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A Unified Gas Chromatography Method for Aromatic Solvent Analysis

Application

Gas Chromatography

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Abstract

A single, easy-to-use GC method for aromatic solvent purity analysis is described that meets the chromatographic requirements of ten separate ASTM methods. This method can be used to obtain identical results on both the Agilent 6890 and 6850 Series Gas Chromatographs designed for the method development lab and the routine production lab respectively. Reproducibility of results between instruments, between labs, and over time are further improved by applying the technique of retention time locking to this unified method.

Introduction

The producers and users of many aromatic hydrocarbons evaluate the product quality by measuring the purity of the material along with specific contaminants. For these types of measurements the most commonly used analysis technique is gas chromatography (GC). In an effort to standardize analysis procedures, the American Society of Testing and Materials (ASTM) has developed and published a number of GC methods specific to an aromatic compound or class of compounds.[1] These methods have evolved over time to meet the requirements of new materials specifications or to incorporate new GC technologies (i.e. capillary columns replacing packed columns). The result of this evolution is a large number of methods that are remarkably alike. In practice, many QA laboratories that support a variety of chemical processes typically devote one GC instrument to each ASTM method they must run.

Recently, there has been a move by many chemical companies to consolidate lab facilities, simplify measurements, and reduce the costs that chemical measurements add to production. Laboratory space is expensive and is becoming limited. Where three or four GCs were operating in the past, there is now only space and budget for one or two. Another part of this trend is to have non-traditional personnel such as plant operators; technicians and engineers perform chemical analyses. Since these personnel are not trained as analytical chemists, simpler methods are needed to perform the analyses without losing measurement performance.

Accommodating these changes in the lab environment makes it necessary to explore alternative approaches to performing GC analyses. One approach is to develop a method that combines the elements of several separate ASTM methods.

A single method has a number of advantages over multiple methods. Fewer GCs could be used in place of a larger number of instruments previously dedicated to individual methods; thus reducing required lab space. By running one method, any GC could also serve as a backup for instruments that are undergoing maintenance or repair. This would result in shorter down times and better utilization of lab space. A single method would also eliminate the need to stock multiple columns and supplies. Plant operators would also find it easier to use since they would only need to be trained once on a single procedure.

Another important advantage to a single aromatics method lies in the use of retention time locking (RTL). RTL is a technique that allows any Agilent 6890 or 6850 GC systems running the same method to obtain nearly identical retention times. Comparing data between instruments, between laboratories, or over time can be difficult due to variations in retention times. This is further complicated when using multiple methods since the different columns and operating conditions result in different retention times for the same compound. For instance, there are eight ASTM methods that measure p-xylene; however, p-xylene retention times range from 6 to 16 minutes depending on the method's operating conditions (column, flow, temperature). By using one method for all aromatic samples, retention time variations can be reduced to less than 0.5 minutes. Then by applying RTL to this method, system-to-system retention time variations can be further reduced to less than 0.03 minutes. Retention time precision on this order greatly simplifies comparison of data between systems, between laboratories, and over time.

This application note describes a GC method that is chromatographically suitable for a wide range of samples typically analyzed by ten different ASTM methods. Table 1 lists these ten methods along with the ASTM recommended columns and reporting specifications.

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

ASTM Method	Title	Liquid phase	Column type	Report specifications
D2306	Std Test for C8 Aromatic Hydrocarbons	0.25 µm Carbowax	Capillary 50 m × 0.25 mm	wt% of individual C8 HC
D2360	Std Test for Trace Impurities in Monocyclic Hydrocarbons	0.32 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual aromatic impurities, total impurities, purity
D3760	Std Test for Cumene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, cumene purity (wt%)
D3797	Std Test for o-Xylene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, o-xylene purity (wt%)
D3798	Std Test for p-Xylene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, total impurities, p-xylene purity (wt%)
D4492	Std Test for Benzene	0.25 µm Carbowax	Capillary 50 m × 0.32 mm	wt% of individual impurities, benzene purity(wt%)
D4534	Std Test for Benzene in Cyclic Products	10%TCEPE on Chromasorb P	Packed 3.7 m × 3.175 mm	wt% of benzene
D5060	Std Test for Impurities in Ethylbenzene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, ethylbenzene purity
D5135	Std Test for Styrene	0.5 µm Carbowax	Capillary 60 m × 0.32 mm	wt% of individual impurities, styrene purity
D5917	Std Test for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)	0.25 µm Carbowax	Capillary 60 m × 0.32 mm	wt% individual impurities, wt% total non-aromatics, wt% total C9 aromatics, purity of main component

Experimental

Two Agilent 6890 Plus Series gas chromatographs and four Agilent 6850 gas chromatographs were used for this work. Each GC was equipped with a split/splitless capillary inlet, a flame ionization detector (FID) and an Agilent 7683 Automatic Liquid Sampler (ALS). The split/splitless inlets were fitted with high-pressure Merlin Microseal Septa (Agilent Part no. 5182-3442) and spilt-optimized liners (Agilent Part no. 5183-4647). Injections were made using 10 µL gas-tight syringes (Agilent Part no. 5181-8809) designed for use with the Merlin Microseal. Table 2 lists the instrument conditions used for this method. An Agilent Chemstation was used for all instrument control, data acquisition and data analysis.

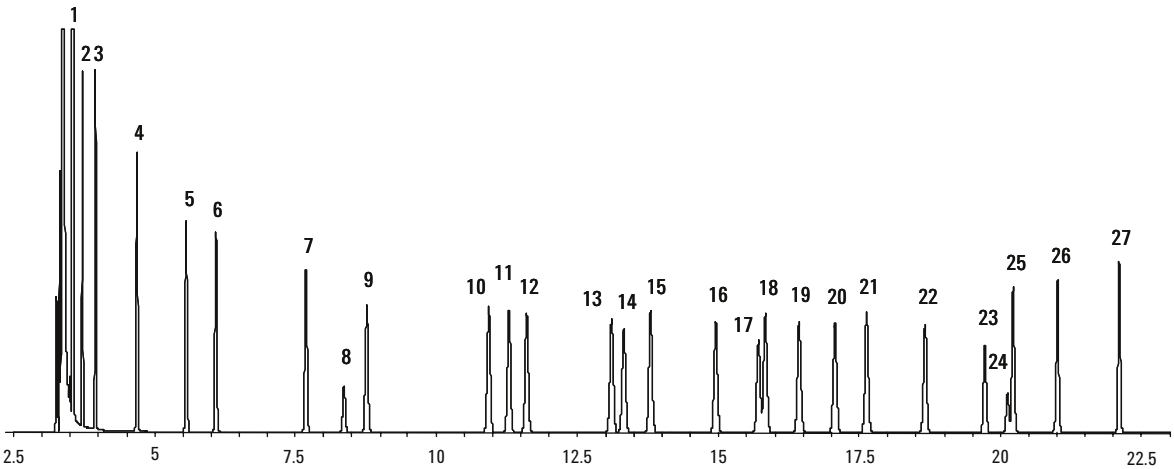
Table 2. Conditions for Unified Aromatic Solvents Method

Column	HP-Innowax, 60 m × 0.32 mm × 0.5 µm Agilent Part no.19091N-216
Carrier Gas	Helium @ 20.00 psi constant pressure mode
Inlet	Split/Splitless @ 250 °C 100:1 to 400:1 split ratio
Oven Temp	75 °C (10 min); 3 °C/min to 100 °C (0 min) 10 °C/min to 145 °C (0 min)
Detector	FID @ 250 °C Data acquisition rate @ 20 Hz
Injection Size	0.1 to 1.0 µL

An n-hexane solution was prepared containing 0.1 wt% of all the aromatic solvents and impurities specified for analysis by the ten ASTM methods listed in Table 1. This standard was used to develop the RTL calibration and to assess the separation of each compound. Final evaluation of this unified method was done by running the recommended standards specified in each of the ten ASTM methods.

Results and Discussion

Figure 1 shows a chromatogram of the hexane solution containing an aggregate of aromatic solvents and impurities. For most compounds, baseline resolution was achieved. There are two pairs that are only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, are also not resolved in the original ASTM method (D-5060 Impurities in ethylbenzene) and, along with o-ethyltoluene, are reported as total ethyltoluene. Therefore, the results presented here represent the same result obtained with the original ASTM method. A second pair, diethylbenzene and n-butylbenzene are also only partially resolved. Again, this does not present a problem since these two components are not typically found together in the same material. Diethylbenzene is sometimes found as a contaminant in ethylbenzene (ASTM D-5060) while n-butylbenzene is used as the internal standard for cumene analysis (ASTM D3760).



1	heptane	8	1,4-dioxane	15	o-xylene	22	tridecane
2	cycohexane	9	undecane	16	proplybenzene	23	diethylbenzene isomer
3	octane	10	ethylbenzene	17	p-ethyltoluene	24	diethylbenzene isomer
4	nonane	11	p-xylene	18	m-ethyltoluene	25	n-butylbenzene
5	benzene	12	m-xylene	19	t-butylbenzene	26	α-methylstyrene
6	decane	13	cumene	20	s-butylbenzene	27	phenylacetylene
7	toluene	14	dodecane	21	styrene		

Figure 1. Separation of the 27 compounds analyzed by the ten ASTM aromatics methods listed in Table 1.

Retention Time Locking (RTL)

Retention time locking calibration was performed using t-butylbenzene as the target peak. Figure 2 shows the five RTL calibration runs with the retention times of t-butylbenzene indicated and Figure 3 shows the RTL calibration. These calibration runs do not have to be repeated by anyone wishing to lock this method on their Agilent 6890 or 6850 GC systems. To use this RTL calibration, simply create a new method with conditions outlined in Table 2, then use the Chemstation RTL software to create a new RTL calibration and enter the data shown in Figure 3. The GC can then be locked by running a sample containing t-butylbenzene and using the RTL software to re-lock the method. The general theory and use of RTL is detailed in previous publications.[2]

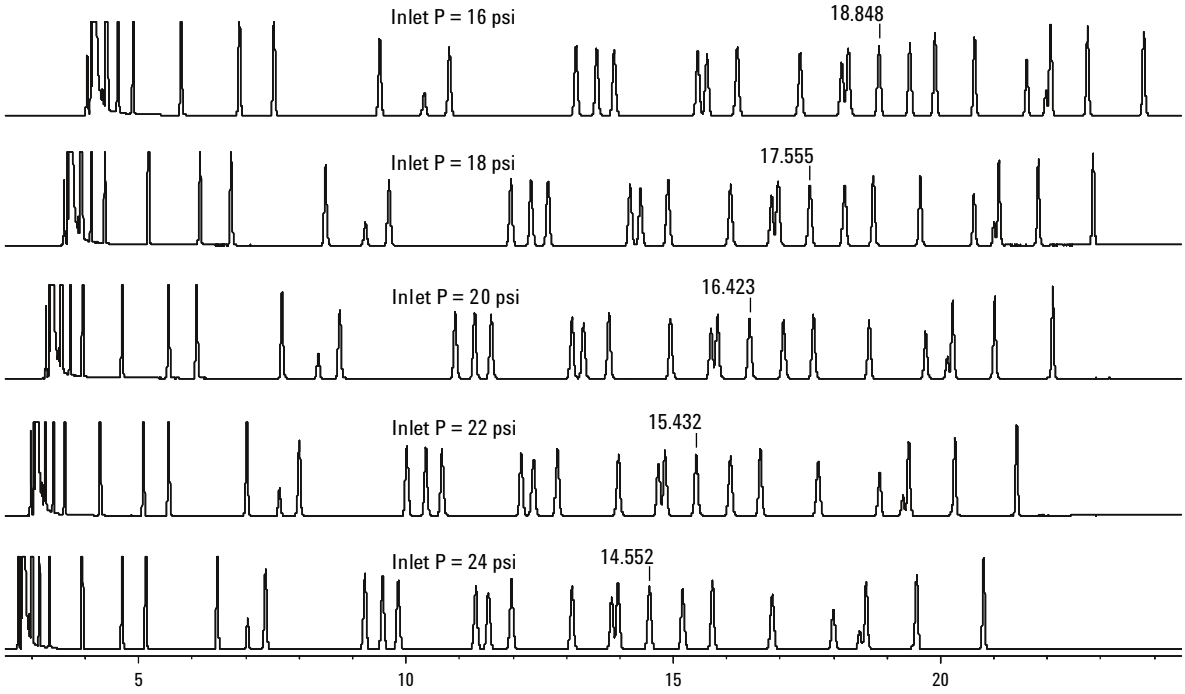


Figure 2. Retention time locking calibration runs using t-butylbenzene as the RTL target peak.

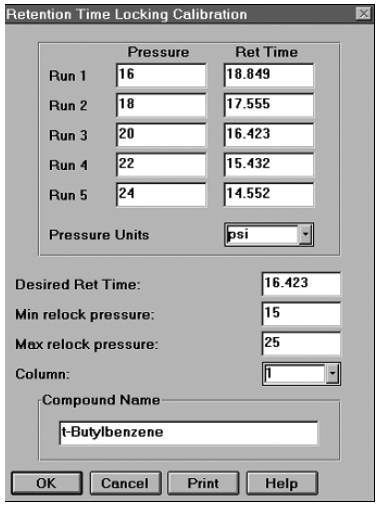


Figure 3. Retention time locking calibration using t-butylbenzene as the RTL target peak.

A total of six GC systems, two 6890s and four 6850s, were configured to run this unified method. Each GC was retention time locked using a t-butylbenzene target retention time of 16.423 minutes. Figure 4 shows an overlay of the locked chromatograms from each of the six GCs. Table 3 lists the retention times and precision of each compound in the standard mix. Excellent retention time precision was observed for the 6890 and 6850 instruments across the entire time range of the chromatographic run. Peaks falling within the initial 10-minute isothermal time had a standard deviation of about 0.02 minutes. Those peaks eluting during the 3 °C/min program ramp had a standard deviation of 0.01 minutes and those eluting in the 10 °C/min ramp showed a standard deviation of 0.03 minutes

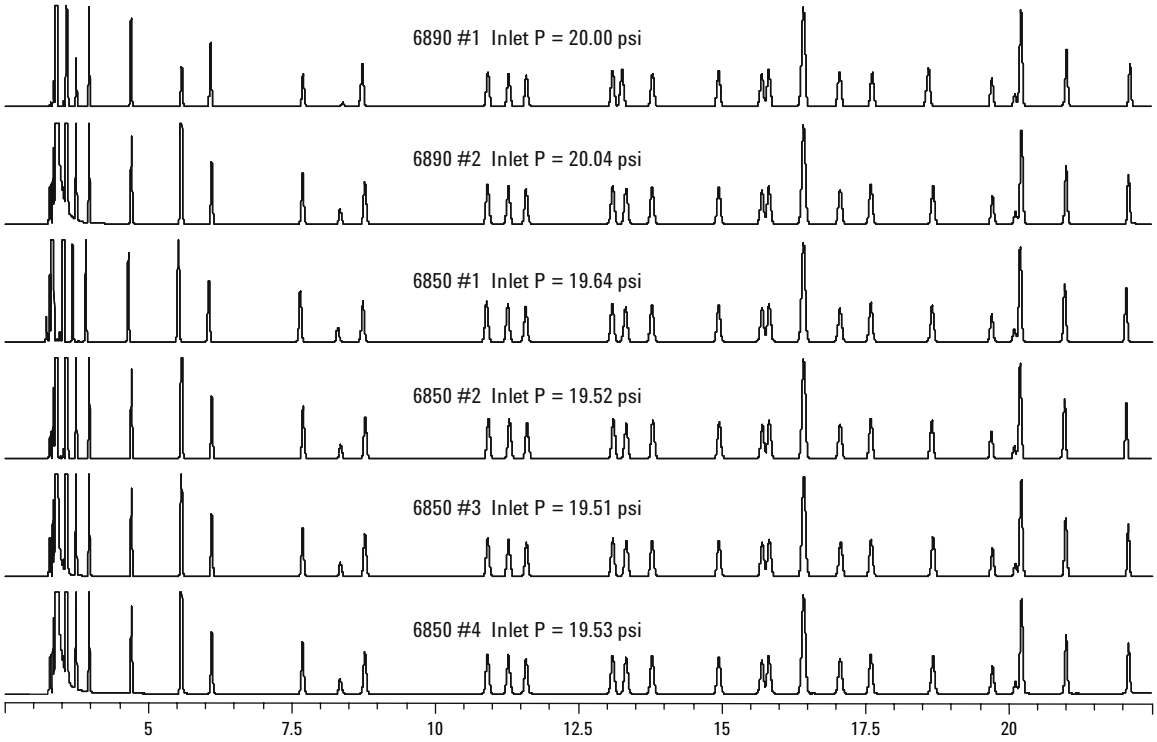


Figure 4. Using RTL, excellent retention time precision was observed for all 27 compounds analyzed using the unified aromatics method. Details of retention time precision are listed in Table 3.

Table 3. Retention Time Precision for Each Compound Analyzed by the Unified Method

	Retention time (min)							
Compound	**6890 #1**	**6890 #2**	**6850 #1**	**6850 #2**	**6850 #3**	**6850 #4**	**Std Dev**	**Range**
heptane	3.572	3.568	3.508	3.569	3.566	3.568	0.025	0.064
cyclohexane	3.745	3.742	3.682	3.743	3.741	3.742	0.025	0.063
octane	3.969	3.971	3.911	3.972	3.970	3.971	0.024	0.061
nonane	4.696	4.704	4.646	4.705	4.703	4.704	0.023	0.059
benzene	5.581	5.572	5.518	5.576	5.572	5.572	0.023	0.063
decane	6.084	6.105	6.053	6.106	6.104	6.105	0.021	0.053
toluene	7.694	7.686	7.646	7.695	7.687	7.686	0.018	0.049
1,4-dioxane	8.386	8.342	8.306	8.350	8.346	8.342	0.025	0.080
undecane	8.732	8.776	8.741	8.782	8.777	8.776	0.022	0.050
ethylbenzene	10.922	10.915	10.899	10.932	10.918	10.915	0.011	0.033
p-xylene	11.282	11.278	11.267	11.295	11.280	11.278	0.009	0.028
m-xylene	11.592	11.587	11.577	11.604	11.589	11.587	0.009	0.027
cumene	13.097	13.097	13.089	13.110	13.098	13.097	0.007	0.021
dodecane	13.264	13.334	13.323	13.337	13.333	13.334	0.028	0.073
o-xylene	13.790	13.781	13.778	13.795	13.782	13.781	0.007	0.017
propybenzene	14.940	14.943	14.939	14.951	14.945	14.943	0.004	0.012
p-ethyltoluene	15.696	15.699	15.699	15.706	15.702	15.699	0.003	0.010
m-ethyltoluene	15.819	15.820	15.820	15.827	15.823	15.820	0.003	0.008
t-butylbenzene	16.423	16.424	16.420	16.426	16.426	16.424	0.002	0.006
s-butylbenzene	17.049	17.060	17.053	17.059	17.063	17.060	0.005	0.014
styrene	17.623	17.600	17.600	17.600	17.603	17.600	0.009	0.023
tridecane	18.602	18.683	18.665	18.661	18.681	18.683	0.031	0.081
diethylbenzene	19.707	19.718	19.701	19.700	19.713	19.718	0.008	0.018
diethylbenzene	20.111	20.123	20.101	20.101	20.116	20.123	0.010	0.022
n-butylbenzene	20.217	20.225	20.201	20.203	20.219	20.225	0.011	0.024
α-methylstyrene	21.011	21.003	20.976	20.975	20.994	21.003	0.015	0.036
phenyacetylene	22.115	22.090	22.050	22.050	22.081	22.090	0.025	0.065
						Avg	***0.015***	***0.039***

For this method it is not always necessary to use t-butylbenzene to perform retention time locking. Analysts who want to use this method for samples not containing t-butylbenzene can select another compound as the RTL target peak. Compounds that do not elute near temperature program transitions can serve as RTL target peaks. Table 4 lists the other suitable RTL target compounds along with the retention time data for constructing alternate RTL calibrations for this method. For instance, if one were preparing the benzene standard prescribed by ASTM method D4492, the toluene in that standard could serve as the RTL target compound. It is not necessary to perform the five RTL calibration runs. Simply create a new RTL calibration using the inlet pressures and toluene retention times from Table 4. This example of an RTL calibration using toluene is shown in Figure 5.

Table 4. Retention Time Locking Calibration Data for Unified Aromatics Method

	Retention time (min) at each inlet pressure				
Compound	**16.00 psi**	**18.00 psi**	**20.00 psi**	**22.00 psi**	**24.00 psi**
nonane	5.794	5.174	4.682	4.279	3.943
benzene	6.880	6.143	5.558	5.080	4.681
toluene	9.507	8.489	7.680	7.018	6.468
cumene	15.460	14.188	13.100	12.148	11.305
o-xylene	16.189	14.897	13.791	12.825	11.969
propylbenzene	17.370	16.064	14.646	13.968	13.100
t-butylbenzene*	18.849	17.555	16.423	15.432	14.552
s-butylbenzene	19.424	18.201	17.061	16.063	15.176
n-butylbenzene	22.054	21.090	20.220	19.404	18.607
styrene	19.891	18.743	17.620	16.621	15.733
α-methylstyrene	22.745	21.824	21.010	20.261	19.552
phenylacetylene	23.795	22.852	22.097	21.421	20.800

*t-butylbenzene used as RTL target peak for this publication (target RT = 16.423 min).

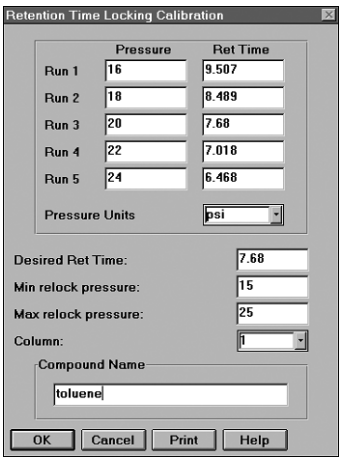


Figure 5. Alternate retention time locking calibration for the unified aromatics method that uses toluene as the locking target compound.

Evaluation of Calibration Standards

The calibration standards specified by each of the ten ASTM methods were prepared and run using this unified method. Each standard was run with Agilent 6890 and Agilent 6850 series gas chromatographs that were retention time locked using t-butylbenzene as the target peak (RT = 16.423 min.).

D2306 - Standard Test for C8 Aromatic Hydrocarbons

Figure 6 shows the chromatograms of the D2306 calibration standard run on Agilent 6890 and 6850 gas chromatographs. The injection size for both runs was 0.1 µL and the split ratio was 400:1.

D2360 - Standard Test for Trace Impurities in Monocyclic Hydrocarbons

The standard calibration mix specified by D2360 was prepared in p-xylene. Figure 7 shows the chromatograms of the D2360 calibration standard. Injection size was 1.0 µL and the split ratio was 100:1. The ethylbenzene peak (RT = 10.98 min) elutes just before p-xylene and was much broader than the other contaminants. This peak shape was due to a reverse solvent effect caused by the overloaded p-xylene along with an oven starting temperature (75 °C) that was much lower than the p-xylene boiling point (138 °C). A broad ethylbenzene peak was also observed in the original ASTM D2360 method.[3]

D3760 - Standard Test for Analysis of Isopropylbenzene (Cumene)

Figure 8 shows the chromatograms of the D3760 calibration standard. The injection size for both runs was 1.0 µL and the split ratio was 100:1. The xylene isomers' concentrations were not listed because they were not added to the standard, but were present as trace contaminants in the cumene used to prepare the standard. Since both GCs are retention time locked, the identification of each xylene isomer could be easily made.

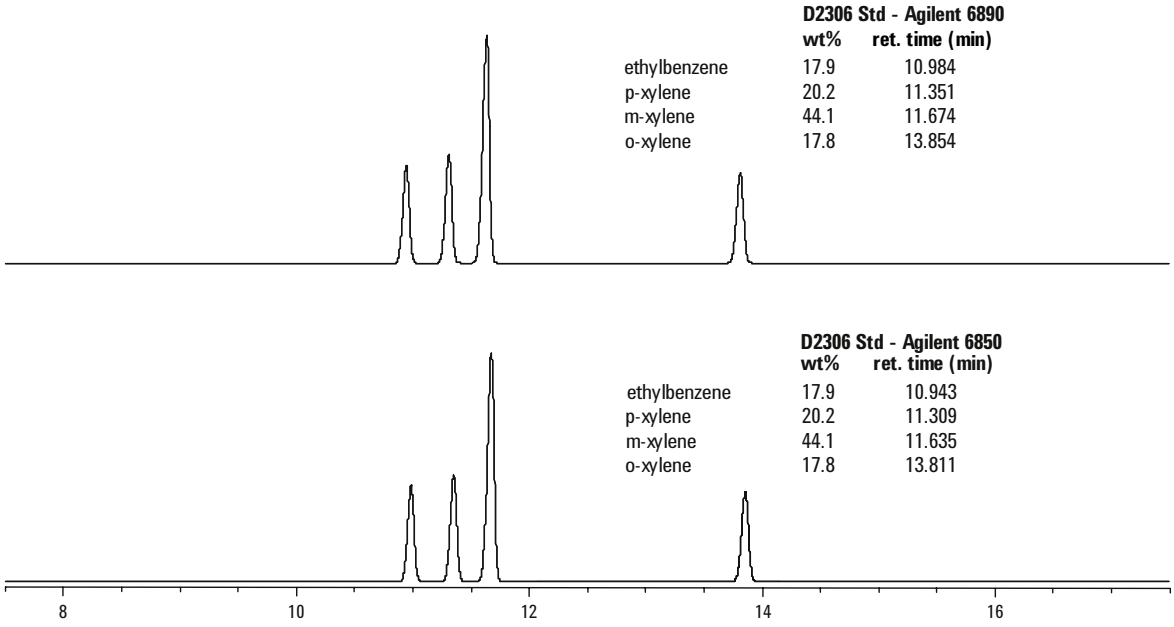


Figure 6. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

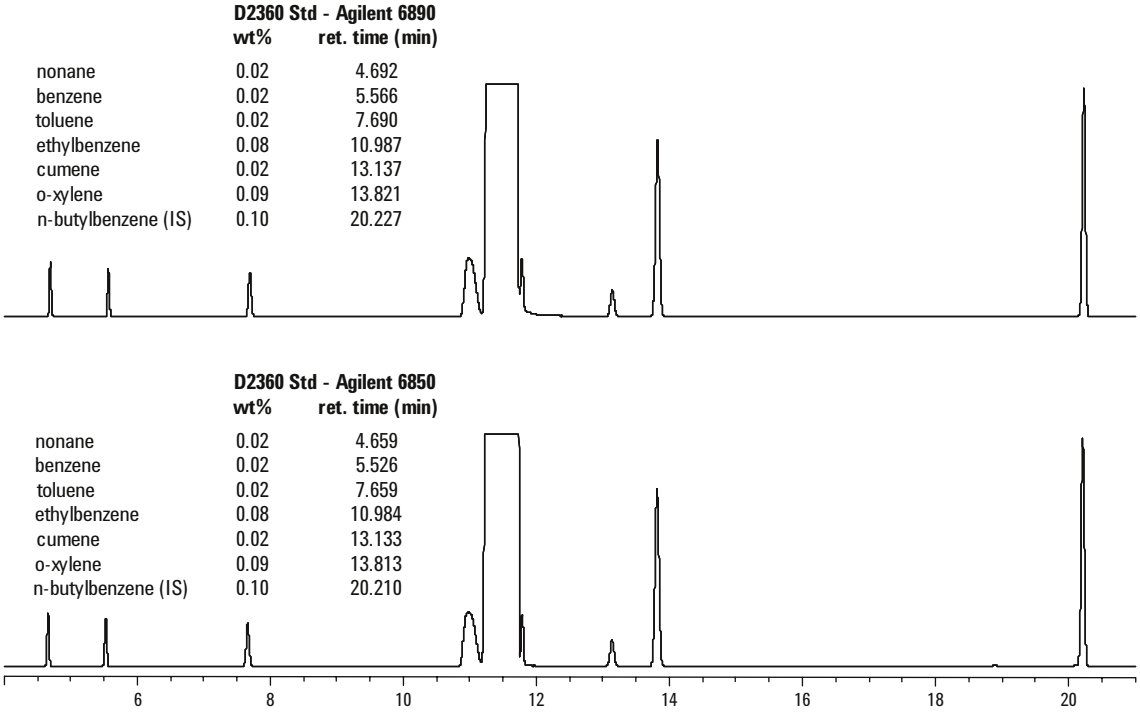


Figure 7. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

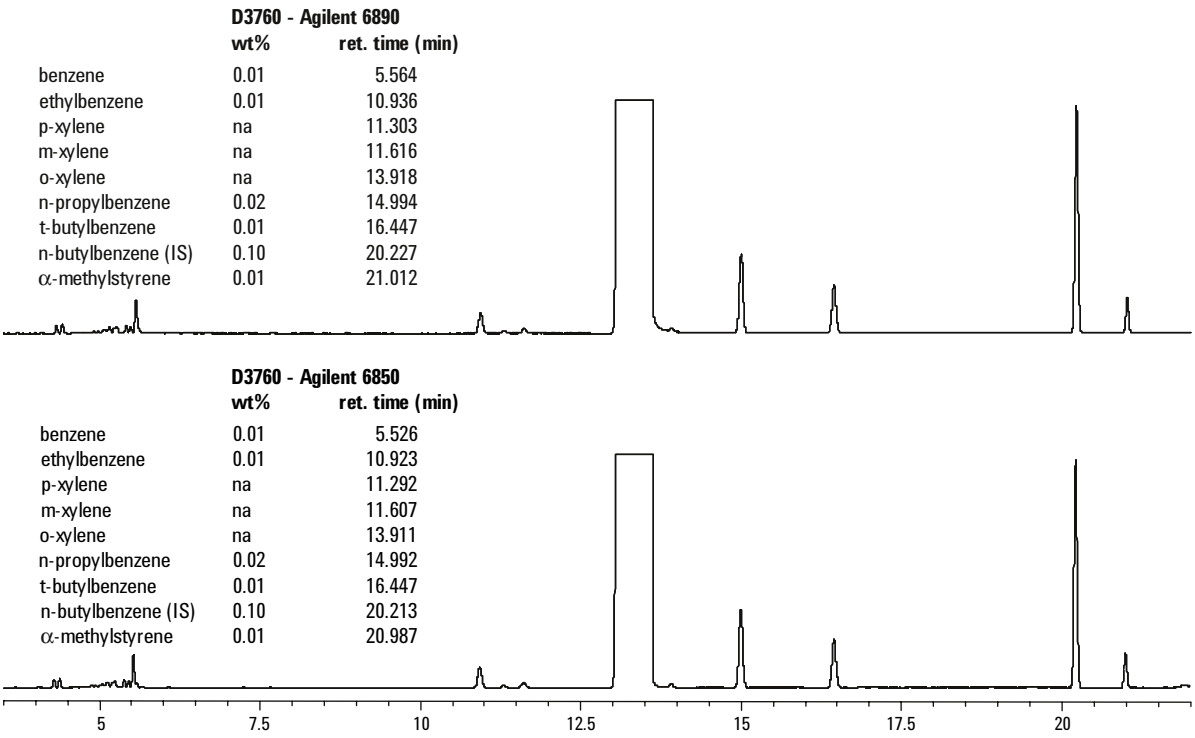


Figure 8. ASTM D3760 isopropylbenzene (cumene) quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D3797 - Standard Test Method for Analysis of o-Xylene

Figure 9 shows the chromatograms of the D3797 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1. The broadening of the cumene peak (RT = 13.28 min) was due to the reverse solvent effect of the overloaded o-xylene peak. This was also observed in the original ASTM D3797 method.

D3798 - Standard Test Method for Analysis of p-Xylene

Figure 10 shows the chromatograms of the D3798 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1. The ethylbenzene peak shows the same broadening observed in the D2360 standard. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Figure 11 shows the details of this separation using the unified method. For each GC this requirement was met for both the ethylbenzene and the m-xylene.

D4492 - Standard Test for Analysis of Benzene

Figure 12 shows the chromatograms of the D4492 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

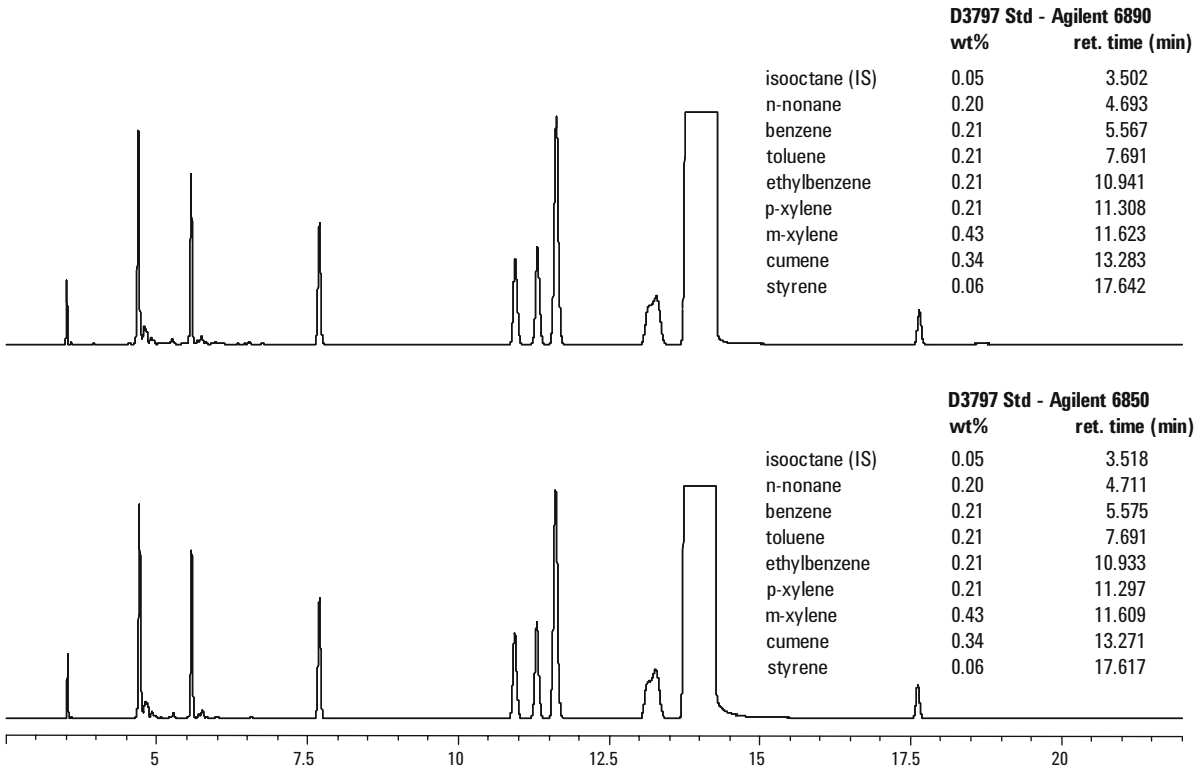


Figure 9. ASTM D3797 o-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

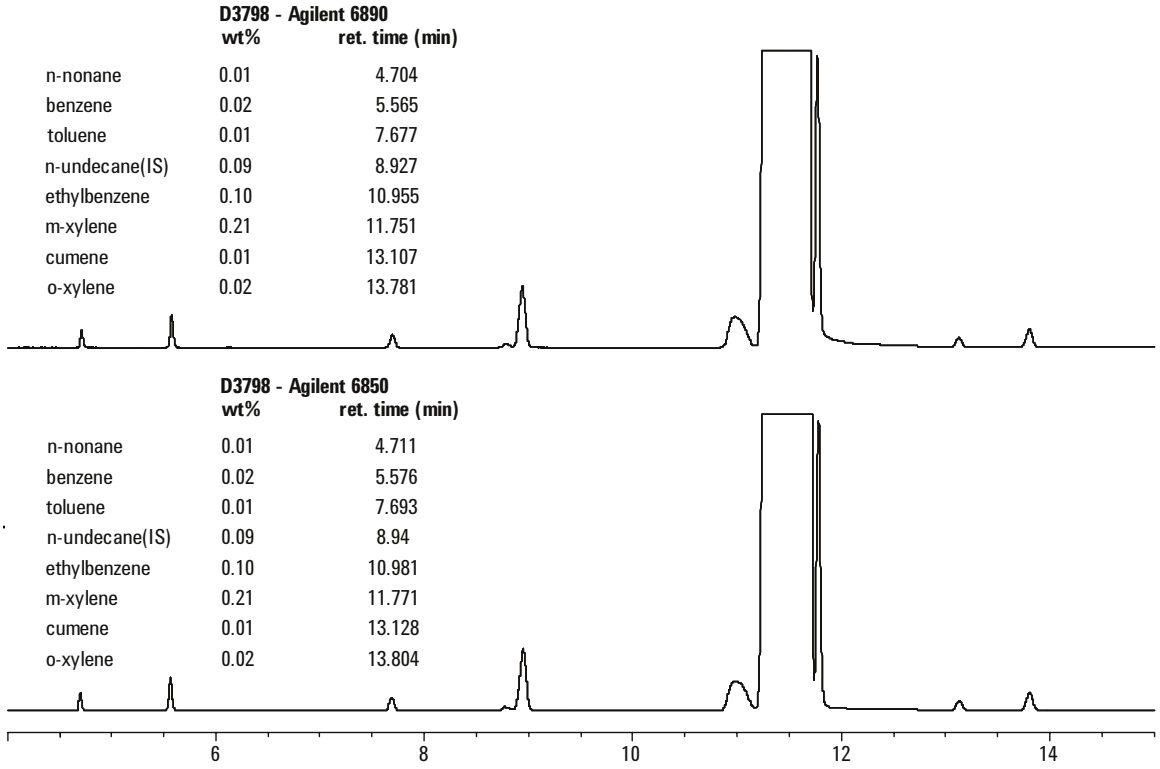


Figure 10. ASTM D3798 p-xylene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

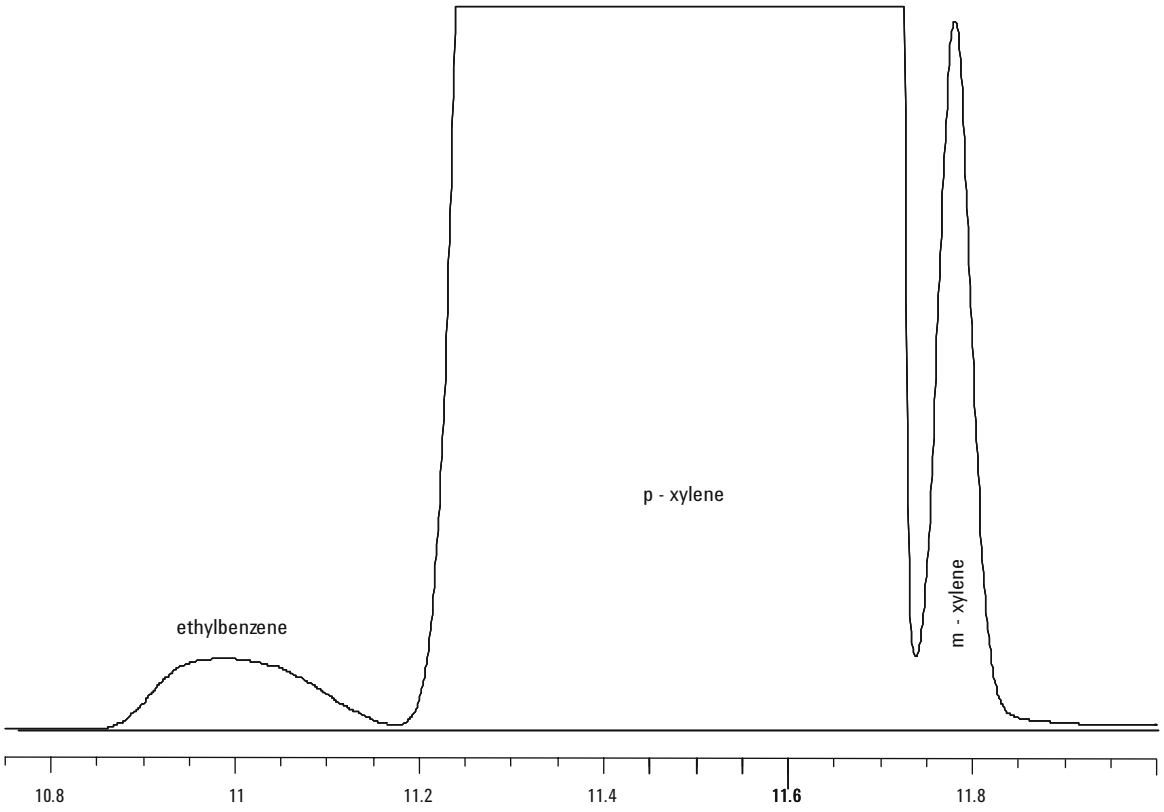


Figure 11. Expanded view from Figure 10 shows excellent separation of m-xylene from p-xylene peak using the unified aromatics method.

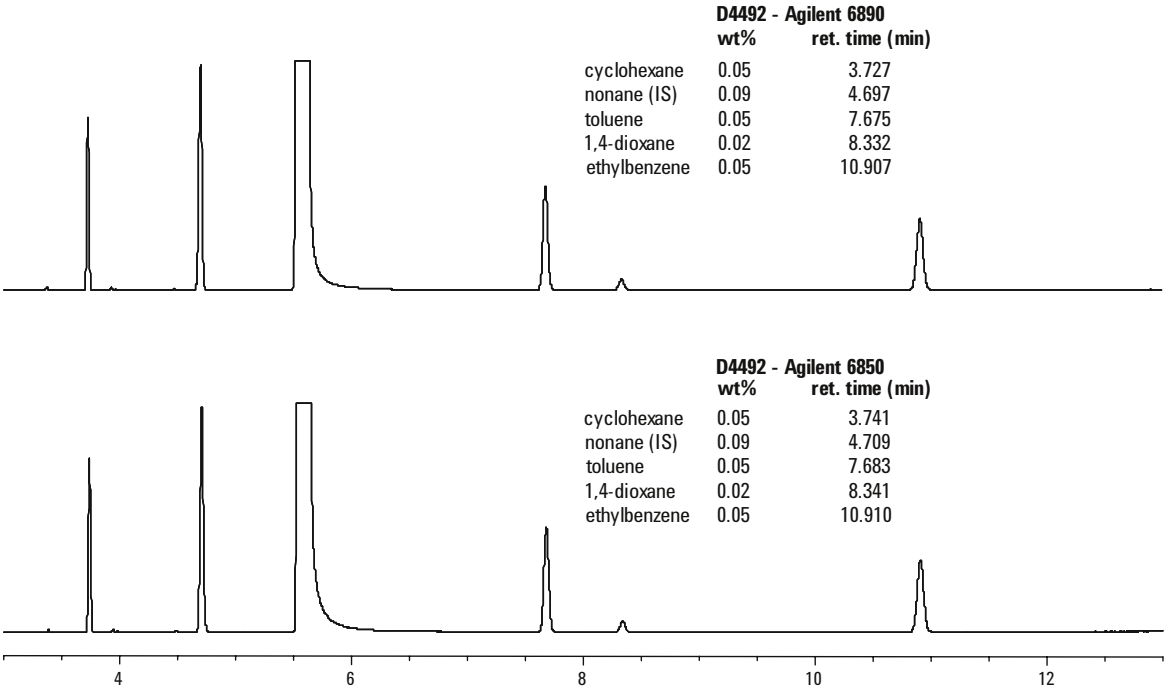


Figure 12. ASTM D4492 benzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cyclohexane

Figure 13 shows the chromatograms of the D4534 calibration standard containing 8 mg/kg (ppm) benzene in cyclohexane. The injection size was 1.0 µL and the split ratio was 100:1.

D4534 Standard Test Method of Benzene Content of Cyclic Products - Toluene

Figure 14 shows the chromatograms of the D4534 calibration standard containing 9 mg/kg (ppm) benzene in toluene. The injection size was 1.0 µL and the split ratio was 100:1. Several contaminants were found in the toluene used to prepare this standard. Most of these contaminants were identified, but the peak at 15.3 minutes did not correspond to the retention times of those listed in Table 3. If the GC systems were not retention time locked, one might assume that this contaminant could be n-propylbenzene or p-ethyltoluene. However, given the retention time precision expected with RTL, it is clear that this contaminant is an unknown.

GC/MS is the best approach to identify this unknown, but under the same GC conditions, GC/MS retention times are often considerably faster than those obtained using atmospheric detectors. However, by combining retention time locking with a technique called method translation, one can obtain GC/MS retention times nearly identical to those found with conventional GC.[4] This makes identifying unknown peaks much easier. Figure 15 shows the D4534 toluene standard run on both the Agilent 6850 and the Agilent 5973 GC/MS. A mass spectral library search of the unknown peak at 15.320 minutes identifies this compound as chlorobenzene. The source of the chlorobenzene was found to be the toluene used to prepare the standard.

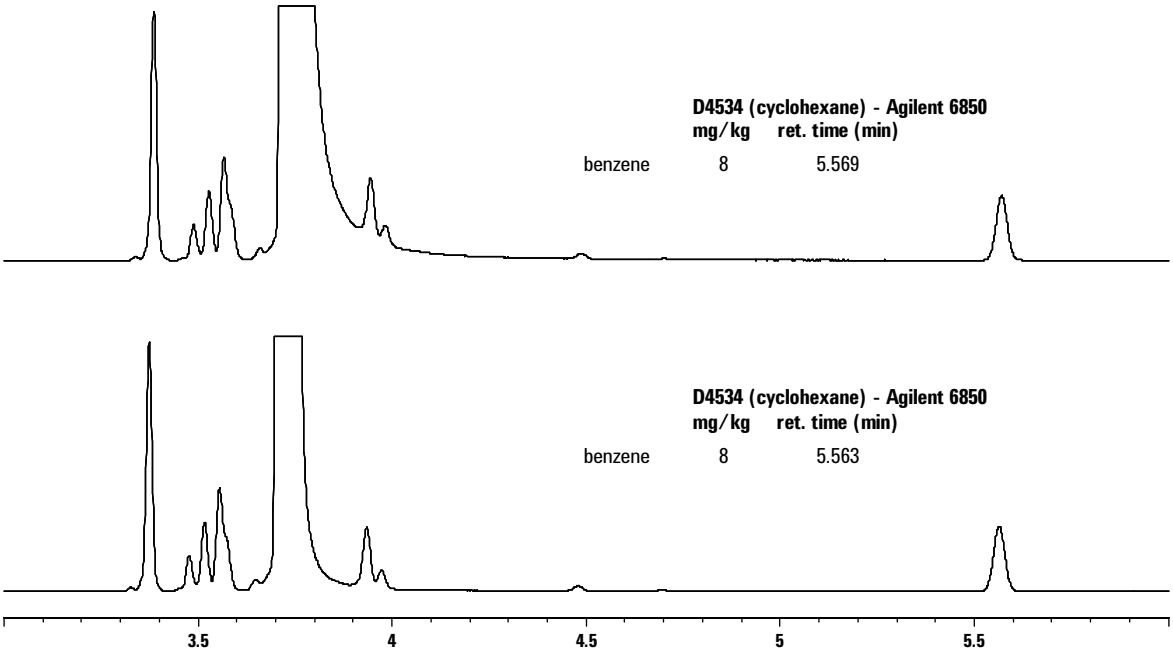


Figure 13. ASTM 4534 cyclohexane quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

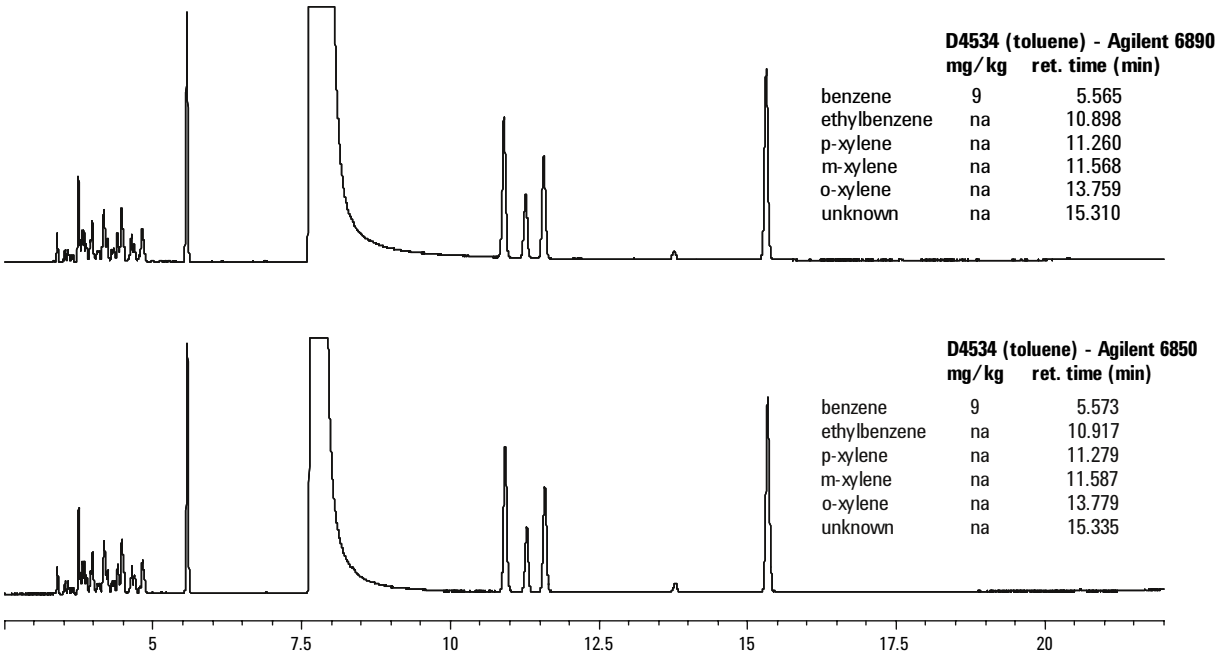


Figure 14. ASTM D4534 toluene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

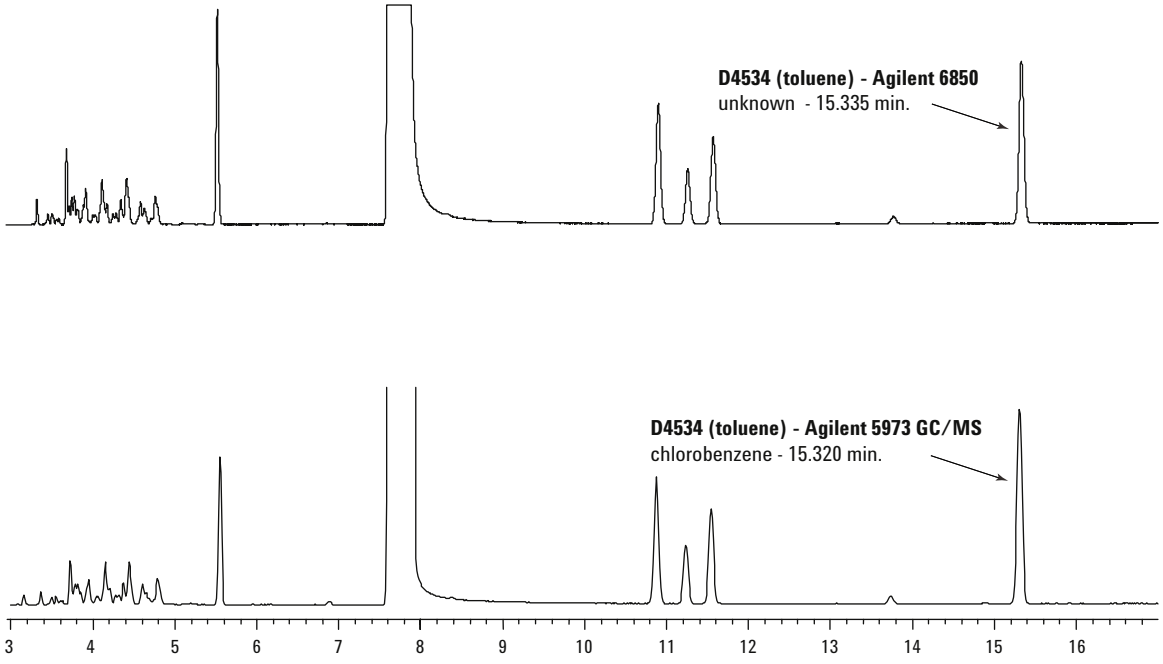


Figure 15. Unknown contaminant found in D4534 toluene standard (top) was identified as chlorobenzene using the retention time locked unified aromatics method run on the Agilent 5973 GC/MS (bottom).

D4534 Standard Test Method of Benzene Content of Cyclic Products - Cumene

Figure 16 shows the chromatograms of the D4535 calibration standard containing 5 mg/kg (ppm) of benzene in cumene. The injection size was 1.0 µL and the split ratio was 100:1. Details of these chromatograms are shown in Figure 17. Although benzene is well resolved, there are still some C9 hydrocarbons that elute near benzene. These compounds represent a potential source of interference when measuring small amounts of benzene (less than 5 mg/kg) in cumene.

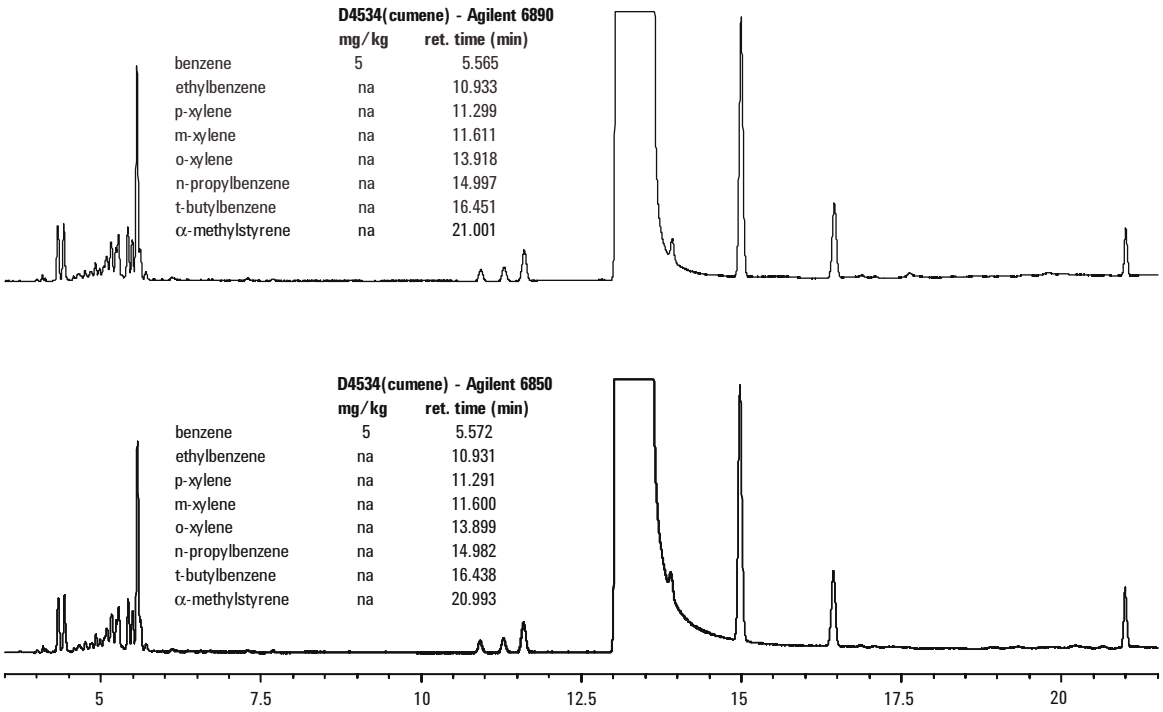


Figure 16. ASTM D4534 cumene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

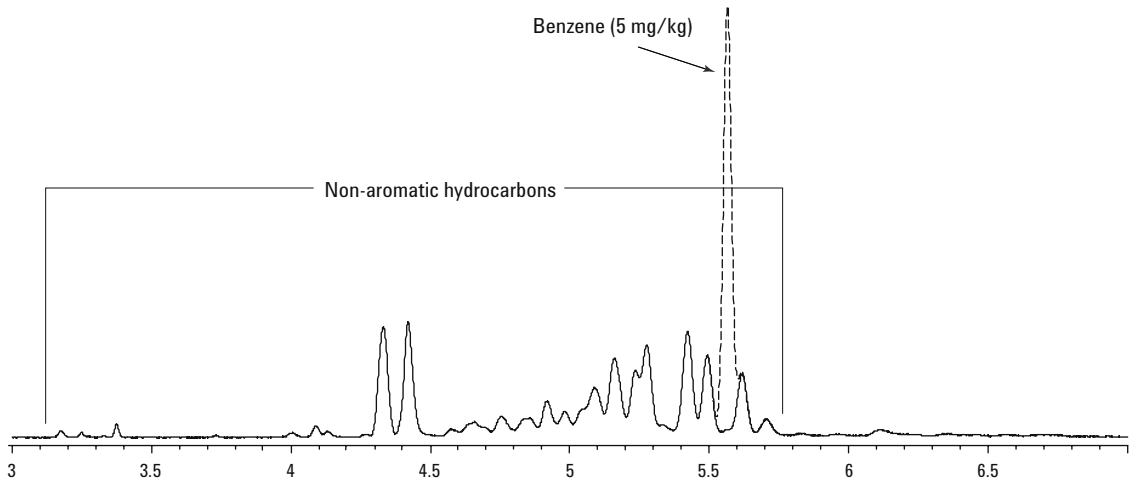


Figure 17. Details of the D4534 cumene standard showing the separation of 5 mg/kg of benzene from non-aromatic hydrocarbons typically found in cumene.

D5060 Standard Test Method for Determining Impurities in High-Purity Ethylbenzene

Figure 18 shows the chromatograms of the D5060 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

D5135 Standard Test Method for Analysis of Styrene by Capillary Gas Chromatography

Figure 19 shows the chromatograms of the D5135 calibration standard. The injection size was 1.0 µL and the split ratio was 100:1.

D5917 Standard Test for Trace Impurities in Monocyclic Hydrocarbons (ESTD Cal)

This method is identical to D2360 without the addition of the internal standard, n-butylbenzene, so that the chromatogram shown in Figure 7 is a good representation of an expected result. However, since n-butylbenzene is not included in the standard or samples for D5917, the run time of the unified method can be reduced to approximately 15 minutes.

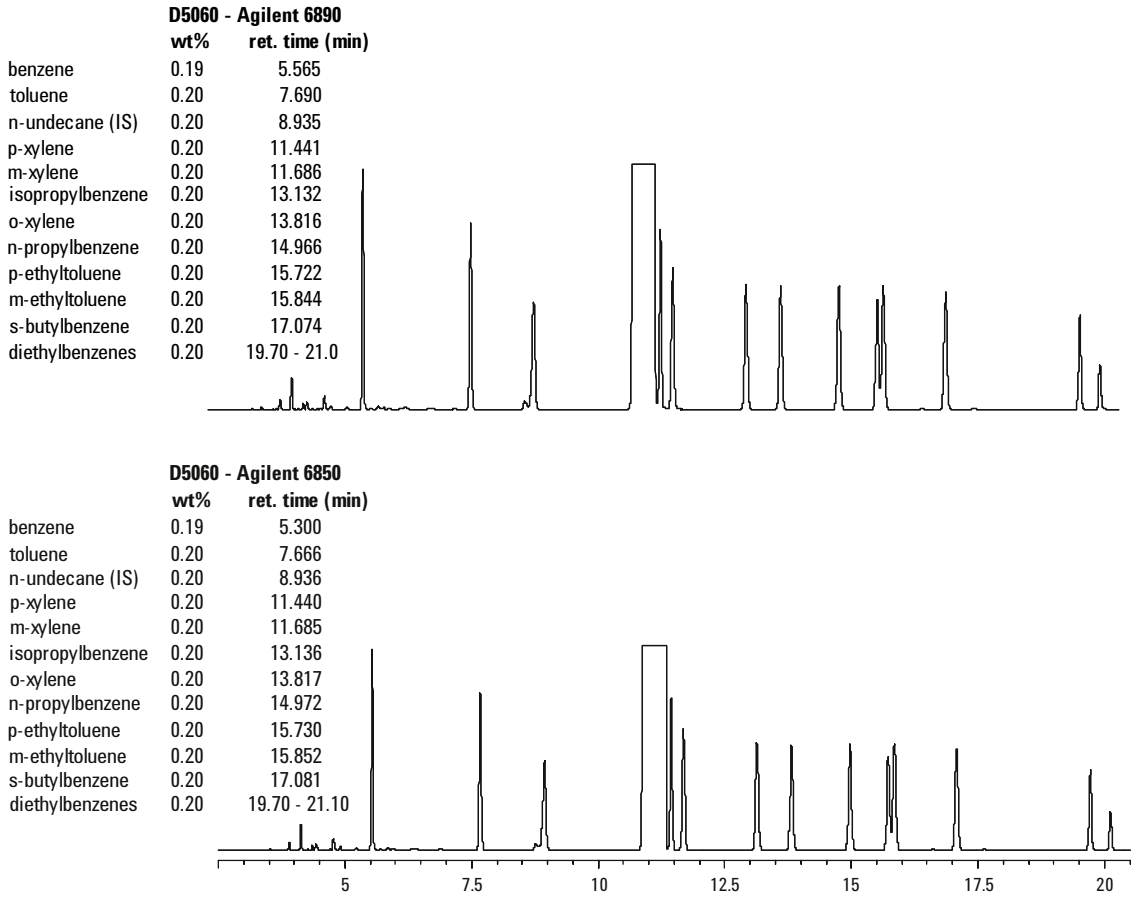


Figure 18. ASTM D5060 ethylbenzene quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

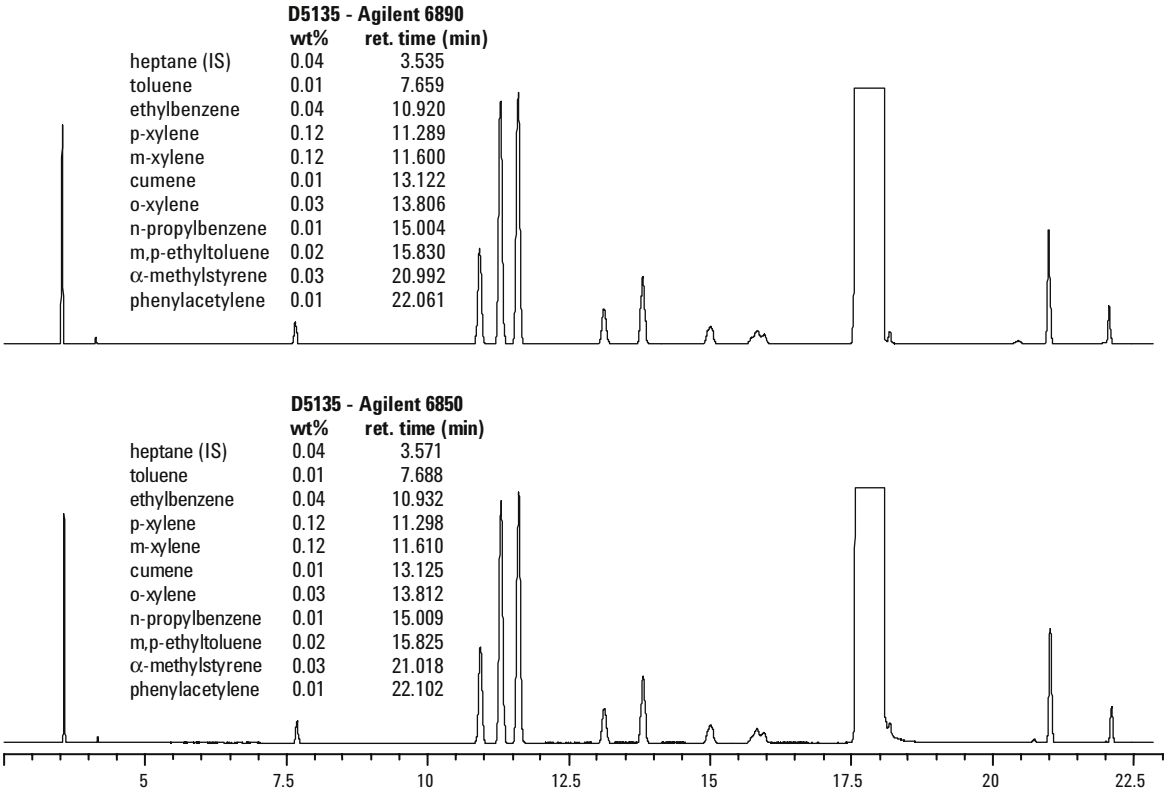


Figure 19. ASTM D5135 quantitative calibration standard run on Agilent 6890 (top) and 6850 (bottom) using the retention time locked unified aromatics method.

Conclusions

The analysis of many different bulk aromatic solvents in the QA/QC laboratory presents the analyst with an array of ASTM methods specific to each material. In an effort to simplify these measurements for today's laboratory environment, the chromatographies of ten ASTM methods were consolidated into one method. This unified method can resolve the 27 compounds found in aromatic materials and can successfully run the calibration standards used by each ASTM method to determine solvent purity. This versatile method can be run on both the Agilent 6890 and 6850 GC to yield consistent results between the method development lab and the plant production lab. To further improve performance, retention time locking (RTL) was applied to the unified method so that retention time standard deviation for each compound in any sample is less than 0.03 minutes. This allows easy comparison of results between instruments, laboratories and over time. The retention time locked unified method meets the need for a fast, simple method that can be run in today's production laboratories.

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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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Increasing Sample Throughput with High-Speed Megabore Columns

Application

Greater than 20% More Plates Per Meter

Improved Resolution and/or Faster Run Times Compared to 0.53-mm ID Columns

No Special Hardware Required

Decreasing the diameter of a capillary column is an effective way of increasing column efficiency. This increase in the number of theoretical plates per meter (N/m) can be utilized to improve compound resolution. A significant decrease in analysis time can also be achieved by adjusting the analysis conditions or shortening the column length.

For the chromatographer using Megabore (that is, 0.53-mm ID) columns, going to smaller internal diameter columns has not always been an option, due in part to capacity issues and injector and/or detector hardware incompatibilities. The 0.45-mm ID, High-Speed Megabore column introduces the traditional Megabore chromatographer to a column that can increase the resolution of analytes and/or reduce some analysis times by as much as 45%. Because Agilent's High-Speed Megabore columns retain the same outer diameter as 0.53-mm ID columns, no special ferrules or adaptors are required.

High-Speed Megabore columns also have the same phase ratio (ß) as 0.53-mm ID columns, making it very easy to translate the method conditions. Methods can easily be optimized for speed or resolution using free method translation software available from the Agilent Web site or by speaking with our Technical Support Department (call 800-227-9770 in the U.S. or Canada or visit www.agilent.com/chem).

On average, the High-Speed Megabore provides 24% more theoretical plates per meter than the comparable 0.53-mm ID column (Table 1). At some point, increasing a column's length can begin to work against chromatographic efficiency gain due to high carrier gas pressure drop in long capillaries. This is exemplified with the 105 m, DB-502.2. Figure 1 compares the two DB-502.2 columns for the analysis of volatile organics by purge and trap (for example, EPA Method 502.2). Most notable in these chromatograms are the essentially identical resolution of analytes and the 23-minute decrease in run time with the High-Speed Megabore column.

High-Speed Megabore columns are ideally suited to applications where dual 0.53-mm columns are currently being used. Figure 2a and 2b show one such application.

Table 1. Column Efficiencies

Column phase	Column length	Internal diameter	Film thickness [1]	Plates/meter (% increase) [2]
DB-VRX	75 meters	0.449 mm	2.55 µm	1997 (28)
	75 meters	0.540 mm	3.00 µm	1447
DB-624	75 meters	0.446 mm	2.55 µm	1402 (22)
	75 meters	0.546 mm	3.00 µm	1090
DB-502.2	75 meters	0.453 mm	2.55 µm	1526 (20)
	105 meters	0.544 mm	3.00 µm	873
DB-WAX	30 meters	0.447 mm	0.85 µm	1656 (25)
	30 meters	0.544 mm	1.00 µm	1357
DB-1	30 meters	0.455 mm	1.30 µm	1477 (27)
	30 meters	0.551 mm	1.50 µm	1357
DB-5	30 meters	0.446 mm	1.30 µm	1895 (23)
	30 meters	0.540 mm	1.50 µm	1454
DB-608	30 meters	0.450 mm	0.71 µm	1477 (23)
	30 meters	0.535 mm	0.83 µm	1134

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

[2] Average 24%

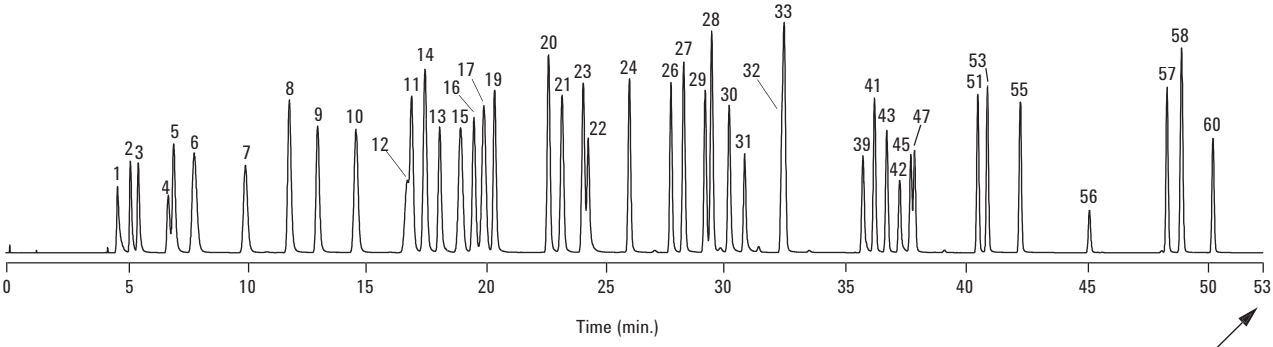
Compound List for all Chromatograms

1. Dichlorodifluoromethane
2. Chloromethane
3. Vinyl chloride
4. Bromomethane
5. Chloroethane
6. Trichlorofluoromethane
7. 1,1-Dichloroethene
8. Methylene chloride
9. trans-1,2-Dichloroethene
10. 1,1-Dichloroethane
11. cis-1,2-Dichlorethene
12. 2,2-Dichloropropane
13. Bromochloromethane
14. Chloroform
15. 1,1,1-Trichloroethane
16. 1,1-Dichloropropene
17. Carbon Tetrachloride
18. Benzene
19. 1,2-Dichloroethane
20. Silica trichloroethene
21. 1,2-Dichloropropane
22. Dibromomethane
23. Bromodichloromethane
24. cis-1,3-Dichloropropene
25. Toluene
26. trans-1,3-Dichloropropene
27. 1,1,2-Trichloroethane
28. Tetrachloroethene
29. 1,3-Dichloropropane
30. Dibromochloromethane
31. 1,2-Dibromomethane
32. Chlorobenzene
33. 1,1,1,2-Tetrachloroethane
34. Ethylbenzene
35. meta-Xylene
36. para-Xylene
37. ortho-Xylene
38. Styrene
39. Bromoform
40. Isopropylbenzene
41. 1,1,2,2-Tetrachloroethane
42. Bromobenzene
43. 1,2,3-Trichloropropane
44. n-Propylbenzene
45. 2-Chlorotoluene
46. 1,2,3-Trimethylbenzene
47. 4-Chlorotoluene
48. tert-Butylbenzene
49. 1,2,4-Trimethylbenzene
50. sec-Butylbenzene
51. 1,3-Dichlorobenzene
52. para-Isopropyltoluene
53. 1,4-Dichlorobenzene
54. n-Butylbenzene
55. 1,2-Dichlorobenzene
56. 1,2-Dibromo-3-chloropropane
57. 1,2,4-Trichlorobenzene
58. Hexachlorobutadiene
59. Naphthalene
60. 1,2,3-Trichlorobenzene

Conditions

Column: **DB-502.2, 105 m x 0.53-mm ID, 3.0 µm**
Part no.: 125-14A4
Carrier: Helium at 10 mL/min, measured at 35 °C
Oven: 35 °C for 10 min
35 °C - 200 °C at 4 °C/min
200 °C for 5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax™/Silica gel/Charcoal
Detector: Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat™ reaction tube in the halogen mode



Conditions

Column: **DB-502.2, 75 m x 0.45-mm ID, 2.55 µm**
Part no.: 124-1474
Carrier: Helium at 9 mL/min, measured at 35 °C
Oven: 35 °C for 6 min
35 °C - 200 °C at 8 °C/min
200 °C for 3.5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax/Silica gel/Charcoal
Detector: ELCD (OIA 4420) with NiCat reaction tube in the halogen mode

High-Speed Megabore saves 23 minutes!

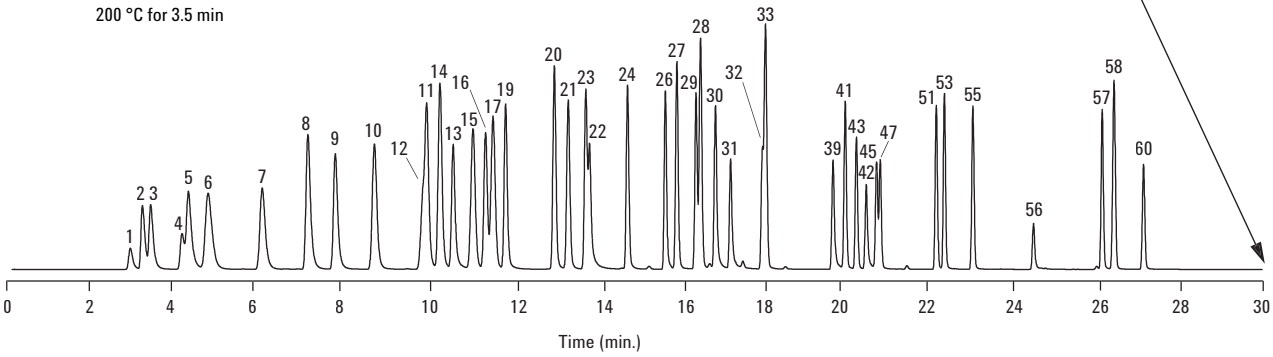


Figure 1. Analysis time comparison

Conditions
Figure 2a and 2b

Columns:	**DB-624** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1374 **DB-VRX** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1574
Guard Column:	5m x 0.53-mm ID deactivated fused silica tubing 3-way universal glass union
Carrier:	Helium at 9 mL/min (18 mL/min total), measured at 35 °C
Oven:	35 °C for 12 min 35 °C - 60 °C at 5 °C/min 60 °C for 1 min 60 °C - 200 °C at 17 °C/min 200 °C for 4 min
Injector:	Purge and trap (OIA 4560) 40 ppb per component in 5 mL water
Trap:	Tenax/Silica gel/Charcoal
Detector A:	Photoionization detector (PID) (OIA 4430) at 220 °C
Detector B:	Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat reaction tube in the halogen mode

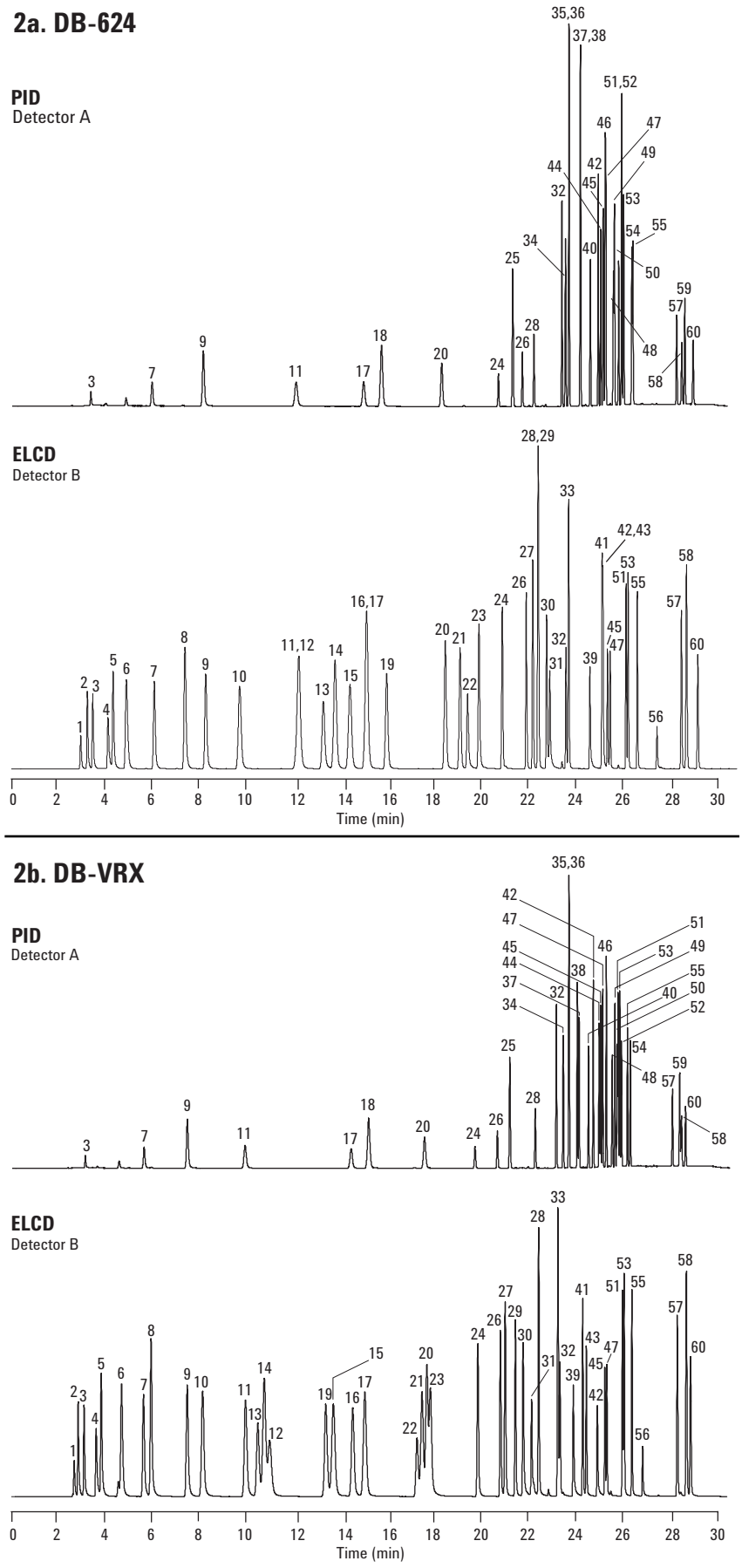


Figure 2a and 2b. High-Speed Megabore dual column applications.

0.45-mm ID High-Speed Megabore Column Order Guide

Phase[1]	Inner diameter (mm)	Length (meter)	Film thickness (µm)	Temperature limits (°C)	Part number
DB-1	0.45	15	1.27	-60 to 300/320	124-1012
DB-1	0.45	15	2.55	-60 to 260/280	124-1014
DB-1	0.45	30	0.42	-60 to 300/320	124-1037
DB-1	0.45	30	1.27	-60 to 300/320	124-1032
DB-1	0.45	30	2.55	-60 to 260/280	124-1034
DB-1	0.45	30	4.25	-60 to 260/280	124-1005
DB-1	0.45	60	1.27	-60 to 300/320	124-1062
DB-5	0.45	15	1.27	-60 to 300/320	124-5012
DB-5	0.45	30	0.42	-60 to 300/320	124-5037
DB-5	0.45	30	1.27	-60 to 300/320	124-5032
DB-5	0.45	30	4.25	-60 to 260/280	124-5035
DB-17	0.45	15	0.85	40 to 260/280	124-1712
DB-17	0.45	30	0.85	40 to 260/280	124-1732
DB-1701	0.45	30	0.42	-20 to 260/280	124-0737
DB-1701	0.45	30	0.85	-20 to 260/280	124-0732
DB-200	0.45	30	0.85	30 to 280/300	124-2032
DB-210	0.45	30	0.85	45 to 220/240	124-0232
DB-2887	0.45	10	2.55	-60 to 350	124-2814
DB-502.2	0.45	75	2.55	0 to 260/280	124-1474
DB-502.2	0.45	105	2.55	0 to 260/280	124-14A4
DB-608	0.45	30	0.42	40 to 260/280	124-6837
DB-608	0.45	30	0.70	40 to 260/280	124-1730
DB-624	0.45	30	2.55	-20 to 260	124-1334
DB-624	0.45	75	2.55	-20 to 260	124-1374
DB-FFAP	0.45	15	0.85	40 to 250/250	124-3212
DB-FFAP	0.45	30	0.85	40 to 250	124-3232
DB-MTBE	0.45	30	2.55	35 to 260/280	124-0034
DB-TPH	0.45	30	1.00	-10 to 290/290	124-1632
DB-VRX	0.45	30	2.55	-10 to 260	124-1534
DB-VRX	0.45	75	2.55	-10 to 260	124-1574
DB-WAX	0.45	60	0.85	20 to 230/240	124-7062
DB-WAX	0.45	15	0.85	20 to 230/240	124-7012
DB-WAX	0.45	30	0.85	20 to 230/240	124-7032
DB-WAXetr	0.45	5	1.70	50 to 230/250	124-7304
DB-XLB	0.45	30	1.27	30 to 320/340	124-1232

[1]Additional phases, lengths, and film thickness can be made with a 0.45-mm ID High-Speed Megabore column. If you do not find the column you are looking for, ask for a custom column quote (order part number 100-2000 and specify the phase, ID, length, and film thickness).

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Agilent Technologies

DuraGuard Columns: GC Columns with Built-In Protection

Application

Guard columns/retention gaps without the use of unions

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

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

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

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

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

Guard Columns

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

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

Retention Gaps

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

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

MSD Transfer Line

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

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

Results

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

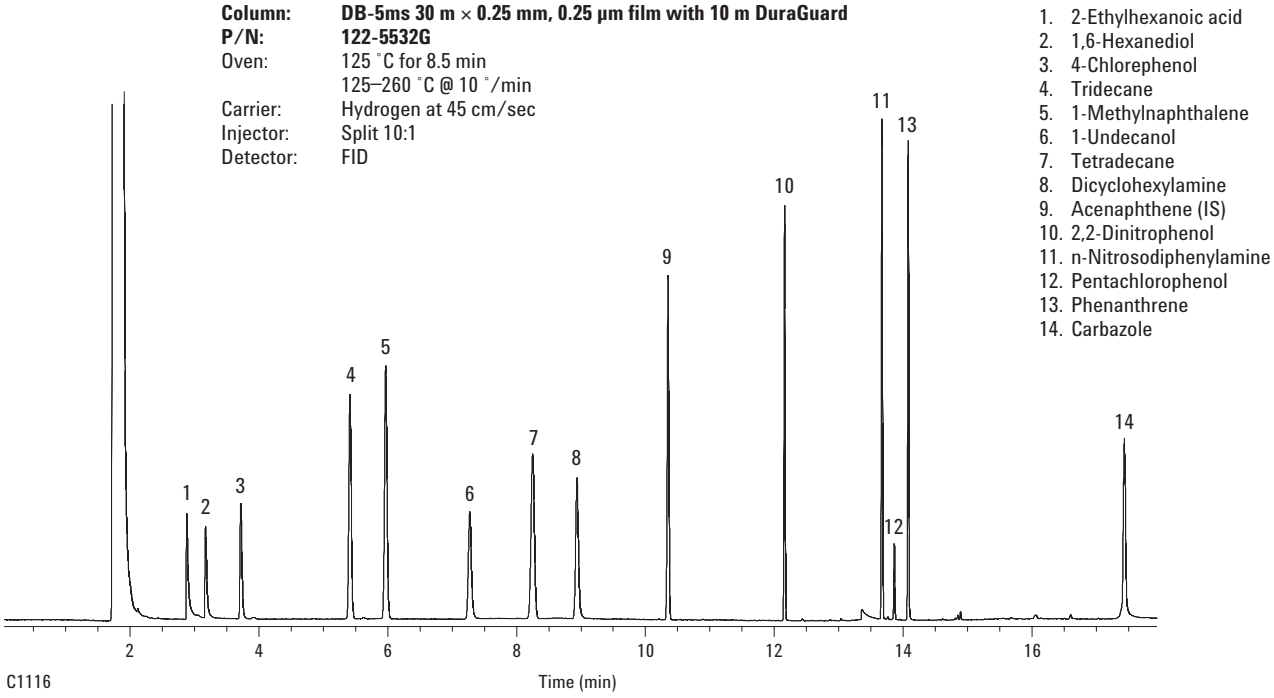

Figure 1. Chromatogram of test mixture using combination guard and analytical columns.

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

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

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

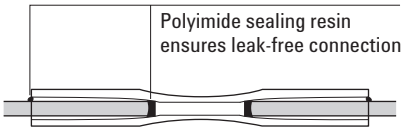

Figure 2. Detail of glass press fit union with polyimide sealing resin.

DuraGuard Column Order Guide

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

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

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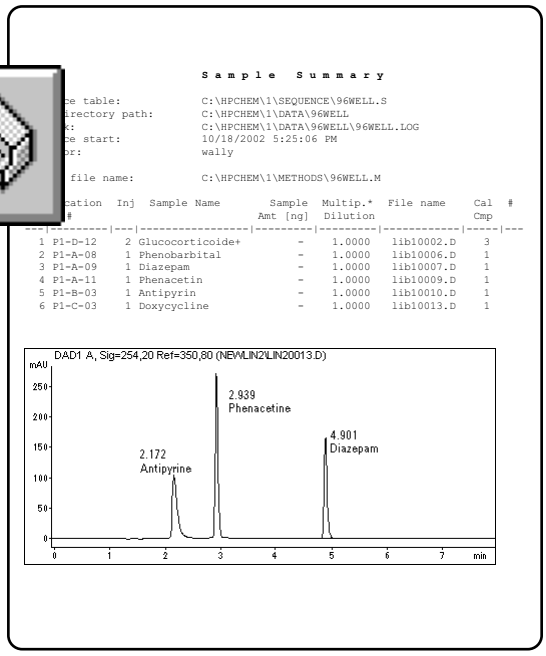
Agilent Technologies

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

Application

Angelika Gratzfeld-Huesgen



Sample Summary

ce table:	C:\HPCHEM\1\SEQUENCE\96WELL.S
irectory path:	C:\HPCHEM\1\DATA\96WELL
t:	C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
ce start:	10/18/2002 5:25:06 PM
or:	wally
file name:	C:\HPCHEM\1\METHODS\96WELL.M

cation #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal Cmp #
1 P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3
2 P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1
3 P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1
4 P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1
5 P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1
6 P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1

Introduction

The Agilent ChemStation base software includes a wide range of built-in report styles and types. For example, it provides standard reports such as area percent (AREA%), external standard (ESTD), internal standard (ISTD), and normalized (NORM) reports as well as system suitability reports and sequence summary reports with statistical evaluation of retention times, areas, heights and more.

For each type of report the user can determine the amount of information that is included in the report. The ChemStation base software also provides a report editor for customizing reports — a topic that is beyond the scope of this note.

This Application Note describes how to set up the different report types, explaining the software screens and giving example reports. The main objective is to give guidelines and to provide strategies on how to use the different built-in reports in the ChemStation base software.

Agilent Technologies

Equipment

The data for the report examples was generated using an Agilent 1100 Series HPLC system comprising the following modules.

- high pressure gradient pump
- micro-vacuum degasser
- well plate sampler
- thermostatted column compartment
- diode array detector

The Agilent ChemStation base software including the 3D data evaluation module, revision A.08.04, was used for instrument control, data acquisition, data handling, sample tracking, and reporting.

Report setup on ChemStation

The standard reporting function in the ChemStation base software provides for single run reports or sample-set reports for a full sequence of runs, whereby these so-called sequence summary reports can only be generated after completion of the sequence. The content of the sequence summary reports is defined by the acquisition sequence.

Further, the ChemStation base software includes a wide range of built-in standard reports that allow users to define the content and amount of printed information. Whereas this functionality meets the requirements of most standard applications to a large extent, it does not have the flexibility to create additional table elements for non-chromatographic information, charts or custom calculations.

If such extended reporting capabilities are required, it is recommended to use the ChemStation Plus data system including the ChemStore data organization module.

The ChemStation base software offers four types of report.

- Individual run reports, which can be generated automatically after each run or sequence, provide quick and easy printouts of results.

- Sequence summary reports provide comprehensive infomation for a full set of samples, including full GLP/GMP details. They are generated automatically at the end of a sequence and may include individual reports as well as statistical summary reports.

- Batch reports provide direct printouts of first-pass review modifications and results. They are generated during reprocessing of data from a complete sequence or of a subset of one sequence using ChemStation batch review.

- Advanced custom reports for requirements that go beyond the scope of the previous types. These include customized reports for individual runs or complete sequences and can also be obtained automatically after each run or sequence.

The following sections focus on the individual-run and sequence-summary report types, which are built-in as standard in the ChemStation base software, and explain in detail how to use and set up these report types.

Qualitative reports for individual runs

Qualitative reports are used mainly during the development of a separation or when a quick decision is needed as to whether a compound is present or not. Here the separation of peaks is of primary interest and a short AREA% report is sufficient. Particularly during method development it does not make sense to obtain reports with quantitative results.

Setup

To obtain an automated printout of an individual report such as a short AREA% report, the item *Standard Data Analysis* must be selected in the *Run Time Checklist*, which is part of the overall method for acquisition, data analysis and reporting, see figure 1. This screen is part of the *Edit Entire Method* dialog or can be accessed directly from the *Method* menu of the *Method and Run Control* view.

The item shown in figure 1 must be selected when the calculation of results is required, such as for printing reports, including sequence summary reports, with or without individual run reports.

Configuration

To obtain qualitative reports the item *Calculate* in the group *Quantitative Results* must be set to *Percent* as shown in figure 2.

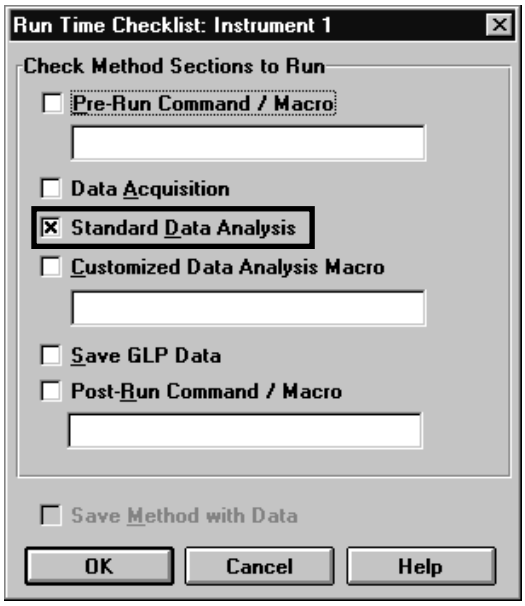


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

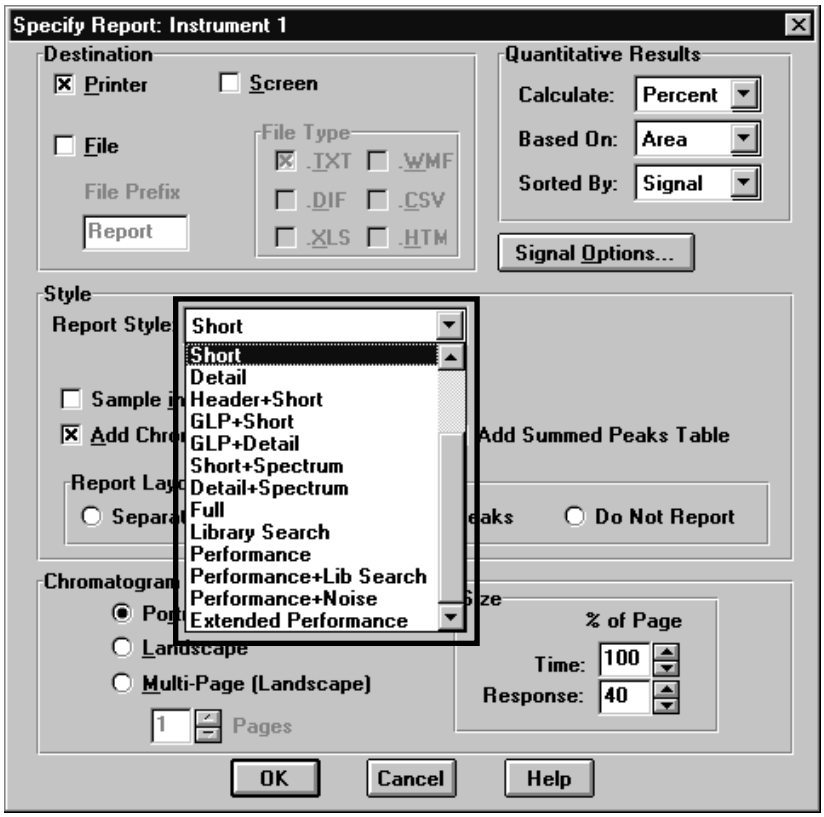


Figure 2
Specifying individual run reports

There are three ways to set up reports for individual runs.

1 Using the report smart icon in the *Method and Run Control* view.
2 Using part of the *Edit Entire Method* wizard
3 Using the *Data Analysis* view by selecting *Report* and then *Specify Report*.

Figure 2 shows the setup screen for run reports. Several report styles are available, covering a broad spectrum of report types. The report output can be sent to a printer, displayed on the screen or saved to a file. Multiple report destinations can be selected at a time. Other report parameters allow to include chromatograms, in landscape or portrait format or even distributed over several pages, and to define the way unknown compounds are reported.

An example of an AREA% report is given on page 12, containing information about the used method, data filename, time of injection, chromatogram and report.

The report styles that are available depend on the installed software modules. For example, the report styles Short+Spectrum, Detail+Spectrum and Library Serach are only available when the 3D data evaluation module is installed.

During method development the combination of *Percent* and *Performance* in reporting can be a valid tool to find out about k', resolution, selectivity, peak width and, for isocratic runs, the number of plates. An example is given on page 19.

Calculation procedures such as **Percent** (for others such as ESTD and ISTD, see section "Quantitative reports for individual runs") can be combined with any of the available standard reports shown in figure 2.

Qualitative reports can not use calculations based on standards such as ESTD and ISTD.

Quantitative reports for individual runs

Quantitative reports offer compound identification and compound quantification. They are mainly used with known samples or reference results in method optimization and quality control areas.

Setup

Before a quantitative report can be generated, standard samples with known compound concentrations have to be run and a calibration table has to be set up.

Peak integration should always be optimized before a peak is used as a reference in the calibration table and before the calibration tasks are done. To optimize integration, load a sample file with known sample concentration and then use the *Integration* tool set in the *Data Analysis* screen. When integration is optimized and saved, the calibration table can be created.

The calibration table is set up in *Data Analysis* from the *Calibration* menu, see figure 3.

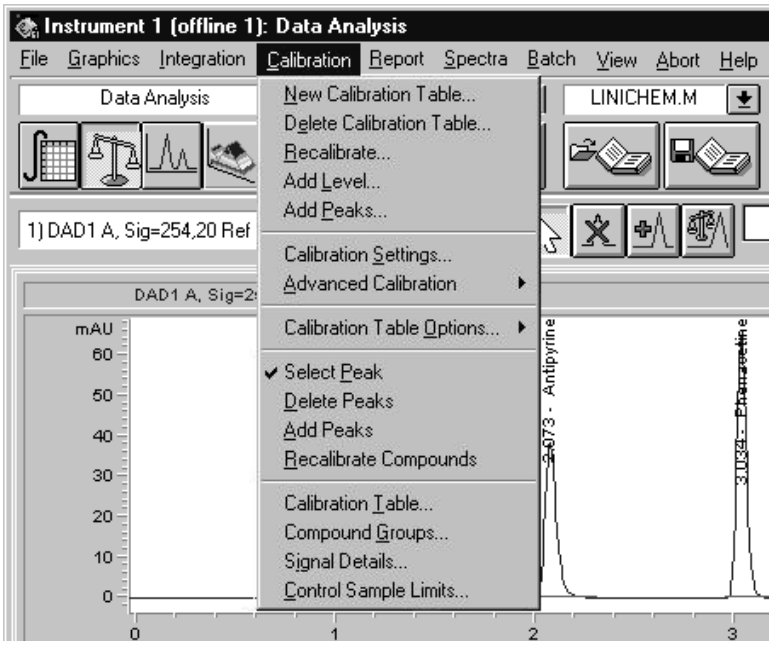


Figure 3
Calibration setup menu

In the following example we set up a multilevel calibration with four calibration levels. Multilevel calibrations use multiple files to complete the calibration. One file defines one level—completion of a four-level calibration thus requires four files. The steps involved are as follows.

1 Load the first file and click on *New Calibration Table*.

2 Calibrate each peak by selecting the peak (left mouse click), and filling in compound name and compound amount.

3 Repeat step 2 for all peaks.

4 When all peaks in the file are calibrated, load the next file with the next concentration. Use the *Add Level* tool to fill in the amounts for the next concentration level (level two).

5 Repeat step 4 for level three and four.

The calibration is stored as part of the ChemStation method. It is saved by simply saving the method. Every calibration update is easily accessible by loading the method, modifying (for example, updating) the calibration files and saving the new method revision.

Setup

When the calibration is complete all prerequisites for generating a quantitative report are met. The first step in generating a report is to specify the report style as described in the section "Qualitative reports for individual runs." The calibration of the method now offers access to all predefined report styles such as standards reports or normalized reports or, when running a sequence, to sequence summary reports (see separate section later.)

The calculation of results can be a normalized (NORM) area determination or based on an external standard (ESTD) or internal standard (ISTD). Result calculations can be based on area or height. Figure 4 shows selection of *External Standard Method* as calculation procedure and *Short* as *Report Style*. An example is given on page 13.

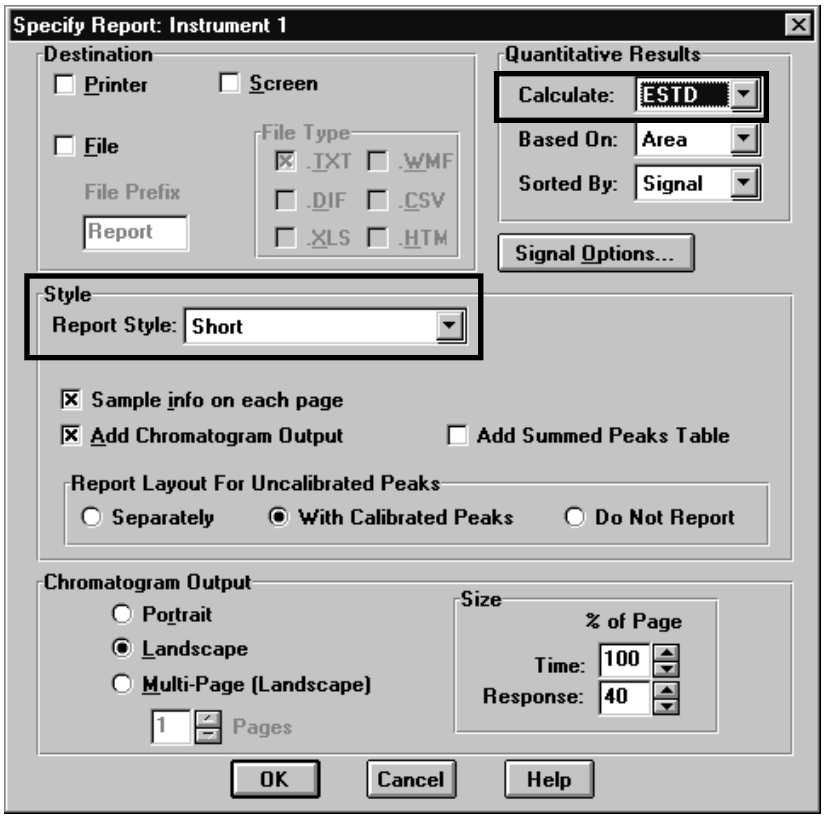


Figure 4
Selection of external standard report and short report style

Configuration

Additional report features can be specified such as output format for the chromatogram (including multipage outputs), picture size and the documentation of uncalibrated (which means unknown) peaks in the *Specify Report* screen as shown in figure 4. Any report style (see figure 2) can also be combined with any calculation procedure. Examples are given on pages 13 through 21.

- ESTD combined with report style *Short* (p 13)
- ESTD combined with report style *Library Search* (p 14)
- ESTD combined with report style *GLP+Short* (p 16)
- ESTD combined with report style *Performance* (p 19)
- ESTD combined with report style *Detail* (p 20)

Similar to the calibration, the report configuration is saved with the ChemStation method. Thus all data analysis steps for integration, calibration, result calculation and reporting are saved under one "umbrella" tool. Once setup, reuse of all steps is automated by simply reapplying the method to any sample under investigation.

The method that has been set up for data acquisition, integration, calibration and reporting has to be saved under a unique name to ensure that samples are analyzed and evaluated using the correct conditions.

Final report output

Final report outputs are quick and easy to obtain with ChemStation. Both qualitative and quantitative reports offer the same options and use identical tools to generate the final report.

Reports can be
- sent to a printer
- displayed on the screen for a quick review or preview when setting up report options
- saved to a file in HTML, CSV, XML, TXT, WMF, or DIF format

It is possible to combine all output types, for example, to get a printed copy on paper, an online report display on the screen and a file copy on the local hard disk.

The user can choose either
- automated report output at the end of each sample analysis (or reanalysis), or
- interactive report output at user request

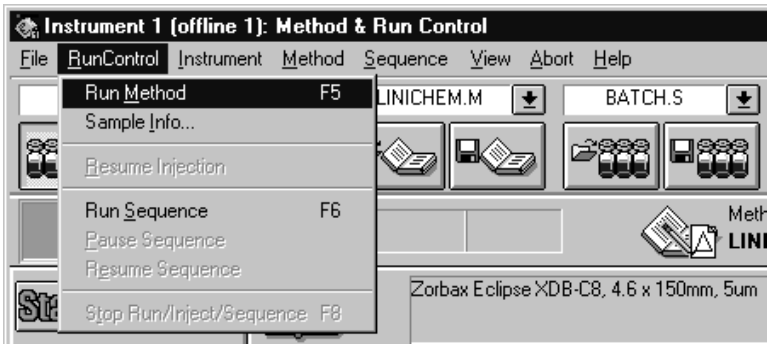


Figure 5
Run method for automated method execution and result output

Automated report output

An automated report is output whenever the ChemStation method is executed and at least one report destination is selected in the *Specify Report* screen, see figure 4. If no report output is desired, simply leave all report destination check boxes blank.

Method execution typically is used to analyze a sample or to reapply changes in calculations or calibration during data analysis. To execute a method, simply press *F5* or select *Run method* from the ChemStation *Run control* menu as shown in figure 5.

If the user wants to re-analyze data without data acquisition, *Data Acquisition* must be disabled in the *Run Time Checklist*, see figure 1.

Interactive report printout

Manual report output is available from the ChemStation *Data Analysis* view. It is designed to preview report outputs on the screen during report configuration or to get an individual sample report during interactive result analysis or result review.

The *Data Analysis* view is designed to set up advanced reports such as library searches, detailed spectrum reports and others. It has a separate report menu and additional smart icons for report setup, preview and output to a printer as shown in figure 6.

When the user wants a report during their data review session, they simply press the preview or print button and immediately get the report on the screen or on paper.

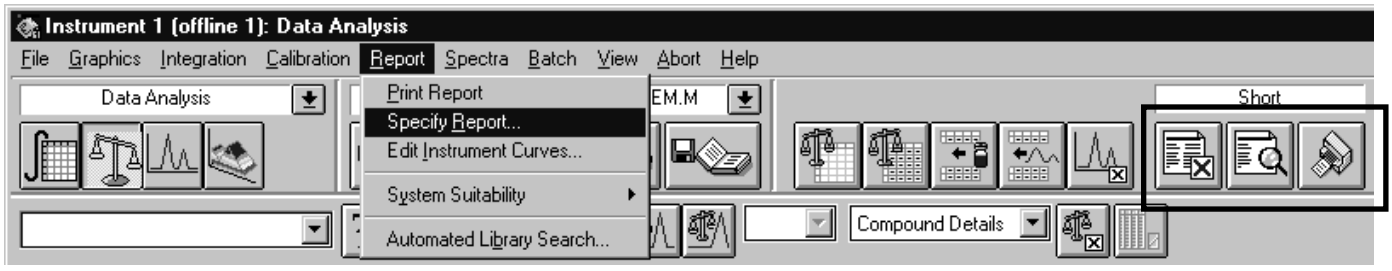


Figure 6
Report menu and smart icons (far right) in ChemStation Data Analysis view

Sequence summary reports

In contrast to individual run reports, sequence summary reports can only be generated for a complete set of samples that have been analyzed in one continuos sequence. The sequence summary report (also referred to as a system suitability report) is designed to meet the specific needs of GLP and GMP regulations in the pharmaceutical industry as well as comparable ISO and DIN regulations in other industries.

In addition to result calculation and result documentation, all regulations require additional documentation on how the results have been obtained and how "well" the analytical system behaved during analysis. The sequence summary report is a single all-inclusive report style, combining the analytical result with full documentation of how the result was obtained and the system suitability information, thereby providing a comprehensive report that addresses all regulatory requirements.

Sequence summary reports are frequently used in quality control work. These reports include the analytical results along with documented evidence of the system's suitability for the analytical purpose. System suitability is defined in the various Pharmacopoeia guidelines and it typically includes system performance information based on parameters such as peak width, theoretical plate number, resolution and others.

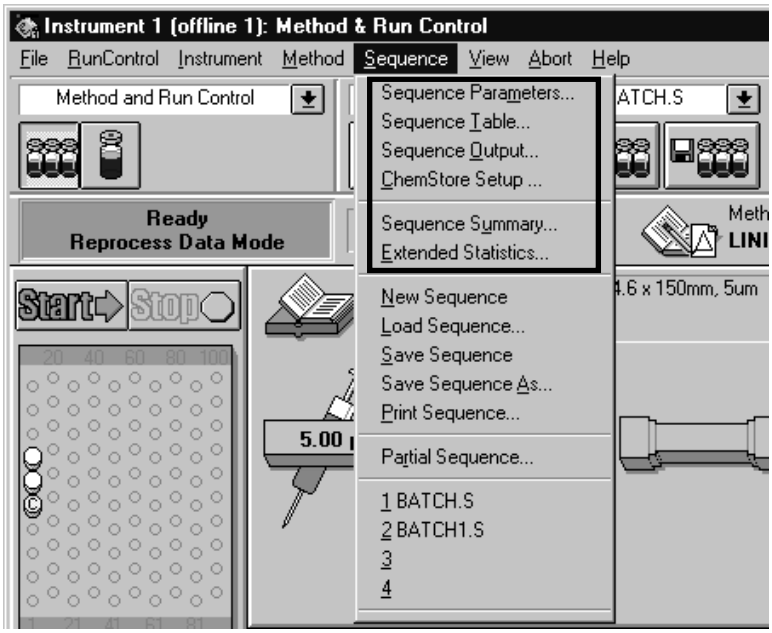


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

All these parameters are available in the report style, but the user must configure the report to suit their own specific needs. The following section describes setup and configuration of a sequence summary report in ChemStation.

Setup and configuration

After each sequence of runs a sequence summary report can be printed. Typically this is done to obtain statistical results and determine system suitability. In addition to the entries in the sequence table and before the report can be calculated and printed, several data inputs for sequence parameter and sequence output are required, see figure 7.

In the *Sequence Parameters* screen (figure 8) the item *Parts of Method to Run* must be set to *According to Runtime Checklist*. This entry determines which part of a method is executed during a sequence and *According to Runtime Checklist* refers to the run-time checklist configuration that was previously edited as part of the method in order to obtain integration and quantitative results.

If data acquisition is completed and the user wants to reanalyze a sequence of samples without data acquisition, the option *Reprocessing Only* allows to recalculate the sequence summary report easily.

In the *Sequence Output* screen the report destination and the content of a sequence summary report are defined by selecting the appropriate check boxes, see figure 9.

The content of the sequence summary report is defined by the items on the right side of the scrreen shown in figure 9. Selecting *Setup* in the *Sequence Output* dialog box accesses this configuration screen. The sequence summary report allows a variety of informations to be printed in one continuously enumerated report.

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

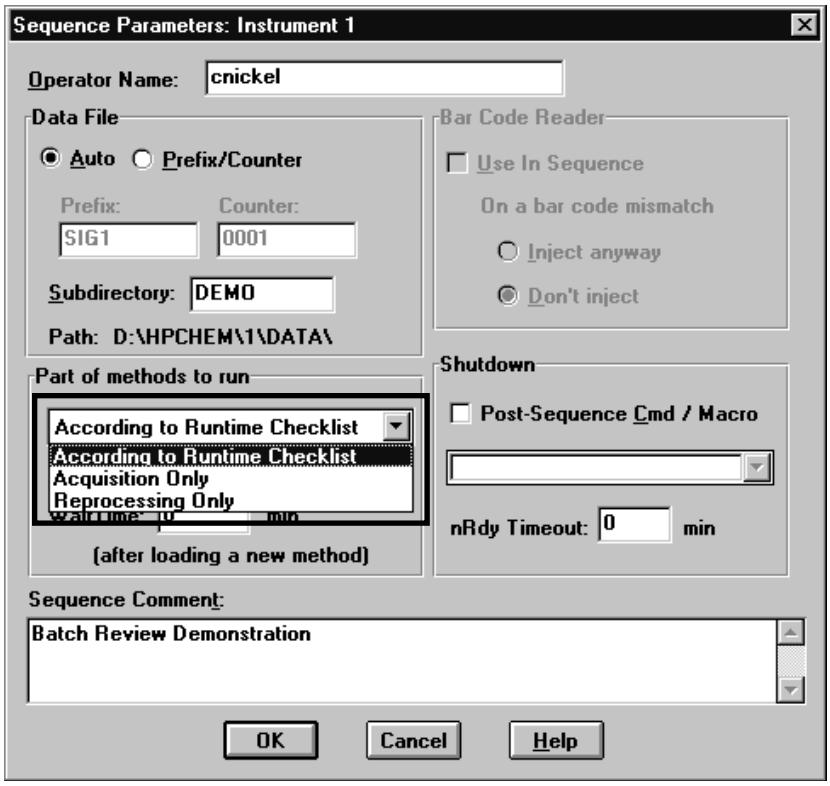


Figure 8
Sequence parameters screen

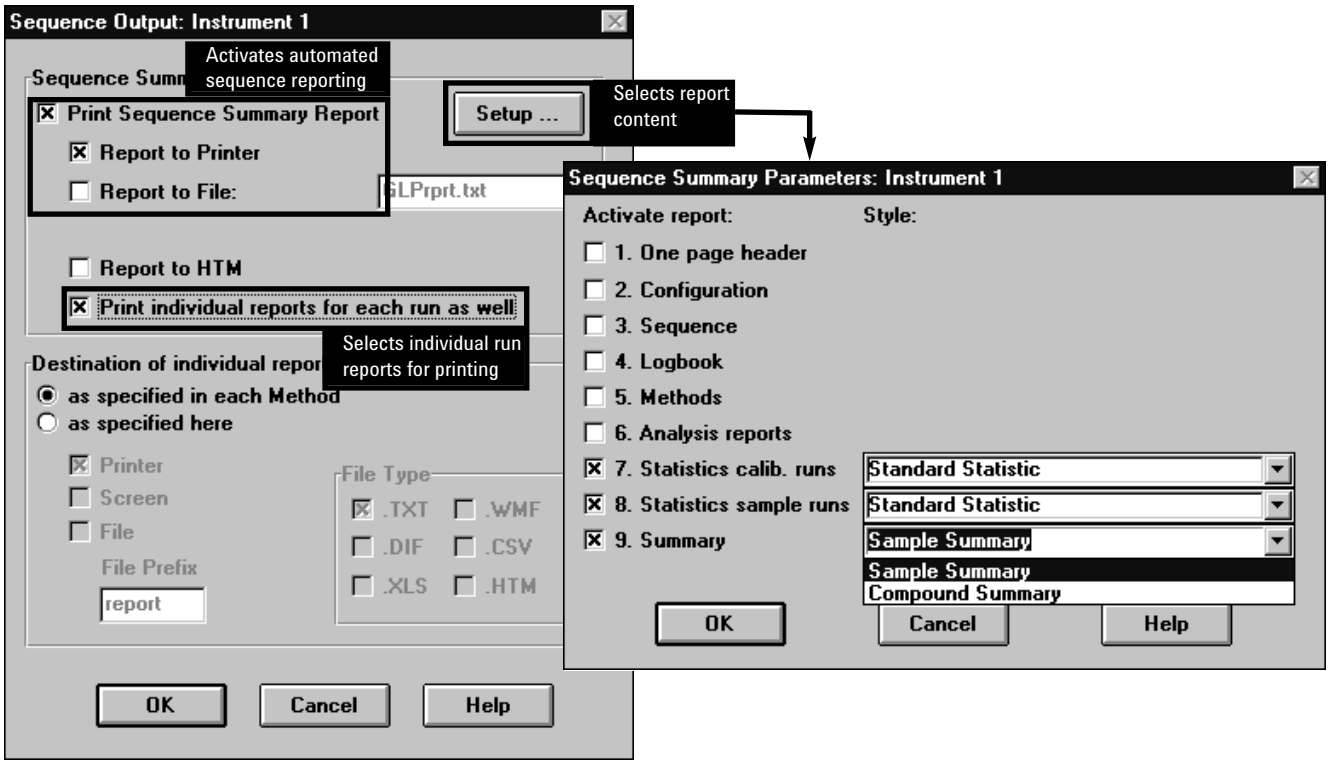


Figure 9
Selection of report destination and content of a sequence summary report

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

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

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

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

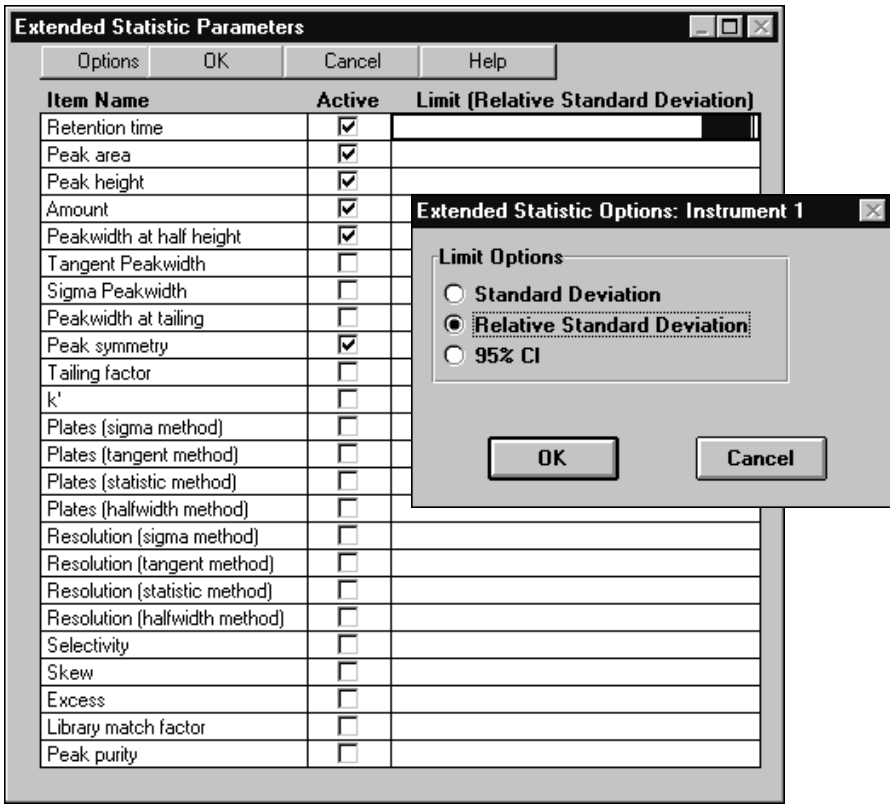


Figure 10
Setup of statistical calculations for sequence runs

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

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

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

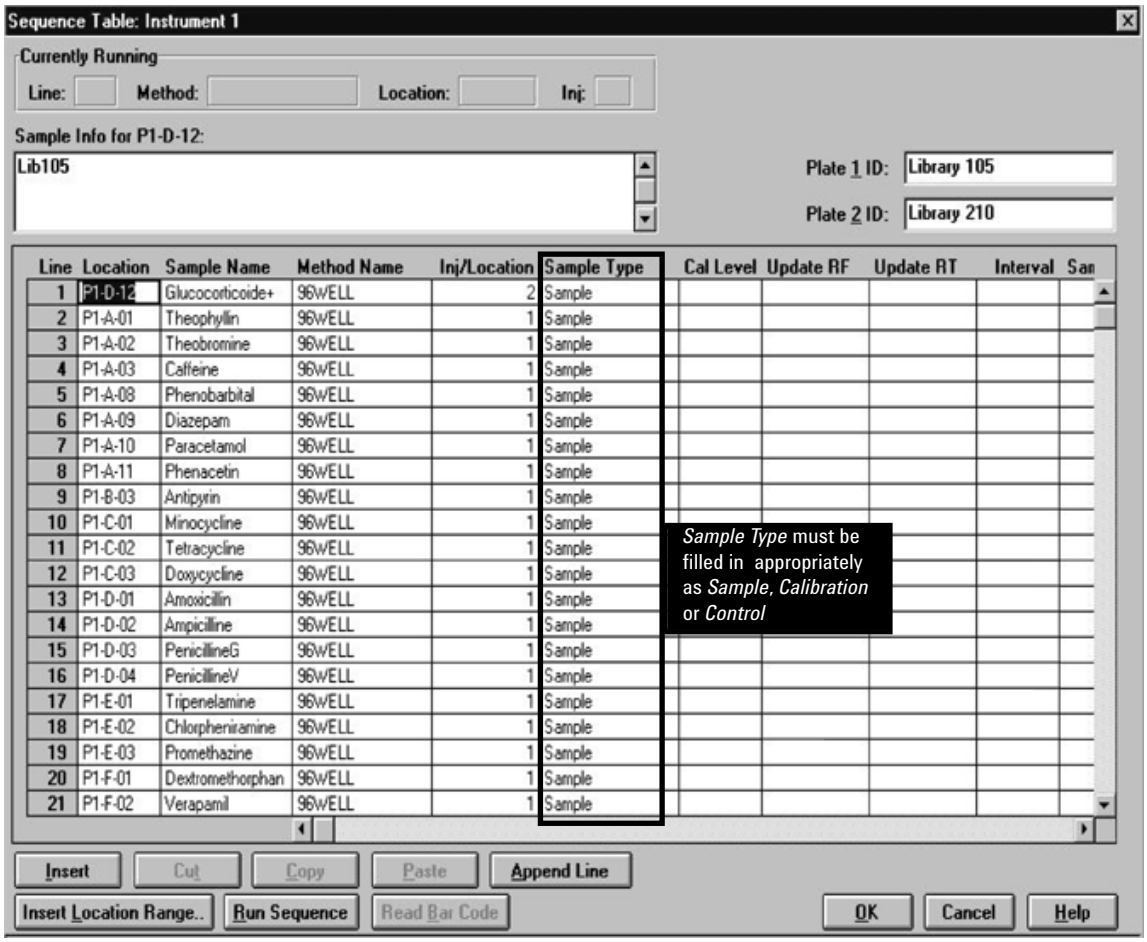


Figure 11
The Sequence Table screen

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

Sequence table: C:\HPCHEM\1\SEQUENCE\96WELL.S
Data directory path: C:\HPCHEM\1\DATA\96WELL
Logbook: C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
Sequence start: 10/18/2002 5:25:06 PM
Operator: agratz

Method file name: C:\HPCHEM\1\METHODS\96WELL.M

Run #	Location #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal #	Cmp
1	P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3	
2	P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1	
3	P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1	
4	P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1	
5	P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1	
6	P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1	

Figure 12
Example of a sequence sample summary report

Conclusion

The built-in single-run and sequences summary reports that are available in the ChemStation base software offer a wide range of reporting capabilities. The various reports give access to all important sample-related information quickly and easily. For all report types the user can select the amount of information to be included, from a simple qualitative report on one page through detailed quantitative reports to comprehensive and powerful sequence summary reports. Knowledge of a report editor is not required to be able to set up the ChemStation reports.

Reports can be obtained after each run or at the end of a sequence. With the ChemStation Method concept users starting from scratch can have a printed result copy of any type in less than 10 minutes – once set up the report is available within seconds after run completion. ChemStation reports are easy to configure, fast to obtain and quickly stored and managed.

Appendix

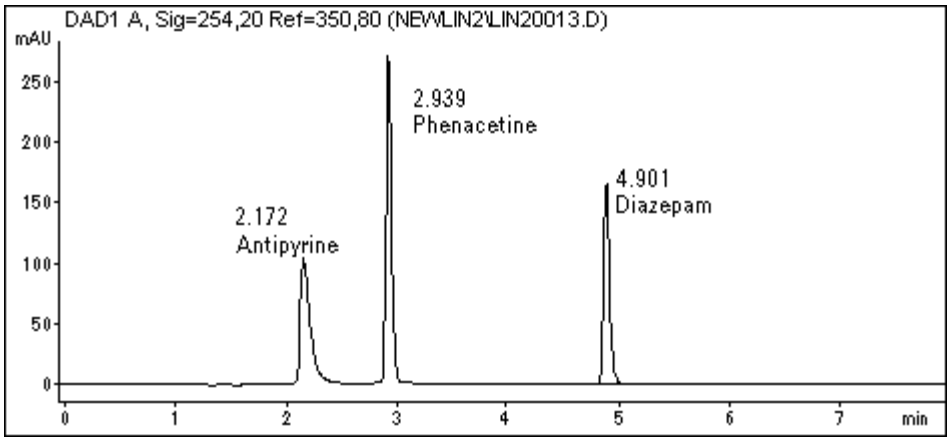
The following pages show examples of summary reports that can be generated with the ChemStation base software. The examples were generated using the print-to-file function and may have different pagination than a report printed directly from the ChemStation. Reports shown include:

- Short Area Percent Report
- Short ESTD Report
- Spectral Library Search Report
- Short GLP Report
- Performance Report
- Detail Report
- Extended Performance Report
- Sequence Summary Report – Compound Summary
- Sequence Summary Report – Standard Statistics for Sample Runs

Short Area Percent Report

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

==================================================================
Injection Date  : 10/25/00 8:47:20 AM            Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                             Inj :   1
                                                 Inj Volume :  1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 8:53:08 AM by agratz
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
```




```
                          Area Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 8:52:20 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

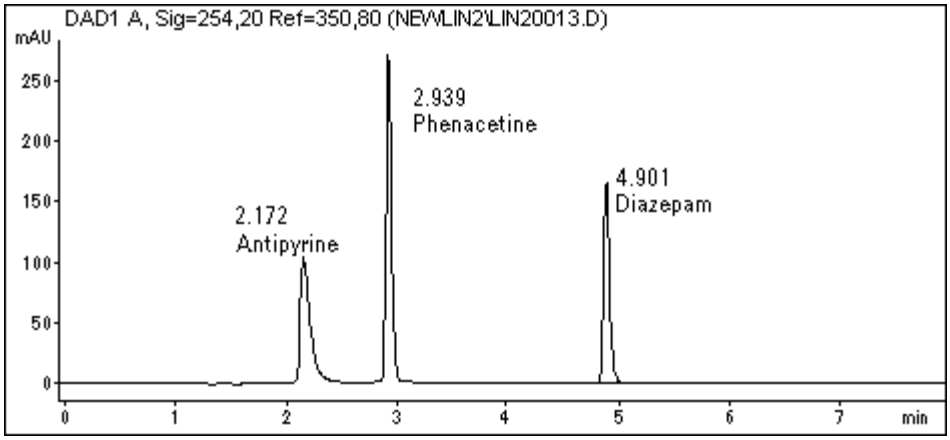
Peak RetTime  Type    Width      Area       Area     Name
  #    [min]           [min]    [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
  1  1.424    BV     0.0829    10.51506   0.4743 ?
  2  2.172    BB     0.0933   661.70422  29.8443 Antipyrine
  3  2.939    BB     0.0535   934.32690  42.1402 Phenacetine
  4  4.901    BB     0.0566   610.64050  27.5412 Diazepam
Totals :                    2217.18669

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

Short ESTD Report

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

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                          Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !       Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:09:14 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                        External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

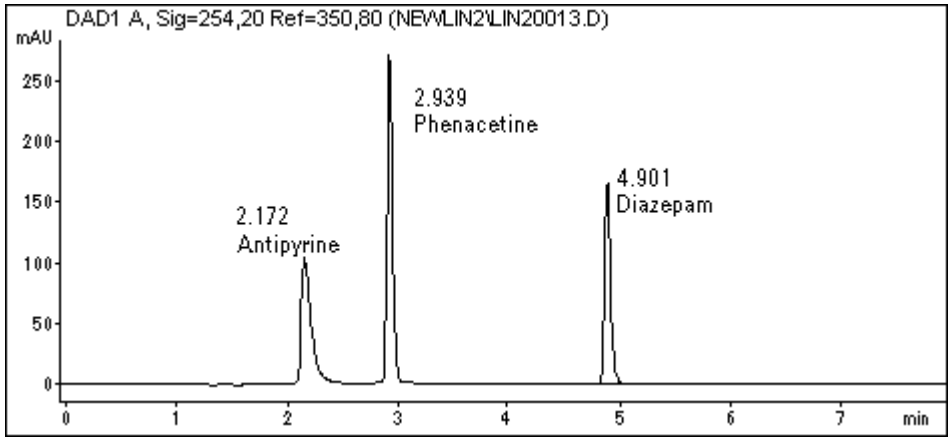
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB      661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB      934.32690    1.00317   937.28787     Phenacetine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

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

Spectral Library Search Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:28:46 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:28:26 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                    External Standard Report
=====================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Library search mode:     Automatic library search

Library file No.       : 1
Library file name      : D:\HPCHEM\1\METHODS\LINICHEM.M\PHARMA.UVL
Match threshold        :     950        Purity threshold: Calculated
Time window  left [%] :    5.00         Case sensitive : No
Time window right [%] :    5.00             Whole word : No
Wavelength shift       :     0.0       Compare spectrum : Yes
Absorbance threshold   :     0.0           Search logic : OR
Search range           :     All
```

Spectral Library Search Report (continued)

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!
Calibrated compounds:

Meas. RetTime [min]	Library RetTime [min]	CalTbl RetTime [min]	Sig	Amount [ng]	Purity Factor	#	Library Match	Name
2.172	2.177	2.071	1	438.70069	1000	1	1000	Antipyrine
2.939	2.944	3.038	1	937.28787	1000	1	1000	Phenacetine
4.901	4.904	5.090	1	599.59734	1000	1	1000	Diazepam

Note(s):
u: compound identified at upslope. Purity factor exceeds threshold.
d: compound identified at downslope. Purity factor exceeds threshold.

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

Short GLP Report

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

This is a special file, named RPTHEAD.TXT, in the directory of a method
which allows you to customize the report header page.
It can be used to identify the laboratory which uses the method.

This file is printed on the first page with the report styles:

   Header+Short, GLP+Short, GLP+Detail, Short+Spec, Detail+Spec, Full


                  XXXX    XXX
                 XX  XX    XX
                XX         XX        XXXXX    XXX XX
                XX         XX XXX   XX     X   XX X XX
                XX     X   XXX XX   XXXXXXX   XX X XX
                 XX  XX    XX  XX   XX         XX    XX
                  XXXX    XXX  XXX   XXXXX    XXX   XXX


    XXXXXX      X                   X          XX
   XX     X     XX                  XX
   XX          XXXXX     XXXXX     XXXXX      XXX       XXXX     XX XXX
    XXXXX      XX            X      XX          XX      XX  XX    XXX XX
        XX     XX       XXXXXX      XX          XX      XX  XX    XX  XX
   X    XX     XX XX   X    XX      XX XX       XX      XX  XX    XX  XX
   XXXXXX       XXX    XXXXX X      XXX       XXXX      XXXX     XX  XX


                                                                  X
         XX XXX     XXXXX    XX XXX      XXXX    XX XXX     XXXXX
          XXX XX   XX     X    XX  XX    XX  XX    XXX XX     XX
          XX       XXXXXXX    XX  XX    XX  XX    XX         XX
          XX       XX          XXXXX     XX  XX    XX         XX XX
         XXXX       XXXXX     XX          XXXX    XXXX         XXX
                              XXXX


         XXX                                XXX
          XX                                 XX
          XX         XXXXX     XXXXX         XX     XXXXX    XX XXX
          XX XXX   XX     X         X      XXXXX    XX     X    XXX XX
          XXX XX   XXXXXXX   XXXXXX     XX  XX    XXXXXXX    XX
          XX  XX   XX         X    XX    XX  XX    XX          XX
         XXX  XXX   XXXXX    XXXXX X    XXXX X    XXXXX    XXXX
```

Short GLP Report (continued)

```
==================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                               Inj :   1
                                                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:31:10 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
Module                                Firmware revision  Serial number
-----------------------------------|------------------|---------------
1100 Wellplate Autosampler            A.04.08            DE02700294
1100 Column Thermostat                A.04.06            DE53400174
1100 Diode Array Detector             S.03.91            DE00900051
1100 Binary Pump                      A.04.06            DE53500104
1100 Sample Thermostat                n/a                DE82203241

Software Revisions for:
- Acquisition:   Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies
==================================================================

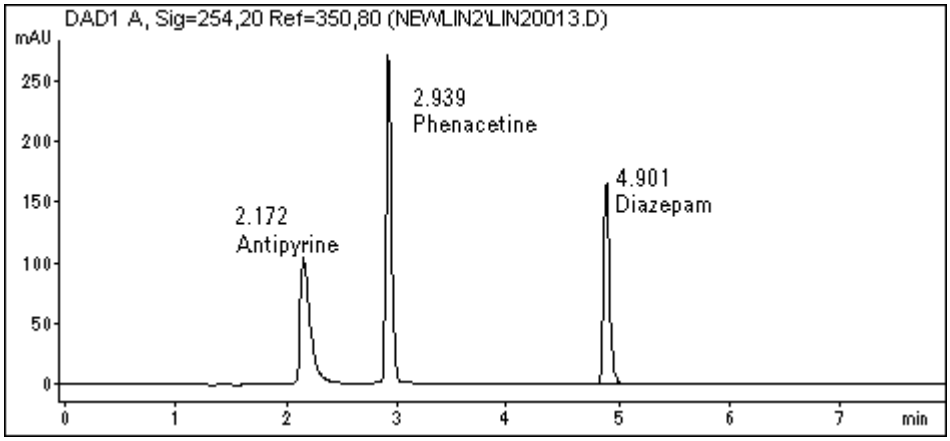
Instrument Conditions   :    At Start            At Stop
Air Temperature (Tray)  :      20.1   °C
Column Temp. (left)     :      40.0                40.0   °C
Column Temp. (right)    :      40.0                40.0   °C
Pressure                :      69.8                75.7   bar
Flow                    :       1.200               1.200 ml/min

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp          :       2.44              454.9   h
DAD 1, Visible Lamp     :       2.44              424.1   h

Solvent Description     :
PMP1, Solvent A         : Water
PMP1, Solvent B         : acn
==================================================================
```

Short GLP Report (continued)

```
                                Run Logbook
=====================================================================
Method        Method started:  line# 7 vial# 2 inj# 1    10:46:18 10/25/00
Method        Instrument running sample Vial 2           10:46:18 10/25/00
1100 ALS    1 Air temperature (tray) = 20.1 °C           10:47:21 10/25/00
1100 PMP    1 Pressure = 69.8 bar                        10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:55:21 10/25/00
1100 PMP    1 Pressure = 75.7 bar                        10:55:21 10/25/00
Method        Instrument run completed                  10:55:23 10/25/00
Method        Method completed                           10:55:23 10/25/00
=====================================================================
=====================================================================
```




```
                    External Standard Report
=====================================================================

Sorted By             :       Signal
Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

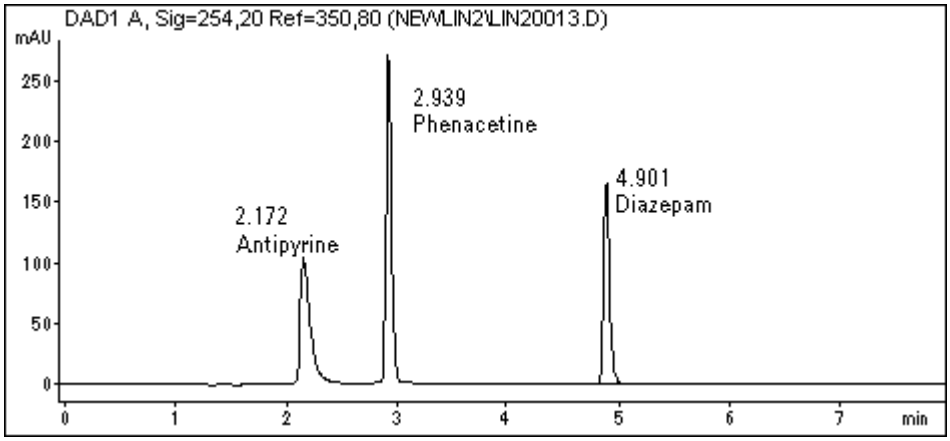
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB       934.32690     1.00317  937.28787     Phenacetine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

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

Performance report

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

================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:36:32 AM by agratz (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150mm, 5µm
================================================================
```




```
     External Standard Report with Performance
================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!

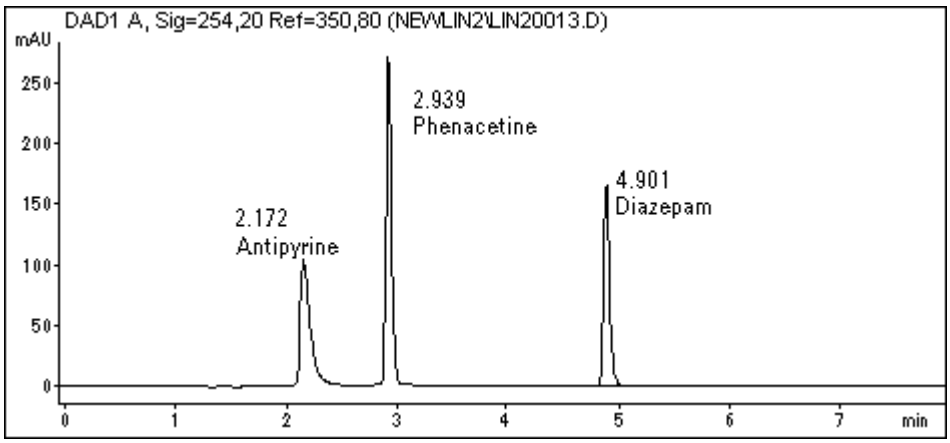
RetTime     k'  Sig    Amount     Symm.  Width    Plates Resol  Name
 [min]                  [ng]             [min]           ution
-------|------|---|----------||-----|-------|-------|-----|----------
 2.172    0.81  1    438.70069    0.44  0.0883     3351  4.47 Antipyrine
 2.939    1.45  1    937.28787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

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

Detail report

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

================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
================================================================
```




```
                  External Standard Report
================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

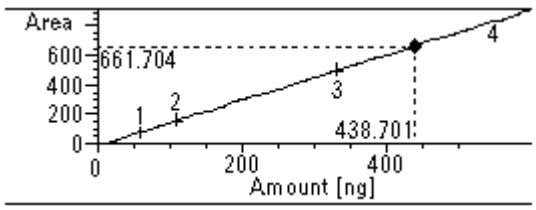
Signal 1: DAD1 A, Sig=254,20 Ref=350,80

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

Detail report (continued)

```
======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================

======================================================================
----------------------------------------------------------------------
                        Calibration Curves
======================================================================
```




```
Antipyrine at exp. RT: 2.071
DAD1 A, Sig=254,20 Ref=350,80
Correlation:          1.00000
Residual Std. Dev.:   0.00000
Formula: y = ax^3 + bx^2 + cx + d
      a:     1.00818e-7
      b:     9.51014e-5
      c:     1.57593
      d:   -19.85331
      x: Amount (ng)
      y: Area
```

:
:
:

The header information and calibration curve is repeated for each peak

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

Extended Performance Report

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

                  Extended Performance Report

Instrument: Instrument 1

Module                          Firmware revision         Serial number
-------------------------------------------------------------------------
1100 Quaternary Pump            A.04.11                   DEl 1116042
1100 Wellplate Autosampler      A.04.13                   DE02700294
1100 Column Thermostat          A.04.11                   DE53400174
1100 Diode Array Detector       A.04.11                   DEO0900051
1100 Sample Thermostat          n/a                       DE82203241

Specials:
micro column switching valve installed in oven

Software Revisions for:
-Acquisition: Rev. A.08.04 [982] Copyright @ Agilent Technologies
-Data Analysis: Rev. A.08.04 [1008] Copyright @ Agilent Technologies

Column Description: XDB-C8
Product# Zorbax Batch#: b99024
Serial# USLLO00162
Diameter 2.1 mm Length: 30.0 mm
Particle size 3.5 mm Void volume 0.08 ml
Maximum Pressure 350 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment: system suitability

Analysis method: D:\HPCHEM\l\METHODS\SYSSUIP.M

Sample information for vial#: 21

  Sample Name:           calanti+     Multiplier:  1.00
  Injection#:            5            Dilution:    1.00
  Injection volume:      3 µl

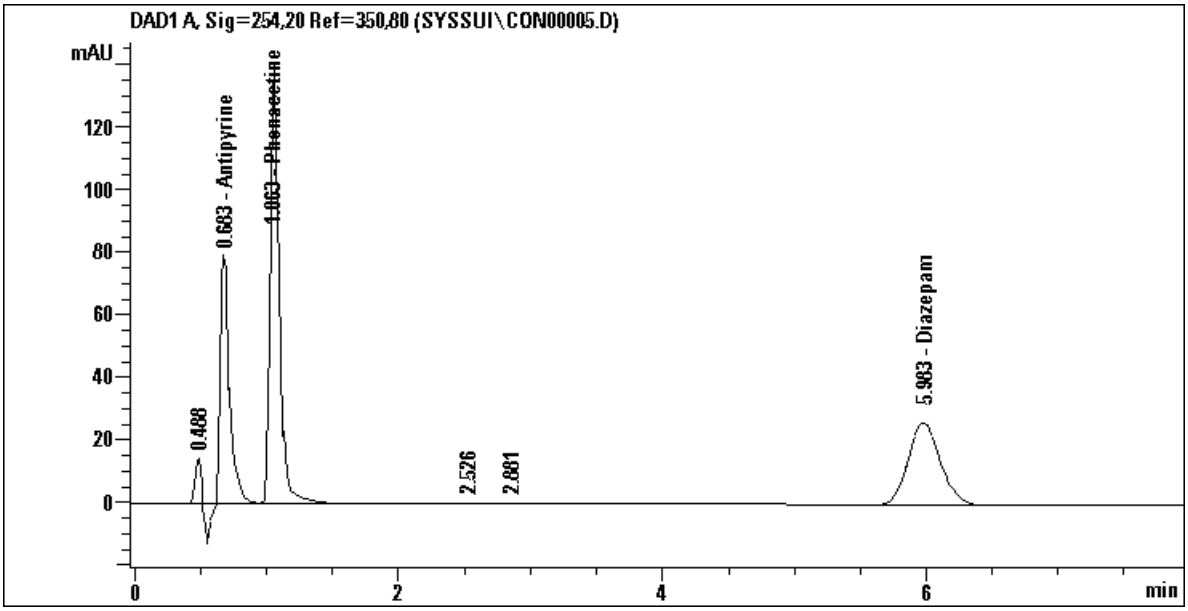
Acquisition information:
  Operator:              agratz
  Date/Time:             2/11/029:06:34 AM
  Data file name:        D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D
  Method file name:      D:\HPCHEM\l\METHODS\SYSSUIP.M

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

Extended Performance Report (continued)

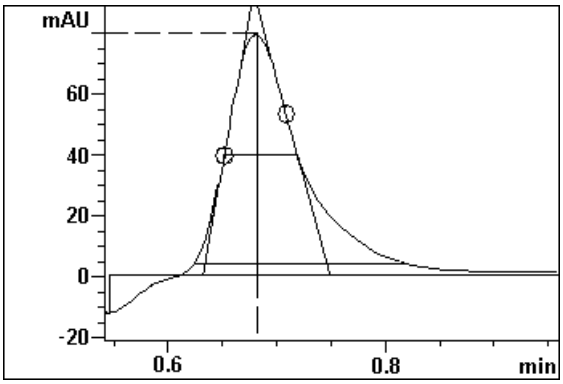
```
Solvents:              PMP1, Solvent A     water
                       PMP1, Solvent B     ACN
                       PMP1, Solvent C
                       PMP1, Solvent D

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




```
Compound# 2    :  Antipyrine
Amount [ng]:         51.1385

Peak description [min]:
      Signal: DAD1 A, Sig=254,20 Ref=350,80
      RetTime: 0.583      K':    0.706
      Height:  79.78  Area:      371.2
      Start:      0.546   End:   0.956
      Skew:    0.898   Excess:   1.643
      Width at half height:      0.067
            5 sigma:             0.196
            tangent:             0.117
            tailing:             0.190
      Symmetry:                  0.483
      USP Tailing:               1.657
      Integration type:            HV
      Time increment [macc]:     400.0
      Data points:                  66
```



Extended Performance Report (continued)

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

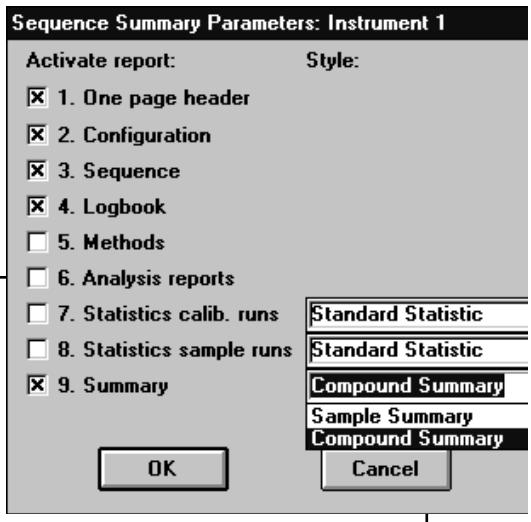
Relationship to preceeding peak:            Selectivity: 3.217
Resolution Tangent method: 2.015            5 sigma method 1.700
Halfwidth method 2.034                      Statistical method 1.067

                                        :
                                        :
                                        :

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

The peak description and statistical moments are repeated for each compound

Sequence Summary Report – Compound Summary




```
  XXXXXX      XX        XXXXXX
 XX    XX     XX        XX    XX
 XX           XX        XX    XX
 XX           XX        XXXXXX
 XX    XX     XX        XX
 XX    XX     XX        XX
 XX    XX     XX        XX
  XXXXXX      XXXXXX    XX
```

S E Q U E N C E

S U M M A R Y

R E P O R T

A.G Huesgen

.....
Date/Signature

I n s t r u m e n t C o n f i g u r a t i o n

Instrument: Instrument 1

Module	Firmware revision	Serial number
1100 Wellplate Autosampler	A.04.08	DE02700294
1100 Column Thermostat	A.04.06	DE53400174
1100 Diode Array Detector	S.03.91	DE00900051
1100 Binary Pump	A.04.06	DE53500104
1100 Sample Thermostat	n/a	DE82203241

Software Revisions for:
- Acquisition: Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies

Sequence Summary Report – Compound Summary (continued)

S e q u e n c e

Sequence Parameters:

Operator:	agratz
Data File Naming:	Prefix/Counter
Signal 1 Prefix:	Lin2
Counter:	0001
Data Directory:	D:\HPCHEM\1\DATA\
Data Subdirectory:	NEWLIN2
Part of Methods to run:	Reprocessing only
	Use SAMPLE.MAC
Wait Time after loading Method:	0 min
Barcode Reader:	not used
Sequence Timeout:	0 min
Shutdown Cmd/Macro:	none
Sequence Comment:	Linearity Test

Sequence Table:

Sample Information Part:

Line	Location	Sample Information
1	Vial 1	1:10 diluted stock solution
2	Vial 1	1:10 diluted stock solution
3	Vial 1	1:10 diluted stock solution
4	Vial 1	1:10 diluted stock solution
5	Vial 1	1:10 diluted stock solution
6	Vial 1	1:10 diluted stock solution
7	Vial 2	1:100 diluted stock solution
8	Vial 2	1:100 diluted stock solution
9	Vial 2	1:100 diluted stock solution
10	Vial 2	1:100 diluted stock solution
11	Vial 2	1:100 diluted stock solution

Sequence Summary Report – Compound Summary (continued)

```
     Method and Injection Info Part:

     Line Location SampleName        Method   Inj SampleType InjVolume DataFile
     ==== ======== ================ ======== === ========== ========= ==========

     1    Vial 1   1:10dil.         LINICHEM 2   Sample        0.1
     2    Vial 1   1:10dil.         LINICHEM 2   Sample        0.5
     3    Vial 1   1:10dil.         LINICHEM 2   Sample        1
     4    Vial 1   1:10dil.         LINICHEM 2   Sample        3
     5    Vial 1   1:10dil.         LINICHEM 2   Sample        5
     6    Vial 1   1:10dil.         LINICHEM 2   Sample        10
     7    Vial 2   1:100dil.        LINICHEM 2   Sample        25
     8    Vial 2   1:100dil.        LINICHEM 2   Sample        50
     9    Vial 2   1:100dil.        LINICHEM 2   Sample        75
     10   Vial 2   1:100dil.        LINICHEM 2   Sample        100
     11   Vial 2   1:100dil.        LINICHEM 2   Sample        0.1


     Calibration Part:

     Line Location SampleName        Method   CalLev Update RF Update RT Interval
     ==== ======== ================ ======== ====== ========= ========= =========



     Quantification Part:

     Line Location SampleName        SampleAmount ISTDAmt   Multiplier Dilution
     ==== ======== ================ ============ =======   ========== =========

     1    Vial 1   1:10dil.
     2    Vial 1   1:10dil.
     3    Vial 1   1:10dil.
     4    Vial 1   1:10dil.
     5    Vial 1   1:10dil.
     6    Vial 1   1:10dil.
     7    Vial 2   1:100dil.
     8    Vial 2   1:100dil.
     9    Vial 2   1:100dil.
     10   Vial 2   1:100dil.
     11   Vial 2   1:100dil.


Sequence Output Parameters:

      Print Sequence Summary Report (SSR):              Yes
           SSR to Printer:                              Yes
           SSR to File:                                 Yes
           SSR File Name:                               GLPrprt.txt
           SSR to HTML:                                 No
           Print individual reports for each run:       No
```

Sequence Summary Report – Compound Summary (continued)

```
Sequence Summary Parameters:

One page header:                        Yes
Print Configuration:                    Yes
Print Sequence:                         Yes
Print Logbook:                          Yes
Print Method(s):                        No
Print Analysis reports:                 No
Print Statistics for Calib. runs:       No
Statistic Sample runs style:            No
Summary style:                          Compound Summary


                              L o g b o o k
24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                          Time      Date
-------------------------------------------------------------------------------------
Sequence      LIN2.S started                                         10:47:06 01/24/02
Method        Loading Method LINICHEM.M                              10:47:07 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 1                10:47:08 01/24/02
CP Macro      Analyzing rawdata Lin20001.D                           10:47:08 01/24/02
Method        Method completed                                       10:47:10 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 2                10:47:11 01/24/02
CP Macro      Analyzing rawdata Lin20002.D                           10:47:11 01/24/02
Method        Method completed                                       10:47:13 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 1                10:47:14 01/24/02
CP Macro      Analyzing rawdata Lin20003.D                           10:47:14 01/24/02
Method        Method completed                                       10:47:16 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 2                10:47:17 01/24/02
CP Macro      Analyzing rawdata Lin20004.D                           10:47:18 01/24/02
Method        Method completed                                       10:47:19 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 1                10:47:21 01/24/02
CP Macro      Analyzing rawdata Lin20005.D                           10:47:21 01/24/02
Method        Method completed                                       10:47:22 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 2                10:47:24 01/24/02
CP Macro      Analyzing rawdata Lin20006.D                           10:47:24 01/24/02
Method        Method completed                                       10:47:26 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 1                10:47:27 01/24/02
CP Macro      Analyzing rawdata Lin20007.D                           10:47:27 01/24/02
Method        Method completed                                       10:47:29 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 2                10:47:30 01/24/02
CP Macro      Analyzing rawdata Lin20008.D                           10:47:30 01/24/02
Method        Method completed                                       10:47:32 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 1                10:47:33 01/24/02
CP Macro      Analyzing rawdata Lin20009.D                           10:47:34 01/24/02
Method        Method completed                                       10:47:35 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 2                10:47:37 01/24/02
CP Macro      Analyzing rawdata Lin20010.D                           10:47:37 01/24/02
Method        Method completed                                       10:47:39 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 1                10:47:40 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

```
CP Macro      Analyzing rawdata Lin20011.D                          10:47:40 01/24/02
Method        Method completed                                      10:47:42 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 2               10:47:43 01/24/02
CP Macro      Analyzing rawdata Lin20012.D                          10:47:43 01/24/02
Method        Method completed                                      10:47:45 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 1               10:47:46 01/24/02
CP Macro      Analyzing rawdata Lin20013.D                          10:47:47 01/24/02
Method        Method completed                                      10:47:48 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 2               10:47:50 01/24/02
CP Macro      Analyzing rawdata Lin20014.D                          10:47:50 01/24/02

24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                         Time      Date
-------------------------------------------------------------------------------------
Method        Method completed                                      10:47:51 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 1               10:47:53 01/24/02
CP Macro      Analyzing rawdata Lin20015.D                          10:47:53 01/24/02
Method        Method completed                                      10:47:55 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 2               10:47:56 01/24/02
CP Macro      Analyzing rawdata Lin20016.D                          10:47:56 01/24/02
Method        Method completed                                      10:47:58 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 1               10:47:59 01/24/02
CP Macro      Analyzing rawdata Lin20017.D                          10:47:59 01/24/02
Method        Method completed                                      10:48:01 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 2               10:48:02 01/24/02
CP Macro      Analyzing rawdata Lin20018.D                          10:48:03 01/24/02
Method        Method completed                                      10:48:04 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 1              10:48:06 01/24/02
CP Macro      Analyzing rawdata Lin20019.D                          10:48:06 01/24/02
Method        Method completed                                      10:48:08 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 2              10:48:09 01/24/02
CP Macro      Analyzing rawdata Lin20020.D                          10:48:09 01/24/02
Method        Method completed                                      10:48:11 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 1              10:48:12 01/24/02
CP Macro      Analyzing rawdata Lin20021.D                          10:48:13 01/24/02
Method        Method completed                                      10:48:14 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 2              10:48:16 01/24/02
CP Macro      Analyzing rawdata Lin20022.D                          10:48:16 01/24/02
Method        Method completed                                      10:48:18 01/24/02
Sequence      LIN2.S completed                                      10:48:19 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

C o m p o u n d S u m m a r y

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

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

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

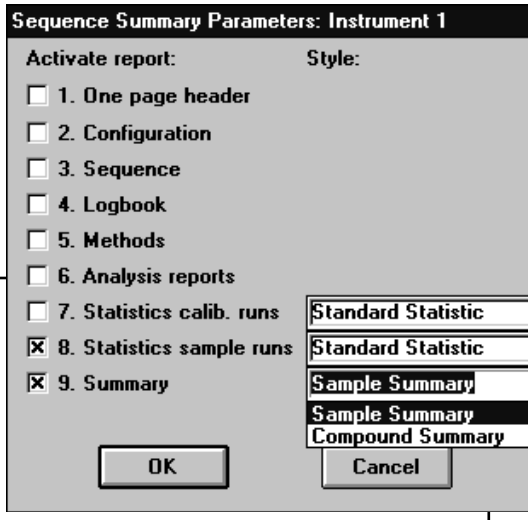
Sequence Summary Report – Compound Summary (continued)

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

==========

*** End of Report ***

Sequence Summary Report – Standard Statistics for Sample Runs




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

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

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

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

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

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BV	2.071	26.23064	834.52417	215.75279	0.0594	0.74
2	BV	2.071	26.28149	836.14185	216.26503	0.0594	0.74
3	BV	2.070	26.22879	834.46539	215.85945	0.0594	0.74
4	BV	2.070	26.27553	835.95233	216.52124	0.0594	0.74
5	BV	2.070	26.21720	834.09644	215.51944	0.0594	0.74
6	BV	2.070	26.19317	833.33203	216.02470	0.0593	0.74
7	BV	2.070	26.27779	836.02423	216.93185	0.0592	0.74
8	BV	2.072	26.29524	836.57941	216.89178	0.0593	0.74
9	BV	2.072	26.22549	834.36017	216.09763	0.0593	0.74
10	BV	2.071	26.21184	833.92590	216.06882	0.0593	0.74
Mean:		2.071	26.24372	834.94019	216.19327	0.0594	0.74
S.D.:		6.81e-4	3.53636e-2	1.12509	4.66512e-1	6.63e-5	1e-3
RSD :		0.033	1.34751e-1	1.34751e-1	2.15784e-1	0.1117	0.20
95% CI:		4.87e-4	2.52976e-2	8.04838e-1	3.33722e-1	4.74e-5	1e-3

Sequence Summary Report – Standard Statistics for Sample Runs

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

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

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

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

Sequence Summary Report – Standard Statistics for Sample Runs

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


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

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

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


================================================================================
                           *** End of Report ***
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Agilent Technologies

Applying the 5975 inert MSD to the Higher Molecular Weight Polybrominated Diphenyl Ethers (PBDEs)

Application

Environmental, Component Testing

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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.

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].

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).

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.

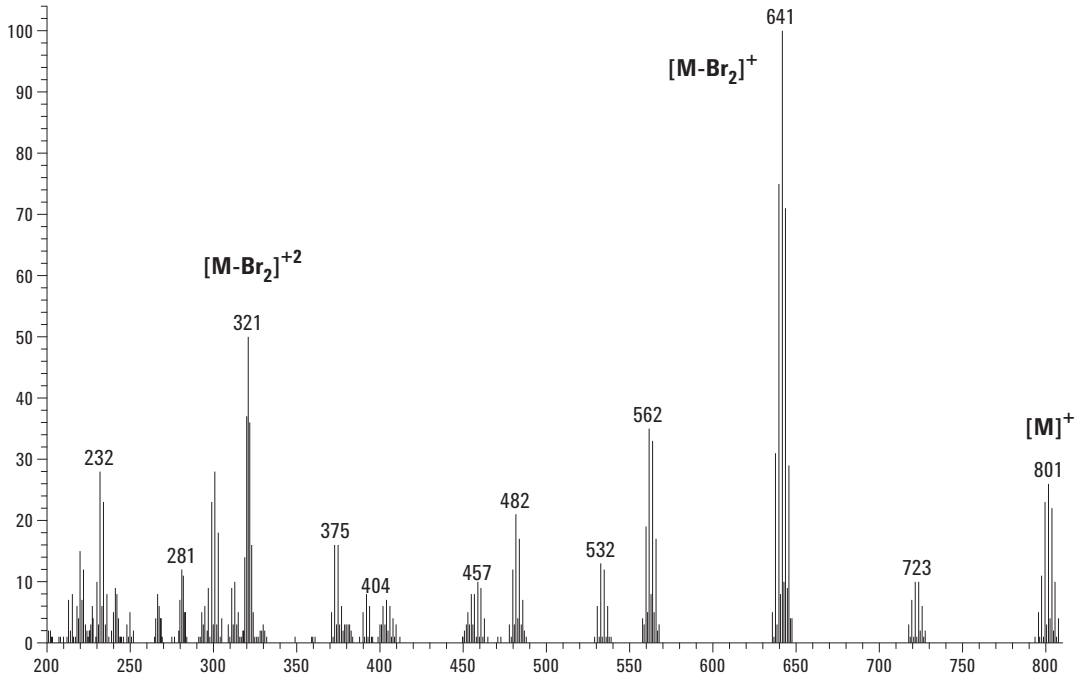

Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 *m/z*.

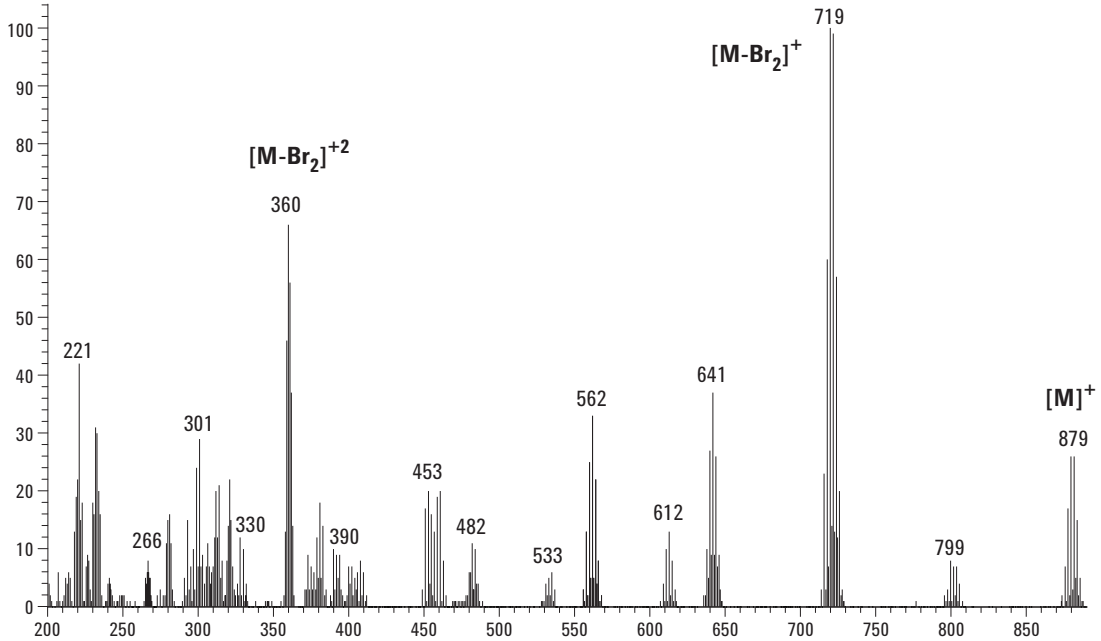

Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 *m/z*.

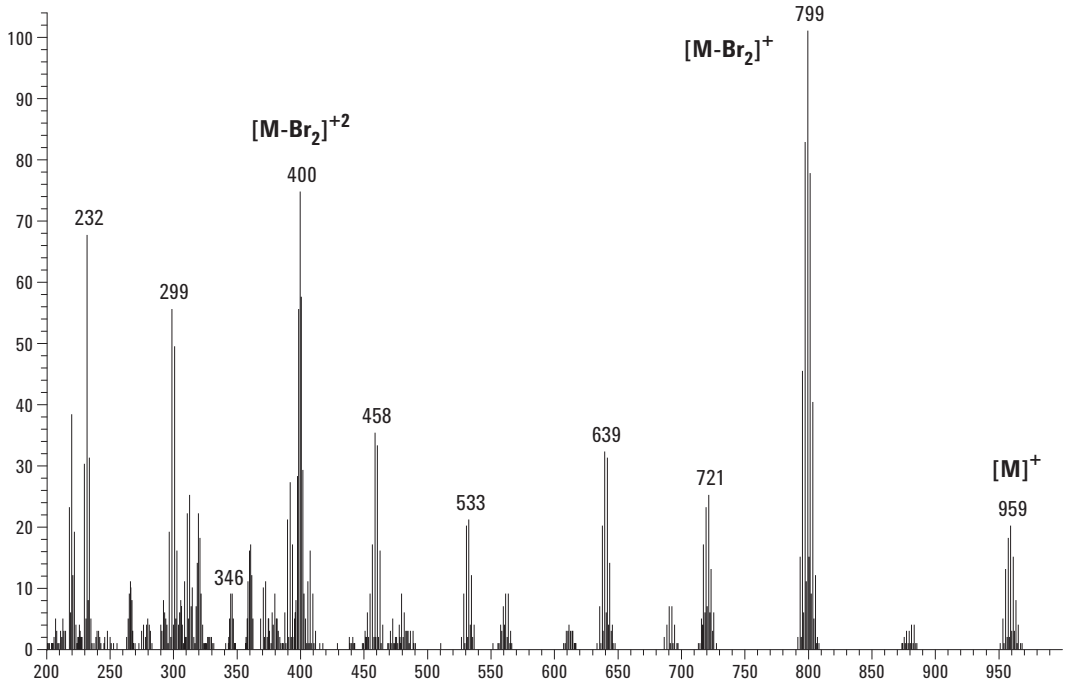

Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 *m/z*.

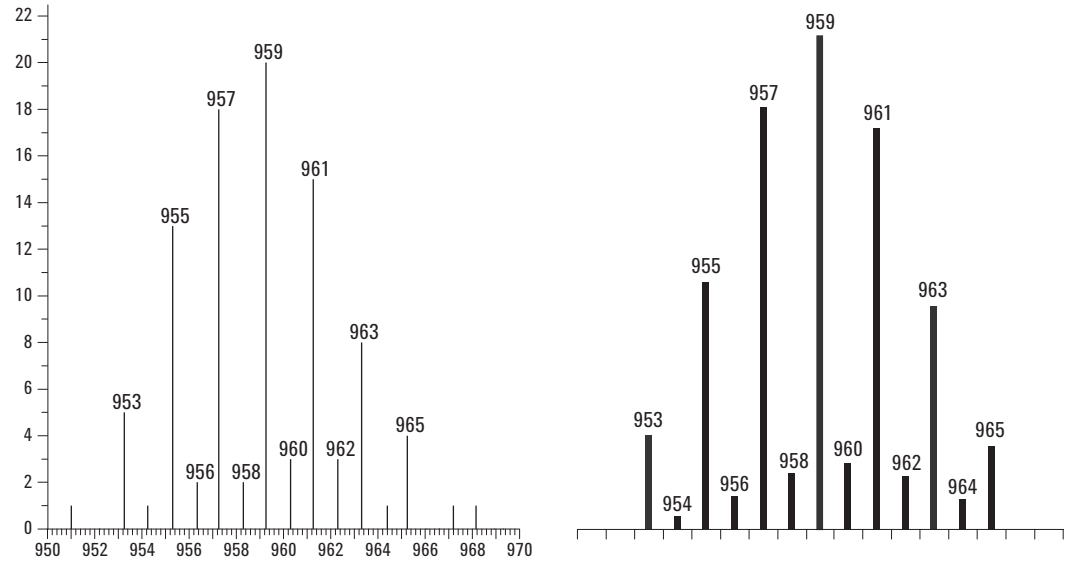

Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

Table 2. Important Ions for the PB_nDEs (n>2)

PBDE bromination	$[M]^+$	$[M\text{-}Br_2]^+$	$[M\text{-}Br_2]^{+2}$
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.

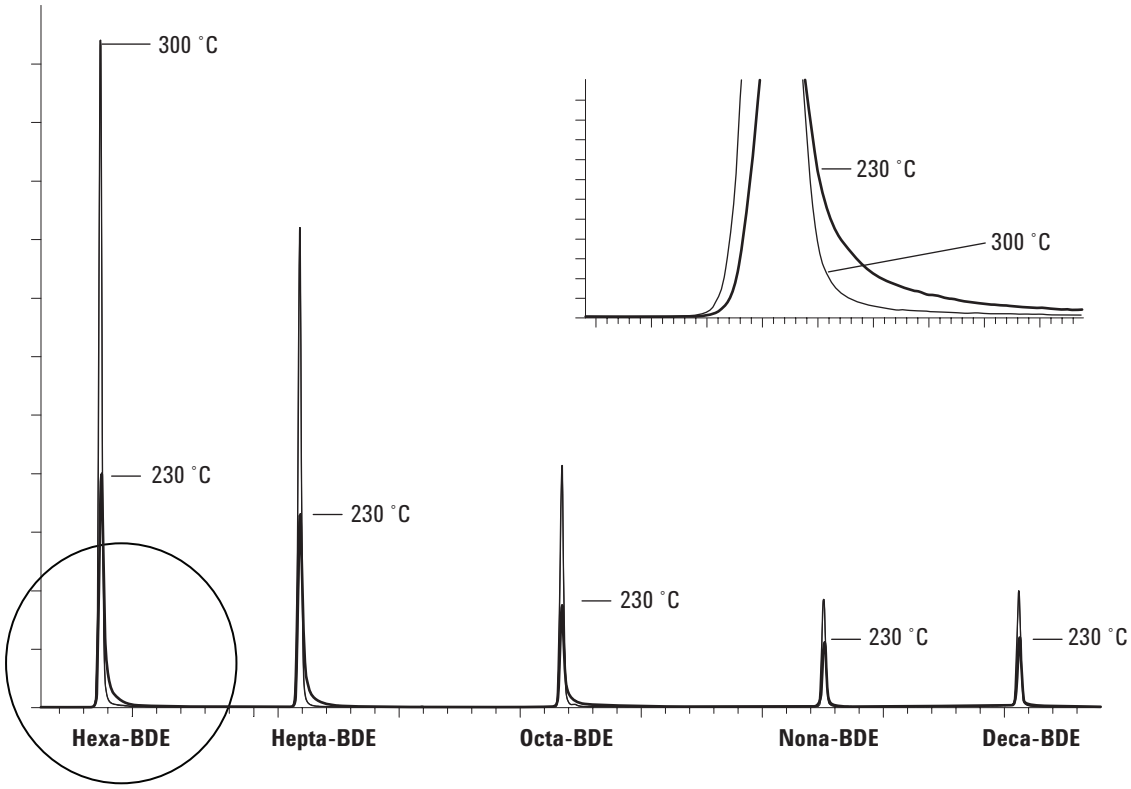

Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

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

Application Note

Michael Frank

Abstract

The need to increase the daily throughputs of LC systems is a constant desire. Now, with the Agilent 1200 Series Rapid Resolution LC system highest throughputs are possible, and in combination with the Agilent ZORBAX RRHT columns and the increased pressure and temperature range of the LC system, excellent chromatographic resolution can be achieved even at run times below one minute.

This Application Note describes the correct set-up of the instrument which is the key for optimal results with narrow bore columns, such as a 2.1 mm x 50 mm column packed with sub two micron particles. Peak capacities in the range of fifty in analysis times as short as 24 seconds and peak widths as narrow as 200 milliseconds are shown. The well-balanced use of all possible module options to achieve shortest cycle times with throughputs far beyond 1500 samples per day is described.

Agilent Technologies

Introduction

Particularly analytical service laboratories in the pharmaceutical industry, responsible for analyzing chemical libraries[1] or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates[2] are faced with the challenge to increase their throughput, but also to maintain a high chromatographic resolution. In 2003 Agilent Technologies introduced sub two micron particles in their RRHT column series. Because of the small particle size, the chromatographic resolution obtainable with these columns is superior to standard particle sizes such as 3.5 µm or even 5 µm. Due to a unique silica manufacturing process, Agilent ZORBAX RRHT columns show a significantly reduced backpressure, if compared to similar column dimensions of other manufacturers. Excellent chromatographic results are achieved in a very short analysis time with the Agilent 1200 Series Rapid Resolution LC system, which facilitates an increased pressure range and flow rates from 0.05 up to 5 mL/min using column diameters ranging from 2.1-mm id up to 4.6-mm id. This Application Note will focus on 2.1-mm id columns only.
Not only are the run times of the analyses important for high throughput, but also the overhead time. The Agilent 1200 Series Rapid Resolution LC system can be optimized to achieve highest throughputs with exceptionally good overall system performance.

Experimental

An important issue when dealing with narrow bore columns, especially in gradient mode where smallest peak widths can be achieved, is to have small extra column volumes. This also includes any volumes in front of the sampling device, because any volume after the solvent mixing point will increase the time for the gradient composition to reach the column. This results in an increased run time. The Agilent 1200 Series Rapid Resolution LC system can be reconfigured within a few minutes to provide appropriate system volumes for different column ids. Here, the pumps are set-up in the low delay volume configuration with an internal volume of approximately 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744). Consequently, only capillaries of 0.12 mm id are used beyond the injection valve. In the Agilent 1200 Series thermostatted column compartment SL the newly introduced low dispersion heat exchangers with 1.6 µL internal volume were used. In some experiments, the Agilent 1200 Series Rapid Resolution LC is set up for alternating column regeneration to achieve highest throughput using the ACR-capillary kit (G1316-68721) and 2.1-mm id columns[3]. The high pressure rated 2-position/10-port valve in the thermostatted column compartment was only placed into the flow path if alternating column regeneration was used indeed.

The instrument set-up is as follows (figure 1):

- Agilent 1200 Series binary pump SL with the new Agilent 1200 Series micro vacuum degasser
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration
- Agilent 1200 Series diode-array detector SL with a 2-µL/3-mm cell
- ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm

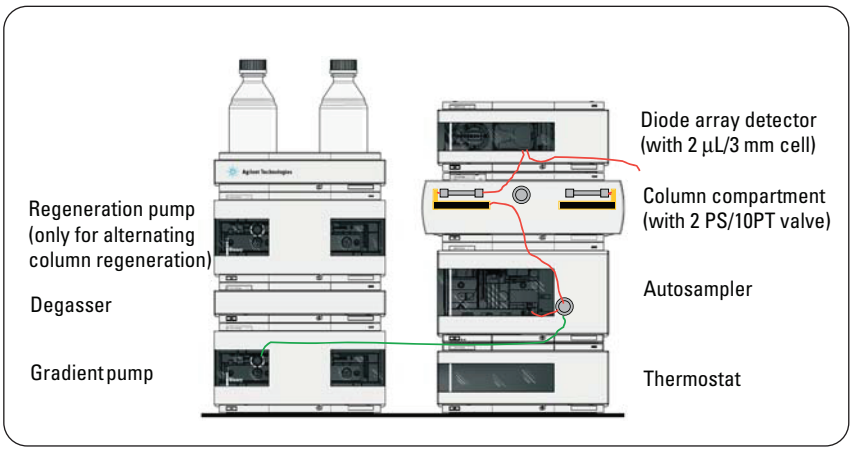

Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and lowest pump ripple. The pump hardware is significantly different from the standard binary pump. In the Agilent 1200 Series binary pump SL the pressure transducer is separate from the damper which has been modified to have a lower delay volume (pressure dependent ranging from 80-280 µL). In this study the pumps were used in the low delay volume configuration without the mixer and damper in the flow path. In contrast to the standard binary pump the pump heads of the binary pump SL have an additional damping coil (500 µL volume each) to allow damping in the low delay volume configuration. This does not add to the gradient delay volume because it is before the mixing point. Anyhow, pressure ripples are also strongly suppressed by the Electronic Damping Control (EDC). The pressure range of the pump and all other modules is increased to 600 bar.

Only one sample, the so-called "phenone-mix", was used in the course of this study to keep variations low. The sample consists of nine compounds: acetanilid, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. Unless otherwise stated, the concentration was 0.1 µg/µL for each compound except butyrophenone which was 0.2 µg/µL. The solvent was water-acetonitril 2:1.

Results and discussion

The most frequently sold particle size in chromatographic columns today is 5 µm. Of course, fast and ultra fast LC is also possible with columns packed with particles of these larger diameters – the reduced back pressure is even beneficial to allow higher flow rates. However, resolution will be sacrificed because conditions are usually far on the right side of the van-Deemter-optimum. Here, the big advantage of the RRHT columns with particles of less than 2 µm diameter is proven. The van Deemter optimum is shifted further to the right and the curve is much flatter at the onset because the "resistance of mass transfer" term is diminished (figure 2). In figure 3 the analysis on a 2.1-mm id column with 1.8-µm particles is compared to the linear scaled analysis on the same stationary phase but on 5 µm particles packed in a 4.6-mm id-column. The gain in resolution is obvious – from Rs = 2.1 up to Rs = 3.5 for the critical pair which matches the theoretically expected value of a 1.66 fold increase in resolution. Also note that there is a saving in solvent consumption of 8.6 mL in the "standard" HPLC analysis and only 1.8 mL in the ultra fast HPLC analysis.

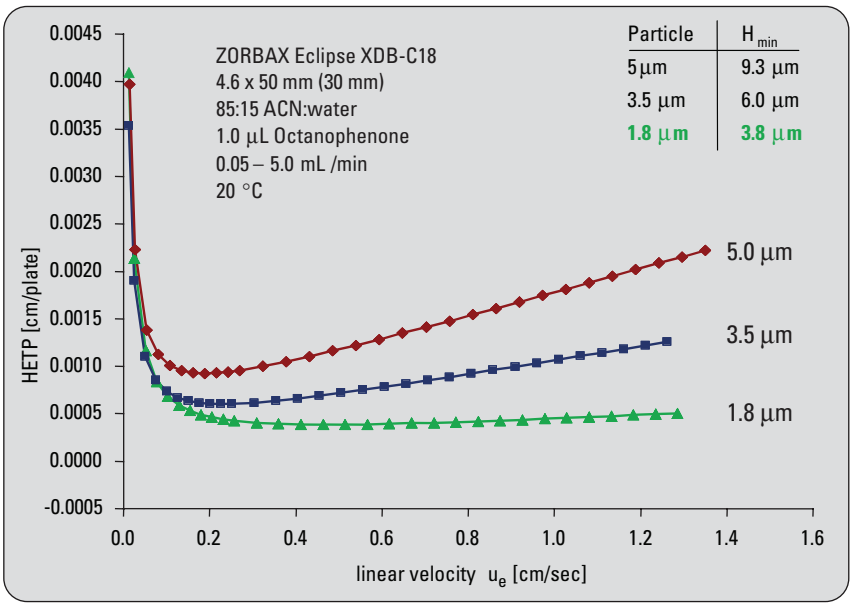

Figure 2
Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.

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

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

(tg = gradient time, F = flow rate, Vm = column void volume, △%B = gradient steepness, S = solvent and solute dependent factor)

If the product of the gradient time and flow rate, the so-called gradient volume, is kept constant together with all other parameters, the gradient time might be decreased while the flow rate is increased. Thus, the capacity factors of two compounds will stay constant and if no large alteration of the plate height occurs, the resolution will not change significantly, either. The final point is the big advantage of the sub two micron particles – the van-Deemter curve is nearly flat on the right side of the minimum (figure 2) and flow rates can be increased with only little increase in plate heights. However, the equation is an empirical one and deviations may occur especially under extreme conditions.

With a two-step approach, highest gradient speeds with virtually no loss or only little loss in resolution can be achieved. In the first step, start from a medium temperature and begin to increase the flow rate up to the pressure maximum. Subsequently the temperature should be increased to lower the viscosity of the solvent and then the flow rate is increased again. It may be worthwhile to check the resolution with two identical gradients but with different temperatures to see the influence of the temperature change on the resolution which may be very compound dependent. In figure 4 the result of this approach is shown. A nearly 7-fold increase in separation speed could be achieved with still baseline separation of the critical pair before meeting the pressure and temperature limit (the maximum temperature is a function of flow, temperature, number of controlled Peltier elements and of the heat capacity of the solvent used).

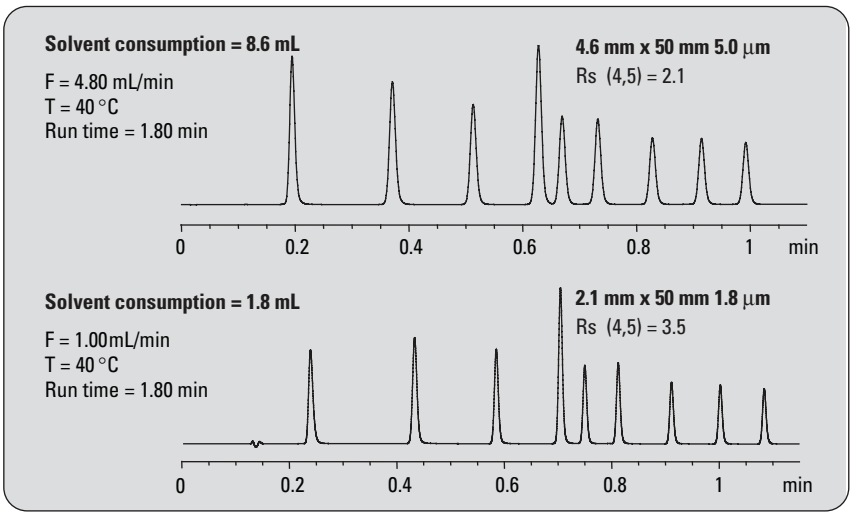

Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

Conditions:	4.6-mm id column used on standard Agilent 1200 system	
Solvent:	A = Water, B = ACN	
Temperature:	40 °C	
Column:	**2.1 mm x 50 mm, 1.8 µm**	**4.6 mm x 50 mm, 5.0 µm**
Flow:	1.0 mL/min	4.8 mL/min (scaled from 2.1 mm col.)
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 % B	1.11 min 35 % B
Stoptime:	1.15 min	1.15 min
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	245 nm (8), ref. 450 nm (80)
Peakwidth:	>0.0025 min (0.05 s res.time), 80 Hz	>0.01 min (>0.2 s), 20 Hz
Injection volume:	1 µL	5 µL (not scaled)

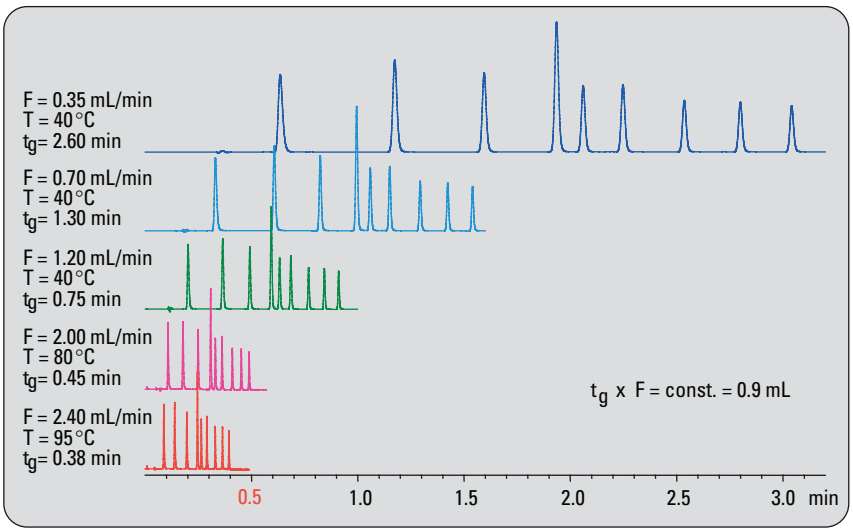

Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

Conditions:	
Solvent:	A = water, B = ACN
Temp.:	40 °C, 80 °C, 95 °C
Flow:	0.35, 0.70, 1.20, 2.00, 2.40 mL/min
Gradient:	0.00 min 35 %B
	2.60 min 95 %B
	3.20 min 95 %B
	3.21 min 35 %B
	Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Wavelength:	245 nm (8), Ref. 450 nm (100)
Peak width:	>0.0025 min (0.05 s response time), 80 Hz

The last chromatogram is enlarged in figure 5 and reveals the details of this separation. The first peak is eluted after only five seconds and peaks with a width at half height of less than 200 ms are achievable. Within twenty-four seconds nine compounds are separated with a peak capacity in the range of fifty.

Retention time precision at highest analysis speed

High analysis speed is meaningless without precision. One basic performance criteria for HPLC pumps is the precision of gradient formation measured by the precision of retention times of repeated gradients. However, the stability of the column temperature must also be taken into consideration, because temperature fluctuations will also influence the retention times of a given sample. In table 1 and figure 6 the results from the 10-fold repeated analysis of a standard sample are listed and since the deviation between individual runs is so small, the octanophenone peak is enlarged in a separate window. This sample contains compounds that are both not retained and refer to isocratically eluted compounds found at the starting conditions of the gradient, as well as highly unpolar and strongly retained compounds. The analyses

Conditions:

Solvent:	A = Water, B = ACN
Temp.:	40 °C, 80 °C
Flow:	0.35 mL/min, 1.20 mL/min, 2.0 mL/min
Gradient:	0.00 min 35%B
	2.60 min 95%B
	3.20 min 95%B
	3.21 min 35%B
	Time values for F = 0.35 mL/min.
	For all other flow rates times are
	scaled so that (time x flow) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Injection vol.:	1.0 µL

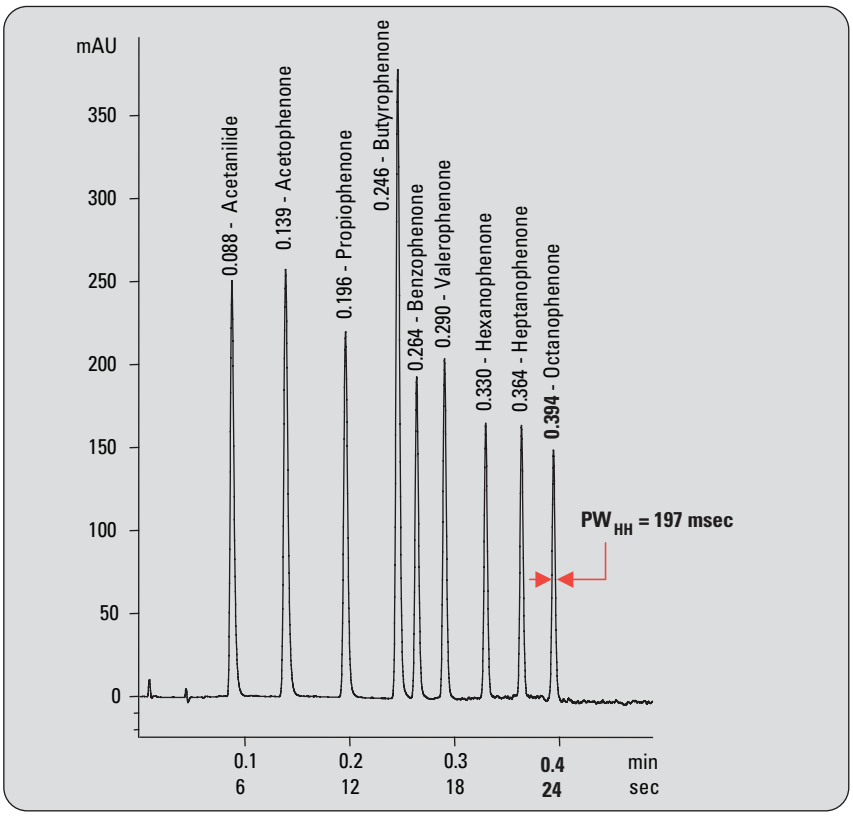

Figure 5
Separation of a nine compound mixture under ultra fast conditions.

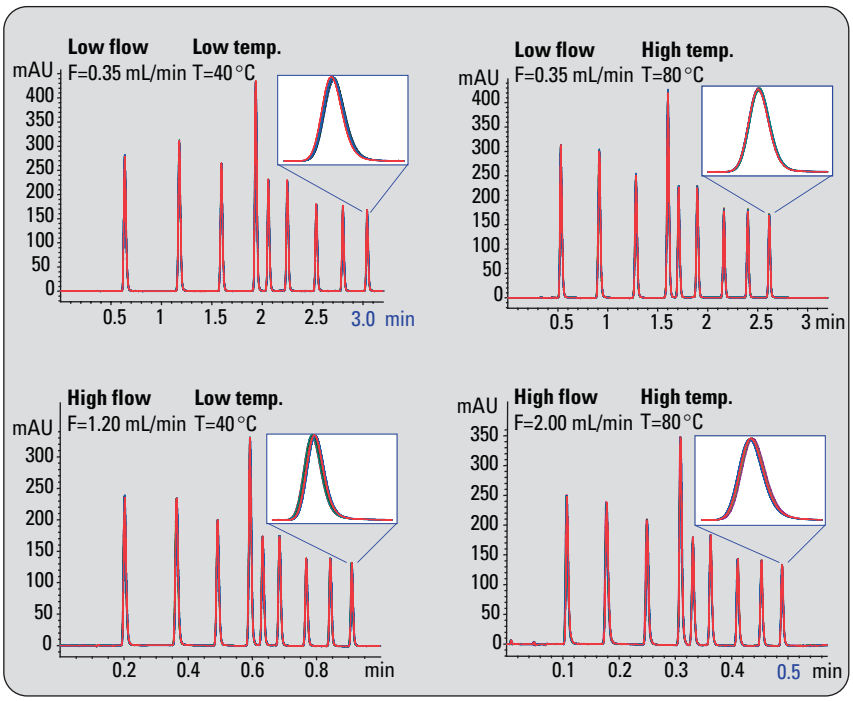

Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

were done at high and low flow rates as well as with high and low temperatures as in the examples shown earlier. In all cases the mean retention time precision is below 0.3 % RSD, which was the specification of the Agilent 1100 Series LC system. Of course, the results are also in line with the specifications for the new Agilent 1200 Series Rapid Resolution LC system which is < 0.07 % RSD or < 0.02 min SD, whichever is met first. At these high gradient speeds, the SD criteria are always met. The RSD criteria are also met for both fast-LC gradients of 2.6 min duration (0.35 mL/min flow rate). Even at ultra-fast gradient speeds, the retention time precisions are still below or only slightly higher than 0.1% RSD (table 1).

Improving the cycle-time

Not only is the gradient speed important when dealing with high-throughput analysis but furthermore the over all cycle time of the entire system, which is the time between two consecutive analyses. A good method to measure the cycle time is by using the time stamp the data file is assigned by the operating system of the computer. Clearly, optimizing the cycle time has some drawbacks. For example, extensive needle cleaning procedures are in contradiction with a high sampling speed. Table 2 gives an overview of important parameters influencing the cycle time. Using 1.8-µm particle size columns together with an optimized HPLC system very short run times can be achieved without sacrificing chromatographic resolution. Combining short run times together with low overhead times will result in a high daily throughput. In figure 7 the cycle time and daily throughput is shown for two

	0.35 mL/min, 40°C		0.35 mL/min, 80°C		1.20 mL/min, 40°C		2.00 mL/min, 80°C	
	SD	% RSD	SD	% RSD	SD	% RSD	SD	% RSD
Average	0.00107	0.067	0.00084	0.070	0.00048	0.098	0.00031	0.134

Table 1
Standard deviations (mAU) and %RSD (n=10) of the retention times under different chromatographic conditions in temperature and flow.

Module	Parameter	Effect on cycle time	Other effects
Pump	Low delay volume setting	Reduced retention times, run time can be shortened, reduced cycle time	Increased pressure ripple, slightly increased mixing noise if modifiers such as TFA are used.
Autosampler	Automatic Delay Volume Reduction (ADVR) – activated	Reduced delay volume, reduced retention times, run time can be shortened, reduced cycle time	Increased carry-over
	ADVR activated and Overlapped Injection (OI)	Enables parallel sampling, thus reduces the cycle time independently of the below listed settings (as long as the overall sampling speed does not exceed the gradient and post time)	Increased carry-over
	no OI – Needle Wash	Increased sampling time with increasing wash time	Reduced carry-over with longer needle wash time
	no OI – Equilibration time	Increased sampling time with increased equilibration time	Better injection precision with longer equilibration time
	no OI – Draw/Eject speed	Low speed causes increased sampling time	Low speed results in better injection precision
Column compartment	Alternating column regeneration	Saves column wash-out and equilibration time, reduces cycle time enormously	Additional hardware required, slightly increased extra column volume, slightly different retention times between columns possible
Detector	Pre-run and/or post-run balance	Increased cycle time	Baseline drifts possible if not applied
	Spectral data acquisition with high data rate, small band width and broad wavelength range large data files	Depending on computer power and additional processes running might increase cycle time because of writing speed	Reduced information content if no spectral data acquired or with lower resolution
Software	Data analysis with acquisition	Increased cycle time, depending on computer power and number of peaks	Data analysis has to be done offline is no set
	Save method with data	Slightly increased cycle time	Information is missing if method is not saved
	Execution of pre-run or post-run macros	Increased cycle time, depending on macro	Depending on macro
System	LC controlled over local network between computer and LC (and MS) only	Faster data and method transfer between computer and LC because of reduced net work traffic reduced cycle time	Additional hardware might be necessary (use independent acquisition computer)
	Number of detectors	More detectors produce a higher data amount and lower the data transfer speed, resulting in higher cycle times	More detectors higher information content

Table 2
Influence of various parameters on the overall cycle time.

different methods – both giving virtually the same resolution. The first method (0.45 min gradient) utilizes alternating column regeneration and high temperatures to allow high flow rates and speed optimized settings. A cycle time of 49 s could be achieved, resulting in a theoretical daily throughput of more than 1700 samples per day. The second method (0.90 min gradient) does not use high temperatures or alternating column regeneration and the time saving of some simple and often forgotten method options are shown. By optimizing these parameters the real cycle time gets as close to 8 s to the run time (stop time plus post time) and allows a daily throughput of more than 700 samples per day. By sub-optimal method set up this can easily drop to below 500 samples per day if options like automatic delay volume reduction, overlapped injection or offline data-analysis are not used.

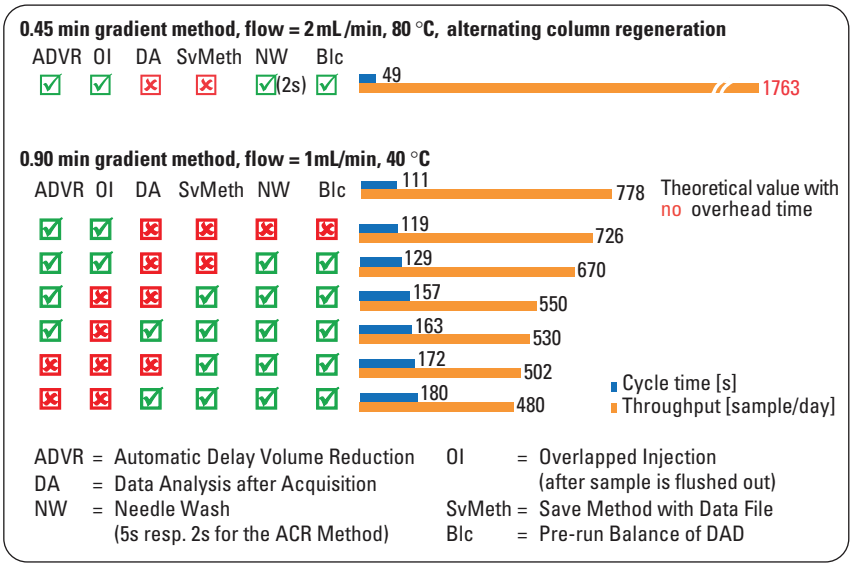

Figure 7
Cycle time and daily throughput optimization.

Chromatographic conditions:

Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	80 °C	
Flow:	2.0 mL/min	
ADVR:	Yes	
Gradient:	**Gradient-Pump**	**Regeneration-Pump**
	0.00 min 35 %B	0.00 min 35 %B
	0.45 min 95 %B	0.01 min 95 %B
	0.46 min 35 %B	0.11 min 95 %B
	0.57 min 35 %B	0.12 min 35 %B
Stoptime:	0.57 min	no limit
Posttime:	off	off
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	none	
Injection volume:	1.0 µL	
Injector:	Overlapped injection, 2 s needle wash, sample flush-out factor = 10, draw/eject speed = 100 µL/min	
Valve:	next position	

No Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	40 °C	
Flow:	1.0 mL/min	
ADVR:	Yes	No
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 %B	1.11 min 35 %B
Stoptime:	1.15 min	*1.40 min (add. 300 µL extra column volume, increased retention times)*
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	all, 190-500 nm, BW = 1 nm	
Injection volume:	1.0 µL	
Injector:	See figure 7, 2 s equilibration time	

Conclusion

The Agilent 1200 Series Rapid Resolution LC system is a powerful tool to achieve highest chromatographic resolutions and also highest throughputs. The extended pressure range allows the usage of columns packed with stationary phases with particles sizes below 2 µm, for example, Agilent RRHT columns with particle sizes of 1.8 µm. These columns not only allow an increase in linear flow rates with virtually no loss in resolution but also have an inherently higher resolution compared to 3.5 µm or even 5.0 µm particle sizes. The possibility to switch the pump into its low delay volume configuration allows the use of the entire bandwidth of today's widely used column ids – from 4.6 mm down to 2.1 mm and even 1.0 mm. As illustrated above, the system has uncompromised performance characteristics even at highest gradient speeds.

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Agilent Technologies

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

Application

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Abstract

The increased availability of sub-2-micron (STM) columns and increased demand for methods friendly to mass spectrometers has led to strong trend toward conversion of existing HPLC methods to smaller diameter and smaller particle size columns. While the conversion is a simple mathematical exercise requiring the scaling flow rates, gradient times and injection volumes, many users observe less than perfect results. Here we look closely at the problem and propose calculations that improve the speed and/or resolution in a more predictable and beneficial way.

Introduction

Methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by replacing these columns with smaller dimension columns packed with smaller particle sizes. The potential benefits include reduced analysis time and solvent consumption, improved sensitivity and greater compatibility with mass spectrometer ionization sources.

Simplistically, a column of 250-mm length and containing 5-µm particles can be replaced by a 150-mm length column packed with 3-µm particles. If the ratio of length to particle size is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2, here about 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

LC/MS (Liquid Chromatography/Mass Spectrometry) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal LC/UV (UltraViolet UV/VIS optical detection) methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected on the smaller column, a proportional increase in peak response. We normally scale the injection mass to the size of the column,

though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column.

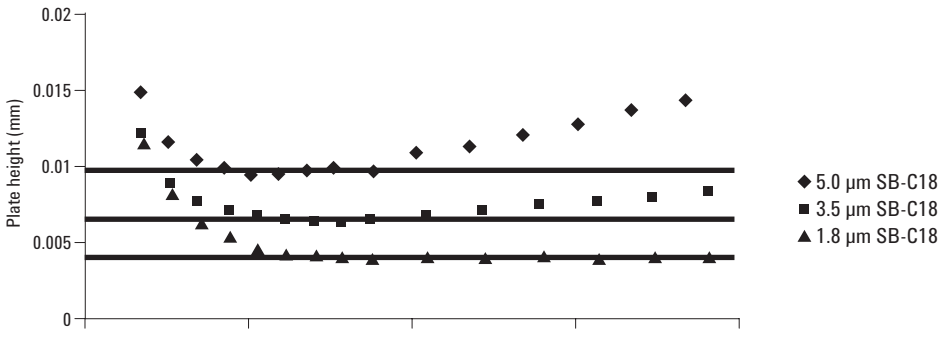
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. If one wishes to improve the outcome of the method conversion, though, there are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, or more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, this is often represented in van Deemter plots as shown in Figure 1, a modified version of the original plot [2].

In Figure 1 we observe that the linear velocity at which 5-µm materials are most efficient, under the conditions used by the authors, is about 1 mm/sec. For 3.5-µm materials the optimum linear velocity is about 1.7 mm/sec and has a less distinct optimum value, suggesting that 3.5-µm materials would give a more consistent column efficiency over a wider flow range. For the 1.8-µm materials, the minimum plate height, or maximum efficiency, is a broad range beginning at about 2 mm/sec and continuing past the range of the presented data. The practical application of this information is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity which results in a further reduction in analysis time. This increase in elution speed will decrease absolute peak width and may require the user to increase data acquisition rates and reduce signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings which will cause a measurable amount of bandspreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest



Lin. vel.	mm/sec	1	2	3	4	5
4.6 mm	mL/min	0.7	1.4	2.1	2.8	3.5
3 mm	mL/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	mL/min	0.14	0.29	0.44	0.58	0.73
1 mm	mL/min	0.033	0.066	0.1	0.133	0.166

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

possible peak volumes. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of % change per minute, it is more useful to express it as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End}\% - \text{Start}\%)}{\#\text{Column volumes}} \right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C autosampler SL, with thermostatic temperature control
G1316B Thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 150 mm, 3.5 µm

Mobile phase conditions

Organic solvent: Acetonitrile
Aqueous solvent: 25 mm phosphoric acid in Milli-Q water

Gradient Conditions

Gradient slope: 7.8% or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

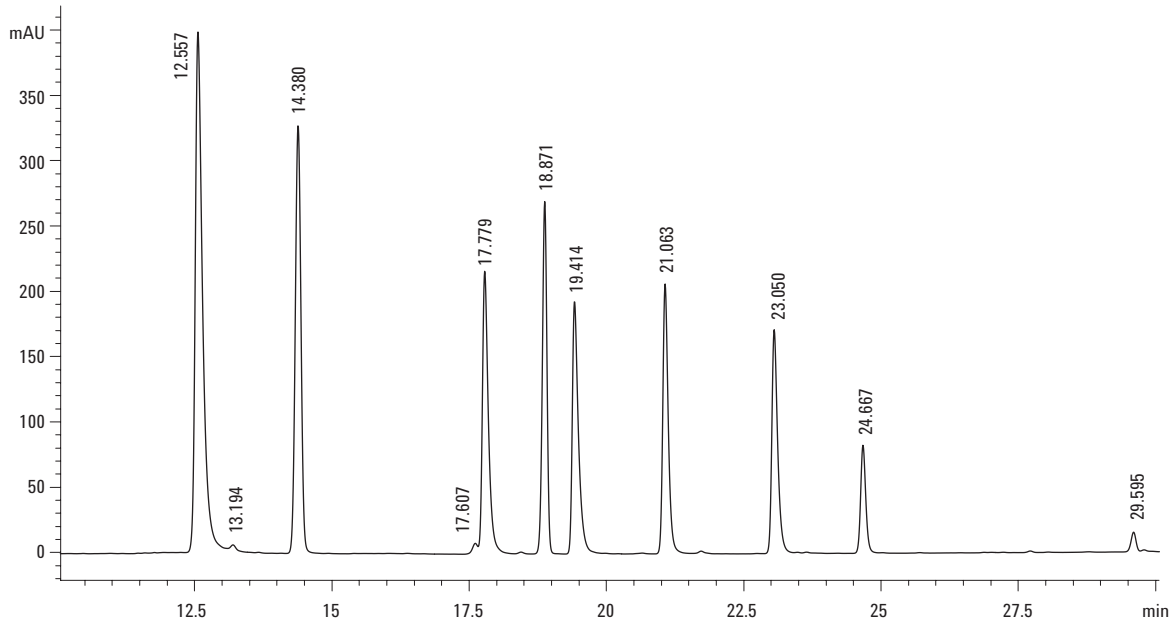
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

Results

The separation was initially performed on a standard 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column thermostatted to 25 °C (Figure 2) using conditions referenced in US EPA Method 555. The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm, 3.5-µm column (Figure 3). Solvent consumption is reduced from 60 mL to 15.5 mL per analysis.

The separation was then re-optimized for faster separation with the identical slope, 7.8%, by increasing the flow rate from 0.43 to 1.42 mL/min, and proportionately reducing the gradient time (Figure 4). Finally, increased resolution is demonstrated by keeping the original times used in Figure 3 with the increased flow rate (Figure 5). This yields a gradient with identical time but a reduced slope of 2.3%. The increased resolution of peaks 4 and 5 is readily apparent.

The conditions in Figure 4, 7.8% slope at increased linear velocity on 3.0 × 150 mm, 3.5-µm material, yield a separation with comparable resolution to the original 4.6 × 250 mm method, but with only a 12-minute total analysis time. This is excellent for

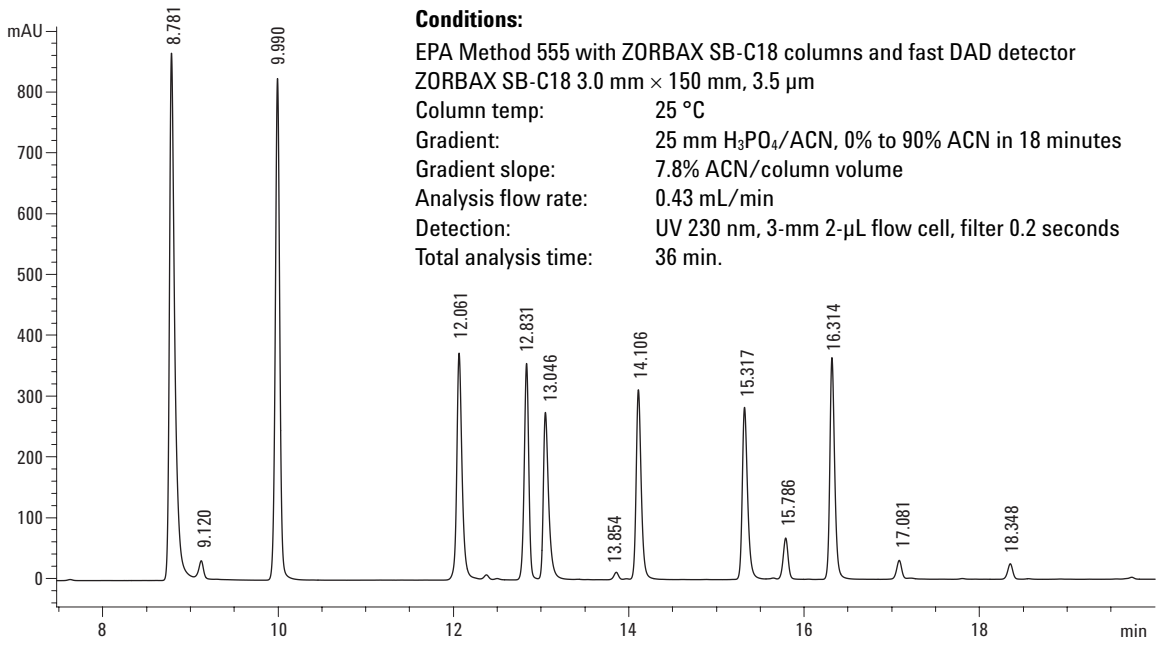


Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm	
Column temp:	25 °C
Gradient:	10% to 90% ACN vs. 25 mM H_3PO_4
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min
Group A Compounds	
Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 2. Gradient separation of herbicides on 4.6 × 250 mm 5-µm ZORBAX SB-C18.



Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 µm	
Column temp:	25 °C
Gradient:	25 mm H_3PO_4/ACN, 0% to 90% ACN in 18 minutes
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min.

Figure 3. Gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

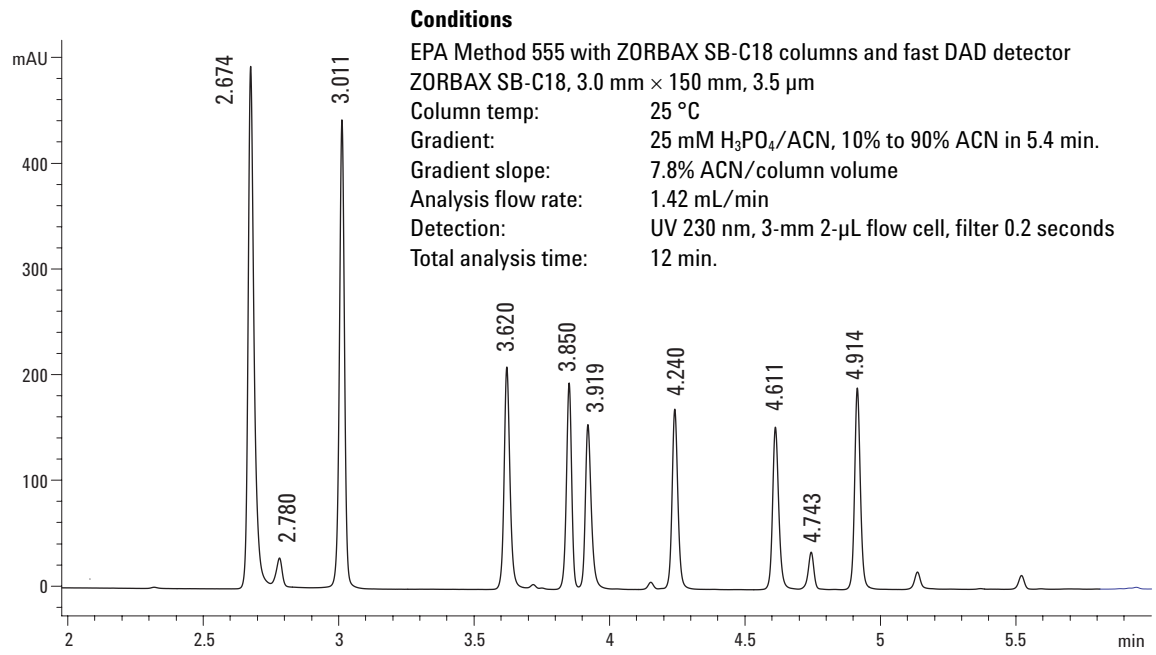


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

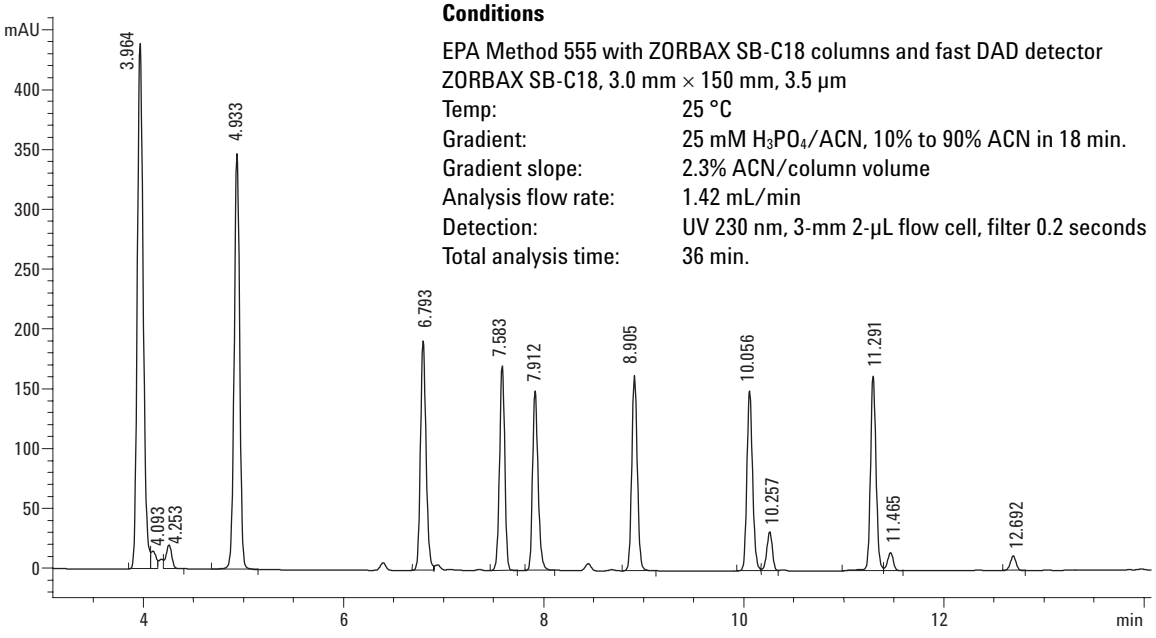


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

high throughput screening and quantitation of a large number of samples. Figure 5, with the gradient slope reduced to 2.3%, results in a high-resolution separation with a calculated R value of 3.3 vs. the standard 3.0 × 150 mm separation value of 1.9, for the critical pair seen in Figure 5 at 7.5 to 8 minutes.

In Table 1 the column has been replaced with a low dead volume connecting union in a system fitted with 0.12-mm id capillary tubing at all points of sample contact. A 1-µL injection of dilute actone is made to determine the bandspreading contribution of the system, with various flow cells. Multiple flow cells were tested, and the average result reported, where possible. The elution volume summarizes the total volume of all tubing in the system. While the absolute volume from the 2-µL to the 13-µL flow cells is 11 µL, we observe an increase of 15 to 16 µL because of the larger diameter inlet tubing integral to the larger volume flow cells.

Table 1. Volumetric Measurements of Various Flow Cells

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

Conclusion

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small and high resolution columns, will ensure good column efficiency which is critical to a successful translation of the method.

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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.

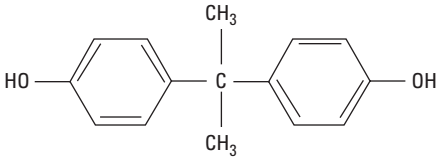

Figure 1. Bisphenol A

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.

Method Optimization and Scalability

The existing HPLC method was proven and robust; however, it was complicated. We sought a similar chromatogram, based on the original method, but using simpler method parameters. Because of the challenge of changing many chromatographic parameters, essentially redeveloping the method, we chose a 4.6 × 50 mm, 1.8-µm Eclipse XDB-C18 column for experiments to reduce the time required. Smaller particles packed in shorter columns increase the speed of analysis and still provide enough efficiency to maintain resolution equivalent to longer columns packed with larger particles. After several trials, we developed a method that produced a chromatogram similar to the original. The short analysis time is a major advantage of Rapid Resolution High Throughput (RRHT) technology. Whereas a handful of experimental runs would take an entire work day using a typical analytical-sized column (50 min/run), the series of runs took about an hour (7.5 min/run), using an RRHT column.

We incrementally scaled up to a 4.6 × 250 mm column. Figure 2 shows an overlay of the sample analyzed by three 4.6-mm id columns of different lengths and particle sizes. Injection volume was also changed proportionally to length. The smaller ZORBAX particles speed up the analysis while maintaining resolution. In fact, resolution increased when using the RRHT columns despite their shorter length.

One reason this method can be easily scaled (up or down) is the uniform spherical Eclipse XDB-C18 packing. It has a proprietary engineered particle size distribution, based on ZORBAX silica with a controlled surface area and pore size. The robust proprietary packing material and proven column manufacturing techniques consistently yield reproducible columns with similar chromatographic performance, independent of the column dimensions.

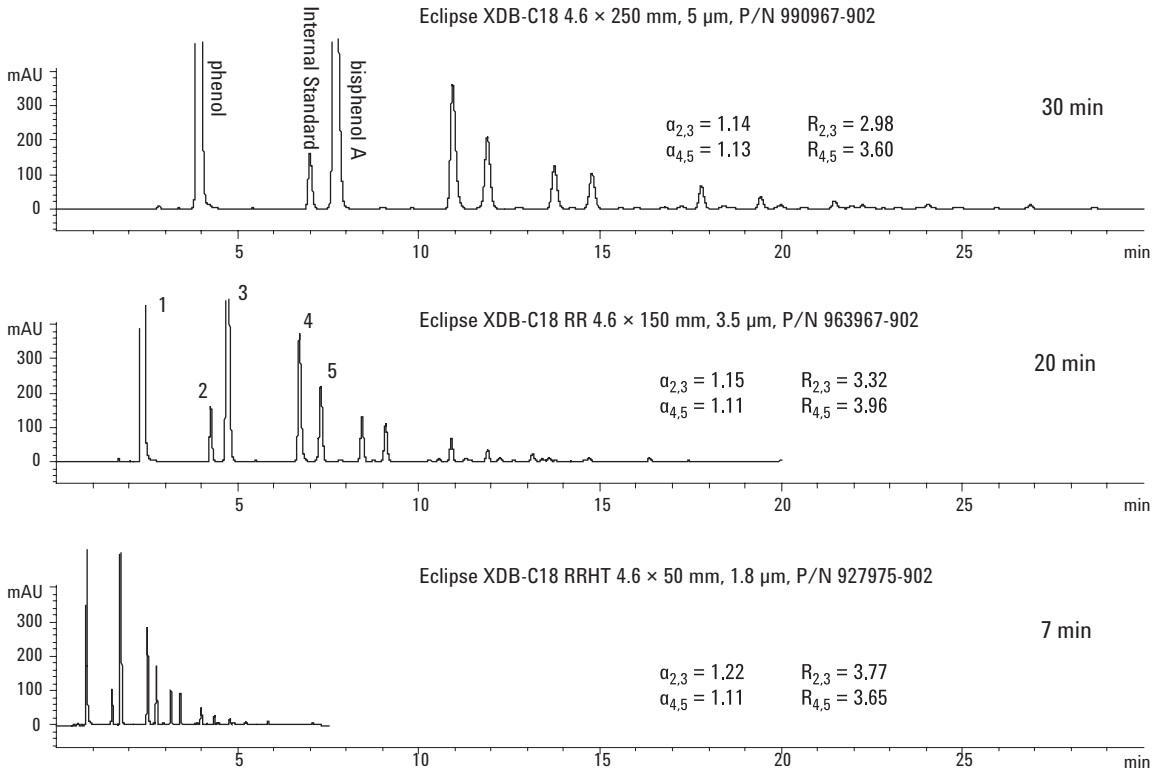

Figure 2. RRHT column configuration increased both speed and resolution.

Particle size does influence resolution. The influence can be noticed when comparing columns of identical dimensions, packed with three different particle sizes. Figure 3 shows the shortened Bisphenol-A analysis using different particle-sized Eclipse XDB-C18 columns. Resolution (Rs) is related to selectivity (α), efficiency (N) and retention (k´):

$$Rs = (1/4)(\alpha-1)\sqrt{N}\,[k'/(1+k')]$$

Factors affecting the selectivity term (stationary phase, mobile phase) and retention term (mobile phase, temperature) are constant for the three chromatograms. The efficiency term is influenced by column length, linear velocity of the mobile phase (both constant), and particle size (varied in Figure 3). N increases as particle size decreases. In Figure 3 the selectivity factors (α) and retention remain about the same, but resolution actually increases. The increase in resolution due to the decrease in particle size highlights the advantage of using smaller particles. The similar selectivity and retention highlight the suitability of ZORBAX Eclipse XDB-C18 columns for scaling methods, especially to more rapid, high-throughput methods.

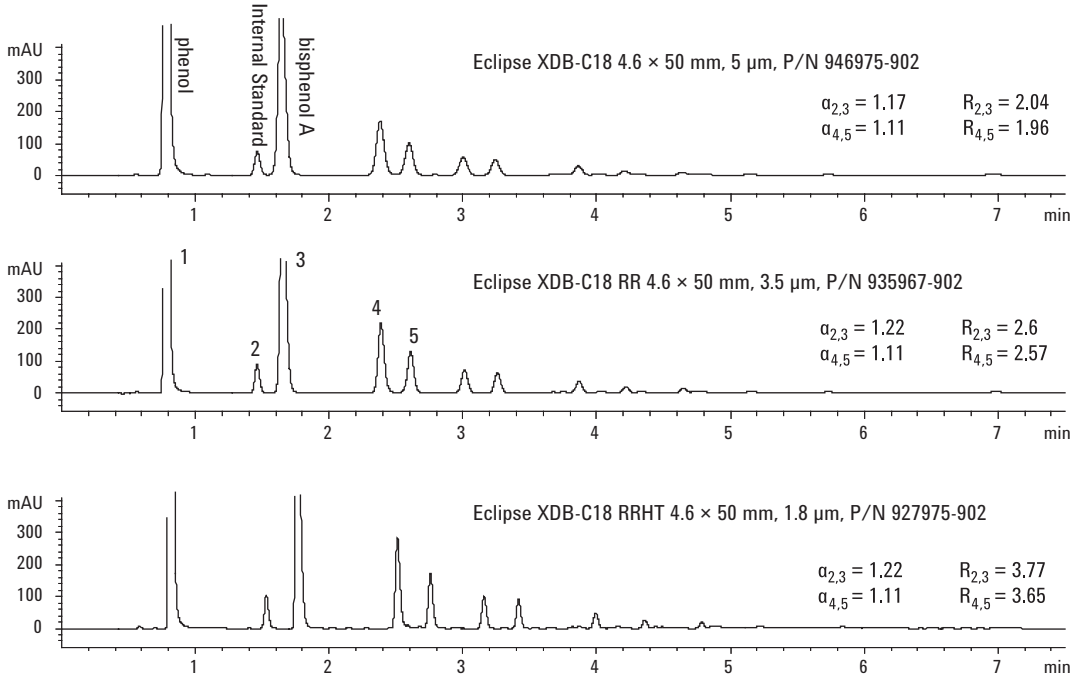

Figure 3. Effect of particle size on resolution and selectivity.

Comparing the Existing Method to the RRHT Method

Figure 4 compares the original BPA separation to the RRHT separation. The top chromatogram is an example of the analysis using the original commercial method, and the bottom is an example of the process sample analyzed with the RRHT method. The method developed with the new column technology clearly increases productivity.

Analysis time is reduced at least six-fold; solvent consumption is reduced about 12.5 times, from 100 mL/analysis to only 7.5 mL/analysis. Interestingly, the peak shape of Bisphenol-A is more symmetrical using Eclipse XDB-C18 as compared to the current C18 column used in the original analysis. The more Gaussian peak shape eluted by the Eclipse XDB-C18 column is important for accurate quantification. Other method improvements such as a simplified gradient and a binary mobile phase are listed in Table 1.

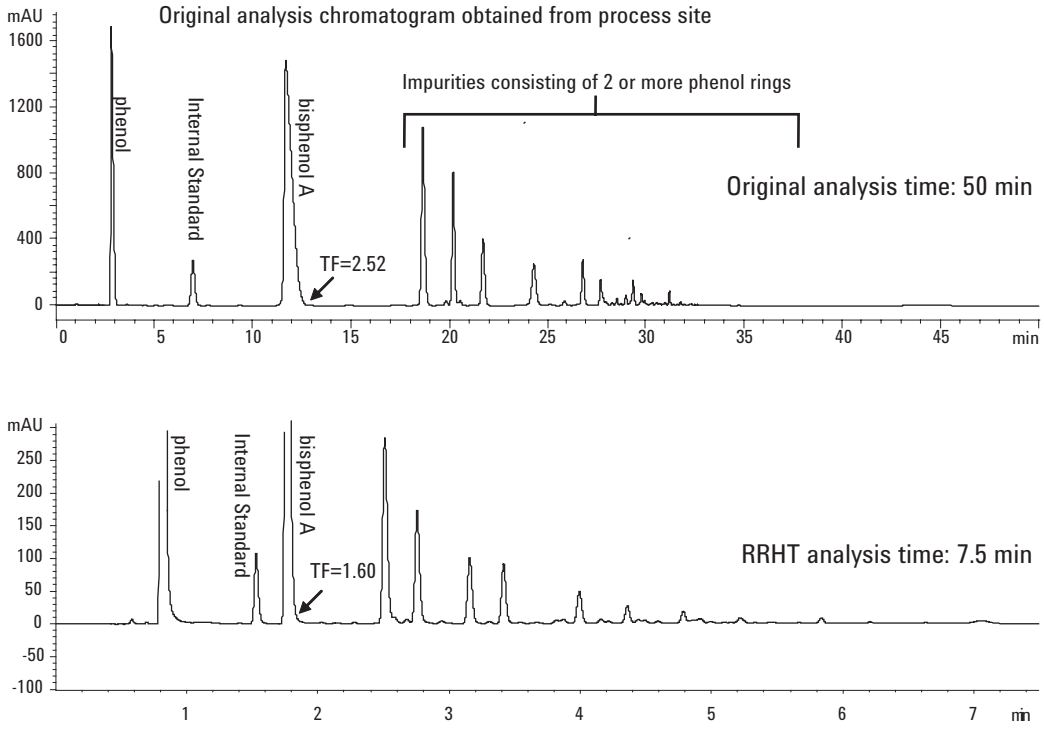

Figure 4. Comparison of methods; original to RRHT.

Table 1. Current and Improved Method Parameters

Original

- Column: Supelco LC-18, 4.6 × 250 mm, 5 µm
- Mobile phase: A: 0.025% H_3PO_4, B: ACN, C: MeOH
- Flow: 2 mL/min
- Temperature: 35 °C
- Sample size: 20 µL
- Gradient: segmented, has isocratic holds

Time	% A:B:C
0	65:25:10
13	65:25:10
18	50:40:10
23	50:40:10
27	30:50:20
32	0:70:30
35	0:70:30
36	0:60:40
40	0:50:50
43	0:20:80
48	65:25:10

RRHT

- Column: ZORBAX XDB-C18, 4.6 × 50 mm, 1.8 µm
- Mobile phase: A: 0.1% formic acid, B: ACN: MeOH (200:800)
- Flow: 1 mL/min
- Temperature: 25 °C
- Sample size: 2 µL
- Gradient: linear, no isocratic holds

Time	% B
0	60
6	95
6.01	60
8	60

Conclusion

Converting an existing method to a high-throughput method is one way to improve lab productivity. Using RRHT columns initially for method development also improves productivity. Eclipse XDB-C18 RRHT columns are a good choice for converting existing C18 methods into high-throughput methods. Smaller particles packed into shorter columns provide comparable resolution to larger particles packed into longer columns in a fraction of the time. RRHT columns are advantageous for gradient method development because gradient re-equilibration is time-consuming and often overlooked in the total analysis time. Methods developed on Agilent RRHT columns can be scaled easily because of the highly uniform particles, bonded phase chemistry, and column manufacturing techniques. An existing method developed on a "traditional analytical-sized" column was easily converted to a high throughput method using an Eclipse XDB-C18 RRHT column. The method was incrementally scaled up to an analytical-sized column, and it performed with predictable results on various column dimensions and particle sizes. The predictability of the results supports Eclipse XDB-C18 RRHT columns' ability to easily improve applications and transfer them into high-throughput and high-resolution applications.

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Fast Analysis of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator

Application

Hydrocarbon Processing

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Abstract

Vitamin E (tocopherol), phenolic antioxidants and erucamide slip additives in polypropylene homopolymer formulations were resolved and detected using liquid chromatography with ultraviolet/visible detection, under guidelines suggested by ASTM Method D6042. Using the Agilent 1200 Rapid Resolution LC system with Agilent ZORBAX RRHT columns, the antioxidants could be rapidly separated with the same or improved resolution. The Agilent method translator was used to transfer the ASTM method into new methods based on the instrument parameters, column dimensions, and particle size in three modes: simple conversion and speed optimized and resolution optimized methods.

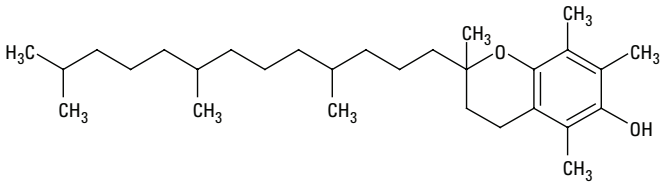
Introduction

Polymers are very popular all over the world owing to their unprecedented physical properties. Various additives are blended into polymeric materials to modify certain properties of the polymer formulation. Erucamide, Irganox 3114, Irganox 1010, Vitamin E (tocopherol), Irganox 1076, and Irgafos168 are often used as antioxidants to prevent the degradation of polypropylene homopolymer formulations by light, heat, and oxygen. In this work, with the goal to shorten the analysis time and reduce solvent consumption without losing separation quality, the existing ASTM method was recalculated for new operating conditions based on columns packed with smaller particle sizes. The chemical information of the antioxidants and Tinuvin P as internal standard is displayed in detail in Table 1.

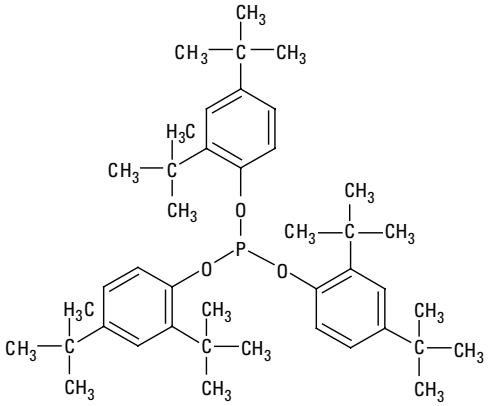
Specific additives and their concentrations in polymer formulations are critical to the properties of polymer, and careful analysis is required to ensure that the additives and levels are appropriate for the intended use. This application will compare two different stationary phases according to analyte retention characteristics and peak shape, show the influence of different injection volume of real sample on the peak shape, and then will focus on showing how to use the method translator. The latter is used to transfer the conventional method to new methods using smaller size columns to perform simple conversion and to extend the method to greater speed and higher resolution.

Table 1. Chemical Information of Antioxidants and Tinuvin P

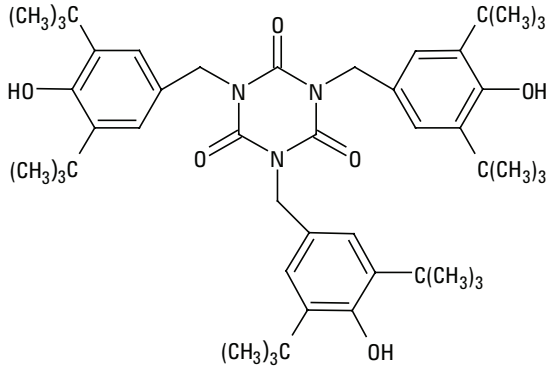
Name:	Vitamin E	DL-all-rac-α-Tocopherol
Formula:	$C_{29}H_{50}O_2$	
Molecular Weight:	430.71	
CAS No.:	10191-41-0	



Name:	Irgafos 168	Tris(2,4 di-tert-butylphenyl) phosphite
Formula:	$[[(CH_3)_3C]_2C_6H_3O]_3P$	
Molecular Weight:	646.92	
CAS No.:	31570-04-4	



Name:	Irganox 3114	Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate
Formula:	$C_{48}H_{69}N_3O_6$	
Molecular Weight:	784.08	
CAS No.:	27676-62-6	



Name:	Erucamide	cis-13-docosenamide
Formula:	$CH_3(CH_2)_7CH=CH(CH_2)_{11}CONH_2$	
Molecular Weight:	337.58	
CAS No.:	112-84-5	

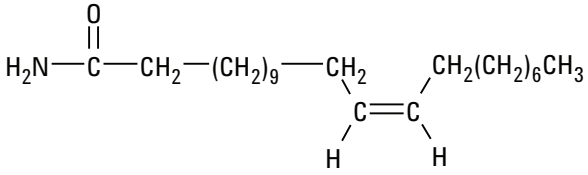
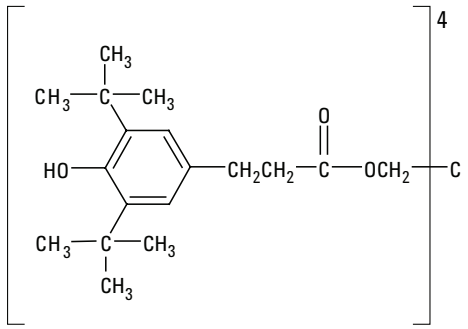
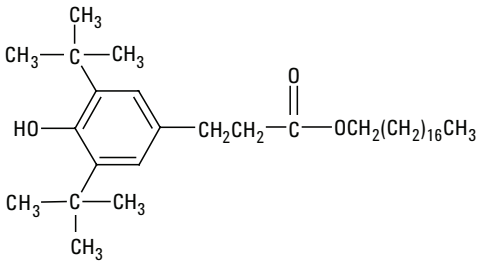
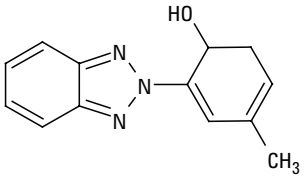


Table 1. Chemical Information of Antioxidants and Tinuvin P (Continued)

Name:	Irganox 1010	Pentaerythritol tetrakis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)
Formula:	$[HOC_6H_2[C(CH_3)_3]_2CH_2CH_2CO_2CH_2]_4C$	
Molecular Weight:	1177.63	
CAS No.:	6683-19-8	
Name:	Irganox 1076	Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate
Formula:	$[(CH_3)_3C]_2C_6H_2(OH)CH_2CH_2CO_2(CH_2)_{17}CH_3$	
Molecular Weight:	530.86	
CAS No.:	2082-79-3	
Name:	Tinuvin P	2-(2-hydroxy-5-methylphenyl)benzotriazole
Formula:	$C_{13}H_{11}N_3O$	
Molecular Weight:	225.25	
CAS No.:	2440-22-4	

Experimental

System

Agilent 1200 Series Rapid Resolution LC (RRLC), consisting of:
G1379B micro vacuum degasser
G1312B binary pump SL
G1367C high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL with 3 mm, 2 µL flow cell
ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 3.0 mm × 50 mm, 1.8 µm

Mobile Phase

Gradients:	A: water B: acetonitrile (ACN)
Gradient conditions:	See individual chromatograms
Column temperature	See individual chromatograms

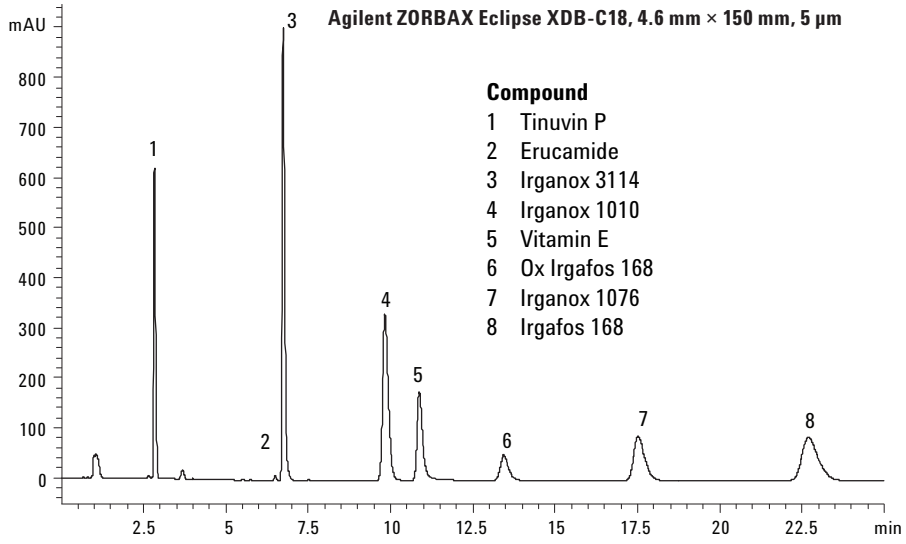
Samples

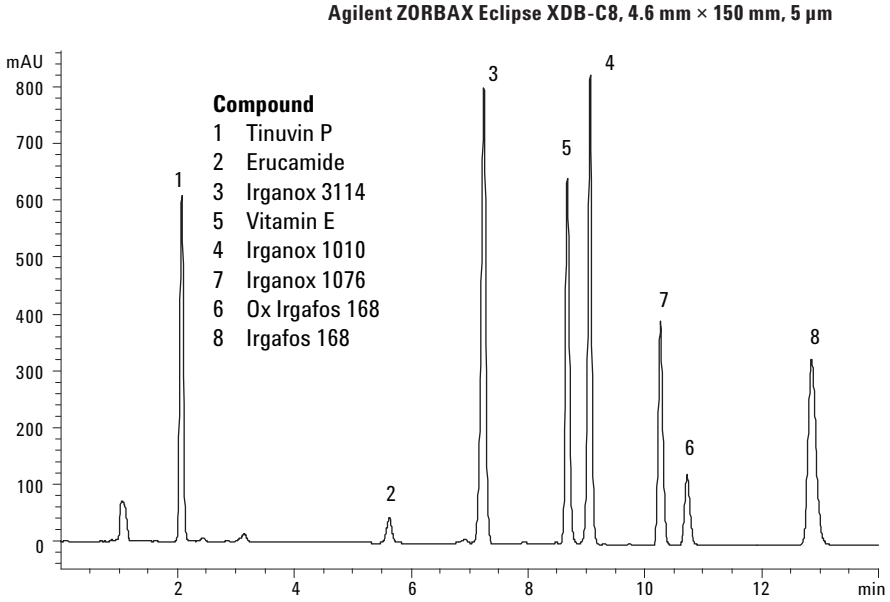
1. Standard mixture of Tinuvin P, Erucamide, Irganox 3114, Irganox 1010, Vitamin E, Irganox 1076, and Irgafos168, all 200 µg/mL in isopropanol
2. Polypropylene Homopolymer Formulation, from customer, extracted by ultrasonic according to the method ASTM D6042-04
3. Polypropylene extract spiked with 20 µg/mL standard mixture

Results and Discussion

Selection of Stationary Phase for the Separation of Antioxidants

It is desirable during method development to select a column that will provide the optimal analyte separation and shortest analysis time. Reversed phase C18 columns are recommended by ASTM D6042-04; however, in our application we determined that the retention characteristics of ZORBAX XDB-C18 columns were too strong for the specified solvents, resulting in broad peak shape and quantitation difficulties for late-eluting peaks. Compared with ZORBAX XDB-C18 columns, ZORBAX XDB-C8 columns showed better retention capability and peak shape. Therefore, we chose the ZORBAX XDB-C8 column for further method development. The different separations with ZORBAX XDB-C18 and ZORBAX XDB-C8 columns are shown in the Figure 1.





Conditions

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

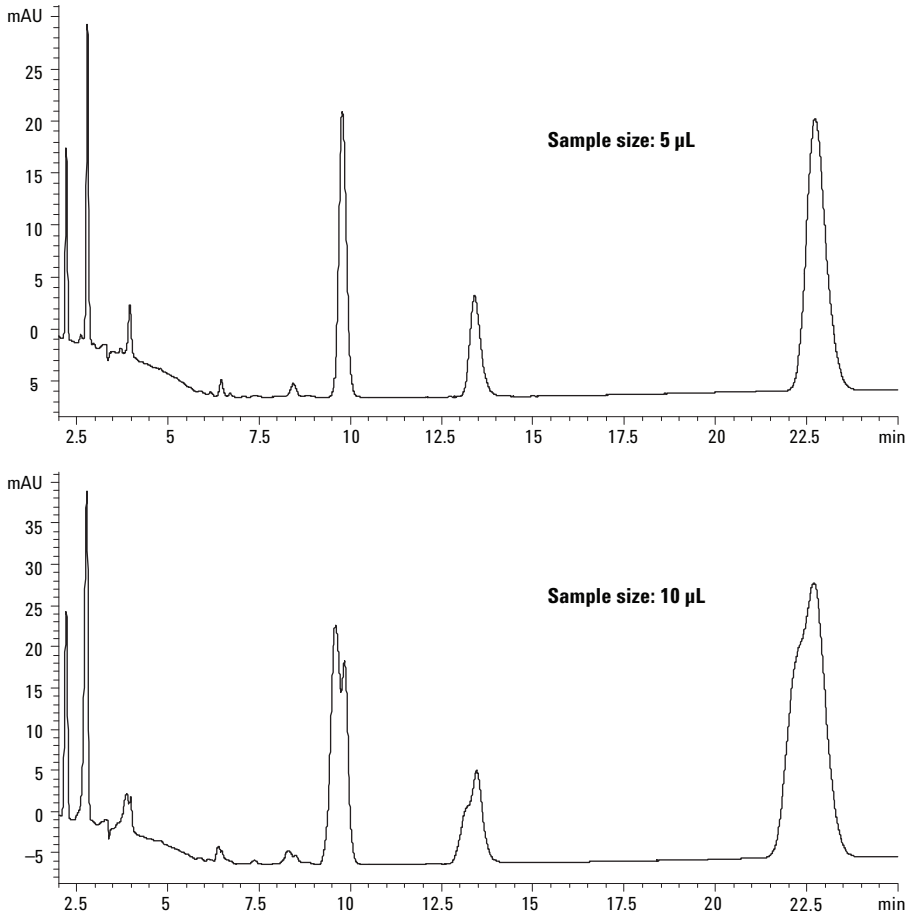
Figure 1. ZORBAX stationary phase comparison for antioxidants.

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 µL. We decreased the sample size of real sample and found that the volume of 5 µL was suitable and free of solvent influence. The split and nonsplit peaks are shown in Figure 2. At the same time, the influence of injection volume was not found in the standard solution, which was dissolved in isopropanol per ASTM method guidance.

Fast Method Developed Based on New 1200 RRLC with Method Translator

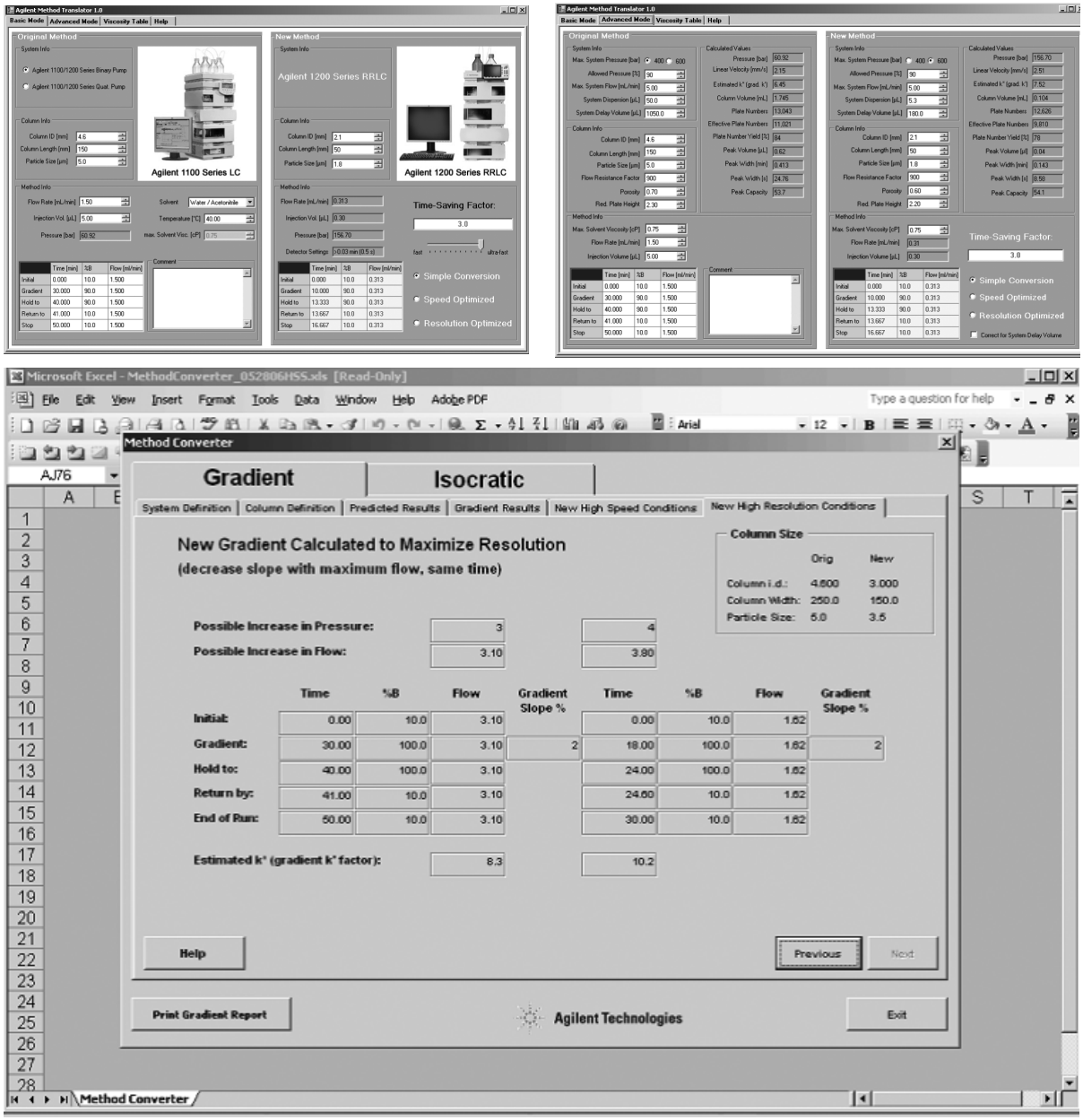
Due to the appearance of sub-two-micron columns and LC systems with higher pressure capabilities, the research of ultra-fast separation is more and more popular. Therefore, it is important to quickly and easily transfer conventional methods to fast or high-resolution methods. Agilent provides the users of RRLC systems with two versions of method translators; one is a Microsoft.net version, which requires that Net-Framework 2.0 be resident on the computer, the other is a Microsoft Excel version, which requires that Excel be resident on the PC. The interface of the two translators is displayed in Figure 3.



Conditions

		Gradient:	
Mobile phase:	A: water; B: ACN	Min	%B
Flow rate:	1.5 mL/min	0.00	75
Wavelength:	200 nm	5.00	100
Injection volume:	5 or 10 µL	25.00	100
Column temperature:	50 °C	25.10	75
Column:	ZORBAX Eclipse XDB-C18 4.6 mm × 150 mm, 5 µm		
Sample:	Polypropylene extraction solution	30.00	75

Figure 2. Injection volume influence of real sample extraction solution on the peak shape.



The upper one is the Microsoft.net version, the lower one is the Microsoft Excel version.

Figure 3. Two different method translators.

Sample Preparation

The two versions of method translators provide three modes of method conversion; the first is the simple conversion, which has the same gradient slope as the conventional method, and changes the flow rate according to equation 1:

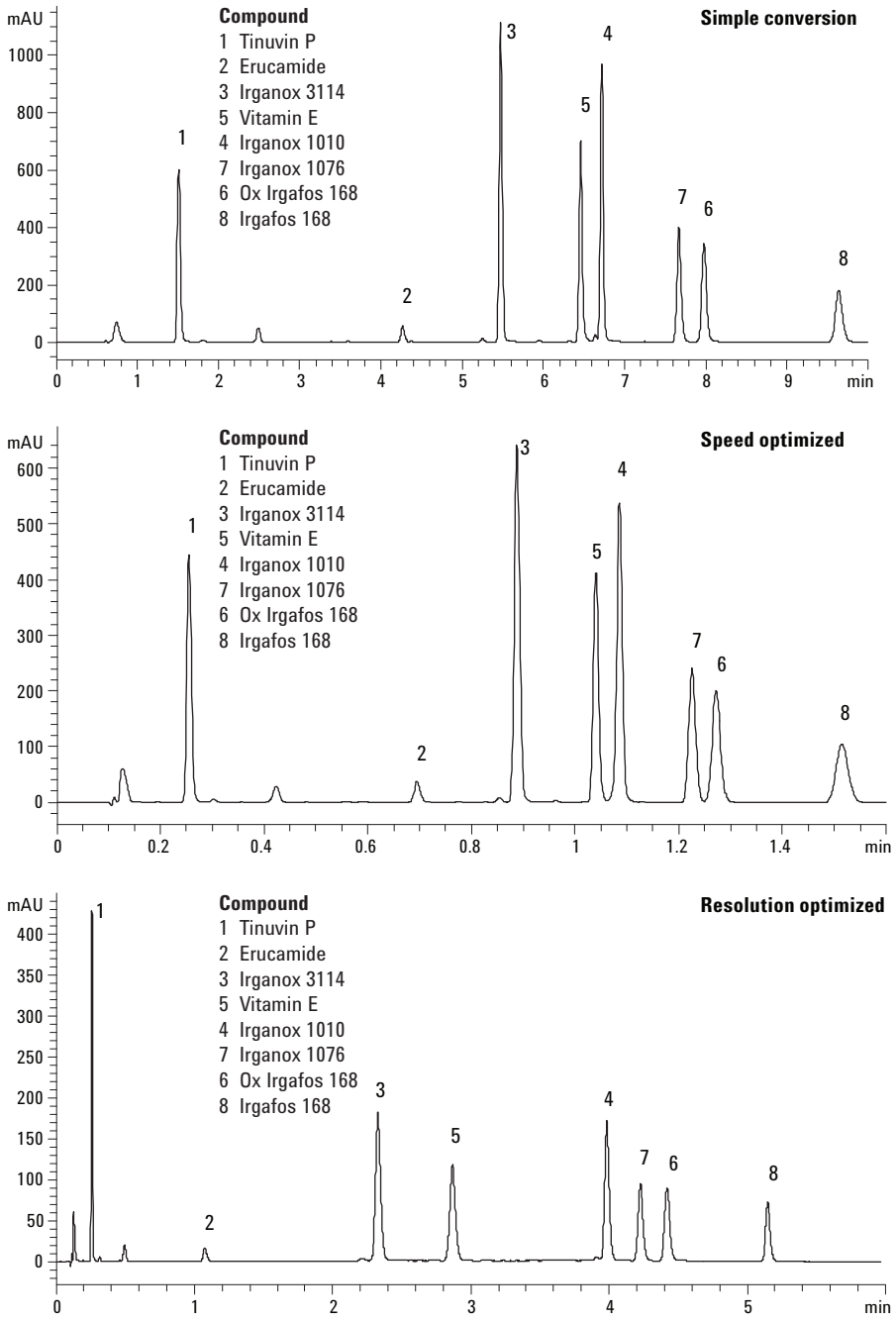
$$Flow_{Col.\,2} = \left[\frac{Diam_{Col.\,2}}{Diam_{Col.\,1}}\right]^2 \times Flow_{Col.\,1} \quad \text{(eq. 1)}$$

The second is the speed optimized conversion, which has the same gradient slope as the conventional method and maximizes the flow rate according the LC system pressure capability. The last is the resolution optimized conversion, which maximizes the flow rate according the LC system pressure capability and has the same gradient time as the simple converted mode, resulting in a reduced gradient slope that normally yields higher peak resolution. For the different columns, the injection volumes should be changed according to the relationship displayed in equation 2.

$$Inj.\ vol._{Col.\,2} = \left[\frac{Volume_{Col.\,2}}{Volume_{Col.\,1}}\right] \times Inj.\ vol._{Col.\,1} \quad \text{(eq. 2)}$$

As mentioned above, the method based on the ZORBAX Eclipse XDB-C8 4.6 mm x 150 mm, 5 µm, was selected as the initial method. Afterwards, the initial method was transferred with the method

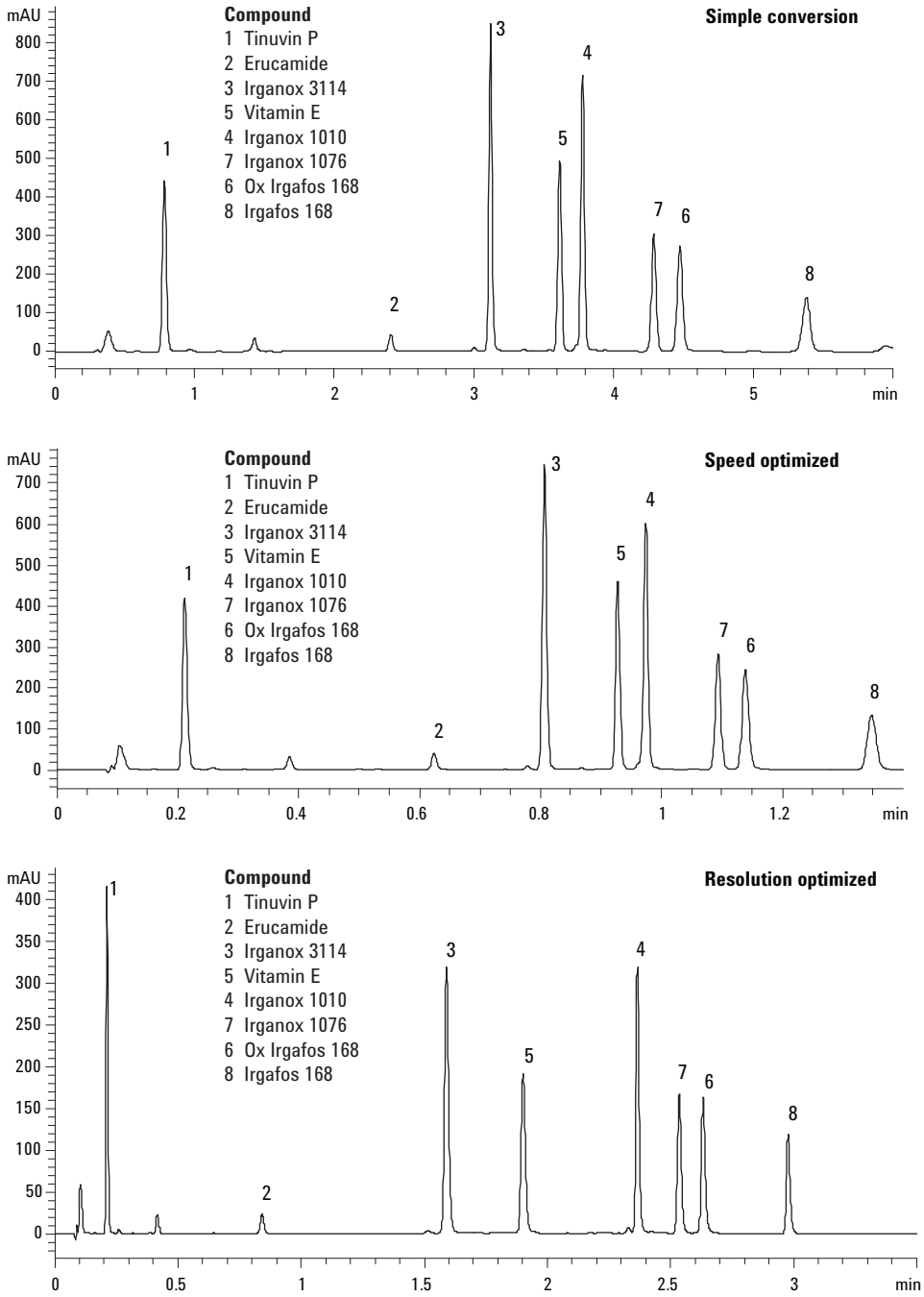
translator into three modes on different column lengths (100, 50 mm) and particle sizes (3.5, 1.8 µm), respectively. Figures 4 and 5 show the separation of antioxidants in smaller particle size columns with the recalculated methods.



Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol			
Mobile phase:	A: water; B: ACN			
Temperature:	50 °C			
Wavelength:	200 nm			
Injection volume:	6.7 µL			
Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 100 mm, 3.5 µm			
Mode:	Simple converted	Speed optimized	Resolution optimized	
Flow rate:	0.64 mL/min	4.00 mL/min	4.00 mL/min	
	Pressure:	110 bar	460 bar	460 bar
	Gradient slope:	3.1%	3.1%	0.5%
	Analysis time:	10 min	1.6 min	5.5 min

Figure 4. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 100 mm, 3.5 µm.

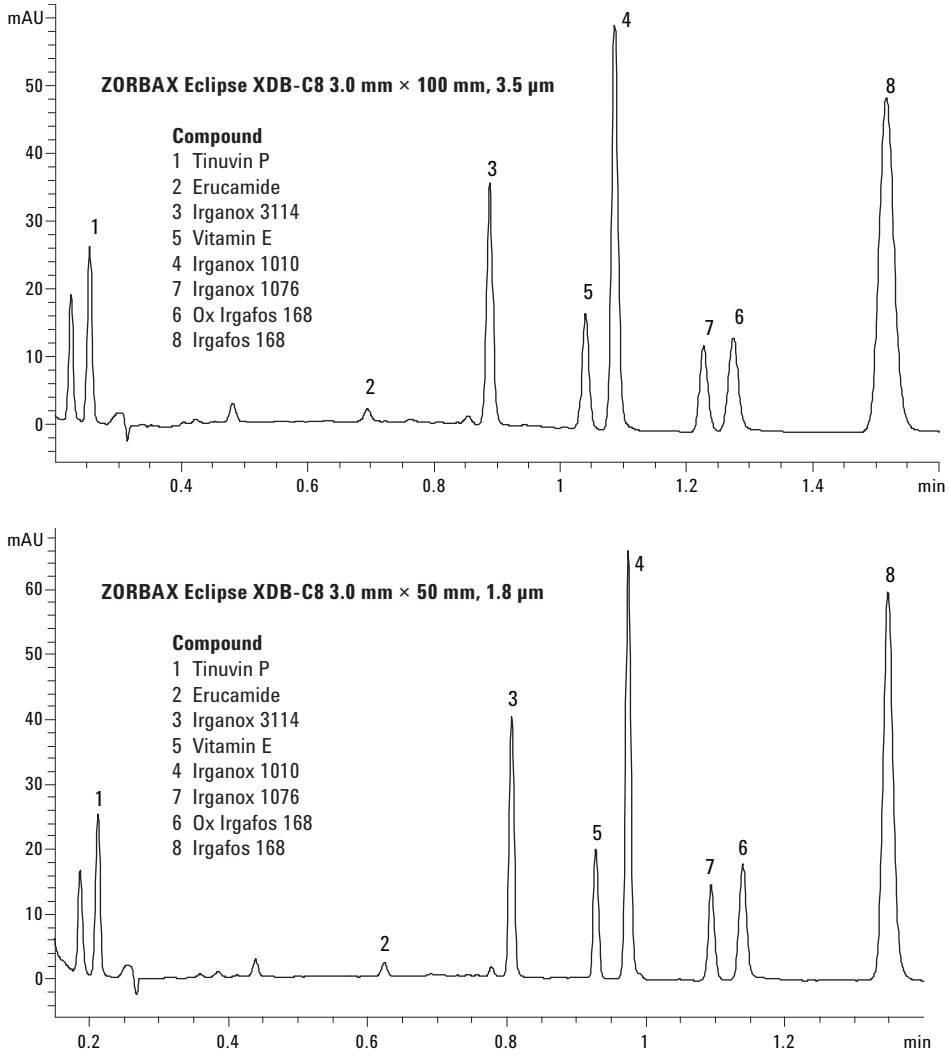


Conditions

Sample:	Standard mixture, 200 µg/mL in isopropanol		
Mobile phase:	A: water; B: ACN		
Temperature:	50 °C		
Wavelength:	200 nm		
Injection volume:	3.3 µL		
Column:	ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm		
Mode:	Simple converted	Speed optimized	Resolution optimized
Flow rate:	0.64 mL/min	2.50 mL/min	2.50 mL/min
Pressure:	160 bar	460 bar	460 bar
Gradient slope:	3.1%	3.1%	0.8%
Analysis time:	6 min	1.4 min	3 min

Figure 5. Separation of antioxidants on ZORBAX Eclipse XDB-C8 3.0 mm × 50 mm, 1.8 µm.

To identify the matrix influence on the separation, the polypropylene extract was spiked with 20 µg/mL standard mixture and injected into the LC system. Figure 6 depicts the separation of spiked sample with the speed optimized method, which shows a sufficient separation of antioxidant in polymer matrix with about 10 times faster speed than the conventional method mentioned above.



Conditions

Sample:	Polypropylene extract spiked with 20 µg/mL standard mixture	
Mobile phase:	A: water; B: ACN	
Temperature:	50 °C	
Wavelength:	200 nm	
Stationary phase:	ZORBAX Eclipse XDB-C8	
Column size:	3.0 mm × 100 mm, 3.5 µm	3.0 mm × 50 mm, 1.8 µm
Injection volume:	3 µL	1 µL
Mode:	Speed optimized	Speed optimized
Flow rate:	4.00 mL/min	2.50 mL/min
Pressure:	460 bar	460 bar
Gradient slope:	3.1%	3.1%
Analysis time:	1.6 min	1.4 min

Figure 6. Separation of spiked polypropylene extract by the speed optimized method.

Conclusions

As an important innovation in the advancement of liquid chromatography, the Agilent 1200 Rapid Resolution LC system provides the customer not only a rapid separation with the same or similar resolution, but also includes a method translator to convert any initial conventional method to a fast or high-resolution method according to the requirements of the user. This note applies the method translation tool in the separation of polymer additives and demonstrates the ease-of-use and power of the method translator using separations of a standard mixture and spiked real sample.

References

1. ASTM D6042-04, “Standard Test Method for Determination of Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using Liquid Chromatography (LC)”
2. Michael Woodman, “Improving the Effectiveness of Method Translation for Fast and High Resolution Separations”
3. Michael Woodman, “Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection”

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Agilent Technologies

Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC

Application

Hydrocarbon Processing

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Abstract

Liquid chromatography with ultraviolet/visible (UV/VIS) detection is a powerful approach for analyzing additives in polymer formulations. This application illustrates the use of the Agilent 1200 Series Rapid Resolution LC (RRLC) system for the separation of antioxidants and erucamide. The system can operate significantly faster than conventional HPLC without sacrificing resolution, precision, or sensitivity. The column chemistry and temperature influence on the separation and the sample preparation method are also discussed.

Introduction

Additives are incorporated into various polymeric materials to retard the degradation caused by ultraviolet light, heat, and oxygen or to modify processing characteristics. A rapid and accurate analytical method is required to ensure that the specified amount of an additive or combination of additives is incorporated into a polymer after the extrusion process. Conventional HPLC methods for additives [1,2] often require more then 30 minutes per analysis, while the application described here can achieve comparable results in as few as 3 minutes.

Agilent has developed an easy-to-use method conversion tool for transferring existing methods for higher speed and/or higher resolution. The tool was used for the method optimization in this application. [3]

This application examines additives mentioned in ASTM Methods D5815 and D1996. The chemical structures are shown in Table 1.

Table 1. Polymer Additives in ASTM Methods D5815 and D1996

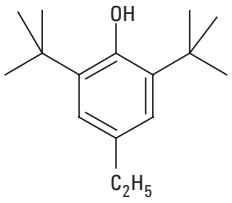
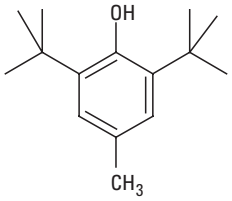
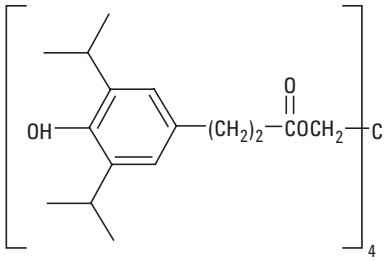
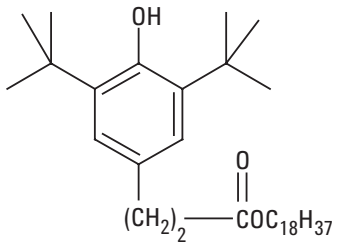
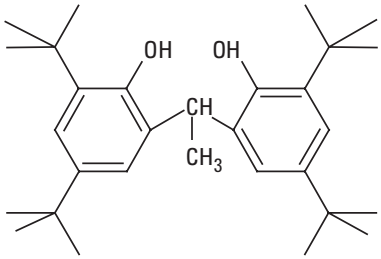
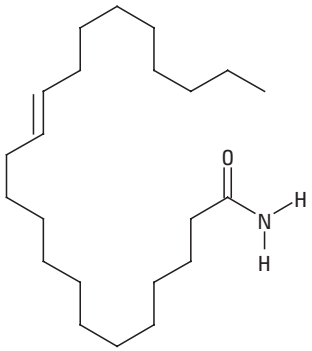
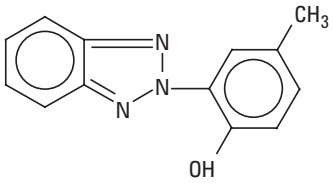
Registered trade name	CAS no.	Chemical name	Chemical structure
BHEB	4310-42-1	2,6-di-tert-butyl-4-ethyl-phenol or butylated hydroxyethyl benzene	
BHT	128-37-0	2,6-di-t-butyl-cresol or butylated hydroxy toluene	
Irganox 1010	6683-19-8	Tetrakis[methylene(3,5-di-t-butyl-4-hydroxy hydrocinnamate)] methane	
Irganox 1076	2082-79-3	Octadecyl-3,5-di-t-butyl-4-hydroxy hydrocinnamate	
Isonox 129	35958-30-6	2,2-ethylidene bis (4,6-di-t-butyl phenol)	

Table 1. Polymer Additives in ASTM Methods D5815 and D1996 (Continued)

Registered trade name	CAS no.	Chemical name	Chemical structure
Kemamide-E	112-84-5	Cis-13-docosenamide or Erucamide or Fatty acid amide ($C_{22}H_{43}NO$)	
Tinuvin P	2440-22-4	2(2'-hydroxy-5'-methyl phenyl) benzotriazole	

Experimental

System

Agilent 1200 Series rapid-resolution LC configured with
G1379B microvacuum degasser
G1312B binary pump SL
G1367B high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL
ChemStation 32-bit version B.02.01

Column

ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm
ZORBAX SB-C18, 4.6 mm × 150 mm, 5 µm
ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm

Mobile phase

Gradients:	A: water B: acetonitrile (ACN)
Gradient slope:	See individual chromatograms for flow rate and gradient time
Column temperature:	See individual chromatograms

Samples

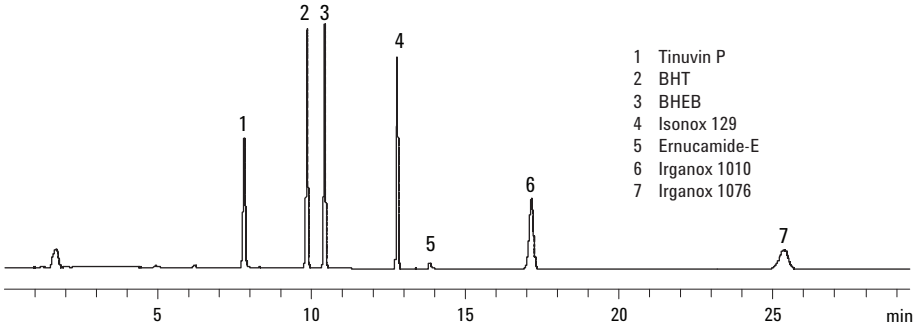
1. Standard mixture described in ASTM D5815 and D1996, 50 µg/mL, 200 µg/mL in isopropanol
2. Linear low-density polyethylene from customer, ground to 20 mesh, extracted by ultrasonic or reflux method

Results and Discussion

Fast Method Conversion

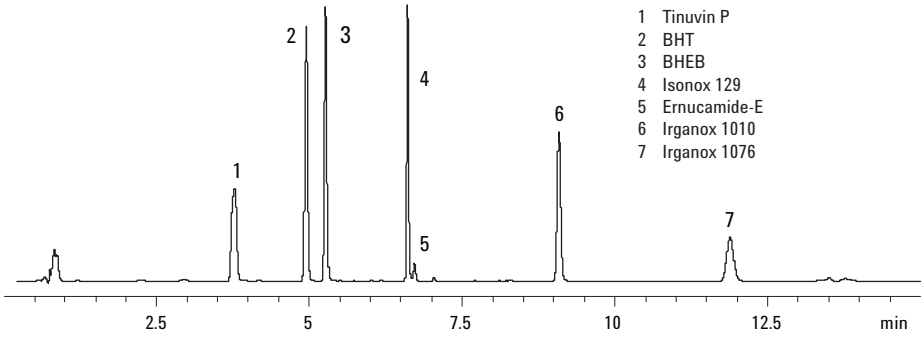
The separation was initially performed on a standard 4.6 mm × 150 mm, 5-µm ZORBAX Eclipse XDB-C18 column thermostatted to 60 °C (Figure 1) following the conditions in ASTM D5815 (or D1996). The method was then scaled in flow and time for exact translation to a 2.1 mm × 50 mm, 1.8-µm column (Figure 2). The analysis time was reduced from 25.5 to 12.5 minutes, and the solvent consumption was reduced from 25 to 2.5 mL.

The separation was then re-optimized for faster separation with the same gradient slope by increasing the flow rate from 0.21 to 0.9 mL/min and proportionately reducing the gradient time (Figure 3), achieving up to 10 times faster than conventional HPLC without sacrificing resolution, precision (showed in Table 2), or sensitivity. Figure 4 demonstrates that 1 ppm of additives can be determined with very good signal-to-noise response using the same condition in Figure 3, which exceeds the specification of 2 ppm of ASTM D5815 (or D1996). Peak 6, Irganox 1010, for example has a signal-to-noise response of 88 at 1 ppm.



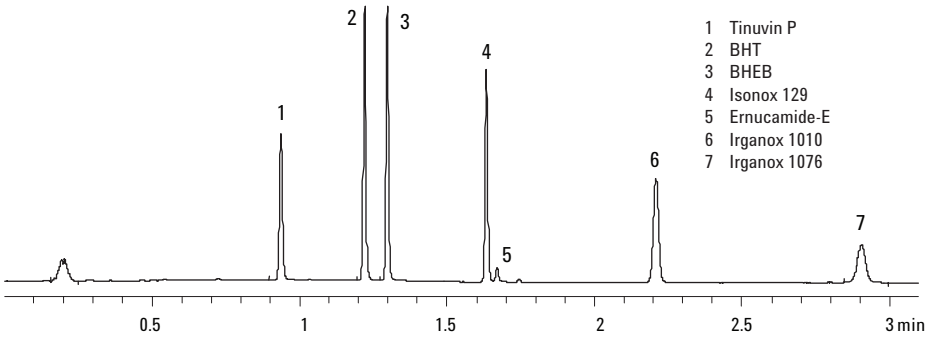
		Gradient	
Conventional method:	Follow ASTM D5815 (or D1996) method with		
	ZORBAX Eclipse XDB-C18,		%B
	4.6 mm × 150 mm, 5 µm	0	50
Sample:	Standard 50 µg/mL	11	100
Sample size:	10 µL	28	100
Detector:	UV 200 nm	28.1	50
Column temperature:	60 °C		
Mobile phase:	A: water		
	B: acetonitrile		
Flow rate:	1 mL/min		

Figure 1. Separation of additives standards on Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm.



		Gradient	
Simple-converted:	Translate the conventional method to a		
	ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm		%B
Sample:	Standard 50 µg/mL	0	50
Sample size:	2 µL	5.2	100
Detector:	UV 200 nm	12	100
Column temperature:	60 °C	12.1	50
Mobile phase:	A: water	15	50
	B: acetonitrile		
Flow rate:	0.21 mL/min (73 bar)		

Figure 2. Separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

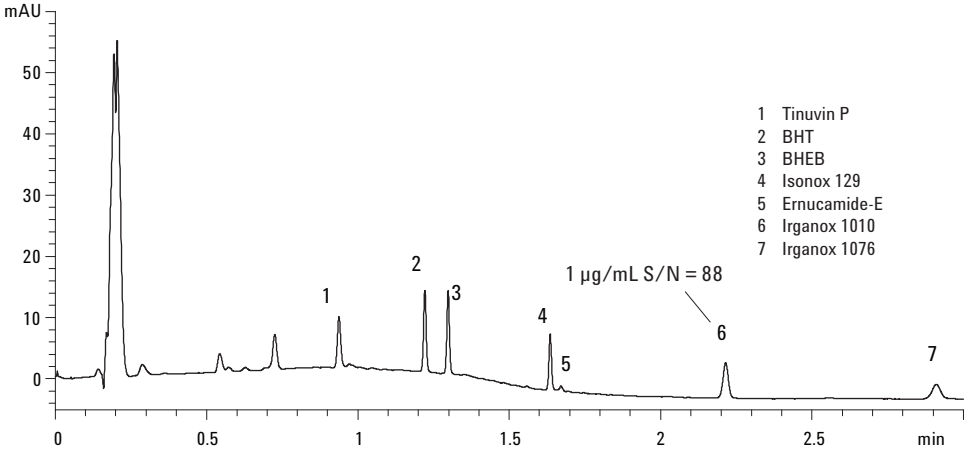


		Gradient	
Speed-optimized:	Optimize the conventional method for speed with ZORBAX Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm		%B
		0	50
Sample:	Standard 50 µg/mL	1.3	100
Sample size:	2 µL	3	100
Detector:	UV 200 nm	3.1	50
Column temperature:	60 °C	3.5	50
Mobile phase:	A: water B: acetonitrile		
Flow rate:	0.9 mL/min (357 bar)		

Figure 3. Fast separation of additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Table 2. Repeatability for the Methods of Conventional, Simple-Converted, and Speed-Optimized Methods (n = 5)

	Area, RSD%		
Compounds (50 ppm)	**Conventional**	**Simple-converted**	**Speed-optimized**
Tinuvin P	0.37	0.39	0.09
Erucamide	0.40	0.57	0.13
Irganox 3114	0.44	0.49	0.22
Irganox 1010	0.38	0.39	0.26
Vitamin E	0.58	0.80	0.68
Irganox 1076	0.58	1.49	0.17
Irgafos 168	0.53	0.77	0.32



Speed-optimized method for analysis of additives standards with concentration of 1 µg/mL LC conditions is identical to that in Figure 3

Figure 4. Fast separation of 1 µg/mL additives standards on Eclipse XDB-C18, 2.1 mm × 50 mm, 1.8 µm.

Optimized Column Temperature

Increasing column temperature can lower both solvent viscosity and nonspecific column/analyte interactions. The new ZORBAX StableBond RRHT columns can operate at temperatures up to 90 °C. We tested operating temperatures at 60, 75, 85, and 90 °C with a ZORBAX SB-C8 4.6 mm × 150 mm, 5-µm column. The results (Figure 5) show that the analysis time obtained from 60 °C to 85 °C is reduced from 23.5 minutes to 17 minutes; at 90 °C, only an additional 0.5 minute is saved. Based on the combined speed reduction and optimized resolution of peaks 4 and 5, 85 °C is chosen as a suitable column temperature.

The method was then scaled in flow and time for exact translation to a 4.6 mm × 50 mm, 1.8-µm column (Figure 6). Finally, the separation was optimized for faster separation by increasing the flow rate from 1 mL/min to 3.5 mL/min, with only a 1.7-minute analysis time (Figure 7). This is really an excellent procedure for high-throughput screening and quantitation of a large number of samples. Figure 8, the separation of an extract of linear low-density polyethylene (LLDPE) spiked with 20 µg/mL of standard solution, shows excellent separation with real sample matrix.

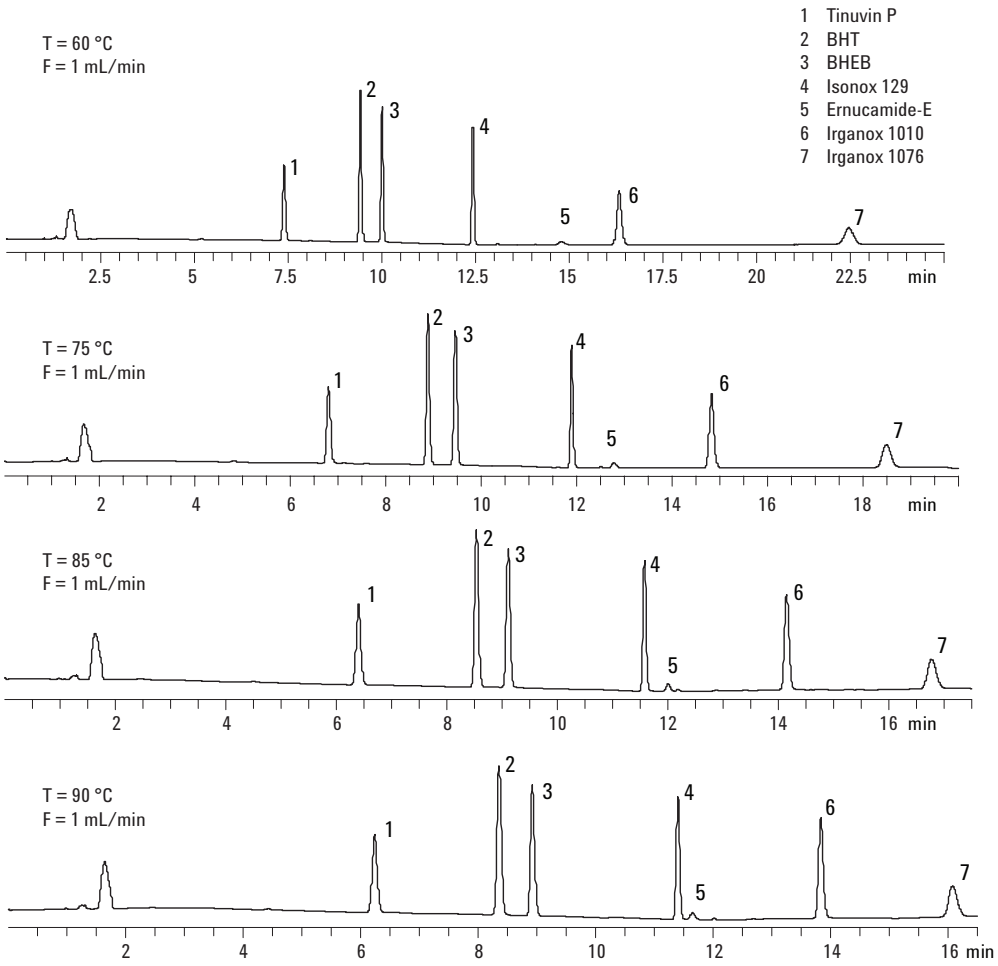
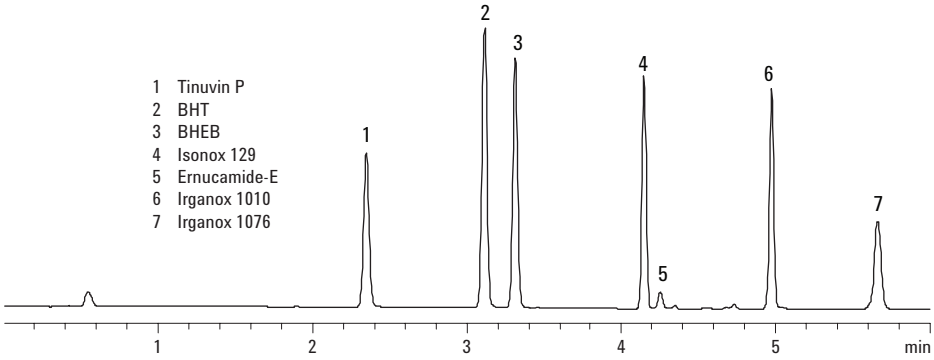
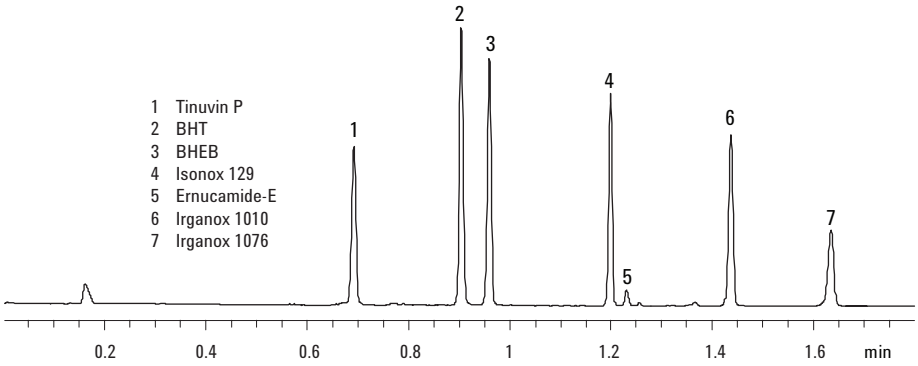


Figure 5. Separation of additives standards on ZORBAX StableBond RRHT SB-C18, 4.6 mm × 150 mm, 1.8 µm.



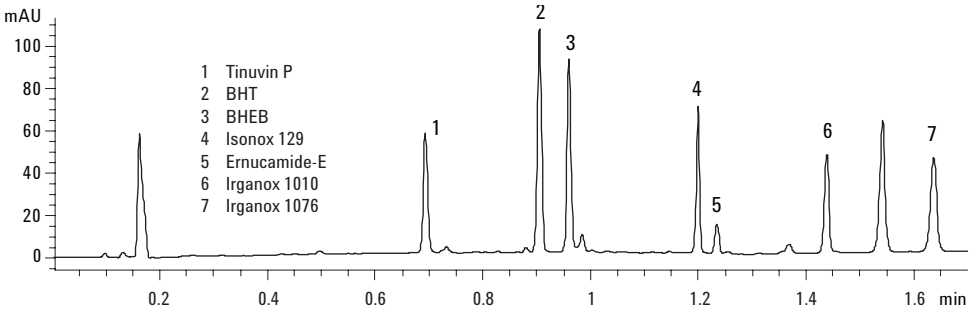
Sample:	Standard 200 mg/mL
Sample size:	2 µL
Detector:	UV 200 nm
Mobile phase:	A: water
	B: acetonitrile
Gradient slope:	6.8%
Flow rate:	1mL/min

Figure 6. Separation of additives standards on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.



Sample:	Standard_200 mg/mL
Sample size:	2 µL
Detector:	UV 200 nm
Mobile phase:	A: water
	B: acetonitrile
Gradient slope:	6.8%
Flow rate:	3.5 mL/min

Figure 7. Fast separation of additives standards on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.



LC conditions are identical with those in Figure 7.

Figure 8. Fast separation of spiked real sample-LLDPE (20 µg/mL) on ZORBAX SB-C18, 4.6 mm × 50 mm, 1.8 µm, at 85 °C.

Sample Preparation

ASTM D5815 (or D1996) method recommends using a reflux apparatus for extracting additives in polymer. This requires periodic operator intervention over the 1.5-hour-long extraction period. To find a time-saving sample-preparation method, ultrasonic extraction was also tested, producing comparable results in 30 minutes. In terms of extraction efficiency, there is not much difference between these two methods. Figure 9 shows very good overlays of extractions by reflux and ultrasonic extraction methods for a LLDPE. Conditions are identical to those in Figure 1.

Conclusions

Liquid chromatography with ultraviolet/visible detection is an effective tool for analyzing additives in polymer formulations. The Agilent 1200 Series RRLC system equipped with RRHT 1.8-µm columns was used to achieve up to 10 times faster than the conventional HPLC method. The ultrasonic extraction method allowed fast extraction without user intervention for a significant reduction in overall analysis time. Total time saved was more than 80 minutes per sample when compared to the conventional analysis and extraction methods.

References

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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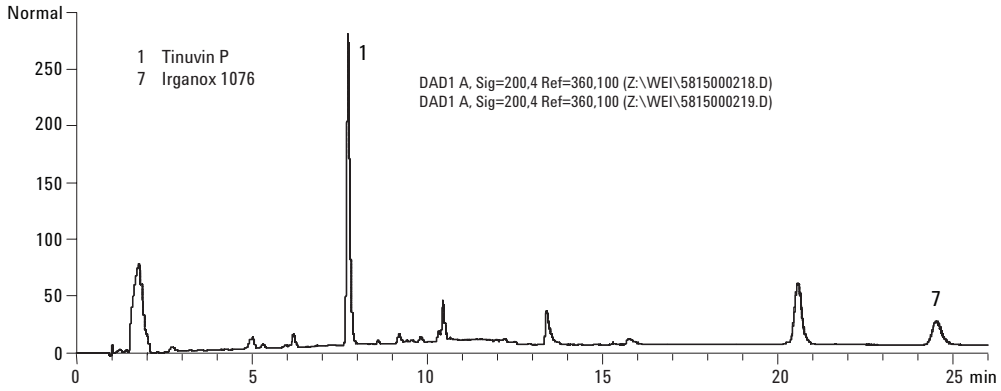

Figure 9. Chromatogram Overlays of extractions by reflux and ultrasonic extraction methods for LLDPE.

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Fast Analysis Method for Rubber Chemical Antidegradants Using 1200 Rapid Resolution Liquid Chromatography (RRLC) Systems with Rapid Resolution High Throughput (RRHT) Columns

Application

Hydrocarbon Processing

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Abstract

***p*-Phenylenediamine (PPD), a chemical antidegradant for rubber, and its analogs were found to be optimally determined using liquid chromatography with ultraviolet/visible detection according to the guidelines of ASTM Method D5666. Using the Agilent 1200 RRLC system with ZORBAX 1.8-µm columns, the PPDs could be separated in one run with a total analysis time up to 6.4 times faster than the conventional method based on a 5-µm column.**

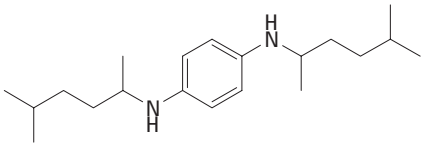
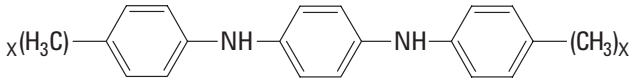
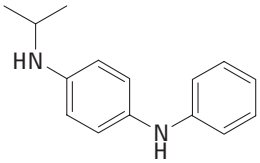
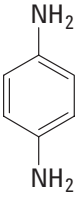
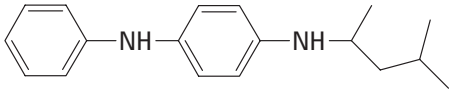
Introduction

Various additives are artificially incorporated into polymeric materials to modify certain properties of the polymer. Therefore, the additives and their concentration in the formulation are crucial to the properties of the end product. 77PD, DTPD, IPPD, PPD, and 6PPD (see Table 1) are often used as chemical antidegradants for rubber materials. The chemical information for five PPDs is displayed in detail in Table 1.

Liquid chromatography with ultraviolet\visible detection is a powerful approach to the qualitative and quantitative analysis of chemical antidegradants in rubber. The isocratic LC method for five PPDs is introduced by ASTM D5666. In this method, the five PPDs are divided into three groups and determined by three different methods (Table 1, ASTM Method D5666-95, 2004).

Agilent 1200 RRLC systems use conventional or sub-two-micron columns, in various lengths up to 300 mm, and can typically provide ultra-fast separations with the same or better resolution as the original method. This application will compare the retention capability and peak shape of the two different stationary phases and will focus on showing the separation of five PPDs in one run, within five minutes, using the 1200 RRLC system with Agilent RRHT reversed phase columns.

Table 1. Chemical Information of Five PPDs

Trade Name	CAS Number	Chemical Structure and Chemical Name
77PD	3081-14-9	N,N'-bis-(1,4-dimethylpentyl)-*p*-phenylenediamine 
DTPD	27417-40-9	N,N'-ditolyl-*p*-phenylenediamine 
IPPD	101-72-4	N-isopropyl-N'-phenyl-*p*-phenylenediamine 
PPD	106-50-3	*p*-phenylenediamine 
6PPD	793-24-8	N-(1,3 dimethylbutyl)-N'-phenyl-*p*-phenylenediamine 

Experimental

System

Agilent 1200 Series Rapid Resolution LC, consisting of:
G1379B micro vacuum degasser
G1312B binary pump SL
G1367C high-performance autosampler SL
G1316B thermostatted column compartment SL
G1315C UV/Vis diode array detector SL with 3-mm, 2-µL flow cell
ChemStation 32-bit version B.02.01-SR1

Columns

Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 100 mm, 3.5 µm
Agilent ZORBAX Eclipse XDB-C8, 4.6 mm × 50 mm, 1.8 µm

Mobile Phase Conditions

A: Water with 0.1 g/L ethanolamine
B: Acetonitrile (ACN) with 0.1 g/L ethanolamine

Samples

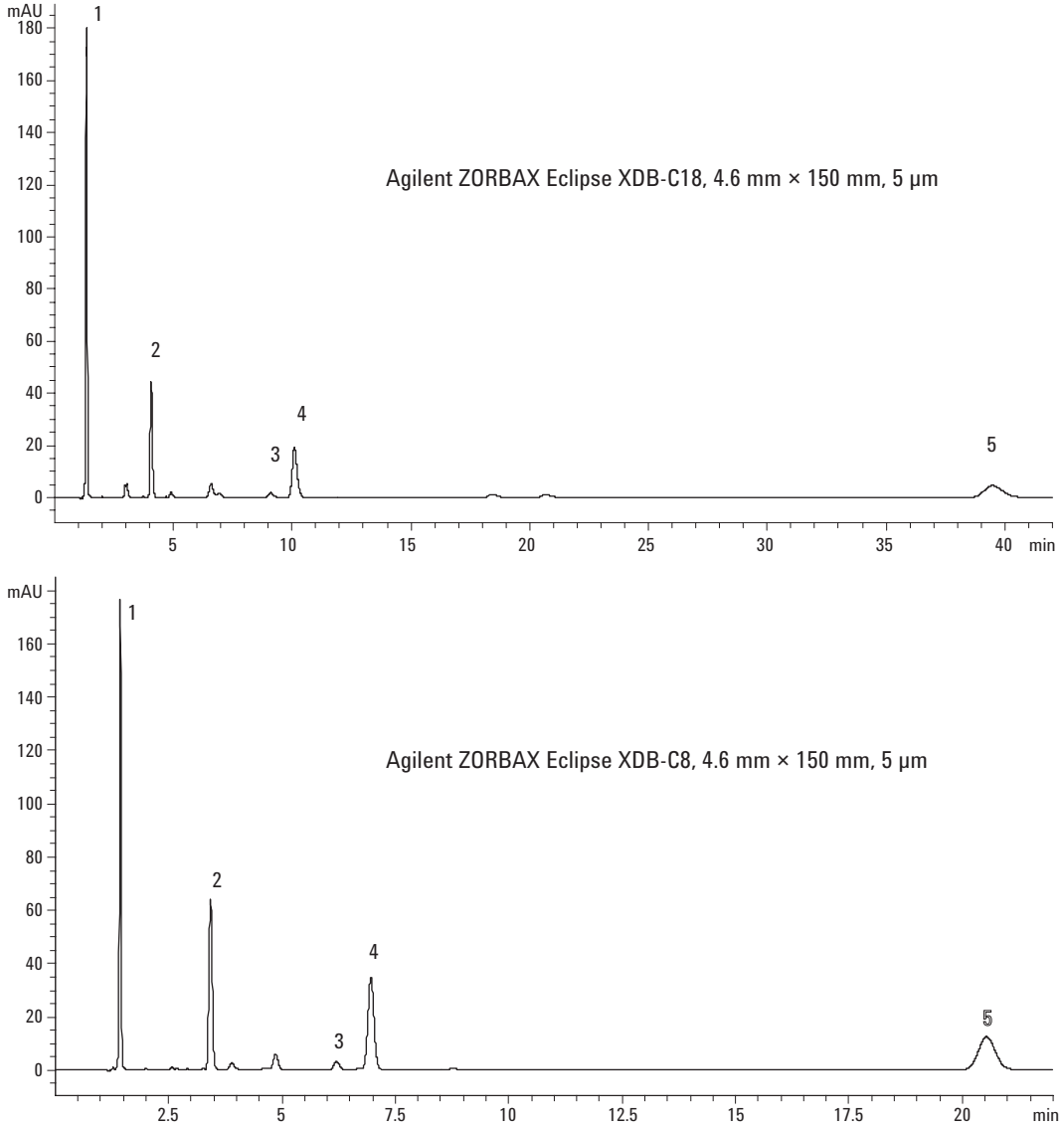
Mixture of 77PD, IPPD, PPD, DTPD, and 6PPD, all 50 µg/mL in acetonitrile. 77PD, IPPD, and PPD were standards from Sigma-Aldrich (St. Louis, Missouri, USA). DTPD and 6PPD were provided by a customer.

Results and Discussion

Selection of Stationary Phase for the Separation of Five PPDs

ASTM D5666-95 recommends a 10- to 15-cm long column packed with C18 grafted silica and 3- to 5-µm particle sizes. In our investigation, however, we observed that the retention characteristics of

ZORBAX Eclipse XDB-C18 were so strong that the total analysis time would be about 40 minutes or more. ZORBAX Eclipse XDB-C8 columns were found to have adequate resolution, and the retention time was only about half of the C18 column. Therefore, we chose the C8 column for further method development. The separations are shown in Figure 1.



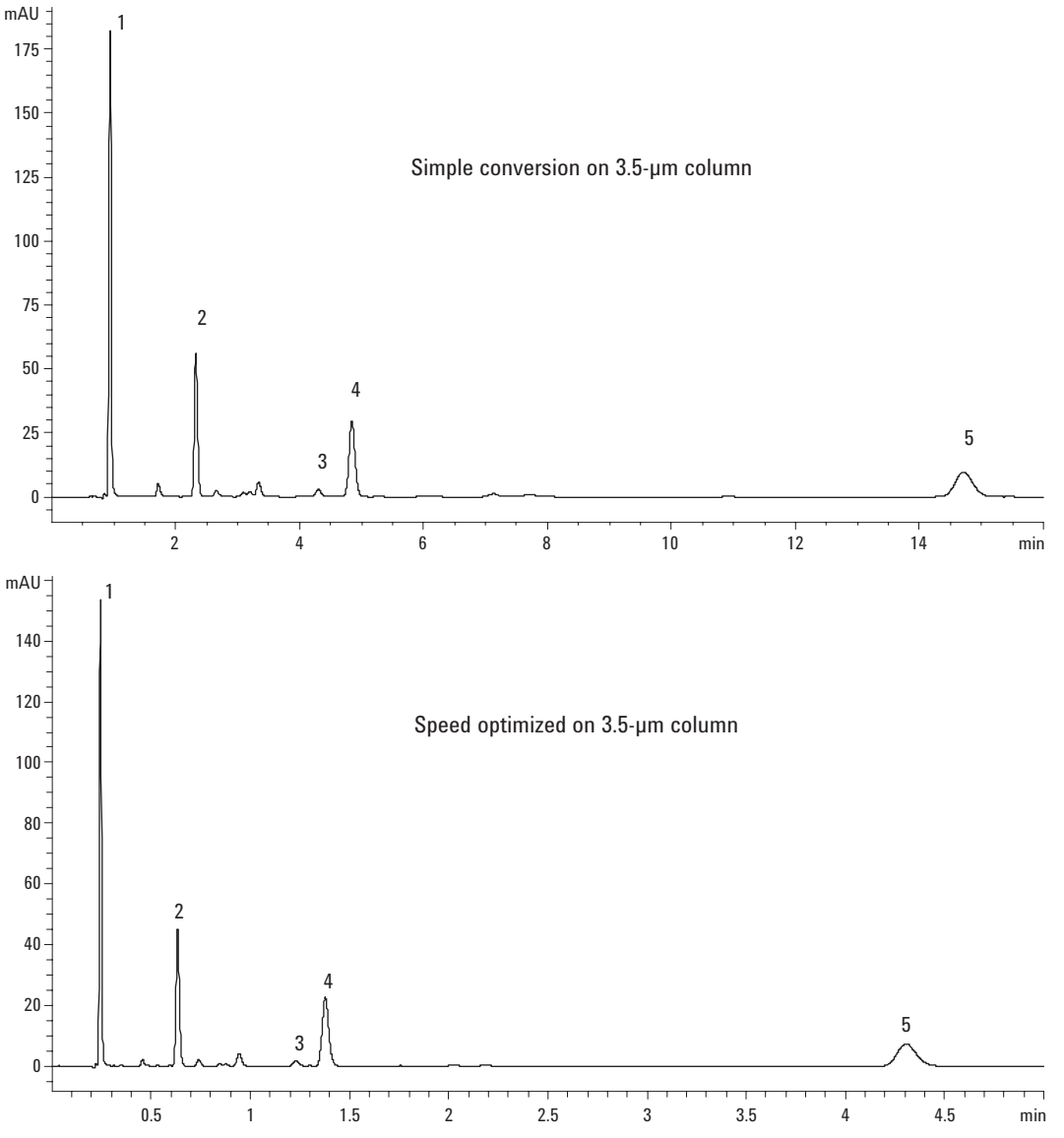
Compound	Conditions		
1 PPD	Mobile phase:	A: water with 0.1 g/L ethanolamine B: ACN with 0.1 g/L ethanolamine	
2 IPPD	Flow rate:	1.0 mL/min	
3 DTPD	Wavelength:	260 nm	
4 6PPD	Injection volume:	10 µL	
5 77PD	Column temperature:	35 °C	
	Sample:	Mixture, 50 µg/mL in ACN	
	Isocratic composition:	30% A, 70% B (v/v)	
	Column size:	4.6 mm × 150 mm, 5 µm	
	Stationary phase:	Agilent ZORBAX Eclipse XDB-C18	Agilent ZORBAX Eclipse XDB-C8
	Analysis time:	42 min	22.5 min

Figure 1. Column stationary phase comparison for five PPDs.

Fast Method Developed Based on New 1200 RRLC

The popular desire of chromatographers is to decrease the analysis time and increase the daily throughput with the same or similar resolution. Nowadays, the Agilent 1200 RRLC system with higher pressure capability and a higher temperature range can provide excellent chromatographic resolution with much shorter run times. Furthermore, a constant concern is how to quickly and easily transfer conventional methods to fast methods. Agilent provides two versions of method translators: one is a Microsoft.net version requiring that Net-Framework 2.0 be resident on the computer, and the other is a Microsoft Excel version requiring that Excel be resident on the PC. When the initial method is an isocratic method, the method translator can provide two modes of faster methods. One is simple conversion, with the scaled flow rate according to the column diameter; the other is speed optimized conversion, with the maximum flow rate and pressure. In gradient mode, an additional option is a resolution optimized conversion.

This application uses a set of Agilent ZORBAX Eclipse XDB-C8 columns, including 4.6 mm × 150 mm (5 µm), 4.6 mm × 100 mm (3.5 µm), and 4.6 mm × 50 mm (1.8 µm). The method translator is used to transfer the initial method on a 5-µm column to two fast methods on 3.5-µm and 1.8-µm columns, respectively. The resulting separation of five PPDs is depicted in Figure 2.



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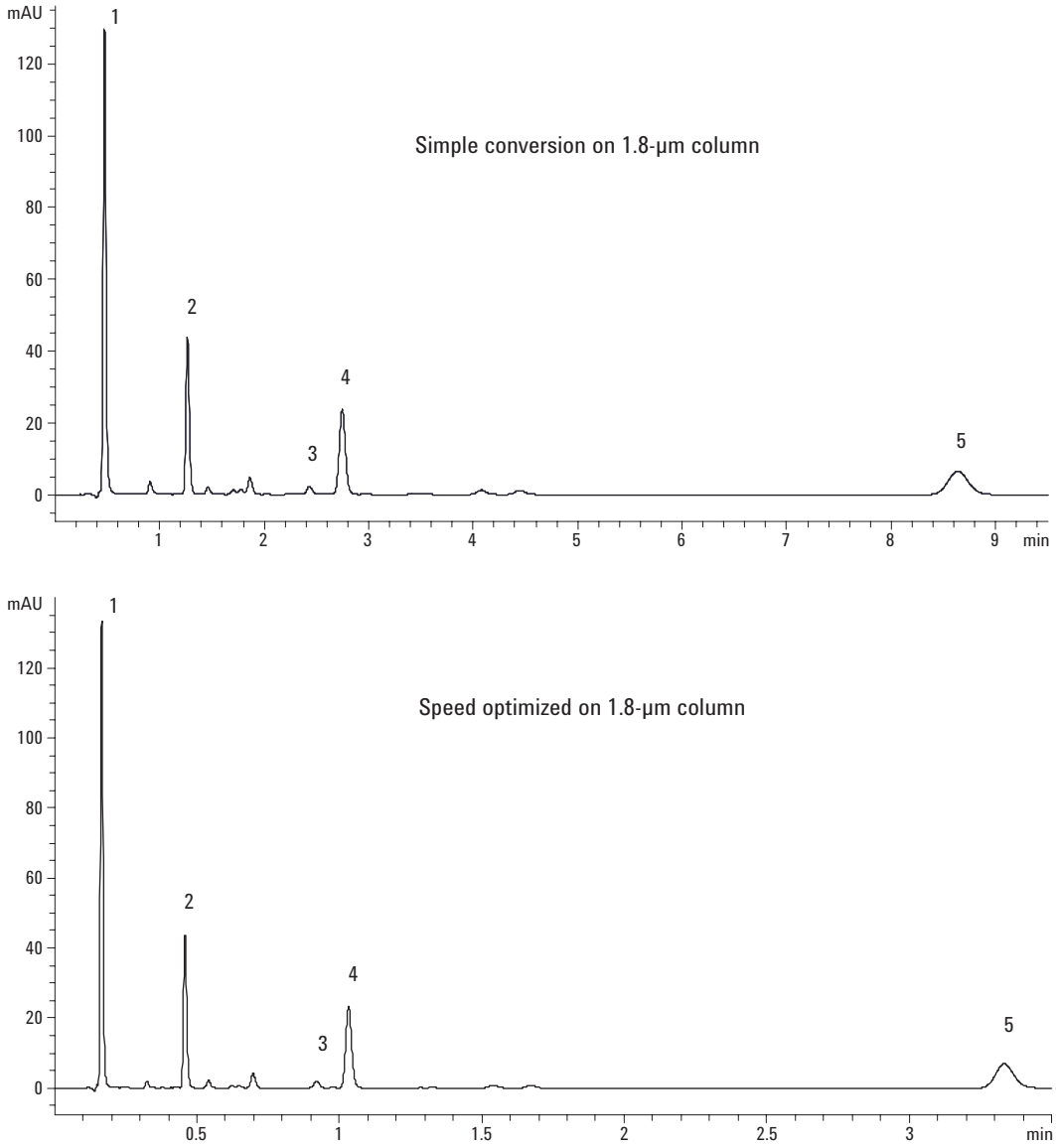

Compound	Conditions				
1 PPD	Stationary phase:	Agilent ZORBAX Eclipse XDB-C8			
2 IPPD	Mobile phase:	A: water with 0.1 g/L ethanolamine			
3 DTPD		B: ACN with 0.1 g/L ethanolamine			
4 6PPD	Isocratic composition:	30% A, 70% B (v/v)			
5 77PD	Column temperature:	35 °C			
	Wavelength:	260 nm			
	Column size	4.6 mm × 100 mm, 3.5 µm		4.6 mm × 50 mm, 1.8 µm	
	Conversion mode:	Simple	Speed optimized	Simple	Speed optimized
	Flow rate:	1.0 mL/min	4.0 mL/min	1.0 mL/min	3.0 mL/min
	Injection volume:	6.7 µL	6.7 µL	3.3 µL	3.3 µL
	Analysis time:	15 min	5 min	10 min	3.5 min

Figure 2. Separation of five PPDs on a smaller particle size column using the transferred methods.

Conclusions

As a powerful approach, liquid chromatography with ultraviolet\visible detection is often used to determine the chemical antidegradants in rubber. Agilent 1200 RRLC systems typically provide the customer with a rapid separation having the same or similar resolution. The method translator can convert any isocratic or gradient method to fast method according to customer requirements. This application details the selection of stationary phases for the separation of five PPDs, separates five PPDs with the RRLC system in one run, and applies the method translator to develop fast methods based on smaller particle size columns. With 1.8-µm column, the total analysis time of five PPDs in one run is about 6.4 times faster than the original 5-µm column method.

References

1. ASTM D5666-95 (Reapproved 2004) "Standard Test Method for Rubber Chemical Antidegradants - Purity of *p*-Phenylenediamine (PPD) Antidegradants by High Performance Liquid Chromatography"
2. Michael Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations," Agilent Technologies publication 5989-5177EN, 2006.

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Improving Productivity and Extending Column Life with Backflush

Application Brief

Chin-Kai Meng

All Industries

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, µECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

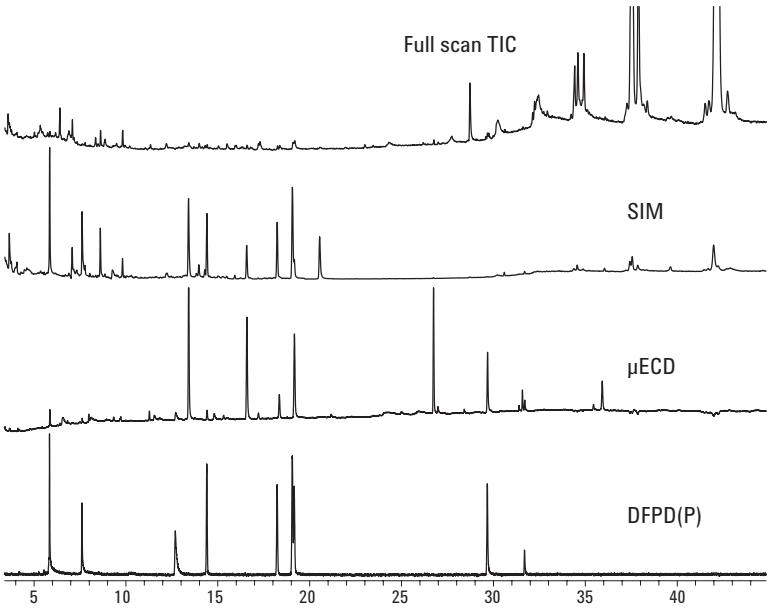

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

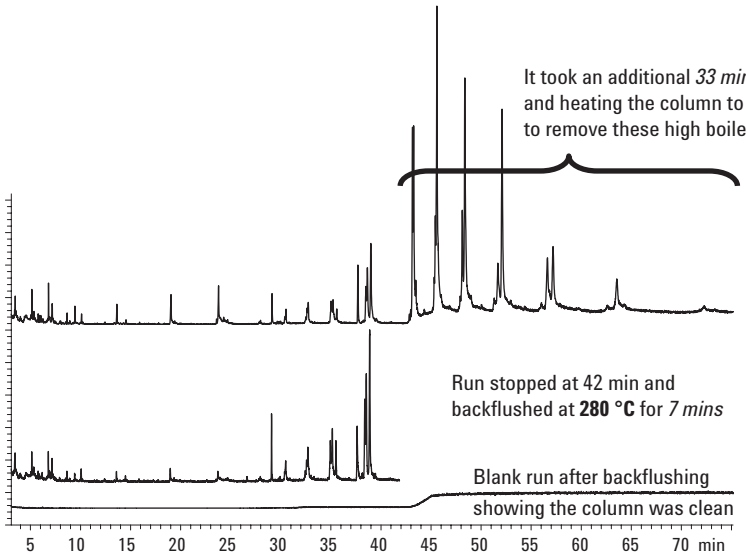

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

References

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

Acknowledgement

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

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Comprehensive Flow Modulated Two-Dimensional Gas Chromatography System

Application Brief

Roger L. Firor

Two-dimensional GC (GC x GC), a relatively new gas chromatographic technique, is being recognized for its powerful separation capabilities for the analysis of complex mixtures. The methodology involves the use of two capillary columns of usually very different polarities installed in series. Between the two columns a device known simply as a flow modulator is installed and interfaced to an auxillary programmable control module (PCM) on an Agilent 7890A gas chromatograph through a three-way solenoid valve. In the flow modulator, analyte bands from the first column are collected in a fixed volume channel and successively injected very quickly into the short second column in very narrow bands.[1] Any separation that occurs on the first column is preserved during transfer to the second column. In summary, GC x GC can greatly increase peak resolution and peak capacity.

The unique flow modulator used in this system is based on Agilent's Capillary Flow Technology hardware and does not require the use of cryogenics for focusing. The modulator consists of a planar structure where flow splitters and collector channel are all incorporated internally to the device. All external connections are made through Agilent CPM fittings (ultimate union technology) incorporated into the plate for zero unswept volumes and leak proof seals. A three-way solenoid valve is installed on top of the GC oven and is interfaced to a PCM module. Experimental conditions used are shown below.

Experimental

GC:	Agilent 7890A, FID at 200 Hz data collection rate, split/splitless inlet
Carrier:	Hydrogen
Column 1:	30 m x 0.25 mm x 0.25 μm HP-5ms, 19091S-433
Column 1 Pressure:	21.5 psig at 50 °C, constant flow mode
Column 2:	5 m x 0.25 mm x 0.15 μm HP-INNOWax
Column 2 Flow:	20 mL/min, constant flow mode
Oven Program:	50 °C (1.0 min) to 260 °C (4 min) @ 8 °C/min.
Modulator Period:	1.4 seconds collect, 0.12 seconds flush typical

GC x GC analysis software: GC Image[2]

An illustration of the modulator is given in Figure 1. The precisely timed and synchronized periodic switching between collect and flush states directs sample pulses continuously to the second column for additional separation during the

Highlights

- Flow modulation offers a viable alternative to thermal modulation without the burdens imposed by cryo requirements for comprehensive GC x GC.
- Modulation, timing (collect and inject), and synchronization are all integrated into the 7890A GC system for easy setup and operation.
- Agilent's fifth-generation electronic pneumatic control with setting to three decimal points combined with Capillary Flow Technology hardware forms the basis for an easy-to-use GC x GC system.

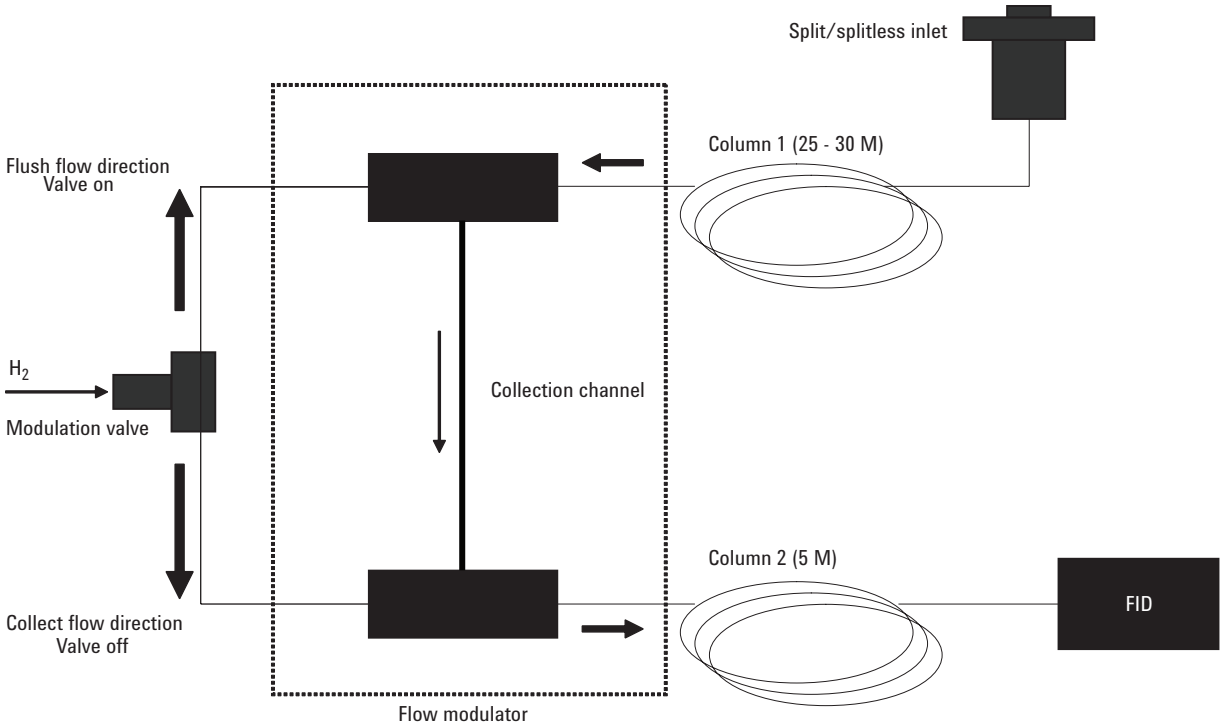

Figure 1. Flow modulator design – differential flow system.

length of the chromatographic run.

Discussion

Figures 2a and 2b show unmodulated and modulated peaks, respectively, of a pure analyte. In this example, n-butylbenzene is shown with approximately three modulations across the first-column peak. The areas of the modulated peaks should ideally equal the area of the un-modulated peak. In other words, no material should be lost in the transfer to the second column. Area agreement was within 3% for this test. Peak widths at half height for modulated butylbenzene are approximately 100 ms. Very narrow peaks as required for the technique are

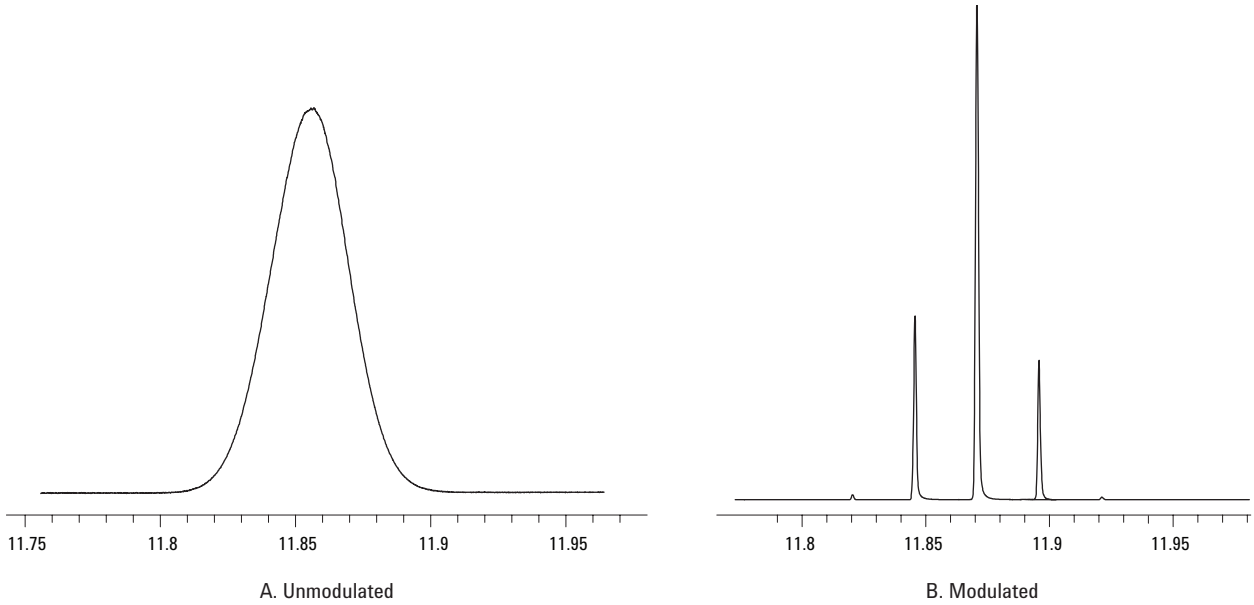

Figure 2. Modulated and unmodulated n-butylbenzene (not to same scale).

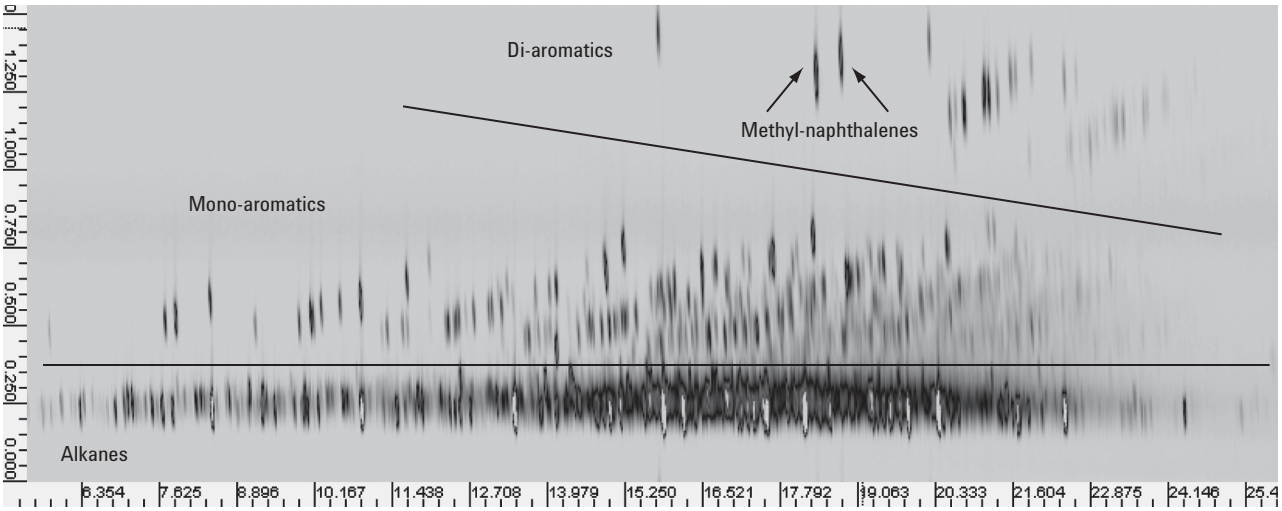

Figure 3. A 2D image of No. 2 kerosene.

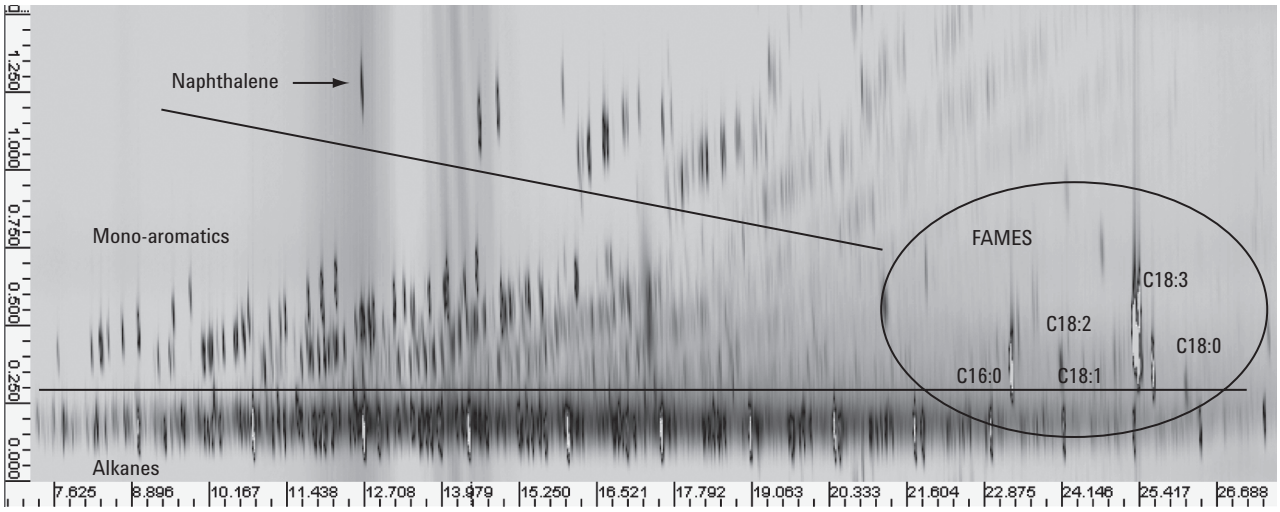

Figure 4. A B20 soy-based biodiesel (20% methyl ester, 80% diesel).

seen that approach the peak widths obtained by thermal modulation systems. Flow modulation has the distinct advantage of not requiring cryo fluid for focusing.

Use of a nonpolar column followed by a polar column produces hydrocarbon-type retention in the following order: alkanes, cyclic alkanes, olefins, single-ring aromatics, and multi-ring aromatics. An example of a 2D image of No. 2 kerosene is shown in Figure 3. Chemical classes are clearly discernable, with good resolution seen for the aromatics. Another example, B20 soy-based biodiesel (20% methyl ester, 80% diesel) is shown in Figure 4. Here the C16 and C18 fatty acid methyl esters are indicated. Data processing for all samples was performed using GC Image[2].

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

Application

Hydrocarbon Processing

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Abstract

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

Introduction

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

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

Experimental

Injection Device

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

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

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

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

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

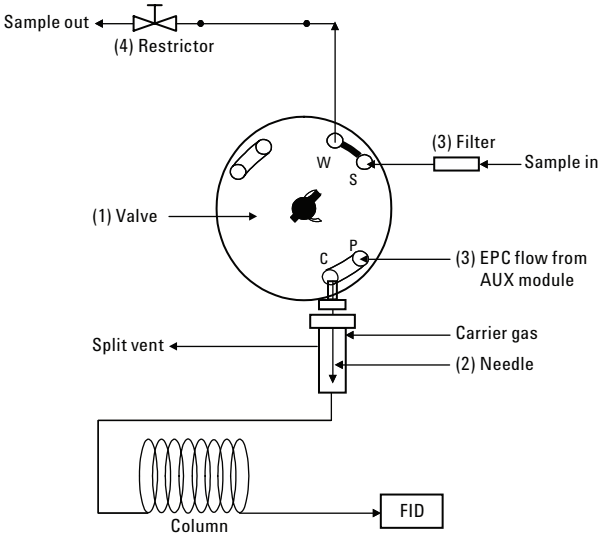


Figure 1. Flow diagram of the HPLI device.

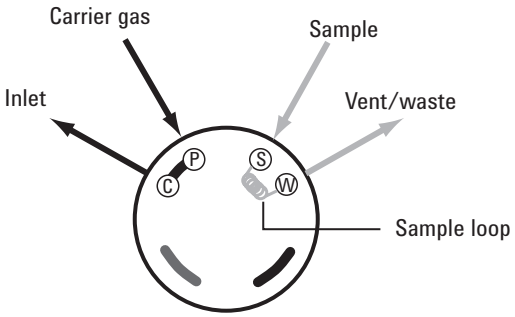
Samples for System Evaluation

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

Operating Process

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

Load



Inject

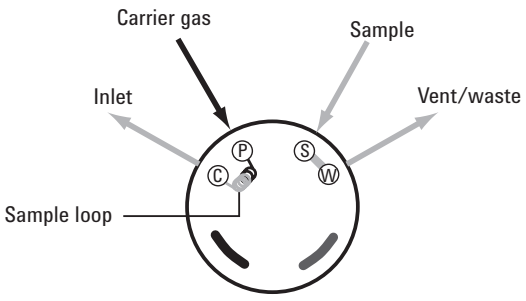


Figure 2. Pneumatic and sample paths in load and inject positions.

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

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

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

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

Table 1. Instrumental Conditions

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

Table 2. Columns and Parameters

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

Results and Discussion

Check for Carryover

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

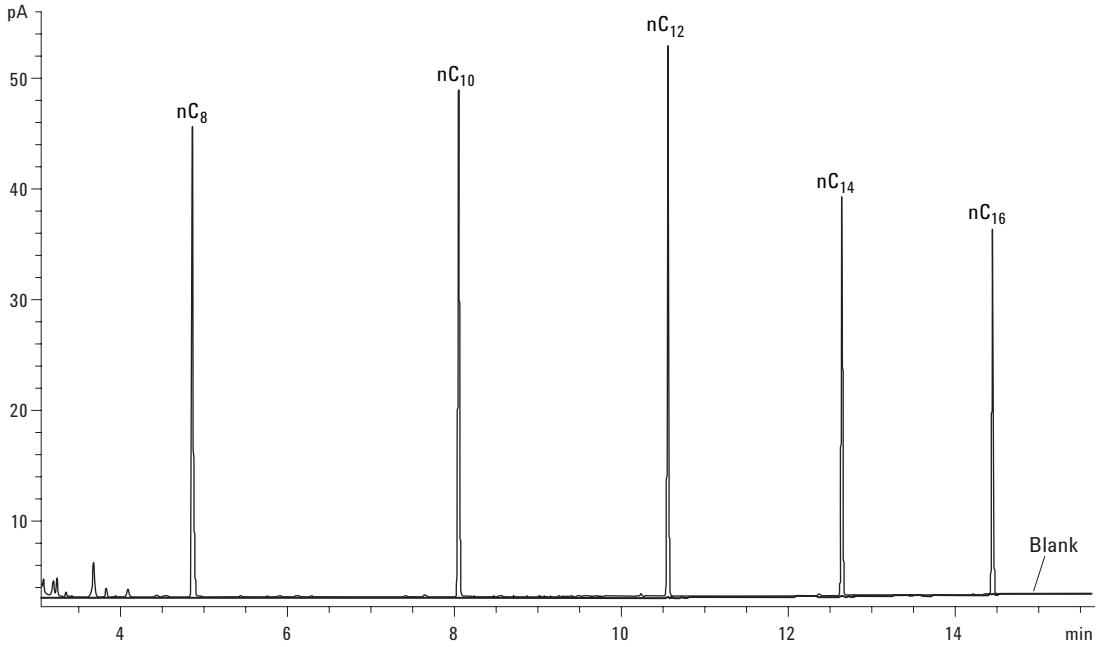


Figure 4. Overlay of standard versus blank (100 ppm each in cyclohexane).

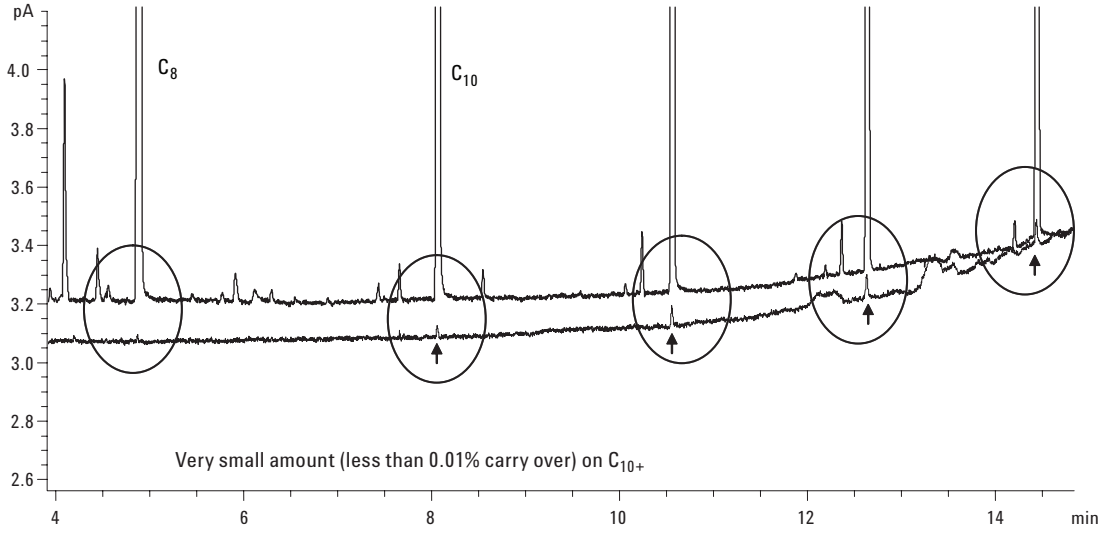


Figure 5. Carryover less than 0.01% on C_{10+}.

Sample Analysis

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

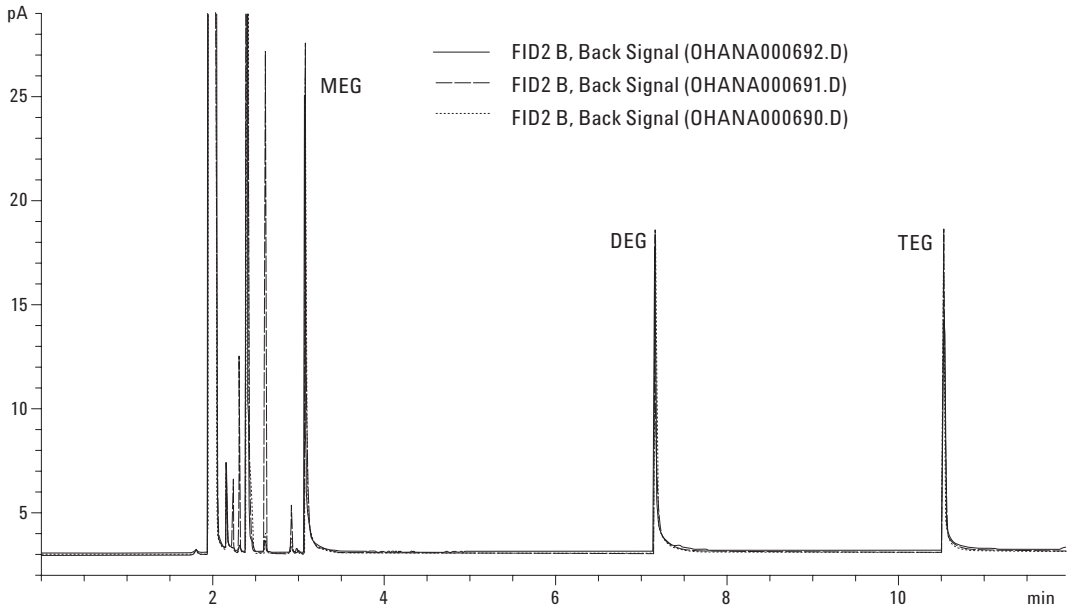


Figure 6. Triplicate run of 100 ppm each of MEG, DEG, and TEG in IPA.

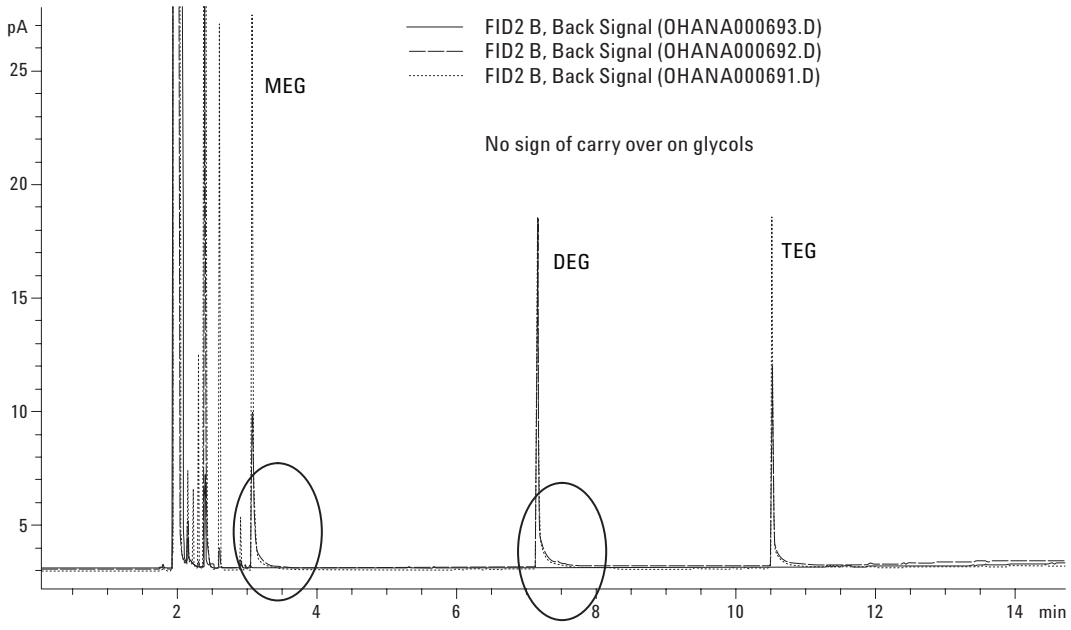


Figure 7. Glycols versus blank. Two standard duplicates, blank run immediately after injection of standard.

A. Liquefied Natural Gas

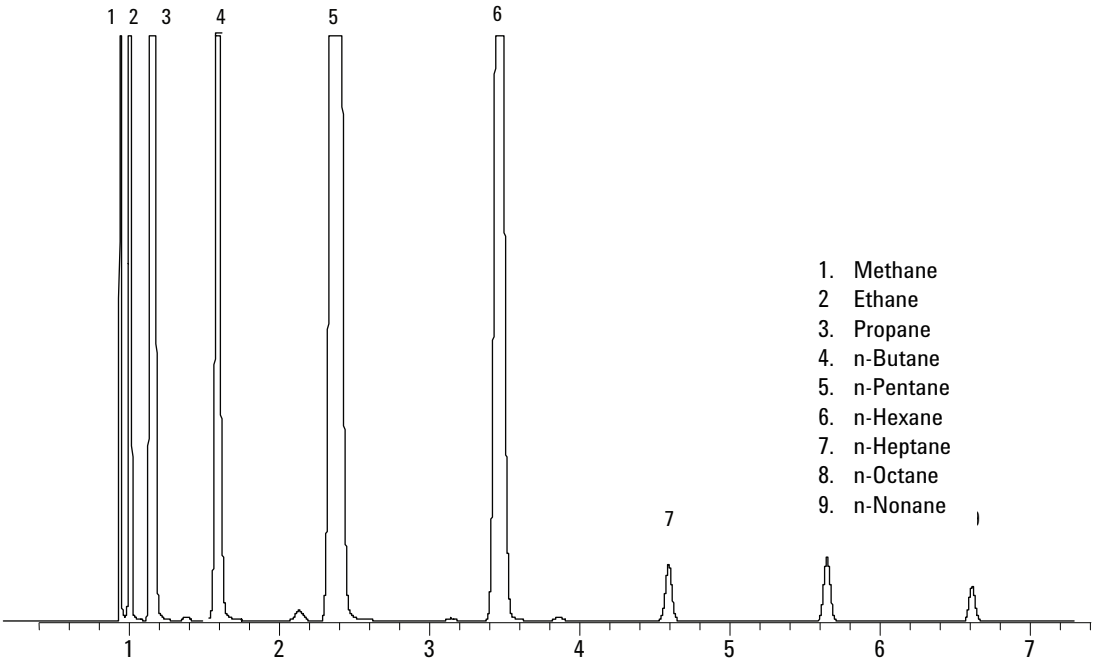


Figure 8. Chromatogram of liquefied natual gas (calibration standard).

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

B. Liquefied Ethylene

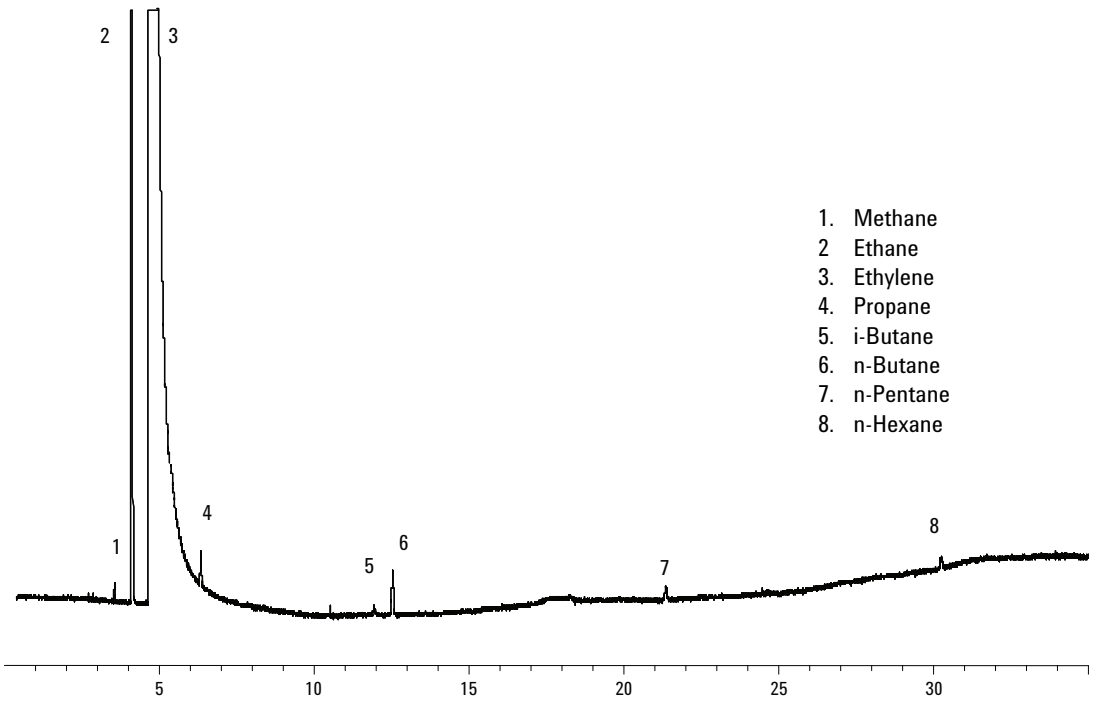


Figure 9. Chromatogram of liquefied ethylene.

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

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

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

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

C. Pressurized Propylene

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

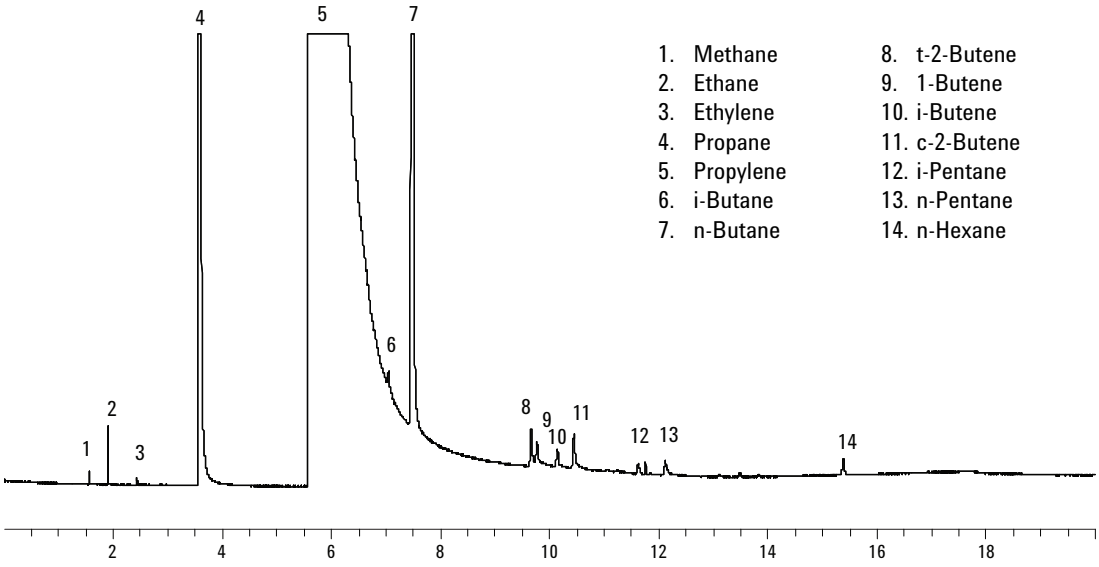


Figure 10. Chromatogram of pressurized propylene.

D. Pressurized 1,3-Butadiene

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

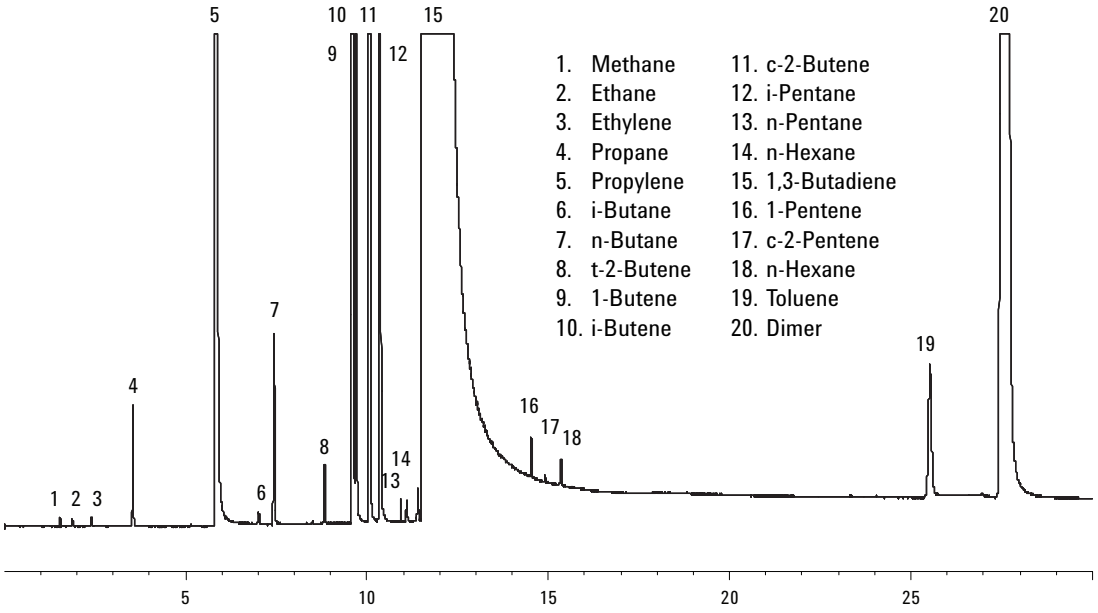


Figure 11. Chromatogram of pressurized 1,3-butadiene.

E. Pressurized Propane + n-Butane

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

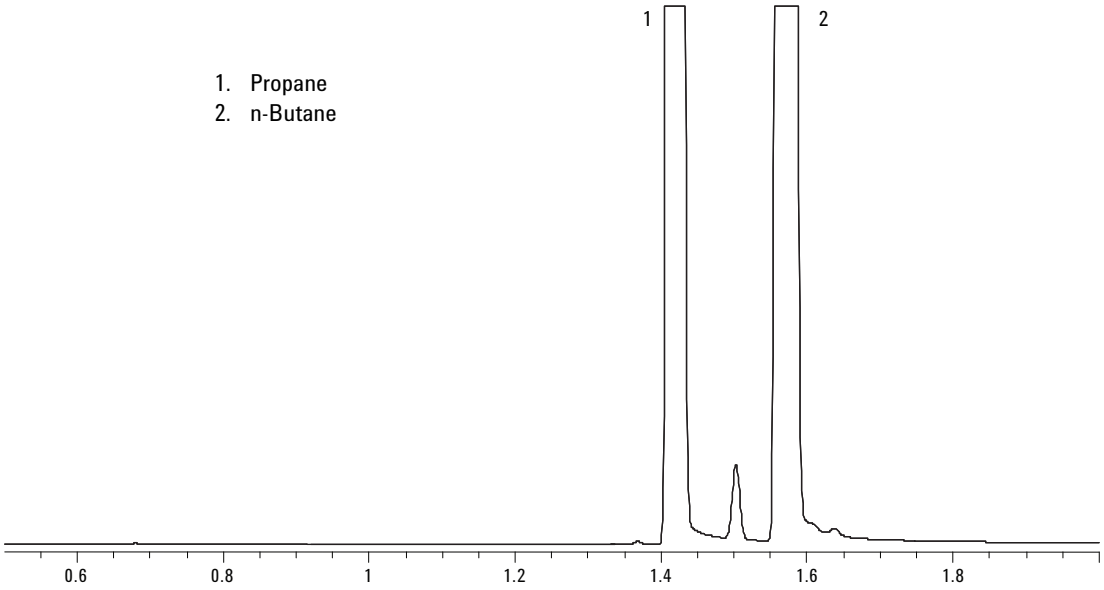


Figure 12. Chromatogram of pressurized propane + n-butane.

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

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

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

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

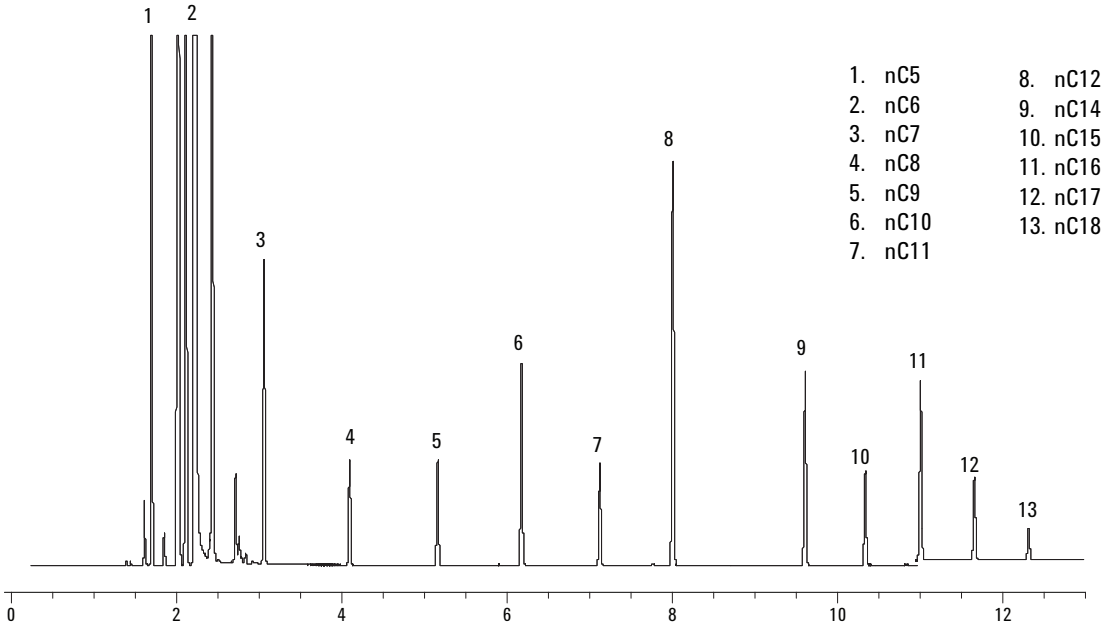


Figure 13. Chromatogram of n-hexane + 1.0% BP standard.

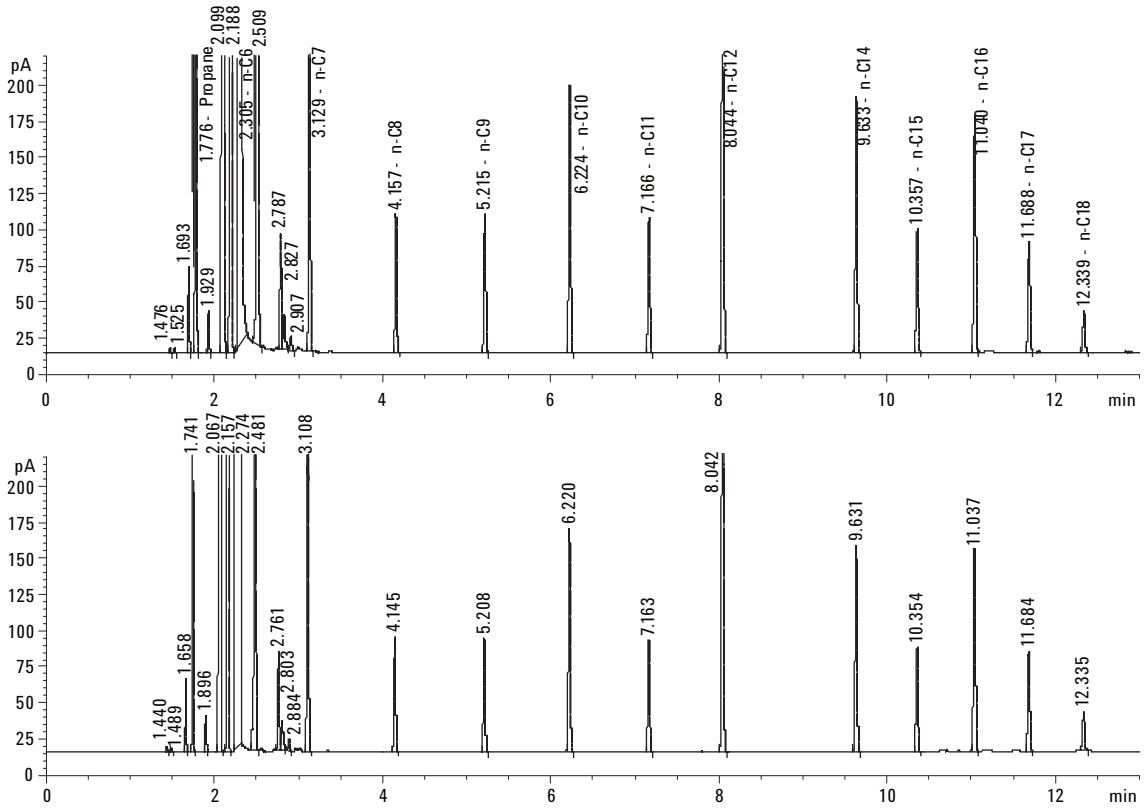


Figure 14. Chromatograms of n-hexane + 1.0% BP standard. Top: HPLI. Bottom: ALS (syringe).

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

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

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

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

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

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

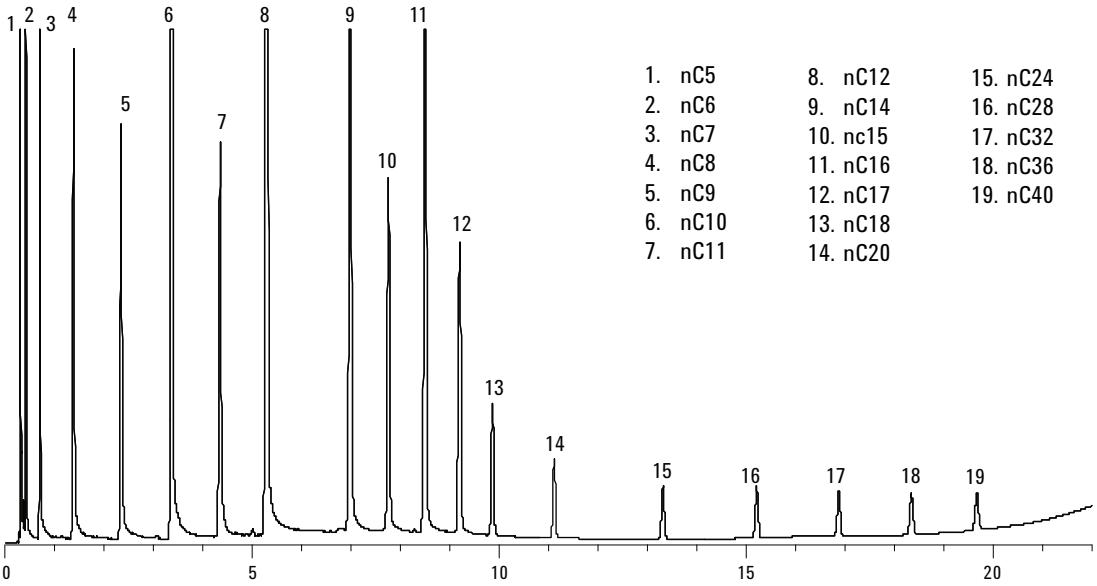


Figure 15. Chromatogram of nC5-nC40 (D2887 BP standard diluted by CS_2).

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

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

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

Conclusions

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

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Acknowledgement

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

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Agilent Technologies

Simultaneous Analysis of Trace Oxygenates and Hydrocarbons in Ethylene Feedstocks Using Agilent 7890A GC Capillary Flow Technology

Application Brief

James McCurry

The presence of trace hydrocarbons in ethylene can have damaging effects on both the process catalysts and the final polymer products. Test methods such as ASTM D6159 are used to ensure the quality of these feedstocks [1]. However, the analysis of other key contaminants, such as oxygenates, requires GC methods that run on separate instruments. This can be time consuming and expensive for the process analysis lab.

The Agilent 7890A GC serves as the ideal platform when analyzing different classes of trace compounds in ethylene. Maximum productivity can be realized by:

- Using Capillary Flow Technology to perform analysis of trace oxygenates and hydrocarbons in a single run through 2-D Deans switch chromatography.
- Automating the preparation of multilevel calibration standards using the new auxiliary electronic pneumatics control (EPC) modules.
- Protecting the sensitive and expensive alumina PLOT column by preventing polar oxygenates from entering the column.

Enhancing ASTM Method D6159 with Capillary Flow Technology 2-D GC

ASTM Method D6159 uses a methyl silicon column in series with an alumina PLOT column to resolve light hydrocarbons in ethylene. Polar oxygenated compounds cannot be analyzed on this column set because methyl silicon has insufficient selectivity and the alumina column will adsorb oxygenates, resulting in column damage. Wax-type liquid phases such as HP-INNOWax can easily separate polar compounds from light hydrocarbons using 2-D GC [2]. A wax column placed before an alumina column will retain polar compounds while the light hydrocarbons elute near the void volume. Therefore, if a Deans switch is placed between the columns, the hydrocarbons can be heart-cut from the wax to the alumina columns while oxygenates are held by the wax column. The optimized thermal and pneumatic performance of the Agilent 7890A Deans switch is a result of Capillary Flow Technology. This provides the high levels of retention time precision and narrow peak shape needed for optimal heart-cutting 2-D GC (Figure 1).

Highlights

- The Agilent 7890A GC Capillary Flow Technology combined with enhanced electronic pneumatics control (EPC) provide greater productivity and flexibility in the analysis of trace contaminants in ethylene.
- Multiple auxiliary EPC channels provide the ability to automatically generate gas calibration standards for trace level impurities.
- Enhancement of ASTM D6159 method with 2-D GC Deans switching measures trace oxygenates and hydrocarbons in a single run.

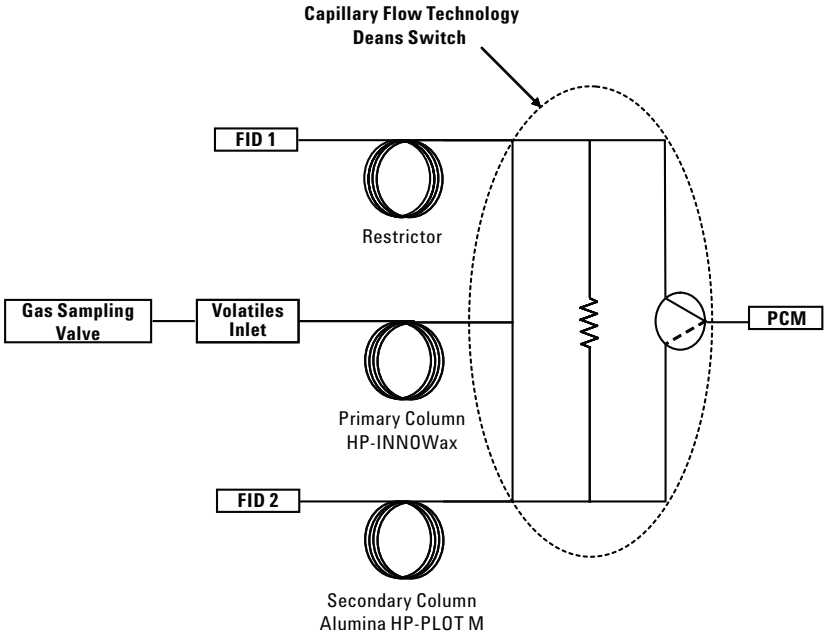

Figure 1. Configuration of Agilent 7890A for the 2-D GC analysis of trace oxygenates and hydrocarbons in ethylene.

Method Parameters for Enhanced ASTM D6159 Method

Primary column:	HP-INNOWax, 30 m × 0.32 mm id × 0.5 µm film (19091N-213)
Primary column flow:	Helium at 2.5 mL/min
Secondary column:	Alumina HP-PLOT M, 30 m × 0.53 mm id × 15 µm (19095P-M23)
Secondary column flow:	Helium at 6 mL/min
Oven temperature program:	40 °C for 6 min, 4 °C/min to 125 °C
Volatiles inlet conditions:	150 °C, 5:1 split
Sample loop:	250 µL at 65 °C
Detector temperature:	250 °C
Capillary Flow Technology: Deans switch cut time	2.3 to 4.5 min

Automating the Preparation of Trace-Level Calibration Standards

Another advantage of the Agilent 7890A GC is the expanded capabilities in EPC. These extra channels of auxiliary EPC are used with the dynamic blending system hardware to allow automated preparation of ppmV gas standards for calibration. This approach has been described for the automated preparation of trace sulfur compounds in various gas matrices [3].

Results

Figure 2 shows the 2-D GC analysis of methanol and C1 to C4 hydrocarbons in a sample of technical grade ethylene. The HP-INNOWax column first separates the polar methanol from the unresolved hydrocarbon peaks. The Deans switch transfers the hydrocarbons to the Agilent alumina HP-PLOT M column, where the C1 to C4 hydrocarbons are easily separated. This column is also shown to provide better separation of trace hydrocarbons from the large ethylene peaks, while maintaining excellent peak shape and intensity for the acetylene. The performance of this alumina column is maintained over many injections since the HP-INNOWax column prevents polar oxygenates (water, alcohols) from damaging the sensitive stationary phase. Table 1 shows very good precision using this method for a sample containing approximately 2 ppmV.

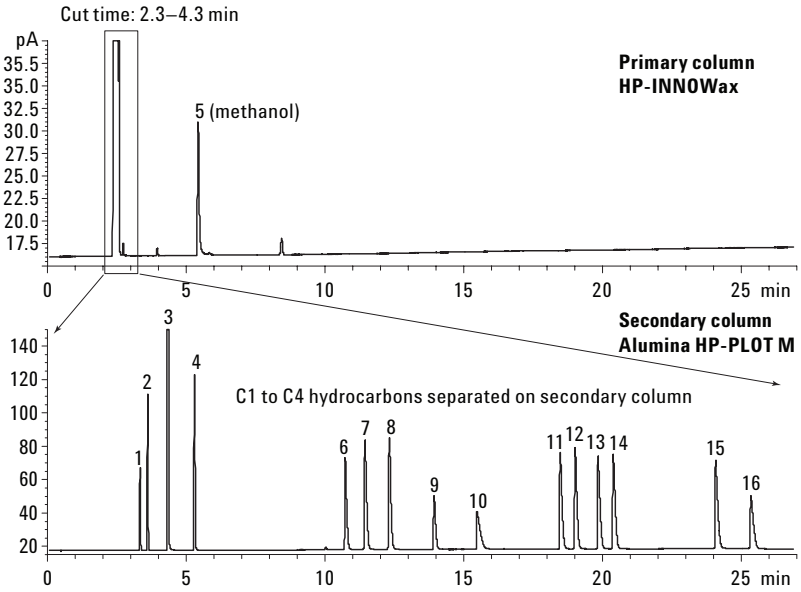

Figure 2. Capillary Flow Technology Deans switch used to separate 100 ppmV oxygenate and hydrocarbon impurities in ethylene.

Table 1. Method Precision for 2-D GC Analysis of Ethylene Impurities

Peak No.	Name	Avg. (ppmV)*	Std Dev*	%RSD*
1	Methane	2.1	0.011	0.5
2	Ethane	21.5	0.049	0.2
3	Ethylene	Balance	Balance	Balance
4	Propane	2.1	0.062	3.0
5	Methanol	2.1	0.081	3.8
6	Propylene	2.1	0.023	1.1
7	Isobutane	2.1	0.015	0.7
8	n-Butane	2.0	0.011	0.5
9	Propadiene	2.1	0.025	1.2
10	Acetylene	1.9	0.036	1.9
11	Tran-2-butene	2.1	0.011	0.5
12	1-Butene	2.0	0.013	0.7
13	Isobutylene	2.1	0.016	0.8
14	cis-2-butene	2.1	0.017	0.8
15	1,3-Butadiene	2.1	0.018	0.9
16	Methylacetylene	2.0	0.015	0.7

*Sample run 20 times

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A Column-Flow Independent Configuration for QuickSwap

Application

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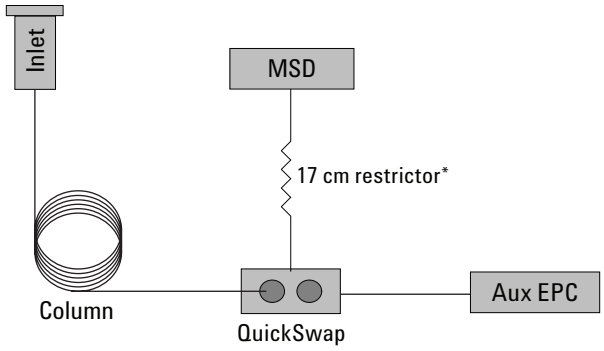
Abstract

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

Introduction

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

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



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

Figure 1. General concept of QuickSwap.

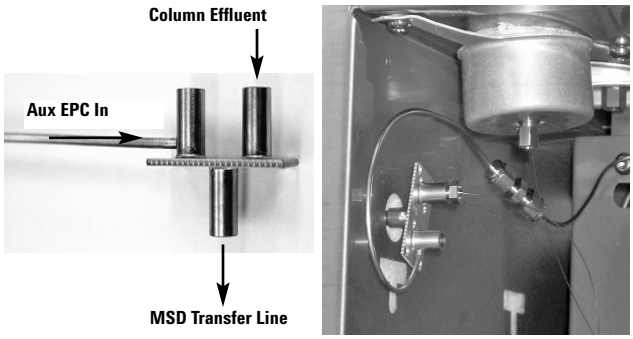


Figure 2. QuickSwap is pictured on the left showing permanent (Aux EPC In) and temporary connections. A picture of a normal QuickSwap installation is shown on the right.

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

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

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

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

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

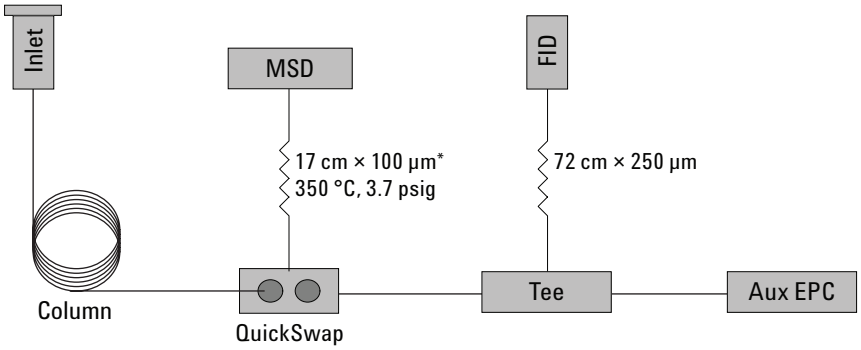
In general, when flow to the MSD changes,

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

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

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

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



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

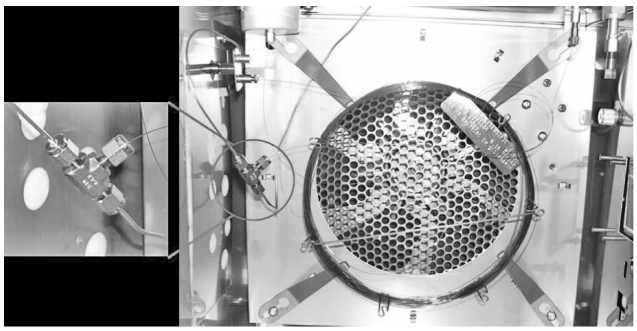
Figure 3. Flexible configuration includes addition of a split vent path on the Aux EPC line leading to QuickSwap.

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

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

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

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



Experimental

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

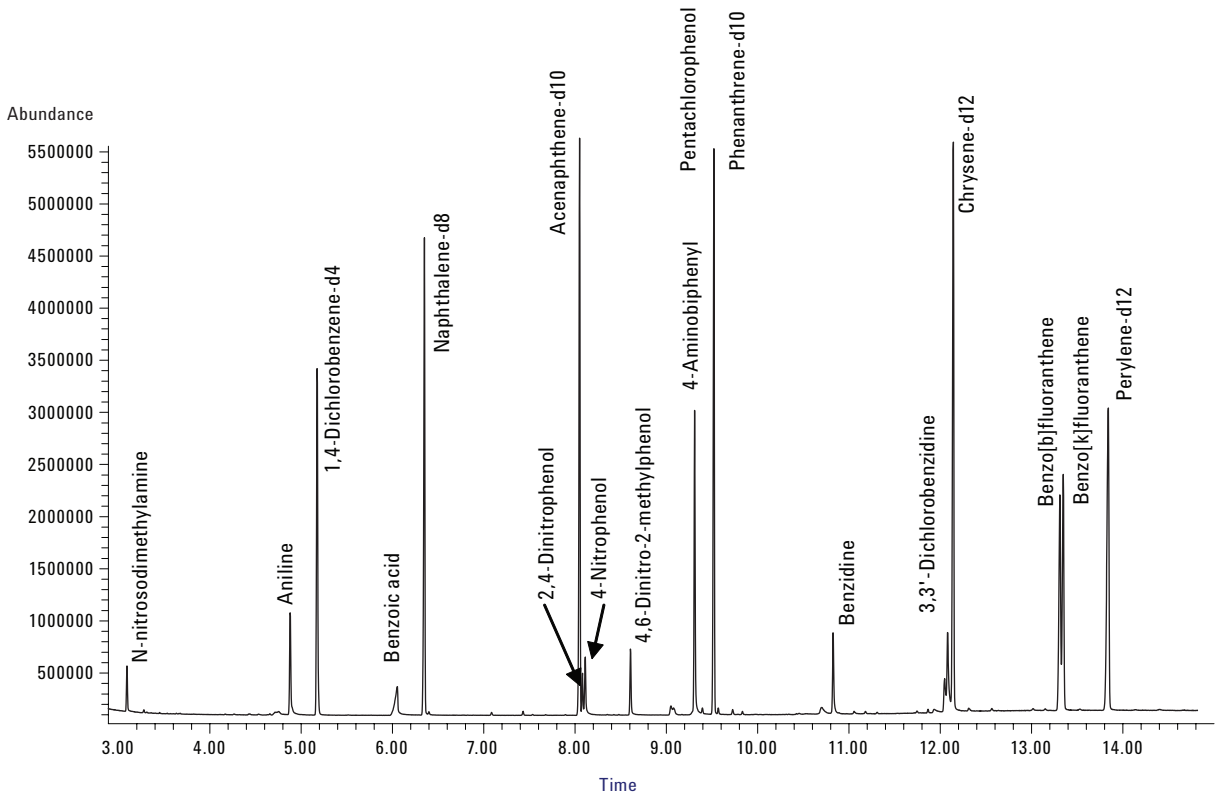


Figure 4. Reference chromatogram for Fast 8270 method.

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

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

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

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

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

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

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

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

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

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

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

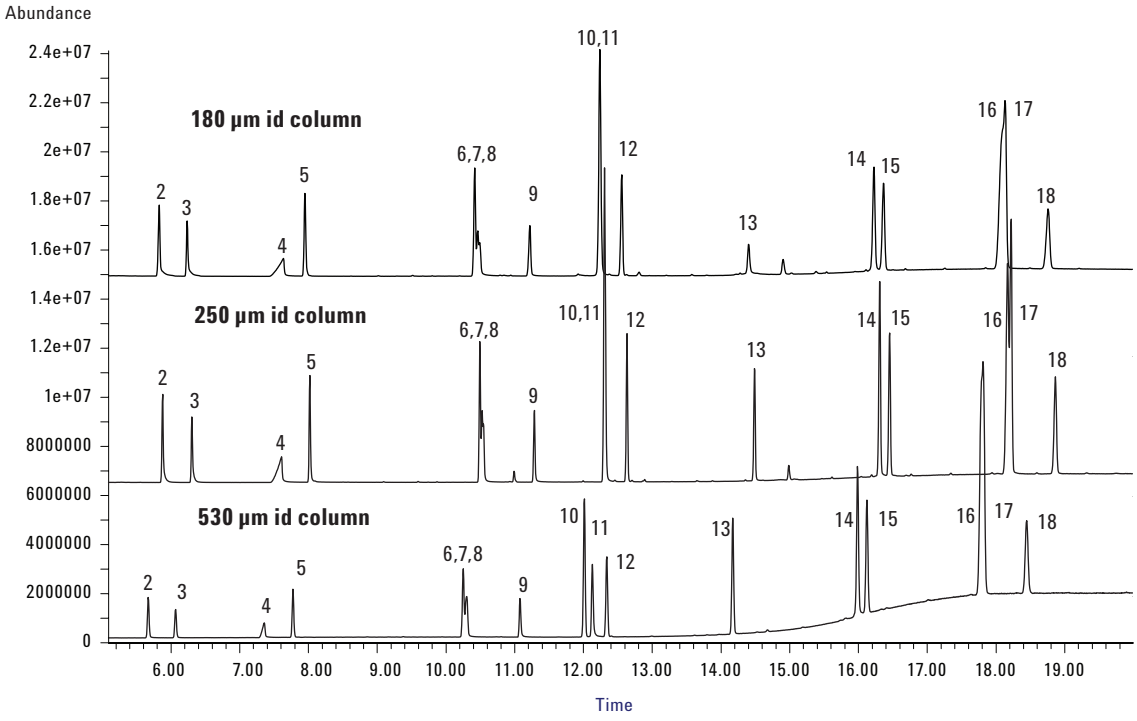


Figure 5. Constant pressure mode analysis with three different column dimensions; 0.5-µL splitless injections of 80-ppm semivolatiles test sample, with flow conditions from Table 2.

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

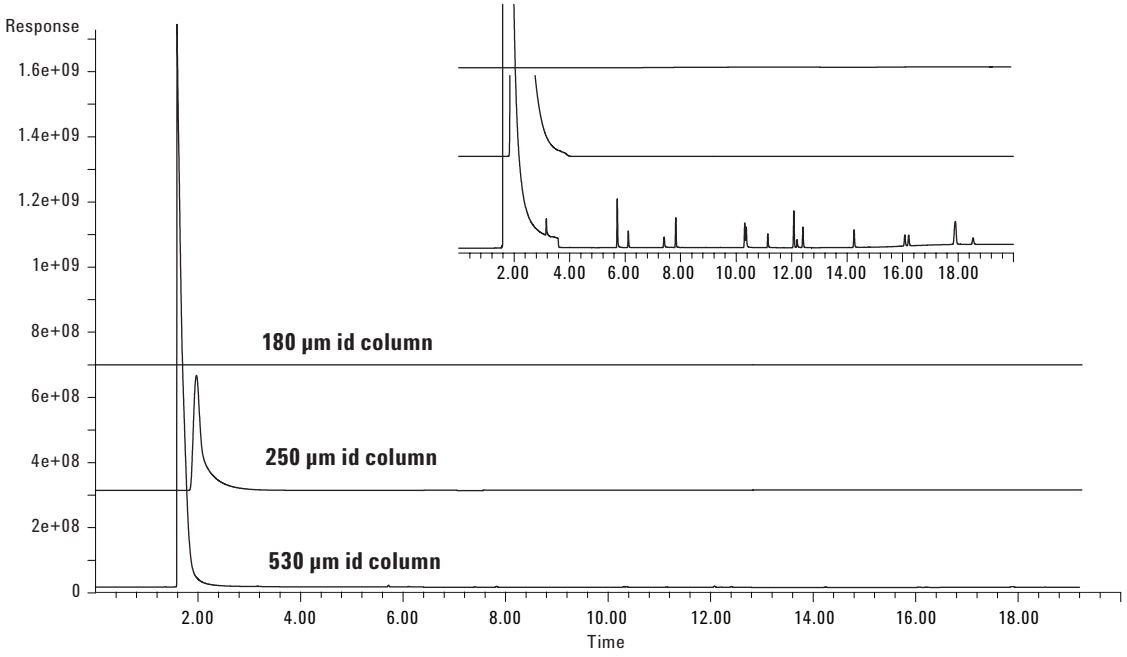


Figure 6. FID signal of vent stream shows what is vented when column flow exceeds flow to MSD.

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

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

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

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

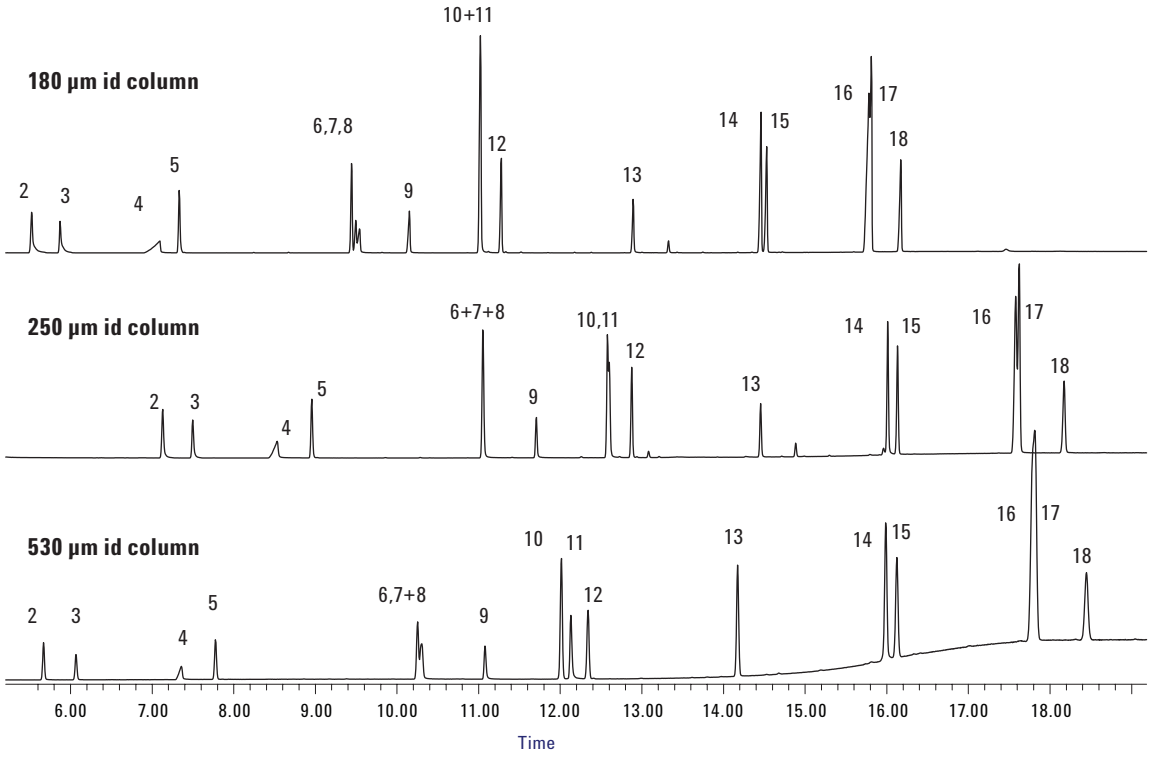


Figure 7. TIC chromatograms for the three columns under optimal constant flow mode conditions.

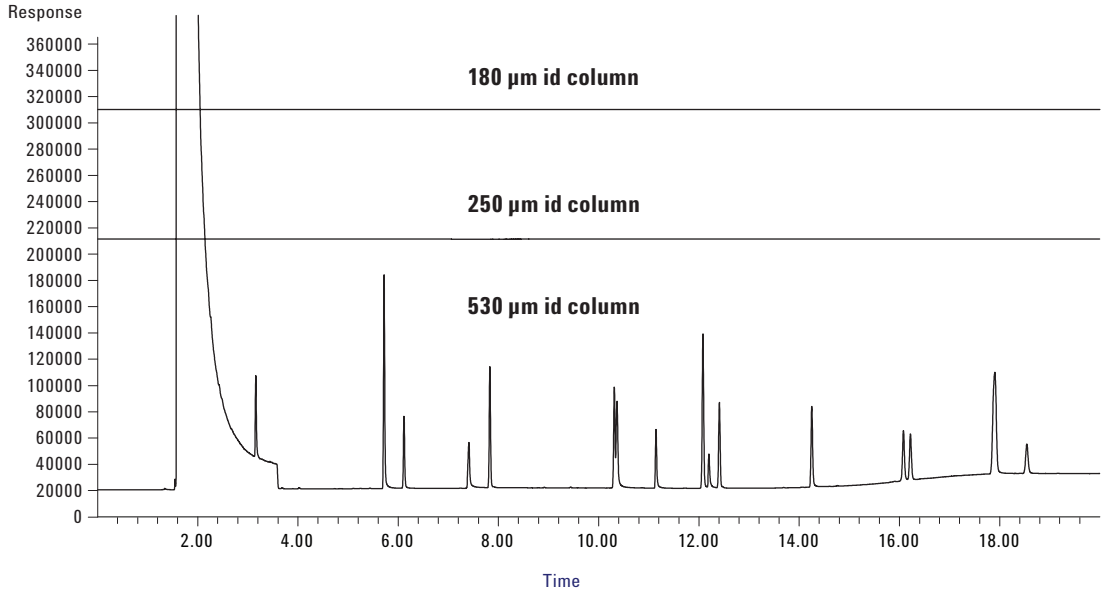


Figure 8. FID vent signal for three columns under optimal flow conditions. Only the 530-µm id column has a flow that exceeds the 1 mL/min flow to the MSD.

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

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

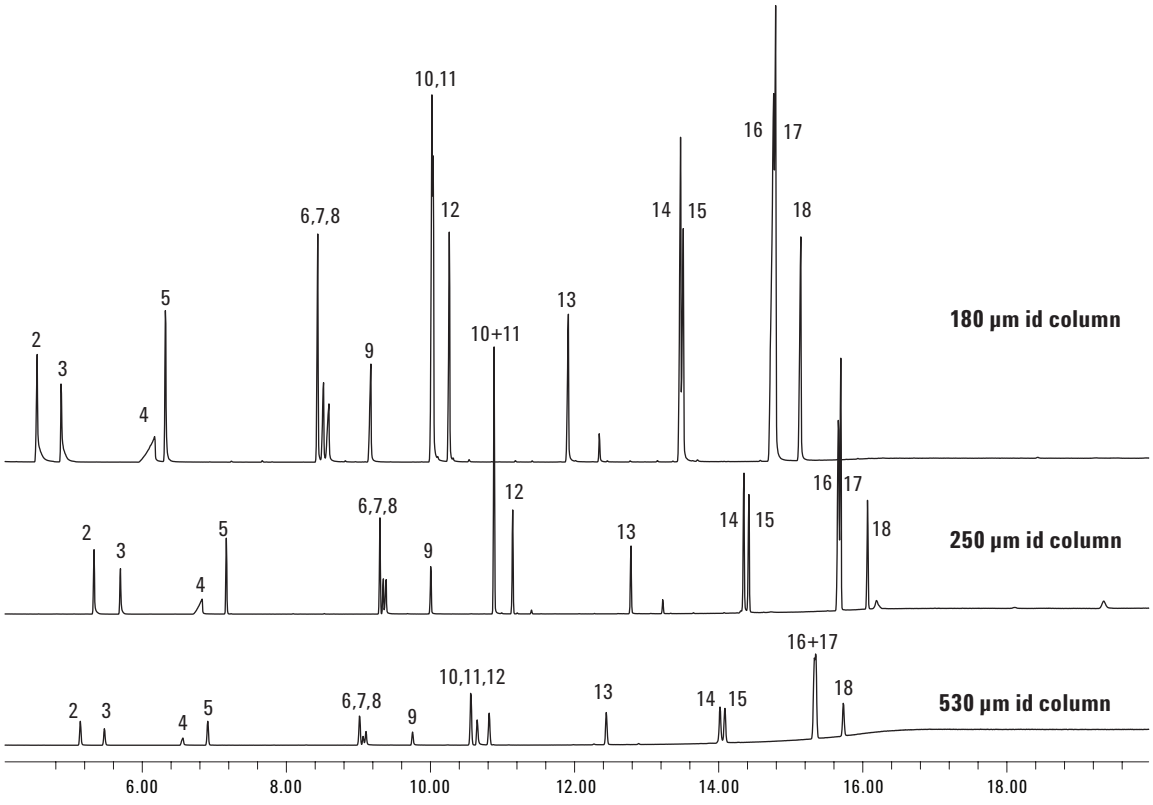


Figure 9. Comparison of MSD TIC chromatograms for three columns run at 2X optimal constant flow mode. Scale is constant for the three, showing the absolute amount of sample reaching the MSD.

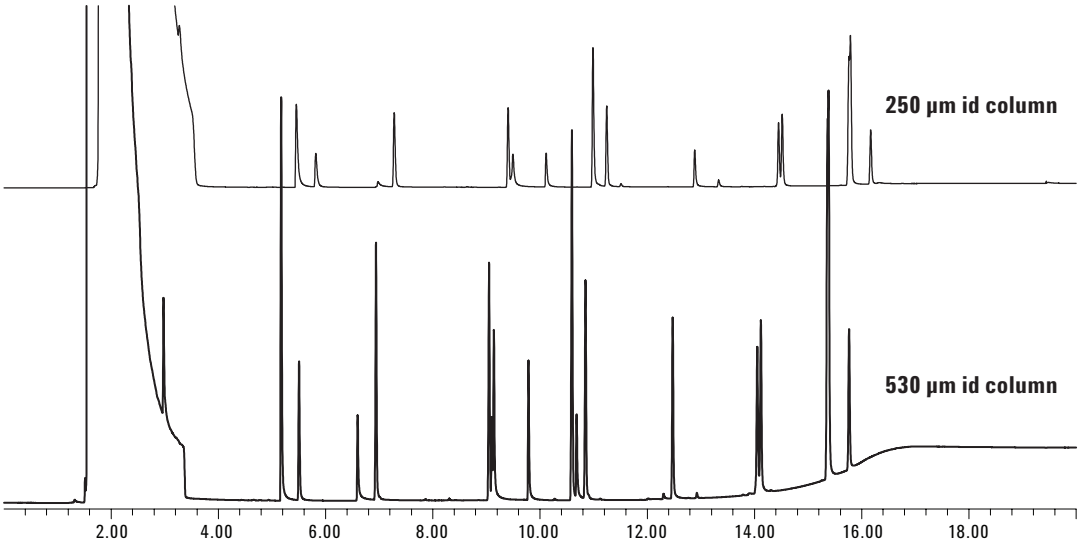


Figure 10. FID vent signals for the two largest columns operated at 2X optimal constant flow rate conditions.

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

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

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

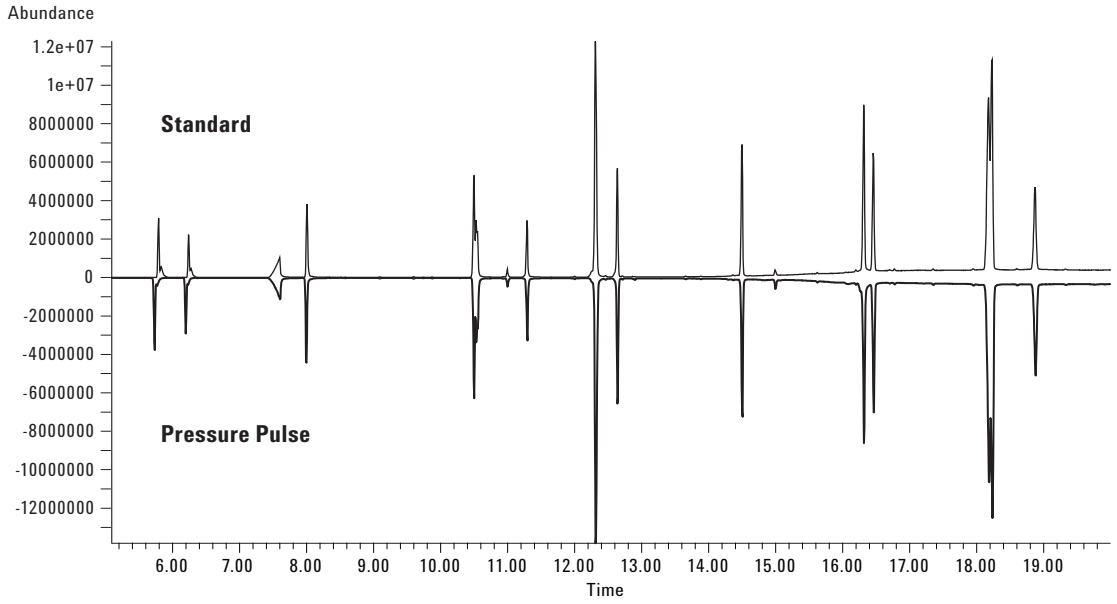


Figure 11. Comparison of standard and pressure-pulse injection modes. No adjustment of QuickSwap pressure was required for the pressure-pulse mode—a benefit of using QuickSwap split configuration.

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

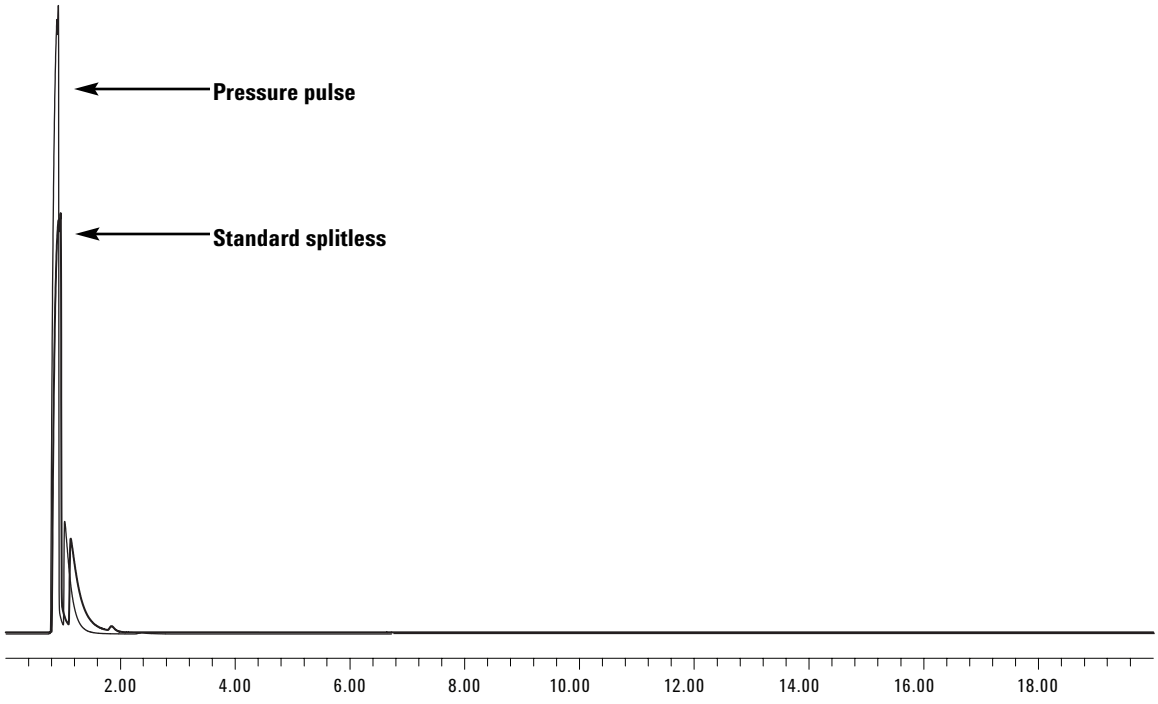


Figure 12. FID vent signal for pressure-pulse injection versus standard splitless injection.

Conclusions

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

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

Parts List

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

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Agilent Technologies

Parallel GC for Complete Refinery Gas Analysis

Application

Hydrocarbon Processing

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Abstract

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

Introduction

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

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

Experimental

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

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

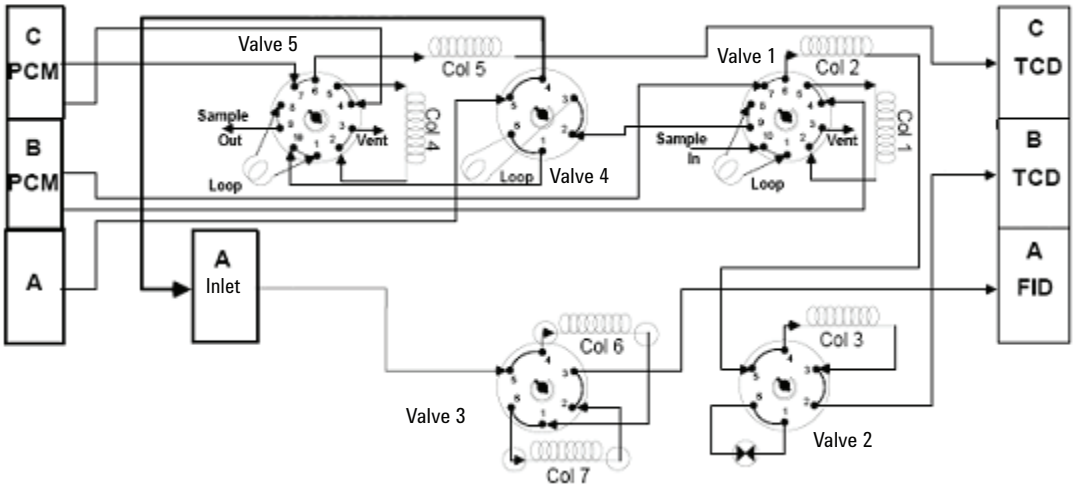
flush of the grouped heavier hydrocarbons (normally C_6+). Valve 3 is a sequence reversal with a short DB1 (column 6) for separating the hexane plus fraction (C_6+) from the lighter components. C_1 through C_5 hydrocarbons are separated on a PLOT alumina column. As soon as the light components C_1 through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

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

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

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

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



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

Figure 1. RGA valve system.

Table 1. Typical GC Conditions for Fast Refinery Gas Analysis

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

Table 2. RGA Calibration Gas Standards

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

Results and Discussion

Enhance Gas Analysis with Union Connector

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

Fast Refinery Gas Analysis (RGA)

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

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

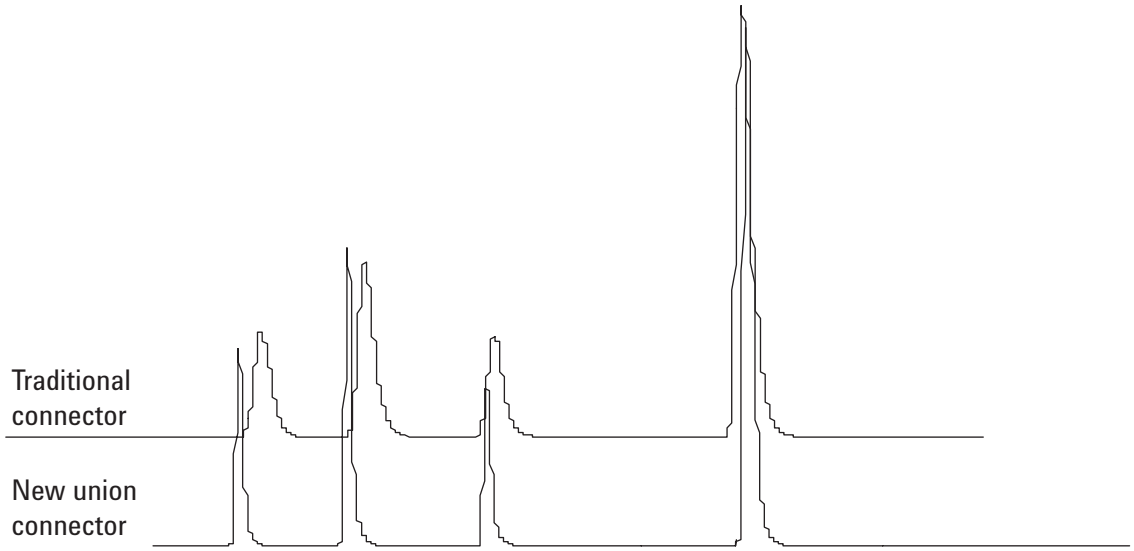


Figure 2. Hydrocarbon peaks obtained from traditional tube connector and new union connector.

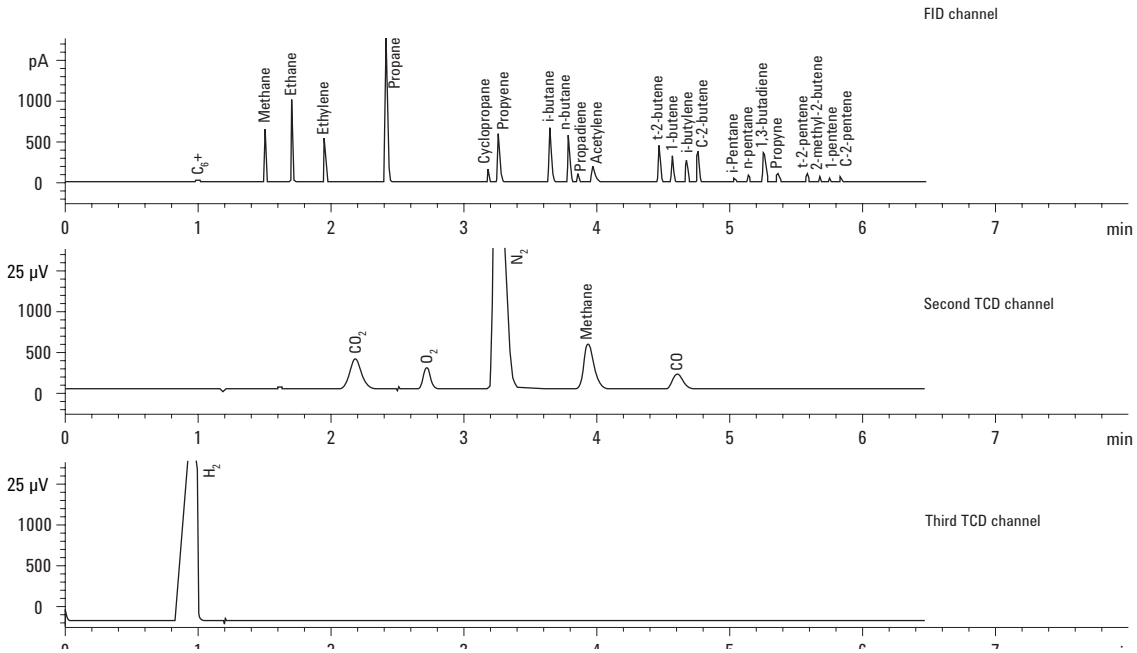


Figure 3. Refinery gas calibration standards analysis. The concentrations for each compound are shown in Table 2.

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

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

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

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

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

Flexibility for Hydrocarbon Analysis

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

H_2S and COS Analysis

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

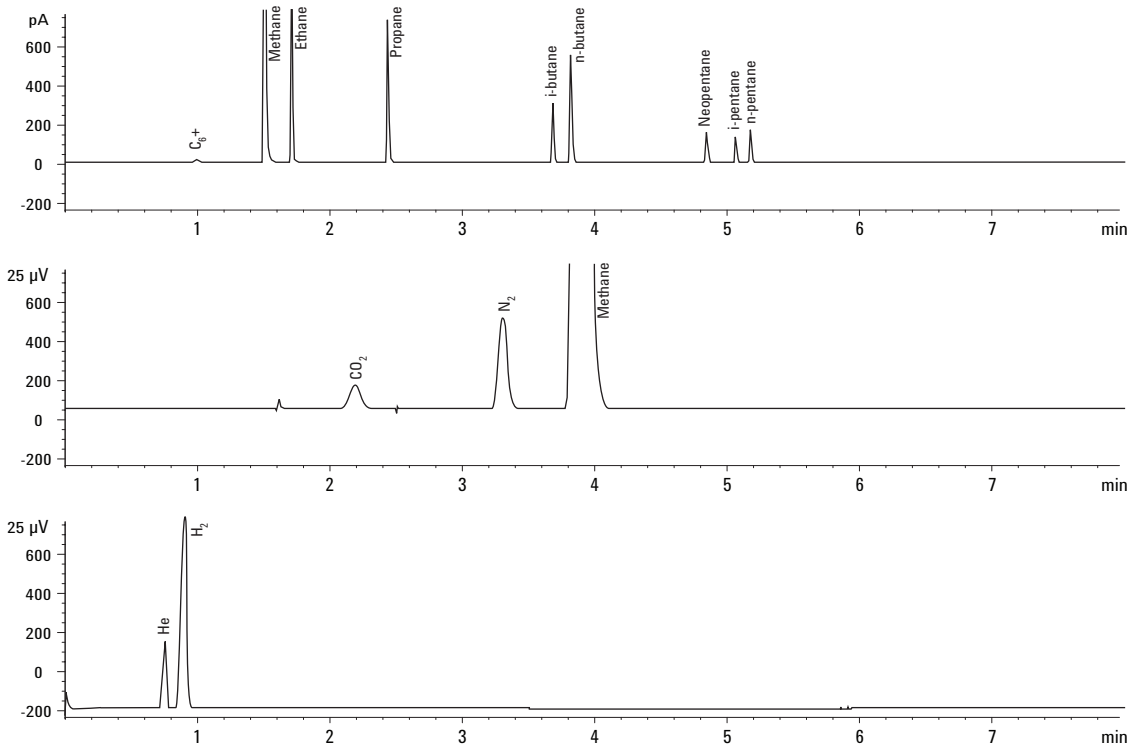


Figure 4. Natural gas analysis of a calibration gas.

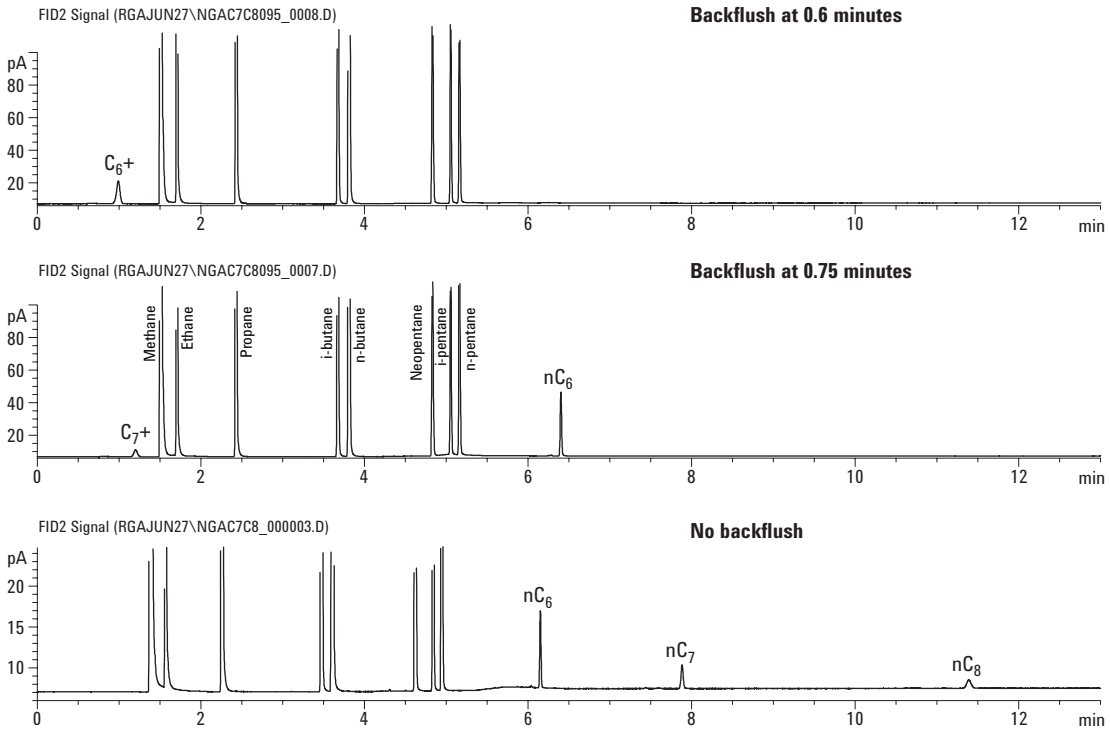


Figure 5. Chromatograms of light hydrocarbons on FID channel with different backflush times .

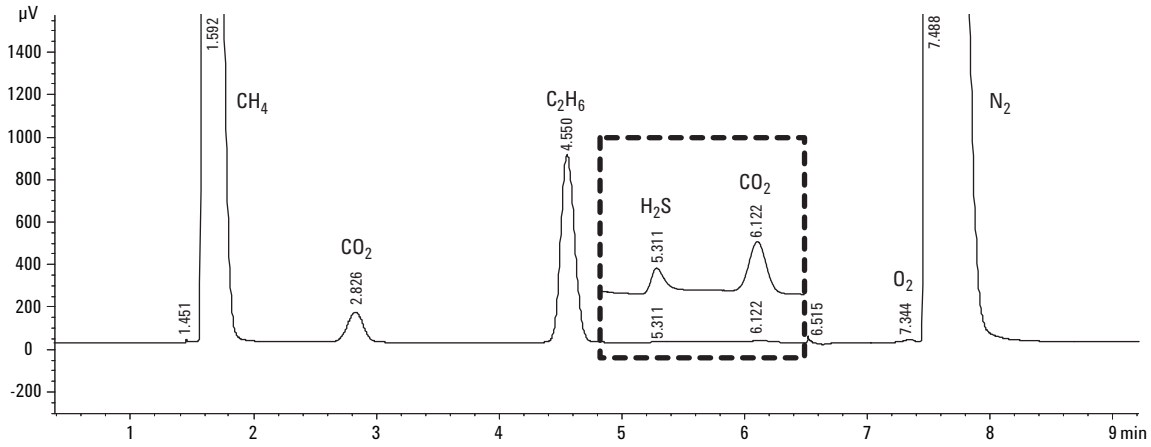


Figure 6. H_2S at approximately 500 ppm and COS 300 ppm on second TCD channel.

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

Reporting

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

Conclusions

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

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

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

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

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Agilent Technologies

Parallel GC for Complete RGA Analysis

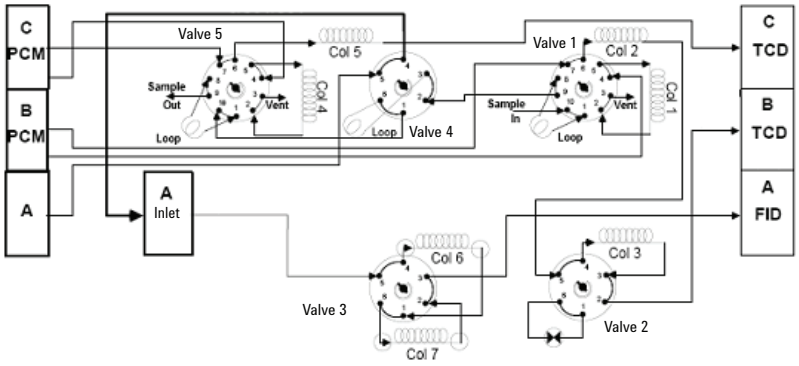
Application Brief

Chunxiao Wang

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

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

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



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

Figure1. RGA valve system.

Highlights

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

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

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

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

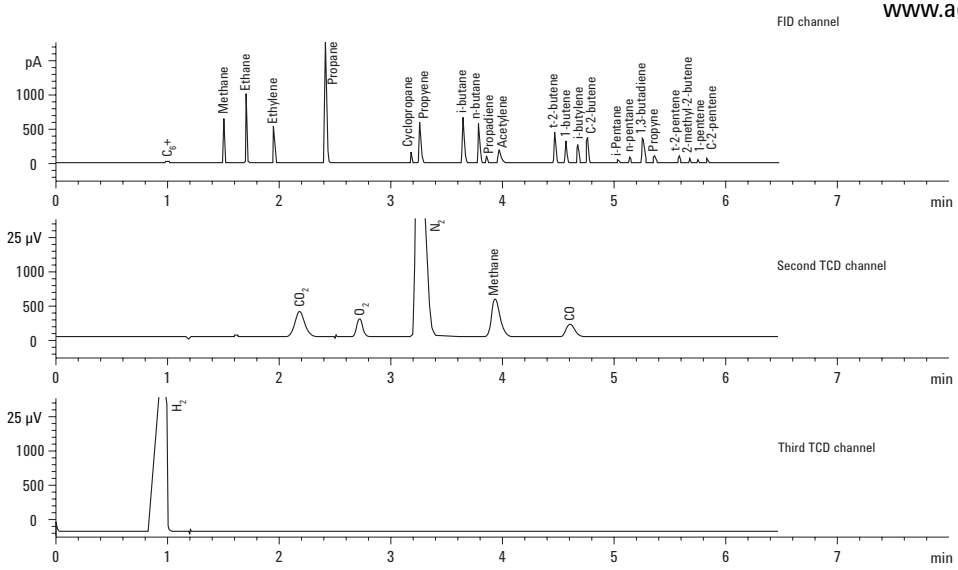


Figure 2. Refinery gas calibration standards analysis.

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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 ($SiOH$-H_2SO_4/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

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 bakeout, they would need to travel through the full length of the column to be removed. In addtion to more effective removal of contaminants, cycle time is significantly reduced, columns are spared from exposure to the high temperatures typical of bakeouts, 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 µL was applied to a series-combination of two cartridges: a 3 mL cartridge filled with 500 mg of silica treated with H_2SO_4 + 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 × 0.25 µm 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 Run pressure Backflush pressure	Helium 150 kPa constant pressure 28 kPa	2 mL/min initial flow rate
QuickSwap Restrictor	GC option number 113 or 17 cm × 110 µm id restrictor	Accessory kit G3185B Part number G3185-60363
Column outlet (QuickSwap) Run pressure Backflush pressure	Helium 28 kPa He using AUX EPC 150 kPa	 Through elution of PCBs 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.

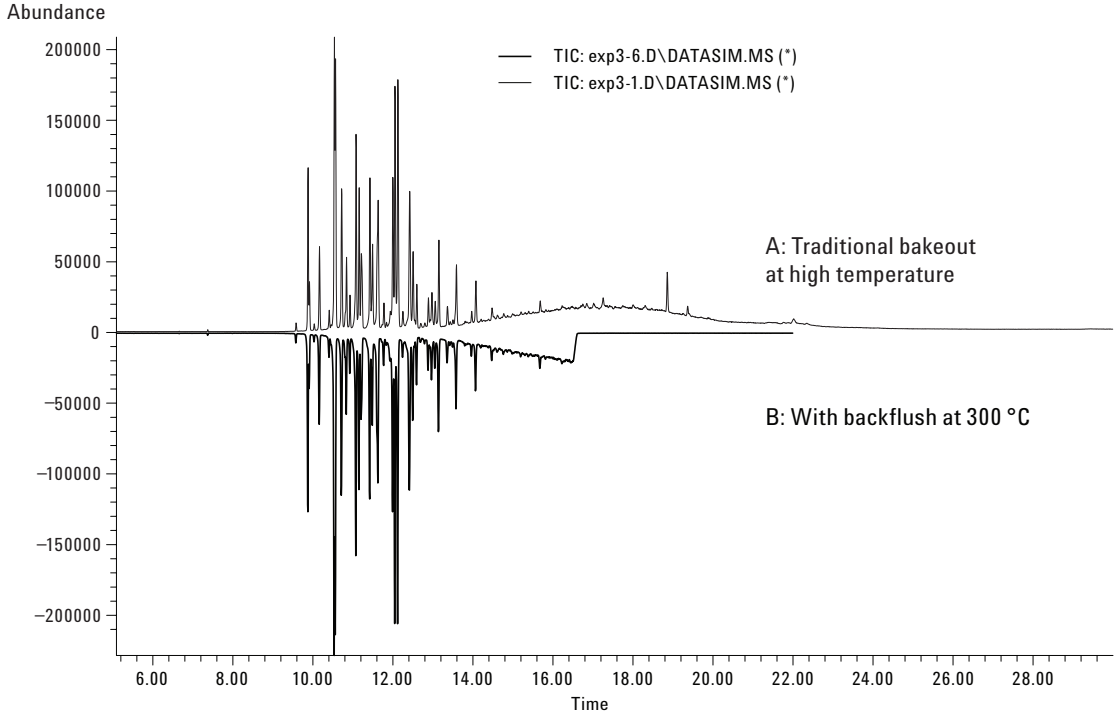

Figure 1. GC/MS total ion chromatograms (TICs) obtained for the analysis of PCBs in waste mineral oil without backflush (A) and with backflush (B)

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.

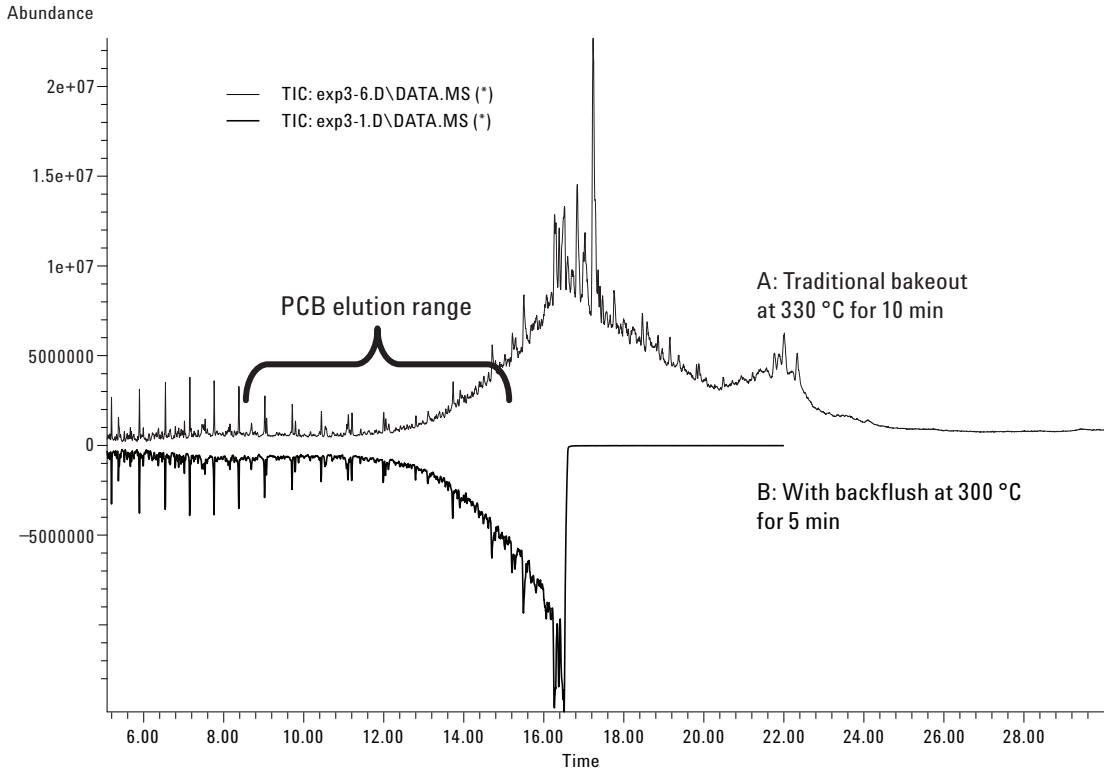

Figure 2. GC/MS TIC scan chromatograms obtained for the analysis of PCBs in waste mineral oil without backflush (A) and with backflush (B).

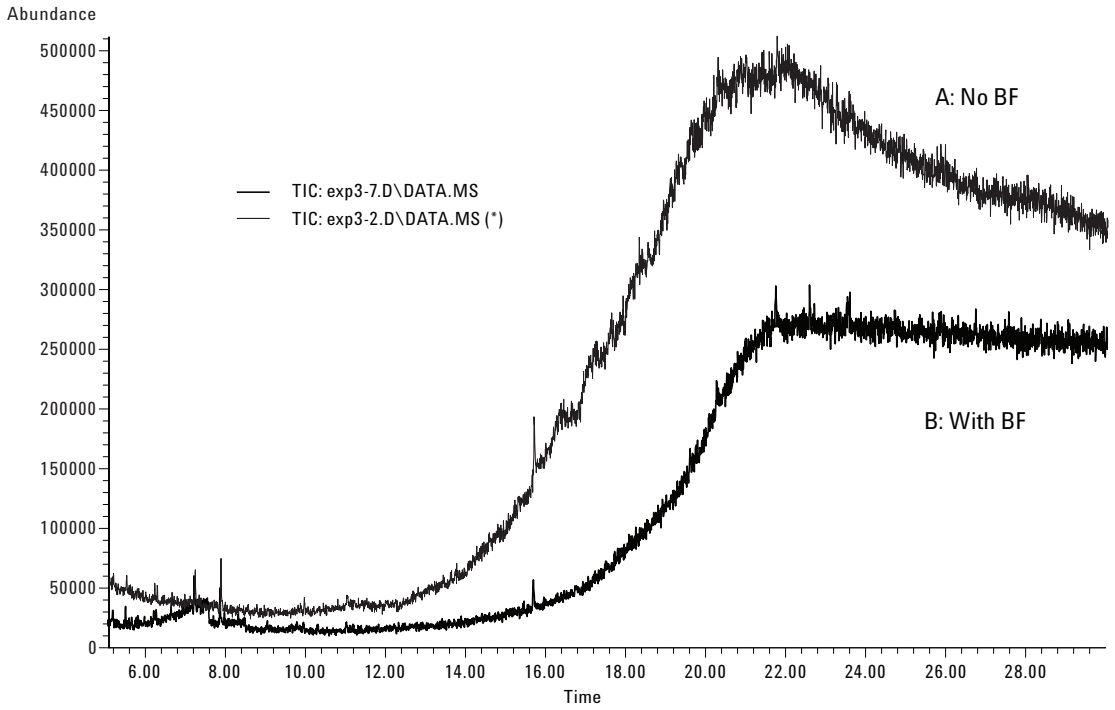

Figure 3. GC/MS TIC chromatograms obtained from scan data for a blank run after the analysis of a sampling with traditional bakeout (no backflush) (A) and with the use of backflush (B).

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.

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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.

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

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

Experimental

High-Efficiency Capillary GC Columns

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

While it is true that an even smaller id column, such as 0.1 mm id, could lead to higher efficiency per meter, routine analysis with such a column imposes high demands on instrumentation. It requires higher inlet pressures, better split control, and faster oven temperature heating rates. On the contrary, 0.18 mm id columns are conveniently compatible with existing standard GC equipment without the need for system modifications. Smaller id, shorter length columns require less carrier flow to achieve separations, thus reduce carrier gas usage. Therefore, high-efficiency 0.18 mm id columns can provide an easy and inexpensive way to speed up GC analysis without compromising resolution.

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

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

Results and Discussion

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

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

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

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

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

The experiment was then repeated with a high-efficiency 20 m × 0.18 mm × 0.18 µm HP-INNOWax column (Agilent part no. 19091N-577) (Method 2). Agilent GC Method Translation Software (http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm) was used to translate Method 1 to Method 2. Three translation modes, namely the "translate only," "best efficiency," and "fast analysis," were attempted with the new column dimensions. However, co-elution of dodecane and o-xylene was observed for all three translated methods. According to ASTM methods, some modifications of the temperature programs were therefore necessary to achieve a similar resolution to Method 1. The resulting experimental conditions are provided in Table 3 along with the chromatogram in Figure 2.

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

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

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

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

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

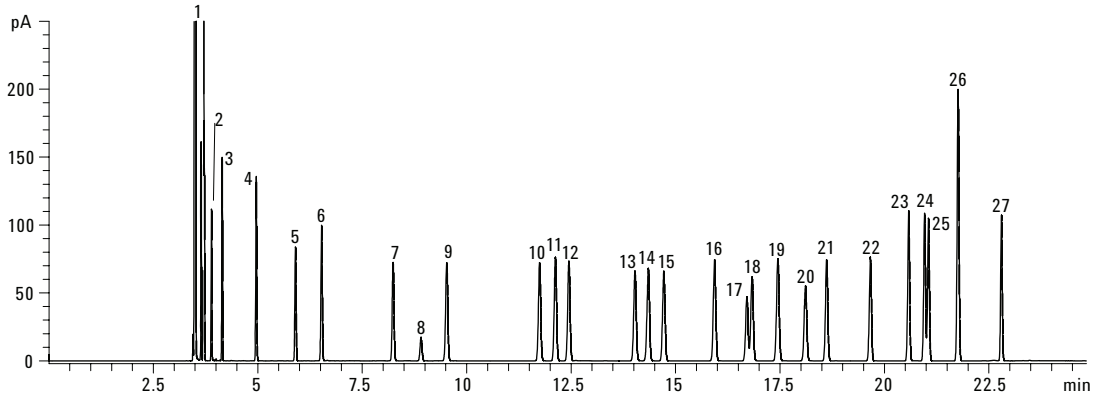


Figure 1. Unified aromatic solvent method with a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column.

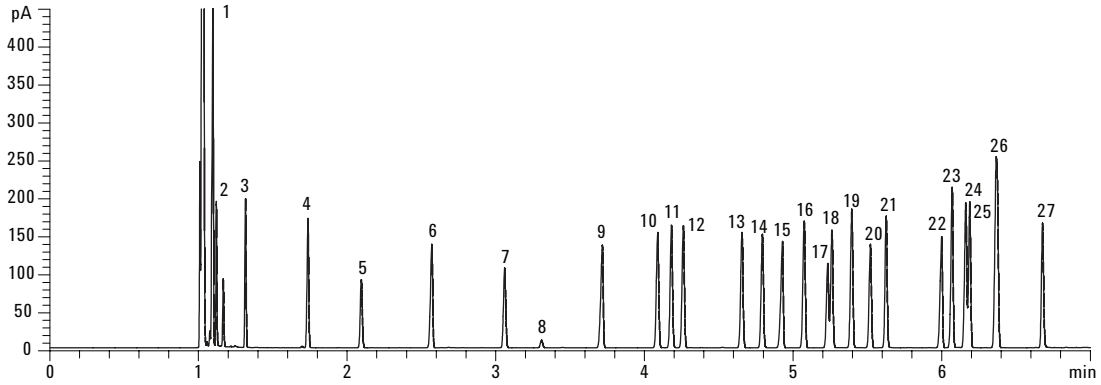


Figure 2. Separation of the same aromatic solvent with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column.

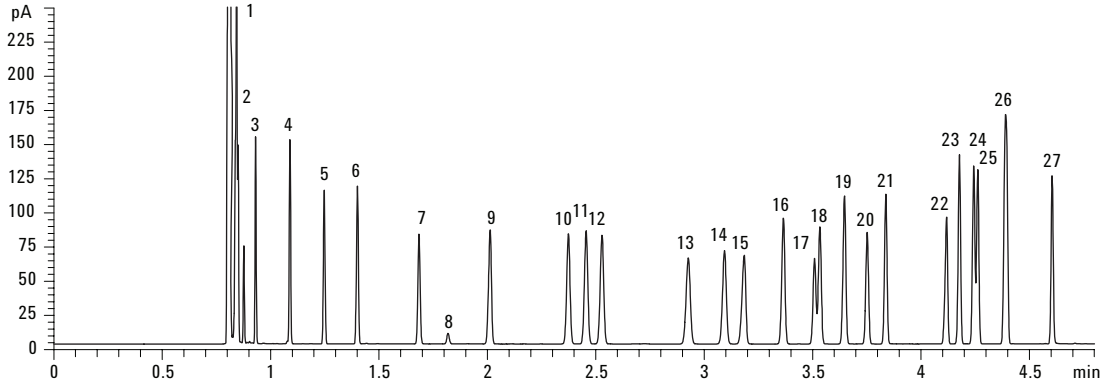


Figure 3. Optimized unified aromatic solvent method with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column.

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

Figures 1, 2, and 3 show the chromatograms of the hexane solution containing an aggregate of aromatic solvents and impurities for Method 1, Method 2, and Method 3, respectively. As indicated in the three chromatograms, baseline resolution was achieved for most of the compounds of interest except for two compound pairs, which were only partially resolved. The first pair, p-ethyltoluene and m-ethyltoluene, was also not resolved in the original ASTM method (D-5060, impurities in ethylbenzene) and, along with o-ethyltoluene, was reported as total ethyltoluene. A second pair, diethylbenzene and n-butylbenzene, was also partially resolved. However, this should not present a

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

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

Influence of Carrier Gas on Analysis Time

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

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

Nitrogen vs. Helium Carrier Gas

To investigate the effect of carrier gas on sample analysis time, Agilent GC method translation software was used where “translate only” mode was chosen so that all experimental conditions were held constant except for the carrier gas. Method 1 was translated to Method 4 where a nitrogen carrier was used (see Figure 4 and Table 6). As shown in Figure 5, the run time for a nitrogen carrier was about 60 minutes compared to 23 minutes with a helium carrier when using a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column.

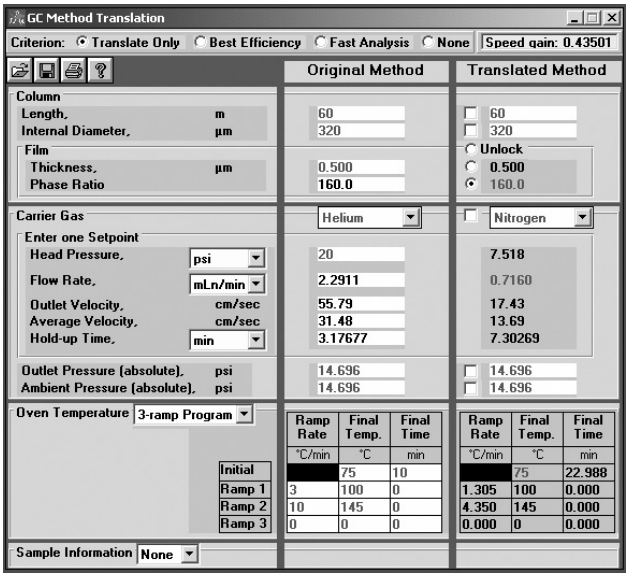


Figure 4. Method translation software input screen for a nitrogen carrier.

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

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

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

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

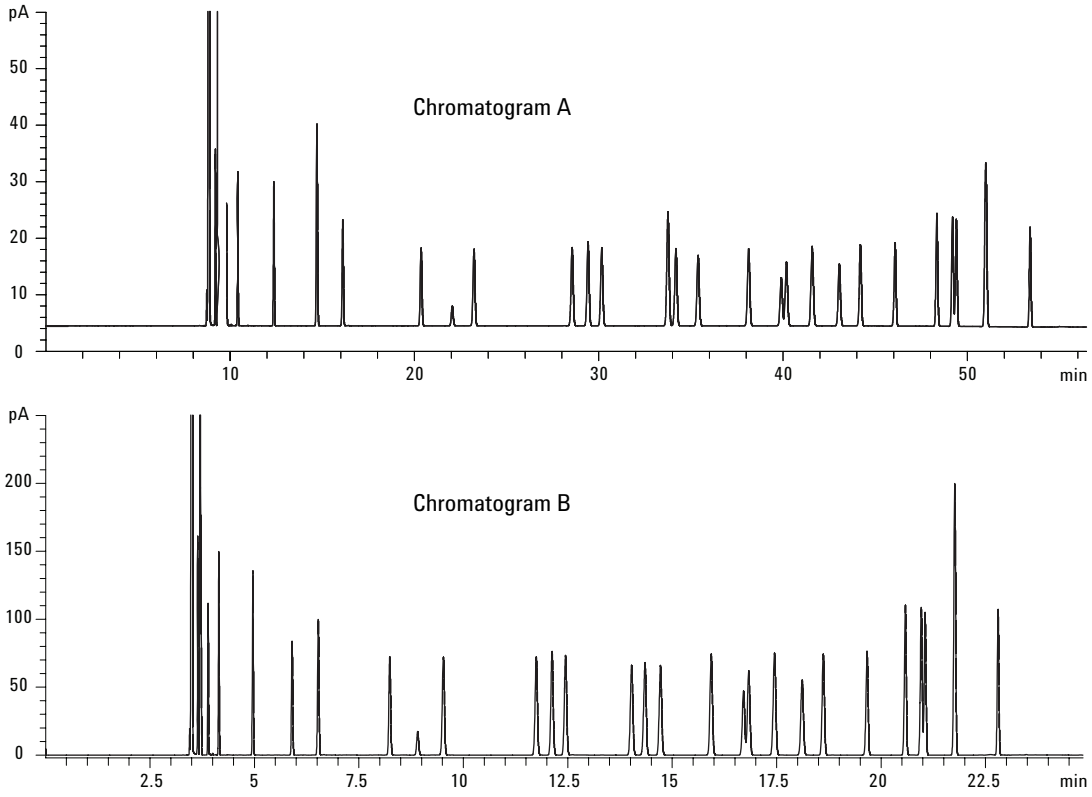


Figure 5. Comparison of unified aromatic solvent analysis using nitrogen and helium carrier gases with a 60 m × 0.32 mm × 0.5 µm HP-INNOWax column. 5a. Nitrogen carrier gas (Method 4). 5b. Helium carrier gas (Method 1).

Hydrogen vs. Helium Carrier Gas

A faster analysis can be achieved by switching the carrier gas from helium to hydrogen on the same coumn. Method 3 was translated to Method 5 using the method translation software (see Figure 6); the detailed experimental condition is provided in Table 7. As shown in Figure 7, the total run time was decreased from 5 to 3 minutes by changing the carrier gas from helium to hydrogen while keeping the peaks well separated.

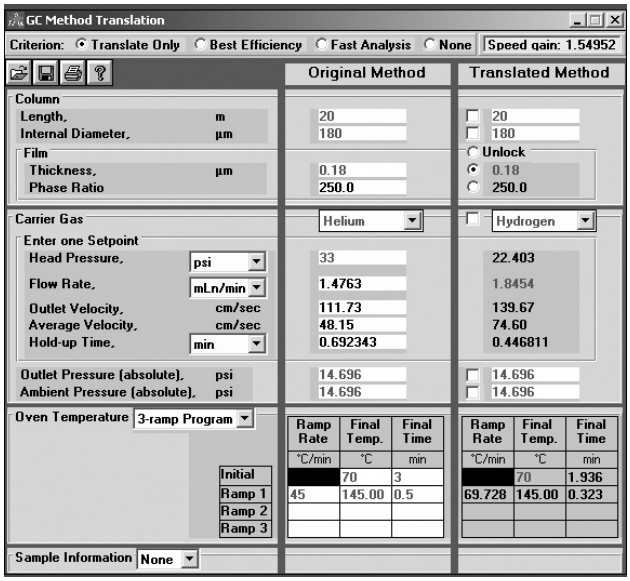


Figure 6. Method translation software input screen for a hydrogen carrier.

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

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

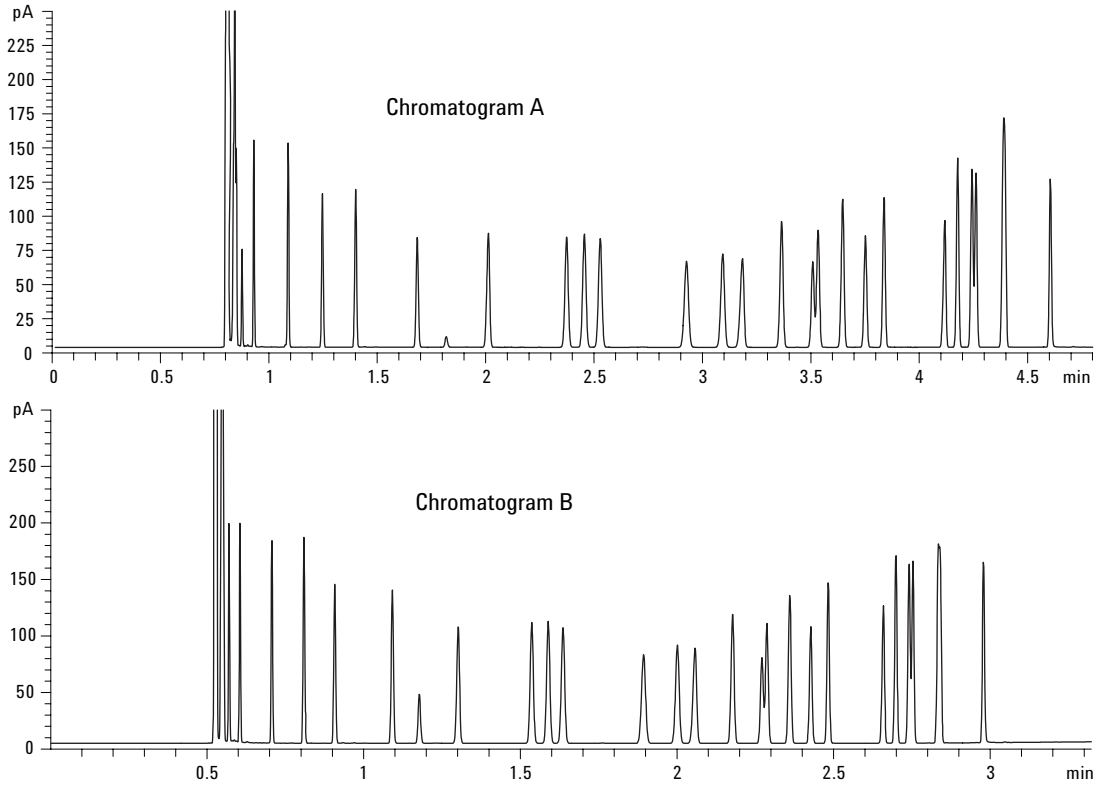


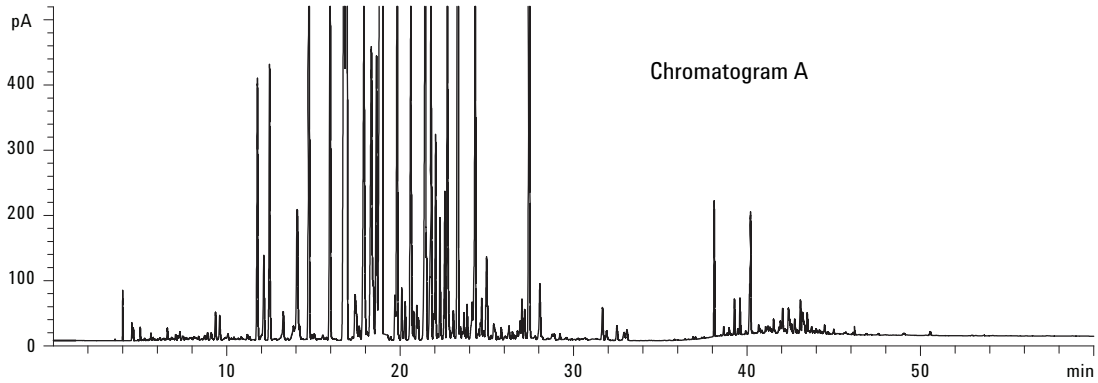
Figure 7. Comparison of unified aromatic solvent analysis using helium and hydrogen carrier gases with a 20 m × 0.18 mm × 0.18 µm HP-INNOWax column. 7a. Helium carrier gas (Method 5). 7b. Hydrogen carrier gas (Method 3).

Complex Matrix Sample

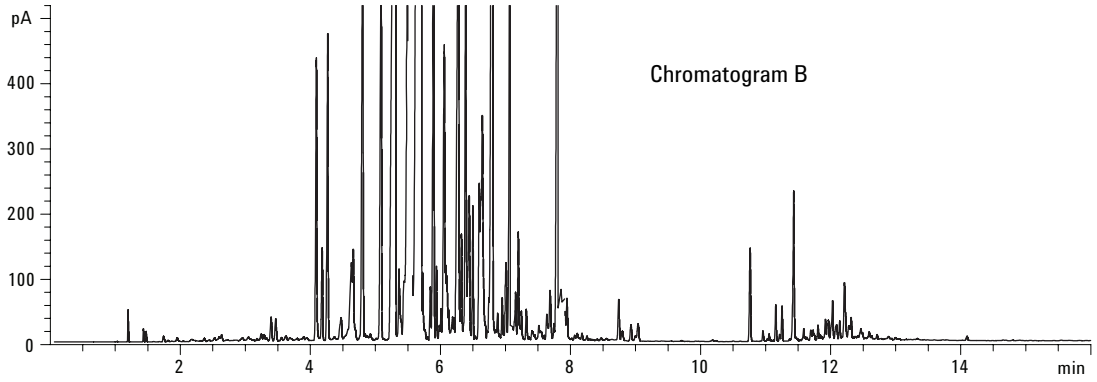
To validate the practicality of fast GC application using high-efficiency GC columns, a real aromatic solvent sample offered by a large-scale integrated petrochemical company was analyzed using the same experimental conditions as those for the standards (Methods 1, 2, and 3); the chromatograms are provided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

Although the analysis time is a bit longer with Method 2 compared to Method 3, the overall resolution obtained is slightly better for Method 2 (see Figures 8e and 8f). On the other hand, all the key compounds, including benzene, toluene, ethylbenzene, m-xylene, p-xylene, o-xylene, propylbenzene, and a-methylstyrene, were well separated with all three methods.

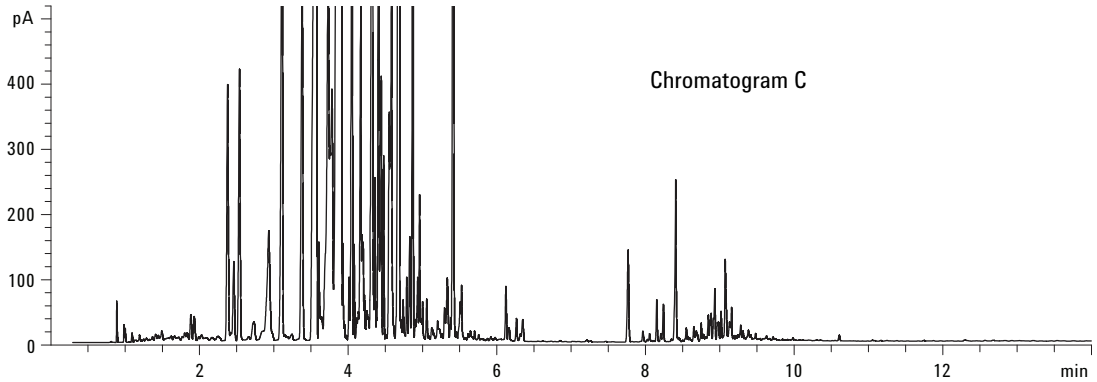
For complex matrix samples, a balance between speed and resolution must be selected according to the laboratory goals. In this case, it demonstrates that a complex matrix sample can be separated well on a high-efficiency 0.18 mm id GC column, where a more than 3x improvement in run time was accomplished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



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



Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 25.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C; 150:1 split ratio
Oven temp	50 °C (2 min); 15 °C/min to 90 °C (0 min); 20 °C/min to 145 °C (3 min), 80°C/min to 220 °C (8 min)
Detector	FID at 250 °C
Injection size	0.2 µL



Column	HP-INNOWax, 20 m × 0.18 mm × 0.18 µm
Carrier gas	Helium at 33.00 psi constant pressure mode
Inlet	Split/splitless at 250 °C; 150:1 split ratio
Oven temp	70 °C (3 min); 45 °C/min to 145 °C (3 min), 80 °C/min to 220 °C (8 min)
Detector	FID at 250 °C
Injection size	0.2 µL

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

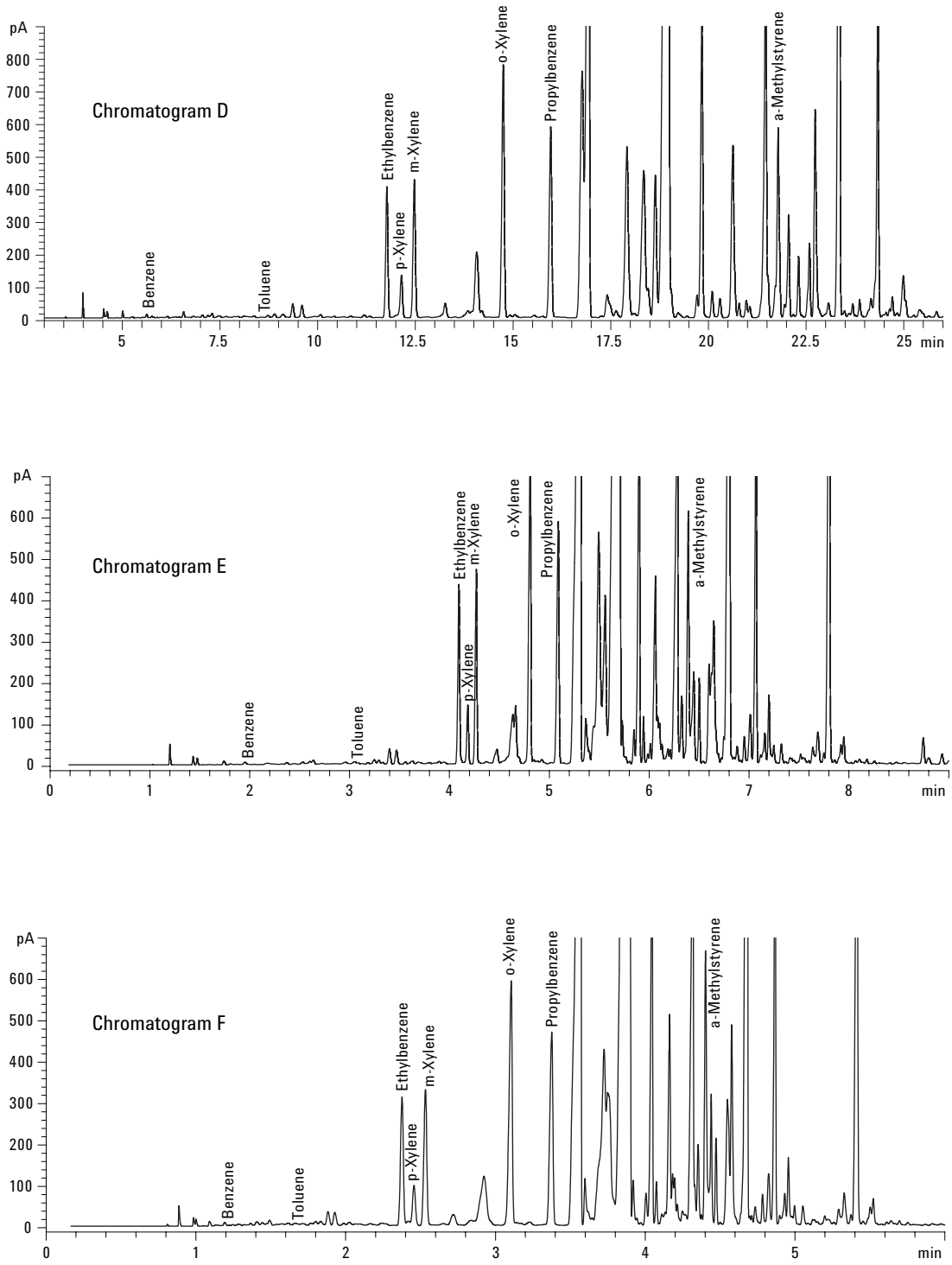


Figure 8. Comparison of real aromatic solvent sample separations (a) and (d) Method 1, (b) and (e) Method 2, and (c) and (f) Method 3. (continued)

Evaluation of Individual ASTM Calibration Standards

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

D2306 – Standard Test for C8 Aromatic Hydrocarbons

Concentration of ASTM D2306 standard calibration mix is quite high. It is therefore a challenge regarding the capacity of the high-efficiency 0.18 mm id column. The workaround is to inject a small volume with a high split ratio. In this experiment, the injection size was 0.2 µL and the split ratio was 600:1. As shown in Figure 9, the run time for the high-efficiency GC column was about 4.5 times shorter than that of the traditional one. The resolution is acceptable in spite of the high concentration of the calibration standard (see Table 8).

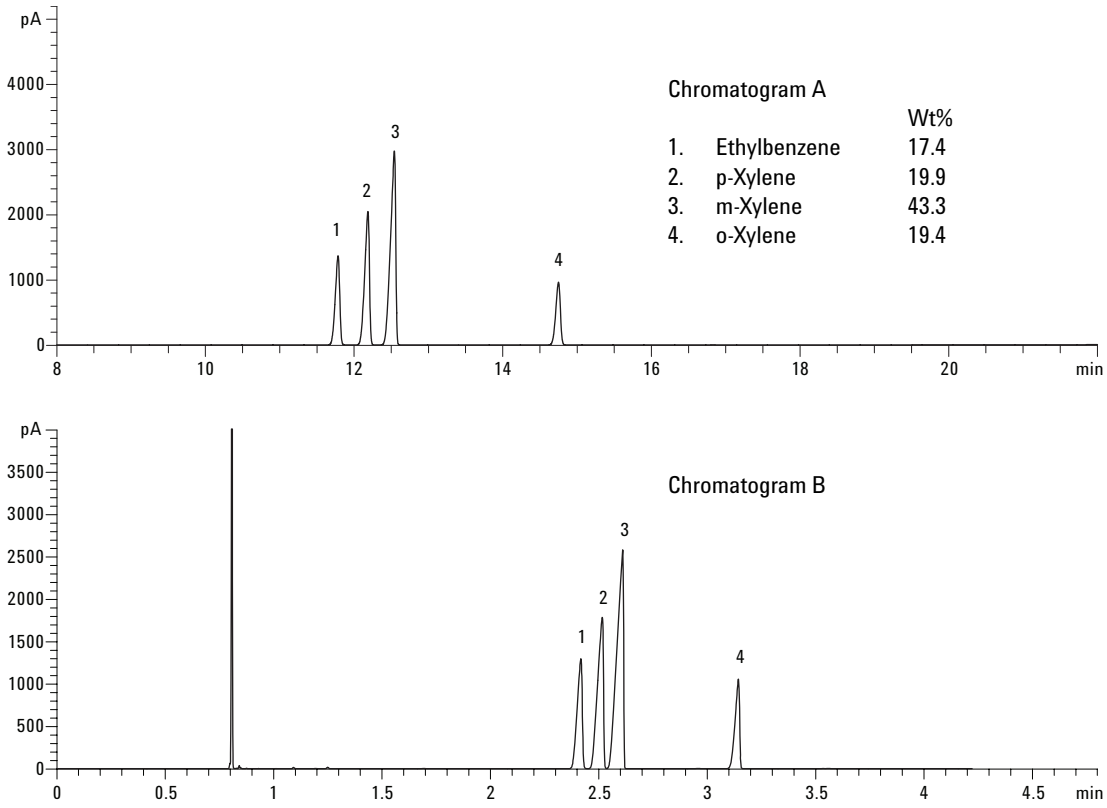


Figure 9. ASTM D2306 C8 aromatic hydrocarbon quantitative calibration standards (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (method 3).

Table 8. Comparison of Resolution Under Different Experimental Condition

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

D2360 – Standard Test for Trace Impurities in Monocyclic Hydrocarbons

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

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

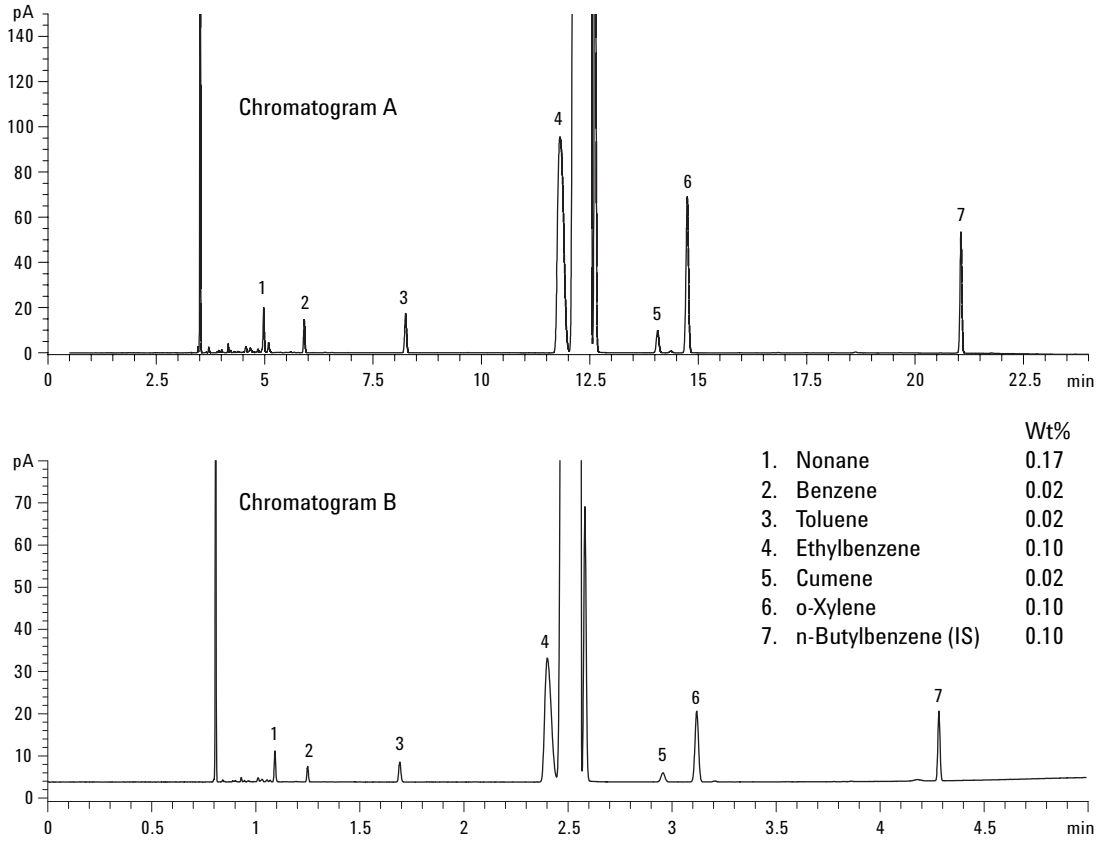
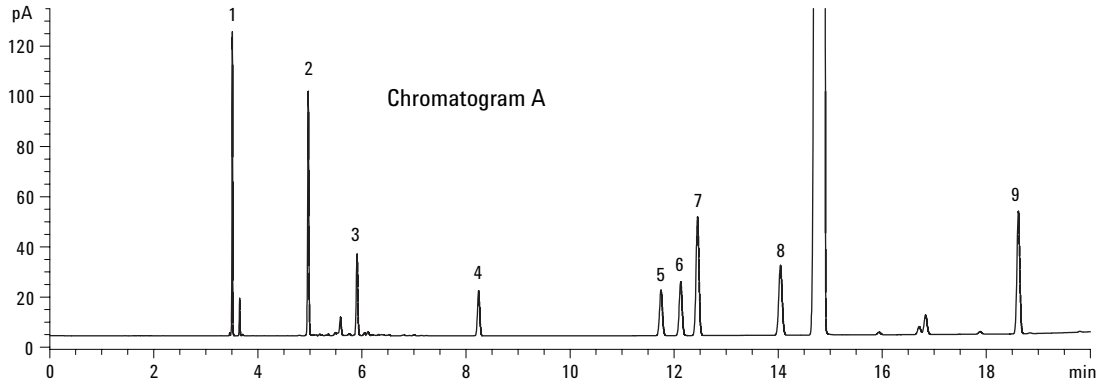


Figure 10. ASTM D2360 monocyclic hydrocarbon quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

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

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



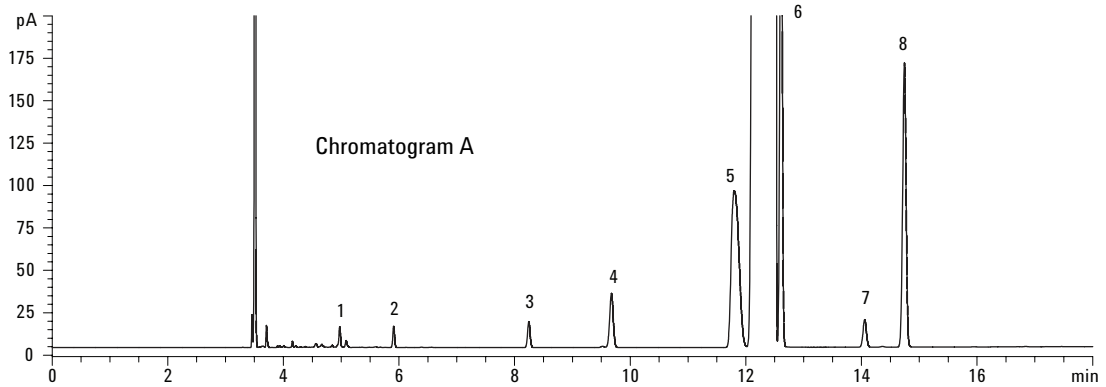
		Wt%			Wt%
1.	Isooctane (IS)	0.05	6.	p-Xylene	0.21
2.	Nonane	0.21	7.	m-Xylene	0.42
3.	Benzene	0.20	8.	Cumene	0.31
4.	Toluene	0.21	9.	Styrene	0.12
5.	Ethylbenzene	0.21			

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

D3798 – Standard Test Method for Analysis of p-Xylene

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

Figure 12 shows the chromatograms of the D3798 calibration standard. Injection size for this run was 0.2 µL and the split ratio was 100:1. The original ASTM D3798 method specifies that the valley points between the large p-xylene peak and the ethylbenzene and m-xylene contaminants should be less than 50% of the contaminants' peak height. Excellent separation was obtained for the critical compounds (Figure 13) with great reproducibility (Figure 14) when using a high-efficiency GC column.



		Wt%			Wt%
1.	Nonane	0.01	5.	Ethylbenzene	0.10
2.	Benzene	0.02	6.	m-Xylene	0.20
3.	Toluene	0.01	7.	Cumene	0.01
4.	Undecane (IS)	0.08	8.	o-Xylene	0.10

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

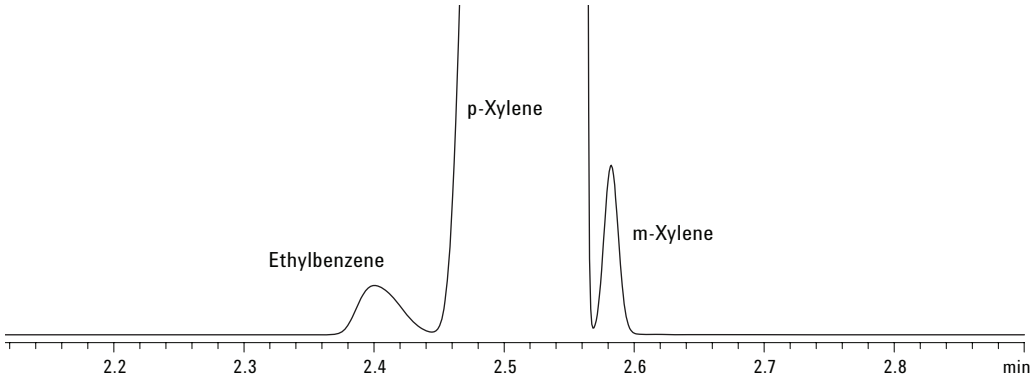


Figure 13. Expanded view from Figure 7 shows excellent separation of m-Xylene peak from p-Xylene peak using the fast GC method.

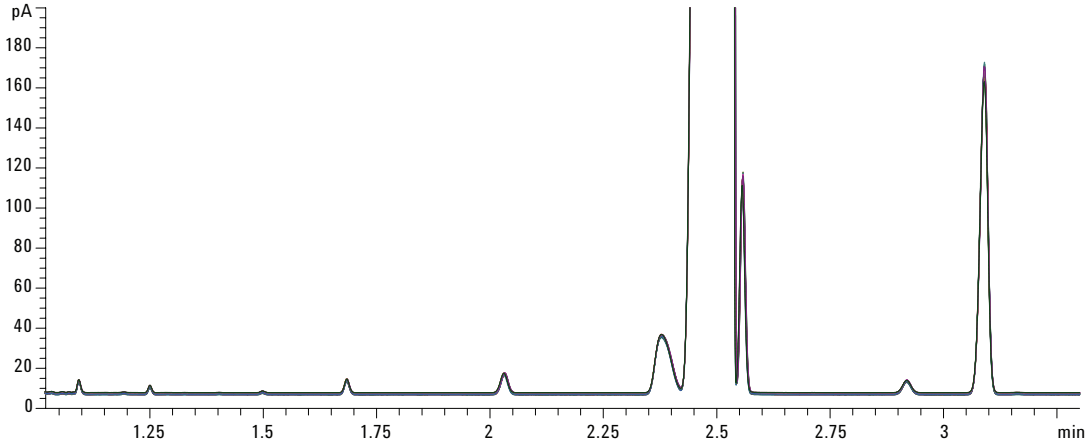


Figure 14. D3798 standard 30th run overlaid using a high-efficiency GC column.

D4492 – Standard Test for Analysis of Benzene

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

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

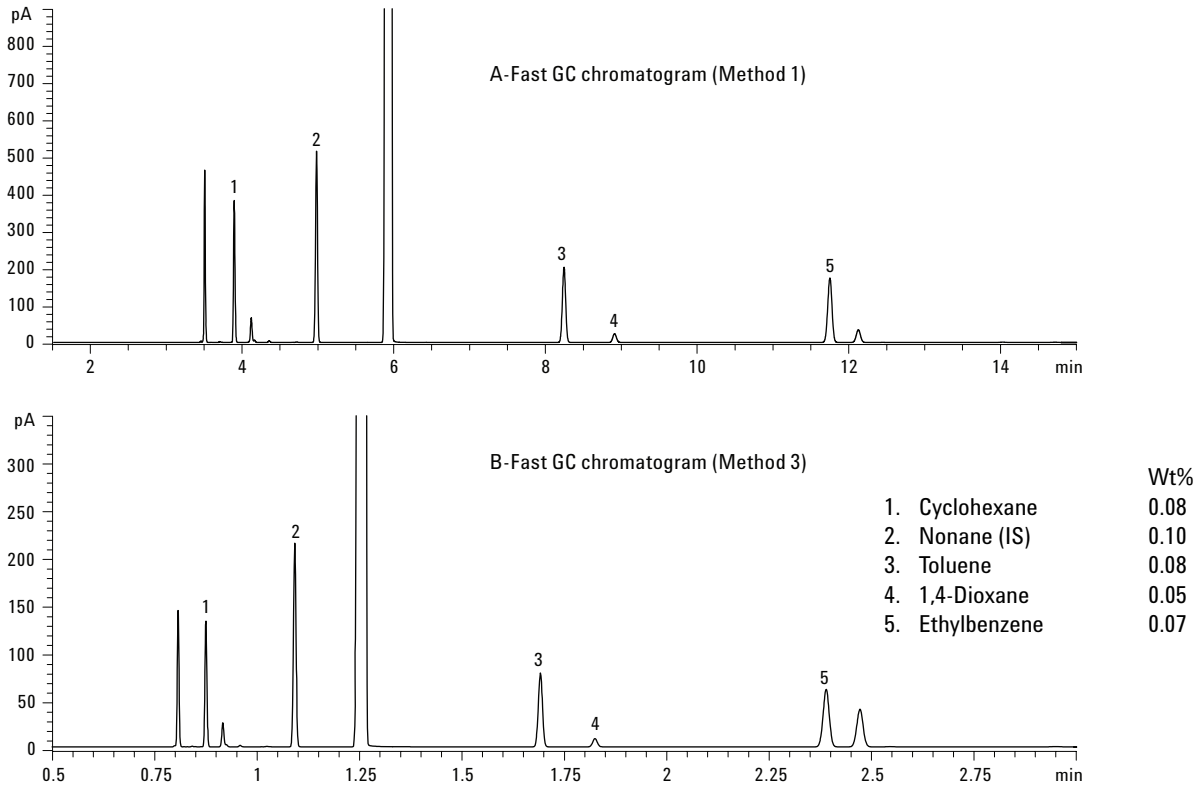


Figure 15. ASTM D4492 benzene quantitative calibration standard run (a) on a standard column (Method 1) and (b) on a high-efficiency GC column (Method 3).

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

Conclusions

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

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5. Ken Lynam and Yun Zou, "A Faster Solution for Unified Volatile Organic Analysis with 0.18 mm ID GC Columns," *Separation Times*, Vol 20, Number 5, 2007

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Agilent Technologies

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

Accessory G3505A

Introduction

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

Injection Device

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

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

Ordering Information

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

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

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

Guideline for choosing Aux flow source

7890AGC

G3471A Pneumatic Control Module (PCM) or

G3470A Aux EPC module

6890GC

G1570A Aux EPC or

G2317A PCM module

The PCM is the preferred source for both GCs.

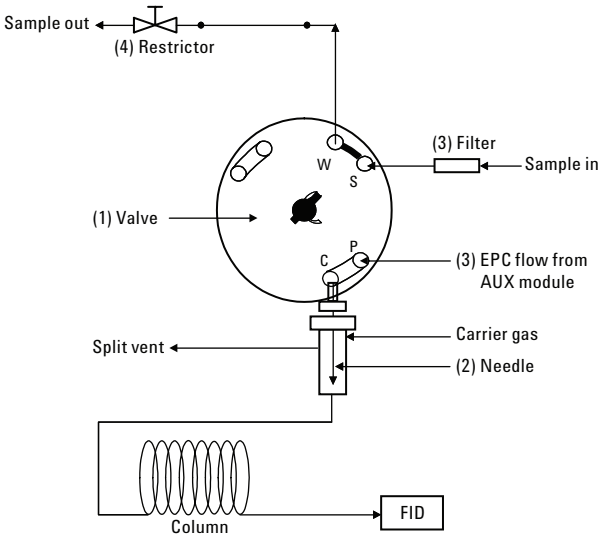
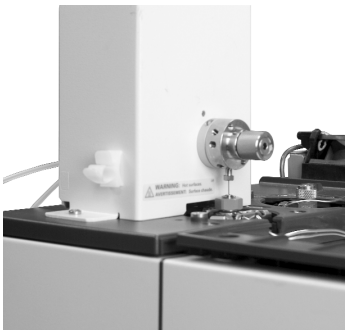


Figure 1. Flow diagram of the high-pressure injection device (HPLI).

Typical Instrumental Conditions

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

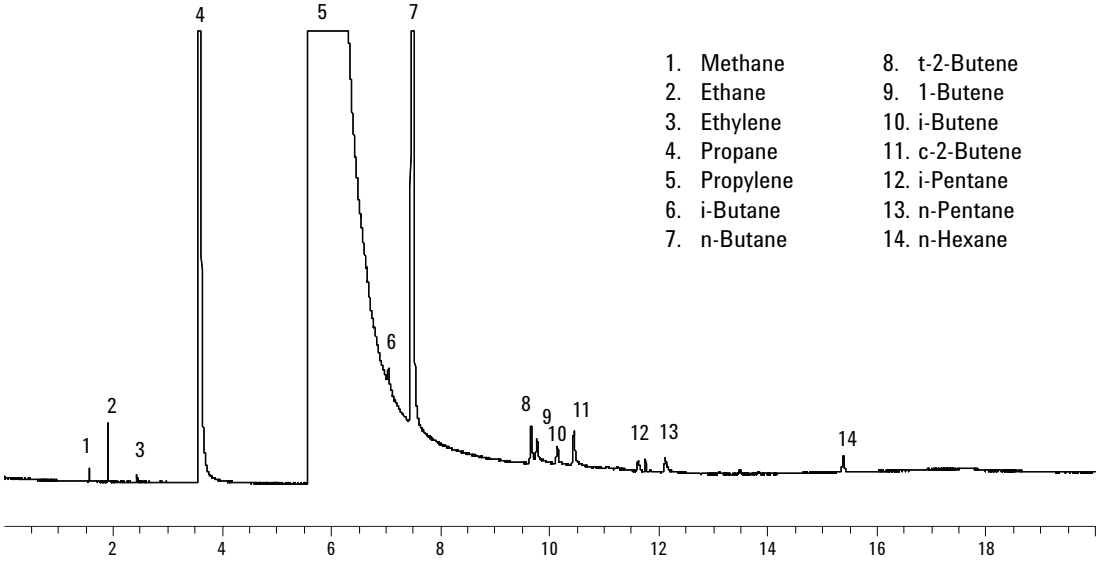


Agilent pneumatic air actuator/valve assembly installed on the 7890A.

Sample Chromatograms

Pressurized Propylene

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



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

Figure 2. Chromatogram of pressurized propylene.

Pressurized 1,3-Butadiene

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

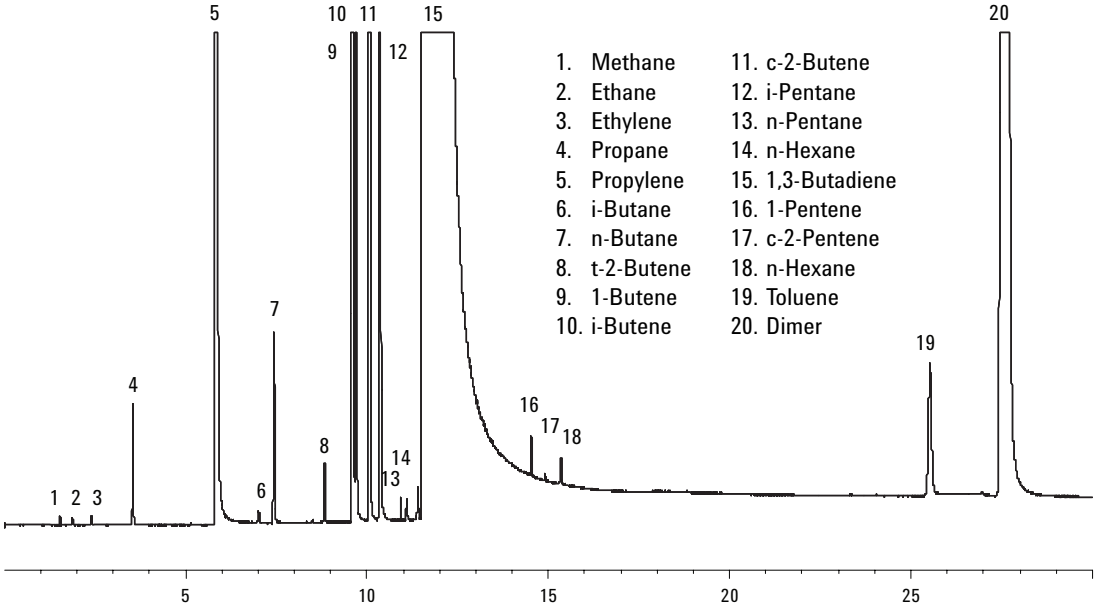


Figure 3. Chromatogram of pressurized 1,3-butadiene.

Summary

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

Competitive Advantages

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

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

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Agilent Technologies

Comprehensive GC System Based on Flow Modulation for the 7890A GC

Application Brief

Introduction

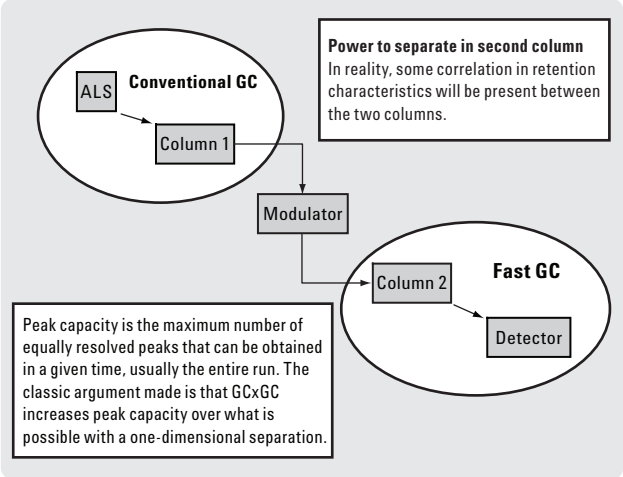
A hardware solution is available on the 7890A for the practice of comprehensive GC. The system uses a capillary flow modulator controlled by the 7890A GC. The system is offered with factory checkout using an FID detector. Other detectors, preferably those operating at 50 Hz or greater, can be used.

Comprehensive two-dimensional (2D) GC, or GCxGC, is a powerful technique that can be used to separate very complex mixtures, such as those found in the hydrocarbon processing, environmental, and food/fragrance industries.

The method uses two columns, typically of very different polarities, installed in series with a modulator in between. The second column is much shorter than the first column to effect a fast separation. The entire assembly is located inside the GC oven.

The modulator performs three functions:

1. It collects effluent from the first column for a fraction of the time equal to peak width. For example, if a peak from column one is six seconds wide, the modulator will accumulate material every two to three seconds, thereby dividing the peak from the first column into two or three "cuts."
2. It focuses the material collected from each cut into a very narrow band through flow compression.
3. It introduces the bands sequentially onto the second column, resulting in additional separation for each band injected onto the second column.



Comprehensive 2D GC uses a primary column (conventional separation), a flow modulator, a second column (very fast separation), and a fast detector.

This technique provides a second dimension of information that can increase the peak resolution and capacity.

A number of different modulator designs have been described in the literature, most relying on thermal cycling to focus the bands from the first column and release them into the second column. Some disadvantages to this approach are:

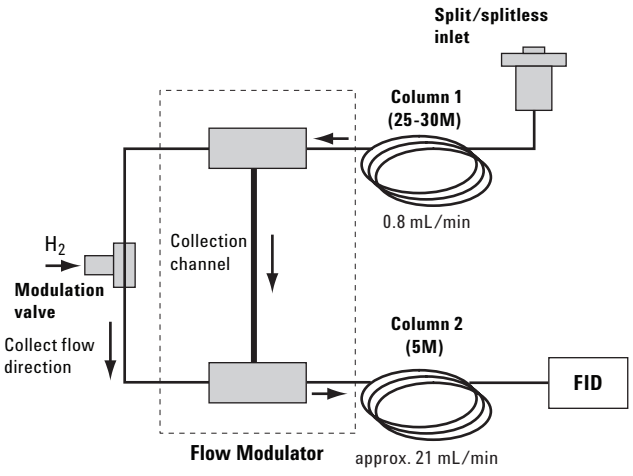
- Large usage of expensive cryogenic gases leading to a high cost of analysis
- Complexity of the hardware
- Longer analysis times

Agilent's proprietary Capillary Flow Technology and fourth-generation Electronic Pneumatics Control (EPC) enable the use of a differential flow modulator to conduct comprehensive 2D-GC without the use of cryogenic gases or complex hardware.

The key to operation is the flow differential (typically 20 to 1) between the second and first columns, respectively. This compresses and focuses the analytes present in any given modulation "inject" pulse into the second column. Precise timing of the modulator is made possible by installing a driver board in the Aux det 2 detector slot of the 7890A mainframe.

The Capillary Flow Technology modulator uses a deactivated, stainless steel structure with all flow splitters and the collector channel incorporated internally in the device. It has low thermal mass so it can track the oven temperature very closely, and its GC oven location allows precise temperature control without lag during programmed runs. All external connections are made using Agilent's Ultimate Union technology for leak-free operation and extremely small, well-swept volumes. A micro three-way solenoid valve, installed on the side of the gas chromatograph, connects to a pneumatics control module (PCM) to accurately and precisely control the flows through the modulator.

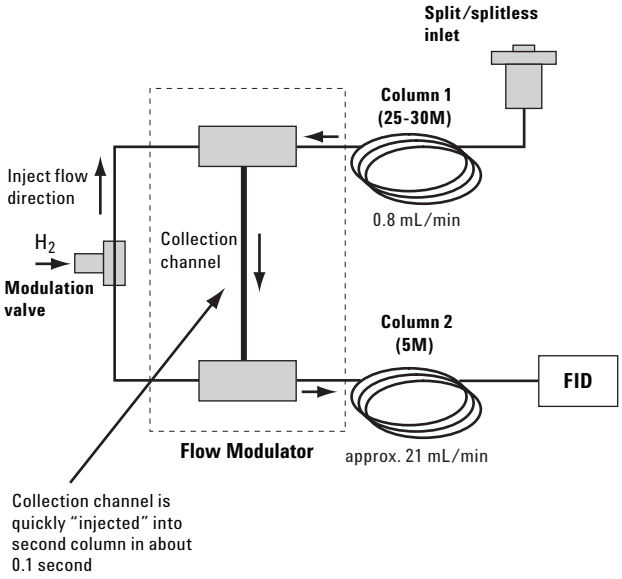
The figures below illustrate the modulator. A three-way solenoid valve receives a controlled supply of hydrogen gas from a PCM. The periodic switching of this three-way valve drives the modulator. The precisely timed and synchronized switching between the collect and inject states directs discrete sample pulses continuously to the second column for additional fast separation throughout the chromatographic run. Both columns are run in constant flow mode. For optimal performance, injection size and split ratio should be carefully adjusted to avoid overloading, which can lead to excessive peak tailing.



Flow rates and flow directions during the load or collect portion of the modulation cycle

Load or collect state (above): At the beginning of this state, the collection channel is filled with hydrogen gas from a previous injection cycle flush.

The primary column effluent enters the modulator's top tee connection and flows into the collection channel. The analytes from this column enter one end of the collection channel. Hydrogen flow from the PCM/three-way micro valve exits the modulator at the bottom tee and is sent to the second column.



Flow rates and flow directions during the transfer or inject portion of the modulation cycle

Inject or flush state (above): Hydrogen gas flow from the three-way solenoid valve is directed to the top tee. A high flow of typically 20 mL/min for about 0.1 second rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes collected in the channel undergo rapid separation.

What is required:

- Agilent 7890A GC with firmware version A.04.06 or higher
- FID with 200 Hz data collection rate or other fast detector
- Split/splitless inlet
- Capillary Flow Technology modulator option or accessory
- Capillary Flow Technology modulator checkout kit
- Pneumatics control module (PCM)
- Agilent GC ChemStation B.03.02 or other data collection and analysis system that can control the flow modulator cycle
- 30-m × 0.25-mm × 0.25-µm DB-5ms column (included with option or accessory)
- 5-m × 0.25-mm × 0.15-µm INNOWax column (included with option or accessory)
- 2D data analysis software, GC Image recommended (not provided by Agilent)
- Internal column nuts and SilTite ferrules

Ordering Information

Description	Part number
7890A GC with Capillary Flow Technology Modulator (requires checkout kit)	G3440A Option 887 or accessory G3486A
7890A GC with 200 Hz FID	G3440A Option 211 or accessory G3462A
7890A GC with split/splitless inlet	G3440A Option 112 or accessory G3452A
Capillary Flow Technology modulator checkout kit	G3487A
PCM for 7890A GC	G3440A Option 309 or accessory G3471A
SilTite metal ferrules, 1/16-in × 0.4-mm id, 10/pk, includes 2 column nuts	5184-3569
Agilent 32-bit ChemStation for 1 GC	G2070BA
Agilent 32-bit ChemStation Bundle for 1 GC includes: – G2070BA 32-bit ChemStation software – Computer with monitor and Windows operating system – Printer	G1875BA
2D GC software Recommend GC Image software, which can be purchased from Zoex Corporation	www.zoex.com

Application Examples

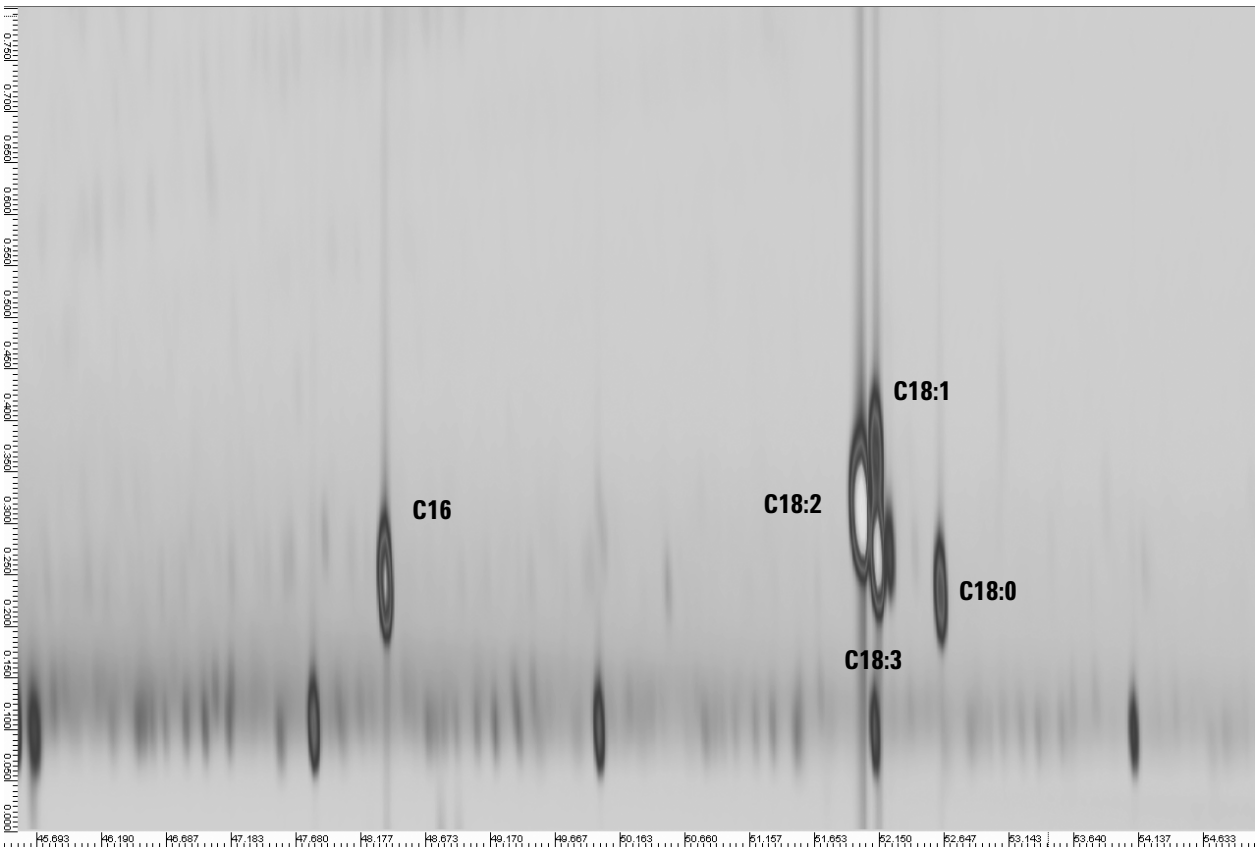
Several applications are shown. Note that primary column lengths have been chosen to give optimal results. While the 30M column that is shipped with the system is an excellent choice for a wide range of applications, other lengths can be used to optimize a given separation. Various columns have been used in these examples to illustrate some of the possibilities. The GC Image software package was used for processing the ChemStation data.

1. B20 biodiesel based on soy FAMES. Section of the 2D image showing the C16 and C18 FAMES is shown.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

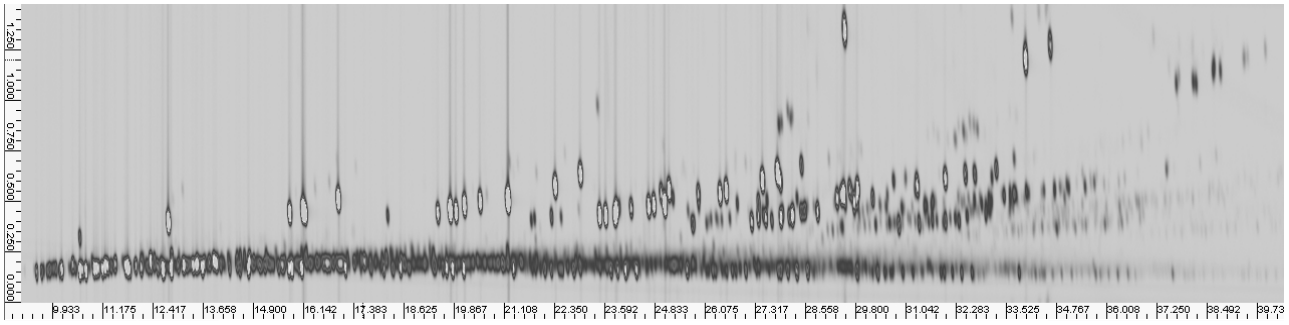


2. Complete 2D image of a sample of heavy gasoline. Each series of substituted 1-ring aromatics is well separated, making hydrocarbon class grouping possible.

   Column 1: 60 m × 0.25 mm × 0.10 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm INNOWax

   Modulation: 1.40 s load, 0.10 s inject

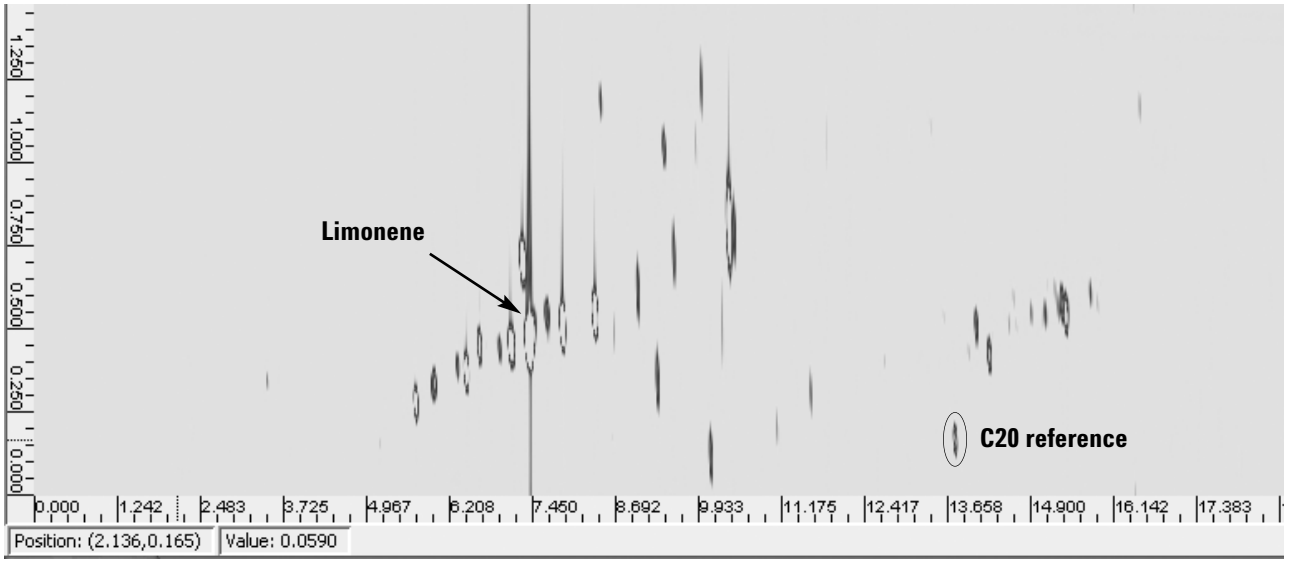


3. Lime oil 2D image.

   Column 1: 15 m × 0.25 m × 0.25 µm DB-5ms

   Column 2: 5 m × 0.25 mm × 0.15 µm DB-17HT

   Modulation: 1.40 s load, 0.10 s inject



Thermal vs. Flow Modulation

Since competitors offer only systems based on thermal modulation, the following table summarizes the key points about the respective approaches of thermal vs. flow modulation.

Thermal modulation	Differential Flow modulation
Cryo-focusing provides potentially narrower peaks in second dimension	Peak widths comparable to thermal. Usually no more than 20% wider. Many users want to sum regions of peaks where peak width is not as critical
Lower flows – Can be used with high-vacuum detectors (TOF)	MSD can be used with a splitter over limited scan range
Large consumption of cryogen	No cryogen required
Complex hardware design, set-up, and maintenance	Simple, reliable Capillary Flow Technology based hardware; small thermal foot print
Long chromatographic runs required for best performance	Run times comparable to a 1D separation
System price (estimate) $60 to $70K	Agilent system approximately $60K (list)

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Agilent Technologies

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

Setup Example Using Biomarkers

Application

Hydrocarbon Processing

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Abstract

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

Introduction

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

The benefits of backflush in capillary gas chromatography are myriad:

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

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

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

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

Experimental

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

Table 1. Original Analytical Method Conditions

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

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

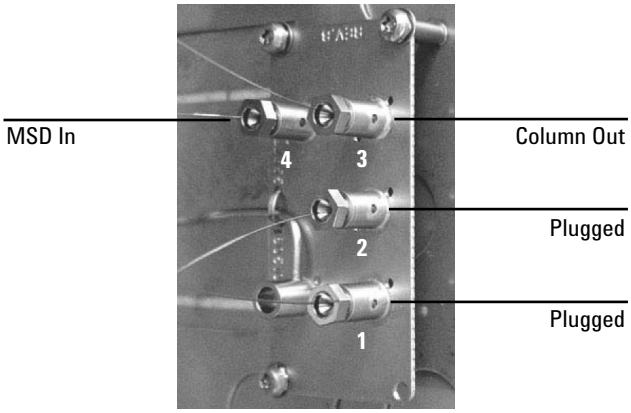

Figure 1. 3-way purged splitter. The column outlet was attached to port 3 and the MSD restrictor was attached to port 4. Ports 1 and 2 were plugged.

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

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

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

Table 2. Backflush Analytical Method Conditions

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

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

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

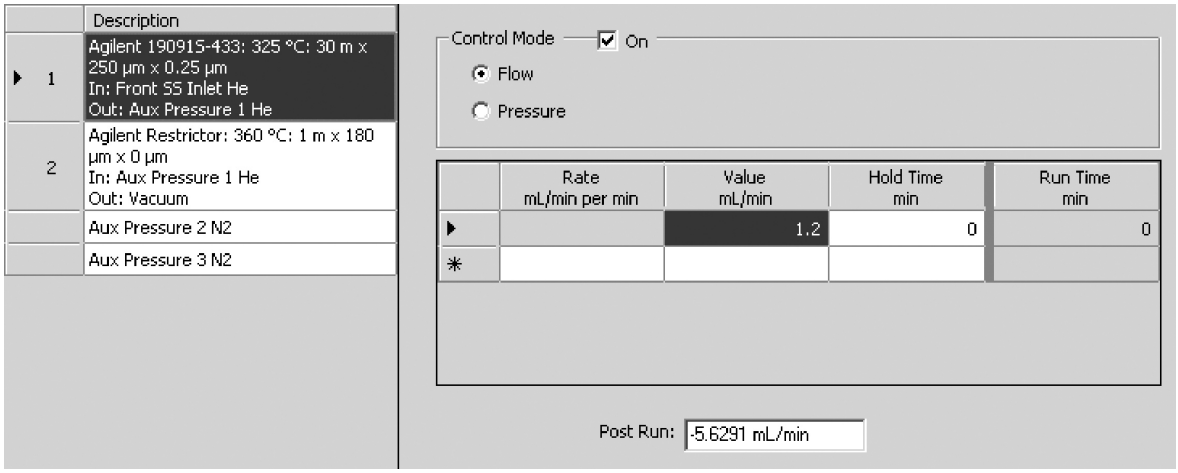

Figure 2. Software setpoints for the analytical column.

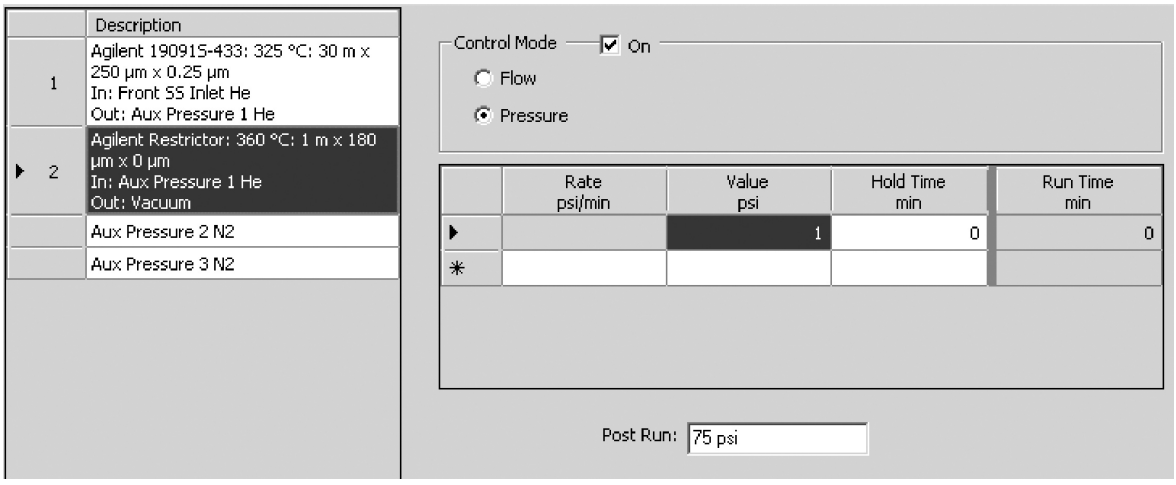

Figure 3. Software setpoints for the restrictor to the MSD.

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

Figure 4. Column inlet and outlet conditions.

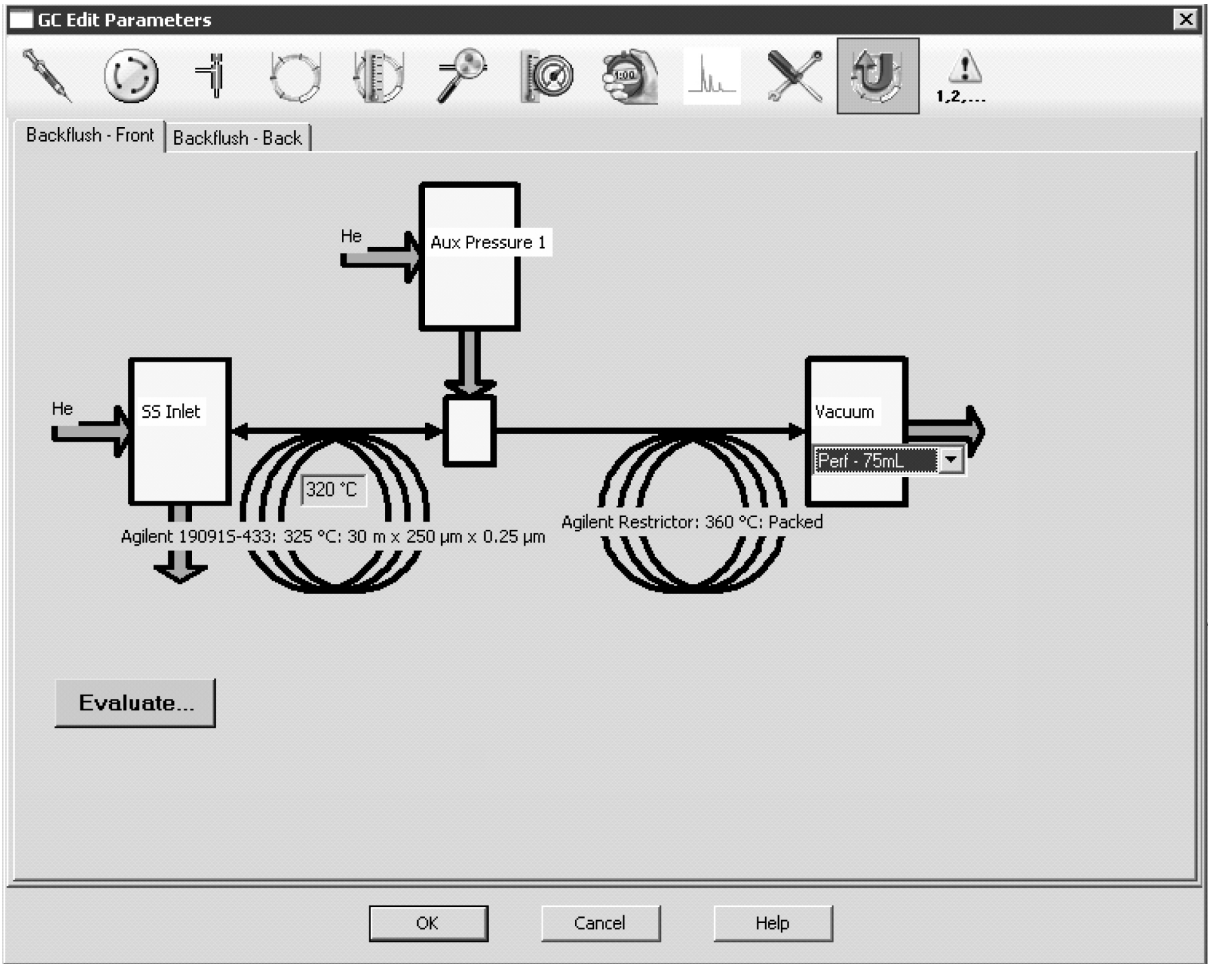

Figure 5. Interactive setup for backflush conditions in ChemStation.

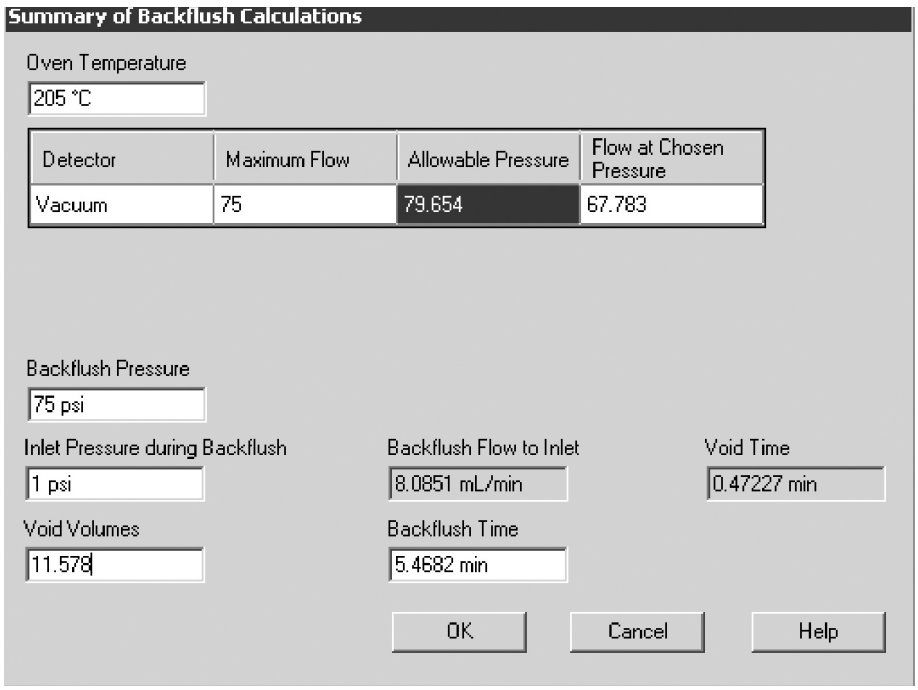

Figure 6. Conditions uploaded to method setpoints.

Results and Discussion

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

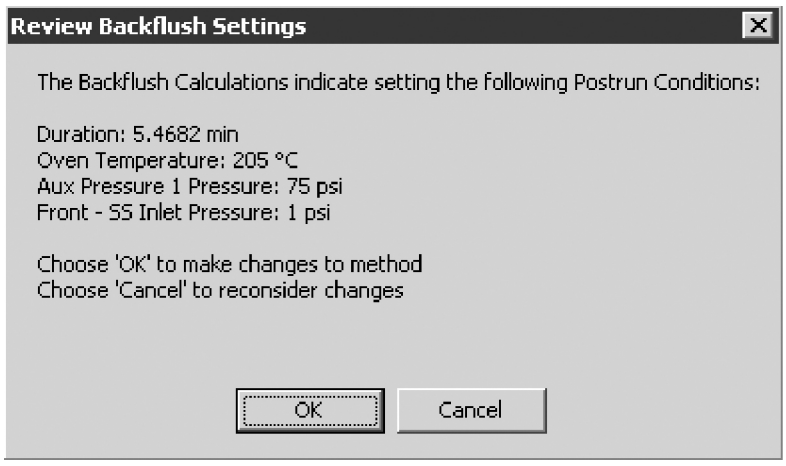

Figure 7. Conditions uploaded to method setpoints.

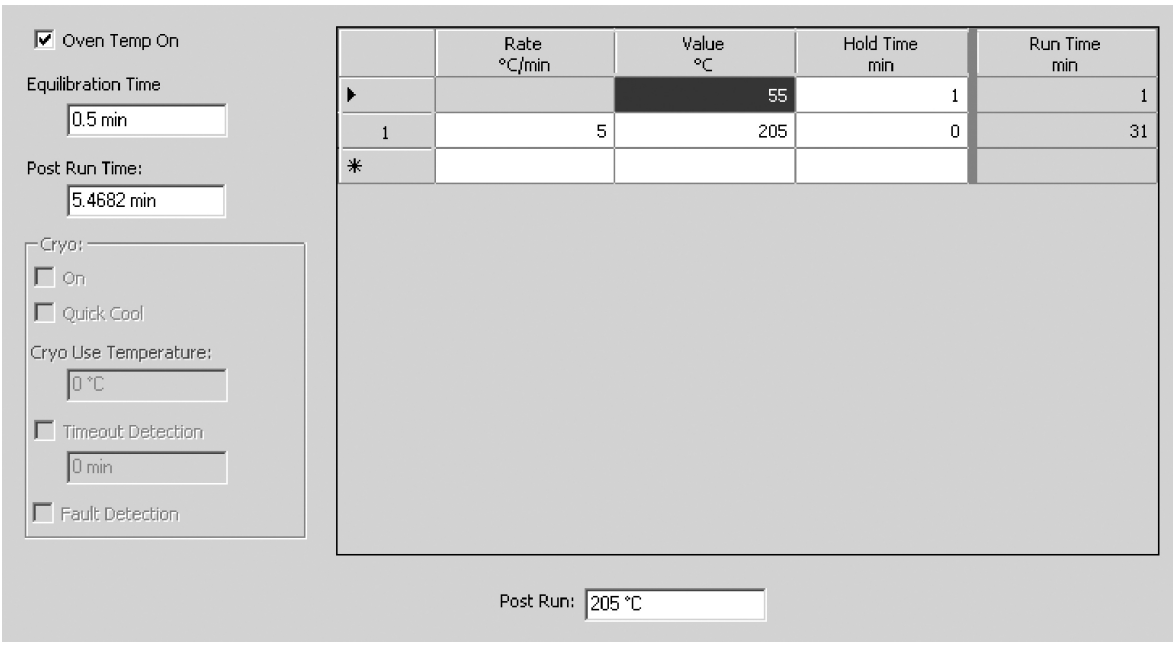

Figure 8. Note that the post-run time has been updated automatically.

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

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

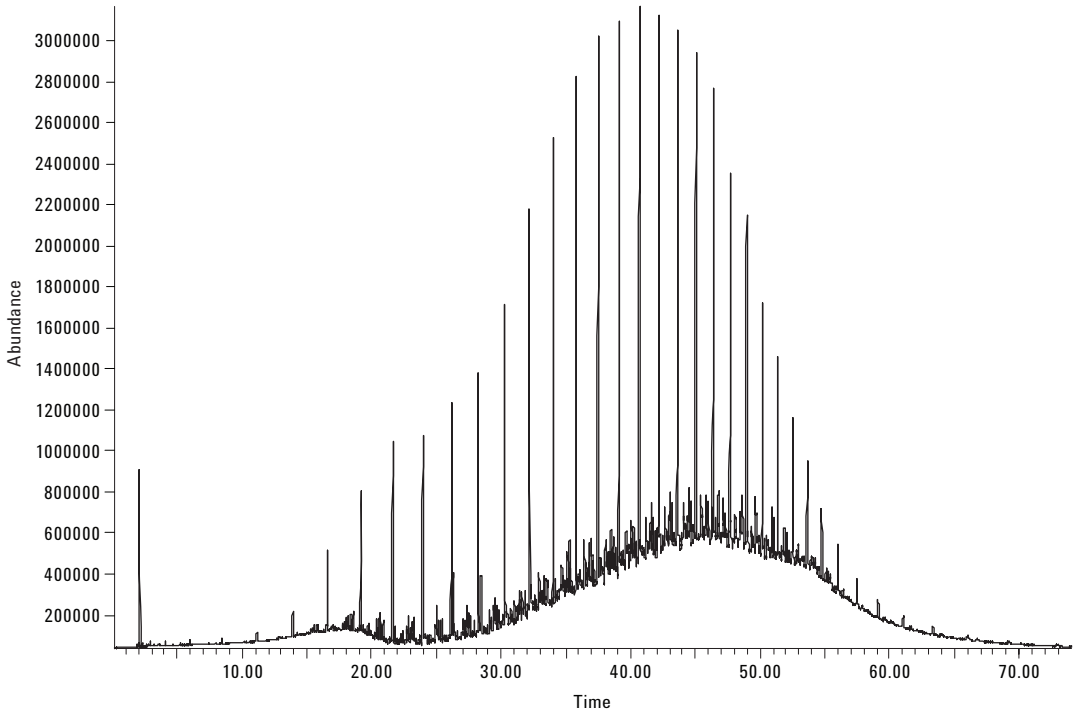

Figure 9. Total ion chromatogram (TIC) of normal analysis. Peaks of interest (benzothiophenes) are obscured by the high concentration of hydrocarbons.

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

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

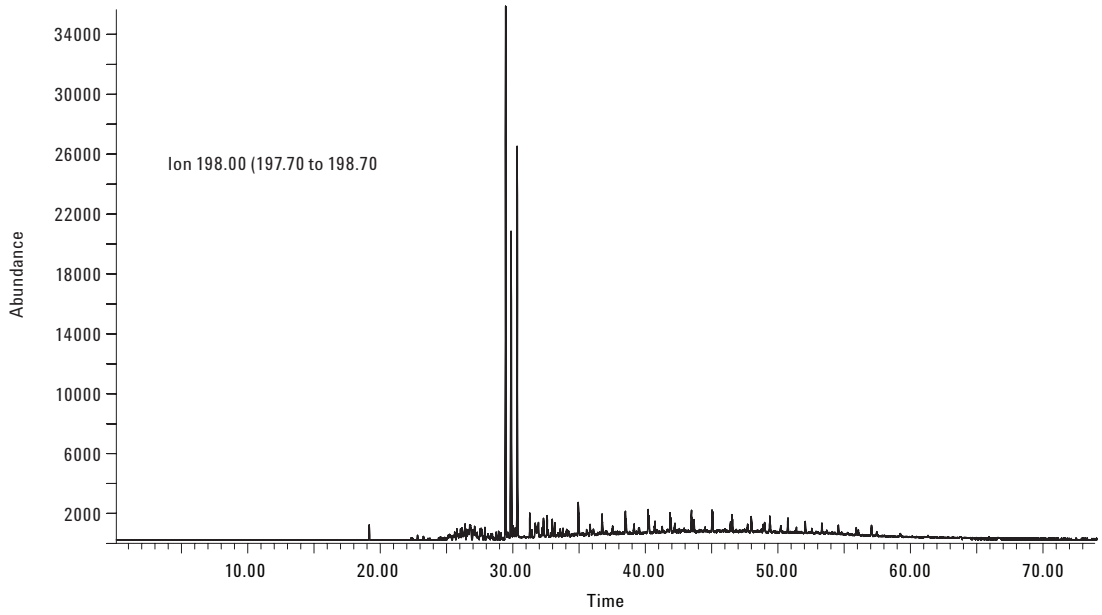

Figure 10. EIC of *m/z* 198 ion. The three methylbenzothiophene peaks of interest at approximately 30 minutes are easily visualized.

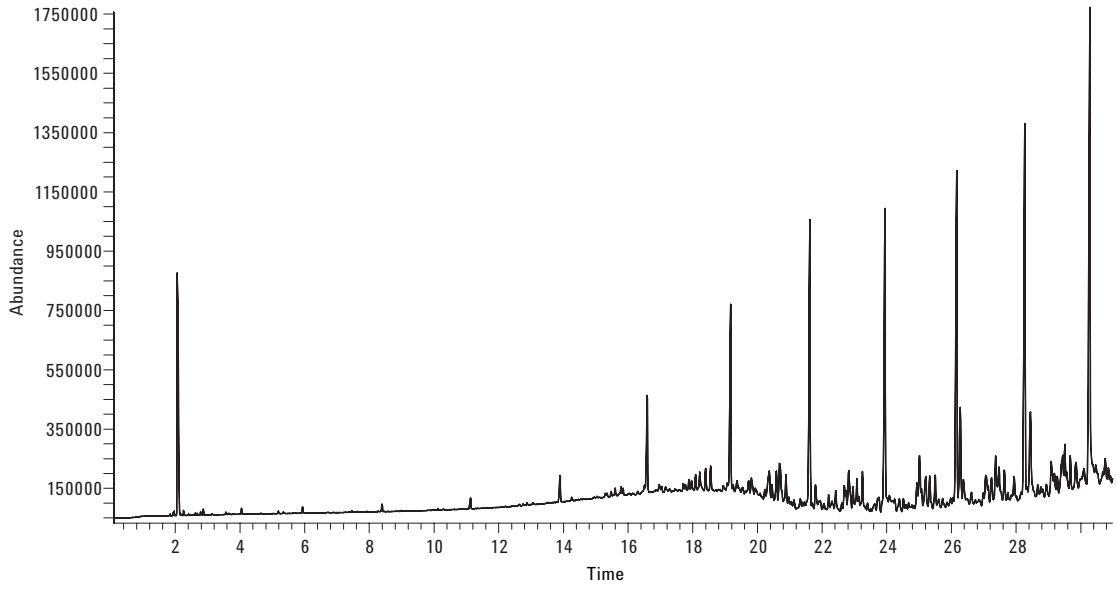

Figure 11. TIC of backflush run; run switched to backflush mode at 31 minutes.

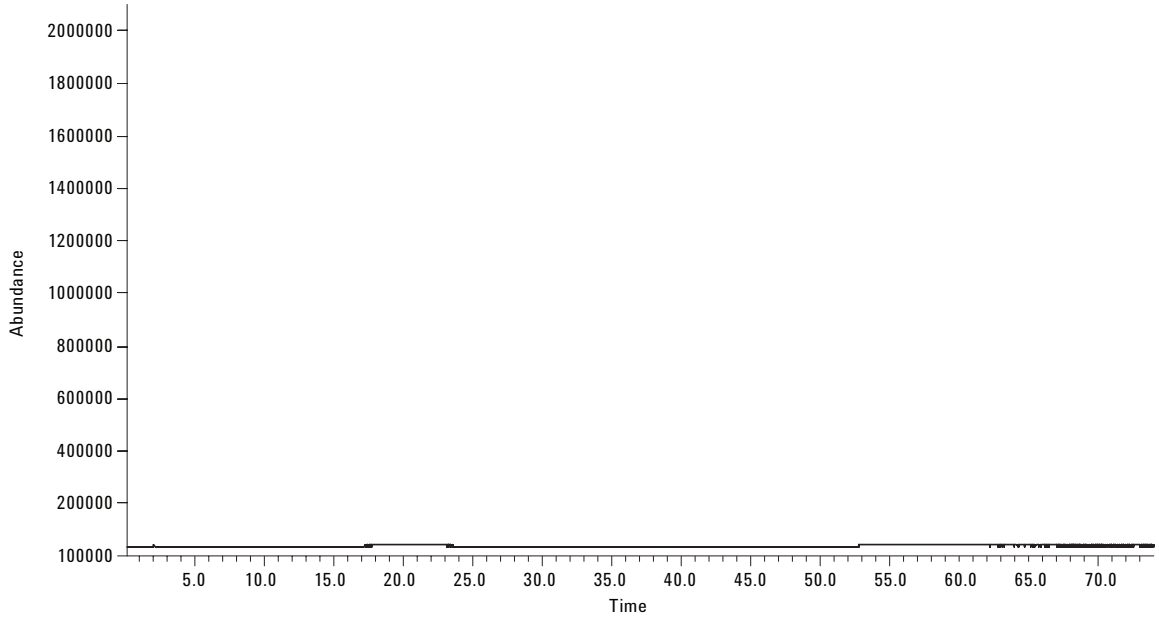

Figure 12. TIC of full run after the backflush with inset of the EIC of *m/z* 198.

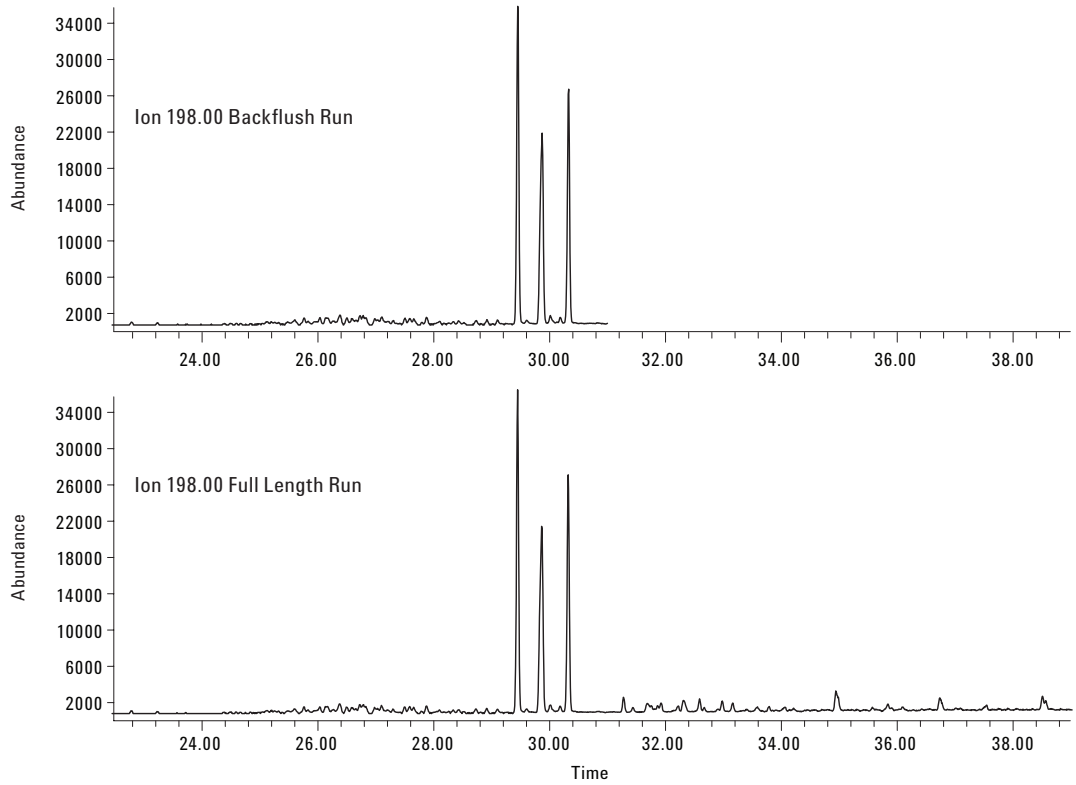

Figure 13. Overlay of EIC of *m/z* 198 from full run and backflush run, showing the exact matching of the analytical portion of each run for the three methylbenzothiophene biomarkers.

Conclusions

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

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

Application

Gas Chromatography

Authors

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Abstract

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

Introduction

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

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

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

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

Experimental

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

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

Results and Discussion

Analysis of Normal Hydrocarbons and Methanol

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

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

Table 1. Conditions for Normal Hydrocarbons and Methanol Analysis

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

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

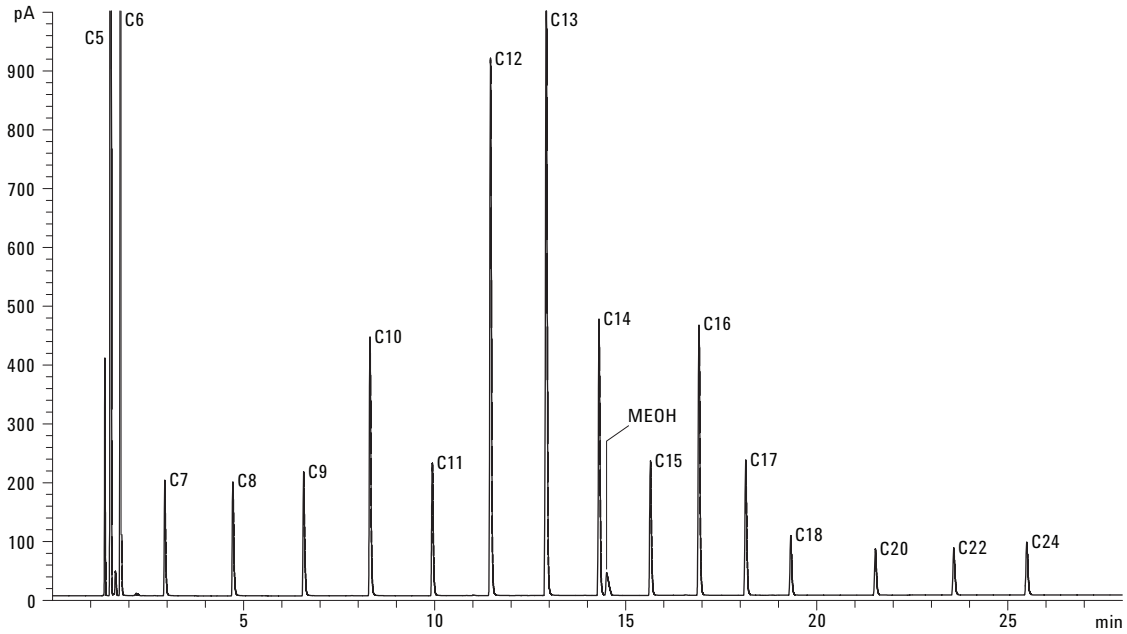

Figure1. Analysis of methanol and normal hydrocarbons on a GS-OxyPLOT column, 10 m × 0.53 mm × 10 µm.

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

Analysis of Alcohols

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

Sample

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

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

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

Table 2. Conditions for Alcohols Analysis

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

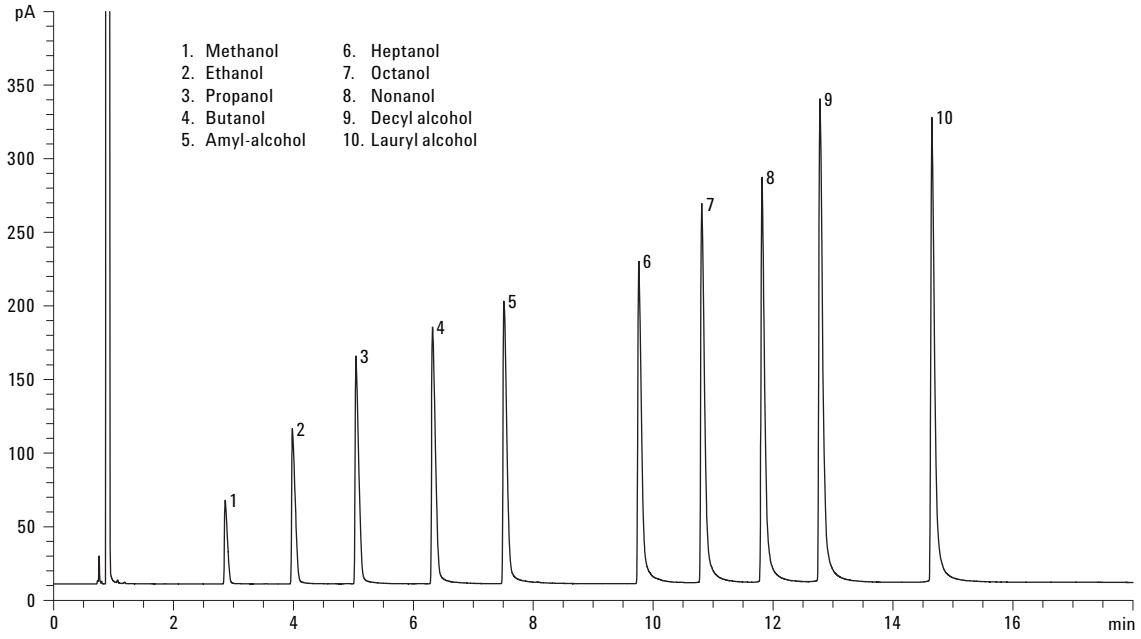

Figure 2. Separation of alcohols using GS-OxyPLOT, 10 m × 0.53 mm × 10 µm.

Influence of Temperature on the Selectivity of GS-OxyPLOT

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

Table 3. Conditions for Kovats Retention Indices Test

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

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

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

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

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

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

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

Influence of Moisture on GS-OxyPLOT

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

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

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

Table 5. Conditions for Moisture Test

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

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

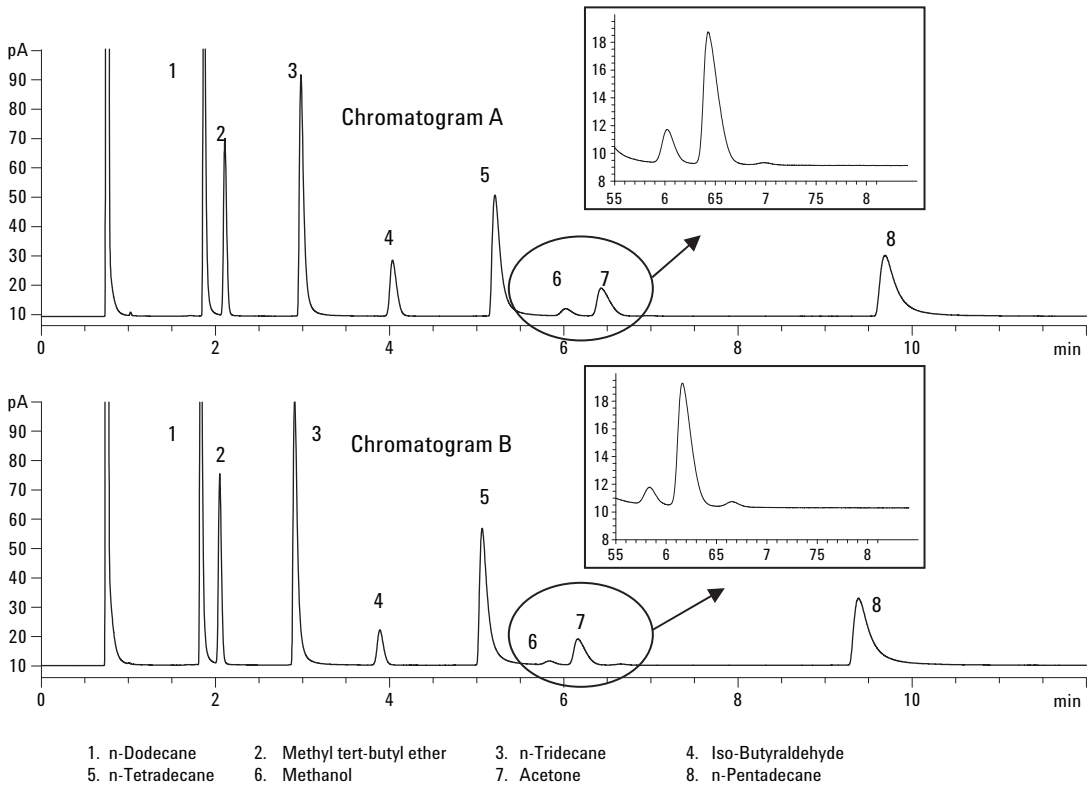

Figure 3. Comparison of test mixture separation before (A) and after (B) 100 injections of water.

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

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

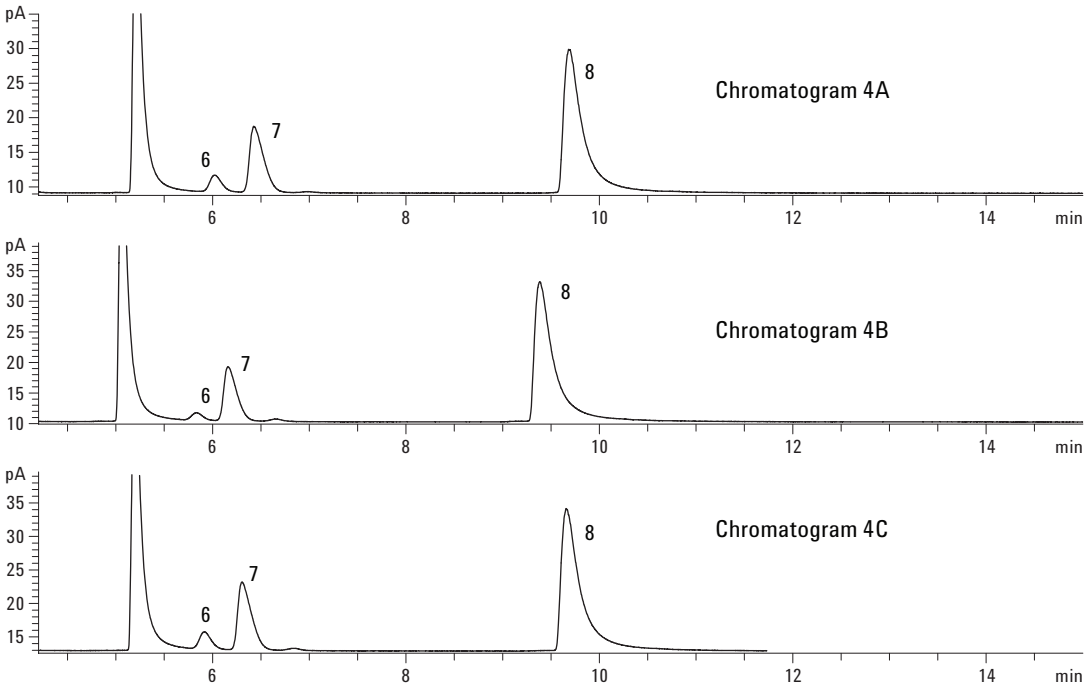

Figure 4. Expanded view shows comparison of test mixture separation on GS-OxyPLOT.
4A. Before injection of water. 4B. After 100 injections of water. 4C. After conditioning the column.

Table 6. Comparison of Test Mixture Separation

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

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

Determination of Methanol

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

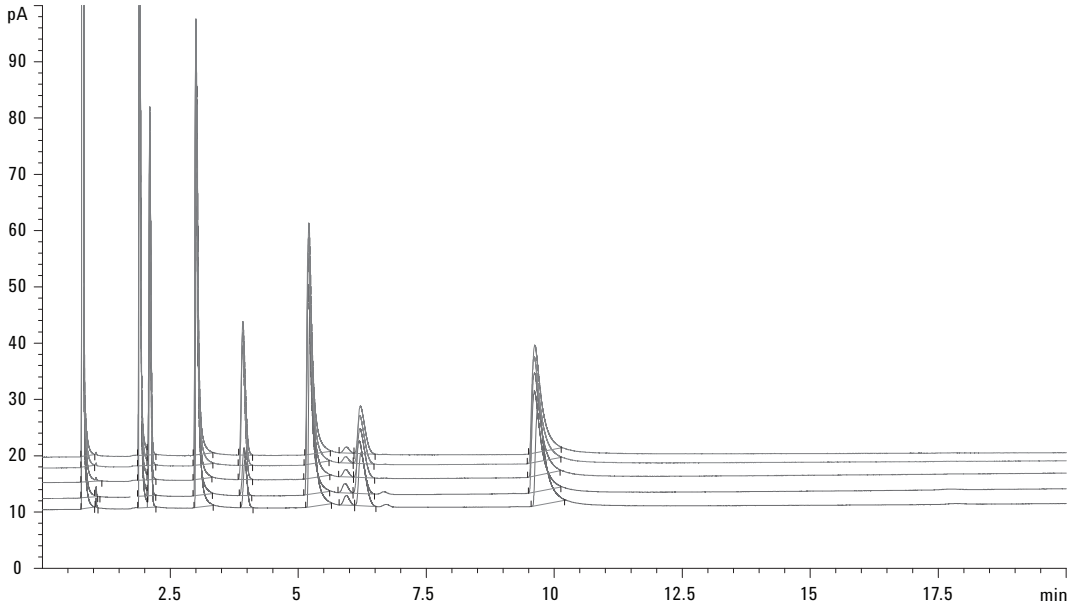

Figure 5. Fifth run overlaid using GS-OxyPLOT (after conditioning column).

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

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

Reagents and Materials

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

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

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

Linearity

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

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

Table 8. System Settings for the Calibration Curve

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

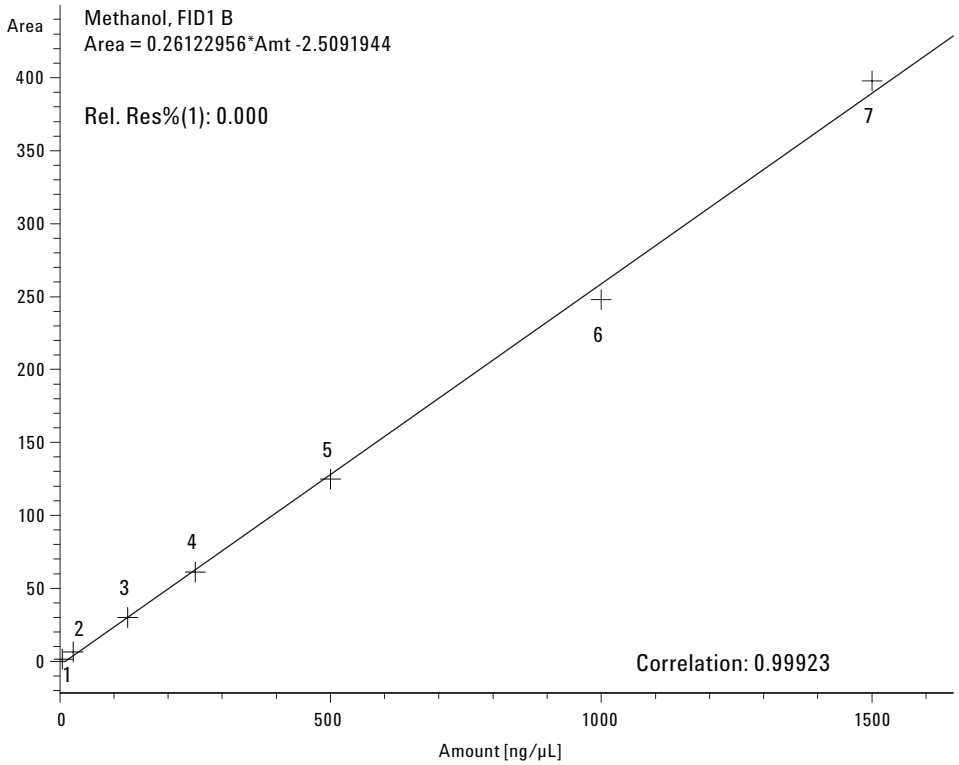

Figure 6. The calibration curve of methanol in toluene.

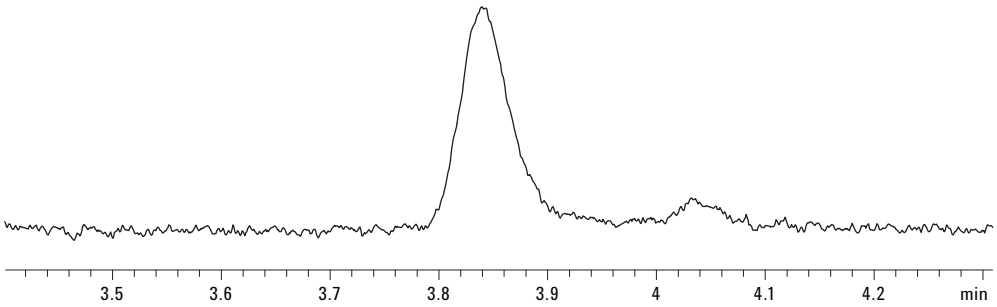

Figure 7. Test mixture of 5 ppm methanol in toluene.

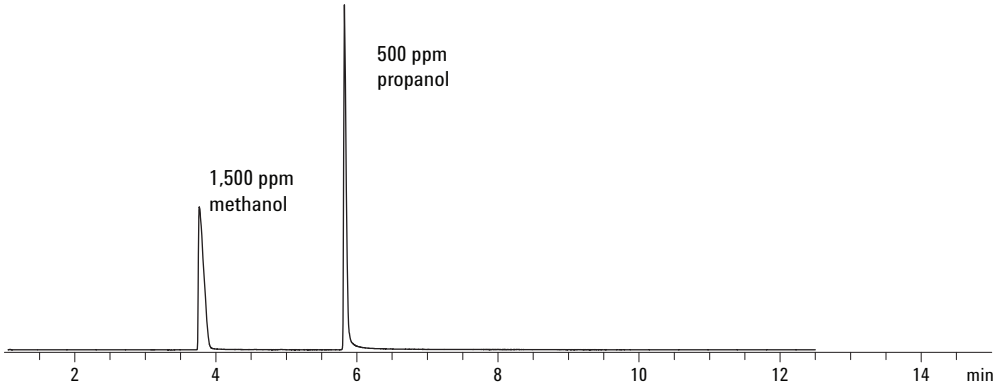

Figure 8. Test mixture of 1,500 ppm methanol in toluene.

Repeatability

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

Life Span

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

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

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

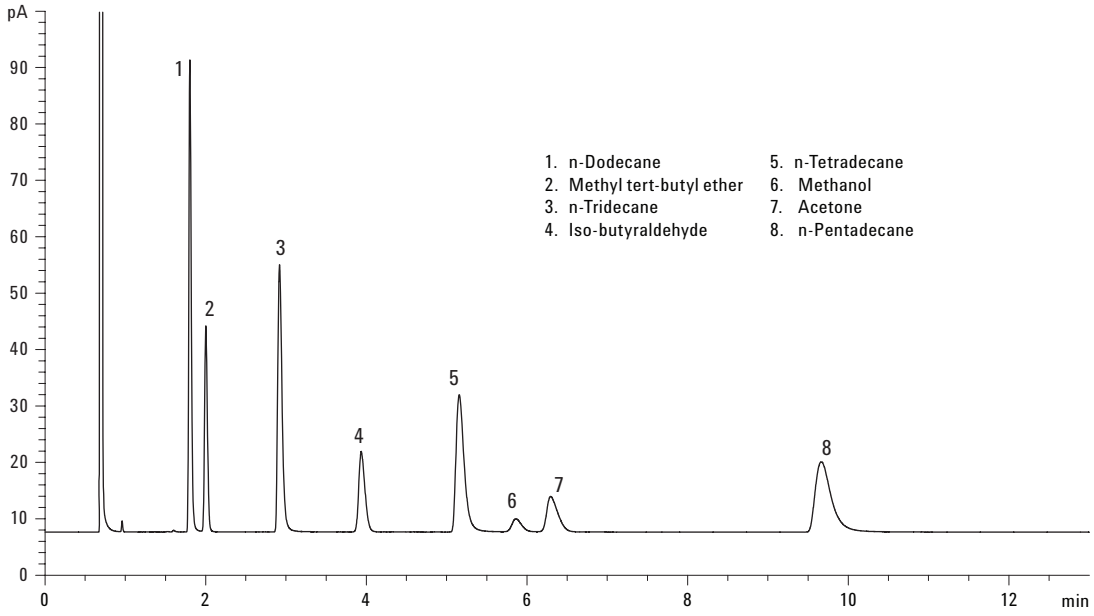

Figure 9. Chromatogram of performance mixture after 1,500 injections.

Conclusions

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

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

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

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

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

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Application Brief

HPI

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

Experimental

Table 1. Typical GC Conditions

Inlet settings	250 °C, Split ratio: 100:1 to 30:1
Injection volume	0.5 µL
Column	HP-INNOWax 60 m × 0.32 µm × 0.5 µm
Column flow (He)	2.6 mL/min (21.8 at 75 °C), constant flow mode
Oven temperature program	For impurities in benzene: 75 °C (10 min); 3 °C/min to 100 °C For aromatic solvent: 75 °C (10 min); 3 °C/min to 100 °C 10 °C/min to 145 °C
FID setting	
Temperature	250 °C
H2 flow	40 mL/min
Air flow	400 mL/min
Make up (N2)	25 mL/min
Data acquisition rate:	20 Hz

Highlights

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

Agilent Technologies

Discussion

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

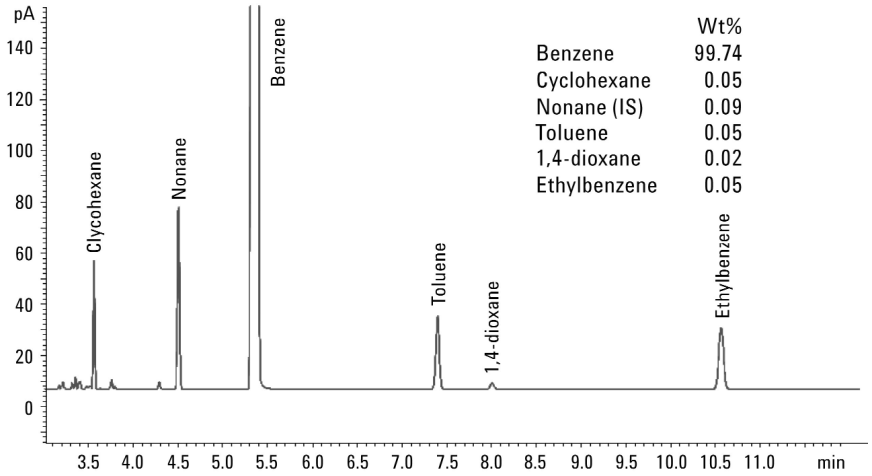


Figure 1. ASTM D4492 benzene calibration standard. *Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 100:1.*

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

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

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

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

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

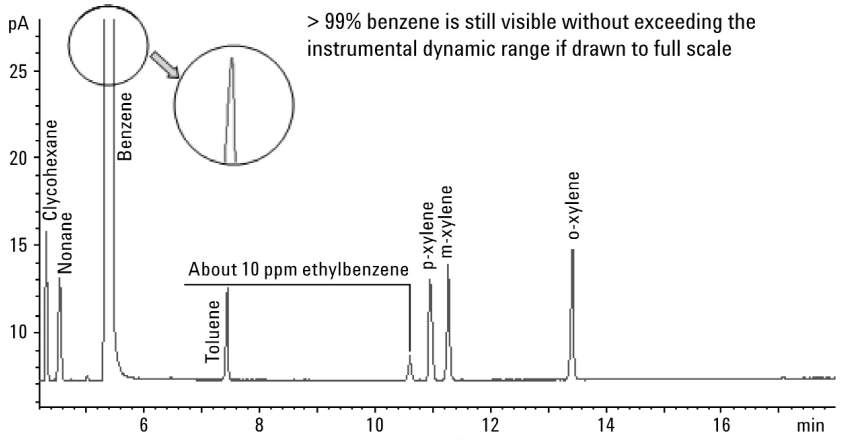
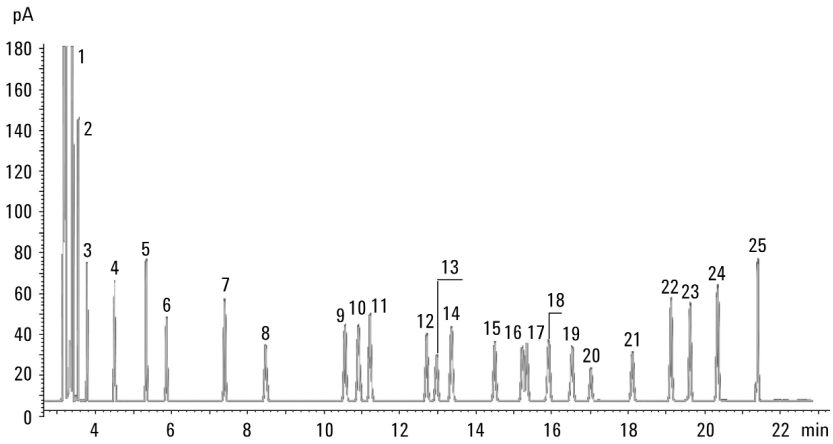


Figure 2. ***Analysis of trace impurities spiked in benzene.*** *Oven temperature program: 75 °C (10 min); 3 °C/min to 100 °C. Sample size: 0.5 µL, Split ratio: 30:1.*

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



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

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

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

Application Note

All Industries

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Abstract

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

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Introduction

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

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

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

Experimental

MMI Operational Modes

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

Hot Splitless (for 1 – 3 µL injections)

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

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

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

Cold Splitless (for 1 – 10 µL injections)

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

Solvent Vent (for 5 – 1000 µL injections)

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

LVI Method Development

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

Backflush

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

Instrument Parameters

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

Oven

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

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

Multimode Inlet (MMI)

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

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

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

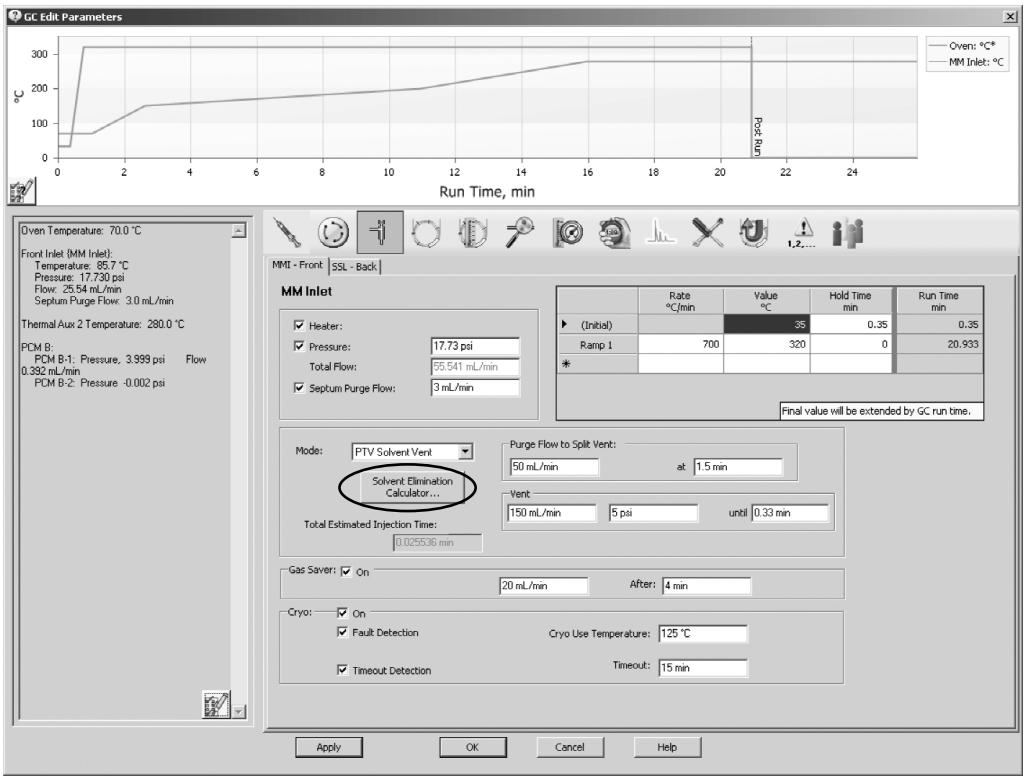

Figure 1. Multimode Inlet "Solvent Elimination Calculator" imbedded in ChemStation for easy method development.

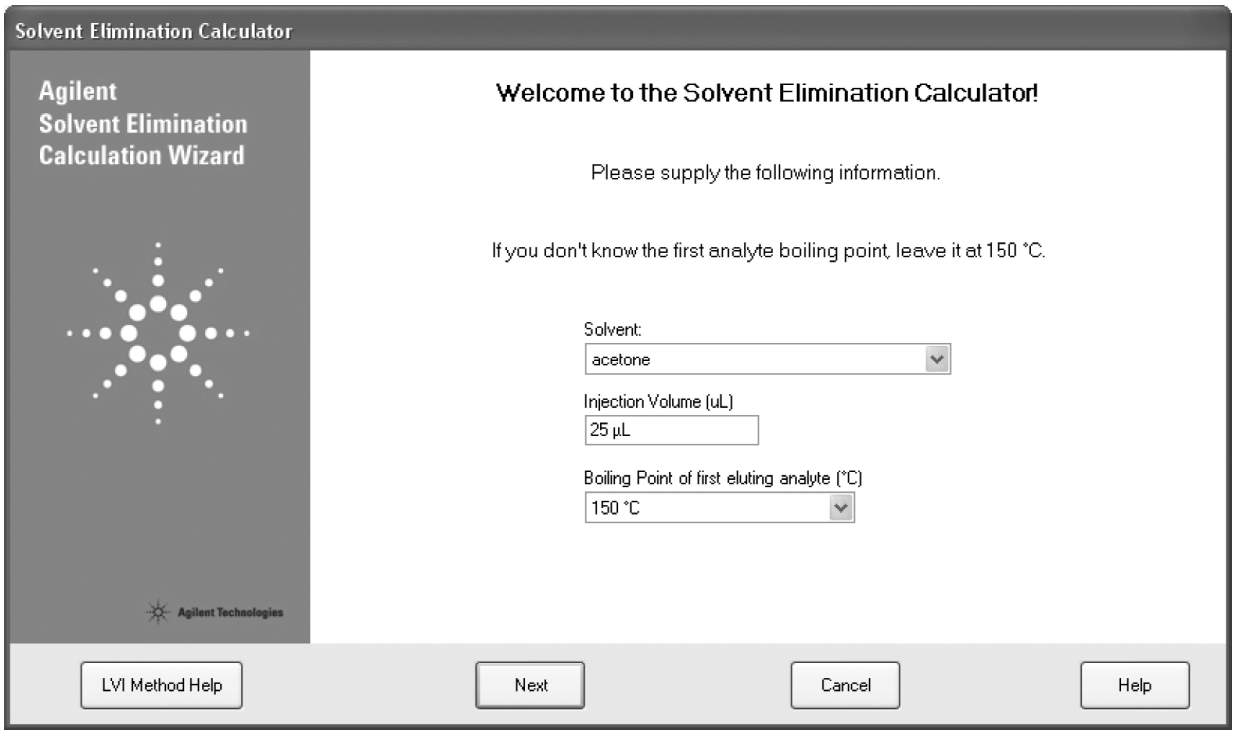

Figure 2. Select solvent of choice and enter the injection volume to start the calculation.

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

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

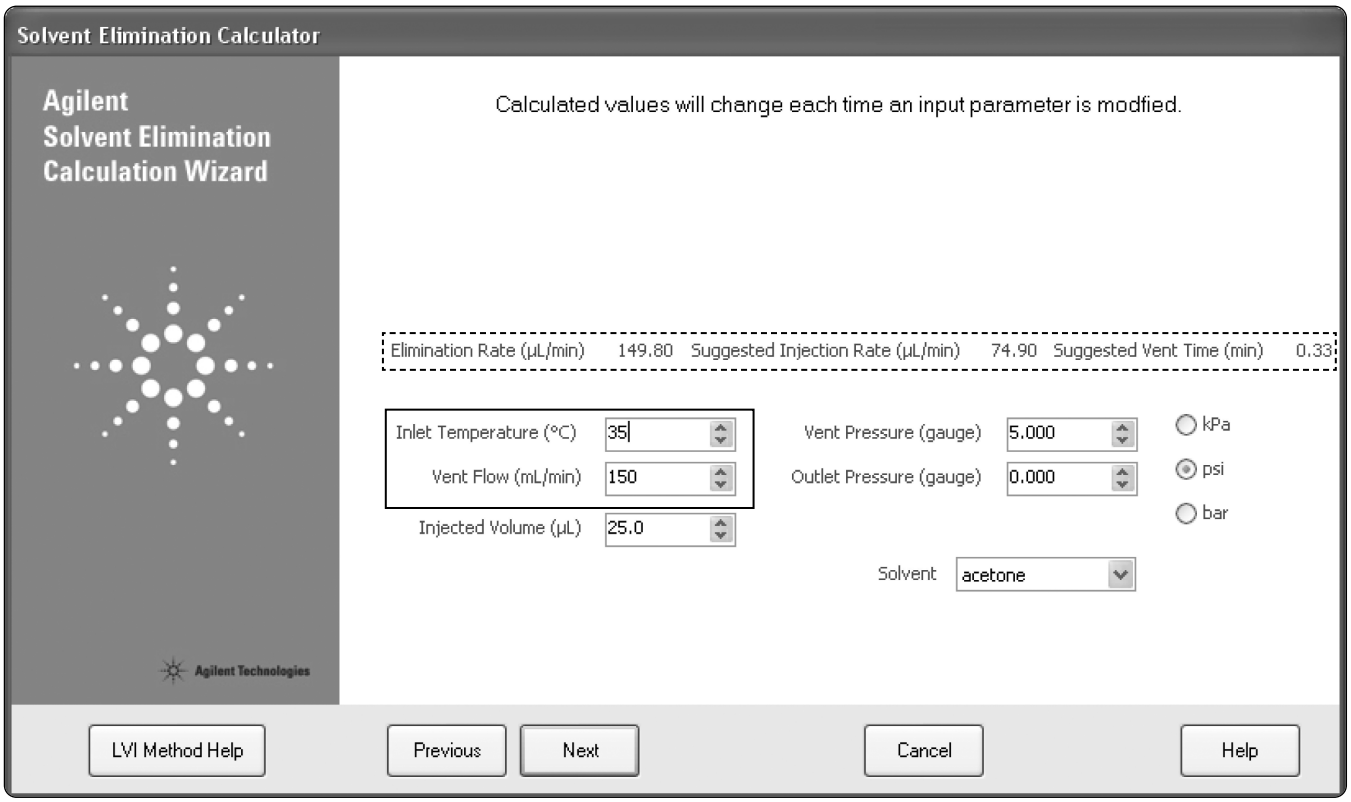

Figure 3. The calculator calculates the injection rate and vent time according to the selected inlet temperature and vent flow.

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

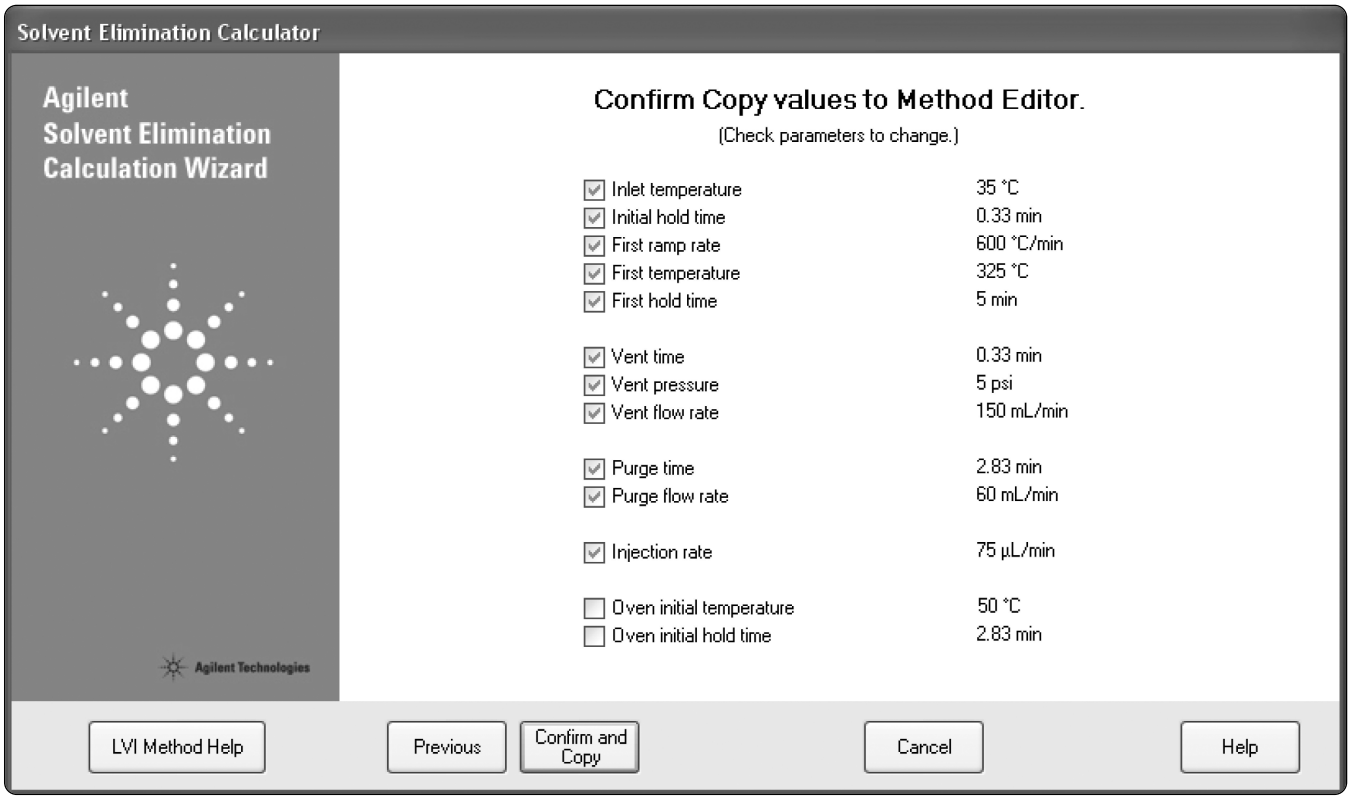

Figure 4. Confirm values suggested by the Calculator and download to ChemStation.

Results and Discussion

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

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

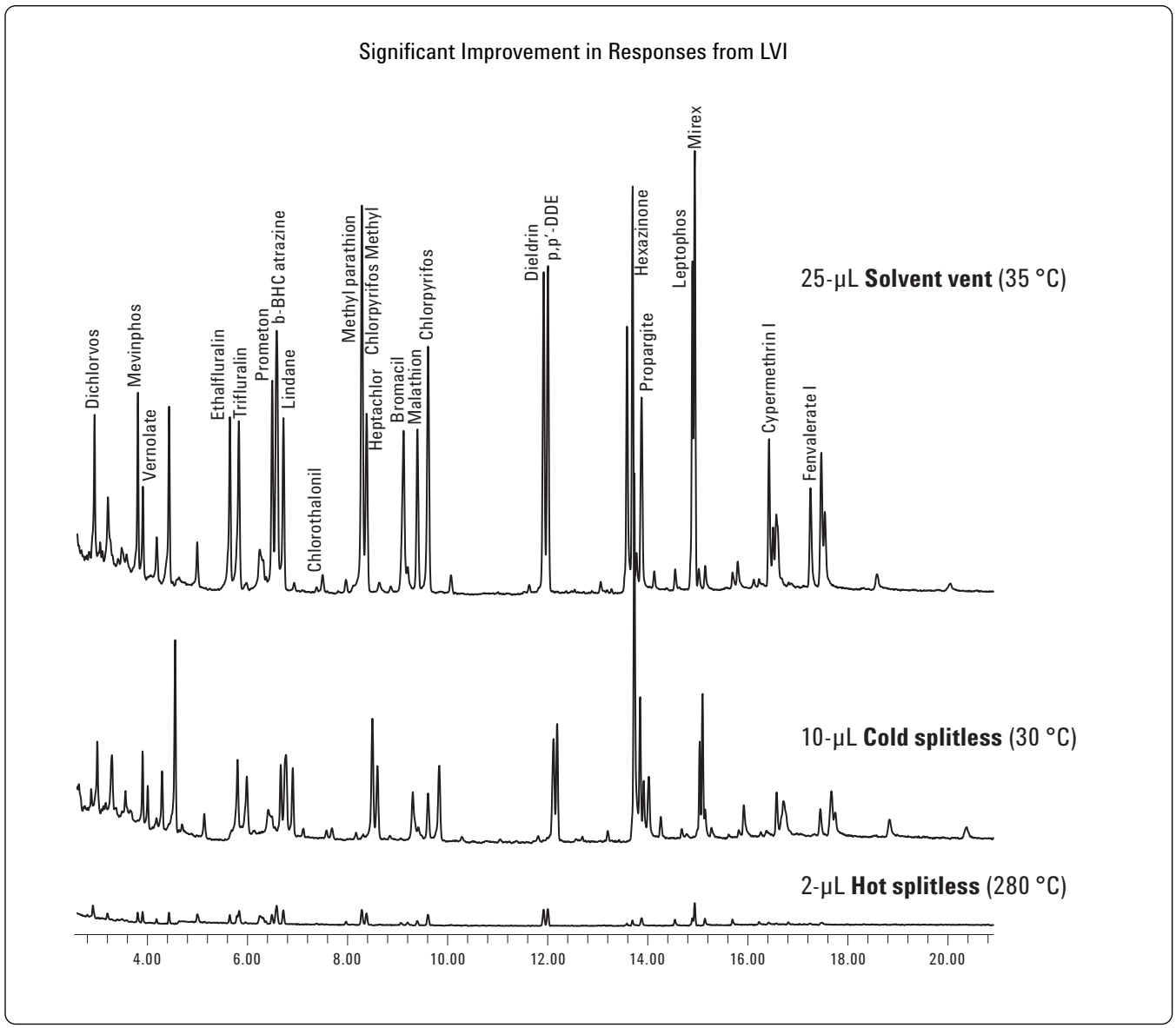

Figure 5. Overlay of total ion chromatograms (TICs) from three injection modes, plotted on the same scale.

Conclusion

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

References

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

Application Note

HPI

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Highlights

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

Abstract

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

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Introduction

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

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

Experimental

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

Table 1. Gas Chromatographic Conditions

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

Table 2. Time Events

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

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

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

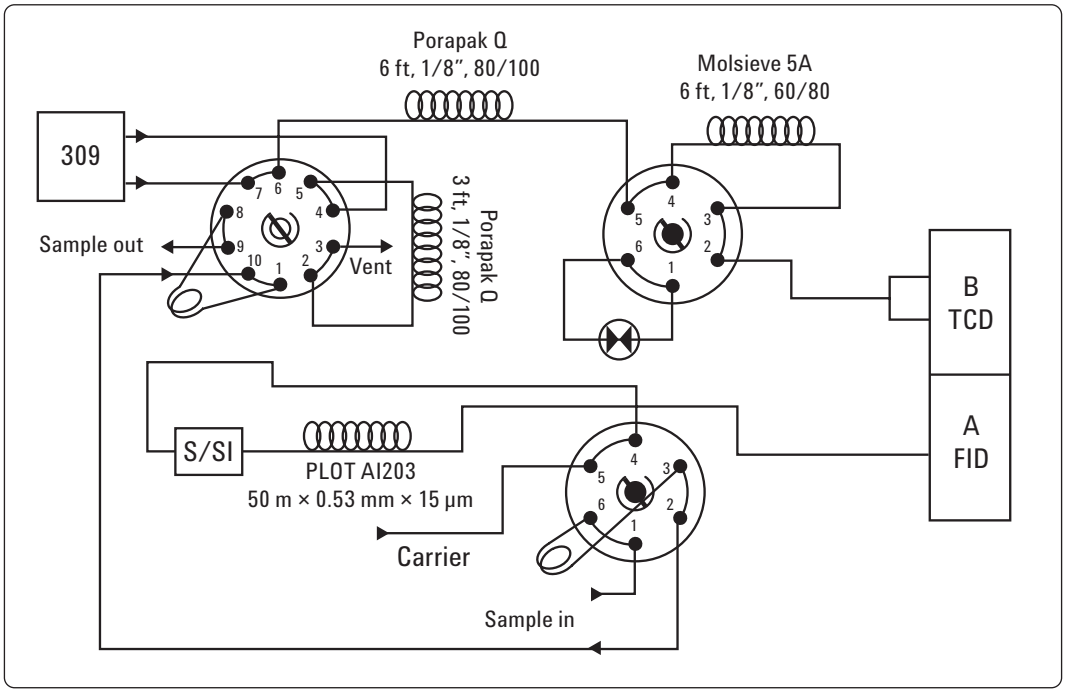

Figure 1. Valve diagram for dual-channel natural gas analysis.

Table 3. Concentrations of the Standard Gases

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

Results

Chromatograms

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

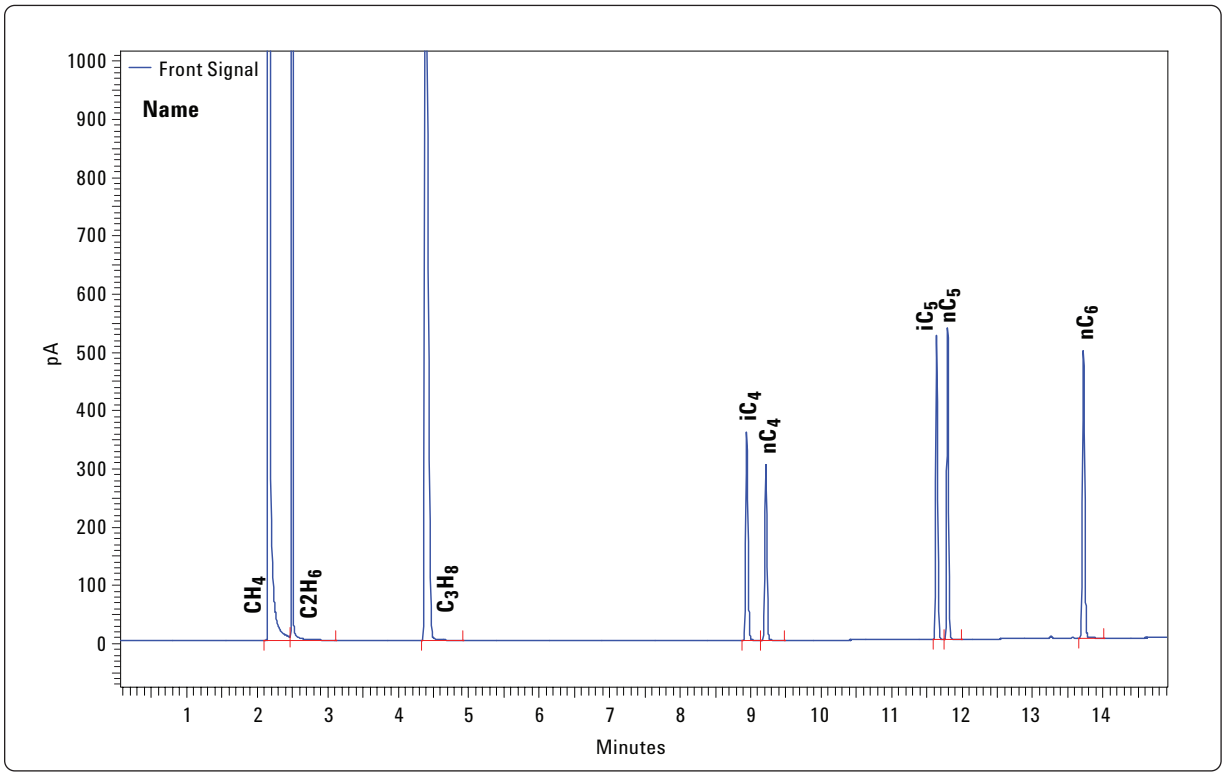

Figure 2. FID Channel chromatogram of CH_4, C_2H_6, C_3H_8, iC_4, nC_4, iC_5, nC_5, and nC_6.

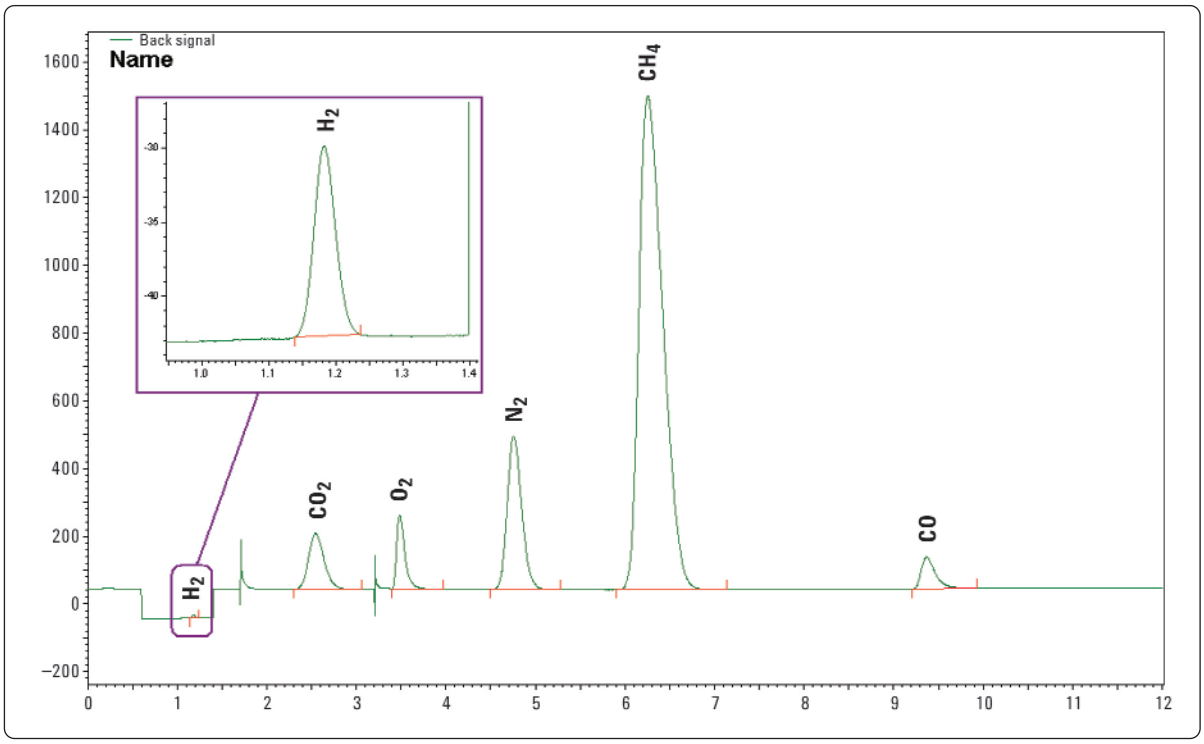

Figure 3. TCD Channel chromatogram of H_2, O_2, CO_2, N_2, CH_4, CO.

Linearity

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

Table 4. Linearity Results of TCD Channel

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

Repeatability

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

Table 5. TCD Channel Repeatability

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

Low Level Permanent Gases

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

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

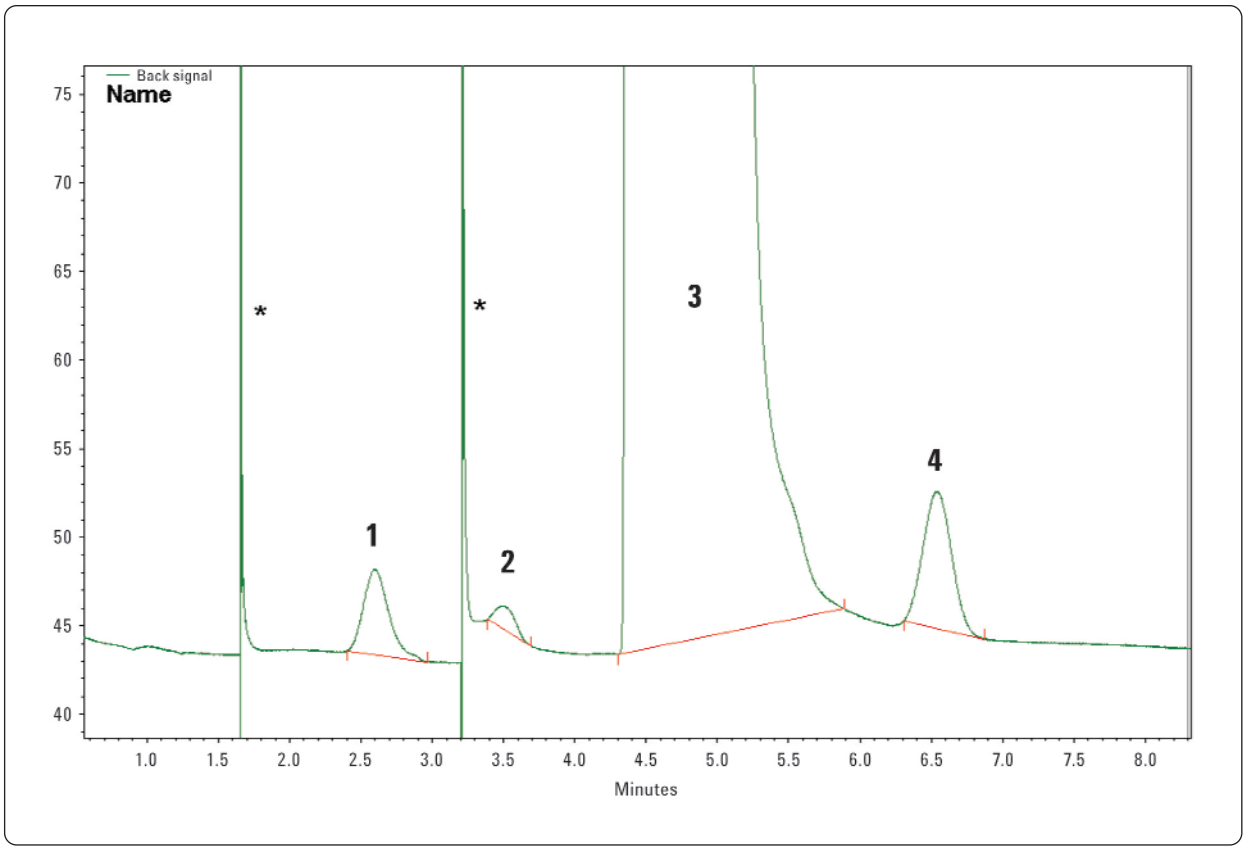

Figure 4. Chromatogram of low level permanent gas standard mix.

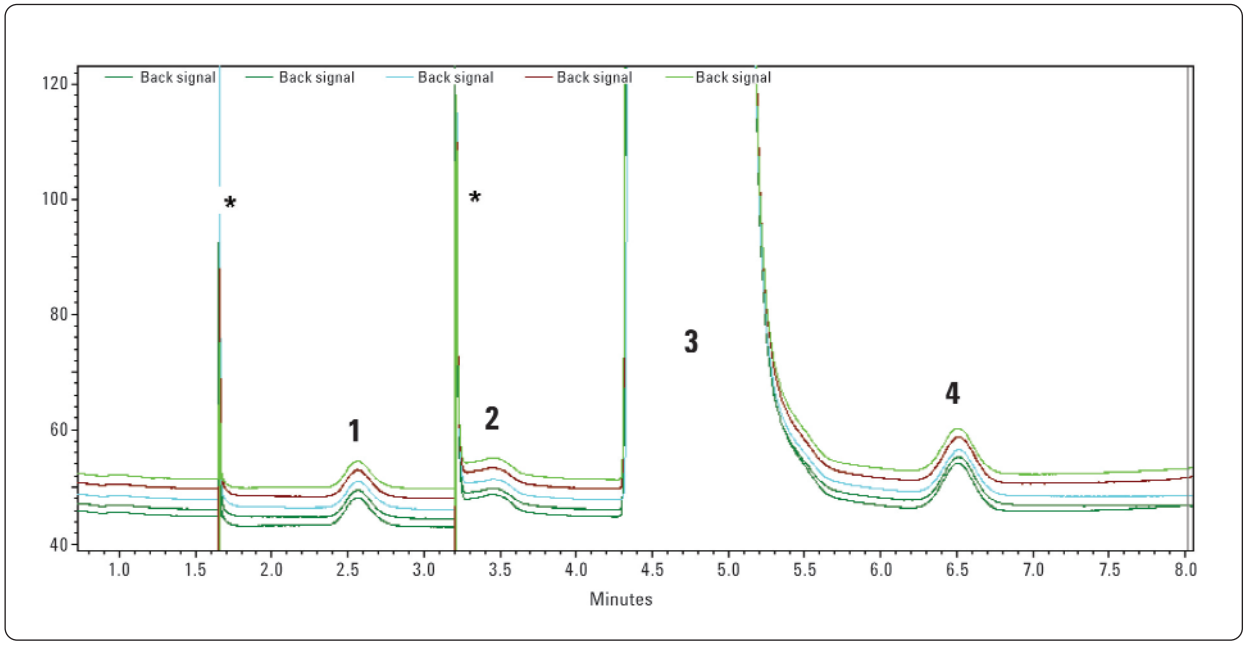

Figure 5. Overlapped chromatograms of five runs.

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Agilent Technologies

Fast analysis of polyaromatic hydrocarbons using the Agilent 1290 Infinity LC and Eclipse PAH columns

Application Note

Environmental

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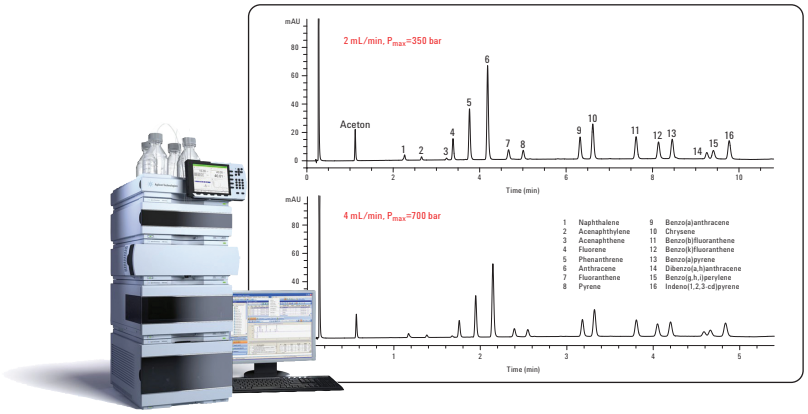
Belgium



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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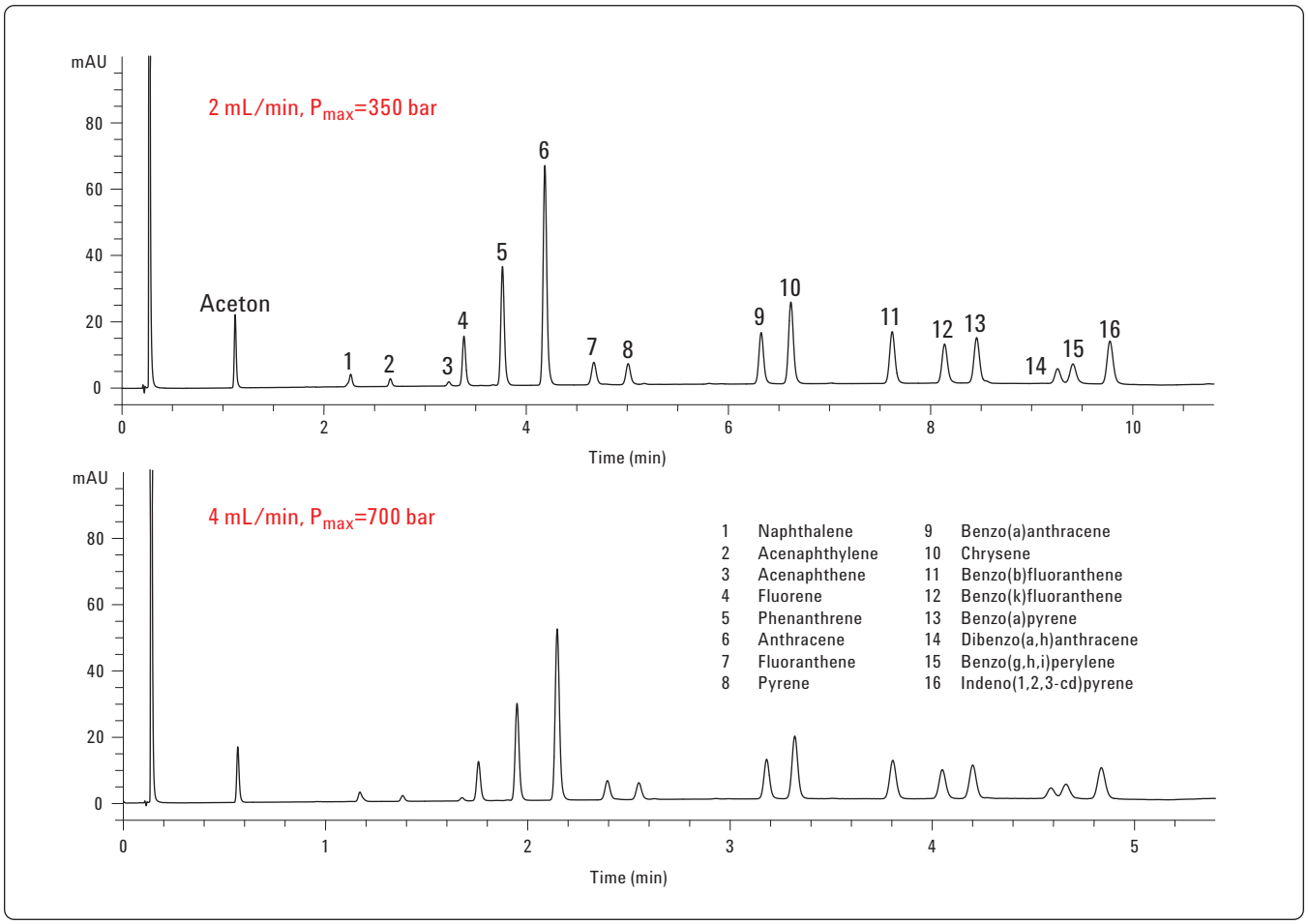

Figure 1
Analysis of 16 PAHs on the 1290 Infinity LC. Sample: standard solution of 16 PAHs, 50 µg/mL each.

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.

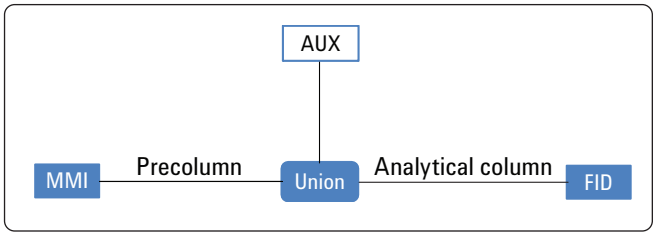

Figure 1. Basic precolumn backflush configuration with purged union.

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 x 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 x 0.53 mm deactivated retention gap
Column 1 Flow:	9 mL/min
Column 2:	5 m x 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.

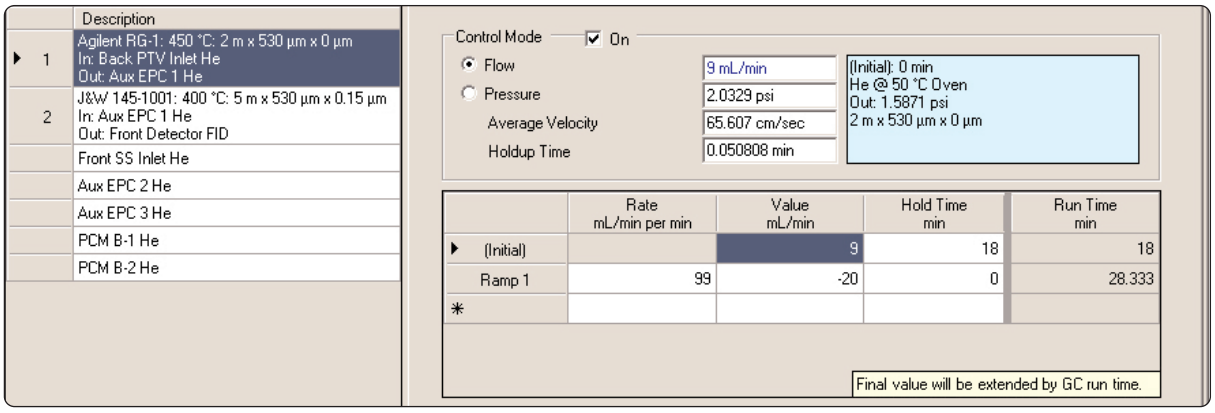

Figure 2A. Precolumn flows. Backflush starts at 18 min in this example.

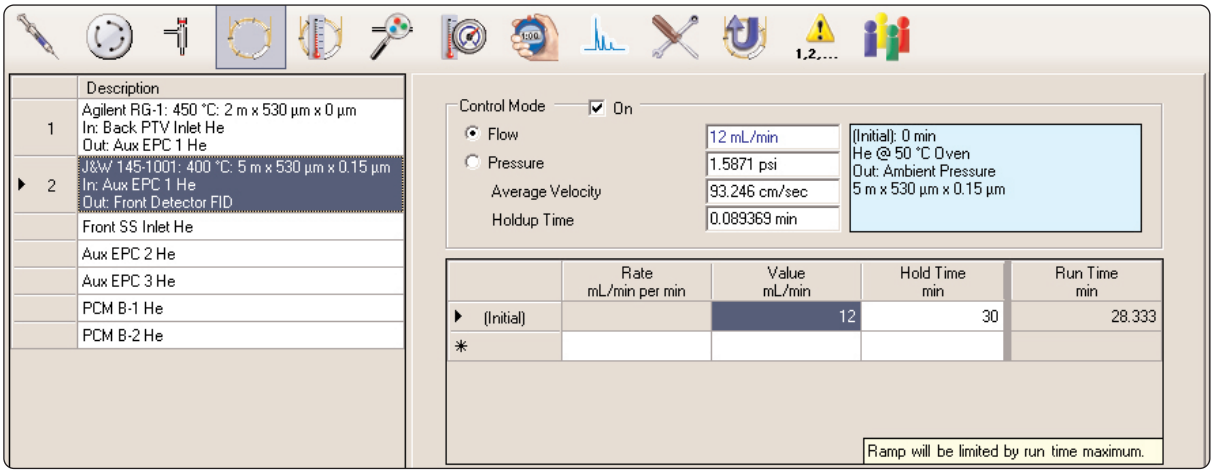

Figure 2B. Analytical column flow set at 12 ml/min for the entire run.

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).

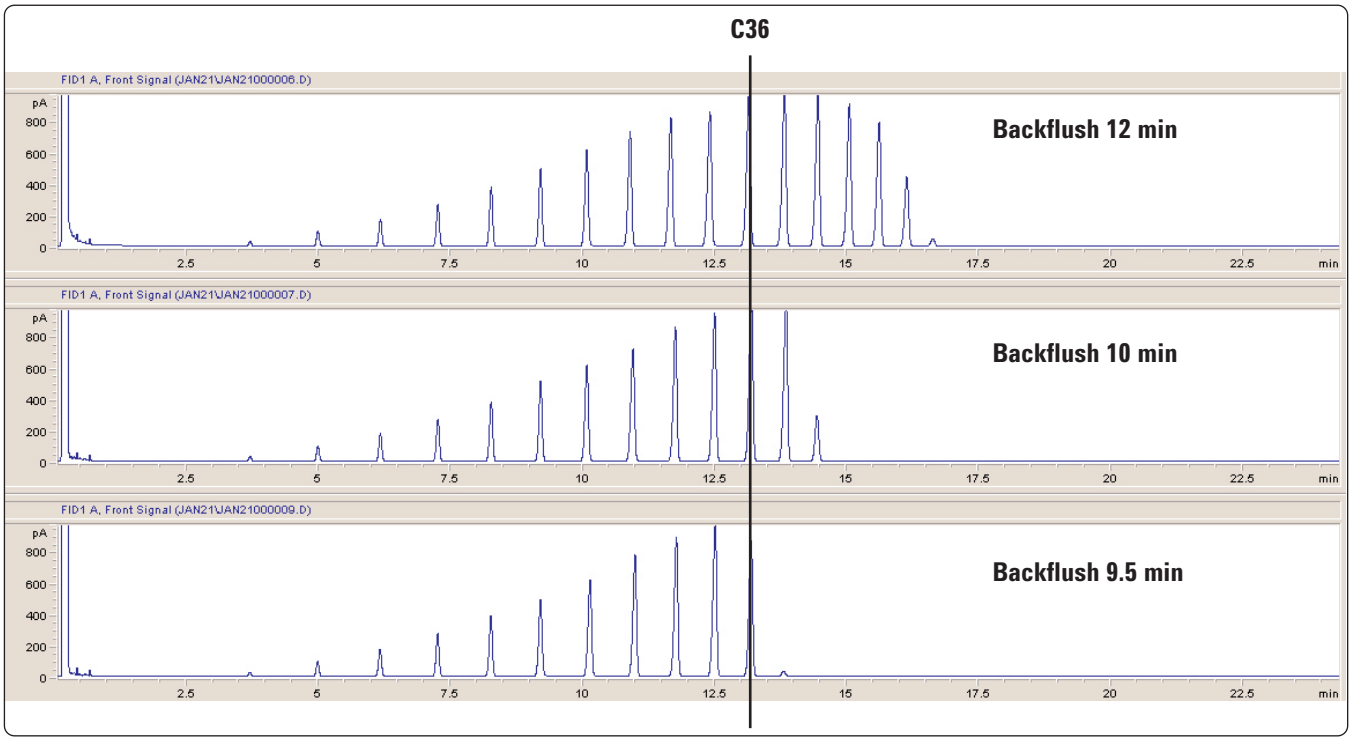

Figure 3. Polywax 500 chromatograms at three backflush times.

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

BF Time = (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 applicatin 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.

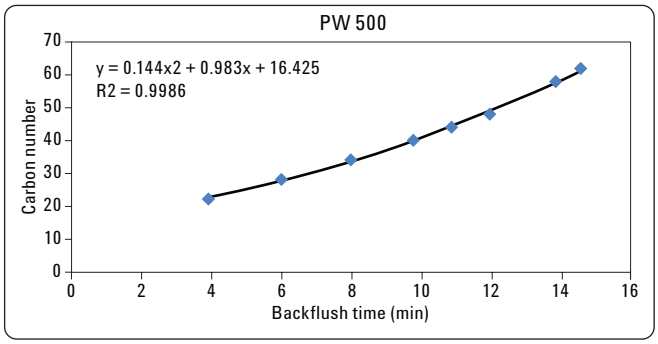

Figure 4A. Polynomial fit.

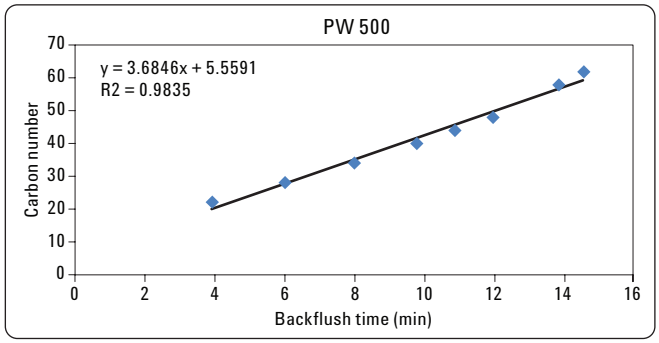

Figure 4B. Linear regression.

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).

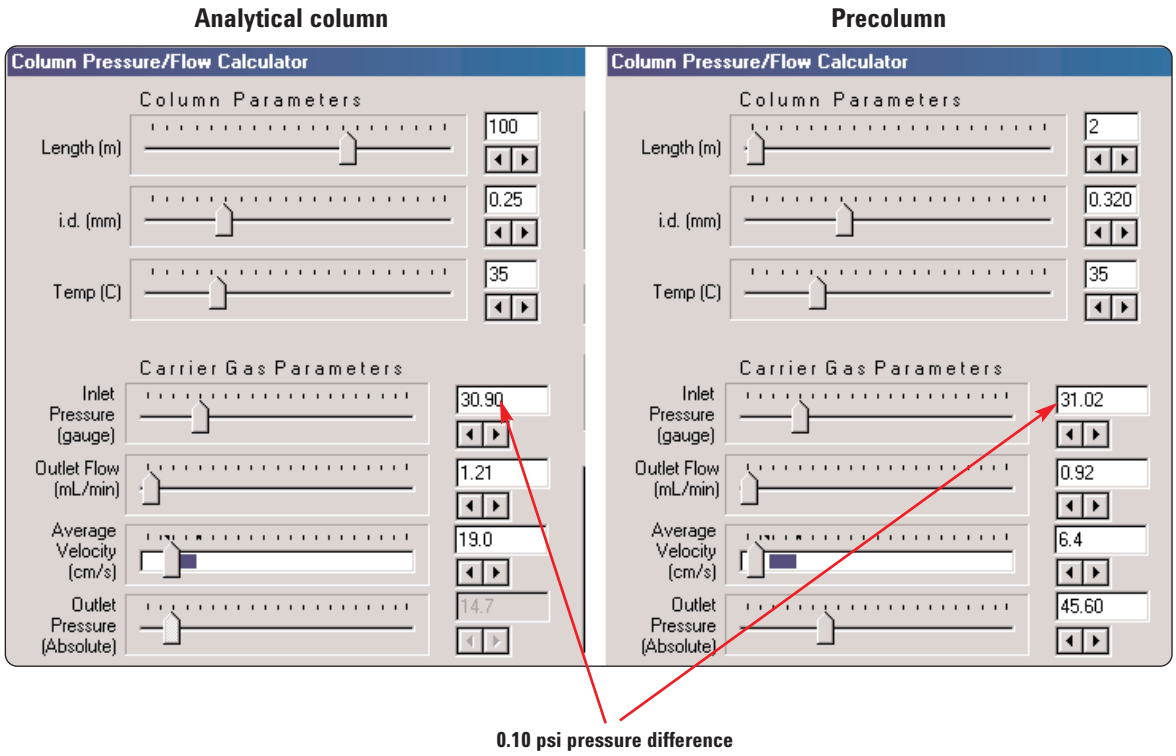

Figure 5. Pressure and flow setting for the analytical column (left pane) and precolumn (right pane).

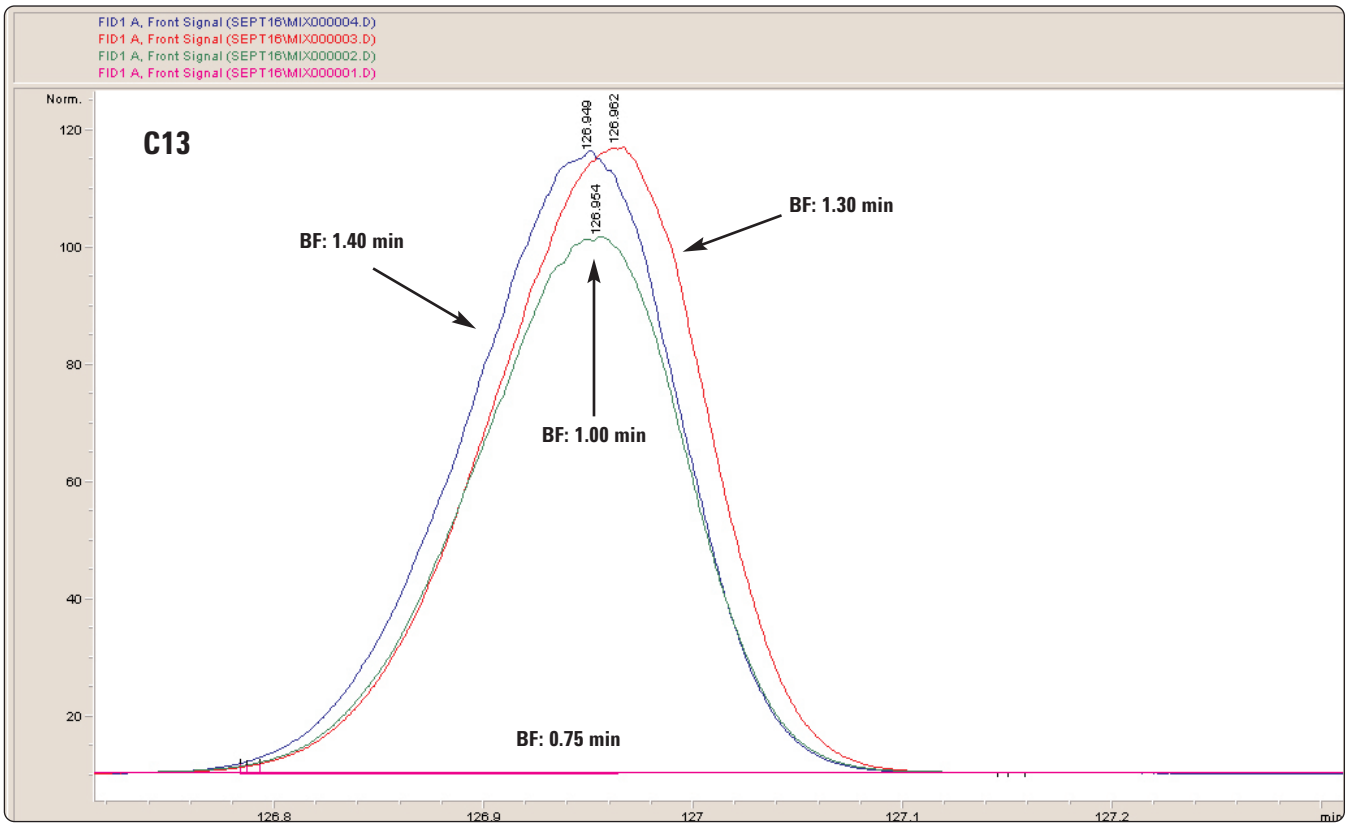

Figure 6. Fine tuning backflush time for ending transfer at C13. Trace at baseline: BF = 0.75 min, Peak at height of 100pa: BF = 1.00 min, Peaks at 117 pa: BF = 1.3 min and 1.4 min.

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 heaver 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.

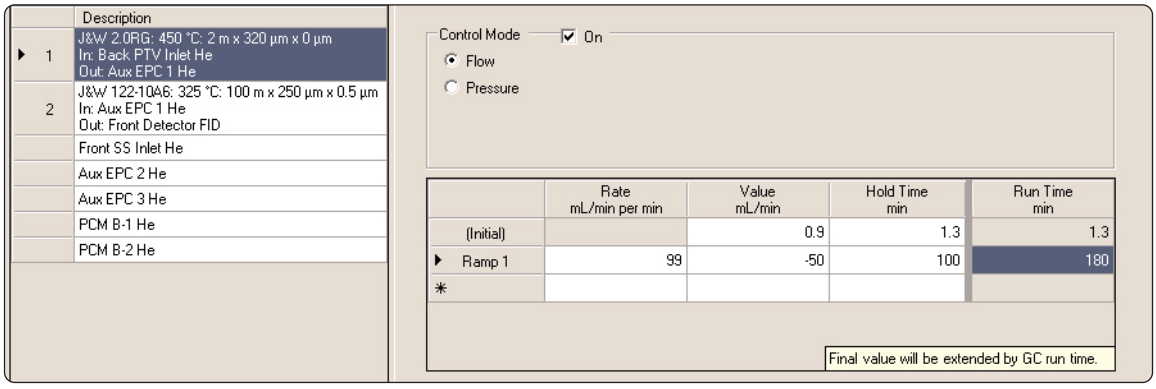

Figure 7A. Precolumn set to backflush at 1.3 min.

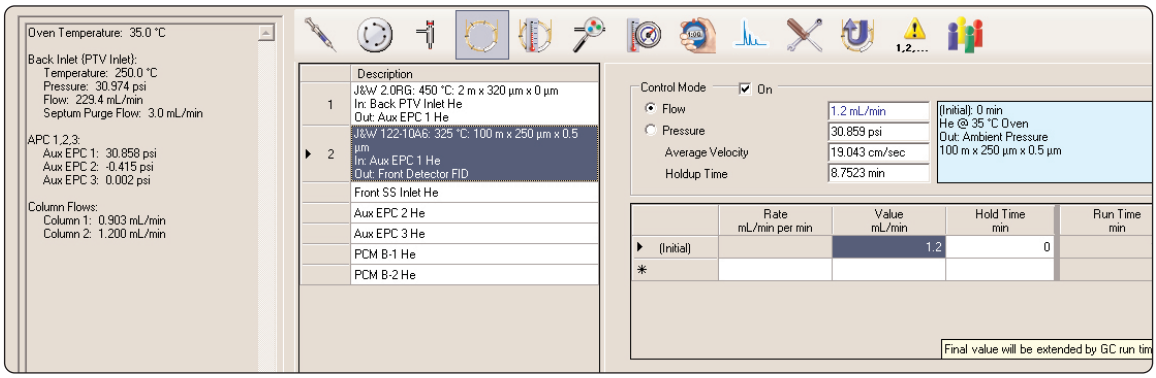

Figure 7B. ChemStation panes for configuring backflush and column flow.

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.

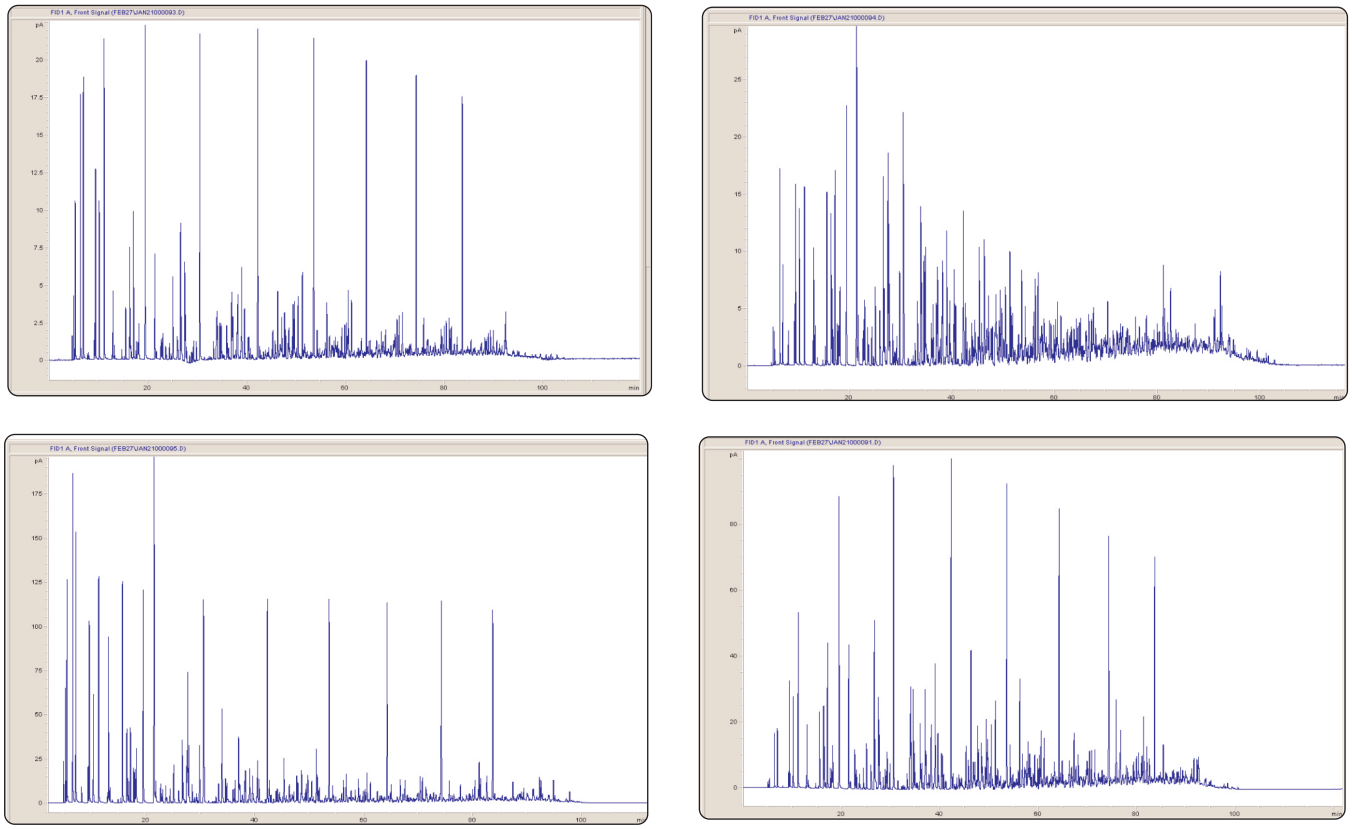

Figure 8. Four crude oils from different regions. Backflushed between C12 and C13.

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.

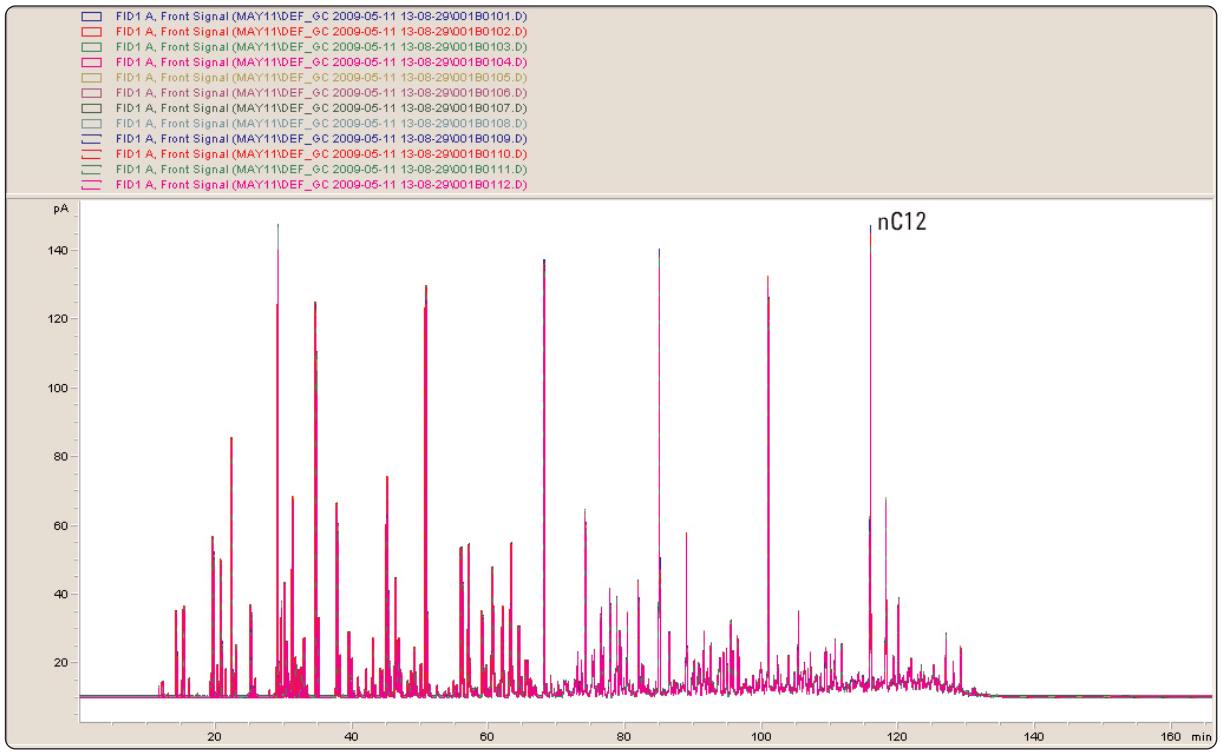

Figure 9. Overlay of twelve runs of crude oil backflushed between C12 and C13.

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 sofware: www.agilent. com/chem/flowcalculator

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Simultaneous Analysis of Greenhouse Gases by Gas Chromatography

Application

Environmental

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China

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 (CO_2), methane (CH_4) and nitrous oxide (N_2O) 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 CH_4, N_2O and CO_2 [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 CO_2, CH_4, N_2O, and SF_6 in air samples. Low concentrations of CO_2 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 CO_2, CH_4, N_2O, and SF_6 in air samples. CO_2 can be analyzed at wide concentration levels. High levels of CO_2 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 N_2 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 CH_4 and CO_2, while the micro-ECD detects N_2O. 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.

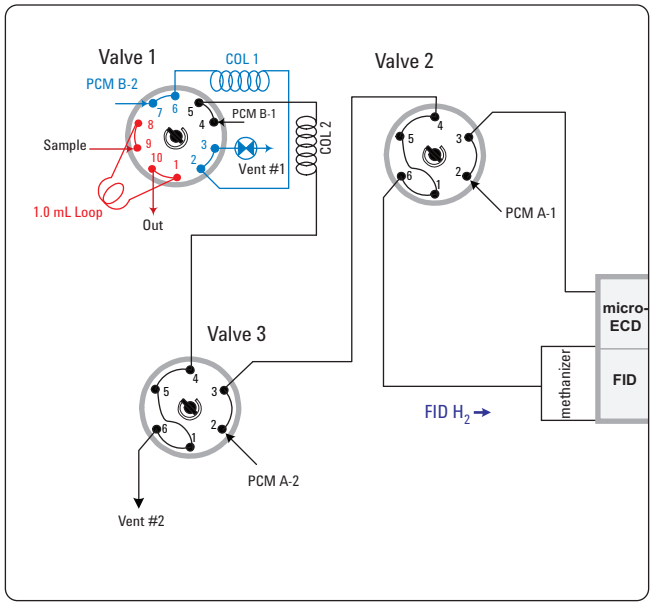

Figure 1. Configuration for SP1 7890-0468.

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_2O:	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 CH_4, CO_2, and N_2O 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_2O	8253.96	11.06	0.13

To improve the sensitivity of micro-ECD, Ar-5% CH_4 is recommended as the make-up gas, which can lower the detection of N_2O 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.

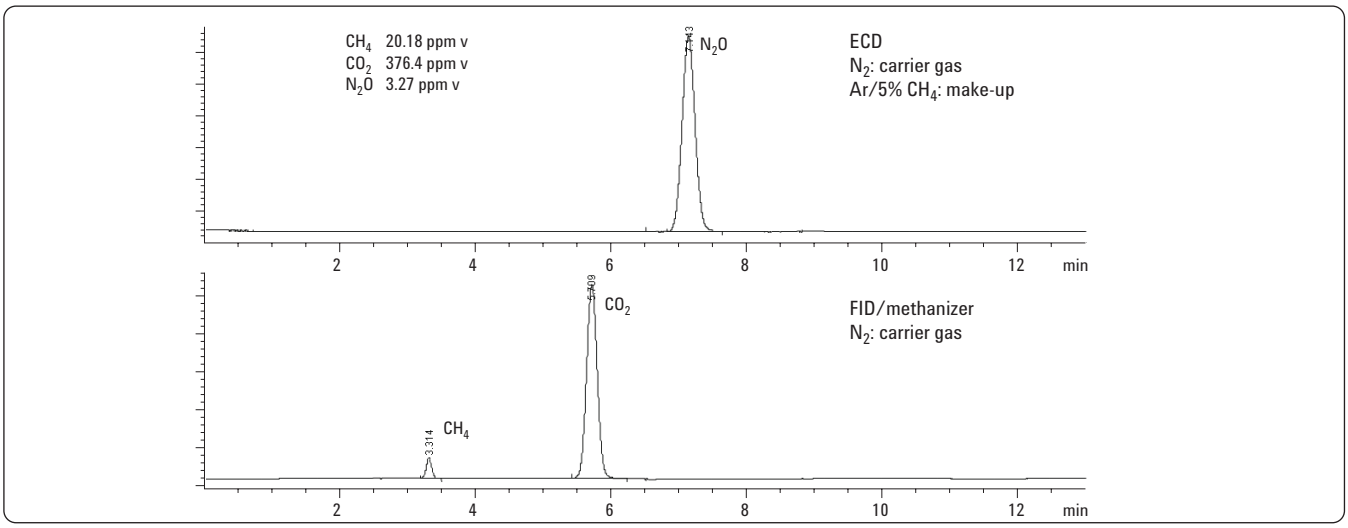

Figure 2. Analysis of greenhouse gases standards using Method 1.

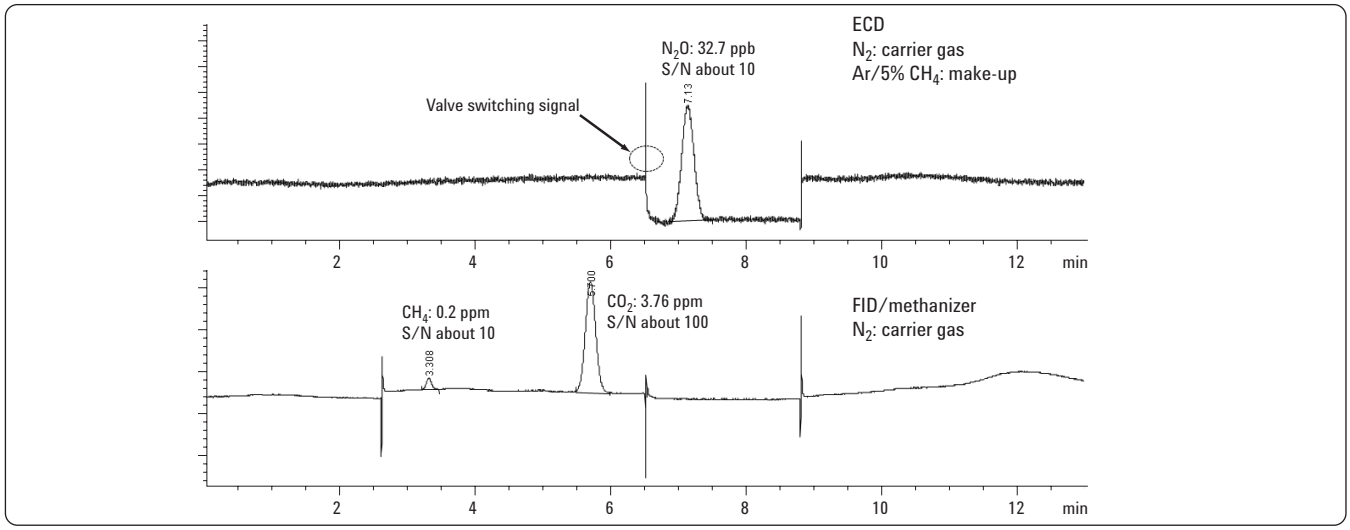

Figure 3. Chromatogram using Method 1 for CH_4, CO_2, and N_2O standards with a 100-times dilution.

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 (precolumn). 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).

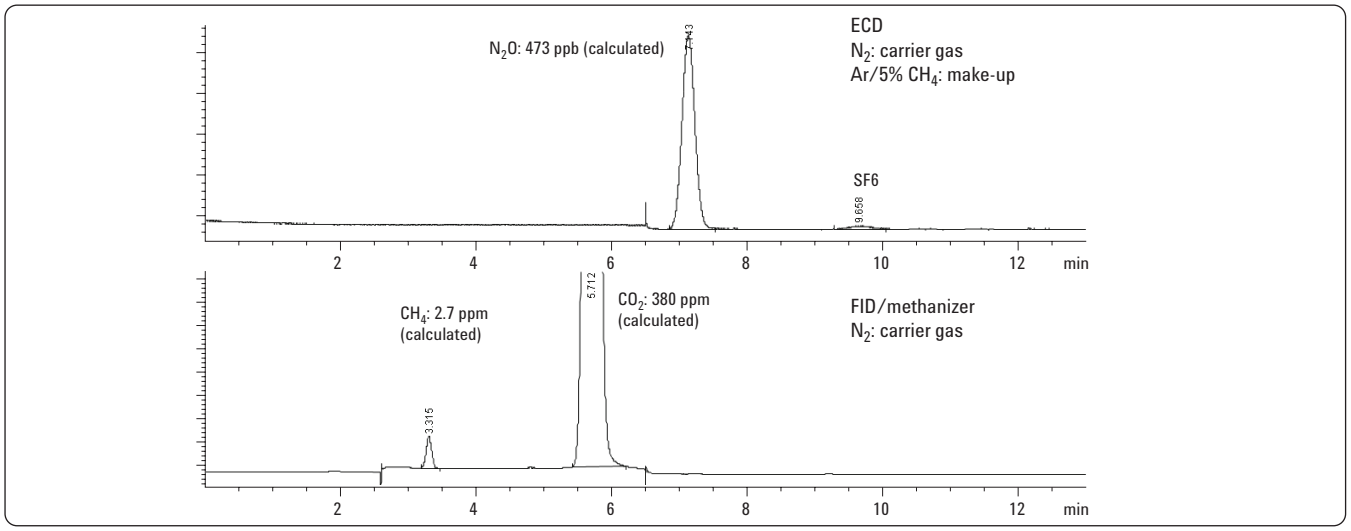

Figure 4. Chromatogram of real sample (laboratory air).

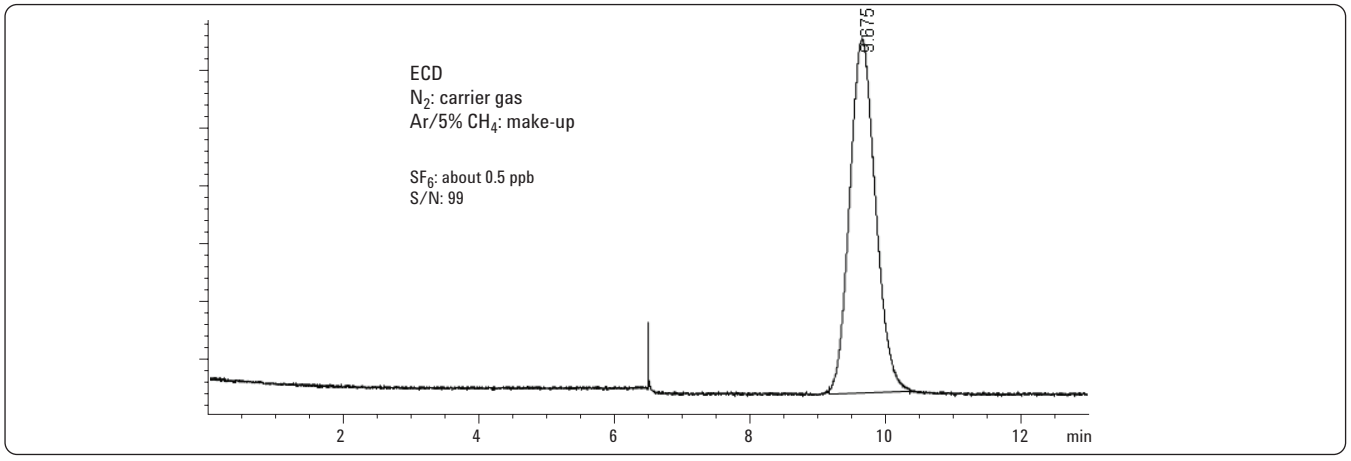

Figure 5. Chromatogram of SF_6 standard at approximately 0.5 ppb.

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 CH_4 and CO_2. This channel provides the flexibility for CO_2 in varying levels. Low level CO_2 can be converted to CH_4 through the methanizer and measured by FID. The system is flexible depending on the requirements. The TCD can be used for high concentrations of CO_2. If only higher levels of CO_2 (higher than 50 ppm) analysis is required, the methanizer can be removed. This channel can be expanded to include O_2 and N_2 analysis by adding an additional Molsive column.

Another micro-ECD channel with two valves is dedicated to measuring N_2O and SF_6. Precolumns (column 1 and 2) direct heavier components (mainly water) to be backflushed to vent 1 and vent 4. O_2 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

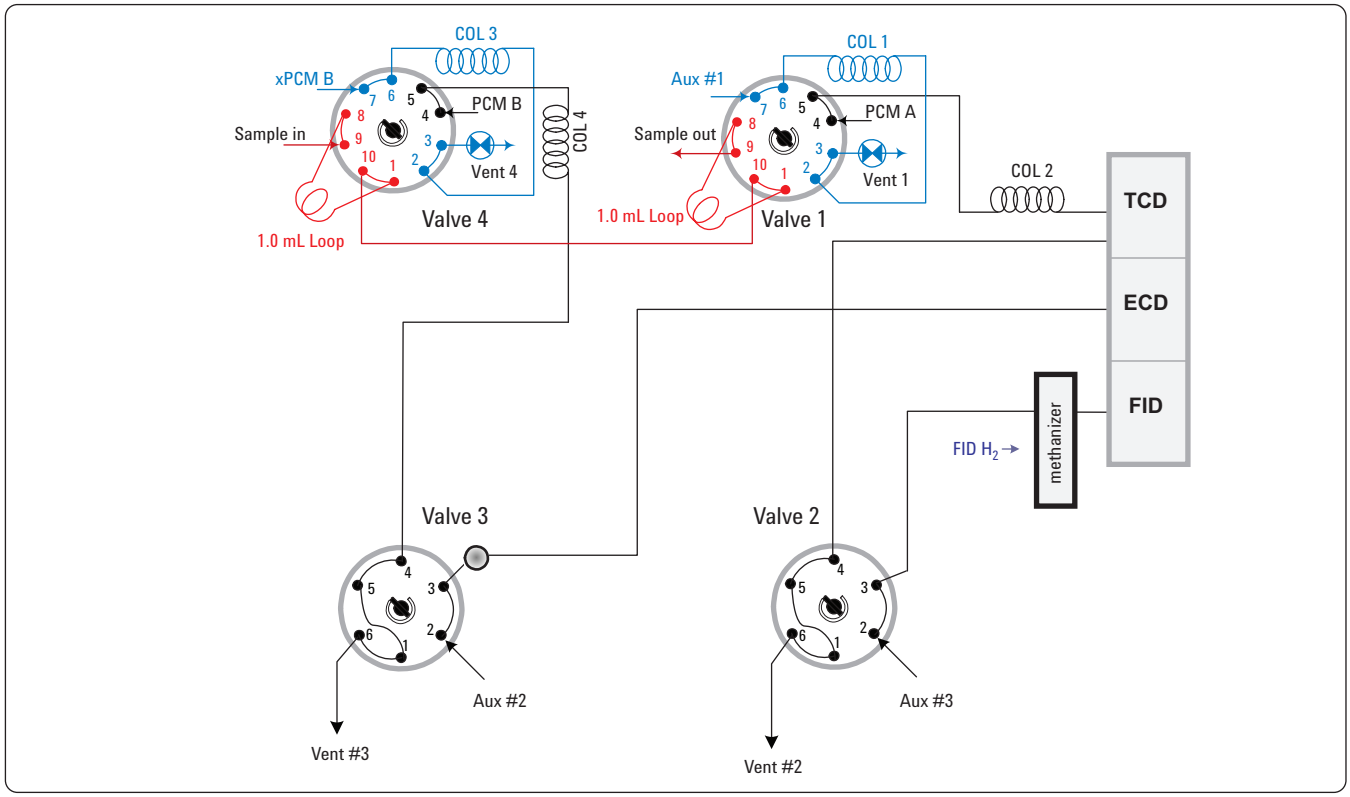

Figure 6. Valve configuration for Method 2.

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
CO_2(FID)	2788.51	14.72	0.53
N_2O	7467.92	13.91	0.19
CO_2(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.

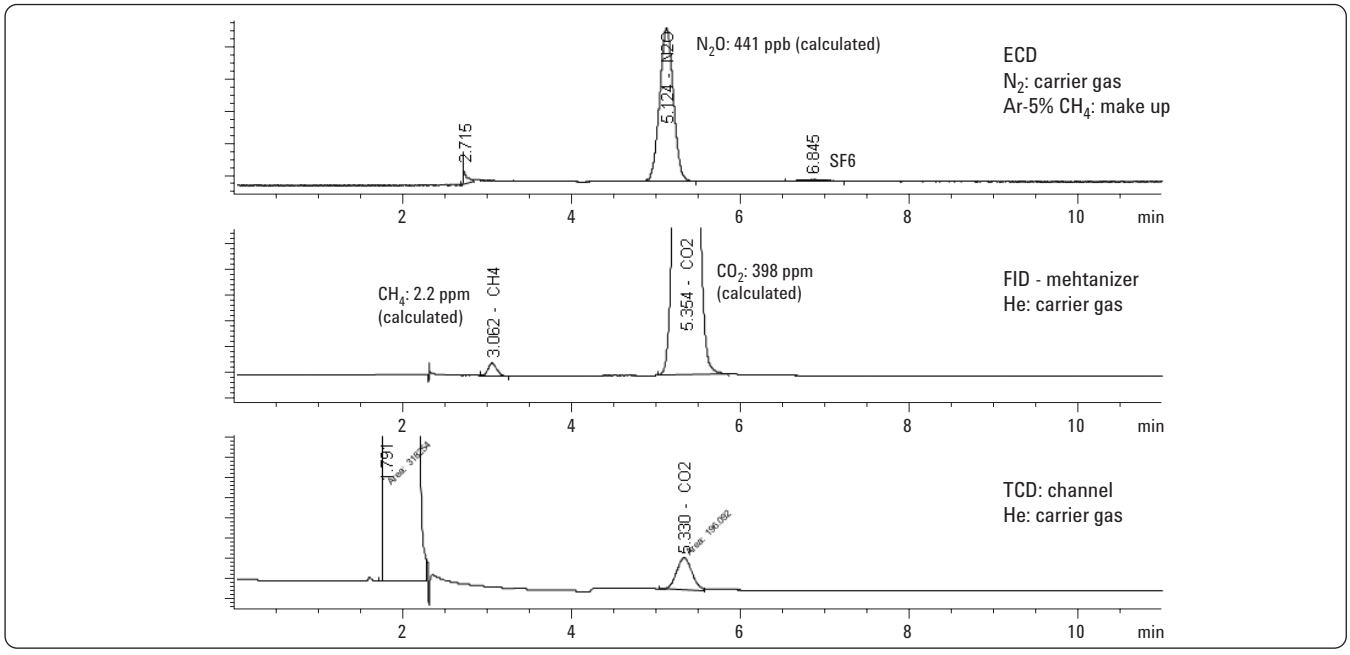

Figure 7. Chromatogram for real sample (laboratory air) using Method 2.

Conclusion

Two Agilent 7890A GC systems have been developed to meet the different requirements for simultaneous analyses of greenhouse gases including CH_4, CO_2, and N_2O in air samples.

Method 1 (SP1 7890-0468) has a simpler valve configuration and with minor modifications, accomodates 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 CO_2 (0.2 ppm to 20%).

Results obtained on both analyzers are the same for greenhouse gases (N_2O, CH_4, CO_2 and SF_6).

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 N_2O, CH_4 and CO_2 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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Agilent Technologies

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

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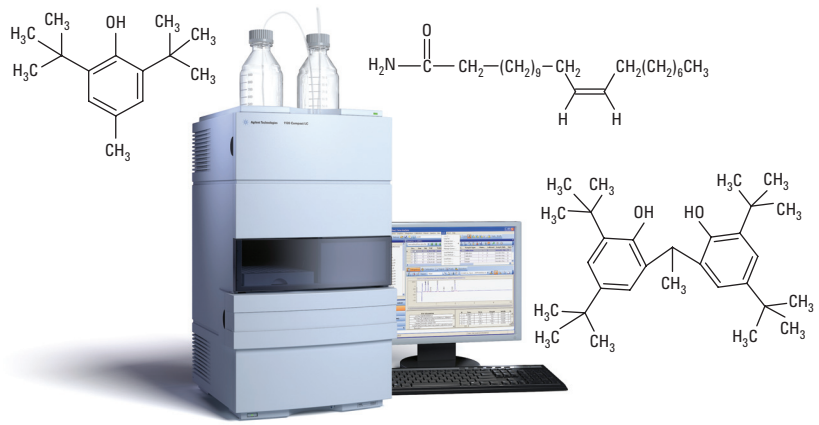
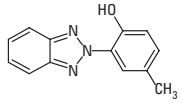
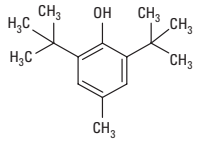
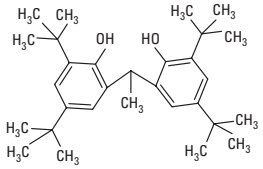
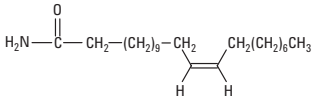
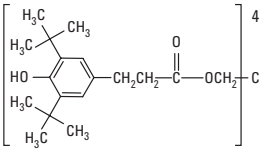
Bangalore, India



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.

Agilent Technologies

Name:	Tinuvin P	2-(2-Hydroxy-5-methylphenyl)benzotriazole
Formula:	$C_{13}H_{11}N_3O$	
Molecular weight:	225.25	
CAS No:	2440-22-4	
Name:	BHT	2,6-Di-*tert*-butyl-*p*-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-*tert*-butylphenol)
Formula:	$C_{30}H_{46}O_2$	
Molecular weight:	438.69	
CAS No:	35958-30-6	
Name:	Erucamide	*cis*-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-*tert*-butyl-4-hydroxyhydrocinnamate)
Formula:	$C_{73}H_{108}O_{12}$	
Molecular weight:	1117.63	
CAS No:	6683-19-8	
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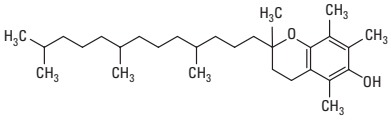
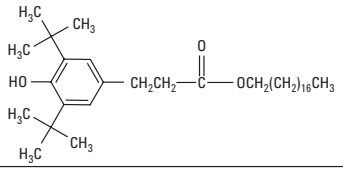
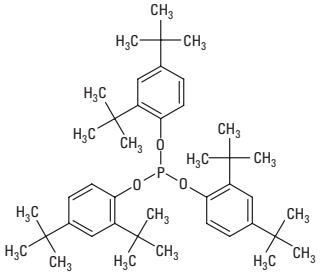
Formula:	$C_{29}H_{50}O_2$	
Molecular weight:	430.71	
CAS No:	10191-41-0	
Name:	Irganox 1076	Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate
Formula:	$C_{35}H_{62}O_3$	
Molecular weight:	530.86	
CAS No:	2082-79-3	
Name:	Irgafos 168	Tris(2,4-di-*tert*-butylphenyl) phosphate
Formula:	$C_{42}H_{63}O_3P$	
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 6042-09[1] 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:cyclohexane[1]. 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 poly propylene 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.

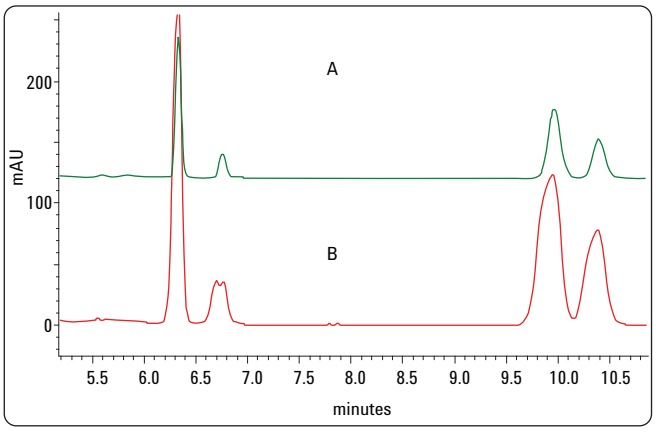


Figure 1
Effect of injection volume on peak shape. The lower trace (A) is with 10 µL injection volume and the upper trace (B) is with 5 µL.

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.

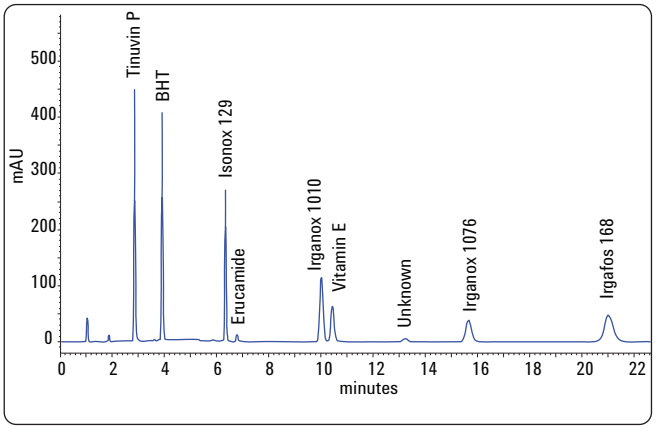


Figure 2
Representative chromatogram of a standard mix as per ASTM method (all at 125 µg/mL in IPA). (Column used: Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm).

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.

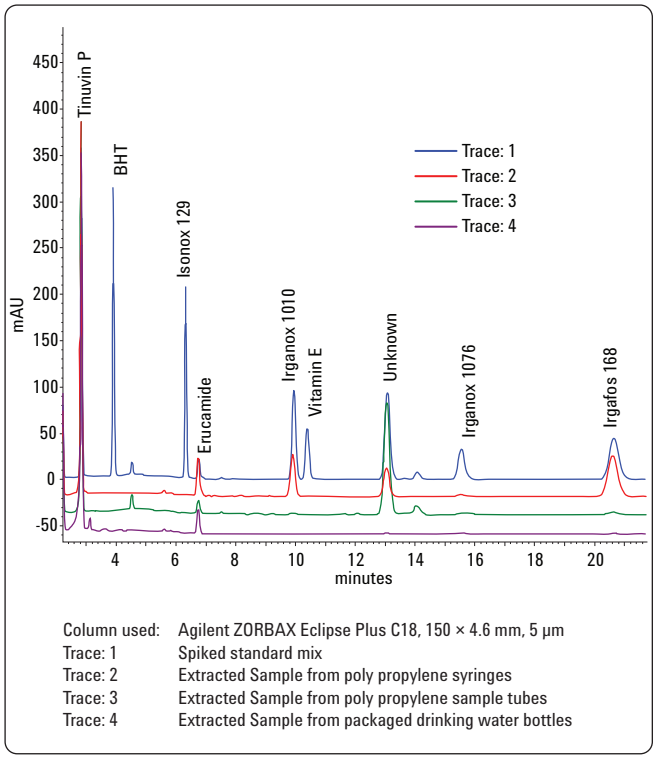


Figure 3
Chromatographic overlay of three extracted samples with spiked standard mixture.

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.

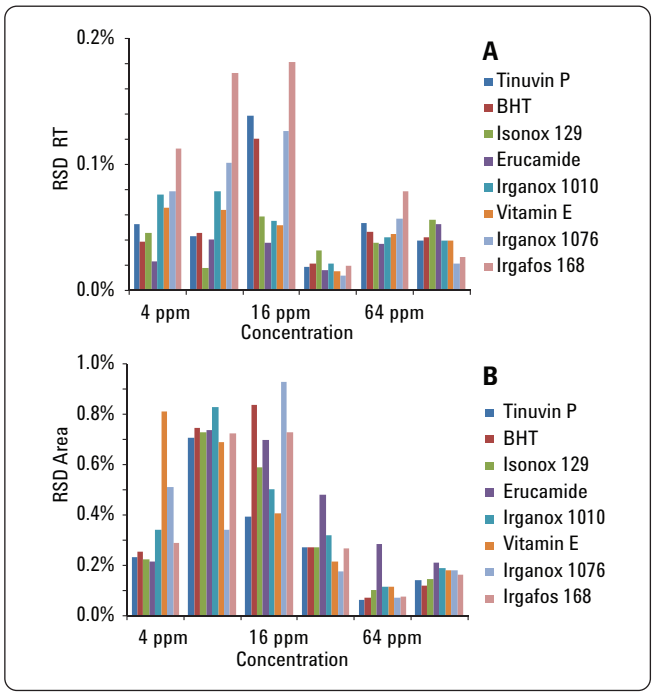


Figure 4
Graphical representation of RSD for (A) RT and (B) area.

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.

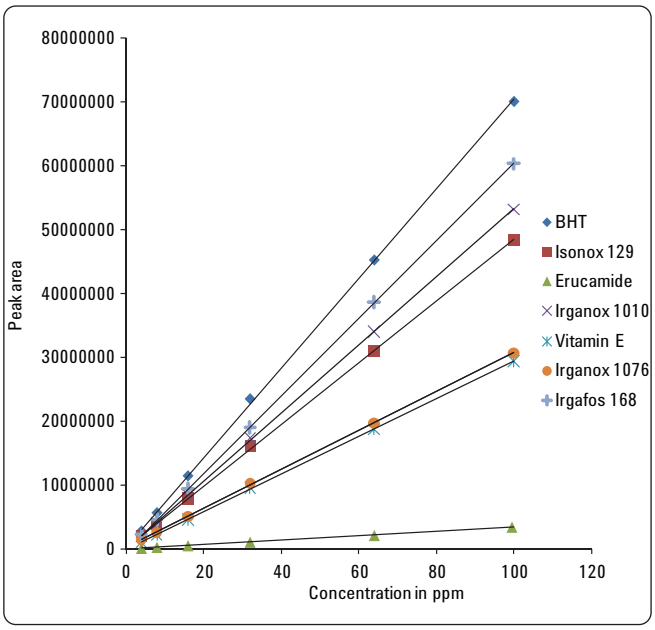


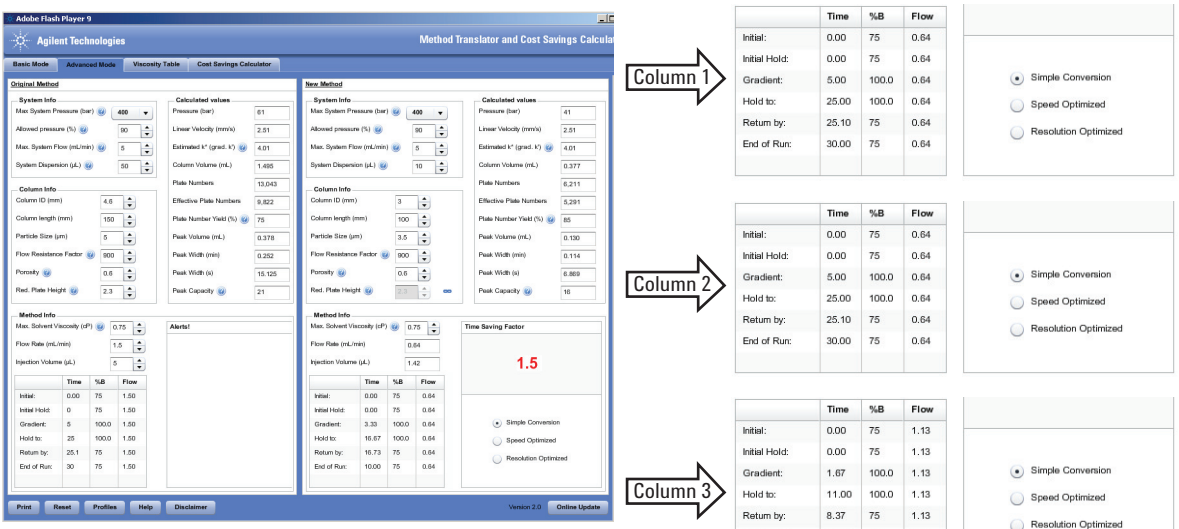
Figure 5
Linearity curves for all standards.

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.

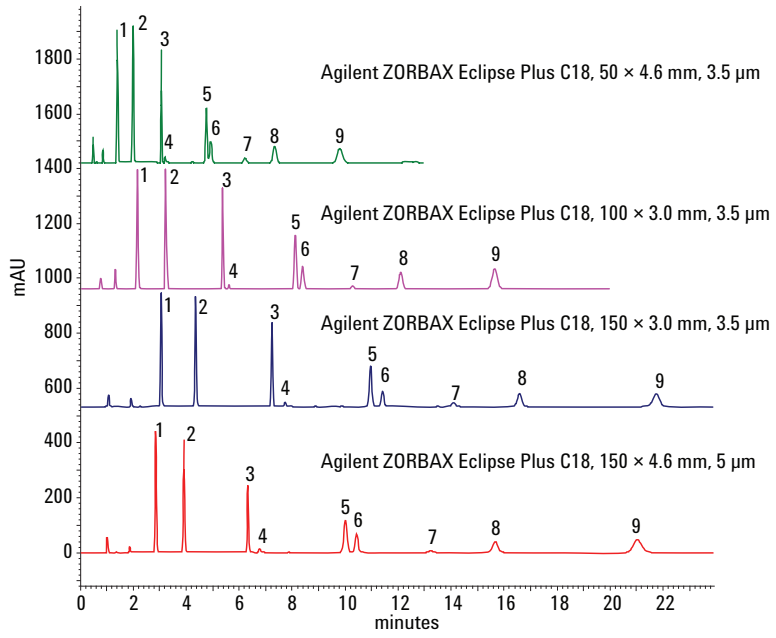
Trials with reducing column dimension and particle size

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: 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.



1. TinuvinP
2. BHT
3. Isonox 129
4. Erucamide
5. Irganox 1010
6. Vitamin E
7. Unknown (Impurity from Irgafos 168)
8. Irganox 1076
9. Irgafos 168

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 Original method	150 × 3.0 mm 3.5 µm	100 × 3.0 mm 3.5 µm	50 × 4.6 mm 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.

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Agilent Technologies

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 derivates with the Agilent 1290 Infinity LC system. The method, which used acetone as organic co-solvent separates the analytes within 3.5 minutes.

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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.

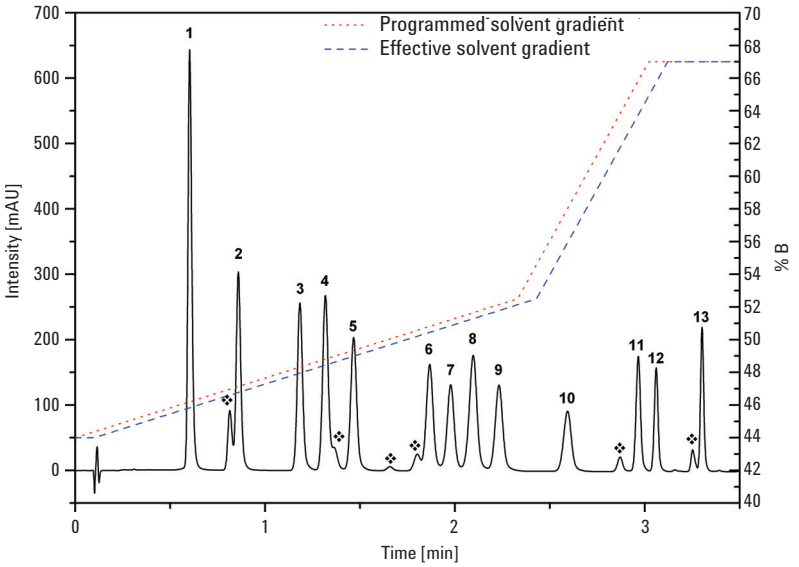
The elution order for all analytes depicted in all figures is:

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 gradient. 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 develped method.



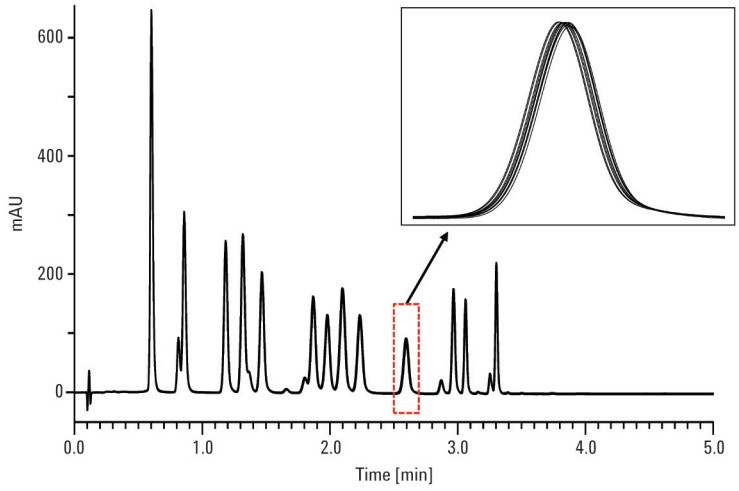
Chromatographic conditions:	
Stationary phase:	Agilent ZORBAX StableBond RRHD C18 (2.1 mm × 50 mm, 1.8 µm)
Mobile phase:	A: deionized water, B: acetone;
Solvent gradient:	0–2.33 min from 44% to 52.5% B, 2.33–3.02 min from 52.5% to 67% B, 3.02–3.50 min isocratic at 67% B;
Flow rate:	1.2 mL/min
Detection:	UV at 360 nm
Injection volume:	1 µL
Temperature:	33.4 °C
Pressure drop:	1100 bar.
Elution order of the analytes:	See experimental section; ❖impurities

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 derivates 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.



See Figure 1 for Chromatographic conditions.

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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Agilent Technologies

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)pyren (BeP), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), benzo(j)fluoranthene (BjFA), benzo(k)fluoranthene (BkFA) and dibenzo(a,h)anthracene (DBAhA). 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,
Post run:	320 °C (4 min)
MS Conditions	
Solvent delay:	2.8 min
MS temp:	250 °C (Source); 180 °C (Quad)
Transfer line:	290 °C
MS:	EI, 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)anthracen	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, acenaphthlene-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

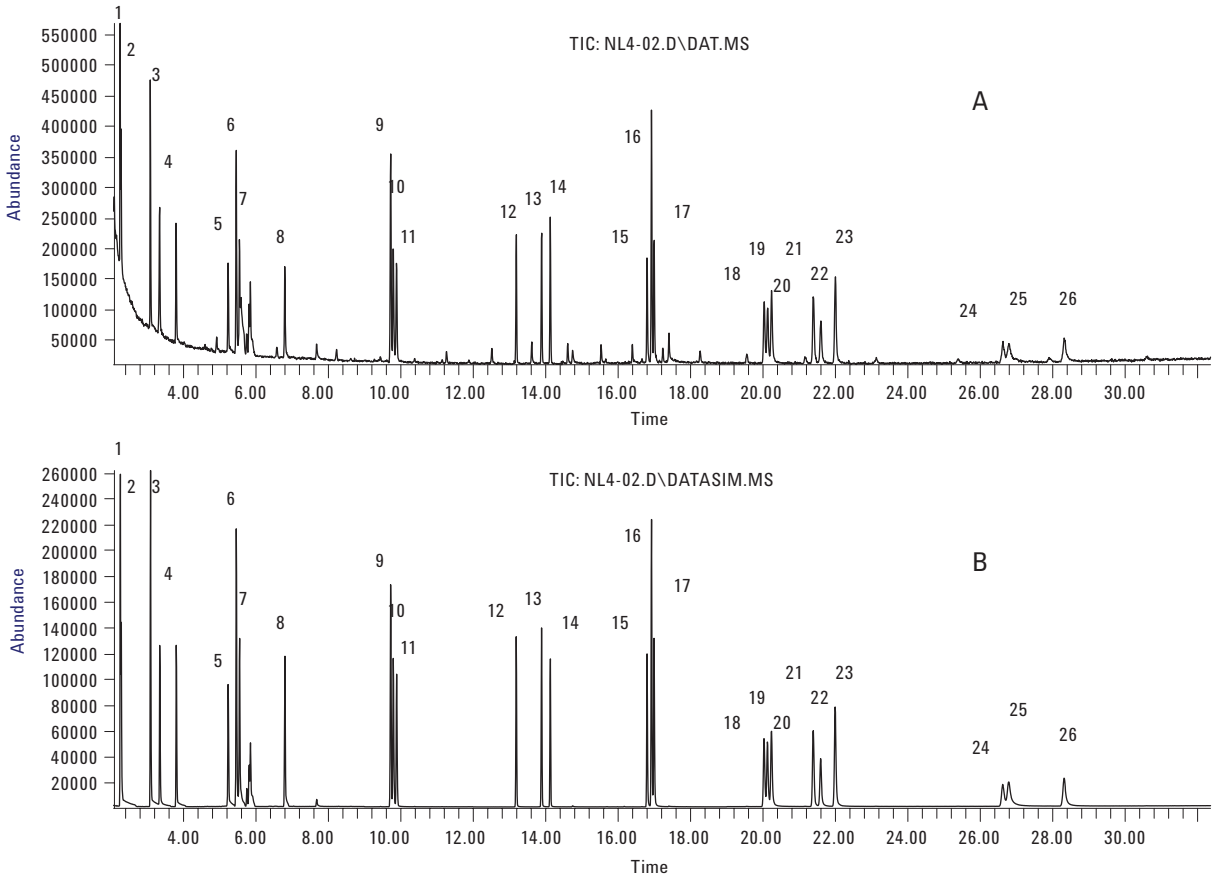
Figure 1 shows the GC/MS total ion chromatograms for the compounds of interest. Due to the presence of isomers, some compounds listed in Table 2 with the same quantitation ions could be eluting quite closely. Therefore good resolution is very important for these isomers to achieve accurate quantitative 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-µL 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.



1. Naphthalene-d8
2. Naphthalene
3. 2-methylnaphthalene
4. 1-methylnaphthalene
5. Acenaphthylene
6. Acenaphthene-d10
7. Acenaphthene
8. Fluorene
9. Phenanthrene-d10
10. Phenanthrene
11. Anthracene
12. Fluoranthene
13. Pyrene
14. p-Terphenyl-d14
15. Benzo(a)anthracene
16. Chrysene-d12
17. Chrysene
18. Benzo(b)fluoranthene
19. Benzo(k)fluoranthene
20. Benzo(j)fluoranthene
21. Benzo(e)pyrene
22. Benzo(a)pyrene
23. Perylene-d12
24. Indeno(1,2,3-cd)pyrene
25. Dibenzo(a,h)anthracen
26. Benzo(g,h,i)perylene

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.

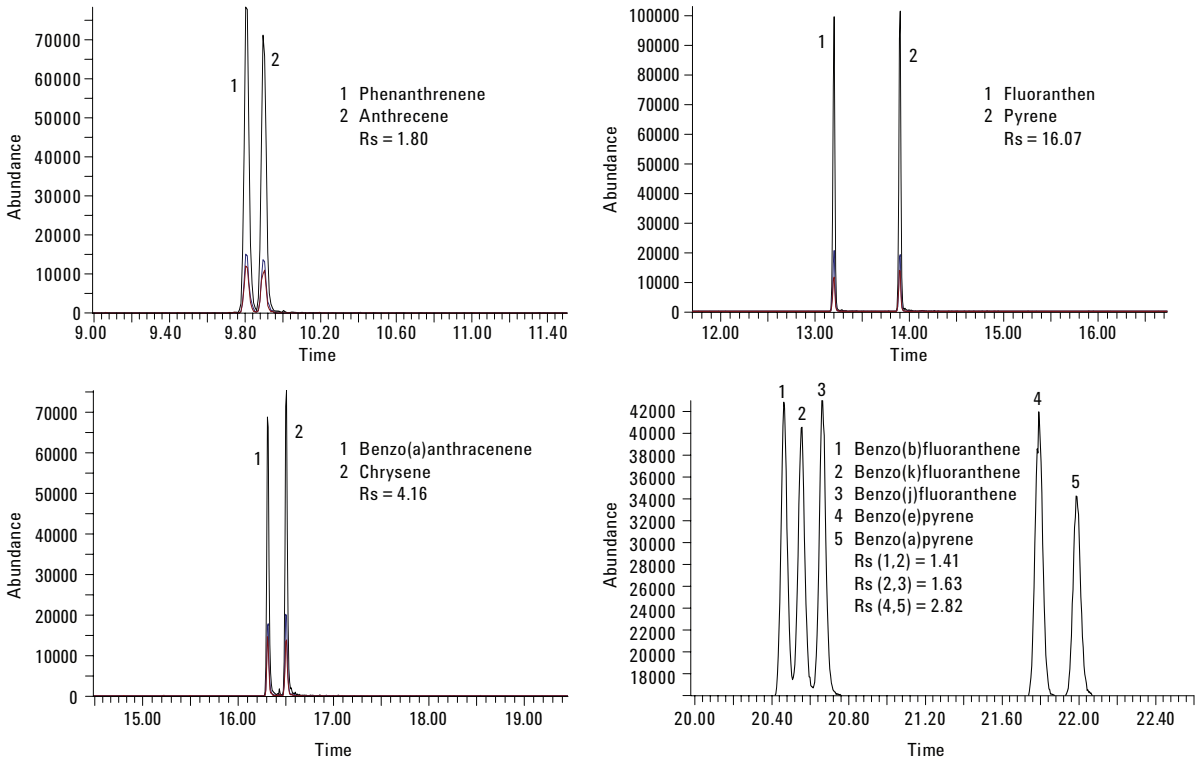


Figure 2. Resolution of Critical isomer Pairs with the DB-EUPAH column, 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627).

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

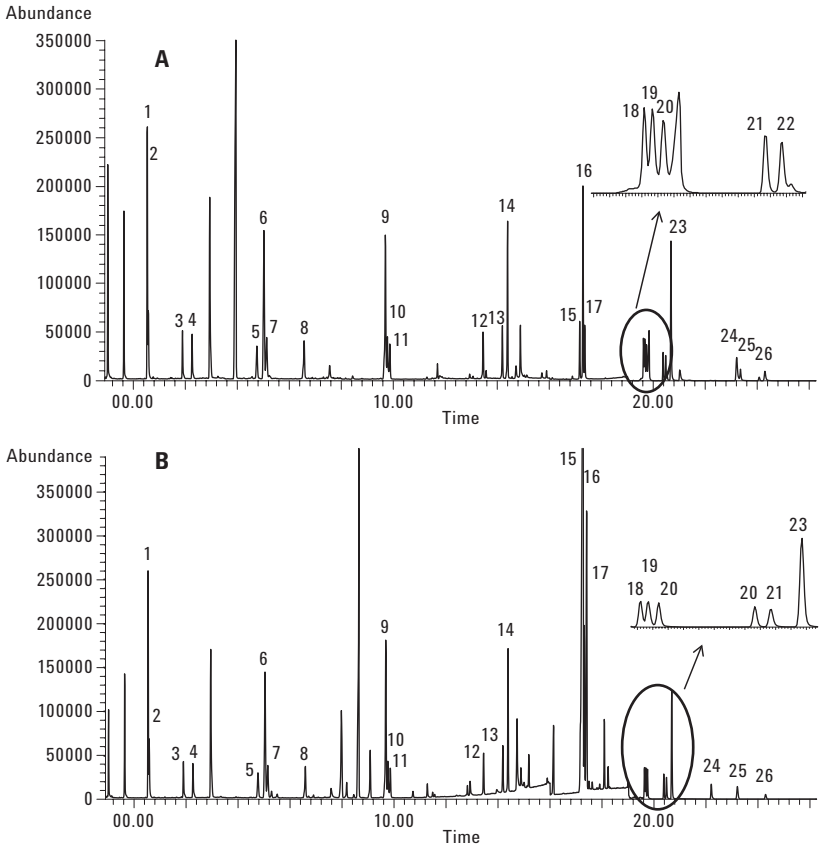
This application demonstrates a highly sensitive and selective GC/MS method for PAH analysis in rubber and plastic products using an Agilent J&W DB-EUPAH GC column. The DB-EUPAH column can effectively separate the 20 PAHs of interest, resolving all the critical, difficult-to-separate pairs. The system 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
Indenol(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
2. Naphthalene
3. 2-methylnaphthalene
4. 1-methylnaphthalene
5. Acenaphthylene
6. Acenaphthene-d10
7. Acenaphthene
8. Fluorene
9. Phenanthrene-d10
10. Phenanthrene
11. Anthracene
12. Fluoranthene
13. Pyrene
14. p-Terphenyl-d14
15. Benzo(a)anthracene
16. Chrysene-d12
17. Chrysene
18. Benzo(b)fluoranthene
19. Benzo(k)fluoranthene
20. Benzo(j)fluoranthene
21. Benzo(e)pyrene
22. Benzo(a)pyrene
23. Perylene-d12
24. Indeno(1,2,3-cd)pyrene
25. Dibenzo(a,h)anthracen
26. Benzo(g,h,i)perylene

Figure 3. TIC of matrix spiked extract using Agilent GC/MS system and Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm column (Agilent p/n 121-9627) A: plastic, B: rubber.

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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.

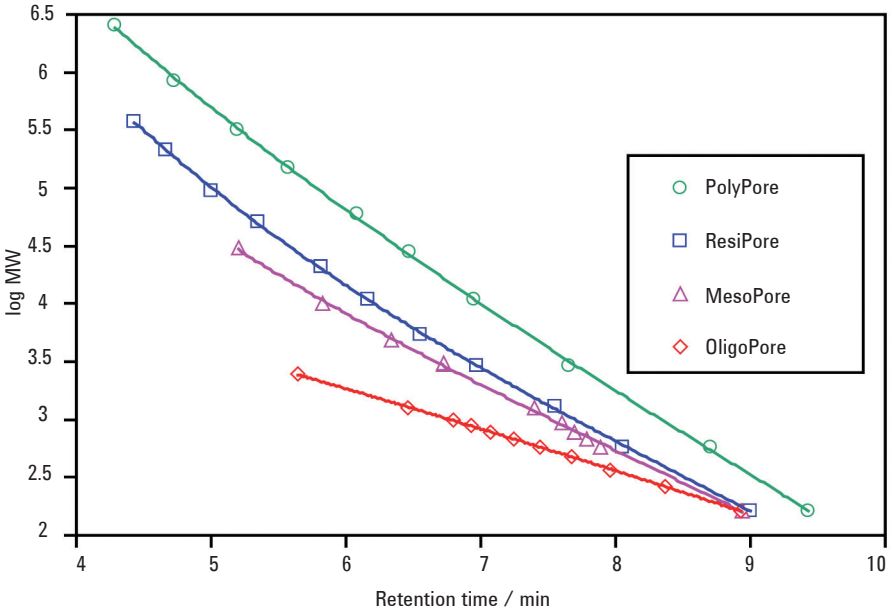


Figure 1. *PlusPore calibration curves showing the resolving ranges and near linear calibrations of the columns*

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.

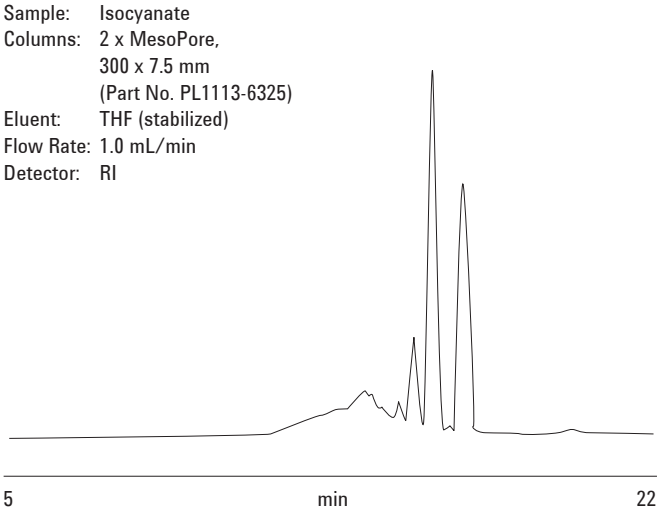


Figure 2. *Chromatogram of an isocyanate sample showing polymer and oligomeric detail*

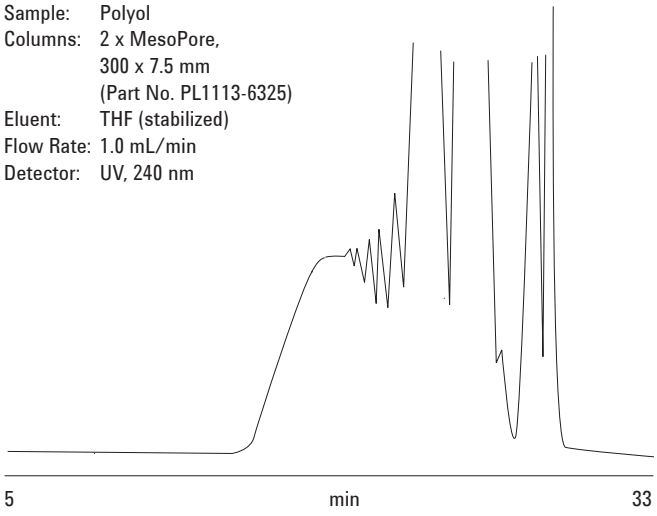


Figure 3. *Chromatogram of a polyol sample showing the presence of large amounts of oligomers*

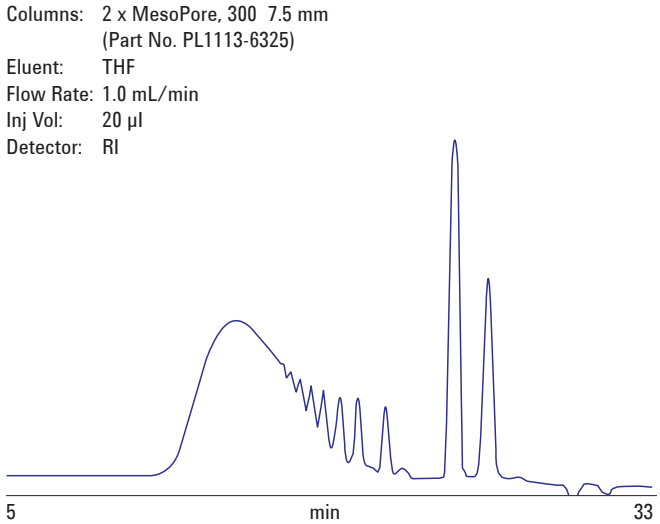


Figure 4. *Chromatogram of a polyurethane sample with oligomers and residual monomers*

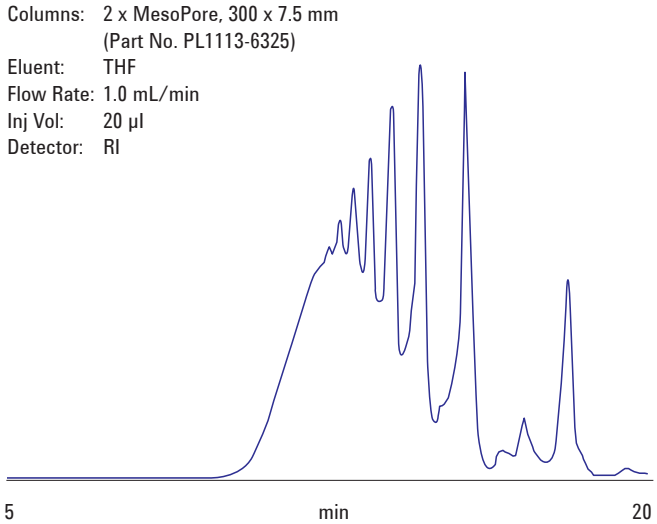


Figure 5. *Chromatogram of an epoxy resin sample showing oligomers dominating the distribution*

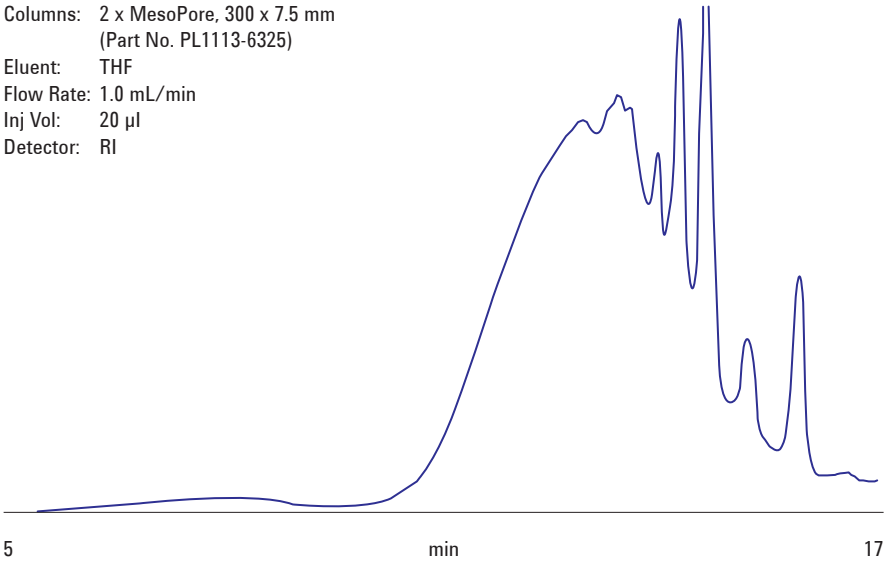


Figure 6. *Chromatogram of a polyesterimide sample with a complex distribution of polymer and oligomer content*

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.

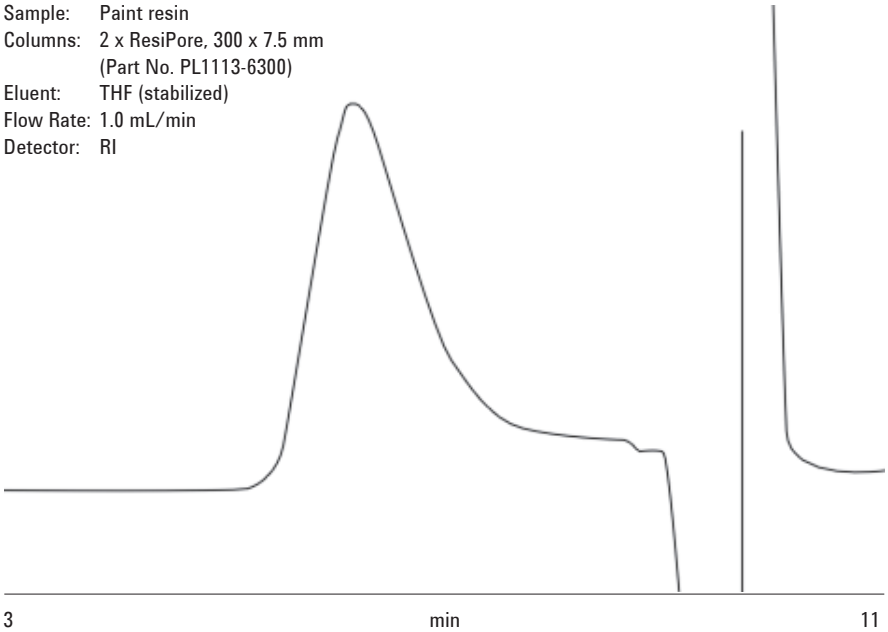


Figure 7. *Chromatogram of a paint resin sample showing low molecular weight content with the polymer*

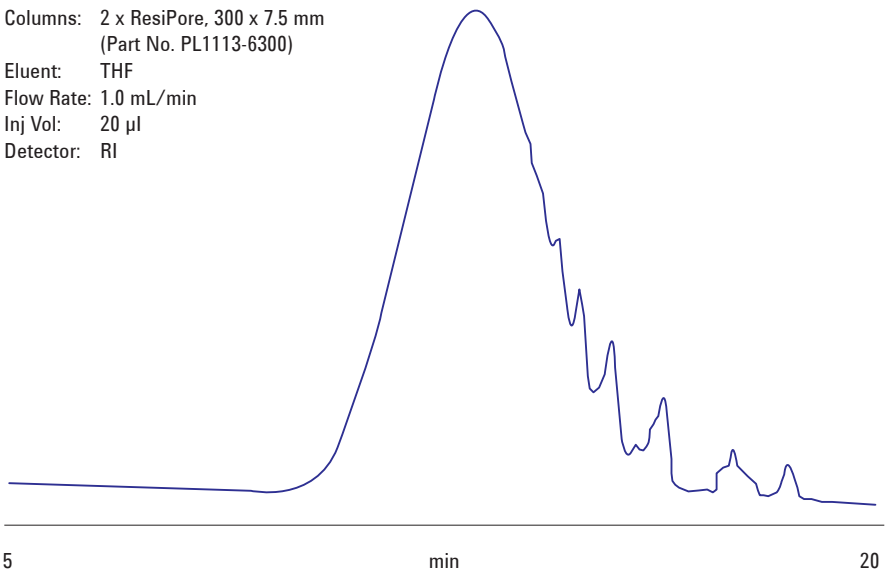


Figure 8. *Chromatogram of higher molecular weight epoxy resin sample with some oligomer content*

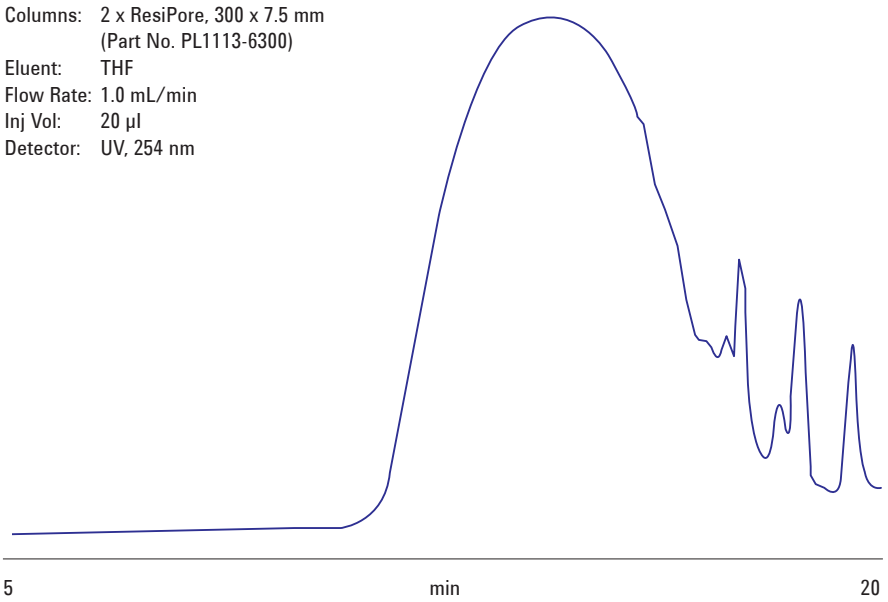


Figure 9. *Chromatogram of an alkyd resin sample with a broad molecular weight distribution*

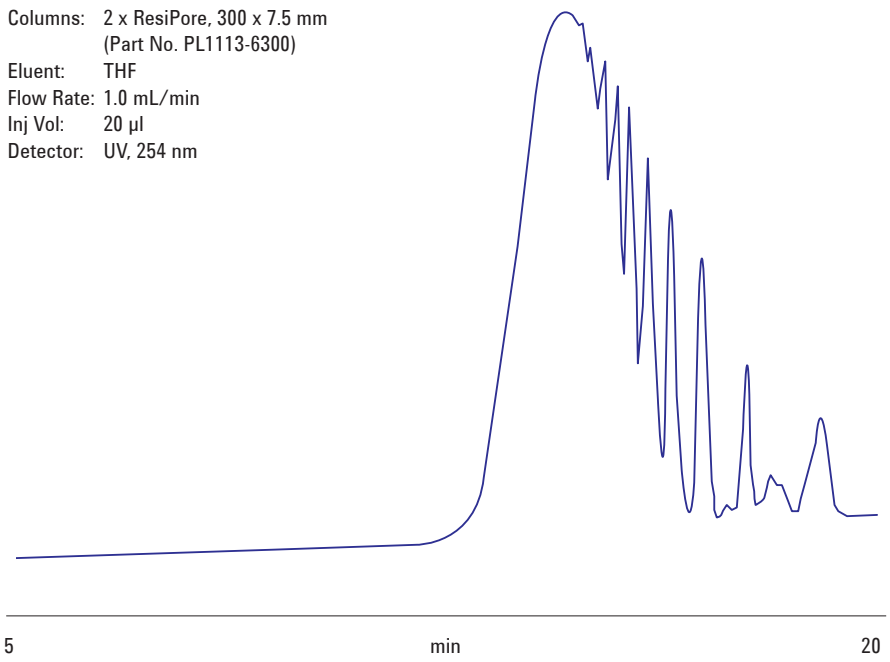


Figure 10. *Chromatogram of a polyester sample showing oligomeric detail*

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 µm MIXED-E columns.

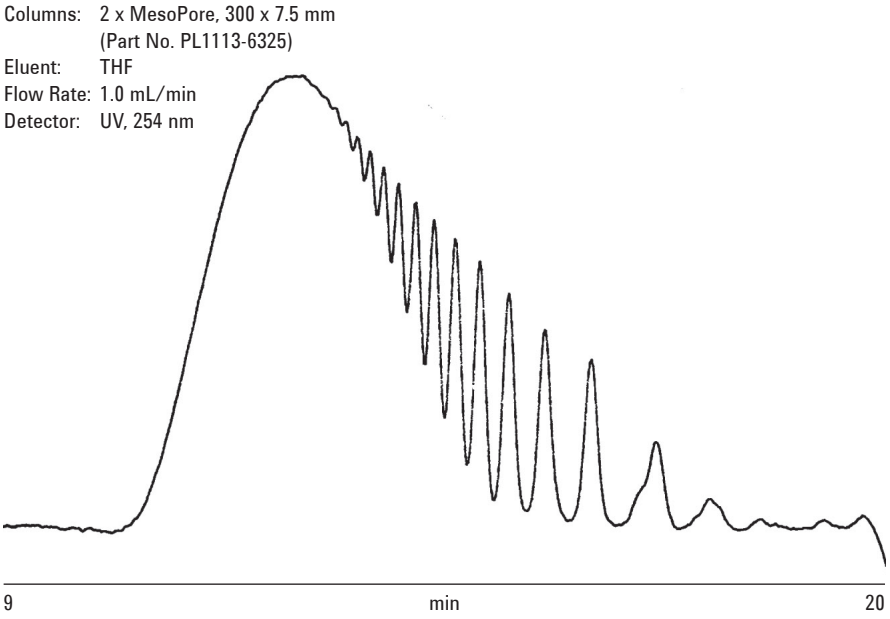


Figure 11. *Chromatogram of a polyester polyol sample, a complex material with a UV chromophore*

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.

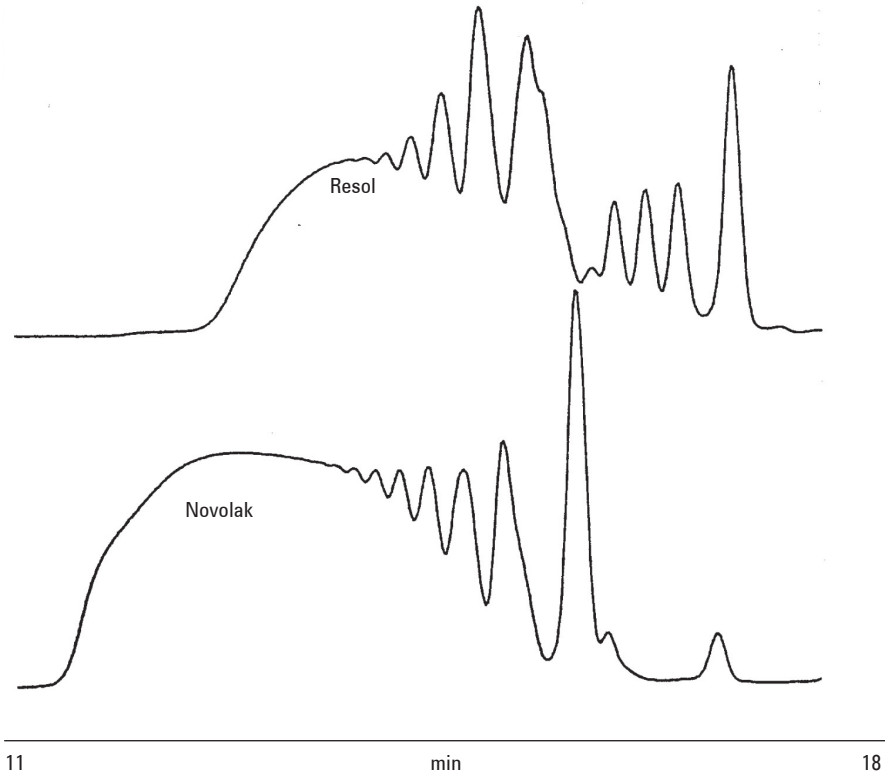


Figure 12. *Overlaid chromatograms of two phenolic resin samples showing differences as a result of synthesis conditions*

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.

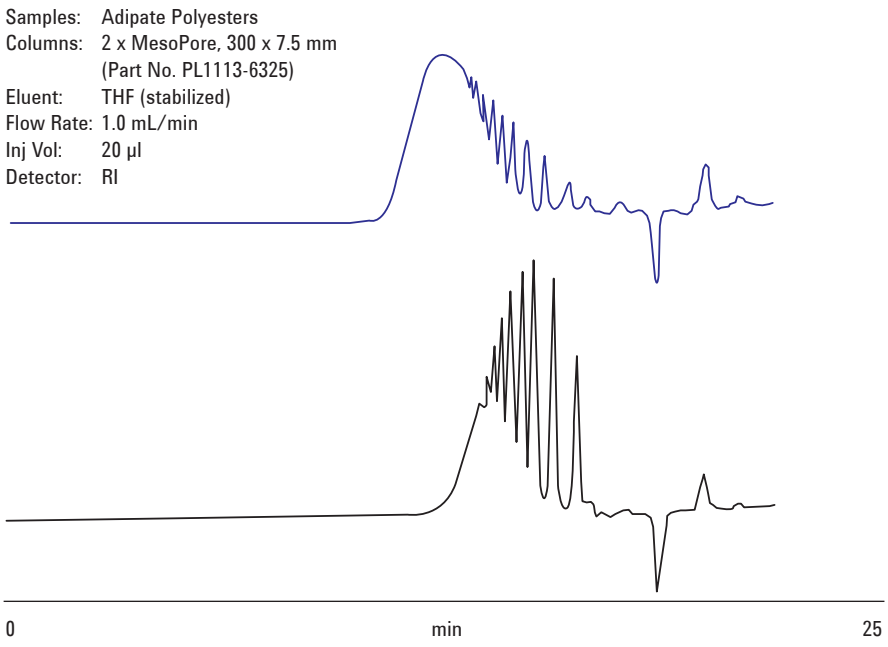


Figure 13. *Overlaid chromatograms of adipate polyester samples showing clear differences between two batches*

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

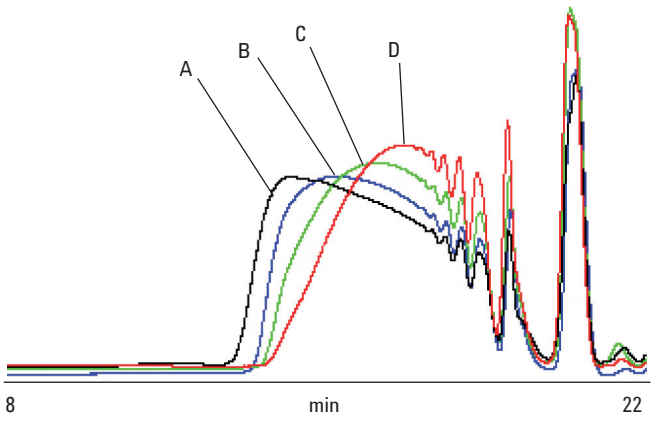


Figure 14. *Overlaid GPC chromatograms obtained from four samples of phenolic resin showing differences in elution behavior*

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

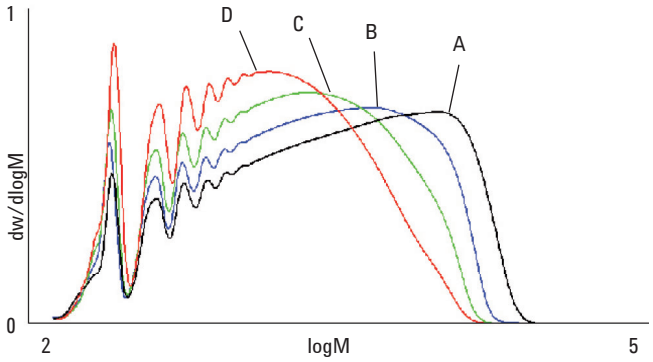


Figure 15. *Overlaid molecular weight distributions from the GPC of four samples of phenolic resin - the same oligomers are present, but the overall molecular weights are very different*

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.

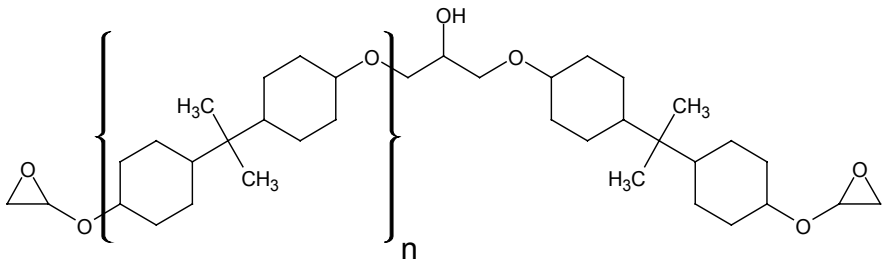


Figure 16. *General structure of Epikote 828 epoxy resin oligomers*

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 reduction.

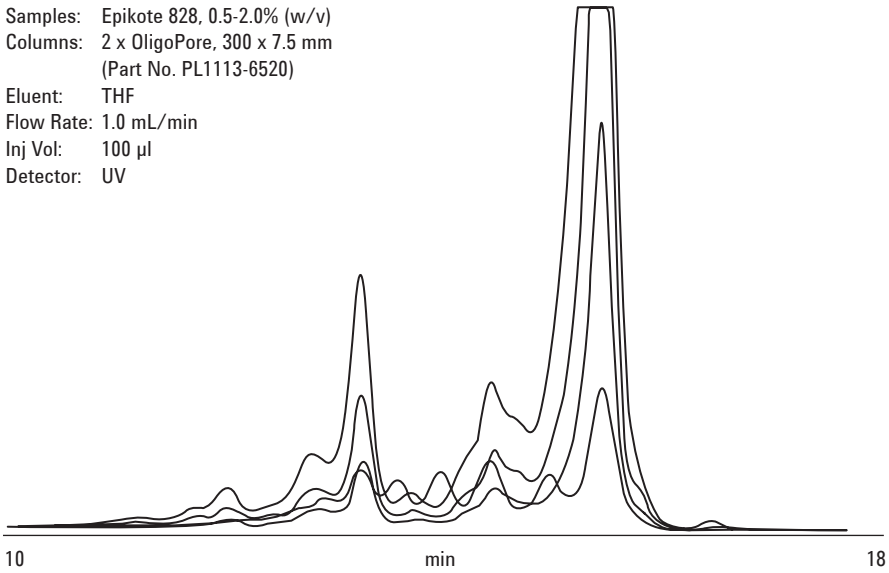


Figure 17. *Overlaid chromatograms of epikote epoxy resin samples at different loadings*

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.

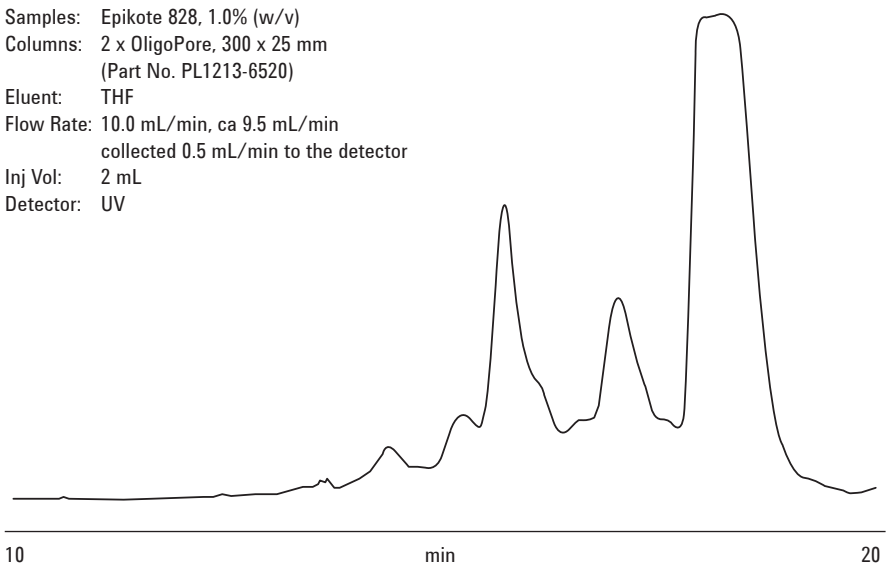


Figure 18. *Chromatogram of an epikote 828 sample on a preparative scale column showing which oligomers could be isolated*

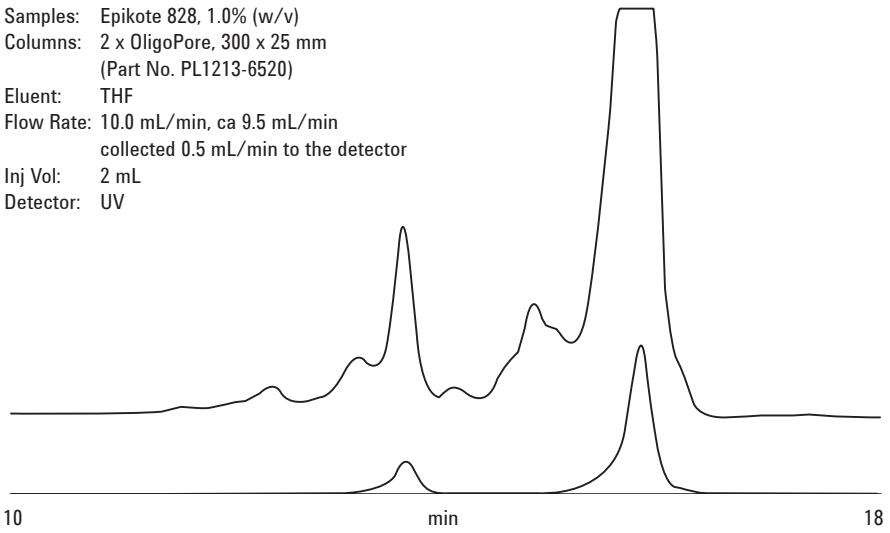


Figure 19. *Overlaid chromatograms of an epikote 828 sample (top) and two separated fraction samples (bottom) showing the isolation of components through preparative GPC*

Separation of epoxy resin oligomers

Epoxy resin prepolymers consist of oligomeric and polymeric diepoxides that are cured to form the finished product by the addiction 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)

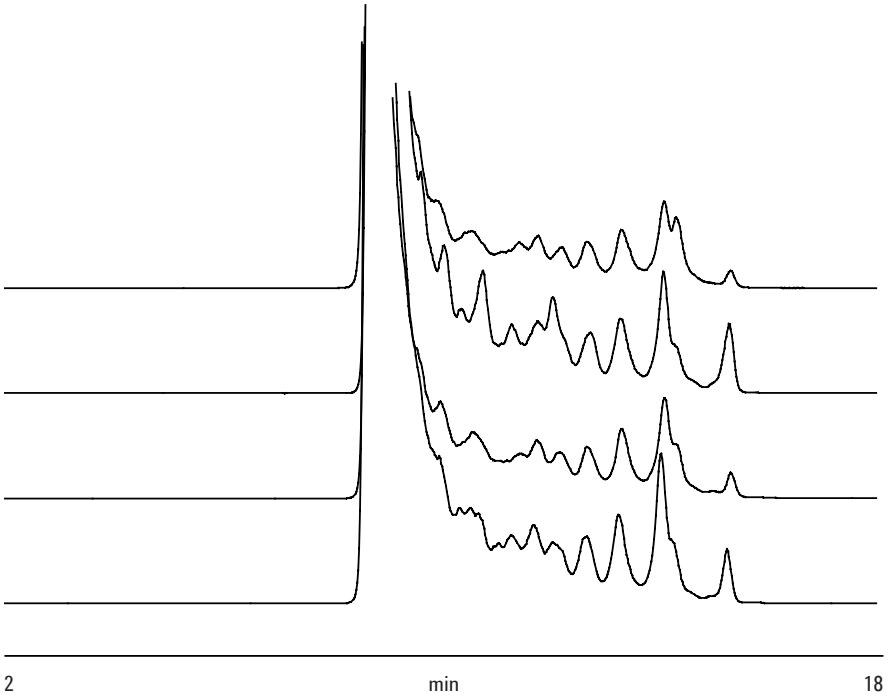


Figure 20. *Overlaid chromatograms of epoxy resin samples, with excluded polymer components to focus on the oligomeric region*

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.

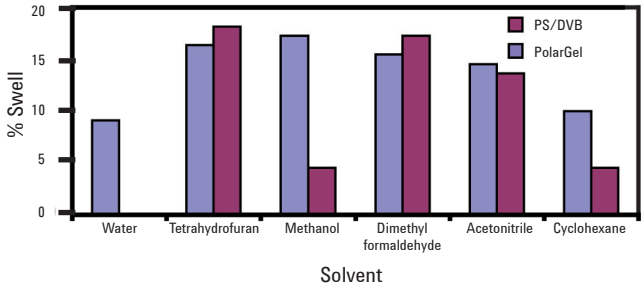
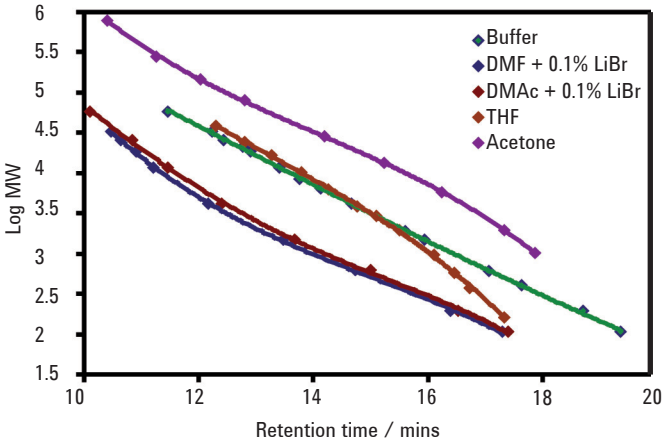


Figure 21. *Swell data for PolarGel and a PS/DVB material showing the low swell of PolarGel*

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 µl
Temp: 50 °C
Detectors: Agilent PL-GPC 50 Plus
Integrated GPC/SEC System, RI

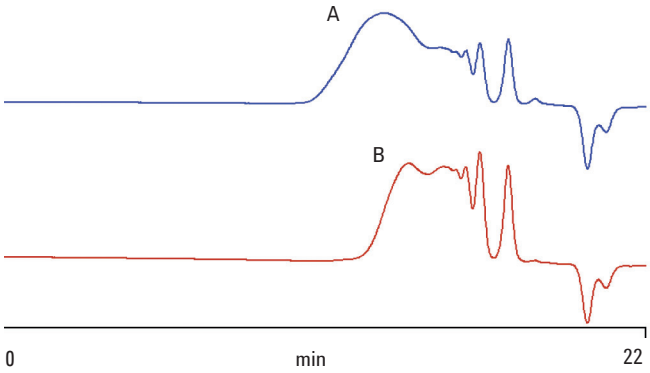


Figure 23. *PolarGel-M reveals the composition of two phenol-formaldehyde resins, with clear differences between the materials*

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 µl
Temp: 50 °C
Detectors: Agilent PL-GPC 50 Plus
Integrated GPC/SEC System, RI

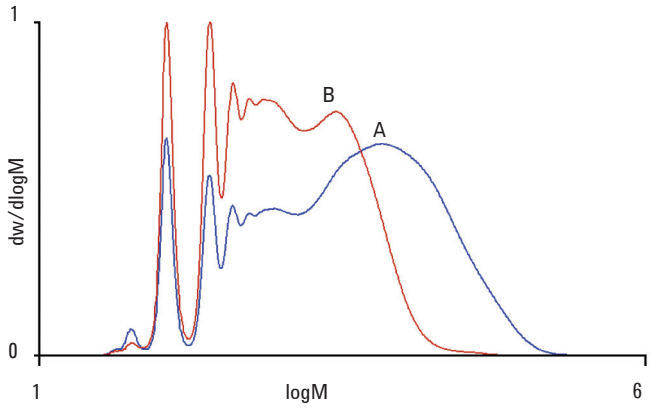


Figure 24. *Overlaid molecular weight distributions of two phenol-formaldehyde resins show the same oligomers are present*

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 that 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 °C
Detectors: Agilent PL-GPC 50 Plus Integrated GPC/SEC System, RI

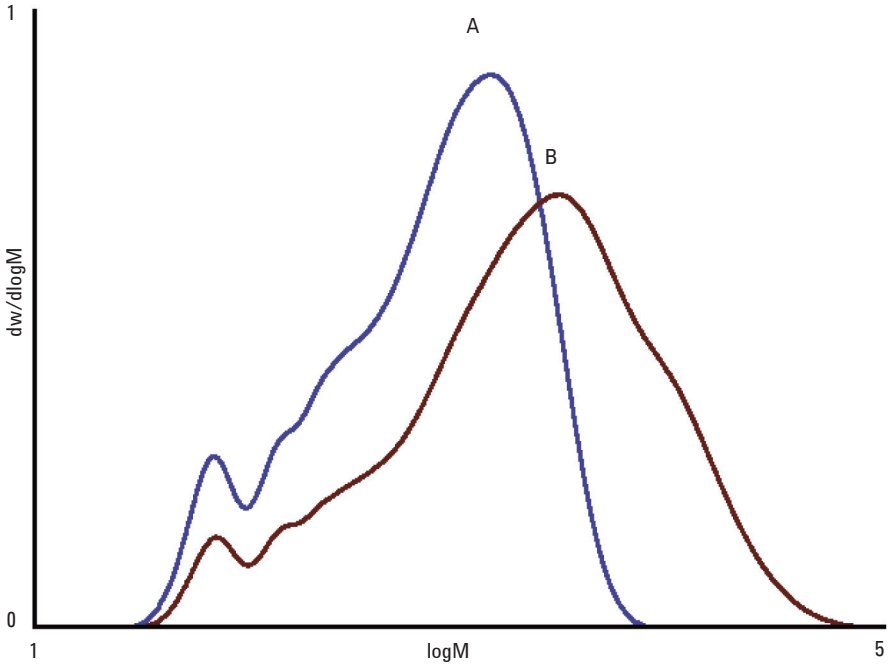


Figure 25. *Overlaid molecular weight distributions of two novolac resins with very different molecular weights*

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

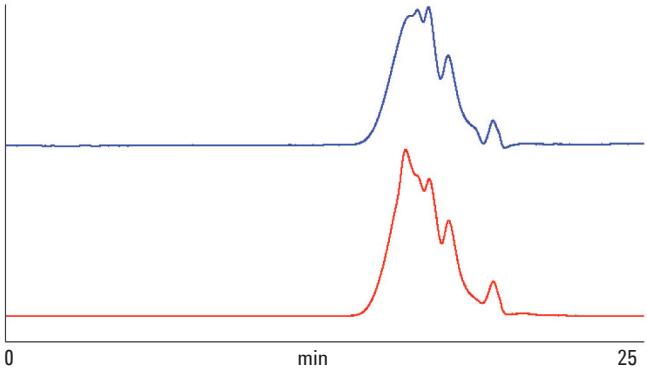


Figure 26. *Chromatograms for two melamine samples showing obvious differences*

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

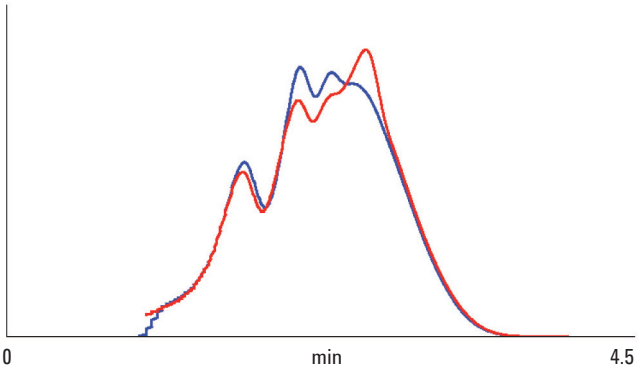


Figure 27. *Overlaid molecular weight distributions for two melamine resin samples highlighting changes in the distributions*

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 Ultimetal. 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 CO_2 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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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 × 0.035" id). One end was filed to create a sharp inner edge. The holes created were about 0.5 mm id.

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

Results

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

A = 0
B = 0.3
C = 0.9

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

A = 0
B = 0.1
C = 0.3

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

Conclusions

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

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

Solvent: hexane, bp = 70 °C

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

Solvent: isooctane, bp = 100 °C

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

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

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Agilent Technologies

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

Application Note

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Introduction

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

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

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Experimental

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

Hardware Configuration

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

Sample Preparation

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

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

Resource Layout:

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

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

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

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

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

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

Results

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

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

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

* Not measured.

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

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

Reproducibility of FID performance

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

Table 2. FID MDL

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

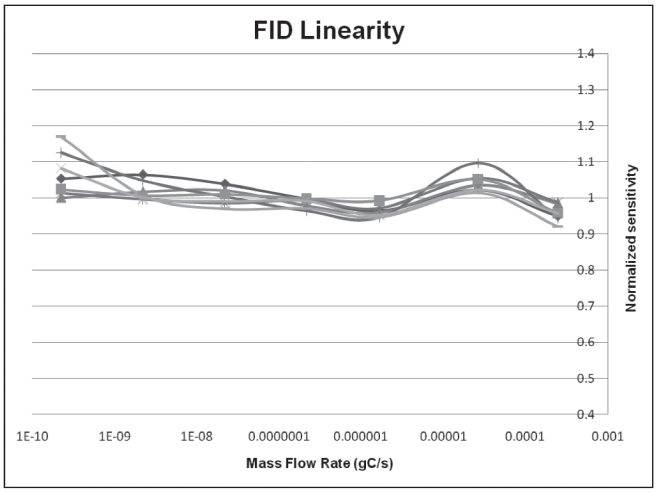

Figure 1. Linearity Plots for all eight runs overlaid.

Conclusion

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

Appendix

500 µL syringe parameters:

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

100 µL syringe parameters:

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

Reference

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

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Improved Data Quality Through Automated Sample Preparation

Application Note

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Abstract

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

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Introduction

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

Figure 1. The Agilent 7696 Sample Prep WorkBench.

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

Experimental

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

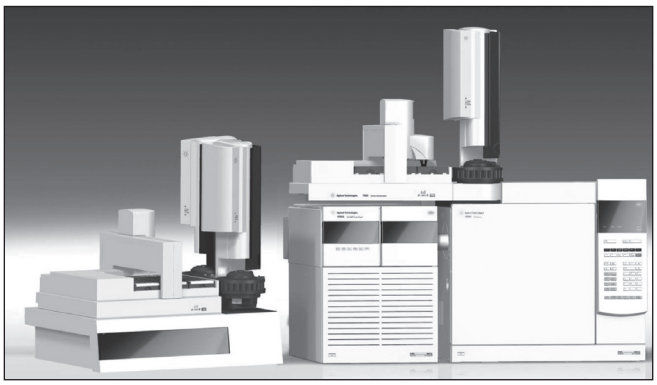

Figure 2. The Agilent 7696 Sample Prep WorkBench with a gas chromatograph and mass spectrometer.

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

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

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

Results and Discussion

GC and LC Sample Dilution

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

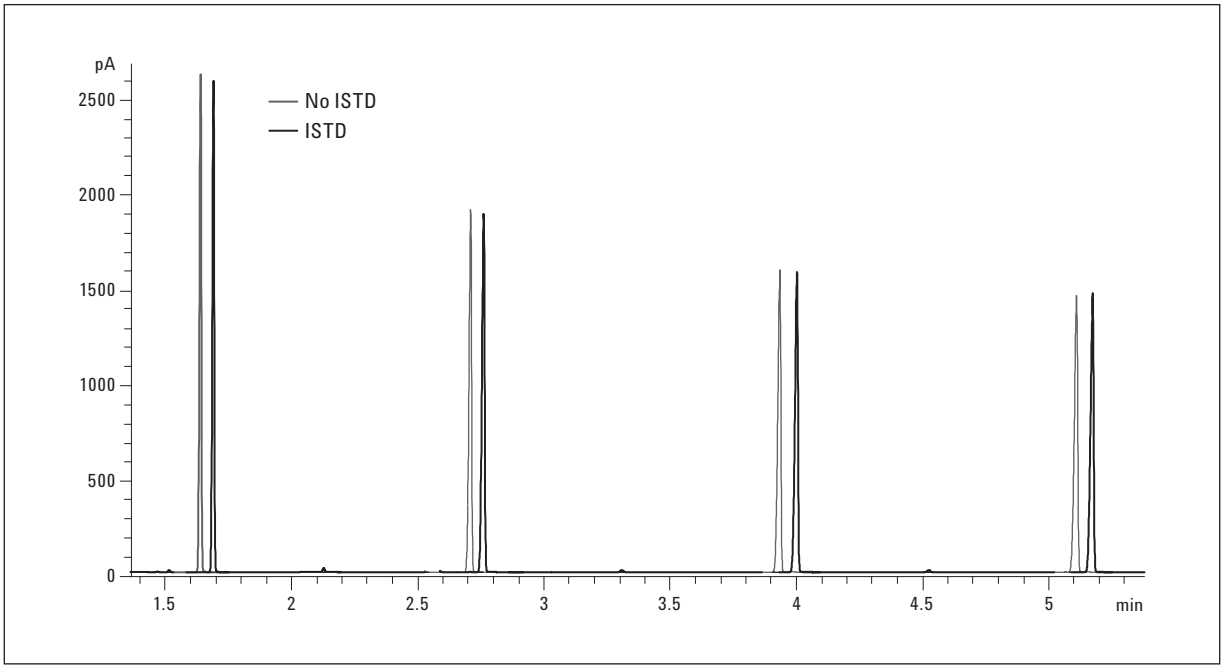

Figure 4. GC chromatograms (slightly offset) are shown for a standard solution dispensed and diluted with and without an ISTD added. No difference in peak areas are observed.

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

Calibration Curve Standard Preparation

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

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

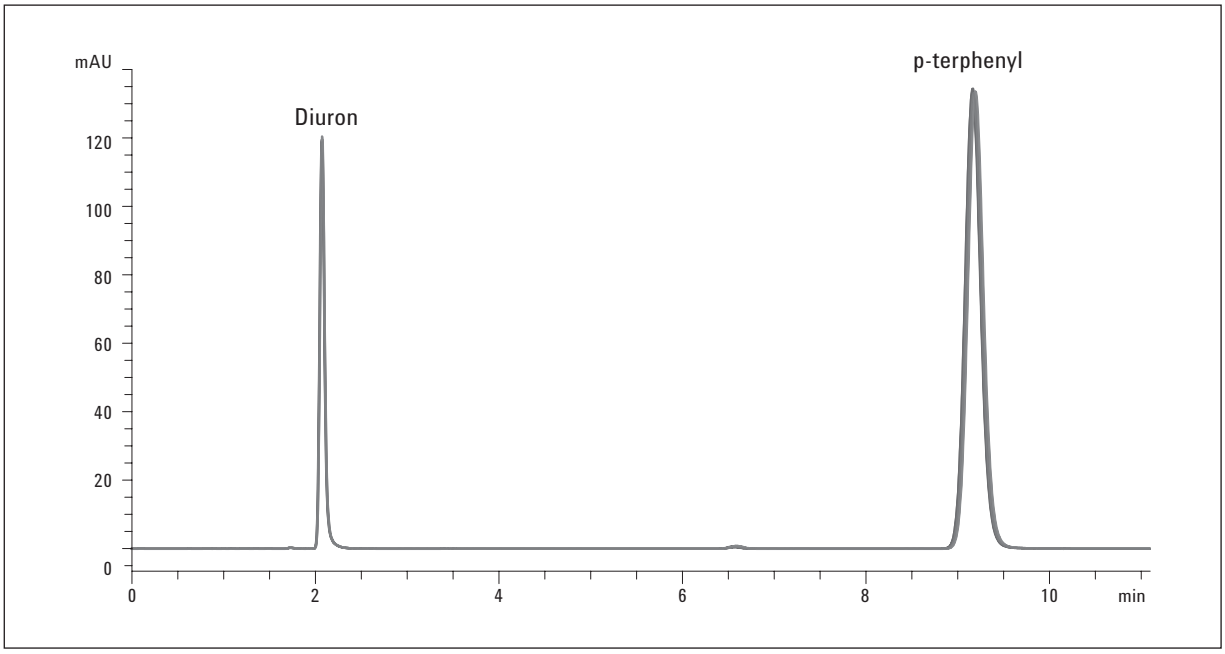

Figure 5. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

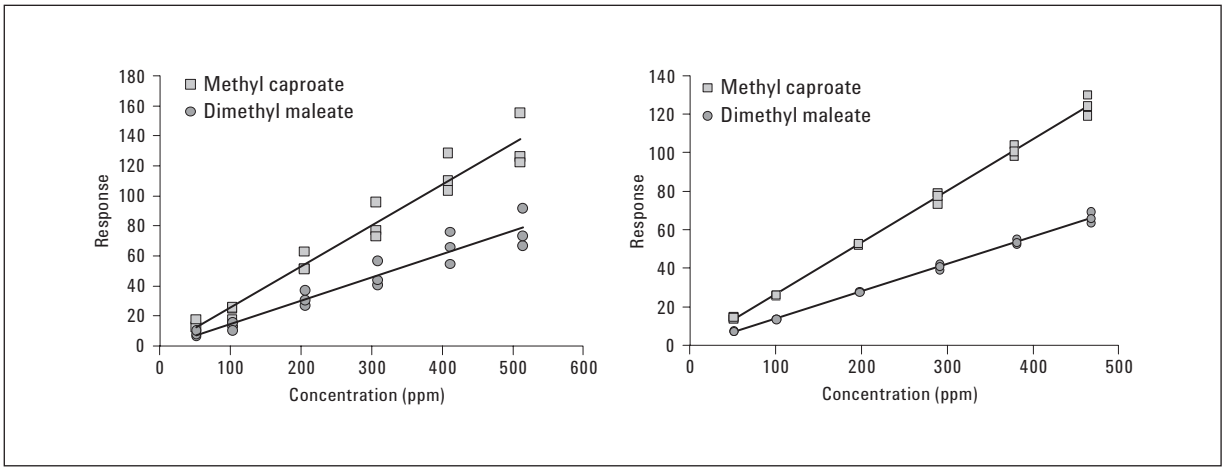

Figure 6. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with the Agilent 7696 Sample Prep WorkBench, are visibly more reproducible than the curves made manually on the left.

Fatty Acid Derivatization

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

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

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

Conclusions

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

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

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

Reference

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

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Agilent Technologies

Biodegradable polymers - analysis of biodegradable polymers by GPC/SEC

Application compendium

Authors

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The Measure of Confidence

Agilent Technologies

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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 biodegrabale 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 µm 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 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent: Chloroform
Flow Rate: 1.0 mL/min
Detector: Agilent ELSD

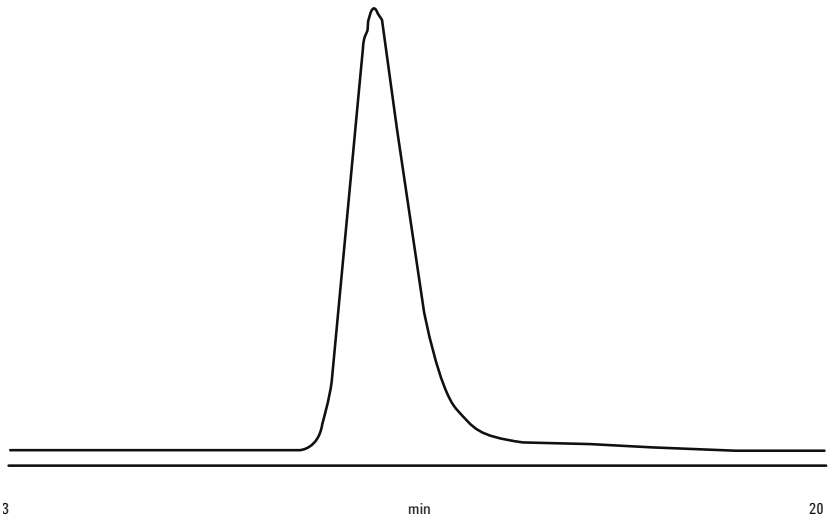


Figure 1. *Typical raw data chromatogram for a poly(lactide-co-glycolide) sample showing a typical gaussian peak shape*

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 µm 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 µm MIXED-C, 300 x 7.5 mm (Part No. PL1110-6500)
Eluent:	THF (stabilized)
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Detector:	RI

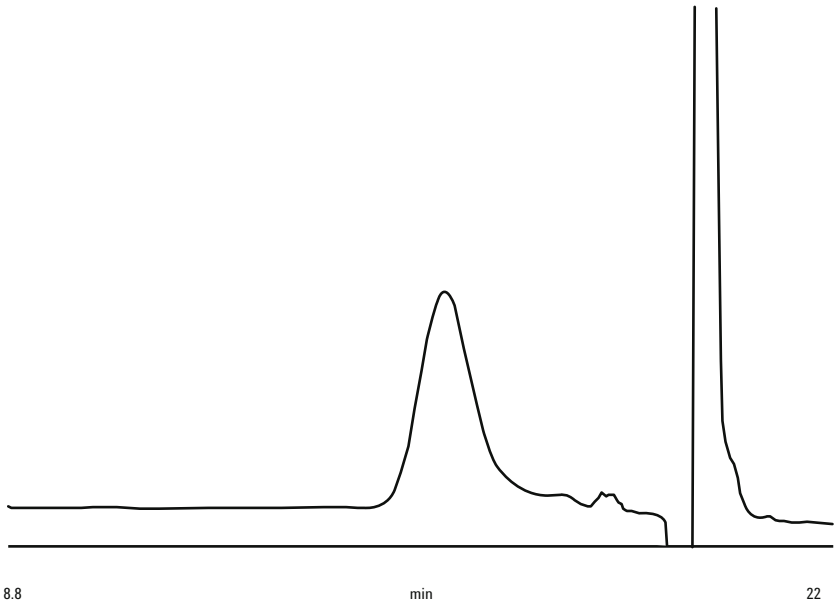


Figure 2. *Typical raw data chromatogram for a sample of polycaprolactam containing low molecular weight components and showing a large system peak*

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 8 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

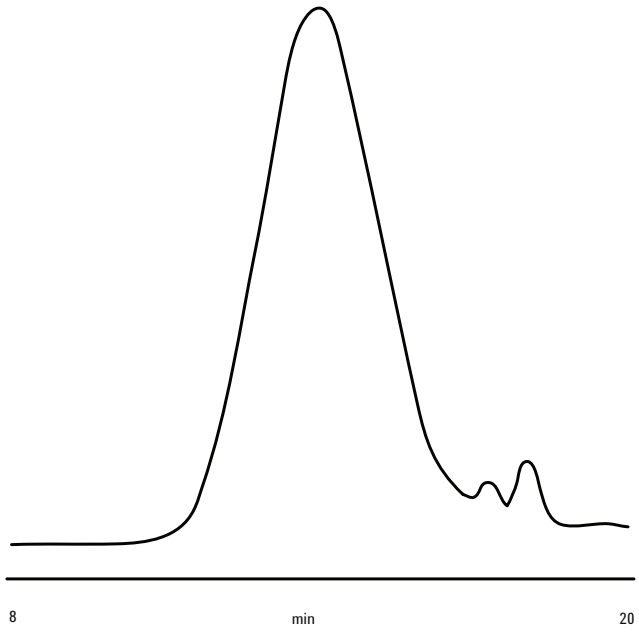


Figure 3. *Raw data chromatogram (sample A) showing the presence of low molecular weight components along with the polymer peak*

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

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

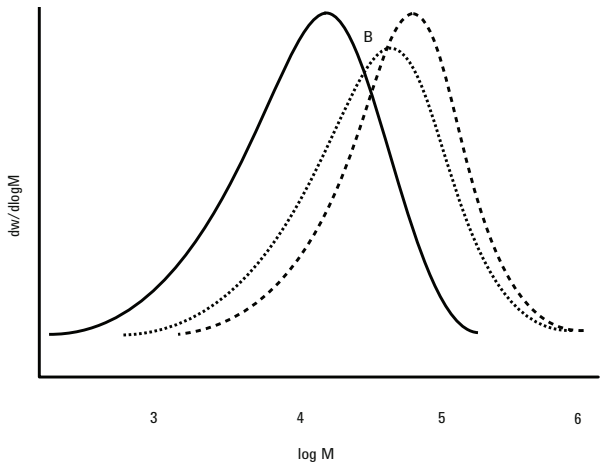


Figure 4. *Example molecular weight distributions of the three polyvinyl alcohols with very different physical properties*

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

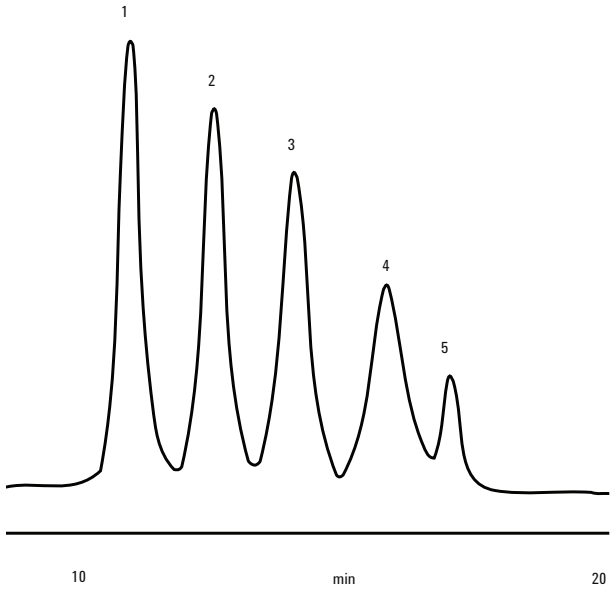


Figure 5. *Resolution achieved in the separation of five PEG samples used for calibration*

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.

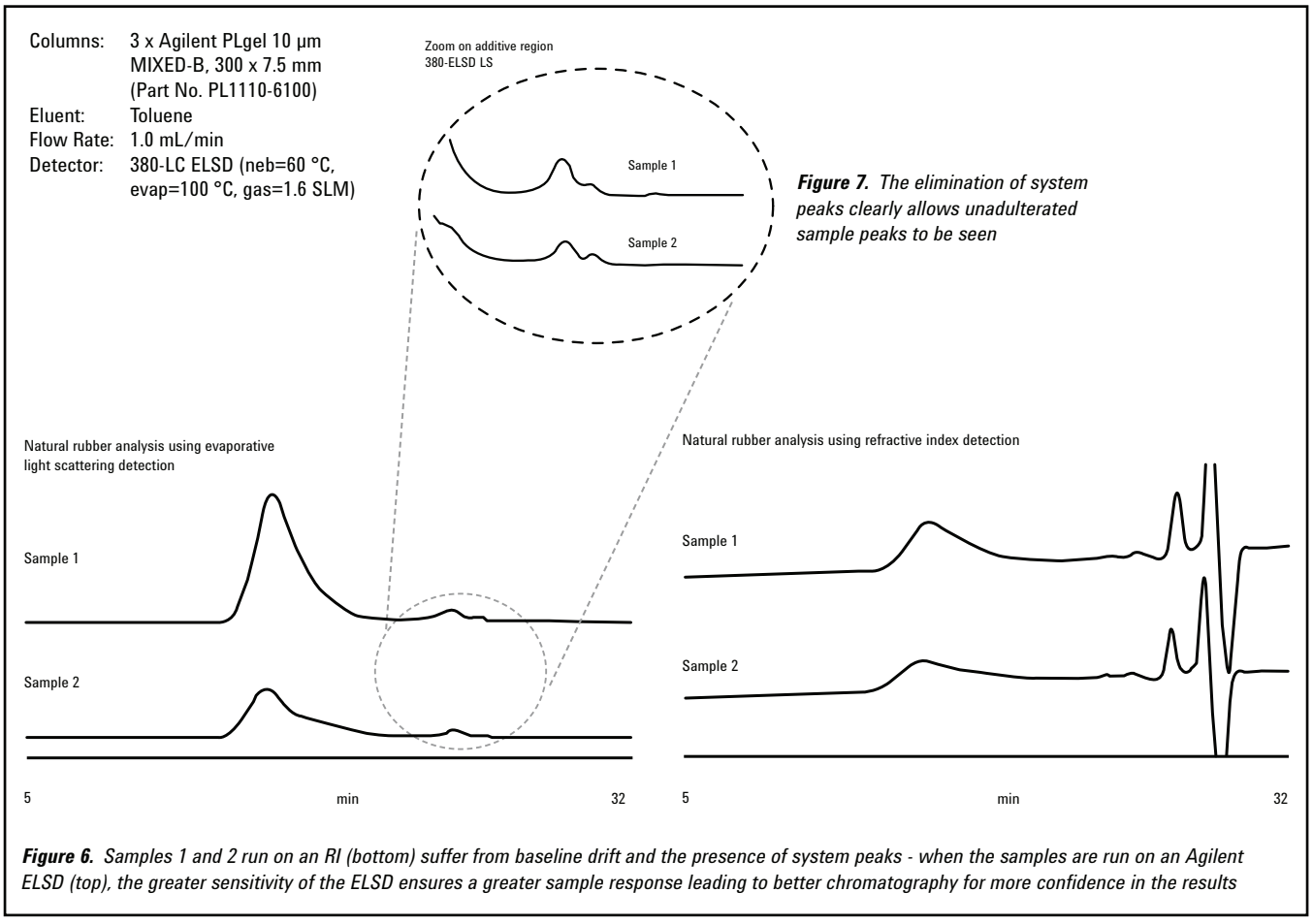


Figure 7. *The elimination of system peaks clearly allows unadulterated sample peaks to be seen*

Figure 6. *Samples 1 and 2 run on an RI (bottom) suffer from baseline drift and the presence of system peaks - when the samples are run on an Agilent ELSD (top), the greater sensitivity of the ELSD ensures a greater sample response leading to better chromatography for more confidence in the results*

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 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Sample Conc:	PEO standards: 0.1-0.5 mg/mL Polyacrylic acid: approx 0.2% w/v
Detector:	RI

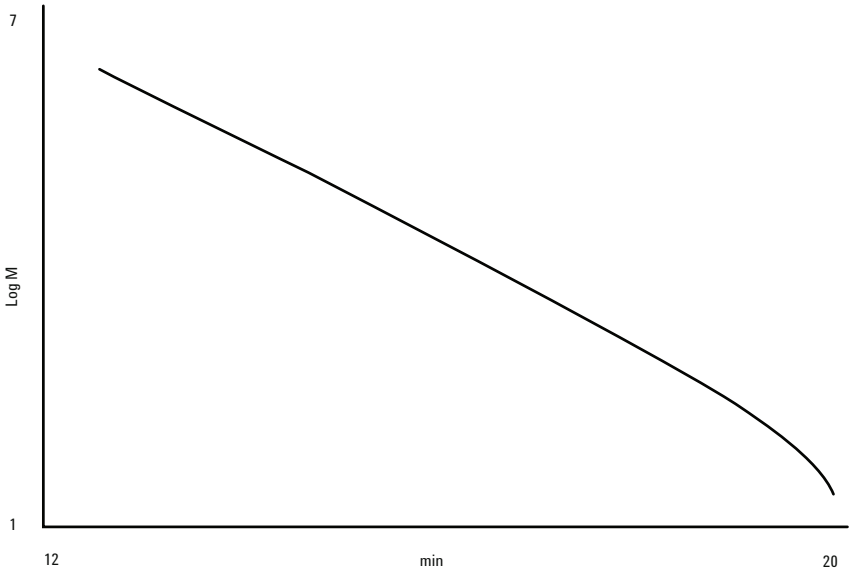


Figure 8. *PL aquagel-OH MIXED-H 8 µm column calibration using PEO EasiVial standards showing the relationship between retention time and the log of molecular weight*

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 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate: 1.0 mL/min
Inj Vol: 200 µL
Sample Conc: PEO standards: 0.1-0.5 mg/mL
Polyacrylic acid: approx 0.2% w/v
Detector: RI

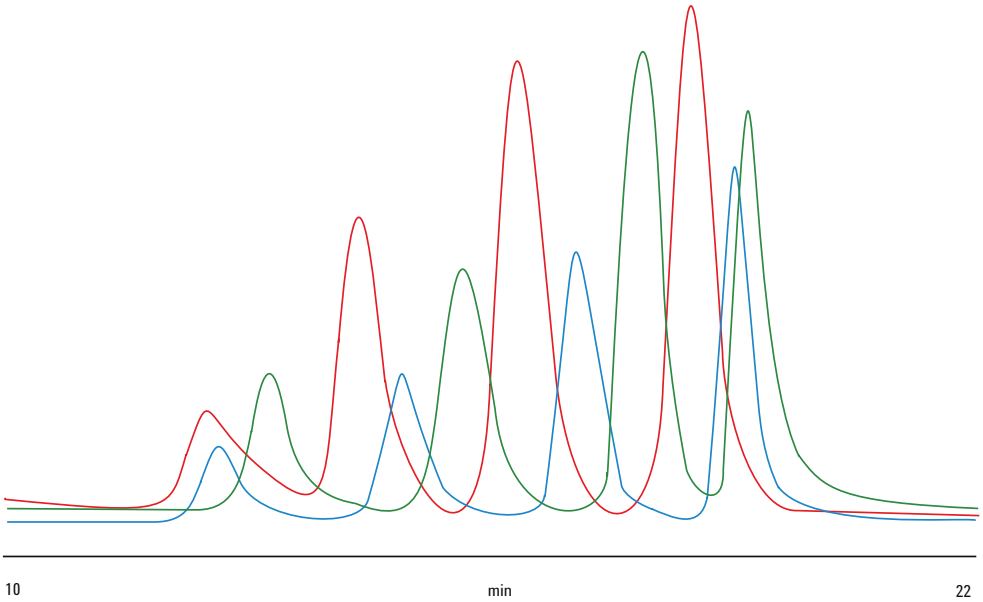


Figure 9. *Example chromatograms of PEO EasiVial standards used to create the calibration*

Columns:	2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, adj to pH 7
Flow Rate:	1.0 mL/min
Inj Vol:	200 µL
Sample Conc:	PEO standards: 0.1-0.5 mg/mL
	Polyacrylic acid: approx 0.2% w/v
Detector:	RI

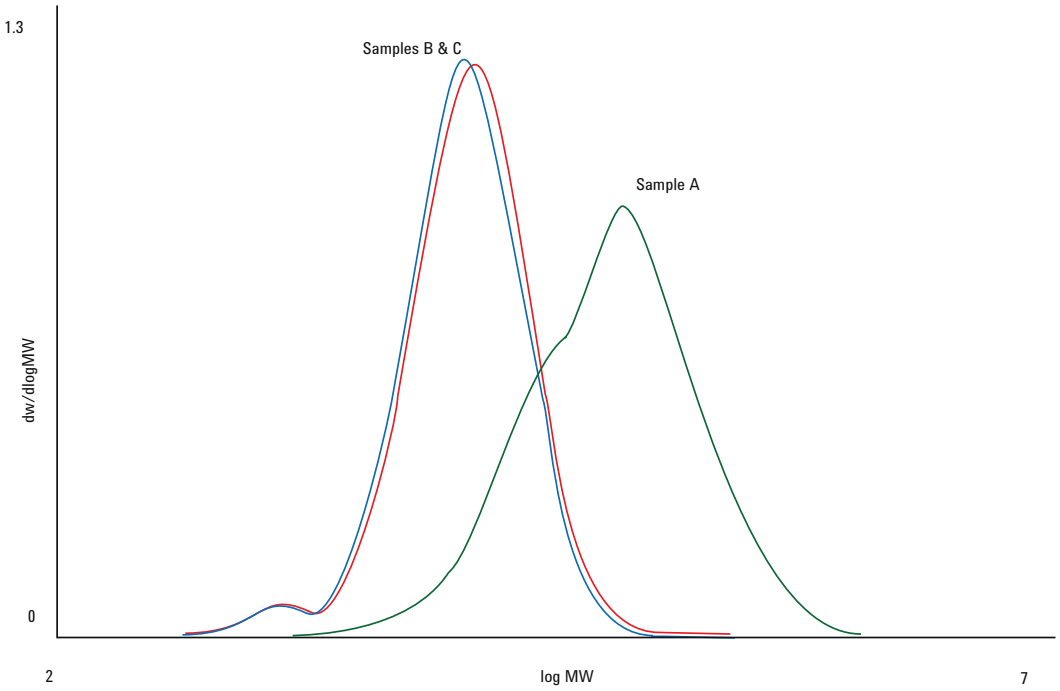


Figure 10. *Molecular weight distribution of three polyacrylic acid samples showing a clear difference between one of the samples (A) and the other two (B and C)*

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 µm 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 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.5M $NaNO_3$, 0.01M NaH_2PO_4, pH 2
Flow Rate: 1.0 mL/min
Detector: RI

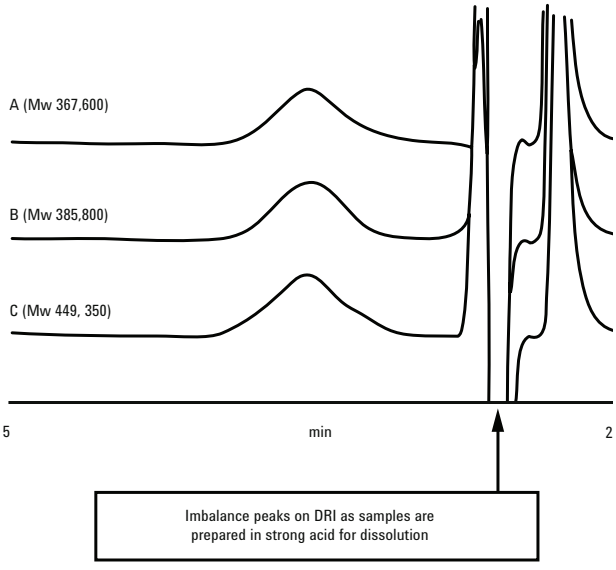


Figure 11. *Raw data chromatograms of three chitosan samples showing typical peak shapes with strong imbalance peaks due to dissolution conditions*

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent: 0.5M $NaNO_3$, 0.01M NaH_2PO_4, pH 2
Flow Rate: 1.0 mL/min
Detector: RI

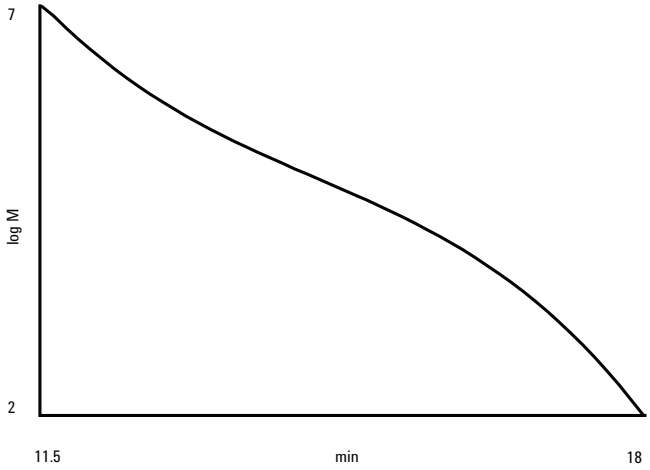


Figure 12. *SEC calibration showing the resolving range of the PL aquagel-OH MIXED-H 8 µm column set*

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 carboxymethl 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 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:	0.5M Na_2SO_4
Flow Rate:	1.0 mL/min
Detector:	RI

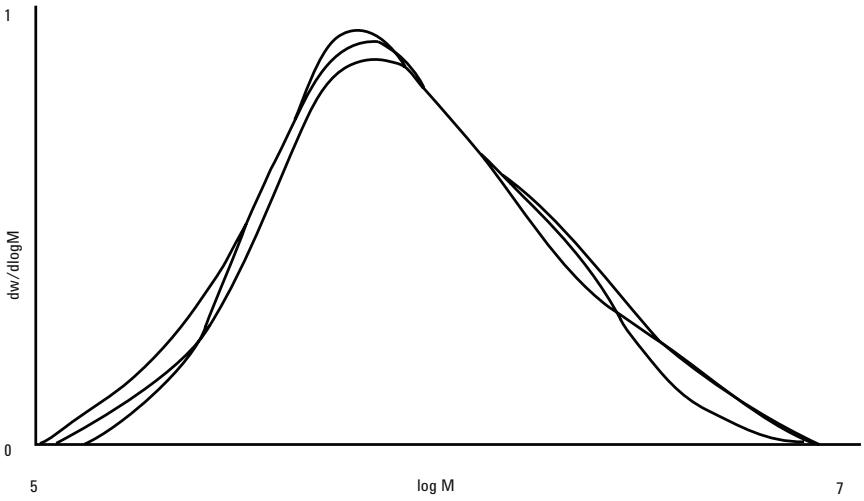


Figure 13. *Example molecular weight distributions of three samples of carboxymethyl cellulose with subtle differences between samples*

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 NaH_2PO_4, 0.25M NaCl, pH 7
Flow Rate:	1.0 mL/min
Detector:	RI

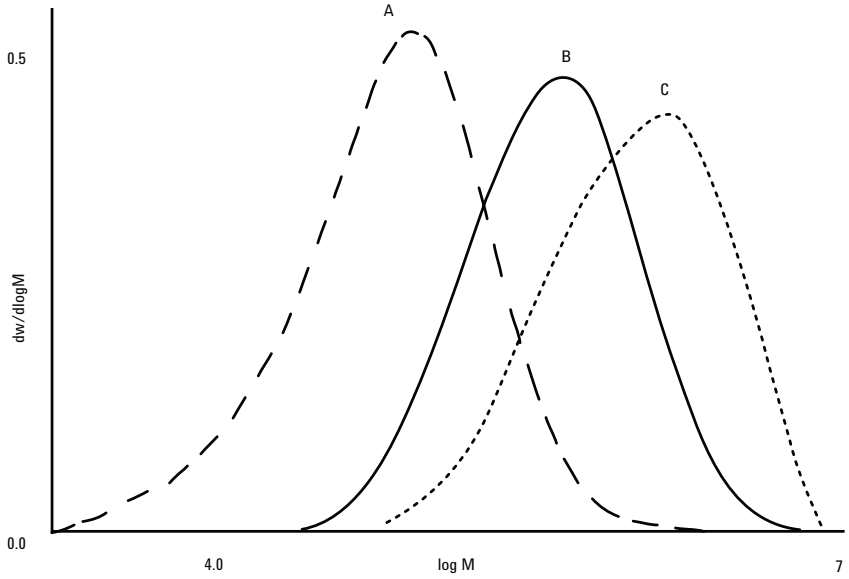


Figure 14. *Example molecular weight distributions of three samples, A, B and C with very different properties*

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 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 NaH_2PO_4, 0.25M NaCl, pH 7
Flow Rate:	1.0 mL/min
Detector:	RI

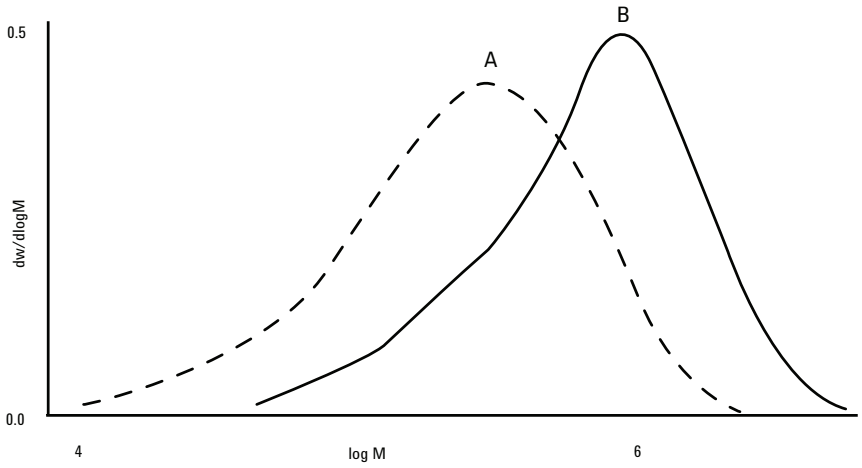


Figure 15. *Example molecular weight distributions for two samples of methyl cellulose which behave very differently*

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 µm 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 µL
Temp:	60 °C
Detector:	GPC (RI)

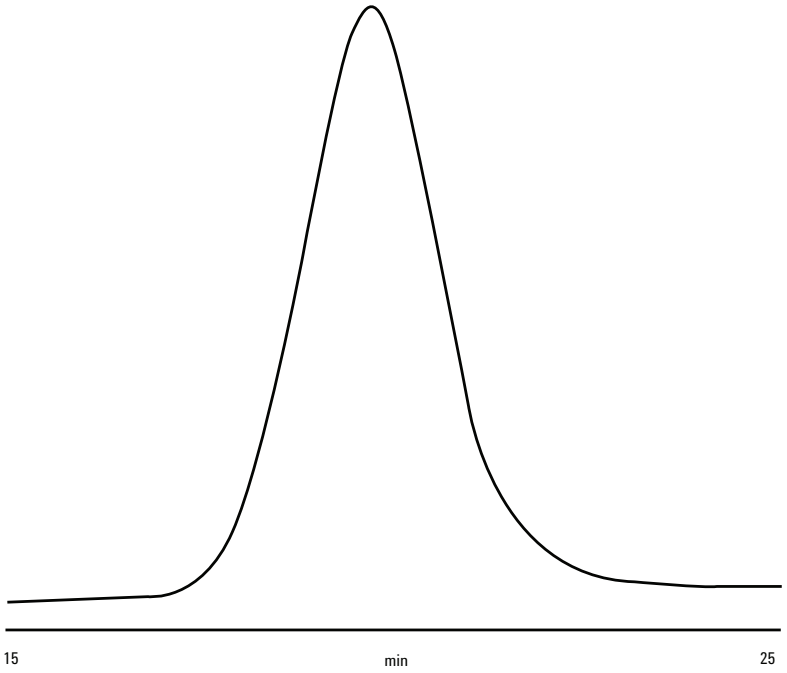


Figure 16. *Example chromatogram for a sample of cellulose acetate analysis showing a typical polymer peak shape*

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 heterogenous 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 $NaNO_3$, 0.01M NaH_2PO_4, 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 $NaNO_3$ + 0.01M NaH_2PO_4, 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

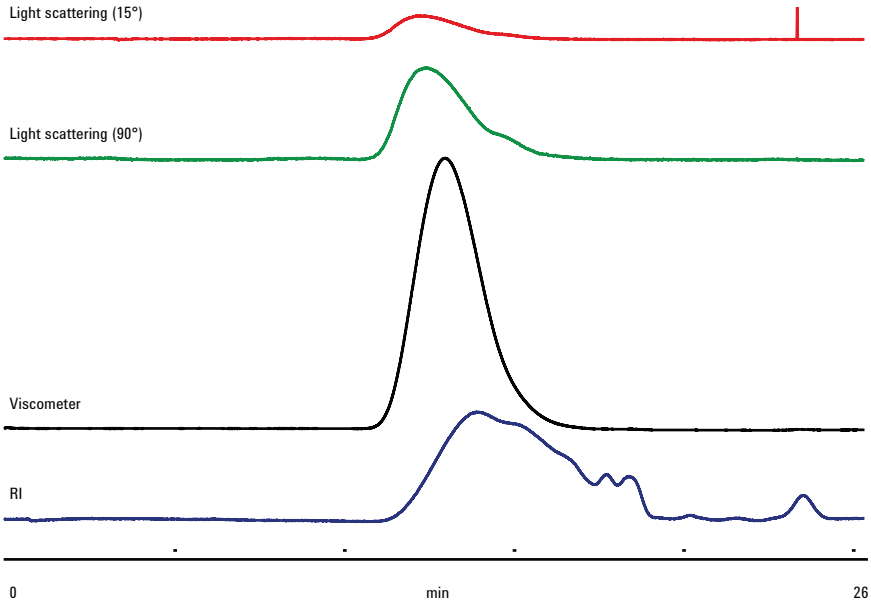
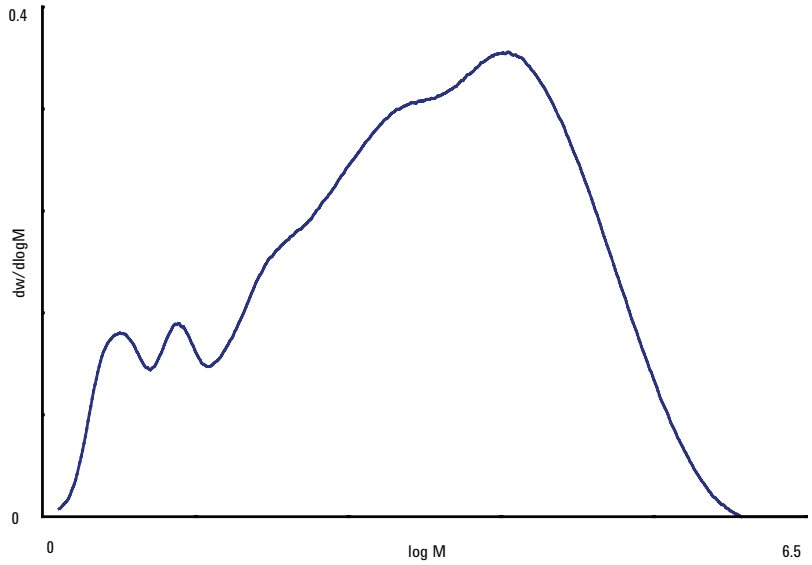


Figure 17. *A typical multi detector overlay of chromatograms for a sample of pectin, showing the different responses of the detector*

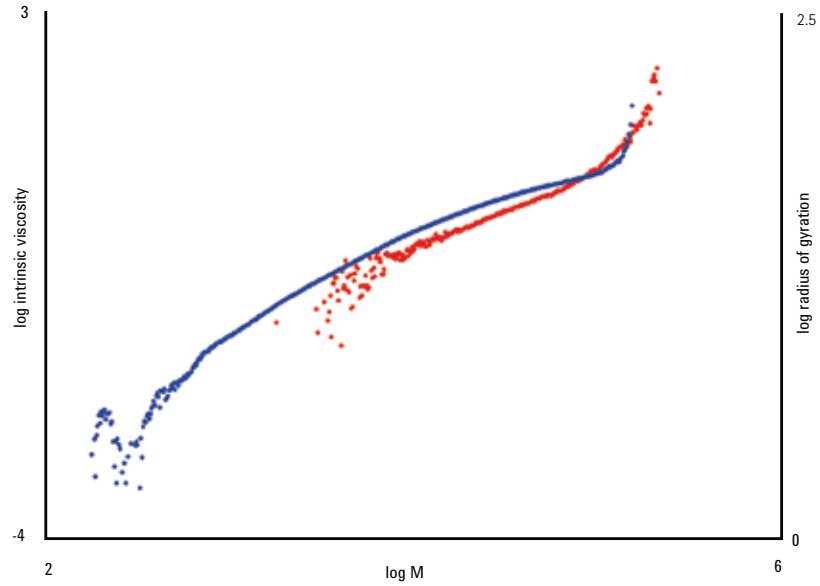
From the viscometry and light scattering data, Mark-Houwink (log intrinsic viscosity versus log M) and conformation (log radius of gyration versus log M) plots were generated for the pectin, shown overlaid in Figure 19.

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 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, 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 18. *Molecular weight distribution calculated for the pectin showing a complex shape*



Columns:	2 x PL aquagel-OH MIXED 8 µm, 300 x 7.5 mm (Part No. PL1149-6800)
Eluent:	0.2M $NaNO_3$ + 0.01M NaH_2PO_4, 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 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.

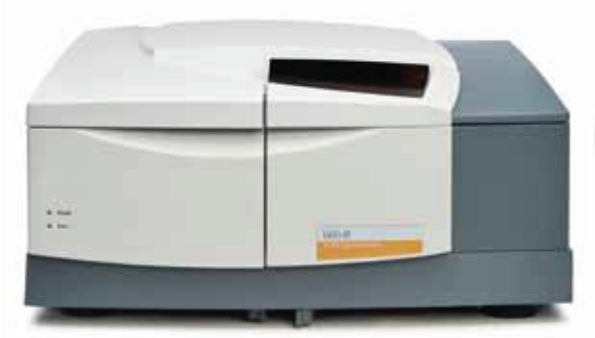
FTIR spectroscopy
The compositional analysis of polymers is made easy with Agilent's FTIR spectrometers and microscopes which extract specific chemical information from extremely small sample areas.

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.



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Agilent Technologies

Analysis of engineering polymers by GPC/SEC

Application Compendium

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Agilent Technologies, Inc.

The Measure of Confidence

Agilent Technologies

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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.

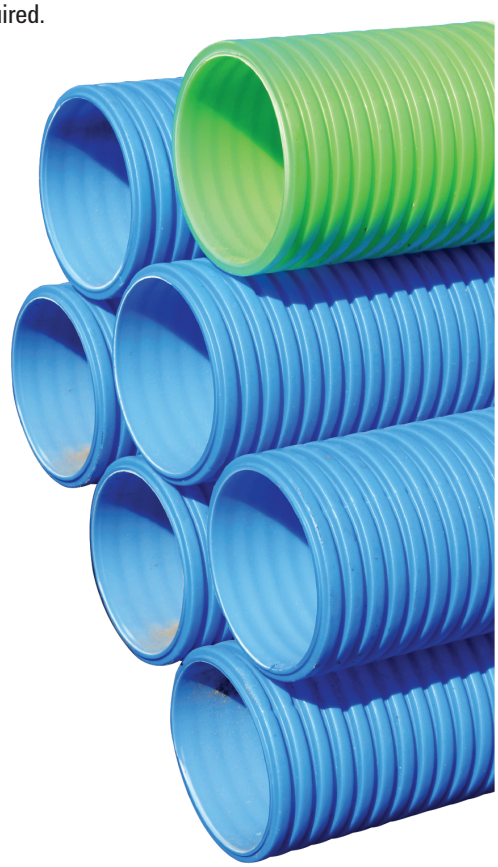

Table 1. *Effects of molecular weight distribution on the properties of engineering polymers*

	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 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

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

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

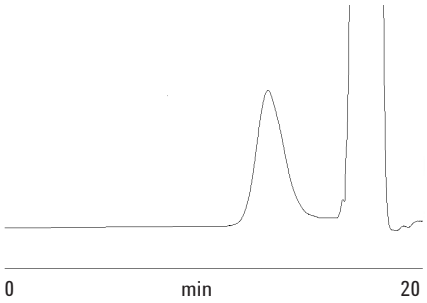

Figure 1. *Chromatogram of a PEEK sample*

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

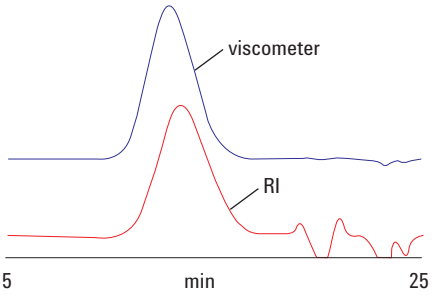

Figure 2. *Example overlay of a dual-detector chromatogram of the virgin PBT resin before molding*

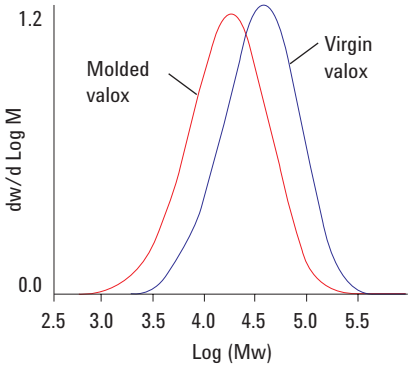

Figure 3. *Molecular weight distributions of the two samples*

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 µm 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

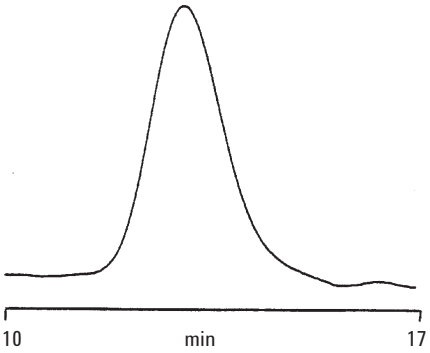

Figure 4. *Chromatogram of a PET sample*

Peak Identification
1. IV=0.72
2. IV=0.75
3. IV=0.84

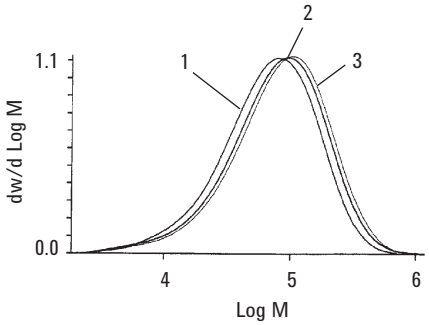

Figure 5. *Molecular weight distributions of the PET samples*

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 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6320)
Eluent:	TCB
Flow Rate:	0.8 mL/min
Inj Vol:	20 µL
Temp:	145 °C
Detector:	PL-GPC 220

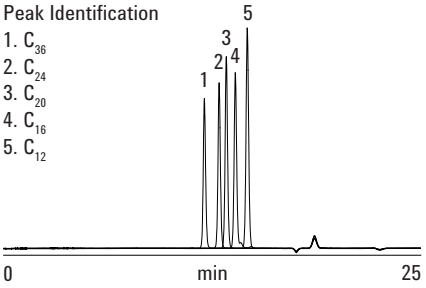

Figure 6. *Separation of a selection of low molecular weight linear hydrocarbons analyzed using two PLgel 3 µm 100Å columns*

Conditions

Sample:	Hydrocarbon wax
Columns:	2 x Agilent PLgel 5 µm MIXED-D, 300 x 7.5 mm (Part No. PL1110-6504)
Eluent:	TCB
Flow Rate:	1.0 mL/min
Inj Vol:	100 µL
Temp:	160 °C
Detector:	PL-GPC 220

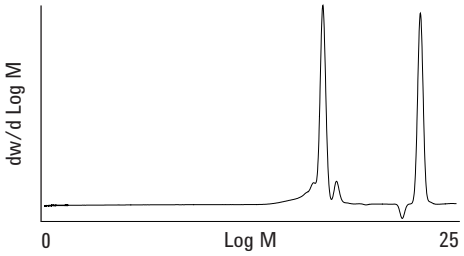

Figure 7. *Chromatogram of a relatively low molecular weight hydrocarbon wax obtained on two PLgel 5 µm MIXED-D columns*

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

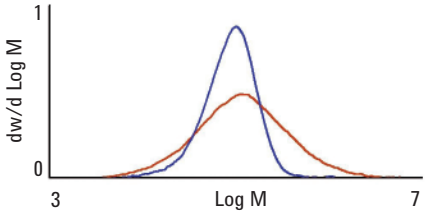

Figure 8. *Overlaid molecular weight distributions of medium and high polydispersity polyethylene samples*

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.

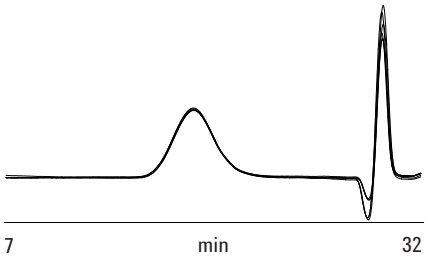

Figure 9. *Overlay of the raw data chromatograms obtained for six consecutive injections of polypropylene*

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 TCB[1] $K=12.1x10^{-5}$ $\alpha=0.707$

Polypropylene in TCB[2] $K=19.0x10^{-5}$ $\alpha=0.725$

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

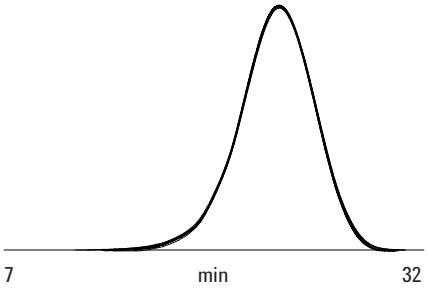

Figure 10. *Overlay of the molecular weight distribution calculated for six consecutive injections of the polypropylene sample*

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

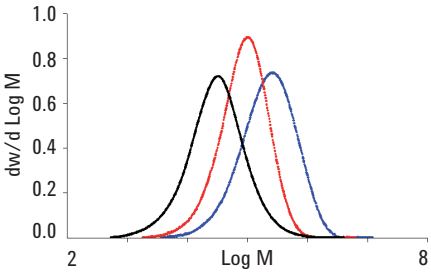

Figure 11. *Molecular weight distribution plots for the three polyethylene samples – the black plot is for the unbranched sample*

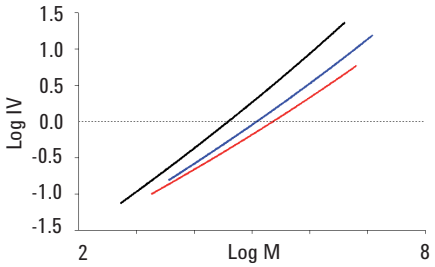

Figure 12. *Mark-Houwink plots for three samples of polyethylene*

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.

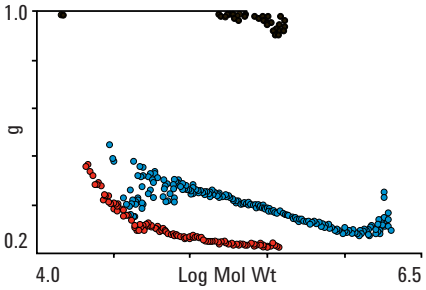

Figure 13. *Branching ratio g plots for the three polyethylene samples – the black plot is the unbranched sample*

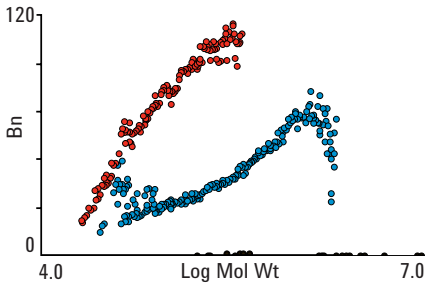

Figure 14. *Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample*

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 processibility 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

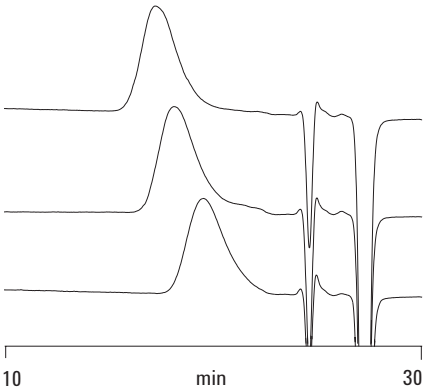

Figure 15. *Overlaid chromatograms of three samples of polyphenylene sulfide*

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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Agilent Technologies

Analysis of polyolefins by GPC/SEC

Application Compendium

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The Measure of Confidence

Agilent Technologies

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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 distributon 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.

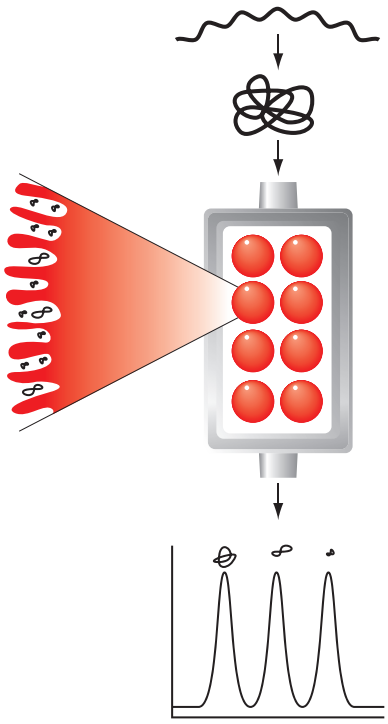
With the acquisition of PL, Agilent offers an even wider range of GPC and SEC solutions for all types of polymer characterization of synthetic and bio-molecular polymers, with options for conventional GPC all the way up to complex determinations using multi-column and multi-detection methods.

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.

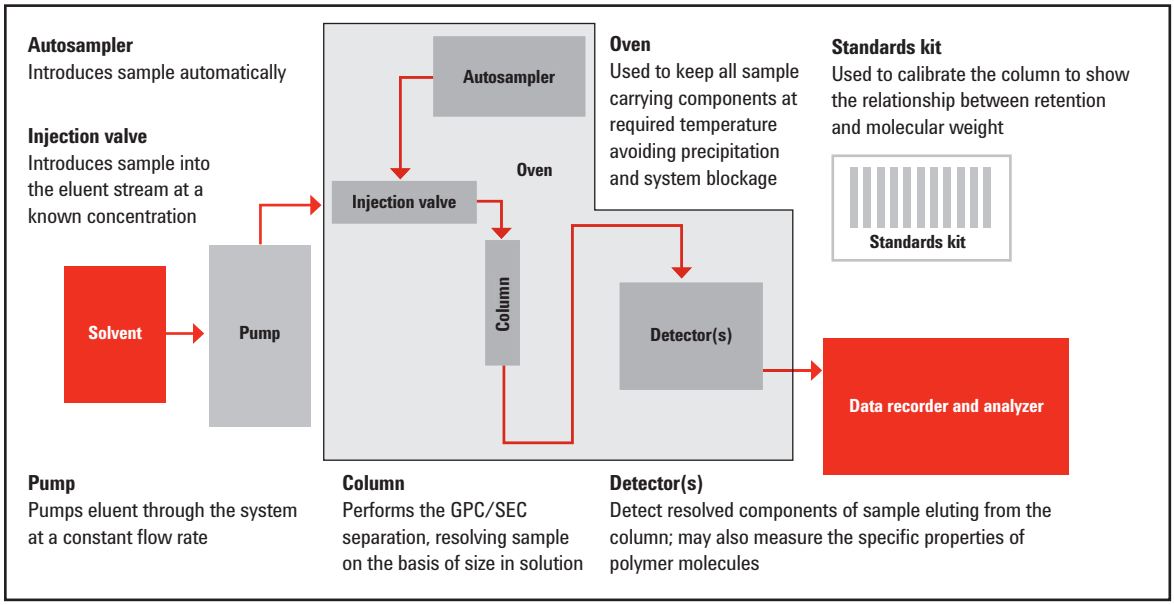


Figure 1. *Schematic of a GPC system for polyolefin analysis*

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 (± 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)

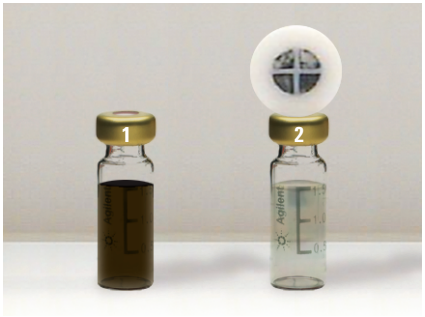


Figure 2. *Filtering a carbon black polyethylene solution – 1. without filtration, 2. after filtration using a 1 µm glass-fiber filter*

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 µm MIXED-B, 300 x 7.5 mm (Part No. PL1110-6100)
Flow Rate: 1 mL/min
Inj Vol: 200 µL
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

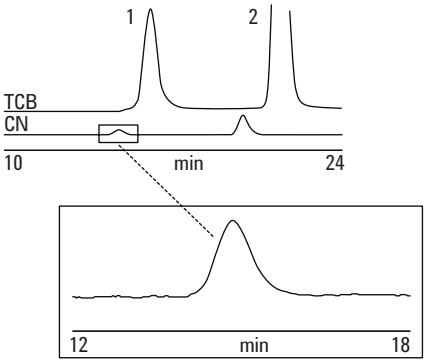


Figure 3. *Excellent signal-to-noise demonstrated in the separation of polystyrene standards*

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).

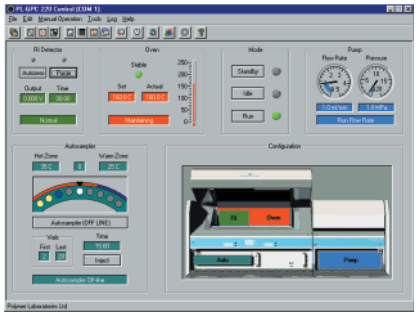


Figure 4. *Software control of the PL-GPC 220*

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? *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.*	High (up to several millions)	**PLgel Olexis**	PLgel Olexis is specifically designed for polyolefin analysis, offers optimal performance, also suitable for light scattering
		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? *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.*	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 efficiences – PLgel Olexis is 13 µm
		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? *Depending on analysis there are two options.*		**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

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 °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.

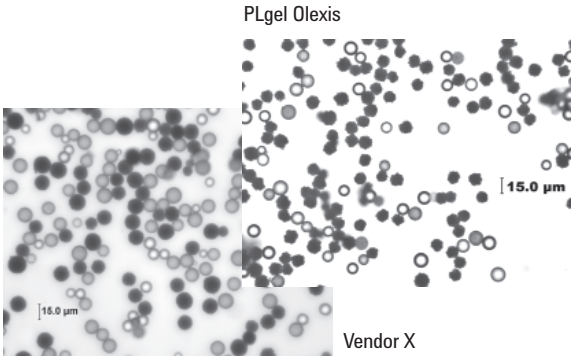


Figure 6. *The superior size consistency of PLgel Olexis particles is clearly evident*

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.

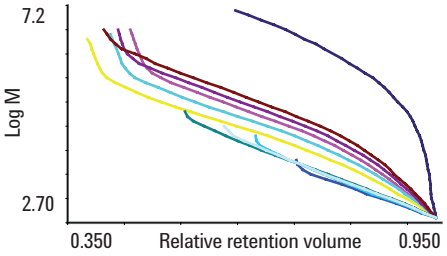


Figure 7. *Some of the components of PLgel Olexis that contribute to its lack of artifacts*

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.

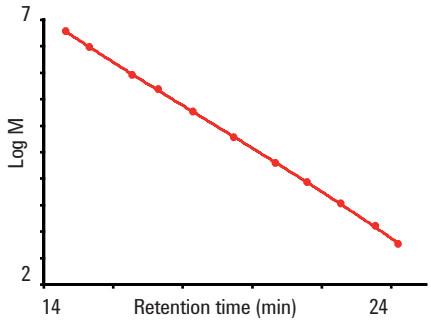


Figure 8. *Careful blending delivers highly linear polystyrene calibrations with PLgel Olexis in TCB*

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 µL
Temp:	160 °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.

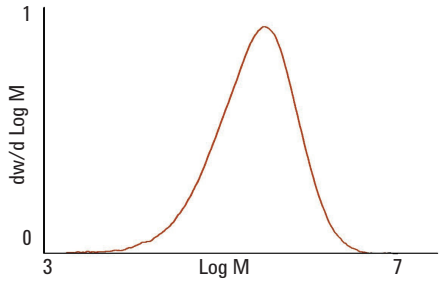


Figure 9. *True representation of polyolefin molecular weight distribution with PLgel Olexis*

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.

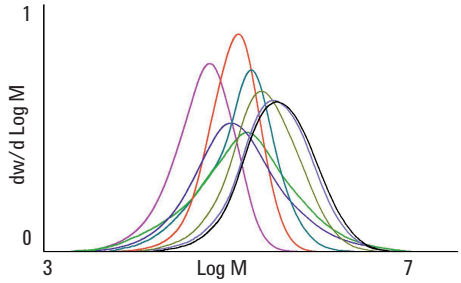


Figure 10. *PLgel Olexis reveals true modalities across the range of polyolefins*

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).

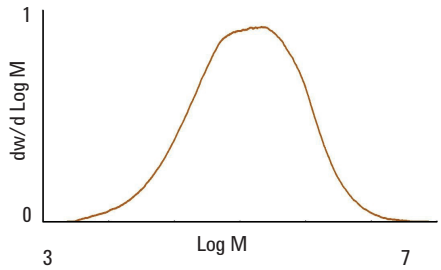


Figure 11. *A true change in peak shape revealed by PLgel Olexis of a multi-modal material manufactured from a multi-site catalyst*

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 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6520)
Eluent:	TCB
Flow Rate:	1 mL/min
Temp:	145 °C
Detector:	PL-GPC 220

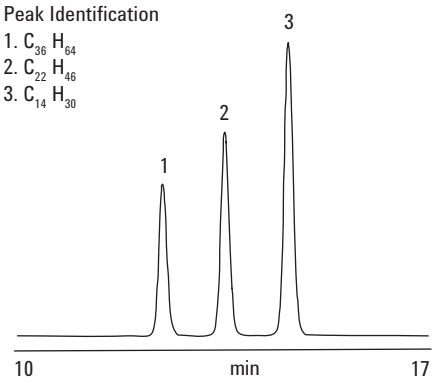


Figure 12. *Linear hydrocarbons separated to base-line on a PLgel column set*

Figure 13 shows the separation of a selection of low-molecular-weight linear hydrocarbons.

Conditions

Samples:	Linear hydrocarbons
Columns:	2 x Agilent PLgel 3 µm 100Å, 300 x 7.5 mm (Part No. PL1110-6320)
Eluent:	TCB
Flow Rate:	0.8 mL/min
Inj Vol:	20 µL
Temp:	145 °C
Detector:	PL-GPC 220

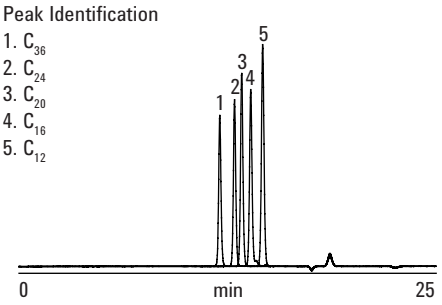


Figure 13. *Separation of low-molecular-weight hydrocarbons*

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 µm 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

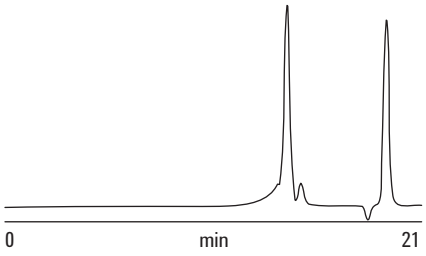


Figure 14. *A low-molecular-weight wax*

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

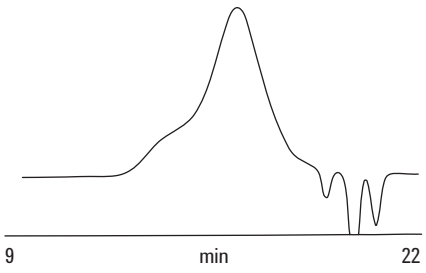


Figure 15. *Fast analysis of asphalt on PLgel 5 µm MIXED-D columns*

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

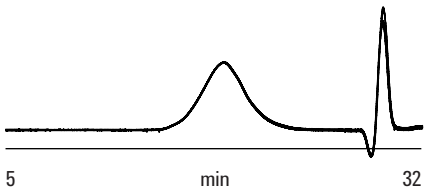


Figure 16. *Overlay of the raw data chromatograms obtained for eight consecutive injections of HDPE*

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 TCB[1] $K = 12.1 \times 10^{-5}$ $\alpha = 0.707$

Polyethylene in TCB[2] $K = 40.6 \times 10^{-5}$ $\alpha = 0.725$

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 µm MIXED-B columns.

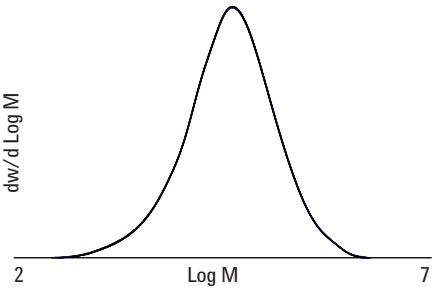


Figure 17. *Molecular weight overlay of eight consecutive injections of HDPE*

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.

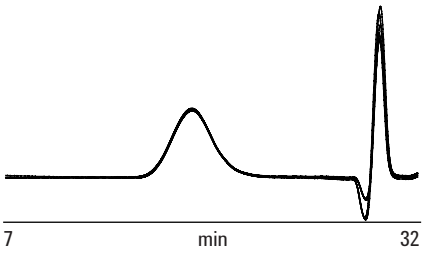


Figure 18. *Overlay of the raw data chromatograms obtained for six consecutive injections of HDPP*

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 TCB[1] $K = 12.1 \times 10^{-5}$ $\alpha = 0.707$

Polypropylene in TCB[2] $K = 19.0 \times 10^{-5}$ $\alpha = 0.725$

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 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 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 µm MIXED-B columns.

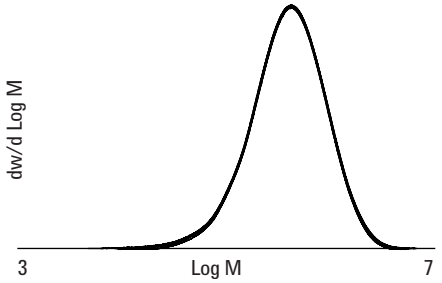


Figure 19. *Molecular weight overlay of six consecutive injections of HDPP*

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 (molecular weight x 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 α molecular weight x intrinsic viscosity

Equation 2:

Log (MW x intrinsic viscosity) versus retention time ≃ log (hydrodynamic volume) versus retention time

PLgel Olexis columns are separated and calibrated in terms of size and so a Universal Calibration is obtained (Figure 20).

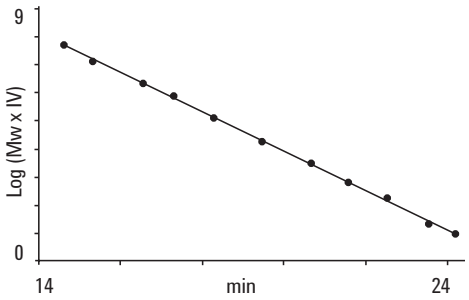


Figure 20. *Multi-detector GPC Universal Calibration of a PLgel Olexis column*

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 x 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 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 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

$$g = \left(\frac{\text{Rg branched}}{\text{Rg linear}} \right)_{MW}$$

Intrinsic viscosity contraction factor

$$g' = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)_{MW}$$

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 µL
Temp:	160 °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.

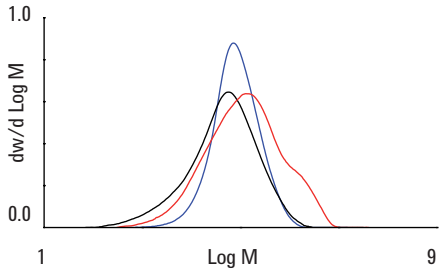


Figure 21. *Overlaid molecular weight distributions for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red*

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.

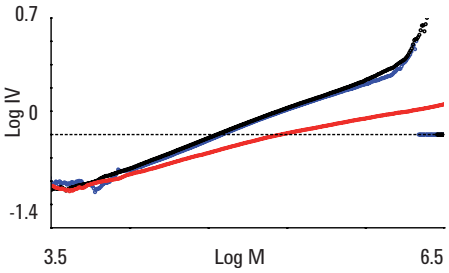


Figure 22. *Overlaid Mark-Houwink plots for three samples of polyethylene, HDPE – black, LLDPE – blue, LDPE – red*

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:

$$g = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)^{1/\epsilon}$$

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 (RI) + viscometer

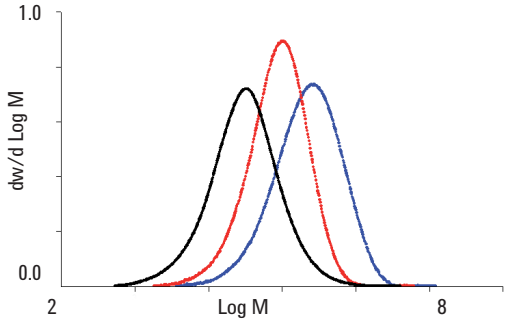


Figure 23. *Molecular weight distribution plots for three polyethylene samples – the black plot is the unbranched sample*

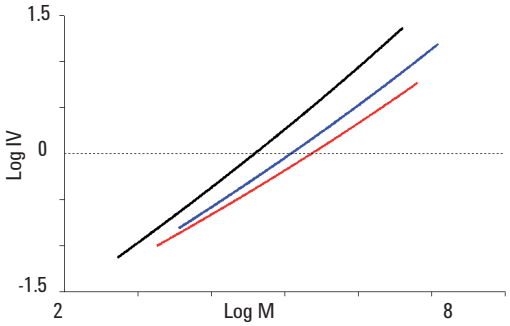


Figure 24. *Mark-Houwink plots for three samples of polyethylene*

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.

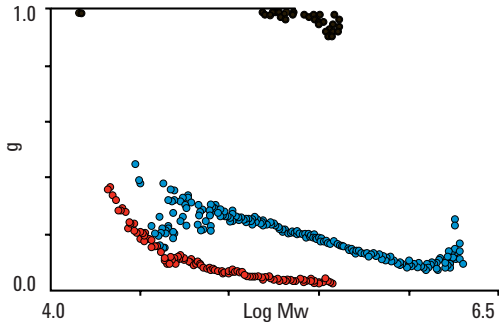


Figure 25. *Branching ratio g plots for three polyethylene samples – the black plot is the unbranched sample*

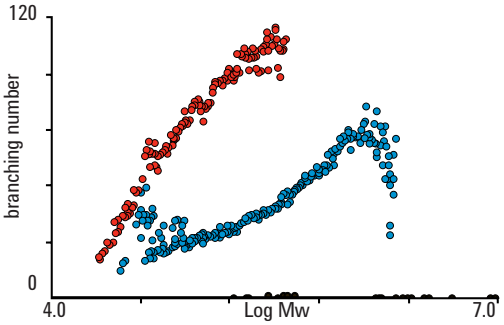


Figure 26. *Calculated branching numbers as a function of molecular weight for three samples of polyethylene – the black plot is the unbranched sample*

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 processibility 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 >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 >C-H stretching region, differences in the proportions of $>CH_2$ and $-CH_3$ 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 $-CH_3$ and $>CH_2$ groups can be used to measure the level of short-chain branching (SCB) in polyethylene[1]. 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 $-CH_3$ and $>CH_2$ 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 hexane 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.

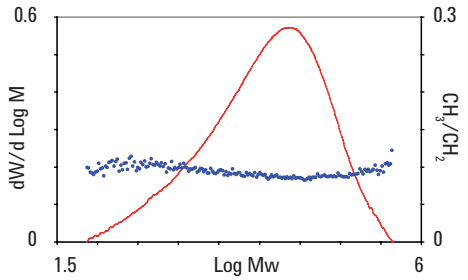


Figure 27. *Overlaid chromatogram of polymer weight and short-chain branching distribution for a sample of ethylene-hexene copolymer*

Reference

[1] P.J. DesLauriers, D.C. Rohlfing and E.T. Shieh (2002) Quantifying short chain branching microstructures in ethylene-1-olephin 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

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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.

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Agilent Technologies

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.

Agilent Technologies

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_2S and CH_3SH, ~500 ppb COS
Injection volume:	1 mL
Injection:	Gas sampling valve

Results and Discussion

The stationary phase shows good selectivity between H_2S, 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 H_2S, COS and CH_3SH 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.

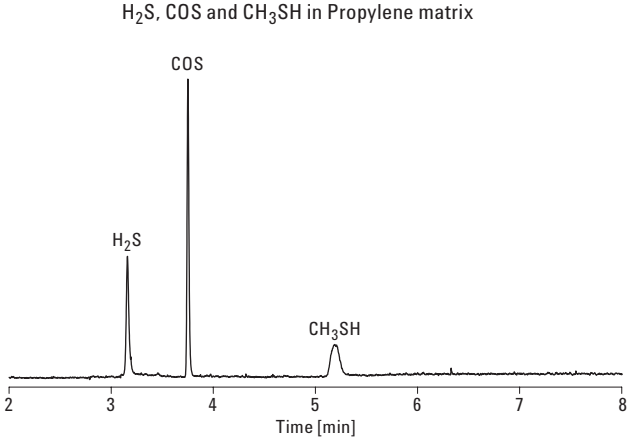

Figure 1. Chromatogram of H_2S, COS and CH_3SH in Propylene matrix, using the Agilent J&W Select Low Sulfur with GC-SCD.

Conclusion

The Agilent J&W Select Low Sulfur used in a GC with a sulfur specific detector, such as an SCD, can detect H_2S, COS and CH_3SH 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 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 H_2S 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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Agilent Technologies

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_2S, COS, and CH_3SH
Injection volume:	1 mL
Injection:	Gas sampling valve

Results and Discussion

The stationary phase shows good selectivity between H_2S, 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 H_2S, COS, and CH_3SH 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.

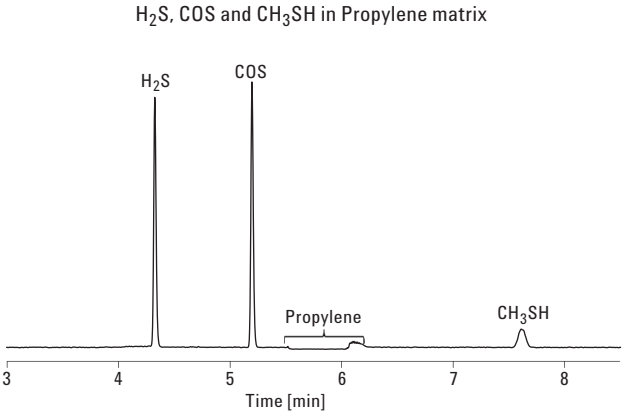

Figure 1. Chromatogram of sulfur compounds H_2S, COS and CH_3SH in a propylene matrix, using the Agilent J&W Select Low Sulfur with GC-PFPD.

Conclusion

The Agilent J&W Select Low Sulfur used in a GC with a sulfur specific detector, such as a PFPD, can detect H_2S, COS and CH_3SH 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 H_2S, 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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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 10^4Å, 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 10^4Å, 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

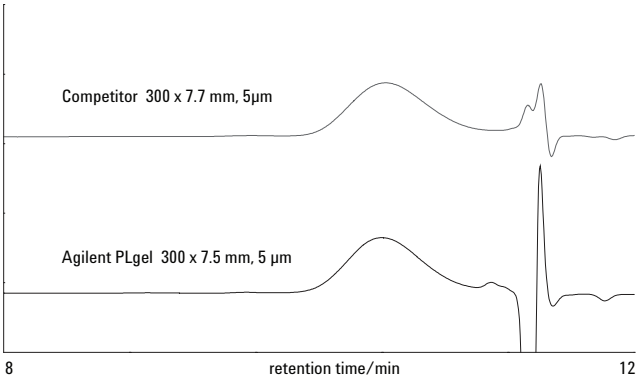

Figure 1. Overlaid chromatograms showing indistinguishable performance in the analysis of polycarbonate on an Agilent PLgel 7.5-mm diameter column compared to a 7.7-mm diameter column.

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.

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.

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Agilent Technologies

Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy

Fast, easy antioxidant measurement

Application Note

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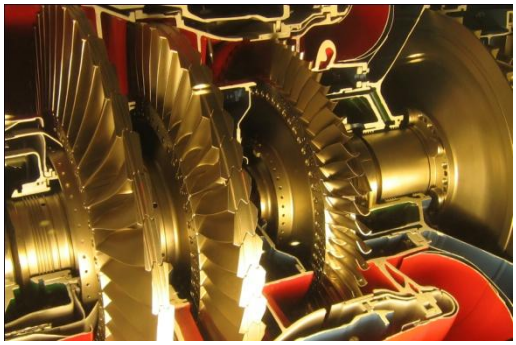

Abstract

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.

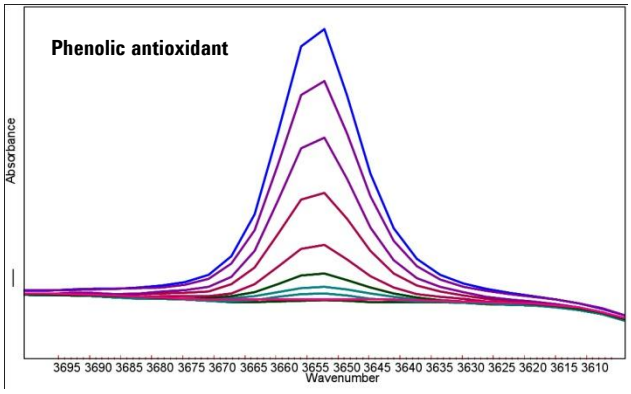

Figure 1. FTIR spectral overlay of the phenolic antioxidant functional group bands depleting as a function of time. The strongest band (light blue) is that of new ISO 32 turbine oil and the weakest absorbance (light green) is from turbine oil that has started to show some oxidation.

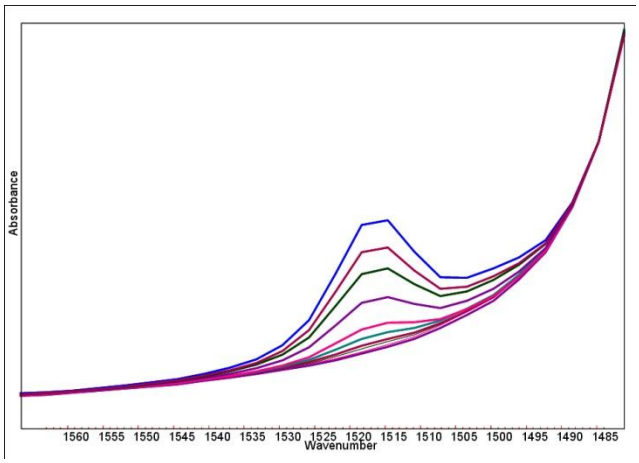

Figure 2. FTIR spectral overlay of the aminic antioxidant functional group depleting as a function of time. The strongest absorbance (red) is aminic antioxidant in new ISO 32 turbine oil and the weakest bands (blue and green) are from turbine oil with spent antioxidant.

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.

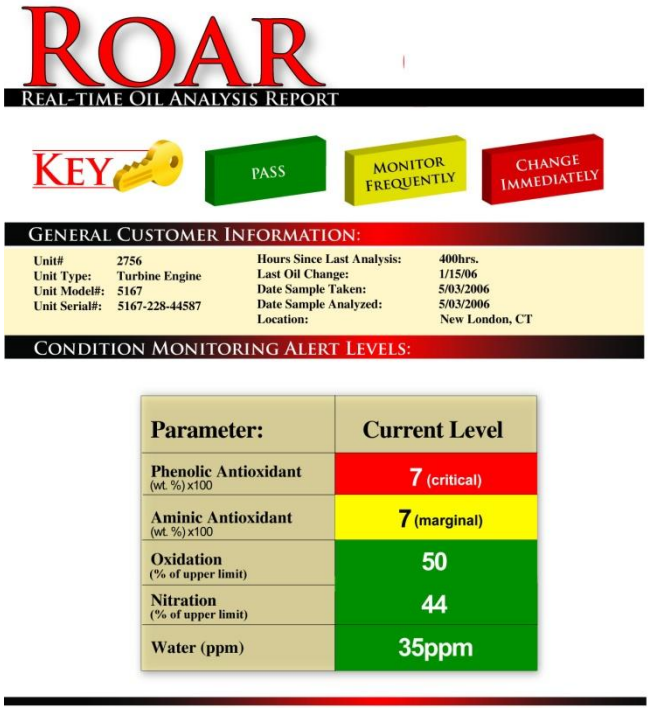

Figure 3. Agilent 5500t FTIR software presents the user with the specific concentration of phenolic and aminic antioxidants as well as crucial information about oxidation by-products and level of water contamination

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.

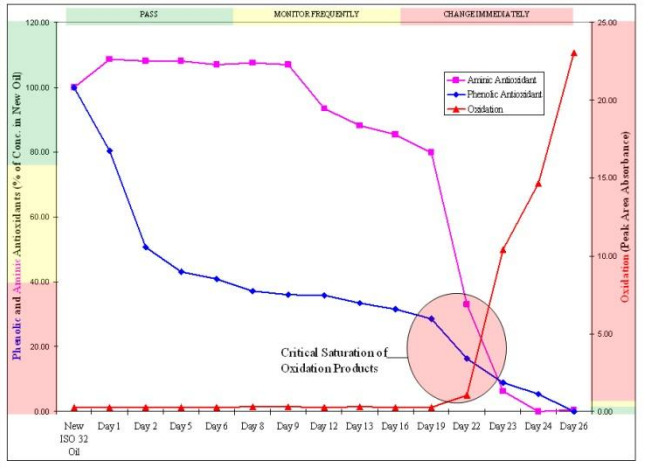

Figure 4. The additive depletion (% relative to new oil concentrations, left scale) and oxidation formation (right scale) trend analysis in thermally stressed ISO 32 turbine oil generated using the Agilent 5500t FTIR spectrometers

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

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 R2=0.977 and a standard error of validation (SEV) of ~40 ppm (20-270 ppm range). The addition of higher water concentration standards to the calibration improves the correlation coefficient to R2=0.996.

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

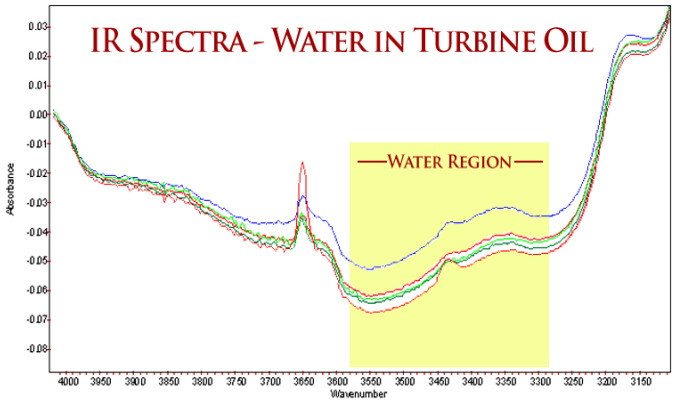

Figure 1. The overlaid IR spectra of turbine oil with the water absorbance region expanded, water values from bottom to top are 30 ppm (red), 80 ppm (dark green), 217 ppm (light green), 533 ppm (red), and 1460 ppm (blue)

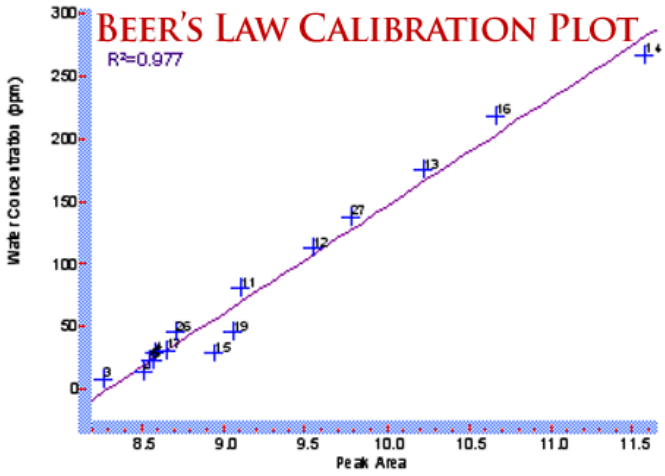

Figure 2. The calibration plot of KF water values (ppm) versus peak absorbance area for water in turbine oil using a Beer's Law peak area method

Pls model

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

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

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

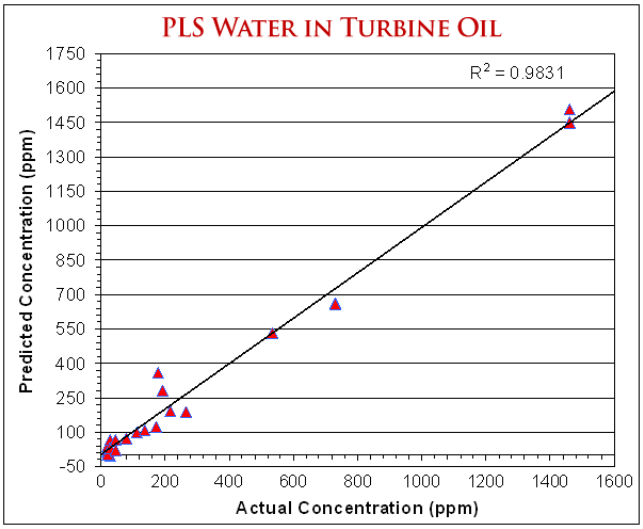

Figure 3. The PLS predicted versus actual plot of KF values using Agilent 4500 Series FTIR spectrometer

Predictions

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

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

Conclusions

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

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

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

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Agilent Technologies

Polyurethane Analysis on Agilent PLgel 5 µm 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 (-NH-CO-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 µm 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 µm 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 × Agilent PLgel MIXED-D, 300 × 7.5 mm, 5 µm (p/n PL1110-6504)
Eluent	THF
Flow Rate	1.0 L/min
Detector	RI
System	Agilent PL-GPC 50

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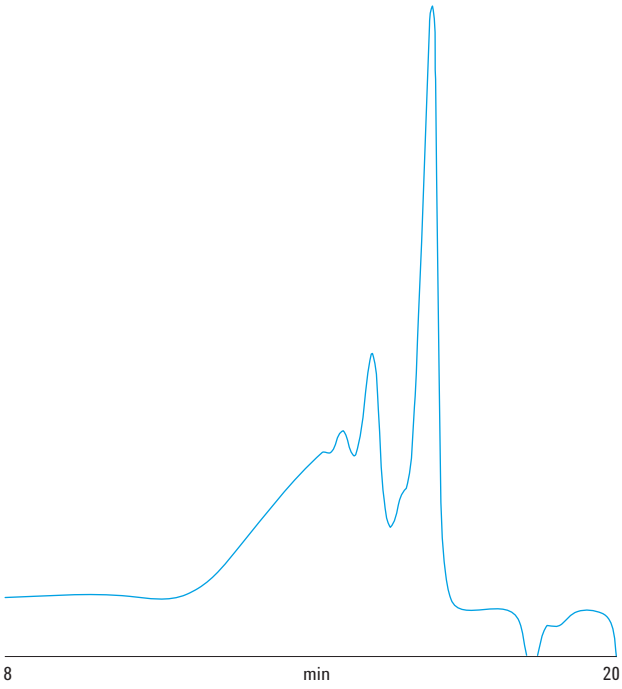

Figure 1. High and low molecular weight components in a sample of polyurethane separated on an Agilent PLgel 5 µm MIXED-D two-column set.

Conclusion

The results show it is possible to deternine the molecular weight distribution of polyurethane with Agilent PLgel 5 µm MIXED-D columns.

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Polycarbonate Analysis on Agilent PLgel 5 µm 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 µm 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 × Agilent PLgel 5 µm MIXED-D, 300 × 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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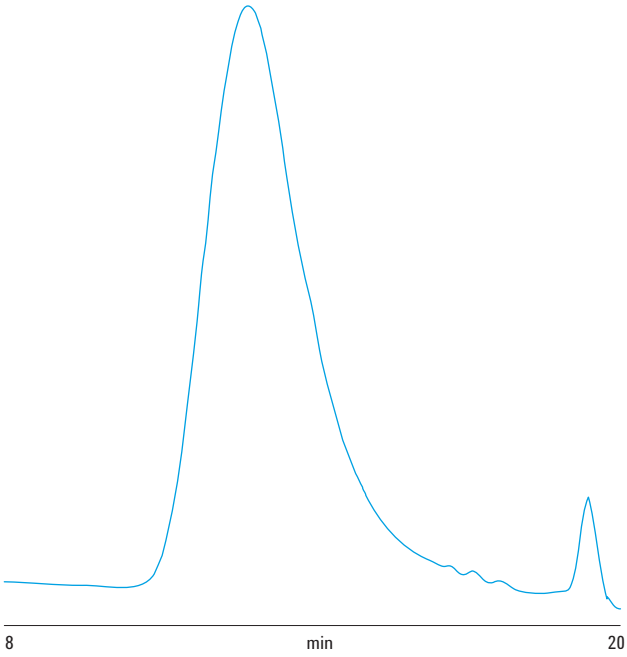

Figure 1. A relatively low molecular weight polycarbonate analyzed on an Agilent PLgel 5 µm MIXED-D two-column set.

Conclusion

The data illustrate the analysis of polycarbonate molecular weight distribution by gel permeation chromatography using Agilent PLgel 5 µm MIXED-D columns.

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Epoxy Resin Analysis on Agilent PLgel 5 µm MiniMIX-D and Gel Permeation Chromatography

Application Note

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Introduction

Epoxies 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 epoxies, while maintaining good resolution of oligomeric components. Figure 1 shows the analysis of three epoxy resins.

Conditions

Columns	2 × Agilent PLgel 5 µm MiniMIX-D, 250 × 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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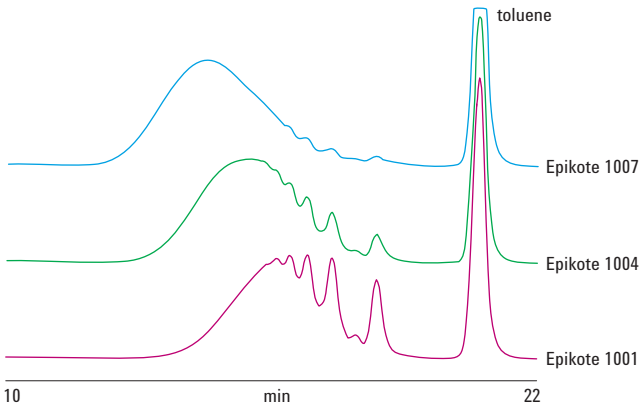

Figure 1. Good resolution of oligomers and no exclusion of higher molecular weight epoxy resins analyzed on an Agilent PLgel 5 µm MiniMIX-D two-column set.

Conclusion

The data illustrate the analysis of epoxy resins at low flow with Agilent PLgel 5 µm MiniMIX-D columns.

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Agilent Technologies

Polysiloxane Analysis on Agilent PLgel 5 µm MIXED-E using GPC/SEC with RI and ELS Detection

Application Note

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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 × Agilent PLgel 5 µm MIXED-E, 300 mm × 7.5 mm (p/n PL1110-6504)
Eluent	Toluene THF
Flow rate	1.0 mL/min
Inj vol	100 µL
Detector	Agilent ELSD (neb = 40 °C, evap = 80 °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).

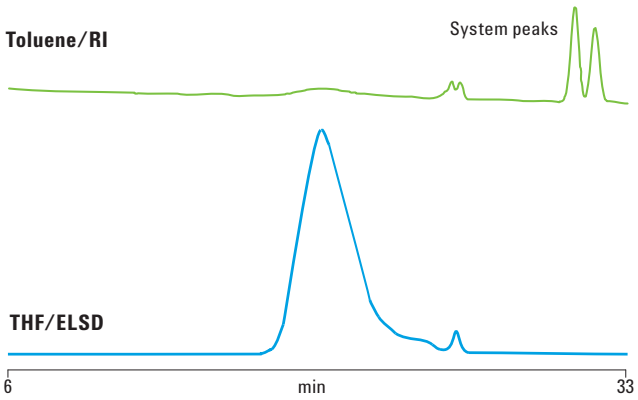

Figure 1 Analysis of a polysiloxane showing the improved data quality from evaporative light scattering detection compared to refractive index detection.

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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Agilent Technologies

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

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Introduction

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

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

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

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Background and the use of fixed pathlength cells

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

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

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

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

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

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

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

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

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

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

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

Practical alternatives for fixed pathlength infrared measurements

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

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

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

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

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

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

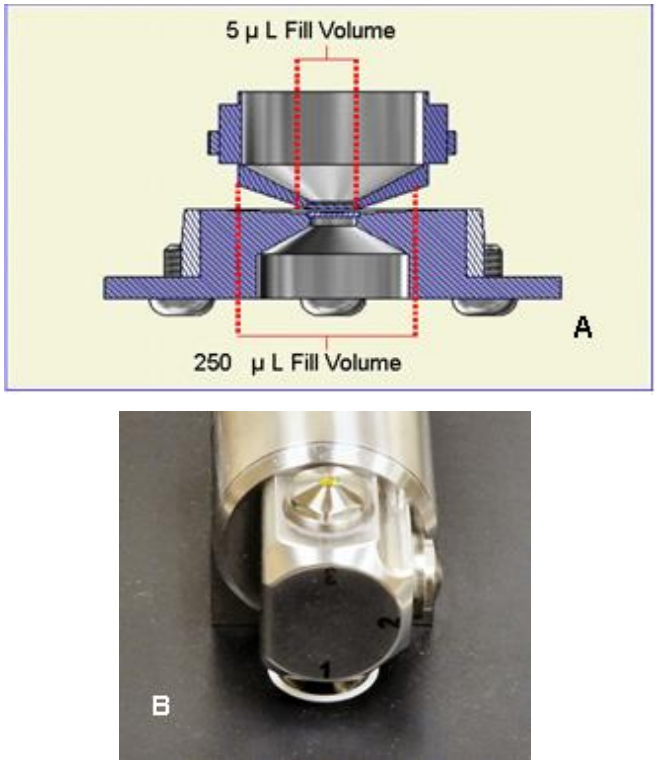


Figure 1. The 5500 DialPath FTIR sampling point concept (A); provides a user selectable pathlength, with one of three fixed/calibrated optical paths, designated 1, 2 and 3 (B)

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

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

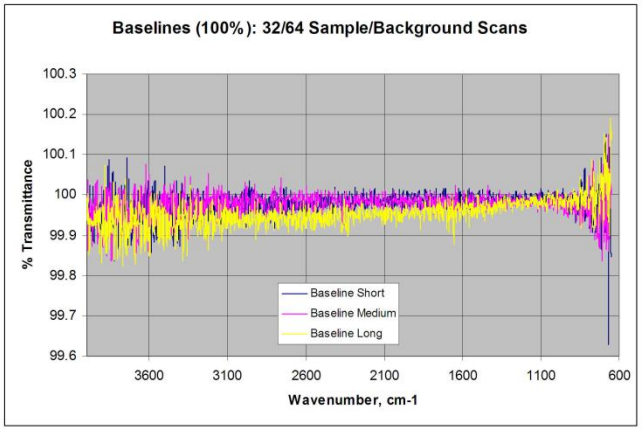


Figure 2. 100% Baseline performance; spectra from long, medium and short pathlengths presented with an average SNR of 8000:1 (2100 cm^{-1} to 1100 cm^{-1})

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

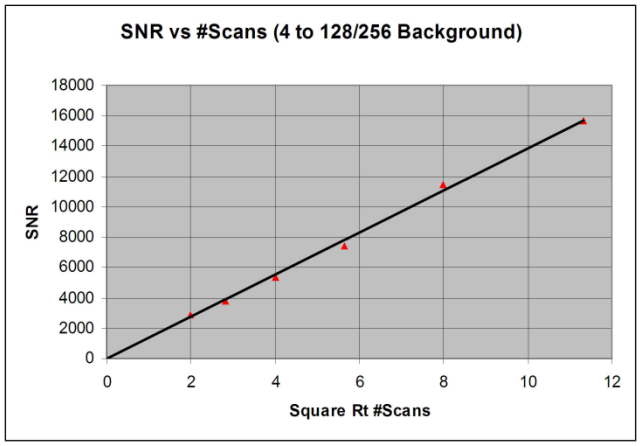


Figure 3. The adherence of the measurement system to the square root law of measured SNR

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

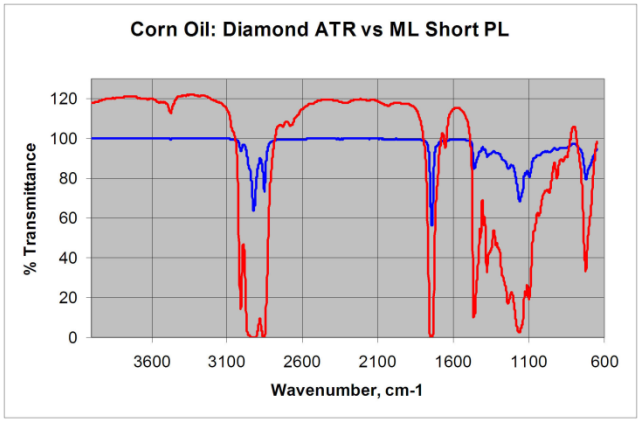
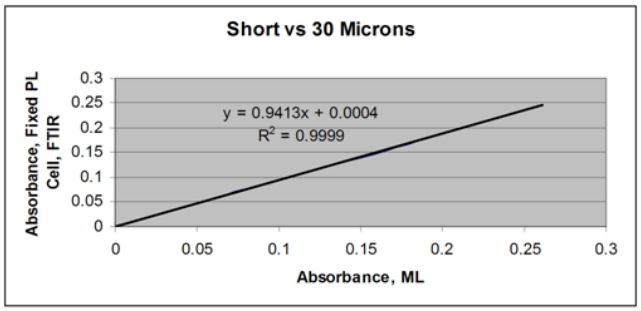


Figure 4. Comparison of the effective pathlength of a diamond ATR integrated system with the short fixed pathlength (~30 µm) transmission spectrum for corn oil

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

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

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



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

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

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

Practical applications of a fixed pathlength measurement system

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

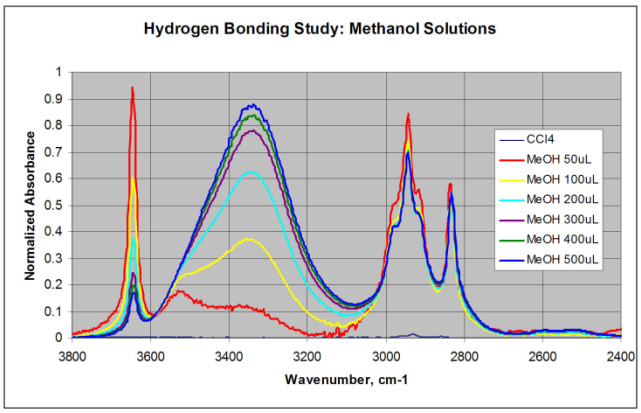


Figure 6: Dilute solutions of methanol in carbon tetrachloride; a study of the effects of hydrogen bonding in non-polar solvents

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

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

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

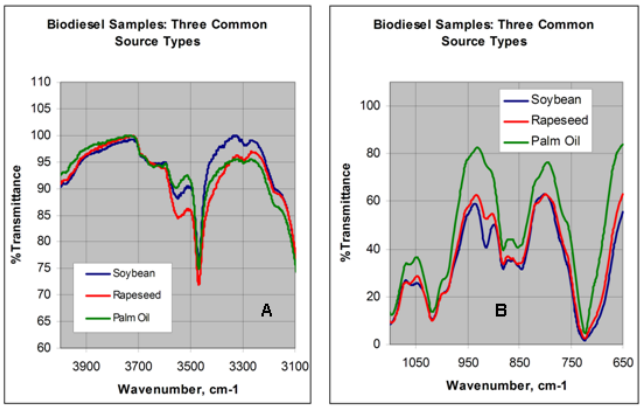


Figure 7. Detailed information from the base ester components used in the production of biodiesel methyl esters; hydroxyl (A) and unsaturation (B) functionalities

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

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

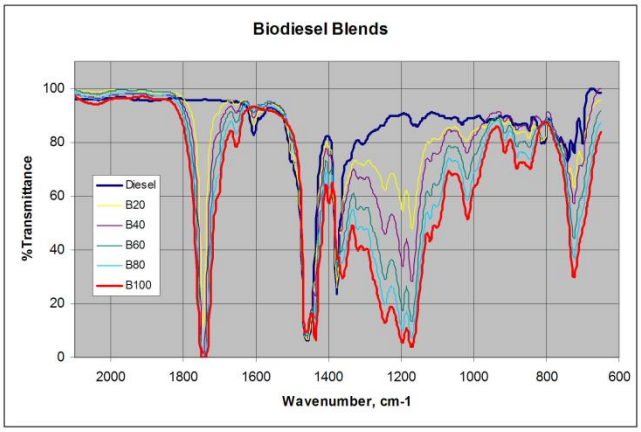


Figure 8. Measurement of biodiesel blends, experimental data from B0 (diesel fuel) to B100 (biodiesel) and intermediate biodiesel/diesel blends

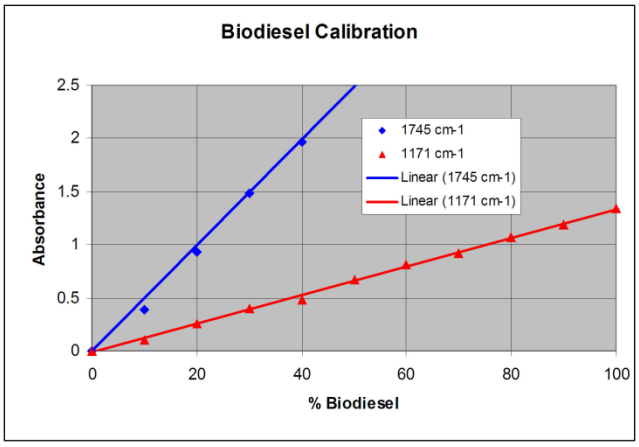


Figure 9. Quantitative measurement of biodiesel blends, B0, B10 to B90 and B10

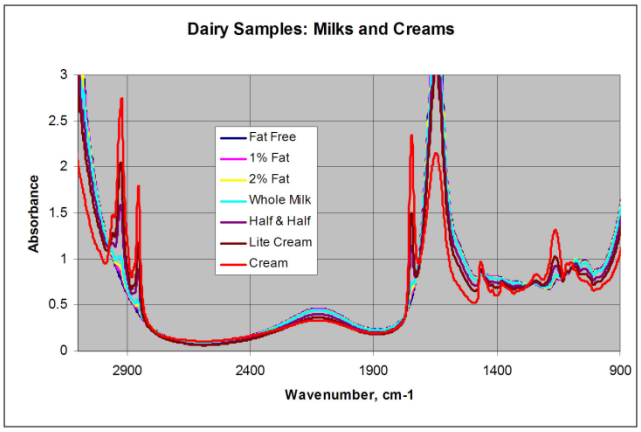


Figure 10. Dairy product spectra; short fixed pathlength (~30 mm), from fat free skim milk to standard heavy cream

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

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

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

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Agilent Technologies

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 µm 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 µm particles gives baseline resolution of the sample components with the lowest MW component eluting within 8 minutes.

Conditions

Columns	Agilent PLgel 3 µm MIXED-E, 300 × 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

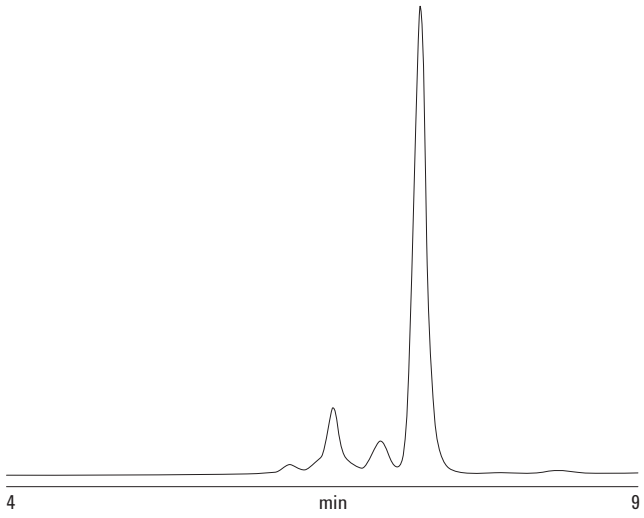

Figure 1 Fast elution of low molecular components of an epoxy resin on an Agilent PLgel 3 µm MIXED-E column.

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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 µm 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 µm MIXED-E columns to improve resolution.

Conditions

Column	2 × Agilent PLgel 3 µm MIXED-E, 300 × 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

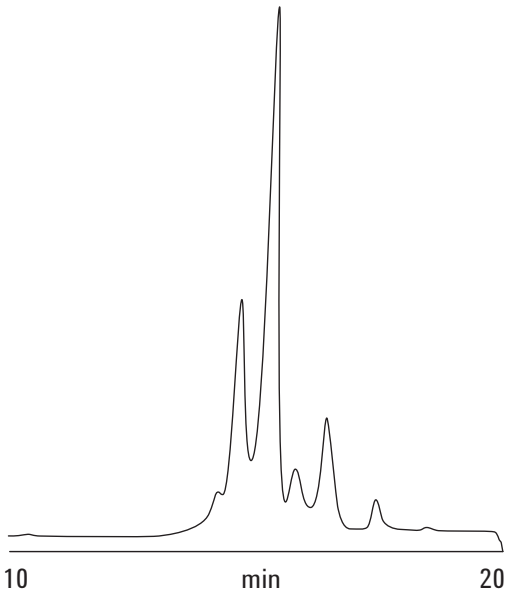

Figure 1. Separation of oligomers of a carboxylic acid ester on an Agilent PLgel 3 µm MIXED-E two-column set.

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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 µm 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.

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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 × PLgel 3 µm MIXED-E, 300 × 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	UV, 254 nm
System	Agilent PL-GPC 50

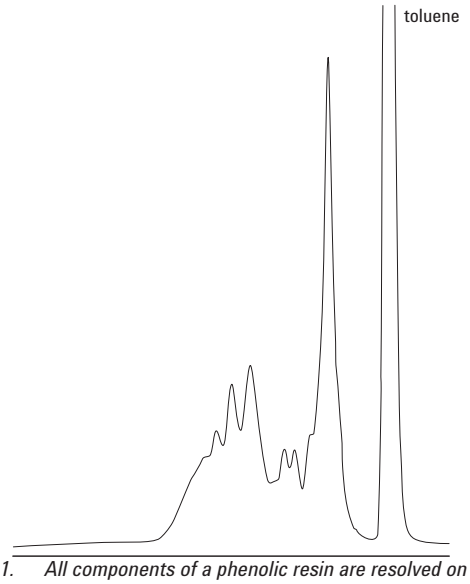

Figure 1. All components of a phenolic resin are resolved on an Agilent PLgel 3 µm MIXED-E two-column set.

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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 µm 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.

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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 µm MIXED-E columns.

Conditions

Column	2 × Agilent PLgel 3 µm MIXED-E, 300 × 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

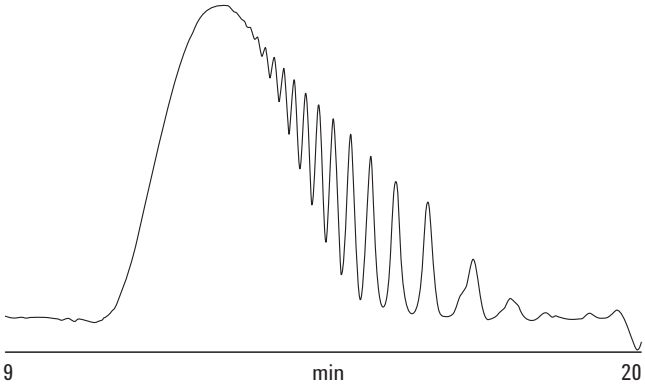

Figure 1. Separating oligomers in a polyester polyol on an Agilent PLgel 3 µm MIXED-E two-column set.

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Agilent Technologies

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.

Agilent Technologies

To construct these materials, adhesive (tie) layers are often required between two adjacent but chemical 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 (Figure1). 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).

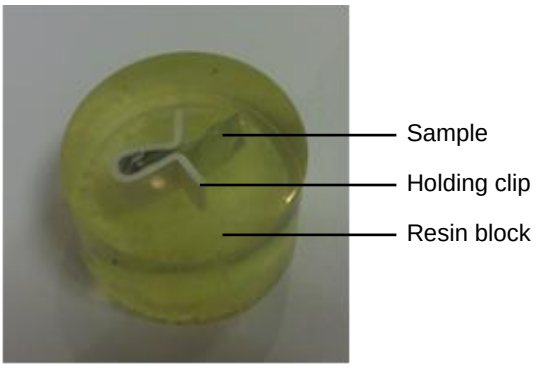


Figure 1. An example of a polymer film, held by a clip and embedded in a resin block

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.

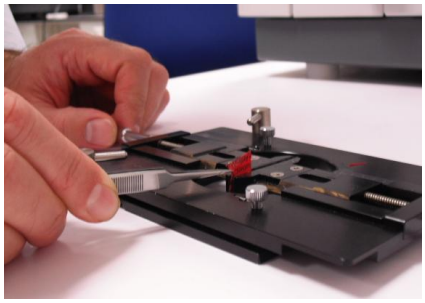
However, having a 2-D FPA alone does not provide for enough contrast to determine the moment of sample contact with the ATR. To overcome these issues Agilent Technologies has recently developed a unique "Live ATR imaging" mode, which significantly enhances the chemical contrast of the real-time FPA image, 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.

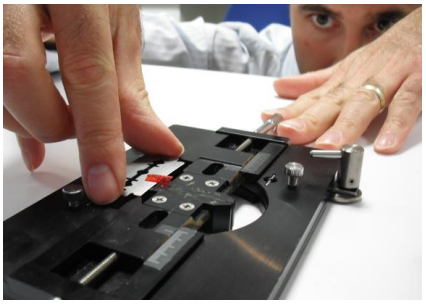
Sample measurement with "Live ATR imaging"

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!

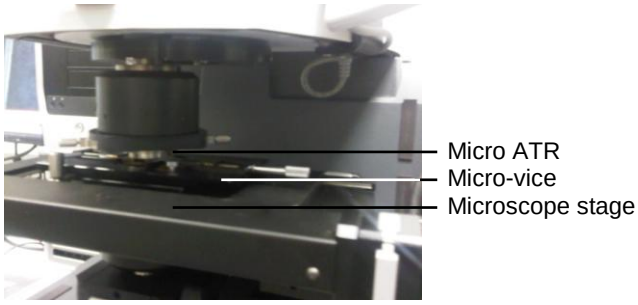
Step 1. Cut a small piece of sample.



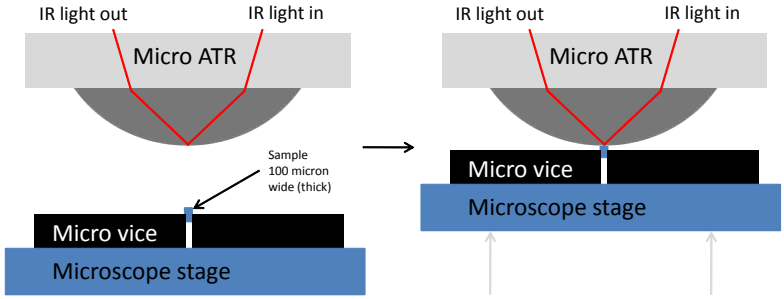
Step 2. Place sample into micro-vice.



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

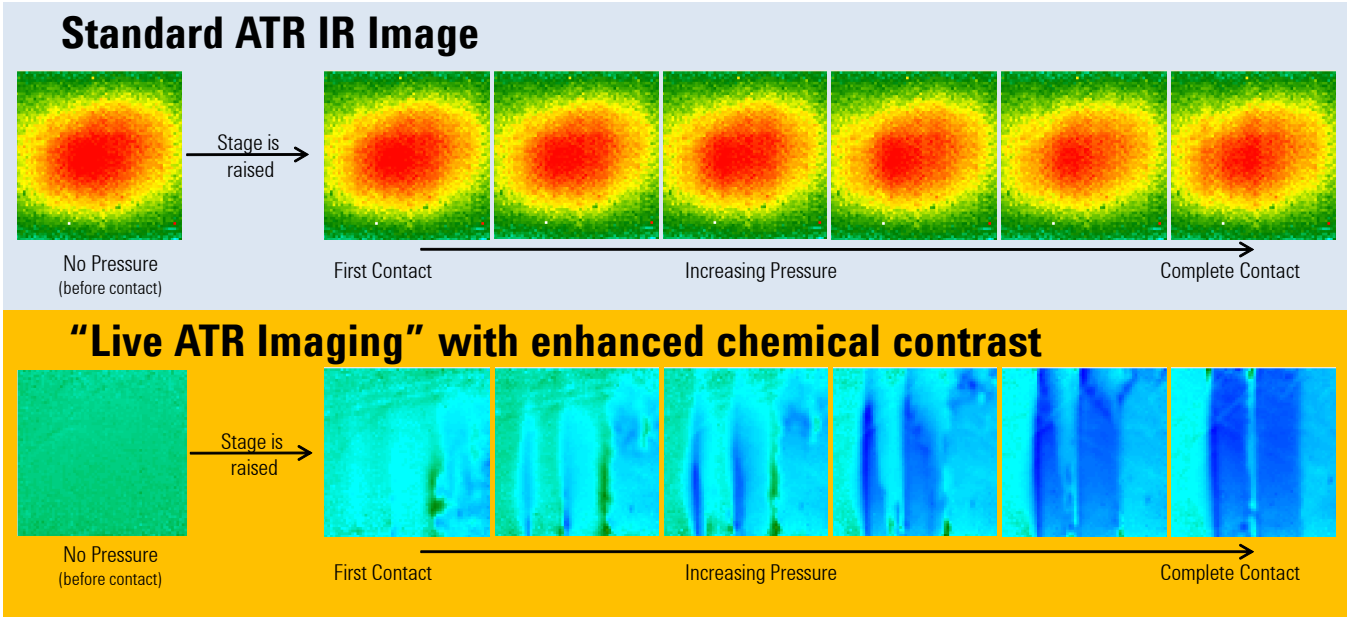


Figure 3. Comparison of a standard ATR IR image and Agilent's "Live ATR imaging" with enhanced chemical contrast—clearly showing the latter can detect first contact of the ATR crystal with the sample and that contact quality can be monitored real-time as the pressure is increased and before any data 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

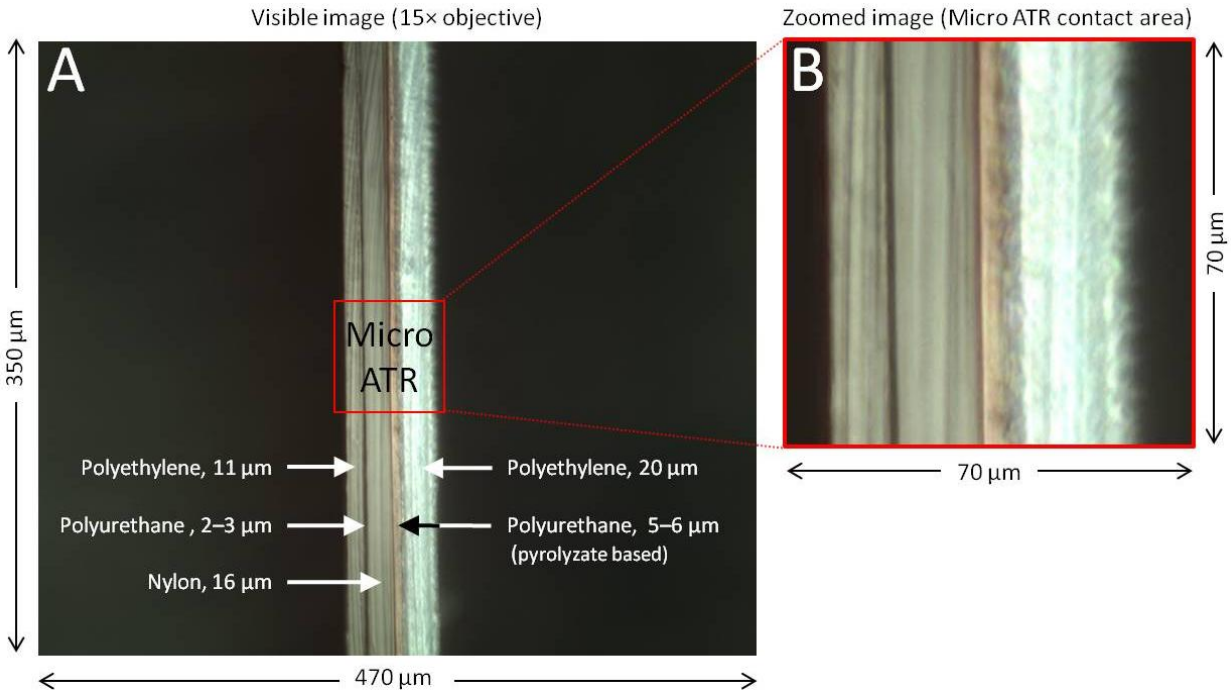


Figure 4. Optical images: A—the full field of view visible through microscope, annotated with the chemical composition and approximate thickness of the various layers in the sample; and B—zoomed image corresponding to the contact area of the Micro ATR

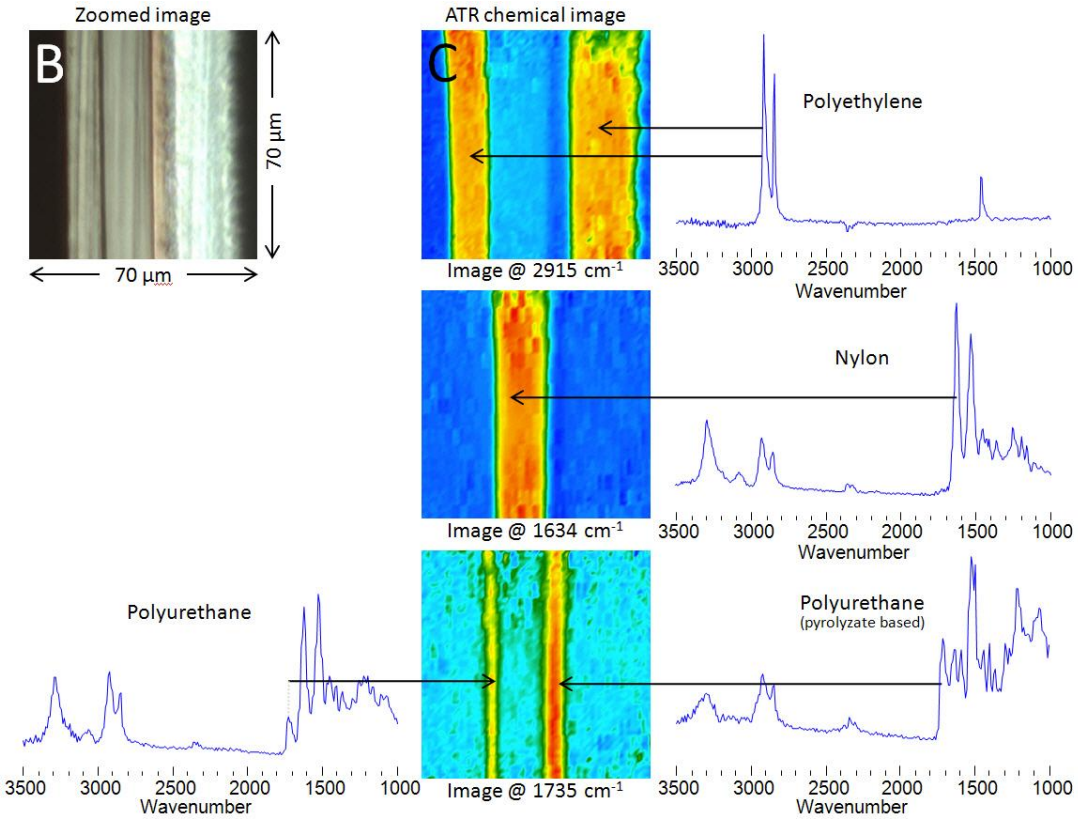


Figure 5. Identifying layers: B—as per Figure 4, above; and C–three chemical images created with different wavenumbers to highlight the main layers and tie layers with corresponding representative spectra as indicated by the arrows. Note: All spectra are shown in absorbance units, with axes omitted for 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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Agilent Technologies

Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns

Application Note

Authors

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Agilent Technologies, Inc.

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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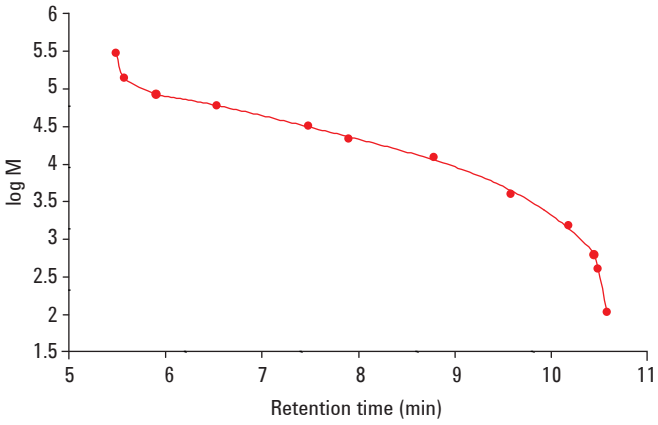
Methods and Materials

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 °C
Detection:	UV at 280 nm

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.

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.

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Agilent Technologies

Sensitive Analysis of Oligo/ polyethylene Glycol by Reversed-phase HPLC with ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

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.

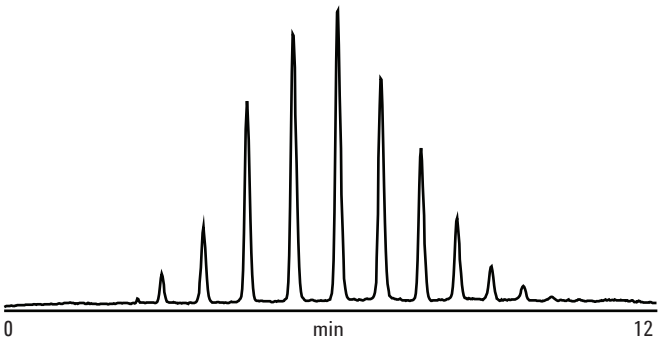

Figure 1a. PEG 400 oligomers detected by the 385-ELSD evaporative light scattering detector.

Figure 1b shows a sample with a nominal molecular weight of 600.

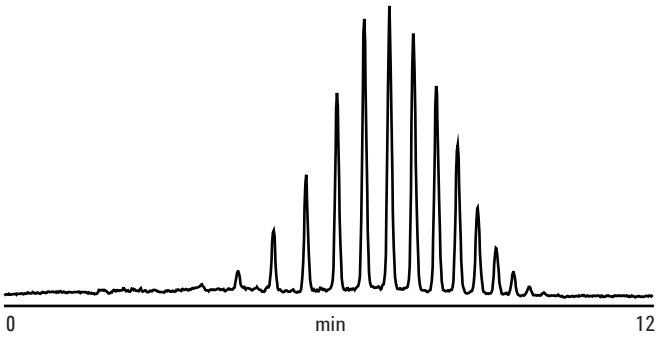

Figure 1b. PEG 600 oligomers detected by the 385-ELSD evaporative light scattering detector.

As the molecular weight increases, it is no longer possible to separate the individual oligomers, as shown in Figures 2a and 2b.

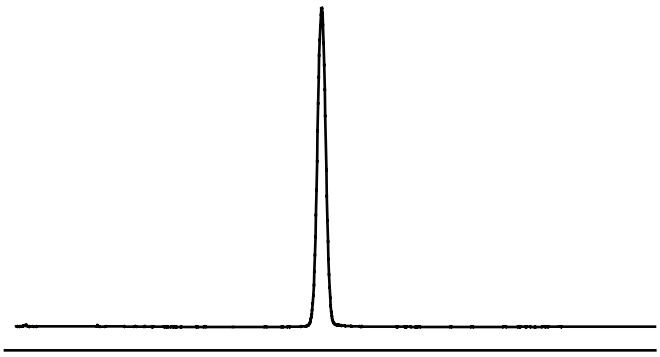

Figure 2a. No separation of oligomers in PEG 4,120.

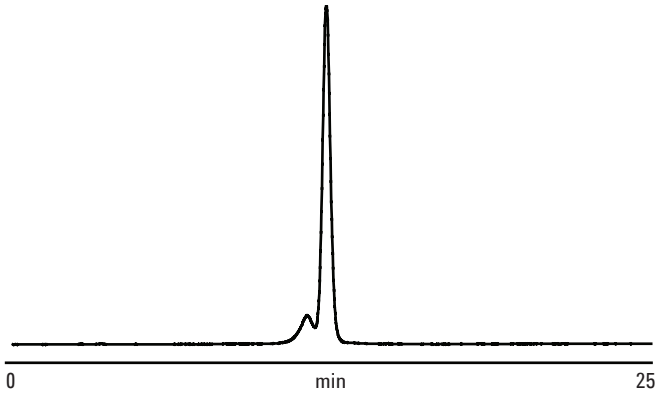

Figure 2b. PEG 22,800 oligomers not visible.

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.

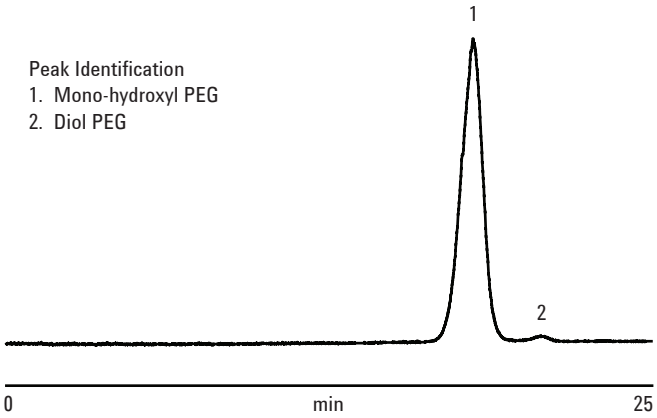

Figure 3. The presence of unwanted diol derivative of PEG revealed by the 385-ELSD evaporative light scattering detector.

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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Agilent Technologies

Sensitive Analysis of Polystyrene 960 and 580 by Reversed Phase HPLC with ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

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

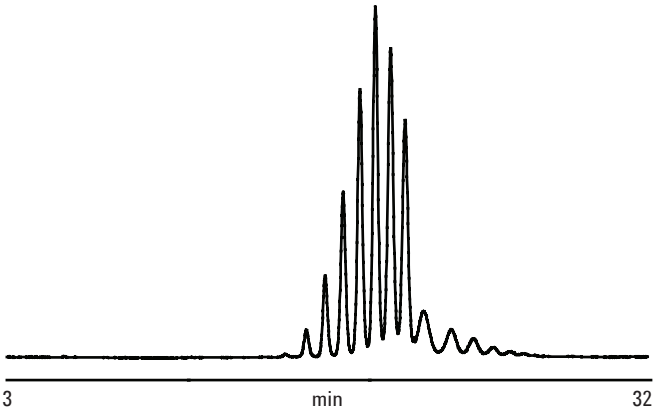

Figure 1. Separation of individual oligomers in polystyrene 960 with a PLRP-S column and 380-LC ELSD.

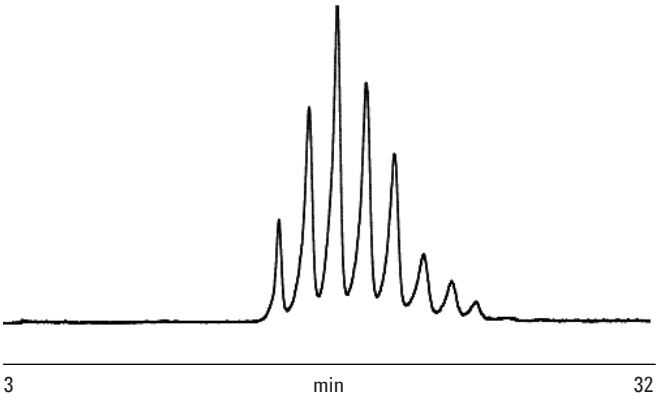

Figure 2. Separation of individual oligomers in polystyrene 580 with a PLRP-S column and 380-LC ELSD.

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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Agilent Technologies

Fingerprinting Isocyanate Pre-polymer Fractions by Agilent PLgel MIXED-E and GPC

Application Note

Materials Testing and Research, Polymers

Authors

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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 µm MIXED-E, 300 ×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 µm MIXED-E columns could be used to fingerprint a desired oligomer/polymer distribution in a sample as part of a rigorous quality control environment.

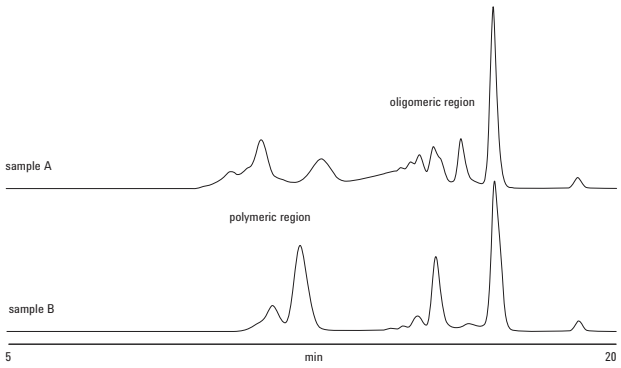

Figure 1. Oligomer and polymer fractions of an isocyanate revealed by an Agilent PLgel 3 µm MIXED-E, three-column set

Conditions

Samples	Isocyanate pre-polymers, 0.4% (w/v)
Column	2 × Agilent PLgel 3 µm MIXED-E, 300 × 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.

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Agilent Technologies

Polybutadiene Terephthalate Analysis by Agilent PL HFIPgel and GPC

Application Note

Materials Testing and Research, Polymers

Authors

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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

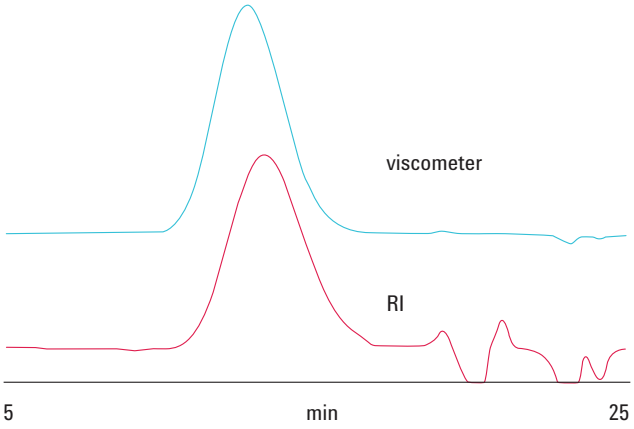

Figure 1. An example overlay of a dual-detector chromatogram of the virgin polybutadiene terephthalate resin before molding.

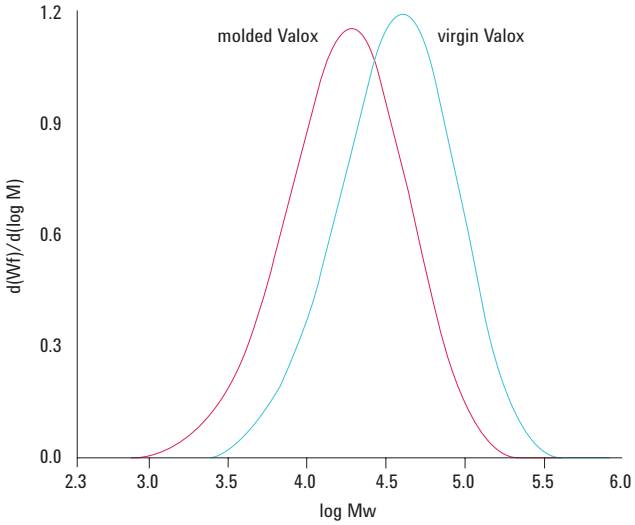

Figure 2. The resulting molecular weight distributions for polybutadiene terephthalate.

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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Agilent Technologies

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 10^4Å, 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

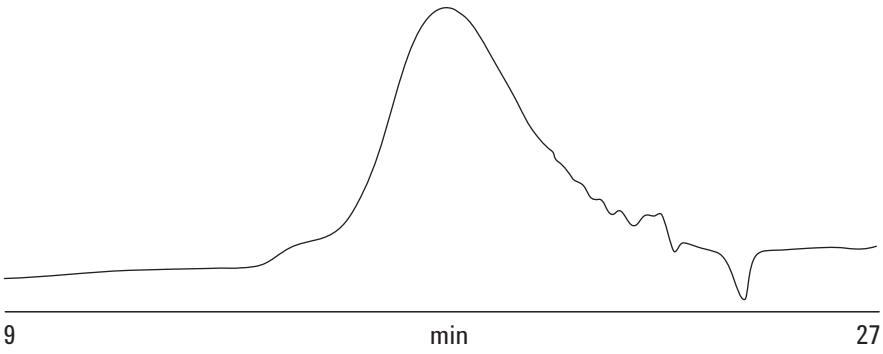

Figure 1. The molecular weight distribution of a polydisperse crystalline polyester revealed by Agilent PLgel 5 µm columns.

Conclusion

Agilent PLgel columns are suitable for analysis of the molecular weight distribution of crystalline polymers in polar solvents.

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Agilent Technologies

Unsaturated Polyester Resins on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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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 10^4Å, 300 × 7.5 mm (p/n PL1110-6540)
	Agilent PLgel 5 µm 500Å, 300 × 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

Mp	4,065
Mn	2,221
Mw	5,846
Mz	13,776
Mw/Mn	2.63

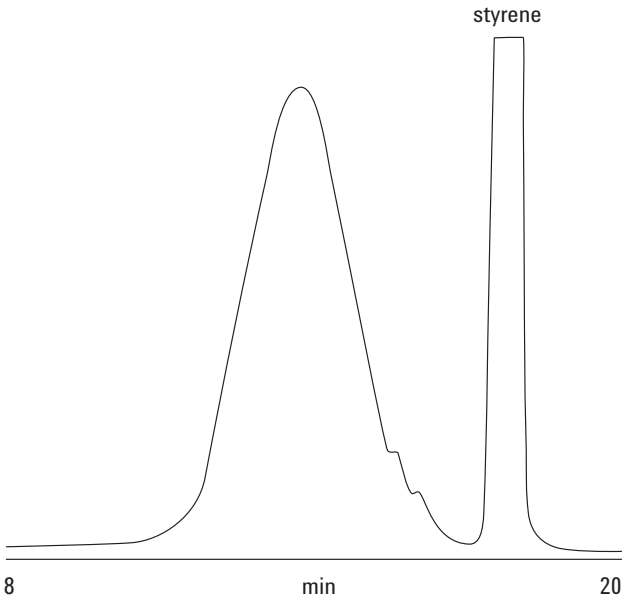

Figure 1. The distribution of an unsaturated polyester resin on Agilent PLgel 5 µm columns.

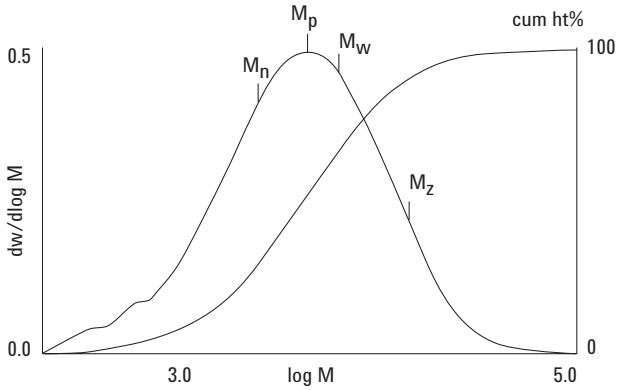

Figure 2. Molecular weights of a sample of an unsaturated polyester resin.

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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Agilent Technologies

Styrene/Isoprene Block Copolymers with Agilent PLgel MIXED-D and GPC/SEC

Application Note

Materials Testing and Research, Polymers

Authors

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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

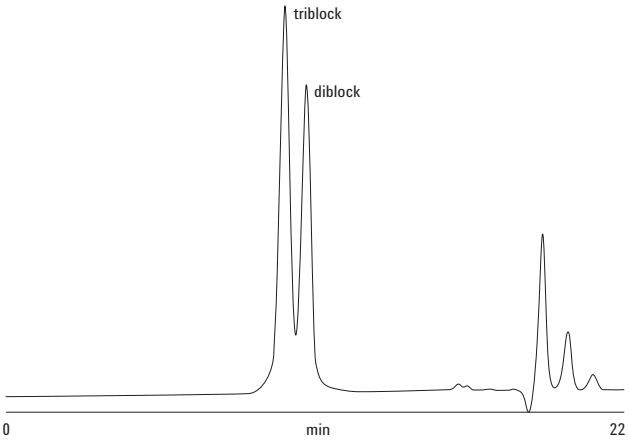

Figure 1. Chromatogram of a styrene isoprene di- and triblock copolymer blend on an Agilent PLgel 5 µm MIXED-D, column.

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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Agilent Technologies

Polyurethane Analysis on an Agilent PLgel Column with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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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 × 7.5 mm (p/n PL1110-6515)
	Agilent PLgel 5 µm, 100Å, 300 × 7.5 mm (p/n PL1110-6520)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

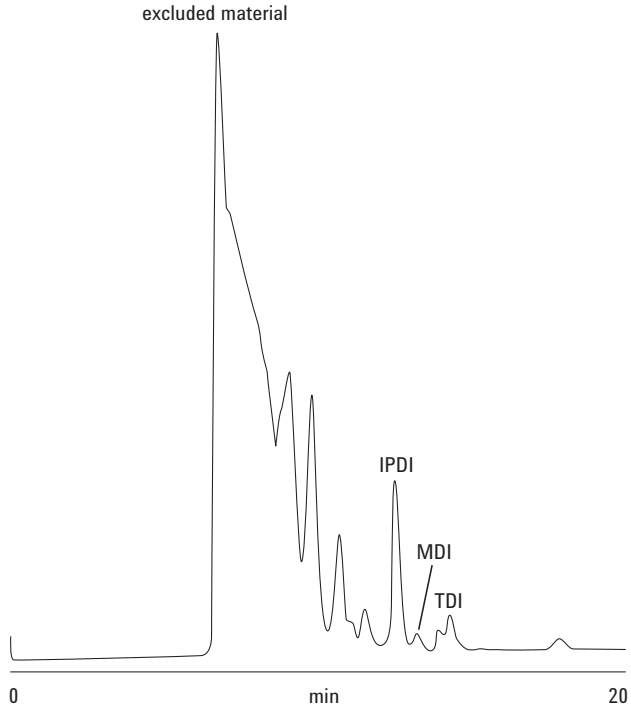

Figure 1. Identifying isocyanates in a polyurethane using Agilent PLgel 5 µm columns.

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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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 $H(OCH_2CH_2)_nOH$ 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.

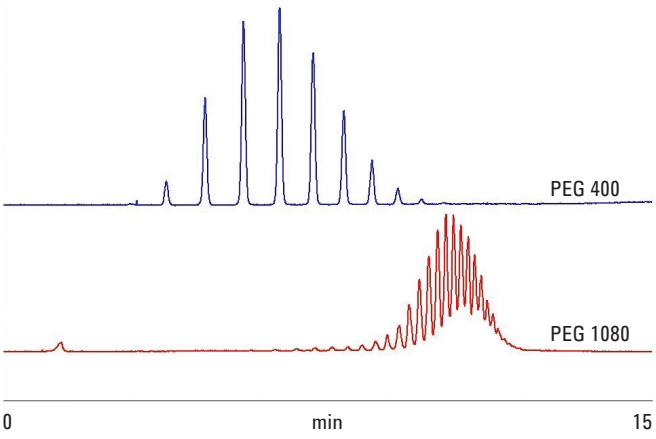

Figure 1. High resolution of PEGs with different molecular weights using a PLRP-S column and Agilent 380-ELSD.

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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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

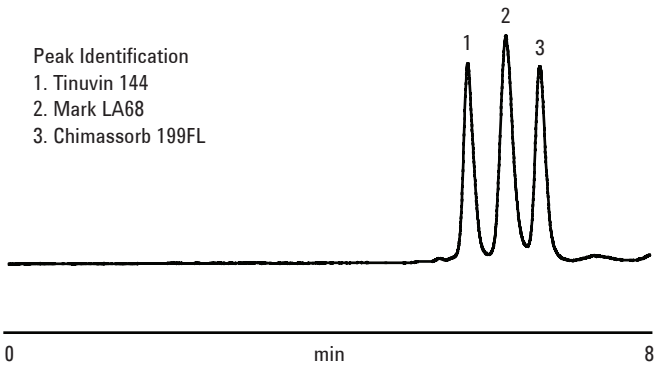

Figure 1. Chromatogram showing good separation of the three light stabilizers.

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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Agilent Technologies

Sensitive Polymer Analysis using Critical Point Chromatography and ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

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 Δ G is the change in Gibbs' free energy, Δ H is the change in enthalpy and Δ S is the change in entropy. Adsorption of the polymer by a reversed/normal-phase mechanism will result in a negative Δ H and, therefore, a negative Δ G, whereas if a size exclusion mechanism occurs then Δ S will be positive and again Δ G will be negative. Separation of any components of the polymer occurs if the reduction in Δ G 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 Δ H and Δ S are balanced and there is no change in Δ G 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.

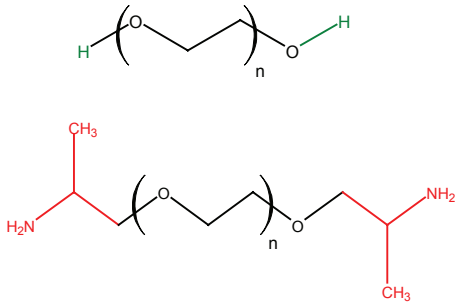

Figure 1. The structure of the original and the modified PEG materials.

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.

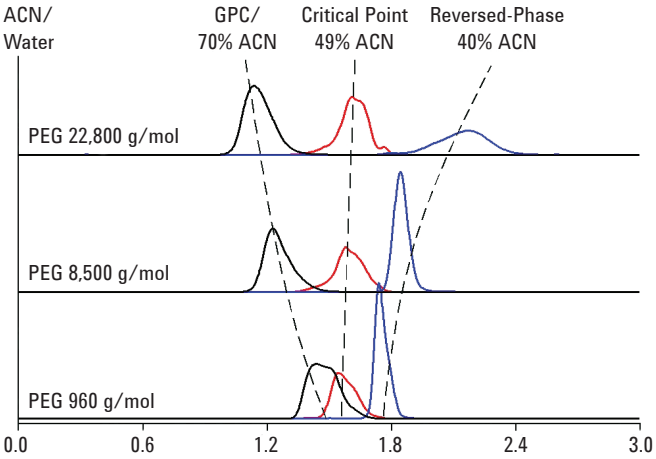

Figure 2. Analysis of PEG in SEC and reversed-phase to reveal the critical point.

Figure 3 shows a chromatogram of the amine-modified PEG material, before and after neutralization of the amine functionality with hydrochloric acid.

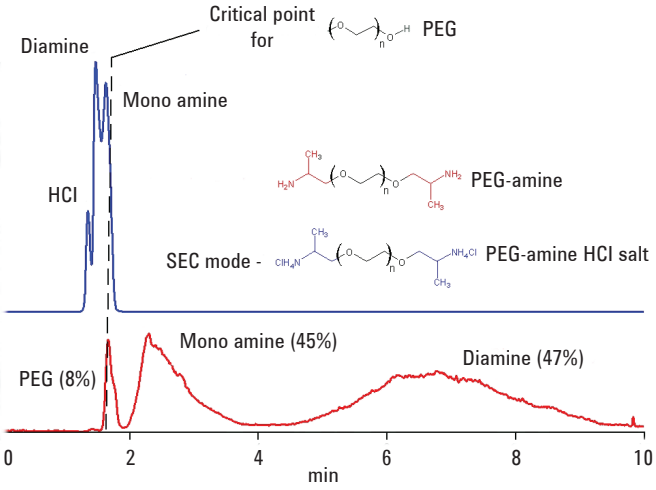

Figure 3. Amine modified PEG before and after neutralization with HCL.

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.

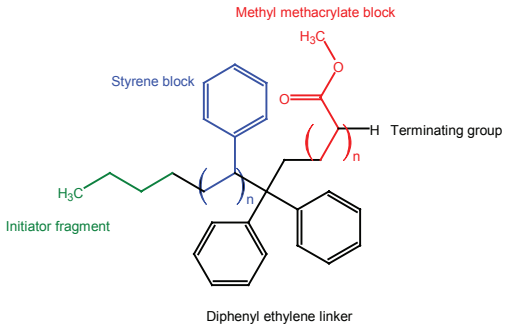

Figure 4. Structure of poly(styrene-b-methyl methacrylate).

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.

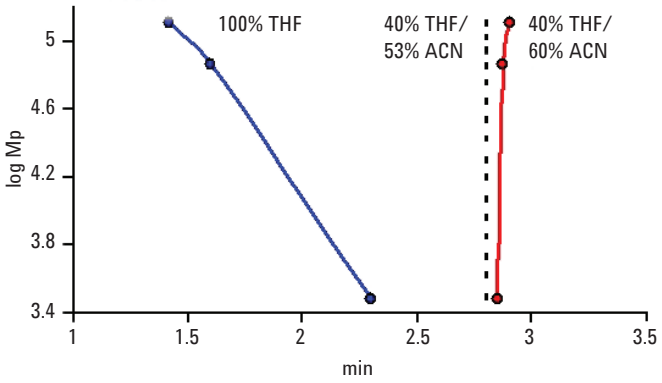

Figure 5a. Critical point diagrams for PS.

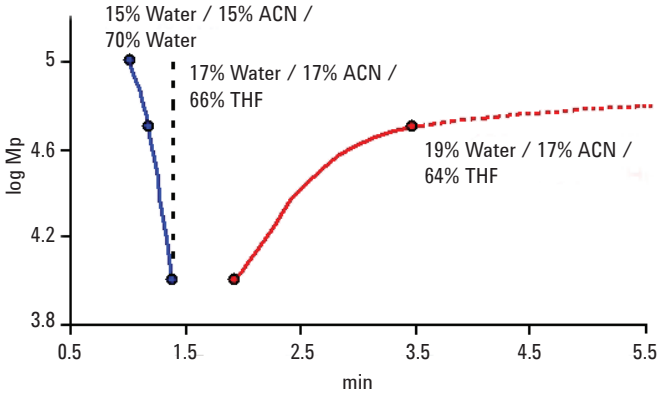

Figure 5b. Critical point diagrams for PMMA.

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.

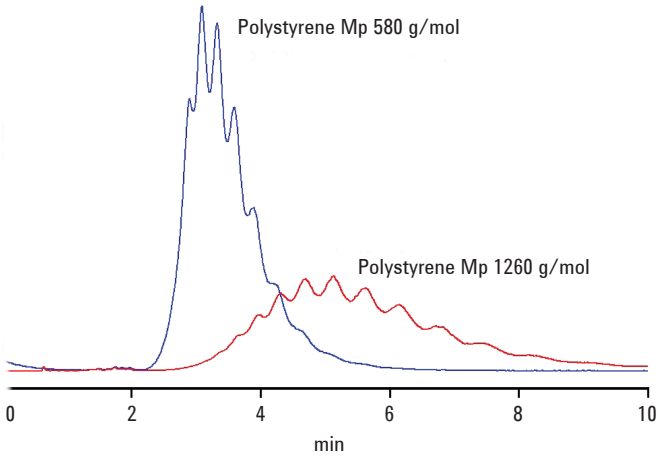

Figure 6. Chromatograms of polystyrene standards under polymethyl methacrylate critical conditions.

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.

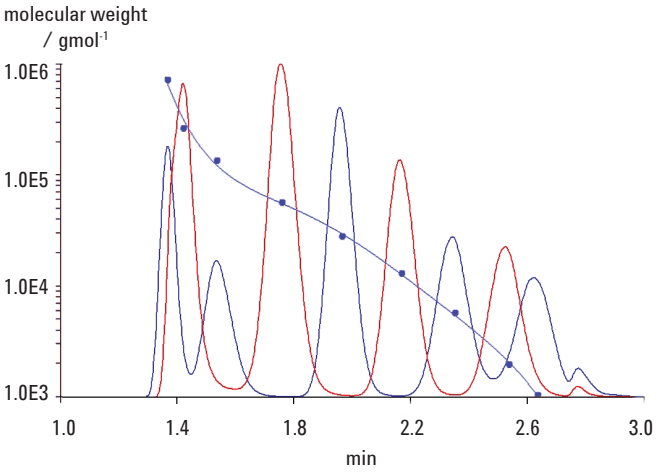

Figure 7. PMMA standards and SEC calibration curve run under PS critical conditions.

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.

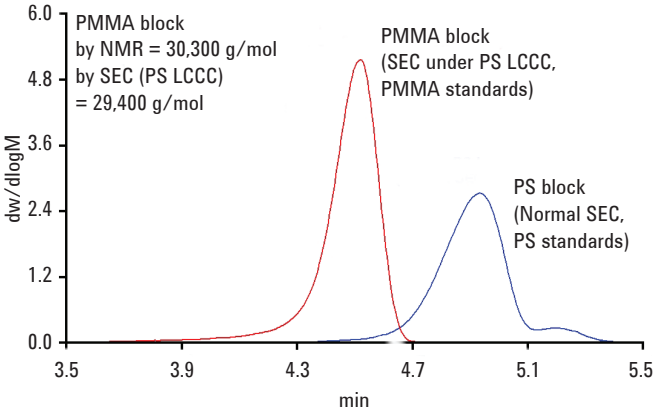

Figure 8. Molecular weight distributions of the styrene block before reaction with PMMA, and the PMMA block under critical conditions for polystyrene.

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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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).

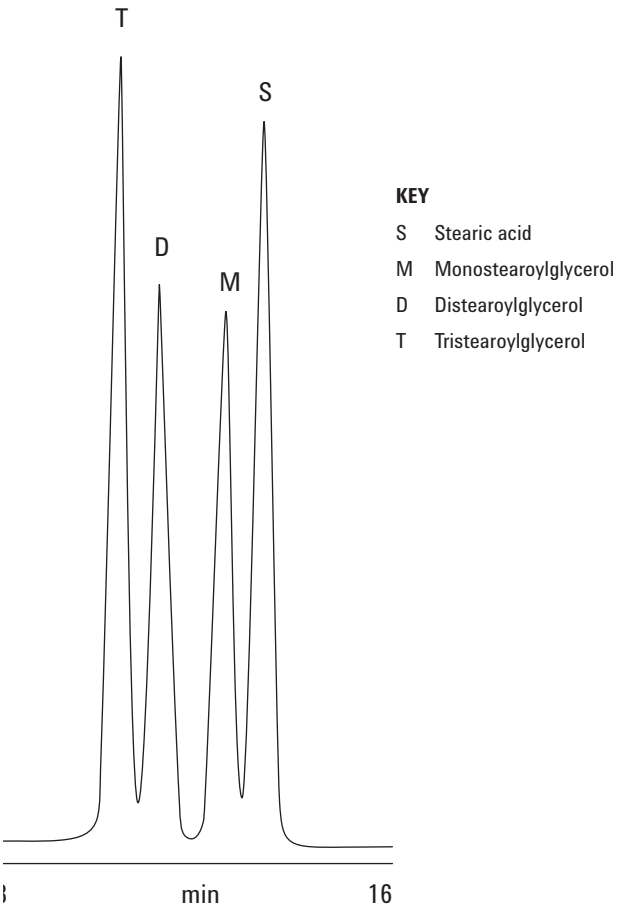

Figure 1. Stearoylglycerols separated on an Agilent PLgel 5 µm column.

Conditions

Column	Agilent PLgel 5 µm 50Å, 300 × 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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Agilent Technologies

Polystyrene Oligomer Analysis on Agilent PLgel with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

Authors

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SY6 6AX

UK

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).

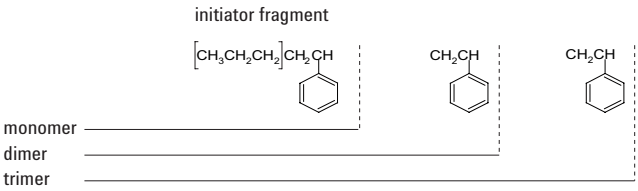

Figure 1. Structures of low molecular weight polystyrene oligomers.

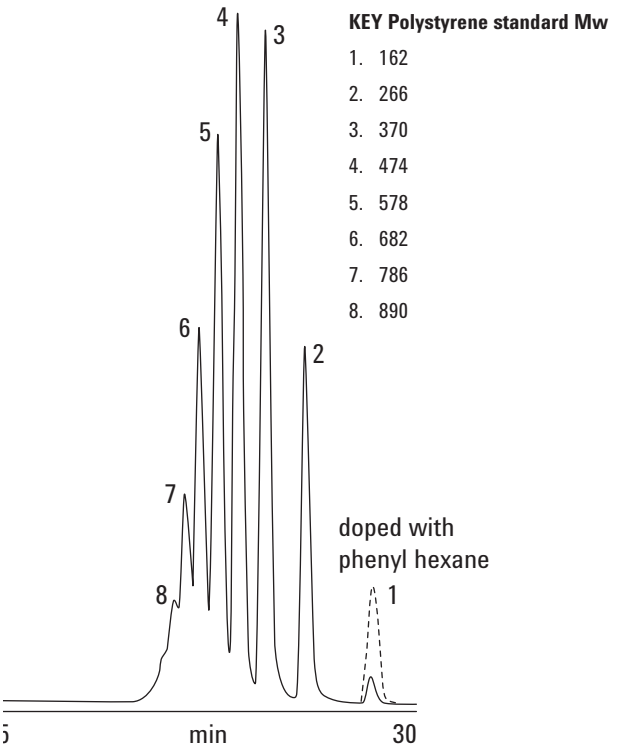

Figure 2. Low molecular weight oligomers of polystyrene on an Agilent PLgel 5 µm two-column set.

Conditions

Columns	2 × Agilent PLgel 5 µm 100Å, 600 × 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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Agilent Technologies

Dialkyl Phthalate Analysis on Agilent PLgel 5 µm 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.

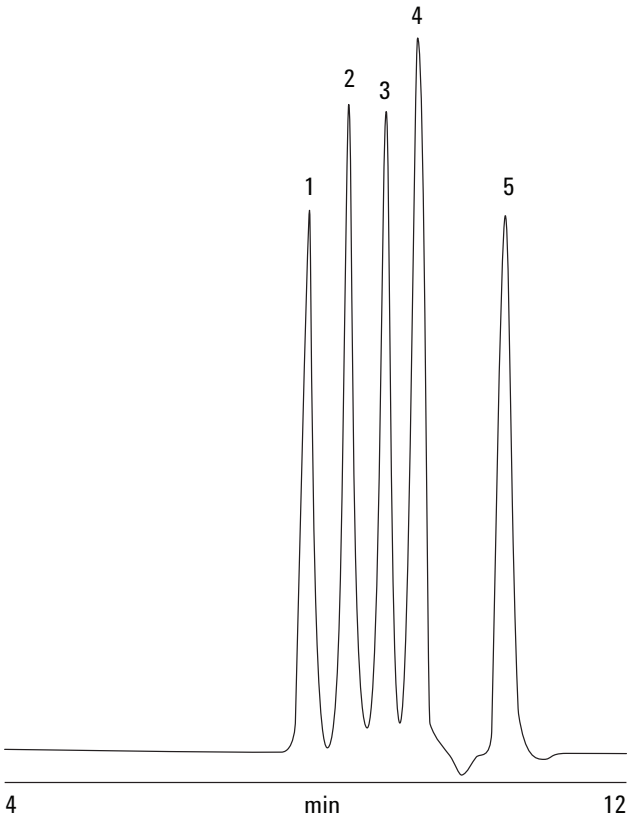

Figure 1. Four dialkyl phthalates separated on an Agilent PLgel 5 µm column.

Conditions

Column	Agilent PLgel 5 µm 50Å, 300 × 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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Agilent Technologies

Polyester Polyol Analysis on Agilent PLgel 5 µm using Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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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.

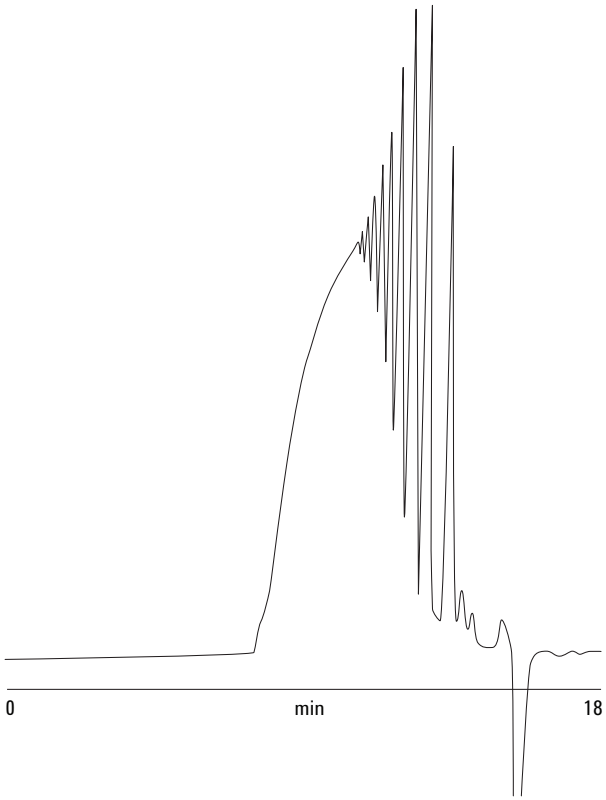

Figure 1. Excellent separation of oligomers in a polyester polyol using Agilent PLgel columns.

Conditions

Column	2 × Agilent PLgel 5 µm 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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Agilent Technologies

Dialkyl Phthalate Analysis on Agilent PLgel 3 µm 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 µm 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 Phthalatea

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 µm 100Å columns with low molecular weight species.

Conditions

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

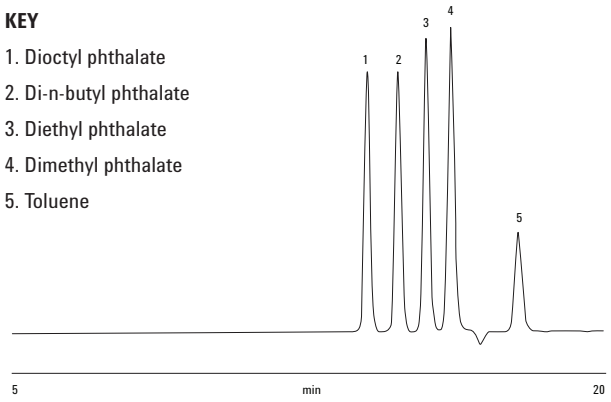

Figure 1. Four dialkyl phthalates separated on an Agilent PLgel 3 µm two-column set.

Conclusions

Agilent low-pore-size PLgel columns can be used to separate low molecular weight molecules with very high resolution.

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Agilent Technologies

Polyvinylidene Fluoride Analysis on Agilent PLgel 10 µm MIXED-B and GPC/SEC

Application Note

Materials Testing and Research, Polymers

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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 µm MIXED-B, 300 × 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.

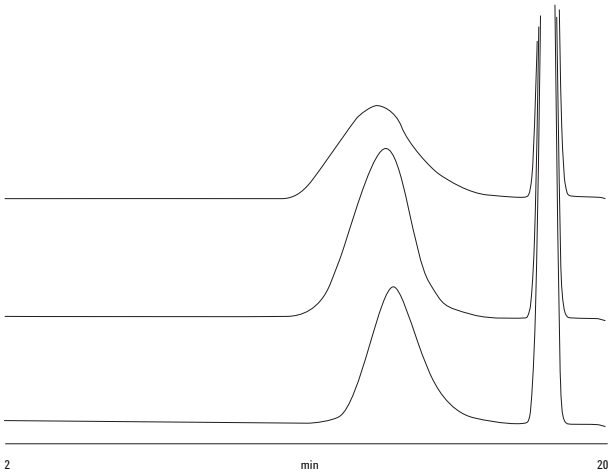

Figure 1. Three samples of polyvinylidene fluoride separated on an Agilent PLgel 10 µm MIXED-B two-column set.

Figure 2 shows overlaid molecular weight distributions of the three samples based on polymethylmethacrylate standards.

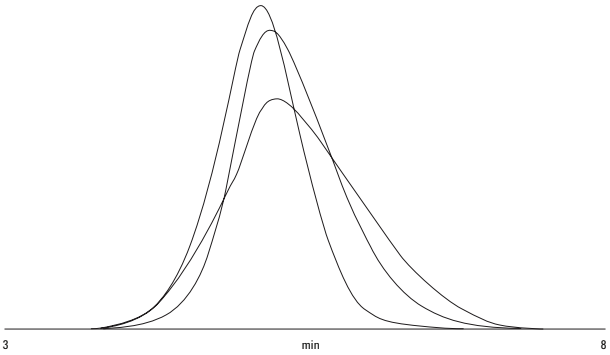

Figure 2. Overlaid molecular weight distributions of three samples of polyvinylidene fluoride based on polymethylmethacrylate standards.

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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Agilent Technologies

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

Application note

Authors

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Abstract

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

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

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Introduction

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

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

Description

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

Figure 1. OneNeb nebulizer

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

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

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

Other nebulizer designs

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

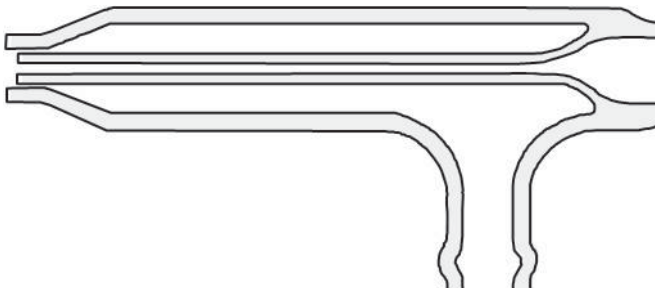

Figure 2. Concentric glass nebulizer

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

Experimental

Instrumentation

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

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

Operating parameters

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

Pump tubing

Two cases of pump tubing were used:

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

Results and discussion

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

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

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

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

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

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

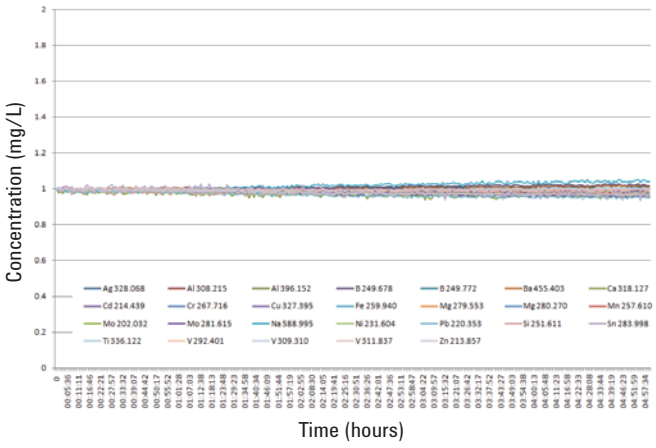

Figure 3. Long-term stability of the OneNeb nebulizer with diisobutyl ketone

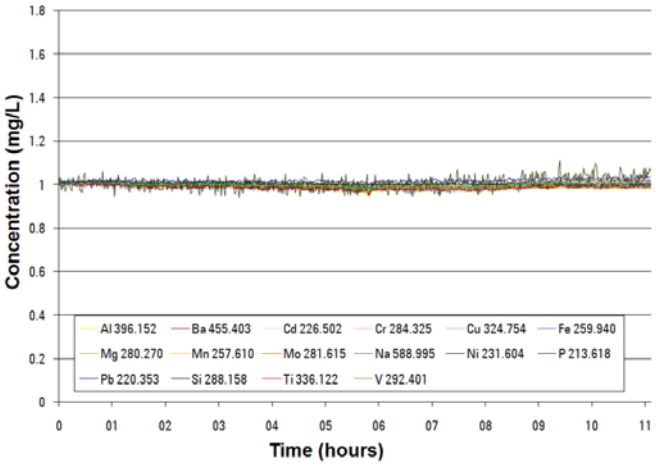

Figure 4. Long-term stability of the OneNeb nebulizer with ShellSol

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

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

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

Conclusion

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

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

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

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Agilent Technologies

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

Application Note

Application Area Identifier

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Abstract

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

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Introduction

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

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

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

Experimental

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

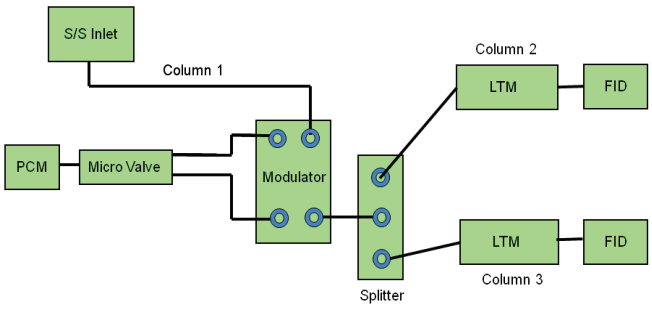

Figure 1. Diagram of the dual LTM GCxGC system.

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

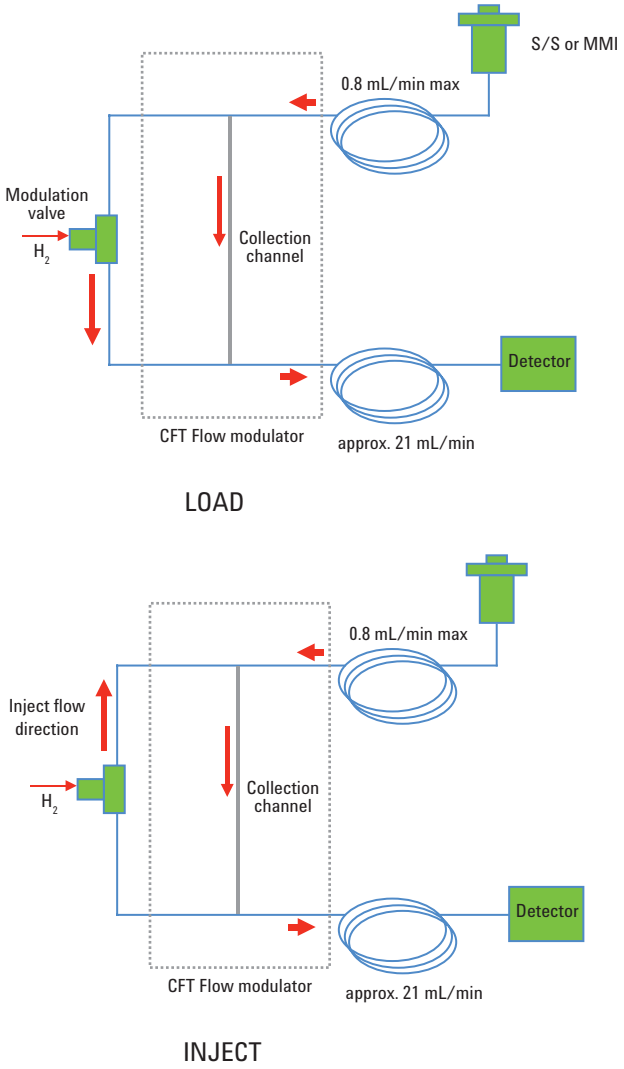

Figure 2. Operational detail of the flow modulator showing load and inject states.

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

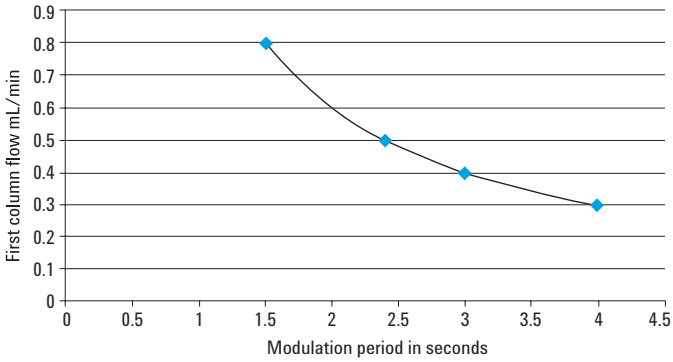

Figure 3. Relationship between modulation period and first dimension column flow rate.

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

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

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

Hardware

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

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

Firmware and Chemstation

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

Typical Parameters

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

GCxGC Parameters

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

GCxGC Data Processing Software

GC Image, Version 2.1b4

Results and Discussion

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

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

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

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

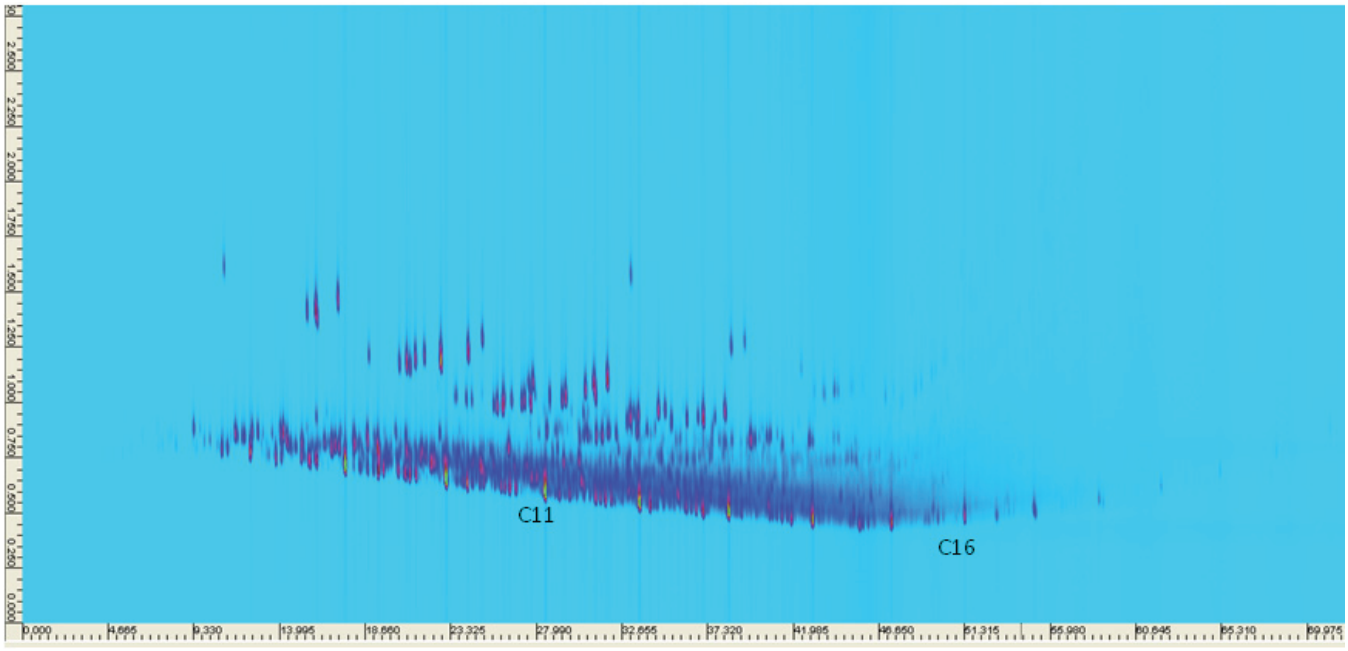

Figure 5a. Jet fuel 2D image. 7 m × 0.25 mm × 0.24 µm HP-INNOWax, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

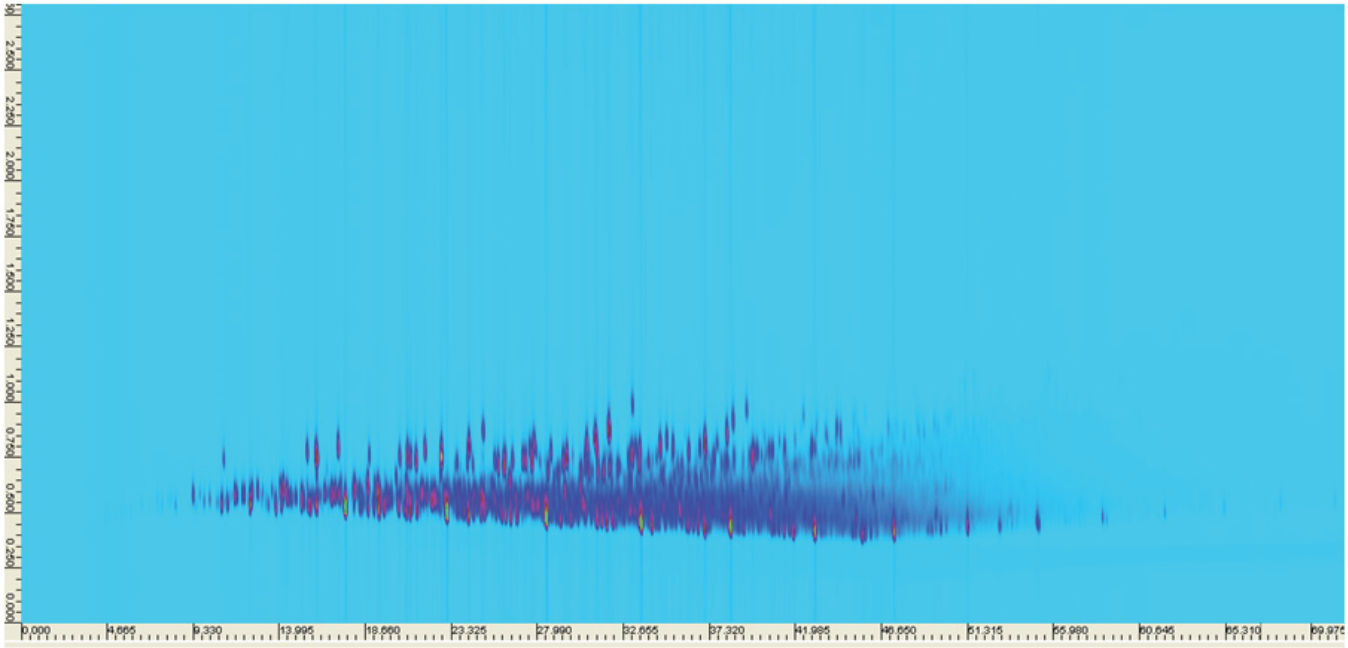

Figure 5b. Jet fuel on 5 m × 0.25 mm × 0.15 µm DB17HT, LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

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

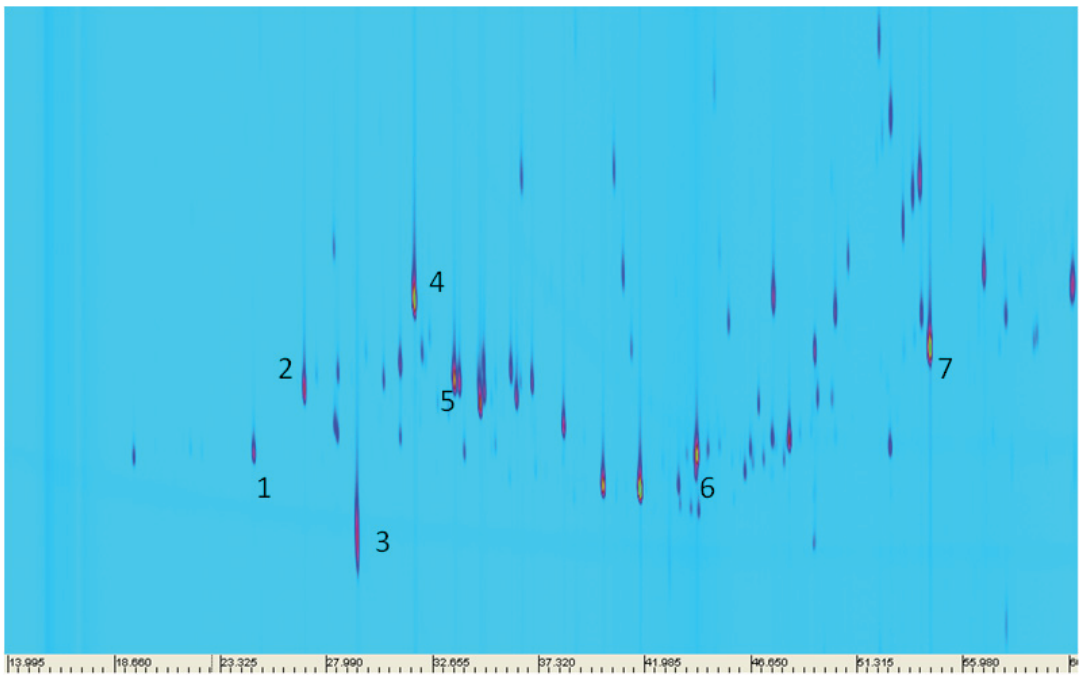

Figure 6a. Fragrance additive using 7 m INNOWax for 2nd dimension, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl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

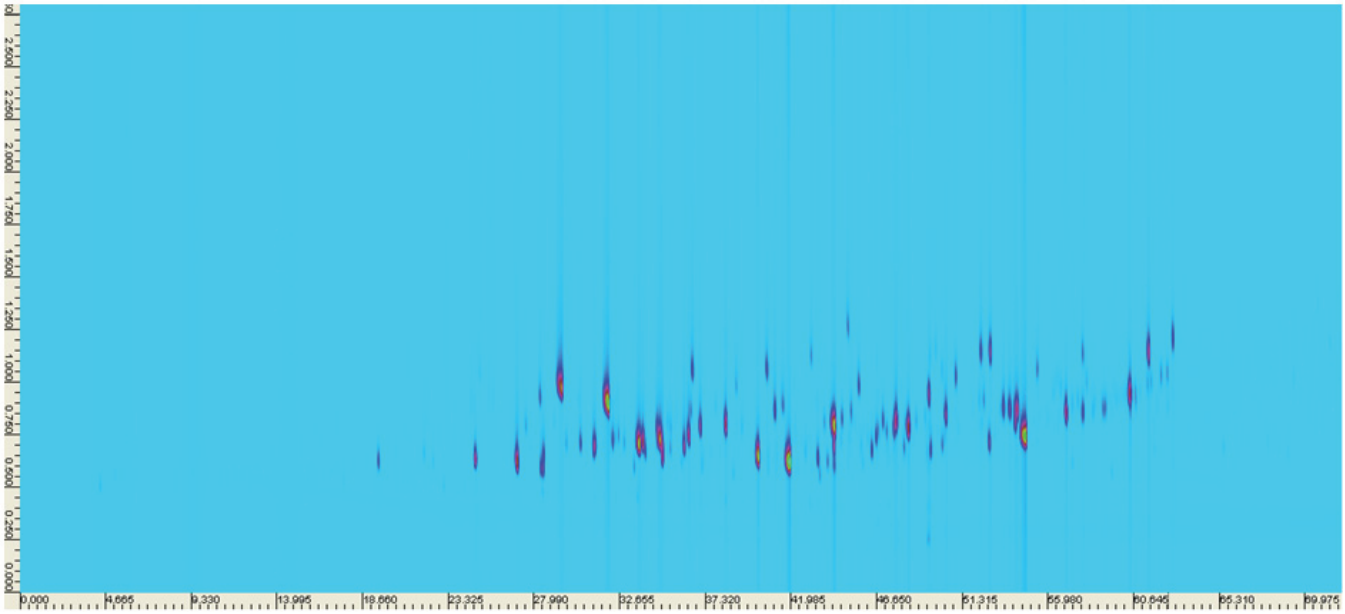

Figure 6b. Fragrance additive using 5m DB17HT for 2nd dimension separation. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

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

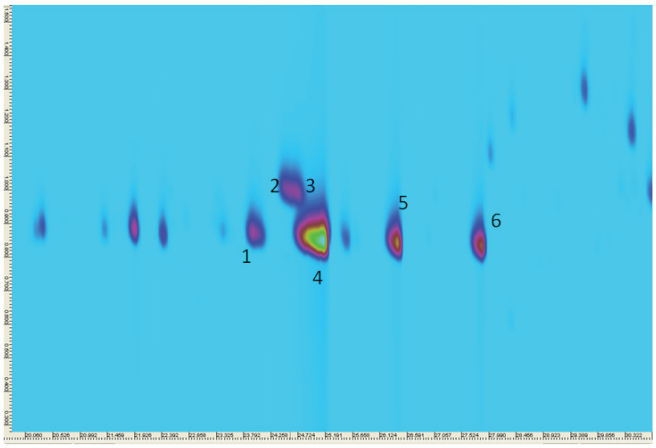

Figure 7a. Lime oil on the 7 m INNOWax. LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate 1.beta pinene,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

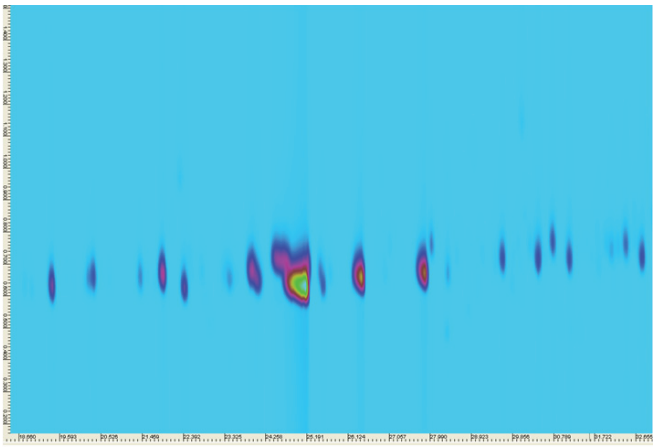

Figure 7b. Lime oil on the 5 m DB17HT. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

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

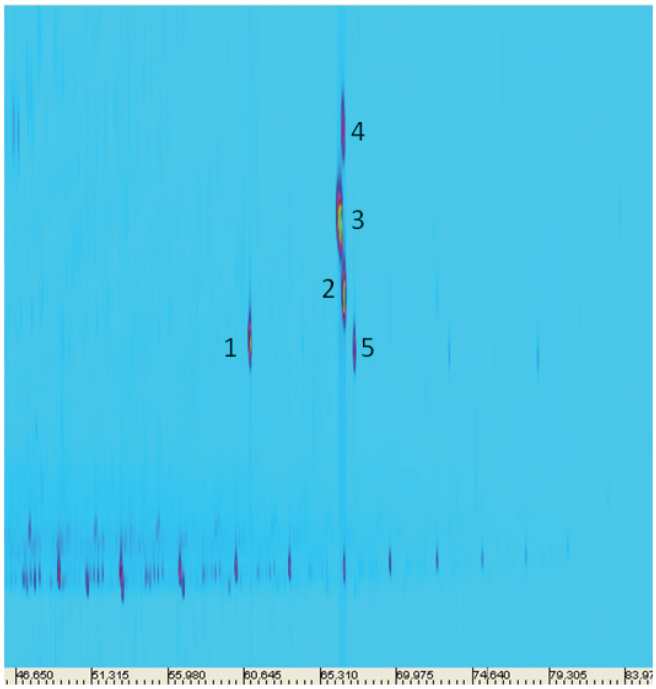

Figure 8. Separation of C16 and C18 fatty acid methyl esters in B20 biodiesel on a 5 m × 0.25 mm × 0.15 µm LTM INNOWax column in the 2nd dimension. LTM program: 30 °C (0 min) to 270 °C (5 min) @ 3 °C/min. 1. C16:0, 2. C18:1, 3. C18:3, 4. C18:3, 5. C18:0.

Conclusions

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

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

References

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

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Agilent Technologies

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 µm particle size columns.

Agilent Technologies

Conditions

Columns: PLgel 5 µm 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 µm MIXED-C column provides sufficient scope to resolve the polymer distribution and the four phthalate plasticizers for quantitative analysis.

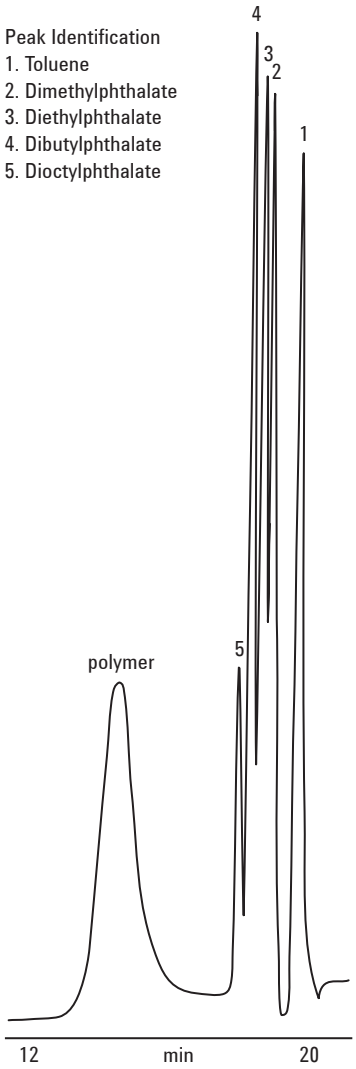

Figure 1. Plasticized PVC analysis.

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 µm 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 µm MIXED-C also provide the versatility essential for the modern R&D laboratory.

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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 µm 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 µL

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.

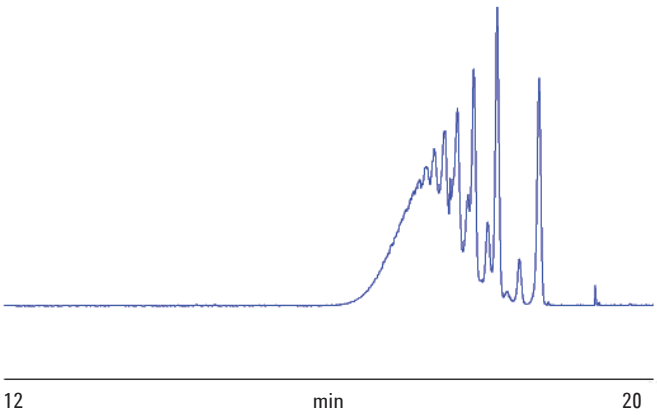

Figure 1. Chromatogram showing the detection of the main sample peak and two contaminants.

Conclusion

The Agilent 380-ELSD was successfully used to elucidate molecular weight distribution of an epoxy resin. Coupled with a PLgel 3 µm 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 µm 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 µm 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.

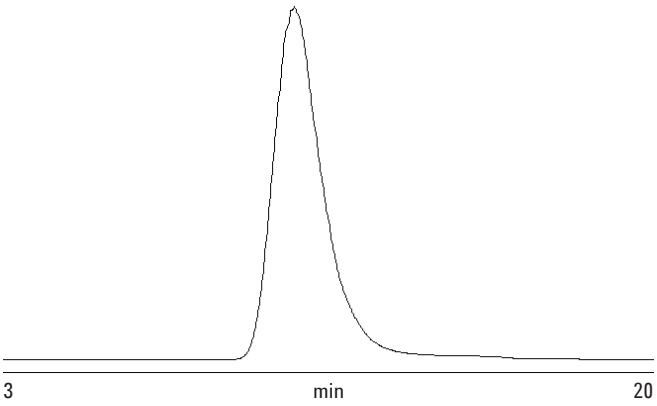

Figure 1. Excellent base line stability in poly(lactide-co-glycolide) analyzed by the Agilent ELSD.

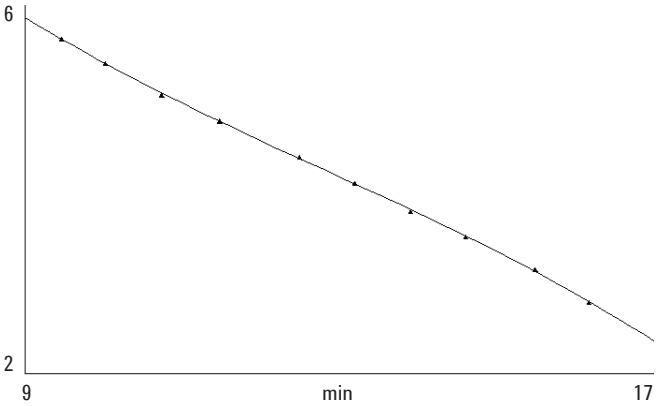

Figure 2. System calibration using EasiCal PS-2 standards.

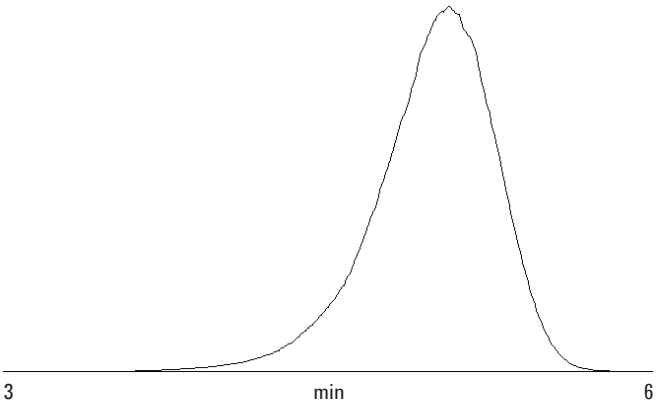

Figure 3. Molecular weight averages (Mw 110626 and Mn 70766) of poly(lactide-co-glycolide).

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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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 µm 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 µm 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.

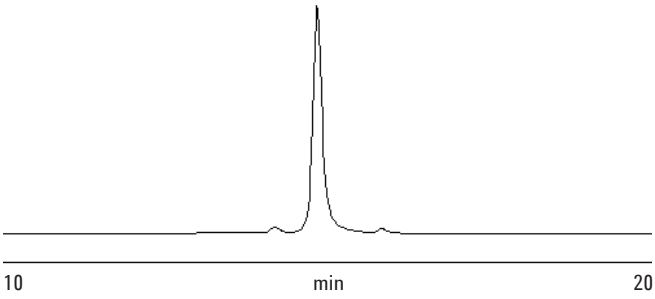

Figure 1. Typical full scale GPC chromatogram of a sample of AKD using Agilent evaporative light scattering detection.

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.

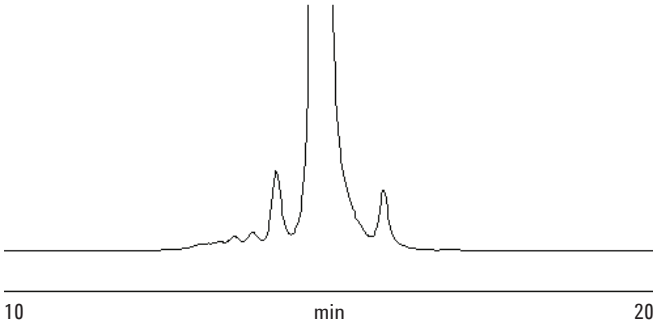

Figure 2. A close up of the region around the main AKD peak revealing the presence of smaller quantities of other oligomers.

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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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 µm 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 µm MIXED-C columns with the Agilent ELSD provides a good system for the discrimination of polymers and additives.

Agilent Technologies

Instrumentation

Column: PLgel 5 µm 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.

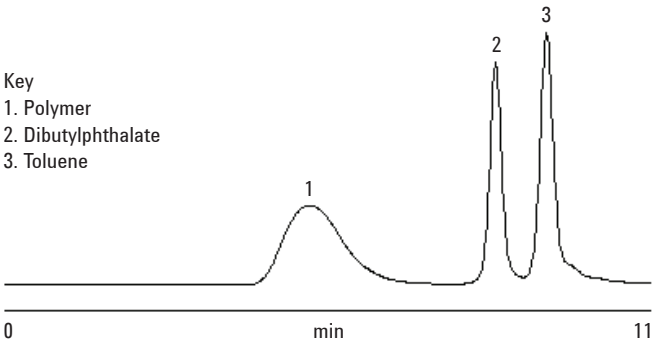

Figure 1. Separation of polymer containing dibutylphthalate using RI.

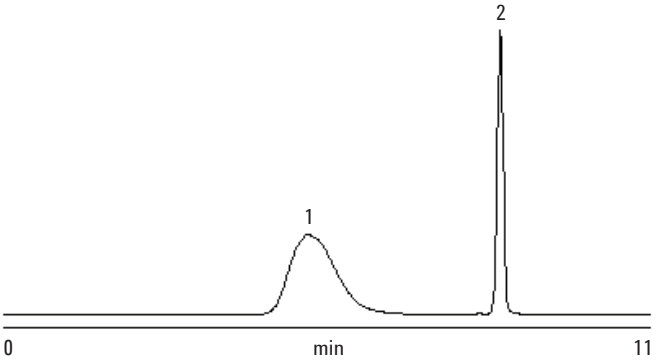

Figure 2. Separation of polymer containing dibutylphthalate using the Agilent ELSD.

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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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 µm 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.

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Instrumentation

Columns: 2 x PLgel 20 µm 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 µL

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 µm MiniMIX-A columns, as shown in Figure 1.

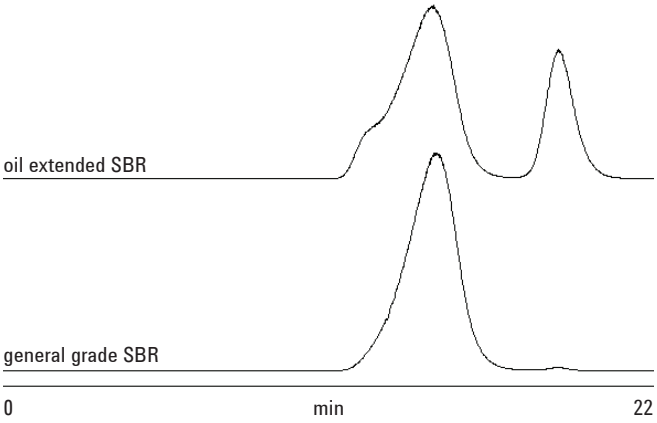

Figure 1. Oil extended SBR (above) and general grade SBR with no oil modifier (below), as revealed by the Agilent ELSD.

Conclusion

PLgel 20 µm 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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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.

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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.

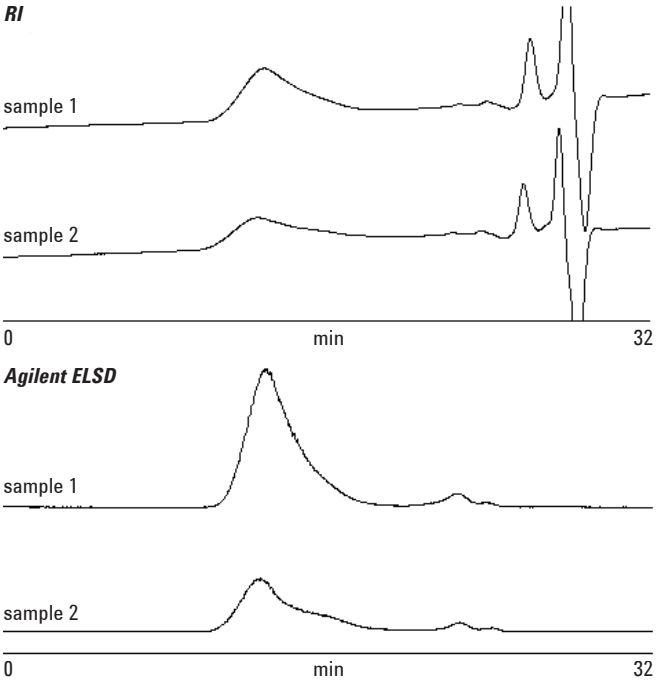

Figure 1. Stable base line and no interference from system peaks using the Agilent ELSD (below) compared to RI detection (above).

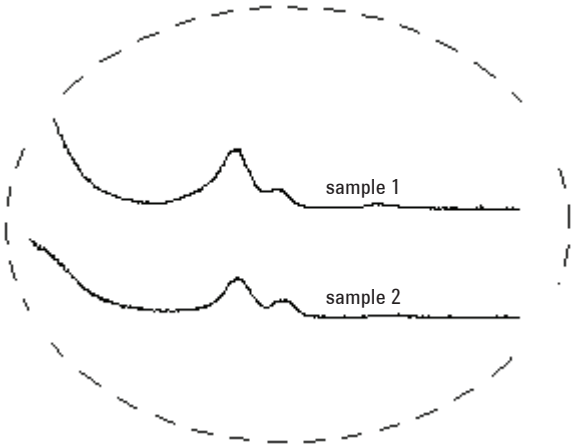

Figure 2. Magnified view of the Agilent ELSD plots showing the additive region.

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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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 µm 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 µm MiniMIX-D column with the Agilent ELSD comprises an excellent system for the quantification of bisphenol-A.

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Instrumentation
Column: PLgel 5 µm 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 µL 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.

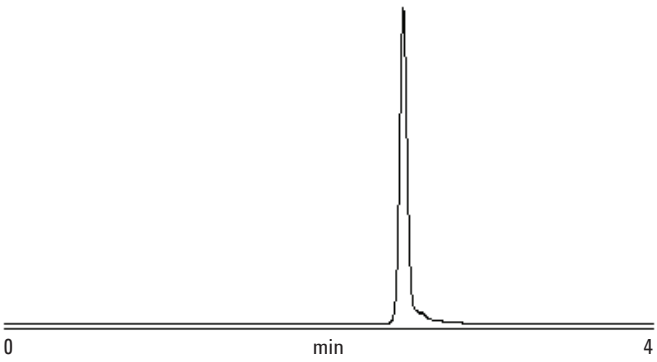

Figure 1. Raw data chromatogram of 0.5 mg bisphenol-A/µL.

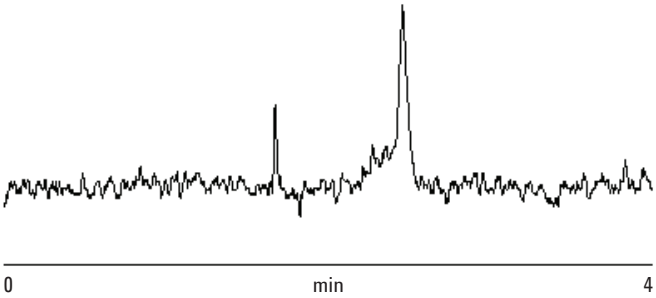

Figure 2. Raw data chromatogram of 3.9 µg bisphenol-A/mL.

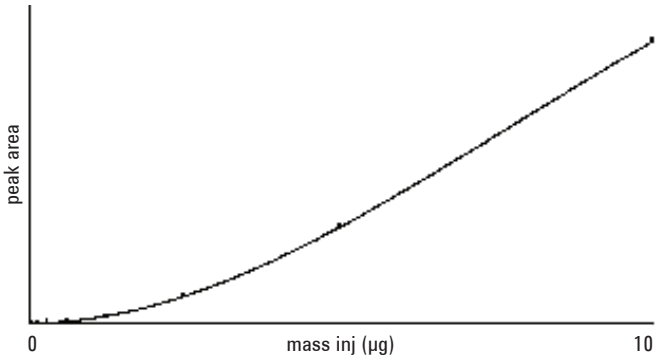

Figure 3. Calibration plot of bisphenol-A.

Conclusion

PLgel 5 µm 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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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 µm 500Å, 7.5 × 300 mm (p/n) PL1110-6525) Agilent PLgel 5 µm 100Å, 7.5 × 300 mm (p/n PL1110-6520)
Eluent	DMF
Flow rate	1.0 mL/min
Temp	50 °C
Detector	RI
System	Agilent PL-GPC 50

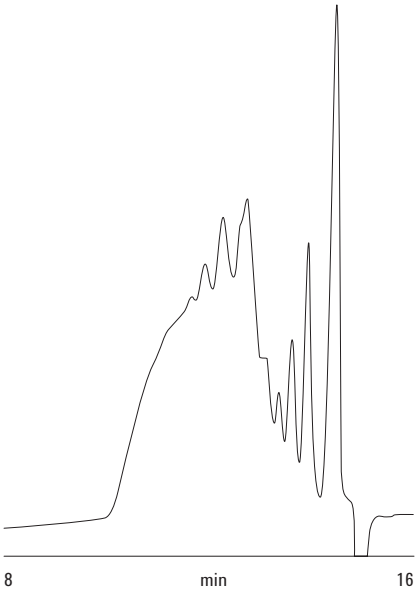

Figure 1. Oligomers in a phenolic resin separated on an Agilent PLgel 5 µm two-column set.

Conditions for Figure 2

Columns	Agilent PLgel 5 µm 100Å, 7.5 × 300 mm
Eluent	DMF
Flow rate	1.0 mL/min
Temp	50 °C
Detecto:	RI
System	PL-GPC 50

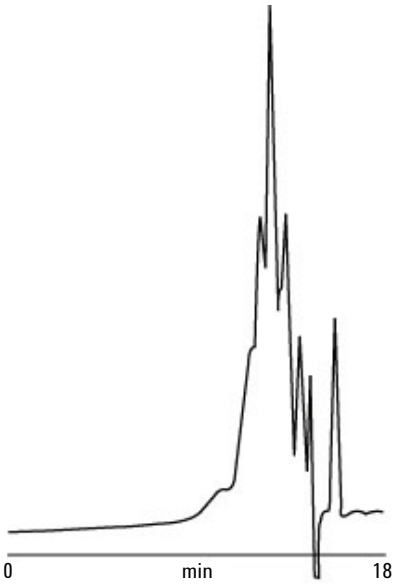

Figure 2. A phenolic resin analyzed on a single Agilent PLgel 5 µm column.

Conclusion

Agilent PLgel columns in DMF can be used to investigate different samples of phenolic resin by gel permeation chromatography.

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Agilent Technologies

Polystyrene Stars on Agilent PLgel 5 µm 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

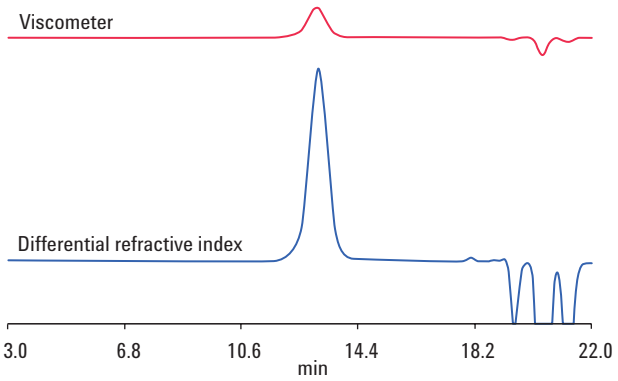

Figure 1 *A 14-star-branched polystyrene detected by viscometry and refractive index.*

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).

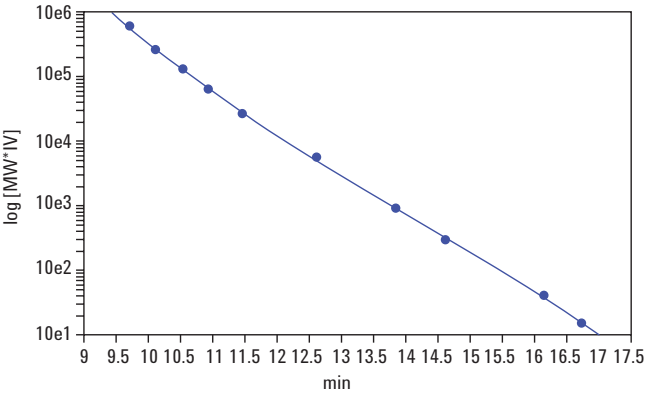

Figure 2 *Linear polystyrene standards produce a Universal Calibration curve.*

Based on this calibration, the molecular weight averages and weight average intrinsic viscosity (IVw) 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*

	Molecular weight averages/gmol^{-1}							
Polystyrene	**Mp**	**Mn**	**Mw**	**Mz**	**Mz+1**	**Mv**	**PD**	**IVw**
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.

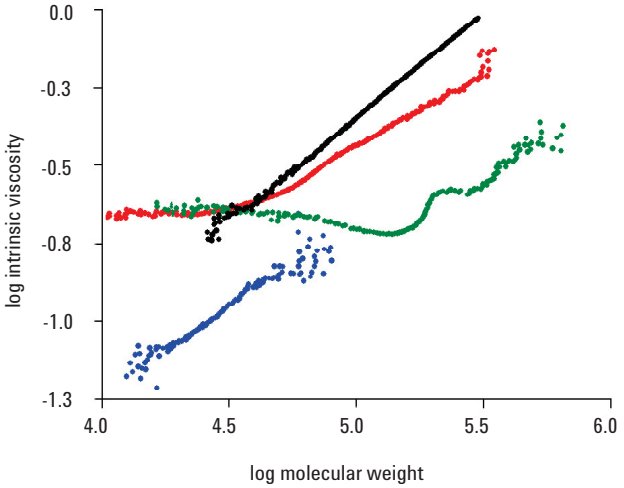

Figure 3 *Overlaid Mark-Houwink plots for the linear PS (black) and the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.*

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.

$$g' = [a + (1a)g^p]g^b$$

Where a = 1.104, p = 7 and b = 0.906 (Weissmuller & Burchard (1997) Polymer Internat. 44, 380).

Figure 4 shows an overlay of Rg 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)

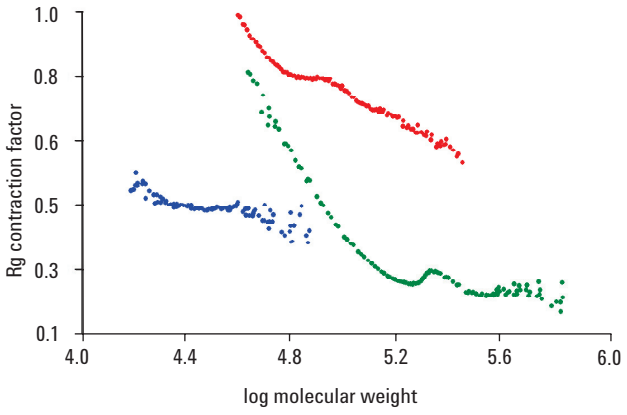

Figure 4. Rg contraction plots for the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.

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.

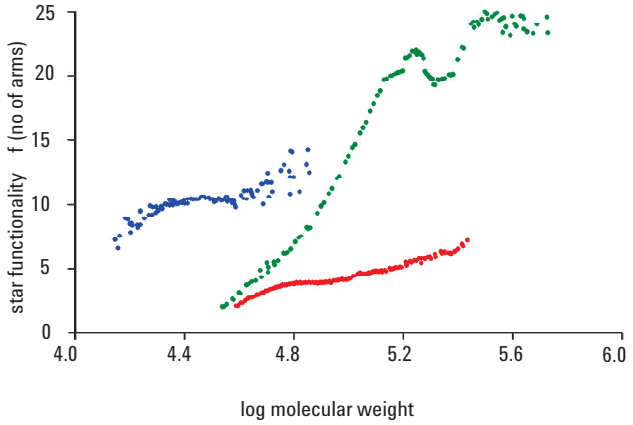

Figure 5. Functionality f plots for the 5-arm (red), 14-arm (blue) and 21-arm (green) star-branched polymers.

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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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

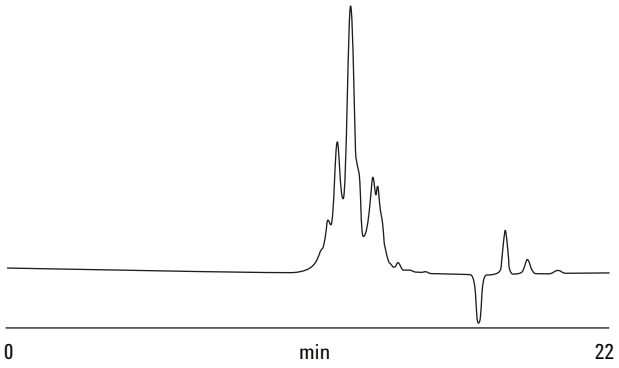

Figure 1. Chromatogram of a microcrystalline wax on an Agilent PLgel 3 µm two-column set.

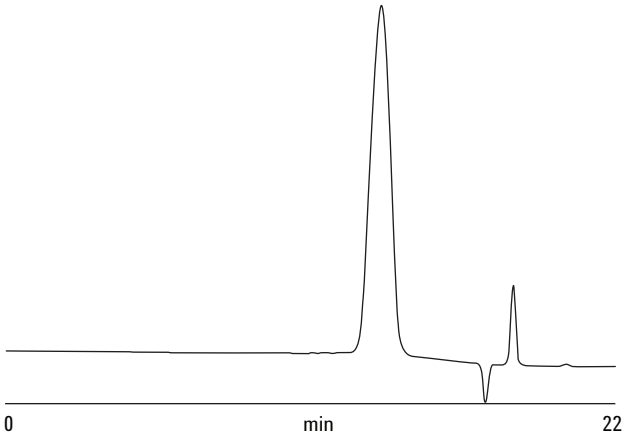

Figure 2. Chromatogram of beeswax on an Agilent PLgel 3 µm two-column set.

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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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 µm 500Å, 7.5 × 300 mm (p/n PL1110-6525)
Eluent	Toluene
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

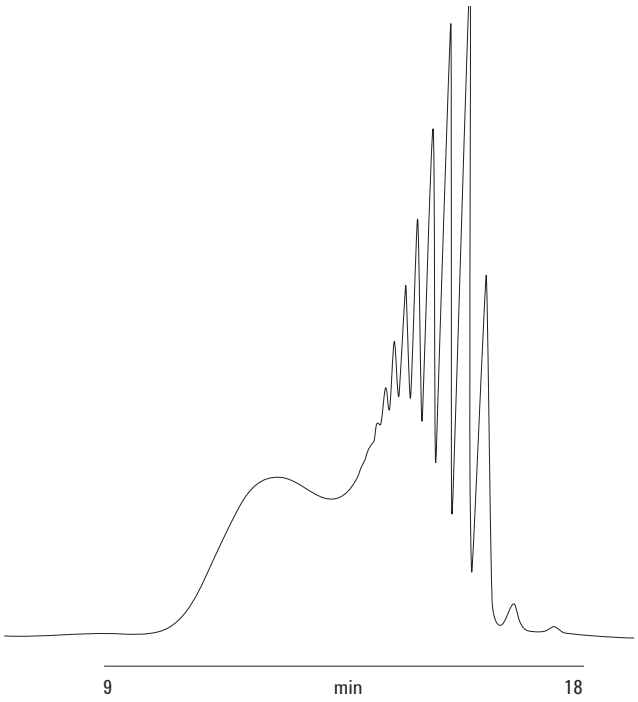

Figure 1. Separation of silicone with high oligomer content on an Agilent PLgel 5 µm column set.

Conclusions

Using toluene as a preferred solvent, Agilent PLgel columns effectively separate silicones with high oligomer content.

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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 10^3Å, 7.5 × 300 mm (p/n PL1110-6530)
	Agilent PLgel 5 µm 100Å, 7.5 × 300 mm (p/n PL1110-6520)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

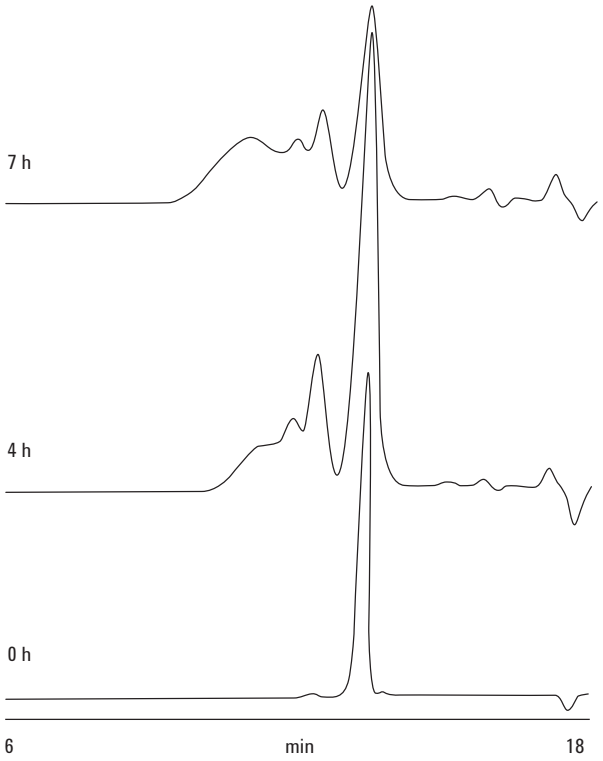

Figure 1. Increasing polymerization of a soya oil over a seven hour period revealed by Agilent PLgel 5 µm columns.

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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Agilent Technologies

Asphalt Analysis with Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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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

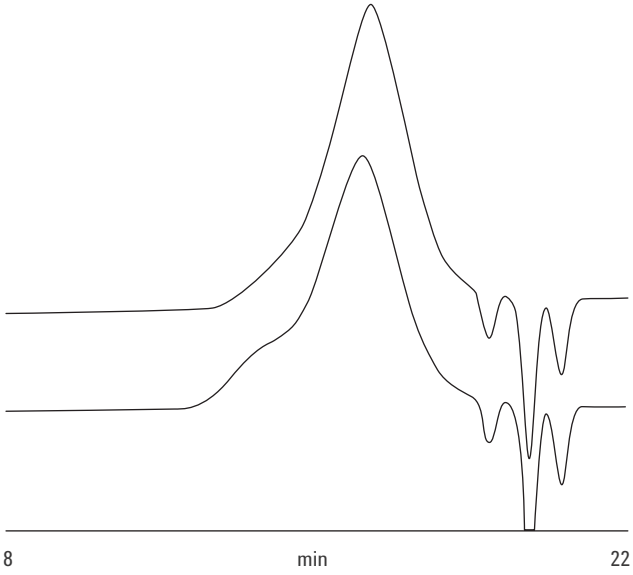

Figure 1. Comparing two batches of asphalt using an Agilent PLgel 5 µm MIXED-D two-column set with tetrahydrofuran as eluent.

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

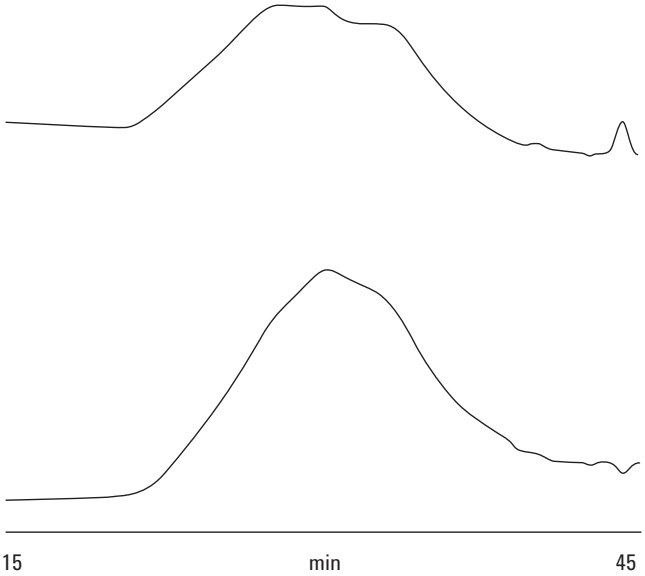

Figure 2. Comparing two batches of asphalt using an Agilent PLgel 5 µm MIXED-E two-column set with o-xylene as eluent.

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.

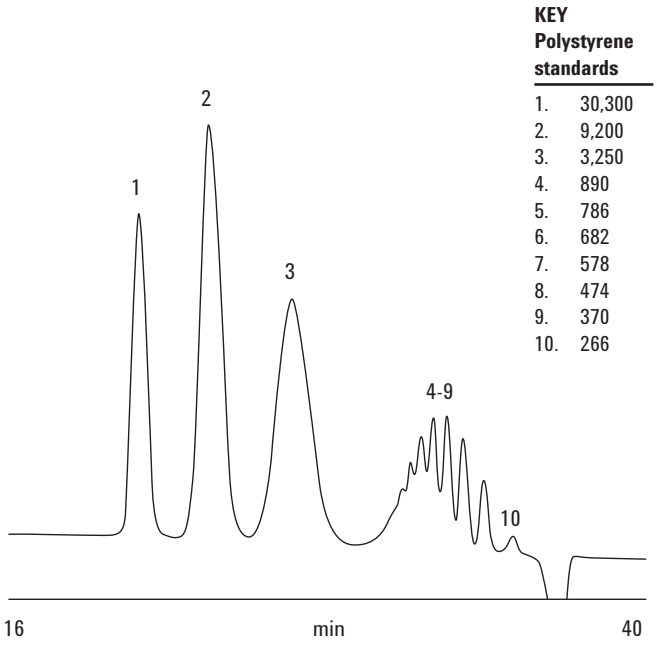

Figure 3. Separation of low molecular weight polystyrene standards on Agilent PLgel 3 µm MIXED-E columns.

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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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

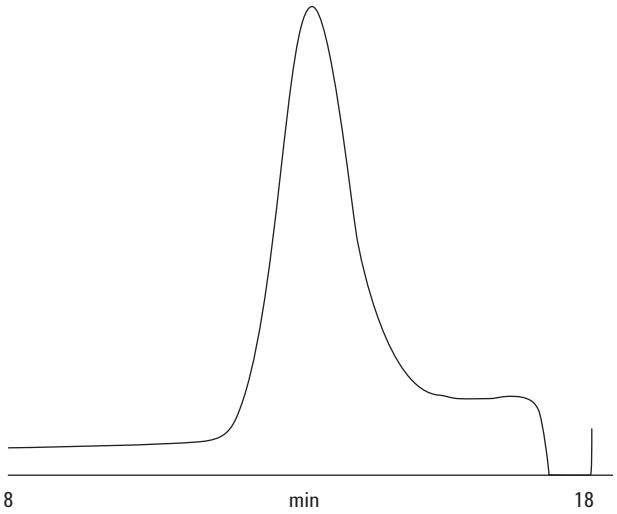

Figure 1. Polyurethane copolymer analysis using an Agilent PLgel 10 µm MIXED-B two-column set.

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

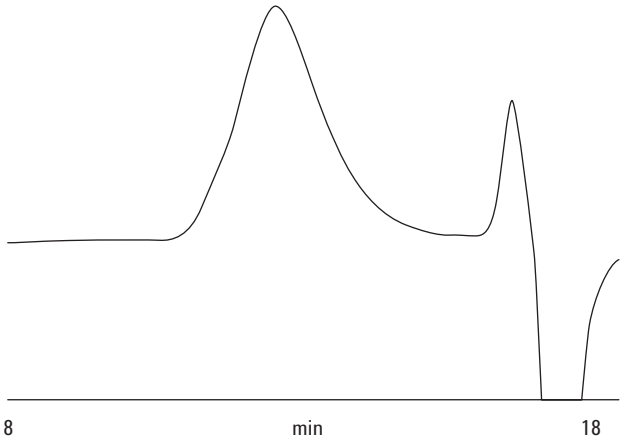

Figure 2. Acrylonitrile butadiene styrene analysis on an Agilent PLgel 5 µm MIXED-D two-column set.

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

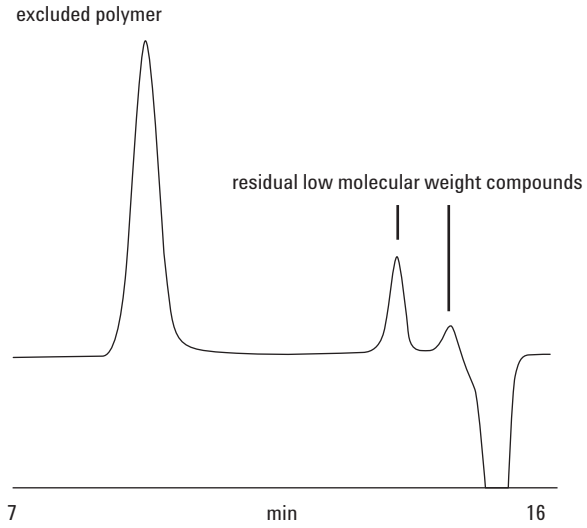

Figure 3. Analysis of residual monomers in acrylonitrile butadiene styrene on an Agilent PLgel 5 µm two-column set.

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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Agilent Technologies

Polyurethane Prepolymer Analysis on Agilent PLgel MIXED-E by GPC

Application Note

Materials Testing and Research

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Introduction

The term polyurethane encompasses a range of materials that contains a urethane (-NHC00-) 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 µm 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 × Agilent PLgel 3 µm MIXED-E, 7.5 × 300 mm (p/n PL1110-6300)
Eluent	DCM
Flow rate	1.0 mL/min
Inj vol	20 µL
Detector	RI
System	Agilent PL-GPC 50

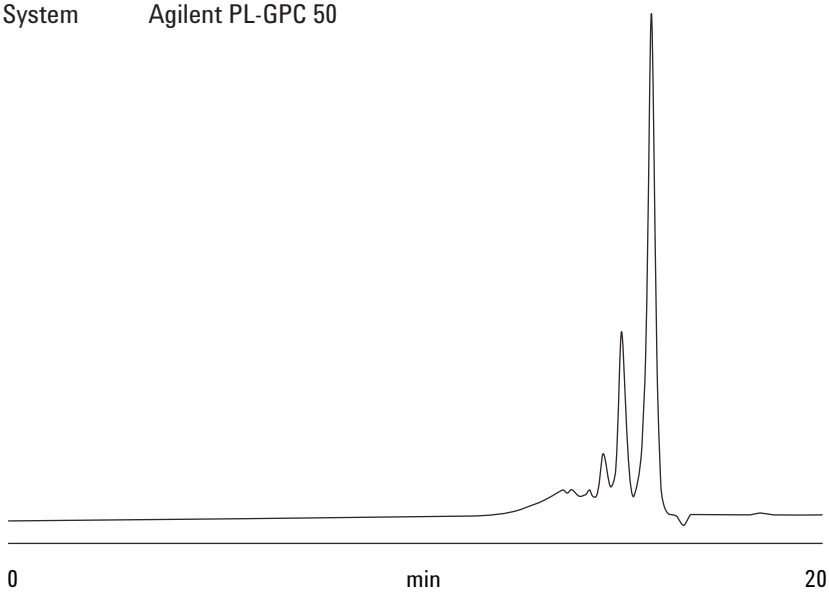

Figure 1. Oligomeric components of a prepolymer on Agilent PLgel MIXED-E columns.

Conclusions

The analysis of low molecular weight prepolymers by gel permeation chromatography using Agilent PLgel 3 µm MIXED-E columns allows a detailed investigation of the oligomeric components of the sample to be determined.

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Agilent Technologies

Analyze Injection-Molding Polymers on Agilent PLgel 5 µm 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 µm 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 Mw 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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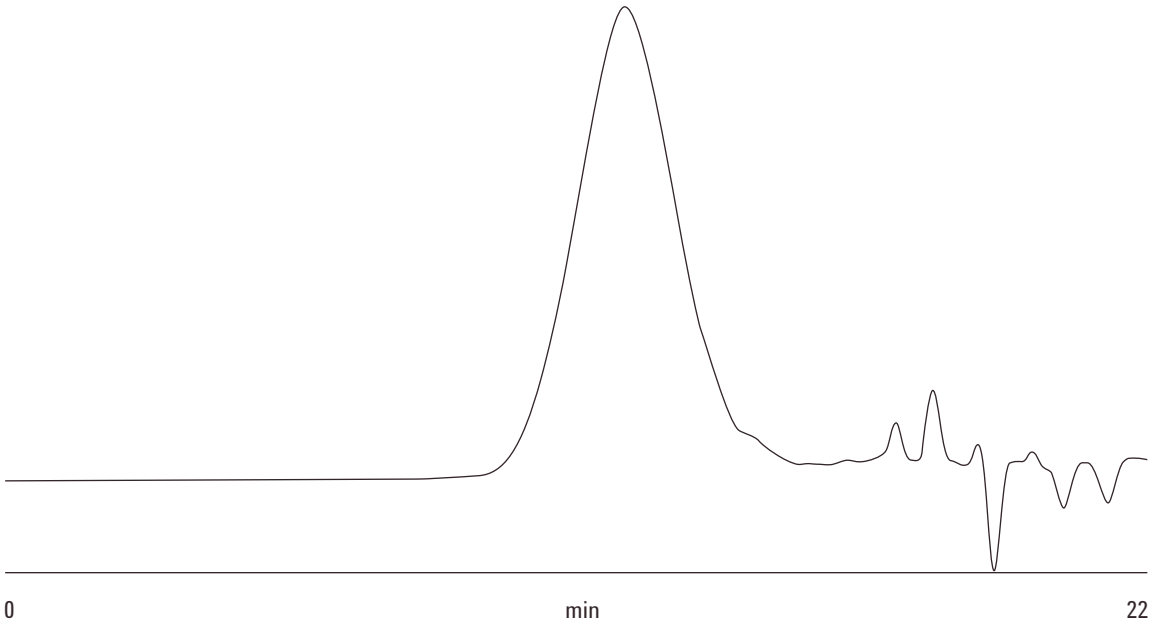

Figure 1. Individual components of an injection-molding polymer elute as a single peak, showing their similarity in molecular size.

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Agilent Technologies

Adipate Polyester Analysis on Agilent PLgel 3 µm 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 µm 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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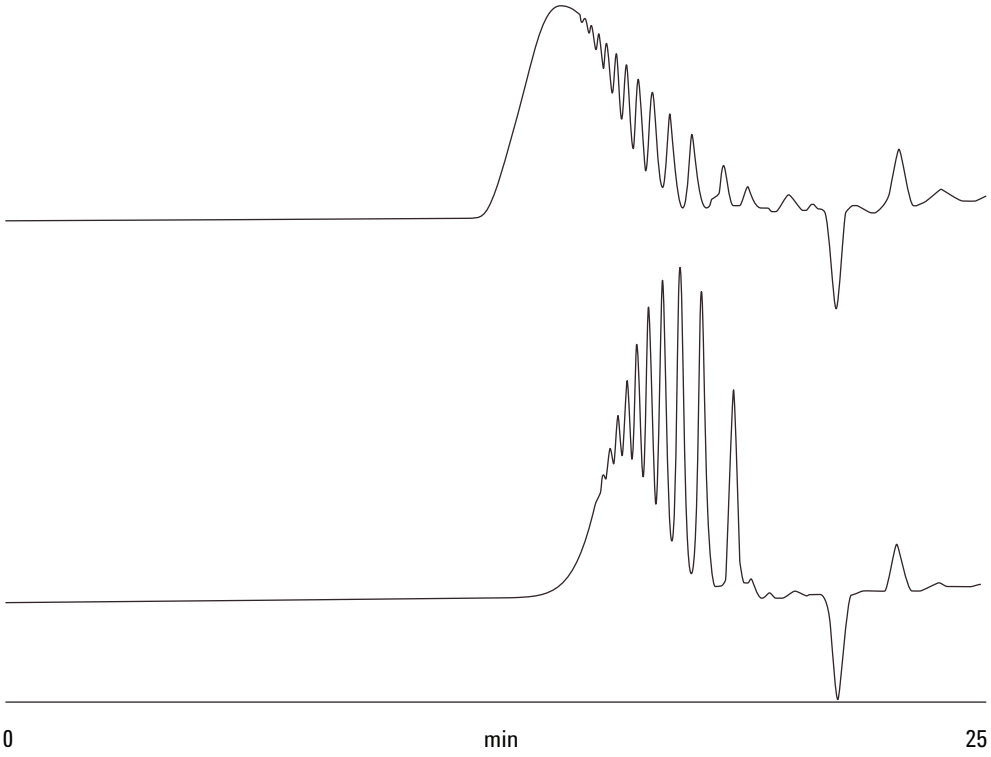

Figure 1. Using gel permeation chromatography with an Agilent GPC system to fingerprint adipate polyesters.

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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 µm 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 µm 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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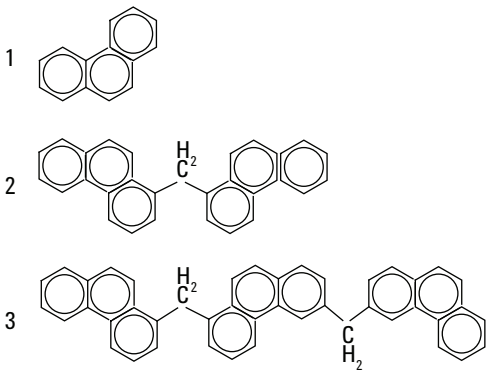

Figure 1. Structure of three fractions of a phenanthrene-formaldehyde resin.

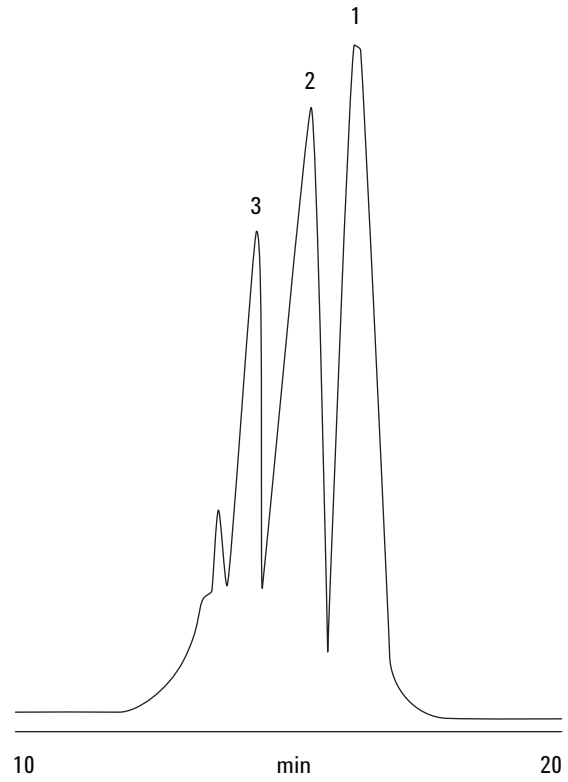

Figure 2. Separation of three fractions of phenanthrene-formaldehyde on Agilent PLgel 10 µm columns.

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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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.

Agilent Technologies

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

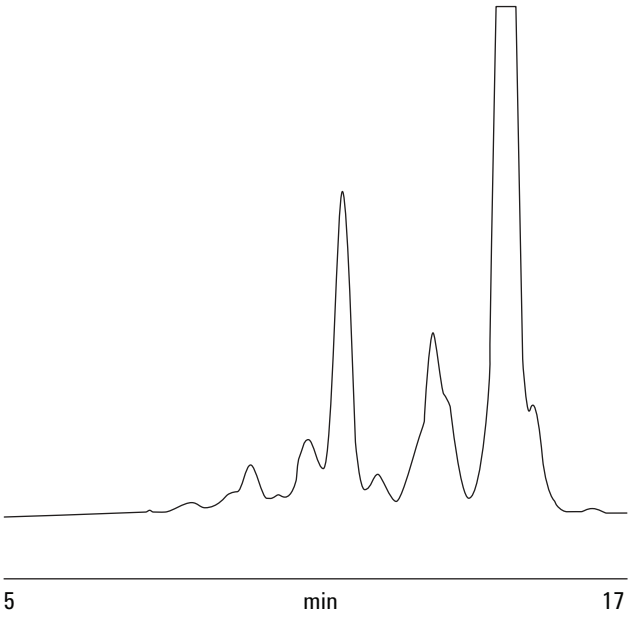

Figure 1. Epikote 828 analyzed on an Agilent OligoPore two-column set to reveal the dominance of a single oligomer.

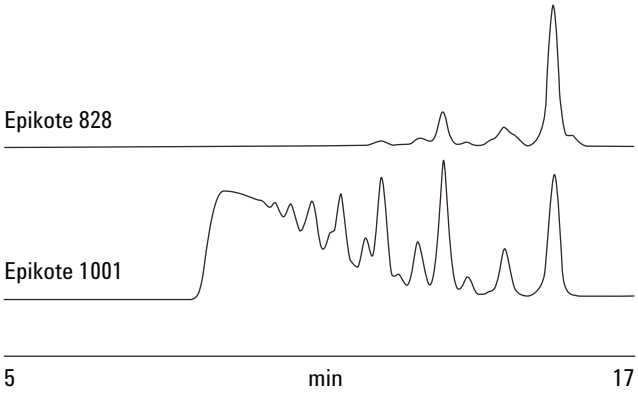

Figure 2. Comparison of Epikote 828 and 1001 epoxy resins on Agilent OligoPore columns shows that some of the oligomers are identical.

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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Agilent Technologies

Silane Oligomer Analysis with Agilent OligoPore and Gel Permeation Chromatography

Application Note

Materials Testing and Research

Authors

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UK

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 Olgomer 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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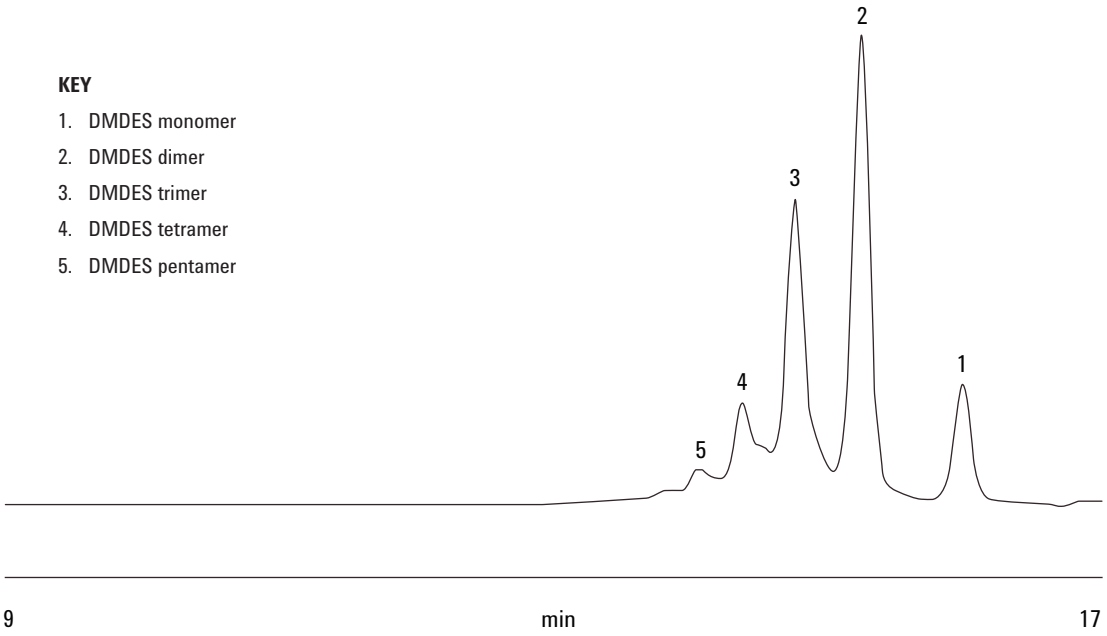

Figure 1. Fingerprinting silane oligomers with an Agilent OlgioPore column.

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Separate Epoxy Resin Oligomers with Agilent Preparative Gel Permeation Chromatography

Application Note

Materials Testing and Research

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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 Olgomer 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.

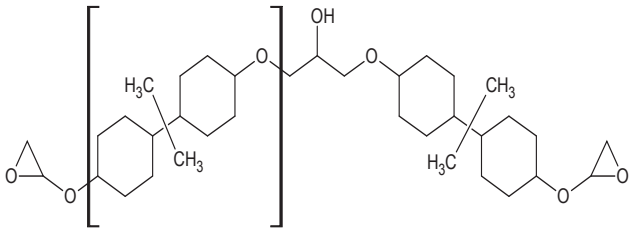

Figure 1. General structure of Epikote 828 epoxy resin oligomers.

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 µL
Detector	UV
System	Agilent PL-GPC 50

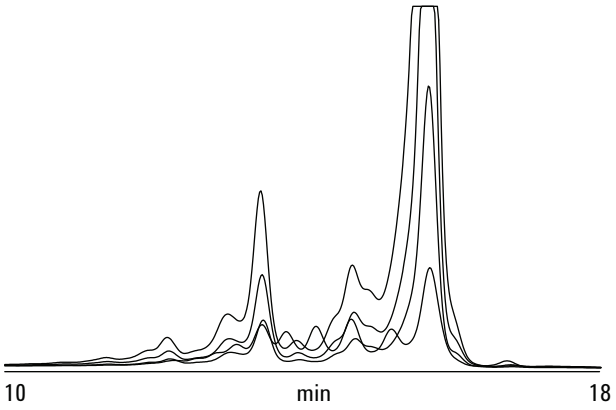

Figure 2. Analytical separation of Epikote 828 on Agilent OligoPore columns indicates that a 2.0% w/v concentration is appropriate for preparative analysis.

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

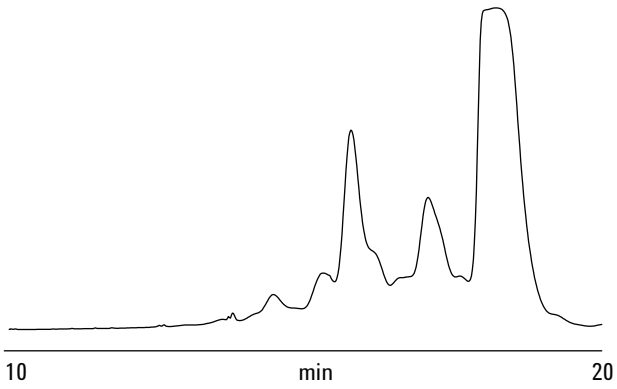

Figure 3. Epikote 828 separated on an Agilent OligoPore two-column set.

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.

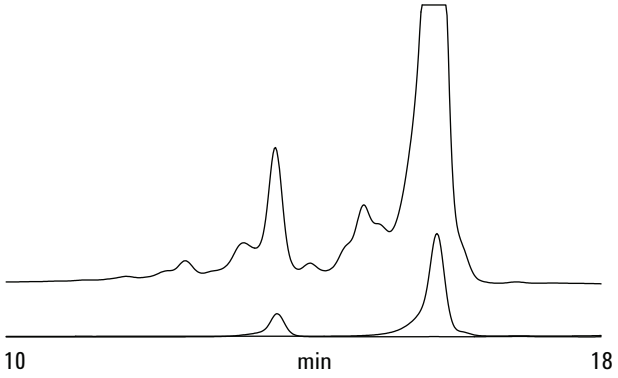

Figure 4. Epikote 828 analytical chromatogram from Figure 1 run at 2.0% (w/v) compared to overlaid analytical chromatograms of the n = 0 and n = 1 oligomers collected from the Agilent preparative GPC system.

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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Agilent Technologies

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.

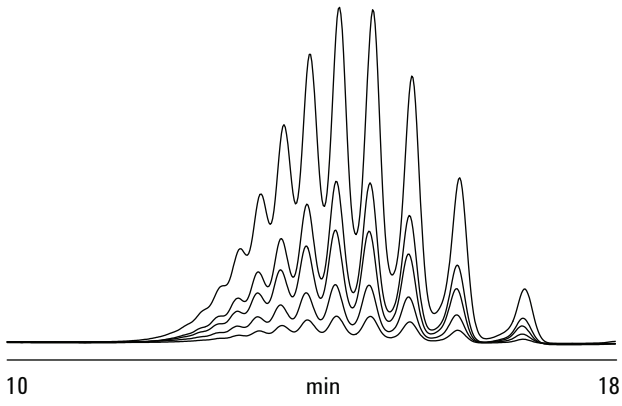

Figure 1. Analytical separation of polystyrene 580 on Agilent OligoPore indicates that a 2.0% w/v concentration is appropriate for preparative analysis.

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.

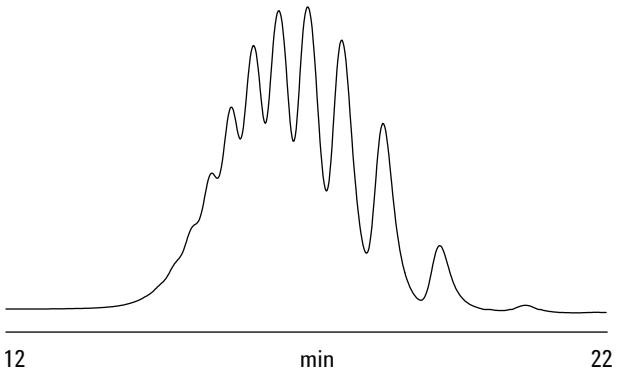

Figure 2. Polystyrene 580 separated on an Agilent OligoPore two-column set.

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.

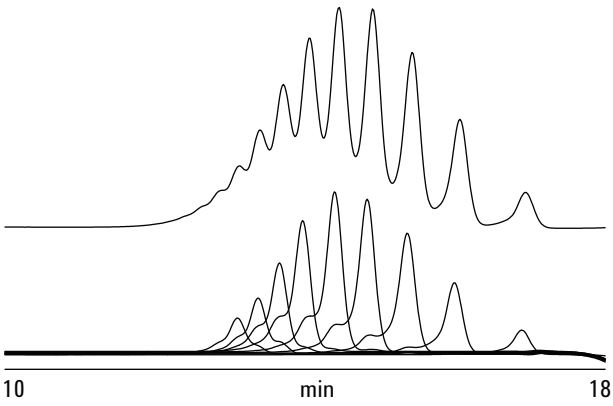

Figure 3. Polystyrene 580 from Figure 1 run at 2.0% (w/v) compared to nine overlaid analytical chromatograms collected from the Agilent OligoPore prep columns.

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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Agilent Technologies

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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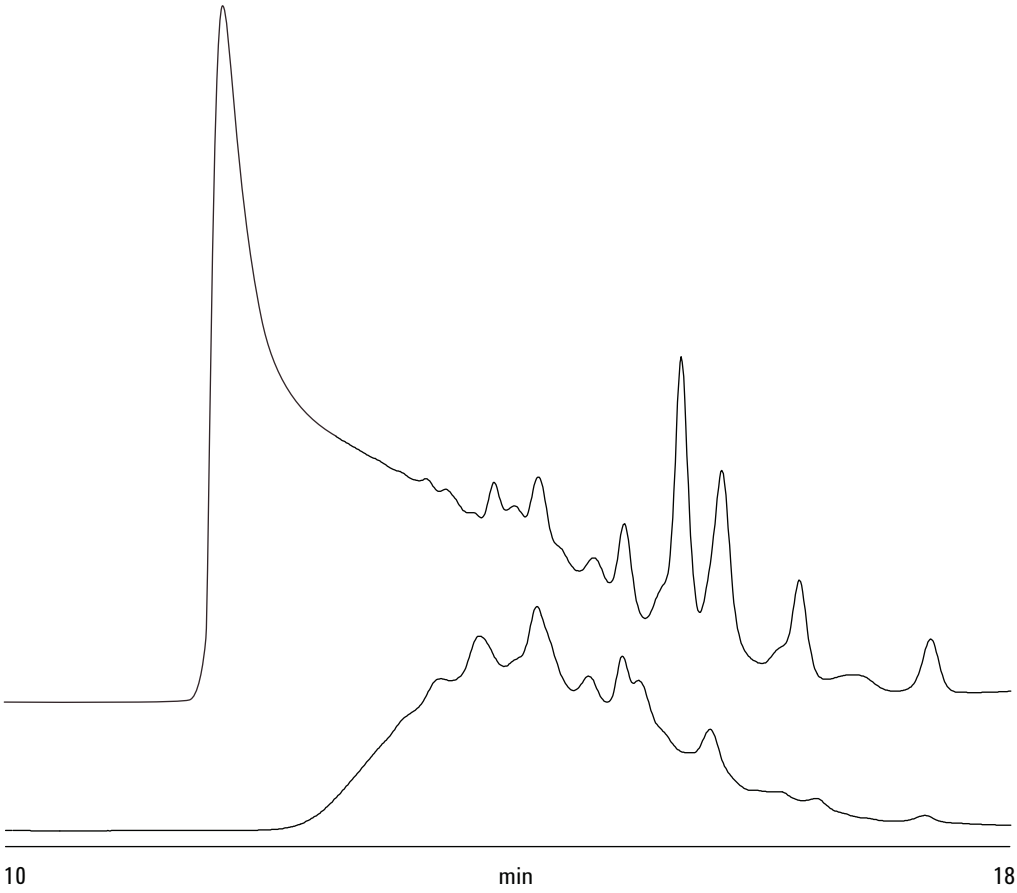

Figure 1. Analysis of two epoxy resins on Agilent OligoPore columns reveals very different oligomer distributions despite the presence of some oligomers common to both samples.

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Agilent Technologies

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

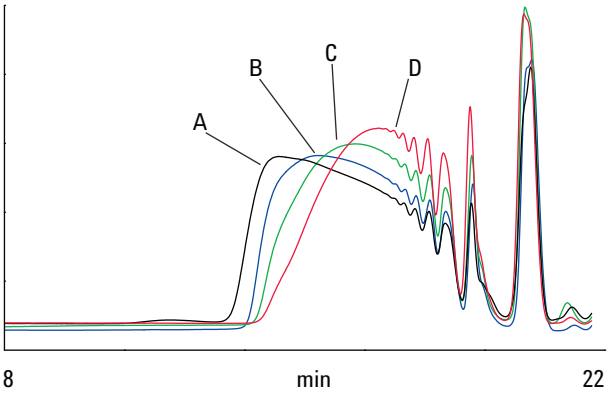

Figure 1. Overlay of GPC chromatograms obtained from four samples of phenolic resin on Agilent ResiPore columns.

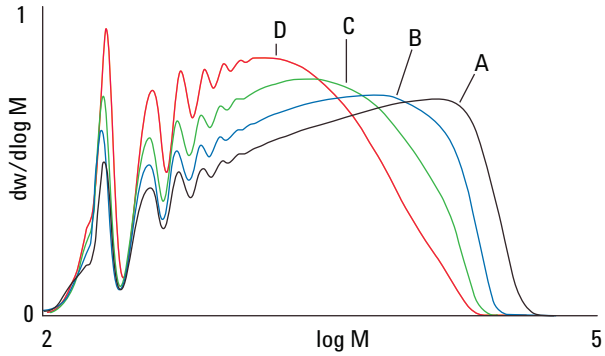

Figure 2. Overlaid molecular weight distributions of four phenolic resins reveals the relative amounts of the oligomer components with significant differences in molecular weight distribution.

Conclusions

High resolution Agient 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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Agilent Technologies

Quantitative analysis of copolymers using the Cary 630 FTIR spectrometer

Application note

Materials testing and research

Author

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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.

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.

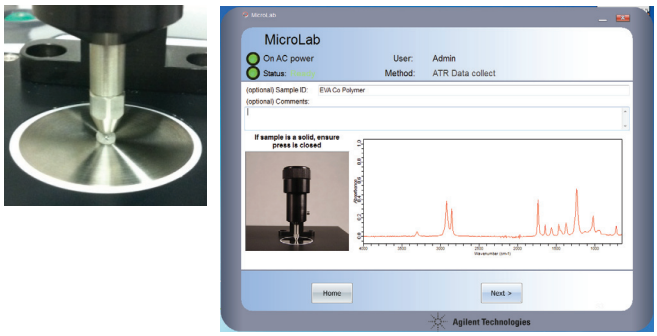

Figure 2. Polymer is placed directly on ATR sampling accessory. Uniform, constant pressure is provided by the sample press, ensuring that high quality spectra are obtained. Real-time analysis software provides an immediate indicator of spectral quality

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 (StDev= 0.03% PS).

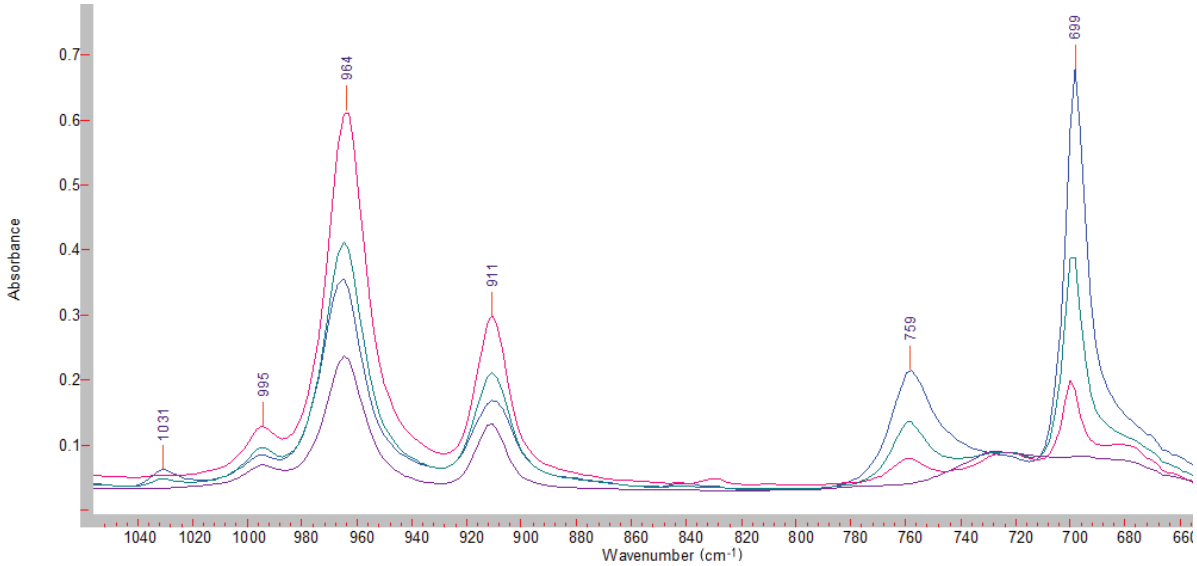

Figure 3. The FTIR spectra of four SBR rubber standards with increasing polystyrene concentrations: 0% (purple), 5% (red), 23% (green), and 45% (blue)

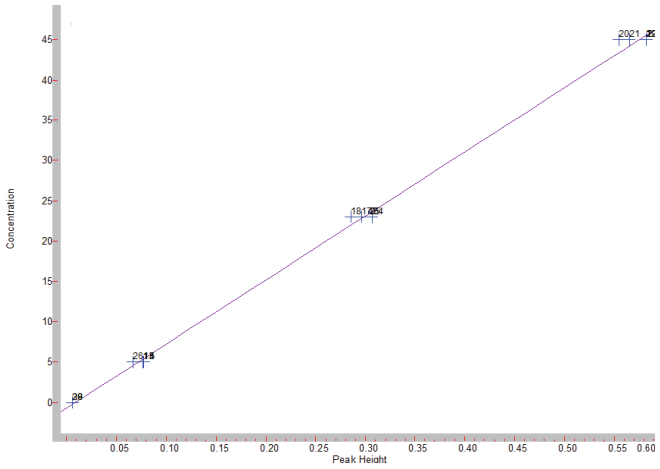

Figure 4. FTIR calibration curve for polystyrene in SBR rubber using the 699 cm^{-1} peak height absorbance; R^2=0.999.

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 2921cm $^{-1}$, 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.

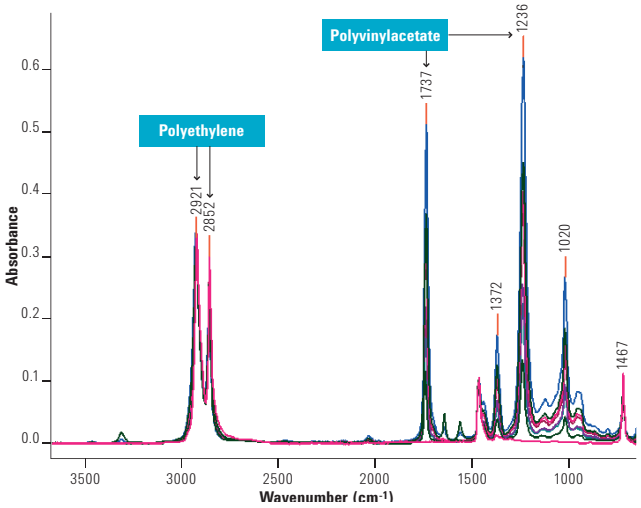

Figure 5. Spectral overlay of the calibration standards for polyethylene vinyl acetate). The spectra are all scaled to the polyethylene absorbance. The blue spectrum is 40 wt% VA, and the red spectrum is 0% VA

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

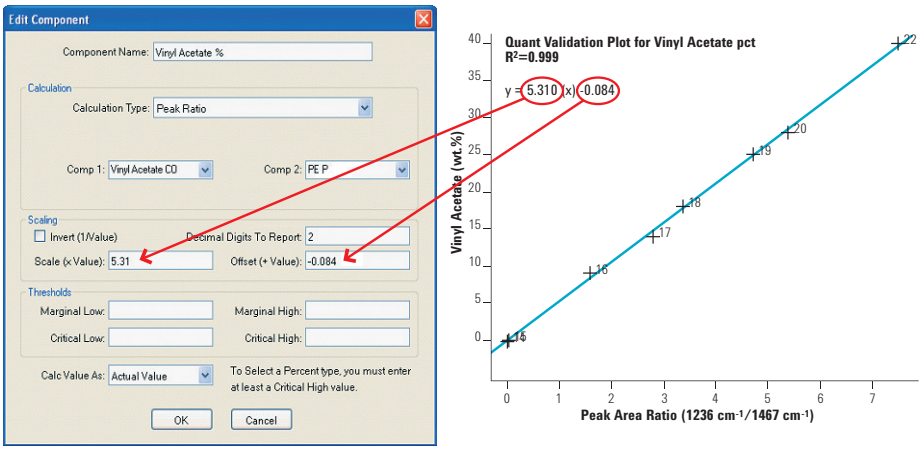

Figure 6. The method editor in the MicroLab FTIR software and the calibration plot for VA in PE

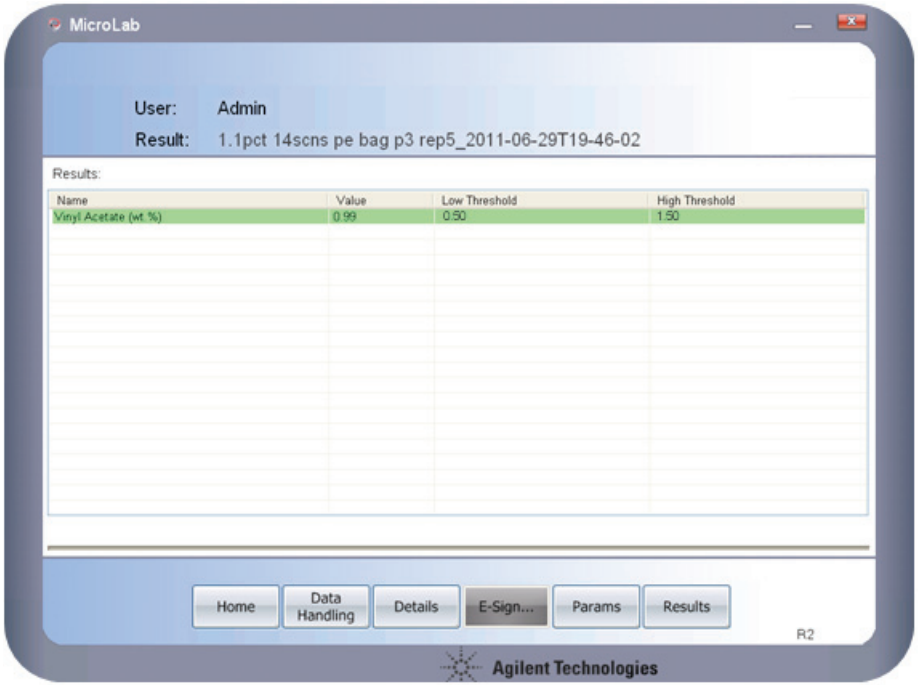

Figure 7. The result for the 1% VA validation standard — green color indicates an in-spec sample

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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Agilent Technologies

Analysis of wear metals and contaminants in engine oils using the 4100 MP-AES

Application note

Energy and fuels

Authors

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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.

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 cuves

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

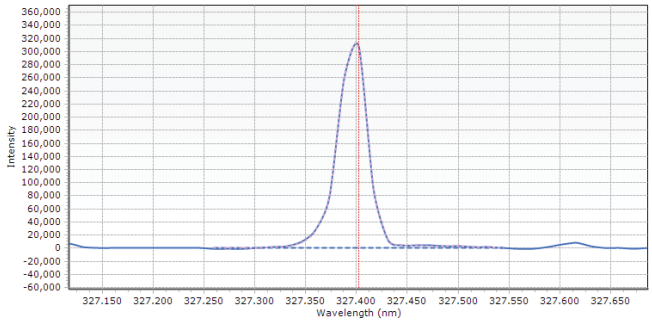

Figure 1. The signal from Cu 327.395 nm at 5 ppm shows the excellent sensitivity of the Agilent 4100 MP-AES

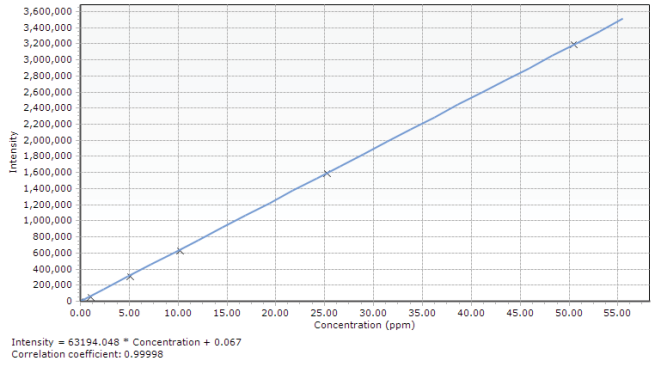

Figure 2. The calibration curve for Cu 327.395 nm up to 50 ppm shows excellent linearity across the calibrated range, with a correlation coefficient of 0.99998

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.

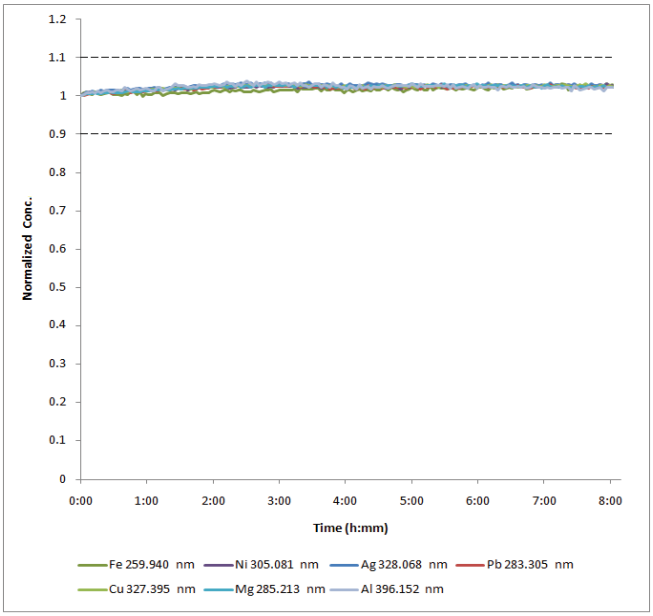

Figure 3. Normalized stability plot for 10 ppm S21+K solution run repeatedly over an 8 hour period

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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Agilent Technologies

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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The Netherlands

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 CO_2 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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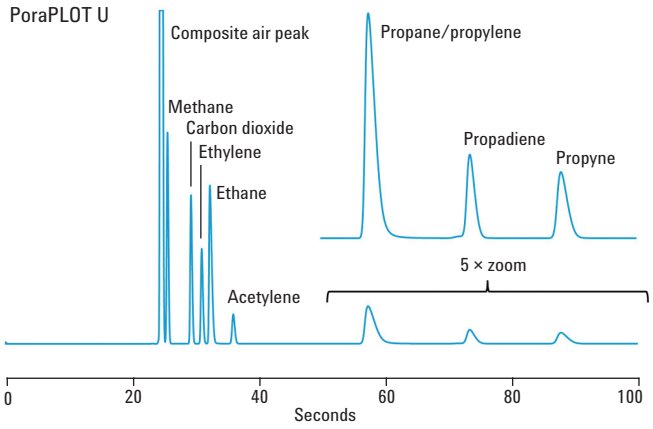
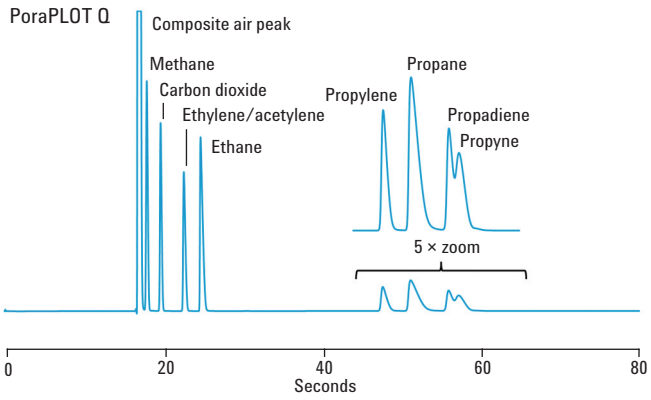
If you want to the ability to measure anywhere and get the results you need in seconds, the Agilent 490 Micro GC is the ideal solution. With its rugged, compact, laboratory quality gas analysis platform, the 490 Micro GC generates more data in less time for faster, and better, business decisions.

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 %
Etylene	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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Agilent Technologies

The Analysis of Polyvinylchloride

Application Note

Atomic Absorption

Authors

D. Nicolas

J. B. Sanders

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.

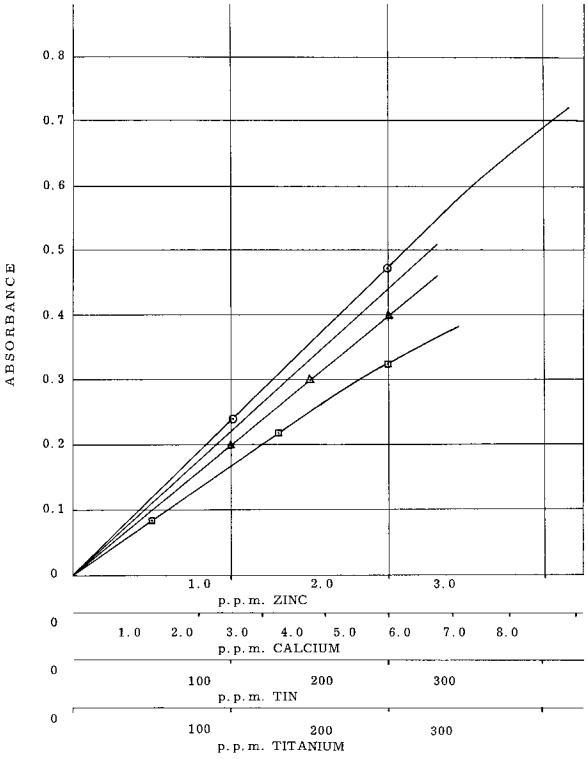

Figure 1. Calibration graphs for calcium, tin, titanium and zinc.

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_2O-C_2H_2	N_2O-C_2H_2	N_2O-C_2H_2	Air-C_2H_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 TiO_2 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 N_2O-C_2H_2 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% $HClO_4$).

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, N_2O-C_2H_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 × scale expansion down to approximately 4 ppm Sn.

Titanium

Titanium absorbance is measured at the 3642.7 Å resonance line, using a N_2O-C_2H_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 × 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 × 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.

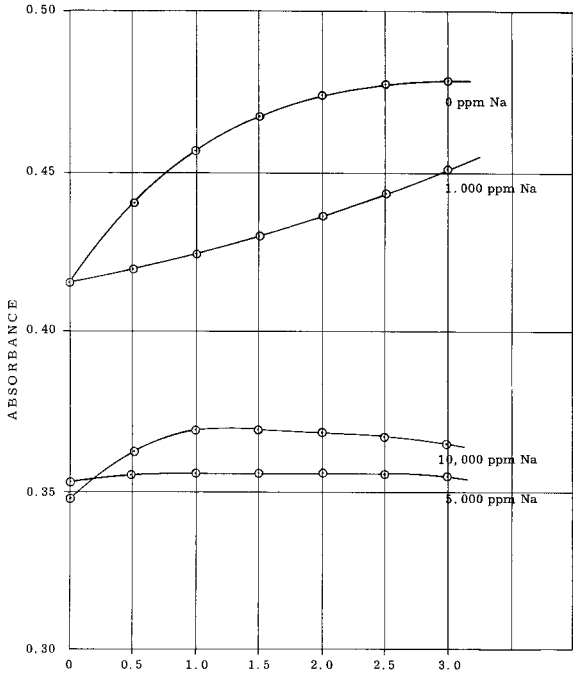

Figure 2. Effect of sodium on titanium absorbance in the zinc presence of hydrofluoric acid.

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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Agilent Technologies

Obtaining Optimum Performance When Using the SIPS Accessory

Application Note

Atomic Absorption

Introduction

The SIPS accessory, which was introduced in December 1994, was the first practical dilution system for flame AA to provide calibration from a single standard and fast, on-line dilution of over range samples. A few simple procedures, outlined in this information sheet, ensure reliable and productive operation of this accessory.

The Agilent SIPS pump tubing is manufactured from a composite material known as Santoprene. The pump tubing commonly used on VGA and ICP pumps is a single-mix polymer. All types of pump tubing, but especially composite tube materials, can sometimes show signs of "spalling" under normal operation. This is a variable effect in which very small particles of the tubing material break away. If severe spalling occurs, these particles can stick together and cause blockage of the nebulizer.

Spalling occurs in various degrees with all peristaltic pump tubing manufactured from composite materials. It is not unique to SIPS.

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The Effect of Spalling

The symptom of severe spalling is an initial increase in the absorbance followed by a decrease as the nebulizer capillary becomes increasingly blocked. A totally blocked nebulizer will cause the sample to be pumped into the diluent bottle thus contaminating the diluent. Sometimes the blockage may clear without intervention.

The extent of the blockage can depend on the nature of the solutions being pumped. It has been found that very dilute solutions are more likely to induce spalling and block the nebulizer than are concentrated solutions.

Why Use Composite Materials?

Composite materials produce long-wearing tubes that have consistent performance. Spalling usually has no noticeable effect. Some formulations, however, display a higher level of spalling. Naturally these are not recommended for use with SIPS.

Achieving Reliable SIPS Operation

There are four easy steps required to minimize spalling effects and to achieve reliable operation. These are:

1. Use only Agilent-supplied SIPS pump tubing
2. Determine, and use the correct arm pressure for each unit
3. Condition new pump tubes, and re-condition (used) tubes before a run
4. Add a detergent to the diluent

A brief summary of these procedures follow. The complete procedures are outlined in publication no. 85-101710-00, which is supplied with all batches of pump tubes.

Use Only Agilent-Supplied SIPS Pump Tubing

It is recommended that SIPS users obtain their pump tubing from Agilent only. Agilent supplied pump tubing is guaranteed to achieve our specified performance and this minimizes batch to batch variations. As with graphite tubes, individual batches of pump tubes are tested to ensure satisfactory operation. Only those batches passing our tests are accepted. Stretching and other problems have been noted with tube batches sampled from a range of vendors.

Determine the Correct Arm Pressure

When the SIPS is first installed, the user must determine the optimum arm pressure setting for that particular unit. This setting does vary from one SIPS unit to another. By optimizing the arm pressure setting, tube life is maximized and the optimum pumping efficiency is achieved.

In practice, this calibration does not have to be repeated when new tubes are installed as there is little variation from one batch of tubes to another.

The procedure need only be repeated if the SIPS unit is repaired or changed (for example, if a SIPS-10 is upgraded to a dual pump SIPS-20).

Condition the Pump Tubing

Before each use of a new pump tube, the pump tubing should be cleaned and conditioned, using the following procedure. Briefly, a dilute detergent solution (such as a 1% solution (mass/volume) of Triton X-100) is pumped through the tube for 15 minutes. Then distilled water is pumped for 30 minutes to rinse it. Once this time has elapsed, the SIPS unit is ready for regular operation.

If the pump tubing has been used previously, it is recommended that before use of the SIPS, the pump tubing is reconditioned. This is achieved by pumping a solution of 0.01 % Triton X-100 (mass/volume) through the tube for 15 minutes. This procedure can be completed while waiting for the hollow cathode lamp and the burner to warm-up and stabilize. Once this time has elapsed, the SIPS unit is ready for regular operation.

Add a Detergent

To minimize nebulizer blockage from spalling, it is recommended that all SIPS users add Triton X-100 (a readily available laboratory detergent) at a concentration of 0.01% (mass/volume) to the Rinse and Make-up (Diluent) solutions. The Triton X-100 evidently alters the surface of the particles so that the particles do not stick together, but pass through the nebulizer and disappear in the flame.

Summary

The SIPS accessory offers real time-saving and cost-saving benefits to users. Completing the simple procedures described above ensures users can achieve the best performance and the maximum benefit from their SIPS.

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Routine Maintenance for Atomic Absorption Spectrophotometers

Application Note

Atomic Absorption

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Introduction

Instruments in good operating condition are a necessity in any analytical laboratory. This level of integrity can be achieved by a regular maintenance schedule with minimal work. The four main areas of such a program for atomic absorption spectrophotometers include:

- General instrument maintenance
- Gas supply maintenance
- Flame component maintenance
- Furnace component maintenance

The benefits of routine maintenance include:

- Increased instrument lifetime
- Reduced downtime
- Overall improvement in instrument performance; giving the operator greater confidence in the validity of his analytical results

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General Instrument Maintenance

Dust and condensed vapors can accumulate on the instrument case, and corrosive liquids can be spilled on the instrument. To minimize damage, wipe off the instrument with a damp, soft cloth using water or a mild detergent solution. DO NOT USE ORGANIC SOLVENTS. The sample compartment windows and the lamp windows can accumulate dust or fingerprints. In such cases, clean the windows with a soft tissue moistened with a methanol or ethanol and water solution. If the windows are not clean, the operator will observe noisy lamp signals and non-reproducible analytical results.

The remaining optical components are sealed, but they should not be exposed to corrosive vapors or a dusty atmosphere. In laboratories where high concentrations of dust or vapors are unavoidable, schedule a yearly check by a service engineer to maintain the efficiency of optical light transmission in the instrument. There is no need for an operator to clean the sealed optical components.

Gas Supply Maintenance

Three gases are suitable for flame M. Air and nitrous oxide are used as combustion support gases (oxidants). Acetylene is used as the fuel gas. Each gas is supplied to the instrument through piped supply systems and rubber hoses. Copper or copper alloy tubing may be used for the oxidant gases. Acetylene should only be supplied through stainless steel or black iron pipe. Check connections regularly between the supply and instrument for leaks, especially when tanks are changed using a soap solution or commercial leak detector. Check the rubber hoses connected to the instrument for fraying and cracking. In addition, each time a tank is changed, check the regulators and valves for proper operation.

Because potentially toxic gases are used or produced in the flame, it is necessary to use a suitable exhaust system with a minimum capacity of 6 m^3/min (200 cfm). A simple smoke test will indicate if it is functioning properly.

Compressed Air Supply

Air may be supplied to the instrument from cylinders, a house air system, or small compressor. Cylinders are the most expensive source of air, particularly where large amounts are consumed and cylinders must be changed frequently. If compressed air from an in-house supply is used, a filter/regulator assembly must be installed in the input line to the instrument. An acceptable “Air Service Unit” (Part No. 01 102093 00) may be ordered from any Agilent sales office.

Whatever source is used, the supply must be continuous and have a delivery pressure of 420 kPa (60 psi). The air must be clean, dry and oil free. Approximately 50% of all gas unit failures are caused by moisture or other impurities inthe air supply.

Excessive noise in the readout has also been attributed to contaminated air. An air filter assembly is therefore an essential component of the atomic absorption spectrophotometer, and its inclusion in the air supply installation is mandatory. Weekly, check the air filter for particle and moisture accumulation. When necessary, dismantle the air filter assembly and clean the filter element, bowl, and drain valve components. Use the following procedure for dismantling and cleaning the air filters supplied with the instrument.

1. Shut off the air supply and allow the system pressure to bleed off.
2. Unscrew the filter bowl, complete with automatic drain valve.
3. Unscrew the retaining ring and push the drain valve back into the bowl.
4. Unscrew the baffle carefully, and remove the filter and filter shield.
5. Clean the filter bowl, drain valve components, baffle, and filter shield by washing in a solution of soap and water. DO NOT USE ORGANIC SOLVENTS AS THEY WILL DESTROY THE BOWL AND VALVE COMPONENTS. Rinse thoroughly in fresh water.
6. Clean the filter element by washing in ethyl alcohol or similar solvent.
7. Ensure that all components are properly dried before reassembly.

Nitrous Oxide Supply

The nitrous oxide used for atomic absorption spectrophotometry must be oil free. If a heated regulator is not used, loss of regulation can occur due to the expansion cooling effect encountered when nitrous oxide is drawn from a cylinder. This can lead to erratic results and create a potential flashback situation with manual gas control units: An acceptable heated regulator may be ordered from any Agilent sales office. The consumption rate is dependent on the application, but is usually 10–20 liters per minute.

Acetylene Supply

Acetylene is the only combustible gas which is normally used in MS. The gas must be supplied packed in acetone. Some companies supply acetylene packed in proprietary solvents, but unfortunately the disadvantages outweigh the advantages. The major disadvantage is that the solvent may be carried over into the instrument and corrode the internal tubing, causing a potential explosion hazard. Ensure that the acetylene is at least 99.6% pure "M Grade" and packed in acetone.

The delivery pressure must be regulated and never exceed 105 kPa (15 psi). Check the instrument operation manual for the correct delivery pressure for the particular instrument being used. In addition, check the acetylene cylinder pressure daily, and maintain in excess of 700 kPa (100 psi) to prevent acetone from entering the gas line and degrading analytical results or causing damage to the instrument.

Flame Component Maintenance

The flame component section of the instrument can be divided into three areas; the nebulizer, spray chamber and burner. Each requires routine maintenance to assure optimum performance.

Nebulizer

The nebulizer area of the flame component consists of the capillary tubing and the nebulizer body. Always ensure that the plastic capillary tubing used for aspirating solutions is correctly fitted to the nebulizer capillary. Any leakage of air, tight bends, or kinks will cause unsteady, non-reproducible readings.

At times the plastic capillary tubing can become clogged and it will be necessary to cut off the clogged section or fit a new piece of capillary tubing (about 15 cm long). in any event, make sure the plastic capillary tubing fits tightly on the nebulizer capillary. The nebulizer capillary can also become clogged. If this occurs, proceed as follows:

1. TURN THE FLAME OFF.
2. Remove the plastic capillary tubing from the nebulizer.
3. Remove the nebulizer from the bung.
4. Dismantle the nebulizer as described in the instrument operation manual or the instruction manual supplied with the nebulizer.
5. Place the nebulizer in an ultrasonic cleaner containing 0.5% liquid soap solution such as Triton X-100 for 5 to 10 minutes. If the ultrasonic bath fails to clear the blockage, pass a burr-free nebulizer wire CAREFULLY through the nebulizer and then repeat the ultrasonic cleaning procedure.
6. Re-assemble the nebulizer in accordance with the instructions.
7. Install the cleaned nebulizer.

   Replace the plastic capillary tubing.

   If blockages are allowed to build up and are not removed, the analytical signal will steadily drop until no absorbance is observed.
8. Check the nebulizer body, capillary, and venturi occasionally for corrosion. Nebulizer problems can be minimized by taking care to always aspirate 50–500 mL of distilled water at the end of each working day.

Spray Chamber

As the sample leaves the nebulizer it strikes the glass bead and breaks into an aerosol of fine droplets. The efficiency of the glass bead can be degraded by surface cracks, pitting and the accumulation of solid material. The reduction in bead efficiency can cause lower absorbance readings and noisy signals. When removing the nebulizer for inspection, always check the glass bead. Look for pitting, cracks, breakage, ensure that the adjusting mechanism operates properly and that the bead is correctly positioned over the nebulizer outlet (venturi).

While the nebulizer and glass bead are removed from the instrument for inspection, the spray chamber and liquid trap should be removed, dismantled, and cleaned. Discard the liquid in the liquid trap and wash both the spray chamber and liquid trap thoroughly with laboratory detergent and warm water. Rinse completely with distilled water and dry all components. Refill the liquid trap and reassemble the spray chamber, checking for any distortion of O-rings or blockages in the gas inlets. Reconnect the drain hose. If a bottle or jug is used to collect the waste solutions, check that the hose is not below the level of the waste. If the hose is below that level, absorbance readings will steadily decrease with occasional abrupt increases as intermittent drainage of the spray chamber occurs. Therefore, it is necessary to daily check the level of the waste and to dispose of it frequently. This is imperative when using organic solvents because of the potential hazards introduced by flammable liquids. Only wide necked, plastic containers can safely be used to collect the waste solutions.

Burner

The final area of concern in the flame component is the burner. During aspiration of certain solutions, carbon and/or salt deposits can build up on the burner causing changes in

the fuel/oxidant ratio and flame profile, potential clipping of the optical beam, and degradation of the analytical signal. To minimize the accumulation of salts, a dilute solution of acid (HNO_3) may be aspirated between samples. However, if salts continue to build up, turn off the flame and use the brass cleaning strip supplied with the instrument. Insert the strip in the burner slot and move it back and forth through the slot. This should dislodge any particles which will then be carried away once the flame is lit and water aspirated.

DO NOT USE SHARP OBJECTS such as razors to clean the burner as they can nick the slot and form areas where salt and carbon can accumulate at an accelerated rate.

If this type of cleaning is inadequate, remove the burner, invert, and soak it in warm soapy water. A scrub brush will facilitate cleaning. Soaking may also be done in dilute acid (0.5% HNO_3). Ultrasonic cleaners containing dilute non-ionic detergent only are another alternative for cleaning. After cleaning, thoroughly rinse the burner with distilled water and dry before installing in the instrument. NEVER DISASSEMBLE THE BURNER FOR CLEANING. IMPROPERLY RE-ASSEMBLED BURNERS WILL LEAK COMBUSTIBLE GAS MIXTURES, POTENTIALLY CAUSING EXPLOSIONS.

Each day after all analyses are completed, 50–100 mL of distilled water should be aspirated to clean the nebulizer, spray chamber, and burner. This is even more important after aspirating solutions containing high concentrations of Cu, Ag, and Hg, since these elements can form explosive acetylides. The entire burner/nebulizer assembly should be disassembled and thoroughly cleaned after analyzing these types of solutions. The burner should be removed weekly, scrubbed with a laboratory detergent, and rinsed with distilled water.

Furnace Component Maintenance

The graphite furnace accessory maintenance can be divided into three major areas; the gas and water supplies, the workhead, and the autosampler. Each plays an important role in obtaining valid analytical results. The following general maintenance program refers to the GTA-95.

Gas and Water Supplies

Normally the gases used in FAAS are inert gases such as N_2 and Ar. Either one may be used, but must be clean, dry, and of high purity. The regulated pressure should be 100–340 kPa (15–50 psi). At times the incorporation of air may be useful to fully ash a sample. However, air should not be used at ash temperatures higher than 500 °C because of the accelerated rate of graphite component deterioration at elevated temperatures.

The water supply, used to cool the furnace, may be supplied either from a laboratory tap or a cooling-recirculating pump. If a recirculating pump is used the water must be kept below 40 °C. The water used must be clean and free of corrosive contamination. The flow should be 1.5–2 liters/minute. Maximum permissible pressure is 200 kPa (30 psi).

Workhead

The workhead is a closed assembly with quartz windows on either end. Before starting an analysis, check the windows for dust or fingerprints. If needed, clean both sides of the quartz windows with a soft tissue moistened with an alcohol/water solution. Never use coarse cloths or abrasive cleaning agents. While the windows are removed, inspect the gas inlets on the window mountings. If the graphite components have deteriorated extensively, graphite particulates may have dropped into the gas inlets, blocking the proper flow of gas. This will cause further graphite deterioration at an accelerated rate and lead to poor analytical performance. To clean, carefully blow out the particulates with a supply of air. Inspect the inside of the window mountings and clean off any sample residue which may have deposited over time.

In the center of the workhead are the graphite components. At frequent, regular intervals, remove the graphite tube atomizer and inspect the inside of the graphite shield. Ensure that the bore and the injector hole area are free of loose carbon or sample residue. Check the electrodes on either end of the graphite shield for proper tapering. If the tapering is worn or burnt, the electrodes will not make the correct contact with the graphite tubing, causing fluctuations in applied power resulting in irreproducibility. The electrodes also have a series of gas inlets which must be free of loose carbon or sample residue.

Above the graphite shield is the titanium chimney. Injected sample or sample residue from the ash/atomize cycles may deposit in this area. A cotton swab soaked with alcohol can be used to clean both the inside and outside of the chimney. Alternatively, the titanium chimney may be soaked in dilute acid to remove deposits.

Autosampler

The components of the autosampler requiring routine maintenance are the rinse bottle, syringe, and capillary tubing, the proper care of which will minimize contamination and improve reproducibility of analytical results.

Regularly remove the rinse bottle for cleaning. This involves soaking the bottle in 20% HNO_3 followed by rinsing with distilled-deionized water. Refill the bottle with a solution of 0.01–0.05% HNO_3 in distilled-deionized water. The solution

may also include 0.005% v/v Triton X-100 R. The Triton helps maintain the sample capillary in clean condition and assists in obtaining good precision.

At times, graphite particulates may accumulate on the capillary tip and should be carefully removed with a tissue. If these particulates are not removed, the dispensing characteristics of the capillary may change. Contamination of the capillary may become a problem when using some matrix modifiers. In such cases, direct the capillary to a vial containing 20% HNO_3, draw up 70 µL, and stop the autosampler while the capillary is in the vial. After a period of a few minutes, the autosampler RESET should be utilized to rinse out the acid solution. This will clean the internal and external areas of the capillary. Similarly, organic residues can be removed by directing the capillary to a vial of acetone and repeating the above procedure. The PTFE capillary should be treated carefully during cleaning and operation. If bends or kinks appear, it can take time to reshape, and while doing so the repeatability of injection may be degraded. If the capillary tip is damaged, the damaged portion should be cut off at a 90° angle with a sharp scalpel or razor blade.

The final area of the autosampler maintenance schedule is the syringe. Daily, check for bubbles in both the capillary and syringe. Any bubbles in the system can cause dispensing errors and lead to erroneous results. Follow the instructions in the operating manual to free the system of bubbles. If the bubbles continue to cling to the syringe, it may need cleaning. The syringe can be washed with a mild detergent solution and thoroughly rinsed with deionized water. Ensure that contamination is not introduced through the syringe. Be particularly careful not to bend the plunger while washing the syringe.

Conclusion

Attached is a routine maintenance schedule for atomic absorption spectrophotometers (Figure 1). By adhering to this program, the overall integrity of the atomic absorption spectrophotometer can be maintained and the laboratory analyst will reap the benefits of increased instrument lifetime, reduced downtime, and gain greater confidence in the analytical results.

Maintenance Schedule (Flame AA)	
Daily	**Completed**
1. Check Gas	
2. Check Exhaust system with smoke test	
3. Empty the drain receptacle	
4. Clean lamp and sample compartment windows	
5. Rinse spray chamber with 50-100 mL of distilled water	
Weekly	
1. Disassemble spray chamber	
(a) Check glassbead	
(b) Check nebulizer components	
(c) Wash the spray chamber and liquid trap	
(d) Scrub the burner	
(e) Change the liquid in the liquid trap	
(f) Check the O-rings	
2. Check air filter assembly	
3. Wipe off instrument	
4. At Time of Gas Tank Change	
5. Check for leaks	
6. Check for operation of the regulators	
7. Check for operation of the shut off valves	
8. Check the gas supply hoses	
Yearly	
1. Schedule an Agilent service engineer to perform Preventive Maintenance	

Figure 1. Routine maintenance schedule for atomic absorption spectrophotometers.

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Guidelines for Using Non-Aqueous Solvents in Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Author

Jonathan Moffett

Introduction

Much of our environment consists of water. Therefore the bulk of AA methodology deals with water as a solvent. The use of water also has advantages:

- Restricted density range
- Relatively constant viscosity
- Constant specific heat
- Nonflammable
- Transparent in UV and visible region

The relatively constant physical properties allow optimized design of nebulizers, spray-chamber and burner. Background correction is not necessary for many applications.

Some disadvantages of water as a solvent include:

- Potentially corrosive action towards metal
- Dissolved solids levels can be very high
- Flame characteristics affected by cooling

The first can be controlled by careful selection of instrument construction materials. Correct instrument setup (such as glass bead adjustment) can substantially minimize flame perturbation caused by the last two.

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The use of non-aqueous (mainly organic) solvents for AA is necessary for certain applications. These include:

- Solvent extraction of metal chelates
- Direct analysis of petroleum products like oil
- Direct analysis of edible oil products
- Direct analysis of pharmaceuticals

The use of organic solvents introduces many complicating aspects including:

- Wide range of densities
- Differing viscosities
- Flammability
- Major effect on flame stoichiometry
- Relatively low flashpoints
- Effect on plastics
- Irritating and noxious fumes
- Increased care required for safe disposal

This wide range of physical and chemical properties (Table 1) makes it difficult to anticipate all the requirements of a particular application. An instrument used with organic solvents must be more flexible than one used for aqueous solvents. The operator also requires more training, especially with the safety aspects. Materials used to protect an instrument from corrosive aqueous solutions are often attacked by organic solvents. Sometimes expensive alternative materials must be used in instrument construction.

Safety Aspects

Organic solvents generally used in AA include the following:

- Hydrocarbon (kerosene, white spirit, xylene)
- Ketone (MIBK, DIBK)
- Alcohol (butanol)
- Ester (isobutylacetate)

The most widely used solvents are usually either a hydrocarbon or a ketone. Further information may be found in Table 1.

Table 1. Physical Properties of Some Organic Solvents

Solvent	Flash point °C	Boiling point °C	Specific gravity
4-Methylpentan-2-one (MIBK)	22	118	0.79
2-Methylpropan-2-ol	23	148	0.83
m-Xylene	29	139	0.86
Cyclohexanone	34	155	0.95
Kerosene (Jet-A1)	39-74	175-325	0.78
3-Heptanone	46	148	0.82
Shellsol T	50	186-214	0.75
White spirit (Pegasol)	55	179-194	0.76
2,6-Dimethylheptan-4-one (DIBK)	60	166	0.81
Cyclohexanol	68	161	0.96
Tetrahydronapthalene (Tetralin)	71	207	0.76

Note: The flash point is the lowest temperature at which the liquid gives sufficient vapor to form an ignitable mixture with air and to produce a flame when an ignition source is brought near the surface of the liquid.

To varying degrees, all organic solvents are both flammable and toxic. The use of organic solvents requires great care.

Organic solvents should be kept in glass bottles. The bottles should be stored in a metal cabinet or in a separate storage area well away from flames and other ignition sources. When using solvents only a relatively small quantity (less than 2 L) should be open to the atmosphere at any one time. In addition most countries have legislation which applies to the storage and handling of flammable liquids. These legal aspects must also be considered.

Prolonged exposure to organic solvent fumes is a health risk. All work with them should be carried out in a fume cupboard which has adequate venting. Samples not being analyzed should be covered. If a sampler is used, it should be placed in an venting system which removes the vapors from the area.

There is always a risk of fire from fumes reaching the flame and adequate ventilation must be provided for the instrument itself. These vapors also absorb ultraviolet radiation and if present in the sample beam light path, can cause a significant background signal.

The plastics materials and paints used in the instrument and its accessories should be protected from direct contact with any solvents. Nearly all plastics except fluorinated plastics are affected to some degree by organic solvents and will swell and distort. Instrument parts are made to close tolerances and such changes may cause malfunctions. Generally if allowed to dry thoroughly these parts will return to their original shape.

A plastic waste container must be used for the instrument wastes. A flashback may shatter a glass waste container with potentially dangerous results. The waste container must be emptied often. All wastes including those from the instrument must be stored in approved containers. Legislation should be consulted for proper disposal of all waste liquids.

The following should never be used as solvents for AA (especially flame):

- Halogenated hydrocarbons (chloroform, Freon)
- Very low boiling point hydrocarbons (petroleum spirit)
- Ethers and acetone
- Tetramethylfuran (TMF)
- Dimethylsulphoxide (DMSO)

Halogenated hydrocarbons are toxic. If aspirated into a flame, even more dangerous gases (phosgene is the most common) are produced.

The other solvents in the list are extremely hazardous in the vicinity of a naked flame because they are volatile. Some are so flammable that they could support a spectrometer flame without acetylene.

Standards

Atomic absorption spectrometric measurement and calibration is based on comparison. Care is needed in preparing standards to obtain accurate results. The amount of care and time needed depends on how accurate the results must be.

Aqueous standard solutions are not generally suitable to calibrate an instrument for organic work. Hydrated metal cations in water have different physical and chemical properties to metallo-organic compounds in an organic solvent.

Metal compounds soluble in organic solvents are commercially available. These can either be dry powders or else dissolved in a matrix oil.

The oil-based standards are easy to use. Single element standards can be weighed out and blended together. This multi-element standard can then be weighed into a clean base matrix. If it is not known whether the base matrix is free of the analyte of interest, then the calibration should be treated as a standard additions calibration. This prepared standard is then diluted by an organic solvent to give a working standard to calibrate the instrument. This approach allows the matrix and concentration range to be adapted to specific requirements. Companies such as Conostan (Ponca City, OK USA) and National Spectrographic Laboratories (Cleveland, OH USA) offer a range of single and multi-element standards that only need dilution to the required levels. Most countries have agents who represent these companies.

The dry standards are typically the cyclobutyrate salts of most metals. The powders are stable and can be stored for long periods. Dissolving the powders can be time consuming and may require two or three liquids. Once dissolved, they may be used in the same way as the oil-based standards. Chemical companies supplying atomic absorption standards also offer the dry powder standards.

Some ways of checking standards accuracy and instrument calibration are:

- Recovery studies
- Measure reference materials
- Inter-laboratory studies

A recovery study is done by spiking a sample with a known amount of standard. The absorption of the sample and spiked sample are measured and the respective concentration calibrated. Percent recovery is calculated by the following equation (US EPA abbreviations are used):

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

where:
SSR = spiked sample result
SR = sample result
SA = spike added

Reference materials are check samples which have accurately known compositions. There are organizations which supply reference materials. A list of these is given in later in this document. Consult their catalogs for further information. Reference materials should be treated in the same way as the other samples. A measured result should be within experimental error of the certified result. These materials could also be used as calibration standards. This is not recommended for two reasons:

- Cost is very high
- Calibration standards and quality control (QC) samples should have different sources to reduce systematic errors

Inter-laboratory studies require the cooperation of laboratories doing the same type of analyses. A sample is divided among the laboratories and measured. The results are all collated and compared. When done as a long term project, this method can monitor a laboratory's performance and allows any necessary remedial action to be taken.

Calculations

Units

Concentration of oil standards are generally expressed as µg/g or ppm (mass).

For solutions presented to the instrument for aspiration, the range is generally in mg/L or ppm (volume).

The term ppm (parts per million) in particular must be very carefully defined. An oil standard may contain 500 µg/g of the element of interest. If diluted 1:10, the solution contains 50 mg/L. To allow direct comparison of oil samples, the concentration of the standard can be entered as 500 in the instrument software. However, when comparing absorbances with other studies, it must be remembered that the solution concentration is 50 mg/L. The unit part per million (ppm) is therefore somewhat ambiguous and will not be used in this discussion.

Dilution

Very often organic samples cannot be presented directly to an instrument's nebulizer. For example an oil sample is too viscous to be aspirated directly without dilution. A gasoline sample is too flammable to be used with a flame instrument. These must be diluted in a suitable miscible liquid. Dilution must be done to allow meaningful measurement of the analyte in question. A 1:5 or 1:10 dilution is usually appropriate for the determination of copper or iron in used oil analysis. The determination of zinc or sodium may require a greater dilution and/or selection of a suitably sensitive resonance line. Burner rotation may also be necessary to reduce sensitivity.

Remember that when the sample has been diluted, the analyte concentration must be carefully defined. It must be very clearly stated whether the concentration refers to the analyte in the original sample or in the diluted solution.

Some examples of typical dilutions are given below.

Case 1: Preparation of oil standards using an oil-soluble metallo-organic salt.

Mass (in grams) of salt to be weighed out, m, can be calculated by equation 1.

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

where M is mass of oil standard required (g)
C is concentration of analyte in oil (µg/g))
P is percent analyte in salt

Example 1: Prepare a 500 µg/g Si standard in 100 g oil. The silicon was assayed at 14.29% in the salt. Using equation 1,

$$\text{mass salt} = \frac{100 \times 500}{10{,}000 \times 14.29} = 0.3499\ \text{g}$$

Method: Weigh out 0.3499 g salt. Dissolve in xylene and organic solubilizers (refer to the instructions provided by the chemical supplier) with warming. Add 80–90 g warm base oil with stirring. Cool. Make up to 100.00 g.

Case 2: Preparation of an oil standard using an oil dissolved standard and clean base oil.

Mass of oil standard (in grams) to be weighed out, m, can be calculated by equation 2.

$$\text{mass oil standard} = \frac{M\ C}{S}\ \text{grams} \qquad (2)$$

where M = mass of standard to be prepared
C = concentration of analyte required
S = stock oil concentration

Example 2: Prepare 10 g of multi-element oil containing 120 µg/g Cu and 300 µg/g Al starting with 5000 µg/g standards.

Using equation 2,

$$\text{Cu}\quad m = \frac{10 \times 120}{5000} = 0.2400\ \text{g} \qquad \text{Al}\quad m = \frac{10 \times 300}{5000} = 0.6000\ \text{g}$$

Method: Weigh out 0.2400 g of the copper standard and 0.6000 g of the aluminium standard. Dissolve in about 8–9 g of warm base oil. Cool. Make up to 10.000 g.

Case 3: Prepare 20 g of a standard to analyze an oil sample with less than or equal to 1.5% Zn.

In this case, there are two possible methods. One method is to make up a standard from the cyclobutyrate salt (assayed at 16.18% Zn) as shown in Case 1.

Method 1: 1.5% Zn = 1.5 × 10,000 µg/g Zn
From equation 1: $m = \frac{20 \times 1.5 \times 10\ 000}{10{,}000 \times 16.18} = 1.854$ g

Dissolve the salt in xylene and organic solubilizer as recommended by the chemical supplier. Add about 18 g warmed clean base oil with stirring. Make up to 20.000 g.

To reduce the amount of diluent required, the 307.6 nm resonance line could be used in this analysis. A 1:5 or 1:10 dilution

would be sufficient. Note that the signal to noise ratio for the 307.6 line is not as good as the 213.9 line, but would still give acceptable results.

Another method is to use a variation of Case 2 and make up a standard from a more easily handled oil-based standard. However the sample (15 000 µg/g) is more concentrated than the standard (usually 5 000 µg/ g). So this method uses a different dilution for the sample compared to that for the standard. If the very sensitive 213.9 nm zinc line is used, then a 1:10 000 dilution of sample is necessary to obtain about 1.5 mg/L. Such a large dilution would mean that the sample solution would have almost the same physical properties as the solvent.

If a 5000 µg/g standard is used, a 150 µg/g working standard can be made which only has to be diluted 1:100. At a 1:100 dilution the physical properties of the standard solution would also be similar to the solvent.

Method 2:

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

Weigh out the oil standard. Add about 12 g warm clean base oil with stirring. Cool. Make up to 20.000 g.

Dilute the sample by weighing out 1.000 g and dissolving in 100 mL solvent solution. Pipette out 1 mL of the solution and make up to 100 mL. This is the solution to be analyzed.

Dilute the standard by weighing out 1.000 g and dissolve in 100 mL solvent solution. This standard is equivalent to 1.5% Zn in the original oil sample.

Ionic Suppression

A nitrous oxide-acetylene flame is recommended for the measurement of the Group II elements (magnesium, calcium, strontium, barium). Under these conditions, the analytes are partially ionized and require the use of an ionization suppressant for their accurate measurement. An organic soluble potassium or sodium salt is added to the standards and samples to give a final concentration of 2000–5000 ppm. The salts are either napthenates, sulphonates or cyclobutyrates.

A branched capillary to aspirate an ionization suppressant and sample simultaneously has been described [1] and it has been claimed to work with organic samples. This has not yet seen wide application.

Hardware

Spraychamber: Check that the components are resistant to solvent attack and do not distort. Removable components should be checked to ensure they are not binding or tight.

O-Rings: Inspect these frequently. KALREZ O-rings are resistant to solvent attack and are available as sets.

Liquid Trap: This should be filled with the liquid being aspirated or a liquid miscible with the solvent being aspirated.

It is recommended that the spraychamber and liquid trap be dismantled and cleaned at the end of each working day. Wash with hot water and detergent or acetone and allow to dry. Reassemble while checking the O-Rings.

Nebulizer: An adjustable nebulizer which allows control of the uptake rate is necessary. The uptake can be continuously varied from zero up to about 10 mL/min.

An adjustable nebulizer does not have a thimble like the standard preset nebulizer. Instead it has a housing with an uptake control. Refer to the instructions on initial setup.

Setting the correct uptake rate should be done using an air-acetylene flame and the selected solvent:

1. Check nebulizer is set for zero uptake rate
2. Light flame and adjust gas flows to give a very lean flame
3. Place capillary in solvent
4. Slowly rotate uptake control clockwise until flame is beginning to become fuel-rich (some yellow may be seen)
5. Measure and record uptake

   Generally, MIBK, DIBK and xylene - 2 mL/min white spirit, kerosene - 4 mL/min. The nitrous oxide-acetylene flame can tolerate higher uptake rates (MIBK - 6 mL/min).

   A high uptake rate is not desirable for a number of reasons: the flame may be extinguished between samples because of insufficient fuel; the risk of background and inter-element interferences is increased; the gains in signal are usually not significant enough.

Burner: An air-acetylene burner should only require periodic cleaning. The use of organic solvents however increases the possibility of carbon buildup with the nitrous oxide-acetylene flame. More frequent cleaning of the nitrous oxide-acetylene burner may be needed.

A carefully cleaned burner gives the best performance and

reduces salt blocking and carbon build-up. The use of a brass strip is no longer recommended. Studies revealed that a metal strip does not clean sufficiently well and that it does not polish the jaws [2]. For optimum performance, any burner should be cleaned as follows:

1. Use a card (for example, business card) and a brass polish (for example, "Brasso")
2. Wet card on both sides with polish
3. Slide card into slot
4. Move card up and down to polish inside of burner jaws
5. Rub card along top of slot
6. Scrub with a soft nylon brush (for example, toothbrush) using hot water and detergent
7. Use ultrasonic bath if available
8. Rinse with hot running water
9. Rinse with distilled water
10. Allow to dry or use a card to remove water from inside slot

Background correction: The organic nature of the matrix means that UV absorption is significant. Background correction is more likely to be required for most elements. Background studies are recommended to determine if correction is needed.

Programmable Gas Box: The sample uptake rate affects the flow of oxidant through the nebulizer into the spraychamber. At low sample uptake rates in the air-acetylene flame, the oxidant flow must be set somewhat higher than the default 13.0 L/min. It is suggested the flow should be about 19 L/min.

Graphite Furnace Operation

Many of the practical precautions of flame are not needed for graphite furnace operation. For example the fire potential is greatly reduced because there is no naked flame and the volumes involved are very small. However some precautions are still necessary. Guidelines for handling, storing and disposing organic solvents must still be observed.

The chemical nature of the metallo-organic compounds means that organic standards may still be required for calibration.

The solvent used for dilution should not be too volatile. A furnace run can take a long time. The solution concentrations could be affected because of evaporation. The ketones (MIBK and DIBK) are probably the most suitable general purpose solvents for furnace work. They are miscible with many organic compounds and solvents. DIBK is also immiscible with water.

The organic phase is very mobile. When injected into a furnace, this mobility may cause more spreading than is desirable. To control droplet spreading in the furnace, a partition graphite tube should be used. Some analyzes of volatile elements like lead and cadmium may require the use of a platform [3]. The platform controls droplet spreading provided no more than about 20 mL is injected. For both types of atomization (wall and platform), the hot injection facility can also be used to control spreading. For example, using DIBK as a solvent the inject temperature on the sampler page can be set to 130 °C and the injection rate slowed down to 5. This facility also helps shorten the time needed to dry the injected solution and allows faster furnace cycles [4].

The solution in the rinse bottle of the sampler does not have to be organic. The rinse solution can be distilled water with 0.01% nitric acid and 0.1% Triton X-100 (a non-ionic detergent)3. If the samples are such that the dispenser tip is not being cleaned, a slightly higher concentration of Triton X-100 may be tried. A small amount (0.5 - 1%) of propan-2-ol in the rinse solution as well can assist with keeping the tip free of grease and oil.

Safety Checkpoints

Choose a Suitable Solvent Which Has the Following Properties

- Miscible with sample
- Uitably high flashpoint
- Density greater than 0.75
- No toxic by-products formed

Handling Solvents

- Use small volumes near instrument
- Keep solutions covered when not in use
- Do not inhale vapors
- Empty waste vessel often
- Use fume cupboard for solution preparation
- Dispose of all wastes carefully and responsibly
- Do not mix with nitric or perchloric acids or wastes

Instrument

- Fill liquid trap with suitable solvent before starting
- Attach tube to spraychamber vent and allow other end to vent safely away from flame
- Install an efficient exhaust system above instrument
- Keep burner clean
- Do not clean burner while flame is on
- Drain liquid trap at the end of each day
- Wash spraychamber and allow to dry overnight; check condition of O-rings often

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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

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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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Agilent Oil Analyzer: customizing analysis methods

Application Note

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Introduction

Traditionally, the analysis of used oils has been conducted by physical and wet chemical methods. FTIR spectroscopy has become a routinely used technique to analyze used oils, providing the following major advantages[1]:

- Ability to simultaneously determine several parameters from a single experiment
- Increase in speed of analysis
- More cost effective than traditional techniques
- Mobility and portability allowing remote on-site analysis

The Agilent FTIR Oil Analyzer is designed to meet the requirements of the US Department of Defense Joint Oil Analysis Program (JOAP)[2] for use in their condition monitoring program as well as commercial applications. It is optimized for monitoring relative changes in various indicators of oil conditions (oil failure symptoms) using a standardized protocol developed by the Joint Oil Analysis Program Technical Support Center (JOAP-TSC). This protocol sets the data extraction algorithm for several types of petroleum and synthetic-based lubricants and hydraulic fluids, and eliminates the need for reference samples as spectral subtraction is no longer required.

The Agilent Oil Analyzer software allows users to readily customize existing methods as well as create new methods to measure other parameters and properties of lubricants defined by the user. The methods can be easily adjusted for performing analysis of samples where spectral subtraction is required.

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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:

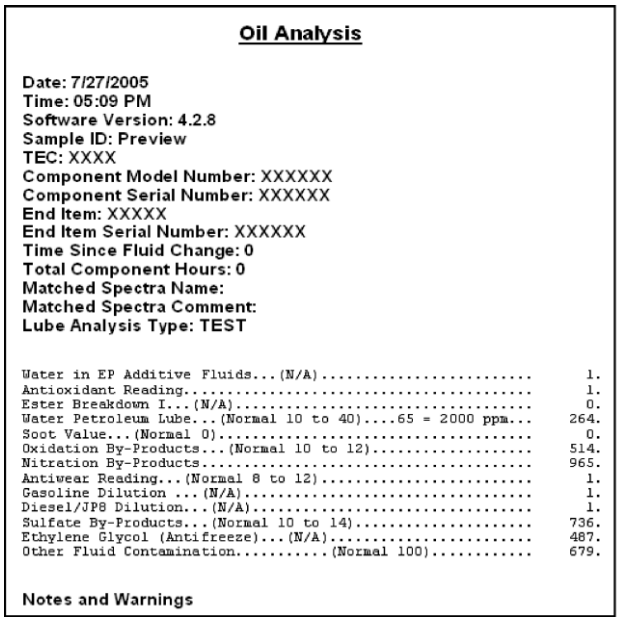
- Aircraft hydraulic (Mil-H-83282)
- Aircraft hydraulic (Mil-H-83282_350 ppm limit for water)
- Dextron transmission fluid
- Engine crankcase (Diesel_gasoline_natural gas)
- Fire retardant hydraulic (Mil-H-46170)
- Gas turbine or Helo Gbx (Mil-L-23699)
- Ground equipment hydraulic (Mil-L-2104_10W)
- 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/JP8 Dilution...(N/A)	1.
Sulfate By-Products...(Normal 10 to 14)	736.
Ethylene Glycol (Antifreeze)...(N/A)	487.
Other Fluid Contamination.....(Normal 100)	679.

Notes and Warnings

Figure 1. Typical standard Oil Analysis report

Calibration

All analysis methods in the Agilent FTIR Oil Analyzer consist of a set of calibration models (procedures) in the form of corresponding files with an indication of the calibration model's type (univariate, or multivariate, or a combination). The analysis method may be composed of one or several calibration files.

The construction of calibration models in quantitative spectral analysis is a two-step procedure: calibration and validation. In the calibration step, indirect instrumental measurements (spectra) are obtained from standard samples in which the value of the parameter of interest has been determined by a standard reference method (an accurate direct measurement method). The set of spectra and results from the reference method, referred to as the calibration set or training set, is used to construct a model that relates parameter values to the spectra. Before the calibration model is accepted and used for prediction, it should be validated by a set of independent (not used in the calibration set) samples of known parameter concentrations (validation set). If parameters from the validation set fall within acceptable accuracy limits using the model derived from the calibration set, an acceptable model has been constructed that can be used to predict for new "unknown" samples.

To build a univariate calibration model, it is necessary to specify a single measurement from a spectrum, such as peak area or height that demonstrates the most distinctive spectral response for the parameter of interest. The univariate calibration and prediction procedures are available as a standard part of Resolutions/Resolutions Pro software and are defined as a simple quantitative analysis. The analysis is described in detail in the Resolutions online help and the corresponding system reference manuals for previous software versions (Win-IR Pro and Merlin). The user must generate a quantitative calibration document and save it as *.BSQ file using Resolutions/Resolutions Pro (Win-IR Pro or Merlin) software.

Where spectral responses attributed to different parameters overlap and the selective spectral measurements for the parameter of interest is very difficult, univariate models may not be reliable. Multivariate methods such as Principal Component Regression (PCR) and Partial Least Squares (PLS) allow multiple responses at the selected wavenumbers to be used. These methods are better suited to extracting spectral information where bands overlap and it is difficult to discern the relevant spectral regions attributable to a particular parameter. The main advantage of multivariate methods is the ability to calibrate for a parameter of interest when it correlates in a complicated (non-specific) way with multiple spectral regions, while minimizing background matrix interferences in the lubricants.

The Agilent Oil Analysis software allows multivariate calibration models created with the use of third party software to be incorporated in analysis methods. The PLSplus IQ package available as an additional application in the Galactic GRAMS/AI (GRAMS/32) software suite must be used. The "PLSplus IQ User's guide" gives step-by-step instructions on how to construct and validate a multivariate calibration model

as well as theory of advanced statistical analysis in spectroscopic quantitative analysis. The user must build an accurate calibration model and save it into a *.CAL file using PLSplus IQ.

The validity of empirically-built calibration models depends heavily on how well the standard samples (calibration set) represents the unknown samples to be analyzed (prediction set). In all cases, the selection of standard samples to be used for calibration must adequately cover the expected range of measurement parameters in the prediction set. This means that the expected extreme values for each parameter of interest in unknown samples must be included in the calibration set, as extrapolation outside the calibrated value range can be unreliable. It is important to ensure that any phenomena that influence the spectral measurements (e.g., not only the total amount of soot but its particle size distribution) also vary in the calibration set over ranges that span the levels of the phenomena occurring in the prediction set. It is also very important to minimize the errors in the standard sample parameters that are used to construct the empirical calibration model, as any calibration model can only be as accurate as the reference measurements from which it was constructed.

Many conditions can affect the results obtained from FTIR lubricant monitoring such as lubricant type, engine type, operational conditions, environmental conditions, etc. When the conditions are changed significantly, new calibration models and methods may be required to ensure accurate prediction of oil properties. For instance, new calibrations may be required when a new oil type with a different base stock and additive chemistries comes for the analysis.

Care must be taken when measuring overall oil quality parameters such as Total Acid Number (TAN) and Total Base Number (TBN) using FTIR spectroscopy. The secondary formation of acidic products in lubricants is characterized by TAN or indirectly by TBN, which assesses the consumption of basic reserve additives in the oil. While the various acids or bases present in a lubricant could, in principle, be individually quantified based on their characteristic absorption bands, no unique absorption bands can be directly related to TAN or TBN. Thus, only indirect FTIR spectroscopic methods for TAN and TBN have been standardized to date. In addition, there is a large discrepancy in new lubricant TAN values, from less than 0.1 mg KOH/g for R&O type oils to 9 or higher for some synthetic oils in industrial applications. On the other hand, the incremental decrease in TBN used to indicate that a product is failing, varies in broad ranges: some oils may have a new TBN value of 12, but rapidly decrease to a value of 3, whereas other synthetic oils may have the beginning TBN of 40.

A calibration model for TBN is currently available in Agilent Oil Analyzer. The calibration is intended for prediction of the values in gasoline and diesel engine oils having typical baseline numbers not higher than 12 mg KOH/g.

Note that in many individual cases, in order to estimate TAN and TBN satisfactorily the user needs to construct a multivariate calibration model that would cover the higher range of values as well as take into account any other factors that could influence the accuracy and the reproducibility of spectral measurements.

Method editor

Once the univariate or multivariate calibration models are built, the corresponding *.BSQ or *CAL files must be moved or copied into the directory C [Local Disk]:\ Program Files\Varian\Resolutions\Oil Analyzer\Methods. This is the storage location for the available calibration and method files. Then, log in as Administrator to the Agilent Oil Analysis software and enter the Method editor. Follow the Chapter 11 “Method Editor” in “Agilent Oil Analyzer operational manual” to incorporate the calibrations to an existing method or to develop a new method.

Note that spectral subtraction is available in the Agilent Oil Analyzer but was not utilized in JOAP protocol. It is not considered to be practical in view of the deployability aspect of many JOAP laboratories and that the required sample volume would increase because of the necessity of new oil samples to act as references. In order to apply the spectral subtraction procedure, the user needs to select "Use spectral subtraction" option in the Sampling method group in the General option dialog and edit the relevant analysis method, by clearing the "Zero less than Zero" check box in all the associated calibration models. Refer to Chapter 4 "General Options–Setup" and Chapter 11 "Method Editor" of "Agilent Oil Analyzer operational manual" for more information.

Conclusion

FTIR spectroscopy has been gaining increased acceptance as a method of choice for used oil analysis. Designed and optimized as a complete system for predictive maintenance programs, according to JOAP standards, the Agilent FTIR Oil Analyzer combines specific capabilities with the flexibility to be successfully used in any oil analysis laboratory.

The Oil Analyzer software allows new and improved analysis methods to be built and ensures that new types of lubricating oils and fluids used in a variety of different machinery are timely and reliably monitored and tested.

The software allows the user to include PCR/PLS methods to measure oil parameters and convert the units of spectral absorbance into physical results (ppm, wt.%, cSt, mg KOH/g oil, etc.) applying spectral subtraction if needed.

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[3] Available from www.astm.org

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Infrared imaging – the evolution of infrared microscopy

Application Note

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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 ~20 µm 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).

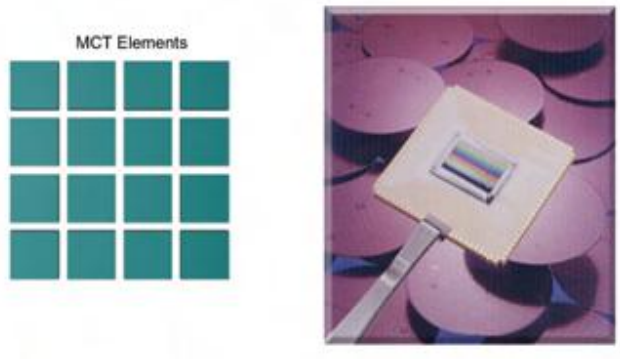

Figure 1. The Focal Plane Array detector: square grid of MCT detector elements (left) and finished FPA detector assembly (right)

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.

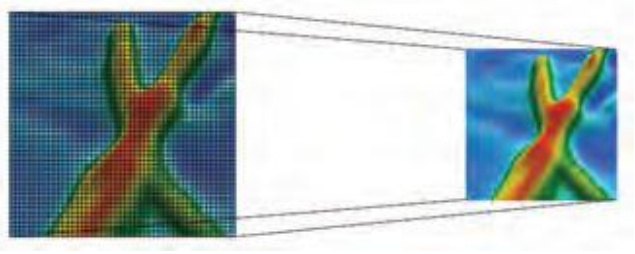

Figure 2. The sample is imaged on to a detector array, with each detector pixel collecting a full infrared spectrum

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.

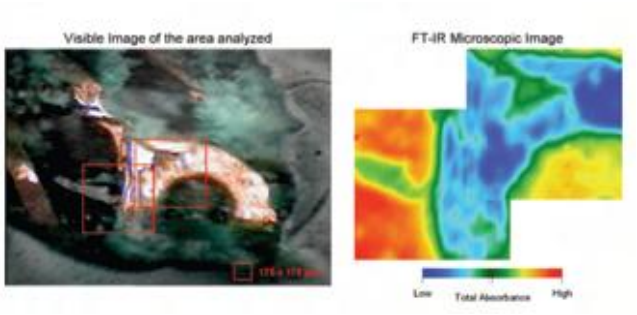

Figure 3. Defect on a metal surface: visible image (left) and total infrared image (right) of the sample

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 8cm^{-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.

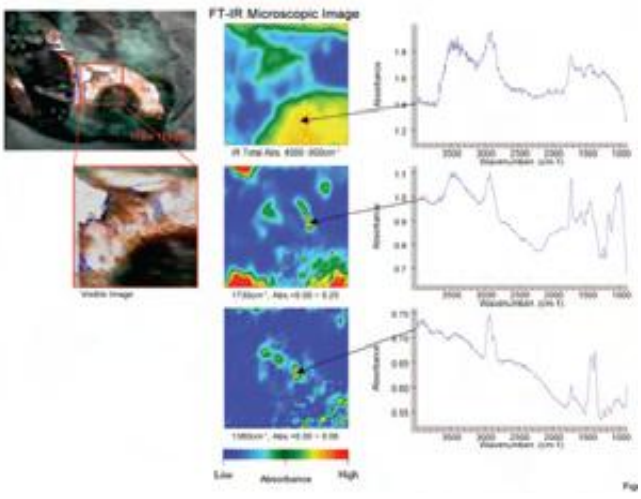

Figure 4. Defect on a metal surface: a complete infrared spectrum is recorded at each point on the detector array. From these individual spectra, functional group images (centre) can be generated

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.

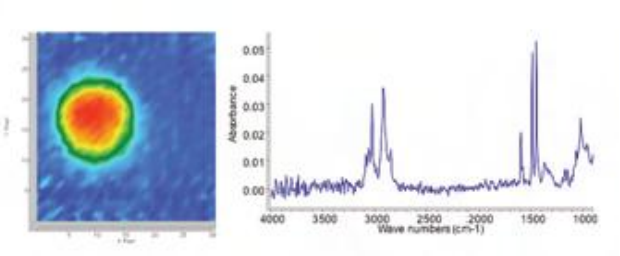

Figure 6. ATR image of polystyrene bead confirming chemical identity (left – total IR image; right – spectrum of bead centre region) and diameter (bead is 13 pixels wide, equating to 18.2 µm).

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

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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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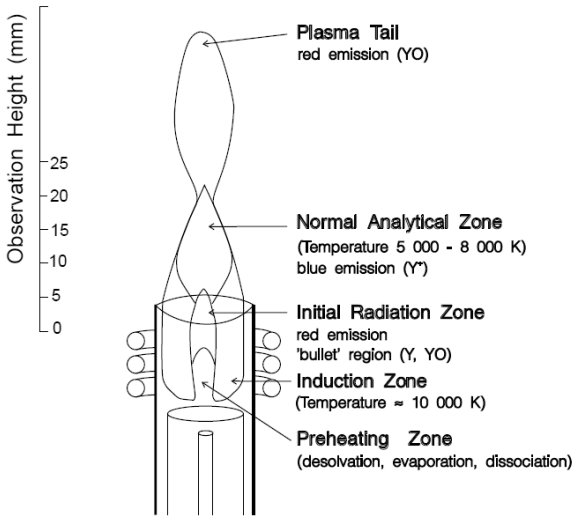

Figure 1. A plasma used for emission spectrometry. The regions refer to those seen when a Yttrium solution is introduced.

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 Short term Long term (over 8 hrs)	0.3 – 2% Less than 5%	0.1 – 1%	0.5 – 5%
Interferences			
Spectral	Many	Virtually none	Minimal
Chemical	Virtually none	Some	Many
Ionization	Minimal	Some	Minimal
Operating costs	High	Low	Relatively high
Combustible gases	No	Yes	No

Table 1. Guide to ICP/AAS Analytical Values

				ICP	Flame AA			Zeeman Furnace AA			
Element		AA λ (nm)	ICP λ (nm)	Detection limit µg/L	Characteristic conc µg/L	Detection limit µg/L	Flame type	Characteristic** conc µg/L	Mass pg	MSR %	EI
Silver	Ag	328.1	328.068	3	30	2	Air	0.035	0.7	97	Ag
Aluminium	Al	309.3	167.081	1.5	800	30	N_2O	0.25	5	100	Al
Arsenic	As	193.7	188.985	12	500	300	N_2O	0.5	10*	86	As
Gold	Au	242.8	267.595	5.5	100	10	Air	0.22	4.4	94	Au
Boron	B	249.8	249.773	1.5	8000	500	N_2O	43	855*	70	B
Barium	Ba	553.6	455.403	0.07	200	20	N_2O	0.85	17	100	Ba
Beryllium	Be	234.9	313.042	0.2	15	1	N_2O	0.025	0.5	64	Be
Bismuth	Bi	223.1	223.061	12	200	50	Air	0.45	9	88	Bi
Bromine	Br		163.340	6000							Br
Carbon	C		247.856	65						–	C
Calcium	Ca	422.7	393.366	0.03	10	1	N_2O	0.03	0.6	94	Ca
Cadmium	Cd	228.8	228.802	1.5	10	2	Air	0.01	0.2*	87	Cd
Cerium	Ce	520.0	418.660	7.5	100000	100000	N_2O			–	Ce
Chlorine	Cl		725.665	200000						–	Cl
Cobalt	Co	240.7	228.616	5	50	5	Air	0.21	4.2	98	Co
Chromium	Cr	357.9	267.716	4	50	6	N_2O	0.075	1.5	100	Cr
Cesium	Cs	852.1	455.531	3200	20	4	Air	0.55	11	58	Cs
Copper	Cu	324.7	324.754	2	30	3	Air	0.3	6	84	Cu
Dysprosium	Dy	421.2	353.170	0.3	600	30	N_2O	2.3	45	100	Dy
Erbium	Er	400.8	337.271	0.7	500	50	N_2O	5	100	100	Er
Europium	Eu	459.4	381.967	0.3	300	1.5	N_2O	1.3	25	100	Eu
Iron	Fe	248.3	259.940	1.5	50	6	Air	0.06	1.2	97	Fe
Gallium	Ga	294.4	417.206	6.5	800	100	Air	0.23	4.5*	80	Ga
Gadolinium	Gd	368.4	342.247	2.5	20000	2000	N_2O			–	Gd
Germanium	Ge	265.1	265.118	13	1000	200	N_2O	0.45	9*	100	Ge
Hafnium	Hf	307.3	264.141	4	10000	2000	N_2O			–	Hf
Mercury	Hg	253.7	184.950	8.5	1500	200	Air	7.5	150*	69	Hg

*Modifier used to obtain these results.

**20 µL injection

***The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Table 1. Guide to ICP/AAS Analytical Values (continued)

Element		AA λ (nm)	ICP λ (nm)	ICP Detection limit µg/L	Flame AA Characteristic conc µg/L	Flame AA Detection limit µg/L	Flame type	Zeeman Furnace AA Characteristic** conc µg/L	Zeeman Furnace AA Characteristic*** Mass pg	MSR %	EI
Holmium	Ho	410.4	345.600	0.5	700	40	N_2O			–	Ho
Iodine	I		178.276	60							I
Indium	In	303.9	325.609	18	150	40	Air	0.35	7.0*	100	In
Iridium	Ir	208.9	224.268	3.5	800	500	Air	6.8	135	97	Ir
Potassium	K	766.5	766.490	10	7	3	Air	0.02	0.4	90	K
Lanthanum	La	550.1	379.478	0.02	40000	2000	N_2O			–	La
Lithium	Li	670.8	670.784	0.6	20	2	Air	0.2	4	49	Li
Lutetium	Lu	336.0	261.542	0.05	7000	300	N_2O			–	Lu
Magnesium	Mg	285.2	279.553	0.1	3	0.3	Air	0.01	0.2	75	Mg
Manganese	Mn	279.5	257.610	0.3	20	2	Air	0.03	0.6	92	Mn
Molybdenum	Mo	313.3	202.030	4	300	20	N_2O	0.35	7	96	Mo
Nitrogen	N		174.272	50 000							N
Sodium	Na	589.0	588.995	1	3	0.2	Air	0.005	0.1	92	Na
Niobium	Nb	334.9	309.418	4	20000	2000	N_2O			–	Nb
Neodymium	Nd	492.5	401.225	2	6000	1000	N_2O			–	Nd
Nickel	Ni	232.0	231.604	5.5	70	10	Air	0.24	4.8	98	Ni
Osmium	Os	290.9	225.585	5	1000	100	N_2O			–	Os
Phosphorous	P	213.6	177.499	18	120000	40000	N_2O	110	2200*	69	P
Lead	Pb	217.0	220.353	14	100	10	Air	0.28	5.5	92	Pb
Palladium	Pd	244.8	340.458	7	50	10	Air	0.43	8.6	100	Pd
Praseodymium	Pr	495.1	417.939	0.8	20000	10000	N_2O			–	Pr
Platinum	Pt	265.9	265.945	20	1000	100	Air	3.5	70	82	Pt
Rubidium	Rb	780.0	780.023	35	50	10	Air	0.05	1	90	Rb
Rhenium	Re	346.1	227.525	11	8000	1000	N_2O			–	Re
Rhodium	Rh	343.5	343.489	5	100	5	Air	0.4	8	95	Rh
Ruthenium	Ru	349.9	267.876	5.5	400	100	Air	0.75	15	100	Ru
Sulphur	S		180.734	20						–	S
Antimony	Sb	217.6	217.581	18	300	40	Air	0.5	10	96	Sb
Scandium	Sc	391.2	361.384	0.4	300	50	N_2O			–	Sc
Selenium	Se	196.0	196.026	37	1000	500	N_2O	0.7	14*	92	Se
Silicon	Si	251.6	251.611	5	1500	300	N_2O	0.75	15	100	Si
Samarium	Sm	429.7	442.434	7	6000	1000	N_2O			–	Sm
Tin	Sn	235.5	242.949	15	700	100	N_2O	0.5	10*	93	Sn
Strontium	Sr	460.7	407.771	0.02	40	2	N_2O	0.1	2	94	Sr
Tantalum	Ta	271.5	268.517	9	10000	2000	N_2O			–	Ta
Terbium	Tb	432.7	350.917	5	7000	700	N_2O	0.18	3.5	90	Tb
Tellurium	Te	214.3	214.281	27	200	30	Air	0.45	9*	93	Te
Thorium	Th		274.716	17						–	Th
Titanium	Ti	364.3	334.941	0.6	1000	100	N_2O	2.5	50	100	Ti
Thallium	Tl	276.8	351.924	16	200	20	Air	0.75	15	63	Tl
Thulium	Tm	371.8	346.220	1.5	300	20	N_2O			–	Tm
Uranium	U	358.5	385.958	18	100000	40000	N_2O			–	U
Vanadium	V	318.5	309.311	2	700	100	N_2O	1.1	22	79	V
Tungsten	W	255.1	239.709	17	5000	1000	N_2O			–	W
Yttrium	Y	410.2	371.030	0.2	2000	200	N_2O			–	Y
Ytterbium	Yb	398.8	328.937	0.3	60	4	N_2O	0.15	3	97	Yb
Zinc	Zn	213.9	213.856	0.9	8	1.0	Air	0.0075	0.15	92	Zn
Zirconium	Zr	360.1	339.198	1.5	9000	1000	N_2O			–	Zr

*Modifier used to obtain these results.

** 20 µL injection

*** The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Analytical Requirements

Before deciding which technique is appropriate, the chemist must define both present and future analytical requirements. That is:

- Number of samples/week?
- What matrices need to be analyzed? For example, steels, bronzes, effluents, soils.
- How many elements need to be determined for each sample type?
- What are the typical sample volumes?
- What elements need to be determined?
- What concentration ranges are present in the matrices?
- Would an Internal Standard be useful? For example, where the samples may change in viscosity from sample to sample, for example, battery acid analysis.
- What expertise do the operators have?
- How much money is available to purchase or lease costs/month?
- Cost of ownership and running costs. Can the user afford an automated AAS or ICP-ES, or is a simple AAS sufficient?

The answers to these questions will help you to decide which is the preferred technique. Sometimes the answer is further complicated by the fact that neither flame AAS nor ICP-ES will satisfy all requirements. You may find, as many do, that both an ICP-ES and a furnace AAS will be necessary to meet the analytical requirements.

For Deuterium Furnace systems, the equivalent Characteristic Concentration and Characteristic Mass is easily calculated using the following conversion:

CMn = CMz X MSR (%)/100 CCn = CCz X MSR (%)/100

where:

CMn = Characteristic Mass for Deuterium Furnace Systems

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

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

CCn = Characteristic Concentration for Deuterium Furnace Systems

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

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Improving Throughput for Oils Analysis by ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

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Introduction

Trend analysis of wear metals in lubricating oils is a proven, cost-effective predictive maintenance technique. The presence and levels of various metal elements in lubricating oils gives an indication of the type of wear occurring in an engine. For example, an increase in the level of copper may indicate increased wear of bushings. Non-metals such as silicon, boron and phosphorus elements can also be determined. Monitoring the levels of wear metals and other elements in lubricating oils provides many benefits apart from predicting engine failure. For example, machinery can be kept up and running until maintenance becomes necessary, avoiding premature maintenance. Potential problems can be associated with specific components, eliminating complete teardowns.

The inductively coupled plasma optical emission spectroscopy (ICP-OES) technique for monitoring wear metals is the method of choice for trend analysis because it is fast and accurate. For the busy laboratory, not only is accuracy and long-term stability important; sample throughput is often a vital factor. The most significant contributor to the time taken for an analysis is the sample introduction system; the actual measurement time is most often less than one tenth of the total analysis time. This work shows that the use of a novel pump tubing arrangement can improve the speed of analysis. Using an improved sample introduction system, it was possible to accurately determine key wear metals and other elements in less than 50 seconds per sample using one simple method.

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Experimental

Instrumental

A Vista-PRO simultaneous ICP-OES with a radially viewed plasma was used. The radial plasma configuration is the accepted standard for the oils industry. The radial plasma orientation allows direct venting of combustion products, thereby reducing carbon build-up on the torch. The highly efficient 40 MHz free-running RF generator is easily able to cope with solvents to produce a stable, robust plasma with excellent long term stability. The instrument was fitted with a 3 channel peristaltic pump to allow a modified pump tubing configuration for faster sample uptake and washout. A glass concentric nebulizer with wide internal bore size was used to better handle particulates, and a glass double-pass spraychamber was used to prevent overloading the plasma with sample. Optimized instrument operating conditions are set out in Table 1.

Table 1. Instrument Operating Conditions

Parameter	Setting	Part number (where applicable)
Power	1.35 kW	
Plasma gas flow	15.0 L/min	
Auxilliary gas flow	2.25 L/min	
Nebulizer pressure or flow	110 kPa or 0.60 L/min	
Viewing height	10 mm	
Pump speed	12 rpm	
Sample uptake delay	15 s	
Stabilization time	5 s	
Rinse time	10 s	
Replicate read time	1 s	
Replicates	2	
Nebulizer type	Slurry glass concentric	20-100976-00
Torch type	Radial fully demountable torch kit (includes bracket and clamp)	99-101064-00
Spraychamber	Twister double pass	79-100437-00
Sample tubing to nebulizer	Grey/grey solvent flex	37-100352-00
Sample tubing to waste	Black/black solvent flex	37-100348-00
Tubing to waste from spraychamber	Solvent flex waste tubing	37-100354-00
Transfer tubing	Solvent flex transfer tubing ¼"internal diameter	37-100378-00
Drain tubing	Purple/black solvent flex	37-100470-00
Autosampler	AIM 1250*	

* Manufactured by A.I. Scientific, Scarborough, Qld, Australia

Standards and Reagents

Calibration solutions of 5, 10, 25, 50, 100, and 250 mg/L were prepared from Conostan S-21 certified standard, which contains 21 elements (Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Ti, V, Zn) at 500 mg/kg in oil. These calibration solutions were viscosity matched using Conostan base oil 75. Single element standards of Ca, Fe, Pb, P, and Zn were prepared from certified 5000 mg/kg Conostan standards (Conostan Division, Conoco Specialty Products Inc., Ponca City, OK, USA). The single element standard concentrations prepared were 10, 25, 50, 100, 250, 500, 1000 and 2500 mg/L. Jet-A1 kerosene (Mobil, Melbourne, Australia) was used as diluent.

Results

Detection Limits

In general, sensitive emission line wavelengths have lower detection limits than less sensitive emission line wavelengths for any given element. This is because sensitive emission lines produce a larger signal for a given concentration than less sensitive emission lines. Thus, low concentrations can be better detected using a sensitive emission line wavelength than an insensitive one. Frequently, detection limits improve with increasing read time because readout noise is reduced. The detection limits of various elements in kerosene are shown in Table 2. All detection limits in the table are below 1 mg/L, which easily allows trace levels of wear metals to be detected and a trend to be observed, even at low levels.

Table 2. Detection Limits of Elements in Kerosene at 2, 5 and 10 Seconds Integration Time

Element and emission line wavelength	3 σ Detection limits (mg/L) 1 s	2 s	3 s
Ag 328.068	0.006	0.003	0.002
Al 308.215	0.05	0.02	0.02
Al 396.152	0.05	0.02	0.01
B 249.772	0.021	0.007	0.005
Ba 455.403	0.003	0.002	0.001
Ba 493.408	0.0010	0.0007	0.0005
Ca 317.933	0.02	0.01	0.01
Ca 396.847	0.002	0.002	0.002
Cd 226.502	0.023	0.003	0.002
Cr 284.325	0.012	0.005	0.003
Cu 327.395	0.011	0.004	0.003
Fe 259.940	0.014	0.006	0.005
Fe 274.932	0.06	0.02	0.02
Mg 280.270	0.001	0.001	0.001
Mn 257.610	0.002	0.001	0.000
Mo 202.032	0.072	0.009	0.005
Na 589.592	0.004	0.002	0.002
Ni 230.299	0.08	0.02	0.01
P 213.618	0.26	0.03	0.02
Pb 220.353	0.39	0.05	0.03
Si 251.608	0.05	0.02	0.02
Sn 283.998	0.11	0.04	0.02
Ti 336.122	0.003	0.002	0.001
V 311.837	0.012	0.004	0.003
Z n 206.200	0.063	0.007	0.005
Zn 213.857	0.017	0.002	0.002

Linear Range

In general, the maximum accurately measurable concentration of an element is obtained by using a less sensitive emission line wavelength for that element. Although sensitive emission line wavelengths have lower detection limits than insensitive ones, insensitive emission line wavelengths can measure higher maximum concentrations. Some elements, such as calcium and phosphorus, may be present at high concentrations in oils, so a high maximum measurable concentration is desirable. The wavelengths chosen for analysis reflect a compromize between best detection limits and desired concentration range.

Table 3. Maximum Measurable Concentration of Selected Elements at Specified Emission Line Wavelengths

Element and emission line wavelength	Maximum concentration (mg/L)
Ag 328.068	250+
Al 308.215	250+
Al 396.192	100
B 249.772	250+
Ba 455.403	100
Ba 493.408	250+
Ca 317.933	2500
Ca 396.847	100
Cd 226.502	250+
Cr 284.325	250+
Cu 327.395	250+
Fe 259.940	250+
Fe 274.932	1000
Mg 280.270	100
Mn 257.610	250+
Mo 202.032	250+
Na 589.592	250+
Ni 230.299	250+
P 213.618	2500
Pb 220.353	1500
Si 251.608	250+
Sn 283.998	250+
Ti 336.122	250+
V 311.837	250+
Zn 206.200	2500
Zn 213.857	250

* Note that 250+ designates an accurately measurable concentration that may surpass 250 mg/L.

Modified Pump Tubing Setup

To speed up sample delivery to the plasma, the flow rate of sample through the autosampler probe was increased based on the "rapid flow" concept conceived by Shane Elliott and investigated as applied to organic solutions by Ross Ashdown (both from Agilent). The idea is to increase the flow rate of sample from the autosampler to the peristaltic pump. To increase the sample flow rate, a wider internal diameter peristaltic pump tubing could have been used, but this would overload the nebulizer, adversely affecting nebulization. Instead, an additional sample peristaltic pump tube was introduced to the system via a T-piece inserted between the end of the autosampler line and the start of the sample peristaltic pump tubing so that sample would flow through two sample perstaltic pump tubings instead of one. One of the peristaltic pump tubes was directed to the nebulizer, and the other to waste, which avoided overloading the nebulizer with sample. By having sample flow through two pump tubings, the sample flow rate through the autosampler probe up to the point where the T-piece was inserted was increased, thus reducing sample uptake time.

To measure sample uptake time, kerosene was introduced to the autosampler probe manually after aspirating air, and the time taken for the plasma to turn bright green (which indicates that organic solution is being aspirated into the plasma) was measured by stopwatch. Table 4 shows that using the modified pump tubing setup, the sample uptake time was decreased by approximately 10 seconds. An added benefit of decreasing sample uptake time is that the time taken to achieve a fixed degree of washout is also reduced.

Table 4. Time Saved Using Modified Pump Tubing Setup

Pump tubing configuration	Acutal sample uptake time (s)	Sample uptake time in method (s)
Standard	24	25
Modified	15	15

Washout

To determine the washout achieved in an autosampler run, an analysis was performed where a blank kerosene solution was measured immediately following a solution containing 1000 mg/L of Fe. These two solutions were then measured in pairs six times each. Table 5 shows that three orders of reduction in sample concentration was achieved in an autosampler run with a rinse time of 10 seconds. If a more thorough rinse was required, then SmartRinse could have been used. The SmartRinse feature of the ICP Expert software optimizes the rinse time for each sample, ensuring that the rinse time is only as long as required to return the signal to that of a blank for each wavelength in the analysis [1]. This means that high concentration samples will take longer to analyze than low concentration samples. For this work, a washout of three orders was acceptable, so a short, fixed rinse time was used.

Table 5. Blank Results After Measuring 1000 mg/L Iron. This Demonstrates that Three Orders of Washout is Achieved with a Rinse Time of 10 Seconds.

Kerosene blank measurement number	Measured Fe conc. (mg/L)
2	0.66
4	0.77
6	0.79
8	0.79
10	0.80
12	0.64

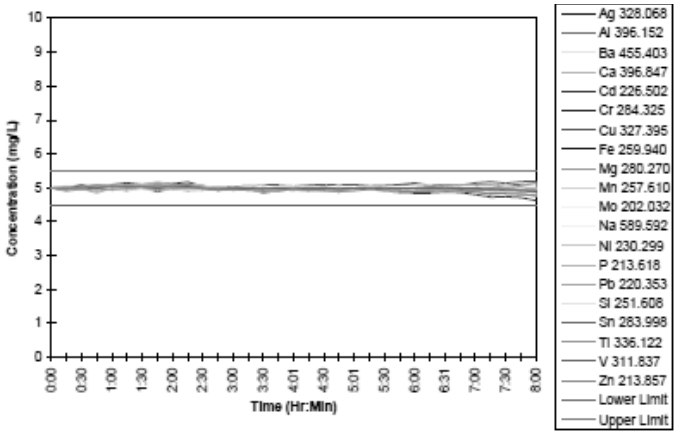

Figure 1. Stability of the Vista-PRO radial instrument over 8 hours. Results remained within ±10% for all elements in the 5 mg/L S21 kerosene solution without internal standardization or recalibration.

Long-Term Stability

A 5 mg/L solution of S21 elements in Jet-A1 kerosene was analysed continuously over an eight hour period. No recalibrations were performed, and no internal standard was used. Figure 1 shows that results remained within 10% of the true value over the entire 8 hours. Precision was typically better than 2 %RSD.

Conclusion

The Vista-PRO radial ICP-OES provides excellent throughput at 47 seconds per sample using a simple optimized sample introduction system. The detection limits and maximum measurable concentration of selected wavelengths allows typical oil samples to be analysed, while the excellent stability allows continuous running without recalibration, providing a saving on costs by reducing analysis time and the amount of standard solution used.

Acknowledgements

The author would like to thank Shane Elliott (Varian Australia) for the initial concept and his advice with alternative sample pump tubing configurations, Ross Ashdown (Varian U.K.) for his early work with fast throughput for organics, Barry Sturman, Alan Wiseman and Kate Pearson-Santiago (Varian Australia) for editing, and Glyn Russell (Varian Australia) for his input, encouragement and review of this work.

Reference

1. I. Szikla, SmartRinse - the latest advance in maximizing

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Agilent Technologies

Analysis of Phenol-formaldehyde Resins by GPC and Agilent PolarGel-M

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

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, comprizing 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 µL
Temperature:	50 °C
Detectors:	Agilent PL-GPC 50, RI

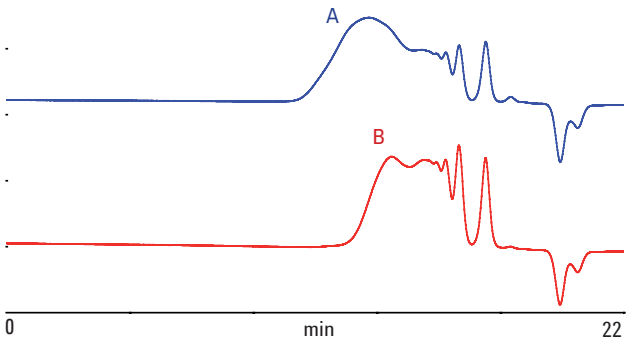

Figure 1. Overlaid molecular weight distributions of two novolac resins

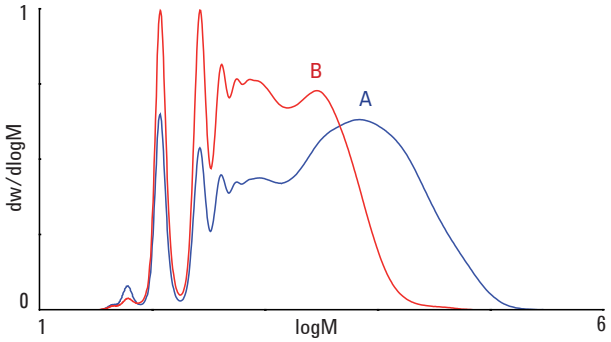

Figure 2. Overlaid molecular weight distributions of two phenol-formaldehyde resins

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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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.

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Conditions

Technique:	GC-FID	Carrier Gas:	Hydrogen, constant flow mode 10 mL/min	Injection Volume:	1.0 µl
Column:	VF-5ht UltiMetal, 15 m x 0.32 mm Df = 0.1 µm + 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

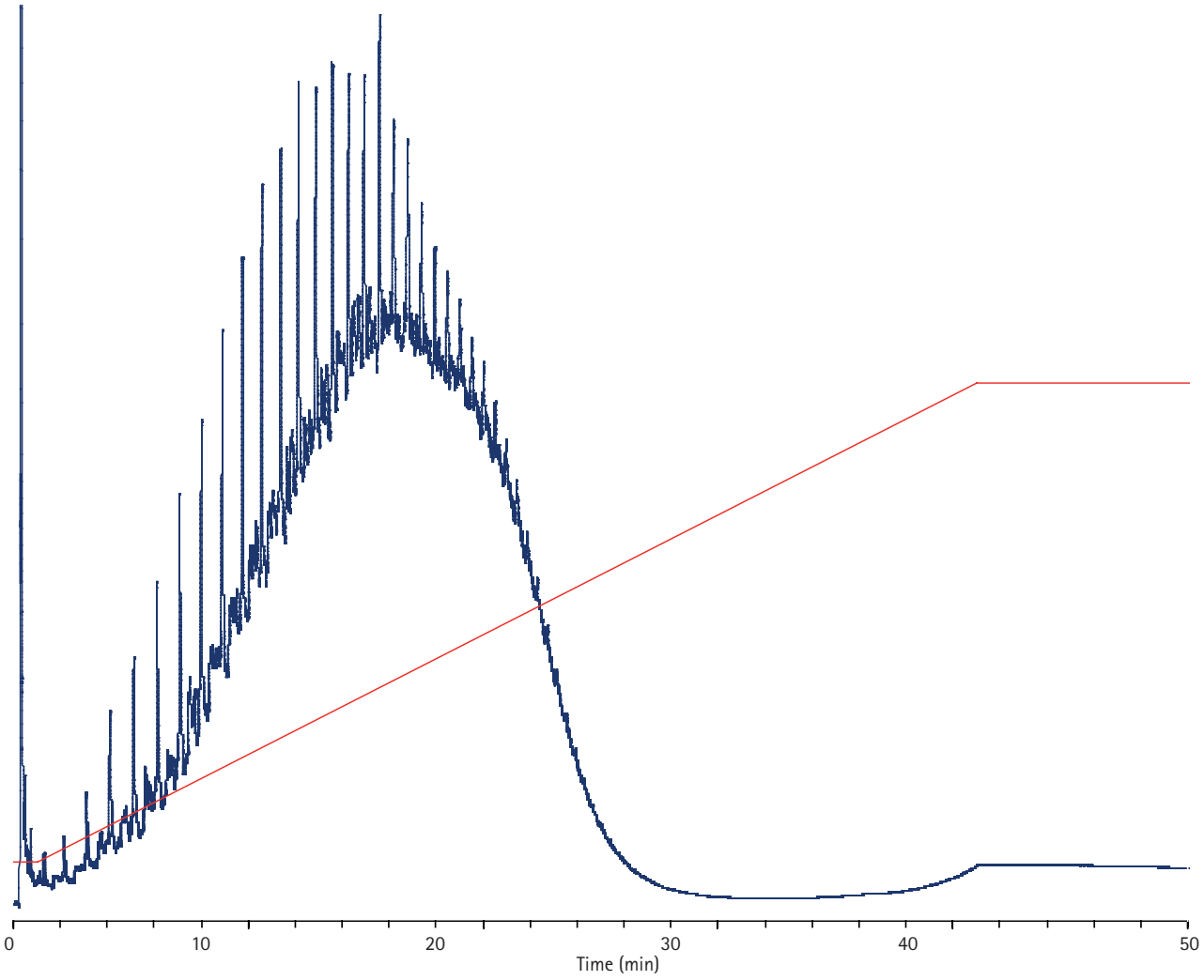

Figure 1. Analysis of fluid cat cracker feed using a VF-5ht UltiMetal column

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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.

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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 µm MIXED-B columns. These columns provide high resolution of polymers that have high molecular weights, even in demanding eluents.

Columns:	3 x PLgel 10 µm 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 µL

Results and Discussion

Figure 1 is a chromatogram of a natural rubber sample showing reponses 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).

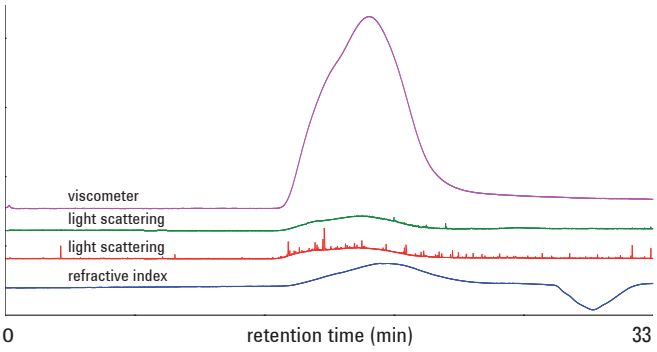

Figure 1. Raw triple detection data for one of the natural rubbers

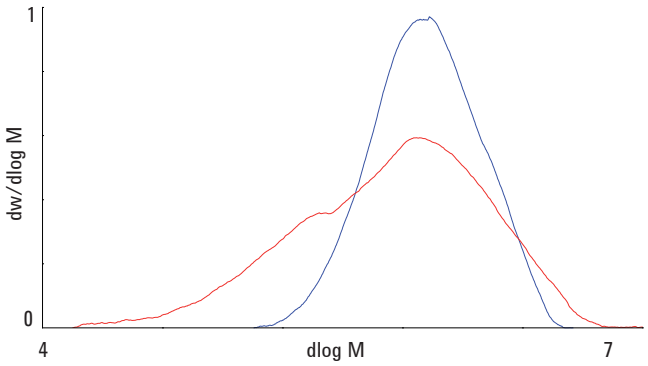

Figure 2. Overlaid triple detection molecular weight distributions of two natural rubbers

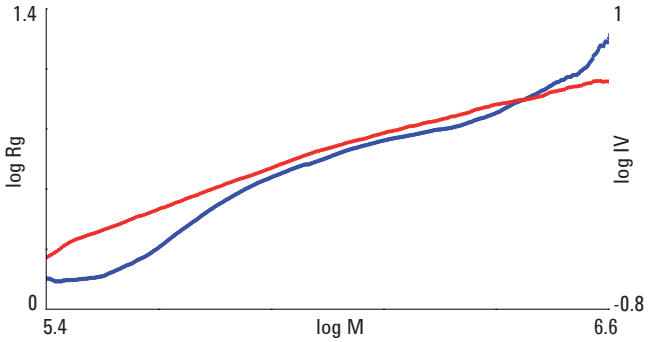

Figure 3. Overlaid Mark-Houwink (log instrinsic viscosity versus log M) for two natural rubbers

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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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 µm MIXED-C columns, which provide high resolution of polymers that have 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 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.

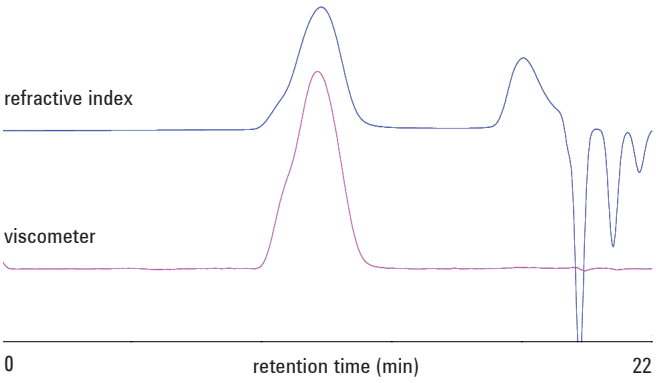

Figure 1. Chromatograms of a poly(styrene-co-butadiene)

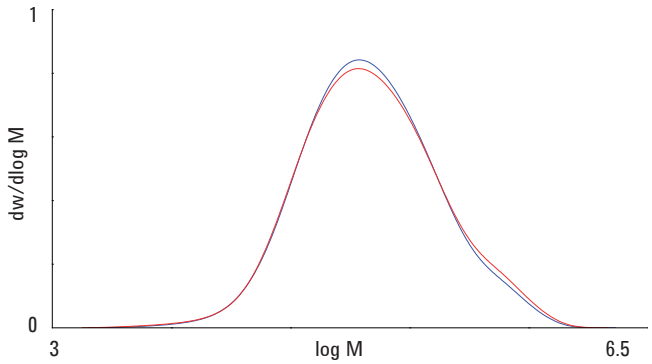

Figure 2. Overlaid molecular weight distributions for two poly(styrene-co-butadiene) samples

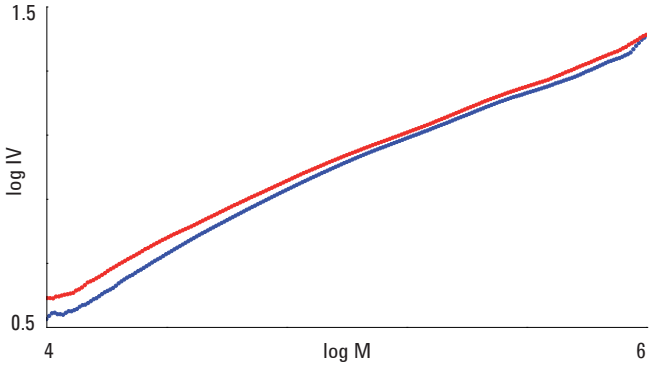

Figure 3. Overlaid Mark-Houwink plots for two poly(styrene-co-butadiene) samples

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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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 °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).

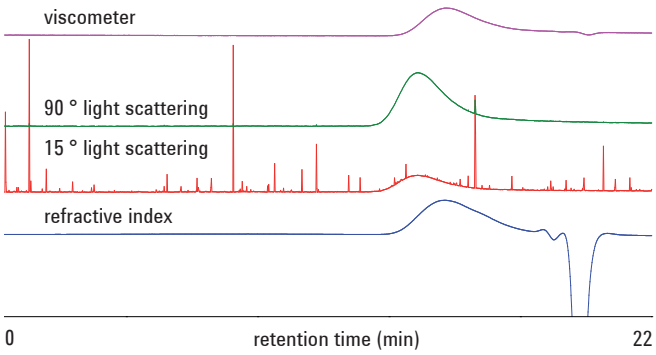

Figure 1. Raw triple detection data for a polyacrylamide

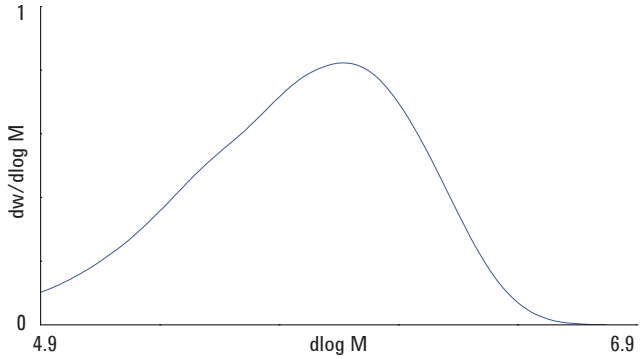

Figure 2. Triple detection molecular weight distribution for a polyacrylamide

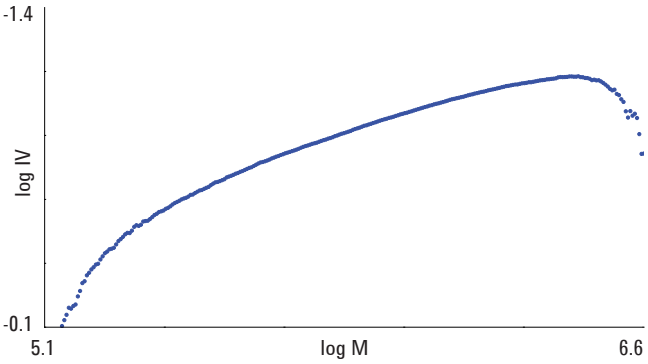

Figure 3. Mark-Houwink plot for the polyacrylamide

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °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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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).

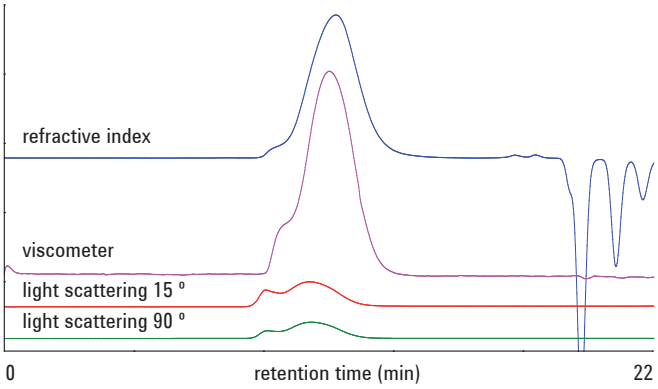

Figure 1. Chromatograms of a polybutadiene sample

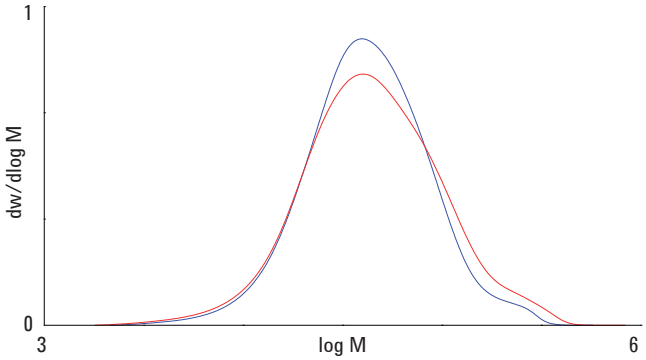

Figure 2. Overlaid molecular weight distributions for two polybutadiene samples

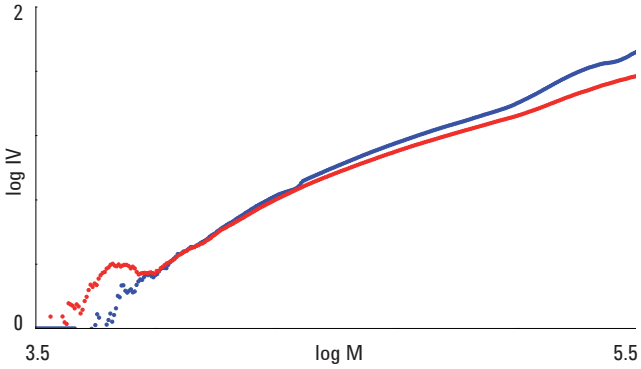

Figure 3. Overlaid Mark-Houwink plots for two polybutadiene samples

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 5um 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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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 µm MIXED-C columns, which provide high resolution of polymers with high molecular weights, even in demanding eluents.

Columns:	2 x PLgel 5 µm 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.

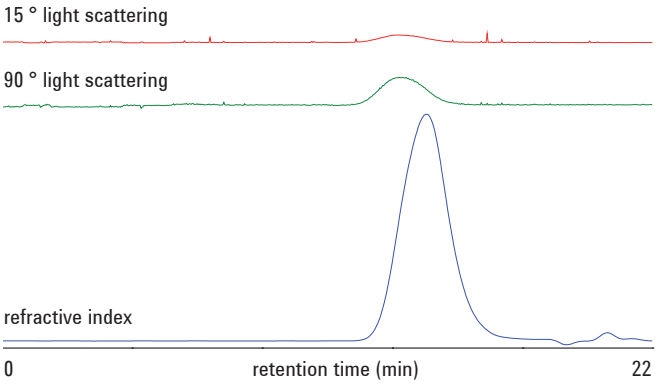

Figure 1. Chromatograms of a polyester sample

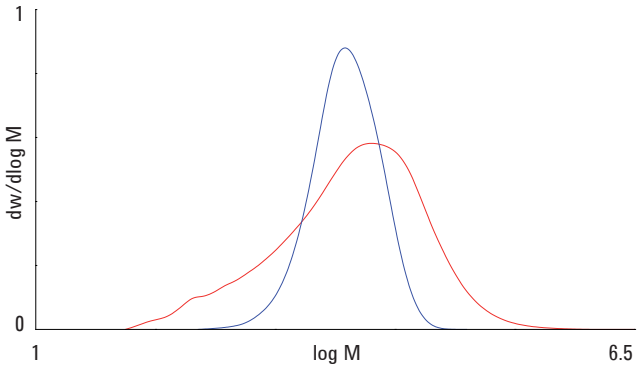

Figure 2. Overlaid molecular weight distributions for two polyester samples

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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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.

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Conditions

Samples: Polyacrylic acids
Columns: 2 x PL aquagel-OH 50 8 µm, 300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.25 M $NaNO_3$ + 0.01 M NaH_2PO_4 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.

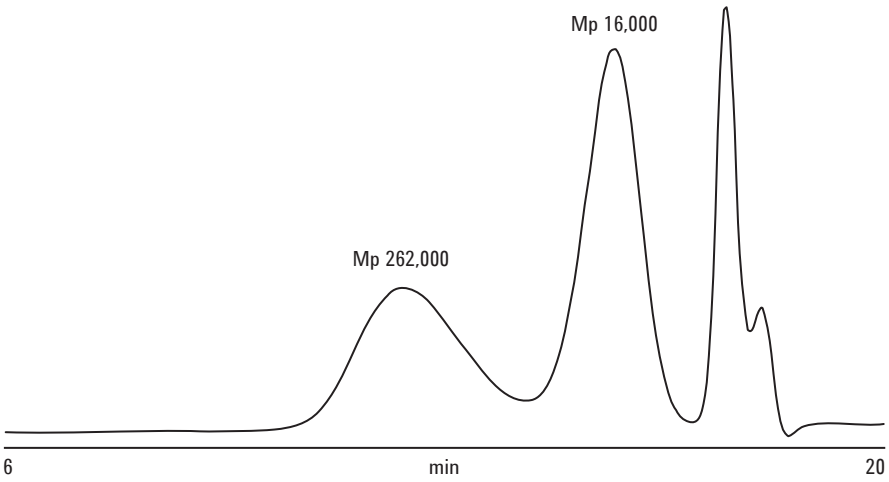

Figure 1. Separation of two polyacrylic acids

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.

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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 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

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.

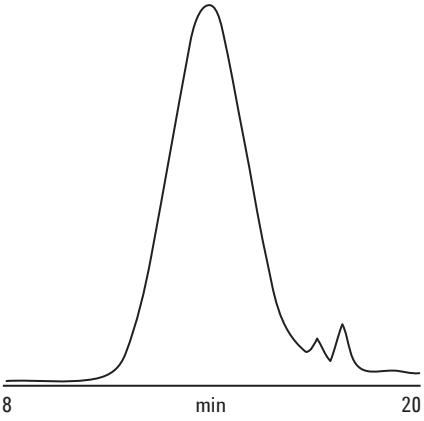

Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

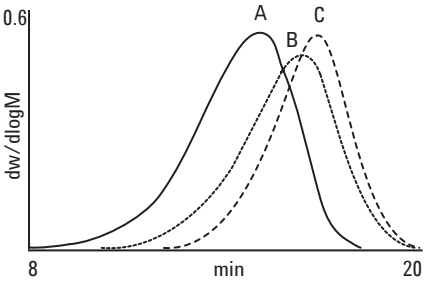

Figure 2. Comparison of molecular weights of three polyvinyl alcohols

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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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 µm, 300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

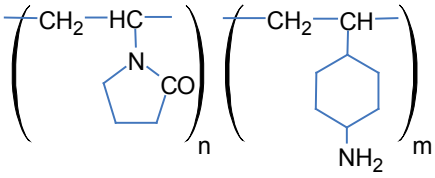

Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

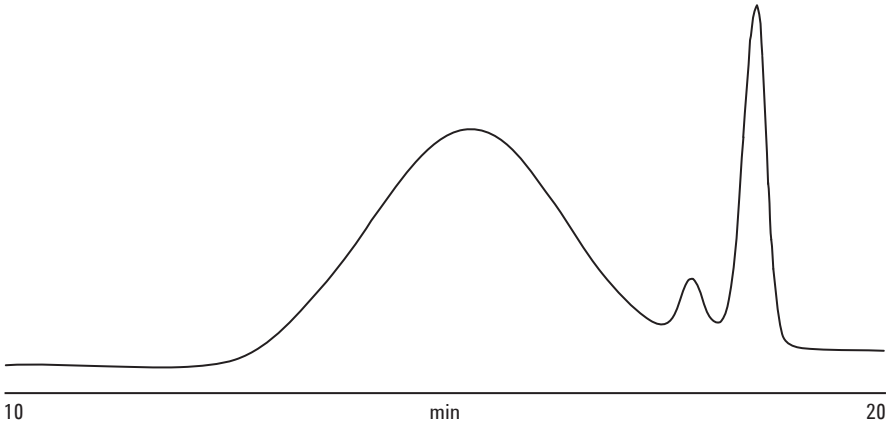

Figure 2. Raw data chromatogram of N-vinyl pyrrolidone / p-amino styrene copolymer

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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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.

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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

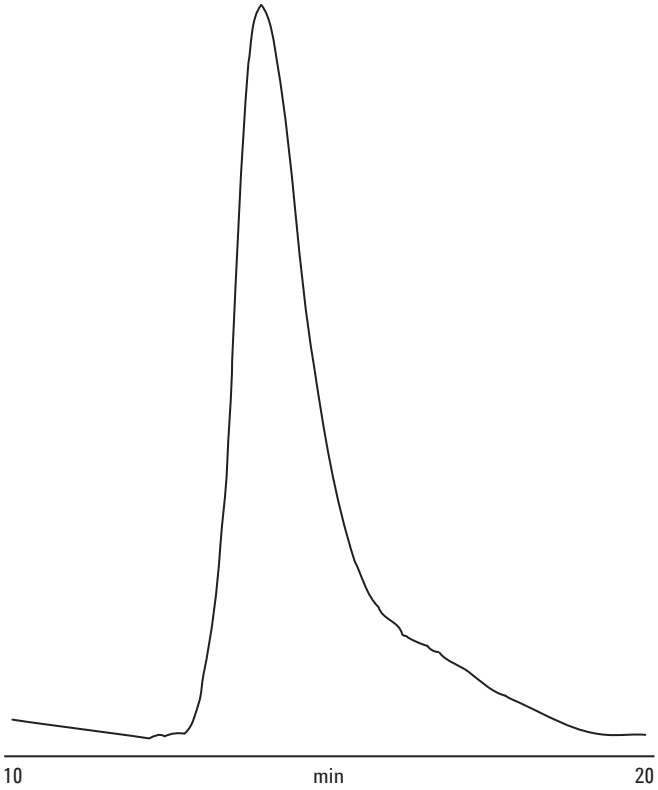

Figure 1. Raw data chromatogram of an acrylamide co-polymer

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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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.

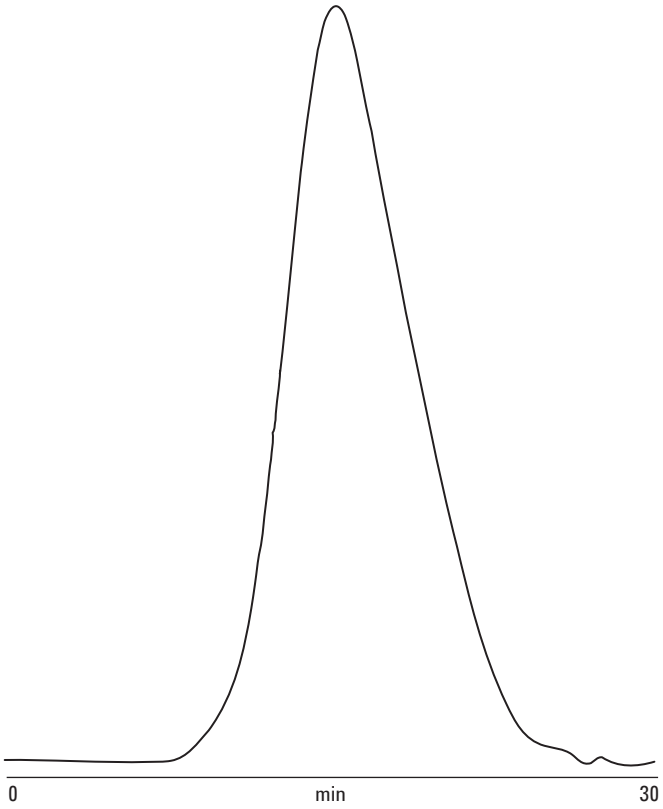

Figure 1. Raw data chromatogram of polyvinyl pyrrolidone

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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 µm, 300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.25 M $NaNO_3$ + 0.01 M NaH_2PO_4 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

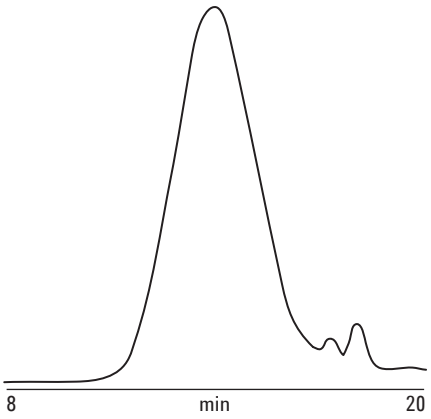

Figure 1. Chromatogram of a polyvinyl alcohol

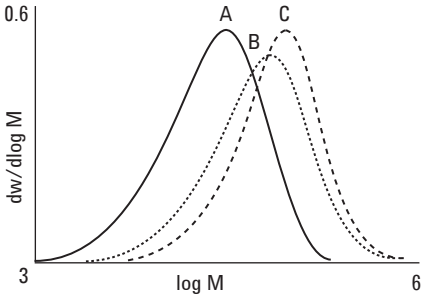

Figure 2. Overlaid molecular weights of three polyvinyl alcohols

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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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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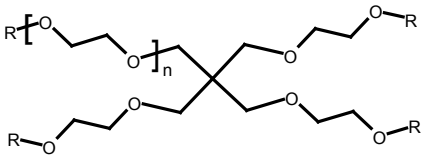

Figure 1. Structure of the star-branched polyethylene glycol

Conditions

Samples:	Star branched PEG
Columns:	2 x PL aquagel-OH 30 8 µm, 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 µL
Detection:	RI

Results and Discussion

The resulting chromatograms are shown in Figures 2 and 3.

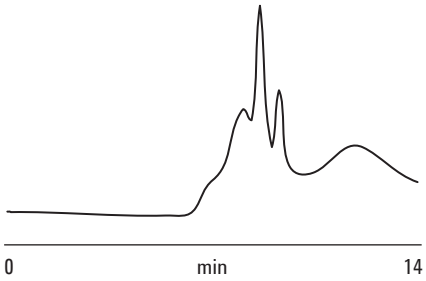

Figure 2. Chromatogram of the star-branched polyethylene glycol obtained in buffer at pH 7

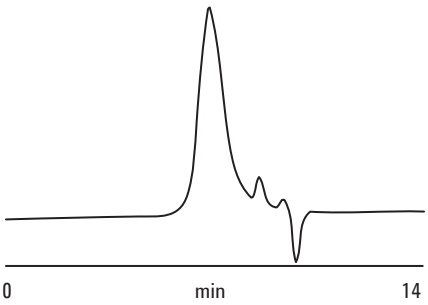

Figure 3. Chromatogram of the start-branched polyethylene glycol obtained in buffer at pH 7 with 30 % methanol

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 hydrophicity 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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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.

Agilent Technologies

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 $NaNO_3$ + 0.01 M NaH_2PO_4 adjusted to pH 7
Flow Rate:	1.0 mL/min
Injection Volume:	200 µL
Detection:	RI

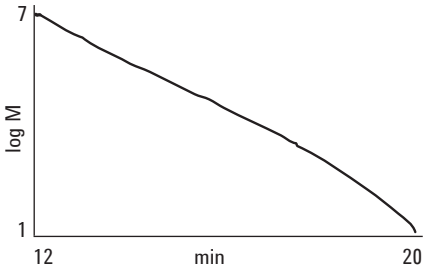

Figure 1. Calibration of PL aquagel-OH MIXED 8 µm column using EasiVial standards

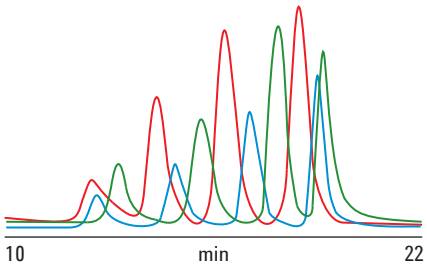

Figure 2. RI chromatograms from EasiVial PEOs

Results and Discussion

Three polyacrylic acid samples (A, B and C) were chromatographed and their corresponding molecular weight distribution compared (Figure 3).

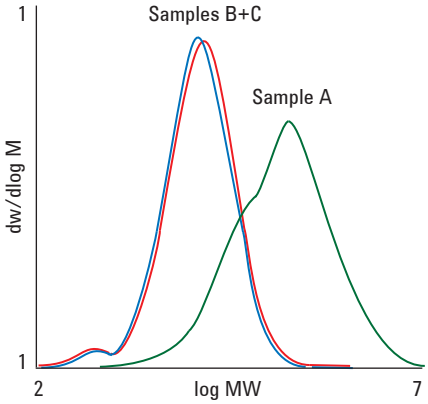

Figure 3. Comparison of three polyacrylic acids

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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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

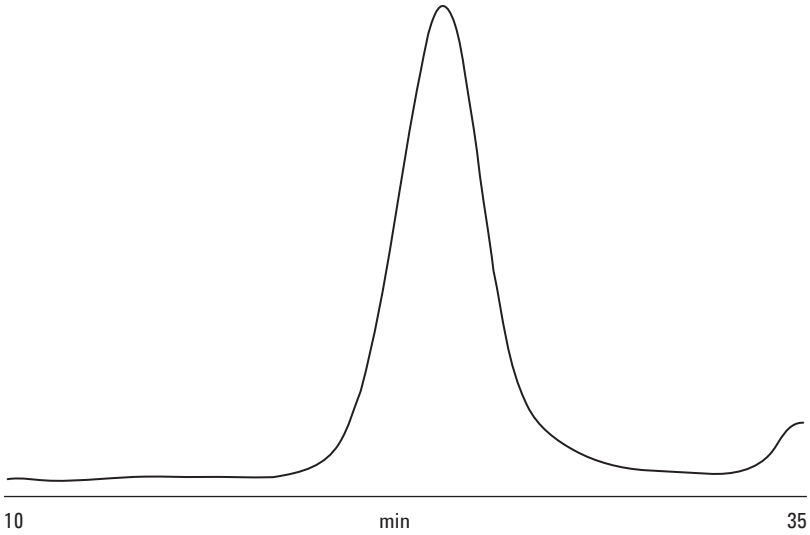

Figure 1. GPC analysis of polybutadiene using PLgel MIXED-A columns

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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

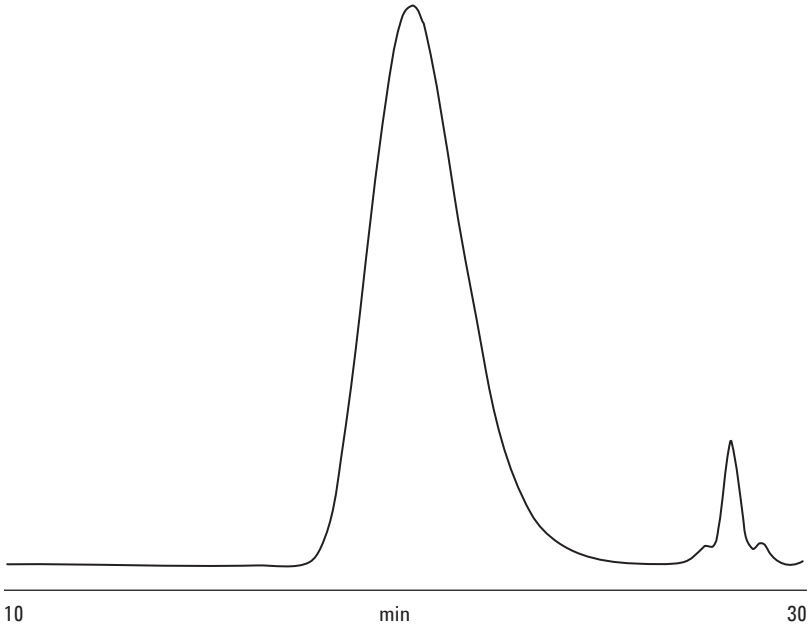

Figure 1. Analysis of polystyrene using PLgel 10 µm MIXED-B columns

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Agilent Technologies

Analysis of Nitrocellulose

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

The operating range of Agilent PLgel 10 µm 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 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: 2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (p/n PL1110-6100)
Eluent: THF
Flow Rate: 1.0 mL/min
Loading: 0.1%, 200 µL
Detection: RI

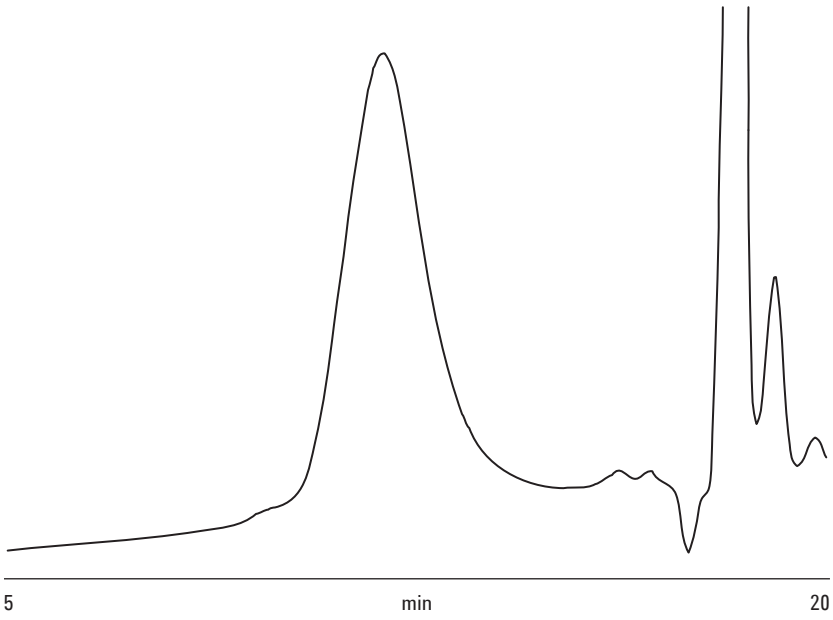

Figure 1. Analysis of nitrocellulose using PLgel 10 µm MIXED-B columns

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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.

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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: 2 x PLgel 10 µm MIXED-B, 300 x 7.5 mm (p/n PL1110-6100)
Eluent: THF
Flow Rate: 1.0 mL/min
Loading: 0.25%, 100 µL
Detection: RI

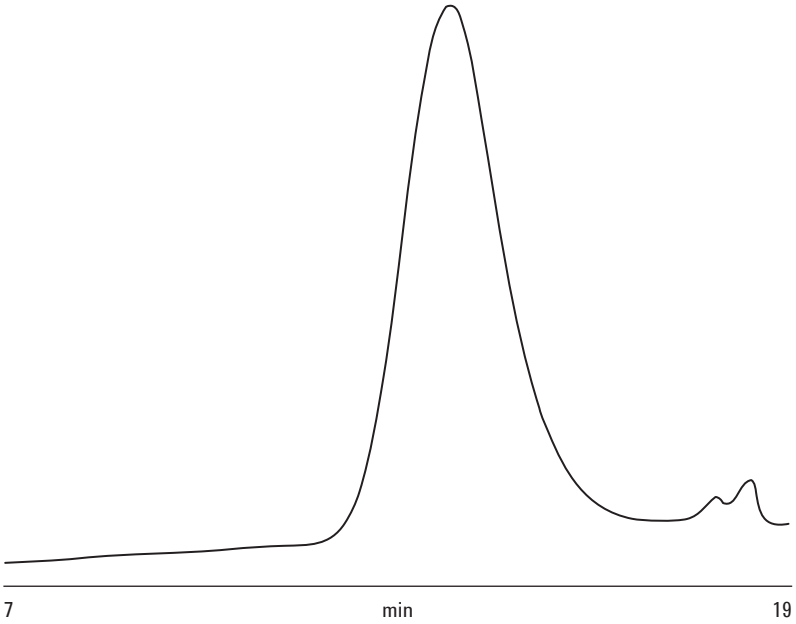

Figure 1. Analysis of reacetylated poly(vinyl) alcohol using PLgel MIXED-B columns

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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

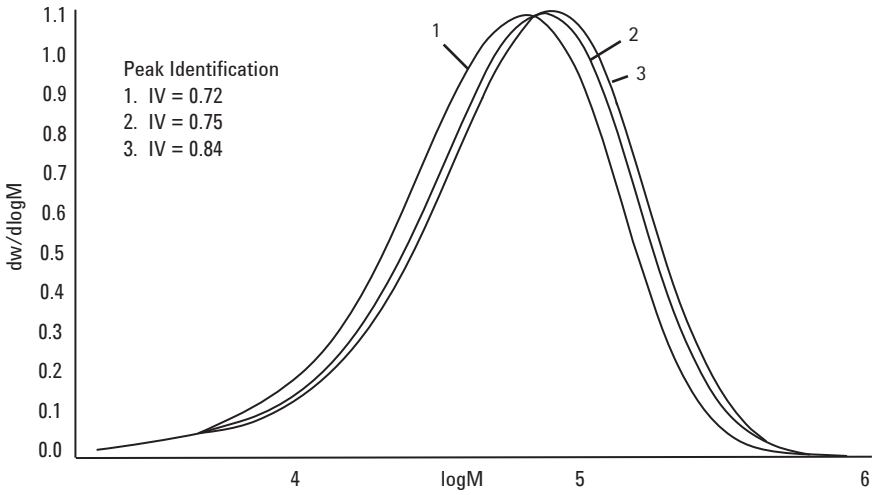

Figure 1. Molecular weight overlays of three polyethylene terephthalates

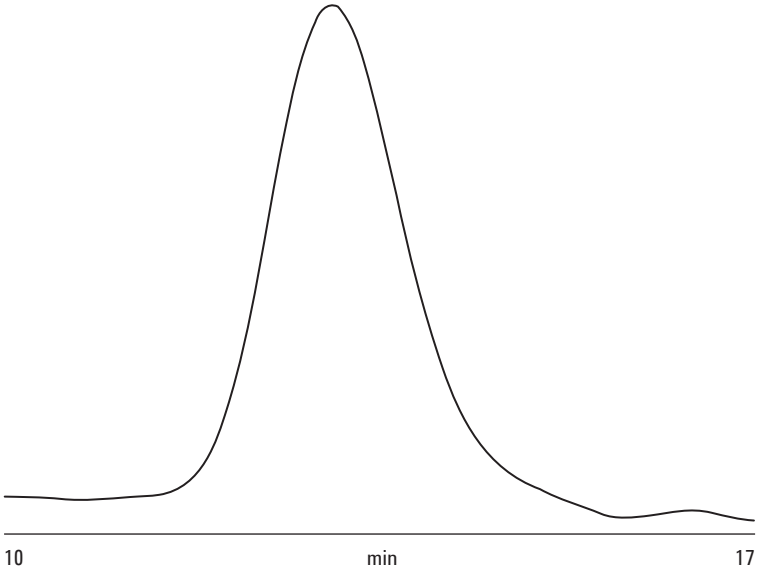

Figure 2. Chromatogram of a polyethylene terephthalate

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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 Mw=250,000 and Mn=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.

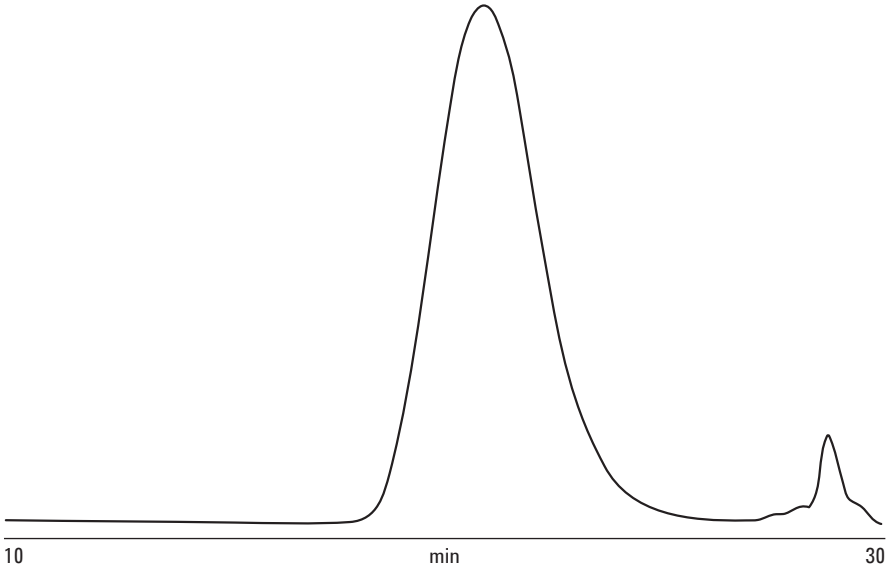

Figure 1. Analysis of polystyrene using PLgel 10 µm MiniMIX-B columns

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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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 µm 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 µm MIXED-C also provide the versatility essential for today's R&D laboratory.

Conditions

Columns:	2 x PLgel 5 µm 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)

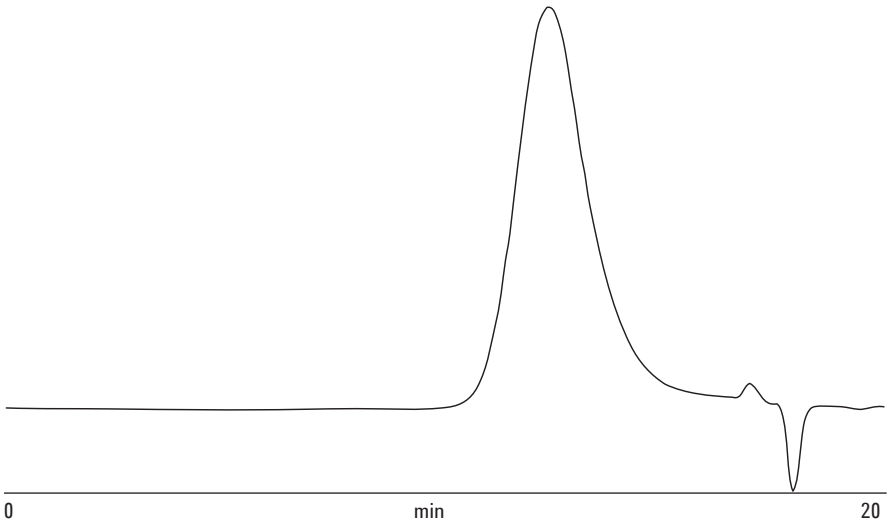

Figure 1. Analysis of polyether sulfone using PLgel 5 µm MIXED-C columns

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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 µm 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 µm MIXED-C also provide the versatility essential for today's R&D laboratory.

Conditions

Columns:	2 x PLgel 5 µm 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)

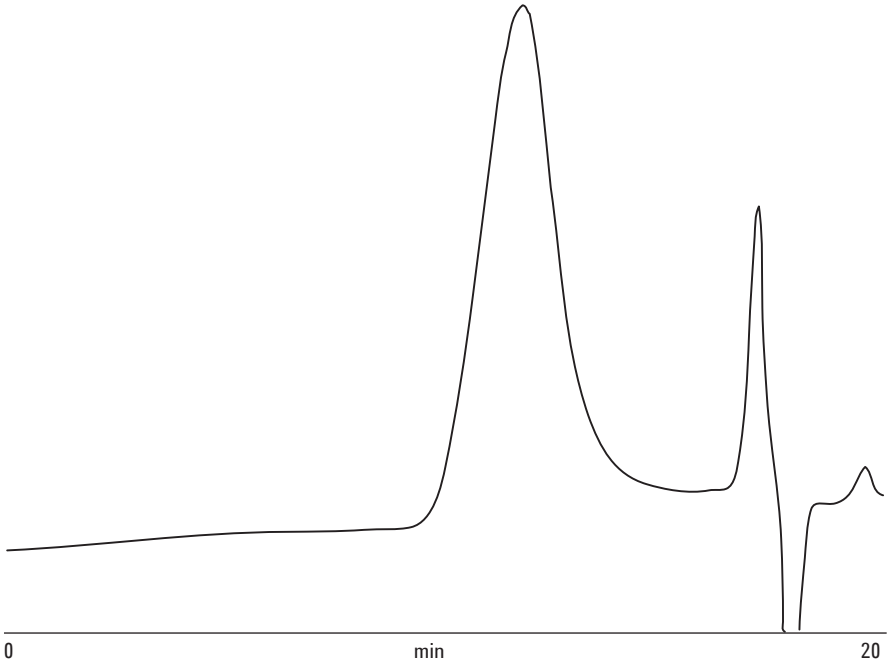

Figure 1. Analysis of polyurethane using PLgel 5 µm MIXED-C columns

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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 µm particle size columns.

Three different grades of PVC tubing containing different plasticizers were analyzed by GPC using a column set comprising three PLgel 5 µm MIXED-C columns.

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Conditions

Columns: 3 x PLgel 5 µm 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.

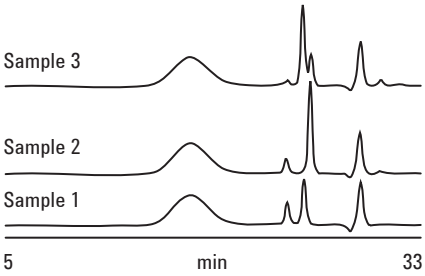

Figure 1. Overlaid raw data chromatograms for three PVC samples

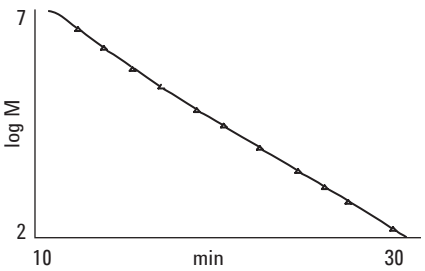

Figure 2. Calibration curve derived from polystyrene standards

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 µm 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 µm MIXED-C also provide ideal versatility for the R&D laboratory.

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Agilent Technologies

Analysis of Polyaniline

Application Note

Materials Testing & Research, Polymers

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

The Agilent PLgel 5 µm 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 10^4/500 Å or 10^4/10^3/500Å/100Å column combinations.

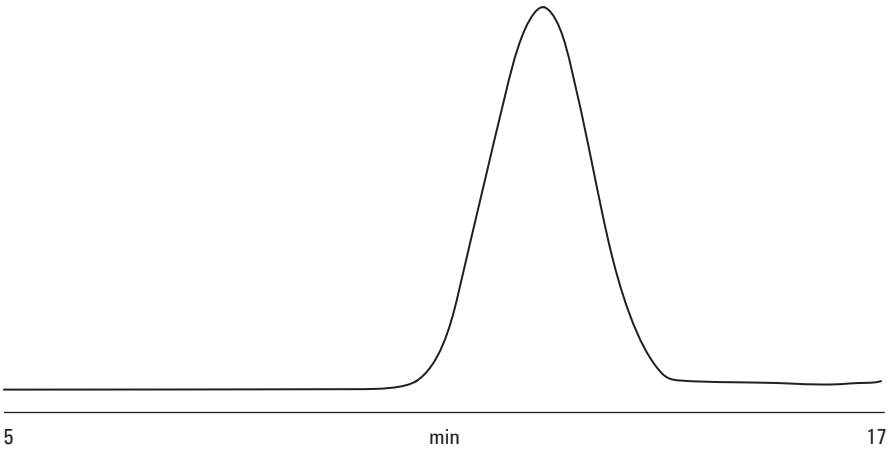

Figure 1. Analysis of emeraldine polyaniline using PLgel 5 µm MIXED-D columns

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Agilent Technologies

Characterization of Block Copolymers Synthesized via Transition Metal Mediated Living Radical Polymerization

Application Note

Authors

Greg Saunders, Ben MacCreath
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 psuedo-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 µm MIXED-C, 300 x 7.5 mm (part number PL1110-6500)
Eluent:	Tetrahydrofuran
Flow Rate:	1 mL/min
Inj. Vol:	100 µL
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.

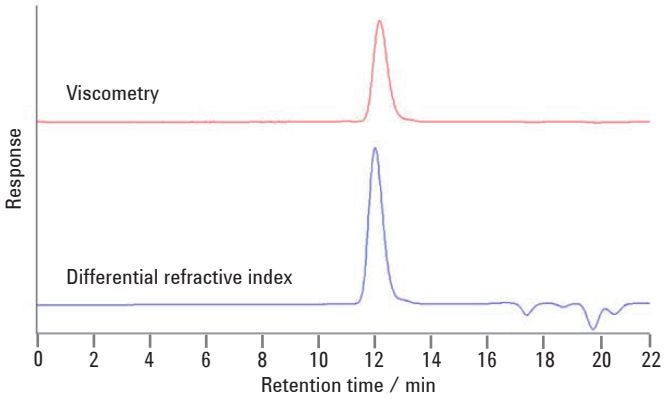

Figure 1. Overlaid dual detector raw data chromatograms for a block copolymer sample

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.

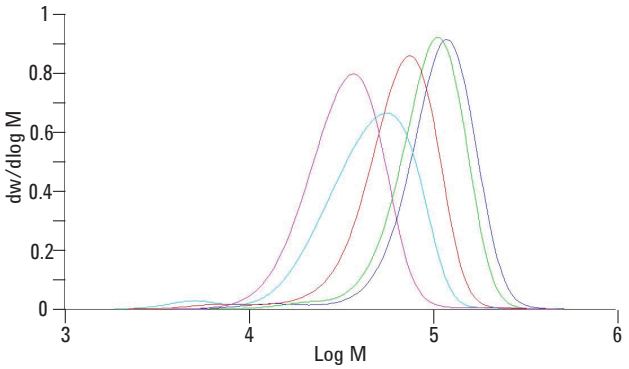

Figure 2. Overlaid molecular weight distributions calculated by universal calibration analysis of all samples

The Mark-Houwink plots for the four materials are shown in Figure 3.

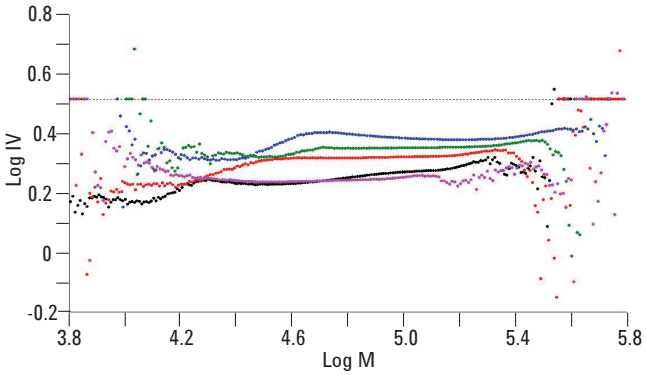

Figure 3. Overlaid molecular weight distributions calculated by universal calibration analysis of all samples

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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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 µm MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Inj Vol:	100 µL
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 µm 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.

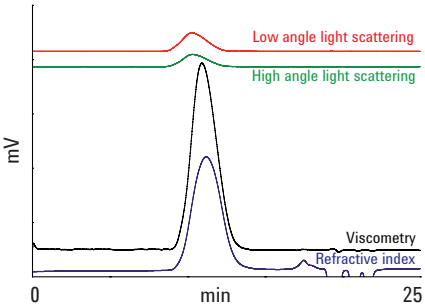

Figure 1. Triple detection of a polybutadiene

Figure 2 reveals the molecular weight distribution calculated for the sample of polybutadiene.

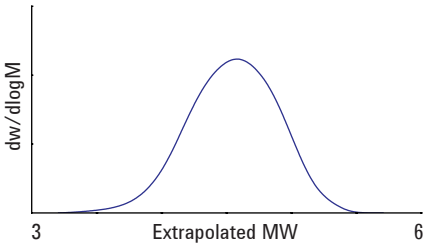

Figure 2. Molecular weight distribution of a polybutadiene

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.

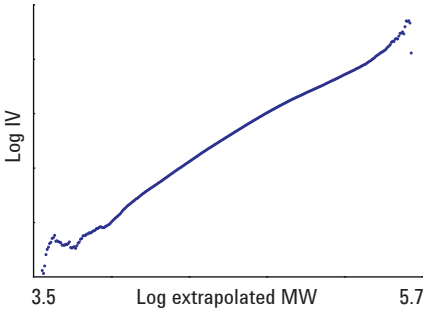

Figure 3. Mark-Houwink plot of a polybutadiene

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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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.

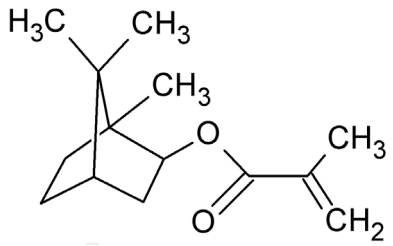

Figure 1. Structure of isobornyl methacrylate monomer

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.

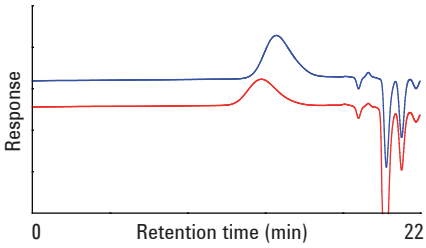

Figure 2. Overlaid chromatograms of two samples of poly(isobornyl methacrylate)

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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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 µm MIXED-D columns.

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Conditions

Sample:	Petroleum jelly A and B
Columns:	2 x PLgel 5 µm MIXED-D, 300 x 7.5 mm (p/n PL1110-6504)
Inj Vol:	100 µL
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).

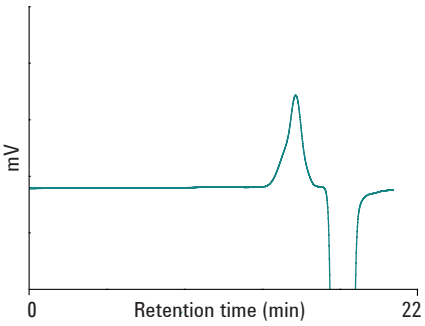

Figure 1. Chromatogram of Petroleum jelly brand A

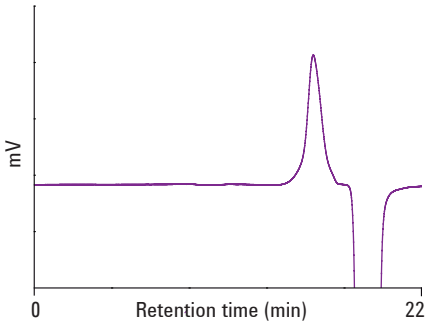

Figure 2. Chromatogram of Petroleum jelly brand B

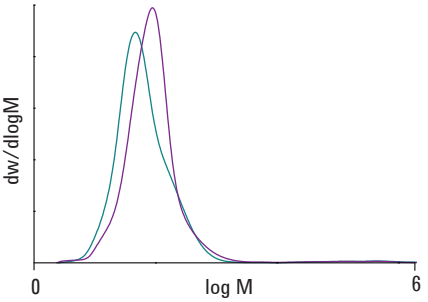

Figure 3. Overlaid chromatograms of two Petroleum jellies reveal slight differences in molecular weight distribution

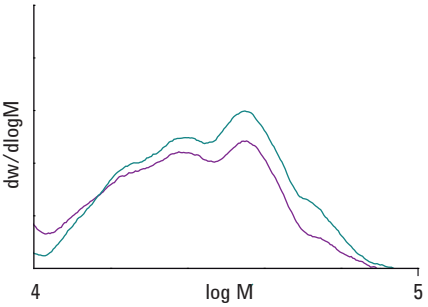

Figure 4. High molecular weight components of two different Petroleum jellies

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 5µm 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 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 10E4/500Å or 10E4/10E3/500Å/100Å column combinations.

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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.

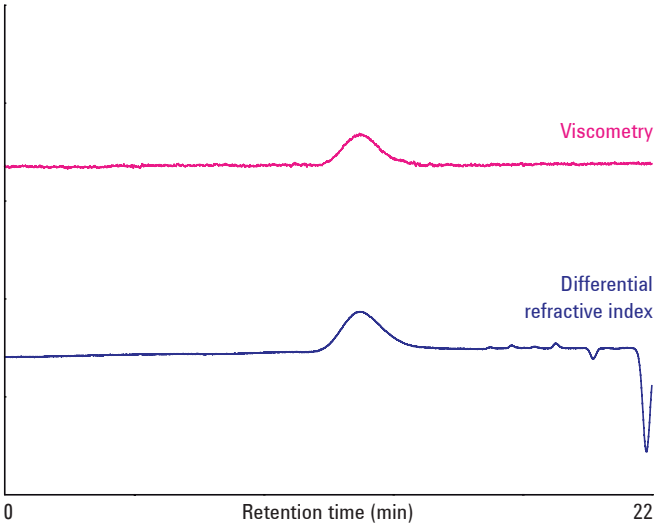

Figure 1. Dual detection chromatograms of a sample of polylactide

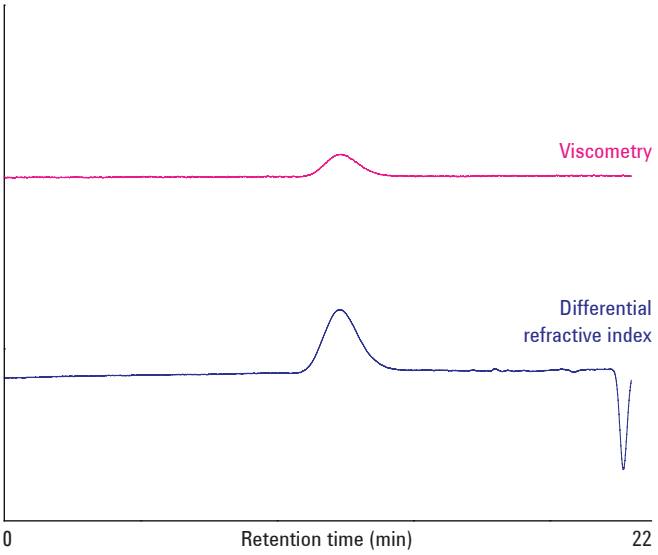

Figure 2. Dual detection chromatograms of a sample of polycaprolactone

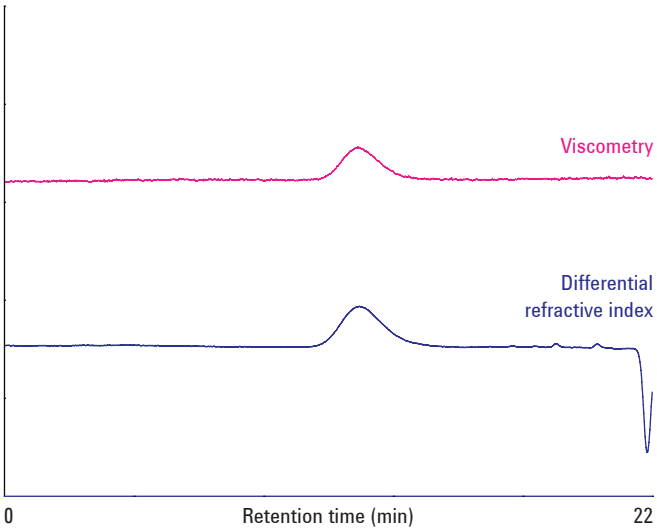

Figure 3. Dual detection chromatograms of a sample of polylactide-glycolide

The overlaid molecular weight distribution plots are shown in Figure 4.

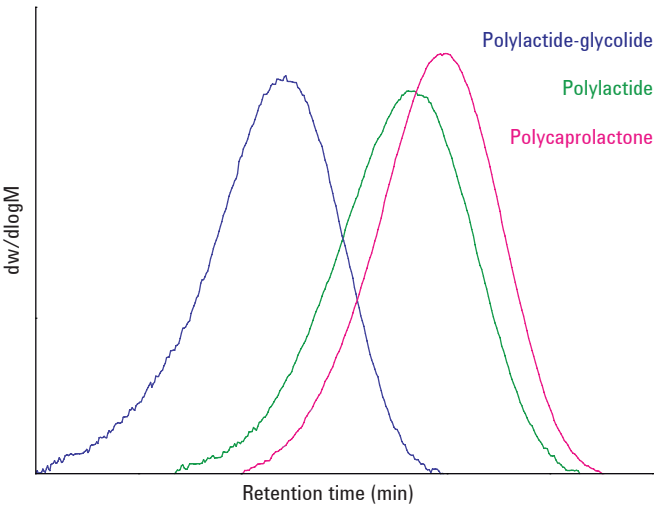

Figure 4. Molecular weight distibutions of three biodegradable polymers

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 (IVw) was calculated for the biodegradable polymers. The table shows the molecular weight averages for a selection of such polymers.

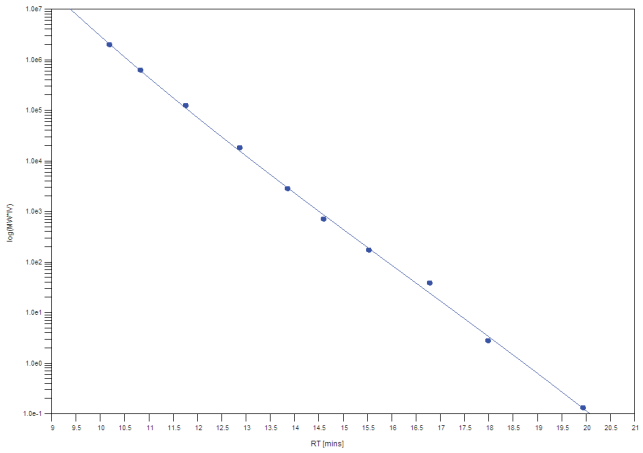

Figure 5. Universal calibration curve

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 distirbutions of the samples, and calculate several of their characterization parameters.

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

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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 µm MIXED-C, 300 x 7.5 mm (part number PL1110-6500)
Eluent:	Tetrahydrofuran
Flow Rate:	1 mL/min
Inj. Vol:	100 µL
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.

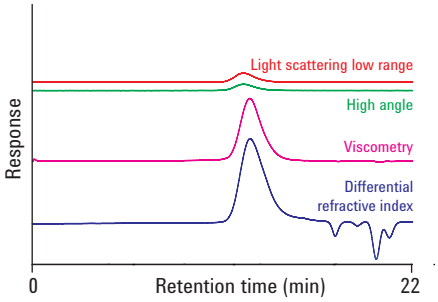

Figure 1. Overlaid triple detector raw data chromatograms for a polycarbonate sample showing the data collected from the individual detectors

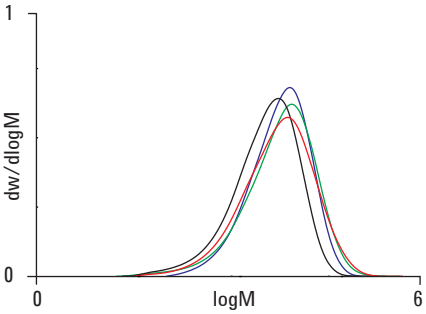

Figure 2. Overlaid molecular weight distributions calculated from triple detection analysis of all samples

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.

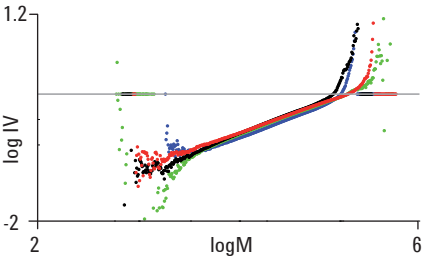

Figure 3. Overlaid Mark Houwink plots calculated from triple detection analysis of all samples

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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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 occuring 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 µm MIXED-D, 300 x 7.5 mm (part number PL1110-6504)
Eluent:	Tetrahydrofuran
Flow Rate:	1 mL/min
Inj. Vol:	100 µL
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

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.

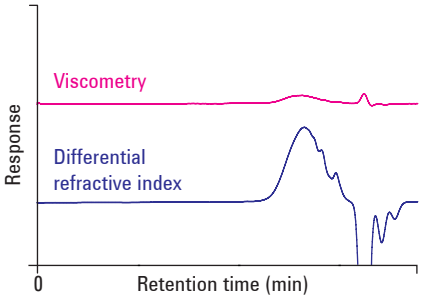

Figure 1. Overlaid dual detector raw data chromatograms for an epoxy resin sample

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.

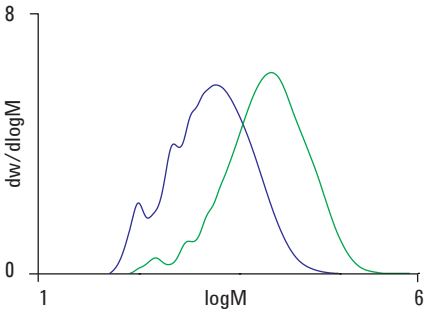

Figure 2. Overlaid molecular weight distributions calculated by universal calibration analysis of the two samples

The Mark-Houwink plots for the two materials are shown in Figure 3.

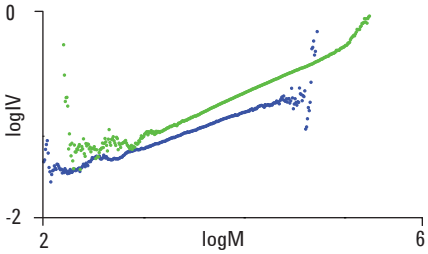

Figure 3. Overlaid Mark-Houwink plots calculated from universal calibration analysis of both samples

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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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 µm 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 µm, 300 x 7.5 mm (p/n PL1149-6800)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 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.

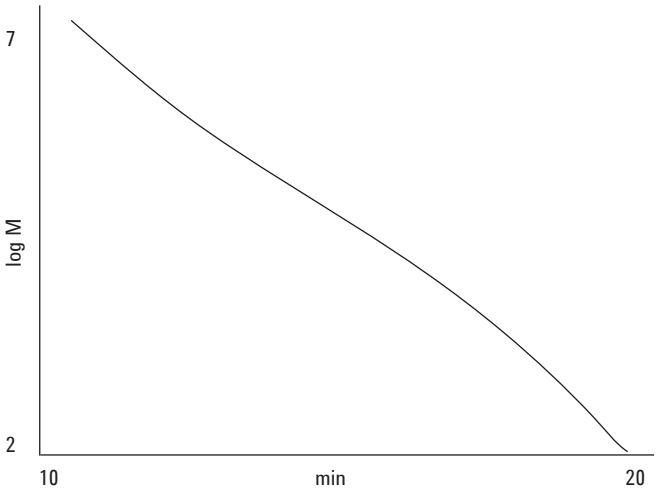

Figure 1. SEC PEG/PEO calibration using EasiVial standards

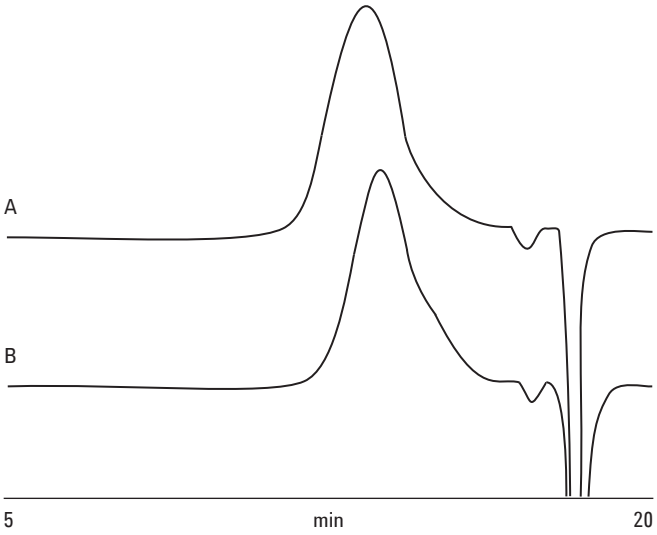

Figure 2. Raw data chromatograms of two dental polymers

The molecular weight distribution plots are presented in Figure 3 and clearly indicate the differences between the two batches.

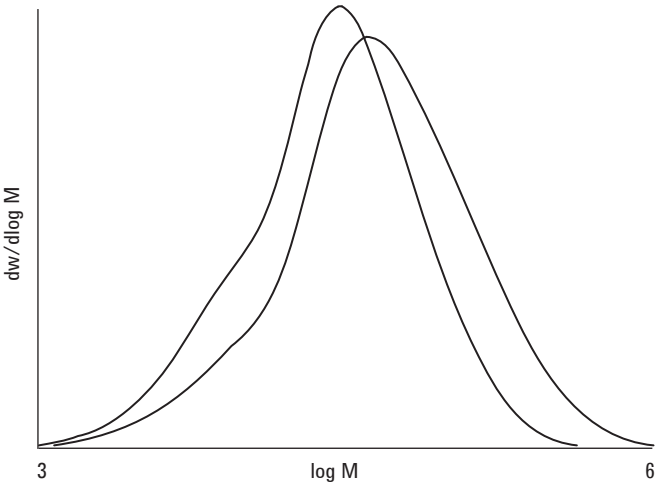

Figure 3. Overlay of the molecular weight distributions of two dental polymers

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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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.

Agilent Technologies

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.

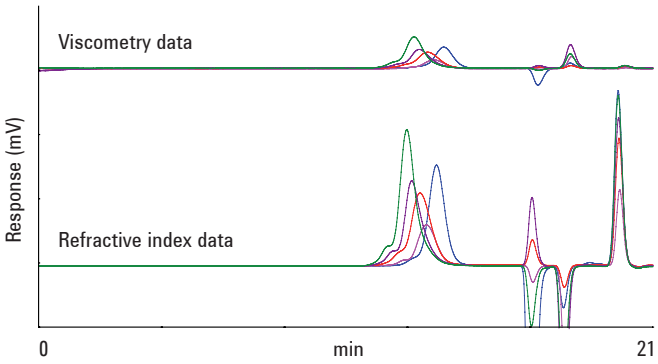

Figure 1. Overlaid raw data chromatograms obtained from a series of linear and branched polyethylene glycol samples

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 °C
Calibrants:	Agilent EasiVial PEG/PEO
Detector:	PL-GPC 50 (Differential Refractive Index + Agilent PL-BV 400RT Viscometer)

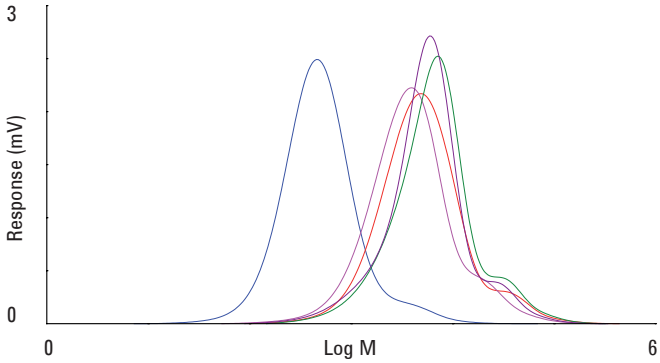

Figure 2. Overlaid raw data chromatograms obtained from a series of linear and branched polyethylene glycol samples

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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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 µm MIXED-D columns, in conjunction with the Agilent PL-GPC 220 instrument.

Conditions

Sample:	Polyethylene
Column:	2 x PLgel 5 µm 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 µL
Sample Conc:	2.0 mg/mL
Temp:	160 °C
Calibrants:	Agilent EasiVial PS-M
Detector:	PL-GPC 220 (Differential Refractive Index)

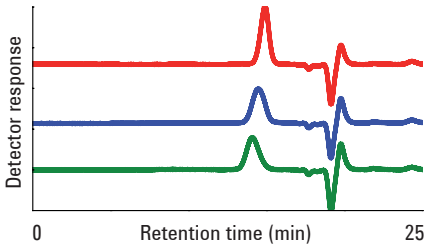

Figure 1. Overlaid raw data chromatograms obtained from series of low molecular weight polyethylene samples

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.

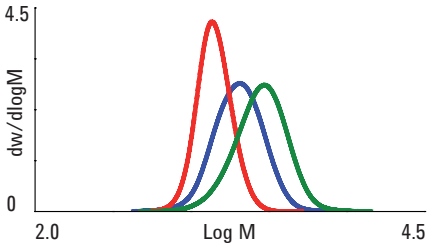

Figure 2. Overlaid molecular weight distributions obtained from series of low molecular weight polyethylene samples

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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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 paticular interest as a result of the their ability to conduct when electrons are doped into or from the conjugated backbone pi-orbitals.

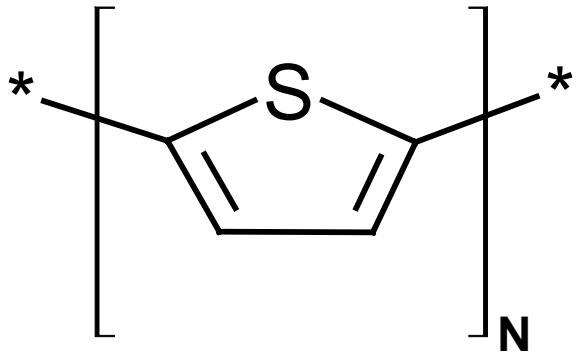

Figure 1. General structure of the thiophene monomeric repeat unit

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 µm 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 µm 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 µL
Sample Conc:	2.0 mg/mL
Temp:	120 °C
Calibrants:	Agilent PS-M EasiVial
Detector:	PL-GPC 220 (Differential Refractive Index)

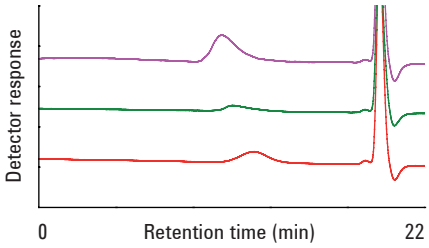

Figure 2. Overlaid RI raw data chromatograms obtained from a series of polythiophene samples

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.

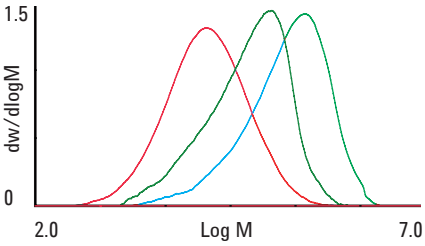

Figure 3. Overlaid molecular weight distributions obtained from a series of polythiophene samples

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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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.

Agilent Technologies

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_2O
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.

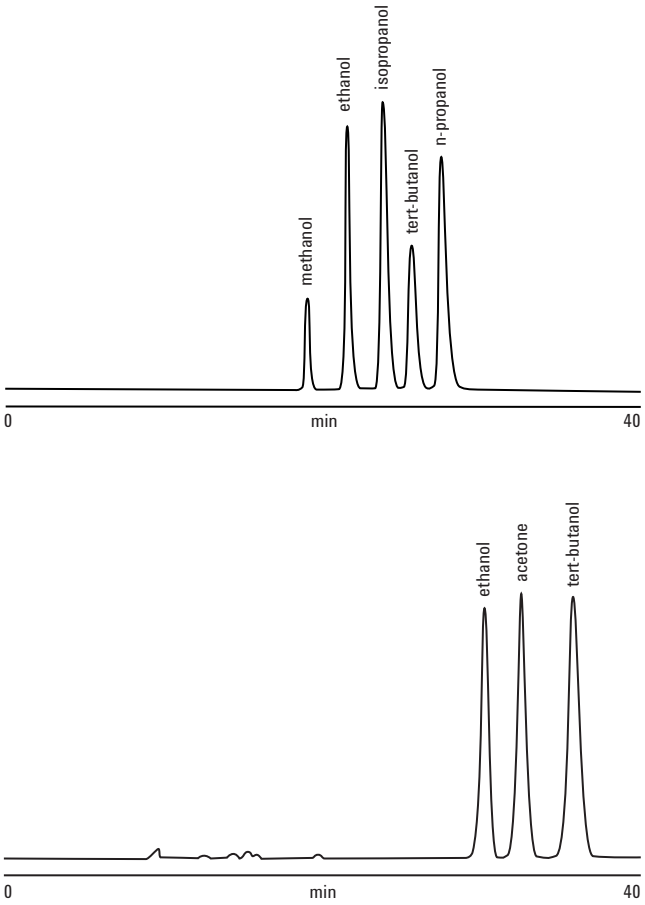

Figure 1. Separation of different aliphatic compounds on an Agilent Hi-Plex H column.

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Agilent Technologies

Separation of Permanent Gases on a Liquid Phase

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

Application Note

Authors

Rick Hamerlinck and Norbert Reuter
Agilent Technologies, Inc.

Introduction

Normally permanent gases are separated by PLOT (porous layer open tubular) columns with their high retentive phases. With WCOT (wall coated open tubular) columns sub-ambient temperatures are normally necessary. Thick films, like the 8 µm film thickness of the Agilent J&W Select CP-Sil 5CB for Formaldehyde, allow the use of high-inert liquid phases for the (pre-) separation of the standard permanent gases from carbon dioxide for possible column switching at normal ambient temperatures.

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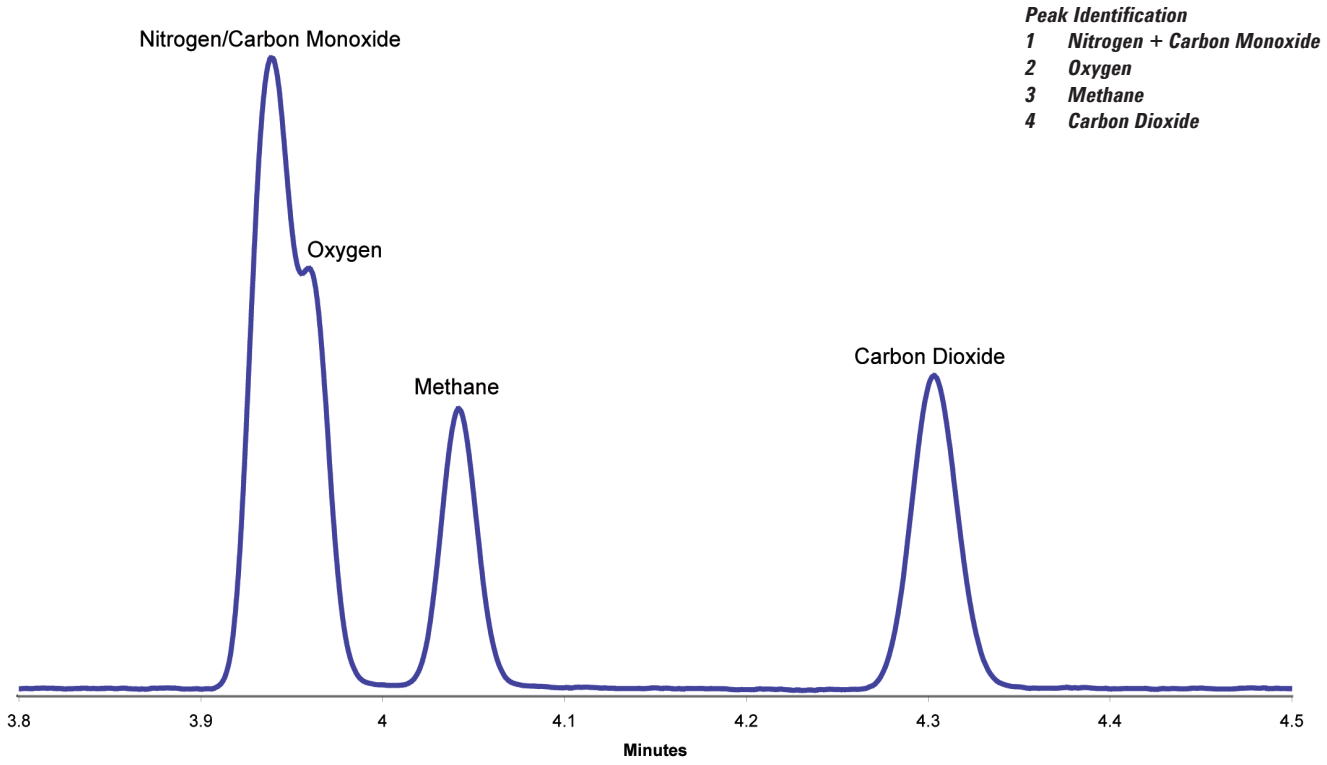
Materials and Methods

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)		



Peak Identification

1. ***Nitrogen + Carbon Monoxide***
2. ***Oxygen***
3. ***Methane***
4. ***Carbon Dioxide***

Analysis of permanent gases at 35°C

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Agilent Technologies

Fast Refinery Gas Analysis Using the 490 Micro GC QUAD

Application Note

Authors

Coen Duvekot
Agilent Technologies, Inc.

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	
3	Oxygen	
4	Nitrogen	
5	Methane	Bal
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.

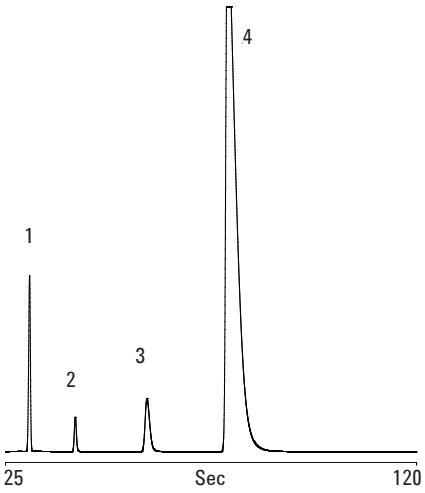

Figure 1. Standard gas on the Molsieve column, channel 1

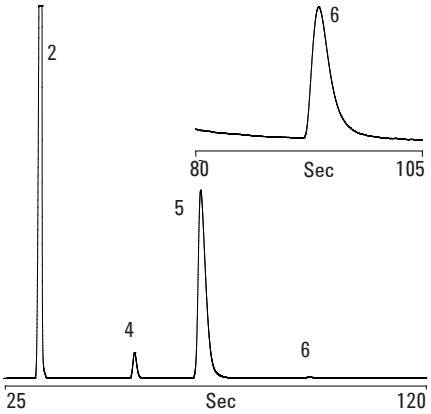

Figure 2. Refinery gas on the Molsieve column, channel 1

Hydrogen or helium, oxygen, nitrogen methane and carbon monoxide were separated and analyzed. Later eluting components were back flushed to vent.

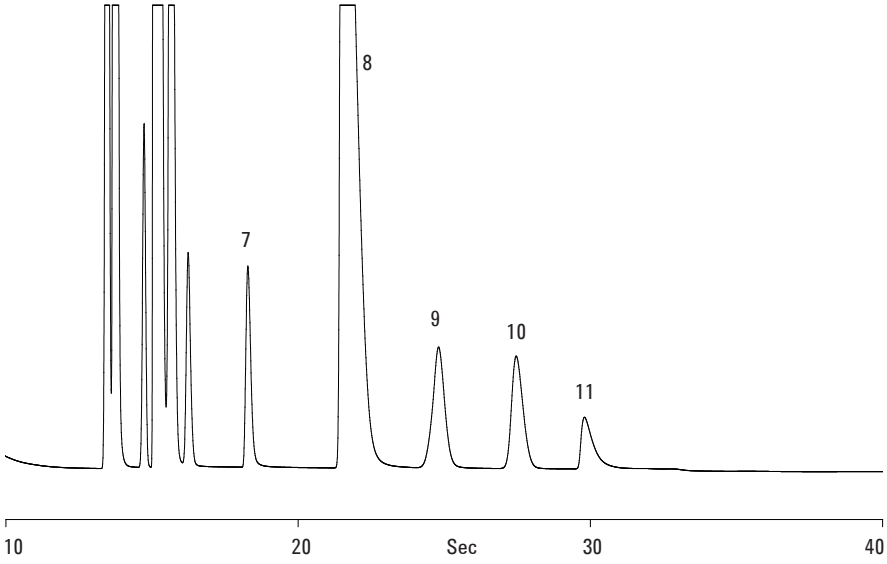

Figure 3. Refinery gas on the CP-PoraPLOT U column, channel 2

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.

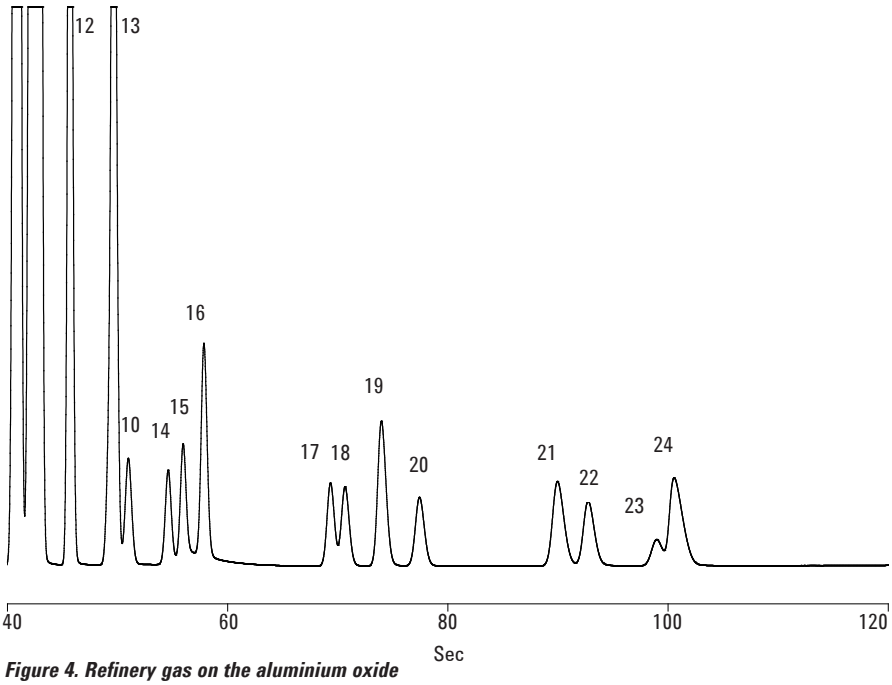

Figure 4. Refinery gas on the aluminium oxide column, channel 3

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.

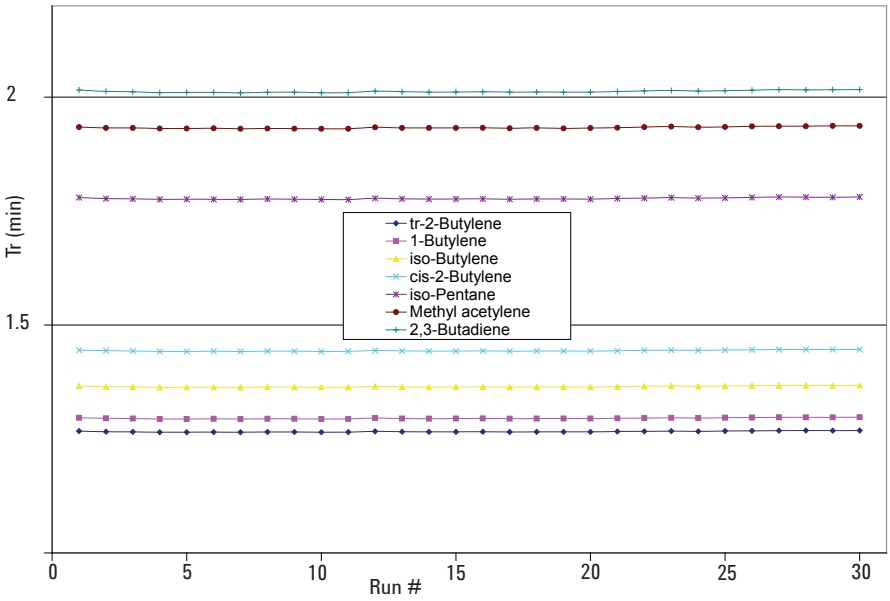

Figure 5. Repeatability figures for the aluminium oxide channel, channel 3

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.

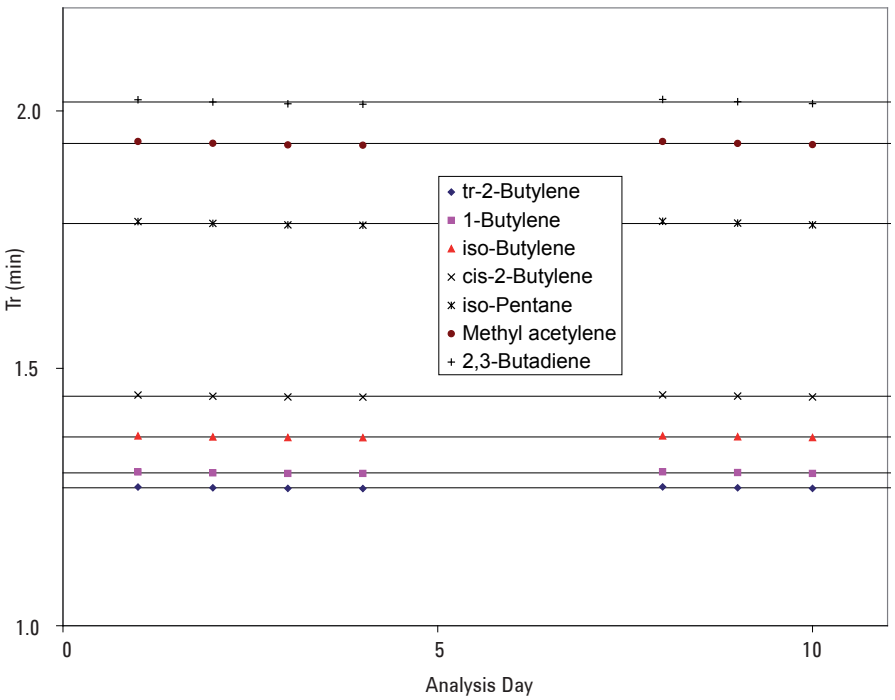

Figure 6. Reproducibility of the aluminium oxide channel, channel 3

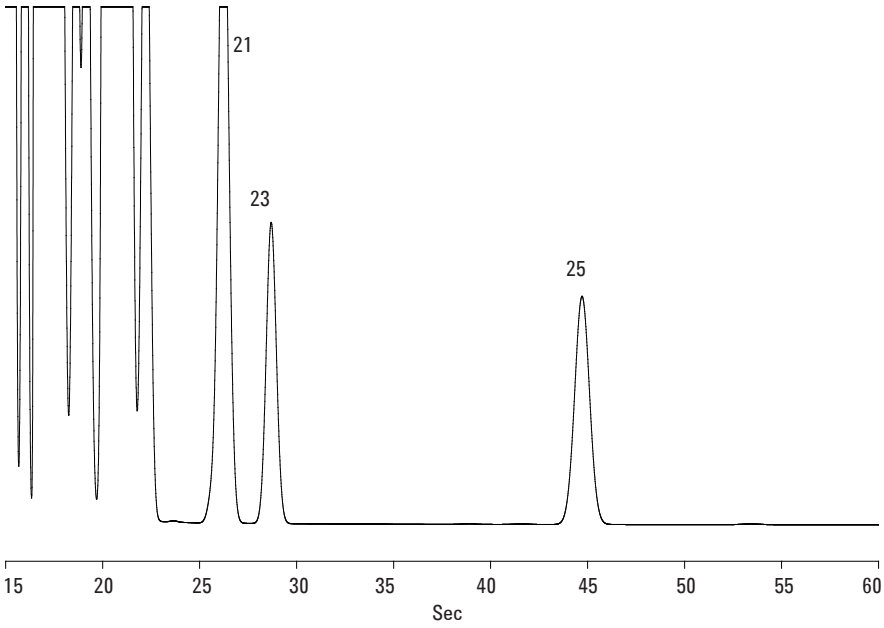

Figure 7. Refinery gas on the CP-Sil 5 CB column, channel 4

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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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.

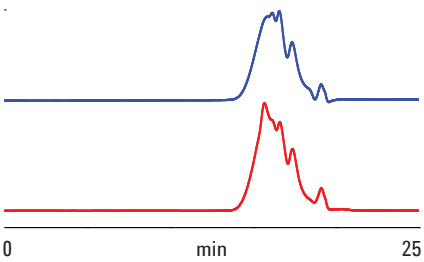

Figure 1. Chromatograms for two melamine samples with different oligomer ratios

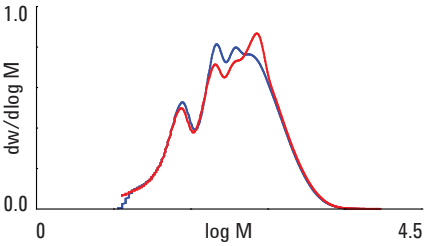

Figure 2. Overlaid molecular weight distributions for two melamine resin samples suggest differences in performance

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 oven ensured that a low column pressure and stable baselines were observed throughout the analysis.

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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 µm 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 µL
Temperature:	40 °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.

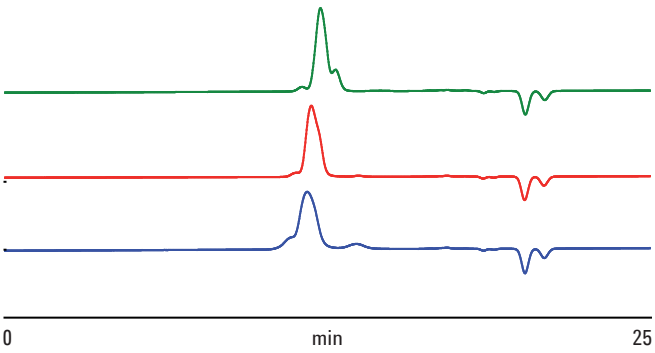

Figure 1. Chromatograms for three samples of poly(styrene/butadiene) – each sample eluted as a multi-modal distribution of relatively sharp peaks

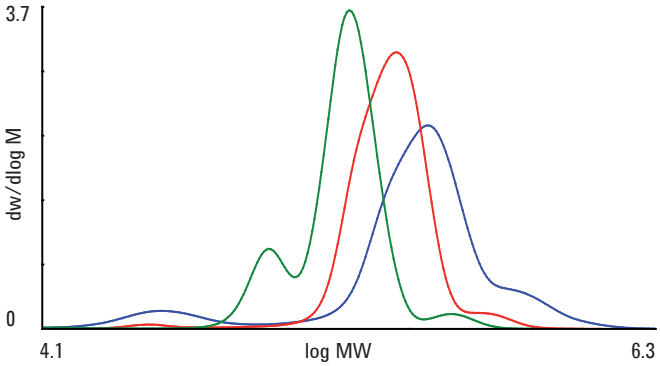

Figure 2. Overlaid molecular weight distributions for the poly(styrene/butadiene) samples

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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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 µm MIXED-C, 300 x 7.5 mm (p/n PL11110-6500)
Injection Volume:	100 µL
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.

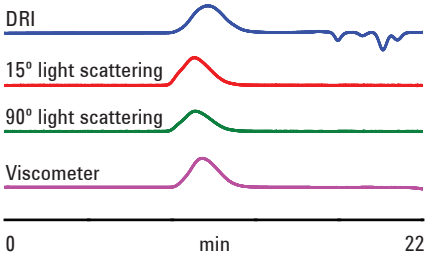

Figure 1. Overlaid multi-detector chromatogram for a sample of polybutadiene

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.

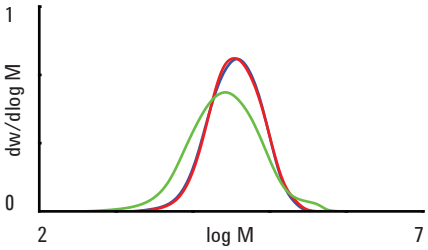

Figure 2. Overlaid triple detector molecular weight distributions of three samples of polybutadiene

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.

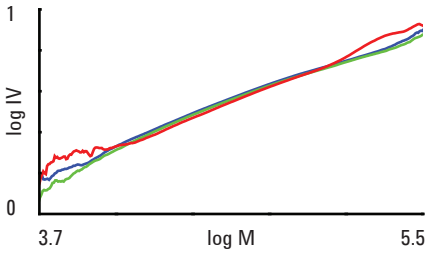

Figure 3. Overlaid Mark-Houwink plots for three polybutadiene samples

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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Agilent Technologies

Analysis of Polyvinyl Butyral by GPC Triple Detection with the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

Greg Saunders, Ben MacCreath
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 µm MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	100 µL
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.

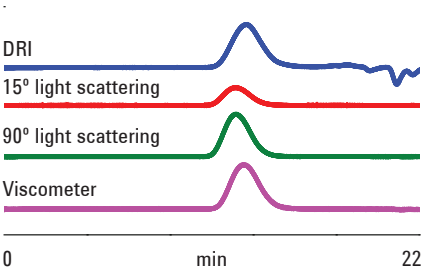

Figure 1. Overlaid multi-detector chromatogram for an example of polyvinyl butyral

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.

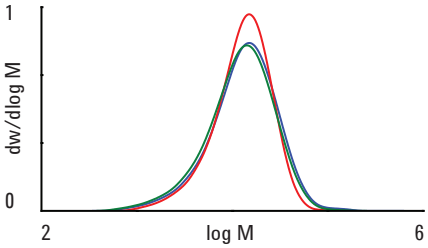

Figure 2. Overlaid triple detector molecular weight distributions of three polyvinyl butyrals

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.

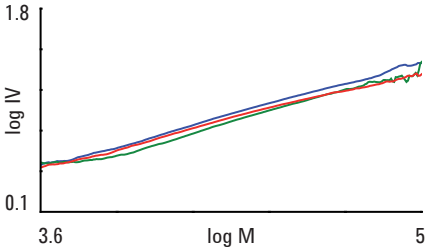

Figure 3. Overlaid Mark-Houwink plots for three samples of polyvinyl butyral

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 a provide a powerful tool for GPC.

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Agilent Technologies

Analysis of Polystyrene Stars by GPC Viscometry with the Agilent 390-MDS Multi Detector Suite

Application Note

Author

Greg Saunders, Ben MacCreath
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 µm 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.

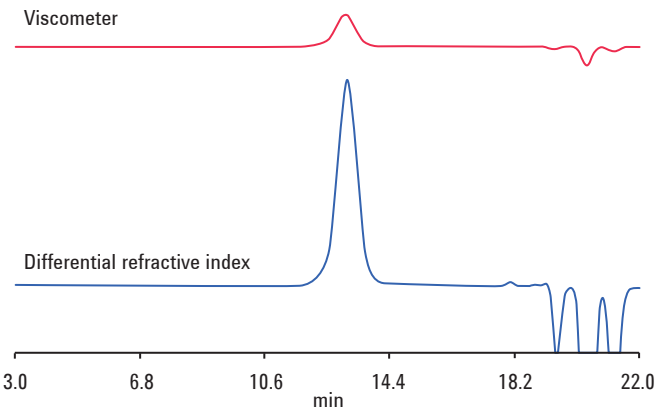

Figure 1. Viscometer and refractive index detection of a 14-arm, star-branched polystyrene

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.

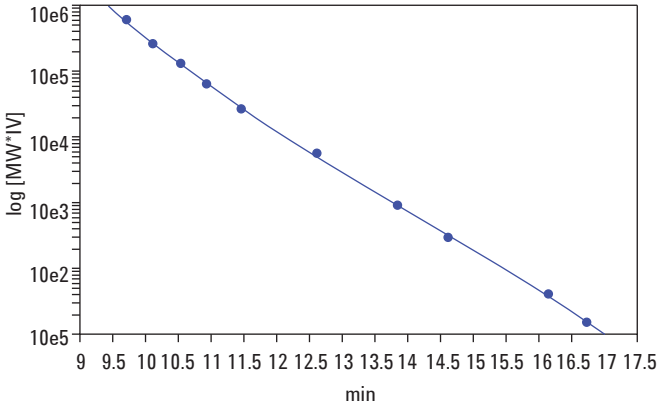

Figure 2. Universal calibration curve using polystyrene standards

Based on this calibration, the molecular weight averages and weight average intrinsic viscosity (IVw) 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	IVw
	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.

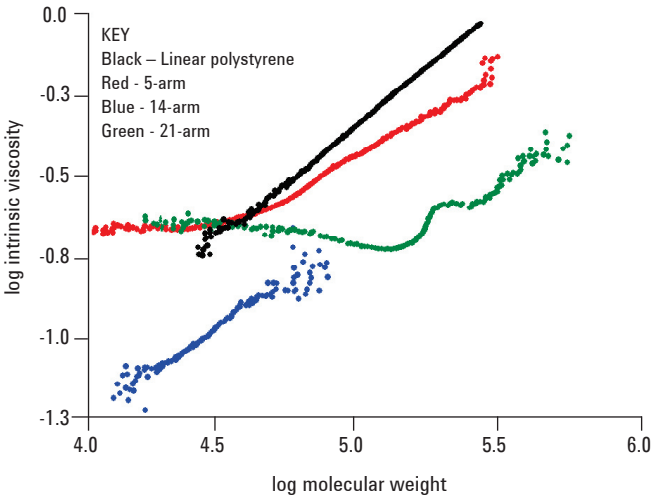

Figure 3. Overlaid Mark-Houwink plots for different star-branched polymers

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.

$$g' = \frac{[\eta]\ \text{star}}{[\eta]\ \text{linear}} \qquad \textbf{Equation 1}$$

From the g′ data, the radius of gyration contraction factor g was calculated using the empirical relationship in Equation 2

$$g' = [a+(1-a)g^{p}]g^{b} \qquad \textbf{Equation 2}$$

where a = 1.104, p = 7, and b = 0.906 (Weissmüller *et al.* 1997).

Figure 4 shows an overlay of Rg 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).

$$g = \frac{3f}{(f+1)2} \qquad \textbf{Equation 3}$$

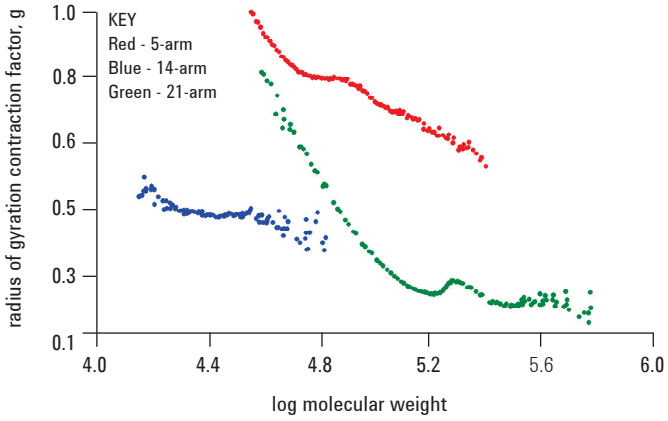

Figure 4. Overlay of Rg contraction g plots for different star-branched polymers

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.

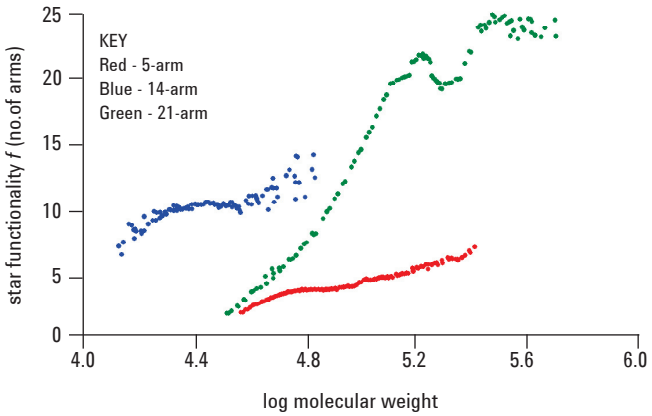

Figure 5. Functionality f plots for the different star-branched polymers

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.

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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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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 µm MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	200 µL
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.

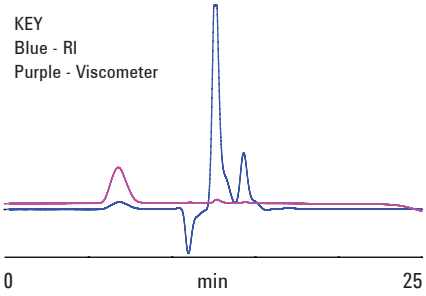

Figure 1. Overlaid multi-detector chromatogram for an example of polyvinyl alcohol

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.

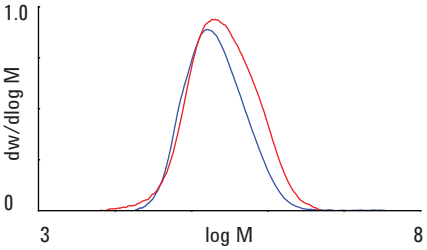

Figure 2. Overlaid multi-detector molecular weight distributions of two samples of polyvinyl alcohol

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.

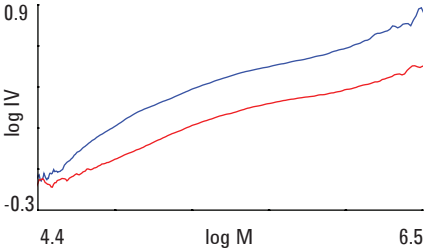

Figure 3. Overlaid Mark-Houwink plots for two samples of polyvinyl alcohol

Conclusion

The results show that the 390-MDS, combined with two PLgel 5 µm 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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Agilent Technologies

Analysis of Bromostyrene by GPC Triple Detection using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

Ben MacCreath, Greg Saunders
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 µm MIXED-C, 300 x 7.5 mm (p/n PL1110-6500)
Injection Volume:	100 µL
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.

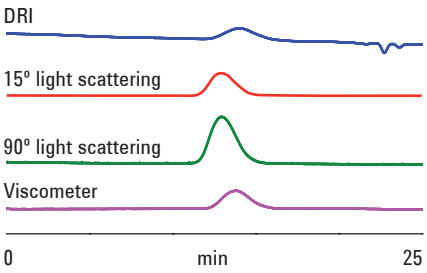

Figure 1. Overlaid multi-detector chromatogram for an example of polybromostyrene

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.

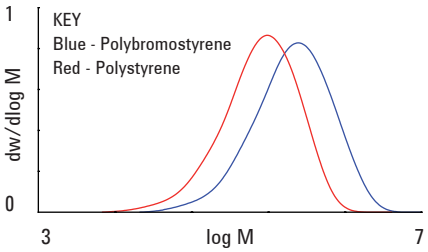

Figure 2. Overlaid triple detector molecular weight distributions of the two samples

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.

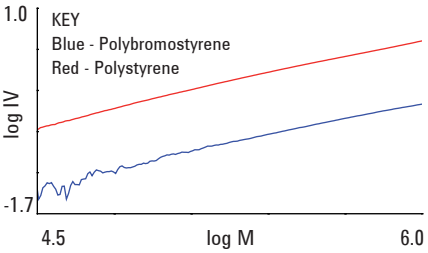

Figure 3. Overlaid Mark-Houwink plots for two samples

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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Agilent Technologies

Analysis of Epoxy Resins by GPC Viscometry using the Agilent 390-MDS Multi Detector Suite

Application Note

Authors

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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 µL
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.

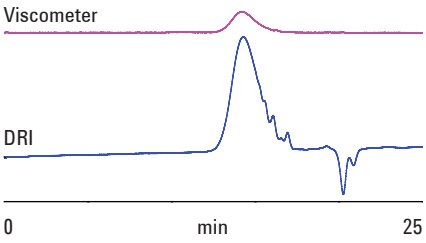

Figure 1. Overlaid multi-detector chromatogram for an example epoxide pre-polymer

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.

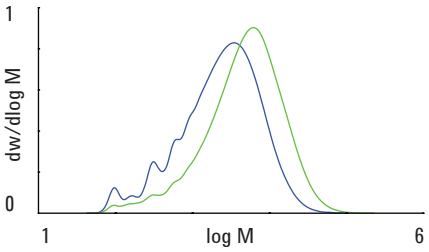

Figure 2. Overlaid multi detector molecular weight distributions of two samples of epoxide pre-polymer

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.

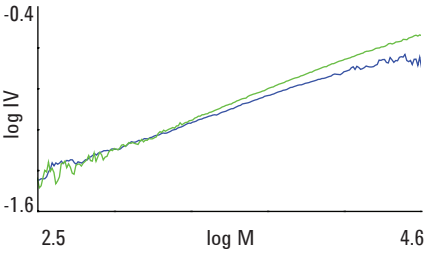

Figure 3. Overlaid Mark-Houwink plots for the two samples of epoxide pre-polymer

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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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 anti-oxidants, 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.

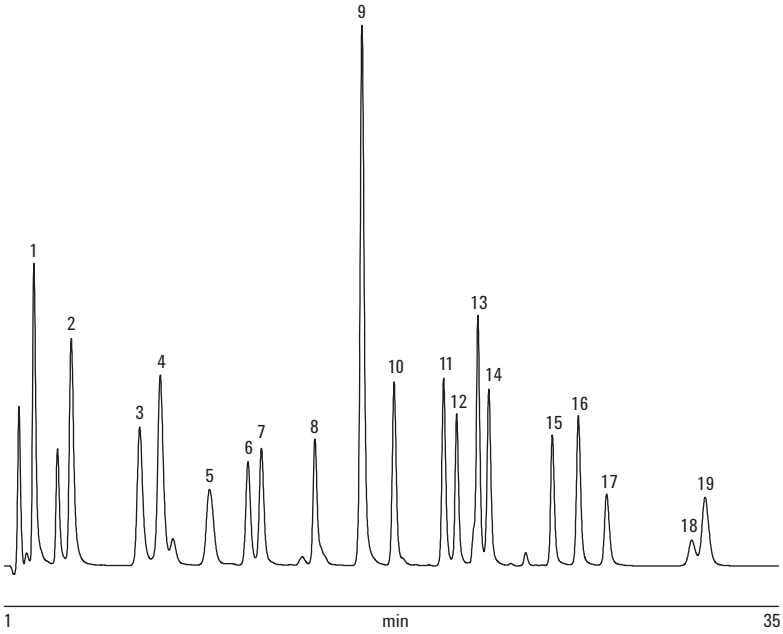

Figure 1. Nineteen UV stabilizers (UVA) and antioxidants (AO) separated by an Agilent Pursuit HPLC column.

Peak identification

1. Lowilite 24 (UVA)
2. Lowilite 20 (UVA)
3. Tinuvin P (UVA)
4. 2,4-di-tert-butylphenol
5. Irganox 245 (AO)
6. 2,6-di-tert-butyl-4-methylphenol (BHT, butylhydroxytoluene) (AO)
7. Irganox 1098 (AO)
8. Hostanox 03 (AO)
9. Irganox 1081 (AO)
10. Lowilite 22/Chimassorb 81 (UVA)
11. Tinuvin 234 (UVA)
12. Irganox 259 (AO)
13. Irganox 3114 (AO)
14. Tinuvin 327 (UVA)
15. Irganox 1010 (AO)
16. Irganox 1330 (AO)
17. Irgafos 168-phosphate (AO)
18. Irganox 1076 (AO)
19. Irgafos 168 (AO)

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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Agilent Technologies

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 CP-Al_2O_3/Na_2SO_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 CP-Al_2O_3/Na_2SO_4 GC column.

Materials and Methods

Technique:	GC-FID
Column:	CP-Al_2O_3/Na_2SO_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 CP-Al_2O_3/Na_2SO_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	*trans*-2-Butene	0.50
12	1-Butene	0.50
13	Isobutene	1.00
14	*cis*-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

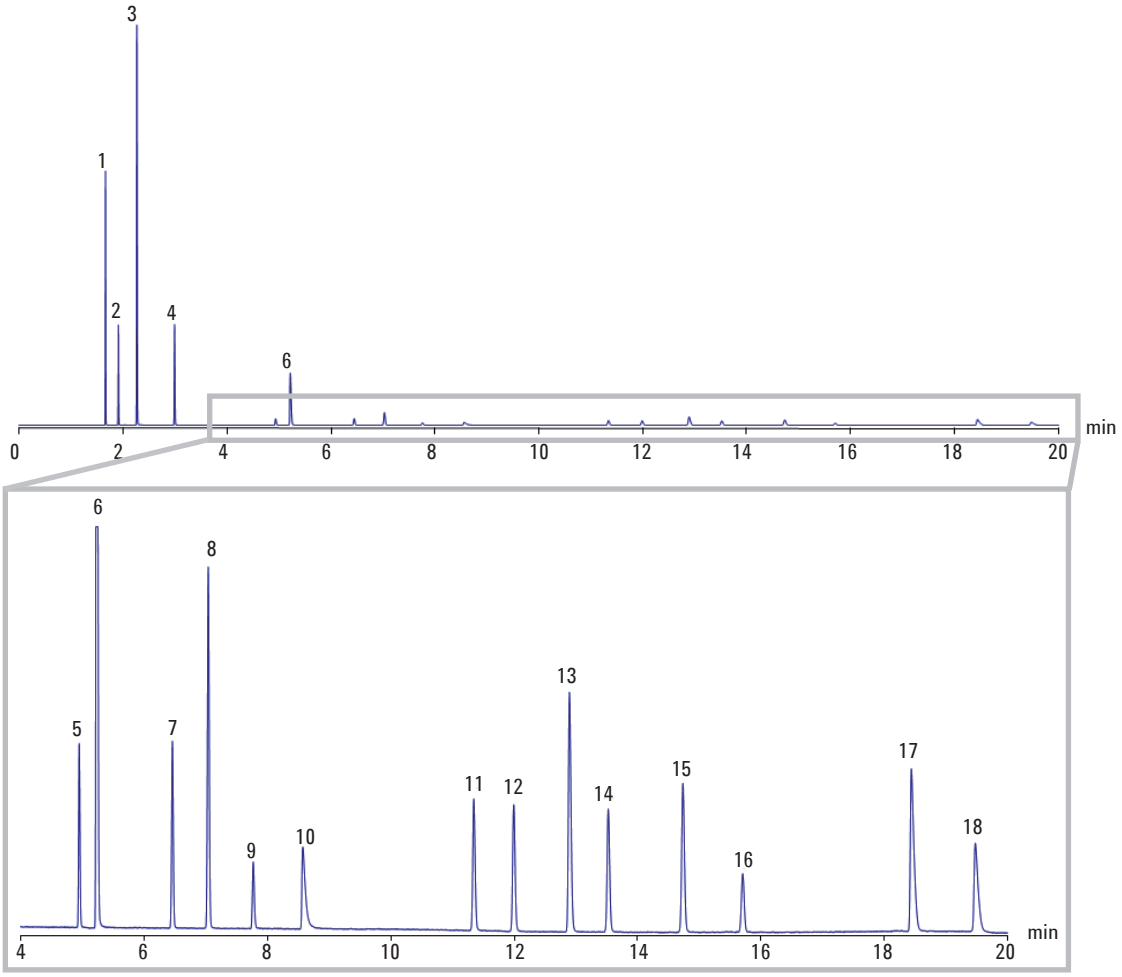

Figure 1. C_1-C_5 hydrocarbons analyzed on a CP-Al_2O_3/Na_2SO_4 column

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.

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Agilent Technologies

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.

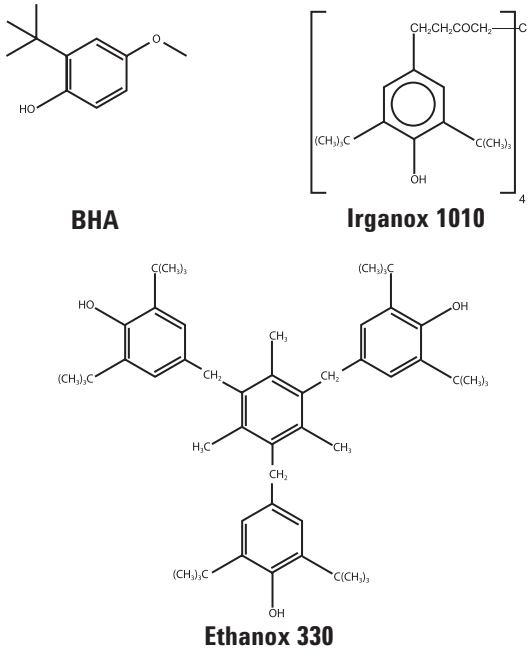

Figure 1. Structure of studied antioxidant additives.

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/µL.

HPLC Conditions

Column	Agilent Microsorb-MV, C8, 4.6 × 50mm, 3.5 µm 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 µL			

MS Parameters

Ionization mode	APCI
Ion polarity	Negative
Trap damping gas	0.8 mL/min
Corona current	–10 µA
API drying gas	14 psi at 400 °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 → 164.5	1.75	100–175
Ethanox 330	773.8 → 717.0	1.70	256–784
Irganox1010	1176 → 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.

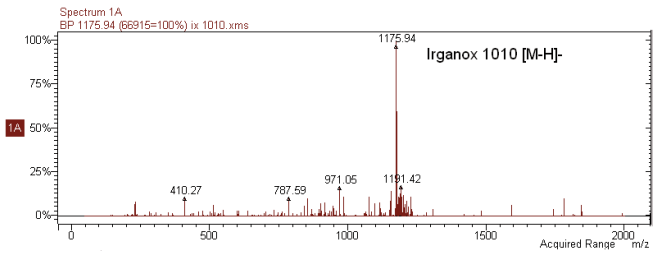

Figure 2. Irganox 1010 infused at 500 pg/µL on the Agilent 500 Ion Trap in full scan mode (50–2000 amu).

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.

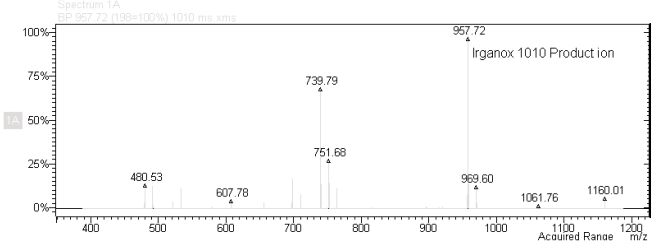

Figure 3. MS/MS product ion spectrum of Irganox 1010 on the 500 Ion Trap. 500 pg/µL infusion.

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.

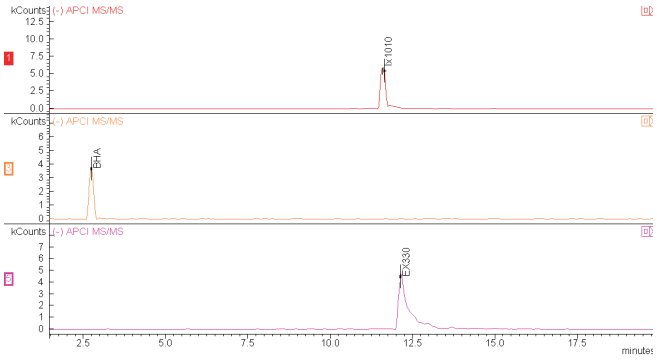

Figure 4. MS/MS chromatograms of Irganox 1010, BHA and Ethanox 330 on Microsorb-MV C8 column (200 pg/µL each component).

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.

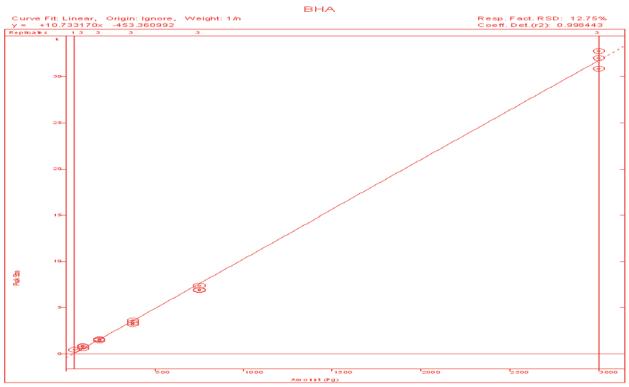

Figure 5. BHA Calibration curve (full scan MS/MS negative APCI) 40–3000 pg on column.

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.

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

Application Note

Atomic Absorption

Author

Jonathan Moffett

Abstract

A simple attachment to enhance the sensitivity of flame atomic absorption spectrometry (FAAS) is described along with some performance results and practical applications. An historical review is also presented.

Introduction

In theory, atomic absorption spectrometry (AAS), is very simple: introduce ground state (metal) atoms into the appropriate instrument's optical path and measure the absorption of light at an appropriate wavelength [1]. The device that generates the atoms is called an atomizer and there are several types:

- Flame
- Vapor generation (cold and heated)
- Graphite furnace
- Cathodic discharge [2,3]

The flame atomization system offers several advantages:

- Relative freedom from interference
- Low capital cost
- Low running cost
- Rapid and simple operation

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Flame atomic absorption spectrometry (FAAS) is routinely used to measure solutions at the parts per million level–equivalent to one gram of element per 1000 kg of solution–which is suitable for a wide range of analyses. The other atomizers offer such benefits as greater sensitivity or minimal sample preparation. However the initial outlay and running expenses can be higher. Much closer attention to the chemistry of the samples is also required. Consequently various schemes have been devised to enhance the sensitivity of FAAS without incurring the expense associated with the other techniques. Some of the more commonly used methods as well as some speculative ideas will be outlined.

Enhancements in FAAS

All methods to improve the sensitivity of FAAS must involve at least one of the following stages:

- Sample preparation/preconcentration
- Nebulization
- Atomization

Each of these techniques is discussed in turn.

Sample

The simplest and cheapest methods for improving sensitivity rely on increasing the concentration of the sample solution. After sample dissolution, one of the following methods of sample preconcentration may be applied:

- Solvent evaporation
- Solvent extraction (for example, APDC/MIBK)
- Ion-exchange (for example, Chelex-100)
- Co-precipitation

While all are used [4], the method of solvent extraction (chelating the analyte and extracting with an organic solvent) is probably the most common. All of the methods are slow, increase the possibility of contamination and need a sample volume of at least 10 to 100 mL. The ion-exchange technique is the only one which could be developed into an automated online system and may overcome the speed and contamination problems.

Nebulization

Nebulization is the physical process of changing the bulk solution into a spray of fine droplets and mixing the droplets with the combustion gases.

The premix (laminar flow) burner assembly is invariably used in commercial FAAS instruments (Figure 1). A venturi is used to create a low pressure zone which draws up and causes nebulization of the solution. An impact bead breaks up the droplets even further. Mixing paddles or baffles may also be used to improve gas mixing and to remove larger droplets. The gas mixture is then passed into the burner and the combustion zone.

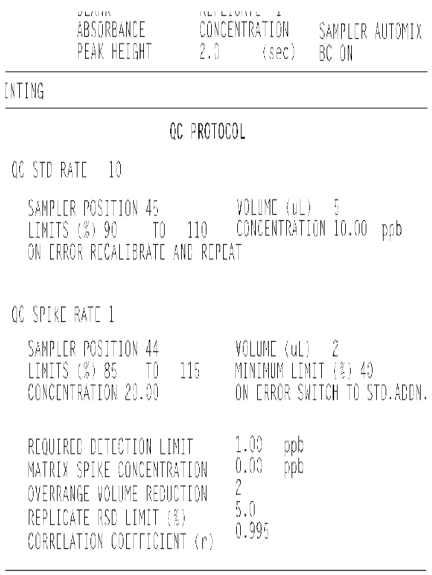

Figure 1. The Agilent Mark-VI spraychamber: (1) nebulizer, (2) ceramic faceplate, (3) adjustable glass bead, (4) drainage tube, (5) dual-head mixing paddle, (6) enhanced slope floor.

The main advantage of the premix burner assembly is its low noise and reproducibility. Agilent Technologies has introduced a new nebulizer [5], spraychamber [6], and a burner [7] to enhance further these benefits. However these improvements were not intended to improve the sensitivity significantly.

The difficulty of improving sensitivity can be demonstrated by using some typical numbers from this process. The nebulization process is only about 10% efficient so an uptake rate of 5 mL/min implies 0.5 mL/min passes through the burner. In most instruments 15–20 L/min of gas also flows through the burner. The effective dilution of the sample is therefore approximately 0.5/15000 or 1/30000.

The spraychamber would appear to be the obvious area to look for improvements in sensitivity. However even after decades of research and experimentation further significant improvements have yet to be made.

A heated spraychamber has been described which improves sensitivity for dilute, low solid solutions [8,9]. It appears likely that the premix spraychamber has been refined to its optimum

performance.

Logically the next potential area for improvement would be the nebulizer. Indeed it is possible to adjust the standard Agilent nebulizer to improve substantially the sensitivity for aqueous copper solutions. However the penalty of this mode of operation is an increased uptake rate and larger droplets in the flame. This would be perfectly acceptable if all samples behaved like aqueous copper solutions. In practice, under these conditions most solutions are known to cause unacceptable problems such as inter-element interferences, signal noise and blocking of the burner or nebulizer. Therefore obtaining sensitivity by increasing uptake rate is not recommended. Other nebulization schemes have been proposed. For example, it is quite feasible to use ultrasonic vibrations for improved nebulization. A different approach is to use electrostatic precipitation of the solid solutes in the aerosol [10-12]. However both techniques have yet to find wide acceptance in FAAS.

Atomization

The physical changes occurring to the solution aerosol in a flame are summarized in Reference 1. Work has been done on trying to understand the process better [8,13,14] but knowledge is still somewhat empirical, even without considering the chemical aspects or interferences. The number of analyte atoms present should in principle depend only on the volume of liquid reaching the combustion zone and the efficiency of atom formation. The flame sensitivity is determined by the number of ground state analyte atoms present in the optical path.

If the removal rate of the atoms from the optical path could be reduced, then an improvement in sensitivity should be observed. Such an approach was pioneered by Robinson [15] on a total combustion burner. Watling [16,17] experimented using a laminar flow burner with a slotted tube above the flame and Brown *et al* [18–20] have done additional work. (It should be mentioned that the Delves cup technique [21] also uses a tube.) This scheme is discussed in more detail in the following section.

A closely related approach pioneered by Lau [22] and investigated by several others [23–31] is to trap the atoms physically on the surface of a narrow diameter water-cooled silica tube placed just above the cone of the flame. After a suitable collecting period, the atom-trap tube is allowed to heat up (by stopping the flow and removing the water) and atoms are released to give an enhanced transient signal. Enhancements of 10 to 30 times have been reported. Practical difficulties have limited the application of this technique.

Atom Concentrator Tube, ACT 80

Watling, in 1977, described a slotted quartz tube which he placed over a conventional AA-6 air-acetylene burner and observed an improvement in analytical sensitivity [16,17].

The commercially available ACT 80 is a quartz tube 150 mm long with two lengthwise cuts. The longer slot is 100 mm × 2 mm, the shorter 80 mm × 2 mm. These cuts are angled at 120 degrees to each other relative to the tube's axis. The ACT 80 is installed in a standard Agilent Vapor Generation Accessory (VGA 76) cell holder and fits on a burner as does the VGA 76 cell. The longer slot is aligned over the burner slot; the shorter faces towards the rear of the instrument away from the holder. As with the VGA 76 cell, only the air-acetylene flame can be used as a hotter flame would destroy the tube. Figure 2 shows the tube in its holder.

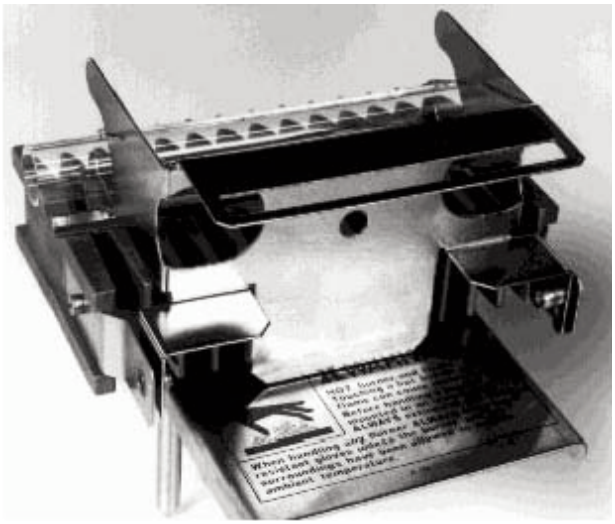

Figure 2. The ACT 80 Atom Concentrator Tube.

The ACT 80 tube must also be optically aligned so that the long axis of the tube coincides with the light beam. It was found in practice that the burner and ACT 80 needed to be lowered about 7 mm (equivalent to the radius of the tube).

Experimental

The performance of the ACT 80 was evaluated using SpectrAA-300/400 spectrometers fitted with a Mark VI spray-chamber and a Mark VA or a Mark VI air-acetylene burner. A VGA cell holder clamp was attached to the burner. Instrument default conditions were used for all measured elements. Where nitrous oxide-acetylene was the default flame, air-acetylene was used instead. Oxidant flow was 13.5 L/min and

acetylene flow 2.0 L/min. Delay time was 20 s and the read time period was 10 s integrated. All measurements were made after the system had been operated at least ten minutes to reach equilibrium.

Results and signal graphics were sent out to a printer. In addition, sample absorbances were sent to an ASCII file for further data manipulation.

Standard solutions were made from BDH (Poole, England) Spectrosol 1000 mg/L standards. Solutions and blanks were acidified with Analar grade concentrated nitric acid to give 0.5% v/v in final volume. Water was distilled from a Pyrex still and deionized with a Waters Milli-Q system to 18 MOhms conductivity.

Practical Points

The ACT 80 must be tilted back out of the way when lighting the flame. Otherwise for tongue-of-flame igniters a significant amount of acetylene builds up inside the ACT 80 with subsequent noisy ignition. Mechanical igniters would physically damage the ACT 80.

Flame composition is also an important factor. It was found that a lean to stoichiometric flame was needed. A rich flame causes soot formation and the signal noise becomes unacceptably high. Elements requiring a rich flame such as arsenic, chromium or molybdenum are therefore not usefully measured using the ACT 80. It was noted with arsenic that each blank signal increased and the blank and solution absorbances tended to give the same value. While this observation is not strong evidence for a memory effect, it cannot yet be eliminated. Alkali and alkaline earth (Group I and II) metals which etch heated silica [22] are also not usefully measured with this technique.

Devitrification of the tube inevitably occurs and starts initially around the inlet slot. The presence of Group I and II metals tends to accelerate this process. However it is possible to aspirate strong solutions (1000 mg/L or greater) of aluminium or lanthanum which provide a protective coating [23] and so retard the devitrification process. This should be done each time the tube is used and must be repeated on a regular basis. Tube lifetimes for samples with simple acidified matrices for example, water or dilute solutions of solids should typically be several hours of continuous operation. At a rate of approximately 200 samples/hour many samples may be determined using one tube.

Lifetime is maximized by continuous operation because cooling and reheating stresses the quartz.

Results and Discussion

Performance

As a guide to performance, improvements in characteristic concentration and detection limit were measured for selected air-acetylene elements. For both values the absorbance of a dilute solution of the analyte must be measured. The absorbance must be determined on a linear portion of the calibration graph and so concentrations were selected to be approximately equal to the characteristic to determine the characteristic concentration (determined using values previously published by Agilent). In practice ten measurements of the solution were made interspersed by measurement of the blank solution. Measurements of each series were done without the ACT-80 and repeated with the ACT-80 fitted (the burner height was reoptimized as needed).

Each element required a large number of readings and to avoid transcription errors the measurements were also printed to an ASCII file. This file was subsequently read by a BASIC program written to extract the absorbance values and perform the necessary calculations. Each solution absorbance was corrected by subtracting the mean of the two adjacent blank readings. The mean and standard deviation of the ten corrected absorbances were used to determine the characteristic concentration and detection limit values. These values were then loaded into a a LOTUS1-2-3 spreadsheet to generate Table 1.

Table 1 also lists, for reference only, Agilent data on detection limit and characteristic concentration values. The values found from this study were obtained using fixed air-acetylene flows and should not be directly compared with values obtained by optimizing conditions for each element.

The following points are drawn from Table 1:

1. All the elements listed showed some improvement in sensitivity. These tended to be consistent as indicated by duplicate runs. Copper was repeated on different systems.
2. All improvements appear to be about 2X to 3X, which reflects the findings of Watling [16,17] and Brown [18–20].
3. Generally there was a corresponding improvement in detection limit. The statistical nature of detection limit means direct comparisons should be interpreted cautiously but since the improvement factor is almost always greater than unity it is inferred that the ACT-80 does improve detection limits. Gold, cadmium and lead appear to show the best improvements.
4. Iron and platinum showed no significant improvements in characteristic concentration or detection limit.

Table 1. Comparison of Detection Limits and Characteristic Concentrations for Selected Air-Acetylene Flame Elements

	Characteristic concentration				Detection limit			
Element	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor
Ag	0.030	0.0134	0.0049	2.7	0.002	0.0019	0.0020	1.0
Au	0.100	0.1226	0.0451	2.7	0.010	0.0148	0.0036	4.1
Bi	0.200	0.2647	0.0919	2.9	0.050	0.0766	0.0177	4.3
Bi		0.2498	0.0903	2.8		0.0414	0.0211	2.0
Cd	0.010	0.0123	0.0054	2.3	0.002	0.0047	0.0011	4.3
Cu	0.030	0.0422	0.0214	2.0	0.003	0.0055	0.0056	1.0
Cu		0.0496	0.0212	2.3		0.0047	0.0034	1.4
Cu *		0.0448	0.0189	2.4		0.0066	0.0065	1.0
Fe	0.050	0.0538	0.0362	1.5	0.006	0.0110	0.0102	1.1
Hg	1.500	2.4278	0.8581	2.8	0.150	0.3094	0.1121	2.8
Mn	0.029	0.0291	0.0141	2.1	0.002	0.0025	0.0019	1.3
Pb	0.100	0.1182	0.0404	2.9	0.010	0.0301	0.0090	3.3
Pt	1.000	2.0064	1.9328	1.0	0.100	0.1220	0.0967	1.3
Sb	0.300	0.3866	0.1244	3.1	0.040	0.0678	0.0462	1.5
Se	1.000	0.3356	0.1010	3.3	0.500	0.1381	0.0927	1.5
Te	0.200	0.2476	0.0903	2.7	0.030	0.0760	0.0492	1.5
Tl	0.200	0.1509	0.0588	2.6	0.020	0.0112	0.0052	2.2

Notes:
-Ten readings were taken and the mean calculated for each value.
-Uptake rate was fixed at 6 mL/min.
-All conditions constant except for burner height ("Ht").
-"Ht" is burner position as shown on the instrument's burner vertical scale.
-Concentrations are about 10 times detection limit (except for Cu which was 100 times).*
-Quoted results for Se used nitrous oxide-acetylene flame. This study used an air-acetylene flame.
-Some elements show replicate results. With Cu, results were from different burners.

The following definitions apply:

$$\text{Detection limit} = \frac{2 \times \text{Standard Deviation} \times \text{Concentration}}{\text{Mean Absorbance}}$$

(IUPAC now recommend detection limit to be 3 times standard deviation, for comparison with literature values 2 times is used here.)

$$\text{Characteristic concentration} = \frac{0.0044 \times \text{Concentration}}{\text{Mean Absorbance}}$$

As an illustration, signal graphics for a standard lead solution measured with and without the ACT-80 tube in place are shown in Figure 3.

Variation in tube dimensions were not investigated, however Brown used a tube 8 mm id (Watling did not specify dimensions). The similarity between the results of this study and the published data indicates that the enhancement is not influenced greatly by the tube dimensions.

Watling suggested the flame characteristics are being affected in a way to encourage atom residence time in the optical path. Whether the flame has less entrained air or the reducing interconal zone is broadened or the diffusion of atoms is slowed down requires more work to elucidate. However, it appears that atoms are not trapped but merely delayed.

The sensitivity of the nitrous oxide-acetylene flame would perhaps also benefit from this technique but its higher temperature (2600 °C) means that the tube would need to be very refractory. The Delves cup method has been applied to the nitrous oxide-acetylene flame [32] so a refractory atom concentrator tube may be feasible.

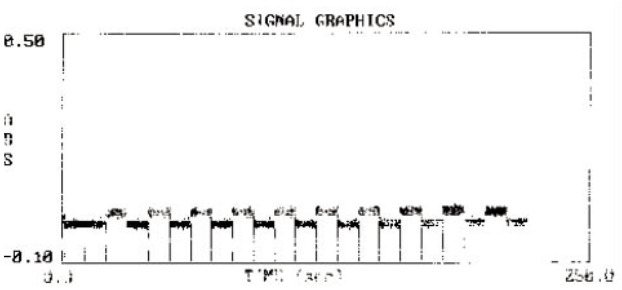

Figure 3(a). Pb signal compared to blank without ACT-80 tube.

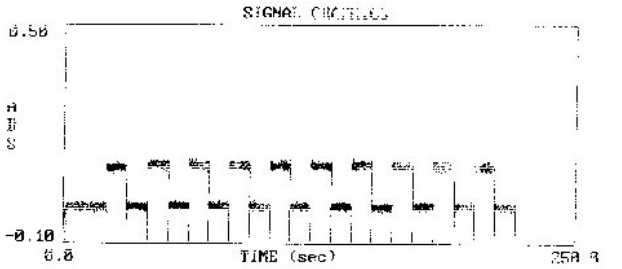

Figure 3(b). Pb signal compared to blank with ACT-80 tube.

Calibration Graphs

Calibration graphs were generated for four selected elements. The highest standard was selected to give about 0.3 Abs without the ACT-80 tube. As shown in Figure 4 the slope is clearly increased as would be anticipated from the improvements seen for the characteristic concentration. The graph for selenium shows that curvature is apparently more pronounced with the ACT-80 in place. However the same curvature is seen with higher solution concentrations without the tube in place. To corroborate this, the highest standard concentration used with the ACT-80 gave an absorbance equivalent to a standard three times the concentration without the tube.

Practical Applications

To illustrate the use of the tube in practical applications, quality control samples supplied by the United States Environmental Protection Agency (US EPA) were measured against aqueous standards. The levels of cadmium, copper and lead in EPA samples #4 and #5 are at or below the quoted detection limits for normal flame operation. A limited amount of National Bureau of Standards SRM 1643b water was also available and used for cadmium determinations.

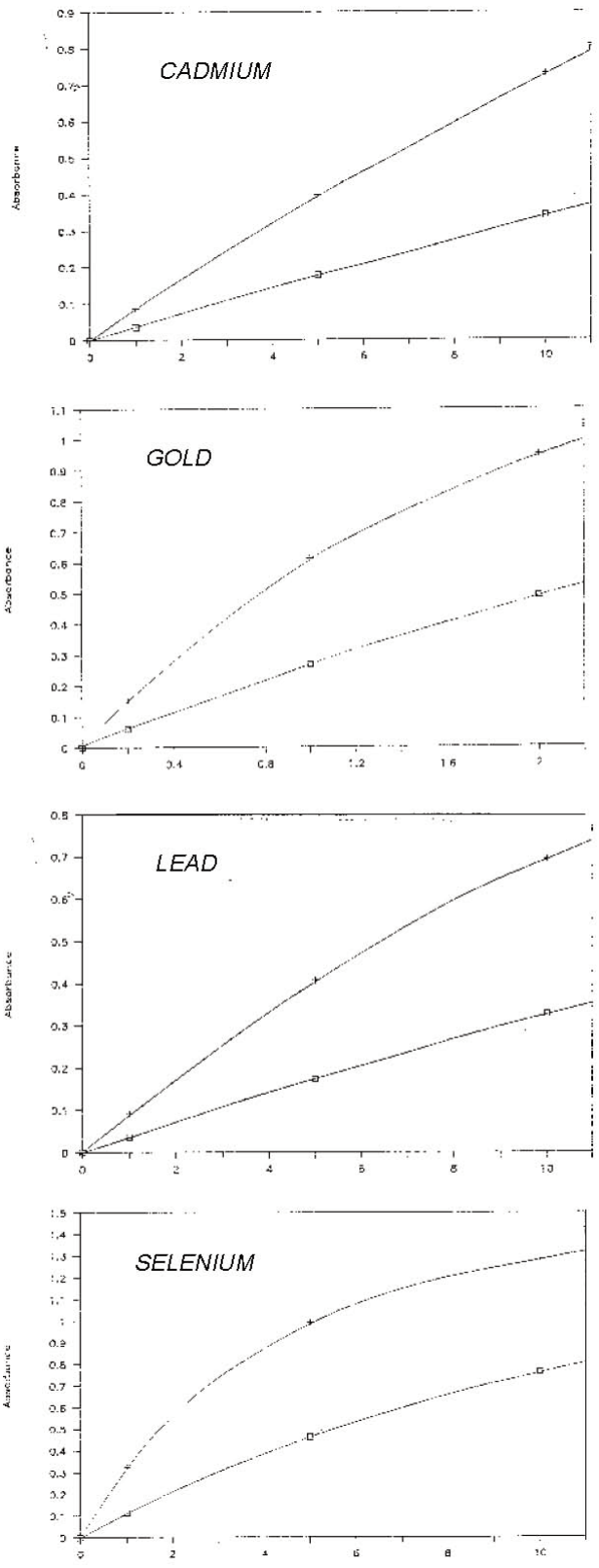

Figure 4. Calibration graphs of selected elements showing improvement in sensitivity. (+ = ACT-80, □ = normal FAAS)

The recommended instrument settings were used for each element. A delay time of five seconds and a read time of three seconds with three replicates were used. With these conditions about 200 solutions could be measured per hour. At least ten readings were taken for each sample to calculate standard deviations. The calibration graphs obtained are shown in Figure 5. A summary of the measured means and standard deviations are listed in Table 2. It can be seen that the measured results agree closely with the certified values even when working at the quoted detection limit for normal flame operation.

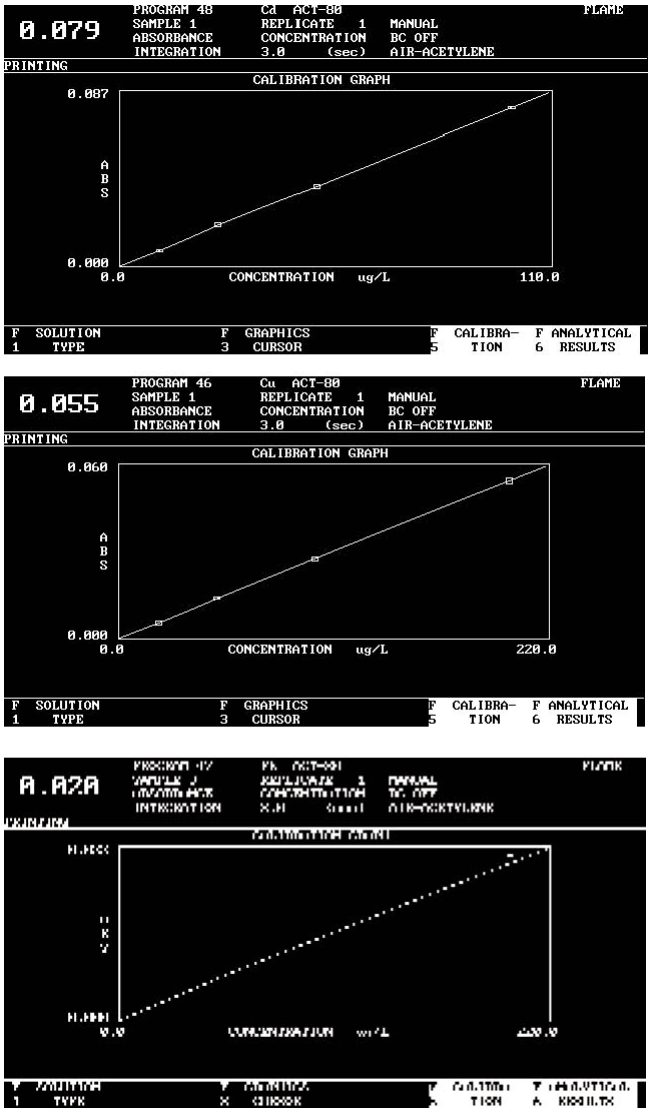

Figure 5. Calibration graphs used to measure quality control samples.

Table 2 Results for Quality Control Samples

Material	Mean ng/g	SD	Mean abs	Comments
Results for Cd using ACT-80				
US EPA sample 4	2.38	0.17		
Found	1.5	0.3	0.001	At quoted detection limit
US EPA sample 5	12.3	1.4		
Found	12.1	0.2	0.009	
NBS SRM 1643b	20	1		
Found	20.6	1.0	0.017	
Results for Cu using ACT-80				
US EPA sample 4	11.3	2.6		
Found	11.7	0.2	0.003	
US EPA sample 5	49.4	3.5		
Found	49.6	0.5	0.014	
Results for Pb using ACT-80				
US EPA sample 4	24.7	3.7		
Found	23.8	2.8	0.002	Twice quoted detection limit
US EPA sample 5	122	14.8		
Found	127.6	2.2	0.013	

Notes: Ten or more readings were taken for each solution.
SD is the standard deviation.

Conclusion

There is a measurable improvement in signal using the ACT-80. The improvements seen are comparable with those previously published. This study shows that there is an improvement in characteristic concentration between two and three times that of the normal FAAS. Detection limits generally show somewhat similar improvements. The ACT-80 is simple, cost effective and offers benefits in low level analyses.

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Sensitivity Enhancement for Flame AAS Using an Atom Concentrator Tube for Elements Dissolved in Organic Solvents

Application Note

Atomic Absorption

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Introduction

The application of a slotted tube placed on an ordinary atomic absorption burner head in order to increase the sensitivity and detection limit for a number of elements in flame-atomic absorption spectrometry (FAAS) was first demonstrated by Watling [1,2]. A very similar technique had been used before in combination with either a nickel "cup" [3] or a tantalum "boat"[4] for the same purpose. The enhancement effect using the combination of a slotted tube and an ordinary acetylene/air flame was later confirmed by several authors who demonstrated that the sensitivity and the detection limit could typically be improved by a factor of 2–5 for easily atomized elements [5–11].

Extraction of aqueous samples into a small volume of an organic solvent after addition of a complexing agent in order to enhance the detection limit is a well established method [12–14]. A concentration factor of at least 20 times can easily be achieved.

Moreover, it is also well known that atomizing organic solutions (especially those rich in oxygen, for example, ketones) can result in 3–5 times better sensitivity for many elements [15] and references therein. Thus the improvement in sensitivity for flame-AAS after extraction should be about 20 x (3–5) = 60 – 100 times.

A combination of extraction into an organic solvent and the atom concentrator tube should thus theoretically result in a total improvement in sensitivity and detection limit of (60 to 100) × (2 to 3) = 120 to 300 times.

Surprisingly, the possibility of combining these techniques has not been investigated. The present paper therefore reports results from a number of experiments using the atom concentrator tube for organic solutions of some metals. For comparison the same solutions have been analyzed without the concentrator tube.

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Experimental

Apparatus

An Agilent SpectrAA-10BQ Atomic Absorption Spectrometer equipped with a Mark VI burner head was used together with an Agilent Atom Concentrator Tube (ACT 80) including a special metal holder constructed to fit the quartz tube to this particular burner–the holder being identical with that used for the quartz tube of the Agilent Vapor Generation Accessory (VGA-77). The quartz tube was 150 mm long with two lengthwise cuts 2 mm wide by 100 and 80 mm long respectively, angled at 120 degrees relative to each other. New tubes were conditioned in the flame by nebulizing a 1% lanthanum nitrate solution for 10–15 min before use in order to prolong the tube life.

The built-in instrument graphics together with an Epson RX-80 printer were used for the recording of the signals and for construction of the calibration graphs.

Gas flow-rates of acetylene for the organic and aqueous solutions were 1.2 and 1.8 L/min respectively. The air flow-rate was 12 L/min in both cases.

The instrument parameters were as follows:

Measurement time	4 sec
Delay time	4 sec
Replicates	3
Recommended SBW and wavelength for each element	Background correction was not used

Experiments

Test solutions containing mixtures of Ag, Cu, Fe Ni and Pb made by appropriate dilutions of a metallo-organic standard mixture of the elements (Conostan S-12 100 ppm (Wt)) with methyl isobutyl ketone (MIBK) were used. A corresponding series of aqueous metal standards were made by diluting a stock solution made from the appropriate amounts of the respective metal nitrates (of A.R. grade) dissolved in water.

The following concentrations were measured: 0, 2, 4, 6, 8 and 10 mg/L of each metal.

The instrument calculated and displayed the calibration graph for each element. From the four graphs: for example, water, MIBK, water + ACT and MIBK + ACT the relative enhancement factors were calculated for each element using the absorbance values for 6 mg/L. The factors are given in Table 1.

Results and Discussion

Both the aqueous and the MIBK-solutions were measured with and without the ACT tube. The No.1 value in the table should be compared with those obtained for No. 4. Both series demonstrated the enhancement factors that can be expected when the ACT is used and that the tube indeed has almost the same effect for organic solutions. Comparison of No. 2 and No. 6 confirms this.

Experiment No. 3 illustrates the total enhancement obtained using an organic solution combined with the concentrator tube relative to aqueous solutions without the tube.

No. 5 shows that atomizing MIBK-solutions without the tube is always more effective than atomizing aqueous solutions with the tube.

The results in Table 1 also confirm that the enhancement effect using the tube is best for the easily atomized elements.

Conclusion

The results show that using a quartz atom concentrator tube for metal compounds in methyl isobutyl ketone solutions will result in the same enhancement of the sensitivity as for aqueous solutions multiplied with a factor of 3–4 due to the beneficial (exothermal) atomizing conditions for organic solvents (see above). This can be utilized in the application of extraction methods for the determination of ions present in water samples thus achieving a much better detection limit relative to that obtained for aqueous samples without extraction.

It is evident that the enhancement effect is caused mostly by the prolonged residence time of the atoms in the light path and is most pronounced for the easily atomized elements. Thus for iron (and nickel) the tube does not seem to offer any advantage at all. This can be explained by the lower temperature inside the quartz tube, this being too low for an effective atomization of the more refractive elements. For such elements it is better to atomize an organic solution without tube.

In many cases, the combination of extraction of metal complexes into organic solvents using an atom concentrator tube for flame-AAS could be an alternative to the graphite furnace technique, for instance for sea-water samples. This approach can be even more attractive if using the extraction equipment recently described for a fast, non-manual extraction of large volumes which can solve the problems associated with the use of the conventional and inconvenient separatory funnels [15].

Alternatively, programmable probe height of the SPS-5 Flame Sampler may be used to advantage in the extraction procedure.

The SPS-5 probe operates through a range of 160 mm. When two immiscible liquids are in a test tube, the probe may be programmed to descend into the upper liquid layer. Thus, the extraction procedure could be as follows:

- Pipette a volume of sample into a stopped test tube, and add a known volume of extractant
- Then pipette a volume of organic solvent into the tube, stopper and shake it
- Remove the stopper, start the SPS-5 Flame Sampler
- The probe will then descend into the upper organic layer. This eliminates the use of separatory funnels.

Table 1. Enhancement Factors for Pb, Cu, Ag, Fe and Ni

	Pb	Cu	Ag	Fe	Ni
MIBK/ACT / MIBK	2.4	1.6	2.8	0.6	1.1
MIBK/ACT / AQ/ACT	3.3	4.0	3.8	2.1	n.d.
MIBK/ACT / aq	8.6	6.0	10.9	2.2	n.d.
AQ/ACT / aq	2.7	1.5	2.8	1.0	n.d.
MIBK / aq/ACT	1.3	2.5	1.3	3.5	n.d.
MIBK / aq	3.6	3.8	3.6	3.6	n.d.

n.d. = Not determined

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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 spectrophot-ometer was passed through a cuvette of polymer pellets.

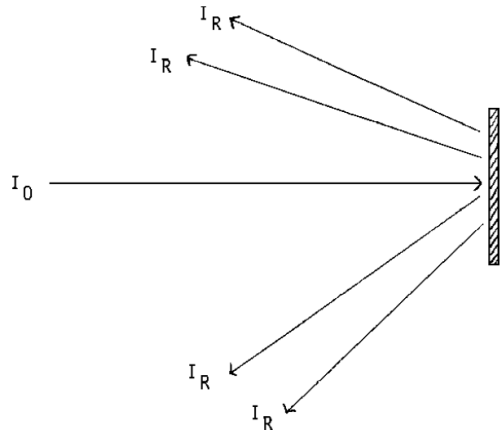

Figure 1A. Reflection off a sample surface.
I_R represents the reflected radiation,
I_0 represents the incident beam

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.

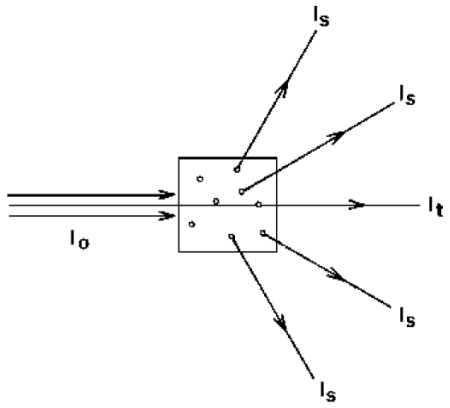

Figure 1B. Scattered transmittance of light through a turbid or opaque sample
I_0 represents the incident radiation
I_S represents the scattered transmittance
I_t represents the transmitted light which has not been scattered

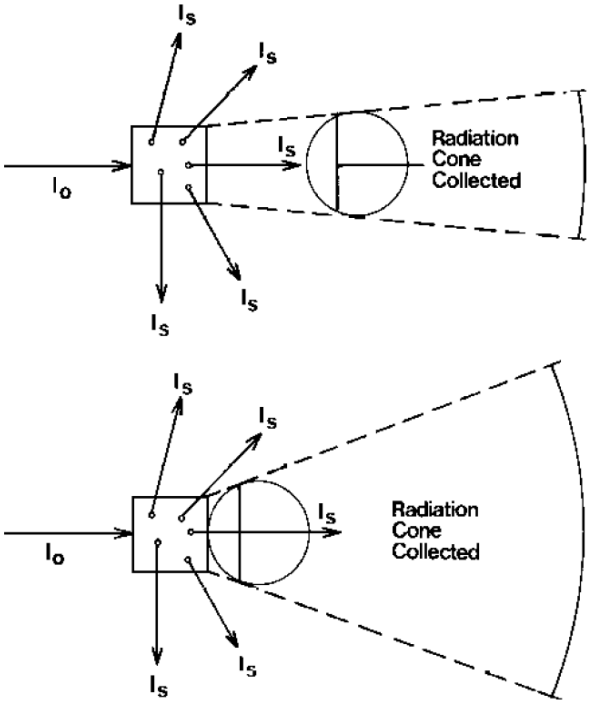

Figure 2A. The light collection efficency is increased by placing the sample so as to be part of the wall of the sphere

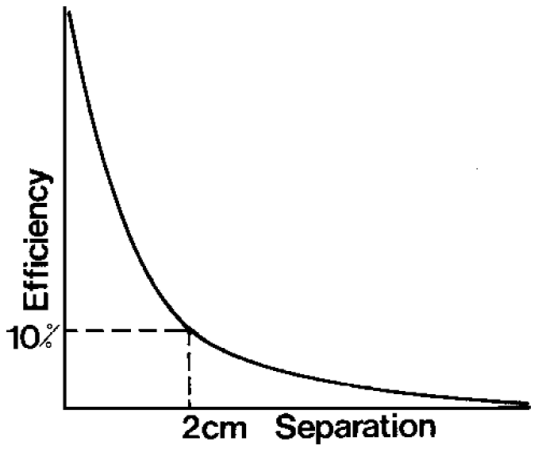

Figure 2B. The relationship between collection efficiency and the separation between the sample and the detector

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.

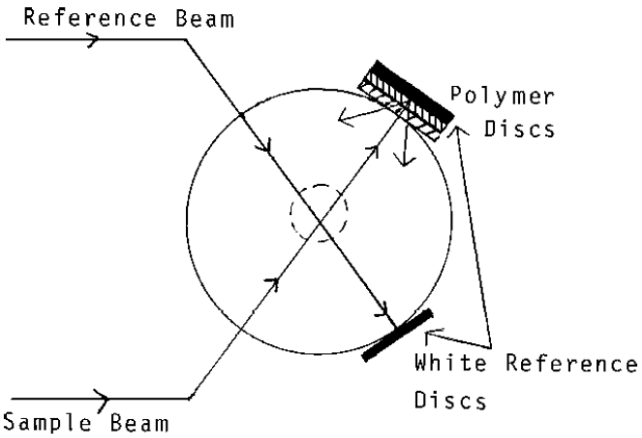

Figure 3A. The sphere configuration for the measurement of the molded polymer discs

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 spectrophoto-meter 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.

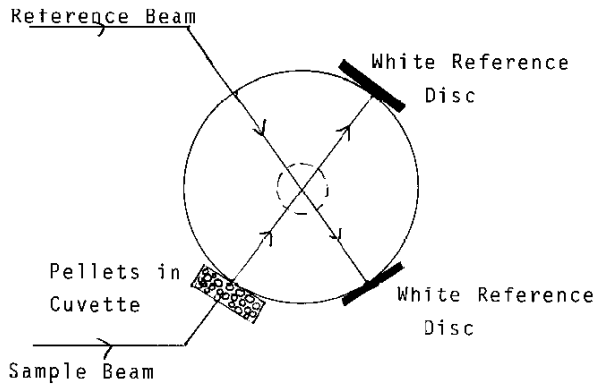

Figure 3B. The sphere configuration for the measurement of the polymer pellets

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.

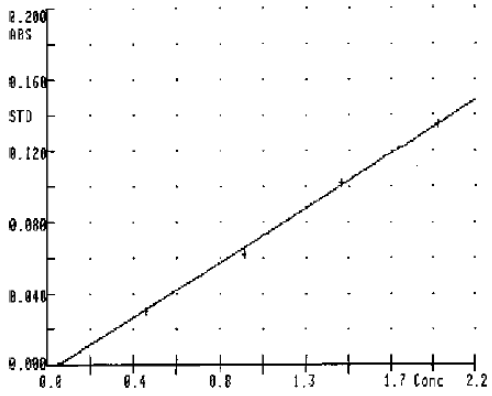

Figure 4. The calibration graph for the analysis of the dye content of the polymer pellets

Table 1. Standard data and statistics for the calibration graph in Figure 4

Standards	Factor	Nom	WL	WL1	WL2
	1.000	Weight	Mode	xxx	xxx
		1.000	2		

	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	Nom	WL	WL1	WL2
	1.000	Weight	Mode	xxx	xxx
		1.000	2		

	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.

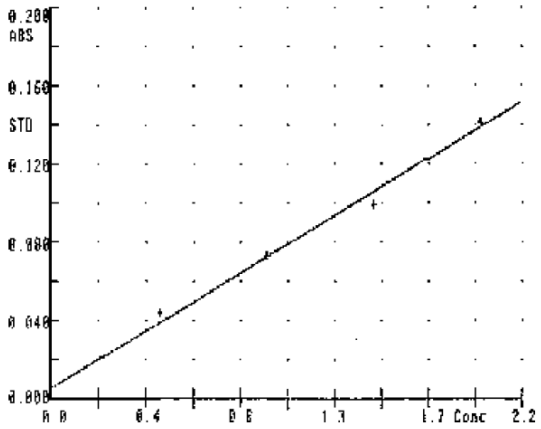

Figure 5. The calibration graph for the molded polymer discs

Table 3. Standard data and statistics for the calibration graph in Figure 5

Standards	Factor	Nom	WL	WL1	WL2
	1.000	Weight	Mode	xxx	xxx
		1.000	2		

	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	Nom	WL	WL1	WL2
	1.000	Weight	Mode	xxx	xxx
		1.000	2		

	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.

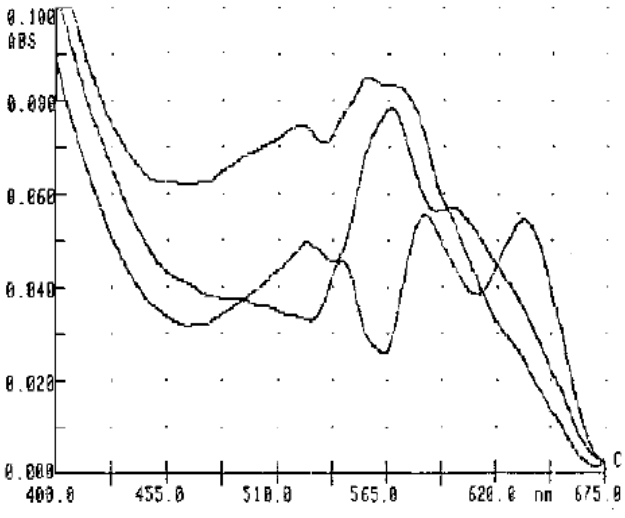

Figure 6. Wavelength scans of various polymer samples with different tints

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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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 technique[1]. 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 Perot[2] 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 (v_1 - v_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 elsewhere[3]. 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.

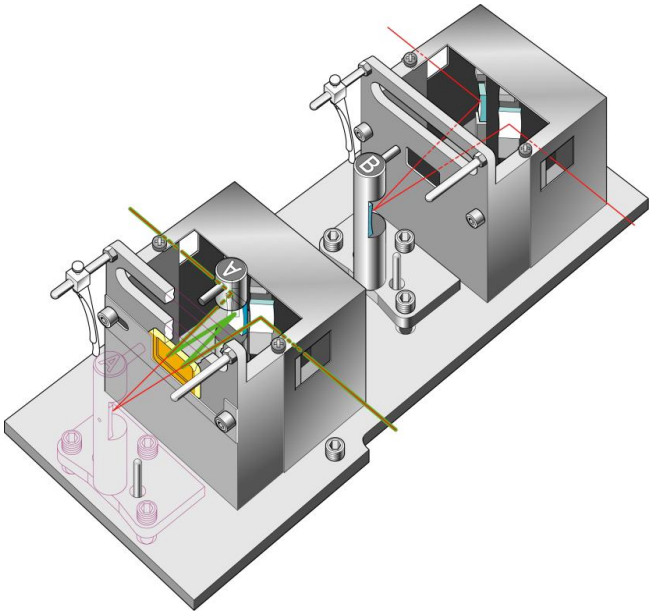

Figure 1. Optical diagram of the Cary VW absolute specular reflectance accessory

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 aligned[6]. 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.

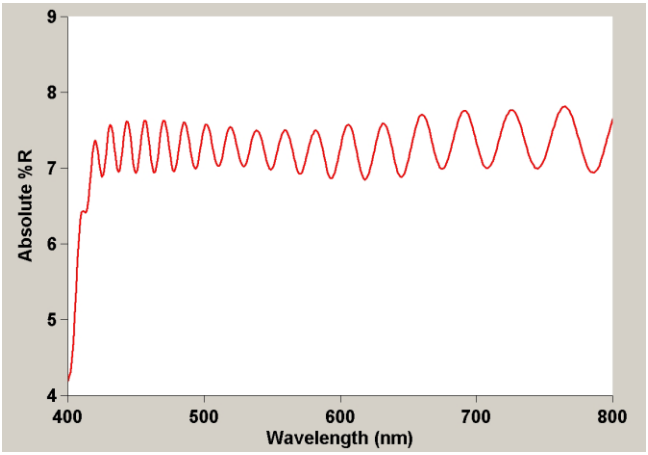

Figure 2. Absolute reflectance spectrum of coated polycarbonate sample showing interference pattern (or fringes) attributable to the coating (thin film) itself

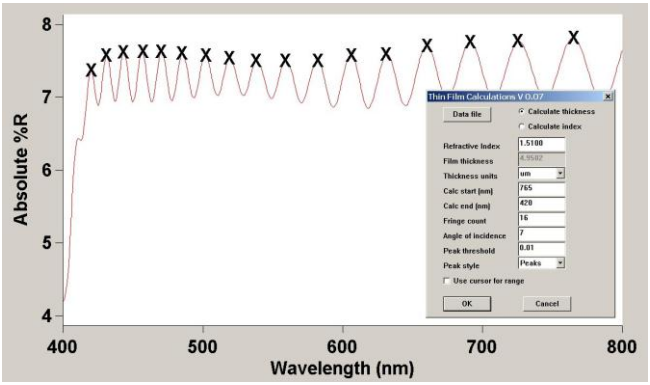

Figure 3. Interference fringes (16) identified between 420 and 756 nm using the Thin Film ADL

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)

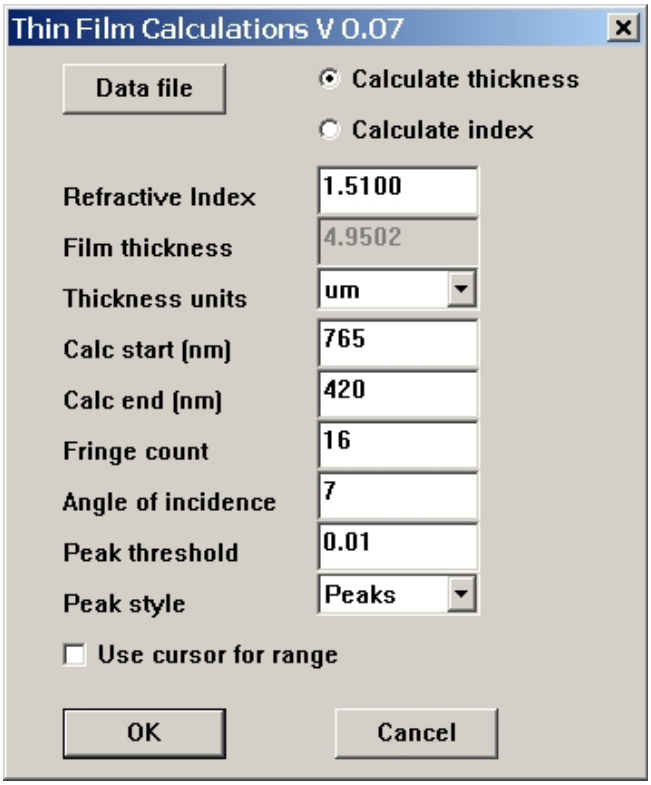

Figure 4. The Thin Film ADL dialog box showing parameters used for film thickness calculation. By selecting the 'Calculate index' option, refractive index may also be determined if film thickness is known

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.

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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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