer incorporation was uniform across the distribution.

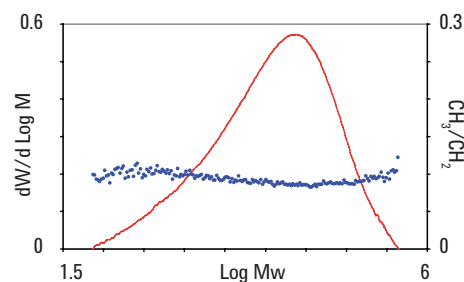


Figure 27. Overlaid chromatogram of polymer weight and short-chain branching distribution for a sample of ethylene-hexene copolymer

Reference

¹ P.J. DesLauriers, D.C. Rohlffing and E.T. Shieh (2002) Quantifying short chain branching microstructures in ethylene-1-olefin copolymers using size exclusion chromatography and Fourier transform infrared spectroscopy (SEC-FTIR). *Polymer*, 43, 159-170.

Ordering Information

Columns	
Description	Part No.
Agilent PLgel 3 µm 100Å, 300 x 7.5 mm	PL1110-6320
Agilent PLgel 5 µm 100Å, 300 x 7.5 mm	PL1110-6520
Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm	PL1110-6504
Agilent PLgel 10 µm MIXED-B, 300 x 7.5 mm	PL1110-6100
PLgel 10 µm MIXED-B LS, 300 x 7.5 mm	PL1110-6100LS*
PLgel 20 µm MIXED-A, 300 x 7.5 mm	PL1110-6200
PLgel 20 µm MIXED-A LS, 300 x 7.5 mm	PL1110-6200LS*
Agilent PLgel Olexis, 300 x 7.5 mm	PL1110-6400

Standards	
Description	Part No.
Agilent PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	PL2010-0301
Agilent E-M-10 polyethylene calibration kit, 10 x 0.2 g	PL2650-0101
Agilent E-MW-10 polyethylene calibration kit, 10 x 0.1 g	PL2650-0102
Agilent E-SCB polyethylene short-chain branching calibration kit, 10 x 0.1 g	PL2650-0103

Instruments	
Description	Part No.
Agilent PL-SP 260VS Sample Preparation System**	
Agilent PL-GPC 220 Integrated GPC/SEC System	PL0820-0000
Agilent PL-HTGPC-FTIR**	
Agilent PL-BV 400HT Online Integrated Viscometer	PL0810-3050
Agilent PL-HTLS 15/90 Light Scattering Detector	PL0640-1200
Agilent custom accessory kit**	

Software	
Description	Part No.
Agilent Cirrus GPC Multi Detector Software	PL0570-2020
Agilent Cirrus GPC Software	PL0570-2000
Agilent GPC-FTIR SCB Software	PL0570-2300

* Low shedding for light scattering applications

** Contact your local sales office or distributor for different options

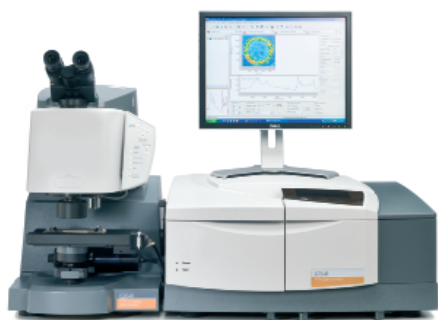
More Agilent solutions for polyolefin analysis

As well as high-temperature GPC, Agilent offers other solutions for the analysis of polyolefins.

FTIR

Fourier transform-infrared spectroscopy is an essential tool in the analysis of polymer films and other materials. Applications range from quality testing of raw materials to failure analysis of large objects. Our solutions feature Agilent's high performing 600-IR Series spectrometers and microscopes, software and accessories.

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The Agilent 600-IR series provides the highest level of sensitivity combined with detailed structural and compositional information for information-rich detection

NMR

Agilent NMR has long been an effective tool for the characterization of polymers. 1D and 2D NMR methods have been routinely used for many years. A more advanced method developed at Agilent uses pulsed-field gradient-heteronuclear multiple-bond correlation with 2D NMR to detect weak signals in the presence of much larger resonances. This technique permits assignment of signals from minor structures such as chain ends and defects, essential information for a full understanding of these complex synthetic compounds.

The Agilent 400-MR provides unmatched productivity for diverse chemical applications by combining easy-to-use software with the outstanding performance of DirectDrive and DirectDigital spectrometer architecture. Push-button experiments, along with straightforward processing and data export capabilities, make the 400-MR the best choice for compound detection, quantification and structure confirmation.

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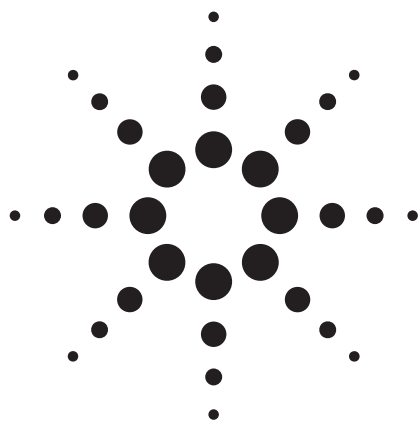
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GC Analysis of Sulfur Components in Propylene using a Sulfur Chemiluminescence Detector

Application Note

Hydrocarbon Processing

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Abstract

The Agilent J&W Select Low Sulfur column measures trace levels of target components in C3 hydrocarbon streams without any matrix interference.

Introduction

Hydrogen sulfide (H_2S), carbonyl sulfide (COS), and methyl mercaptan (CH_3SH) are common components in light hydrocarbon streams. They have corrosive and toxic properties, causing damage to pipes and equipment. The emission of undesired odors caused by volatile sulfur compounds in intermediates and final products have serious economic and environmental impact. In addition, the presence of sulfur can affect the performance of industrial processes, causing undesired chemical reactions, loss of catalyst activity (catalyst poisoning), and ultimately lower yield.

These sulfur components must be quantified at low ppb levels. They can be measured with sulfur specific detection devices such as the Sulfur Chemiluminescence Detector (SCD) but large sample volumes are needed to reach the desired low parts per billion (ppb) detection limits. This creates matrix overload and quenching effects (decreased signal/sensitivity due to background interferences) on most sulfur specific detectors, limiting the detector's sensitivity and linearity and raising quantification limits. The capillary PLOT column, Agilent J&W Select Low Sulfur column, with a novel stationary phase was developed for the analysis of sulfur species such as H_2S , COS and CH_3SH in light hydrocarbon C3 matrices, with high loadability properties and unique selectivity giving baseline resolution for sulfur components and matrix components.



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Experimental

Technique:	GC-SCD
Column:	Agilent J&W Select Low Sulfur, 60 m × 0.32 mm (p/n CP8575)
Oven:	65 °C for 4 minutes, 30 °C/min to 120 °C for 5 minutes
Carrier gas:	Helium, constant flow, 2.0 mL/min
Injector:	200 °C, split 1:10
Detector:	SCD, 200 °C
Sample:	Propylene matrix containing ~300 ppb H ₂ S and CH ₃ SH, ~500 ppb COS
Injection volume:	1 mL
Injection:	Gas sampling valve

Results and Discussion

The stationary phase shows good selectivity between H₂S, COS and low mercaptans in various C3 hydrocarbon matrices. Therefore co-elution of the sulfur components and the matrix, which causes “quenching”, is avoided.

The system was equipped with a gas sampling valve. The gas sampling valve event table is shown in Table 1. The detector settings are shown in Table 2.

Table 1. Gas Sampling Valve Event Table

Time (min)	Gas sampling valve
Initial	Fill
0.01	Inject
1.00	Fill

Table 2. Detector SCD Settings

SCD settings	
Burner temperature	800 °C
Vacuum of burner	370 torr
Reactor hydrogen flow	40 mL/min
Reactor air flow	65 mL/min
Attenuation	1
Ozone air pressure	5 psig

Figure 1 shows a chromatogram of sulfur compounds H₂S, COS and CH₃SH in a propylene matrix. Methyl mercaptan shows peak broadening from column overloading by the large amount of propylene matrix. The propylene matrix elutes between COS and methyl mercaptan.

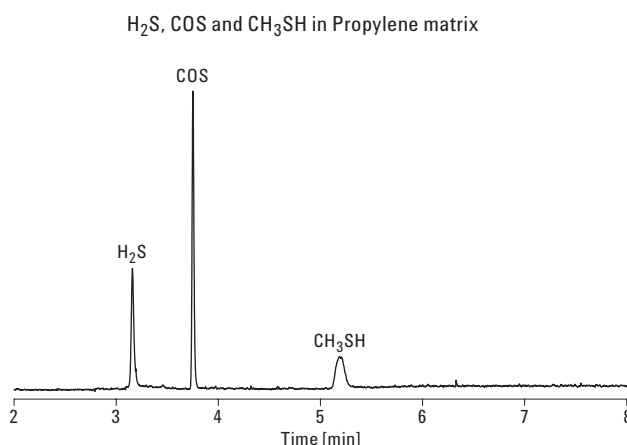


Figure 1. Chromatogram of H₂S, COS and CH₃SH in Propylene matrix, using the Agilent J&W Select Low Sulfur with GC-SCD.

Conclusion

The Agilent J&W Select Low Sulfur used in a GC with a sulfur specific detector, such as an SCD, can detect H₂S, COS and CH₃SH at trace level in a propylene matrix as a result of excellent separation of the sulfur compounds and the matrix. Separating the matrix from the sulfur compounds eliminates the “quenching” effects caused by the matrix. This provides a better response for the sulfur compounds. The column provides a good response for reactive sulfur compounds, such as H₂S making detections of 20 ppb possible.

Although this is a PLOT column, no spikes will be observed because the column does not shed particles. It can therefore be used safely in combination with switching valves.

References

1. W. Wardencki (1998) Review “Problems with the determination of environmental sulphur compounds by gas chromatography.” J. Chromatog. A. 793: 1-19.
2. Roger L. Firor and Bruce D. Quimby, “Comparison of Sulfur Selective Detectors for Low-Level Analysis in Gaseous Streams,” Agilent Technologies publication 5988-2426EN.

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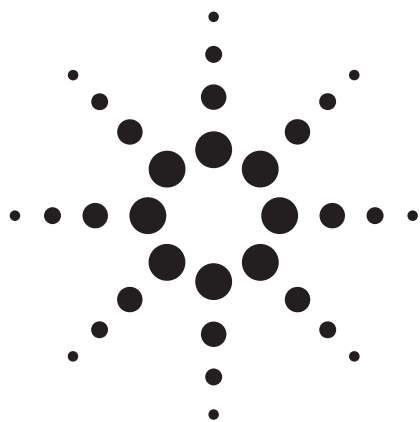
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Agilent Technologies



Gel Permeation Chromatography - Keep the Method but Change the Column

Application Note

Materials Testing and Research, Polymers

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Introduction

Chromatographers can be wary of using a column of different dimensions because they fear it could disrupt a standard method. However, an alternative column may offer significant performance or economic advantages that may outweigh any changes that result from the move to a new in column dimension. This note shows how the Agilent PLgel 5 μm gel permeation chromatography column matches the performance of another leading column, even though it has slightly different dimensions. Polycarbonate is used as the test analyte to demonstrate the indistinguishable performance of the columns.



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Conditions

Columns	Agilent PLgel 10 ⁴ Å, 300 x 7.5 mm, 5 µm (p/n PL1110-6540) Competitor 300 x 7.7 mm, 5 µm
Eluent	THF
Flow Rate	1.0 mL/min
Inj Vol	100 µL
Temp	Room temperature
Detector	RI
System	PL-GPC 50

Results

The identical results (within the 3-5% run-to-run reproducibility of gel permeation chromatography) are evident from the overlaid chromatograms in Figure 1 and the data in Table 1. There is slightly later elution of the peak on the 7.7 mm column, but once calibrated the difference disappears and the calculated results are indistinguishable.

Table 1. Polycarbonate Analysis - Molecular Weight Averages on Columns of Different Dimensions

Column	Dimensions	Mp	Mn	Mw	Mz	Pd
Agilent PLgel 10 ⁴ Å, 5 µm	300 x 7.5 mm	51,154	33,709	54,682	77,175	1.62
Competitor 5 µm	300 x 7.7 mm	49,778	34,215	52,555	71,295	1.53

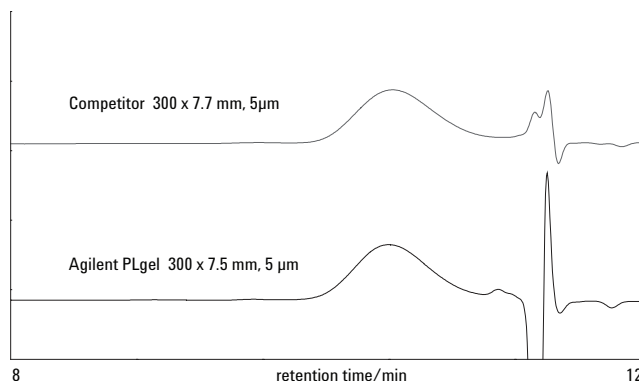


Figure 1. Overlaid chromatograms showing indistinguishable performance in the analysis of polycarbonate on an Agilent PLgel 7.5-mm diameter column compared to a 7.7-mm diameter column.

Conclusion

It is evident that analysts working with gel permeation chromatography can interchange 7.5 mm columns for 7.7 mm columns without changing the method, as the results will be indistinguishable. This expands column options and lets analysts take advantage of Agilent columns that deliver performance or economic advantage, or both, with no risk to established methods.

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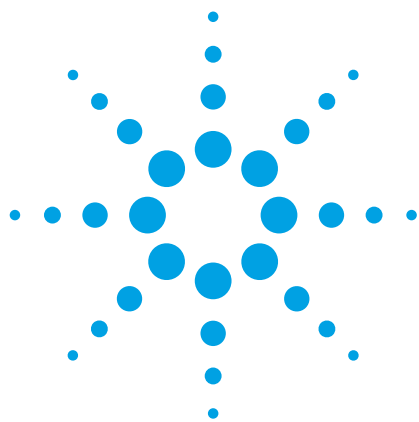
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Agilent Technologies



Polyurethane Analysis on Agilent PLgel 5 μ m MIXED-D with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Polyurethanes are synthetic resins containing urethane links ($-\text{NH}-\text{CO}-\text{O}-$) manufactured by polyaddition from isocyanate esters and polyhydric alcohols. These durable polymers are widely used in solid foams, plastics, and paints.

The resolving range of the Agilent PLgel 5 μ m MIXED-D column (200–400,000 MW) allows excellent resolution of both the high molecular weight portion of a polyurethane sample and the lower MW components.

Polyurethane Analysis

The Agilent PLgel 5 μ m MIXED-D column is specifically designed for the analysis of polymers, paints, and resin systems where material above 400,000 MW is unlikely to be present. The columns are ideal for the analysis of polyurethanes, as shown in Figure 1.

Conditions

Columns	2 $\times$ Agilent PLgel MIXED-D, 300 $\times$ 7.5 mm, 5 μ m (p/n PL1110-6504)
Eluent	THF
Flow Rate	1.0 L/min
Detector	RI
System	Agilent PL-GPC 50



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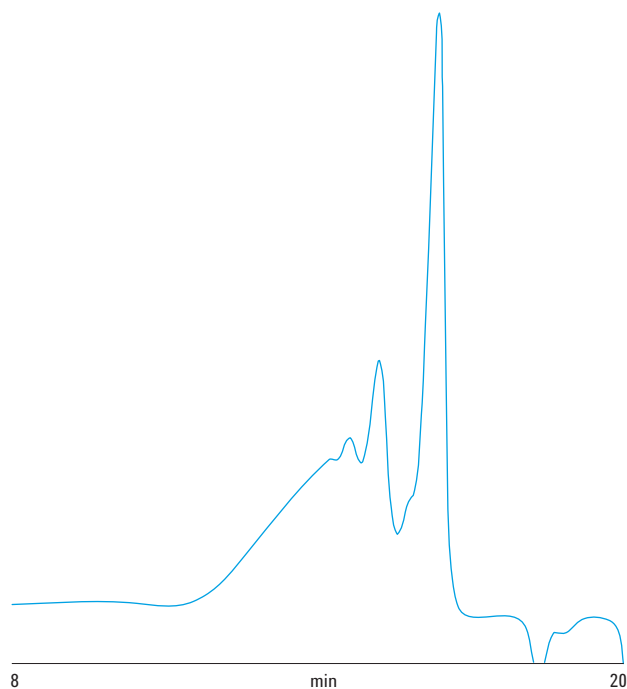


Figure 1. High and low molecular weight components in a sample of polyurethane separated on an Agilent PLgel 5 μ m MIXED-D two-column set.

Conclusion

The results show it is possible to determine the molecular weight distribution of polyurethane with Agilent PLgel 5 μ m MIXED-D columns.

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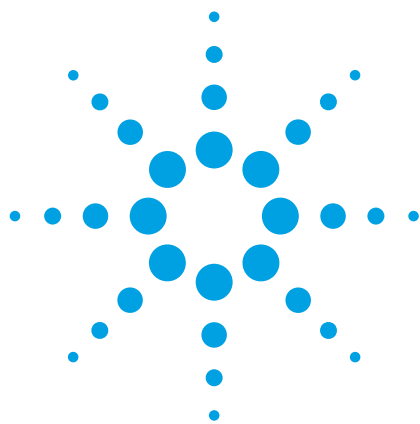
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Agilent Technologies



Polycarbonate Analysis on Agilent PLgel 5 μ m MIXED-D using Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Polycarbonates are synthetic thermoplastic polymers containing carbonate links ($-O-(C=O)-O-$) manufactured from bisphenol-A and phosgene. Polycarbonate is a good electrical insulator, resistant to flame and high temperature. It is mainly used in electronics and telecommunications. It is also used in the construction industry and lens making. Despite its durability and high impact resistance, polycarbonate lenses have low scratch resistance and must be coated with a durable surface.

Dichloromethane is a good solvent for polycarbonate, making it easy to analyze by gel permeation chromatography.

Polycarbonate Analysis

The Agilent PLgel MIXED-D 5 μ m column is specifically designed for the analysis of polymers, paints, and resin systems where material above 400,000 MW is unlikely to be present. Figure 1 shows the analysis of a relatively low molecular weight polycarbonate that elutes well within the range of PLgel MIXED-D columns.

Conditions

Columns	2 $\times$ Agilent PLgel 5 μ m MIXED-D, 300 $\times$ 7.5 mm (p/n PL1110-6504)
Eluent	Dichloromethane
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50



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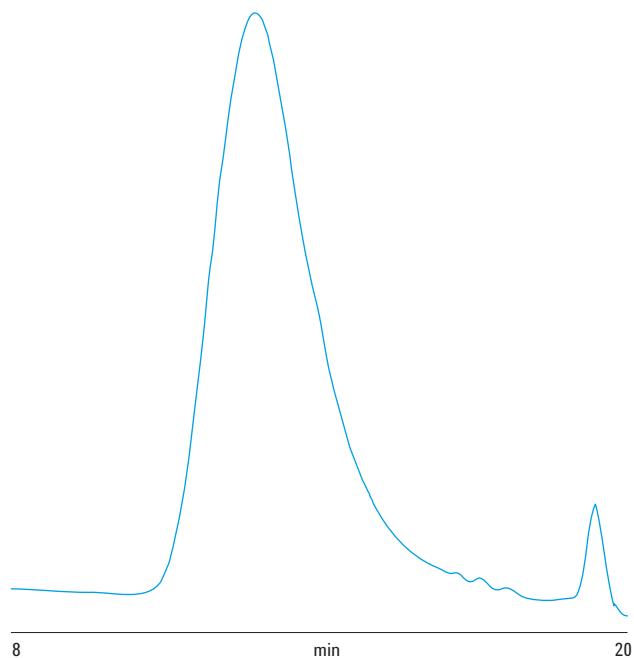


Figure 1. A relatively low molecular weight polycarbonate analyzed on an Agilent PLgel 5 μ m MIXED-D two-column set.

Conclusion

The data illustrate the analysis of polycarbonate molecular weight distribution by gel permeation chromatography using Agilent PLgel 5 μ m MIXED-D columns.

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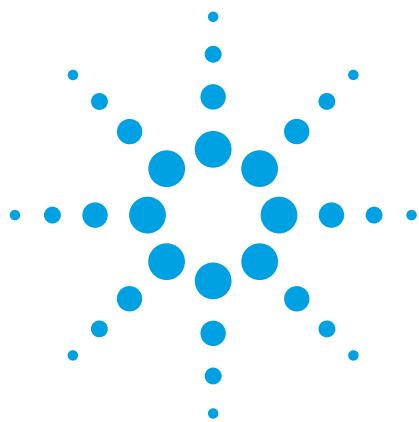
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Agilent Technologies



Epoxy Resin Analysis on Agilent PLgel 5 μ m MiniMIX-D and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Epoxyes are thermosetting copolymers formed by mixing epoxide with polyamine. When cured, epoxy resins are extremely durable, leading to their wide application as general-purpose adhesives, coatings, and composites.

Analysis of epoxy resins by gel permeation chromatography is straightforward with Agilent PLgel MiniMIX columns, which are selected because of their low solvent use compared to the PLgel MIXED version.

Epoxy Resin Analysis

The resolving range and high efficiency of the PLgel MiniMIX-D packing prevents exclusion of higher molecular weight epoxyes, while maintaining good resolution of oligomeric components. Figure 1 shows the analysis of three epoxy resins.

Conditions

Columns	2 $\times$ Agilent PLgel 5 μ m MiniMIX-D, 250 $\times$ 4.6 mm (p/n PL1510-5504)
Eluent	THF
Flow rate	0.3 mL/min
Detector	UV, 254 nm
System	Agilent PL-GPC 50



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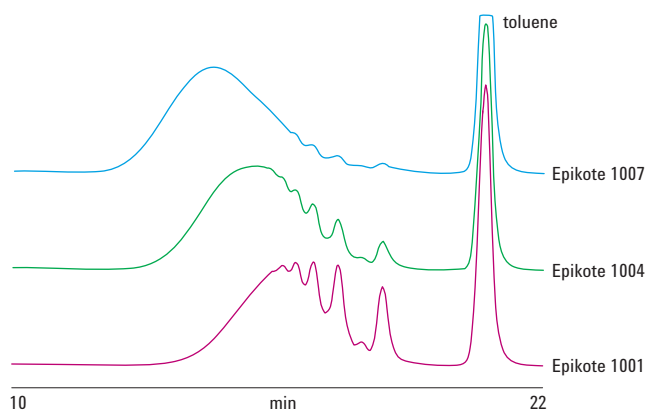


Figure 1. Good resolution of oligomers and no exclusion of higher molecular weight epoxy resins analyzed on an Agilent PLgel 5 μ m MiniMIX-D two-column set.

Conclusion

The data illustrate the analysis of epoxy resins at low flow with Agilent PLgel 5 μ m MiniMIX-D columns.

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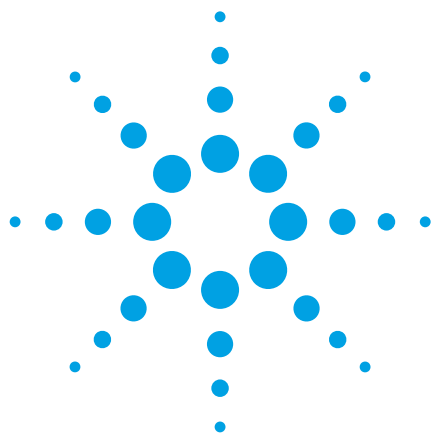
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Agilent Technologies



Polysiloxane Analysis on Agilent PLgel 5 μ m MIXED-E using GPC/SEC with RI and ELS Detection

Application Note

Materials Testing and Research, Polymers

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Introduction

Polysiloxanes are important commercial polymers used in applications such as lubricating oils, defoaming agents and sealants. Analysis of these materials by gel permeation chromatography (GPC) is an important part of process and quality control procedures. Although polysiloxane is soluble in tetrahydrofuran (THF), it is isorefractive with this solvent. As a result, refractive index (RI) detection cannot be used with THF.

Conditions

Sample	Polysiloxane, 0.2% 9w/v)
Column	3 $\times$ Agilent PLgel 5 μ m MIXED-E, 300 mm $\times$ 7.5 mm (p/n PL1110-6504)
Eluent	Toluene THF
Flow rate	1.0 mL/min
Inj vol	100 μ L
Detector	Agilent ELSD (neb = 40 $^{\circ}$ C, evap = 80 $^{\circ}$ C, gas = 1.0 SLM)



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Analysis of Polysiloxane

There are two options for the analysis of polysiloxane - change the solvent and use RI detection or use THF with an Agilent evaporative light scattering detector (ELSD). ELS detection does not rely on the refractive index of the polymer and provides a positive response proportional to sample concentration in all solvents. Another advantage of ELS over RI detection is that it does not generate system peaks due to solvent imbalance. The solvent is evaporated in the detector, which gives the Agilent ELSD excellent baseline stability.

Figure 1 shows chromatograms of a polysiloxane obtained in toluene with RI detection and THF with ELS detection. The ELS chromatogram has no system peaks and a flat, stable baseline. The RI chromatogram shows large system peaks and a drifting baseline. The ELS also shows a very large response to the polymer compared to the RI; in this application, the ELS sensitivity is about 250 times higher than the RI detector (RI full scale output is 1 V whereas the ELS full scale output is 10 V).

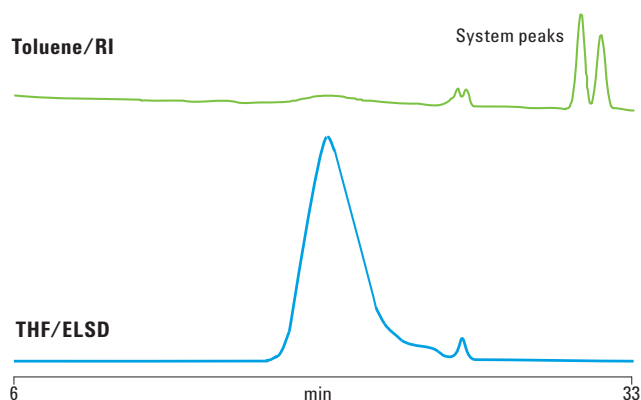


Figure 1 Analysis of a polysiloxane showing the improved data quality from evaporative light scattering detection compared to refractive index detection.

Conclusions

In the case of polysiloxanes, the Agilent ELS detector provides more information about the sample. The improved detector response and baseline stability compared to RI allow a much greater accuracy of analysis of the molecular weight distribution of the polymer to be achieved.

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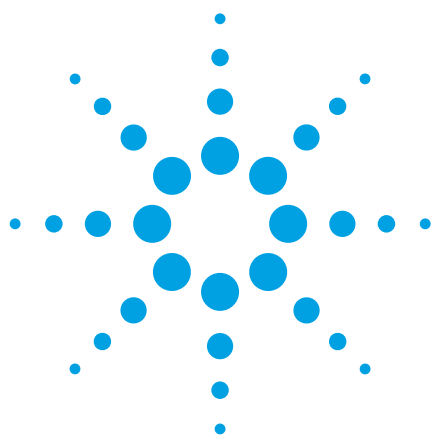
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Agilent Technologies



Epoxy Resin Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Epoxy resins are thermosetting copolymers formed by mixing epoxide with polyamine. They are extremely durable after curing, and so are widely used as general-purpose adhesives, coatings and composites.

Analysis of epoxy resins by gel permeation chromatography is straightforward with Agilent PLgel 3 μ m MIXED-E columns, which are ideal for low molecular weight samples that contain oligomeric fractions, as well as polymers, up to 30,000 MW.



Agilent Technologies

Analysis of polysiloxane

In this analysis of epoxy resin, the ultra high efficiency achieved with Agilent PLgel 3 μ m particles gives baseline resolution of the sample components with the lowest MW component eluting within 8 minutes.

Conditions

Columns	Agilent PLgel 3 μ m MIXED-E, 300 $\times$ 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

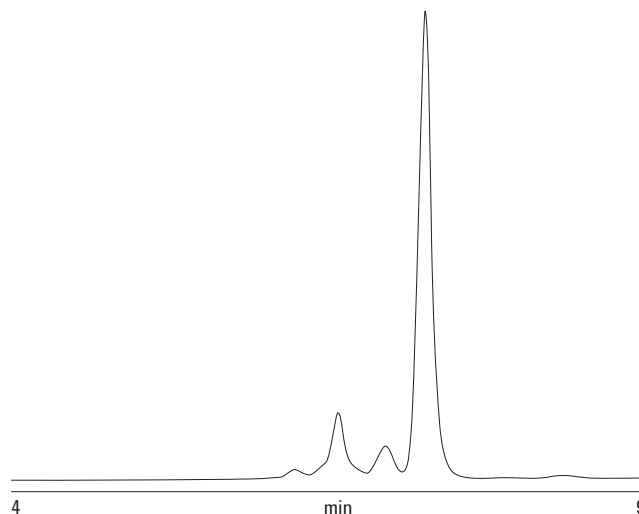


Figure 1 Fast elution of low molecular components of an epoxy resin on an Agilent PLgel 3 μ m MIXED-E column.

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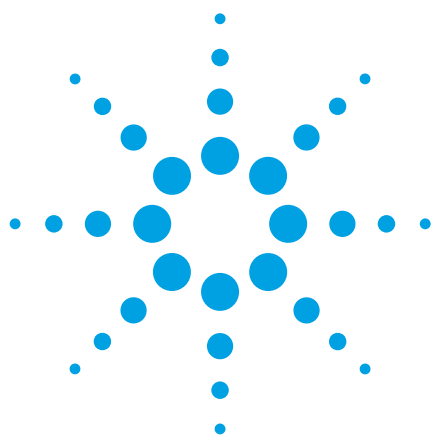
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Agilent Technologies



Carboxylic Ester Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Carboxylic acid esters are made by Fischer esterification, in which a carboxylic acid is treated with an alcohol in the presence of a dehydrating agent. Esters are widely found in fruit and vegetable odors, and in insect pheromones. Commercially they are used in fragrances and as flavorings in the food industry.

Agilent PLgel 3 μ m MIXED-E columns simplify the analysis of carboxylic esters by gel permeation chromatography. These columns are ideal for low molecular weight samples that contain oligomeric fractions, as well as polymers, up to 30,000 MW.



Agilent Technologies

Analysis of a carboxylic acid ester

Figure 1 shows the rapid separation of carboxylic acid ester oligomers using two Agilent PLgel 3 μ m MIXED-E columns to improve resolution.

Conditions

Column	2 $\times$ Agilent PLgel 3 μ m MIXED-E, 300 $\times$ 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

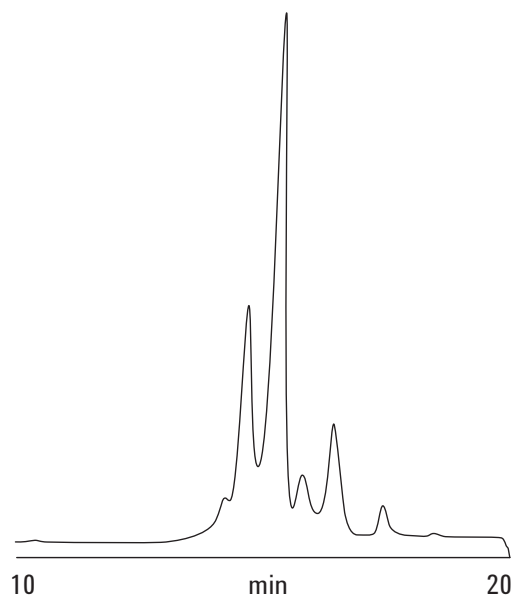


Figure 1. Separation of oligomers of a carboxylic acid ester on an Agilent PLgel 3 μ m MIXED-E two-column set.

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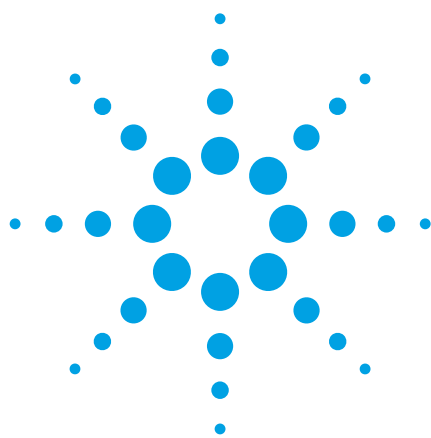
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Agilent Technologies



Phenolic Resin Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Phenol formaldehyde resins are manufactured by reacting phenol with formaldehyde, though precursors occasionally include other phenols or aldehydes. These resins are mainly used to make printed circuit boards, or for adhesives, coatings and lab bench tops. The earliest commercial phenol formaldehyde resin was Bakelite, developed by the Belgian chemist Leo Baekeland in 1909.

The gel permeation chromatography of phenolic resins is easily accomplished using Agilent PLgel 3 μ m MIXED-E columns. These columns are ideal for low molecular weight samples that contain oligomeric fractions, as well as polymers, up to 30,000 MW.



Agilent Technologies

Analysis of a phenolic resin

Excellent oligomer resolution is obtained using the Agilent PLgel MIXED-E 3 μm packing for the analysis of phenolic resins (Figure 1). The sample is partially excluded when lower pore size columns were used. However, the higher exclusion limit of the PLgel MIXED-E column (30,000) fully resolves all the sample components.

Conditions

Column	Agilent 2 $\times$ PLgel 3 μm MIXED-E, 300 $\times$ 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	UV, 254 nm
System	Agilent PL-GPC 50

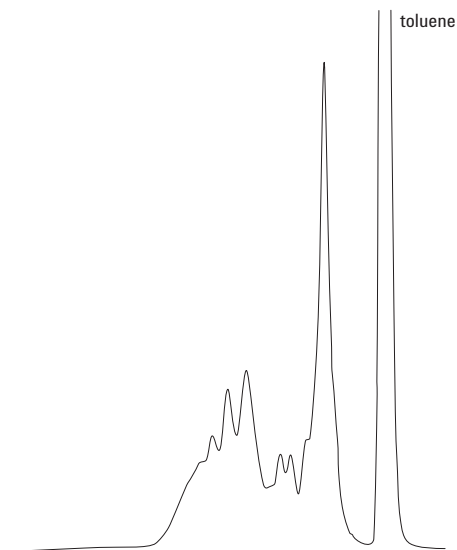


Figure 1. All components of a phenolic resin are resolved on an Agilent PLgel 3 μm MIXED-E two-column set.

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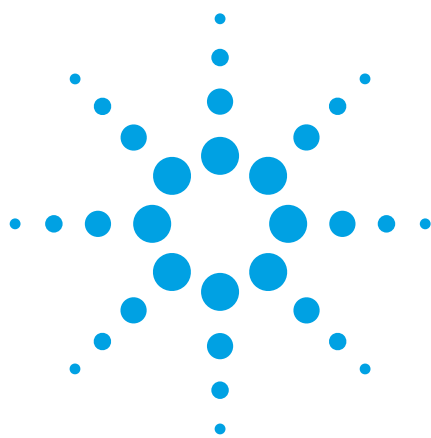
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Agilent Technologies



Polyester Polyol Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Polyols are alcohols containing multiple hydroxyl groups. Their main use is as reactants to make other polymers such as polyester polyol. This is formed by condensation or step-growth polymerization of a diol polyol with dicarboxylic acid. Polyester polyols are reacted with polyisocyanates in the manufacture of polyurethanes for rigid-foam, flame-retardant building board. Natural oil polyester polyols from vegetable oils are beginning to replace some epoxide-based polyols.

Analysis of polyester polyols is straightforward with gel permeation chromatography using Agilent PLgel 3 μ m MIXED-E columns. These columns are ideal for low molecular weight samples that contain oligomeric fractions, as well as polymers, up to 30,000 MW.



Agilent Technologies

Analysis of a polyester polyol

This separation (Figure 1) demonstrates the excellent resolution of the oligomeric species in a polyol sample prepared from adipic acid and butandiol using Agilent PLgel 3 μ m MIXED-E columns.

Conditions

Column	2 $\times$ Agilent PLgel 3 μ m MIXED-E, 300 $\times$ 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

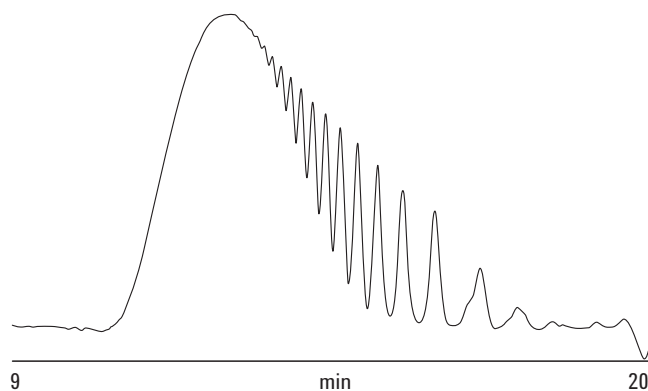


Figure 1. Separating oligomers in a polyester polyol on an Agilent PLgel 3 μ m MIXED-E two-column set.

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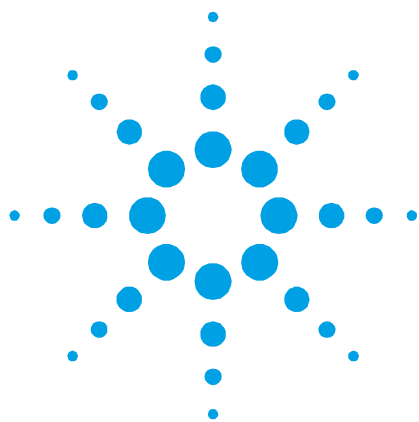
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A new approach to sample preparation free micro ATR FTIR chemical imaging of polymer laminates

Application Note

Materials Testing & Research

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Abstract

Micro ATR chemical imaging of polymers and in particular polymer laminates typically requires significant application of pressure to ensure good contact between the ATR crystal and the sample. To ensure that such thin samples can withstand the pressure without buckling, elaborate sample preparation procedures are often required to support cross-sectioned materials: embedding of sample in resin, cutting the resin and polishing the contact surface. Such procedures are tedious, require overnight resin curing and carry the added risk of cross-contamination. Presented here is a novel method of ultralow pressure micro ATR FTIR chemical imaging that removes the need for any structural support. This allows samples to be measured "as-is" using direct contact with the ATR crystal. This unique capability is made possible through the use of Agilent's "Live ATR imaging" technique which provides enhanced chemical contrast, and enables the exact moment of contact between the sample and ATR crystal to be determined and provides a visual measure of the quality of contact. Adhesive layers as thin as a few microns can be clearly observed in 50-micron thick polymer laminates without sample preparation.

Introduction

What are polymer laminates and what are they used for?

Polymer laminates are film structures consisting of two or more layers adhered together to make a structure. The polymeric materials forming these laminates have varying thickness—from a few microns to tens of microns. This can influence a variety of properties, such as chemical, mechanical and barrier (e.g., impervious to oxygen and/or moisture) properties.



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To construct these materials, adhesive (tie) layers are often required between two adjacent but chemically incompatible layers. Typically these incompatibilities are between materials with differing polarities, such as nylon and polyethylene.

The adhesives typically have intermediate polarity or contain functional groups with an affinity to both polar and non-polar layers and hence act as good binding material. Such adhesive layers in laminates can be very thin, e.g., between 2 to 10 microns.

Polymer laminates can range in complexity and thickness from those containing only two layers to more than 10 layers (not including adhesive layers). With total cross-sectional thicknesses ranging from <50 microns to >200 microns, polymer laminates are used in a variety of packaging applications, which are employed in industries such as food and pharmaceuticals.

What are the analytical challenges/requirements for polymer laminates?

With ever increasing manufacturing sophistication enabling more complex and thinner laminate structures to be produced, the analytical challenges to ensure good product quality control, troubleshooting or the reverse engineering of competitive products are also increasing in complexity.

The analytical tools available to analyze such laminates are wide and varied and include a range of optical microscopy techniques, thermal techniques (such as differential scanning calorimetry) and various spectroscopic techniques.

In particular, Fourier Transform Infrared (FTIR) microscopy has proven most useful for the analysis of polymer laminates. This has resulted from the core application of FTIR spectroscopy in the identification and characterization of polymers, combined with the ability to obtain this information from small areas.

When applied to polymer laminate analysis, FTIR microscopy is typically performed in transmission mode and requires that the total sampled thickness be within a certain limit. For polymeric materials, this is typically 10–20 microns. Preparing thinly sliced polymer and polymer laminate materials at a thickness of 10–20 microns presents some challenges. Typically, dedicated (and often expensive) specialized cutting devices such as microtomes are required. Even then, the cut samples are often difficult to handle due to curling or difficulties with static stick. To minimize these effects, samples can be embedded in resin before cutting and microtomed together within the resin support (Figure 1). This unfortunately adds another material with a complex IR spectrum to the sample. Once cut, if the sample is flat, it can be placed in a sandwich between infrared transparent windows and sampled in transmission mode. However, because of internal reflections between the front and back surfaces of the sample, “fringing effects” can commonly be observed. This results in a sinusoidal baseline during such measurements.

With these issues and sampling preparation steps aside, transmission FTIR microscopy is a relatively simple technique to obtain spectra from small areas. It does however suffer from one major limitation: spatial resolution is relatively poor, especially when compared to optical microscopy techniques. Typical spatial resolution limits for transmission mode FTIR microscopy are about 10–15 microns.

In comparison to transmission mode, the use of micro attenuated total reflectance (ATR) as the mode of analysis removes the requirement for samples to be a certain thickness, so samples no longer need to be thinly cut. However, as ATR requires intimate contact with the samples, there are still some important sample preparation requirements. Primarily, the sample must be flat and smooth to ensure that there is full and complete contact across the ATR measurement’s field of view. Additionally, and of paramount importance to the detection of ultrathin layers, micro ATR FTIR

microscopy provides for a factor of four spatial resolution enhancement over transmission mode.

To ensure complete and intimate contact, a significant amount of pressure must be applied between the sample and ATR crystal. Many micro ATR imaging systems rely on indirect methods of ensuring good contact, by using coarse pressure sensors, often with preset pressure levels.

The inability to directly monitor the exact moment and quality of contact in most micro ATR imaging systems is also another factor that requires the use of higher pressures to ensure good contact. For naturally hard materials, the pressures needed to ensure a good contact between the ATR and surface is typically not an issue. However, given samples may have cross-sectional thicknesses of only 50–200 microns, even very slight pressures will cause an unsupported polymer laminate to buckle or deform in a way that prevents good contact.

Therefore, to avoid buckling or other structural distortions of delicate and thin samples under applied ATR pressure, it is mandatory to provide some degree of support. This is most commonly achieved by resin embedding of the sample, followed by cutting and polishing of the surface (Figure 1).

The process of resin embedding is tedious and time consuming (>12 hours), typically consisting of the following steps:

1. Cut a small piece of sample and place it vertically in a holding clamp.
2. Place sample and clamp into a mold and pour in resin to fully cover sample.
3. Allow resin to cure, typically overnight, and then remove the resin-embedded sample from mold.
4. Cut the top surface of resin, so as to expose a cross section of the sample.
5. Polish the cut surface with successively finer and finer lapping paper (from 30 microns to 1 micron).

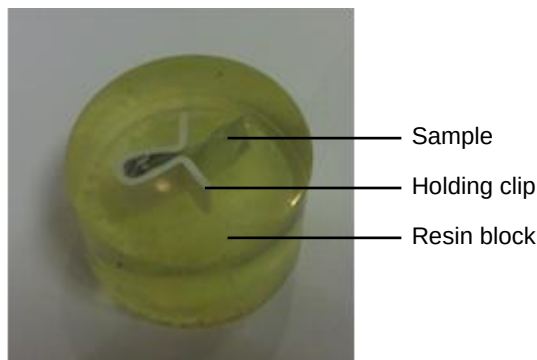


Figure 1. An example of a polymer film, held by a clip and embedded in a resin block

Cutting and polishing also introduces the risk that resin and polishing material may contaminate the sample or complicate the image and spectral interpretation.

Once prepared, resin-embedded samples are brought into contact with the micro ATR and pressure is applied. Often, the levels of pressure applied—even at lower settings—are enough to produce indentations at the surface of the samples, potentially preventing the subsequent analysis of the sample with other analytical techniques. This technique is then potentially destructive.

A new approach to “pressure free” micro ATR imaging

Agilent Technologies has developed a radically new approach that removes the need for resin embedding or any other sample preparation. This enables delicate and thin samples to be measured “as-is”. The new approach hinges upon the fact that the infrared detector in an Agilent FTIR imaging system is a focal plane array (FPA*) and so affords simultaneous two-dimensional (2-D) data collection. And, most importantly and uniquely, it utilizes the “Live ATR imaging” feature with enhanced chemical contrast to ensure that the minimum pressure necessary for good contact is applied. This results in a non-destructive measurement—a remarkable capability.

Unlike linear array IR detectors, which must be scanned across an area to generate a 2-D image, FPAs provide instantaneous “real-time” imaging of the sample's surface, as it is in contact with the ATR. Such real-time imaging permits a visual assessment of the quality of sample contact before any data collection.

However, having a 2-D FPA alone does not provide for enough contrast to determine the moment of sample contact with the ATR. To overcome these issues Agilent Technologies has recently developed a unique “Live ATR imaging” mode, which significantly enhances the chemical contrast of the real-time FPA image, so the exact moment of sample contact can be visualized and contact monitored as the pressure is increased.

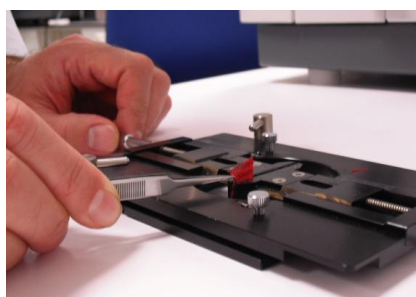
This mode provides for direct and real-time monitoring of the quality of contact (i.e., has the sample made complete contact across the entire field of view), which allows for extremely low levels of pressure to be applied. And it is this extremely low level of pressure that now allows for delicate and thin samples to be mounted, cross-sectioned end on, without any need for sample support using resin.

Sample measurement with “Live ATR imaging”

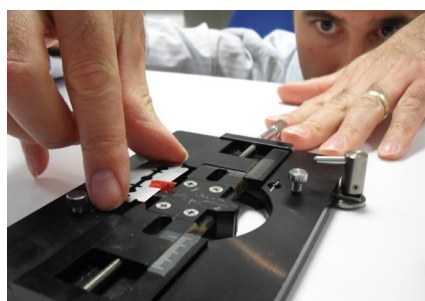
In five simple steps which only take only a few minutes (Figure 2), a sample of polymer laminate (i.e., a sausage wrapper) can be prepared for measurement using “Live ATR imaging”—removing the need to spend hours embedding, cutting, and polishing!



Step 1. Cut a small piece of sample.



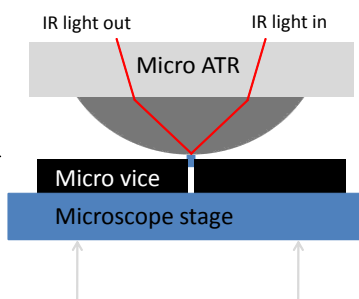
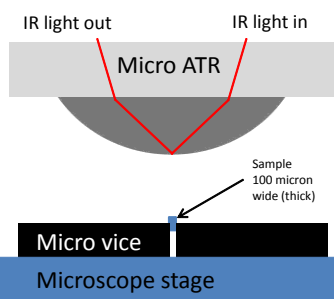
Step 2. Place sample into micro-vice.



Step 3. Cross-section sample with razor.



Step 4. Place micro-vice onto microscope stage.



Step 5. Raise stage to make contact and then collect data.

Figure 2. Easy five-step process—from raw sample to data collection—allows sample measurement of polymer laminates to be achieved in minutes using “Live ATR imaging” with enhanced chemical contrast. Note: Micro ATR and sample are drawn to scale

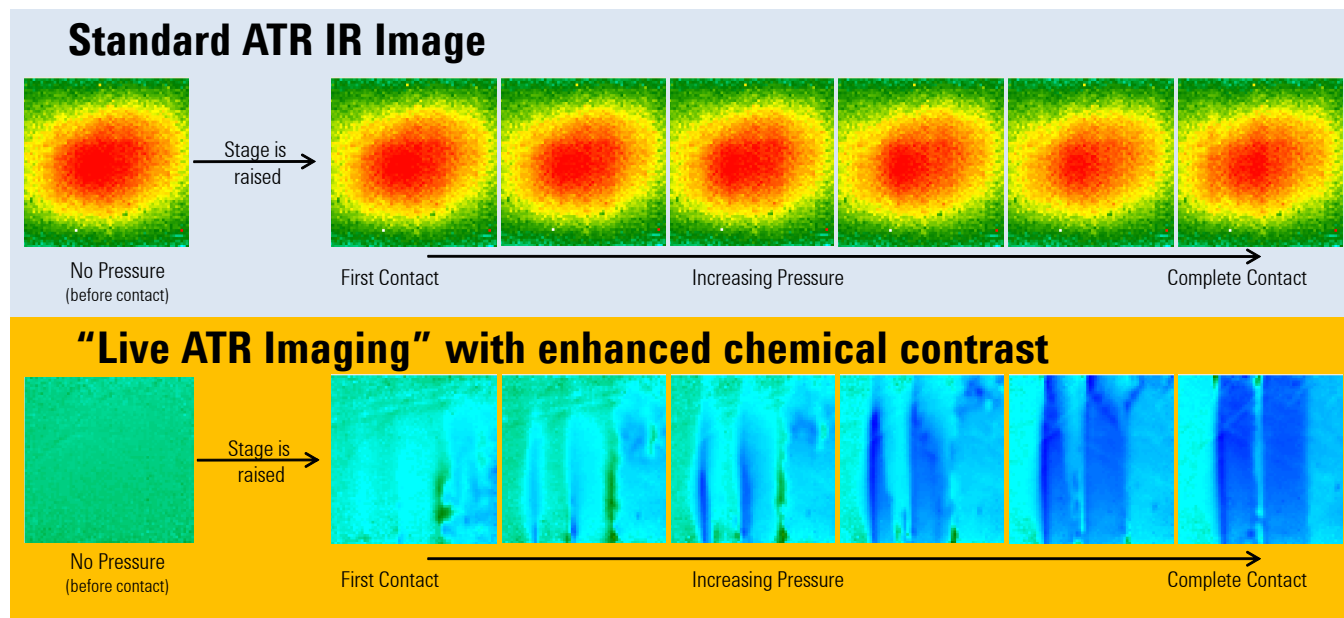


Figure 3. Comparison of a standard ATR IR image and Agilent’s “Live ATR imaging” with enhanced chemical contrast—clearly showing the latter can detect first contact of the ATR crystal with the sample and that contact quality can be monitored real-time as the pressure is increased and before any data collection

Figure 3 shows a side-by-side comparison of Agilent’s unique “Live ATR imaging” with enhanced chemical contrast and a standard ATR IR image.

Reviewing the upper series of images, the similarity of all the standard ATR IR images makes it impossible to determine when the ATR crystal makes contact with the sample’s surface or make any reasonable assessment of the quality of the contact as the pressure being applied increases.

Whereas, as seen in the lower series of images, Agilent’s unique “Live ATR imaging” with enhanced chemical contrast enables real-time monitoring of the sample contact as the sample is being raised and pushed up against the germanium crystal of the Micro ATR. The real-time monitoring allows for a near “pressure free” contact to be made between the sample’s surface and the Micro ATR, this means unsupported cross-sections of ultrathin polymer laminates can be measured directly—even very thin samples of less than 50 microns—without the need for being embedded in resin!

Results

To further demonstrate the capabilities of “Live ATR imaging”, a polymer laminate sample was obtained from the plastic wrapper of a sausage (~55 microns total thickness).

The results below were collected using the following conditions:

FTIR Spectrometer	Agilent Cary 670 FTIR
FTIR Microscope	Agilent Cary 620 FTIR
Focal Plane Array*	64 × 64 MCT
Spectral Resolution	4 cm ⁻¹
Number of Scans	64 (2 mins)
Spatial Resolution	1.1 microns (pixel size)
Collection mode	Micro ATR (Ge)
Sample Type:	Sausage wrapper

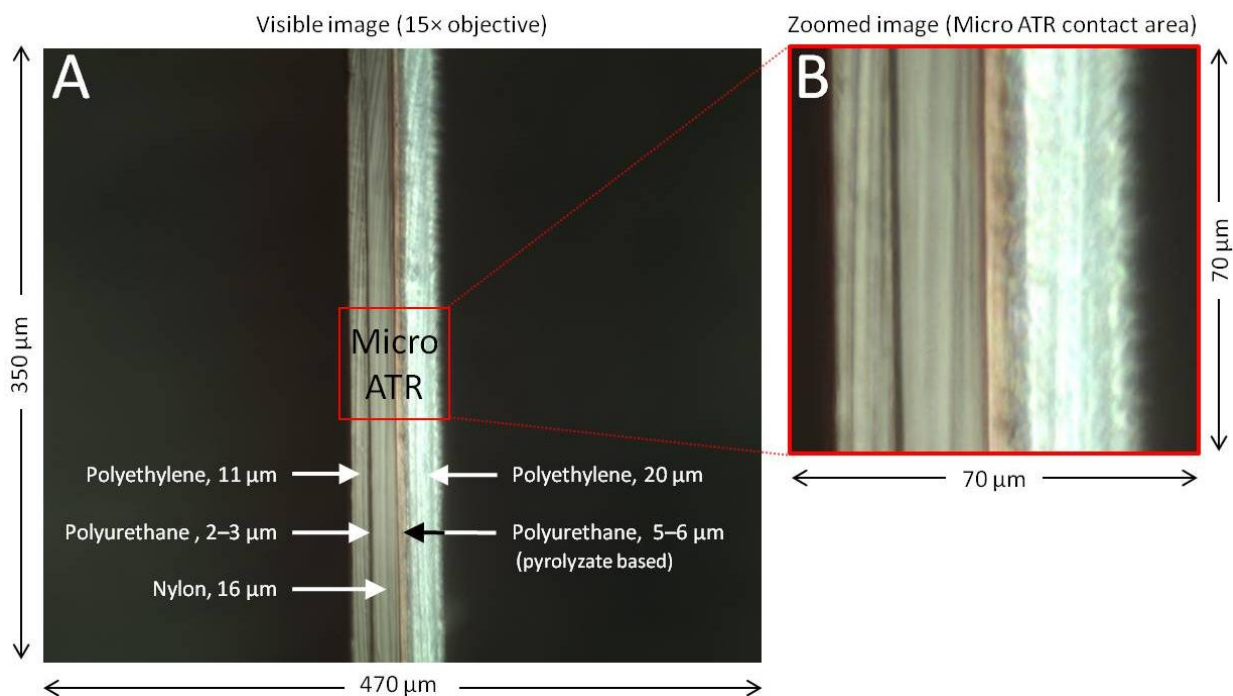


Figure 4. Optical images: A—the full field of view visible through microscope, annotated with the chemical composition and approximate thickness of the various layers in the sample; and B—zoomed image corresponding to the contact area of the Micro ATR

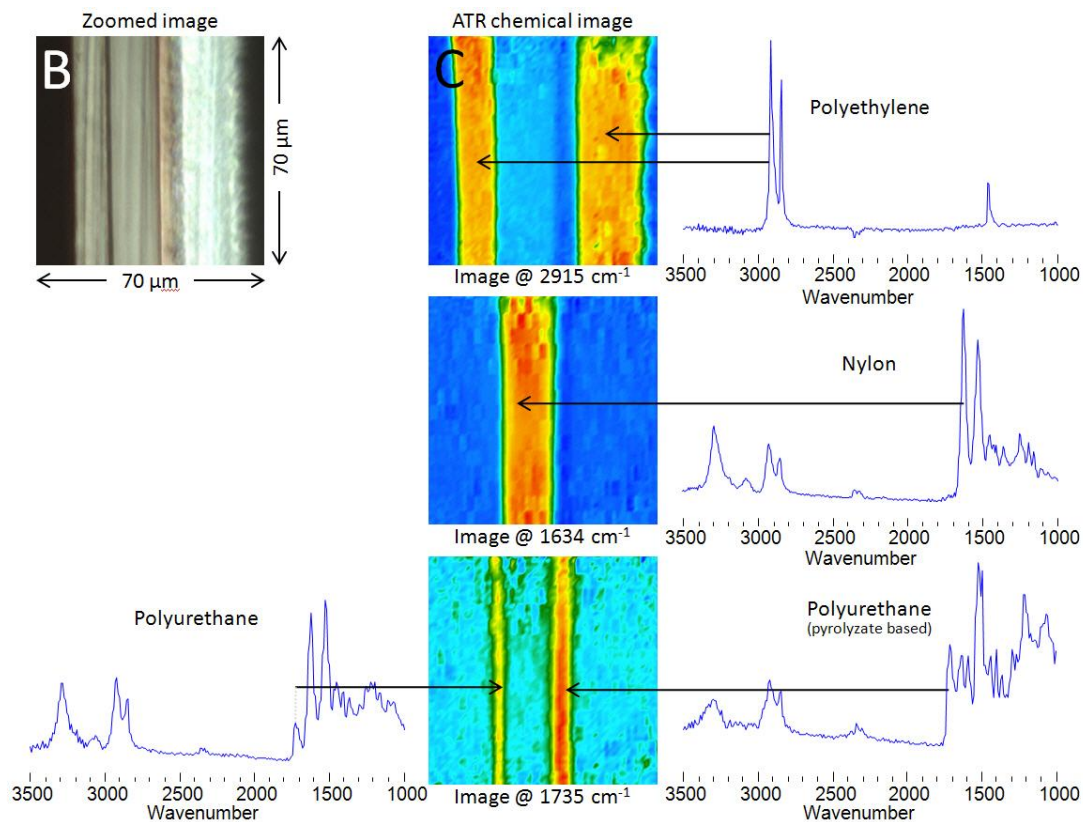


Figure 5. Identifying layers: B—as per Figure 4, above; and C—three chemical images created with different wavenumbers to highlight the main layers and tie layers with corresponding representative spectra as indicated by the arrows. Note: All spectra are shown in absorbance units, with axes omitted for clarity

A visual inspection of the sample using the standard binocular or internal visible camera revealed the sample to be a polymer film containing three main layers with two adhesive layers (Figure 4).

A summary of the results is presented in Figure 5. This shows how the three main layers are clearly identified: a 16-micron thick layer of nylon sandwiched between two layers of polyethylene, 11 and 20 microns in thickness.

However, as demonstrated, the power of Micro ATR chemical imaging with an Agilent FPA detector is in its ability to measure layers as thin as a few microns. Two tie layers were clearly identified and easily determined as being composed of subtly different polyurethane adhesives. The thinner of the two polyurethane layers was only 2–3 microns across and the pyrolyzate-based layer was thicker at 5–6 microns. The measurement of both these layers would be impossible with any other technique other than micro ATR chemical imaging on the Agilent Cary 620 FTIR chemical imaging system.

Summary

There are two clear benefits to analyzing polymeric laminates using Agilent's Cary 620 FTIR chemical imaging system:

1. Analyze ultra-thin samples without resin embedding

Through the use of Agilent's unique "Live ATR imaging", ultra-thin films of 50 microns or less can be measured as-is with Micro ATR chemical imaging. This avoids the need for any of the traditional and complicated resin embedding requirements. As such, instead of waiting hours for resin to cure and then spending time cutting and then polishing the surface, multiple samples or multiple locations on one sample can be measured in a few minutes.

2. Unrivalled spatial resolution

In combination with the use of a FPA detector*, Agilent's unique Micro ATR design provides for a pixel size of 1.1 microns that allows ultra-thin adhesive layers as narrow as two microns to be identified. This level of spatial resolution provides unrivalled levels of detail and chemical information to assist in the most complicated and difficult sample measurements.

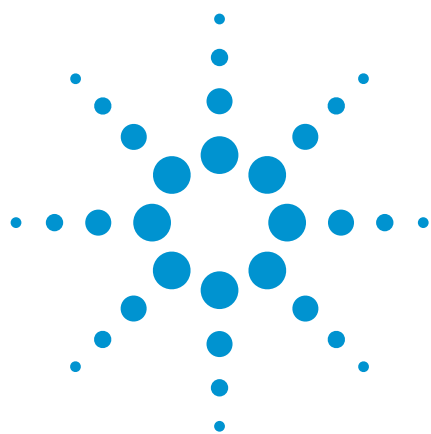
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Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns

Application Note

Authors

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Introduction

Obtaining good quality size exclusion data for a protein sample requires a column with a suitable molecular weight resolving range, such as ProSEC 300S. The easiest way to probe the resolving range of an SEC column is to analyze a series of chemically and structurally homologous polymers differing only in the molecular dimensions. In the case of a column for use in water or buffers such as those used in protein analysis, polyethylene glycol/oxide standards (which differ only in the nature of the chain end groups) are excellent candidates.

To investigate the molecular weight resolving range of the ProSEC 300S column a series of polyethylene glycol/oxide standards were analyzed and their elution order plotted as a function of molecular weight.



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Methods and Materials

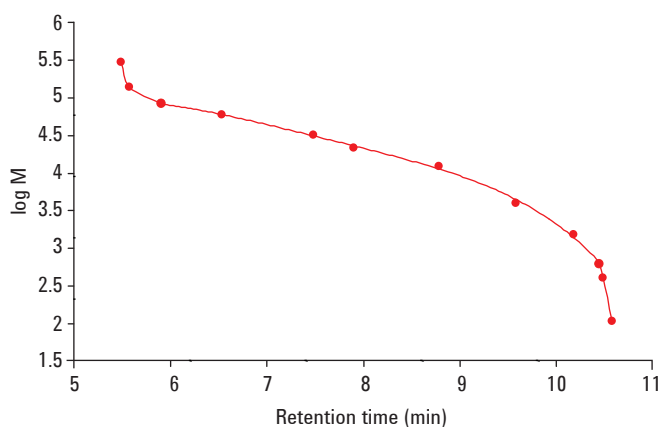
Conditions

Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent:	0.3M: 50mM KH_2PO_4 - K_2HPO_4 , pH 6.8, containing 0.3M NaCl
Flow Rate:	1.0 mL/min
Inj Vol:	20 μL
Sample Conc:	4 mg/mL
Temp:	25 $^{\circ}\text{C}$
Detection:	UV at 280 nm

Conclusion

The wide resolving range of the ProSEC 300S column is suitable for the analysis of a wide variety of proteins on the basis of their size in solution and molecular weight. ProSEC 300S columns contain a packing with a surface modified for compatibility with proteins, ensuring that true size exclusion is obtained with minimal unwanted interaction effects. The pore size of the packing has been specifically selected to allow the analysis of a wide range of small to medium-sized proteins.

Results and Discussion



The calibration curve shows the relationship between the log of the molecular weight and the retention time, illustrating the wide resolving range of the ProSEC 300S column. The column resolves up to around 70,000 Daltons, which would equate to the molecular dimensions of a large sized globular protein.

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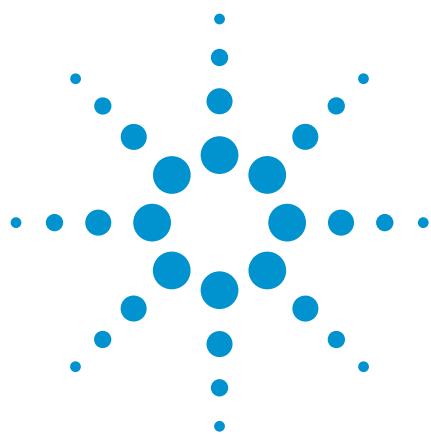
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Agilent Technologies



Sensitive Analysis of Oligo/ polyethylene Glycol by Reversed- phase HPLC with ELSD

Application Note

Author

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Introduction

Oligo/polyethylene glycol is a hydrophilic water-soluble material which has many uses in biotechnology and biomedicine as it is non-toxic and non-immunogenic. In aqueous solutions, it is heavily hydrated and can be used as an additive or shield to minimize non-specific interactions with proteins. Molecular size information and molecular size distributions can be obtained using gel permeation chromatography (GPC) which utilizes an isocratic system with refractive index (RI) detection. However, with GPC, there is insufficient resolution for the quantitation of individual oligomers. Reversed phase HPLC separates on the basis of differences in solute hydrophobicity but requires gradient elution if the analysis is to be accomplished in a reasonable time frame. This means that an RI detector cannot be used. Also oligo/polyethylene glycol does not have a UV chromophore and therefore this method of detection is unsuitable unless derivatization is first performed.

A better approach is to combine the polymeric reversed-phase HPLC column, PLRP-S 100Å, with the 385-ELSD evaporative light scattering detector. The 385-ELSD offers greater sensitivity than UV detection. Solvent peaks are absent and excellent baseline stability is present. The 385-ELSD is renowned for its rugged design and ability in delivering high performance for demanding HPLC or GPC applications. PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds because the small pore sizes have extremely high surface areas available to the solutes. Coupling PLRP-S columns with the 385-ELSD evaporative light scattering detector creates an excellent system for separating oligo/polyethylene glycol.



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Instrumentation

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)

Detector

Figure 1a, 1b and 3:

385-ELSD (neb = 85 °C, evap = 80 °C, gas = 1.0 SLM)

Figure 2a:

385-ELSD (neb = 85 °C, evap = 75 °C, gas = 1.0 SLM)

Figure 2b:

385-ELSD (neb = 85 °C, evap = 78 °C, gas = 1.0 SLM)

Materials and Reagents

Eluent A: Water

Eluent B: Acetonitrile

Conditions

Flow Rate: 0.5 mL/min

Gradient:

Figures 1a and 1b:

10-30% B in 15 min, 30-100% clean up 5 min

Figures 2a and 3:

30-50% B in 15 min, 50-100% clean up 5 min

Figure 2b:

40-60% B in 15 min, 60-100% clean up 5 min

Temp: 40 °C

Results and Discussion

Examples are given in Figure 1a of the separation of polyethylene glycol of 400 nominal molecular weight.

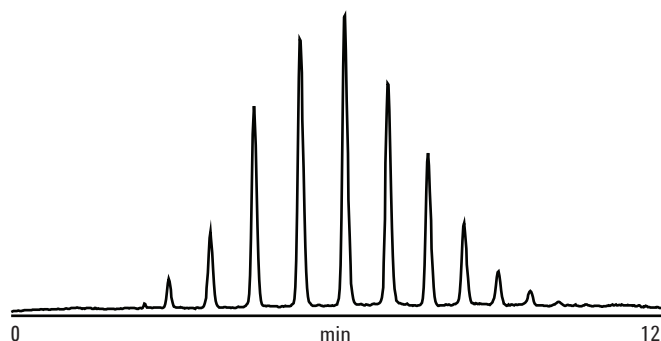


Figure 1a. PEG 400 oligomers detected by the 385-ELSD evaporative light scattering detector.

Figure 1b shows a sample with a nominal molecular weight of 600.

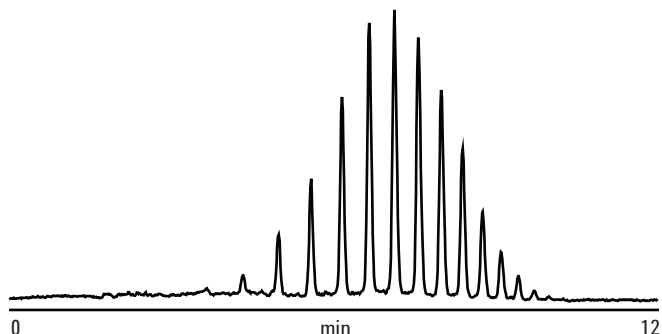


Figure 1b. PEG 600 oligomers detected by the 385-ELSD evaporative light scattering detector.

As the molecular weight increases, it is no longer possible to separate the individual oligomers, as shown in Figures 2a and 2b.

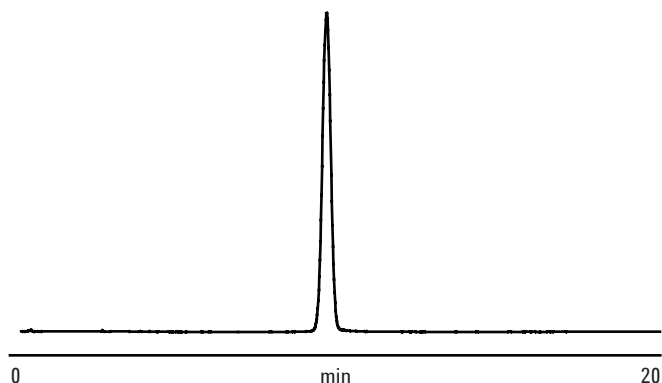


Figure 2a. No separation of oligomers in PEG 4,120.

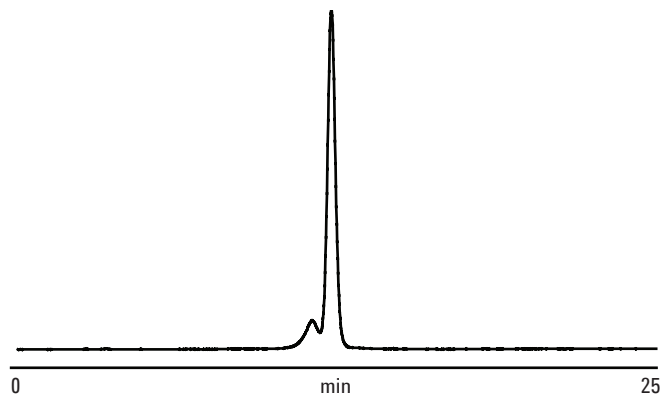


Figure 2b. PEG 22,800 oligomers not visible.

Reversed-phase HPLC with ELS detection can also be used to look for contaminants in polyethylene glycol samples which have slightly different hydrophobicities.

Figure 3 shows the separation of a sample of mono-hydroxyl polyethylene glycol containing a small amount of the unwanted diol derivative.

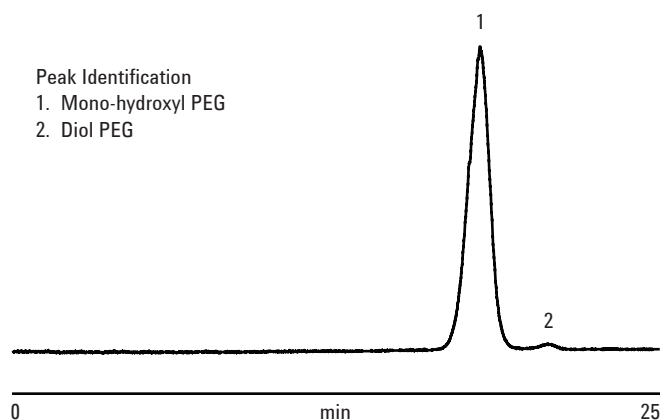


Figure 3. The presence of unwanted diol derivative of PEG revealed by the 385-ELSD evaporative light scattering detector.

Conclusion

Reversed-phase HPLC with the 385-ELSD evaporative light scattering detector and PLRP-S columns successfully separated oligo/polyethylene glycols and revealed the presence of unwanted derivatives.

PLRP-S columns are the preferred choice for the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules may be retained for much longer, resulting in greater resolution.

A PLRP-S column used with the 385-ELSD detector is an ideal combination for the separation of low molecular weight compounds that have no chromophores.

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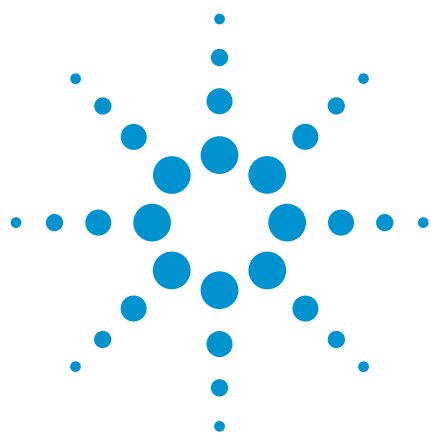
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Agilent Technologies



Sensitive Analysis of Polystyrene 960 and 580 by Reversed Phase HPLC with ELSD

Application Note

Author

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Introduction

Polystyrene 960 is a high impact polystyrene extruded or thermoformed into containers for foodstuffs, such as salad dressing, because it is highly resistant to degradation from fats and oils. Polystyrene 580 is designed for injection molding. It is used for high quality electrical components, and medical and office supplies since it has very good sparkle and stiffness. The physical characteristics are related to the molecular weight of the polymers.

Using reversed phase chromatography, it is possible to separate individual oligomers of these low molecular weight polystyrenes. The combination of PLRP-S columns and the 380-LC evaporative light scattering detector is ideal for this analysis. The 380-LC detector evaporates the mobile phase before it reaches the detection chamber, eliminating problems of baseline drift, which would be observed with a UV detector due to changes in the UV absorbance of the eluent during the THF gradient. The 380-LC offers greater sensitivity than UV detection with no solvent peaks. It is also renowned for its rugged design and ability in delivering high performance for demanding HPLC or GPC applications. PLRP-S columns are ideally suited to the analysis of low molecular weight polymers because the small pore sizes have extremely high surface areas available to the solutes.



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Instrumentation

Column

Figure 1: PLRP-S 300Å 8 µm, 250 x 4.6 mm (p/n PL1512-5801)

Figure 2: PLRP-S 100Å 8 µm, 250 x 4.6 mm (p/n PL1512-5800)

Detector: 380-LC (neb=80 °C, evap=75 °C, gas=1.0 SLM)

Materials and Reagents

Eluent A: Water

Eluent B: THF

Conditions

Flow Rate: 0.5 mL/min

Gradient: 65-85% B in 20 min

Results and Discussion

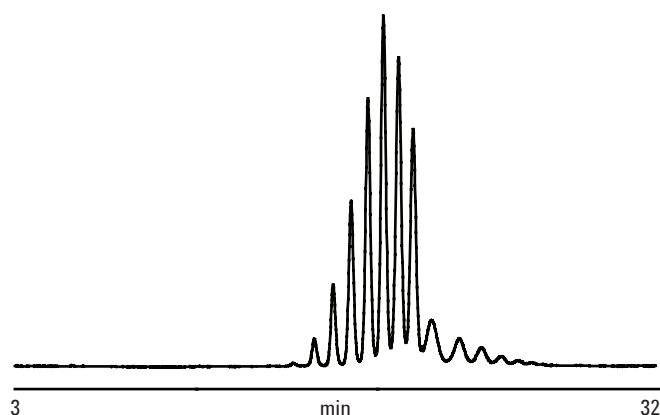


Figure 1. Separation of individual oligomers in polystyrene 960 with a PLRP-S column and 380-LC ELSD.

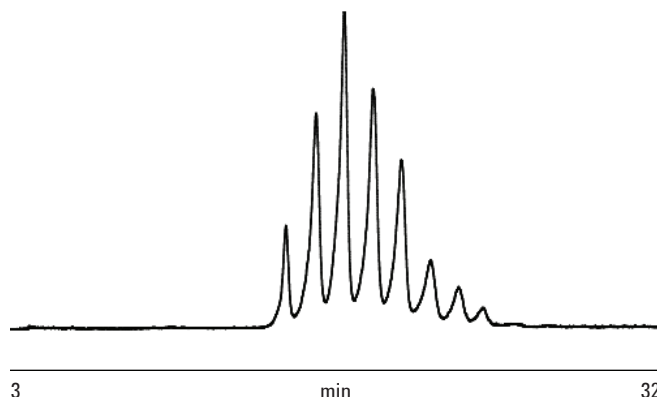


Figure 2. Separation of individual oligomers in polystyrene 580 with a PLRP-S column and 380-LC ELSD.

Conclusion

The 380-LC evaporative light scattering detector and PLRP-S columns successfully separated individual oligomers in two, low molecular weight polystyrenes.

PLRP-S columns are the preferred choice for the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules may be retained for much longer, resulting in greater resolution.

PLRP-S columns used with the 380-LC is an ideal combination for the accurate and sensitive separation of small molecule components.

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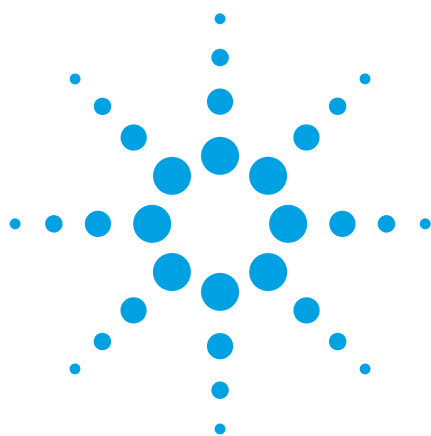
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Agilent Technologies



Fingerprinting Isocyanate Pre-polymer Fractions by Agilent PLgel MIXED-E and GPC

Application Note

Materials Testing and Research, Polymers

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Introduction

Isocyanate polymers are important commercial materials. The finished isocyanate resins are produced by setting pre-polymer formulations containing oligomers and low molecular weight polymers with an appropriate hardener. Unsurprisingly, the formulation of pre-polymer mixtures can have a profound effect on the setting characteristics of the oligomers and therefore the physical properties of the final product. Quality and process control requires that the isocyanate pre-polymer formulations be analyzed, and the oligomer distribution and polymer molecular weight distribution characterized accurately.



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For the analysis of samples where both the oligomeric and polymeric components are of interest, high efficiency Agilent PLgel 3 μ m MIXED-E, 300 $\times$ 7.5 mm columns, with an exclusion limit of 30,000 g/mol, are ideal.

Analysis of Isocyanate Pre-Polymers

Figure 1 shows two overlaid chromatograms of isocyanate pre-polymer A and B. Differences can be detected between the oligomeric distribution of both samples. The presence of different peaks due to polymers can also be identified between the samples, demonstrating how PLgel 3 μ m MIXED-E columns could be used to fingerprint a desired oligomer/polymer distribution in a sample as part of a rigorous quality control environment.

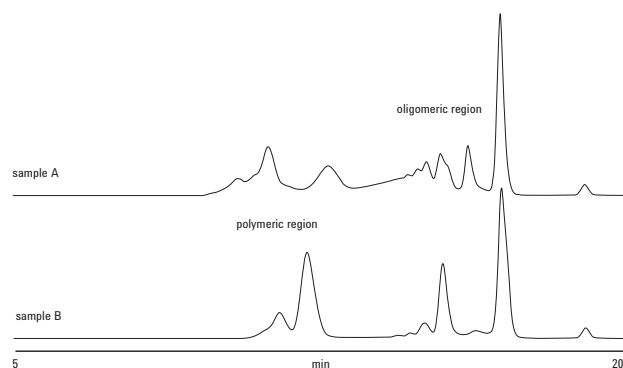


Figure 1. Oligomer and polymer fractions of an isocyanate revealed by an Agilent PLgel 3 μ m MIXED-E, three-column set

Conditions

Samples	Isocyanate pre-polymers, 0.4% (w/v)
Column	2 $\times$ Agilent PLgel 3 μ m MIXED-E, 300 $\times$ 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow Rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

Agilent PLgel columns with small pore sizes can be used to analyze low MW isocyanates to reveal details of their oligomeric distribution, a property that affects many of their applications in end-use.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com.

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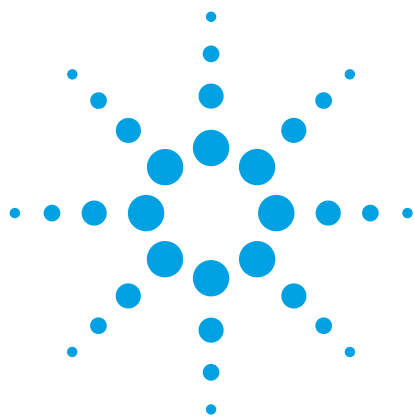
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Polybutadiene Terephthalate Analysis by Agilent PL HFIPgel and GPC

Application Note

Materials Testing and Research, Polymers

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UK

Introduction

Polybutadiene terephthalate (PBT) resins are used in a wide variety of applications in which toughness and resistance to damage are highly advantageous. Mechanical and thermal stress during the production of molded parts can cause degradation, giving a reduction in desirable physical properties. The molecular weight distribution of the resin is a key measure of the onset of degradation and estimating the mechanical strength of the final product. Molecular weight distributions of polymers such as PBT are determined by gel permeation chromatography.

Analysis of Polybutadiene Terephthalate

PBT is soluble in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), a polar organic solvent, which is excellent for dissolving polar polymers such as polyamides and polyesters. The analysis was carried out in HFIP modified by the addition of 20 mM sodium trifluoroacetate to prevent aggregation. Two Agilent PL HFIPgel columns, designed specifically for HFIP applications, were employed for the analysis at a temperature of 40 °C. The Agilent PL-GPC 220 integrated chromatograph was used with differential refractive index and viscometry detection.

GPC coupled with a molecular weight sensitive viscometer allowed calculation of molecular weights based on hydrodynamic volume using the Universal Calibration approach, leading to molecular weights independent of the standards used to generate the column calibration. Agilent polymethylmethacrylate standards were employed for the Universal Calibration.



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Table 1 shows the molecular weight averages and intrinsic viscosity for the Valox PBT sample before and after molding as determined by GPC/viscometry. Clearly, the molecular weight distribution indicates that after molding the material has suffered from degradation and is less robust than the virgin material.

Table 1. Molecular Weight Averages and Intrinsic Viscosity of a Polybutadiene Terephthalate Resin Before and After Molding

	Mn/g mol ⁻¹	Mw/g mol ⁻¹	Intrinsic viscosity/g ⁻¹
Virgin resin	24,400	48,600	0.535
Molded part	11,200	24,000	0.306

Conditions

Samples	Polybutadiene terephthalate resin
Columns	2 × Agilent PL HFIPgel, 300 × 7.5 mm (p/n PL1114-6900HFIP)
Eluent	HFIP + 20 mM NaTFA
Flow Rate	1.0 mL/min
Inj Vol	200 µL
Temp	40 °C
Detectors	Agilent PL-GPC 220 (RI), Viscometer

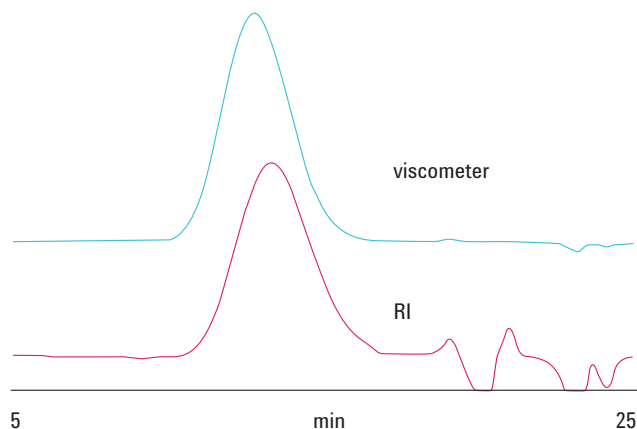


Figure 1. An example overlay of a dual-detector chromatogram of the virgin polybutadiene terephthalate resin before molding.

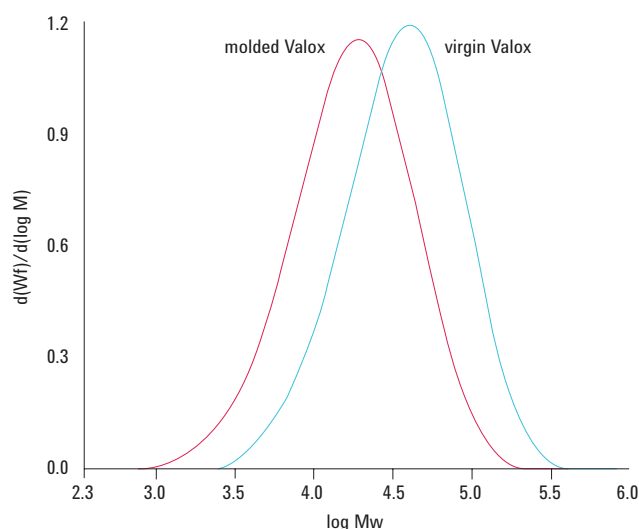


Figure 2. The resulting molecular weight distributions for polybutadiene terephthalate.

Conclusions

A sample of polybutadiene terephthalate resin was successfully analyzed on an Agilent PL HFIPgel two-column set, revealing degradation of the PBT after a molding process. These columns use a novel dispersion-polymerization process, giving near-uniform bead size and characteristics. This technology avoids the excessive calibration curvature, dislocations, and poor low molecular weight resolution associated with conventional columns that use styrene/divinyl benzene when using HFIP as solvent.

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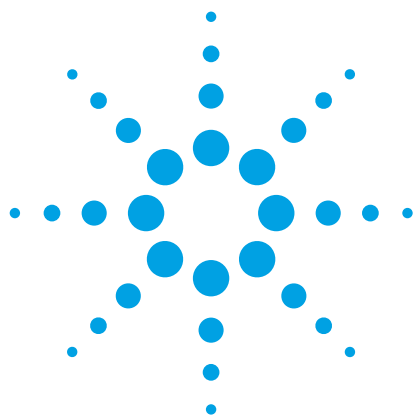
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Crystalline Polyester Analysis on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Crystalline polyesters find use in a variety of thermosetting molding compounds and are usually available in a range of molecular weight grades. Their analysis by gel permeation chromatography is straightforward using Agilent PLgel columns, with a three-column set of different pore sizes to cover a broad range of molecular weight from such a polydisperse material.



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Analysis of a Crystalline Polyester

The unsaturated crystalline polyester is soluble in dimethyl formamide (DMF) at 60 °C.

Columns Agilent PLgel 5 μm 10⁴Å, 300 × 7.5 mm
(p/n PL1110-6540)

Agilent PLgel 5 μm 500Å, 300 × 7.5 mm
(p/n PL1110-6525)

Agilent PLgel 5 μm 50Å, 300 × 7.5 mm
(p/n PL1110-6515)

Eluent DMF + 0.1% LiBr

Flow Rate 1.0 mL/min

Temp 70 °C

Detector RI

System Agilent PL-GPC 50

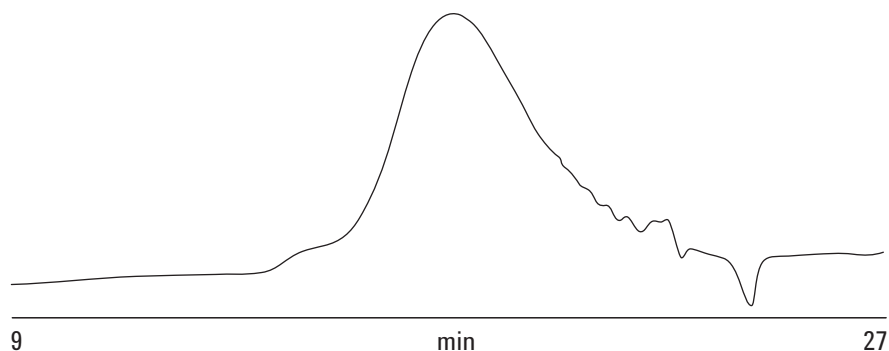


Figure 1. The molecular weight distribution of a polydisperse crystalline polyester revealed by Agilent PLgel 5 μm columns.

Conclusion

Agilent PLgel columns are suitable for analysis of the molecular weight distribution of crystalline polymers in polar solvents.

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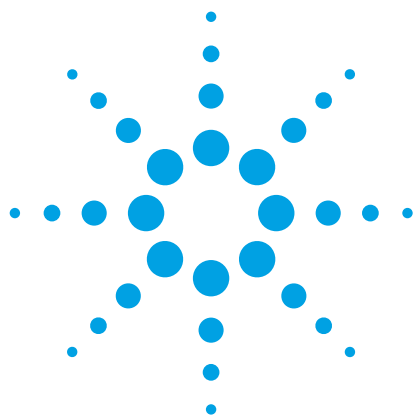
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Agilent Technologies



Unsaturated Polyester Resins on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Unsaturated polyester resins are used extensively with glass and other reinforcements in the production of composite materials. Gel permeation chromatography of these materials is employed for product development and quality control.



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Analysis of Unsaturated Polyester Resins

The distribution of a polyester resin is shown in Figure 1, with the calculated molecular weights indicated in Table 1 and Figure 2.

Calibrants	Agilent Polystyrene
Columns	Agilent PLgel 5 μm 10 ⁴ Å, 300 $\times$ 7.5 mm (p/n PL1110-6540) Agilent PLgel 5 μm 500Å, 300 $\times$ 7.5 mm (p/n PL1110-6525)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Table 1. Calculated Molecular Weights for a Sample of Unsaturated Polyester Resin

M _p	4,065
M _n	2,221
M _w	5,846
M _z	13,776
M _w /M _n	2.63

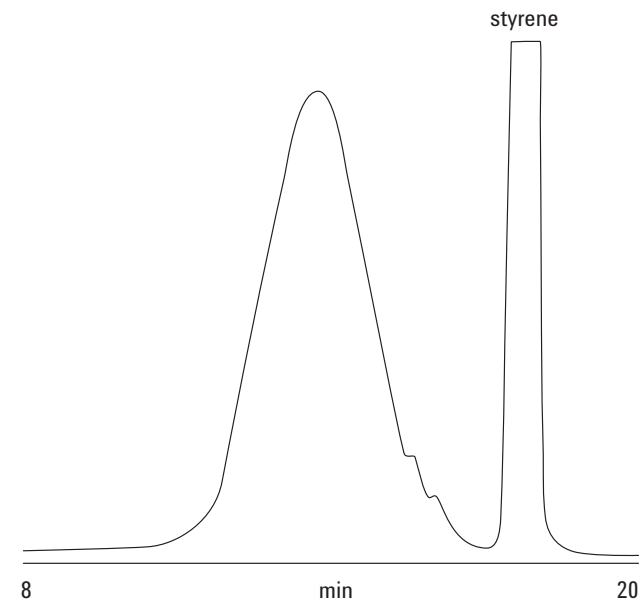


Figure 1. The distribution of an unsaturated polyester resin on Agilent PLgel 5 μm columns.

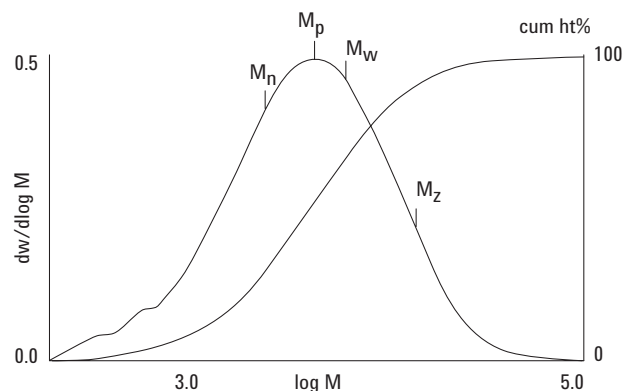


Figure 2. Molecular weights of a sample of an unsaturated polyester resin.

Conclusion

Gel permeation chromatography with Agilent PLgel columns can be used to confirm the molecular weight of polyester resins, a property that affects application in the manufacture of composite materials.

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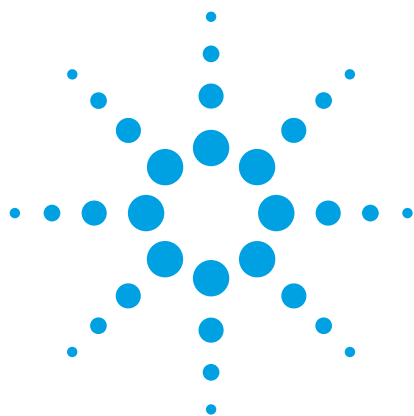
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Agilent Technologies



Styrene/Isoprene Block Copolymers with Agilent PLgel MIXED-D and GPC/SEC

Application Note

Materials Testing and Research, Polymers

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Introduction

Unlike random copolymers where two or more monomers are included in a polymer chain in a statistically random manner, block copolymers contain isolated blocks of what are essentially homopolymers linked at one or more terminal positions. This regulated structure produces materials with novel properties not observed in random copolymer analogues, typically associated with phase behaviour at interfaces. Such copolymers have been exploited for their surfactant characteristics and for their propensity to form isolated domains in thin films. The simplest block copolymers are AB diblocks consisting of two chains of different polymers (A and B) joined at one terminal. Triblock polymers contain three separate polymer chains connected in series, typically composed of two (ABA triblocks) or three (ABC) different types of polymer chain. Producing block copolymers from blocks with widely differing chemistries results in materials with very unusual properties.

A family of block copolymers of styrene (S) and isoprene (I) have been developed for their excellent adhesive properties. In these materials, SI diblock and SIS triblock copolymers are blended to give a product with the required viscosity and modulus for particular applications.

Gel permeation chromatography (GPC) can be used to estimate the relative proportion of the two components in an SI/SIS product.



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Analysis of Styrene/Isoprene Block Copolymers

In this example, the relative styrene chain lengths in the diblock and triblock materials are the same and so the diblock will have a reduced molecular weight and therefore size in solution compared to the triblock.

Using high efficiency GPC columns, the two components can be resolved based on this size difference. Figure 1 shows a chromatogram of a commercial SI/SIS blended product. The SI and SIS components have been resolved allowing quantification to take place. In this case, the SI content is approximately 42%, typical of a material that finds application in reducing the modulus of polyolefins.

Sample	Styrene isoprene block copolymer blend
Columns	2 × Agilent PLgel 5 µm MIXED-D, 300 × 7.5 mm (p/n PL1110-6504)
Eluent	THF (stabilized)
Flow rate	1.0 mL/min
Inj Vol	100 µL
Detector	RI
System	Agilent PL-GPC 50

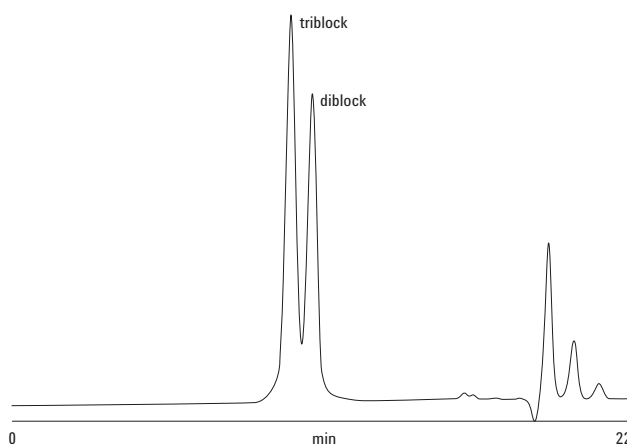


Figure 1. Chromatogram of a styrene isoprene di- and triblock copolymer blend on an Agilent PLgel 5 µm MIXED-D, column.

Conclusions

High efficiency Agilent PLgel MIXED-D columns resolve components of an isoprene/styrene block copolymer based on their size in solution. This allows quantification to take place. Typically, an increase in the proportion of diblock in the blend reduces the viscosity and modulus of the product, a feature that is particularly suited to pressure-sensitive label applications, for example. In this case, the styrene/isoprene content is approximately 42%, typical of a material used to reduce the modulus of polyolefins.

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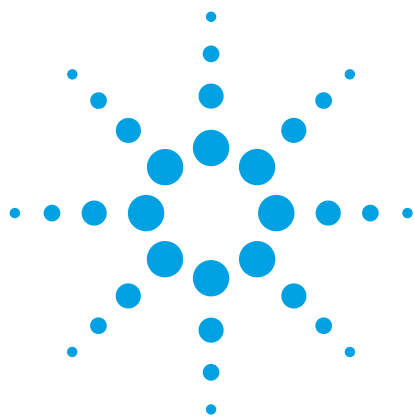
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Agilent Technologies



Polyurethane Analysis on an Agilent PLgel Column with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Low-pore-size, high efficiency Agilent PLgel columns were chosen for an analysis of polyurethane, specifically to exclude the bulk of the sample and give maximum resolution of the low molecular weight components.

Analysis of Polyurethane

Base line resolution of residual isocyanate monomers is almost achieved. The individual isocyanates (IPDI, MDI, and TDI) are identified (Figure 1).



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Conditions

Columns	Agilent PLgel 5 μm , 50Å, 300 $\times$ 7.5 mm (p/n PL1110-6515) Agilent PLgel 5 μm , 100Å, 300 $\times$ 7.5 mm (p/n PL1110-6520)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

Low-pore-size Agilent PLgel columns are ideally suited to high resolution separations of oligomers, the presence of which affects the end-use application of many materials.

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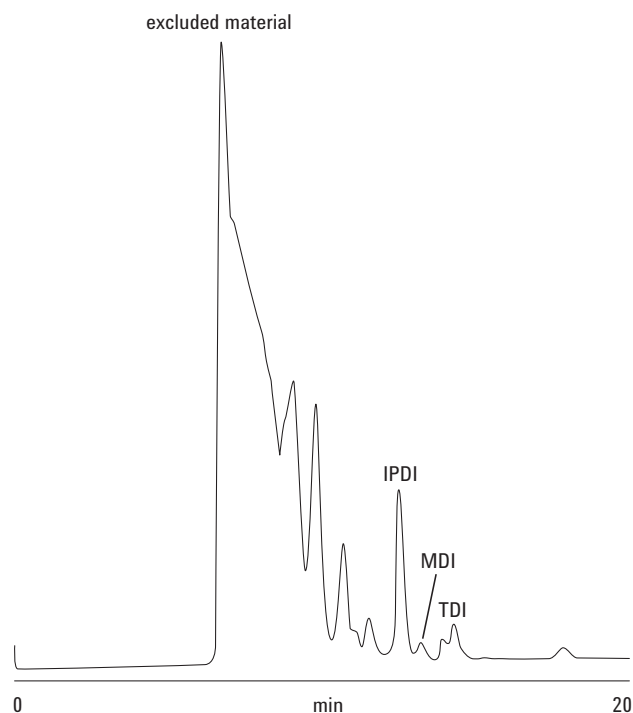


Figure 1. Identifying isocyanates in a polyurethane using Agilent PLgel 5 μm columns.

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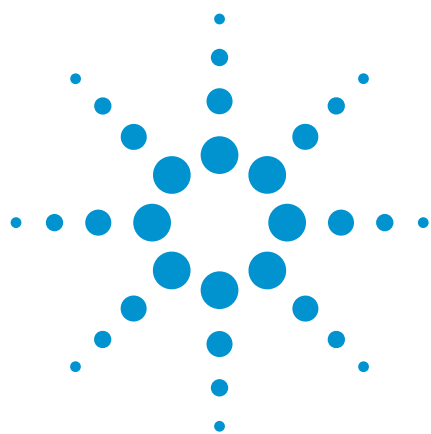
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Agilent Technologies



High Resolution Analysis of Polyethylene Glycol using HPLC with ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

PEGs are low molecular weight polymers of the general formula $\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$ and they are widely used as excipients or drug delivery agents in the pharmaceutical industry, as well as additives in cosmetics and home care products.

Oligomeric separation of low molecular weight PEGs by gradient reversed-phase HPLC is widely used to verify the composition of the polymer. However, PEG has no UV chromophore and, as a gradient elution is required, RI detection is not a viable alternative.

For non-UV absorbing compounds, the Agilent evaporative light scattering detector is the primary choice since the principle of detection does not rely on the optical properties of the solute. RI detectors generally suffer from relatively low sensitivity and poor baseline stability. In this respect, the Agilent 380-ELSD offers significant benefits as it operates over very rapid changes in eluent composition and temperature with no effect on the baseline stability, and offers very high sensitivity.

PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds because the very small pore sizes have extremely high surface areas available to the solutes. An excellent demonstration of the capability of the Agilent 380-ELSD with this type of column is illustrated by the analysis of polyethylene glycols.



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Instrumentation

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm
(p/n PL1111-3500)
Detection: Agilent 380-ELSD (neb=50 °C, evap=70 °C,
gas=1.6 SLM)

Materials and Reagents

Eluent A: Water
Eluent B: ACN

Conditions

Gradient: 10-30% B in 12 min
Flow Rate: 1.0 mL/min

Results and Discussion

Figure 1 is an overlay of the chromatograms obtained for two PEGs, with molecular weights of 400 and 1080. The high resolution achieved for the separation of individual PEG oligomers present in the two samples is clearly evident.

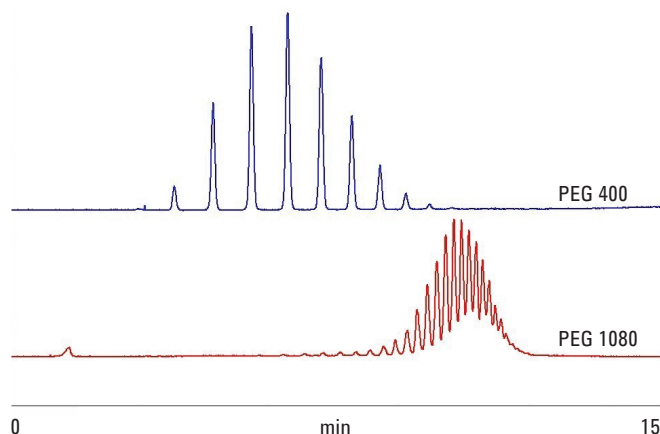


Figure 1. High resolution of PEGs with different molecular weights using a PLRP-S column and Agilent 380-ELSD.

Conclusion

The Agilent 380-ELSD combined with a PLRP-S 100Å column successfully responded to all of the PEG samples' components and offered high sensitivity and low limits of detection because of the optimum match of column, detector and sample.

PLRP-S columns are ideally suited to the analysis of many small molecules. The 100Å pore size has an exceptionally high surface area that is accessible to the solutes. It is more retentive for small molecules than the majority of alkyl bonded silicas.

PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules such as carboxylic acids may be retained much longer, resulting in greater resolution.

The Agilent 380-ELSD evaporative light scattering detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Agilent 380-ELSDs 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. The novel design of the Agilent 380-ELSD provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

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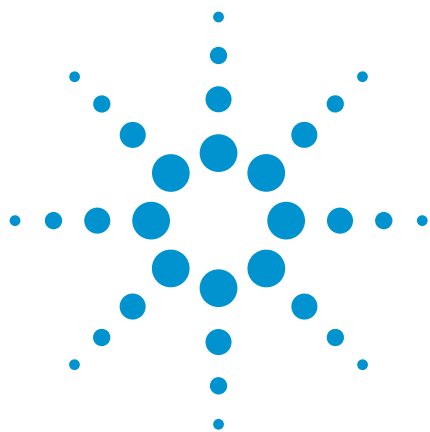
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Agilent Technologies



Fast Analysis of Light Stabilizers by HPLC with ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

Synthetic additives are added to plastics to protect them from degradation by light. There has been a lot of concern about the presence of these compounds in food packaging materials. These molecules contain radical traps in the form of CH_3 and OH groups and, consequently, are important antioxidants, as they are able to eliminate free radicals, which would otherwise cause polymer chain cleavage. Several reviews and papers have been published discussing the possibility that additives may have detrimental effects on health^{1,2}. The additives are discrete, complex, organic compounds, some of which have strong UV chromophores, whereas others do not. The compounds analyzed in this study contain good chromophores and are therefore also suited to UV detection.

However, the Agilent evaporative light scattering detector provides even greater sensitivity than detection by UV. The instrument also gives no solvent peaks and exhibits excellent baseline stability. The Agilent ELSD is renowned for its rugged design and ability to deliver high performance for demanding HPLC or GPC applications. PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds because the very small pore sizes have extremely high surface areas available to the solutes.



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Instrumentation

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm
(p/n PL1512-5500)
Detection: Agilent ELSD (neb=40 °C, evap=80 °C,
gas=1.0 SLM)

Materials and Reagents

Eluent: 90% THF, 10% Water

Sample Preparation

Tinuvin 144 (0.8 µg), Mark LA68 (8.2 µg) and Chimassorb 199FL (1 µg)

Conditions

Flow Rate: 0.5 mL/min

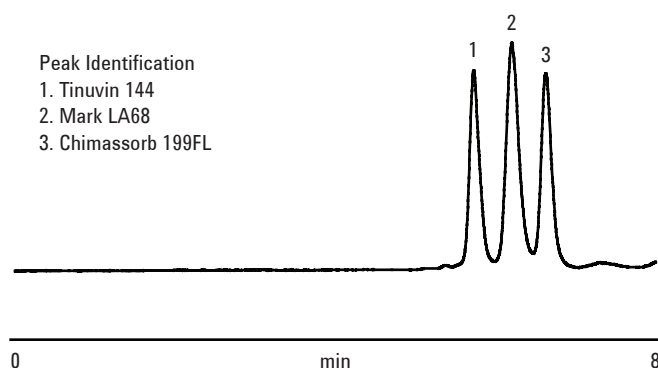


Figure 1. Chromatogram showing good separation of the three light stabilizers.

Conclusion

The Agilent evaporative light scattering detector and a PLRP-S column efficiently resolved three light stabilizing polymer additives. PLRP-S columns are ideally suited to the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules such as carboxylic acids may be retained much longer, resulting in greater resolution. PLRP-S columns used with the Agilent ELSD is an ideal combination for these challenging applications.

References

- [1] Arvanitoyannis, I.S and Bosnea, L. 2004. Migration of substances from food packaging materials to foods. *Crit. Rev. Food Sc. Nutr.* 44: 63-76
- [2] Barnes, K. Sinclair, R. and Watson, D. (eds) 2006. Chemical Migration and Food Contact Materials. *Woodhead Publishing Ltd*, Cambridge, UK. 480pp.

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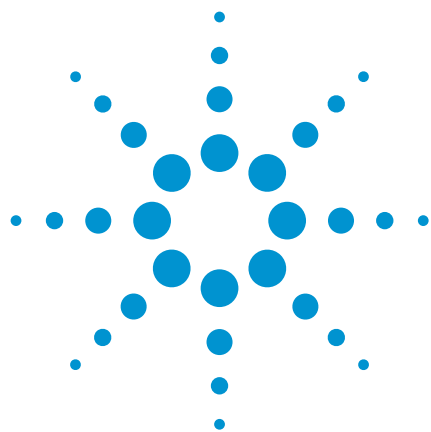
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Agilent Technologies



Sensitive Polymer Analysis using Critical Point Chromatography and ELSD

Application Note

Author

Stephen Ball
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Introduction

Liquid chromatography under critical conditions (LCCC), or critical point chromatography, is a technique used to investigate very small differences between the chemical structures of polymers. These differences could arise through the use of co-monomers or through the introduction of end-group functionality. Traditional interactive chromatographic techniques are often insensitive to small changes in structure and critical point chromatography has become the method of choice for these analyses.



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LCCC relies on carrying out isocratic chromatography at the so-called critical point for the polymer under investigation. In liquid chromatography of polymers, the samples are introduced into an eluent flowing through a column packed with porous media. Any retention of the polymer on the column media results from a reduction in the free energy of the polymer in solution, which can be described by:

$$\Delta G = \Delta H - T \Delta S$$

where ΔG is the change in Gibbs' free energy, ΔH is the change in enthalpy and ΔS is the change in entropy. Adsorption of the polymer by a reversed/normal-phase mechanism will result in a negative ΔH and, therefore, a negative ΔG , whereas if a size exclusion mechanism occurs then ΔS will be positive and again ΔG will be negative. Separation of any components of the polymer occurs if the reduction in ΔG differs between the individual components. For many polymers, controlling the choice of chromatographic eluent and column determines whether or not the retention mechanism is primarily or exclusively adsorption or size exclusion. Critical point conditions are reached for a given polymer/solvent combination when ΔH and ΔS are balanced and there is no change in ΔG during the analysis. Under these conditions, all components of a polymer with the same chemical composition will elute at the total permeation limit of the column, regardless of the molecular weight. At the critical point, the polymer is said to be 'chromatographically invisible', as no separation is obtained. For the majority of polymers, the critical point can be determined by making the appropriate selection of column, temperature and eluent. Critical point chromatography is very useful for obtaining compositional information for polymers with differing end groups or those containing co-monomers. If critical conditions are applied for one component of a sample, that component becomes chromatographically invisible and any separation observed is controlled by other components.

Critical point chromatography is carried out under isocratic conditions and, therefore, can be performed on a standard LC system composed of a pump, injection valve, a reversed/normal-phase HPLC column and concentration detector, without the need for complex equipment. LCCC is well illustrated using a PLRP-S column and the Agilent ELSD for the analysis of modified polyethylene glycol (PEG) and poly(styrene-*b*-methyl methacrylate) (PMMA).

Instrumentation

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm
(p/n PL1111-3500)

Detection: Agilent ELSD

Materials and Reagents

Analysis of PEG

Eluent: 49% Acetonitrile in Water

Analysis of PMMA

Eluent: Polystyrene - 47% ACN in THF;

Polymethylmethacrylate - 17% ACN, 17% Water, 66% THF

Conditions

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Results and Discussion

LCCC analysis of end-group modified polyethylene glycol

Critical point chromatography was used to analyze a PEG that had been modified with amine end groups (Figure 1). Critical point conditions for PEG were established by analyzing a series of PEG narrow standards of different molecular weights using different isocratic combinations of acetonitrile and water.

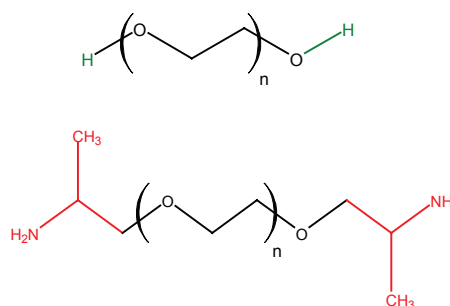


Figure 1. The structure of the original and the modified PEG materials.

Figure 2 shows chromatograms of the standards in SEC and reversed-phase mode, and at the critical point where elution is independent of molecular weight.

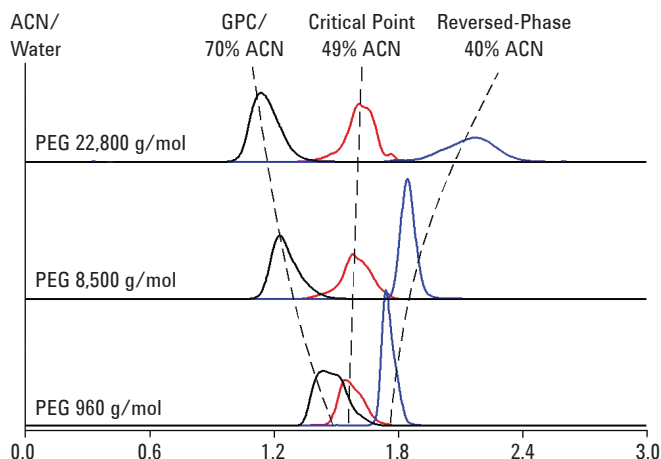


Figure 2. Analysis of PEG in SEC and reversed-phase to reveal the critical point.

Figure 3 shows a chromatogram of the amine-modified PEG material, before and after neutralization of the amine functionality with hydrochloric acid.

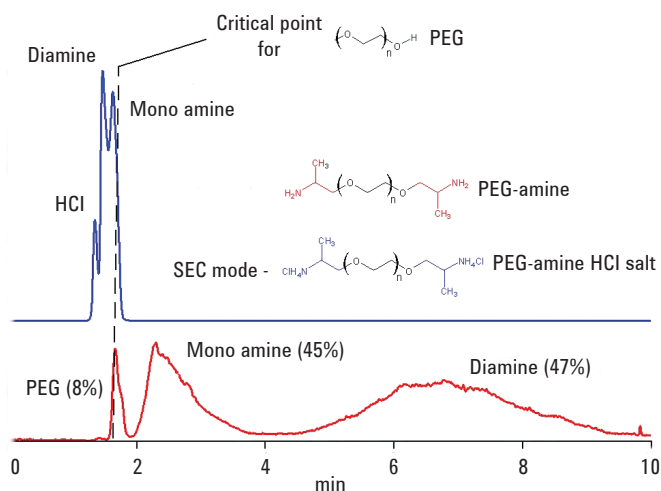


Figure 3. Amine modified PEG before and after neutralization with HCL.

Before the addition of the acid, one peak was observed at total permeation (corresponding to unmodified PEG) and two peaks were observed eluting in interactive mode (after total permeation of the column). The two peaks eluting in interactive mode were assigned as the mono and di-amine end-group modified PEGs. Based on the peak areas, the ratio of components assigned as 8% PEG, 45% mono-amine and 47% di-amine. Addition of the hydrochloric acid changed the elution to SEC mode (elution before the PEG peak), indicating the sensitivity of the chromatography at critical conditions to sample chemistry.

LCCC Analysis of Poly(styrene-*b*-methyl methacrylate)

Critical point chromatography was used to analyze a sample of poly(styrene-*b*-methyl methacrylate) block copolymer, whose structure is shown in Figure 4.

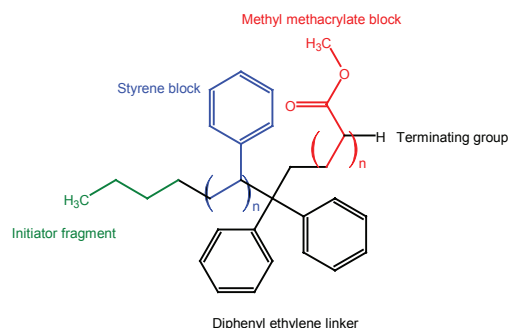


Figure 4. Structure of poly(styrene-*b*-methyl methacrylate).

Critical conditions were established for both polystyrene and polymethyl methacrylate by running narrow standards of varying molecular weight using different isocratic mixtures of solvents.

Figure 5 shows critical point diagrams for the polystyrene and polymethyl methacrylate standards.

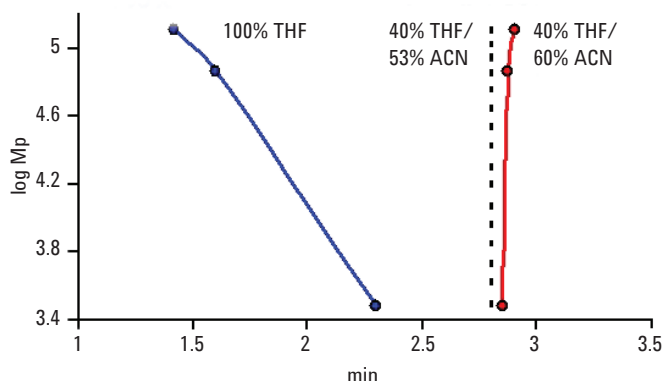


Figure 5a. Critical point diagrams for PS.

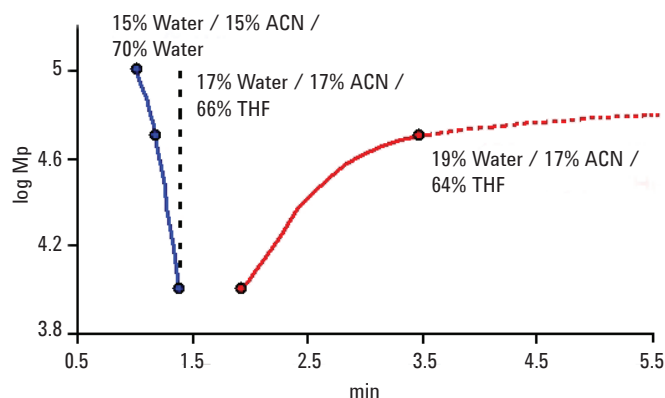


Figure 5b. Critical point diagrams for PMMA.

Under the critical conditions for PMMA, chromatography of PS resulted in elution based on adsorption mode.

Chromatograms of two polystyrene narrow standards eluting under polymethyl methacrylate critical conditions are shown in Figure 6.

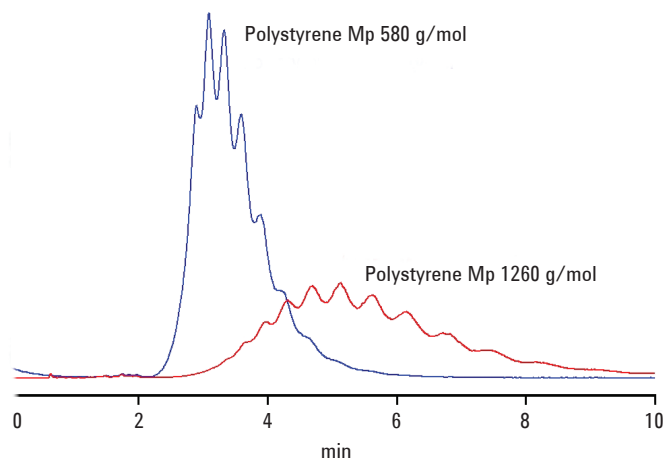


Figure 6. Chromatograms of polystyrene standards under polymethyl methacrylate critical conditions.

Under the critical conditions for PS, PMMA resulted in elution based on SEC.

Figure 7 shows chromatograms of a series of polymethyl methacrylate narrow standards and a SEC calibration curve obtained under the polystyrene critical conditions.

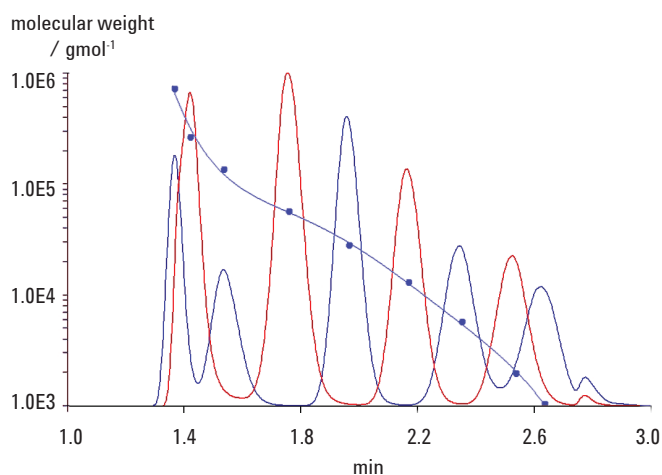


Figure 7. PMMA standards and SEC calibration curve run under PS critical conditions.

The poly(styrene-*b*-methyl methacrylate) sample was analyzed under the critical conditions for polystyrene. Using these conditions, the elution is controlled purely by the molecular weight of the methyl methacrylate block of copolymer, the polystyrene block is 'chromatographically invisible'.

Figure 8 shows the molecular weight distributions of the polystyrene block by conventional GPC using polystyrene standards before introduction of the methyl methacrylate and growth of the PMMA block, and the PMMA block under critical conditions for polystyrene using PMMA standards.

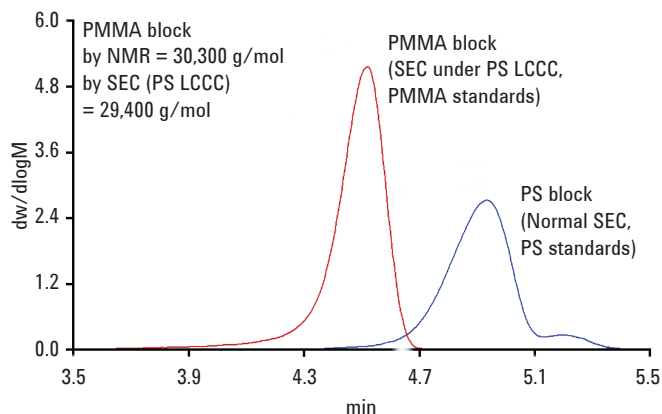


Figure 8. Molecular weight distributions of the styrene block before reaction with PMMA, and the PMMA block under critical conditions for polystyrene.

Using these results, the molecular weight of the total polymer and the comparative block lengths could be determined as approximately 71% polystyrene, 29% polymethyl methacrylate. The results showed good agreement with the results from NMR experiments.

Conclusion

Critical point chromatography is a powerful tool for analyzing small chemical changes in the structure of polymers, such as the inclusion of co-monomers to a polymer backbone or a change in end-groups.

In many cases, traditional chromatography techniques are not sufficiently sensitive to show these changes, and critical point chromatography offers a cheaper and easier route to structural analysis compared to other techniques that have been used in the past, such as NMR.

However, critical point chromatography is extremely sensitive to the chemistry of the sample and column and so a specific methodology must be developed for each application.

PLRP-S columns and the Agilent ELSD is an ideal combination for these challenging applications.

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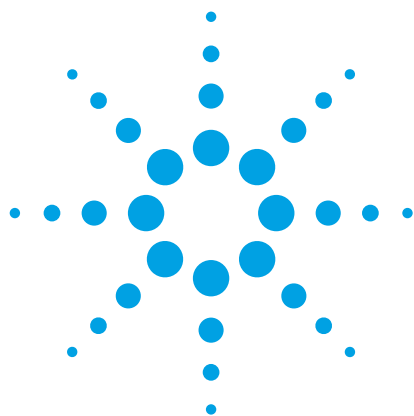
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Agilent Technologies



Mono-, Di-, and Tristearoylglycerol Analysis on Agilent PLgel by GPC

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Stearoylglycerols are esters of glycerol and stearic acid. They can be produced as stable intermediates during the microbial metabolism of vegetable oils and animal fats. Microbial transformation of natural oils is used in the search for commercially valuable compounds.

Analysis of stearoylglycerols is straightforward using gel permeation chromatography (GPC) with Agilent PLgel columns.



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Analysis of Stearoylglycerols

GPC with an Agilent PLgel 5 μm 50Å column separates mono-, di, and tristearoylglycerol from stearic acid in less than 16 minutes (Figure 1).

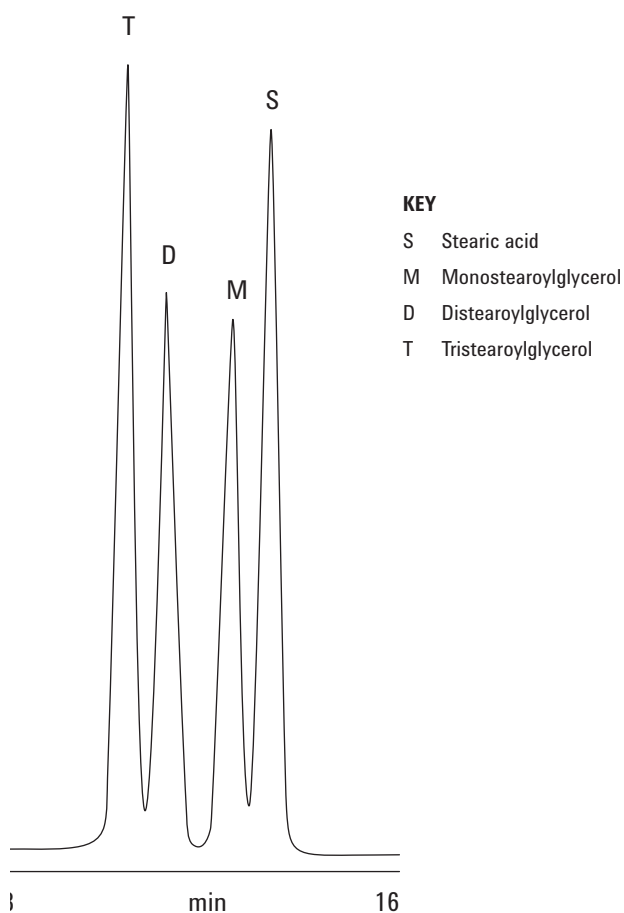


Figure 1. Stearoylglycerols separated on an Agilent PLgel 5 μm column.

Conditions

Column	Agilent PLgel 5 μm 50Å, 300 $\times$ 7.5 mm (p/n PL1110-6515)
Eluent	THF
Flow rate	0.5 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

Low-pore-size PLgel columns are well suited to the separation of complex esters produced by metabolic processes.

Acknowledgment

Data supplied by Dr G J Jones, Dept of Agricultural and Environmental Science, University of Newcastle upon Tyne, UK.

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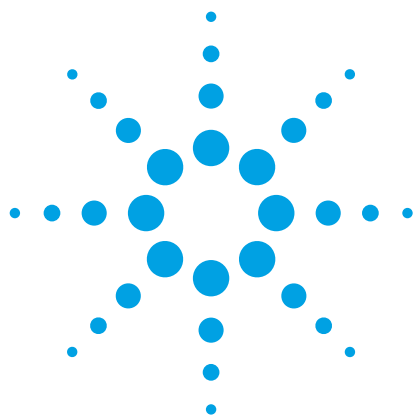
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Agilent Technologies



Polystyrene Oligomer Analysis on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

Greg Saunders and Ben MacCreath
Agilent Technologies (UK) Ltd
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Introduction

High performance, low pore size Agilent PLgel columns are ideal for resolving low molecular weight polystyrene standards into their oligomeric components, indicating several peaks for one standard.



Agilent Technologies

Analysis of Polystyrene Oligomers

Structures of low molecular weight polystyrene oligomers are shown in Figure 1. The lowest MW peak can be assigned using phenyl hexane as a marker. This is styrene monomer plus the initiator fragment, with a MW of 162. Successive peaks increase in molecular weight by the MW of the styrene repeat unit, 104 (Figure 2).

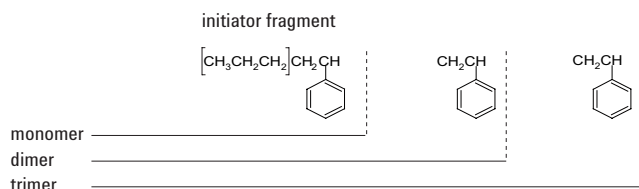


Figure 1. Structures of low molecular weight polystyrene oligomers.

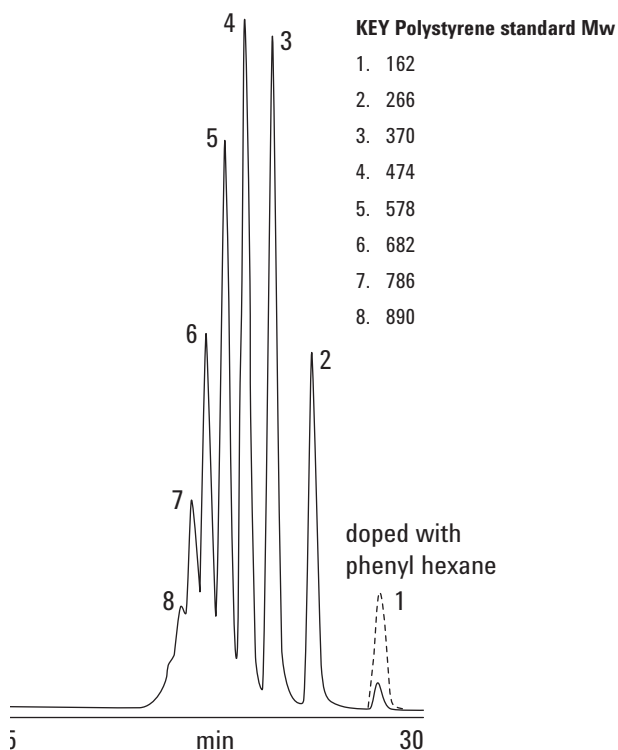


Figure 2. Low molecular weight oligomers of polystyrene on an Agilent PLgel 5 μ m two-column set.

Conditions

Columns	2 $\times$ Agilent PLgel 5 μ m 100Å, 600 $\times$ 7.5 mm (p/n PL1110-8520)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

High resolution GPC with low-pore-size PLgel columns can be used to resolve individual oligomers of polystyrene.

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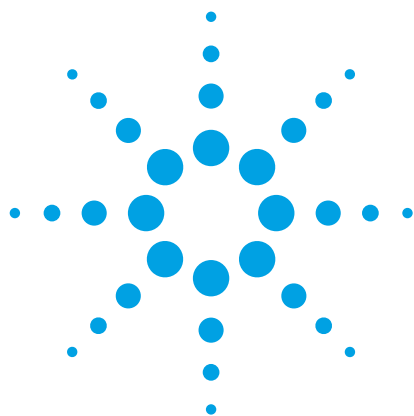
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Dialkyl Phthalate Analysis on Agilent PLgel 5 μ m using Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Phthalates are phthalic acid esters mainly used as softeners of polyvinylchlorides, to increase their flexibility, transparency, durability, and longevity. Although phthalates have been very valuable in this role, they are being phased out because of health concerns. This has increased attention on their analysis, which is straightforward using gel permeation chromatography and an Agilent PLgel column.



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Analysis of Dialkyl Phthalate

A single Agilent PLgel high performance, low pore size column achieves rapid analysis of dialkyl phthalates, as shown in Figure 1. Resolution would be further improved by increasing the number of columns.

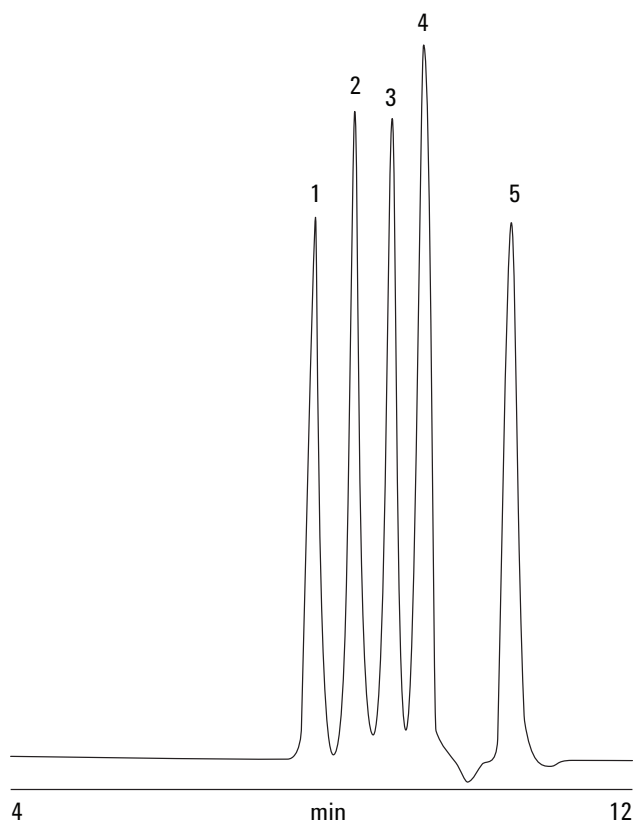


Figure 1. Four dialkyl phthalates separated on an Agilent PLgel 5 μ m column.

Conditions

Column	Agilent PLgel 5 μ m 50Å, 300 $\times$ 7.5 mm (p/n PL1110-6515)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

Small-molecule separations are possible on low-pore-size PLgel GPC columns, showing good resolution.

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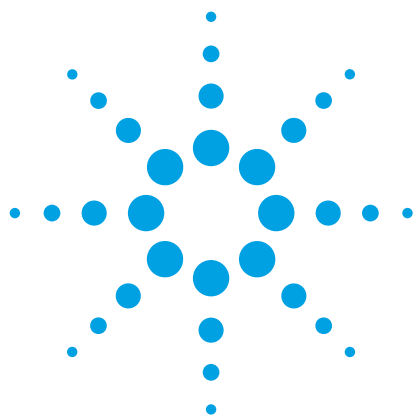
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Polyester Polyol Analysis on Agilent PLgel 5 μ m using Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Polyols are alcohols containing multiple hydroxyl groups. They are mainly used as reactants to make polymers such as polyester polyol, by condensation or step-growth polymerization of a diol polyol with dicarboxylic acid. Polyester polyols are themselves reacted with polyisocyanates to make polyurethanes for rigid-foam, flame-retardant building board. Natural oil polyester polyols from vegetable oils are beginning to replace some epoxide-based polyols.

Analysis of polyester polyols is easy with gel permeation chromatography and an Agilent PLgel column.



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Analysis of Polyester Polyol

Figure 1 shows the excellent oligomer resolution achieved for a relatively low molecular weight polyester polyol.

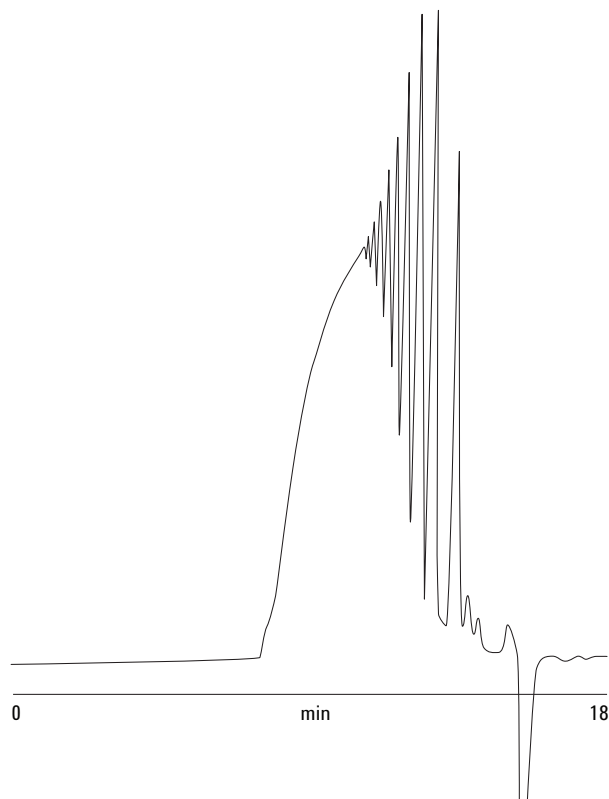


Figure 1. Excellent separation of oligomers in a polyester polyol using Agilent PLgel columns.

Conditions

Column	2 × Agilent PLgel 5 μ m 500Å, 300 × 7.5 mm (p/n PL1110-6525)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusion

Low-pore-size PLgel columns can resolve oligomeric content from polymer samples, an important component that determines the behaviour of many polymers.

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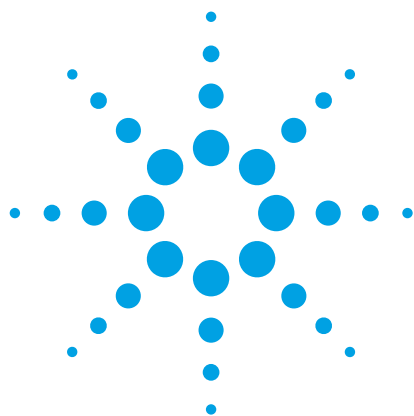
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Agilent Technologies



Dialkyl Phthalate Analysis on Agilent PLgel 3 μ m with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Many commercial polymers contain additives that improve the physical properties of the bulk material. The analysis and quantitation of polymer additives is of vital importance for quality and process control. One such group of additives are the dialkyl phthalates, which act as plasticizers in many polymers.

Agilent PLgel 3 μ m 100Å columns have been specifically designed for the analysis of low molecular weight discrete molecules such as dialkyl phthalates. Using these columns, polymers with molecular weights over 4,000 g/mol are excluded so only the low molecular weight additives are resolved.



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Analysis of Dialkyl Phthalates

Analyte samples were made up at 0.2% (w/v) in tetrahydrofuran and injected without further treatment. Figure 1 shows a chromatogram of four dialkyl phthalates and toluene, demonstrating the base line resolution of Agilent PLgel 3 μ m 100Å columns with low molecular weight species.

Conditions

Samples	Dialkyl phthalates, 0.2% (w/v)
Columns	2 $\times$ Agilent PLgel 3 μ m 100Å, 300 $\times$ 7.5 mm (p/n PL1110-6320)
Eluent	THF
Flow rate	1.0 mL/min
Injection volume	20 μ L
Detector	RI
System	Agilent PL-GPC 50

KEY

1. Dioctyl phthalate
2. Di-n-butyl phthalate
3. Diethyl phthalate
4. Dimethyl phthalate
5. Toluene

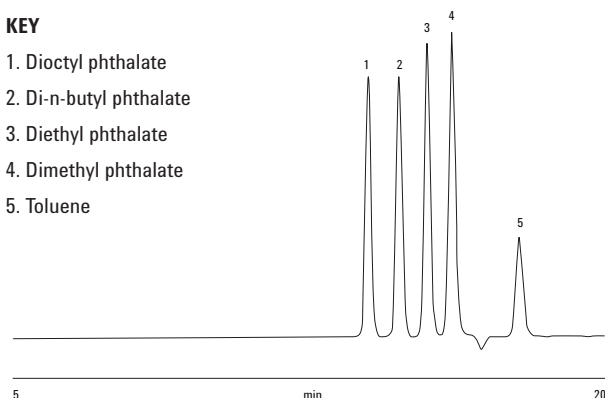


Figure 1. Four dialkyl phthalates separated on an Agilent PLgel 3 μ m two-column set.

Conclusions

Agilent low-pore-size PLgel columns can be used to separate low molecular weight molecules with very high resolution.

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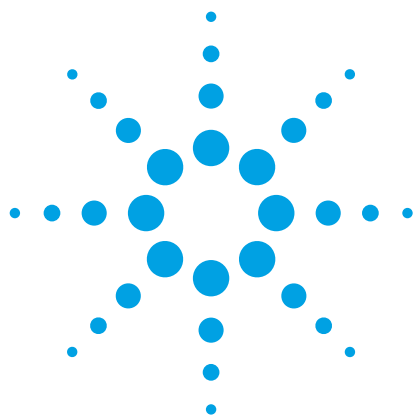
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Agilent Technologies



Polyvinylidene Fluoride Analysis on Agilent PLgel 10 μ m MIXED-B and GPC/SEC

Application Note

Materials Testing and Research, Polymers

Authors

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Introduction

Fluoropolymers are important materials with a wide range of uses from non-stick coatings to inert packaging materials. However, fluoropolymers can be difficult to analyze by gel permeation chromatography due to their low solubility in common solvents. This concern can be overcome by using dimethyl sulfoxide as solvent, Agilent PLgel MIXED-B columns, and operating the GPC system at elevated temperature with appropriate high temperature sample preparation.

Analysis of Polyvinylidene Fluoride

Three samples of polyvinylidene fluoride (PVDF) were analyzed in dimethyl sulfoxide at 95 °C using the Agilent PL-GPC 220 instrument with two Agilent PLgel 10 μ m MIXED-B, 300 $\times$ 7.5 mm columns. For this application, elevated temperatures are required to dissolve the samples and to reduce the viscosity of the solvent that would otherwise result in high back pressures at flow rates of 1.0 mL/min, and poor chromatography.

The samples were prepared at a nominal 3 mg/mL. An appropriate amount of each sample was weighed into a vial, the eluent added and the solution heated at 95 °C for 4 h in the Agilent PL-SP 260 sample preparation unit.

The solutions were then filtered and dispensed into the PL-GPC 220 autosampler vials. The samples were analyzed on the Agilent PL-GPC 220 with the autosampler carousel temperature at 95 °C and 40 °C in the hot and warm zones, respectively.



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The system was calibrated using Agilent narrow polydispersity polymethylmethacrylate (PMMA) standards. When using polar organic solvents, polystyrene standards may be soluble but they exhibit hydrophobic interactions with the column, resulting in non-meaningful molecular weight assignments.

Conditions

Samples	Polyvinylidene fluoride
Columns	2 × Agilent PLgel 10 µm MIXED-B, 300 × 7.5 mm (p/n PL1110-6100)
Eluent	Dimethyl sulfoxide
Flow rate	1.0 mL/min
Injection volume	100 µL
Temp	95 °C
System	PL-GPC 220

Figure 1 shows raw data chromatograms of the three samples. The positive peaks that elute after the samples are due to imbalances between the solvent in which the sample is prepared and the eluent.

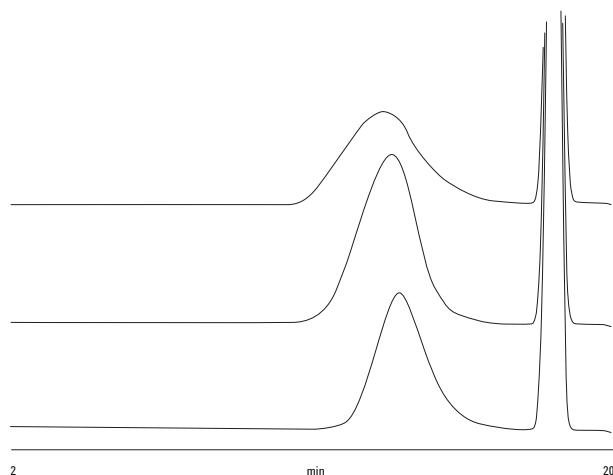


Figure 1. Three samples of polyvinylidene fluoride separated on an Agilent PLgel 10 µm MIXED-B two-column set.

Figure 2 shows overlaid molecular weight distributions of the three samples based on polymethylmethacrylate standards.

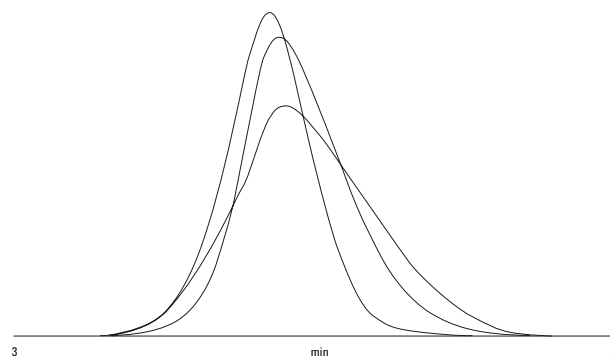


Figure 2. Overlaid molecular weight distributions of three samples of polyvinylidene fluoride based on polymethylmethacrylate standards.

Conclusions

Agilent PLgel columns can be used in a wide range of solvents, including polar organics, which facilitate the analysis of very polar samples such as polyvinylidene fluoride.

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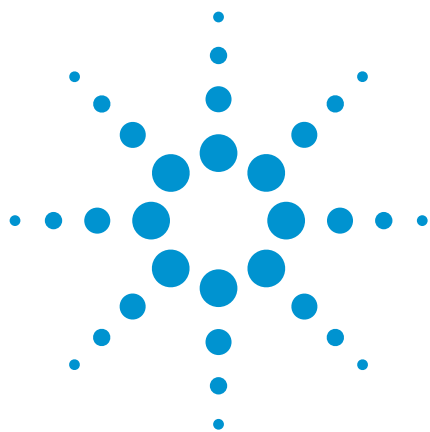
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Quantitative GPC Analysis of PVC and Plasticizers

Application Note

Author

Greg Saunders
Agilent Technologies, Inc.

Introduction

Poly(vinyl chloride) is a thermoplastic widely encountered in everyday life. It is light, non-flammable, robust and durable. PVC is permeable, does not deteriorate, is easy to maintain and its physical and mechanical characteristics make it ideal for many different uses. PVC application areas include the toiletry, food, water and car industries.

Unplasticized PVC has a high melt viscosity leading to some difficulties in processing. The finished product is also too brittle for many applications. In order to overcome these problems, it is routine to incorporate additives to the PVC. In addition to acting as impact modifiers, a number of polymeric additives may be considered as processing aids. Such materials are primarily included to ensure more uniform flow and hence improve surface finish. The properties of the final material are dependent on the molecular weight distribution of the PVC and the type and level of the added plasticizers. The analysis of the compounded material is, therefore, of primary importance, and GPC is the ideal analytical tool for its characterization.

With their linear resolving capability over a wide molecular weight range, PLgel MIXED columns provide resolution of both polymer and additives, particularly with the high efficiency 5 μ m particle size columns.



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Conditions

Columns: PLgel 5 μ m MIXED-C, 600 x 7.5 mm (p/n: PL1110-8500)
Eluent: THF
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Figure 1 shows the analysis of plasticized PVC. The high efficiency PLgel 5 μ m MIXED-C column provides sufficient scope to resolve the polymer distribution and the four phthalate plasticizers for quantitative analysis.

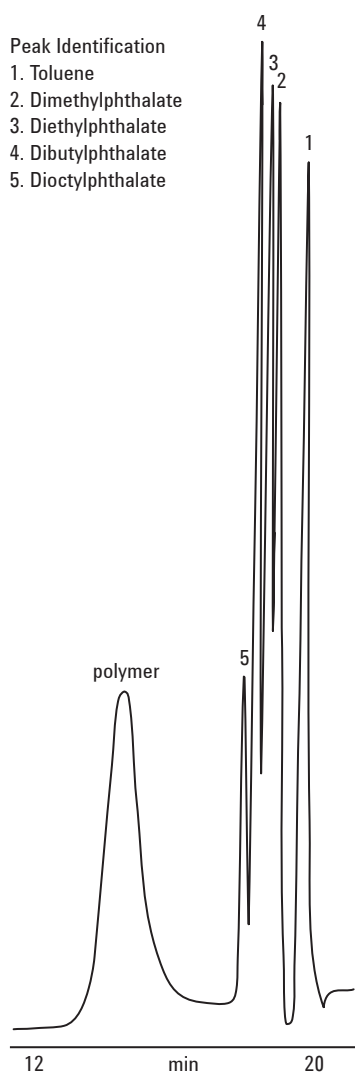


Figure 1. Plasticized PVC analysis.

Conclusion

GPC using PLgel MIXED-C columns permits the determination not only of the molecular weight distribution of PVC samples, but also the identification and quantification of their plasticizers.

PLgel 5 μ m MIXED-C columns are designed for rapid polymer analysis. With its linear calibration up to 2 million MW, this is the column of choice for highest resolution and accuracy in molecular weight distribution analyses. Rapid solvent change capability, excellent temperature stability and the high resolution of the PLgel 5 μ m MIXED-C also provide the versatility essential for the modern R&D laboratory.

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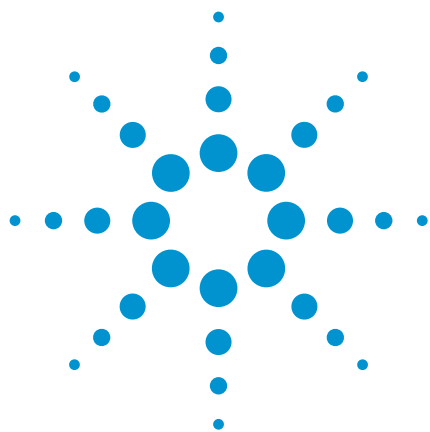
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Agilent Technologies



Optimal Analysis of Epoxy Resins by GPC with ELSD

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Epoxy resins are used widely in industry because of their strong adhesive properties, chemical resistance, mechanical toughness and high electrical insulation. Consequently, they are employed in adhesives, stabilizers, sealants, varnishes and paints. There are various types of epoxy resins that correspond to differences in molecular weight distribution and oligomeric profile. The distribution of oligomers in epoxy resins dictates their physical nature and so the characterization of epoxy resins is highly important in their quality and process control. The analysis of low molecular weight epoxy resins can be achieved by GPC or HPLC using RI or UV detection. RI detection is typically used for isocratic separations, whereas for gradient analysis UV detection is usually favored. While these detection methods are suitable for simple epoxy resin determination, they are insufficient for analyzing complex mixtures of epoxy resins and non-UV absorbing impurities. Evaporative light scattering detection (ELSD) offers significant benefits over RI and UV, as it is not dependent on the optical properties of the compound. ELSD can detect any compound that is less volatile than the mobile phase; it is compatible with a wide range of solvents, is insensitive to solvent gradients and displays excellent baseline stability. A PLgel MIXED column of appropriate pore size is the optimum choice for polydisperse compounds. In this case, the preferred option is a PLgel 3 μm MIXED-E column, an ultra high efficiency column for low molecular weight compounds. The benefits of combining ELSD with a PLgel 3 μm MIXED-E column are highlighted in the analysis of epoxy resins, such as epikotes.



Agilent Technologies

Instrumentation

Columns: 2 x PLgel 3 μ m MIXED-E, 300 x 7.5 mm (p/n PL1110-6300)
Detection: Agilent 380-ELSD (neb=50 °C, evap=90 °C, gas=0.8 SLM)

Materials and Reagents

Eluent: THF

Sample Preparation

Sample: Epikote 1001 (Hexion Specialty Chemicals, Columbus OH, USA)

Conditions

To achieve optimum sensitivity on the Agilent 380-ELSD, the evaporator temperature was set to 90 °C as the analyte is non-volatile. In addition, the gas flow was turned off, because the mobile phase of THF was easily removed at this temperature.

Flow Rate: 1.0 mL/min
Injection Volume: 10 μ L

Results and Discussion

The low dispersion of the Agilent 380-ELSD produced peak shapes comparable to those obtained by UV, and more responsive than RI detection, as shown in Figure 1.

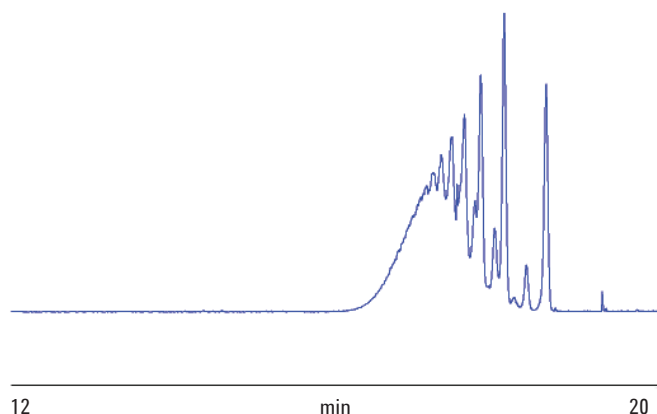


Figure 1. Chromatogram showing the detection of the main sample peak and two contaminants.

Conclusion

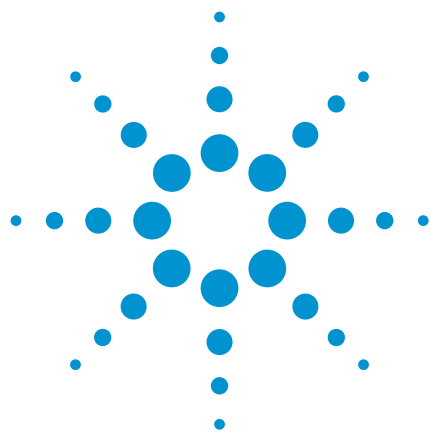
The Agilent 380-ELSD was successfully used to elucidate molecular weight distribution of an epoxy resin. Coupled with a PLgel 3 μ m MIXED-E column, optimized for the rapid analysis of low molecular weight materials below 30,000 MW, the ELS detector was more responsive than RI because it is independent from the optical characteristics of the analyte. This system is also suited to the analysis of prepolymers, oils and additives.

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Agilent Technologies



Stable Baselines in the Analysis of Poly(lactide-*co*-glycolide) Polymers by GPC with ELSD

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Poly(lactide-*co*-glycolide) copolymers are extensively used in the pharmaceutical and medical industries, for example, as absorbable sutures, surgical clips and staples. The molecular weight distribution of the polymer can affect the properties of the end product, and is therefore of interest in the areas of development and quality control. The copolymer is quite polar in nature, but can be dissolved in several solvents suitable for gel permeation chromatography, notably tetrahydrofuran and chloroform. Low boiling solvents like chloroform can suffer from outgassing effects. When employing refractive index detection, this can lead to chromatograms with noisy or drifting baselines. The Agilent ELSD always delivers baselines that are stable and drift-free. Furthermore, due to its evaporative nature, it provides chromatograms that are free from system peaks around total permeation that are commonly associated with RI detectors. The Agilent ELSD also offers superior sensitivity compared to RI. Poly(lactide-*co*-glycolide) copolymers are relatively low in molecular weight. PLgel 5 μ m MIXED-D columns, with their high efficiency (>50,000 plates/meter) and broad resolving molecular weight range (up to 400,000 daltons relative to polystyrene), are the columns of choice for this application.



Agilent Technologies

Instrumentation

Columns: 2 x PLgel 5 μ m MIXED-D, 300 x 7.5 mm (p/n PL1110-6504)
Detection: Agilent ELSD

Materials and Reagents

Eluent: Chloroform

Conditions

Flow Rate: 1.0 mL/min

Results and Discussion

Figure 1 shows a typical raw data chromatogram for a poly(lactide-co-glycolide) sample. The system was calibrated with narrow EasiCal PS-2 polystyrene standards and the calibration curve is presented in Figure 2. The molecular weight distribution plot and calculated molecular weight averages for the sample are illustrated in Figure 3.

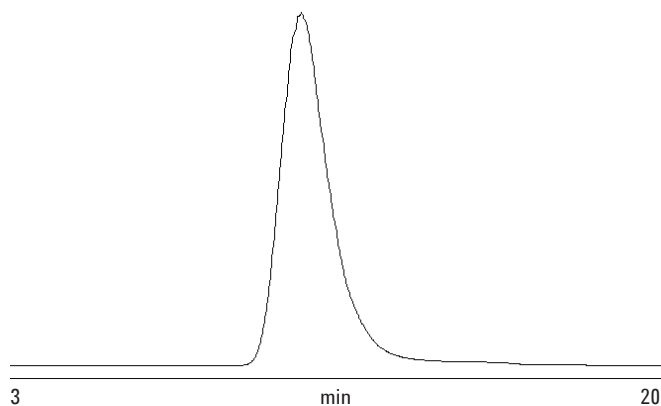


Figure 1. Excellent base line stability in poly(lactide-co-glycolide) analyzed by the Agilent ELSD.

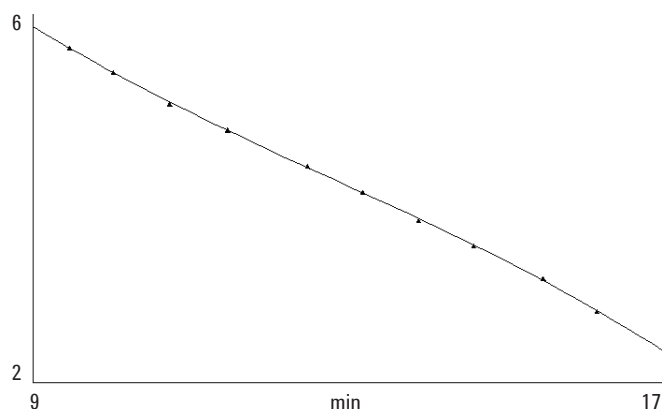


Figure 2. System calibration using EasiCal PS-2 standards.

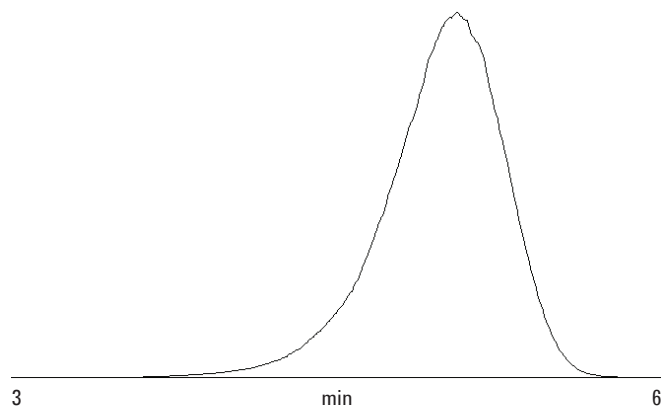


Figure 3. Molecular weight averages (M_w 110626 and M_n 70766) of poly(lactide-co-glycolide).

Conclusion

PLgel columns and the Agilent ELSD are ideal combinations for the determination of poly(lactide-*co*-glycolide) because of their very low signal to noise ratios and excellent baseline stability.

Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage.

PLgel columns and the Agilent ELSD are well suited to the separation of compounds that have no chromophores, under isocratic or gradient conditions.

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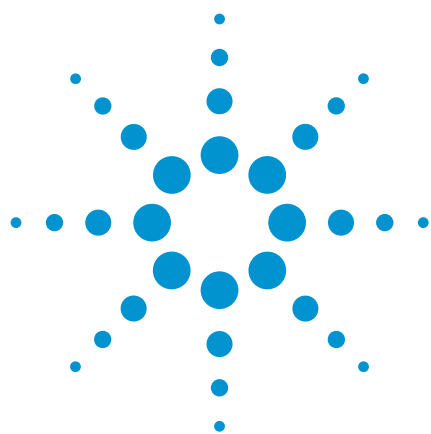
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Agilent Technologies



Superior Analysis of Alkylketene Dimers by GPC with Evaporative Light Scattering Detection

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Alkylketene dimers (AKD) are widely used as sizing agents in paper production. AKD is not a pure material because it is prepared from commercial grades of stearic acid that contain a range of fatty acid impurities with chain lengths varying from C14 to C20, hence the pluralization to dimers.

Gel permeation chromatography is used for both qualitative and quantitative analysis of AKD. The level of AKD present in the sizing fluid can be monitored before, during and after the sizing procedure. The Agilent ELSD evaporative light scattering detector is an excellent detector for AKD since it provides the benefit of significantly improved signal to noise ratio as the detector response is almost independent of dn/dc effects.

The PLgel 3 μ m MIXED-E columns, with their high efficiency (>80,000 plates/meter) and broad resolving molecular weight range (up to 30,000 daltons relative to polystyrene), are the columns of choice for low molecular weight prepolymers.

Coupling PLgel MIXED-E columns with the Agilent ELSD provides an ideal combination for the analysis of alkylketene dimers.



Agilent Technologies

Instrumentation

Columns: 2 x PLgel 3 μ m MIXED-E, 300 x 7.5 mm (p/n PL1110-6300)
Detection: Agilent ELSD (neb=40 °C, evap=90 °C, gas=1.5 SLM)

Materials and Reagents

Eluent: THF

Conditions

Flow Rate: 1.0 mL/min

Results and Discussion

As the dimer species is predominant in AKD, quantitation via peak area measurements is performed (Figure 1). AKD can be present in variable quantities and the high sensitivity of the Agilent ELSD facilitates quantitation even at very low concentration.

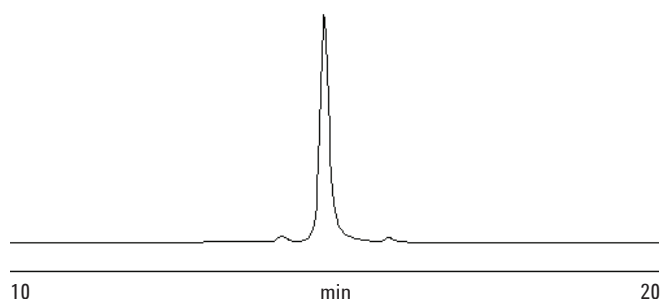


Figure 1. Typical full scale GPC chromatogram of a sample of AKD using Agilent evaporative light scattering detection.

The effectiveness of AKD as a sizing agent is related to the distribution of oligomers, which form by condensation reactions after hydrolysis in the sizing fluid. GPC separates according to molecular size in solution and is therefore ideal for monitoring the extent of the reaction. High resolution GPC columns permit separation of individual AKD oligomers as illustrated in Figure 2.

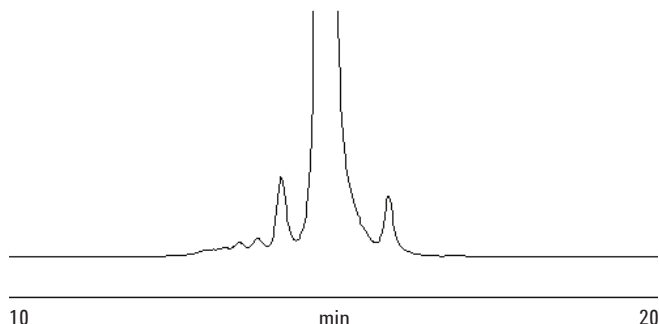


Figure 2. A close up of the region around the main AKD peak revealing the presence of smaller quantities of other oligomers.

The high sensitivity of the Agilent ELSD permits detection of the low level components and would permit a quantitative analysis of AKD products in extracts of sized paper.

Conclusion

PLgel columns and Agilent ELSD are ideal for the determination of AKD because of the system's very low signal to noise ratios and excellent baseline stability.

Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage.

PLgel columns and Agilent ELSD are well suited to the separation of compounds that have no chromophores, under isocratic or gradient conditions.

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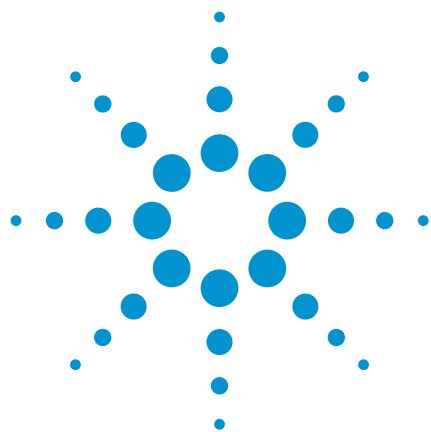
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5990-8402EN



Agilent Technologies



Effective Analysis of Plasticizers by GPC and Evaporative Light Scattering Detection

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

A common application for GPC is the analysis of polymers that contain plasticizers, where the polymer molecular weight distribution is to be determined as well as some qualitative information about the additives.

RI is often used for separation of additives, however, the Agilent ELSD is a good alternative for this application since it provides the benefit of significantly improved signal to noise ratio as the detector response is almost independent of dn/dc effects.

The PLgel 5 μ m MIXED-C columns, with their high efficiency (>50,000 plates/meter) and broad resolving molecular weight range (up to 2,000,000 daltons relative to polystyrene), are the columns of choice for mid molecular weight polymers and demanding eluents.

Identification of the plasticizer dibutylphthalate reveals that the combination of PLgel 5 μ m MIXED-C columns with the Agilent ELSD provides a good system for the discrimination of polymers and additives.



Agilent Technologies

Instrumentation

Column: PLgel 5 μ m MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Detector: Agilent ELSD (neb=40 °C, evap=90 °C, gas=1.5 SLM)

Materials and Reagents

Eluent: THF

Conditions

Flow Rate: 1.0 mL/min

Results and Discussion

Figures 1 and 2 show chromatograms from the analysis of a polymer containing dibutylphthalate (DBP) using RI and ELS detection. With relatively gentle evaporation conditions, the DBP is easily recovered from the system permitting identification and possibly quantification through calibration of peak response factor, although the more volatile toluene added as a flow rate marker is lost with the ELS.

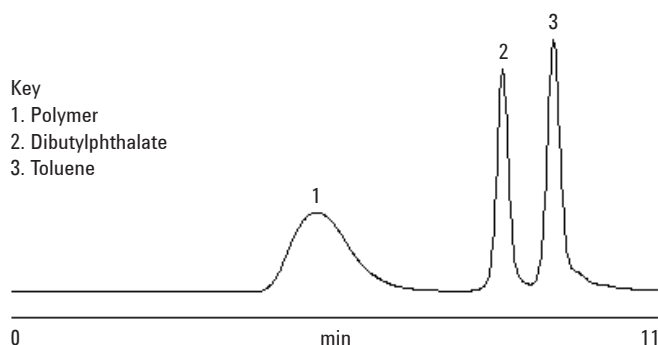


Figure 1. Separation of polymer containing dibutylphthalate using RI.

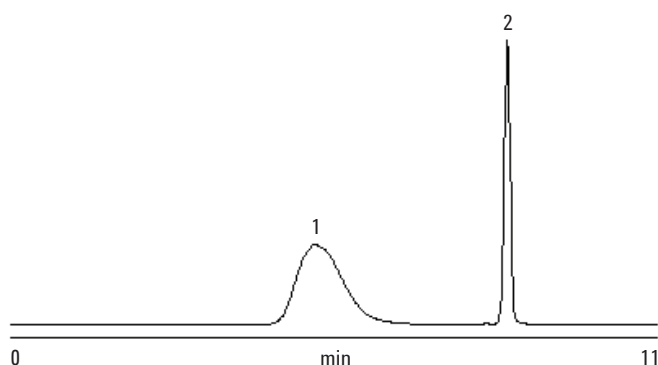


Figure 2. Separation of polymer containing dibutylphthalate using the Agilent ELSD.

Conclusion

PLgel columns and the Agilent ELSD provide a good combination for the separation of polymer additives because of the system's very low signal to noise ratios and excellent base line stability.

Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage.

PLgel columns and the Agilent ELSD are well suited to the separation of compounds that have no chromophores, under isocratic or gradient conditions.

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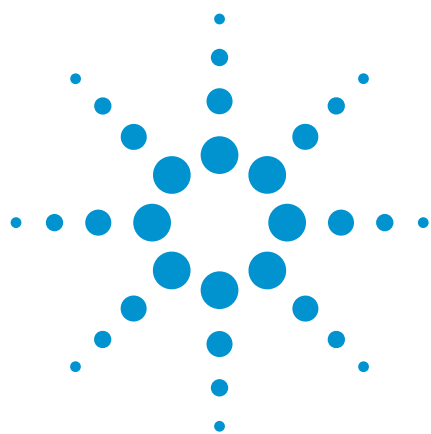
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Agilent Technologies



Sensitive Analysis of Styrene Butadiene Rubber by GPC with ELSD

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Commercial grades of styrene butadiene rubber (SBR) can contain very high molecular weight fractions and therefore, for successful GPC separations, the sample concentration must be minimized in order to avoid viscous streaming effects. Some grades of SBR can also contain low molecular weight mineral oil as a modifier (so-called oil extended grades) that can be resolved from the polymer peak, thus permitting quantification using the Agilent ELSD.

The Agilent ELSD is a good choice for this application since it provides the benefit of significantly improved signal to noise ratio as the detector response is almost independent of dn/dc effects. The PLgel 20 μ m MiniMIX-A columns, with their high efficiency (>17,000 plates/meter) and broad resolving molecular weight range (up to 40,000,000 daltons relative to polystyrene), are the columns of choice for ultra high molecular weight polymers. Identification of styrene butadiene rubber reveals that the combination of PLgel MiniMIX-A columns with the Agilent ELSD comprises an excellent system for the discrimination of polymers and additives.



Agilent Technologies

Instrumentation

Columns: 2 x PLgel 20 μ m MiniMIX-A, 250 x 4.6 mm (p/n PL1510-5200)
Detector: Agilent ELSD (neb=45 °C, evap=90 °C, gas=0.7 SLM)

Materials and Reagents

Eluent: THF

Conditions

Flow Rate: 0.3 mL/min
Loading: 1 mg/mL, 100 μ L

Results and Discussion

The high sensitivity of the Agilent ELSD permitted the polymers to be chromatographed at low loadings using the narrow bore PLgel 20 μ m MiniMIX-A columns, as shown in Figure 1.

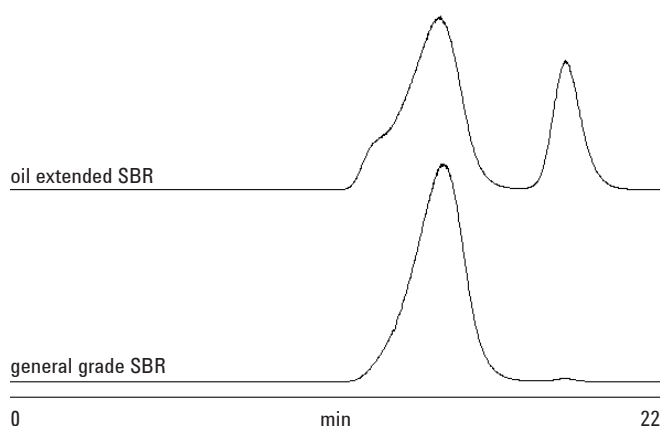


Figure 1. Oil extended SBR (above) and general grade SBR with no oil modifier (below), as revealed by the Agilent ELSD.

Conclusion

PLgel 20 μ m MiniMIX-A columns and the Agilent ELSD provide an excellent combination for the separation of styrene butadiene rubber because of the system's very low signal to noise ratios and excellent base line stability. Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. In addition, the MiniMIX variants permit low column loadings. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage. PLgel columns and Agilent ELSD are well suited to the separation of compounds that have no chromophores, under isocratic or gradient conditions.

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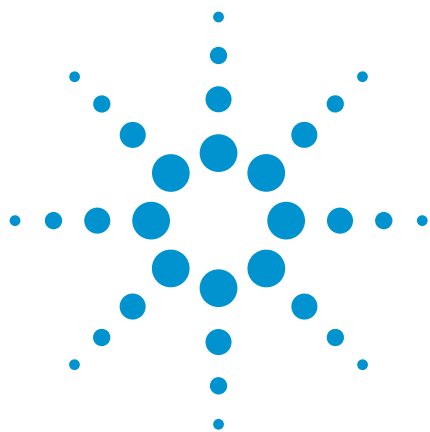
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Agilent Technologies



High Sensitivity Analysis of Natural Rubber by GPC with Evaporative Light Scattering Detection

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Solutions of natural rubber samples are generally very difficult to prepare for GPC due to the fact that the polymer contains relatively high levels of 'gel' that is partially crosslinked. Normally, an aliquot of the eluent is added to the weighed sample. This is allowed to swell and dissolve overnight, and then the gel material is filtered out (0.5 μm) prior to GPC analysis.

In this case, the actual polymer concentration can be significantly lower than the original concentration prepared depending on the gel content of the sample, and therefore detector response, usually RI, tends to be quite poor. The Agilent ELSD exhibits significantly increased sensitivity compared to an RI and gives much greater response for this application. In addition, RI baseline drift, which commonly occurs, is very much emphasized when the actual peak response is so small. The Agilent ELSD always gives a flat baseline which, together with the improved response, makes baseline and peak setting much more reliable for GPC calculations.

RI is also sensitive to system peaks around total permeation that usually occur even when samples are prepared in an aliquot of the eluent. These system peaks can interfere with low molecular weight components that are commonly found in natural rubber samples. With the Agilent ELSD system peaks are eliminated due to evaporation, leaving unadulterated sample peaks in the additives region.

The PLgel 10 μm MIXED-B columns, with their high efficiency (>35,000 plates/meter) and broad resolving molecular weight range (up to 10,000,000 daltons relative to polystyrene), are the columns of choice for high molecular weight polymers and demanding eluents.

Separation of natural rubber reveals that the combination of PLgel 10 μm MIXED-B columns with the Agilent ELSD comprises a highly sensitive system for the discrimination of additives.



Agilent Technologies

Instrumentation

Columns: 3 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (p/n PL1110-1120)
Detector: Agilent ELSD (neb=50 °C, evap=90 °C, gas=1.0 SLM)

Materials and Reagents

Eluent: Toluene

Conditions

Flow Rate: 1.0 mL/min

Results and Discussion

Figure 1 shows chromatograms of two samples of natural rubber on RI and Agilent ELSD detectors. Figure 2 is a magnified view of the additive area revealing the unadulterated peaks in this region of interest.

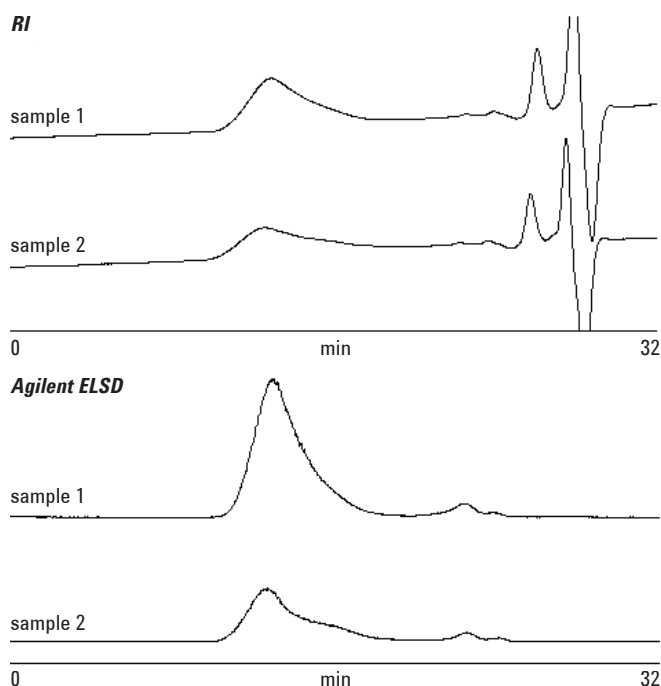


Figure 1. Stable base line and no interference from system peaks using the Agilent ELSD (below) compared to RI detection (above).

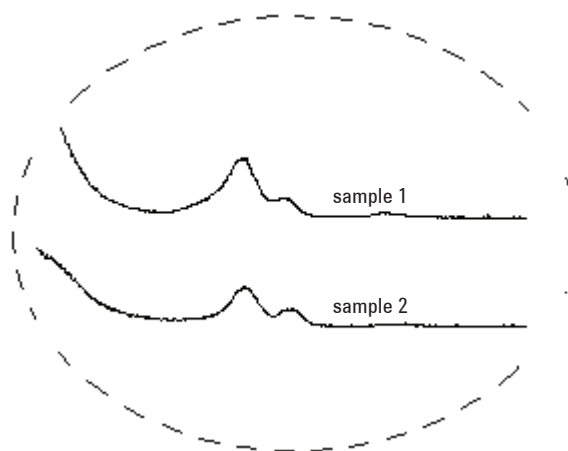


Figure 2. Magnified view of the Agilent ELSD plots showing the additive region.

Conclusion

PLgel 10 µm MIXED-B columns and the Agilent ELSD provide an excellent combination for the molecular weight determination of natural rubbers because of the system's high sensitivity, very low signal to noise ratios and excellent base line stability.

Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage.

PLgel columns and the Agilent ELSD are well suited to the separation of compounds that have no chromophores under isocratic or gradient conditions.

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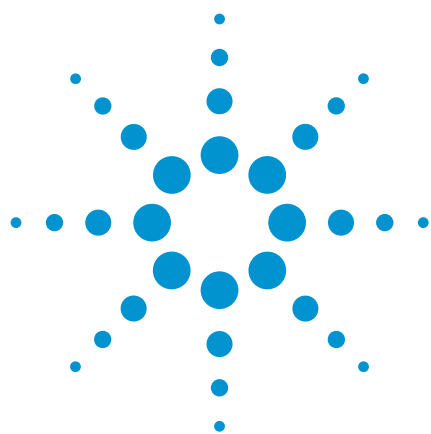
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Agilent Technologies



Rapid Quantification of Bisphenol-A by GPC with Evaporative Light Scattering Detection

Application Note

Authors

Stephen Ball and Greg Saunders
Agilent Technologies, Inc.

Introduction

Bisphenol-A is an intermediate material in the production of epoxy resins. A fast GPC method for monitoring residual levels of bisphenol-A uses a narrow bore column at relatively high eluent flow rate. Although the separation method is by GPC, the data handling and peak area quantification are carried out using a conventional LC approach. As there was a requirement to detect very low levels of the compound, evaporative light scattering detection, using the Agilent ELSD, is used in preference to RI or UV.

Agilent ELSD is a good choice for this application since it provides the benefit of significantly improved signal to noise ratio as the detector response is almost independent of dn/dc effects.

The PLgel 5 μ m MiniMIX-D column, with its high efficiency (>50,000 plates/meter) and broad resolving molecular weight range (up to 400,000 daltons relative to polystyrene), is the column of choice for resins. The combination of a PLgel 5 μ m MiniMIX-D column with the Agilent ELSD comprises an excellent system for the quantification of bisphenol-A.



Agilent Technologies

Instrumentation

Column: PLgel 5 μ m MiniMIX-D, 240 x 4.6 mm (p/n PL1510-5504)
Detector: Agilent ELSD (neb=50 °C, evap=70 °C, gas=1.0 SLM)

Materials and Reagents

Eluent: THF

Sample Preparation

A calibration curve of peak area versus mass injected was generated by preparing successive dilutions of a master solution and making flushed, full loop 20 μ L injections.

Conditions

Flow Rate: 1.0 mL/min

Results and Discussion

Figures 1 and 2 are chromatograms for the injections of the highest and lowest concentration of bisphenol-A. Figure 3 is the final calibration curve.

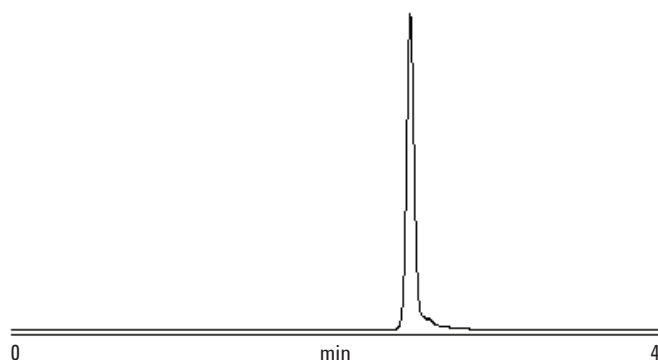


Figure 1. Raw data chromatogram of 0.5 mg bisphenol-A/ μ L.

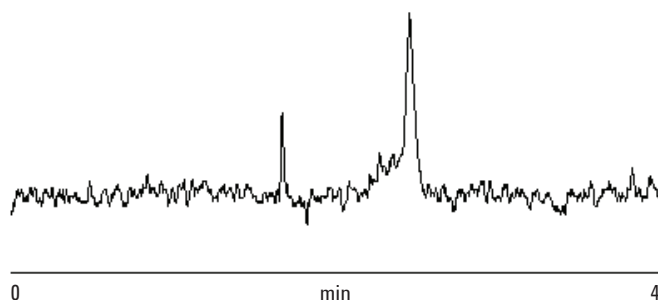


Figure 2. Raw data chromatogram of 3.9 μ g bisphenol-A/mL.

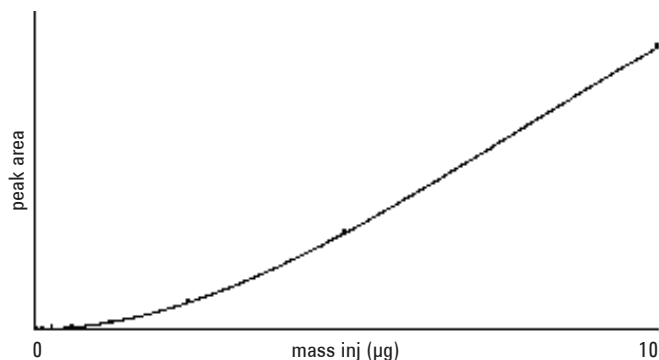


Figure 3. Calibration plot of bisphenol-A.

Conclusion

PLgel 5 μ m MiniMIX-D columns and the Agilent ELSD provide an excellent combination for the separation and quantification of bisphenol-A because of the system's high sensitivity, very low signal to noise ratios and excellent base line stability.

Mixed pore size PLgel columns offer high resolution over a specific molecular weight range. In addition, the MiniMIX variants permit low column loadings. The robust design of the Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage.

PLgel columns and the Agilent ELSD are well suited to the separation of compounds that have no chromophores under isocratic or gradient conditions.

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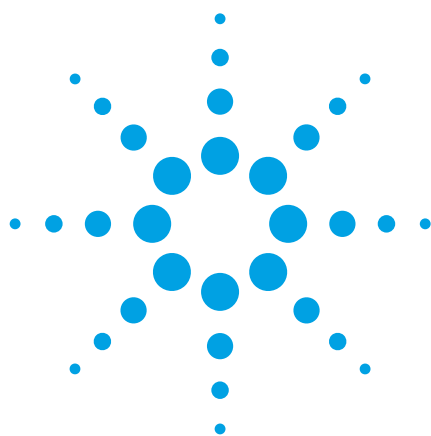
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Agilent Technologies



Phenolic Resin Analysis with Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Environmental

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Introduction

Phenol formaldehyde resins are made by the reaction of phenol and formaldehyde, though precursors occasionally include other phenols or aldehydes. Phenolic resins are mainly used to make printed circuit boards, or for adhesives, coatings, and lab bench tops.

Dimethylformamide (DMF) is a polar eluent, and is therefore suitable for the analysis of polar phenolic resins. Elevated temperature is recommended to reduce operating pressures and improve resolution. Good resolution of oligomeric species is achieved using gel permeation chromatography with Agilent PLgel 5 μm 500Å columns.

Analysis of Phenolic Resin

Two different phenolic resins were analyzed on PLgel 5 μm columns. The sample shown in Figure 1 was run on a two-column set at a lower temperature than the second sample, which was run at an elevated temperature (Figure 2). The approach adopted reflects the composition of these simple samples. For more difficult resins, Agilent PolarGel columns are recommended.



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Conditions for Figure 1

Columns Agilent PLgel 5 μ m 500Å, 7.5 $\times$ 300 mm
(p/n) PL1110-6525)
Agilent PLgel 5 μ m 100Å, 7.5 $\times$ 300 mm
(p/n PL1110-6520)
Eluent DMF
Flow rate 1.0 mL/min
Temp 50 °C
Detector RI
System Agilent PL-GPC 50

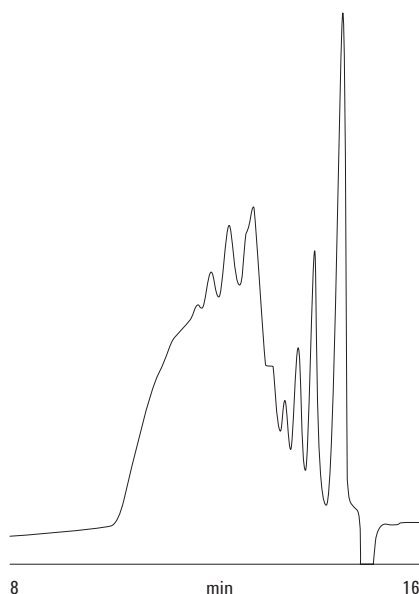


Figure 1. Oligomers in a phenolic resin separated on an Agilent PLgel 5 μ m two-column set.

Conditions for Figure 2

Columns Agilent PLgel 5 μ m 100Å, 7.5 $\times$ 300 mm
Eluent DMF
Flow rate 1.0 mL/min
Temp 50 °C
Detector: RI
System PL-GPC 50

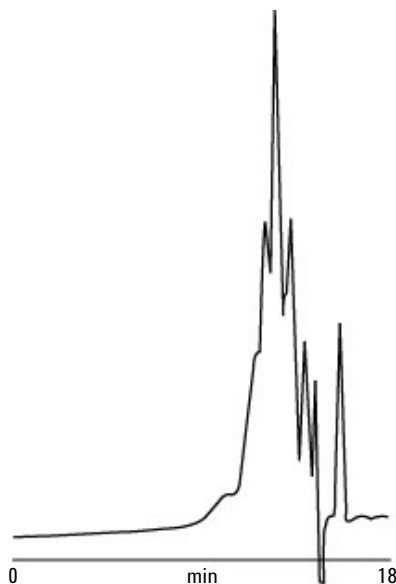


Figure 2. A phenolic resin analyzed on a single Agilent PLgel 5 μ m column.

Conclusion

Agilent PLgel columns in DMF can be used to investigate different samples of phenolic resin by gel permeation chromatography.

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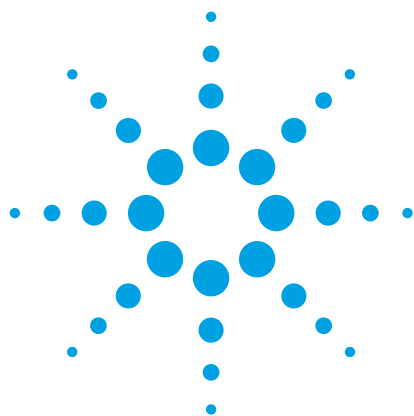
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Agilent Technologies



Polystyrene Stars on Agilent PLgel 5 μ m MIXED-C using Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Recently, there has been increasing interest in the synthesis of star-branched polymers due to their unusual flow and viscosity properties compared to linear analogues. Star-branched polymers are constructed with several arms radiating from a central core, either by preparing the individual arms and attaching them to a central molecule, the arms first approach, or by growing the polymer arms from a central core, the core first approach. Many commercial polymers can be constructed with a star-branched morphology relatively easily, but their characterization is still a challenge to the analytical chemist. Gel permeation chromatography (GPC) employing a concentration detector (typically, a refractive index detector) combined with a viscometer can be used to measure not only the molecular weight of the materials, but also to investigate the star-branched structure.

Analysis of Polystyrene Stars

GPC/viscometry was used to analyze a series of star-branched polystyrenes that had been synthesized by a core first approach, giving theoretical 5-, 14- and 21-arm structures. Figure 1 shows a dual detection chromatogram of the 14-arm star-branched polystyrene.



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Conditions

Columns	2 × Agilent PLgel 5 µm MIXED-C, 7.5 × 300 mm (p/n PL1110-6500)
Eluent	THF
Flow rate	1 mL/min
Temp	40 °C
Detector	Agilent PL-GPC 220

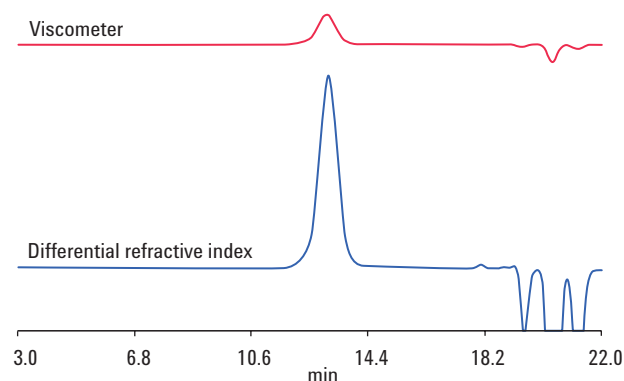


Figure 1 A 14-star-branched polystyrene detected by viscometry and refractive index.

The Universal Calibration approach was used to calculate the molecular weight averages for the star-branched polymers. The universal calibration curve was generated using linear polystyrene (PS) standards with narrow polydispersity (Figure 2).

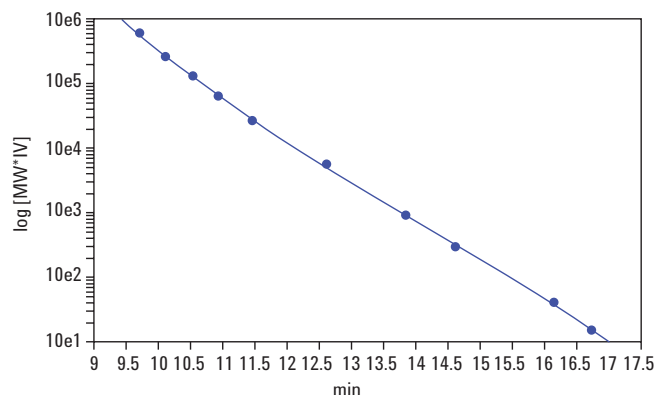


Figure 2 Linear polystyrene standards produce a Universal Calibration curve.

Based on this calibration, the molecular weight averages and weight average intrinsic viscosity (IV_w) calculated for the PS star-branched polymers are given in Table 1.

Table 1. Molecular Weight Averages and Weight Average Intrinsic Viscosity for a 14-Star-Branched Polystyrene

Polystyrene	Molecular weight averages/gmol ⁻¹						PD	IV _w
	Mp	Mn	Mw	Mz	Mz+1	Mv		
5-arm	56,120	10,460	64,856	98,594	134,877	46,292	6.20	0.28
14-arm	27,436	26,812	29,310	32,425	36,542	28,687	1.10	0.10
21-arm	149,752	111,377	157,884	201,225	256,977	141,293	1.42	0.21

Mark-Houwink plots of log intrinsic viscosity as a function of log molecular weight were calculated for the PS star-branched polymers and for a broad PS material that was known to contain no branching. Figure 3 shows an overlay of the Mark-Houwink plots obtained, indicating that increasing the number of arms on the star-branched PS results in a decrease in intrinsic viscosity at any given molecular weight when compared to the broad linear PS. However, for the 21-arm star-branched polymer, the change in intrinsic viscosity relative to the linear PS varied strongly with the molecular weight.

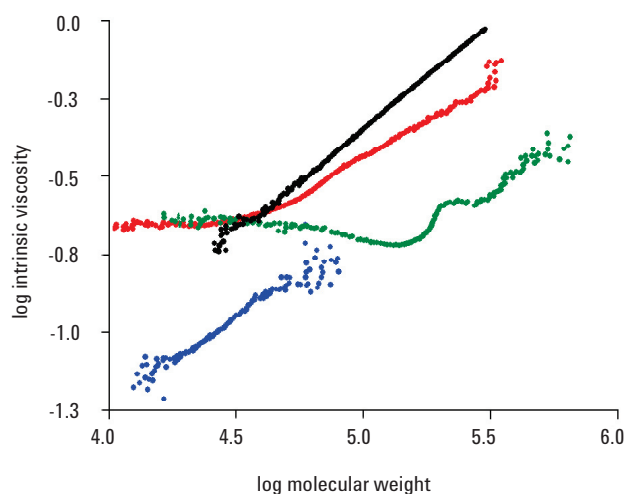


Figure 3 Overlaid Mark-Houwink plots for the linear PS (black) and the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.

Based on the linear regions in the Mark-Houwink plots for the stars, the intrinsic viscosity contraction factor for the stars, the intrinsic contraction factor g' was calculated as a function of molecular weight.

$$g' = \frac{[\eta]_{\text{star}}}{[\eta]_{\text{linear}}}$$

From the g' data, the radius of gyration contraction factor g was calculated.

$$g' = [a + (1/a)g^p]g^b$$

Where $a = 1.104$, $p = 7$ and $b = 0.906$ (Weissmuller & Burchard (1997) Polymer Internat. 44, 380).

Figure 4 shows an overlay of R_g contraction g plots. Using the calculated g values, the functionality f for the stars (the theoretical number of arms) was calculated using a model based on an assumption that the arms were random, that is, polydisperse in molecular weight. For random stars with f number of polydisperse arms:

$$g = \frac{3f}{(f+1)^2}$$

(Burchard (1983) Adv. Polym. Sci. 48, 1; Burchard (1997) Macromolecules 10, 919)

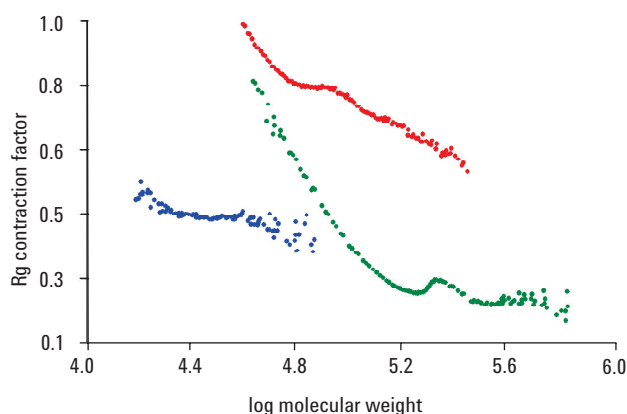


Figure 4. R_g contraction plots for the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.

Figure 5 shows an overlay of f for the stars as a function of the log molecular weight. The random model gave a prediction of the functionality f , the number of arms, which was in good agreement with the value expected from the synthesis. However, for all of the star-branched polymers, especially the 21-arm PS, the calculated value of f increased sharply with molecular weight, indicating that a considerable portion of the sample at low molecular weight contained components with fewer than expected arms.

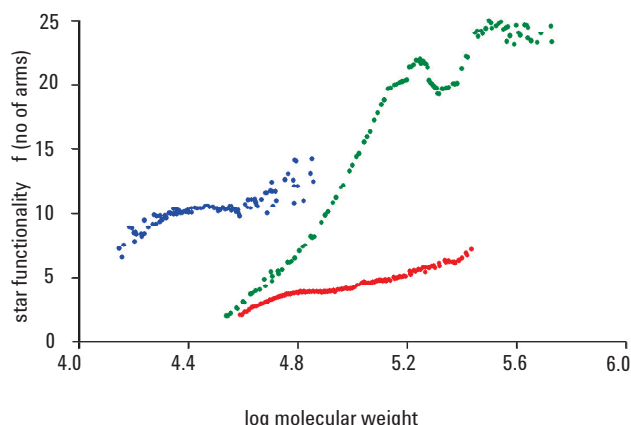


Figure 5. Functionality f plots for the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.

Conclusions

The results show that gel permeation chromatography employing refractive index and viscometry detectors can be used to investigate the structure of star-branched polymers. The variation in the functionality f (the number of arms on the polymers) with molecular weight gives valuable insight into the mechanism of the core first approach used to synthesize these materials.

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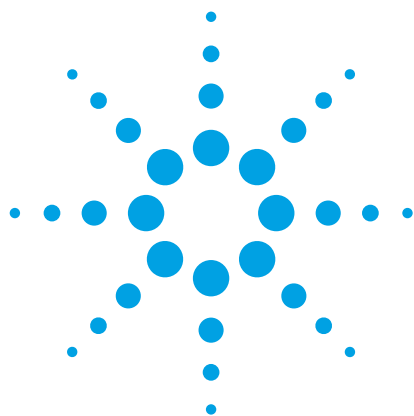
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Agilent Technologies



Natural and Synthetic Wax Analysis on Agilent PLgel and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymer

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Introduction

The term wax is used to describe a wide range of materials that share a similar appearance and consistency. Typically, waxes are white or tan in color and range from soft, readily pliable materials to harder, more resistant products. Waxes are generally of two types; natural, renewable waxes such as beeswax, and crude oil products such as paraffin waxes. This note describes the analysis of a crude oil wax and a renewable wax, and contrasts the results, using gel permeation chromatography with Agilent PLgel 3 μm 100Å columns.

Analysis of Waxes

The solubility of waxes is very dependent on molecular weight. Lower molecular weight waxes are soluble in tetrahydrofuran. However, as the molecular weight increases, the wax becomes harder and more brittle, due to higher crystallinity, and more aggressive solvents, such as trichlorobenzene, may be required for dissolution.

Microcrystalline wax is a refined mixture of solid, saturated hydrocarbons, mainly branched paraffin, obtained from the heavy lubricating oil fraction of crude oil during distillation. The wax is pure white in color with characteristics that closely resemble those of natural waxes, and is used as a substitute for other waxes in laminating paper, foil, and polishes. Microcrystalline wax typically contains C30-C70 hydrocarbon chains, including paraffins.



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Beeswax is a more complex product made from the honeycomb of bees. The wax is firm and yellow in color and has been used for thousands of years, correspondingly, with a wide range of applications. The main commercial use of the wax in recent times is in the production of candles and in cosmetic formulations. Beeswax typically contains about 15% partially unsaturated hydrocarbons, 15% free fatty acids, and 70% monohydroxesters, and di- and tripolyesters.

Both waxes were analyzed in tetrahydrofuran and the chromatograms are shown in Figures 1 and 2.

Conditions

Samples	Hydrocarbon waxes
Columns	2 × Agilent PLgel 3 µm 100Å, 7.5 × 300 mm (p/n PL1110-6320)
Eluent	THF (stabilized)
Flow rate	1.0 mL/min
Inj vol	20 µL
Detector	RI
System	Agilent PL-GPC 50

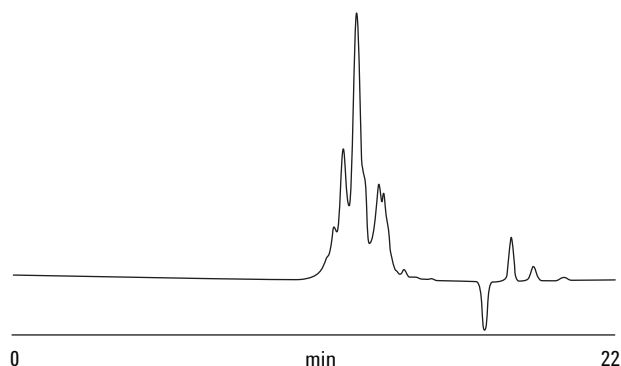


Figure 1. Chromatogram of a microcrystalline wax on an Agilent PLgel 3 µm two-column set.

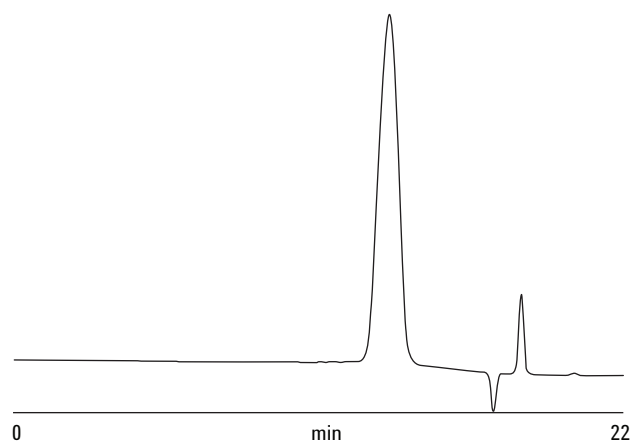


Figure 2. Chromatogram of beeswax on an Agilent PLgel 3 µm two-column set.

Conclusions

The chromatogram of the microcrystalline wax showed that a number of components could be resolved using the high efficiency columns. In comparison, the beeswax eluted as a broad polymer peak indicating that the various components had a similar size in solution. Although similar in properties, the two materials can be clearly differentiated by gel permeation chromatography.

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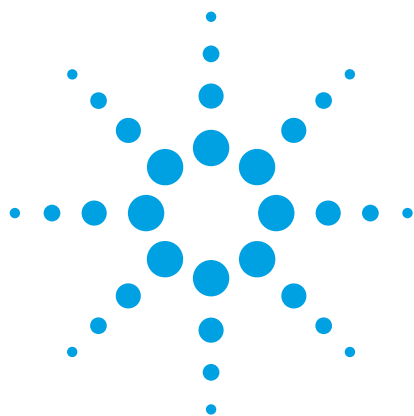
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Agilent Technologies



Silicone Analysis on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Silicones are polymers of siloxane. They are widely used as oils, sealants, waxes, and rubbers. Because of their resistance to temperature and chemical degradation, they are used particularly in the electronic, construction, and motor industries.

In gel permeation chromatography, many silicones are analyzed with toluene as eluent as they show very little response in tetrahydrofuran with a refractive index detector.

Analysis of Silicone

Toluene is a preferred solvent for the analysis of silicone materials as it has a significantly higher refractive index, resulting in good response when using a refractive index detector. Figure 1 shows a relatively low molecular weight silicone oil, which contains a high proportion of oligomers.



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Conditions

Column	2 × Agilent PLgel 5 μ m 500Å, 7.5 × 300 mm (p/n PL1110-6525)
Eluent	Toluene
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

Conclusions

Using toluene as a preferred solvent, Agilent PLgel columns effectively separate silicones with high oligomer content.

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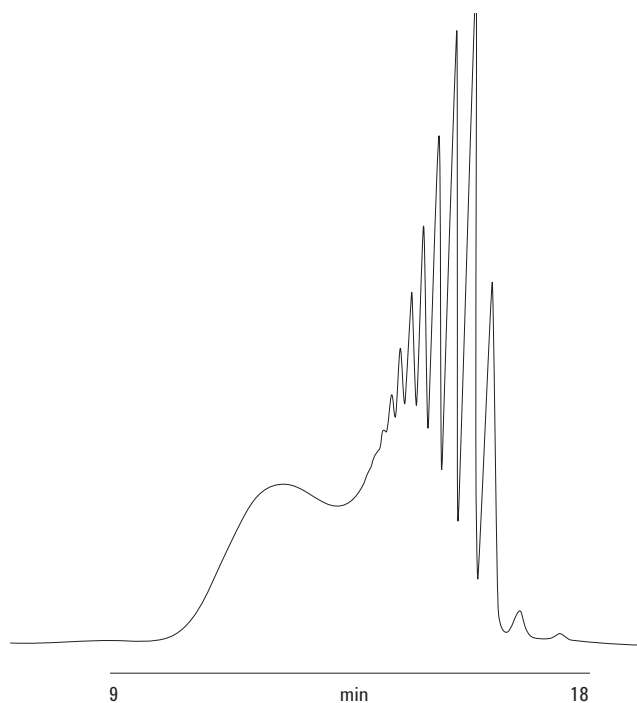


Figure 1. Separation of silicone with high oligomer content on an Agilent PLgel 5 μ m column set.

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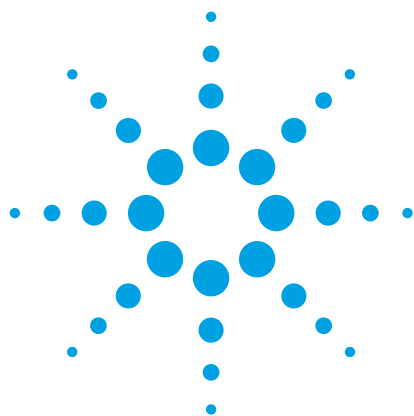
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Agilent Technologies



Polymerization Study using Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Gel permeation chromatography (GPC) can be used to monitor the effect of process variables on materials. As the mechanism in GPC separates molecules according to their size in solution, it is ideal for monitoring polymerization reactions and the effect of external influences on the synthesis of polymers, or both. Oligomeric species may be completely resolved and the formation of higher molecular weight species can be detected as an increasing broad polymer distribution.

Polymerization Study

This example features a study of the polymerization of soya oil. The original GPC chromatogram at 0 h shows essentially a monomeric species present corresponding to the starting sample of soya oil. An initiator was then mixed with the oil and a thin film was spread onto a glass plate which was exposed to light. After a period of 4 h and 7 h, a sample of the oil film was removed and analyzed by GPC. The resultant chromatograms were compared with that of the starting oil (Figure 1). Increasing amounts of high molecular weight species eluting at earlier retention times are quite clearly evident in the aged samples. Furthermore, the polymerization reaction can be seen to be progressing with increasing time.

An indication of the polymerization kinetics could be derived from normalized peak area determinations for the monomer and the higher molecular weight components.



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Conditions

Column	Agilent PLgel 5 μm 10 ³ Å, 7.5 $\times$ 300 mm (p/n PL1110-6530) Agilent PLgel 5 μm 100Å, 7.5 $\times$ 300 mm (p/n PL1110-6520)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

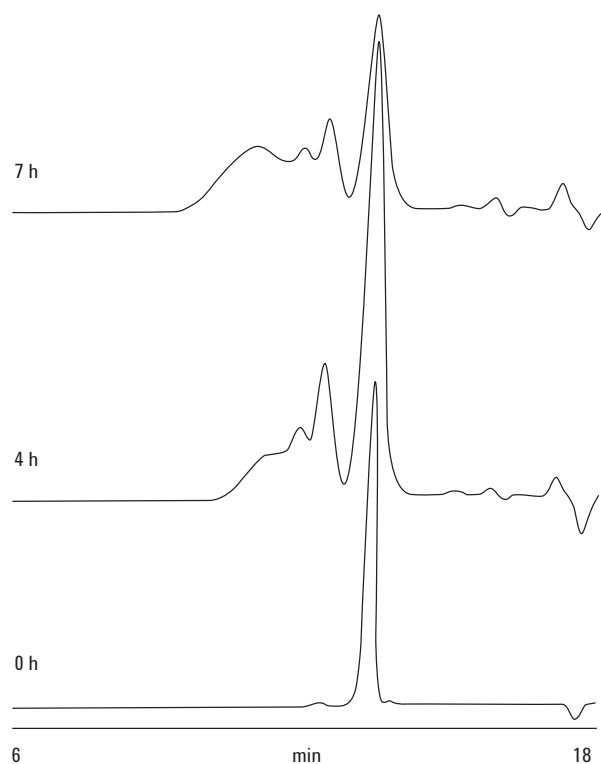


Figure 1. Increasing polymerization of a soya oil over a seven hour period revealed by Agilent PLgel 5 μm columns.

Conclusions

Gel permeation chromatography with Agilent PLgel columns can be used to follow the evolution of molecular weight that occurs during polymerization reactions.

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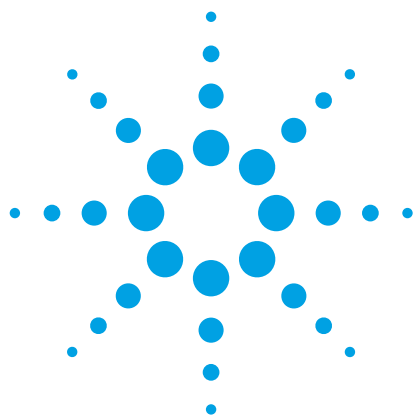
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Agilent Technologies



Polymer Analysis with Polar Organic Solvents using Agilent PLgel Columns and GPC

Application Note

Materials Testing and Research, Polymers

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Introduction

Increasingly, the choice of solvent for use as an eluent in gel permeation chromatography (GPC) is becoming more diverse since the polymers to be analyzed are more demanding in terms of solubility. Polar organic solvents are often the most suitable choice. However, such solvents usually exhibit relatively high viscosity, and demand the application of elevated temperature in order to improve the separation and reduce the column operating pressure. Agilent PLgel columns are used extensively in these application areas.

Polymer Analysis using Polar Organic Solvents

Agilent PLgel 10 μm MIXED-B columns are typically employed for the analysis of high molecular weight, polydisperse materials. Table 1 shows some of these compounds with associated solvents for analysis by GPC. Figures 1 and 2 illustrate typical examples of these types of application.



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Table 1. Appropriate Solvents for a Range of Polymer Types

Polymer	Solvent
Acrylonitrile butadiene styrene (ABS)	Dimethyl formamide (DMF)
Cellulose	Dimethyl sulfoxide/dimethylacetamide (DMSO/DMAC)
Poly(acrylates)	DMF/DMAC
Poly(acrylonitrile)	DMF
Poly(ethylene oxide)	DMF
Poly(urethane)	DMF/DMAC
Poly(vinyl pyrrolidone)	DMF/DMAC

Conditions for Figure 1

Columns 2 × Agilent PLgel 10 µm MIXED-B, 7.5 × 300 mm (p/n PL1110-6100)
 Eluent DMAC + 0.02 M LiBr
 Flow rate 1.0 mL/min
 Temp 60 °C
 Detector 390-MDS Multi Detector Suite RI

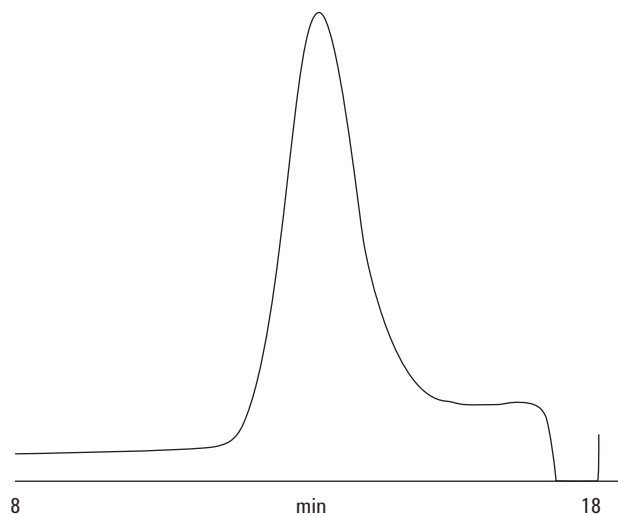


Figure 1. Polyurethane copolymer analysis using an Agilent PLgel 10 µm MIXED-B two-column set.

Conditions for Figure 2

Columns 2 × Agilent PLgel 5 µm MIXED-D, 7.5 × 300 mm (p/n PL1110-6504)
 Eluent THF
 Flow rate 1.0 mL/min
 Temp 40 °C
 Detector 390-MDS Multi Detector Suite RI

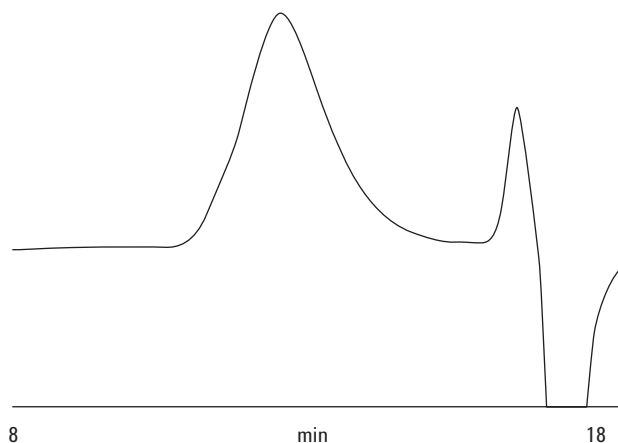


Figure 2. Acrylonitrile butadiene styrene analysis on an Agilent PLgel 5 µm MIXED-D two-column set.

Low pore size PLgel columns are also fully compatible with polar organic solvents, and have applications in resin analysis and the determination of low molecular weight species in polymers, as illustrated in Figure 3.

Conditions for Figure 3

Columns	2 × Agilent PLgel 5 µm 50Å, 7.5 × 300 mm (p/n PL1110-6515)
Eluent	DMF + 0.1% LiBr
Flow Rate	1.0 mL/min
Temp	60 °C
Detector	390-MDS Multi Detector Suite RI

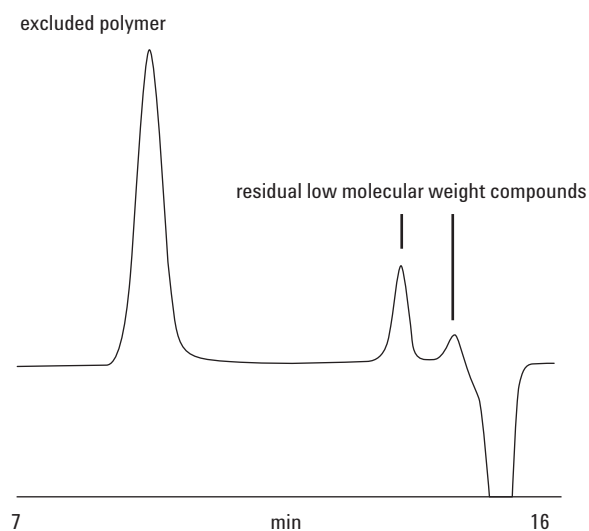


Figure 3. Analysis of residual monomers in acrylonitrile butadiene styrene on an Agilent PLgel 5 µm two-column set.

Conclusions

The compatibility of packing materials for gel permeation chromatography with polar organic solvents assumes increasing importance in high performance separations of modern polymer systems. Column performance should be unaffected by solvent transfer, which demands a high degree of chemical and physical stability in the column bed, as delivered by Agilent PLgel packing.

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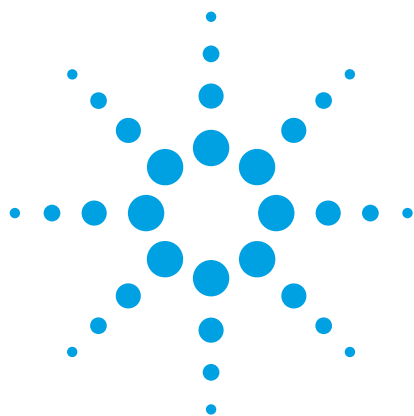
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Agilent Technologies



Polyurethane Prepolymer Analysis on Agilent PLgel MIXED-E by GPC

Application Note

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Introduction

The term polyurethane encompasses a range of materials that contains a urethane (-NHC(=O)-) group in the backbone repeat unit. Typically, these materials also contain ester or amide groups and are usually produced by the reaction of a diisocyanate with a diol or polyol in a step-growth reaction. Polyurethanes are among the most versatile polymers, with a wide range of applications ranging from elastomers, fibers and rigid/semi-rigid foams to solid plastics, coatings, and adhesives. Many polyurethanes are produced in a one-shot process, but some are manufactured by a two-stage synthesis, involving the formation of a prepolymer. In these processes, a polyester or polyester polyol is reacted with a diisocyanate to produce a stable isocyanate-terminated prepolymer. This can then be further reacted with chain extenders or crosslinking agents to produce the final polyurethane. The prepolymer process has often been used to manufacture rigid-polyurethane foams due to low reactivity of many polyols.

In these reactions, the properties of the prepolymer must be carefully controlled to ensure complete reaction. Gel permeation chromatography (GPC) is often used to characterize prepolymers. This application note describes the fingerprinting of the oligomer distribution of a polyurethane prepolymer by GPC.



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Polyurethane Prepolymer Analysis

Two high resolution Agilent PLgel 3 μ m MIXED-E columns are used, with dichloromethane as eluent and differential refractive index (DRI) detection. As seen in Figure 1, the MIXED columns resolve the individual components of the oligomer distribution of a prepolymer, allowing the reactivity of the prepolymer to be assessed.

Conditions

Columns	2 $\times$ Agilent PLgel 3 μ m MIXED-E, 7.5 $\times$ 300 mm (p/n PL1110-6300)
Eluent	DCM
Flow rate	1.0 mL/min
Inj vol	20 μ L
Detector	RI
System	Agilent PL-GPC 50

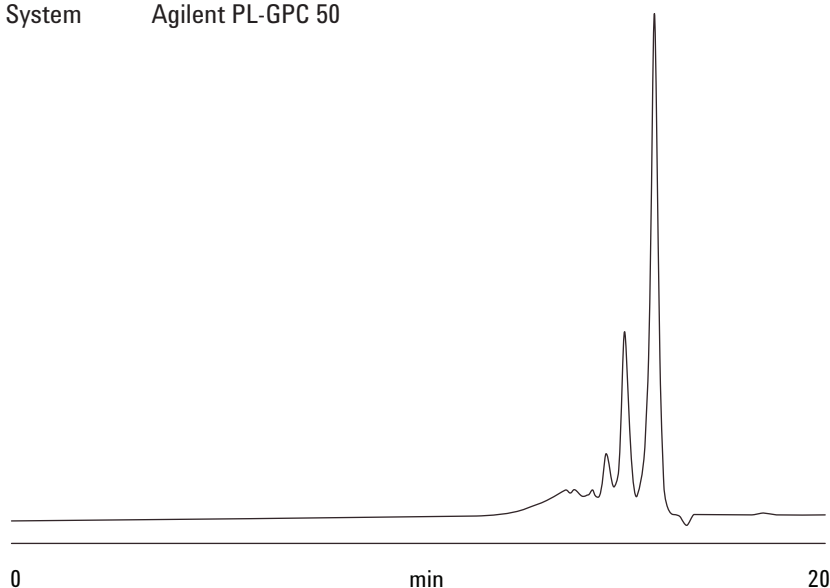


Figure 1. Oligomeric components of a prepolymer on Agilent PLgel MIXED-E columns.

Conclusions

The analysis of low molecular weight prepolymers by gel permeation chromatography using Agilent PLgel 3 μ m MIXED-E columns allows a detailed investigation of the oligomeric components of the sample to be determined.

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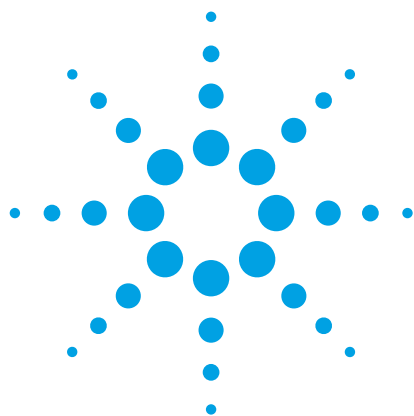
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Agilent Technologies



Analyze Injection-Molding Polymers on Agilent PLgel 5 μ m MIXED-C by GPC

Application Note

Materials Testing and Research

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Introduction

Injection-molding thermoplastics are used in a wide variety of applications, including panels for plastic doors, casings for scientific instruments, refrigerator linings, and telephone cases. Ideally, the materials should display excellent weather and impact resistance, good color retention and consistency, resistance to environmental stress, cracking, and high heat resistance. A number of copolymers have been developed that display these properties, such as copolymers of acrylonitrile/styrene/acrylate (ASA) and acrylonitrile/butadiene/styrene (ABS). Typically, these materials are either layered or blended to meet the requirements of a specific application.

This application note describes the analysis of a sample of an ASA/ABS-blended material from a car door panel by gel permeation chromatography (GPC), using Agilent PLgel 5 μ m MIXED-C columns.

Injection-Molding Polymer Analysis

Figure 1 shows the blended material that eluted as a single monomodal peak, indicating that the two components of the blend were very similar in molecular size, with an M_w of 160,000 g/mol and a polydispersity of 2.6.



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Conditions

Samples	Blend of ASA and ABS
Columns	2 × Agilent PLgel 5 µm MIXED-C, 7.5 × 300 mm (p/n PL1110-6500)
Eluent	THF (stabilized)
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	RI
System	Agilent PL-GPC 50

Conclusions

Gel permeation chromatography with Agilent PLgel 5 µm MIXED-C columns permits the molecular weight distribution of polymers to be assessed, and the size of the molecular components in a polymer blend to be investigated.

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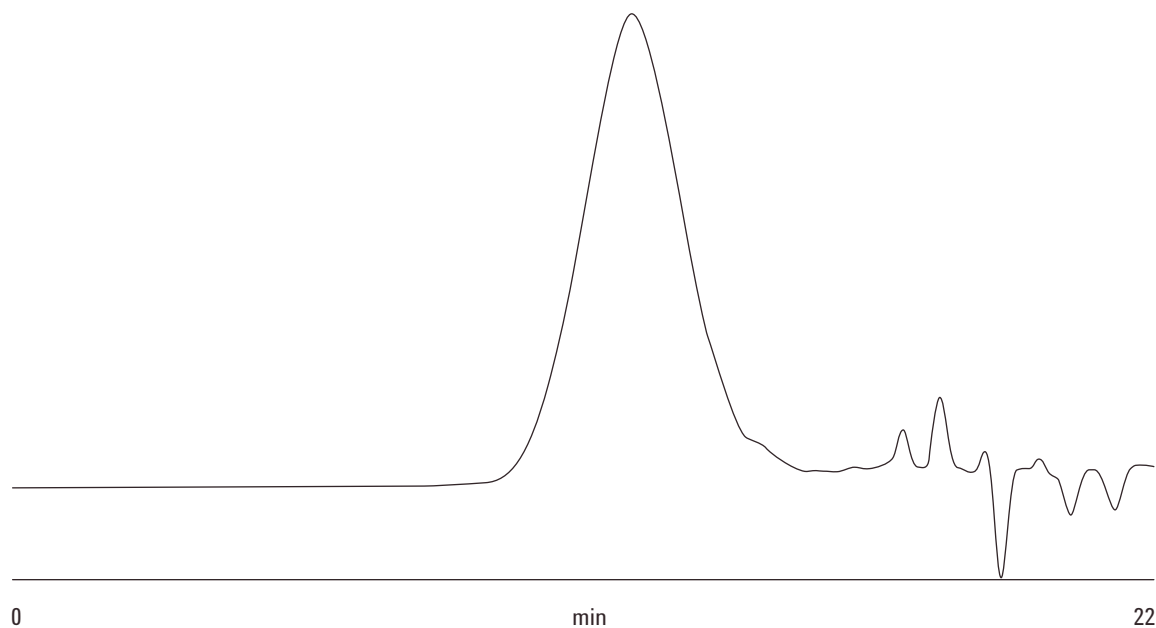


Figure 1. Individual components of an injection-molding polymer elute as a single peak, showing their similarity in molecular size.

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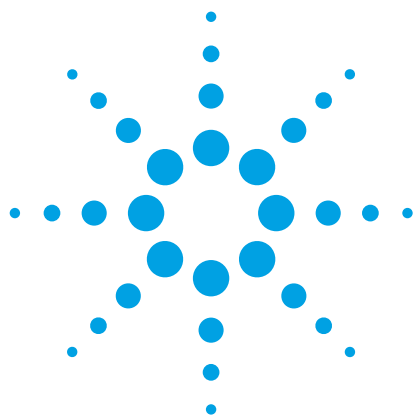
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Adipate Polyester Analysis on Agilent PLgel 3 μ m MIXED-E by Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Polyesters are produced from the condensation of a diacid with a dialcohol, eliminating water in the process. Depending on the acid and alcohol used, polyester can have a wide range of properties including flexibility or hardness, stability to hydrolytic degradation and solvent, and abrasion and shock resistance. All these properties are useful for a wide range of applications.

Adipate esters are produced by the condensation of a dialcohol with adipic acid. Saturated adipate polyesters are used as cast elastomers; depending on the dialcohol used in the synthesis, linear or branched polyesters may be obtained. These polyesters are reacted with isocyanates to produce prepolymers with residual NCO groups that are precursors to mixed polyurethanes, a very important commercial class of material.

In this case, gel permeation chromatography (GPC) with Agilent PLgel 3 μ m MIXED-E columns is used to investigate two adipate polyester samples.

Adipate Polyester Analysis

Figure 1 shows the analysis of two adipate polyester materials. The high resolution MIXED columns resolve the polymers into individual oligomers, giving a characteristic peak shape that can be used to identify and fingerprint different batches of polymer.



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Conditions

Columns	2 × Agilent PLgel 3 µm MIXED-E, 7.5 × 300 mm (p/n PL1110-6300)
Eluent	THF (stabilized)
Flow rate	1.0 mL/min
Inj vol	20 µL
Detector	RI
System	Agilent PL-GPC 50

Conclusions

Low pore size, high resolution Agilent PLgel 3 µm MIXED-E columns can be used to fingerprint the oligomeric distribution of resins such as adipate polyester.

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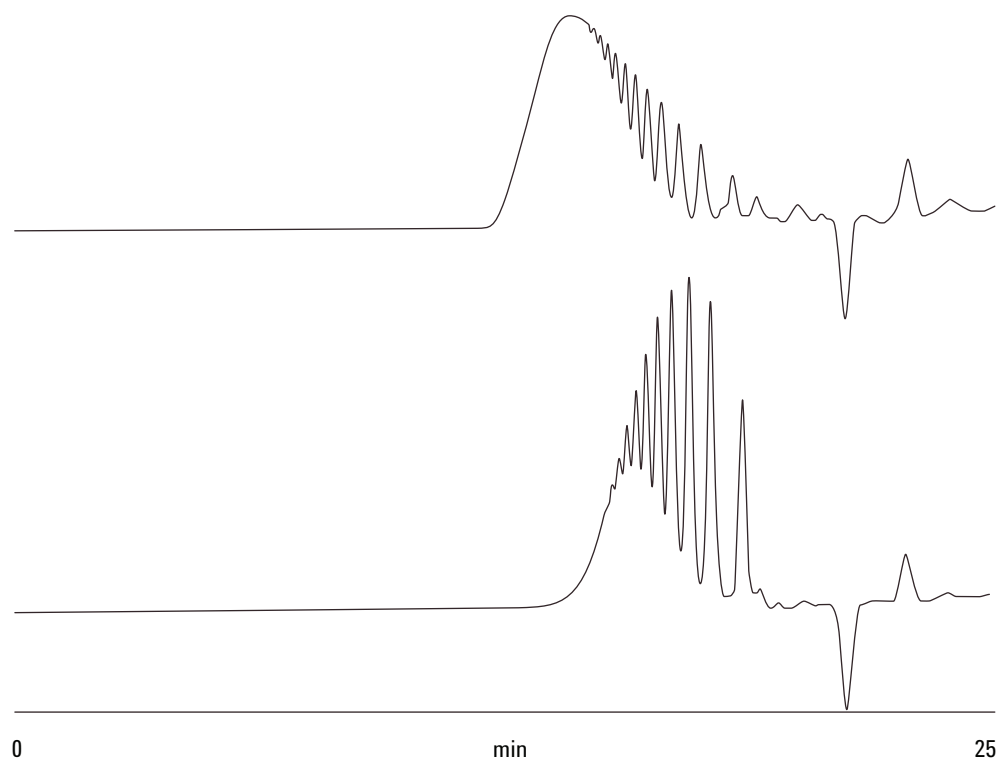


Figure 1. Using gel permeation chromatography with an Agilent GPC system to fingerprint adipate polyesters.

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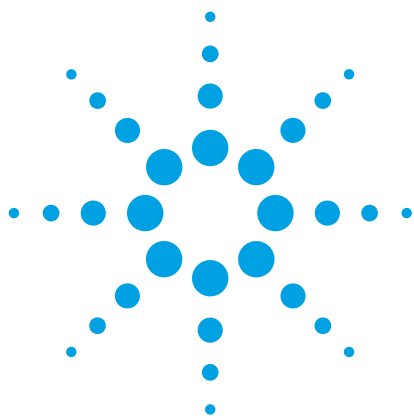
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Agilent Technologies



Fractionation of Formaldehyde Resin on Agilent PLgel by Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Resolving closely eluting fractions in highly disproportionate concentrations is achieved by using a heart-cutting technique. The same technique can also be used to avoid cross-fraction contamination by removing the sample matrix. In this example, heart-cutting was employed in an identification of fractions of a phenanthrene-formaldehyde resin. The resin fractions were separated by gel permeation chromatography with Agilent PLgel 10 μ m 500Å columns.

Fractionation of Phenanthrene-Formaldehyde Resin

Figure 1 shows the phenanthrene-formaldehyde resin monomer, dimer and trimer fractions. After separation of the fractions (Figure 2), identification was done using mass spectroscopy and infra-red spectroscopy.

Conditions

Column	Agilent PLgel 10 μ m 500Å, 25 × 300 mm (p/n PL1210-6125)
Eluent	Dichloromethane
Flow rate	9.0 mL/min
Conc	10%
Inj vol	2 mL
Detector	RI
System	Agilent PL-GPC 50



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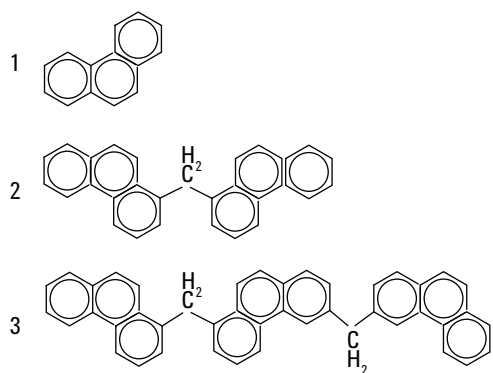


Figure 1. Structure of three fractions of a phenanthrene-formaldehyde resin.

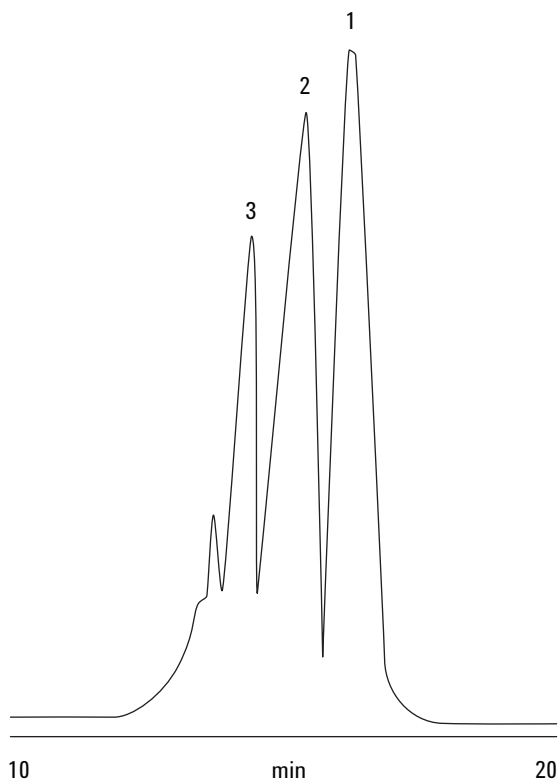


Figure 2. Separation of three fractions of phenanthrene-formaldehyde on Agilent PLgel 10 μ m columns.

Conclusions

Preparative gel permeation chromatography employing a heart-cut technique can be used to separate and isolate components of a polymer on the basis of size in solution.

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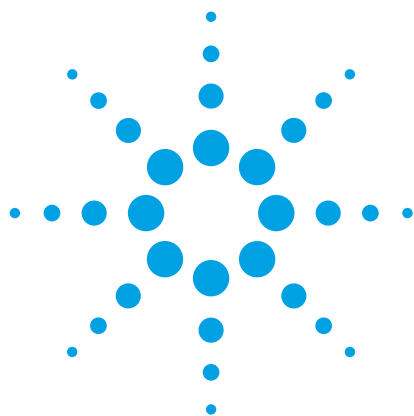
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Fingerprinting Epoxy Resins with Agilent OligoPore and Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Epoxy resins are commercially important materials that consist of bridged diepoxide oligomers of increasing molecular weight. The distribution of oligomers in individual resins dictates the physical nature of the finished materials such as curing time and mechanical strength.

The analysis of the oligomer distribution by gel permeation chromatography (GPC) allows different grades of resin to be fingerprinted and is of vital importance for quality and process control.

Agilent's OligoPore columns have been specifically designed for the analysis and fingerprinting of oligomeric samples, as shown in this example with two commercial epoxy resins, Epikote 828 and Epikote 1001.

Epoxy Resin Analysis

The Epikote samples were made up at 0.2% (w/v) in tetrahydrofuran and injected without further treatment. Figure 1 shows a close up of the Epikote 828 chromatogram, which was dominated by a single oligomer peak with only small quantities of other oligomers, while Figure 2 is a comparison of the chromatograms of Epikote 828 and 1001. Epikote 1001 contains many oligomers with increasing molecular weight up to the exclusion limit of the column at about 4,000 g/mol. The presence of oligomers with the same retention times in both samples indicates that some of the oligomers in the two samples are identical.



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Conditions

Samples	Epikote 828 and 1001, 0.2% (w/v)
Columns	2 × Agilent OligoPore, 7.5 × 300 mm (p/n PL1113-6520)
Eluent	THF
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	RI
System	Agilent PL-GPC 50

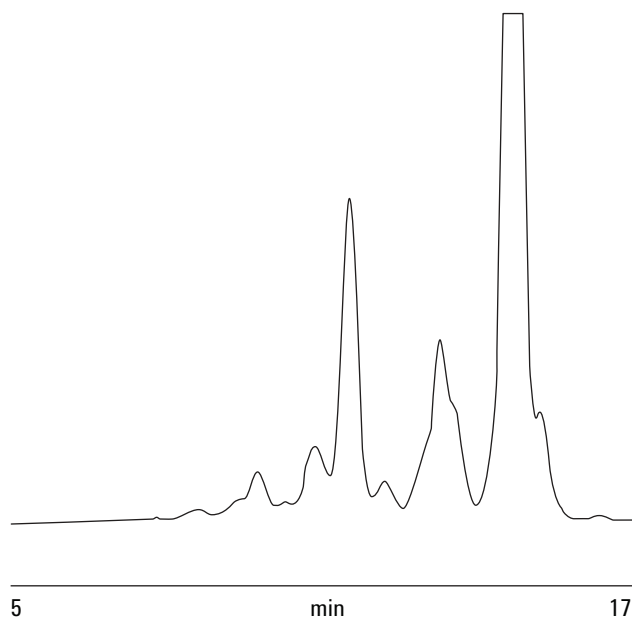


Figure 1. Epikote 828 analyzed on an Agilent OligoPore two-column set to reveal the dominance of a single oligomer.

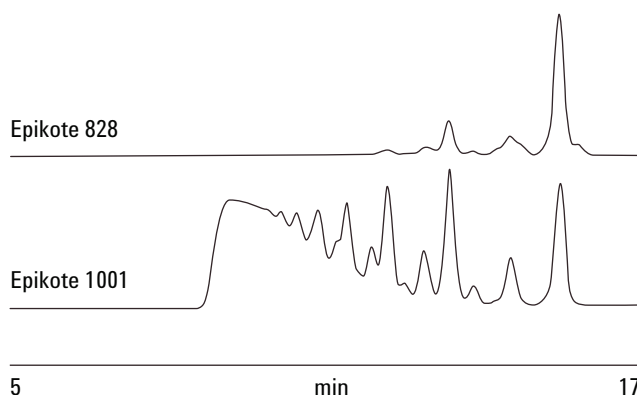


Figure 2. Comparison of Epikote 828 and 1001 epoxy resins on Agilent OligoPore columns shows that some of the oligomers are identical.

Conclusions

Low pore size Agilent OligoPore columns allow the oligomeric fingerprint of samples such as epoxy resins to be investigated quickly and accurately.

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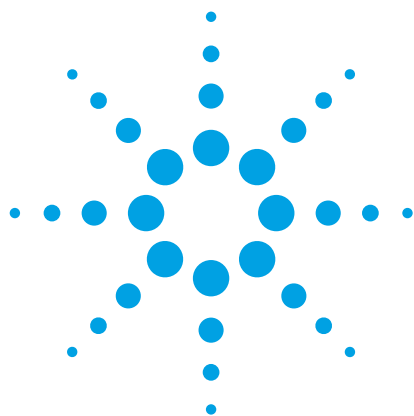
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Agilent Technologies



Silane Oligomer Analysis with Agilent OligoPore and Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Alkyl silanes are a class of reactive molecules that can be hydrolyzed and polymerized by condensation reactions to form oligomeric and polymeric materials, including silica glasses and gels. This application note illustrates the use of Agilent OligoPore columns and gel permeation chromatography for the analysis of silane oligomers generated by the controlled hydrolysis of silanes under acidic conditions.

Silane Oligomer Analysis

Dimethyldiethyl (DMDE) silane was mixed with a 0.1 N hydrochloric acid solution in 50:50 ratio and left to react for 12 hours. After the reaction period, the sample was made up at 0.2% (w/v based on the silane) in tetrahydrofuran and injected without further treatment. Figure 1 is the resulting chromatogram of dimethyldiethyl silane after the hydrolysis reactions.



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Conditions

Samples	Hydrolyzed dimethyldiethyl silane (DMDES), 0.2% (w/v)
Columns	2 × Agilent OligoPore, 7.5 × 300 mm (p/n PL1113-6520)
Eluent	THF
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	RI
System	Agilent PL-GPC 50

Conclusions

Agilent OligoPore columns resolve the individual low molecular weight DMDE silane oligomers after hydrolysis. Individual oligomers can be identified allowing a fingerprint of the silane oligomer distribution after hydrolysis for different times and under different conditions, a vital tool for quality and process control.

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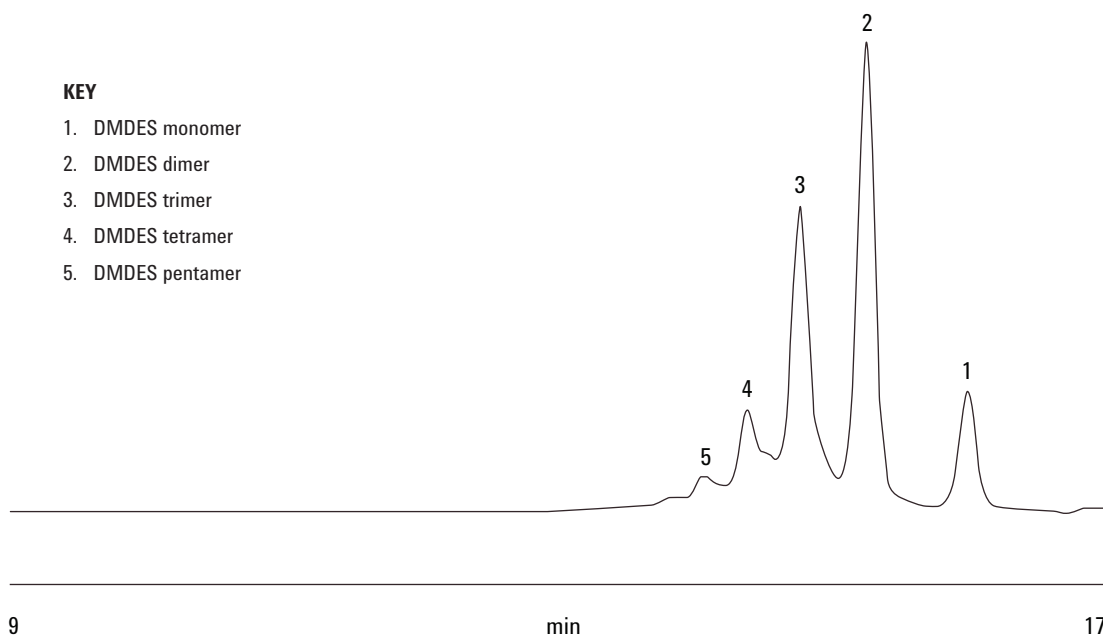


Figure 1. Fingerprinting silane oligomers with an Agilent OligoPore column.

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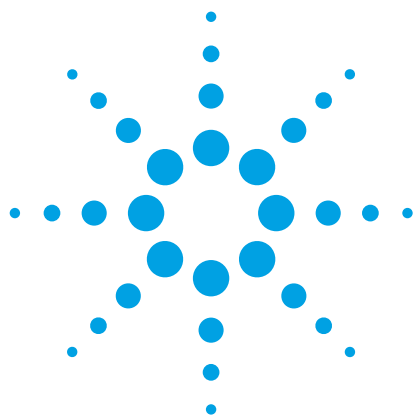
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Separate Epoxy Resin Oligomers with Agilent Preparative Gel Permeation Chromatography

Application Note

Materials Testing and Research

Authors

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Introduction

Preparative gel permeation chromatography (GPC) can be used to separate and isolate individual components of a sample based on size exclusion. By scaling up analytical separations, preparative GPC isolates practical quantities of individual components that can be used in further analysis. The Agilent OligoPore preparative GPC column is ideally suited to the separation and isolation of individual oligomers from oligomer distributions and complex mixtures. In this example, the columns are employed for the fractionation of epoxy oligomers.



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Epoxy Resin Oligomer Analysis

Figure 1 shows the general structure of an epoxy oligomer such as Epikote 828. This commercial epoxy resin is composed of two main epoxy oligomers where $n = 0$ and $n = 1$, and small amounts of the mono- and di-epoxy water adducts.

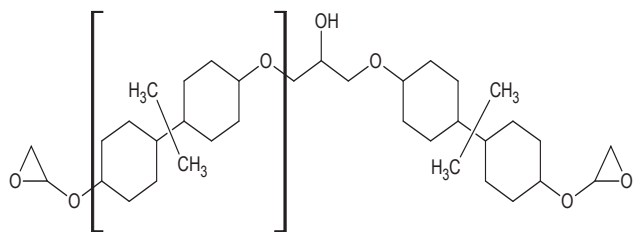


Figure 1. General structure of Epikote 828 epoxy resin oligomers.

Initially, the optimum loading of Epikote 828 on the OligoPore columns was analyzed on an analytical scale. Figure 2 shows analytical chromatograms at concentrations of 0.5% to 2.0% (w/v). They indicate that Epikote 828 could be analyzed at a concentration of 2.0% (w/v) without serious loss of reduction.

Conditions - Analytical

Samples	Epikote 828, 0.5-2.0% (w/v)
Columns	2 × Agilent OligoPore, 7.5 × 300 mm (p/n PL1113-6520)
Eluent	THF
Flow rate	1.0 mL/min
Inj vol	100 μ L
Detector	UV
System	Agilent PL-GPC 50

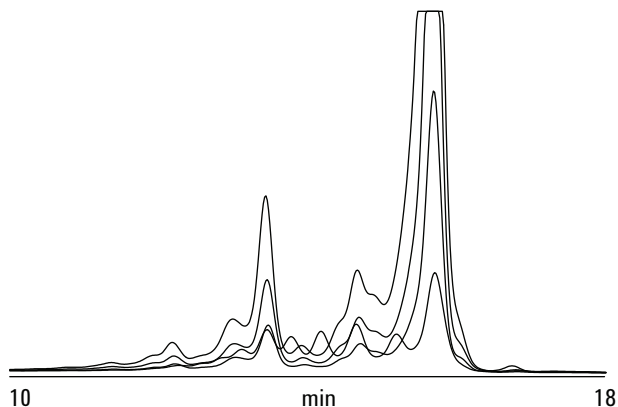


Figure 2. Analytical separation of Epikote 828 on Agilent OligoPore columns indicates that a 2.0% w/v concentration is appropriate for preparative analysis.

OligoPore preparative columns were then used to fractionate and purify the two oligomers from the resin. A preparative GPC system was set up with a 2 mL injection loop, two Agilent OligoPore 25 × 300 mm columns and a flow rate of 10.0 mL/min, an approximate ten-fold scale-up over the analytical separation. The flow rate from the columns was split into two lines, about 0.5 mL/min went to a UV detector, the remainder of the flow to a waste/fraction collector. The epoxy resin sample was injected at a concentration of 1.0% (w/v).

Figure 3 shows a chromatogram of Epikote 828 obtained on the preparative columns indicating the resolution. The sample was re-run and the two oligomers $n = 0$ and $n = 1$ were collected. The fractions were then analyzed on two Agilent OligoPore analytical columns.

Conditions - Preparative

Samples	Epikote 828, 1.0% (w/v)
Columns	2 × Agilent OligoPore, 25 × 300 mm (p/n PL1213-6520)
Eluent	THF
Flow rate	10.0 mL/min, about 9.5 mL/min collected; 0.5 mL/min to the detector
Inj vol	2 mL
Detector	UV
System	PL-GPC 50

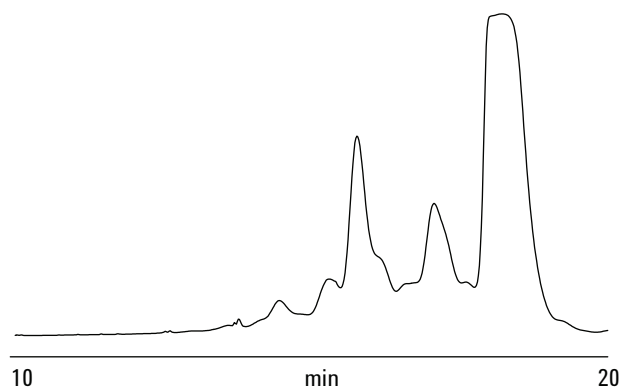


Figure 3. Epikote 828 separated on an Agilent OligoPore two-column set.

Figure 4 shows the original analytical chromatogram of Epikote 828 run at a concentration of 2.0% (w/v) and an overlay of analytical chromatograms of the $n = 0$ and $n = 1$ oligomers collected from the Agilent OligoPore preparative GPC columns.

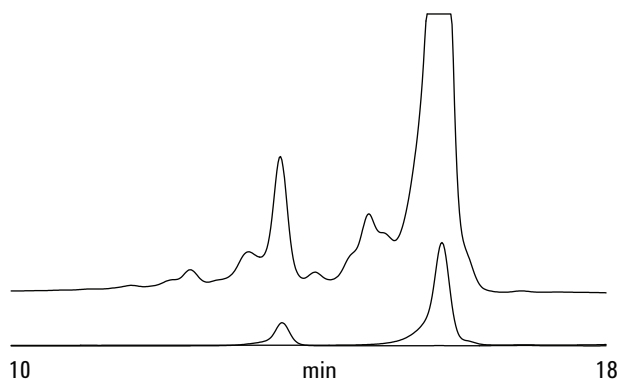


Figure 4. Epikote 828 analytical chromatogram from Figure 1 run at 2.0% (w/v) compared to overlaid analytical chromatograms of the $n = 0$ and $n = 1$ oligomers collected from the Agilent preparative GPC system.

Conclusions

Low pore size preparative GPC columns from Agilent can be used to isolate individual oligomers from complex samples after method development with an equivalent analytical-scale column.

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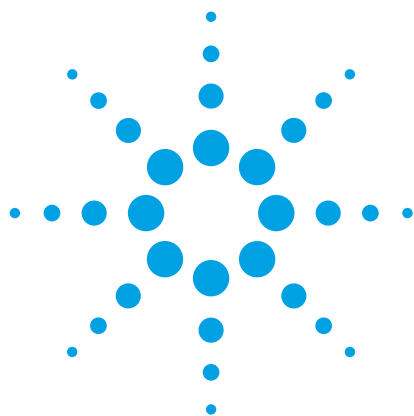
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Separation of Styrene Oligomers by Agilent Preparative Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Preparative gel permeation chromatography separates and isolates individual components of a sample based on size exclusion. If based on an analytical separation, prep GPC can be used to isolate practical quantities of individual components, which are thus made available for further analysis. The preparative separation of individual oligomers from oligomer distributions and complex mixtures is easily achieved with the Agilent OligoPore preparative GPC column. This application note illustrates the use of Agilent OligoPore preparative columns in the fractionation of polystyrene oligomers.



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Styrene Oligomer Analysis

Initially, the optimum loading of polystyrene 580 on the Agilent OligoPore columns was analyzed at an analytical scale. The Agilent polystyrene narrow standard Mp 580 (p/n PL2012-2001) is composed of a distribution of styrene oligomers differing by a repeat unit of relative molecular mass 104.

Conditions - Analytical

Sample	Polystyrene 580, 0.5 to 2.0% (w/v)
Columns	2 × Agilent OligoPore, 7.5 × 300 mm (p/n PL1113-6520)
Eluent	THF
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	UV
System	Agilent PL-GPC 50

Figure 1 shows a series of analytical chromatograms at concentrations ranging from 0.2% to 2.0% (w/v). The chromatograms indicate that polystyrene 580 could be analyzed at a concentration of 2.0% (w/v) without serious loss of resolution.

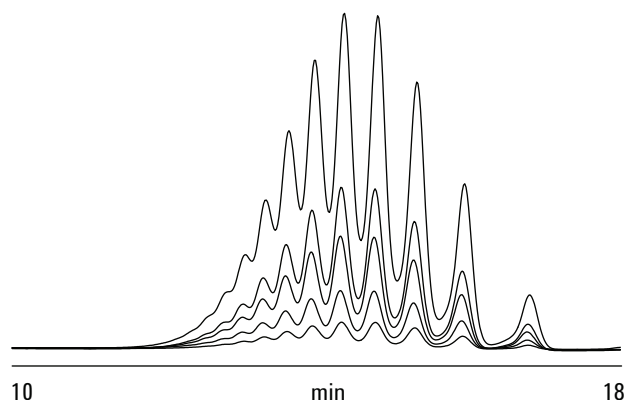


Figure 1. Analytical separation of polystyrene 580 on Agilent OligoPore indicates that a 2.0% w/v concentration is appropriate for preparative analysis.

Agilent OligoPore preparative columns were used to fractionate and collect the individual oligomers. A preparative GPC system was set up with a 2 mL injection loop, two Agilent OligoPore 25 × 300 mm columns and a flow rate of 10.0 mL/min, an approximate ten-fold scale up over the analytical separation. The flow from the columns was split into two lines, approximately 0.5 mL/min went to a UV detector, the remainder of the flow to a waste/fraction collector. The polystyrene sample was injected at a concentration of 1.0% (w/v).

Conditions - Preparative

Sample	Polystyrene 580, 1.0% (w/v)
Columns	2 × Agilent OligoPore, 25 × 300 mm (p/n PL1213-6520)
Eluent	THF
Flow rate	10.0 mL/min, about 9.5 mL/min collected; 0.5 mL/min to the detector
Inj vol	2 mL
Detector	UV
System	PL-GPC 50

Figure 2 is a chromatogram of polystyrene 580 obtained on the preparative columns indicating the resolution obtained. The sample was re-run and the individual oligomer fractions collected. Each oligomer fraction was then analyzed on two Agilent OligoPore analytical columns.

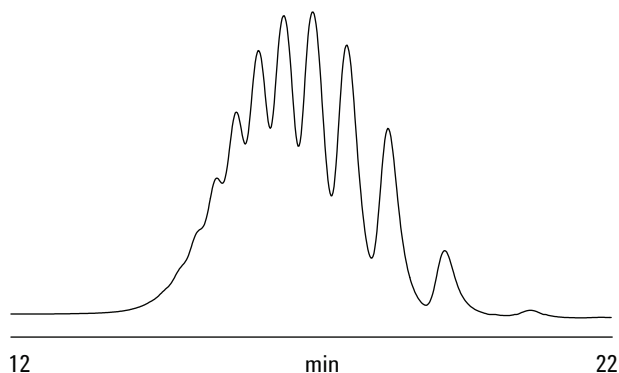


Figure 2. Polystyrene 580 separated on an Agilent OligoPore two-column set.

Figure 3 shows the original analytical chromatogram of polystyrene 580 run at a concentration of 2.0 (w/v) and an overlay of nine analytical chromatograms of the individual oligomer fractions collected from the Agilent OligoPore preparative GPC columns. The individual oligomers can be identified by comparison with the retention times in the chromatogram of polystyrene 580.

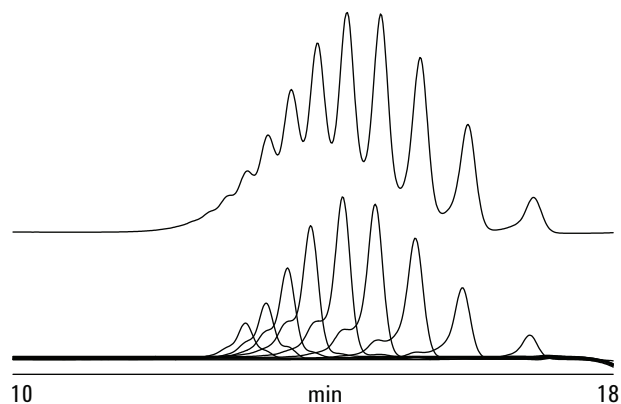


Figure 3. Polystyrene 580 from Figure 1 run at 2.0% (w/v) compared to nine overlaid analytical chromatograms collected from the Agilent OligoPore prep columns.

Conclusions

Preparative gel permeation chromatography can be used to separate and isolate individual oligomers from complex samples once method development has been performed on analytical column equivalents.

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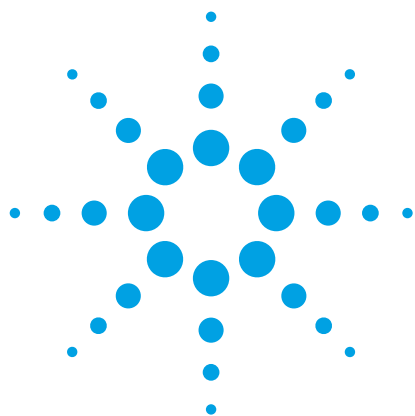
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Epoxy Resin Analysis with Agilent OligoPore Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

Epoxy resin prepolymers consist of oligomeric and polymeric diepoxides that are cured to form the finished product by the addition of a fixing or hardening agent. The formulation of the prepolymer is vital to controlling the physical properties of the final product. High resolution gel permeation chromatography (GPC) can be used to investigate the oligomeric distributions of epoxy resin prepolymers for formulation and quality control. This application note outlines the analysis of two grades of epoxy resin using Agilent OligoPore columns. Epoxy resins contain strong chromophores therefore, UV detection can be employed.

Epoxy Resin Oligomer Analysis

Figure 1 shows chromatograms of two epoxy resin samples. The first resin contains some high molecular weight material that is excluded on the Agilent OligoPore columns. However, the oligomeric distribution can be seen. The second resin contains no material with a molecular weight greater than about 5,000 g/mol, the exclusion limit of the column. A number of oligomers are resolved. Although the presence of some peaks, with similar retention times, indicates that there are some oligomers common to both samples, the oligomer distributions of the two resins are clearly very different.



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Conditions

Columns	2 × Agilent OligoPore, 7.5 × 300 mm (p/n PL1113-6520)
Eluent	THF + 250 ppm BHT
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	UV, 254 nm
System	Agilent PL-GPC 50

Conclusions

The relative oligomeric content of two grades of epoxy resin can be compared by high resolution gel permeation chromatography using Agilent OligoPore columns.

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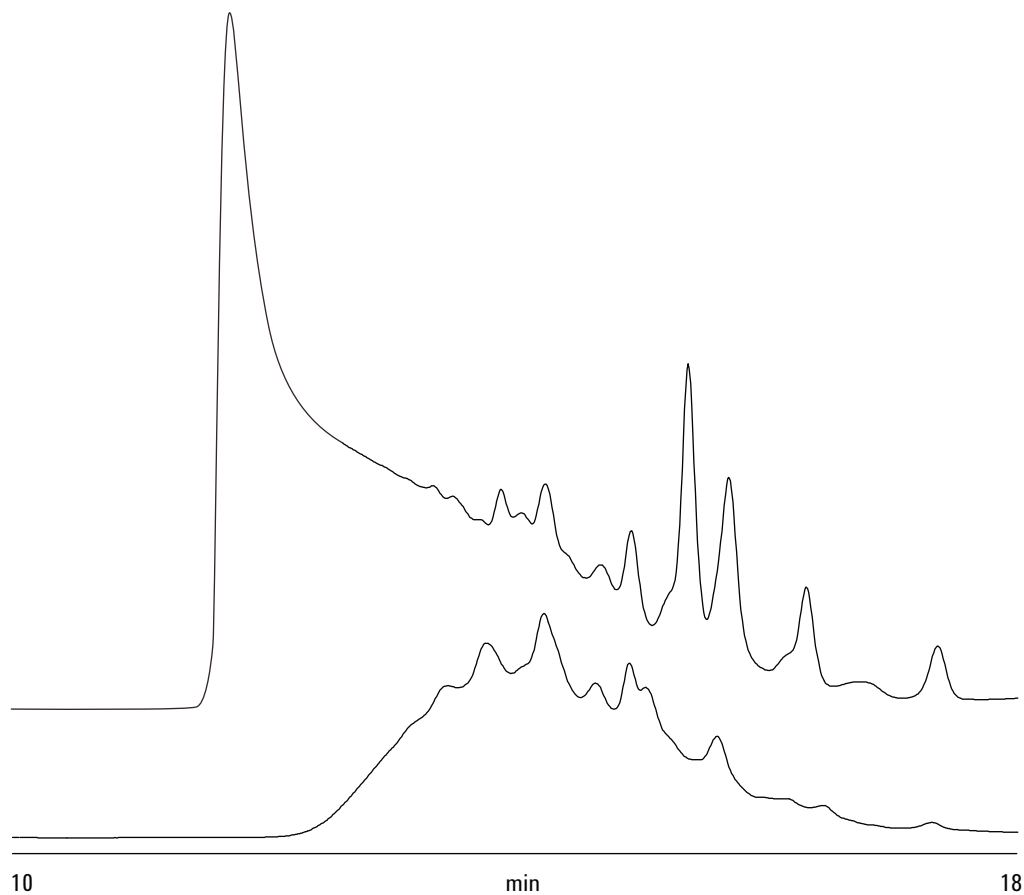


Figure 1. Analysis of two epoxy resins on Agilent OligoPore columns reveals very different oligomer distributions despite the presence of some oligomers common to both samples.

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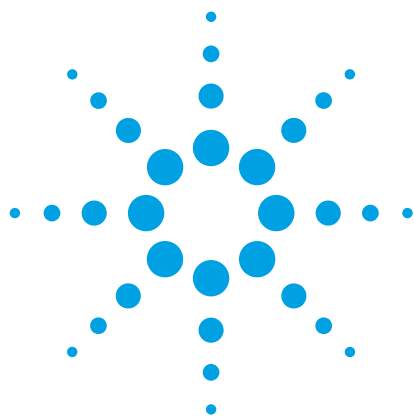
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Phenolic Resin Analysis by Agilent ResiPore Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research

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Introduction

The term phenolic resin is used to describe a group of thermosetting resins produced through the reaction of phenol with an aldehyde. Phenolic resins were the earliest synthetic polymers to be developed, by Leo Baekeland in 1907, and possess useful mechanical and physical properties. Applications of phenolic resins include electrical insulation, molding, lamination, and adhesives. Due to the relatively low cost and favorable properties of phenolic resins, they are produced in the greatest volume of all thermosetting polymers. Key characteristics of phenolic resins are their molecular weight distribution and oligomeric fingerprint, as these both have significant effects on the end use properties of the resin.

Gel permeation chromatography (GPC) is an ideal analytical tool for the examination of both of these characteristics. In this case, the use of high resolution Agilent ResiPore columns is advantageous, since these allow an optimized oligomeric separation, and provide detailed information regarding the oligomeric sample composition.

Phenolic Resin Analysis

Four distinct grades of phenolic resin are analyzed by GPC using an Agilent ResiPore column set. Resulting from the small particle size (3 μm) and optimized pore size distribution of this column packing material, good resolution is obtained in the molecular weight range of interest.

The chromatography obtained from the GPC of each phenolic resin sample is shown in Figure 1. Differential molecular weight distributions are given in Figure 2. This plot clearly shows significant differences in molecular weight distribution and the relative amounts of oligomeric material.



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Conditions

Columns	2 × Agilent ResiPore, 7.5 × 300 mm (p/n PL1113-6300) (conditioned with 10 injections of a typical sample solution at 10 mg/mL)
Eluent	THF (stabilized with 250 ppm BHT)
Flow rate	1.0 mL/min
Inj vol	20 µL
Detector	RI
System	Agilent PL-GPC 50

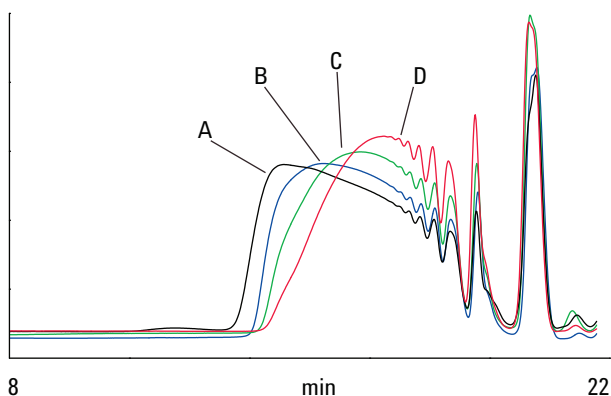


Figure 1. Overlay of GPC chromatograms obtained from four samples of phenolic resin on Agilent ResiPore columns.

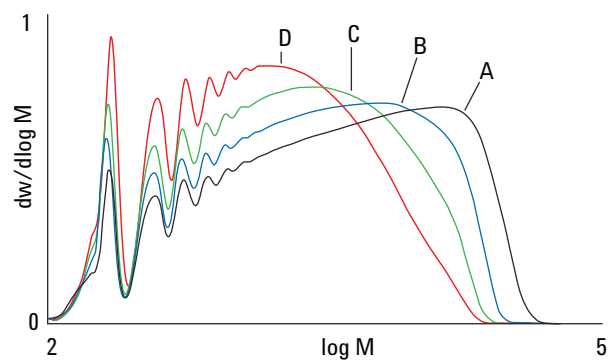


Figure 2. Overlaid molecular weight distributions of four phenolic resins reveals the relative amounts of the oligomer components with significant differences in molecular weight distribution.

Conclusions

High resolution Agilent ResiPore columns permit different grades of phenolic resin to be compared and their relative oligomeric content to be assessed, a property that affects the end-use applications of these materials.

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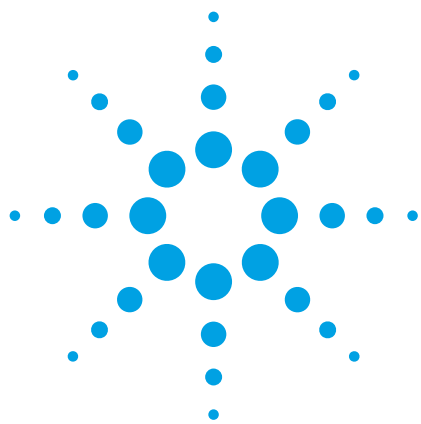
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Agilent Technologies



Quantitative analysis of copolymers using the Cary 630 FTIR spectrometer

Application note

Materials testing and research

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Introduction

FTIR spectrometers employing attenuated total reflectance (ATR) sampling interfaces are a proven and powerful tool for the analysis of polymeric materials. Because of its unique combination of features and class-leading performance, the new Agilent Cary 630 FTIR spectrometer makes quantitative analysis of polymers especially fast and easy.

In this application note, the amount of key components in two important copolymeric materials are measured — the styrene content in styrene butadiene rubber (SBR) and the ratio of polyethylene to vinyl acetate in polyethylene vinyl acetate (PEVA) polymer. The Cary 630 FTIR equipped with its single reflection Diamond ATR sampling accessory (Figure 1) is used for these measurements.



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Figure 1. Agilent Cary 630 FTIR spectrometer equipped with single reflection Diamond ATR sampling accessory

Styrene concentration in SBR polymer

Styrene butadiene rubber (SBR) is the most common synthetic rubber material and its main use is in the manufacture of tires, which accounts for nearly 70% of its production. The properties of SBR rubber can be altered by varying the ratio of styrene to butadiene monomers in the manufacturing process. The normal ratio is 3:1 butadiene to styrene (25% styrene). Higher styrene concentrations make the material harder, but less elastic. Most performance industries, such as racing tires and specialty military applications, are requiring more consistent SBR product, which drives the need for better quality assurance and control by both end users and manufacturers.

The measurement of a polymer sample by the Cary 630 FTIR equipped with an ATR accessory is extremely straightforward. The polymer material is placed on the diamond crystal and the sample pressure press is rotated downward until adequate pressure is placed on the sample to observe a spectrum in the Cary 630's real-time analysis MicroLab FTIR software (Figure 2). The real-time analysis mode provides instantaneous spectral update and makes it easy for even novice users to get highly repeatable results. The sample press on the Cary 630 is designed so that it cannot be over-tightened, thus protecting the diamond crystal against over-pressure.

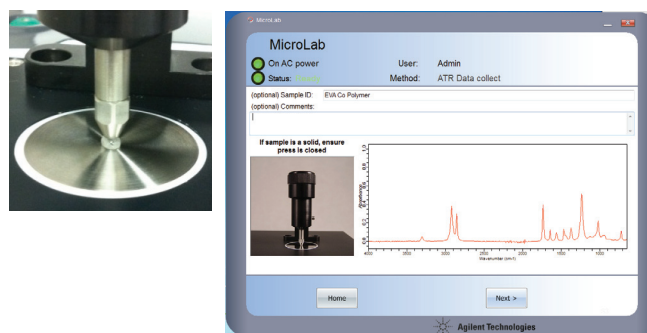


Figure 2. Polymer is placed directly on ATR sampling accessory. Uniform, constant pressure is provided by the sample press, ensuring that high quality spectra are obtained. Real-time analysis software provides an immediate indicator of spectral quality

To develop a quantitative FTIR method, four commercial SBR calibration standards, with polystyrene concentrations of 0%, 5%, 23%, and 45%, were measured in triplicate using the Cary 630 FTIR. The spectra reveal the expected polystyrene (PS) absorbance bands (Figure 3) at 699 cm^{-1} , 759 cm^{-1} , and a weaker band at 1031 cm^{-1} . Spectral bands at 911 cm^{-1} , 964 cm^{-1} , and 995 cm^{-1} arise from unsaturations (*vinyl* and *trans* CH wag) in polybutadiene, which decrease as the PS bands increase. The exception is the pure polybutadiene, which has far more *cis* unsaturations relative to the other polymers, since it is not cross-linked and in liquid form. The PS absorbance bands appear to follow Beer's Law by increasing proportionately with concentration, and therefore are excellent candidates for quantitative analysis.

The plot of the peak height absorbance for the strongest IR band of PS at 699 cm^{-1} as a function of concentration indicates great linearity and a strong correlation coefficient of $R^2=0.999$ in the calibration (Figure 4). Using the linear regression slope and offset from this calibration, a method is added to the MicroLab FTIR software that enables the polystyrene percentage in an unknown sample to be automatically displayed. The limit of detection for the quantitative analysis of PS in SBR is 0.09%, calculated as three times the standard deviation of the 0% replicate data ($\text{StDev} = 0.03\% \text{ PS}$).

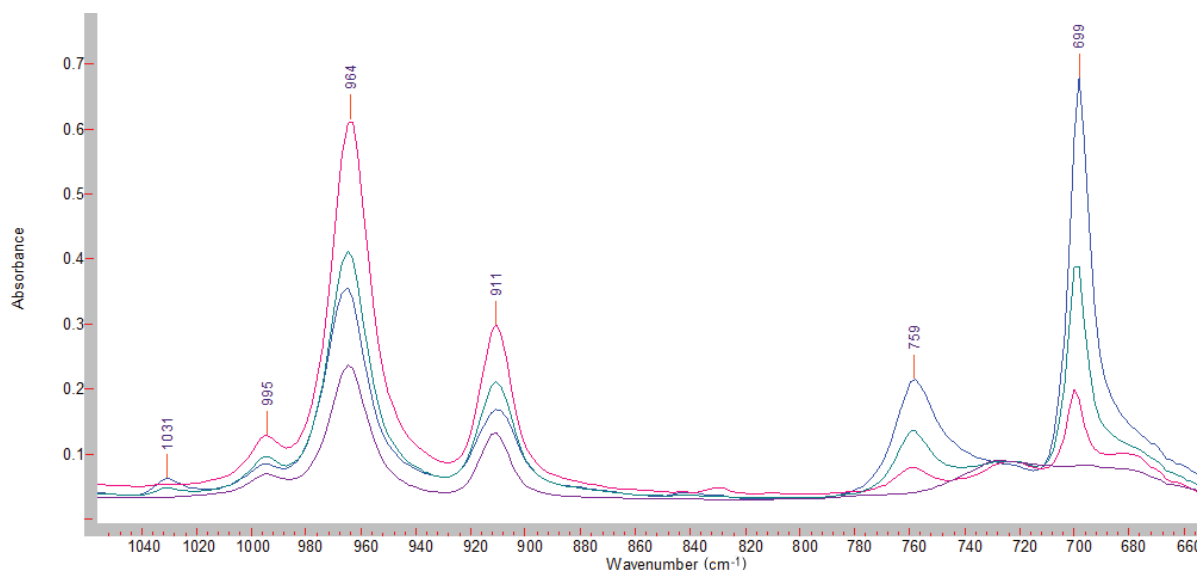


Figure 3. The FTIR spectra of four SBR rubber standards with increasing polystyrene concentrations: 0% (purple), 5% (red), 23% (green), and 45% (blue)

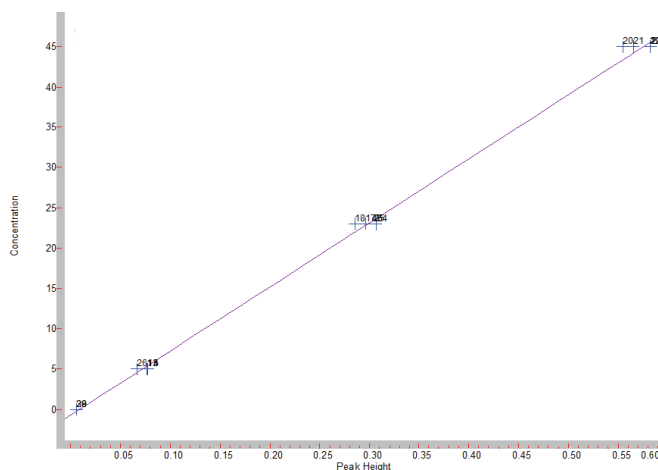


Figure 4. FTIR calibration curve for polystyrene in SBR rubber using the 699 cm^{-1} peak height absorbance; $R^2=0.999$.

Ratio of polyethylene to vinyl acetate in PEVA

Polyethylene vinyl acetate (PEVA) is very common in everyday products used in the home, sports equipment, industrial and medical applications. In the latter applications, medicines can be mixed in solution with PEVA and then the mixture dried to produce biologically-inert, slow-release plastic implants and transdermal patches.

Since the ratio of polyethylene (PE) to vinyl acetate (VA) in PEVA can affect the physical properties of the final product, it is important for manufacturers to have a fast, easy measurement procedure for these components. As in the previous example, the Cary 630 FTIR spectrometer with single reflection diamond ATR is ideal for this measurement.

In this example, seven commercially-available standards of PEVA were measured with the Cary 630 FTIR system. The calibration standards used were:

- Polyethylene, low density (0% vinyl acetate)
- Ethylene/vinyl acetate copolymer #506 (9 wt% vinyl acetate)
- Ethylene/vinyl acetate copolymer #243 (14 wt% vinyl acetate)
- Ethylene/vinyl acetate copolymer #244 (18 wt% vinyl acetate)
- Ethylene/vinyl acetate copolymer #245 (25 wt% vinyl acetate)
- Ethylene/vinyl acetate copolymer #316 (28 wt% vinyl acetate)
- Ethylene/vinyl acetate copolymer #326 (40 wt% vinyl acetate)

The calibration samples were measured with one minute collection times, at a resolution of 4 cm^{-1} . The FTIR spectra exhibit strong acetate ester carbonyl bands at 1737 cm^{-1} and an ester C-O stretch band at 1236 cm^{-1} (Figure 5) arising from polyvinyl acetate (VA). Both of these bands are ideal for quantitative analysis of the VA in the polyethylene (PE) matrix. The characteristic PE absorbance bands are located at 2921 cm^{-1} , 2852 cm^{-1} , 1467 cm^{-1} and 720 cm^{-1} . The best calibration is obtained by a peak area ratio of the 1236 cm^{-1} VA absorbance band ratioed to the PE absorbance at 1467 cm^{-1} . This IR absorbance ratio technique corrects for random variables that may affect the measurement, such as contact pressure or contact area of the polymers on the ATR diamond crystal. This is important since reliable ATR measurements require the sample to make good optical contact with the diamond, and hard, round polymer beads may not contact the whole diamond surface.

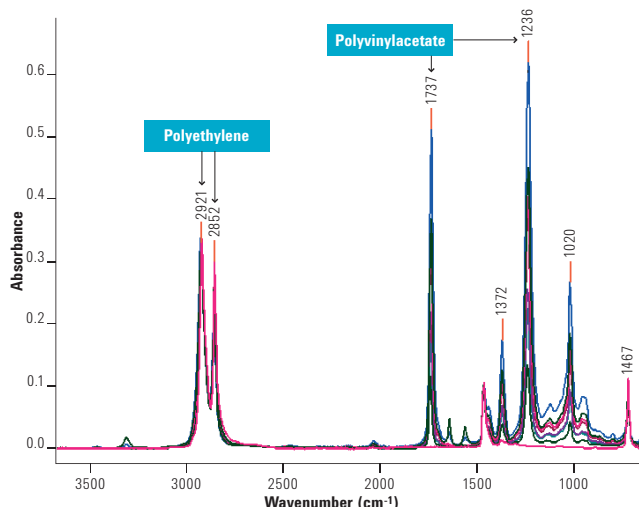


Figure 5. Spectral overlay of the calibration standards for polyethylene vinyl acetate). The spectra are all scaled to the polyethylene absorbance. The blue spectrum is 40 wt% VA, and the red spectrum is 0% VA

The resulting linear regression calibration curve from the above peak area ratio is excellent (Figure 6) with a correlation coefficient of $R^2 = 0.999$. The slope and offset for the linear regression is easily inserted into the MicroLab FTIR method editor (Figure 6), and the resultant method is now permanently calibrated. To test the robustness of the method, validation standards

were made by diluting (by weight) the 9% VA with the pure PE (0% VA) standards to make 1% and 0.55% VA samples. The polymer validation samples were then dissolved in toluene and heated to 75 °C until all the polymer dissolved. The toluene mixtures were then cast as thin films onto aluminum foil over a 60 °C hotplate and allowed to dry. The resulting polymer validation samples were then measured with the stored method. These validation samples were measured with a much shorter scan time (5 seconds) than the calibration set of spectra (60 seconds). This allows for multiple measurements of incoming raw materials in a very short time; this fast sample analysis is important for quality assurance and quality control (QA/QC) analysis. The speed of this analysis is also a benefit for incoming raw materials analysis in which a batch of PEVA can have some uniformity differences, requiring sampling from multiple areas of the container or on a molded part. The results of this fast analysis (5 second) yield exceptional repeatability and accuracy (Table 1) on the validation samples. A standard deviation of nominally 0.01% VA was obtained with limits of detection (LOD) and limits of quantitation (LOQ) of 0.03 wt% VA and 0.10 wt% VA, respectively. When a sample is run using this calibrated FTIR method, the results can also be displayed in color-coded format (Figure 7), indicating that the sample is in-spec (green), marginal (yellow), or out of spec (red). This enables an operator to get a rapid, visual indicator of the quality of the material.

Table 1. VA prediction values from the calibrated VA FTIR method for validation standards at 0.55% VA and 1.00% vinyl acetate in polyethylene. These validation samples were run with only 5 second collection times

Validation sample	0.55% VA	1.00% VA
Rep 1	0.53	0.97
Rep 2	0.54	0.96
Rep 3	0.55	0.96
Rep 4	0.56	0.96
Rep 5	0.55	0.99
Standard deviation	0.0114	0.0130
Average	0.55	0.97

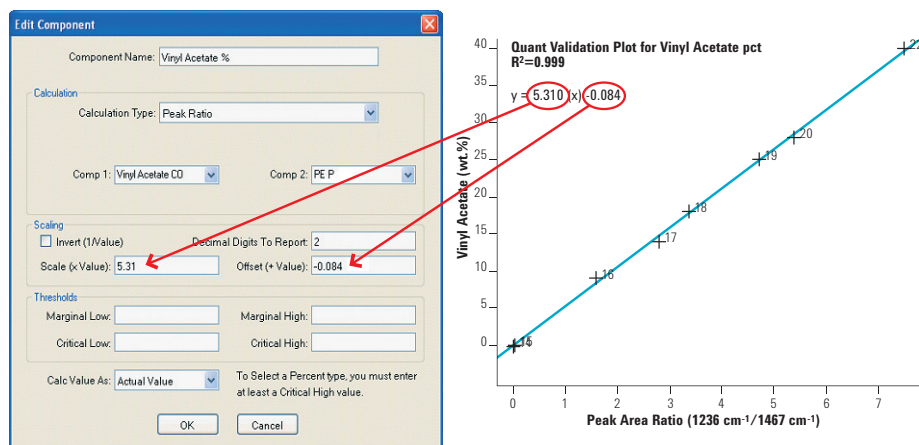


Figure 6. The method editor in the MicroLab FTIR software and the calibration plot for VA in PE

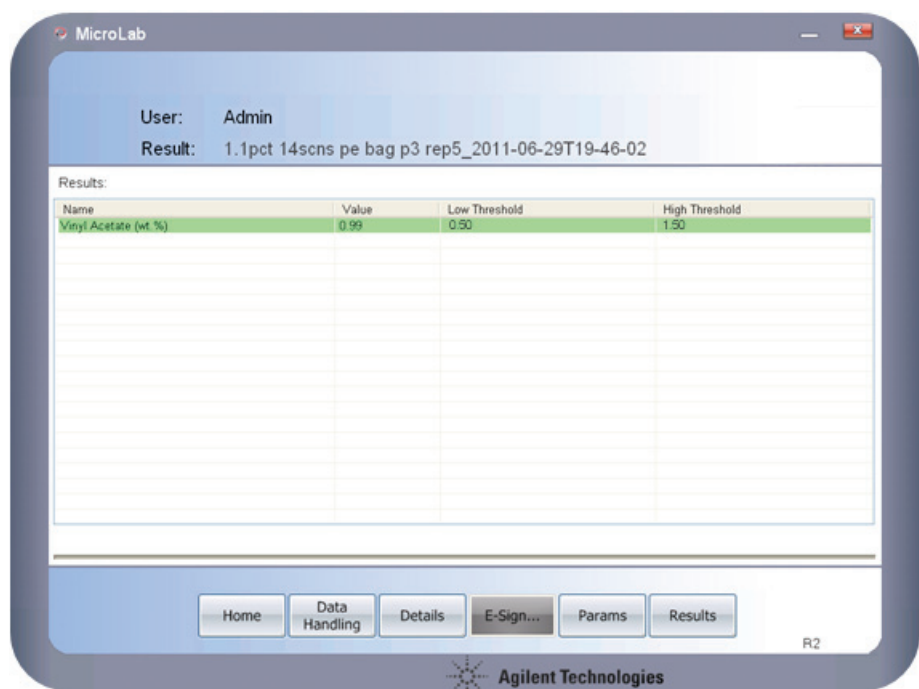


Figure 7. The result for the 1% VA validation standard — green color indicates an in-spec sample

Conclusion

The Agilent Cary 630 FTIR equipped with ATR sampling technology is an exceedingly effective spectrometer for analyzing copolymer blends. The combination of its compact size, sampling technology, performance, speed of analysis, and intuitive software enables quantitative methods for polymers to be rapidly developed and deployed in quality assurance and quality control applications. The measurement of both SBR and PEVA copolymers yields highly linear calibrations with excellent quantitative accuracy and reproducibility.

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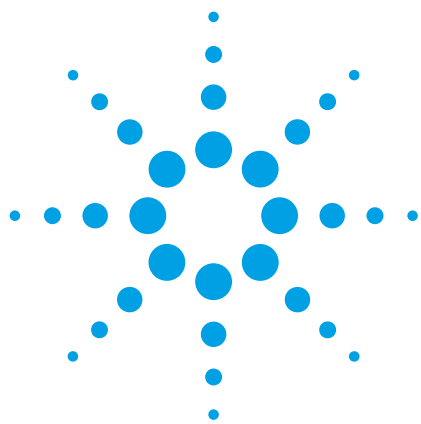
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Analysis of wear metals and contaminants in engine oils using the 4100 MP-AES

Application note

Energy and fuels

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Melbourne, Australia



Introduction

The regular tracking of the metals present in oils used to lubricate machinery is a vital preventive maintenance task used to gauge the condition of the lubricant and machine over time. Analysts are particularly interested in the elements found in engines, such as Cu, Fe and Al, which are present in the oil as a result of wear and tear, and elements like Na and Si, which are present as a result of contamination from water or road dust. The trend analysis of these metals is performed on the oils so that any action required to keep the engine in service can be taken and costly repairs and downtime can be avoided.

With engines and machinery being central to most transport and manufacturing industries, many laboratories are required to analyze a high volume and variety of oil samples a day, for multiple elements. While flame atomic absorption spectrometry (FAAS) has been used extensively to study trace wear metals in used oils, the sheer number of samples has forced many laboratories to consider a faster, multi-element technique that is capable of high sample throughput.



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This can now be effectively achieved using fast sequential atomic emission spectroscopy in the form of the Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES). The 4100 MP-AES uses magnetically-coupled microwave energy to generate a robust and stable plasma using nitrogen gas. Both aqueous and organic samples can be introduced into the MP-AES, which has good tolerance to the organic solvent load.

Experimental

Instrumentation

An Agilent 4100 MP-AES was used with an External Gas Control Module (EGCM) allowing air injection into the plasma to prevent carbon deposition in the torch, overcome any plasma instability that may arise from the analysis of organic samples, and to reduce background emissions. The instrument was set up with the Organics kit comprising the EGCM, the inert OneNeb nebulizer [1] and solvent resistant tubing, along with a double pass spray chamber. The OneNeb nebulizer offers superior performance for this application over other comparable nebulizers as it offers increased nebulization efficiency and a narrow distribution of small droplets. This allows the analysis to be performed at lower flow rates, reducing the solvent loading on the plasma, while maintaining excellent sensitivity. An Agilent SPS 3 Sample Preparation System was used for automatic sample delivery.

The instrument is controlled using Agilent's unique worksheet-based MP Expert software, which runs on the Microsoft® Windows® 7 operating system, and features automated optimization tools to accelerate method development by novice operators. For example, the software automatically adds the recommended wavelength, nebulizer pressure, and EGCM setting when elements are selected.

Instrument operating conditions and analyte settings are listed in Tables 1a and 1b. Viewing position and nebulizer pressure settings were optimized using the auto-optimization routines in MP Expert. Rational fit is a non-linear curve fit and allows an extended working range so that sample analysis can be carried out using a single wavelength without further dilutions being required.

Samples and sample preparation

Standards were prepared at concentrations of 5 ppm, 10 ppm, 25 ppm and 50 ppm from a 500 ppm oil-based metal calibration standard S21+K (Conostan). Shellsol 2046 (Shell) was used as the diluent. All standards were matrix-matched with 10% Blank Oil (Conostan).

NIST SRM 1085b Wear Metals in Lubricating Oil was prepared by performing a 1:10 dilution in Shellsol.

A sample consisting of a mix of used gear oils was diluted 1:10 with Shellsol and spiked with S21+K, giving a final spike concentration of 10.2 ppm.

Table 1a. Agilent 4100 MP-AES operating conditions

Instrument parameter	Setting
Nebulizer	Inert OneNeb
Spray chamber	Double-pass glass cyclonic
Sample tubing	Orange/green solvent-resistant
Waste tubing	Blue/blue solvent-resistant
Read time	3 s
Number of replicates	3
Stabilization time	15 s
Rinse time	45 s
Fast pump (80 rpm) during sample uptake	On
Background correction	Auto
Pump speed	5 rpm

Table 1b. Analyte nebulizer pressures and calibration curves

Element & wavelength (nm)	Nebulizer pressure (kPa)	Calibration curve
Cd 228.802	140	Rational
Mn 259.372	120	Rational
Fe 259.940	100	Rational
Cr 276.653	140	Rational
Pb 283.305	220	Rational
Sn 303.411	240	Rational
Ni 305.081	180	Linear
V 310.229	220	Rational
Mo 319.398	240	Rational
Ti 323.452	220	Rational
Cu 327.395	200	Linear
Ag 328.068	200	Linear
Al 396.152	240	Rational
Na 589.592	240	Linear
Si 251.611	140	Linear

Results and discussion

Analysis of standard reference materials

To test the validity of the method, NIST SRM 1085b was analyzed. The results presented in Table 2 show excellent agreement (accuracy) between the MP-AES measured results and the certified values.

Table 2. Measured results versus certified values

Element & wavelength (nm)	Measured (mg/kg)	Certified (mg/kg)	Recovery (%)
Fe 259.940	314.7 ± 0.3	301.2 ± 5.0	104
Mn 259.372	289.9 ± 0.2	300.7 ± 2.0	96
Cd 226.502	290.9 ± 2.9	302.9 ± 5.1	96
Cr 276.653	305.2 ± 0.1	302.9 ± 3.9	101
Si 251.611	295.7 ± 1.9	300.2 ± 5.0	99
Ni 305.081	291.6 ± 0.1	295.9 ± 7.4	99
Cu 327.395	300.9 ± 0.1	295.6 ± 8.5	102
Ag 328.068	308 ± 0.2	304.6 ± 8.9	101
Pb 283.305	296.1 ± 0.1	297.7 ± 6.8	99
V 310.229	287.6 ± 0.1	297.8 ± 4.6	97
Ti 323.452	293.9 ± 0.1	301.1 ± 2.9	98
Sn 303.411	295.3 ± 0.3	299.4 ± 4.8	99
Mo 319.398	296.9 ± 0.1	300.6 ± 3.2	99
Al 396.152	291.7 ± 0.2	300.4 ± 9.3	97
Na 589.592	297.4 ± 0.1	305.2 ± 7.0	97

Spike recoveries

The recoveries obtained for the spiked mixed gear oil sample are presented in Table 3. Excellent recoveries were obtained for all elements analyzed, demonstrating the validity of the analytical method. The signal graph and calibration curve for Cu are shown in Figures 1 and 2 respectively.

Table 3. Accurate recovery for all analytes of 10 ppm spikes in a mixed gear oils sample

Element	Wavelength (nm)	Unspiked gear oil (ppm)	Spiked gear oil (ppm)	Spike recovery (%)
Ag	328.068 nm	0.27	11.01	105
Al	396.152 nm	0.32	10.31	98
Cd	228.802 nm	0.14	9.85	95
Cr	276.653 nm	0.25	9.92	95
Cu	327.395 nm	2.68	13.14	103
Fe	259.940 nm	10.41	20.09	95
Mn	259.372 nm	0.80	11.54	105
Mo	319.398 nm	9.02	19.34	101
Na	589.592 nm	0.46	10.70	100
Ni	305.081 nm	0.07	10.13	99
Pb	283.305 nm	0.25	11.36	109
Si	251.611 nm	2.23	11.60	92
Sn	303.411 nm	0.16	10.62	103
Ti	323.452 nm	0.01	10.87	106
V	310.229 nm	0.15	10.71	104

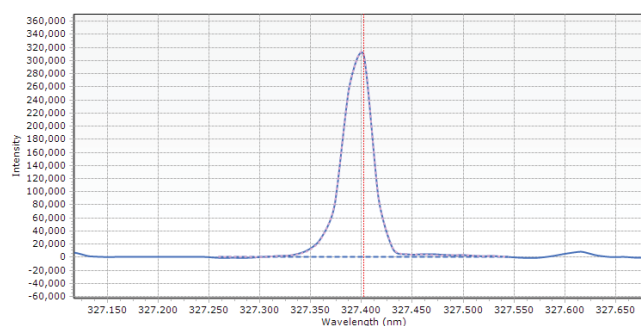


Figure 1. The signal from Cu 327.395 nm at 5 ppm shows the excellent sensitivity of the Agilent 4100 MP-AES

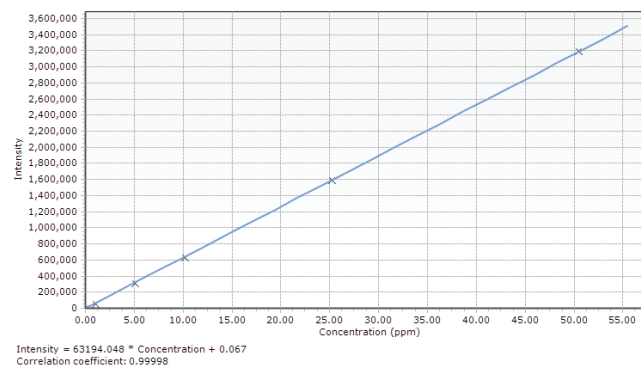


Figure 2. The calibration curve for Cu 327.395 nm up to 50 ppm shows excellent linearity across the calibrated range, with a correlation coefficient of 0.99998

Using the Agilent SPS 3 Sample Preparation System, the sample throughput time for the analysis was under 5 minutes per sample, or about 13 samples per hour. With the ability to run unattended, the 4100 MP-AES is capable of greater sample throughput than FAAS.

Long-term stability

Long-term stability of the MP-AES was investigated by continuously aspirating a 10 ppm S21+K solution over an 8 hour period. The resulting stability plot is shown in Figure 3, and the %RSDs for each element are listed in Table 4.

The sample handling capability of the vertically-oriented plasma in the 4100 MP-AES, combined with the air injection from the EGCM and the solids handling of the inert OneNeb nebulizer [1] means that excellent long-term stability (< 1% RSD) can be achieved, even when analyzing challenging organic samples.

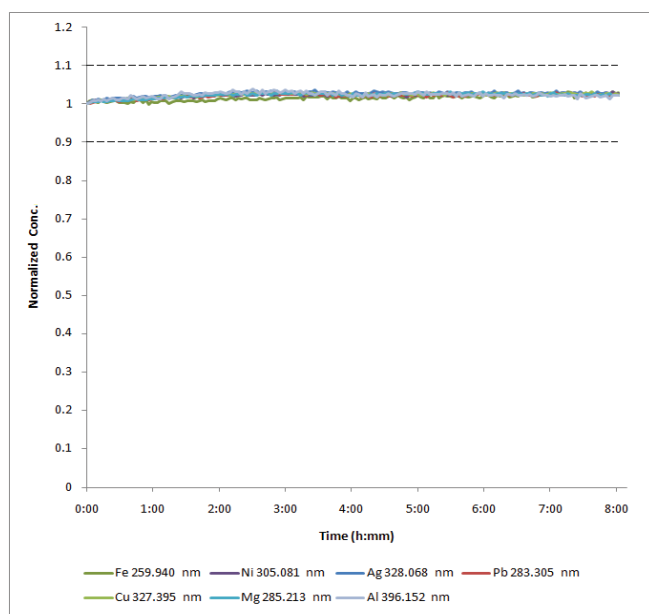


Figure 3. Normalized stability plot for 10 ppm S21+K solution run repeatedly over an 8 hour period

Table 4. %RSDs for each element spiked at 10 ppm level over an 8 hour sampling period

Element	Wavelength (nm)	%RSD
Fe	259.940	0.7
Ni	305.081	0.5
Ag	328.068	0.5
Pb	283.305	0.6
Cu	327.395	0.6
Al	396.152	0.6

Conclusions

The Agilent 4100 MP-AES equipped with a OneNeb nebulizer and fitted with the EGCM is an ideal solution for the routine multi-element analysis of wear metals in oils. Furthermore, the Agilent 4100 MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide. By injecting a controlled flow of air into the plasma via the EGCM to prevent carbon buildup in the injector, excellent recoveries were achieved for SRM samples and on spiked solutions at the 10 ppm level. Excellent long-term stability was also achieved.

Reference

1. J. Moffett and G. Russell, "Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer", Agilent Application Note 5990-8340EN

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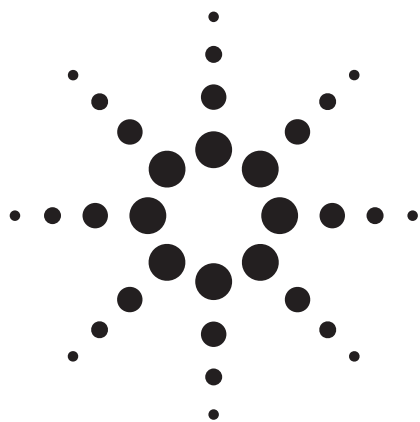
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Agilent Technologies



The Analysis of Polyvinylchloride

Application Note

Atomic Absorption

Authors

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Introduction

The presence of various metals in synthetic fibres and plastic materials can arise from catalyst residues, stabilizing agents, and other sources, however, excessive concentrations can have a deleterious effect upon the characteristics of such materials.

The technique of atomic absorption spectrophotometry is eminently suitable for the rapid and accurate analysis of metals in such polymeric materials.

The choice of solvents or the method used for dissolving polymeric materials depends on the type of polymer. Olivier [1] has described the determination of a wide range of trace metals in various polymeric materials, and the determination of germanium in synthetic fibres has also been reported [2].

A description is given of a method employed at Agilent Technologies, Inc. for the analysis of calcium, tin, titanium and zinc in a sheet of polyvinylchloride (P. V. C.) that contains a relatively high amount of titanium.



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Experimental

The calibration graphs generated using the conditions of Table 1 are shown in Figure 1.

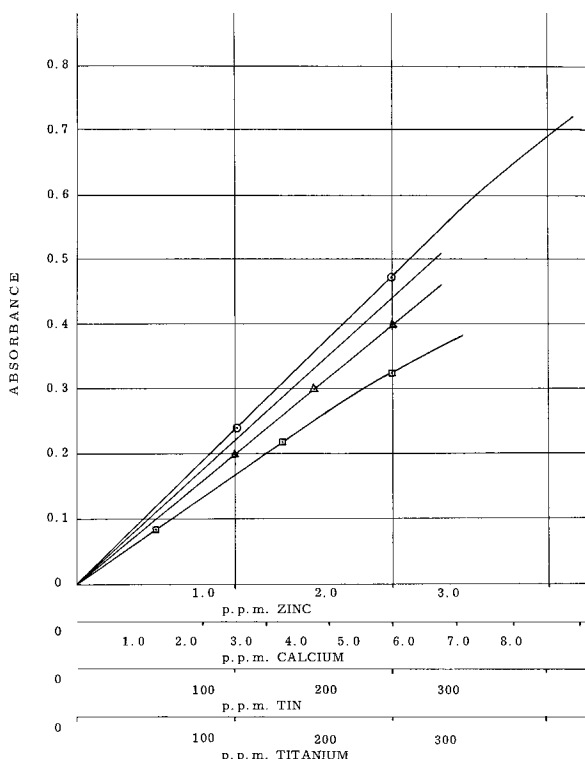


Figure 1. Calibration graphs for calcium, tin, titanium and zinc.

Instrumentation

Table 1. Instrument Parameters

	Calcium	Tin	Titanium	Zinc
Line (Å)	4226.7	2246.1	3642.7	2138.6
Lamp Current	4 mA	8 mA	20 mA	6 mA
Spectral Band Pass (Å)	3.3 Å	3.3 Å	1.7 Å	3.3 Å
Flame	N ₂ O-C ₂ H ₂	N ₂ O-C ₂ H ₂	N ₂ O-C ₂ H ₂	Air-C ₂ H ₂

P. V. C. Sample Solutions

Approximately 2 g of P. V. C. are cut up and weighed out accurately into a 250 mL conical flask.

Twenty mL of concentrated nitric acid are added and the mixture is warmed on a hot-plate in order to initiate the oxidation.

Five mL of 60% v/v perchloric acid are carefully added and the mixture is boiled until white fumes of perchloric acid are evolved.

This procedure is repeated with another 10 mL of nitric and 4 mL of perchloric acids.

When all the nitric acid has been boiled off the mixture is heated strongly until all the organic matter has been removed and only a fine white precipitate of TiO₂ remains.

The contents are transferred quantitatively to a PTFE beaker and evaporated down to a volume of about 10 mL.

8 mL of nitric acid and 4 mL of hydrofluoric acid are added and the mixture is gently heated for one hour in order to complete the dissolution.

The clear solution is then transferred to a volumetric flask and made up to exactly 30 mL with distilled water.

Standard Solutions

Standard solutions are prepared so as to cover the expected concentration of the metals in the sample solution.

Notes on Individual Metal Determinations

Calcium

The atomic absorption measurements are made at the 4226.7 Å resonance line, using a N₂O-C₂H₂ flame.

In order to prevent ionization of the calcium an excess of an ionization suppressant, for example, 5 000 ppm Na, is added to both sample and standard solutions. The standard solutions also contain approximately the same amount of perchloric acid as in the final diluted sample solution (approximately 1.5% HClO₄).

Normal concentration range of standards:

0.5 to 8.0 ppm Ca, with 10 × scale expansion down to approximately 0.1 ppm Ca.

Tin

The atomic absorption measurements are made at the 2246.1 Å resonance line. The use of the hotter, but less sensitive, $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame is preferred to the more sensitive Air- H_2 flame in order to eliminate or minimize possible chemical interferences.

Standard solutions containing the same amount of perchloric acid as in the sample solution are made up (approximately 25% HClO_4)

Normal concentration range of standards:

20 to 300 ppm Sn, with $10 \times$ scale expansion down to approximately 4 ppm Sn.

Titanium

Titanium absorbance is measured at the 3642.7 Å resonance line, using a $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

It has been observed that titanium absorbance is enhanced in the presence of hydrofluoric acid, therefore both sample and standard solutions should be fairly closely matched for hydrofluoric acid content.

It has also been found that sodium causes an interference on titanium absorbance in the presence of hydrofluoric acid (Figure 2), therefore, if sodium is present in the sample solutions it should also be present at the same concentration in the standard solutions.

Normal concentration range of standards:

20 to 300 ppm Ti, with $10 \times$ scale expansion down to approximately 4 ppm Ti.

Zinc

The atomic absorption measurements are made at the 2138.6 Å resonance line, using an air- C_2H_2 flame.

The standard solutions should contain approximately the same amount of perchloric acid as in the final diluted sample solution (approximately 1.5% HClO_4)

Normal concentration range of standards:

0.2 to 3.0 ppm Zn, with $10 \times$ scale expansion down to approximately 0.04 ppm Zn.

The lowest given metal concentration is not the detection limit, but a concentration at which the precision of the determination is still fairly high.

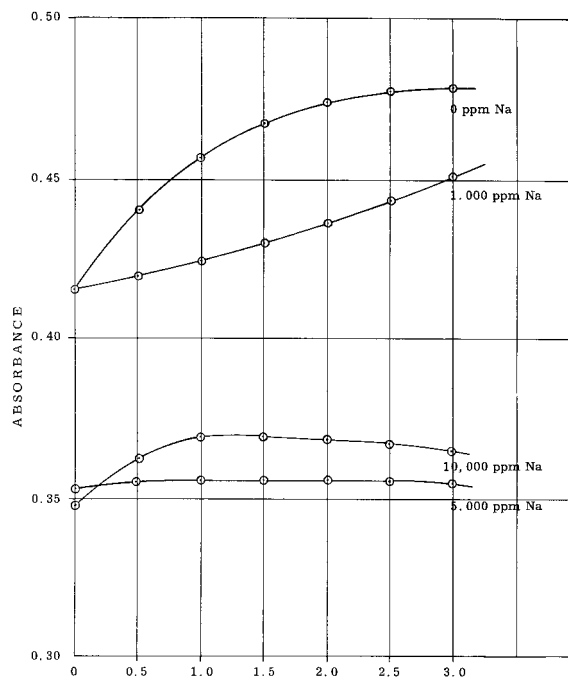


Figure 2. Effect of sodium on titanium absorbance in the zinc presence of hydrofluoric acid.

Results and Discussion

Some typical results are shown in the table.

Table 2. Typical Results

Ca	0.015	ppm
Sn	0.023	ppm
Ti	2.01	ppm
Zn	0.024	ppm

Conclusion

The atomic absorption spectrophotometric technique is highly suitable for the rapid and accurate determination not only of these four elements but other elements as well.

For the determination of much lower levels of these metals in polyvinylchloride a chelation-organic solvent extraction-concentration procedure would have to be carried out prior to the atomic absorption measurements.

References

1. M. Olivier, Z. Anal. Chem., 248, 145-148 (1969)
2. M. Yanagis, M. Suzuki, T. Takeuchi, Anal. Chim. Acta, 46(1), 152-154 (1969)

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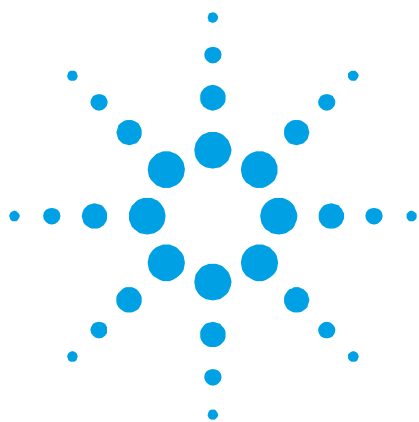
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Infrared imaging – the evolution of infrared microscopy

Application Note

Author

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Agilent Technologies, Inc.

Introduction

For a number of years infrared (IR) microscopy has been an important tool for IR spectroscopists. The IR microscope has developed considerably over the past few years and can now be considered a multifunctional accessory suitable for a wide variety of measurements in transmission, reflectance, grazing angle, and attenuated total reflectance (ATR) modes. IR microscopy is a powerful investigative technique and can be used to solve problems in fields as diverse as mineralogy (oil inclusions in rocks), polymer manufacturing (surface defects) and forensics (identification of paint chips). In fact, IR microscopy has almost become the ubiquitous trouble shooting technique when it comes to surface defects and contaminants, and wherever small samples are encountered. Using a conventional IR microscope it is possible to collect an IR spectrum from an area as small as 7-10 μm in transmission and reflection, or $\sim 20 \mu\text{m}$ using an ATR contact technique. The minimum area from which a sample can be measured is defined by the diffraction limit. That is the minimum area from which a measurement can be made approaches the wavelength of light with which the spectral analysis is made.

An intrinsic application of IR microscopy is the determination of the chemical distribution of a compound, component or defect, on or within a sample. To date, much of this work has been done by IR mapping. In a mapping experiment, a grid or line is defined across a sample surface and a series of spectra collected sequentially. Using these sequential spectra, a map of the sample can be generated and, from the spectral information obtained, a contour or 3D map depicting the distribution of a chemical functional group and/or component can be prepared.



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Whilst still a powerful investigative technique, IR mapping suffers from a number of disadvantages and, whilst still useful in many cases, is limited with respect to time and efficiency. In a mapping experiment, data is collected sequentially and, with each data point requiring several minutes to co-add spectra, even relatively small maps can require long acquisition times (~1 hr). Using a smaller sampling area, and/or higher spectral resolution further increases the analysis time required, meaning detailed investigations can require 8 hours (or more) scanning time (introducing additional problems where liquid nitrogen cooled detectors are concerned).

Since the launch of the first FTIR microscope in the early 1980's (Digilab UMA-100), continuous innovation and development has extended the functionality and efficacy of IR microscopy. The late 1990's saw the release of the first FTIR imaging system using focal plane array (FPA) detector technology (Digilab Stingray). In combination, FTIR microscope and FPA detector make for a powerful analytical tool. FTIR imaging provides all the functionality of mapping, but does so faster and with better spatial resolution, and most importantly, with considerably better sensitivity.

Focal plane arrays

Based on technology first developed with astronomy and military applications in mind, an FPA essentially performs like a camera – but with a spectral advantage. Instead of collecting one data point at a time, or collecting a row of data points (as is the case with linear arrays), the imaging is accomplished by collecting spectra from all detector pixels simultaneously. Less time consuming and more efficient than mapping, true FPA imaging permits the fast acquisition of a spectrochemical snapshot of the sample being investigated.

Spectrochemical imaging

IR imaging (or spectrochemical imaging as it has become known) employs a focal plane array detector consisting of a square array of Mercury Cadmium Telluride (MCT) detector elements (Figure 1). Using an IR microscope, an image of the sample is imaged onto the MCT detector array and, at each element of the array a complete IR spectrum of the corresponding region of the sample is obtained (Figure 2).

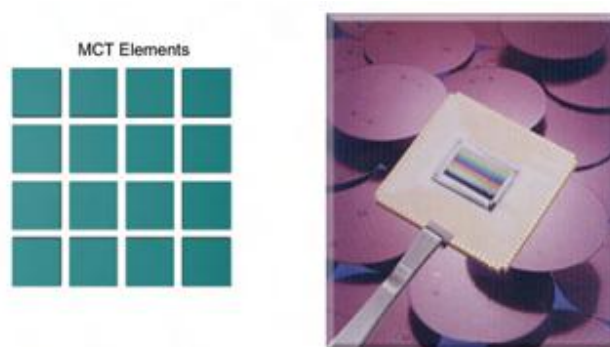


Figure 1. The Focal Plane Array detector: square grid of MCT detector elements (left) and finished FPA detector assembly (right)

The total IR energy across the array (or total IR response at each detector element) is used to create the primary IR image of the sample, with this IR image corresponding to the visible image of the sample. The power of the technique stems from the fact that from each pixel of the array an entire infrared spectrum can be extracted. Each individual spectrum can then be used to create secondary (or functional group) images of the area sampled. There may be as many as 200 secondary images behind the primary image, with spectral information extractable as peak heights, peak areas or peak ratios within each separate spectrum.

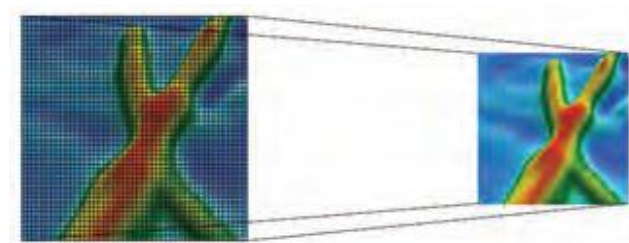


Figure 2. The sample is imaged on to a detector array, with each detector pixel collecting a full infrared spectrum

Arrays can range in size from 16 x 16 MCT pixels up to 256 x 256 pixels, and comprise between 256 and 65,536 individual IR spectra. The area of sample imaged is dependent on the size of the array used, with areas between 88 μm^2 and 1.2 mm^2 typically measurable in a time span of approximately 0.5 to 9.0 seconds (at 8 cm^{-1} spectral resolution).

The sheer speed of data collection offers enormous advantages over conventional IR mapping, with sample throughput increased at least fifty-fold. Combined with improved spatial resolution and signal-to-noise, FTIR imaging is a technology with wide ranging applications. In fact, wherever an IR microscope is being used today, FTIR imaging will offer significant advantages to the analyst.

Applications of spectrochemical imaging

FTIR imaging is an information-rich investigative technique and, as such, finds application in many branches of the physical sciences. Be it semiconductor defect analysis or biomedical bacterial screening, spectroscopists and non-spectroscopists alike are quickly discovering the power of FTIR imaging. In some cases it is simply the increased speed of analysis that sets imaging apart from mapping. In others it is the improved spatial resolution and signal-to-noise that, in combination, provide new insights into areas as diverse as drug dissolution and the science of adhesion, or simply permit the acquisition of an infrared spectrum of a sample that has previously proved 'unanalysable'.

An indicative example of the power of spectrochemical imaging is the identification of surface contamination. Figure 3 shows the visible image of a metal surface with an unidentified surface defect.

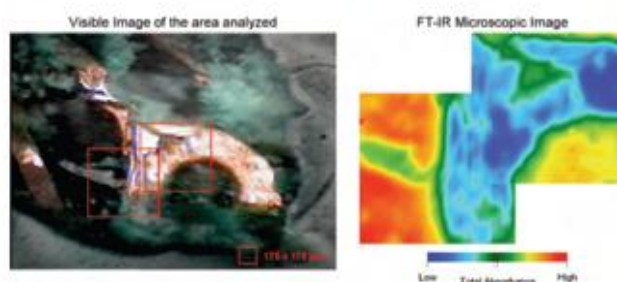


Figure 3. Defect on a metal surface: visible image (left) and total infrared image (right) of the sample

In a matter of seconds the surface defect can be imaged using the focal plane array, and an IR map of the sample surface displayed. As described previously, the IR image is created from the total IR reflection across the area of the sample imaged.

At each point (or pixel) on the IR image (corresponding to a 5.5 μm^2 point on the sample), an entire infrared spectrum is available. In this particular image, there are 4096 (64 x 64) points with 4096 associated spectra. The area of the image is 35 μm^2 and, at a spectral resolution of 8 cm^{-1} , a single image is collected in less than one second. From the individual spectra, peak heights can be used to generate the secondary images shown in Figure 4.

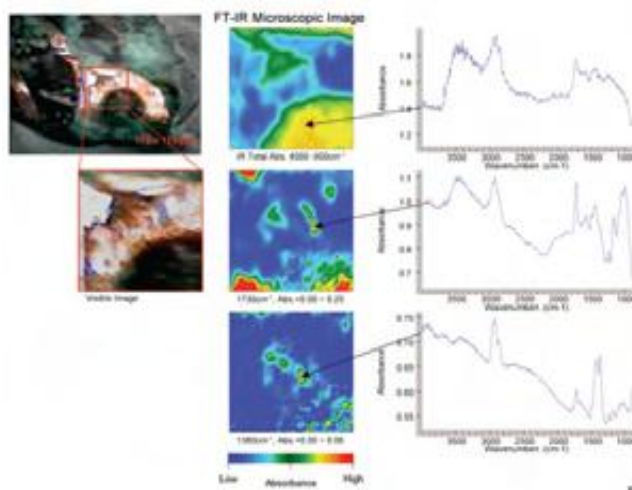


Figure 4. Defect on a metal surface: a complete infrared spectrum is recorded at each point on the detector array. From these individual spectra, functional group images (centre) can be generated

These secondary images allow the analyst to determine the nature of the defect or contaminant and/or image the distribution of the contaminant (in this case organic) across the surface in question.

An extension of FTIR imaging involves the use of attenuated total reflectance (ATR). ATR imaging has a number of advantages over conventional FTIR imaging, the most significant of these being the ability further improve spatial resolution. ATR is a contact sampling technique which requires the use of an IR transparent, high refractive index medium (such as Germanium). Where imaging is concerned, this has the double advantage of reducing the diffraction limited minimum area from which a sample can be measured whilst also simplifying sample preparation.

Figure 5 shows the visible image of a number of polystyrene beads known to have an approximate diameter of 15 μm .



Figure 5. Visible image of a number of polystyrene beads known to have an approximate diameter of 15 μm

The corresponding ATR image of one of these beads (Figure 6), confirms (a) the chemical identity of the bead and (b) the diameter of the bead. Where samples are too thick for transmission and/or poor reflectors, ATR imaging is the tool of choice.

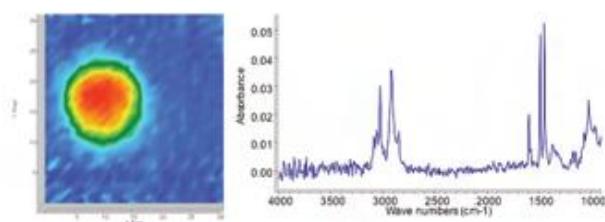


Figure 6. ATR image of polystyrene bead confirming chemical identity (left – total IR image; right – spectrum of bead centre region) and diameter (bead is 13 pixels wide, equating to 18.2 μm).

Conclusion

Spectrochemical imaging is a powerful and information rich technique applicable to a variety of disciplines and applications. The combination of FTIR microscopes with focal plane array detectors overcomes many of the limitations of IR mapping, and the use of square arrays greatly reduces the time required to perform imaging analyses.

Once solely the domain of the 'nuts & bolts' spectroscopist, the IR microscope has now become an almost ubiquitous FTIR accessory, finding application in laboratories the world over. Taking the power of IR microscopy one step further, FTIR imaging using FPA technology is poised to do the same. 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.

About the authors

Andrew Hind has a PhD in physical/analytical chemistry, using predominantly FTIR, and more than 10 years experience using molecular spectroscopy in fundamental and applied research and industrial problem solving.

John Wilson has a PhD in analytical chemistry. Specialising in molecular spectroscopy, he has 30 years experience in the application of molecular spectroscopic techniques.

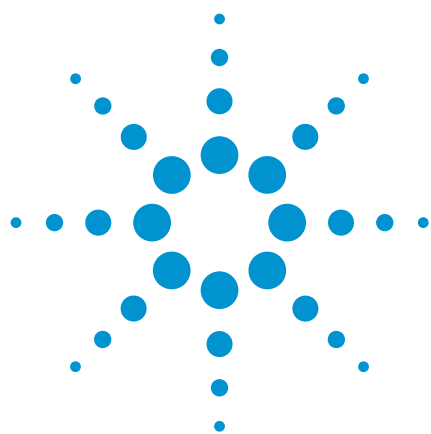
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Analysis of Phenol-formaldehyde Resins by GPC and Agilent PolarGel-M

Application Note

Authors

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Introduction

Phenol formaldehyde (P-F) resins are thermoplastic materials made with an excess of phenol in an acid catalyzed reaction with formaldehyde. P-F resins are commonly used as precursors to varnishes and other surface finish products.



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GPC Analysis

PolarGel-M GPC columns are packed with low swell, macroporous copolymer beads that have a surface of balanced polarity, comprising hydrophobic and hydrophilic components. These allow PolarGel-M to be used in the analysis of high polarity polymers that are insoluble in water to give a more accurate representation of the molecular weight distribution of the polymer. If these polar polymers were to be analyzed with traditional styrene/divinyl benzene columns, interactions would cause artifacts in the peak shape and longer retention times, which would translate into apparently much lower molecular weight averages.

Sample Preparation

Two types of phenol-formaldehyde resin were analyzed to obtain an indication of differences in molecular weight, if any. The samples were made up in 0.2 % (w/v) DMF, with 0.1 % LiBr added to reduce sample aggregation, and injected without further treatment.

Conditions

Columns:	2 x PolarGel-M, 300 x 7.5 mm (p/n PL1117-6800)
Eluent:	DMF & 0.1 % LiBr
Flow Rate:	1.0 mL/min
Injection Volume:	100 μ L
Temperature:	50 $^{\circ}$ C
Detectors:	Agilent PL-GPC 50, RI

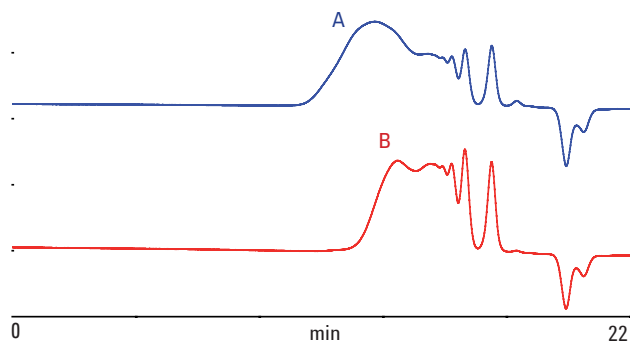


Figure 1. Overlaid molecular weight distributions of two novolac resins

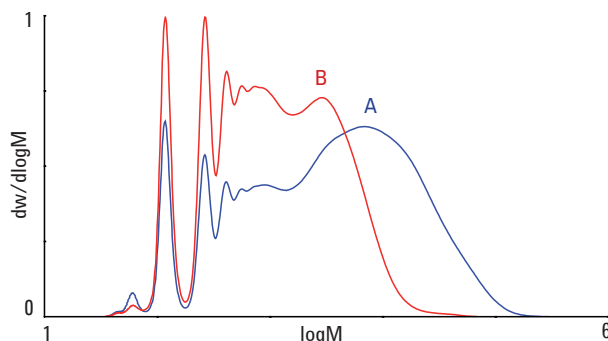


Figure 2. Overlaid molecular weight distributions of two phenol-formaldehyde resins

Conclusion

GPC with PolarGel-M columns allows for the artifact, interaction free calculation of the composition and molecular weight distributions of phenol-formaldehyde resins that are difficult to analyze on traditional, organic (PS/DVB) GPC columns.

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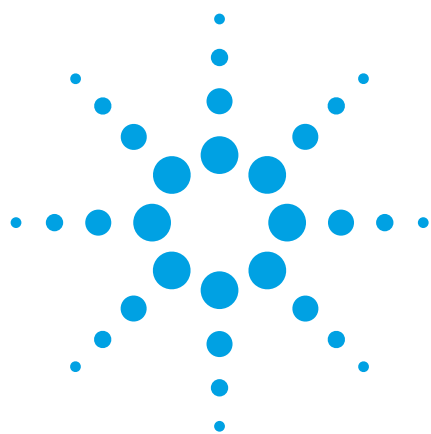
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Published in UK, September 2, 2010

SI-00949



Agilent Technologies



Analysis of Fluid Cat Cracker Feed using an Agilent J&W FactorFour VF-5ht UltiMetal Column

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

This analysis of fluid cat cracker feed is performed using a VF-5ht UltiMetal column. The column has been developed using proprietary UltiMetal technology that provides a virtually unbreakable metal column material with excellent inertness properties similar to fused silica tubing. The UltiMetal tubing is coated with the VF-5 low bleed arylene stabilized liquid phase, resulting in a highly temperature stable and durable column perfectly suited for a variety of high temperature applications.



Agilent Technologies

Conditions

Technique:	GC-FID	Carrier Gas:	Hydrogen, constant flow mode	Injection Volume:	1.0 μ l
Column:	VF-5ht UltiMetal, 15 m x 0.32 mm Df = 0.1 μ m + Retention Gap, 2 m x 0.53 mm (p/n CP9095)	Injector:	On-column (1093), reversed liner, 100 °C (0 min) to 400 °C with 15 °C/min	Temperature:	50 °C (1 min) to 450 °C (20 mins) with 10 °C / min
Sample:	1 % CDU5 FCC Feed, 1 % in CS2			Detection:	FID (HT), 400 °C

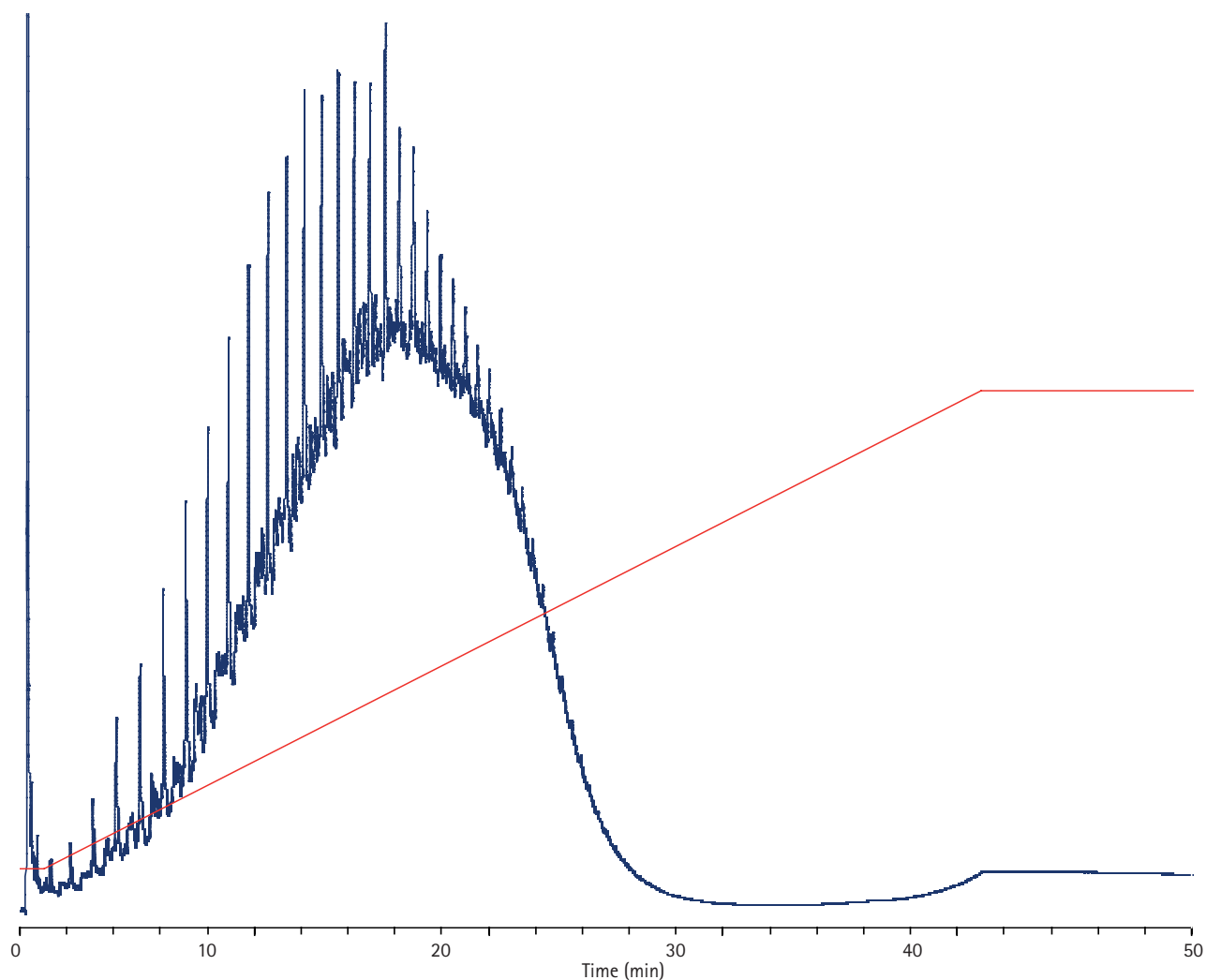


Figure 1. Analysis of fluid cat cracker feed using a VF-5ht UltiMetal column

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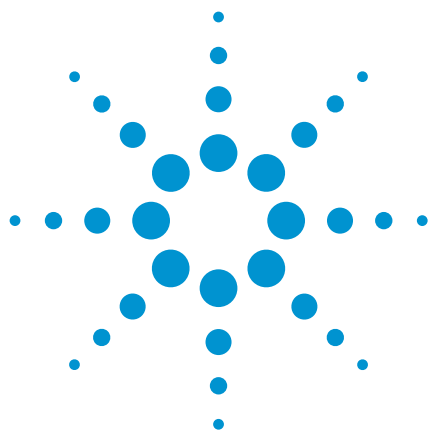
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Published in UK, October 07, 2010

SI-01130



Agilent Technologies



Analysis of Natural Rubber by GPC with Triple Detection

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

The para rubber tree, *Hevea brasiliensis* (A. Juss.) Muell. Arg. (Euphorbiaceae) is the major commercial source of natural rubber latex, essentially a polymer of isoprene units. The material properties of natural rubber make it an elastomer and a thermoplastic. Rubber materials are used in a wide variety of household and industrial applications, either at the intermediate stage or as final products. The largest use of natural rubber is in the manufacture of tires and inner tubes, accounting for around 56% of the total world use.

Two samples of natural rubber were analyzed by gel permeation chromatography with triple detection, yielding molecular weight independent of a column calibration. The objective was to determine why one of the materials had failed in end use. An integrated GPC system was used for the analysis.



Agilent Technologies

Instrumentation

The samples were assessed by a Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer, Agilent PL-LS 15°/90° dual angle light scattering detector, and Agilent PLgel 10 μ m MIXED-B columns. These columns provide high resolution of polymers that have high molecular weights, even in demanding eluents.

Columns: 3 x PLgel 10 μ m MIXED-B, 300 x 7.5 mm
(p/n PL1110-6100)

Materials and Reagents

Samples: 2 x Natural rubber
Eluent: Toluene

Conditions

Flow Rate: 1.0 mL/min
Temperature: 50 °C
Injection Volume: 200 μ L

Results and Discussion

Figure 1 is a chromatogram of a natural rubber sample showing responses from the different detectors. Figure 2 indicates that one of the samples has a considerably broader molecular weight distribution than the other, although the Mark-Houwink plots show that the two materials are structurally similar (Figure 3).

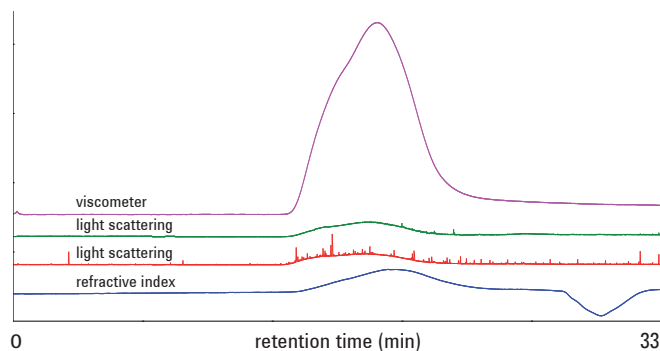


Figure 1. Raw triple detection data for one of the natural rubbers

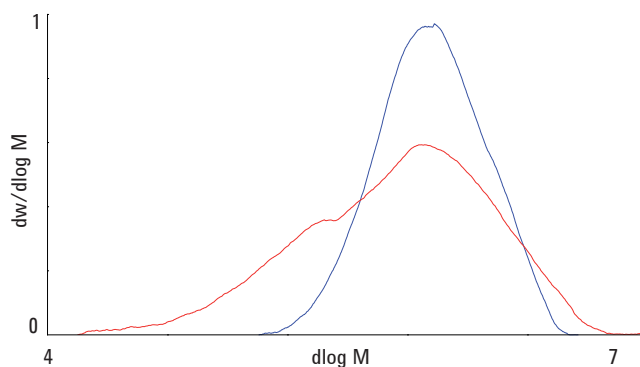


Figure 2. Overlaid triple detection molecular weight distributions of two natural rubbers

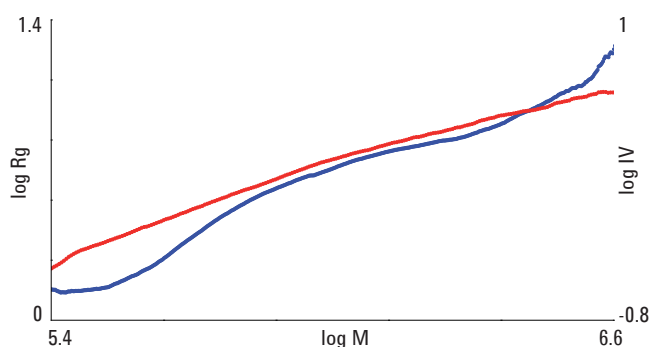


Figure 3. Overlaid Mark-Houwink (log intrinsic viscosity versus log M) for two natural rubbers

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel columns, a PL-BV 400RT viscometry detector and a PL-LS 15°/90° dual angle light scattering detector, the PL-GPC 50 Plus uses triple detection for the accurate determination of molecular weight information.

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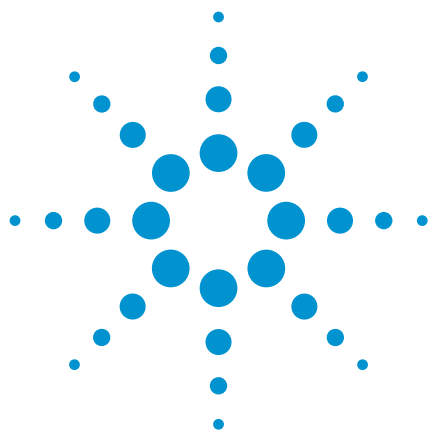
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SI-01513



Agilent Technologies



Poly(styrene-*co*-butadiene) GPC with Viscometry

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Styrene/butadiene rubber copolymers are elastomeric materials that show excellent abrasion and aging properties and are used in a wide variety of commercial and industrial applications. For these materials, the molecular weight of the polymer determines many of its final properties and therefore, the end use suitability. The accurate molecular weight distributions of two different samples of polybutadiene were investigated by gel permeation chromatography with viscometry. Light scattering could not be used with these samples as they are copolymers and therefore not of uniform chemistry across the molecular weight distribution. This could lead to errors in the light scattering calculations. However, the Universal Calibration technique employing a viscometer can be used with copolymers to give molecular weight that is independent of the standards used in the column calibration. To investigate the molecular structure of the materials, they were analyzed on an integrated GPC system.



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Instrumentation

The copolymers were analyzed by a Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer and Agilent PLgel 5 μ m MIXED-C columns, which provide high resolution of polymers that have mid-range molecular weights.

Columns: 2 x PLgel 5 μ m MIXED-C, 300 x 7.5 mm
(p/n PL1110-6500)

Materials and Reagents

Samples: 2 x Poly(styrene-*co*-butadiene)
Eluent: Tetrahydrofuran

Conditions

Flow Rate: 1 mL/min
Temperature: 40 °C

Results and Discussion

Figure 1 is an example chromatogram of a poly(styrene-*co*-butadiene). Slight differences in the molecular weight distributions of the two samples are clearly shown in Figure 2, but the Mark-Houwink plots in Figure 3 indicate that the materials are structurally very similar.

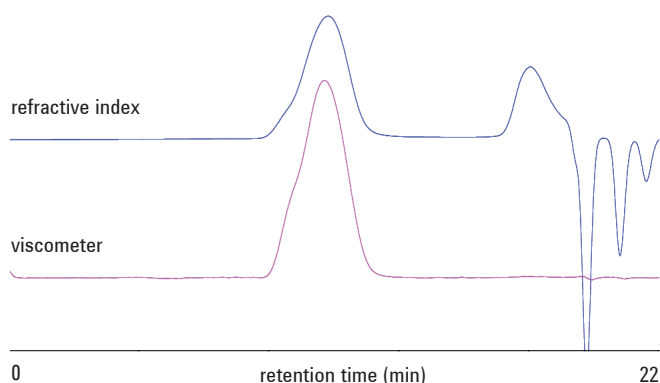


Figure 1. Chromatograms of a poly(styrene-*co*-butadiene)

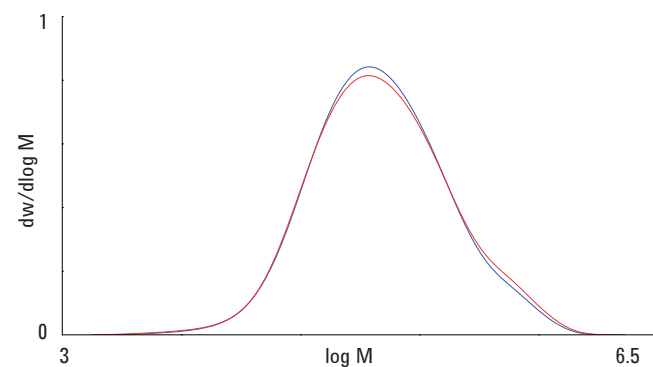


Figure 2. Overlaid molecular weight distributions for two poly(styrene-*co*-butadiene) samples

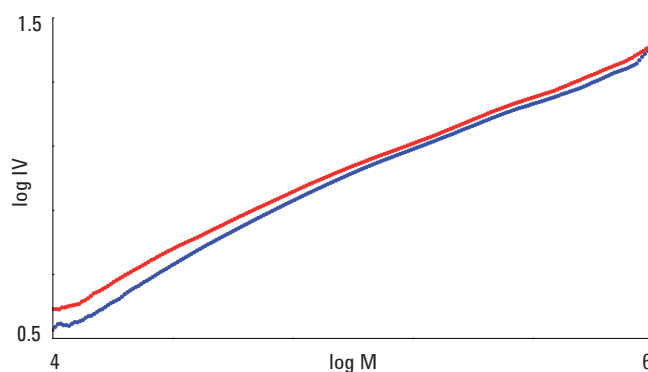


Figure 3. Overlaid Mark-Houwink plots for two poly(styrene-*co*-butadiene) samples

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel columns and a PL-BV 400RT viscometry detector, the PL-GPC 50 Plus provides accurate molecular weight determination for all polymer types based on the Universal Calibration principle, such as poly(styrene-*co*-butadiene) rubber.

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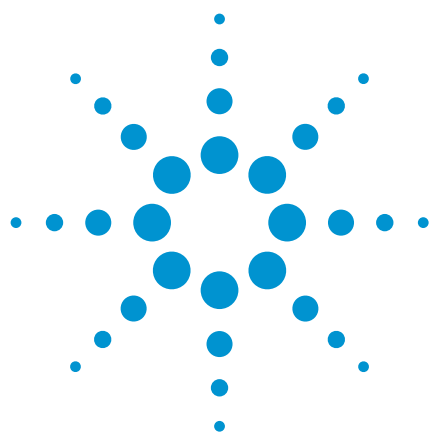
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SI-01515



Agilent Technologies



Analysis of Modified Polyacrylamide by Aqueous SEC with Triple Detection

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyacrylamides find many commercial uses, mainly in water treatment, pulp and paper production and mineral processing. These applications rely on the polymer's ability as a flocculant. Supplied in dry or liquid form, the most common liquid polyacrylamide is available as an emulsion with 10-40 % actives in a carrier fluid containing surfactants and latex. These emulsion polymers require activation to invert the emulsion and allow the electrolyte groups to be exposed.

A sample of a modified polyacrylamide was analyzed by triple detection in order to obtain an accurate molecular weight for the material, a critical parameter controlling flocculation properties. An integrated GPC system was used for the analysis.



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Instrumentation

The polyacrylamide was assessed by an Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer, Agilent PL-LS 15/90 dual angle light scattering detector and Agilent PL aquagel-OH MIXED-H 8 μm columns. These columns provide high resolution over a very wide range of molecular weights, simplifying column selection and producing a versatile analytical system.

Columns: 2 x PL aquagel-OH MIXED-H 8 μm , 300 x 7.5 mm
(part number PL1149-6800)

Materials and Reagents

Samples: Polyacrylamide
Eluent: 0.2 M NaNO_3 + 0.1 M NaH_2NO_3 , pH 7

Conditions

Flow Rate: 1 mL/min
Temperature: 40 $^\circ\text{C}$
Injection Volume: 100 μL

Results and Discussion

Using triple detection, the sample could be analyzed without the need to perform a column calibration (Figure 1). The triple detection molecular weight distribution for the sample appeared Gaussian (Figure 2) and the linearity of the Mark-Houwink plot indicated that the material had a uniform structure across the majority of the molecular weight range (Figure 3).

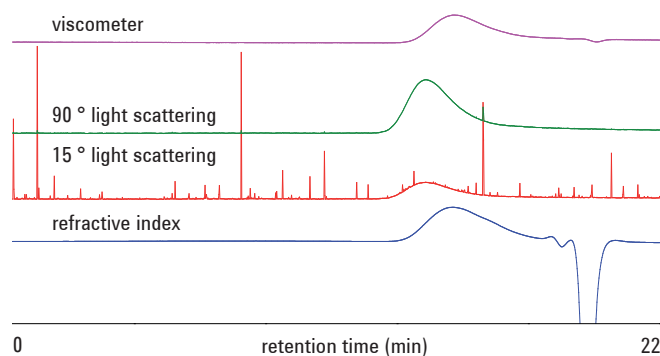


Figure 1. Raw triple detection data for a polyacrylamide

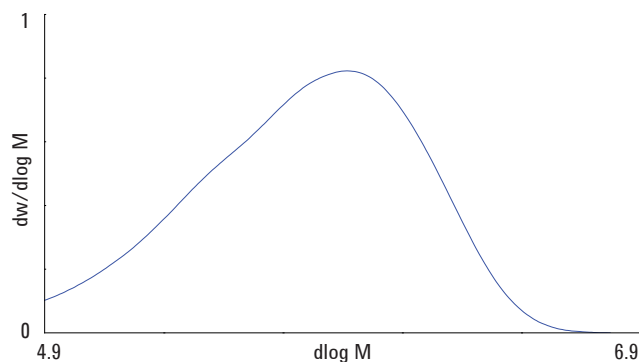


Figure 2. Triple detection molecular weight distribution for a polyacrylamide

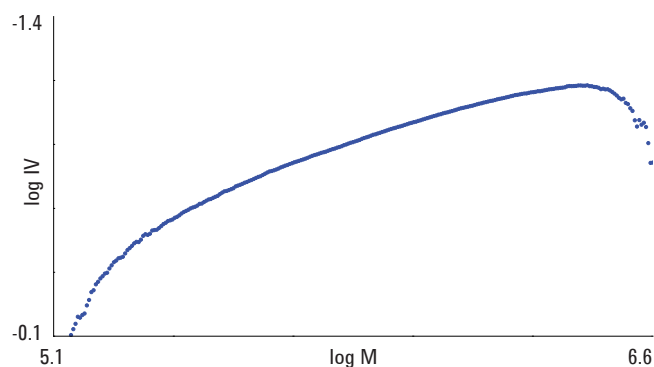


Figure 3. Mark-Houwink plot for the polyacrylamide

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 $^\circ\text{C}$. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PL aquagel MIXED-H 8 μm columns, a PL-BV 400RT viscometry detector and a PL-LS 15/90 dual angle light scattering detector, the PL-GPC 50 Plus uses triple detection for the accurate determination of polymer molecular weights.

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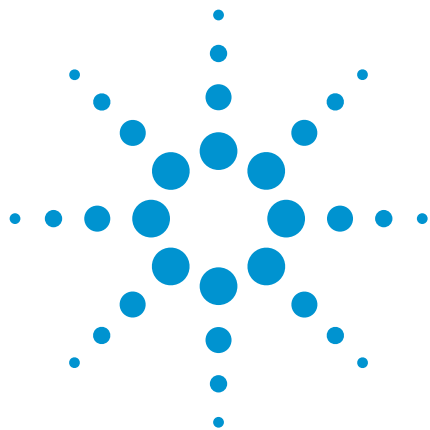
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SI-01517



Agilent Technologies



Analysis of Polybutadiene by GPC with Triple Detection

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polybutadiene is a synthetic rubber polymer manufactured from the polymerization of 1,3-butadiene. It is used extensively in commercial applications that require elastomeric properties, the most obvious being in the production of car tires. For these applications, the molecular weight of the material determines many of the final properties of the polymer and therefore the end use suitability.

The accurate molecular weight distributions of two different samples of polybutadiene were investigated by gel permeation chromatography with triple detection, yielding molecular weight independent of a column calibration. An integrated GPC system was used for the analysis.



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Instrumentation

The samples were assessed by an Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer, Agilent PL-LS 15/90 dual angle light scattering detector and Agilent PLgel 5 μm MIXED-C columns. These columns provide high resolution of polymers with mid range molecular weights.

Columns: 2 x PLgel 5 μm MIXED-C, 300 x 7.5 mm
(p/n PL1110-6500)

Materials and Reagents

Samples: 2 x Polybutadiene
Eluent: Tetrahydrofuran

Conditions

Flow Rate: 1 mL/min
Temperature: 40 °C

Results and Discussion

Both samples eluted as clear Gaussian peaks with a small high molecular weight component that was visible on all the detectors (Figure 1). The molecular weight distributions of the samples were similar but some differences were observed between the samples (Figure 2). Similarly, the Mark-Houwink plots indicated that there may be slight structural differences between the two samples (Figure 3).

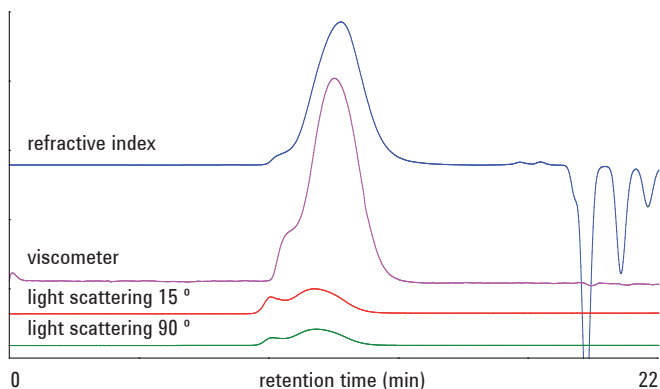


Figure 1. Chromatograms of a polybutadiene sample

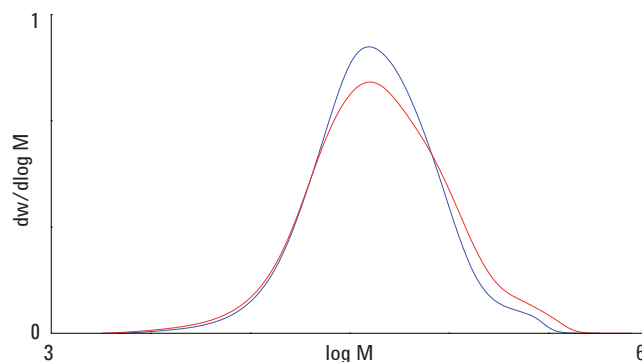


Figure 2. Overlaid molecular weight distributions for two polybutadiene samples

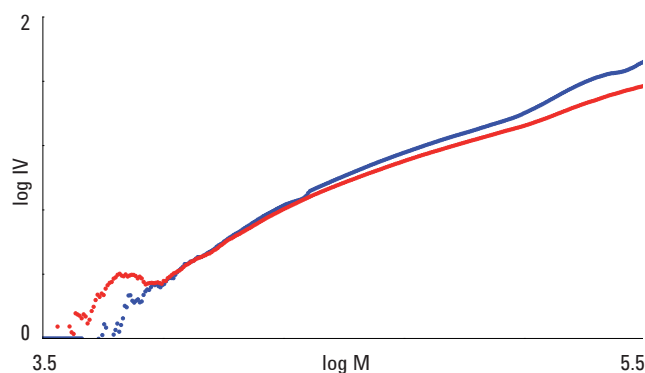


Figure 3. Overlaid Mark-Houwink plots for two polybutadiene samples

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel 5 μm MIXED-C columns, a PL-BV 400RT viscometry detector and a PL-LS 15/90 dual angle light scattering detector, the PL-GPC 50 Plus uses triple detection for the accurate determination of molecular weight information.

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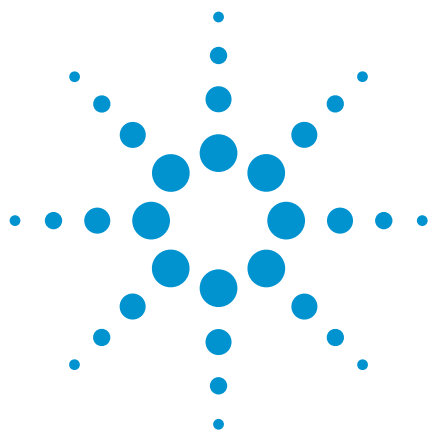
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SI-01518



Agilent Technologies



Analysis of Polyesters by GPC with Light Scattering Detection

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyesters are a class of polymer containing ester linkages in the backbone. A wide range of polyesters are available and they are used in many common consumer products, from plastic bottles to polished coatings for wide-grained wood. Typically synthesized from condensation esterification and transesterification reactions or from ring opening polymerizations, polyesters are an important class of synthetic materials. Some natural polyesters do exist, however, such as the cutin found in plant cuticles.

The accurate molecular weight distributions of two different samples of polyester were investigated by gel permeation chromatography with light scattering detection, using an integrated GPC system.



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Instrumentation

The polyesters were analyzed by an Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-LS 15/90 dual angle light scattering detector and Agilent PLgel 5 μ m MIXED-C columns, which provide high resolution of polymers with high molecular weights, even in demanding eluents.

Columns: 2 x PLgel 5 μ m MIXED-C, 300 x 7.5 mm
(p/n PL1110-6500)

Materials and Reagents

Samples: 2 x Polyester
Eluent: Tetrahydrofuran

Conditions

Flow Rate: 1 mL/min
Temperature: 50 °C

Results and Discussion

Figure 1 is a chromatogram of one of the polyester samples. It is apparent from Figure 2 that the two samples had very different molecular weight distributions, indicating that they would perform very differently in final application.

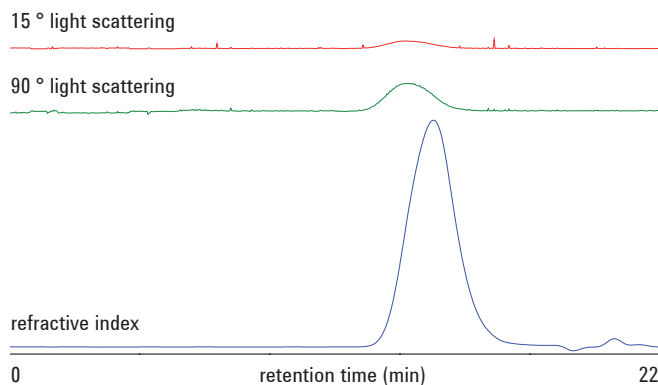


Figure 1. Chromatograms of a polyester sample

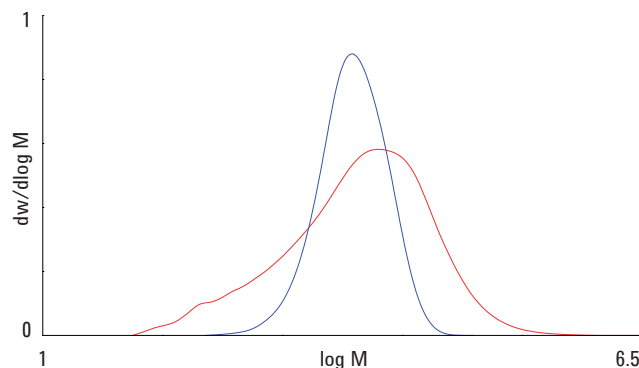


Figure 2. Overlaid molecular weight distributions for two polyester samples

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel MIXED-C columns and a PL-LS 15/90 dual angle light scattering detector, the PL-GPC 50 Plus is ideal for the accurate determination of polymer molecular weights.

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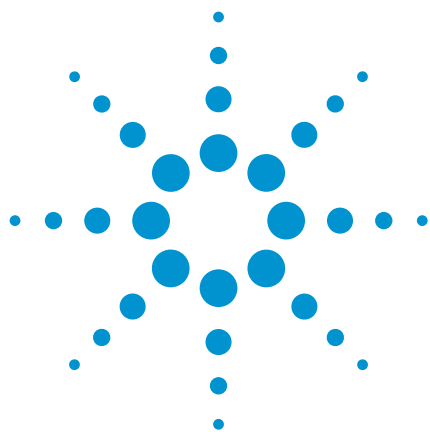
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SI-01519



Agilent Technologies



SEC Analysis of Polyacrylic Acid

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyacrylic acid is a resin formed by the polymerization of acrylic acid. It is water soluble and used as a suspending agent for sizing cloth, and in hydraulic fluids, adhesives and paints. A sample of the polymer was analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. A salt/buffer eluent was necessary as polyacrylic acids are polyelectrolytes.



Agilent Technologies

Conditions

Samples: Polyacrylic acids
Columns: 2 x PL aquagel-OH 50 8 μ m,
300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.25 M NaNO₃ + 0.01 M NaH₂PO₄ at
pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Figure 1 shows a chromatogram of a separation of two polyacrylic acids that have typical polydispersities of 1.3-1.7.

Conclusion

SEC using PL aquagel-OH columns successfully analyzed samples of polyacrylic acid. Aqueous SEC not only provides molecular weight data but also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

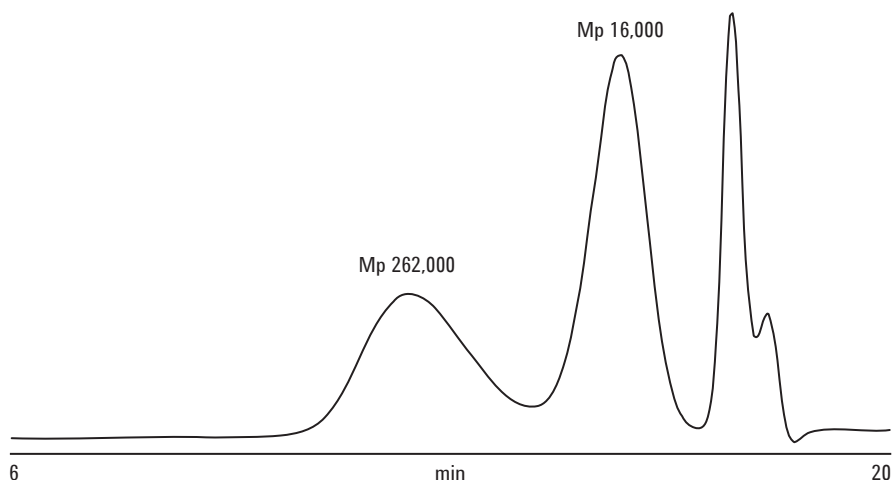


Figure 1. Separation of two polyacrylic acids

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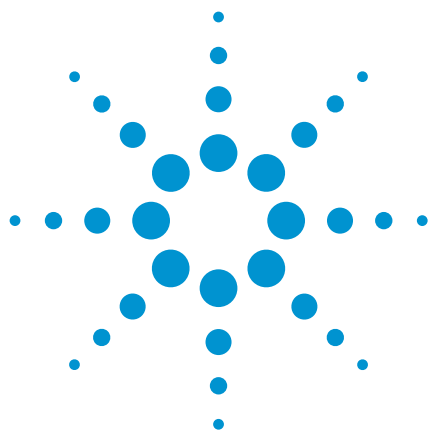
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SI-01573



Agilent Technologies



SEC Fingerprinting of Polyvinyl Alcohols

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Three samples of polyvinyl alcohol were analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. The calculated molecular weight averages were compared with manufacturers' quoted viscosity values. Calibration was done using pullulan polysaccharides.



Agilent Technologies

Conditions

Samples: Three polyvinyl alcohols
Columns: 2 x PL aquagel-OH 40 8 μ m,
300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at
pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Sample	Viscosity (mPa.s)	Mn	Mw
A	4	9771	29,470
B	10	23,339	80,174
C	20	31,210	102,309

Figure 1 is a chromatogram of one of the samples and Figure 2 shows overlaid molecular weight distributions of all three samples. Molecular weight distribution overlays provide a convenient method of fingerprinting for quality control and are more informative in production control and end-use performance evaluation than are single point viscosity measurements.

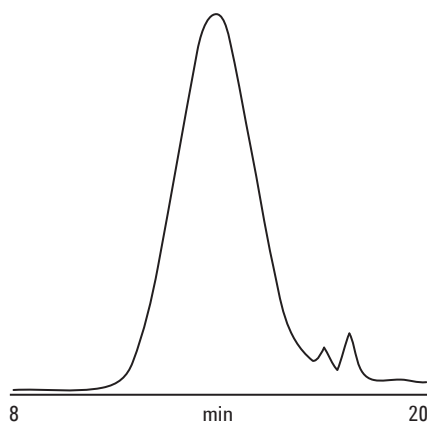


Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

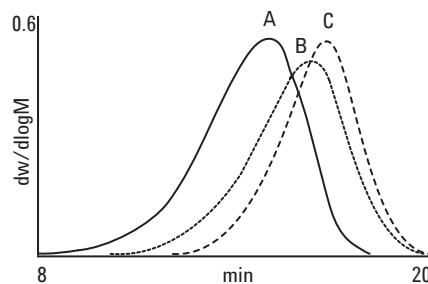


Figure 2. Comparison of molecular weights of three polyvinyl alcohols

Conclusion

Size exclusion chromatography using PL aquagel-OH columns highlighted the advantages of molecular weight values over single point viscosity measurements in the analysis of polyvinyl alcohols. Aqueous SEC also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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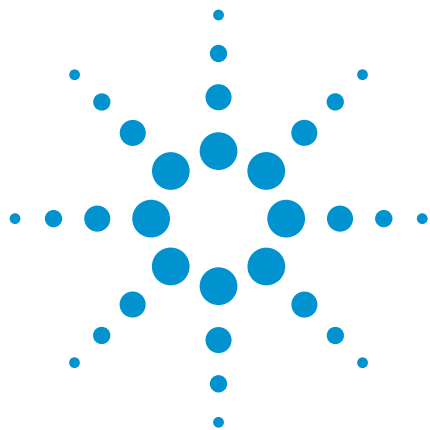
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SI-01576



Agilent Technologies



SEC Analysis of a Water Soluble Copolymer

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

This sample is a copolymer of N-vinyl pyrrolidone / p-amino styrene. It was assessed by aqueous SEC with Agilent PL aquagel-OH 50 8 μm columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution.



Agilent Technologies

Conditions

Sample: Water soluble copolymer
Columns: 2 x PL aquagel-OH 50 8 μ m,
300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at
pH 7
Flow Rate: 1.0 mL/min
Detection: RI

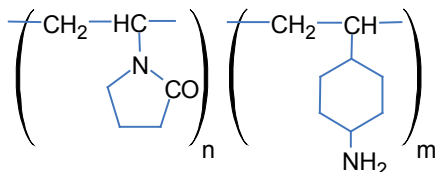


Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

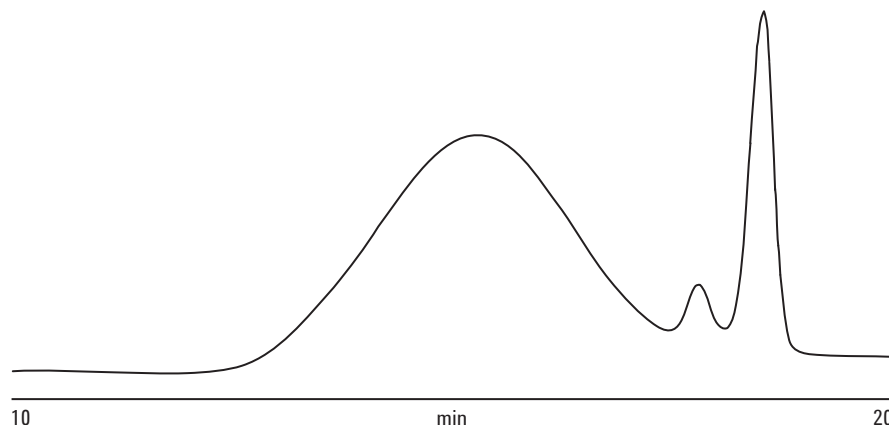


Figure 2. Raw data chromatogram of N-vinyl pyrrolidone / p-amino styrene copolymer

Conclusion

Size exclusion chromatography using PL aquagel-OH columns successfully analyzed a sample of water soluble copolymer. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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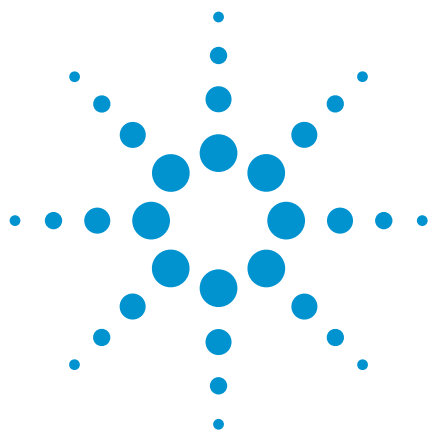
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SI-01577



Agilent Technologies



SEC Analysis of a Acrylamide Copolymer

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

This sample is a copolymer of acrylamide and dimethylaminoethyl acrylate quaternized with methyl chloride. It was necessary to maintain a low sample concentration (0.1 %) to minimize problems of shear with this high molecular weight polymer. The sample was assessed by aqueous SEC with Agilent PL aquagel-OH 40 and 60 15 μm columns. These columns were employed in order to avoid on-column shear degradation, and cover a molecular weight range from 10^4 to 10^7 .



Agilent Technologies

Conditions

Samples: Sodium polyacrylate
Columns: 2 x PL aquagel-OH 60 15 μm , 300 x 7.5 mm (p/n PL1149-6260)
+ 1 x PL aquagel-OH 40 15 μm , 300 x 7.5 mm (p/n PL1149-6240)
Eluent: 0.2 M NaNO_3 + 0.01 M NaH_2PO_4 at
pH 7
Flow Rate: 1.0 mL/min
Detection: RI

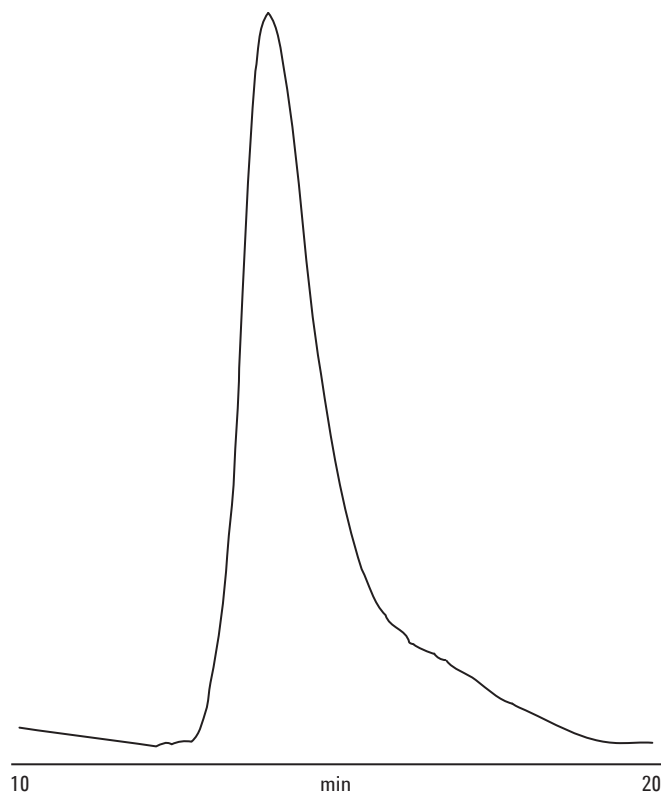


Figure 1. Raw data chromatogram of an acrylamide co-polymer

Conclusion

Size exclusion chromatography using PL aquagel-OH 40 and 60 15 μm columns successfully analyzed a sample of acrylamide copolymer. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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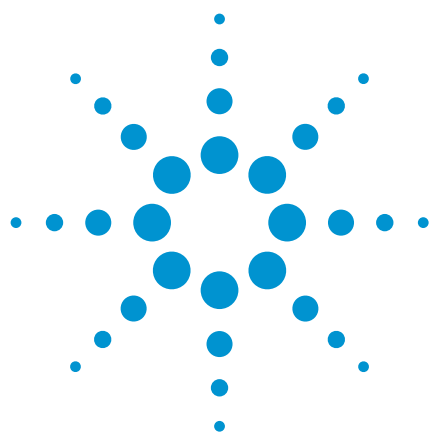
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SI-01578



Agilent Technologies



SEC Analysis of Polyvinyl Pyrrolidone

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

PVP (polyvinyl pyrrolidone, povidone, polyvidone) is a water-soluble polymer made from N-vinyl pyrrolidone monomer. PVP is soluble in aqueous solvents and polar organics. It has excellent wetting properties and readily forms films, and so it performs well as a coating or coating additive. PVP binds to polar molecules exceptionally easily, owing to its polarity. This has led to applications in coatings for tablets and photo-quality ink-jet paper and transparencies, as well ink-jet inks. In addition, PVP has uses in personal care products, contact lens solutions, hair sprays and gels. Industrial applications include paints, wettable adhesives and food additives. A sample of PVP was analyzed using Agilent PL aquagel-OH 50 and 60 8 μm columns. These columns are ideal for characterizing PVP because they combine high pore volume and high column efficiency ($>35,000$ plates/meter) for maximum resolution. In this instance, they were employed in a column set, covering a molecular weight range from about 5×10^5 to 10^7 .



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Results and Discussion

The chromatogram reveals that the PVP sample has a broad distribution.

Conditions

Sample: Polyvinyl pyrrolidone
Columns: 1 x PL aquagel-OH 60 8 μm , 300 x 7.5 mm (p/n PL1149-6860)
+ 2 x PL aquagel-OH 50 8 μm , 300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.2 M NaNO_3 + 0.01 M NaH_2PO_4 at pH 3
Flow Rate: 1.0 mL/min
Detection: RI

Conclusion

SEC using PL aquagel-OH columns successfully analyzed a sample of polyvinyl pyrrolidone. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

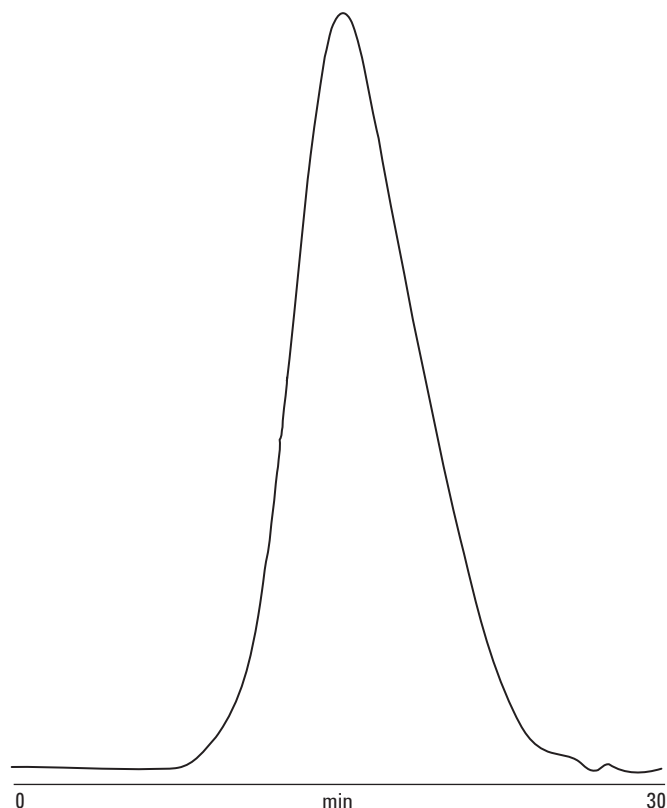


Figure 1. Raw data chromatogram of polyvinyl pyrrolidone

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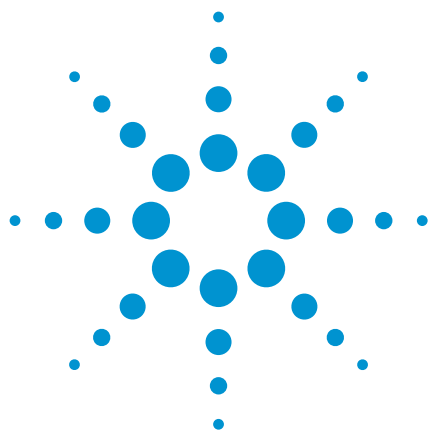
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SI-01580



Agilent Technologies



Characterizing Polyvinyl Alcohol by SEC

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Fully or partially hydrolyzed grades of polyvinyl alcohol (PVA) are normally specified according to their viscosity in solution. Aqueous SEC can be used to characterize these polymers in terms of molecular weight distribution. Three samples with the same degree of hydrolysis were compared by overlaying their molecular weight distributions. This is a convenient method of fingerprinting materials for quality control, and is more informative in production control and end-use performance evaluation than single point viscosity measurements. Agilent PL aquagel-OH columns are ideal for characterizing PVA because they combine low exclusion limit, high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. Column calibration was done using pullulan standards.



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Conditions

Samples: Three polyvinyl alcohols
Columns: 2 x PL aquagel-OH 40 8 μ m,
300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.25 M NaNO₃ + 0.01 M NaH₂PO₄ at
pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Figure 1 shows the raw data chromatogram of Sample A, with the calculated molecular weights of all three samples in Figure 2. A correlation of the SEC results with the polymer specification is shown in Table 1.

Table 1. Correlation of the SEC results with the polymer specification

Sample	Viscosity	Mn	Mw
A	4	9,771	29,470
B	10	23,339	80,174
C	20	31,210	102,309

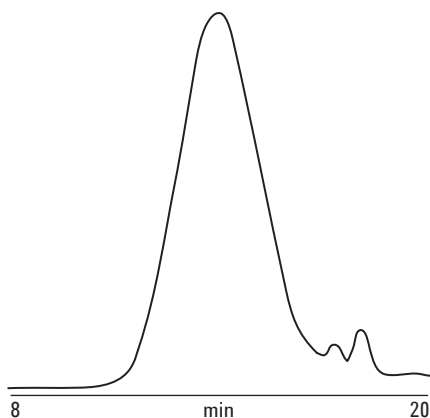


Figure 1. Chromatogram of a polyvinyl alcohol

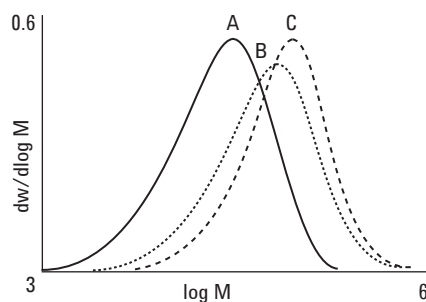


Figure 2. Overlaid molecular weights of three polyvinyl alcohols

Conclusion

SEC and PL aquagel-OH columns successfully fingerprinted three polyvinyl alcohols. The 'neutral' surface and ability to operate across a wide range of eluent conditions equip PL aquagel-OH for the high performance analysis of analytes with neutral, ionic and hydrophobic moieties, singly or combined.

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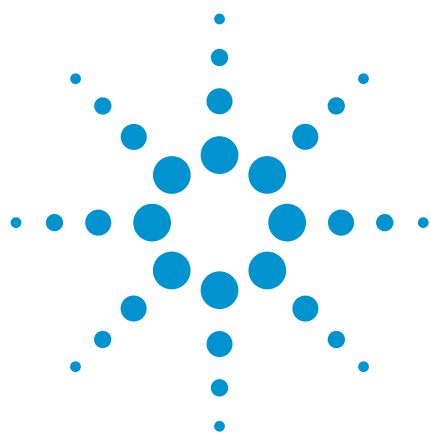
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SI-01626



Agilent Technologies



SEC Analysis of Star Branched Polyethylene Glycol without Column Interaction

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Recent advances in polymer chemistry have led to the development of novel materials with both controlled functionality and structure resulting in unusual rheological properties. Examples include the many di- and triblock copolymer architectures and dendritic systems. These materials often include regions of different polarity and hydrophobicity and therefore represent a challenge to the analytical chemist. This application note illustrates the analysis of a star-branched polyethylene glycol with a significant hydrophobic component by aqueous SEC.

The basic structure of the star-branched polyethylene glycol is shown in Figure 1, where R represents an aromatic hydrophobic component. Unmodified polyethylene glycol can be analyzed in aqueous solution at pH 7. However, the star-branched material contained a significant hydrophobic component that could interact with the column packing material resulting in non-SEC effects.

To investigate the effect of the hydrophobic component on the elution profile, the sample was analyzed in buffer at pH 7 both before and after the inclusion of 30% methanol (by volume). The presence of a miscible organic solvent such as methanol significantly lowers the polarity of the eluent and serves to minimize hydrophobic interactions between the sample and column packing material. Agilent PL aquagel-OH columns were chosen because they can be used with up to 50 % methanol in the eluent. These high performance columns operate across a wide range of eluent conditions for high performance analysis of analytes with neutral, ionic and hydrophobic moieties, singly or combined.



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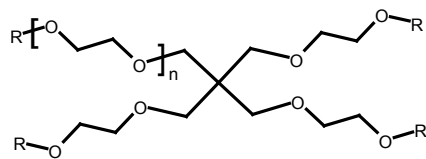


Figure 1. Structure of the star-branched polyethylene glycol

Conditions

Samples:	Star branched PEG
Columns:	2 x PL aquagel-OH 30 8 μ m, 300 x 7.5 mm (part number PL1120-6830)
Eluent:	Water + 0.2 M NaNO_3 + 0.01 M NaH_2PO_4 + 30 % methanol
Flow Rate:	1.0 mL/min
Injection Volume:	100 μ L
Detection:	RI

Results and Discussion

The resulting chromatograms are shown in Figures 2 and 3.

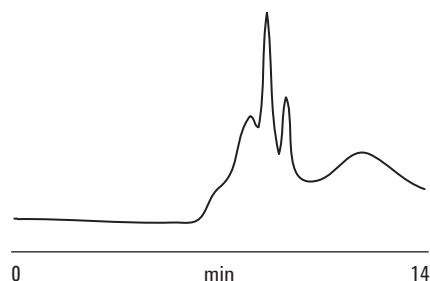


Figure 2. Chromatogram of the star-branched polyethylene glycol obtained in buffer at pH 7

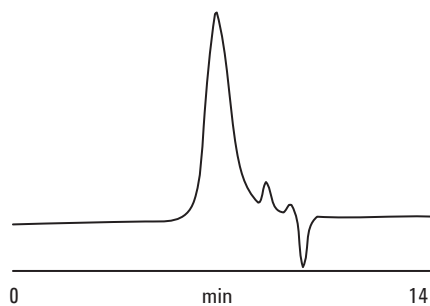


Figure 3. Chromatogram of the star-branched polyethylene glycol obtained in buffer at pH 7 with 30 % methanol

Clearly, the chromatogram obtained without methanol shows that the sample is interacting strongly with the column, with some material eluting after the total permeation limit. However, the presence of 30% methanol has inhibited the interaction and a normal Gaussian peak shape has been obtained, which can be integrated for SEC calculations.

Conclusion

The presence of significant hydrophobicity in a star branched polymer is no barrier to its resolution by SEC with PL aquagel-OH columns. In addition, the column's ability to handle eluents containing up to 50% methanol means that star-branched polymers can be resolved without interactions.

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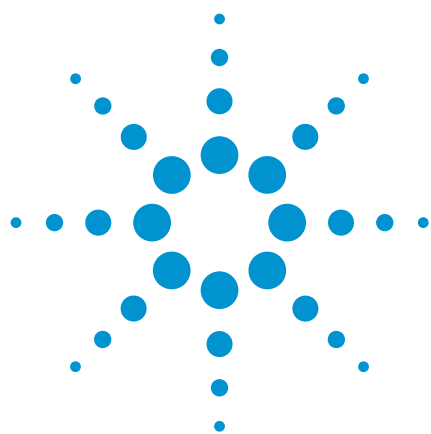
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SI-01649



Agilent Technologies



Accurate Determination of the MWD of Polyacrylic Acid

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyacrylic acid is a biodegradable water soluble polymer with numerous industrial applications, including as a super adsorbent (eg in disposable nappies), in water treatment as a metal ion scavenger and in the treatment of metal surfaces prior to coating. The molecular weight distribution (MWD) of this material is an important parameter, as it strongly affects its end use properties. Aqueous SEC is an ideal analytical tool for the measurement of the MWD of polyacrylic acid. Since polyacrylic acid is a polyelectrolyte, care must be taken in selecting the appropriate SEC conditions. In the method described below, a buffered mobile phase with a high electrolyte content was used to minimize non-size exclusion effects. Agilent PL aquagel-OH MIXED 8 μm columns were selected to provide good resolution over a wide molecular weight range. Column calibration was achieved using Agilent EasiVial PEG/PEO standards see Figure 1. EasiVials provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). Each vial contains a mixture of four individual, highly characterized, narrow dispersity standards. The amount of each individual standard is carefully controlled during manufacture, allowing their use in SEC-viscometry, which requires accurate concentrations. Refractive index chromatograms obtained from each EasiVial PEO are shown in Figure 2.



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Conditions

Samples: Polyacrylic acid, approx
0.2 % w/v; EasiVial PEG/
PEO standards at
0.1-0.5 mg/mL

Columns: 2 x PL aquagel-OH
MIXED-H 8 µm,
300 x 7.5 mm

r PL1149-6800)

Eluent: 0.2 M NaNO₃ + 0.01 M
NaH₂PO₄ adjusted to pH 7

Flow Rate: 1.0 mL/min

Injection Volume: 200 µL

Detection: RI

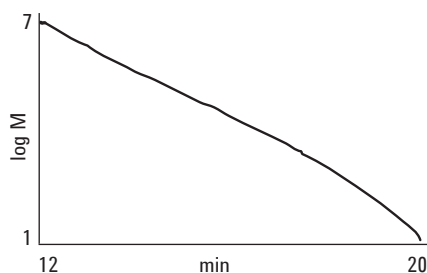


Figure 1. Calibration of PL aquagel-OH MIXED 8 µm column using EasiVial standards

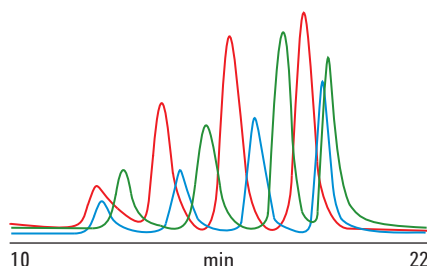


Figure 2. RI chromatograms from EasiVial PEOs

Results and Discussion

Three polyacrylic acid samples (A, B and C) were chromatographed and their corresponding molecular weight distribution compared (Figure 3).

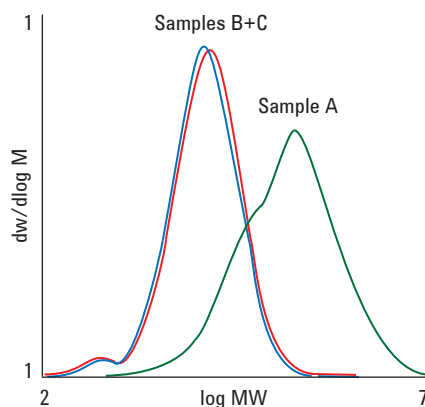


Figure 3. Comparison of three polyacrylic acids

Sample	Mn (g/mol)	Mw (g/mol)	PD
A	33,450	89,430	2.67
B	7990	14,930	1.87
C	7880	13,490	1.71

Sample A had a significantly higher molecular weight distribution compared to Samples B and C, which were similar. Consequently, Sample A was expected to possess significantly different rheological properties compared to the other two samples. Closer examination showed that Sample A was significantly more viscous than Samples B and C, which were similar. In addition, the MWD of Sample A was bi-modal, which suggests that it may be a blend of more than one component.

Conclusion

Size exclusion chromatography using PL aquagel-OH MIXED 8 µm columns calibrated with EasiVial standards accurately identified molecular weight distributions of polyacrylic acids. These differences were corroborated through visual examination of the samples' bulk viscosity.

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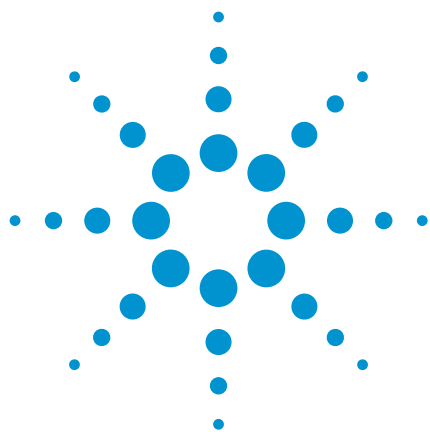
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SI-01652



Agilent Technologies



Analysis of Polybutadiene

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Agilent PLgel 20 μm MIXED-A columns are designed for the analysis of ultra high MW polymers. These columns minimize the potential effects of shear degradation and are ideal for regular high temperature operation. The capability of PLgel MIXED-A columns is demonstrated in the analysis of polybutadiene by gel permeation chromatography (GPC). This high molecular weight compound was prepared as a 0.1% solution. The calculated MW was 2,766,700 relative to polystyrene standards.



Agilent Technologies

Specific features of the PLgel 20 μm MIXED-A include a unique linear calibration to 40 million MW, rigid, high pore size PLgel matrix, particle size of 20 μm , large frit porosity of 10 μm , and long lifetimes under low pressure.

Conditions

Columns: 4 x PLgel 20 μm MIXED-A,
300 x 7.5 mm (p/n PL1110-6200)

Eluent: THF

Flow Rate: 1.0 mL/min

Detection: RI

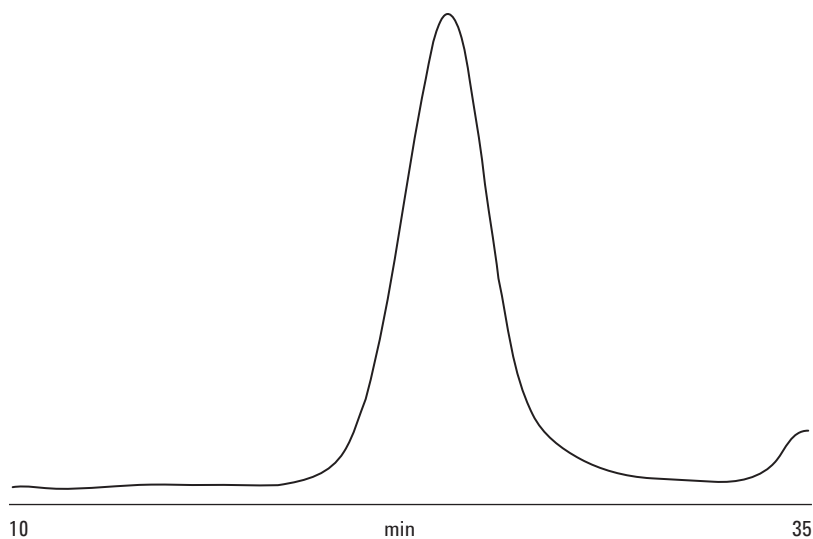


Figure 1. GPC analysis of polybutadiene using PLgel MIXED-A columns

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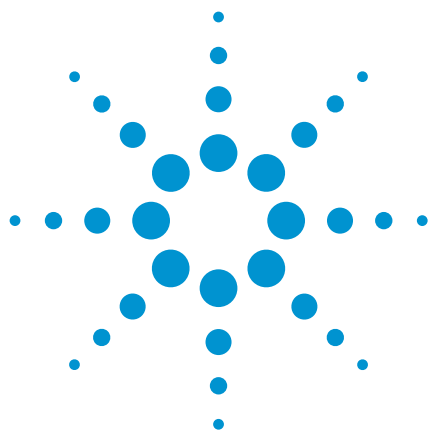
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SI-01758



Agilent Technologies



Routine Analysis of Polystyrene

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

With a minimum of 35,000 plates per meter, a three column Agilent PLgel MIXED-B set is ideal for routine analysis of polystyrene. This polystyrene has a MW 250,000 and a polydispersity of 2.5. Some low MW components are detected with UV at 254 nm.



Agilent Technologies

PLgel 10 μm MIXED-B columns are designed for high MW polymer analysis and demanding eluent conditions. The PLgel 10 μm MIXED-B spans a wide range of molecular weights, up to 10 million, with a linear calibration curve. It is particularly useful for molecular weight distributions where slightly higher than average MWs are encountered. The 10 μm particle size provides good resolution with relatively low pressures for enhanced lifetimes in demanding conditions.

Conditions

Columns: 3 x PLgel 10 μm MIXED-B,
300 x 7.5 mm (p/n PL1110-6100)

Eluent: THF

Flow Rate: 1.0 mL/min

Detection: UV, 254 nm

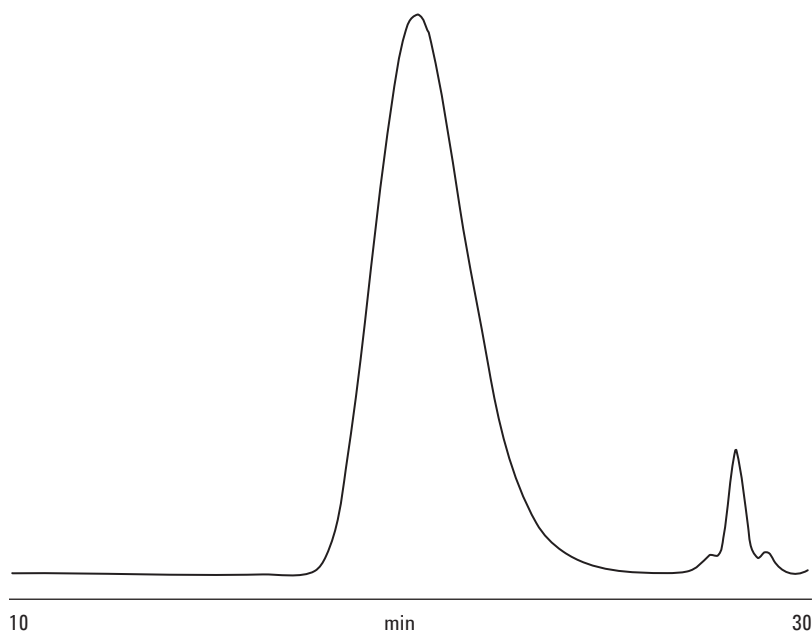


Figure 1. Analysis of polystyrene using PLgel 10 μm MIXED-B columns

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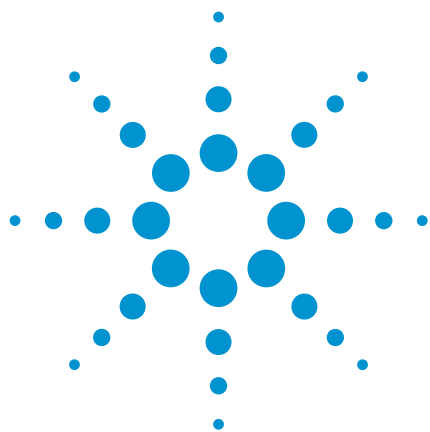
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SI-01775



Agilent Technologies



Analysis of Nitrocellulose

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

The operating range of Agilent PLgel 10 μ m MIXED-B columns makes them ideal for the separation of polymers that contain slightly higher than average molecular weight, such as nitrocellulose. The filtering of such samples prior to injection is recommended and sample concentrations should be low to avoid viscous shearing effects.



Agilent Technologies

PLgel 10 μ m MIXED-B columns are designed for high MW polymer analysis and demanding eluent conditions. The PLgel 10 μ m MIXED-B spans a wide range of molecular weights, up to 10 million, with a linear calibration curve. It is particularly useful for molecular weight distributions where slightly higher than average MWs are encountered. The 10 μ m particle size provides good resolution with relatively low pressures for enhanced lifetimes in demanding conditions.

Conditions

Columns: 2 x PLgel 10 μ m MIXED-B,
300 x 7.5 mm (p/n PL1110-6100)

Eluent: THF

Flow Rate: 1.0 mL/min

Loading: 0.1%, 200 μ L

Detection: RI

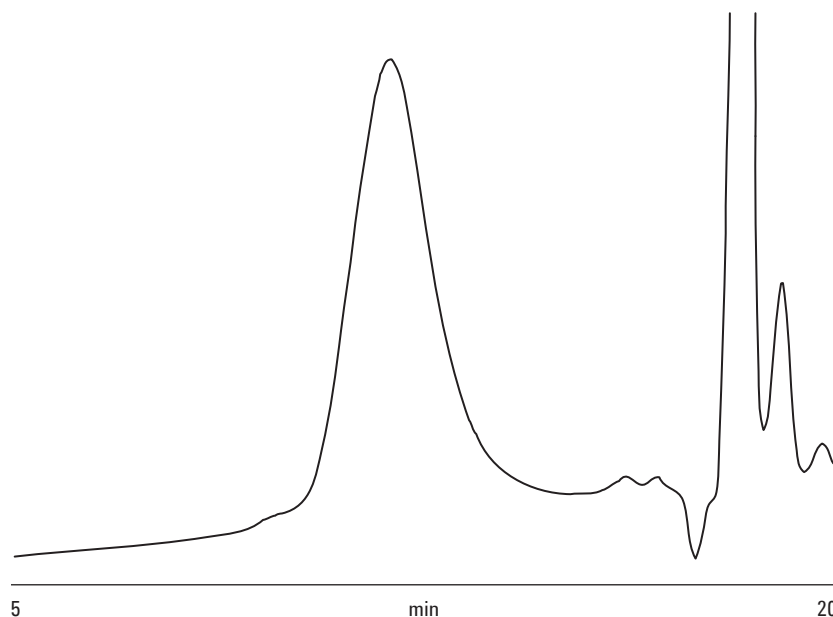


Figure 1. Analysis of nitrocellulose using PLgel 10 μ m MIXED-B columns

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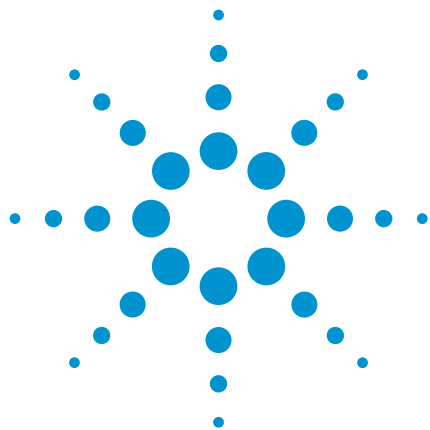
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SI-01776



Agilent Technologies



Analysis of Reacetylated Polyvinyl Alcohol

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyvinyl alcohols (PVOH) are water soluble polymers, which can be analyzed by aqueous size exclusion chromatography (SEC). However, if reacetylated, the resultant polyvinyl acetate (PVAc) is rendered THF soluble, and so Agilent PLgel MIXED-B columns are the columns of choice.



Agilent Technologies

PLgel 10 μ m MIXED-B columns are designed for high MW polymer analysis and demanding eluent conditions. The PLgel 10 μ m MIXED-B spans a wide range of molecular weights, up to 10 million, with a linear calibration curve. It is particularly useful for molecular weight distributions where slightly higher than average MWs are encountered. The 10 μ m particle size provides good resolution with relatively low pressures for enhanced lifetimes in demanding conditions.

Conditions

Columns: 2 x PLgel 10 μ m MIXED-B,
300 x 7.5 mm (p/n PL1110-6100)

Eluent: THF

Flow Rate: 1.0 mL/min

Loading: 0.25%, 100 μ L

Detection: RI

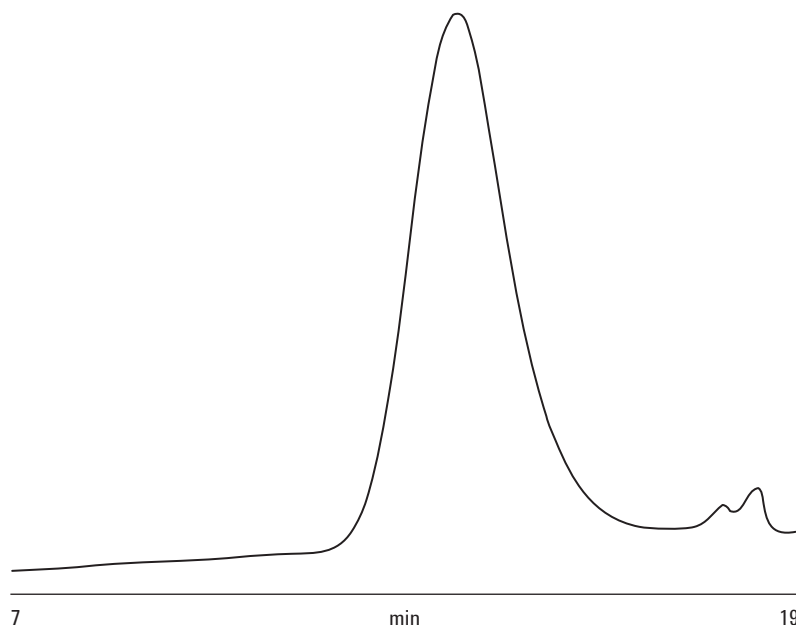


Figure 1. Analysis of reacetylated poly(vinyl) alcohol using PLgel MIXED-B columns

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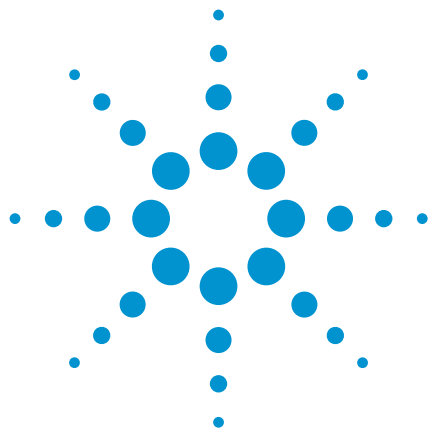
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SI-01779



Agilent Technologies



High Temperature Analysis of Polyethylene Terephthalates

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Agilent PLgel 10 μm MIXED-B columns are designed for high MW polymer analysis under demanding eluent conditions such as high temperature gel permeation chromatography (GPC).



Agilent Technologies

Samples of polyethylene terephthalate (PET) were dissolved by heating to 110 °C for 30 min. The polymer remains in solution at room temperature but the high viscosity of the eluent means that high temperature GPC is necessary. Three grades of PET, with different intrinsic viscosities, were analyzed and compared on PLgel MIXED-B columns (Figures 1 and 2).

The PLgel 10 µm MIXED-B spans a wide range of molecular weights, up to 10 million, with a linear calibration curve. It is particularly useful for molecular weight distributions where slightly higher than average MWs are encountered. The 10 µm particle size provides good resolution with relatively low pressures for enhanced lifetimes in demanding conditions.

Conditions

Columns:	2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (p/n PL1110-6100)
Eluent:	Chlorophenol
Flow Rate:	1.0 mL/min
Temperature:	100 °C
Detection:	Agilent PL-GPC 220

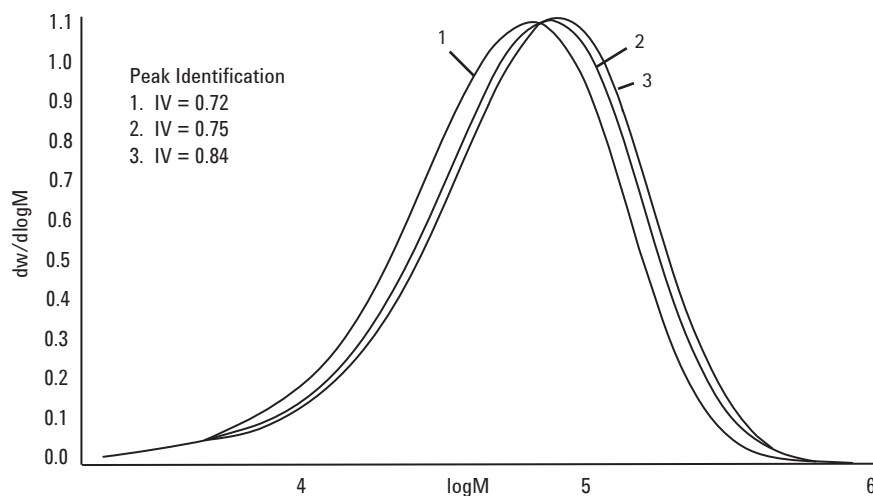


Figure 1. Molecular weight overlays of three polyethylene terephthalates

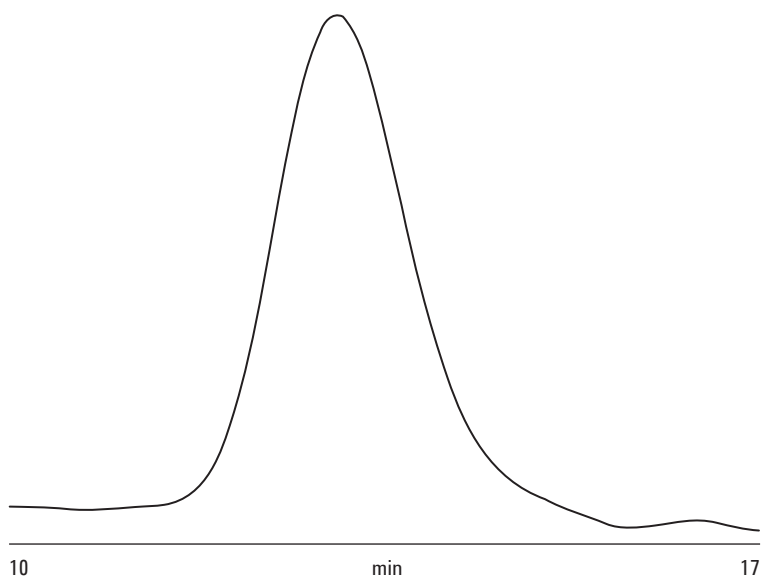


Figure 2. Chromatogram of a polyethylene terephthalate

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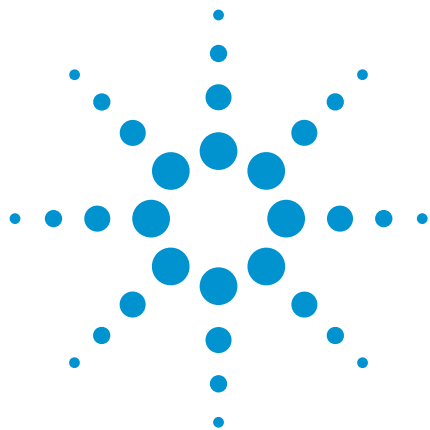
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SI-01780



Agilent Technologies



Analysis of Polystyrene

Application Note

Materials Testing and Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

A set of three Agilent PLgel MiniMIX-B columns is ideal for routine analyses of polystyrene in tetrahydrofuran.



Agilent Technologies

This sample has $M_w=250,000$ and $M_n=100,000$, and some low molecular weight components are detected with a UV at 254 nm.

PLgel 10 μm MiniMIX-B columns are designed for high MW polymer analysis and demanding eluent conditions. The PLgel 10 μm MiniMIX-B spans a wide range of molecular weights, up to 10 million, with a linear calibration curve. It is particularly useful for molecular weight distributions where slightly higher than average MWs are encountered. The 10 μm particle size provides good resolution with relatively low pressures for enhanced lifetimes in demanding conditions.

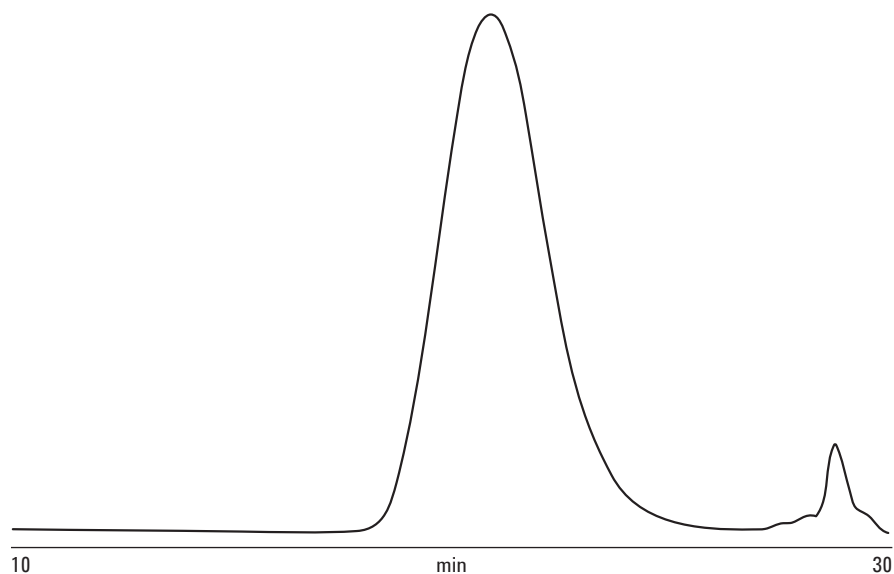


Figure 1. Analysis of polystyrene using PLgel 10 μm MiniMIX-B columns

Conditions

Column:	3 x PLgel 10 μm Mini-MIX-B, 300 x 7.5 mm (part number PL1510-5100)
Eluent:	THF
Flow Rate:	0.3 mL/min
Detection:	UV, 254 nm

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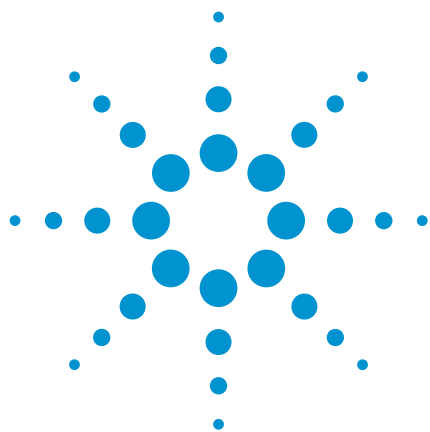
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SI-1793



Agilent Technologies



High-Temperature Analysis of Polyether Sulfone

Application Note

Materials Testing and Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Elevated temperature is preferred for the analysis of polyether sulfones to reduce operating pressure and improve resolution by reducing solvent viscosity. This particular sample was readily soluble in DMF at room temperature. Agilent PLgel 5 μm MIXED-C columns are well suited to the analysis of polyether sulfones.



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PLgel 5 μ m MIXED-c columns are designed for rapid polymer analysis. With its linear calibration up to 2 million MW, this is the column of choice for highest resolution and accuracy in molecular weight distribution analyses. Rapid solvent change capability, excellent temperature stability and the high resolution of the PLgel 5 μ m MIXED-C also provide the versatility essential for today's R&D laboratory.

Conditions

Columns:	2 x PLgel 5 μ m MIXED-C, 300 x 7.5 mm (part number PL11110-6500)
Eluent:	DMF + 0.1% LiBr
Flow Rate:	1.0 mL/min
Temperature:	60 °C
Detection:	390-MDS Multi Detector Suite (differential refractive index)

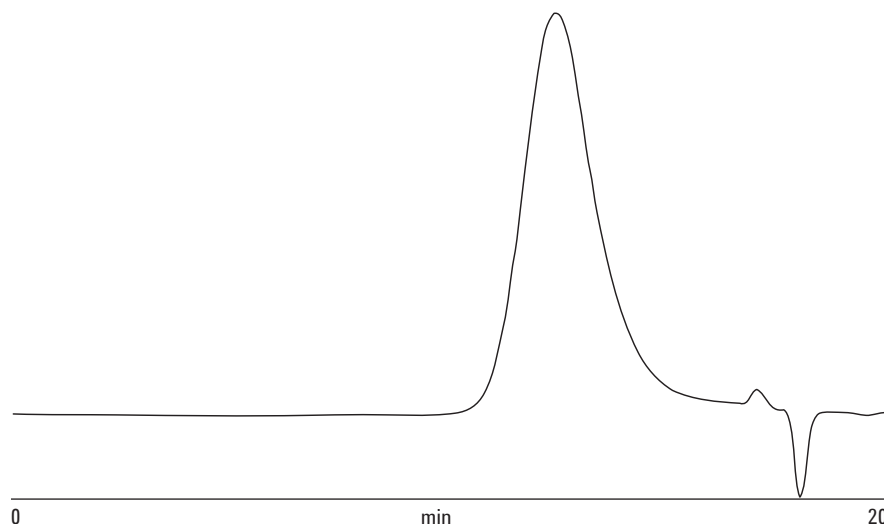


Figure 1. Analysis of polyether sulfone using PLgel 5 μ m MIXED-C columns

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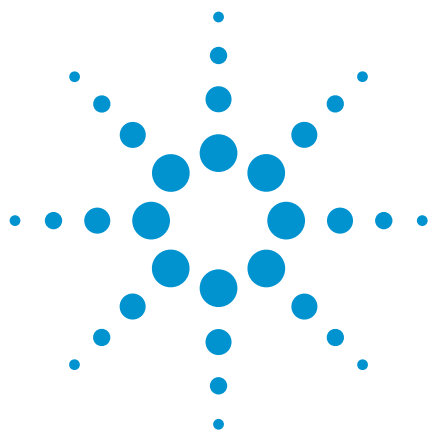
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SI-1822



Agilent Technologies



Reduced Viscosity Analysis of Polyurethane

Application Note

Materials Testing and Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyurethanes are soluble in several medium to high polarity solvents. In DMF, the temperature is increased to reduce eluent viscosity so reducing operating pressure. Agilent PLgel 5 μm MIXED-C columns are well suited to the analysis of polyurethanes.



Agilent Technologies

PLgel 5 μ m MIXED-c columns are designed for rapid polymer analysis. With its linear calibration up to 2 million MW, this is the column of choice for highest resolution and accuracy in molecular weight distribution analyses. Rapid solvent change capability, excellent temperature stability and the high resolution of the PLgel 5 μ m MIXED-C also provide the versatility essential for today's R&D laboratory.

Conditions

Columns:	2 x PLgel 5 μ m MIXED-C, 300 x 7.5 mm (part number PL1110-6500)
Eluent:	DMF + 0.1% LiBr
Flow Rate:	1.0 mL/min
Temperature:	60 °C
Detection:	390-MDS Multi Detector Suite (differential refractive index)

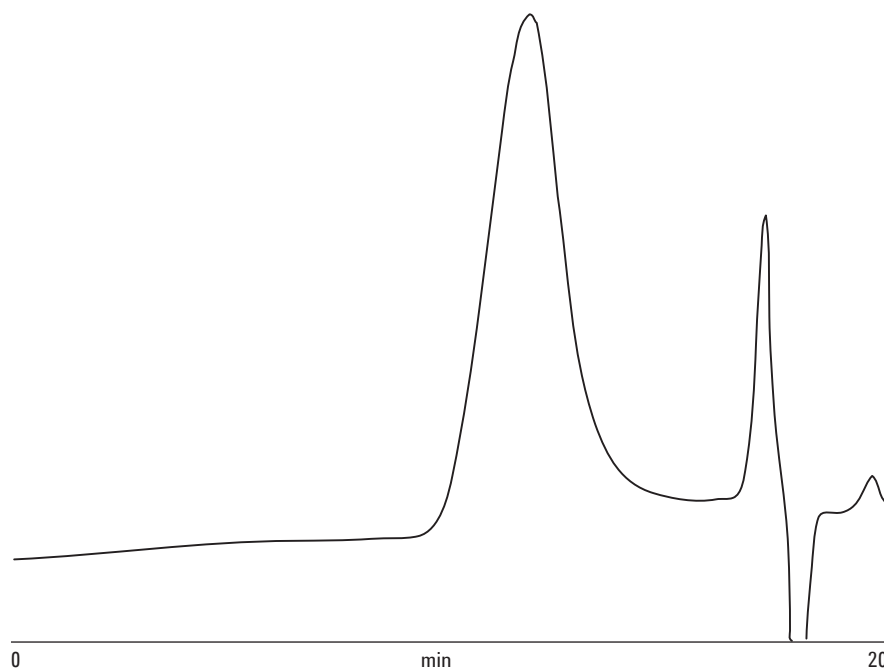


Figure 1. Analysis of polyurethane using PLgel 5 μ m MIXED-C columns

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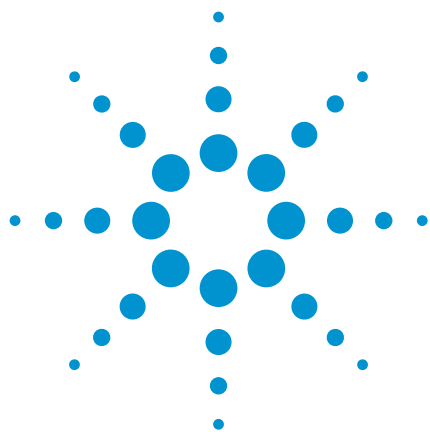
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SI-1823



Agilent Technologies



GPC Analysis is Ideal for Characterizing PVC

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Poly(vinyl) chloride is a thermoplastic widely encountered in everyday life. It is light, non-flammable, robust and durable. PVC is permeable, does not deteriorate, is easy to maintain and its physical and mechanical characteristics make it ideal for many different uses. PVC application areas include the toiletry, food, water and car industries.

Unplasticized PVC has a high melt viscosity leading to some difficulties in processing. The finished product is also too brittle for many applications. In order to overcome these problems, it is routine to incorporate additives to the PVC. In addition to acting as impact modifiers, a number of polymeric additives may be considered as processing aids. Such materials are primarily included to ensure more uniform flow and hence improve surface finish. The properties of the final material are dependent on the molecular weight distribution of the PVC and the type and level of the added plasticizers. The analysis of the compounded material is, therefore, of primary importance, and GPC is the ideal analytical tool for its characterization. With their linear resolving capability over a wide molecular weight range, Agilent PLgel MIXED columns provide resolution of both polymer and additives, particularly with the high efficiency 5 μ m particle size columns.

Three different grades of PVC tubing containing different plasticizers were analyzed by GPC using a column set comprising three PLgel 5 μ m MIXED-C columns.



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Conditions

Columns: 3 x PLgel 5 μ m MIXED-C,
300 x 7.5 mm (p/n PL1110-6500)
Eluent: THF (stabilized)
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Sample 1 contained an aliphatic plasticizer, while Sample 2 contained an aromatic plasticizer. Sample 3 contained both. The common peak eluting at approximately 29 minutes was due to toluene, which was included in the samples for flow rate correction.

All three samples displayed a broad peak at the same retention time, which was due to the PVC (approx 17 min), but also displayed other peaks with varying retention times which were attributed to the different plasticizers (Figure 1). Polystyrene standards and the resultant calibration is illustrated in Figure 2.

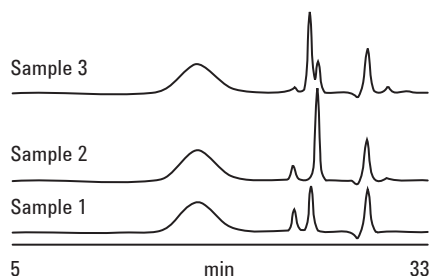


Figure 1. Overlaid raw data chromatograms for three PVC samples

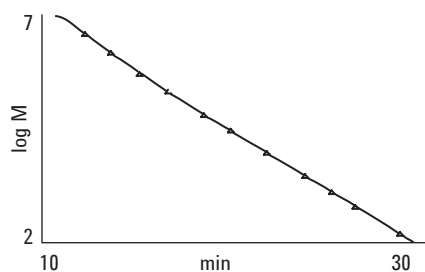


Figure 2. Calibration curve derived from polystyrene standards

Conclusion

GPC using PLgel MIXED-C columns permits the determination not only of the molecular weight distribution of PVC samples, but also the identification and quantification of their plasticizers.

PLgel 5 μ m MIXED-C columns are designed for rapid polymer analysis. With its linear calibration up to 2 million MW, this is the column of choice for highest resolution and accuracy in molecular weight distribution analyses. Rapid solvent change capability, excellent temperature stability and the high resolution of the PLgel 5 μ m MIXED-C also provide ideal versatility for the R&D laboratory.

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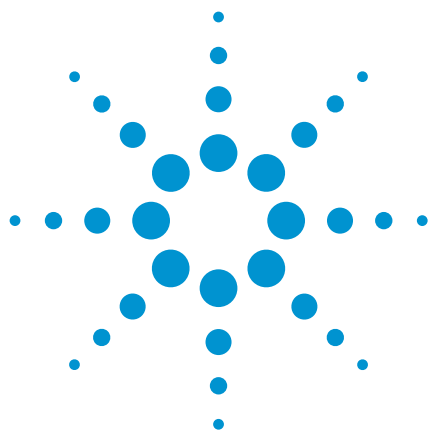
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SI-01824



Agilent Technologies



Analysis of Polyaniline

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

The Agilent PLgel 5 μ m MIXED-D column is specifically designed for the analysis of polymers, paints and resin systems where material above 400,000 MW is unlikely to be present. These columns are ideal for the analysis of polyanilines.

Polyaniline exists in two forms: the emeraldine form, which contains alternating secondary amines and imine groups, and the leucoemeraldine form, which contains only secondary amines. The emeraldine polymer is semi-conjugated and so is an intense blue color and is of interest as a polymeric dyestuff (the leucoemeraldine is colorless). The analysis of polar polymers, such as polyaniline, requires the use of polar organic solvents as eluents, for example: dimethylformamide, dimethyl sulfoxide and N-methyl-2-pyrrolidinone (NMP). Often these solvents require high temperature GPC, due to their increased viscosity compared to tetrahydrofuran, and the addition of salts reduces interactions between the polymer and the column packing material.



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A sample of the emeraldine form was dissolved in the eluent to produce a solution at 0.05% (w/v) and left for 12 hours to dissolve. Any undissolved material was removed from the sample by filtration through a 0.45 µm syringe filter prior to injection.

The figure shows a chromatogram of the emeraldine polyaniline. The polymer elutes as a broad distribution after about 12 minutes.

Conditions

Columns:	2 x PLgel 5 µm MIXED-D, 300 x 7.5 mm (part number PL1110-6504)
Eluent:	NMP + 0.1% (w/v) LiBr
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Temperature:	80 °C

High pore volume combined with the 5 µm efficiency provides excellent resolution for low MW polymers and oligomers. Two, or even three, PLgel 5 µm MIXED-D columns are the perfect replacement for the popular 10⁴/500 Å or 10⁴/10³/500Å/100Å column combinations.

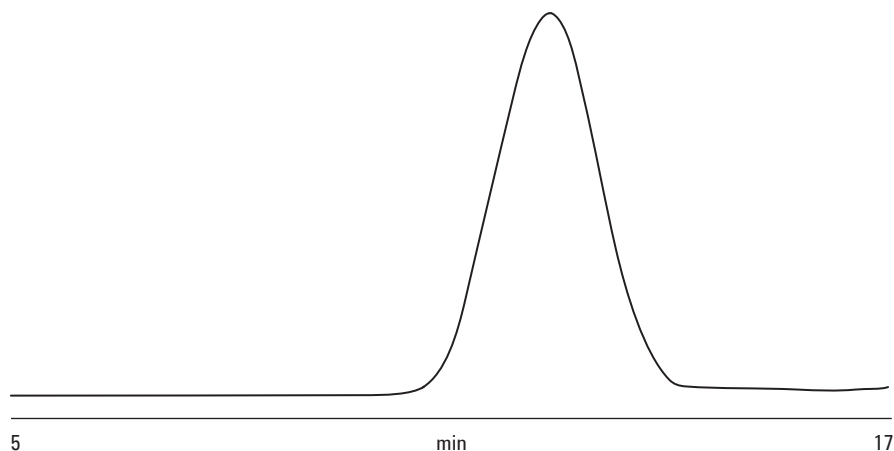


Figure 1. Analysis of emeraldine polyaniline using PLgel 5 µm MIXED-D columns

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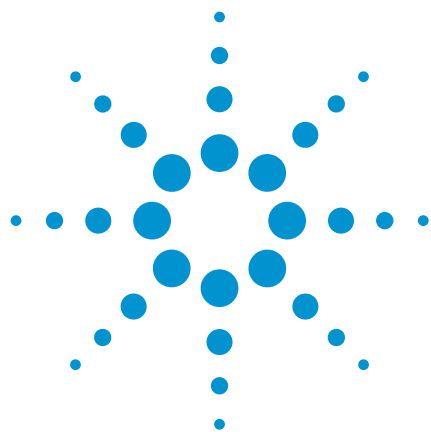
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Agilent Technologies



Characterization of Block Copolymers Synthesized via Transition Metal Mediated Living Radical Polymerization

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Polymerization reactions mediated by the presence of a transition metal have received much interest in both literature and the commercial world. These processes allow the controlled synthesis of polymers of predetermined molecular weight, polydispersity and well-defined architecture using cheap and synthetically simple procedures. Generation of a carbon radical initiates free-radical polymerization in the presence of a monomer and a suitable solvent. Termination steps are minimized by the reversible nature of the radical formation reaction. Careful tailoring of the relative reaction rates can lead to well-controlled polymerizations where termination steps are sufficiently controlled to allow polymers of polydispersity of 1.1 to be produced from the reaction. These reactions typically follow pseudo-first order kinetics. The ability to readily convert hydroxyl functional groups to active initiator groups ready for polymerization leads to a convenient synthetic pathway for the synthesis of architecturally diverse materials such as stars, blocks and grafts.

One such class of polymer structures that may be prepared using this synthetic process are block copolymers. These copolymers are made up of two or more chains that contain different repeat units that are known as blocks, for example a block of polystyrene connected to a block of polymethyl methacrylate repeat units. The materials are of interest because under certain conditions they facilitate phase separation, forming nanostructures with properties that differ from the equivalent blended material or from random copolymers of the monomers used in the two blocks.

Determining the molecular weight of block copolymers is not straightforward as the composition of the material affects the molecular dimensions, which in turn means that molecular weights determined by conventional GPC using only a refractive index detector are inaccurate. However, using viscometry, it is possible to determine the molecular weights of these materials by the universal calibration approach.



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This methodology makes use of the relationship between molecular weight and molecular density, allowing accurate molecular weights to be determined for materials irrespective of their chemistry. This note describes the analysis of a series of five poly(styrene-co-methyl methacrylate) copolymers manufactured with different molecular weights by GPC viscometry.

Results and Discussion

Conditions

Columns: 2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (part number PL1110-6500)
 Eluent: Tetrahydrofuran
 Flow Rate: 1 mL/min
 Inj. Vol: 100 μ L
 Sample Conc: 2 mg/mL
 Temp: 40 °C
 Detectors: Agilent 390-MDS Multi Detection Suite comprising a differential refractive index and a four capillary bridge viscometer
 Calibration Standards: Agilent Polystyrene EasiVials

Figure 1 shows overlaid dual detector raw data chromatograms for a poly(styrene-co-methyl methacrylate) sample showing the data collected from the individual detectors. The polymers all eluted as narrow near-Gaussian peaks.

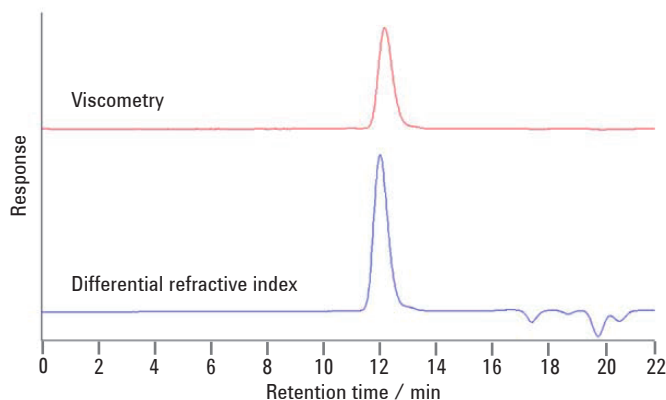


Figure 1. Overlaid dual detector raw data chromatograms for a block copolymer sample

The samples were all then analyzed by GPC with viscometry, employing the universal calibration method to determine molecular weights that were not dependent on calibrant chemistry. The overlaid molecular weight distributions are shown in Figure 2.

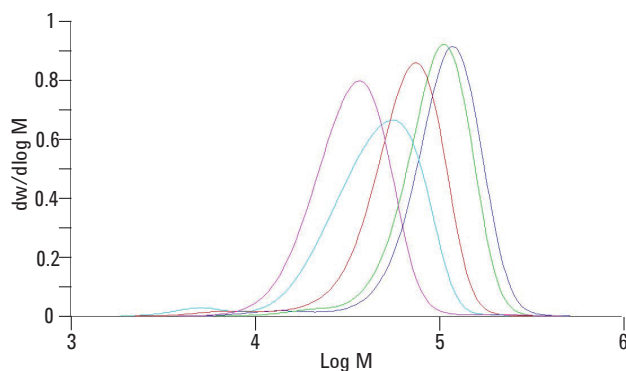


Figure 2. Overlaid molecular weight distributions calculated by universal calibration analysis of all samples

The Mark-Houwink plots for the four materials are shown in Figure 3.

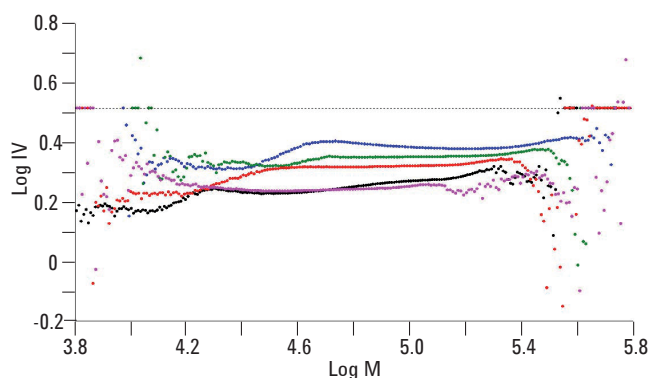


Figure 3. Overlaid molecular weight distributions calculated by universal calibration analysis of all samples

The Mark-Houwink relationship describes the scaling behavior of the intrinsic viscosity of polymers as a function of molecular weight. Assuming that materials have the same molecular density, they will follow the same Mark-Houwink plot. In these samples the variations in the block lengths for the different samples have caused shifts in the Mark-Houwink plots.

Conclusion

The 390-MDS multi detection suite successfully determined the molecular weights of some block copolymers and revealed differences in the block lengths. Viscometry detection delivered by the 390-MDS is a powerful tool for investigating the molecular weight and structural properties of polymers, irrespective of their chemistry.

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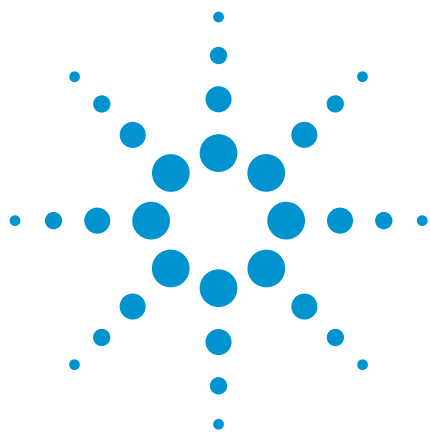
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SI-01876



Agilent Technologies



Analysis of Polybutadienes by GPC with Triple Detection

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polybutadienes are a type of synthetic rubber, commonly used to make tires along with other car parts such as belts, hoses and gaskets, because of their resilience to temperature and wear. They also offer a high level of electrical resistivity. Polybutadiene was one of the first types of synthetic elastomer to be invented and now synthetic rubber has largely replaced natural rubber in a wide variety of industrial applications.

Triple detection size exclusion chromatography (SEC) employs a concentration detector, a viscometer and a light scattering detector to assess the molecular weight distribution and molecular structure of polymers without having to rely on column calibrations. This can be important when analyzing complex materials for which no structurally similar standards are available.



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Conditions

Sample: Polybutadiene
Columns: 2 x Agilent PLgel 5 μ m MIXED-C,
300 x 7.5 mm (p/n PL1110-6500)
Inj Vol: 100 μ L
Eluent: THF
Flow Rate: 1 mL/min
Detector: Agilent PL-GPC 50 Plus, RI, Agilent
PL-BV 400, Agilent PL-LS

Results and Discussion

A sample of polybutadiene was analyzed on a PL-GPC 50 Plus integrated GPC system running at 30 °C. The system was fitted with a refractive index detector, a PL-BV 400 four capillary bridge viscometer and a PL-LS dual angle light scattering detector (collecting scattered light at low and high angle).

Two PLgel 5 μ m MIXED-C columns were used for this analysis. The polybutadiene sample was prepared accurately at a nominal concentration of 2 mg/mL in tetrahydrofuran and injected into the system without further treatment. For the purpose of light scattering calculations, an average dn/dc was used for the sample.

Figure 1 shows an overlay of the triple detector chromatograms for the sample.

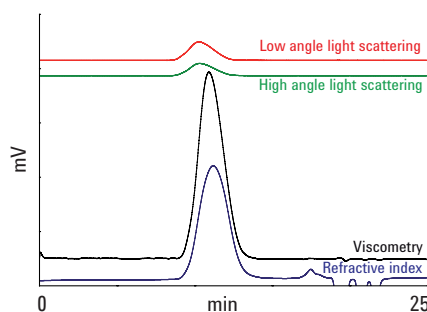


Figure 1. Triple detection of a polybutadiene

Figure 2 reveals the molecular weight distribution calculated for the sample of polybutadiene.

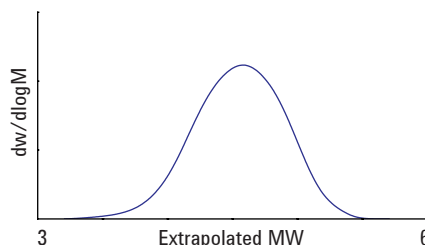


Figure 2. Molecular weight distribution of a polybutadiene

Mark-Houwink (log intrinsic viscosity versus log M) plots were generated from the viscometry and light scattering data (Figure 3). The curvature in the Mark-Houwink Plot may be a result of structural changes in the polymer as a function of molecular weight.

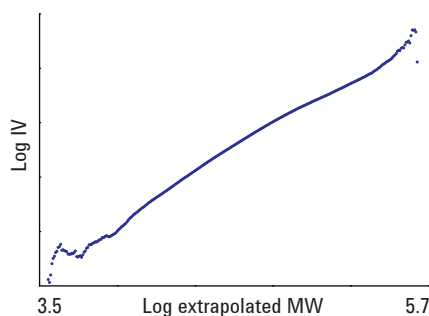


Figure 3. Mark-Houwink plot of a polybutadiene

Conclusion

The PL-GPC 50 Plus is ideal for the analysis of structurally complex but commercially important materials by multi detector GPC. By using triple detection the molecular weight distribution and molecular structure of polymers can be ascertained without having to rely on column calibrations which may not be possible because of

a lack of suitable standards.

The enhanced PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises a precision solvent delivery system, a sample injection system, a high performance differential refractive index detector and a column oven, with fully integrated software control. For maximum flexibility and applicability, a choice of system enhancements is also available.

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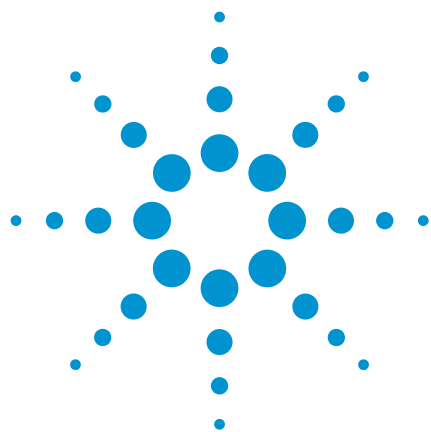
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Agilent Technologies



Analysis of Poly(isobornyl methacrylate) in Tetrahydrofuran by GPC

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Methacrylic monomers have the advantage that they are generally easy to polymerize through simple and well-understood free radical processes. Polymerization is mediated through the vinyl group, leaving the esterified carboxylic group unaffected. As a consequence, it is possible to introduce a wide variety of molecular structures and functional groups to the monomers by coupling different molecules to the ester linkage of the carboxylic group. This has led to the synthesis and commercialization of a huge number of monomers that can be used alone, or in combination, to produce polymers with widely varying physical properties.



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An example of a modified methacrylate monomer is isobornyl methacrylate, shown in Figure 1.

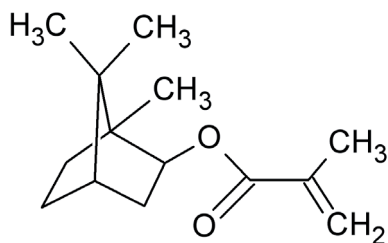


Figure 1. Structure of isobornyl methacrylate monomer

In this species an isobornyl cage has been attached to a methacrylate molecule through esterification. The resulting poly(isobornyl methacrylate) produced by polymerization of the monomer is a hard material with a high glass transition temperature and a high chemical and water resistance, useful properties in the adhesives and coatings industries. Furthermore, isobornyl methacrylate can be used as a reactive diluent for oligomers as the cyclic group can be cross linked through free radical curing to make highly resistant insoluble materials. For applications of this type the molecular weight distribution of the polymer is of key importance, as properties such as chemical resistance and rate of cross linking are dependent on the length of the polymer chains. This application describes the analysis of two samples of poly(isobornyl methacrylate) by GPC (gel permeation chromatography).

Conditions

Samples: Poly(isobornyl methacrylate),
2 mg/mL
Columns: 2 x Agilent PolyPore, 300 x 7.5 mm
(part number PL1113-6500)
Inj Vol: 100 µL
Eluent: Tetrahydrofuran
Flow Rate: 1 mL/min
Detector: Agilent PL-GPC 50 Plus with DRI

Results and Discussion

Two PolyPore columns from the PlusPore range, which resolve up to around 2,000,000 g/mol (polystyrene in tetrahydrofuran), were fitted in the oven of a PL-GPC 50 Plus integrated GPC system. The poly(isobornyl methacrylate) was fully soluble in tetrahydrofuran and so this solvent was chosen for the analysis. Differential refractive index, standard in the PL-GPC 50 Plus, was chosen as the method of detection. Figure 2 shows the overlaid chromatograms for the two materials. In this case it can be seen that the two materials have differing molecular weight distributions and will therefore have considerably different properties in their final application.

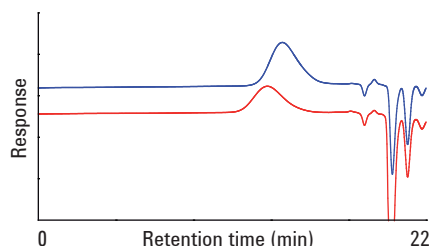


Figure 2. Overlaid chromatograms of two samples of poly(isobornyl methacrylate)

Conclusion

The PL-GPC 50 Plus, when combined with PolyPore columns, is ideal for investigating the molecular weight distributions of methacrylic monomers.

The enhanced PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises a precision solvent delivery system, a sample injection system, a high performance differential refractive index detector and a column

oven, with fully integrated software control. For maximum flexibility and applicability, a choice of system enhancements is also available.

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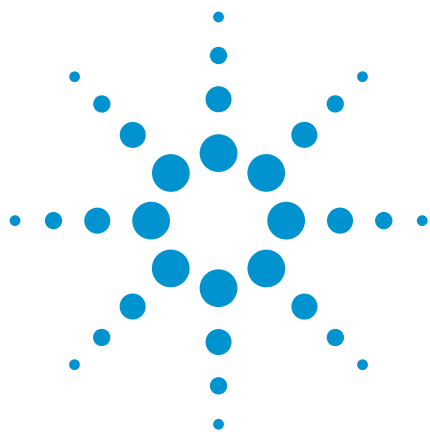
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SI-01911



Agilent Technologies



Analysis of Petroleum Jelly using Conventional GPC

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Petroleum jelly is a colorless, translucent gel, usually without taste or smell, that is commonly found as an ingredient in skin care products and cosmetics. It is a purified mixture of semi-solid, saturated hydrocarbons, similar to paraffin in nature and obtained from petroleum. Petroleum jelly has been used for over a hundred years, having first been marketed in 1870 when Richard Chesebrough discovered that by distilling the lighter, thinner oil products from unrefined rod wax he could create the useful gel. Since then many brands, grades and varieties have been created. Analysis of these low molecular weight materials can easily be achieved by gel permeation chromatography (GPC) with high efficiency Agilent PLgel 5 μ m MIXED-D columns.



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Conditions

Sample: Petroleum jelly A and B
Columns: 2 x PLgel 5 μ m MIXED-D,
300 x 7.5 mm (p/n PL1110-6504)
Inj Vol: 100 μ L
Eluent: THF
Flow Rate: 1 mL/min
Detector: DRI

Results and Discussion

Two varieties of Petroleum jelly were analyzed from different manufacturers to obtain an indication of any differences in molecular weight. The samples were made up at 0.2% (w/v) in tetrahydrofuran and injected without further treatment. Figure 1 shows the chromatogram given by Petroleum jelly brand A and Figure 2 shows the chromatogram for brand B.

The two chromatograms are overlaid in Figure 3 to show the subtle differences in molecular weight distribution between the two varieties.

The samples both contain a small amount of high molecular weight material, which can be seen in the molecular weight distribution (Figure 4).

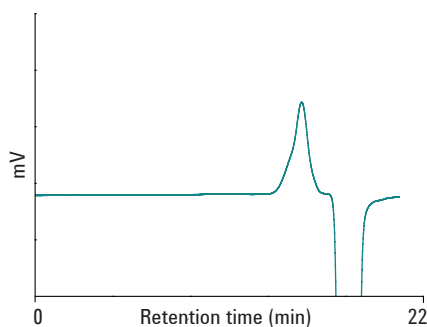


Figure 1. Chromatogram of Petroleum jelly brand A

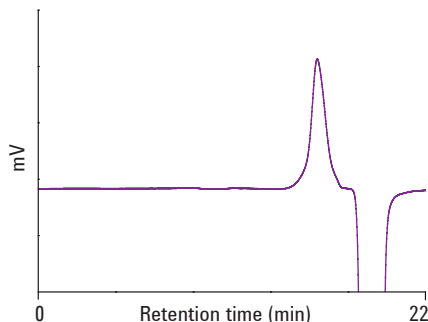


Figure 2. Chromatogram of Petroleum jelly brand B

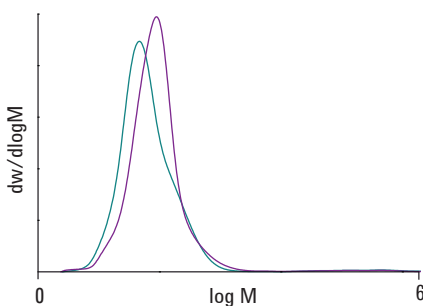


Figure 3. Overlaid chromatograms of two Petroleum jellies reveal slight differences in molecular weight distribution

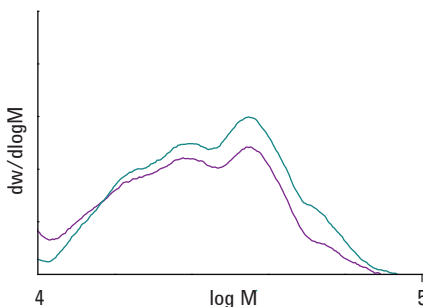


Figure 4. High molecular weight components of two different Petroleum jellies

Conclusion

A two column set of PLgel MIXED-D columns revealed slight differences in the molecular weight distribution of two commercial brands of petroleum jelly.

PLgel 5 μ m MIXED-D columns are specifically designed for the analysis of polymers, paints and resin systems where material above 400,000 MW is unlikely to be present. High pore volume, concentrated in this operating range, combined with the 5 μ m efficiency, provides excellent resolution for low MW polymers and oligomers. Two, or even three, PLgel 5 μ m MIXED-D columns are the perfect replacement for the popular 10E4/500Å or 10E4/10E3/500Å/100Å column combinations.

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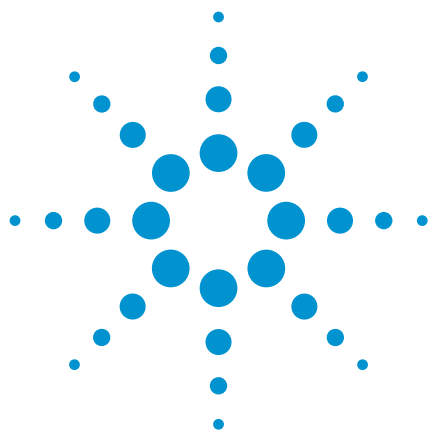
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SI-01913



Agilent Technologies



Analysis of Biodegradable Polymers by GPC

Application Note

Author

Greg Saunders
Agilent Technologies, Inc.

Introduction

Polymers have a wide range of uses in society because of their durability and resistance. This durability, however, has its drawbacks, especially when it comes to the disposal of polymers once they are no longer useful. An accumulation of degradation resistant polymers in landfill sites has become a serious problem. The solution is a polymer that can be degraded by natural means without losing the functional properties that make the polymer so useful.

A biodegradable polymer can be broken down into simpler substances by the activities of living organisms and is, therefore, unlikely to persist in the environment. Biodegradable polymers are also used in medicine, for such things as drug and gene delivery or bio-absorbable stents. Polycaprolactones and polylactides are good examples of biodegradable polymers with a wide range of industrial and biomedical applications. Polycaprolactones are fully biodegradable thermoplastic polymers, though they are derived from the chemical synthesis of non-renewable crude oil. Polylactides (PLA) are biodegradable polymers derived from lactic acid. Gel permeation chromatography is an ideal method for the analysis of biodegradable polymers. The approach adopted here employs refractive index and viscometry detection.



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Conditions

Sample: Polylactide, polycaprolactone and polylactide-glycolide
Columns: 2 x Agilent ResiPore, 300 x 7.5 mm (p/n PL1113-630)
Eluent: THF
Flow Rate: 1 mL/min
Temp: 40 °C
Detector: Agilent PL-GPC 50 Plus (DRI and Agilent PL-BV 400RT)

Results and Discussion

Figures 1 to 3 show examples of dual detection chromatograms for some biodegradable polymers. Figure 1 shows a polylactide sample, Figure 2 is a polycaprolactone sample and Figure 3 is a polylactide-glycolide.

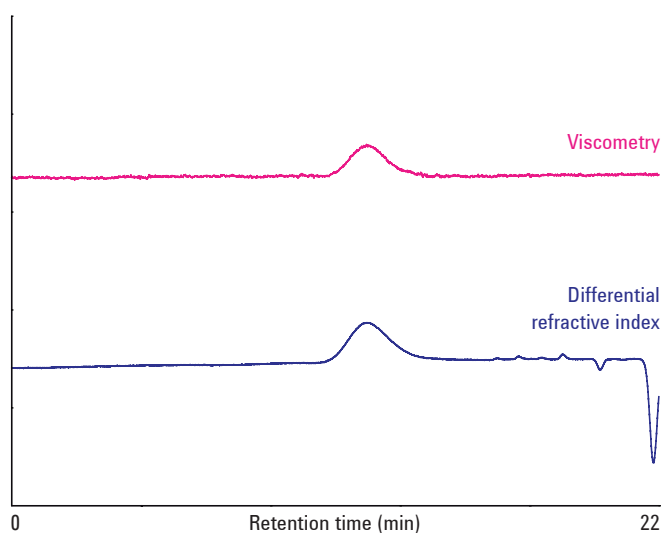


Figure 1. Dual detection chromatograms of a sample of polylactide

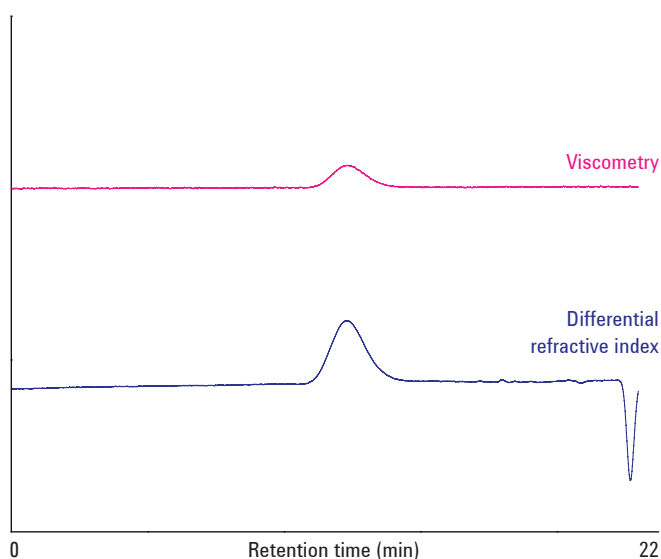


Figure 2. Dual detection chromatograms of a sample of polycaprolactone

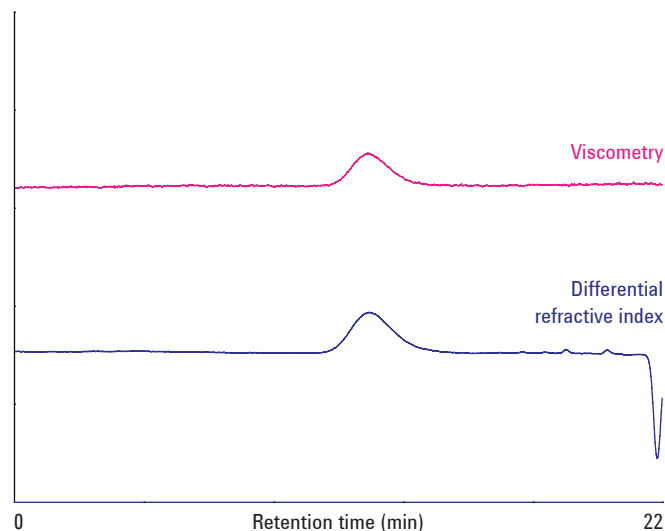


Figure 3. Dual detection chromatograms of a sample of polylactide-glycolide

The overlaid molecular weight distribution plots are shown in Figure 4.

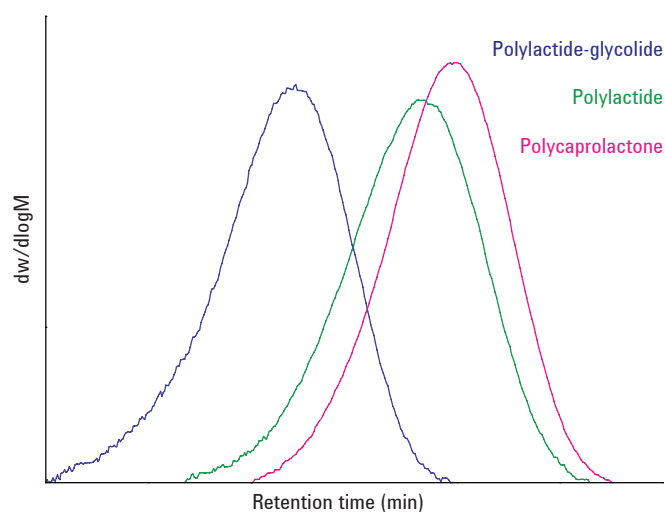


Figure 4. Molecular weight distributions of three biodegradable polymers

The universal calibration curve was generated using linear PS standards with narrow polydispersity (Figure 5).

Based on this calibration, the molecular weight averages and weight average, intrinsic viscosity (IV_w) was calculated for the biodegradable polymers. The table shows the molecular weight averages for a selection of such polymers.

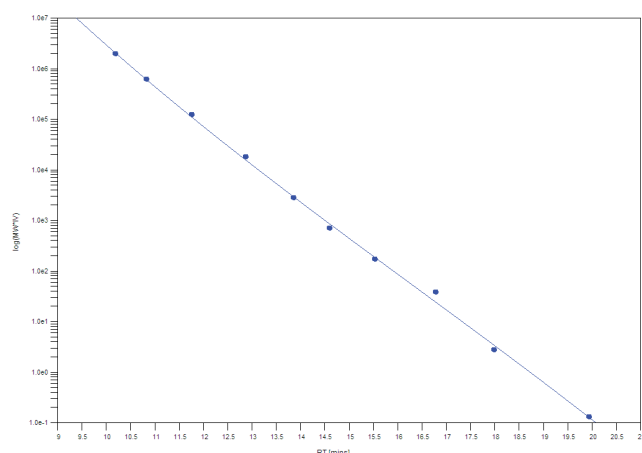


Figure 5. Universal calibration curve

Table 1. Molecular weight averages for a selection of biodegradable polymers

Sample	Molecular Weight Averages gmol^{-1}						PD
	Mp	Mn	Mw	Mz	Mz+1	Mv	
Poly(dl-lactide)	70,863	42,039	73,904	115,032	160,338	68,604	1.758
	69,596	41,967	74,148	114,767	158,539	68,860	1.7668
Poly(dl-lactide)-glycolide	72,153	44,926	77,077	118,849	164,761	71,687	1.7156
	70,863	43,821	76,555	118,849	164,761	71,687	1.747
50:50 d,l-PLGA	43,010	24,729	42,860	63,021	84,231	40,121	1.7332
	42,259	24,183	41,822	62,163	83,542	390,774	1.7294
65:35 d,l-PLGA	64,762	36,471	63,183	96,758	133,397	58,812	1.7324
	59,209	33,999	61,212	96,217	135,076	56,698	1.8004
75:25 d,l-PLGA	72,153	43,984	75,487	116,184	160,925	70,231	1.7162
	72,153	42,852	74,164	114,689	158,620	68,914	1.7307
95:5 d,l-PLGA	16,447	9,114	16,488	24,968	33,641	15,339	1.8091
	16,447	9,231	16,280	24,477	32,753	15,167	1.7636
Polycaprolactone	100,091	67,340	105,978	153,033	203,984	99,736	1.5738
	100,091	67,310	105,871	154,173	206,777	99,514	1.5729

Conclusion

The GPC system successfully characterized some biodegradable polymers. Using RI and viscometer detection, and universal calibration, it was possible to derive the molecular weight distributions of the samples, and calculate several of their characterization parameters.

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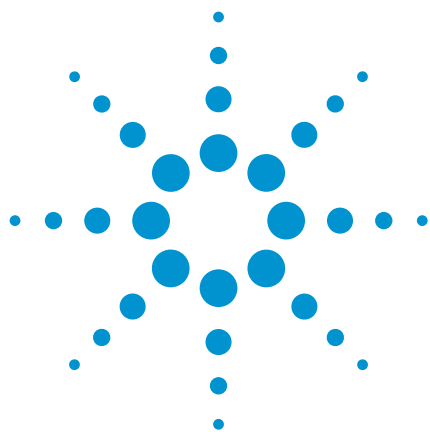
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SI-01914



Agilent Technologies



Triple Detection Characterization of Polycarbonates with the Agilent 390-MDS

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polycarbonates are a class of tough, strong, high-performance engineering thermoplastics. With an amorphous structure, these materials are characterized by the presence of carbonate groups ($-O-(C=O)-O-$) in the polymer backbone, although the chemistry of the rest of the polymer can vary considerably.

Polycarbonates typically exhibit high transparency to visible light and have better light transmission characteristics than many kinds of glass, leading to their use in applications such as lighting and eyeglass lenses. In recent years polycarbonates have become more widely used in houseware based applications due to the high impact and temperature resistance properties.

In the manufacturing of polycarbonates used for engineering applications the final properties of the polymer must be carefully controlled to ensure the final performance. Triple detection allows the molecular weight and structural properties of different batches of polymer to be assessed and compared, and is an excellent quality control methodology. The use of light scattering gives molecular weights that are independent of the structure of the polymers, whereas the Mark-Houwink plot can be used to probe the structural properties of the materials.

This note describes the triple detection characterization of a series of four polycarbonate samples from different batches of product manufactured with slight changes to the production protocol. The intention of the analysis was to determine if the production changes caused any change to the molecular weight and structural characteristics of the materials.



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Materials and Methods

Conditions

Columns:	2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (part number PL1110-6500)
Eluent:	Tetrahydrofuran
Flow Rate:	1 mL/min
Inj. Vol:	100 μ L
Sample Conc:	2 mg/mL
Temp:	40 °C
Detectors:	390-MDS Multi Detector Suite comprising differential refractive index, four capillary viscometry and dual-angle light scattering detection
Calibration Standards:	Agilent Polystyrene Individual Narrow Standard (Mp 197,300)

Results and Discussion

Figure 1 shows an example overlaid triple detection chromatogram of one of the materials.

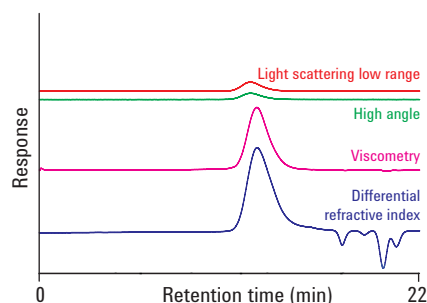


Figure 1. Overlaid triple detector raw data chromatograms for a polycarbonate sample showing the data collected from the individual detectors

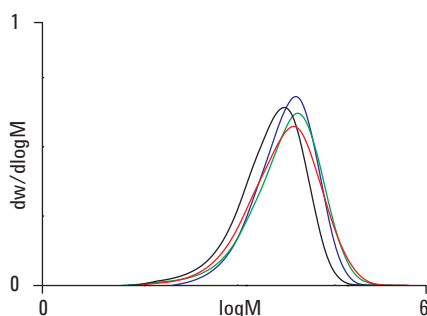


Figure 2. Overlaid molecular weight distributions calculated from triple detection analysis of all samples

The samples were all then analyzed by triple detection, employing the light scattering detector to determine molecular weights that were not dependent on a column calibration. The overlaid molecular weight distributions are shown in Figure 2.

The results showed that the changes to the production protocol had affected the molecular weight distributions of the four materials, which would result in differing characteristics in the final product. The Mark-Houwink plots for the four materials are shown in Figure 3.

The Mark-Houwink relationship describes the scaling behavior of the intrinsic viscosity of polymers as a function of molecular weight. Assuming that two materials have the same molecular density, they will follow the same Mark-Houwink plot. Typically, changes in molecular density result either from differing sample chemistries or from the presence of long chain branching.

The Mark-Houwink plots of these materials show their relative structural similarities, with deviations in the relative positions of the plots indicative of structural changes. In the case of the four samples, the plots overlay, indicating that the production change has not affected the structural properties of the materials.

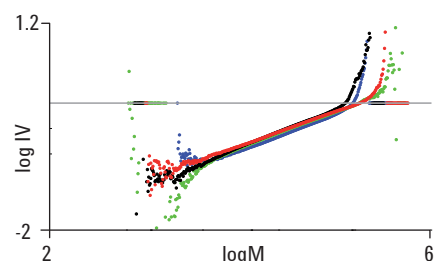


Figure 3. Overlaid Mark Houwink plots calculated from triple detection analysis of all samples

Conclusion

Triple detection GPC using the 390-MDS Multi Detector Suite is a powerful tool for investigating the molecular weight and structural properties of polymers. In this application the effect of changes to a production protocol on a series of polycarbonates could be easily observed using the 390-MDS.

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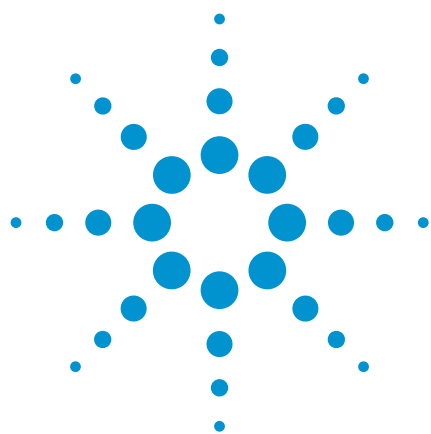
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SI-01957



Agilent Technologies



Gel Permeation Chromatography with Viscometry for the Molecular Weight Characterization of Epoxy Resins

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Synthetic resins are a polymeric class of materials designed to mimic the properties of naturally occurring resin materials. Typically viscous liquids that are capable of hardening, they are routinely synthesized via esterification reactions or soaping of organic compounds. First developed in the 1930s and commercialized in the 1940s, epoxy resins are manufactured through the reaction of polyols with epichlorohydrin, such as the largest production epoxy resin, called diglycidyl ether of bisphenol-A (DGEBA). The molecules of epoxy resins are low in molecular weight until cured, when they form a network of large cross-linked structures. Epoxy resins exhibit strong heat and chemical resistance as well as high mechanical strength and therefore find use in many different application areas from adhesives to high performance sports equipment.

The molecular weight and composition of the pre-cured materials is important as these parameters control how the material will cure and the properties of the final product. In their non-cured state epoxy resins may be analyzed by gel permeation chromatography (GPC). Determining the molecular weight of these complex materials is not straightforward as the composition of the material affects the molecular dimensions, which in turn means that molecular weights determined by conventional GPC using only a refractive index detector are inaccurate. However, using viscometry, it is possible to determine the molecular weights of these materials by the universal calibration approach. This note describes the use of GPC with viscometry to determine the molecular weight profiles of two different epoxy resin materials. Employing the universal calibration method, it is possible to determine accurate molecular weights for the materials regardless of composition, and also probe their structure via Mark-Houwink plots.



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Materials and Methods

Conditions

Columns: 2 x Agilent PLgel 5 μ m
MIXED-D, 300 x 7.5 mm
(part number PL1110-6504)
Eluent: Tetrahydrofuran
Flow Rate: 1 mL/min
Inj. Vol: 100 μ L
Sample Conc: 2 mg/mL
Temp: 40 °C
Detectors: Agilent 390-MDS
Multi Detector Suite
comprising a refractive
index detector and a four
capillary bridge viscometer
Calibration Standards: Agilent Polystyrene
EasiVials

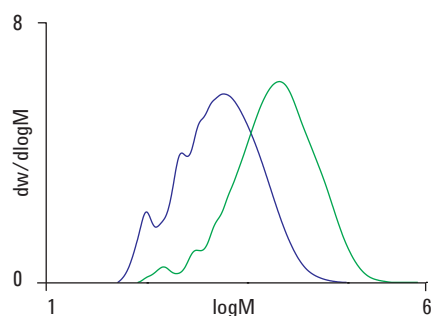


Figure 2. Overlaid molecular weight distributions calculated by universal calibration analysis of the two samples

The Mark-Houwink plots for the two materials are shown in Figure 3.

Results and Discussion

Figure 1 shows overlaid dual detector raw data chromatograms for an epoxy resin sample showing the data collected from the individual detectors. Each polymer eluted as a broad peak.

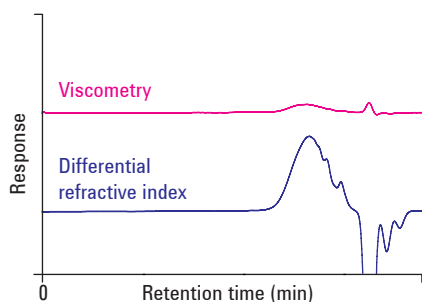


Figure 1. Overlaid dual detector raw data chromatograms for an epoxy resin sample 22

The samples were all then analyzed by GPC with viscometry, employing the universal calibration method to determine molecular weights that were independent of calibrant chemistry. The overlaid molecular weight distributions are shown in Figure 2.

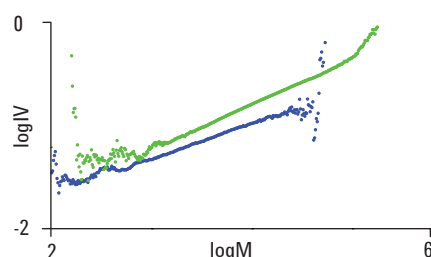


Figure 3. Overlaid Mark-Houwink plots calculated from universal calibration analysis of both samples

Conclusion

The structure of some epoxy resins was elucidated by using gel permeation chromatography with the 390-MDS. The 390-MDS detected differences in the chemistry of the resins, demonstrating the effectiveness of the instrument when investigating the molecular weight and structural properties of polymers.

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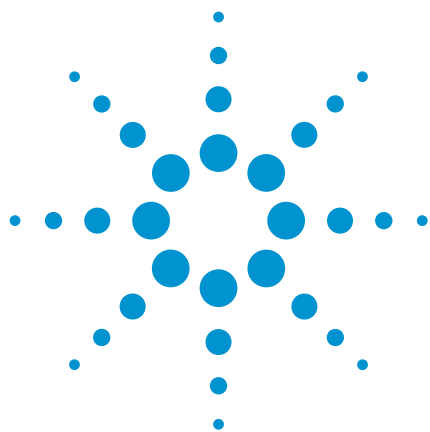
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SI-01960



Agilent Technologies



Size Exclusion Chromatography for the Analysis of Dental Polymers

Application Note

Author

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Copolymers of vinylmethyl ether and maleic anhydride are widely used in a range of dental applications. These include their use as a denture bioadhesive, as well as a toothpaste additive which helps the toothpaste to remain active between brushing. Batch to batch variations of such copolymer systems strongly influence performance in dental applications. Here, two batches of a commercial copolymer were analyzed by size exclusion chromatography (SEC). One of them had worked well in formulation but the other had failed.



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Materials and Methods

A column set comprising 2 x Agilent PL aquagel-OH MIXED 8 μ m columns were used for the analysis. These versatile columns offer resolution over a wide range of molecular weight (up to 10,000,000 relative to PEG/PEO), simplifying column selection. Column calibration was achieved using Agilent EasiVial PEG/PEO standards. EasiVials provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). The samples were made up in sodium nitrate buffer at neutral pH.

Conditions

Samples: Two samples of dental polymers
Columns: 2 x PL aquagel-OH MIXED 8 μ m, 300 x 7.5 mm (p/n PL1149-6800)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7
Flow Rate: 1.0 mL/min
Detection: Agilent PL-GPC 50 Plus (DRI)

Results and Discussion

Figure 1 shows the Agilent EasiVial PEG/PEO calibration curve. Figure 2 shows overlaid raw data chromatograms for the two batches, indicating large differences in molecular weight and molecular weight distribution.

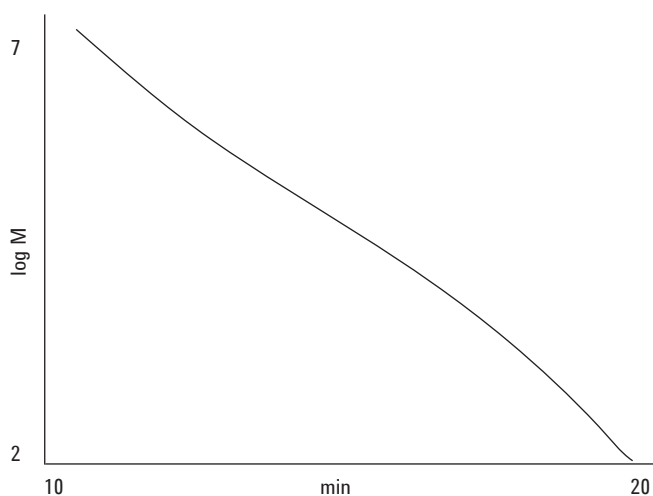


Figure 1. SEC PEG/PEO calibration using EasiVial standards

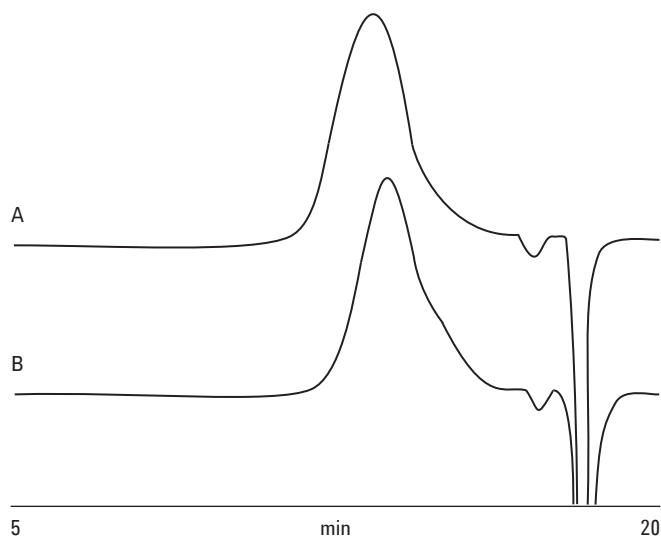


Figure 2. Raw data chromatograms of two dental polymers

The molecular weight distribution plots are presented in Figure 3 and clearly indicate the differences between the two batches.

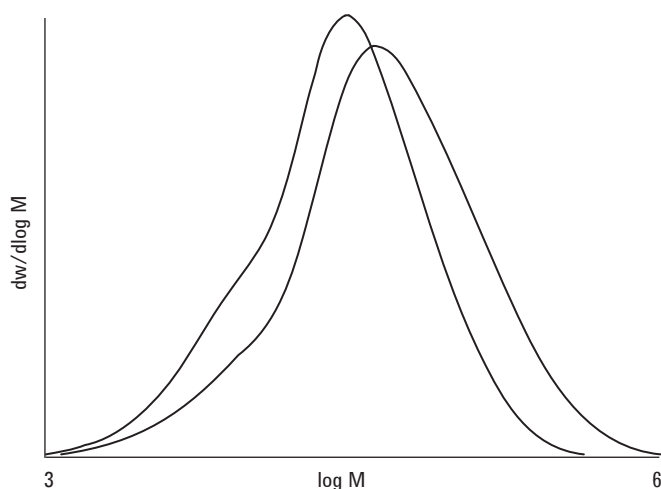


Figure 3. Overlay of the molecular weight distributions of two dental polymers

Table 1. Mp, Mw, Mn and polydispersity values for the two dental polymers

Batch	Mp	Mw	Mn	Polydispersity
A	169,330	100,070	258,200	2.6
B	124,100	69,550	152,060	2.2

Conclusion

Size exclusion chromatography using the PL-GPC 50 Plus in combination with PL aquagel-OH MIXED 8 μm columns was able to distinguish between successful and failed batches of copolymer for a dental application, illustrating the usefulness of the system for the quality control of polymers.

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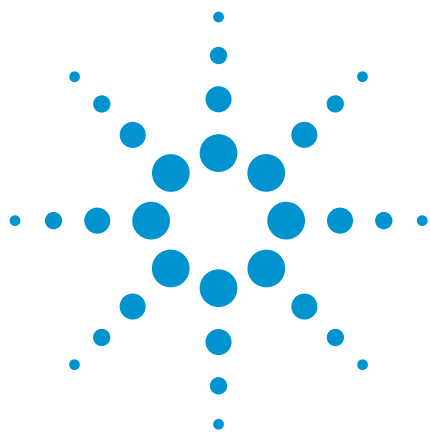
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SI-01965



Agilent Technologies



Analysis of Linear and Branched Polyethylene Glycols via GPC Viscometry

Application Note

Author

Ian Willoughby, Ben MacCreath,
Greg Saunders
Agilent Technologies, Inc.

Introduction

Polyethylene glycol is a biocompatible polymer used in many different commercial products because of its low toxicity. It forms the basis of many cosmetic materials and lubricants. More recently, through its conjugation to different protein and therapeutic materials, it finds use in a variety of biomedical application areas. The biomedical interest in branched type PEG materials has increased as a result of their lower viscosity in comparison with equivalent linear type polymers. Investigation of PEGs by gel permeation chromatography (GPC) can be used to elucidate their relatively compact nature in solution.



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Materials and Methods

Analysis of low molecular weight polyethylene materials is easily achieved by GPC with high efficiency Agilent PLgel 5 μm MIXED-D (300 x 7.5 mm) columns and the Agilent PL-GPC 50 Plus instrument. The PL-GPC 50 Plus was equipped with both differential refractive index and viscometry detection. The PEG samples were prepared at 0.2% (w/v) in dimethylformamide at room temperature for 2 hours and injected without further treatment.

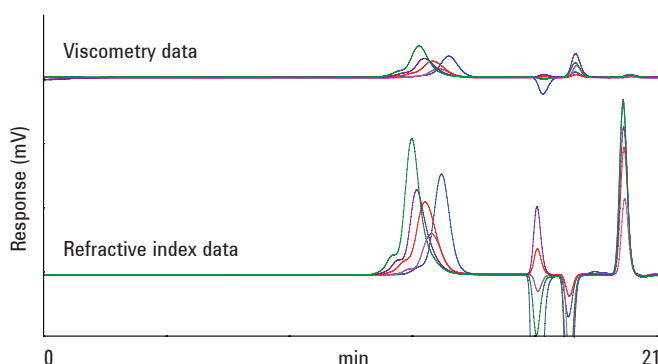


Figure 1. Overlaid raw data chromatograms obtained from a series of linear and branched polyethylene glycol samples

Conditions

Sample: Polyethylene glycol
Column: 2 x PLgel 5 μm MIXED-D (300 x 7.5 mm)
(part number PL1110-6504)
Eluent: Dimethylformamide (+ 0.1% LiBr)
Flow Rate: 1.0 mL/min
Inj Vol: 100 μL
Sample Conc: 2.0 mg/mL
Temp: 50 $^{\circ}\text{C}$
Calibrants: Agilent EasiVial PEG/PEO
Detector: PL-GPC 50 (Differential Refractive Index +
Agilent PL-BV 400RT Viscometer)

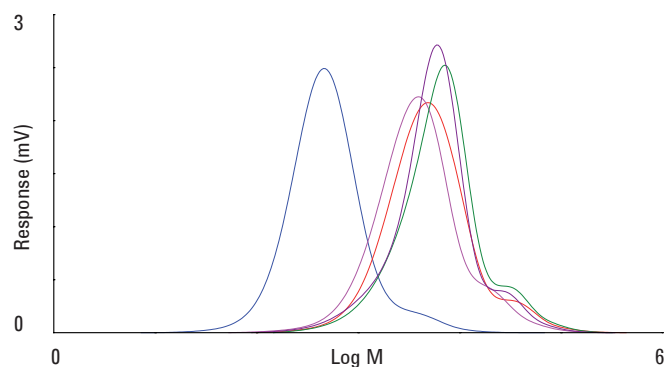


Figure 2. Overlaid raw data chromatograms obtained from a series of linear and branched polyethylene glycol samples

Results

Figure 1 shows the overlaid chromatograms given by the samples and Figure 2 the calculated molecular weight distributions.

Conclusion

This investigation demonstrates how gel permeation chromatography with viscometry detection, delivered by the PL-GPC 50 Plus and PL-BV 400RT, can be used for the analysis of pharmaceutically and commercially interesting materials such as linear and branched polyethylene glycols.

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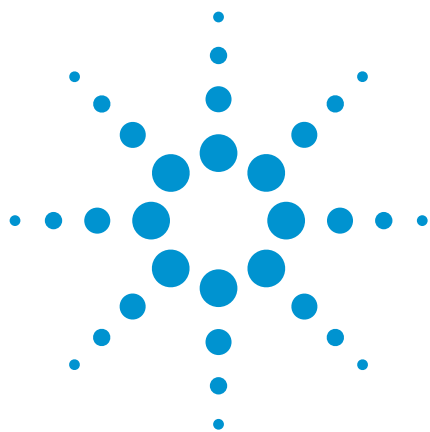
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Agilent Technologies



Analysis of Low Molecular Weight Polyethylene via Conventional GPC

Application Note

Author

Ian Willoughby, Ben MacCreath,
Greg Saunders
Agilent Technologies, Inc.

Introduction

Polyethylene is a structurally simple material with a high commercial relevancy. Currently over 60 million tons of polyethylene is produced worldwide every year. Despite its relatively simple structure, it is the most widely used thermoplastic in the world and has a range of final end user applications from grocery bags to bullet proof vests.



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Methods and Materials

Analysis of low molecular weight polyethylene materials can be easily achieved by gel permeation chromatography (GPC) with high efficiency Agilent PLgel 5 μ m MIXED-D columns, in conjunction with the Agilent PL-GPC 220 instrument.

Conditions

Sample:	Polyethylene
Column:	2 x PLgel 5 μ m MIXED-D, 300 x 7.5 mm (part number PL1110-6504)
Eluent:	1,2,4-Trichlorobenzene
Flow Rate:	1.0 mL/min
Inj Vol:	100 μ L
Sample Conc:	2.0 mg/mL
Temp:	160 °C
Calibrants:	Agilent EasiVial PS-M
Detector:	PL-GPC 220 (Differential Refractive Index)

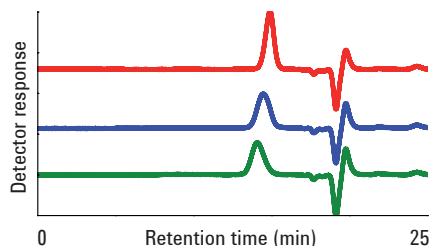


Figure 1. Overlaid raw data chromatograms obtained from series of low molecular weight polyethylene samples

The polyethylene samples were prepared using a Varian PL-SP260 sample preparation module at 0.2% (w/v) in trichlorobenzene at 150 °C for 2 hours and injected without further treatment.

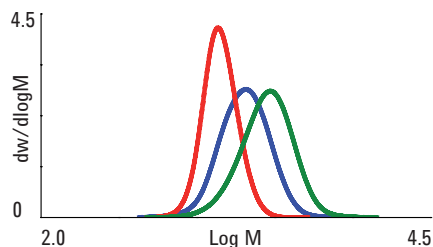


Figure 2. Overlaid molecular weight distributions obtained from series of low molecular weight polyethylene samples

Results

Figure 1 shows the overlaid chromatograms given by the samples and Figure 2 shows the calculated molecular weight distributions.

Conclusion

PLgel MIXED columns and the PL-GPC 220 successfully analyzed samples of polyethylene, demonstrating how the investigation of these structurally simple but commercially important materials can be accomplished by GPC.

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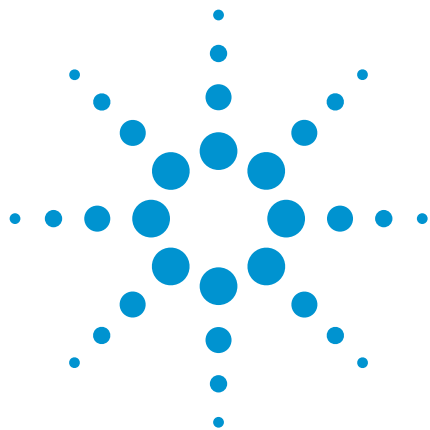
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Agilent Technologies



Analysis of Polythiophenes via Conventional GPC

Application Note

Author

Ian Willoughby, Ban MacCreath,
Greg Saunders
Agilent Technologies, Inc.

Introduction

Polythiophene is a class of polymer that contains a sulfur heterocycle in the polymer backbone. Figure 1 shows the chemical structure of the general thiophene monomeric repeat unit. Polythiophenes are of particular interest as a result of their ability to conduct when electrons are doped into or from the conjugated backbone pi-orbitals.

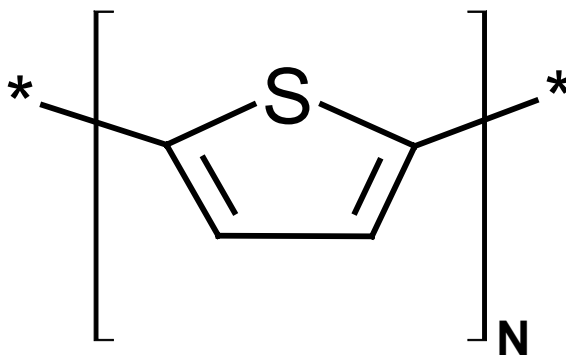


Figure 1. General structure of the thiophene monomeric repeat unit

A recent highlight of the study of this class of materials was the award of the 2000 Nobel Prize in Chemistry to Heeger, MacDiarmid and Shirakawa for “The discovery and development of conductive polymers”.



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Methods and Materials

Analysis of polythiophene materials can easily be achieved by gel permeation chromatography (GPC) with high efficiency Agilent PLgel 5 μ m MIXED-D (300 x 7.5mm) columns in conjunction with the Agilent PL-GPC 220 instrument equipped with RI detection.

The polythiophene samples were prepared using a Agilent PL-SP 260VS sample preparation module at 0.2% (w/v) in trichlorobenzene at 150 °C for 2 hours and injected without further treatment.

Conditions

Sample:	Polythiophene
Column:	2 x PLgel 5 μ m MIXED-D, 300 x 7.5 mm (part number PL1110-6504)
Eluent:	1,2,4-Trichlorobenzene
Flow Rate:	1.0 mL/min
Inj Vol:	100 μ L
Sample Conc:	2.0 mg/mL
Temp:	120 °C
Calibrants:	Agilent PS-M EasiVial
Detector:	PL-GPC 220 (Differential Refractive Index)

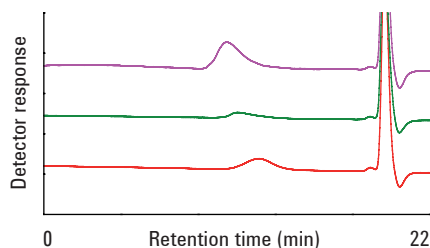


Figure 2. Overlaid RI raw data chromatograms obtained from a series of polythiophene samples

Results

Figure 2 shows the overlaid chromatograms obtained via RI detection for the series of polythiophene samples and Figure 3 is the overlaid calculated molecular weight distribution from these samples.

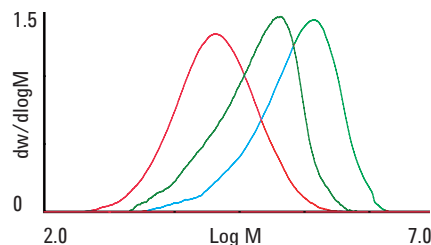


Figure 3. Overlaid molecular weight distributions obtained from a series of polythiophene samples

Conclusion

This note demonstrates how the structure of this new class of important materials can be elucidated using GPC with PLgel columns and the PL-GPC 220.

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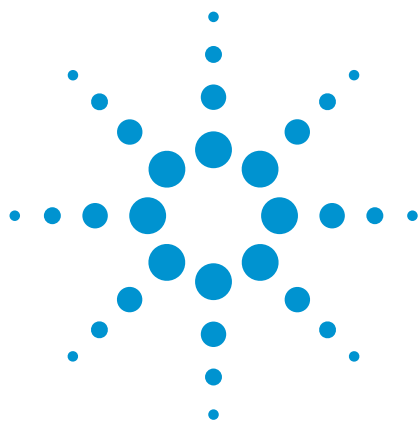
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SI-02025



Agilent Technologies



Analysis of Aliphatic Alcohols by Ligand-Exchange Chromatography

Application Note

Chemical

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

This application note demonstrates how an Agilent Hi-Plex H column can be used to separate aliphatic alcohols.



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Materials and Reagents

Column	Agilent Hi-Plex H (8% crosslinked), 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	100% DI H ₂ O
Flow rate	0.6 mL/min
Temperature	40 °C
Detector	RI

Conclusion

Using only pure HPLC-grade water as eluent, the Agilent Hi-Plex H column is capable of separating a range of aliphatic alcohols. In addition to those shown in Figure 1, it may also be possible to separate a much wider range of this type of compound. Molecular weight and degree of branching are critical factors in determining the amount of retention on a Hi-Plex H 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.

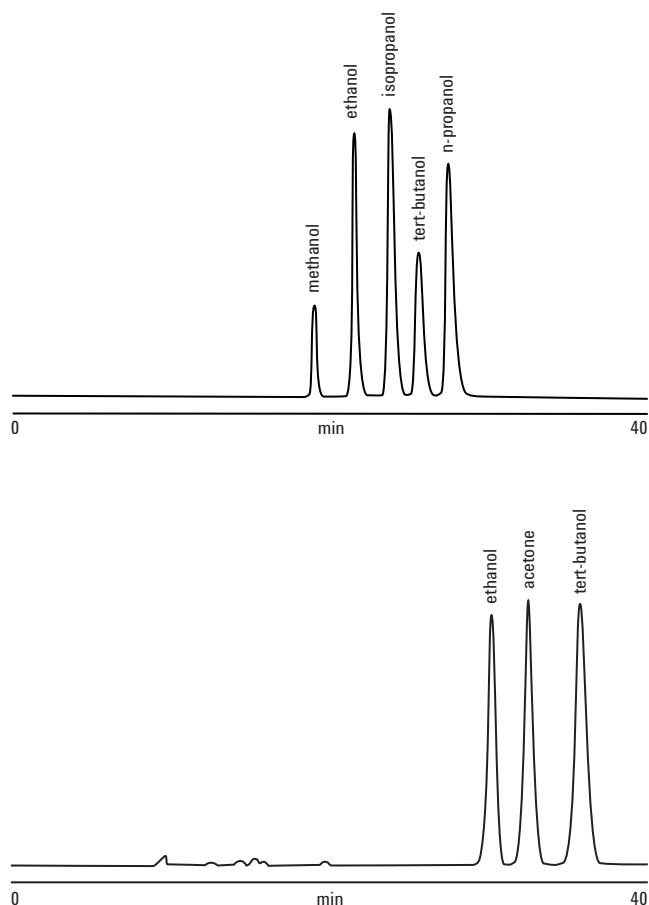


Figure 1. Separation of different aliphatic compounds on an Agilent Hi-Plex H column.

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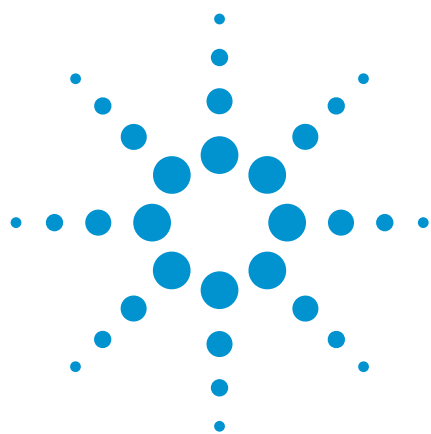
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Agilent Technologies



Analysis of Melamine Resins by Conventional GPC using Agilent PolarGel-L Columns and the Agilent PL-GPC 50 Plus

Application Note

Authors

Ben MacCreath, Ian Willoughby,
Greg Saunders
Agilent Technologies, Inc.

Introduction

Melamine resins are durable thermosetting plastics formed by the condensation polymerization of melamine with formaldehyde. They are commonplace in the home as they are employed to laminate chipboard, creating inexpensive furniture, as well as being used in the manufacturing of kitchen tableware and food packaging. The molecular weight distribution of melamine resins determines many of the final properties of the polymer and therefore their end-use suitability for particular applications. Subtle differences in the molecular weight distributions of these materials can have large consequences on their final properties, and so accurate characterization of the molecular weight distribution of melamine resins is essential.



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Methods and Materials

Conditions

Samples:	Two samples of melamine resin
Columns:	2 x PolarGel-L, 300 x 7.5 mm (part number PL1117-6830)
Eluent:	Dimethylacetamide + 0.1% LiBr
Flow Rate:	1.0 mL/min
Temperature:	50 °C
Detection:	PL-GPC 50 Plus with DRI

Results and Discussion

It is apparent from the chromatograms (Figure 1) and overlaid molecular weight distributions (Figure 2) that the two samples of melamine resins had quite different molecular weight distributions, with differing ratios of the various oligomers present. These differences suggest that the samples will have markedly dissimilar properties, which will affect their end use.

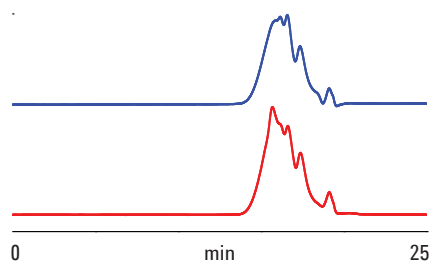


Figure 1. Chromatograms for two melamine samples with different oligomer ratios

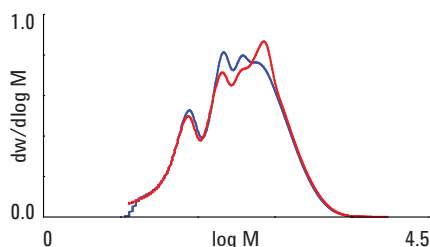


Figure 2. Overlaid molecular weight distributions for two melamine resin samples suggest differences in performance

Conclusion

The PL-GPC 50 Plus fitted with two PolarGel-L columns successfully analyzed two samples of melamine resin, indicating clear differences between the samples. The PolarGel-L columns are well suited to operation in highly polar solvents such as dimethylacetamide. The stability of the PL-GPC 50 Plus column even ensured that a low column pressure and stable baselines were observed throughout the analysis.

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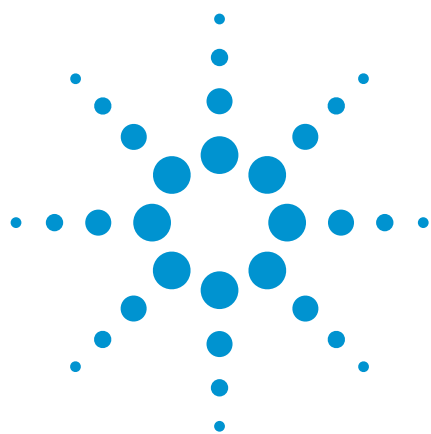
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SI-02367



Agilent Technologies



Analysis of Poly(styrene/butadiene) Copolymers by Conventional Gel Permeation Chromatography on the Agilent PL-GPC 50 Plus

Application Note

Authors

Ben MacCreath, Ian Willoughby,
Greg Saunders
Agilent Technologies, Inc.

Introduction

A poly(styrene/butadiene) block copolymer (SBR) mimics many of the properties of natural rubber and has applications in a wide variety of industrial areas. The characteristics are provided by the hard polystyrene chains being surrounded by a network of rubbery polybutadiene, which provides strength and flexibility over a large temperature range. The copolymer is a thermoplastic elastomer and therefore can easily be used in manufacturing by injection moulding, or blended into an existing product to increase elasticity or impart toughness. The molecular weight distribution is critical, as any homopolymer will significantly affect the resultant end properties.



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Methods and Materials

Conditions

Columns: 2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm
(part number PL1110-6500)
Eluent: Tetrahydrofuran (250 ppm BHT)
Flow Rate: 1.0 mL/min
Sample Concentration: 2.0 mg/mL
Injection Volume: 100 μ L
Temperature: 40 $^{\circ}$ C
Calibration Standards: Agilent Polystyrene EasiVial
Detection: PL-GPC 50 Plus, DRI

Results and Discussion

Chromatograms for three poly(styrene/butadiene) block copolymer samples are shown in Figure 1. Figure 2 reveals the copolymer overlaid molecular weight distributions.

Each peak eluted as a relatively narrow main peak indicative of ionic polymerization. The molecular weights of the components of the samples were different, due to a change to the synthesis conditions employed. The main peak of each sample was the block copolymer. However, one of the samples also contained a smaller peak eluting after the main peak, indicating the presence of homopolymer at lower molecular weight. Evidence of high molecular weight termination products is provided by the small peaks eluting before the main peak. The presence of these peaks is a result of imperfections in the polymerization processes used to manufacture the materials.

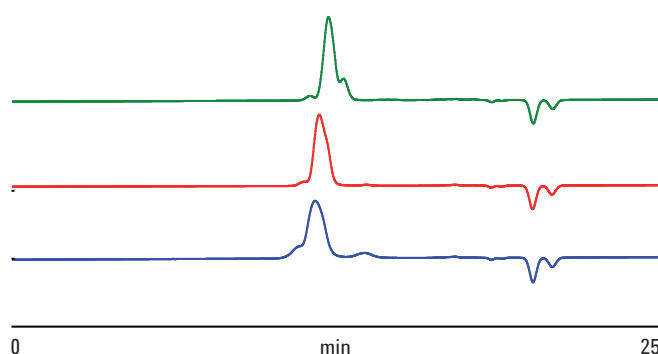


Figure 1. Chromatograms for three samples of poly(styrene/butadiene) – each sample eluted as a multi-modal distribution of relatively sharp peaks

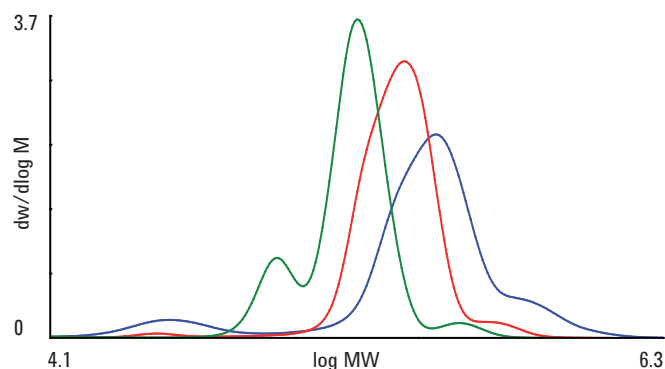


Figure 2. Overlaid molecular weight distributions for the poly(styrene/butadiene) samples

Conclusion

Three samples of poly(styrene/butadiene) were analyzed by conventional gel permeation chromatography on the PL-GPC 50 Plus. Distinct differences were observed arising from presence of homopolymers, along with the anticipated copolymer. This application note demonstrates how GPC may be used to assess the products of complex synthesis reactions to gain mechanistic insights into polymerization processes.

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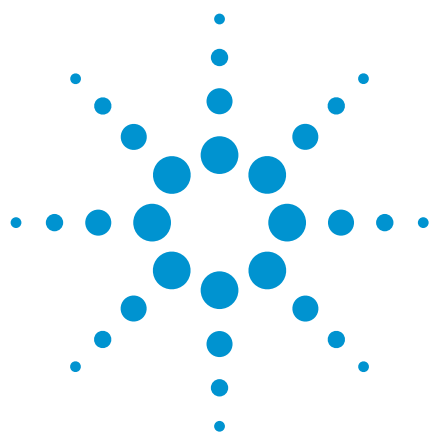
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Agilent Technologies



Analysis of Polybutadiene by GPC Triple Detection with the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polybutadiene is an elastomer – a polymer that exhibits elasticity. Elasticity is the ability to deform under external stress but return to the original form after removal of the stress. Polybutadiene is a synthetic rubber manufactured from the monomer 1,3-butadiene. Polybutadiene exhibits 80% recovery after stress, one of the highest values of a synthetic material. It is commonly used to coat electronic assemblies due to its extremely high electrical resistivity. Polybutadiene displays a high wear resistance, low heat build-up after repeated flexing and a low rolling resistance, making it suitable for applications such as tires. It is often used in combination with other materials to produce rubber blends with differing properties.

The 390-MDS offers triple detection GPC by employing a light scattering detector and a viscometer in combination with a differential refractive index detector, to determine accurate molecular weights for polymers for which narrow standards are not available, such as polybutadiene.



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Methods and Materials

Conditions

Samples:	Polybutadienes
Columns:	2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (p/n PL11110-6500)
Injection Volume:	100 μ L
Eluent:	THF (stabilized)
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Agilent 390 Dual angle light scattering, Viscometer and DRI options
Detector Temp:	All detectors set at 40 °C

Results and Discussion

Figure 1 shows an overlaid multi-detector chromatogram for a sample of polybutadiene. The polymer delivered strong, fairly broad signals in all the detectors, indicating the high polydispersity of the materials.

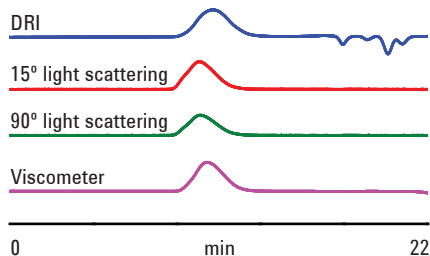


Figure 1. Overlaid multi-detector chromatogram for a sample of polybutadiene

Figure 2 is an overlay of the molecular weight distributions of the three polybutadiene samples. Two of the samples displayed similar molecular weights with only slight differences across the distributions. However, the third sample showed an appreciable difference in molecular weight distribution, especially at high molecular weight where there was slight exclusion of the sample. This change was thought to be responsible for the difficulty in processing this material.

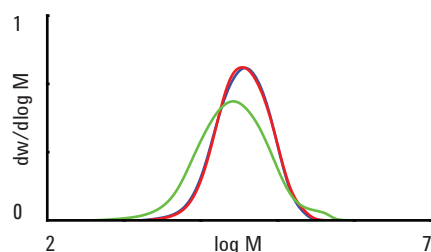


Figure 2. Overlaid triple detector molecular weight distributions of three samples of polybutadiene

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight. All of the samples gave the same relationship between increasing log intrinsic viscosity and increasing log molecular weight. This indicates that the polymers were structurally identical and, therefore, that the difference in molecular weight observed was as a result of the synthesis methods employed rather than any changes to the nature of the polymers themselves.

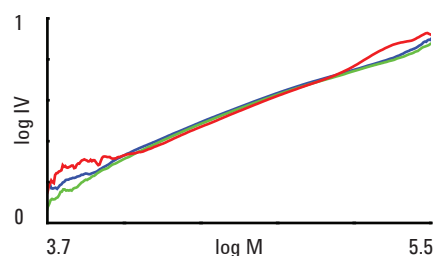


Figure 3. Overlaid Mark-Houwink plots for three polybutadiene samples

Conclusion

Triple detection GPC revealed that samples of polybutadiene were structurally similar and that differences in molecular weight resulted from the synthesis method rather than any changes to the nature of the polymers.

The triple detection 390-MDS when combined with the use of PLgel columns provides a powerful system for the characterization of polybutadienes.

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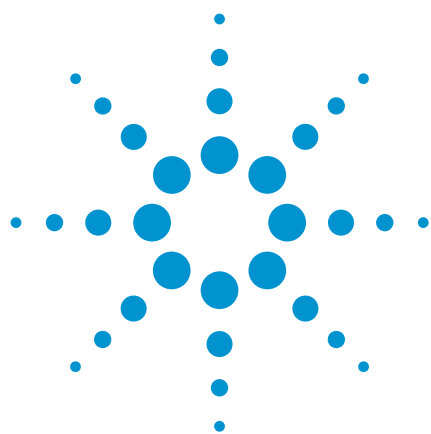
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Agilent Technologies



Analysis of Polyvinyl Butyral by GPC Triple Detection with the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Polyvinyl butyral (PVB) is a resin prepared from polyvinyl alcohol by reaction with butyraldehyde. PVB is an important material that has a wide range of uses in applications that require strong binding, optical clarity, excellent surface adhesion, toughness and flexibility. The most well-known application of PVB is in laminated safety glass, for example, in the windscreens of vehicles. In such products, PVB is used as an interface layer between two sheets of toughened glass. The interface layer absorbs the energy of impacts, binding broken shards of glass together and distributing the force of the impact over a wide area. The use of laminated glass has greatly reduced injuries resulting from automobile accidents. As it is the viscoelastic properties of PVB that contribute to its performance in glass laminates, the molecular weight distribution of the material must be closely monitored to ensure optimum performance.

Gel permeation chromatography (GPC) with triple detection, employing a light scattering detector and viscometer, may be used to determine accurate molecular weights for polymers such as PVB. Three PVB materials were analyzed using these techniques, one of which did not perform as expected in application testing.



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Methods and Materials

Conditions

Samples:	Polyvinyl butyrals
Columns:	2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	100 μ L
Eluent:	THF (stabilized)
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Agilent 390 Dual angle light scattering, Viscometer and DRI options
Detector Temp:	All detectors set at 40 °C

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for a sample of PVB. All of the samples exhibited strong signals that were fairly broad in all the detectors, indicating the high polydispersity of the materials.

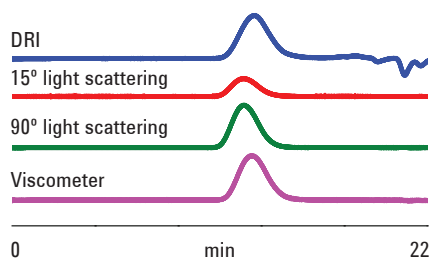


Figure 1. Overlaid multi-detector chromatogram for an example of polyvinyl butyral

Figure 2 is an overlay of the molecular weight distributions of three PVBs. Two of the samples displayed similar molecular weights with only slight differences across the distributions. However, the third sample showed an appreciable difference in molecular weight distribution, especially in the high molecular weight region where there was less material present than the other samples. This change was thought to be responsible for the change in the performance of the material in comparison to the other polymers.

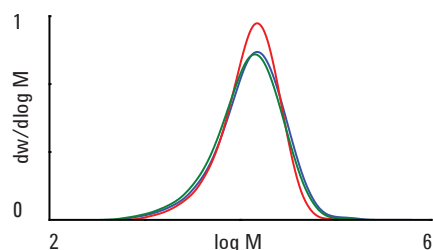


Figure 2. Overlaid triple detector molecular weight distributions of three polyvinyl butyrals

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight. All of the samples had a similar relationship between increasing molecular weight and increasing intrinsic viscosity, indicating that the PVBs were of a comparable structure. Therefore, the difference in molecular weight observed was as a result of the synthesis methods rather than any changes to the nature of the polymers themselves.

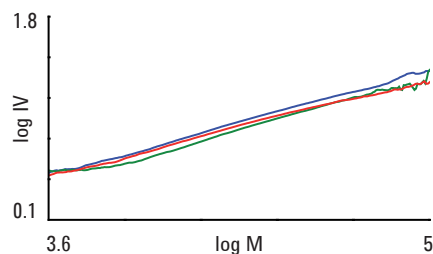


Figure 3. Overlaid Mark-Houwink plots for three samples of polyvinyl butyral

Conclusion

GPC is a well-known technique for assessing polymer molecular weight distribution, a parameter that influences many of their physical properties such as the toughness of polyvinyl butyral interface layers in laminated glass. The 390-MDS is designed for such applications using organic solvents. The 390-MDS can be linked with any liquid chromatography system to provide a powerful tool for GPC.

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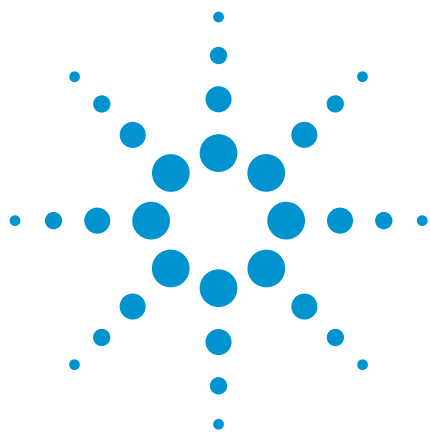
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Agilent Technologies



Analysis of Polystyrene Stars by GPC Viscometry with the Agilent 390-MDS Multi Detector Suite

Application Note

Author

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Agilent Technologies, Inc.

Introduction

There has been increasing interest in the synthesis of star-branched polymers due to their unusual flow and viscosity properties compared to linear analogs. Star-branched polymers are constructed with several 'arms' radiating from a central core, either by preparing the individual arms and attaching them to a central molecule - the 'arms first' approach - or by growing the polymer arms from a central core - the 'core first' approach. Many commercial polymers can be constructed with a star-branched morphology relatively easily, but their characterization is still a challenge to the analytical chemist. Gel permeation chromatography (GPC) employing a concentration detector (typically a refractive index detector) combined with a viscometer can be used to measure not only the molecular weight of the materials but also to investigate the star-branched structure. The 390-MDS is ideal for this type of application since it was specifically designed to extract additional information from polymers by GPC.

A series of PS star-branched polymers was analyzed, which had been synthesized by a 'core first' approach giving theoretical 5-, 14- and 21-arm structures.



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Materials and Methods

Conditions

Samples: Polystyrene star-branched polymers
Columns: 2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm
(part number PL1110-6500)
Eluent: THF
Flow Rate: 1.0 mL/min
Temperature: 40 °C
Detector Train: 390-MDS incorporating Viscometer and DRI
Detector Temp: All detectors set at 40 °C

Results and Discussion

Figure 1 shows the dual-detection chromatogram of the 14-arm polymer.

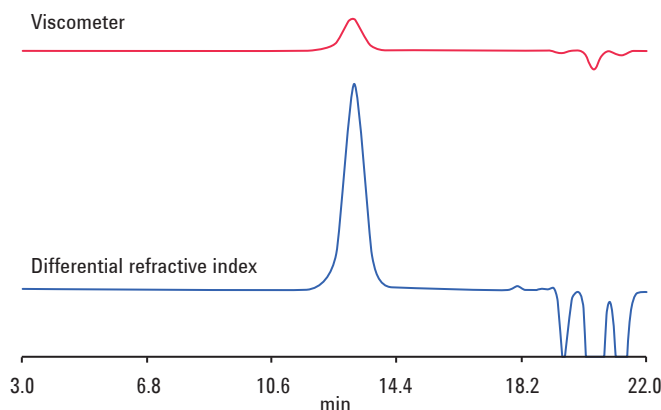


Figure 1. Viscometer and refractive index detection of a 14-arm, star-branched polystyrene

The universal calibration approach was used to calculate the molecular weight averages for the star-branched polymers. The universal calibration curve was generated using linear PS standards with narrow polydispersity and is shown in Figure 2.

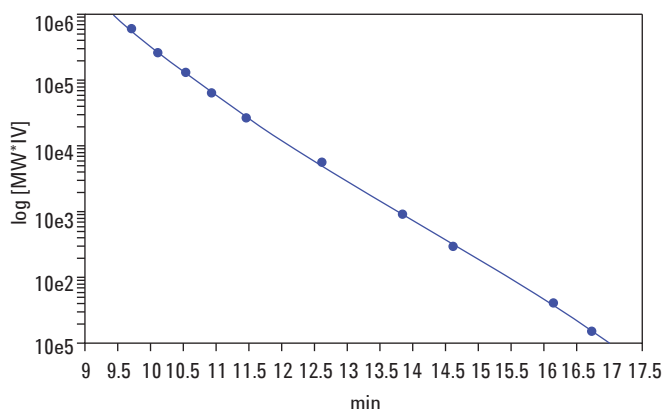


Figure 2. Universal calibration curve using polystyrene standards

Based on this calibration, the molecular weight averages and weight average intrinsic viscosity (IV_w) calculated for the PS star-branched polymers are given in Table 1.

Table 1. Molecular weight averages and weight average viscosities for star-branched polymers

Sample	Molecular Weight Average (g.mol ⁻¹)						PD	IV _w
	Mp	Mn	Mw	Mz	Mz+1	Mv		
5-arm	56,120	10,460	64,856	98,594	134,877	46,292	6.20	0.28
14-arm	27,436	26,812	29,310	32,425	36,542	28,687	1.10	0.10
21-arm	149,752	111,377	157,884	201,225	256,977	141,293	1.42	0.21

Mark-Houwink plots of log intrinsic viscosity as a function of log molecular weight were calculated for the PS star-branched polymers and for a broad PS material that was known to contain no branching. Figure 3 shows an overlay of the Mark-Houwink plots obtained, indicating that increasing the number of arms on the star-branched PS resulted in a decrease in intrinsic viscosity at any given molecular weight when compared to the broad linear PS. However, for the 21-arm star-branched polymer, the change in intrinsic viscosity relative to the linear PS varied strongly with the molecular weight.

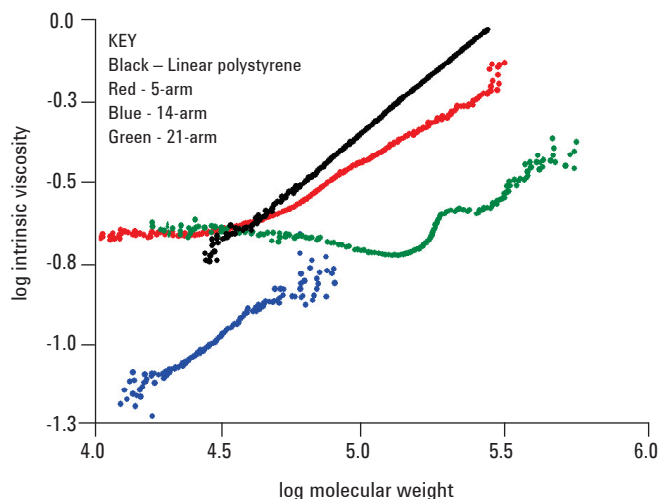


Figure 3. Overlaid Mark-Houwink plots for different star-branched polymers

Based on the linear regions in the Mark-Houwink plots for the stars, the intrinsic contraction factor g' was calculated as a function of molecular weight using Equation 1.

$$g' = \frac{[\eta]_{\text{star}}}{[\eta]_{\text{linear}}} \quad \text{Equation 1}$$

From the g' data, the radius of gyration contraction factor g was calculated using the empirical relationship in Equation 2

$$g' = [a + (1-a)g^p]g^b \quad \text{Equation 2}$$

where $a = 1.104$, $p = 7$, and $b = 0.906$ (Weissmüller *et al.* 1997).

Figure 4 shows an overlay of R_g contraction g plots for 5-, 14- and 21-arm star-branched polymers. Using the calculated g values and the functionality f for the stars, the theoretical number of arms was calculated using the model in Equation 3, based on an assumption that the arms were 'random', that is, polydisperse in molecular weight (Burchard 1977, 1983).

$$g = \frac{3f}{(f+1)^2} \quad \text{Equation 3}$$

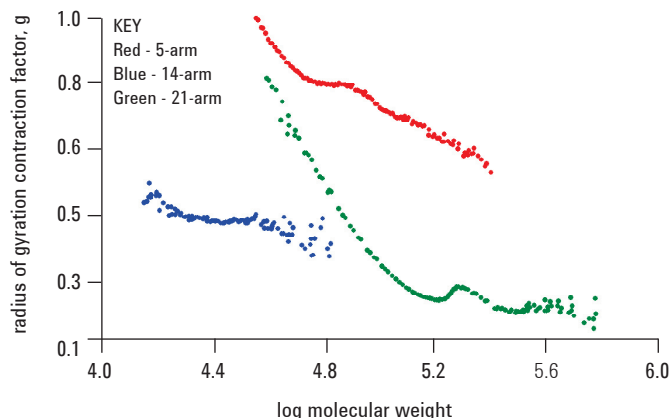


Figure 4. Overlay of R_g contraction g plots for different star-branched polymers

Figure 5 shows an overlay of f for the stars as a function of the log molecular weight. The random model gave a prediction of f , the number of arms, which was in good agreement with the value expected from the synthesis. However, for all of the star-branched polymers, especially the 21-arm PS, the calculated value of f increased sharply with molecular weight, indicating that a considerable portion of the sample at low molecular weight contained components with fewer than expected arms.

The variation in the functionality f with molecular weight provided valuable insight into the mechanism of the 'core first' approach used to synthesize the materials.

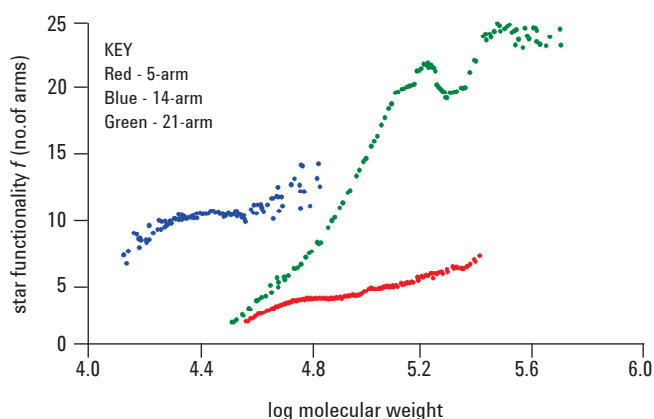


Figure 5. Functionality f plots for the different star-branched polymers

Conclusion

The results show that GPC employing refractive index and viscometry detectors in the 390-MDS can be used to investigate the structure of star-branched polymers, and provide valuable information on synthesis mechanisms used during their manufacture.

References

Burchard, W. (1977) Particle scattering factors of some branched polymers. *Macromolecules*, 10, 919-927.

Burchard, W. (1983) Static and dynamic light scattering from branched polymers and biopolymers. *Adv. Polym. Sci.*, 48, 1-124.

Weissmüller, M. and Burchard, W. (1997) Molar mass distributions of end-linked polystyrene star macromolecules. *Polymer Internat.*, 44, 380-390.

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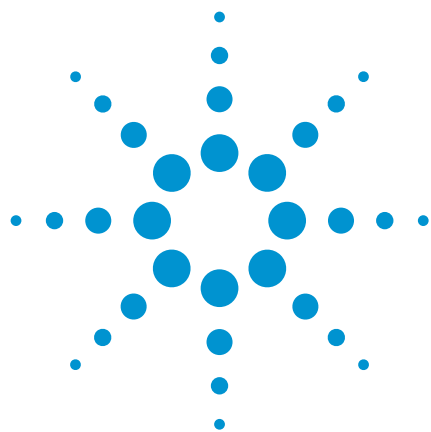
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Agilent Technologies



Analysis of Modified Polyvinyl Alcohol by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polyvinyl alcohol (often abbreviated as PVOH) is a water-soluble synthetic material used as an emulsifier and adhesive. PVOH can be modified by reaction of pendant –OH groups with various reagents to form new polymers with novel surfactant properties.

Gel permeation chromatography (GPC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many of their physical characteristics. GPC viscometry employing a viscometer in combination with a differential refractive index detector may be used to determine accurate molecular weights for polymers based on the universal calibration approach, useful for materials for which narrow standards are not available. GPC viscometry can also be used to investigate the solution behavior of modified polymers, a useful tool in the analysis of surfactant materials. This application note describes the analysis of two PVOH materials by GPC viscometry – one pure PVOH, and one that had been modified by reaction with imidazole. This second material was insoluble in water so for comparison both materials were analyzed in a polar organic solvent, dimethyl sulfoxide.



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Methods and Materials

Conditions

Sample:	Modified polyvinyl alcohols
Columns:	2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	200 μ L
Eluent:	Dimethyl sulfoxide + 0.1% LiBr
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Viscometer and DRI
Detector Temp:	All detectors set at 60 °C

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for the PVOH sample. The material produced fairly broad, strong signals in all the detectors, indicating the high polydispersity of the polymer.

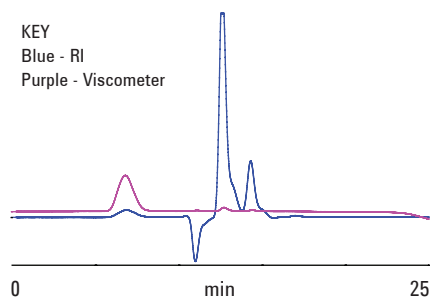


Figure 1. Overlaid multi-detector chromatogram for an example of polyvinyl alcohol

Figure 2 shows an overlay of the molecular weight distributions of the two samples under investigation. As can be seen, the PVOH samples showed an appreciable difference in molecular weight distribution, especially at high molecular weight.

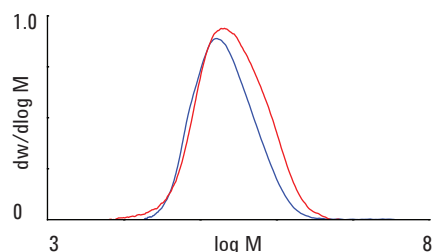


Figure 2. Overlaid multi-detector molecular weight distributions of two samples of polyvinyl alcohol

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight. The two samples gave a very different relationship between increasing molecular weight and increasing intrinsic viscosity, indicating that the polymers were of differing sizes in solution. This resulted from the differing degrees of solvation in DMSO due to the modification of the PVOH backbone. This analysis shows that the surfactant properties of the PVOH had been significantly altered by grafting of imidazole.

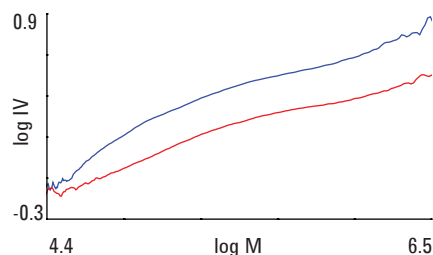


Figure 3. Overlaid Mark-Houwink plots for two samples of polyvinyl alcohol

Conclusion

The results show that the 390-MDS, combined with two PLgel 5 μ m MIXED-C columns, can be used to investigate the structural and solution properties of polymers, greatly increasing the potential of GPC over using a single detector alone.

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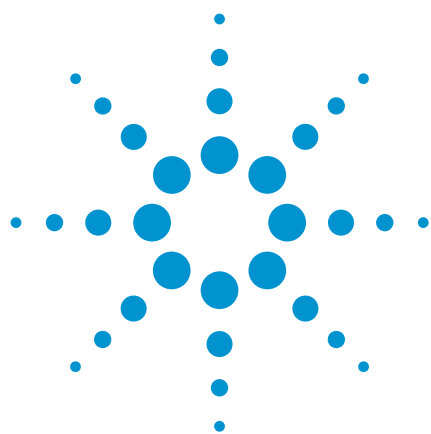
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Agilent Technologies



Analysis of Bromostyrene by GPC Triple Detection using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

The structure of many common types of polymer may be modified by either post-polymerization reaction or by the use of modified monomers, producing materials with new and novel properties. Understanding and assessing the effect of these modified structures on the behavior of polymers in various applications is crucial to developing novel uses for such materials. Polystyrene is one of the most commonly seen polymers, used primarily for the manufacture of packing materials. Modified forms of polystyrene may be produced by the use of novel styrene monomers.

Gel permeation chromatography (GPC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many physical characteristics. Importantly, increasing the sophistication of the GPC experiment by the use of additional detectors allows the structure and solution properties of polymers to be assessed, of interest when studying the affect of structural modifications on a polymer. Triple detection employing a light scattering detector and a viscometer in combination with a refractive index detector may be used to determine accurate molecular weights for polymers for which narrow standards are not available, and to assess their solvation properties. This application note describes the analysis of a sample of polystyrene and a modified polybromostyrene material by triple detection.



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Methods and Materials

Conditions

Samples:	Polystyrene and polybromostyrene
Columns:	2 x Agilent PLgel 5 μ m MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	100 μ L
Eluent:	THF (stabilized)
Flow Rate:	Agilent 290-LC PIM at 1.0 mL/min
Detector Train:	390-MDS incorporating Agilent 390 Dual angle light scattering, Viscometer and DRI options
Detector Temp:	All detectors set at 40 °C

Polystyrene and polybromostyrene are both soluble in tetrahydrofuran, which is an excellent solvent for GPC and was therefore chosen for this analysis. The 390-MDS was chosen as part of the system in its powerful triple detection set up, as this would allow the most detailed analysis of the polymer samples.

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for the sample of polybromostyrene. Each of the polymers gave strong signals in all the detectors that were fairly broad, indicating the high polydispersity of the materials.

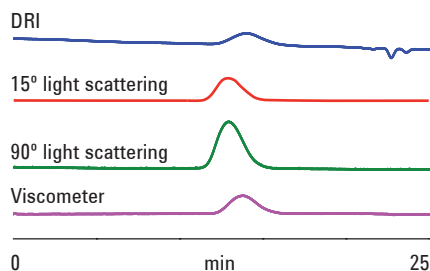


Figure 1. Overlaid multi-detector chromatogram for an example of polybromostyrene

Figure 2 shows an overlay of the molecular weight distributions of the two samples under investigation. As can be seen, the polybromostyrene was considerably higher in molecular weight than the polystyrene sample.

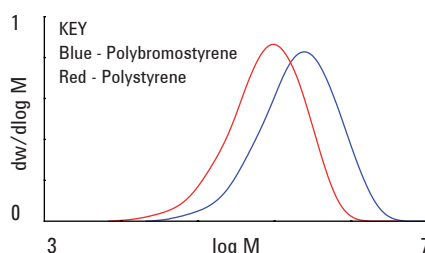


Figure 2. Overlaid triple detector molecular weight distributions of the two samples

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight for the two samples. Compared to the polystyrene, the polybromostyrene shows a marked shift of the Mark-Houwink plot to lower intrinsic viscosity values at any given molecular weight. This indicates that polybromostyrene is smaller in solution than polystyrene across the molecular weight range, a result of changes in the level of interaction between the polymer coils and the solvent. The plot parallels that of polystyrene, indicating that the structure is identical across the range of molecular weight, as expected when a modified monomer unit is used to create the new polymer.

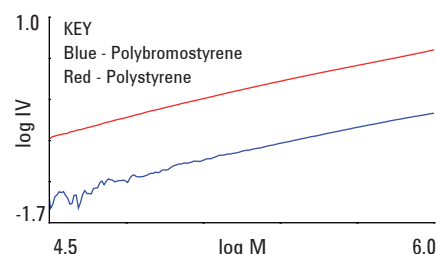


Figure 3. Overlaid Mark-Houwink plots for two samples

Conclusion

The data in this application note illustrate how multi-detector GPC employing the 390-MDS can be used to clearly see structural differences between polystyrene and a related polymer with a modified structure.

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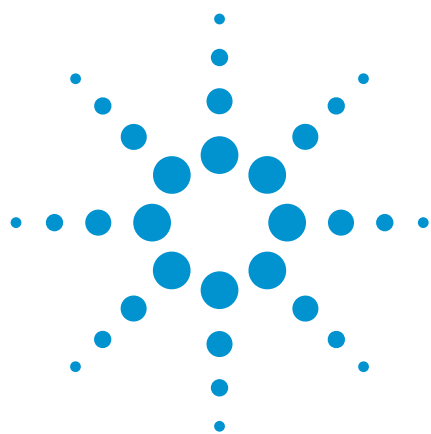
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Agilent Technologies



Analysis of Epoxy Resins by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Epoxy resins are complex materials manufactured by the reaction of a 'resin' with a 'hardener'. This produces a cross-linked polymer that is extremely strong, tough and may be used to bond materials together. The resin component of the mixture is an epoxide pre-polymer, typically produced by the reaction of an epoxide with bisphenol-A. The resulting short-chain material contains pendant $-OH$ and epoxide functionalities and is polar in nature. The molecular weight of the epoxide chains in the resin is important as this influences the curing rate of the final epoxy resin formulation when mixed with the hardener, however, accurate molecular weights are difficult to obtain due to the complex, inhomogeneous nature of the epoxide polymer chains.

Gel permeation chromatography (GPC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many physical characteristics such as the curing time of epoxy resins. GPC viscometry employing a viscometer in combination with a differential refractive index detector has the advantage of allowing the determination of accurate molecular weights for structurally complex polymers and co-polymers via the Universal Calibration approach. Using this methodology, the same molecular weights are determined for samples regardless of the standards used to create the calibration. This application note describes the analysis of two samples of epoxide pre-polymers by GPC viscometry.



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Methods and Materials

Conditions

Samples:	Epoxide pre-polymers
Columns:	2 x Agilent PolarGel-L, 300 x 7.5 mm (p/n PL1117-6830)
Injection Volume:	200 μ L
Eluent:	Dimethyl formamide + 0.1% LiBr
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Viscometer and DRI
Detector Temp:	All detectors set at 60 °C

Epoxide pre-polymers are polar in nature and can be run in polar organic solvents such as dimethyl formamide using suitable columns such as a set of PolarGel-L columns. The 390-MDS was chosen as part of the system as it is capable of multi-detector GPC in polar solvents.

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for the sample of one of the epoxide pre-polymers. The appearance of oligomers resolved by the PolarGel-L columns is clearly apparent.

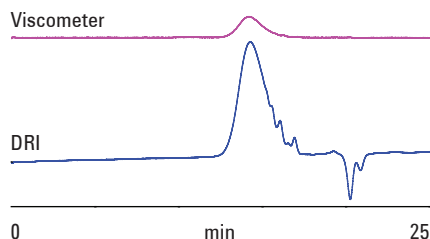


Figure 1. Overlaid multi-detector chromatogram for an example epoxide pre-polymer

Figure 2 shows an overlay of the accurate molecular weight distributions of the two samples under investigation. As can be seen, they have very different distributions indicating that the materials are two different grades of epoxide pre-polymer that will display differing hardening rates when mixed with the hardener component of the epoxy resin mixture.

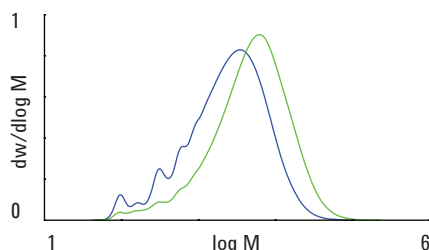


Figure 2. Overlaid multi detector molecular weight distributions of two samples of epoxide pre-polymer

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of log molecular weight for the two samples. Both grades of epoxide pre-polymer display a similar relationship only showing deviation at high molecular weight. This would indicate that the molecular dimensions of the two materials are approximately the same at low molecular weight, however, as the molecular weight increases there is a structural disparity between the two materials, most likely due to a change in the chemistry of the samples.

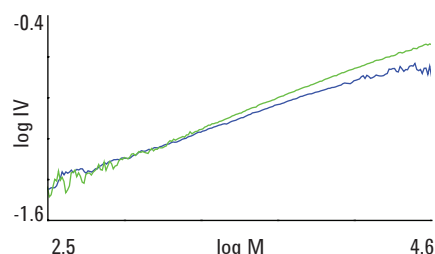


Figure 3. Overlaid Mark-Houwink plots for the two samples of epoxide pre-polymer

Conclusion

The structure of some epoxide pre-polymers was determined using gel permeation chromatography with the Varian 390-MDS Multi Detector Suite. Mark-Houwink plots provided by the 390-MDS revealed differences in the chemistry of the resins, demonstrating the effectiveness of the instrument when investigating the molecular weight and structural properties of polymers.

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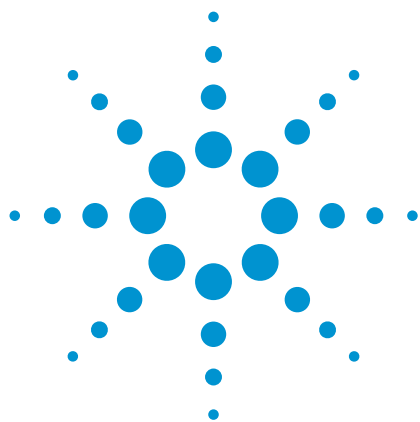
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SI-02387



Agilent Technologies



Separation of Antioxidants and UV Stabilizers Used in Plastics

Application Note

Chemical

Introduction

Commercial plastics often contain small quantities of UV stabilizers and antioxidants, introduced to alter the physical properties of the finished product and protect it from environmental degradation. Accurately controlling the inclusion of these additives in the manufacturing process is vital to ensure the quality of the finished plastic. The analysis of plastic additives in different batches of material forms an integral part of quality control protocols.

This application note describes the HPLC analysis of 19 commercially available antioxidants and UV stabilizers on an Agilent Pursuit column.



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Conditions

Sample	Standard mix
Sample solvent	THF
Column	Agilent Pursuit C18, 2.0 × 150 mm, 5 µm (p/n A3000150X020)
Mobile phases	A: 0.001 M methane sulfonic acid B: Acetonitrile
Gradient	0 min 60% B, 2 min 60% B, 18 min 100% B, 35 min 100% B
Flow rate	0.3 mL/min
Temperature	40 °C
Injection volume	10 µL
Detector	UV, 230 nm

Results and Discussion

The chromatogram in Figure 1 shows the separation of 19 commercially available UV stabilizers and antioxidants in about 30 minutes.

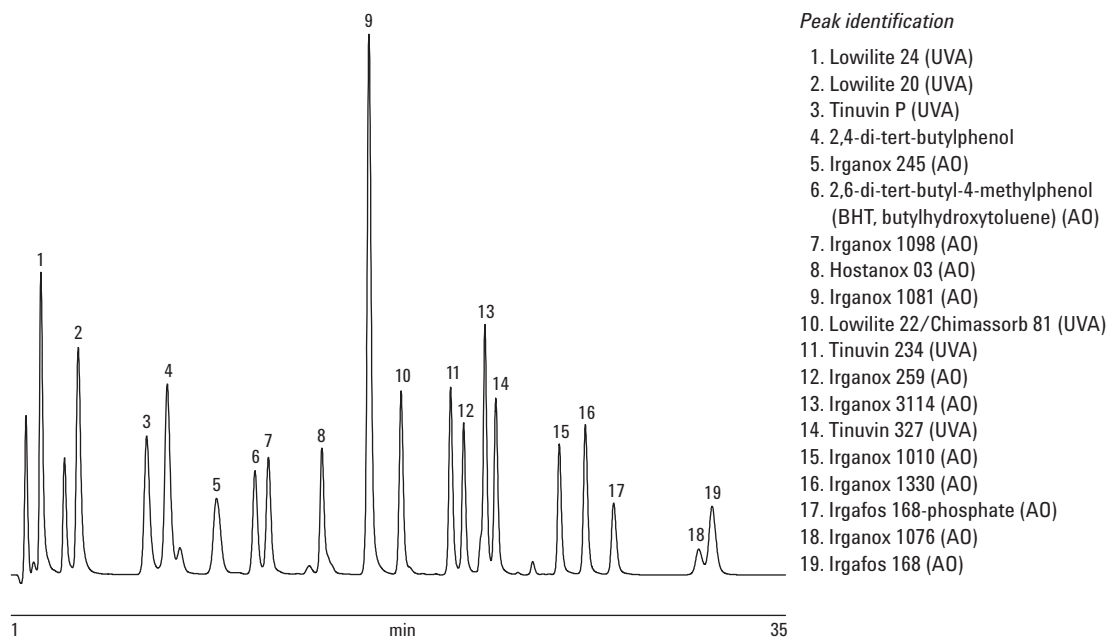


Figure 1. Nineteen UV stabilizers (UVA) and antioxidants (AO) separated by an Agilent Pursuit HPLC column.

Conclusion

A range of plastics additives was successfully separated using HPLC with an Agilent Pursuit column. The ultrahigh silica purity, extensive end-capping, and high bonded phase density of the Pursuit C18 are key to its performance in reversed-phase separations.

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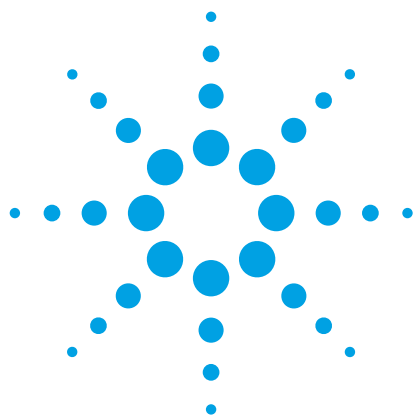
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Analysis of Polymer Antioxidant Additives on the Agilent 500 Ion Trap LC/MS

Application Note

Materials Testing and Research

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Abstract

This note demonstrates the quantitative analysis of three commonly used antioxidant polymer additives, namely butylated hydroxyl anisole (BHA), Ethanox 330, and Irganox 1010 using the Agilent 500 Ion Trap LC/MS. This instrument provides excellent MS/MS and full scan sensitivity.

Introduction

Plastics are widely used and they vary in their application, ranging from automobile parts, components for houses and buildings, and packaging for everything from food to electronic parts. The diverse applications of plastics are credited to the incorporation of additives. These additives improve the performance characteristics of the polymer resins.

As the structure of polymers has become more and more complex, there has been an increasing need for reliable analysis of additives to meet more exacting performance demands. Accurate and precise analytical methods are required for the manufacture of high quality products. The analytical needs for additives analysis are qualitative identification, screening for potential contaminants (non-target analysis), and reliable, accurate quantitative determination of additive concentration in a complex matrix (typically down to 0.1 wt% or less in the plastic material). A considerable analytical challenge is the ability to provide all of this information in a single analytical run.

An ion trap mass spectrometer is well suited for this analytical challenge, as it provides excellent full scan and high mass sensitivity along with true MS/MS capability. Both target additives and non-target contaminants can be reliably detected and quantitated in a complex matrix. This application note uses the Agilent 500 Ion Trap LC/MS to demonstrate the quantitative analysis of three commonly used antioxidant polymer additives, namely butylated hydroxyl anisole (BHA), Ethanox 330, and Irganox 1010. Figure 1 shows the chemical structures for these common additives.



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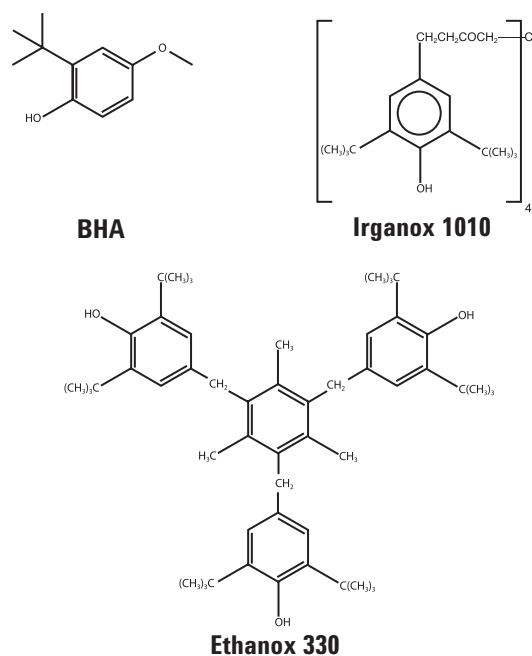


Figure 1. Structure of studied antioxidant additives.

Instrumentation

- Agilent 500 Ion Trap LC/MS, equipped with an APCI source and built-in syringe pump.
- Agilent ProStar 210 Binary Solvent Delivery Modules
- Agilent ProStar 430 AutoSampler

Materials and Reagents

BHA (B1253) was purchased from Sigma-Aldrich, Milwaukee, WI. Ethanox 330 (PLAS-CAL-002-3) was purchased from AccuStandard, New Haven, Connecticut and Irganox 1010 was provided by Ciba Specialty Chemicals, Tarry Town, New York. Methanol, dichloromethane and isopropanol are HPLC grade and provided by Fisher Scientific Co, Fair Lawn, NJ.

Sample Preparation

A stock solution of BHA was prepared in methanol and dilutions for the calibration curve were made in methanol. Ethanox 330 was supplied in isopropanol and the dilutions for the calibration curve were made in isopropanol. The stock solution of Irganox 1010 was made in dichloromethane and it was further diluted in methanol for calibration. The concentration ranges prepared were typically from 5–800 pg/ μ L.

HPLC Conditions

Column	Agilent Microsorb-MV, C8, 4.6 $\times$ 50mm, 3.5 μ m Agilent p/n R0086300F3			
Solvent A	Water			
Solvent B	Methanol			
LC program	Time (min:sec)	%A	%B	Flow (mL/min)
	00:00	40	60	1
	10:00	0	100	1
	15:00	0	100	1
	15:01	40	60	1
	20:00	40	60	1
Injection Volume	5 μ L			

MS Parameters

Ionization mode	APCI
Ion polarity	Negative
Trap damping gas	0.8 mL/min
Corona current	–10 μ A
API drying gas	14 psi at 400 $^{\circ}$ C
API nebulizing gas	Air at 60 psi
Shield voltage	–600 Volts

MS/MS Parameters

Analyte	Transition (m/z)	Amplitude Excitation (volts)	Scan range (m/z)
BHA	179.5 $\rightarrow$ 164.5	1.75	100–175
Ethanox 330	773.8 $\rightarrow$ 717.0	1.70	256–784
Irganox1010	1176 $\rightarrow$ 958	2.0	389–1000

Results and Discussion

The additive content of plastics needs to be monitored for quality and regulatory reasons. Some of the regulations with limits are food-contact plastic articles intended for repeated use (21 CFR 177.2600) and food contact packaging for irradiated food (21 CFR 179.45)

When it comes to identifying these antioxidants, it is obviously important to obtain spectra that verify the molecular weight and demonstrate that the starting material is free from degradation. The Agilent 500 Ion Trap LC/MS has excellent full scan sensitivity as demonstrated in Figure 2. Irganox 1010 has an $[M-H]^{-}$ ion at m/z 1175.9 and is very intense in the spectrum generated on the 500 Ion Trap.

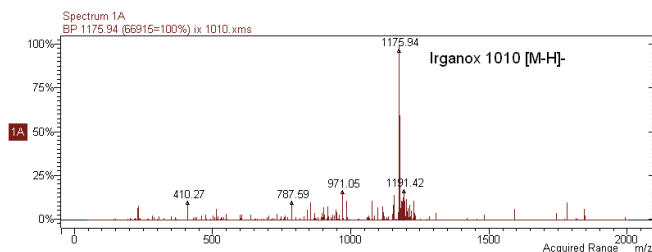


Figure 2. Irganox 1010 infused at 500 pg/μL on the Agilent 500 Ion Trap in full scan mode (50–2000 amu).

Figure 3 is an example of the type of product ion spectrum that can be obtained with the 500 Ion Trap, which is rich in detail and provides the user with structural information and confirmation of its identity in a complex matrix.

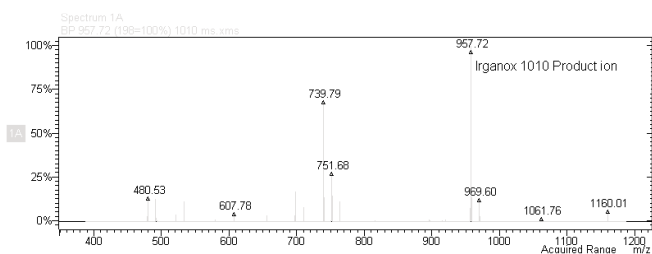


Figure 3. MS/MS product ion spectrum of Irganox 1010 on the 500 Ion Trap. 500 pg/μL infusion.

Although in-source CID can be used for generating fragments on single quadrupole instruments, one must be concerned with the quality of the spectra obtained, since the skimmer-CID is not truly selective against the matrix. The 500 Ion Trap provides true MS/MS, totally eliminating the effect of matrix and therefore adding increased confidence in the analytical results.

The MS/MS chromatograms of the three antioxidant additives studied are given in Figure 4. Many of these compounds have similar structures and co-elute on HPLC columns, thus increasing the need for selective MS/MS detection.

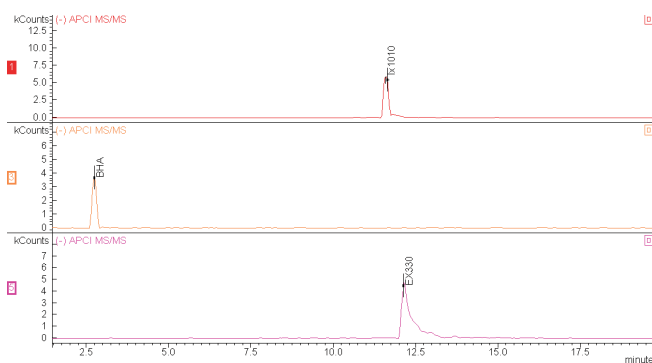


Figure 4. MS/MS chromatograms of Irganox 1010, BHA and Ethanox 330 on Microsorb-MV C8 column (200 pg/μL each component).

Calibration linearity is demonstrated in Figure 5 for BHA in MS/MS mode. The curve has an R^2 value of 0.998 and an RSD of 12.3%. LOQ values for pg quantities on column are shown in Table 1.

The antioxidant additive concentration incorporated in plastics is typically 0.1 % by weight (K. Figge and Freytag, Food Additives and Contaminants V.1, n 4, 1984) - quite high in concentration relative to the very low detection limits reported here (40 pg on column). This excellent sensitivity enables the user to have more flexibility in the sample preparation process by allowing the use of smaller sample size and/or the ability to dilute the sample prior to analysis. In addition, the superior full scan sensitivity of the 500 Ion Trap provides reliable information about potential impurities.

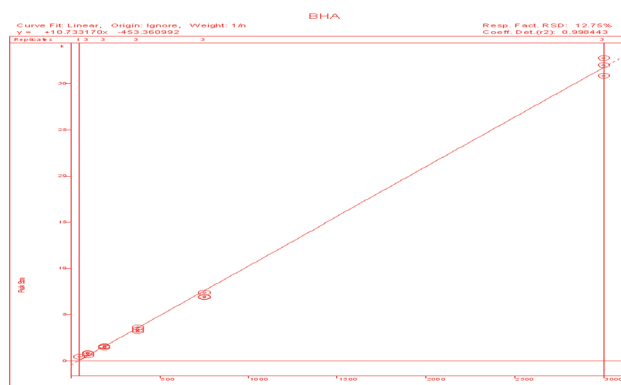


Figure 5. BHA Calibration curve (full scan MS/MS negative APCI) 40–3000 pg on column.

Table 1. LOQ for Antioxidant Additives

Compound	LOQ
BHA	100 pg
Ethanox	330 78 pg
Irganox 1010	250 pg

Conclusion

- The Agilent 500 Ion Trap LC/MS provides excellent full scan sensitivity and MS/MS making it an ideal tool for the screening and quantitative analysis of antioxidant polymer additives.
- Information rich MS/MS spectra eliminate false positives.
- Low limits of quantitation and excellent linearity have been demonstrated.

References

1. K. Figge and W. Freytag, Additive migration from various plastics with different processing or properties into test fat HB 307., Food Additives and Contaminants v.1, n.4, 1984
2. 21 CFR 177.2600
3. 21 CFR 179.45

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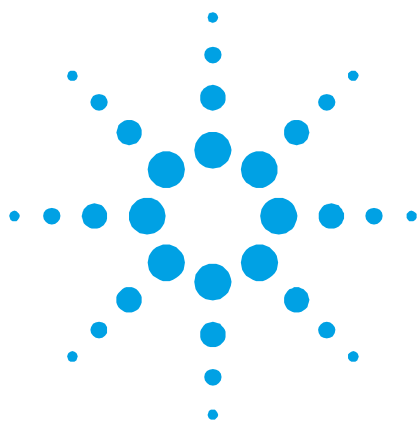
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Quantitative analysis of tint in polymer pellets and disks

Application Note

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Introduction

The natural color (usually yellow) of many polymers such as acrylic, polycarbonate, and styrene–acrylonitrile (SAN) is unattractive for commercial purposes. Therefore, manufacturers add tint, or a dye to the polymer to mask the natural color. The final color and clarity of the polymer can determine the commercial value of the final product.

Traditional measurements of tint concentration require molding polymer pellets into disks. The color of the disks is then measured in a spectrophotometer or colorimeter. The time required to melt a representative sample of pellets, mold the melt into a disk, followed by a cooling down period can be one to two hours.

A fast and convenient method for quantitating the amount of tint directly in polymer pellets and molded disks is described.

This method could prevent the production of large amounts of incorrectly dyed polymer by detecting any problems quickly, allowing corrective action to be taken.



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Equipment

- DMS 300 UV–Visible Spectrophotometer
- Diffuse Reflectance Accessory (Integrating Sphere)
- Sekonic Printer/Plotter
- Concentration Application Module

Procedure

Note: Because of the nature of the polymer samples described in this paper, the name of the manufacturer, the polymer, the tint and wavelengths used, must remain proprietary.

Note: This method can also be performed with a Cary 1,3,4 or 5 instrument, fitted with a Diffuse Reflectance accessory and a Concentration application.

A Diffuse Reflectance Accessory (DRA) was used to measure both pellets and molded disks. The DRA consists of a 73 mm diameter integrating sphere with a built-in photomultiplier detector. The sphere's internal surface is coated with a white diffusing material - barium sulfate. The sphere can measure either diffuse reflectance (Figure 1A) or scattered transmittance (Figure 1B) where I_R and I_S represent reflected and scattered radiation, respectively. The sphere was chosen for this work because of its ability to collect a high percentage of the light scattered by the polymer pellets. The samples themselves were translucent, irregular-shaped spheroids of approximately 5 mm in diameter.

As single pellets were too small for representative absorbance measurements, a 1 cm square cuvette was chosen to hold a volume of pellets. Due to their irregular shape and size the pellets did not pack perfectly. Therefore, scattering of the incident radiation was observed when light from a spectrophotometer was passed through a cuvette of polymer pellets.

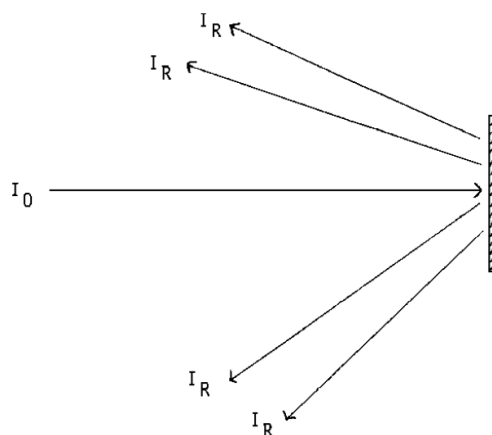


Figure 1A. Reflection off a sample surface.

I_R represents the reflected radiation,
 I_0 represents the incident beam

This effect is illustrated in Figure 1B where I_0 is the incident radiation and is the scattered radiation. In a normal spectrophotometer arrangement the detector may be some distance from the cuvette. The light collection efficiency of such a system is thus very low. When the detector is much closer to the scattering matrix a much greater proportion of scattered radiation is collected. The integrating sphere acts as a highly efficient collector of scattered radiation. The sphere can be used in this manner if the cuvette containing the pellets is located so as to be part of the wall of the sphere as in Figure 2A. Therefore, all of the scattered radiation which passes through the rear face of the cuvette is collected by the sphere. Figure 2B illustrates the relationship between collection efficiency and distance between the sample and detector.

Once the integrating sphere is installed it can also be used to measure the absorbance of molded polymer disks. Molded disks are approximately 120 mm in diameter and 1/8" thick. The molded disks were measured by placing two disks back-to-back followed by a white reflectance disk. Light was passed through the two molded disks, reflected off the white disk, and passed back through the two molded disks into the sphere where it was detected. See Figure 3A.

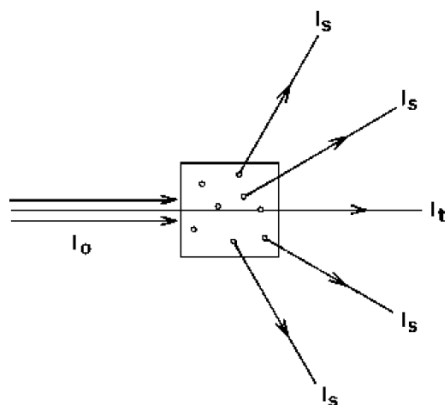


Figure 1B. Scattered transmittance of light through a turbid or opaque sample

I_o represents the incident radiation

I_s represents the scattered transmittance

I_t represents the transmitted light which has not been scattered

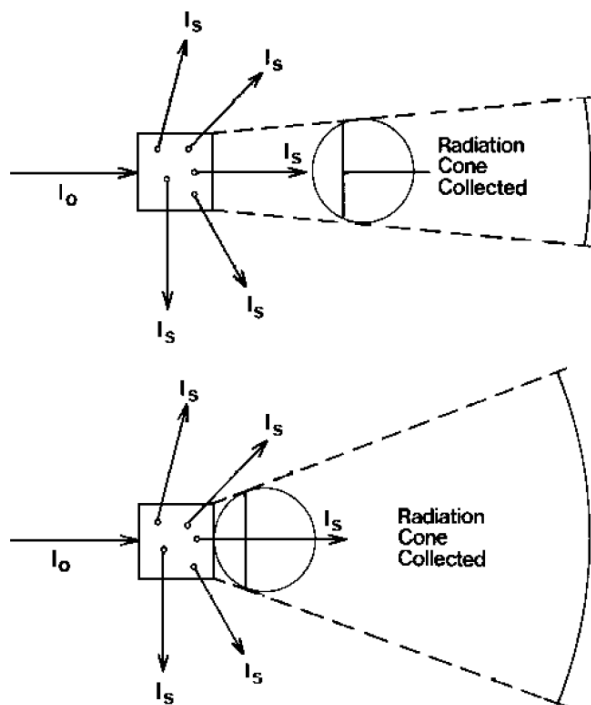


Figure 2A. The light collection efficiency is increased by placing the sample so as to be part of the wall of the sphere

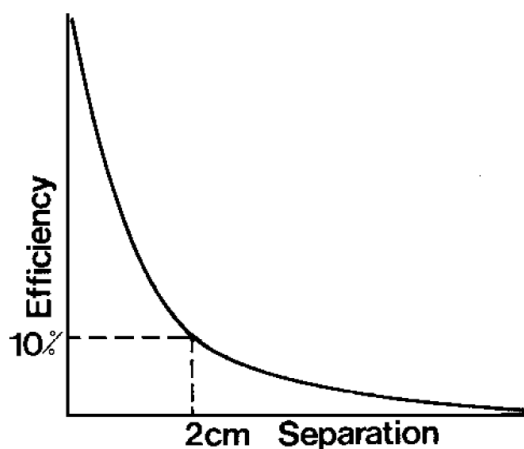


Figure 2B. The relationship between collection efficiency and the separation between the sample and the detector

Quantitative calculations were determined using a Concentration Application Module. The application module plugs into the back of the instrument and contains software for performing quantitative calculations. Access and interaction with the module is performed by following softkey instructions on the CRT of the instrument. Pellet and disk methods can be setup and stored in the module in non-volatile memory. Once a method is set up it can be recalled and executed automatically.

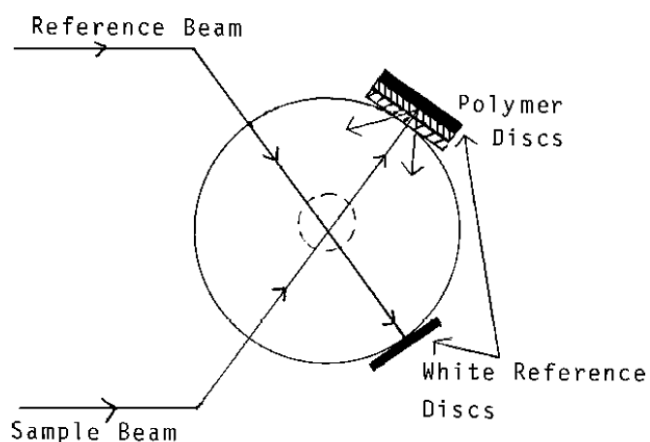


Figure 3A. The sphere configuration for the measurement of the molded polymer discs

Pellet analysis

The pellets were packed into a 1 cm square cuvette and placed in the light path before the sphere. See Figure 3B.

The concentration of tint in the polymer pellets was calculated by measuring the absorbance of the tint at its peak wavelength and a secondary wavelength. These wavelengths were chosen by utilizing a scanning UV-Visible spectrophotometer. The peak wavelength was determined by the spectrophotometer by scanning a sample of moderate concentration (ie. a sample containing enough tint to exhibit a well defined peak). A secondary wavelength was required because of the varying degrees of scattered radiation (or background) from sample to sample. The secondary wavelength was chosen from a series of scans as the wavelength where absorbance was independent of concentration of tint.

Since each pellet-filled cuvette will pack slightly differently, causing varying degrees of background scatter, the net sample absorbance was determined by subtracting the absorbance at the secondary wavelength from the absorbance at the peak wavelength. The sample net absorbance was then compared against a predetermined calibration curve stored in the Concentration Module. The Concentration Module automatically slews to each wavelength, performs the background correction, determines and then prints the final concentration. Five replicates on each sample can be determined in less than 3 minutes.

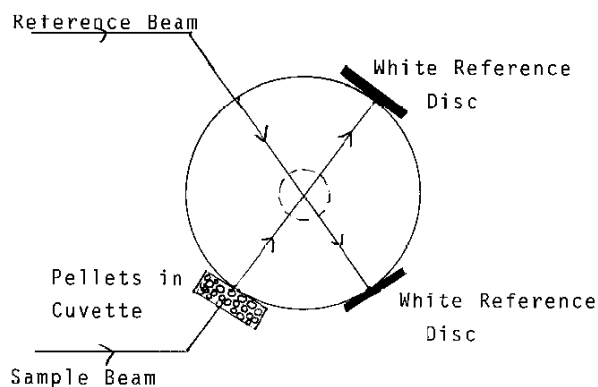


Figure 3B. The sphere configuration for the measurement of the polymer pellets

A calibration graph was determined by measuring, as described above, a series of polymer samples (or standards) of known dye content. Each standard was measured five times. Each measurement represented a different volume of pellets. The Concentration Module calculated the average of the five replicates and the final result was stored in memory. Because of the varying degrees of pellet size and homogeneity, five replicates were chosen to provide the best representative sample. Once the average standard values were determined the Concentration Module calculated a linear least squares calibration graph for the number of standards chosen. The calibration was stored in the Concentration Module in non-volatile memory. Subsequent samples were then measured as outlined previously, and their corresponding concentrations calculated from the calibration graph.

Molded disk analysis

The concentration calculations and calibration graph for tint in molded disks were determined as described in the pellet analysis section. Since disks do not exhibit scattering effects only one replicate was required.

Discussion

This method provides a fast, accurate way to quantitatively determine the concentration of tint in polymer pellets and disks. The calibration graph for pellets is shown in Figure 4 with a printout of the standard data and statistics indicated in Table 1.

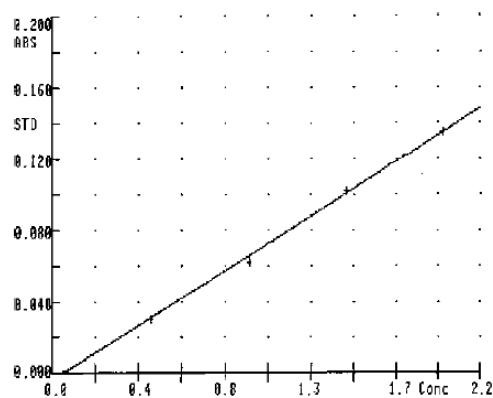


Figure 4. The calibration graph for the analysis of the dye content of the polymer pellets

Table 1. Standard data and statistics for the calibration graph in Figure 4

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	14.44	0.056	0.999	0.000 2.000
	ABS	CONC			
1	-0.002	0.000			
2	0.030	0.500			
3	0.042	1.000			
4	0.101	1.500			
5	0.135	2.000			

Each concentration value is listed in parts per million (ppm) along with corresponding absorbance values. The correlation coefficient (the 'goodness' of the fit of the standard points) of 0.999 is excellent. The results of the same polymer pellets randomly remeasured as samples are shown in Table 2. The theoretical concentrations for each sample are listed in the last column.

Table 2. The results of the pellet samples analysis

Samples	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	14.44	0.056	0.999	0.000 2.000
	ABS	CONC	Weight	Theoretical Conc	
1	0.135	2.006	1.000	2.0	
2	0.033	0.536	1.000	0.5	
3	0.098	1.476	1.000	1.5	
4	0.069	1.055	1.000	1.0	
5	-0.007	-0.039	1.000	0.0	

The calibration graph for molded disks is indicated by Figure 5 with the printout of standard data and statistics shown in Table 3. The correlation coefficient for the disks' standard graph was 0.993.

(The results of the polymer disks randomly measured as samples are shown in Table 4.) Theoretical values are listed in the last column.

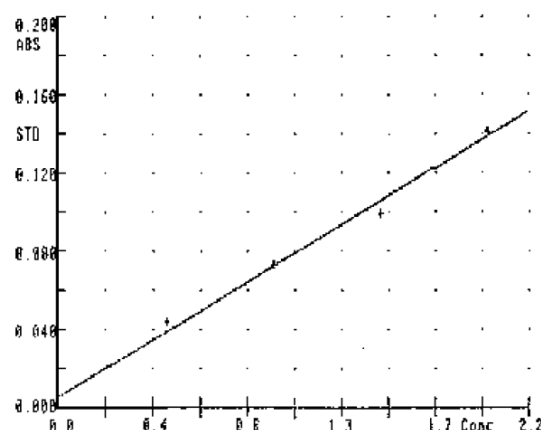


Figure 5. The calibration graph for the molded polymer discs

Table 3. Standard data and statistics for the calibration graph in Figure 5

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	15.03	-0.079	0.993	0.000 2.000
	ABS	CONC			
1	0.003	0.000			
2	0.044	0.500			
3	0.072	1.000			
4	0.099	1.500			
5	0.141	2.000			

Table 4. The results of the analysis of the molded polymer discs

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	15.03	-0.079	0.993	0.000 2.000
	ABS	CONC	Weight	Theoretical Conc	
1	0.094	1.301	0.000	1.5	
2	0.069	0.952	0.000	1.0	
3	0.142	2.056	0.000	2.0	
4	0.038	0.497	0.000	0.5	
5	0.004	-0.011	0.000	0.0	

Additional applications

Another application of this technique uses the ability to perform wavelength scans on pellets and disks. This application offers the capability to fingerprint the tint used by various competitive manufacturers. Figure 6 shows wavelength scans of various polymer samples with different tints. UV-Visible wavelength scans also allow screening for possible contaminants.

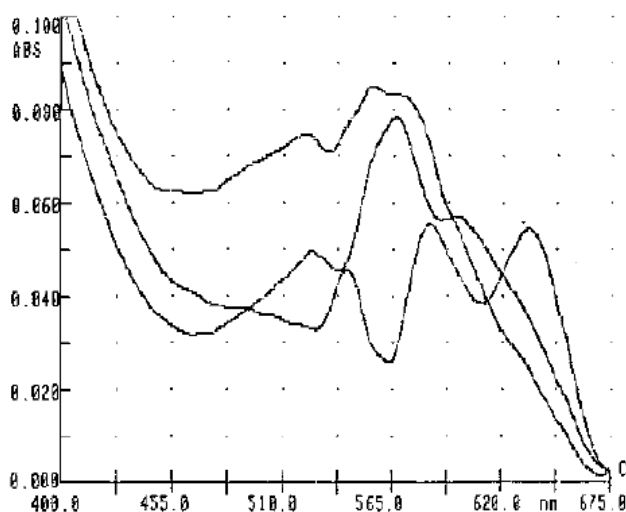


Figure 6. Wavelength scans of various polymer samples with different tints

Conclusion

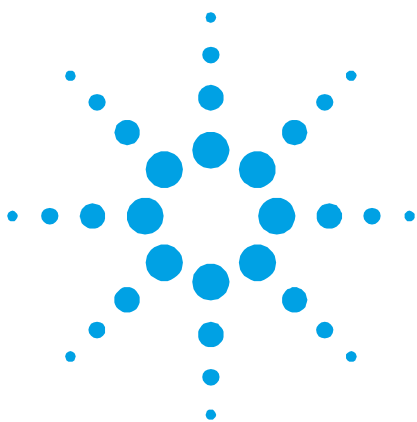
The DMS 300 in conjunction with the Diffuse Reflectance Accessory and Concentration Application Module performs an automated analysis of polymer tint in pellets and disks. The analysis of tint in a polymer pellet sample can be accomplished in a matter of a few minutes, versus one to two hours by traditional methods. Potentially this can avoid large wastage of polymer material.

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Agilent Technologies



The determination of thin film thickness using reflectance spectroscopy

Application Note

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Abstract

The reflectance spectrum of a coated polycarbonate sample was used to determine the film thickness of a polymeric coating. Absolute reflectance spectra were acquired over the range 400–800 nm using a Cary 5000 UV-Vis-NIR spectrophotometer and 'VW' absolute specular reflectance accessory. Based on the interference fringes observed, the thickness of the film was calculated to be 4.95 μm .

Introduction

Thin films, layers of one material deposited on another material, are significant in many high-technology industries. Thin films are used in a wide variety of applications including antireflection coatings, beam splitters, color filters, narrow bandpass filters, semi-transparent mirrors, heat control filters, high reflectivity mirrors, polarizers and reflection filters. The characterization of thin films is thus extremely important in many optics/photonics applications (semiconductor, micro-machining, defence, architectural glass and flat panel displays to name but a few), with parameters of interest including film thickness, refractive index, coating homogeneity and reflectivity.

The measurement of film thickness using reflected light is a well-established technique¹. Such optical techniques for the determination of thin film characteristics rely upon the interaction of the film with light, and can be used to determine not only thickness, but also roughness and optical constants. They are dependent upon the interference pattern (or fringes) resulting from partial reflection/transmission through two partially reflecting surfaces. This phenomenon was first observed over a century ago by Fabry and Perot² and, importantly, provides an investigative tool that is accurate, nondestructive, and requires little in the way of sample preparation.



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In this instance, an absolute specular reflectance measurement was used to determine the thickness of the coating on a polycarbonate substrate. The exact composition of the coating cannot be revealed, however it can be generally described as being polymeric in nature and having a refractive index of 1.51. This particular substrate/coating combination has application in the glass and automotive industries.

Theory

In the case of a thin film on the surface of another material, both the top and bottom surfaces of the film reflect light, with the total amount reflected being dependent upon the sum of these two reflections. Furthermore, these two reflections may add together constructively or destructively depending upon their phase relationship. This phenomenon is due to the wavelike nature of light, with the phase relationship determined by the difference in optical path lengths of the two reflections.

The resulting interference pattern (interference fringes) can be used to determine the thickness of the film in question, assuming that refractive index and angle of incidence are both known. Conversely, refractive index can be determined if film thickness is known. Film thickness can thus be calculated using the following expression:

$$d = \frac{m}{2D_n \sqrt{(n^2 - \sin^2 \theta)}}$$

Where:

d = film thickness

m = number of fringes in wavenumber region used

n = refractive index

θ = angle of incidence

D_n = wavenumber region used ($\nu_1 - \nu_2$; cm^{-1})

Specular reflectance measurements were made using a 'VW' absolute specular reflectance accessory

(SRA; see Figure 1). The 'VW' SRA is designed to measure 'mirror-like' reflectance from a sample surface, and has been described elsewhere³. The accessory uses a modification of the 'VW' configuration first described by Strong,⁴ which calculates absolute specular reflectance using a pair of matched mirrors to perform the calibration and measure the sample reflectance. The Cary 'VW' absolute SRA eliminates the need for expensive, perfectly matched reference mirrors by using one movable mirror for both the calibration and sample reflectance measurements.

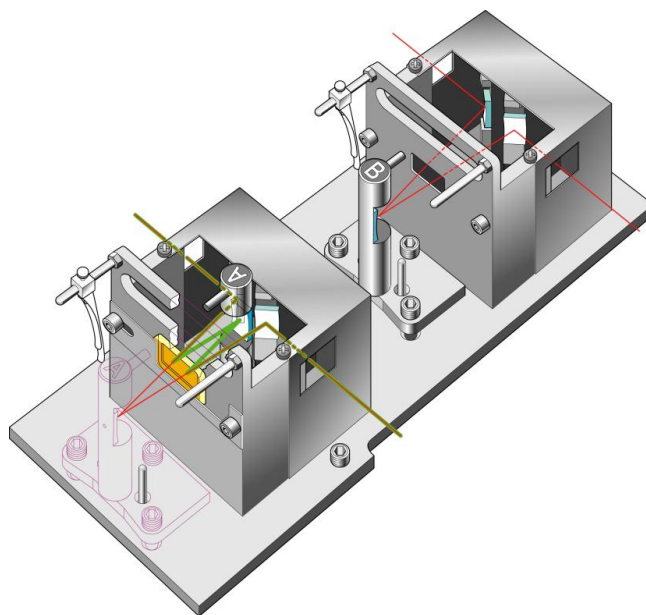


Figure 1. Optical diagram of the Cary VW absolute specular reflectance accessory

Materials and methods

For part numbers please see Reference 5.

Equipment

- Agilent Cary 5000 UV-Vis-NIR Spectrophotometer
- "VW" Absolute Specular Reflectance accessory

Protocol

The 'VW' SRA was installed into the spectrophotometer and aligned⁶. Reflectance spectra were collected between 400 nm and 800 nm using a spectral bandwidth of 2 nm and a scan rate of 600 nm/min (0.1 sec signal averaging time and 1 nm

data interval). All measurements were made in double beam mode, using reduced slit height and zero/baseline correction.

In each case, the sample was positioned using the sample clip supplied with the 'VW' accessory. The 'Zero SRA' baseline correction was performed prior to the acquisition of sample spectra in order to set 0 and 100 %T values. This is particularly important when measuring samples with low reflectance (zero SRA baseline correction can be performed automatically by the Cary WinUV software).

Film thickness calculations were performed automatically using the Agilent 'Thin Film' ADL (Applications Development Language). The Applications Development Language is a spectroscopy programming language built into the Cary software. ADL uses simple programming terms to perform common spectroscopic functions and uses a commercial programming language, SAXBasic, to provide basic functionality with additional Cary-specific commands. ADL is a powerful spectroscopy tool that can perform everything from simple calculations on raw data to the production of a fully customized Cary interface for instrument setup, data collection, storage and retrieval of data, calculation of results, and report creation. The film thickness ADL and others are available for free on the Agilent web site.

Results and discussion

The absolute reflectance spectrum of the coated polycarbonate sample can be seen in Figure 2. The interference pattern due to the thin film is plainly evident over the entire wavelength range scanned, with the fringe spacing increasing with wavelength as expected.

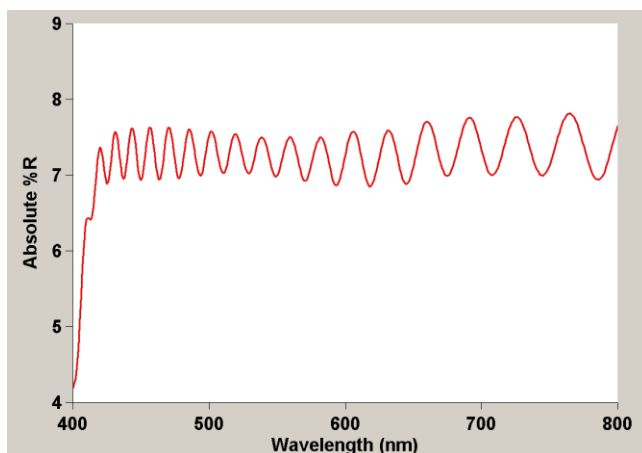


Figure 2. Absolute reflectance spectrum of coated polycarbonate sample showing interference pattern (or fringes) attributable to the coating (thin film) itself

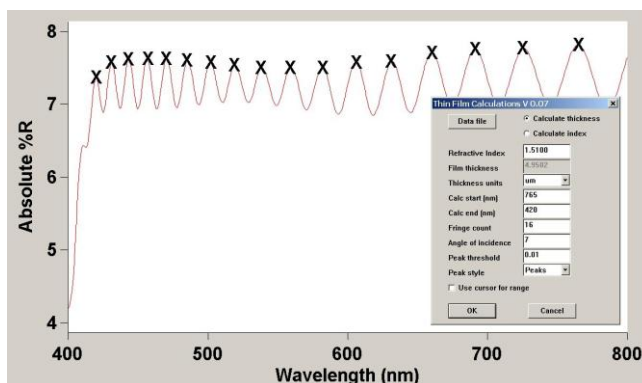


Figure 3. Interference fringes (16) identified between 420 and 756 nm using the Thin Film ADL

Using the aforementioned Thin Film ADL, 16 fringes were identified between 420 and 765 nm (Figure 3). Based upon an angle of incidence of 7 degrees and a thin film refractive index of 1.51, this fringe count resulted in a calculated film thickness of 4.95 μm (Figure 4)

Thin Film Calculations V 0.07

☒ Calculate thickness ☐ Calculate index

Refractive Index

Film thickness

Thickness units

Calc start (nm)

Calc end (nm)

Fringe count

Angle of incidence

Peak threshold

Peak style

☐ Use cursor for range

Figure 4. The Thin Film ADL dialog box showing parameters used for film thickness calculation. By selecting the 'Calculate index' option, refractive index may also be determined if film thickness is known

Conclusion

The thickness of a thin polymeric film deposited on polycarbonate has been calculated to be 4.95 μm . Measurement was based on the interference fringes observed in the reflectance spectrum of the coated polycarbonate sample. Absolute reflectance spectra were acquired over the range 400–800 nm using a Cary 5000 UV-Vis-NIR spectrophotometer and 'VW' absolute specular reflectance accessory.

References

1. Huibers, P. D. T. and Shah, D. O., Langmuir 13 1997 5995.
2. Fabry, C. and Perot, A., Ann. Chim. Phys. 16 1899 115.
3. Hind, A.R. and Soebekti R., 'The deep ultraviolet spectroscopic properties of a next generation photoresist', UV At Work 82, www.agilent.com.
4. Strong, J., 'Procedures in Experimental Physics', 1st Ed., Prentice-Hall, Inc., New York, 1938, 376.

5. Part numbers

Product	Part Number
Agilent Cary 5000 UV-Vis-NIR Spectrophotometer	0010079300
'VW' Specular Reflectance Accessory	0010043800
Cary WinUV Analysis Pack Software	8510195000

6. Cary WinUV Software, 'Cary Help' and videos, Version 3.0.

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POLYMERS

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Analysis of Antioxidants and UV Stabilizers in Polymers using HPLC

Angelika
Gratzfeld-Huesgen

Polymer
/chemical industry

Abstract

Additives are frequently used to protect polymers against thermo-oxidative degradation and destruction caused by UV irradiation. The following antioxidants and UV stabilizers were analyzed using reversed phase liquid chromatography and diode-array detection in technical styrene.

- Uvinol 3000 • Tinuvin P • Irganox 1098 • Uvinol 3008 • Lavinix BHT • Tinuvin 320 • Irganox 1010 • Irganox 1076 • Irgafos 168

Irganox 1010 for example, is a highly effective, non-discoloring stabilizer for organic substrates such as plastics, synthetic fibers, elastomers, waxes, oils and fats. Tinuvin P can be used to protect plastics against UV irradiation as it absorbs the UV light and transfers it into thermal energy which cannot destroy the polymer. Both compound classes have a wide ranging molecular structure and molecular weight. Irganox 1010 has a molecular weight of 1178 and its chemical structure is [3-(3,5-di-tert.butyl-4-hydroxyphenyl)-propionate]. Tinuvin P has a much lower molecular weight of 225 and its chemical structure is 2-(2'-hydroxy-5'-methyl-phenyl)-benzotriazol.

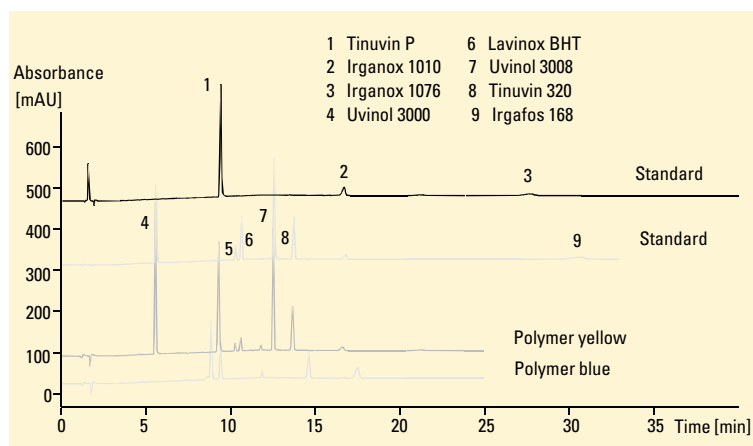


Figure 1
Analysis of antioxidants and stabilizers with the same conditions

Conditions

Column 125 x 3 mm BDS, 3 μ m

Mobile Phase A = Water + 0.001 m Tetrabutylammoniumhydrogensulfate, pH = 3.0 with H₂SO₄, B = Acetonitrile

Gradient

Start with 30 % B, to 98 % B in 10 min

Flow Rate 0.5 ml/min

Injection Vol 5 μ l

Oven Temp 40 $^{\circ}$ C

UV-Detector DAD, 280/20 nm

Reference 900/50 nm

Sample preparation

Polymer samples were dissolved in Tetrahydrofuran and filtered after extraction with ultra-sonic bath for 30 min



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Innovating the HP Way

Antioxidants and UV stabilizers are typically added to polymers as a mixture of several compounds, which also includes costabilizers and antistatic agents. The application range of these additives is broad and can be found in most polymers.

All mentioned compounds are soluble in organic solvents and can be analyzed using reversed phase HPLC with ion-pairing modifier. In addition to the identification by retention time, UV spectra were used.

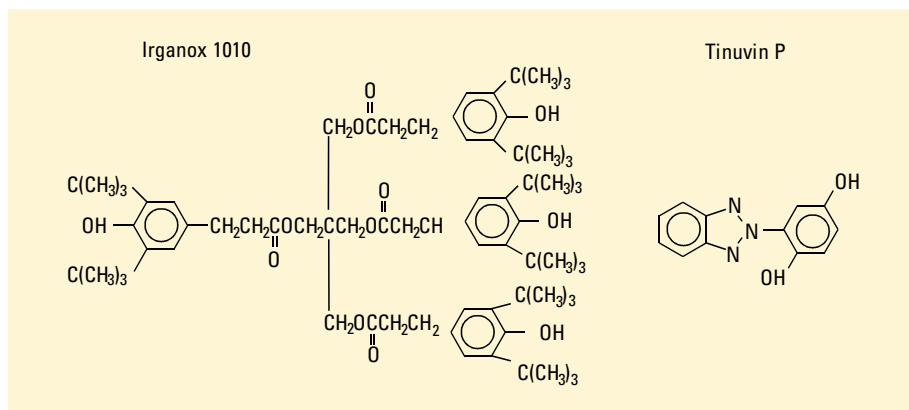


Figure 2
Formula of Antioxidants

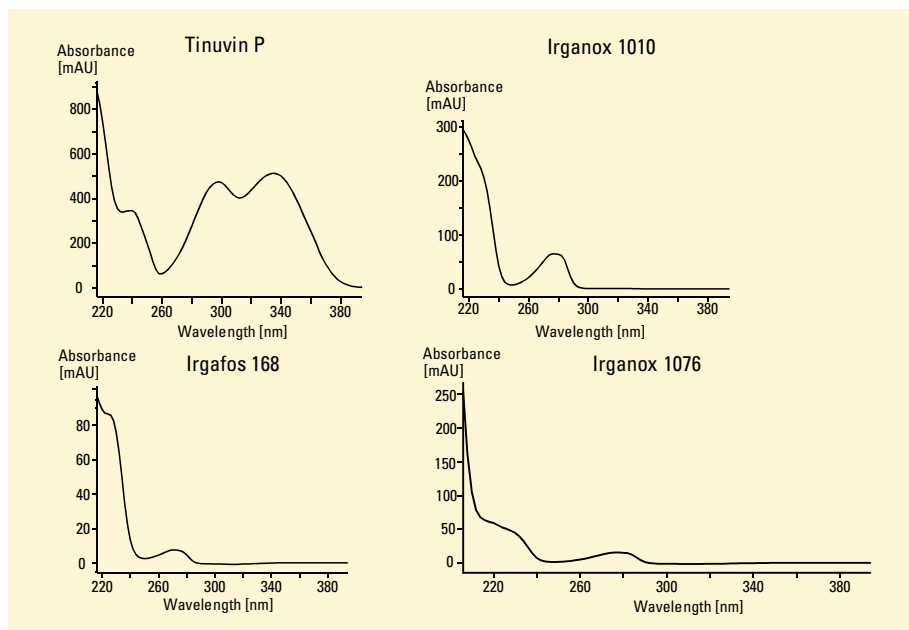


Figure 3
Spectra of antioxidants

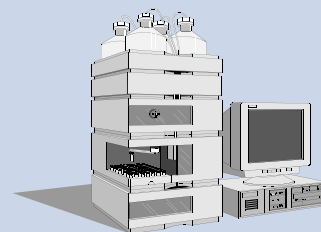
Method performance

Limit of Detection (LOD) = < 1 ng
Precision of retention times (rsd) = 0.2 %
Precision of areas (rsd) = < 3 %

Equipment

Agilent 1100 Series

- degasser
 - binary pump
 - autosampler
 - thermostatted column compartment
 - diode array detector
- Agilent ChemStation + software



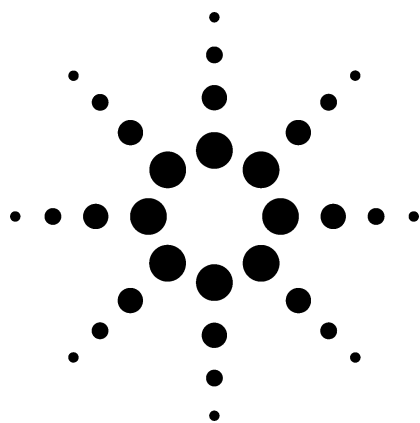
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Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection

Application

Consumer Products

Author

Michael Woodman
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Abstract

Liquid chromatography with ultraviolet/visible spectroscopy and mass selective detection is a powerful approach to antioxidant analysis and identification. Examples illustrate that mobile-phase conditions affect the quality and usability of the acquired data. Unknown compounds can be identified with sufficient MS data and additive degradation can be quickly evaluated.

Introduction

Plastic products are an essential part of our lives today. Whether they are used for automotive components, CDs, toys, or biocompatible replacement parts for humans, they are the subjects of intense research into new and improved polymers and blends. Equally important is the selection and quantity of chemical additives which are used to provide color, density, opacity, stiffness, flexibility, resistance to heat, light and air, flame retardance, and to improve processing properties during pellet creation and final product fabrication.

This application note examines several antioxidant (AO) types, their chemical composition, and suitable high-performance liquid chromatography (HPLC) conditions for assessing their concentration and identity, as well as their degradation products.

AOs arise from various compound classes including small hindered phenols, large hydrophobic hindered phenols, and phosphite or phosphonate linked aromatics. Examples appear in Tables 1 and 2.



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Table 1. AO Studied with Structures

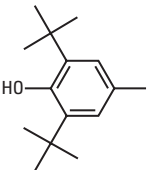
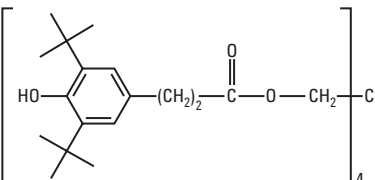
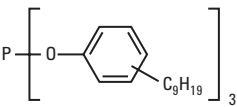
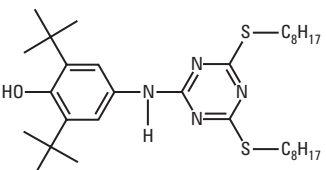
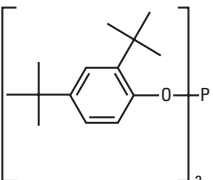
Name:	BHT	Butylated hydroxytoluene
Formula:	$C_{15}H_{24}O$	
Molecular Weight: (MW)	220.2	
Trade name:	Irganox 1010 (CibaGeigy)	Pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate)
Formula:	$C_{73}H_{108}O_{12}$	
Molecular Weight: (MW)	1176.8	
Trade name:	Naugard P (Uniroyal)	Tris nonylphenyl phosphite
Formula:	$(C_{15}H_{23}O)_3P$	
Molecular Weight: (MW)	688.5	
Trade name:	Irganox 565 (CibaGeigy)	
Formula:	$C_{33}H_{56}N_4OS_2$	
Molecular Weight: (MW)	588.4	
Trade name:	Irgafos 168	
Formula:	$C_{42}H_{63}O_3P$	
Molecular Weight: (MW)	646.5	

Table 2. Other Common AOs

Name	Formula	MW
BHA	$C_{11}H_{16}O_2$	180.1
t-BHQ	$C_{10}H_{14}O_2$	166.1
Cyanox 1790	$C_{42}H_{57}N_3O_6$	699.4
Ethanox 330	$C_{54}H_{76}O_3$	772.6
Irganox 1076	$C_{35}H_{62}O_3$	530.5
Sandostab P-EPQ	$C_{68}H_{92}O_4P_2$	1034.6

Gas chromatographs with conventional detectors or mass spectrometers (MS) can readily analyze many small molecules; however, the increased molecular weight (MW) and decreased volatility of many AOs makes gas chromatography (GC) generally unsuitable. Liquid chromatography (LC) is a common choice because it can analyze materials exhibiting a wide MW range and varied solubility. Since LC is generally a nondestructive technique, it offers the possibility of compound isolation and recovery.

Many AOs contain functionalized aromatic groups and offer distinctive ultraviolet/visible spectroscopy (UV/VIS) spectral opportunities. This detector type is an essential part of an additive analysis system. Since UV/VIS detectors are relatively insensitive to the chromatographic mobile phase, they are readily compatible with gradient-elution separation methods.

The presence of functionalized aromatic rings, oxygen, nitrogen, phosphorous, and sulfur in many of the AOs also makes them ideal candidates for investigation by atmospheric pressure ionization mass spectrometry (API-MS). Compound identity can be supported by matching retention data, UV/VIS spectra, and from the MS, a molecular ion (essentially giving the molecular weight of the compound). Depending on the type of ionization and MS chosen, further identification can be made where higher energy is employed, causing fragmentation of the molecules. These fragments help experienced users propose chemical structures.

Instrumentation and General Method

Agilent 1100 LC system:

- Quaternary gradient pump with low volume degasser
- Binary gradient pump with degasser, for pre-MSD reagent addition
- ALS automatic sampler with 2-mL vial tray
- Thermostatted column compartment with automated 6-port, 2-position switching valve
- Diode array UV/VIS spectrophotometer

General chromatographic conditions:

- Gradient elution of increasing organic-solvent strength with combinations of:
Water/Acetonitrile (ACN)
Water/Methanol (MeOH)
Water/Methanol/Tetrahydrofuran (THF), HPLC grade
- UV/VIS spectral-data collection from 200–400 nm, 1-nm slit, 4 nm resolution
- UV/VIS single-wavelength collection for 210 and 280 nm, at 4 nm resolution

ChemStation PC Data and Control System

Mass selective detector (MSD) SL single quadrupole MS with APCI interface

Fragmentor: 100 V, positive and negative ionization

Vaporizer: 400 °C

Nebulizer: 50 psi nitrogen

Drying gas: 6 LPM Nitrogen

Column: Zorbax XDB-C8, 4.6 mm id × 50 mm L, 3.5 µm particles

Gradients:

Method 1. "MeOH/THF", Column 30 °C, 25 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	50	0	10
1	15	0	90	0	10
1	20	0	90	0	10
1	21	40	50	0	10

Method 2. "MeOH", Column 40 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	60	0	0
1	10	0	100	0	0
1	15	0	100	0	0
1	16	40	60	0	0

Method 3. "ACN", column 50 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	0	60	0
1	10	0	0	100	0
1	15	0	0	100	0
1	16	40	0	60	0

Experimental Results

Figures 1 through 3 are overlaid UV chromatograms for nine AOs, using three different gradients.

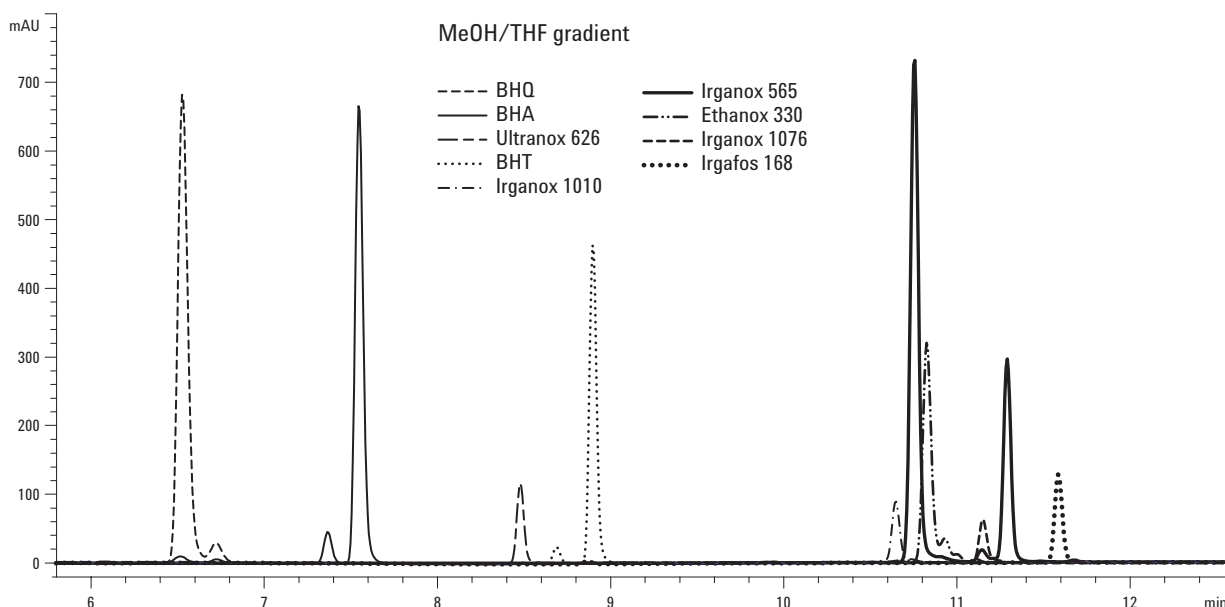


Figure 1. Overlaid UV chromatograms for the selected AOs using the methanol/THF gradient.

Many samples have minor peaks originating from impurities or degradation products having structures similar to the parent molecules. For the smaller molecules like BHA, BHQ, and BHT, there is no problem with resolution. For larger

molecules, there is reduced resolution in the 10- to 12-minute region. These molecules have unique MWs, though, and can be analyzed using selective MS detection.

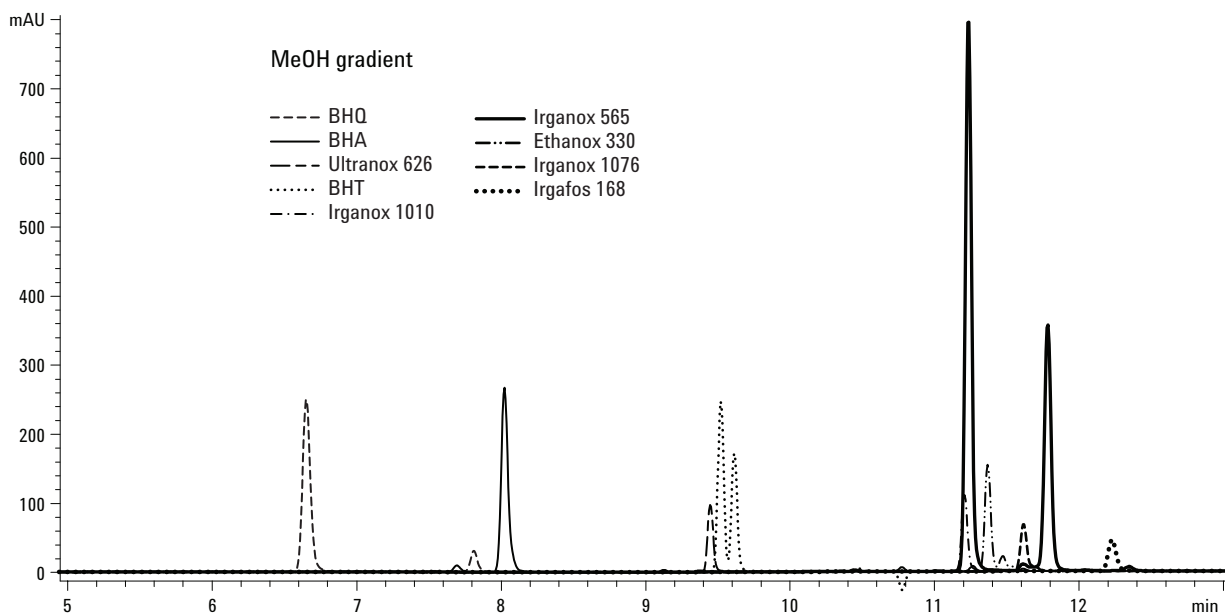


Figure 2. Separation of AOs using the MeOH gradient.

Using the MeOH gradient, relative separation is somewhat different, and as before, the smaller molecules are well resolved. The larger molecules in the 11- to 12-minute region exhibit reduced resolution, but can be analyzed using selective MS detection.

Figure 3 shows the separation of the same AOs using the ACN gradient.

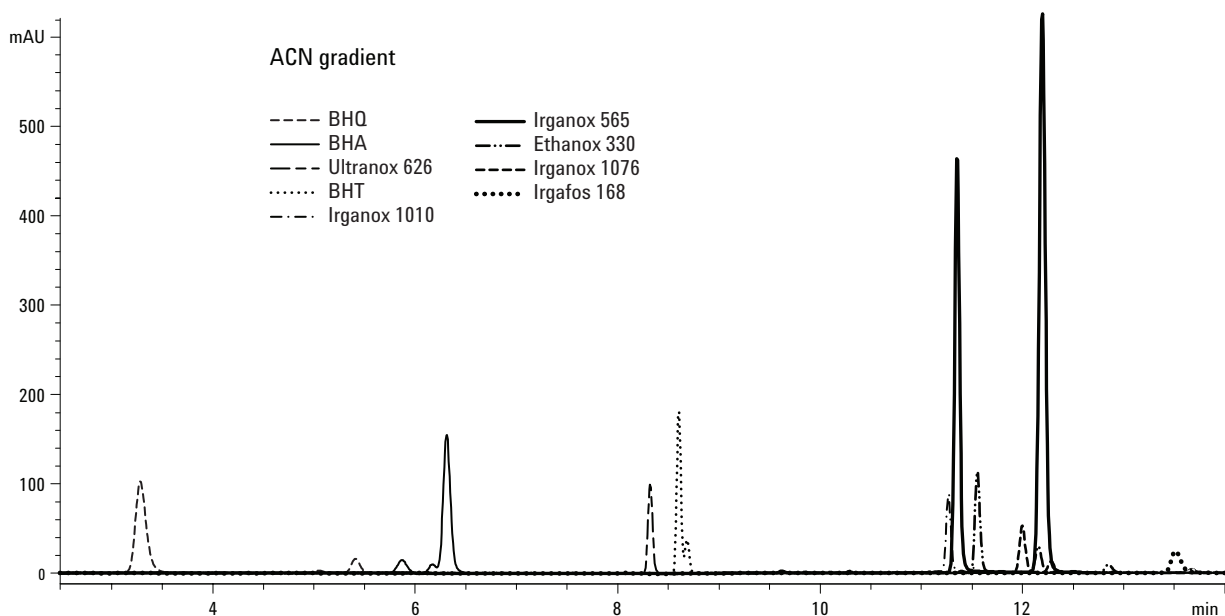


Figure 3. Separation of AOs using the ACN gradient.

Once again, no problem exists with resolution of the smaller molecules. For larger molecules in the 11- to 12-minute region there is somewhat better resolution. ACN has the best UV transparency at low wavelengths, maximizing baseline stability in the wavelength range where UV response would be observed for the AOs.

It is often attractive to use UV/VIS libraries to tentatively identify components in the sample

mixture. This approach is especially useful when the various analytes have distinct spectra. Where many AOs have phenolic rings with characteristic UV/VIS spectra, distinguishing analytes by this approach is difficult and the user must rely on retention time data to support any identification attempt.

As we investigate various AO molecules, it is useful to note the general mass range for single- and multiple-ring structures. See Figure 4.

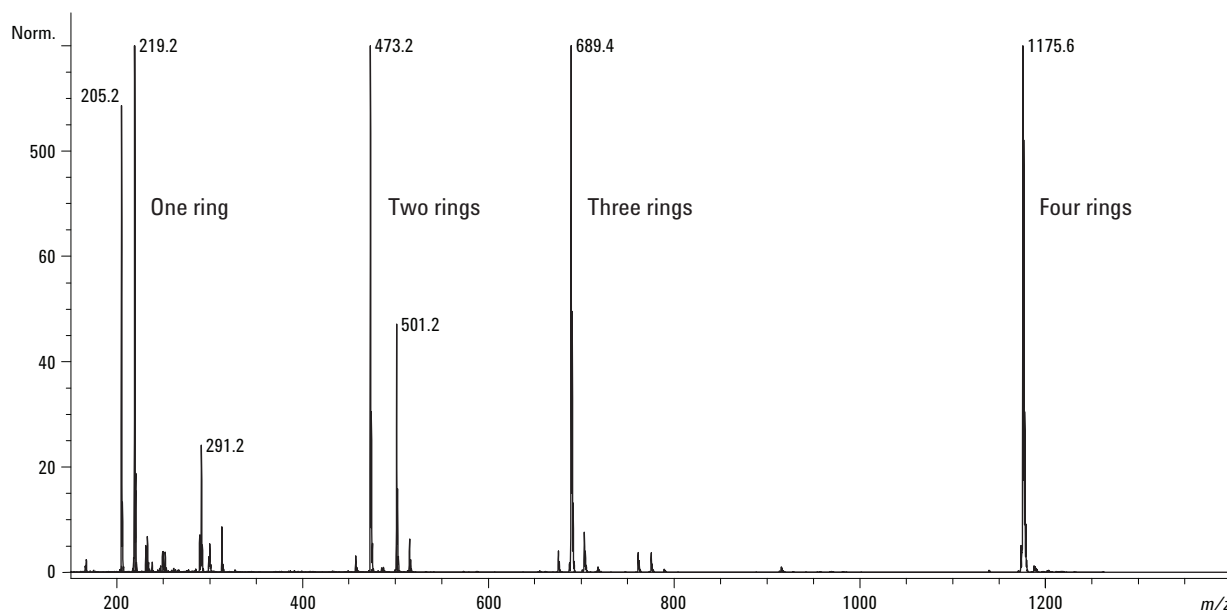


Figure 4. Overlaid AO mass spectra, illustrating effect of ring number on observed mass range.

In Figure 4 we see intact and fragmentation ions representing structures from one to four aromatic rings. The m/z 219 is $[M-H]^-$ for BHT while m/z 205, less one CH_2 , is a fragmentation ion of a larger molecule having the hindered phenolic feature. The m/z 473 and m/z 501 are fragments discussed later in this text. The m/z 689 is Naugard P, $(C_{15}H_{23}O)_3P$. The m/z 1176, Irganox 1010, $(C_{73}H_{108}O_{12})$ has four rings and long alkyl chains that increase the mass and remind us that it is important to acquire mass data well over 1000 Da for general AO screening and analysis.

The mobile phase absorbance background invariably affects UV/VIS spectra. See Figure 5. In this example, the UV/VIS spectra for Irgafos 168 are shown for the three previously described solvent conditions.

Significant differences in response, especially in the important low UV range, are generally observed. This interference is also found with many ionic modifiers added to the mobile phase to control ionization of analytes, possibly improving the separation or enhancing ionization of the compounds in the MS.

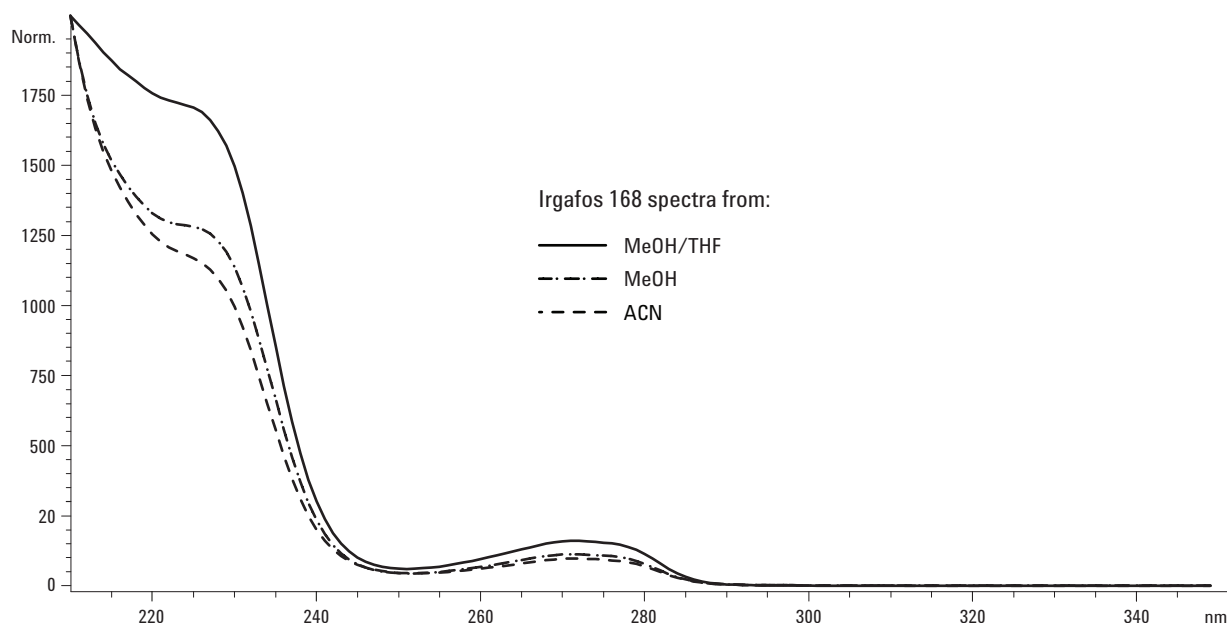


Figure 5. Solvent effects on UV/VIS spectra for Irgafos 168.

Ionization, and thus ion abundance in the MS, may also be affected by the mobile phase composition.

In Figure 6, the extracted positive-ion spectra for Irgafos 168 (molecular weight 646.5, detected as the $[M+H]^+$ ion) appear in the three previously described solvent conditions, where it elutes in high organic concentrations. Observe the significant differences in response, with the lowest response in ACN. Reduced response from the molecular ion may be from decreased ionization or increased fragmentation. It may be possible to add

modifiers after the UV, and prior to the MSD inlet, to enhance MS response in circumstances where the solvent offers chromatographic or UV/VIS advantages but negatively impacts ionization in the MS.

The degree of fragmentation in the MS may also be affected by the mobile-phase composition. In Figure 7, the extracted negative-ion spectra for Irgafos 168 appear in the three previously described solvent conditions.

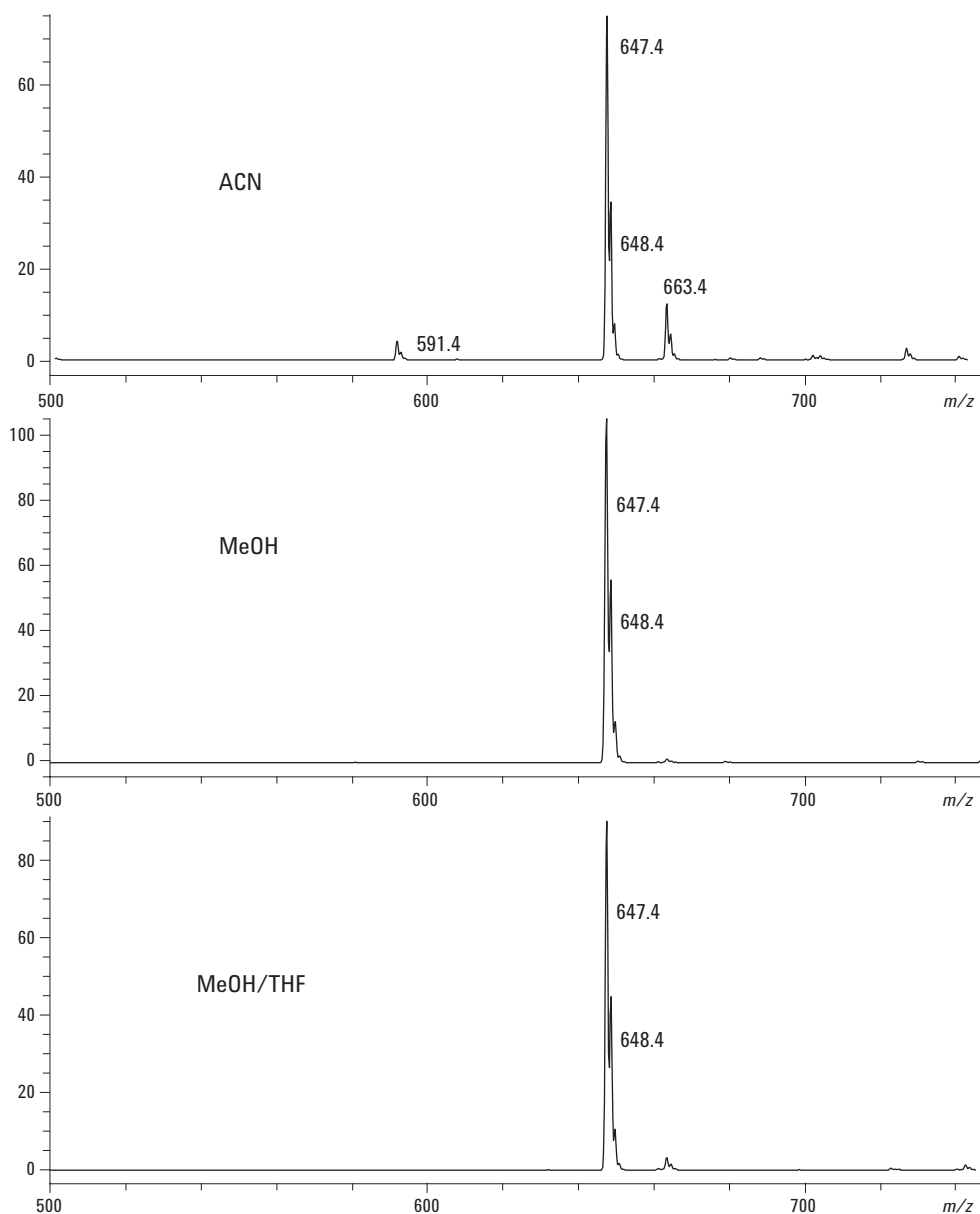


Figure 6. Solvent effects on positive-ion MSD spectra for Irgafos 168.

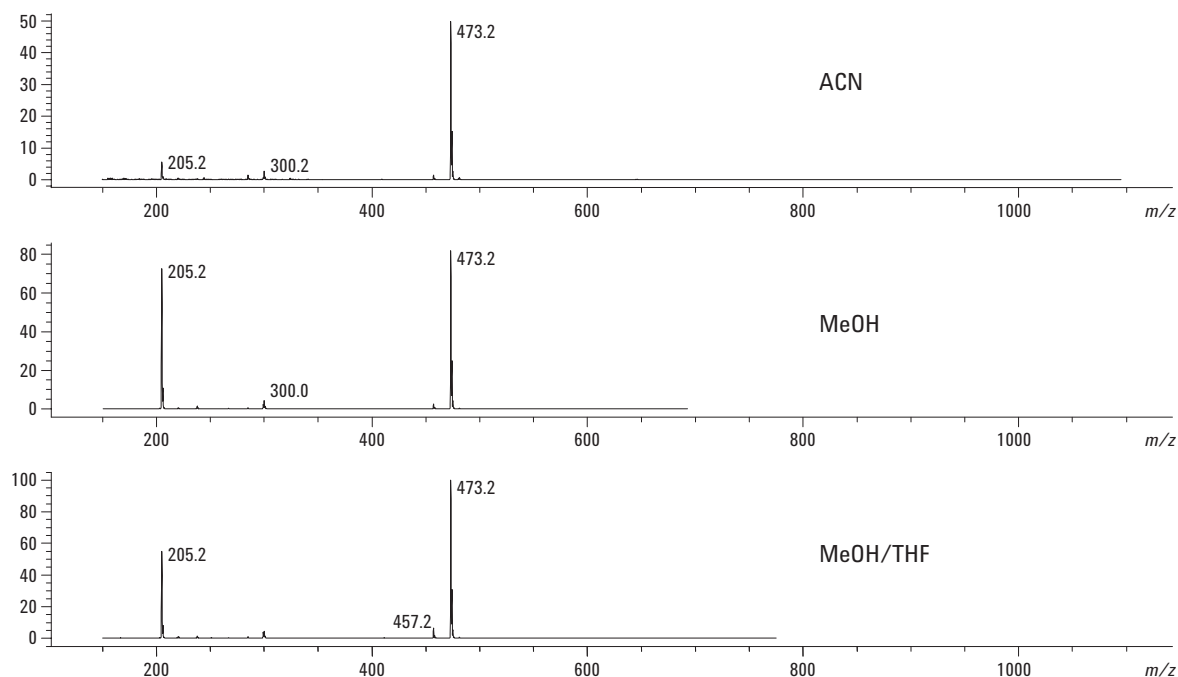


Figure 7. Solvent effects on negative-ion MSD spectra for Irgafos 168.

Note the significant differences in response with the lowest response in ACN. Reduced response for the molecular ion and fragment ions suggests that the ACN response is simply reduced ionization. Based on known degradation chemistry of Irgafos 168 and similar compounds, the m/z 473 fragment is likely $[C_{28}H_{42}O_4P]^-$ where an “arm” is lost (m/z 205) and an oxygen remains on phosphorous as $P=O$.

Identification of Unknowns

Retention data may allow experienced chromatographers to suggest how an unknown peak might differ structurally from a group of knowns run under the same conditions, but identification invariably takes far more resources than simple elution patterns provide. From UV/VIS data, we

can often suggest molecule class, especially so in our discussion of compounds commonly having the phenoxy group in the chemical structure. UV/VIS spectra may be suggestive but, when used without significant prior knowledge, lack sufficient resolution to confirm identity. MS data, on the other hand, have the spectral resolution necessary to infer structural details leading to actual chemical identification. The following examples describe several situations in which either detector would be helpful.

In the simple case of an unknown containing either BHA or BHT, the UV spectra (Figure 8) are sufficiently unique to allow a reasonable identification along with characteristic retention data. Nearly 1.5 minutes separate these two peaks in the conditions above and little doubt would remain.

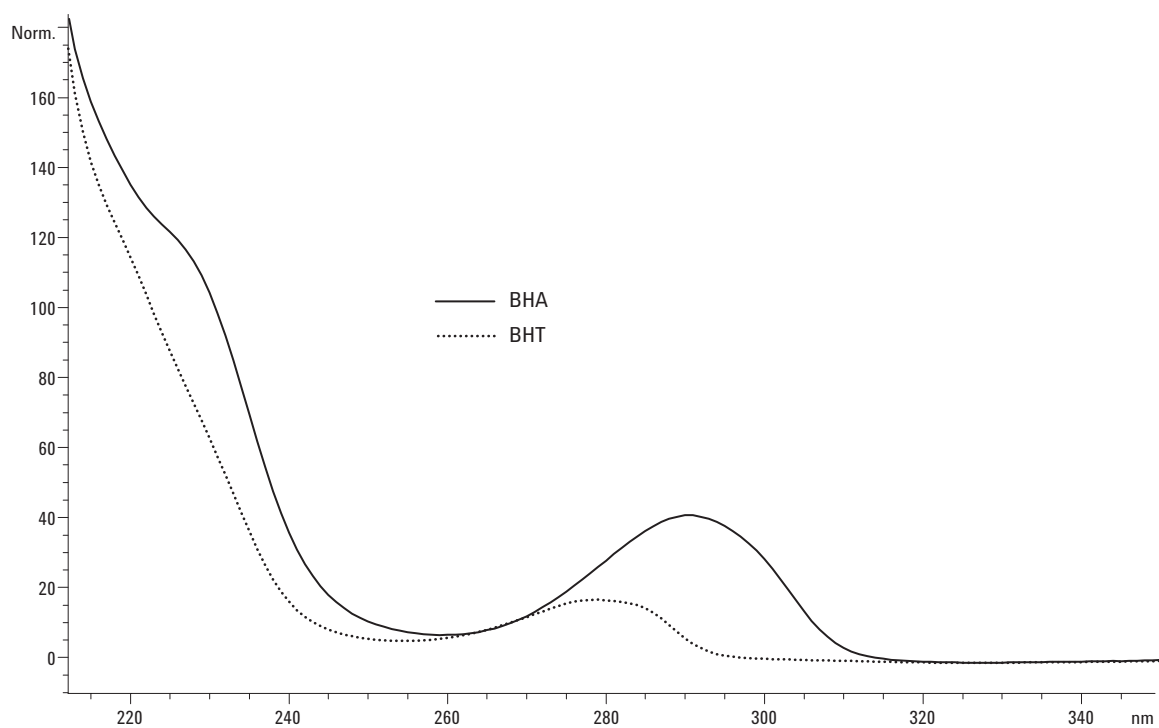


Figure 8. Extracted UV spectra from mixture containing only BHA and BHT.

Using MS data for the same sample, we would reach similar conclusions. See Figure 9.

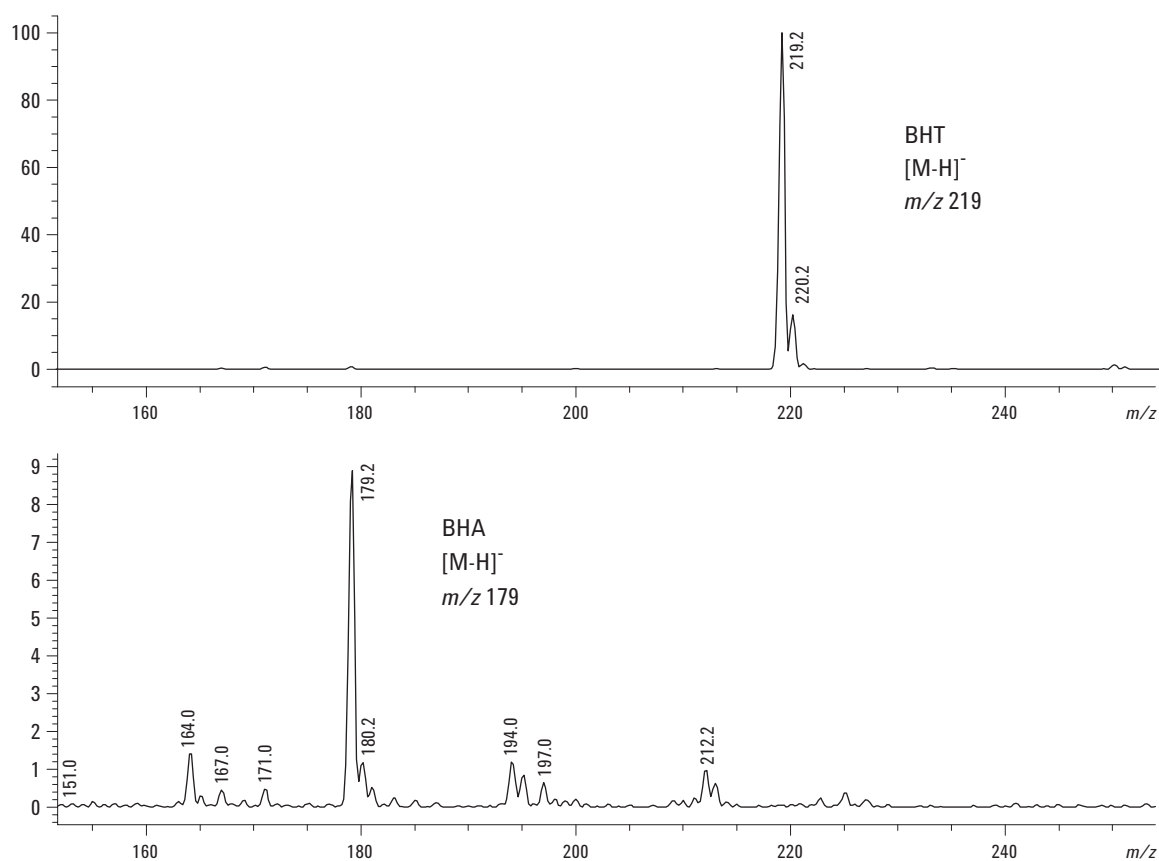


Figure 9. Extracted negative-ion MS spectra from mixture containing only BHA and BHT.

Retention data suggests two distinct molecules leading to an unambiguous identification without any need for MS fragmentation data.

When examining MS data, we generally expect to see classic molecular ions, either molecular mass+1 in positive-ion mode or mass-1 in negative-ion mode. These conditions, in the absence of significant adduct or fragment ion formation, often yield the best sensitivity and quantitative result. Such is the case in the Irganox 565 example shown in Figure 10.

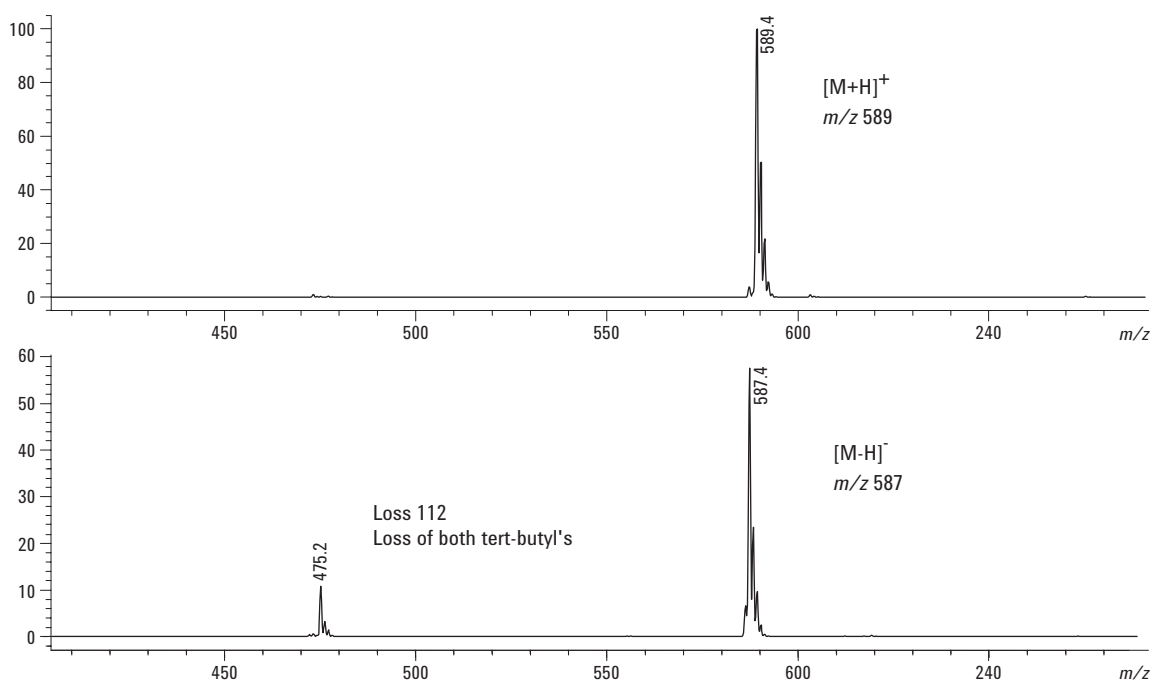


Figure 10. Positive- (upper) and negative- (lower) ion spectra for Irganox 565, using MeOH/THF gradient.

Only minor amounts of fragmentation are seen in the negative-ion spectrum, corresponding to the loss of both tert-butyl groups. In some cases, a radical ion is formed and the MS ion observed will correspond to the mass of the parent molecule. It is difficult to predict when this may occur, but the user must be prepared to interpret the spectral data with this situation in mind.

Irganox 1010 was run under the same conditions and produced minimal fragmentation in the negative-ion spectrum. An $[M-H]^-$ ion at m/z 1175.6 is detected for the expected MW 1176.8. See Figure 11.

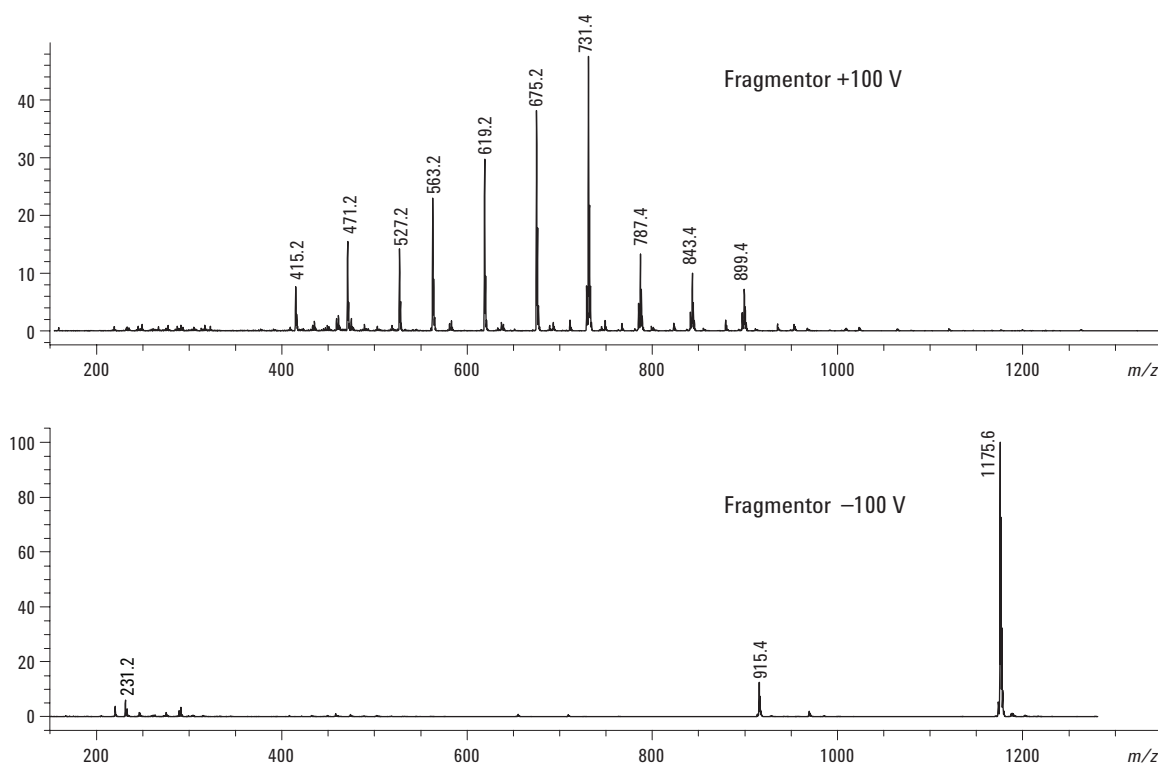


Figure 11. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH/THF gradient.

The positive-ion spectrum, however, is devoid of any useful amount of the molecular ion. The resulting fragmentation pattern suggests a molecule with a significant number of tert-butyl structures which, with the molecular ion from negative ionization, is consistent for a tentative identification for the named compound.

Little change is observed in the fragmentation pattern by reducing the fragmentor voltage to 25 V, though overall ion production is reduced from the 100 V experiments. See Figure 12.

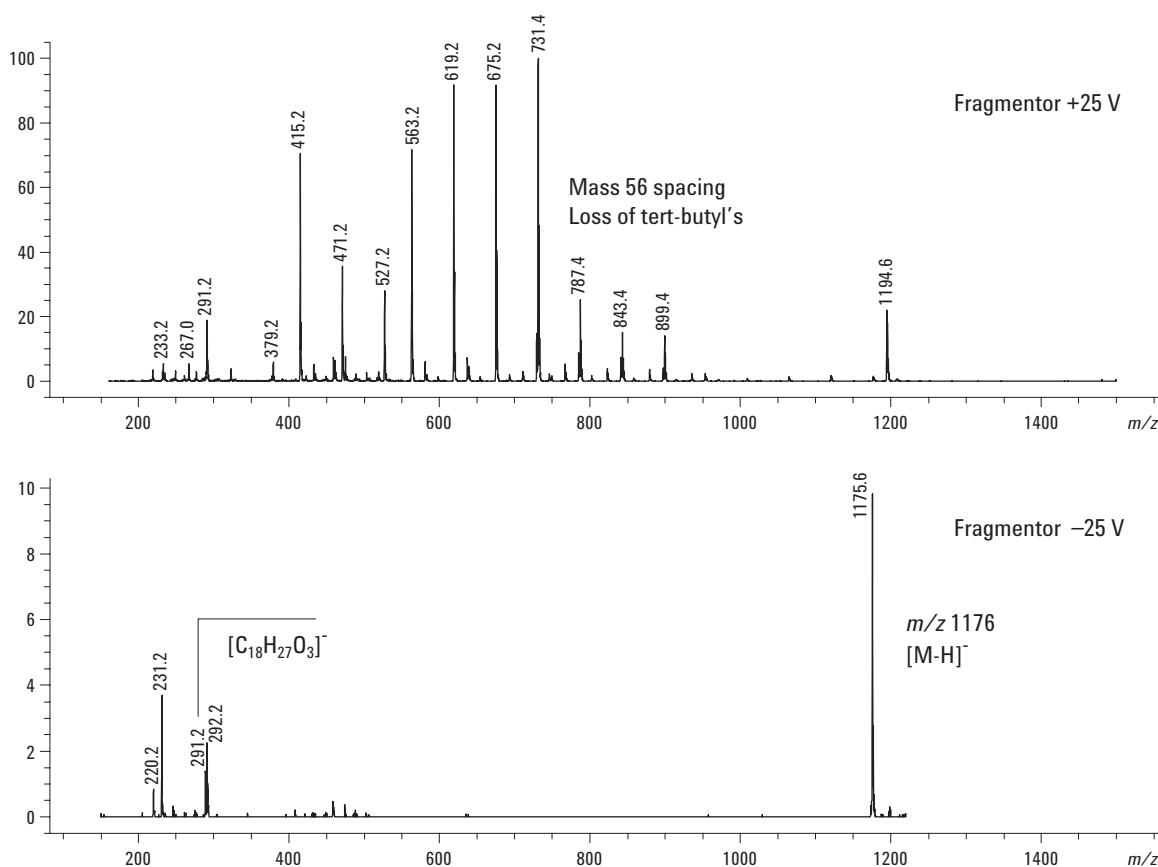


Figure 12. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH gradient.

An m/z 291 fragment ion can be observed, which corresponds to one of the symmetrical “arms” of the molecule.

The positive- and negative-ion spectra extracted from the main peak in a degraded standard of Naugard P appear in Figure 13. Naugard P responds comparably to the Irganox 1010 in positive-ion mode, yielding an easily observed molecular ion.

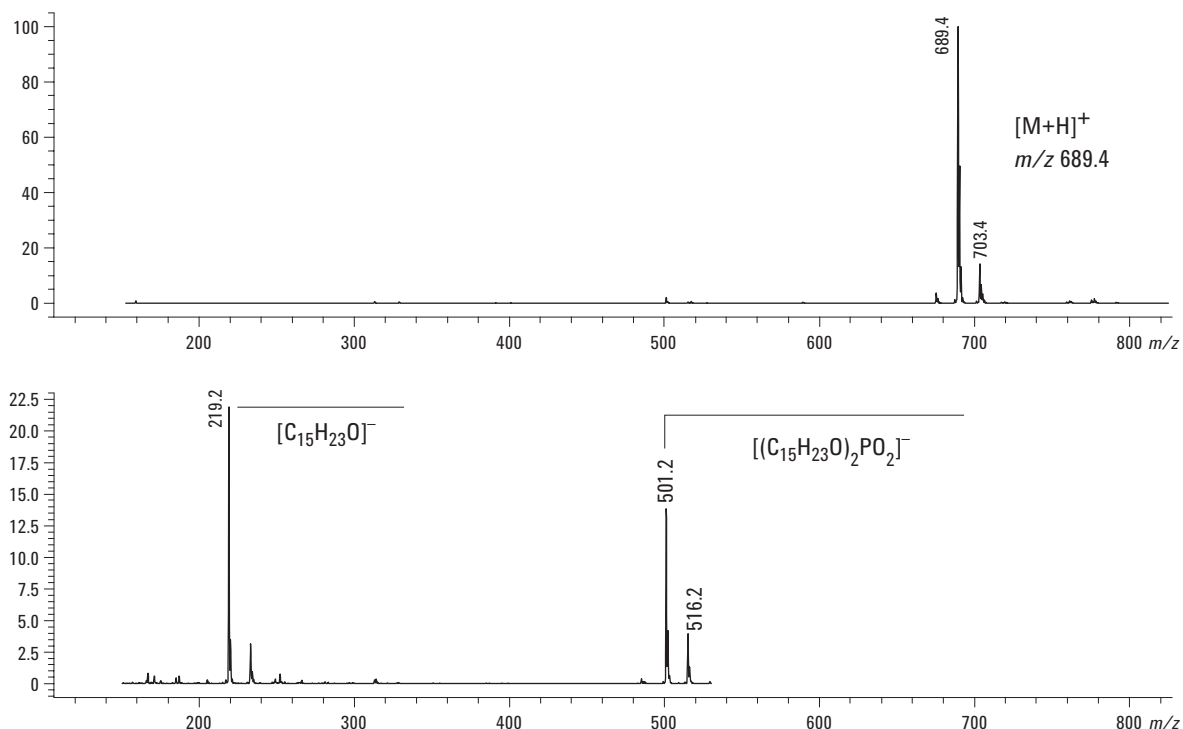


Figure 13. Extracted positive- (upper) and negative- (lower) ion spectra from the main peak in a degraded Naugard P standard.

Poor response in negative-ion mode is presumably due to excessive fragmentation, and no molecular ion is observed. Fragments and minor rearrangements found under these conditions are excellent markers for this sample type and would be good indicators if unknown samples were analyzed.

Peaks in the degraded Naugard P analysis have characteristic positive- and negative-ion spectra which could be studied to confirm typical or propose unknown degradation products. All the peaks seem to have the alkyl side chain present. The other variations presumably lie with the number of oxygen atoms attached to the phosphorous, as proposed in the spectra of the peak at 11.6 min in Figure 14.

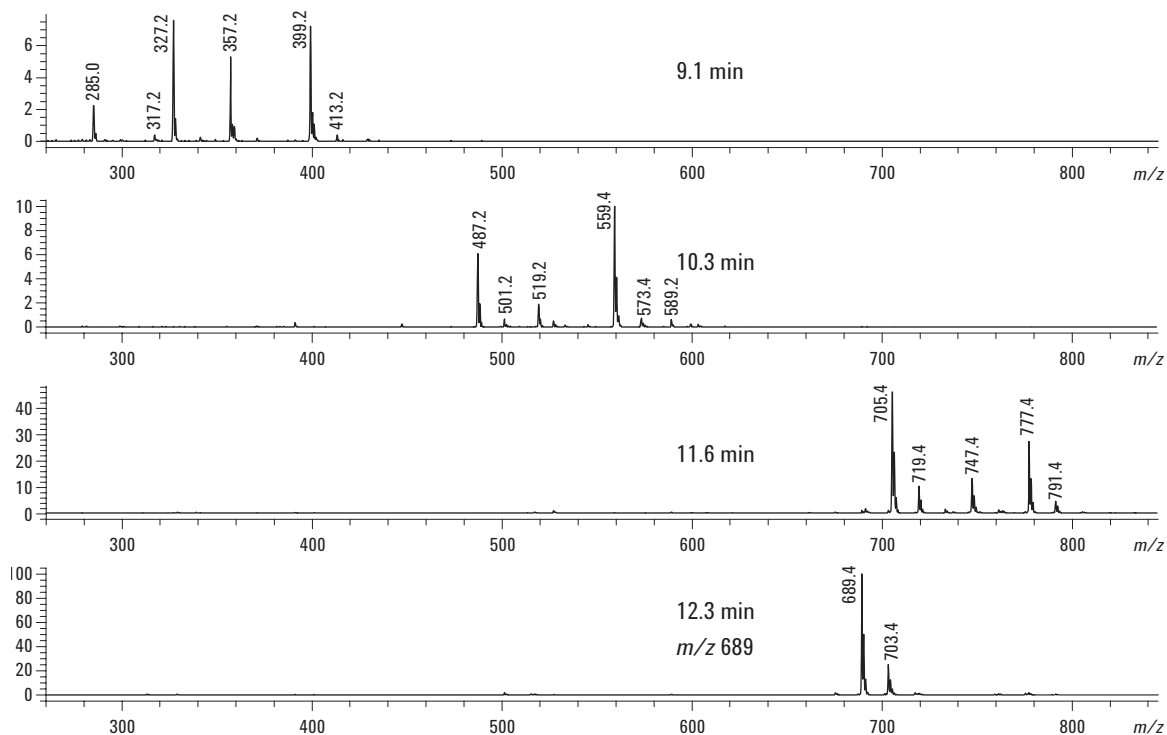


Figure 14. Extracted positive-ion spectra for Naugard P.

Likewise, the negative-ion fragmentation patterns shown in Figure 15 help simplify the investigation by showing differences in the alkyl chain or P-O bonds.

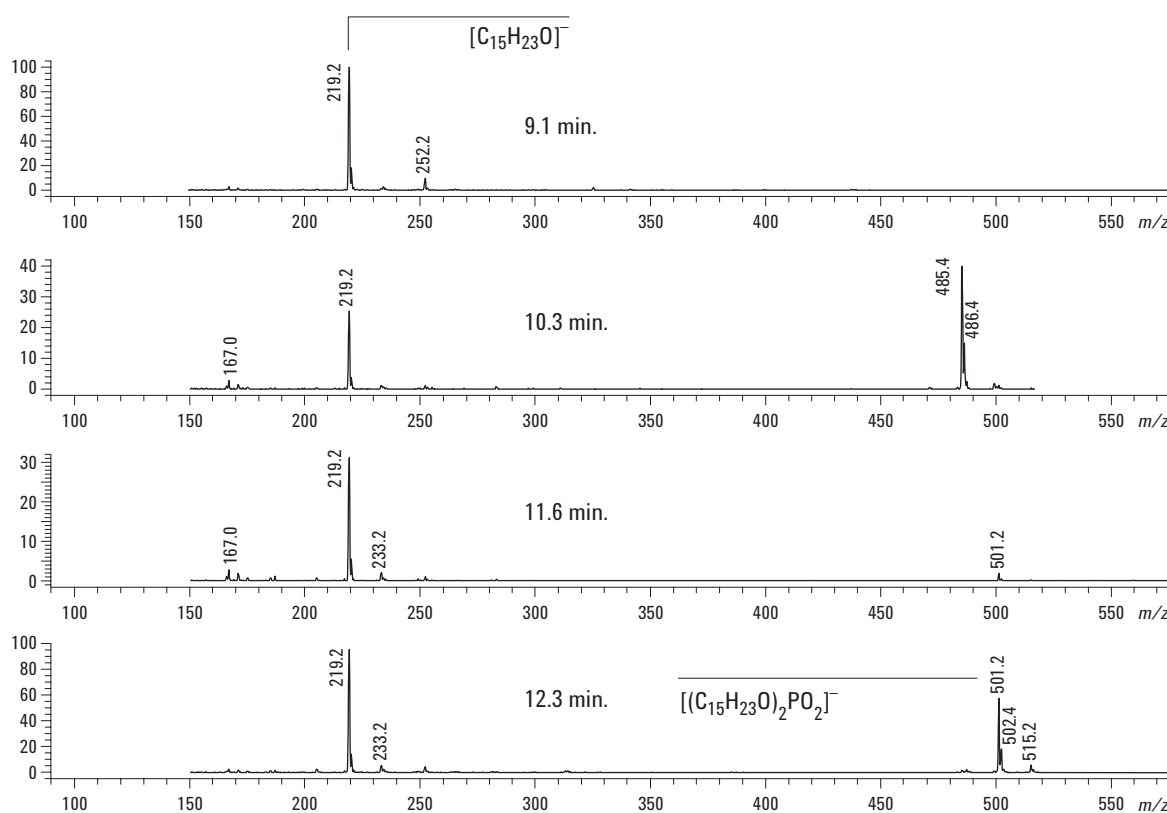


Figure 15. Extracted negative-ion spectra for Naugard P.

We received several unknown samples containing polymer additives. The prepared solutions were analyzed with a wide variety of known standards of AOs and other additive classes. Of all the analyzed standards, Naugard P chromatographic patterns, as shown in Figure 16, most closely matched the unknown samples. Additional spectral investigations followed.

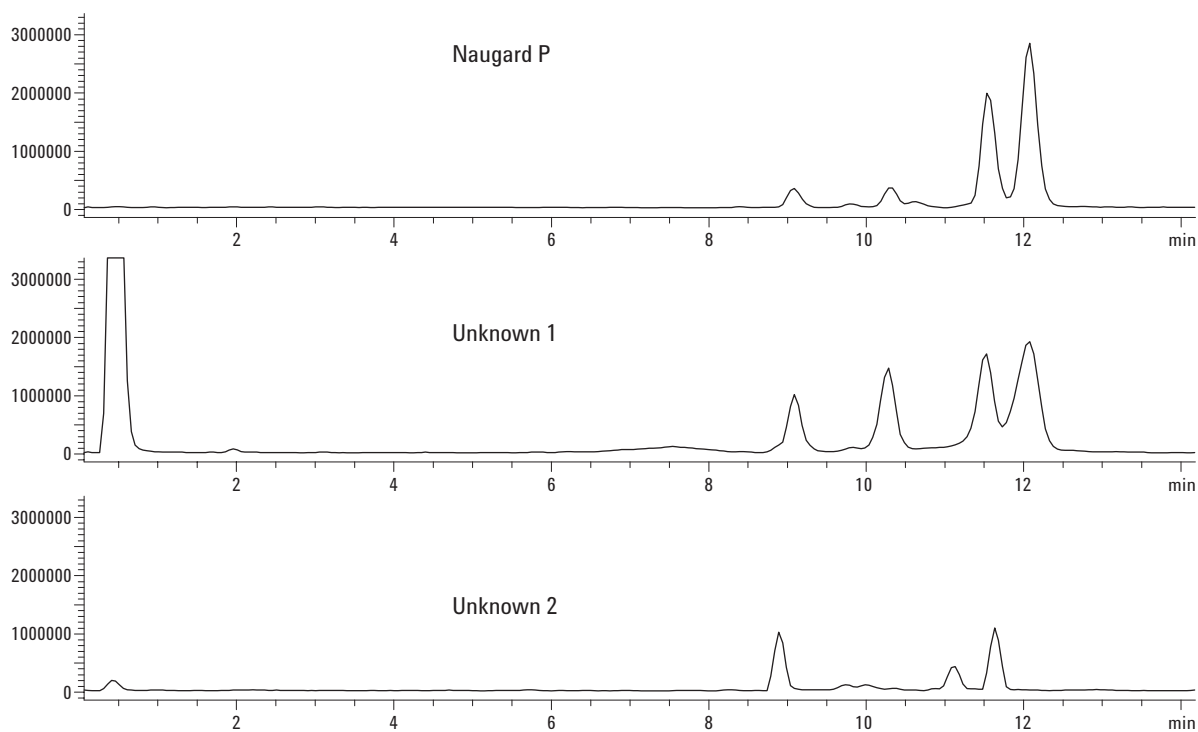


Figure 16. Total positive-ion chromatograms of Naugard P and two unknowns are compared.

The UV spectra for these same samples shown in Figure 17 are similar, though still generally characteristic of many aromatic compounds having minimal ring substitution. These data are interesting, but not conclusive.

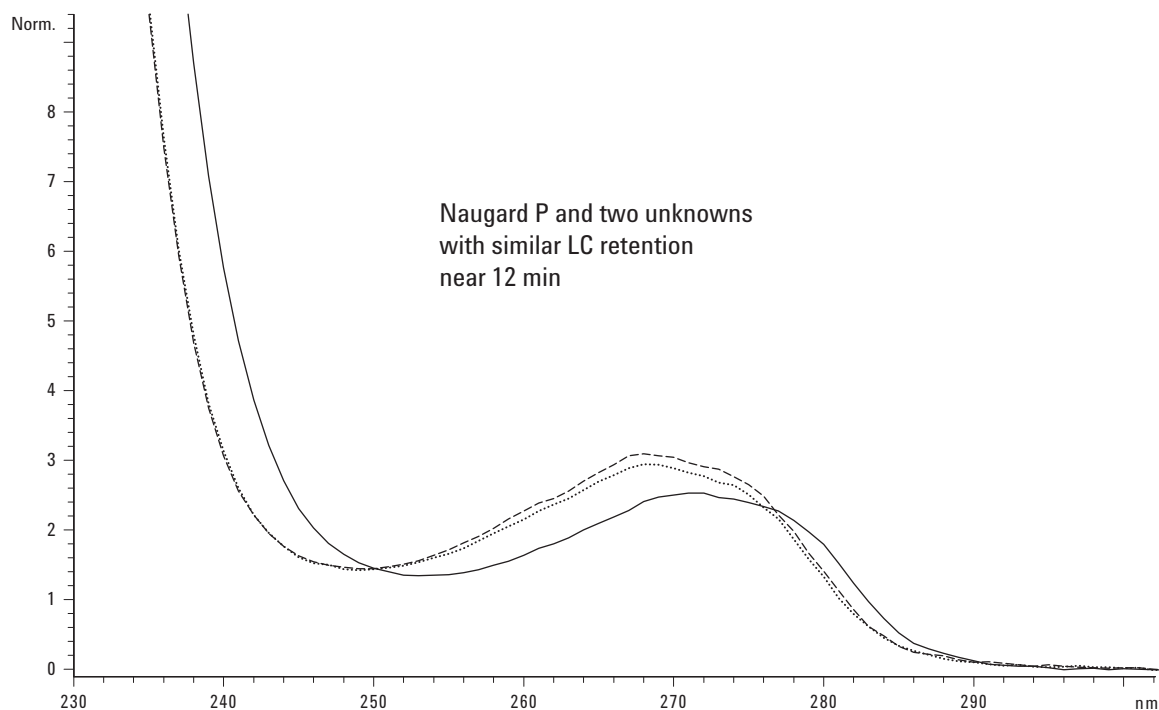


Figure 17. UV spectra of Naugard P and the two unknowns.

The positive-ion mass spectrum of Unknown 1, shown in Figure 18, is an excellent match to that of Naugard P, showing slightly more alkyl variation than the standard. This could be a different lot of Naugard P or a product from a different supplier. Unknown 2 has the primary positive-ion at m/z 647, reasonably due to a shorter alkyl chain, C_8H_{17} , compared to the C_9H_{19} alkyl chain on Naugard P.

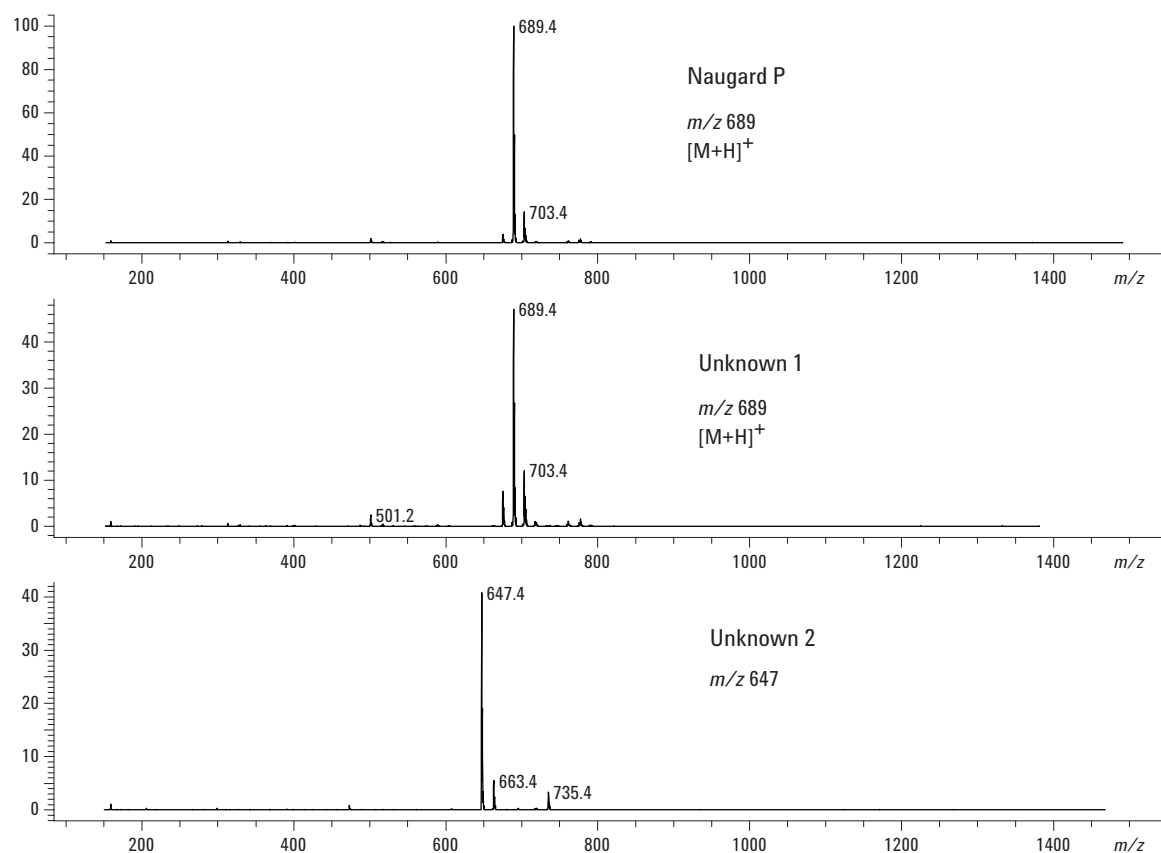


Figure 18. The positive-ion mass spectra of Naugard P and the two unknowns.

In negative-ion mass chromatograms, we see similarities to Naugard P in Unknown 1 and quite dissimilar data in Unknown 2. Recalling from earlier discussions that Naugard P is highly fragmented in negative-ion mode, the negative-ion mass spectra should be extremely helpful in supporting our initial thoughts taken from the positive-ion spectra. See Figure 19.

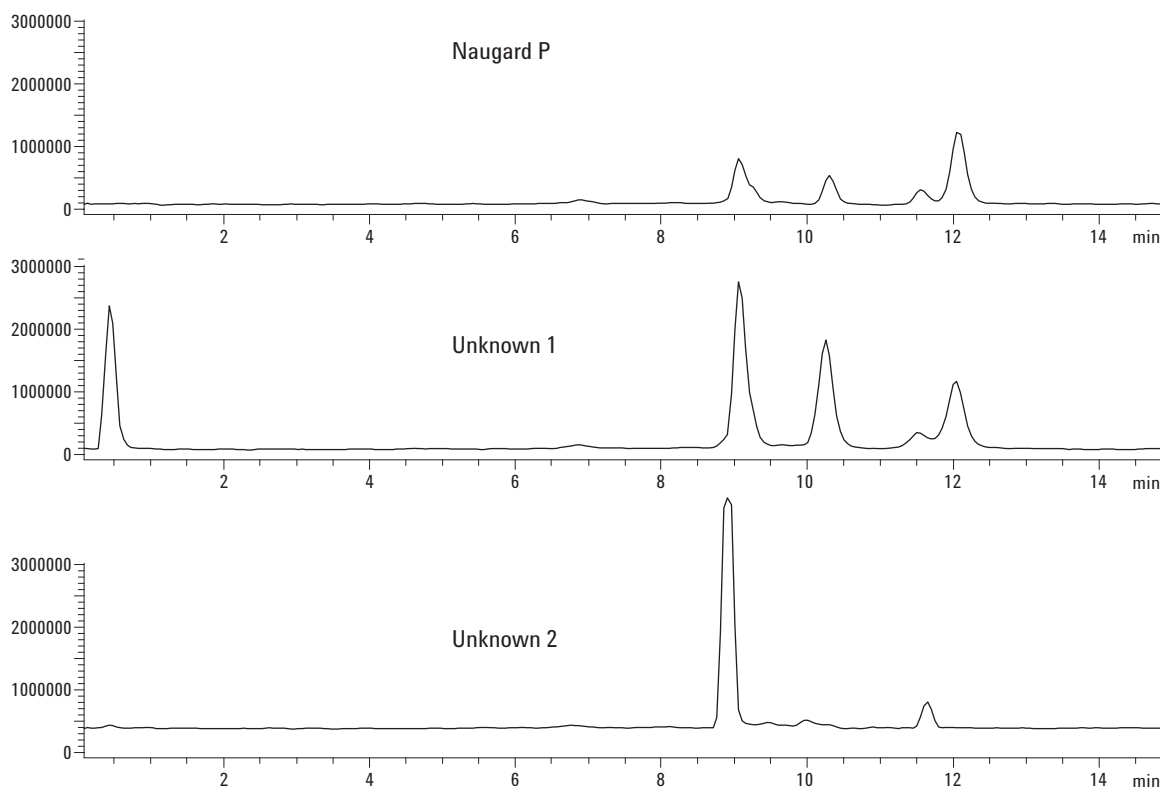


Figure 19. Total negative-ion chromatograms of Naugard P and two unknowns are compared.

The negative-ion spectra for Naugard P and Unknown 1 are an excellent match and probably offer the best support of that chemical identity and structural details. Unknown 2, however, speculatively presents two CH_2 's less in the m/z 501 fragment and one CH_2 less the m/z 219 fragment. See Figure 20. This is highly supportive of the proposed structure from the positive-ion data and allows us to conclude that, while similar to Naugard P, it is a unique product whose structure is most likely $(\text{C}_6\text{H}_4\text{-C}_8\text{H}_{17}\text{-O})_3\text{P}$.

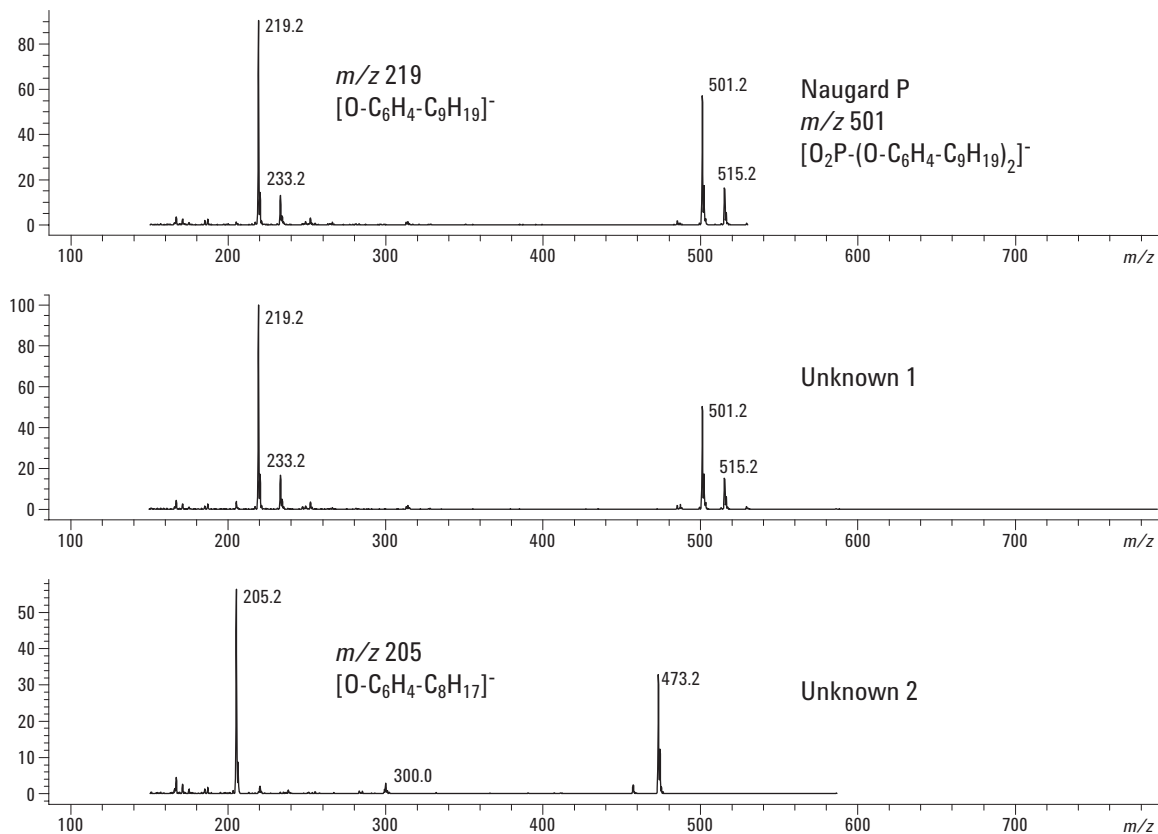


Figure 20. Negative-ion fragmentation mass spectra of Naugard P and the two unknowns.

Conclusions

- LC with UV/VIS and MSD detection is a powerful approach to compound analysis and identification.
- Mobile phase conditions affect the quality and usability of the acquired data.
- Unknown compounds can be tentatively identified with MS data.
- Additive degradation can be quickly evaluated to optimize formulations for better performance.

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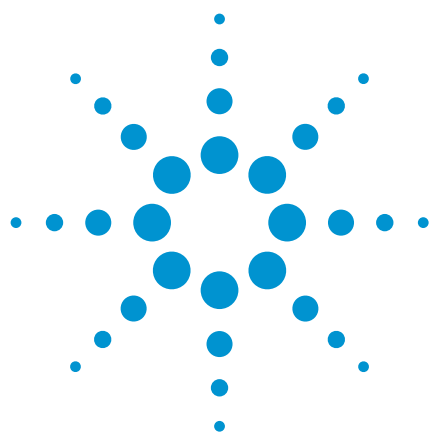
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Agilent Technologies



Artifact Free Analysis of Lignins by GPC using Agilent PolarGel-M

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Lignin is commonly derived from wood and is one of the most abundant organic compounds on earth, being an integral part of the cell wall of plants. It is a large, cross-linked biopolymer with several unusual but useful properties. For example, wood with a high abundance of lignin is durable and therefore makes a good raw material for construction. However, the presence of lignin is detrimental to the paper making industry and the biopolymer must be removed during the chemical process of pulping the wood to form the paper, an expensive activity. In this chemical pulping - or Kraft – process, the lignin is removed from wood pulp by the breaking down of the cross-links and through sulfonation. The resultant sulfates are considered to be environmentally friendly and have many uses, including forming the bases of many other chemicals such as ethanol and vanillin. They can also be used as additives in agricultural chemicals, as dispersants in water treatment formulations and as textile dyes.



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GPC Analysis

PolarGel-M GPC columns are packed with low swell, macroporous copolymer beads that have a surface of balanced polarity, comprising hydrophobic and hydrophilic components. These allow PolarGel-M to be used in the analysis of high polarity polymers that are insoluble in water to give a more accurate representation of the molecular weight distribution of the polymer. If these polar polymers were to be analyzed with traditional styrene/divinyl benzene columns, interactions would cause artifacts in the peak shape and longer retention times, which would translate into apparently much lower molecular weight averages.

Sample Preparation

Three varieties of lignin from different sources were analyzed to obtain an indication of differences in molecular weight, if any. The samples were made up at 0.2 % (w/v) in DMSO, with 0.1 % LiBr added to reduce sample aggregation, and injected without further treatment.

Conditions

Columns:	2 x PolarGel-M, 300 x 7.5 mm (p/n PL1117-6800)
Eluent:	DMSO & 0.1 % LiBr
Flow Rate:	1.0 mL/min
Injection Volume:	100 μ L
Temperature:	50 $^{\circ}$ C
Detectors:	Agilent PL-GPC 50, RI

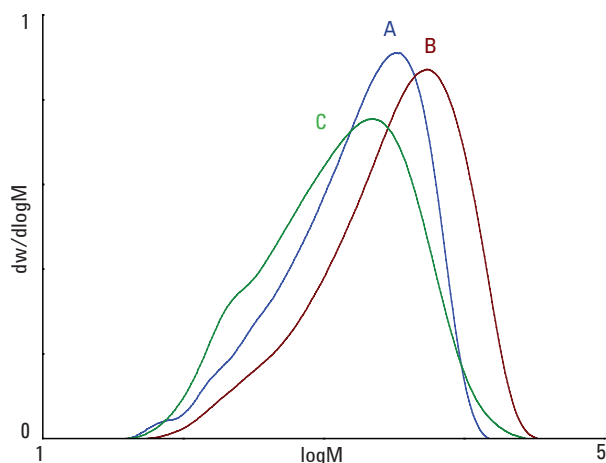


Figure 1. Overlaid molecular weight distributions of the three lignins

Results

Figure 1 shows overlaid molecular weight distributions of three lignins.

Conclusion

GPC with PolarGel-M columns allows for the artifact, interaction free analysis of a wide range of high polarity polymers that are difficult to analyze on traditional, organic (PS/DVB) GPC columns.

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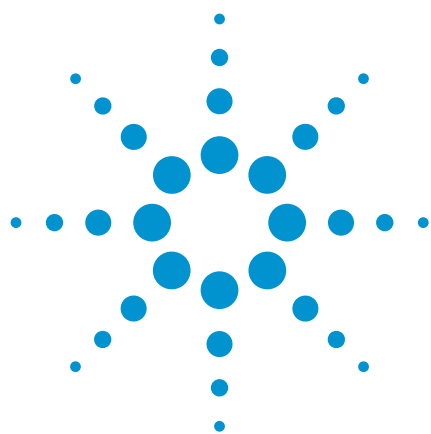
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SI-00947



Agilent Technologies



GPC and Agilent PolarGel-M Columns for the True Representation of Novolac Resins

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Novolac resins are thermoplastic materials made with an excess of phenol in an acid catalyzed reaction with formaldehyde. Novolacs are commonly employed as photoresists (light-sensitive materials used to form patterned surface coatings) and in varnishes. They have higher heat distortion temperatures and tend to be more expensive than regular epoxy resins.



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GPC Analysis

PolarGel-M GPC columns are packed with low swell, macroporous copolymer beads that have a surface of balanced polarity, comprising hydrophobic and hydrophilic components. These allow PolarGel-M to be used in the analysis of high polarity polymers that are insoluble in water to give a more accurate representation of the molecular weight distribution of the polymer. If these polar polymers were to be analyzed with traditional styrene/divinyl benzene columns, interactions would cause artifacts in the peak shape and longer retention times, which would translate into apparently much lower molecular weight averages.

Sample Preparation

Two novolac resins were analyzed to obtain an indication of differences in molecular weight, if any. The samples were made up at 0.2 % (w/v) in DMSO, with 0.1 % LiBr added to reduce sample aggregation, and injected without further treatment.

Conditions

Columns:	2 x PolarGel-M, 300 x 7.5 mm (p/n PL1117-6800)
Eluent:	DMSO & 0.1 % LiBr
Flow Rate:	1.0 mL/min
Injection Volume:	100 μ L
Temperature:	50 $^{\circ}$ C
Detectors:	Agilent PL-GPC 50, RI

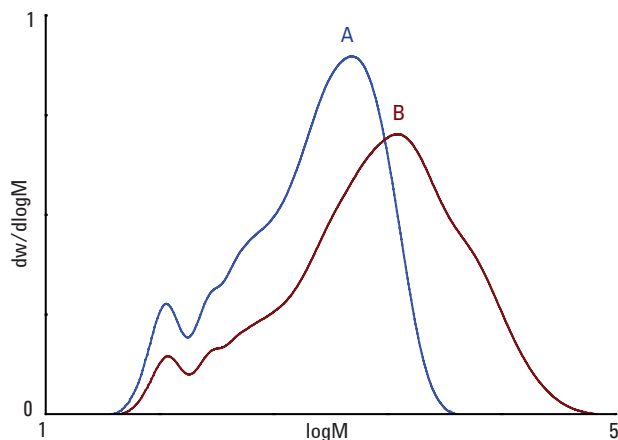


Figure 1. Overlaid molecular weight distributions of two novolac resins

Results

Figure 1 shows the overlaid molecular weight distributions of two novolac resins.

Conclusion

GPC with PolarGel-M columns allows for the artifact, interaction free calculation of the composition and molecular weight distributions of novolac resins that are difficult to analyze on traditional, organic (PS/DVB) GPC columns.

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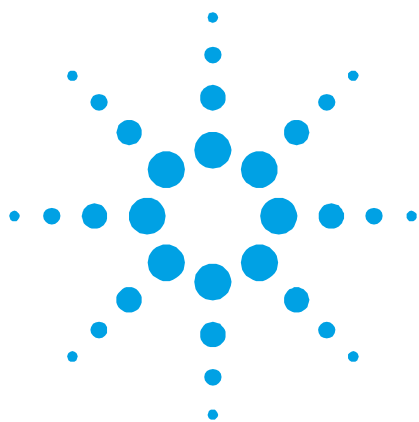
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Agilent Technologies



Material analysis by infrared mapping: A case study using a multi-layer paint sample

Application Note

Author

Dr. Jonah Kirkwood, Dr. John Wilson and Dr. Mustafa Kansiz

Agilent Technologies, Inc.

Introduction

Agilent's 610 FTIR fourier transform infrared (FTIR) microscopes are routinely used for the analysis of heterogeneous materials. They provide an ability to characterize the spatial distribution of components as well as the ability to identify the specific chemical nature of a sample. Agilent's infrared microscopes can be used on both the microscopic and macroscopic scale using multiple measurement modes including:

- transmission
- reflection
- attenuated total reflectance (ATR)
- grazing angle reflection analysis
- 'large sample' mode using Agilent's large sampling side-port accessory

They are ideal for advanced materials characterization as they are simple to use, provide the best sensitivity and versatility, and can be customized to suit a desired area of analysis. By adding a motorized sample stage to an Agilent Cary 610 FTIR single-element detector microscope system, the capabilities can be extended to include automated infrared mapping analysis.



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Infrared mapping allows for multiple infrared spectra to be sequentially acquired from different spatially-resolved points on the same sample and provides both spectral and spatial information, thereby facilitating the study of within-sample chemical heterogeneity. Common infrared mapping applications in material sciences include simple material characterization, the analysis of the homogeneity of coating materials, the investigation of multi-layer sample interfaces such as polymer laminates and paint cross-sections, the automated screening of samples for defects or contamination, the characterization of the total reflectance of optical surfaces and other process control applications.

This paper highlights the simplicity and power of Agilent's Agilent Cary 610 infrared mapping microscope for the rapid and automated analysis of a multi-component paint sample.

Instrumentation

The infrared mapping experiment was conducted using a Cary 610 FTIR spectrometer, equipped with a 610 FTIR infrared microscope (containing a 250 micron single-element, narrow-band Mercury Cadmium Telluride detector and a motorized sample stage) operating under Resolutions Pro 5.0 software. A constant flow of dry air was used to purge the system, limiting the contributions from carbon dioxide and atmospheric water vapor.

The infrared map was collected in reflection-mode using a pre-loaded grid mapping template that was customized to collect a 19×19 grid (totaling 361 spectra) using a $20\ \mu\text{m}$ step size from an area measuring 380×380 microns. The infrared spectra were sequentially recorded over the range of $4000\text{--}700\ \text{cm}^{-1}$ at a spectral resolution of $8\ \text{cm}^{-1}$ by co-adding 16 scans per point (~ 40 mins for the entire infrared map).

Sample preparation

The paint chip cross sections were prepared from vehicle paint fragments provided by a police forensic laboratory. Samples were mounted in a clear casting polyester resin, and then polished using a 12,000-mesh Micromesh polishing cloth. The embedded paint fragments were microtomed to a thickness of $\sim 10\ \mu\text{m}$, and the samples transferred to a standard glass microscope slide that was covered with aluminum foil to allow for reflection/absorption analysis.

Results and discussion

Infrared mapping using Agilent's Cary 610 FTIR Microscope allows for the automated sequential acquisition of hundreds of high-quality infrared spectra from analytical samples. Using Resolutions Pro software, mapping experiments are extremely flexible. Users can either select individual spectral collection locations themselves or use one of several grid mapping templates that can be customized to a sample, saved and re-applied later. In this experiment, a paint fragment found at an automobile crime scene was embedded in a polymer resin, then microtomed to obtain an appropriate sample thickness. This sample was deposited onto the surface of a reflective infrared support slide which was then placed on the motorized stage of the microscope. A visual image of the paint sample was acquired, followed by the sequential collection of the 361 spectra (19×19 grid map; $380 \times 380\ \mu\text{m}$ area) using automated infrared mapping. The visual image of the sample and the spectral acquisition locations are shown in Figure 1. Each spectrum in the infrared map results from a spatial resolution of $20\ \mu\text{m}$.

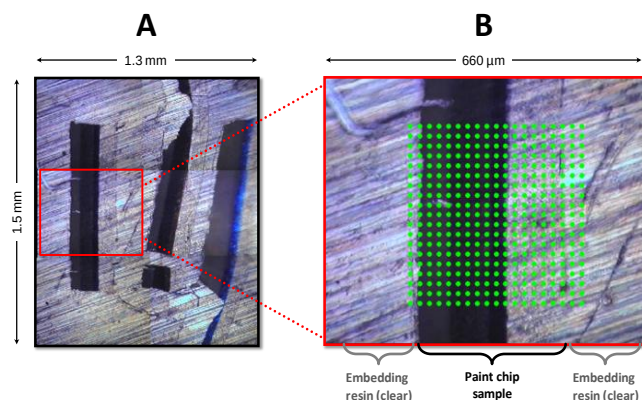


Figure 1. (A) Visual image of 3 sections of a paint chip sample (vertical bars), which were embedded into a polyester resin (clear). The reflective aluminum IR-slide upon which the samples are deposited can be seen through the resin. (B) Higher resolution view of a paint chip sample overlaid with the locations of spectral acquisition (represented by the grid of green circles). The overall area of analysis for the spectral map was $380 \times 380 \mu\text{m}$, yielding a total of 361 spectra.

The investigation and interpretation of the infrared data was simplified by several intuitive software features. For example, the grid of green circles that is overlaid on the surface of the visual image of the sample can be used to extract spatially resolved data. Simply clicking on a desired sample location (or multiple locations) will fill in the green circle(s) and will display the corresponding IR spectra in the software's 'spectrum' display panel. Spectral peaks of interest can then be compared or used for quantitative analysis, and the selected spectra can be overlaid or stacked to facilitate visual interpretation. Upon cursory visual examination of the forensic evidence in Figure 1, the vertical black strip appeared to be uniform in composition with only minimal variations. However, infrared investigation revealed that the sample is heterogeneous and composed of multiple spatially-resolved vertical layers. Exploratory investigation of the spectra in the map revealed the presence of four chemically distinct layers. In addition, the high spatial resolution of the infrared map allowed for the identification of localized areas with different chemical compositions within the stratified layers. Figure 2 illustrates selected absorbance spectra from the paint chip sample.

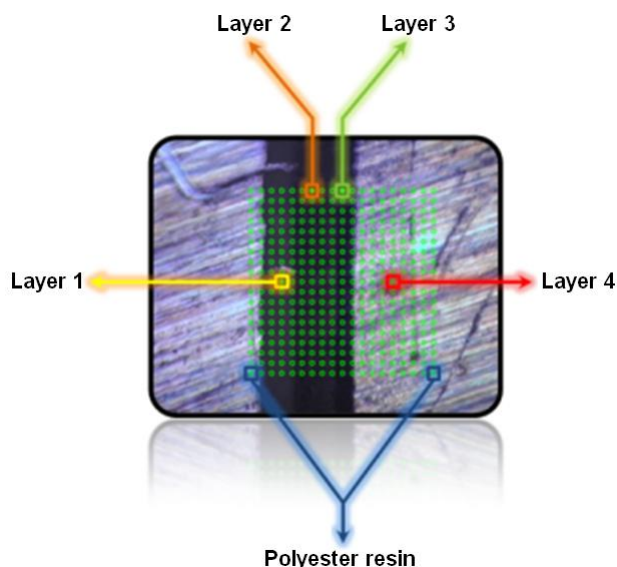
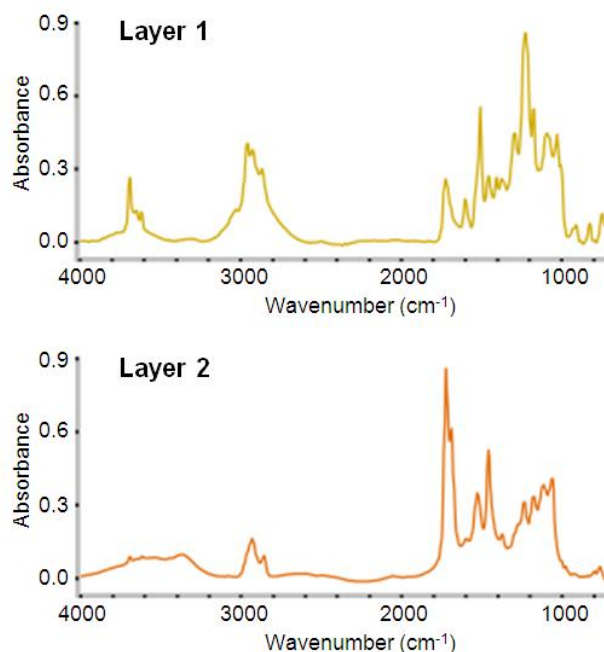
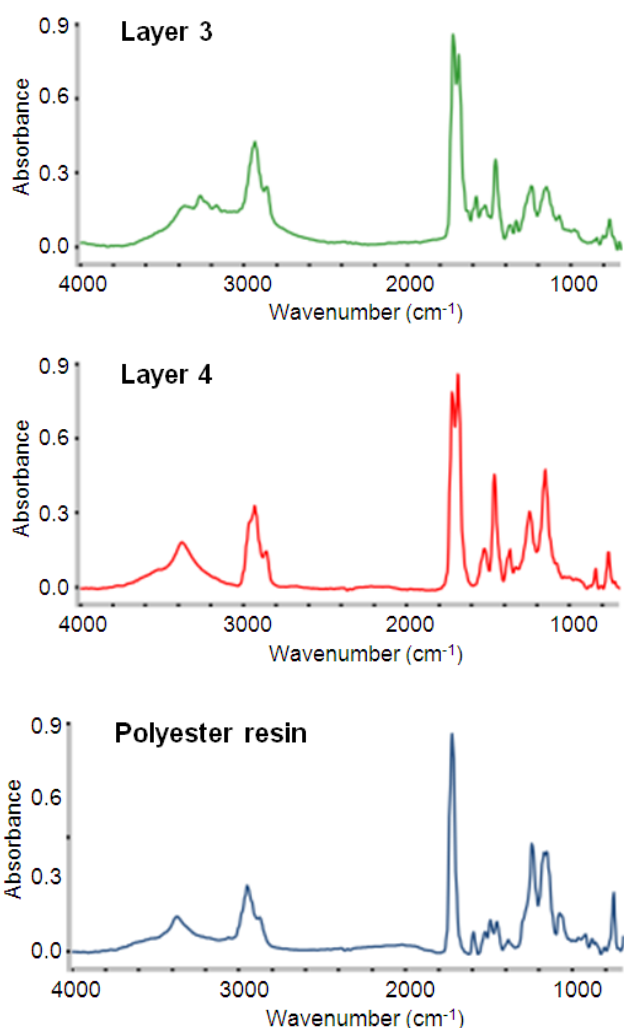


Figure 2. Representative FTIR spectra from the four layers of the paint chip sample as well as a spectrum of the embedding resin. Three of the spatially-resolved layers are in the black vertical bar, while one layer is transparent, as is the polyester resin. See layer spectra in the five images below.





The spectra in Figure 2 are visually distinct and contain sufficient information to allow for the characterization of each individual layer. Based on these spectra, forensic scientists are able to search spectral databases of paint and coating samples to identify the vehicle's make, model, year, and color. In this instance, the ability to detect trace materials in the evidence proved to be very useful in extending the knowledge of the sample's composition far beyond that which could have been obtained by in-bench FTIR experiments or by other analytical techniques.

Without a clear delineation of the layers, it is difficult to study the variations in sample chemistry across the infrared map by using the spectrum display alone. Resolutions Pro software makes it easy to view chemical differences across an entire infrared map of a sample. One means of probing a sample is to generate a feature image based on one or multiple spectral peaks (one or multiple functional groups of interest). A feature image assigns a color to the absorbance value of a selected peak (or spectral region) and plots the intensity across the infrared map to easily view spatially-resolved chemical differences on the visual image of a sample. The color red indicates a high absorbance value, while the color blue indicates a lower absorbance value. Figure 3 shows a feature image generated from a spectral peak that is unique to one layer of the paint chip. It is equally possible to view the feature image without displaying the locations of spectra acquisition, or to view it as a '3D' chemical image as shown in Figure 3.



Figure 3. A feature image generated from a spectral peak that is unique to one layer of the paint chip (left), the same feature image shown without the spectral acquisition grid for clarity (center), and the 3-dimensional view of the feature image (right). These images were generated by plotting the intensity of the peak at 3692 cm^{-1} in the spectrum from each pixel across the entire infrared map.

Advantageously, feature images can be generated in real-time using any spectral range or absorbance peak to provide users with a better understanding of a sample's composition. Figure 4 illustrates the feature images generated from the four chemically distinct paint chip layers.

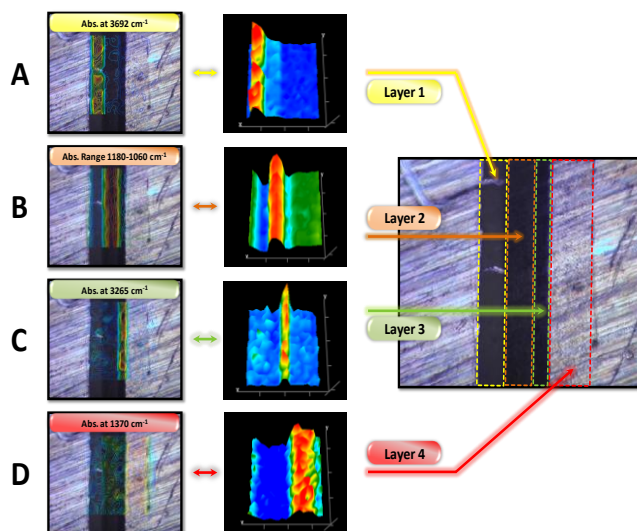


Figure 4. Feature images based on spectral peaks that are unique to each layer in the four-layer paint chip sample. The feature image in 'A' is based on the absorbance of the peak centered at 3692 cm^{-1} , which is primarily found in layer 1 of the paint chip; while the feature image in 'B' was generated from the absorbance peaks between $1180\text{--}1060\text{ cm}^{-1}$, which are largely found in the second layer; 'C' shows the spatial distribution of the absorbance peak centered at 3265 cm^{-1} ; while 'D' shows the feature image of the clear coating layer of the paint sample based on the absorbance at 1370 cm^{-1} . Legend for feature images: red = high intensity, green = medium intensity, blue = low intensity.

The chemical image display of the infrared mapping software was particularly useful to highlight the clear external coating of the paint sample, designated by layer 4 in Figure 4D. Depending on the visible contrast of a sample, it is occasionally easier to view the distribution of a selected spectral peak (or range) in different feature image views. From the feature images it is a simple task to estimate the approximate width of each stratified vertical layer; layer 1 is $\sim 80\text{ }\mu\text{m}$, layer 2 is $\sim 80\text{ }\mu\text{m}$, layer 3 is $\sim 40\text{ }\mu\text{m}$, while layer 4 is $\sim 120\text{ }\mu\text{m}$. It is equally possible to probe the heterogeneity within each layer for an improved characterization of the sample. For example, layer 1 in Figure 4A is not uniform in chemical composition and has a number of visible defects that can also be observed in the visible and feature images. With Resolutions Pro software, it is simple to investigate the chemical differences between adjacent spectra by displaying spectra simultaneously. However, for a more in-depth understanding of the samples' heterogeneity on the

micro-scale, a higher spatial resolution infrared image would be required.

An alternate approach to acquiring IR spectra with a significantly higher spatial resolution involves the use of an infrared imaging system equipped with a focal plane array (FPA*) detector. An FPA-FTIR system would provide a superior means of investigating the subtle chemical differences found in each layer of the paint sample. Unlike infrared mapping using a single-element detector, an FPA* detector collects hundreds to thousands of spectra simultaneously within seconds, thereby providing dramatic savings in spectral acquisition time compared to infrared mapping techniques that perform sequential data collection. In practical terms, this infrared map required ~ 40 minutes acquisition time to collect 361 spectra for the area of $380 \times 380\text{ }\mu\text{m}$ using a $20\text{ }\mu\text{m}$ spatial resolution; comparatively, Agilent's 128×128 FPA-FTIR system could acquire over 16,000 spectra with an identical signal-to-noise ratio from an area of $700 \times 700\text{ }\mu\text{m}$ within a few seconds using an even higher spatial resolution of $5.5\text{ }\mu\text{m}$ per spectrum.

In addition, Agilent's FPA-FTIR imaging spectrometers have a number of easily user-changeable spatial resolution modes including: $1.1\text{ }\mu\text{m}$ (ATR Analysis), $5.5\text{ }\mu\text{m}$, $11\text{ }\mu\text{m}$, $22\text{ }\mu\text{m}$ and even larger sizes with pixel binning or macro imaging (for example, $>40\text{ }\mu\text{m}$). FPA-FTIR analysis would involve the same minimal sample preparation and could be used to reveal even the smallest features of the forensic evidence sample.

While this experiment focused on the characterization of a sample obtained from a crime scene, the application of FTIR microscopy and mapping in paint analysis extends far beyond forensic applications. They are commonly used for the characterization of historical art works, and for the development of conservation and preservation strategies for paintings and photographs. FTIR microscopy and mapping are equally important in the QC analysis of raw materials used in the manufacture of paints and inks, and are routinely applied to the analysis of resins, pigments, solvents and additives.

Conclusion

Agilent's Cary 610 FTIR Microscope provides the ability to collect high quality chemical information from multi-layer samples with a high spatial resolution. It provides an excellent means of probing a sample's chemistry as it can be used to visualize the relative distribution of specific components across a sample area of several centimeters. In this experiment, a $380 \times 380 \mu\text{m}$ infrared map was automatically collected using a pre-defined acquisition grid to investigate the chemical heterogeneity of a paint chip sample. Four chemically distinct layers were resolved in the forensic evidence, including a miniscule layer measuring $\sim 40 \mu\text{m}$.

Feature images also were used to highlight each layer within the infrared map and to probe localized areas with varying chemical compositions within the stratified layers. The rapid nature and the simplicity of automated infrared mapping make it a key technique for the advanced characterization of material and polymer samples.

References

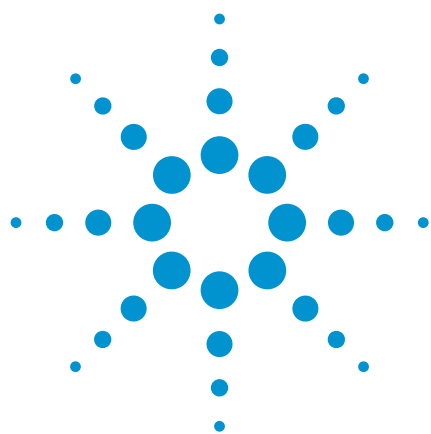
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Agilent Technologies



Analysis of Polysaccharides by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

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Agilent Technologies, Inc.

Introduction

Polysaccharides are complex polymers constructed from sugar units. There is a wide range of polysaccharides, many of which show large structural differences due to the manner in which they are synthesized. This is most commonly seen in the presence of branches on the polymer chains of some polysaccharides, which strongly influences properties such as solution viscosity. Pullulan polysaccharide is composed of maltotriose units in the polymer backbone, produced from starch by the action of a fungus. Pullulan has a linear structure, whereas in contrast dextran is a complex glucan with many differing components manufactured from sucrose by bacterial action that has a highly branched structure. Investigating the structure of polysaccharides is of interest for determining their properties in applications such as their use as food additives.

Gel permeation chromatography (GPC) is a well-known technique for assessing the molecular weight distribution of polymers, a property that influences many of the physical characteristics of these materials. GPC viscometry, employing a viscometer in combination with a differential refractive index detector, has the advantage of allowing the accurate determination of molecular weights for structurally complex polymers and co-polymers regardless of their structure, via the Universal Calibration approach. GPC viscometry also reveals information about the solution viscosity of polymers, a property related to molecular size. Using this information, the branched structure of polymers can be investigated. This application note describes the analysis of two samples of polysaccharide by GPC viscometry, pullulan with a linear structure, and a highly branched dextran.



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Methods and Materials

Conditions

Samples:	Polysaccharides
Columns:	2 x Agilent PL aquagel-OH MIXED-M 8 μ m, 300 x 7.5 mm (p/n PL1149-6801)
Injection Volume:	200 μ L
Eluent:	0.2 M NaNO ₃ + 0.01 M NaH ₂ PO ₄
Flow Rate:	1.0 mL/min
Detector Train:	390-MDS incorporating Viscometer and DRI
Detector Temp:	All detectors set at 40 °C

The 390-MDS was chosen as part of the system as it is capable of multi-detector GPC in aqueous solvents and therefore allows the complex nature of these materials to be investigated.

Results and Discussion

Figure 1 shows an example overlaid multi-detector chromatogram for a sample of pullulan polysaccharide. The material eluted as a broad peak.

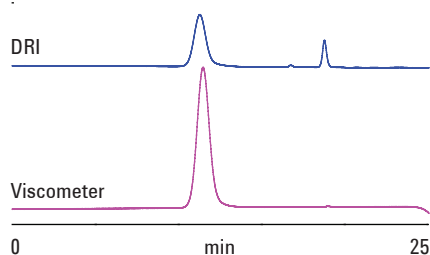


Figure 1. Overlaid multi-detector chromatogram for an example of pullulan polysaccharide

Figure 2 shows an overlay of the accurate molecular weight distributions of the two samples under investigation. As can be seen, they have very different molecular weight distributions.

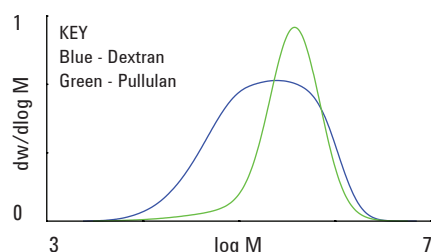


Figure 2. Overlaid multi-detector molecular weight distributions of two samples of polysaccharide

Figure 3 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight for the two samples. Compared to the pullulan, the dextran shows a marked shift of the Mark-Houwink plot to lower intrinsic viscosity values at any given molecular weight. This indicates that dextran is smaller in solution than pullulan across the molecular weight range, a result of the presence of branching on the dextran molecules. The dextran plot is complex and shows some changes in slope, indicating that the degree of branching varies across the range of molecular weight, as expected for a complex material.

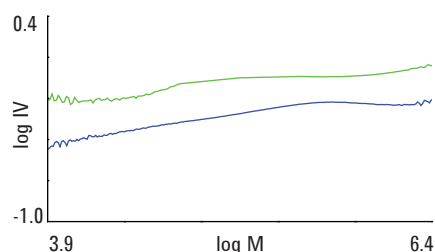


Figure 3. Overlaid Mark-Houwink plots for the two samples of polysaccharide

Conclusion

The data in this application note illustrates how multi-detector GPC employing the 390-MDS can be used to clearly see structural differences between pullulan and dextran with a highly branched structure.

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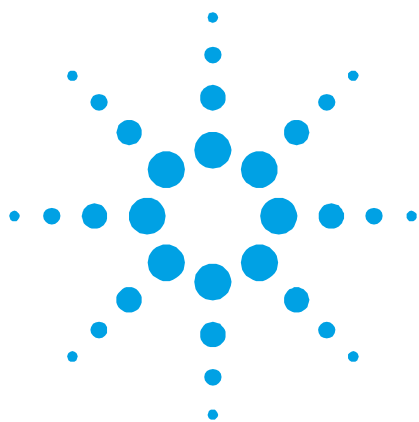
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Agilent Technologies



Quantitative analysis of tint in polymer pellets and disks

Application Note

Author

Alan Marks

Agilent Technologies Ltd.

Introduction

The natural color (usually yellow) of many polymers such as acrylic, polycarbonate, and styrene–acrylonitrile (SAN) is unattractive for commercial purposes. Therefore, manufacturers add tint, or a dye to the polymer to mask the natural color. The final color and clarity of the polymer can determine the commercial value of the final product.

Traditional measurements of tint concentration require molding polymer pellets into disks. The color of the disks is then measured in a spectrophotometer or colorimeter. The time required to melt a representative sample of pellets, mold the melt into a disk, followed by a cooling down period can be one to two hours.

A fast and convenient method for quantitating the amount of tint directly in polymer pellets and molded disks is described.

This method could prevent the production of large amounts of incorrectly dyed polymer by detecting any problems quickly, allowing corrective action to be taken.



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Equipment

- DMS 300 UV–Visible Spectrophotometer
- Diffuse Reflectance Accessory (Integrating Sphere)
- Sekonic Printer/Plotter
- Concentration Application Module

Procedure

Note: Because of the nature of the polymer samples described in this paper, the name of the manufacturer, the polymer, the tint and wavelengths used, must remain proprietary.

Note: This method can also be performed with a Cary 1,3,4 or 5 instrument, fitted with a Diffuse Reflectance accessory and a Concentration application.

A Diffuse Reflectance Accessory (DRA) was used to measure both pellets and molded disks. The DRA consists of a 73 mm diameter integrating sphere with a built-in photomultiplier detector. The sphere's internal surface is coated with a white diffusing material - barium sulfate. The sphere can measure either diffuse reflectance (Figure 1A) or scattered transmittance (Figure 1B) where I_R and I_S represent reflected and scattered radiation, respectively. The sphere was chosen for this work because of its ability to collect a high percentage of the light scattered by the polymer pellets. The samples themselves were translucent, irregular-shaped spheroids of approximately 5 mm in diameter.

As single pellets were too small for representative absorbance measurements, a 1 cm square cuvette was chosen to hold a volume of pellets. Due to their irregular shape and size the pellets did not pack perfectly. Therefore, scattering of the incident radiation was observed when light from a spectrophotometer was passed through a cuvette of polymer pellets.

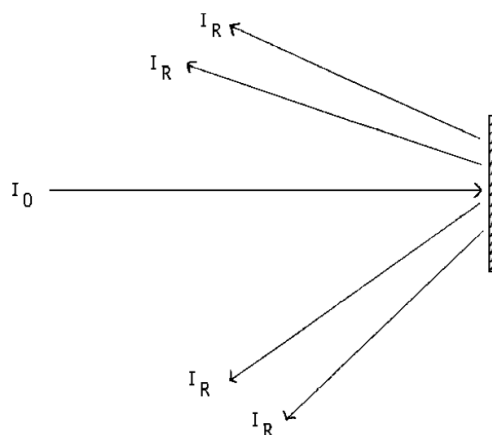


Figure 1A. Reflection off a sample surface.

I_R represents the reflected radiation,
 I_0 represents the incident beam

This effect is illustrated in Figure 1B where I_0 is the incident radiation and is the scattered radiation. In a normal spectrophotometer arrangement the detector may be some distance from the cuvette. The light collection efficiency of such a system is thus very low. When the detector is much closer to the scattering matrix a much greater proportion of scattered radiation is collected. The integrating sphere acts as a highly efficient collector of scattered radiation. The sphere can be used in this manner if the cuvette containing the pellets is located so as to be part of the wall of the sphere as in Figure 2A. Therefore, all of the scattered radiation which passes through the rear face of the cuvette is collected by the sphere. Figure 2B illustrates the relationship between collection efficiency and distance between the sample and detector.

Once the integrating sphere is installed it can also be used to measure the absorbance of molded polymer disks. Molded disks are approximately 120 mm in diameter and 1/8" thick. The molded disks were measured by placing two disks back-to-back followed by a white reflectance disk. Light was passed through the two molded disks, reflected off the white disk, and passed back through the two molded disks into the sphere where it was detected. See Figure 3A.

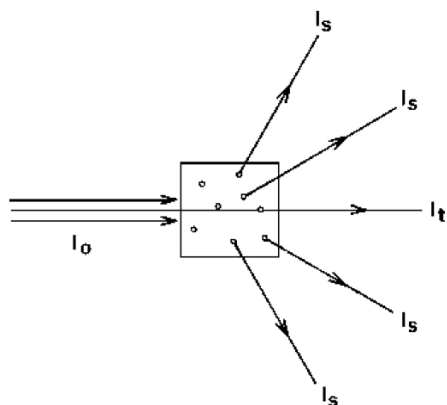


Figure 1B. Scattered transmittance of light through a turbid or opaque sample

I_o represents the incident radiation

I_s represents the scattered transmittance

I_t represents the transmitted light which has not been scattered

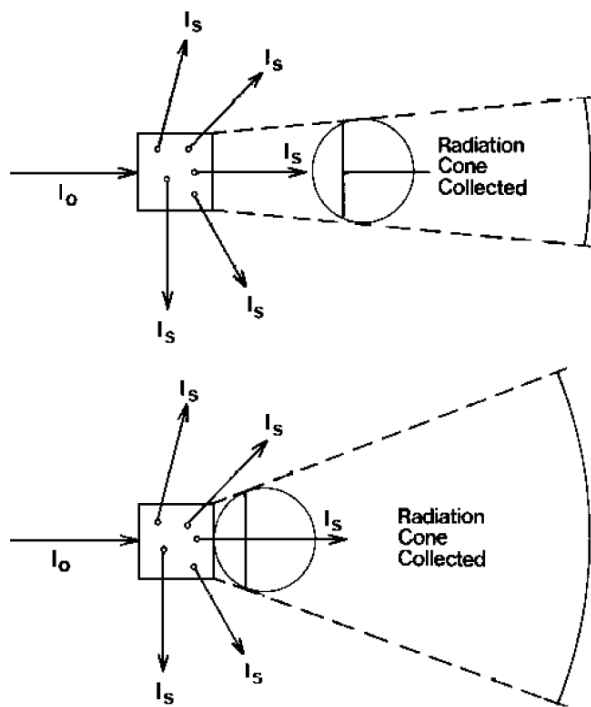


Figure 2A. The light collection efficiency is increased by placing the sample so as to be part of the wall of the sphere

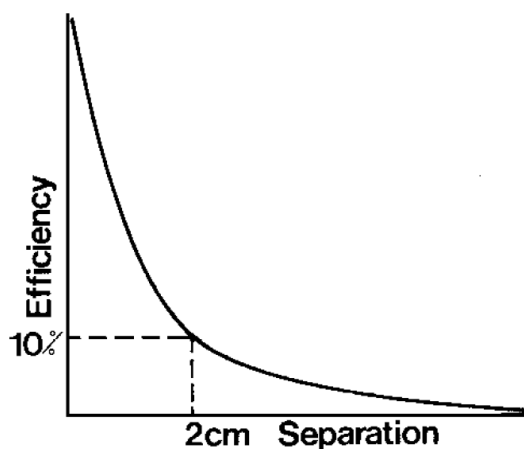


Figure 2B. The relationship between collection efficiency and the separation between the sample and the detector

Quantitative calculations were determined using a Concentration Application Module. The application module plugs into the back of the instrument and contains software for performing quantitative calculations. Access and interaction with the module is performed by following softkey instructions on the CRT of the instrument. Pellet and disk methods can be setup and stored in the module in non-volatile memory. Once a method is set up it can be recalled and executed automatically.

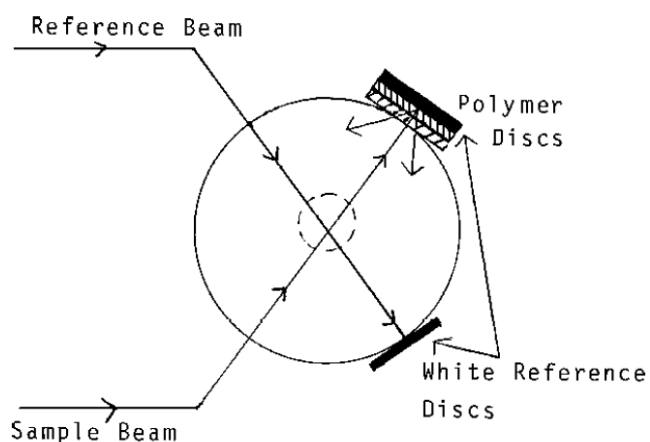


Figure 3A. The sphere configuration for the measurement of the molded polymer discs

Pellet analysis

The pellets were packed into a 1 cm square cuvette and placed in the light path before the sphere. See Figure 3B.

The concentration of tint in the polymer pellets was calculated by measuring the absorbance of the tint at its peak wavelength and a secondary wavelength. These wavelengths were chosen by utilizing a scanning UV–Visible spectrophotometer. The peak wavelength was determined by the spectrophotometer by scanning a sample of moderate concentration (ie. a sample containing enough tint to exhibit a well defined peak). A secondary wavelength was required because of the varying degrees of scattered radiation (or background) from sample to sample. The secondary wavelength was chosen from a series of scans as the wavelength where absorbance was independent of concentration of tint.

Since each pellet-filled cuvette will pack slightly differently, causing varying degrees of background scatter, the net sample absorbance was determined by subtracting the absorbance at the secondary wavelength from the absorbance at the peak wavelength. The sample net absorbance was then compared against a predetermined calibration curve stored in the Concentration Module. The Concentration Module automatically slews to each wavelength, performs the background correction, determines and then prints the final concentration. Five replicates on each sample can be determined in less than 3 minutes.

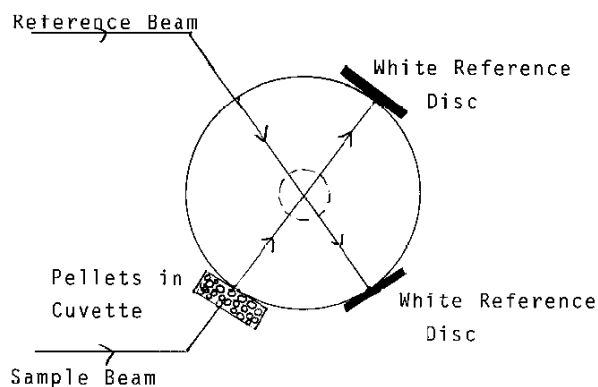


Figure 3B. The sphere configuration for the measurement of the polymer pellets

A calibration graph was determined by measuring, as described above, a series of polymer samples (or standards) of known dye content. Each standard was measured five times. Each measurement represented a different volume of pellets. The Concentration Module calculated the average of the five replicates and the final result was stored in memory. Because of the varying degrees of pellet size and homogeneity, five replicates were chosen to provide the best representative sample. Once the average standard values were determined the Concentration Module calculated a linear least squares calibration graph for the number of standards chosen. The calibration was stored in the Concentration Module in non-volatile memory. Subsequent samples were then measured as outlined previously, and their corresponding concentrations calculated from the calibration graph.

Molded disk analysis

The concentration calculations and calibration graph for tint in molded disks were determined as described in the pellet analysis section. Since disks do not exhibit scattering effects only one replicate was required.

Discussion

This method provides a fast, accurate way to quantitatively determine the concentration of tint in polymer pellets and disks. The calibration graph for pellets is shown in Figure 4 with a printout of the standard data and statistics indicated in Table 1.

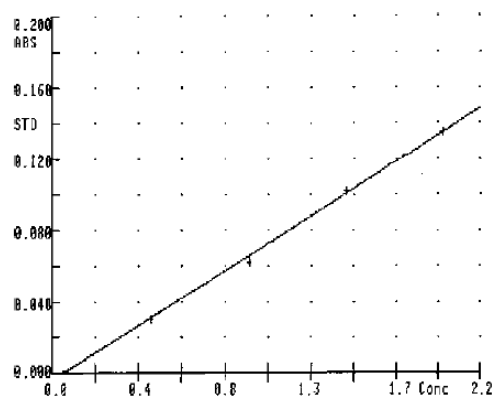


Figure 4. The calibration graph for the analysis of the dye content of the polymer pellets

Table 1. Standard data and statistics for the calibration graph in Figure 4

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	14.44	0.056	0.999	0.000 2.000
	ABS	CONC			
1	-0.002	0.000			
2	0.030	0.500			
3	0.042	1.000			
4	0.101	1.500			
5	0.135	2.000			

Each concentration value is listed in parts per million (ppm) along with corresponding absorbance values. The correlation coefficient (the 'goodness' of the fit of the standard points) of 0.999 is excellent. The results of the same polymer pellets randomly remeasured as samples are shown in Table 2. The theoretical concentrations for each sample are listed in the last column.

Table 2. The results of the pellet samples analysis

Samples	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	14.44	0.056	0.999	0.000 2.000
	ABS	CONC	Weight	Theoretical Conc	
1	0.135	2.006	1.000	2.0	
2	0.033	0.536	1.000	0.5	
3	0.098	1.476	1.000	1.5	
4	0.069	1.055	1.000	1.0	
5	-0.007	-0.039	1.000	0.0	

The calibration graph for molded disks is indicated by Figure 5 with the printout of standard data and statistics shown in Table 3. The correlation coefficient for the disks' standard graph was 0.993.

(The results of the polymer disks randomly measured as samples are shown in Table 4.) Theoretical values are listed in the last column.

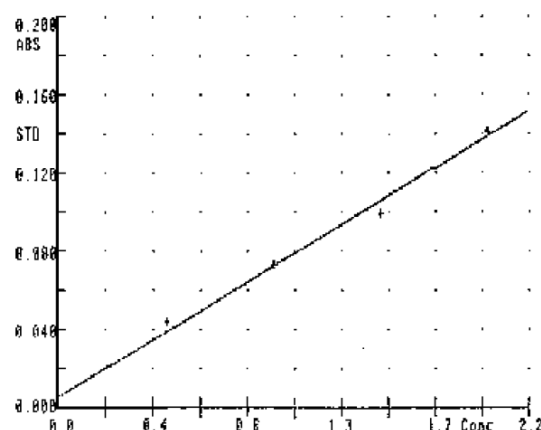


Figure 5. The calibration graph for the molded polymer discs

Table 3. Standard data and statistics for the calibration graph in Figure 5

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	15.03	-0.079	0.993	0.000 2.000
	ABS	CONC			
1	0.003	0.000			
2	0.044	0.500			
3	0.072	1.000			
4	0.099	1.500			
5	0.141	2.000			

Table 4. The results of the analysis of the molded polymer discs

Standards	Factor 1.000	Nom Weight 1.000	WL Mode 2	WL1 xxx	WL2 xxx
	A	B	C	Rsquared	Low High
	0.000	15.03	-0.079	0.993	0.000 2.000
	ABS	CONC	Weight	Theoretical Conc	
1	0.094	1.301	0.000	1.5	
2	0.069	0.952	0.000	1.0	
3	0.142	2.056	0.000	2.0	
4	0.038	0.497	0.000	0.5	
5	0.004	-0.011	0.000	0.0	

Additional applications

Another application of this technique uses the ability to perform wavelength scans on pellets and disks. This application offers the capability to fingerprint the tint used by various competitive manufacturers. Figure 6 shows wavelength scans of various polymer samples with different tints. UV-Visible wavelength scans also allow screening for possible contaminants.

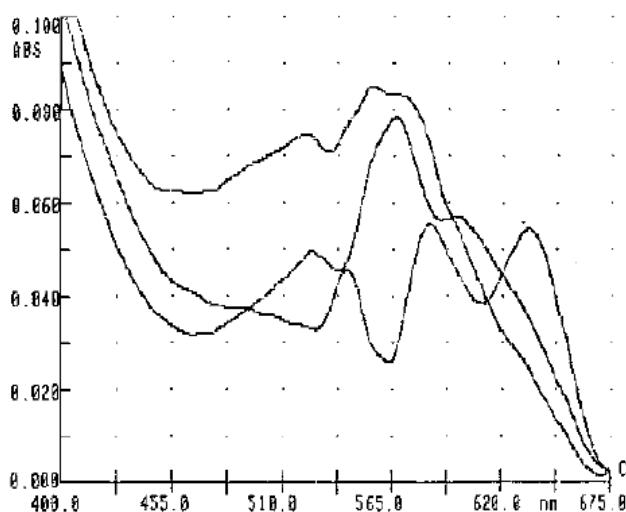


Figure 6. Wavelength scans of various polymer samples with different tints

Conclusion

The DMS 300 in conjunction with the Diffuse Reflectance Accessory and Concentration Application Module performs an automated analysis of polymer tint in pellets and disks. The analysis of tint in a polymer pellet sample can be accomplished in a matter of a few minutes, versus one to two hours by traditional methods. Potentially this can avoid large wastage of polymer material.

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POWER GENERATION

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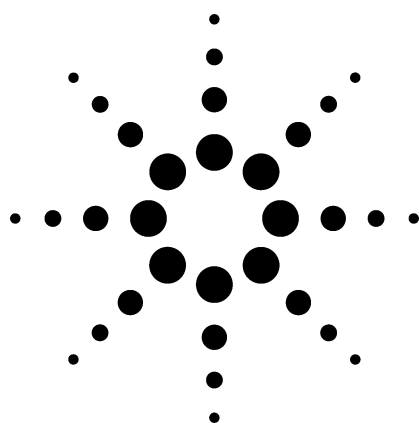
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The Measure of Confidence

- TOGA Analyzer SP1 2310-0047
- Permanent Gases on a COX Module Using a Agilent 490 Micro GC
- Using the Agilent 490 Micro GC for the Monitoring of a Circulating Fluidized Bed Biomass Gasifier
- Fast Separation of Oxygen and Nitrogen on a MolSieve 5A Channel Using the Agilent 490 Micro GC
- Permanent Gas Analysis - Separation of Argon and Oxygen on a MolSieve 5A Column using the Agilent 490 Micro GC
- C1 - C5 Hydrocarbon Analysis Using the Agilent 490 Micro GC - Separation Characteristics for PoraPLOT U and PoraPLOT Q Column Channels
- Analysis of Biogas Using the Agilent 490 Micro GC Biogas Analyzer

TOGA Analyzer SP1 2310-0047

Technical Overview



Application Highlights

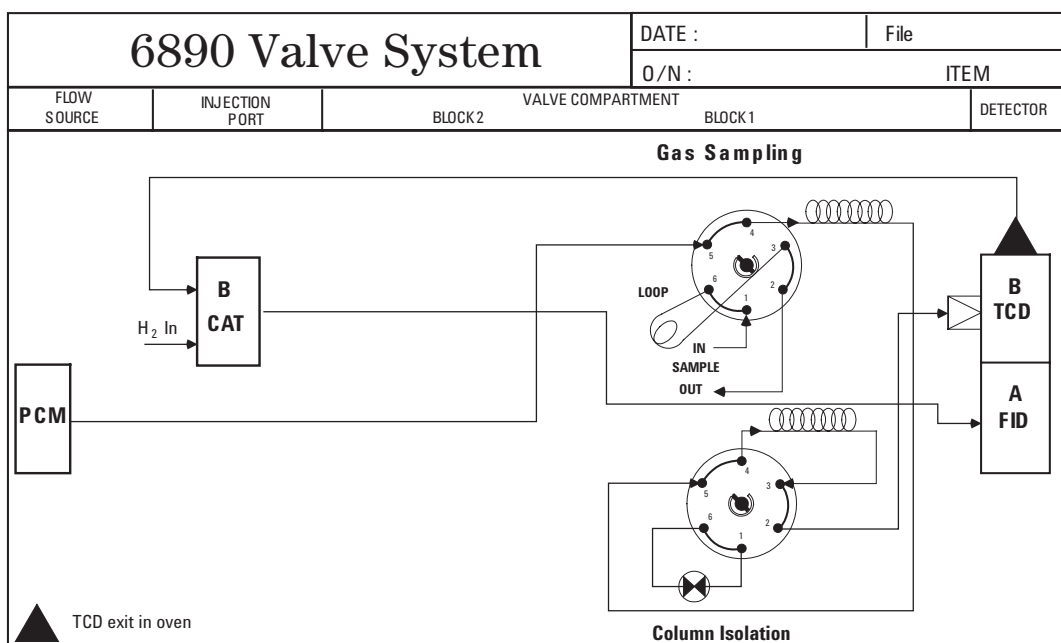
- A Thermal Conductivity Detector (TCD) and a Flame Ionization Detector (FID) working with a methanizer to identify hydrogen, oxygen, nitrogen, carbon monoxide, carbon dioxide, ethane, ethylene, acetylene, 1-Butene, and propane/propylene.
- Analysis time is approximately 20 minutes.

Configurations

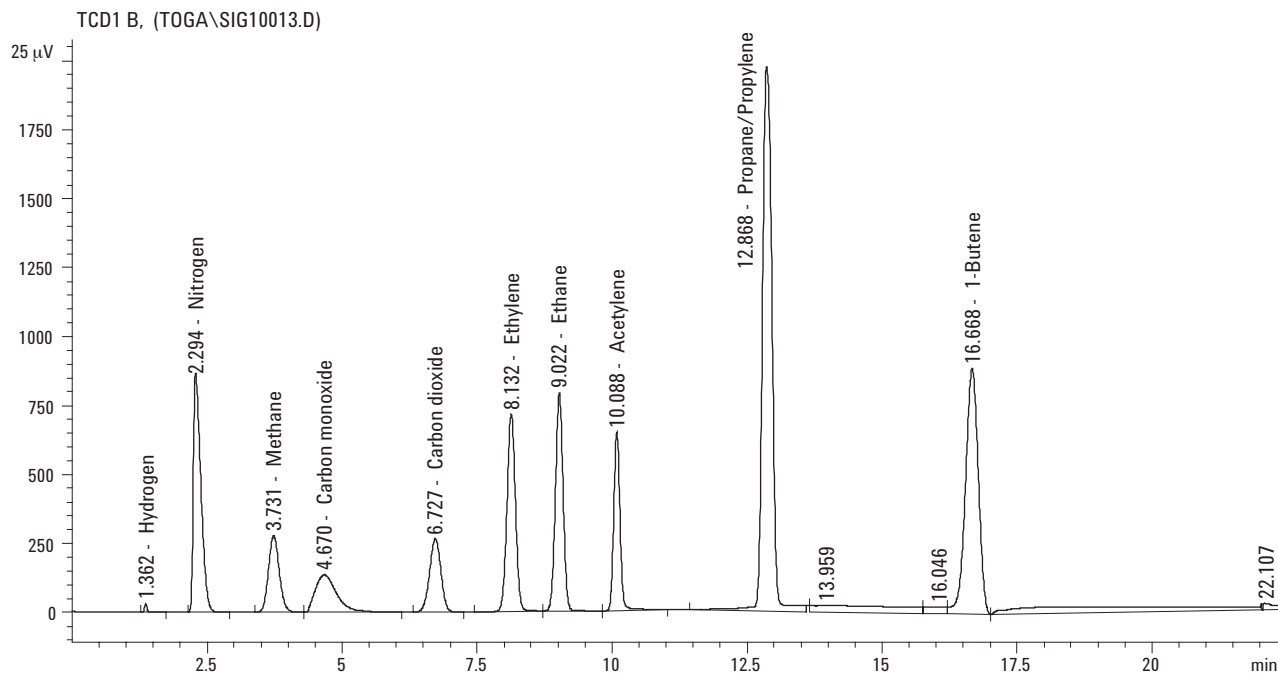
- GC must have options packed inlet (102) or pneumatics control module (PCM) (309), FID (210), TCD (220), methanizer (307), 1-mL sample loop (504), valve box (752), 6-port valve (701) and 6-port valve (702).
- Special includes columns, checkout sample for valve timing, factory checkout with chromatograms, method supplied on disk for ChemStation and user manual.

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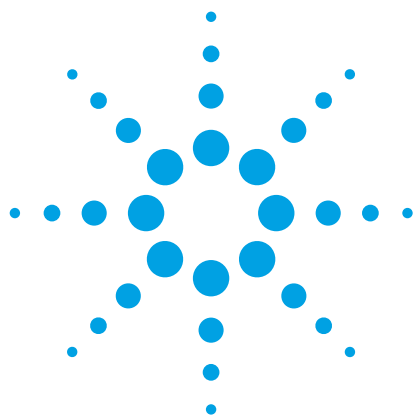
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5988-9300EN



Agilent Technologies



Fast Separation of Oxygen and Nitrogen on a MolSieve 5A Channel Using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Authors

Mohamed Bajja and Remko van Loon
Agilent Technologies, Inc.
Middelburg
The Netherlands



Introduction

When a really fast separation of Oxygen and Nitrogen is required, the Agilent 490 Micro GC, equipped with a short MolSieve 5A column channel, delivers the speed you need.

This application note shows the fast separation of Oxygen and Nitrogen using a 4 m MolSieve 5A column channel instead of using the standard 10 m MolSieve 5A column channel. The advantage of the Agilent 490 Micro GC, in combination with this 4 m MolSieve 5A column channel, is the ease-of-use and the speed of analysis. Nitrogen will elute in less than 20 s.

Argon and Oxygen will not be separated on the 4 m MolSieve 5A column. These compounds will coelute. The separation of Argon and Oxygen requires the use of a 20 m MolSieve 5A column channel on a low temperature.

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 chromatography.



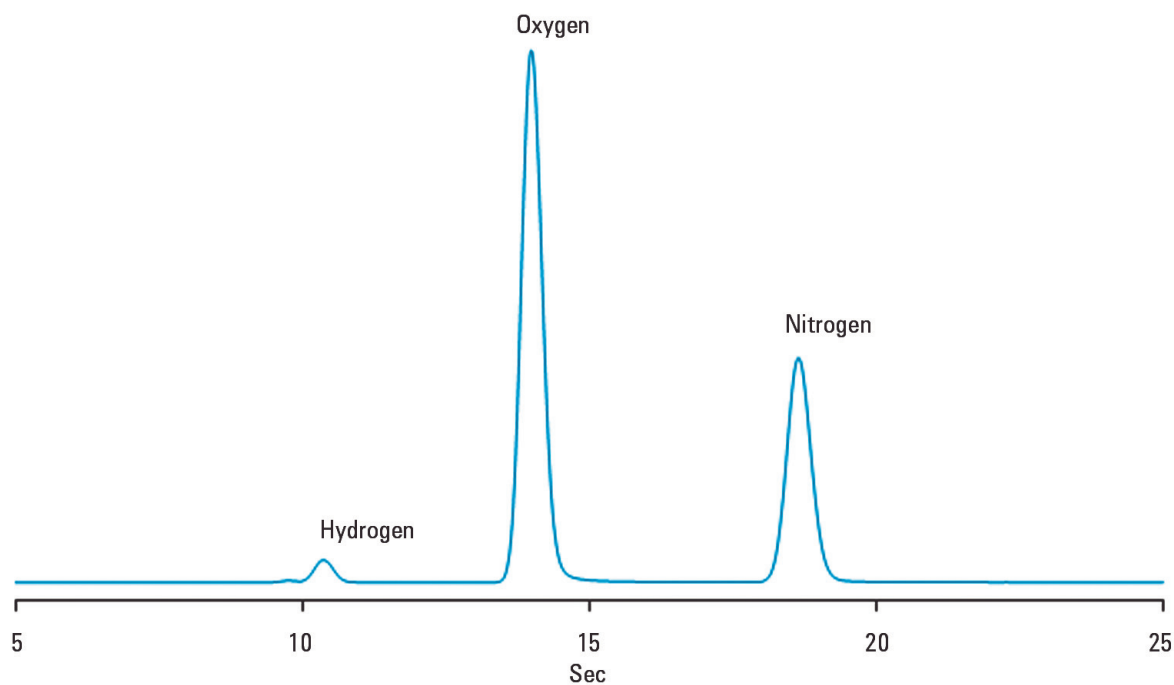
Agilent Technologies

Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	MolSieve 5A, 4 m
Column temperature	100 °C
Carrier gas	Helium, 100 kPa
Injection time	40 msec

Sample information

Hydrogen	1.0%
Oxygen	0.4%
Nitrogen	0.2%



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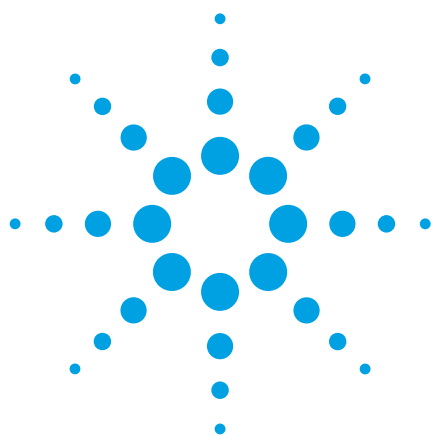
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5990-8529EN



Agilent Technologies



Permanent Gas Analysis – Separation of Argon and Oxygen on a MolSieve 5A Column using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Authors

Mohamed Bajja and Remko van Loon
Agilent Technologies, Inc.
Middelburg
The Netherlands



Introduction

This application note shows an example of the analysis of permanent gases, including the separation of Argon and Oxygen, using the Agilent 490 Micro GC. For the separation of Argon and Oxygen, a High Resolution 20 m MolSieve 5A column is used.

The advantage of the Agilent 490 Micro GC is speed of analysis. Even with the 20 m HR MolSieve 5A column, you get the results fast. Total analysis time for the permanent gases until Nitrogen is approximately 3 minutes. The Agilent 490 Micro GC delivers lab-quality separations in an ultra-compact, portable instrument.



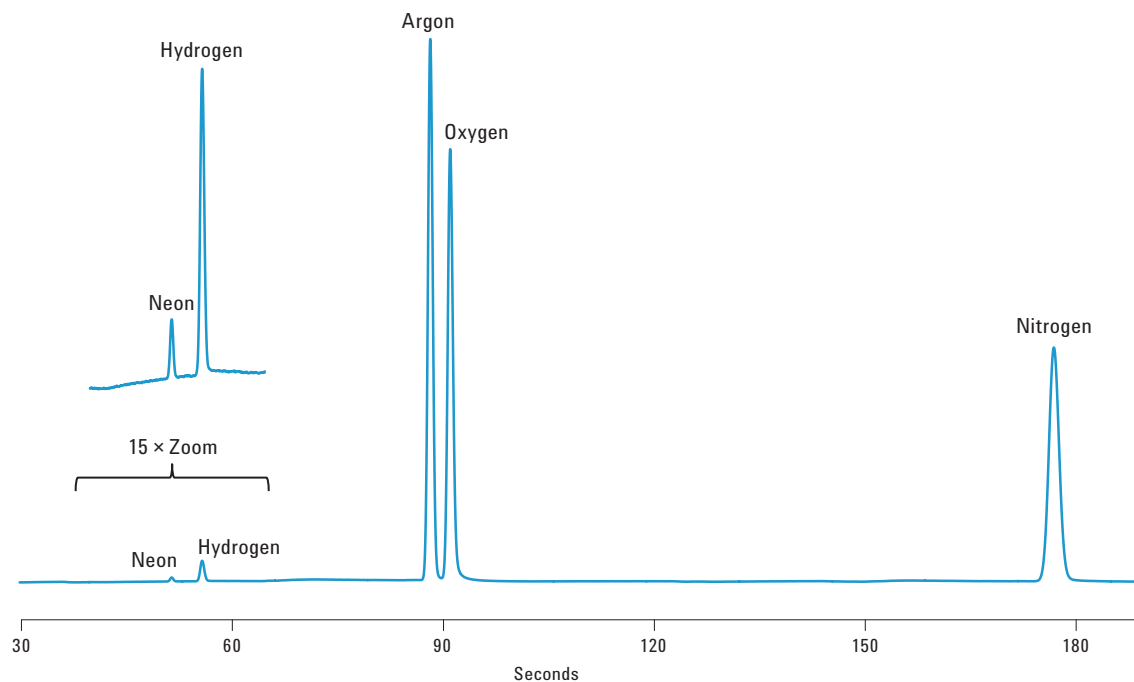
Agilent Technologies

Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	20 m MolSieve 5A
Column temperature	40 °C
Carrier gas	Helium, 200 kPa
Injection time	40 msec

Sample information

Neon	18 ppm
Hydrogen	1.0 %
Argon	0.2 %
Oxygen	0.2 %
Nitrogen	0.2 %
Helium	matrix



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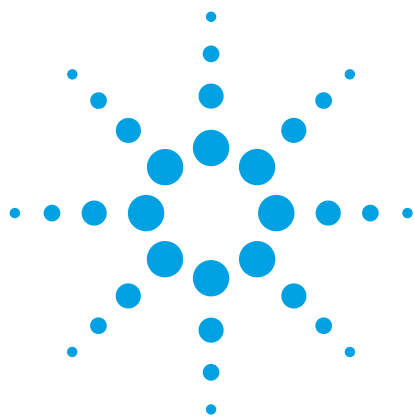
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Analysis of Biogas Using the Agilent 490 Micro GC Biogas Analyzer

Application Note

Micro Gas Chromatography, Hydrocarbon Processing, Renewable Energy, Biogas Analysis

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Middelburg, the Netherlands

Abstract

Biogas is considered a renewable and sustainable energy source and therefore is of great interest worldwide. This application note shows the analysis of biogas, and related samples, using the Agilent 490 Micro GC Biogas Analyzer. Depending on the biogas composition two configurations are available; the Agilent 490 Micro GC Biogas Analyzer for pure biogas analysis and the Agilent 490 Micro GC Biogas Analyzer Extended when biogas is mixed with other hydrocarbon streams, such as natural gas or liquefied petroleum gas (LPG).

Introduction

Biogas is a gas mixture produced through biological processes; from anaerobic fermentation or digestion of organic material such as biomass, manure or sewage, municipal waste and energy crops. The composition of biogas is related to the origin of the organic material; the main components of biogas are methane and carbon dioxide, with some other permanent gases, hydrogen and hydrogen sulfide.

Biogas has a role in modern waste management and can fuel any type of heat engine to generate either mechanical or electrical power. To increase its caloric values it is sometimes necessary to remove some of the carbon dioxide or blend it with other hydrocarbon streams. Biogas can be compressed, much like liquefied natural gas, and used to power motor vehicles. For this purpose, it is essential to remove hydrogen sulfide if present. Biogas is a renewable fuel, and so it qualifies for renewable energy subsidies in some parts of the world.

The increased interest in biogas has resulted in a growing demand for fast and efficient analysis technology to determine its composition. The Agilent Micro GC Biogas Analyzers can play a significant role in achieving this goal.



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The Agilent 490 Micro GC Biogas Analyzers are shipped as a total solution; the analyzers are factory tuned, for optimal separation, and come with final test data, analytical method parameters, a user manual and a check-out sample.

Biogas Analyzer setup and conditions

Based on the Agilent 490 Micro GC, two Biogas Analyzers are available; the configuration required for biogas analysis depends on the sample composition.

For pure biogas analysis, including permanent gases and hydrogen sulfide, the Agilent 490 Micro GC Biogas Analyzer (p/n G3582A#110) is recommended, even ethane and propane can be analyzed with this setup. This Biogas Analyzer consists of a dual channel cabinet including a 10-meter CP-Molsieve 5A with argon as a carrier gas, providing excellent sensitivity and linearity for hydrogen, and a 10-meter CP-PoraPLOT U column channel with helium as carrier gas.

When biogas is mixed with other hydrocarbon streams such as natural gas or liquefied petroleum gas (LPG), the sample contains higher boiling hydrocarbons. To analyze these hydrocarbons the Agilent 490 Micro GC Biogas Analyzer Extended is the analyzer of choice. This Biogas Analyzer Extended (p/n G3582A#111) is a quad channel cabinet Micro GC including three column channels; a 10-meter CP-Molsieve column on argon as carrier gas, a 10-meter CP-PoraPLOT U column and an additional 6-meter CP-Sil 5 CB column on helium as carrier gas for the analysis of higher boiling hydrocarbons. Figure 1 shows the quad and dual cabinet housing for the Agilent 490 Micro GC Biogas Analyzers.



Figure 1. Agilent 490 Micro GC Biogas Analyzers.

Both Biogas Analyzers are equipped with heated sample line and injectors to eliminate any cold spot and prevent possible condensation of moisture, to ensure the integrity of the sample is maintained throughout the sample flow path. Both CP-Molsieve 5A and CP-PoraPLOT U columns have a back-flush to vent option, moreover the CP-Molsieve 5A is equipped with the retention time stability (RTS) option. This RTS option consists of additional in-line filters between the electronic gas control and the column module to ensure moisture and carbon dioxide free carrier gas. Moreover the use of the RTS option enables a more efficient backflush of carbon dioxide. This enhances column lifetime and, most importantly, leads to more stable retention times.

Table 1 gives an overview of typical conditions used for the Biogas Analyzers.

Table 1. 490 Micro GC Biogas Analyzer Instrument Conditions

	CP-Molsieve 5A, 10 m	CP-PoraPLOT U, 10 m	CP-Sil 5 CB, 6 m
Column temperature	80 °C	80 °C	60 °C
Carrier gas	argon, 200 kPa	helium, 150 kPa	helium, 150 kPa
Injector temperature	110 °C	110 °C	110 °C
Injection time	40 ms	40 ms	40 ms
Backflush time ¹	11	14	no backflush
Detector sensitivity	auto	auto	auto
Invert signal	yes	no	no
Sample line temperature	110 °C		
Sampling time	30 seconds		

Note ¹ Backflush time is column channel dependent and should be fine tuned for each new column.

The sample can be introduced to the Agilent 490 Micro GC Biogas Analyzer either pressurized (maximum limit 1 bar), through a Tedlar sampling bag using the internal sampling pump, or by using a continuous flow sampling mode. When the sample pressure exceeds the 1 bar limit, for example with a liquefied natural gas or liquefied petroleum gas, the pressure should be reduced. The use of the Agilent Micro-Gasifier, a heated pressure reducer, is recommended here.

Results and Discussion

The first column channel, a CP-Molsieve 5A, is used to analyze the permanent gases, including hydrogen, oxygen, nitrogen, methane, and carbon monoxide. Figure 2 shows a chromatogram for this column channel.

As biogas and related samples may contain larger amounts of carbon dioxide, moisture, and higher hydrocarbons it is necessary to backflush these components to maintain the separation efficiency of the Molsieve 5A column. Moisture and carbon dioxide tend to adsorb quickly to the Molsieve 5A stationary phase and change its chromatographic properties. This would result, over time, in retention shifts and loss of separation. Higher hydrocarbons will eventually elute, but will cause higher detector noise levels and lead to reduced sensitivity. The backflush to vent functionality on the Molsieve 5A column channel prevents this from happening.

Table 2 shows excellent repeatability figures for both retention time, below RSD 0.05 %, and peak area below RSD 0.1 %, for the compounds analyzed on the Molsieve column channel.

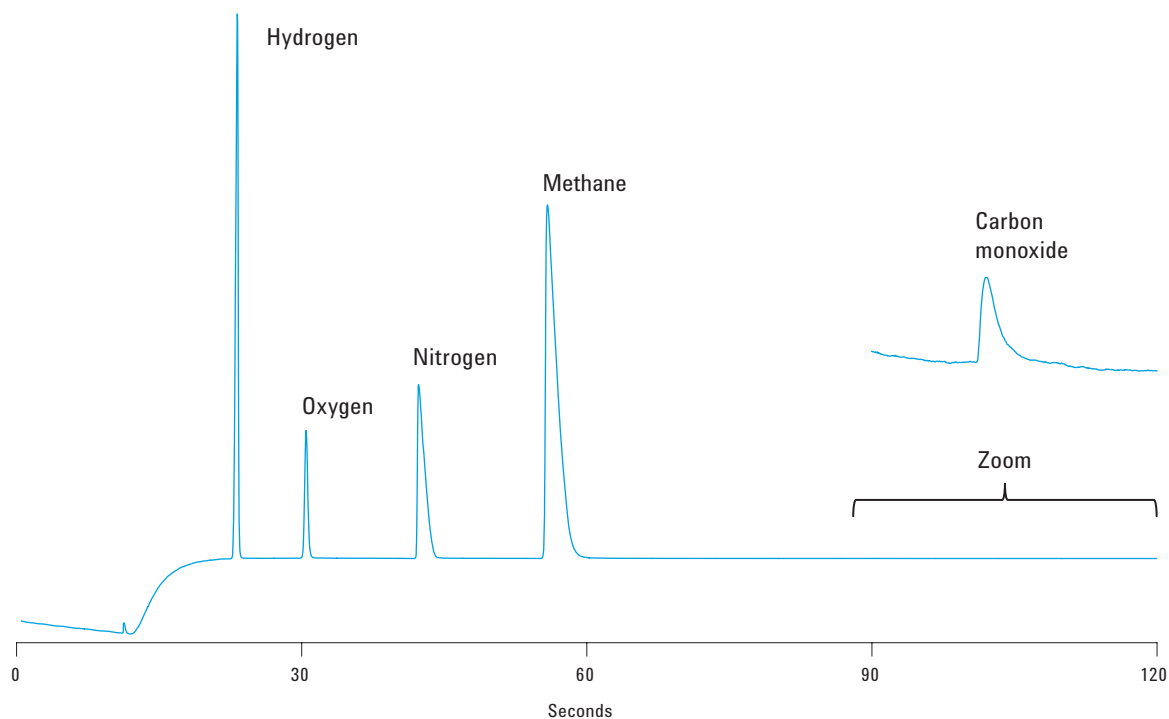


Figure 2. Chromatogram for permanent gases on the CP-Molsieve 5A column channel.

Table 2. Repeatability Figures for Retention Time and Peak Area on the CP-Molsieve Column

Run no.	Hydrogen Rt (sec)	Oxygen Rt (sec)	Nitrogen Rt (sec)	Methane area	Hydrogen area	Oxygen area	Nitrogen area	Methane area
1	23.23	30.46	42.31	55.85	5852426	1594746	4855956	15750694
2	23.22	30.46	42.31	55.85	5852402	1594913	4856189	15752646
3	23.22	30.45	42.30	55.85	5849806	1594074	4853402	15749892
4	23.22	30.45	42.30	55.85	5857044	1596055	4859671	15769519
5	23.22	30.46	42.31	55.86	5853222	1595289	4856426	15762840
6	23.23	30.46	42.30	55.85	5847437	1593546	4853332	15742096
7	23.22	30.45	42.30	55.85	5855831	1596512	4860136	15768153
8	23.23	30.46	42.31	55.86	5846434	1594241	4854710	15745279
9	23.22	30.46	42.30	55.85	5860122	1597659	4864955	15785858
10	23.22	30.45	42.30	55.85	5852819	1595989	4860359	15768762
Average	23.22	30.46	42.30	55.85	5852754	1595302	4857514	15759574
Std. dev.	0.0048	0.005	0.005	0.004	4210	1258	3691	13699
RSD (%)	0.021	0.017	0.012	0.008	0.072	0.079	0.076	0.087

For pure biogas, carbon dioxide and hydrogen sulfide are analyzed on a CP-PoraPLOT U column channel. When biogas is mixed with other hydrocarbon streams, ethane and propane can also be analyzed on this channel. Baseline separation of

carbon dioxide, ethane, hydrogen sulfide, and propane is obtained, shown in Figure 2. Higher hydrocarbons present in the sample are backflushed to vent; which prevents late eluting components from interfering in the next analysis.

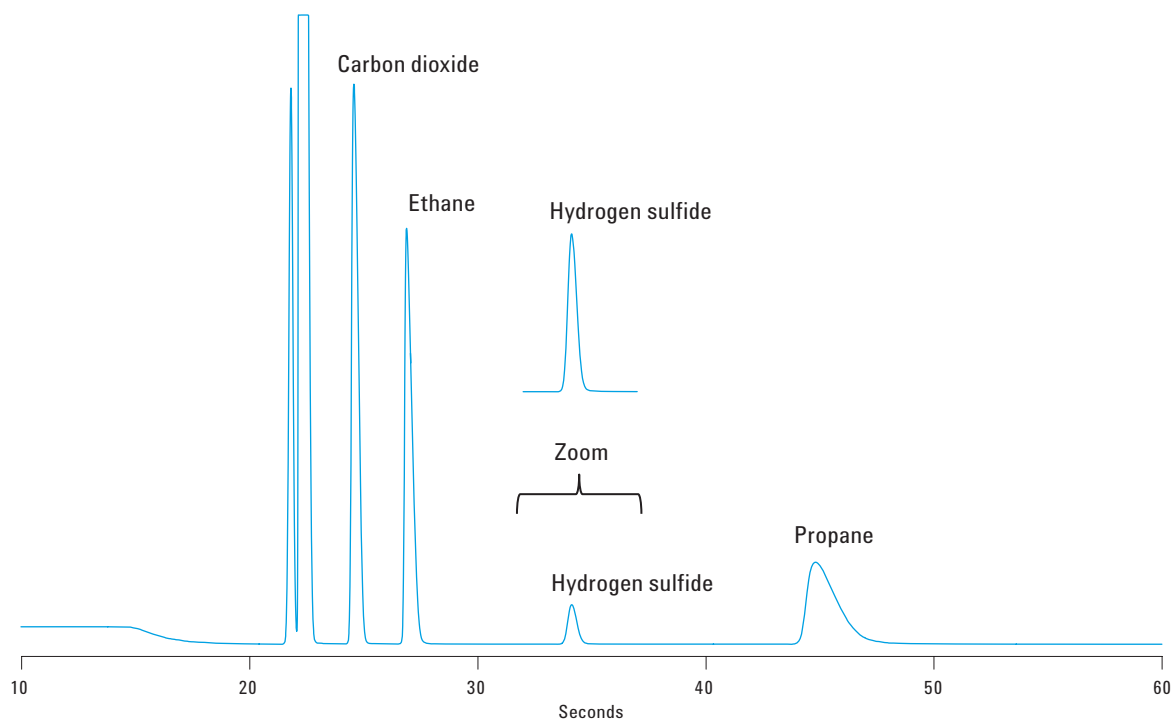


Figure 3. Chromatogram for carbon dioxide, hydrogen sulfide, ethane, and propane on the CP-PoraPLOT U channel.

The stainless steel tubing in the CP-PoraPLOT U channel and the sample inlet of the Micro GC have an UltiMetal deactivation layer, which results in an inert sample flow path and better performance for hydrogen sulfide analysis. Results presented in Table 3 shows very good repeatability figures for hydrogen sulfide and the other compounds (carbon dioxide, ethane, and n-propane) analyzed on the CP-PoraPLOT U channel. Relative standard deviation (RSD %) below 0.02 % for retention time and below 0.15 % based on area illustrates the system's suitability for this type of analysis. Moreover the UltiMetal deactivated sample inlet tubing provides an excellent peak shape for hydrogen sulfide, see Figure 3.

The CP-Molsieve and CP-PoraPLOT U channel, chromatograms as shown in Figure 3, are part of both the Biogas and Extended Biogas Analyzer.

Table 3. Retention Time and Peak Area Repeatability Results for the CP-PoraPLOT U Column

Run no.	Carbon dioxide Rt (sec)	Ethane Rt (sec)	Hydrogen sulfide Rt (sec)	n-Propane Rt (sec)	Carbon dioxide area	Ethane area	Hydrogen sulfide area	n-Propane area
1	24.56	26.87	34.11	44.80	3240882	2662227	320047	2175181
2	24.56	26.88	34.12	44.80	3239148	2660569	319969	2178315
3	24.56	26.87	34.12	44.80	3240617	2662025	320273	2181300
4	24.56	26.87	34.11	44.79	3239973	2661327	320031	2180366
5	24.56	26.87	34.11	44.79	3239006	2661163	319909	2178141
6	24.56	26.87	34.11	44.80	3240134	2661385	319833	2174648
7	24.55	26.87	34.11	44.79	3239972	2661379	320000	2173550
8	24.55	26.87	34.11	44.79	3238407	2660348	319721	2177678
9	24.56	26.87	34.11	44.79	3238332	2660512	320024	2179891
10	24.55	26.87	34.11	44.79	3237012	2659615	319789	2176390
Average	24.56	26.87	34.11	44.79	3239348	2661055	319960	2177546
Std. dev.	0.0048	0.0032	0.0042	0.0052	1197	797	157	2578
RSD (%)	0.020	0.012	0.012	0.012	0.037	0.030	0.049	0.12

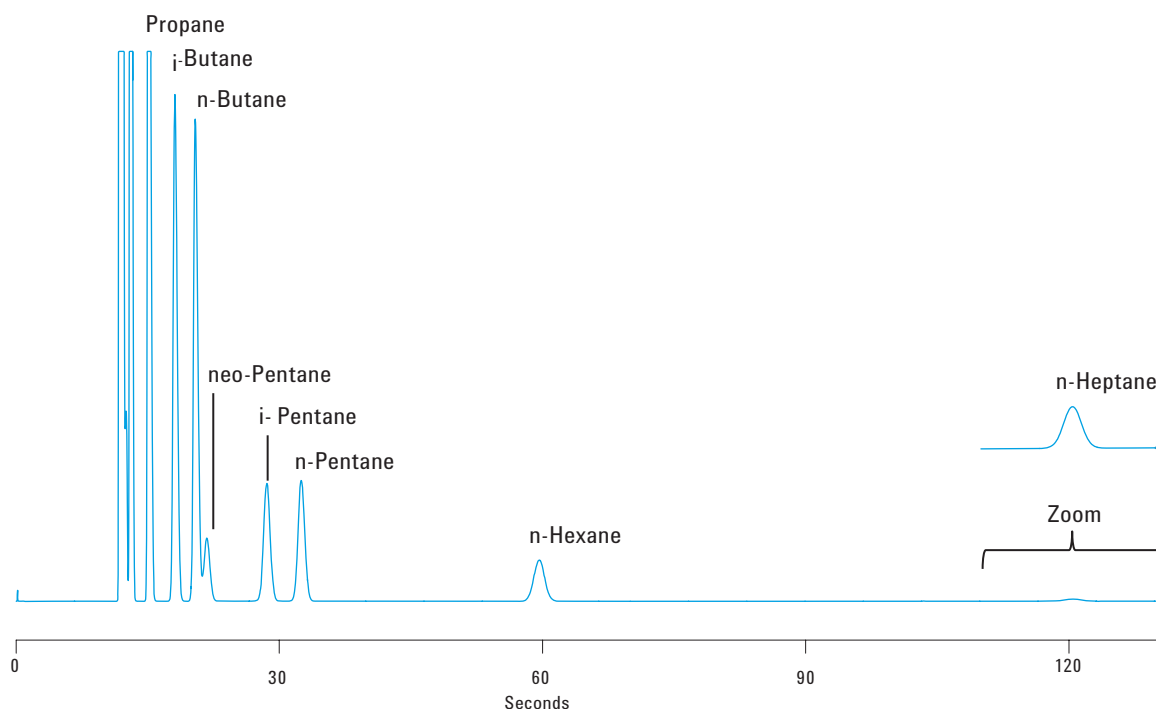


Figure 4. Chromatogram on the CP-Sil 5 CB, separating the hydrocarbons from butanes to n-heptane.

In Figure 4, the chromatogram illustrates the separation and quantification of the higher boiling hydrocarbons as part of the Extended Biogas Analyzer setup; the column used is a CP-Sil 5 CB. This additional channel expands the application range of biogas analysis to blends with natural gas or liquefied petroleum gas (LPG). In this particular case, the biogas was mixed with natural gas.

Tables 4a and 4b show repeatability on the CP-Sil 5 CB channel for the hydrocarbons. The repeatability data of approximately 0.05% for retention times and below the 0.2% mark for peak area can be considered as excellent. Even the partially separated neo-pentane shows a good peak area repeatability performance.

Table 4a. Retention Time Reproducibility Data for the CP-Sil 5 CB Channel

Run no.	i-Butane Rt (sec)	n-Butane Rt (sec)	neo-Pentane Rt (sec)	n-Pentane Rt (sec)	i-Pentane Rt (sec)	n-Hexane t (sec)	n-Heptane Rt (sec)
1	18.10	20.43	21.75	28.58	32.52	59.67	120.66
2	18.10	20.43	21.75	28.58	32.52	59.67	120.69
3	18.10	20.42	21.74	28.58	32.51	59.66	120.70
4	18.10	20.42	21.74	28.57	32.51	59.66	120.71
5	18.09	20.42	21.74	28.57	32.50	59.64	120.72
6	18.09	20.42	21.74	28.57	32.50	59.64	120.72
7	18.09	20.41	21.73	28.56	32.49	59.63	120.72
8	18.08	20.41	21.72	28.55	32.48	59.61	120.73
9	18.08	20.40	21.72	28.55	32.48	59.60	120.72
10	18.08	20.40	21.72	28.54	32.47	59.59	120.74
Average	18.09	20.42	21.74	28.57	32.50	59.64	120.71
Std. dev.	0.0088	0.0107	0.0118	0.014	0.018	0.029	0.023
RSD (%)	0.048	0.053	0.054	0.050	0.054	0.049	0.019

Table 4b. Reproducibility Data, Based on Peak Area, for the CP-Sil 5 CB Column

Run no.	i-Butane area	n-Butane area	neo-Pentane area	n-Pentane area	i-Pentane area	n-Hexane area	n-Heptane area
1	7014680	7186850	1265110	2702141	2781533	1552255	133755
2	7018181	7190966	1264813	2703703	2783345	1553847	133682
3	7018469	7187273	1269047	2704327	2783935	1554441	133642
4	7017302	7188209	1269045	2705176	2784640	1554809	133920
5	7017858	7190794	1264914	2705022	2784520	1554963	133951
6	7024447	7196790	1265962	2707439	2787091	1556518	133959
7	7025658	7196118	1269229	2708459	2787981	1557169	133959
8	7019982	7188645	1270146	2706467	2785715	1555951	133880
9	7018355	7189383	1267352	2706536	2785636	1556096	134091
10	7018173	7190297	1266144	2706696	2785947	1555806	134130
Average	7019311	7190533	1267176	2705597	2785034	1555186	133897
Std. dev.	3315	3418	2043	1888	1865	1439	162
RSD (%)	0.047	0.048	0.16	0.070	0.067	0.093	0.12

Conclusion

The Agilent 490 Micro GC Biogas Analyzer type required depends on biogas sample type. Regular biogas contains methane, carbon dioxide, nitrogen, and sometimes some hydrogen, hydrogen sulfide, and carbon monoxide. For this type of sample, the 490 Micro GC Biogas Analyzer is perfectly suited.

The first column channel, configured with a CP-Molsieve 5A column with argon as carrier gas, will separate and analyze hydrogen, oxygen, nitrogen, methane, and carbon monoxide. Moisture and carbon dioxide, as well as higher hydrocarbons present in the sample, are backflushed to vent, ensuring trouble free operation, perfect repeatability, and a long column lifetime without the need for extensive conditioning procedures. Moreover, this column channel is equipped with a Retention Time Stability option (RTS) to ensure stable retention time on the CP-Molsieve 5A column over time.

The second channel, equipped with a CP-PoraPLOT U column, analyzes carbon dioxide and hydrogen sulfide as part of the biogas sample. This column can even be used when ethane and propane are present in the sample. The sample inlet of the Micro GC and the CP-PoraPLOT U channel are treated with an UltiMetal deactivation process to guaranty good performance for hydrogen sulfide analysis.

When butanes and higher hydrocarbons need to be analyzed, the Agilent 490 Micro GC Biogas Analyzer Extended is recommended. This analyzer, suited for analysis of biogas mixed with other hydrocarbon streams such as natural gas or LPG, is equipped with an additional CP-Sil 5 CB column channel.

All results clearly illustrate that both analyzer configurations are perfectly capable of analyzing biogas and related sample streams. Typical repeatability figures show RSD's around 0.05 % for retention time and RSD's less than 0.2 % for peak area, while the factory specification for peak area repeatability is specified on 0.5% RSD (based on 1 % concentration level propane).

The Agilent 490 Micro GC Biogas Analyzers are factory tuned, including the appropriate settings for the backflush times for the CP-MolSieve 5A and CP-PoraPLOT U columns. The Agilent Biogas Analyzers are shipped with final test data, optimized analytical method, Biogas Analyzer User Manual, and a check out sample kit to have all information available at installation.

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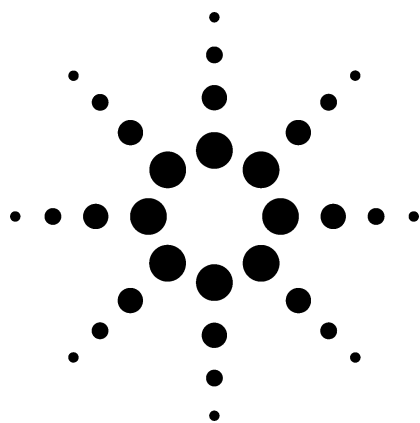
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Agilent 6850 Sub-Ambient Oven Performance for ASTM D3710 Simulated Distillation of Gasoline

Application

Gas Chromatography

September 2000

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Abstract

The Agilent 6850 gas chromatograph is now offered with sub-ambient column oven capabilities using liquid carbon dioxide as the cryogenic gas. This extends the 6850 oven temperature from -20 °C to 350 °C. The sub-ambient oven performance of the 6850 was tested using the ASTM D3710 method for simulated distillation of gasoline range materials. This method requires starting oven temperatures of -20 °C followed by programming to 180 °C. Four chromatographic performance tests required by the ASTM method were used to evaluate 6850 system performance. The same tests were also run on the Agilent 6890 GC to offer a comparison with an industry standard system. Both the 6850 and the 6890 results exceeded the specifications for peak shape, resolution, and

retention time repeatability and area percent repeatability. In most cases both GCs showed performance that was 10 times better than the ASTM requirements. The 6850 and the 6890 also showed nearly identical performance when compared to each other. Additionally, the 6850 used about one-half of the CO₂ cryogenic gas compared to the 6890 when running this method.

Introduction

Most gas chromatographic separations can be performed with GC oven temperatures starting above ambient conditions. However, there are some instances that require GC oven temperatures that are below ambient. For example, separation of gases, low boiling components, and solvent focusing are all examples where the chromatographer needs to use cold oven temperatures. For modern instruments, sub-ambient temperatures are achieved by controlled introduction of a cryogenic gas into the oven. Typically, liquid carbon dioxide (CO₂) or liquid nitrogen are used as the cryogenic gas.

The Agilent 6850 gas chromatograph is a rugged, easy-to-use, single chan-

nel instrument especially suited for production laboratories where space is a premium. The 6850 occupies 50% of the linear bench space compared to the Agilent 6890, while still providing the same chromatographic performance. For applications requiring sub-ambient oven temperatures down to -20 °C, the 6850 now offers a CO₂ cryogenic cooling option.

The 6850 cryogenic oven performance was evaluated using the ASTM D3710 Method for Boiling Range Distribution of Gasoline and Gasoline Fractions by Gas Chromatography¹. This simulated distillation method was slightly modified by using a fused silica capillary column in place of a packed column. Instrument conditions for this method are listed in table 1. The sample run on these instruments was a D3710 Qualitative Calibration Mix (part # 506427, Supelco, Bellefonte, PA, USA) which contains the nineteen compounds required for system performance evaluation. Five consecutive runs of this mix were made on each GC. The quantity of CO₂ cryogenic gas used for each run was also measured to compare consumption differences between the 6850 and 6890.



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Table 1. Instrument conditions for ASTM D3710 Simulated Distillation

Autoinjector	Agilent 7683 ALS 10 μ L Teflon-tipped plunger syringe
Inlet	Split/splitless operated in split mode Temperature: 200 °C Liner part # 5183-4647
Column	HP-1, 3.0 μ m film thickness, 15 m $\times$ 530 μ m (part # 19095Z-421E)
Oven	-20 °C for 2 min, 20 °C/min to 180 °C, hold 2 min
Detector	Flame ionization detector (FID) Temperature: 300 °C FID hydrogen flow rate: 40 mL/min FID air flow rate: 450 mL/min FID make-up flow rate: 45 mL/min nitrogen Data acquisition rate: 10 Hz
Data system	Agilent Chemstation

This ASTM D3710 method is useful for evaluating GC cryogenic oven performance. It is a widely used application and is familiar to many GC analysts. D3710 also has stringent chromatographic performance specifications that can be used to assess the sub-ambient oven performance. These requirements are listed below:

1. *Resolution* - compounds lighter than isopentane must be separated such that the valley above the baseline is less than 5% of the height of the smaller peak.
2. *Peak Shape* (Skew) - peak skew must be not less than 0.5 and not more than 2.0.
3. *Retention Time Repeatability* - the retention time difference between consecutive runs must not be not more than 3 seconds for isopentane and lighter compounds. For compounds heavier than n-pentane, the maximum difference in retention time

between successive runs must not be greater than the time equivalent of 3 °C. Additionally, the minimum retention time of the propane must be greater than 15 seconds.

4. *Area Percent Repeatability* - duplicate area percent results for each compound from consecutive runs must not differ by more than 0.1 area percent.

Results

Resolution. Figure 1 shows the chromatograms of the D3710 qualitative calibration sample run on both the 6850 and 6890 GCs. The nineteen compounds in this sample are baseline resolved on both GCs in approximately 12 minutes. Figure 2 shows the resolution of the compounds that are lighter than isopentane. For all four peaks, the valley above the baseline is much less than 5% of the height of the smaller peak.

Peak Shape. Table 2 lists the symmetry of each peak in the chromatograms shown in figure 1. A skew value of 1.0 would indicate perfectly symmetrical peaks. Skews of less than 0.5 would indicate tailing, and values greater than 2.0 would indicate fronting (overload). Both the 6850 and 6890 show peaks that are almost all perfectly symmetrical.

Retention Time Repeatability. Both the 6850 and 6890 show excellent retention time repeatability as shown in table 3. For isopentane and lighter compounds, the retention time repeatability on both GCs is about ten times better than the ASTM specification. For compounds heavier than n-pentane, the ASTM specification calls for repeatability of less than the time equivalent of 3 °C. For this method, that translates into a value of ≤ 2.0 seconds. Both the 6850 and the 6890 show retention time repeatability that is 20 times better. Additionally, the retention time of the propane on each GC is greater than 15 seconds (figure 2).

Area Percent Repeatability. Table 4 shows the area percent repeatability of the 6850 and 6890. Both instruments show 5-10 times better performance than what is required by the ASTM specification.

CO₂ Cryogenic Gas Usage. The 6850 required about 1.5 lbs. of CO₂ cryogen for each run of D3710. The 6890 GC used about 3 lbs. of carbon dioxide. The smaller oven design of the 6850 makes it easier to cool below ambient conditions and hold that temperature. Oven cycle times after temperature programming were approximately the same for both instruments (10 minutes).

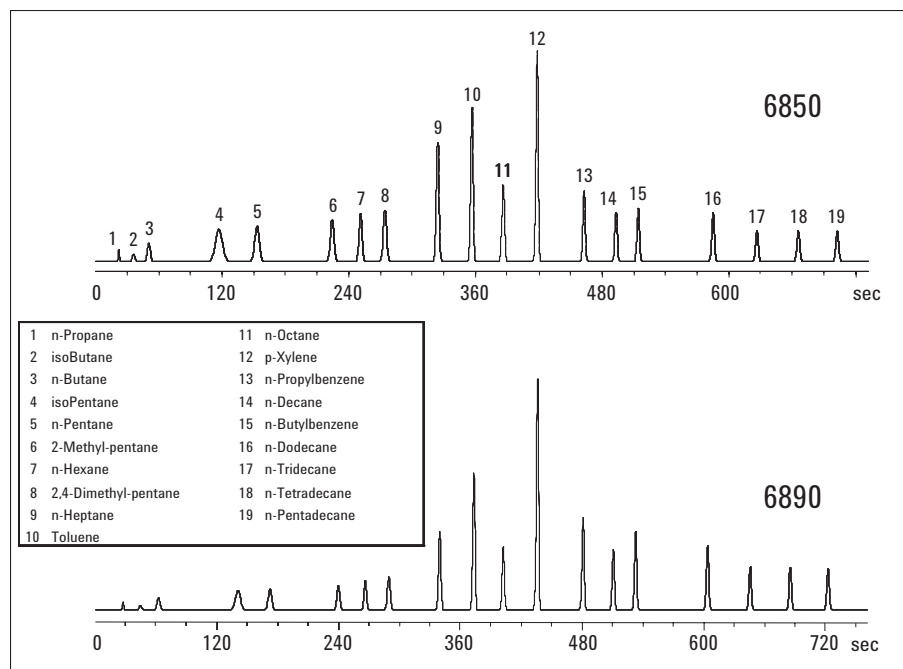


Figure 1. Separation of nineteen compounds used to evaluate GC system performance of the ASTM D3710 method for the simulated distillation of gasoline. Each run was made using CO₂ as the cryogenic gas for sub-ambient oven temperatures on both the 6850 and 6890.

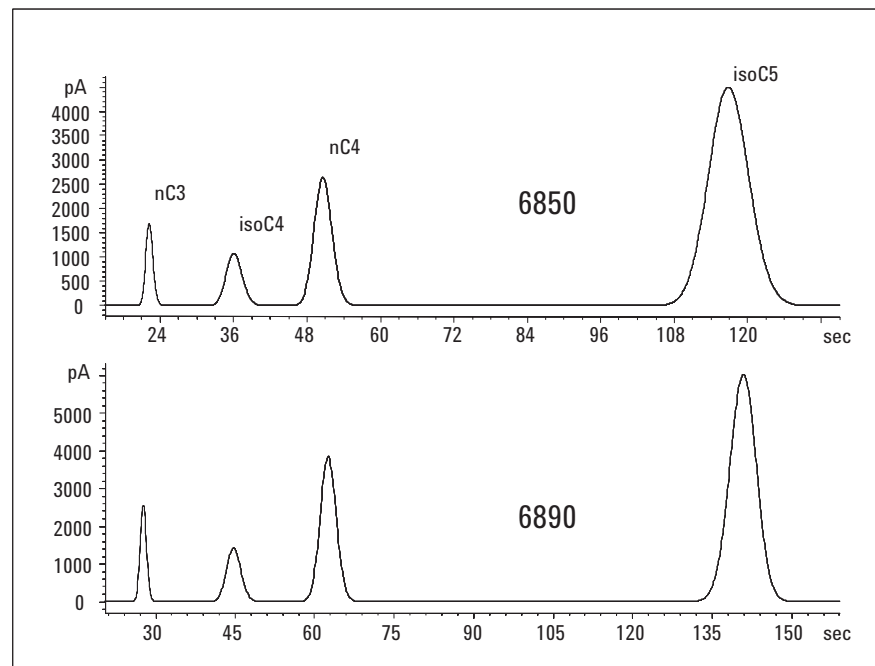


Figure 2. Baseline resolution is achieved for isopentane and lighter compounds on both the 6850 and 6890.

Table 2. ASTM D3710 Peak Symmetry Test (Skew)

Compound	ASTM Specification	Observed*	
		6890	6850
n-Propane	0.5-2.0	0.9	0.9
IsoButane	0.5-2.0	0.9	0.9
n-Butane	0.5-2.0	1.0	0.9
isoPentane	0.5-2.0	1.0	1.0
n-Pentane	0.5-2.0	1.0	1.0
2-Methyl-pentane	0.5-2.0	1.0	1.0
n-Hexane	0.5-2.0	1.0	1.0
2,4-Dimethyl-pentane	0.5-2.0	1.0	1.0
n-Heptane	0.5-2.0	1.1	1.1
Toluene	0.5-2.0	1.2	1.1
n-Octane	0.5-2.0	1.1	1.1
p-Xylene	0.5-2.0	1.5	1.2
n-Propylbenzene	0.5-2.0	1.2	1.1
n-Decane	0.5-2.0	1.1	1.0
n-Butylbenzene	0.5-2.0	1.2	1.1
n-Dodecane	0.5-2.0	1.2	1.1
n-Tridecane	0.5-2.0	1.1	1.0
n-Tetradecane	0.5-2.0	1.1	1.0
n-Pentadecane	0.5-2.0	1.1	1.0

*Average from 5 consecutive runs

Table 3. ASTM D3710 Retention Time Repeatability Test

Compound	ASTM Specification	Observed (sec)*	
		6890	6850
n-Propane	≤ 3.0s	0.1	0.1
isoButane	≤ 3.0s	0.1	0.1
n-Butane	≤ 3.0s	0.1	0.0
isoPentane	≤ 3.0s	0.2	0.3
n-Pentane	≤ 2.0s	0.2	0.2
2-Methyl-pentane	≤ 2.0s	0.2	0.1
n-Hexane	≤ 2.0s	0.2	0.1
2,4-Dimethyl-pentane	≤ 2.0s	0.1	0.1
n-Heptane	≤ 2.0s	0.1	0.1
Toluene	≤ 2.0s	0.1	0.1
n-Octane	≤ 2.0s	0.1	0.1
p-Xylene	≤ 2.0s	0.1	0.1
n-Propylbenzene	≤ 2.0s	0.1	0.1
n-Decane	≤ 2.0s	0.1	0.1
n-Butylbenzene	≤ 2.0s	0.1	0.1
n-Dodecane	≤ 2.0s	0.1	0.1
n-Tridecane	≤ 2.0s	0.1	0.1
n-Tetradecane	≤ 2.0s	0.1	0.1
n-Pentadecane	≤ 2.0s	0.1	0.1

*Average from 5 consecutive runs

Table 4. ASTM D3710 Area Percent Repeatability Test

Compound	ASTM Specification	Observed (area%)*	
		6890	6850
n-Propane	≤ 0.10%	<0.01	<0.01
isoButane	≤ 0.10%	<0.01	<0.01
n-Butane	≤ 0.10%	0.01	<0.01
isoPentane	≤ 0.10%	0.02	<0.01
n-Pentane	≤ 0.10%	0.01	<0.01
2-Methyl-pentane	≤ 0.10%	0.01	<0.01
n-Hexane	≤ 0.10%	0.01	<0.01
2,4-Dimethyl-pentane	≤ 0.10%	0.01	<0.01
n-Heptane	≤ 0.10%	0.02	<0.01
Toluene	≤ 0.10%	<0.01	0.01
n-Octane	≤ 0.10%	<0.01	<0.01
p-Xylene	≤ 0.10%	0.03	0.01
n-Propylbenzene	≤ 0.10%	0.02	<0.01
n-Decane	≤ 0.10%	0.01	<0.01
n-Butylbenzene	≤ 0.10%	0.02	<0.01
n-Dodecane	≤ 0.10%	0.02	<0.01
n-Tridecane	≤ 0.10%	0.01	<0.01
n-Tetradecane	≤ 0.10%	0.02	<0.01
n-Pentadecane	≤ 0.10%	0.02	<0.01

*Average from 5 consecutive runs

Summary

The Agilent 6850 gas chromatograph can now use liquid carbon dioxide to achieve sub-ambient column oven temperatures down to -20 °C. The CO₂ cryogenic performance characteristics of the 6850 were tested using the ASTM D3710 method for simulated distillation of gasoline. The 6850 showed ten-times better chromatographic performance.

These results were also identical to the Agilent 6890 equipped with CO₂ cryogenic oven cooling. The 6850 also used 50% less CO₂ for each run of D3710 when compared to the 6890.

References

1. ASTM Method D3710, "Standard Test Method for Boiling Range Distribution of Gasoline and Gasoline Fractions by Gas Chromatography", ASTM Book of Standards, Volume 5.02, ASTM, 500 Barr Harbor Drive, West Conshohocken, PA 19428, USA.

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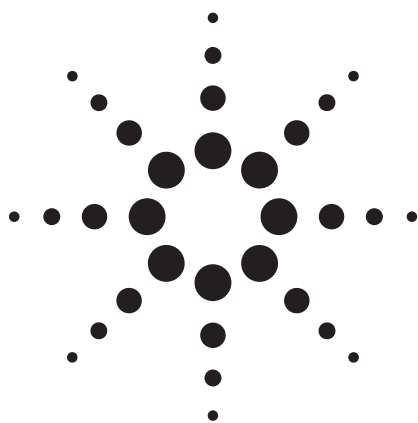
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Detection of Sulfur Compounds According to ASTM D5623 in Gasoline with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) and an Agilent 7890A Gas Chromatograph

Application

Hydrocarbon Processing

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Abstract

Sulfur components in gasoline samples are detected utilizing ASTM D 5623 with an Agilent 7890A GC configured with Agilent 355 dual plasma sulfur chemiluminescence detector (DP SCD). The 355 SCD response is linear, equimolar, and provides a linear range from 0.1 to 10 ppm. The coelution of hydrocarbon and sulfur peaks does not present a problem for the detector. The detection limits of sulfur compounds in gasoline are down to 20 ppb, while no quenching was found in the gasoline sample analysis.

Introduction

Gas chromatography with sulfur chemiluminescence detection (SCD) provides a rapid and highly specific means to identify and quantify various sulfur compounds that may be present in petroleum feed stocks and products, such as gasoline.

Frequently, petroleum feeds and products contain varying amounts and types of sulfur compounds. Many sulfur compounds can be corrosive to equipment, can inhibit or destroy catalysts employed in downstream processing, and can impart undesirable odors to products. The ability to speciate sulfur compounds in various petroleum liquids is useful in controlling sulfur compounds in finished products and is frequently more important than the ability to simply measure total sulfur content alone.

The SCD burner easily mounts on the 6890 and 7890A GCs, and incorporates features for easier and less frequent maintenance. The DP technology harnesses the power of dual flame plasma combustion, optimizing combustion of the sample matrix and formation of sulfur monoxide (SO).

The 355 DP SCD response is inherently linear, equimolar, and far less susceptible to hydrocarbon interference. These advantages eliminate the need for linearizing data or determining separate response factors for individual sulfur compounds. Furthermore, hydrocarbons are virtually invisible to the DP SCD. The coelution of hydrocarbon and sulfur peaks does not show quenching. Frequent column changes are required for analysis of various hydrocarbon products by flame photometric detectors (FPD) to avoid serious quenching and inaccurate results. ASTM Method D5623 utilizes the sulfur chemiluminescence detector (SCD) for the detection of sulfur compounds in gasoline.



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Experimental

An Agilent 7890A GC configured with split/splitless inlet (Sulfinert-treated), 7683B autosampler, and Agilent 355 DP SCD were used. The sulfur standards in toluene and iso-octane (10:90) were purchased from Supelco (Bellefonte, PA). The component information is in Table 1.

Table 1. Sulfur Standards Components

Components	Formula	Concentration (ppm) (w/w)
1 Ethyl mercaptan	CH ₃ CH ₂ SH	11.62
2 Dimethyl sulfide	(CH ₃) ₂ S	11.92
3 Carbon disulfide	C ₂ S	17.84
4 Isopropyl mercaptan	(CH ₃) ₂ CHSH	34.32
5 T-butyl mercaptan	(CH ₃) ₃ CSH	11.28
6 n-propyl mercaptan	CH ₃ CH ₂ CH ₂ SH	5.93
7 Methylethyl sulfide	CH ₃ CH ₂ SCH ₃	11.87
8 Thiophene	C ₄ H ₄ S	14.81
9 Sec-butyl mercaptan	CH ₃ CH ₂ CH(SH)CH ₃	23.26
10 n-butyl mercaptan	CH ₃ (CH ₂) ₃ SH	5.89
11 Dimethyl disulfide	CH ₃ SSCH ₃	14.75
12 2-methyl thiophene	C ₅ H ₆ S	14.29
13 3-methyl thiophene	C ₅ H ₆ S	21.35
14 Diethyl disulfide	(C ₂ H ₅) ₂ S ₂	27.99
15 Benzo(b)thiophene	C ₈ H ₆ S	40.49

NIST Standard Reference Material 2299, Sulfur in Gasoline, was used. The total sulfur in gasoline is 13.6 ± 1.5 µg/g based on analyses by isotope dilution thermal ionization mass spectrometry (ID-TIMS). Homogeneity testing was performed using X-ray fluorescence spectrometry.

Experimental Conditions

7890A GC Conditions

Front inlet	Split/splitless (Sulfinert-treated capillary inlet system)
Heater	275 °C
Pressure	10.951 psi
Septum purge flow	3 mL/min
Mode	Split
Gas saver	20 mL/min after 2 min
Split ratio	10 :1
Split flow	15 mL/min
Oven	30 °C (1 min) 10 °C/min 250 °C (1 min)
Column	HP-1 30 m × 0.32 mm × 4 µm (P/N 19091Z-613)

SCD Conditions – Agilent G6603A

Burner temperature	800 °C
Vacuum of burner	372 torr
Vacuum of reaction cell	5 torr
H ₂	40 mL/min
Air	53 mL/min

Results and Discussion

Several commercially available sulfur detectors are available for the determination of sulfur compounds in various matrices. When compared to flame photometric detection (FPD), pulsed flame photometric detection (PFPD), atomic emission detection (AED), and inductively coupled plasma-mass spectrometry (ICP-MS), the SCD shows the best performance based on stability, cost, and quantification. [2,3]

With DP technology, the performance of Agilent 355 SCD has been further enhanced, for unsurpassed stability, selectivity, and lack of quenching. Table 2 lists the DP SCD stability at different sulfur levels.

Table 2. Repeatability of Sulfur Compounds at Different Concentrations (Refer to Table 1 for peak identification.)

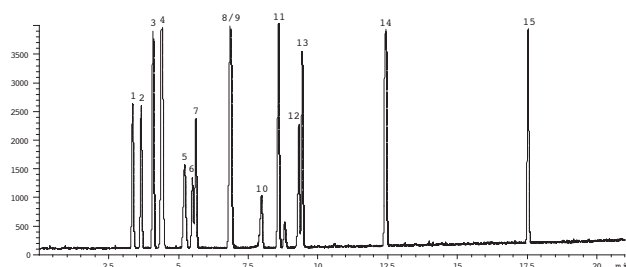
	1	2	3	4	5	6	7	8	10	11	12	13	14	15
Con. ppm	1.16	1.79	1.78	3.42	1.13	0.59	1.19	3.80	0.59	1.47	1.43	2.14	2.80	4.0
RSD (%)	2.8	3.6	3.1	1.9	3.0	2.7	3.9	3.9	2.9	2.1	2.2	2.9	0.4	3.7
Con. ppm	0.12	0.18	0.18	0.34	0.11	0.06	0.12	0.38	0.06	0.15	0.14	0.21	0.28	0.4
RSD (%)	5.7	7.4	3.4	3.7	6.6	4.8	5.7	4.8	8.0	4.0	3.3	4.7	7.3	3.1

Correlation coefficients of the tested sulfurs over three orders of magnitude were better than 0.99 (R^2). Table 3 shows the linearity of each sulfur

compound. Figure 1 shows the chromatogram of sulfurs in a hydrocarbon matrix without interference (1 to approximately 2 $\mu\text{g}/\text{kg}$).

Table 3. Linear Ranges of Tested Sulfurs (Refer to Table 1 for peak identification.)

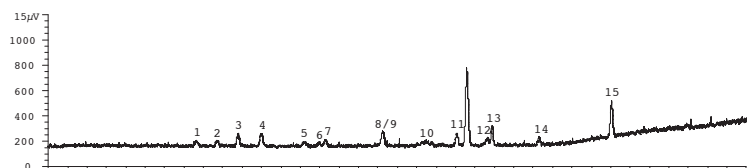
Analytes	Concentration Range	Linearity (R^2)
1	0.1ppm to 10ppm	0.9975
2	0.1ppm to 10ppm	0.9982
3	0.1ppm to 10ppm	0.9991
4	0.1ppm to 10ppm	0.9992
5	0.1ppm to 10ppm	0.9995
6	0.1ppm to 10ppm	0.9996
7	0.1ppm to 10ppm	0.9998
8/9	0.1ppm to 10ppm	0.9998
10	0.1ppm to 10ppm	0.9994
11	0.1ppm to 10ppm	0.9999
12	0.1ppm to 10ppm	0.9997
13	0.1ppm to 10ppm	0.9995
14	0.1ppm to 10ppm	0.9997
15	0.1ppm to 10ppm	0.9999

**Figure 1. Chromatogram of sulfur standard in hydrocarbon matrix. (Refer to Table 1 for peak identification.)**

The data in Table 4 illustrate the sensitivity (S/N) of 355 SCD for trace-level analysis (approximately 20 ng/kg) of sulfurs in a hydrocarbon matrix. Figure 2 shows the chromatogram of trace sulfurs, which also indicates no interferences in the analysis.

Table 4. Sulfur Sensitivity (Refer to Table 1 for peak identification.)

Peak No.	1	2	3	4	5	6	7	8/9	10	11	12	13	14	15
S/N	2.0	2.5	5.0	4.6	1.8	1.6	2.4	5.0	1.5	3.6	2.0	4.6	3.2	5.2

**Figure 2. Chromatogram of sulfurs at trace levels (20 ng/g). (Refer to Table 1 for peak identification.)**

Gasoline Samples Analysis

NIST Standard Reference Material 2299 was detected on an Agilent SCD and the mass concentration of total sulfur in sample was calculated by summing the sulfur content of all sulfur components (known and unknown) in the sample to arrive at its total sulfur value as recommended by ASTM 5623.

Figure 3A shows the chromatogram of the sulfur standard and Figure 3B shows the chromatogram of the standard gasoline sample. The total amount of sulfur is calculated by summing all the peak areas in Figure 3B and quantitated as thiophene 11.8 $\mu\text{g/g}$ of total sulfur with an RSD 2.7% ($n = 5$) was calculated in the gasoline sample. This was within the specified range of $13.6 \pm 1.5 \mu\text{g/g}$.

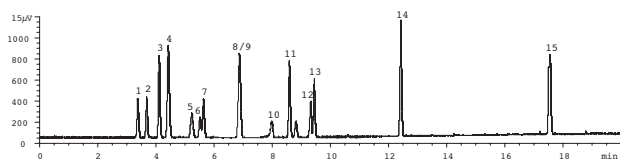


Figure 3A. Chromatogram of sulfur standard.

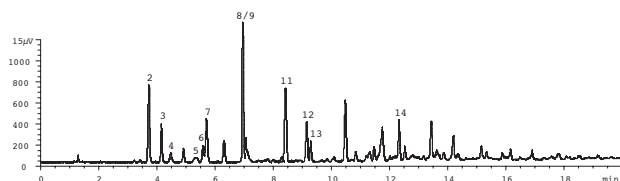


Figure 3B. Chromatogram of gasoline standard sample. (Refer to Table 1 for peak identification.)

Conclusions

The Agilent DP SCD is used for the detection of sulfur compounds in a complex hydrocarbon matrix. The results show that the DP SCD has linear response to sulfur compounds without quenching, yielding MDLs down to 20 ng/g. This solution is available as an Agilent preconfigured system; please refer to Agilent SP1 7890-0365 for ordering information.

References

1. ASTM 5623: Standard test method for sulfur compounds in light petroleum liquids by gas chromatography and sulfur selective detection
2. Roger L. Firor and Bruce D. Quimby, "Analysis of Trace Sulfur Compounds in Beverage-Grade Carbon Dioxide," Agilent Technologies publication 5988-2464EN
3. Roger L. Firor and Bruce D. Quimby, "A Comparison of Sulfur Selective Detectors for Low-Level Analysis in Gaseous Streams," Agilent Technologies publication 5988-2426EN

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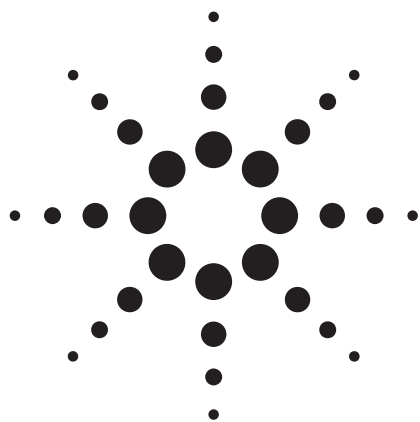
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Agilent Technologies



Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems

Application Note

Hydrocarbons

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Abstract

The performance of diesel fuel is predominantly determined by its ignition quality. This parameter is known as the Cetane number. The Cetane number describes the volume % Cetane (hexadecane) present in a mixture of Cetane and 1-Methylnaphthalene. Generally, in order to provide the best performance and lifetime of an engine, the amount of aromatics in diesel should be as low as possible. For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range 150 °C to 400 °C, there exists an IP Method (391/07), which uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) are separated using normal phase HPLC and a column which has little affinity for non-aromatic but pronounced selectivity for aromatic hydrocarbons [1]. Recent growth in biodiesel production created a demand for analysis of petrodiesel and petrodiesel/biodiesel blends. In this method revision, fatty acid methyl esters (FAME) originating from biodiesel sources must elute after a tetra-aromatic marker peak, chrysene, which facilitates improved accuracy of large PAH molecules without interference from FAME. The refractive index detector is used because this detector responds to both non-aromatic and aromatic hydrocarbons.



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About Standard Method IP391/07

"This European Standard specifies a test method for the determination of the concentration of mono-aromatic, di-aromatic and tri+-aromatic hydrocarbons in diesel fuels that may contain fatty acid methyl esters (FAME) up to 5 % (v/v) and petroleum distillates in the boiling range from 150 °C to 400 °C. The polycyclic aromatic hydrocarbon content is calculated from the sum of di-aromatic and tri+-aromatic hydrocarbons and the total content of aromatic compounds is calculated from the sum of the individual aromatic hydrocarbon types. Compounds containing sulfur, nitrogen and oxygen may interfere in the determination; mono-alkenes do not interfere, but conjugated di-alkenes and polyalkenes, if present, may do so.

The precision statement of the test method has been established for diesel fuels with and without FAME blending components, with a mono-aromatic content in the range from 6 % (m/m) to 30 % (m/m), a di-aromatic content from 1 % (m/m) to 10 % (m/m), a tri+-aromatic content from 0 % (m/m) to 2 % (m/m), a polycyclic aromatic content from 1 % (m/m) to 12 % (m/m), and a total aromatic content from 7 % (m/m) to 42 % (m/m)." [2]

This method, also known as EN12916:2006, is an official method of the Energy Institute (United Kingdom, www.energyinst.org.uk) which maintains IP (Institute of Petroleum) standards since their acquisition of the IP. Earlier IP391 revisions are similar to ASTM D-6591-06 and include a column backflush-capable instrument configuration and analysis scheme. This requirement was discontinued in the current IP391/07 revision due to erroneous reporting of tri+-aromatic hydrocarbons when FAME were present. The main IP391 changes from earlier revisions include the elimination of the backflushing valve, allowing compatibility with biodiesel/ petrodiesel fuel blends (up to 5% v/v FAME) and modifications to calibrants to improve data accuracy.

The various methods associated with middle distillate fuel analysis are shown in Table 1.

Table 1. Middle Distillate Fuel Analysis Methods

IP method and revision	Method overview	Special parameters	ASTM method	Comments
IP391/07	150-400 °C diesel fuel petro/bio blends up to B-5	No backflush, amino and/or cyano column	No current equivalent available	Same as method EN12916:2006 *MAH, DAH, Tri+AH are reported
IP436/01	50-300 °C aviation fuel, kerosene	No backflush, amino and/or cyano column	D-6379-04	MAH and DAH reported not for samples with Tri+AH
IP548/06	150-400 °C diesel fuel	Backflush required, amino and/or cyano column	D-6591-06	MAH, DAH, Tri+AH reported FAME interferes with result

*MAH – monoaromatic hydrocarbon, DAH – diaromatic hydrocarbon, Tri+AH – tri and higher ring aromatic hydrocarbons

Equipment and Conditions

LC:	Agilent 1200 Series LC including
G1312B:	Binary pump, used isocratically with pump head seals for normal phase, Agilent p/n 0905-1420
G1367C:	Autosampler with needle wash
G1316C:	Thermostatted column compartment
G1362A:	Refractive index detector
Software:	Agilent ChemStation with version B.04.01 software
Columns:	ZORBAX NH ₂ 4.6 mm × 150 mm, 5 µm (p/n 883952-708) and ZORBAX SB-CN 4.6 mm × 150 mm, 5 µm (p/n 883975-905) connected in series using 0.12 × 70 mm ss connector tubing, Agilent p/n G1316-87303
Mobile phase:	n-heptane, HPLC grade
Flow rate:	1 mL/min
Injection volume:	10 µL
Oven temperature:	25 °C
Detection:	Refractive index

Sample preparation

Samples and standards were prepared according to Standard Method IP391/07, using heptane as the diluent. Final quantitative results were reported using Agilent IP391/07 standard mixtures (p/n 5190-0485 system calibration standards SCS1 and SCS2, and p/n 5190-0484 quantitative calibration standards A-D).

Results and Discussion

The first step in implementing of IP391/07 is the analysis of calibrants that establish overall separation selectivity and resolution, and confirmation of the elution order of the calibrant components. (Sections 8.6, 8.7, 8.9 IP391/07). Figure 1 shows the results of running these calibrants on the Agilent system.

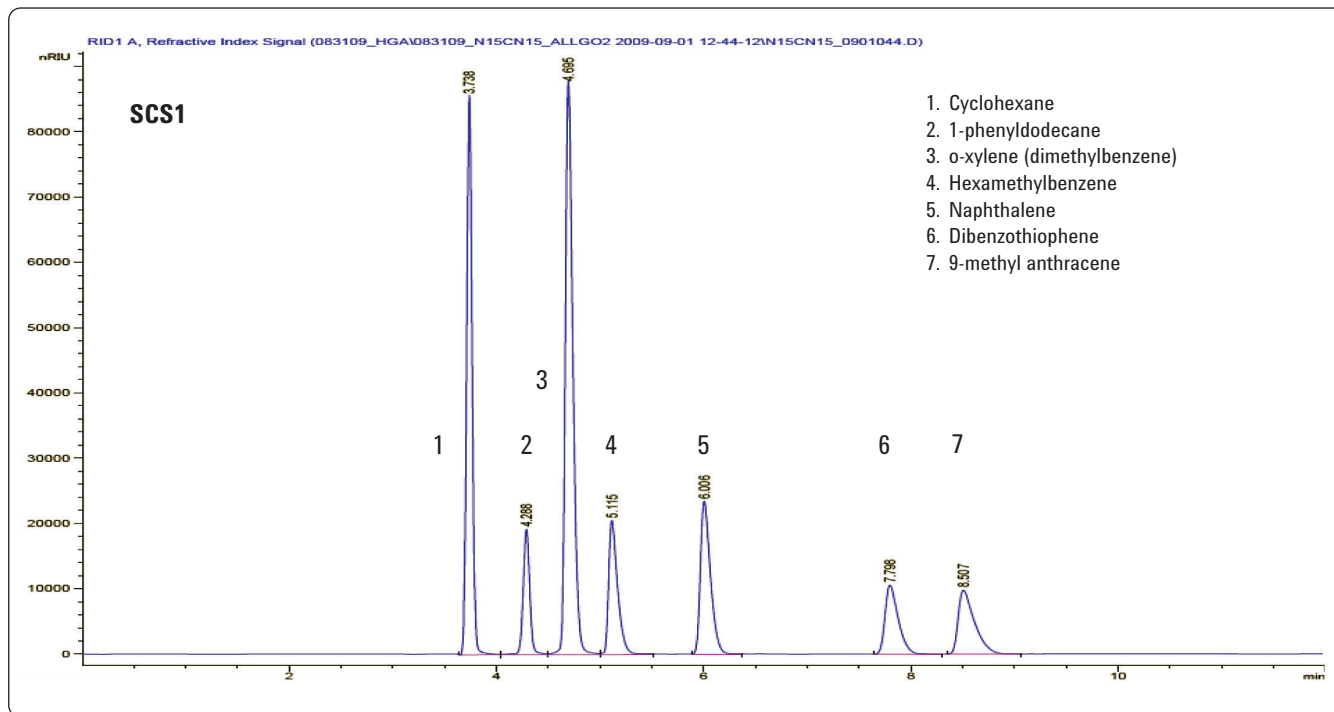


Figure 1. Standard chromatogram of SCS1.

The system calibration standard 1 (SCS1) determines selectivity and retention data for the saturate and aromatic markers used for method acceptance criteria. The SCS1 also determines retention time grouping parameters for sample reporting. Resolution between cyclohexane and o-xylene (1,2-dimethylbenzene) is part of the method specification and must conform to a minimum and maximum value.

System calibration standard 2 (SCS2) establishes the selectivity for components present in petro/bio diesel blends, demonstrating that there is no interference with tri+aromatic components by FAME. Petro/bio fuel blends require longer analysis time to elute all FAME peaks before the next analysis is

begun. This applies whether there is an interest in quantifying the FAME or not. When FAME is present in the sample, but does not need to be quantified, it is possible to reduce analysis time by programming the flow rate through the column to increase with time. This will rapidly wash FAME components from the column.

The method requirements state that chrysene, a tetra-aromatic marker peak, must elute with or before the first FAME peak. As shown in Figure 2, the selected operating conditions provide ample separation of the chrysene from the first FAME peak (C16:0 and C18:0 partially resolved).

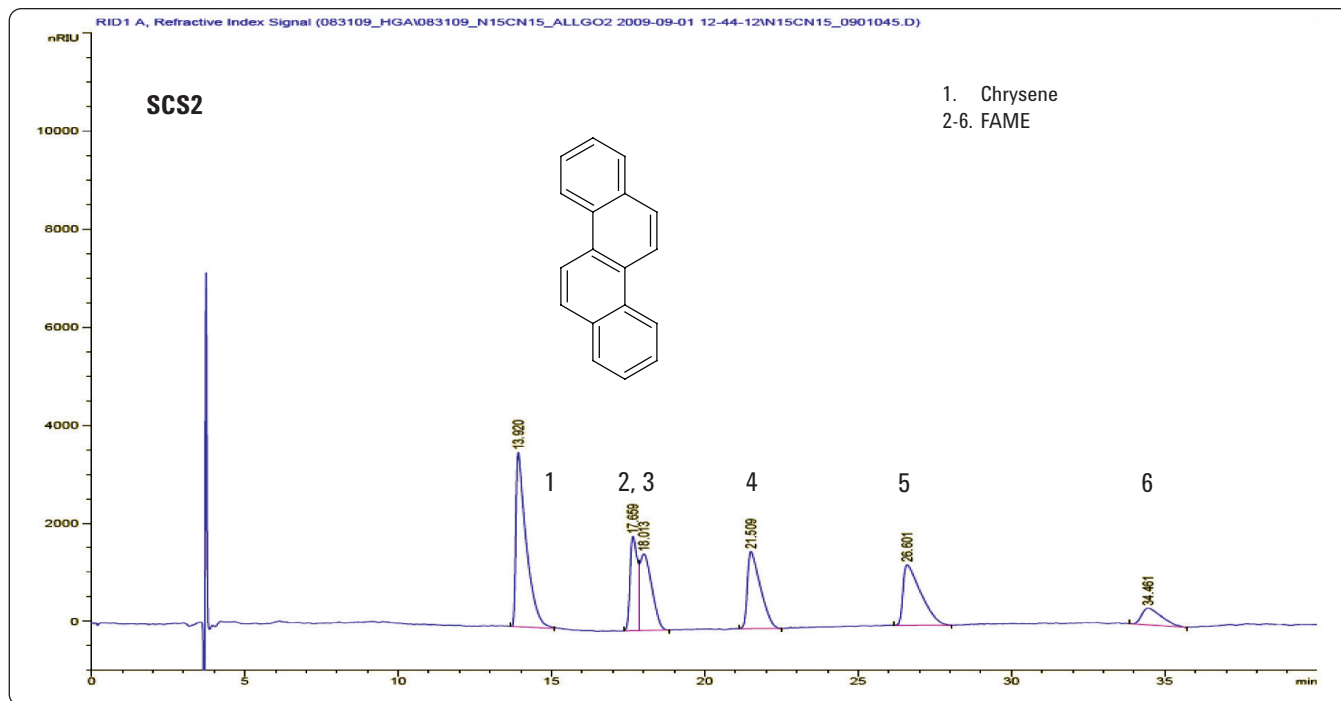


Figure 2. Standard chromatogram of SCS2.

With a genuine fuel sample, in this case retail quality petrodiesel, we can see greater complexity and overlapping of the various compound class regions (Figure 3). Within the

method definitions there are specific "cut" points defining the grouping to be performed in the quantitative reports. These are calculated from retention and peak width data in SCS1.

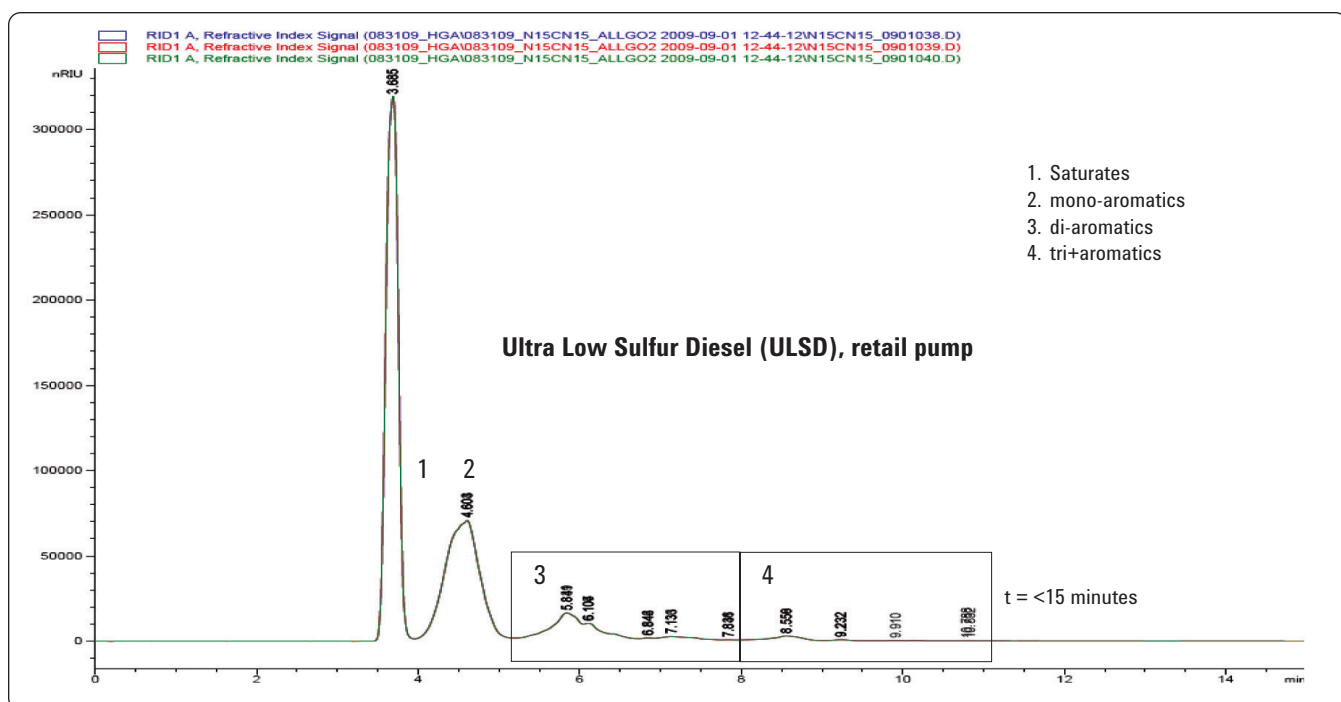


Figure 3. Petroleum diesel sample showing cut points for the various compound groups typically present in these samples.

Results and Discussion

Method Performance

As with most official methods, there are specific performance criteria that allow qualification of the separation system and its subsequent use for reporting quantitative results of diesel fuel analysis.

- 6.4 Column system, consisting of a stainless steel HPLC column(s) packed with a commercial 3 µm, 5 µm or 10 µm amino-bonded (or amino/cyano-bonded) silica stationary phase meeting the resolution requirements given in 8.6, 8.7 and 8.9.
- 8.6 Ensure the components of the SCS1 are eluted in the order: cyclohexane, phenyldodecane, 1,2, dimethylbenzene, hexamethylbenzene, naphthalene, dibenzothioephene and 9-methylanthracene.
- 8.7 Ensure that baseline separation is obtained between all components of the SCS1.
- 8.9 Ensure that the resolution between cyclohexane and 1,2 dimethylbenzene is between 5.7 and 10. [calculated as described in 11.2]
- 11.2 Column Resolution

Calculate the resolution, R, between cyclohexane and 1,2 dimethylbenzene using the following equation.

$$R = \frac{2(t_3 - t_1)}{1.699(y_1 + y_3)}$$

(difference in retention time)
(averaging of peak widths)

Name	Ret Time [min]	Resolution	W hh [min]	Cut ref
1. Cyclohexane	3.738	—	0.0558	t1
2. 1-phenyldodecane	4.288	—	0.0648	t2
3. 1,2-dimethylbenzene	4.695	8.57	0.0756	t3

- 8.11 Ensure the retention time peak of chrysene is higher than the ... 9-methylanthracene peak....and check that the chrysene peak elutes just before or with the first peak of FAME.

In Figure 4, we see that there is distinct separation between the markers specified in section 8.11 of the method. With this information in hand, it is possible to proceed to the evaluation of calibration standards.

- 9.4 R = >0.999, Intercept <0.01 g/100 mL)

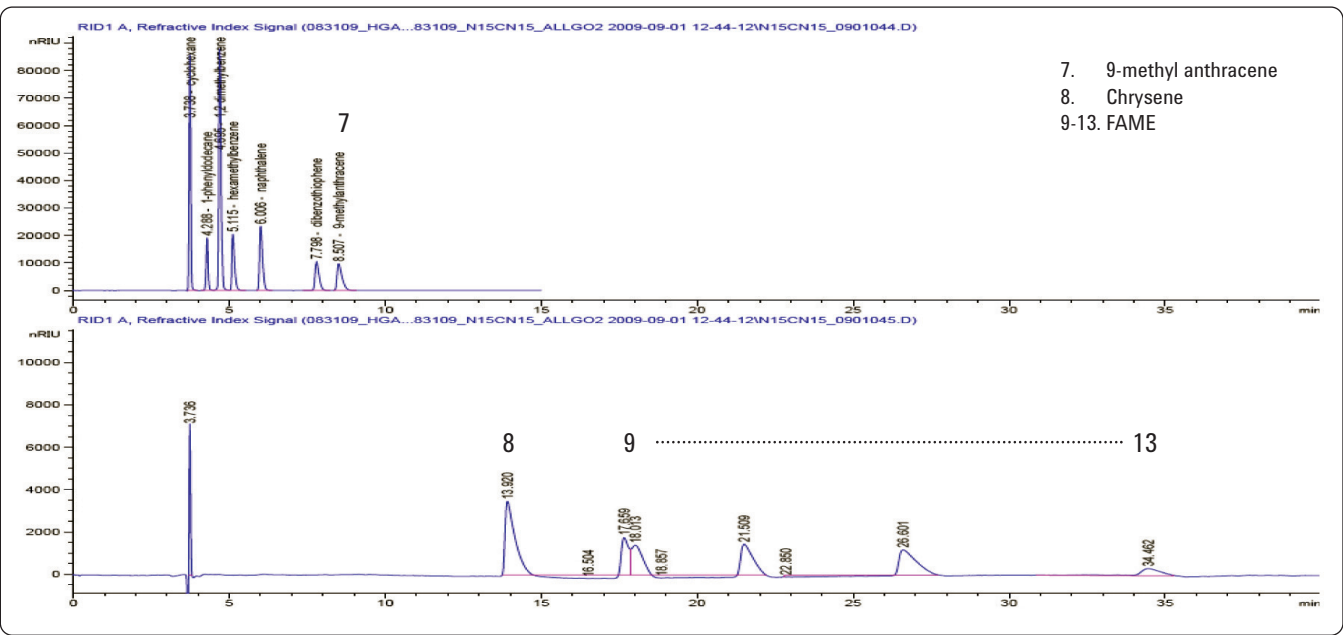


Figure 4. Stack plot of SCS1 and SCS2 showing proof for section 8.11.

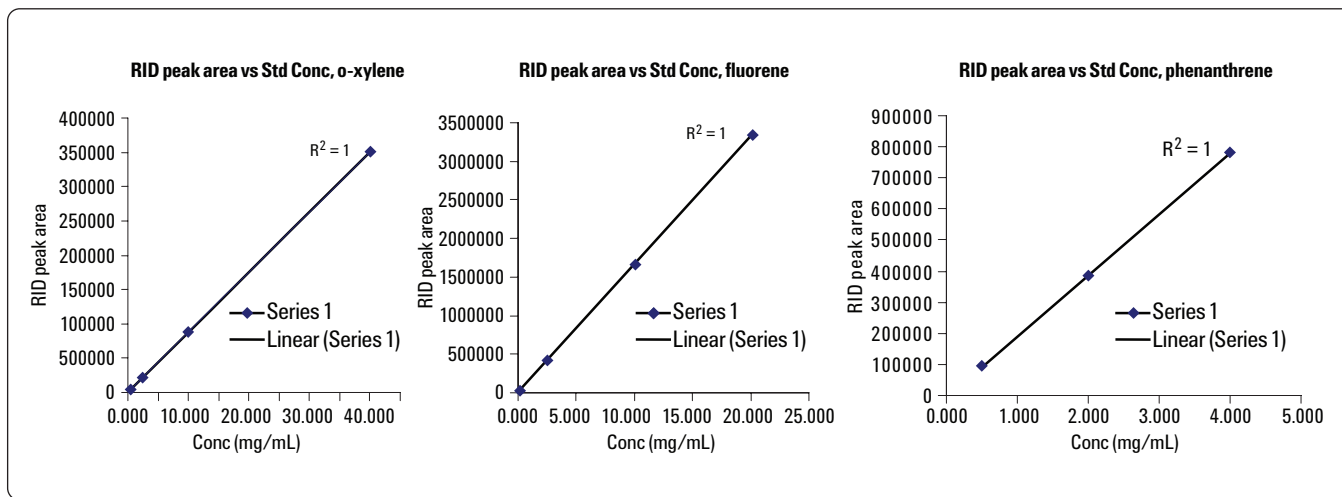


Figure 5. Calibration plots for o-xylene, fluorene and phenanthrene, the three components of the four calibration levels specified in the method.

In the calculated results, all calibration plots exceed linearity of 0.9999 and have calculated intercepts well below 0.01 g/100 mL, the method specification of section 9.4.

Retention time and peak area precision can be found in Table 2, in which we see that the overall performance of the method is excellent.

Table 2. Retention Time and Peak Area Precision

Calibrant A

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.57	0.003	0.06	3.54E+06	5829.9	0.16
Fluorene	6.82	0.004	0.05	3.38E+06	2500.5	0.07
Phenanthrene	8.32	0.004	0.04	8.05E+05	594.03	0.07

Calibrant B

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.65	0.001	0.02	9.23E+05	636.28	0.07
Fluorene	6.95	0.001	0.02	1.70E+06	1731.17	0.10
Phenanthrene	8.44	0	0.00	4.00E+05	473.79	0.12

Calibrant C

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.70	0.002	0.03	2.24E+05	474.36	0.21
Fluorene	7.15	0.002	0.03	4.29E+05	507.38	0.12
Phenanthrene	8.62	0.002	0.02	1.00E+05	291.04	0.29

Calibrant D

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.72	0.001	0.02	4.45E+04	321.90	0.72
Fluorene	7.36	0.002	0.02	1.70E+04	145.91	0.86
Phenanthrene	8.70	0.002	0.02	3.80E+04	551.43	1.45
Average RSD% All Runs			0.029	0.355		

Results for Specific Petrodiesel and Petro/Biodiesel Blends

Various samples were collected from local commercial and retail fuel delivery points. An overlay of four samples is shown in Figure 6.

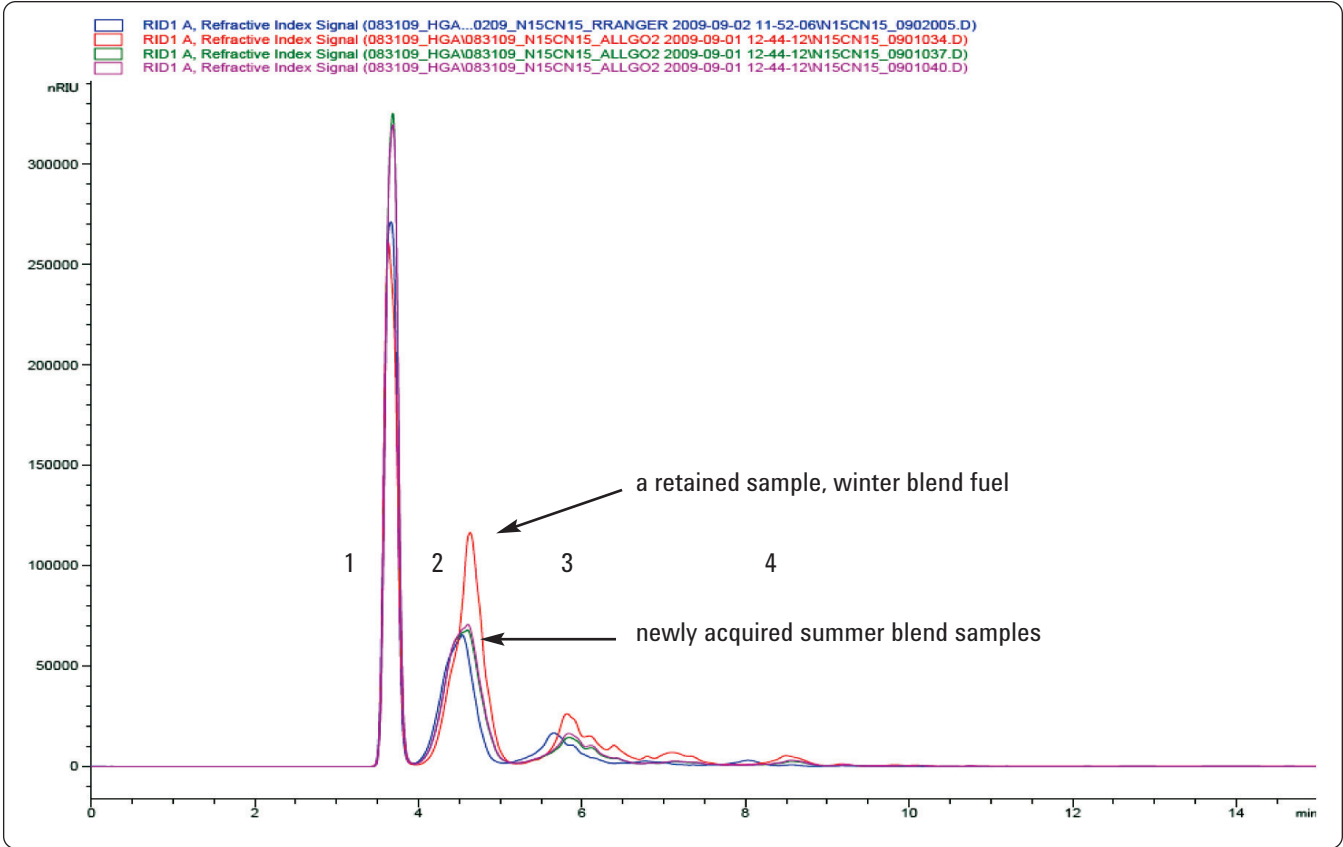


Figure 6. Overlay of four samples.

Results (n=3 each sample) and precision data are reported in Table 3, where vendor 4 results are n=3 based on one sampling of each of the three B-11 biodiesel delivery points (commercial heavy truck, commercial auto/light truck and retail auto/light truck).

Table 3. Results (n=3 each sample) and Precision Data for Four Vendors.

Vendor	Group	Avg, n=3		RSD%	Std dev
1	MAH	36.0	g/100 mL	0.06	0.022
	DAH	8.4	g/100 mL	0.09	0.008
	Tri+AH	1.2	g/100 mL	2.78	0.033
2	MAH	28.5	g/100 mL	0.05	0.016
	DAH	4.8	g/100 mL	0.48	0.023
	Tri+AH	0.6	g/100 mL	3.24	0.020
3	MAH	29.0	g/100 mL	0.07	0.021
	DAH	5.3	g/100 mL	0.37	0.020
	Tri+AH	0.7	g/100 mL	6.81	0.047
4	MAH	24.7	g/100 mL	5.06	1.252
	DAH	5.1	g/100 mL	3.38	0.174
	Tri+AH	0.7	g/100 mL	4.58	0.033

Because of very low level response and broadened peaks, it is much more difficult to get high precision for the tri+aromatic hydrocarbons group of components. If no biodiesel components are present, it would be practical to consider using Method IP548/01, which uses a backflush valve to elute the tri+aromatic hydrocarbons group as a single peak via the backflush configuration.

Ruggedness and Stability of the IP391/07 Method

As with most normal phase methods the column is susceptible to the adsorption of highly polar components which can affect overall separation performance. Water present in samples or the mobile phase also adsorbs into the column and somewhat predictably causes reduced elution times for all sample components. Using a high quality HPLC grade mobile

phase is essential, and the user may consider using a drying agent such as molecular sieve to dehydrate the mobile phase. While this is often done by adding molecular sieve to the solvent container, it is also possible and preferable to prepare a high pressure compatible column with pre-washed drying agent and placing it inline between the pump and injector.

Conclusion

The performance of the Agilent 1200 Series HPLC with normal phase separation and refractive index detection meets or exceeds the requirements of IP391/07 within the range of samples defined in the method. The user should take care identifying samples of petrodiesel that may contain biodiesel components to ensure adequate analysis time before proceeding to the next analytical run.

References

1. ASTM 6591-06 and ASTM 6379-04, www.astm.org
2. IP391/07, Energy Institute (formerly Institute of Petroleum Test Methods)
3. EN12916/06, Energy Institute (formerly Institute of Petroleum Test Methods)
4. Agilent Technologies publication 5965-9044EN, (1997)

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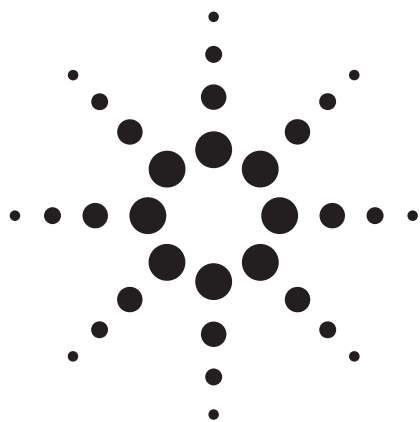
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Meeting the requirements of ASTM D 6591-06 (IP548/06) Using Agilent 1200 Series HPLC Systems

Application

Hydrocarbons

Abstract

The performance of diesel fuel is predominantly determined by its ignition quality. This parameter is known as the Cetane number. The Cetane number describes the volume % Cetane (aliphatic hexadecane) present in a mixture of Cetane and (aromatic) 1-Methyl-naphthalene. Generally, in order to provide the best performance and maximize the lifetime of an engine, the amount of aromatics in diesel should be as low as possible. For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range of 150 °C to 400 °C, there exists an ASTM Method (D 6591-06), and identical method IP548/06 that uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) are separated using normal phase HPLC and a column that has little affinity for non-aromatic but has pronounced selectivity for aromatic hydrocarbon classes [1]. The refractive index detector is used because this detector responds to both non-aromatic and aromatic hydrocarbons.

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About Standard Method ASTM D 6591-06

“This test method covers a high performance liquid chromatographic test method for the determination of monoaromatic, di-aromatic, tri+-aromatic, and polycyclic aromatic hydrocarbon contents in diesel fuels and petroleum distillates boiling in the range of 150 to 400 °C. The total aromatic content in % m/m is calculated from the sum of the corresponding individual aromatic hydrocarbon types.

NOTE 1—Aviation fuels and petroleum distillates with boiling points that range from 50 to 300 °C are not determined by this test method and should be analyzed by Test Method, D 6379 or another suitable equivalent test method.

- 1.2 The precision of this test method has been established for diesel fuels and their blending components, containing from 4 to 40 % (m/m) mono-aromatic hydrocarbons, 0 to 20 % (m/m) di-aromatic hydrocarbons, 0 to 6 % (m/m) tri+-aromatic hydrocarbons, 0 to 26 % (m/m) polycyclic aromatic hydrocarbons, and 4 to 65 % (m/m) total aromatic hydrocarbons.
- 1.3 Compounds containing sulfur, nitrogen, and oxygen are possible interferents. Mono-alkenes do not interfere, but conjugated di- and poly-alkenes, if present, are possible interferents.
- 1.4 By convention, this standard defines the aromatic hydrocarbon types on the basis of their elution characteristics from the specified liquid chromatography column relative to model aromatic compounds. Quantification is by external calibration using a single aromatic compound, which may or may not be representative of the aromatics in the sample, for each aromatic hydrocarbon type. Alternative techniques and methods may classify and quantify individual aromatic hydrocarbon types differently.
- 1.5 Fatty Acid Methyl Esters (FAME), if present, interfere with tri+-aromatic hydrocarbons. If this method is used for diesel containing FAME, the amount of tri+-aromatics will be overestimated.”[2]

This method, also known as IP548/06, is an official method of the American Society of Testing Methods (United States, www.astm.org). The method requires a column backflush-capable instrument configuration and analysis scheme, and is similar to other hydrocarbon group analysis methods. Because of this similarity, with respect to mobile phase and detection strategy, the instrument configuration is readily adaptable to those other methods.

The various methods associated with middle distillate fuel analysis are shown in Table 1.

Equipment and Conditions

LC:	Agilent 1200 Series LC
Binary pump:	G1312B used isocratically with pump head seals for normal phase, Agilent p/n 0905–1420
Autosampler:	G1367C with needle wash
Therm. Column Compartment:	G1316C with 6 port 2 position switching valve
Refractive Index Detector:	G1362A
Software:	Agilent ChemStation with version B.04.02 software
Columns:	Agilent ZORBAX NH ₂ 4.6 × 250 mm, 5 µm (p/n 880952-708)
Mobile Phase:	n-heptane, HPLC grade
Flow Rate:	1 ml/min
Injection Volume:	10 µl
Oven Temperature:	20 °C
Detection:	Refractive index

Sample preparation

Samples and standards were prepared according to guidance published in the method, using heptane as the diluent. System qualification and final quantitative results were reported using Agilent ASTM D 6591-06 standard mixtures (p/n 5190-0483 system performance solution SPS, and p/n 5190-0482 quantitative calibrant solutions A-D, respectively).

Table 1. Fuel Analysis Methods

IP Method and Revision	Method Overview	Special Parameters	ASTM Method	Comments
IP391/07	150-400 °C diesel fuel petro/bio blends up to B-5	no backflush, amino and/or cyano column	No current equivalent available	same as method EN12916:2006 *MAH, DAH, Tri+AH are reported
IP436/01	50-300 °C aviation fuel, kerosene	no backflush, amino and/or cyano column	D-6379-04	MAH and DAH reported not for samples with Tri+AH
IP548/06	150-400 °C diesel fuel	backflush required, amino and/or cyano column	D-6591-06	MAH, DAH, Tri+AH reported FAME interferes with result

*MAH – monoaromatic hydrocarbon, DAH – diaromatic hydrocarbon, Tri+AH – tri and higher ring aromatic hydrocarbons

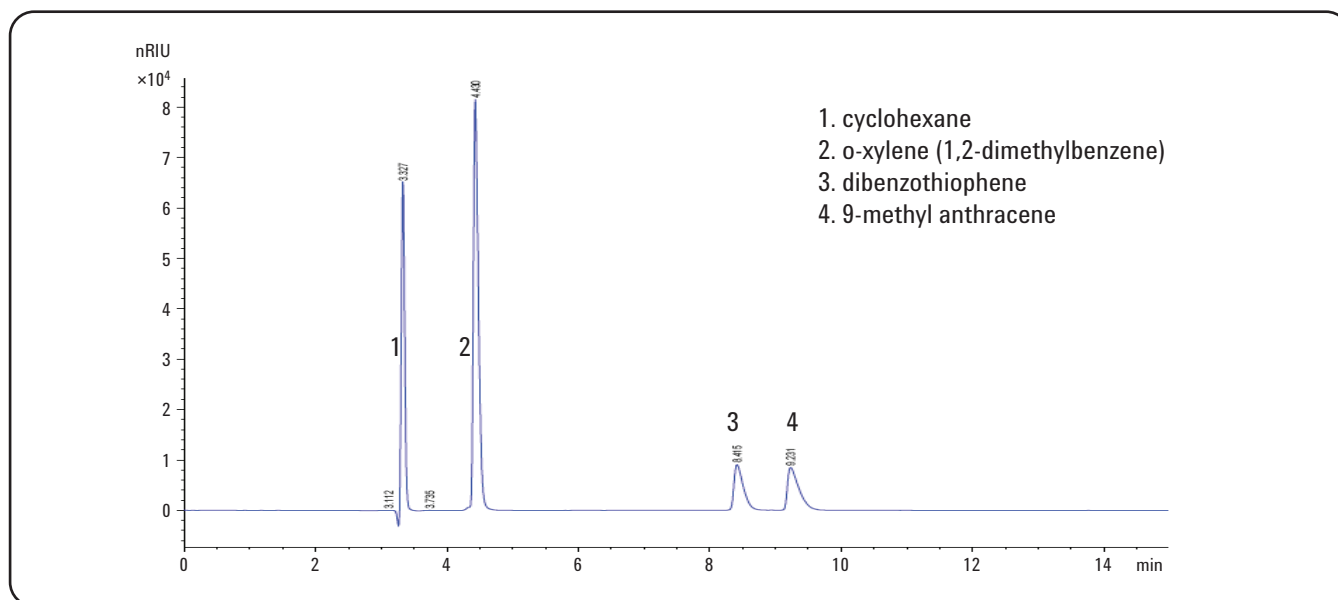


Figure 1. Standard chromatogram of system performance solution (SPS).

Results and Discussion

The first steps in method implementation are to analyze a system performance solution (SPS) that establishes overall separation selectivity and resolution, and to establish the event time table for column backflushing during the analysis. (Sections 9.4 and 9.6 of the method). Figure 1 illustrates the

results of running the performance solution on the Agilent system without a backflush event.

The SPS is used to determine selectivity and retention data for the saturate and aromatic markers that are used for method acceptance criteria. It is also used to determine the backflush time for eluting tri+aromatics as a single peak.

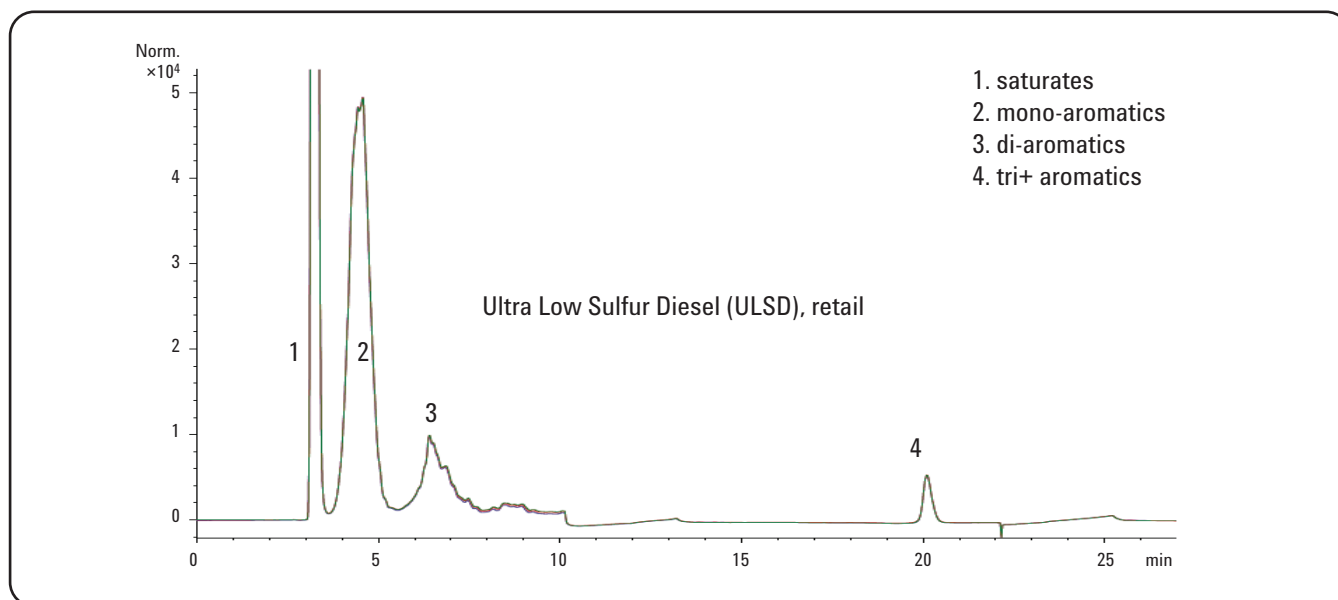


Figure 2. Petroleum diesel sample, n=3 overlay, showing cutpoints for the various compound groups typically present in these samples.

Resolution between cyclohexane and o-xylene (1,2-dimethylbenzene) is part of the method specification and must attain a minimum value of 5.

With a genuine fuel sample, in this case retail quality petrodiesel, greater complexity and overlapping of the various compound class regions are evident. Within the method definitions there are specific “cutpoints” defining the grouping to be performed in the quantitative reports. Manual peak integration is specified in the method for setting the baseline, and inserting valley drop points.

Results and Discussion

Method Performance

As with most official methods, there are specific performance criteria that allow qualification of the separation system and its subsequent use for reporting quantitative results of diesel fuel analysis.

- 6.4 Column System—Any stainless steel HPLC column(s) packed with an approved amino-bonded (or polar amino/cyano-bonded) silica stationary phase is suitable, provided it meets the resolution requirements laid down in 9.4.3. [2]
- 9.4.1 Ensure that baseline separation is obtained between all components of the SPS.
- 8.9 Ensure that the resolution between cyclohexane and 1,2 dimethylbenzene is at least 5 as described in 9.4.3.

- 9.4.3.1 Column Resolution
Calculate the resolution, R, between cyclohexane and 1,2 dimethylbenzene using the following equation.

$$R = \frac{2(t_2 - t_1)}{1.699(y_1 + y_2)}$$

difference in retention time
averaging of peak widths

- 10.1.5: R = >0.999, Intercept <0.01 g / 100 ml)

Table 2.

Name	R. Time [min]	width (hh)	Resolution
1. cyclohexane	3.307	0.059	8.79
2. 1,2-dimethylbenzene)	4.477	0.097	
3. dibenzothiophene	8.907	0.186	
4. 9-methyl anthracene (r.t. with backflush)	18.905	0.282	

In Figure 1 there is distinct separation between the markers specified in sections 9.4 and 9.6 of the method. Table 2 confirms the minimum resolution requirement of section 9.4 and shows retention time data obtained with the programmed backflush calculated as defined in section 9.6. With this information, it is possible evaluate calibration standards.

An overlay of calibrant solutions A-D is shown in Figure 3. The backflush time was determined from injections of SPS at the beginning of the analysis sequence.

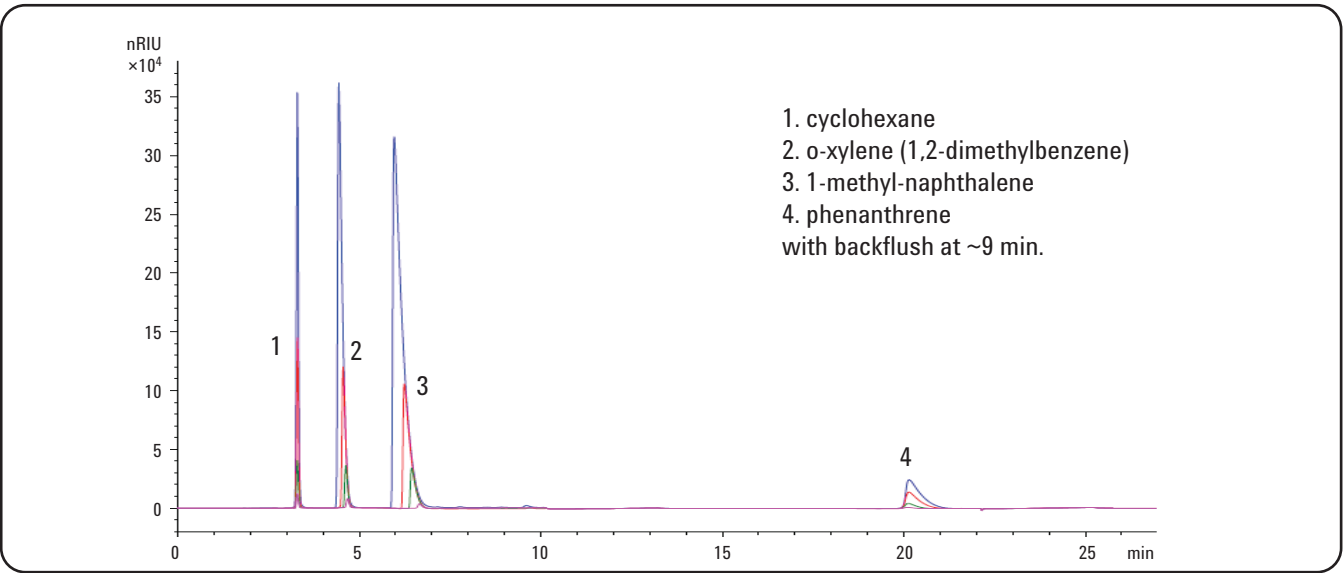


Figure 3. Overlay of calibrant solutions A-D.

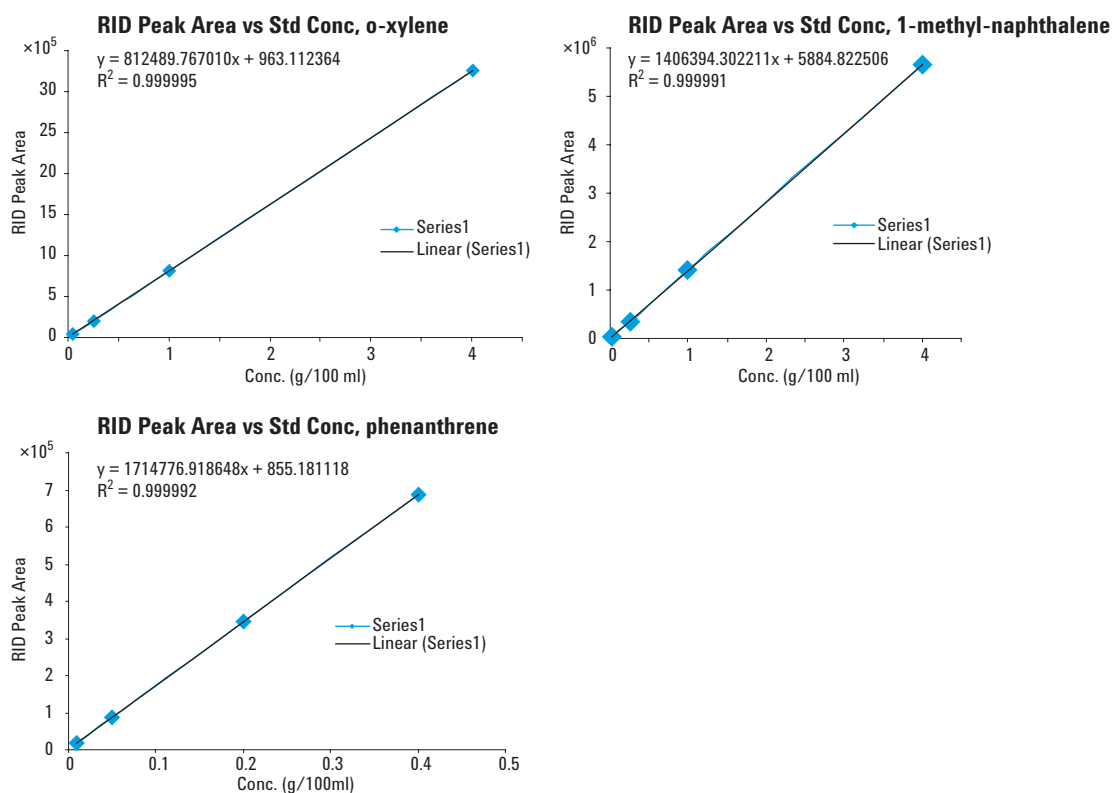


Figure 4. Calibration plots for o-xylene, 1-methyl-naphthalene, and phenanthrene which, are the three components of the four calibration levels specified in the method.

In the calculated results, all calibration plots exceed linearity of 0.9999 and have calculated intercepts well below 0.01 g/100 mL, which are the method specifications of section 10.1.5.

Retention time and peak area precision can be found in Table 3, illustrating that the overall performance of the calibration method is excellent.

Table 3. Calibration Precision

Calibrant A

Analyte	RT, Avg, n=3	RT, Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.44	0.0005	0.01%	3.29E+06	755.9	0.02%
1-Methyl-naphthalene	5.96	0.001	0.02%	5.76E+06	2299.2	0.04%
phenanthrene	20.14	0.0020	0.01%	7.12E+05	8351.8	1.17%

Calibrant B

Analyte	RT, Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.55	0.0020	0.05%	8.33E+05	5263.9	0.63%
1-Methyl-naphthalene	6.24	0.0041	0.07%	1.46E+06	14197.7	0.97%
phenanthrene	20.13	0.0023	0.01%	3.55E+05	849.5	0.24%

Calibrant C

Analyte	RT, Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.63	0.0017	0.04%	2.06E+05	536.3	0.26%
1-Methyl-naphthalene	6.44	0.0036	0.06%	3.66E+05	1830.7	0.50%
phenanthrene	20.12	0.0040	0.02%	8.87E+04	139.0	0.16%

Calibrant D

Analyte	RT Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.67	0.0005	0.01%	4.03E+04	214.7	0.53%
1-Methyl-naphthalene	6.65	0.0020	0.03%	2.96E+04	334.1	1.13%
phenanthrene	20.10	0.0025	0.01%	1.76E+04	176.5	1.00%

Average RSD% All Runs

0.028%

0.555%

Results for specific petrodiesel and petro/biodiesel blends

Various samples were collected from local commercial and retail fuel delivery points. An overlay of three samples is shown in Figure 5.

Despite some apparent compositional differences among the samples, the general resolution and valley points are consistent. This should ensure relatively straightforward data reduction.

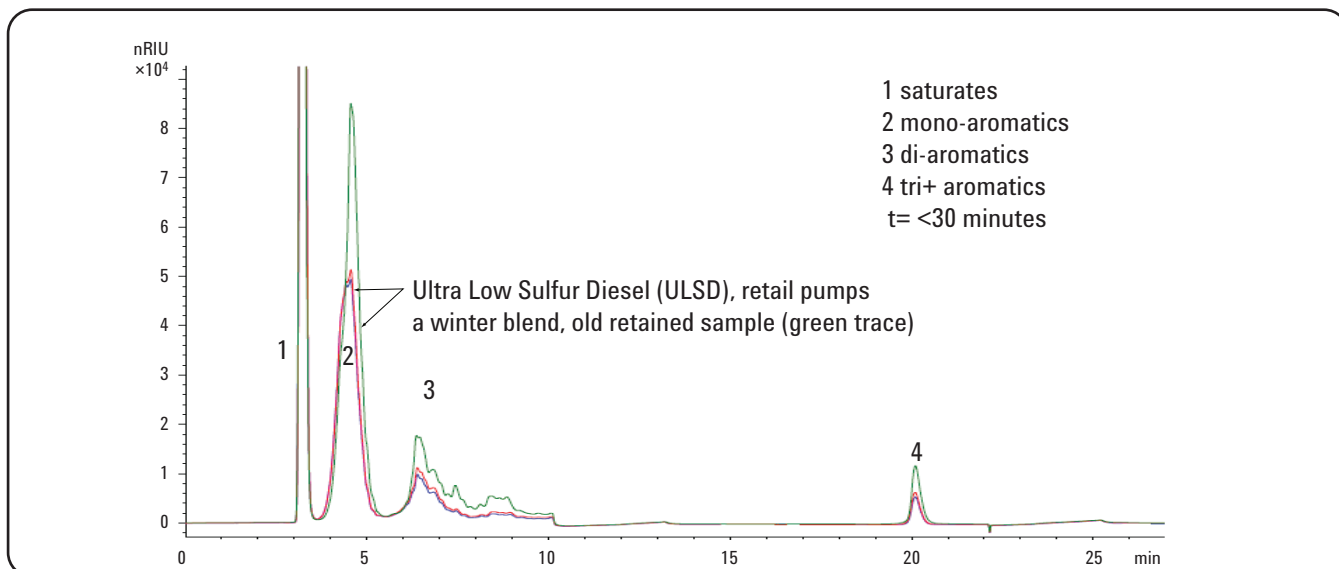


Figure 5. Overlay of three samples.

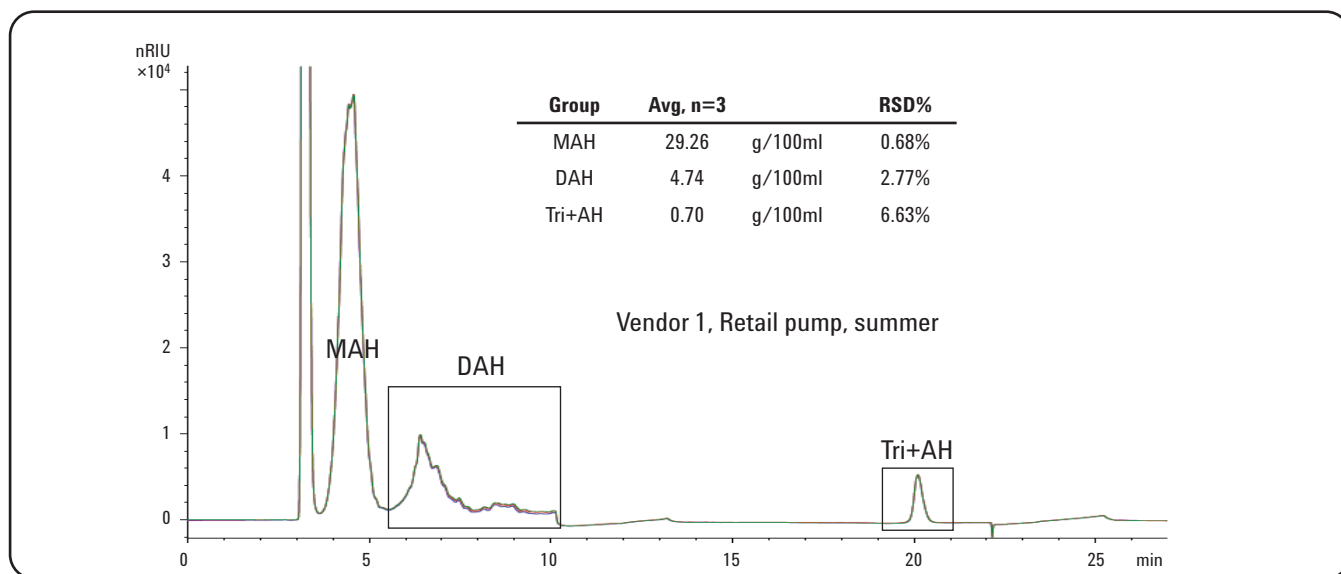


Figure 6. Results and precision for sample designated "Vendor 1".

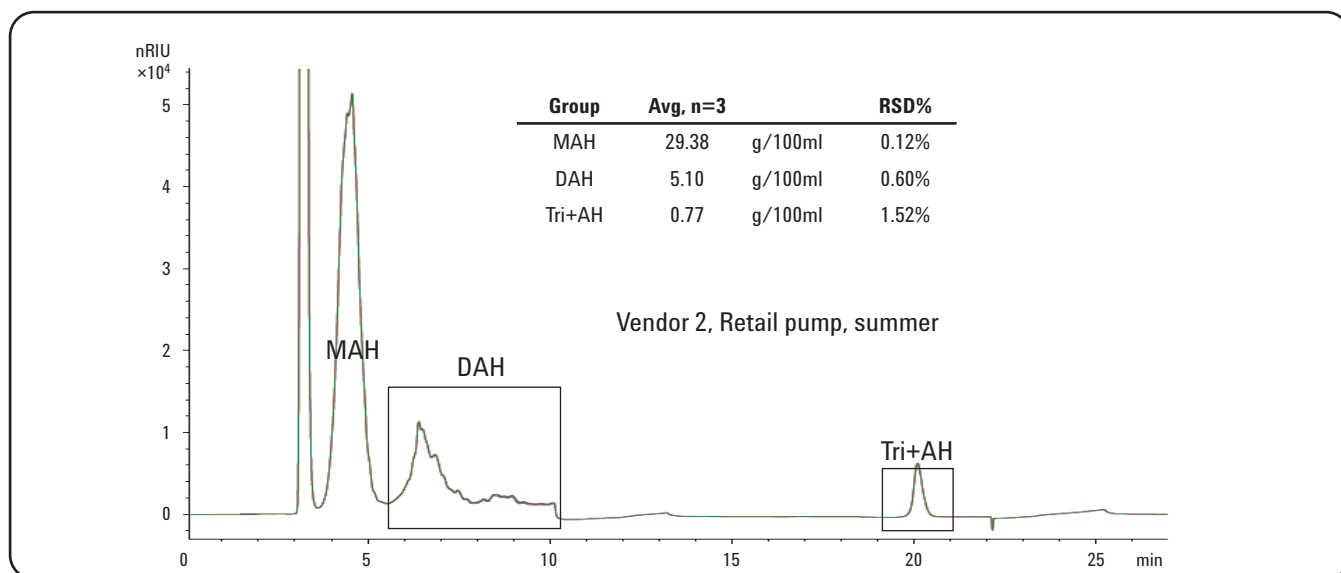


Figure 7. Results and precision for sample designated "Vendor 2".

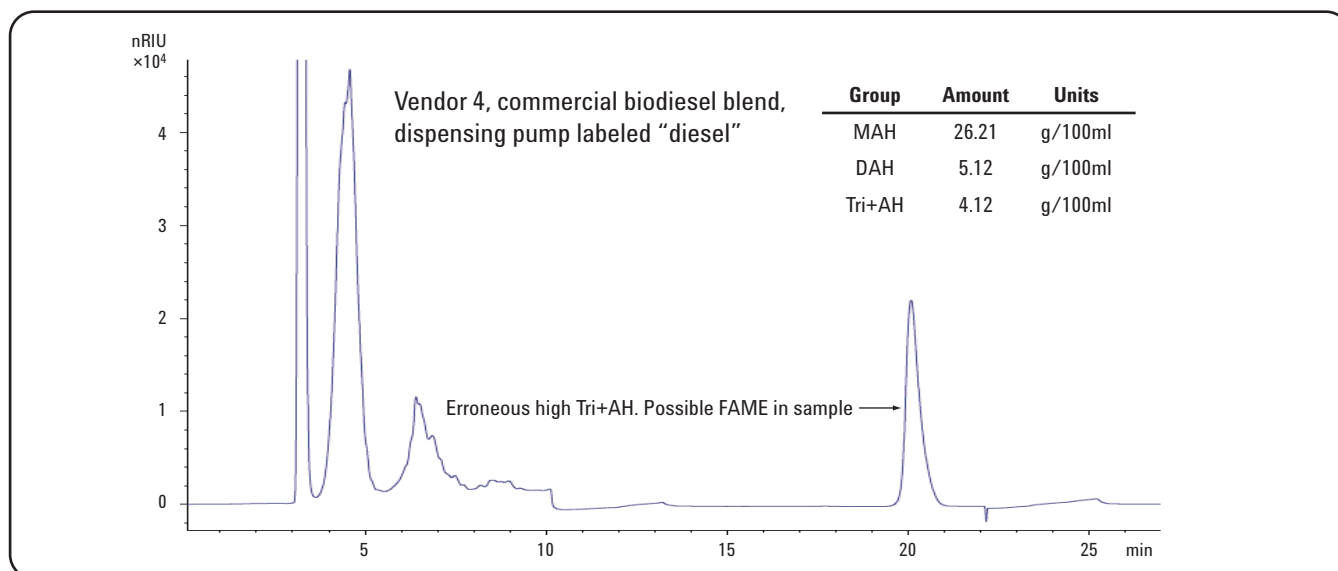


Figure 8. This sample was represented as diesel and was analyzed by the method. Suspiciously high tri+aromatic values compelled an analysis by the alternate, biodiesel approved, method IP391/07.

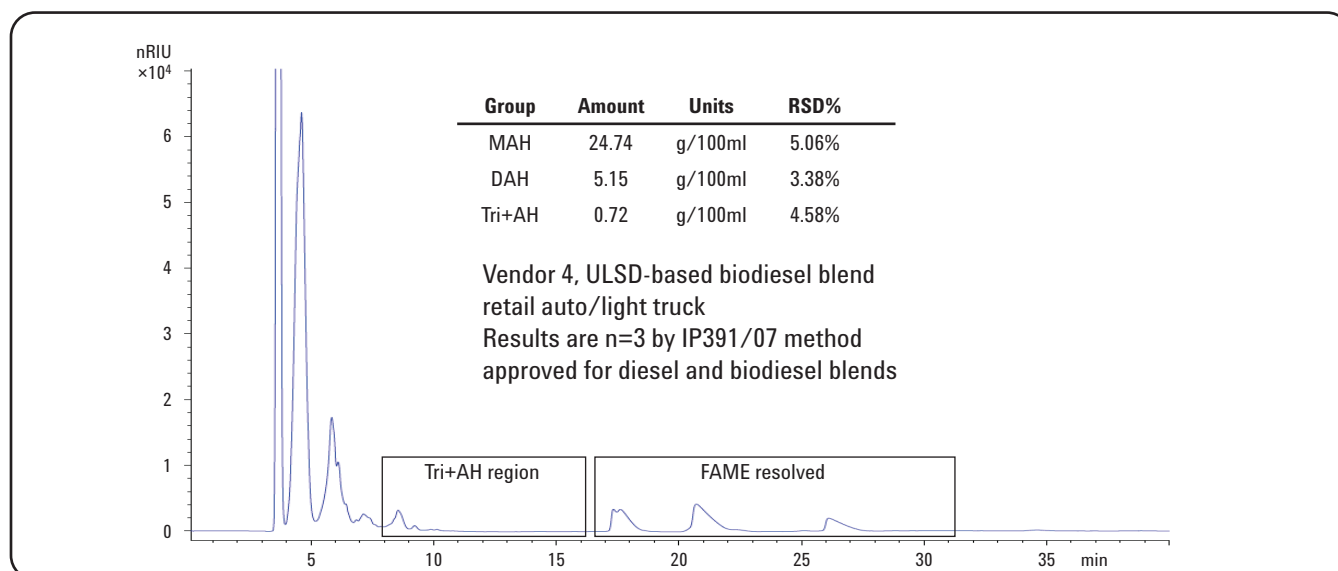


Figure 9. Analysis of suspect biodiesel sample by IP391/07 conditions confirms the contamination or dispensing pump mislabeling and yields a more expected result for typical diesel motor fuel. For further details on the performance and utility of this method, please refer to Agilent application note 5990-4789EN. [3]

Ruggedness and Stability of the ASTM D 6591-06 method

As with most normal phase methods the column is susceptible to adsorption of highly polar components that can affect overall separation performance. Water present in samples or mobile phase also adsorb to the column and somewhat predictably cause reduced elution times for all sample components. Using a high quality anhydrous HPLC grade mobile phase is essential, and the user may consider using a drying agent such as molecular sieve to dehydrate the mobile phase. While this is often done by adding molecular sieve to the solvent container, it is also possible and preferable to prepare a high pressure compatible column with prewashed drying agent and placing it inline between the pump and injector.

Conclusion

The performance of the Agilent 1200 Series High Performance LC system with normal phase separation and refractive index detection meets or exceeds the requirements of ASTM D 6591-06 within the range of samples defined in the method. The user should take care to identify samples of petrodiesel containing biodiesel components to ensure adequate analysis modifications are made to prevent erroneous high tri+aromatic values. IP391/07 (EN12916:2006) is required for samples found to contain biodiesel FAME components, and any results showing suspiciously high Tri+aromatics values with ASTM D 6591-06 should be re-analyzed by IP391/07.

References

1. Angelika Gratzfeld-Huesgen, "Analysis of Aromatic Hydrocarbons in Middle Distillates with HPLC using IP Standard Method 391/95", Agilent Application Note, 5965-9044E, 1997.
2. ASTM D 6591-06 "Standard Test Method for Determination of Aromatic Hydrocarbon Types in Middle Distillates—High Performance Liquid Chromatography Method with Refractive Index Detection".
3. Michael Woodman and Malgorzata Sierocinska, "Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems", Agilent Application Note 5990-4789EN, 2009.

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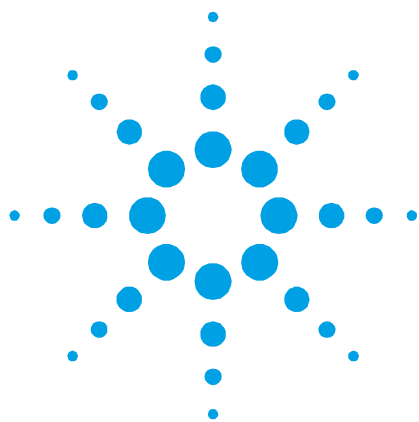
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Agilent Technologies



Rapid measurement of gasoline (petrol) in diesel fuel using portable FTIR spectroscopy

Application Note

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Introduction

Gasoline contamination in diesel fuel is a growing problem as diesel and alternative biodiesel blends are becoming more popular for commercial and personal fuel consumption. Gasoline can contaminate diesel fuel stocks in transit from the refinery to the final destination via tanker trucks, railcars, pipelines, or cargo ship. Gasoline contamination of diesel fuel can also occur in underground storage tanks at distribution facilities or end user filling stations. Some South American countries are experiencing problems with gasoline dilution of diesel to increase profits due to the lower cost of gasoline relative to diesel in certain markets. Some individual diesel owners mix gasoline into diesel to prevent gel formation in very cold winter locations.

Gasoline consists of light distillates with hydrocarbons in the C7-C11 range. Most hydrocarbons in gasoline are straight or branched chained aliphatics; although, 25-30 % of the hydrocarbons are aromatics consisting of hexagonal rings. Aromatics are more volatile and have lower flashpoints than their aliphatic counterparts, and high octane gasoline contains more aromatics. Additionally, gasoline in North America also contains 7-10 % ethanol (oxygenate), which lowers the smog emissions of gasoline engines.



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Diesel fuels are middle distillates from the refining process and consist of hydrocarbon chains greater than C12. These longer hydrocarbon chains result in more energy per unit of volume; furthermore, the diesel combustion process is about 20 % more efficient than a spark ignition combustion engine. Both these properties make turbo diesel powered vehicles travel ~40 % further than an equal sized gasoline powered vehicle on the same volume of fuel. A gasoline contaminant in diesel fuel creates a mixture with less energy content, lower cetane value, and lower lubricity compared to straight diesel. This can cause coke formation (carbonization) on diesel fuel injectors and can also cause excessive wear on injectors, pistons, and other fuel contact engine parts. This is especially problematic for ultra low sulfur diesel (ULSD) due to its lower lubricity compared to agricultural diesel. Furthermore, gasoline can cause varnish deposits on diesel fuel filters to be washed off the filter and into the engine, which could clog injectors and contaminate other critical engine parts.



The chemical differences between gasoline and diesel can be seen in the infrared spectrum of each. Figure 1 shows a comparison between the infrared spectra of gasoline and diesel highlighting the ethanol and aromatics in gasoline. Neither ethanol or light aromatics present in gasoline are observed in the diesel fuel spectrum; in fact, the spectrum of diesel fuel is similar to mineral oil.

Diesel fuel does contain heavy aromatics, such as naphthalenes and other condensed ring compounds, but their infrared bands do not overlap the aromatics present in gasoline. The intensity of an infrared absorbance band is proportional to the concentration of that component in a mixture, as stated in Beer's Law. This relationship allows the Agilent 5500t FTIR spectrometer to accurately measure gasoline in diesel fuels. The sensitivity of the 5500t FTIR allows gasoline to be measured down to 0.025 % in diesel. To demonstrate this, several concentrations of gasoline (87 octane) are carefully prepared in ultra low sulfur diesel (US, Danbury CT). The samples were prepared with 0 %, 0.0269 %, 0.2669 %, and 1.0586 % gasoline in diesel. The FTIR spectra were measured on the 5500t spectrometer and the gasoline absorbance results are plotted against their concentrations in Figure 2. This gasoline absorbance plot indicates a very good linear correlation with concentration. This linear correlation is common in spectroscopy and can be easily added to 5500 FTIR methods. Multiple components can be reported from a single 3 minute analysis, such as gasoline in diesel, biodiesel in diesel, oxidation, and water.

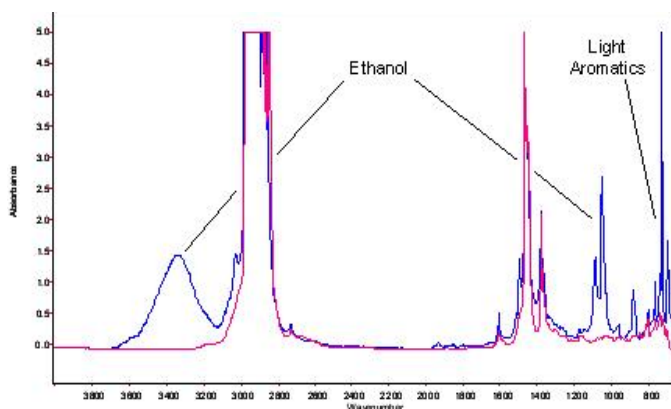


Figure 1. The IR spectral overlay of gasoline (Blue) and diesel fuel (Red) using the Agilent 5500t FTIR spectrometer, 100 μ m pathlength

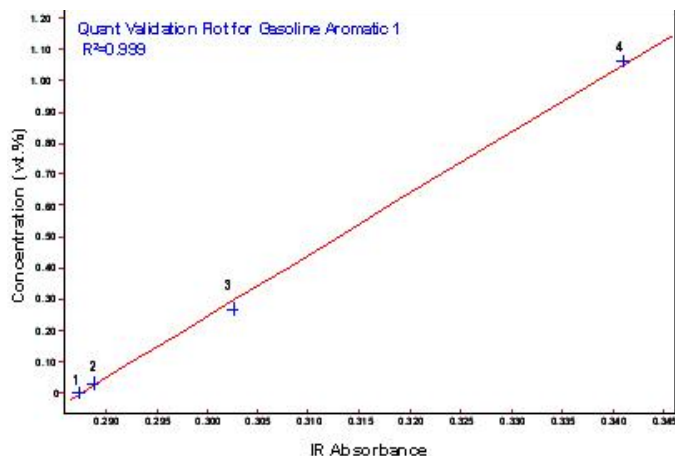


Figure 2. The IR absorbance vs. concentration plot for gasoline in diesel, Agilent 5500t FTIR 100 um pathlength

Conclusion

Fuel analysis using the Agilent 5500t FTIR spectrometer has been shown to accurately measure gasoline in diesel fuel from 0.025-100 % gasoline. This ability coupled with the 5500t FTIR's industry established measurement of biodiesel in diesel, provides highly sensitive on-site and field portable diesel contamination analysis. Oxidation and water contamination are also accurately measured using the same 5500t instrument.

The instrument can be operated from a laptop (5500t) or the Agilent 4500t FTIR which is a fully field portable version with an onboard battery and operated from a hand held computer (PDA). The instrument software is simple to use with little to no sample preparation. The instrument is not harmed by humidity or other outdoor conditions, weighs 8lbs, and takes up less bench space than a laptop computer.



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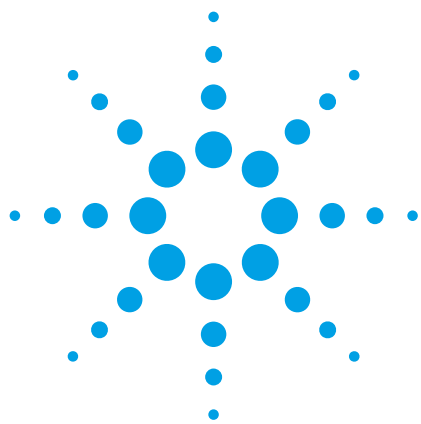
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Agilent Technologies



Measurement of additives in lubricating oils using the Agilent 4100 MP-AES

Application note

Energy and fuels

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Introduction

The regular tracking of the additives present in oils used to lubricate machinery is a vital preventive maintenance task used to gauge the condition of the lubricant and machine over time. Several compounds such as Zn, P, Ca, Ba and Mg are typically added to lubricating oils. These metal-containing additives act as detergents, oxidation and corrosion inhibitors, dispersants, anti-wear agents, viscosity index improvers, emulsifiers and anti-foaming agents etc.

With engines and machinery being central to most transport and manufacturing industries, many laboratories are required to analyze a high volume and variety of oil samples per day, for multiple elements. While flame atomic absorption spectrometry (FAAS) has been used extensively to study additives used in oils, the sheer number of samples has forced



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many laboratories to consider a faster, multi-element technique that is capable of high sample throughput.

This can now be effectively achieved using fast sequential atomic emission spectroscopy in the form of the Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES). The 4100 uses magnetically coupled microwave energy to generate a robust and stable plasma using nitrogen gas. Both aqueous and organic samples can be introduced into the MP-AES with a good tolerance to organic solvent load.

Experimental

Instrumentation

An Agilent 4100 MP-AES was used with an External Gas Control Module (EGCM) for air injection into the plasma to prevent carbon deposition in the torch, overcome any plasma instability that may arise from the analysis of organic samples, and to reduce background emissions. The instrument was set up with the Organics kit comprising the EGCM, the inert OneNeb nebulizer [1] and solvent resistant tubing, along with a double pass spray chamber. The OneNeb nebulizer offers superior performance for this application over other comparable nebulizers as it offers increased nebulization efficiency and a narrow distribution of small droplets. This allows the analysis to be performed at lower flow rates, reducing the solvent loading on the plasma, while maintaining excellent sensitivity. An Agilent SPS 3 Sample Preparation System was used for automatic sample delivery.

The instrument is controlled using Agilent's unique worksheet-based MP Expert software, which runs on the Microsoft® Windows® 7 operating system, and features automated optimization tools to accelerate method development by novice operators. For example, the software automatically adds the recommended wavelength, nebulizer pressure, and EGCM setting when elements are selected.

Instrument operating conditions and analyte settings are listed in Tables 1a and 1b. Viewing position and nebulizer pressure settings were optimized using the auto-optimization routines in MP Expert.

Samples and sample preparation

Standards were prepared at concentrations of 5 ppm, 10 ppm, 25 ppm and 50 ppm from a 500 ppm oil-based metal calibration standard S21+K (Conostan). Shellsol 2046 (Shell) was used as the diluent. All standards were matrix matched with 10% Blank Oil (Conostan).

NIST SRM 1085b Wear Metals in Lubricating Oil was prepared by performing a 1:10 dilution in Shellsol.

A sample of mixed gear oils were diluted 1:100 with Shellsol and a spiked with S21+K giving a final spike concentration of 10.1 mg/kg.

Table 1a. Agilent 4100 MP-AES operating conditions

Instrument parameter	Setting
Nebulizer	Inert OneNeb
Spray chamber	Double-pass glass concentric
Sample tubing	Orange/green solvent-resistant
Waste tubing	Blue/blue solvent-resistant
Read time	3 s
Number of replicates	3
Stabilization time	15 s
Rinse time	45 s
Fast pump during sample uptake	On
Background correction	Auto
Pump speed	5 rpm

Table 1b. Analyte nebulizer pressures and EGCM settings

Element & wavelength (nm)	Nebulizer pressure (kPa)	EGCM setting
Mg 285.213	180	High
Ca 422.673	240	High
Zn 481.053	120	High
Ba 614.171	240	High
P 213.618	120	Medium

Calibration parameters

The calibration fit and correlation coefficients for the elements analyzed are shown in Table 2. Rational fit is a non-linear curve fit and allows an extended working range so that sample analysis can be carried out using a single wavelength without further dilutions being required. The excellent correlation coefficients demonstrate the capability of the MP-AES to cover the

range of concentrations expected in this analysis. The calibration curve for Zn is shown in Figure 1.

Table 2. Analyte calibration fits and correlation coefficients

Element & wavelength (nm)	Calibration fit	Correlation coefficient
Ba 614.171	Rational	0.99908
Ca 422.673	Linear	0.99958
Mg 285.213	Rational	0.99933
Zn 481.053	Linear	0.99999
P 213.618	Rational	0.99998

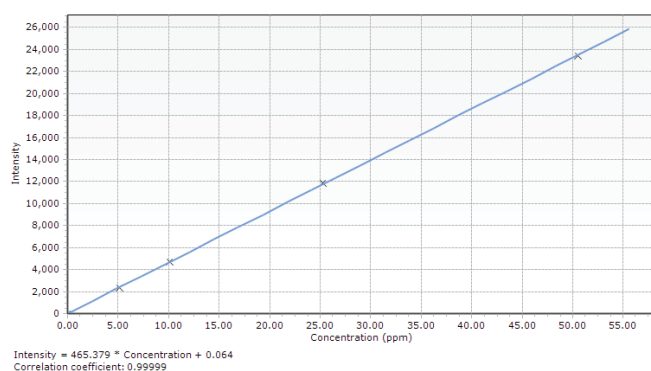


Figure 1. Calibration curve for Zn 481.053 nm showing excellent linearity up to 50 ppm with a correlation coefficient of 0.99999

Results and discussion

Analysis of standard reference materials

To test the validity of the method, NIST SRM 1085b was analyzed. The results given in Table 3 show excellent agreement (accuracy) between the MP-AES measured results and the certified values.

Table 3. Measured results versus certified values

Element & wavelength (nm)	Measured concentration (mg/kg)	Certified (mg/kg)	Recovery (%)
P 213.618	301.5 ± 0.1	299.9 ± 7.2	101
Zn 481.053	314.9 ± 0.3	296.8 ± 6.8	106
Mg 285.213	300.6 ± 0.2	297.3 ± 4.1	101
Ca 422.673	279.6 ± 0.1	(298)	94
Ba 614.171	281.2 ± 0.1	300.1 ± 2.4	94

Spike recoveries

The recoveries obtained for the spiked mixed gear oil sample are given in Table 4. Excellent recoveries were obtained for all elements analyzed, demonstrating the validity of the analytical method. The spectrum for Zn is shown in Figure 2.

Table 4. Accurate recovery for all analytes of 10 ppm spikes in a mixed gear oils sample

Element & wavelength (nm)	Unspiked gear oil (ppm)	Spiked gear oil (ppm)	Spike recovery (%)
P 213.618	17.16	26.71	95
Zn 481.053	6.99	17.17	101
Mg 285.213	1.53	11.32	97
Ca 422.673	8.89	19.69	107
Ba 614.171	0.00	9.16	91

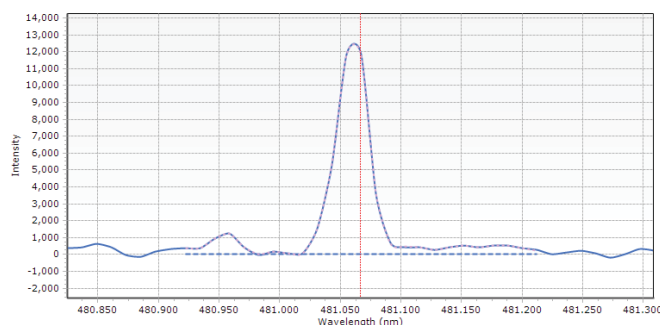


Figure 2. The spectrum for Zn 481.053 nm corrected with Auto background correction

Conclusions

The new Agilent 4100 MP-AES equipped with a OneNeb [1] nebulizer and fitted with the EGCM is an ideal solution for the routine multi-element analysis of additives in oils. The nitrogen-based plasma excitation source exhibits a high tolerance to organic solvent load. Furthermore, the Agilent 4100 MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide. By injecting a controlled flow of air into the plasma via the EGCM to prevent carbon buildup in the injector, excellent recoveries were achieved on SRM samples and on spike solutions at the 10 ppm level.

Reference

1. J. Moffett and G. Russell, "Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer", Agilent Application Note 5990-8340EN

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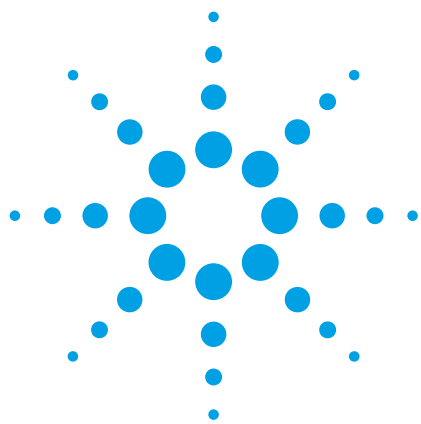
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Agilent Technologies



Direct measurement of metallic impurities in petroleum fuels using the 4100 MP-AES

Application note

Energy and fuels

Authors

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Introduction

The performance of engine or turbine components can be compromised over time through exposure to certain trace elements that may be present in gasoline (petrol) and petro-diesel fuels. It is important to monitor these elements in order to ensure the quality of the fuel and to guard against corrosion and deposition on moving parts. For example, ASTM method D6751 specifies a maximum limit of 5 ppm for the combined concentration of Ca and Mg, and 5 ppm for the combined concentration of Na and K [1]. Even though the method relates to biofuels, it is equally relevant to other petroleum fuels such as gas turbine fuel oil [2].

This application note describes the determination of trace elements in gasoline, without dilution or digestion, using the innovative Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES). The 4100 is a



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fast sequential atomic emission spectrometer that uses magnetically-coupled microwave energy to generate a robust and stable nitrogen plasma. This stable plasma is capable of analyzing both aqueous and challenging organic matrices. By using a nitrogen plasma, the 4100 MP-AES eliminates the need for expensive and dangerous gases, such as acetylene, resulting in lower running costs, unattended operation, and improved productivity when compared with traditional elemental analysis techniques like flame atomic absorption spectrometry.

Experimental

Instrumentation

The Agilent 4100 MP-AES was fitted with an optional External Gas Control Module (EGCM) allowing air injection into the plasma to prevent carbon deposition in the torch, overcome any plasma instability that may arise from the analysis of organic samples, and reduce background emissions. The instrument was set up with Organics kit comprising the EGCM, the inert OneNeb nebulizer [3], along with a double-pass glass cyclonic spray chamber. The OneNeb nebulizer offers increased nebulization efficiency and a narrow distribution of small droplets. This allows the analysis to be performed at lower flow rates, reducing the solvent loading on the plasma, while maintaining excellent sensitivity.

Due to the high volatility of the gasoline sample, an IsoMist cooled spray chamber from Glass Expansion was used to reduce the solvent loading on the plasma, resulting in a more stable plasma and further reducing background emissions.

The instrument was controlled using Agilent's unique worksheet-based MP Expert software, which runs on the Microsoft® Windows® 7 operating system, and features automated optimization tools to accelerate method development by novice operators. For example, the software automatically adds the recommended wavelength, nebulizer pressure, and EGCM setting when elements are selected.

MP Expert also provides Standard Addition Calibration to allow the analysis of samples where finding a matrix matched set of standards is difficult. A further feature

of the software, which simplifies analysis and method development, is the easy-to-use off-peak background correction markers that can be directly modified on the spectra in real time.

Instrument operating conditions and analyte settings are listed in Tables 1a and 1b.

Table 1a. Agilent 4100 MP-AES operating conditions

Instrument parameter	Setting
Nebulizer	Inert OneNeb
Spray chamber	Double-pass glass cyclonic
Sample tubing	Orange/green solvent-resistant
Waste tubing	Blue/blue solvent-resistant
Read time	3 s
Number of replicates	3
Stabilization time	45 s
Fast pump during sample uptake	On
Background correction	Off-peak
Pump speed	5 rpm
Calibration	Standard additions
Cooled spray chamber temperature	-10 °C

Table 1b. Analyte viewing positions, nebulizer pressures and EGCM settings

Element & wavelength (nm)	Nebulizer pressure (kPa)
Mg 285.213	240
Ca 422.673	240
Na 588.995	240
K 766.491	240

Samples and sample preparation

Gasoline fuel standards were prepared by spiking a sample with an oil-based metal calibration standard, S21+K (Conostan), giving final concentrations of 0.89 ppm, 1.92 ppm and 3.94 ppm.

The gasoline samples were directly analyzed, without any sample preparation.

For the spike recovery test, gasoline samples were spiked with S21+K to give spike concentrations of 1.1 ppm.

Results and discussion

Detection limits

Method detection limits were calculated as the concentration equivalent to 3 standard deviations of 10 blank gasoline measurements. The detection limits for gasoline are sufficiently low for the requirements of the analysis. These detection limits demonstrate the ability of the Agilent 4100 MP-AES to handle tough organic samples when coupled with the EGCM, the OneNeb nebulizer and the cooled spray chamber.

Table 2. Method detection limits (ppb) for Mg, Ca, Na and K in gasoline

Element	Wavelength (nm)	Gasoline MDL (ppb)
Mg	285.213	2.7
Ca	422.673	4.3
Na	588.995	5.3
K	766.491	29.4

Calibration

When measuring gasoline samples, the high volatility of the samples makes finding matching standards difficult, even if samples have been diluted with kerosene. For this reason, standard additions calibration was used for this analysis.

Standard addition also means that the gasoline samples can be directly analyzed, without the need for further sample preparation. The stable nitrogen plasma of the MP-AES can easily handle these volatile samples and, as shown in Table 3, excellent correlation coefficients were found for the elements measured in this analysis. A typical spectrum for Mg 285.213 with off-peak background positions, and a calibration curve for Mg 285.213 are shown in Figures 1 and 2.

Table 3. Calibration correlation coefficients for Mg, Ca, Na and K

Element	Wavelength (nm)	Correlation coefficient
Mg	285.213	0.99993
Ca	422.673	0.99934
Na	588.995	0.99939
K	766.491	0.99975

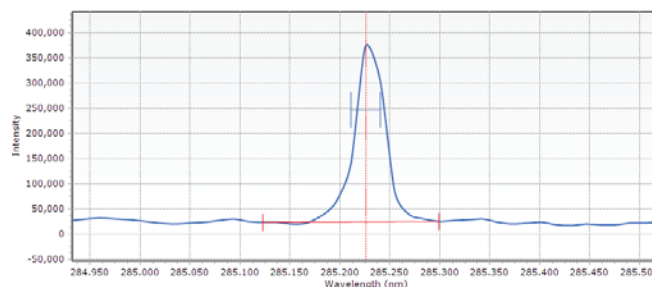


Figure 1. Representative spectrum for Mg 285.213 in the spiked sample

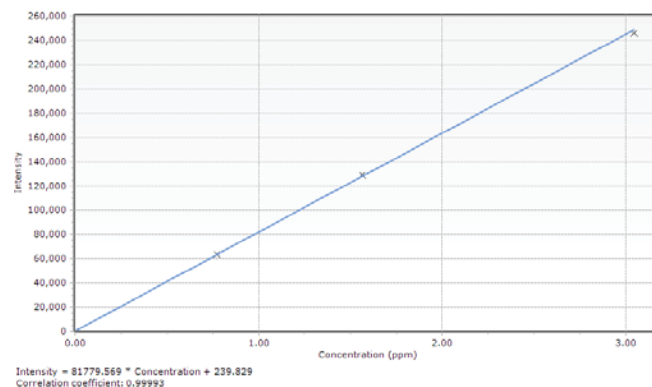


Figure 2. Calibration curve for Mg 285.213

Spike recoveries

The spike recovery results for the gasoline samples are shown in Table 4. The spike concentration was 1.1 ppm and all recoveries were within $\pm 10\%$ of the target value. The excellent recoveries demonstrate the ability of the 4100 MP-AES to accurately determine Mg, Ca, Na and K at the levels required in the gasoline samples.

Table 4. Spike recovery results for Mg, Ca, Na and K in gasoline

Element & wavelength (nm)	Sample (ppm)	Spiked sample (ppm)	Recovery (%)
Mg 285.213	< MDL	1.11	100
Ca 422.673	< MDL	1.06	95
Na 588.995	< MDL	1.11	100
K 766.491	0.05	1.12	96

Conclusions

The Agilent 4100 MP-AES equipped with a OneNeb nebulizer, the EGCM and the IsoMist cooled spray chamber provides an ideal solution for the routine and direct analysis of highly volatile gasoline. The nitrogen-based plasma excitation source exhibits a high tolerance level to organic solvent loading, and the powerful features of the MP Expert software such as standard addition enables analysis of tough samples. By injecting a controlled flow of air into the plasma via the EGCM to prevent carbon buildup in the injector, excellent calibrations, detection limits, and recoveries were achieved in spiked gasoline samples at levels likely to be encountered in this analysis (low ppm).

Furthermore, the Agilent 4100 MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide. The Agilent 4100 MP-AES also improves sample throughput and removes the need for hollow cathode lamps.

References

1. ASTM D6751 – 11b, Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels, ASTM International.
2. ASTM D2880 – 03, Standard Specification for Gas Turbine Fuel Oils, ASTM International.

3. J. Moffett and G. Russell, "Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer", Agilent Application Note 5990-8340EN

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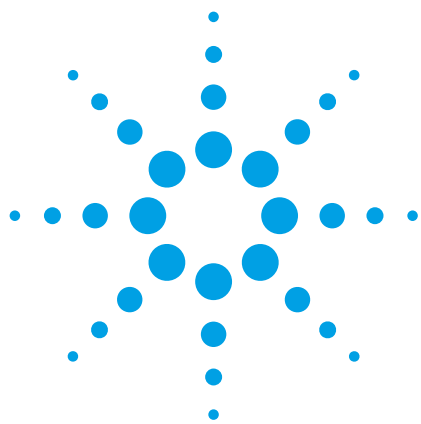
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Analysis of diesel using the 4100 MP-AES

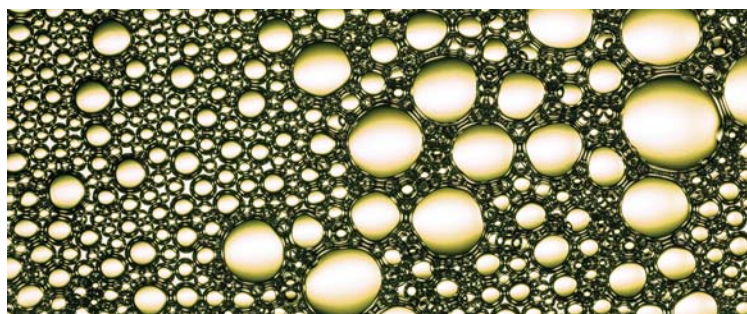
Application note

Energy and fuels

Authors

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Introduction

The presence of certain trace elements in petro-diesel and biodiesel fuels can cause corrosion and deposition on engine or turbine components, especially at elevated temperatures. Some diesel fuels therefore specify the maximum levels of these elements to guard against the occurrence of engine deposits. For instance ASTM method D6751 specifies a limit of 5 ppm for the combined concentration of Ca and Mg, and 5 ppm for the combined concentration of Na and K [1]. Trace elemental analysis is used to determine the level of contamination of diesel fuels.

The Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) uses magnetically-coupled microwave energy to generate a robust and stable plasma using nitrogen gas. This stable plasma is capable of analyzing not only aqueous solutions, but also challenging organic matrices. When compared to conventional flame AA, the 4100 MP-AES eliminates expensive and dangerous gases such as acetylene, resulting in lower running costs, unattended operation, and improved productivity.

This application note describes the determination of trace elements in diesel fuels using the Agilent 4100 MP-AES.



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Experimental

Instrumentation

The Agilent 4100 MP-AES was fitted with an External Gas Control Module (EGCM) allowing air injection into the plasma to prevent carbon deposition in the torch, overcome any plasma instability that may arise from the analysis of organic samples, and reduce background emissions. The instrument was set up with the Organics kit comprising of the EGCM, the inert OneNeb nebulizer [2] and solvent resistant tubing, along with a double pass spray chamber. The OneNeb nebulizer offers increased nebulization efficiency and a narrow distribution of small droplets. This allows the analysis to be performed at lower flow rates, reducing the solvent loading on the plasma, whilst maintaining excellent sensitivity.

The instrument was controlled using Agilent's unique worksheet-based MP Expert software, which runs on the Microsoft® Windows® 7 operating system, and features automated optimization tools to accelerate method development by novice operators. For example, the software automatically adds the recommended wavelength, nebulizer pressure, and EGCM setting when elements are selected. Also, the powerful Auto background correction mode easily and accurately corrects for the emission background arising from the organic matrix.

Instrument operating conditions and analyte settings are listed in Tables 1a and 1b.

Table 1a. Agilent 4100 MP-AES operating conditions

Instrument parameter	Setting
Nebulizer	Inert OneNeb
Spray chamber	Double-pass glass cyclonic
Sample tubing	Orange/green solvent-resistant
Waste tubing	Blue/blue solvent-resistant
Read time	3 s
Number of replicates	3
Sample uptake delay	15 s
Stabilization time	30 s
Fast pump during sample uptake	On
Background correction	Auto
Pump speed	5 rpm

Table 1b. Analyte viewing positions, nebulizer pressures and EGCM settings

Element & wavelength (nm)	Nebulizer pressure (kPa)	EGCM setting
Mg 285.213	240	High
Ca 422.673	240	High
Na 588.995	240	High
K 766.491	240	High

Samples and sample preparation

Method EN 14538 [3] was followed for the analysis of the diesel samples. Calibration standards were prepared at concentrations of 0.5 ppm, 1 ppm, 5 ppm and 10 ppm by diluting a 500 ppm S21+K solution (Conostan) with Shellsol (Shell). All standards were matrix matched with Blank Oil 75 (Conostan).

A commercial diesel sample was spiked with S21+K at the 0.5 ppm level and the spikes were measured to validate the method.

Results and discussion

Detection limits

Method detection limits were calculated as the concentration equivalent to 3 standard deviations of 10 blank diesel measurements. The detection limits reported in Table 2 are in solution, and are sufficiently low for the requirements of the analysis. These detection limits demonstrate the ability of the 4100 MP-AES to handle tough organic samples, provide excellent detection limits at low sample flow rates, and handle the challenging background from carbon emissions using the power and simplicity of auto background correction.

Table 2. Method detection limits (ppb) for Mg, Ca, K, and Na

Element	Wavelength (nm)	MDL (ppb)
Mg	285.213	2.7
Ca	422.673	8.2
Na	588.995	18.7
K	766.491	2.7

Spike recoveries

The spike recoveries in diesel fuel are shown in Table 3. The spike concentration was 0.55 ppm and all recoveries were within $\pm 10\%$ of the target value. The excellent recoveries demonstrate the ability of the 4100 MP-AES to accurately determine Mg, Ca, Na and K at the levels required in the diesel fuel samples. A typical spectrum and calibration graph for K are shown in Figures 1 and 2 respectively.

Table 3. Results of spike recovery test

Element and wavelength (nm)	Sample (ppm)	Spike (ppm)	Recovery (%)
Mg 285.213	< MDL	0.53	97
Ca 422.673	< MDL	0.51	93
Na 588.995	< MDL	0.51	93
K 766.491	< MDL	0.51	93

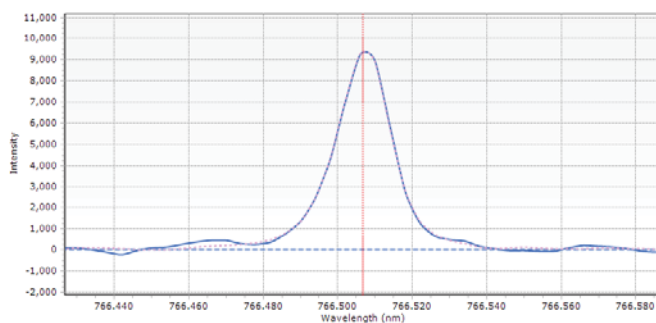


Figure 1. Signal for K 766.491 at 0.5 ppm showing the excellent sensitivity of the 4100 MP-AES when analyzing fuel samples

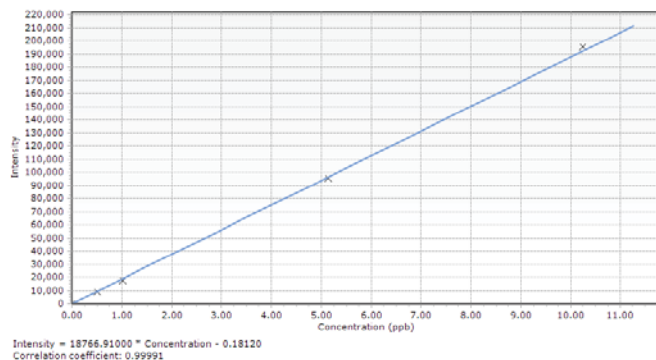


Figure 2. Calibration curve for K 766.491 showing excellent linearity across the calibrated range and a correlation coefficient of 0.99991

Conclusions

The Agilent 4100 MP-AES equipped with the OneNeb nebulizer and the EGCM provides an ideal solution for the routine analysis of semi-volatile organic samples such as diesel. The nitrogen-based plasma excitation source exhibits a high tolerance to the organic solvent load and the easy-to-use yet powerful features of the MP Expert software, such as the auto background correction mode, ensure excellent detection limits. By injecting a controlled flow of air into the plasma via the EGCM to prevent carbon buildup in the injector, excellent calibrations, detection limits, and recoveries were achieved in spiked diesel fuel samples at levels likely to be encountered in this analysis (low ppm).

Furthermore, the Agilent 4100 MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide. The 4100 MP-AES also improves sample throughput and removes the need for consumables like hollow cathode lamps.

Reference

1. ASTM D6751 – 11b, Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels, ASTM International, www.astm.org
2. J. Moffett and G. Russell, "Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer", Agilent Application Note 5990-8340EN
3. EN 14538:2006, Fat and oil derivatives – Fatty acid methyl ester (FAME) – Determination of Ca, K, Mg and Na content by optical emission spectral analysis with inductively coupled plasma (ICP OES), European Committee for Standardization, www.cen.eu

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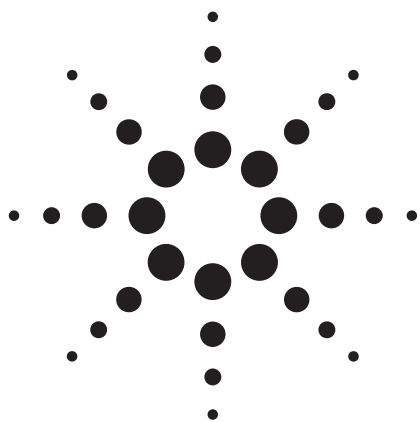
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Agilent Technologies



The Determination of Lead in Unleaded Gasoline Using the Agilent 55 AA Atomic Absorption Spectrophotometer

Application Note

Atomic Absorption

Author

John Sanders

Introduction

The reduction of lead levels in gasoline has been a priority of environmental agencies around the world since the early eighties.

In this paper we describe the use of Agilent's 55B AA atomic absorption spectrophotometer when applied to the analysis of unleaded gasoline for trace lead levels.

The methodology used in sample preparation was taken from the ASTM D3237-79 [1], with a change in the sample solvent from methyl isobutyl ketone (MIBK) to di-isobutyl ketone (DIBK).

A National Institute of Standards and Technology (NIST, Gaithersburg, MD, U.S.A.) Standard Reference Material (SRM) 2712 Pb in Reference Fuel was used to establish the accuracy and precision of the method.

Experimental

Instrumentation

An Agilent 55B AA atomic absorption spectrometer, air/acetylene burner, organics solvent "O" ring kit for the spray chamber, and a serial printer.

This system was also operated with a simulated LIMS connection through Hyperterminal under Windows®95.

A series of analyses was also conducted using the Agilent 55 AA software.



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The instrument operating conditions are shown in Table 1.

Table 1. Instrument operating conditions

Element	Pb
Instrument mode	Absorbance
Active current mA	10.0
Standby current mA	0.0
D ₂ background correction	Yes
Flame type	Air acetylene
Wavelength nm	217.0
Slit nm	1.0
Measurement parameters	
Measurement mode	Integration
Read time sec	5.0
Replicates sec	3
Pre-read delay sec	3.0
Calibration parameters	
Standard concentrations	
Cal 0	0.000
Std 1	20 mg/USgal
Std 2	50 mg/USgal
Std 3	100 mg/USgal
Nebulizer uptake rate	5 mL/min

Reagents and Solutions

All reagents were Analytical Reagent grade.

- Di-isobutyl ketone, DIBK (818831, Merck, Schuchardt, Germany)
- Lead free gasoline: Retail grade (containing less than 0.005 g/USgal)
- Aliquat 336 (tricaprylmethylammonium chloride, Aldrich, Milwaukee, WI, U.S.A.)
- 10% (v/v) Aliquat 336/DIBK solution Dissolve Aliquat 336 (88 g) in DIBK and dilute to 1000 mL
- 1% (v/v) Aliquat 336/DIBK solution: Dissolve Aliquat 336 (8.8 g) in DIBK and dilute to 1000 mL
- Iodine Solution: Dissolve iodine crystals (3.0 g) in toluene and dilute to 100 mL
- 5g/USgal lead standard solution: Dissolve anhydrous lead chloride (PbCl₂, 0.4433 g), previously dried at 105 °C for 3 hours, in about 200 mL of 10% Aliquat 336/DIBK solution in a 250-mL volumetric flask. Dilute to the mark with 10% Aliquat 336/DIBK. This solution contains 1321 µg Pb/mL and is equivalent to 5.0 g Pb/USgal
- 1g/USgal lead standard solution: Accurately pipette 50.0 mL of the 5.0 g Pb/USgal solution to a 250-mL volumetric flask and dilute to the mark with 1% (v/v) Aliquat 336/DIBK

- Lead standard solutions: (0.02, 0.05, and 0.1 g Pb/USgal.) Accurately pipette 2.0, 5.0, and 10.0 mL of the 1 g/USgal Lead Standard solution to 100-mL volumetric flasks respectively and add 5.0 mL of 1% (v/v) Aliquat 336 solution to each flask. Dilute to the mark with DIBK.

Calibration Solutions

Prepare working standards and a blank using the 0.02, 0.05 and 0.10 g Pb/USgal standard lead solution.

1. Add 30 mL DIBK and 5.0 mL of lead free gasoline to each of four 50-mL volumetric flasks. add 5.0 mL of standard solution respectively to three flasks. The last flask represents a blank.
2. Add immediately 0.1 mL of iodine/toluene solution by means of a 100 µL Eppendorf pipet, mix well and allow to stand for 5 minutes.
3. Add 5 mL of 1% (v/v) Aliquat 336/DIBK solution and mix well.
4. Dilute to volume with DIBK and mix well.

Sample Preparation

1. To each of four 50 mL volumetric flasks containing 30 mL DIBK add 5.0 mL the gasoline sample.
2. Add immediately 0.1 mL of iodine/toluene solution using a 100 µL digital (Eppendorf or similar) pipette, mix well and allow to stand for 5 minutes.
3. Add 5 mL of 1% (v/v) Aliquat 336/DIBK solution and mix well.
4. Dilute to volume with DIBK and mix well.

Instrument Optimization

Set up the spectrophotometer and adjust the nebulizer uptake to approximately 5 mL/min.

On the optimize page, maximize the lamp signal intensity and the background lamp intensity.

Then adjust the signal intensity, by adjusting the burner rotation, lateral controls, and the gas flows while continuously aspirating the 0.1 g Pb/USgal standard.

Instrument Measurement

Set the instrument zero while aspirating the blank. Read the calibration solutions from Cal 0 to Standard 3 respectively. Read the samples relative to the calibration.

Results and Discussion

The Agilent 55 AA provides a simple and accurate means of determining lead in unleaded gasoline. Output can be fed either to a LIMS system or to a printer. By using computer control sample identification and data manipulation can be accomplished.

The use of the Agilent 55 AA v2 software and computer control provides access to result storage and archival retrieval.

Table 2 shows the results obtained from this study for the certified reference fuel. The experimental results compare well with the certified values.

Table 2. SRM 2712 Result Summary

SRM 2712	g/USgal
Measured value	0.0302 ± 0.0001
Certified value	0.0297 ± 0.0010

Samples of regular unleaded gasoline and premium grade unleaded gasoline were also analyzed. The results in Table 3 show the lead level in the regular grade was close to the detection limit, while the level in the premium unleaded gasoline was easily measured.

Table 3 expresses the results in mg/USgal. In using these units the Agilent 55B AA provides an increased number of significant figures to enable the results to be expressed to the full capability of the instruments performance.

Table 3. Analytical Results Agilent 55 AA 217.0nm

	mg/USgal	mg/L	Mean Abs
Unleaded regular gasoline	1.0	0.10	0.006
Premium unleaded gasoline	3.0	0.80	0.012
SRM 2712	30.2	8.00	0.071
SRM 2712 certified value	29.7 ± 0.10	7.9 ± 0.3	
Instrument detection limit 3σ	0.28	0.07	

Tables 4 and 5 illustrate the results obtained using the Agilent 55 AA Windows®95 software, using the two common wavelengths for lead. Both wavelengths gave the same result for the analysis.

Table 4. Agilent 55 AA Results Using the 283.3 nm Line

	g/USgal	g/L	Mean Abs
Cal zero	0	0	0.0001
Standard 1	0.0200	0.0053	0.0228
Standard 2	0.0500	0.0132	0.0542
Standard 3	0.1000	0.0265	0.1013
Regular unleaded	0.0002	0.0001	0.0002
Premium unleaded	0.0024	0.0006	0.0027
SRM-2712	0.0303	0.0080	0.0338
SRM-2712 certified value	0.0297 ± 0.0010	0.0079 ± 0.0003	
Instrument detection limit 3σ	0.0007	0.0002	

Table 5. Agilent 55 AA Results Using the 217.0 nm Line

	g/USgal	g/L	Mean Abs
Cal zero	0	0	-0.0019
Standard 1	0.0200	0.0053	0.0508
Standard 2	0.0500	0.0132	0.1217
Standard 3	0.1000	0.0265	0.2262
Regular unleaded	0.0011	0.0003	0.0028
Premium unleaded	0.0028	0.0007	0.0073
SRM-2712	0.0302	0.0080	0.0757
SRM-2712 certified value	0.0297 ± 0.0010	0.0079 ± 0.0003	
Instrument detection limit 3σ	0.0006	0.0002	

No significant difference was found between the values measured at the 217.0 nm and 283.3 nm wavelengths.

The 283.3 nm line is preferred due to the higher signal intensity and lower non-specific background absorbance at the higher wavelength.

Conclusion

The Agilent 55B AA is able to measure lead in unleaded gasoline with precision of better than 0.0001 g/USgal using the ASTM method D3237. Accuracy is excellent and is shown to be better than 0.0005 g/USgal.

Both the 217.0 nm and 283.3 nm lines can be used.

Safety Notice

Solvents used in this methodology present a hazard risk to users; all operators should consult the relevant Material Safety Data Sheet, and local hazardous substances precautions, before carrying out this procedure.

Use of flame atomic absorption systems with gasoline samples requires complete observation of all relevant safety practices for the presence of flammable materials in the presence of flames.

References

1. ASTM D3237-79 (Re-approved 1984); current version D3237-97

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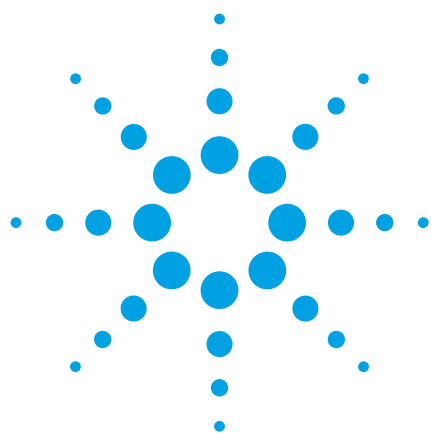
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Analysis of Diesel using an Agilent J&W FactorFour VF-5ht UltiMetal Column

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

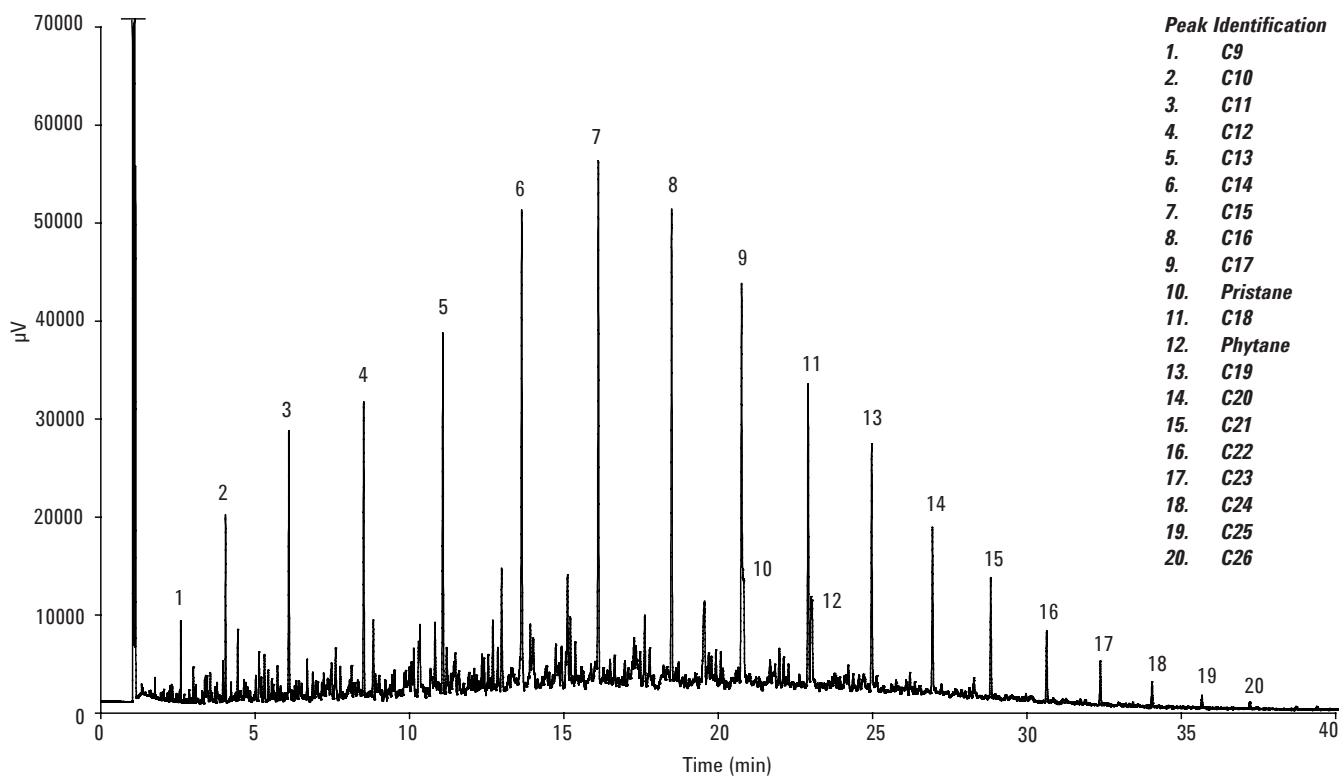
This analysis of diesel is performed using a VF-5ht UltiMetal column. The column has been developed using proprietary UltiMetal technology that provides a virtually unbreakable metal column material with excellent inertness properties similar to fused silica tubing. The UltiMetal column tubing is coated with the VF-5ms low bleed arylene stabilized liquid phase, resulting in a highly temperature stable and durable column perfectly suited for a variety of high temperature applications.



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Conditions

Technique:	GC	Carrier Gas:	Hydrogen, 65 kPa (9 psi)
Column:	VF-5ht UltiMetal, 30 m x 0.25 mm (part number CP9093) Df = 0.1 µm + Retention Gap, 2 m x 0.53 mm	Injector:	Split, 325 °C, split ratio 1:100
		Injection Volume:	2.0 µl
		Temperature:	50 °C to 400 °C with 5 °C/ min
Sample:	Diesel, 0.1 % (Pentane)	Detection:	FID, 340 °C



Analysis of Diesel using a VF-5ht UltiMetal column

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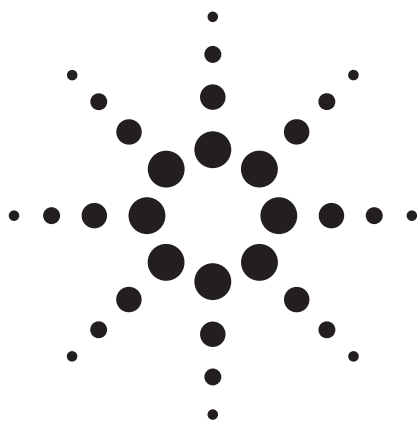
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Agilent Technologies



Determination of Rhodium in Catalyst Oil and Aqueous Samples Using the Agilent 710 ICP-OES

Application Note

Energy and Fuels

Author

Jin-Na Chang

Introduction

A series of oil catalyst samples and an unknown aqueous sample were supplied for analysis. The rhodium content in these samples was determined using the Agilent 710 ICP-OES axially viewing ICP-OES. The oil catalyst samples were prepared using microwave digestion.

The axially viewing ICP-OES was selected for measurement as the expected rhodium content was low, and this system provides better sensitivity than that achieved by the radially viewed configuration, independent of the complexity of the sample medium [1]. The improvement in sensitivity with the axially viewed configuration is typically up to a 20 fold improvement in detection limits [1,2].



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Instrumentation

All sample measurements were performed on a Agilent 710 ICP-OES axially viewing ICP-OES with simultaneous CCD detection.

The Agilent 710 ICP-OES is a simultaneous ICP-OES featuring an Echelle polychromator and a megapixel CCD detector, which provides the benefit of simultaneous measurement and continuous wavelength coverage over the range from 177 to 785 nm. The polychromator can be purged with a low flow of either argon or nitrogen for improved detection capability when measuring emission lines at low UV wavelengths.

The system is supplied as standard with a 3-channel peristaltic pump and manual pressure control of the nebulizer gas flow. The standard sample introduction system consists of a glass concentric nebulizer (Conikal) and a glass cyclonic spray chamber. Agilent ICP Expert II software was used for instrument operation. The operating parameters of the system are listed in Table 1.

Table 1. Instrument Operating Parameters

Condition	Setting
Power	1.15 kW
Plasma gas flow	16.5 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Glass cyclonic (single-pass)
Torch	Standard one piece axial torch
Nebulizer pressure	200 kPa
Nebulizer type	Conikal
Replicate read time	5 s
Auto-integration	On
Number of replicates	3
Stabilization time	15 s
Pump tubing	Sample: white-white (1.05 mm ID) Waste: blue-blue (1.65 mm ID) Buffer/Reference element: black-black (0.76 mm ID)
Sample uptake delay time	15 s
Pump speed	15 rpm
Rinse time	10 s
Fast pump	On
Background correction	Fitted

Sample Preparation and Instrument Conditions

All chemicals and reagents used were of high purity grade.

- HNO₃, Ultrapure, 60%, Merck.
- 40 and 100 mg/L Rh standard solutions as supplied by the client.
- Milli-Q water with resistivity less than 18 Mohm-cm⁻¹.

The oil catalyst samples were prepared using a microwave digestion system with temperature and pressure control (Shanghai EU Microwave Chemistry Technology Co. Ltd. model WX-4000).

0.3 g of sample was accurately weighed and placed into a digestion vessel. The digestion vessel was heated on a conventional hot plate at 80 °C for approximately 20 minutes. The aim of this sample pre-treatment was to remove the volatile organic components from the sample. The digestion vessel was removed from the hot plate and allowed to cool.

This solution was quantitatively transferred into a microwave digestion vessel and 4 mL of nitric acid (HNO₃) was added. The digestion vessel was sealed and placed into the microwave digestion system. The microwave digestion method used is summarized in Table 2.

Table 2. Microwave Digestion Settings

Step	Temperature (°C)	Pressure (atm.)	Time (min.)
1	130	10	5
2	160	16	5
3	180	20	5
4	200	25	5

The digestion vessels were removed from the microwave digestion system and left to cool to room temperature. The digest was transferred to a volumetric flask and diluted on a mass basis. A summary of the initial sample weights, the final weights after dilution and observations on the digest obtained are summarized in Table 3.

As the expected concentration of rhodium in the aqueous sample was around 60 mg/L, no preparation was required for analysis. The aqueous sample was measured directly.

All the prepared samples were analyzed directly for rhodium.

Table 3. Actual Sample Weights Used for Digestion Together with Observations on the Samples Before and After Microwave Digestion

No.	Sample ID	Sample type	Expected Rh concentration	Observations	Sample weight (g)	Weight of predetermined volume (g)	Digest obtained
1	Q22321 2007-08-04	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3033	23.8461	Clear
2	Q22321 2007-08-05	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3260	27.5567	Clear
3	Q22321 2007-08-06	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3442	23.2292	Clear
4	Q22321 2007-08-07	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3386	22.7784	Clear
5	Q22321 2007-08-08	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3043	24.2429	Clear
6	Q22321 2007-08-09	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3380	24.9658	Clear
7	Q22321 2007-08-10	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3025	22.2151	Clear
8	Q21011 2007-08-06	Oil catalyst	Est. 100-200 mg/L	Yellow, transparent oil sample with pungent odor	0.3096	52.3151	Light yellow color, but basically clear
9	Rhodium acetate solution	Aqueous solution	Est. 60 mg/L	Light yellow, transparent sample	N/A	N/A	N/A

Calibration Solutions

Conventional aqueous Rh standard solutions of 40 and 100 mg/L were provided by the client together with the samples.

For the determination of the aqueous sample, which was expected to be at a concentration of 60 mg/L, these standard solutions were used directly to calibrate the instrument.

For the determination of the digested oil samples, for which the Rh content was expected to be low, these standard solutions were diluted by a factor of 10 to concentrations of 4 and 10 mg/L respectively.

Results and Discussion

The calibration graphs obtained are shown in Figures 1 and 2.

The weight/dilution corrected sample results are listed in Table 4. These results have been converted back to report the actual rhodium content contained in the original samples. This takes into account the sample weight used for digestion and the applied dilution ratio during preparation.

The measured concentrations for rhodium in a number of the samples were close to the calibration blank solution. Accordingly, these results have been reported as “Not Detected” (ND).

Signal traces for the measured solutions where the Rh content could be quantified are included in Figures 3 to 5.

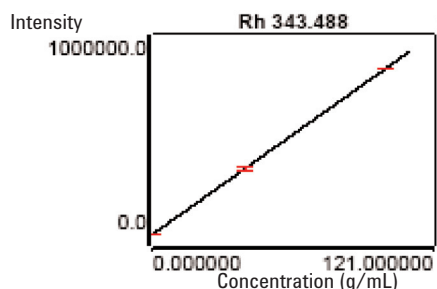


Figure 1. Calibration graph used for the determination of the aqueous sample at the Rh 343.488 nm emission line.

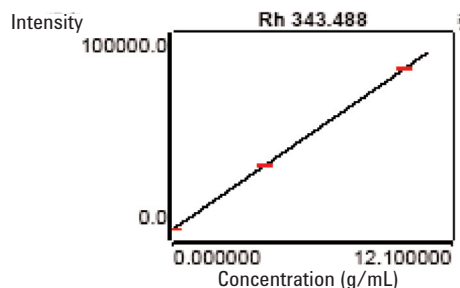


Figure 2. Calibration graph used for determination of the digested oil catalyst samples at the Rh 343.488 nm emission line.

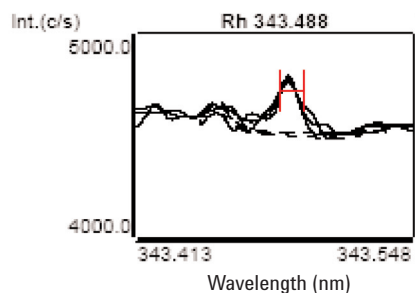


Figure 3. Signal trace for oil sample Q22321 2007-08-07.

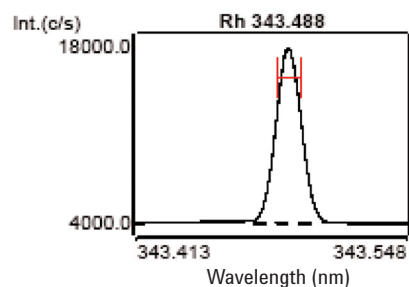


Figure 4. Signal trace for oil sample Q21011 2007-08-06.

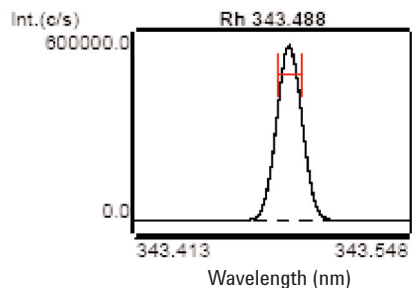


Figure 5. Signal trace for the rhodium acetate aqueous solution.

Table 4. Measured Rhodium Concentrations in Each of the Samples

Sample Type	Sample ID	Expected Rh Concentration (mg/L)	Measured Rh Concentration (mg/L)	Precision (% RSD)
Oil samples	Q22321 2007-08-04	Very low	ND	N/A
	Q22321 2007-08-05	Very low	ND	N/A
	Q22321 2007-08-06	Very low	ND	N/A
	Q22321 2007-08-07	Very low	3.08	4.15
	Q22321 2007-08-08	Very low	ND	N/A
	Q22321 2007-08-09	Very low	ND	N/A
	Q22321 2007-08-10	Very low	ND	N/A
	Q21011 2007-08-06	Est. 100–200 mg/L	216.20	0.197
Aqueous samples	Rhodium acetate	Est. 60 mg/L	55.26	0.842

Conclusion

Using the Agilent 710 ICP-OES axially viewing ICP-OES, it was possible to determine the rhodium content in both the oil catalyst and the aqueous samples without interferences. The oil catalyst samples were prepared using a microwave digestion system with temperature and pressure control. The digests obtained were clear, confirming complete digestion. The method demonstrated good results, although the Rh concentration in most samples was not detectable.

The results demonstrate this method can be readily applied to the routine determination of rhodium in oil catalyst and aqueous samples.

References

1. F.V. Silva, L.C. Trevizan, C.S. Silva, A.R.A. Nogueira and J.A. Nobrega, Evaluation of inductively coupled plasma optical emissions spectrometers with axially and radially viewed configurations, *Spectrochim. Acta*, 2002, 57B, 1905.
2. I.B. Brenner and A.T. Zander, Axially and radially viewed inductively coupled plasmas – A critical review, *Spectrochim. Acta*, 2000, 55B, 1195.

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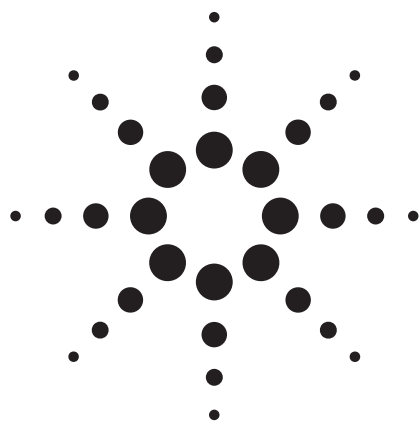
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Increased Identification of Impurities in Octyl Dimethyl p-Aminobenzoic Acid Using the Agilent 6140 High Throughput LC/MS



Application

Chemical Analysis

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Abstract

The 6140 Single Quadrupole LC/MS High Throughput Mass Spectrometer is used to analyze octyl dimethyl para-aminobenzoic acid (OD-PABA) for the presence of impurities. The Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system is used for the chromatographic separation of the compound from its impurities on a 3.0 mm id C18 column with a 1.8 μ m-particle size. The LC/MS interface used in this work is a G1948B electrospray ionization source (ESI) in positive ion mode. While many compounds can be analyzed at the standard scan rate of 5400 amu/sec, one impurity is only clearly seen at the scan speed of 10,000 amu/sec, which is a unique capability of the 6140 system. This impurity is

identified as p-dimethylbenzoic acid, a known degradate of octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Introduction

Para-aminobenzoic acid (PABA) has historically been used as an ultraviolet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer, a derivative in the form of OD-PABA is currently and more commonly used. However, as PABA may be formed as a degradate of OD-PABA, it is important to monitor its potential presence in neat standards of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, not only for the purpose of safety, but for economics as well. In this work we investigate the capability of the Agilent 6140 Single Quadrupole Mass Spectrometer to detect impurities that are seen above 0.1% of the OD-PABA absorbance level in UV.

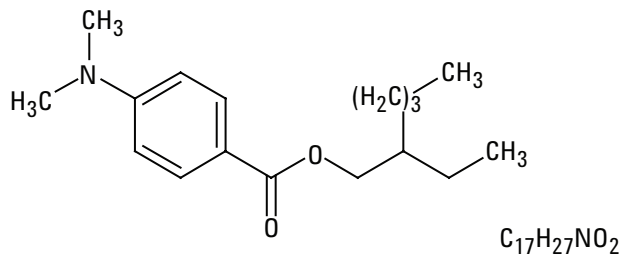


Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).



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The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

Experimental

Sample Preparation

The OD-PABA is obtained at a concentration of 1 mg/mL in methanol. Injection volumes of 5 μ L at this concentration are made into the LC/MS system.

LC/MS Method Details

LC Conditions

Agilent 1200 Series binary pump SL, wellplate sampler, thermostatted column compartment

Column: Agilent ZORBAX SB-C18, 3 $\times$ 30 mm, 1.8 μ m (p/n 824975-302)

Column temp: 45 $^{\circ}$ C

Mobile phase: A = 0.1% formic acid in water
B = 0.1% formic acid in acetonitrile

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

Gradient:	Time (min)	%B
	0	25
	7	75

Stop time: 7 min

Post-run time: 2 min

UV Conditions

Sample: 320 nm; Bw, 5 nm; reference off

MS Conditions

Mode: Positive ESI using the Agilent G1948B ionization source

Nebulizer: 60 psig

Drying gas flow: 12 L/min

Drying gas temp: 350 $^{\circ}$ C

V_{cap} : 3000 V

MS Scan: m/z 100–450

Cycle times (sec/cycle), 0.09 in Standard Fast Scan mode; 0.04 in Ultra Fast Scan mode

Table 1. Integration Results of Three Significant Peaks Found in OD-PABA Chromatogram of Figure 2

Peak #	Time (min)	Area	Height	Area %
1	0.707	76.4	44.1	0.4
2	5.184	176.6	50	0.925
3	6.005	18847.7	2911.3	98.676

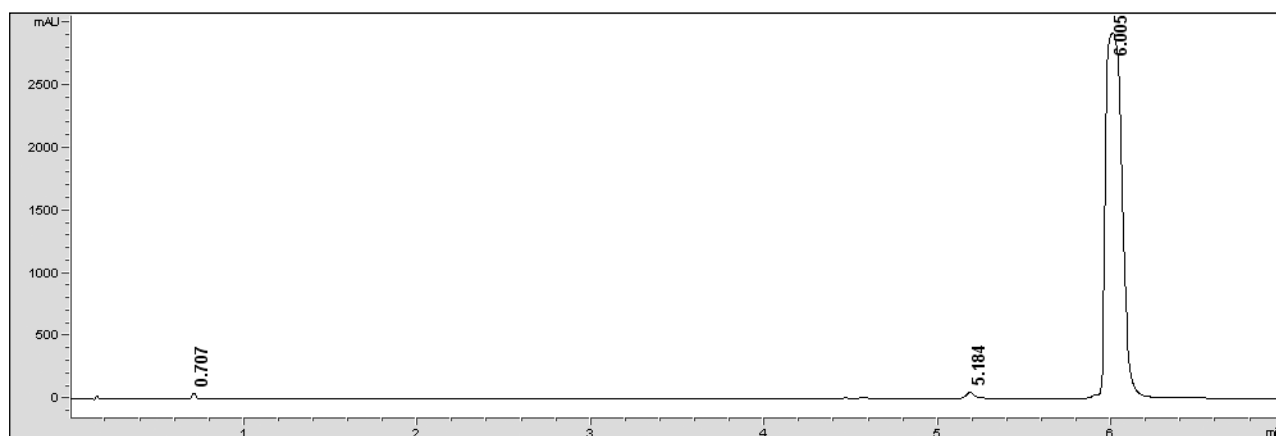


Figure 2. UV chromatogram of OD-PABA at 320 nm absorbance.

Results and Discussion

The UV absorbance of OD-PABA is shown at a retention time of 6.005 minutes in Figure 2. The tabulated integration results for each of the peaks shown are given in Table 1.

The highest data acquisition speed in the Standard Fast Scan mode (5,400 amu/sec) is 0.09 sec/cycle. The total ion chromatogram (TIC) corresponding to this mode is shown in Figure 3A. The Ultra Fast Scan mode (10,000 amu/sec) is only available in the Agilent 6140 mass spectrometer and has a corresponding cycle time of 0.04 sec/cycle. The total ion chromatogram corresponding to the Ultra Fast Scan mode is shown in Figure 3B.

While the Standard Fast Scan is adequate for detecting the peaks at 5.184 and 6.005 minutes in the UV chromatogram, and a few more peaks are detected as well (4.379, 4.478, 4.594, and 5.616 min in the TIC of Figure 3A), the peak at 0.707 minutes in the UV chromatogram is much more easily seen

in the Ultra Fast Scan mode of Figure 3B. This is because the earlier eluting peak at 0.707 minutes has a relatively narrower peak width so that the scan speed must be higher to adequately detect signal in such a narrow window of time.

It should be noted that while the faster scan speed in Ultra Fast Scan mode results in the acquisition of more data points across the ion chromatogram, the variation in amount of signal from scan to scan is larger because the amount of time involved with collecting signal is reduced. When less ions are collected during each cycle, the variation in signal from one cycle to the next is larger. As a result, Figure 3B shows more variation of the baseline signal in comparison to Figure 3A.

In Figure 4 an overlay of the two TICs is shown with the region around the 0.707 min peak (0.732 min in the MS) expanded. With more data points acquired in the Ultra Fast Scan mode of the 6140 Single Quadrupole, the impurity peak is more readily seen.

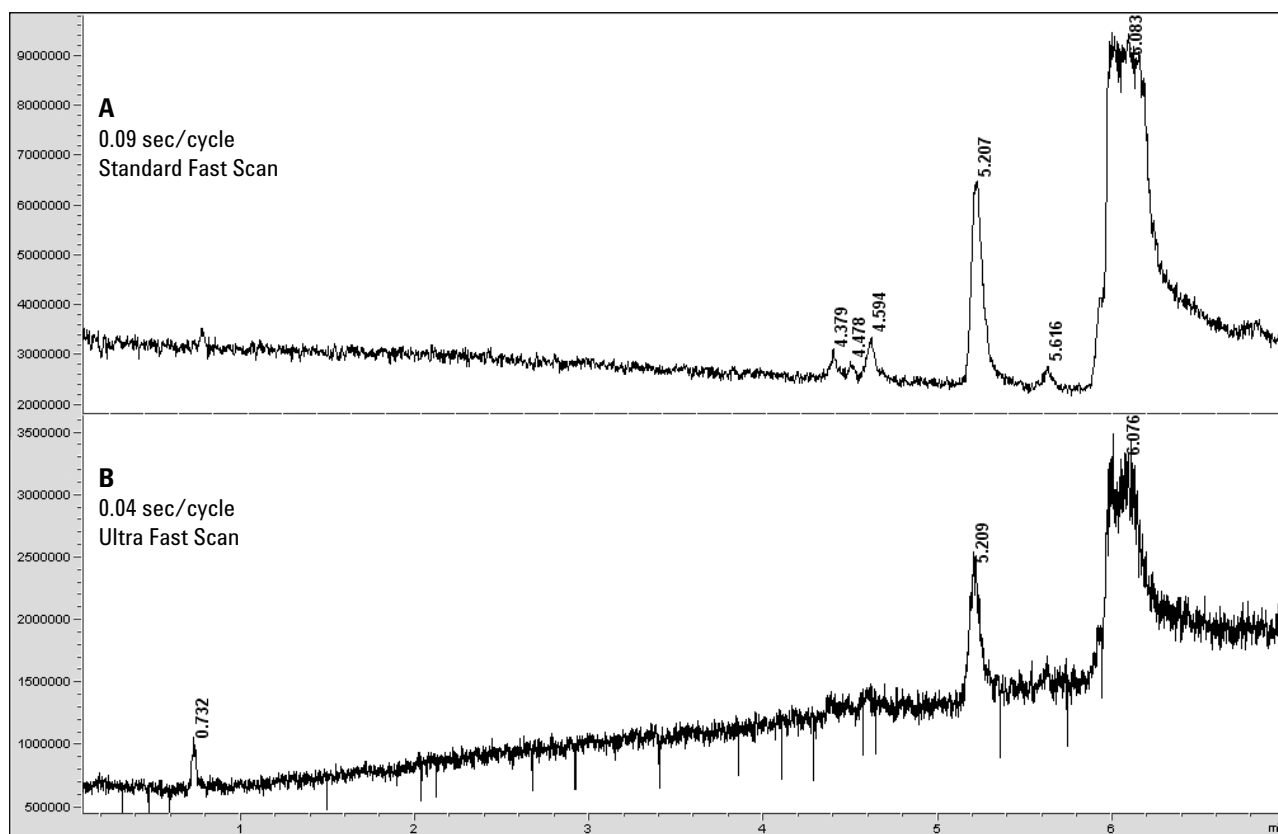


Figure 3. The total ion chromatograms (TICs) corresponding to the highest acquisition speed of the Standard Fast Scan mode (A) and the Ultra Fast Scan mode (B).

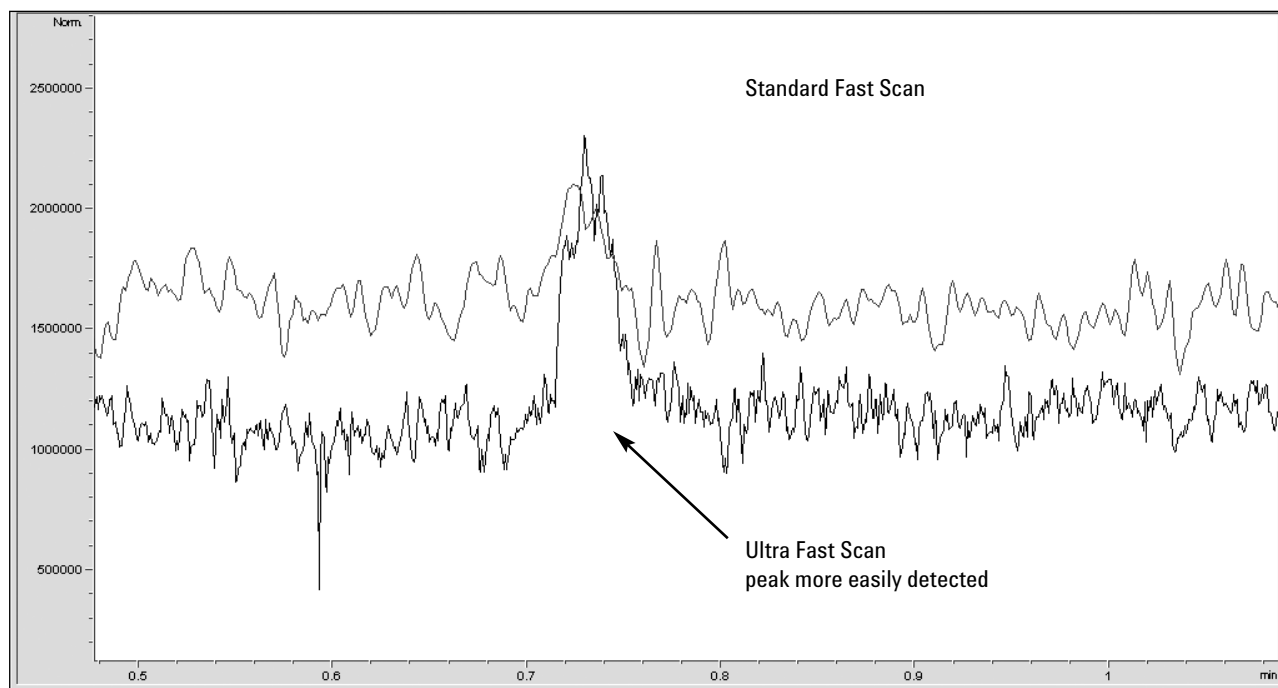


Figure 4. An overlay of the TICs in the expanded region around the peak seen at 0.707 min in the UV chromatogram (Figure 2). The peak is more easily detected in the Ultra Fast Scan mode.

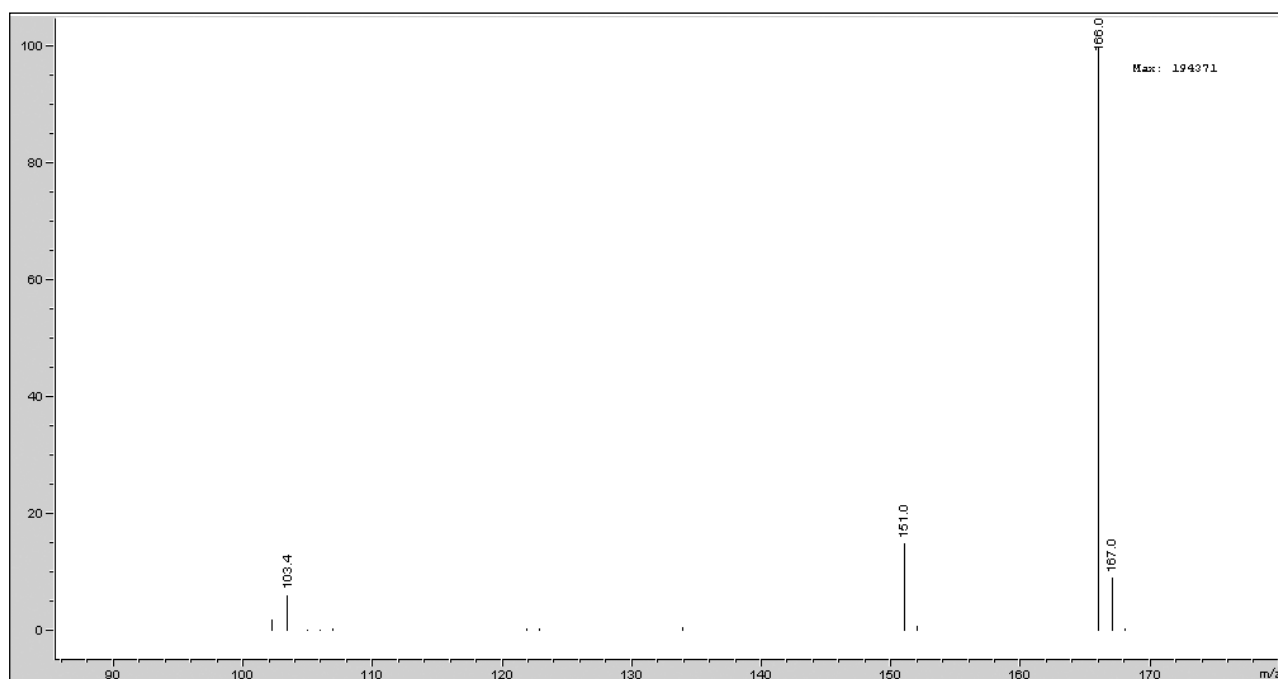


Figure 5. Background subtracted averaged spectrum in Ultra Fast Scan mode of peak at ion chromatogram peak at 0.732 minutes (0.707 minutes in UV).

According to the integration results in Table 1, the peak at 0.707 minutes of the UV chromatogram has a percent relative area of 0.4 % and should be considered an impurity requiring further investigation. A background subtracted spectrum of this

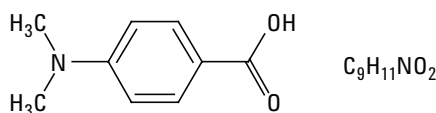


Figure 6. Structure of p-dimethylaminobenzoic acid, which has a protonated ion mass $[M + H]^+$ of 166.0 in positive ion mode using electrospray.

peak is derived from the Ultra Fast Scan mode acquisition and shown in Figure 5.

The m/z 166.0 peak clearly dominates the spectrum of Figure 5. A possible structure corresponding to this m/z value is shown in Figure 6. This structure corresponds to p-dimethylaminobenzoic acid, which is a known degradate of OD-PABA.

Conclusions

Detection of impurities is enhanced at higher acquisition speeds in mass spectrometry. This work demonstrates the usefulness of the Ultra Fast Scan mode (10,000 amu/sec) in detecting a relatively narrow peak impurity, eluting early (0.707 minutes) in the analysis of the OD-PABA neat standard. The peak, which clearly surpasses the 0.1% area cutoff in the UV chromatogram, is easily detected in the Ultra Fast Scan mode of the Agilent 6140 Single Quadrupole Mass Spectrometer. Upon analysis of the background subtracted averaged spectrum under this peak, an m/z 166 ion is clearly observed and believed to be p-dimethylaminobenzoic acid, a known degradate of the OD-PABA compound.

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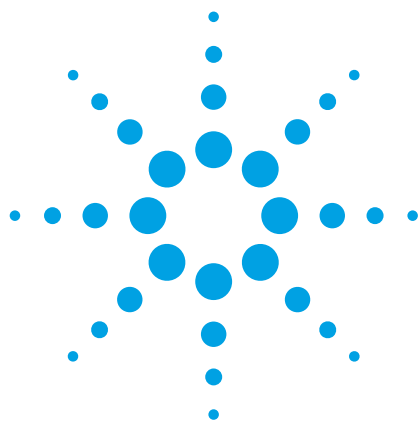
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Monitoring of electroless plating baths by capillary electrophoresis

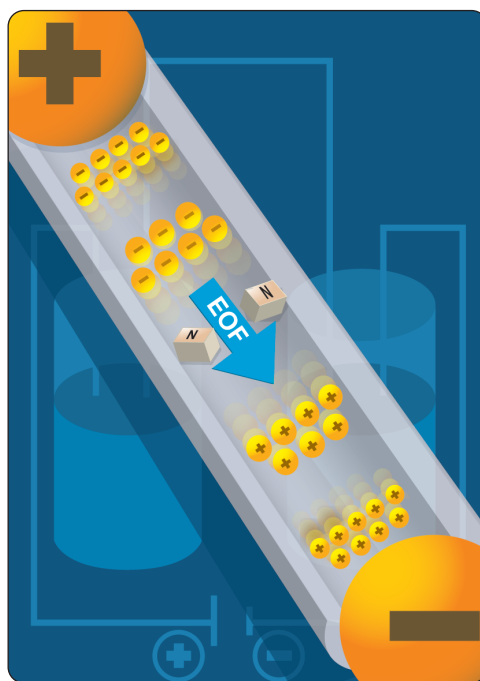
Application Note

Chemical

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Abstract

Electroless plating is mainly used for the plating of non-metals, for example, ceramics and plastics, and allows the plating of complex shaped parts with a uniform film-thickness. In addition to metal cations, the bath solutions contain additives such as reducing agents (which drive the plating reaction) and organic acids (as buffering and/or metal complexing agents). Inorganic anions are also present as counter-ions of the plating metals. These ions can easily be monitored using capillary electrophoresis (CE) with indirect UV detection.



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Experimental

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and Agilent ChemStation software. The analysis uses the Agilent Plating Bath Analysis Kit (Agilent part number 5064-8228).

Prior to first use, a new capillary was flushed with run buffer for 15 minutes (at 1 bar). Between analyses the capillary was flushed for 4 minutes from an extra buffer vial into waste. Buffer vials were replaced after 10 runs when using 2 mL-vials and after 5 runs when using 1 mL-vials. Sample preparation consisted simply of dilution with water.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation
- Agilent Plating Bath Analysis Kit

Results and discussion

Figure 1 shows the analysis of two different plating baths. Electroless nickel-plating baths contain nickel sulfate or nickel chloride, together with hypophosphite as the reducing agent. Formate, present in the electroless copper-plating bath, is an oxidation product of formaldehyde, which is used as a reducing agent. The assay was linear over the range 10–100 ppm with $r^2 > 0.999$. The method detection limit was 1–2 ppm. For the analysis of the electroless nickel-plating bath repeatability ($n = 8$) was $< 0.1\%$ RSD for migration times and $< 4.5\%$ RSD for peak area. The assay also allows the analysis of iron (II) and iron (III) in electro-plating with direct UV detection at 230 nm (data not shown).

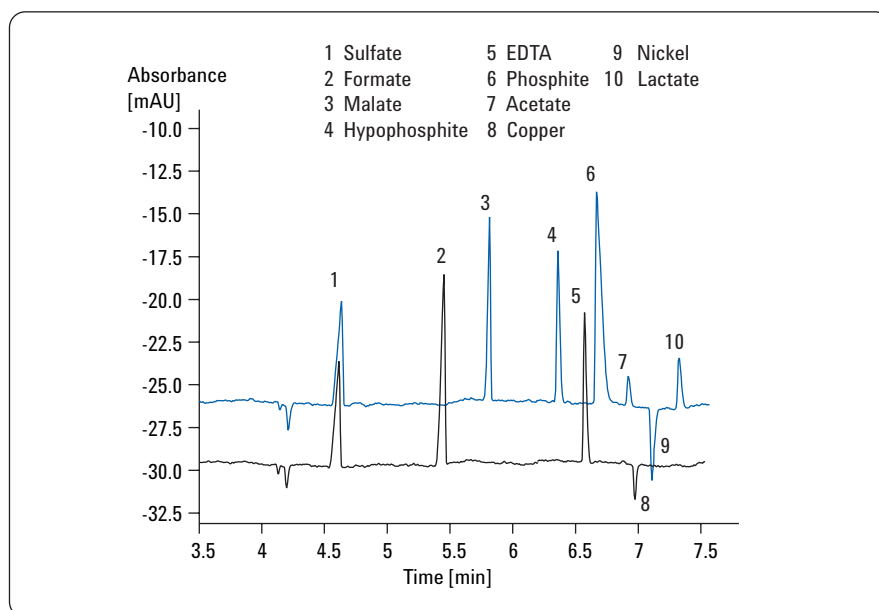


Figure 1
Analysis of electroless nickel- or copper-plating baths.

Chromatographic conditions

Sample: Electroless nickel- and copper-plating bath, 1:500 diluted with water
Injection: 8 seconds at 50 mbar
Capillary: Fused silica capillary, total length 80.5 cm, effective length 72 cm, internal diameter 50 μm (Agilent part number G1600-62211)
Buffer: Agilent Plating Bath Analysis Buffer (Agilent part number 5064-8236)
Voltage: -25 kV
Temperature: 20 $^{\circ}\text{C}$
Detection: Signal 350/20 nm, reference 275/10 nm

In the plating bath industry, the monitoring of additives in bath solutions or waste is essential for quality control, cost saving and environmental concerns. Electroless plating bath samples have presented a number of challenges to ion chromatography. CE, in contrast, allows a quick determination of all major components with only minor sample preparation.

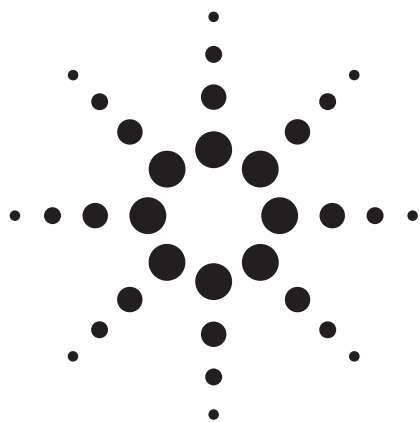
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Analysis of Suspected Flavor and Fragrance Allergens in Perfumes Using Two-Dimensional GC with Independent Column Temperature Control Using an LTM Oven Module

Application Note

Food and Flavors

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Abstract

Several different analytical methods based on GC/MS are used for the determination of flavor and fragrance allergens in raw materials and cosmetic products in accordance with EU Directive 2003/15/EC. For complex perfume samples with possible coelution of target compounds with other solutes, two-dimensional GC with heartcutting is preferred.

In this application note, a multidimensional capillary GC method is presented coupling Deans switch heartcutting with GC/MS and a low thermal mass (LTM) column module for optimal separation and quantitation of regulated allergens in complex samples. The method was applied to a perfume sample containing several regulated allergens. By using an LTM column module, the temperature of the second column could be controlled independently from the primary column in the main GC oven. Allergens were heartcut to the LTM at 50 °C, where they were focused and then later separated in an independent temperature program, resulting in optimum selectivity and better resolution of target compounds from sample matrix.



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Introduction

Recent European regulation requires allergen compounds to be monitored in fragranced products [1]. The target compounds include some common organic compounds such as limonene, citral, and cinnamic aldehyde. These compounds are often detected in natural products but can cause irritation to sensitive skin. According to the regulation, cosmetic products should therefore be labeled if the allergens are present above specified concentrations (10 ppm in "leave-on" and 100 ppm in "rinse-off" products). Consequently, effective methods are needed for qualitative and quantitative determination of the targeted compounds in these complex matrices.

The official target compound list includes 24 compounds. Some of the solutes consist of more than one chemical identity. Citral consists of two isomers: neral (Z citral) and geranial (E citral). Lylal also contains two isomers: (3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde). Farnesol consists of at least four possible isomers, of which the Z,E (farnesol 1) and E,E isomer (farnesol 2) are the predominant compounds observed. In addition, some related compounds, such as phenylacetaldehyde, estragole, methyl 2-nonynoate, and methyleugenol are also monitored [2]. In total, 31 target compounds are analyzed. The list of solutes is given in Table 1 and the first dimension separation is shown in Figure 1.

Table 1. Target Allergen List in Order of Elution on the Agilent J&W HP-5MS Column

Peak number	Compound
1	Limonene
2	Benzyl alcohol
3	Phenyl acetaldehyde
4	Linalool
5	Estragol
6	Methyl 2-octynoate (= folione)
7	Citronellol
8	Neral
9	Geraniol
10	Geranial
11	Cinnamaldehyde
12	Anisyl alcohol
13	Hydroxy citronellal
14	Methyl 2-nonynoate (methyl octane carbonate)
15	Cinnamic alcohol
16	Eugenol
17	Methyleugenol
18	Coumarin
19	Isoeugenol
20	Alpha isomethyl ionone
21	Lilial (BMHCA)
22	Amyl cinnamaldehyde
23	Lylal 1
24	Lylal 2
25	Amyl cinnamyl alcohol
26	Farnesol 1
27	Farnesol 2
28	Hexyl cinnamaldehyde
29	Benzyl benzoate
30	Benzyl salicylate
31	Benzyl cinnamate

The range of matrices in which the target compounds have to be measured is very broad and includes natural essential oils, synthetic mixtures of flavor and fragrance compounds, natural product extracts, and finished products, such as soaps, gels, shower gels, lipsticks, and other cosmetic products.

Moreover, the range of concentrations of the fragrance compounds in these matrices is very wide (from high ppb to percent). It is clear that to analyze all target compounds in all classes of matrices using one single method would be impossible. Therefore we have proposed classifying the different matrices into four classes [3]. For each class, dedicated analytical methods have been developed and validated. Direct injection of a diluted sample and analysis by one-dimensional GC/MS either in scan mode [4] or selected ion monitoring (SIM) mode is effective for samples that contain solutes that elute on an apolar column between decane (retention index 1000) and docosane (retention index 2200), providing that the sample complexity and analyte concentration range are not high, and that no nonvolatile matrix compounds are present [2]. One such method was developed using an Agilent J&W HP-5MS (apolar) column. The conditions and corresponding retention time locked information [5] and a complete allergens deconvolution reporting software (DRS) database with peak deconvolution are available from the Agilent Technologies Web site (www.agilent.com).

For highly complex samples (> 100 solutes) containing only volatile and semivolatile solutes, or for samples with a very broad concentration range of components (for example: very low concentrations of target compounds in a very high concentration of matrix compounds), a single-dimension GC separation is not effective. For these, the added power of two-dimensional capillary GC (GC/GC, 2D GC) has been shown to be helpful [3]. Using multiple heartcuts from a primary apolar column, target compounds can be isolated and resolved from interfering sample components on a polar secondary column, making accurate quantification possible even in cases where MS deconvolution of one-dimensional GC/MS data fails.

In this paper, the application of capillary flow technology Deans switching is demonstrated for the 2D GC analysis of a complex perfume sample. For even more method flexibility and separation power, the second-dimension column was housed in a low thermal mass (LTM) oven module for independent control of the column temperature. With this configuration, multiple heartcuts could be focused on the cooler secondary column and then released with an independent temperature program, which could be independently optimized for best separation of target compounds from complex sample matrix.

Experimental

The perfume sample was diluted to 5% (50 mg/mL) in acetone. Standard solutions were prepared from pure compounds at 100 ng/ μ L in acetone.

The analyses were performed on a 7890A GC/5975 MSD combination. The GC was equipped with an SSL inlet, FID detector, a capillary flow technologies based Deans switching system (p/n G2855B), a PCM flow module (option #309), and an LTM system controller bundle (p/n G6579A).

As illustrated in Figure 2, the primary column was installed in the GC oven and configured from the split/splitless inlet to the Deans switch. "Long leads" were requested when

ordering the column for the LTM so that the inlet end could be connected directly to the Deans switch. The outlet of the column was cut close to the column module and connected to the MSD via uncoated but deactivated fused silica (FS) tubing using an Agilent Ultimate Union (p/n G3182-61580). This configuration results in better method translation of conditions than when the long lead is left on the outlet end of the column because this 1 m extends into the GC column oven and becomes an isothermal (third) separation zone that broadens peaks and can alter the relative retention and resolution achieved at the exit of the LTM module. A restrictor (uncoated but deactivated retention gap) was also connected between the second output of the Deans switch and a monitoring FID. The conditions are summarized in Table 2.

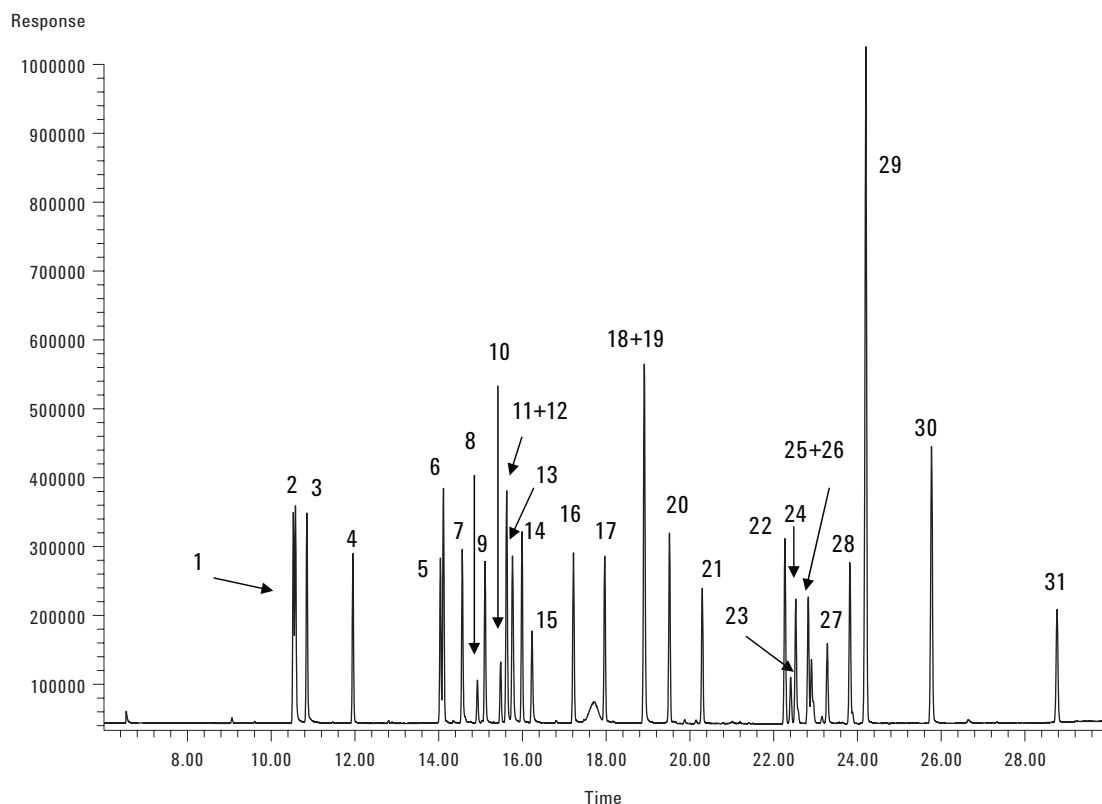


Figure 1. Separation of flavor and fragrance allergen test mixture (100 ppm) on the first dimension column (Agilent J&W HP-5MS) and FID detection. Peak identification is given in Table 1.

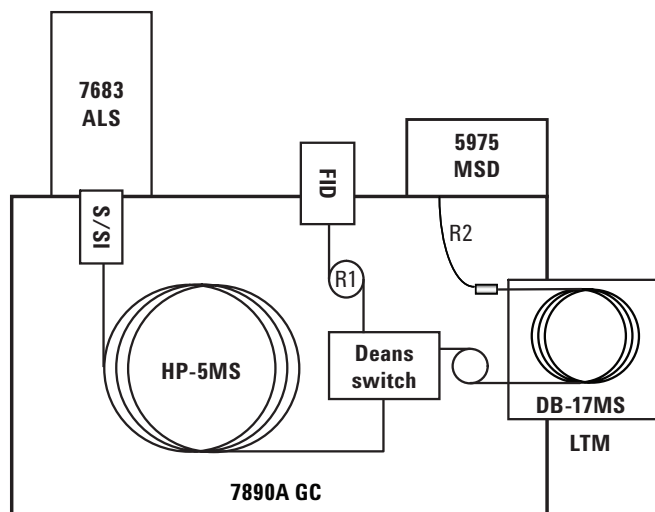


Figure 2. System configuration.

Results and Discussion

First, a standard mixture containing all target compounds at 100 ng/μL was analyzed. No heartcutting was used. The resulting chromatogram from the separation on the J&W HP-5MS column on the monitor FID is given in Figure 2. A good separation was obtained. Some coeluting pairs can effectively be resolved by mass spectral deconvolution (specific ions), as is done with DRS methods.

Next, the perfume sample was run under the same conditions. The chromatogram from the monitor FID detector shown in Figure 3A shows that the perfume is very complex, making determination of target compounds difficult. Some target solutes, such as linalool (peak 4) and alpha-isomethyl ionone (peak 20) are clearly resolved and can be determined. However, the elution window between 22 and 24.5 min, is quite complex. In this window, several target allergens elute,

Table 2. Analytical Conditions

Injection	1.0 μL
Inlet	S/SI, 250 °C, split ratio = 1:25
Column 1 (Carrier gas = He)	30 m × 0.25 mm id × 0.25 μm Agilent J&W HP-5MS, p/n 19091S-433 Flow = 1.4 mL/min; constant flow mode (185 kPa at 50 °C) Inlet = SSL; outlet = PCM1
Column 2 (LTM) (Carrier gas = He) Flow (PCM1)	30 m × 0.25 mm id × 0.25 μm Agilent J&W DB-17ms, p/n 122-4732LTM with "long leads" (1 m at each end not wrapped) 2 mL/min constant flow mode (120 kPa at 50 °C) for first experiment, 120 kPa (1 min) → 256 kPa (28 min) at 4.35 kPa/min for second experiment
Restrictors	R1 = 63 cm × 100 μm id deactivated FS (cut from, for example, p/n 160-1010-5) R2 = 1 m × 250 μm id deactivated FS (p/n 160-2255-1)
GC oven temperature	50 °C (1 min) → 300 °C (27.75 min) at 8 °C/min Total run time = 60 min
LTM oven	50 °C (25 min, after last heartcut) → 250 °C (1 min) at 6 °C/min (Total run time = 60 min)
FID monitor detector	300 °C, 30 mL/min H ₂ , 400 mL/min air
Deans switch heartcutting	Initially OFF Cut 1: ON at 10.2 min, OFF at 11.0 min Cut 2: ON at 15.3 min, OFF at 16.4 min Cut 3: ON at 22.0 min, OFF at 24.5 min
MS data acquisition	Autotune, scan mode, 41–300 u, samples = 2 ²
MSD transfer line	300 °C
MS solvent delay	5 min
MS temperatures	Source = 300 °C, quad = 150 °C

including amyl cinnamaldehyde, lylal (two isomers), amyl cinnamyl alcohol (with a related impurity), farnesol (two isomers), hexyl cinnamaldehyde, and benzyl benzoate. Within the same window, interfering perfume constituents such as methyl dihydrojasmonate, ionones, and sesquiterpenes elute. Most of these have mass spectra with strong fragmentation, resulting in many nonspecific low mass ions, interfering significantly with target ion spectra and ion ratios. Traditional selective detection and quantification using SIM data or deconvolved scan data from DRS that are effective with simpler samples would therefore be problematic with this sample.

For example, confirming the presence of lylal in this sample was difficult with the simpler approach. With GC-SIM-MS, it was not possible to accurately quantify lylal, and its qualifier

ions did not fall within the specified range. Review of the scan data clearly showed the presence of coeluting interferences.

Next, the sample was rerun with three heartcuts, including the problematic region between 22 and 24.5 minutes, which were heartcut to the second column. Propylene glycol, used as "keeper" in some perfumes, is a potential interferent in the first window that contains limonene, benzylalcohol, and phenylacetaldehyde. Quantification and identification of hydroxycitronellal in the second heartcut window is another component that, in the presence of interferences, is sometimes problematic to quantify using standard methods. The chromatogram obtained on the monitor detector is shown in Figure 3B, wherein the three heartcut windows show up as flat sections in the baseline.

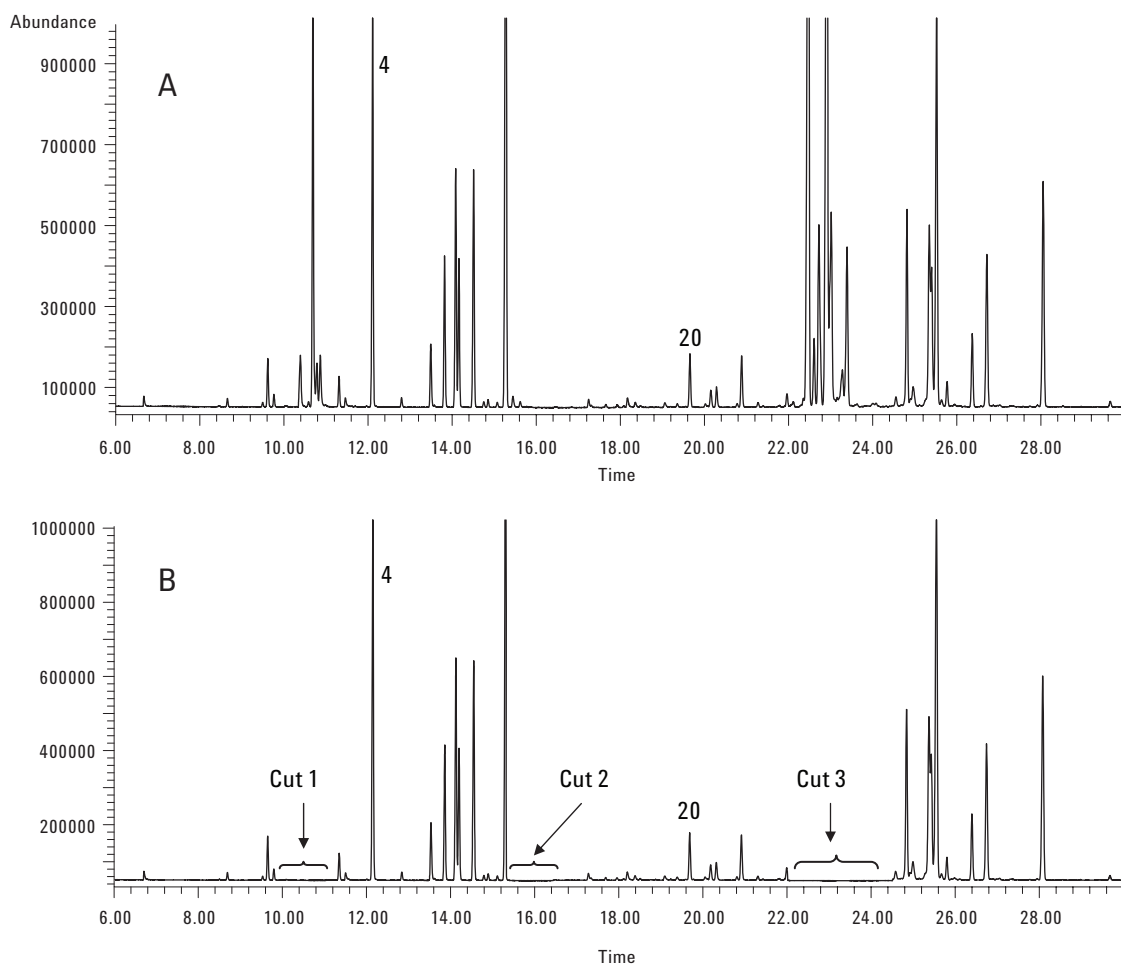


Figure 3. A) Separation of a perfume sample on the first-dimension column (Agilent J&W HP-5MS) using FID detection without heartcutting. Peaks: 4. Linalool; 20. Alpha-isomethyl ionone. B) Separation of a perfume sample on the first-dimension column (Agilent J&W HP-5MS) using FID detection with heartcutting (fractions: 10.2–11.0, 15.3–16.4, and 22.0–24.5 min).

The TIC chromatogram obtained after separation on the second-dimension column of the lyral fraction (heartcut 3) is shown in Figure 4A. First the analysis was performed using the same temperature program for the second column as for the first column (LTM program = 7890A oven program), emulating what would happen if the secondary column were housed in the GC oven (traditional configuration 2D GC). At least eight peaks were detected. The lyral isomers elute at 25.4 and 25.5 minutes. The second isomer, however, coelutes with another solute, and confirmation and quantification are not possible. The elution temperature of the lyral isomers in this case was around 240 °C. Both retention and selectivity at this temperature are low.

The experiment was repeated, this time with the J&W DB-17ms secondary column kept at 50 °C until the last heart-

cut was completed, and then the temperature was increased (at 6 °C/minute). Using this approach, the solutes are first focused at the head of the LTM column, and then elute at lower temperature (200 °C) during the temperature ramp, allowing both retention and selectivity to play more important roles. An added benefit is that the peak widths are narrowed due to the focusing, which improves peak resolution. Under these conditions, the isomers elute at 49.25 and 49.4 minutes and can be quantified without interference. The chromatogram of heartcut fraction 3 (22 to 24.5 minutes from column 1) is shown in Figure 4B. In contrast to Figure 4A, at least 20 peaks spanning a wide concentration range are clearly resolved. The presence of lyral isomers in the sample could thereby be confirmed and accurately quantified.

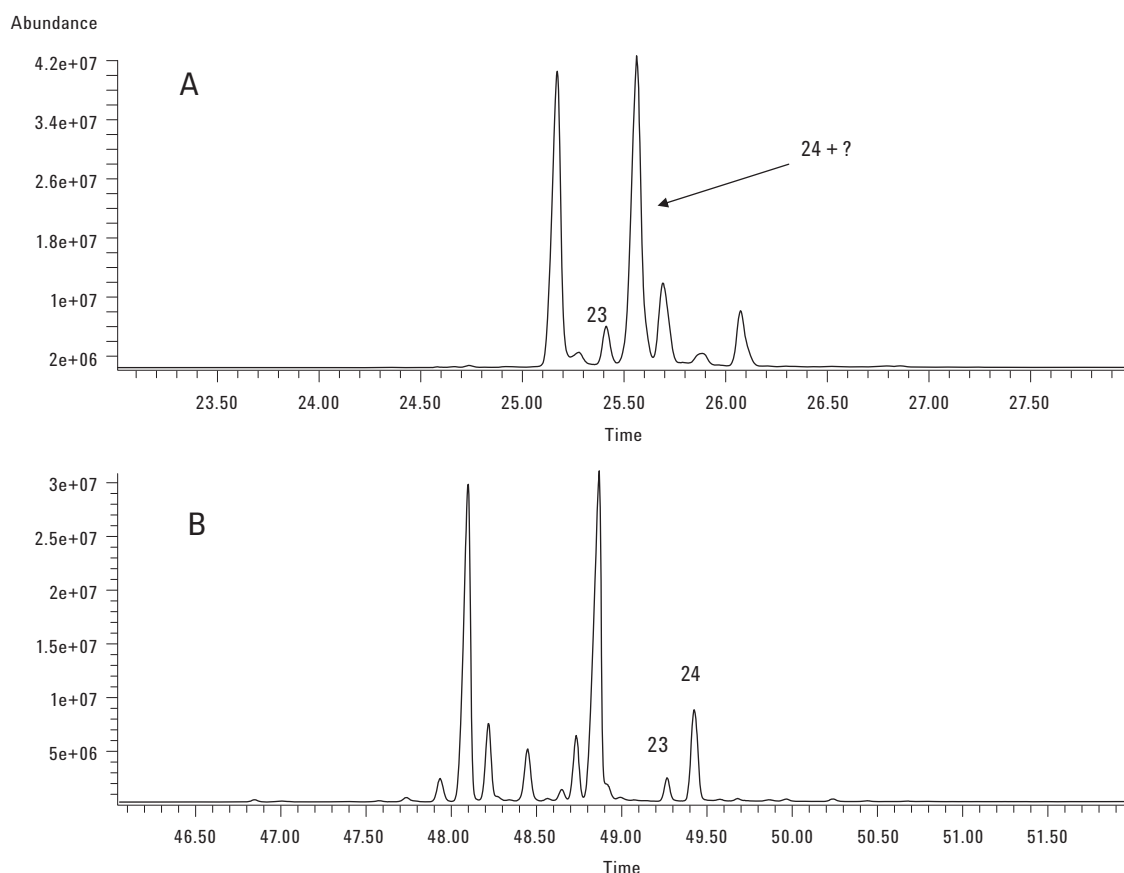


Figure 4. A) Separation of fraction 3 on the second-dimension column (Agilent J&W DB-17ms) using MS detection. Column 1 temperature = column 2 temperature: 50 °C (1 min) → 270 °C at 8 °C/min. Peaks: 23. Lyral 1; 24. Lyral 2. B) Separation of fraction 3 on the second-dimension column (Agilent J&W DB-17ms) using MS detection. Column 2 temperature: 50 °C (25 min) → 250 °C at 6 °C/min. Peaks: 23. Lyral 1; 24. Lyral 2.

By comparing the chromatograms in Figure 4, it is obvious that the independent temperature control of the second column in a 2D GC greatly increases the ability to optimize selectivity and resolution. This point was also demonstrated in the analysis of enantiomers using a chiral second-dimension column [6].

In addition to perfume samples, the approach presented herein can also be used for the determination of flavor and fragrance allergens in finished products. In these applications, any nonvolatile or late-eluting matrix compounds could be backflushed from the first-dimension column, as discussed in a manner similar to that described in an earlier application note [7].

Conclusions

Two-dimensional GC using Deans switch heartcutting in combination with MS can be used for the determination of flavor and fragrance allergens in complex perfume and cosmetic samples. Using LTM technology, the second dimension column temperature can be optimized independently from the primary column, resulting in better selectivity and resolution of target solutes from matrix interferences. Addition of an LTM module is more cost-effective, less cumbersome to configure, and takes up less space than if using a second GC as the independent zone.

References

1. Directive 2003/15/EC, Official Journal of the European Union, 6 66/26, 11.3.2003
2. A. Chaintreau, D. Joulain, C. Marin, C.-O. Schmidt, and M. Vey, *J. Agric. Food Chem.*, 2003, 51: 6398–6403
3. F. David, C. Devos, and P. Sandra, *LC.GC Europe* 19, Nov 2006, 602–616
4. H. Leijts, J. Broekmans, L. van Pelt, and C. Mussinan, *J. Agric. Food Chem.*, 2005, 53: 5487–5491
5. W. Luan, C. Sandy, and M. Szelewski, “Determination of Allergens in Fragrance Products Using Agilent Deconvolution Reporting Software,” Agilent Technologies publication 5989-8724EN, June 2008
6. F. David and M.S. Klee, “Independent Column Temperature Control Using an LTM Oven Module for Improved Multidimensional Separation of Chiral Compounds,” Agilent Technologies publication 5990-3428EN, January 2009
7. F. David and M.S. Klee, “Analysis of Suspected Flavor and Fragrance Allergens in Cosmetics Using the 7890A GC and Capillary Column Backflush,” Agilent Technologies publication 5989-6460EN, March 2007

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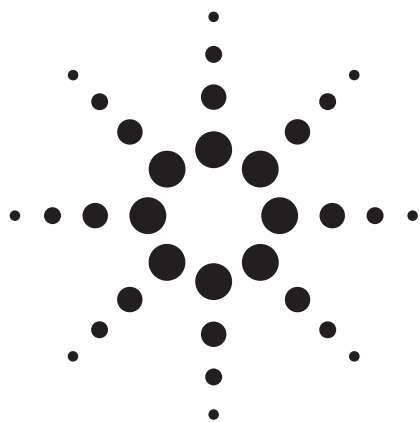
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Screening Impurities in Fine Chemicals Using the Agilent 1290 Infinity LC System

Application Note

Fine Chemical

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Abstract

The Agilent 1290 Infinity LC System with ultra violet/visible (UV/VIS) Diode Array detection (DAD) is used to analyze octyl-dimethyl-p-aminobenzoic acid for the presence of impurities. The system is used for the chromatographic separation of the compound from its impurities on 3.0 and 2.1 mm id C18 columns, of various lengths, with 1.8-um packing materials prepared in 600-bar (9000 psi) or special 1200-bar (18,000 psi) configurations. The ability of the 1290 Infinity LC System to operate with long, high resolution columns under conditions of rapid analysis is demonstrated with low viscosity acetonitrile (ACN) and higher viscosity methanol (MeOH) solvent conditions.



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Introduction

The analysis of impurities in starting materials, intermediates and finished products intended for a wide range of final uses is essential for ensuring product quality, performance, and consumer safety. The general conditions for successful analysis of impurities by high-performance liquid chromatography (HPLC) include gradient elution and multi-wavelength monitoring of the overall separation and may benefit from other detectors including evaporative light scattering (ELSD) and mass spectrometers (MS). Because impurity determination is the primary goal, one needs to ensure that mobile phase, vials, and HPLC components are free of minor impurities that might lead to confusing results during the analysis. Careful preparation of diluent blanks and blanks that might represent contamination sources due to additional sample preparation, such as filtration, are also appropriate. The analysis sequence is likely to include runs of the production material, solvent or diluent blank runs. It is also typical to include limit standards prepared by diluting the primary component to the lowest level where detection of impurities might be required. Finally, it is generally essential to include an authentic high purity reference standard.

Para-aminobenzoic acid (PABA) has historically been used as an ultra-violet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer a derivative in the form of octyl-dimethyl-p-aminobenzoic acid (OD-PABA), is currently and more commonly used. However, PABA may be formed as a degradate of OD-PABA, so it is important to monitor its potential presence in samples of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, for the purposes of safety and economics. In this work we investigate the capability of the Agilent 1290 Infinity LC system (UHPLC system with 1200 bar pressure limit) to detect impurities in OD-PABA samples with UV/VIS Diode Array detection.

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

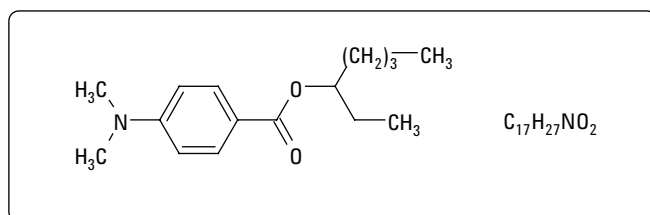


Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Experimental

Sample Preparation

The primary OD-PABA solution was prepared at a concentration of 1 mg/mL in 2-propanol and subsequently diluted to lower concentrations as needed. Injection volumes of 0.2–2 μ L were made into the LC/DAD system.

LC Method Details

LC Conditions

Agilent 1290 Infinity LC system binary pump G4220A,
Agilent 1290 Infinity LC system autosampler G4226A
Agilent Thermostatted Column Compartment G1316C with switching valve
Agilent 1290 Infinity system diode array UV/VIS detector G4212A with 10 mm path fiber optic flow cell

Columns: (See individual figures for specific usage)
Agilent ZORBAX SB-C18 RRHT, 3 mm $\times$ 50 mm, 1.8 μ m
600 bar p/n 827975-302
Agilent ZORBAX SB-C18 RRHD, 2.1 mm $\times$ 100 mm, 1.8 μ m
1200 bar, p/n 858700-902
Agilent ZORBAX SB-C18 RRHD, 2.1 mm $\times$ 150 mm, 1.8 μ m
1200 bar, p/n 859700-902

Column temp: 40 $^{\circ}$ C

Mobile phase: A = HPLC grade water
B = Acetonitrile (ACN) or methanol (MeOH)
(See individual figures)

Flow rate: See individual figures

Gradient: Gradient: the gradient conditions were either 40% to 90% ACN or 50% to 100% MeOH. The gradient slope was maintained at 3.5% organic phase increase per column volume, altering gradient time and flow rate accordingly. This is based on calculations using a modification of the Agilent Method Translator. [1]

UV Conditions

Monitoring 210, 254, 280 and 320 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

The UV response of OD-PABA, with four wavelengths monitored, is shown at a retention time of 2 min in Figure 2. Multi-wavelength monitoring of the separation provides a simple way to account for multiple impurities and assist in the selection of a final wavelength condition that can maximize sensitivity for all detected analytes.

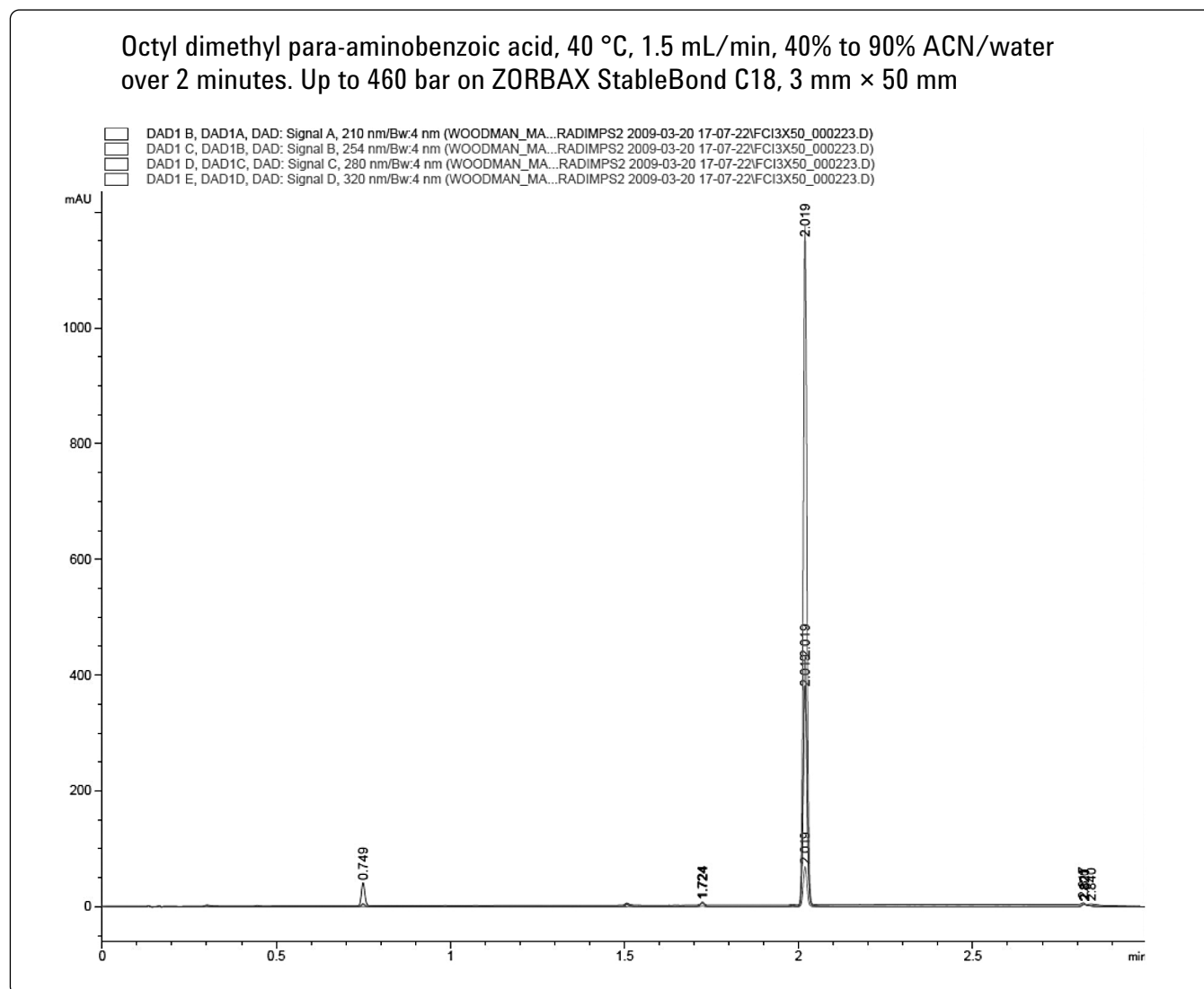


Figure 2. Multi-wavelength UV chromatogram of OD-PABA production material on a 3 mm × 50 mm ZORBAX Rapid Resolution High Throughput (RRHT) column. The chromatogram demonstrates the typical difficulties encountered with this type of separation, which are a need for wide dynamic range detection and sensitive impurity measurement. The peak at 0.75 minutes is confirmed by retention time matching and UV spectra to be PABA, the primary impurity in the mixture.

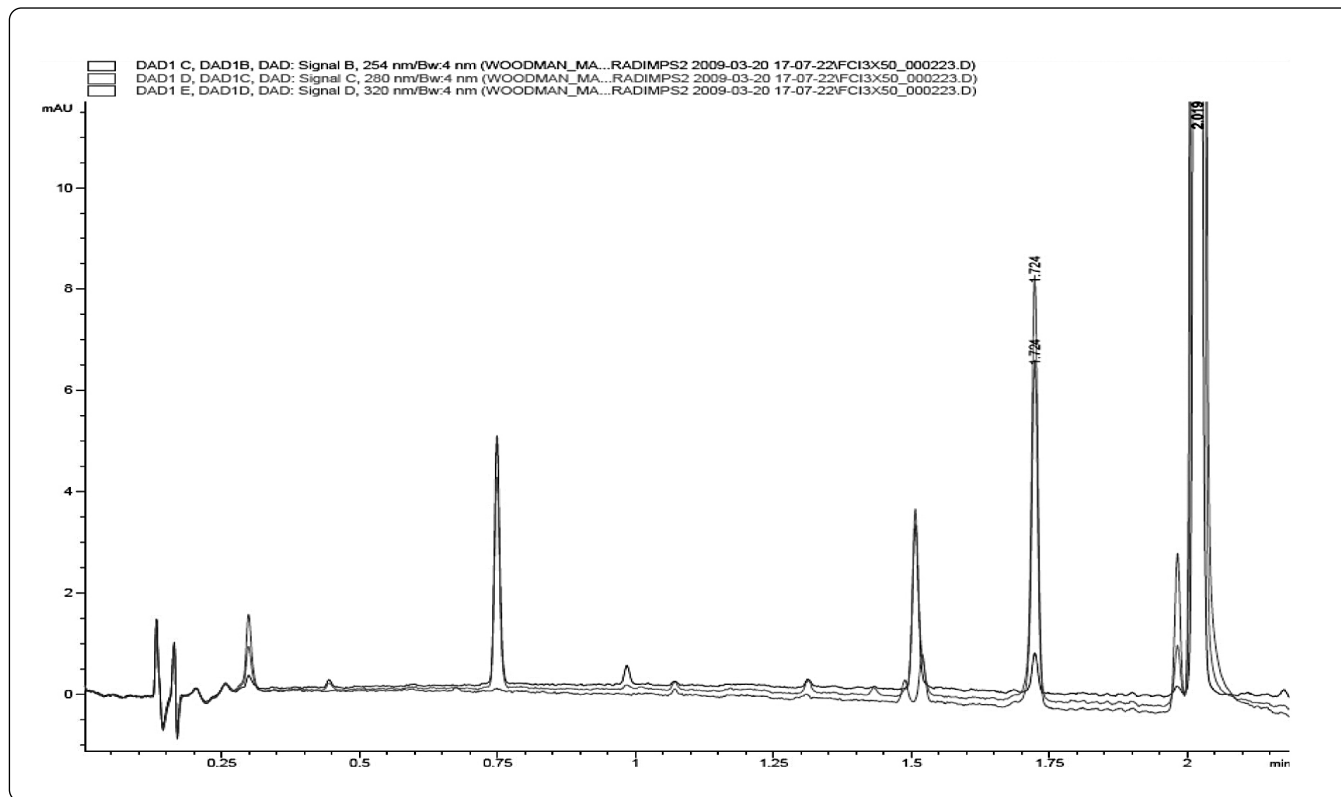


Figure 3. An expanded presentation of the chromatogram shown in Figure 2 based on the 3 mm × 50 mm gradient separation.

In Figure 3 the expanded multi-wavelength chromatogram allows us to see close detail and shows the number of impurities, as well as several areas where chromatographic resolution is clearly inadequate for individual component measurement. Despite the small particle size used in this column, the relatively short length limits the total resolution. As we move to longer column dimensions we will often reduce column diameter to reduce overall solvent consumption at the same time.

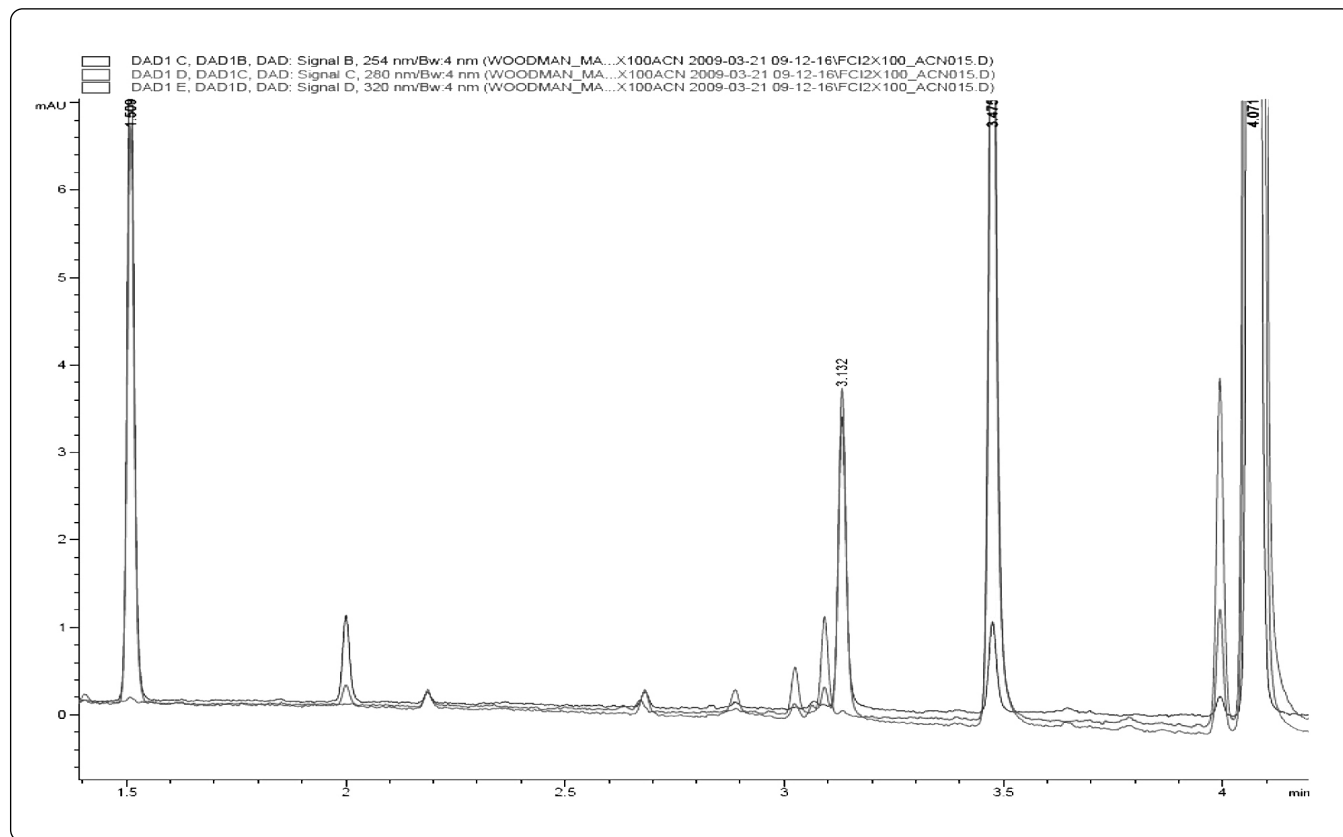


Figure 4. Analysis of the standard material on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18 column prepared for operation at 1200 bar pressure limit. Acetonitrile water gradient, 0.74 mL per minute, gradient time 4.0 minutes.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution it would be practical to explore longer columns or explore alternative mobile phase or column chemistries.

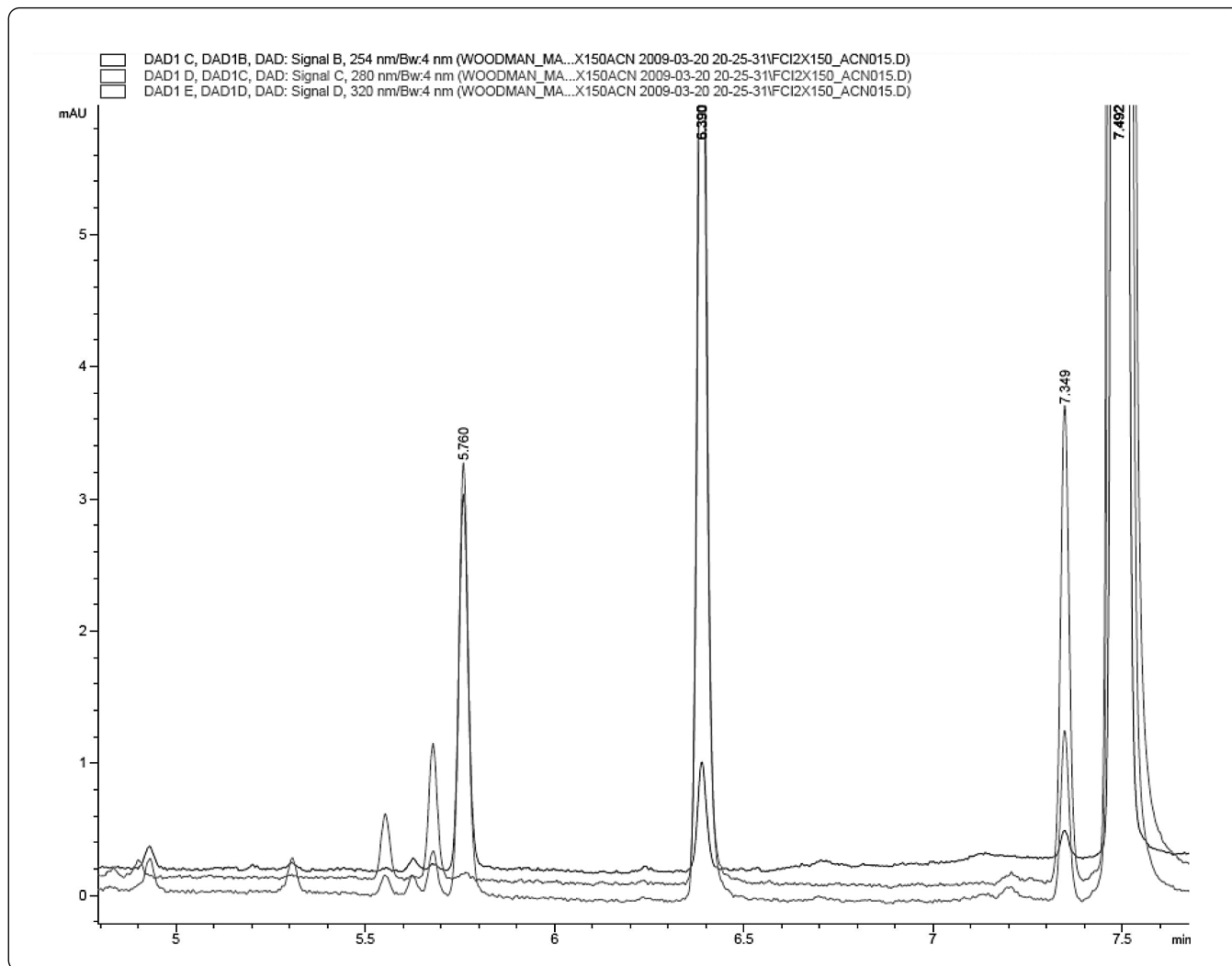


Figure 5. An expanded view of the acetonitrile separation using the same gradient slope on a 2.1 mm × 150 mm column rated for 1200 bar operating pressure. Agilent ZORBAX StableBond C18, 1.8 μm.

The increased column length clearly gives more resolution, however the increased back pressure also limits the flow rate if one is to operate in a conservative range of operating pressure. The Agilent 1290 Infinity LC system and associated ZORBAX Rapid Resolution High Definition (RRHD) chemistries are capable of operating pressures up to 1200 bar, approximately 18,000 psi. To ensure robust and rugged system operation many users typically specify the upper pressure limit for a method at a value less than 80% of the rated operating pressure.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes.
Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm.

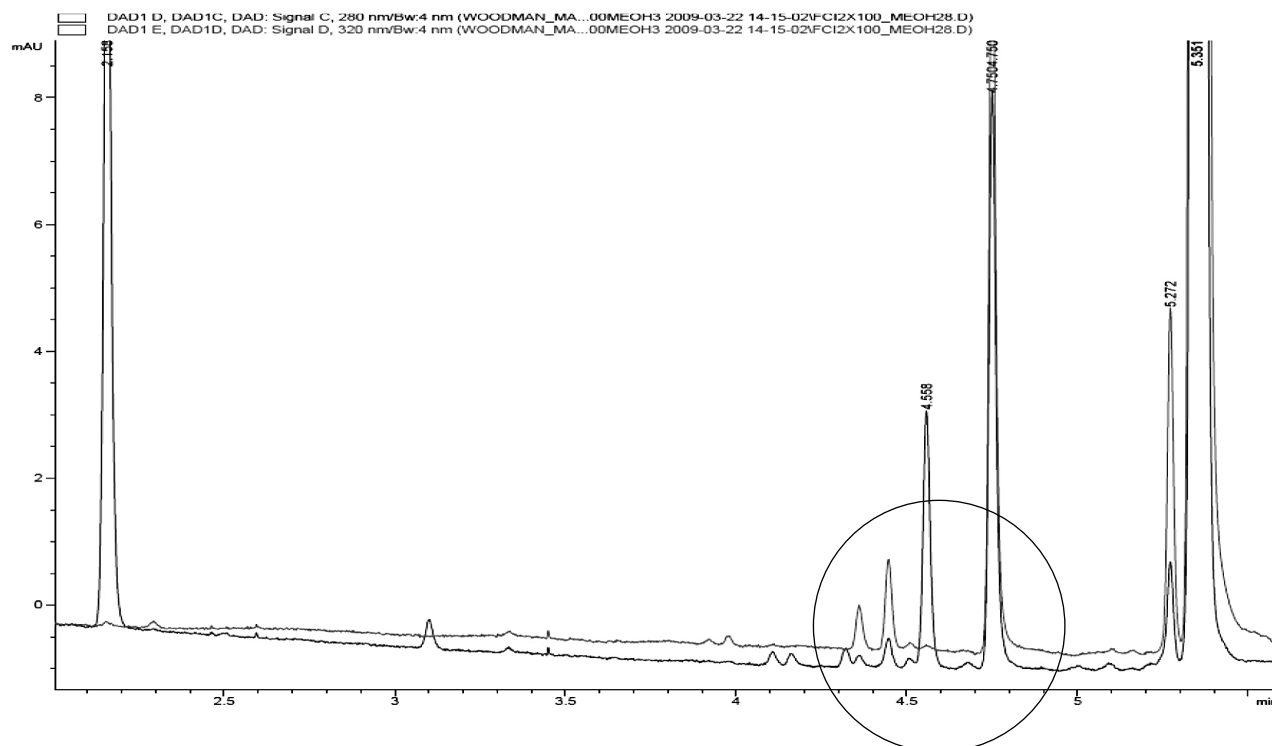


Figure 6. Separation of the sample mixture on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18, using methanol as the organic phase. Flow rate 0.52 mL/min gradient time 5.7 min, for a gradient of 5% to 100% methanol

When considering the fundamental components of the resolution equation we are all quite familiar with the concepts of capacity, selectivity, and efficiency. Increasing the column length, like decreasing the particle size of the packing material, will increase the efficiency of the overall separation. Because the increase in efficiency yields a relatively low return in terms of resolution, users often need to ensure that the capacity factor is optimized by exploring alternative chemical variables that could promote increased selectivity in the separation.

In Figure 6 we see the dramatic results achieved by changing the separation conditions from using acetonitrile as the

organic phase to methanol. If this separation was highly dependent on monitoring the separation at very low wavelengths one might find the UV cutoff of the methanol, 205 nm, to be problematic. In this example, however, the highly conjugated structures of the parents and related impurity structures allow sensitive detection at wavelengths well above the UV cutoff of common organic solvents used in reversed phase chromatography. In about the same amount of analysis time as the example in Figure 5, we achieve significantly higher selectivity leading to more resolved impurities while reducing overall solvent consumption and eliminating the need for expensive acetonitrile as the organic phase.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes.
Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm, 40 °C.

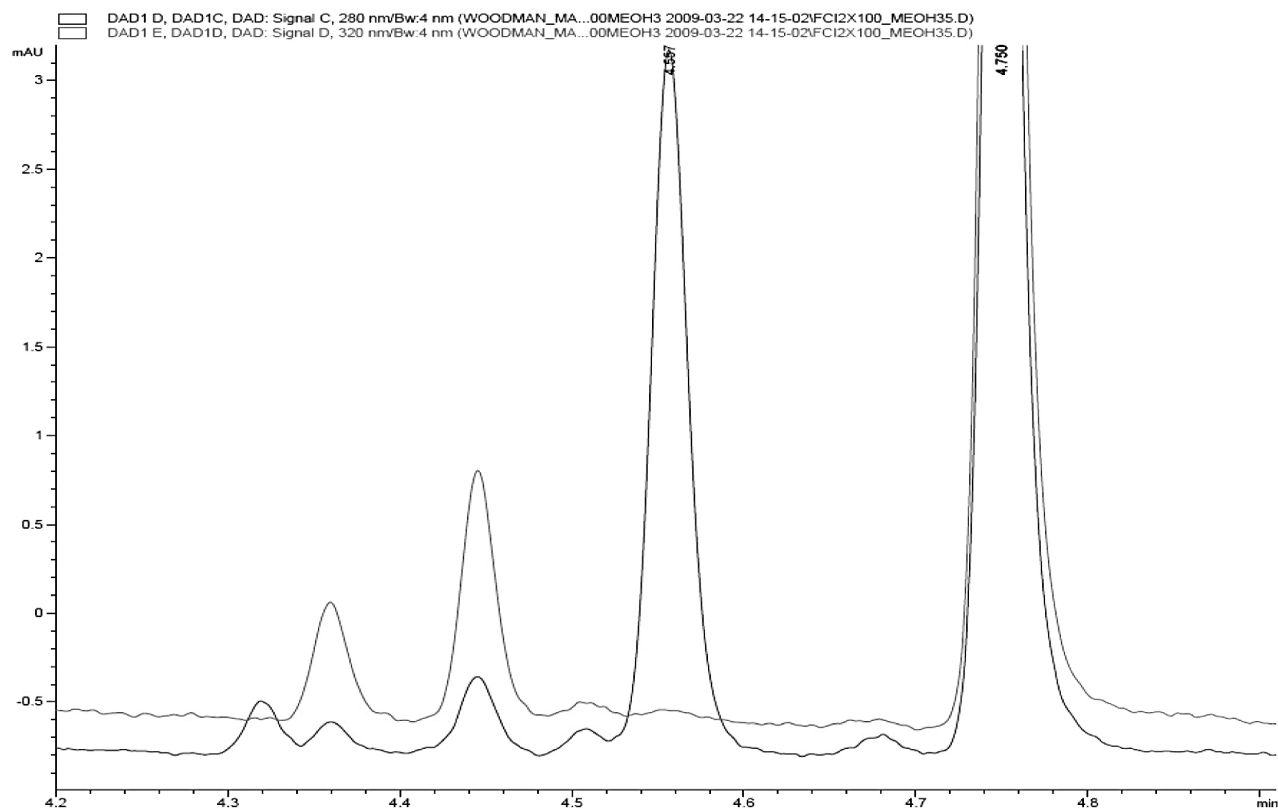


Figure 7. An expanded view of the small region of the chromatogram in Figure 6 showing a significant number of low concentration impurities. Conditions as in Figure 6. Estimated impurity concentrations for the smallest peaks in this figure are less than 0.02%.

Conclusions

The detection of low-level impurities in synthetic materials and highly refined natural products is of critical importance to the ultimate utility of these substances. Rapid analysis by HPLC using high-resolution columns and appropriately chosen organic phases ensures consistent results with rapid analysis turnaround time. Using the Agilent 1290 Infinity LC system, we were able to easily demonstrate UHPLC capabilities well within the operating range of the designed system. Higher throughput could still be achieved with this system by increasing flow rate and simultaneously reducing the gradient segment time to reproduce the gradient slope in a shorter total analysis time.

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References

1. <http://www.chem.agilent.com/en-US/products/instruments/lc/pages/gp60931.aspx>

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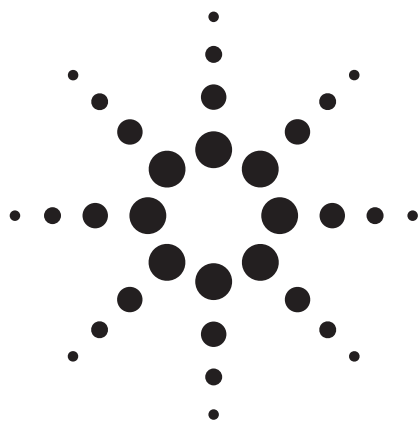
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Determination of Formic Acid in Acetic Acid for Industrial Use by Agilent 7820A GC

Application Brief

Wenmin Liu, Chunxiao Wang

HPI

With rising prices of crude oil and a future shortage of oil and gas resources, people are relying on the development of the coal chemical industry.

Acetic acid is an important intermediate in coal chemical synthesis. It is used in the production of polyethylene, cellulose acetate, and polyvinyl, as well as synthetic fibres and fabrics. The production of acetic acid will remain high over the next three years. In China, it is estimated that the production capacity of alcohol-to-acetic acid would be 730,000 tons per year in 2010.

The purity of acetic acid determines the quality of the final synthetic products. Formic acid is one of the main impurities in acetic acid. Many analytical methods for the analysis of formic acid in acetic acid have been developed using gas chromatography. For example, in the GB/T 1628.5-2000 method, packed column and manual sample injection is used with poor separation and repeatability which impacts the quantification of formic acid.

In this application brief, a new analytical method was developed on a new Agilent GC platform, the Agilent 7820A GC System. The GC was configured with a micro thermal conductivity detector (μ TCD) which provides an easy to use method for the determination of formic acid in acetic acid. To achieve a better separation, an Agilent J&W DB-FFAP (30 m $\times$ 320 μ m, 0.25 μ m) capillary column was used as the analytical column.

Highlights

- The Agilent 7820A GC coupled with a μ TCD provides a simple method for analysis of formic acid in acetic acid.
- ALS and EPC ensure good repeatability and ease of use which makes the 7820GC appropriate for routine analysis in QA/QC labs.
- Using a capillary column as the analytical column ensures better separation of formic acid in acetic acid compared to the China GB method.



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Experimental

Analytical conditions

Inlet	150 °C, Split ratio: 10:1
Injection volume	1 µL
Column	Agilent J&W DB-FFAP, 30 m × 320 µm, 0.25 µm (p/n 123-3232)
Carrier gas	He, Constant flow: 1.5 mL/min
Oven	80 °C (3 min) 8 °C/min 150 °C (5 min)
Detector	µTCD: 200 °C, Reference gas: 15 mL/min, Makeup gas: 6.5 mL/min FID: 300 °C, H ₂ : 30 mL/min; Air: 350 mL/min; Makeup flow (N ₂): 60 mL/min
Autosampler	Agilent 7693A automatic liquid sampler
Data analysis system	EZChrom Elite Compact

Results

Figure 1 shows the chromatogram of the analysis of formic acid in acetic acid at 1% (weight to weight) on the Agilent 7820A GC. From the chromatogram it could be seen that formic acid elutes after acetic acid on the Agilent J&W DB-FFAP column, which is confirmed by the Agilent 6890 GC and the Agilent 5975C series GC/MSD. In this experiment, a flame ionization detector (FID) was also used to confirm the detection of formic acid. The results are shown in Figure 2.

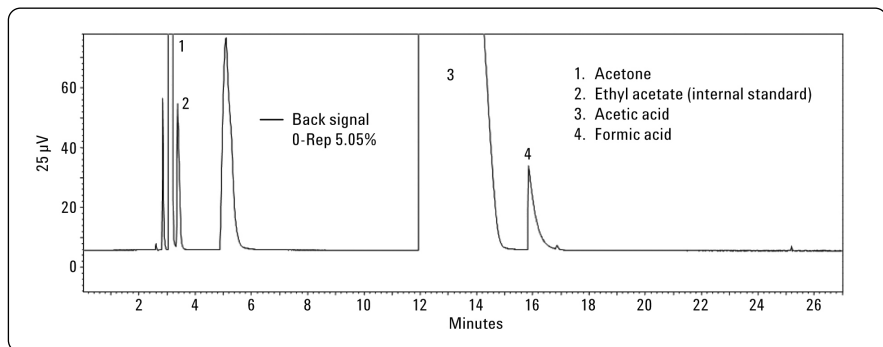


Figure 1. Chromatogram of formic acid analysis in acetic acid on the TCD channel.

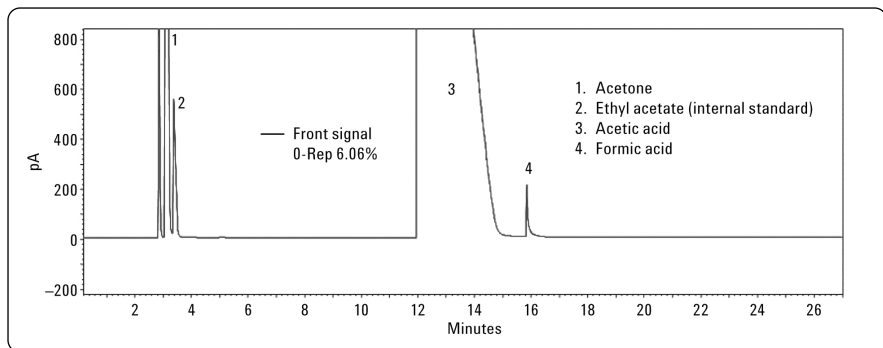


Figure 2. Chromatogram of formic acid analysis in acetic acid on the FID channel.

Agilent μ TCD is a proprietary designed single-filament flow switching detector. This design eliminates the need for a reference column, by exposing the filament to column effluent and reference flows at a frequency of 5 Hz. There is no other reference column that assures a stable baseline even with a ramped temperature program. Compared to the typical TCD, the smaller volume cell of μ TCD provides higher sensitivity.

The detection limits of formic acid on μ TCD was tested using a series of diluted formic acid samples. The method has a signal-to-noise of 6.8 for a formic acid concentration of 0.05 wt%. A method precision of 1.75% RSD for five injections was also calculated for the 0.05 wt% concentration. These excellent results were attributed to the Electronic Pressure Control (EPC) and precision auto sampler that are the key features of the Agilent 7820A GC.

In this method, ethyl acetate was used as the internal standard according to the GB/T 1628.5-2000. Concentrations of 0.1%, 0.5%, 1%, 10% (weight to weight) formic acid in acetic acid standard solution were made with ethyl acetate as an internal standard. The results show that from 0.1% to 10% (weight to weight), formic acid response to concentration was linear with an $r^2 = 0.9917$.

Conclusions

The Agilent 7820A GC coupled with a μ TCD provides a simple method for analysis of formic acid in acetic acid. Use of a capillary column ensures good separation of impurities and acetic acid in both high concentrations and low concentrations. The stable and sensitive μ TCD is a good choice for formic acid analysis compared to an FID which has a relatively low response. The 7693A autosampler with a capacity of 16 sample vials and 7820A GC EPC control ensure good repeatability and ease of operation, which is suitable for the fast growing coal to chemical industry and routine analysis labs where feedstock and intermediate quality control is important. The data processing system, EZChrom Elite Compact software specially designed for Agilent 7820 GC, is easy to use and provides commonly used report templates.

References

1. GB/T 1628.5-2000. Determination of formic acid in acetic acid by gas chromatography.

Wenmin Liu, Chunxiao Wang are application chemist based at Agilent Technologies, Shanghai, China.

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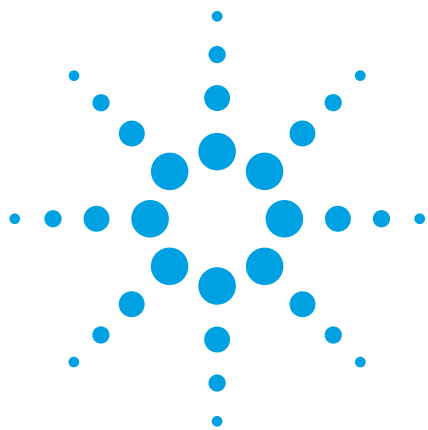
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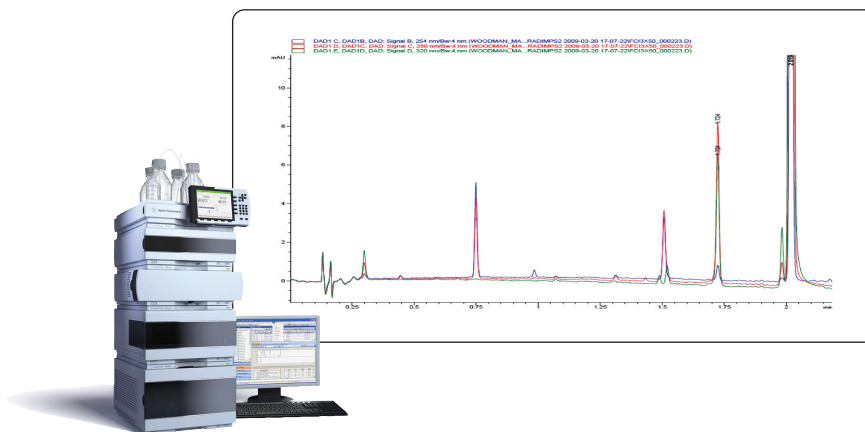
Analysis of impurities in fine chemical octyl-dimethyl-4-aminobenzoate using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 μm columns

Application Note

Fine Chemicals

Author

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USA



Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system and has the flexibility to operate with a wide range of column dimensions and particle sizes. Additionally, advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of small impurities in fine chemicals.

Introduction

The combined benefits are demonstrated by a separation of impurities found in a sample of octyl-dimethyl-4-aminobenzoate (Figure 1). The high pressure capability of the system allows the use of methanol, as well as acetonitrile, to explore the selectivity of the two solvents. At 1.5 mL/min, using a simple 2-min gradient and a 3.0 mm $\times$ 50 mm 1.8 μm column, the analysis time is only 3 min. The separation of the main components is shown in Figure 2.



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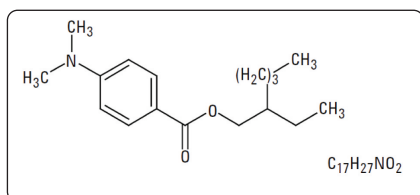


Figure 1
Structure of the cited compound.

The speed, resolution and flexibility of the system are further demonstrated by a separation of the sample using methanol or acetonitrile with low solvent consumption 2.1 mm id, 1.8 μ m columns. The flow rate and gradient conditions are optimized for each solvent, to produce a gradient separation with maximum pressure of approximately 850 bar, a conservative setting for the 1200-bar capability of the Agilent 1290 Infinity LC. The separation of the main components, with the two organic solvents, is shown in Figure 3a (acetonitrile, top panel) and 3b (methanol, lower panel), where the chromatograms are zoomed to the region of peaks shown from approximately 1.2-2.5 min in Figure 2.

Configuration

- G4220A 1290 Infinity Binary Pump with Integrated Vacuum Degasser
- G4226A 1290 Infinity Autosampler
- G1316C 1290 Infinity Thermostatted Column Compartment
- G4212A 1290 Infinity Diode Array Detector

Conclusion

The combined high flow and high pressure capability of the system allows one to use high efficiency columns, producing rapid separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns. Impurity detection, due to high detector sensitivity and stability, is estimated to be < 0.01%.

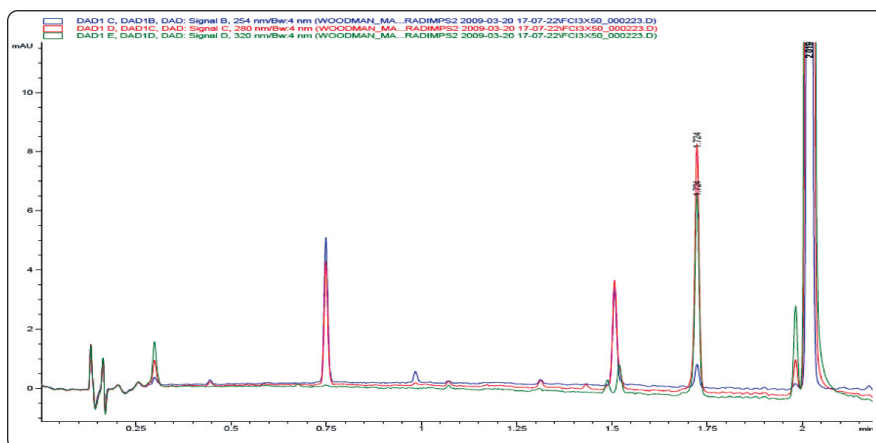


Figure 2
Initial separation conditions showing a need for greater resolution and selectivity. Sample: Octyl dimethyl para-aminobenzoate, 1 mg/mL. Gradient: 1.5 mL/min, 40% to 90% ACN/water over 2 minutes. Up to 460 bar on ZORBAX StableBond RRHT C18, 3 mm $\times$ 50 mm, 1.8 μ m, 40 $^{\circ}$ C. 0.75 minute retention: 4-amino-benzoic acid; 2.1 minute retention: Octyl dimethyl para-aminobenzoate.

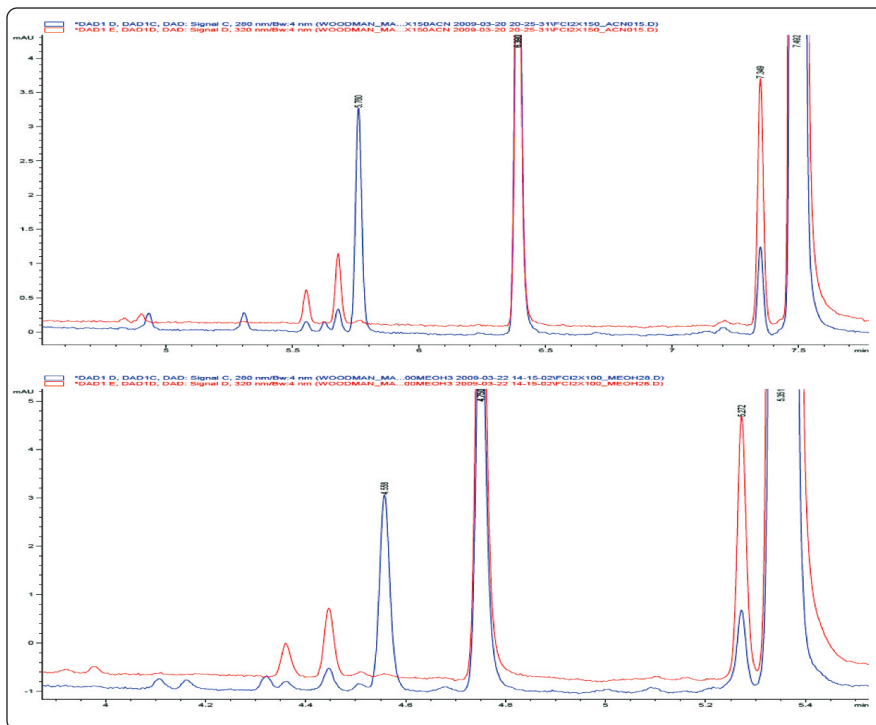


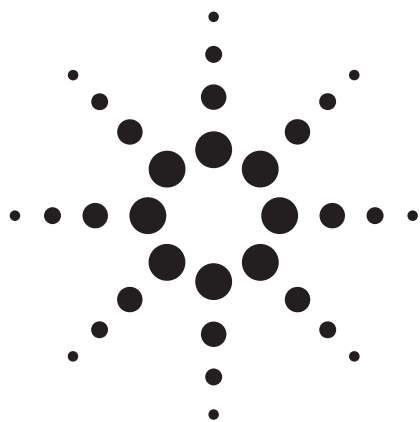
Figure 3
Results using ACN vs. MeOH with the same gradient slope on the 1290 Infinity LC. Sample: ODPABA working standard, 1 mg/mL. Conditions: ACN gradient 0.6 mL/min, 40% to 90% ACN/water over 7.4 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm $\times$ 150 mm, 1.8 μ m, 40 $^{\circ}$ C. Methanol gradient 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm $\times$ 100 mm 1.8 μ m, 40 $^{\circ}$ C. The increased selectivity of methanol allowed a shorter column to be used, decreasing run time and avoiding the use of more expensive acetonitrile mobile phase.

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Basic Performance of the Agilent 7700s ICP-MS for the Analysis of Semiconductor Samples

Application Note

Semiconductor

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Japan

Abstract

Agilent ICP-MS systems have become the benchmark for accurate low-level analysis of trace contaminants across a wide range of high-purity chemicals used in the semiconductor industry. As the first commercial ICP-MS to offer reliable and routine cool plasma operation, the Agilent 4500 Series ICP-MS set the standard for low level analysis of the previously problematic elements Na, K, Ca and Fe. Building on the success of the 4500 Series, the Agilent 7500s ICP-MS provided additional sensitivity, stability and matrix tolerance, while the Agilent 7500cs ICP-MS introduced new levels of performance through the provision of an Octopole Reaction System (ORS) operating in both collision and reaction modes.

The newly-developed Agilent 7700s ICP-MS further extends the performance of the technique for routine semiconductor applications by combining unmatched sensitivity, matrix tolerance, interference removal and stability in a single reliable and easy to use system.



Agilent Technologies

Introduction

The Agilent 7700 Series ICP-MS product line includes the 7700s model, which is configured specifically for semiconductor applications, with a PFA nebulizer, Pt interface and high-transmission ion lens. Development of the 7700 Series was focused on ensuring “ease of use”, while improving on the sensitivity and interference-removal capability of the 7500cs. To meet these goals, many new features have been introduced in the 7700s, including much smaller size, easier maintenance, and lower cleanroom service requirements (20% lower exhaust flow and 3.5x lower pressure drop than the 7500cs). The 7700s also introduces a new, robust, frequency matching RF generator for improved performance in volatile organic solvents, and a 3rd generation ORS³ collision reaction cell.

Basic Performance of the 7700s

The 7700s incorporates a new interface, ion lens and the newly-developed ORS³, to deliver better ion transmission and lower backgrounds than the 7500cs. In general, the sensitivity (cps/ppt) of the 7700s is approximately 40% higher than that of 7500cs, and the new off-axis lens configuration reduces random background noise by half. The result is that the 7700s has much lower Detection Limits (DLs) and Background Equivalent Concentrations (BECs) than its predecessor. Table 1 shows the normal plasma DLs and BECs obtained on the 7700s in a matrix of 1% HNO₃. Elements with a * may also be measured in cool plasma (BEC and DL in brackets). Figure 1 shows the preferred-mode DL of the 7700s compared to the 7500cs. In all cases, the 7700s DL was lower, notably for elements with plasma-based interferences (for example ⁵⁶Fe, ⁷⁸Se).

Improved Interference Removal

The newly developed ORS³ has longer rods and a smaller internal diameter, and operates at higher frequency and higher cell gas pressure than the cell fitted to the 7500cs. As a result, higher bias voltages may be used, which improves the removal of polyatomic interferences in helium (He) mode with Kinetic Energy Discrimination (KED), and also promotes Collision Induced Dissociation (CID) for relatively weakly-bound polyatomic ions such as ArO⁺ (dissociation energy 0.6 eV) and ArAr⁺ (dissociation energy 1.3 eV). More effective removal of these background (plasma-based) polyatomic ions provides much lower DL and BEC for many elements, although reaction mode (H₂ cell gas) provides the lowest DL and BEC for several elements including Fe and Se in the highest purity semiconductor samples.

Table 1. 7700s ICP-MS BECs and DLs in 1% HNO₃

Mass	Element	BEC ppt	DL ppt	Mode
7	Li*	0.2 (0.01)	0.06 (0.01)	No gas
9	Be	0.04	0.05	No gas
11	B	6	1	No gas
23	Na*	200 (0.5)	4 (0.3)	No gas
24	Mg	0.2	0.07	No gas
27	Al*	20 (0.2)	0.6 (0.3)	No gas
39	K*	250 (0.5)	10 (0.3)	H ₂
40	Ca*	5 (2)	2 (1)	H ₂
48	Ti	0.2	0.2	He
51	V	0.1	0.1	He
52	Cr*	10 (0.03)	0.7 (0.09)	He
55	Mn*	0.6 (0.05)	0.6 (0.1)	He
56	Fe*	7 (0.5)	0.6 (0.2)	H ₂
59	Co*	0.08 (0.01)	0.09 (0.06)	He
60	Ni*	1 (0.03)	0.6 (0.1)	He
63	Cu*	6 (0.6)	0.8 (0.4)	He
64	Zn	0.6	0.5	He
71	Ga*	0.07 (0.01)	0.05 (0.01)	He
74	Ge	0.01	0.05	He
75	As	0.07	0.4	He
78	Se	0.5	0.9	H ₂
85	Rb*	0.2 (0.01)	0.2 (0.03)	He
88	Sr	0.01	0.04	He
90	Zr	0.06	0.05	He
93	Nb	0.01	0.02	He
98	Mo	0.04	0.07	He
107	Ag	0.04	0.03	He
114	Cd	0.02	0.06	He
118	Sn	0.5	0.2	He
121	Sb	0.03	0.04	He
138	Ba	0.01	0.02	He
178	Hf	0.01	0.01	He
181	Ta	0.01	0.01	He
182	W	0.01	0.03	He
197	Au	0.05	0.05	He
205	Tl	0.01	0.03	He
208	Pb	0.1	0.09	He
209	Bi	0.02	0.04	He
232	Th	0.01	0.01	He
238	U	0.01	0.01	He

*Elements may also be measured in cool plasma (BEC and DL in brackets).

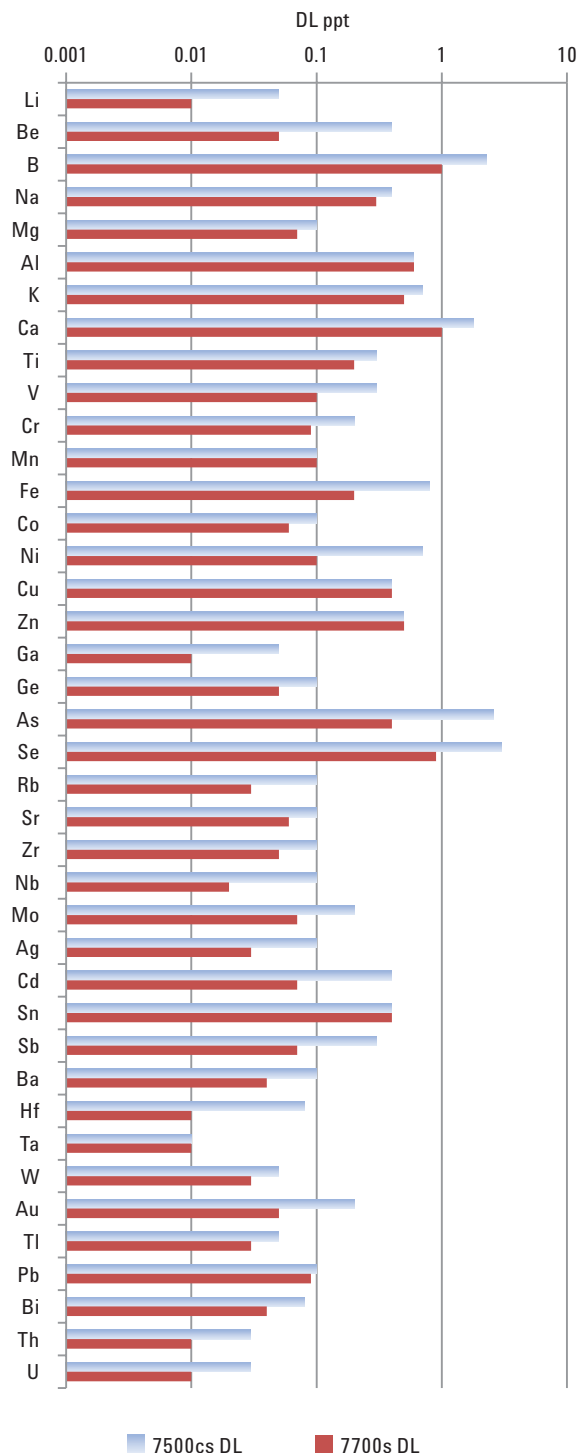


Figure 1. DL comparison for Agilent 7700s and 7500cs ICP-MS.

The enhanced He mode operation of the ORS³ on the 7700s delivers dramatically improved detection of phosphorus, with DLs and BECs improved by a factor of 10 to 50 compared with conventional collision/reaction cell ICP-MS. The 7700s He mode calibration curve for phosphorus in 1% HNO₃ is shown in Figure 2.

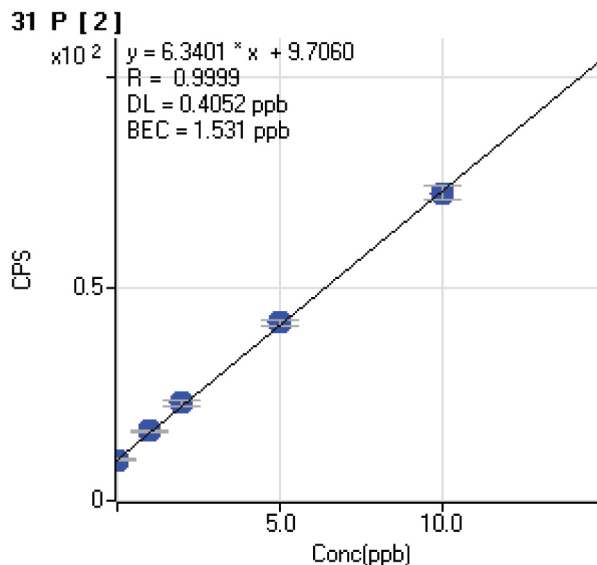


Figure 2. 7700s ICP-MS He mode calibration for ³¹P in 1% HNO₃.

The new ORS³ also delivers better performance in reaction mode with H₂ cell gas, due to the higher cell gas density and the higher energy of the collisions between the interfering ions and the cell gas. This gives lower DLs for elements such as Si (measured directly at mass 28), and reduces the need for highly reactive cell gases such as NH₃, which has a strong tendency to form multiple cluster ions in the cell and is therefore not generally suitable for variable or complex process chemical matrices, or for multi-element analysis. The performance of the 7700s in H₂ reaction mode is illustrated in Figure 3, which shows the calibration curve, DL and BEC for ²⁸Si in a matrix of 1% HNO₃. It should be noted that the pure water used in the examples in Figures 2 and 3 was normal Milli-Q water and not further purified. Silicon is expected to be present at high concentration in such water and blank contamination would therefore have contributed to the BEC.

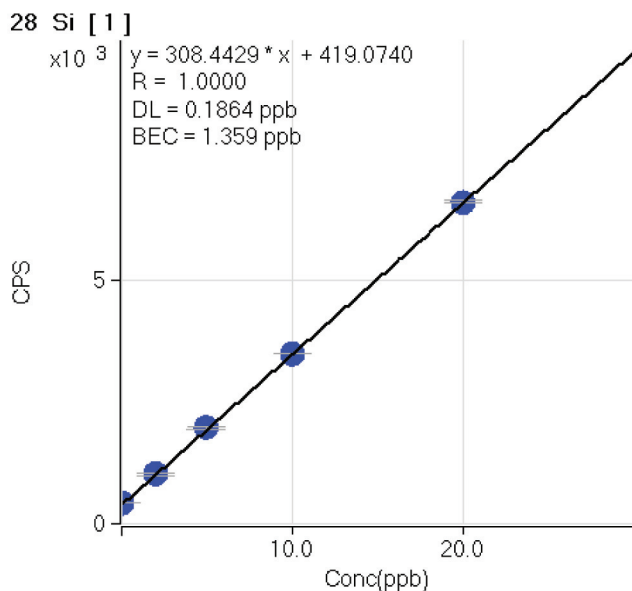


Figure 3. 7700s ICP-MS H_2 mode calibration for ^{28}Si in 1% HNO_3 .

Long Term Stability

Long term (9 hours) stability was measured for several elements spiked at 100 ppt in 1% HNO_3 . Sampling and data acquisition was done every 35 minutes. Raw count rate signal drift was within $\pm 5\%$ over the 9 hour sequence, and the %RSD was $< 3\%$ for all analytes. The excellent long term stability of the 7700s operating in He mode is shown in Figure 4.

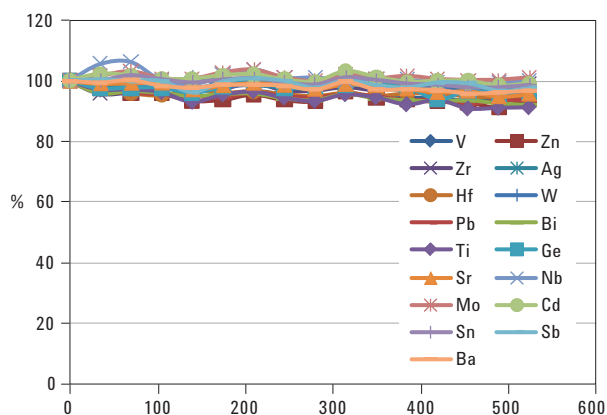


Figure 4. Nine hours stability of 7700s ICP-MS operating in He mode (100 ppt spike in 1% HNO_3).

Other Reaction Gases

The 7700s has both collision (He) and reaction (H_2) cell gas lines fitted as standard, but can also have an optional 3rd cell gas line fitted for specialized applications. In the semiconductor industry, this includes analyses where optimum interference removal requires a highly reactive cell gas such as NH_3 . While the requirements for such highly reactive cell gases are very small, there are some specific cases where such gases offer the lowest DLs.

One example of the use of highly reactive cell gases is illustrated in Figure 5, which shows the calibration for V in concentrated HCl, using NH_3 mode. The intense ClO interference which affects the only useful isotope of V at mass 51 is not very reactive with H_2 , so H_2 cell mode does not give sufficiently good interference removal for the lowest DL to be achieved in the highest purity HCl, such as (TAMAPURE-AA100 (20%)). As can be seen in Figure 5, the 7700s ICP-MS operating in NH_3 mode gives effective removal of the ClO interference in undiluted (20%) HCl, providing a BEC and DL of only 2.3 ppt for V.

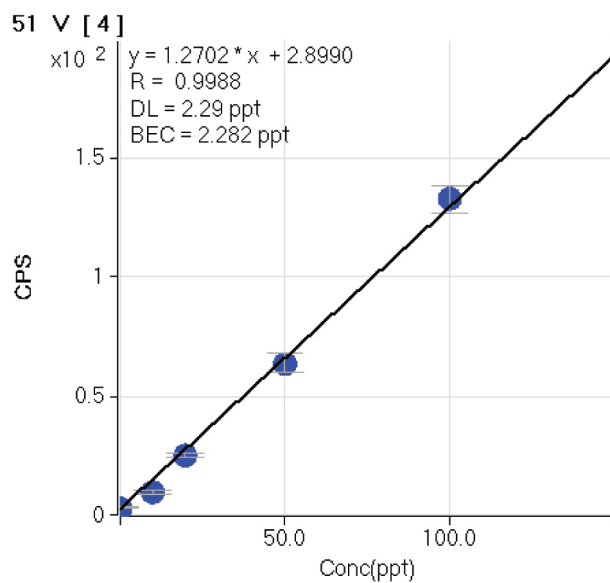


Figure 5. Vanadium calibration in undiluted HCl, using NH_3 mode.

Improved Analysis of Organic Solvents

The 7700s incorporates a newly developed frequency matching RF generator, which responds more quickly to any change of impedance in the plasma, compared to a conventional fixed frequency matching system. A wide range of organic solvents can therefore be introduced without causing disturbance of the plasma.

The most important organic solvent in the semiconductor industry is isopropyl alcohol (IPA). IPA is frequently used to clean silicon wafers and must be analyzed periodically to check for contamination by trace metallic elements. For most organic solvents (including IPA), an organics torch with a 1.5 mm internal diameter (id) injector is used, but a torch with a 1.0 mm id injector is also available for the most volatile solvents.

Figure 6 illustrates the single-ppt and sub-ppt DLs and BECs achieved on the 7700s ICP-MS for the analysis of undiluted IPA. The elements Ti, V, Co, Zn, As, Sr, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Ba, W, Ir, Au, Tl, Bi, Th and U were measured in normal hot plasma conditions (in He, H₂ or no gas mode) and the remaining elements were measured in cool plasma.

Figure 7 shows the excellent raw count rate stability for a 100 ppt spike of several elements in undiluted IPA, measured continuously over 4 hours. Total signal variation was < 5 %RSD.

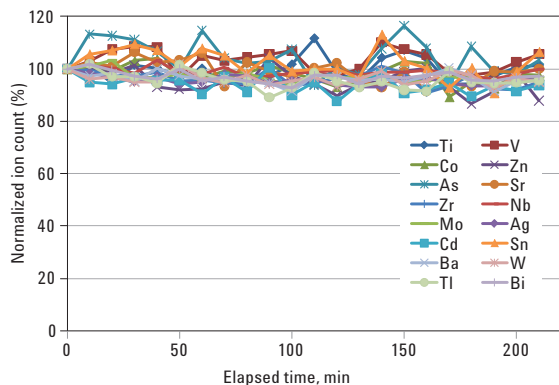


Figure 7. Four hours stability of He mode (100 ppt spiked into IPA).

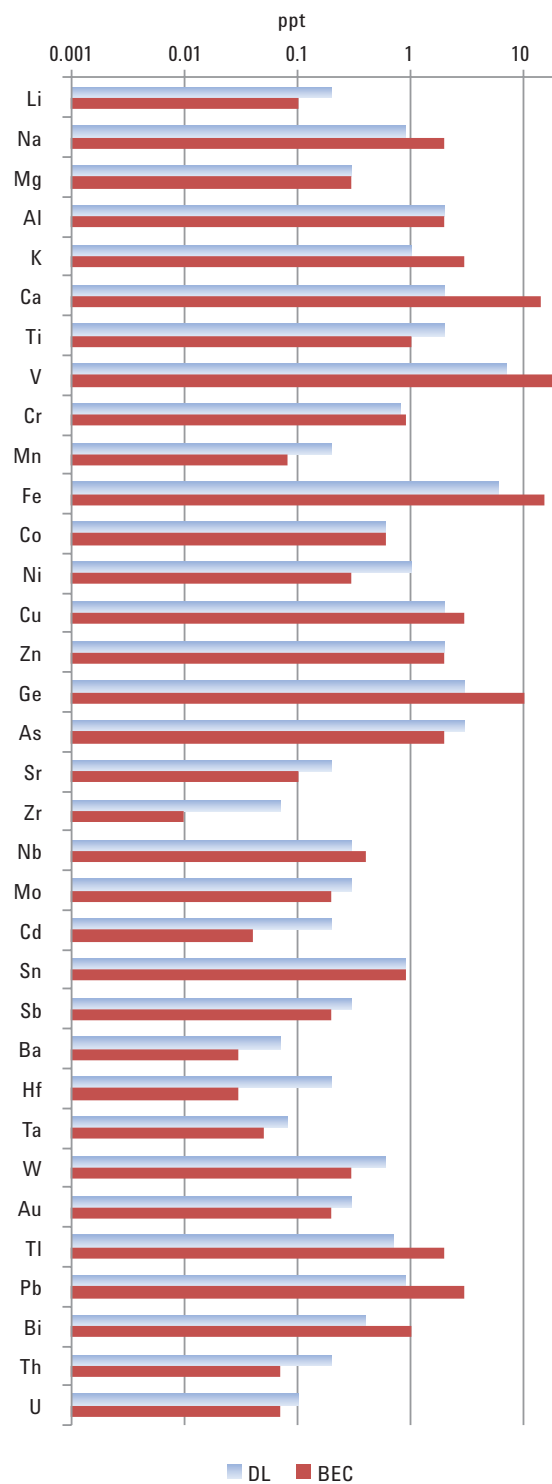


Figure 6. 7700s ICP-MS DLs and BECs in undiluted IPA.

Cool Plasma Operation

As the manufacturer responsible for the development of the first ICP-MS capable of routine operation under cool plasma conditions (4500 Series), Agilent was largely responsible for the initial acceptance and widespread use of ICP-MS in the semiconductor industry. In each subsequent generation of Agilent ICP-MS instrument, cool plasma has been further refined, allowing analysts to make use of the widest range of analytical techniques for interference removal. The 7700s provides a further enhanced cool plasma mode of operation, with improved robustness and stability due to the use of the new frequency matching RF generator.

The exceptional cool plasma performance of the 7700s is demonstrated in Figure 8 which shows the long term stability of several elements in 1% HNO_3 .

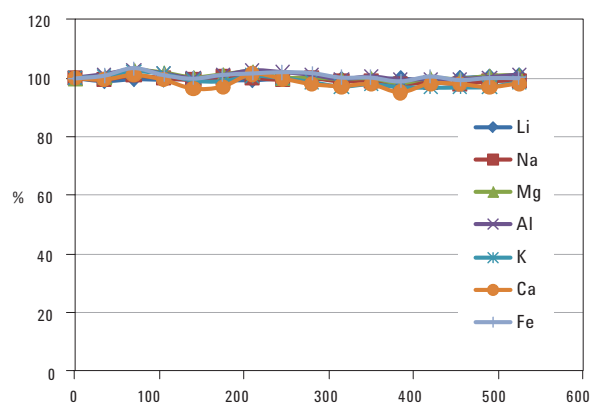


Figure 8. Nine hours stability of cool plasma mode (100 ppt spike in 1% HNO_3).

Cool plasma on the 7700s is also suitable for the measurement of organic solvents. Due to the high volatility of IPA (boiling point 82.4 °C), it may be difficult to keep the ICP stable, particularly when switching between cool and hot plasma modes within an analysis. This issue is resolved with the new frequency matching generator of the 7700s, as demonstrated in the data shown in Figure 9. The elements in Figure 9 were measured in cool plasma, while the corresponding data shown in Figure 7 were measured in normal hot plasma conditions. The plasma conditions were switched automatically between cool plasma and hot plasma for each sample in the analytical sequence, illustrating the robustness and matrix tolerance of the new 7700s RF generator.

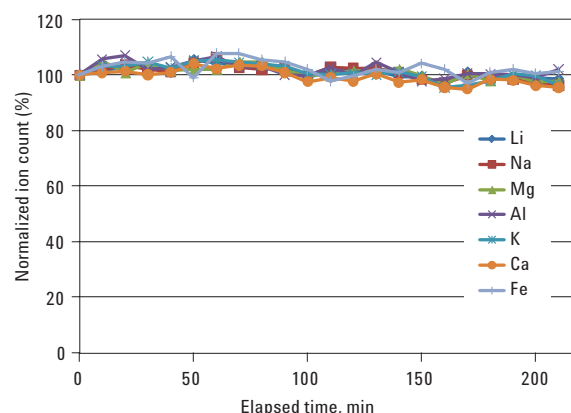


Figure 9. Four hours stability of cool plasma mode (100 ppt spiked into IPA).

Conclusions

In addition to greatly reduced cleanroom setup and operating costs due to its small size and low service requirements, the Agilent 7700s ICP-MS provides lower DLs and BECs, and higher sensitivity than the Agilent 7500cs ICP-MS.

Moreover, the newly developed, 3rd generation ORS³ can be fitted with up to 3 cell gas lines (2 are included as standard), allowing total flexibility. The new ORS³ cell improves performance for several critical elements by increasing the efficiency of both collision and reaction mode, and providing enhanced CID. These developments now allow several elements like phosphorus to be determined at lower concentrations than previously possible.

The robust RF generator of the 7700s also improves the analysis of volatile organic solvents, simplifying the analysis of a variety of process chemicals. Improved matrix tolerance and stability is provided, both for conventional analysis and cool plasma operation.

Details regarding the operating conditions and performance achieved in the analysis of specific semiconductor chemicals are provided in the tuning guide that ships with every Agilent 7700s ICP-MS instrument.

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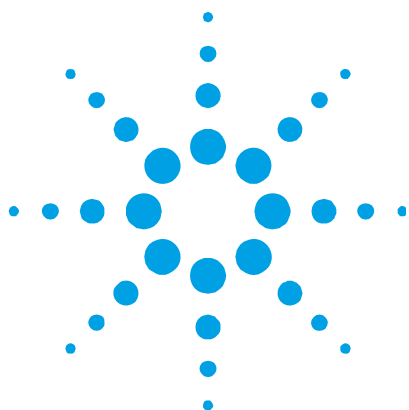
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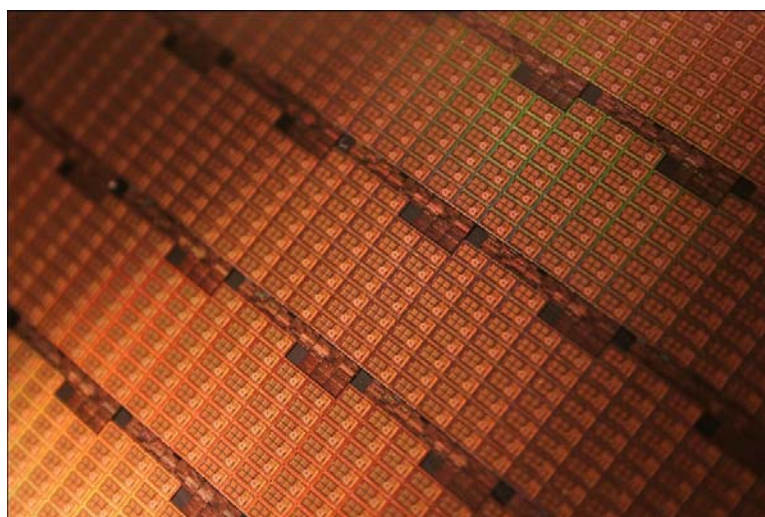
Direct analysis of trace metallic impurities in high purity hydrochloric acid by Agilent 7700s ICP-MS

Application Note

Semiconductor

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Abstract

This application note illustrates the advanced analytical performance and robustness of the Agilent 7700s ICP-MS for the direct determination of metallic impurities in high purity concentrated hydrochloric acid (HCl). The 7700s incorporates a third generation Octopole Reaction System, the ORS³, which effectively removes polyatomic interferences, allowing ultimate detection limits to be achieved for elements that suffer from severe chloride-based interferences. For example, the polyatomic ion $^{40}\text{Ar}^{35}\text{Cl}^+$ can be eliminated by the ORS³ allowing the direct measurement of As at mass 75, and permitting accurate analysis of As at trace levels in undiluted concentrated HCl. Enabling direct analysis of concentrated acids eliminates the dilution step from the sample preparation procedure, and so significantly reduces the potential for sample contamination.



Agilent Technologies

Introduction

Hydrochloric acid is frequently used to remove metallic impurities on the surface of silicon wafers. Together with hydrogen peroxide, this cleaning method is well known as RCA Standard Clean 2 (SC-2). The manufacturing process of semiconductor devices requires routine monitoring of contaminants in HCl, and ICP-MS is the accepted tool for this purpose. Although HCl is diluted prior to use for SC-2, the concentration of industrial grade HCl is usually 20% or 35%, depending on the method of production. Because HCl is highly corrosive; direct introduction of concentrated HCl into an ICP-MS is normally avoided. Moreover, introduction of HCl at high concentration leads to the formation of a large number of polyatomic ions in the ICP, which cause significant spectral interferences with some key elements of interest, for example, $\text{H}_2^{37}\text{Cl}^+$ on $^{39}\text{K}^+$, $^{35}\text{Cl}^{16}\text{O}^+$ on $^{51}\text{V}^+$, $^{35}\text{Cl}^{16}\text{OH}^+$ on $^{52}\text{Cr}^+$, $^{35}\text{Cl}^{37}\text{Cl}^+$ on $^{72}\text{Ge}^+$, $^{37}\text{Cl}_2^+$ on $^{74}\text{Ge}^+$, and $^{40}\text{Ar}^{35}\text{Cl}^+$ on $^{75}\text{As}^+$. Consequently, some methodology for the analysis of high purity HCl by ICP-MS has recommended sample pre-treatment steps to remove the chloride matrix, which can lead to analyte loss and sample contamination. However, the Agilent 7700s ICP-MS is manufactured using robust and anti-corrosive materials, which means that undiluted HCl can be analyzed directly, while the ORS³ drastically improves the efficiency of removing polyatomic ions, allowing many elements to be determined at lower detection limits than were previously possible.

Experimental

Instrumentation

An Agilent 7700s ICP-MS fitted with an optional third cell gas mass flow controller (in addition to the standard collision (helium) and reaction (hydrogen) gas lines) was used throughout. The optional cell gas line is required for specialized applications, including analyses where optimum interference removal requires a highly reactive cell gas such as ammonia. The standard 7700s sample introduction system was used, consisting of the following parts (part numbers for replacements are shown in brackets):

- Pt sampling cone (G3280-67036)
- Pt skimmer cone with Cu base (G3280-67064)
- PFA nebulizer with uptake rate of 200 $\mu\text{L}/\text{min}$ (G3139-65100)
- Quartz torch with 2.5 mm internal diameter injector (G3280-80001)
- Quartz spray chamber (G3280-80008).

Materials and reagents

High purity hydrochloric acid, TAMAPURE-AA100 (20%), was purchased from TAMA Chemicals, Japan. Undiluted HCl was introduced directly into the ICP-MS, to eliminate any sample preparation steps and thereby significantly reduce the potential for sample contamination.

Calibration standard solutions were prepared by spiking a mixed multielement standard (SPEX Certiprep) into an acid blank at 10, 20, 50 and 100 ppt.

Results and discussion

Detection limits and background equivalent concentrations

Forty two elements were measured using the 7700s operating in multiple tune modes. Data was acquired in an automated sequence of cool plasma, no gas and gas modes, during a single visit to the sample vial. Sample-to-sample run time was approximately 6 minutes. Data for each of the modes was combined automatically into a single report for each sample. Detection Limits (DL) and Background Equivalent Concentrations (BEC) are shown in Table 1. DLs were calculated from 3σ of 10 measurements of the acid blank.

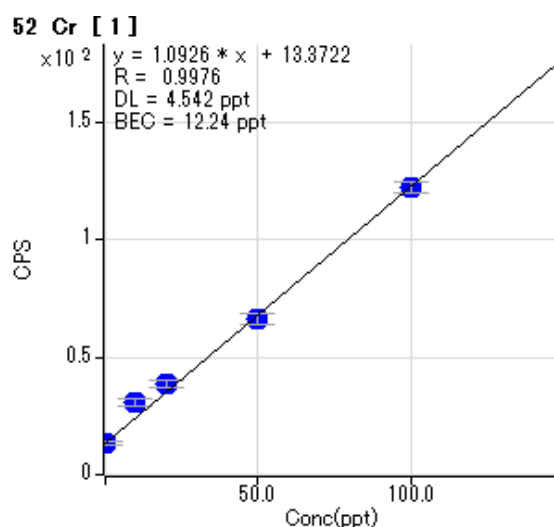
Table 1. 7700s ICP-MS DLs and BECs in 20% high purity HCl

Element	m/z	Mode	DL ppt	BEC ppt
Li	7	cool	0.016	0.004
Be	9	no gas	0.13	0.11
B	11	no gas	4.5	9.7
Na	23	cool	0.44	1.3
Mg	24	cool	0.11	0.22
Al	27	cool	0.79	1.1
K	39	cool/NH ₃	0.40	0.50
Ca	40	cool/NH ₃	1.1	2
Ti	48	He	0.71	0.68
V	51	NH ₃	2.1	2.0
Cr	52	cool/He	4.5	12
Mn	55	He	1.57	2.84
Fe	56	cool	2.4	4.2
Co	59	He	0.20	0.13
Ni	60	He	3.03	4.43
Cu	63	cool	0.49	0.59
Zn	64	He	2.1	2.9
Ga	71	He	0.47	0.31
Ge	74	He	2.1	13
As	75	He	4.0	16
Se	78	He	5	5.5
Sr	88	He	0.21	0.061
Zr	90	He	0.11	0.03
Nb	93	He	0.34	0.43
Mo	98	He	0.52	0.67
Ru	101	He	0.05	0.01
Pd	105	He	0.57	0.51
Ag	107	He	0.056	0.033
Cd	114	He	0.41	0.52
Sb	121	He	2	2.8
Te	125	He	5.4	1.1
Ba	138	He	0.076	0.067
Hf	178	He	0.06	0.015
W	182	He	0.094	0.13
Re	185	He	0.49	0.54
Ir	193	He	0.1	0.07
Au	197	He	0.15	0.4
Tl	205	He	0.054	0.024
Pb	208	He	0.37	0.56
Bi	209	He	0.44	0.33
Th	232	He	0.01	0.003
U	238	He	0.032	0.013

Cr and K determination

Cool plasma is a proven technique used to remove plasma-based interferences. Although it has been largely superseded by Collision Reaction Cell (CRC) methodology, cool plasma remains the most effective analytical mode for some elements in certain matrices. Furthermore, the 7700s provides an enhanced cool plasma mode of operation, delivering improved robustness and stability due to the use of the new frequency matching RF generator. Used together with the ORS³, cool plasma has recently been demonstrated to provide a new, powerful mode to remove interferences¹.

Because the major isotope of chromium ($^{52}\text{Cr}^+$) suffers an interference from $^{35}\text{Cl}^{16}\text{O}^+\text{H}^+$, chromium was determined using cool plasma and He mode. With cool plasma (low plasma RF power), production of ClOH^+ ions is suppressed because of its high ionization potential (I.P.) of 11 eV². For further analytical improvement, He mode was used in combination with the cool plasma conditions to completely eliminate any remaining $^{35}\text{Cl}^{16}\text{OH}^+$ ions. The resultant calibration curve for ^{52}Cr is shown in Figure 1.

**Figure 1.** ^{52}Cr calibration curve obtained using He mode and cool plasma

The approach of using the ORS³ with cool plasma is also effective for other elements such as potassium. In order to suppress the interference of $\text{H}_2^{37}\text{Cl}^+$ on $^{39}\text{K}^+$, ammonia was selected as the cell gas with cool plasma. While there are very few cases where such highly reactive cell gases are required, there are some specific cases where such gases offer the lowest DLs. The intense $\text{H}_2^{37}\text{Cl}^+$ interference that affects K at mass 39 is not very reactive with H_2 , so H_2 cell mode does not give sufficiently good interference removal for the lowest DL to be achieved in the highest purity HCl. The calibration curve for K (shown in Figure 2) illustrates the effective removal of the $\text{H}_2^{37}\text{Cl}^+$ interference using this novel mode of acquisition, providing a K BEC of 0.5 ppt and DL of 0.4 ppt in undiluted (20%) HCl.

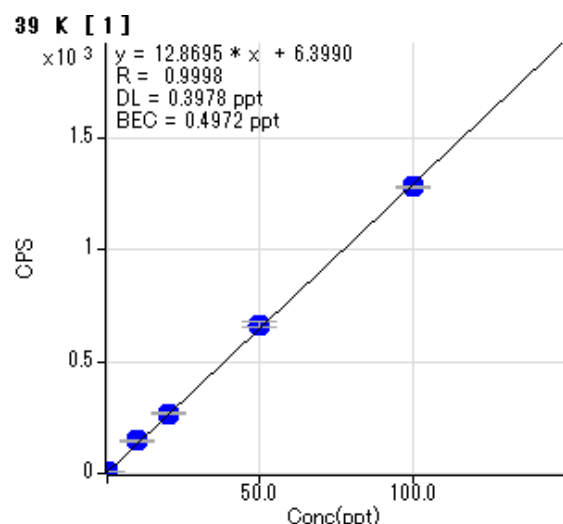


Figure 2. ^{39}K calibration curve obtained using NH_3 mode and cool plasma

Ge and As determination

The newly developed ORS³ of the 7700s improves the removal of polyatomic interferences using He mode with Kinetic Energy Discrimination (KED), and also promotes Collision Induced Dissociation (CID) for relatively weakly bound polyatomic ions.

Germanium has 3 major isotopes at 70, 72 and 74 amu that suffer from Cl-based polyatomic interferences, namely $^{35}\text{Cl}^{35}\text{Cl}^+$, $^{35}\text{Cl}^{37}\text{Cl}^+$ and $^{37}\text{Cl}^{37}\text{Cl}^+$. As the dissociation energy of Cl_2^+ is approximately 4 eV

(3.95 eV for $^{35}\text{Cl}^{35}\text{Cl}^+$), CID of Cl_2^+ would be unlikely to happen with the previous generation ORS, which provided a collision energy of only 0.9 eV in He mode. In contrast, in the ORS³ of the 7700s, the collision energy is increased to 5 eV, facilitating CID of several polyatomic ions including Cl_2^+ . The performance of the 7700s with ORS³ operating in high energy He mode is illustrated in Figure 3, which shows the calibration curve, DL and BEC for ^{74}Ge in a matrix of 20% HCl.

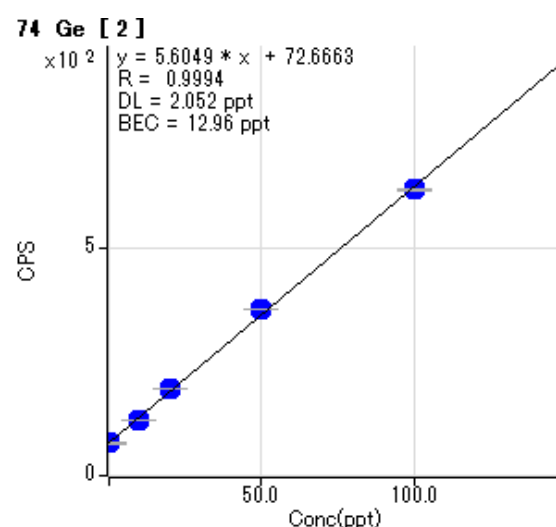


Figure 3. ^{74}Ge calibration curve obtained using high energy He mode

Arsenic has a single isotope at m/z 75, that can suffer an interference from the polyatomic ion $^{40}\text{Ar}^{35}\text{Cl}^+$ that readily forms in a chloride matrix, making it extremely difficult to determine ^{75}As at low levels directly at mass 75. The ArCl interference on As can be avoided by indirectly measuring As at 91 amu as the AsO^+ ion, which is formed either by applying cool plasma conditions or via the use of O_2 cell gas in the CRC. The latter approach utilizes hot plasma conditions but the measurement of As at mass 91 can still be affected by a CaClO^+ interference that forms from CaCl^+ when O_2 cell gas is used. Furthermore, AsO^+ at mass 91 suffers an isobaric interference from $^{91}\text{Zr}^+$, an overlap that does not occur under cool plasma conditions, since Zr is not ionized in a cool plasma. However, as with Cl_2^+ , the higher collision energy of He mode in the ORS³ of the 7700s means that the ArCl^+ ion can also be dissociated by CID. This allows As to be determined at low levels

directly at 75 amu in 20% HCl, thus avoiding the use of both cool plasma and O₂ cell gas. A typical calibration curve for As in 20% HCl is shown in Figure 4.

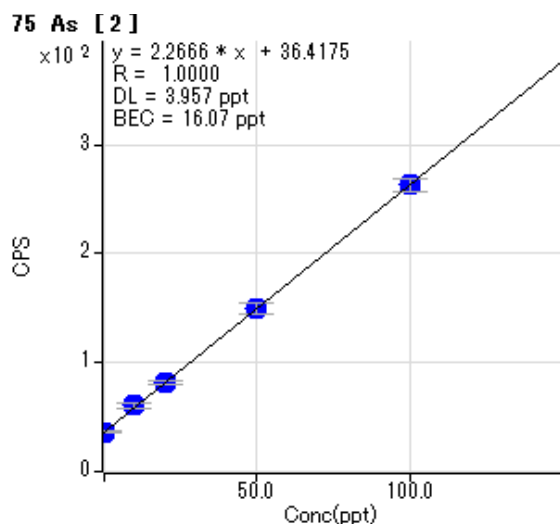


Figure 4. ⁷⁵As calibration curve obtained using high energy He mode

V determination

The ³⁵Cl¹⁶O⁺ interference on ⁵¹V⁺ can also be eliminated using NH₃ as the cell gas, but under normal hot plasma conditions (1600 W). The increased collision energy due of the ORS³ improves the reaction efficiency and gives a significant improvement in the DL and BEC, as shown in Figure 5.

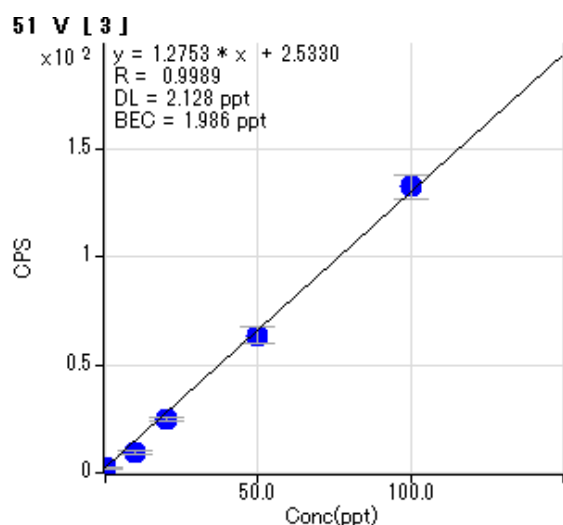


Figure 5. ⁵¹V calibration curve obtained using NH₃ and normal plasma mode

Conclusions

The high performance of Agilent ICP-MS systems for the analysis of trace metallic impurities in concentrated HCl has been described previously⁴. Now, the Agilent 7700s ICP-MS with unparalleled cool plasma performance and ORS³ collision/reaction cell further improves the detection limits for the analysis of high purity acids. The newly developed ORS³ can be fitted with up to 3 cell gas lines (2 are included as standard), allowing total flexibility in both collision and reaction modes. The ORS³ cell improves performance for several critical elements by increasing the efficiency of both collision and reaction mode, and providing enhanced dissociation of certain polyatomic ions by CID. These developments now allow several elements like Cr, K, Ge, As and V to be determined at lower concentrations than previously possible in a chloride matrix.

References

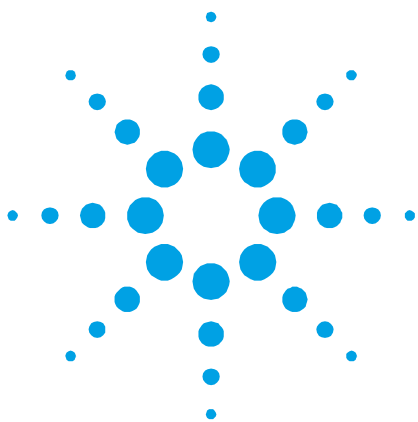
1. "Use of Collision Reaction Cell under Cool Plasma Conditions in ICP-MS", Junichi Takahashi and Katsuo Mizobuchi, 2008 Asia Pacific Winter Conference on Plasma Spectroscopy
2. Colbourne, D., Frost, D.C., McDowell, C.A., Westwood, N.P.C., J. Chem. Phys., 1978, 68, 3574
3. Huber, K. P. and Herzberg, G., Constants of Diatomic Molecules, Van Nostrand Reinhold Co., 1979
4. "Determination of Impurities in Semiconductor Grade Hydrochloric Acid Using the Agilent 7500cs ICP-MS", Junichi Takahashi, Agilent Application Note, 5989-4348EN

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Application Note

Chemicals

Author

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Summary

The Agilent Cary 60 UV-Vis spectrophotometer is the new, improved successor to the award-winning Cary 50 UV-Vis. In this short review, this instrument platform was evaluated for its potential to measure small (40 μ L) samples of methylene blue *in situ* during exposure to high-intensity UV irradiation. Methylene blue is combined with other compounds used in a variety of applications, including use in cosmetics and sun screen products and environmental remediation in contaminated air and polluted water.

Introduction

By virtue of its unique optical design, we have previously demonstrated that the Cary 60 UV-Vis has no measureable effect on photobleaching of the polyaromatic hydrocarbon probe methylene blue¹, which has been shown to be particularly susceptible to photobleaching during continuous readings in UV-Vis instruments from other manufacturers². In the present study, we extend our observations to applications in which photobleaching is desirable, induced by an external, high-intensity UV lamp. This is of significant benefit when analyzing the photochemical properties of electron-quenching compounds, such as methylene blue, that can have protective properties against a broad range of ailments associated with UV exposure, including cancer³.



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Using the fiber optics microprobe accessory with the Cary 60, the purpose of this study was to develop an automated method of analysis to study the induced photobleaching of samples *in situ*, as opposed to conventional approaches that require sampling be made manually using a cuvette resulting in less accurate results and significant increase in time per analysis (Wang et al⁴).

In the present study, we use fiber optics in aqueous samples at 20 °C under conditions of normal laboratory fluorescent ambient lighting. This approach allows users to take the instrument to the sample, rather than the conventional approach in spectroscopy in which the sample is presented to the instrument. The unique optical configuration of the Cary 60 makes this possible mainly by virtue of the high-intensity xenon flash lamp combined with the latest electronics, enabling the system to effectively monitor small changes in absorbance without any effect of ambient light. Key benefits of this approach are discussed further in this document.

Apparatus and materials

Part Number	Description
G6860AA	Cary 60 UV-Vis with WinUV software and PC
7910035600	Fiber optic microprobe
G6866A	Fiber optic probe coupler

Methods and results

The Cary 60 instrument platform was fitted with the fiber optics coupler and microprobe as shown in Figure 1. Baseline readings were taken using purified water.

A solution of methylene blue (12.5 ppm, 60 mL) was prepared by diluting stock solution (400 ppm) in purified water. Incrementally, small aliquots of a solution of titanium (0.50 g/L, 200 μ L) were added in order to assess and evaluate the effects of the photodegradation rate of methylene blue against time in the presence of titanium. Samples were placed in a Class 2 safety cabinet where they were irradiated with

high-intensity UV light to induce photochemical reaction, being stirred continuously during the analysis, and the absorbance measured at 20 °C using a fiber optic microprobe.



Figure 1. The Cary 60 UV-Vis fitted with the fiber optics microprobe accessory provides a simple mechanism to measure a sample *in situ* and remote to the instrument

Using the Scanning Kinetics application module in the Cary WinUV software package, scans were taken at 2-min intervals from 400–800 nm over a period of 20 min. The effect of the high-intensity UV lamp on the photokinetic properties of the methylene blue solution was assessed in terms of peak height and blue-shifting of the spectra (Figure 2).

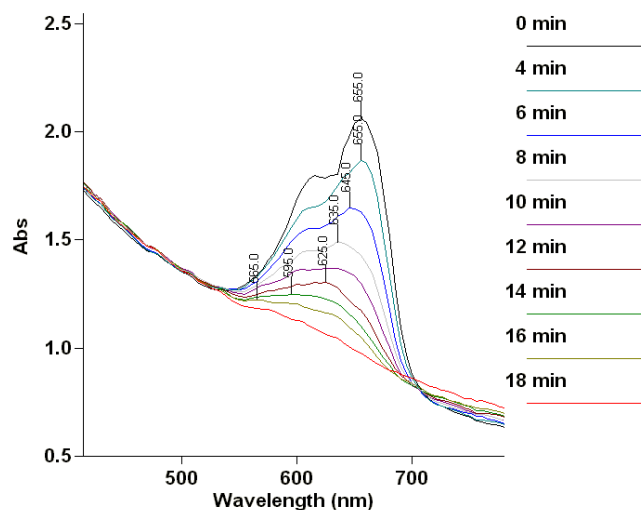


Figure 2. Scanning kinetics using fiber optics *in situ* of methylene blue under the exposure of a high-intensity UV lamp (Oriell 500 W Hg(Xe) lamp) over a period of 20 minutes within the range 400 to 800 nm. Labels reflect maximum absorbance wavelengths

Discussion

Results in Figure 2 show significant photobleaching of the methylene blue solution and a blue-shift of the maximum absorbance peak at 655 nm over the 20-min period.

These results are comparable with those taken in a cuvette at room temperature, which conventionally demands transfer of aliquots of the sample to an instrument remote from the reaction chamber. This approach results in inaccurate data, particularly so when dealing with photosensitive samples such as methylene blue².

Conclusion

Results discussed above demonstrate that the Agilent Cary 60 instrument fitted with the fiber optics microprobe accessory provides a simple, cost-effective, rapid and versatile system for the automated measurement of photocatalytic reactions *in situ*. As far as the authors are aware, this is the first time this approach (i.e., with fiber optics) has been successfully accomplished.

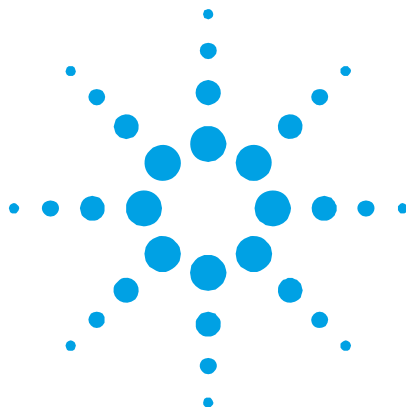
1. Fyfe, DJ, and Wang, X. (2011) Cary 60 Optics Inhibit Photodegradation of Aromatic Markers in Applications for UV/VIS Spectroscopy. Agilent Application Note #5990-7862EN. www.agilent.com
2. Kok, C. et al 2005. Study of Photobleaching Mechanism in Methylene Blue Sensitized Gelatin Using a Single Beam UV-Vis Fiber Optics Spectrophotometer. *Pertanika J. Sci. & Technol.* **2005**, 13(1), 23-30.
3. Sturmey et al (2009) Removal of red light minimizes methylene blue-stimulated DNA damage in oesophageal cells: implications for chromoendoscopy *Mutagenesis* **24** (3); 253-8.
4. Wang, W., et al (2008) Gold Nanoparticle Incorporation into Porous Titania Networks Using an Agarose Gel Templating Technique for Photocatalytic Applications. *Chem Mater.* **2008**, 20, 3917-3926

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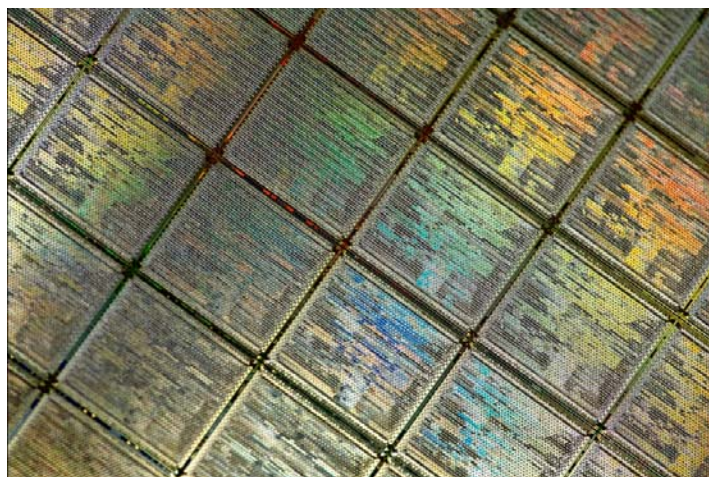
Direct measurement of metallic impurities in 20% ammonium hydroxide by Agilent 7700s ICP-MS

Application Note

Semiconductor

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Abstract

Ammonium hydroxide (NH_4OH) is a chemical used in the manufacture of semiconductor devices, and must therefore be analyzed for trace metal impurities. The direct analysis of undiluted (20%) NH_4OH using ICP-MS is challenging, because its high vapor pressure causes plasma instability. However, dilution of the samples would degrade detection limits, so the ability to directly analyze 20% NH_4OH is important. The Agilent 7700s ICP-MS employs a unique, high-speed frequency-matching ICP RF generator that can maintain a stable plasma even when 20% NH_4OH is aspirated. The 7700s also features the most effective technologies for removing spectral interferences in high-purity chemicals, making it ideally suited to semiconductor chemical analysis. An Agilent 7700s, using an inert sample introduction system, was used to measure trace elements in high purity 20% NH_4OH . Detection limits in the single digit ppt or sub-ppt range were obtained for 48 elements, and long term (~7 hours) stability of approximately 5% RSD was achieved for a spike level of 100 ppt in 20% NH_4OH , demonstrating the ability of the 7700s to routinely measure trace contaminants in high purity 20% NH_4OH .



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Introduction

Many chemicals are used throughout the manufacturing process of semiconductor devices. Metallic impurities present in the chemicals and chemical mixes used can cause contamination and give rise to defects in the final product, so their levels must be strictly controlled. ICP-MS is the standard technique used for the measurement of metallic impurities in semiconductor chemicals. Among all the process chemicals, those that are used as part of the silicon wafer cleaning process are particularly important, as they are in direct contact with the wafer and can therefore impart impurities to the wafer surface. There are over 100 cleaning steps throughout the manufacture of a typical semiconductor device, and one of the typical solutions used for wafer cleaning is a chemical mix developed by RCA, commonly referred to as Standard Clean 1 (SC-1). SC-1 is a mixture of NH_4OH , hydrogen peroxide (H_2O_2) and ultra-pure water (UPW) in the ratio 1:1:5, and is used to remove surface particles by lightly etching the wafer. There is a clear requirement for a highly sensitive and reliable analytical methodology to measure metallic impurities in the high purity chemicals from which SC-1 is produced.

While UPW and H_2O_2 are easy matrices to analyze, the analysis of high purity NH_4OH by ICP-MS is very difficult for two reasons. Firstly, NH_4OH is a strong alkali, which causes some metals to readily precipitate as insoluble hydroxides. This presents difficulties for determination using the method of standard additions (MSA), because MSA is performed by sequentially spiking an acid-based multi-element standard into the sample. At higher concentrations, some metals will precipitate when spiked into the undiluted NH_4OH , making accurate determination by MSA impossible. However, the NH_4OH used in SC-1 is high purity grade with a maximum metallic impurity level of 100 ppt, so higher level spikes can be avoided if the ICP-MS detection limits are sufficiently low. If MSA spikes are at the low ppt level (<100 ppt), minimal precipitation should occur, and MSA can in fact be used to analyze undiluted NH_4OH . To be able to produce good MSA

calibrations below 100 ppt of course requires the interference removal technology employed in the ICP-MS to be extremely effective for all interferences.

The Agilent 7700s ICP-MS, with the third generation Octopole Reaction System (ORS³), has the widest range of interference removal technologies of any ICP-MS instrument. In addition to conventional no gas mode, the ORS³ operates in both collision (He) mode and reaction (e.g. H_2) mode, and cool plasma mode is also available. The interference removal approach selected depends on the analytical requirement: for this application, calibration down to the 10 ppt level is required for all analytes and therefore the most efficient interference removal mode is required for every analyte. Switching between modes is fully automated and all analytes are measured with a single visit to the sample vial. The small size of the ORS cell allows very fast switching between cell gas modes so the additional time required for multi-mode operation is minimized.

The second challenge when analyzing undiluted NH_4OH is that the plasma becomes unstable due to the high vapor pressure of undiluted NH_4OH , so routine, direct analysis of undiluted NH_4OH has not previously been possible. The accepted method of analyzing NH_4OH is to remove the matrix by heating to near dryness and then re-dissolving the residue in 1% HNO_3 prior to measurement by ICP-MS [1]. Although this method is widely used, laboratories favor elimination of the sample preparation step, to shorten analysis time and reduce the risk of sample contamination and loss of volatile analytes. The Agilent 7700 Series features a unique RF plasma generator design with high-speed frequency-matching that can instantaneously adjust to changes in plasma load – for example when switching from aqueous to a high vapor pressure solvent. This produces a very stable plasma capable of tolerating the direct aspiration of undiluted NH_4OH .

The combination of high sensitivity, effective removal of interferences, and high-speed frequency-matching ICP RF generator enables the Agilent 7700s to measure low ppt level metallic impurities directly in undiluted high purity NH_4OH .

Experimental

An Agilent 7700s was fitted with an inert sample introduction system (Agilent part # G4912-68002) comprising a PFA double pass spray chamber and demountable torch fitted with a 1.5 mm ID sapphire injector. Standard Pt interface cones and PFA concentric nebulizer were used. A solution of Li, Zn, Sn and Pb prepared in 2% NH₄OH was used to tune the instrument. These elements were chosen since they form amphoteric oxides or hydroxides (which can display both acidic and basic properties), and are therefore stable in NH₄OH. The normal tuning solution containing Li, Y, Ce and Tl in 2% nitric acid may also be used, but a thorough rinsing with UPW prior to analysis is necessary to prevent residual acid mixing with the NH₄OH standard and samples. Once the tuning conditions have been established, the system should be rinsed with UPW and then 20% NH₄OH for an hour to ensure that the sample introduction system is free from acid. Operating parameters are shown in Table 1. The acquisition used a multi tune mode method, performed with a single visit to the sample vial, and data for each of the modes (cool plasma, no gas, He mode and H₂ mode) was combined automatically into a single report for each sample. Total run time per sample, including uptake and rinse, was 8m 20s.

High purity grade NH₄OH (20% as NH₃) was used (TAMAPURE-AA100, TAMA Chemicals, Kawasaki, Japan), and the MSA calibration standard solutions were prepared by spiking a mixed multi-element standard (SPEX CertiPrep, Metuchen, NJ, USA). Calibration levels, added sequentially into a blank of undiluted NH₄OH, were at 10, 20, 50 and 100 ppt.

Results

DLs and BECs

3 σ detection limits (DLs) and background equivalent concentrations (BECs) obtained for 48 elements in high purity NH₄OH are shown in Table 2. For Se and Te, the lowest detection limits were obtained using H₂ mode, while for Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni and Cu, cool plasma mode was used. For the remainder, He mode was used except for Be and B which were run using no gas mode. The DLs were calculated from 10 measurements of the blank. All DLs were single figure ppt or lower, as were the BECs, aside from Ca at 11 ppt. This demonstrates the ability of the 7700s to remove spectral interferences, and also the high quality of this NH₄OH product. Since no individual analysis mode gives the ultimate DLs for every element, the 7700s offers the ultimate performance for semiconductor chemical analysis by allowing the user to automate analysis methods with no gas, He and H₂ modes, plus cool plasma. This flexibility, coupled with pre-defined methods and simple, automated optimization routines makes the 7700s the most powerful ICP-MS for semiconductor analysis.

Table 1: 7700s Operating Parameters

	Normal Plasma	Cool Plasma
Forward power (W)	1600	600
Carrier gas (L/min)		0.8
Make up gas (L/min)	0.1	0.5
Sampling depth (mm)	7	18
Cell gas flow (mL/min)		
He mode	5	-
H ₂ mode	6	-
Cell KED (V)	3	-
Uptake time (s)		60
Acquisition time (s)		350
Rinse time (s)		90

Table 2: 7700s ICP-MS DLs and BECs in 20% NH₄OH

Element	m/z	Mode	DL/ppt	BEC/ppt
Li	7	cool	0.014	0.003
Be	9	no gas	0.33	0.1
B	11	no gas	2.6	16
Na	23	cool	0.43	0.38
Mg	24	cool	0.17	0.32
Al	27	cool	0.26	0.67
K	39	cool	0.25	0.38
Ca	40	cool	1.9	11
Ti	48	He	2.4	1.4
V	51	He	0.67	0.31
Cr	52	cool	0.3	0.4
Mn	55	cool	0.078	0.026
Fe	56	cool	1.5	2.1
Co	59	cool	0.23	0.052
Ni	60	cool	0.88	0.42
Cu	63	cool	3	1.8
Zn	66	He	1.7	0.8
Ga	71	He	1.7	0.68
Ge	72	He	4.3	1.6
As	75	He	6.5	3.8
Se	78	H ₂	8.4	4.6
Rb	85	He	0.022	0.028
Sr	88	He	0.86	0.29
Zr	90	He	0.35	0.2
Nb	93	He	0.057	0.076
Mo	98	He	0.24	0.16
Ru	101	He	0.26	0.1
Rh	103	He	0.41	1.4
Pd	105	He	0.18	0.092
Ag	107	He	0.11	0.12
Cd	111	He	0.66	0.35
Sn	118	He	2.3	1.5
Sb	121	He	2.3	0.92
Te	125	H ₂	1.3	1.3
Cs	133	He	0.41	0.21
Ba	138	He	0.27	0.13
Hf	178	He	0.24	0.086
Ta	181	He	0.047	0.036
W	182	He	0.16	0.071
Re	185	He	0.14	0.061

Element	m/z	Mode	DL/ppt	BEC/ppt
Ir	193	He	0.15	0.16
Pt	195	He	0.39	0.48
Au	197	He	0.4	0.17
Tl	203	He	0.21	0.27
Pb	208	He	0.75	1.1
Bi	209	He	0.16	0.15
Th	232	He	0.085	0.025
U	238	He	0.064	0.013

Calibration linearity

Figures 1a and 1b show examples of the calibration plots achieved for elements in two different modes: V (He mode) and Fe (cool plasma mode). Excellent linearity at the ppt level was achieved in each case, demonstrating the interference removal power of the 7700s in both modes.

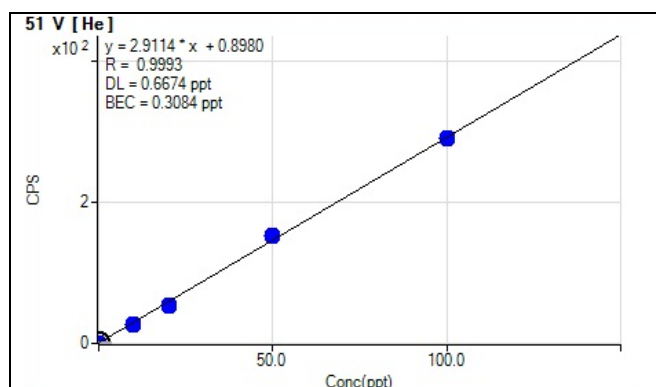


Figure 1a: Calibration plot showing spikes at 0, 10, 20, 50 and 100 ppt for V in 20% NH₄OH

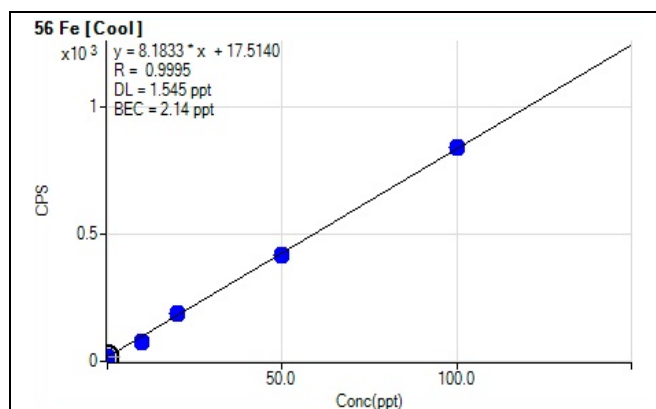


Figure 1b: Calibration plot showing spikes at 0, 10, 20, 50 and 100 ppt for Fe in 20% NH₄OH

Long term stability

If present at a high enough concentration in an alkaline solution, many metals form insoluble hydroxides which easily form precipitates or can be adsorbed on to the surface of the vessel wall, and the likelihood of precipitation or adsorption increases with time. Keeping metal concentrations low (at a maximum of 100 ppt) reduces the risk of precipitation, and monitoring signal stability over an extended period is an effective way to confirm the chemical stability of the analytes. A long term (just under 7 hours) stability test was performed for 22 elements spiked at 100 ppt in 20% NH_4OH . Signal intensity was measured every 45 min while blank samples of 20% NH_4OH were introduced between each spike measurement. Figure 2 shows the excellent stability (approx. 5% RSD) of the spikes over the test period, confirming the applicability of direct analysis using MSA at spike concentrations up to the 100 ppt level in 20% NH_4OH .

Conclusions

The direct analysis of 20% NH_4OH was performed successfully using an Agilent 7700s ICP-MS. The high speed frequency-matching RF generator of the 7700 produces a stable plasma when 20% NH_4OH is aspirated, while the availability of multiple interference removal technologies assures low DLs and BECs for all 48 elements measured. The routine applicability of direct measurement using MSA has been demonstrated by limiting spike concentrations to 100 ppt, which avoids precipitation in the alkaline matrix. As a result, labs are no longer forced to carry out matrix removal in order to analyze high purity 20% NH_4OH by ICP-MS, and direct analysis is routinely possible.

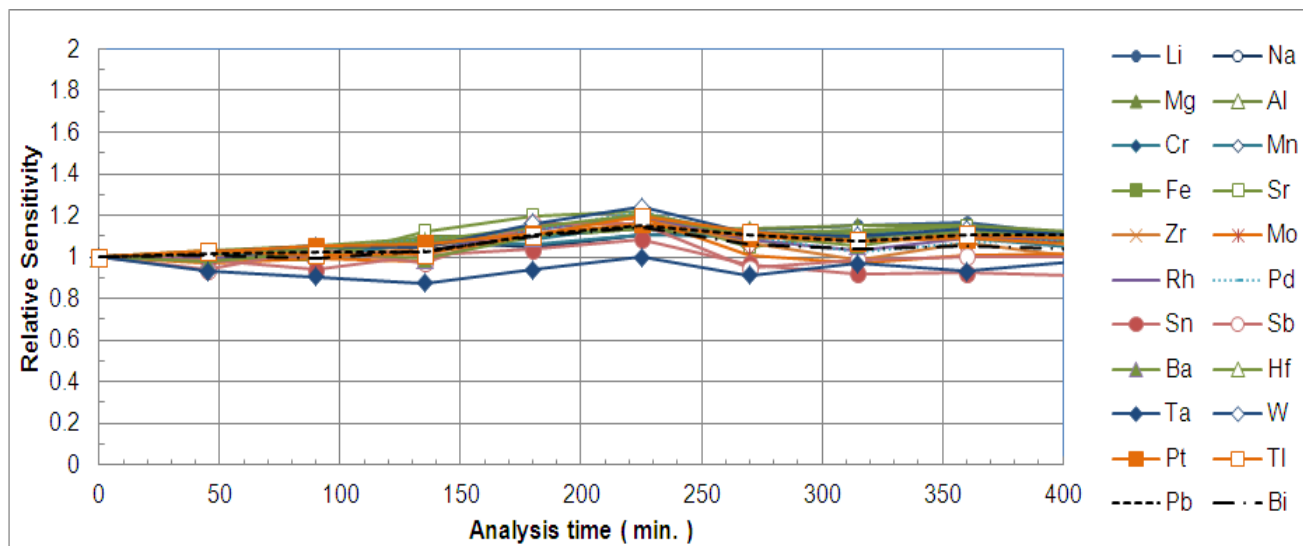


Figure 2: Signal stability of a representative selection of 22 elements spiked at 100 ppt in 20% NH_4OH

References

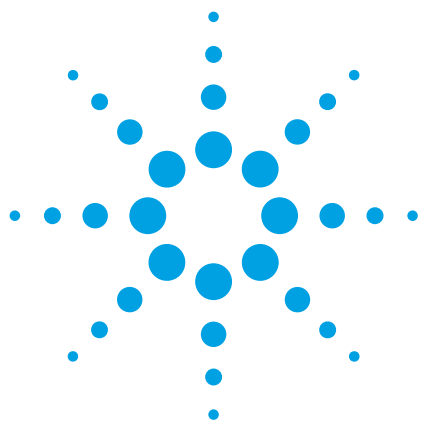
1. Takeda K., Ikushima S., Okuzaki J., Watanabe S., Fujimoto T., Nakahara T., *Anal. Chim. Acta* 426, 1, 105, 200

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The ultratrace determination of iodine 129 in aqueous samples using the 7700x ICP-MS with oxygen reaction mode

Application note

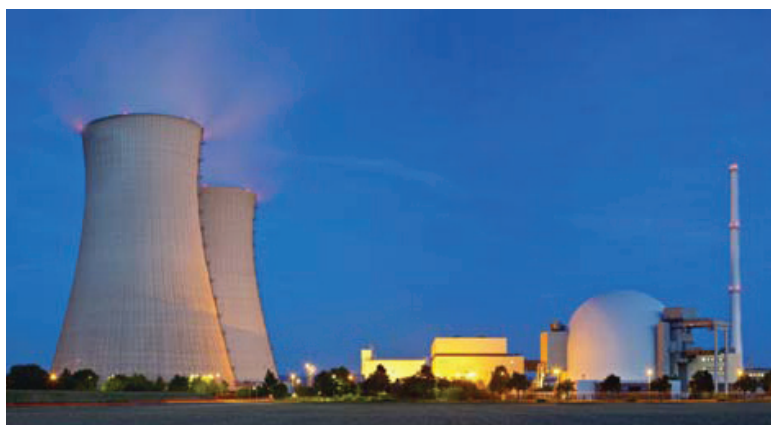
Nuclear

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Abstract

Determination of the radionuclide iodine 129 using ICP-MS has been improved by the use of an Octopole Reaction System (ORS) ICP-MS, operated in oxygen cell mode. Oxygen reaction gas removes the background signal at m/z 129, which is due to the presence of Xe as an impurity in the Ar plasma gas and to the polyatomic ion $^{127}\text{IH}_2^+$. NIST SRM standards with different $^{129}\text{I}/^{127}\text{I}$ ratios and at multiple concentrations were successfully measured, and a ^{129}I detection limit of 1.1 ppt was obtained. No sample preparation is required for aqueous samples and the method is capable of high sample throughput.



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Introduction

Iodine 129 (^{129}I) is a long-lived radionuclide (half-life of 15.7 million years), which has been released into the environment as a result of nuclear weapons testing and accidental releases from nuclear power plants and spent nuclear fuel reprocessing plants. Neutron activation analysis (NAA) and accelerator mass spectrometry are commonly used techniques for the measurement of ^{129}I at ambient (pre-nuclear age) levels, but these techniques are costly and time consuming (several weeks for NAA) involving radiochemical separation. Less sensitive but faster and more routine techniques used to monitor for ^{129}I release are liquid scintillation counting and gamma spectrometry, though measurements still take several hours and significant radiochemical sample preparation is required. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can also be used to measure ^{129}I , however detection limits are compromised by a spectral overlap from ^{129}Xe , due to the Xe impurity present in the argon plasma gas.

Since ^{129}Xe is an elemental, rather than a polyatomic interference, magnetic sector ICP-MS does not have sufficient resolution to separate the 2 isobars, so a different approach is necessary. In this study, a quadrupole ICP-MS (Agilent 7700x) with an Octopole Reaction System (ORS) was used to remove the Xe interference on ^{129}I by reaction with oxygen gas in the ORS cell.

Instrumentation

The Agilent 7700x ICP-MS features a 3rd generation ORS cell (ORS³), which features a longer, narrower octopole, and operates at higher frequency, higher cell gas flow rates, and higher collision energy than in previous ORS versions [1]. In combination, these enhancements give better interference removal efficiency in both collision and reaction modes. The ORS³ cell operates effectively in both collision mode, using an inert collision gas (helium) with kinetic energy discrimination (KED), and reaction mode, using a reactive gas such as hydrogen. In this study, the ORS³ was operated in reaction mode, using oxygen as a reaction gas. Oxygen reacts with Xe, converting Xe^+ ions to Xe atoms by charge transfer,

and also removes the $^{127}\text{IH}_2$ overlap on ^{129}I by chemical reaction, thereby removing both the interferences on ^{129}I . Helium was also added to the cell to thermalize the ion beam. This minimizes the high mass tail of the ^{127}I peak, which might otherwise contribute to the signal measured at m/z 129 when ^{127}I is present at high concentration. The two cell gases (oxygen and helium) can be added as a pre-mixed blend or, for greater flexibility, added separately using independent mass flow controllers. The latter approach was used in this work, utilizing an optional low-flow 3rd cell gas controller. A standard Agilent glass concentric nebulizer and double-pass quartz spray chamber cooled to 2 °C were used for sample introduction. In order to ensure the chemical stability of iodine in aqueous solutions, samples are typically prepared in a basic diluent, such as tetramethyl ammonium hydroxide (TMAH). This ensures that iodine is not converted to volatile chemical forms that would rapidly be lost from the solution. Plasma parameters were automatically optimized to give 'Robust Plasma' conditions, using the pre-set plasma autotune feature in the Agilent ICP-MS MassHunter software. Robust plasma conditions are defined by a CeO^+/Ce^+ ratio of 1% or less and provide high matrix tolerance for routine use. Ion lens voltages were also autotuned for maximum sensitivity.

Optimization of oxygen flow rate

The optional low-flow 3rd cell gas mass flow controller of the 7700 Series ICP-MS can be used for several different gases and, when used for oxygen cell gas, the flow rate range is 0 to 1.12 mL/min (expressed as 0 to 100% flow, where 100% = 1.12 mL/min). Oxygen flow rate was optimized while aspirating a ^{127}I standard prepared in 0.5% TMAH (Tama Chemicals, Kawasaki, Japan) and monitoring m/z 127 and 129 (Figure 1). Assuming equal signal response for the 2 isotopes of I (127 and 129), the optimum cell gas flow rate was identified from the rate that gave the lowest background equivalent concentration (BEC) at m/z 129, using the measured ^{127}I sensitivity to calculate the BEC for ^{129}I . The lowest ^{129}I BEC achieved was 0.6 ng/L (ppt), obtained at an oxygen gas flow rate of 90% of maximum (1.01 mL/min), demonstrating the effective removal of the Xe and IH_2 interferences. Figure 1 shows the cell gas optimization plot.

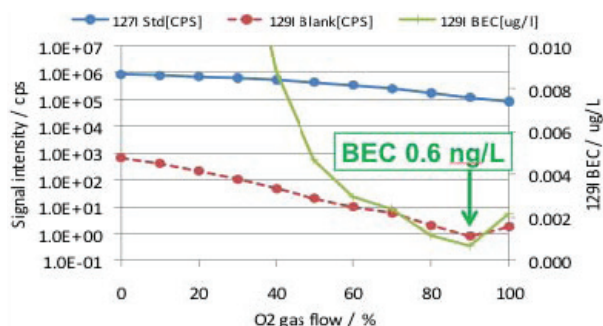


Figure 1. Profile of ^{127}I sensitivity, m/z 129 background and ^{129}I BEC versus oxygen flow rate

The Xe concentration in high purity argon varies depending on the Ar source (Xe is generally higher in bottled Ar gas than in liquid Ar tanks), but Xe peaks are always present at significant levels in ICP-MS spectra. The effectiveness of the ORS³ in removing this Xe signal is demonstrated visually from the log scale spectra shown in Figure 2.

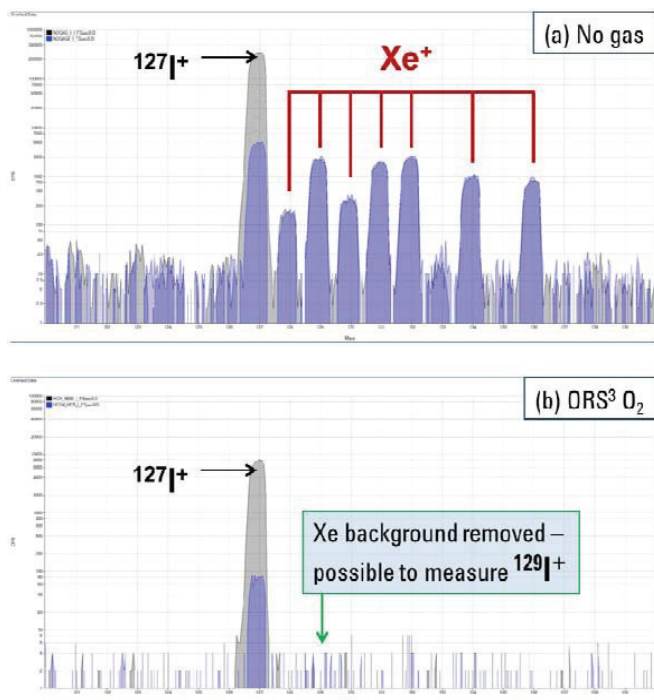


Figure 2. Mass spectra at mass range m/z 120–140 in (a) no gas mode and (b) oxygen reaction mode. In each case, the grey spectrum is 10 $\mu\text{g/L}$ ^{127}I and the overlaid blue spectrum is the blank.

The upper spectrum (a) in no gas mode (no oxygen cell gas flow) clearly shows the Xe background arising from the Ar plasma gas and the background contribution from the polyatomic ion $^{127}\text{IH}_2^+$ that is produced in the plasma during the analysis of real samples. The grey spectrum is from a 10 $\mu\text{g/L}$ ^{127}I solution, and the overlaid blue spectrum is from a blank. Switching on the cell gases (b) decreases sensitivity but the Xe^+ peaks disappear, as oxygen converts Xe^+ to Xe via a thermodynamically favorable charge transfer reaction ($\text{Xe}^+ + \text{O}_2 \rightarrow \text{Xe} + \text{O}_2^+$; $k = 1.1 \times 10^{-10}$), freeing m/z 129 for the measurement of ^{129}I . Again, the grey spectrum is from the 10 $\mu\text{g/L}$ ^{127}I solution, while the overlaid blue spectrum is from the blank, showing a small amount of memory from the iodine standard previously run. In oxygen reaction mode, m/z 131 also becomes available, allowing for the trace measurement of ^{131}I , though this is not particularly useful since its half-life is only 8 days.

Table 1 shows the ICP-MS operating parameters (at optimum oxygen flow rate). Total cell gas flow rate was 5.01 mL/min (4 mL/min He plus 1.01 mL/min oxygen).

Table 1. ICP-MS operating parameters

Parameter	Value
RF power (W)	1550
Sampling depth (mm)	8
Carrier gas (L/min)	1.05
Spray chamber temperature ($^{\circ}\text{C}$)	2
He gas flow (mL/min)	4
Oxygen flow (mL/min)	1.01
KED (V)	10

Calibration standards

Calibration standards were prepared by diluting ^{129}I isotopic standards NIST SRM 3231 Level I and II (NIST, Gaithersburg MD, USA) with 0.5% TMAH in deionized water. The certified value for the $^{129}\text{I}/^{127}\text{I}$ ratio is $0.981 \times 10^{-6} \pm 0.012 \times 10^{-6}$ in Level I, and $0.982 \times 10^{-8} \pm 0.012 \times 10^{-8}$ in Level II. An intermediate standard with a $^{129}\text{I}/^{127}\text{I}$ ratio at the 10^{-7} level was prepared by spiking the Level I standard solution with ^{127}I at the appropriate concentration using a potassium iodide solution. The rinse solution was 1% TMAH.

Analytical data

Measurement of the NIST 3231 level I standard

In order to confirm the applicability of the method to measurement of ^{129}I at varying concentration levels, the $^{129}\text{I}/^{127}\text{I}$ ratio was measured in 4 different solutions containing NIST 3231 at different concentrations, as shown in the data in Table 2. In the case of the highest dilution (NIST 3231 diluted x100), ^{129}I was present at the single figure ppt level. For each solution, 5 replicate measurements of the $^{129}\text{I}/^{127}\text{I}$ ratio were acquired, with a total data acquisition time of 10 minutes for the 5 replicates. After subtracting the reagent blank, the measured $^{129}\text{I}/^{127}\text{I}$ ratio corresponded well with the certified value of 0.981×10^{-6} (9.81×10^{-7}) at all concentrations. RSD values were higher than is typically achieved for a more conventional isotope ratio measurement, due to the very low count rate measured for ^{129}I , although the RSD improved to 1.5% in the x10 diluted standard as the ^{129}I count rate increased to 70 cps.

Table 2. NIST 3231 Level I — measured iodine 129/127 ratio for each dilution. (Integration time was 0.3 s for $m/z=127$ and 15 s for $m/z=129$.)

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}\text{I}/^{127}\text{I}$	$^{129}\text{I}/^{127}\text{I}$ (average)	RSD (%)
100	6224116	6.1	9.80×10^{-7}	9.81×10^{-7}	8.6
	6092355	6.3	1.03×10^{-6}		
	6073353	6.4	1.05×10^{-6}		
	6125790	6.2	1.01×10^{-6}		
	6099791	5.1	8.37×10^{-7}		
50	14044748	12.3	8.76×10^{-7}	9.93×10^{-7}	7.8
	13933138	13.4	9.64×10^{-7}		
	13475103	13.6	1.01×10^{-6}		
	14128483	15.0	1.06×10^{-6}		
	14144548	15.0	1.06×10^{-6}		
20	36305910	33.8	9.32×10^{-7}	9.62×10^{-7}	3.9
	35573975	32.9	9.24×10^{-7}		
	36062147	36.4	1.01×10^{-6}		
	36295813	36.0	9.93×10^{-7}		
	36050890	34.3	9.51×10^{-7}		
10	75347525	72.6	9.64×10^{-7}	9.68×10^{-7}	1.5
	75216132	74.5	9.90×10^{-7}		
	73965391	71.2	9.62×10^{-7}		
	73792267	71.9	9.74×10^{-7}		
	74307176	70.7	9.52×10^{-7}		

Calibration plots for ^{127}I and ^{129}I

In order to confirm the calibration linearity for both iodine isotopes, the different dilutions of NIST 3231 Level I were processed as calibration standards, and the calibration plots generated are shown in Figure 3. Excellent linearity was achieved for both isotopes. The BECs for ^{127}I and ^{129}I were $0.65 \mu\text{g/L}$ and 1.9 ng/L , while the detection limits (3σ , $n=10$) for ^{127}I and ^{129}I were $0.14 \mu\text{g/L}$ and 1.1 ng/L , respectively. The BEC and DL for ^{127}I were higher due to carryover from previous measurements.

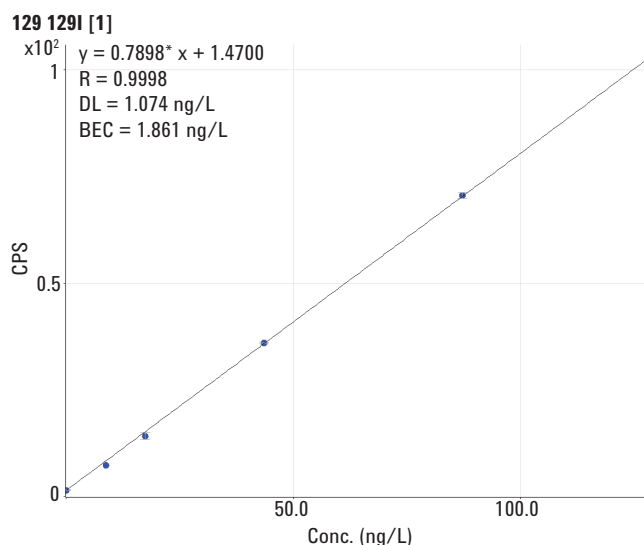
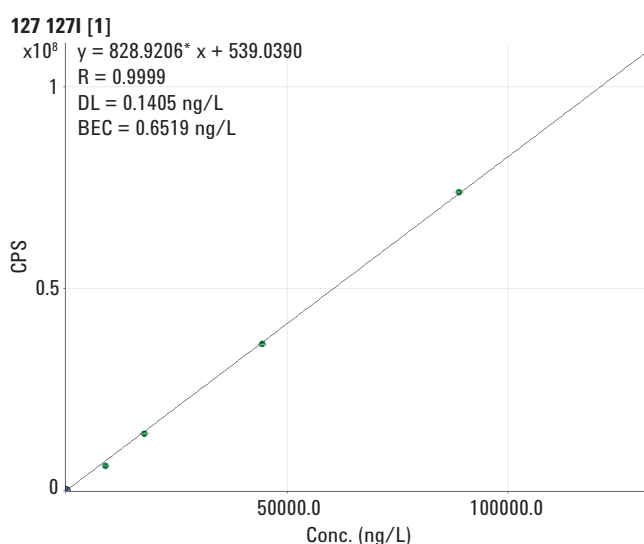


Figure 3. Calibration plots for ^{127}I (top) and ^{129}I (bottom) obtained from multiple dilutions of NIST 3231 Level I

Analysis of an 'intermediate' NIST 3231 standard and NIST 3231 level II

In order to validate the method, an 'intermediate' standard (nominal $^{129}\text{I}/^{127}\text{I}$ ratio 10^{-7}) was prepared by diluting NIST 3231 Level I 1:100 in a solution of potassium iodide (88.9 mg/L iodine). Measurement of the $^{129}\text{I}/^{127}\text{I}$ ratio in this intermediate standard gave good agreement with the target $^{129}\text{I}/^{127}\text{I}$ value of 1×10^{-7} (Table 3). NIST 3231 Level II ($^{129}\text{I}/^{127}\text{I} = 0.982 \times 10^{-8}$) was also measured and good agreement was again obtained (Table 4), though with higher RSD as the ^{129}I concentration approached the detection limit of the method.

Nevertheless, the fast, direct measurement at the 10^{-8} level for $^{129}\text{I}/^{127}\text{I}$ using standard liquid sample introduction and without sample preparation impressively demonstrates the capability of this method for rapid screening of samples for ^{129}I .

To confirm that overlap of ^{127}I on ^{129}I does not occur when ^{127}I is present at high concentration, spectra of NIST 3231 Level I, the prepared intermediate NIST 3231 standard, and a blank containing ^{127}I at the same concentration as in the standards (88.9 mg/L (ppm)) were acquired. The overlaid linear scale spectra are shown in Figure 4. Note there is no overlap of the ^{127}I high mass side peak tail on m/z 129. While some iodine hydride ($^{127}\text{I}^+\text{H}$) can be seen at m/z 128, there is no IH_2 observed at m/z 129, as had been proposed in a previous study [2].

Table 3. Measurement of the 'intermediate' NIST 3231 standard

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}\text{I}/^{127}\text{I}$	$^{129}\text{I}/^{127}\text{I}$ (average)	RSD (%)
I 88.9 mg/L added 1/100 diluted NIST 3231 (expected 10^{-7})	76548859	7.3	0.95×10^{-7}	0.99×10^{-7}	7.9
	76618521	8.3	1.08×10^{-7}		
	76523125	6.8	0.89×10^{-7}		
	76849756	8.1	1.05×10^{-7}		
	76052388	7.6	1.00×10^{-7}		

Table 4. Measurement NIST 3231 Level II

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}\text{I}/^{127}\text{I}$	$^{129}\text{I}/^{127}\text{I}$ (average)	RSD (%)
NIST 3231 Level II $^{129}\text{I}/^{127}\text{I} = 0.982 \times 10^{-8}$	536908532	4.0	0.74×10^{-8}	1.07×10^{-8}	22.4
	526648579	5.9	1.13×10^{-8}		
	518477906	7.3	1.41×10^{-8}		
	508547526	5.2	1.03×10^{-8}		
	503530493	5.3	1.05×10^{-8}		

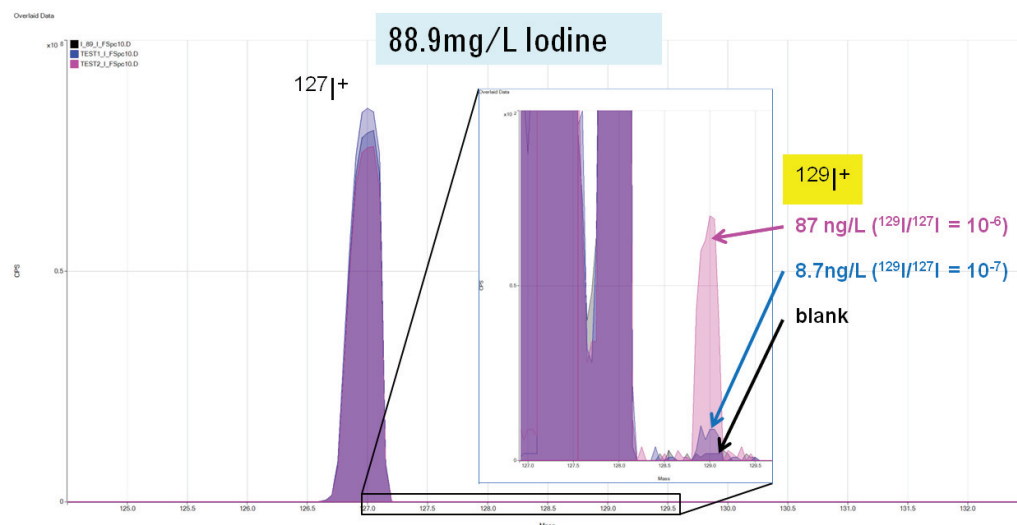


Figure 4. Overlaid spectra of 88.9 mg/L ^{127}I blank, NIST 3231 Level I (87 ng/L ^{129}I), and the intermediate standard (8.7 ng/L ^{129}I)

Conclusions

Improvements in the performance of ICP-MS for the direct measurement of ^{129}I in solution have been made possible by the use of an Agilent 7700x ICP-MS, featuring an ORS³ cell operated in oxygen reaction mode. The new design features of the ORS³ cell offer improved interference removal capability such that the Xe background due to the presence of Xe in the Ar plasma gas could be completely removed. A ^{129}I detection limit of 1.1 ppt was obtained and excellent abundance sensitivity prevented spectral overlap on ^{129}I from the presence of ppm levels of ^{127}I . NIST standards with different $^{129}\text{I}/^{127}\text{I}$ ratios and at multiple concentrations including the low level NIST 3231 Level II with a $^{129}\text{I}/^{127}\text{I}$ ratio of 10^{-8} were accurately measured without any sample preparation and with a standard liquid sample introduction system. The method is applicable to routine use and is capable of high sample throughput.

References

1. Enhanced Helium Mode Cell Performance for Improved Interference Removal in ICP-MS. Agilent publication, 5990-7573EN, February 2011, available from www.agilent.com/chem/icpms
2. Bienvenue et al, CJASS, Volume 49, No. 6, 423 (2004)

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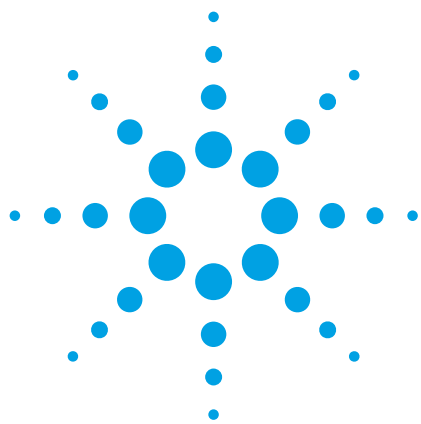
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Agilent Technologies



Trace elemental analysis of trichlorosilane by Agilent 7700s ICP-MS

Application note

Semiconductor analysis

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Abstract

Metallic impurities in trichlorosilane (TCS), an intermediate product used in the production of photovoltaic (PV) silicon, must be strictly controlled in order to produce the high purity PV silicon necessary for the manufacture of solar cells. A successful analytical method was developed, featuring measurement of impurities in TCS by an Agilent 7700s ICP-MS, following a sample preparation approach developed by Agilent. A spike recovery test demonstrated the validity of the method for 33 elements including boron and phosphorus, and two TCS samples were also analyzed. The ability to analyze TCS allows PV silicon manufacturers to check the TCS intermediate chemical for metallic impurities prior to the manufacture of PV silicon.



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Introduction

The search for alternative energy resources has intensified due to the exhaustion of natural fossil fuel resources and their contribution to global warming, together with associated geological, environmental, and political concerns. Among the many alternative methods of generating electricity, solar power or photovoltaic (PV) has experienced the highest annual growth since 2004. Photovoltaic panels, which are generally silicon wafer-based, convert energy from the sun into electricity, but the efficiency of this process is relatively low, so improving conversion efficiency is a key goal. The metallic impurity level in the polycrystalline silicon (polysilicon) used in the manufacture of the wafers that go into the PV panels must be strictly controlled, as impurities degrade the conversion efficiency. An effective method for the analysis of PV grade silicon by ICP-MS has already been developed by Agilent [1]. In an effort to further increase device efficiency, PV silicon manufacturers want to measure impurities in the chemicals used in the manufacture of polysilicon. This application note describes the application of the Agilent 7700s Octopole Reaction System (ORS) ICP-MS to the analysis of trichlorosilane, which is commonly used in the manufacture of ultrapure polysilicon.

Experimental

Instrumentation

A standard Agilent 7700s ICP-MS was used, with the addition of an Agilent Inert Sample Introduction kit consisting of a PFA concentric nebulizer, PFA double pass spray chamber and a demountable torch with 2.5 mm inner diameter platinum injector.

The 7700s has the widest range of available interference removal technologies of any ICP-MS instrument. In addition to conventional no gas mode, the 7700s can be operated in cool plasma mode and in ORS mode, which provides effective interference removal in both collision mode using an inert cell gas (He), and in reaction mode using a reactive cell gas (such as H₂). The 7700 Series ICP-MS features a third generation ORS cell (ORS³) that features a longer, smaller internal diameter octopole, operating at higher frequency than in previous ORS versions. The ORS³ can also be operated at higher cell

gas flow rates and with higher bias voltages, which promotes greater collision energy. In combination, these features give improved interference removal efficiency in both collision and reaction modes.

The interference removal approach is selected depending on the analytical requirement. For this application, ultimate sensitivity is required for all analytes and therefore the most effective interference removal mode is required for every analyte/interference. A data acquisition protocol featuring four steps was used, and all instrument operating parameters are given in Table 1. During method development, some analytes were run in multiple modes, and the mode that gave the best detection limits in the sample matrix was determined for each analyte.

Table 1. ICP-MS operating parameters

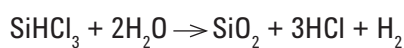
	Method step			
	Step 1	Step 2	Step 3	Step 4
Plasma	Cool	Normal		
RF power (W)	600	1600		
Sample uptake rate (μL/min)	~160 (free aspiration)			
Sampling depth (mm)	16	8		
Carrier gas flow rate (L/min)	0.7			
Makeup gas (L/min)	0.8	0.5		
He cell gas flow rate (mL/min)	0		5	2
KED (V)	13	3		135
Analytes:	Step 1	⁷ Li, ²³ Na ²⁴ Mg, ²⁷ Al, ³⁹ K, ⁴⁰ Ca, ⁵⁵ Mn, ⁵⁶ Fe, ⁶³ Cu, ⁷¹ Ga		
	Step 2	¹⁰ B, ¹³⁸ Ba, ¹⁷⁸ Hf, ¹⁸¹ Ta, ¹⁸² W		
	Step 3	⁴⁸ Ti, ⁵¹ V, ⁵² Cr, ⁵⁹ Co, ⁶⁰ Ni, ⁶⁴ Zn, ⁷⁵ As, ⁸⁸ Sr, ⁹⁰ Zr, ⁹³ Nb, ⁹⁸ Mo, ¹⁰⁷ Ag, ¹¹⁴ Cd, ¹¹⁸ Sn, ¹²¹ Sb, ²⁰⁵ Tl, ²⁰⁸ Pb, ²⁰⁹ Bi, ²³² Th, ²³⁸ U		
	Step 4	³¹ P		

The analytes measured in each step in the final method are also shown in Table 1. Step 1 employed cool plasma mode, and all remaining steps used normal or hot plasma. Step 2 used conventional no gas mode, Step 3 used He collision mode, and Step 4 was a modified He collision mode optimized for phosphorus determination. In the past, P has been measured indirectly as ³¹P¹⁶O at m/z 47, using cool plasma or O₂ cell gas. The improved performance of the ORS³ enables a greater reduction of the interferences ¹⁵N¹⁶O and ¹⁴N¹⁶O¹H that overlap P at

mass 31, lowering the detection limit of P in He collision mode by a factor of 50, and making direct measurement of P possible at the required levels. Switching between modes is fully automated and all analytes were measured with a single visit to the sample vial, which helps to minimize sample contamination. The small size of the ORS cell allows for very fast switching between modes, so the additional time required for multi mode operation is minimized. The total analysis time was 8 minutes per sample.

Sample preparation

Trichlorosilane (TCS) is an intermediate compound used in the manufacture of high purity polysilicon. Since TCS is a volatile liquid that can easily be purified by distillation, it can be made from low-grade metallurgical grade silicon, purified, and then converted to high purity polysilicon. TCS is liquid at room temperature with high volatility (BP 31.8 °C). It easily decomposes to SiO₂ in air by hydrolysis, as shown below:



The direct sampling and on-line ICP-MS analysis of TCS in the manufacturing line is not feasible, because SiO₂ would deposit in the transfer tube and on the ICP-MS sample introduction and interface components. In addition, TCS must be chilled and handled in an inert environment to avoid evolution of HCl gas, and so an ICP-MS inside a clean mini-environment would need to be installed at each sampling point. The only practical approach is therefore to transfer liquid TCS to the lab for analysis. In this work, liquid TCS was transferred to a clean laboratory following appropriate safety precautions, and analyzed after careful sample preparation using the following procedure: Liquid TCS was converted to SiO₂ via gentle hydrolysis in an inert gas atmosphere, dissolved in HF solution and Si removed (as SiF₄ gas) by heating to dryness. The dry residue was then re-dissolved in 0.4% HCl solution before analysis by ICP-MS. Caution! The sample preparation step must be performed with great care and all appropriate safety precautions followed*.

Calibration standards

External calibration in a matrix of 0.4% HCl was used for quantification of the trace elements. Since the Si matrix is removed during the sample preparation, the sample residue re-dissolved in 0.4% HCl, is essentially free of any matrix. Four multielement calibration standard solutions were prepared at 0, 1, 2 and 5 ppb in 0.4% HCl. No internal standardization was used, to avoid the possibility of adding contamination.

Results and discussion

Detection limits

Detection limits (DLs) were calculated from 3 sigma of the calibration blank and are shown in Table 2. Low ppt DLs for V and As demonstrate the efficient removal of the ClO (on ⁵¹V) and ArCl (on ⁷⁵As) polyatomic interferences in the HCl matrix. Also the phosphorus DL of 0.1 ppb shows the effectiveness of the optimized He collision mode parameters used in Step 4 for the removal of NO/NOH on P. DLs in the original sample were also calculated, by multiplying by a dilution factor of 7.5*, and all DLs were under 1 ppb in the original TCS sample.

Quantitative analysis

Table 2 also shows the results from the quantitative analysis of two TCS samples, after reagent blank subtraction. Sample A was a high purity TCS sample obtained from a semiconductor company and stored in a glass vial. Sample B was also obtained from a semiconductor company, but shipped in a stainless steel pressure vessel. As is clearly shown in Table 2, Sample B contained significantly higher levels of Fe, Ni and Cr than Sample A, indicating metallic contamination from the steel container. Sample A was found to be of high purity with only four elements above 1 ppb in the original sample.

*Agilent ICP-MS users requiring more detailed information on TCS sampling and analysis should contact their local Agilent ICP-MS applications team.

Table 2. Detection limits and quantitative analysis

m/z	Element	DL (final solution) (ppb)	DL (original sample) (ppb)	Analysis — Sample A (ppb)	Analysis — Sample B (ppb)
7	Li	0.0003	0.002	0.007	0.007
10	B	0.08	0.60	1.4	5.5
23	Na	0.002	0.01	0.53	15
24	Mg	0.001	0.010	2.5	1.4
27	Al	0.006	0.04	0.75	8.5
31	P	0.1	0.7	2.7	4.2
39	K	0.02	0.15	0.23	3.6
40	Ca	0.006	0.05	0.83	26
48	Ti	0.001	0.008	0.08	2.3
51	V	0.008	0.06	0.08	0.6
52	Cr	0.02	0.12	0.12	22
55	Mn	0.001	0.008	0.01	1.6
56	Fe	0.01	0.08	1.9	180
59	Co	0.0001	0.001	0.02	0.3
60	Ni	0.001	0.008	0.08	14
63	Cu	0.002	0.01	0.08	0.8
64	Zn	0.001	0.01	0.38	3.5
71	Ga	0.001	0.006	0.01	0.03
75	As	0.02	0.14	0.14	0.02
88	Sr	0.0001	0.0004	0.01	0.1
90	Zr	0.0002	0.001	0.08	1.0
93	Nb	0.0002	0.001	0.007	0.02
98	Mo	0.0003	0.002	0.08	1.3
107	Ag	0.004	0.03	0.03	0.02
111	Cd	0.0001	0.001	0.007	0.04
118	Sn	0.003	0.02	0.38	1.7
121	Sb	0.001	0.01	0.08	0.5
138	Ba	0.0002	0.002	0.007	1.4
181	Ta	0.0001	0.0004	0.007	0.2
182	W	0.0003	0.002	0.007	0.3
208	Pb	0.0002	0.002	0.007	0.8
232	Th	0.0001	0.001	0.007	0.007
238	U	0.0001	0.0002	0.007	0.007

Recovery test

In order to test the recovery efficiency of the method, and in particular to check for loss of volatile elements during the sample preparation procedure, a high purity sample of TCS was purchased (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). The TCS sample was divided into four aliquots, each containing 1.5 g of TCS, and sample preparation was performed as previously described. After hydrolysis and HF addition, but prior to the heating step to remove Si, one of the aliquots was spiked at the 5 ppb level with a multielement standard (Spex, Metuchen, NJ, USA). The samples were then evaporated to dryness and the dry residue was taken up in 0.4% HCl and analyzed. Figure 1 shows the recoveries achieved for the 5 ppb spiked sample. All elements gave good recoveries, including B, which can be problematic due to its volatility, demonstrating the validity of both the sample preparation and analytical methods.

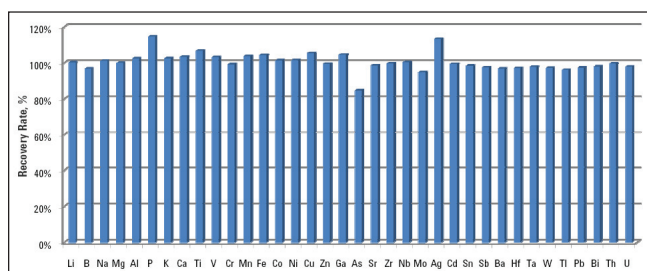


Figure 1. 5 ppb spike recovery test in TCS, confirming no loss of volatile elements during sample evaporation, and accurate recovery (between 80 and 120%) for all analytes.

Conclusions

Trichlorosilane has been successfully analyzed using the Agilent 7700s ICP-MS, following a sample preparation approach developed by Agilent. The ORS³ cell improves He collision cell performance significantly, achieving a DL of 0.1 ppb for phosphorus from a direct measurement at m/z 31. A spike recovery test demonstrated the validity of the sample preparation and analytical methods for all elements including boron. The ability to analyze TCS allows PV silicon manufacturers to check the TCS intermediate product for metallic impurities prior to the manufacture of PV silicon.

References

1. Ultratrace Analysis of Solar (Photovoltaic) Grade Bulk Silicon by ICP-MS. Agilent Application Note, 5989-9859EN, Oct 2008.

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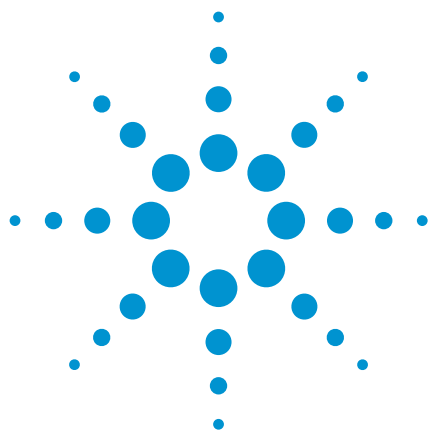
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Agilent Technologies



Identification of Oxidation Products of L-Ascorbic Acid by HPLC

Application Note

Author

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Introduction

Ascorbic acid is a water soluble sugar acid with antioxidant properties. The L-isomer of ascorbic acid is commonly known as vitamin C and is found naturally in fruits and vegetables. It is also added to fruit juices and other processed products as an antioxidant. Vitamin C is an essential nutrient in the human diet in the manufacture of collagen. In humans, absence of the vitamin leads to scurvy, a deficiency disease.

The acid has strong reducing power but when oxidized is converted to several compounds that do not have the same antiscorbutic or reducing properties. Given the importance of the vitamin in human health and its widespread use as an antioxidant in processed foods, study of its degradation products is merited. This note describes aspects of the rate of degradation of L-ascorbic acid and the nature of some of its degradation products using PLRP-S columns. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase with outstanding chemical and physical stability. The high surface area of the 100Å pore size enables retention of water soluble solutes.



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The aim of this study was to develop a rapid HPLC method for the quantitative and qualitative analysis of L-ascorbic acid.

Materials and Reagents

Reference samples: commercial L-ascorbic acid, oxalic acid, L-dehydroascorbic acid (DHAA) dimer.

Conditions

Columns: 2 x PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
 Eluent: 0.2 M NaH₂PO₄, pH 2.14
 Flow rate: 0.5 mL/min
 Inj Vol: 20 µL
 Detector: UV, 268 and 220 nm

Results and Discussion

Reference materials

The retention of reference compounds is shown in Figure 1. L-ascorbic acid was well resolved from its possible degradation products.

Bromine oxidation of L-ascorbic acid

HPLC of freshly prepared solutions of L-ascorbic acid treated with bromine produced the curves shown in Figure 2. Peak 1 is a high response at 220 nm, whereas peak 2 is a smaller response later identified as DHAA monomer with a free side chain. Peak height intensity was proportional to the amount of bromine added. Further addition of bromine was made to a cold solution of L-ascorbic acid (2 °C) to control degradation of DHAA. Homocysteine was then added and the reduction reaction went ahead at room temperature.

Effect of homocysteine on DHAA

The reaction of homocysteine with DHAA was examined to distinguish the DHAA peak from surrounding peaks in the commercial samples (Figure 3). *Inter alia*, L-ascorbic acid (peak 4a) increased very rapidly after reaction with homocysteine, and peaks 4 and 6 represent monomeric forms of DHAA.

The complete data set and analysis is available in Kennedy *et al.* (1989).

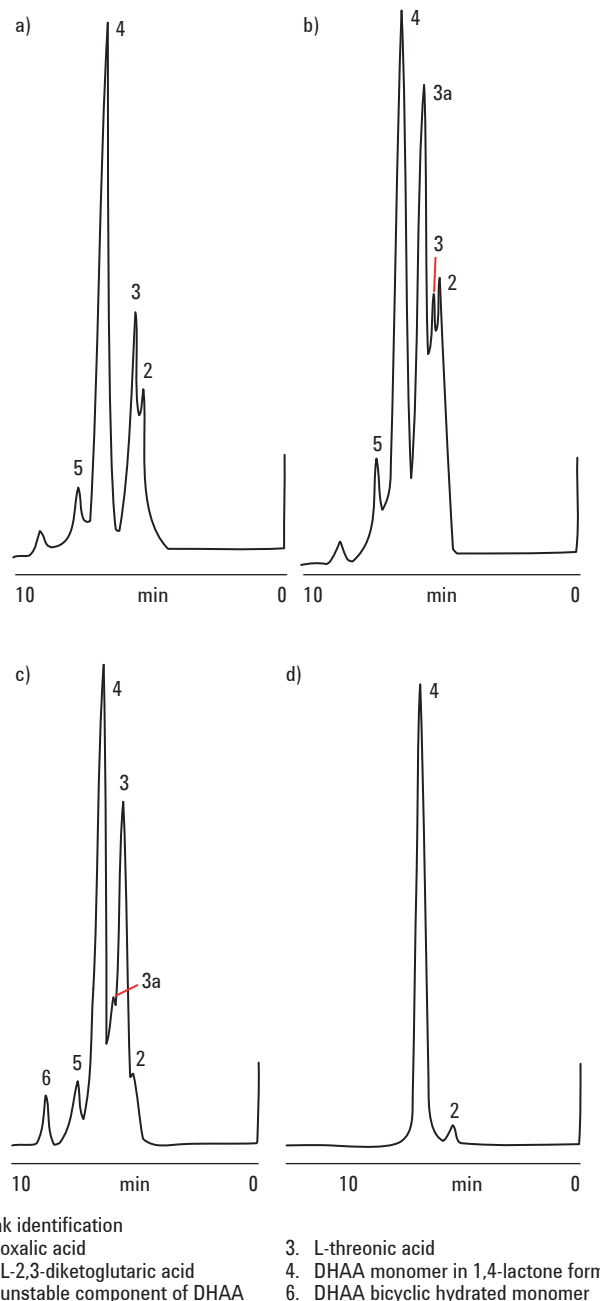


Figure 1. HPLC separation of a) L-dehydroascorbic acid solution spiked with b) potassium L-2,3-diketoglutaric acid and c) calcium threonate reference materials. d) is L-dehydroascorbic acid produced by Dietz's method, at 220 nm (0.2 AUFS).

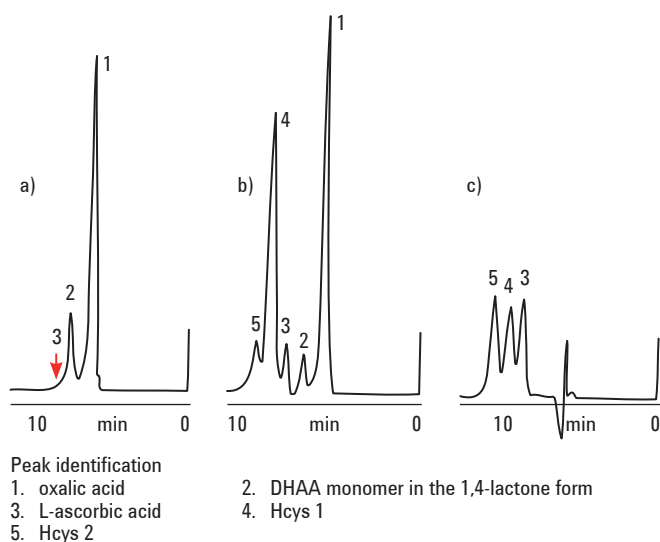


Figure 2. HPLC separation of a) bromine-oxidized L-ascorbic acid, at 220 nm (0.2 AUFS), and b) after addition of homocysteine (1:1), at 220 nm (0.1 AUFS) and c) at 268 nm (0.02 AUFS).

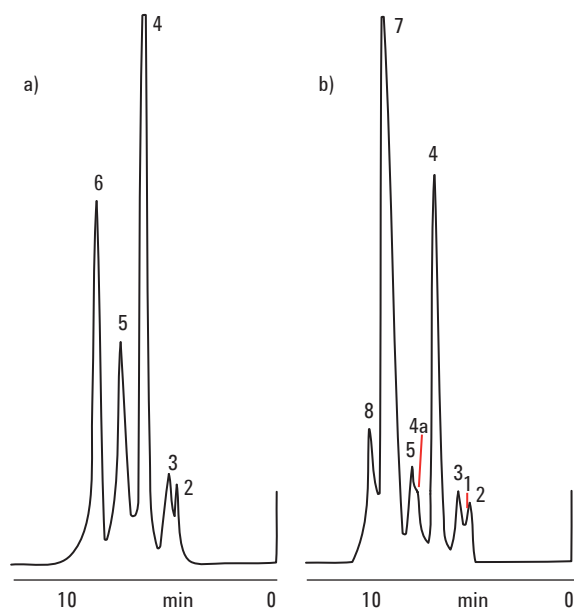


Figure 3. HPLC separation of L-dehydroascorbic acid solution a) before and b) after reduction reaction with homocysteine, at 220 nm (0.05 AUFS), using optimized conditions.

Conclusion

PLRP-S columns successfully revealed the identities of some degradation products of vitamin C in the development of a qualitative and quantitative HPLC method for the fast analysis of L-ascorbic acid.

Reference

Kennedy, JF, White, CA, Warner, FP, Lloyd, LL and Rivera, ZS (1989) The identification and analysis of the oxidation products of L-ascorbic acid by HPLC *C. J. Micronut. Anal.*, 5, 91-109.

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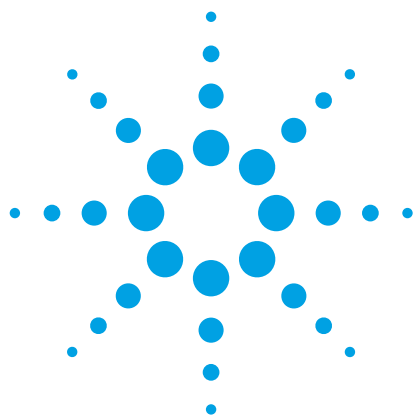
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5990-8217EN



Agilent Technologies



Fullerene Analysis using Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Fullerenes are normally prepared by evaporating graphite rods in arc reactors under a helium atmosphere to produce soot that contains C_{60} , C_{70} , higher fullerenes, and uncharacterized insoluble material. The soluble fractions may be extracted using a suitable solvent and further purification may be carried out by gel permeation chromatography (GPC). Low pore size Agilent PLgel 50Å GPC columns are most appropriate for these applications. As an analytical tool, high efficiency columns can be used to screen fullerene compounds. The behavior of preparative separations can be predicted by running an analytical separation using the same column packing.

Analysis of Fullerene

One of the difficulties in fullerene chromatography is the relatively poor solubility of the compounds. However, toluene exhibits favorable properties as a GPC solvent since it provides reasonable solubility (6 to 8 mg/mL), good chromatographic selectivity, and is amenable to solvent removal and recovery by distillation in preparative separations.

Figure 1 illustrates the separation achieved for a material whose basic structure was believed to be that indicated in Figure 2.



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Conditions for Figure 1

Columns	2 × Agilent PLgel 5 μm 50Å, 7.5 × 300 mm (p/n PL1110-6515)
Eluent	Toluene
Flow rate	1.0 mL/min
Loading	2 mg/mL, 200 μL
Detector	RI
System	Agilent PL-GPC 50

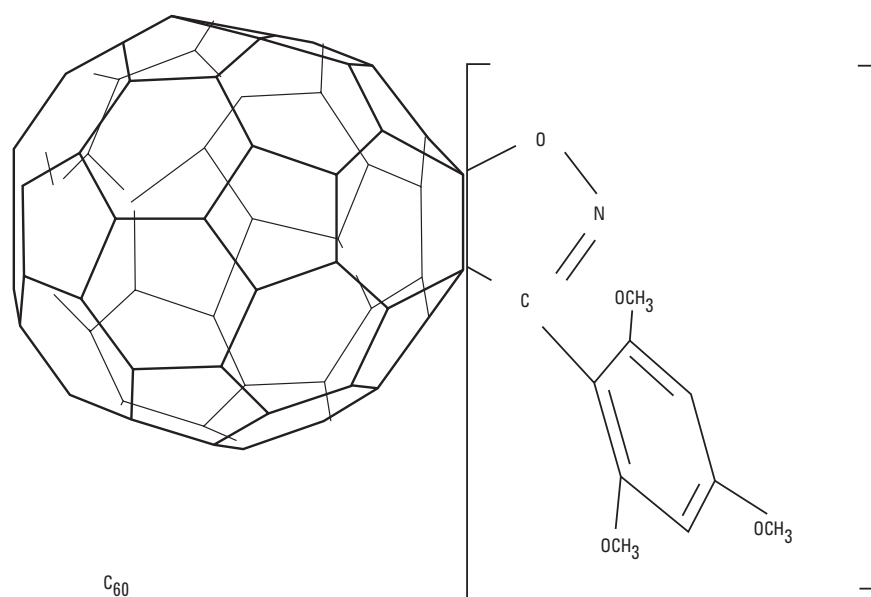


Figure 1. Separation of a fullerene on an Agilent PLgel two-column set.

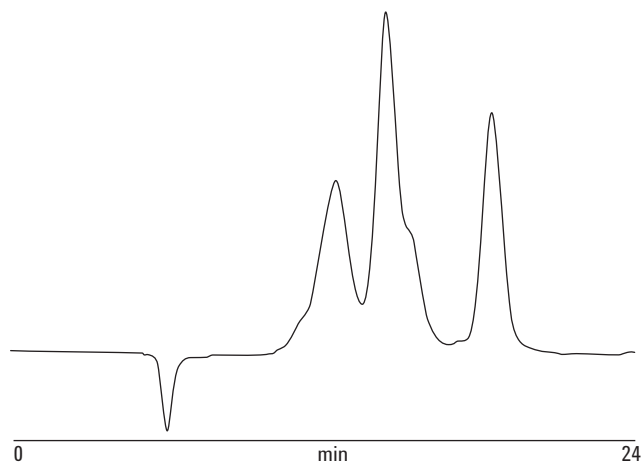


Figure 2. Suspected structure of the fullerene under investigation.

In preparative work, columns packed with larger particle size packings are preferred for increased loading. Preparative column separations can easily be predicted by running samples on analytical columns packed with the same type of beads. Figure 3 illustrates this approach; if this separation was scaled up to a preparative system it would require 25-mm id columns with a subsequent tenfold increase in flow rate and loading.

Conditions for Figure 3

Columns	4 × Agilent PLgel 10 μ m 50Å, 7.5 × 300 mm (p/n PL1110-6115)
Eluent	Toluene
Flow rate	1.0 mL/min
Loading	2 mg/mL, 200 μ L
Detector	RI
System	PL-GPC 50

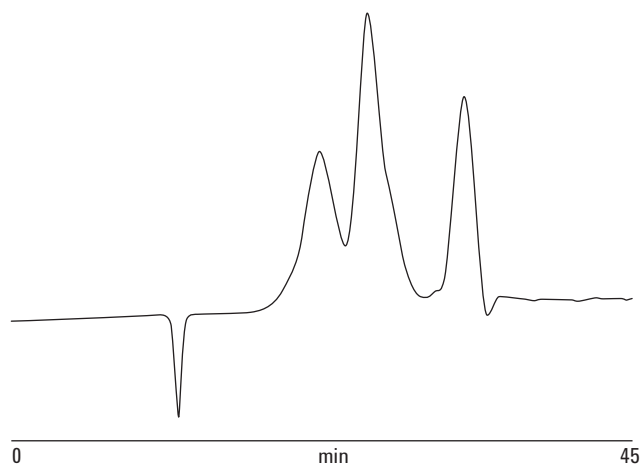


Figure 3. Separation of fullerene using an Agilent PLgel 10 μ m analytical packing to predict a preparative scale separation using the same packing.

Conclusion

Soluble fullerene fractions extracted using a suitable solvent can be further purified by gel permeation chromatography using low pore size Agilent PLgel 5 μm columns. The behavior of preparative separations can be predicted by first running an analytical separation with larger pore sized Agilent PLgel 10 μm columns, using the same column packing.

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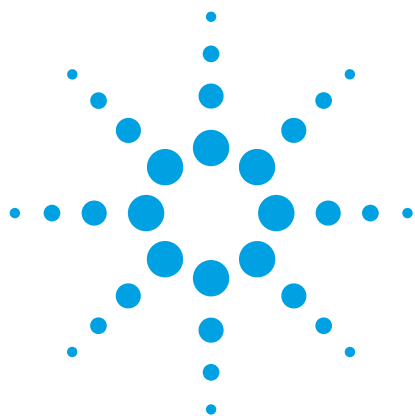
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Agilent Technologies



Permanent Gas Analysis – Separation of Helium, Neon and Hydrogen a MolSieve 5A column using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Author

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Middelburg
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Introduction

This application note shows an example of the permanent gas analysis in a sample with high % level of Oxygen and Nitrogen (Air) on an Agilent 490 Micro GC, including the separation of Helium, Neon, and Hydrogen (ppm level). The separation of these compounds is done on a 10 m MolSieve 5A column and requires the use of Argon as carrier gas to detect all potential other carrier gases like Helium, Hydrogen, and Nitrogen.

The advantage of the Agilent 490 Micro GC, is the ease-of-use and the speed of analysis, resulting in a total analysis time of less than 40 seconds.

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 chromatography.



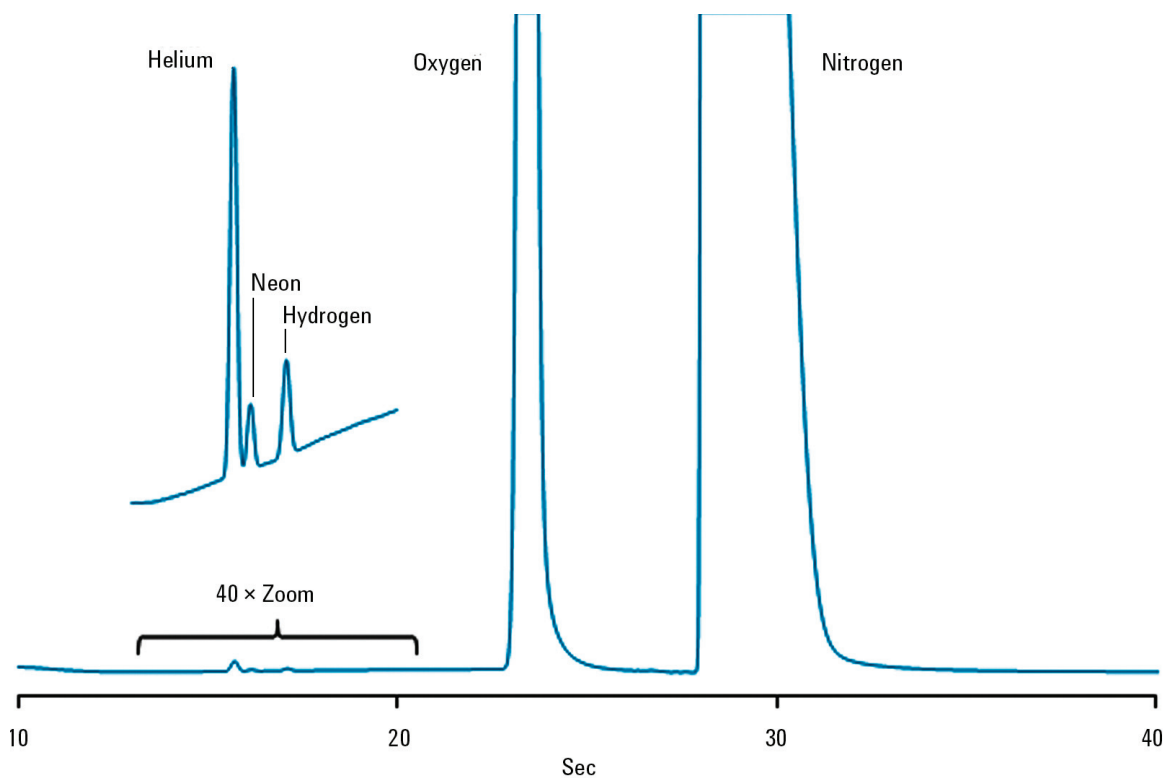
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Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	MolSieve 5A, 10 m
Column temperature	80 °C
Carrier gas	Argon, 240 kPa
Injector temperature	60 °C
Injection time	60 msec

Sample information

Helium	ppm level
Neon	ppm level
Hydrogen	ppm level
Oxygen	high % level
Nitrogen	high % level



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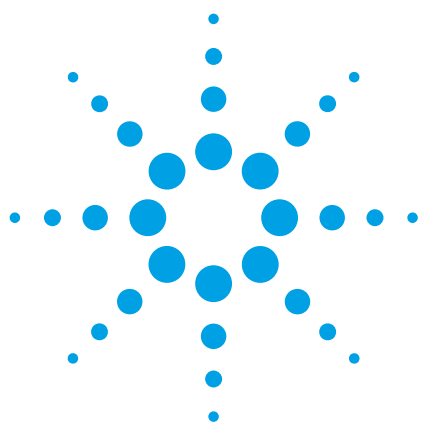
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Agilent Technologies



New methodology for determination of gold and precious metals using the Agilent 4100 MP-AES

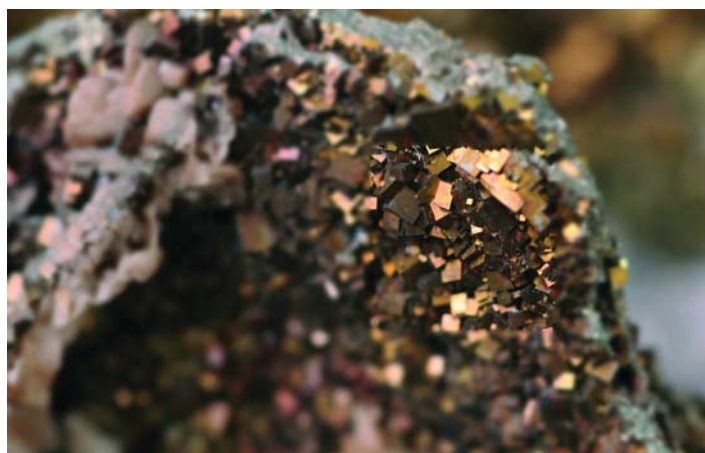
Application note

Geochemistry, metals and mining

Author

Craig Taylor

Agilent Technologies
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Introduction

The accurate and precise determination of gold and other precious metals (PMs) is a vital task for companies involved in PM production and the contract or service laboratories that support them. This might involve the processing of hundreds of samples typical of exploration and mining activities, or supplying the quality control assay of the final product from PM producers or recyclers. PMs are also produced as a by-product of copper or nickel processing.

Today's analysts have access to a range of elemental analytical techniques including flame and graphite furnace atomic absorption spectroscopy (AA), inductively coupled plasma optical emission spectroscopy (ICP-OES) and ICP mass spectroscopy (ICP-MS). The choice of method will depend on a number of factors, for example, the technology that is available, the skill of the operator, sample type, cost of analysis, sample throughput requirements and the objective of the analytical result.



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Agilent has identified an increased need for multi-element determinations over a wide dynamic range at an attractive overall cost of analysis, taking into account instrument supplies, consumables, power and labor, and has developed a new instrument to meet this need.

The Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (Figure 1) is a low-cost, highly automated technique that is suitable for the trace analysis of PMs in samples typically found in mining and exploration activities. As MP-AES relies on the generation of a microwave plasma using nitrogen, no flammable gases such as acetylene are required. This reduces running costs and improves lab safety. Nitrogen can be supplied from bottled gas or the Agilent 4107 Nitrogen Generator. This alleviates the difficulty and costs in sourcing gases such as acetylene, especially in remote locations.



Figure 1. Agilent 4100 MP-AES

This application note describes the analysis of PM samples prepared by fire assay using the Agilent 4100 MP-AES.

Experimental

Instrumentation

The Agilent 4100 MP-AES is a fast sequential multi-element analytical technique that has a microwave-induced nitrogen plasma at its heart. As a result running costs are significantly reduced as only nitrogen is required for plasma operation.

The 4100 MP-AES uses Agilent's unique Microwave Excitation Assembly to create a concentrated axial magnetic field around a conventional torch. This focuses and contains the microwave energy where it is needed to produce a toroidal plasma with a cooler central channel that is suitable for stable introduction of liquid samples using a conventional sample introduction system.

Samples and sample preparation

A series of samples that are normally analyzed by flame AA were prepared using fire assay. A 30 g rock sample was heated with flux to over 1,000 °C. The process yields a small silver sphere, which was then dissolved in 4 mL of 25% aqua regia. The 4100 MP-AES operating parameters were then optimized, as shown in Table 1.

Table 1. Agilent 4100 MP-AES operating parameters

Instrument parameter	Setting
Analytes (wavelength)	Au (267.595), Pt (265.945), Pd (363.470)
Nebulizer pressure	140–240 kPa
Read time	3 s
No. of replicates	3
Sample uptake delay	10 s
Stabilization time	5 s
Background correction	Auto

Method detection limits

Method Detection Limits (MDLs) for gold, platinum and palladium were determined by measuring two sets of ten method blanks twice, on non-consecutive days, using the conditions as defined in the analytical method. The MDL was calculated as the 3 sigma standard deviation of the twenty concentration results.

The MDLs listed in Table 2 are sufficiently low for this type of analysis.

Table 2. MDLs for Au, Pt and Pd in fire assay samples

Analyte	Wavelength (nm)	MDL (µg/L)
Au	267.595	4
Pt	265.945	13
Pd	363.470	0.7

Linear range

The concentration or working range of an analytical technique is the range of concentrations that can be measured accurately without the need to recalibrate or dilute the sample. The linear range for Au, Pt and Pd was investigated using the 4100 MP-AES. A series of standards was prepared at concentrations of 2, 7, 70, 90, 100, 110 and 120 mg/L, and analyzed using the 4100 MP-AES. The calibration graphs obtained are shown in Figures 2, 3 and 4. These show that the linearity for all three analytes was acceptable up to a concentration of 120 mg/L, which exceeds the requirements of the application.

Sample volume

The analysis of PMs is volume-sensitive. The typical total sample volume available for analysis is around 4 mL. By using an Agilent SPS 3 Sample Preparation System connected to the 4100 MP-AES, the method cycle time (sample to sample) was 55 s and the sample volume consumed during analysis was 1.8 mL.

Accuracy

In order to test the ability of the 4100 MP-AES to analyze PMs at variable concentrations, a batch of Certified Reference Materials (CRMs) was analyzed.

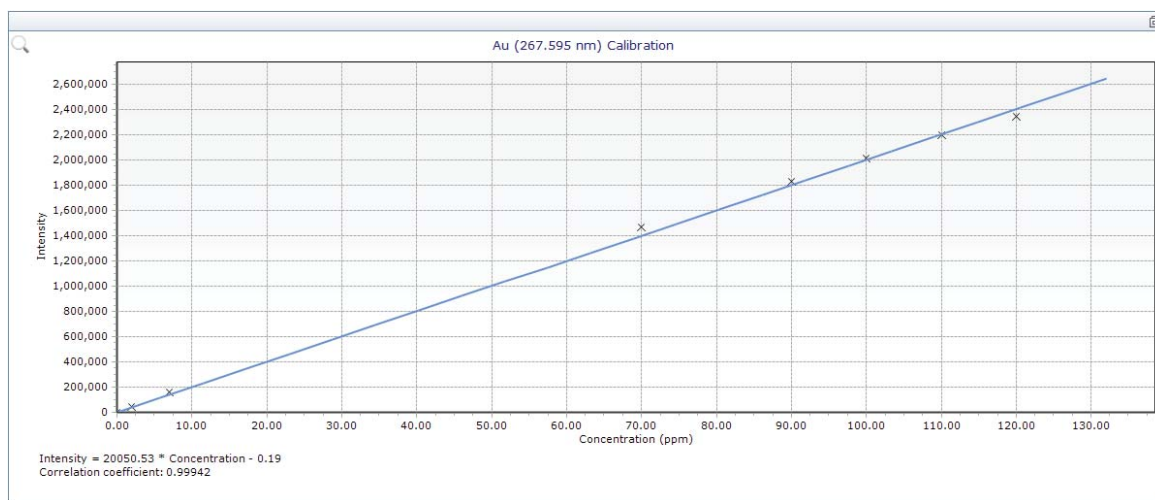


Figure 2 Calibration graph for Au at the 267.595 nm wavelength

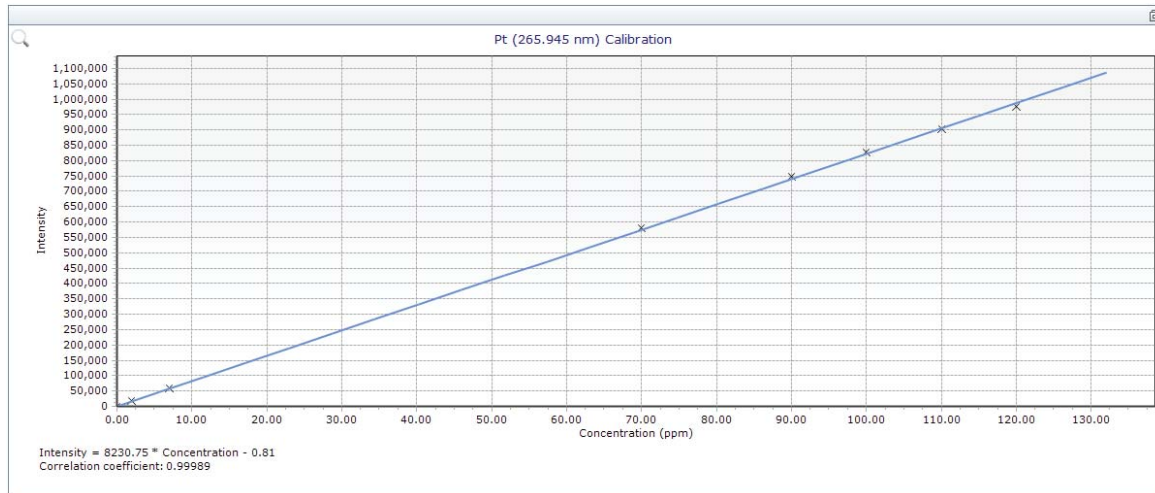


Figure 3. Calibration graph for Pt at the 265.945 nm wavelength

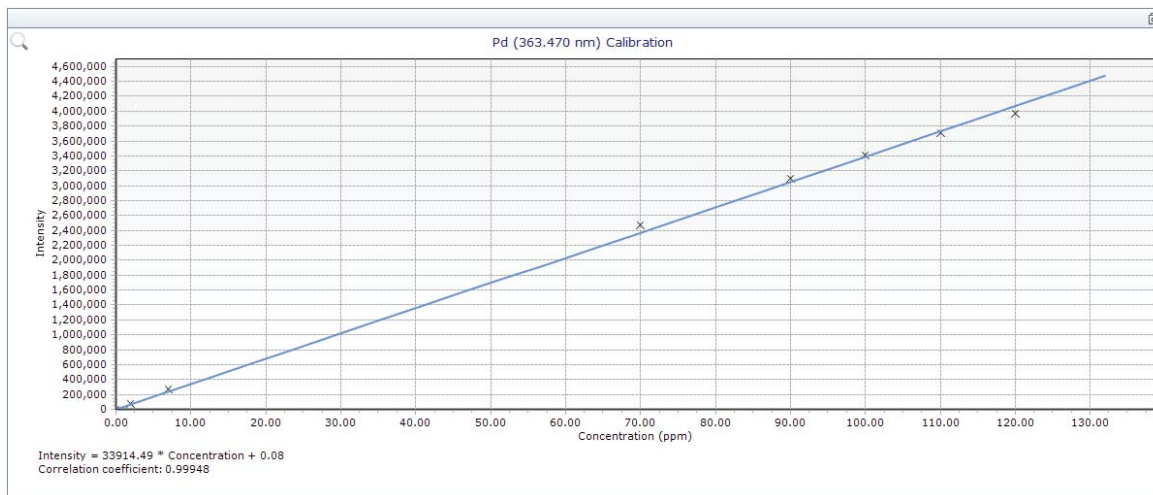


Figure 4. Calibration graph for Pd at the 363.469 nm wavelength

These were custom CRMs that had been professionally prepared from solid ore samples and certified through a round robin test process. They are not commercially available. The results listed in Table 4 show excellent agreement (accuracy) between the 4100 MP-AES measured results and the certified values.

Table 4. Results for Au, Pt and Pd obtained using the 4100 MP-AES compared to certified reference values

Gold	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 1	19.8	19.3
CRM 2	7.9	7.4
CRM 3	23.1	22.7
CRM 4	5.6	5.7
CRM 5	57.8	55.3
CRM 6	3.1	3.3
CRM 7	35.9	35.4
CRM 8	8.4	8.9
Platinum	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 6	0.74	0.75
CRM 7	35.6	35.9
CRM 8	9.0	9.5
Palladium	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 6	3.21	3.4
CRM 7	44.4	44.0
CRM 8	35.0	36.5

Recovery of unknown samples

A batch of unknown samples was analyzed for gold content using the 4100 MP-AES, and the results compared with the data obtained from analysis using conventional flame AA. The comparison can be seen in Table 5.

A typical spectrum of a sample containing approximately 40 ppm of gold can be seen in Figure 5. This demonstrates excellent signal to noise ratio with the flat baseline and the narrow emission peak confirming there are no spectral interferences.

Table 5. Results for gold in unknown samples, comparing the 4100 MP-AES with flame AA

Sample	MP-AES result (mg/L)	Flame AA result (mg/L)	Agreement with AA result (%)
1	0.09	0.09	100
2	0.85	0.84	101
3	5.3	5.1	104
4	13.7	14.4	95
5	20.8	21.8	95
6	4.3	4.1	105
7	1.0	1.0	100

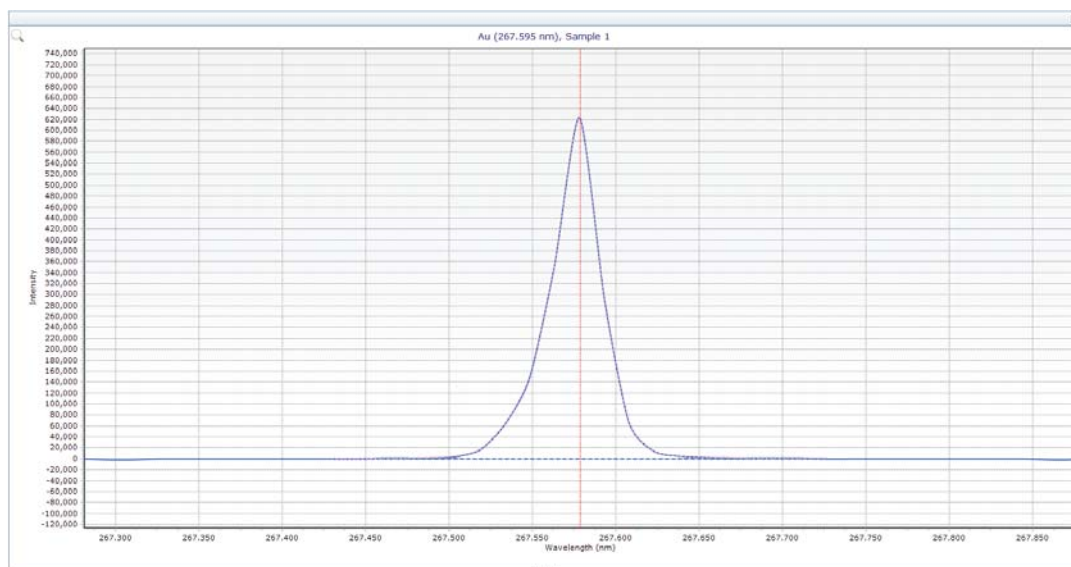


Figure 5. A typical PM sample spectrum for Au at the 267.595 nm wavelength

Conclusions

Following a thorough evaluation of the performance of the Agilent 4100 MP-AES, it is apparent that the 4100 MP-AES offers many advantages for the analysis of gold and other precious metals compared to conventional analysis techniques such as flame AA. The 4100 MP-AES offers superior sensitivity, an increased linear dynamic range and improved speed of analysis. In addition, the 4100 MP-AES more than doubles the measurement speed of conventional AA systems. It also offers exceptional accuracy as demonstrated by the excellent agreement with the certified values for several CRMs and real samples. Furthermore, the 4100 MP-AES has the lowest running costs of all of today's atomic spectroscopy techniques, due to reduced gas costs. Its use of non-combustible nitrogen also ensures that, unlike flame AA, the 4100 MP-AES can provide safer, multi-element unattended overnight operation.

The 4100 MP-AES also offers the option of installation at remote locations. This enables laboratories to analyze samples at the source rather than shipping the samples to a central laboratory for analysis, as is the current practice. In other instances where remote analysis is already performed using flame AA, the MP-AES provides the user with capability to analyze the samples in a safer environment, without the need for flammable gases such as acetylene.

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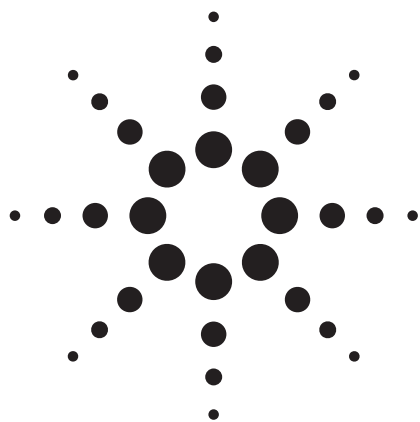
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Agilent Technologies



The Analysis of Cement

Application Note

Atomic Absorption

Author

P. E. Thomas

Introduction

The atomic absorption technique may be used for the determination of a wide range of elements in various samples of cement.

Several methods can be used for the preparation of sample solutions, which are suitable for analysis, by atomic absorption spectrophotometry.

When silicon has to be determined the cement samples can be fused with an alkali metal salt, but a hydrochloric acid leach is adequate when the silicon content is not required.

Both of the above procedures for obtaining cement sample solutions have been tested and are employed at Agilent Technologies, Inc.



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Experimental

Fusion Technique

Fuse 0.5 g of the sample of cement with 2 g of either NaOH or Na_2O_2 in either a platinum or a zirconium crucible over a Meker burner.

If a platinum crucible is used do not raise the temperature above a dull red heat or prolong the fusion unnecessarily.

Cool the melt and dissolve the fused cake in 100 mL of warm distilled water and acidify by the addition of 15 mL of conc. HCl, and finally add 5 mL of a 3% H_2O_2 solution. Do not boil the solution.

Transfer the solution quantitatively to a 250 mL volumetric flask and make up to volume with distilled water.

This sample solution is suitable for the determination of the following elements:

Silicon, Aluminium, Magnesium and Calcium.

Notes on the Individual Determinations

Silicon

In the determination of silicon it is necessary to match standards with samples by the addition of both sodium and aluminium, since both of these metals enhance silicon absorption additively by approximately 10%. The standards should also contain an equivalent amount of HCl to that used in the sample preparation.

It should be noted that silica does not precipitate readily from acid solutions, provided that the normality does not exceed 3N and provided that the acidic solution is not boiled.

The atomic absorption measurements are made at the 2516.1 Å resonance line, using a fuel-rich (reducing) $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Aluminium

In the determination of aluminium the standards are matched with sample solutions for sodium, calcium and silicon. Both sodium and calcium enhance aluminium absorption, while silicon depresses it.

The atomic absorption measurements are made at the 3092.7 Å, 3092.8 Å doublet, using a $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Magnesium

In the determination of magnesium the standards are matched with sample solutions with respect to their sodium content, since sodium acts as an ionization suppressant and hence its presence overcomes the slight ionization interference. If an air-acetylene flame is used the standards are also matched for aluminium and silicon content.

The atomic absorption measurements are made at the 30 times less sensitive 2025 Å resonance line rather than at the more sensitive 2852.1 Å line to avoid time-consuming dilutions, and in this case it is recommended that the lamp is operated at 8 mA.

Generally, the air- C_2H_2 flame is used in magnesium determinations, although the $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame is especially recommended if aluminium and/or silicon is present. However, in these types of matrices, the N_2O -propane flame (~2400 °C) also appears to be quite satisfactory, from the point of view of ionization and interferences.

Calcium

As with magnesium, so with calcium, the standards are matched with the sample solutions with respect to their sodium content. If an air-acetylene flame is used they should also be matched for aluminium and silicon content.

The atomic absorption measurements are made at the 2398.6 Å resonance line, rather than at the 200 times more sensitive 4226.7 Å line, using a $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame. It is recommended that the lamp should be operated at 10 mA since the 2398.6 Å line is weak in signal intensity.

Acid Leaching Technique

Digest in a tall beaker 2 g of a finely divided cement sample with 10 mL of 1:1 v/v HCl at just below the boiling point for 5–10 minutes. Transfer the contents of the beaker quantitatively to a 100-mL volumetric flask and make up to volume with distilled water.

Allow the sediment to settle and sample the supernatant liquid, or either filter or centrifuge the solution prior to making measurements upon the solution.

The following metals may be determined in this solution:

Iron, Sodium and Potassium (as well as Nickel or Chromium if desired).

Notes on the Individual Determinations

Iron

The atomic absorption measurements are carried out on a diluted solution (~10 times) at the 3719.9 Å line, rather than the 9 times more sensitive 2483.3 Å line, using an air-C₂H₂ flame.

The sample solution obtained from the fusion procedure could also be used. However, it is then necessary to match standards and samples for both sodium and HCl content.

Sodium

The atomic absorption measurements are made on a diluted solution (~10 times) at the 5890 Å resonance line, using the air-C₂H₂ flame and the burner rotated at 90 degrees to the optical path in order to reduce the analytical sensitivity by a factor of about fifteen.

Dilution may be avoided by the use of the much less sensitive (550 times) 3302.3 Å, 3303.0 Å doublet without burner rotation. In either case the cooler air-propane flame may also be used.

Potassium

The atomic absorption measurements are made on a diluted solution (~10 times) at the 7664.9 Å resonance line, using an air-C₂H₂ flame together with a very small rotation of the burner. Rotation may be avoided by using an air-acetylene flame burning at the 5 cm nitrous oxide-acetylene burner. This is quite safe.

Some Typical Results

Table 1. Typical Results

Component	Sample A		Sample B	
	Supplied analysis%	A. A. S.%	Supplied analysis%	A. A. S.%
SiO ₂	24.17	24.20	21.03	20.59
Al ₂ O ₃	3.3	3.10	5.38	5.02
Fe ₂ O ₃	3.07	3.05	2.07	2.11
CaO	64.43	65.5	66.6	66.8
MgO	1.39	1.37	1.12	1.12
Na ₂ O	0.2	0.17	0.08	0.07
K ₂ O	0.32	0.32	0.26	0.26

Conclusion

An estimated time of analysis for all seven elements in two cement samples, in a routine laboratory, is approximately 2½ hours from the time of receipt of the samples. The efficiency can be greatly increased by carrying out the atomic absorption measurements on a larger number of samples.

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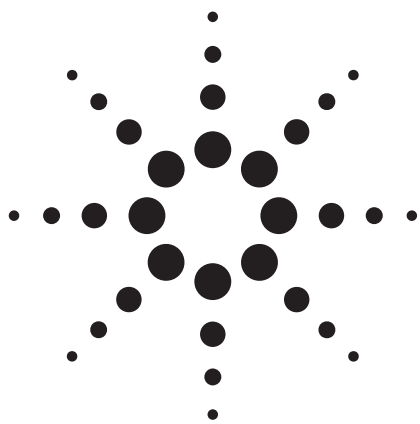
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Agilent Technologies



The Determination of Selenium in Steels

Application Note

Atomic Absorption

Authors

B. J. Lowings

S. Skujins

Introduction

The determination of selenium by atomic absorption spectroscopy [1,2] and also by atomic fluorescence spectroscopy [3] has been reported by several workers. Improvements in detection limits have been achieved both with the use of an argon-hydrogen-entrained air flame [4], as well as with a nitrogen separated air-acetylene flame [5].

The relatively poor sensitivity and detection limit for selenium together with the large chemical interference from iron and acids make the accurate determination of the very low concentrations of selenium, found in steels, very difficult.

In the procedure described below interferences are largely overcome by carrying out a separation of the selenium from steel, as well as maintaining a closer control over the final acidity of the resultant sample solutions.

Experimental

Instrumental

Standard selenium solutions are prepared, each containing 15% vol./vol. HClO_4 (S.G. 1.54).

The atomic absorption measurements are made at the selenium 1960.3 Å resonance line using a spectral band pass of 6.6 Å and the hollow cathode lamp operating at 8 mA. An argon-hydrogen entrained-air flame together with scale expansion are employed. Other flames may also be employed, for example, nitrogen-hydrogen entrained-air, air-hydrogen, and air-acetylene flames.



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Samples

Approximately 2 g of the steel sample are weighed out accurately, transferred to a 250-mL conical flask and dissolved in 30 mL of a 2 vol. HClO_4 (S. G. 1.54) - 2 vol. HNO_3 (S. G. 1.42) - 1 vol. H_3PO_4 (S. G. 1.75) acid mixture.

This mixture is gently heated until all of the sample has dissolved and is then evaporated to fumes.

The solution is allowed to fume gently for 2 minutes, cooled and 20 mL of HCl (S. G. 1.16) are added and then boiled for 5 minutes.

The solution is cooled once more to 20 °C and 10 mL of a 35% SnCl_2 solution are added and the whole mixture is allowed to stand for 15 minutes.

Note: The stannous chloride solution is prepared by dissolving 35 g of SnCl_2 in 50 mL HCl (S.G. 1.16) with beating, followed by cooling and dilution to 100 mL with distilled water. The addition of SnCl_2 reduces the selenium to the metallic state thus enabling its separation from iron to take place.

The solution is filtered on a tight pulp pad and the precipitate is washed with 50% HCl until it is free from iron.

The precipitate and the pad are transferred to a beaker, 20 mL of HNO_3 and 15 mL of HClO_4 are added and the whole evaporated to fumes in order to destroy the organic matter. The solution is then cooled and diluted to exactly 100 mL with distilled water.

2 g of high purity iron are treated in exactly the same manner in order to obtain a selenium free blank solution.

Typical Results

Component		% Selenium	
		By atomic absorption	By colorimetric method
MAC 89 Steel	(a)	0.018	0.013
	(b)	0.0175	0.013
MAC 103 Steel	(a)	0.0385	0.039
	(b)	0.0390	0.039

Conclusion

For the MAC 103 steel the atomic absorption results are in excellent agreement with the colorimetric method results.

The high results obtained for MAC 89 steel may be due to chemical interference from tellurium (0.018% Te in MAC 89). Chakrabarti 2 has shown that 160 ppm Te cause the absorbance of a 1 ppm Se solution to be enhanced by about 15%. Thus, in order to compensate for this enhancement both the sample and standard solution should contain approximately equal amounts of tellurium.

This method for the determination of selenium in steels is readily adaptable to the determination of tellurium in steels, using the 2142.8 Å tellurium resonance line.

References

- 1 W. Slavin, *Atomic Absorption Spectroscopy*, Interscience Publ. 1968, 156–7
- 2 C. L. Chakrabarti, *Analytica Chimica Acta*, 1968, 42, 379–387.
- 3 M. S. Cresser and T. S. West, *Spectroscopy Letters*, 1969, 2(1), 9-12.
- 4 H. L. Kahn, J. E. Schallis, *Atomic Absorption Newsletter*, 1968, 7, 5–9.
- 5 G. K. Kirkbright, M. Sargent and T. S. West, *Atomic Absorption Newsletter*, 1969, 8(2), 34– 37.

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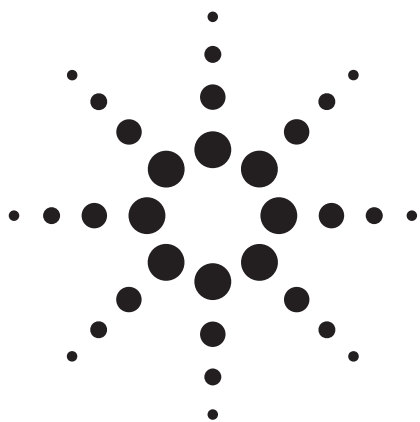
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Agilent Technologies



The Analysis of Iron Ores

Application Note

Atomic Absorption

Authors

J. B. Sanders
P. E. Thomas

Introduction

The atomic absorption technique is ideally suited for the rapid analysis of a wide range of elements in ores. A description is given of the methods employed at Agilent Technologies in the analysis of iron ore samples for fifteen different elements.



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Experimental

Fe, Al and Si Sample Solutions

Fuse 0.5 g of the finely ground sample of iron ore with 2 g of NaOH pellets in a zirconium crucible.

Cool the melt and dissolve it in a solution of 100 mL of distilled water plus 2 mL of 10 vol. H_2O_2 and 9.5 mL of conc. HNO_3 . Boil until a clear solution is obtained and then cool.

Transfer the solution quantitatively into a 200 mL volumetric flask and make up to volume with distilled water.

Aluminium and silicon can be determined directly on this solution.

For the determination of iron the above solution must be diluted 50-fold.

Notes on the Individual Determinations

Aluminium

No serious interferences occur provided that the standard and the sample solutions are matched with respect to their Na and Fe content.

Standard solutions are prepared to cover the range 0–150 ppm Al, for example, for Al concentrations of less than 6% in the original ore sample.

The atomic absorption measurements are made at the 3092.7, 3092.8 Å doublet, using the $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Silicon

Standard solutions are prepared to cover the range 0–100 ppm Si, containing similar concentrations of Na and Fe as are found in the sample solution.

A standard Si stock solution containing 1000 ppm Si may be prepared by fusing 1.069 g of ignited SiO_2 with 2 g of NaOH, dissolving and making up to 500 mL with distilled water.

It should be noted that silica does not precipitate readily from acid solutions, provided that the normality does not exceed 3N and provided that the acidic solution is not boiled.

The atomic absorption measurements are made at the 2516.1 Å resonance line, using a slightly fuel-rich (reducing) $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Iron

The use of the less sensitive (9 times) 3719.9 Å line is recommended because of the high concentration of iron in solution and because of its much higher intensity than the more commonly used 2483.3 Å line, resulting in a better signal to noise ratio and thus improved precision.

Care should be taken to ensure, when any free acids are present (particularly HCl and HClO_4), that a fuel-lean flame is used in order to overcome the suppressing effect of these acids. It is advisable to match the standard and sample solutions with respect to their acid content.

Trace Metals

The relatively low concentrations of other elements generally found in iron ores necessitate the use of sample solutions which contain approximately 2 g of ore per 25 mL of solution. At such high concentrations (~8%) severe blockage of both the nebulizer and the burner can occur. In order to overcome this problem the bulk of the iron is first removed by extraction into iso-butyl acetate.

Extraction Procedure

2 g of finely ground ore are digested with 25 mL of conc. HCl. The mixture is evaporated to dryness and the residue is redissolved in a minimum amount of 50% v/v HCl.

This acidic solution is filtered and the residue (A) is washed with minimum amounts of hot 20% v/v HCl and distilled water.

Treatment of Filtrate (Plus Washings)

The filtrate is evaporated down to a paste and redissolved in 20 mL of conc. HCl.

This solution is oxidized by the dropwise addition of conc. HNO_3 . After cooling it is washed into a 100 mL separating funnel with conc. HCl, giving a final total volume of 40 mL.

50 mL of iso-butyl acetate are added and the mixture is shaken for 30 seconds. The lower aqueous layer is run into a second separating funnel.

The residual organic layer is washed with 5 mL of conc. HCl and the washings added to the second funnel.

The above extraction procedure, using 30 mL of iso-butyl acetate followed by a 5 mL conc. HCl wash, is repeated on the aqueous acid solution in the second separating funnel.

The combined aqueous layer and washings are evaporated almost to dryness and the paste is redissolved in 5 mL of 50% v/v HNO_3 (Solution B).

- **Treatment of Residue (A)**

The residue (A) is ignited and then treated with a mixture of conc. HF (5 mL) and conc. H₂SO₄ (2–3 drops) in order to remove SiO₂.

The residue is ignited once more before fusion with 4 times its weight of anhydrous sodium carbonate. The cooled melt is extracted with 10 % v/v HNO₃ and combined with solution (B).

Finally, the combined solutions are transferred to a 25 mL graduated flask and made up to volume with distilled water.

The atomic absorption determination of each element by comparison with prepared standard solutions is carried out, using the conditions mentioned below.

Magnesium

The atomic absorption measurements are made at the 2852.1 Å line, using the air-C₂H₂ flame.

It has been reported that Si causes a depression of the Mg absorption in the air-C₂H₂ flame. It is recommended that either the hotter N₂O-C₂H₂ be used (together with excess Na as an ionization suppressant) or that when the air-C₂H₂ flame is used that both the standard and sample solutions are matched with respect to the Si content.

Calcium

In the determination of Ca it is recommended that the N₂O-C₂H₂ flame, rather than the cooler air-C₂H₂ flame, be used. This flame gives an increase in sensitivity and virtually eliminates interferences.

Sufficient Na is available from the fusion step to suppress Ca ionization in this flame.

The atomic absorption measurements are made at the 4226.7 Å line.

Sodium

It is obvious that Na cannot be determined on this solution. Treatment of another sample must be carried out in which the Na fusion of residue (A) is omitted. The residue (A) is dissolved in 5 mL of 10% v/v HNO₃. A blank should be run with the sample to compensate for sodium pick-up from reagents and glass-ware.

The atomic absorption measurements are made at the 5890.0 Å line, using either an air-C₂H₂ or an air-propane flame.

Chromium

The atomic absorption measurements are made at the 3578.7 Å line, using either a fuel-rich air-C₂H₂ or a N₂O-C₂H₂ flame.

All the other elements are determined in an air-C₂H₂ flame.

Element	Resonance line
Pb	2170.0 Å
Ni	2320.0 Å
Zn	2138.6 Å
Cu	3247.5 Å
K	7664.9 Å
Bi	2230.6 Å
Mn	2794.8 Å
Co	2407.3 Å

Other metals which may also be determined in such sample solutions are: As, Mo, Sb, Sn, Ti, V.

Some typical results

Element	Concentration	Unit
Fe	60.0	%
Si	3.50	%
Al	2.31	%
Bi	3.0	ppm
Ca	41	ppm
Co	4.5	ppm
Cr	35	ppm
Cu	18	ppm
K	40	ppm
Mg	10	ppm
Mn	39	ppm
Na	110	ppm
Ni	3.5	ppm
Pb	20	ppm
Zn	28	ppm

Conclusion

It is estimated that a suitably equipped laboratory set up for routine analysis of iron, silicon and aluminium could carry out the determination, of these elements within 3 hours of receipt of the sample. For twelve elements the time required would be approximately 6 to 8 hours.

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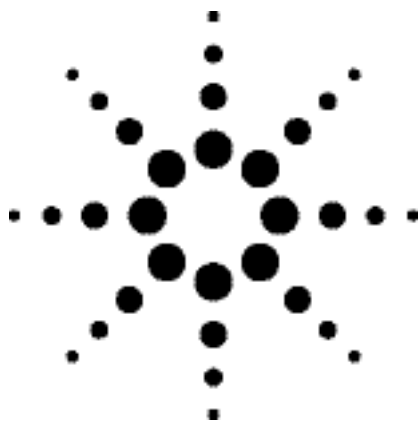
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Determination of Low Levels of Arsenic using Flame AAS and Agilent UltrAA Lamps

Application Note

Atomic Absorption

Author

Dr. Jon Hall

Introduction

Nickel ore can be profitable to mine and process depending on the prevailing market value of the nickel. It can also be profitable depending on the levels of more valuable base metals such as cobalt that may be present and that can be extracted by the processing method. The value of the ore may be decreased however depending on the levels of "penalty" elements. Arsenic presents a unique case in that it is an unwanted impurity and at high levels (generally greater than 200 ppm in ore) may inhibit the efficiency of modern processing technologies.

Rapid screening of the ore is therefore required. Base metals such as iron, nickel, cobalt, copper, zinc can be quickly and easily determined by flame AAS. Arsenic presents a unique challenge because it is usually present at levels that make it difficult to determine by flame AAS.

The matrix to be analyzed and the relative slowness of both hydride and graphite atomizer techniques make them unattractive for this application. The UltrAA boosted discharge hollow cathode lamps allow arsenic to be determined by flame to the low levels required for the screening application in the presence of high levels of the other base metals, without the need to resort to separate extraction procedures for arsenic or the use of a different technique. That is, all elements – high level base metals and low level arsenic – can be determined from a single extracted solution by a single analytical technique.



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Experimental

Instrumentation

The following were used for this study:

- Agilent SpectrAA 55B Atomic Absorption Spectrometer, connected to a PC
- Agilent UltrAA lamp control module
- Arsenic UltrAA lamp
- Sample Introduction Pump System accessory (SIPS) for actual samples

The instrument parameters for sample measurement are listed in Table 1.

Table 1. Instrument Parameters Used for Sample Measurements

Parameter	Value
Instrument mode	Absorbance
Sampling mode	Manual
Calibration mode	Concentration
Measurement mode	Integrate
Replicates standard	10
Replicates sample	10
Wavelength	189.0 nm
Slit width	1.0 nm
Lamp current	10.0 mA
Background correction	BC On
SIPS	On
Number SIPS standards	4
Measurement time	1.0 s
Pre-read delay	5 s
Flame type	N ₂ O/Acetylene
N ₂ O flow	9.00 L/min
Acetylene flow	6.80 L/min

Reagents and Solutions

Standards used were diluted from 1000 ppm AA standards supplied by BDH (Poole, England).

All acids used were AR grade supplied by BDH (Poole, England).

Sample Preparation

The general procedure is that the milled ore sample (1 g) is treated with a mixed acid reagent (nitric acid, hydrochloric acid, hydrofluoric acid, perchloric acid) with hydrofluoric

acid being used to remove silicates and the perchloric used to dissolve sulfates.

Warning: Observe all precautions when using hydrofluoric acid or perchloric acids.

The digest solution is taken to dryness, re-dissolved in 10 mL of 10% hydrochloric acid and made up to 100 mL with distilled water.

Calibration

A study was carried out to determine the best resonance line to use. Arsenic has three resonance lines, 189.0 nm, 193.7 nm and 197.2 nm. The UltrAA lamp boosts all three lines; the intensities as measured (without flame) by the relative instrument gain are similar (Table 2). The respective calibration graphs for each of the resonance lines using air-acetylene flame are compared in Figure 1.

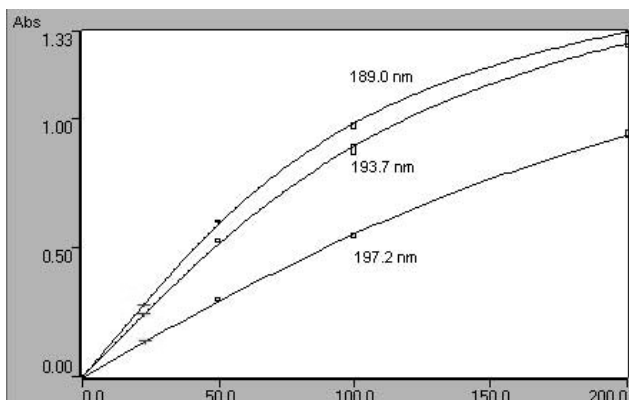


Figure 1: Calibration graphs at the three resonance lines for arsenic using air-acetylene flame.

The respective calibration graphs for each of the resonance lines using nitrous oxide-acetylene flame are compared in Figure 2.

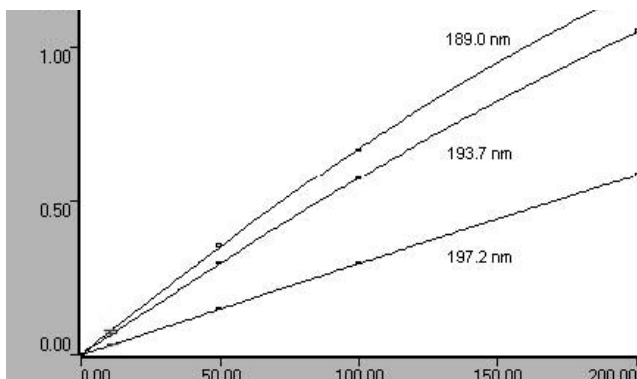


Figure 2: Calibration graphs at the three resonance lines for arsenic using nitrous oxide-acetylene flame.

The characteristic concentrations (C.C.) for each resonance line using air-acetylene and nitrous oxide-acetylene flames were determined and are reported in Table 2.

Table 2. Characteristic Concentrations of Arsenic at Different Wavelengths and Different Flame Conditions

Wavelength (nm)	189.0	193.7	197.2
Gains (%)	54	52	47
C.C. mg/L (air-C ₂ H ₂)	0.37	0.42	0.74
C.C. mg/L (N ₂ O-C ₂ H ₂)	0.62	0.74	1.47

Although the most sensitive conditions are in the air-acetylene flame at 189.0 nm, the nitrous oxide-acetylene flame is generally preferred for the nickel ore samples. The nitrous oxide-acetylene flame provides the normal advantages of reduced interferences from the sample matrix because of its hotter temperature. Additionally, the nitrous oxide-acetylene flame tends to be more transparent than air-acetylene flame in the extreme ultra-violet region of the spectrum.

A variety of high nickel ores which contained low levels of arsenic (10–2500 ppm in ore) were analyzed (using SIPS). The instrument parameters are shown in Table 1. The results are shown in Table 3.

Table 3: Extract of Report for 26 Samples

Sample id	Concentration mg/L	Mean Abs	Recovery
Cal zero	0.0	-0.0033	
Standard	25.0	0.1717	
Standard	50.0	0.3342	
Standard	75.0	0.4805	
Standard	100.0	0.6186	
50 ppm Standard 2	49.1	0.3281	
Blank	0.2	0.0017	
Sample 001	13.3	0.0916	
Sample 001 dup	13.8	0.0950	104%
50ppm Standard 2	45.6	0.3064	91%
Sample 013	11.9	0.0816	
Sample 014	5.7	0.0395	
Sample 013 dup	11.5	0.0791	97%
Sample 014 dup	5.4	0.0372	95%
Sample 017	0.1	0.0007	
100 ppm Standard 4	92.6	0.5790	93%
Sample 023	23.4	0.1604	
Sample 024	12.2	0.0841	
Sample 025	0.6	0.0043	
Sample 026	0.9	0.0065	
100 ppm Standard 4	97.3	0.6029	97%
Sample 023 dup	23.6	0.1613	101%
Sample 024 dup	12.0	0.0828	98%
Sample 024 dup	11.6	0.0801	95%
Sample 023 dup	23.9	0.1634	102%
Blank	0.3	0.0019	

The limits of detection ranged from 0.05 to 0.14 mg/L in final solution. This equates to 5 to 14 ppm in ore.

Notable are the quality of the repeats, the recoveries of QCs and the ability to discriminate between samples with very low levels of analyte (< 1ppm in solution) without the need to modify the extraction procedure specifically for arsenic or to have a second extraction procedure for this element.

Conclusion

The combination of nitrous oxide-acetylene flame and UltrAA lamps with any SpectrAA instrument allows rapid and simple determination of low levels of arsenic in nickel ores on extractions that are common for the determination of the base metals. The high intensity UltrAA lamp allows full use of the more sensitive 189.0 nm and 193.7 nm lines not normally available on other systems.

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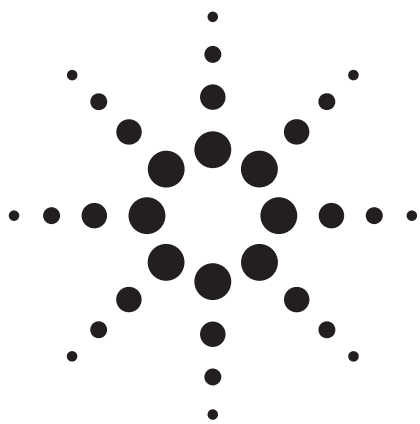
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The Investigation of Fertilizer Analyses Using Microwave Digestion and the Agilent 720-ES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Authors

Christine M. Rivera
Doug Shrader

Introduction

Fertilizers play a vital role in sustaining crop yields by supplying essential plant nutrients such as macronutrients nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O). The optimum “fertilizer ratio” of these three elements can vary according to the type of plant material being fertilized. Secondary nutrients such as calcium, magnesium and sulfur and micronutrients boron, copper, iron, manganese, molybdenum and zinc also play important roles in plant growth.

The purpose of these investigations was to evaluate microwave digestion procedures for two analyses of fertilizer samples. The experiment was divided into two phases.

Phase 1 was to evaluate using microwave digestion to prepare fertilizers for the determination of As, Ca, Cd, Cu, Cr, Fe, K, Na, Mg, Mn, P, Pb, Se and Zn by simultaneous ICP-OES.

Phase 2 was to evaluate using microwave digestion to prepare fertilizers for the determination of available phosphorus (P_2O_5) and potassium (K_2O) by simultaneous ICP-OES. Traditionally, the preparation of samples for phosphorus and potassium is done by extraction with ammonium citrate—EDTA. The samples are placed in a heated water bath and shaken for 1 hour. The reagents are added to warmed samples and the shaking must be continuous. The number of samples typically required for preparation is large and this process is extremely time-consuming.

In this field, phosphorus is traditionally determined using an auto-analyzer, which can be tedious to set up and run. Potassium is typically determined by flame photometry. To simplify sample preparation and analysis, the CEM MARS Xpress microwave digestion system with stirring option and Agilent 720-ES simultaneous ICP-OES were used for both analysis phases.



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Instrumentation

Agilent 720-ES

The Agilent 720-ES with axial torch configuration is a truly simultaneous ICP-OES with solid-state, Charge Couple Device (CCD) detection system. The custom-designed and patented CCD detector incorporates IMAP technology, whereby pixels are arranged in continuous angled arrays matched exactly to the image produced by the echelle optics. This provides true simultaneous measurement and full wavelength coverage from 167 nm to 785 nm.

Microwave Digestion System

CEM, Corp. MARSXpress is an ultra-high throughput microwave digestion system designed to make high-throughput sample preparation and research applications quick and easy.

Forty high-pressure vessels, available in 55 mL or 75 mL sizes, can be processed per run with temperature control of every vessel.

Sample Preparation

Phase 1

To verify the method, two Magruder fertilizer check standards (200204 and 200206), which form part of the association of American Plant Feed Control Officials (AAPFCO) round robin laboratory checks, and a certified reference material, Industrial Sludge (CRM-S-I) from High Purity Standards, were prepared for analysis. Approximately 0.5 g of sample was accurately weighed and transferred to 55 mL MARSXpress vessels. Then, 9 mL of HNO₃ and 1 mL of HCl was added to each sample vessel. Samples were digested in duplicate.

The microwave digestion method is summarized in Table 1.

Table 1. Microwave Digestion Settings

Stages	Max power (W)	% power	Ramp (min.)	Pressure (PSI)	Temp. (°C)	Hold (min)
1	1200	100	15:00	120	200	15:00

The total digestion time was 30 minutes. Figure 1 represents the average digestion temperature over time in the vessels during sample preparation.

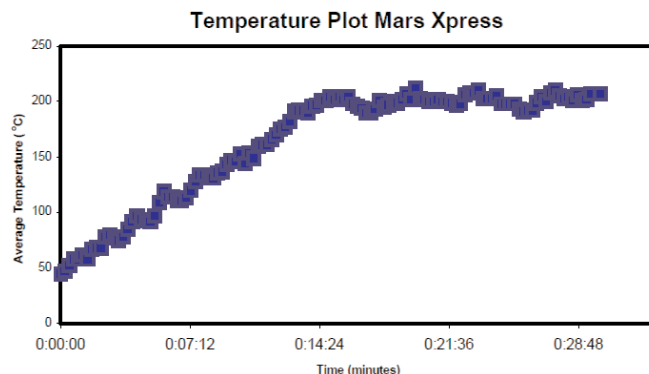


Figure 1. MarsXpress digestion temperature profile.

All samples were diluted to 50.00 mL in plastic disposable tubes and filtered with 2 micron Teflon FilterMate. This high dirt trapping FilterMate is especially suitable for trace level analysis and is supplied with lot certification for trace metals. The filtered samples were transferred directly to the Agilent SPS3 autosampler for analysis on the Agilent 720-ES.

Calibration Solution Preparation

The calibration summary for Phase 1 is listed in Table 2.

Table 2. Phase 1 Calibration Summary

Elements	Concentration (mg/L)
As, Cd, Cr, Cu, Mn, P, Pb, Se, Zn	0.5, 5, 10 and 50
Ca, Fe, K, Na, Mg	5, 10, 100 and 1000

Conditions

Instrument operating conditions are shown in Table 3.

Table 3. Instrument Operating Conditions

Parameter	Setting
Spraychamber type	Double-pass, glass cyclonic
Nebulizer type	SeaSpray
Nebulizer flow	0.75 L/min
RF Power	1.25 kW
Plasma gas flow	15 L/min
Auxiliary gas flow	1.5 L/min
Uptake delay	20 s
Stabilization delay	10 s
Rinse time	15 s
Internal standard	2 mg/L yttrium
Ionization buffer	0.4% caesium
Integration time*	60 s
Replicates	2
Total analysis time#	165 s

*If Se is not required at detection limit concentrations, a 5 s integration time is adequate. # 55 s for sample sets not requiring low concentrations of Se to be measured.

Results and Discussion

Phase 1

The results for Phase 1 are summarized in Table 4. Many of the samples encountered in feed and fertilizer laboratories consist of high concentrations of nutrients. Some of these elements cause ionization interferences while others cause

spectral overlap problems, for example, iron. The use of an ionization buffer, for example, 0.4 % caesium minimized the ionization interferences. The spectral overlap can be overcome by the advanced background correction techniques of the ICP Expert II software, such as fitted background correction and FACT (Fast Automated Curve-Fitting Technique) spectral deconvolution.

Table 4. Results Summary

Sample labels units	As 188.980 mg/kg	Expected mg/kg	Ca 370.602 %	Expected %	Cd 214.439 mg/kg	Expected %	Cr 267.716 mg/kg	Expected mg/kg
Sludge B	141	141	0.0233	0.0242	0.64	NA	110	111
Magruder 4B	2.05	1.75	2.71	2.48	12.31	NA	125.2	132.6
Magruder 6B	5.75	5.66	4.93	5.94	1.51	NA	50.88	51.08
Sample labels units	Cu 327.395 %	Expected %	Fe 261.382 %	Expected %	K ₂ O 404.721 %	Expected % K ₂ O	Mg 279.078 %	Expected %
Sludge B	0.0407	0.0398	0.012	0.014	NA	NA	12.6	12.2
Magruder 4B	0.0461	0.0307	0.350	0.400	11.02	10.54	1.62	1.64
Magruder 6B	1.010	0.976	0.500	0.500	21.37	20.54	0.53	0.62
Sample labels units	Mn 294.921 %	Expected %	Na 589.592 %	Expected %	P 214.914 % P ₂ O ₅	Expected % P ₂ O ₅		
Sludge B	0.51	0.48	0.94	0.94	0.51	0.50		
Magruder 4B	0.036	0.039	0.31	0.29	8.1	9.1		
Magruder 6B	0.014	0.015	0.57	0.58	9.1	9.9		
Sample labels units	Pb 220.353 mg/kg	Expected mg/kg	Se 196.026 mg/kg	Expected mg/kg	Zn 213.857 %	Expected %		
Sludge B	6.8	5.7	NA	NA	0.0244	0.0249		
Magruder 4B	1.16	2.18	0.43	0.44	0.043	0.048		
Magruder 6B	1.88	2.15	0.13	0.12	0.003	0.003		

Phase 2 Available K₂O and P₂O₅

The extraction reagent preparation requires 325 g EDTA and 650 g dibasic ammonium citrate dissolved in 19.5 L distilled water. With mixing, 390 mL of a 1:1 solution of NH₄OH:H₂O is then added. When the solution is cooled to room temperature, the pH is carefully adjusted to 7.0 with additional 1:1 solution of NH₄OH:H₂O and the final solution diluted to 26.0 L.

The traditional method requires that 0.25 g of sample undergo extraction in 100 mL ammonium citrate reagent in a Wheaton bath stabilized to 65 °C (the extraction solution is added to the warmed sample). Upon completion of the extraction procedure, the solutions are cooled and diluted to 250 mL with ammonium citrate/EDTA reagent.

The samples are then typically run on the flame photometer for available K₂O and the auto-analyzer for P₂O₅.

The first part of the Phase 2 experiment was to determine if ICP-OES is a viable alternative technique for the determination of K and P. A set of ten fertilizer samples were extracted per the defined method and analyzed by the traditional techniques and ICP-OES.

With the ICP-OES technique, beryllium was selected as the internal standard and 0.8% caesium as an ionization buffer. Optimum instrument conditions were found to be at a power of 1.1 kW and a nebulizer flow of 0.65 L/min. The total sample analysis time was 55 s/sample.

A comparison of the results by technique for the determination of K and P are summarized in Table 5.

Table 5. Traditional Methodology Verses ICP-OES (1 Hour Water Bath Extraction) Results

Calibration solutions (units)	ICP-OES K ₂ O (769.897 nm) (% w/v)	Flame photometer K ₂ O (% w/v)	ICP-OES P ₂ O ₅ (214.914 nm) (% w/v)	Auto-analyzer P ₂ O ₅ (% w/v)
Blank	0		0	
Std 1			0.1145	
Std 2	0.06023		0.4581	
Std 3			0.68709	
Std 4	0.24038		0.91612	
Std 5			1.3742	

Calibration solutions (units)	ICP-OES K ₂ O (769.897 nm) (% w/v)	Flame photometer K ₂ O (% w/v)	ICP-OES P ₂ O ₅ (214.914 nm) (% w/v)	Auto-analyzer P ₂ O ₅ (% w/v)
NIST SRM 200a	34.81	34.64	52.62	52.11
Sample 1	4.51	4.59	21.6	21.59
Sample 2	4.50	4.29, 5.11, 5.19	8.10	8.59
Sample 3	9.22	9.22, 9.30	10.04	10.09
Sample 4	9.89	9.80, 9.71	10.04	9.75, 9.95
Sample 5	1.80	1.48, 2.46, 2.16	19.87	20.07
Sample 6	2.50	2.53, 3.03, 3.15	22.47	22.49, 22.69
Sample 7	10.17	9.88	10.58	10.47
Sample 8	8.56	8.58	4.17	4.06
Sample 9	5.47	5.45	9.71	9.73
Sample 10	18.49	18.00	16.56	16.70, 16.45

Note: No internal standard for K₂O.

The final step of the Phase 2 study was to mimic the extraction process using microwave digestion. The microwave is not used for total digestion of the fertilizer sample, but as a way to consistently heat the extraction to 65 °C.

Three Magruder fertilizers and NIST SRM 200a potassium dihydrogen phosphate (KH₂PO₄) were carefully weighed to

0.1 g into HP 5000 vessels. Using the 75 mL vessels, 75 mL of ammonium citrate/EDTA extraction fluid was added. Stirring bars were added to each sample to simulate the shaking process and the contents were heated to 65 °C for 1 hour.

Table 6 summarizes the results collected by ICP-OES for available K₂O and P₂O₅.

Table 6. Summary of Results for Microwave Extraction and Determination of K_2O and P_2O_5 by ICP–OES

Calibration solutions(units)	K_2O 404.721 (% w/w)	K_2O 769.897 (% w/w)		P_2O_5 185.878 (% w/w)	P_2O_5 214.914 (% w/w)	Calibration
Blank	0	0		0	0	
Std 1				0.01139	0.01139	
Std 2		0.012				
Std 3		0.03038		0.05785	0.05785	
Std 4	0.06022	0.06022		0.11449	0.11449	
Std 5	0.1223	0.1223		0.22881	0.22881	
Std 6	0.2431	0.2431				
Std 7	0.49789					

Samples(units)	K_2O 404.721 (% w/w)	K_2O 769.897 (% w/w)	Expected (% w/w)	P_2O_5 185.878 (% w/w)	P_2O_5 214.914 (% w/w)	Expected (% w/w)
NIST_SRM 200a	34.42	34.58	34.61	52.44	52.56	52.11
NIST SRM duplicate	34.93	34.34		52.70	52.44	
MAG # 4	11.66	10.45	10.54	20.71	20.36	20.82
MAG # 4 duplicate	10.04	10.48		20.90	20.75	
MAG # 6	26.65	25.41	25.42	22.33	22.07	22.54
MAG # 6 duplicate	23.99	24.88		22.25	22.10	
MAG # 9	12.57	13.66	13.00	14.04	13.85	13.00
MAG # 9 duplicate	12.65	13.66		14.09	13.86	

Conclusion

The preparation of fertilizer samples by microwave digestion/extraction for the determination of macro, secondary and micro nutrients by simultaneous ICP-OES was evaluated and found to compare well with more traditional methods. The combination of microwave and ICP-OES techniques resulted in significantly faster and simpler sample preparation and analysis, requiring only a single analytical system to measure all elements of interest.

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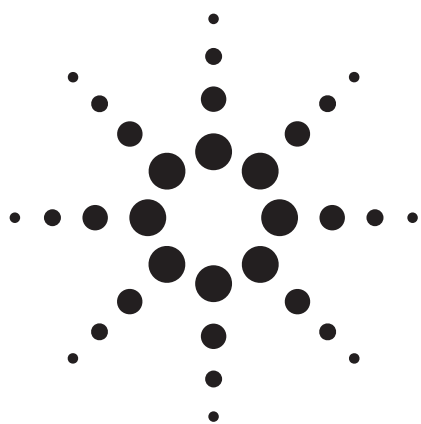
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Determination of Mercury With On-line Addition of Stannous Chloride Using an Axial ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Tran T. Nham

Introduction

Mercury is one of the most toxic heavy metals in the environment. It is therefore important to routinely monitor the Hg level in many types of samples. The US EPA approved methodology for the determination of Hg uses the cold vapor technique with stannous chloride as the reducing agent [1]. This methodology is applicable to Hg determinations in a range of waters (drinking, ground, surface, sea and brackish waters) plus domestic and industrial wastes. It allows mercury detection down to the sub- $\mu\text{g/L}$ range.

While Hg can be determined with direct aspiration using the ICP-OES technique, the performance achieved is not sensitive enough for many environmental applications.

Ultra-trace level determination of Hg therefore requires the use of a vapor generation accessory such as the Agilent VGA-77. The detection limit of Hg achieved with this accessory using an axially-viewing ICP-OES is $0.02 \mu\text{g/L}$ [2]. However, if the required determination level of Hg is not so low, it is feasible to use on-line addition of reductant to achieve the required signal enhancement. This work demonstrates a simple and convenient way to determine Hg in the $\mu\text{g/L}$ range using an axially-viewing ICP-OES.



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Instrumentation

All measurements were performed on an Agilent 720-ES axially-viewing ICP-OES. The Agilent 720-ES is a simultaneous ICP-OES featuring an Echelle polychromator incorporating a quartz prism and a custom-designed and patented CCD detector, which provides the benefit of simultaneous measurement and continuous wavelength coverage over the range from 167 to 785 nm. The system is available with a choice of sample introduction system; either a 3 or 4 channel peristaltic pump for sample introduction and mass flow control or manual pressure control of the nebulizer gas flow. For this application, the system was fitted with a mass flow controller and a four channel peristaltic pump. The four channel peristaltic pump allows the sample, SnCl_2 reductant and the waste to be simultaneously pumped. To enable on-line addition of the reductant to the sample, a Y-piece (Agilent p/n 1610132400) was used to combine the sample and reductant flows prior to the sample introduction system. The mixture was then nebulized into the plasma.

A conventional one piece axial torch was used. The sample introduction system consisted of a concentric glass nebulizer and a glass cyclonic chamber. Agilent ICP Expert II software was used for instrument operation. The operating parameters of the system are listed in Table 1.

Table 1. Instrument Operating Parameters

Conditions	Settings
Power	1.2 kW
Plasma gas flow	15 L/min
Auxiliary gas flow	1.5 L/min
Nebulizer flow	0.65 L/min
Pump speed	12 rpm
Pump tubing	White-white (inlet) for both sample and reductant (1.02 mm id) Blue-blue (outlet) (1.65 mm id)
Sample uptake rate	1.0 mL/min
Sample uptake rate	1.0 mL/min
Replicate read time	30 s
Sample uptake delay time	80 s
Fast pump	Off
Rinse time	120 s
Sampling mode	Manual
Background correction	Fitted
Number of replicates	3 10 for detection limit measurements

The on-line addition of reductant results in an unusually large quantity of liquid being nebulized. It is recommended that the fast pump option be disabled during the sample uptake delay, to minimize droplet condensation in the injector tube of the torch.

Materials and Reagents

All chemicals and reagents used were of high-purity grade.

- HCl, Tracepur, 36%, Merck.
- HNO_3 , Ultrapure, 60%, Merck.
- 1000 mg/L Hg certified standard solution, EM Science.
- SnCl_2 , Analar, BDH.
- Milli-Q water.

Sample Preparation

Preparation of SnCl_2 Solutions

Stannous chloride (SnCl_2) can dissolve in less than its own mass of water without apparent decomposition, but as the solution is diluted, hydrolysis occurs to form an insoluble basic salt ($\text{Sn}(\text{OH})\text{Cl}$) which is readily oxidized by air. Therefore HCl must be added to suppress hydrolysis and to help prevent the oxidation of SnCl_2 by air. An insufficient amount of HCl will result in a yellow-colored solution. In this work, all SnCl_2 solutions were prepared in 20% HCl. It is recommended to dissolve SnCl_2 in concentrated HCl prior to mixing with water.

Sample Preparation for NIST 1641D Mercury in Water [3]

The certified reference material NIST 1641D mercury in water is supplied in an ampoule. After the ampoule was opened, a 0.2 mL aliquot was transferred to a 100 mL volumetric flask to make a 1 in 500 dilution with 2% v/v HNO_3 .

Calibration solutions and blank were prepared in 2% v/v HNO_3 .

Cleaning of the Torch After Analysis

After the analysis, it was observed that the end of the outer tube of the torch was covered by a layer of white powder, which is presumably SnO_2 . This can be removed by simply soaking the torch directly in concentrated HCl to dissolve the white deposit. After soaking, rinse the torch with de-ionized water and allow to dry.

Results and Discussion

Effect of SnCl_2 on Hg Signal Intensity

The effect of different SnCl_2 concentrations on the signal intensity of a 50 $\mu\text{g/L}$ mercury solution was studied. As illustrated in Figure 1, on-line addition of SnCl_2 has enhanced the Hg signal over 30 times.

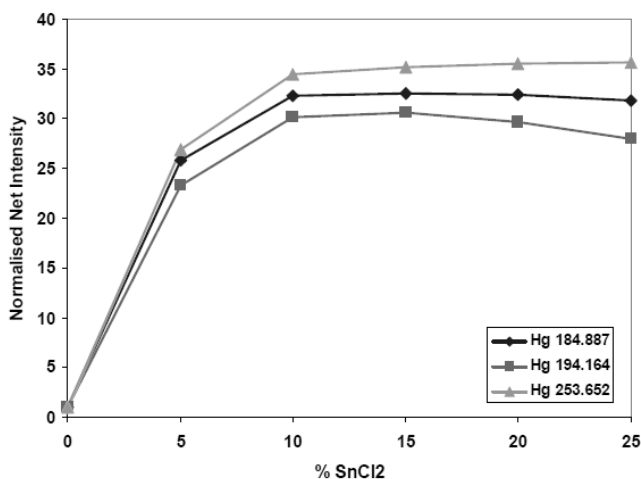


Figure 1. Effect of different SnCl_2 concentrations on the signal intensity of 50 $\mu\text{g/L}$ Hg.

As the concentration of SnCl_2 increases, the Hg signal intensity increases, and then plateaus out at about 10–15% SnCl_2 . Therefore, the concentration of SnCl_2 used in this work was 15%.

It is noted that for both the Hg 184 nm and Hg 194 nm lines, the Hg signal increases up to 15% SnCl_2 , then slowly decreases as the concentration of SnCl_2 increases. This is caused by the spectral interference of Sn on both the Hg 184 nm and Hg 194 nm lines, respectively. The potential spectral interferences on various Hg lines are listed in Table 2.

Table 2. Potential Spectral Interferences on Hg Emission Lines

Wavelength (nm)	Potential interferences
184.887	Sn 184.821 nm
194.164	Sn 194.205 nm
253.652	Cr 253.634 nm
	Cr 257.692 nm

Figures 2 to 4 show the signal graphics for Hg at the 184, 194 and 253 nm lines with various SnCl_2 concentrations. The sloping background at the Hg 184 nm and Hg 194 nm lines is caused by the presence of high levels of Sn. The Hg 253 line is free of interference from Sn, but there is the potential for interference

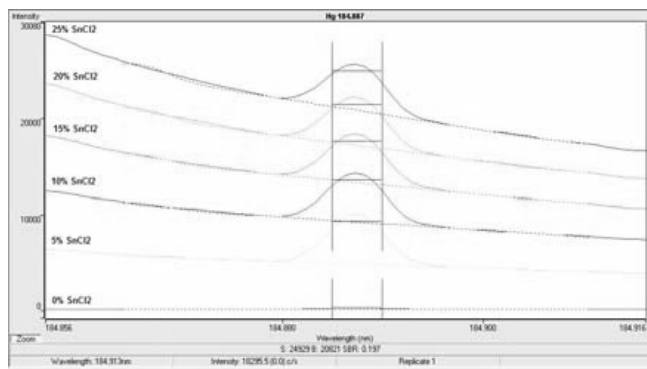


Figure 2. Signal traces of 50 $\mu\text{g/L}$ Hg 184 nm at various SnCl_2 concentrations.

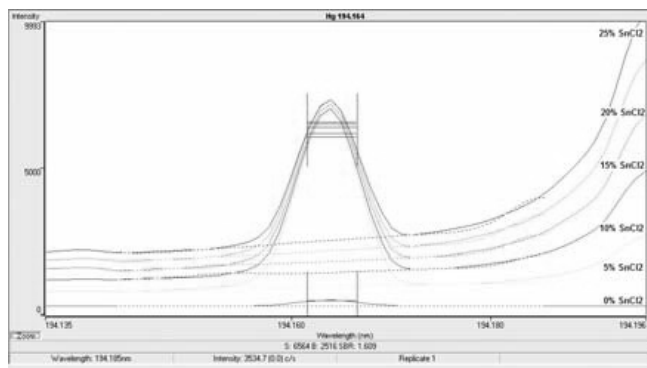


Figure 3. Signal traces of 50 $\mu\text{g/L}$ Hg 194 nm at various SnCl_2 concentrations

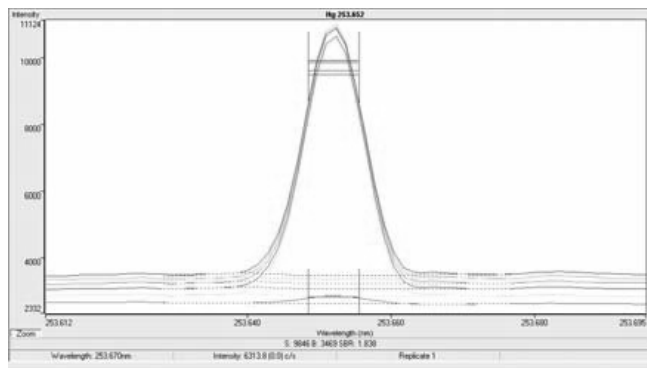


Figure 4. Signal traces of 50 $\mu\text{g/L}$ Hg 253 nm at various SnCl_2 concentrations.

from Cr if the sample has been prepared in $\text{K}_2\text{Cr}_2\text{O}_7$.

Detection Limit

Detection limit (DL) is expressed as three times the standard deviation of the blank intensity in concentration units. The detection limits for Hg with and without the addition of SnCl_2 are listed in Table 2. Generally speaking, an order of magnitude improvement in detection limit is achieved with the addition of SnCl_2 as a reductant.

However it is also noted that these detection limits are around a factor of 10 higher than those that can be achieved

with the use of the cold vapor technique using the VGA-77 ($0.02 \mu\text{g}/\text{L}^2$).

Table 2. Detection Limits for Hg Achieved with On-line Addition of SnCl_2

Wavelength (nm)	DL ($\mu\text{g}/\text{L}$) with 15% SnCl_2	Without SnCl_2
184.887	0.4	2.0
194.164	0.2	2.0
253.652	0.2	3.0

Analysis of NIST 1641D Mercury in Water

Conventional aqueous Hg standards of 1, 5 and 10 $\mu\text{g}/\text{L}$ were used to calibrate the instrument at each of the respective Hg wavelengths. The calibration graphs obtained for each Hg emission line are shown in Figure 5. The NIST 1641d mercury in water certified reference material was

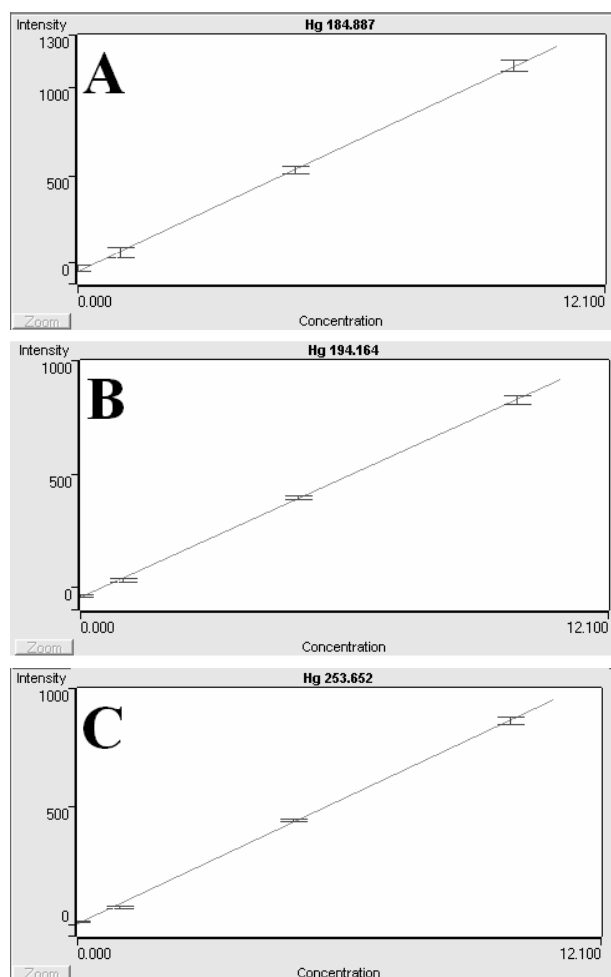


Figure 5. Calibration curves for Hg at (A) the 184 nm emission line, (B) the 194 nm emission line and (C) the 253 nm emission line.

measured against these calibrations. The results are listed in Table 3. The measured values are in good agreement with the certified values.

Table 3. Results for Analysis of the NIST 1641D Mercury in Water Certified Reference Material

Wavelength (nm)	Concentration (mg/kg) measured	Certified
184.887	1.592 ± 0.010	1.590 ± 0.018
194.164	1.585 ± 0.010	1.590 ± 0.018
253.652	1.596 ± 0.012	1.590 ± 0.018

Conclusion

The determination of Hg by on-line addition of SnCl_2 using the Agilent 720-ES axial ICP-OES instrument has been described. The on-line addition of SnCl_2 reductant for Hg determination can provide a tenfold improvement in detection limit compared with routine determination. This on-line reduction method allows Hg determinations at $\mu\text{g}/\text{L}$ levels without the use of a cold vapor generation technique. It is also simple and easy to implement.

References

1. US EPA Publication No. EPA-600/4-79-020, "Method for chemical analysis of water & wastes", (1979), Method 245.1.
2. P. Doidge, "Determination of mercury in a certified reference sludge material using the Varian 710-ES",

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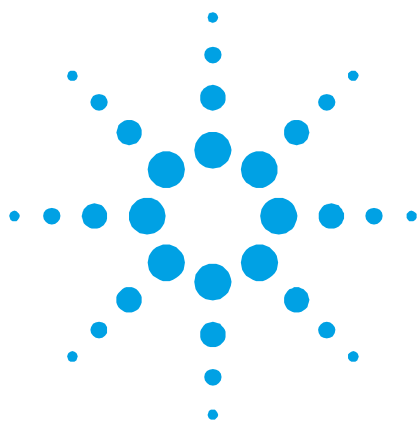
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Using the Agilent Cary 670 FTIR Spectrometer to observe rotational and isotopic bands in CO through high resolution FTIR Spectroscopy

Application Note

Author

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Introduction

The advent of Fourier transform infrared (FTIR) spectroscopy has permitted the observation of spectra at higher spectral resolutions previously unattainable with traditional scanning spectrometers. Resolution of rotational-vibrational bands within gas-phase samples using modern FTIR spectrometers is now commonplace.

The observation of rotational-vibrational bands at high resolution requires the use of small apertures to limit the divergence of the IR radiation through the interferometer, leading to a reduced throughput in power at the sample focus. This translates to a degradation of signal-to-noise (S/N) performance, thereby necessitating longer sampling times to resolve weak bands.

To overcome this, an instrument designed specifically for high optical performance is required. The Agilent Cary 670 FTIR spectrometer incorporates several design enhancements, including large collection optics and a retro-reflected source, which result in increased infrared energy at the sample focus. The results discussed in this application note demonstrate that weakly-abundant isotopic peaks can be resolved in a relatively short time using the Agilent Cary 670 FTIR spectrometer.



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Instrumentation

An Agilent Cary 670 FTIR spectrometer was used for all measurements with the settings listed in Table 1. The instrument was purged with nitrogen prior to spectral collection.

Table 1. Instrument parameters in Resolutions Pro software used in all collections

	Instrument Parameters	Settings
Detector	MCT narrow band	
	Speed (kHz)	25.0
	Filter (kHz)	17.4
Source	MIR	Normal
Collection	Sample scans	4
	Background scans	4
	Resolution (cm ⁻¹)	0.09
	Aperture (cm ⁻¹)	0.10
	Symmetry	asym
Computation	Apodisation type	boxcar
	Zero filling factor	16
	UDR	2

Materials and reagents

A carbon monoxide cell (KBr windows, 10 cm path length) was obtained from Pike Technologies, Madison WI, USA. The cell contains carbon monoxide at a reduced pressure of 4 Torr to ensure that the resolution of the obtained spectra are instrument-limited and not physically limited by the sample. The cell was mounted on the slide-on sample holder and placed in the left hand side position of the sample compartment to account for the extra length of the gas cell.

Results and discussion

Isotopomers of carbon monoxide

Sample spectra were recorded (Figure 1) and the region between 2250–2000 cm⁻¹ analyzed to reveal the rotational-vibrational transitions of the C≡O stretching band centered at 2143.3 cm⁻¹. These transitions are grouped into two branches reflecting a simultaneous rotational transition: the lower energy P-branch where the change in rotational state (ΔJ) = -1, and the higher energy R-branch where ΔJ =1.

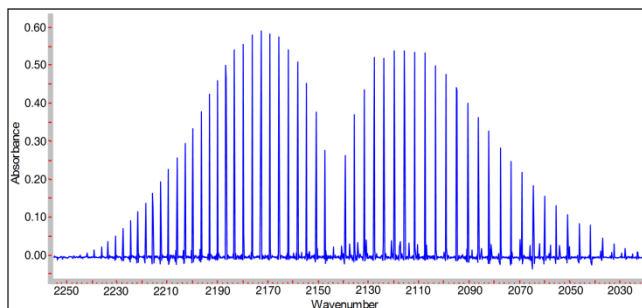


Figure 1. Sample spectrum displayed between 2250–2000 cm⁻¹ revealing the P (right) and R (left) branching of the C≡O stretching band centered at 2143.3 cm⁻¹

These bands are due to transitions of the C¹²O¹⁶ isotopomer. Atmospheric carbon monoxide is known to contain the isotopomers C¹³O¹⁶ and C¹²O¹⁸ at abundances of 1.1% and 0.2% respectively. The frequency of the fundamental transition, $\nu_0 \rightarrow \nu_1$, $\Delta J=0$ of these isotopomers is given by the ratio of the bond strength for the C≡O bond, k , and the reduced mass, μ , of the molecule (Equation 1 below).

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad (1)$$

The reduced mass of the various isotopomers of this molecule are given by:

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \quad (2)$$

where m_1 and m_2 are the atomic masses of each atom. The fundamental frequency of the $\nu_0 \rightarrow \nu_1$ transition in the C¹²O¹⁶ molecule¹ is 2143.3 cm⁻¹, while those of the other isotopomers can be estimated by considering the ratio of their reduced masses with C¹²O¹⁶ (as given by Equation 3 below).

$$\frac{\nu_a}{\nu_b} = \sqrt{\frac{u_b}{u_a}} \quad (3)$$

Using the above equations, the fundamental frequency of the $\nu_0 \rightarrow \nu_1$ transition can be calculated for each isotopomer.

Table 2. Calculated fundamental frequency for the isotopomers of C≡O

	Reduced Mass	$\bar{\nu}$ (cm ⁻¹)
C ¹² O ¹⁶	6.8571	2143.3
C ¹³ O ¹⁶	7.1724	2095.7
C ¹² O ¹⁸	7.2000	2092.1

By focusing on the region between 2170 and 2095 cm⁻¹ (as highlighted in Figure 2), the R branch of C¹³O¹⁶ is clearly visible extending right to left from the forbidden $\Delta J=0$ transition centered at 2095.7 cm⁻¹.

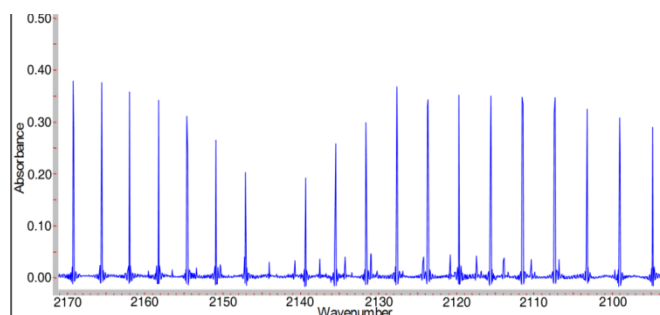


Figure 2. Spectrum of C≡O between 2170–2095 cm⁻¹ highlighting the fine detail of the R branches of C¹³O¹⁶

Figure 3 exhibits some visually discernable differences between the transitions of C¹²O¹⁸ and the other isotopomers of carbon monoxide. The most intense of these is C¹³O¹⁶ (at 2114.0 cm⁻¹) followed by C¹²O¹⁸ (at 2113.4 cm⁻¹) reflecting the natural abundances of these isotopomers within atmospheric carbon monoxide.

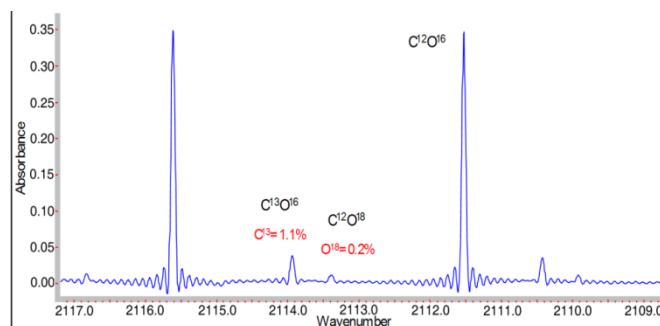


Figure 3. Expanded view of the same spectrum of carbon monoxide between 2117 and 2109 cm⁻¹, illustrating transitions of the C¹²O¹⁶, C¹³O¹⁶ and C¹²O¹⁸ isotopomers

The observation of the peaks from the weakly abundant species requires good S/N performance at high resolution. Typically, resolution of these peaks would

require a large number of co-additions in order to average out noise within the spectrum.

Due to its superior optical design, the Agilent Cary 670 FTIR delivers excellent signal-to-noise performance even at very high spectral resolutions and facilitates fast analysis of weak bands with a high degree of precision and accuracy.

Spectra recorded in this study were collected with just 4 scans which correlate to a 40 second collection time. The high throughput and performance are attributable to a number of design features including:

- 57 mm optics that ensure a larger collection area
- A novel source with retro-reflector to ensure the collection of a greater portion of the source's output
- An AC-powered source to prevent the wandering of the hot spot within the source and to ensure that perfect alignment of the source is maintained.

All of these factors combine to deliver more power to the sample focus translating to better S/N performance. In addition, the frictionless air-bearing interferometer and dynamic alignment system of the Agilent Cary 670 FTIR spectrometer deliver superb velocity control of the moving mirror. This leads to superior wavelength precision and accuracy and permits faster collection times, especially at higher spectral resolutions.

Wavelength resolution, accuracy and precision

The wavelength resolution, accuracy and precision values reported in Table 3 were determined. From three replicate collections of carbon monoxide using the collection parameters outlined in Table 1. The wavelength resolution, accuracy and precision of the spectrometer was calculated based on the R-5, R-6 and R-7 transitions of C¹²O¹⁶ in the C≡O spectra.

Table 3. Wavenumber accuracy, precision and spectral resolution results for the Agilent Cary 670 FTIR spectrometer using the R-5, R-6 and R-7 transitions of $C^{12}O^{16}$

	wavenumber reference ² (cm^{-1})	wavenumber actual (cm^{-1})	precision (st dev) (cm^{-1})	resolution (cm^{-1})
R-5	2165.6010	2165.6010	0.0016	0.0572
R-6	2169.1979	2169.1986	0.0010	0.0598
R-7	2172.7588	2172.7586	0.0008	0.0552
R-5	2165.6010	2165.6010	0.0016	0.0572

The wavelength of maximum absorption for each of the R-5, R-6 and R-7 transitions was calculated using the mean of the three replicate scans of carbon monoxide. These were compared with reference values from the HITRAN database² to assess the wavelength accuracy of the spectrometer. All three transitions were found to lie within 0.001 cm^{-1} of reference values, highlighting the superior performance of the Agilent Cary 670 FTIR spectrometer.

Also calculated was the wavelength precision of the spectrometer, defined as the standard deviation of the peak wavelength from the mean of the three replicate scans. This was determined for each of the R-5, R-6 and R-7 transitions. In each case the precision was found to be better than 0.001 cm^{-1} again demonstrating the stability and performance of the Agilent Cary 670 FTIR spectrometer.

The instrument-limited spectral resolution was determined by calculating the full width at half maximum (FWHM) for each of the three transitions as illustrated in Figure 4. The resolution of the absorbance bands was determined to be better than 0.06 cm^{-1} for each transition, hence enabling well-resolved observation of rotational structure within gaseous samples.

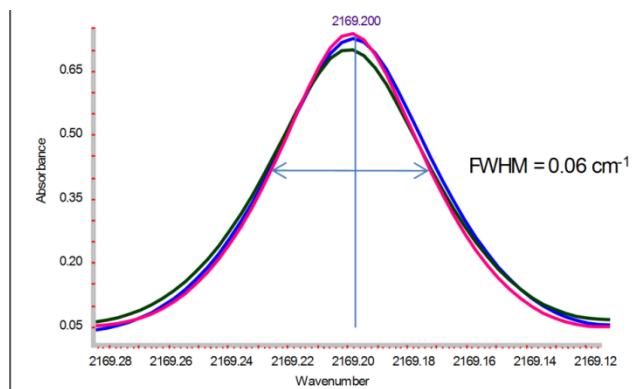


Figure 4. Spectra of three replicates of the R-6 transition of $C^{12}O^{16}$. Such spectra were used to calculate the wavelength precision, accuracy and resolution of the Agilent Cary 670 FTIR spectrometer

Conclusion

The rotational structure of the $C\equiv O$ stretching band centered at 2143.3 cm^{-1} was observed with $<0.06\text{ cm}^{-1}$ spectral resolution for the R-5, R-6 and R-7 transitions. Isotopic splitting of the stretching band was also observed, with the isotopomers $C^{13}O^{16}$ and $C^{12}O^{18}$ being resolved with just a 40 second collect reflecting the high optical performance of the Agilent Cary 670 FTIR spectrometer. The spectrometer's wavelength accuracy was determined to be better than 0.001 cm^{-1} against reference values, while the precision was found to be better than 0.001 cm^{-1} . These performance attributes are the result of a number of design features which have been incorporated into the Agilent Cary 670 FTIR spectrometer and demonstrate the suitability of the instrument for high resolution gas phase applications.

References

1. Rao, K.N. and Mathews, C.W., editors, *Molecular Spectroscopy: Modern Research*, Academic Press, New York, 1972.
2. Rothman L.S. et al. 2004. The HITRAN 2004 molecular spectroscopic database. *J. Quantitative Spectroscopy & Radiative Transfer*, 96(2): 241-250.

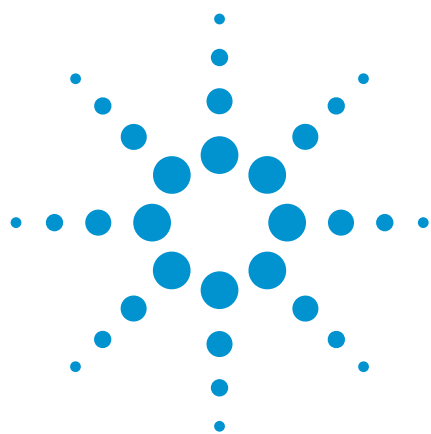
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Analysis of Polylactide and Poly(lactide-*co*-glycolide) by GPC with Viscometry

Application Note

Authors

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Introduction

Polylactide (PLA) and copolymers with glycolic acid (PLGA) are polyesters derived from corn starch and sugar cane. Synthesized by the tin-mediated ring-opening polymerization of the dimeric acid formed from bacterial fermentation, these materials can be manufactured to reasonably high molecular weights. PLA and PLGA have received considerable attention due to their non-toxic, biocompatible and biodegradable properties. The polymers are used in a number of biomedical applications, including sutures, stents and as dialysis media. Fibers of PLA are also employed in the manufacture of upholstery and diapers.

The accurate molecular weight distributions of a sample of polylactide and of poly(lactide-*co*-glycolide) were compared by gel permeation chromatography with viscometry. Light scattering could not be used with these samples as one was a copolymer and therefore not of uniform chemistry across the molecular weight distribution. This could lead to errors in the light scattering calculations. However, the Universal Calibration technique employing a viscometer can be used with copolymers, to provide molecular weights that are independent of the standards used in the column calibration. To investigate the molecular structure of the materials they were analyzed on an integrated GPC system.



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Instrumentation

The copolymer was assessed on an Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer and Agilent PLgel 5 μ m MIXED-D columns, which provide high resolution of resin and condensation polymers.

Columns: 2 x PLgel 5 μ m MIXED-D, 300 x 7.5 mm
(part number PL1110-6504)

Materials and Reagents

Samples: Polylactide and poly(lactide-co-glycolide)
Eluent: Tetrahydrofuran

Conditions

Flow Rate: 1 mL/min
Temperature: 40 °C

Results and Discussion

Figure 1 shows chromatograms for the polylactide. Figure 2 reveals that the samples were clearly quite different in molecular weight, the copolymer having a lower molecular weight than the homopolymer. The Mark-Houwink plot indicated that there might also be some structural differences between the homopolymer and the copolymer, with the copolymer showing a deviation to lower intrinsic viscosities as a function of molecular weight, indicating a smaller and more compact structure in solution (Figure 3).

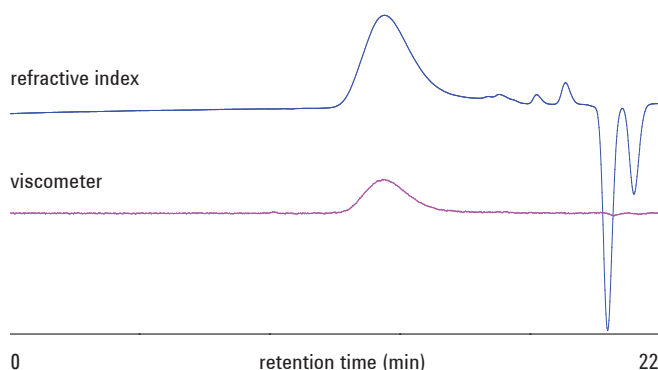


Figure 1. Chromatograms for the polylactide sample

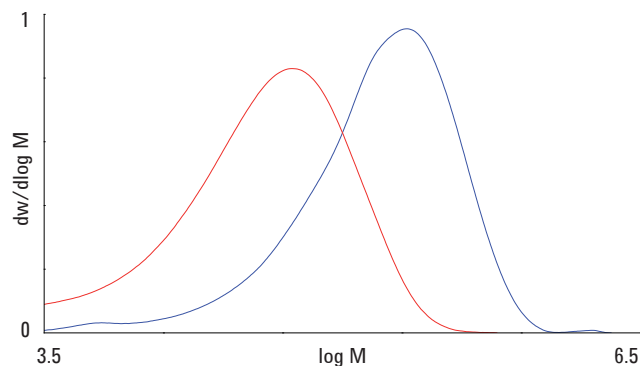


Figure 2. Overlaid molecular weight distributions for polylactide (blue) and poly(lactide-co-glycolide) (red) samples

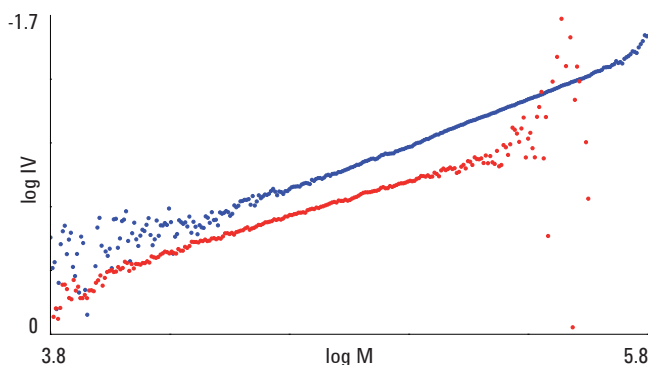


Figure 3. Overlaid Mark-Houwink plots for polylactide (blue) and poly(lactide-co-glycolide) (red) samples

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel 5 μ m MIXED-D columns and a PL-BV 400RT viscometry detector, the PL-GPC 50 Plus provides accurate molecular weight determination for all polymer types based on the Universal Calibration principle, such as polylactide and its copolymers.

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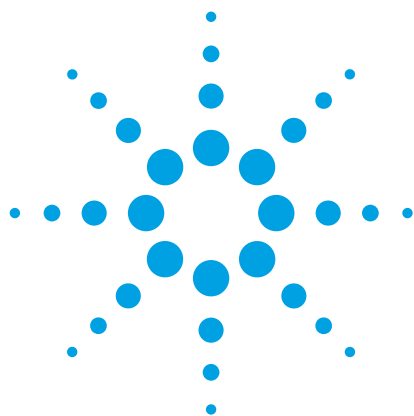
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SI-01520



Agilent Technologies



Analysis of Surfactants Using the Agilent 500 Ion Trap LC/MS

Application Note

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Abstract

Four surfactants were identified in rolling oil used to roll out large sheets of aluminum using the Agilent 500 Ion Trap LC/MS. The full scan sensitivity of the 500 Ion Trap makes it ideal for identification of unknown components in a mixture at an unknown concentration.

Introduction

In this analysis, surfactants were identified in rolling oil used to roll out large sheets of aluminum. It was suspected that surfactant contaminants were causing the oil to foam up over time, and in order to identify the surfactants in the oil, samples were analyzed by LC/MS using the Agilent 500 Ion Trap LC/MS. In addition to using the 500 Ion Trap for initial confirmation of oil contaminants, continuous monitoring of the oil for presence of surfactants maintains optimal operation and efficiency of these machines over time. The full scan sensitivity of the 500 Ion Trap makes it ideal for this type of analysis, where the analysis must identify unknown components in a mixture at an unknown concentration. In addition, the MS/MS capabilities of the instrument make it a powerful tool for structural elucidation and identification of unknown compounds.



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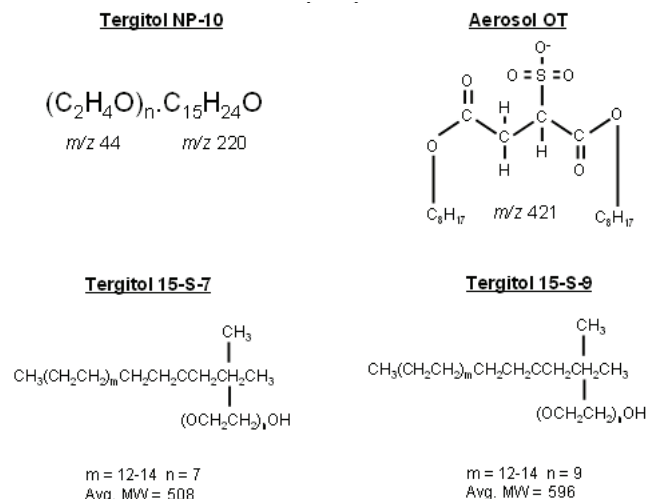


Figure 1. Chemical structures of Tergitol NP-10, Tergitol 15-S-7, Tergitol 15-S-9, and Aerosol OT surfactants.

Instrumentation

The following instruments were used in this study:

- Agilent 500 Ion Trap LC/MS with ESI source
- Agilent 212-LC Binary Solvent Delivery Modules
- Agilent ProStar 430 AutoSampler

Materials and Reagents

A clean sample of Hygold 100 petroleum distillate rolling oil was supplied for analysis, along with neat standards of Tergitol NP-10, Tergitol 15-S-7, Tergitol 15-S-9, and Aerosol OT.

Sample Preparation

ESI positive compounds

- Neat rolling oil** (blank) was Hygold 100 petroleum distillate, Ergon Refining MSDS, CAS #64742-52-5. The oil was agitated in water (acidified) to release any water soluble components into the aqueous phase. The aqueous layers were injected and analyzed by LC/MS.

- Neat surfactant materials** included Tergitol NP-10 in Hygold 100, Dow MSDS 1985; Tergitol 15-S-7 surfactant, Union Carbide MSDS 1917; Tergitol 15-S-9 surfactant, Union Carbide MSDS 1912. The neat surfactant materials dissolved in rolling oil were added to acidified aqueous (0.2% acetic acid) and agitated to release surfactants into the aqueous phase. The aqueous layer was analyzed by LC/MS.

ESI negative compounds

- Neat rolling oil** (blank) was Hygold 100 petroleum distillate, Ergon Refining MSDS, CAS #64742-52-5 and was agitated in water (alkaline) to release surfactants into the aqueous phase. The aqueous layer was injected and analyzed by LC/MS.
- Surfactant material** was Aerosol OT surfactant in Hygold 100, Alcoa MSDS #120778. The surfactant dissolved in rolling oil was added to alkaline aqueous (0.2% ammonium hydroxide) and agitated to release surfactants into the aqueous phase. The aqueous layer was injected and analyzed by LC/MS.

HPLC Conditions

Column:	Inertsil ODS-3, 100 × 4.0 mm, 3 μm (Pursuit C18 Recommended Agilent Alternative p/n A3001100X020)			
Solvent A:	0.2% acetic acid in water (ESI+) 0.2% ammonium hydroxide in water (ESI-)			
Solvent B:	Methanol			
LC program:	Time (min:sec)	%A	%B	Flow (μL/min)
	00:00	95	5	200*
	01:00	95	5	200*
	11:00	0	100	200
	31:00	0	100	200
	31:01	95	5	200*
	36:00	95	5	200*
	* diverted			
Injection volume:	10 μL			

MS parameters

Ionization mode:	ESI (positive) and ESI (negative)
API drying gas:	15 psi at 300 °C
API nebulizing gas:	20 psi
Needle:	4500 V –5500 V
Shield:	600 V –600 V

Table 1. MS Segment Parameters

Analyte	Scan range	Retention time (min)	Capillary voltage (V)	RF load
Tergitol NP-10	200–700	13.48	100	85
Tergitol 15-S-7	329 >			
	280–320	11.12	75	82
Tergitol 15-S-9	315 >			
	280–320	11.68	75	82
Aerosol OT	313 >			
	280–320	12.30	75	82

Results and Discussion

The surfactants analyzed in this method were spiked into blank Hygold rolling oil. Figure 2 shows a chromatogram that compares a full scan blank injection with the subsequent spiked injections containing the surfactants of interest. The blank chromatogram is the extracted ion chromatogram for all of the ions of interest for all the surfactants tested. As the chromatogram shows, no background signal for the surfactants was observed in the blank rolling oil.

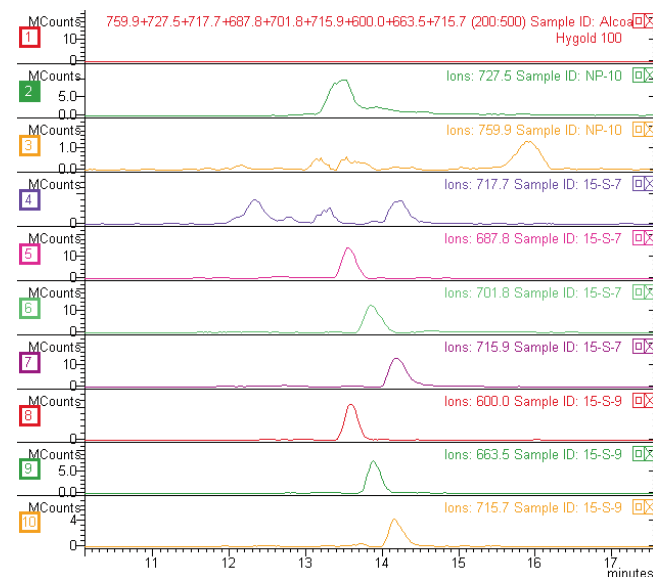


Figure 2. Chromatogram of a blank injection (top) followed by the same blank spiked with Tergitol NP-10, Tergitol 15-S-7, and Tergitol 15-S-9.

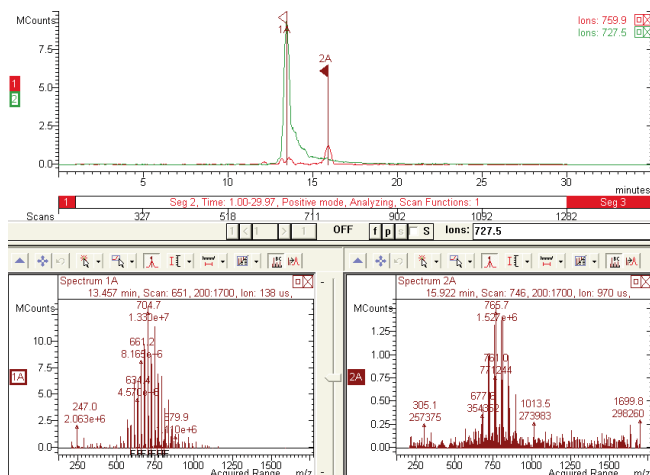


Figure 3. Extracted ion chromatogram and mass spectra for Tergitol NP-10 surfactant in Hygold 100 rolling oil, full scan analysis.

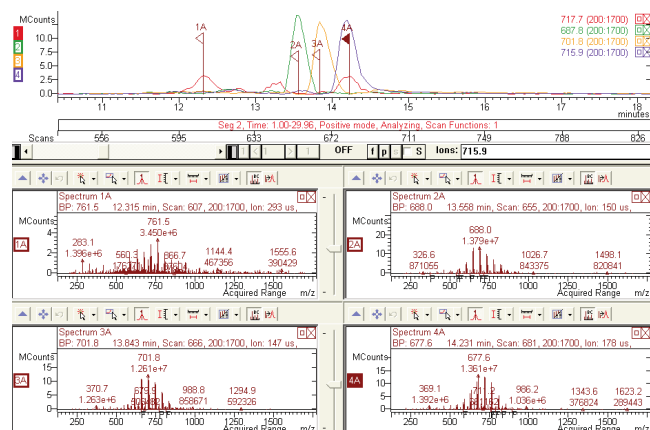


Figure 4. Extracted ion chromatogram and mass spectra for Tergitol 15-S-7 surfactant full scan analysis.

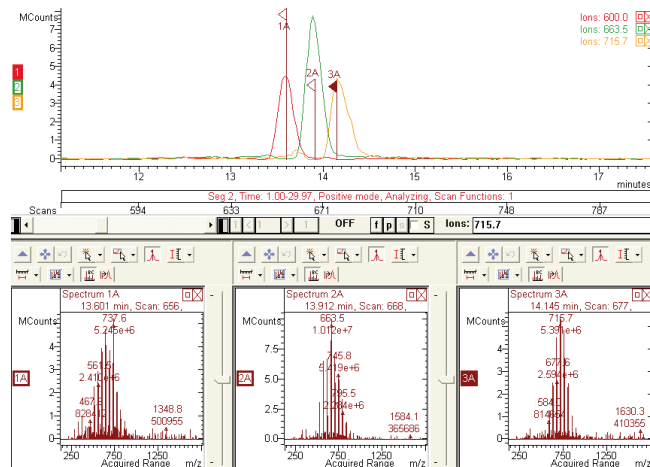


Figure 5. Extracted ion chromatogram mass spectra for Tergitol 15-S-9 surfactant full scan analysis.

The surfactant samples have interesting mass spectra with repeated losses of 44 mass units, representing a loss of C_2H_4O groups from the polymeric chain. Figure 6 shows the mass spectrum of Tergitol NP-10. The fragmentation of NP-10 is observed and a characteristic cluster is seen.

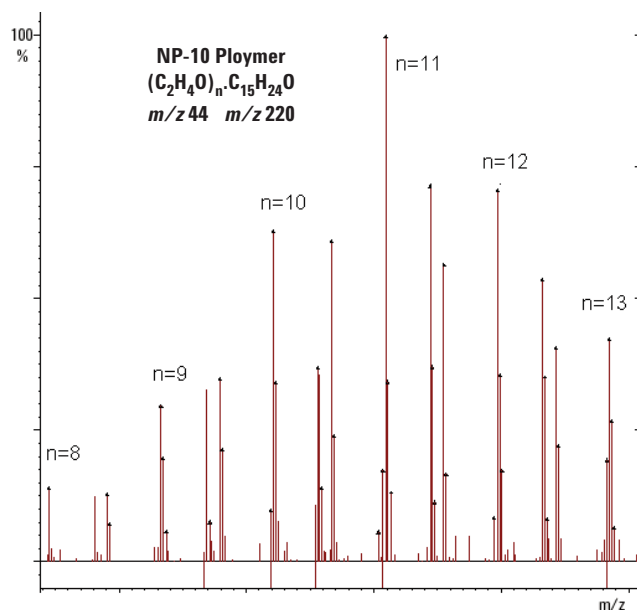


Figure 6. Mass spectrum of Tergitol NP-10.

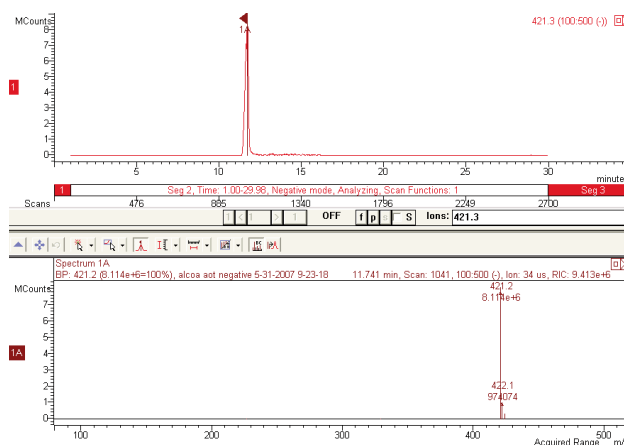


Figure 7. Extracted ion chromatogram of full scan analysis of Aerosol AOT surfactant.

Conclusion

Four surfactants were extracted from rolling oil for machines that roll out large aluminum sheets. The components of the oil were unknown, which made the full scan sensitivity of the Agilent 500 Ion Trap LC/MS ideal for this analysis. By extracting the ions of interest, clear chromatographic peaks are observed and the unique mass spectra are identified.

For More Information

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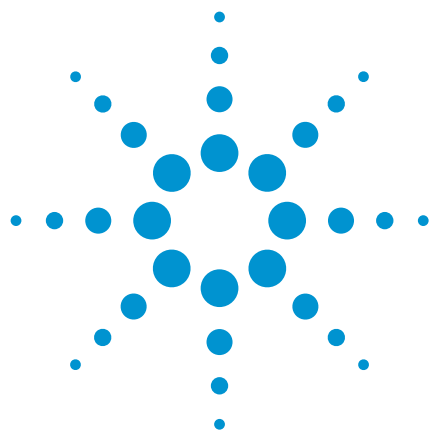
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Agilent Technologies



SEC Analysis of Sodium Polystyrene Sulfonate

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Sodium polystyrene sulfonate is an ion-exchange resin used to treat high levels of blood potassium by substituting potassium in the diet and body for sodium in the resin. The potassium is then excreted and levels in the body are reduced. A sample of sodium polystyrene sulfonate was analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. As the polymers are both ionic and relatively hydrophobic, eluent conditions are chosen to minimize sample to column interaction, which would otherwise result in late elution times.



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Conditions

Samples: Sodium polystyrene sulfonates
Columns: 2 x PL aquagel-OH 40 8 μ m,
300 x 7.5 mm (p/n PL1149-6840)
Eluent: 80 % 0.3 M NaNO_3 + 0.01 M
 NaH_2PO_4 at pH 9 + 20 % Methanol
Flow Rate: 1.0 mL/min
Detection: RI

Conclusion

SEC using PL aquagel-OH columns successfully analyzed samples of sodium polystyrene sulfonate. Aqueous SEC not only provides molecular weight data but also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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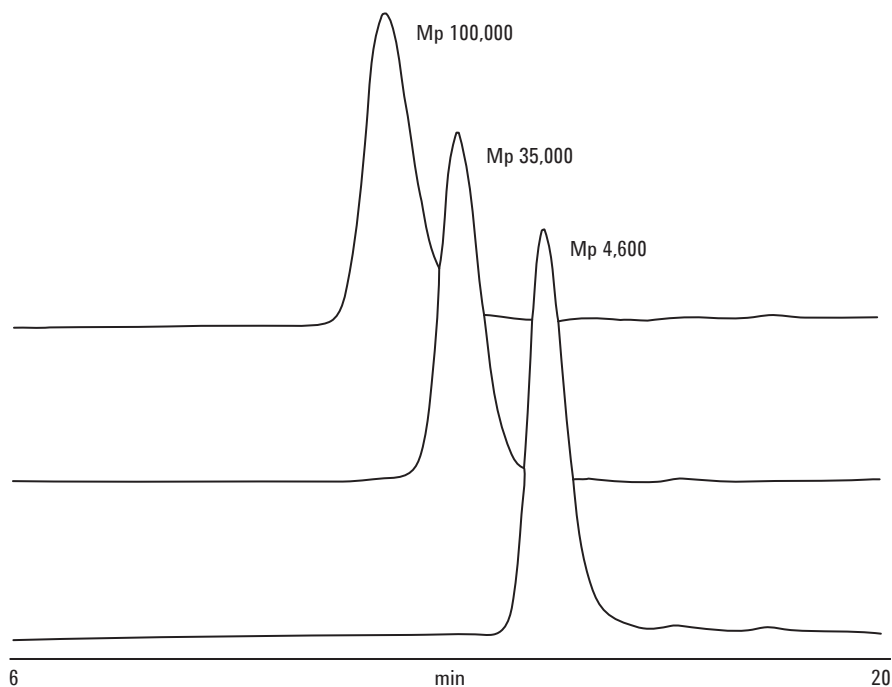


Figure 1. Raw data chromatograms of three samples of sodium polystyrene sulfonate

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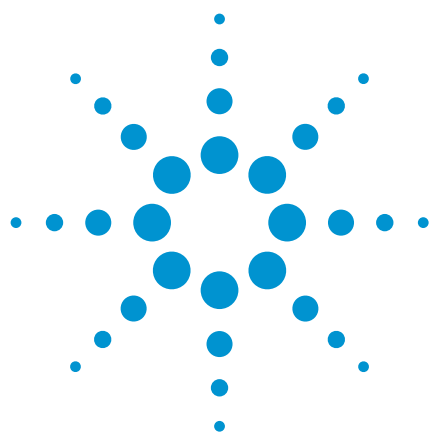
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SI-01574



Agilent Technologies



SEC Analysis of Poly 2-Vinyl Pyridine

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Poly 2-vinyl pyridines are non-toxic, water soluble, cationic polymers with the ability to chelate heavy metals. This makes them valuable in removing metals found in poisoning and contaminated environments. They also have useful electrical conductivity properties when reacted with halogens such as iodine. This property is made use of in heart pacemakers. A sample of poly 2-vinyl pyridine was analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. The pH of the salt buffer eluent was reduced to 3 in order to minimize ionic interaction between the sample and column. In order to dissolve the sample, the solvent pH had to be reduced further to pH 1.5 but the polymer remained in solution at pH 3 for analysis.



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Conditions

Sample: Poly 2-vinyl pyridine
Columns: 2 x PL aquagel-OH 50 8 μ m,
300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.8 M NaNO₃ + 0.01 M NaH₂PO₄ at
pH 3
Flow Rate: 1.0 mL/min
Detection: RI

Conclusion

SEC using PL aquagel-OH columns successfully analyzed a sample of poly 2-vinyl pyridine. Aqueous SEC not only provides molecular weight data but also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

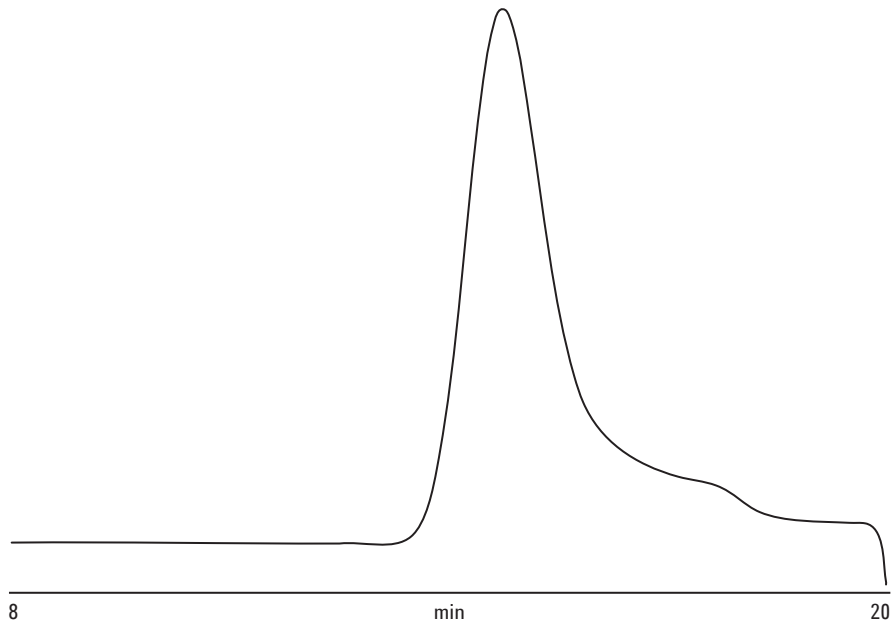


Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

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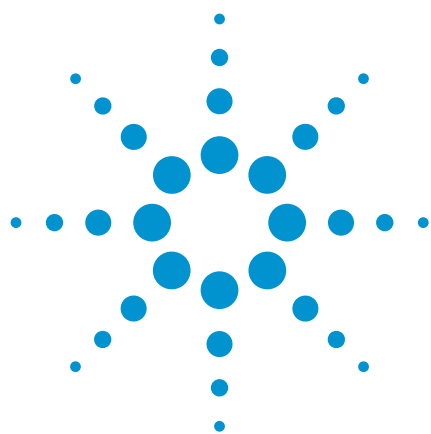
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Agilent Technologies



SEC Analysis of Sodium Polyacrylate

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Sodium polyacrylate ($-\text{CH}_2-\text{CH}(\text{COONa})_n$) is an acrylic acid polymer sodium salt. The value of this polymer lies in its ability to absorb up to 1,000 times its weight in distilled water, giving it a high gel capacity. Such polymers are referred to as 'super absorbents' or 'water crystals'. Unsurprisingly, sodium polyacrylate finds applications that make use of its superabsorbency, such as diapers and incontinence pads, and in agriculture for spray drift control, seed germination, soil conditioning, hydroponics and as a water storage agent for soils, where it soaks up soil moisture and releases it when the soil dries out. It is also used by many industries as a thickener and stabilizer. SEC using Agilent PL aquagel-OH columns is an excellent system for characterizing this polymer. The columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution.



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Conditions

Samples: Sodium polyacrylate
Columns: 2 x PL aquagel-OH 40 8 μm , 300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.2 M NaNO_3 + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

The chromatogram shows a relatively low molecular weight sample with Mw approximately 13,000. The sample was readily soluble and the salt/buffer eluent was selected to minimize ionic effects.

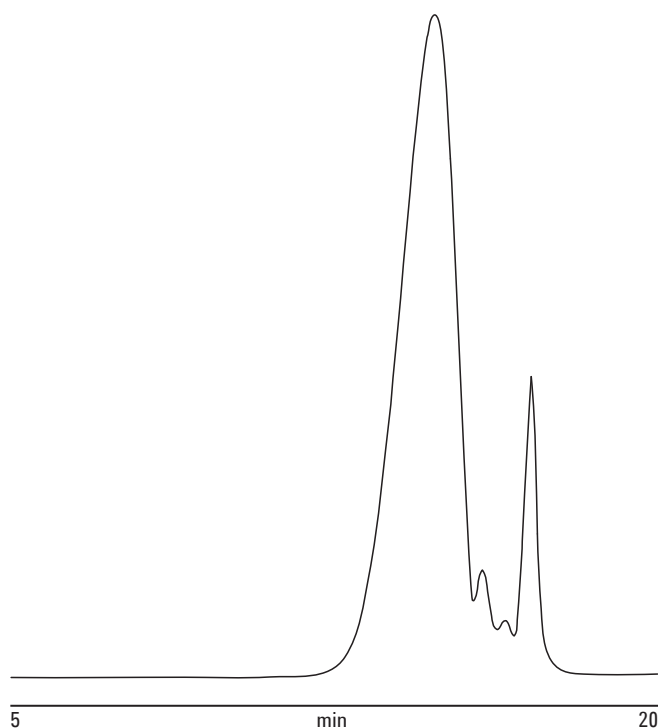


Figure 1. Raw data chromatogram of sodium polyacrylate

Conclusion

SEC using PL aquagel-OH columns successfully analyzed a sample of sodium polyacrylate. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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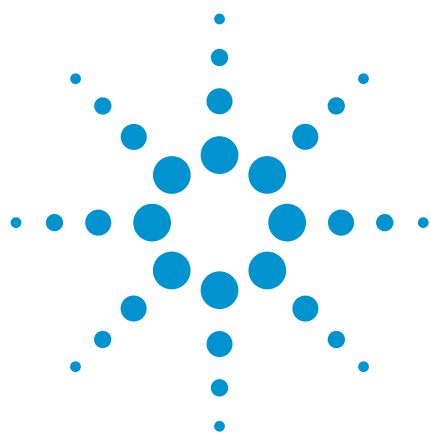
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SI-01579



Agilent Technologies



SEC for the Analysis of Non-ionic Surfactants

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Non-ionic surfactants are used in a variety of applications ranging from cleaning and personal care products to industrial processing. The hydrophobic and the hydrophilic portions of the surfactant molecule can be incrementally varied and manipulated to any molecular weight, in order to adjust its properties to suit a particular application. The determination of the surfactant molecular weight distribution is therefore extremely important in terms of performance as well as batch to batch reproducibility. This type of sample is normally relatively low in terms of molecular weight. Agilent PL aquagel-OH 30 8 μm columns are ideal for these analyses, because they combine low exclusion limit, high pore volume and high column efficiency ($>35,000$ plates/meter) for maximum resolution. Column calibration was achieved using polyethylene oxide Agilent EasiVial standards (Figure 1). EasiVials provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). Each vial contains a mixture of four individual, highly characterized, narrow dispersity standards. The amount of each individual standard is carefully controlled during manufacture, allowing their use in SEC-viscometry, which requires accurate concentrations.



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Conditions

Non-ionic surfactants contain hydrophobic species so the addition of 30% of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions between the sample and the column packing. The PL aquagel-OH packing material allows such eluent modifications while retaining the high column efficiency.

Samples: Surfactants; EasiVial PEO standards
Columns: 2 x PL aquagel-OH 30 8 μ m,
300 x 7.5 mm (p/n PL1120-6830)
Eluent: Water + 0.2 M NaNO_3 + 0.01 M
 NaH_2PO_4 at pH 7 + 30 % methanol
Flow Rate: 1.0 mL/min
Detection: RI

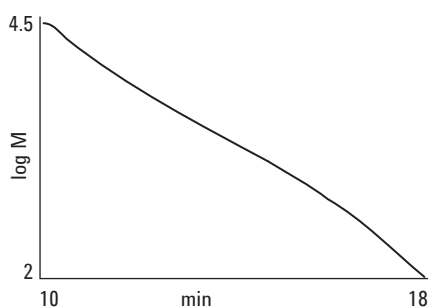


Figure 1. EasiVial calibration of PL aquagel-OH columns

Results and Discussion

A typical raw data chromatogram for the sample is illustrated in Figure 2. The sample displays a low molecular weight shoulder.

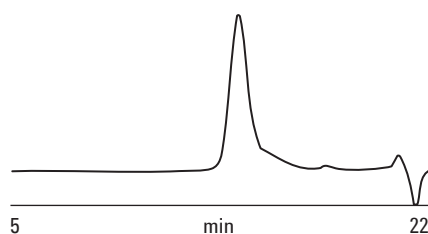


Figure 2. Chromatogram of a non-ionic surfactant

Conclusion

The presence of significant hydrophobicity in a surfactant is no barrier to its resolution by SEC with PL aquagel-OH columns. The column's ability to handle eluents containing up to 50% methanol means that such compounds can be resolved without interactions.

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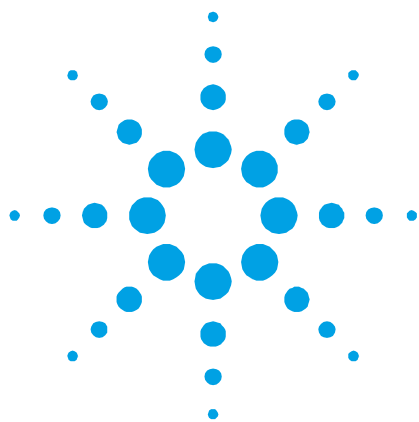
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SI-01642



Agilent Technologies



Monitoring fast reactions using Stopped Flow Kinetics on the Cary 50/60 UV-Vis

Application Note

Chemical

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Introduction

There are many factors which control the rate of a chemical reaction. These include the type of metal center, the size and charge of the ligands, the concentration of the reactants, and environmental conditions such as pH and temperature. All these factors can produce systems with half lives in the region of milliseconds to hours to days.

One method of monitoring a reaction is through UV-Vis spectrophotometry. If the reactant or product exhibits a change in absorbance as a function of reaction time, this method can usually be employed. A conventional cell is adequate for reactions with half lives greater than a couple of minutes, however, reactions that are over within a second or milliseconds need specialized equipment. A Rapid Kinetics Accessory (or Stopped-Flow Apparatus) can be used to measure such fast reactions.



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Theory

Conventional spectrophotometric techniques cannot be used when investigating reactions that occur at a sub-second rate. If reactants are added manually, and then stirred for a few seconds to allow adequate mixing, the reaction is over and no changes in spectra are recorded. This is overcome by using a Stopped-Flow apparatus (Figure 1) that provides instantaneous mixing and recording of data on a tens-of-millisecond time scale. The technique rapidly mixes two solutions in a flow cell and starts recording data when the mixing ceases. The Cary 50 and Cary 60 spectrophotometer's can record a data point every 12.5 ms.



Figure 1. Rapid Mix Accessory - SFA20

The stopped flow apparatus is connected to the spectrophotometer through a remote-sense cable that is attached to an accessory controller port on the instrument. The reactants are placed in two syringes, labelled Solution A and Solution B, as shown in Figure 2. Upon pressing the plunger, the solutions travel separately to the cuvette and only mix upon entering the cell. Previously reacted solution is ejected into a waste syringe, which moves back until hitting a micro switch. Solution then stops flowing into the cell and data collection begins instantaneously, which eliminates any delay caused by a manual start.

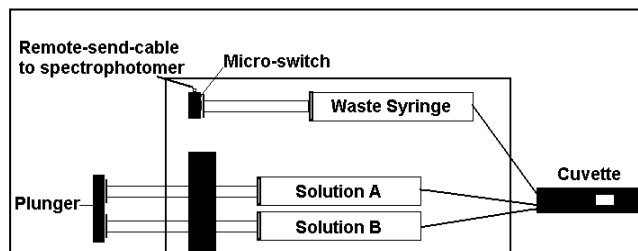


Figure 2. Schematic of Rapid Mix Accessory

The data are processed in much the same way as when using conventional techniques. The in-built algorithms, available in the Cary WinUV kinetics application, allows zero, first and second order fits to be applied to the experimental data. The fitted curve is displayed on the graph and the calculated parameters appear in the report. It is critical that there are a sufficient number of data points in the initial stage of the reaction as this is where most of the changes in the spectra occur. Enough data spanning 3-4 half lives should be collected. The Cary 50/60 UV-Vis spectrophotometers coupled to a Stopped-Flow apparatus can measure reactions that are over within a couple of hundred milliseconds!

Equipment

- Cary 50 UV-Vis spectrophotometer¹
- Rapid Mix Accessory (SFA-20)²
- Green food dye
- Bleach in the form of White King³

Experimental

To demonstrate the extremely fast data collection capabilities of the Cary 50/60 UV-Vis, the rate of bleaching of green food dye was investigated. The rate of the reaction was controlled by varying the concentration of bleach, until the limits for measuring this reaction were achieved. First-order kinetic fits were then applied to the data.

A solution of green dye in de-ionized distilled water was prepared so as to give an absorbance of ca. 0.4 (Solution A).

Solution B was prepared by diluting White King (0.655 mL) with 30 mL of de-ionized distilled water.

The instrument parameters for the Cary 50/60 were set up as follows:

Wavelength (nm)	414
Ave Time (s)	0.0125
Y Min	0
Y Max	0.5
Cycle (min)	0
Stop (min)	0.2

Results

The color green is composed of the 2 primary colors yellow and blue, which is reflected in the UV-Vis spectrum of green food dye in water, Figure 3. The yellow component has an absorbance at 414 nm and the blue component at 629 nm. The addition of bleach causes a rapid change in the absorbance at 414 nm, following a first order decay path, the rate of which depends on the concentration of bleach. The reaction was monitored at 414 nm.

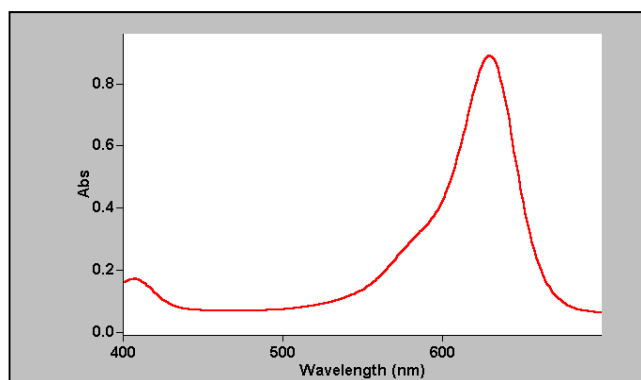


Figure 3. UV-Vis spectrum of green food dye in water

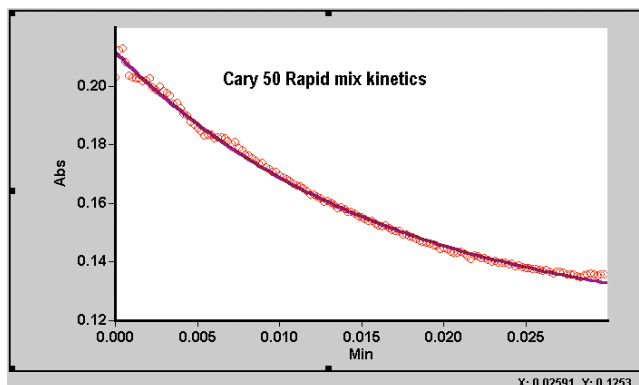


Figure 4. Change in absorbance at 414 nm of green dye and bleach on a Cary 50

Figure 4 shows the change in absorbance over time for the bleaching of green food dye collected on a Cary 50. This experiment could also be done on a Cary 60 UV-Vis. The reaction is over within 2 seconds, and the rate constant for a first order decay was calculated to be 60.320 min^{-1} with a SD of 0.0013.

Discussion

In order to accurately monitor reactions with sub-second half-lives, a spectrophotometer must have the following features:

1. Minimum delay time between mixing of reagents and recording the first data point.
2. The ability to acquire enough data points per second to allow accurate fitting of the data.
3. Remote read plug to accommodate a Rapid Mix Accessory.

The Cary 50/60 UV-Vis has minimized the waiting period between mixing and collecting the first data point by incorporating a 'Synch Start' function in its software. This essentially 'primes' the software, preparing it for its first reading. The overall delay is less than 50 ms on a Cary 50/60, which means that valuable data are not lost during the initial part of the reaction.

The WinUV kinetics software provides all the necessary tools to analyze and display the data. Curve fitting is completed within seconds and the fitted curve is overlaid on the experimental data. The option of displaying the experimental data as points and the fitted curve as a solid line results in a professional report, which can be customized by the user.

The instrument parameters used in the data collection and the results of the curve fitting are also presented in the report.

Conclusion

The Cary 50/60 is the fastest wavelength scanning UV-Vis spectrophotometer currently on the market. The minimum delay between mixing of the reagents and collecting the first data point allows for the collection of more data during the initial part of the reaction, where the greatest change in absorbance occurs. This enables accurate monitoring and analysis of reactions that occur on a sub-second level.

References

1. Part numbers for ordering:

Product	Part Number
Cary 60 UV-Vis with WinUV software and PC	G6860AA

2. Part numbers for ordering:

Product	Part Number
SFA-20	7910030400
Mounting bracket (Cary 300)	7910030500
Country Kit	depends on country

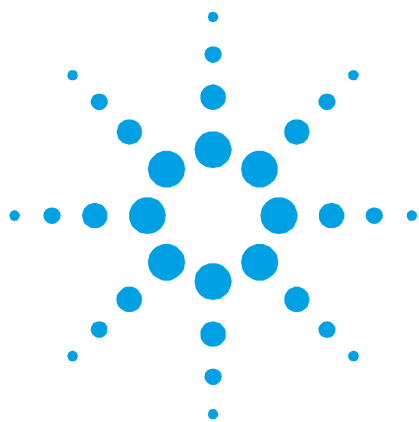
3. Household laundry bleach liquid with 40 g/L available chlorine present as sodium hypochlorite.

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Investigation of photochemical reactions using the Cary 50/60 UV-Vis

Application Note Chemical

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Introduction

A photochemical reaction is a reaction that is initiated by the absorption of light. The reaction may then proceed with or without continuous irradiation. The reactivity arises from the absorption of light, which places the reactant molecules into an excited state. These molecules may then undergo a variety of subsequent reactions to form products. This reactivity is generally not observed in the ground state, i.e. when no light has been absorbed, however, very slow thermal reactions may occur¹.

In order to investigate a photochemical reaction *in situ*, using UV-Vis spectrophotometry, it is essential that the analyzing beam from the instrument does not degrade the sample, especially if the irradiating wavelength differs from that of the analyzing wavelength/s. This is a problem with commercial diode array spectrophotometers, where the sample is analyzed with white light. Significant photodegradation occurs over time.

Another requirement of the spectrophotometer is that it must accommodate a second external source used for irradiation. Light from this source may be introduced to the sample via a fiber optic bundle, which means that the instrument must be able to operate with the sample compartment open. A black cloth may be placed over the instrument and sample to prevent unwanted degradation from room light. A subsequent consideration then becomes stray light interference from the irradiating beam with the analyzing measurement from the spectrophotometer. If the instrument is not room light immune, then the intensity of the irradiating beam can cause major problems, particularly with deviations from photo-linearity instrument specifications. This can result in significant errors with the data collected.



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The Cary 50 and Cary 60 UV-Vis takes into consideration all of the aforementioned points. It is room light immune, so stray light is not a problem, and it does not degrade even the most photo-sensitive samples. Figure 1 shows the absorbance at 366 nm over time for the irradiation of a photoactive platinum compound in acetonitrile². The experiment, performed on a Cary 50 and a commercial spectrophotometer, shows significant photo-degradation by the other instrument.

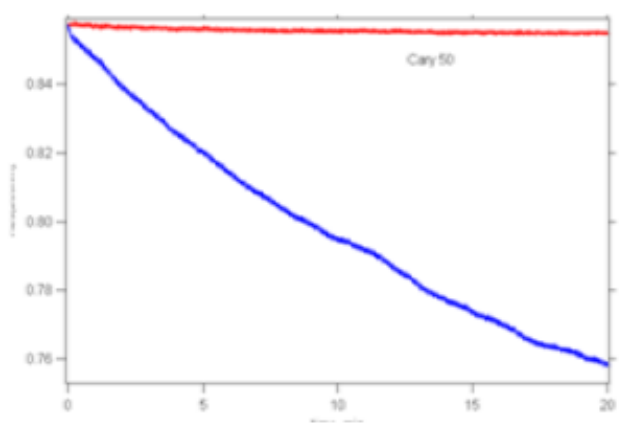


Figure 1. Absorbance(366 nm) vs time of platinum complex

This paper demonstrates the advantages of the Cary 50 and Cary 60 when used to monitor photochemical reactions *in situ*, by investigating the photochemical degradation of the compound potassium ferrioxalate ($K_3[Fe(C_2O_4)_3]$).

Background

The efficiency of a photochemical reaction is defined by its quantum yield of product formation, i.e. how much product is produced with respect to how much light is absorbed. For a reaction where $A \rightarrow B$ via the absorption of light, the quantum yield of photo-product formation (Φ_{PR}) is defined by Equation 1, where I_{abs} is the amount of light absorbed by the reactant, A, and $d[A]/dt$ is the change in concentration of A with time.

$$-\frac{d[A]}{dt} = \Phi_{PR} I_{abs} \quad \text{Equation 1}$$

The change in concentration is followed by measuring the absorbance at a specific wavelength and using the Beer-Lambert law to convert this to a concentration. The intensity of absorbed light is calculated using Equations 2-5, which require the measurement of the absolute intensity, I_0 , of the irradiating source.

$$I_{abs} = \int_0^t I_{abs}(t) dt \quad \text{Equation 2}$$

$$I_{abs}(t) = I_{sol}(t) \alpha(t) \quad \text{Equation 3}$$

$$I_{sol}(t) = I_0 \left(1 - 10^{-Abs_{\lambda,t} l}\right) \frac{S}{V} \quad \text{Equation 4}$$

$$\alpha(t) = \frac{\epsilon_A [A]_t l}{Abs_{\lambda,t}} \quad \text{Equation 5}$$

Where :

I_{sol} = the amount of light absorbed by the solution

$\alpha(t)$ = the fraction of light absorbed by **1a**

S = the area exposed to irradiation (cm²)

V = the volume of solution irradiated (dm³)

The absolute light intensity of a source can be determined through actinometry. An actinometer is a substance or device that responds, in some measurable way, to the amount of light it absorbs. In the case of a chemical actinometer, the absorption of light leads to a photochemical transformation that is monitored by some analytical technique. The amount of reactant transformed is then used to calculate the intensity of irradiating light in units of Einsteins per unit time and area.

The sensitivity of the actinometer depends on the quantum yield for the photolysis reaction and the method of analysis. The latter is the most important as some analysis methods can cause significant degradation of the actinometer solution. Also, since most actinometers are extremely sensitive to room light, they must be protected and analyzed in a darkroom under red or yellow safelights.

Experimental

Reagents:

Potassium ferrioxalate (0.0114 M)

Buffer (CH₃COONa/H₂SO₄ pH 3.5)

1:10 phenanthroline (0.1% w/v)

Deionized distilled water

Apparatus:

Cary 50 (or Cary 60) UV-Vis

WinUV Software

Single cell Peltier temperature accessory

10 mm pathlength quartz cuvette

100 W Hg arc lamp/housing (Oriel)

366 nm bandpass filter

3' quartz fiber optic bundle (Dolan Jenner)

Potassium ferrioxalate (0.0114 M, 1.30 cm³) was added to the quartz cuvette, immediately followed by CH₃COONa/H₂SO₄ buffer solution (pH 3.5, 0.70 cm³) and 1:10 phenanthroline (0.1% w/v, 0.50 cm³). The cuvette was placed in the thermostatted cell holder (25 °C) and allowed to equilibrate for 5-10 minutes in the dark. The solution was then irradiated with 366 nm Hg light and the absorbance at 510 nm monitored. The instrument parameters used are listed below and Figure 2 shows the setup used for irradiation.

Wavelength (nm)	366
Av. Time (s)	0.1
Y Min	0
Y Max	1.0
Cycle (min)	0
Stop (min)	30

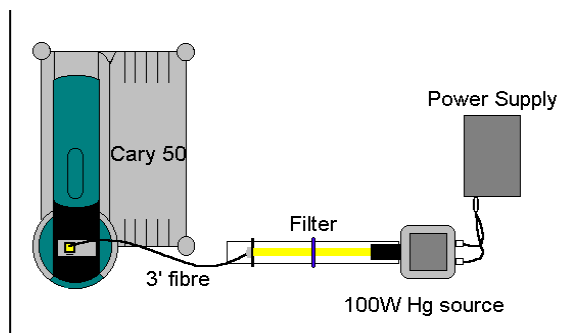
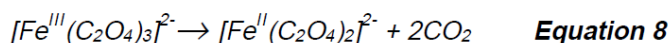
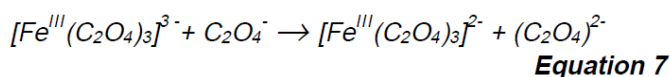
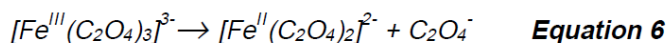


Figure 2. Setup of apparatus used for irradiating sample

Light from a 100 W Hg source passes through a bandpass filter isolating the intense 366 nm Hg line, and is directed via a 3 foot fiber optic bundle into the top of a quartz cuvette. The solution of K₃[Fe(C₂O₄)₃] in the cuvette, which is continuously stirring, immediately undergoes photochemical reduction upon irradiation from the Hg source and the change in absorbance is measured at 510 nm. The solution is analyzed using a Cary 50 UV-Vis with the Cary WinUV Kinetics software package. A Cary 60 UV-Vis can also be used for this experiment.

Discussion

Potassium ferrioxalate is an extremely sensitive actinometer. Investigated by Hatchard and Parker³ in the mid 1950s, K₃[Fe(C₂O₄)₃] undergoes photochemical reduction from Fe³⁺ to Fe²⁺ upon absorbing UV-Vis ($\lambda < 500$ nm) radiation, as shown in Equations 6-8. The quantum yield of Fe²⁺ formation is reported in Hatchard and Parker's paper, and the change in amount of Fe²⁺ produced during irradiation is followed by UV-Vis spectrophotometry at 510 nm. The addition of 1:10 phenanthroline to the irradiated solution results in the formation of [Fe(phen)]²⁺, which has an absorption maximum at 510 nm.



In the past, the amount of Fe^{2+} produced has been determined by measuring the absorbance of $\text{K}_3[\text{Fe}(\text{III})(\text{C}_2\text{O}_4)_3]$ at 510nm before irradiation, irradiating the sample for a known time, adding 1:10 phenanthroline in a dark room, and then measuring the absorbance of $[\text{Fe}(\text{II})(\text{phen})_3]^{2+}$ at 510 nm again.

The intensity of the irradiating source, I_0 , is then calculated from the amount of Fe^{2+} produced during irradiation.

The Cary 50/60 allows the above procedure to be slightly modified. Due to the room light immunity of the instrument, the change in absorbance at 510 nm can be measured *in situ* during irradiation of the $\text{K}_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ solution. This reduces the handling of solution during the reaction which, in turn, eliminates unnecessary exposure to ambient light. In order to avoid degradation during the reaction due to room light, a thick black cloth was placed over the entire instrument.

Figure 3 shows the change in absorbance at 510 nm in the absence (0 - 1.8 min) and presence (1.8 - 4 min) of the irradiating beam.

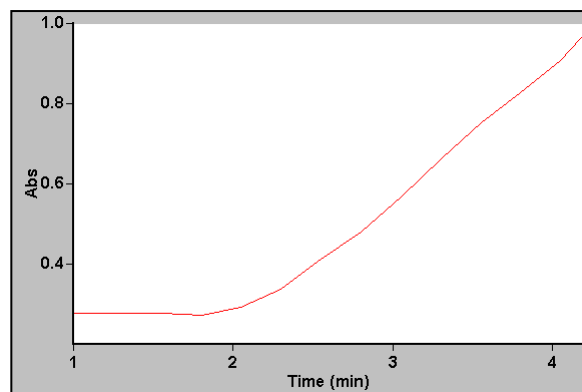


Figure 3. Absorbance(510 nm) vs time during irradiation with 366 nm Hg light

The change in absorbance at 510 nm only occurs during irradiation and is linear. This linearity and absence of noise in the data, shows that stray light has little effect on the measurements and also confirms that stirring was extremely efficient.

The intensity of the irradiating beam, in Einsteins $\text{s}^{-1} \text{dm}^{-3}$, was calculated from the change in absorbance using Equations 9 and 10⁴.

$$\frac{n[\text{Fe}(\text{phen})_3]^\text{II}}{dt} = \frac{d\text{Abs}_{510}}{dt} \frac{V}{\epsilon_{510}l} \quad \text{Equation 9}$$

Where

V = volume of solution irradiated (dm^3)

ϵ_{510} = molar extinction coefficient at 510 nm of $[\text{Fe}(\text{phen})_3]^{2+}$ ($\text{M}^{-1}\text{cm}^{-1}$)

l = pathlength (cm)

$$I_0 = \frac{n[\text{Fe}(\text{phen})_3]^\text{II}}{\phi_{\text{Fe}^{2+},\lambda} \frac{dt}{\epsilon_{510}l}} \quad \text{Equation 10}$$

Equation 9 calculates the change in the number of moles of $[\text{Fe}(\text{phen})_3]^{2+}$ with respect to irradiating time, which is used in Equation 10 to determine the intensity of the irradiating beam, I_0 . Using the value of 1.21 for the quantum yield of Fe^{2+} formation when $\text{K}_3\text{Fe}(\text{C}_2\text{O}_4)_3$ is irradiated with 366 nm light³, the intensity of the irradiating beam incident upon the cuvette is $(9.32 \pm 0.02) \times 10^{-10} \text{ Einstein s}^{-1} \text{cm}^{-2}$.

Conclusion

The Cary 50 and Cary 60 UV-Vis allow measurements to be performed whilst using an external light source to irradiate the sample. The problems of noise and deviations from photo-linearity, associated with stray light, are not observed. The Cary 50/60 is able to study even the most light-sensitive of compounds *in situ* and does not cause photodegradation from the analyzing beam, which generally occurs when white light is used for analysis.

References

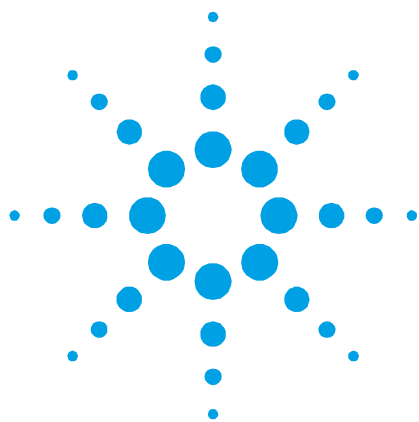
1. N. J. Turro, *Modern Molecular Photochemistry*, Benjamin/Cummings Pub. Co., California, **1978**.
2. J. Comerford and P. A. Tregloan, *Personal Communication*, The University of Melbourne, Australia, **1997**.
3. C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc. A*, **278**, 518, **1956**.
4. J. F. Rabek, *Experimental methods in photochemistry and photophysics (Part 2)*, Wiley, New York, p944, **1982**.

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Agilent Technologies



The investigation of the photo-kinetics of a platinum organoamine complex using the Cary 50/60

Application Note

Chemical

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Introduction

A photochemical reaction is a reaction that is initiated by the absorption of light. The reaction may then proceed with or without continuous irradiation. Reactivity arises from the absorption of light, which places the reactant molecules into an excited state. These molecules may then undergo a variety of subsequent reactions to form products. This reactivity is generally not observed in the ground state, i.e., when no light has been absorbed, however, very slow thermal reactions may occur¹.

In order to investigate a photochemical reaction *in situ*, using UV-Vis spectrophotometry, it is essential that the analyzing beam from the instrument does not degrade the sample, especially if the irradiating wavelength differs from that of the analyzing wavelength/s. This exemplifies the problem encountered with commercial diode array spectrophotometers, where the sample is analyzed with white light.

Another requirement of the spectrophotometer is that it must accommodate a second external source used for irradiation. Light from this source may be introduced to the sample via a fiber optic bundle, which means that the instrument must be able to operate with the sample compartment open. A black cloth is usually used to prevent unwanted degradation from room light. A subsequent consideration then becomes the stray light interference from the irradiating beam with the analyzing beam from the spectrophotometer. If the instrument is not room light immune, then the intensity of the irradiating beam can cause instrument problems, such as deviations from instrument photo-linearity specifications.



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The Agilent Cary 50 and Cary 60 takes into consideration all of the aforementioned points. It is room light immune, so stray light is not a problem, and it does not degrade even the most photo-sensitive samples. Figure 1 shows the absorption at 366 nm vs time profile for the irradiation of a photoactive platinum compound, [N,N'-Bis(2,3,5,6-tetrafluorophenyl)ethane-1,2-diaminato(2-)]dipyridineplatinum(II) in acetonitrile (quantum yield of photosubstitution in acetonitrile of 0.96)², performed on a Cary 50 and a commercial instrument. As can be seen, significant photo-degradation is caused by the other spectrophotometer.

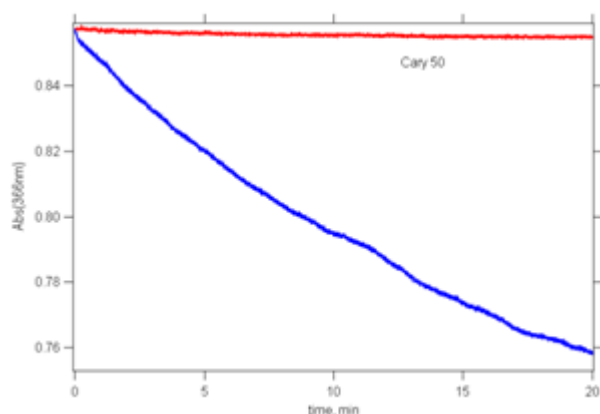


Figure 1. Absorbance(366 nm) vs time of platinum complex

This paper demonstrates the advantages of the Cary 50/60 UV-Vis when used to monitor photochemical reactions, by investigating the kinetics of [N,N'-Bis(2,3,5,6- tetrafluorophenyl)ethane-1,2-diaminato(2-)]dipyridineplatinum(II) in acetonitrile.

Background

The photochemistry of [N,N'-Bis(2,3,5,6-tetrafluorophenyl)ethane-1,2-diaminato(2-)]dipyridineplatinum(II)ⁱ, referred from now on as **1a**, has been investigated in previous studies^{2,4}. When irradiated with UV/blue light, solutions of **1a** undergo photochemical substitution in which a pyridine ligand is replaced by a solvent molecule (Figure 2). Continued irradiation sees the loss of a second pyridine and

finally the formation of a tetrakis-platinum solvent species.

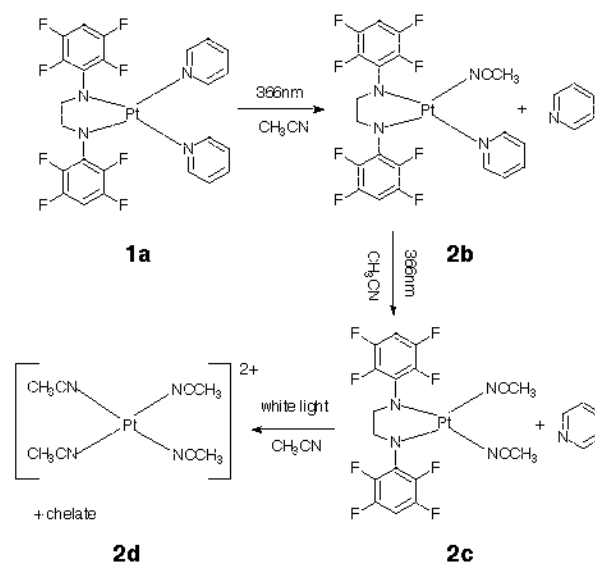


Figure 2. Schematic for the photosubstitution of **1a** in acetonitrile

The quantum yield of photoproduct formation for the mono-solvento complex has been determined in acetonitrile, DMSO and THF². In fact, the quantum yield measured in acetonitrile is the highest ever reported for a platinum photosubstitution reaction. The mechanism of photosubstitution is shown in Figure 3.

Irradiation with light of 366 nm populates a ligand-ligand charge transfer (LLCT) excited state, which is in thermal equilibrium with a ligand field (LF) excited state. High pressure photochemical experiments support a dissociative mechanism, in which the formation of the mono-solvento species occurs via a three coordinate transition state intermediate².

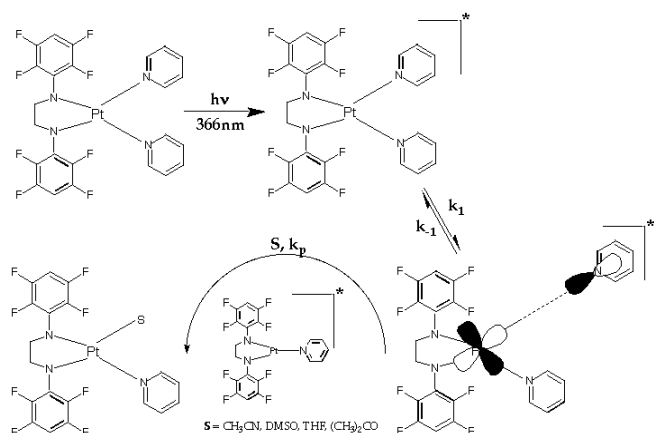


Figure 3. Mechanism of photosubstitution of 1a in solution

Experimental

Reagents:

- Acetonitrile (BDH, HPLC grade)
- [N,N'-Bis(2,3,5,6- tetrafluorophenyl)ethane-1,2-diaminato(2-)] dipyrindineplatinum(II)
- Potassium ferrioxalate (0.0114 M)
- Buffer (CH₃COONa/H₂SO₄ pH 3.5)
- 1:10 phenanthroline (0.1% w/v)
- MilliQ water

Apparatus:

- Agilent Cary 50 (Cary 60) UV-Vis
- WinUV Software
- Single cell Peltier temperature accessory
- 10mm pathlength quartz cuvette
- 100W Hg arc lamp/housing
- 366 nm bandpass filter
- 3' quartz fibre optic bundle

Figure 4 shows the setup used for irradiation.

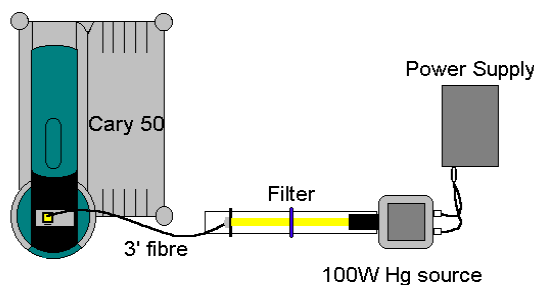


Figure 4. Setup of apparatus used for irradiating sample

White light from a 100W Hg source passes through a bandpass filter isolating the intense 366 nm Hg line, and is directed via a 3 foot fibre optic bundle into the top of a quartz cuvette. The solution in the cuvette, which is continuously stirred, immediately undergoes photochemical change upon irradiation from the Hg source. The solution is analysed using a Cary 50 UV-Vis with the WinUV Scanning and Kinetics software packages. A Cary 60 UV-Vis can also be used for this experiment.

Discussion

The intensity of the 366 nm irradiating beam from the Hg lamp was measured through chemical actinometry⁵. The method used is a modification from Hatchard and Parker's original procedure⁵, the details of which are fully described in References 2 and 4 and summarised below.

Potassium ferrioxalate (0.0114M, 1.30 cm³) was added to the quartz cuvette, immediately followed by CH₃COONa/H₂SO₄ buffer solution (pH 3.5, 0.70 cm³) and 1:10 phenanthroline (0.1% w/v, 0.50 cm³). The cuvette was placed in the thermostatted cell holder (25 °C) and allowed to equilibrate for 5-10 minutes in the dark. The solution was then irradiated with 366 nm Hg light and the absorbance at 510 nm monitored in the absence (no change in Abs, Figure 5) and presence (change in Abs, Figure 5) of the irradiating beam.

The instrument parameters used are outlined below.

Wavelength (nm)	510
Ave Time (s)	0.1
Y Min	0
Y Max	1.0
Cycle (min)	0
Stop (min)	30

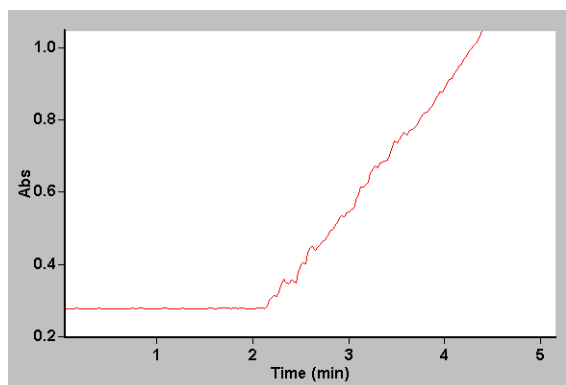


Figure 5. Absorbance(510 nm) vs time during irradiation with 366 nm Hg light

The change in absorbance only occurs in the presence of the irradiating beam. The intensity of the irradiating beam, in einsteins s⁻¹ dm⁻³, is calculated from this change in absorbance with time using Equations 1 and 2 below⁶.

$$\frac{n[\text{Fe(phen)}_3]^{2+}}{dt} = \frac{d\text{Abs}_{510}}{dt} \frac{V}{\epsilon_{510} l} \quad \text{Equation 1}$$

Where :

V = volume of solution irradiated (dm³)

ϵ_{510} = molar extinction coefficient at 510 nm of [Fe(phen)₃]²⁺ (M⁻¹cm⁻¹)

l = pathlength (cm)

$$I_0 = \frac{n[\text{Fe(phen)}_3]^{2+}}{\phi_{\text{Fe}^{2+}, \lambda} \frac{dt}{dt}} \quad \text{Equation 2}$$

Equation 1 calculates the change in the number of moles of [Fe(phen)₃]²⁺ with respect to irradiating time, which is used in Equation 2 to determine the intensity of the irradiating beam, I₀. The quantum yield for Fe²⁺ formation is 1.21, as determined by Hatchard and Parker⁵. The intensity of the irradiating source is determined before and after the photolysis of **1a**, and the result averaged to account for any intensity fluctuations in the Hg lamp over the course of the experiment. The lamp used is relatively stable over a period of days. The average intensity of the irradiating beam incident on the platinum solution is 9.32(2) × 10⁻¹⁰ einstein sec⁻¹ cm⁻².

Compound **1a** in acetonitrile (2.5 cm³) was then irradiated with the Hg source. The change in spectra over a 250 nm range (Figure 6) and the absorbance at 366 nm (Figure 7) were recorded in two separate experiments using the WinUV Scan and Kinetics packages respectively.

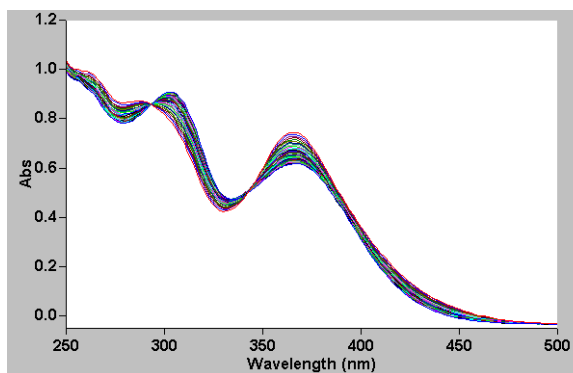


Figure 6. Change in absorption spectra of 1a in CH₃CN during irradiation with Hg light

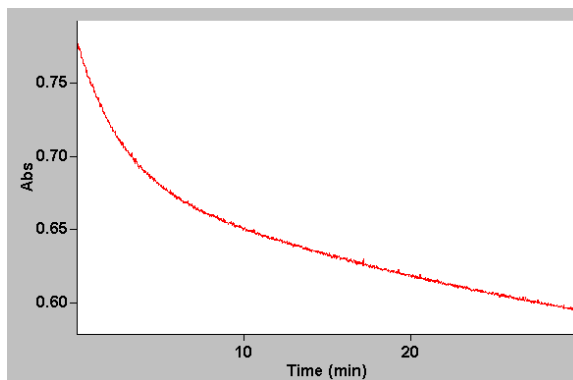


Figure 7. Change in absorbance(366 nm) as a function of time during irradiation

Figure 6 shows the formation of 2 isosbestic points, at 293 nm and 344 nm, during the initial stage of photolysis. During this time, only the mono-CH₃CN complex is being formed. The extremely fast scanning speed of the Cary 50/60 allows the fifty overlaid spectra in Figure 6 to be collected in less than 2 minutes.

The change in absorbance(366 nm) with time, $Abs_{\lambda,t}$, was used in Equation 3 to calculate the change in concentration of **1a**, $[A_t]$. The molar extinction coefficients at 366 nm of **1a** (ϵ_A) and **2b** (ϵ_B) have been previously determined², hence, $d[1a]/dt$ can be calculated.

$$[A_t] = \frac{Abs_{\lambda,t} - (\epsilon_B [A_0] l)}{l(\epsilon_A - \epsilon_B)} \quad \text{Equation 3}$$

Where :

l = pathlength (cm)

$[A_0]$ = the initial concentration of **1a**

The quantum yield of photoproduct formation (Φ_{PR}) of **2b** is defined as the amount of **2b** formed (or the amount of **1a** depleted), with respect to the amount of light absorbed by **1a**. The amount of light absorbed by **1a** over the course of the experiment (I_{abs}) is given by Equations 4-7⁷.

$$I_{abs} = \int_0^t I_{abs}(t) dt \quad \text{Equation 4}$$

$$I_{abs}(t) = I_{sol}(t) \alpha(t) \quad \text{Equation 5}$$

$$I_{sol}(t) = I_0 \left(-10^{-Abs_{\lambda,t} l} \right) \frac{S}{V} \quad \text{Equation 6}$$

$$\alpha(t) = \frac{\epsilon_A [A]_t l}{Abs_{\lambda,t}} \quad \text{Equation 7}$$

Where:

I_{sol} = the amount of light absorbed by the solution

$\alpha(t)$ = the fraction of light absorbed by **1a**

S = the area exposed to irradiation (cm²)

V = the volume of solution irradiated (dm³)

The quantum yield of formation of **2b** is then determined by plotting the change in concentration of **1a** against I_{abs} (Figure 8), and fitting a linear regression over the first 10% of the reaction. The quantum yield is given by the gradient, as defined in Equation 8.

$$-\frac{d[A]}{dt} = \Phi_{PR} I_{abs} \quad \text{Equation 8}$$

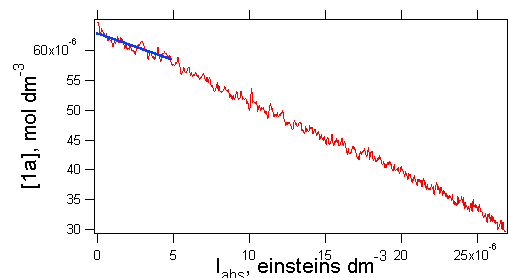


Figure 8. [1a] vs I_{abs} in acetonitrile

The WinUV software allows data to be exported as ASCII *.csv files. These files were imported into the mathematical analysis program IGOR PRO⁸, where Equations 3-8 were calculated. Figure 8 was also generated in IGOR PRO. A calculated quantum yield of (0.92 ± 0.04) molecules per photon of light absorbed is in excellent agreement with the literature value of (0.96 ± 0.03) ².

Conclusion

The Agilent Cary 50 and Cary 60 UV-Vis, with its room light immunity and fast scan speeds, has revolutionized the world of UV-Vis spectroscopy. Coupled with the powerful and versatile WinUV Kinetics and Scanning software, the Cary 50/60 UV-Vis is ideal for the study of photochemical kinetics. In analysing photochemical reactions, it allows the use of an irradiating light source without the problems associated with stray light. Also, studying even the most light-sensitive of compounds *in situ*, does not cause photodegradation from the analysing beam, which generally occurs when white light is used for analysis.

References

1. P. C. Ford, *Inorganic Reactions and Methods*, VCH, Deerfield Beach, Florida, 1986 Strong, J., 'Procedures in Experimental Physics', 1st Ed., Prentice-Hall, Inc., New York, 1938, 376.
2. J. Comerford, *PhD Thesis*, The University of Melbourne, 1997 Cary WinUV Software, 'Cary Help' online help, Version 3.0.
3. D. P. Buxton, G. B. Deacon, B. M. Gatehouse, I. L. Grayson, R. J. Thomson and D. St.C. Black, *Aust. J. Chem.*, **39**, 2013-26, 1986
4. J. Comerford, G. B. Deacon, P. A. Tregloan, "*Manuscript in progress*"
5. C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc. A*, **278**, 518, 1956
6. J. F. Rabek, *Experimental methods in photochemistry and photophysics (Part 2)*, Wiley, New York, p944, 1982
7. V. Balzani, V. Carassiti, *Photochemistry of Coordination Compounds*, Academic Press Inc., London, 1970
8. *Igor Pro User's Guide*, Wavemetrics Inc., 1996

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 Sensitive Analysis of Polystyrene 960 and 580 by Reversed Phase HPLC with ELSD
 Fingerprinting Isocyanate Pre-polymer Fractions by Agilent PLgel MIXED-E and GPC
 Polybutadiene Terephthalate Analysis by Agilent PL HFIPgel and GPC
 Crystalline Polyester Analysis on Agilent PLgel with Gel Permeation Chromatography
 Unsaturated Polyester Resins on Agilent PLgel with Gel Permeation Chromatography
 Styrene/Isoprene Block Copolymers with Agilent PLgel MIXED-D and GPC/SEC
 Polyurethane Analysis on an Agilent PLgel Column with Gel Permeation Chromatography
 High Resolution Analysis of Polyethylene Glycol using HPLC with ELSD
 Fast Analysis of Light Stabilizers by HPLC with ELSD
 Sensitive Polymer Analysis using Critical Point Chromatography and ELSD
 Mono-, Di-, and Tristearoylglycerol Analysis on Agilent PLgel by GPC
 Polystyrene Oligomer Analysis on Agilent PLgel with Gel Permeation Chromatography
 Dialkyl Phthalate Analysis on Agilent PLgel 5 using Gel Permeation Chromatography
 Polyester Polyol Analysis on Agilent PLgel 5 using Gel Permeation Chromatography
 Dialkyl Phthalate Analysis on Agilent PLgel 3 with Gel Permeation Chromatography

Petrochemicals (continued)

Polyvinylidene Fluoride Analysis on Agilent PLgel 10 MIXED-B and GPC/SEC
 Quantitative GPC Analysis of PVC and Plasticizers
 Optimal Analysis of Epoxy Resins by GPC with ELSD
 Stable Baselines in the Analysis of Poly(lactide-co-glycolide) Polymers by GPC with ELSD
 Superior Analysis of Alkylketene Dimers by GPC with Evaporative Light Scattering Detection
 Effective Analysis of Plasticizers by GPC and Evaporative Light Scattering Detection
 Sensitive Analysis of Styrene Butadiene Rubber by GPC with ELSD
 High Sensitivity Analysis of Natural Rubber by GPC with Evaporative Light Scattering Detection
 Rapid Quantification of Bisphenol-A by GPC with Evaporative Light Scattering Detection
 Phenolic Resin Analysis with Agilent PLgel Columns and Gel Permeation Chromatography
 Polystyrene Stars on Agilent PLgel 5 MIXED-C using Gel Permeation Chromatography
 Natural and Synthetic Wax Analysis on Agilent PLgel and Gel Permeation Chromatography
 Silicone Analysis on Agilent PLgel with Gel Permeation Chromatography
 Polymerization Study using Agilent PLgel Columns and Gel Permeation Chromatography
 Asphalt Analysis with Agilent PLgel Columns and Gel Permeation Chromatography
 Polymer Analysis with Polar Organic Solvents using Agilent PLgel Columns and GPC
 Polyurethane Prepolymer Analysis on Agilent PLgel MIXED-E by GPC
 Analyze Injection-Molding Polymers on Agilent PLgel 5 MIXED-C by GPC
 Adipate Polyester Analysis on Agilent PLgel 3 MIXED-E by Gel Permeation Chromatography
 Fractionation of Formaldehyde Resin on Agilent PLgel by Gel Permeation Chromatography
 Fingerprinting Epoxy Resins with Agilent OligoPore and Gel Permeation Chromatography
 Silane Oligomer Analysis with Agilent OligoPore and Gel Permeation Chromatography
 Separate Epoxy Resin Oligomers with Agilent Preparative Gel Permeation Chromatography
 Separation of Styrene Oligomers by Agilent Preparative Gel Permeation Chromatography
 Epoxy Resin Analysis with Agilent OligoPore Columns and Gel Permeation Chromatography
 Phenolic Resin Analysis by Agilent ResiPore Columns and Gel Permeation Chromatography
 Quantitative analysis of copolymers using the Cary 630 FTIR spectrometer
 Analysis of wear metals and contaminants in engine oils using the 4100 MP-AES
 C1 - C3 Hydrocarbon Analysis Using the Agilent 490 Micro GC - Separation Characteristics for PoraPLOT U and PoraPLOT Q Column Channels
 The Analysis of Polyvinylchloride
 Infrared imaging - the evolution of infrared microscopy
 Analysis of Phenol-formaldehyde Resins by GPC and Agilent PolarGel-M
 Analysis of Fluid Cat Cracker Feed using an Agilent J&W FactorFour VF-5ht UltiMetal Column
 Analysis of Natural Rubber by GPC with Triple Detection
 Poly(styrene-co-butadiene) GPC with Viscometry
 Analysis of Modified Polyacrylamide by Aqueous SEC with Triple Detection
 Analysis of Polybutadiene by GPC with Triple Detection
 Analysis of Polyesters by GPC with Light Scattering Detection
 SEC Analysis of Polyacrylic Acid
 SEC Fingerprinting of Polyvinyl Alcohols
 SEC Analysis of a Water Soluble Copolymer
 SEC Analysis of a Acrylamide Copolymer
 SEC Analysis of Polyvinyl Pyrrolidone
 Characterizing Polyvinyl Alcohol by SEC
 SEC Analysis of Star Branched Polyethylene Glycol without Column Interaction
 Accurate Determination of the MWD of Polyacrylic Acid
 Analysis of Polybutadiene

Petrochemicals (continued)

Routine Analysis of Polystyrene
 Analysis of Nitrocellulose
 Analysis of Reacetylated Polyvinyl Alcohol
 High Temperature Analysis of Polyethylene Terephthalates
 Analysis of Polystyrene
 High-Temperature Analysis of Polyether Sulfone
 Reduced Viscosity Analysis of Polyurethane
 GPC Analysis is Ideal for Characterizing PVC
 Analysis of Polyaniline
 Characterization of Block Copolymers Synthesized via Transition Metal Mediated Living Radical Polymerization
 Analysis of Polybutadienes by GPC with Triple Detection
 Analysis of Poly(isobornyl methacrylate) in Tetrahydrofuran by GPC
 Analysis of Petroleum Jelly using Conventional GPC
 Analysis of Biodegradable Polymers by GPC
 Triple Detection Characterization of Polycarbonates with the Agilent 390-MDS
 Gel Permeation Chromatography with Viscometry for the Molecular Weight Characterization of Epoxy Resins
 Size Exclusion Chromatography for the Analysis of Dental Polymers
 Analysis of Linear and Branched Polyethylene Glycols via GPC Viscometry
 Analysis of Low Molecular Weight Polyethylene via Conventional GPC
 Analysis of Polythiophenes via Conventional GPC
 Analysis of Aliphatic Alcohols by Ligand-Exchange Chromatography
 Fast Refinery Gas Analysis Using the 490 Micro GC QUAD
 Analysis of Melamine Resins by Conventional GPC using Agilent PolarGel-L Columns and the Agilent PL-GPC 50 Plus
 Analysis of Poly(styrene/butadiene) Copolymers by Conventional Gel Permeation Chromatography on the Agilent PL-GPC 50 Plus
 Analysis of Polybutadiene by GPC Triple Detection with the Agilent 390-MDS Multi Detector Suite
 Analysis of Polyvinyl Butyral by GPC Triple Detection with the Agilent 390-MDS Multi Detector Suite
 Analysis of Polystyrene Stars by GPC Viscometry with the Agilent 390-MDS Multi Detector Suite
 Analysis of Modified Polyvinyl Alcohol by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite
 Analysis of Bromostyrene by GPC Triple Detection using the Agilent 390-MDS Multi Detector Suite
 Analysis of Epoxy Resins by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite
 Separation of Antioxidants and UV Stabilizers Used in Plastics
 Detailed Separation of C1-C5 Light Hydrocarbons on CP-Al2O3/Na2SO4 PL0T GC Column
 Analysis of Polymer Antioxidant Additives on the Agilent 500 Ion Trap LC/MS
 Quantitative analysis of tint in polymer pellets and disks
 The determination of thin film thickness using reflectance spectroscopy

Polymers

Analysis of Antioxidants and UV Stabilizers in Polymers using HPLC
 Screening and Qualitative Identification of Antioxidants Polymer Additives by HPLC with UV/VIS and APCI-MS Detection
 Quantitative analysis of copolymers using the Cary 630 FTIR spectrometer
 The Analysis of Polyvinylchloride
 Artifact Free Analysis of Lignins by GPC using Agilent PolarGel-M
 GPC and Agilent PolarGel-M Columns for the True Representation of Novolac Resins
 Analysis of Natural Rubber by GPC with Triple Detection
 Size Exclusion Chromatography for the Analysis of Dental Polymers
 Materials analysis by infrared mapping: A case study using a multi-layer paint sample
 Analysis of Epoxy Resins by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Polymers (continued)

Analysis of Polysaccharides by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite
Quantitative analysis of tint in polymer pellets and disks

Power Generation

TOGA Analyzer SP1 2310-0047
Permanent Gases on a COX Module Using a Agilent 490 Micro GC
Using the Agilent 490 Micro GC for the Monitoring of a Circulating Fluidized Bed Biomass Gasifier
Fast Separation of Oxygen and Nitrogen on a MolSieve 5A Channel Using the Agilent 490 Micro GC
Permanent Gas Analysis - Separation of Argon and Oxygen on a MolSieve 5A Column using the Agilent 490 Micro GC
C1 - C5 Hydrocarbon Analysis Using the Agilent 490 Micro GC - Separation Characteristics for PoraPLOT U and PoraPLOT Q Column Channels
Analysis of Biogas Using the Agilent 490 Micro GC Biogas Analyzer

Refining

Agilent 6850 Sub-Ambient Oven Performance for ASTM D3710 Simulated Distillation of Gasoline
A Unified Gas Chromatography Method for Aromatic Solvent Analysis
High-Pressure Liquid Injection Device for the Agilent 7890A and 6890 Series Gas Chromatographs
Parallel GC for Complete Refinery Gas Analysis
Parallel GC for Complete RGA Analysis
GC/MS Analysis of PCBs in Waste Oil Using the Backflush Capability of the Agilent QuickSwap Accessory
Fast Analysis of Aromatic Solvent with 0.18 mm ID GC column
Investigation of the Unique Selectivity and Stability of Agilent GS-OxyPLOT Columns
Detection of Sulfur Compounds According to ASTM D5623 in Gasoline with Agilent's Dual Plasma Sulfur Chemiluminescence Detector (G6603A) and an Agilent 7890A Gas Chromatograph
Fast Hydrocarbon and Sulfur Simulated Distillation Using the Agilent Low Thermal Mass (LTM) System on the 7890A GC and 355 Sulfur Chemiluminescence Detector
Dual Channel Simulated Distillation of Carbon and Sulfur with the Agilent 7890A GC and 355 Sulfur Chemiluminescence Detector
Analysis of Trace Hydrocarbon Impurities in Benzene by Agilent 7820A Gas Chromatograph
Automated Preparation of Simulated Distillation Samples for ASTM Methods D2887, D7213, D7398 and D6352 using a Dual Tower 7693A and Tray System
Analysis of Permanent Gases and Light Hydrocarbons Using Agilent 7820A GC With 3-Valve System
Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems
Fast analysis of polyaromatic hydrocarbons using the Agilent 1290 Infinity LC and Eclipse PAH columns
Prefractionator for Reliable Analysis of the Light Ends of Crude Oil and other Petroleum Fractions
Simultaneous Analysis of Greenhouse Gases by Gas Chromatography
High-throughput method development for aldehydes and ketones using an Agilent 1290 Infinity LC system and an Agilent ZORBAX StableBond HD column
Meeting the requirements of ASTM D 6591-06 (IP548/06) Using Agilent 1200 Series HPLC Systems
Automated Preparation of Simulated Distillation Standards and Samples for ASTM Methods D2887, D7213, D7398 and D6352 using the 7693A System with Easy SamplePrep Software
Analysis of Trace 2-Ethylhexyl Nitrate in Diesel Using Chemiluminescence Detector (NCD)
Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance
Improved Data Quality Through Automated Sample Preparation
GC Analysis of Sulfur Components in Propylene using a Sulfur Chemiluminescence Detector
GC Analysis of Sulfur Components in Propylene using a Pulsed Flame Photometric Detector

Refining (continued)

Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy
 Low level detection of biodiesel in diesel fuel using the Agilent 5500t FTIR spectrometer
 Test method for low level detection of biodiesel in diesel using the Agilent 5500t FTIR spectrometer
 Rapid measurement of gasoline (petrol) in diesel fuel using portable FTIR spectroscopy
 Onsite quantitative FTIR analysis of water in turbine oil
 Portable measurement of biodiesel in diesel fuels by ASTM D7371-07 (FTIR-ATR-PLS method) with the Agilent 5500t FTIR spectrometer
 Asphalt Analysis with Agilent PLgel Columns and Gel Permeation Chromatography
 Petroleum Analysis with Agilent PLgel Columns and Gel Permeation Chromatography
 Analysis of wear metals and contaminants in engine oils using the 4100 MP-AES
 Measurement of additives in lubricating oils using the Agilent 4100 MP-AES
 Direct measurement of metallic impurities in petroleum fuels using the 4100 MP-AES
 Analysis of diesel using the 4100 MP-AES
 C1 - C3 Hydrocarbon Analysis Using the Agilent 490 Micro GC - Separation Characteristics for PoraPLOT U and PoraPLOT Q Column Channels
 The Determination of Lead in Unleaded Gasoline Using the Agilent 55 AA Atomic Absorption Spectrophotometer
 Analysis of Fluid Cat Cracker Feed using an Agilent J&W FactorFour VF-5ht UltiMetal Column
 Analysis of Diesel using an Agilent J&W FactorFour VF-5ht UltiMetal Column
 Determination of Rhodium in Catalyst Oil and Aqueous Samples Using the Agilent 710 ICP-OES
 Fast Refinery Gas Analysis Using the 490 Micro GC QUAD
 Detailed Separation of C1-C5 Light Hydrocarbons on CP-Al2O3/Na2SO4 PLOT GC Column

Specialty Chemicals

Applying the 5975 inert MSD to the Higher Molecular Weight Polybrominated Diphenyl Ethers (PBDEs)
 Process Monitoring of Bishphenol-A in Industrial Feedstock using High Throughput HPLC
 Increased Identification of Impurities in Octyl Dimethyl p-Aminobenzoic Acid Using the Agilent 6140 High Throughput LC/MS
 Monitoring of electroless plating baths by capillary electrophoresis
 Analysis of Suspected Flavor and Fragrance Allergens in Perfumes Using Two-Dimensional GC with Independent Column Temperature Control Using an LTM Oven Module
 Screening Impurities in Fine Chemicals Using the Agilent 1290 Infinity LC System
 Determination of Formic Acid in Acetic Acid for Industrial Use by Agilent 7820A GC
 Analysis of impurities in fine chemical octyl-dimethyl-4-aminobenzoate using the Agilent 1290 Infinity LC ZORBAX RRHT and RRHD 1.8 columns
 Fast analysis of polyaromatic hydrocarbons using the Agilent 1290 Infinity LC and Eclipse PAH columns
 High-throughput method development for aldehydes and ketones using an Agilent 1290 Infinity LC system and an Agilent ZORBAX StableBond HD column
 Basic Performance of the Agilent 7700s ICP-MS for the Analysis of Semiconductor Samples
 Permanent Gases on a COX Module Using a Agilent 490 Micro GC
 Direct analysis of trace metallic impurities in high purity hydrochloric acid by Agilent 7700s ICP-MS
 Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy
 Onsite quantitative FTIR analysis of water in turbine oil
 Simple, automated measurements of the photocatalytic properties of colorimetric species using the Agilent Cary 60 UV-Vis spectrophotometer with fiber optics
 Direct measurement of metallic impurities in 20% ammonium hydroxide by Agilent 7700s ICP-MS
 Carboxylic Ester Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography
 The ultratrace determination of iodine 129 in aqueous samples using the 7700x ICP-MS with oxygen reaction mode
 Trace elemental analysis of trichlorosilane by Agilent 7700s ICP-MS
 Identification of Oxidation Products of L-Ascorbic Acid by HPLC

Specialty Chemicals (continued)

Fullerene Analysis using Agilent PLgel Columns and Gel Permeation Chromatography
Permanent Gas Analysis - Separation of Helium, Neon and Hydrogen a MolSieve 5A column using the Agilent 490 Micro GC
Fast Separation of Oxygen and Nitrogen on a MolSieve 5A Channel Using the Agilent Micro GC
New methodology for determination of gold and precious metals using the Agilent 4100 MP-AES
Permanent Gas Analysis - Separation of Argon and Oxygen on a MolSieve 5A Column using the Agilent 490 Micro GC
The Analysis of Cement
The Determination of Selenium in Steels
The Analysis of Iron Ores
Determination of Low Levels of Arsenic using Flame AAS and Agilent UltrAA Lamps
The Investigation of Fertilizer Analyses Using Microwave Digestion and the Agilent 720-ES
Determination of Mercury With On-line Addition of Stannous Chloride Using an Axial ICP-OES
Determination of Rhodium in Catalyst Oil and Aqueous Samples Using the Agilent 710 ICP-OES
Using the Agilent Cary 670 FTIR Spectrometer to observe rotational and isotopic bands in CO through high resolution FTIR Spectroscopy
Analysis of Polylactide and Poly(lactide-co-glycolide) by GPC with Viscometry
Analysis of Surfactants Using the Agilent 500 Ion Trap LC/MS
SEC Analysis of Sodium Polystyrene Sulfonate
SEC Analysis of Poly 2-Vinyl Pyridine
SEC Analysis of Sodium Polyacrylate
SEC for the Analysis of Non-ionic Surfactants
Analysis of Aliphatic Alcohols by Ligand-Exchange Chromatography
Material analysis by infrared mapping: A case study using a multi-layer paint sample
Monitoring fast reaction using Stopped Flow Kinetics on the Cary 50/60 UV-Vis
Investigation of photochemical reactions using the Cary 50/60 UV-Vis
The investigation of the photo-kinetics of a platinum organoamine complex using the Cary 50/60