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Fast Analysis of Landfill Gas by Agilent Micro 3000 GC with Performance-Enhanced System

Application Note

Hydrocarbon Processing

Authors

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Abstract

A fast and accurate solution is provided for landfill gas component analysis by the Agilent 3000 Micro GC configured with two performance-enhanced modules. The individual components are baseline separated in less than 30 seconds by a two-channel configured Micro GC. With this solution, landfill gas can be monitored and controlled easily.

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Introduction

Throughout the world, millions of tons of waste are deposited into landfills. That waste in landfills should be monitored for many years. The waste within the landfill will consist of a wide variety of substances, but a large proportion will be biodegradable, including animal and vegetable matter, paper, and wood. Landfill gas is a complex mixture of gases, but a few gases predominate. Generally, methane, carbon dioxide, and nitrogen are the main components that contribute more than 90 percent of landfill gas. Many other gases can be produced in trace amounts and the exact composition of the landfill gas will vary between different landfill sites, different parts of the same site, and over time. Clearly this gas has to be monitored and controlled. A level of 5 percent of methane in air is explosive. Some other components of landfill gas are dangerous to the health of people living nearby the landfill sites. Landfill operators are also legally required to monitor and control landfill gas on their site.

The Agilent 3000 Micro GC is a powerful tool for the fast analysis of gaseous analytes [1–3], and its robust construction and multiple channel configurations make analyzing complex samples quick and easy. The Agilent 3000 Micro GC with its newly developed performance-enhanced module is specially designed for the analysis of low-concentration components. In this application, an efficient solution is developed to analyze gas components in landfill gas by the Agilent 3000 Micro GC with performance-enhanced modules.

Experimental

Sample

The landfill gas standard was provided by Beijing AP BAIF Gas Industry Co. Ltd. (Beijing, China). The compositions and concentrations are shown in Table 1.

Table 1. Standard Compositions and Their Concentrations

Component	Concentration (µL/L)
CH_4	399.5
C_2H_6	174.5
CO_2	132.5
O_2	100
H_2S	254
N_2	Balance gas

Configurations and Analytical Conditions

The Agilent 3000 Micro GC configured with two performance-enhanced modules is used for the fast analysis of landfill gas. The PLOT U column channel (PLOT U, 8 m × 0.32 mm × 30 µm) is responsible for the separation of CO_2, C_2H_6, and H_2S. The second channel is a molecular sieve (MS) column (PLOT-U, 3 m × 0.32 mm/MolSeive 5Å PLOT, 10 m × 0.32 mm) for the separation of O_2, N_2, and CH_4. The injectors used are the variable-volume type for PLOT U channel and backflush mode for MS channel; helium was used as the carrier gas. The analytical conditions are shown in Table 2.

Table 2. Analytical Conditions of Two-Channel Micro GC

Channel	Molecular sieve (PLOT-U, 3 m × 0.32 mm/ MolSeive 5Å PLOT, 10 m × 0.32 mm)	PLOT U (8 m × 0.32 mm × 30 µm)
Sample inlet temp. (°C)	60	60
Injector temp. (°C)	80	80
Column temp. (°C)	70	70
Inject time (ms)	100	100
Run time (s)	100	100
Column pressure (psi)	30	30

Results and Discussion

CO_2, C_2H_6, and H_2S were separated within 60 seconds. The analytical time for separation of O_2, N_2, and CH_4 was also less than 60 seconds. Figure 1A shows the chromatogram of channel A, and Figure 1B shows the chromatogram of channel B.

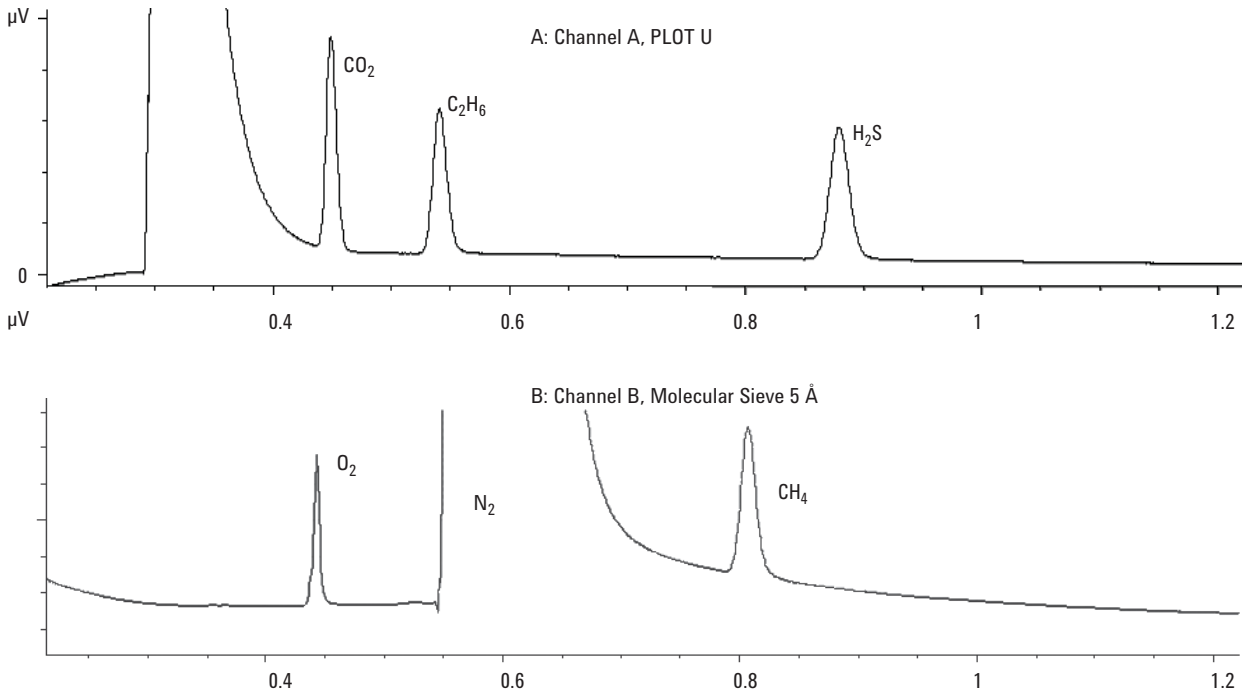

Figure 1. Chromatograms of landfill gas on PLOT U channel (A) and MS channel (B).

Table 3 shows the run-to-run repeatability by relative standard deviations (RSDs) of the peak height. The RSDs of separated components are all less than 1%, showing that the Micro GC is stable and reliable.

Table 3. Reproducibility of Two-Channel Micro GC Solution

Compound	Avg. R.T.	Avg. peak height	Peak height S. D.	R. S. D. (N = 10)
O_2	0.443	193963.28	1671.26	0.86%
N_2	0.553	1211399.24	6496.73	0.54%
CO_2	0.447	1868.49	17.61	0.94%
CH_4	0.807	1878.01	6.44	0.34%
H_2S	0.811	1336.20	18.35	1.37%

Conclusions

The Agilent 3000 Micro GC with performance-enhanced modules provides a fast and reliable method for the analysis of landfill gas samples. Six key components can be separated in less than
60 seconds with good repeatability, H_2S, the sulfur compound existing in landfill gas, can also be detected on such a two-module micro GC with the detection limit of 20 ppm (V/V).

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Ultra-Fast Total Petroleum Hydrocarbons (TPH) Analysis with Agilent Low Thermal Mass (LTM) GC and Simultaneous Dual-Tower Injection

Application Note

Environmental

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Abstract

This application note is targeted for ultra-high productivity of total petroleum hydrocarbons (TPH) analysis in environmental laboratories. Agilent's Low Thermal Mass (LTM) technology is employed here to perform ultra-fast gas chromatographic (GC) separations. The LTM technology uses a column module combining a fused silica capillary column with heating and temperature-sensing components wound around it, which can be heated and cooled very efficiently. In this application note, the speed of analysis for the hydrocarbon group eluting between C_{10} and C_{44} can be dramatically increased to about 13 times faster than a conventional method. In addition, the ultra-fast cooling function of an LTM module can reduce the total GC cycle time to 5.1 minutes. The simultaneous dual-tower injection from Agilent is used to further double productivity. The final result for TPH analysis productivity is 5.1 minutes per two samples.

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Introduction

Total petroleum hydrocarbons (TPH) is a term used to describe a large family of several hundred chemical compounds that originally came from crude oil. Many environmental laboratories in the world are analyzing the total amount of TPH at a site to evaluate the water or soil contamination by TPH, such as oil, gasoline, diesel fuel, etc.

The Agilent Low Thermal Mass (LTM) system (except for an external power supply) is built into a replacement GC oven door, which is mounted as an add-on to an Agilent 7890A GC. A version is also available for the Agilent 6890 GC. The key component of LTM system is the LTM column module combining a fused silica capillary column with heating and temperature-sensing components wound around it. The LTM system can heat and cool the column very efficiently for significantly shorter analytical cycle times as compared to conventional air bath GC oven techniques involving much higher thermal mass.

The GC method translation software from Agilent is a calculator used to scale a method between different column dimensions with equal or increased speed. In this application note, a 40-minute separation with a 30-meter column is translated into a 20-minute separation with a 15-meter column at first, without LTM technology. Then the method is further translated for LTM use with a 5-meter column within 3.1 minutes.

As a base for the LTM system, the Agilent 7890A can provide dual complete analysis channels. With a configuration of dual injection towers, single sample tray, dual split/splitless inlets, and dual detectors, the simultaneous TPH analysis can be accomplished to double lab productivity, in addition to the speed gains realized with LTM.

Experimental

Standard Preparation

The custom alkanes mix (cus-908) from Ultra Scientific (North Kingstown, Rhode Island, U.S.) contains n-alkanes from n-decane (C_{10}) to n-tetratetracontane (C_{44}) in hexane at the concentration listed in Table 1. Dilutions in dichloromethane are made up at 1.0, 5.0, 10.0, 50.0, and 100.0 µg/mL concentrations.

Table 1. Custom Alkanes Mix

Component	Concentration, mg/mL	Component	Concentration, mg/mL
n-decane	0.2	n-tetracosane	0.1
n-dodecane	0.1	n-hexacosane	0.1
n-tetradecane	0.2	n-octacosane	0.1
n-hexadecane	0.1	n-triacontane	0.1
n-octadecane	0.1	n-dotriacontane	0.1
n-eicosane	0.1	n-hexatriacontane	0.1
n-docosane	0.1	n-tetracontane	0.1
n-tricosane	0.2	n-tetratetracontane	0.1

Sample Preparation

Soil samples are mixed with sodium sulfate to remove excess moisture and then sonicated with 60-mL aliquots of dichloromethane, three times. Water samples are placed in a 2-L separate funnel. A 100-mL aliquot of dichloromethane is added and the mixture is shaken automatically for about 2 minutes. The liquid-liquid extraction is repeated two more times. For both matrices, the extract is concentrated on a steam bath to either 5 mL for a soil sample or 1 mL for a water sample. The extracts are not routinely treated with silica gel, unless specified.

Instrumentation and Conditions

Agilent 7890A GC with LTM system, consisting of:

G3440A	7890A Series GC system
#112	Split/splitless inlet with EPC (2)
#211	Capillary FID with EPC (2)
	Autoinjector modules (2)
	Autosampler tray module
G6579A	LTM system bundle for 2-channel LTM operation, for use with standard size LTM column modules (100–2000LTM DB-5 5 M × 0.32 mm id, 1.0 µm standard 5-inch LTM column module)

ChemStation 32-bit version B.04.01

Table 2. Gas Chromatograph Conditions

	Original 1X Method	2X Method	LTM Method
GC			
Agilent Technologies 7890A			
Inlet	EPC split/splitless	EPC split/splitless	EPC split/splitless
Mode	Constant pressure	Constant pressure	Ramp pressure
Injection type	Split	Split	Split
Injection volume (µL)	1.0	1.0	1.0
Inlet temp (°C)	300	300	300
Pressure, nominal (psig)	30	14.319	13.1 (0.1 min), 11.27 psi/min to 30 (1.5 min)
Liner	Helix liner, open ended, deactivated (p/n 5188-5396)	Helix liner, open ended, deactivated (p/n 5188-5396)	Helix liner, open ended, deactivated (p/n 5188-5396)
Split ratio	2:1	2:1	2:1
Gas saver	20 mL/min after 2 min	20 mL/min after 2 min	20 mL/min after 2 min
Gas type	Helium	Helium	Helium
Sample overlap	2 min after end of GC run	2 min after end of GC run	2 min after end of GC run
Oven	GC Oven	GC Oven	LTM module (p/n G6579A) with GC oven 300 °C for 3.1 min
Initial oven temp (°C)	40	40	40
Initial oven hold (min)	1	0.5	0.1
Ramp rate (°C/min)	10	20	200
Final temp (°C)	320	320	340
Final hold (min)	11	6.5	1.5
Run time (min)	40	21	3.1
Cooldown time (min)	5.4	5.4	2
Cycle time (min)	45.4	25.4	5
Column			
Type	DB-5 (p/n 123-5032)	DB-5 (p/n 123-5012)	DB-5 (p/n*)
Length (m)	30	15	5
Diameter (mm)	0.32	0.32	0.32
Film thickness (um)	0.25	0.25	1.0
FID			
Telperature (°C)	300	300	300
H_2 flow (mL/min)	30	30	30
Air flow (mL/min)	400	400	400
Makeup flow (mL/min)	25	25	25
Sampling rate (Hz)	50	50	50

*100–2000LTM DB-5 5M x 0.32 mm id, 1.0 µm standard 5-inch LTM column module

Results and Discussion

Ultra-Fast Separation of n-alkanes Mixture with LTM System and Scale-Up Using the GC Method Translator

The application is started with the analysis of a standard mixture of n-alkanes, containing n-C_{10}, n-C_{12}, up to n-C_{44}. Figure 1 compares the chromatogram of the standard mixture using three different methods in the same time scale. With the LTM system, the GC run time can be more than 10 times faster than conventional methods. In terms of cooling down, the classical GC oven such as 7890 fast oven will take about 5.4 minutes from 320 to 40 °C. Relatively, the LTM system has a much lower thermal mass, which can perform ultra-fast cooling. In this case, the LTM system will take about 2 minutes from 340 to 40 °C, for dual parallel LTM modules. In addition, sample overlap of the 7890 sample tray can prepare the sample after the end of the last GC run parallel with GC oven cooldown. The resulting cycle time for LTM is 5.1 minutes, which means about nine times faster than the conventional method.

Resolution is also a concern with fast analysis. Figure 2 is the expanded view of Figure 1 with the nominal time scale, which demonstrates that all the peaks of n-alkanes are baseline separated, even with the nine-times-faster LTM method (speed calculated by total cycle time). The result is calculated by total amount of TPH, not by the individual peak amount; peak-grouping of ChemStation is employed here. The calibration is checked by injecting the standard mixture in different concentration levels, ranging from 1 to 100 µg/mL. The calibration curve of the LTM method is displayed in Figure 3, with average n-alkanes response factor by peak-grouping.

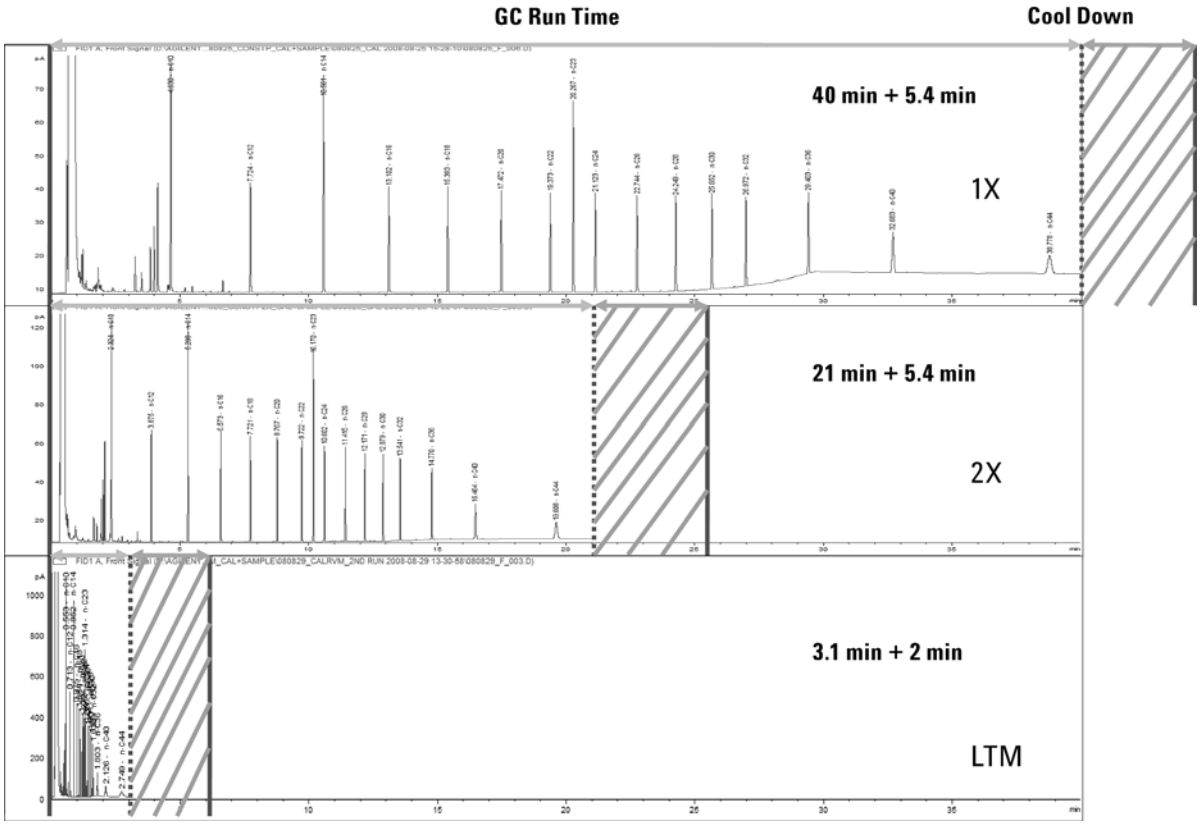

Figure 1. Comparison of conventional method and LTM method.

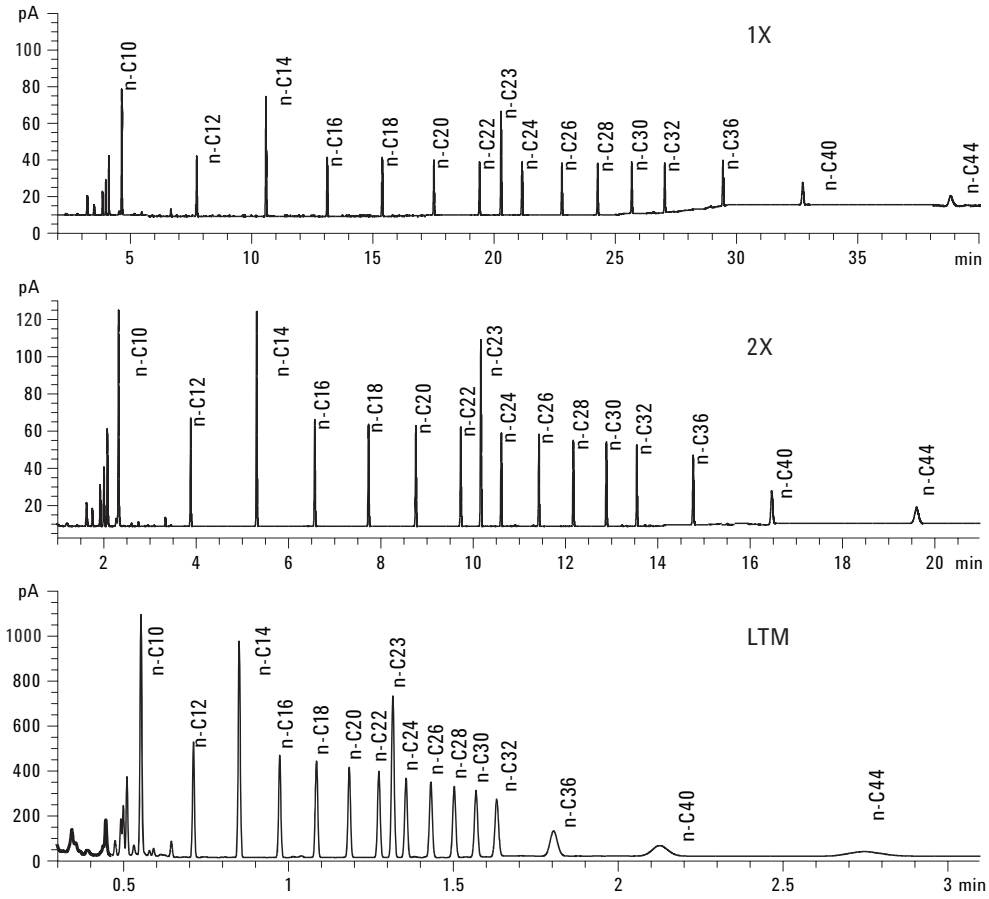

Figure 2. Expanded view of Figure 1, with the nominal time scale.

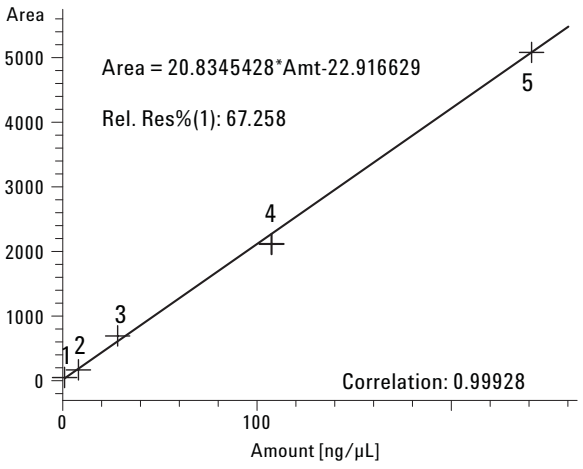

Figure 3. Calibration curve of LTM method after peak-grouping.

Simultaneous Dual-Channel Analysis with Agilent Dual-Tower Injection

Agilent 7890A and 6890 GCs make dual-channel analysis possible, with the configuration of a single sample tray and dual injection towers, inlets, columns, and detectors. Typically, a dual-channel configuration is used to identify target compounds in one GC run, using different retention time in columns of different polarity. The purpose here is to double lab productivity using dual identical channels at a much lower cost compared to two single-channel instruments. ChemStation can provide different choices for final data file generation. Figure 4 shows one option of detection signal setting for separating the dual-tower injection into two individual data files. Figure 5 is the chromatogram of two real samples with simultaneous dual-tower injection.

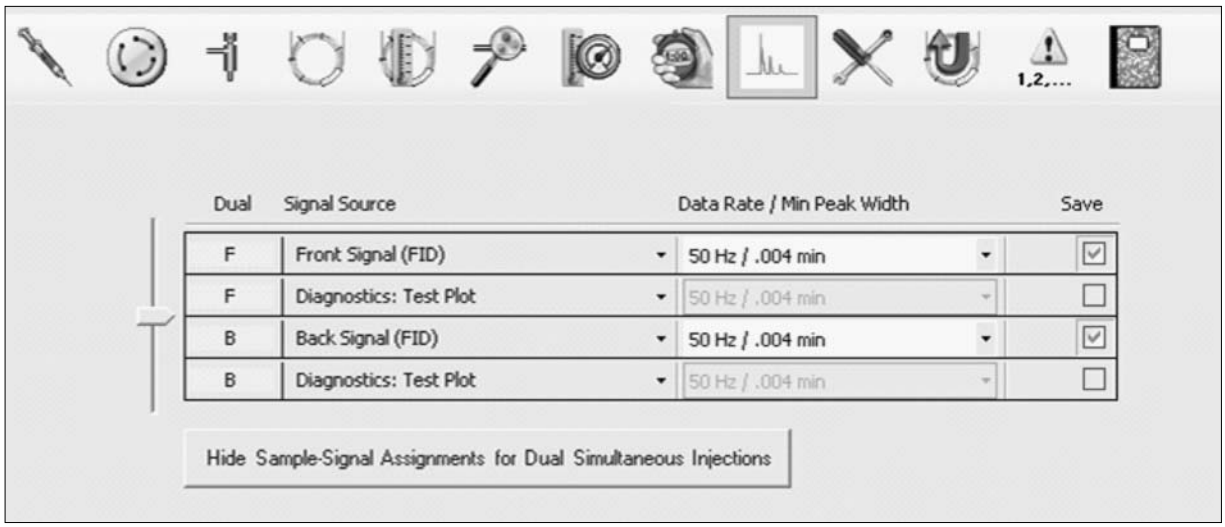

Figure 4. Signal setting for dual-tower injection to generate two individual data files.

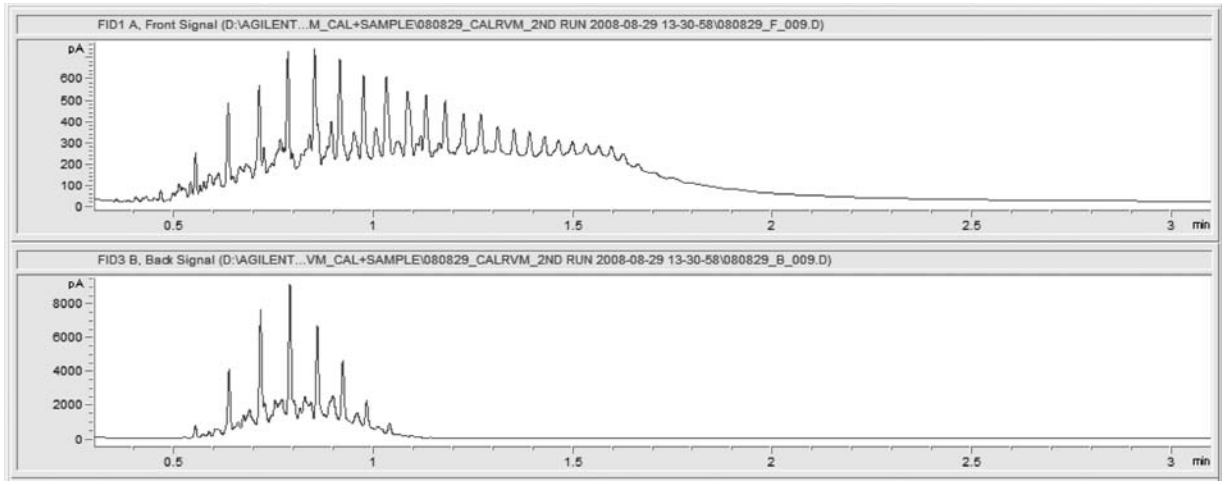

Figure 5. Chromatogram of two real samples with simultaneous dual-tower injection.

Quantitative Analysis of TPH with Peak-Grouping and Peak-Summing

Peak-grouping is used to average each n-alkane response factor. With this average response factor, the nominal calibration curve can be used for quantitation of each peak, including unidentified peaks eluting between n-C_{10} and n-C_{44}. In this case, the compound peak-grouping details and unidentified peak calibration settings can be seen in Figure 6.

Another requirement for TPH analysis is quantitation across the whole eluting time range between n-C_{10} and n-C_{44} to calculate all petroleum hydrocarbons not only n-alkanes. Baseline-holding and peak-summing in the ChemStation integration events table are necessary to meet this requirement; the related setting can be seen in Figure 7. For example, the integration result of real sample is shown in Figure 8.

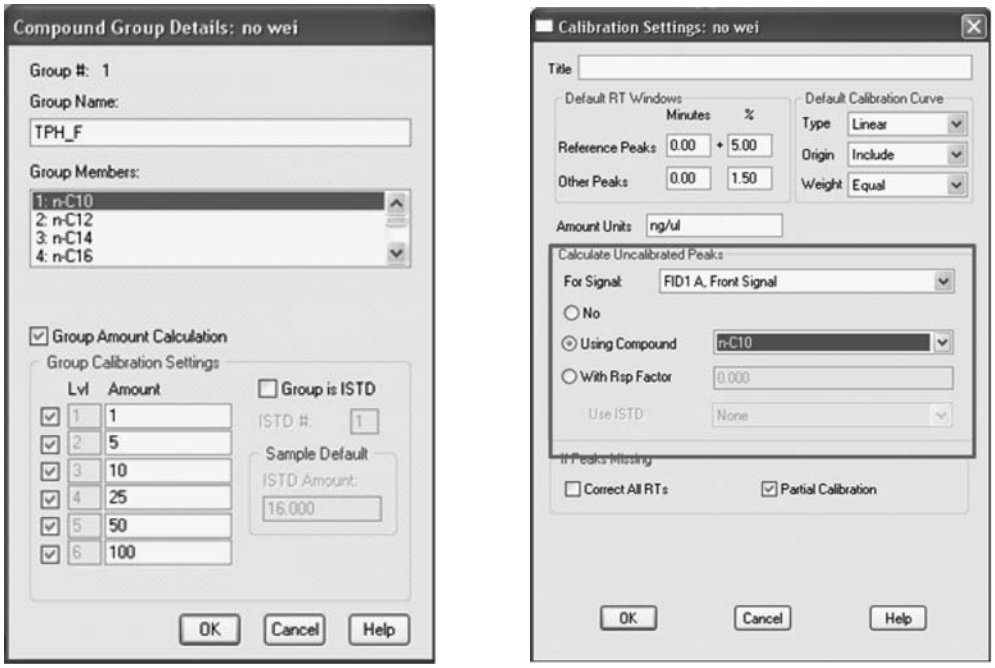

Figure 6. Peak-grouping (left) and unidentified peak calibration setting (right) in ChemStation.

Time	Integration Events	Value
Initial	Slope Sensitivity	10
Initial	Peak Width	0.04
Initial	Area Reject	1
Initial	Height Reject	1
Initial	Shoulders	OFF
0.000	Integration	OFF
4.560	Area Sum	ON
4.560	Baseline Hold	ON
4.560	Integration	ON
39.100	Integration	OFF
39.100	Area Sum	OFF

Figure 7. Baseline-holding and peak-summing setting in the ChemStation integration events table.

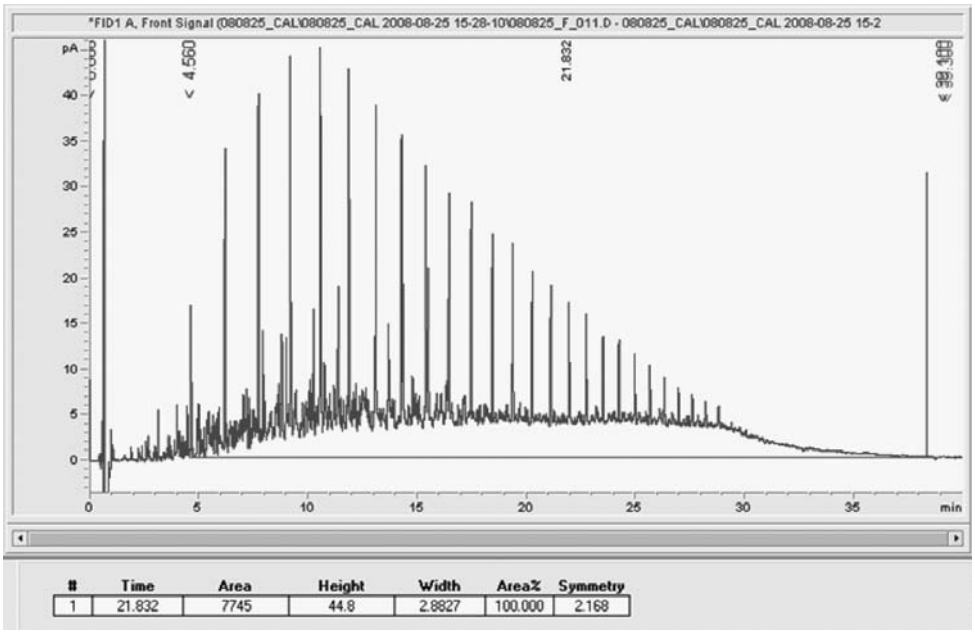

Figure 8. Integration result of real sample after baseline-holding and peak-summing.

Real Sample Analysis

After calibration by peak-grouping and integration through peak-summing, the quantitation result can be reported as the total amount of TPH in a real sample. As a comparison of quantitation results with three different acquired methods, Table 3 demonstrates that the real sample analysis result by the ultra-fast LTM method is comparable with conventional methods.

Table 3. Comparison of Quantitation Result with Three Different Acquired Methods

	TPH Concentration (µg/mL)
Original method (30 m)	1097
2X method (15 m)	920
LTM method (5 m)	909

Conclusions

The low thermal mass of the Agilent LTM system can perform very efficient column heating and cooling, and is used here to develop an ultra-fast TPH analysis to meet the requirement for high lab productivity. Dual-tower injection is also used to further double the productivity with much less cost. The final solution with the LTM system and dual-tower injection can perform TPH analyses at a rate of 5.1 minutes per two samples. The total productivity increase is 18x compared to a conventional analysis on a single-channel system.

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Improving GC-MS Method Robustness and Cycle Times Using Capillary Flow Technology and Backflushing

Application Note

Environmental

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Abstract

This application note demonstrates the customer benefits from using Capillary Flow Technology to provide backflushing of high-boiling materials in GC and GC/MS analyses. Benefits include reduction in chromatographic cycle times, a reduction in system column maintenance, and extended GC column life. If a GC/MS system is utilized, the author has experienced an increase in the number of samples analyzed before ion source maintenance is required.

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Introduction

A critical component of the GC/MS analysis of any sample that contains large amounts of matrix material is the sample preparation. Environmental samples such as soils and sediments require not only extraction, but may also require multiple cleanup steps in order to present as clean an extract as possible for injection in to the GC/MS system.

Any remaining matrix in the sample extract can have deleterious effects on the GC sample inlet, column, and the ion source of the mass spectrometer. Traditionally, these high-boiling matrix materials are removed from the capillary column by a long bake-out period after the analytes of interest have eluted. This long bake-out process causes thermal stress to the column and also drives the matrix material towards the ion source, where it will eventually affect system performance. Moreover, should any material remain in the column after the bake-out process, it can cause loss of chromatographic peak shape and retention time shifting of target analytes. This shifting of retention time is particularly troublesome if the mass spectrometer is being used in the selected ion monitoring (SIM) mode (as with a single quadrupole GC/MS) or in the multiple reaction monitoring (MRM) mode (as with a triple quadrupole GC/MS).

This paper demonstrates how high-boiling matrix materials can be removed from the column quickly and effectively – between sample injections – by using capillary flow technology and capillary column backflushing.

Figure 1 shows a schematic diagram of the GC/MS system used. The 15-m analytical column was connected to the EPC split/splitless inlet and a capillary flow technology two-way splitter (p/n G3180B or G1540 option number 889).

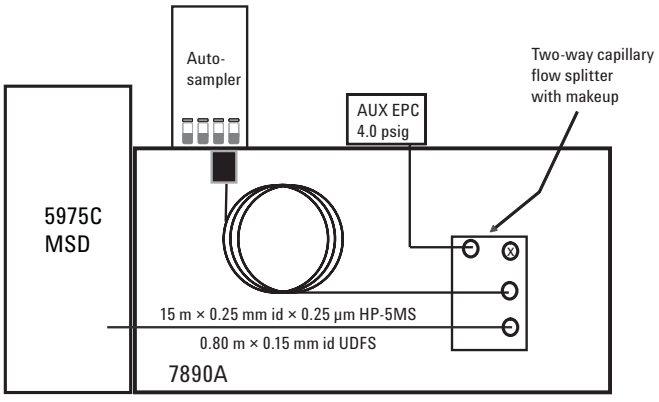

Figure 1. Schematic diagram of GC-MS system.

A short length of uncoated, deactivated fused silica (UDFS) capillary column is used as a restrictor between the splitter and the MS. Note carefully how the connections are made at the splitter. The X represents a port on the splitter plate that is closed off with a SilTite metal ferrule and stainless steel wire plug.

Backflushing in this example was accomplished during a post-run period by a combination of increasing oven temperature, reducing the inlet pressure of the analytical column, and increasing the pressure applied to the splitter plate.

Experimental

The full analytical conditions, both with and without post-run backflush set-points, are shown in Table 1.

Table 1. GC/MS Analysis Conditions

Gas chromatograph	Agilent 7890A
Columns	(1) 15.0 m × 0.25 µm id × 0.25 µm HP-5MS Ultra Inert (19091S-431SI) Inlet Front split/splitless, outlet 2-way Capillary Flow Device
	(2) 0.80 m × 0.15 mm id uncoated deactivated fused silica inlet two-way capillary flow device at 4.0 psig outlet vacuum
Carrier gas	Helium
Carrier gas mode	Constant pressure
Flow rate	17.18 psi
Injection port	EPC split/splitless
Autosampler	Agilent 7683A
Injection mode	Splitless, purge delay 0.5 min Purge flow 50.0 mL/min at 0.5 min
Injection volume	2.0 µL
Injection port liner	4 mm single-taper splitless liner (5181-3316)
Oven program °C (min)	70 (1) – 50 °C /min – 150 (0) 6 – 200 (0) – 16 – 280 (0) °C
Mass spectrometer	Agilent 5975C MSD
MS interface	280 °C
MS source	230 °C
MS quad 1	150 °C
Backflush conditions (1)	Post-run, 10 min, AUX 60 psig, oven 320 °C
Backflush conditions (2)	Post-run, 6 min, AUX 80 psig, oven 320 °C
Detection mode	EI full scan; mass range 40:550 amu
EI tune	Gain factor = 1

Results and Discussions

Experiment 1: No Backflushing Employed

In the first experiment, an extracted sediment sample was analyzed in full-scan mode to show the extent of the matrix problem. No backflushing was employed.

Before any sediment was injected, a system blank (no injection) followed by a 2-µL solvent blank was made. In the absence of the actual hexane solvent used to prepare the sediment extract, hexane that was not particularly clean was used. The TICs are shown overlaid in Figure 2, system blank in black, and solvent blank in gray. These chromatograms show that the system is free from high-boiling matrix material.

Following the blanks, a single injection of the sediment extract was made without backflushing; the TIC is shown in Figure 3. Note the very high abundance of the matrix and that when the analysis finishes, there is still a significant amount of matrix material to elute from the column.

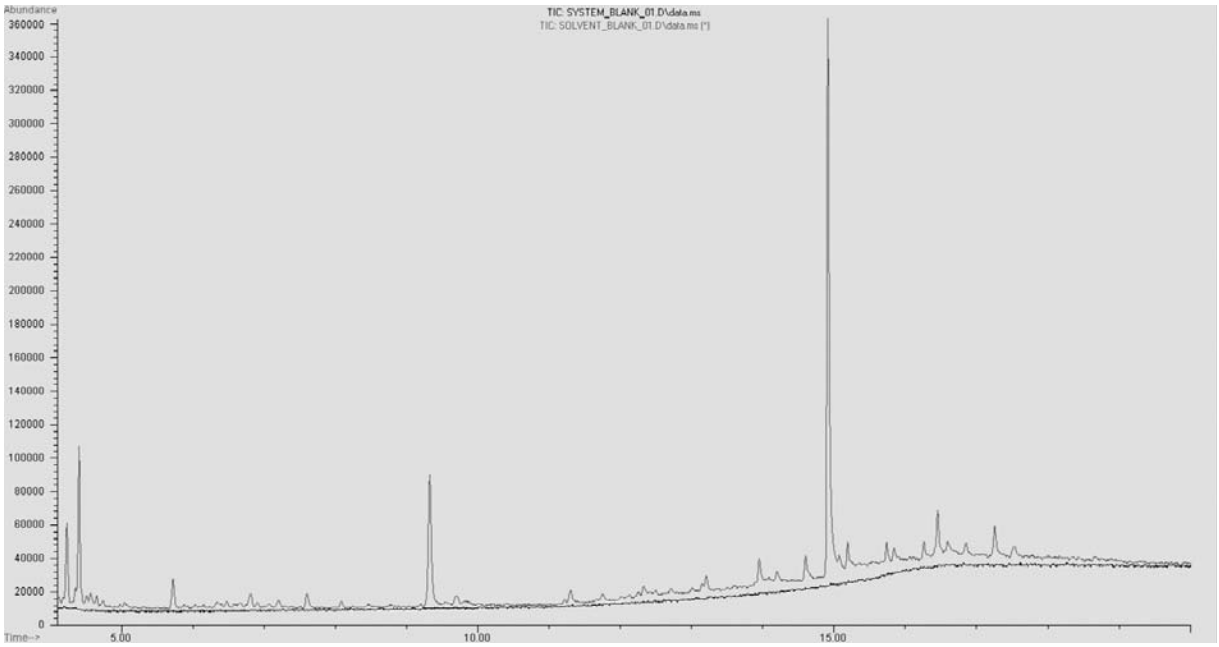

Figure 2. System blank and solvent blank TICs.

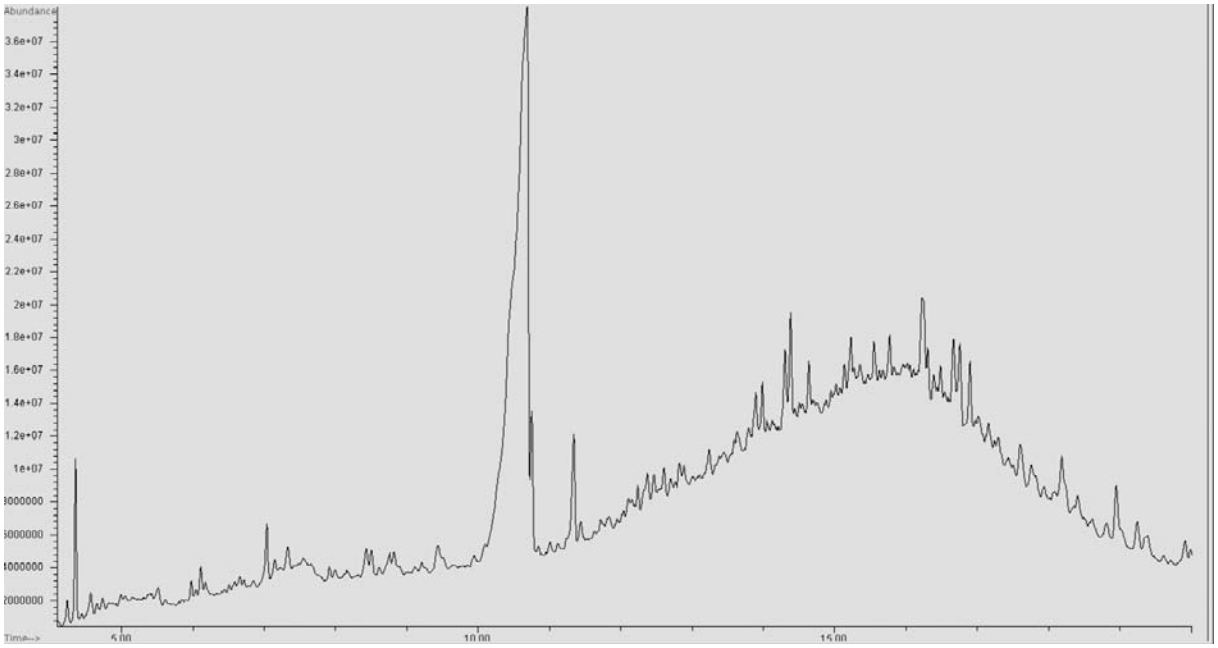

Figure 3. Sediment extract TIC.

The sediment extract injection was followed by a series of hexane blank injections. The first seven hexane blank TICs are shown overlaid in Figure 4 with the solvent blank before the sediment was injected into the GC/MS system.

Figure 5 shows that after the eighth solvent blank injection, the system has almost recovered to the level of background before the sediment sample was injected.

The original solvent blank TIC is shown in black, the eighth solvent blank TIC after the sediment injection is shown in gray.

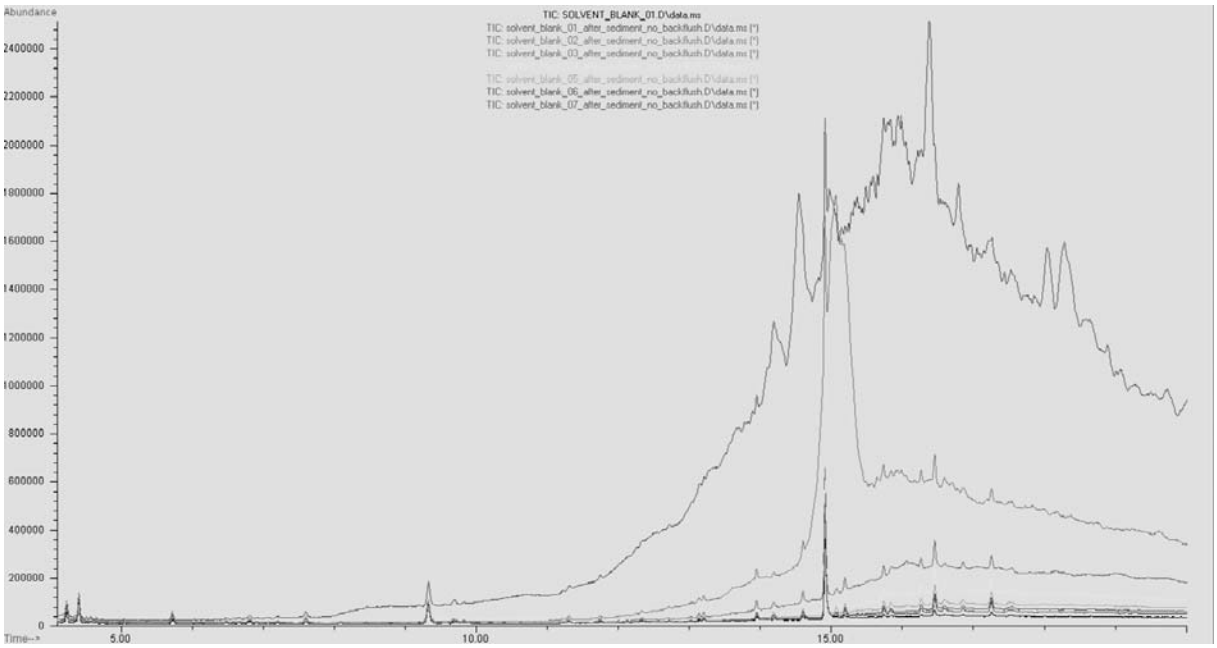

Figure 4. Successive solvent blank injections.

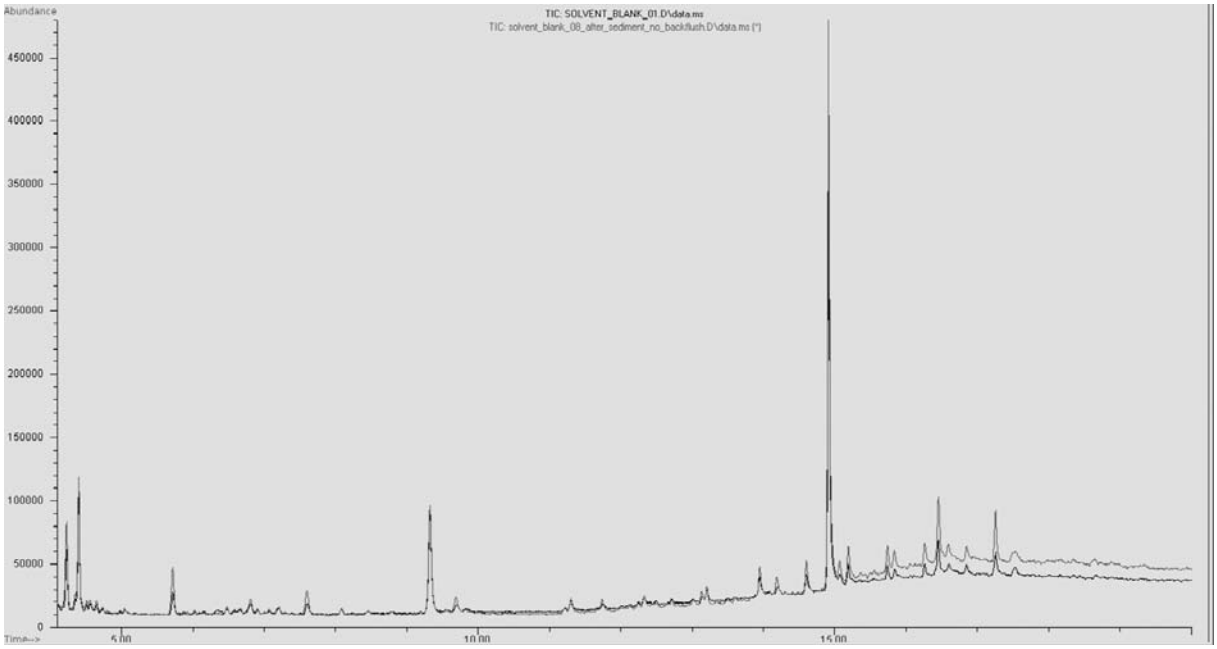

Figure 5. Eighth solvent blank and original solvent blank TICs

Experiment 2: Backflushing Employed

Backflushing was enabled during a post-run period by increasing column oven temperature, reducing the inlet pressure of the analytical column, and increasing the gas pressure applied to the splitter plate.

The 7890A instrument control software includes simple and easy-to-use screens to help set up post-run backflushing conditions. Figure 6 shows the configuration of columns and connections with the GC oven.

Figure 7 shows the actual backflushing conditions, namely the post-run oven temperature (320 °C), post-run inlet pressure for the analytical column (1 psig), post-run pressure applied to the splitter device (60 psig), and post-run time (10 minutes). The figure also shows the number of column-volumes of carrier gas that will backflush the analytical column.

Note that using the backflushing conditions shown in Figure 7 (320 °C, column pressure 1 psig, and splitter pressure 60 psig for 10 minutes), that 59.4 column volumes of carrier gas was used to backflush the column during the post-run period. This backflush time may have been more than necessary. Alternate conditions were also investigated and are presented later.

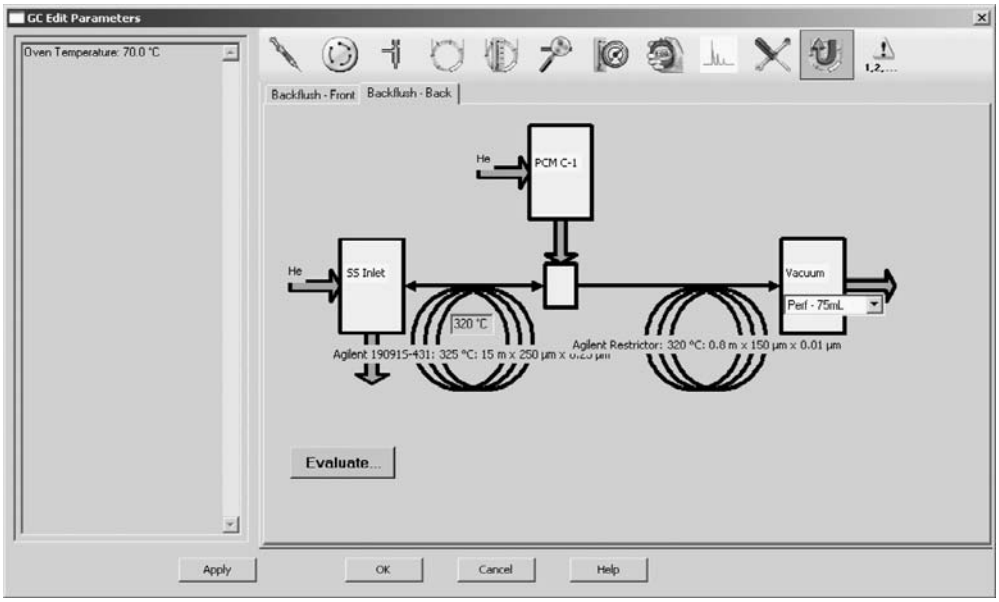

Figure 6. Post-run backflushing screen number 1.

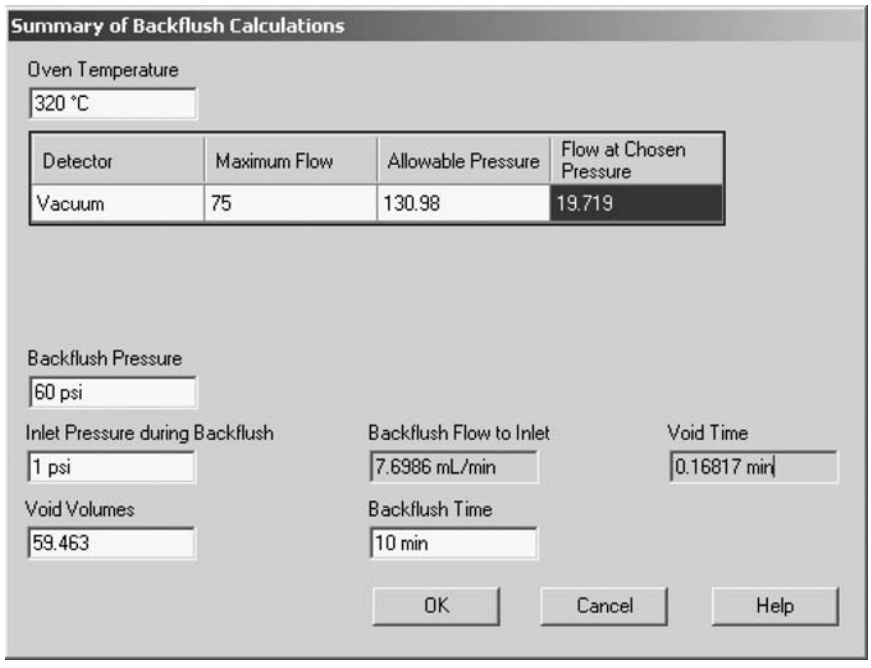

Figure 7. Post-run backflushing screen number 2.

Before applying the backflush conditions to the method the user is presented with a convenient summary of the back-flush conditions. See Figure 8.

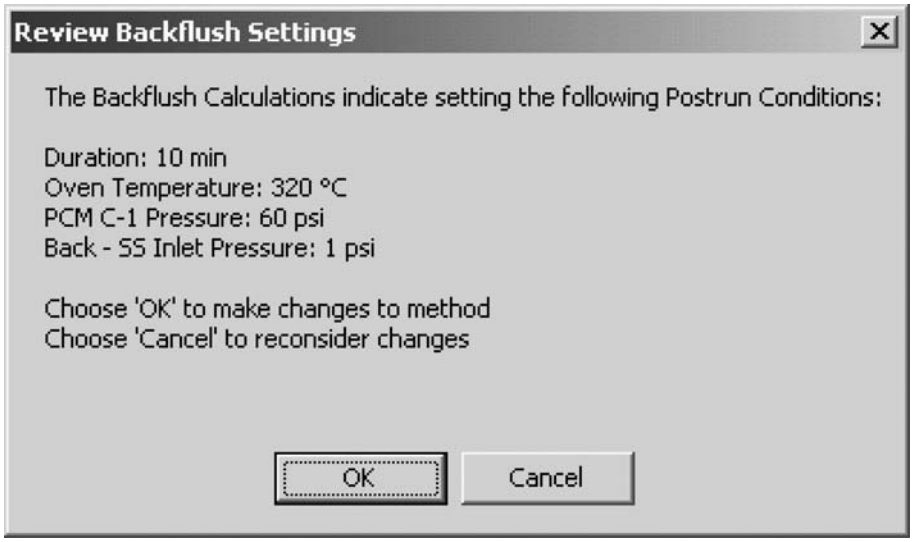

Figure 8. Post-run backflushing screen number 3.

Another injection of the sediment including backflush was made followed by a blank injection of solvent. Figure 9 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material had been effectively removed by backflushing.

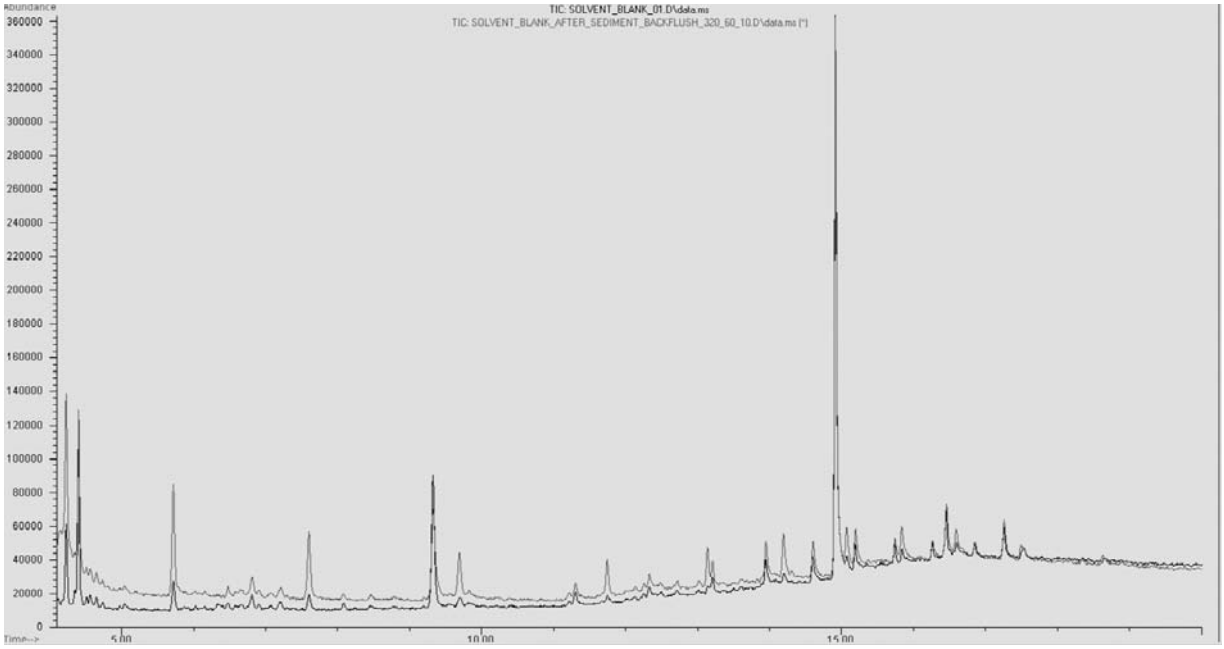

Figure 9. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (1).

Experiment 3: Backflushing Employed

In order to reduce cycle time for the method, the backflush conditions were modified by increasing the backflush pressure to 80 psig and holding for 6 minutes.

Note that using the backflushing conditions shown in Figure 10 (320 °C, column pressure 1 psig, and splitter pressure 80 psig for 6 minutes), that 46.6 column volumes of carrier gas was used to backflush the column during the post-run period.

Another injection of the sediment was made, followed by a blank injection of solvent. Figure 11 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material has been removed by backflushing with the more aggressive conditions as well. These conditions reduced the cycle time for this method 4 minutes compared to the backflushing conditions used in Experiment 1.

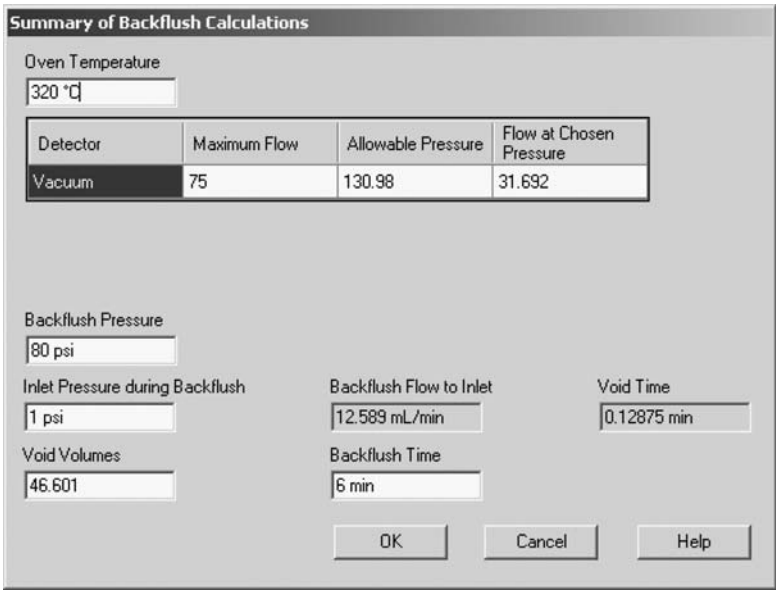

Figure 10. Post-run backflushing screen conditions number 2.

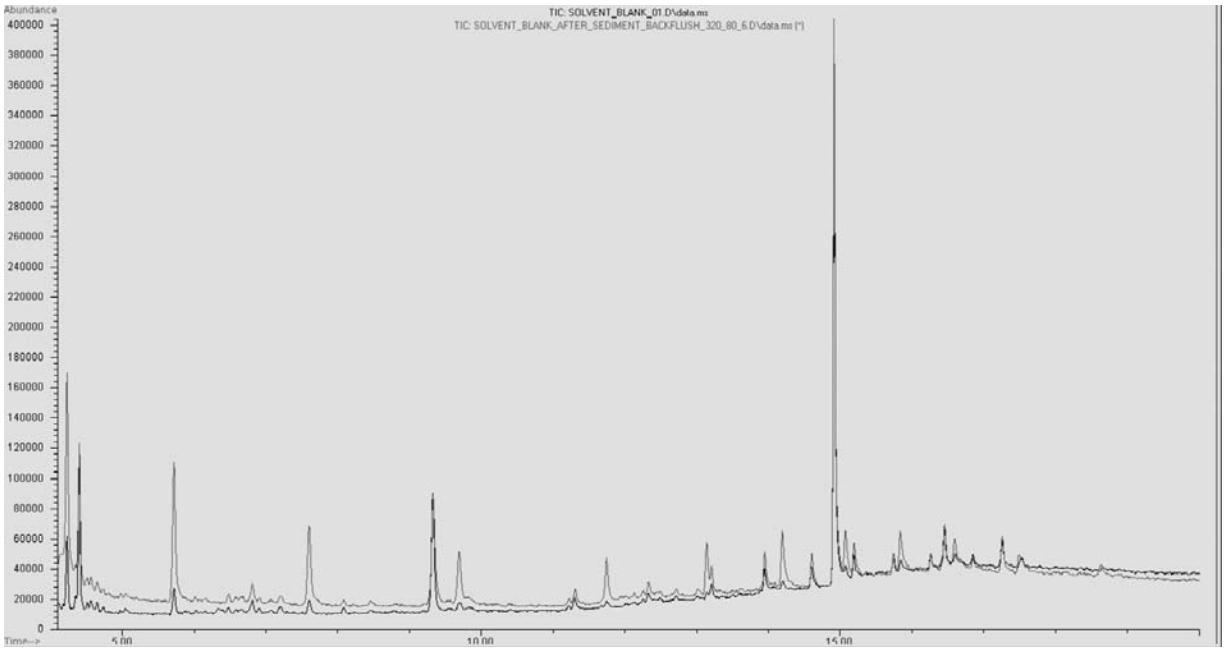

Figure 11. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (2).

Conclusions

Post-run backflushing was shown to effectively eliminate high-boiling sample matrix in a short amount of time. The major benefits of GC capillary column post-run backflushing include:

- Agilent's capillary flow technology and GC software enable easy and robust setup of GC backflushing.
- Compared to long bake-out periods with flow in the forward direction, a short period of backflushing can remove high-boiling matrix materials more effectively without contaminating the MS ion source.
- Chromatographic cycle time is reduced, columns stay clean, and the integrity of target analyte peak shapes and retention times are maintained.
- For this particular sediment extract the GC column was free of sample matrix after a backflush period of 6 minutes.
- Less system maintenance (ion source cleaning) is required.

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Evaluation of Total Petroleum Hydrocarbon in Soil Using LC with Fraction Collector and GC/MS

Application

Environmental

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Abstract

Normal-phase liquid chromatography (NPLC) and capillary gas chromatography with mass spectrometry are employed to evaluate the total petroleum hydrocarbon (TPH) in the soil contaminated by crude oil. In this paper, paraffins and mono-aromatic and multi-aromatic compounds present in the sample were first separated by NPLC into different classes of compounds according to their individual polarities, and fractions were collected for subsequent analysis by GC/MS, separated by boiling point, and identified by their unique mass spectra.

Introduction

Pollution due to oil spills happens frequently all over the world. Positive identification of the source is a critical part of establishing liability for cleanup costs and environmental damages. Because the spill is subject to time-based alteration by weathering (dissolution or evaporation), chemical degradation (effects of sunlight, heat, air, and soil chemistry), and biological alteration (impact of microorganisms) it has become more and more important to map these effects. Scientists have developed diverse technologies to perform the comprehensive evaluation analysis of TPH in environmental matrices. DIN 38 409 H18 [1] is the official method using infrared spectrometry. Robert [2] introduced a comprehensive two-dimensional gas chromatography to track the weathering of an oil spill. A portable GC/MS method was presented to determine the concentration of TPH from unresolved signals in short test runs in the field.

Sjaak [3] introduced group-type characterization of mineral oil samples by two-dimensional comprehensively coupled LC x GC-ToF MS. The interface between LC and GC/MS consisted of a 100-µL syringe, with two side entrances/exits in the upper part of the barrel, installed in an injection robot. A stop-flow mode of LC was adopted during the GC/MS analysis.

In this paper, we employed a fraction collector to replace the complex interface between HPLC and GC/MS and applied a combination of NPLC and GC/MS to evaluate the TPH in the soil contaminated by crude oil.

Experimental

Instrumentation and Conditions

Agilent 1200 Series LC, consisting of:

G1379B	Micro vacuum degasser
G1312B	Binary pump SL
G1367C	High-performance autosampler SL
G1316B	Thermostatted column compartment SL with 6- or 10-port 2-position switching valve
G1315C	UV/VIS diode array detector SL
G1364C	Fraction collector (analytical scale)

ChemStation 32-bit version B.02.01-SR1

Agilent 6890GC with 5975B MSD, consisting of:

G1540N	6890N network GC system with options: 201 MSD interface
G3243A	5975B inert MSD/DS perf turbo EI bundle
G3397A	Ion gauge/controller for use with 5975 MSD
G2913A	7683B autoinjector module
G2614A	7683 autosampler tray module

MSD Chemstation version D.03.00 with NIST 05 MS Library version 2.0d

The LC and GC/MS operating conditions are listed in Table 1.

Sample Preparation

The crude oil sample was from the Daqing, China, oil field and contributed by Sinopec Shanghai Gaoqiao Petrochemical Corporation.

The sample was prepared by mixing a 1 g oil sample with a blank soil sample and depositing the mixture in a fume hood for 2 days. Next, 50 mL of hexanes was added and the sample was extracted in an ultrasonic water bath for 1 hour. The extract was filtered, and 10 mL of filtrate was pipetted and then evaporated under a nitrogen stream to less than 1 mL. The extract was then made up with hexanes to 1 mL, and the solution was injected into NPLC for analysis.

Operation of Column Switching Valve and Fraction Collector of NPLC

The crude oil sample was so complex that a column switching valve was employed to backflush the analysis column in the NPLC system. To approximately evaluate the retention time of every group of compounds, a system calibration standard was used, which was composed of cyclohexane, o-xylene, dibenzothiophene and 9-methylanthracene, as generally outlined in ASTM Methods D6379 and D6591. The separation of the system calibration standard is shown in Figure 1. To minimize the total analysis time, the LC eluate of the first 3 min was sent to waste. Afterwards, fractions were collected every 0.5 min by the fraction collector. After collecting the fractions that contained the compounds of interest, the column was switched to backflushing mode for cleaning and the LC run was closed after the baseline stabilized.

Results and Discussion

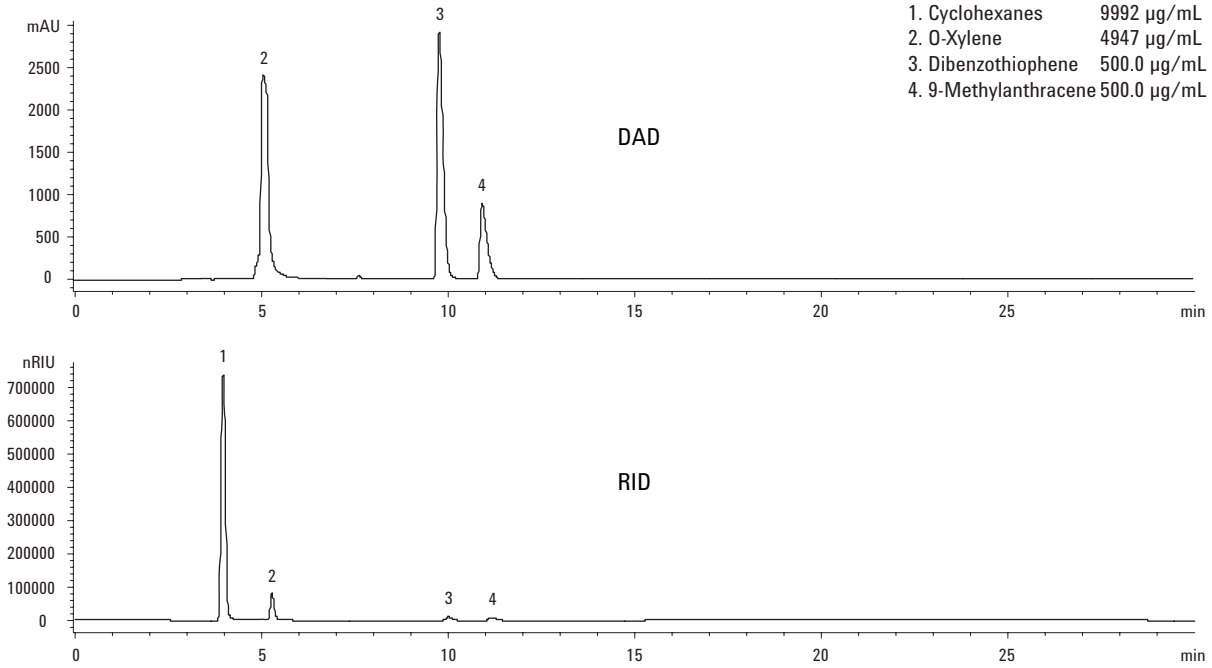
The soil sample extract was separated into different groups by normal phase liquid chromatography according to their polarities, as displayed in Figure 2. A total of 23 fractions were collected, which were injected into the GC/MS system for subsequent separation according to their boiling point and identification according to their characteristic mass fragments. A total ion chromatogram (TIC) of typical paraffins and mono-aromatic, bi-aromatic, and tri-aromatic compounds is depicted, respectively, in Figure 3. Through the identification by mass spectra, the first group with a retention time range of 3.7 to 4.7 min in LC chromatography contained paraffins; the second group, with a retention time range of 4.7 to 6.2 min, contained mono-aromatic compounds; the third group, with a retention time range of 6.2 to 11.2 min, contained bi-aromatic compounds; and the fourth group, with a retention time range of 11.2 to 13.7 min, contained tri-aromatic compounds. No aromatic compounds eluted at the retention time range from 13.7 min to the end.

Table 1. LC and GC/MS Operating Conditions

LC	**Agilent Technologies 1200SL**		
Mobile phase	Hexanes		
Flow rate	0.8 mL/min		
Wavelength	210 nm		
Injection volume	100 µL		
Mode	Isocratic		
Column	Agilent ZORBAX NH_2		
	4.6 mm x 250 mm, 5 µm		
Analysis time	30 min		
Column temperature	35 °C		
Column switching valve	Backflushing off		
Column switching timetable	Time	Column	
	15.00 min	Backflushing on	
	30.00 min	Backflushing off	
Fraction trigger mode	Use timetable		
Fraction collector timetable	Time	Trigger mode	Time slices
	3.70 min	Time-based	0.5 min
	15.00 min	Off	–

GC	**Agilent Technologies 6890N**
	7683 autoinjector and tray
Autoinjector	
Sample washes	3
Sample pumps	6
Injection volume	1 µL
Syringe size	5 µL
Preinjection solvent A	0
Preinjection solvent B	3
Post-injection solvent A	0
Post-injection solvent B	3
Viscosity delay	0 s
Plunger speed	Fast
Preinjection dwell	0 min
Post-injection dwell	0 min
Sampling depth	Disable

Inlet	**EPC**
Injection type	Splitless
Inlet temperature	250 °C
Pressure	7.61 psi
Purge flow	50.0 mL/min
Purge time	0.75 min
Total flow	54.0 mL/min
Gas saver	On
Saver flow	20.0 mL/min
Saver time	2.00 min
Gas type	Helium
Oven	
Initial temperature	50 °C
Initial time	1.00 min
Ramp rate	30.00 °C/min
Final temperature	300 °C
Final hold	2.00 min
Total run time	11.33 min
Equilibration time	0.5 min
Column	
Type	HP 5-ms
Length	30 m
Diameter	0.25 mm
Film thickness	0.25 µm
Mode	Constant flow
Initial flow	1.0 mL/min
MSD	**Agilent Technologies 5975B inert**
Solvent delay	4 min
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45.0 amu
High mass	450.0 amu
Threshold	150
Sampling	2
Scans	3.54
Quad temperature	150 °C
Source temperature	230 °C
Transfer line temperature	280 °C



Mobile phase	Hexanes
Flow rate	0.8 mL/min
Detection	DAD and RID
UV wavelength	210 nm
Analysis time	30 min (backflush @ 15 min)
Column	Agilent ZORBAX NH_2 4.6 x 250 mm, 5 µm
Method	Follow ASTM D6379 (or D6591)
Sample	System Calibration Standard
Sample size	10 µL
Column temperature	35 °C

Figure 1. Chromatogram of standard solution.

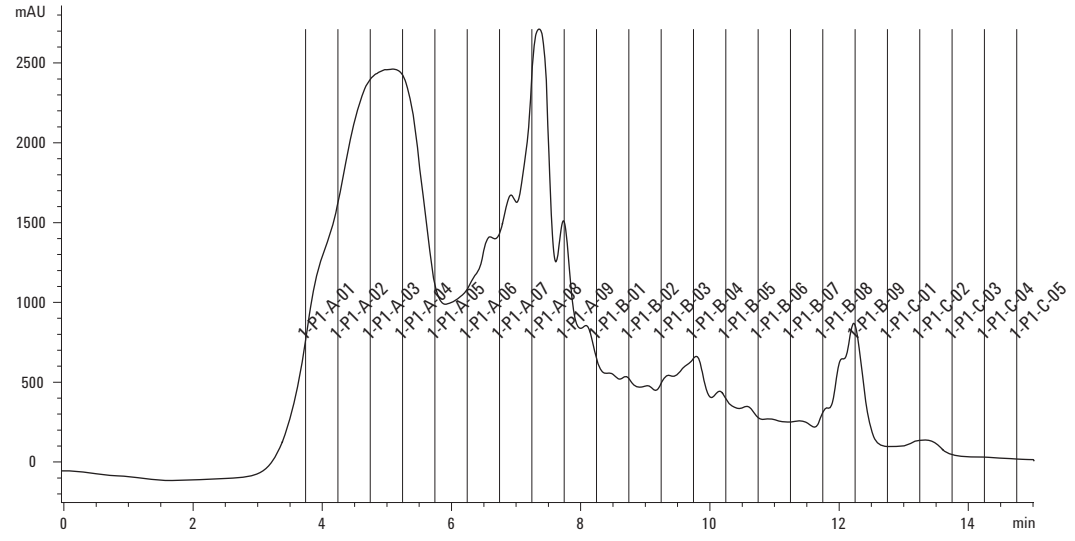


Figure 2. Chromatogram of soil sample extract and factions collected in different vials.

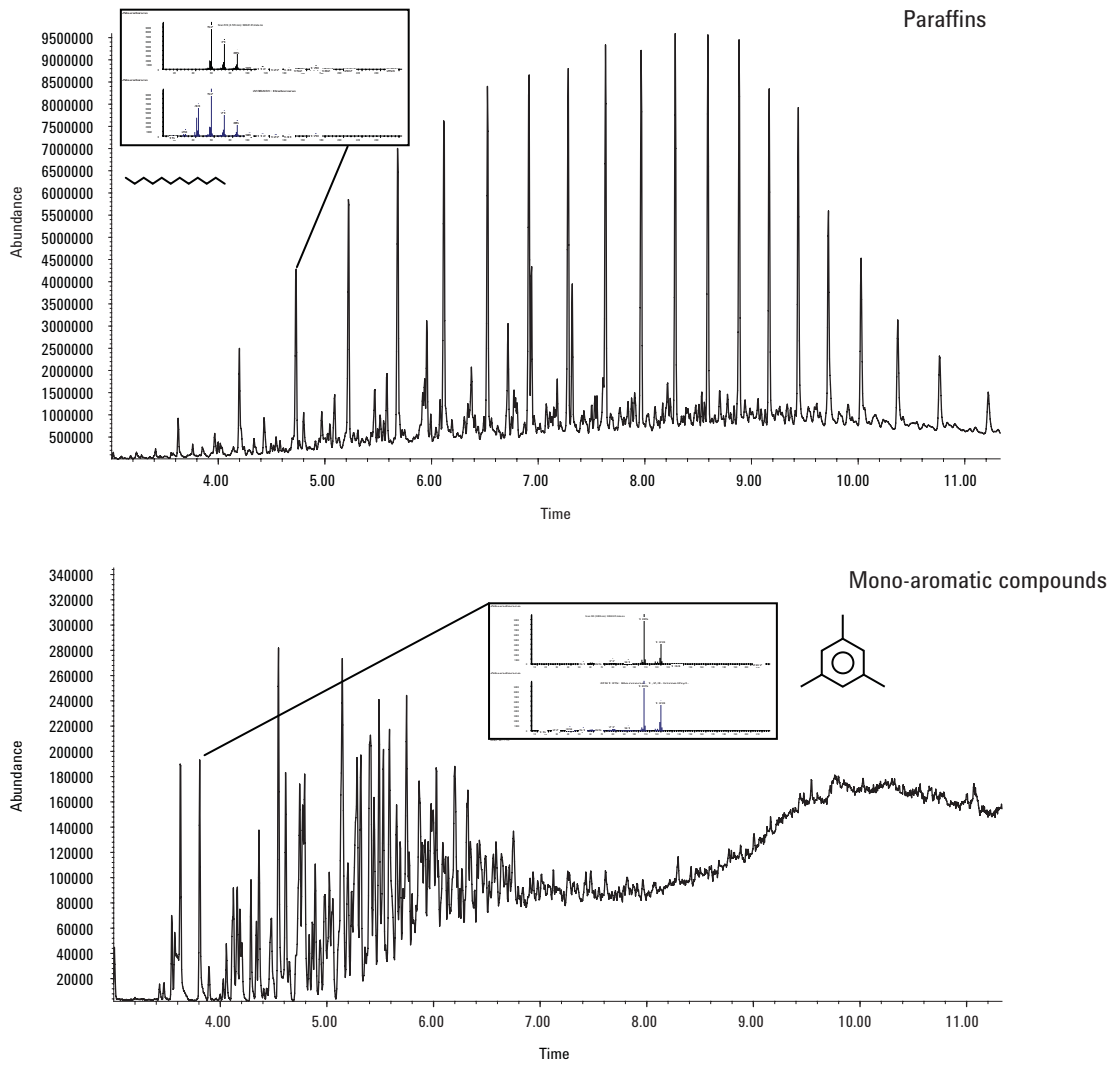


Figure 3. Total ion chromatogram of typical fractions including paraffins and mono-, bi-, and tri-aromatic compounds.

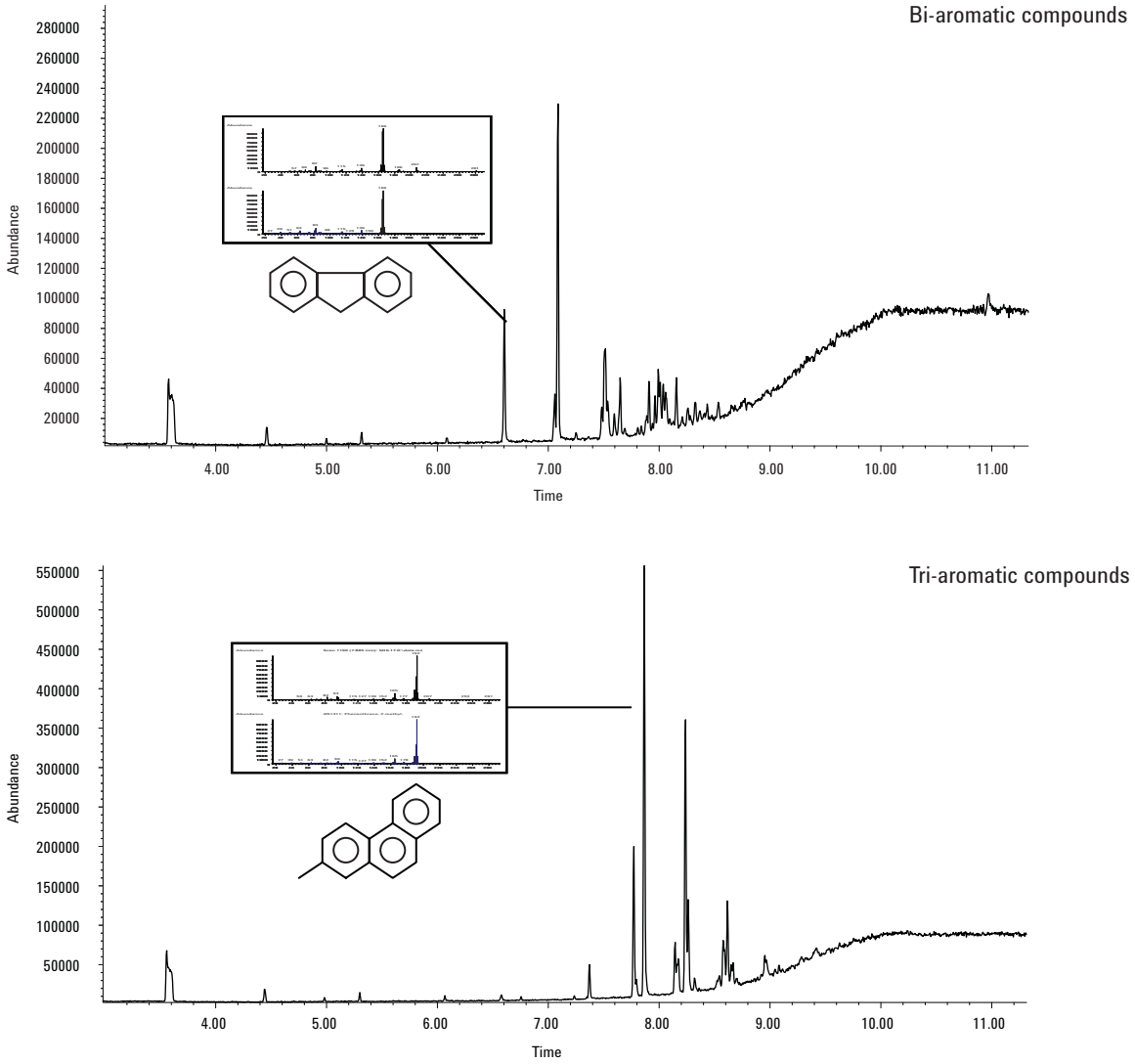


Figure 3. Total ion chromatogram of typical fractions including paraffins and mono-, bi-, and tri-aromatic compounds. (continued)

	Vial Position	Time Slices
Paraffin	1-P1-A-01	3.7 to 4.2 min
Mono-aromatic compounds	1-P1-A-04	5.2 to 5.7 min
Bi-aromatic compounds	1-P1-B-04	9.7 to 10.2 min
Tri-aromatic compounds	1-P1-B-08	11.7 to 12.2 min

Conclusions

The separations by NPLC and GC are based on polarity and boiling point, respectively. Mass spectra could provide the information on the molecular structure; therefore, the combination of NPLC and GC/MS could be used to evaluate the complex matrix. In this work, an LC with a fraction collector performed the separation of classes of paraffins and mono-, bi-, and tri-aromatic compounds and collected time-based fractions into individual sample vials. The fractions were injected into the GC/MS for identification. A soil sample contaminated by crude oil was analyzed by this method and the results showed the detailed component information of every typical class, based on fractionation by polarity, to evaluate the total petroleum hydrocarbon in soil.

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Techniques for Optimizing the Analysis of Volatile Organic Compounds in Water Using Purge-and-Trap/GC/MS

Application

Environmental

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Abstract

The analysis of volatile organic compounds in water is normally accomplished by purge-and-trap/gas chromatography/mass spectrometry. U.S. EPA Method 8260B with purge and trap sample introduction is widely used for the analysis of aqueous samples other than drinking water. This application note discusses problems that can arise and some easy solutions for them. These techniques have resulted in robust calibrations that meet Method 8260B calibration requirements over the range of 1–200 µg/L.

Introduction

U.S. EPA Method 8260B [1] is a general purpose method for the analysis of volatile organic compounds (VOCs) in matrices such as ground and surface water, sludges, soils and sediments, filter cakes, spent carbons, and spent catalysts. This method is only used for the analyses of target VOCs by gas chromatography with mass spectral detection (GC/MS). It refers analysts to other U.S. EPA sample introduction methods that are appropriate for the matrix to be analyzed. This paper focuses on the analysis of VOCs in water using purge and trap (P&T) sample introduction according to U.S. EPA Method 5030C [2] coupled to GC/MS for separation and analysis (P&T/GC/MS). For simplicity, the combination of Methods 5030C with 8260B is referred to as just Method 8260B.

This P&T/GC/MS procedure is widely used in environmental laboratories for the analysis of VOCs in surface, ground, and wastewater samples. A similar method for the analysis of drinking water is described in EPA Method 524.2 [3]. Though well established, P&T/GC/MS methods can be a challenge to run successfully. There are numerous P&T, GC, and MS variables to optimize in order to obtain good recoveries for the target VOCs without undo disturbance from water and methanol that are inevitably transferred to the GC during trap desorption.

This application note describes techniques for optimizing Method 8260B using the Agilent 6890N GC and new 5973 inert mass selective detector (MSD) coupled to the new Teledyne Tekmar Velocity XPT P&T system. Included, in the paper, are suggestions for MSD tuning, sample preparation, instrument setpoints, and maintenance techniques that lead to a robust method for the analysis of VOCs in water. The discussion is applicable to most other P&T/GC/MS methods.

Experimental

Chemical Standards, Reagents, and Vials

High purity B&J brand methanol was obtained from Honeywell Burdick & Jackson Co. (Muskegon, MI). Standard mixtures used for the preparation of calibration samples, spiking solutions, tune evaluation, and stability test samples were purchased from AccuStandard (New Haven, CT). These include the following: Part no. M-502-10X-Pak containing 60 VOC target analytes (54 liquids and 6 gases) at 2000 µg/mL each in methanol; Part no. M-8260A/B-IS/SS-10X-PAK containing p-bromofluorobenzene (BFB), chlorobenzene-d_5, dibromofluoromethane, 1,4-dichlorobenzene-d_4 (DCB-d_4) , 1,2-dichloroethane-d_4, fluorobenzene (FBz), and toluene-d_8 at 2000 µg/mL each in methanol; and part no. M-524-FS-PAK containing BFB, 1,2-dichlorobenzene-d_4, and fluorobenzene (FBz) at 2000 µg/mL each in methanol.

VOC-free water was used for the preparation of standards and test samples. TraceClean 40-mL (nominal volume, actual volume is 43 mL) VOA vials (part no. 15900-022) were purchased from VWR Scientific (West Chester, PA).

Preparation of Calibration and Spiking Solutions

Secondary spiking solutions were prepared in methanol for each calibration level so that each 43-mL water sample could be spiked with 10 µL of the calibration solution (containing 60 VOCs) and 10 µL of the internal standard/surrogate mixture. Table 1 provides details on how the eight calibration standards were prepared.

Table 1. Procedure for Preparing Calibration Samples

A	B	C	D	E
Calibration level (µg/L)	Volume of 2000 µg/mL VOC Standard (µL)	Diluted to this volume in methanol (mL)	Results in this secondary standard concentration (µg/L)	Amount to spike into 43-mL vial (µL)
1	53.75	25.00	4.3	10.00
2	43.00	10.00	8.6	10.00
5	53.75	5.00	21.5	10.00
20	43.00	1.00	86	10.00
50	43.00	0.40	215	10.00
100	43.00	0.20	430	10.00
200	43.00	0.10	860	10.00
300	*	*	2000*	6.45**

Column A. Concentration of each analyte in the final aqueous calibration solution.

Column B. Volume of the 2000 µg/mL 60-component VOC standard solution which was diluted to the volume shown in column C.

Column C. Final volume of VOC solution after dilution in methanol.

Column D. Concentration of the calibration spiking solution prepared by diluting the amount of 2000 µg/mL standard in column B to the volume shown in column C.

Column E. Amount of the secondary standard solution (column D) added to 43 mL of water to prepare the calibration standard at the level shown in column A.

*The undiluted VOC standard (2000 µg/mL) was used for spiking.

**The 300 µg/L aqueous calibration standard was prepared by adding 6.45 µL of the 2000 µg/mL AccuStandard VOC solution and 3.55 µL of methanol to 43 mL of water in a VOA vial.

As discussed below, containers for storing the secondary standards (column C, Table 1) were chosen to minimize the headspace. Larger volumes were transferred to 2-mL screw top vials, while smaller volumes were transferred to crimp cap microvials of the appropriate size.

A solution of the internal standards (ISTDs) and surrogates was prepared at 215 ppm in methanol by diluting 43 µL of the 2000-µg/mL AccuStandard solution to a volume of 400 µL. Each 43-mL water sample was spiked with 10 µL of this solution so that all samples and standards contained 50 µg/L of each compound.

Preparation of Solutions for Repeatability Studies

Two kinds of spiked water samples were prepared for use in repeatability studies.

- System blanks consisted of clean water spiked with fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 10 µg/L each.
- VOC spikes consisted of clean water with fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 10 µg/L and the 60 VOC target compounds at 20 µg/L each.

Replicate samples were prepared as follows.

- Secondary dilution standards containing fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 50.0 µg/mL were prepared in 2-mL autosampler vials by diluting 25 µL of the 2000-µg/mL AccuStandard solution with 975 µL of methanol.
- Secondary dilution standards of the 60-component VOC solution were prepared at 100 µg/mL in 2-mL autosampler vials by diluting 50 µL of the 2000 µg/mL AccuStandard solution with 950 µL of methanol.

System blanks were prepared by adding 100 µL of the 50.0 µg/mL three component solution and 100-µL methanol to 500 mL of water in a 1.0-L screw-cap bottle. After inverting to mix thoroughly, this bottle was attached to the apparatus shown in Figure 1 and 11 VOA vials were filled by transferring the spiked water solution under nitrogen pressure.

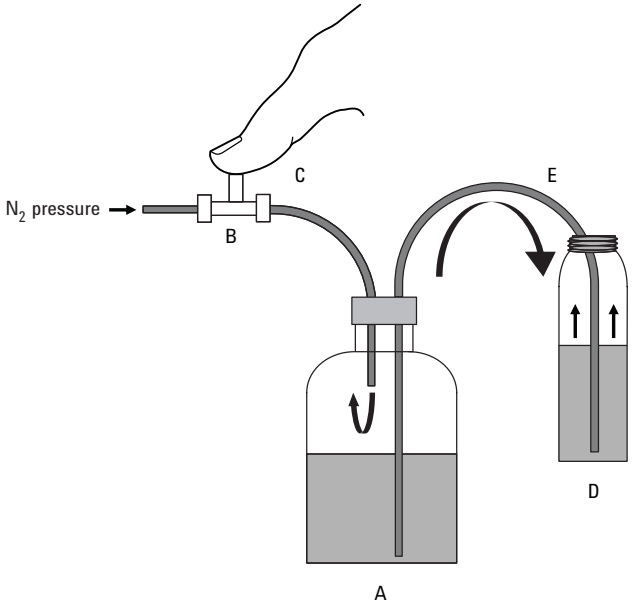

Figure 1. Apparatus used to fill multiple VOA vials with the same spiked water solution.
A) 1-L liquid chromatography solvent bottle
B) Swagelok Tee with nothing connected to one fitting
C) Finger used to cap fitting in order to pressurize the reservoir bottle
D) VOA vial
E) 1/8-inch PTFE tubing

VOA spiked samples were prepared by adding 100 µL of the 50.0-µg/mL three component solution and 100 µL of the 100-µg/mL 60-component VOC standard to 500 mL of water in a 1.0-L screw cap bottle. After inverting to mix thoroughly, this bottle was attached to the apparatus shown in Figure 1 and 11 VOA vials were filled by transferring the spiked water solution under nitrogen pressure.

Instrumentation and Analytical Conditions

The P&T instrumentation and setpoints are listed in Table 2. The following P&T options were not used: DryFlow trap, automatic ISTD addition, sample heating, dry purging, and sample cryofocusing. The method shown in Table 2 was derived using the wizard that is provided in the TekLink 2.2 P&T control software.

Table 2. Purge and Trap Instrumentation and Setpoints

P&T Instrument	**Teledyne Tekmar Velocity XPT**
Automatic sampler	**Teledyne Tekmar Aquatek 70**
Software control	Teledyne Tekmar VOC Teklink version 2.2
Trap	Vocarb 3000
P&T-GC interface	P&T transfer line spliced into the GC split/splitless inlet carrier gas line and GC carrier gas plumbed to the Velocity XPT
Sample size	5 mL
Valve oven temperature	150 °C
Transfer line temperature	150 °C
Sample mount temp	90 °C
Purge ready temp	45 °C
DryFlow standby temperature	175 °C
Standby flow	10 mL/min
Pressurize time	0.25 min
Fill I.S. time	0.00 (ISTDs added by hand)
Sample transfer time	0.25 min
Pre-purge time	0.00 min
Pre-purge flow	40 mL/min
Sample heater	Off (Samples not heated)
Sample preheat time	1.00 min
Preheat temperature	40 °C
Purge time	11.00 min
Purge temperature	0 °C (That is, less than the purge ready temp of 45 °C)
Purge flow	40 mL/min
Purge rinse time	0.25 min
Purge line time	0.25 min
Dry purge time	0.00 min (Dry purge not used)
Dry purge temp	40 °C
Dry purge flow	200 mL/min
GC start	Start of desorb
Desorb preheat temperature	245 °C
Desorb drain	On
Desorb time	1.00 min
Desorb temperature	250 °C
Desorb flow	200 mL/min
Bake rinse	On
Number of bake rinses	3
Bake drain time	0.50 min
Bake drain flow	400 mL/min
Bake time	3.00 min
Bake temperature	270 °C
Dry flow bake temperature	300 °C
Bake flow	400 mL/min
Focus temperature	Not used
Inject time	1.00 min
Inject temperature	180 °C
Standby temperature	100 °C

Table 3. GC/MS Instrumentation and Setpoints

Gas Chromatograph	**Agilent 6890N**
Inlet	Split/Splitless
Inlet liner	Single taper, deactivated (Agilent part no. 5181-3316)
Inlet temperature	250 °C
Split ratio	50:1
Column	20 m × 0.18 mm × 1.0 µm DB-VRX (Agilent part no. 121-1524)
Carrier gas	Helium at 1.0 mL/min constant flow
Oven temperature program	40 °C (3 min), 10 °C/min to 100 °C (0 min), 25 °C/min to 225 °C (3 min)
Mass Spectrometer	**Agilent 5973 Inert MSD**
Transfer line temperature	260 °C
Quad temperature	150 °C
Source temperature	230 °C
EM voltage	2035 volts
Scan range	35–260 *m/z*
Threshold	0
Samples	3
Solvent delay	0 min
Software	MSD Productivity ChemStation Software (Part no. G1701DA version D.01.00)

Results and Discussion

Section 1.3 of Method 8260B can be used to quantitate most VOCs that have boiling points below 200 °C. It lists 123 compounds that can be determined by the method using various sample prep and sample introduction methods. Of these, seven are ISTDs or surrogates, nine are not recommended for P&T sample introduction, and three must be purged at 80 °C for efficient recovery. The remaining analytes vary considerably in their water solubility and volatility making this a challenging method to optimize. The intent of this application note is to share several techniques that one can use to optimize Method 8260B or any other P&T/GC/MSD method employed for water analysis.

For this study, the 60 VOCs listed in EPA Method 502.2 were analyzed along with three ISTDs and four surrogates (Table 4).

Table 4. Compound List with Average Response Factors (RF) and the RF %RSDs for Two Calibration Ranges: 1–300 and 1–200 µg/L

Type*	Compound	Retention time (min)	Minimum average response factor**	Maximum %RSD of calibration response factors***	Average RF 1–300 µg/L	RF %RSD 1–300 µg/L	Average RF 1–200 µg/L	RF %RSD 1–200 µg/L
T	Dichlorodifluoromethane	1.25		15	0.283	8.21	0.289	5.44
T,SPCC	Chloromethane	1.34	0.1	15	0.324	9.62	0.328	9.38
T,CCC	Vinyl chloride	1.42		30	0.220	2.47	0.220	2.66
T	Bromomethane	1.60		15	0.099	14.11	0.096	12.30
T	Ethyl chloride	1.67		15	0.152	5.57	0.154	4.27
T	Trichloromonofluoromethane	1.97		15	0.372	11.38	0.386	3.49
T,CCC	1,1-Dichloroethene	2.29		30	0.330	5.31	0.336	1.45
T	Methylene chloride	2.40		15	0.299	5.02	0.301	4.95
T	*trans*-1,2-Dichloro-ethene (E)	2.92		15	0.323	2.54	0.325	1.36
T,SPCC	1,1-Dichloroethane	3.14	0.1	15	0.444	4.93	0.446	5.22
T	*cis*-1,2-Dichloroethene (Z)	3.68		15	0.360	1.28	0.361	1.17
T	Bromochloromethane,	3.83		15	0.234	1.82	0.234	1.84
T,CCC	Chloroform	3.89		30	0.442	0.92	0.443	0.60
T	2,2-Dichloropropane	3.96		15	0.202	9.87	0.209	4.19
Sur	Dibromofluoromethane	4.01		15	0.248	0.83	0.248	0.89
Sur	1,2-Dichloroethane-d_4	4.47		15	0.298	1.76	0.299	1.79
T	1,2-Dichloroethane	4.55		15	0.359	1.57	0.359	1.66
T	1,1,1-Trichloroethane	4.64		15	0.388	7.99	0.398	1.43
T	1,1-Dichloropropene	4.86		15	0.336	12.44	0.351	3.16
T	Carbon tetrachloride	5.01		15	0.309	13.88	0.322	7.66
T	Benzene	5.08		15	1.063	7.10	1.077	6.52
ISTD	Fluorobenzene	5.34		15		1.34		1.41
T	Dibromomethane	5.68		15	0.198	1.86	0.198	2.01
T,CCC	1,2-Dichloropropane	5.75		30	0.266	1.58	0.268	0.77
T	Trichloroethylene	5.81		15	0.288	6.79	0.295	2.14
T	Bromodichloromethane	5.85		15	0.334	5.47	0.331	5.60
T	1,3-Dichloropropene (Z)	6.64		15	0.383	5.49	0.381	5.74
T	1,3-Dichloropropene (E)	7.18		15	0.322	8.76	0.318	8.93
T	1,1,2-Trichloroethane	7.32		15	0.236	1.57	0.237	1.67
Sur	Toluene-d_8	7.47		15	0.945	0.50	0.945	0.51
T,CCC	Toluene	7.55		30	1.098	7.47	1.126	2.07
T	1,3-Dichloropropane	7.62		15	0.428	1.28	0.428	1.20
T	Dibromochloromethane	7.86		15	0.254	12.10	0.249	11.88
T	1,2-Dibromoethane	8.15		15	0.244	1.88	0.244	2.03
T	Tetrachloroethylene	8.40		15	0.307	18.72	0.327	5.07
T	1,1,1,2-Tetrachloroethane	9.15		15	0.254	8.79	0.254	9.49
ISTD	Chlorobenzene-d_5	9.19		15		0.98		0.81
T,SPCC	Chlorobenzene	9.22	0.3	15	0.981	5.00	0.997	2.14
T,CCC	Ethylbenzene	9.51		30	1.559	11.66	1.623	1.90
T,SPCC	Bromoform	9.72	0.1	15	0.246	14.57	0.242	15.08
T	m- & p-Xylene	9.73		15	2.510	11.97	2.614	2.75
T	Styrene	10.03		15	1.008	5.68	1.022	4.25
T,SPCC	1,1,2,2-Tetrachloroethane	10.08	0.3	15	0.395	3.41	0.394	3.46
T	o-Xylene	10.10		15	1.289	9.27	1.330	1.89
T	1,2,3-Trichloropropane	10.21		15	0.347	2.90	0.346	2.94
Sur	BFB	10.44		15	0.381	0.93	0.382	0.82
T	Isopropylbenzene	10.44		15	1.474	17.44	1.562	4.13
T	Bromobenzene	10.58		15	0.643	5.20	0.653	3.12
T	n-propylbenzene	10.82		15	1.840	17.38	1.950	3.60
T	2-Chlorotoluene	10.85		15	1.124	10.66	1.166	1.93
T	4-Chlorotoluene	10.92		15	1.184	10.23	1.224	3.75

Table 4. Compound List with Average Response Factors (RF) and the RF %RSDs for Two Calibration Ranges: 1–300 and 1–200 µg/L (Continued)

Type*	Compound	Retention time (min)	Minimum average response factor**	Maximum %RSD of calibration response factors***	Average RF 1–300 µg/L	RF %RSD 1–300 µg/L	Average RF 1–200 µg/L	RF %RSD 1–200 µg/L
T	1,3,5-Trimethylbenzene	11.08		15	1.275	14.63	1.340	3.02
T	Tertbutylbenzene	11.26		15	1.196	18.98	1.274	4.24
T	1,2,4-Trimethylbenzene	11.36		15	1.353	12.22	1.411	2.35
T	sec-Butylbenzene	11.43		15	1.729	21.91	1.858	5.67
T	1,3-Dichlorobenzene	11.44		15	1.529	10.75	1.579	5.61
T	1,4-Dichlorobenzene	11.49		15	1.597	9.97	1.643	5.99
ISTD	1,4-Dichlorobenzene-d_4	11.47		15		1.09		1.17
T	p-Isopropyltoluene	11.58		15	2.587	19.00	2.757	3.52
T	1,2-Dichlorobenzene	11.73		15	1.485	6.33	1.516	2.74
T	Butylbenzene	11.87		15	2.355	20.68	2.522	4.81
T	1,2-Dibromo-3-chloropropane	12.06		15	0.186	13.90	0.180	11.56
T	1,2,4-Trichlorobenzene	12.95		15	1.211	12.42	1.250	8.76
T	Naphthalene	13.10		15	2.879	5.54	2.852	5.32
T	Hexachlorobutadiene	13.16		15	0.750	24.53	0.809	10.56
T	1,2,3-Trichlorobenzene	13.22		15	1.196	11.09	1.226	9.06
	Average %RSD of targets				9.07		4.60	
	Average %RSD of all compounds					8.22		4.23

*Compound designations as follows: T (target); SPCC (system performance check compound); CCC (calibration check compound); Surr (surrogate); ISTD (internal standard). Target compounds may also be designated as SPCCs or CCCs.

**The minimum average RF that must be met for the SPCCs.

***The maximum %RSD of the RFs. If any one or more of the CCC RF RSDs exceeds 30%, instrument maintenance is required. If the RF %RSD for any target compound exceeds 15%, other curve fits must be substituted for the average RF.

Method 8260B Requirements

Below is a summary of the most significant requirements of Method 8260B. If you are already very familiar with this method, you may want to skip this section.

ISTDs and surrogates: The ISTDs and surrogates listed in Table 4 are the recommended compounds for this method, although other compounds may be used instead.

Tuning requirements: Prior to running samples, the MSD must be adjusted so as to pass Method 8260B's BFB tuning specifications [1]. However, the method allows users to substitute CLP [4], Method 524.2 [3] or manufacturers' instructions for the specified BFB ion ratios. Table 5 lists the BFB tuning specifications for all three EPA methods. A scan range of 35–260 m/z is recommended.

Table 5. Criteria for BFB Tuning for Three Capillary GC/MS Volatiles Methods

Mass (*m/z*)	Relative abundance criteria Method 524.2	Method 8260B*	CLP-SOW
50	15%–40% of 95	Same**	8%–40% of 95
75	30%–80% of 95	30%–60% of 95	30%–66 % of 95
95	Base Peak, 100%	Same	Same**
96	5%–9% of 95	Same	Same
173	<2% of 174	Same	Same
174	>50% of 95	Same	50%–120% of 95
175	5%–9% of 174	Same	4%–9% of 174
176	>95% but <101% of 174	Same	93%–101% of 174
177	5%–9% of 176	Same	Same

*Alternative tuning criteria may be used (for example, CLP or Method 524.2) including manufacturer's instructions provided that method performance is not adversely affected.

**"Same" implies that this requirement is the same as that shown for Method 524.2. Note, however, that alternative tuning criteria may be used for Method 8260B (see previous footnote).

System Performance Check Compounds (SPCCs): The SPCCs are used to check the performance of the system after calibration and before analysis of samples. These compounds are known to be sensitive to active sites and instrument contamination. They must meet a minimum RF that is specified in Table 4.

Calibration Requirements: As a minimum, Method 8260B requires a five-point calibration curve. In order to assume linearity of the calibration curve, the RF RSD of all target compounds must be less than or equal to 15%. Six analytes are designated as Calibration Check Compounds (CCCs) (Table 4). If the RF RSDs for any of these compounds exceeds 30%, it is indicative of instrument problems and repairs must be made. Compounds that exceed 15% RSD for their RFs can use alternative curve fitting methods as specified in EPA Method 8000B [5].

GC/MS Calibration Verification for Each 12-hour Shift: The P&T/GC/MSD performance must be re-evaluated every 12 hours. The most significant requirements are:

- The BFB tune must be rechecked and pass the original tuning requirements.
- A sample near the midpoint of the calibration curve must be analyzed using P&T sample introduction, demonstrating that:
  - Each SPCC meets its minimum RF.
  - The percent difference (between current and original response) must be less than 20% for each CCC.
  - The retention time of each ISTD must not drift by more than 30 s.
  - The ISTD areas must not change by more than a factor of 2 from the original mid-point calibration level (50% to 200%).
  - A method blank must be run to show that there is no carryover or contamination of the system.

Calibration Results

Many laboratories employing Method 8260B generate five-point calibration curves between 5 and 200 µg/L. Knowing that laboratories often try to extend this range at both ends, an eight-point calibration was run at 1, 2, 5, 20, 50, 100, 200, and 300 µg/L. The signals for all analytes at 1 µg/L were sufficient to allow calibration at even lower levels. However, the lowest calibration level run for this work was 1 µg/L. Figure 2 shows a chromatogram of the targets, surrogates, and ISTDs at 50 µg/L each.

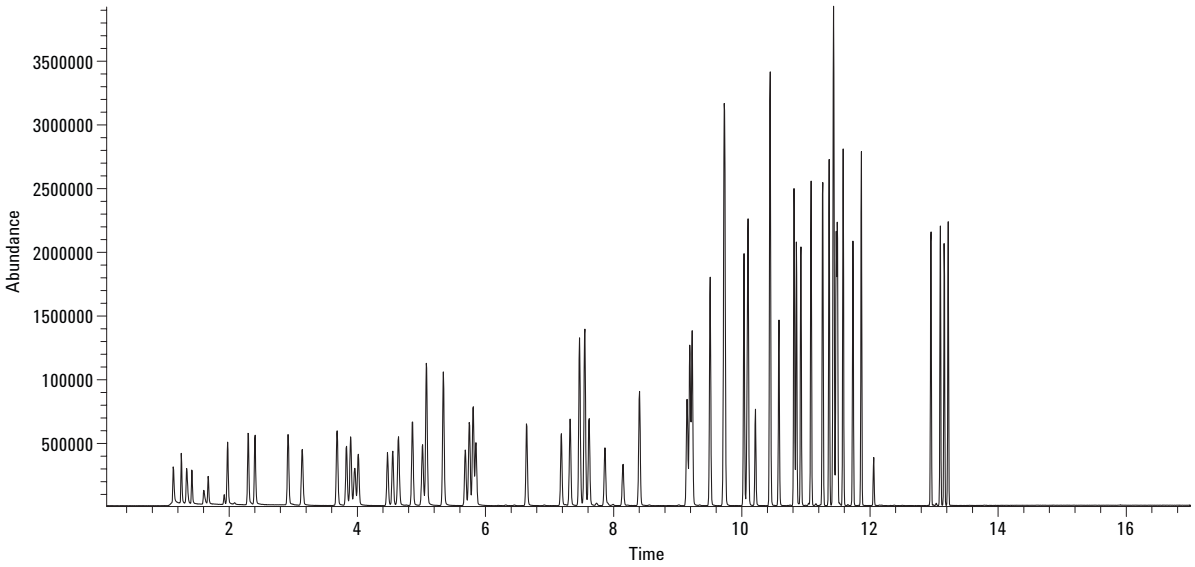

Figure 2. P&T/GC/MS analysis of a standard containing all of the compounds listed in Table 4, each at 50 µg/L in VOC-free water.

The average RF and %RSD of the RFs were calculated for each compound over the 1–300 µg/L and 1–200 µg/L ranges. As seen in Table 4, all five of the SPCCs exceeded their minimum RFs by a comfortable margin for both calibration ranges.

As mentioned above, the CCC RF RSDs must not exceed 30%. Table 4 shows that all six CCCs were significantly less than this for both calibration ranges. In fact, the average %RSD of the CCCs was only 4.90% for the 1–300 µg/L calibration and a remarkably small 1.58% in the narrower 1–200 µg/L range.

Only eight compounds exceeded the 15% RSD requirement in the 1–300 µg/L calibration range. In all cases, the RF fell off significantly for the 300 µg/L standard, suggesting that the strong target ion response overloaded the MSD at that very high concentration.

In the 1–200 µg/L calibration range, the average RF could be used for all targets except, perhaps, bromoform which exceeded the 15% limit by 0.08%. If one justifies only two significant figures, even bromoform could use an average RF for calibrations. The average of the %RSDs for all targets was 8.9% for the 1–300 µg/L calibration and only 4.5% for the 1–200 µg/L range (Table 4). Figure 3 shows a plot of the RFs for each target compound over the 1–200 µg/L calibration range.

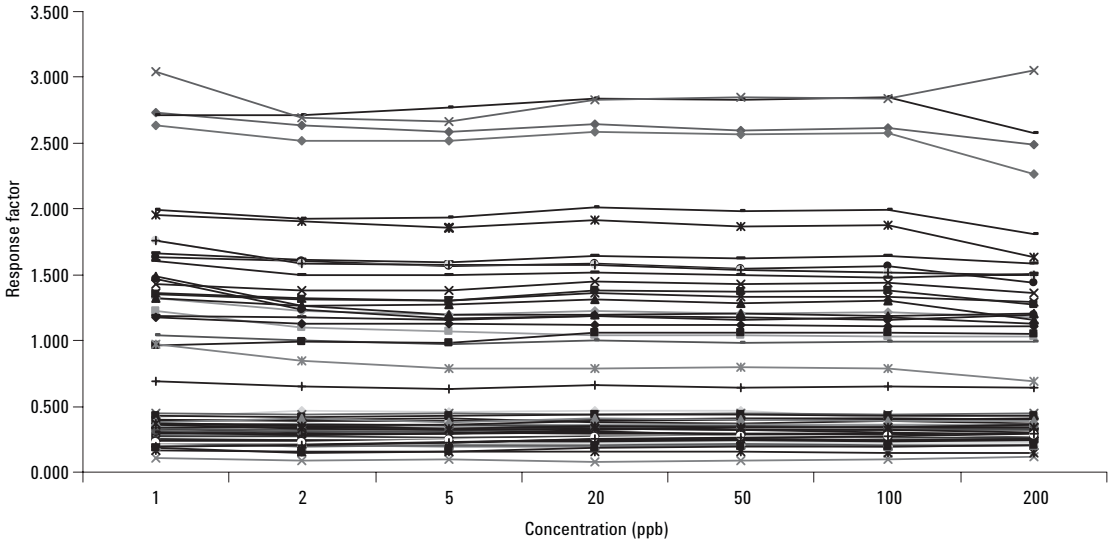

Figure 3. Plot of the RFs from a seven-level calibration for all of the target compounds listed in Table 4. Concentrations were at 1, 2, 5, 20, 50, 100, and 200 µg/L.

Figure 4 plots a distribution of the RF %RSD values for the 59 calibrated peaks (m- and p-Xylene were not resolved). It shows that most compounds have RFs over the 1–200 µg/L calibration range with less than six percent RSD. More than 91% of the compounds have RSD values of 10% or less.

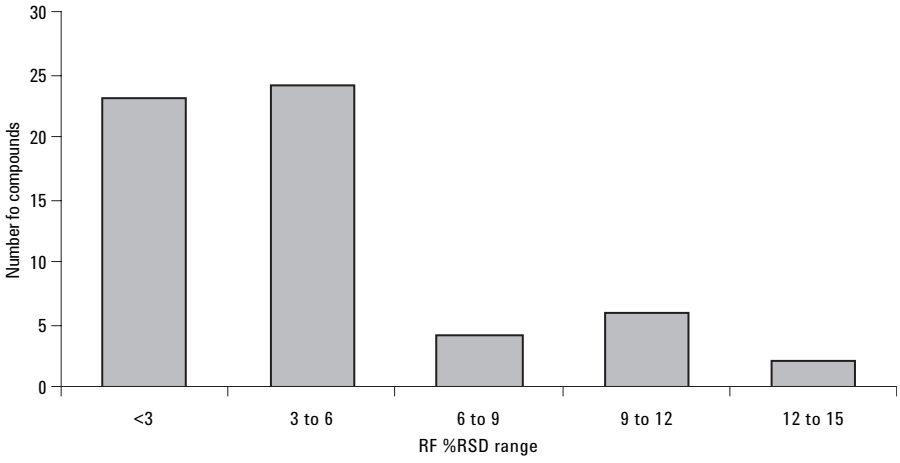

Figure 4. Distribution of the RF RSDs for the 59 calibrated peaks (m- and p-xylene were not resolved).

Response Stability

The longevity of any calibration depends upon having a consistent response for all compounds, even when running samples almost continuously over the course of several days, weeks, or even months. Some laboratories have observed a falloff in response over time that can jeopardize the calibration. Moreover, it has been observed that the recoveries for certain compounds may be dependent upon the presence or absence of other VOCs in the sample. A complete discussion of this problem and some simple solutions for it may be found in the "Optimization Techniques" section below.

In order to assess instrument stability over time, two types of samples were prepared. "System Blanks" contained only FBz, BFB, and 1,2-dichlorobenzene-d_4 (DCB-d_4) at 10 µg/L in water. The first compound was used as the ISTD while the latter two were chosen as surrogates. "Spiked" samples were the same as the system blanks but with the 60 target VOCs added at 20 µg/L each. These samples were analyzed alternately, typically for 22 runs, but sometimes many more runs over several days.

Figure 5 is a plot of the normalized recoveries for FBz, BFB, and DCB-d_4. It illustrates the two problems that can be observed when instrument parameters are not optimized. First, there is a gradual drop in response for all three compounds as illustrated by the sloping arrows. Superimposed upon this is a reduction in surrogate recovery in the absence of added VOCs. Because system blanks and spiked samples were alternated in the sequence, there was a "zigzag" appearance to the plot.

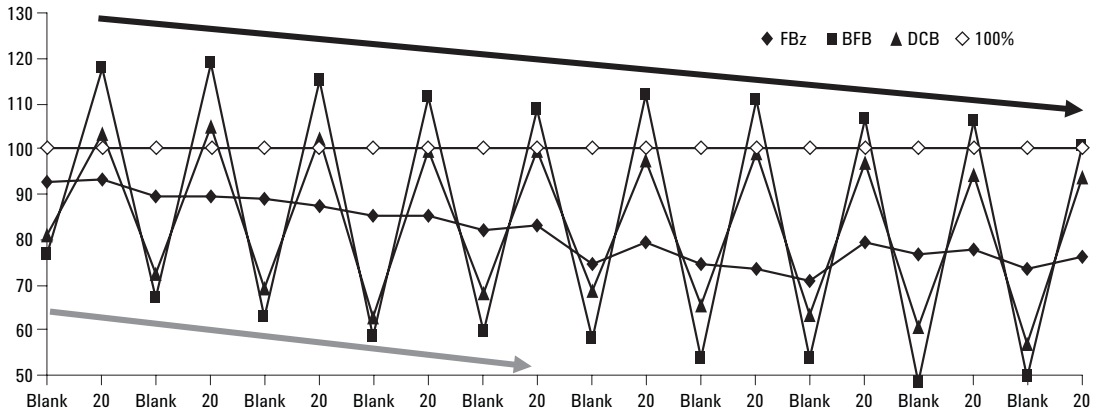

Figure 5. Normalized recoveries for FBz, BFB, and 1,2-DCB-d_4. System blanks (containing only FBz, BFB, and DCB-d_4 at 10 µg/L each) were analyzed alternately with system blanks spiked with an additional 60 VOCs at 20 µg/L each. Arrows show a gradual loss of response over the course of the sequence. The zigzag pattern arises because the recovery of BFB and DCB-d_4 is higher in the presence of other VOCs.

The problems illustrated in Figure 5 can be avoided rather easily by not overloading the MSD's electron multiplier (EM) and by ensuring that there are no active sites in the sample flow path. Figure 6 shows normalized recovery plots for BFB and DCB-d_4 that are typical when the instrument parameters are set correctly. Once again, system blanks and spiked samples were alternated, but this time there was no drop in response over time. Surrogate recovery was independent of sample spiking. Simple solutions for resolving these problems are discussed below.

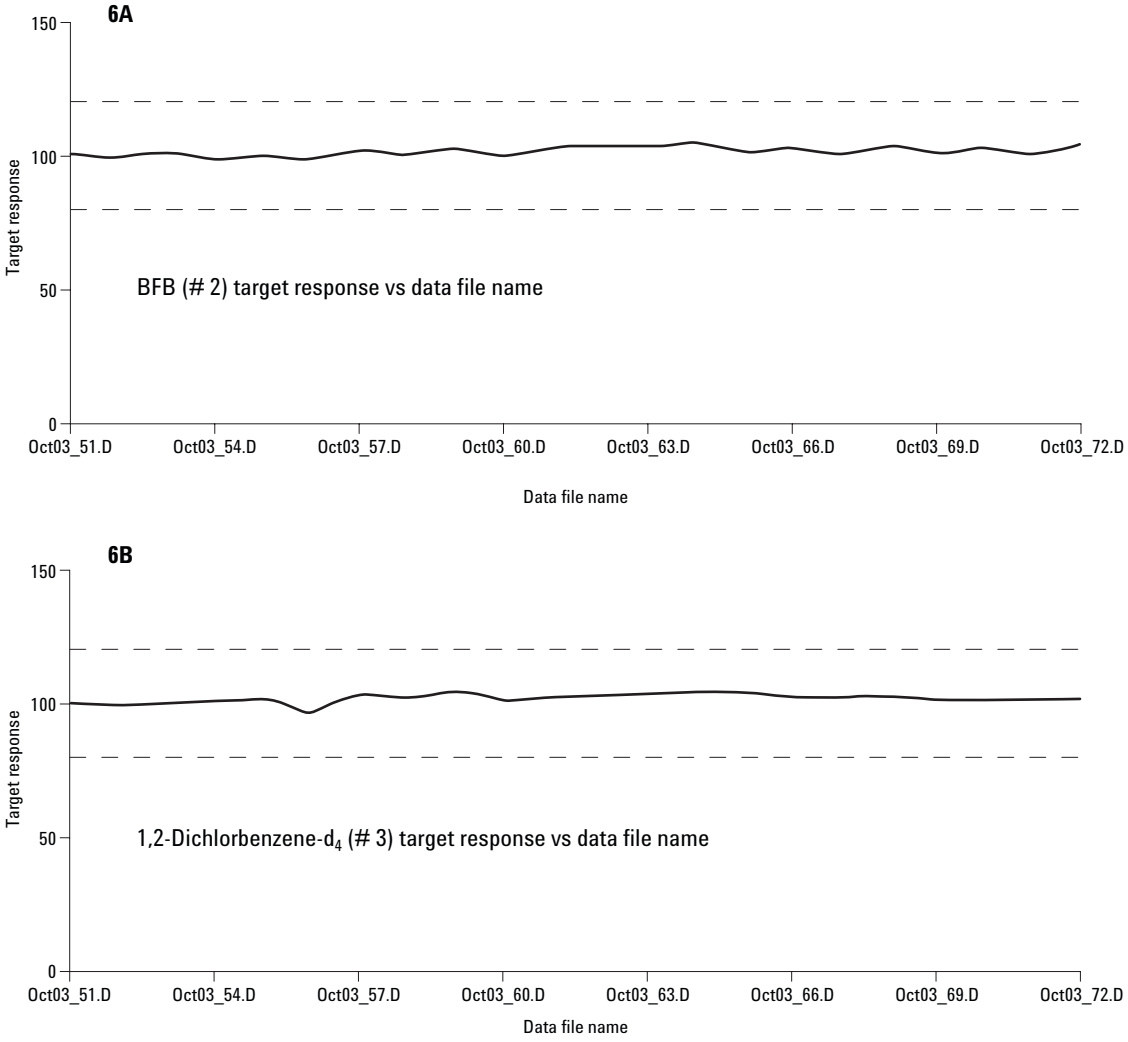

Figure 6. Normalized recovery for BFB (6A) and DCB-d_4 (6B) using the Agilent 6890N/5973 inert GC/MS coupled to the Velocity XPT P&T with optimized system parameters.

Optimization Techniques

MSD Tuning: Application Note 5988-4373EN [6] discusses three different ways to tune Agilent's 5973N MSD in order to meet BFB requirements. With the recent introduction of the 5973 inert MSD, these procedures still apply, though it is helpful to turn off the variable entrance lens setting when using the BFB autotune. The CLP Statement of Work specifications (Table 5) offers more latitude than the 8260B tuning requirements. Most importantly, ion 174 can be up to 120% of ion 95 (the reference ion). It is helpful to tune the MSD so as to produce a 174/95 ion ratio that is in the 90%–120% range because this improves the signal for bromoform (base peak = 173), which purges with poor efficiency. For this work, the "modified autotune" method was used and the 174/95 ratio was about 105%. It has been our experience that once the Agilent 5973 inert has been tuned to meet BFB requirements, the tune is stable for many weeks. It is impossible to say how long, because once tuned, it never failed to pass the BFB requirements.

MSD Parameter Optimization: When ISTD or surrogate responses fall off with repeated injections, overloading the Agilent 5973 MSD's high energy dynode (HED) EM may be the cause. The 5973 was designed to be significantly more sensitive than its predecessors and incorporates an HED in the EM. This reduces the noise and increases the signal, especially for ions of higher mass. However, this highly sensitive detector can be overloaded by continuous ion bombardment or by operating it at too high a voltage. The symptom is an unusually large loss of response over time.

Many GC/MS users erroneously believe that they can increase the sensitivity of their MSD by increasing the EM voltage. This can be done by raising the target value during tuning or by adding voltage to the tune value in the "MS SIM/Scan Parameters" window. However, in the electron impact mode, the noise increases at approximately the same rate as the signal. So, the true sensitivity (signal/noise) does not increase. The main consequence is to reduce the EM's lifetime. This can show up as a reduced response over time that might even be noticeable after several runs. (Note that these statements about signal/noise ratios do not necessarily apply to chemical ionization techniques.)

The solution to this "problem" is relatively simple. The easiest way is to reduce the EM voltage, which reduces the signal and noise, but not the signal/noise ratio. It may also be necessary to reduce the threshold value in the "Edit Scan Parameters" window in order to see the smaller ions. The default EM voltage values from an Autotune or BFB tune are usually correct, but these can be decreased somewhat if the above-mentioned symptoms occur.

It is easier to overload the EM in the selected ion monitoring (SIM) mode, because only a few ion fragments are monitored. During peak elution in the scan mode, there are "blank" spaces in all spectra where the signal is small or zero. With SIM, the signal is almost continuous and the ions monitored are usually the most abundant ones. Here again, the solution is relatively simple. One can reduce the EM voltage, decrease the SIM dwell time, and/or reduce the peak width by choosing the "High Resolution" option. The latter two values are set in the "Edit SIM Parameters" window. In any case, it is important to remember that both signal and noise are roughly proportional to the EM voltage and nothing is sacrificed by making small reductions in its value. Just remember to lower the threshold value or set it to 0 at the same time.

Reducing System Activity: When surrogate recoveries are higher in the presence of other analytes, as illustrated in Figure 5, active sites in the sample flow path are a likely cause. Surrogates can adsorb on these active sites, reducing their recovery. Surrogate recoveries improve when other analytes are present that compete for the active sites. To prevent such problems, one must use a highly inert P&T/GC/MS system and maintain its cleanliness by avoiding contamination from foaming samples. The Agilent 6890N/5973 inert GC/MS coupled to the Velocity XPT P&T showed no signs of sample adsorption. As seen in Figure 6, surrogate recoveries were highly stable with this system. If target or surrogate recoveries vary depending upon the presence of other analytes, it may be helpful to increase the temperature of the MSD source or upgrade an older 5973A or N with the new "Inert" source.

The P&T Method and Water Management: The VOC Teklink software used to control the new Teledyne Tekmar Velocity XPT concentrator and Aquatek 70 autosampler offers a "wizard" tool to help the user choose parameters for the method. Only minor modifications were made to the wizard-generated method. ISTDs were added manually to each sample so the "Fill I.S. Time" was set to 0.00 min. The bake time was increased to 3 minutes and the number of bake rinses was increased to three. The wizard chose all other parameters after the user provided information about the system configuration.

One of the primary concerns of P&T/GC/MS methods is the management of water that is inevitably purged along with the analytes. Since calibration, surrogate, and ISTD solutions are prepared in methanol, some of this solvent is also purged and retained by the trap. By starting the scan at 40 µ, methanol and water ions were not detected by the MSD. Nevertheless, transferring large amounts of water or methanol from the P&T to the GC/MS can result in poor reproducibility for those compounds that co-elute with them. Using the Velocity XPT with the Agilent 6890N/5973 inert system there were no problems that could be attributed to water. Because the P&T was configured with a Vocarb 3000 trap, the DryFlow trap was not required. Various dry purge times and flow rates were tried, but the only affect this had was to distort the peak shape of one or more early eluting peaks. Therefore, the dry purge option was not used. It is likely that some problems attributed to an excess of water actually result from overloading the MSD EM.

Standard preparation: The careful preparation of standards for calibration cannot be overemphasized. As with most laboratories, the initial dilutions were purchased as 2000 µg/mL/component concentrates, which were stored without problem in a refrigerator. Experience in this laboratory showed that best results were obtained when observing the following guidelines:

- Prepare secondary dilutions used for sample spiking from freshly opened standards.
- Transfer secondary dilutions to appropriately sized glass containers so that there is little or no headspace in the vial. Store small quantities in microvials.
- Mininert vial closures were tried for sample storage but were prone to leakage and their use was discontinued. In addition, they were not available for microvials.
- It works well to prepare calibration standards by spiking methanolic solutions into pure water through the septum of the VOA vial. It works equally well to prepare standards in 50- or 100-mL volumetric flasks and pour the aqueous solutions into VOA vials.
- If several VOA vials of the same solution are being prepared at one time, do not prepare the solution in a single large volumetric flask. There will be some VOC loss by pouring repeatedly from the flask. Instead, spike vials individually or use the apparatus described in Figure 1 for sample transfer.
- When preparing calibration standards, transfer the same amount of methanolic solution to each VOC sample. This requires preparing secondary dilutions in methanol for each calibration level instead of spiking different amounts of a single standard.

Leaks: Leaks anywhere in the system can result in poor precision, loss of sample, and calibration failure. Leaks in the carrier gas flow path can easily be detected by the MSD as a high background of oxygen and nitrogen. To correct leaks, tighten or replace the offending fittings after finding the leaks using established techniques. A more difficult problem to detect results from leaks in the fittings that connect the purge vessel to the P&T instrument. Even the smallest leaks during the purge cycle can result in the loss of VOCs and cause poor precision. Leaks that a helium leak detector might miss, can still cause VOC loss. If all the RFs for a given calibration level seem to be low by a similar amount, or if the RF RSDs are all very similar (but too large), then P&T leaks are the likely cause. Tighten or replace the fittings associated with the purge vessel.

Conclusions

EPA Method 8260B with P&T sample introduction is one of the most widely used water analysis methods. There are numerous P&T, GC, and MS variables to optimize in order to obtain long-lasting linear calibration curves and good analytical results. This application note summarizes much of Agilent's experience in optimizing all facets of this VOC method. Most analysts know how to prepare calibration and check samples, tune the MSD, and set instrument parameters; and they find this method to be very rugged with infrequent need for retuning and recalibration. The suggestions in this paper are designed to help in case problems do arise or when an analyst runs this method for the first time. Though the focus was on Method 8260B, these techniques apply to almost any P&T/GC/MS method.

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Speciation of Volatile Selenium Species in Plants Using GC/ICP-MS

Application

Environmental

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Abstract

Gas chromatography/inductively coupled plasma mass spectrometry coupled with solid phase micro-extraction can provide a simple, extremely selective and sensitive technique for the analysis of volatile sulfur and selenium compounds in the headspace of growing plants. In this work, the technique was used to evaluate the volatilization of selenium in wild-type and genetically-modified *Brassica juncea* seedlings. By converting toxic inorganic selenium in the soil to less toxic, volatile organic selenium, *B. juncea* might be useful in bioremediation of selenium contaminated soil [1].

Several instrumental methods can be used for the detection and determination of volatile selenium (Se) and sulfur (S) compounds. These rely primarily on gas chromatography (GC) for separation and different detection techniques, including atomic emission detection (AED), mass spectrometric detection (MS), and flame photometric detection (FPD). The present work addresses the use of gas chromatography/inductively coupled plasma mass spectrometry (GC/ICP-MS) in conjunction with an attractive sample introduction method for volatile species, solid-phase microextraction (SPME). Sampling and preconcentration can be combined into a single step using the SPME technique, which allows simultaneous adsorption and up to 1000-fold preconcentration of low molecular weight analytes. To achieve the elemental speciation, an element specific detection with high sensitivity and selectivity is necessary. The ICP-MS meets these requirements.

GC combined with selective SPME minimizes the possibility of co-eluting matrix-based interferences, eliminating the need for conventional sample preparation steps. Therefore, headspace SPME with GC separation and ICP-MS is the hyphenated system of choice for volatile Se and S volatiles from plants. This system allows performing speciation analysis without significant changes in analyte composition. This will be useful for the extraction and further characterization of volatile Se and S species formed in several plants (for example, garlic, onion, etc.). In this application note, the applicability of the developed approach is demonstrated with Indian mustard, *Brassica juncea,* seedlings.

Phytoremediation and Phytovolatilization

Phytoremediation is defined as the use of living plants to remediate contaminated soil or groundwater through removal, degradation, or containment of the pollutants. It is an *in situ* technique that is gaining public acceptance.

It is known that Se is mainly volatilized as dimethyl selenide, which is 500–700 times less toxic than its inorganic forms. This is beneficial in comparison

to the biovolatilization of volatile organic compounds (VOCs), which are released to the atmosphere without any changes. Volatilization studies of Se from plant tissues may provide a mechanism of Se detoxification.

Experimental Conditions

This study was carried out with two different *Brassica juncea* lines: a transgenic line transformed with tDNA including the Se-cysteine methyl transferase (SMT) gene and wild-type plants. Homozygous SMT and wild-type seeds were sterilized by rinsing them on a rocking platform in 96% ethanol for 30 seconds, then in 0.65% sodium chlorate(i) (NaOCl) solution for 30 minutes, and finally in sterile deionized water for 5–10 minutes.

Sterilized seeds were sown in a grid pattern in each magenta box on half-strength MS-medium (Sigma) with 10 g/L sucrose and 5 g/L phytagar (Sigma). After 2 days at 4 °C, the seeds were gently placed in 20 mL vials (approximately 5 cm high and 2 cm diameter) with half-strength hydroponic solution and left to stand for 1 day.

The next day, Se-containing solutions were added as Na_2SeO_3, Na_2SeO_4, Se-methionine, and KSeCN to a final concentration of 200 µmol Se/L, and a rubber septum was placed on the top and capped to seal (see Figure 1). Control plants received the same treatment, but without the Se-spike. The vials were placed under constant light in a controlled environment room maintained at 25 °C for 1 week.

Figure 1. Photograph of the plant in the glass vial.

Afterwards, the SPME fiber was exposed for 10 minutes to the vial headspace (HS), then immediately inserted into the GC injection port and left to stand for 3 minutes to thermally desorb the analytes. To ensure that there was no memory effect from previous extractions or condensation of larger analytes inside the pores of the Carboxen phase [2], the SPME fiber was conditioned between runs for approximately 5 minutes in a hot injector port.

Results and Discussion

Separation and Identification of the Se Compounds and Stability of the Different Species

GC conditions for GC/ICP-MS were optimized by direct injection of a daily-prepared solution of the standards (DMeSe, DMeDSe, and DEtDSe) in methanol and pentane. The final optimum separation conditions are summarized in Table 1. Fresh standard solutions must be used since several-day-old solutions stored in the refrigerator showed the presence of a few unknown species. Figure 2 shows the GC/ICP-MS chromatogram obtained from a 1-week-old solution containing 100 ppb each (as individual compounds) of DMeS, DMeDS, and DEtDS, and the corresponding Se analogs (DMeSe, DMeDSe, and DEtDSe). Samples were introduced using the HP-SPME technique. However, seven different species can be observed (in the Se trace), one of them with the intensity comparable to the standards. The compound eluting at about 8.5 minutes, peak 8, corresponds to the ethylmethyl diselenide (EtMeDSe), formed from the cross-interaction of DMeDSe and DEtDSe. This was observed in aqueous and pentane or methanol solutions. Further characterization of the unknown compounds was performed using GC/MS.

Table 1. Operating Conditions for GC/ICP-MS

GC	
Model	Agilent 6890A
Column	Capillary column DB-5 (5% dimethylpolisiloxane)
Split ratio	5:1
Column size	30 m × 0.320 mm × 0.25 µm
Inlet liner	SPME injection sleeve, 0.75 mm id
Carrier gas	He, 2.4 mL/min
Injection port temperature	200 °C
Column temperature	35 °C, 4 min isothermal; then 15 °C/min to 125 °C; then 5 min isothermal
ICP-MS	
Model	Agilent 7500s
Optional gas	5% N_2 or O_2
RF power	1100 W
Ar plasma gas flow rate	15 L/min
Ar carrier gas flow rate	0.50 L/min
Ar auxiliary gas flow rate	1.00 L/min
Isotope monitored	^{77}Se, ^{78}Se and ^{33}S, ^{34}S
Interface	
	Uncoated, deactivated, approx. 1 m, 0.320 mm id, in stainless-steel tube surrounded by Ar/N_2 gas flow. Heating via two auxiliary ports at 200 °C.

The mass spectra of Et-Se-S-Et can be easily misinterpreted as that of diethyl selenone, Et-Se(O_2)-Et (as observed in the literature with Me-Se-S-Me and dimethyl selenone). However, simultaneous capabilities of ICP-MS to selectively monitor S and Se signals allow the confirmation of the compound (see Figure 2 where both elements, S and Se, can be observed). Other cross-products have been also observed when S and Se volatile species are mixed together for a certain period of time, namely Me-S-Se-Me, Et-S-S-Me, Et-S-Se-Me, and Me-S-Se-Et. The last two compounds cannot be resolved chromatographically on either DB-1 or DB-5 capillary columns.

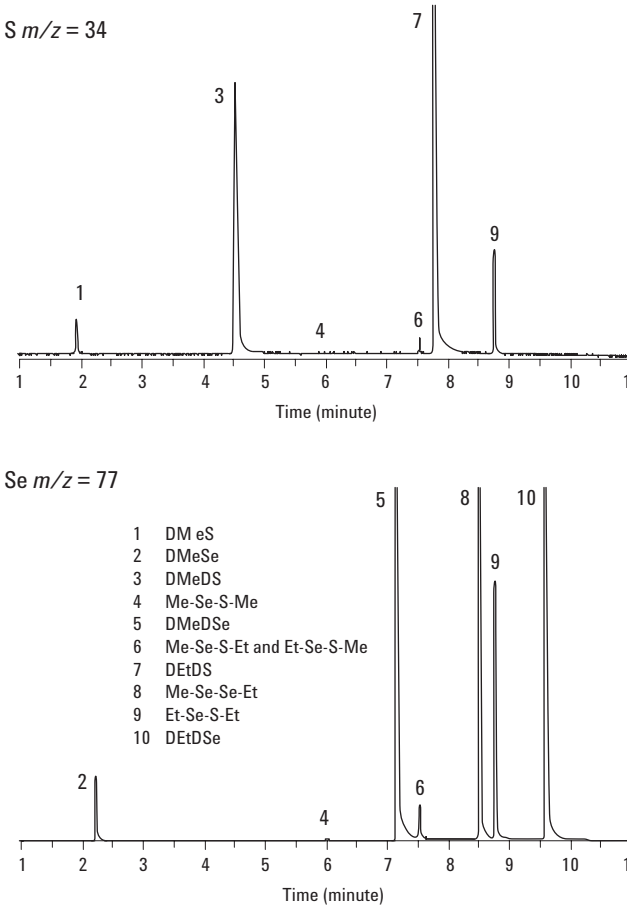

Figure 2. GC/ICP-MS chromatogram of mixed volatiles containing selenium and sulfur.

Analytical Figures of Merit

Detection limits (DLs), precision for five manual injections, and linearity up to 0.5 ppm were evaluated for DMeS, DMeDS, DEtDS and the corresponding Se analogs; these parameters were evaluated using both direct injection and HS-SPME sampling. DLs were calculated using the following formula.

$$\mathrm{DL} = \lim_{\phi_i \to \mathrm{DL}} \left\{ \phi_i \frac{3 s_b}{A_i} \sqrt{n} \right\}$$

Here, ϕ_i is volume fraction of analyte (ppt, ppb), s_b is the average standard deviation of background near the peak of analyte (~200 data points), A_i is the peak area of analyte corrected for the background signal, and n is the number of data points in the analyte peak (usually n = 20–100). Observe the results in Table 2 that were obtained for optimal conditions of RF power and carrier gas flow using 5% N_2 as optional gas. This table also illustrates retention time (RT), peak width at half-height, and precision of the isotope ratio (78/77 for Se and 34/33 for S). As seen in the table, because of the preconcentration on the SPME fiber, the increase in detection capability for all the species when using SPME as sample introduction is dramatic (about three orders of magnitude). This shows high promise for detection of volatile Se species at sub-picogram and S species at picogram levels, a significant improvement over literature values for S [3]. The precision in both cases (five manual injections and fiber exposure to five different vials) is adequate for this approach (see Table 2). The responses indicate linearity of up to 4000 ppb in the case of SPME. Note that due to the traces of ^{82}Kr present in the N_2, ^{82}Se could not be monitored, and the results expressed in the table correspond to the ^{77}Se isotope (although ^{78}Se was always simultaneously monitored to be sure of the presence of Se).

Table 2. Analytical Performance Characteristics for Test Compounds by SPME/GC/ICP-MS

Compound	DL	RT, min	Peak width at half height, s	Method precision** (RSD)	Precision of the isotope ratio***
DMeS	300 ppt	1.94 ± 0.02	1.8	7%	2.1%
DMeDS	80 ppt	4.56 ± 0.02	2.3	12%	1.7%
DEtDS	25 ppt	7.78 ± 0.02	1.7	10%	2.2%
DMeSe	7 ppt (65 ppb)*	2.21 ± 0.01	2.4	7%	1.1%
DMeDSe	1 ppt (7 ppb)*	7.13 ± 0.01	2.0	8%	2.3%
DEtDSe	1 ppt (7 ppb)*	9.58 ± 0.01	1.6	13%	2.2%

*DL for direct injection (1 µL solutions in pentane)
**Evaluated at the level of 100DL (n = 4)
***34/33 for S and 78/77 for Se

Detection of Volatile Species in *Brassica juncea*

Brassica juncea plants were grown hydroponically in nutrient solutions inside a glass vial with a rubber septum cover through which the SPME needle was pierced. Two main Se compounds are present from *Brassica juncea:* DMeSe and DMeDSe. Due to the ultratrace DLs available with this instrumentation (GC/ICP-MS), it was possible to observe minor additional Se containing species that were not identifiable at that level by GC/MS. Enrichment of samples with different Se sources: Na_2SeO_3, Na_2SeO_4, Se-Methionine, and KSeCN did not provide any significant difference in terms of the species released by the plants (mainly DMSe and DMDSe), but important differences were observed in the concentration of these species. Previous studies in this field have shown that wild type Indian mustard treated with selenate accumulate Se mainly as inorganic forms in root and shoot tissues, whereas selenite and Se-methionine treated plants accumulate Se in the form of Se-methyl selenocysteine. The Se-methionine is volatilized much more readily than selenate or selenite to form mainly DMSe through an intermediate selenonium compound (Se-methyl-selenomethionine). On the other hand, DMDSe could be produced through Se-methyl-selenocysteine, but to a much lesser extent than DMSe in *Brassica* tissues. Very little literature exists describing the presence of this species. This could be ascribed to the lack of highly sensitive/selective techniques for the determination of this species at very low levels.

According to the results previously published, the production of DMeSe is dramatically increased when the Se source is Se-methionine, described above.

The enrichment with KSeCN did not produce any significantly different species to those obtained with any of the other Se enrichments (mainly DMeSe and minimum amount of DMeDSe). Further identification studies by GC/MS indicated that the main volatile species present in the *Brassica juncea* headspace are allyl isothiocyanate and 3-butenyl isothiocyanate. The ratio of these two isothiocyanates is found to be 2:1, and the ratio is not affected by the different Se treatment procedures. Allyl isothiocyanate is the volatile S compound released during the decomposition of leaf tissues of *Brassica juncea*. These isothiocyanates have been observed on each one of the plants analyzed, and their levels have proved to be constant despite the treatment or modification used. Therefore, these species could be used as an *in vivo* internal standard for normalization of the Se signal for day-to-day variations.

When the plants are left unrefrigerated and natural decomposition starts to occur, the production of H_2S and DMeS seems to increase. Figure 3 shows the comparison of the Se and S traces in the fresh plant and in the old one.

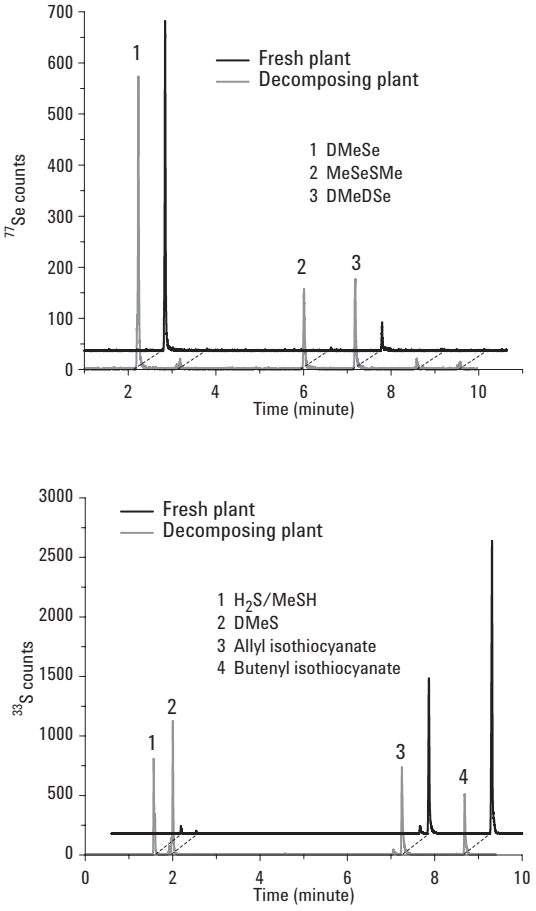

Figure 3. S- and Se-containing volatile species in wild-type *Brassica juncea* treated with $SeCN^-$. Fresh and decomposing plants are compared.

Conclusions

The coupled technique of HS-SPME/GC-ICP-MS has proven suitable for the speciation of volatile Se species in plants. The ultratrace DLs achieved permits the speciation of these compounds at very low levels in biological samples, such as plants, and requires minimal sample treatment.

Use of auxiliary gases, such as oxygen and nitrogen, increases the sensitivity of GC/ICP-MS for Se. Maximum sensitivity for volatile Se species can be achieved by mixing 5% nitrogen with the argon carrier gas. Although the use of oxygen or nitrogen leads to spectral interferences, the ^{77}Se minor isotope is not affected and, therefore, can be used for quantification. Monitoring xenon, which is a common impurity in the argon plasma gas, may be used for ICP-MS optimization studies, as the behavior of ^{131}Xe in the plasma is similar to that of ^{77}Se.

The use of GC/MS in combination with SPME allows the identification of several unknown species found as decomposition products in the standards and also as volatilization products from the *Brassica* seedlings.

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Optimized Analysis of Gasoline (BTEX) in Water and Soil Using GC/FID with Purge and Trap

Application Note
228-324

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Abstract

Gas chromatography with purge and trap analysis using their HP-1 capillary column and the Agilent 5890 Series II gas chromatograph/flame ionization detector was done to determine gasoline components in contaminated water and soil in accordance with modified EPA Methods 8015/8020. Purge and trap and gas chromatograph parameters were optimized for accurate quantitation of gasoline range organics (aliphatics, aromatics, and oxygenates) and to increase analysis speed.

Key Words

- EPA Methods 8015/8020
- Gasoline
- BTEX
- Purge and Trap
- GROs
- GC/FID analysis
- LUST

Introduction

Modified EPA Methods 8015/8020 are used to determine gasoline and gasoline components in water and soil by capillary gas chromatography (GC) with a flame ionization detector (FID) or photoionization detector (PID). The hydrocarbons in gasoline encompass a wide range, from butane to decane and benzene to naphthalene, and cover a boiling point range of 50°C to 281°C. For such complex mixtures, an efficient purge and trap (P and T) system is required to concentrate samples for high-resolution gas chromatography. Detection is achieved using an FID, and quantitation is based on FID response to a gasoline standard. Other light petroleum products that can be determined in the same manner include paint stripper, Stoddard solvent, mineral spirits, petroleum naphtha, and aviation jet fuels using the pattern recognition technique.

The analysis of gasoline components, e.g., gasoline range organics (GROs), and benzene, toluene, ethylbenzene, and xylenes (BTEX) in particular is of great importance because BTEX is frequently used as a marker in the identification of gasoline-type products. Subsequently, the analysis of BTEX is often used to determine the composition and the origin of such products including weathered fuels leaking from underground storage tanks (LUST), spills in pipe lines, and run-off from surface transportation.

For the analysis of gasoline with BTEX, the sample is introduced into a sparge tube on the P and T autosampler or purge vessel or the P and T unit. The P and T concentrates the volatiles in the sample and transfers them onto the capillary column.

Parameters affecting the efficiency of P and T sample concentration include time and temperature for sample purge, dry purge, desorption of trapped volatile organics and trap baking. Most P and T system manufacturers recommend 11 minutes of purge or a total of 440 ml purge gas through the sample. Many laboratories use the manufacturer's set purge flow of 40 ml/min which corresponds to 11 minutes of purge time, to achieve a minimum of 440 ml purge gas through the sample. In this study a Vocarb-3000 trap was used because it can provide higher trapping efficiency and allow for higher desorption and baking temperature.

A typical analysis can usually be completed in 35 to 40 minutes. In this application both P and T parameters and GC conditions were optimized for accurate quantitation and analysis speed.

Experimental

Samples were concentrated using an Agilent 7695A P and T system with a Vocarb-3000 trap (part no. 5182-0775) and a 5-ml frit sparger (part no. 5182-0852). Using an HP-1 column (30 m x 0.53 mm x 5.0 µm, (part no. 19095Z-623), hydrocarbons were analyzed on an Agilent 5890 Series II GC with EPC and FID. Instrument requirements and optimal GC and P and T conditions are listed in **Table 1**.

Working solutions were prepared from diluting commercial gasoline, LUST-modified GROs (part no. 5182-0860), and internal standard and surrogate (part no. 8500-6007) with GC-grade methanol (Burdick and Jackson). Concentrations of GROs, gasoline, and jet fuel standards are listed in **Table 2**.

Samples were prepared from spiking 5 ml of organic-free reagent water using a 5-ml sample syringe with a luer connector (part no. 9301-1185) with standard solutions using 5-µl to l00-µl fixed needle syringes (HP part nos. 9301-0810, 9301-0818, 9301-0059, 9301-0063, respectively).

Results and Discussion

To obtain accurate and reproducible results, complete sample purging, managing water adequately from the P and T system, and preventing carry-over from the trap are essential. Many environmental laboratories analyze gasoline with BTEX using long sample purge (11 to 15 minutes), dry purge (2 to 4 minutes), trap desorb (2 to 4 minutes), and trap bake (10 to 20 minutes) times. Therefore, a typical run usually takes 40 to 48 minutes including 3 to 5 minutes for trap cool-down.

Figure 1 shows a GC/FID analysis of a gasoline standard and a GC/PID chromatogram of a GROs standard using an OI 4460A P and T system with a BTX trap and DB-1 column (30 m x 0.53 mm x 5 µm). GC and P and T conditions are listed in **Table 3**. Although the GC runs were completed in 27 minutes, the actual cycle time for each run was 37 to 40 minutes.

Table 1. Instrument Requirements and Optimized Conditions

A. Recommended Instrumentation

Gas chromatograph:	5890 Series II
Injection port:	Split/splitless inlet
Column:	HP-1, 30 m x 0.53 mm x 5.0 µm (Part no. 19095Z-623)
Detector:	FID
Injection technique:	7695A P and T
Data system:	3365 ChemStation and HP Vectra 486/100MX

B. Experimental Conditions

GC Parameters

Inlet:	220°C, split injection (split ratio 5:1)
Carrier:	Helium, 10 ml/min, constant flow (6.5 psi at 40°C)
Oven parameters:	40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min
Detector:	FID, 300°C; nitrogen makeup gas, 25 ml/min; H_2, 30 ml/min; and air, 350 ml/min PID, 250°C

P and T Parameters

Line temperature:	200°C	Purge time:	11 min
Valve temperature:	200°C	Dry purge time:	1 min
Mount temperature:	40°C	Desorb time:	2 min
MCS line temperature:	100°C	Bake time:	5 min
Purge ready temperature:	30°C	BGB time:	2 min
MCS desorb temperature	40°C		
Desorb preheat temperature:	245°C		
Desorb temperature:	250°C		
Bake temperature:	265°C		
MCS bake temperature:	300°C		

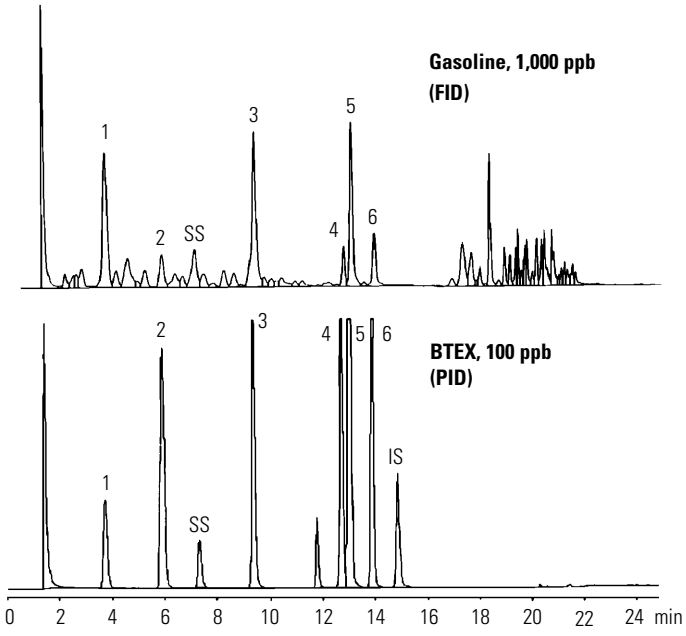


Figure 1. Typical chromatograms of gasoline and GROs standards using a DB-1 column under the GC and P and T conditions (Table 3) used in environmental testing laboratories (see Table 2 for peak identification).

Optimized GC Run Time

With the HP-1 column (30 m x 0.53 mm id x 5 µm) and a faster oven temperature, the GC run time was initially reduced to 21 minutes for GROs and gasoline (see **Figure 2**). Good baseline separations and sharp symmetric peaks **(Figure 2B)** were obtained for all GROs, including surrogate (α,α,α-trifluorotoluene) and internal (4-bromofluorobenzene) standard. The oven temperature program used was 40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min and a constant carrier flow of 10 ml/min. Under these conditions (**Table 1**), both pentane and MtBE were clearly separated from the large solvent peak (menthanol).

Even though the last GROs component (naphthalene) eluted below 200°C at 17.8 minutes, the oven temperature was increased to 250°C to bake out the high-boiling material purged from the sample. As a result, no carryovers were found even with repeated injections of gasoline standard in the 23,000-ppb level.

GC run times were further lowered by using a thinner-film HP-1 column and/or faster oven temperature programs. **Table 4** shows the benefits of using various column thicknesses, temperature ramps, and carrier flows to achieve the optimal GC run time of 17 minutes. Analytes generally elute faster from a thin-film column (**Figure 4**). In **Figure 3**, the thick-film column retained hydrocarbons longer initially until the faster oven temperature ramp (15°C/min) sped up the elution of all GROs components from the column. To avoid potential coelution (peaks 4 and 5), a comparative smaller carrier flow (4.5 ml/min) was used instead of the optimal 10 ml/min carrier flow. Reducing the GC run time, however, would be counterproductive because the total run time is dependent on the P and T cycle.

Table 2. Analytes in Working Standards

Standards	Peak No.	Components	Concentration
GROS mix	1	MtBE	100 ppm each
	2	Benzene	
	3	Toluene	
	4	Ethylbenzene	
	5	m-/p-Xylene	
	6	o-Xylene	
	7	1,2,4-Trimethylbenzene	
	8	1,3,5-Trimethylbenzene	
	9	Naphthalene	
	10	α,α,α-Trifluorotoluene (SS)	
	11	4-Bromofluorobenzene (IS)	
Gasoline standard		Gasoline	500 ppm
Gasoline		Gasoline	2,500 ppm
Jet fuel		Aviation jet fuel	1,000 ppm

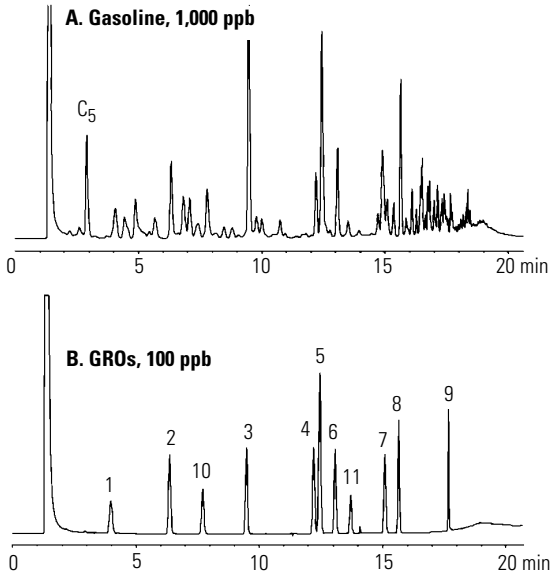


Figure 2. Chromatograms for gasoline and GROs standards using an HP-1 column under the optimal GC and P and T conditions listed in Table 1. (See Table 2 for peak identification.)

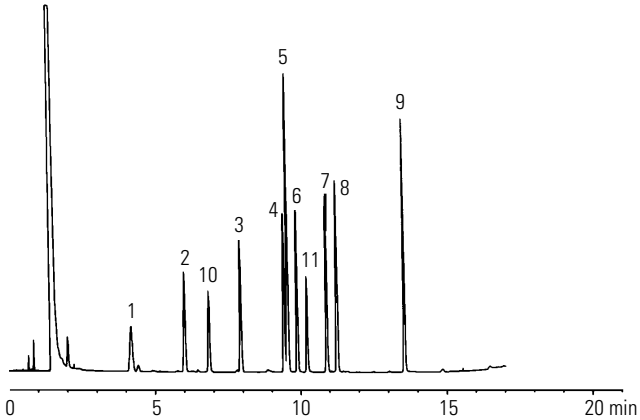
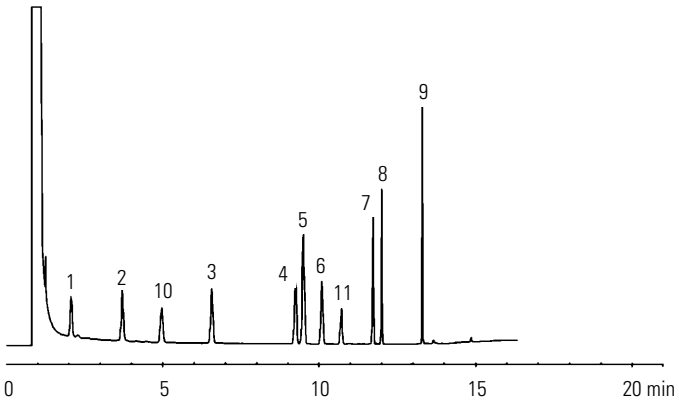


Figure 3. Chromatogram of GROs standards using a thick-film HP-1 (30 m x 0.53 mm x 5 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)

Table 3. Typical GC and P and T Conditions for Gasoline and BTEX Analysis

GC Parameters			
Injection:	Direct injection		
Carrier flow:	Initially 10 ml/min, constant pressure mode		
Oven temperature:	50°C (hold 3 min) to 125°C at 5°C/min to 240°C (5 min) at 45°C/min		
Detector:	PID (250°C) in series with FID (300°C)		
P and T Parameters			
Trap:	BTX trap		
Purge temperature:	Ambient	Purge time:	11 min
Dry purge temperature	22°C	Dry purge time:	2 min
Desorb preheat temperature	150°C	Desorb time:	4 min
Desorb temperature	180°C	Bake time:	15 min
Bake temperature:	200°C		

Figure 4. Chromatogram of GROs standards using a thin-film HP-1 (30 m x 0.53 mm x 3 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)



Optimized P and T Cycle Time

Further optimization of the run was dependent on obtaining the most efficient parameters for the P and T cycle. Each aspect of the cycle was optimized as follows.

Sample Purge

Experimentation showed 11 minutes of purge time, or 440 ml of helium purge gas, to be the most efficient time for analyses of gasoline and GROs because shorter purge times (8 minutes or 320 ml of purge gas) were not sufficient to purge all GROs from the sample solution. **Figure 5** shows a comparative analysis of the same GROs standard shown in **Figure 2B** using 8 minutes of purge time instead of 11 minutes of purge time. The conditions for both analyses were the same and are shown in **Table 1**. By comparison, hydrocarbon recoveries (including aromatics) for the GC runs with 8 minutes of sample purge were not as good particularly for the high-boiling fractions, such as trimethylbenzenes and naphthalene (compare peaks 7, 8, and 9 in **Figure 5** and **Figure 2B**). The naphthalene peak in **Figure 5** (8 minutes of purge) was remarkably small, and area counts were lower than 1% of that recovered in **Figure 2B**. Based on this finding, 11 minutes is the optimal sample purge time for the determination of gasoline with BTEX.

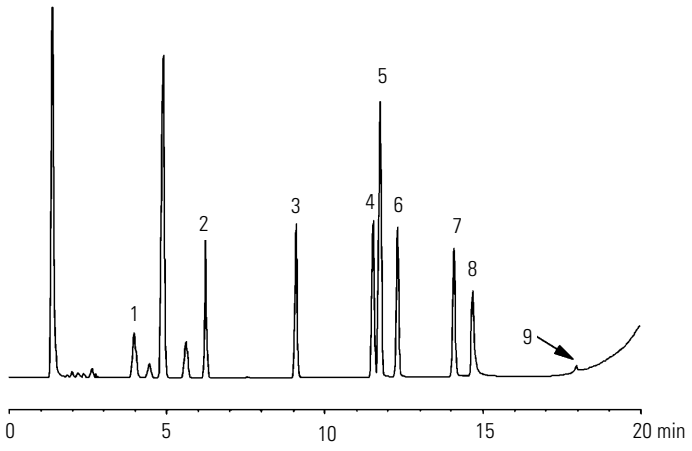
Dry Purge

During sample purge, a larger amount of water is purged along with the volatile organics and is collected on the trap sorbent. Sorbent material in the Vocarb-3000 trap is designed to minimize water trapping and reduce the release of excessive water onto the GC column during the thermal desorption process. A 1-minute dry purge of the Vocarb trap was selected because the early-eluting peaks (such as pentane, MtBE, and benzene in **Figure 2**) were not skewed by water released from the trap onto the column.

Desorption

According to Klee[1], a fast and reproducible desorption temperature is the key to good chromatography using the P and T concentration technique. The higher the desorption temperature and desorption rate, the faster the volatile analytes can be moved to the GC column, and the narrower the peak widths of the early-eluting analytes. Therefore, a short desorption time is preferred. In addition,

Figure 5. Chromatogram of GROs standard using an 8-minute sample purge. (See Table 2 for peak identification and Table 1 for GC and P and T conditions.)



Doherty[2] reported that peak heights and peak areas of volatile organics, including those in the GROs mix, were virtually unchanged when the desorb time changed from 4 minutes to 1 minute. Several manufacturers of P and T systems also recommend a 1-minute desorb time for the routine analysis of volatile organics. However, experimentation (**Figure 2**) using a 2-minute desorb time at 250°C accommodated sharp initial peaks as well as good separation. This study applied a 2-minute desorption time at 250°C to all analyses.

Trap Baking

Three different bake times were evaluated for the Vocarb-3000 trap (used a bake temperature of 265°C, recommended for the Vocarb-3000 trap): 10, 8, and 5 minutes. At each bake time, the gasoline sample (1000-ppb concentration) was run using an 11-minute purge time followed by a run of reagent water with no sample purge. Chromatograms of these two runs were evaluated for carryover. In all three cases (bake times of 5, 8, and 10 minutes), no carryover was observed for any gasoline component. Therefore, a 5-minute bake time at 265°C was selected as an optimal bake time for the analysis of gasoline and GROs aromatics.

For samples containing 46,000 ppb of gasoline, no carry over from the trapped analytes was observed at the 5-minute bake time. This is based on the comparison of chromatograms of reagent water (0-minute purge) run immediately after each sample. However, carry over from the purge vessel was found. Repeated rinsing of the purge vessel with reagent water reduced the amount of carry over but did not eliminate it. Therefore, after a high level sample is run, it is advisable to remove and clean the purge vessel prior to the next run.

Heavier petroleum products, such as diesel and jet fuel (**Figure 6**), that often contain volatile components are also detectable by this method. Again, carry over is a problem. Carry over was observed in the reagent water (used an 11-minute purge) run immediately after the jet fuel sample.

Carry over ranged from 10 ppb to 60 ppb jet fuel and was high enough to cause a false-positive identification in subsequent runs.

As demonstrated by **Figure 7B** (a chromatogram of reagent water, 0-minute purge, run immediately after a jet fuel sample), carry over from the Vocarb trap was found to be negiligible. Clearly the carry over was the result of contamination from the purge vessel (see **Figure 7A**). Although repeated rinsing reduced the amount of carry over, it did not eliminate it completely. Purge vessel carry over was eliminated completely when the purge vessel and the purge needle were removed and cleaned (see **Figure 7C**).

Table 4. GC Run Time of 17 Minutes

HP-1 Column Thickness	Oven Ramp	Carrier Flow Time
30 m x 0.53 mm x 5 µm	40°C (3 min) at 15°C/min to 250°C	4.5 ml/min (see **Figure 3**)
30 m x 0.53 mm x 3 µm	40°C (3 min) at 7°C/min to 95°C to 250°C (2 min) at 45°C/min	10 ml/min (see **Figure 4**)

Figure 6. Chromatogram of 1,000-ppb aviation jet fuel standard. (See Table 1 for GC and P and T conditions.)

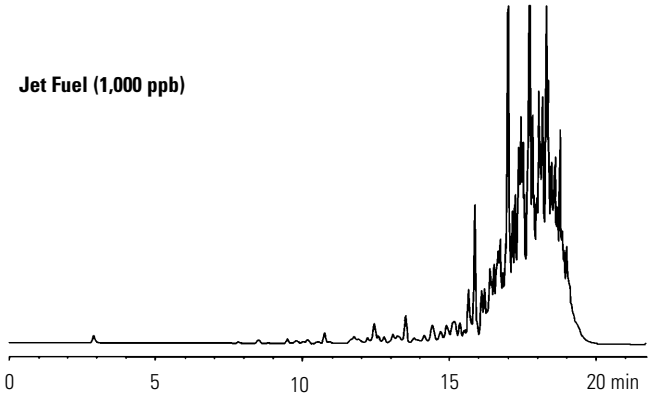
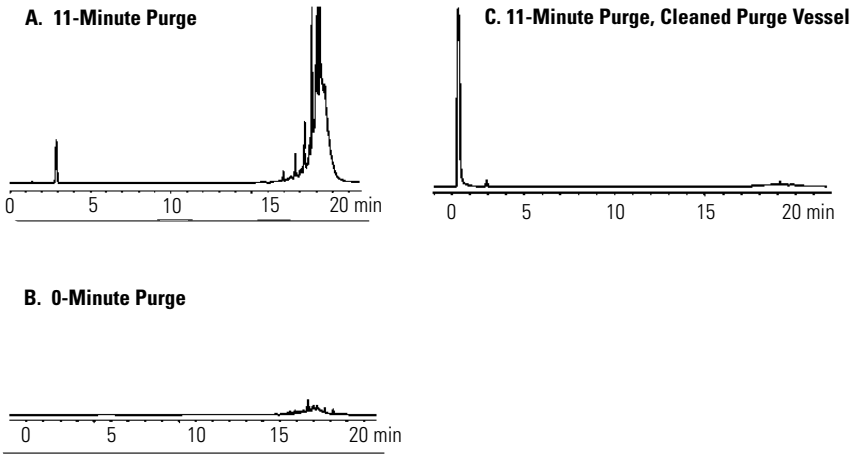


Figure 7. Chromatograms of reagent water following the analysis of the 1,000-ppb aviation jet fuel sample. (See Table 1 for GC and P and T conditions.) Note: The chromatograms were plotted on the same FID response scale.



Conclusion

Determination of optimized P and T parameters is critical in establishing optimized run times for the analysis of gasoline/BTEX. By reducing the P and T bake time to 5 minutes and selecting shorter dry purge (1 minute) and desorption times (2 minutes), the overall P and T cycle was shortened to 25-26 minutes. This is compatible with the run time of 21-22 minutes established for optimized GC conditions. When carry over from the purge vessel is controlled, this same application can be used successfully for the analysis of samples containing in excess of 46,000 ppb of gasoline and other volatile organics in light petroleum products.

Acknowledgment

The authors wish to than Mr. Robert Remlinger at the Pace Laboratory in Petaluma, California for providing his GC/FID and GC/PID chromatograms.

Reference

1. Mattew S. Klee, *GC Inlets—An Introduction*, Hewlett-Packard Company, 1991, Part No. 5958-9468.
2. Linda Doherty, "The analysis of volatile organics in drinking water with an OI 4460A purge and trap and an HP GC/MS system," Hewlett-Packard Company, GC/MS Application Note, August 1991, Publication No. (23) 5091-2477E.

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GC Analysis of Polybrominated Flame Retardants

Application

Environmental

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Abstract

Polybrominated diphenyl ethers (PBDE) are used as flame retardants in such diverse products as textiles, circuit boards, and computer covers. Through the disposal of those products in landfills, PBDEs have found their way into the environment. Studies have shown that PBDEs have detrimental health effects.

Detection and quantitation of these compounds is complicated by their intrinsic properties: high boiling points and low thermal stability. This application note describes development of suitable gas chromatography/mass spectrometry, gas chromatography micro electron capture detection, and gas chromatography inductively coupled plasma mass spectrometry methods to analyze PBDEs. The Agilent DB-XLB is the column of choice for this demanding analysis. The detection limit with micro electron capture detector was 100 ppt for most congeners.

Introduction

The presence of polybrominated diphenyl ethers (PBDE) throughout the environment has attracted the attention of scientists around the world. PBDEs are used as flame retardants in many commercial products, such as textiles and furniture, and in circuit boards in consumer electronics, such as TVs and computers. As more and more of these abundant consumer products find their way into landfills, PBDEs have been found in our drinking water supplies [1]. One alarming study predicts that the levels found in human breast milk of North American women appear to double every 2 to 5 years [2]. Exposure of personnel working with computers is also a concern [3]. While the toxicology of PBDE is still under investigation, research has established that it is persistent, bioaccumulative, and toxic. There is evidence that

PBDE can cause neurotoxic effects similar to the now-banned polychlorinated biphenyls (PCB). As a result, California has just signed legislation banning the use of PBDEs [4]. Like PCBs, there are 209 PBDE congeners (Figure 1), and they are named in analogy to PCBs [5]. However, only seven congeners comprise about 95% of all detected peaks [6]. These major congeners are (by IUPAC number): 28, 47, 99, 100, 153, 154, and 209.

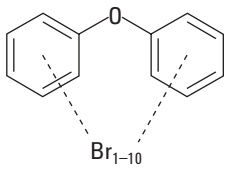


Figure 1. Structure of PBDE.

Until recently, the lack of available standards and individual congeners has made accurate quantitation difficult [7]. Now, practically all individual congeners are commercially available. For analysis by GC, several different stationary phases have been used. However, analysis times are generally quite long, and often not all critical congeners are sufficiently resolved. This study investigates two different columns and three detection modes. DB-XLB (Agilent Technologies, Folsom CA), a proprietary low-polarity stationary phase and DB-35ms (Agilent Technologies, Folsom CA), a mid-polarity phase, are both columns that have very low bleed and high thermal stability. DB-XLB has shown to be an excellent choice for detailed, high-resolution analysis of PCB congeners by GC/MS [8]. The structural similarities between PCBs and PBDEs suggest that DB-XLB should be an excellent choice for separation of PBDEs as well. DB-35ms has shown to be a suitable confirmatory column to DB-XLB [9]. The detection modes evaluated were mass selective detector (MSD), micro electron capture detector (μECD), and inductively coupled plasma mass spectrometry (ICP-MS). Method optimization efforts for speed, sensitivity, and resolution included different column dimensions, inlet conditions, detector settings, and temperature programs.

Results and Discussion

Baseline separation of all 14 critical congeners (Table 1) in a standard mixture including decabromodiphenylether (BDE-209) could be accomplished by DB-XLB in about 20 minutes with excellent peak shape and response of the decabromodiphenylether [10].

Table 1. PBDE Congeners in Test Mix EO-5103 Elution Order on DB-XLB

Peak	Congener (2.5 mg/mL)
1	2,2',4-TriBDE (BDE-17)
2	2,4,4'-TriBDE (BDE-28)
3	2,3',4',6-TetraBDE (BDE-71)
4	2,2',4,4'-TetraBDE (BDE-47)
5	2,3',4,4'-TetraBDE (BDE-66)
6	2,2',4,4',6-PentaBDE (BDE-100)
7	2,2',4,4',5-PentaBDE (BDE-99)
8	2,2',3,4,4'-PentaBDE (BDE-85)
9	2,2',4,4',5,6'-HexaBDE (BDE-154)
10	2,2',4,4',5,5'-HexaBDE (BDE-153)
11	2,2',3,4,4',5'-HexaBDE (BDE-138)
12	2,2',3,4,4',5',6-HeptaBDE (BDE-183)
13	2,3,3',4,4',5,6-HeptaBDE (BDE-190)
14	DecaBDE (BDE-209) (12.5 mg/mL)

A more demanding mixture (Table 2a,b) containing 39 of the most common and important congeners at very low concentration could be separated by DB-XLB in about 14 minutes (Figure 2a, b). This is much faster than analysis times typically reported with other columns. Although two of the tetra isomers are very close with this column, they were baseline resolved with DB-35ms. By contrast, there were two co-elutions with the DB-35ms, which were both baseline resolved on DB-XLB. This demonstrates that these two stationary phases are an excellent choice as a pair of confirmation columns. For baseline resolution of all congeners on a single column, as well as for separation of more complex mixtures, a column with more theoretical plates and/or a higher phase ratio may be necessary. Using

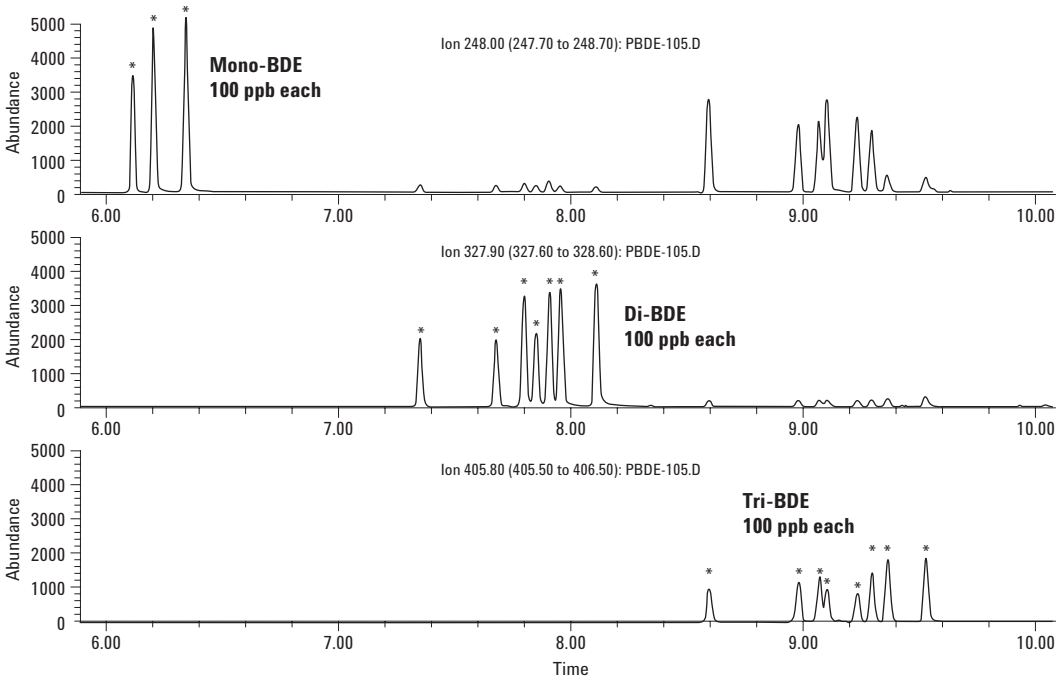
a DB-XLB, 30 m × 0.18 mm × 0.18 µm gave complete baseline separation of the tetra isomers, as did a DB-5ms, 60 m × 0.25 mm × 0.25 µm. However, the higher substituted isomers, in particular BDE-209, showed relatively low response. The lower phase ratio results in longer retention times for all congeners. This longer residence time on the column at high temperature may lead to on-column break down of these thermally labile compounds.

Table 2a. PBDE Congeners in Test Mix EO-5113 Elution Order on DB-XLB

2-MonoBDE (#1)	2',3,4-TriBDE (#33)	2,3,4,5,6-PentaBDE (#116)
3-MonoBDE (#2)	2,4,4'-TriBDE (#28)	2,3′,4,4′,5-PentaBDE (#118)
4-MonoBDE (#3)	3,3′,4-TriBDE (#35)	2,2',4,4',6,6'-HexaBDE(#155)
2,6-DiBDE (#10)	3,4,4'-TriBDE (#37)	2,2′,3,4,4′-PentaBDE (#85)
2,4-DiBDE (#7)	2,4,4′,6-TetraBDE (#75)	3,3',4,4',5-PentaBDE (#126)
3,3′-DiBDE (#11)	2,2',4,5'-TetraBDE (#49)	2,2',4,4',5,6′HexaBDE(#154)
2,4′-DiBDE (#8)	2,3′,4′,6-TetraBDE (#71)	2,2',4,4',5,5'-HexaBDE(#153)
3,4-DiBDE (#12)	2,2',4,4'-TetraBDE (#47)	2,2',3,4,4',5'-HexaBDE(#138)
3,4′-DiBDE (#13)	2,3′,4,4′-TetraBDE (#66)	2,3,4,4',5,6-HexaBDE (#166)
4,4'-DiBDE (#15)	3,3',4,4'-TetraBDE (#77)	2,2',3,4,4',5',6-HeptaBDE (#183)
2,4′,6-TriBDE (#32)	2,2',4,4′,6-PentaBDE (#100)	2,2',3,4,4',5,6-HeptaBDE(#181)
2,4,6-TriBDE (#30)	2,3′,4,4′,6-PentaBDE (#119)	2,3,3',4,4',5,6-HeptaBDE (#190)
2,2',4-TriBDE (#17)	2,2',4,4',5-PentaBDE (#99)	
2,3′,4-TriBDE (#25)		

Table 2b. PBDE Congeners in Test Mix EO-5113 Elution Order on DB-35ms

3-MonoBDE (#2)	2,4,4'-TriBDE (#28)	2,3′,4,4′,5-PentaBDE (#118)
2-MonoBDE (#1)	2',3,4-TriBDE (#33)	2,3,4,5,6-PentaBDE (#116)
4-MonoBDE (#3)	3,3′,4-TriBDE (#35)	2,2',4,4',6,6'-HexaBDE(#155)
2,6-DiBDE (#10)	3,4,4'-TriBDE (#37)	3,3',4,4',5-PentaBDE (#126)
2,4-DiBDE (#7)	2,2',4,5'-TetraBDE (#49)	2,2′,3,4,4′-PentaBDE (#85)
3,3′-DiBDE (#11)	2,4,4′,6-TetraBDE (#75)	2,2',4,4',5,6′HexaBDE(#154)
2,4′-DiBDE (#8)	2,3′,4′,6-TetraBDE (#71)	2,2',4,4',5,5'-HexaBDE(#153)
3,4-DiBDE (#12)	2,2',4,4'-TetraBDE (#47)	2,2',3,4,4',5'-HexaBDE(#138)
3,4′-DiBDE (#13)	2,3′,4,4′-TetraBDE (#66)	2,3,4,4',5,6-HexaBDE (#166)
4,4'-DiBDE (#15)	3,3',4,4'-TetraBDE (#77)	2,2',3,4,4',5',6-HeptaBDE (#183)
2,4′,6-TriBDE (#32)	2,2',4,4′,6-PentaBDE (#100)	2,2',3,4,4',5,6-HeptaBDE(#181)
2,4,6-TriBDE (#30)	2,3′,4,4′,6-PentaBDE (#119)	2,3,3',4,4',5,6-HeptaBDE (#190)
2,3′,4-TriBDE (#25)	2,2',4,4',5-PentaBDE (#99)	
2,2',4-TriBDE (#17)		

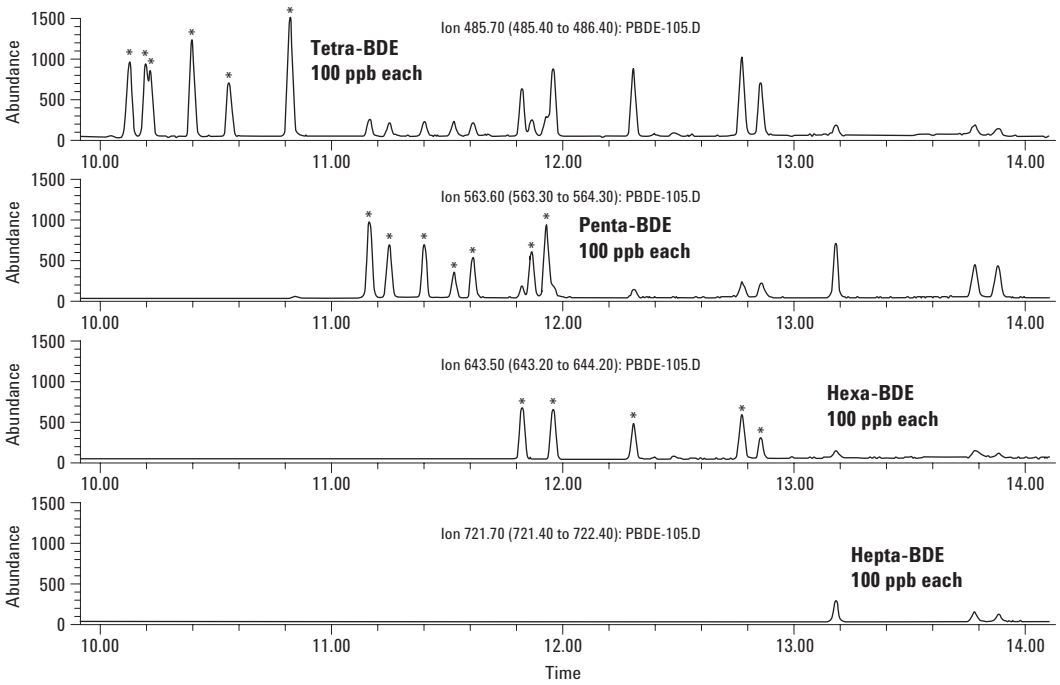


Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas:	Helium at 38 cm/s at 100 °C (1.2 mL/min), constant flow mode
Oven:	100 °C for 1 min; 100 °C to 340 °C at 20 °C/min , 340 °C for 12 min
Injector:	Cool-on-column, oven-track mode, 0.5 µL
Detector:	Agilent 5973 MSD; transfer line at 325 °C, EI
SIM:	(Ions monitored: 231.8, 248.0, 327.9, 398.6, 400.5, 405.8, 845.7, 563.6, 643.5, 721.4, 799.3)

Note:

Mono-through octa-substituted homologs detected using selected ion monitoring (SIM) at the most intense of the M^+, $(M+2)^+$, $(M+4)^+$, $(M+6)^+$, or $(M+8)^+$ masses, with a data acquisition rate of approxroximately 3 cycles/second. Monitoring the molecular ion was not possible above octa-substituted PBDEs due to the limitations of the mass range of the Agilent 5973 instrument (maximum of *m/z* 800). Decabromodiphenylether was detected by monitoring significant fragments of high abundance: *m/z* 231.8, 398.6, 400.5, and 799.3.

Figure 2a. Gas chromatography/mass spectrometry (GC/MS) of PBDE congener mixture (EO-5113).



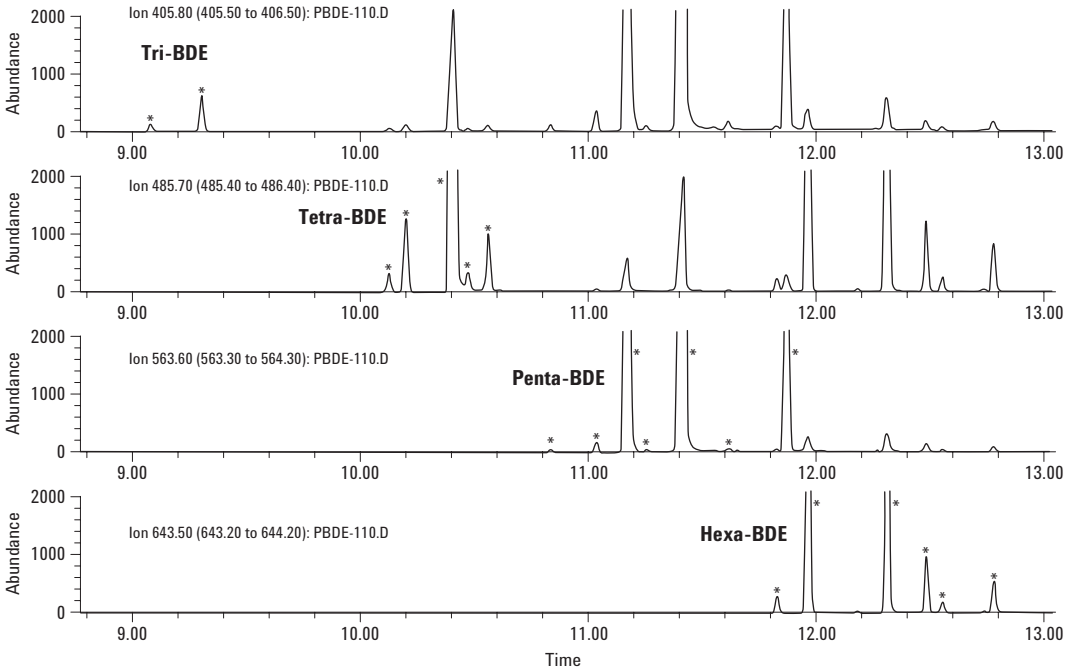
Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas:	Helium at 38 cm/s at 100 °C (1.2 mL/min), constant flow mode
Oven:	100 °C for 1 min; 100 °C to 340 °C at 20 °C/min, 340 °C for 12 min
Injector:	Cool-on-column, oven-track mode, 0.5 µL
Detector:	Agilent 5973 MSD; transfer line at 325 °C, EI
SIM:	(Ions monitored: 231.8, 248.0, 327.9, 398.6, 400.5, 405.8, 845.7, 563.6, 643.5, 721.4, 799.3)

Note:

Mono-through octa-substituted homologs detected using SIM at the most intense of the M^+, $(M+2)^+$, $(M+4)^+$, $(M+6)^+$, or $(M+8)^+$ masses, with a data acquisition rate of approxroximately 3 cycles/second. Monitoring the molecular ion was not possible above octa-substituted PBDEs due to the limitations of the mass range of the 5973 instrument (maximum of *m/z* 800). Decabromodiphenylether was detected by monitoring significant fragments of high abundance: *m/z* 231.8, 398.6, 400.5, and 799.3.

Figure 2b. GC/MS of PBDE congener mixture (EO-5113).

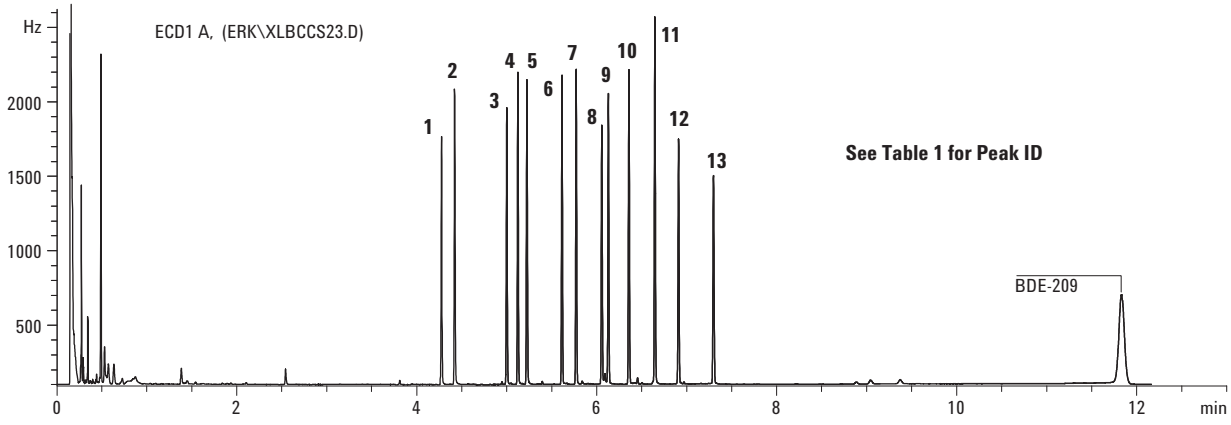
Figure 3 shows a chromatogram of a commercial flame retardant mixture. While commercial samples are typically classified as “penta”, “octa”, or “deca”, they contain other congeners as well. Again, the congeners in this mixture are well resolved, and the run time is very short (13 minutes).



Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas:	Helium at 38 cm/s at 100 °C (1.2 mL/min), constant flow mode
Oven:	100 °C for 1 min; 100 °C to 340 °C at 20 °C/min , 34 °C for 12 min
Injector:	Cool-on-column, oven-track mode, 0.5 µL
Detector:	Agilent 5973 MSD; transfer line at 325 °C, EI
SIM:	(Ions monitored: 231.8, 248.0, 327.9, 398.6, 400.5, 405.8, 845.7, 563.6, 643.5, 721.4, 799.3)

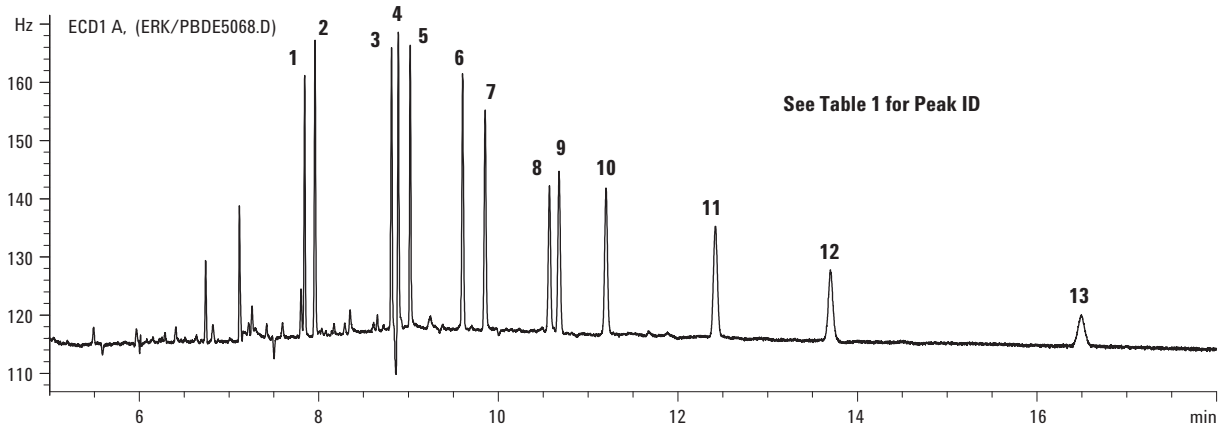
Figure 3. Commercial flame retardant penta DE71-R.

Analysis times could be reduced even further by using hydrogen carrier gas and an Electron Capture Detector (ECD). This combination allows for faster flow rates, while improving sensitivity and lowering the detection limit. With the same column dimensions as above, run times of around 15 minutes are possible. With a custom-made column (DB-XLB, 15 m × 0.18 mm id × 0.07 µm) the run time was less than 12 minutes (Figure 4), with no signs of degradation of the 209 congener. Break down of the higher congeners was, however, dependent on the run conditions. An inlet temperature of 250 °C worked best, while the µECD gave best results at 300 °C. At higher detector temperature, degradation was noticeable, while lower ECD temperatures resulted in tailing peaks (likely due to cold trapping). As expected, sensitivity for PBDEs with a µECD is excellent (Figure 5). In the splitless injection mode, the detection limit under those run conditions for the tri and higher substituted PBDEs was around 100 ppt, with a signal-to-noise ratio of >20. The calibration curve for 2,2',4,4',6-PentaBDE (BDE-100) was linear from 1 ppm to 100 ppt.



Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 15 m × 0.18 mm id × 0.07 µm (Agilent Technologies, custom column)
Carrier gas:	Hydrogen at 72 cm/s at 100 °C (4.0 mL/min), constant flow mode
Oven:	100 °C for 0.5 min; 100 °C to 300 °C at 30 °C/min, 300 °C for 5 min
Injector:	250 °C, split 20:1, 1 µL
Detector:	ECD at 300 °C

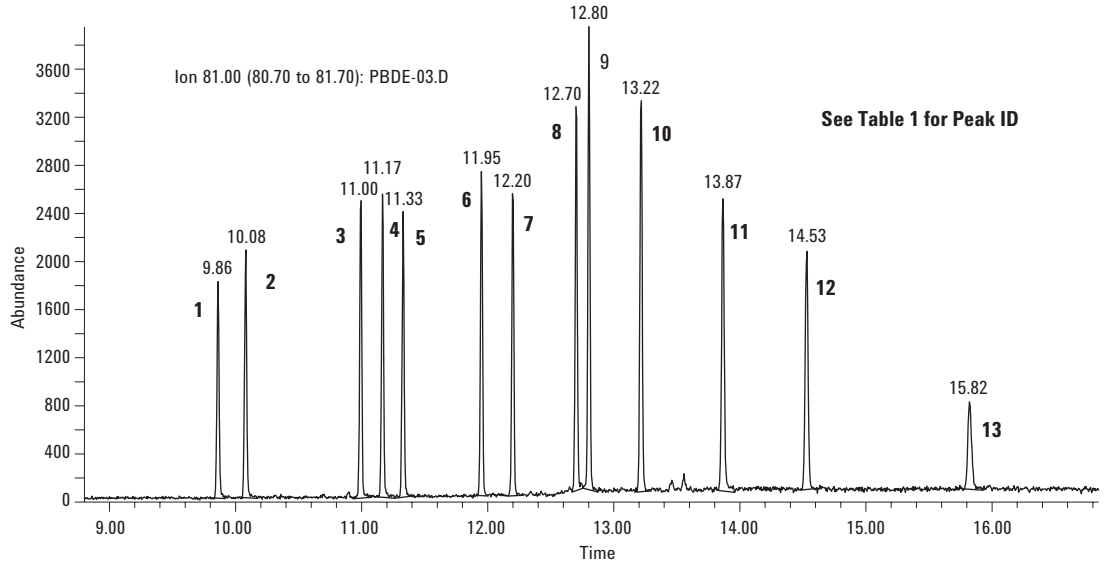
Figure 4. GC-µECD of PBDE congener mixture (EO-5103).



Instrument: Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column: DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas: Hydrogen at 72 cm/s at 100 °C (4.0 mL/min), constant flow mode
Oven: 100 °C for 1 min; 100 °C to 300 °C at 25 °C/min, 300 °C for 10 min
Injector: 300 °C, splitless, 1 µL
Detector: ECD at 300 °C

Figure 5. GC-µECD of PBDE mixture EO-5103 at 500 ppt.

The same sensitivity could be achieved with GC-ICP-MS. Figure 6 shows congener mixture EO-5103 diluted to 10 ppb. Calibration curves of individual congeners from 1 ppm to 1 ppb were linear (R^2 = 1.000), and the lower detection limit is calculated at 150 ppt. The system setup conditions for the ICP-MS, such as torch position, may not be fully optimized yet, so detection limits may be even lower.



Instrument: Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column: DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier Gas: Helium at 36 cm/s at 100 °C (1.5 mL/min), constant flow mode
Oven: 100 °C for 1 min; 100 °C to 300 °C at 20 °C/min, 320 °C for 13 min
Injector: 320 °C, splitless, 1 µL
Detector: Agilent 7500cs ICP-MS, monitoring Br at *m/z* = 81

Figure 6. GC-ICP-MS of PBDE mixture EO-5103 at 10 ppb.

Conclusions

DB-XLB is the column of choice for GC analysis of PBDEs. The high upper temperature limit and very low bleed characteristics of this column make it ideal for this class of large molecules. While the high upper temperature limit allows for fast run times - complete analyses, including BDE-209, can be run in about 20 minutes, the extremely low bleed at those temperatures increases sensitivity, thus providing lower detection limits. The DB-35ms is an excellent secondary column that has the same outstanding bleed and thermal properties as DB-XLB, yet a different selectivity required for a confirmation column. In general, short columns with a high phase ratio (thin film) yield better response for the higher congeners, since the shorter residence times on the column reduce the exposure to high temperatures, therefore reducing on-column break down.

Due to the high bromine content of PBDEs, sensitivity on an ECD is very high. With splitless injection, the lower detection limit that we achieved is approximately 100 ppt. This limit might be pushed even lower with a programmable temperature vaporization (PTV) inlet, where larger injection volumes are possible. However, in real samples, for example, marine wildlife, other halogenated compounds, like PCBs, may be present. Since an ECD cannot distinguish between halogens, it is impossible to determine if a PCB co-elutes with a PBDE, thus quantitation may not be accurate. GC/MS offers secondary confirmation of the identity of the eluted peak, but sensitivity is not as great. In SIM mode, the detection limit for PBDEs is estimated at about 10 ppb. GC-ICP-MS offers both – high sensitivity and ion selectivity. It can be tuned for Cl or Br. Thus, by monitoring for example *m/z* 81, only PBDE would be detected, and PCB would not interfere with quantitation.

Acknowledgements

Individual PBDE congeners were a generous gift from AccuStandard (New Haven, CT). Special thanks to Mehran Alaee and Ivy D'Sa of the Aquatic Ecosystem Protection Research Branch, National Water Research Institute, Burlington, Canada, for helpful discussions, sharing of data, and providing samples of industrial brominated flame retardants. Thanks also to Harry Prest and Emmett Soffey of Agilent Technologies and Robert Trengrove of Murdoch University for helpful discussions and providing data.

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Agilent Technologies

Determination of Polybrominated Diphenylethers (PBDE) in Sediment and Sewage Sludge

Application

Environmental

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Abstract

Generally used as flame retardants, polybrominated diphenylethers (PBDE) have become chemicals of significant environmental concern. While little toxicological information is available, PBDEs have been determined to be persistent and bio-accumulative substances, similar to well-known environmental contaminants such as polychlorinated biphenyls (PCBs). Therefore, environmental laboratories are asked to analyze polybrominated diphenylethers (flame retardants) in sediment and sewage sludge. This application note describes the successful separation of all PBDEs, including the most difficult, decabrominated diphenylether. Examples include standards as well as real samples of sewage sludge with quantitative data.

Introduction

With increasing frequency, environmental laboratories are asked to analyze PBDEs (flame retardants) in sediment and sewage sludge. See Figure 1.

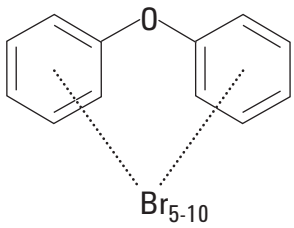

Figure 1. Structure of PBDEs.

Brominated flame retardants (BFRs) are a group of chemicals added to many products, including computers, TVs, and household textiles, in order to reduce fire risk. Two substances, decabromodiphenyl ether (DecaBDE) and tetrabromobisphenol A (TBBP-A), account for about 50% of world use of brominated flame retardants. Two other polybrominated diphenyl ethers (PolyBDE) - octabromodiphenyl ether (OctaBDE) and pentabromodiphenyl ether (PentaBDE) - are used commercially, but in much smaller quantities than DecaBDE.

Heating (for example, during manufacture of plastics) and burning of materials containing PBDEs and other BFRs can produce polybrominated

dibenzo-p-dioxins and dibenzofurans, which have similar toxicological effects to chlorinated dioxins. Research has shown that low-level exposure of young mice to PBDEs causes permanent disturbances in behavior, memory, and learning (Eriksson et al., 1998) [1]. PBDEs have also been shown to disrupt the thyroid hormone system in rats and mice; these systems are a crucial part of the development of the brain and body (Darnerud and Thuvander, 1998 [2]; Hallgren and Darnerud, 1998) [3].

The release of these organic pollutants can be revealed by analyses of sewage sludge produced by municipal waste-water treatment plants. Therefore, the European community has given a directive (2000/60/CE) [4] for water to analyze four PBDEs (BDE-99, BDE-100, BDE-205, BDE-209) and is now working on an ISO norm ISO/CD 22032 to analyze eight PBDEs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-205, BDE-209).

This analysis starts with an extraction of brominated diphenyl ethers (BDEs) from the dried sample of sediment or sewage sludge by a solvent (for example, hexane or other solvents suitable to get high extraction rates). The extract is cleaned, with silica, for example, if necessary. After concentration, the BDEs are separated by capillary gas chromatography (GC) and detected with a suitable system. A calibration over the total procedure using an internal standard (ISTD) mix is used to calculate the concentration in the sample.

When analyzing PBDE with GC, a number of problems arise: [5]

- Adsorption to glass surfaces
- Discrimination of high molecular weight compounds
- Degradation of the heavier congeners
- Irreproducible results
- Disappearing peaks

This application note gives analysts the necessary tools to attempt low-level detection of PBDE by gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Samples

All sewage sludge and sediment samples were provided by municipal waste-water treatment plants. Ten grams of sediment or 1 g of sewage sludge is liquid extracted. The extract is cleaned on silica and the clean extract is concentrated in 1-mL hexane prior to GC analysis.

Standards and ISTDs

The project for the European norm 22032 (2000/60/CE) is requesting analysis of four PDBE (BDE-99, BDE-100, BDE-205 and BDE-209) and recommends TetraBDE (BDE-77) as ISTD. (See Table 1.) These standards were purchased commercially and were of the highest grade available. A test mixture of pentaBDE (BDE-99, BDE-100), octaBDE (BDE-205), and decaBDE (BDE-209) was used for the evaluation in order to obtain a GC analysis with little or no discrimination. BDE-77 was used as ISTD. Standard solutions containing 0.01; 0.05; 0.1; 0.2; 0.25; 0.5 ng/µL of pentaBDE, 0.5; 1; 2; 3; 4; ng/µL of decaBDE and octaBDE, and 0.2 ng/µL of ISTD were prepared in hexane.

Table 1. Selected BDEs

Name	Formula	Abbreviation	Molar mass g/mol
3,3',4,4'-tetraBDE	$C_{12}H_6Br_4O$	BDE-77	481.715
2,2',4,4',5-pentaBDE	$C_{12}H_5Br_5O$	BDE-99	564.6911
2,2',4,4',6-pentaBDE	$C_{12}H_5Br_5O$	BDE-100	564.6911
2,3,3',4,4',5,5',6-octaBDE	$C_{12}H_2Br_8O$	BDE-205	801.3804
DecaBDE	$C_{12}Br_{10}O$	BDE-209	959.1714

GC Conditions

The selection of column and injection parameters is of great importance for the GC analysis of PBDE, especially for the high molecular weight congeners. See Table 2.

The temperature of the GC is of great importance since some congeners decompose at temperatures just above 300 °C. Thermal degradation is a function of temperature and time; thus, by choosing a column with as little retention for the BDE congeners as possible and shortening the column to the minimum length required for the separation, thermal degradation can be minimized. In addition, pulsed injection allows shorter injection time and also helps to minimize risk of thermal degradation.

A pulsed splitless injection and a DB-1 30 m, 0.32 mm, thin film, 0.1 µm, really minimizes the time each PDBE stays in both the injector and in the column and avoids degradation.

Table 2. Optimized Run Conditions

Column: **Part number:**	**DB-1** **123-1031**
Length:	30 m
Diameter:	0.32 mm
Film thickness:	0.1 µm
Carrier:	Helium at 58 cm/s Flow rate 2.5 mL/min
Injector:	2 µL Pulsed splitless at 250 °C
Oven:	60 °C for 2 minutes 60 °C–200 °C at 10 °C/min 200 °C for 2 minutes 200 °C–300 °C at 20 °C/min 300 °C for 25 minutes
Detector	MS
Agilent 5973 inert MSD	
SIM mode	Group 1 / 3 min / *m/z* 486; 484; 326 Group 2 / 20 min / *m/z* 406; 564; 566 Group 3 / 24 min / *m/z* 642; 644; 562 Group 4 / 28 min / *m/z* 799; 797
Quad temperature	150 °C
Source temperature	230 °C
Transfer line temperature	300 °C

Results and Discussion

The chromatograms (Figures 2 and 3) show very good peak shapes for each PBDE and a high response for the most critical decaBDE (BDE-209) (see Figure 4) using the optimized run conditions listed in Table 2.

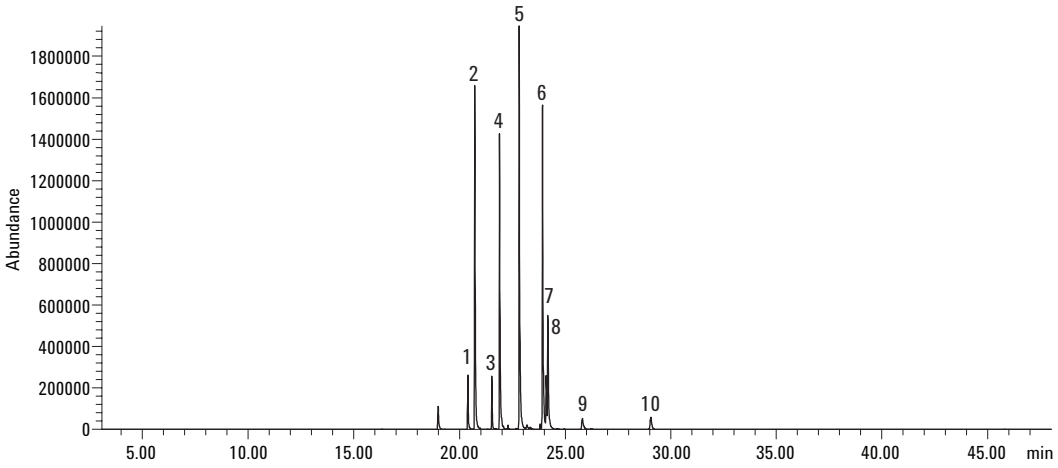

Figure 2. Total Ion Chromatogram (TIC) of a standard mixture at 2–20 ng/µL
1–2: pentaBDE
3–9: octaBDE
10: decaBDE

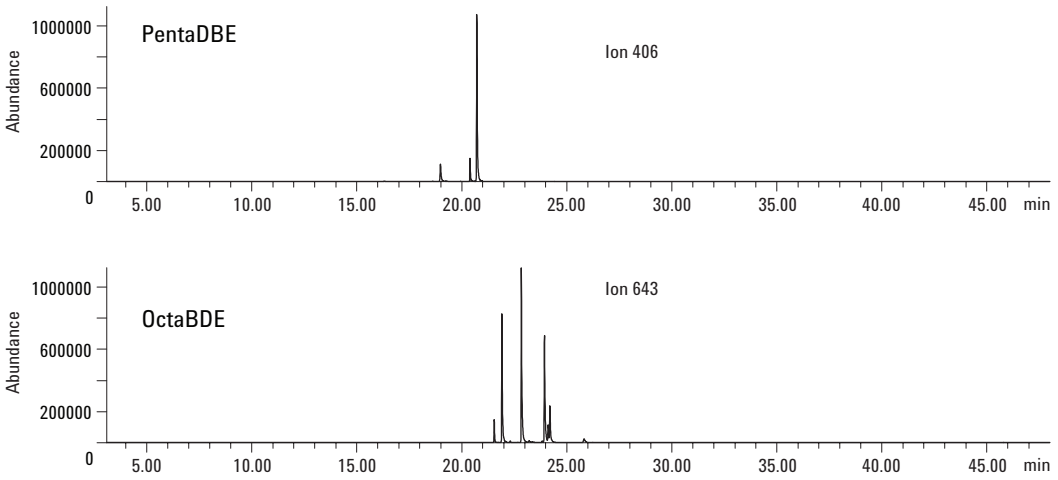

Figure 3. Selected Extracted Ion Chromatograms (EICs) of a standard mixture at 0.5–5 ng/µL.

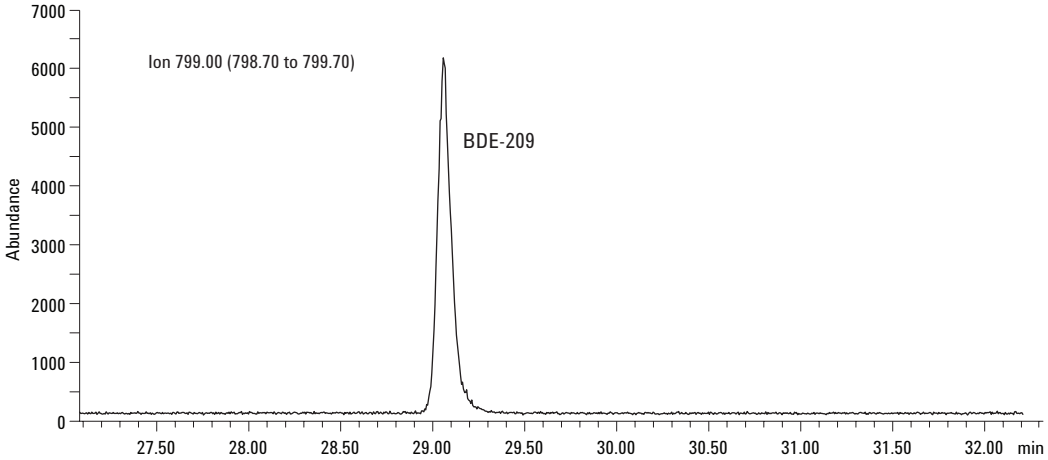

Figure 4. EIC of BDE-209 in a standard mixture at 5 ng/µL.

In order to have a precise quantitation, five point calibration curves from 0.01 to 0.25 ng/µL for pentaBDE and from 0.5 to 4 ng/µL of octa and decaBDE were achieved with ISTD BDE-77 at 0.2 ng/µL. (See Figure 5.) For all components, the R^2 values range from 0.996 to 1, meeting the AFNOR requirements for valid quantitation (See Table 3).

Table 3. Calibration Curve Summary Using Optimized Analysis Conditions with GC/MS

Compound	Calibration range (ng/µL)	Target ion *m/z*	Qualifier ion *m/z*	R^2 value
BDE-77	*ISTD–0.2*	486.0	326.0	ISTD
BDE-99	*0.01–0.25*	405.8	563.6	1
BDE-100	*0.01–0.25*	405.8	563.6	1
BDE-205	*0.5–4*	641.6	643.6	0.990
BDE-209	*0.5–4*	799.4	797.4	0.996

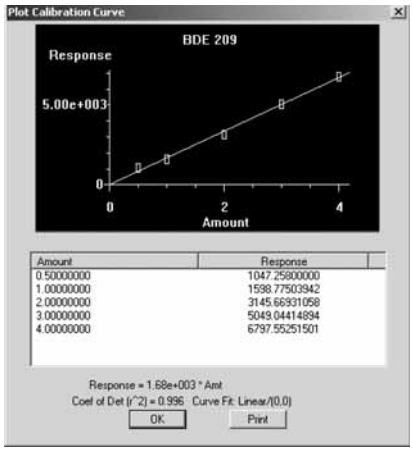

Figure 5. Calibration curve for decaBDE (BDE-209) by GC/MS.

Concerning the limit of detection (LOD), the lower level at 1 µg/kg (10 µg/kg for sewage sludge\) for pentaBDE and 50 µg/kg (500 µg/kg for sewage sludge) for octaBDE and decaBDE in sediment, which is 10 pg/µL of pentaBDE or 0.5 ng/µL of octa and decaBDE in solution, is easily achieved. This is the case even for decaBDE because a very good signal-to-noise ratio was achieved, as shown in Figure 6.

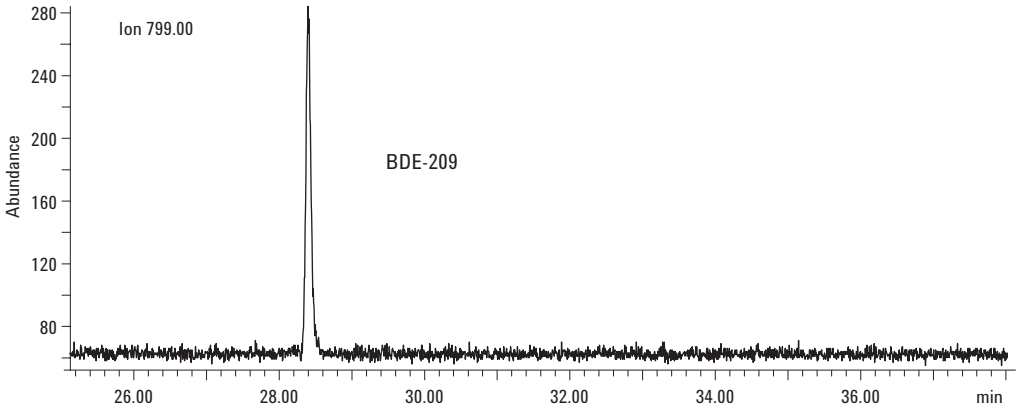

Figure 6. EIC of BDE-209 in a standard mixture at 0.5 ng/µL, which is the required LOD 50 µg/kg of sediment.

Real sewage sludge samples were analyzed using the run conditions listed in Table 2. Figure 7 shows one example. The EICs of the different PDBE show that only one pentaBDE and the decaBDE are present in this sample, and they were quantified in a quantitation report showed on Table 4.

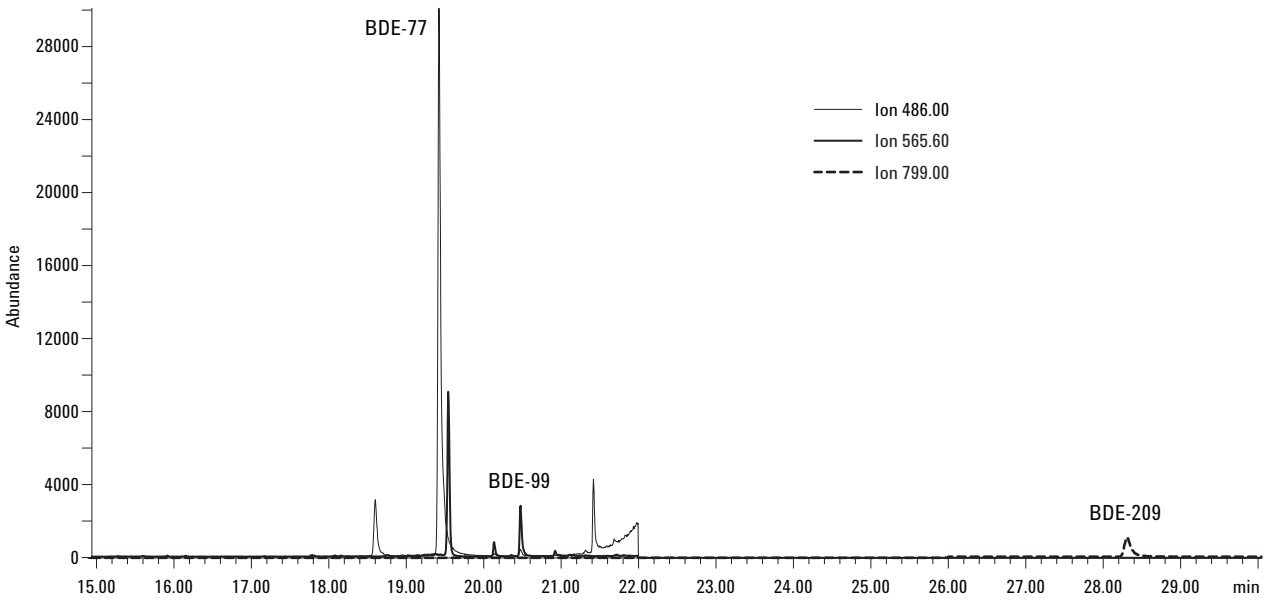

Figure 7. Overlaid EICs for BDE-77, 99, and 209 from sewage sludge.

Table 4. Quantitation Report of Real Sewage Sludge

ISTDs	RT	QIon	Response	Conc units	Dev(min)
BDE 77	19.43	486	823207	0.20 ng/µL	0.02
Target compounds					**Qvalue**
BDE 100	20.14	406	22687	0.0072 ng/µL	11
BDE 99	20.48	406	107372	0.0405 ng/µL	87
BDE 205	23.91	642	17336	0.3417 ng/µL	57
BDE 209	28.31	799	64526	5.2530 ng/µL	90

Summary

By combining the highly inert thin film DB-1 with the Agilent 6890 gas chromatograph and the Agilent 5973 inert MSD, laboratories can achieve accurate quantitation of PBDE in sediments and sewage sludge.

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Agilent Technologies

Fast USEPA 8270 Semivolatiles Analysis Using the 6890/5973 inert GC/MSD with Performance Electronics

Application

Environmental Analysis

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Abstract

The analysis of semivolatiles using EPA Method 8270 presents challenges due to the simultaneous measurement of acids, bases, and neutrals over a wide concentration range. Due to productivity demands, laboratories want to run faster while maintaining linearity and sensitivity for even the most active compounds. The 6890/5973 *inert* GC/MSD system with Performance Electronics is designed to meet the criteria for fast analysis, while minimizing activity and maintaining linearity.

Introduction

USEPA Method 8270 for semivolatiles analysis is used to concurrently measure a mixture of acids, bases, and neutrals. Most laboratories analyze for 70–100 compounds with a chromatographic run time of 25–40 min. Laboratories want to reduce this run time for productivity increases. The calibration range required for the analysis varies depending on a particular laboratory's statement of work (SOW). Historically, a range of 20–160 ng has been used. With the increased sensitivity of newer gas chromatograph/mass spectrometer (GC/MS) systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the calibration range down to 1 ng.

The Agilent 6890/5973 *inert* GC/MSD (Gas Chromatograph/Mass Selective Detector) system with Performance Electronics was designed to meet the demand for faster runs and lower MDLs. Faster scan rates without loss of signal are now possible. This allows the use of smaller diameter columns, such as 0.18-mm id, resulting in shorter runs while maintaining sufficient data points across narrower chromatographic peaks.

The inert source allows for less material injected onto the column while maintaining mass spectrometer performance. Injection volume, therefore, can be matched to the 0.18-mm column. Performance comparisons using the inert source were published previously [1, 2].

This application note will demonstrate the use of the Agilent 6890/5973 *inert* with Performance Electronics for USEPA Method 8270. Smaller id columns with faster scan rates yield run times of 15 min while meeting Method 8270 criteria.

Experimental

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890
Inlet	EPC Split/Splitless
Mode	Pulsed splitless, 0.5 µL injection
Inlet temp	250 °C
Pressure	21.48 psi
Pulse pres	40.0 psi
Pulse time	0.20 min
Purge flow	50.0 mL/min
Purge time	1.00 min
Total flow	54.0 mL/min
Gas saver	Off
Gas type	Helium
Inlet Liner	Agilent splitless, single taper, 4-mm id, p/n 5181-3316
Oven	240 V

Oven ramp	**°C/min**	**Next °C**	**Hold min**
Initial		55	1.00
Ramp 1	25	100	0.00
Ramp 2	30	280	0.00
Ramp 3	25	320	4.60

Total run time	15 min
Equilibration time	0.5 min
Oven max temp	325 °C
Column	Agilent Technologies DB-5.625, p/n 121-5622
Length	20.0 m
Diameter	0.18 mm
Film thickness	0.36 µm
Mode	Constant Flow = 1.0 mL/min
Inlet	Front
Outlet	MSD
Outlet pressure	Vacuum
MSD	Agilent Technologies 5973 *inert* with Performance Electronics
Drawout lens	6-mm Large Aperture Drawout lens, p/n G2589-20045
Solvent delay	1.90 min
EM voltage	Run at DFTPP tune voltage - 153 V = 1012 V
Low mass	35 amu
High mass	500 amu
Threshold	10
Sampling	1
Scans/s	5.92
Quad temp	150 °C
Source temp	230 °C
Transfer line temp	280 °C
Emission current	DFTPP tune @ 25 µA

Calibration Standards were obtained from Accustandard, New Haven, CT, (p/n M-8270-IS-WL-0.25x to 10x). They contain 74 target compounds at nine concentration levels with six ISTDs at 40 ppm.

Pulsed splitless injection was used to minimize residence times of analytes in the liner, thereby reducing loss of active compounds. The column flow rate alone, without using a pulsed injection, would take too long to sweep the 900-µL liner volume.

The inlet liner (p/n 5181-3316) is the most commonly used liner for Method 8270 analysis. It does not contain glass wool which would contribute to active compound degradation. Other liners can be used and a detailed discussion of these can be found in Reference 1.

The Agilent 6890 240 V oven was necessary for the 25 °C/min Ramp 3 used.

A 120 V oven will achieve 20 °C/min at higher temperatures and could be used, resulting in slightly longer run times.

The DB-5.625 column was recently introduced in the dimensions listed. A 0.5-µL injection volume is well suited to this column. The excellent resolution from this column allows a higher than normal initial temperature, 55 °C vs 40 °C. This higher temperature shortens cool-down time by more

than 5 min, resulting in productivity increases for the laboratory. Benzo[b]fluoranthene and benzo[k]flouranthene met Method 8270 resolution requirements at the 80-ppm calibration level and lower, using the operating parameters in Table 1.

Previous work has shown improved linearity across a wide calibration range using a 6-mm drawout lens instead of the standard 3-mm lens [1]. Although not shown here, that comparison was repeated on this Performance Electronics system and is still valid. The 6-mm lens is also included in Agilent Kit p/n G2860A.

The 5973 *inert* was tuned using the automatic DFTPP target tune. The following steps were taken before executing DFTPP tune to insure that Method 8270 DFTPP criteria were met on injection.

1. Using the Tune Wizard, set the Mass 50 Target Abundance to 1.3% and the Emission Current to 25, as shown in Figures 1a–1f.
2. Edit the tuning macro as follows:
   a Copy atune73.mac from the MSDChem\msexe folder.
   b Paste the copy of atune73.mac into the MSDChem\msexe folder. The file name should be Copy of atune73.mac. This preserves an original copy of the file.
   c Open atune73.mac in Notepad. Refer to Figures 2a–2h.
   d Click Edit>Find and type samples in the Find What box.
   e Click Find Next.
   f Change the samples value from 3 to 1.
   g Change the averages value from 3 to 6.
   h Save the file and Close Notepad.

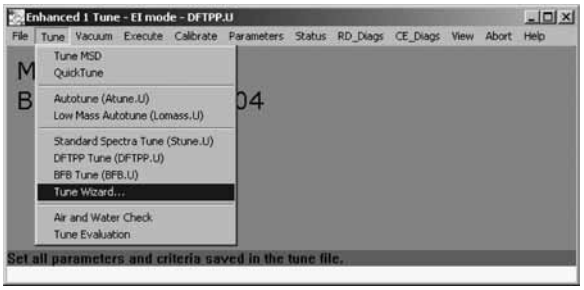

Figure 1a. Starting the Tune Wizard.

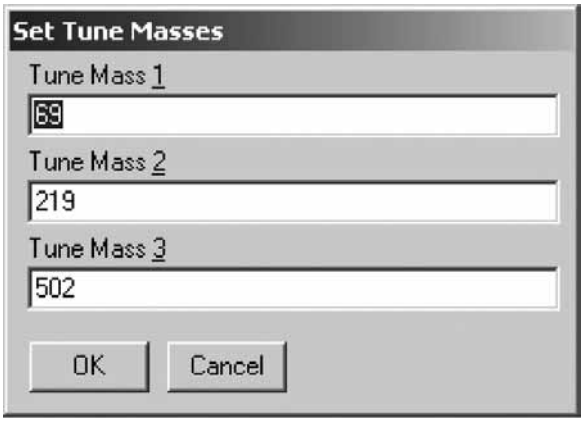

Figure 1b. Accept these masses.

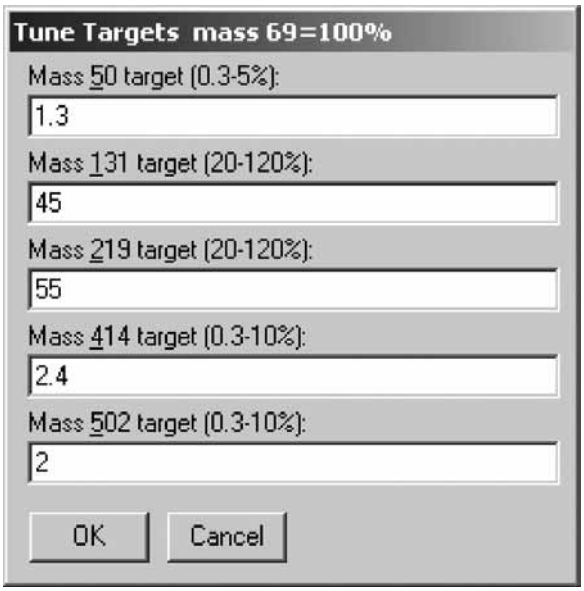

Figure 1c. Set Mass 50 target to 1.3.

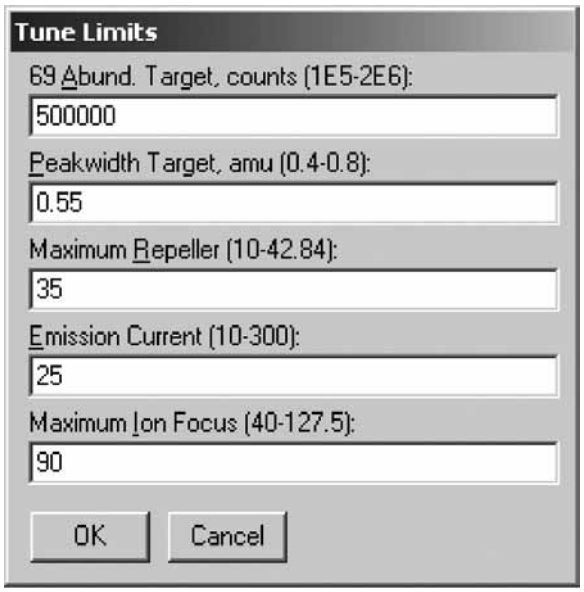

Figure 1d. Set Emission Current to 25.

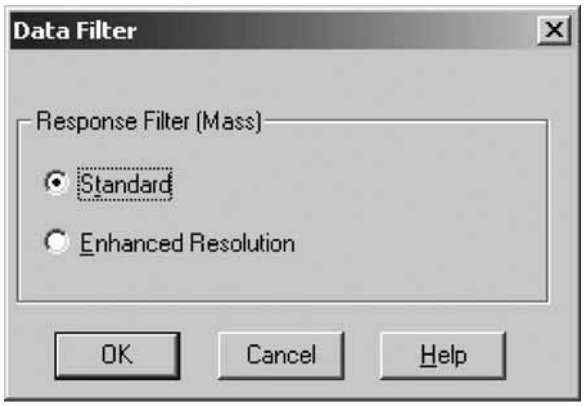

Figure 1e. Accept Standard.

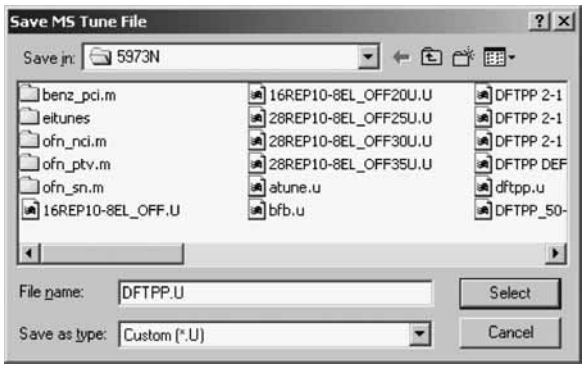

Figure 1f. Type in DFTPP.U if not present and click Select to save.

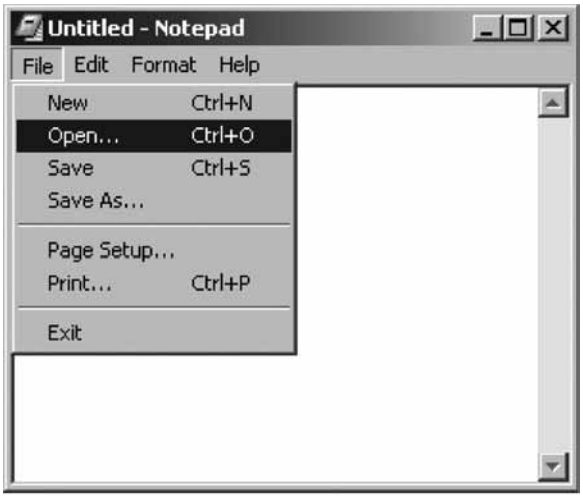

Figure 2a. Select File>Open in Notepad.

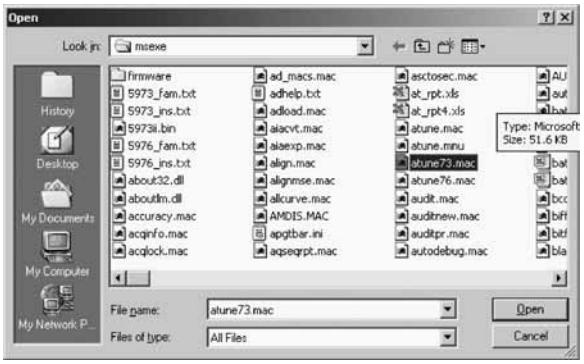

Figure 2b. Select atune.73.mac and click Open.

Figure 2c. Select Edit>Find.

Figure 2d. Type samples into the Find What box, then click Find Next.

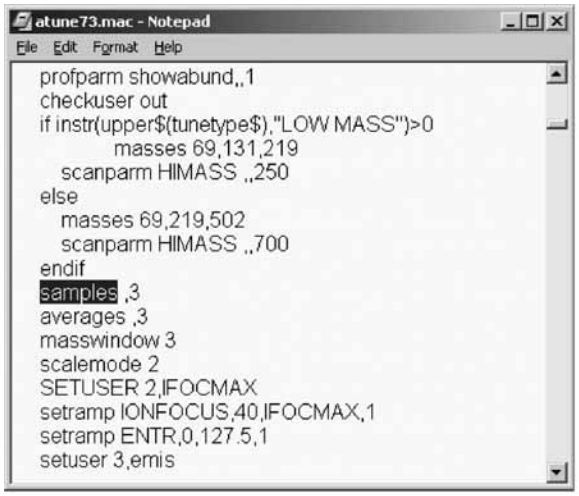

Figure 2e. Results of Find samples.

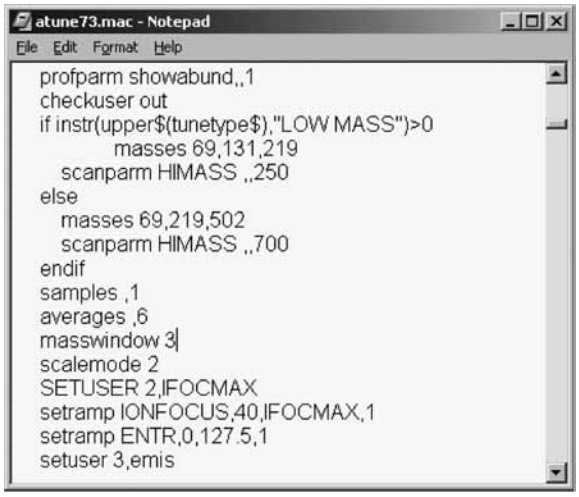

Figure 2f. Change samples from 3 to 1 and averages from 3 to 6.

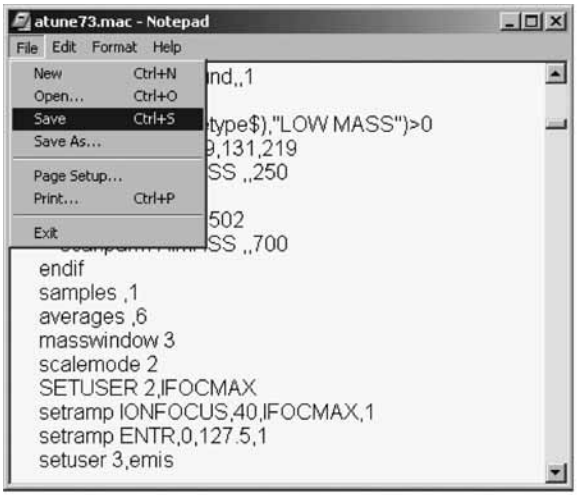

Figure 2g. Select File>Save (do not use Save as).

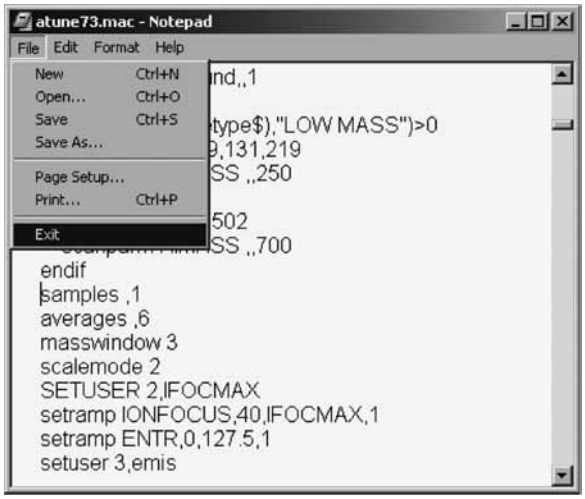

Figure 2h. Select File>Exit to close Notepad.

Previous work has shown improved linearity across a wide calibration range using a 25-µA emission current instead of the 35-µA default. The tuning macro was changed so that the sampling rate during tuning matched the sampling rate during data acquisition. The system was tuned at 2^1 and data were collected at 2^1. These changes resulted in reliably passing Method 8270 criteria on injection of DFTPP.

Remember that the tune macro changes are also reflected if an Autotune is done. The copy of atune73.mac contains the macro without the changes.

The sampling rate for data acqisition was changed-from the usual 2^2 to 2^1, while preserving sufficient sensitivity. The resultant 5.92 scans/s typically yield 10 data points across the peaks that have a width of 1.8 s.

Results

The system was calibrated at nine levels: 1, 2, 5, 20, 50, 80, 120, 160, and 200-ppm. The TIC (Total Ion Chromatogram) for the 5-ppm level is shown in Figure 3. The peak shape is excellent and the run time is less than 15 min. The benzo[b]fluoranthene and benzo[k]flouranthene resolution can be seen at about 11.4 min. Each calibration level contained 74 compounds together with 6 ISTDs at 40 ppm.

The RRF (relative response factor) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the %RSD (percent relative standard deviation) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Excel.

USEPA Method 8270D specifies criteria for suitable RRFs and %RSD. Minimum system performance is determined by four active compounds, the SPCCs (system performance check compounds) and is measured by the average RRF.

Table 2 lists the Method 8270D SPCC criteria and the performance of the 5973 inert. The 5973 *inert* data easily exceeds the 8270D criteria, and are very good considering the low end of the calibration range. This performance margin allows more samples to be run before system maintenance is necessary.

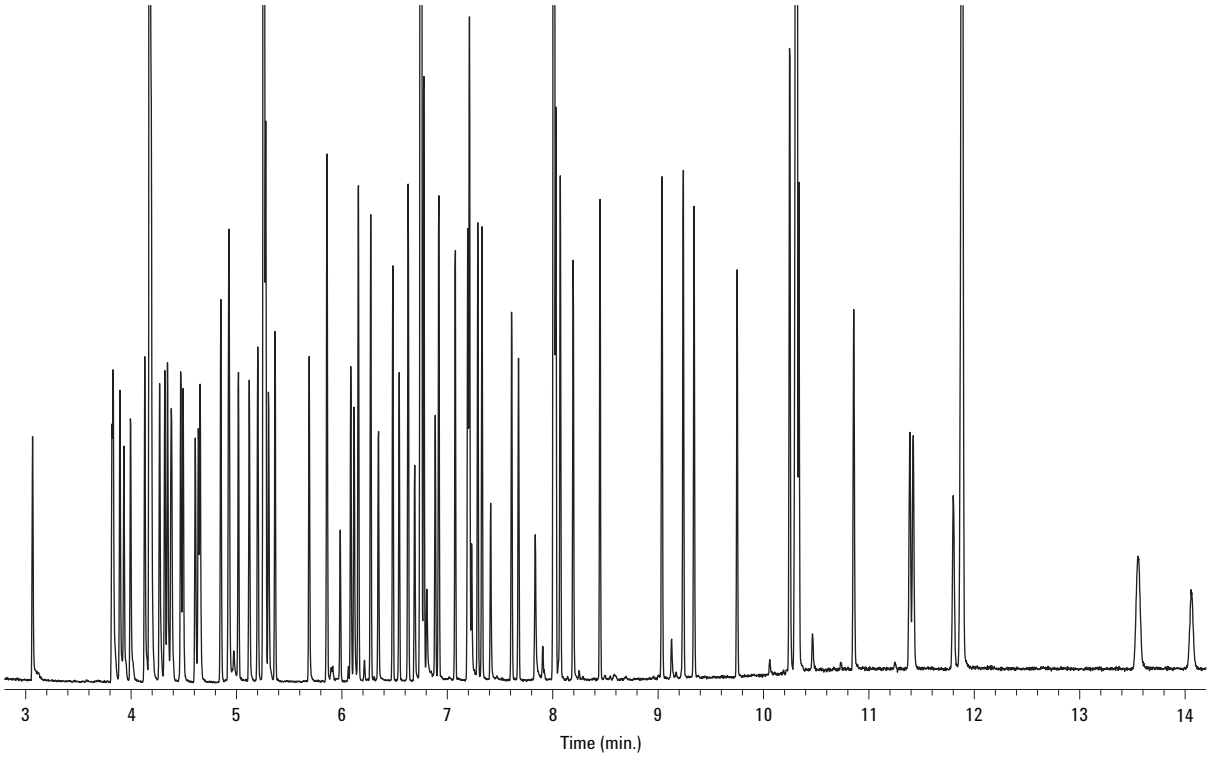

Figure 3. TIC for 5 ppm Method 8270 Semivolatiles.

Table 2. SPCCs and Comparison of Average RRF

	8270D Criteria	1–200 ng 5973 *inert*
N-Nitroso-di-n-propyl amine	0.050	0.963
Hexachlorocyclopentadiene	0.050	0.216
2,4-Dinitrophenol	0.050	0.133
4-Nitrophenol	0.050	0.139

Linearity is shown in Table 3. Method 8270D specifies that this group of Calibration Check Compounds (CCCs) meet a 30% RSD criteria. The %RSD is calculated across the RRFs determined at each calibration level. All CCCs pass criteria using a calibration range of 2–200 ppm. Across a 1–200 ppm range, pentachlorophenol does not pass due to its known activity.

Table 3. CCC %RSD of RRFs from 1–200 ppm and 2–200 ppm

	1–200	2–200
Phenol	6	6
1,4-Dichlorobenzene	7	6
2-Nitrophenol	6	6
2,4-Dichlorophenol	5	4
Hexachlorobutadiene	6	4
4-Chloro-3-methylphenol	5	5
2,4,6-Trichlorophenol	12	10
Acenaphthene	11	10
Diphenylamine	8	8
Pentachlorophenol	36	24
Fluoranthene	8	7
Benzo[a]pyrene	3	3

The excellent system linearity shown here is due to many factors including tuning, the large aperture drawout, and the Performance Electronics. The new electronics allow using a scan rate of 2^1, while maximizing sensitivity. This improved signal/noise together with more data points across a peak yields easier and more reproducible peak integration.

Conclusions

The Agilent 6890/5973 *inert* with Performance Electronics shows improved sensitivity at faster scan rates. The faster scan rates allow using 0.18 mm id columns for faster runs and shorter cool-down times. Analysis of 74 analytes and 6 ISTDs can be accomplished in less than 15 min. EPA Method 8270D tune criteria can be routinely achieved. SPCC performance and CCC linearity can be met over a wider calibration range than that historically used. Productivity increases are possible through shorter runs, faster cool-down, easier peak integration, and use of a wider calibration range.

References

1. M. Szelewski, B. Wilson and P. Perkins, “Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270”, Agilent Technologies, publication 5988-3072EN, www.agilent.com/chem.
2. M. Szelewski, “Fast Semivolatiles Analysis using the Agilent Technologies 6890/5973 *inert* GC/MSD”, Agilent Technologies, publication 5989-0207EN, www.agilent.com/chem.

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Addressing the Challenges of Analyzing Trace Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) Using LC/QQQ

Application

Food, Environmental

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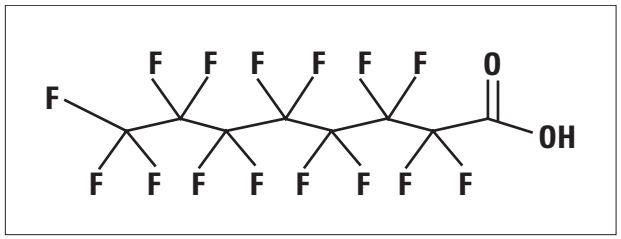
Abstract

An approach to the difficult task of quantifying trace quantities of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in complex matrix was developed using liquid chromatography and tandem mass spectrometry (LC/MS/MS). The technique uses isotopically labeled analytes for accurate quantitation (0.4 to 400 pg on column). It is important to recognize that if using the linear chain sample as standard for calibration, the quantitation results of real-world samples (branched and linear isomers mixed) will be off by as much as 40%.

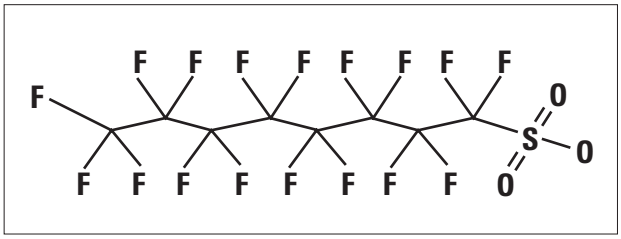
Introduction

Perfluorooctanoic acid (PFOA) is an industrial surfactant and a necessary processing aid in the manufacture of fluoropolymers [1]. Fluoropolymers have many valuable properties, including fire resistance and the ability to repel oil, stains, grease and water. One of the most common uses of PFOA is for processing polytetrafluoroethylene (PTFE), most widely known as Teflon®. PFOA is also a by-product from direct and indirect contact with food packaging (for example, microwave-popcorn bags, bags for muffins or french fries, pizza box liners, boxes for hamburgers, and sandwich wrappers), and in the fabrication of water- and stain-resistant clothes.

Perfluorooctanesulfonic acid (PFOS) is usually used as the sodium or potassium salt and is referred to as perfluorooctane sulfonate. See Figure 1.



PFOA



PFOS

Figure 1. Chemical structures for PFOA and PFOS. Note that both have C8 chains.

Analytical Methodology for PFOA/PFOS

- LC/MS/MS is the preferred detection methodology due to its high sensitivity and specificity in complex matrices.
- Multiple reaction monitoring (MRM) is used to quantitate, using two or more product ions for confirmation.
- The detection limit is typically in the range 1 to 100 pg/mL (ppt), requiring high-sensitivity detection.
- On-column or off-line solid-phase extraction (SPE) and concentration are needed to achieve low-level detection (1 pg/mL).

Measuring PFOS and PFOA

Issue 1: ***What transitions should be used to give the best accuracy when quantifying with a linear standard?***

Quantification of PFOS and PFOA is usually based on a linear standard, but actual samples show a series of branched isomers together with the linear isomer. The ratio of these isomers varies based upon biodegradation and industrial processes in their formation; therefore, it is unlikely that a standard can be formulated to mimic the actual sample. The relative intensities of the MRM transitions will vary based upon branching, making some transitions better than others. Branching impacts ionization efficiency and CID energy; therefore, it affects the accuracy of analytical measurement [2].

Issue 2: ***Can isotopically labeled standards in matrix be used to measure nonlabeled PFOS and PFOA?***

Most biological and environmental matrices have background levels of PFOS and PFOA; although matrix-matched calibrations are providing good results, the accuracy can be enhanced. The method of standard additions is a protocol to address this issue, but it adds several additional injections to the analysis. Matrix may have varying amount of background. Standard addition is not practical in analyzing many different matrices. Solvent calibrations do not correct for matrix effects.

Experimental

Sample Prep

- All solvent standards were prepared in methanol.
- Plasma extracts were prepared by acetonitrile precipitation and centrifuging, with the upper layer taken and spiked with known concentrations of PFOA or PFOS.

LC

- Agilent 1200 Rapid Resolution LC system
- ZORBAX Eclipse Plus C18 Rapid Resolution HT column 2.1 cm × 50 mm, 1.8-µm particles (P/N 959741-902)
- 20-µL injection, 0.4 mL/min column flow
- 0 to 100% B in 10 min, A = water with 2 mM ammonium acetate; B = MeOH

MS/MS

- Agilent QQQ
- Negative-ion detection
- 3500 V_{cap}, drying gas 9.5 L/min at 350 °C, nebulizer 45 psi
- Fragmentor voltages, collision energy (CE), and ion transitions are experimentally determined

Multiple Reaction Monitoring (MRM)

Figure 2 displays a cross-section of the Agilent 6410 QQQ above a hypothetical sequence of spectra characteristic of ion transitions within the instrument.

The ions are generated in the source shown at the far left of the figure. The precursor ion of interest is then selected from this mixture and isolated through the Q1 quadrupole, which acts as a mass filter. This is similar to selected ion monitoring (SIM). After Q1, characteristic fragments that are specific to the structure of the precursor ion are generated in the collision cell (Q2, although not a quadrupole). By using the Q3 quadrupole, these fragments are then selected for measurement at the detector. This is a selective form of collision-induced dissociation (CID), known as tandem MS/MS. By setting Q3 to a specific fragment ion existing in the collision cell, the chemical or background noise is almost totally eliminated from the analyte signal, therefore, significantly increasing the signal-to-noise ratio. Ion 210 is called the precursor ion and ions 158 and 191 are product ions. Each transition (210→191 or 210→158) is a reaction for a particular target. Typically, the QQQ is used to monitor multiple analytes or mass transitions, therefore, the term MRM. The 158 could be considered the quantitation ion, because it is the

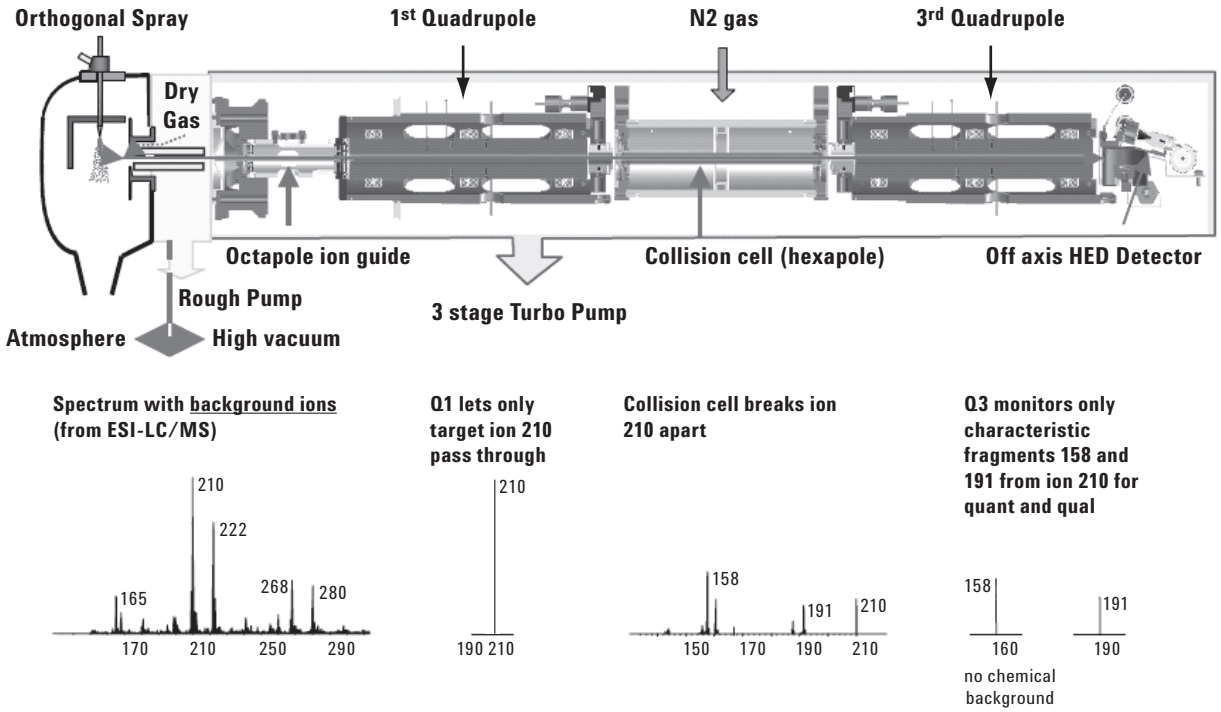


Figure 2. A cross-section of the Agilent 6410 QQQ above a sequence of spectra characteristic of ion transitions within the instrument for a hypothetical sample (*not* PFOA or PFOS). Note that the final spectrum is very clean, containing only the desired target ions. (HED = high-energy dynode electron multiplier)

most intense, and 191 could be used for confirmation by using the area ratio of the 191 qualifier to the 158 quantifier ion as a criterion for confirmation. With MRM, most chemical noise is eliminated in Q1, and again in Q3, allowing us to get ppt detection.

The fragmentor is the voltage at the exit end of the glass capillary where the pressure is about 1 mTorr. Fragmentor and collision energies need to be optimized. A fragmentor that is too small won't have enough force to push ions through the gas. A fragmentor that is too high can cause CID of precursor ions in the vacuum prior to mass analysis, thereby reducing sensitivity. The actual voltage used is compound-, mass-, and charge-dependent, and therefore needs to be optimized to get the best sensitivity. The CE in the collision cell needs to be optimized in order to generate the most intense product ions representative of each target compound. Collision cell voltage will depend on the bond strength, the molecular weight of the compound, and the path by which the ion is formed (directly from the precursor ion or through a series of sequential intermediates). Typically each product ion will exhibit a preferential collision energy that results in the best signal abundance.

The experimental operations required to arrive at optimal conditions are exemplified by the series of experiments shown in Figures 3 to 5.

Optimization of the fragmentor voltages for the $[M-H]^-$ ions of PFOA (*m/z* 413) and PFOS (*m/z* 499) are shown in Figure 3.

Note that there is little signal detected for PFOA at the optimal fragmentor voltage for PFOS (200 V). Ions 413 and 499 are called precursor ions. PFOA is relatively fragile; its precursor signal drops off at 160 V. PFOS shows that it is harder than PFOA to break apart; the best fragmentor voltage for PFOS is 200 V.

The appropriate collision energies for product ions *m/z* 369 $[M-CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$ are experimentally determined and used to quantify PFOA. See Figure 4.

In each case the collision energy producing the most intense peak for each ion is chosen for the analysis. PFOA takes little collision energy to break into ion *m/z* 369 (6 V for highest intensity).

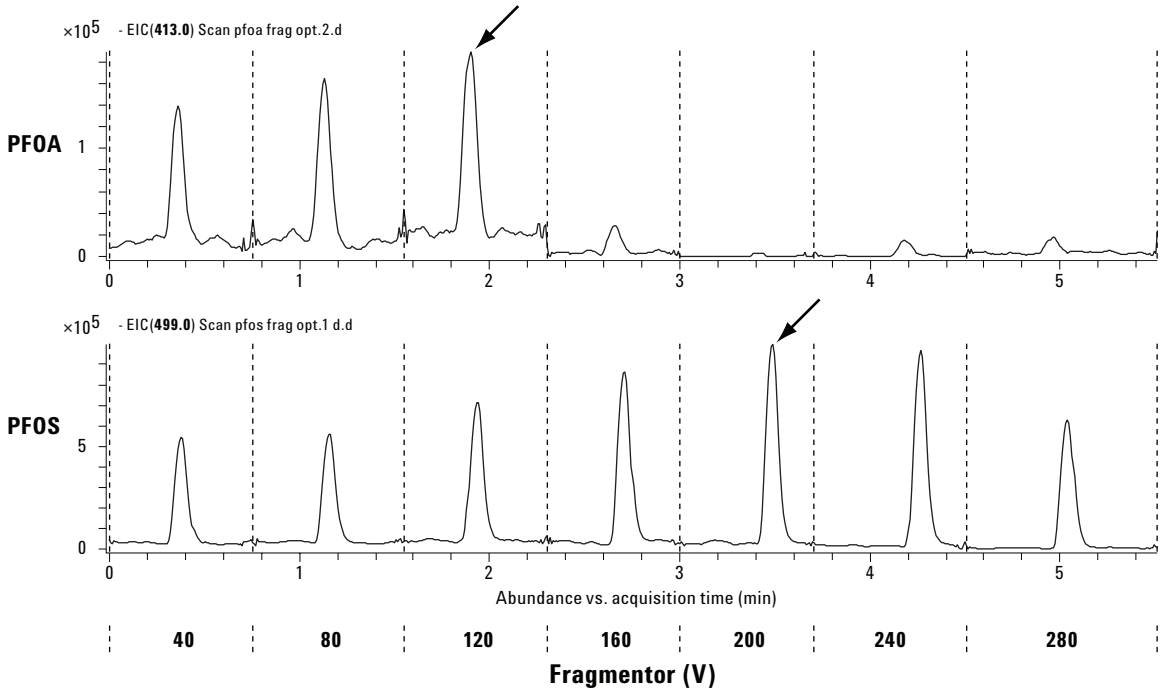


Figure 3. Determination of optimal fragmentor voltage using sequential plots of signal intensity versus applied voltage.

To maximize the intensity of the ion at *m/z* 169, the collision energy needs to go to 16 V.

The QQQ software can switch collision energies very rapidly. So in a method, the optimal collision voltage can be selected for each ion transition.

In the same manner, the appropriate collision energies for PFOS product ions at *m/z* 169, 99, and 80 are experimentally determined and used for its quantitation. The optimal collision energies for the three ion transitions are 45, 50, and 70 V. See Figure 5.

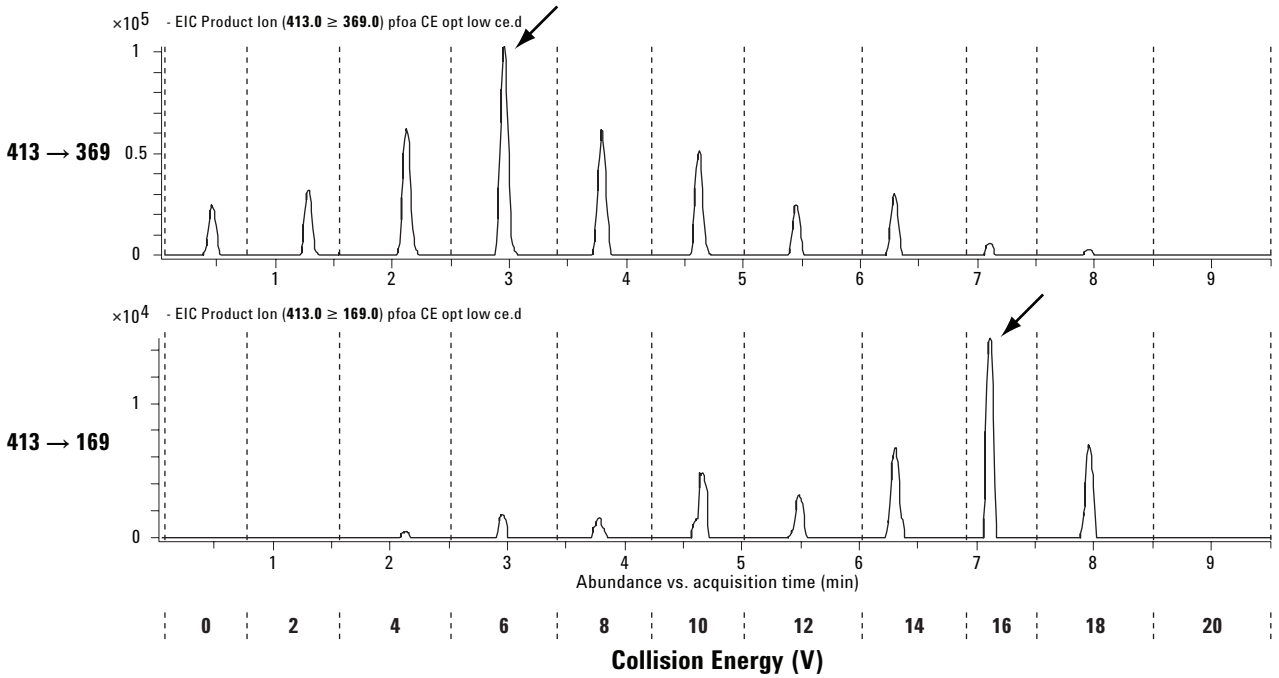


Figure 4. Signal intensity as a function of collision energy for PFOA product ions *m/z* 369 $[M\text{-}CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$.

Notice the big difference in collision energy between PFOA (6 to 16 V) and PFOS (45 to 70 V). We have seen from fragmentor optimization that PFOA is relatively fragile compared to PFOS, in which the optimum fragmentor voltages are 120 and 200 V for PFOA and PFOS, respectively. The CE reinforces that aspect.

Example calibration curves for the specified product ions used to quantitate PFOA and PFOS are shown in Figure 6. The analyst can also sum the intensities of these MRM transitions to get a calibration curve.

These five ion transitions exhibit linear correlation coefficients > 0.998, and are good for quantitation over three orders of magnitude. Notice that the lowest amount on column is 0.4 pg.

Regarding issue 1: ***What transitions should be used to give the best accuracy when quantifying with a linear standard?***

This is addressed using Figures 7 to 9.

Figure 7 exhibits chromatograms from these representative transitions for PFOA and PFOS for the linear standard and samples containing branches (10-min gradient).

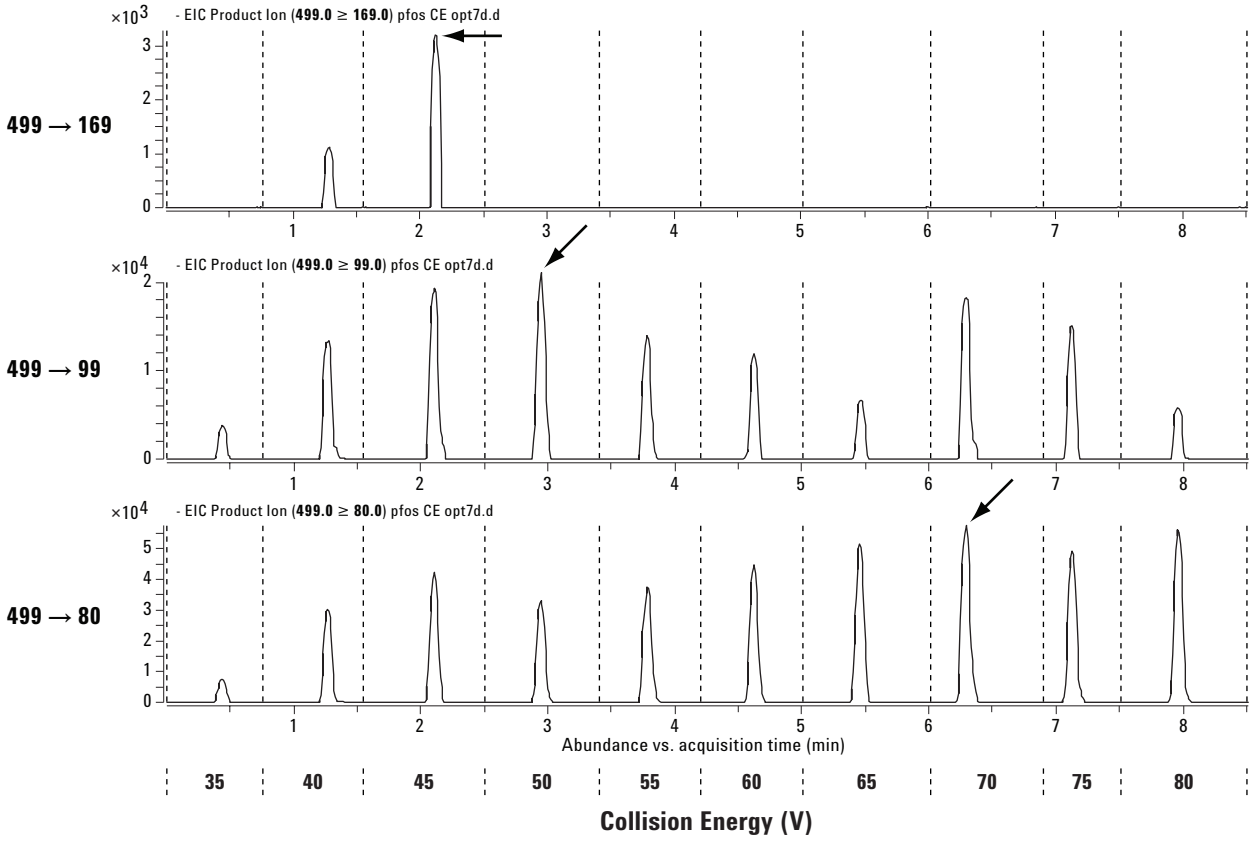
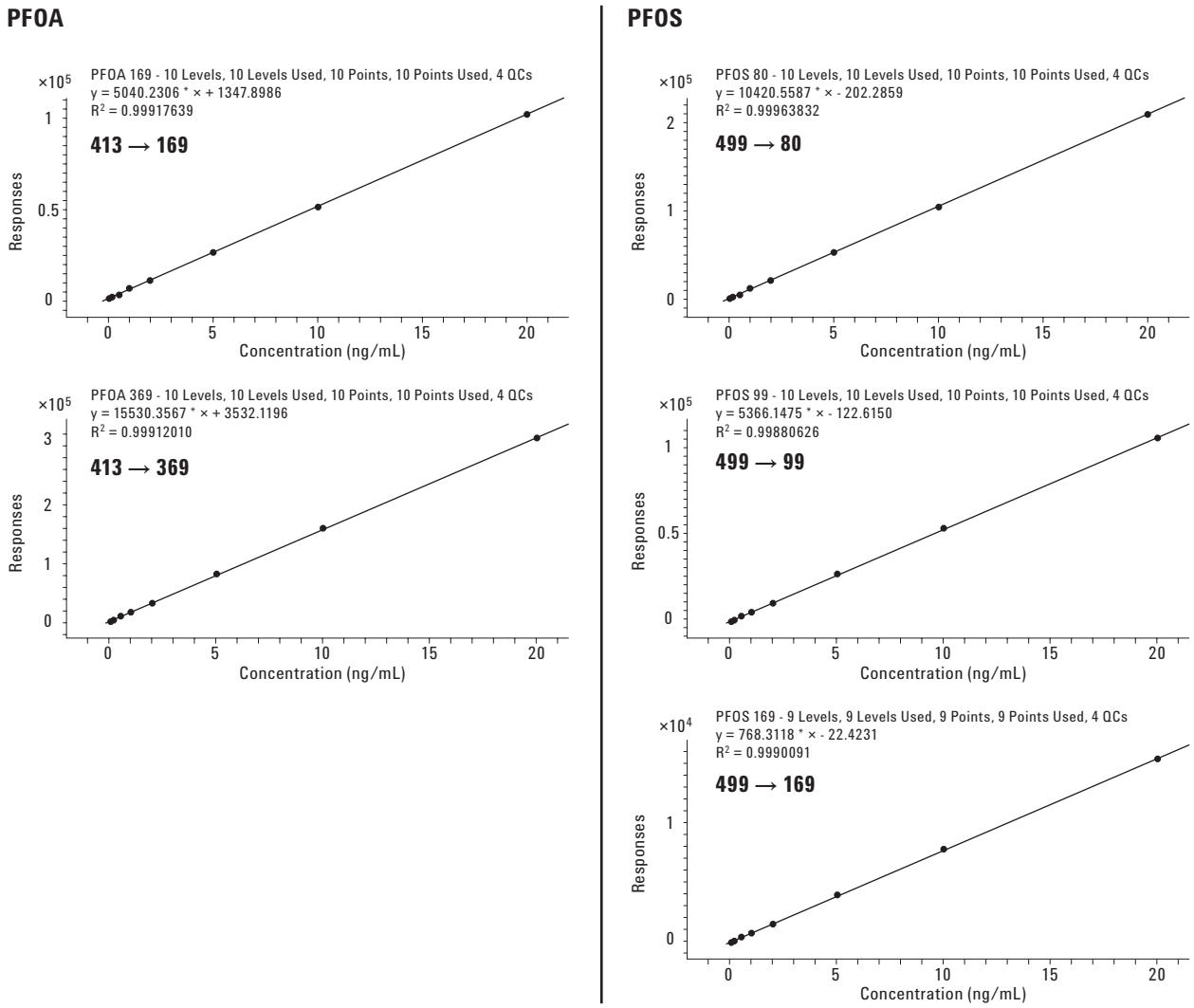


Figure 5. Signal intensity as a function of collision energy for PFOS product ions at *m/z* 169, 99, and 80.



Concentration range 0.02 to 20 ng/mL (0.4 to 400 pg injected on column)

Figure 6. Calibration curves for the product ions used to measure PFOA and PFOS.

Real-world samples have been detected with branched isomers due to manufacturing processes, metabolism, and degradation processes. The top chromatogram of Figure 7 shows only linear chain compounds from a standard. The bottom chromatogram is an actual sample from the environment. It shows additional peaks (shoulders) in the chromatogram resulting from branched isomers.

We examine those peaks in greater detail in Figure 8.

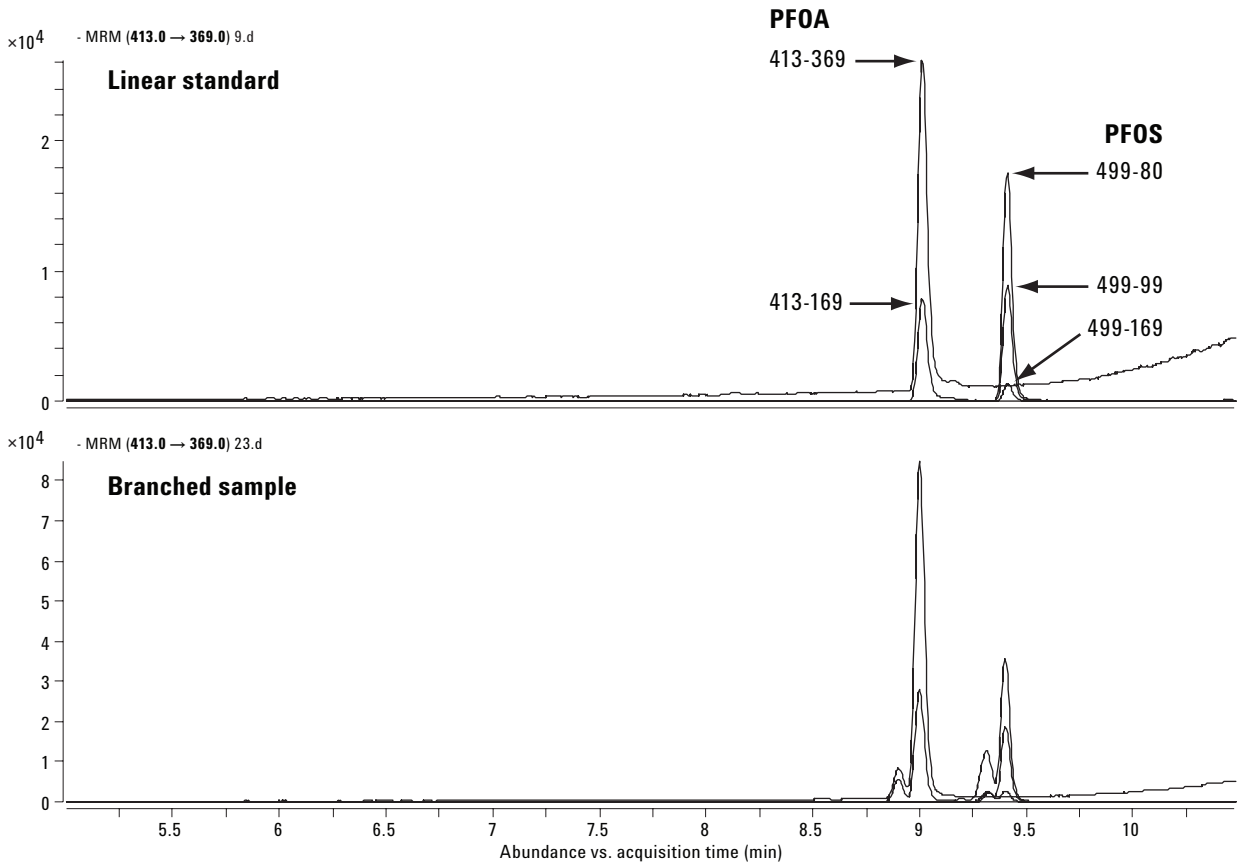


Figure 7. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

The relative abundances for each MRM transition are dependent on the branching locations and the specific mass transitions. Figure 8 shows a 10-minute run. The chromatography can separate the linear from the branched isomers. The branched sample is typically a C7 chain with a methyl side group (isooctyl isomer). The most interesting part of the analysis is that the ion ratios for the branched compounds are very different from the linear chain compounds [3, 4, 5]. For linear PFOA, the ion at *m/z* 169 is about 30 to 40% of ion 369. The branched isomer shows that the ratio changed to 90 to 100%. For linear PFOS, the ion at *m/z* 99 is about 50% of ion 80 and is 500% of ion 169. The branched isomer shows that ion 99 is only 20 to 30% of ion 80, and 100% of ion 169. This is a cause of concern in terms of quantitation accuracy. This shows that CID stability is very different when the analyte is branched.

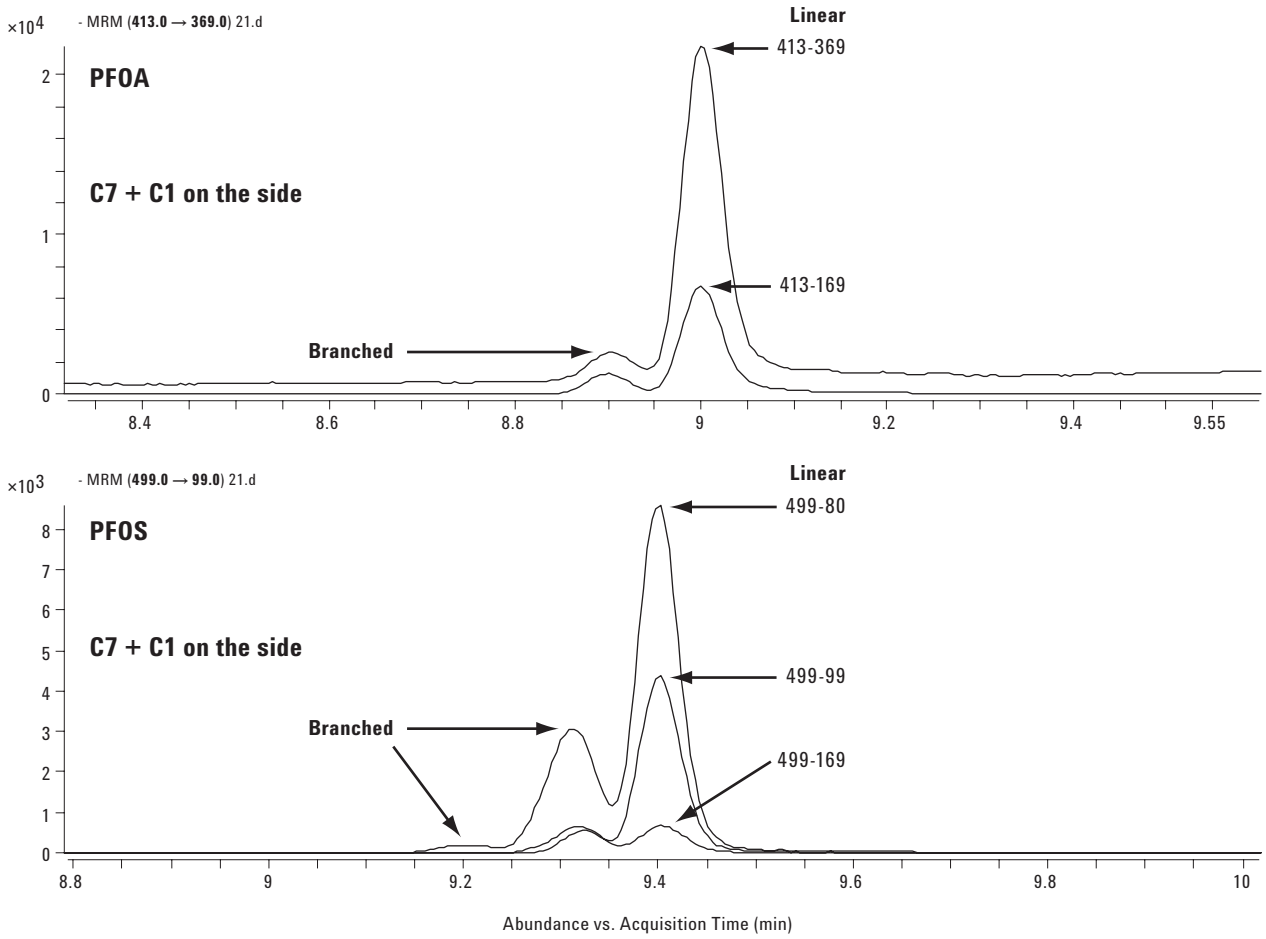


Figure 8. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

Another variable in the analysis is the gradient time. Figure 9 compares the effect of a 3-min versus 10-min gradient.

In the fast gradient case (on the right), the branched isomers (dashed lines) are not resolved from the linear isomers (solid lines), resulting in a significant error in the measured value (most noticeable for PFOS).

The two chromatograms on the left are the same two that are shown in Figure 8. They are used here for comparison against the unresolved analytes shown on the right (3-min run). Although we would like to cut down on the analysis time, the branched and linear isomers need to be resolved in order to get accurate quantitation results.

Two samples of the same concentration. One sample is the pure linear isomer; the other sample has a mixture of branched isomers. If their MRM responses (ion ratios) are the same, they would show the same results as when the isomers are not resolved. This example shows that the responses are not the same when the isomers are not resolved. If you add the responses of the side chain analyte and the linear chain analyte of the same sample, the area of each ion transition is different from the pure linear chain analyte ion transition, as seen in the two chromatograms on the right, most apparent is for PFOS. If using the linear chain sample as standard for calibration, the results of real-world samples (branched and linear isomers mixed) will be off by as much as 40% (see Table 1). The quantitation falls apart.

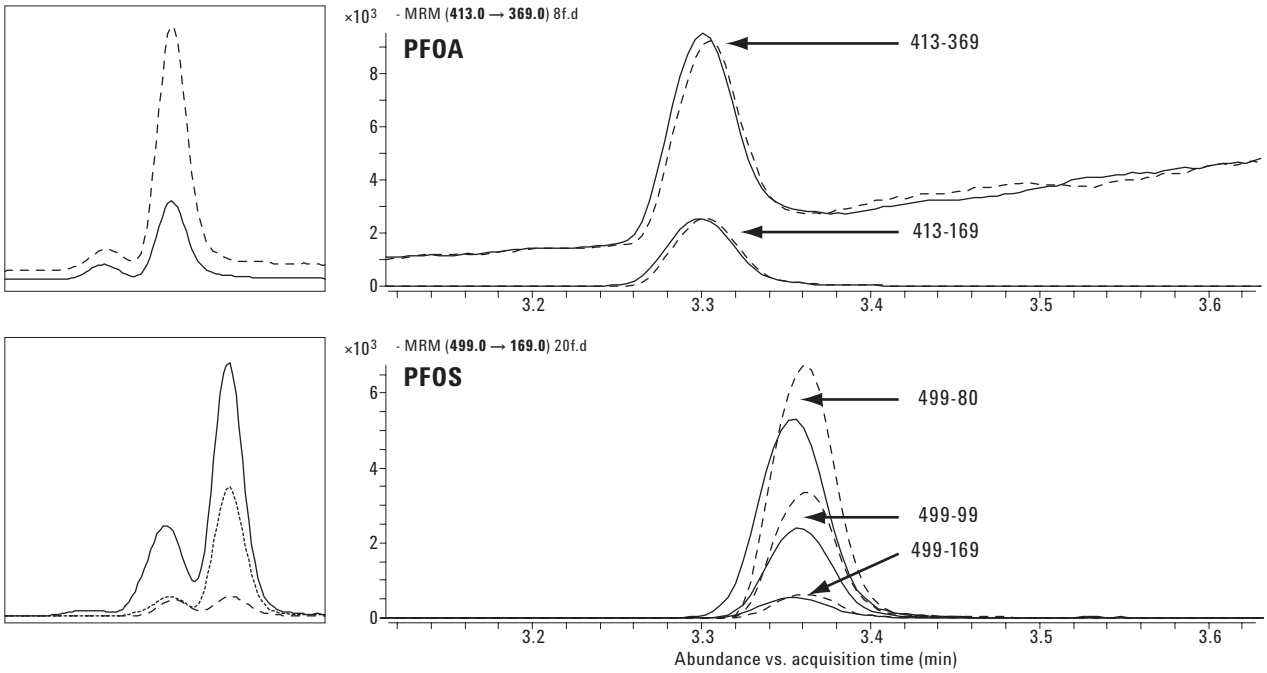


Figure 9. Comparison of PFOA and PFOS MRM chromatograms produced using both 10- and 3-minute gradients. The 3-minute gradient chromatograms are on the right.

The effect of measurement accuracy (*not* ion ratios) of total PFOA and PFOS in branched samples against *a linear standard for each MRM transition* is shown in Table 1.

Table 1. Measurement Accuracy (Target Is 100%) as Function of Compound, Transition, and Run Time

Compound	MRM transition	Percent response (n = 8)	
		10-min run	3-min run
PFOA	**413→369**	**105.9**	**108.2**
	413→169	**96.4**	**89.4**
PFOS	**499→169**	**102.5**	**112.2**
	499→99	75.0	73.3
	499→80	59.3	61.1

The best MRM ions are in bold type. The best results for PFOA can be obtained by averaging the results for the two MRM ions together.

Ion ratios can cause quantitation failure. For PFOA, it does not matter if it's a 3-min run or a 10-min run: the ion 369 transition response is always higher and the ion 169 transition response is always lower. The errors are larger for the 3-min run. The variations are greater for PFOS. In literature, PFOS analysis monitors the ion 80 transition, but it exhibits a large variation. It can be as low as 60%, as seen in Table 1. 499 → 169 is a good transition for quantitation. It is much more accurate, but it is less sensitive compared to 499 → 80 transition.

Regarding issue 2: *Can isotopically labeled standards in matrix be used to measure non-labeled PFOS and PFOA?*

This is addressed using Figures 10 to 12.

Observations regarding the effect of different matrices on signal responses are shown in Figure 10. The taller trace represents the response of PFOA in methanol. The response is lower as the same amount of PFOA is added into a plasma extract.

The matrix effect (common using electrospray ionization) can lead to signal suppression or enhancement; therefore, matrix-matched calibrations are required for accurate quantitation. Due to varying background levels of PFOS and PFOA in matrix, it may not be feasible to use matrix-matched calibrations for quantitating PFOS or PFOA concentrations in study samples. Also, the method of standard additions is not a practical alternative for many matrices with varying levels of target analytes.

As a practical alternative, measuring PFOA using isotopically labeled matrix-matched standards was examined. Results are shown in Figures 11 and 12.

Figure 11 shows that isotopically labeled standards can provide a good linear calibration curve over the quantitation range of 0.02 to 20 ng/mL (0.4 to 400 pg on column). Excellent linear correlation coefficients (≥ 0.9994) were obtained.

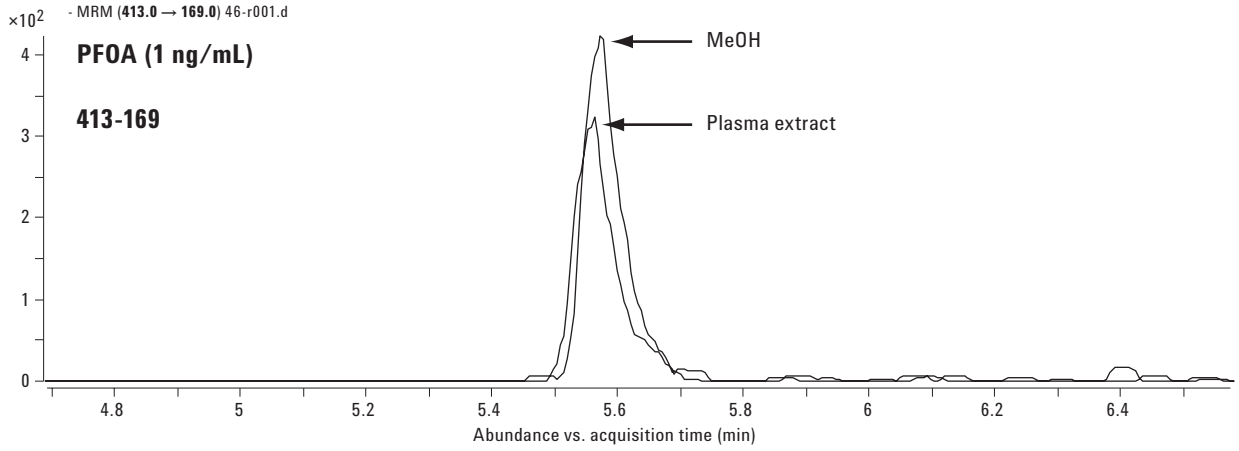
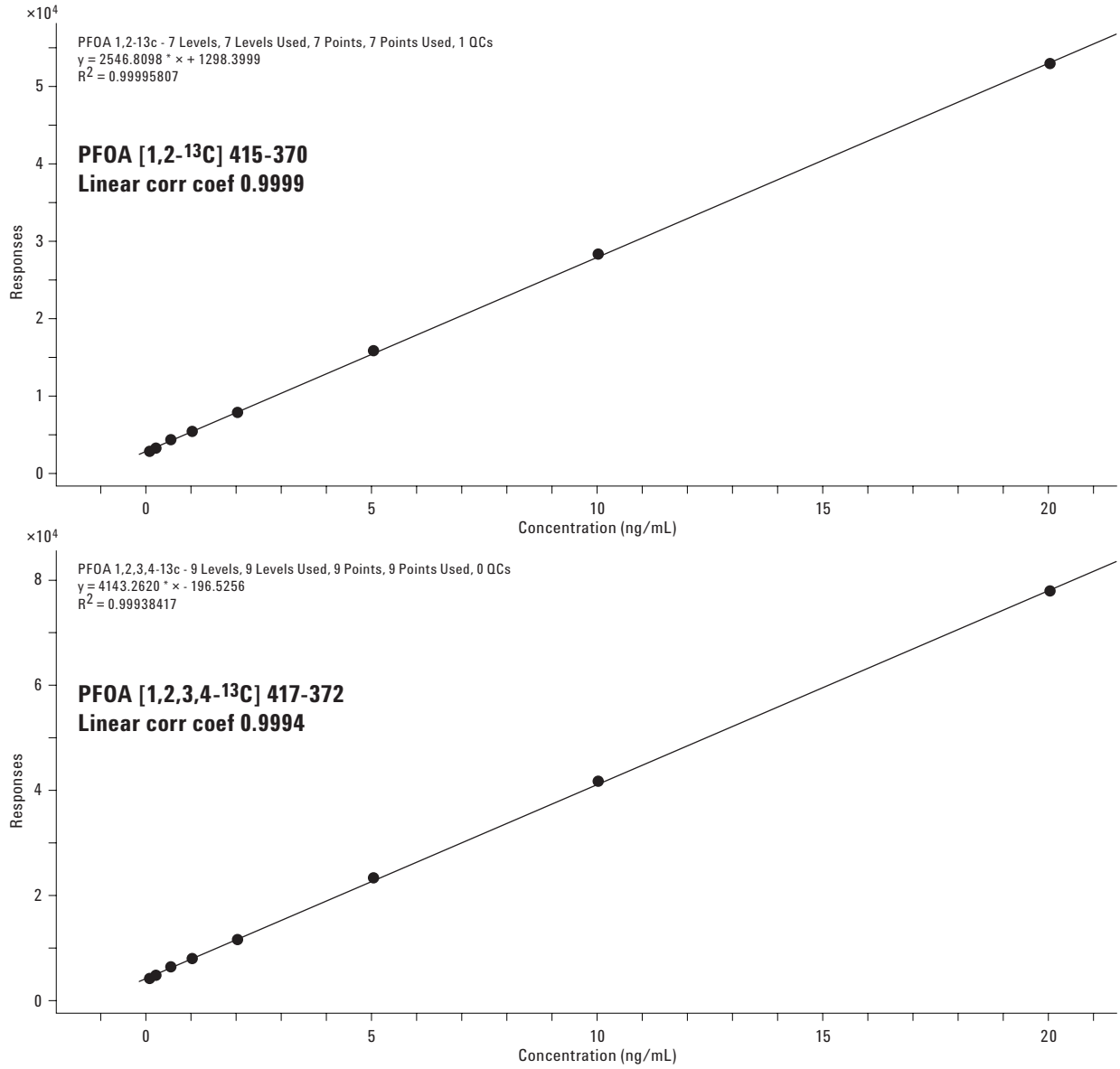


Figure 10. PFOA responses in MeOH and plasma extract at the same concentrations.



Calibration in plasma from 0.02 to 20 ng/mL

Figure 11. Linear correlations for PFOA using two different isotopically labeled calibration standards.

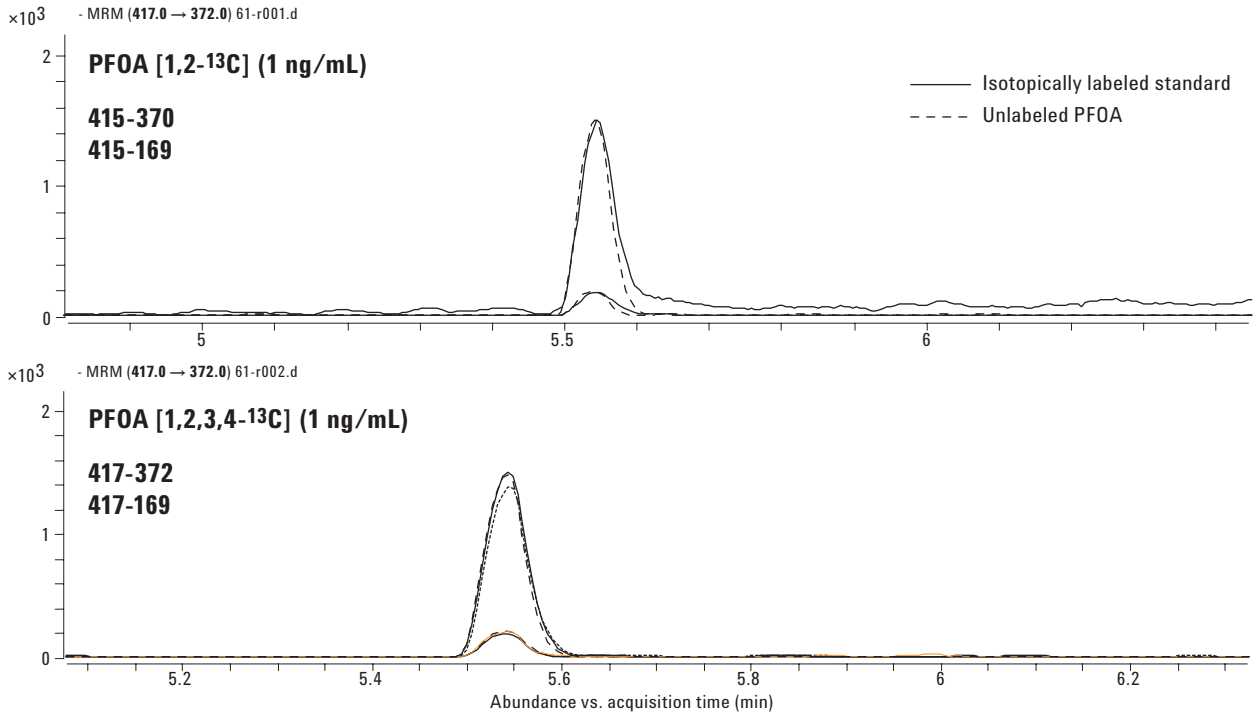


Figure 12. Both isotopically labeled PFOA compounds show good correlation to the unlabeled PFOA. The same transitions for the labeled and native forms of the PFOA were used.

Table 2. Comparison of Different Matrix-Matched Calibrations for Measuring PFOA in Plasma

	Calibration standard	Matrix for calibration	Plasma sample response (Std Dev)
1	PFOA	MeOH	71 (± 33 %)
2	PFOA [1,2-^{13}C]	Plasma	100.4 (± 3.1 %)
3	PFOA [1,2,3,4-^{13}C]	Plasma	97.3 (± 5.1 %)

Matrix-matched calibrations using isotopically labeled PFOA work well.

For row 1, the calibration standard used MeOH as the solvent, and the plasma sample exhibited a 71% response due to matrix suppression. Therefore, we cannot use a calibration standard in MeOH to quantitate samples in matrix; the variation can be as large as 30%. Rows 2 and 3 show that if the calibration is done using an isotopically labeled compound in matrix, the actual plasma sample yields accurate results: 100 and 97%.

Conclusions

- The Agilent LC/QQQ is an excellent instrument for quantifying trace target compounds in complex mixtures.
- The best ion transitions for analysis need to be determined experimentally.
- Fragmentor voltages and collision energies require experimental determination and optimization.
- Using MRM in the QQQ helps achieve the lowest detection limits in complex matrices.
- Branched PFOA/PFOS can affect quantitation accuracy as much as 40% unless it is corrected.
- Matrix suppression can cause the quantitation to be off by as much as 30%. Isotopically labeled analytes work well for accurate quantitation in spite of varying background levels of PFOA/PFOS in matrices.

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Agilent Technologies

Femtogram GC/MSD Detection Limits for Environmental Semivolatiles Using a Triple-Axis Detector

Application

Environmental

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Abstract

The analysis of semivolatiles at very low levels presents challenges due to analyte activity, background contamination, and instrument sensitivity. Method requirements vary worldwide, with the least sensitive specifying 1-µL injections and full-scan data acquisition. The lowest detection limits can be achieved using a programmable temperature vaporizing (PTV) inlet, trace ion detection (TID), and a triple-axis detector (TAD) with the MSD operating in SIM mode.

Introduction

Low-level semivolatiles analysis is used to concurrently measure a mixture of acids, bases, neutrals, and pesticides in drinking water or source water. Most laboratories analyze for > 100 compounds, with a chromatographic run time of 25 to 40 minutes. Sample extraction is accomplished using liquid-solid extraction (LSE) with C_{18} disks or cartridges. Liquid-liquid extraction with a solvent such as dichloromethane is an alternative technique. Extract injection is typically 1 µL hot splitless with the MSD operating in full-scan mode, as specified in some commonly used methods such as USEPA Method 525.2 [1].

Sensitivity is an area where laboratories are seeking improved performance; it can be affected by sample preparation, extract volume injected, instrument tuning, signal acquisition, and overall system activity. Sensitivity is also a confusing term, with all of the following used interchangeably: maximum sensitivity, minimum sensitivity, best sensitivity, lowest detection limit, instrument detection limit (IDL), and method detection limit.

Previous publications have focused on activity/linearity, speed, productivity, and large-volume injection [2–5]. Sensitivity is a factor in all of these, and many times is a trade-off.

This application addresses the parameters that affect the IDL, that is, the "sensitivity" of the GC/MSD system. There are statistical ways to calculate the IDL, but these may not answer the questions, "How much can I actually see?" or "What is the lowest amount that will produce a peak I can integrate?"

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized. For the best sensitivity, parameters should be chosen that transfer the maximum amount of analyte onto the column. Furthermore, the entire system must be inert, as sensitivity is almost always lost on active analytes first.

Many analysts associate the use of PTV only with large-volume injection (LVI) in solvent vent mode [4]. LVI will allow lower levels of calibration, but

Table1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A or 6890N		
Inlet	EPC PTV		
Mode	Splitless		
Temperature ramp	°C/min	Next °C	Hold min
Initial		20	0.05
Ramp 1	600	350	0.90
Ramp 2	10	250	0.00
Cryo	On		
Cryo use temperature	100 °C		
Cryo timeout	10.00 min (On)		
Cryo fault	On		
Pressure	11.40 psi (On)		
Purge flow	30.0 mL/min		
Purge time	1.50 min		
Total flow	34.4 mL/min		
Gas saver	Off		
Gas type	Helium		
PTV Liner	Agilent multi-baffle liner, no packing, p/n 5183-2037		
Oven	120V		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	2.50
Ramp 1	50	110	0.00
Ramp 2	10	320	1.10
Total run time	26 min		
Equilibration time	0.5 min		
Oven max temperature	325 °C		
Column	Agilent Technologies HP 5 MSi, p/n 19091S-433i		
Length	30.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant flow		
Pressure	11.40 psi		
Nominal initial flow	1.4 mL/min		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 12.700 min		

Front Injector	
Sample washes	1
Sample pumps	2
Injection volume	2.0 µL
Syringe size	10 µL
PreInj Solv A washes	0
PreInj Solv B washes	1
PostInj Solv A washes	3
PostInj Solv B washes	2
Viscosity delay	0 seconds
Plunger speed	Fast
PreInjection dwell	0 minutes
PostInjection dwell	0 minutes
MSD	Agilent Technologies 5975C, Triple-Axis Detector
Drawout lens	3 mm standard aperture drawout lens
Solvent delay	4 min
Low mass	45 amu
High mass	450 amu
Threshold	0
Sampling	2
Quad temp	180 °C
Source temp	300 °C
Transfer line temp	280 °C
Tune type	Autotune
EMV mode	Gain factor = 1

MSD-SIM

AutoSIM was used to pick ions, groups and switching times

Number of groups	25
Compounds/group	Varied 1 to 22
Ions/group	Varied 2 to 45
Dwell time, msec	Varied 5 to 50
Cycles/peak	Minimum 10

Calibration Standards

Ultra Scientific, North Kingstown, RI. p/n DWK-5252. Four mixtures, co-diluted in dichloromethane, resulting in 108 compounds at 7 concentration levels: 10, 4, 1, 0.4, 0.1, 0.04, and 0.01 ppm. Each level spiked with 3 Internal Standards at 2 ppm and 4 surrogate standards at 2 ppm. Each level then diluted 1:100 in dichloromethane, resulting in 7 concentration levels: 100, 40, 10, 4, 1, 0.4, and 0.1 ppb (pg/uL) with IS/SS at 2 ppb.

method development is necessary to optimize recovery of compounds while eliminating the solvent. LVI also injects more matrix and may not improve Signal-to-Noise (S/N) due to chemical noise. The PTV has other operating modes; "cold" splitless mode was used here. Splitless injection into a cold inlet instead of a typical hot splitless inlet offers these advantages:

1. Solvent expansion is minimized; analytes do not travel outside the liner and contact metal surfaces, thereby minimizing degradation.
2. Analytes vaporize at the lowest temperature, also minimizing degradation.
3. Volatile solvent is transferred onto the column first; analyte peak shape is improved for injections of 2 to 5 µL.

Figure 1 shows the PTV temperature and flow programs together with the oven program. The PTV is held at 20 °C, a temperature below the boiling point of the solvent dichloromethane, 39.8 °C, during the fast injection period, 0.05 min. At the end of the injection period, the PTV is rapidly heated to 350 °C, transferring analyes onto the column. At the end of the splitless time, 1.5 min, the inlet is purged at 30 mL/min. The PTV is allowed to cool during the run.

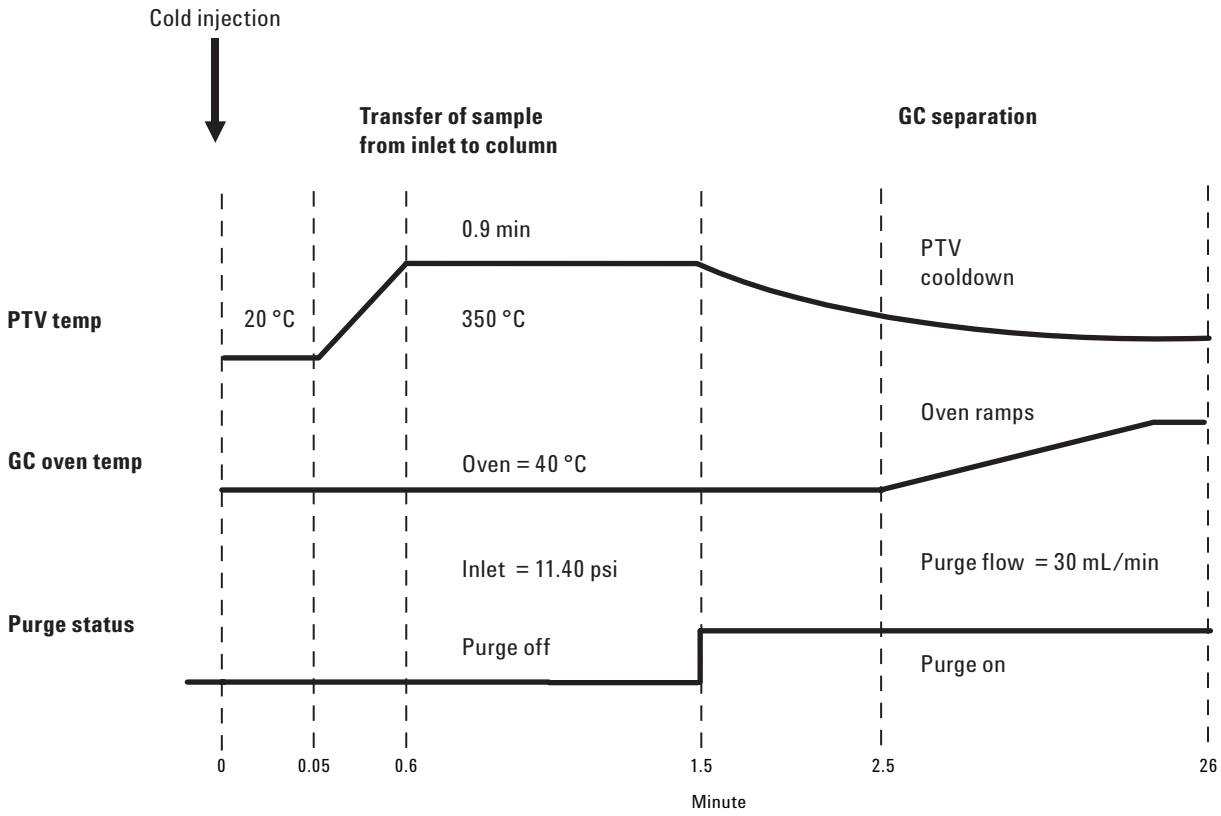

Figure 1. PTV cold splitless temperature and flow programs.

The PTV program ramp can be adjusted and multiple ramps are possible. The PTV inlet liner (p/n 5183-2037) is multi-baffled and deactivated. It does not contain glass wool, which could contribute to active compound degradation. This liner has sufficient capacity to accommodate a 2- to 5-µL injection volume at fast speed. A 2-µL injection was used for all data presented here.

The oven program relationship to the PTV parameters is shown in Figure 1. The oven starts at 40 °C and is held there during the injection cycle and splitless transfer of analytes onto the column. The oven then programs rapidly to 110 °C, followed by a slower ramp for compound separation. There is an extra 1 min of oven hold time at 40 °C, which is between 1.5 and 2.5 min. This maintains the retention time locked (RTL) times for analytes while providing room for the injection to be scaled up to LVI, if desired. The 240V oven was used, but a 120V oven can also achieve the ramp rates found in Table 1.

The HP-5MSi column is designed for inertness and is well suited to this method. This is the latest version of the most popular column in environmental laboratories, the HP-5MS. The column was run in constant-flow mode at 1.4 mL/min to maintain peak shape and sensitivity.

The system was RTLocked to phenanthrene-d10 at 12.700 min. The primary benefit of RTL for this analysis is maintaining constant switching times for SIM groups. After clipping the column, a rerun and analysis of the locking standard is all that is needed to restore shifted peak times. Quantitation database and integration events times also do not have to be changed. Additional RTL applications detailing the numerous benefits of RTL are available at www.agilent.com/chem. It is almost impossible to use a method with this many SIM groups without RTL, in a productive laboratory.

The standard 3-mm drawout lens was used for best sensitivity. Previous work has shown improved linearity across a wide calibration range using the optional 6-mm lens [1]. Using the 6-mm lens will show a typical loss of 2 to 5x in the IDL.

The 5975C MSD was equipped with a Triple-Axis Detector (TAD) [6]. The TAD presents several advantages to the user, one of which is, “Although signal is enhanced, neutral noise is substantially reduced through the off-axis design.” This increase in S/N for clean samples with minimal chemical noise can help reach a lower IDL. Trace ion detection (TID) was switched on during all data acquisition [7]. TID is a filtering routine to minimize noise and is selectable in the software.

Scan parameters are listed and data were collected in either scan mode or in SIM mode. None of the runs was made in synchronous SIM/scan mode. A sampling rate of 2 was used, as it is typical of most methods on a 250-µm id column. This sampling rate, with a 45 to 450 mass range, resulted in at least 10 scans across each peak.

AutoSIM setup was used in combination with the scan quantitation database to pick ions, groups, and switching times. The SIM acquisition table from AutoSIM was used directly with only two modifications. Tebuthiuron (ion 156) and tricyclazole (ion 189) are known for poor peak shape. Their ions were manually added to the groups across which the peaks eluted. A target ion plus one qualifier ion were used for all internal (ISTDs) and surrogate standards (SSs). A target ion plus two qualifier ions were used for all other analytes, if they were present in sufficient abundance in the spectra. A minimum of 10 SIM data points were acquired across each peak.

A source temperature of 300 °C was used instead of the typical 230 to 250 °C range. This higher temperature has been used to minimize peak tailing, and therefore improve sensitivity for PAHs [5].

The compound list was taken from USEPA 525 and is typical of the analytes that laboratories worldwide are interested in analyzing at low levels. The USEPA 8270 list was not used, as it is targeted at higher concentrations of compounds in waste samples that contain high levels of matrix and are not comparable here. The best way to improve sensitivity for solids and waste samples is through extract cleanup. The standards were prepared in dichloromethane only for the single component analytes, except disulfoton sulfoxide and disulfoton sulfone, which were not included in the commercially available mixture. Standards were not prepared for multicomponent toxaphene or the Aroclors.

A typical calibration range for low-level semivolatiles is 0.1 to 10 ppm as defined in USEPA 525. Standards were made from 0.01 to 10 ppm, containing 2 ppm of ISTDs and SSs. A dilution of 1:100 of each of these yields a range of 0.1 to 100 ppb, with ISTDs and SSs at 20 ppb, for a lower working range. Atrazine and alachlor are present in two of the stock mixes, so their concentrations are twice that of other analytes. Pentachlorophenol is present at four times the other analyte concentrations, as described in USEPA 525.

Results

The standard solutions from 0.1 to 100 ppb were run in both SIM and scan modes. Data from the 0.1-ppb scan injections showed insufficient response or were too noisy to reproducibly integrate. The SIM data at 0.1 ppb were significantly improved compared to the scan data and could be routinely used. A listing of selected analytes with S/N measured from 1.0 ppb scan runs (2 pg) are shown in Table 2, together with data from 0.1- (0.2-pg) and 1.0-ppb SIM runs. Each value is an average of three acquisitions on one system, using peak-to-peak noise.

Table 2. Signal-to-Noise for Selected Analytes, SIM and Scan Modes

Compound	Ion	pg → RT	0.2 SIM S/N	2.0 SIM S/N	2.0 Scan S/N
Hexachlorocyclopentadiene	237	7.960	6.3	77	7.5
Trifluralin	264	11.608	4.4	49	7.7
Simazine	201	12.274	1.0	16	2.4
Atrazine	200	12.385	3.1	30	13
Pentachlorophenol	266	12.492	2.4	20	3.7
Chlorothalonil	266	13.146	2.6	26	2.9
Aldrin	66	14.661	1.6	15	1.9
Heptachlor epoxide	353	15.429	6.2	49	3.4
4,4′-DDE	246	16.557	7.0	72	17
Carboxin	143	16.696	2.4	22	4.0
Endrin	263	17.003	2.3	22	4.1
4,4′-DDD	235	17.323	7.5	76	7.5
4,4′-DDT	235	18.000	5.9	60	5.9

There is excellent agreement between the SIM S/N values at the two levels for most compounds. This shows that the responses are real and that the entire system is inert. There is a slight loss of simazine and minimal interference for pentachlorophenol and heptachlor epoxide at the lowest level, 0.2 pg. At the 200 femtogram level, this is no surprise.

The scan S/N at 2.0 pg is lower than SIM, as expected, by 3- to 15-fold. The gains in S/N moving from scan to SIM are related to the dwell time versus the original sampling rate.

Extracted Ion Currents (EICs) from the 1.0-ppb level for both SIM and scan are shown in Figures 2a to 2d. It can clearly be seen that either the SIM or scan signals could be used for quantitation based on S/N and peak shape. Of particular note is the response and very good peak shape for pentachlorophenol, even at an 8-pg full scan.

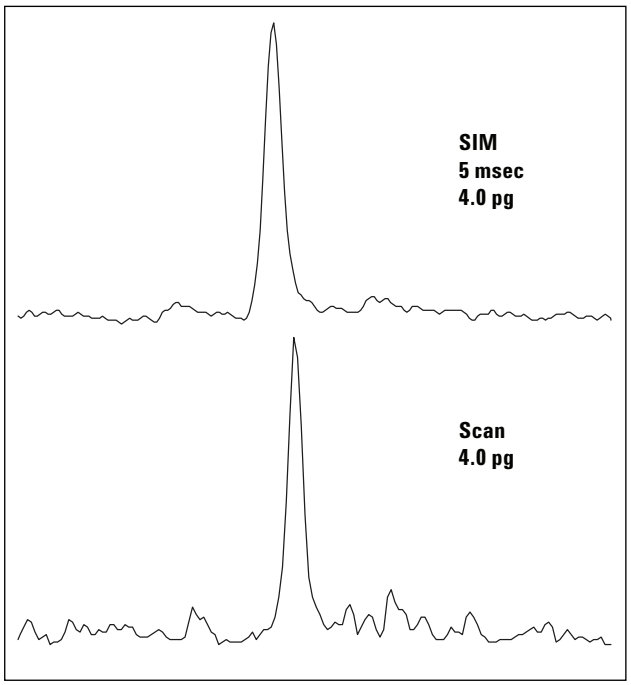

Figure 2a. Atrazine – Extracted Ion 200, RT 12.350 min.

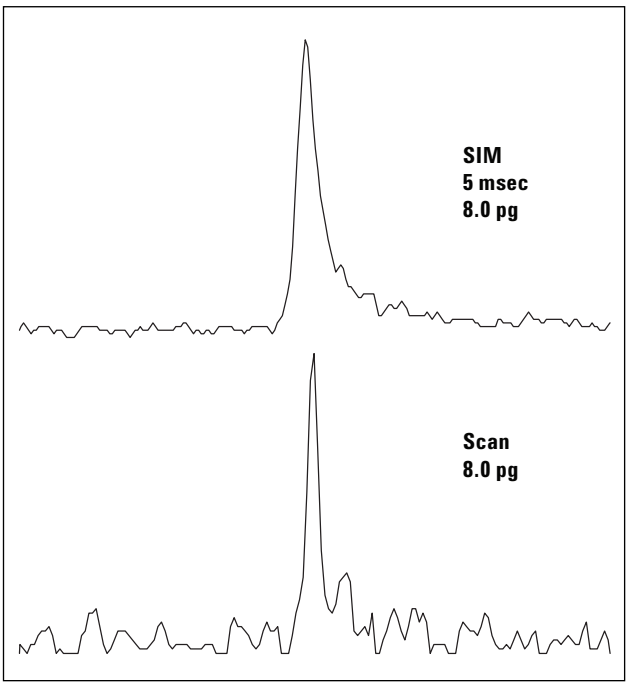

Figure 2b. Pentachlorophenol – Extracted Ion 266, RT 12.445 min.

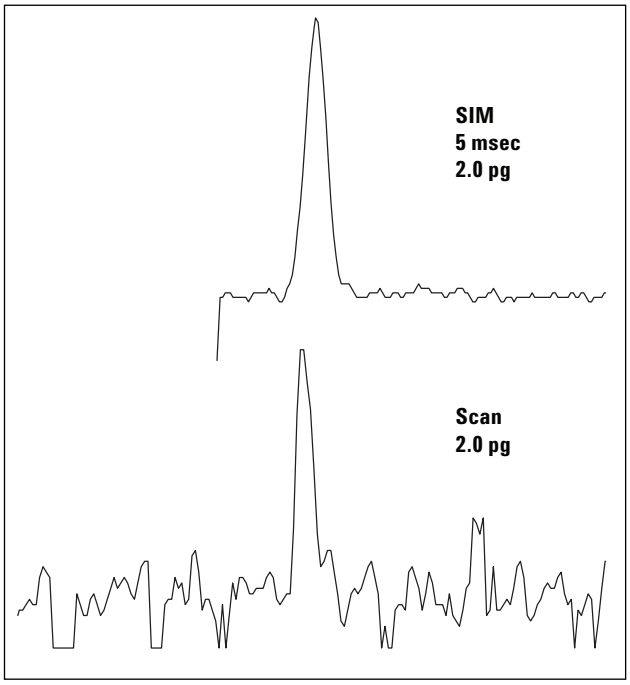

Figure 2c. Aldrin – Extracted Ion 66, RT 14.616 min.

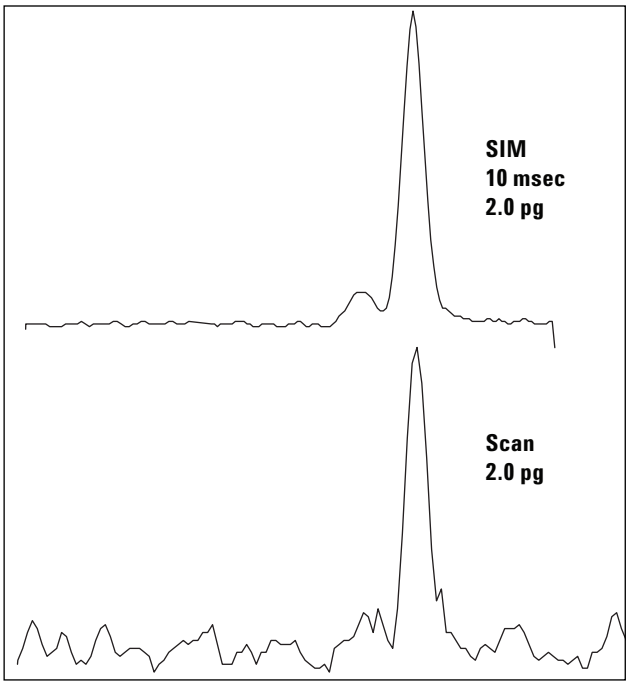

Figure 2d. 4,4′-DDT – Extracted Ion 235, RT 18.00 min.

Although linearity is not the focus of this application, it is a measure of inertness, reproducibility, and sensitivity. Linearity can be determined by the percent relative standard deviation (%RSD) of the relative response factor (RRF) for each compound across the calibration range. The %RSD and the RRF calculations are done automatically by the GC/MSD ChemStation software in conjunction with Excel. There is no correct %RSD, as it is method dependent. The %RSDs of the RRFs for selected compounds are shown in Table 3.

Table 3. Linearity of Selected Analytes

Calibration range pg →	0.2–200 SIM	2–200 Scan
Compound	**%RSD**	**%RSD**
Dichlorvos	1.9	7.0
Mevinphos	10.1	7.0
2,3-Dichlorobiphenyl	5.3	3.0
Atrazine	14.2	14.5
Pentachlorophenol	6.3	33.0
Anthracene	2.2	3.0
Chlorothalonil	7.6	25.0
Heptachlor epoxide	6.6	13.0
4,4′-DDE	4.5	9.0
4,4′-DDD	7.4	8.0
4,4′-DDT	4.0	5.9

At first glance some of the %RSD values appear high, such as pentachlorophenol (PCP) and chlorothalonil. These are calibrated, however, from 2 to 200 pg in scan mode, which is 50-fold lower than USEPA 525 mandates. The SIM data are calibrated from 0.2 to 200 pg, which is 500-fold lower and a 10-fold wider range. This demonstrates both inertness and detectability at the femtogram level.

As an additional overall measure of system linearity, the average of all %RSDs was calculated at 8% for SIM data and 13% for scan data. Not all compounds were calibrated to the 0.1-ppb level, as they did not have a signal that could be reliably measured. The phthalates, easily detected at low levels, were excluded from these averages due to common laboratory contamination.

EICs at the 200-femtogram level, from SIM, are shown for six different compounds in Figures 3. All are easily seen and measured against noise. As an analyst's measure of sensitivity, the question from the introduction was "How much can I actually see?" The answer: very low picogram levels for most environmental semivolatiles in scan mode. The IDL using SIM is even lower, in the femtogram range.

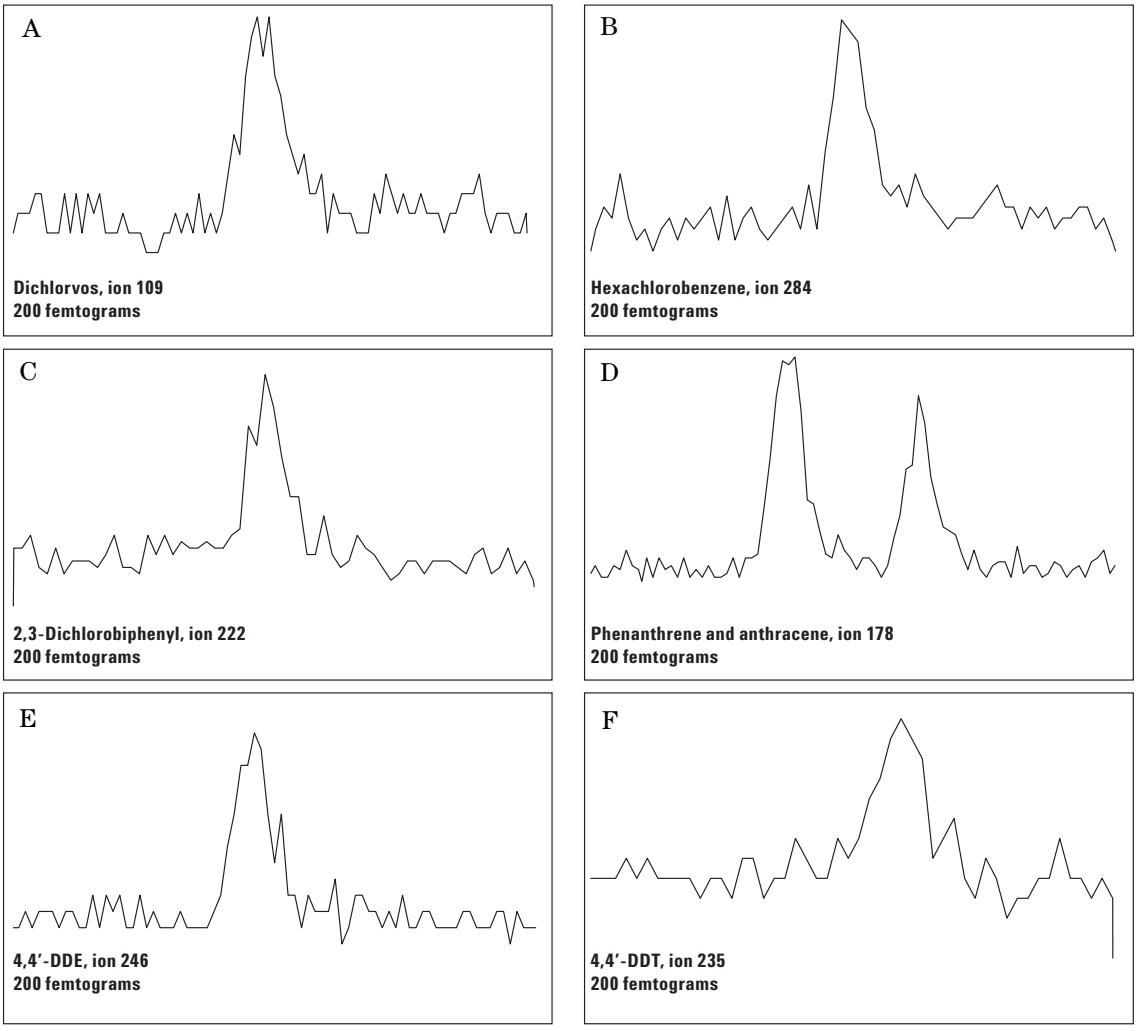

Figure 3. EICs at the 200 femtogram level.

Conclusions

Traditional semivolatiles methods can be altered to achieve better instrument detection limits. There have been advancements in hardware, such as the Triple-Axis Detector (TAD), that improve sensitivity. Signal handling using Trace Ion Detection (TID) provides better S/N through lower noise. The PTV, used in "cold" splitless mode, maximizes the amount of sample on the column, while vaporizing analytes at the lowest possible temperature. Coupled with an inert column and source, the PTV provides an easy way to improve sensitivity. Methods that require only a target ion and a few qualifier ions for identification can often be changed to SIM from scan, improving S/N by 3- to 50-fold. Combining all of these hardware, software, and operating parameters can result in femtogram instrument detection limits (IDLs) and sensitivity you can use.

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Agilent Technologies

Semivolatile Analysis Using an Inertness Performance Tested Agilent J&W DB-5ms Ultra Inert Column

Application

Environmental

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Abstract

Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. The new testing procedure uses deliberately aggressive probes to thoroughly investigate column inertness quality. The value of using probes such as 1-propionic acid, 4-picoline, and trimethyl phosphate to establish a column inertness baseline is discussed. This baseline inertness profile is then extended to a real-world application example with challenging analytes in the semivolatile sample set. Inertness performance with analytes such as 2,4-dinitrophenol, benzoic acid, and benzidine clearly shows the advantage of using the Agilent J&W DB-5ms Ultra Inert columns for semivolatile analysis.

Introduction

Semivolatile analyses using methods similar to USEPA method 8270 [1] are important in environmental laboratories worldwide. A number of very active analytes presents significant challenges for analysts, equipment providers, and column manufactures in terms of inertness. Acidic compounds such as benzoic acid or 2,4-dinitrophenol and strong bases such as pyridine or benzidine are examples of active species found in the semivolatile sample set. These chemically charged species are particularly susceptible to adsorption onto active surfaces in the sample flow path, including the column itself. Both system and column inertness are critical for effective analysis of these active chemical species.

For many years Grob's mix [2] has been the standard mix to evaluate capillary GCs and columns. This mix consists of a series of alkanes, a substituted phenol (acidic component), an amine (basic component), an alcohol, and a diol. Virtually all capillary column manufactures have used Grob's or a very similar test mix to evaluate column performance historically. These mixtures work well to evaluate column efficiency, system suitability against solute discrimination during injection, and potential solute absorption in the chromatographic flow path. Inertness evaluation based on single acidic and basic species in these mixes, though valuable, falls short of the rigorous requirements for inertness that applications on modern capillary GC columns require [3–4]. Modern GC applications demand a more comprehensive approach to properly investigate column inertness performance.

Experimental

Baseline inertness testing of columns was on an Agilent 6890N GC equipped with a 7683B autosampler and an FID. Semivolatile application-specific chromatograms were generated using an Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler.

Tables 1 and 2 list the chromatographic conditions used on each of the chromatographic systems. Table 3 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions 6890N/FID System

GC:	Agilent 6890N
Sampler :	Agilent 7683B, 0.5-µL syringe (Agilent p/n 5188-5246), 0.02-µL split injection, 1 ng each component on column
Carrier:	Hydrogen constant pressure 38 cm/s
Inlet:	Split/splitless; 250 °C, 1.4 mL/min column flow, split flow 900 mL/min, gas-saver flow 75 mL/min. on at 2.0 min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column:	Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven:	65 °C isothermal
Detection:	FID at 325 °C, 450 mL/min air, 40 mL/min hydrogen, 45 mL/min nitrogen makeup

Table 2. Chromatographic Conditions 6890N/5975B MSD System

GC:	Agilent 6890N/5975B MSD
Sampler :	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-5246), 1.0-µL splitless injection, 5 ng each component on column
Carrier:	Helium constant flow 30 cm/s
Inlet:	Split/splitless; 260 °C, 53.7 mL/min total flow, purge flow 50 mL/min on at 0.5 min, gas-saver flow 80 mL/min on at 3.0 min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column:	Agilent J&W DB-5 ms Ultra Inert, 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven:	40 °C (1 min) to 100 °C (15 °C/min), 10 °C to 210 °C (1 min), 5 °C/min. to 310 °C (8 min)
Detection:	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 290 °C, scan range 50–550 AMU

The flow path supplies used in these experiments are listed in Table 3.

Table 3. Flow Path Supplies

Vials:	Amber screw cap (Agilent p/n 5182-0716)
Vial caps:	Blue screw cap (Agilent p/n 5282-0723)
Vial inserts:	100-µL glass/polymer feet (Agilent p/n 5181-1270)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647) for FID Deactivated single taper direct connect (Agilent p/n G1544-80730) for MSD
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier:	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

Test probes for baseline inertness evaluation were purchased from Sigma Alrich (Milwaukee, WI 53201, USA). Dichloroethane used was Burdick and Jackson spectral grade purchased thorough VWR International (West Chester, PA 19380, USA). semivolatile standard (USEPA 8270) solutions were obtained either from Ultra Scientific (North Kingstown, RI 02852, USA) or AccuStandard (New Haven, CT 06513, USA).

Solutions were prepared using dichloroethane solvent and class A volumetric pipettes and flasks.

Results and Discussion

Baseline Inertness Profile for the Ultra Inert Columns

One means of quickly evaluating the suitability of a chromatographic system and the column component of that system is the deliberate injection of challenging analyte mixes on the system. Good sample recoveries and peak shapes quickly show that the injection system is functioning properly and establish a baseline inertness profile for the column. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species like those in the semivolatile sample set. The use of more demanding test mixes to certify column inertness performance is the approach taken for every column offered in the Ultra Inert series of capillary GC columns.

This application illustrates the implementation of more rigorous testing procedures to certify GC capillary column inertness. The baseline test mix selected for inertness contains 1-propionic acid, 4-picoline, trimethyl phosphate, and 1-heptanol. Key column evaluation criteria include efficiency of n-decane elution at a k' of 5, probe peak shapes, and peak height ratios of 4-picoline and trimethyl phosphate relative to closely eluting alkanes. The peak height ratio of active analytes, such as 4-picoline and trimethyl phosphate, relative to less active alkanes indicate the degree of surface activity for the reactive analyte. A higher ratio indicates better inertness. Testing with these aggressive probes provides more probative tools for evaluating inertness with problematic acidic and basic species. This testing procedure raises the bar for column inertness QC testing and sets a new industry standard for consistent column performance.

Figure 1 shows a baseline inertness chromatogram for an Ultra Inert DB-5ms column. Please note the peak shapes for trimethyl phosphate. This compound exhibits minor peak tailing in this example chromatogram and, for this analyte, represents very good peak shape. The observable peak tailing for this analyte is what makes it an excellent tool for evaluating column inertness. On a lesser column this peak may not be seen at all.

Semivolatile Challenging Analytes

The evaluation of column performance went beyond the new baseline testing for inertness and looked at an abbreviated list of compounds specific to the USEPA Method 8270 sample set. The semivolatiles mix [5] contained N-nitrosodimethylamine, aniline, benzoic acid, 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol, 4-aminobiphenyl, benzidine, 3,3'-dichlorobenzidine, benzo [b] fluoroanthene, benzo [k] fluoroanthene as well as recommended internal standards. These species were selected to range in polarity from basic to acidic species and from very early eluting nitrosamine to late eluting polynuclear aromatic hydrocarbons (PAHs). Figure 2 is a total ion chromatogram of the challenging analyte mix with a 5-ng on-column loading of each component.

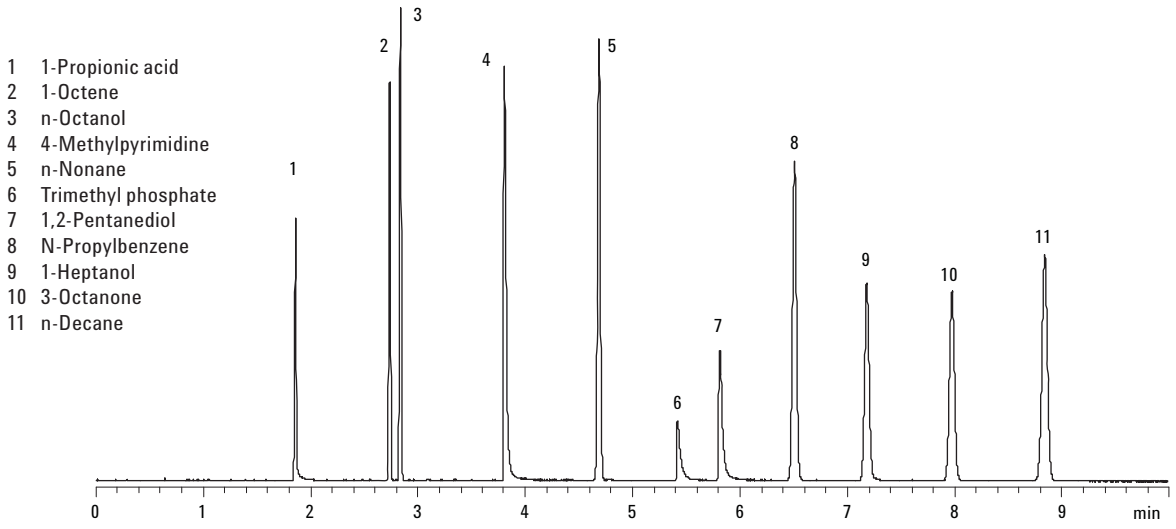

Figure 1. **Baseline inertness test chromatogram,** 1 ng/component load on the Agilent J&W DB-5ms Ultra Inert column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 1, flow path supplies as in Table 3.

1 N-nitrosodimethylamine
2 Aniline
3 1,4-Dichlorobenzene-D4
4 Benzoic acid
5 Naphthalene-D8
6 Acenapthene-D10
7 2,4-Dinitrophenol
8 4-Nitrophenol
9 2-Methyl-4,6-dinitrophenol
10 Pentachlorophenol
11 4-Aminobiphenyl
12 Phenanthrene-D10
13 Benzidine
14 Chrysene-D12
15 3,3′-Dichlorobenzidine
16 Benzo [b] fluoroanthene
17 Benzo [k] fluoroanthene
18 Perylene-D12

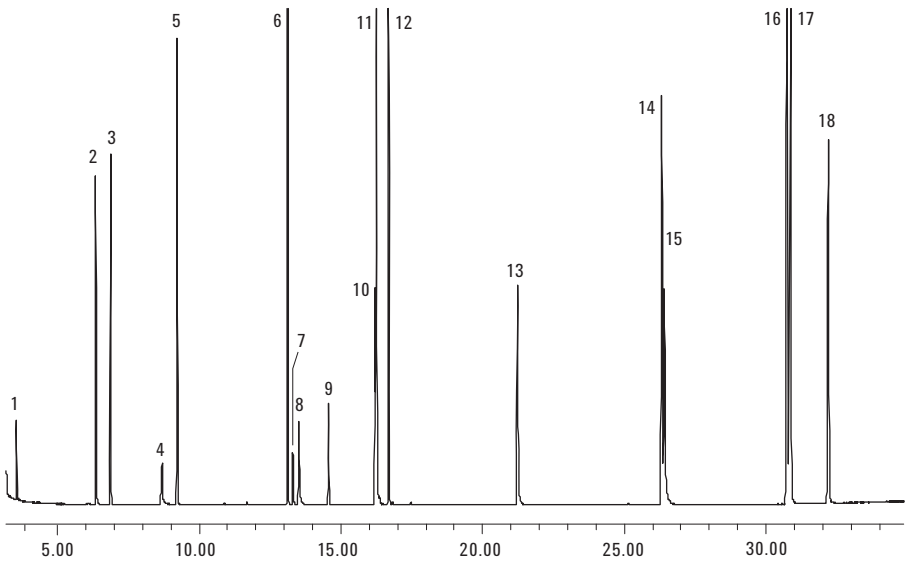

Figure 2. Abbreviated semivolatile test chromatogram, 5 ng/component load on the Agilent J&W DB-5ms Ultra Inert column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 2, flow path supplies as in Table 3.

One key assessment criterion for USEPA 8270 system suitability is the response factor for 2,4-dinitrophenol and its most closely eluting internal standard acenaphthene-d10. The minimum acceptable average response factor (over the entire concentration range) is 0.050 and the typical range is between 0.1 to 0.2. This response tends to decrease at lower concentrations and as the chromatographic system or the standard starts to deteriorate. In Figure 2, response factors for 2,4-dinitrophenol were greater than 0.1, and for 4-nitrophenol, they were greater than 0.2, each at a concentration of 5 µg/mL. These values are indicative of excellent column performance even at low standard concentration.

The recovery of benzidine is another key indicator of inertness performance for semivolatile analysis. This particular base is subject to thermal breakdown in the inlet and to oxidation from standing in solution. Injection temperatures above 260 °C caused benzidine recoveries to drop dramatically. It was necessary to balance benzidine recoveries with the elution of heavier PAHs when setting injection port temperatures. An injection port temperature setting of 260 °C gave good recoveries for benzidine and was still hot enough for higher molecular weight PAHs to volatilize.

Semivolatile Large Mix

Figure 3 shows a 5-ng on-column loading of a broader range of semivolatile analytes. This large mixture was prepared by combing AccuStandard® semivolatile mixes 1, 2, 3, 4a, 4b, 5, and 6 all at a nominal concentration of 5 µg/mL. In total, 93 semivolatile compounds were included in this mix, ranging in boiling points from very low-boiling N-nitrosodimethylamine to high-boiling benzo (g,h,i) perylene. In addition, a wide diversity of analyte polarities was represented in this mix. The highlighted area in Figure 3 shows the elution and peak shape of highly basic benzidine and its response relative to the nearest eluting peak, flouranthene. Even in this large mix, benzidine gave good relative response and peak shape.

1 N-nitrosodimethylamine
2 2- Methyl pyridine
3 Benzidene
4 Fluoranthene
5 Benzo (g,h,i) perylene

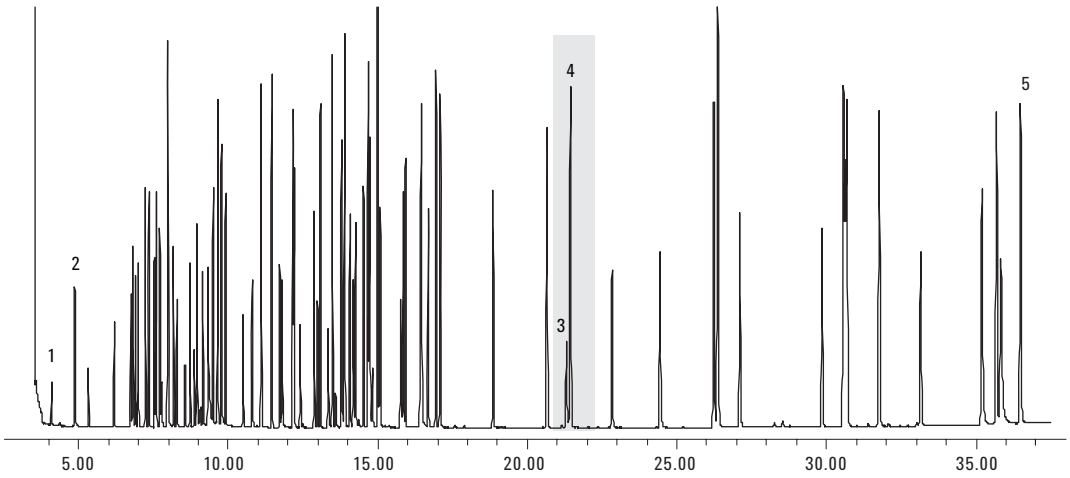

Figure 3. **Semivolatile (large mix) test chromatogram,** 5 ng/component load on the Agilent J&W Ultra Inert DB-5ms column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 2, flow path supplies as in Table 3. Several peaks of interest are labeled to indicate early- and late-eluting species. Benzidine (peak 3) and fluoranthene (peak 4) peaks are shown in the highlighted section.

Conclusions

Rigorous column inertness testing with aggressive probes ensures consistent and reliable column inertness performance for active analytes. Challenging probes such as 1-propionic acid, 4-picoline, and trimethyl phosphate are better predictive indicators of column behavior toward active analytes than traditional Grob style mixes used by many column manufacturers. Inertness testing with these aggressive probes produces columns with well-defined baselines for inertness performance.

Columns with well-defined inertness baselines provide a reliable platform for the analyst to begin analysis of semivolatiles. The Ultra Inert DB-5ms column used in this series of experiments demonstrates excellent inertness performance for some of the most difficult analytes in the semivolatile sample set, including N-nitrosodimethylamine, 2,4-dinitrophenol, 4-nitrophenol, and benzidine. The good recoveries and peak shapes observed for these difficult species, even with a 5-ng on-column loading, are indicative of successful semivolatile analyses on these new Ultra Inert DB-5ms columns.

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Agilent Technologies

Polybrominated Diphenyl Ether (PBDE) Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column

Application

Environmental

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Abstract

Trace and ultra trace-level polybrominated diphenyl ether (PBDE) analyses are important tools for understanding food supply and environmental quality worldwide. In this application, trace-level PBDE analysis is demonstrated using electron impact single quadrupole scanning mass spectrometry. For these challenging separations, knowing that each GC column has been thoroughly tested for column inertness gives the analyst higher confidence in the accuracy of the results.

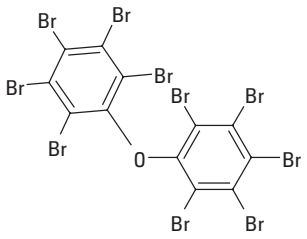
Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate column inertness and quality. These extremely active probes, including 1-propionic acid, 4-picoline, and trimethyl phosphate, are used to verify each column's inertness performance.

Introduction

Polybrominated diphenyl ethers (PBDEs) are both persistent and increasingly common in the environment. These chemicals are typically used as flame retardants in textiles and electronic products such as televisions and computer equipment. There are 209 possible PBDE congeners that vary in the degree of bromination from mono to fully brominated decabromodiphenyl ether. Each of the individual congeners is assigned both an IUPAC name and bromodiphenyl ether (BDE) number, by convention. For example, fully brominated decabromodiphenyl ether is assigned the number BDE-209.

PBDEs as a class of molecules tend to undergo degradation on exposure to heat and light. BDE-209's long retention and susceptibility to thermal breakdown make it a particularly challenging analyte.

BDE-209 Structure



Unfortunately, these chemicals continue to find their way into food supplies and common house dust. [1–5] Similarities between PBDEs and polychlorinated biphenyl (PCBs) compounds include their tendency to persist in the environment and to bioaccumulate in adipose tissues.

The chief routes of human exposure to PBDEs appear to be ingestion of contaminated foods and inhalation of contaminated house dust. Measurable levels of PBDEs have been found in fish, meats,

dairy products, eggs, and vegetables. Higher levels of PBDEs are found more often in fish than in other food sources. House dust studies in the U.S., Belgium, and Singapore have all shown appreciable levels of PBDEs. The need for reliable, sensitive, and robust analytical methods for the analysis of PBDEs is of global concern.

Long-term human toxicities for PBDEs are not well understood, even though a number of studies have found appreciable levels in breast milk and human adipose tissue. These studies suggest a link between long-term exposure of the mother to specific BDEs and neurological effects in the growing fetus. Human heath concerns led to a ban on the use of penta-BDE and octa-BDE within the European Union in 2004.

This application highlights the value of using a 15-m Agilent J&W DB-5ms Ultra Inert capillary GC column for challenging PBDE analysis. Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate column inertness and quality. These extremely active probes, including 1-propionic acid, 4-picoline, and trimethyl phosphate, are used to verify each column's inertness performance. Capillary GC column activity as a potential source of result uncertainty has been all but eliminated with the Ultra Inert series of columns.

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow-path consumable supplies used in these experiments.

Sample Preparation

A seven-level eight-component BDE calibration curve set was purchased from AccuStandard (New Haven, CT). These solutions were transferred directly to amber glass autosampler vials and used as supplied. Concentration ranges were 0.5 to 250 ng/mL for BDEs -47, -100, -99, -154, -153, -183, and -205. BDE-209 concentration ranged from 2.5 to 1,000 ng/mL. The isooctane used was Burdick and Jackson Ultra Resi Grade purchased through VWR International (West Chester, PA, USA). Isooctane was used as a reagent blank and syringe wash solvent.

Table 1. Chromatographic Conditions

GC	Agilent 6890N/5973B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5188-5246), 1.0-µL splitless injection, 5 ng each component on column
Carrier	Helium 72 cm/s, constant flow
Inlet	Pulsed splitless; 325 °C, 20 psi until 1.5 min, purge flow 50 mL/min at 2.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5512UI)
Oven	150 to 325 °C (17 °C/min), hold 5 min
Detection	MSD source at 300 °C, quadrupole at 150 °C, transfer line at 300 °C, scan range 200–1000 amu
SIM program	

			SIM ions			
Time (min)	Group	PBDE bromination	$[M]^+$	$[M–Br_2]^+$	$[M–Br_2]^{+2}$	Confirmation ion
3.00	1	3	405.8	246		247.9
		4	485.7	325.8	162.9	
5.75	2	5	536.6	403.8		565.7
		6	643.6	483.7	241.8	
8.00	3	7	721.5	561.6		563.6
9.25	4	8	801.5	641.5	320.8	643.6
11.50	5	10	959.3	799.4	399.7	797

Table 2. Flow Path Supplies

Vials	Amber glass vials (Agilent p/n 5182-0716)
Vial caps	Blue screw cap (Agilent p/n 5282-0723)
Vial inserts	100 µL glass/polymer feet (Agilent p/n 5181-1270)
Syringe	5 µL (Agilent p/n 5181-1273)
Septum	Advanced Green (Agilent p/n 5183-4759)
Inlet liners	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier	20x magnifier loupe (Agilent p/n 430-1020)

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with highly active probes at low concentration and low temperature. [6] This is a new rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto the column's active sites, particularly at trace levels, like the BDEs in this application example. A detailed description of the test mix and additional application examples are available in references 7 through 9.

PBDE Analyses

PBDE-209 is a particularly challenging analyte due to its long retention and tendency to degrade with high-temperature exposure. High-temperature thermal stability is an issue for this class of compounds, but is more pronounced for BDE-209, as it is highly brominated and well retained. One key to successful BDE analysis is to limit the time that these compounds are exposed to high temperatures. A 15-m long column, as opposed to a typical 30-m long column was used in this case to limit residence time for BDE-209. [10,11] Fortunately, the BDEs resolve well, with symmetrical peak shapes, when using Agilent J&W DB-5ms phase, enabling successful separation on the shorter column. Figure 1 shows a total ion chromatogram of the eight BDEs investigated in this study.

In this application a seven-level eight-component BDE calibration curve set was evaluated over the concentration range of 0.5 to 250 ng/mL for BDEs -47, -100, -99, -154, -153, -183, and -205 and the range of 2.5 to 1,000 ng/mL BDE 209 on an Agilent J&W Ultra Inert DB-5ms 15 m × 0.25 mm × 0.25 µm (p/n 122-5512UI) column. Sensitivity was excellent, even for the more challenging BDE-209 with a 0.025 ng on-column loading, yielding a 3.28 signal-to-noise level. The exploded view of the BDE-209 peak in Figure 2 illustrates the sensitivity observed for a 0.025-ng on-column loading of BDE-209.

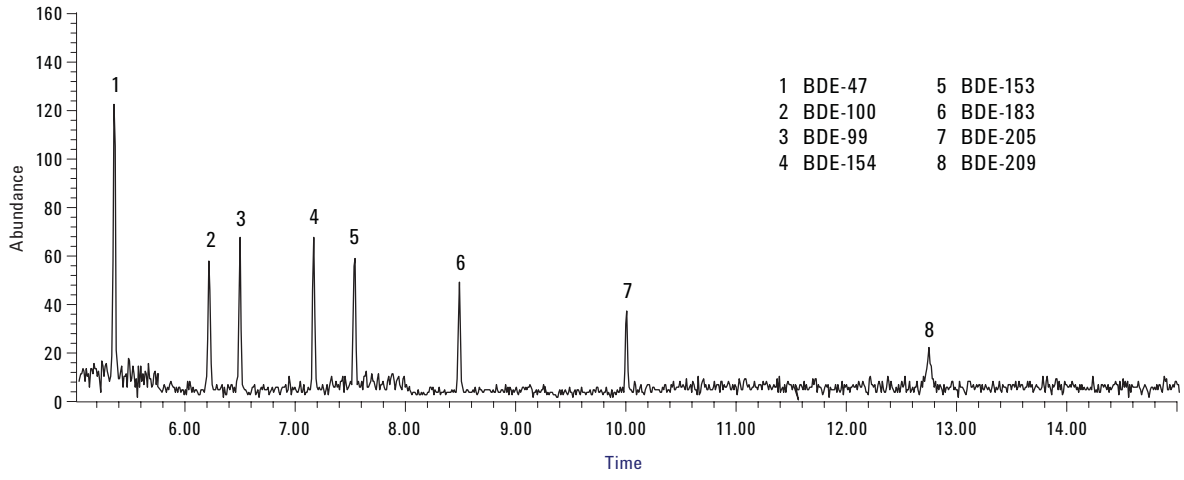


Figure 1. Total ion chromatogram (SIM mode) of a 0.005-ng (BDEs -47, -100, -99, -154, -153, -183, -205, and -209) and 0.025-ng (BDE-209) on-column loading on an Agilent J&W DB-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5512UI).

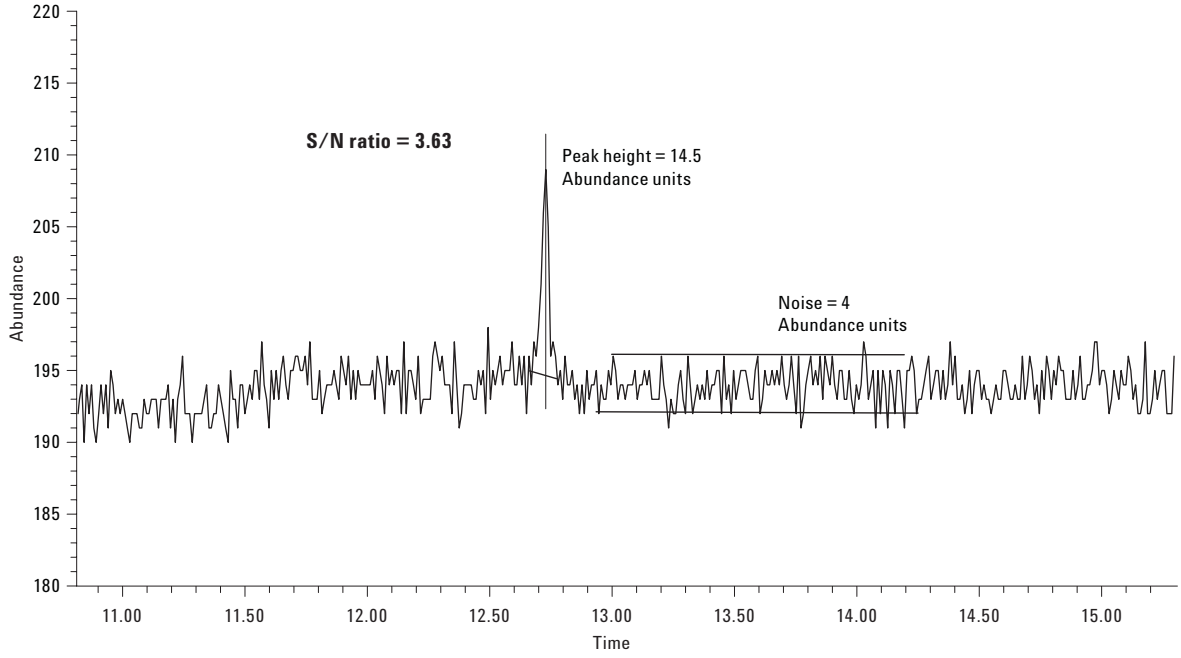


Figure 2. Enlarged section of the total ion chromatogram (SIM mode) of a 0.025-ng BDE-209 on-column loading. The large peak in the figure is BDE-209, a particularly challenging BDE due to its long retention and thermal instability.

Linearity was excellent across the range studied, giving R^2 values of 0.997 or greater in all cases. Figure 3 indicates the correlation coefficients for each of the individual analytes and shows an example linear regression plot for BDE-209.

BDR	R^2
47	0.999
100	0.999
99	0.999
154	0.999
153	1.000
183	0.999
205	0.999
209	0.997

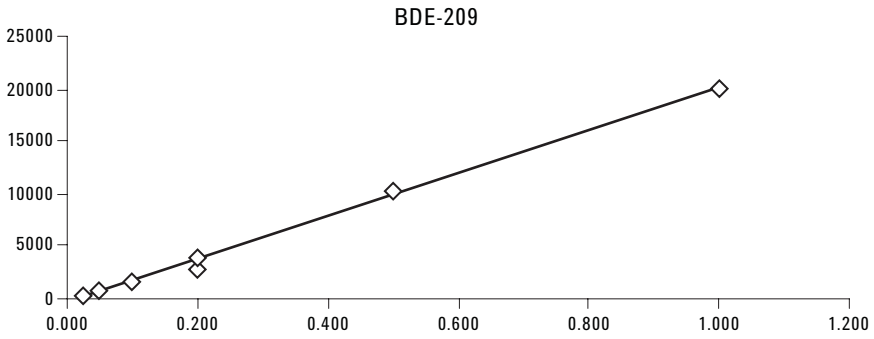


Figure 3. Correlation coefficients for the eight components over the 0.5 ng/mL to 1,000 ng/mL concentration range (BDE-209 2.5 to 1,000 ng/mL) used in this study. An example linear regression plot of particularly challenging BDE-209 is also shown.

Conclusions

This application successfully demonstrates the use of a 15-m Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level BDEs in a 15-minute analysis. Linearity was excellent for all eight BDEs studied, yielding 0.997 or greater R^2 values down to a 0.005 ng (0.025 ng for BDE-209) on-column loading of each component. One of the reasons for the excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for trace-level applications.

The Agilent 6890/5975B GC/MSD (SIM mode) equipped with an inert electron impact source had excellent sensitivity with even the most challenging BDE in this set, PBD-209. The signal-to-noise ratio for a 0.025-ng on-column loading of BDE-209 was greater than three to one with this system. This result shows clearly the power of using an Agilent J&W DB-5ms Ultra Inert column for trace-level BDE analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W DB-5ms Ultra Inert GC capillary column.

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EPA Method 1694: Agilent's 6410A LC/MS/MS Solution for Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS

Application Note

Environmental

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Abstract

An analytical methodology for screening and confirming the presence of 65 pharmaceuticals in water samples was developed using the Agilent G6410A Triple Quadrupole mass spectrometer (QQQ). The method was developed following the guidelines in EPA Method 1694. Four distinct chromatographic gradients and LC conditions were used according to the polarity and extraction of the different pharmaceuticals. Positive and negative ion electrospray were used with two multi-reaction monitoring (MRM) transitions (a quantifier and a qualifier ion for each compound), which adds extra confirmation in this methodology compared with the EPA method. Linearity of response of three orders of magnitude was demonstrated ($r^2 > 0.99$) for all the pharmaceuticals studied. The analytical performance of the method was evaluated for one wastewater sample collected from Boulder Creek, Colorado; positive identifications for carbamazepine and diphenhydramine were found for this sample using the methodology developed in this work.

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Introduction

The analytical challenge of measuring emerging contaminants in the environment has been a major research focus of scientists for the last 20 years. Pharmaceuticals and personal care products (PPCPs) are an important group of contaminants that have been targeted, especially in the last decade. In the area of PPCPs there are several methods addressing the analysis of these analytes, including EPA Method 1694 [1], which was recently published (December 2007). This EPA protocol uses solid-phase extraction (SPE) for water sample preparation [1]. The extracts are then analyzed directly by a tandem mass spectrometer using a single transition for each compound. This application note describes the Agilent solution to this method, which is demonstrated with the Agilent model 6410A LC/MS QQQ. The Agilent initial implementation for EPA Method 1694 consists of 65 analytes (of 75 total analytes) and 17 labeled internal standards (of 20 total), which are a mixture of PPCPs that are analyzed each by a single MRM transition. (Note that the other compounds and internal standards could not be obtained at this time.) The method also uses Agilent C-18 and Hydrophilic Interaction Chromatography (HILIC) columns for all analytes. To provide additional confirmation, a second MRM transition was added for 60 of the 65 analytes analyzed. This gives an even greater assurance of correct identification than prescribed by the EPA. Table 1 shows the list of pharmaceuticals studied here.

Table 1. Analytes Studied in This Work

List of Group 1 Compounds EPA 1694: 46 Analytes

Acetaminophen	Codeine	Flumequine	Penicillin V	Sulfanilamide
Ampicillin	Cotinine	Fluoxetine	Roxithromycin	Thiabendazole
Azithromycin	Dehydronifedipine	Lincomycin	Sarafloxacin	Trimethoprim
Caffeine	Digoxigenin	Lomefloxacin	Sulfachloropyridazine	Tylosin
Carbadox	Diltiazem	Miconazole	Sulfadiazine	Virginiamycin
Carbamazepine	1,7-Dimethylxanthine	Norfloxacin	Sulfadimethoxine	Digoxin*
Cefotaxime	Diphenhydramine	Ofloxacin	Sulfamerazine	
Ciprofloxacin	Enrofloxacin	Oxacillin	Sulfamethazine	
Clarithromycin	Erythromycin	Oxolinic acid	Sulfamethizole	
Cloxacillin	Erythromycin anhydrate	Penicillin G	Sulfamethoxazole	

*Compound formed intractable Na adduct with current conditions.

List of Group 2, 3, and 4 Compounds: EPA 1694: 19 Analytes

Anhydrotetracycline (2)	Doxycycline (2)	Minocycline (2)	Triclocarban (3) Triclosan (3) Warfarin (3)
Chlorotetracycline (2)	4-Epianhydrotetracycline (2)	Tetracycline(2) Meclocycline (2)	Albuterol (4) Cimetidine (4) Metformin (4)
Demeclocycline(2)	4-Epitetracycline(2)	Gemfibrozil (3) Ibuprofen (3) Naproxen (3)	Ranitidine (4)

List of Labeled Internal Standards

$^{13}C_2$-^{15}N-Acetaminophen	$^{13}C_2$-Erythromycin	$^{13}C_6$-Sulfamethazine	$^{13}C_3$-Trimethoprim
$^{13}C_3$-Atrazine	Fluoxetine-d_6	$^{13}C_6$-Sulfamethoxazole	Warfarin-d_5
$^{13}C_3$-Caffeine	Gemfibrozil-d_6	$^{13}C_6$-2,4,5-Tricloro-phenoxyacetic acid	Carbamazepine-d_{10} (Extra compound, not EPA list)
$^{13}C_3$-^{15}N-Ciprofloxacin	$^{13}C_3$-Ibuprofen	$^{13}C_6$-Triclocarban	
Cotinine-d_3	^{13}C-Naproxen-d_3	$^{13}C_{12}$-Triclosan	

Experimental

Sample Preparation

Pharmaceutical analytical standards were purchased from Sigma, (St. Louis, MO). All stable isotope labeled compounds used as internal standards were obtained from Cambridge Isotope Laboratories (Andover, MA). Individual pharmaceutical stock solutions (approximately 1,000 µg/mL) were prepared in pure acetonitrile or methanol, depending on the solubility of each individual compound, and stored at –18 °C. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Water samples were collected from the wastewater treatment plant at the Boulder Creek outfall (Boulder, CO) and extracted as per the EPA method. Agilent has introduced a polymeric SPE sorbent with hydrophilic/lipophilic properties that may also be appropriate for this application. "Blank" wastewater extracts were used to prepare the matrix-matched standards for validation purposes. The wastewater extracts were spiked with the mix of pharmaceuticals at different concentrations (ranging from 0.1 to 500 ng/mL or ppb) and subsequently analyzed by LC/MS/MS.

LC/MS/MS Instrumentation

The analytes were subdivided in groups (according to EPA protocol for sample extraction) and LC conditions for the chromatographic separation of each group are as follows.

LC Conditions for Group 1-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2–0.3 mL/min
Gradient	t_0 = 10% ACN, 0.2 mL/min t_5 = 10% ACN, 0.2 mL/min t_6 = 10% ACN, 0.3 mL/min t_{24} = 60% ACN, 0.3 mL/min t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 2-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2 mL/min
Gradient	t_0 = 10% ACN t_{10} = 10% ACN t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 3-acidic extraction, negative electrospray ionization (ESI–) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	40% MeOH and 60% H_2O with 5 mM ammonium acetate, pH 5.5
Flow rate	0.2 mL/min
Gradient	$t_{0.5}$ = 40% MeOH t_7 = 100% MeOH
Injection volumes	15 µL

LC conditions for Group 4-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX HILIC Plus 2.1 × 100 mm, 3.5 µm (p/n 959793-901 custom order until November 1, 2008)
Column temperature	25 °C
Mobile phase	98% ACN and 2% H_2O with 10 mM ammonium acetate, pH 6.7
Flow rate	0.25 mL/min
Gradient	t_0 = 98% ACN t_5 = 70% ACN t_{12} = 70% ACN
Injection volumes	15 µL

The mass spectrometer conditions were general to all groups and are as follows.

MS Conditions

Mode	Positive and negative (depending on group) ESI using the Agilent G6410A Triple Quadrupole mass spectrometer
Nebulizer	40 psig
Drying gas flow	9 L/min
V capillary	4000 V
Drying gas temperature	300 °C
Fragmentor voltage	70–130 V
Collision energy	5–35 V
MRM	2 transitions for every compound as shown in Table 1
Dwell time	10 msec

Results and Discussion

Optimization of LC/MS/MS Conditions

The initial study consisted of two parts. First was to optimize the fragmentor voltage for each of the pharmaceuticals studied in order to produce the largest signal for the precursor ion. Typically the protonated molecule was used for the precursor ion. Each compound was analyzed separately using an automated procedure (MassHunter Optimizer software, Agilent Technologies, Santa Clara, CA) to check the fragmentor at each voltage. The data was then selected for optimal fragmentor signal and each compound was optimized again to determine automatically the collision energies for both the quantifying and qualifying ions. Optimal collision energies varied between 5 and 35 V. The MRM transitions and optimized energies used for this study are shown in Tables 2A to 2D.

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.)

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Acetaminophen	90	152 → 110 152 → 65	15 35
$^{13}C_2$-^{15}N-Acetaminophen	**90**	**155 → 111** **155 → 93**	**15** **25**
Ampicillin	70	350 → 160 350 → 106	10 15
$^{13}C_3$-Atrazine	**120**	**219 → 177** **219 → 98**	**15** **25**
Azithromycin	130	749.5 → 591.4 749.5 → 158	30 35
Caffeine	110	195 → 138 195 → 110	15 25
$^{13}C_3$-Caffeine	**110**	**198 → 140** **198 → 112**	**15** **25**
Carbadox	80	263 → 231 263 → 130	5 35
Carbamazepine	110	237 → 194 237 → 179	15 35
Carbamazepine-d_{10}	**110**	**247 → 204** **247 → 202**	**15** **35**
Cefotaxime	90	456 → 396 456 → 324	5 5
Ciprofloxacin	110	332 → 314 332 → 231	20 35
$^{13}C_3$-^{15}N-Ciprofloxacin	**110**	**336 → 318** **336 → 235**	**15** **35**

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Clarithromycin	110	748.5 → 158 748.5 → 590	25 15
Cloxacillin	90	436 → 160 436 → 277	15 15
Codeine	130	300 → 215 300 → 165	25 35
Cotinine	90	177 → 98 177 → 80	25 25
Cotinine-d_3	**90**	**180 → 80** **180 → 101**	**25** **25**
Dehydronifedipine	130	345 → 284 345 → 268	25 25
Digoxigenin	90	391 → 355 391 → 337	15 15
Digoxin	No response, Na adduct		
Diltiazem	130	415 → 178 415 → 150	25 25
1,7-Dimethylxanthine	90	181 → 124 181 → 99	15 15
Diphenhydramine	70	256 → 167 256 → 152	15 35
Enrofloxacin	130	360 → 316 360 → 342	15 15
Erythromycin	90	734.5 → 158 734.5 → 576	35 15
$^{13}C_2$-Erythromycin	**90**	**736.5 → 160** **736.5 → 578**	**25** **15**
Erythromycin anhydrate	90	716.5 → 158 716.5 → 116	25 25
Flumequine	90	262 → 174 262 → 244	35 15
Fluoxetine	90	310 → 148	5
Fluoxetine-d_6	**90**	**316 → 154**	**5**
Lincomycin	110	407 → 126 407 → 359	25 15
Lomefloxacin	130	352 → 308 352 → 265	15 25
Miconazole	90	415 → 159 415 → 69	35 25
Norfloxacin	70	320 → 302 320 → 276	15 15
Ofloxacin	110	362 → 318 362 → 261	15 25

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Oxacillin	70	402 → 160 402 → 243	15 5
Oxolinic acid	90	262 → 244 262 → 216	15 25
Penicillin G	90	335 → 160 335 → 176	5 5
Penicillin V	70	351 → 160 351 → 114	5 25
Roxithromycin	130	837.5 → 679 837.5 → 158	15 35
Sarafloxacin	130	386 → 299 386 → 368	25 25
Sulfachloropyridazine	90	285 → 156 285 → 92	10 25
Sulfadiazine	110	251 → 156 251 → 92	15 25
Sulfadimethoxine	80	311 → 156 311 → 92	20 35
Sulfamerazine	110	265 → 156 265 → 92	15 25
Sulfamethazine	90	279 → 156 279 → 186	15 15
$^{13}C_6$-Sulfamethazine	**90**	**285 → 186** **285 → 162**	**25** **25**
Sulfamethizole	80	271 → 156 271 → 92	10 25
Sulfamethoxazole	110	254 → 156 254 → 92	15 25
$^{13}C_6$-Sulfamethoxazole	**110**	**260 → 162** **260 → 98**	**15** **25**
Sulfanilamide	70	173 → 156 173 → 92	5 15
Thiabendazole	130	202 → 175 202 → 131	25 35
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	**110**	**259 → 201** **259 → 165**	**5** **25**
Trimethoprim	110	291 → 230 291 → 261	25 25
$^{13}C_3$-Trimethoprim	**110**	**294 → 233** **294 → 264**	**25** **25**
Tylosin	110	916.5 → 174 916.5 → 772	35 35
Virginiamycin	110	526 → 508 526 → 355	5 15

Table 2B. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 2

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Anhydrotetracycline	90	427 → 410 427 → 154	15 25
Chlorotetracycline	110	479 → 462 479 → 197	15 35
Demeclocycline	130	465 → 430 465 → 448	25 15
Doxycycline	110	445 → 428 445 → 154	15 25
4-Epianhydrotetracycline (EATC)	90	427 → 410 427 → 105	15 35
4-Epitetracycline (ETC)	110	445 → 410 445 → 427	15 5
Minocycline	90	458 → 441	15
Tetracycline (TC)	110	445 → 410 445 → 427	15 5

Table 2C. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 3

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Gemfibrozil	100	249 → 121	5
Gemfibrozil-d_6	**100**	**255 → 121**	**5**
Ibuprofen	75	205 → 161	5
$^{13}C_3$-Ibuprofen	**75**	**208 → 163**	**5**
Naproxen	75	229 → 169 229 → 170	25 5
^{13}C-Naproxen-d_3	**75**	**233 → 169** **233 → 170**	**25** **5**
Triclocarban	100	313 → 160 313 → 126	10 25
$^{13}C_6$-Triclocarban	**90**	**319 → 160** **319 → 132**	**5** **25**
Triclosan	75	287 → 35	5
$^{13}C_{12}$-Triclosan	**75**	**299 → 35**	**5**
Warfarin	125	307 → 117 307 → 161	35 15
Warfarin-d_5	**90**	**312 → 161** **312 → 255**	**15** **25**

Table 2D. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 4

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Albuterol (Salbutamol)	90	240 → 148	15
		240 → 166	5
Cimetidine	100	253 → 159	10
		253 → 95	25
Metformin	80	130 → 60	10
		130 → 71	25
Ranitidine	110	315 → 176	15
		315 → 130	25

Chromatographic separation was done independently for each group and a dwell time of 10 msec was used for every MRM transition. Figures 1A to 1D show the chromatograms corresponding to 100 ppb standard on column for all the pharmaceuticals studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective protonated molecule and product-ion MRM transitions.

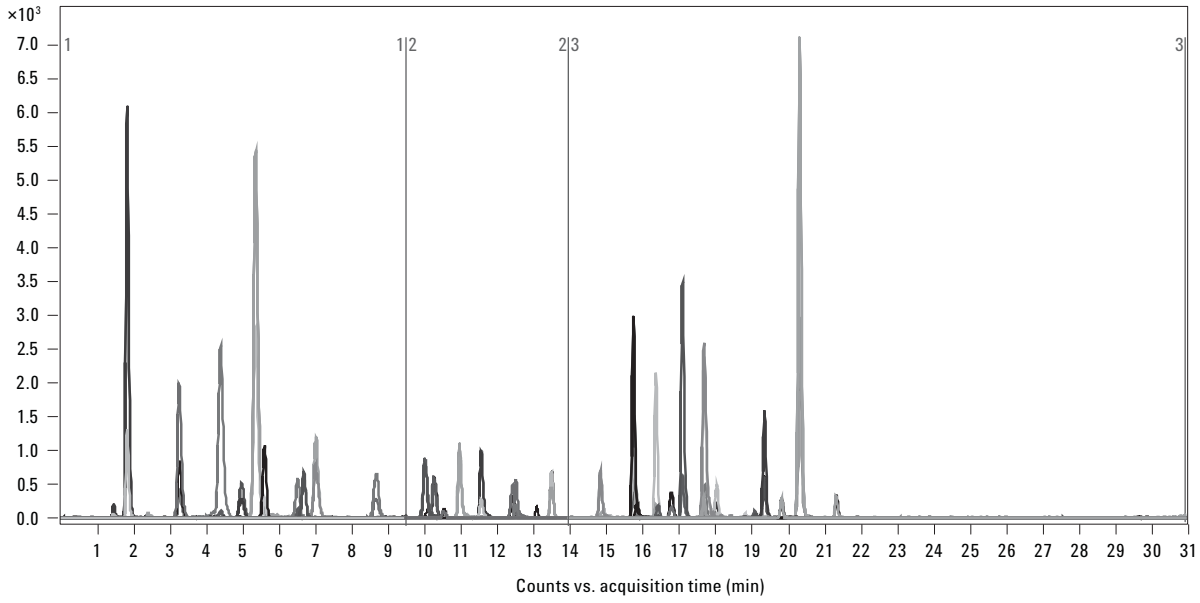

Figure 1A. MRM extracted chromatogram for pharmaceuticals in Group 1. Three time segments were used in this chromatographic separation.

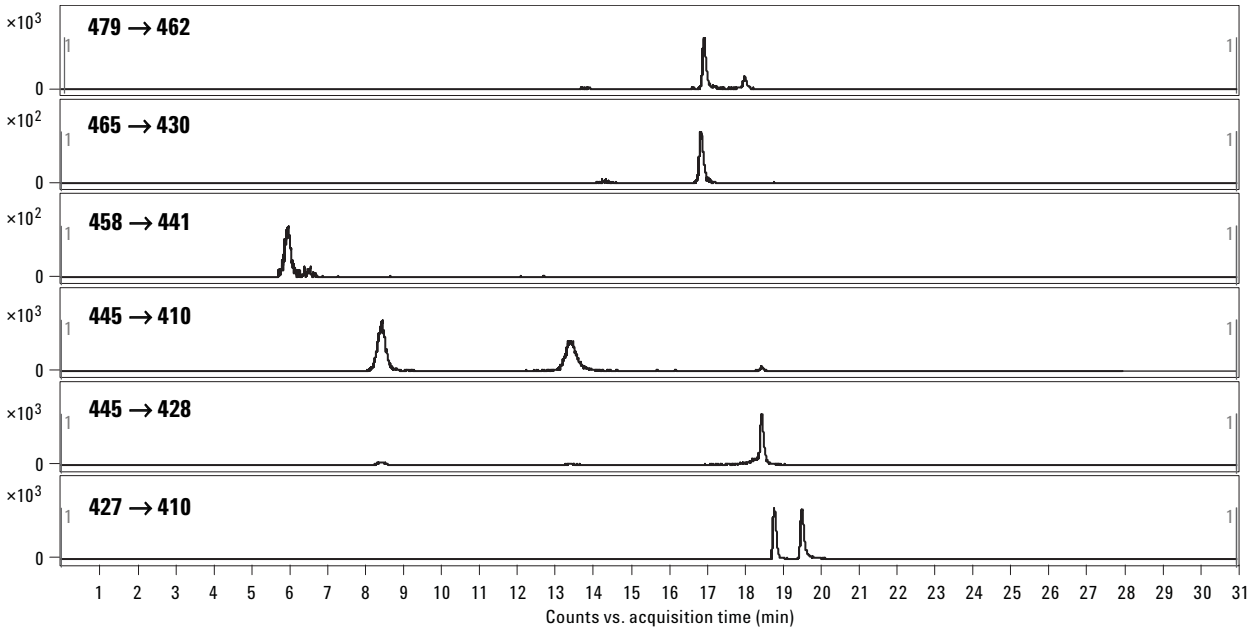

Figure 1B. MRM extracted chromatogram for pharmaceuticals in Group 2. Only one transition shown. See Table 2B for compound identification.

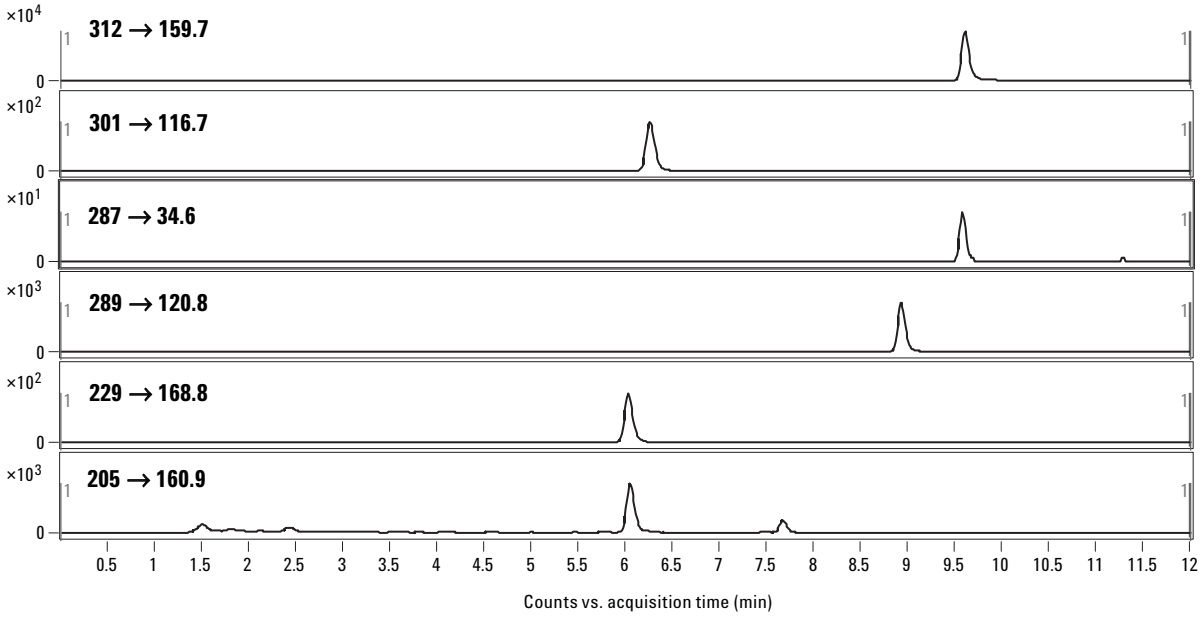

Figure 1C. MRM extracted chromatogram for pharmaceuticals in Group 3. Only one transition shown. See Table 2C for compound identification.

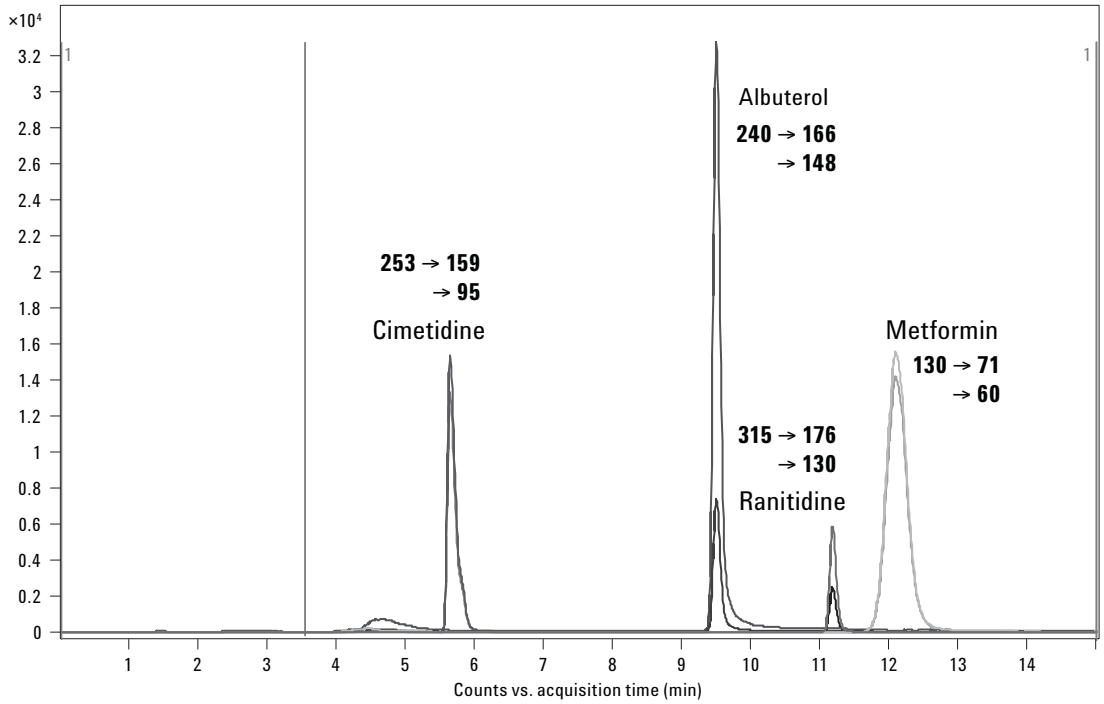

Figure 1D. MRM extracted chromatogram for pharmaceuticals in Group 4.

Application to Wastewater Samples

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were analyzed in a wastewater matrix from an effluent site, at eight concentrations (0.1, 0.5, 1, 5, 10, 50, 100, and 500 ng/mL or ppb concentrations). Figure 2 shows an example standard curve for acetaminophen in the wastewater matrix. In general, all compounds gave linear results with excellent sensitivity over three orders of magnitude, with r^2 values of 0.99 or greater.

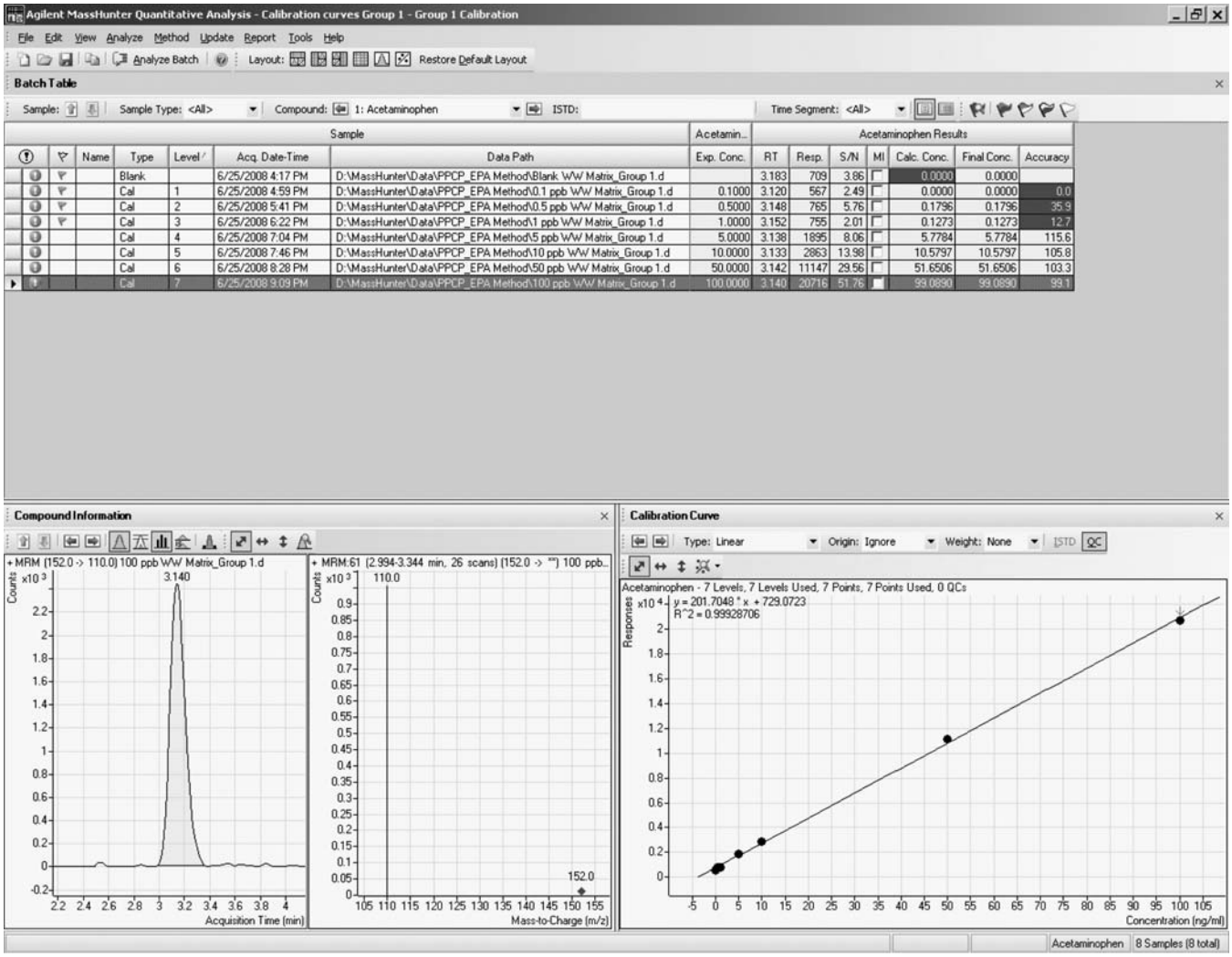

Figure 2. Calibration curve for acetaminophen in a wastewater matrix using a seven-point curve from 0.1 to 100 ng/mL (ppb) using a linear fit with no origin treatment.

Finally, a "blank" wastewater sample was analyzed and the presence of two pharmaceuticals, carbamazepine and diphenhydramine, could be confirmed with two MRM transitions. Figure 3 shows the ion ratios qualifying for these two compounds in a wastewater extract. As shown in Figure 3 in the two ion profiles, both pharmaceuticals were easily identified in this complex matrix due to the selectivity of the MRM transitions and instrument sensitivity.

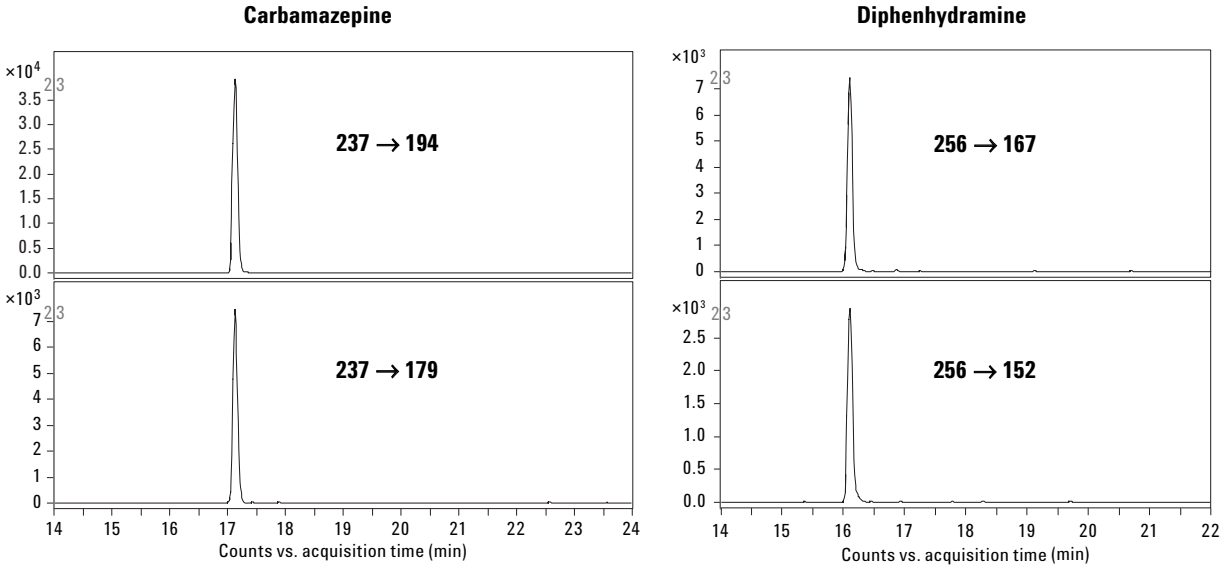

Figure 3. MRM chromatograms of a wastewater sample for carbamazepine and diphenhydramine using two transitions.

Conclusions

The results of this study show that the Agilent 6410A Triple Quadrupole is a robust, sensitive, and reliable instrument for the study of pharmaceuticals in water samples, using high throughput methods. The Agilent 6410A Triple Quadrupole has been shown to be a successful instrument for the implementation of EPA Method 1694.

References

1. EPA Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS, December 2007, EPA-821-R-08-002.

www.agilent.com/chem

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Agilent Technologies

Improving GC-MS Method Robustness and Cycle Times Using Capillary Flow Technology and Backflushing

Application Note

Environmental

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Abstract

This application note demonstrates the customer benefits from using Capillary Flow Technology to provide backflushing of high-boiling materials in GC and GC/MS analyses. Benefits include reduction in chromatographic cycle times, a reduction in system column maintenance, and extended GC column life. If a GC/MS system is utilized, the author has experienced an increase in the number of samples analyzed before ion source maintenance is required.

Agilent Technologies

Introduction

A critical component of the GC/MS analysis of any sample that contains large amounts of matrix material is the sample preparation. Environmental samples such as soils and sediments require not only extraction, but may also require multiple cleanup steps in order to present as clean an extract as possible for injection in to the GC/MS system.

Any remaining matrix in the sample extract can have deleterious effects on the GC sample inlet, column, and the ion source of the mass spectrometer. Traditionally, these high-boiling matrix materials are removed from the capillary column by a long bake-out period after the analytes of interest have eluted. This long bake-out process causes thermal stress to the column and also drives the matrix material towards the ion source, where it will eventually affect system performance. Moreover, should any material remain in the column after the bake-out process, it can cause loss of chromatographic peak shape and retention time shifting of target analytes. This shifting of retention time is particularly troublesome if the mass spectrometer is being used in the selected ion monitoring (SIM) mode (as with a single quadrupole GC/MS) or in the multiple reaction monitoring (MRM) mode (as with a triple quadrupole GC/MS).

This paper demonstrates how high-boiling matrix materials can be removed from the column quickly and effectively – between sample injections – by using capillary flow technology and capillary column backflushing.

Figure 1 shows a schematic diagram of the GC/MS system used. The 15-m analytical column was connected to the EPC split/splitless inlet and a capillary flow technology two-way splitter (p/n G3180B or G1540 option number 889).

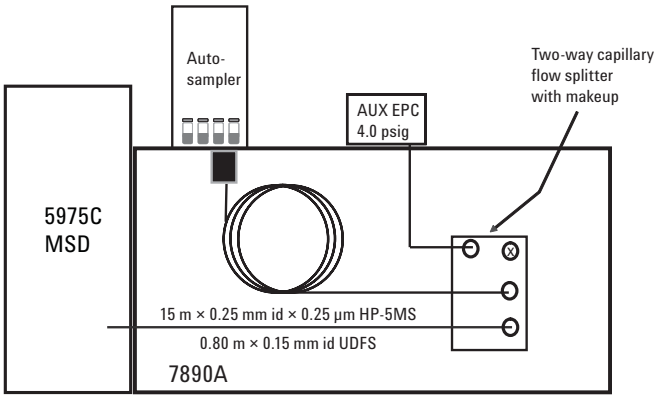

Figure 1. Schematic diagram of GC-MS system.

A short length of uncoated, deactivated fused silica (UDFS) capillary column is used as a restrictor between the splitter and the MS. Note carefully how the connections are made at the splitter. The X represents a port on the splitter plate that is closed off with a SilTite metal ferrule and stainless steel wire plug.

Backflushing in this example was accomplished during a post-run period by a combination of increasing oven temperature, reducing the inlet pressure of the analytical column, and increasing the pressure applied to the splitter plate.

Experimental

The full analytical conditions, both with and without post-run backflush set-points, are shown in Table 1.

Table 1. GC/MS Analysis Conditions

Gas chromatograph	Agilent 7890A
Columns	(1) 15.0 m × 0.25 µm id × 0.25 µm HP-5MS Ultra Inert (19091S-431SI) Inlet Front split/splitless, outlet 2-way Capillary Flow Device
	(2) 0.80 m × 0.15 mm id uncoated deactivated fused silica inlet two-way capillary flow device at 4.0 psig outlet vacuum
Carrier gas	Helium
Carrier gas mode	Constant pressure
Flow rate	17.18 psi
Injection port	EPC split/splitless
Autosampler	Agilent 7683A
Injection mode	Splitless, purge delay 0.5 min Purge flow 50.0 mL/min at 0.5 min
Injection volume	2.0 µL
Injection port liner	4 mm single-taper splitless liner (5181-3316)
Oven program °C (min)	70 (1) – 50 °C /min – 150 (0) 6 – 200 (0) – 16 – 280 (0) °C
Mass spectrometer	Agilent 5975C MSD
MS interface	280 °C
MS source	230 °C
MS quad 1	150 °C
Backflush conditions (1)	Post-run, 10 min, AUX 60 psig, oven 320 °C
Backflush conditions (2)	Post-run, 6 min, AUX 80 psig, oven 320 °C
Detection mode	EI full scan; mass range 40:550 amu
EI tune	Gain factor = 1

Results and Discussions

Experiment 1: No Backflushing Employed

In the first experiment, an extracted sediment sample was analyzed in full-scan mode to show the extent of the matrix problem. No backflushing was employed.

Before any sediment was injected, a system blank (no injection) followed by a 2-µL solvent blank was made. In the absence of the actual hexane solvent used to prepare the sediment extract, hexane that was not particularly clean was used. The TICs are shown overlaid in Figure 2, system blank in black, and solvent blank in gray. These chromatograms show that the system is free from high-boiling matrix material.

Following the blanks, a single injection of the sediment extract was made without backflushing; the TIC is shown in Figure 3. Note the very high abundance of the matrix and that when the analysis finishes, there is still a significant amount of matrix material to elute from the column.

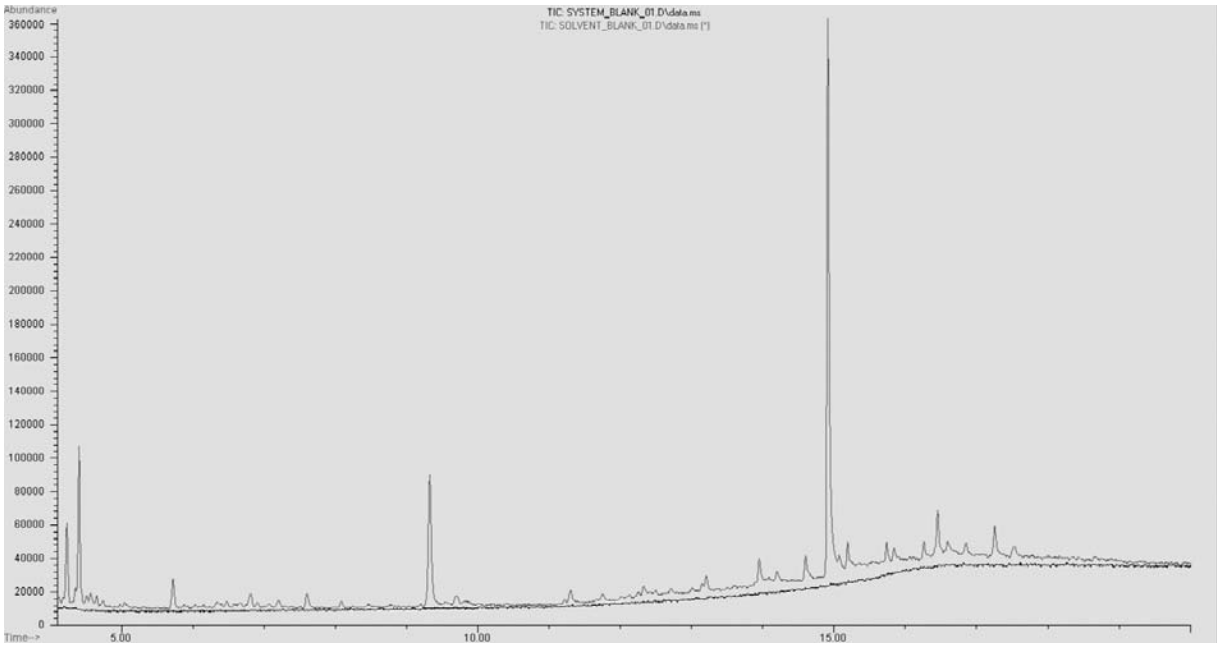

Figure 2. System blank and solvent blank TICs.

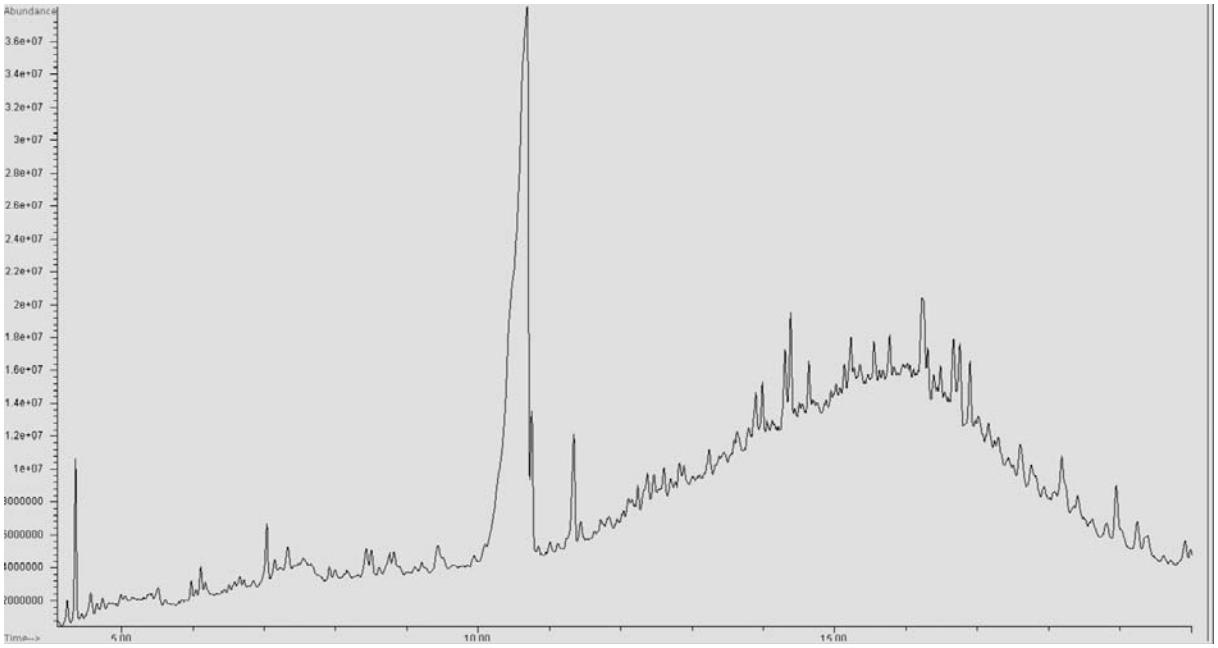

Figure 3. Sediment extract TIC.

The sediment extract injection was followed by a series of hexane blank injections. The first seven hexane blank TICs are shown overlaid in Figure 4 with the solvent blank before the sediment was injected into the GC/MS system.

Figure 5 shows that after the eighth solvent blank injection, the system has almost recovered to the level of background before the sediment sample was injected.

The original solvent blank TIC is shown in black, the eighth solvent blank TIC after the sediment injection is shown in gray.

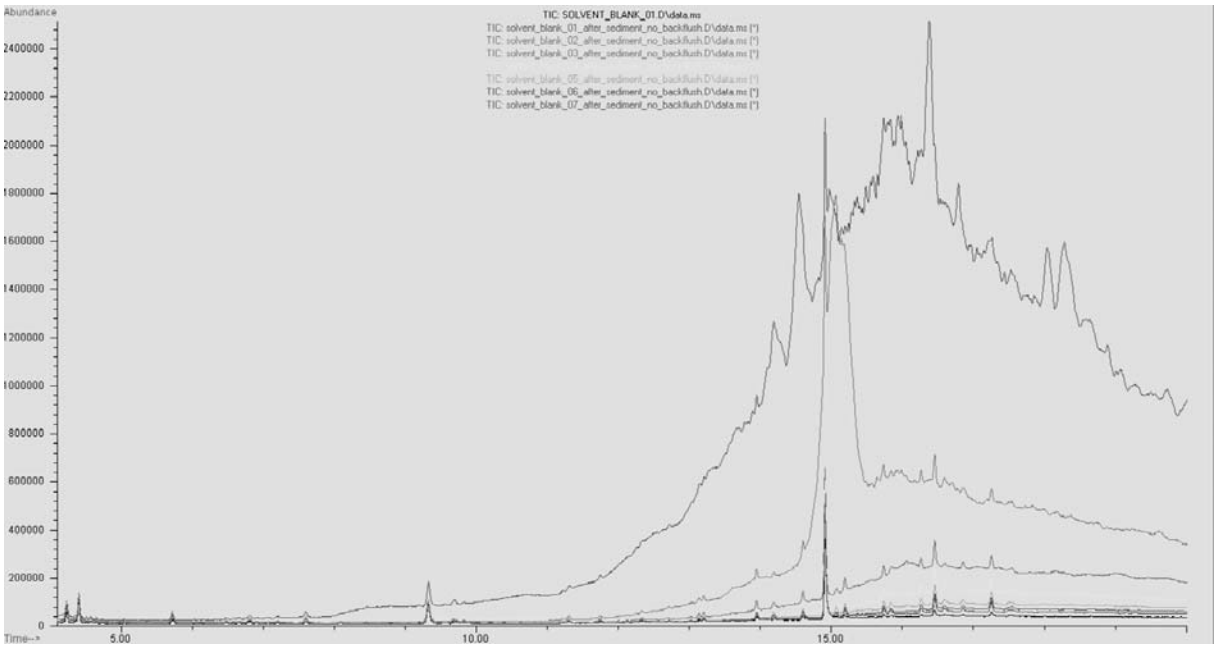

Figure 4. Successive solvent blank injections.

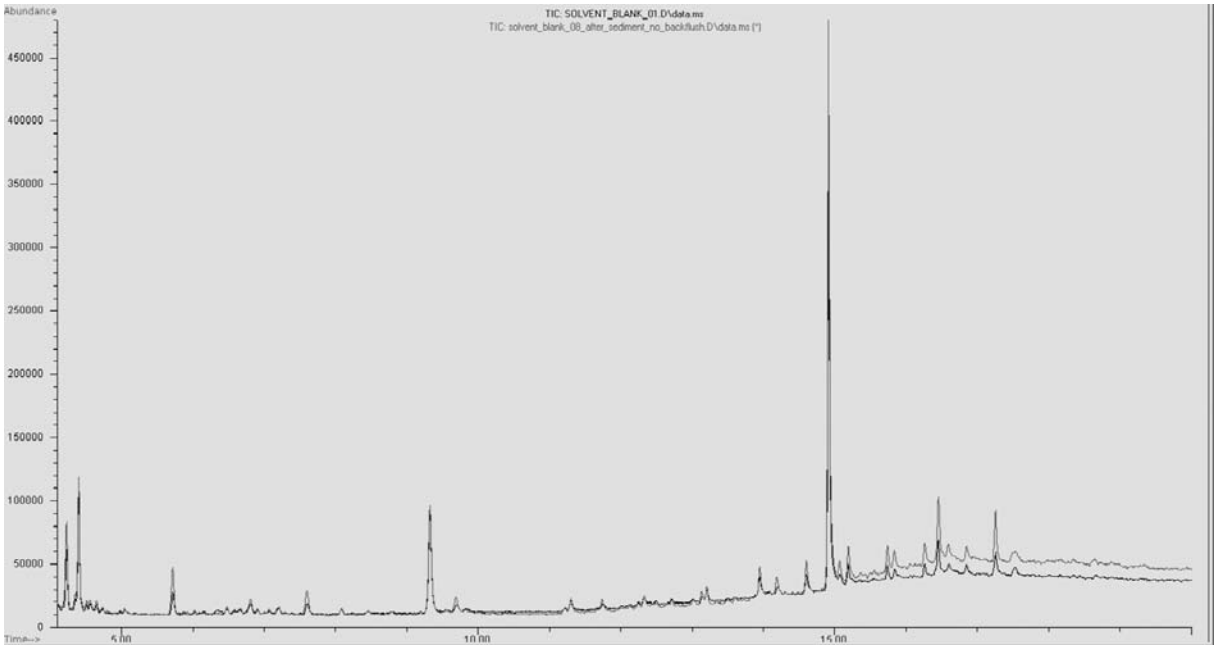

Figure 5. Eighth solvent blank and original solvent blank TICs

Experiment 2: Backflushing Employed

Backflushing was enabled during a post-run period by increasing column oven temperature, reducing the inlet pressure of the analytical column, and increasing the gas pressure applied to the splitter plate.

The 7890A instrument control software includes simple and easy-to-use screens to help set up post-run backflushing conditions. Figure 6 shows the configuration of columns and connections with the GC oven.

Figure 7 shows the actual backflushing conditions, namely the post-run oven temperature (320 °C), post-run inlet pressure for the analytical column (1 psig), post-run pressure applied to the splitter device (60 psig), and post-run time (10 minutes). The figure also shows the number of column-volumes of carrier gas that will backflush the analytical column.

Note that using the backflushing conditions shown in Figure 7 (320 °C, column pressure 1 psig, and splitter pressure 60 psig for 10 minutes), that 59.4 column volumes of carrier gas was used to backflush the column during the post-run period. This backflush time may have been more than necessary. Alternate conditions were also investigated and are presented later.

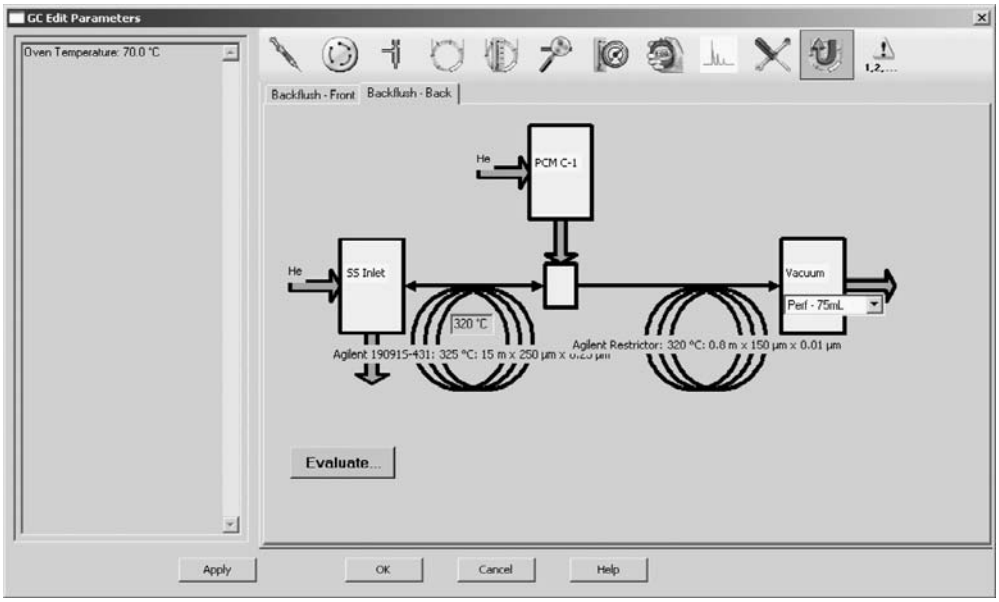

Figure 6. Post-run backflushing screen number 1.

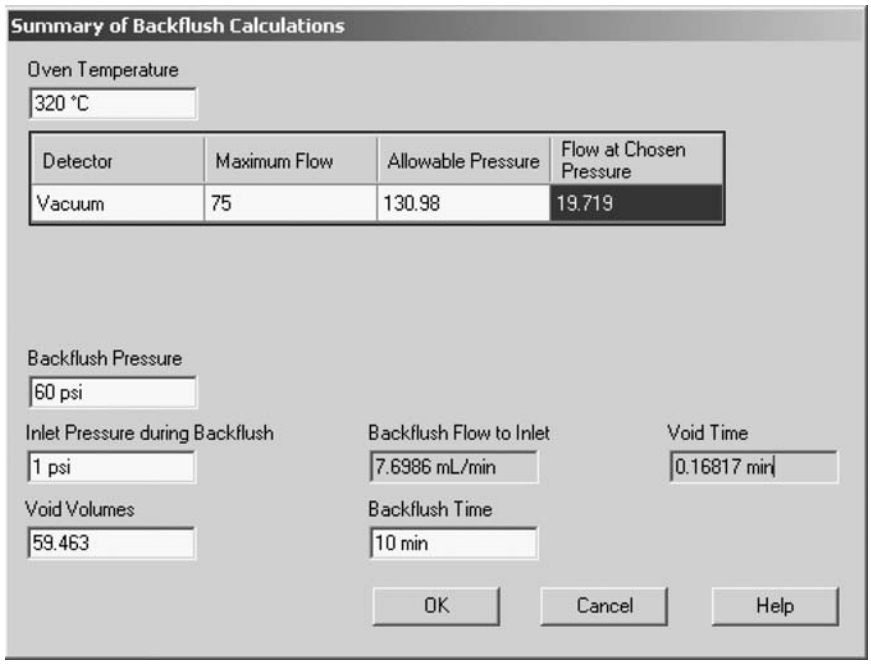

Figure 7. Post-run backflushing screen number 2.

Before applying the backflush conditions to the method the user is presented with a convenient summary of the back-flush conditions. See Figure 8.

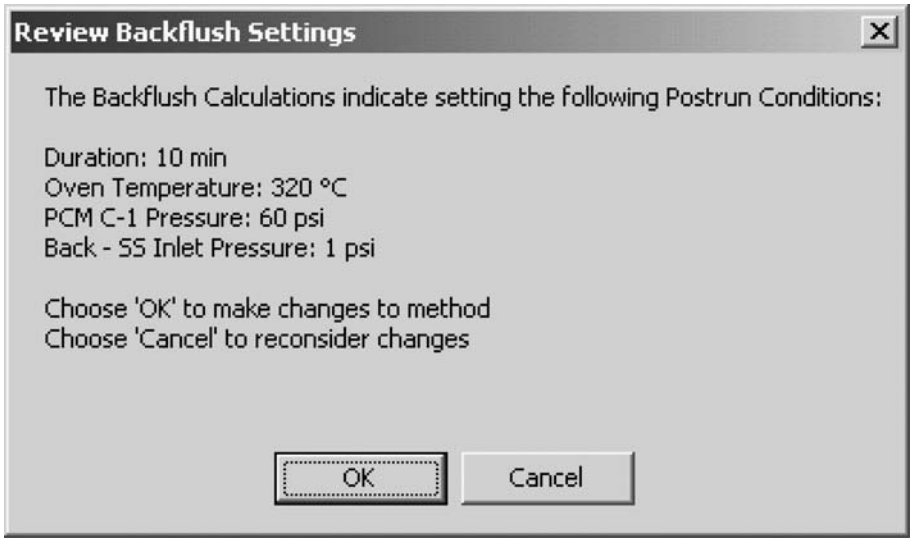

Figure 8. Post-run backflushing screen number 3.

Another injection of the sediment including backflush was made followed by a blank injection of solvent. Figure 9 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material had been effectively removed by backflushing.

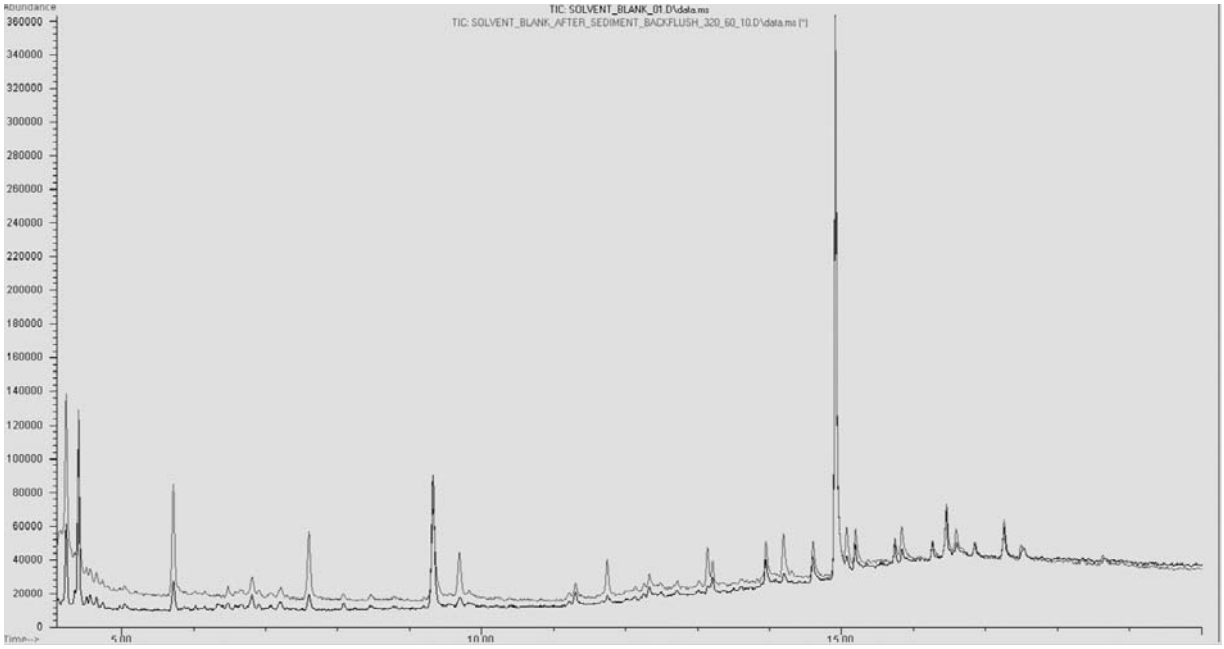

Figure 9. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (1).

Experiment 3: Backflushing Employed

In order to reduce cycle time for the method, the backflush conditions were modified by increasing the backflush pressure to 80 psig and holding for 6 minutes.

Note that using the backflushing conditions shown in Figure 10 (320 °C, column pressure 1 psig, and splitter pressure 80 psig for 6 minutes), that 46.6 column volumes of carrier gas was used to backflush the column during the post-run period.

Another injection of the sediment was made, followed by a blank injection of solvent. Figure 11 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material has been removed by backflushing with the more aggressive conditions as well. These conditions reduced the cycle time for this method 4 minutes compared to the backflushing conditions used in Experiment 1.

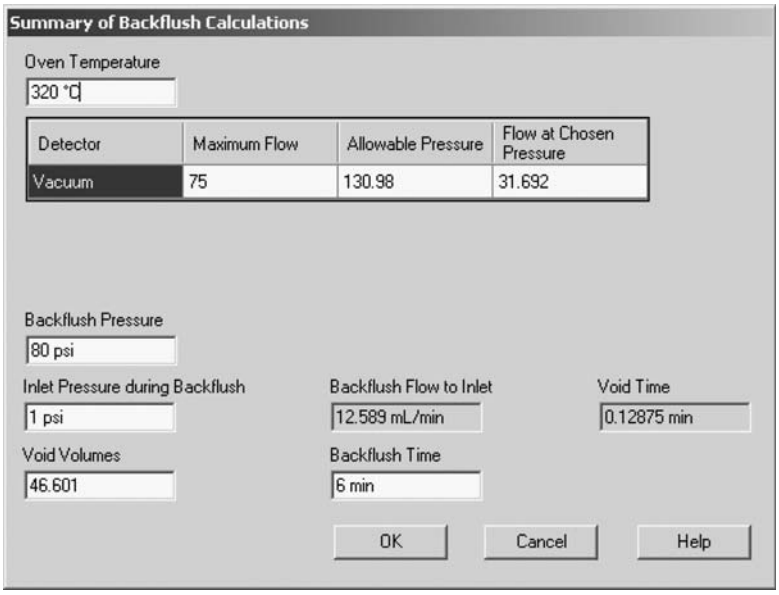

Figure 10. Post-run backflushing screen conditions number 2.

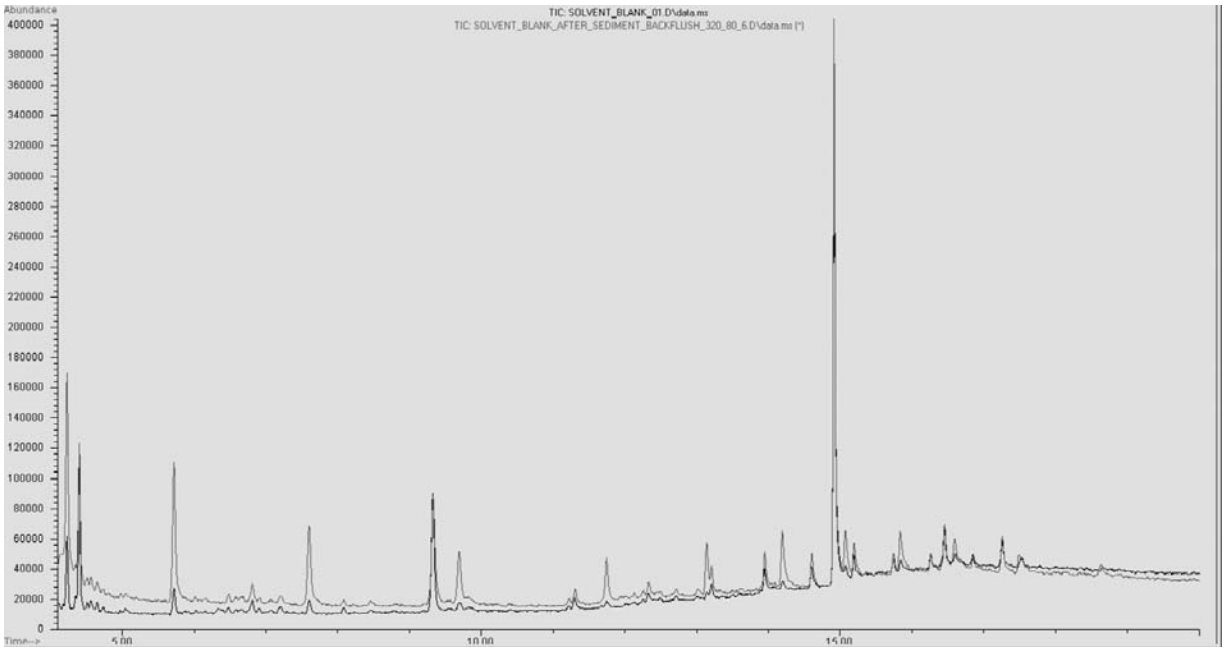

Figure 11. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (2).

Conclusions

Post-run backflushing was shown to effectively eliminate high-boiling sample matrix in a short amount of time. The major benefits of GC capillary column post-run backflushing include:

- Agilent's capillary flow technology and GC software enable easy and robust setup of GC backflushing.
- Compared to long bake-out periods with flow in the forward direction, a short period of backflushing can remove high-boiling matrix materials more effectively without contaminating the MS ion source.
- Chromatographic cycle time is reduced, columns stay clean, and the integrity of target analyte peak shapes and retention times are maintained.
- For this particular sediment extract the GC column was free of sample matrix after a backflush period of 6 minutes.
- Less system maintenance (ion source cleaning) is required.

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Semivolatile Organics Analysis Using an Agilent J&W HP-5ms Ultra Inert Capillary GC Column

Application Note

Environmental

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Abstract

Trace-level semivolatile organics analyses using methods such as USEPA Method 8270 are important tools for assessing environmental contaminants worldwide. The wide-ranging chemical diversity of target semivolatiles can prove chromatographically challenging. This application note demonstrates the benefits of using an Agilent J&W HP-5ms Ultra Inert Capillary GC column with electron impact single quadrupole scanning mass spectrometry for trace-level semivolatiles analysis.

Agilent Technologies has implemented new testing procedures to more effectively evaluate GC column inertness performance. These new testing procedures employ deliberately aggressive probes to thoroughly investigate column inertness and quality. These aggressive probes, including 1-propionic acid, 4-picoline, and trimethyl phosphate, are used to verify each column's inertness performance.

Agilent Technologies

Introduction

USEPA Method 8270 [1] is a commonly used method for detecting semivolatile organic compounds in environmental samples by GC/MS. This method encompasses several classes of analytes, including amines, alcohols, polycyclic aromatic hydrocarbons, and phenols. The acidic and basic nature of many of the analytes makes minimizing any column or instrument activity critical to good chromatography and reliable results.

Minimizing activity in the GC column is essential in maximizing an analyte's response. Nitrophenols are among the most active compounds in semivolatiles series. 2,4-Dinitrophenol in particular is notorious for showing low response through adsorption onto active sites in the flow path during analysis. At low concentrations, the response factor (RF) for 2,4-dinitrophenol can fall below the minimum average RF of 0.050 required by USEPA 8270 due to interaction between the analyte and sample flow path. Capillary GC column activity as a potential source of result uncertainty has been effectively eliminated with the Ultra Inert series of columns.

A custom standard containing an abbreviated list of analytes specific to USEPA Method 8270 was analyzed to evaluate column performance. This semivolatiles "short mix" contained n-nitrosodimethylamine, aniline, benzoic acid, 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol, 4-aminobiphenyl, benzidine, 3,3'dichlorobenzidine, benzo[b]fluoranthene, and benzo[k]fluoranthene, along with the recommended internal standards. These target analytes were chosen based on their chemical activity, as well as their poor chromatographic behavior. The short mix is particularly useful for rapid evaluation of system performance for semivolatiles analysis. Challenging analytes from early-eluting nitrosoamines through late-eluting PAHs are represented in this mix and chromatographic performance can be assessed quickly.

A second "large mix" standard containing a broader selection of semivolatiles was also evaluated to show the Ultra Inert's performance when analyzing a more complex sample. This standard contained a variety of acidic, basic, and neutral groups, which ranged from very low-boiling components to high-boiling polycyclic aromatic hydrocarbons.

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions for EPA Method 8270 Calibration Standards

GC:	Agilent 6890N/5975B MSD
Sampler:	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0 µL splitless injection
Carrier:	Helium 30 cm/s, constant flow
Inlet:	Splitless; 260 °C, purge flow 50 mL/min at 0.5 min
	Gas saver 80 mL/min at 3 min
Inlet liner:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column:	Agilent HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 19091S-433UI)
Oven:	40 °C (1 min) to 100 °C (15 °C/min), 10 °C/min to 210 °C (1 min), 5 °C/min to 310 °C, hold 8 min
Detection:	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 290 °C, scan range 45 to 450 amu

Table 2. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial caps:	Blue screw caps (Agilent p/n 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier:	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

A 12-component custom semivolatiles mix was purchased from Ultra Scientific (Kingston, RI) and used to prepare a seven-level calibration standard set. The stock semivolatiles solution as delivered had a nominal concentration of 2,000 µg/mL. An internal standard mix as recommended by USEPA Method 8270 was purchased from AccuStandard (New Haven, CT). The internal/surrogate solution as delivered had a

nominal concentration of 4,000 µg/mL. The calibration standards were prepared with component and internal standard concentrations of 80, 40, 20, 10, 5, 2, and 1 µg/mL. All solutions were prepared in dichloromethane using class A volumetric pipettes and flasks. The dichloromethane used was Burdick and Jackson spectral grade purchased thorough VWR International (West Chester, PA). Dichloromethane was used as a reagent blank and syringe wash solvent.

The EPA 8270 Calibration Level 2 standard set was purchased from AccuStandard containing 83 semivolatile components and internal standards. The large mix calibration standard was prepared at an analyte concentration of 5 µg/mL.

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with aggressive active probes at low concentration and low temperature. This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level like the semivolatiles in this application example. A more detailed description of the test mix and additional application examples can be found in references 2 through 7.

Semivolatiles Analysis (USEPA 8270)

In this application note a seven-level semivolatile calibration curve set was evaluated over the concentration range of 1 to 80 µg/mL on an Agilent J&W Ultra Inert HP-5ms 30 m × 0.25 mm × 0.25 µm (p/n 19091S-433UI). An example chromatogram of a 1-µL injection of the 1 µg/mL short mix calibration standard is shown in Figure 1. Scanning mode was used exclusively for this analysis.

Pentachlorophenol and benzidine are two components that are used to verify inlet and column inertness. Excessive peak tailing of these components would indicate column activity. Analysis of the short mix standard yielded sharp, symmetrical peak shapes for the problematic analytes as shown in Figure 2. Good separation was obtained in the analysis of the 5-ng on-column 8270 large mix standard for each of the semivolatiles, which is shown in Figure 3.

Semivolatile analysis by USEPA Method 8270 requires a minimum average RF of 0.050 for a system performance check compound such as 2,4-dinitrophenol. 2,4-Dinitrophenol is a highly active analyte that has proven to be one of the most challenging compounds, often yielding lower than expected

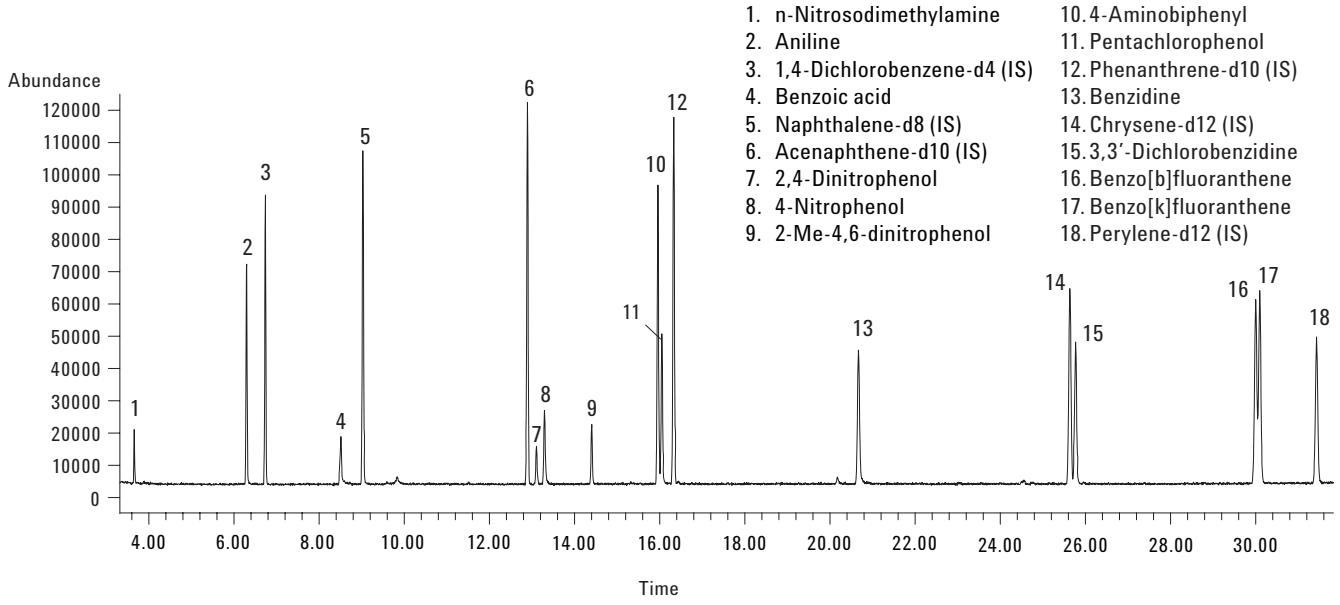

Figure 1. Total ion chromatogram (SCAN mode) of the 1-ng on-column EPA8270 short mix standard solution loading on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). Chromatographic conditions are listed in Table 1.

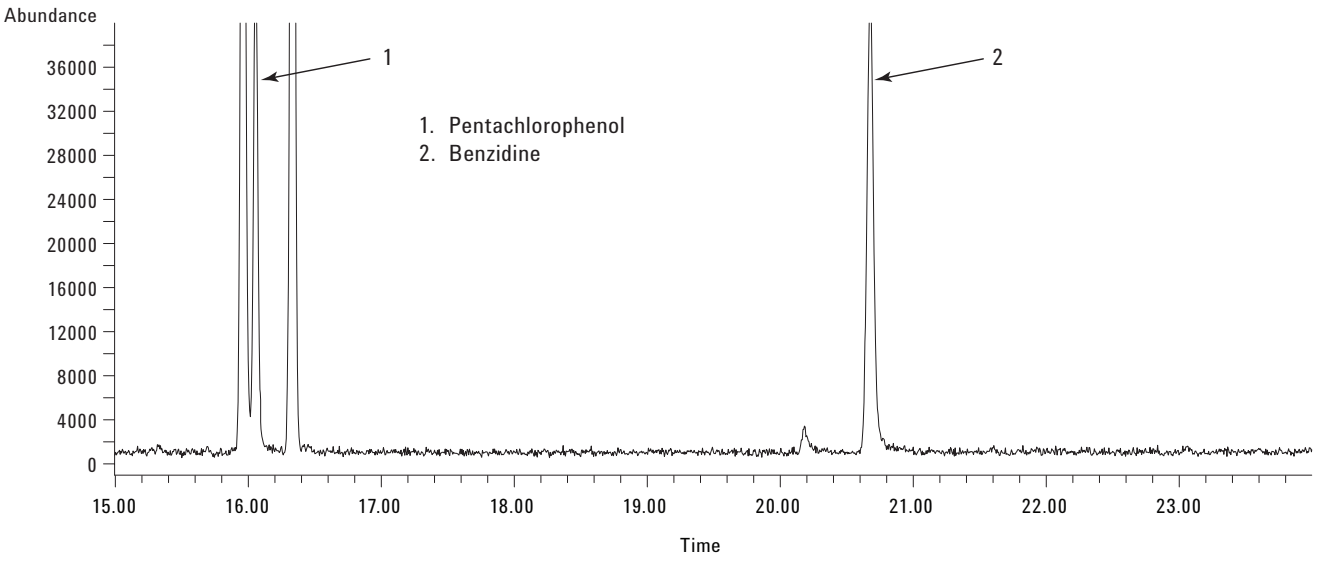

Figure 2. Enlarged section of the total ion chromatogram for a 1-µL injection of 1.0 µg/mL EPA 8270 short mix standard. The peaks of interest noted in the figure are two semivolatiles that are prone to peak tailing. Chromatographic conditions are listed in Table 1.

1. n-Nitrosodimethylamine
2. Pyridine
3. 2-Fluorophenol
4. Phenol-d5
5. Phenol
6. Aniline
7. Bis(2-chloroethyl)ether
8. 2-Chlorophenol
9. 1,3-Dichlorobenzene
10. 1,4-Dichlorobenzene-D4
11. 1,4-Dichlorobenzene
12. Benzyl alcohol
13. 1,2-Dichlorobenzene
14. o-Cresol
15. Bis(2-chloroisopropyl)ether
16. p-Cresol
17. n-Nitroso-di-n-propylamine
18. Hexachloroethane
19. Nitrobenzene-d5
20. Nitrobenzene
21. Isophorone
22. 2-Nitrophenol
23. 2,4-Dimethylphenol
24. Benzoic acid
25. Bis(2-chloroethoxy)methane
26. 2,4-Dichlorophenol
27. 1,2,4-Trichlorobenzene
28. Naphthalene-d8
29. Naphthalene
30. 4-Chloroaniline
31. Hexachlorobutadiene
32. 4-Chloro-3-methylphenol
33. 2-Methylnaphthalene
34. Hexchlorocyclopentadiene
35. 2,4,6-Trichlorophenol
36. 2,4,5-Trichlorophenol
37. 2-Fluorobiphenyl
38. 2-Chloronaphthalene
39. 2-Nitroaniline
40. Dimethyl phthalate
41. 2,6-Dinitrotoluene
42. Acenaphthylene
43. 3-Nitroaniline
44. Acenaphthene-d10
45. Acenaphthene
46. 2,4-Dinitrophenol
47. 4-Nitrophenol
48. Dibenzofuran
49. 2,4-Dinitrotoluene
50. Diethyl phthalate
51. Fluorene
52. 4-Chlorophenyl phenyl ether
53. 4-Nitroaniline
54. 2-Me-4,6-dinitrophenol
55. n-Nitrosodiphenylamine
56. Azobenzene
57. 2,4,6-Tribromophenol
58. 4-Bromophenyl phenyl ether
59. Hexachlorobenzene
60. Pentachlorophenol
61. Phenanthrene-d10
62. Phenanthrene
63. Anthracene
64. Carbazole
65. Dibutylphthalate
66. Fluoranthene
67. Benzidine
68. Pyrene
69. p-Terphenyl-d14
70. Benzyl butyl phthalate
71. 3,3-Dichlorobenzidine
72. Benzo[a]anthracene
73. Chrysene-D12
74. Chrysene
75. Bis(2-ethylhexyl)phthalate
76. Di-n-octyl phthalate
77. Benzo[b]fluoranthene
78. Benzo[k]fluoranthene
79. Benzo[a]pyrene
80. Perylene-d12
81. Indeno[1,2,3,cd]pyrene
82. Dibenzo[a,h]anthracene
83. Benzo[g,h,i]perylene

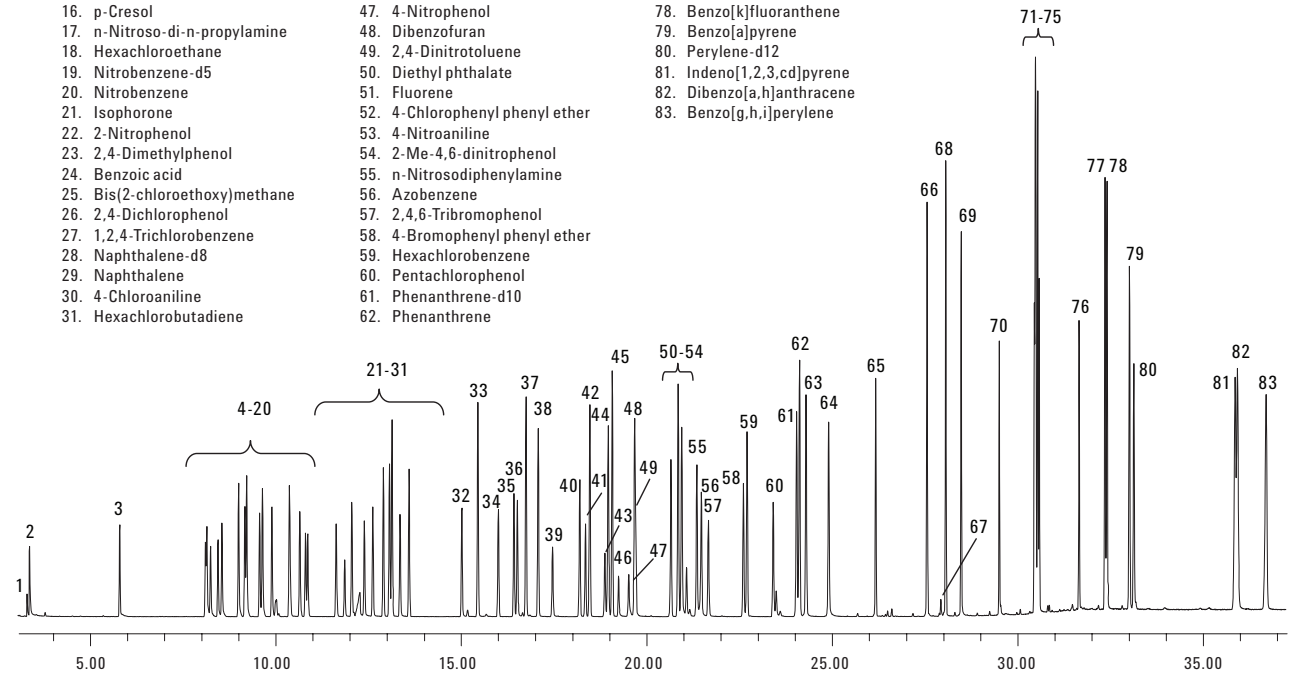

Figure 3. Total ion chromatogram (SCAN mode) of 5-ng on-column loading of EPA 8270 calibration (large mix) standard solution on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). Chromatographic conditions are listed in Table 1.

response factors at lower concentrations. In the analysis of the short mix calibration standard, the response for 2,4-dinitrophenol was greater than 0.1 at the 1-ng level. The average response was 0.15 over the concentration range studied. An example chromatogram for the signal-to-noise ratio for a 1-ng on-column loading of 2,4-dinitrophenol is shown in Figure 4. The signal-to-noise ratio for this difficult analyte was greater than 16 to 1. This demonstrates the excellent performance of the HP-5ms Ultra Inert GC column.

Linearity was excellent across the range studied, giving R^2 values of 0.990 or greater for even the more difficult phenols. Figure 5 indicates the correlation coefficients for several of the more active analytes.

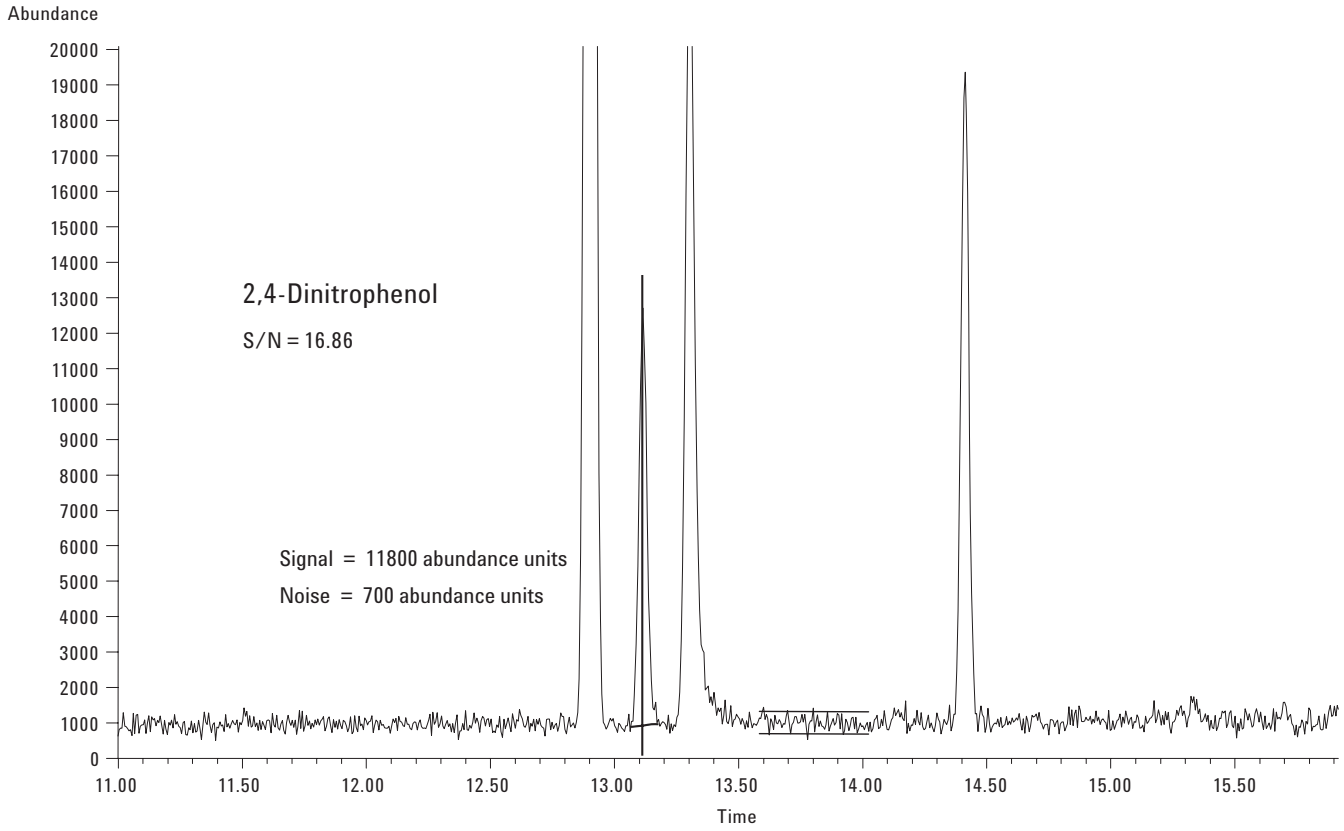

Figure 4 . Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1 µg/mL EPA Method 8270 short mix standard on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). The peak in the figure is 2,4-dinitrophenol, one of the more demanding semivolatiles. This injection represents an on-column loading of 1 ng per component. Chromatographic conditions are listed in Table 1.

	R^2
n-Nitrosodimethylamine	0.995
2,4-Dinitrophenol	0.990
4-Nitrophenol	0.995
Pentachlorophenol	0.995
Benzidine	0.995

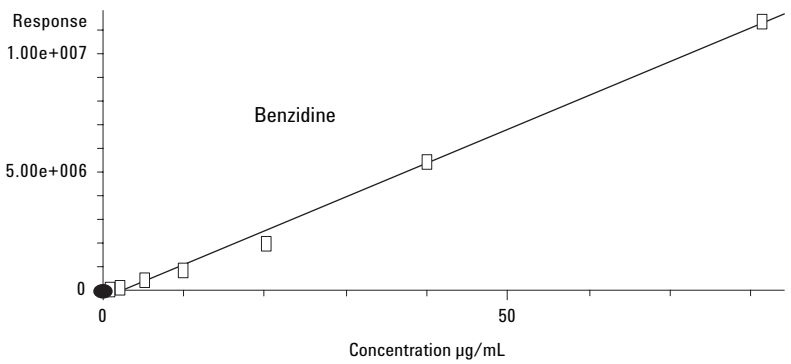

Figure 5. Correlation coefficients for some of the more challenging analytes in the EPA Method 8270 short mix standard over the 1 to 80 µg/mL range of this study and an example linear regression plot for benzidine.

Conclusions

This application successfully demonstrates the use of an Agilent J&W HP-5ms Ultra Inert capillary GC column for low-level semivolatile organics. Linearity was excellent for all semivolatiles studied, yielding 0.99 or greater R^2 values down to a 1-ng column loading of each component. One of the reasons for excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for semivolatiles analyses.

This study was done using SCAN mode on an Agilent 6890N/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 1-ng on-column loading of 2,4-dinitrophenol was greater than 16 to 1 with this system. This result clearly shows the power of using an Agilent J&W HP-5ms Ultra Inert column for low-level semivolatile organics analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890A/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W HP-5ms Ultra Inert GC capillary column.

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Agilent Technologies

Fast Analysis of Polynuclear Aromatic Hydrocarbons Using Agilent Low Thermal Mass (LTM) GC/MS and Capillary Flow Technology QuickSwap for Backflush

Application Note

Environmental

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Abstract

Cycle time for GC-MS analysis of polynuclear aromatic hydrocarbons (PAHs) in soil and sediment samples was improved dramatically through the combined use of a narrow-bore (180-µm id) column installed in an LTM module and capillary column backflushing with QuickSwap. Improvements were achieved while maintaining resolution. Agilent Capillary Flow Technology enabled column backflushing to remove low-volatility material from the capillary column, reducing bakeout and maintenance time. The method resulted in increased sample throughput and lab productivity, while maintaining analytical performance.

Agilent Technologies

Introduction

The analysis of polycyclic (polynuclear) aromatic hydrocarbons (PAHs) by GC-MS is one of the most important applications in environmental analysis. In most reference methods, 16 target PAHs of particular toxicity and carcinogenicity are monitored, ranging from naphthalene (eluting first, MW = 128) to benzo(ghi)perylene (eluting last, MW = 276). Typically, PAH analyses are performed with a 30 m × 0.25 mm id × 0.25 µm 5% phenyldimethyl siloxane columns (for example, Agilent J&W HP-5MS or J&W DB-5MS) using a temperature program from 40–50 °C to 300–320 °C in 20 to 30 minutes of analysis time. The analysis of PAHs, therefore, covers the whole boiling point range of semivolatiles as typically covered in U.S. EPA Methods 525, 625, and 8270. These methods include compounds such as polychlorinated biphenyls (PCBs), phthalates, phenols, and most GC-amenable pesticides (for example, organochlorine, triazines, and organophosphorous). Any combination of these compounds is potentially present in PAH samples, in addition to hydrocarbon fuels and oils, complicating analysis of target PAHs. The use of mass spectrometers is required to facilitate reliable identification and quantitation.

Most labs are interested in maximizing lab efficiency and output. To this end, decreasing method analysis time and increasing sample throughput is often of interest. For this, columns of smaller diameter in combination with fast oven programming are an important option. However, a key challenge in PAH analysis is sufficient separation of key isomers (for example, benzo(b)fluoranthene and benzo(k)fluoranthene) since mass spectrometry is not able to differentiate them. Therefore, these isomers should be chromatographically separated, which requires columns of sufficient separation power and some careful choice in column flow and temperature ramp rates. Hence, it is important to use a tool like method translation software [1] to scale reference conditions appropriately.

Higher molecular-weight PAHs are potentially sensitive to cold spots and adsorption in the sample flow path. Problems with column connections, transfer line, source temperature, or activity in the inlet or column rapidly lead to peak tailing and loss of sensitivity. Although the compounds are considered apolar, PAH analysts find that frequent inlet, column, and source maintenance are needed when analyzing dirty samples (for example, sediment extract). A high source temperature (for example, ≥ 300 °C) and capillary column backflushing can greatly reduce the need for maintenance, thereby improving data quality and further increasing lab productivity.

In this application note, translation of a standard PAH method to a fast method using a 180-µm id column is demonstrated. To maximize sample throughput, fast column heating and cooling were further improved by using a low thermal mass (LTM) oven module. An Agilent Capillary Flow Technology QuickSwap capillary flow module was chosen for its ability to improve column replacement and system maintenance time, while providing the means to backflush the capillary column after the elution of the last PAH. As a result, low-volatility matrix compounds were efficiently removed, and maintenance interval greatly increased.

Experimental

Solutes and Sample

Tests were performed using a PAH standard mixture containing 16 PAHs in dichloromethane. The test mixture was obtained by dilution of a mixture in CH_2Cl_2/benzene (for example, Supelco, Bellefonte, PA, USA, cat no 48905, 2000 mg/mL) to 1 ng/µL (1 ppm) in dichloromethane.

A soil sample contaminated with mineral oil and PAHs was obtained from an environmental laboratory. 1 g of the soil was extracted in 20 mL dichloromethane (30 min ultrasonic treatment). This extract was filtered and analyzed directly as is typical in most high-volume environmental laboratories. The mineral oil concentration in the sample was approximately 5 g/kg. The concentrations of the PAHs were around 150 mg/kg for pyrene and fluoranthene and 5 to 10 mg/kg for the higher molecular-weight PAHs (for example, 9 mg/kg for benzo(a)pyrene). In the extract, the concentrations are 20 times lower (for example, 0.45 ng/µL for benzo(a)pyrene, assuming 100% extraction efficiency).

A sewage sludge sample (BCR-088, IRMM, Geel, Belgium) was also analyzed. 1 g of sludge was extracted in 20 mL of dichloromethane. The extract was filtered and analyzed directly. The sample contained concentrations of approximately 1 mg/kg of the higher molecular-weight PAHs (0.05 ng/µL benzo(a)pyrene in extract).

GC-MS Conditions

Analyses were performed on an Agilent 7890A GC – Agilent 5975C Series MSD system. The GC was equipped with an S/SI inlet, a QuickSwap device using a 17 cm × 110 µm id deactivated fused silica restrictor (G3185-60363), an AUX EPC module, and a LTM column module. The system configuration is diagrammed in Figure 1.

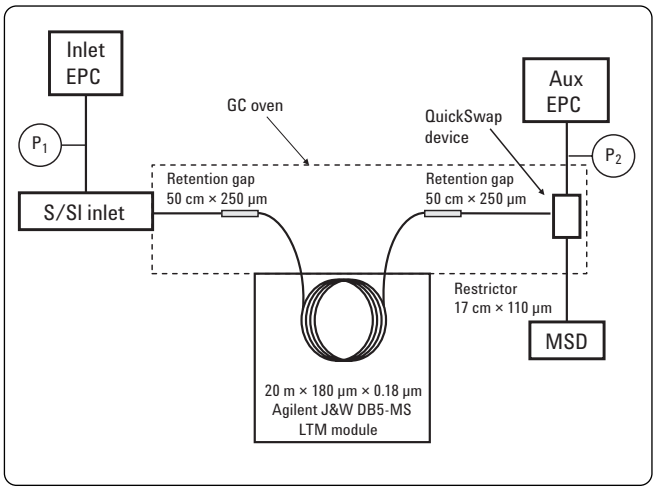

Figure 1. System configuration for fast GC analysis of PAHs using an LTM module and QuickSwap device for backflush.

Reference analyses were performed on a 30 m × 0.25 mm id × 0.25 µm Agilent J&W DB-5MS column, installed in the GC oven. For the fast method, a 20 m × 0.18 mm id × 0.18 µm J&W DB-5MS was used in LTM column format (p/n 121-5522LTM). Two pieces of 50 cm × 250 µm id deactivated fused silica tubing were used to connect the S/SI inlet to the column and from the column to the Quick-Swap device.

The final conditions for the 180-µm id column with backflushing are listed in Table 1.

Table 1. GC-MS Setpoints for Fast PAH Analysis Using a Low Thermal Mass Oven and QuickSwap Device for Backflush

Injection	1 µL, splitless mode, 280 °C
S/SI pressure program (He) (column inlet)	213 kPa (0.9 min), 60 kPa/min → 351 kPa, 7.5 kPa/min → 392 kPa (1.5 min), 600 kPa/min → 10 kPa (6 min)
AUX 1 (QuickSwap device, He) (column outlet)	28 kPa (10 min), 600 kPa/min → 250 kPa (6 min)
GC oven temperature	300 °C isothermal (17 min)
LTM oven program (translation of reference)	40 °C (0.9 min), 87 °C/min → 240 °C, 11 °C/min → 300 °C (8.35 min)
MS	SIM/Scan mode
Scan	50 to 300 *m/z*, samples = 2[1]
SIM	See Table 2
Transfer line	280 °C
Solvent delay	2.70 min
MS temperatures	Source = 300 °C, Quad = 150 °C
Run table events	MS off at 10 min

Table 2. SIM Table

Group	Start time (min)	Ion (*m/z*)	Dwell time (msec)
1	2.70	128	50
2	3.00	152, 153, 154, 165, 166	25
3	3.65	178	50
4	4.20	202	50
5	5.00	228	50
6	6.50	252	50
7	8.50	276, 278	50

Results and Discussion

For reference, a mixture containing 16 PAHs at 1 ppm in dichloromethane was analyzed using the typical 30 m × 0.25 mm id × 0.25 µm J&W DB-5MS column. The column temperature was programmed from 40 °C (2 min) at 40 °C/min to 240 °C and then at 5 °C/min to 300 °C (11 min hold). A low initial temperature is typically applied when a low-boiling solvent such as dichloromethane is used in order to allow recondensation (solvent focusing) in the column. Higher initial temperatures can be used if higher-boiling solvents are used. With higher initial temperatures, both the run time and the cool-down time of the GC are reduced; however, method translations are only valid with the same starting temperature used in the reference method.

The reference chromatogram of the standard mixture is shown in Figure 2. The critical pair benzo(b)fluoranthene/benzo(k)fluoranthene (peaks 11 and 12), eluted at 15 minutes, with sufficient resolution for quantification. The last solute, benzo(ghi)perylene, eluted at 19.7 minutes.

Next, the method was translated using GC Method Translation software [1] for the 20 m × 180 µm id × 0.18 µm column. Since separation power scales with length/diameter, the separation power of this column is very similar to that of the reference column. Through method translation, the inlet pressure and oven temperature program were scaled for the new column in fast analysis mode (one of the possible presets in the software). The resulting conditions are listed in Table 1. The predicted speed gain was a factor 2.17.

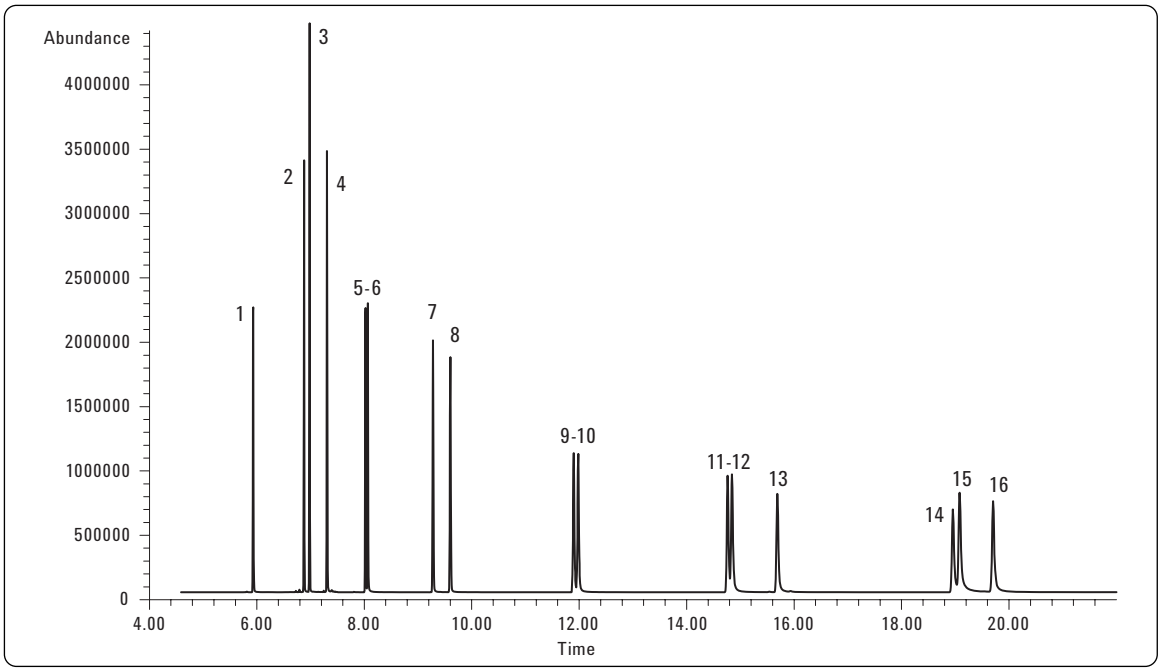

Figure 2. Reference total ion chromatogram for conventional system configuration and method conditions. Peak identities are listed in Table 3.

The resulting chromatogram is shown in Figure 3. Comparing this chromatogram with the one in Figure 2, one can see that very similar resolution is obtained in about half of the retention time. Benzo(ghi)perylene elutes at 9.3 minutes, which corresponds well to the predicted retention time of 9.1 minutes (19.7/2.17). As expected, the resolution of benzo(b)fluoranthene and benzo(k)fluoranthene was similar to the separation obtained on the standard column. Resolution was maintained, while analysis time was reduced by a factor of 2.

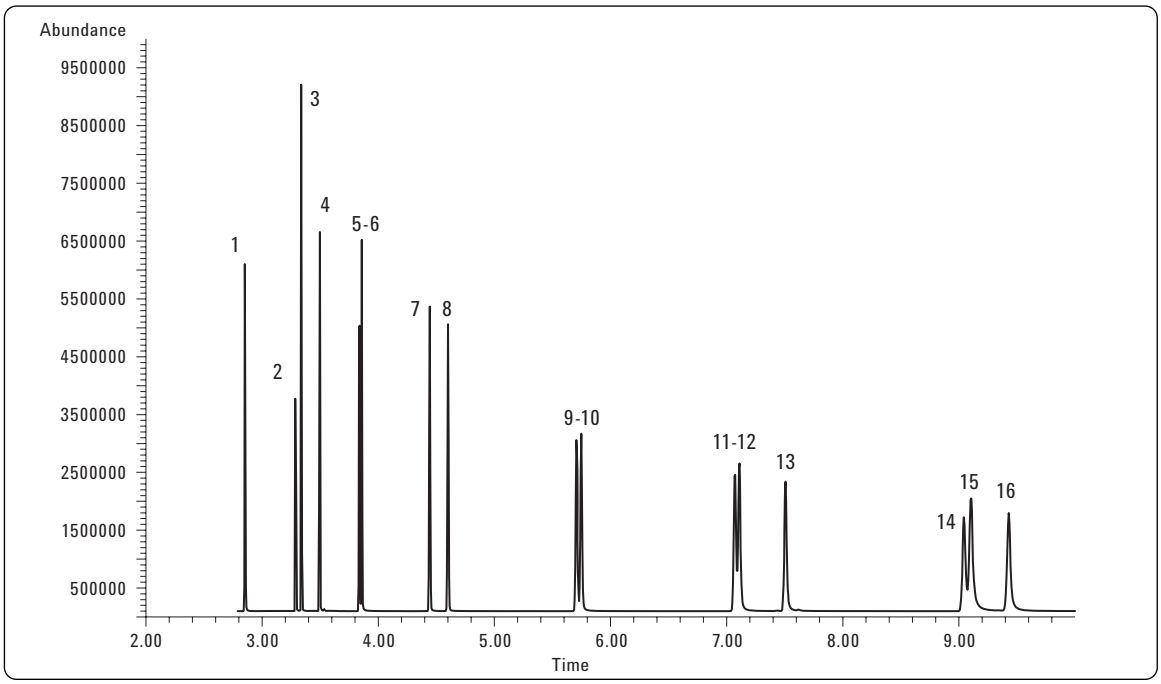

Figure 3. Fast PAH method using 20 m × 0.18 mm id × 0.18 µm Agilent J&W DB-5MS LTM column and BF at 10 minutes. SIM chromatogram shown.

Using these conditions, a backflush can be initiated at 10 minutes. To accomplish backflush, inlet pressure was dropped to 10 kPa and outlet pressure (AUX 1, QuickSwap device) was raised to 250 kPa while the column temperature was maintained at 300 °C. These conditions resulted in efficient backflush of solutes in about 7 minutes. This time was found to be required (under the stated pressure conditions) for effective removal of sample contamination (mineral oil). During backflush, the mass spectrometer detector was switched off.

Retention time and peak area repeatability were tested by analyzing a series of six standard samples using the fast GC-MS (SIM/Scan) method in combination with backflushing. The results are given in Tables 3 and 4. The repeatability of retention times was excellent, with an average standard deviation of 0.001 minute (typically < 0.01% RSD). This confirms that the heating of the capillary column in the LTM oven is uniform. Also, the peak area repeatability was excellent (2% RSD on average) and similar to results obtained using splitless injection in combination with standard GC-MS conditions.

Table 3. Retention Time Repeatability of Target PAH Compounds Using the Fast Analysis Method

Order of elution	Compound name	Mean t_R (min)	σ	% RSD
1	Naphthalene	2.850	0.000	0.00
2	Acenaphthylene	3.280	0.000	0.00
3	Acenaphthene	3.326	0.000	0.00
4	Fluorene	3.481	0.000	0.00
5	Phenanthrene	3.825	0.000	0.01
6	Anthracene	3.846	0.000	0.00
7	Fluoranthene	4.429	0.001	0.01
8	Pyrene	4.583	0.001	0.01
9	Benz(a)anthracene	5.654	0.001	0.02
10	Chrysene	5.693	0.001	0.01
11	Benzo(b)fluoranthene	6.979	0.001	0.02
12	Benzo(k)fluoranthene	7.016	0.002	0.02
13	Benzo(a)pyrene	7.405	0.002	0.02
14	Indeno(123-cd)pyrene	8.910	0.002	0.02
15	Dibenz(ah)anthracene	8.968	0.002	0.02
16	Benzo(ghi)perylene	9.278	0.002	0.02
		Average	**0.001**	**0.01**

Table 4. Area Repeatability for Target PAHs

Order of elution	Compound name	Mean peak area	σ	% RSD
1	Naphthalene	2145022	45438.72	2.12
2	Acenaphthylene	2531734	57007.60	2.25
3	Acenaphthene	3410703	76081.85	2.23
4	Fluorene	3108369	66651.55	2.14
5	Phenanthrene	2341505	50290.78	2.15
6	Anthracene	2304885	46774.62	2.03
7	Fluoranthene	2598819	57961.99	2.23
8	Pyrene	2678698	58471.60	2.18
9	Benz(a)anthracene	2264966	58416.06	2.58
10	Chrysene	2597444	50507.12	1.94
11	Benzo(b)fluoranthene	2245073	50491.19	2.25
12	Benzo(k)fluoranthene	2954729	71902.33	2.43
13	Benzo(a)pyrene	2239967	57434.91	2.56
14	Indeno(123-cd)pyrene	1616395	56133.34	3.47
15	Dibenz(ah)anthracene	3065828	86273.62	2.81
16	Benzo(ghi)perylene	2511598	65387.08	2.60
			Average	**2.37**

Next, a real sample extract was analyzed with the fast method, applying backflushing at 10 minutes after elution of benzo(ghi)perylene. The chromatograms obtained in scan and SIM mode are shown in Figures 4 and 5. The chromatogram (Figure 4) shows the high background from mineral oil contamination of the sample. At 10 minutes, the drop in signal clearly signals the initiation of the backflush. Tests would later show that the mineral oil "hump" extends well after 24 minutes (ending temperature of 300 °C) when backflush is not used.

The SIM chromatogram in Figure 5 clearly shows the presence of PAHs in the sample (high concentration of phenanthrene through pyrene, lower concentrations of later-eluting PAHs; see inset).

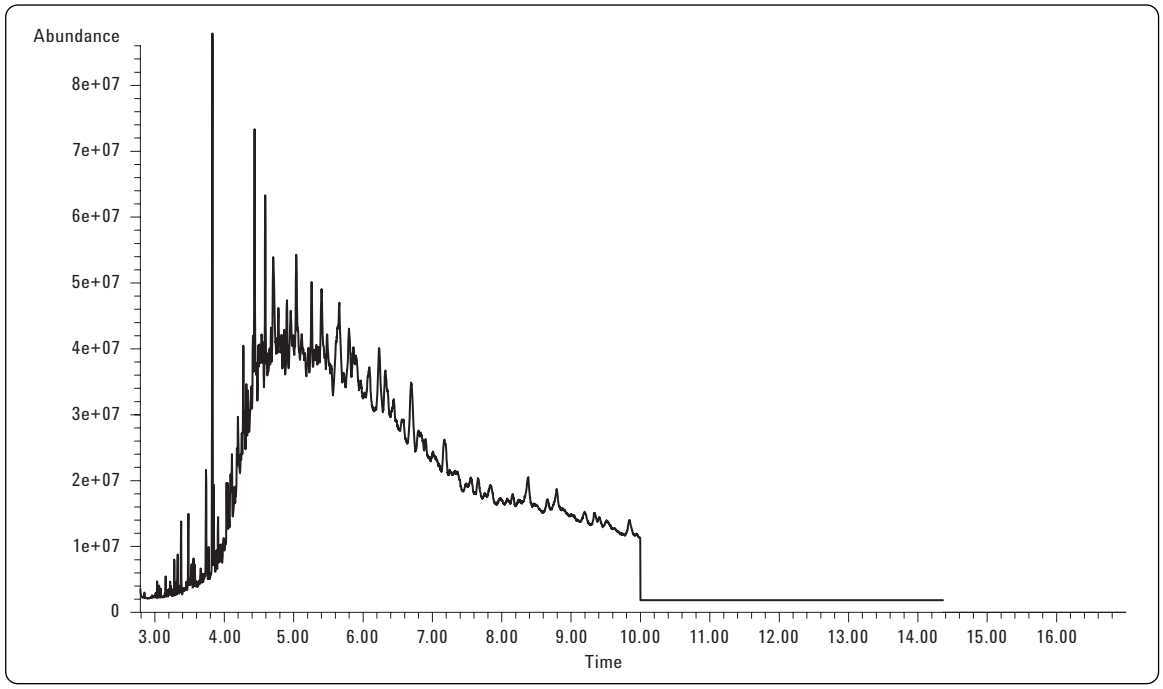

Figure 4. Sample (soil extract) with fast method and backflush at 10 minutes. Scan TIC shown.

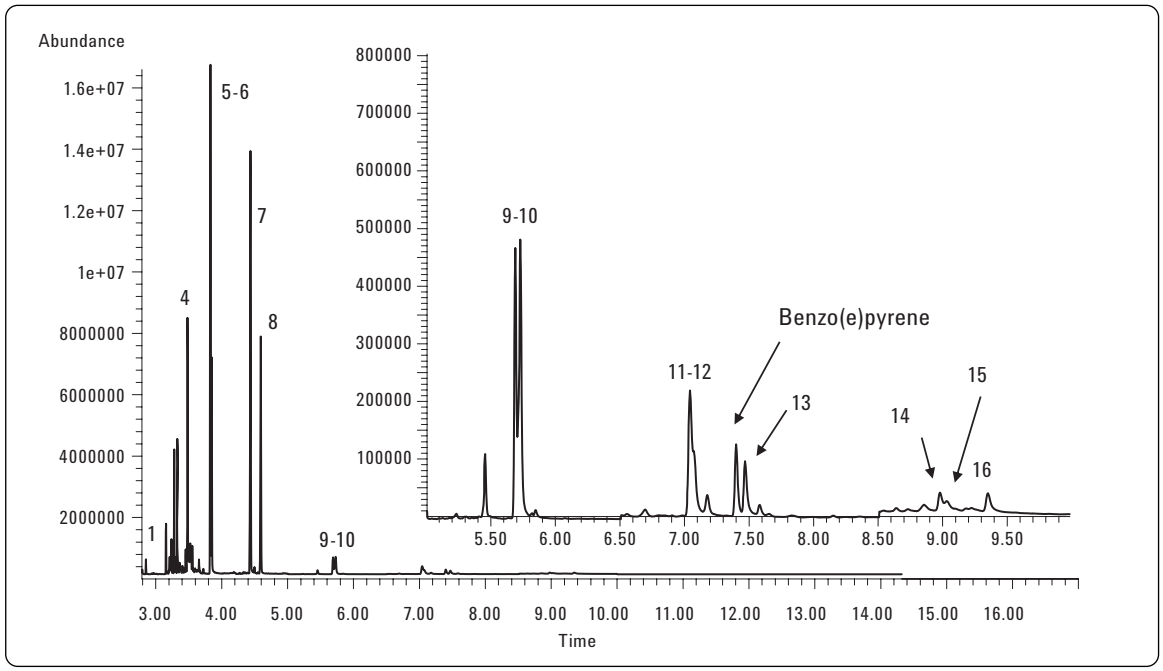

Figure 5. Sample (soil extract) with fast method and backflush at 10 minutes. SIM chromatogram shown with later portion expanded for better visibility.

A blank run was performed after the sample analysis with backflush. The resulting chromatogram in Figure 6 shows that no significant peaks were detected and that the background is flat, indicating that remaining mineral oil contamination was effectively removed with backflush.

One might question if the fast GC analysis (especially with temperature programming at 87 °C/min) in combination with SIM/Scan MS data acquisition mode results in enough data points per peak for accurate identification and quantification. The repeatability of peak areas, shown in Table 4, clearly demonstrates that the speed and repeatability of the Agilent 5975C Series MSD detection is excellent.

This is further illustrated in Figure 7, the AMDIS report for the detection of benzo(a)pyrene in the environmental sample. For this exercise, the scan data (as in Figure 3) were analyzed using AMDIS [2]. The upper window in Figure 7 shows overlaid TIC and extracted ion (*m/z* 252) chromatograms. The middle window confirms > 10 scans across the peak. The lower two windows show the raw spectrum and deconvolved spectrum, allowing unequivocal confirmation of the presence of benzo(a)pyrene in the sample. Even with the high background level, trace-level PAHs were reliably detected and confirmed.

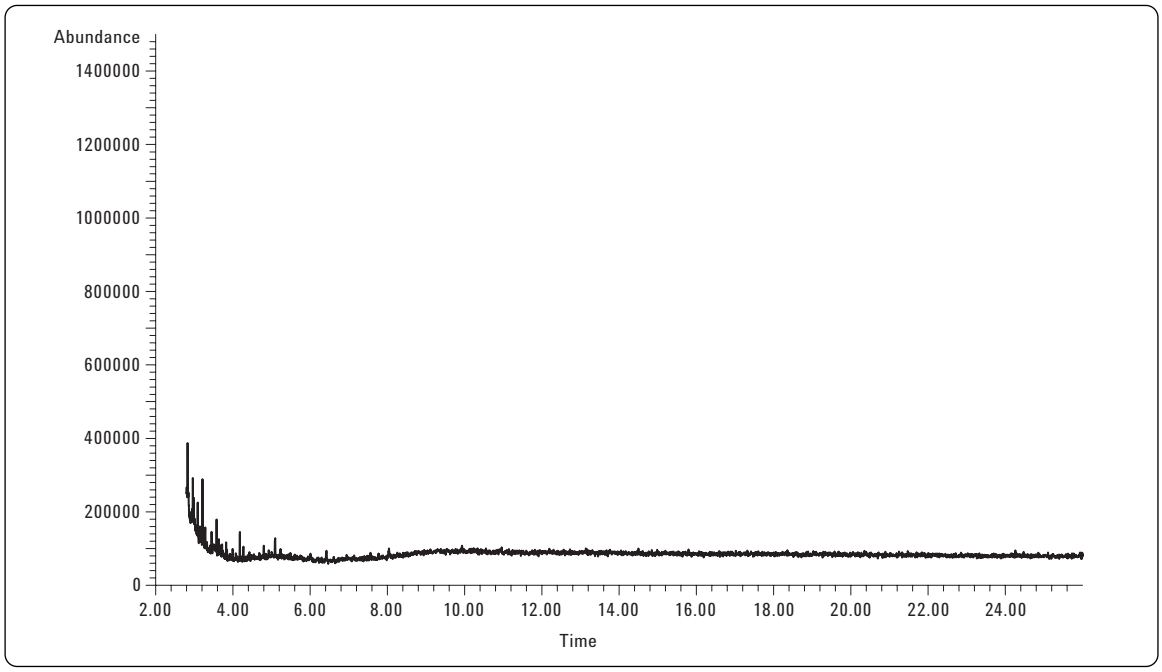

Figure 6. Blank SIM/Scan run after sample extract analysis using backflush. Scan chromatogram shows clean baseline free of contamination.

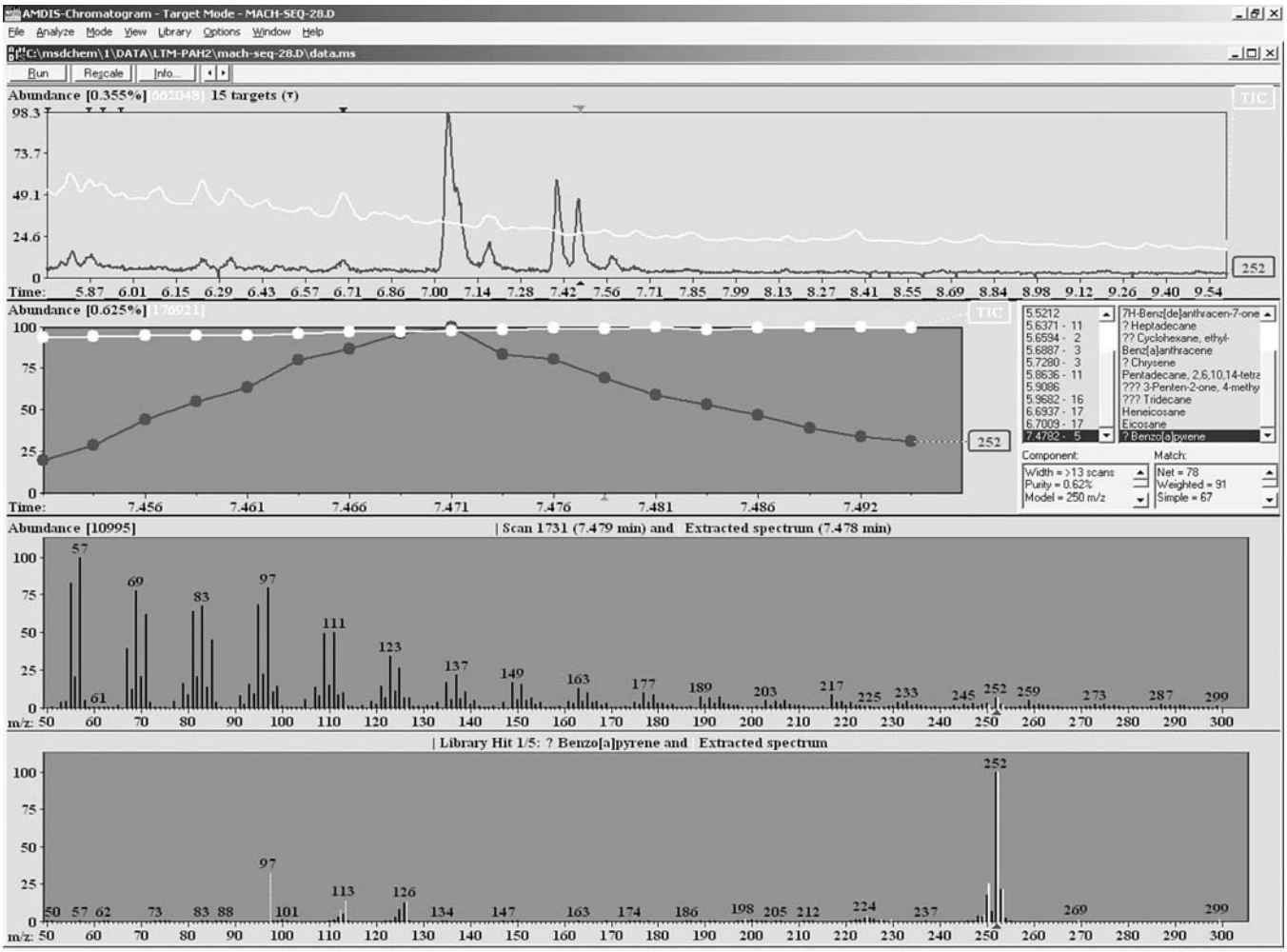

Figure 7. AMDIS report on the detection of benzo(a)pyrene in soil extract in the presence of mineral oil.

An additional sequence of multiple runs without backflush was performed to illustrate the benefits of backflushing for elimination of sample carryover. Sample extract was analyzed with the LTM oven module programmed to 300 °C and held for 11 minutes (17 minutes total run time). An example TIC chromatogram is shown in Figure 8. It can be seen that the mineral oil envelope extends to 17 minutes (and beyond).

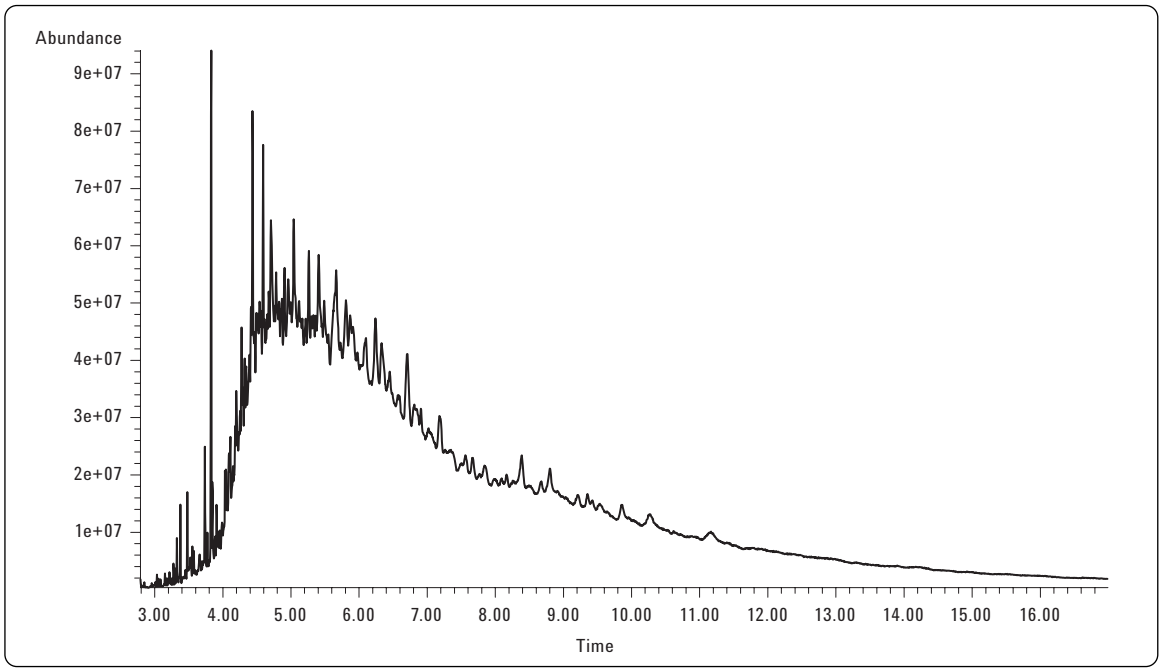

Figure 8. Soil extract analyzed without backflush. Scan chromatogram shows contribution of high mineral oil content in the extract.

Next, a blank run was performed. The chromatogram in Figure 9 shows that a significant portion of the mineral oil remained in the column even after the extended hold at the higher temperature. Comparing the upper and lower chromatograms in Figure 9 (plotted with the same scale) clearly illustrates the efficacy of backflushing.

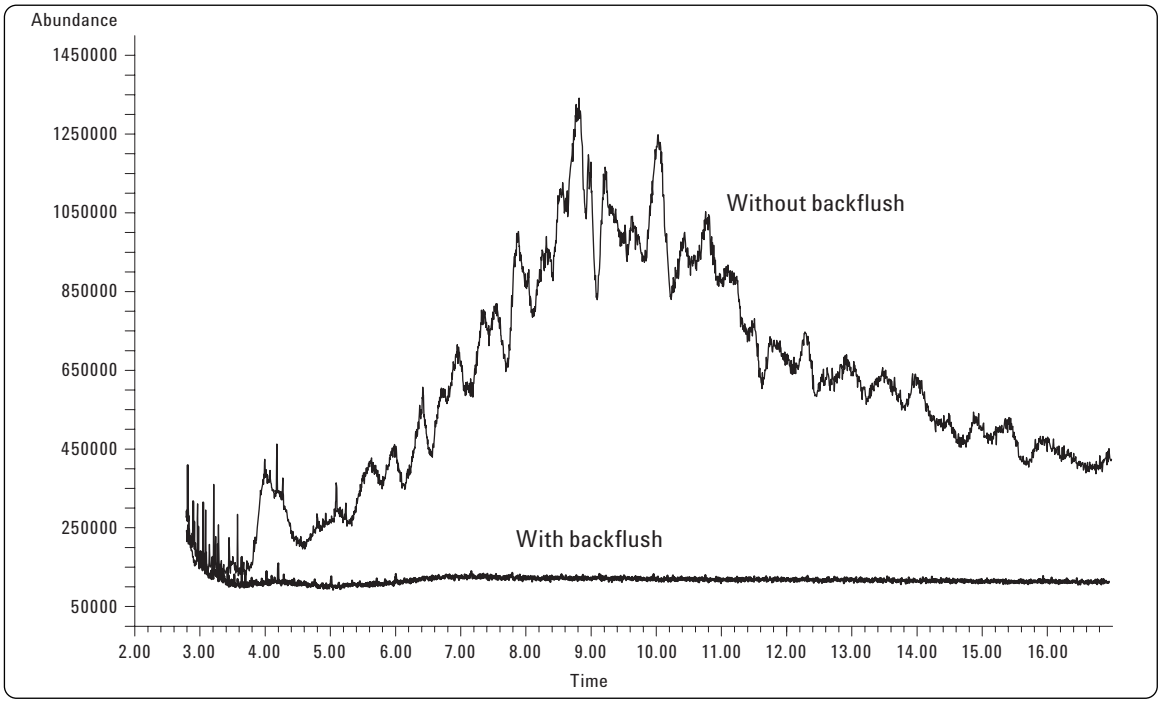

Figure 9. Blank run following the soil extract run without backflush (Figure 7). The upper TIC chromatogram shows high background carryover of matrix remaining on column after run. Bottom chromatogram is from Figure 6 (blank after method with backflush).

The standard mixture was then run after an analysis of sample extract without backflush. The resulting TIC chromatogram of the standard in Figure 10 shows several potential interferences from sample carryover in and among the PAH peaks. These contaminants are absent when backflushing is included in the method.

The extract of a sewage sludge was also analyzed. The sludge was contaminated with low-volatility plant material, phthalates, and linear alkyl sulphonates (LASs). A sequence was programmed wherein a 1-ppm PAH reference standard was analyzed first followed by 10 runs of the sewage sludge extract using backflush, one reference standard, another 10 runs of the sewage sludge extract without backflush, and a final reference standard. The chromatograms of the reference samples are overlaid in Figure 11. The two chromatograms of the references before and after the sample analyses using backflush match perfectly and show a low baseline without ghost peaks. The last reference sample chromatogram (after 10 runs of sample without backflush) shows a significantly higher background, including ghost peaks.

From this test, it is clear that low-volatility material is not removed by the standard process of keeping the column at 300 °C for 7 minutes. By backflushing, this material is effectively removed. This improves not only the quality of the data, but also reduces or eliminates the need to trim the column due to accumulation of contaminants, a typical maintenance procedure for those running extracts without backflush. This demonstration should serve to assuage concerns about the use of LTM modules with dirty samples. This had been a concern in the past because the format does not accommodate column trimming.

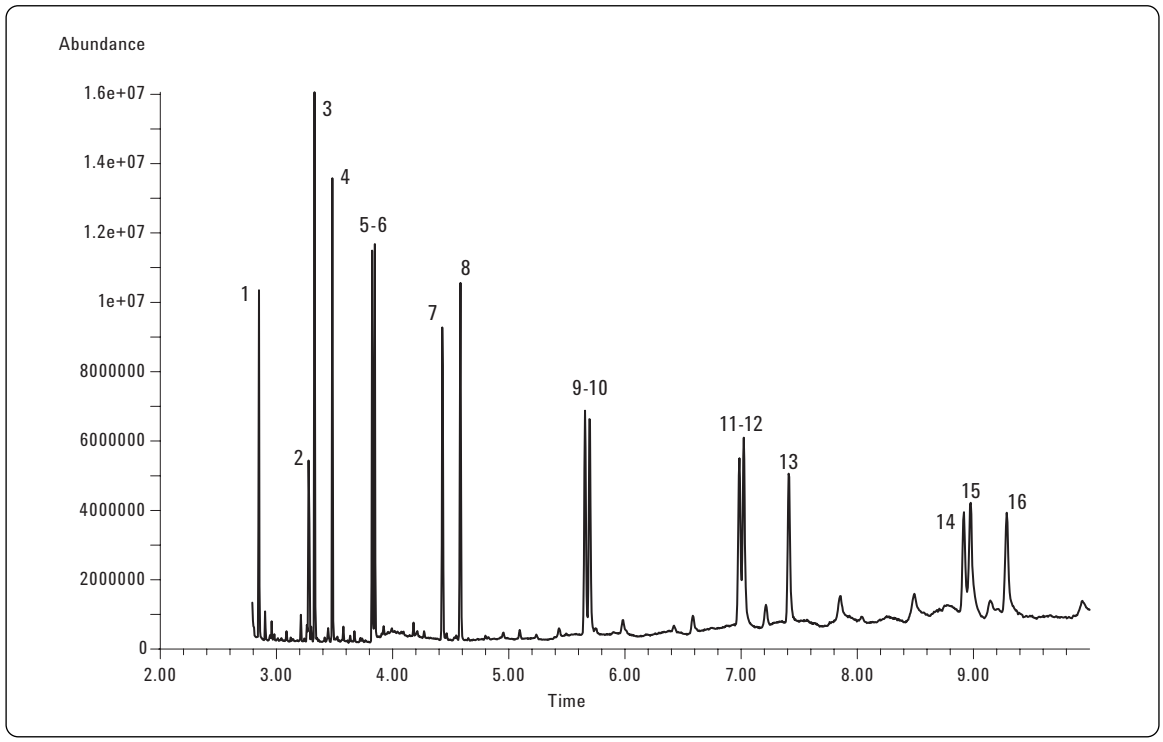

Figure 10. GC-MS scan chromatogram of 1-ppm PAH standard after sample extract analysis with no backflush.

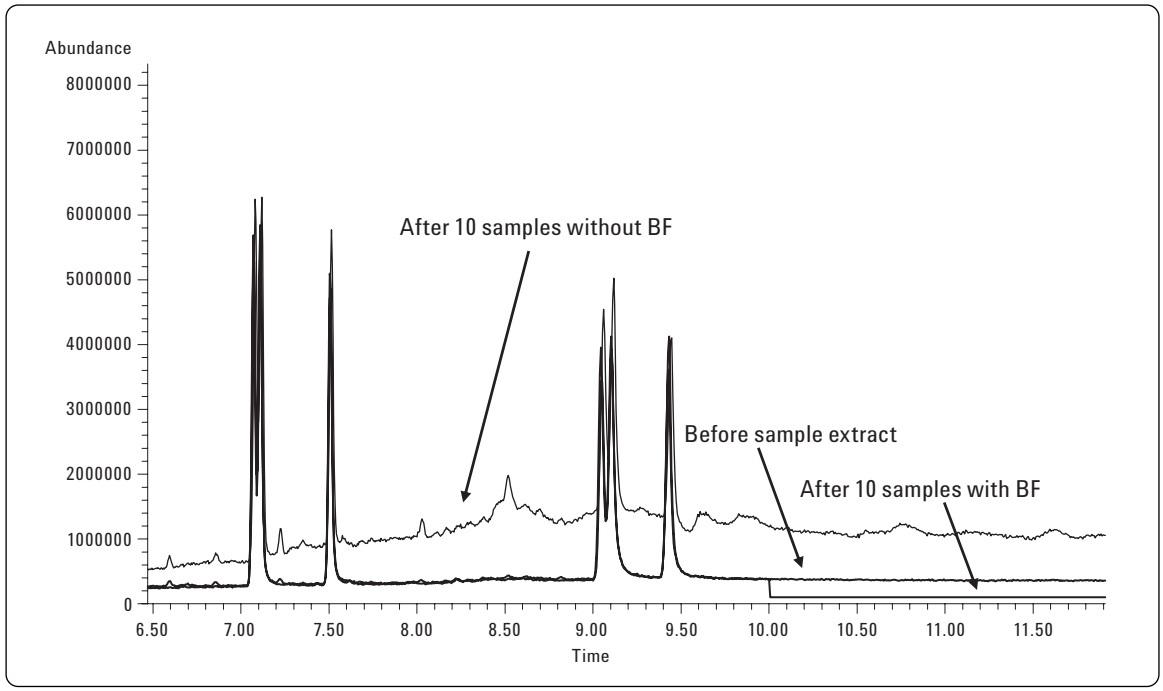

Figure 11. Standard analyses before and after analyzing sewage sludge extract with backflush and after analyzing sewage sludge extract without backflush.

A final review of improvements in analysis time revealed that the LTM column module provides an additional advantage of approximately 30% faster cooldown time compared to the conventional 7890A GC (even with fast cooling on). This further improves overall cycle time by one minute in addition to the savings from fast temperature ramp conditions and potential truncated run time from backflush.

Conclusions

A standard 25-minute method for the analysis of PAHs was translated to a fast GC-MS method using a narrow-bore column in an LTM column module. The resulting fast GC method decreased analysis time with elution of all PAHs within 10 minutes while maintaining resolution of critical isomers and method performance metrics.

The backflush capability afforded by using a Capillary Flow Technology QuickSwap capillary flow module resulted in efficient removal of high molecular-weight sample matrix and addresses prior concerns about column trimming with LTM columns. Faster run time, backflush, and faster cooldown combined in the new fast GC method with LTM significantly increase sample throughput and lab productivity while maintaining or improving data quality.

References

1. GC Method Translation Software available at http://www.chem.agilent.com.
2. AMDIS is an add-on to NIST Mass Spectral libraries. Information is available at http://chemdata.nist.gov/mass-spc/amdis/

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Agilent Technologies

Analysis of Semivolatiles Using High Efficiency Capillary GC Columns

Application Brief

Ken Lynam and Mike Szelewski

Introduction

U.S. EPA Method 8270 is broadly applicable for analysis of semivolatiles using capillary gas chromatography with mass spectral detection. EPA 8270 is widely used in both contract analytical and government environmental laboratories. The method is capable of concurrently measuring a mixture of 70 to 100 acidic, basic, and neutral species. Shifting these important analyses from 0.25-mm id to 0.18-mm id or high efficiency GC columns is a viable means of obtaining faster results and improving laboratory productivity.

In this example, 77 compounds of interest and six internal standards are resolved on a 0.18-mm id high efficiency GC column using 7 minutes of analysis time. The same compounds and internal standards were also resolved using a 0.25-mm id column where 25 minutes of analysis time was required. Analysis speed using the high efficiency column was 8 minutes faster, resulting in a 32% reduction in analysis time.

Experimental

Method translation software available from Agilent Technologies translates chromatographic parameters from an existing method to the new column format with a few simple keystrokes [1].

Column dimensions, flow, and temperature parameters from an existing method are entered into a table along with the desired new column dimensions. The software then generates flow and temperature setpoints for the new translated method. Often these new setpoints yield a successfully translated method with the same separation and elution order with no additional method development. In this example, one-to-one phase-ratio correspondence was maintained between the 0.25-mm and 0.18-mm id column formats, enhancing the reliability of the software's predicted conditions. Keeping the phase ratio constant helps maintain peak elution order on the new column.

Instrument conditions are described in Table 1, and Figures 1 and 2.

Highlights

- 0.18-mm id, also known as high-efficiency GC columns, deliver faster results for U.S. EPA 8270 analyses.
- 32% reduction in analysis time when translating 0.25-mm id column method to the 0.18-mm id format.
- Resolution of 77 peaks of interest is maintained for the faster 0.18-mm id separation.
- DB-5.625 column: Agilent DB-5.625 column in 0.18-mm id provides faster sample analysis without loss of resolution.

Table 1. Experimental Conditions

Column:	Figure 1. 30 m x 0.25 mm x 0.50 µm DB-5.625 column, Agilent Technologies part number 122-5632 Figure 2. DB-5.625 20 m x 0.18 mm x 0.36 µm column, Agilent Technologies part number 121-5622
Carrier:	He constant-flow mode 1.1 mL/min
Oven:	40 °C for 1.00 min, 25 °C/min to 320 °C 4.80 min hold
Injection:	Splitless 0.5 µL injected at 300 °C, QuickSwap pressure 5.0 psi during acquisition, 80.0 psi during backflush with inlet set to 1.0 psi during backflush
Detector:	Agilent Technologies 5975C Performance Turbo MSD equipped with a 6-mm large-aperture draw-out lens, Agilent Technologies part number G2589-20045

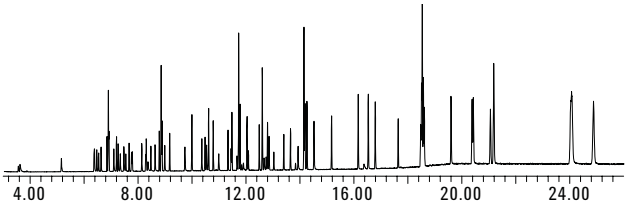

Figure 1. Figure 1. U. S. EPA Method 8270, 5 ng/mL System Performance Check Compounds Chromatogram using a 30-m x 0.25-mm x 0.50-µm DB-5.625 column, Agilent Technologies part number 122-5632. Please refer to Table 1 for instrument conditions.

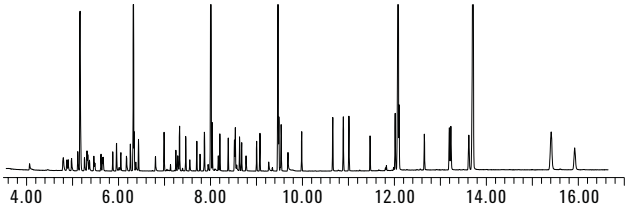

Figure 2. U.S. EPA Method 8270, 5 ng/mL System Performance Check Compounds Chromatogram using a 20-m x 0.18-mm x 0.36-µm DB-5.625 column Agilent Technologies part number 121-5622. Please refer to Table 1 for instrument conditions.

Discussion of Results

Figures 1 and 2 depict the resolution of 77 compounds of interest along with six internal standards first on a 30 m x 0.25 mm x 0.5 µm (Agilent part number 122-5632) standard-bore capillary column (Figure 1) and second on a 20 m x 0.18 mm x 0.36 µm (Agilent part number 121-5622) high efficiency column (Figure 2). Peak resolution and quantification are comparable, and in both cases meet EPA 8270 criteria for System Performance Check Compounds (SPCCs) and Continuous Calibration Compounds (CCCs) over a calibration range from 1 to 200 ppm; 5 ppm SPCC chromatograms were selected for visualization purposes.

Significant improvement in analysis time was achieved by shifting the column used from a 0.25-mm id standard-bore capillary to a 0.18-mm id high efficiency GC column example; the 0.25-mm id column required 25 minutes of run time, and the 0.18-mm id column required 17 minutes. In this semi-volatile analysis example, 25 minutes of run time were required for the 0.25-mm id column, and 17 minutes were required on the 0.18-mm id column. Moving the analysis to a 0.18-mm id column yielded 8 minutes in time savings or 32% faster sample analysis.

Typical run time for EPA 8270 analysis using 0.25-mm id or standard-bore capillary columns is 25 minutes, excluding post-analysis bakeout and system cooldown time often required for dirty samples. When bakeout and subsequent system cooldown periods are accounted for, the overall cycle time climbs to 57 minutes. As shown above, a time saving of 8 minutes was achieved by using a high efficiency column. Further improvements in the cycle time for EPA 8270 analysis are achieved through the use of several advanced features on the Agilent 7890A GC. A QuickSwap device installed in a 7890A can be used to backflush heavy material matrix contaminants back out of the inlet, dramatically reducing matrix bakeout time [2]. Faster cooldown and thermal isolation features available on the 7890A GC also reduce system cycle times for dirty samples. The combination of a high efficiency column and the unique features of the 7890A reduce sample analysis time from 57 minutes to 24.3, a 32.7-minute time saving per sample run.

Conclusions

High efficiency GC columns provide a straightforward way to obtain faster results for EPA 8270 analysis without compromising resolution.

References

1. Method translation software: free download of method translation software available at http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.
2. Mike Szelewski, “Significant Cycle Time Reduction Using the Agilent 7890A/6975C GC/MSD for EPA Method 8270,” Agilent Technologies publication 5989-6026EN

For More Information

For more information on our new line of high efficiency GC columns, please visit this link: http://www.chem.agilent.com/scripts/PDS.asp?lpage=60005.

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Agilent Technologies

Separation of Explosives in EPA 8330: Column Choices Optimize Speed, Resolution, and Solvent Use

Application

Environmental

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Abstract

ZORBAX Extend-C18 columns separate the explosive compounds in EPA method 8330, and the variety of column configurations available allows customized HPLC methods based on resolution, speed, and even solvent usage. For example, a fast method for the explosive-materials standard (EPA 8330) uses 1.8-µm, short length columns. The method was then customized using two other Extend-C18 column configurations. Each column highlights a combination of resolution, speed, and/or solvent savings. The advantage is being able to choose which combination of resolution, speed, and solvent usage is needed by simple column substitution.

Introduction

The ZORBAX Rapid Resolution High Throughput (RRHT, 1.8 µm) LC column line has over 120 column choices, including 11 bonded phases and silica, three column diameters, and six lengths. In addition, there are another 150+ Rapid Resolution (3.5 µm) column choices, allowing customization of HPLC methods to meet the analyst's tailored objectives. Many ZORBAX column choices are available because the stationary phase chemistry (both silica support and bonded phase) between 5-, 3.5- and 1.8-µm particles is uniform.

EPA 8330 explosives residues are typically analyzed by a 4.6 mm × 250 mm, 5 µm C18 column [1] but can be improved by newer technology: smaller 1.8-µm or 3.5-µm ZORBAX particles and Extend-C18 bonded phase. Many different Extend-C18 columns can be chosen (the combination of column length, diameter, and particle size) to provide a satisfactory separation, and each separation exemplifies a newer column technology's benefit and supports the end user's choice of speed, resolution, and solvent usage.

High-efficiency 1.8-µm particles in 100-mm length columns reduce analysis time and have about the same efficiency compared to 5-µm particles in 250-mm columns. Therefore, they are helpful by saving time in method development or generating more data in a limited amount of time. But these columns will generate a higher back pressure that some people may not desire. It is still possible to obtain the same resolution but using a longer 3.5-µm column. The end result is an analysis time still shorter than that achieved with a 250-mm, 5-µm column.

Experimental

The Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase A: 5 mM ammonium formate in water, B: methanol
- G1376C automatic liquid sampler (ALS) SL
- G1316B Thermally Controlled Column (TCC) Compartment SL using the low-volume heat exchanger kit (PN G1316-80003)
- G1365C multiwavelength detector (MWD) at 254 nm, with a G1315-60024 micro flow cell (3-mm path, 2-µL volume), response time setting of 0.5 s

ZORBAX columns:

- Rapid Resolution High Throughput (RRHT) Extend-C18, 4.6 mm × 100 mm, 1.8 µm, PN 728975-902
- Rapid Resolution (RR) Extend-C18, 4.6 mm × 100 mm, 3.5 µm, PN 764953-902
- Solvent Saver Plus Extend-C18, 3.0 mm × 100 mm, 3.5 µm, PN 764953-302

The sample is a 1:1 mix of EPA 8330 Mix A (cat. no. 47283) and EPA 8830 Mix B (cat. no. 47284) from Sigma-Aldrich (Bellefonte, PA), diluted in methanol:water.

Results and Discussion

Selectivity, or the relative band spacing between two peaks, is different among C18 columns. In many cases the difference is small, so adjusting mobile phase organic strength can fine tune the retention to achieve comparable resolution between one C18 column and an alternative C18 column. Temperature may also influence selectivity, and small adjustments in temperature can fine tune the resolution.

For complex mixtures, fine tuning organic strength and temperature could be used to improve resolution and ultimately make a method more robust. Determining the combination of temperature, % organic, and what column (stationary phase) is best is frequently discovered by experimentation. This is time consuming at the very least and often daunting. Fortunately, research narrows the testing.

Consider an explosive residue standard of 14 nitroaromatic and nitramine compounds. Trace residues of these explosives were analyzed by time-of-flight LCMS by Kinghorn et al. using an Extend-C18, 4.6 mm × 250 mm, 5-µm column and a methanol/water gradient at a temperature of 40 °C [2]. Additionally, EPA method 8330 describes an HPLC method for the 14 compounds using an isocratic methanol/water mobile phase and a C18 column. Temperature is not specified, but the method states, *"If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification."* [1]

In both methods a lack of selectivity required a TOF detector or additional analysis by an orthogonal stationary phase to confirm peak identity.

We separated the 14 compounds with enough resolution to make the MS detector or secondary analysis by a different stationary phase redundant.

The above methods narrowed our method-development starting conditions to:

- Extend-C18 (from successful Kinghorn method)
- Isocratic mobile phase A: 5 mM ammonium formate, B: Methanol (so new method is similar to EPA 8330). The ammonium formate was selected based on recommendations from a pre-existing method. The difference between water and 5 mM ammonium formate was not investigated.
- 40 °C controlled temperature (to ensure constant selectivity)
- RRHT column configuration 4.6 mm × 100 mm, 1.8 µm (for rapid analyses with efficiency comparable to the 4.6 mm × 250 mm, 5-µm columns used in the Kinghorn and EPA methods)

The methanol composition of the mobile phase was lowered incrementally from 50 to 25% until all 14 were reasonably resolved. A critical pair (peaks 6 and 7) persisted as partially resolved. Further decreasing organic strength would result in excessive retention of peaks 12, 13, and 14. Temperature was then optimized. A one-degree temperature increase (41°C) provided enough selectivity to resolve the critical pair. Figure 1 demonstrates temperature's selectivity effect on these compounds.

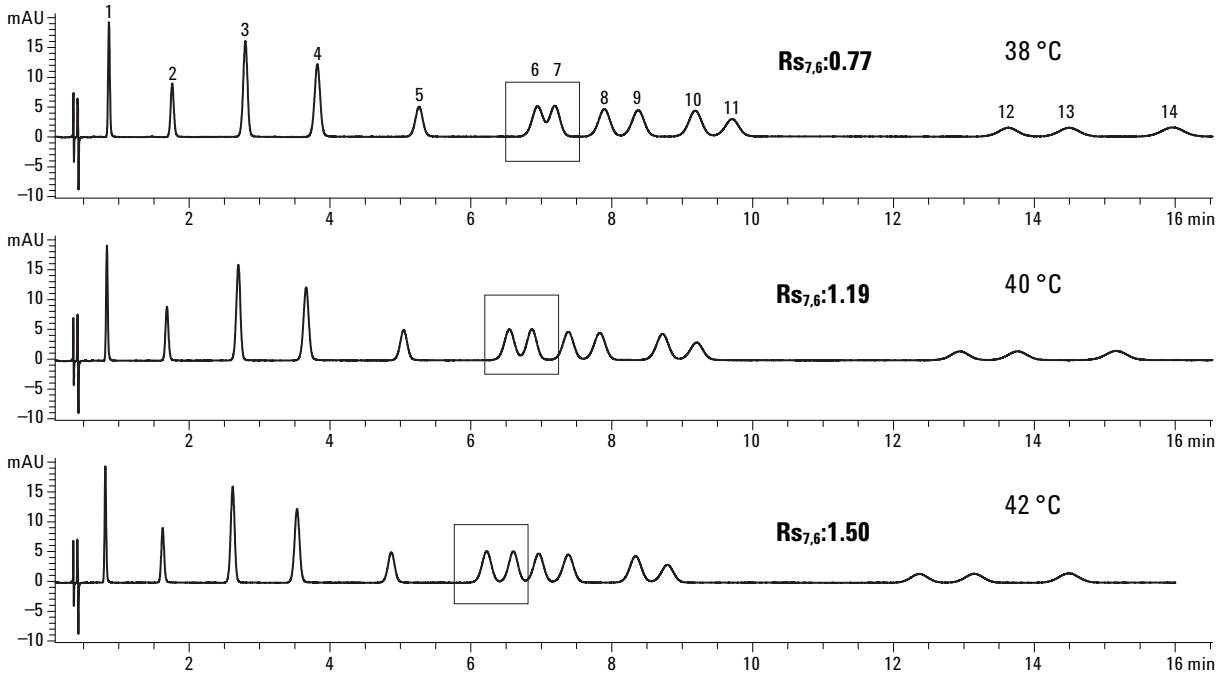
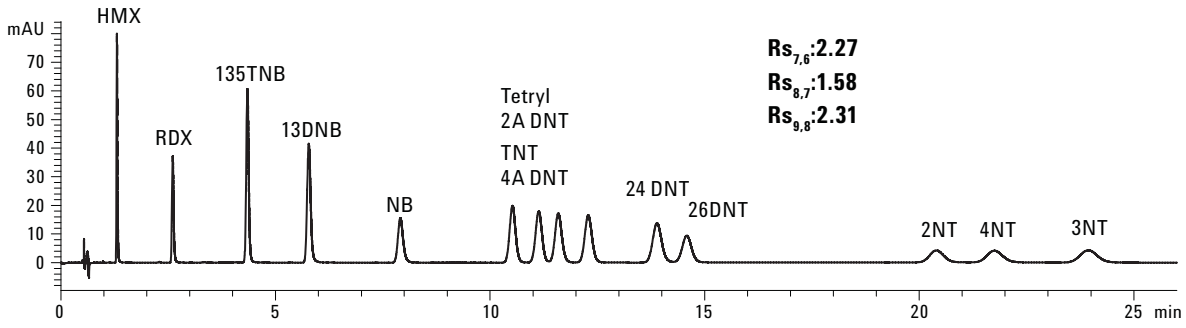


Figure 1. Temperature optimizes critical pair resolution.

Extend-C18 provides ample selectivity for the 14 nitroaromatics and nitramines identified in the EPA 8330 method; excellent resolution is obtained in a reasonable time. Figure 2 shows the separation using a RRHT 4.6 mm × 100 mm, 1.8 µm, Extend-C18. Resolution of all peaks is baseline or better (Rs > 1.5). High resolution makes it easier to quantify the analytes. For example, the EPA 8330 method warns, *"2,4-DNT and 2,6-DNT elute at similar retention times (Rs < 1.5) and a large concentration of one isomer may mask the other; therefore, if it is not apparent that both isomers are present, an isomeric mixture should be reported"* [1]. When baseline resolution is obtained, retention times differ significantly, avoiding peak masking. If higher resolution is the most important objective, then the Extend-C18 4.6 mm × 100 mm, 1.8-µm column using the conditions in Figure 2 is an excellent choice.



Column:	Rapid Resolution HT Extend-C18, 4.6 mm × 100 mm, 1.8 µm
Mobile phase:	A: 5 mM NH_4COOH (ph 6)
	B: MeOH (75A:25B)
Flow rate:	1.7 mL/min
Temperature:	41 °C
Injection:	2 µL × (0.5 µg ea/mL)
Pressure:	520 bar

Figure 2. EPA 8330 explosive standard high-resolution separation on Extend-C18.

Table 1 names the 14 explosives and their abbreviations used in the figures.

Table 1. EPA 8330 Explosives and Their Abbreviations

Name	Abbreviation
Cyclotetramethylene-tetranitramine	HMX
Cyclotrimethylene-trinitramine	RDX
1,3,5-trinitrobenzene	135TNB
1,3-dinitrobenzene	13DNB
Nitrobenzene	NB
2,4,6-trinitrophenyl-N-methylnitramine	tetryl
2,4,6-trinitrotoluene	TNT
2-amino-4,6-dinitrotoluene	2A DNT
4-amino-2,6-dinitrotoluene	4A DNT
2,4-dinitrotoluene	24 DNT
2,6-dinitrotoluene	26 DNT
2-nitrotoluene	2NT
4-nitrotoluene	4NT
3-nitrotoluene	3NT

If higher throughput is important, isocratic methods can be sped up by increasing flow rate. The 25% methanol mobile phase flowing 1.7 mL/min through the 4.6 mm × 100 mm, 1.8-µm column generates a system pressure of about 500 bar, leaving a small range to increase flow rate. An alternative is to substitute the 1.8-µm column with a 3.5-µm column. Pressure decreases substantially, allowing faster flow rates.

Figure 3 overlays two Extend-C18 chromatograms. The top chromatogram is a 4.6 mm × 100 mm column with 3.5-µm particles at a 2.5 mL/min flow rate. Compared to Figure 2, the 32% increase in flow rate reduces analysis time by roughly 40%. The price for the considerable time savings is less resolution of closely neighboring peaks. Resolution is still sufficient, as a resolution factor (Rs) of 1.25 for equally sized peaks means 99.4% of peak area is not overlapped. If one peak is 1/32 as tall as the other, an Rs of 1.0 still means 99.2% of the peak areas do not overlap [3].

Figure 3's bottom chromatogram is a different column substitution, replacing the 4.6-mm-id column with the Solvent Saver 3.0-mm-id column. Flow rate was reduced from 2.5 to 1.1 mL/min. for equivalent mobile phase linear velocity. The outcome is similar retention and resolution, but only half of the solvent is consumed.

Table 2 summarizes the customization benefits.

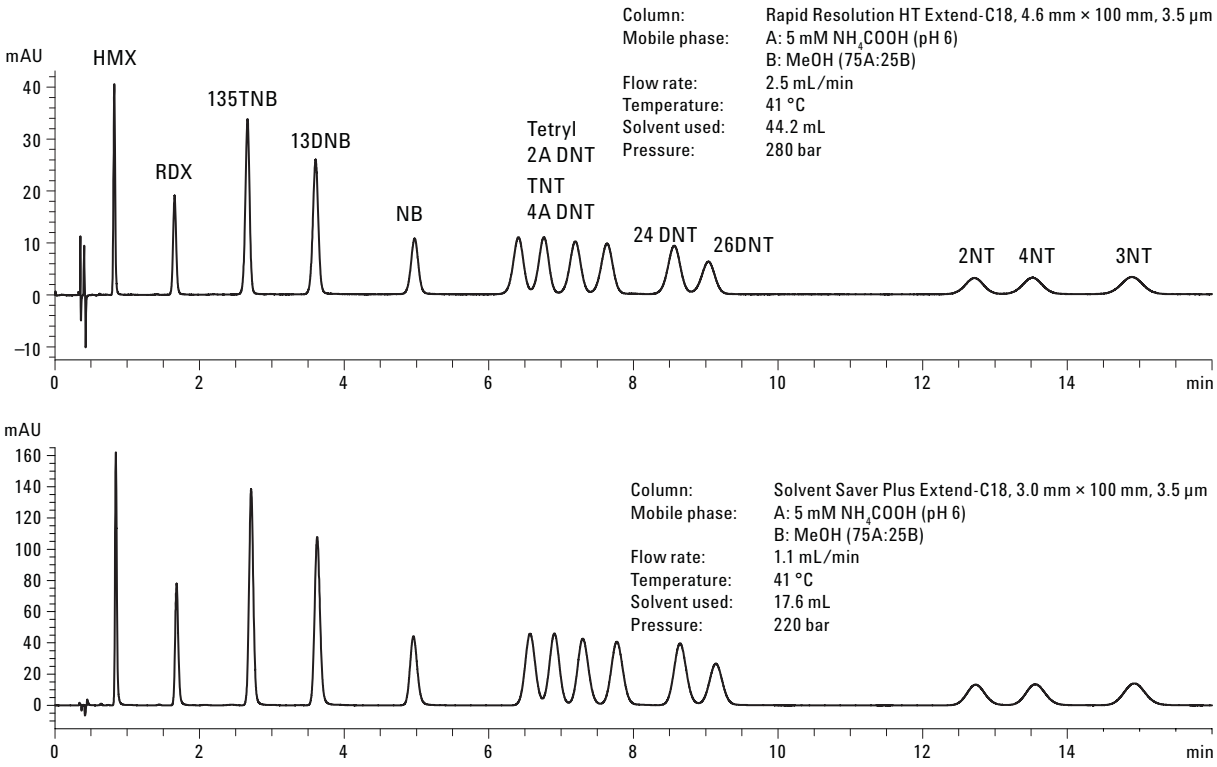


Figure 3. Rapid resolution options for EPA 8330 explosive standard on Extend-C18.

Table 2. Column Dimensions Highlight Resolution, Speed, and Solvent Savings

	RRHT (4.6 mm id, 1.8 µm)	RR (4.6 mm id, 3.5 µm)	Solvent Saver Plus (3.0 mm id, 3.5 µm)
Resolution: Rs 7,6	2.3	1.3	1.3
Resolution: Rs 8,7	1.6	1.6	1.4
Resolution: Rs 9,8	2.3	1.5	1.6
Analysis time	26 min	16 min	16 min
Solvent consumption	44.2 mL/analysis	40 mL/analysis	17.6 mL/analysis

Table 2 suggests that another column configuration could be valuable for this analysis: Solvent Saver HT Extend-C18, 3.0 mm × 100 mm, 1.8-µm column (PN 728975-302). This would produce high resolution like the RRHT column and produce time and solvent savings from the smaller column diameter. The Solvent Saver HT Extend-C18 column was not evaluated in this work.

Conclusions

Highly efficient (1.8 µm) short columns (100 mm) are ideal for method development compared to 5-µm, 150-mm or 250-mm columns because shorter analysis time increases productivity and allows more analyses to be performed in a fixed time frame.

Selectivity is manipulated by changing stationary phase, mobile phase, and temperature.

An isocratic HPLC method for complex mixtures of explosive materials was quickly created from highly efficient 100-mm columns, Extend-C18's unique selectivity, and temperature optimization. The selectivity and column configurations make Extend-C18 a compelling choice for the analysis of explosive substances named in EPA method 8330. Extend-C18's selectivity provides ample resolution with negligible peak coelution; this may eliminate an additional analysis to confirm peak identity.

The ZORBAX column family, including Extend-C18, has consistent stationary-phase chemistry between 3.5- and 1.8-µm particles, enabling simple column substitution for method customization. The high-resolution 4.6 x 100, 1.8-µm configuration, however, requires flexibility to work at operating pressures above 400 bar. The chromatographer can choose benefits such as higher resolution, faster analysis time, or less solvent usage based on column dimensions.

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Agilent Technologies

Synchronous SIM/Scan Low-Level PAH Analysis Using the Agilent Technologies 6890/5975 inert GC/MSD

Application

Environmental

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Abstract

The analysis of Polynuclear Aromatic Hydrocarbons (PAHs) presents challenges due to the tendency of the PAHs to adsorb on surfaces in the chromatographic system. Selected Ion Monitoring (SIM) analysis is needed for low-level analysis, while scan data are desired for confirmation. The 6890/5975 inert GC/MSD system is designed for improved PAH analysis using synchronous SIM/scan while maintaining linearity across a wide calibration range.

Introduction

PAHs are produced during combustion of organic material and are suspected carcinogens. The high amounts and widespread occurrence of these compounds in our environment requires reliable, sensitive, and very robust analytical methods.

PAHs tend to be adsorbed on any active or cold site in a GC/MSD system, such as inlets and ion sources. The 6890/5975 inert includes the inert source with high temperature filaments described previously [1]. Using the proper inlet liner also improves chromatographic peak shape and sensitivity.

Many laboratories calibrate for PAHs from 0.1 ppm to 10 ppm using SIM for low-level work. Historically, SIM has been necessary because of instrument sensitivity and loss of PAHs at the lower concentration levels. Full scan data is preferred for further confirmation of the compounds. The 5975 inert can acquire both SIM and scan data in a single run.

This application note will show the performance of the 6890/5975 inert for PAHs using a calibration range of 0.01 ppm–10.0 ppm in synchronous SIM/scan mode with linearity equal to that of many SIM only methods.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890			
Inlet	EPC Split/Splitless			
Mode	Pulsed Splitless, 1 µL injected			
Inlet temp	300 °C			
Pressure	13.00 psi			
Pulse pressure	40.0 psi			
Pulse time	0.20 min			
Purge flow	30.0 mL/min			
Purge time	0.75 min			
Total flow	34.6 mL/min			
Gas saver	Off			
Gas type	Helium			
Inlet Liner	**Description**			**Agilent part number**
	Direct connect, dual-taper, 4-mm id			G1544-80700
	or Splitless liner, single-taper, 4-mm id			5181-3316
Oven 240 V				
Oven ramp	**°C/min**	**Next °C**	**Hold min**	
Initial		55	1.00	
Ramp 1	25	320	3.00	
Total run time	14.60 min			
Equilibration time	0.5 min			
Oven max temp	325 °C			
Column	Agilent Technologies HP-5MS			19091S-433
Length	30.0 m			
Diameter	250 µm			
Film thickness	0.25 µm			
Mode	Constant Flow = 1.5 mL/min			
Inlet	Front			
Outlet	MSD			
Outlet pressure	Vacuum			
RTL	System Retention Time Locked to Triphenyl phosphate at 10.530 min			
MSD	Agilent Technologies 5975			
Drawout lens	6-mm ultra-large aperture			G2589-20045
Solvent delay	4.00 min			
EM voltage	Run at Autotune voltage = 1294 V			
Low mass scan	45 amu			
High mass scan	450 amu			
SIM	12 groups, 3–6 ions/group, 10 ms dwell/ion			
Threshold	0			
Sampling	1			
Cycles/s	5.55 each, SIM and scan			
Quad temp	180 °C			
Source temp	300 °C			
Transfer line temp	280 °C			
Emission current	Autotune value = 34.6 µamp			

Calibration Standards

Calibration standards were diluted in dichloromethane from a stock mix of 16 PAHs. The 10 levels made were 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02 and 0.01 ppm. The perylene-d12 internal standard and the three surrogate standards, 1,3-dimethyl-2-nitrobenzene, pyrene-d10 and triphenylphosphate, were added to each calibration level at 1.0 ppm.

The 6890 inlet temperature was set to 300 °C, instead of the typical 250 °C, to minimize compounds adsorbing on the liner surface. Pulsed injection was used to facilitate quantitative transfer of the heavier PAHs onto the column, minimizing inlet discrimination. Pulsed injection parameters are easily set in the ChemStation software and are automatically controlled by the EPC (Electronic Pneumatic Control) module.

The Direct Connect inlet liner allows for complete transfer of analytes onto the column. The column inlet end attaches to the liner and minimizes analyte exposure to the stainless steel annular volume in the inlet. The splitless liner, 5181-3316, yields better peak shapes for early eluters at the expense of lower amounts of analytes transferred to the column. Neither of these liners is well suited for split injections. Higher concentration samples requiring split injection would need a cyclosplitter-type liner, also suitable for splitless.

The 6890N 240V oven was necessary for the 25 °C/min ramp used up to the final temperature of 320 °C. A 120 V oven will achieve 20 °C/min at these higher temperatures and could be used, resulting in slightly longer run times.

The HP-5MS column is the most widely used column for environmental analysis. It has excellent lifetime and stability at elevated temperatures.

The system was Retention Time Locked to Triphenyl phosphate at 10.530 min. See the fundamentals of Retention Time Locking (RTL) for GC/MSD systems [2]. The primary benefit of RTL for the environmental laboratory is the ability to maintain retention times after clipping or changing the column. Quant database and integration events times do not have to be changed. For laboratories performing PAH SIM analyses, reproducible retention times are a must so SIM group times remain constant. Additional RTL application notes are available at www.agilent.com/chem, detailing the numerous benefits of RTL.

The 5975 inert was tuned using Autotune. The automatic DFTPP target tune, as required by some government methods, can also be used. The ultra-large aperture drawout lens was used to maintain linearity across the wide calibration range of 0.01–10.0 ppm. Source temperature was set to 300 °C, which is now possible with the high temperature filaments. This higher source temperature in combination with the new source material produces better peak shapes for the PAHs.

Data were collected using the synchronous SIM/scan mode available with the 5975 inert. A quant database is first setup using full scan data. SIM ions and groups are then determined automatically using Generate AutoSIM Method. A checkbox in data acqusition is used to acquire SIM and scan data in the same run. For details of synchronous SIM/scan, see reference 3.

Results

The system was calibrated at 10 levels: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ppm using the SIM data from SIM/scan acquisition. The calibration table allows the user to choose either the SIM or scan data. The TIC (Total Ion Chromatogram) for the 0.2 ppm level is shown in Figure 1, both SIM and scan traces. Each calibration level contained 16 PAHs, perylene-d12 (ISTD) and the three surrogate standards, 1,3-dimethyl-2-nitrobenzene, pyrene-d10, and triphenyl phosphate.

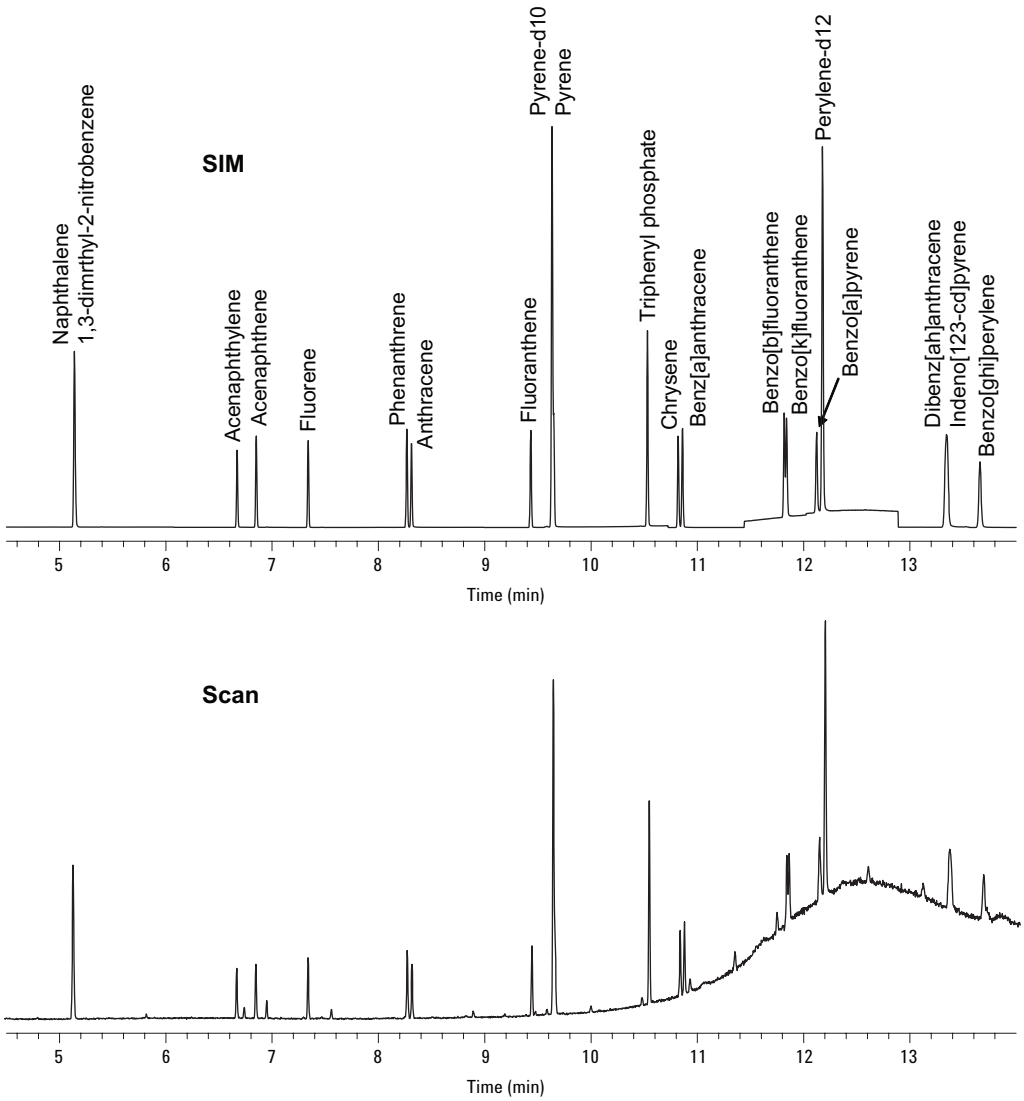

Figure 1. Sixteen PAHs at 0.2 ppm each with surrogates and ISTD at 1.0 ppm each, using synchronous SIM/scan mode.

The RRF (relative response factor) was calculated automatically for each compound at each level by the GC/MSD ChemStation software. Linearity was determined by calculating the %RSD (percent relative standard deviation) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Excel.

Linearity is excellent with the average of all %RSDs = 6 %. This compares favorably with other methods that are SIM only or those that only calibrate down to 0.1 ppm.

There were 5.55 SIM cycles/s and 5.55 scans/s acquired throughout the run. This yields 11 SIM data points and 11 scan data points across a typical peak.

Full scan data are also available for further PAH confirmation using library searching. Figure 2 shows a full scan spectrum from benzo[ghi]perylene, together with its library match. Unknown peaks for which SIM data were not acquired can also be library searched. A more reliable, faster method for identifying all the peaks is the use of Deconvolution Reporting Software [4].

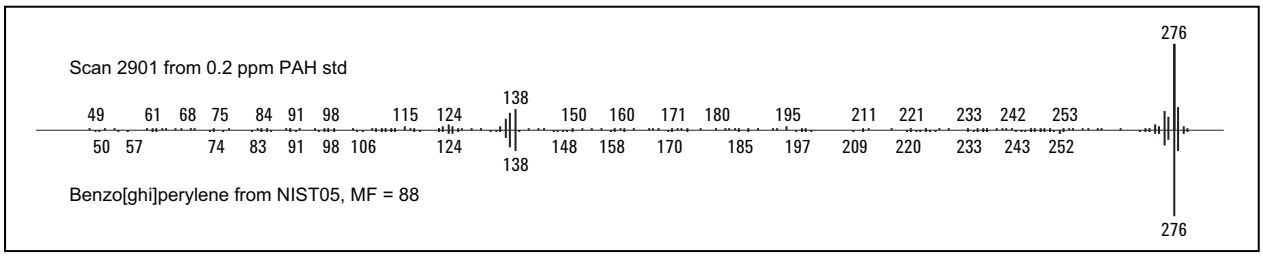

Figure 2. Spectrum from scan at 13.662 min with NIST05 Library match.

Conclusions

The 6890/5975 inert shows much improved response and peak shape for PAHs due to the inert source material and higher allowable source temperature. This improved response gives better linearity across the calibration range. Analysis of PAHs can be accomplished using synchronous SIM/scan data acquisition over a calibration range of 0.01 ppm to 10 ppm, while maintaining performance similar to SIM methods. Sensitivity of SIM is achieved while providing full scan data for confirmation of PAHs and identification of unknowns in a single run.

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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.

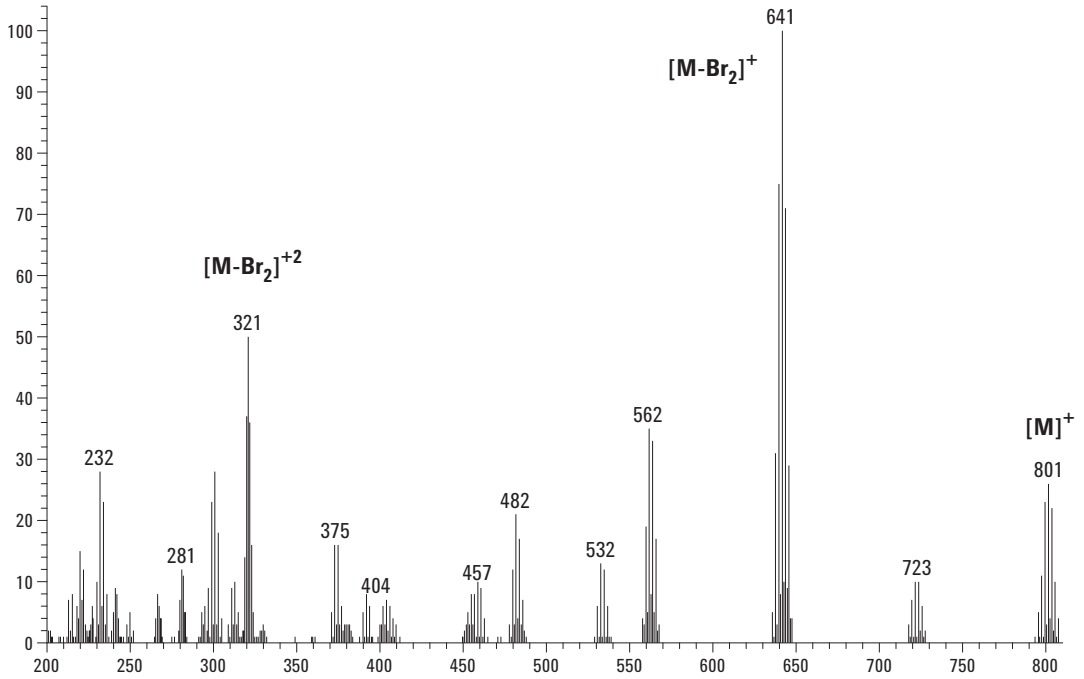

Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 *m/z*.

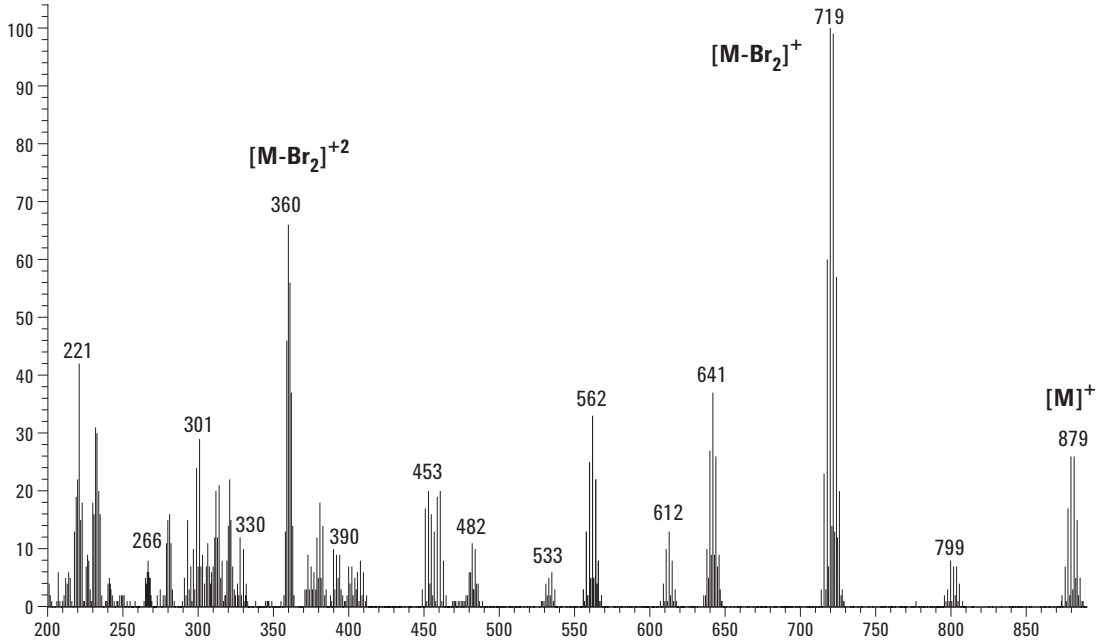

Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 *m/z*.

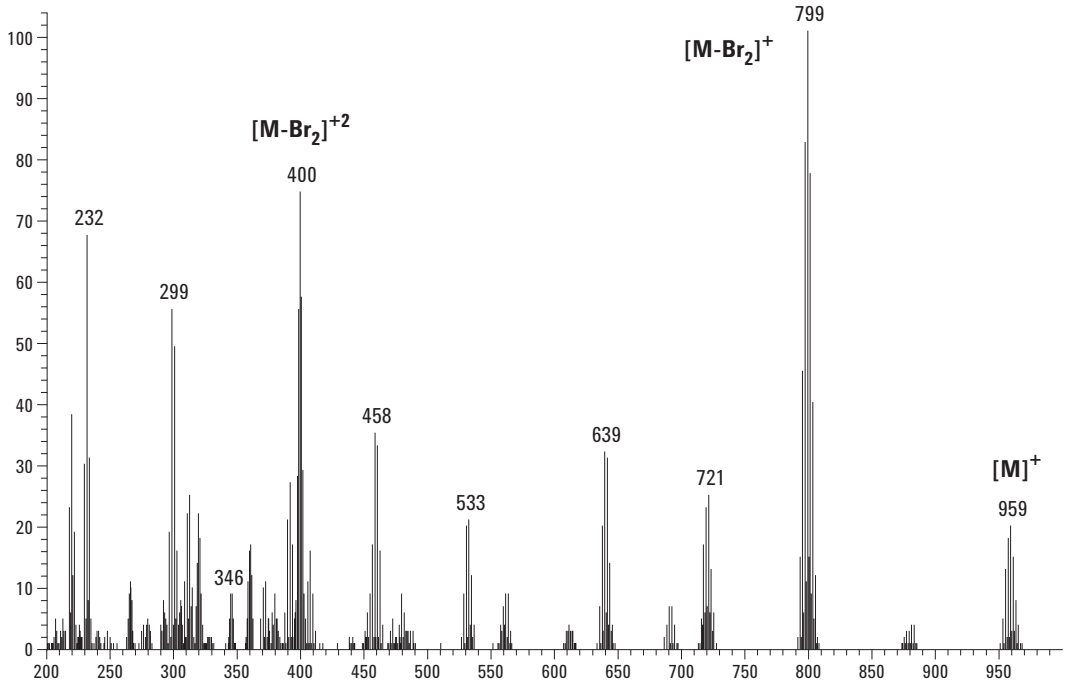

Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 *m/z*.

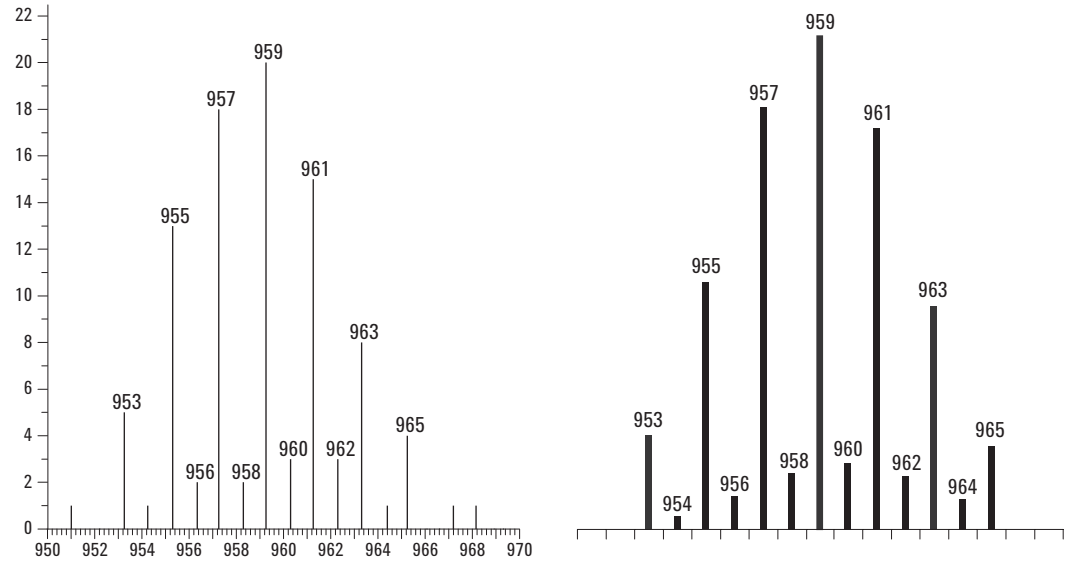

Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

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.

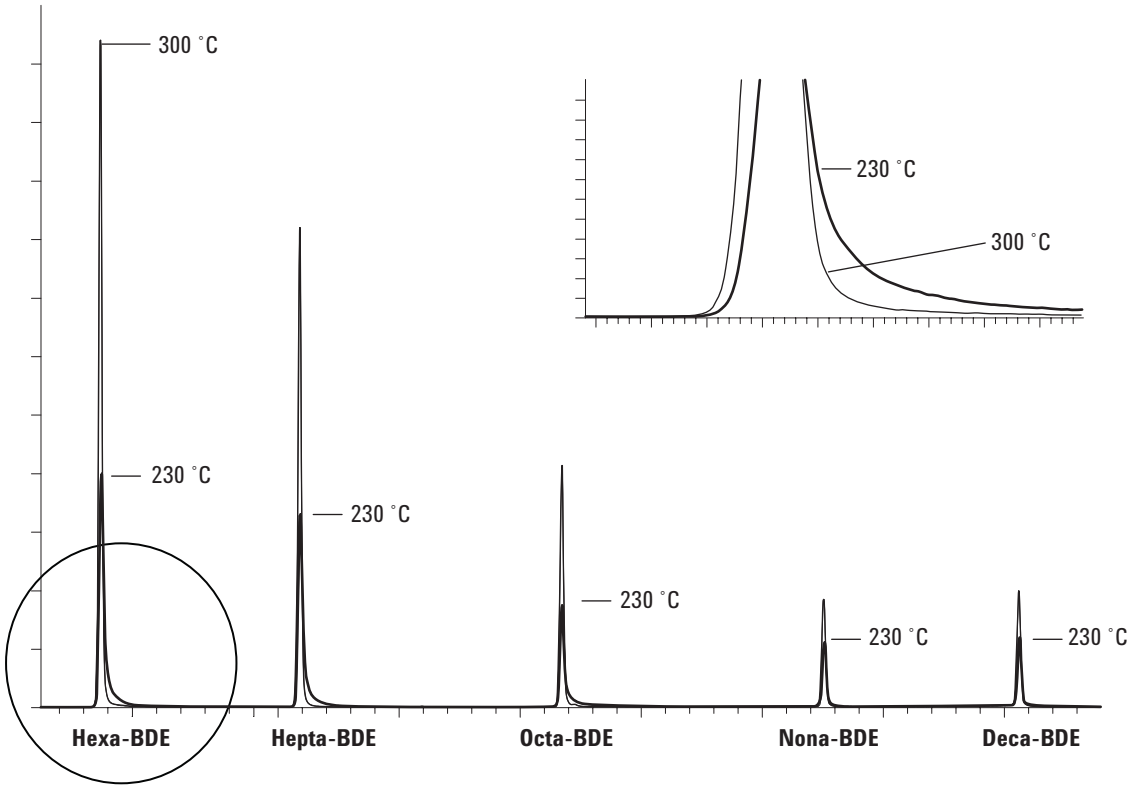

Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

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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Determination of Polybrominated Diphenyl Ethers in Polymeric Materials Using the 6890 GC/5973N inert MSD with Electron Impact Ionization

Application

Environmental, Component Testing

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Abstract

Due to their ubiquitous appearance in the ecosphere, various polybrominated diphenyl ether formulations have been banned. A major application of PBDEs is to impart fire retardancy to plastics used in electronics and electrical applications. This application note details an approach to determining the PBDEs present in the technical formulations in polymers. The instrumental analysis uses GC/MS with selected-ion monitoring (SIM) to determine tri-BDEs through the decaBDE in 15 minutes. Full scan spectra are presented for the PBDEs with interpretation and to provide an explanation of the choices in SIM ions. To insure correct identification of the PBDE isomers and allow rapid and convenient implementation in the laboratory, Retention Time Locking is applied to an internal standard. A sample preparation scheme referenced in this document provides two flexible and simple approaches to processing polymeric materials for this instrumental technique. PentaBDE, OctaBDE and DecaBDE technical formulations are characterized under the method and results for a typical high-impact polystyrene sample are also presented.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a major issue in discussions of persistent organic contaminants. The detection of PBDEs in essentially all compartments of the ecosystem, including human serum and breast milk, has resulted in a ban of the manufacture and use of certain PBDE formulations by the European Union (EU). Some companies have made it a policy not to allow these compounds in their components and have insisted their suppliers comply. Because the PBDEs are added at percent concentrations (as w/w), the usage of these formulations has been prodigious. Global consumption in 2001 was estimated at 7500, 3790, 56100 metric tons, for the PentaBDE, OctaBDE and DecaBDE technical formulations, respectively.

PBDE analysis even at these relatively high concentrations is challenging in several respects. The PBDEs are a complicated class of compounds and their utility in suppressing combustion also makes them relatively fragile and subject to degradation in GC analysis. This was demonstrated by using shorter GC columns to improve PBDE responses, the most significant improvement being for the deca-BDE (BDE 209) [1]. The loss in congener resolution is less important in this application because the technical mixtures most frequently applied in polymers predominantly consist of isomers extending from the tri-BDEs to the deca-BDE and far less than the 209 possible congeners. Distinguishing congeners on the basis of their electron impact (EI) mass spectrum may be possible since there appears to be some differences in their spectra, however the most reliable index remains retention time (RT). For this reason, compound

Retention Time Locking (RTL) is used to simplify identification and reproduction of the method in the user's laboratory.

Another complication is in sample preparation. There are several methods for extracting PBDEs from polymers each with advantages and disadvantages [2]. Of the many methods, the two approaches applied in processing samples for this application note are relatively inexpensive, simple, universal in application and in their acceptance, and allow for high sample throughput with minimal polymeric interferences. They are polymer dissolution and soxhlet extraction.

Experimental

Polymer samples were obtained from Agilent customers in the electrical and electronic component industries. Specific details of the polymer dissolution and soxhlet extraction methods are presented elsewhere [3]. In summary, the methods extract PBDEs from the sample via solvent, a dilution is made into toluene and PCB 209 is added to follow the dilution factor. Prior to injection, PCB 207 is added as an internal (injection) standard. Standards were made taking into account the potential percent concentration range of the PBDEs in polymeric samples and dilution factors used in the method.

PBDE standards were acquired from Cambridge Isotope Laboratories (Andover, MA) and AccuStandard (New Haven, CT). PCBs 209 and 207 were acquired from AccuStandard (New Haven, CT). Solutions were made in toluene of Burdick & Jackson solvent (VWR Scientific, San Francisco, CA).

Instrumental Configuration and Conditions

The 6890 GC and 5793N-inert MSD (mass selective detector) system configuration and conditions are given in Table 1. The GC is operated under constant flow conditions developed by applying RTL to lock the PCB 209 internal standard RT at 9.350 minutes. The 5973N inert MSD was equipped with the new Performance Electronics upgrade and allowed a single SIM group containing 24 ions to be used. The SIM ions are listed in Table 1 and were acquired with a dwell of 10-ms. This single SIM group method can be used to develop a preliminary method that can be further refined into multiple SIM groups by applying the AUTOSIM utility if the user wishes [4]. This is recommended for 5973-MSDs using standard electronics and targeting only congeners known to predominate in the particular technical mixture.

Table 1. GC and MSD Configuration and Parameters

Injection parameters

Injection mode	Pulsed splitless	
Injection volume	1 µL	
Injection port temperature	320 °C	
Pulse pressure and time	15.8 psi	1.80 min
Purge flow and time	50.0 mL/min	2.00 min
Gas saver flow and time	20.0 mL/min	3.00 min

DB-5ms Column and oven parameters

GC column	DB-5ms (15 m × 0.25 mm id, 0.1 µm film) (p/n: 122-5511)		
Flow and mode	1.8 mL/min	Constant flow	
RTL parameters	9.350 min	RTL compound PCB 209	
Detector and outlet pressure	MSD	Vacuum	
Oven temperature program	90 °C 20 °C/min	1.00 min 340 °C	 2.00 min
Oven equilibrium time	1.0 min		
Total program time	15.5 min		
MSD transfer line temp	320 °C		

Mass spectrometer parameters

Tune parameters	Autotune
Electron multiplier voltage	Autotune + 400V
Solvent delay	6.5 min
Quadrupole temperature	150 °C
Inert source temperature	300 °C

Mass spectrometer SIM ions for single group

405.8	246.0	123.0
485.7	325.9	162.9
563.6	403.8	201.9
643.5	483.7	241.9
721.5	561.6	320.8
799.4	641.5	360.7
719.4	461.7	399.7
463.7	497.7	499.7

*Optional addition of *m/z* 280.8

Miscellaneous parts

Septa	5182-0739	BTO septa (400 °C)
Liner	5181-3315	Deactivated 4-mm id double taper
GC column ferrule	5181-3323	250 µm Vespel/Graphite
MSD interface ferrule	5062-3508	0.4-mm id preconditioned vespel/graphite

Results

Chromatography

After evaluating a series of columns the DB-5ms phase seems the best choice overall, which is consistent with the literature [1]. The literature shows that the shorter columns and thinner films are of benefit to improving the PBDE responses, especially deca-BDE (PBDE-209) [1] and this approach is applied here. The benefit appears in both response and also in shorter analysis times; elution of deca-BDE occurs in less than 15 minutes. The separation on the DB-5ms phase seems sufficient for characterizing PBDE additives in polymers since the desire is not so much the complete separation as it is the overall composition and contribution of the various isomers [5]. Nonetheless, the short analysis time makes RT reproducibility and accuracy more critical for correct assignments of the various PBDE isomers and this is greatly enhanced by applying RTL. A list of the Retention Time Locked elutions of the most prominent PBDEs is presented in Table 2. For reference, Figures 1, 2 and 3 present chromatograms of PentaBDE, OctaBDE, and DecaBDE technical mixtures with approximate elution windows of the various isomers.

Table 2. Prominent PBDE Congeners and their Locked RTs

Compound name	RTL RT (min)
PCB 207	8.69
PCB 209 (locking compound)	9.350
PBDE 17 (tri Br)	6.89
PBDE 28 (tri Br)	7.08
PBDE 71 (tetra Br)	7.97
PBDE 47 (tetra Br)	8.09
PBDE 66 (tetra Br)	8.25
PBDE 100 (penta Br)	8.82
PBDE 99 (penta Br)	9.06
PBDE 85 (penta Br)	9.43
PBDE 154 (hexa Br)	9.62
PBDE 153 (hexa Br)	9.93
PBDE 138 (hexa Br)	10.31
PBDE 183 (hepta Br)	10.73
? hepta PBDE	11.07
PBDE 190 (hepta Br)	11.23
PBDE 204 (octa)	11.62
PBDE 203 (octa)	11.78
? PBDE 196 (octa)	11.84
PBDE 205 (octa)	12.00
PBDE 208 (nona)	12.56
PBDE 207 (nona)	12.64
PBDE 209 (deca Br)	13.60

Note - tentative identification of PBDE 196 was based on reference [1]

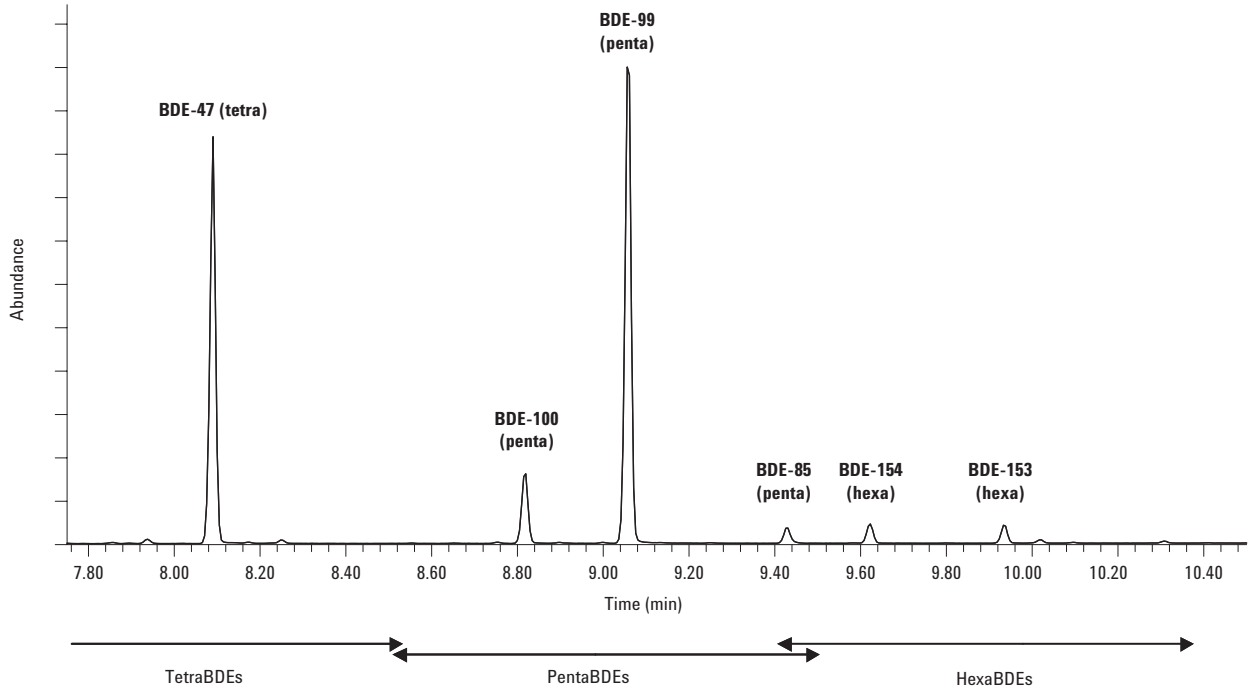

Figure 1 Reconstructed ion chromatogram (RIC) for the GC/MS EI-SIM acquisition of a PentaBDE technical mixture (Cambridge Isotope Laboratories).

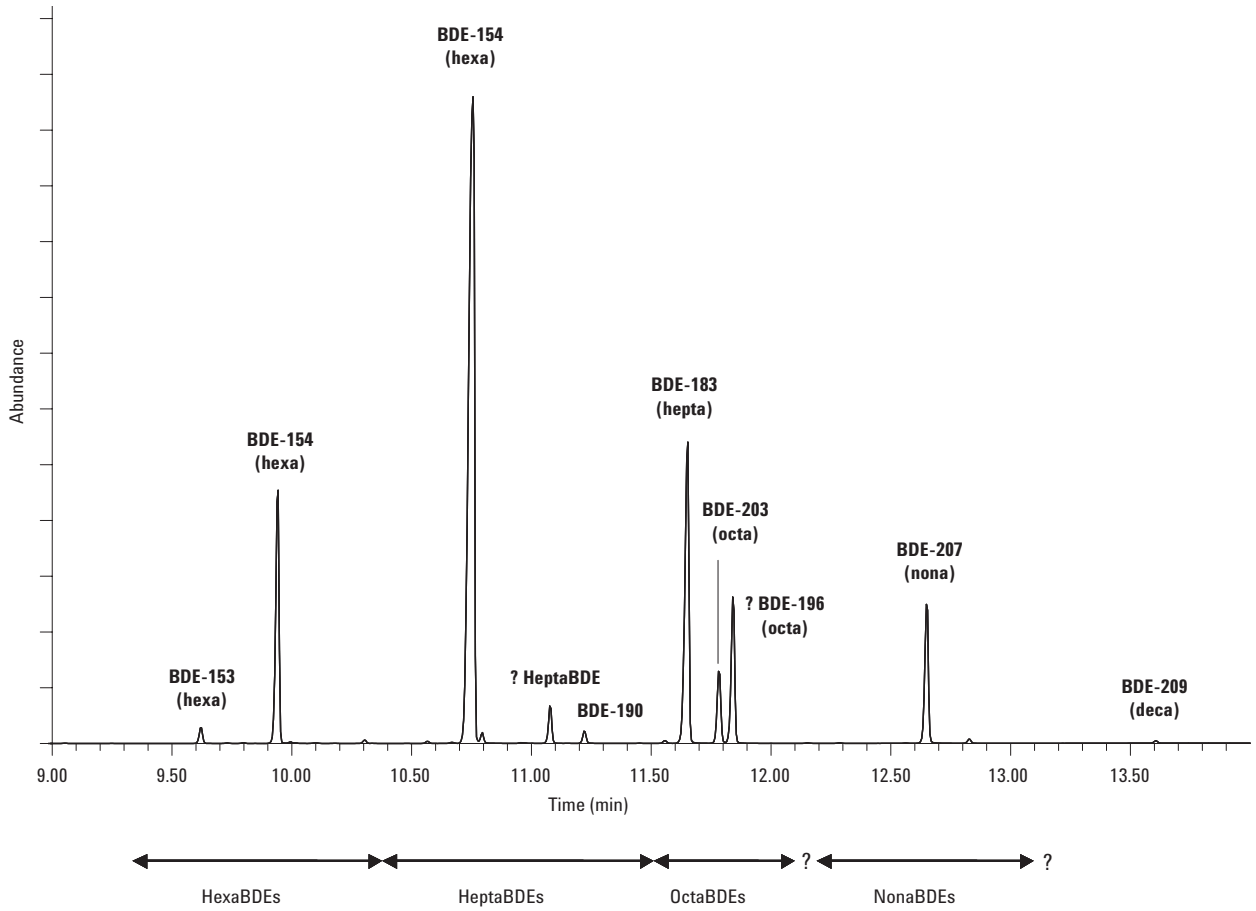

Figure 2 RIC for the GC/MS EI-SIM acquisition of a OctaBDE technical mixture (Cambridge Isotope Laboratories)

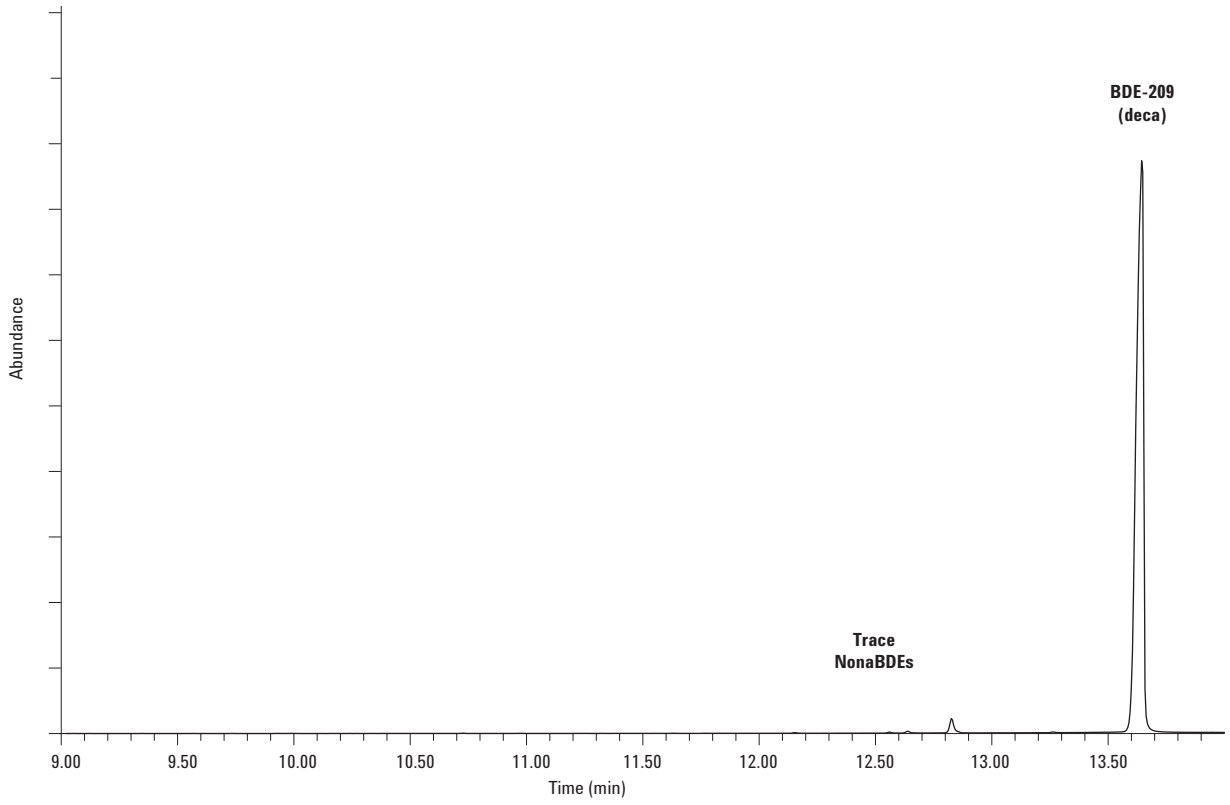

Figure 3 RIC for the GC/MS EI-SIM acquisition of a DecaBDE technical mixture (Cambridge Isotope Laboratories).

PBDE Spectral Interpretation

The EI ionization mass spectra of the PBDE congeners are rich in details and partially described in the literature [7]. Among the isomers the spectra are expected to be approximately identical in pattern and fragmentation pathway. Figure 4 presents a full scan spectrum of a hexabrominated-DE, PBDE-138, obtained at a source temperature of 300 °C. The spectrum shows the isotope cluster due to the molecular ion (643 *m/z*) and an intense cluster (484 *m/z*) consistent with the loss of Br_2. The mass assignment of the *m/z* 484 cluster is consistent with the result of $[M\text{-}Br_2]^+$, that is, $[C_{12}H_4OBr_4]^+$, and shows the tetrabrominated pattern (18 : 69 : 100 : 65 : 16). The next highest abundance isotope cluster appears around 242 *m/z*. Figure 4 shows this cluster and the cluster at *m/z* 484, $[M\text{-}Br_2]^+$. The isotope cluster patterns are similar, which suggests the same degree of bromination, but the fragment mass assignments are half those of the 484 cluster and mass spacing is not 2 but 1 *m/z* unit. While it is possible this is due to overlapping fragments, the close correspondence in patterns lead the authors to propose that this isotope cluster is due to double-charged fragments; that is, $[M\text{-}Br_2]^{+2}$. Recently, this assignment was confirmed by high-resolution MS and the results will be published elsewhere [8]. This $[M\text{-}Br_2]^{+2}$ fragment is common among the PBDEs congeners and grows in relative abundance as the degree of bromination increases: approximately in 10% tetraBDEs; 15% in pentaBDEs; 20%–25% in hexaBDEs and heptaBDEs; 45% in octaBDEs; 60% in nonaBDEs; and > 80% in decaBDE. Figures 5, 6, 7, 8 and 9 show spectra for several PBDEs. We have also observed the same phenomena for the polybrominated biphenyls (PBBs). We also find the ratios vary within an isomeric series more than in PCBs.

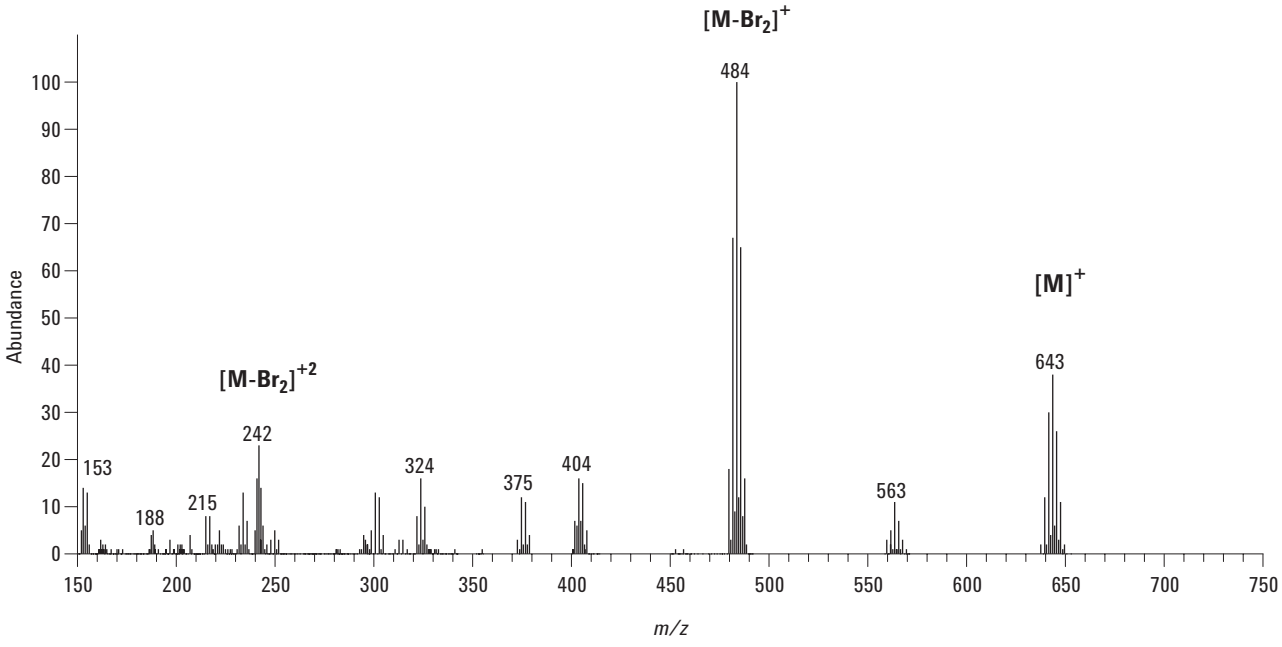

Figure 4 Normalized EI mass spectrum of a hexabrominated-DE, PBDE-138, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

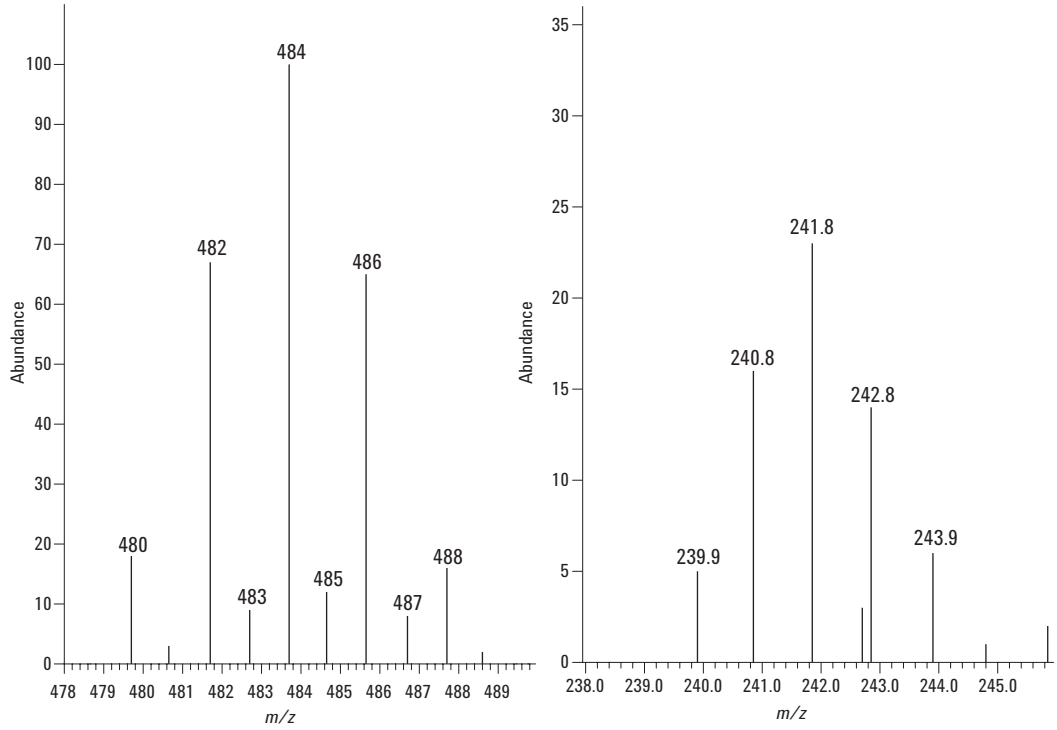

Figure 5 **Sections of the normalized EI mass spectrum of the hexabrominated-DE, PBDE-138, for the $[M\text{-}Br_2]^+$ and proposed $[M\text{-}Br_2]^{+2}$ clusters.**

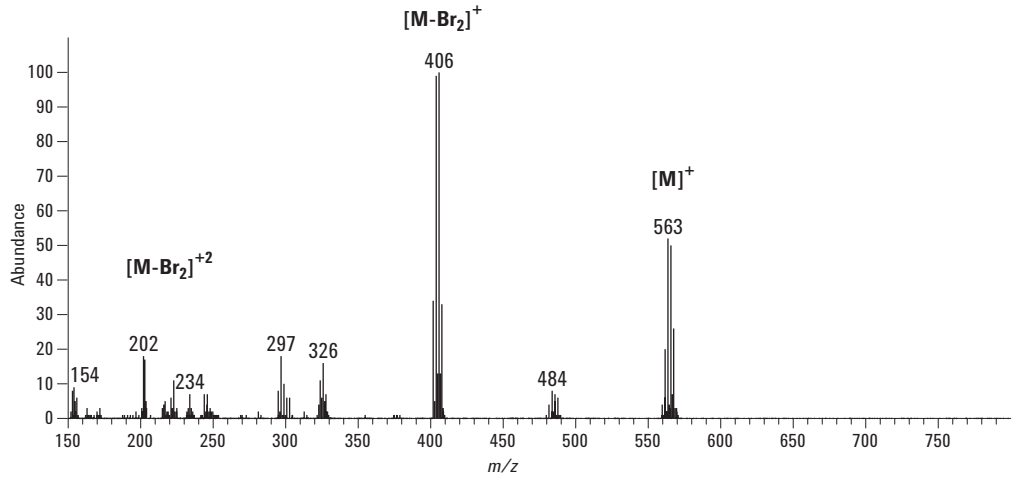

Figure 6 **Normalized EI mass spectrum of a pentabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.**

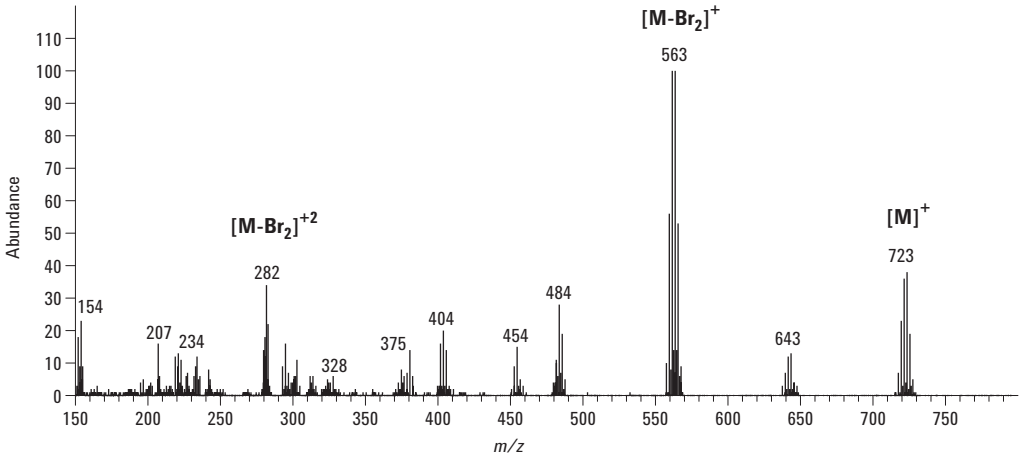

Figure 7 Normalized EI mass spectrum of a heptabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

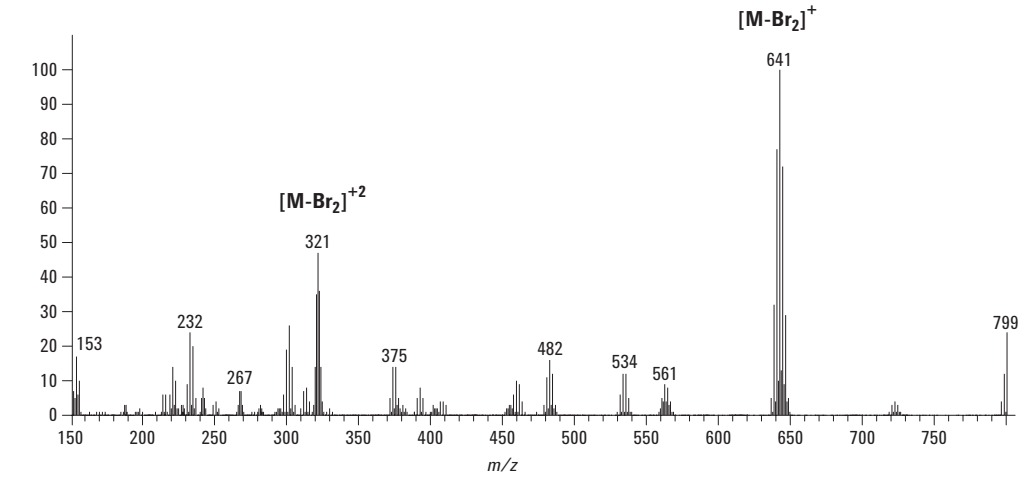

Figure 8 Normalized EI mass spectrum of a octabrominated-DE, PDBE-203, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

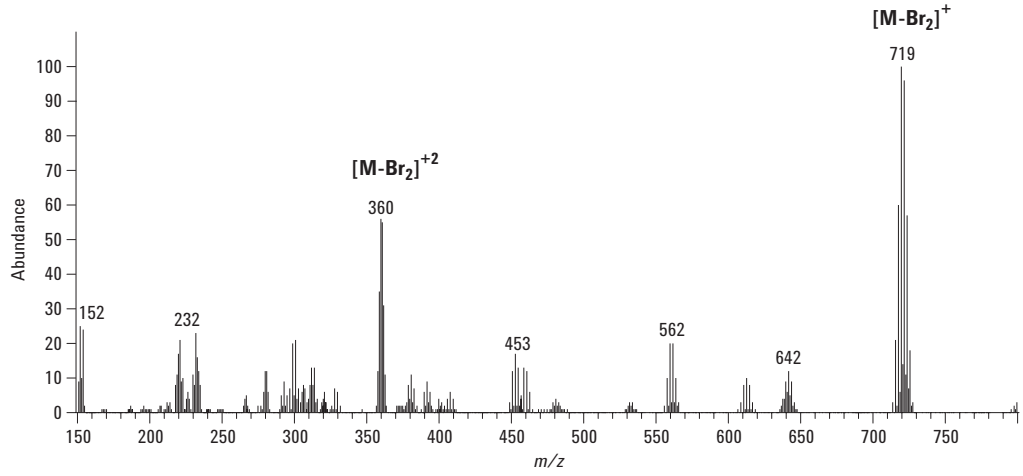

Figure 9 Normalized EI mass spectrum of a nonabrominated-DE, PDBE-208, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

In considering the EI spectrum of the decabromodiphenyl ether, PBDE-209, the same observations apply, Figure 10. Although the cluster of the molecular ion at 959 u, eludes the mass range limitation of the 5973N-MSD, the loss of Br_2 forms an intense isotope cluster at *m/z* 799, $[M\text{-}Br_2]^+$ and the doubly charged fragment(s) for the $[M\text{-}Br_2]^{+2}$ at *m/z* 400 (399.6) as shown in Figure 11. Other data has shown that the intensity of the molecular ion cluster (959 u) is far less than that of the fragments at *m/z* 799 as is the trend for the PBDEs.

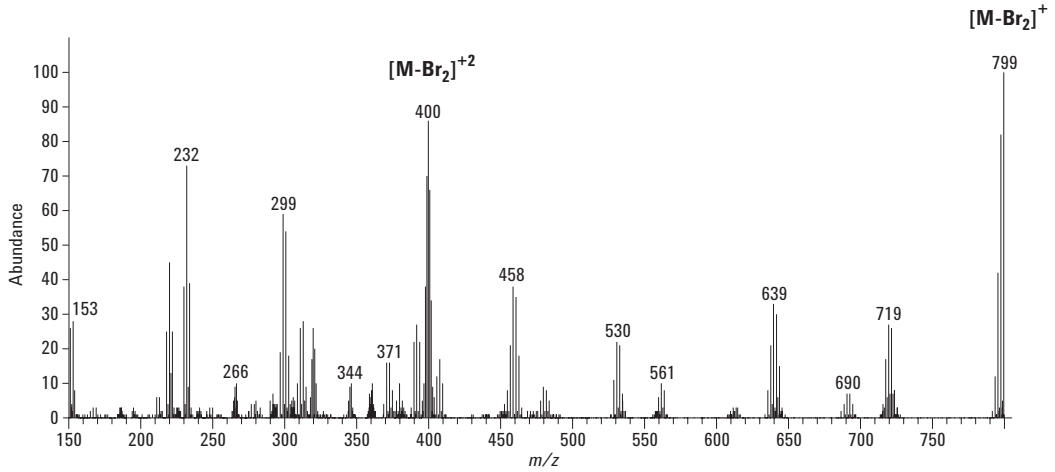

Figure 10 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

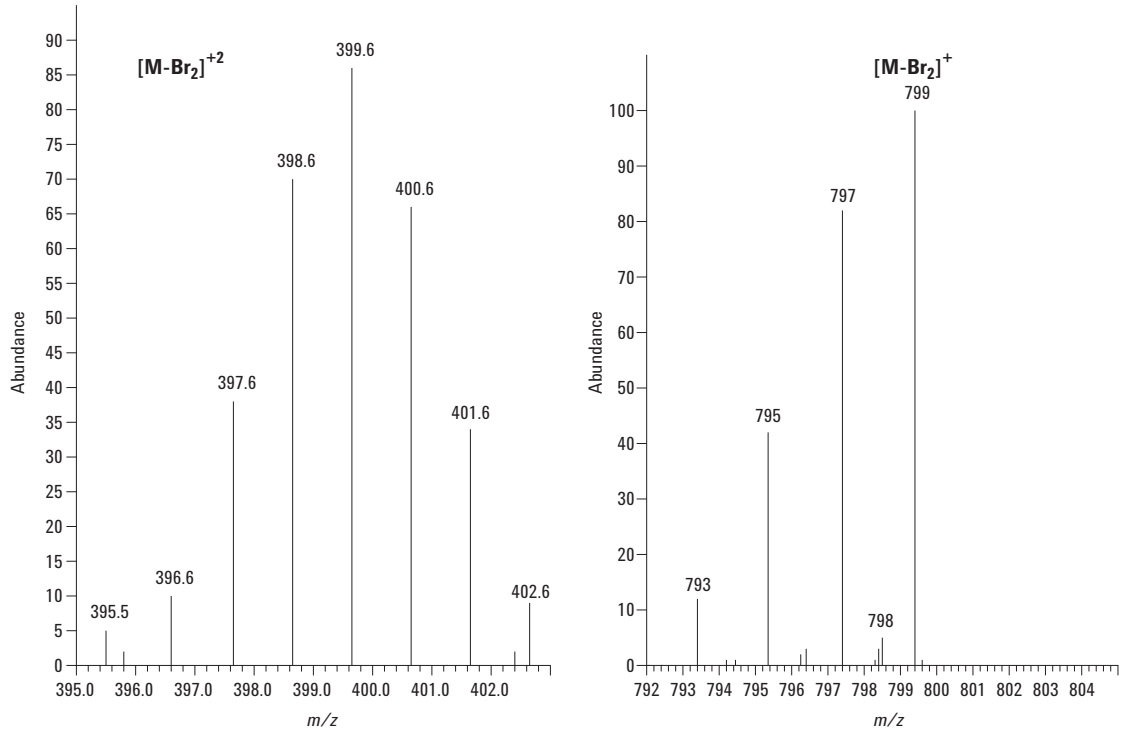

Figure 11 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

Therefore these ions (that is, $[M\text{-}Br_2]^+$, $[M\text{-}Br_2]^{+2}$ and $[M]^+$ where available), and compound RTs, identify and allow determination of the deca-BDE and other PBDEs to the ability of the 15-m column to separate the isomers, which appears quite effective and sufficient for characterizing additives. The monitored ions are given in Table 3 with the ions for the internal standards used in this analysis. Obviously, the bromines provide other ions displaced in mass by two units (except for the doubly-charged ions) that offer other additional ions for quantitation or confirmation.

Using the ions listed in Table 3 to identify the PBDE isomers, the regions in the chromatograms presented in Figures 1, 2 and 3 were labeled with the isomer elution windows. These ions and their ratios were also used to characterize PBDEs not available in the standards but found to occur within the samples and technical mixtures (for example, PBDE 196).

Results for Polymeric Samples

Extracting PBDEs from polymers requires that the entrained PBDEs permeate the polymer into the extracting medium. Apparently "melting" the polymer closes the transport corridors in the polymer and impedes extraction. However, "swelling" the polymer with a proper solvent, greatly improves the kinetics of extraction. Beyond deciding the proper solvent, the optimal time of the extraction must be experimentally determined for each plastic based on its consistency and response to the solvent. For the polymer dissolution and soxhlet extraction methods used here, solvent contact times or the number of soxhlet cycles for near complete extraction was determined by serial extraction. Other concerns are described in the sample preparation protocols [3].

Figure 12 shows the chromatogram for an extracted HIPS (high-impact polystyrene) polymer sample supplied by an Agilent customer and Table 4 shows the results for replicate extractions and analysis. Note the chromatogram and its major components closely resembles the chromatogram for the OctaBDE technical mixture (Figure 2) and indicates the specificity of the selected ions and most importantly, the lack of polymeric interferences. The reproducibility of the component compositions is a testament to the reproducibility of the total method. A good portion of the variance is introduced by the high dilution factors used in the method to bring the polymer extract concentrations with the scale of the PBDE standards and therefore discriminates against the lower abundance components producing a higher degree of variation and absolute detection. A series of 25 replicate injections of an extracted sample showed negligible degradation in response or chromatography. The robust performance is largely due to the high MSD ion source and quadrupole operating temperatures of 300 °C and 150 °C, respectively. These high temperatures mitigate the effect of co-extracted polymeric residues on the ion source optics to render robust performance. The high operating temperature of the quadrupole provides a very long lifetime without cleaning or maintenance even when analyzing very dirty matrices such as these.

Table 3. Quantitation and Confirmation Ions for the PB_nDEs (n>2)

PBDE bromination	$[M]^+$	$[M\text{-}Br_2]^+$	$[M\text{-}Br_2]^{+2}$	Confirmation ion
3	405.8	246.0	123.0	403.8
4	485.7	325.9	162.9	483.7
5	563.6	403.8	201.9	561.6
6	643.5	483.7	241.9	641.5
7	721.5	561.6	(280.8 **)	563.6/719.4
8	799.4	641.5	320.8	643.5
9	–	719.4	360.7	721.5
10	–	799.4	399.7	–
PCB 207	463.7	461.7	–	–
PCB 209	497.7	499.7	–	–

**The 280.8 and 281.8 *m/z* ions can be compromised by column bleed interferences so these were not used in acquisition although they provide a useful diagnostic for column degradation.

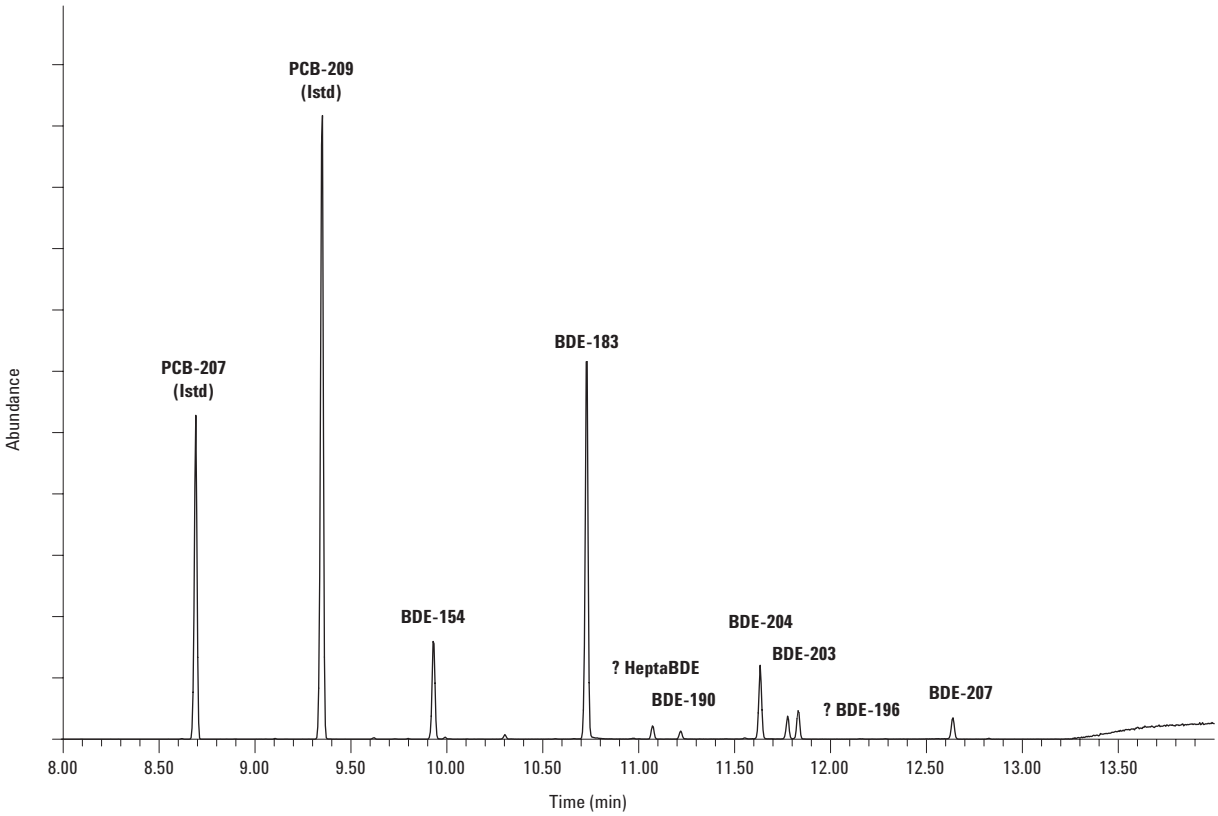

Figure 12 RIC of the GC/MS SIM acquisition of an extracted HIPS polymer sample.

Table 4. Extraction Results for Replicate Analysis of a Polymer Sample for PBDE Composition Using the Two Extraction and Sample Preparation Protocols [3]

Soxhlet polymer extraction protocol results

Sums	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Replicate 4 (%)	Replicate 5 (%)	SD
HexaBDEs	9.1	9.5	8.9	8.7	9.1	0.3
HeptaBDEs	53.3	52.5	51.7	53.1	53.1	0.7
OctaBDEs	29.5	29.5	30.7	29.5	29.8	0.5
NonaBDEs	8.0	8.4	8.6	8.7	8.1	0.3

Polymer Dissolution Extraction Protocol Results*

Sums	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	SD
HexaBDEs	9.9	10.0	9.7	0.2
HeptaBDEs	55.3	56.2	55.9	0.5
OctaBDEs	34.8	33.8	34.4	0.5

SD standard deviation

No tri-DEs, tetraBDEs, pentaBDEs, or decaBDE were detected.

*A difference in analyte lists used to quantitate the soxhlet extracts slightly skews the results, specifically the addition of the nona-BDE analytes. Removing this group, the results agree within 3%.

Remarks

Figure 13 presents two overlaid reconstructed ion chromatograms of the SIM acquisitions of two splits of a single PBDE standard. One of the splits was contained in a clear vial and was exposed to laboratory light for about a week and the other split was stored in amber vial and in a freezer as a reference. The most impressive feature is the dramatic loss of the decaBDE and the possible appearance of another intense nonaBDE (around 11.8 minutes). Note the nonaBDEs in the standard showed no degradation while the octaBDEs and heptaBDEs showed varying degrees of loss in concentration. A number of small peaks appear in the baseline that suggest, on the basis of their fragments, ion ratios, and proximity to existing PBDEs in the standard, the presence of other BDE isomers. Assigning any identification in SIM without a standard reference compound to confirm RT and fragment ratios, or a full scan acquisition, must be considered highly speculative. However, the data does indicate a degradation of the decaBDE and some other PBDEs, and suggests possible isomerization of the some PBDEs under the influence of typical laboratory fluorescent lights. Time and resources do not allow us to pursue this matter, but we provide these observations since there are implications in sample handling and standard preparation and storage.

Conclusions

The 5973N inert MSD equipped with performance electronics allows a single SIM group to survey for PBDE isomers important to characterizing the technical formulations of the PBDEs. Using a single group has the advantages of allowing many formulations to be studied without regard to the particular elution of the congeners (which would require careful maintenance of SIM windows), simplified setup and very rapid analysis. Implementing RTL allows specific congeners to be characterized and quantitated with high confidence. The intense fragmentation of the PBDEs and their universal propensity to form $[M\text{-}Br_2]^+$ and $[M\text{-}Br_2]^{+2}$ ions provides a unique fingerprint for each degree of bromination. The 15-m column used here provides rapid analysis and sufficient class separation. The method is universally applicable regardless of the sample preparation scheme as demonstrated here by replicate polymer analysis by two techniques, soxhlet extraction and polymer dissolution.

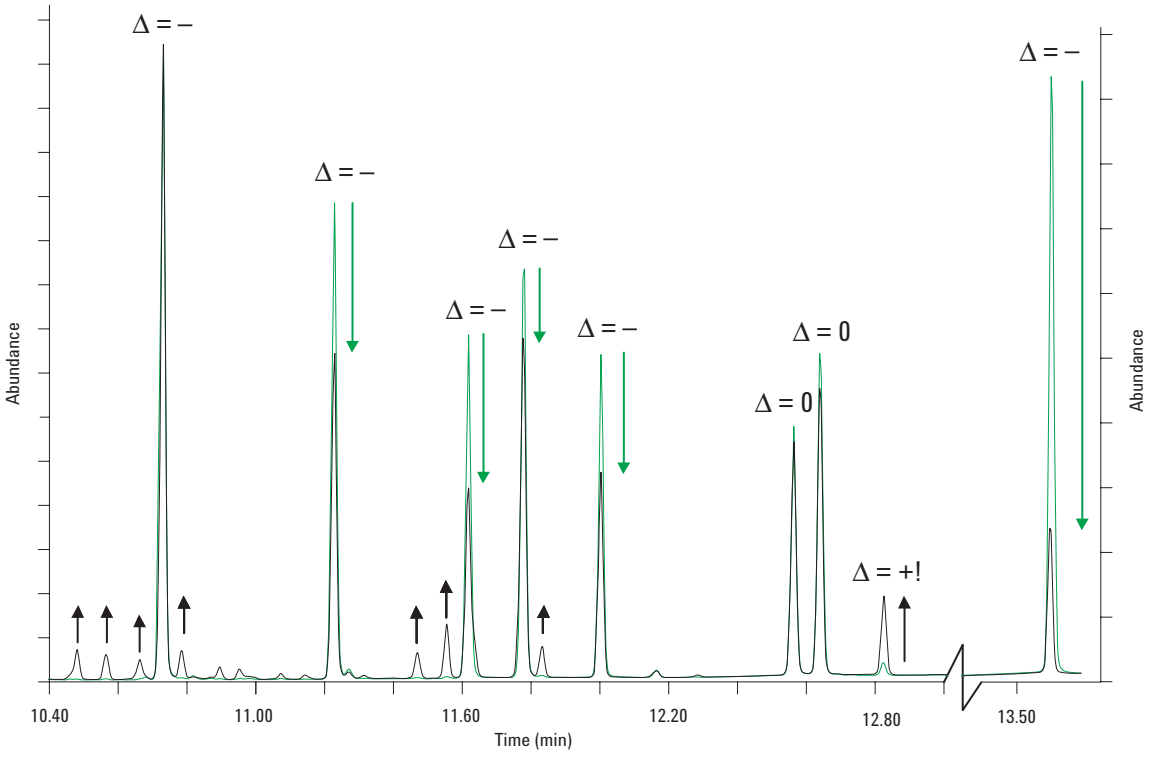

Figure 13. PBDE standard unexposed (green) and exposed to laboratory light. Delta (Δ) indicates change in response as Exposed-Unexposed (with negative signs indicating loss in response and positive an increased response).

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Agilent Technologies

Full-Scan Low-Level Polynuclear Aromatic Hydrocarbon Analysis Using the Agilent Technologies 6890/5973 inert Gas Chromatograph/Mass Selective Detector

Application

Environmental

Author

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Abstract

The analysis of polynuclear aromatic hydrocarbons presents challenges due to the tendency of the polynuclear aromatic hydrocarbons to adsorb on surfaces in the chromatographic system. This results in calibrations that are not linear and the need to run selected ion monitoring for low-level analysis. The Agilent Technologies 6890/5973 inert gas chromatograph/mass selective detector system is designed for improved polynuclear aromatic hydrocarbons analysis using full scan while maintaining linearity across a wide calibration range.

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are produced during combustion of organic material and are suspected carcinogens. The high amounts and widespread occurrence of these compounds in our environment requires reliable, sensitive, and very robust analytical methods.

PAHs, especially the high molecular weight ones, tend to be adsorbed on any active or cold site in a gas chromatographic system. Additionally occurring inlet discrimination often further reduces the number of compounds with higher boiling points that are transferred onto the column. Therefore, typical PAH analyses on a gas chromatography (GC) or gas chromatography/mass selective detector (GC/MSD) system show decreasing response and sensitivity with increasing molecular weight.

The Agilent 6890/5973 inert GC/MSD system has features to overcome this negative trend, including a new uncoated solid-source material and higher temperature filaments. Using a direct-connect inlet liner also improves chromatographic peak shape and sensitivity.

Many laboratories calibrate for PAHs from 0.1 to 10 ppm using Selected Ion Monitoring (SIM) for low level work. Historically, SIM has been necessary because of instrument sensitivity considerations and loss of PAHs at the lower concentration levels, although full scan data is preferred for further confirmation of the compounds.

This application note will show the performance of the Agilent 6890/5973 inert for PAHs using a calibration range of 0.1 to 10.0 ppm in full scan mode with linearity equal to that of many SIM methods.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet	EPC split/splitless		
Mode	Pulsed splitless, 1 µL injected		
Inlet temperature	300 °C		
Pressure	12.64 psi		
Pulse presssure	30.0 psi		
Pulse time	0.30 min		
Purge flow	30.0 mL/min		
Purge time	1.0 min		
Total flow	34.6 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Direct Connect, deactivated, 4-mm id, Agilent part number G1544-80700		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		50	1.00
Ramp 1	25	200	0.00
Ramp 2	8	316	0.00
Total run time	21.50 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS part number 19091S-433		
Length	30.0 m		
Diameter	250 µm		
Film thickness	0.25 µm		
Mode	Constant flow		
Flow	1.5 mL/min		
Initial pressure	12.64 psi		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 inert		
Drawout lens	6-mm ultralarge aperture, Agilent part number G2589-20045		
Solvent delay	3.00 min		
EM voltage	Run at DFTPP tune voltage = 1000 V		
Low mass	45 amu		
High mass	450 amu		
Threshold	0		
Sampling	2		
Scans/s	3.58		
Quad temp	180 °C		
Source temp	300 °C		
Transfer line temperature	280 °C		
Repeller voltage	DFTPP tune value		
Emission current	DFTPP tune value = 34.6 µamp		

Calibration standards

Calibration standards were diluted in dichloromethane from a stock mix of the 13 PAHs. The seven levels made were 10, 5, 2, 1, 0.5, 0.2 and 0.1 ppm. The perylene-d12 internal standard (ISTD) and the two surrogate standards, 1,3-dimethyl-2-nitrobenzene and triphenylphosphate, were added to each calibration level at 2.0 ppm.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions that may have to be optimized.

The Agilent 6890 inlet temperature was set to 300 °C, instead of the typical 250 °C, to minimize compounds adsorbing on the liner surface. Pulsed injection was used to facilitate quantitative transfer of the heavier PAHs onto the column, minimizing inlet discrimination. Pulsed injection parameters are easily set in the ChemStation software and are automatically controlled by the electronic pneumatic control (EPC) module.

The Direct Connect inlet liner allows for complete transfer of analytes onto the column. The column inlet end attaches to the liner and minimizes analyte exposure to the stainless steel annular volume in the inlet.

The Agilent 5973 inert was tuned using the automatic DFTPP target tune, as required by some Government methods. The ultralarge aperture drawout lens was used to maintain linearity across the wide calibration range. Source temperature was set to 300 °C, which is now possible with the high temperature filaments. This higher source temperature in combination with the new source material produces better peak shapes for the PAHs.

Results

The system was calibrated at seven levels, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ppm using full scan data acquisition. The total ion chromatogram (TIC) for the 0.2-ppm level is shown in Figure 1. Each calibration level contained 13 PAHs, perylene-d12 internal standard (ISTD) and the 2 surrogate standards, 1,3-dimethyl- 2-nitrobenzene and triphenylphosphate.

The relative response factor (RRF) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the percent relative standard deviation (%RSD) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Microsoft® Excel.

Linearity is shown in Table 2. The %RSD of the RRFs are shown for each of the PAHs. All RSDs are less than 5%. This level of performance is equal to that of most SIM methods for PAHs.

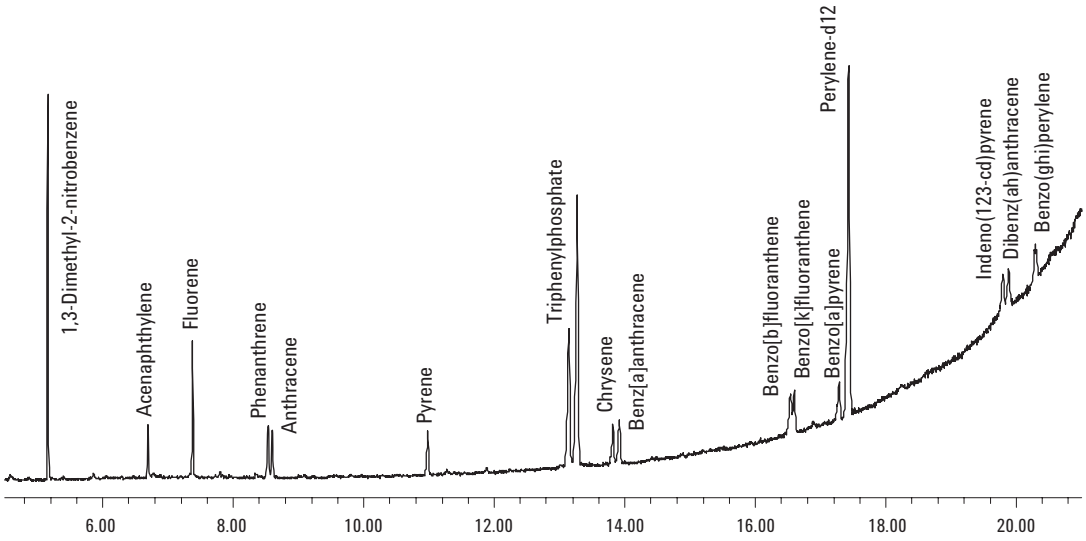

Figure 1. Thirteen PAHs at 0.2 ppm each with surrogates and ISTD at 2.0 ppm each.

As further proof of system inertness and sensitivity, a 0.01-ppm level spike was analyzed. This sample was quantitated against the seven level calibration curve using average response factor. The results are shown in Table 2. These results are excellent considering this is full scan data and the spike level was 10× lower than the lowest calibration point.

Table 2. %RSD of RRF from Seven Level Calibration and 0.01-ppm Spike Results

	%RSD	0.01 ppm Spike
Perylene-d12	3	–
1,3-dimethyl-2-nitrobenzene	1	2.100
Acenaphthylene	3	0.011
Fluorene	3	0.010
Phenanthrene	3	0.010
Anthracene	3	0.011
Pyrene	3	0.010
Triphenylphosphate	1	1.940
Chrysene	2	0.009
Benz[a]anthracene	3	0.010
Benzo[b]fluoranthene	2	0.009
Benzo[k]fluoranthene	4	0.010
Benzo[a]pyrene	2	0.010
Indeno(123-cd)pyrene	4	0.010
Dibenz(ah)anthracene	2	0.007
Benzo(ghi)perylene	3	0.011

Conclusions

The Agilent 6890/5973 inert shows much improved response and peak shape for PAHs due to the inert source material and higher allowable source temperature. This improved response gives better linearity across the calibration range. Analysis of PAHs can be accomplished using full scan data acquisition over a calibration range of 0.1 to 10 ppm, while maintaining performance similar to SIM methods.

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Agilent Technologies

Fast Semivolatiles Analysis using the Agilent Technologies 6890/5973 inert GC/MSD

Application

Environmental

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Abstract

The analysis of semivolatiles presents challenges due to the simultaneous measurement of acids, bases, and neutrals over a wide concentration range. Due to productivity demands, laboratories want to run faster while maintaining linearity and sensitivity for even the most active compounds. The Agilent Technologies 6890/5973 inert gas chromatography/mass selective detector system is designed to meet the criteria for fast analysis, while minimizing activity and maintaining linearity.

Introduction

Semivolatiles analysis concurrently measures a mix of acids, bases, and neutrals. This mix presents a challenge for instrument design due to the interaction of the analytes with the instrument and consumables. Most laboratories analyze for 70–100 compounds with a chromatographic run time of 25–40 minutes. The calibration range required for the analysis varies dependent on a particular laboratory's statement of work. Historically a range of 20–160 ng was used. With the increased sensitivity of newer gas chromatography/mass spectrometry (GC/MS) systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the calibration range down to 5 ng.

The Agilent 6890/5973 inert gas chromatograph/mass selective detector (GC/MSD) system was designed to meet the demand for these lower MDLs. A new uncoated solid source material has shown improved performance for the most active compounds, such as 2,4-dinitrophenol.

This inert source allows for less material injected onto the column while maintaining mass spectrometer performance. Split injections are possible where only splitless would suffice before. The ability to do split injections matches very well with smaller diameter columns such as 100 µm. These smaller columns provide for run times of 10 minutes or less.

This application note will show the performance of the Agilent 6890/5973 inert for semivolatiles using a 100-µm id column with a run time of 7.5 minutes and a calibration range of 5–200 ng.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet	150 psi EPC split/splitless		
Mode	Split, 1 µL injected		
Split ratio	10:1		
Inlet temp	250 °C		
Pressure	118 psi		
Split flow	22.8 mL/min		
Total flow	26.9 mL/min		
Gas saver	Off		
Inlet liner	Siltek™ Cyclosplitter, 4-mm id, Restek part number 20706-214.1		
Oven	240 V		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	0.20
Ramp 1	45	320	1.58
Total run time	8.0 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS Custom		
Length	12.5 m		
Diameter	100 µm		
Film thickness	0.1 µm		
Mode	Ramped flow		
Flow	mL/min[2]	mL/min	Hold min
Initial		2.3	0.10
Ramp 1	10	0.8	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 inert		
Solvent delay	0.95 min		
EM voltage	Run at DFTPP tune voltage = 1200 V		
Low mass	35 amu		
High mass	500 amu		
Threshold	0		
Sampling	1		
Scans/s	5.92		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	280 °C		
Repeller voltage	DFTPP tune value		
Emission current	DFTPP tune at 35 µamp, run at 25 µamp		

Calibration standards

Accustandard, New Haven, CT. Part number M-8270-IS-WL-0.25x to 10x, 77 compounds at eight concentration levels with six ISTDs at 40 ppm.

The Agilent 6890 with a 150 psi inlet (option) is necessary for both the initial high flow during injection and to maintain constant flow during the run. A 10:1 split is used to match the column capacity to the calibration concentration range. Higher splits can be used but splitting less or using splitless will cause peak overload and too much distortion for good integration.

The inlet liner was found to be of low activity, as it does not contain glass wool. Proper mixing for split injections is done by the internal liner geometry. This liner was also found to perform adequately for higher split ratios and for splitless.

The Agilent 6890 240 V oven was necessary for the 45 °C/min oven program ramp used.

The custom order HP-5MS column was obtained in a 20 m length and cut down to 12.5 m. The ramp flow allows for faster transfer of analytes onto the column to minimize exposure to the inlet liner. Ramp flows are easily set by the software and are accomplished with electronic pneumatic control (EPC).

The Agilent 5973 inert was tuned using the automatic DFTPP target tune, as required by some Government methods. After tuning, the emission current was manually set to 25 µamp. This was done to maximize linearity for easily ionized compounds. The sampling rate was changed from the default of 2 to 1, while preserving sufficient sensitivity. The resultant 5.92 scans/s yields a minimum of eight data points across the narrowest peaks.

Results

The system was calibrated at eight levels, 5, 10, 20, 50, 80, 120, 160, and 200 ppm. The total ion chromatogram (TIC) for the 5-ppm level is shown in Figure 1. Each calibration level contained 77 compounds together with six internal standards (ISTDs) at 40 ppm.

The relative response factor (RRF) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the percent relative standard deviation (%RSD) of the RRFs across the calibration range for each compound and was performed automatically by the software in conjunction with Microsoft® Excel.

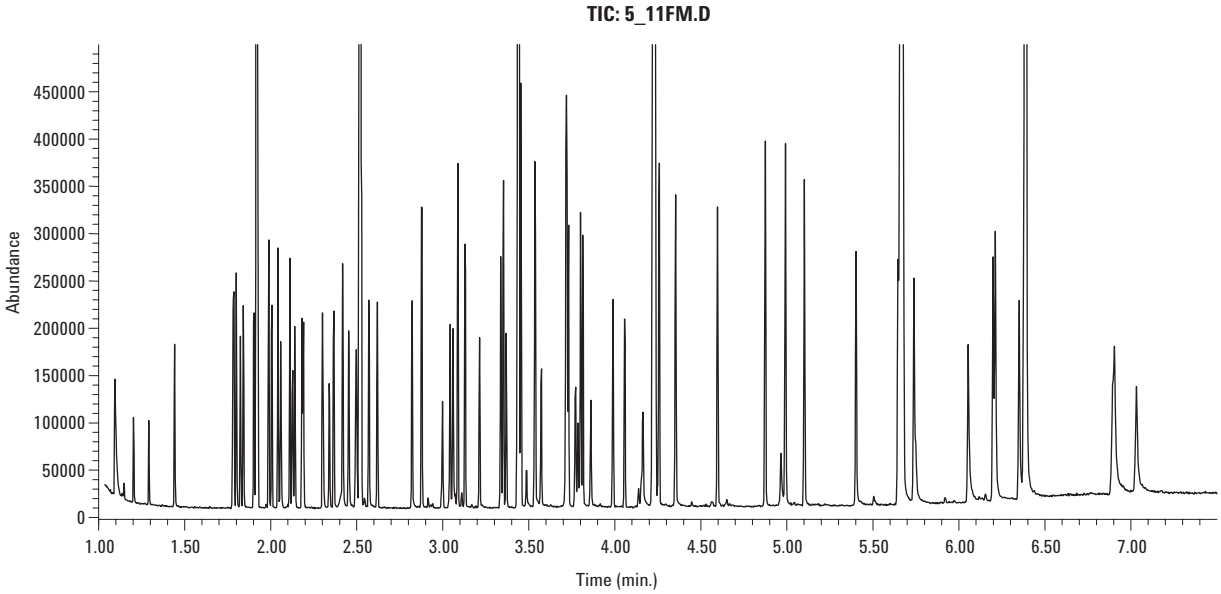

Figure 1. Five ppm each 77 semivolatiles and six ISTDs at 40 ppm each.

There are published Government Methods, such as USEPA Method 8270D for Semivolatiles, that specify criteria for suitable RRFs and %RSD. In Method 8270D, minimum system performance of four active compounds, the system performance check compounds (SPCCs) is measured by the average RRF.

Table 2 lists the Method 8270D SPCC criteria, and performance of the Agilent 5973 inert together with an Agilent 5973 system. The Agilent 5973 inert data exceeds the 8270D criteria. The Agilent 5973 inert also shows exceptional results compared to the Agilent 5973. These results are superior because they were run 10:1 split, putting 10× less compound on column than those run on the Agilent 5973.

Table 2. SPCCs, Comparison of Average RRF

	8270D Criteria	Agilent 5973 inert	Agilent 5973
Calibration range, ppm		0.5–20	5–160
N-Nitroso-di-n-propyl amine	0.050	1.146	0.970
Hexachlorocyclopentadiene	0.050	0.284	0.253
2,4-Dinitrophenol	0.050	0.188	0.075
4-Nitrophenol	0.050	0.236	0.162

Linearity is shown in Table 3. The 77 compounds were grouped as indicated. The RSDs of the RRFs were averaged to show performance for entire compound classes, not just a few selected analytes. The linearity of the Agilent 5973 inert is significantly better than the Agilent 5973 across the same concentration range and across an extended range.

Table 3. Average RSDs of RRFs by Compound Class

	Agilent 5973 inert	Agilent 5973 inert	Agilent 5973
Calibration range, ppm	0.5–20	2–16	20–160
Miscellaneous base neutrals (19)	8	5	11
Acids (17 phenols, dinitrophenols)	8	5	11
Bases (12)	8	6	12
Phthalates, ethers (13)	9	6	12
PAHs (16)	7	5	8

Conclusions

The Agilent 6890/5973 inert shows improved response for active compounds such as nitrophenols at low levels. This improved response gives better linearity across the calibration range. Split injections are now possible while maintaining sufficient response and fast analysis can be done using 100-µm columns. Analysis of 77 analytes and six ISTDs can be accomplished in less than 8 minutes over an extended calibration range of 0.5 ppm to 20 ppm.

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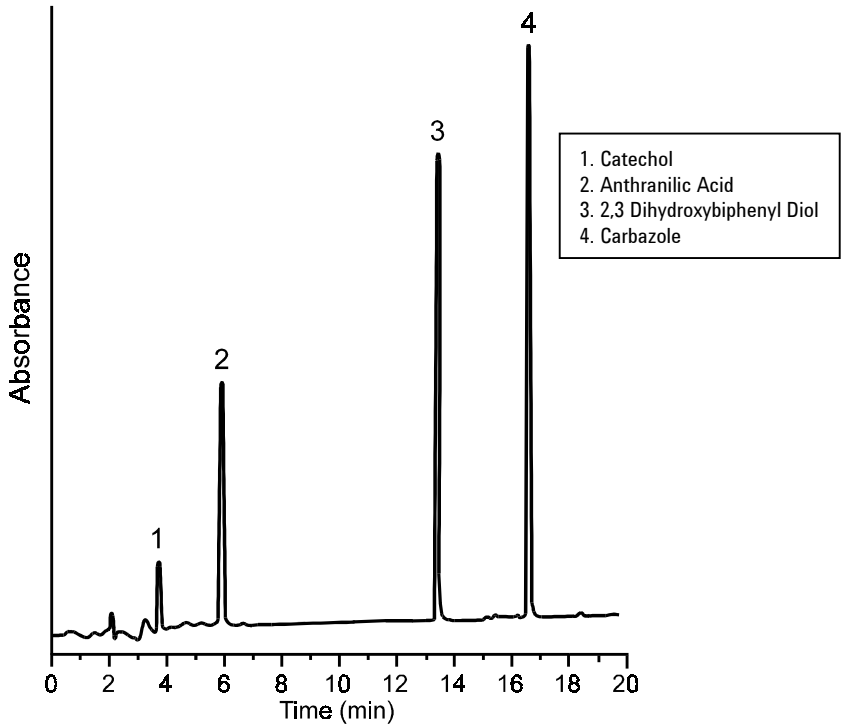
Separation of Catechol and Various Metabolites

Application

Environmental

Robert Ricker

Carbazole is an environmental pollutant of some concern. Sensitive detection of carbazole and related metabolites provides a means of following its breakdown in various sources. The postulated pathway is: **carbazole → 2,3-aminobiphenyl diol → anthranilic acid → catechol**. (The 2nd intermediate is not commercially available and was replaced with 2,3-dihydroxybiphenyl diol, to act as a related marker.)



Courtesy of Phillip Gibbs, Energy Biosystems Corp. The Woodlands, TX;
Rich Willson, University of Houston, Dept. Chemical Engineering, Houston, TX

Conditions:
ZORBAX 300 SB-C18 (4.6 x 150 mm) (Agilent P/N: 883995-902)
Mobile Phase: Gradient 5-60% in 20 min., Wash, 95%B for 2 min.
A) H_2O with 0.1% TFA to pH3 with TEA; B) ACN with 0.085% TFA
Injection 15µL, 250-500 µg/mL each, 1 mL/min, 65°C, Detect. UV (233 nm)

Highlights

- *The resolution of peaks 1 and 2, not previously obtained, was achieved using the ZORBAX 300SB-C18 at low pH.*
- *The steric protecting groups of the ZORBAX SB-C18 stabilize the bonded phase, permitting stable and reproducible chromatography at pH ≤ 1.*

Agilent Technologies

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Agilent Technologies

Retention Time Locked GC-MS Analysis of Phenols

Application

Environmental

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Abstract

Comprehensive retention time locking, gas chromatography-mass spectrometry methods for more than 50 phenols and their common internal standards are available. Retention time locking makes the phenol retention times on DB-XLB and DB-5ms columns universal and permanent. "Universal" means that any analyst using an Agilent 6890 Plus Gas Chromatograph and 5973N Mass Selective Detector can reproduce the retention times of every listed phenol. "Permanent" means that the phenol retention times will remain unchanged with typical column maintenance such as column trimming or replacement. This is particularly useful in making definitive assignments of phenol identity from among the many possible isomers that may be present in the samples but may not be on hand in the laboratory standards. A high resolution method and a more rapid "Fast Quant" method were developed for both columns. The high resolution programs have analysis times of 31 and 21 minutes while the "Fast Quant" method runtimes are reduced to 16 and 14 minutes for DB-XLB and DB-5ms columns, respectively. Both columns produce minimal column bleed resulting in improved mass spectral determinations. They also provide excellent separation of the phenols, the DB-XLB column achieving slightly better separation than the DB-5ms.

Introduction

Phenols are widely used compounds and their substituted derivatives are manufactured for use in plastics, drugs, dyes, preservatives, insecticides, fungicides, antiseptics, and disinfectants. They also occur as by-products of various industrial activities such as paper and pulp processing, coal gas liquification, and coke production. This multi-industry use has lead to widespread environmental contamination by phenolic compounds.

Use of chlorine as a bactericide in water treatment results in chlorinated species that rapidly react with phenol to form various substituted chlorophenols. As the degree of substitution increases, acidity, lipophilicity and tendency to bioaccumulate and become toxic to aquatic life increases. These chlorophenols are also added to provide or enhance anti-bacterial properties of various products. Recently, representatives of the German, Danish and Swedish environmental ministries have advised consumers against use of anti-bacterial soaps, many of which contain chlorophenols, in part because of their environmental effects.

Nitrophenols tend to be among the most toxic of the phenols and their alkylated derivatives are commonly used as herbicides and pesticides. It is a startling and disturbing fact that 60 years ago 2,4-dinitrophenol was ingested to cause weight loss. Reputedly, it is becoming popular again despite well documented risks.

As a family, phenolic compounds exhibit a wide range of properties due to the numerous substitution derivatives possible, the most common of which are chlorinated, alkylated or nitro-group derivatives. Consequently, a variety of methods have been developed to test the presence of this important class of compounds in water, soil, sediment, and biota. Recently, a U.S. EPA method (528) has been promulgated that is dedicated to the analysis of 12 common phenols in drinking water. One of the limitations of this method and others is the lack of characterization of other phenol isomers that may be present and undetected or improperly identified.

This work details a gas chromatography-mass spectrometry (GC-MS) approach to the analysis of underivatized phenols. Retention time locking (RTL) is used to make this method universally applicable [1,2]. In other words, any laboratory analyzing samples for phenols can use this method to confidently identify phenolic compounds. This is important because many of the substituted phenols exist as multiple isomers, which exhibit very similar mass spectra and therefore require retention time information for confirmation. This retention time locking (RTL) method allows for the identification of phenols that might otherwise be undetected or possibly misidentified, simply because they are not present in the particular set of standards in use. Obviously, quantification requires standards, but estimating concentrations is possible using the response factors for related phenol isomers via standards on hand.

Experimental

Phenols were obtained from Ultra Scientific (North Kingstown, RI) and AccuStandard (New Haven, CT) as neat compounds and mixtures. Isotopically labeled phenols were acquired from Cambridge Isotope Laboratories (Andover, MA). Dilutions were made in acetone and in dichloromethane (Burdick and Jackson Grade, VWR Scientific).

The configuration and operating parameters of the Agilent 6890 Plus GC ("fast ramping" 220 V option), 7683 Automatic Liquid Sampler, and 5973N MSD with CI option used for acquiring the data are given in Table 1. The relatively recent EPA Method 528 for determination of phenols in drinking water via GC-MS suggests the DB-5ms column [3]. This column is widely used due to its versatility, robustness and low bleed. For similar reasons, the DB-XLB column is also very popular, particularly for the analysis of PCBs and other organochlorine compounds. Method parameters and compound elutions were explored for both columns under the criteria of resolution and total analysis time. This led to the development of two methods for both the DB-XLB and DB-5ms columns. The method details of the GC and MSD programs are given in Table 1.

Phenols are included in a number of U.S. EPA methods including 604, 1625, 1653, 528, 8270, 8041 and others. Each method utilizes different surrogates and internal standards. Method 528 uses 1,2-dimethyl-3-nitrobenzene and 2,3,4,5-tetrachlorophenol as internal standards and 2-chlorophenol-3,4,5,6-d_4, 2,4-dimethylphenol-3,5,6-d_3 and 2,4,6-tribromophenol as recovery surrogates. All these are included in this study. Isotopically labeled phenols, both deuterated and ^{13}C-substituted, provide the most reliable recovery (surrogate) information and have also been included. A variety of internal standards have been applied in the various methods, such as pentafluorophenol, 2,5-dibromotoluene, and 2,2',5,5'-tetrabromobiphenyl. The tetrabromobiphenyl standard requires extending both the GC oven program slightly and the mass scan range beyond the mass range pertinent to the phenols if measuring the molecular ion(s) is desired (as an example, from 50 to 350 *m/z* to 50 to 476 *m/z*). However, the tetrabromobiphenyl method can be used with the "phenol" scan range if one assigns the intense 150 and 310 *m/z* fragments to identify the internal standard. (Technically, if the phenols alone were of interest, the scan range could be confined from 50 to 275 *m/z*, which has the advantage of more scans over the peak.)

The 2,4-dibromophenol was chosen as the locking compound because of its elution in the middle of the oven program, easily distinguished mass spectrum, and low cost.

Table 1. GC Injection, Oven and MSD Parameters for Both Fast Quant and High Resolution Methods Using the DB-XLB and DB-5ms Columns

Injection Parameters

Injection Mode	Pulsed splitless	
Inlet Temperature	200 °C	
Pulse Pressure and Time	25.0 psi	1.00 min
Purge Flow and Time	50.0 mL/min	0.25 min
Gas Saver Flow and Time	20.0 mL/min	3.00 min

DB-XLB Column and Oven Parameters: Fast Quant Method

GC column (P/N: 122-1232)	DB-XLB 30 m × 0.25 mm I.D., 0.25 µm	
Flow and Mode	1.2 mL/min	Constant Flow
Detector and Outlet Pressure	MSD	Vacuum
Oven Temperature Program	40 °C	2.00 min
40 °C/min	100 °C	0.20 min
2 °C/min	110 °C	0.00 min
30 °C/min	340 °C	0.00 min
Oven Equilibrium Time	0.50 min	
Total Program Time	16.37 min	
MSD Transfer Line Temp	320 °C	

DB-XLB Column and Oven Parameters: High Resolution Method

GC column (P/N: 122-1232)	DB-XLB 30 m × 0.25 mm I.D., 0.25 µm	
Flow and Mode	1.2 mL/min	Constant Flow
Detector and Outlet Pressure	MSD	Vacuum
Oven Temperature Program	40 °C	2.00 min
40 °C/min	100 °C	0.50 min
2 °C/min	140 °C	0.00 min
30 °C/min	340 °C	0.00 min
Oven Equilibrium Time	0.50 min	
Total Program Time	30.67 min	
MSD Transfer Line Temp	320 °C	

DB-5ms Column and Oven Parameters: Fast Quant Method

GC column (P/N: 122-5532)	DB-5 ms 30 m × 0.25 mm I.D., 0.25 µm	
Flow and Mode	1.2 mL/min	Constant Flow
Detector and Outlet Pressure	MSD	Vacuum
Oven Temperature Program	40 °C	2.00 min
40 °C/min	100 °C	0.20 min
2 °C/min	105 °C	0.00 min
30 °C/min	340 °C	0.00 min
Oven Equilibrium Time	0.50 min	
Total Program Time	14.03 min	
MSD Transfer Line Temp	320 °C	

DB-5ms Column and Oven Parameters: High Resolution Method

GC column (P/N: 122-5532)	DB-5 ms 30 m × 0.25 mm I.D., 0.25 µm	
Flow and Mode	1.2 mL/min	Constant Flow
Detector and Outlet Pressure	MSD	Vacuum
Oven Temperature Program	40 °C	2.00 min
40 °C/min	100 °C	0.50 min
2 °C/min	120 °C	0.00 min
30 °C/min	340 °C	0.00 min
Oven Equilibrium Time	0.50 min	
Total Program Time	21.33 min	
MSD Transfer Line Temp	320 °C	

Mass Spectrometer Parameters

Tune Parameters	Autotune
Electron Multiplier Voltage	Autotune +400 V
Solvent Delay	4.20 min
Scan Parameters	50 to 340 *m/z*
Quadrupole Temperature	150 °C
Source Temperature	230 °C

Miscellaneous Parts

Septa	5182-0739	BTO septa (400 °C)
Liner	5181-3315	deactivated 4 mm I.D. double taper
GC column ferrule	5181-3323	250 µm Vespel/graphite
MSD interface ferrule	5062-3508	0.4 mm I.D. preconditioned Vespel/graphite

Results and Discussion

Table 2 presents the locked retention times of the phenols using the DB-5ms and DB-XLB columns under the parameters presented in the experimental section. The absolute retention times of the phenols result as a consequence of locking the 2,4-dibromophenol elution time on both columns under all methods. The Fast Quant methods are intended for customers whose analyte list is a subset of the entire phenol list presented here. The High Resolution methods are given for customers with more extensive lists or concerns over possible coelutions or assignments of phenol identity. Both methods are intended for quantitative work.

Method 528 for phenols states that "Any capillary column that provides adequate resolution, capacity, accuracy, and precision can be used. Medium polarity, low bleed columns are recommended ..." Both the DB-XLB and DB-5ms columns meet these criteria. Bleed is extremely low from the DB-XLB

column making it well suited for many GC-MS analyses. The remarks concerning “adequate resolution” are apparently limited to the list of 17 compounds in the method and further qualified by “complete resolution is not necessary ... if unique ions with adequate intensity are available for quantitation.” They do not cite potential failures among the many possible isomeric phenols that lack unique ions. Coelutions are difficult to avoid due to the many possible phenol isomers.

Table 2. Phenol names, CAS numbers, retention times and identifying ions under the acquisition methods are presented in Table 1. All compound absolute retention times are determined by locking the 2,4-dibromophenol retention time to the specified time under each method. On DB-XLB, dibromophenol is locked at 11.320 and 16.220 minutes in the "Fast Quant" and High Resolution methods, respectively. On DB-5ms, dibromophenol is locked at 8.950 and 13.850 minutes in the "Fast Quant" and High Resolution methods, respectively. Tetrabromobiphenyl is listed twice to emphasize the use of different identifying ions appropriate to different scanning ranges; entry #70 is for the scan range 50 to 340 *m/z*.

			Retention Times				
			DB-XLB Fast Quant	DB-5ms Fast Quant	DB-XLB High Resolution	DB-5ms High Resolution	Identifying Ions
#	Compound Name	CAS #	(min)	(min)	(min)	(min)	(*m/z*)
1	2-fluorophenol	367-12-4	4.345	4.135	4.370	4.125	112; 64; 92; 63
2	pentafluorophenol	771-61-9	5.205	4.940	5.360	4.940	183.9; 135.9; 116.9
3	d_5-phenol		5.315	5.070	5.455	5.085	99; 71
4	phenol	108-95-2	5.350	5.085	5.490	5.100	94; 66; 65
5	2-chlorophenol-3,4,5,6-d_4		5.590	5.270	5.730	5.285	132; 133.9; 68; 96
6	2-chlorophenol	95-57-8	5.620	5.300	5.775	5.315	127.9; 129.9; 64
7	2-methylphenol (o-cresol)	95-48-7	6.405	6.025	6.605	6.050	108; 107; 79; 77
8	4-methylphenol (p-cresol)	106-44-5	6.775	6.320	7.005	6.370	107; 108; 77; 79
9	3-methylphenol (m-cresol)	108-39-4	6.815	6.345	7.045	6.375	108; 107; 79; 77
10	2-chloro-5-methylphenol	615-74-7	7.425	6.715	7.690	6.870	141.9; 107; 143.9; 77
11	2,6-dimethylphenol	576-26-1	.630	6.840	7.905	7.035	122; 107; 121; 77
12	2-ethylphenol (o-ethylphenol)	90-00-6	7.980	7.120	8.265	7.550	122; 107; 77
13	2,4-dimethylphenol-3,5,6-d_3		8.400	7.240	8.720	7.770	125; 124; 110; 109
14	2,4-dimethylphenol	105-67-9	8.425	7.255	8.740	7.795	122; 107; 121; 77
15	2,5-dimethylphenol	95-87-4	8.520	7.275	8.850	7.830	122; 107; 121; 77
16	2-nitrophenol-d_4		8.500	7.135	8.825	7.545	143; 69; 85; 113
17	2-nitrophenol	88-75-5	8.560	7.155	8.885	7.585	138.9; 64.95; 81.1; 108.9
18	4-ethylphenol (p-ethylphenol)	123-07-9	8.750	7.425	9.080	8.170	122; 107; 77
19	3-ethylphenol (m-ethylphenol)	620-17-7	8.825	7.440	9.170	8.205	122; 107; 77
20	3,5-dimethylphenol	108-68-9	9.030	7.445	9.410	8.270	122; 107; 77; 121
21	2,3-dimethylphenol	526-75-0	9.085	7.550	9.505	8.455	122; 107; 77; 121
22	2,4-dichlorophenol-d_3		9.060	7.505	9.475	8.305	164.9; 166.9; 66; 101
23	2,4-dichlorophenol	120-83-2	9.110	7.530	9.545	8.350	161.9; 163.9; 97.9; 63
24	2,5-dichlorophenol	583-78-8	9.155	7.550	9.610	8.410	161.9; 163.9; 63; 98.9
25	2,3-dichlorophenol	576-24-9	9.190	7.600	9.660	8.530	161.9; 163.9; 125.9; 63
26	2-isopropylphenol	88-69-7	9.340	7.740	9.900	8.980	136; 121; 103; 91
27	3-chlorophenol	108-43-0	9.360	7.710	9.935	8.910	127.9; 129.9; 65; 99.9
28	4-chlorophenol	106-48-9	9.400	7.715	10.020	8.915	127.9; 129.9; 65; 99.9
29	3,4-dimethylphenol	95-65-8	9.440	7.700	10.100	8.865	122; 107; 121; 77

Table 2. Continued

#	Compound Name	CAS #	Retention Times DB-XLB Fast Quant (min)	DB-5ms Fast Quant (min)	DB-XLB High Resolution (min)	DB-5ms High Resolution (min)	Identifying Ions (*m/z*)
30	1,2-dimethyl-3-nitrobenzene	83-41-0	9.490	7.675	10.175	7.675	151; 134; 77; 106
31	2,6-dichlorophenol	87-65-0	9.700	7.850	10.585	9.215	161.9; 163.9; 63; 125.9
32	2-n-propylphenol	644-35-9	9.720	7.940	10.645	9.585	136; 107; 77
33	2,4,6-trimethylphenol	527-60-6	9.765	7.845	10.750	9.275	136; 121; 135; 91
34	4-chloro-2-methylphenol	1570-64-5	10.470	8.345	12.730	11.105	141.9; 107; 77; 143.9
35	2,3,5-trimethylphenol	697-82-5	10.535	8.375	12.980	11.215	136; 121; 91; 135
36	4-tertbutylphenol	98-54-4	10.615	8.515	13.300	11.905	150; 135; 107
37	4-chloro-3-methylphenol-d_2	59-50-7	10.670	8.480	13.500	11.745	143.9; 109; 143.9; 79
38	4-chloro-3-methylphenol		10.670	8.480	13.500	11.740	141.9; 107; 77; 143.9
39	2,5-dibromotoluene	615-59-8	10.990	8.680	14.640	12.410	249.7; 251.8; 168.8; 170.9
40	2,3,5-trichlorophenol	933-78-8	11.180	8.845	15.605	13.340	195.8; 197.8; 159.8; 199.8
41	2,4-dibromophenol (lock compound)	615-58-7	11.320	8.950	16.220	13.850	251.7; 253.7; 249.7; 63
42	2,4,6-trichlorophenol-d_2		11.390	8.960	16.760	14.075	197.9; 199.9; 201.8; 133.9
43	2,4,6-trichlorophenol	88-06-2	11.405	8.975	16.825	14.120	195.8; 197.8; 131.9; 199.8
44	2,4,5-trichlorophenol-d_2		11.390	8.990	16.760	14.200	97.9; 199.9; 201.8; 133.9
45	2,4,5-trichlorophenol	95-95-4	11.405	9.005	16.825	14.250	195.8; 197.8; 131.9; 199.8
46	2,3,5,6-tetramethylphenol	527-35-5	11.400	9.000	16.950	14.375	150; 135; 91; 151
47	2,3,4-trichlorophenol	15950-66-0	11.465	9.075	17.105	14.510	195.8; 197.8; 159.8; 199.8
48	3,5-dichlorophenol	591-35-5	11.495	9.135	17.775	15.040	161.9; 163.9; 98.9; 63
49	2,3,6-trichlorophenol	933-75-5	11.625	9.180	18.125	14.900	195.8; 197.8; 159.8; 199.8
50	3,4-dichlorophenol	95-77-2	11.700	9.280	19.005	15.410	161.9; 163.9; 98.9; 63
51	3-nitrophenol	554-84-7	12.140	9.600	22.565	16.150	138.9; 65; 93; 81
52	1-naphthol	90-15-3	12.390	9.850	24.175	16.520	143.9; 114.9; 116; 89
53	4-nitrophenol-d_4		12.450	9.865	24.920	16.625	143; 113; 69
54	4-nitrophenol	93951-79-2	12.470	9.875	24.960	16.640	138.9; 65; 109; 81
55	2,5-dinitrophenol	329-71-5	12.500	9.605	24.995	16.045	183.9; 63; 53
56	2,3,4,5-tetrachlorophenol	4901-51-3	12.655	10.040	25.320	16.790	231.8; 229.8; 233.8; 130.9
57	2,3,5,6-tetrachlorophenol	935-95-5	12.655	10.025	25.370	16.775	231.8; 229.8; 233.8; 130.9
58	2,3,4,6-tetrachlorophenol	58-90-2	12.710	10.065	25.550	16.845	231.8; 229.8; 233.8; 130.9
59	2,4-dinitrophenol-d_3		12.710	9.800	25.745	16.420	186.9; 156.9; 110; 54
60	2,4-dinitrophenol	51-28-5	12.730	9.810	25.785	16.445	183.9; 153.9; 106.9; 91
61	3,4,5-trichlorophenol	609-19-8	12.910	10.315	26.285	17.305	195.8; 197.8; 199.8; 132.9
62	2,4,6-tribromophenol	118-79-6	13.155	10.485	26.650	17.450	329.7; 331.7; 327.6
63	2-methyl-4,6-dinitrophenol-d_2		13.280	10.305	27.045	17.235	199.9; 123; 107; 170
64	2-methyl-4,6-dinitrophenol	534-52-1	13.290	10.310	27.055	17.245	197.9; 120.9; 104.95; 167.9
65	pentachlorophenol-$^{13}C_6$		13.660	10.930	27.595	18.045	271.8; 273.8; 269.8; 169.8
66	Pentachlorophenol	87-86-5	13.665	10.930	27.595	18.045	265.7; 267.7; 263.7; 164.8
67	Dinoseb	88-85-7	13.920	11.090	28.065	18.265	210.9; 239.95; 162.95; 146.9
68	2-cyclohexyl-4,6-dinitrophenol	131-89-5	15.620	12.380	29.625	19.660	230.95; 266; 184.95; 192.9
69	2,2',5,5'-tetrabromobiphenyl	59080-37-4	15.625	12.875	29.995	20.165	469.6; 388.7; 471.6; 390.7
70	2,2',5,5'-tetrabromobiphenyl (2)	59080-37-4	15.625	12.875	29.995	20.165	149.9; 309.8; 311.8; 307.8

The DB-XLB method results

Using the DB-XLB column, a single coelution is apparently unavoidable for reasonable oven programs. The 2,4,6-trichlorophenol and 2,4,5-trichlorophenol isomers completely coelute on the DB-XLB under both the Fast Quant and High Resolution methods. Closely eluting peaks in the DB-XLB High Resolution method are the meta and para cresols (≈ 40% resolved), the 2,5-dichlorophenol, 2,4-dichlorophenol and 2,3-dichlorophenol (≈ 50% resolved) the 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol (> 50% resolved). The DB-XLB Fast Quant Method oven program sacrifices resolution of the 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol. Additional close elutions occur between 3-chlorophenol and 4-chlorophenol, 3,4-dimethylphenol and 2,3-dimethylphenol, and 2-n-propylphenol and 2,4,6-trimethylphenol.

The DB-5ms method results

Using the DB-5ms High Resolution method, complete coelutions are apparently unavoidable for the 3-methylphenol and 4-methylphenol (meta- and para-cresol), and 3-chlorophenol and 4-chlorophenol for reasonable oven programs. Only partial resolution of 3-ethylphenol, 4-ethylphenol and 3,5-dimethylphenol, 2,4-dimethylphenol and 2,5-dimethylphenol, 2,4-dichlorophenol and 2,5-dichlorophenol, and 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol can be obtained. This last coelution is particularly troublesome in view of the critical role of the 2,3,4,5-tetrachlorophenol as an internal standard in Method 528. Shortening the oven program in the DB-XLB Fast Quant Method tightens the window between a few compounds such as 2,4,5-trichlorophenol and 2,3,4-trichlorophenol.

General Considerations

Regardless of which column and oven method are used, there are overlapping ions that can affect quantitation ratios, therefore, the user must exercise caution. Table 2 also suggests ions for each of the phenols, although the exact ratios and choices depend on the choice of tuning criteria. This table shows that some confirming ion ratios must be affected by the presence of closely eluting phenols. For example, using the deuterated trichlorophenols as surrogates produces a 198 *m/z* fragment, which is common to the native trichlorophenols. There are similar concerns for other components such as the native and deuterated dichlorophenols.

Two noteworthy deviations from recommendations in Method 528 exist in these methods. The oven programs begin at 40 °C as opposed to the recommended 35 °C. The method states "...GC conditions may be modified, if all performance criteria...are met." Raising the initial temperature to 40 °C avoids the difficulties associated with trying to reach an oven equilibrium temperature of 35 °C when ambient laboratory temperatures are high and so greatly reduces the oven cycle time. This temperature of 40 °C was also demonstrated to work well with standards in dichloromethane as a concession to the continued use of dichloromethane solvent in methods like Method 528 (despite the mandate to reduce the use of chlorinated solvents).

Method 528 also suggests a constant head pressure of 12 to 15 psi. By using constant flow at 1.2 mL/min, the peak shape of the later eluting peaks is dramatically improved, resulting in increased sensitivity and reduced overall runtime. Method 528 contains a peak tailing factor (PTF) performance criteria that requires the acidic and poorly behaving phenols, the 2,4-dinitrophenol, 4-nitrophenol, pentachlorophenol, and 2-methyl-2,4-dinitrophenol, to demonstrate a tailing factor of less than 5 at a concentration of 5 to 10 µg/mL. Figures 1 through 4 show these factors for two of the difficult compounds, the 2,4-dinitrophenol and pentachlorophenol, using the Fast Quant methods for the DB-XLB and DB-5ms columns. Excellent PTFs are achieved for these compounds under these methods. PTFs were less than 1.5 which is much smaller than the required PTF of 5.

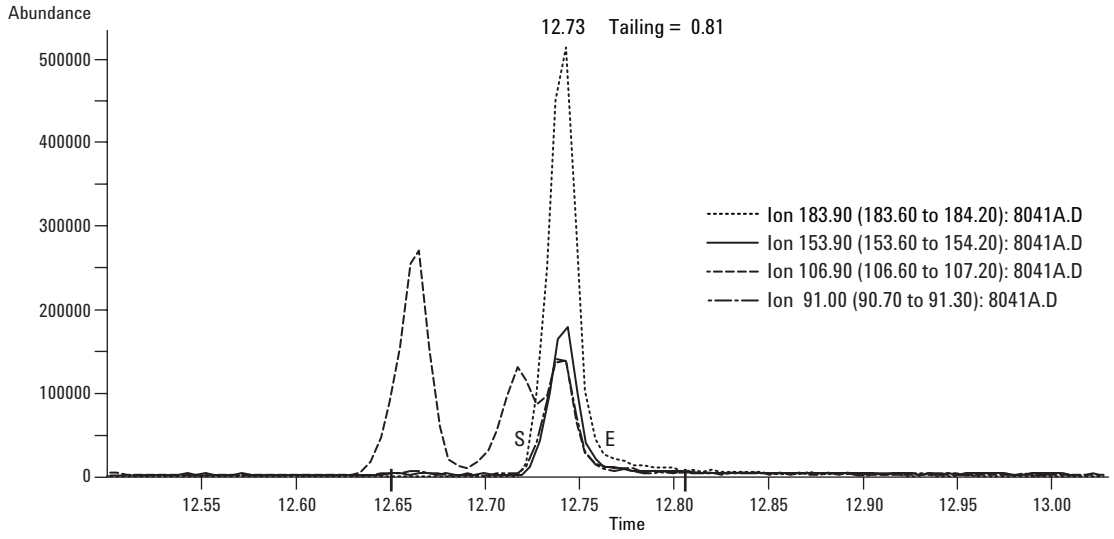

Figure 1. Peak tailing factor 2,4-dinitrophenol at 5 ng/µL on the DB-XLB column with the Fast Quant Method. The PTF is 0.81 (much smaller than the required 5) on a column that had over 100 injections.

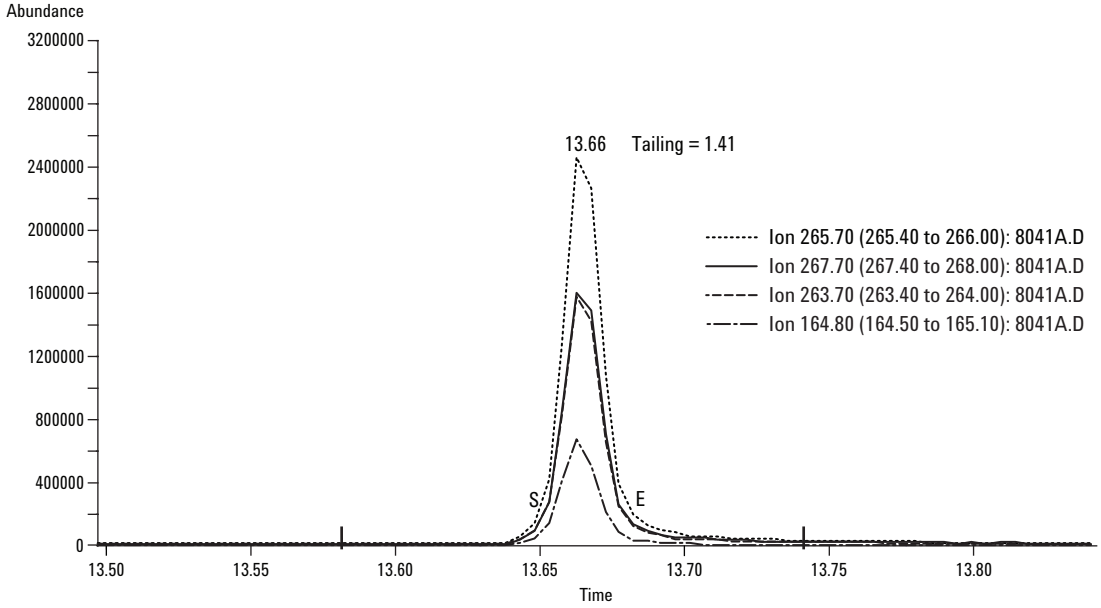

Figure 2. Peak tailing factor for pentachlorophenol at 5 ng/µL on the DB-XLB column with the Fast Quant Method. The PTF is 1.41 (much smaller than the required 5) on a column that had over 100 injections.

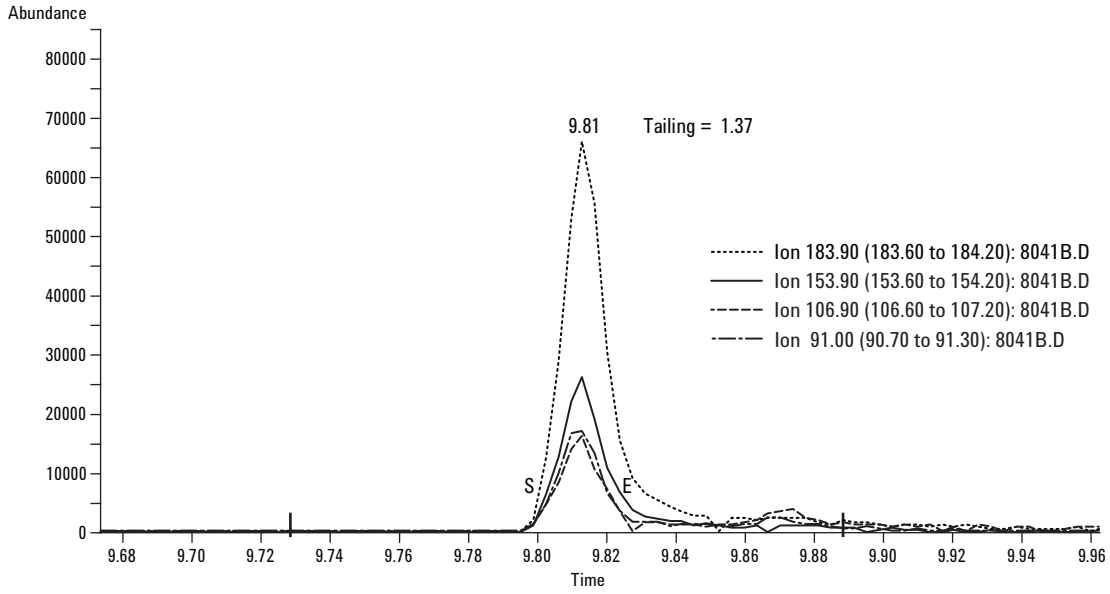

Figure 3. Peak tailing factor for 2,4-dinitrophenol at 5 ng/µL on the DB-5ms column with the Fast Quant Method. The PTF is 1.37 (much smaller than the required 5) on a column that had over 100 injections.

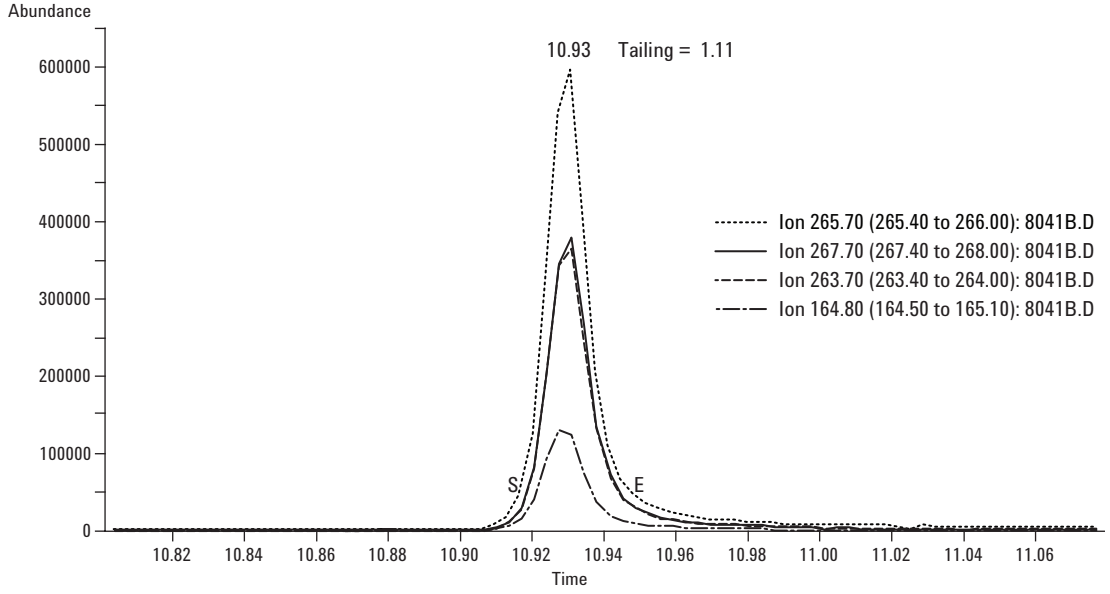

Figure 4. Peak tailing factor for pentachlorophenol at 5 ng/µL on the DB-5ms column with the Fast Quant Method. The PTF is 1.11 (much smaller than the required 5) on a column that had over 100 injections.

"Active" Compounds

The phenols containing nitro-group substituents are well-known to be “active” or difficult compounds that are easily degraded or “lost” in the GC inlet, column, mass spectrometer or GC to MS connection. If the customer wishes to improve the response for these compounds, Agilent supplies liners that directly connect to the GC capillary column and improves the response of the nitrophenols and related poorly performing compounds. These single and double taper direct connect liners (part numbers G1544-80730 and G1544-80700, respectively) have shown a large increase in 2,4-dinitrophenol response over the standard single taper liners. At the minimum, a double taper liner should be used for the phenols. Improvements in response and peak shape are also possible through pressure or flow programming at injection.

Similarly, the new “Ultra” source (part of applications kit G2860A) for the Agilent 5973 and 5973N MSD shows improvement in response and peak shape for the active phenols, especially the dinitro-series. The discussions in the recent Agilent 8270 application note are very pertinent to the analysis of phenols or other active compounds [4].

Conclusions

These methods are completely adaptable to any of the numerous methods requiring phenol quantification. All the phenols and their internal standards used in the U.S. EPA methods, including the recent 528 Method, are listed. A number of phenols not found in any method are also included to offer analysts an opportunity for more complete characterization of phenols and to avoid misidentification. It is apparent that without this precaution there may be mistakes in phenol identifications which will be misleading in environmental studies and limit the usefulness of the data in toxicity assessments. The Fast Quant methods developed on both the DB-XLB and DB-5ms columns are particularly useful when surveying and quantitating a limited number of compounds. The significantly reduced runtimes of 16 minutes (DB-XLB) and 14 minutes (DB-5ms) are quite attractive for rapid, target compound analysis. Obviously, the longer, high-resolution methods provide the best separation. In general, better resolution for the phenols is achieved with the DB-XLB column as compared to the DB-5ms column. Both columns exhibit excellent low bleed characteristics, inertness and robustness, which makes them well-suited to these and related analyses.

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Solid-Phase Extraction and Gas Chromatography/Mass Spectrometry Analysis of Selected Phenols

Application

Environmental

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Abstract

Solid-phase extraction offers a simplified approach to the concentration of compounds present at trace levels in water. Polymeric resins, such as polystyrene-divinylbenzene, offer advantages over the commonly used octadecyl and similar silica-substrate solid-phase extraction adsorbents, especially for more polar compounds. Recently Agilent Technologies has expanded its offering of solid-phase extraction products to include a polystyrene-divinylbenzene solid-phase extraction material specifically for environmental applications, the AccuBond™ ENV cartridge. Using the retention time locked gas chromatography/mass spectrometry analytical method previously described [1], this note makes an initial demonstration of the accuracy and precision that can be achieved for selected phenols at 10 ppb in water using this polystyrene-divinylbenzene solid-phase extraction material. The solid-phase extraction procedure is rapid, uses reduced drying times, and requires only two surrogates. The cartridge design has been optimized to provide increased recoveries for phenol, which typically has low and irreproducible recoveries. Recoveries for phenol exceeded 70% and other phenols were greater than 90%. Precision was better than 5% and accuracy, as indicated by average absolute deviation as percent, was better than 8% for all phenols except 2-cyclohexyl-2,4-dinitrophenol. Sample delivery rate is high (20 to 25 mL/min) so a 1-liter sample can be extracted in less than an hour.

Introduction

Solid-phase extraction (SPE) has evolved to be a powerful tool for isolation and concentration of trace analytes in a variety of sample matrices. SPE has grown to replace liquid/liquid extraction due to the minimal use of solvent, the simplicity and flexibility of the approach, and the increased selectivity for analytes available. Beginning in and throughout the last decade, a large number of SPE applications were developed for compounds in matrices of environmental interest. The major focus of these applications was the collection and concentration of trace analytes from water. Most of the analytes were non-polar and strongly hydrophobic in nature such as polychlorinated biphenyls (PCBs), the organochlorine pesticides, polynuclear aromatic hydrocarbons (PAHs), for example, as these were relatively easy candidates for the technique and of widespread concern. More polar compounds like the phenols offer particular challenges.

SPE exploits the similarity in physicochemical properties of a class of analytes, their interaction with the SPE material, and their differences from the matrix. The phenols encompass a wide range in polarities and solubilities as shown in Table 1.

The pKa values indicate that the dinitrophenol and the tetra- and penta-chloro phenols are fairly acidic and therefore are predominately dissociated in water at near-neutral pHs. Acid-base equilibrium considerations require that the water sample be acidified to at least 2 pH units below that of the lowest pKa value(s) to generate phenols primarily in their non-ionized form. Octanol-water partition constants (K_{ow}) and water solubilities of the undissociated compounds range over a factor of more than several thousand. The high aqueous solubilities and low K_{ow} s of phenol and the monosubstituted phenols make these the most difficult phenols to capture and retain.

Table 1. Physicochemical Properties of Some Phenols

Compound	$\log_{10} K_{ow}$	pKa	Solubility$_{(aq)}$ g/L
Phenol	1.46	9.89	0.0884
4-chlorophenol	2.4	9.18	.027
4-methylphenol	1.96	10.26	.02
3-methylphenol	1.98	10	.022
4-nitrophenol	1.91	7.08	.013
2,4-dichlorophenol	3.2	7.68	.0045
2,4-dimethylphenol	2.35	10.6	.0088
2,4-dinitrophenol	1.67	4.09	.00034
2,4,6-trichlorophenol	3.69	7.42	.00043
2,3,4,6-tetrachlorophenol	4.45	5.38	.00018
Pentachlorophenol	5.05	4.92	0.000014

Polymeric resins were used early in the history of solid-phase extraction. These early materials needed extensive cleanup prior to use to avoid interferences obscuring analytes of interest. New generations of these polymers such as polystyrene-divinylbenzene (PS-DVB) have much lower backgrounds due to improvements in manufacturing processes. The use of PS-DVB polymers as an absorbent material has been demonstrated to provide improved recoveries for phenolic compounds as compared to the traditional and more commonly applied C18 material [2]. The details provided here ensure that analysts will observe less breakthrough of phenol, greatly improving overall recoveries.

The objective of this work was to develop a simple approach to SPE extraction and gas chromatography/mass spectrometry (GC/MS) analysis for selected phenols and perform a preliminary demonstration of accuracy and precision. A previous application note describes the retention-time locked GC/MS method in detail [1].

Experimental

The phenols were obtained from Ultra Scientific (North Kingstown, RI) and AccuStandard (New Haven, CT) as mixtures. Dilutions were made in acetone and in dichloromethane (Burdick and Jackson Grade, VWR Scientific, San Francisco, CA) for surrogates or spiking and standards, respectively. Sodium sulfate (analytical grade, VWR Scientific, San Francisco, CA) was kilned at 500 °C and stored in a desiccator.

Empty 6-mL cartridges and frits were obtained from Agilent Technologies Inc. (Wilmington, DE) for use as drying cartridges. AccuBond^{II} ENV PS-DVB cartridges containing 1000 milligrams of PS-DVB sorbent in a 6-mL cartridge were obtained from Agilent Technologies Inc. (Wilmington, DE). A summary of the equipment and consumables is given in Table 2.

Table 2. Equipment and Consumables Summary

Description	Part Number
Silanized amber vials	5183-4496
Vial crimp caps	5181-1210
AccuBond^{II} ENV PS-DVB polymeric resin as 1000 mg / 6-mL cartridge, box of 30	188-3060
Empty SPE Cartridges Reservoirs, 6 mL, box of 50	700-4006
Frits for 6 mL cartridges reservoirs, 100/pk	700-4031
Stopcock valves, 10/pk	5185-5758
SPE Manifold, 10-port	5185-5754
SPE Manifold, 20-port	5185-7565

Spike and Recovery Experiments

For the initial demonstration of the accuracy and precision of the approach, 1.0 L of deionized RO water was spiked with 21 phenols at 10 pbb each. Deuterated phenol, 2,4-dibromophenol, and 2,4,6-tribromophenol were added at 10 ppb as recovery surrogates. Three “calibrators” were also made at that time by adding the spike and surrogates to a silanized vial containing some dichloromethane (DCM) as a keeper. The solution was mixed and the pH lowered to ≤ 2 with 5N HCl.

The PS-DVB SPE cartridge was conditioned by sequentially rinsing with 9 to 12 mL of DCM, 9 to 12 mL of methanol, and 9 to 12 mL of 0.05N HCl. At no time after the initial addition of DCM was the column allowed to run dry.

The 1-L water sample was then pulled through the SPE cartridge at a flow between 20 and 25 mL/min such that the sample was processed in less than 1 hour. The SPE cartridge was dried briefly by drawing clean laboratory air through the cartridge for about 2 minutes while tapping the cartridge body to dislodge bound water. The SPE cartridge was then eluted with 9 mL of DCM. The DCM eluant was dried using a cartridge filled with anhydrous sodium sulfate.

The dried DCM eluant was evaporated under dry, filtered nitrogen and transferred to a silanized amber vial. At this point, the volumes of the sample and the three calibrators were brought to approximately 0.9 mL and 100 µL of a solution containing 2,5-dibromotoluene and 2,2',5,5'-tetrabromobiphenyl at 0.05 µg/µL in DCM was added as internal (injection) standards. A solvent blank, the three calibrators and the sample were then analyzed using an Agilent 6890 Plus GC and 5973N MSD according to operating parameters given in a previous note [1].

Results and Discussion

Corrected and uncorrected results of inter-day replicates for selected phenols are shown in Table 3. Phenol values were corrected to the deuterated phenol while all other compounds were corrected to 2,4-dibromophenol recoveries. With the exception of the 2-cyclohexyl-2,4-dinitrophenol, all RSD values and deviations are under 5% and 8%, respectively. The average RSD and absolute deviation for all the compounds are 4% and 6%, respectively. These indicate very good reproducibility and accuracy. An anomalously high value in the third trial seems to have inflated the deviation for 2-cyclohexyl-2,4-dinitrophenol.

Table 3. Spike and recovery results for accuracy and precision at 10 ppb using the AccuBond[II] ENV SPE cartridge. The average deviation is calculated as the relative average of the absolute deviations from 10 ppb and expressed as percentages. RSD represents the relative standard deviations.

Trial Number:	Trial #1		Trial #2		Trial #3		RSD	Average
Compound	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	%	Deviation
Phenol	7.8	10.7	7.2	10.3	7.7	11.0	3%	7%
2-chlorophenol	9.8	9.3	9	8.9	10.3	9.3	3%	8%
2-methylphenol	9.9	9.4	9.1	9.0	10.5	9.5	3%	7%
3- & 4-methylphenol	9.8	9.3	9.2	9.1	10.4	9.4	2%	7%
2,4-dimethylphenol	9.9	9.4	9.4	9.3	10.5	9.5	1%	6%
2-nitrophenol	10.0	9.5	9.1	9.0	10.7	9.6	4%	6%
2,4-dichlorophenol	9.9	9.4	9.1	9.0	10.5	9.5	3%	7%
2,6-dichlorophenol	9.7	9.2	9.1	9.0	10.4	9.4	2%	8%
4-chloro-3-methylphenol	10.1	9.6	9.3	9.2	10.7	9.6	3%	5%
2,4-dibromophenol	10.5		10.1		11.1			
2,4,6-trichlorophenol	9.7	9.2	9.1	9.0	10.6	9.5	3%	7%
2,4,5-trichlorophenol	9.8	9.3	9.1	9.0	10.4	9.4	2%	8%
4-nitrophenol	10.0	9.5	9.8	9.7	11.5	10.4	4%	4%
2,3,4,5-tetrachlorophenol	9.7	9.2	9.3	9.2	10.7	9.6	3%	6%
2,3,5,6-tetrachlorophenol	9.9	9.4	9.1	9.0	10.4	9.4	2%	7%
2,3,4,6-tetrachlorophenol	9.8	9.3	9.3	9.2	10.6	9.5	2%	6%
2,4-dinitrophenol	10.6	10.1	9.8	9.7	11.9	10.7	5%	4%
2,4,6-tribromophenol	9.7	9.2	9.6	9.5	10.7	9.6	2%	5%
2-methyl-4,6-dinitrophenol	10.1	9.6	9.5	9.4	11.4	10.3	5%	4%
Pentachlorophenol	9.8	9.3	9.5	9.4	11.1	10.0	4%	4%
Dinoseb	10.2	9.7	9.4	9.3	11.5	10.4	5%	4%
2-cyclohexyl-4,6-dinitrophenol	11.2	10.7	10.6	10.5	14.4	13.0	12%	14%
2,2',5,5'-tetrabromobiphenyl	10.5	10.0	10.5	10.4	12.3	11.1	5%	5%

Typically, a 1-gram sorbent cartridge is considered an excessive use of material. However, work with 500-mg cartridges showed recoveries for phenol near and below 50%. Tandem cartridges revealed substantial phenol on the second cartridge. Increasing the polymer mass to 1 gram reduced breakthrough and consequently increased phenol recoveries. The methylphenols also demonstrated this behavior to a lesser degree and supported the change in sorbent bed mass.

Using a single surrogate to correct all the substituted phenols seems a tremendous simplification since the behavior and chemistries of the phenols differ widely. It is likely that this will become apparent at lower concentrations and most likely for the nitrophenols. Data does imply that the tetrabromobiphenyl (included in Table 3) may allow a better correction of the injection volume for the late eluters.

Conclusions

These preliminary results show that phenols can be extracted from aqueous samples accurately and precisely using AccuBond^{II} ENV PS-DVB polymeric resin. Coupled with a gas chromatographic analysis and retention time locking GC/MS [1], extraction, identification and quantitation of phenolic compounds can be done confidently, accurately and reproducibly. This method is a modification of U.S. EPA Method 528 [3]. The procedure here for the extraction of phenols from drinking water by polymeric SPE results in improved recoveries and greatly reduced drying times (2 minutes compared to 20 minutes), which increases sample throughput. The next steps in developing a full method would be an exploration of detection limits which will require extraction of replicates at lower concentrations. It is expected that the behavior of the more “active” compounds may suggest an expanded suite of surrogates.

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Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270

Application

Environmental

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Abstract

Method 8270 presents challenges due to the simultaneous measurement of acids, bases and neutrals over a concentration range that varies from lab to lab. Laboratories want GC/MS instruments that are linear and inert over a wide concentration range. Changes have been made to the 6890/5973 GC/MSD system in the inlet, column, and source areas based on feedback from our customers. System performance has been improved by maximizing linearity and minimizing activity.

Introduction

USEPA Method 8270 (including versions A, B, C and D) is used to determine the concentration of semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water. The January 1998, revision 4, 8270D lists 240 possible analytes that can be measured. Most laboratories analyze for a significantly smaller number of compounds, usually 70 to 100.

Regardless of the number of analytes, there is usually a mix of acids, bases and neutrals that must be measured concurrently. This mix presents a challenge for instrument design due to the interaction of the analytes with the instrument and consumables.

The calibration range required for the analysis varies depending on a particular laboratory's statement of work. Method 8270 does not specify a calibration range, yet traditionally a range of 20 to 160 ng (nanograms) has been used as a carryover from the USEPA Contract Lab Program (CLP). With the increased sensitivity of newer GC/MS systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the 8270 calibration range down to 5 ng.

The 6890/5973 GC/MSD (Gas Chromatograph /Mass Selective Detector) system was designed to meet demand for these lower MDLs. To further enhance performance, two main areas of improvement were identified in communications with users.

The first was improving the linearity of the GC/MSD system at the high end of the calibration range where roll off or flattening out of the calibration was observed. The relative response factors (RFs) were lower than they should have been at higher concentrations. This was seen for phthalates and for PAHs (Polyaromatic Hydrocarbons).

The second area for improvement was recovery at the low end of the calibration range for active compounds. The most active compounds, the nitrophenols, showed lower RFs at the low end of the calibration range than what was expected on some systems. The most active of these, 2,4-dinitrophenol, showed RFs below method requirements on some systems.

A study was undertaken to address the high end linearity and the low end activity. The primary goals of the study were to meet the following 8270 requirements:

1. Minimum Average RF of 0.050 for the System Performance Check Compounds (SPCCs) (Method 8270D, section 7.3.4.2)
2. Maximum Relative Standard Deviation (RSD) of 30% for the Calibration Check Compounds (Method 8270D, section 7.3.5.2)
3. Maximum Mean Relative Standard Deviation of 15% across all compounds (Method 8000B, section 7.5.1.2.1)

Additional study goals to ensure maximum productivity for the user were:

1. Minimize activity in the entire GC/MSD system to maximize RFs for active compounds—this gives the user a greater margin for system degradation when analyzing dirty samples.
2. Maximize linearity in the GC/MSD system at the high end without losing significant sensitivity at the low end—this improves overall RSDs.
3. Preserve method resolution requirements for benzo[b]fluoranthene and benzo[k]fluoranthene when using thinner film columns for shorter analysis times.

Experiments were done to meet the study goals by dividing the system into three main sections : GC column, GC inlet, and MSD. Each of these areas is treated separately in a following section of this note.

Column

Column test system

To reduce the complexity of the chromatographic system and to provide the best possible sample introduction and detection, a COC/FID (Cool-On-Column/Flame Ionization Detector) system was used to test column performance. On-column injection eliminates any inlet activity while FID gives sensitive and essentially universal response for the analytes. The FID also provides directly comparable response information that can be used to validate analyte introduction between systems.

Test mix

To establish a test mix for evaluating the column and other components in the system, both anecdotal information and suggestions in the method were reviewed. Section 1.4 of 8270D points out the following compounds as potentially troublesome:

1.4.1 Benzidine may be subject to oxidative losses during solvent concentration and its chromatographic behavior is poor.

1.4.4 N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described.

1.4.6 Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, benzoic acid, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, 2-nitroaniline, 3-nitroaniline, 4-chloroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

Furthermore, the method cites several mixes of compounds for evaluating system performance. The DFTPP (decafluorotriphenylphosphine) mix adds 4,4'-DDT to the previously cited compounds. The system performance check compounds add N-nitroso-di-n-propylamine and hexachlorocyclopentadiene. Finally, the calibration check compounds add six more phenols as well as seven base/neutral compounds.

From anecdotal information, the phenols presented the greatest challenge. If the phenols cited are combined, the list is essentially all the phenols in EPA Method 604. While more test solute information was being collected, the phenols were run by COC/FID. Table 1 shows the RSD values for relative response factors from 5 to 160 ng on column. The last four compounds prove to be the most troublesome with 2,4-dinitrophenol being noticeably worse. Even so, the RSD values are all below 8%, indicating that COC/FID can be used to evaluate column performance. When done with unoptimized conditions/consumables in splitless sample introduction, the RSD values become unusable for these difficult compounds. With this information and the anecdotal performance data, a more comprehensive mix was devised.

Table 1. Cool-on-column FID, RSD of RFs from 5 to 160 ng on column

Solute	RSD
Phenol	3.0
2-Chlorophenol	3.1
2-Nitrophenol	3.2
2,4-Dimethylphenol	3.3
2,4-Dichlorophenol	3.1
4-Chloro-3-methylphenol	3.2
2,4,6-Trichlorophenol	3.3
2,4-Dinitrophenol	6.9
4-Nitrophenol	3.8
4,6-Dinitro-2-methylphenol	4.3
Pentachlorophenol	4.5

The "short mix" is comprised of the four phenols from above, several bases, several neutral compounds, and the internal standards at 40 ng/µL. The compounds were selected so that they were easily resolved and unambiguously detected by COC/FID. Figure 1 shows a sample chromatogram of the short mix on a 0.5 µm column.

Column testing

8270D states that a 30 m × 0.25 mm × 1 µm silicone coated capillary column be used in the analysis. However, the method also makes provisions for split injections, allowing a thinner film to be used. Because of the obvious time pressure to perform environmental analyses, thinner film columns are widely used. From customer inputs, film thickness ranged from 0.25 to 1 µm; consequently, 0.25, 0.5, and 1 µm film thickness columns were evaluated. Each of the columns had already passed the Agilent 5MS column checkout and were used as received. In addition, columns from another supplier were also tested, yielding similar results to the Agilent columns.

1 N-Nitrosodimethylamine
2 Aniline
3 2,4-Dinitrophenol
4 4-Nitrophenol
5 4,6-Dinitro-2-methylphenol
6 4-Aminobiphenyl
7 Pentachlorophenol
8 Benzidine
9 3,3-Dichlorobenzidine
10 Benzo[b]fluoranthene
11 Benzo[k]fluoranthene

ISTD 1 Dichlorobenzene-d4
ISTD 2 Naphthalene-d8
ISTD 3 Acenaphthene-d10
ISTD 4 Phenanthrene-d10
ISTD 5 Chrysene-d12
ISTD 6 Perylene-d12

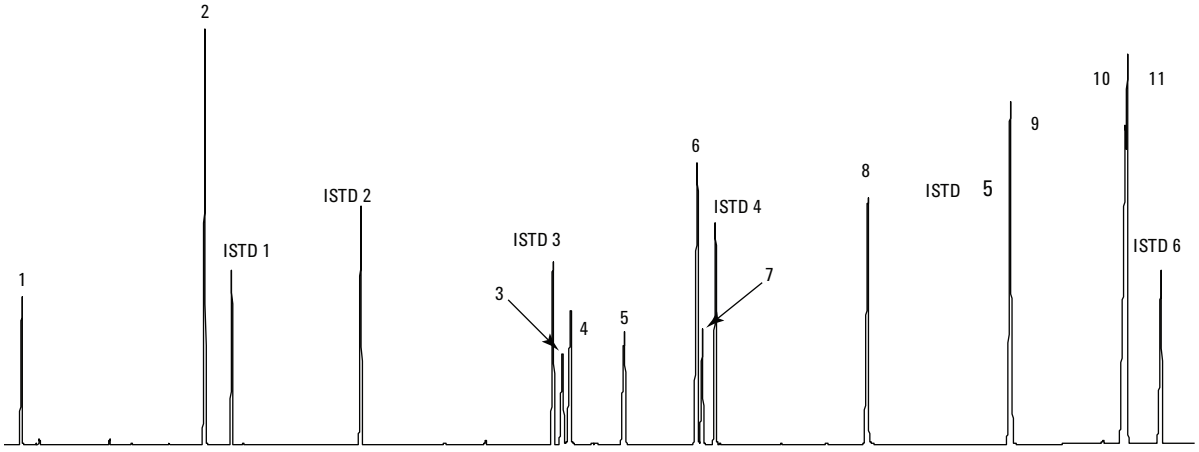

Figure 1. COC-FID Chromatogram and identification of short mix compounds.

The instrument parameters followed the 8270 method operating conditions as closely as possible. The COC inlet was run in oven track mode, the column flow was constant at 1.3 mL/min, and the FID was set to 300 °C. The oven program followed the 8270 method except that the program rate (10 °C/min) was adjusted for the different film thickness to resolve the test compounds.

Numerous columns from each film thickness were tested. It is important to remember that all of these columns passed the standard column testing protocol. An arbitrary metric was set for pass/fail criteria. This value was 10% RSD for the 2,4-dinitrophenol RFs from 5 to 160 ng on column. Of all the columns tested, only a fraction gave results below this metric. Film thickness was not a factor since the same fraction of columns passed for each film thickness. Some of the columns were so active that the column alone could cause the system to fail the method qualifying criteria. For this reason, it is imperative that a stringent test protocol be utilized for evaluating columns and that only specifically tested columns be used for 8270D. A comparison of a column that passed and one that failed the arbitrary criteria is shown in Table 2.

Table 2. COC-FID Results on a "Pass" and a "Fail" 0.5 µm Column, 5 to 160 ng

Solute	"Pass" RSD	Avg RF	"Fail" RSD	Avg RF
N-Nitrosodimethylamine	0.78	0.54	0.30	0.52
Aniline	1.2	1.45	0.59	1.43
2,4- Dinitrophenol	7.5	0.33	21.	0.28
4-Nitrophenol	0.49	0.52	2.9	0.50
4,6-Dinitro-2-methylphenol	4.7	0.40	14.	0.37
4-Aminobiphenyl	1.0	0.94	3.3	1.02
Pentachlorophenol	5.0	0.34	15.	0.26
Benzidine	2.6	0.74	2.8	0.72
3,3'-Dichlorobenzidine	7.9	0.57	0.58	0.62

Column selection

Since all the film thicknesses studied can meet the method objectives, the column selection is typically based on other analysis needs. As in all chromatographic systems, there is a balance between speed of analysis, resolution, and column capacity. The 0.25 µm film thickness columns offer the fastest analysis possible but with a compromise in resolution and capacity. Conversely, the 1.0 µm columns provide the best capacity but at a cost of time. Using the Agilent method translation tool, the 0.5 µm film column is only a factor of two slower than the thin film column while providing a twofold increase in capacity. The 0.5 µm film thickness column offers a good compromise between speed and capacity.

Inlet

There are many inlet related factors that affect 8270 performance. These include: split vs. splitless injection, syringes, injection volume, septa, inlet temperature, inlet seal, liners, using wool or not in the liner, and using a pulsed (flow programmed) vs a normal injection. Some of these parameters were studied to determine their contribution to low end activity and to high end linearity.

Split injection is allowed if the MSD has enough sensitivity. Split injections put less material on column, making it easier to meet resolution requirements on thinner film columns and at the same time improving peak shapes. However less material on column results in noticeable losses of active compounds due to column or MSD activity. High end linearity could be improved using split injections but the issue was solved as described in the source section of this note. Splitless injection is almost universally used and will be the focus of this inlet section.

A syringe experiment was not done as part of this study. Previous data show better reproducibility when using a 5 µL syringe in an ALS (Automatic Liquid Sampler). All injections were made using a 5 µL syringe with a tapered needle.

Injection volume was always 1 µL for the study. 8270 allows for 1 to 2 µL injection volumes, but previous data show worse reproducibility when using 2 µL injections. This is most likely due to expansion outside the liner and subsequent loss of analytes. Additionally, more residue is introduced with larger injection volume, negatively impacting instrument uptime.

Septa types were not studied and green septa were used. Inlet temperature was held at 250 °C. A new gold inlet seal was fitted with each liner, although changing the seal for a direct connect liner may not be necessary. Stainless steel (SS) seals were not used.

The inlet study focussed on liner types, carrier gas flow through the column during injection and the presence or absence of glass wool. The five different liner types that were used are described in Table 3. Two of the liners, the G1544-80700 and G1544-80730 are new designs. The column makes a direct connection into the liner bottom, similar to a capillary column connector.

Table 3. 2,4-Dinitrophenol Average RFs Using Various Inlet Liners, COC-FID

Splitless time			**0.2 min**		**0.2 min**		**0.75 min**		**0.75 min**	
Column flow			**1 mL/min**		**3 mL/min**		**1 mL/min**		**3 mL/min**	
Part number	**Liner**	**ng injected**	Avg RF	RSD	Avg RF	RSD	Avg RF	RSD	Avg RF	RSD
5062-3587	Single taper with glass wool	5	0.007	63	0.023	55	0.017	58	0.072	38
		160	0.122		0.198		0.187		0.228	
5181-3316	Single taper	5	0.092	37	0.136	21	0.105	33	0.125	22
		160	0.279		0.232		0.261		0.207	
5181-3315	Dual taper	5	0.203	14	0.215	11	0.201	15	0.216	12
		160	0.285		0.255		0.296		0.287	
G1544-80730	Single taper direct contact	5	0.287	5	0.269	7	0.272	6	0.229	9
		160	0.311		0.316		0.310		0.285	
G1544-80700	Dual taper direct contact	5	0.289	5	0.280	6	0.275	7	0.278	7
		160	0.331		0.330		0.327		0.328	
	COC	5	0.311	3	0.311	3	0.331	3	0.311	3
		160	0.331		0.331		0.331		0.331	

All liner experiments were performed using an FID to eliminate any affect an MSD would have on the results. RFs are not identical for MSD and FID due to inherent response differences. An approximate conversion is $RF_{fid} \times 0.7 = RF_{msd}$. Cool-on-column injection was done as a baseline for inlet performance.

The short mix was injected at the 5, 20, 80 and 160 ng levels, four replicates at each level. This series of 16 injections was made on each liner at each set of inlet conditions. There were four sets of inlet conditions. The splitless time was either 0.2 minute or 0.75 minute. The carrier flow through the column was either 1.0 mL/min or 3.0 mL/min held for the splitless time + 0.05 minute, then reduced to 1.2 mL/min. This is similar to a “pulsed splitless” injection, however flow programming gives the analyst control over the depressurization rate. The COC injections were made at a fixed column flow and the splitless time is not relevant.

Table 3 shows the results of these analyses. The average RFs for 2,4-dinitrophenol at the 5 ng and 160 ng levels are shown together with the RSD of all 16 RFs for each liner type/inlet conditions.

The 3587 liner shows the worst performance. 2,4-dinitrophenol has been eaten by the glass wool and low end activity is at its worst. Unfortunately most analysts use glass wool in the liner to prevent solids from contaminating the column.

The 3316 liner is the same as the 3587 but without the wool. Loss of 2,4-dinitrophenol can be attributed to contact with the gold inlet seal, the polyimide coating on the column outside and the stainless steel at both the top and bottom of the inlet. There could also be analyte contact with the stainless steel in the annular volume outside the liner.

The 3315 liner is the same as the 3316, but with a narrower opening at the top. This minimizes contact with the top of the inlet and there is an increase in 2,4-dinitrophenol response.

The new 80730 liner minimizes analyte contact with the polyimide on the column outside, the gold inlet seal and the inlet annular volume. Response for 2,4-dinitrophenol was significantly improved using this liner, even though it has a wide top similar to the 3316.

The new 80700 liner has the advantages of the 80730 and has a narrower top opening similar to the 3315. An additional increase is seen in 2,4-dinitrophenol response because analyte contact with inlet surfaces is minimized at both the top and bottom. Performance with this liner is nearly equal to that of COC and low end activity is minimized for the inlet only.

Figure 2 shows performance of the five liners and COC with one set of conditions that was used. The splitless time was 0.75 minute and the column flow was 3 mL/min during the injection. Each of the bars shows the average RF for 2,4-dinitrophenol of the four replicate injections at each level. The order is 5 ng at the top increasing to 160 ng at the bottom. Above each bar is listed the average RF and RSD across all 16 injections. The 3587 liner with the wool shows the worst performance and the new 80700 liner with the direct connect bottom and narrow top shows the best performance. The COC data show column performance isolated from a hot splitless inlet. There is a slight drop-off in COC RFs comparing 5 ng to higher levels. Data for the other three sets of experimental conditions show similar trends and are not presented.

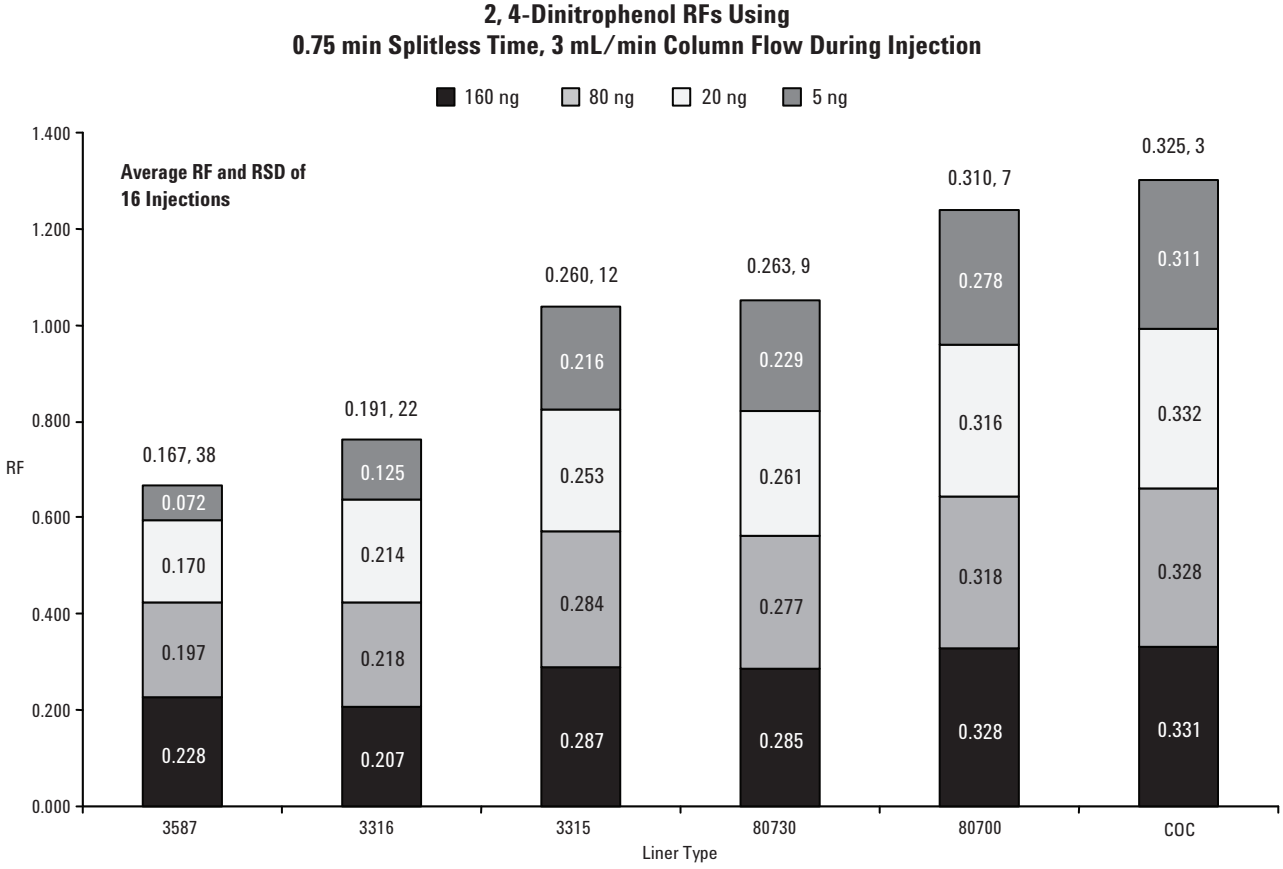

Figure 2. 2,4-Dinitrophenol RFs using five different liners.

Figure 3 shows performance of the 3587 liner at the four sets of experimental conditions. These data are shown as liners with wool are widely used. Comparing the first two bars shows the difference between a column flow of 1 mL/min and 3 mL/min during injection, both with a 0.2 minutes splitless time. Higher flow sweeps the inlet faster minimizing contact with the wool resulting in better 2,4-dinitrophenol performance. The higher column flow also means a higher inlet pressure. Previous work has shown that higher inlet pressures can keep the expanded solvent vapor contained in the liner. This holds true comparing the third and fourth bars with a 0.75 minute splitless time. The longer splitless time also results in a more complete transfer of 2,4-dinitrophenol onto the column at a fixed flow. In all cases performance suffers compared to COC.

In addition to 2,4-dinitrophenol, RFs and RSD were tracked for the other analytes in the short mix. Similar improvements were seen for the other active compounds although the liner effects were not as dramatic due to better performance of these compounds initially. No adverse effects were seen for the neutral or basic compounds when the acidic compounds improved.

Another factor that was monitored was ISTD (internal standard) reproducibility. Using the new direct connect liners showed variability in ISTD areas on some test systems greater than that using the standard liners. These systems still met the -50%/+100% 8270 ISTD criteria. Using a column flow of 3 mL/min during the injection period minimized this ISTD variability.

The high end linearity issue is not caused by the inlet although inlet parameters can affect it. The low end activity issue is directly related to inlet activity, including liner, seal, wool and stainless steel. Activity can be minimized by using pressure programmed flow and optimized splitless time. The liner and the presence of wool have the largest affect on low end activity. The liner must be chosen based on sample type, allowing for a trade-off of activity vs dirtiness of extract.

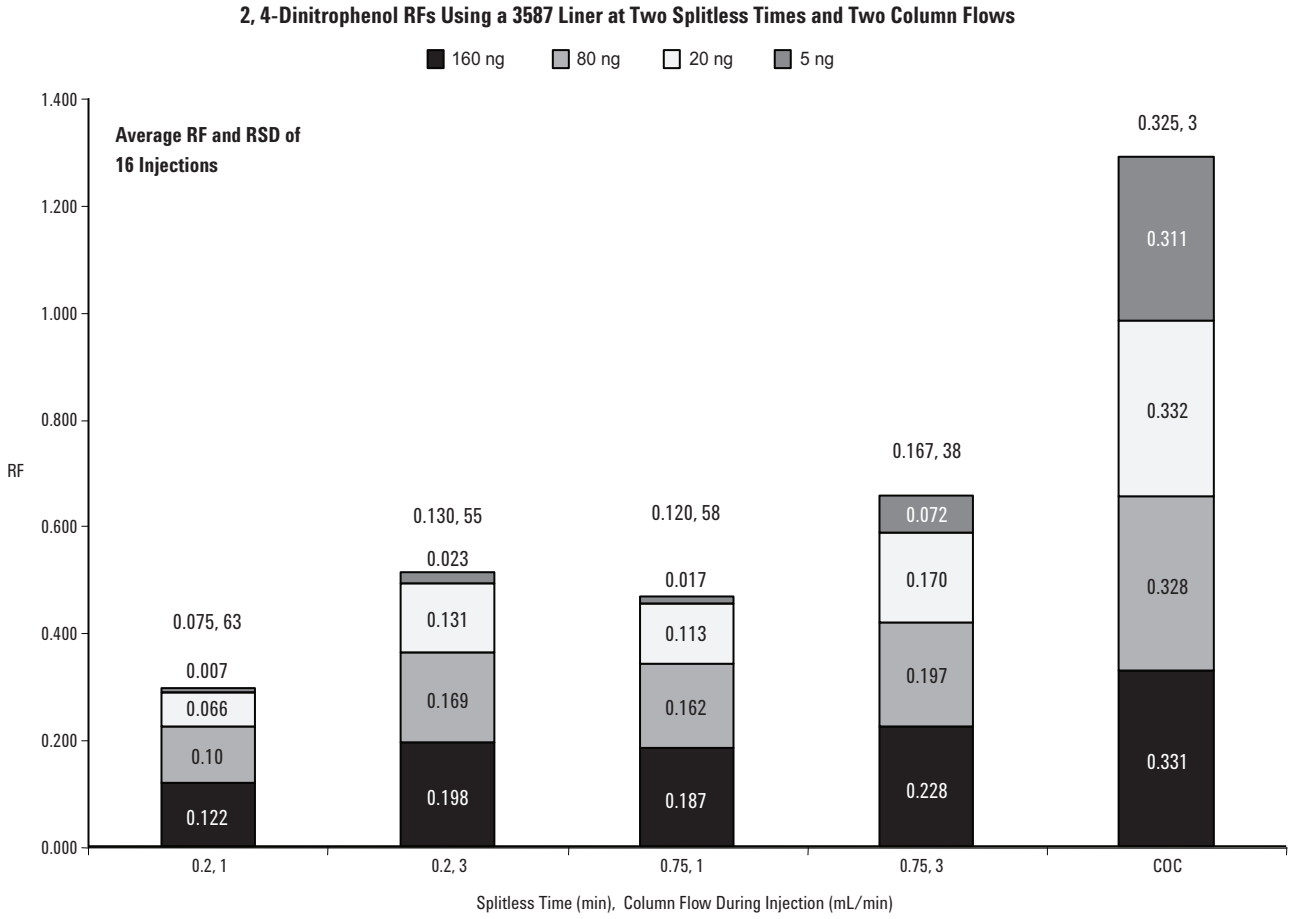

Figure 3. 2,4-Dinitrophenol RFs using a 3587 liner.

Source

The position of the column in the ion source, ion source materials, dimensions of the ion source, and parameters used for operation all affect the response of the system in this method.

In general, the column should be positioned beyond the end of the interface but not too far into the ion source. If the column tip is positioned inside of the interface guide tube, compounds are exposed to the hot metal surface of the interface and may decompose. On the other hand, if a significant length of column is exposed inside the ion source, the polyimide coating of the column can take on a static charge due to the ions and electrons in the ion source, and this charge interferes with the ejection of ions from the ion source chamber. In practice, a 2 to 3 mm extension of the column out of the interface has been found to yield the best results. This position may be set by one of two methods:

1 Put the column nut and ferrule on the column; open the analyzer door; push the column through the interface until 2 to 3 mm is sticking out of the end; then tighten down on the column nut; or

2 Put the column nut and ferrule on the column; open the analyzer door; push the column through the interface until it is just beyond the end of the interface. Tighten the nut only finger tight. Hold the MSD analyzer door closed, and then slide the column in until it just bottoms out (stops). You have now hit the left side of the source. Mark the column with typewriter correction fluid in the oven next to the nut. You can release the MSD analyzer door. Back the column out 12.5 mm (the source i.d.). Tighten the column nut. There should be 2 to 3 mm of column visible at the MSD end of the transfer line. You have now positioned the column just inside the source.

Method 1 above has the advantage that you can see what is happening, but a disadvantage is that it is difficult to measure the 2 to 3 mm column length inside the vacuum manifold. Method 2 has a few more steps but gives reproducible results, if followed exactly.

The material used for the standard MSD ion source may decompose some analytes under some conditions, especially when the source temperature is high. The Ultra source (patent applied for) has been found to reduce low end activity under the conditions typically used for this method. Table 4 shows a comparison of RFs for 2,4-dinitrophenol using different source materials. It also shows that the new Ultra source can be abrasively cleaned with minimal loss in performance.

The high end linearity is a function of the density of ions produced in the ion source. Reducing the ion source pressure improves the high end linearity. Therefore, increasing the size of the holes in the ion source improves the high end linearity, attended by some loss in sensitivity. To improve high end linearity, the hole in the drawout lens is made 6 mm in diameter rather than the standard 3 mm diameter. The change in dimension allows for a better match between the instrument's linear working range and the requirements of the method.

Another way of improving the high end linearity is to alter the operating parameters used in the method. A combination of a lower emission current (20 µA) and a high repeller setting (25 V) was determined to improve high end linearity so that the RSD of the analytes with strong response were single digit values. These analytes are the PAHs and phthalates. The emission current of 20 µA is set by the analyst. The revised tuning macros automatically set the repeller voltage to 25 V.

Table 4. 2,4-Dinitrophenol Average RFs Using Various Ion Sources

	COC-MSD Avg RFs of 2,4-dinitrophenol, n=4				
ng Injected	**5**	**20**	**80**	**160**	**Avg**
Ultra source	0.121	0.185	0.229	0.244	0.194
Ultra source air baked at 150 °C	0.120	0.185	0.228	0.244	0.194
Ultra source cleaned with 400 grit SC paper	0.119	0.181	0.220	0.231	0.188
Ultra source cleaned with metal polish	0.107	0.169	0.209	0.221	0.177
Standard source B	0.036	0.073	0.132	0.152	0.098
Standard source A	0.025	0.036	0.063	0.086	0.052

Data

As a result of this study, the G2860A 8270 Semi-Volatiles Applications Kit has been developed. The kit provides modified and/or pretested components to improve system performance for USEPA Method 8270. The kit includes an Ultra source, specially tested column, inlet liners and tune macros. The data in Table 5 are an average result of four Ultra source/column combinations. These can be considered typical of a 6890/5973 system with the applications kit installed.

The data in Table 5 are from calibrations at 5, 10, 20, 50, 80, 120 and 160 ng. This extended range exceeds the typical range of 20 to160 ng. The data meet the 8270 criteria listed in the Introduction section of this application note. The minimum average RF is well above the required 0.050 for all of the SPCCs. The RSDs for all the CCCs are significantly less than 30% required. The mean RSD of 7% across all compounds easily meets the minimum criteria of 15%.

Table 5. Typical results from a 6890/5973 GC/MSD System with the G2860A Applications Kit Installed, 5 to 160 ng, 1 µL Splitless Injection

	Avg RF	RSD		Avg RF	RSD
ISTDs					
1,4-Dichlorobenzene-d4		8	2-Chloronaphthalene	1.003	5
Naphthalene-d8		7	2-Nitroaniline	0.482	10
Acenaphthene-d10		7	Acenaphthylene	1.512	5
Phenanthrene-d10		8	Dimethylphthalate	1.187	4
Chrysene-d12		9	2,6-Dinitrotoluene	0.272	6
Perylene-d12		9	Acenaphthene (CCC)	0.958	5
			3-Nitroaniline	0.265	8
Analytes			2,4-Dinitrophenol (SPCC)	0.130	25
Pyridine	1.436	6	Dibenzofuran	1.421	4
N-Nitrosodimethylamine	0.799	6	2,4-Dinitrotoluene	0.364	9
2-Fluorophenol	1.189	4	4-Nitrophenol (SPCC)	0.205	11
Aniline	1.576	6	Fluorene	1.152	5
Phenol-d5	1.639	6	4-Chlorophenyl-phenylether	0.566	6
Phenol (CCC)	1.783	4	Diethylphthalate	1.177	5
bis(2-chloroethyl) ether	1.280	5	4-Nitroaniline	0.223	9
2-Chlorophenol	1.293	4	4,6-Dinitro-2-methylphenol	0.135	16
1,3-Dichlorobenzene	1.320	3	Diphenylamine (CCC)	0.518	6
1,4-Dichlorobenzene (CCC)	1.371	3	2,4,6-Tribromophenol	0.109	8
1,2-Dichlorobenzene	1.275	3	Azobenzene	0.177	5
Benzyl alcohol	0.895	7	4-Bromophenyl-phenylether	0.206	5
bis(2-chloroisopropyl)ether	2.273	9	Hexachlorobenzene	0.198	4
2-Methylphenol	1.356	7	Pentachlorophenol (CCC)	0.123	9
Hexachloroethane	0.615	3	Phenanthrene	1.064	4
N-Nitroso-di-n-propylamine (SPCC)	1.508	5	Anthracene	1.017	4
4-Methylphenol	1.243	7	Carbazole	0.734	7
Nitrobenzene-d5	0.489	3	Di-n-butylphthalate	1.248	7
Nitrobenzene	0.452	3	Fluoranthene (CCC)	1.184	5
Isophorone	0.770	3	Pyrene	1.344	5
2-Nitrophenol (CCC)	0.188	7	Benzidine	0.295	9
2,4-Dimethylphenol	0.309	8	Terphenyl-d14	0.963	5
bis(2-Chloroethoxy)methane	0.407	4	Butylbenzylphthalate	0.707	8
Benzoic acid	0.154	39	3,3'-Dichlorobenzidine	0.322	8
2,4-Dichlorophenol (CCC)	0.282	7	Benzo[a]anthracene	1.213	5
1,2,4-Trichlorobenzene	0.289	4	Chrysene	1.168	3
Naphthalene	0.919	4	bis(2-Ethylhexyl)phthalate	0.906	4
4-Chloroaniline	0.340	9	Di-n-octylphthalate (CCC)	1.650	9
Hexachlorobutadiene (CCC)	0.191	5	Benzo[b]fluoranthene	1.197	9
4-Chloro-3-methylphenol (CCC)	0.341	6	Benzo[k]fluoranthene	1.108	8
2-Methylnaphthalene	0.606	4	Benzo[a]pyrene (CCC)	0.995	7
Hexachlorocyclopentadiene (SPCC)	0.267	11	Indeno[1,2,3-cd]pyrene	0.807	8
2,4,6-Trichlorophenol (CCC)	0.370	8	Dibenz[a,h]anthracene	0.689	9
2,4,5-Trichlorophenol	0.368	6	Benzo[g,h,i]perylene	0.741	8
2-Fluorobiphenyl	1.222	4	Average of analyte RSDs		7

Continued

The additional study goals were also met. Method resolution requirements for benzo[b]fluoranthene and benzo[k]fluoranthene can be met when using thinner film columns depending on inlet parameters used. Linearity has been maximized at the high end without losing significant sensitivity at the low end. This improves overall RSDs. Activity in the entire GC/MSD system has been reduced thereby maximizing RFs for active compounds such as the nitrophenols. This gives the user a greater margin for system degradation when analyzing dirty samples.

These system improvements ensure maximum productivity for the analyst using an Agilent Technologies 6890/5973 GC/MSD for USEPA Method 8270.

Instrument Operating Parameters

Two sets of recommended instrument operating parameters are listed in Table 6 and Table 7. These are starting conditions and may have to be optimized.

The ramped flow and splitless times in Table 6 result in less material on column, better peak shape and resolution of benzo[b]fluoranthene and benzo[k]fluoranthene using a 0.5 µm column as provided in the G2860A 8270 Semi-Volatiles Applications Kit. However, less material on column may result in lower response factors for active compounds.

The ramped flow and splitless times in Table 7 result in more material on column, resulting in worse peak shape and benzo[b]fluoranthene and benzo[k]fluoranthene are not resolved. However, more material on column may result in higher response factors for active compounds.

The 0.5 µm film thickness column is a compromise of speed versus resolution. A 1.0 µm film thickness column is recommended in 8270 for best resolution and best peak shape at higher analyte concentrations. Using a 1.0 µm film thickness column also results in the longest run times. A 0.25 µm film thickness column will give shorter run times, but capacity suffers and consequently so does peak shape. Some laboratories meet method resolution requirements using split injections on a 0.25 µm column.

Many users have had success keeping this method running by clipping the front end of the column on a regular basis, daily if needed. The first compounds to suffer degradation from not clipping the column are the phenols.

The 5181-3316 liner is also a compromise. The absence of wool helps to preserve active analytes but potentially subjects the column to degradation from dirty samples. Adding a wisp of wool will help protect the column but active analytes will decompose. The new direct connect liners are the best choice for clean samples or for minimizing inlet activity.

The method operating parameters given here should only be considered a good starting point. Optimization of the parameters by the analyst are dependent on the analytes and calibration ranges required by the individual laboratory's statement of work.

Table 6. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet Liner	5181-3316, single taper, 4 mm i.d., deactivated		
Inlet	EPC Split/splitless		
Mode	Splitless, 1 µL		
Inlet temp	250 °C		
Pressure	9.24 psi		
Purge flow	30 mL/min		
Purge time	0.35 min		
Gas saver	Off		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	15	100	0.00
Ramp 2	20	240	0.00
Ramp 3	10	310	6.00
Total run time	25 min		
Equilibration time	1.0 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, 19091S-133, specially tested		
Length	30 m		
Diameter	0.250 mm		
Film thickness	0.5 µm		
Mode	Ramped flow		
Flow	mL/min	mL/min	Hold min
Initial		1.2	0.00
Ramp 1	99	2.0	0.35
Ramp 2	10	1.2	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 with Ultra Source		
Solvent delay	3.2 min		
EM voltage	DFTPP tune - 75 volts		
Low mass	35 amu		
High mass	500 amu		
Threshold	50		
Sampling	2		
Scans/sec	3.25		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	310 °C		
Repeller voltage	25 V as set by new tuning macro		
Emission current	20 µA set by the analyst		

Table 7. *Alternate* Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet Liner	5181-3316, single taper, 4 mm i.d., deactivated		
Inlet	EPC Split/splitless		
Mode	Splitless, 1 µL		
Inlet temp	250 °C		
Pressure	23.14 psi		
Purge flow	30 mL/min		
Purge time	0.50 min		
Gas saver	Off		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	15	100	0.00
Ramp 2	20	240	0.00
Ramp 3	10	310	6.00
Total run time	25 min		
Equilibration time	1.0 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, 19091S-133, specially tested		
Length	30 m		
Diameter	0.250 mm		
Film thickness	0.5 µm		
Mode	Ramped flow		
Flow	mL/min	mL/min	Hold min
Initial		3.0	0.55
Ramp 1	10	1.2	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 with Ultra Source		
Solvent delay	3.2 min		
EM voltage	DFTPP tune - 200 volts		
Low mass	35 amu		
High mass	500 amu		
Threshold	50		
Sampling	2		
Scans/sec	3.25		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	310 °C		
Repeller voltage	25 V as set by new tuning macro		
Emission current	20 µA set by the analyst		

Conclusion

In this paper, we have demonstrated a process for examining the performance of various components on a complex analytical method composed of many compounds. The challenges of analyzing different classes of compounds in the shortest time while meeting the method requirements are difficult. This study has led to the development of a 25-minute 8270D method suitable for an extended calibration range of 5 to 160 ng. The G2860A 8270 Semi-Volatiles Applications Kit provides the components necessary to convert an existing 6890/5973 to perform this analysis.

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Agilent Technologies

The Analysis of Dioxin Using a Benchtop Mass Spectrometer

Application

6890/5973 Gas Chromatograph/Mass Selective Detector

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Abstract

Currently the analysis of dioxins uses high resolution mass spectrometry, (HRMS); often considered a prohibitively expensive technique. To move to a more cost-effective approach, improvements in the analytical method (sample cleanup and chromatographic separation) as well as improvements in sensitivity of benchtop mass spectrometers were needed.[1, 2]

Compared to earlier generations of benchtop mass spectrometers, the 5973 mass selective detector offers measurably greater sensitivity for electron-impact-based detection due to a number of innovative enhancements.[3]

Analysis via the GC/MSD System

This work focused on determining the detection limit for 2,3,7,8-tetrachlorodibenzo-p-dioxin with a GC/MSD system configured as outlined in this note. The desired analytical goal was to detect 0.2 pg.

A submitted sample [5 pg/µL (ppb); 2,3,7,8-TCDD in 95/5 hexane/ether] was diluted by a factor of 100 with pure hexane. (The hexane was analyzed for response at the appropriate masses prior to use to verify its purity with respect to this analyte.)

For both concentrations, the mass ratio 319.9/321.9 was measured to confirm appropriate isotopic performance. Moreover, the response factors for m/z = 321.9 were determined for both levels and compared to verify linearity over a large concentration range.

Results

The mass ratio of 319.9/321.9 is 78%, correctly reflecting appropriate isotopic abundances. Comparing the response ratios of 0.05 pg and 5 pg injections (1 µL each level), we observed that those were nearly equal: 24.6 and 23.0 (2302.6 ÷ 100).

The signal/noise for the 0.05 pg injection is about 4:1 peak/peak, representing an approximate detection limit on the system used.

The conclusion is that the sensitivity of a 5973 MSD operating as an electron impact instrument is well-suited to trace analysis of dioxins, making it a cost-effective instrument for use in EPA Methods 625 and 613.[4] For 2,3,7,8-TCDD, the detection limit with the 5973 is comparable to using HRMS. Note, however, that the ultimate method detection limits will depend on other factors — e.g., the sample matrix, type of sample cleanup used, etc. Additional sensitivity may be possible by using large volume injection techniques.[3] Future experiments will aim at evaluating the NCI (negative chemical ionization) performance of the 5973 for further gains in sensitivity and selectivity.

This will mean that a laboratory manager can choose configurations of both the chromatograph and the MSD to best match the needs of a laboratory workload. The work on the system described here demonstrated greatly enhanced sensitivity provided by cost-effective benchtop mass spectrometry.

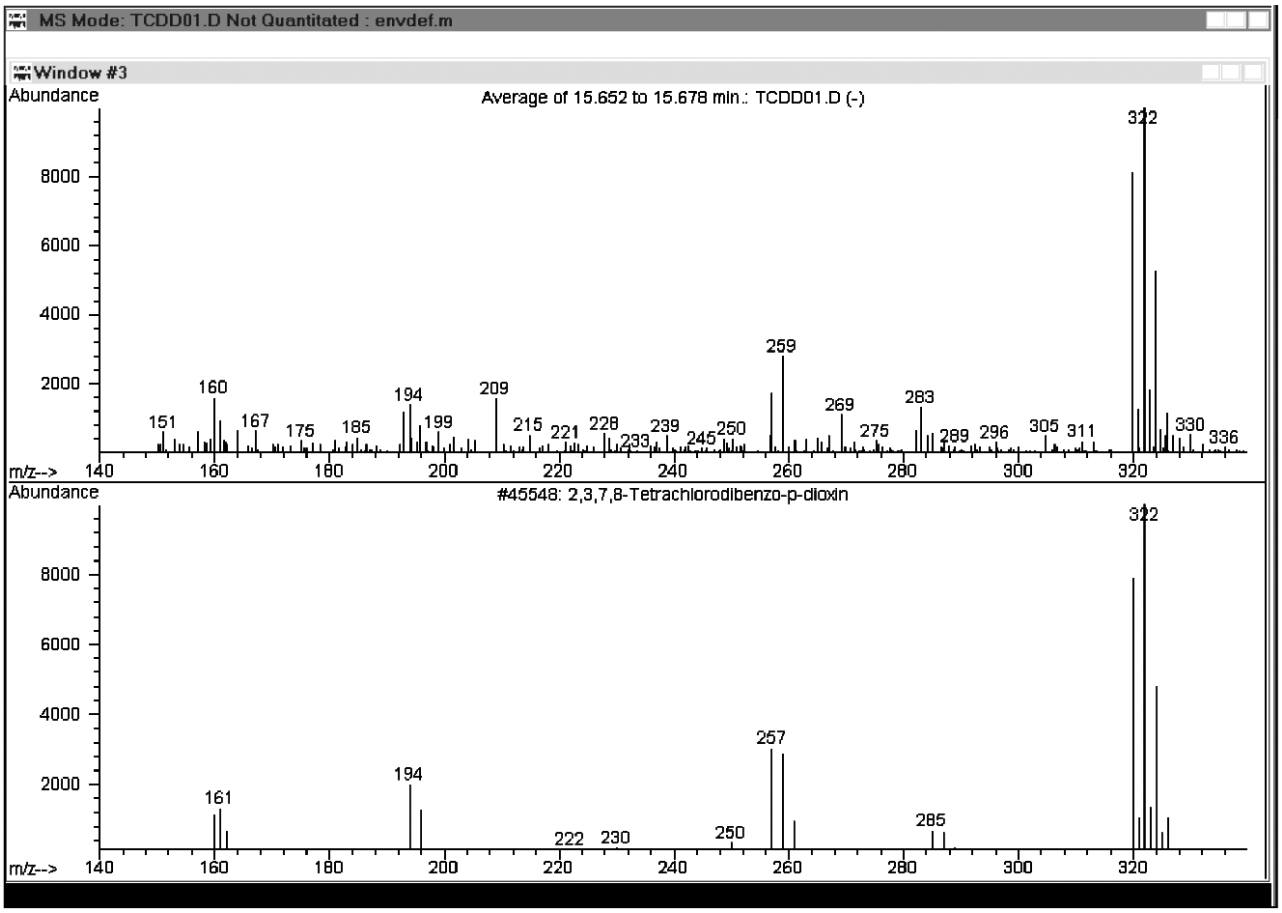

Figure 1. The match of the spectum for 5 pg 2,3,7,8-tetrachlorodibenzo-p-dioxin with the library search (lower panel). The match quality was 90%.

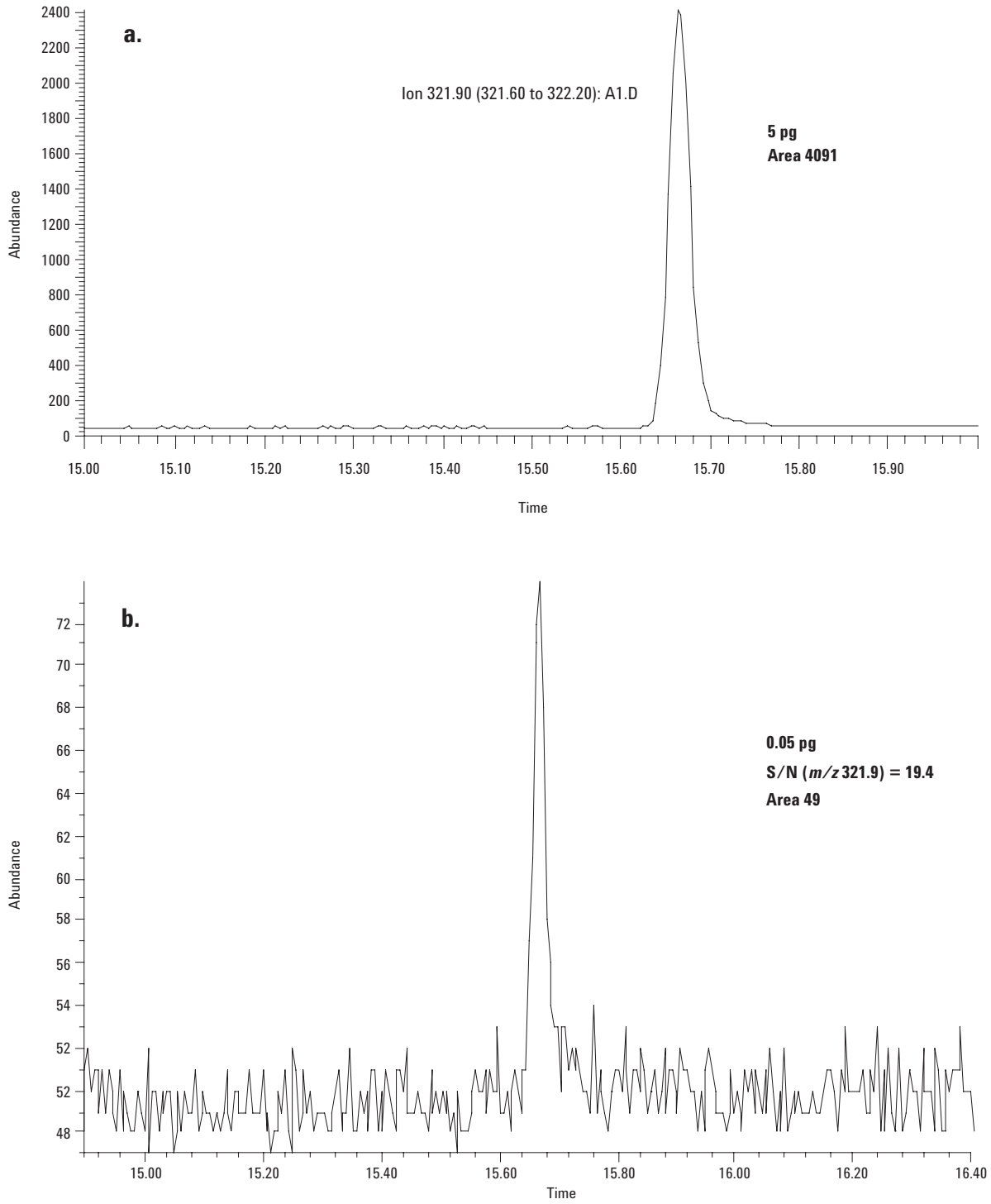

Figure 2. The TIC at *m/z* 321.9 for injection quantities of 5 pg (a.) and 0.05 pg (b.) in SIM mode.

6890 with 5973 MSD

Injection	• Pulsed splitless single taper liner with glass wool plug, P/N 5062-3587. • 250 °C • 1 µL injection volume • Viscosity delay, 1 sec • Sample washes, 3; post-injection solvent washes, 4
Column	HP-5MS: 30 m × 250 µm, 0.25 µm film (crosslinked 5% Ph Me Siloxane), P/N 19091S-433
Carrier	Helium, 37 cm/sec; vacuum compensation, on.
Temperature	Initial: 70 °C for 1.50 min
Program	Rate 1: 25.00 °C/min to 150 °C Rate 2: 10.00 °C/min to 280 °C Final: 280 °C for 0.00 min
Pressure	25.0 psi for 1.50 min; then
Program	1.0 mL/min constant flow rate
MSD	• Temperatures Transfer line = 300 °C Source = 230 °C Quadrupole = 106 °C • Tune = autotune • Emission current = 35 µamp • SIM mode, EMV = Autotune + 400 V • Solvent delay = 14.00 min • Dwell per ion = 125 msec • SIM Ions (*m/z*): 319.9, 321.9
Autosampler	7673B
ChemStation	G1701AA

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1. P. R. Gardinali et al, Chemosphere 32 (1), pp. 1-11 (1996).
2. R. Malisch et al, Chemosphere 32 (1), pp. 31-44 (1996).
3. L. Doherty, "Enhancing Pesticide Analysis with a Highly Sensitive GC/MSD System," Application Note, Pub. No. (23) 5966-0370E (1997).
4. Code of Federal Regulations, Title 40, Vol. 13, Parts 136-149, Appendix A. Revised, July 1, 1997. U.S. Government Printing Office (via GPO Access; CITE: 40CFR136). Method 613 - 2,3,7,8-tetrachloro-dibenzo-p-dioxin by GC/MS (SIM). Method 625 - Base/Neutrals and Acids, Semivolatiles by GC/MS (SCAN).

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Agilent Technologies

A New Approach to the Analysis of Phthalate Esters by GC/MS

Application

Gas Chromatography/Mass Spectrometry

March 2001

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Abstract

A new instrumental method for the determination of 29 phthalate esters, including six recently banned from baby toys by the European Union, using positive chemical ionization and retention-time locking is described. Positive chemical ionization provides a high degree of selective ionization for the phthalates, primarily producing spectra in which the protonated molecule (M+1) is the base peak. This provides easy discrimination among the phthalates on the basis of their molecular weight, while retention-time locking increases confidence in the identification of the various isomers.

In this approach, both pure compounds and technical mixtures are considered. Although this work focuses on the more commonly used 1,2-substituted esters, the 1,3-isomers and 1,4-isomers are also characterized.

The combination of positive chemical ionization and retention-time locking makes the method rugged, durable and applicable to a wide variety of matrices.

Introduction

The widespread use and manufacture of plastics have made the phthalate esters one of the most ubiquitous classes of compounds in our everyday environment. These “plasticizers” increase polymer flexibility due to their function as intermolecular “lubricants”. Because they are additives and not reagents, they are not chemically bound in the polymer and are available to leach from the matrix. Phthalates are also components of cosmetics, detergents, building products (flooring, sheeting, films), lubricating oils, PCB substitutes, carriers in pesticide formulations and solvents. Consequently, the potential for human exposure is very high. Toxicological studies have linked some of these compounds to liver and kidney damage, and to possible testicular or reproductive-tract birth defect problems, characterizing them as endocrine disruptors. Scientists at the U.S. Centers for Disease Control have, for the first time, documented human exposure to phthalates by determinations of the monoester metabolites in human urine [1]. Their work leads to the conclusion that “phthalate exposure is both higher and more common than previously suspected.”

Of particular concern were the significantly higher concentrations of the dibutyl phthalate metabolite in urine of women of childbearing age (20-40 years) than in other portions of the population.

The presence of phthalate esters in polyvinyl chloride (PVC) toys has generated the most

controversy. While regulators in Greece have completely banned soft PVC toys, Austria, Denmark, Finland, France, Germany, Norway and Sweden have unilaterally banned phthalates in PVC toys for children under three years old. In December of 1999, the European Union (EU), concerned with a "serious and immediate risk" to children, placed an emergency ban on six of the phthalate esters in soft PVC toys and childcare products meant to be placed in the mouths of children under the age of three [2]. None of the six banned phthalates may exceed 0.1% by weight.

These heightened concerns suggest the need for an improved method of detecting and characterizing phthalate esters which is applicable to a wide variety of matrices. This application note describes such an analytical method.

Phthalate Structure and Mass Spectra

The three primary structures of phthalates are shown in Figure 1. Although there are three possible positions for the ester linkages, the most commonly used phthalates are based on the 1,2-benzenedicarboxylic acid structure (top). There are an infinite number of possible alkyl side chains, (R) and an infinite number of combinations of the side groups (R and R'). For example, the diisononyl phthalate consists of an array of compounds due to the isomeric branched-chain alkyl groups on both side chains.

For phthalate esters with saturated alkyl side chains (without oxygen), the most intense peak in the electron impact (EI) ionization mass spectrum at 70 eV is always at *m/z* 149 due to the rapid formation and stability of the ion shown in Figure 2. (The only exception is R=R'=CH_3 where the base peak is at *m/z* 163).

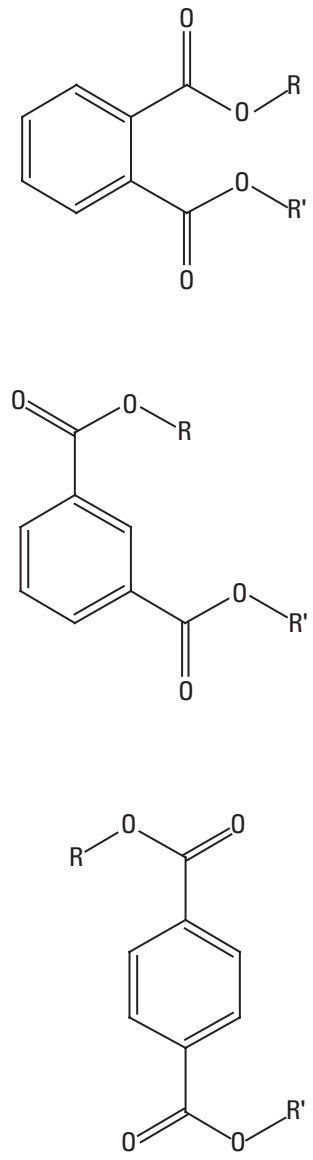

Figure 1. Phthalic ester (top) or the 1,2-benzenedicarboxylic acid ester, isophthalic ester (middle) or the 1,3-benzenedicarboxylic acid ester, and terephthalic ester (bottom) or the 1,4-benzenedicarboxylic acid ester. R and R' represent alkyl side chains which may be branched and contain oxygen.

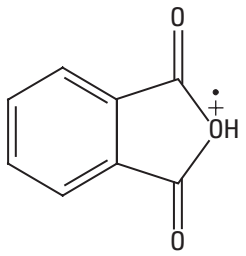

Figure 2. The most abundant ion in the mass spectra of the phthalate esters with saturated alkyl side chains; *m/z* 149. The exception is for dimethyl phthalate where both R and R' are CH_3 and so the H on the oxygen is replaced by CH_3 and consequently *m/z* 163 becomes the base peak.

Invariably, the molecular ion is very weak or altogether absent; other fragments that provide information on the phthalate identity are also of very low abundance. As an example, consider the EI mass spectrum of dibutyl phthalate, one of the six banned by the EU, and bis(4-methyl-2-pentyl) phthalate in Figure 3. Identifying fragments have relative intensities of less than 10%. Gas chromatography provides some separation of the phthalates, but with the array of possible isomers and essentially a single identifying ion (i.e., *m/z* 149), distinguishing the individual phthalates of concern is difficult. More confident identification of the phthalates is possible using chemical ionization mass spectrometry in conjunction with retention-time locking (RTL).

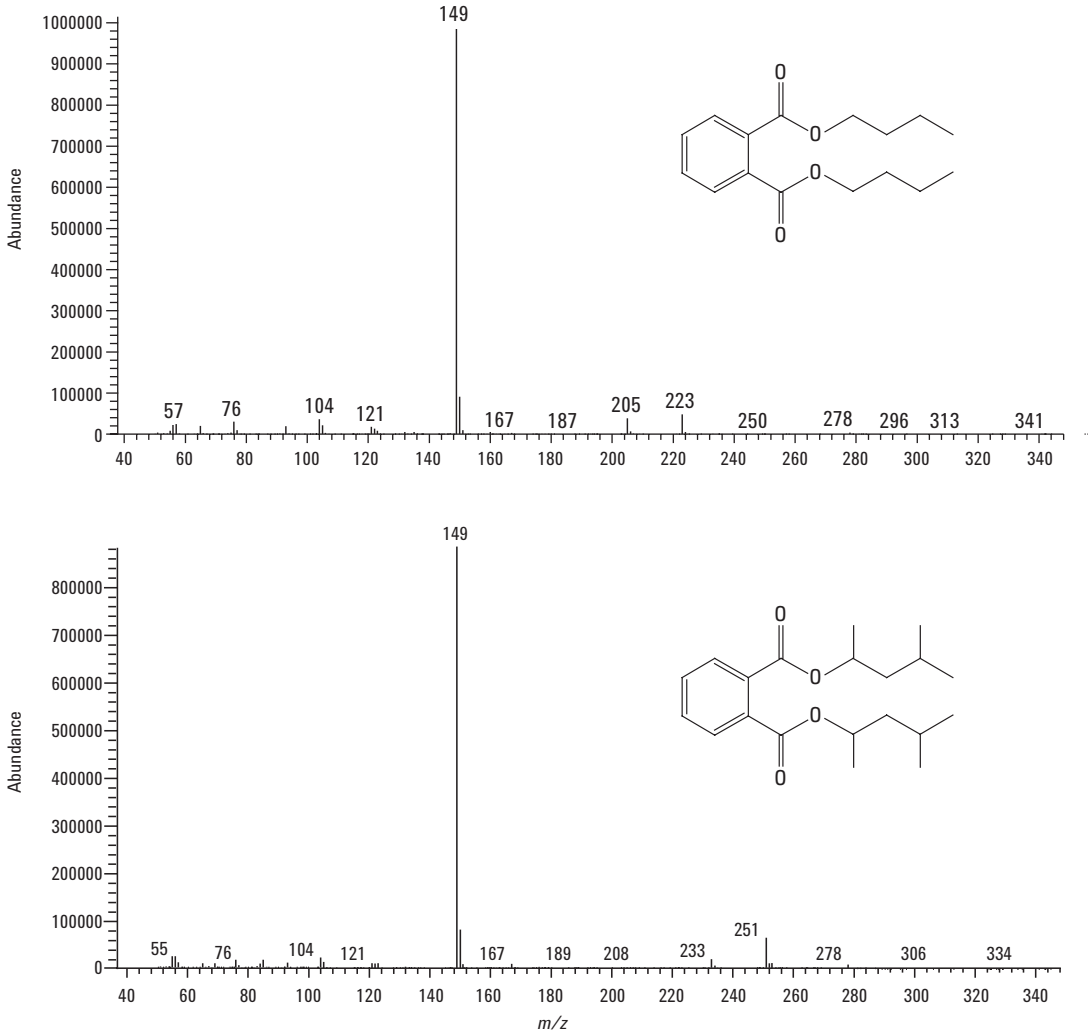

Figure 3. Electron impact ionization mass spectra of di-n-butyl phthalate (upper panel) and bis(4-methyl-2-pentyl) phthalate (lower panel) from *m/z* 50 to 350 at 70 eV. Notice the lack of intense fragments and molecular ions. The molecular weights are 278 and 334 g/mole, respectively.

Retention-time locking allows compound retention times achieved on any one Agilent 6890 gas chromatograph (GC) to be replicated to within a few seconds on any other Agilent 6890 gas chromatograph (GC) applying the same GC method [3-5]. RTL is a powerful approach to compound identification. RTL allows the creation of compound acquisition methods and quantitation databases that can be reproduced in any laboratory, anywhere, because a compound can have a universally fixed and reproducible retention time. It is important that RTL be applied in conjunction with the appropriate detection scheme and sample reparation methods.

Chemical ionization provides a more selective form of ionization than electron impact [6]. By judicious choice of the reagent gases, the degree of compound fragmentation can be controlled to a certain extent. In positive chemical ionization, methane reagent gas usually provides more fragmentation than gases of higher proton affinity such as ammonia. Less fragmentation would be helpful in identifying the phthalates. Instead of all phthalates generating a single, similar ion, positive ionization can provide phthalate ester molecular weights.

Experimental

Phthalate esters were obtained from Ultra Scientific (North Kingstown, RI), AccuStandard (New Haven, CT), and ChemServices (West Chester, PA) as neat compounds and mixtures. Dilutions were made in isooctane (Burdick and Jackson Grade, VWR Scientific).

The configuration and operating parameters of the Agilent 6890Plus GC (standard 120V or "faster ramping" 220V), 7683 Automatic Liquid Sampler and 5973N MSD with CI option used for acquiring the data are given in the following tables. PCI reagent gas purities were 99.99% or higher.

Injection Parameters

Injection Mode	Pulsed Splitless	
Injection Port Temperature	300°C	
Pulse Pressure & Time	25.0 psi	1.00 min
Purge Flow & Time	20.0 mL/min	3.00 min
Gas Saver Flow & Time	20.0 mL/min	3.00 min

Oven Parameters

Temperature Program	80°C	1.00 min
50.00°C/min	200°C	0.00 min
15.00°C/min	350°C	2.00 min
Oven Equilibrium Time	0.25 min	
MSD Transfer Line Temp	325°C	

Column Parameters

GC column (122-5532)	DB-5MS 30 m;	
	0.25 mm i.d.; 0.25 µm film	
Initial Flow & Mode	1.2 mL/min	Constant Flow
Detector & Outlet Pressure	MSD	Vacuum

Mass Spectrometer Parameters

Tune Parameters	PCI Autotune (NH3)
Electron Multiplier Voltage	Autotune + 400V
Solvent Delay	4.00 min
Scan Parameters	194 - 550 *m/z*
Quadrupole Temperature	150°C
Source Temperature	250°C
Ammonia Gas Flow (MFC setting)	0.5 mL/min (10%)

Miscellaneous Parts

Septa	5182-0739	BTO septa (400°C)
Liner	5062-3587	Deactivated 4 mm i.d. single taper
GC column ferrule	5181-3323	250 µm Vespel
MSD interface ferrule	5082-3508	0.4 mm i.d. graphitized Vespel

Results and Discussion

As expected using methane as the reagent gas, the PCI mass spectra of the phthalates show ions corresponding to the protonated molecule $[M+H]^+$ and adducts $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$. Because of the relatively vigorous fragmentation produced by methane, the spectra of the dialkyl phthalate esters still resemble that produced in EI. In most cases, the fragment at *m/z* 149 is the base peak, however ions at *m/z* M+1, M+29 and M+41 are relatively intense with $[M+H]^+$ from 10% to 30% (Figure 5). The dialkyl phthalate spectra also show a fragment corresponding to loss of one of the alkyloxy side chains to produce an ion shown in Figure 4. This is the most intense fragment for the dimethyl and diethyl phthalates and for the dibutyl and dipentyl (diamyl) phthalates, about 75% of the 149 base peak. As the length of ester alkyl chain increases, the intensity of this fragment decreases. (Apparently, in the dialkyl isophthalates, loss of the alkyl side chain not accompanied by the oxygen may be a preferred route.)

Although positive chemical ionization with methane provides more information than EI on phthalate identity, the methane reagent is still rather unselective in ionization and will produce more chemical noise in the background, complicating identification in complex matrices.

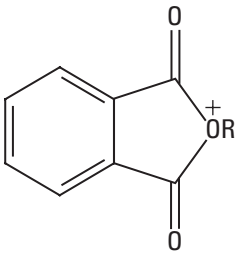

Figure 4. One of the most intense fragments in the methane PCI spectra of the phthalate esters is formed by loss of one of the alkyloxy side groups.

Applying ammonia as the reagent gas in PCI to reduce chemical noise and enhance identification of the phthalates is a more useful approach. The relatively gentle ionization produces protonation of the dialkyl phthalates, with *m/z* M+1 the base peak in their spectra. When combined with retention-time locking, identification of phthalates becomes further simplified. Compare the spectra of the di-n-butyl phthalate acquired using methane versus ammonia as the reagent gas (Figure 5). The protonated molecule is the single dominant peak in the ammonia PCI mass spectrum of the di-n-butyl phthalate, and the adduct at *m/z* 296 ($[M+NH_4]^+$) is relatively small.

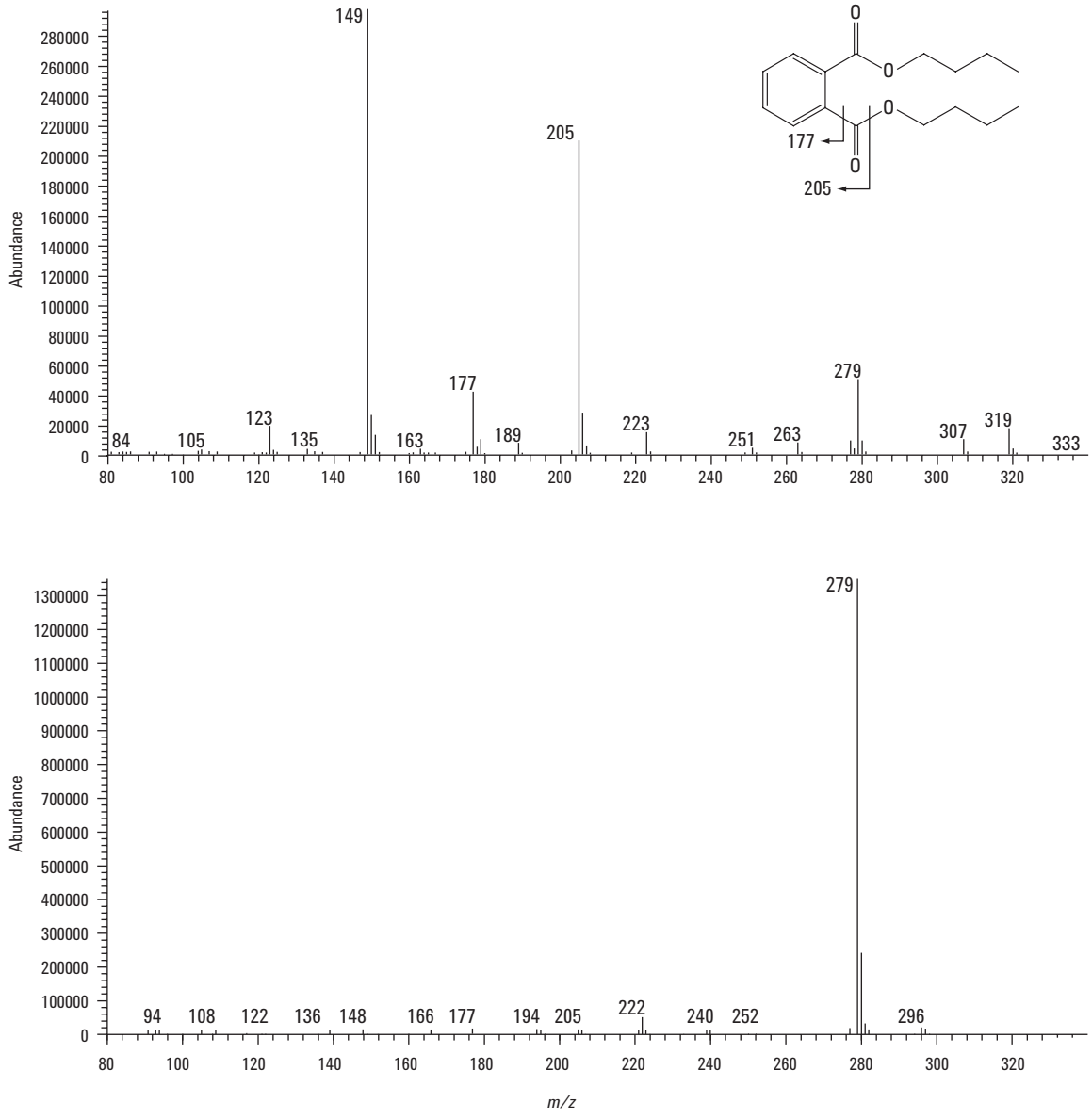

Figure 5. PCI methane (upper panel) and ammonia (lower panel) mass spectra of di-n-butyl phthalate. The PCI methane mass spectrum shows substantial fragmentation but relative to the EI spectrum in Figure 3, high abundance for the higher *m/z* ions such as the protonated molecule at *m/z* 279. The ion at *m/z* 205 is generated by loss of an oxybutyl fragment; a process described in Figure 4. The PCI-ammonia mass spectrum consists almost completely of the protonated molecule.

This implies an easy method for identification. Whereas the EI spectra of the phthalates most frequently result in a base peak at *m/z* 149, the dialkyl phthalate PCI-ammonia spectra have base peaks at *m/z* = M+1. All dialkyl phthalates molecular formulas can be expressed as

$$C_8H_6O_4(CH_2)_y\,(CH_2)_x.$$

These phthalates have (nominal) molecular masses given by

$$M = 166 + (x + y)\cdot 14, \text{ or}$$
$$M = 166 + (w)\cdot 28,$$

where x and y are the side chain lengths, and the second formula applies to symmetrical side chains (i.e., x = y = w). For example, di-n-butyl phthalate has x = y = 4, and therefore a (nominal) molecular mass of 278 which produces *m/z* 279 as the base peak. Interestingly, the PCI-ammonia spectra of the dialkyl isophthalates and terephthalates appear to have base peaks at *m/z* M+18 due to $[M+NH_4]^+$. Because of the greater steric access to the ester linkages, adduct formation may be preferred.

Table 1 gives the phthalate names, CAS numbers, molecular formula, nominal molecular mass, base peak in the PCI-ammonia spectrum and the RTL elution times. These retention times are "locked" relative to diphenyl phthalate, which has been chosen as the RTL locking compound and locked to elute at 9.450 min. Notice that the branched chain isomers elute prior to their straight chain forms on this column phase.

Table 1. Phthalate compound names, Chemical Abstracts Services numbers (CAS), molecular weights (M. Wt.), molecular formulas, nominal base peak in the PCI-ammonia spectrum and retention time (RT) in minutes. Retention times are locked relative to diphenyl phthalate (9.450 min). Retention time ranges are given for the isoalkyl phthalate technical mixtures. Phthalates banned by the EU are indicated by an asterix*. Benzyl benzoate is included since it is used as a surrogate in U.S. Environmental Protection Agency Method 8061.

Name	CAS	M. Wt.	Molecular Formula	Base Peak	RT (min)
dimethyl phthalate	131-11-3	194	$C_8H_4O_4(CH_3)_2$	195	4.32
dimethyl isophthalate	1459-93-4	194	$C_8H_4O_4(CH_3)_2$	212	4.54
diethyl phthalate	84-66-2	222	$C_8H_4O_4(C_2H_5)_2$	223	4.81
diethyl terephthalate	636-09-9	222	$C_8H_4O_4(C_2H_5)_2$	240	5.06
benzyl benzoate	120-51-4	212	$C_{14}H_{12}O_2$	230	5.62
diisobutyl phthalate	84-69-5	278	$C_8H_4O_4(C_4H_9)_2$	279	5.95
di-n-butyl phthalate*	84-74-2	278	$C_8H_4O_4(C_4H_9)_2$	279	6.40
bis(2-methoxyethyl) phthalate	117-82-8	282	$C_8H_4O_4(C_2H_4OCH_3)_2$	283	6.57
diamyl phthalate	131-18-0	306	$C_8H_4O_4(C_5H_{11})_2$	307	6.94
bis(2-ethoxyethyl) phthalate	605-54-9	310	$C_8H_4O_4(C_2H_4OC_2H_5)_2$	311	7.13
butyl benzyl phthalate*	85-68-7	312	$C_8H_4O_4(C_4H_9)(CH_2C_6H_5)$	313	8.42
diphenyl phthalate	84-62-8	318	$C_8H_4O_4(C_6H_5)_2$	319	9.45
diphenyl isophthalate	744-45-6	318	$C_8H_4O_4(C_6H_5)_2$	319	10.30
dicyclohexyl phthalate	84-61-7	330	$C_8H_4O_4(C_6H_{11})_2$	331	9.32
bis(4-methyl-2-pentyl) phthalate	146-50-9	334	$C_8H_4O_4(CH_3C_5H_{10})_2$	335	6.93
diisohexyl phthalates	146-50-9	334	$C_8H_4O_4(C_6H_{13})_2$	335	7.55 - 8.28
dihexyl phthalate	84-75-3	334	$C_8H_4O_4(C_6H_{13})_2$	335	8.34
dibenzyl phthalate	523-31-9	346	$C_8H_4O_4(CH_2C_6H_5)_2$	347	10.51
hexyl-2-ethylhexyl phthalate	75673-16-4	362	$C_8H_4O_4(C_2H_5C_6H_{12})(C_6H_{13})$	363	8.84
bis(2-n-butoxyethyl) phthalate	117-83-9	366	$C_8H_4O_4(C_2H_4OC_4H_9)_2$	367	8.98
bis(2-ethylhexyl) phthalate*	117-81-7	390	$C_8H_4O_4(C_2H_5C_6H_{12})_2$	391	9.32
di-n-octyl phthalate*	117-84-0	390	$C_8H_4O_4(C_8H_{17})_2$	391	10.28
dioctyl isophthalate	137-89-3	390	$C_8H_4O_4(C_8H_{17})_2$	408	10.84
diisononyl phthalates*	28553-12-0	418	$C_8H_4O_4(CH_3C_8H_{17})_2$	419	9.40 - 11.10
dinonyl phthalate	84-76-4	418	$C_8H_4O_4(C_9H_{19})_2$	419	11.19
diisodecyl phthalates*	26761-40-0	446	$C_8H_4O_4(CH_3C_9H_{18})_2$	447	10.16 - 11.86
didecyl phthalate	84-77-5	446	$C_8H_4O_4(C_{10}H_{21})_2$	447	12.05
diundecyl phthalate	3648-20-2	474	$C_8H_4O_4(C_{11}H_{23})_2$	475	12.87
didodecyl phthalate	2432-90-8	502	$C_8H_4O_4(C_{12}H_{25})_2$	503	13.65
ditridecyl phthalate	119-06-2	530	$C_8H_4O_4(C_{13}H_{27})_2$	531	12.01 - 13.81

Technical formulations of the isoalkyl phthalates tended to contain substantial amounts of the straight chain isomer, which may convolute quantitation as well as peaks that may be construed as originating from nonequivalent side chains i.e., $x \neq y$ in equation 1). These impurities can be detected as M±14 around the mass of the nominal isomer. For example, technical grade diisononyl phthalate contains compounds that generate ions at *m/z* 391 (minor), 405, 433, and 447 in addition to the nominal diisononyl phthalate compound at *m/z* 419. The "gentle" ionization of ammonia reagent gas, the elution times and the study of the spectra of other pure isomers, such as the dinonyl phthalate, suggest that these fragments are not formed by the PCI process but are due to these different alkyl side chain impurities (Figure 6). To demonstrate the utility of the PCI-ammonia compared to conventional EI analysis, consider the chromatograms presented in Figure 7. The EI spectra of the phthalates produce *m/z* 149 as the base peak for all the phthalates present; distinguishing ions are minor constituents (<10% relative intensity), making identification complicated. However, by examining the appropriate PCI-ammonia ions, the various phthalates are easily distinguished.

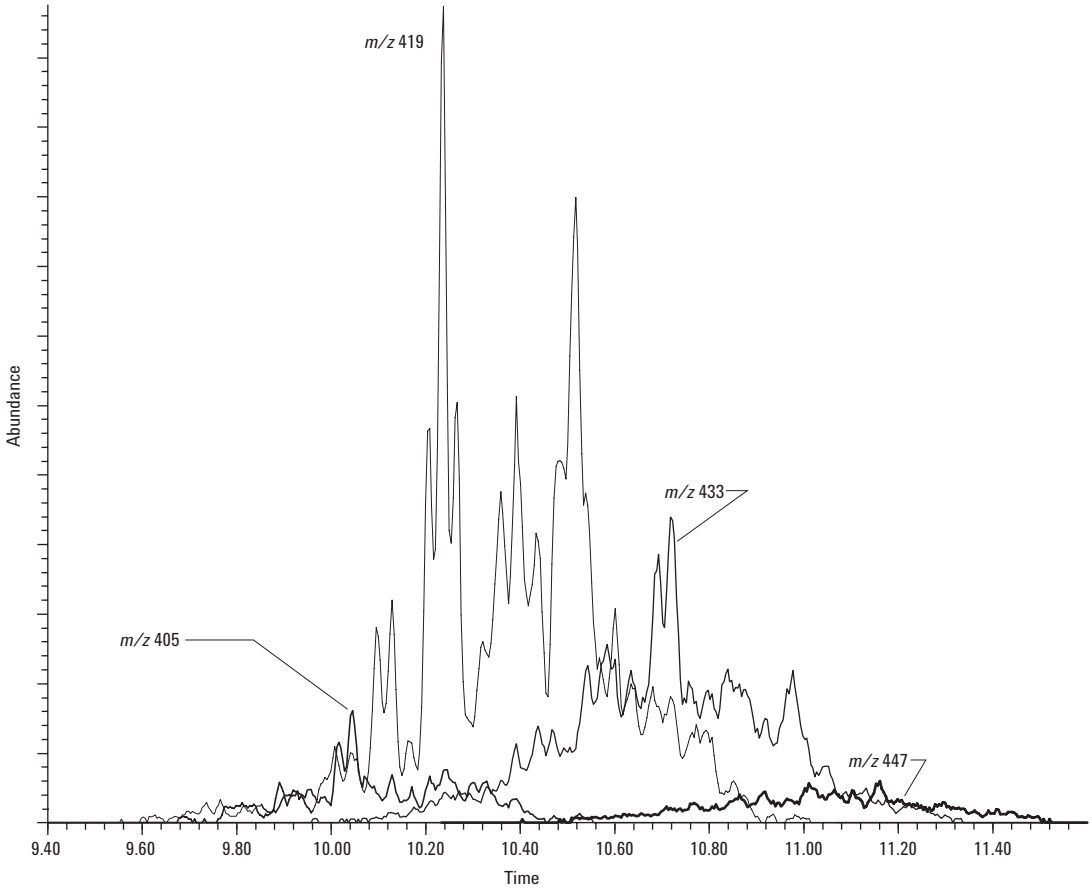

Figure 6. PCI-Ammonia extracted ion chromatogram of technical diisononyl phthalate. The diisononyl appears as the major component at *m/z* 419 while ions at *m/z* 405, 433, and 447 indicate alkyl chains shorter by one CH_2 unit and longer by one and two CH_2 units respectively.

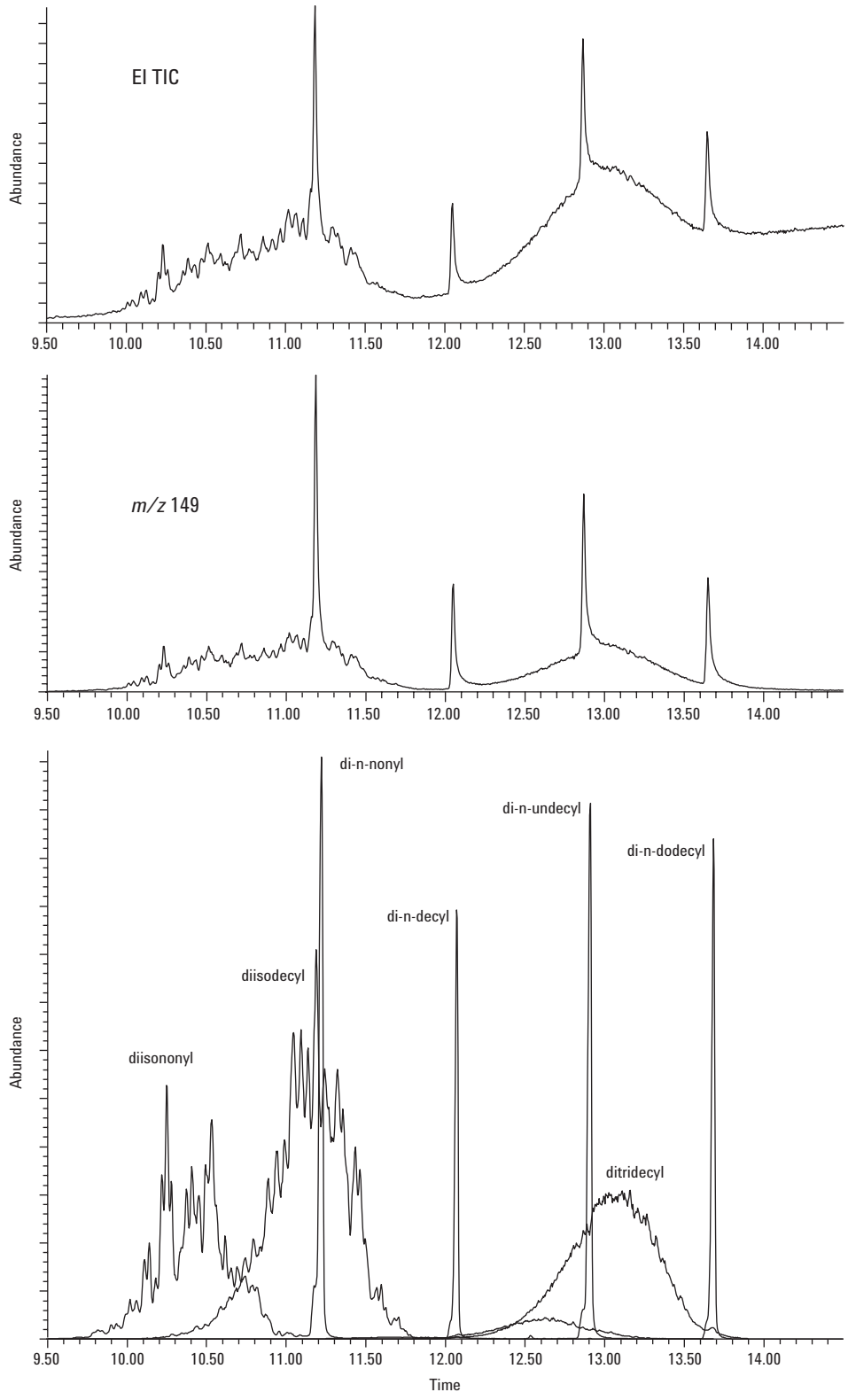

Figure 7. Chromatograms of dinonyl, diisononyl, didecyl, diisodecyl, diundecyl, didodecyl, ditridecyl phthalate esters in EI (upper panel), EI as an extracted ion chromatogram at *m/z* 149 (middle panel), and PCI-extracted ion chromatogram with ions selected for the individual phthalate classes as given in Table 1. The EI information is insufficient to identify coeluting phthalates. For example, the dinonyl and diundecyl phthalates are "buried under" the signals from the isodecyl and ditridecyl phthalates.

Conclusions

Applying GC - electron impact (EI) mass spectrometry to the determination of phthalates requires full chromatographic separation. The EI spectra of the phthalates are distinguished only by ions of very low intensity. In EI, the phthalates produce a single common ion (*m/z* 149) as the most intense spectral peak, regardless of the alkyl side chain substitution. Applying tandem mass spectrometry (i.e., EI/MS/MS) gains nothing, because there is a common parent ion, and therefore any daughter ions would also be non-unique. However, the combination of positive chemical ionization with retention-time locking allows even complex mixtures of phthalates to be characterized. Ammonia reagent gas produces the protonated molecule as the base peak, which immediately allows the phthalates to be distinguished on the basis of their substitution. PCI is also an advantage in complex matrices, where the non selective ionization of EI produces a high chemical background. This method should therefore be suitable for use in phthalate determinations in environmental media, plastics, cosmetics and many other matrices.

"Locking" the retention time enhances confidence in the characterization of the various phthalate isomers on the basis of their definitive retention time. This is especially helpful for determinations using selected ion monitoring (SIM), since SIM groups need not be edited after column maintenance [4]. The data in Table 1 facilitate the development of a SIM method. The extension of the method to phthalates which elute at higher temperatures (>350°C) is also easily accomplished.

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Analysis of Nitroaromatics and Nitro-Polycyclic Aromatic Hydrocarbons by Capillary Gas Chromatography with the Agilent 6890 Micro-ECD

Application

Gas Chromatography

March 1997

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Abstract

A new electron capture detector (ECD) for the Agilent 6890 Series gas chromatograph (GC) allows very sensitive detection of nitroaromatic compounds at low picogram levels with a linear response over three orders of magnitude.

This application note describes the performance of the new 6890 Series Micro-ECD when analyzing two types of nitro-aromatic compounds—explosives and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs).

Introduction

Electron capture detection is most often used for the sensitive and selective detection of halogenated compounds. However, other compound classes also have electron capturing properties and can, therefore, be detected at low levels using an electron capture detector (ECD). Compounds containing a nitro-function—particularly nitroaromatics—are strong electron-capturing molecules. The ECD provides a very sensitive tool for trace analysis of these solutes.

This application note demonstrates that the 6890 Series Micro-ECD provides an extremely sensitive alternative to the typical NPD or MS detection[1, 2] for nitro-PAHs and explosives.

Experimental

The analyses were performed on an 6890 Series GC. Injection was automated splitless using an Agilent 7673 automatic sampler. The instrument configuration and analytical conditions used for the analysis of the nitro-PAHs and explosives are summarized in table 1.

Results and Discussion

The sensitivity of the ECD depends on the makeup flow rate. The 6890 Micro-ECD optimized the argon/5% methane (Ar/CH_4) makeup gas flow rate for the analysis of nitro-PAHs. Nitropyrene was used as test solute. The makeup flow rate was varied from 10 to 80 mL/min; at each setting, five runs were made.

Figure 1 shows the mean peak areas plotted versus the makeup flow rate. The optimum flow rate was obtained between 20 to 30 mL/min. At lower flow rates, the peak area decreased and the detector became less stable, shown in the increasing standard deviation on peak area. At higher flow

Table 1. Instrumental Configuration and Analytical Conditions

Chromatographic System	
Gas chromatograph	6890 Series
Inlet	Split/splitless
Detector	Micro-ECD
Automatic sampler	7673 Series
Liner	Single taper deactivated (part number 5181-3316)
Data handling	ChemStation (DOS Series)
Column	30 m x 0.25 mm id x 0.25 µm HP-5 MS (part number 19091S-433)
Experimental Conditions	
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Splitless
Purge time	0.75 min
Purge flow	50 mL/min
Carrier gas	Hydrogen
Head pressure	58 kPa at 50 °C
Carrier gas mode	Constant flow
Flow, velocity	1.4 mL/min, 40 cm/s
Oven temperature	50 °C, 1 min initial, 20 °C/min to 320 °C, 0.5 min hold
Detector temperature	320 °C
Detector gases	Argon/5% methane: 20 mL/min

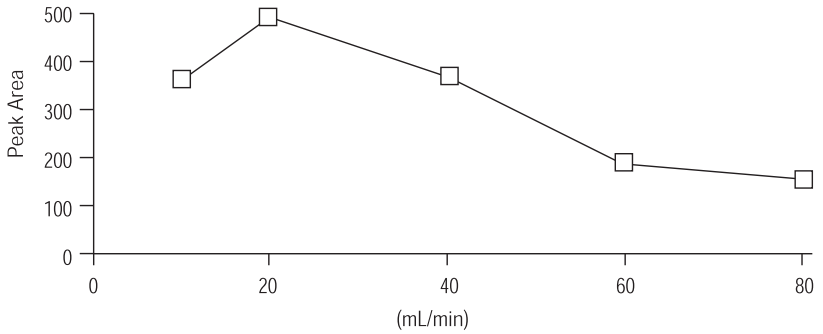

Figure 1. Peak area of 1-nitropyrene versus argon/5% methane makeup gas flow rate.

rates, the detector was stable (exhibiting a small standard deviation), but sensitivity drastically decreased.

Nitrogen is an alternative makeup gas for electron capture detection. It can usually be used interchangeably with Ar/CH_4; similar results for the effect of makeup gas flow rate are expected.

Next, the linearity of the detector response was measured. Using nitropyrene as the test solute, standard solutions of 1, 10, 50, 100 and 1,000 ppb were analyzed. The calibration curve for this compound, as shown in figure 2, exhibits a very good correlation coefficient (r = 0.99996).

Nitrated Polycyclic Aromatic Hydrocarbons

Nitro-PAHs are an important class of environmental pollutants.[3] Polycyclic aromatic compounds are formed during incomplete combustion of organic material. In the presence of nitrogen oxides (NO_x), the neutral PAHs (such as naphthalene or pyrene) are converted into nitro-PAHs.[3–5]

The nitro-PAHs have much higher mutagenic and carcinogenic activity than the neutral PAHs, but their extremely low concentration (measured as pg/m^3) in environmental samples, particularly air particulates, makes them difficult to monitor. Very sensitive detection is needed.

Using the optimized GC conditions, a mixture of 11 nitro-PAHs, each having a concentration of 40 pg/mL (40 ppb), was analyzed. The chromatogram for this analysis is shown in figure 3. Good peak shapes were obtained for all compounds. The detection limit, which varied from 0.1 to 1 pg for the different PAHs, is at least one order of magnitude lower than that obtained by nitrogen-phosphorus detection (NPD), mass spectrometry (MS), or MS-MS.[2] It can, therefore, be concluded that the 6890 Micro-ECD offers greater sensitivity for the detection of these nitro-PAHs than other methods.

Explosives

Explosives can be present as residues at chemical waste sites or on materials close to an explosion. Sensitive and fast methods are needed for analyzing and monitoring these compounds for environmental remediation or forensic evidence.

Although explosives are often analyzed by high pressure liquid chromatography (EPA method 8330), capillary gas chromatography (CGC) can provide a good alternative for most solutes using NPD or MS. Some of the nitro-aromatics are included in the target compound lists of EPA methods 8090 and 8270 (CGC-MS).

Explosives such as TNT (2,4,6-trinitrotoluene) contain one or more nitro-functions. CGC-ECD can provide a very sensitive and fast screening method for detecting these compounds.

The chromatogram in figure 4 shows the results of a standard mixture of explosives using the analytical conditions in table 1. The concentration of the test solutes was 100 pg/mL

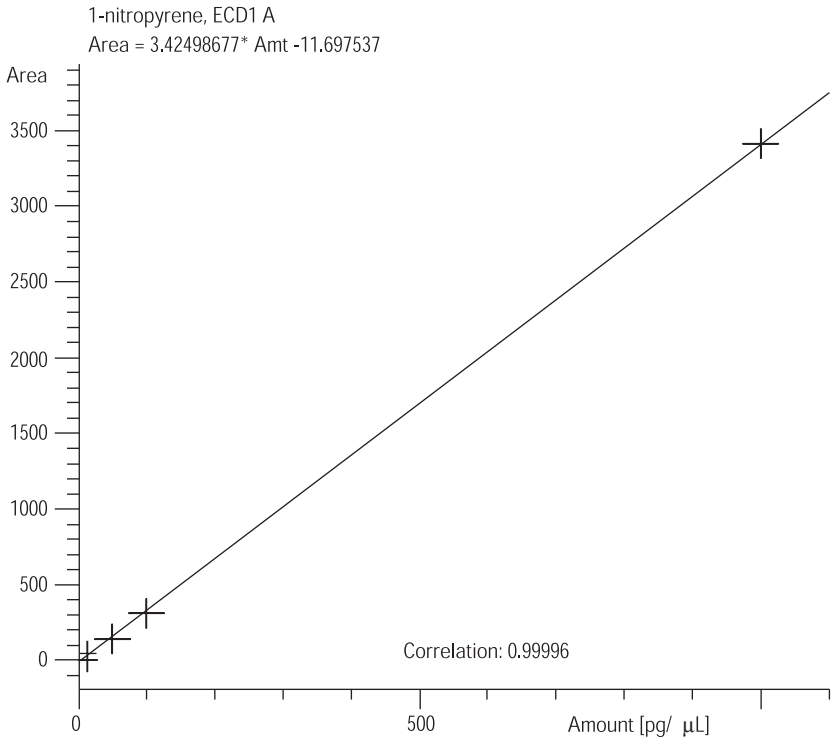

Figure 2. Calibration curve for 1-nitropyrene from 1 to 1,000 ppb

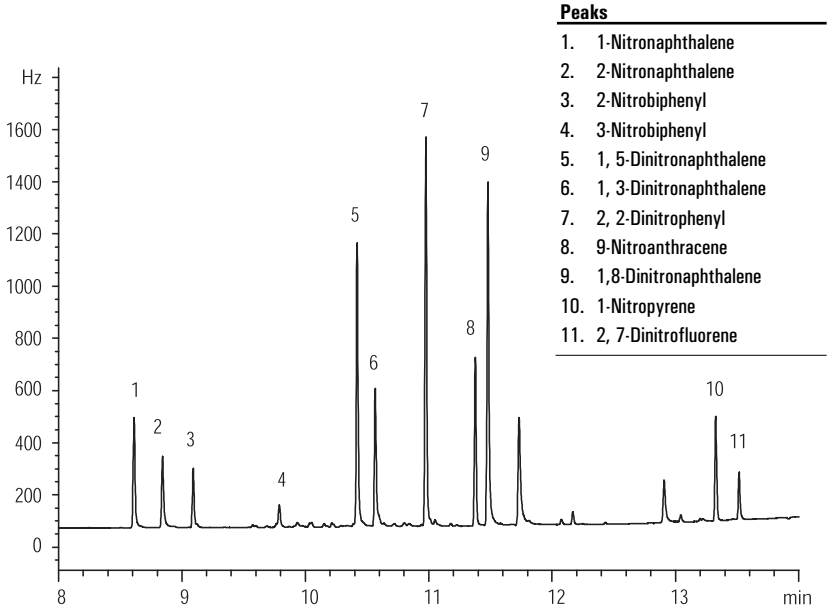

Figure 3. CGC-ECD analysis of nitrated polycyclic aromatic hydrocarbons (solute concentration: 40 ppb)

(100 ppb), except for 1,2-dinitrobenzene, which was present as an impurity. As the chromatogram shows, the different nitro-, dinitro-, trinitro-, and amino-nitro-compounds are well separated and elute with good peak shape.

The ECD response is dependent on the number of nitro-groups. For the mono-nitroaromatics, the detection limit is around 10 pg, while for the di- and tri-nitroaromatics the detection limit is below 1 pg. This example confirms that CGC-ECD can be used as a fast screening method for the analysis of this category of explosives.

Conclusion

The Agilent 6890 Series Micro-ECD allows very sensitive detection of nitroaromatic compounds. The detector was successfully used for the analysis of nitrated polycyclic aromatic hydrocarbons and explosives. Detection limits below 1 pg were obtained, and the detector was found to give a linear response over three orders of magnitude.

Peaks	
1. Nitrobenzene	7. 1,2-Dinitrobenzene (impurity)
2. 2-Nitrotoluene	8. 2,4-Dinitrotoluene
3. 3-Nitrotoluene	9. 1,3,5-Trinitrobenzene
4. 4-Nitrotoluene	10. 2,4,6-Trinitrotoluene
5. 1,3-Dinitrobenzene	11. 4-amino-2,6-Dinitrotoluene
6. 2,6-Dinitrotoluene	12. 2-amino-4,6-Dinitrotoluene

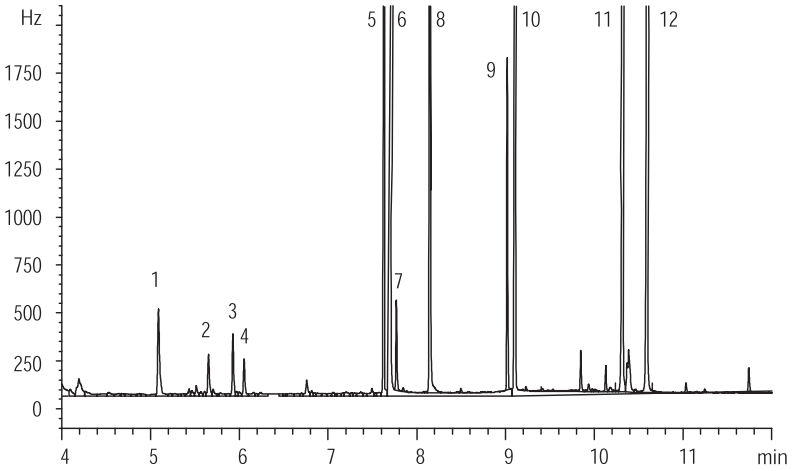

Figure 4. CGC-ECD analysis of explosives (solute concentration: 100 ppb)

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application

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An approach to the determination of N-nitrosodimethylamine at part-per-quadrillion levels using Positive Chemical Ionization and Large-Volume Injection

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Introduction

N-nitrosodimethylamine (NDMA, Figure 1) is one of a series of nitroso compounds known to be carcinogenic. NDMA is found in nitrate-cured or smoked meats,[1] cheeses,[2] tobacco smoke,[3] cooked foods and in beverages such as beer[4] (both foreign and domestic[5–7]). The presence of NDMA in surface waters designated for use drinking water use is of particular concern and the U.S. Environmental Protection Agency (EPA) has promulgated a regulatory standard for these waters of 0.7 ng/l (700 ppq). When in 1998 NDMA was detected in California drinking water, the source was associated with the production and use of a rocket fuel component, unsymmetrical dimethylhydrazine. In response, the California Department of Health Services (DHS) announced an action level in drinking water of 2 ng/l (2 ppt). However, the best available methods in the literature provide *detection* limits on the order of 1–3 ng/l. EPA methods 625 and 1625 specify a detection limit for NDMA of 50 ppb—25,000 times the California DHS action level and 70,000 times the EPA regulatory standard. It follows that using existing methodologies, *any* detection of NDMA represents a violation.

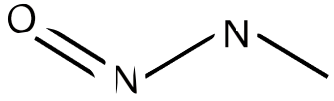

Figure 1. N-nitrosodimethylamine, $(CH_3)_2N_2O$, 74 g/mole, CAS Registry No. 62-75-9

Determining NDMA at ppt or ppq concentrations in water is an analytical challenge. The extraction methods that have been applied, such as liquid-liquid or solid-phase extraction,[8–10] produce concentration factors of 500 to 1000, but overall recoveries are generally low. The high polarity and volatility of NDMA contribute to lowered recoveries and extensive extract concentration by evaporation can lead to high losses.

To increase sensitivity and specificity, one prevalent detection scheme involves use of the chemiluminescent nitrogen detector. Electron impact mass spectrometry has also been used but the fragmentation pattern is not very favorable (Figure 2). While the molecular ion at 74 m/z may be a reliable quantitation ion, the confirming ions at 42 and 43 m/z are hardly unique and are easily compromised by fragments from interferences.

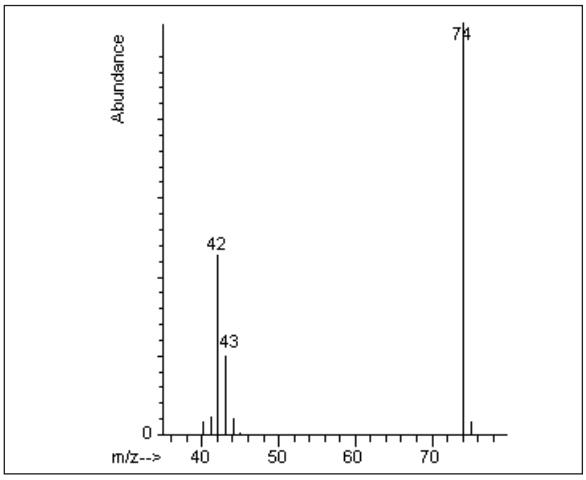

Figure 2. Electron impact ionization mass spectrum of NDMA

One approach to overcoming the unfavorable electron impact (EI) ionization mass spectrum of NDMA is to apply positive chemical ionization (PCI). PCI can provide enhanced analyte selectivity and sensitivity. Utilizing large-volume injection (LVI) should lower the concentration of NDMA that can be detected in an extracted sample. This note describes the combined application of these two techniques as a possible approach to determining NDMA at ppt and ppq concentrations.

Experimental

NDMA standards were made by serial dilution in 1-ml of dichloromethane from a 100 ng/µl standard (Ultra Scientific, Kingstown, RI; part number NS-100). Dichloromethane was chosen as the solvent, because this solvent is used in both liquid-liquid and solid-phase extraction techniques.

Instrumental Section

The 6980 Plus GC / 5973 MSD with chemical ionization option was operated in the selected-ion-monitoring mode (SIM) with ammonia reagent gas. An HP-210 GC column 50%-trifluoropropyl-50%-methyl-siloxane (30-m, .25 mm i.d., 0.5 µm film thickness, Part Number 19091C-733) was used with a 5-m, 0.32 mm i.d. uncoated retention gap (Part Number 19091-60600) joined by a press-fit connector (Part Number 5062-3555). A 100-µl syringe was used in the integrated automated liquid sampler 7683 injector for the 50-µl injections. GC oven conditions and mass selective detector settings are given in Tables 1 and 2.

Table 1. GC and Injector parameters

Oven Temperature Program	Temp	Time
Initial Temperature	45°C	3.00 min
Ramp 50°C / min	180°C	0.50 min
GC Oven Equilibrium Time		3.00 min
MSD Transfer Line		225°C
Inlet Mode		Split
Split Flow		50 ml / min
Gas Saver		Off
Column Flow (Helium carrier gas)		2.0 ml / min
Mode		Constant Flow
Outlet Pressure		Vacuum
Injection Volume		50 µl
Syringe Size		100 µl
Plunger Speed		Slow
Solvent Washes A, B	Methanol*	Dichloromethane

* A solvent that "wets" the glass bore improves syringe life.

Table 2. MSD parameters

Tune File *	PCINH3.U
Ammonia Reagent Gas Flow	10 %
EM Voltage	PCI CH_4 AutoTune + 400V
MS Quadrupole Temp	106°C
MS Source Temp	250°C
Acquisition Mode	SIM
Solvent Delay	5.25 min
SIM Ions	Dwell
75.1 amu	80 msec
92.1 amu	80 msec

* PCI Autotune parameters were used for these experiments. Autotune provides high sensitivity over a large mass range, but even greater sensitivity for these low molecular weight ions can be achieved by manual adjustment of the tuning parameters.

Large-Volume Injections

The APEX ProSep™ 800 Series XT Plus Preseparation System Inlet (APEX Technologies, Cincinnati, OH) was used as the inlet for large-volume injections.[11, 12] Injections were made into a fused-silica preseparation column packed with deactivated fused-silica wool in the top 3 to 7 cm of the column (available from APEX). The ProSep Precolumn Temperature Module and Flow Module parameters that were successful for this particular preseparation column are given in Tables 3 and 4. This is a very flexible device, and the parameters given can be further optimized to provide better performance for particular extracted matrices. For example, a higher final precolumn temperature than 180°C can be applied to remove high-boiling contaminants.

Table 3. ProSep Precolumn Temperature Program

	Target	Duration
Initial	45°C	0.05 min
250°C / min	180°C #	6.00 min

Higher bake-out temperatures are recommended for extracted samples.

Table 4. ProSep Precolumn Mode Program

	Mode	Duration
Initial	Split	0.05 min
1	Splitless	0.07 min*
2	GC Split **	2.50 min*

*These times should be appropriately optimized.

** It is recommended that ProSep Split be implemented instead of simply GC Split due to superior venting.

Results

The application of PCI with ammonia reagent gas to NMDA produces a simplified mass spectrum consisting only of protonated NDMA, $[NDMA+H]^+$, and the ammonium adduct, $[NDMA+NH_4]^+$, which correspond to 75 m/z and 92 m/z, respectively. PCI provides a three-fold advantage over the EI approach. First, the relatively non-unique 74, 43, 42 m/z ions of the EI have been replaced by higher-mass ions. Second, PCI provides increased sensitivity for NDMA and a reduction in low-mass, "background" ions which enhances the signal-to-noise ratio. Third, by manipulating the ammonia gas flow, the abundances of the 92 m/z and 75 m/z ions can be controlled. As the ammonia flow into the source is increased, the abundance of the $[NDMA+NH_4]^+$ adduct also increases, allowing the ratio of 92 m/z to 75 m/z to be controlled by the analyst. For example, at 0.4 ml/min of ammonia—a relatively low flow setting of the reagent gas mass flow controller (8% of the total 5-ml/min provided by the controller)—the ratio of the protonated form to adduct is biased toward the protonated form: $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 4$ **:** 3. At higher flows, the situation reverses and $[NDMA+NH_4]^+$ predominates, e.g., at 0.9 ml/min ammonia (18% flow setting) $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 1$ **:** 5. It is therefore possible to produce an intense confirming ion for quantitative applications. A good compromise between signal intensities and ion abundancies was achieved at a 0.5 ml/min ammonia flow setting. Figure 3 shows the 75 m/z and 92 m/z SIM signals for a 40-fg/µL standard for this flow. Under these conditions, $[NDMA+H]^+$ is 79% of $[NDMA+NH_4]^+$ according to the integrated signal areas.

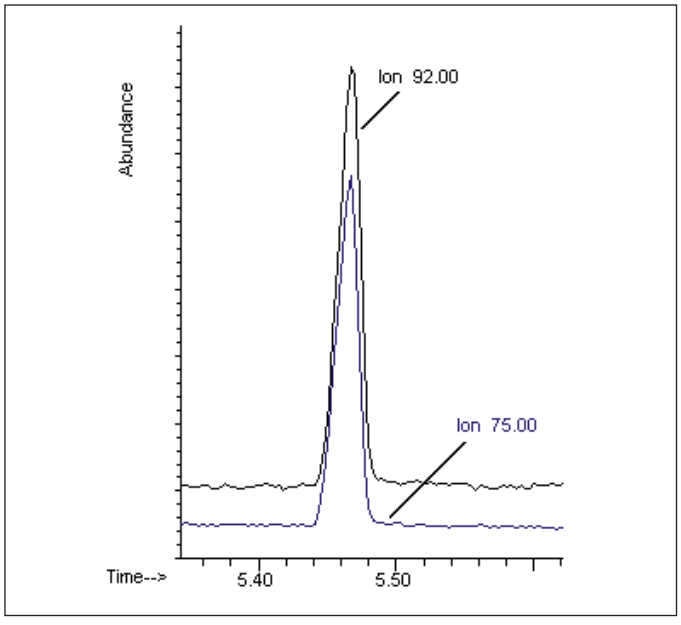

Figure 3. Extracted ion chromatogram for NDMA at 40-fg/µl using PCI-SIM with NH_3 reagent gas.

Figure 4 shows the results of a linear regression of the response of the 92 m/z ion for 50-µl injections of NDMA standards from 20-fg/µl to 4000-fg/µl. The regression fit was very good, $r^2 = 0.999$, considering the propagation of error in the dilutions. The relative standard deviation in the response factors was less than 6% and could be improved by using a perdeuterated or ^{15}N-labeled NDMA surrogate.

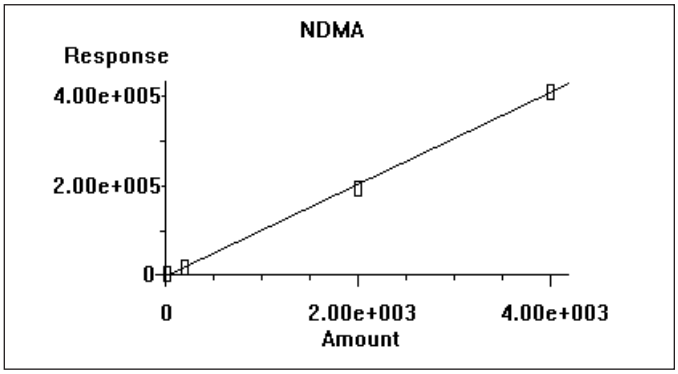

Figure 4. Linear regression of response of the 92 m/z ion versus NDMA concentration from 20-fg/µl to 4000 fg/µl, $r^2 = 0.999$.

Table 5 shows the excellent degree of reproducibility in the ratio of 75 m/z confirming ion to 92 m/z target ion over a wide range of concentrations. The absolute value of the ratio was 0.79, with a relative standard deviation of < 3%. This high precision is important to the degree of confidence in confirming and quantitating NDMA.

Table 5. Reproducibilities of the ratio of the integrated areas of 75 m/z : 92 m/z and the response of the 92 m/z target ion for 5 injections at 5 concentrations.

Concentration as fg NDMA / µl	RSD Ratio 75 mz / 92 m/z	RSD Response by 92 m/z area
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

Table 5 also shows the excellent reproducibility of the response of the 92 m/z ion for replicate 50-µl injections. Even at the 20-fg/µl concentration, precision is better than 3%.

Conclusions

Concentration and recovery factors for NDMA using present published methodologies suggest effective preconcentration of NDMA in samples to be on the order of 500, e.g., 60–70% recovery of NDMA in extraction of a 1-liter water sample. This implies that the low 20-fg/µl NDMA standard corresponds to a sample concentration of 40 pg/l, or 40 parts-per-quadrillion. Alternatively, to quantitate NDMA at 0.5 ppt in water, which is 4 times lower than the California DHS limit and slightly lower than the EPA regulated limit, quantitating at 20 fg/µl is equivalent to requiring the extraction of *only* 80 ml of water even if recoveries are still only 50%. Extracting small volumes presents a significant simplification of the process and offers savings in solvent and related materials, and in processing time.

With NDMA eluting in about 5 1/2 minutes, the analysis is fast, and the run-to-run cycle time is short—less than 13 minutes between injections. The method may be further optimized for even more rapid analysis.

The 5973 MSD provides very stable ratios for the confirming ion that can be optimized for quantitative purposes as described. In contrast to EI, in which many possible interfering fragment ions are possible that may distort the ratio of the target and confirming ion(s), PCI with ammonia is unlikely to cause fragmentation-induced interferences because of the relatively "gentle" nature of ammonia reagent gas. Interferences could occur involving compounds with molecular weights of 74 or 91 g/mole eluting at the same retention time but that is unlikely scenario.

The high degree of reproducibility in the injections, even at very low NDMA concentrations, demonstrates the robustness of large-volume injections using the APEX ProSep with the 6890/5973 MSD. It should be emphasized that the reproducibility of 2.4% for the replicate 50-µl injections of the 20-fg/µl standard reported here was for the *absolute* response. Use of an internal standard should further lower the deviation in response and improve quantitation.

Using this approach it should now be possible to satisfy the 2 ppt action level for NDMA set by the State of California and the 700 ppq regulatory standard promulgated by the U.S. EPA.

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Contract Laboratory Program (CLP) Pesticide Analysis with 0.18 mm ID High-Efficiency GC Columns Utilizing Helium Carrier Gas

Application

Environmental

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Abstract

Contract Laboratory Program (CLP) pesticide analysis is demonstrated on high-efficiency GC columns (20 m × 0.18 mm id × 0.18 µm film thickness) with helium carrier gas. DB-17ms stationary phase is used for primary analysis and DB-XLB stationary phase for confirmation. Primary analysis and confirmation of 22 CLP pesticides in the protocol is achieved in an 11-minute analysis, a 35% reduction in analysis time versus 0.32 mm id columns.

Method translation software is successfully employed to translate an original set of conditions with hydrogen carrier gas to a new set of conditions using helium carrier gas. Elution order and degree of separation are shown to translate precisely from the original method to the new method through use of this software (available for free download) [1].

Introduction

The determination of organochlorine pesticides (OCPs) in environmental remediation samples are important, high-volume analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, adding costs to both the lab and its client. Here, 0.18 mm id high-efficiency GC columns are demonstrated as a means of enhancing laboratory productivity. These columns are fully compatible with standard gas chromatographs and helium carrier gas operation. The high efficiency these columns offer coupled with their full compatibility with existing GCs provide laboratories with a powerful tool for enhancing sample throughput. When analysis times for 30 m × 0.32 id and 20 m × 0.18 mm id columns were compared, a 17-minute analysis was reduced to only 11 minutes and with improved resolution [2].

Helium carrier gas was selected as a means to show the utility of 0.18 mm id columns in doing CLP pesticide analyses and to demonstrate the full compatibility of these columns with standard gas chromatographs. The operating gas pressures for these 20 m × 0.18 mm id columns range from 33 psi initially to 50 psi at the high point of the temperature program. The gas pressure range used with helium carrier gas with these 0.18 mm id columns was well within the operating range for standard chromatographs.

Experimental

This work was accomplished using an Agilent 6890N GC equipped with dual µECDs and a 7683B autosampler. A single split/splitless injection port was used for sample introduction at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the chromatographic conditions are presented in Table 1.

Table 1. Chromatographic Conditions

GC	Agilent 6890N
Sampler:	Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Inlet	Split/splitless; 250 °C pulsed splitless (35 psi for 0.5 min)
Inlet liner	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Carrier	Helium (constant flow, 49.5 cm/sec at 120 °C, purified through big universal trap Agilent p/n RMSH-2)
Retention gap	5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter	Quartz deactivated (Agilent p/n 5181-3398)
Columns:	
1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven	120 °C (0.49 min); 85 °C/min to 160 °C; 20 °C/min to 260 °C (0.20 min); 25 °C/min to 285 °C; 40 °C/min to 300 °C (3.5 min)
Detection	µECD 325 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

The flow path supplies used in these experiments are listed in Table 2 below.

Table 2. Flow Path Supplies

		Agilent p/n
Vials	Amber screw cap	5182-0716
Vial caps	Blue screw cap	5282-0723
Vial inserts	100 µL glass/polymer feet	5181-1270
Syringe	5 µL	5181-1273
Septum	Advanced green	5183-4759
Inlet liner	Deactivated single taper direct connect	1544-80730
Ferrules	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter	Quartz deactivated	5181-3398
Sealing resin	Polyimide sealing resin	500-1200
20x magnifier	20x magnifier loop	430-1020
Tubing cutter	Ceramic wafer column cutter	5181-8836

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard, New Haven, CT 06513-USA. ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchased from J. T. Baker, Phillipsburg, NJ 08865-USA. CLP-023R-160X and CLP-024R-160X concentrates were diluted separately into two 100-mL volumetric flasks in 2,2,4 trimethylpentane and then combined in subsequent serial dilutions. Volumetric flasks and pipettes used were all class A. Standard concentration range for low-level target compounds in the protocol was from 1.6 to 40 ng/mL. On-column loading ranged from 0.4 to 10 pgs for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Tip on Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loop to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach gave tight, reliable connections that have lasted without any difficulty for more than 2 months (to date) and hundreds of oven temperature program cycles.

Results and Discussion

The starting point for this application was a set of conditions for CLP pesticide analyses using hydrogen carrier gas and 0.18-mm high-efficiency columns developed by Wool and Decker [3]. Using hydrogen carrier and flow programming, they were able to achieve primary separation and confirmation analysis of CLP pesticides in a 7-minute analysis. The chromatographic parameters for the hydrogen carrier separation were input as initial setpoints in method translation software to convert the method to use with helium carrier. Helium carrier was selected for use in laboratories reluctant to work with hydrogen carrier due to site safety policy or individual preference. High-efficiency GC columns give the chromatographer the option to work with either helium or hydrogen carrier gases and still achieve faster analyses.

Wool and Decker [3] indicated in their paper that frequent trimming of the front of the column was necessary for use with heavy matrix samples due primarily to the lower sample capacity of 0.18-mm columns. In this work a 5-m 0.25-mm id retention gap and Y connector were installed ahead of the

analytical columns to help offset the diminished sample capacity relative to wider bore capillary columns. Use of a retention gap will also shield the analytical columns from deleterious matrix affects and extend the useful lives of the columns.

Agilent's method translation software simplifies conversion from established laboratory GC methods to parallel sets of conditions suitable for high-efficiency GC columns. Chromatographic conditions from the original method, along with the new column dimensions, are entered into a menu-driven table within the software. The software then generates a translated method table with all the new chromatographic setpoints for the translated method. The new translated method setpoints produced by the software are often all that is required to successfully translate a method.

Three primary modes of method translation are available in the method translation software: translate only, best efficiency, and fast analysis. The "translate only" mode produces a set of conditions that most closely resembles the original method in terms of relative position on the Van Deemter curve, degree of separation, and elution order. The "best efficiency" mode generates a set of conditions where column efficiency is prioritized. The "fast analysis" mode generates a set of conditions where analysis speed is prioritized. By using the various modes available a translated method specific to a particular application can be developed quickly with a few keystrokes and iterative passes through the software.

The software is very useful in porting methods from the use of one carrier gas to another. Translation from the original method using one carrier to a method using another carrier is accomplished by entering the original method setpoints, the new column dimensions, and the desired carrier. The software then generates the translated method setpoints for the new column and carrier. For additional information on Agilent's method translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.

Flow programming is not addressed in the method translation software, so minor adjustments to flow rate parameters may be required to achieve desired results. When translating flow-programmed methods, initial or intermediate flow rates can be entered into the original method parameters table to visualize the effect on the other parameters' output in the translated method table. The operator can then collect data at several different flow rates and select the best set of conditions for the application.

In this CLP pesticide example, the original method used a hydrogen carrier and flow programming. The initial flow parameters were entered into the method translation software, along with the new column dimensions, specifying helium as the carrier gas. Translate-only mode was selected in the software and produced the translated method setpoints that appear in Figure 1.

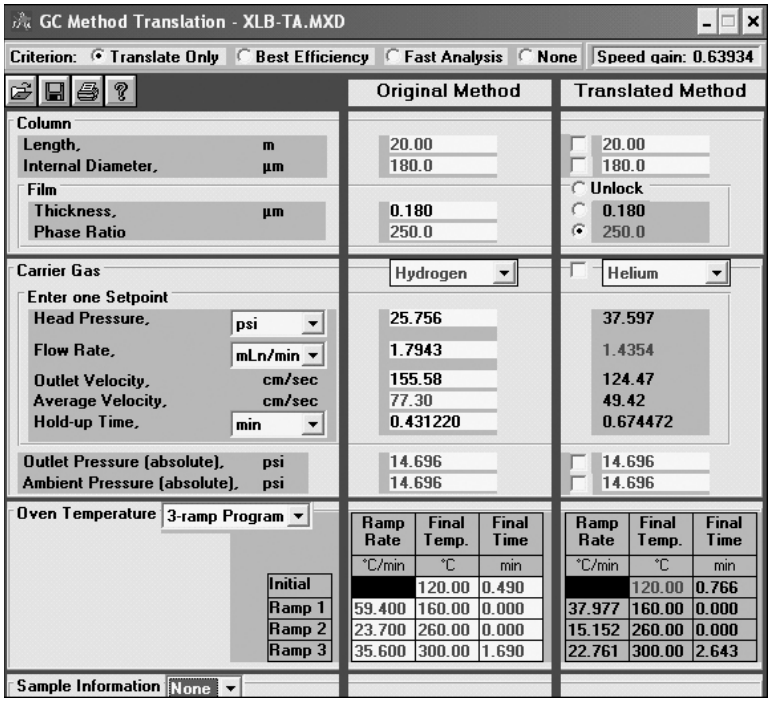

Figure 1. Method translation using translate-only mode.

Figure 2 shows the resulting CLP pesticide separations produced using translate-only mode in the method translation software on the DB-17ms column. Note that all 22 species are baseline resolved on the DB-17ms column where there is a partially separated triplet consisting of gamma chlordane, alpha chlordane, and endosulfan 1 on the DB-XLB column (Figure 3). This partially separated triplet was also observed in the original DB-XLB separation using hydrogen carrier.

Table 3 is a standard compound key for the numbered peaks in the chromatograms. Separation characteristics such as degree of separation and elution order were maintained exactly as they were in the original method using the new translated method with helium carrier. The original method was successfully translated with no additional method development.

Unfortunately, the unresolved triplet on DB-XLB observed in the original method remained unresolved in the translated method. Additional method development attempts focused on resolving the partially separated triplet on the DB-XLB confirmation column and reduction of analysis time. Some success was achieved; however, the trailing two peaks in the triplet remained partially resolved on the DB-XLB confirmation column while analysis time was reduced to 11 minutes. The DB-17ms column resolved all of the species in the protocol throughout these experiments (Figure 4). Triplet resolution on the DB-XLB (Figure 5), though not ideal, is adequate for the purpose of peak confirmation of well-resolved species on the DB-17ms.

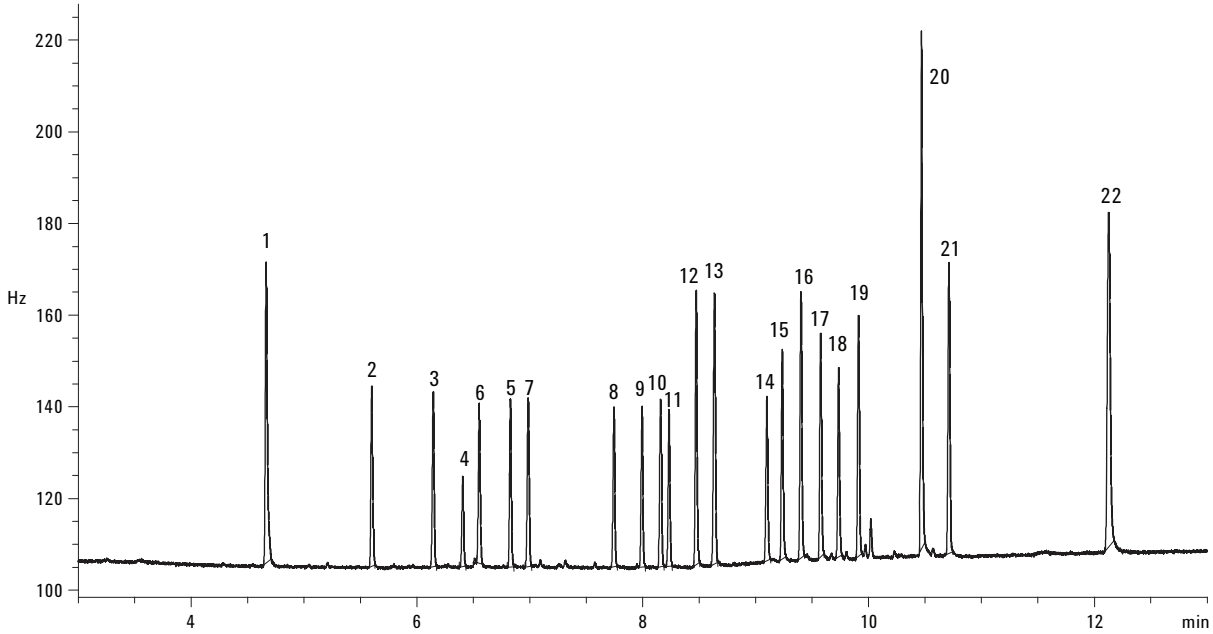

Figure 2. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene
2. Alpha BHC
3. Gamma BHC
4. Beta BHC
5. Delta BHC
6. Heptachlor
7. Aldrin
8. Heptachlor epoxide
9. Gamma chlordane
10. Alpha chlordane
11. Endosulfan I
12. 4,4′ DDE
13. Dieldrin
14. Endrin
15. 4,4′ DDD
16. Endosulfan II
17. 4,4′ DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl

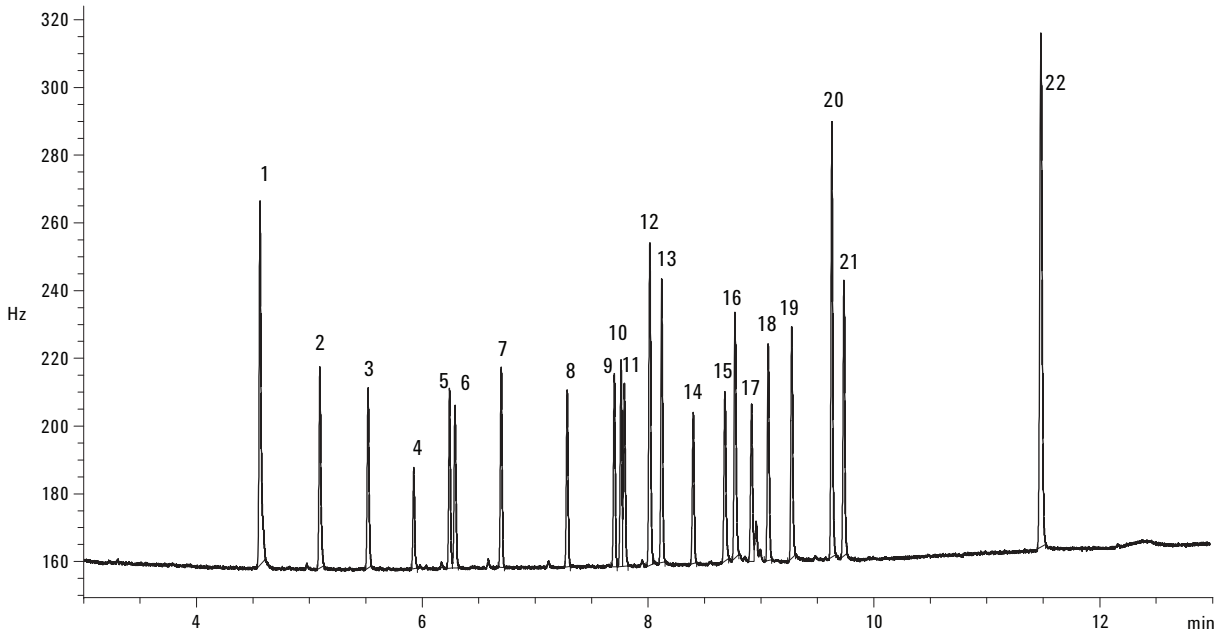

Figure 3. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

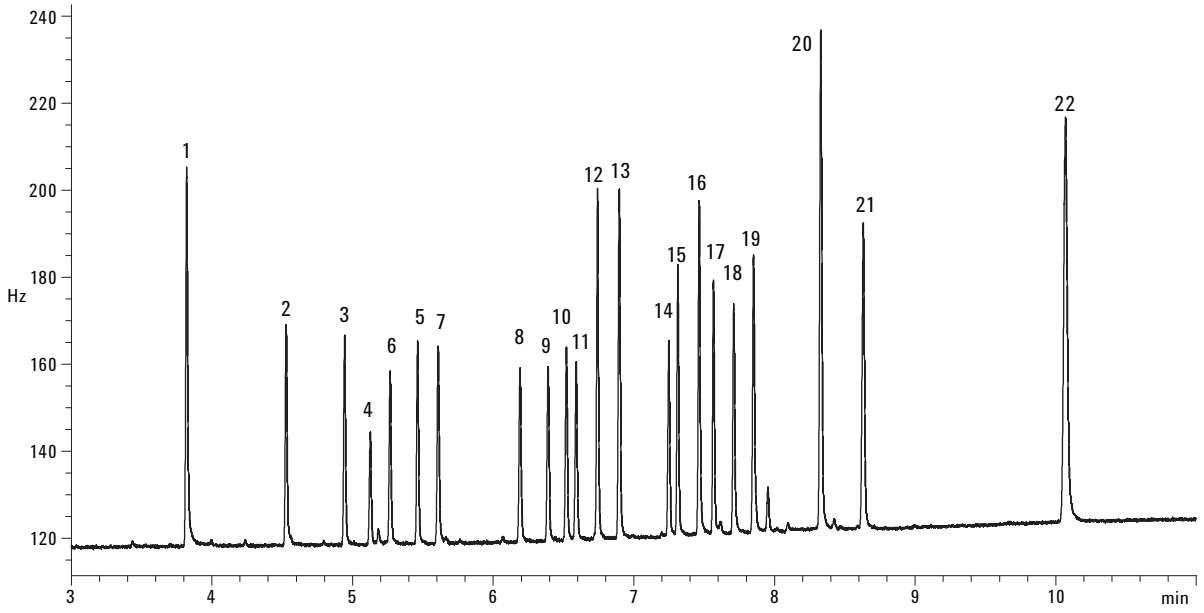

Figure 4. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

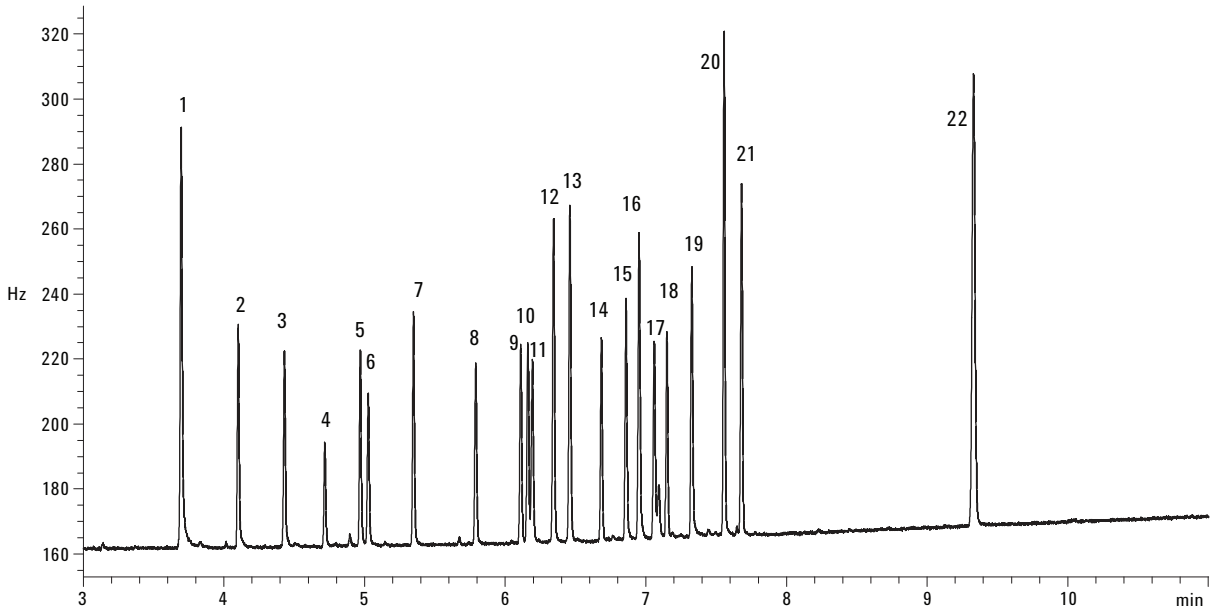

Figure 5. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

Detector Sensitivity and Linearity

The 0.5-µL injections were split between two columns for an on-column loading of 0.4 pg per component of the low-level target compounds. The data suggest that detection limits of at least an order of magnitude lower are possible. Sensitivity and linearity measurements conducted with these chemical species using µECD detection support this assertion [4]. Analyte concentration range investigated here was from 1.6 – 40 ng/mL. This range meets the 16-fold low- to high-check standard criteria for the protocol and appears to cover only the middle of the dynamic range the detector is capable of fielding. Figure 3 shows the DB-17ms separation where low-level component loading was 0.4 pg. Figure 6 shows the same separation with a 10-pg loading for the same components.

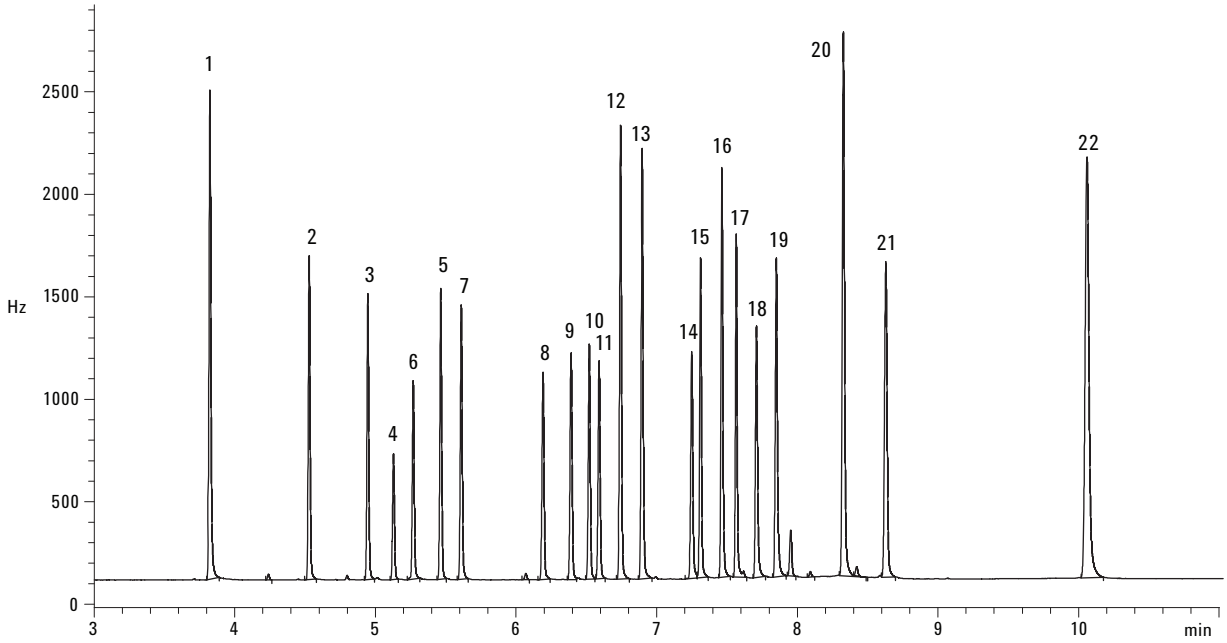

Figure 6. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 10-pg/component loading for low-level target compounds.

Conclusions

Complete separation and confirmation of all 22 species in the CLP pesticide protocol were accomplished in an 11-minute analysis with helium carrier gas. These results demonstrate the utility of these 0.18-mm id high-efficiency GC columns for CLP pesticide analysis. Using a 0.5-µL injection of pesticide standard solutions over a concentration range of 1.6 – 40 ng/mL gave excellent results. These results easily meet the 16x high/low dynamic range requirement for the protocol and suggest that expanding the range to both lower and higher concentrations is certainly possible with these 0.18-mm columns.

Full compatibility for use of these columns with standard GC equipment and helium carrier was also established by this successful separation. Operating pressure for use of these columns at the high point of the temperature program (300 °C) was 50 psi, well within the operation pressure range for standard GC equipment.

Method translation software successfully translated the original method using hydrogen carrier to the new method using helium carrier. Separation characteristics from the original method, such as elution order and degree of separation, were matched exactly in the translated method. This exercise served once again to validate the simplicity of method translation using the software. Method development beyond the translated method setpoint only became necessary when improvements to the original separation were attempted.

References

1. To download Agilent Method Translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.
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Agilent Technologies

A Direct Column-Performance Comparison for Rapid Contract Laboratory Program (CLP) Pesticide Analysis

Application

Environmental

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Abstract

Agilent J&W High Efficiency GC columns with internal diameter of 0.18 mm for Contract Laboratory Program (CPL) pesticide analyses gave superior results for CPL pesticide primary analysis and confirmation. Chromatograms depicting peak shape characteristics, peak resolution, and baseline stability for two sets of 0.18-mm id columns are presented in a head-to-head comparison. Complete primary and confirmatory analysis of the 20 pesticides in the protocol is accomplished in less than 6 minutes using hydrogen carrier gas and flow programming. Successful primary and confirmatory analyses were achievable only on Agilent J&W High Efficiency GC columns.

Introduction

The analyses of organochlorine pesticides (OCPs) in environmental remediation samples are important, high volume, analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, accumulating costs to both the lab and its client. Use of Agilent J&W 0.18-mm id High Efficiency GC columns is a means of enhancing laboratory productivity [1-3].

Wool and Decker [3] reported their findings at the U.S. Environmental Protection Agency (Region VI, Houston, TX) laboratory and described the value of columns in the 20 m × 0.18 mm format for CLP pesticide analysis. Their suggestion to use a retention gap to protect the analytical columns from deleterious matrix effects and to help offset the lower sample capacity of these columns relative to wider bore columns was incorporated into this column comparison. Deactivated 5 m × 0.25 mm id retention gaps were used in this series of experiments on each column set used.

Columns with 0.18 mm id capable of doing CPL pesticide analysis are available from several leading column manufacturers. Agilent's suggested pair for CLP pesticide analysis in the 0.18 mm id format is a DB-17ms column for primary analysis and a DB-XLB column for confirmation. Vendor R's offering is a set of proprietary phase 0.18 mm id columns for both primary and confirmation analysis of CLP pesticides.

Experimental

The chromatograph used was an Agilent 6890N GC equipped with dual electron capture detectors (μECDs) and a 7683B autosampler. Sample introduction was done by a single split/splitless injection port at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the initial chromatographic conditions appear in Table 1.

Table 1. Chromatographic Conditions

GC :		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed, 69 cm/s at 120 °C, ramped at 99 mL/min to 106 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C, pulsed splitless (35 psi for 0.5 min, purge flow of 40 mL/min on at 1 minute, gas saver flow 20 mL/min on 3 minutes
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
	2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven:		120 °C (0.32 min); 120 °C/min to 160 °C; 30 °C/min to 258 °C (0.18 min); 38.81 °C/min to 300 °C (1.5 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow (60 mL/min)

The flow path supplies used in these experiments are listed in Table 2.

Table 2. Flow Path Supplies

	Description	Agilent p/n
Vials:	Amber screw cap	5182-0716
Vial caps:	Blue screw cap	5282-0723
Vial inserts:	100 µL glass/polymer feet	5181-1270
Syringe:	5 µL	5181-1273
Septum:	Advanced green	5183-4759
Inlet liner:	Deactivated single taper direct connect	1544-80730
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter:	Quartz deactivated	5181-3398
Sealing resin:	Polyimide sealing resin	500-1200
20x magnifier:	20x magnifier loupe	430-1020
Tubing cutter:	Ceramic wafer column cutter	5181-8836

Both sets of columns used in this comparison were installed into the GC in the same manner. The same retention gap and inlet liner were used for both sets of columns. The chromatographic conditions (except for the columns) in Tables 1 and 2 were used to evaluate both the proprietary columns recommended by Vendor R and Agilent's columns. The primary analysis column from Agilent was a 20 m × 0.18 mm x 0.18 µm DB-17ms. The column from Vendor R was a 20 m x 0.18 mm × 0.18 µm with a proprietary stationary phase. The confirmatory analysis column from Agilent was a 20 m × 0.18 mm × 0.18 µm DB-XLB. The column from Vendor R was a 20 m × 0.18 mm x 0.14 µm with a proprietary stationary phase.

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard (New Haven, CT 06513 USA). ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchases from J.T. Baker (Phillipsburg, NJ 08865 USA).

CLP-023R-160X and CLP-024R-160X concentrates were diluted first into 50-mL volumetric flasks in 2,2,4 trimethylpentane and then serially diluted. Volumetric flasks and pipettes used were all class A. The standard concentration range for low-level target compounds in the protocol was from 3.2 to 80 ng/mL. On-column loading ranged from 0.8 to 20 pg for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loupe to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach has given tight, reliable connections that have lasted without difficulty for over 2 months and through hundreds of oven temperature program cycles.

Method Translation Software/Path to Successful Conditions

The starting point for this comparison was the conversion of a successful set of separation conditions using helium carrier on Agilent's DB-17ms and DB-XLB 0.18 mm id columns [4] to a set of conditions using hydrogen carrier. The chromatographic parameters for the helium carrier separation were keyed into the translation table in the Agilent GC Method Translation software [5–6] to convert the method to use with hydrogen carrier. In the software, the "Translate Only" mode was used to convert the 11-minute helium carrier method to a 7.3-minute hydrogen carrier method using the same columns.

Method development effort beyond conversion from helium carrier to hydrogen carrier gas became necessary only when the goal of the analysis shifted to emphasize speed of analysis using flow programming. Flow programming is outside the scope of the Method Translation software. In this series of experiments, flow programming helped to elute highly retained peaks faster. Further temperature program modifications also increased the speed of analysis with minimal loss of resolution on the Agilent columns.

Results and Discussion

Successful separation of CLP pesticides using hydrogen carrier was demonstrated on Agilent's DB-17ms and DB-XLB 0.18-mm id columns using the conditions shown in Table 1. Vendor R's 0.18-mm ID columns were evaluated using the following conditions: the conditions shown in Table 1, the conditions obtained on Vendor R's Web site (to the extent practical), and with a set of conditions optimized specifically on Vendor R's columns for this analysis. The goal throughout these experiments was to show as fair and objective a comparison as possible.

To compare chromatograms, injections at a standard concentration of 3.2 ng/mL for low-level target species in the CLP protocol were selected. Using this concentration consistently provides a fixed point of reference and at the same time alleviates the potential for masking deleterious chromatographic effects often seen at higher concentrations. Inclusion of a Y scale in each chromatogram provides another fixed reference within each figure to facilitate comparison. Key aspects to look for in the example chromatograms are peak resolution, indications of peak tailing, and temperature-dependent drift on the μECD.

An Agilent DB-17ms column was used as the primary analysis column in these experiments. An example chromatogram from an injection at a nominal concentration of 3.2 ng/mL for low target level pesticides is shown in the upper portion of Figure 1. This column resolved all the peaks of interest in less than 6 minutes, gave sharp symmetrical peaks, and had minimal background drift on the μECD. A compound label key for the numbered peaks in the chromatogram is located in Table 3.

An Agilent DB-XLB 20 m x 0.18 mm x 0.18 μm column was used for confirmatory analysis on these experiments. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in the lower portion of Figure 1. This column resolved 20 of the peaks of interest in less than 6 minutes and gave near baseline resolution of peaks 10 and 11. Again, sharp symmetrical peaks and minimal temperature-dependent baseline drift were observed on the μECD. Although complete resolution of 20 of the 22 peaks of interest on the confirmatory columns is not ideal, the observed resolution is satisfactory for peak confirmation.

The peak identification table applies to Figure 1, depicting CPL pesticide separation on Agilent's column only. Elution order for these columns with their particular selectivity was established in previous work. To establish elution order on Vendor R's columns with different selectivity, injection of individual standards or mass spectral confirmation is required.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene	12. 4,4' DDE
2. Alpha BHC	13. Dieldrin
3. Gamma BHC	14. Endrin
4. Beta BHC	15. 4,4' DDD
5. Delta BHC	16. Endosulfan II
6. Heptachlor	17. 4,4' DDT
7. Aldrin	18. Endrin aldehyde
8. Heptachlor epoxide	19. Endosulfan sulfate
9. Gamma chlordane	20. Methoxychlor
10. Alpha chlordane	21. Endrin ketone
11. Endosulfan I	22. Decachlorobiphenyl

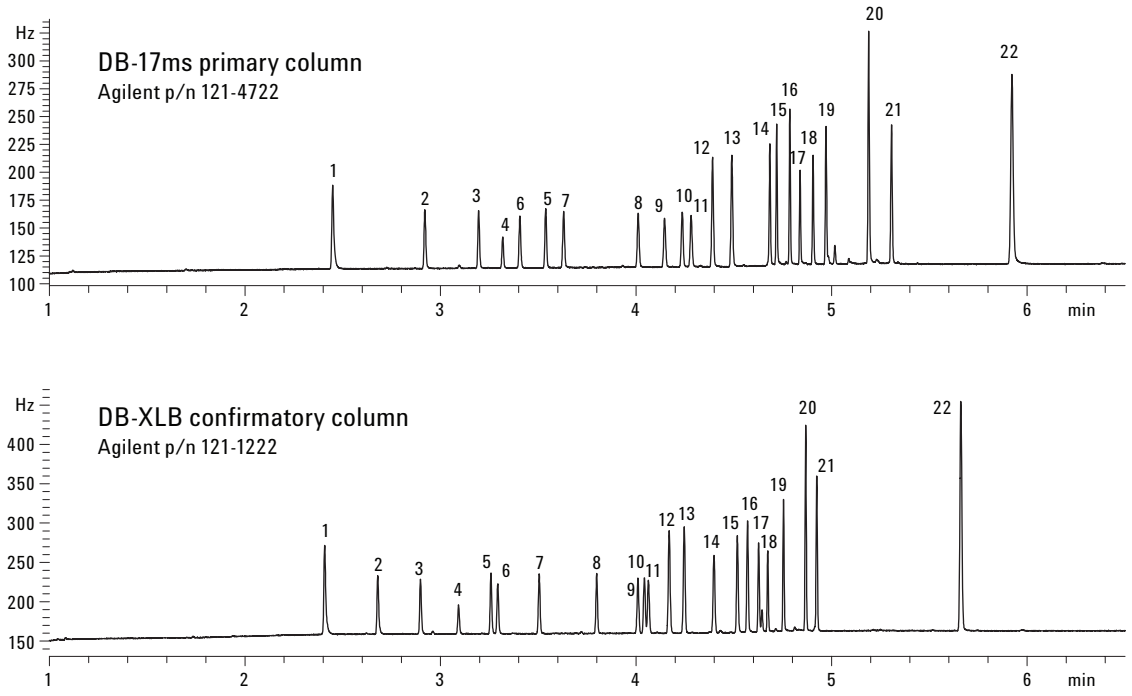

Figure 1. Chromatogram of 0.5-µl injection of 3.2 ng/ml low-level target pesticide standard solution injected through a Y-splitter onto DB-17ms (Agilent p/n 121-4722) primary analysis column and a DB-XLB (Agilent p/n 121-1222) confirmatory analysis column, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column gave resolution of 20 of the 22 peaks of interest, peak tailing for some species, and minimal temperature dependent baseline drift on the µECD. The arrows within the figure point to co-eluting and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift, in this case over 100 Hz.

Vendor R's suggested separation conditions for their column pair were unsuccessful at producing results equivalent to those shown on their Web site. This appears to stem from an oversight on their part. Suggested conditions found in a figure caption on the Web site called for a 2-min hold 10° C above the maximum recommended temperature. A temperature of 330 °C was called for; however, the label on the column box listed the upper temperature program limit as 320 °C for the confirmation column. Vendor R's confirmation column demonstrated significant bleed even with a temperature program that reached only 300 °C, a full 20 °C below the upper limit.

A series of attempts to resolve the co-eluting pair of pesticides on Vendor R's primary analysis column gave improved but still incomplete resolution. It was necessary to substantially reduce flow rate and modify both temperature and flow programming parameters to achieve the results shown in Figure 3. The chromatographic conditions used for these injections appear in Table 4; the flow path supplies were the same as those listed in Table 2.

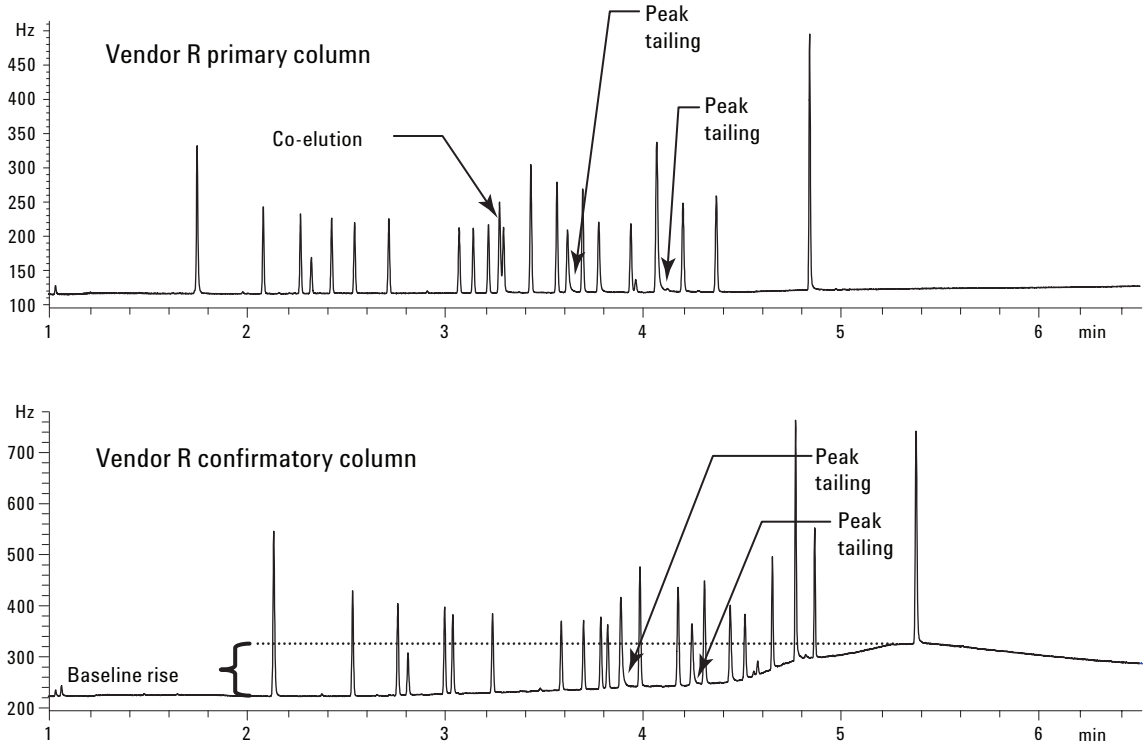

Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1. Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column still gave resolution of 20 of the 22 peaks of interest, indication of peak tailing for some species, and minimal temperature-dependent baseline drift on the µECD. The arrows within the figure point to the unresolved peaks and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift.

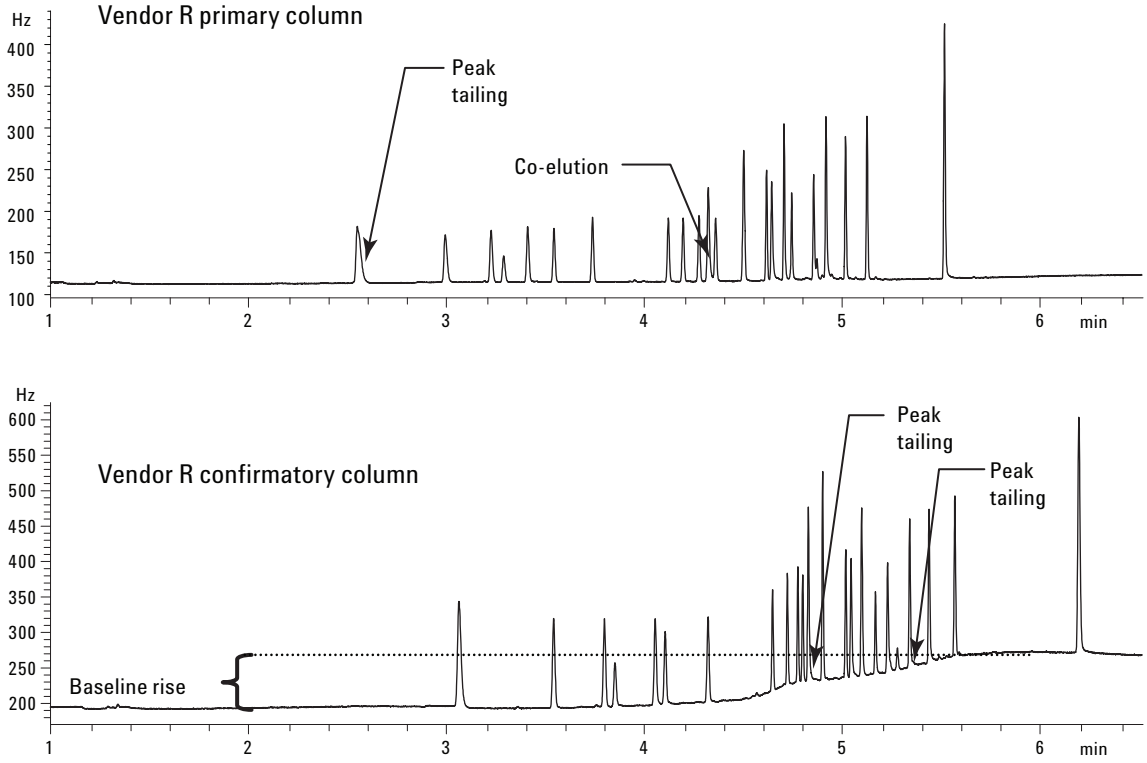

Figure 3. Chromatogram of 0.5-µl-injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary and confirmatory analysis columns, conditions as in Table 4.

Table 4. Chromatographic Conditions

GC:		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed , 45 cm/s at 120 °C, ramped at 99 mL/min to 72 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C pulsed splitless (35 psi for 0.5 min, purge flow 40 mL/min on 1 minute, gas saver 20 mL/min at 3 minutes)
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m x 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm primary analysis column
	2	20 m x 0.18 mm × 0.14 µm confirmatory analysis column
Oven:		120 °C (0.50 min); 60 °C/min to 160 °C; 30 °C/min to 260 °C; 40 °C/min to 300 °C (2.0 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

Conclusions

Agilent's 0.18-mm id primary analysis column is superior to Vendor R's offering. All 22 peaks of interest were resolved on the DB-17ms primary analysis column in less than 6 minutes with sharp, symmetrical peaks and minimal baseline drift. Vendor R's primary analysis column resolved 20 of 22 peaks of interest and displayed evidence of peak tailing for some of the peaks of interest.

Agilent's 0.18-mm id confirmatory analysis column offering is superior to Vendor R's offering. Twenty of 22 peaks of interest were resolved on the DB-XLB, with the other two peaks being almost baseline resolved in less than 6 minutes, with sharp, symmetrical peaks and minimal temperature-dependent baseline drift. Resolution of 20 of 22 peaks is less than ideal but should serve well for peak confirmation. Vendor R's confirmatory column resolved all 22 peaks of interest but showed evidence of peak tailing and an unacceptable level of temperature-dependent baseline drift.

The DB-17ms and the DB-XLB columns used in these experiments gave very low bleed profiles on the µECDs. The stable baselines produced by both of these columns can lead to lower detection limits,

simpler integration and more reliable results over time. These columns also have the versatility of use with other analyses beyond CLP pesticides.

Reliable CLP pesticide primary and confirmation analyses are achievable using Agilent J&W high-efficiency GC columns in less than 6 minutes with standard gas chromatographic equipment.

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Agilent Technologies

Organophosphorus Pesticides Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column

Application Note

Environmental

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Abstract

Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate and verify column inertness and quality. In challenging separations, knowing that the GC column has been thoroughly investigated for column inertness gives analysts higher confidence in the accuracy of their results.

Trace- and ultra trace-level pesticide analyses are important tools for accessing food supply and environmental quality worldwide. In this application note, trace-level organophosphorus pesticide analysis is demonstrated using electron impact single quadrupole scanning mass spectrometry. Agilent's J&W DB-5ms Ultra Inert capillary GC column provides excellent peak shape for even the most problematic pesticides.

Agilent Technologies

Introduction

Pesticides are commonly used in agricultural and residential applications throughout the world. Organophosphorus pesticides make up approximately 70 percent of the insecticides currently in use. Unfortunately, these highly toxic materials have three main routes of human exposure: inhalation, ingestion, and skin penetration. Sources of these exposures include consumption of foodstuff containing pesticide residues, aerosol inhalation, and dermal contact during pesticide application. [1]

Organophosphorus pesticides use the same mechanism of action as deadly nerve agents such as sarin, soman, and VX. These pesticides affect the nervous system of insects, mammals, and wildlife by inhibiting the enzyme cholinesterase, important in helping regulate nerve impulses. Inactivation of cholinesterase leads to the accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous system, which leads to depressed motor function and respiratory depression. Human toxicities for this class of molecules have shown acute as well as chronic effects from pesticide poisoning. [2,3]

Organophosphorus pesticides tend to be difficult to quantify due to poor peak shape, as evidenced by broad, asymmetrical peaks. An EPA Method 525.2 standard containing organophosphorus pesticides along with a custom pesticide mix acquired from Ultra Scientific (North Kingstown, RI) were analyzed to highlight the value of using a 30-m Agilent J&W DB-5ms Ultra Inert capillary GC column for difficult pesticide analysis. Many pesticides are sensitive to chromatographic system activity and will readily breakdown. The Ultra Scientific custom mix contains several types of these pesticides, which are useful in quickly evaluating system performance with particularly challenging pesticide analytes. Capillary GC column activity as a potential source of result uncertainty has been virtually eliminated with the Ultra Inert series of columns. [4]

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1A. Chromatographic Conditions for EPA Method 525.2 Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 44 cm/s, constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	40 °C (1 min) to 110 °C (50 °C/min), 7 °C/min to 190 °C, 12 °C/min to 285 °C, hold 2 min.
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 1B. Chromatographic Conditions for Ultra Scientific Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 52 cm/s, constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	75 °C to 175 °C (15 °C/min), 10 °C/min to 275 °C (1 min)
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 2. Flow Path Supplies

Vials	Amber crimp-top glass vials (Agilent p/n 5183-4496)
Vial caps	Crimp caps with 11-mm septa (Agilent p/n 5181-1210)
Vial inserts	100-µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe	5 µL (Agilent p/n 5181-1273)
Septum	Advanced Green (Agilent p/n 5183-4759)
Inlet liners	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

A six-component EPA Method 525.2 pesticide standard mix and internal/surrogate standard mix were purchased from Accu-Standard (New Haven, CT) and used to prepare a six-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The internal/surrogate solution as delivered had a nominal concentration of 500 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2, 1, 0.5, and 0.1 µg/mL and a constant level of 5 µg/mL of internal/surrogate standard as per EPA Method 525.2. All solutions were prepared in acetone using class A volumetric pipettes and flasks. Acetone used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). Acetone was used as a reagent blank and syringe wash solvent.

An 11-component pesticide standard mix was purchased from Ultra Scientific and used to prepare a seven-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µg/mL. All solutions were prepared in 2,2,4-trimethylpentane using class A volumetric pipettes and flasks. The 2,2,4-trimethylpentane used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). 2,2,4-Trimethylpentane was used as a reagent blank and syringe wash solvent.

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with aggressive active probes at low concentration and low temperature. [5] This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level, like the organophosphorus pesticides in this application example. A more detailed description of the test mix and additional application examples can be found in references 6 through 8.

Organophosphorus Pesticide Analysis

In this application note, a multilevel pesticide calibration curve set was evaluated over the concentration range of 0.1 to 10 µg/mL on an Agilent J&W Ultra Inert DB-5 ms 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI). Separate calibration curves were developed for both the EPA 525.2 organophosphorus and Ultra Scientific standards. The standard levels used for the 525.2 calibration were 0.1, 0.5, 1, 2, 5, and 10 µg/mL, while the Ultra Scientific calibration levels were 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 µg/mL. The custom pesticide standard from Ultra Scientific was used to determine system performance by analyzing difficult pesticides, such as endrin and p,p'-DDT, which are prone to analyte breakdown.

No tailing was observed for any of the organophosphorus pesticide peaks across the range studied in either standard set. Sharp, symmetrical peak shapes were noted for all the organophosphorus pesticides analyzed. Good resolution was obtained for each of the pesticides investigated.

Linearity for the 525.2 standard components was excellent across the range studied, giving R^2 values of 0.997 or greater in all cases but fenamiphos, which had an R^2 value of 0.978. This value increases to 0.991 at the midlevel concentrations as suggested by EPA Method 525.2 Sec. 13.2.3.3. Figure 5 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for disulfoton.

Linearity for the Ultra Scientific standard components was also quite good across the range studied. R^2 values of 0.990 or greater were obtained for the organophosphorus pesticides. Figure 6 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for mevinphos.

1. 1,3-Dimethyl-2-nitrobenzene (SS)
2. Acenapthene-d10 (IS)
3. Cycloate
4. Prometron
5. Phenanthrene-d10 (IS)
6. Disulfoton
7. Ametryn
8. Fenamiphos
9. Tribufos (DEF)
10. Triphenylphosphate (SS)
11. Chrysene-d12 (IS)
12. Perylene-d12 (SS)

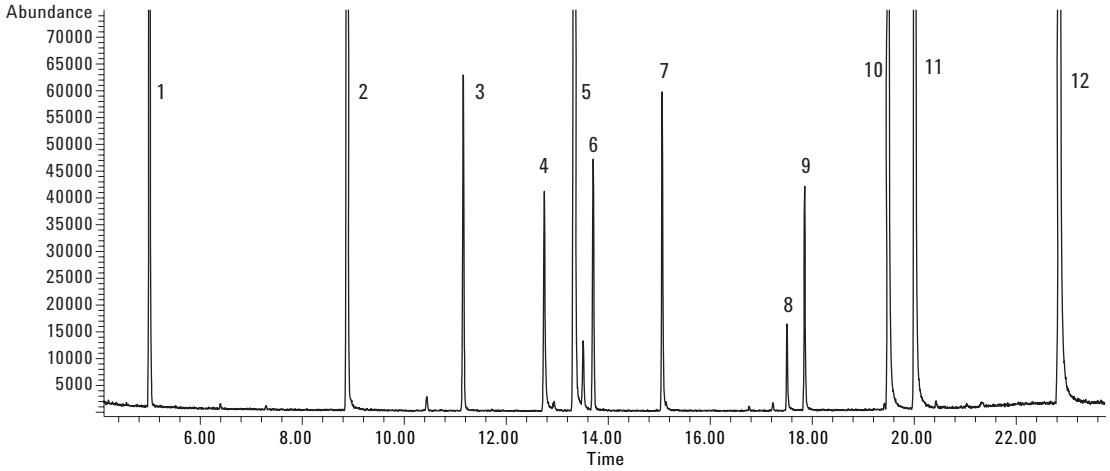

Figure 1. Total ion chromatogram (scan mode) of the 1-ng on-column EPA Method 525.2 standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1A.

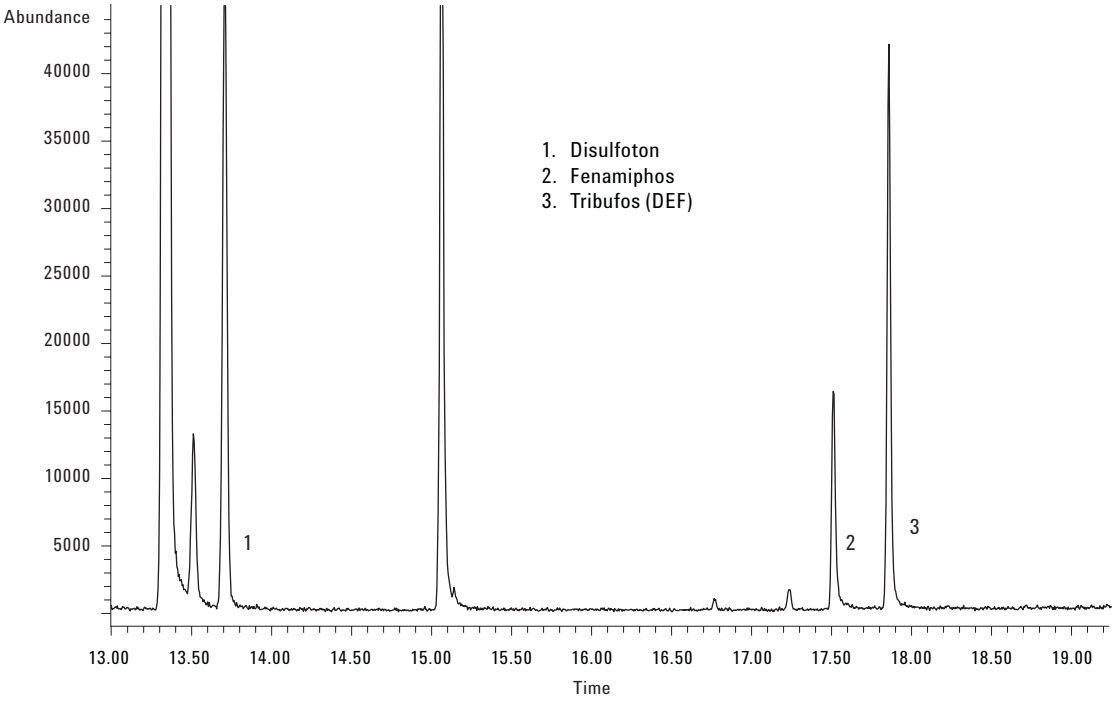

Figure 2. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1.0 µg/mL EPA Method 525.2 standard pesticide mix. The peaks noted in the figure are the three organophosphorus pesticides of interest. Chromatographic conditions are listed in Table 1A.

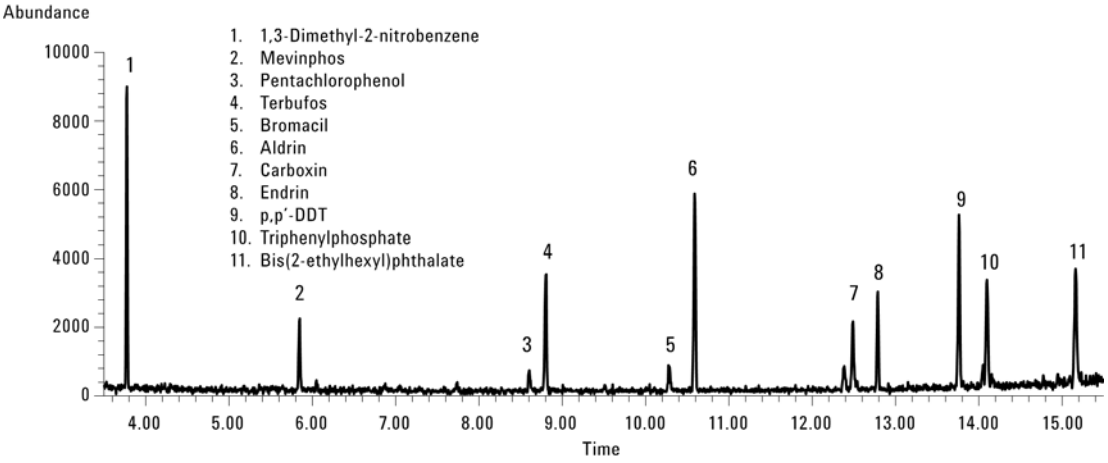

Figure 3. Total ion chromatogram (scan mode) of the 0.1-ng on-column Ultra Scientific standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1B.

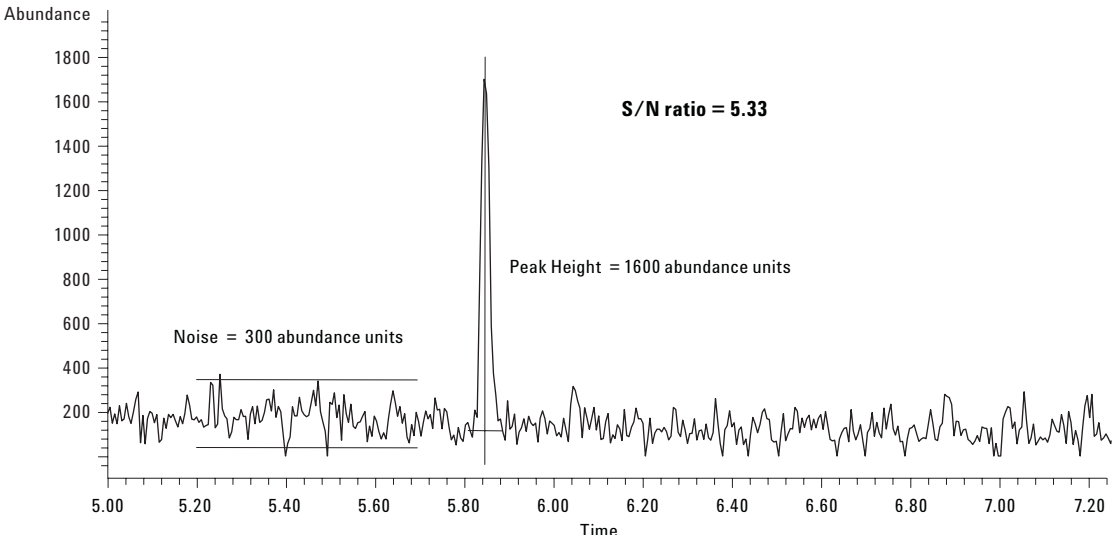

Figure 4. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 0.1 µg/mL Ultra Scientific standard pesticide mix on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). The peak in the figure is mevinphos, an organophosphorus pesticide of interest. This injection represents an on-column loading of 0.1 ng per component. Chromatographic conditions are listed in Table 1B.

Component	R^2
Cycloate	1.000
Prometon	0.999
Disulfoton	0.999
Ametryn	0.999
Fenamiphos	0.978
Tribufos (DEF)	0.997

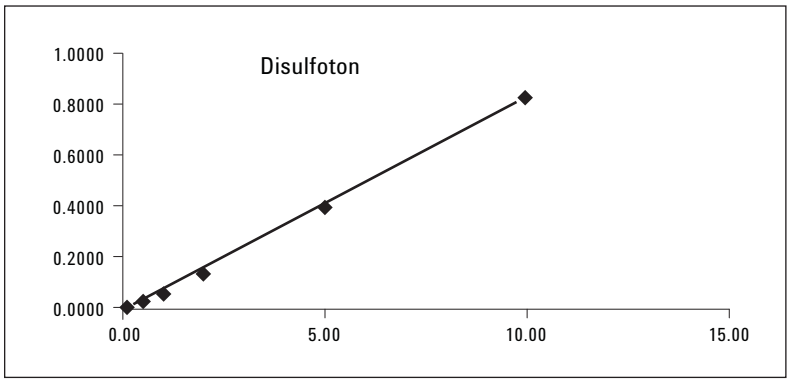

Figure 5. Correlation coefficients for the EPA Method 525.2 pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for disulfoton.

Component	R^2
1,3-Dimethyl-2-nitrobenzene	0.999
Mevinphos	0.990
Pentachlorophenol	0.989
Terbufos	0.996
Bromacil	0.988
Aldrin	0.999
Carboxin	0.996
Endrin	0.998
p,p'-DDT	0.996
Triphenylphosphate	0.997
DEHP	0.996

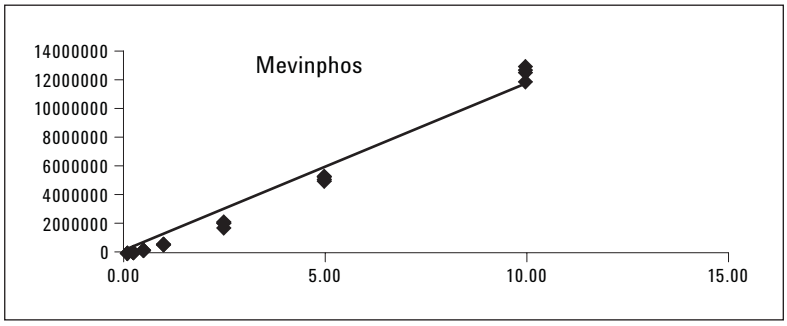

Figure 6. Correlation coefficients for the Ultra Scientific pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for mevinphos.

Conclusions

This application successfully demonstrates the use of an Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level organophosphorus pesticides. Linearity was excellent for all organophosphorus pesticides studied, yielding 0.99 or greater R^2 values down to a 0.1-ng on-column loading of each component. One of the reasons for excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for trace-level applications.

This study was done using scan mode on an Agilent 6890/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 0.1-ng on-column loading of mevinphos was greater than 5 to 1 with this system. This result shows clearly the power of using an Agilent J&W DB-5ms Ultra Inert column for trace-level organophosphorus pesticides analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W DB-5ms Ultra Inert GC capillary column.

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Agilent Technologies

A Direct Column-Performance Comparison for Rapid Contract Laboratory Program (CLP) Pesticide Analysis

Application

Environmental

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Abstract

Agilent J&W High Efficiency GC columns with internal diameter of 0.18 mm for Contract Laboratory Program (CPL) pesticide analyses gave superior results for CPL pesticide primary analysis and confirmation. Chromatograms depicting peak shape characteristics, peak resolution, and baseline stability for two sets of 0.18-mm id columns are presented in a head-to-head comparison. Complete primary and confirmatory analysis of the 20 pesticides in the protocol is accomplished in less than 6 minutes using hydrogen carrier gas and flow programming. Successful primary and confirmatory analyses were achievable only on Agilent J&W High Efficiency GC columns.

Introduction

The analyses of organochlorine pesticides (OCPs) in environmental remediation samples are important, high volume, analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, accumulating costs to both the lab and its client. Use of Agilent J&W 0.18-mm id High Efficiency GC columns is a means of enhancing laboratory productivity [1-3].

Wool and Decker [3] reported their findings at the U.S. Environmental Protection Agency (Region VI, Houston, TX) laboratory and described the value of columns in the 20 m × 0.18 mm format for CLP pesticide analysis. Their suggestion to use a retention gap to protect the analytical columns from deleterious matrix effects and to help offset the lower sample capacity of these columns relative to wider bore columns was incorporated into this column comparison. Deactivated 5 m × 0.25 mm id retention gaps were used in this series of experiments on each column set used.

Columns with 0.18 mm id capable of doing CPL pesticide analysis are available from several leading column manufacturers. Agilent's suggested pair for CLP pesticide analysis in the 0.18 mm id format is a DB-17ms column for primary analysis and a DB-XLB column for confirmation. Vendor R's offering is a set of proprietary phase 0.18 mm id columns for both primary and confirmation analysis of CLP pesticides.

Experimental

The chromatograph used was an Agilent 6890N GC equipped with dual electron capture detectors (μECDs) and a 7683B autosampler. Sample introduction was done by a single split/splitless injection port at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the initial chromatographic conditions appear in Table 1.

Table 1. Chromatographic Conditions

GC :		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed, 69 cm/s at 120 °C, ramped at 99 mL/min to 106 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C, pulsed splitless (35 psi for 0.5 min, purge flow of 40 mL/min on at 1 minute, gas saver flow 20 mL/min on 3 minutes
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
	2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven:		120 °C (0.32 min); 120 °C/min to 160 °C; 30 °C/min to 258 °C (0.18 min); 38.81 °C/min to 300 °C (1.5 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow (60 mL/min)

The flow path supplies used in these experiments are listed in Table 2.

Table 2. Flow Path Supplies

	Description	Agilent p/n
Vials:	Amber screw cap	5182-0716
Vial caps:	Blue screw cap	5282-0723
Vial inserts:	100 µL glass/polymer feet	5181-1270
Syringe:	5 µL	5181-1273
Septum:	Advanced green	5183-4759
Inlet liner:	Deactivated single taper direct connect	1544-80730
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter:	Quartz deactivated	5181-3398
Sealing resin:	Polyimide sealing resin	500-1200
20x magnifier:	20x magnifier loupe	430-1020
Tubing cutter:	Ceramic wafer column cutter	5181-8836

Both sets of columns used in this comparison were installed into the GC in the same manner. The same retention gap and inlet liner were used for both sets of columns. The chromatographic conditions (except for the columns) in Tables 1 and 2 were used to evaluate both the proprietary columns recommended by Vendor R and Agilent's columns. The primary analysis column from Agilent was a 20 m × 0.18 mm x 0.18 µm DB-17ms. The column from Vendor R was a 20 m x 0.18 mm × 0.18 µm with a proprietary stationary phase. The confirmatory analysis column from Agilent was a 20 m × 0.18 mm × 0.18 µm DB-XLB. The column from Vendor R was a 20 m × 0.18 mm x 0.14 µm with a proprietary stationary phase.

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard (New Haven, CT 06513 USA). ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchases from J.T. Baker (Phillipsburg, NJ 08865 USA).

CLP-023R-160X and CLP-024R-160X concentrates were diluted first into 50-mL volumetric flasks in 2,2,4 trimethylpentane and then serially diluted. Volumetric flasks and pipettes used were all class A. The standard concentration range for low-level target compounds in the protocol was from 3.2 to 80 ng/mL. On-column loading ranged from 0.8 to 20 pg for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loupe to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach has given tight, reliable connections that have lasted without difficulty for over 2 months and through hundreds of oven temperature program cycles.

Method Translation Software/Path to Successful Conditions

The starting point for this comparison was the conversion of a successful set of separation conditions using helium carrier on Agilent's DB-17ms and DB-XLB 0.18 mm id columns [4] to a set of conditions using hydrogen carrier. The chromatographic parameters for the helium carrier separation were keyed into the translation table in the Agilent GC Method Translation software [5–6] to convert the method to use with hydrogen carrier. In the software, the "Translate Only" mode was used to convert the 11-minute helium carrier method to a 7.3-minute hydrogen carrier method using the same columns.

Method development effort beyond conversion from helium carrier to hydrogen carrier gas became necessary only when the goal of the analysis shifted to emphasize speed of analysis using flow programming. Flow programming is outside the scope of the Method Translation software. In this series of experiments, flow programming helped to elute highly retained peaks faster. Further temperature program modifications also increased the speed of analysis with minimal loss of resolution on the Agilent columns.

Results and Discussion

Successful separation of CLP pesticides using hydrogen carrier was demonstrated on Agilent's DB-17ms and DB-XLB 0.18-mm id columns using the conditions shown in Table 1. Vendor R's 0.18-mm ID columns were evaluated using the following conditions: the conditions shown in Table 1, the conditions obtained on Vendor R's Web site (to the extent practical), and with a set of conditions optimized specifically on Vendor R's columns for this analysis. The goal throughout these experiments was to show as fair and objective a comparison as possible.

To compare chromatograms, injections at a standard concentration of 3.2 ng/mL for low-level target species in the CLP protocol were selected. Using this concentration consistently provides a fixed point of reference and at the same time alleviates the potential for masking deleterious chromatographic effects often seen at higher concentrations. Inclusion of a Y scale in each chromatogram provides another fixed reference within each figure to facilitate comparison. Key aspects to look for in the example chromatograms are peak resolution, indications of peak tailing, and temperature-dependent drift on the μECD.

An Agilent DB-17ms column was used as the primary analysis column in these experiments. An example chromatogram from an injection at a nominal concentration of 3.2 ng/mL for low target level pesticides is shown in the upper portion of Figure 1. This column resolved all the peaks of interest in less than 6 minutes, gave sharp symmetrical peaks, and had minimal background drift on the μECD. A compound label key for the numbered peaks in the chromatogram is located in Table 3.

An Agilent DB-XLB 20 m x 0.18 mm x 0.18 μm column was used for confirmatory analysis on these experiments. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in the lower portion of Figure 1. This column resolved 20 of the peaks of interest in less than 6 minutes and gave near baseline resolution of peaks 10 and 11. Again, sharp symmetrical peaks and minimal temperature-dependent baseline drift were observed on the μECD. Although complete resolution of 20 of the 22 peaks of interest on the confirmatory columns is not ideal, the observed resolution is satisfactory for peak confirmation.

The peak identification table applies to Figure 1, depicting CPL pesticide separation on Agilent's column only. Elution order for these columns with their particular selectivity was established in previous work. To establish elution order on Vendor R's columns with different selectivity, injection of individual standards or mass spectral confirmation is required.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene	12. 4,4' DDE
2. Alpha BHC	13. Dieldrin
3. Gamma BHC	14. Endrin
4. Beta BHC	15. 4,4' DDD
5. Delta BHC	16. Endosulfan II
6. Heptachlor	17. 4,4' DDT
7. Aldrin	18. Endrin aldehyde
8. Heptachlor epoxide	19. Endosulfan sulfate
9. Gamma chlordane	20. Methoxychlor
10. Alpha chlordane	21. Endrin ketone
11. Endosulfan I	22. Decachlorobiphenyl

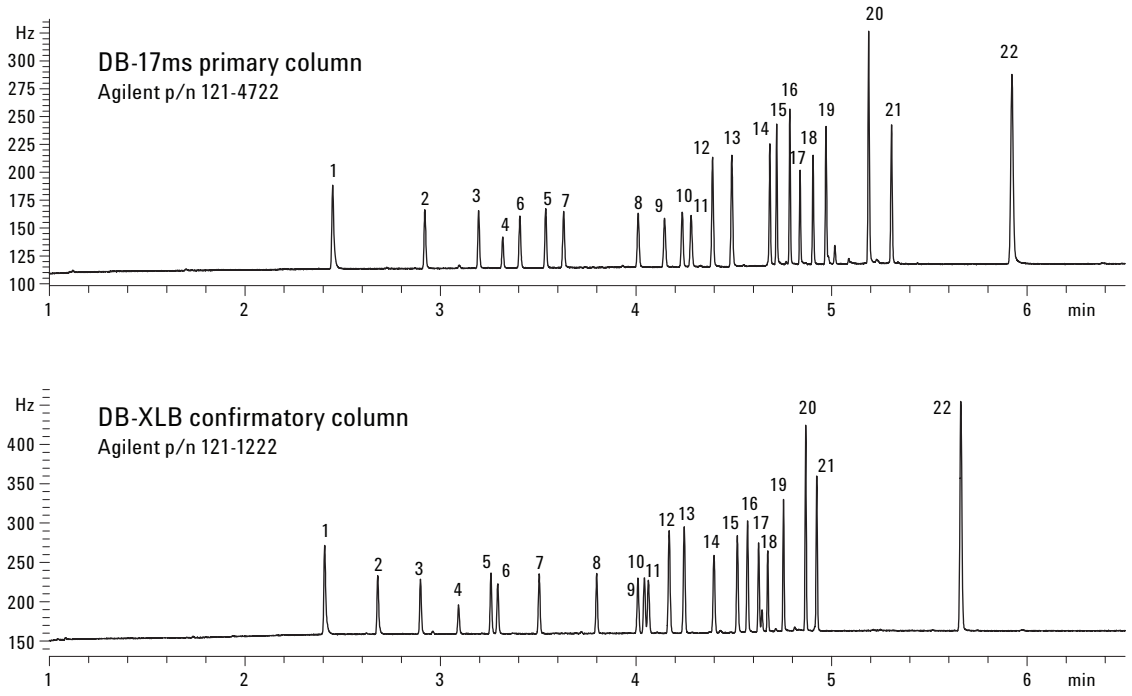

Figure 1. Chromatogram of 0.5-µl injection of 3.2 ng/ml low-level target pesticide standard solution injected through a Y-splitter onto DB-17ms (Agilent p/n 121-4722) primary analysis column and a DB-XLB (Agilent p/n 121-1222) confirmatory analysis column, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column gave resolution of 20 of the 22 peaks of interest, peak tailing for some species, and minimal temperature dependent baseline drift on the µECD. The arrows within the figure point to co-eluting and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift, in this case over 100 Hz.

Vendor R's suggested separation conditions for their column pair were unsuccessful at producing results equivalent to those shown on their Web site. This appears to stem from an oversight on their part. Suggested conditions found in a figure caption on the Web site called for a 2-min hold 10° C above the maximum recommended temperature. A temperature of 330 °C was called for; however, the label on the column box listed the upper temperature program limit as 320 °C for the confirmation column. Vendor R's confirmation column demonstrated significant bleed even with a temperature program that reached only 300 °C, a full 20 °C below the upper limit.

A series of attempts to resolve the co-eluting pair of pesticides on Vendor R's primary analysis column gave improved but still incomplete resolution. It was necessary to substantially reduce flow rate and modify both temperature and flow programming parameters to achieve the results shown in Figure 3. The chromatographic conditions used for these injections appear in Table 4; the flow path supplies were the same as those listed in Table 2.

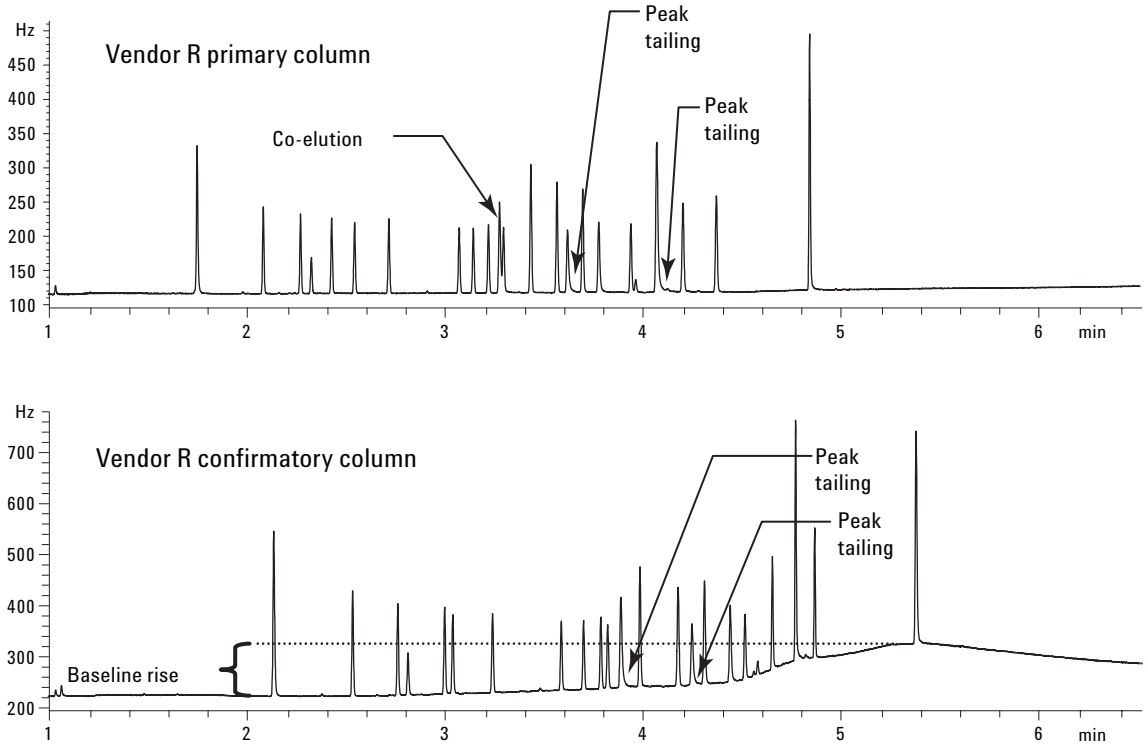

Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1. Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column still gave resolution of 20 of the 22 peaks of interest, indication of peak tailing for some species, and minimal temperature-dependent baseline drift on the µECD. The arrows within the figure point to the unresolved peaks and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift.

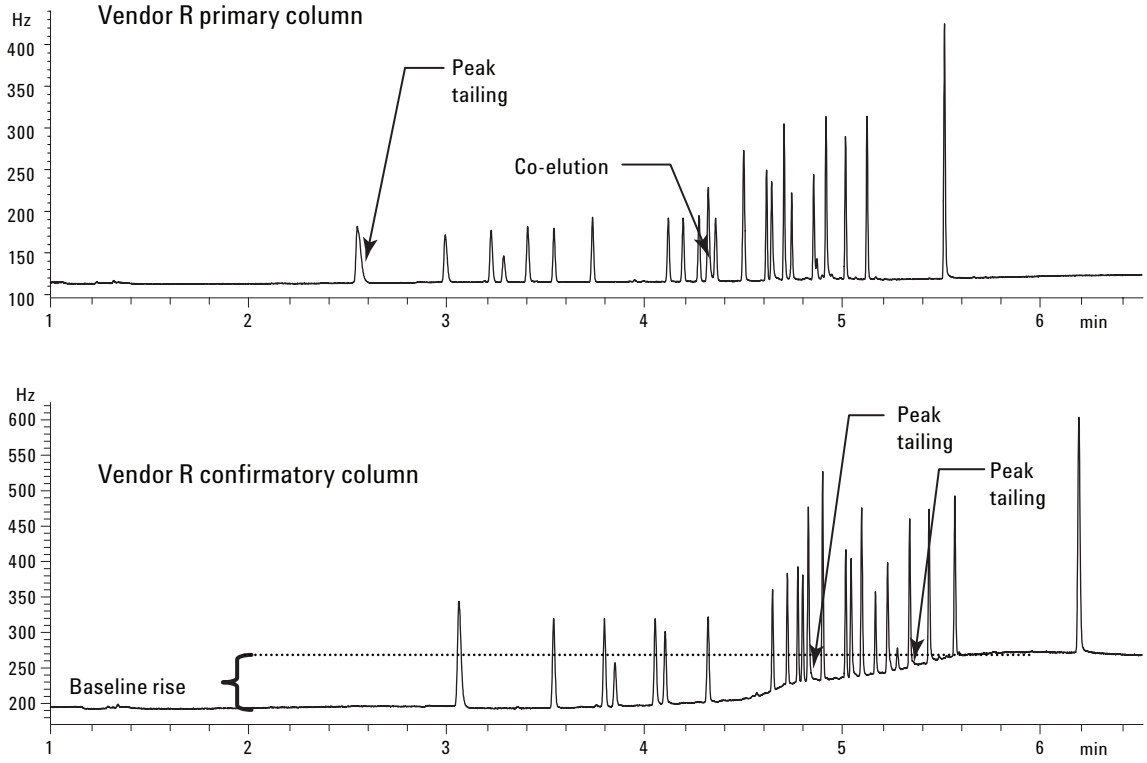

Figure 3. Chromatogram of 0.5-µl-injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary and confirmatory analysis columns, conditions as in Table 4.

Table 4. Chromatographic Conditions

GC:		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed , 45 cm/s at 120 °C, ramped at 99 mL/min to 72 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C pulsed splitless (35 psi for 0.5 min, purge flow 40 mL/min on 1 minute, gas saver 20 mL/min at 3 minutes)
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m x 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm primary analysis column
	2	20 m x 0.18 mm × 0.14 µm confirmatory analysis column
Oven:		120 °C (0.50 min); 60 °C/min to 160 °C; 30 °C/min to 260 °C; 40 °C/min to 300 °C (2.0 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

Conclusions

Agilent's 0.18-mm id primary analysis column is superior to Vendor R's offering. All 22 peaks of interest were resolved on the DB-17ms primary analysis column in less than 6 minutes with sharp, symmetrical peaks and minimal baseline drift. Vendor R's primary analysis column resolved 20 of 22 peaks of interest and displayed evidence of peak tailing for some of the peaks of interest.

Agilent's 0.18-mm id confirmatory analysis column offering is superior to Vendor R's offering. Twenty of 22 peaks of interest were resolved on the DB-XLB, with the other two peaks being almost baseline resolved in less than 6 minutes, with sharp, symmetrical peaks and minimal temperature-dependent baseline drift. Resolution of 20 of 22 peaks is less than ideal but should serve well for peak confirmation. Vendor R's confirmatory column resolved all 22 peaks of interest but showed evidence of peak tailing and an unacceptable level of temperature-dependent baseline drift.

The DB-17ms and the DB-XLB columns used in these experiments gave very low bleed profiles on the µECDs. The stable baselines produced by both of these columns can lead to lower detection limits,

simpler integration and more reliable results over time. These columns also have the versatility of use with other analyses beyond CLP pesticides.

Reliable CLP pesticide primary and confirmation analyses are achievable using Agilent J&W high-efficiency GC columns in less than 6 minutes with standard gas chromatographic equipment.

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Agilent Technologies

Contract Laboratory Program (CLP) Pesticide Analysis with 0.18 mm ID High-Efficiency GC Columns Utilizing Helium Carrier Gas

Application

Environmental

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Abstract

Contract Laboratory Program (CLP) pesticide analysis is demonstrated on high-efficiency GC columns (20 m × 0.18 mm id × 0.18 µm film thickness) with helium carrier gas. DB-17ms stationary phase is used for primary analysis and DB-XLB stationary phase for confirmation. Primary analysis and confirmation of 22 CLP pesticides in the protocol is achieved in an 11-minute analysis, a 35% reduction in analysis time versus 0.32 mm id columns.

Method translation software is successfully employed to translate an original set of conditions with hydrogen carrier gas to a new set of conditions using helium carrier gas. Elution order and degree of separation are shown to translate precisely from the original method to the new method through use of this software (available for free download) [1].

Introduction

The determination of organochlorine pesticides (OCPs) in environmental remediation samples are important, high-volume analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, adding costs to both the lab and its client. Here, 0.18 mm id high-efficiency GC columns are demonstrated as a means of enhancing laboratory productivity. These columns are fully compatible with standard gas chromatographs and helium carrier gas operation. The high efficiency these columns offer coupled with their full compatibility with existing GCs provide laboratories with a powerful tool for enhancing sample throughput. When analysis times for 30 m × 0.32 id and 20 m × 0.18 mm id columns were compared, a 17-minute analysis was reduced to only 11 minutes and with improved resolution [2].

Helium carrier gas was selected as a means to show the utility of 0.18 mm id columns in doing CLP pesticide analyses and to demonstrate the full compatibility of these columns with standard gas chromatographs. The operating gas pressures for these 20 m × 0.18 mm id columns range from 33 psi initially to 50 psi at the high point of the temperature program. The gas pressure range used with helium carrier gas with these 0.18 mm id columns was well within the operating range for standard chromatographs.

Experimental

This work was accomplished using an Agilent 6890N GC equipped with dual µECDs and a 7683B autosampler. A single split/splitless injection port was used for sample introduction at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the chromatographic conditions are presented in Table 1.

Table 1. Chromatographic Conditions

GC	Agilent 6890N
Sampler:	Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Inlet	Split/splitless; 250 °C pulsed splitless (35 psi for 0.5 min)
Inlet liner	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Carrier	Helium (constant flow, 49.5 cm/sec at 120 °C, purified through big universal trap Agilent p/n RMSH-2)
Retention gap	5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter	Quartz deactivated (Agilent p/n 5181-3398)
Columns:	
1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven	120 °C (0.49 min); 85 °C/min to 160 °C; 20 °C/min to 260 °C (0.20 min); 25 °C/min to 285 °C; 40 °C/min to 300 °C (3.5 min)
Detection	µECD 325 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

The flow path supplies used in these experiments are listed in Table 2 below.

Table 2. Flow Path Supplies

		Agilent p/n
Vials	Amber screw cap	5182-0716
Vial caps	Blue screw cap	5282-0723
Vial inserts	100 µL glass/polymer feet	5181-1270
Syringe	5 µL	5181-1273
Septum	Advanced green	5183-4759
Inlet liner	Deactivated single taper direct connect	1544-80730
Ferrules	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter	Quartz deactivated	5181-3398
Sealing resin	Polyimide sealing resin	500-1200
20x magnifier	20x magnifier loop	430-1020
Tubing cutter	Ceramic wafer column cutter	5181-8836

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard, New Haven, CT 06513-USA. ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchased from J. T. Baker, Phillipsburg, NJ 08865-USA. CLP-023R-160X and CLP-024R-160X concentrates were diluted separately into two 100-mL volumetric flasks in 2,2,4 trimethylpentane and then combined in subsequent serial dilutions. Volumetric flasks and pipettes used were all class A. Standard concentration range for low-level target compounds in the protocol was from 1.6 to 40 ng/mL. On-column loading ranged from 0.4 to 10 pgs for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Tip on Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loop to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach gave tight, reliable connections that have lasted without any difficulty for more than 2 months (to date) and hundreds of oven temperature program cycles.

Results and Discussion

The starting point for this application was a set of conditions for CLP pesticide analyses using hydrogen carrier gas and 0.18-mm high-efficiency columns developed by Wool and Decker [3]. Using hydrogen carrier and flow programming, they were able to achieve primary separation and confirmation analysis of CLP pesticides in a 7-minute analysis. The chromatographic parameters for the hydrogen carrier separation were input as initial setpoints in method translation software to convert the method to use with helium carrier. Helium carrier was selected for use in laboratories reluctant to work with hydrogen carrier due to site safety policy or individual preference. High-efficiency GC columns give the chromatographer the option to work with either helium or hydrogen carrier gases and still achieve faster analyses.

Wool and Decker [3] indicated in their paper that frequent trimming of the front of the column was necessary for use with heavy matrix samples due primarily to the lower sample capacity of 0.18-mm columns. In this work a 5-m 0.25-mm id retention gap and Y connector were installed ahead of the

analytical columns to help offset the diminished sample capacity relative to wider bore capillary columns. Use of a retention gap will also shield the analytical columns from deleterious matrix affects and extend the useful lives of the columns.

Agilent's method translation software simplifies conversion from established laboratory GC methods to parallel sets of conditions suitable for high-efficiency GC columns. Chromatographic conditions from the original method, along with the new column dimensions, are entered into a menu-driven table within the software. The software then generates a translated method table with all the new chromatographic setpoints for the translated method. The new translated method setpoints produced by the software are often all that is required to successfully translate a method.

Three primary modes of method translation are available in the method translation software: translate only, best efficiency, and fast analysis. The "translate only" mode produces a set of conditions that most closely resembles the original method in terms of relative position on the Van Deemter curve, degree of separation, and elution order. The "best efficiency" mode generates a set of conditions where column efficiency is prioritized. The "fast analysis" mode generates a set of conditions where analysis speed is prioritized. By using the various modes available a translated method specific to a particular application can be developed quickly with a few keystrokes and iterative passes through the software.

The software is very useful in porting methods from the use of one carrier gas to another. Translation from the original method using one carrier to a method using another carrier is accomplished by entering the original method setpoints, the new column dimensions, and the desired carrier. The software then generates the translated method setpoints for the new column and carrier. For additional information on Agilent's method translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.

Flow programming is not addressed in the method translation software, so minor adjustments to flow rate parameters may be required to achieve desired results. When translating flow-programmed methods, initial or intermediate flow rates can be entered into the original method parameters table to visualize the effect on the other parameters' output in the translated method table. The operator can then collect data at several different flow rates and select the best set of conditions for the application.

In this CLP pesticide example, the original method used a hydrogen carrier and flow programming. The initial flow parameters were entered into the method translation software, along with the new column dimensions, specifying helium as the carrier gas. Translate-only mode was selected in the software and produced the translated method setpoints that appear in Figure 1.

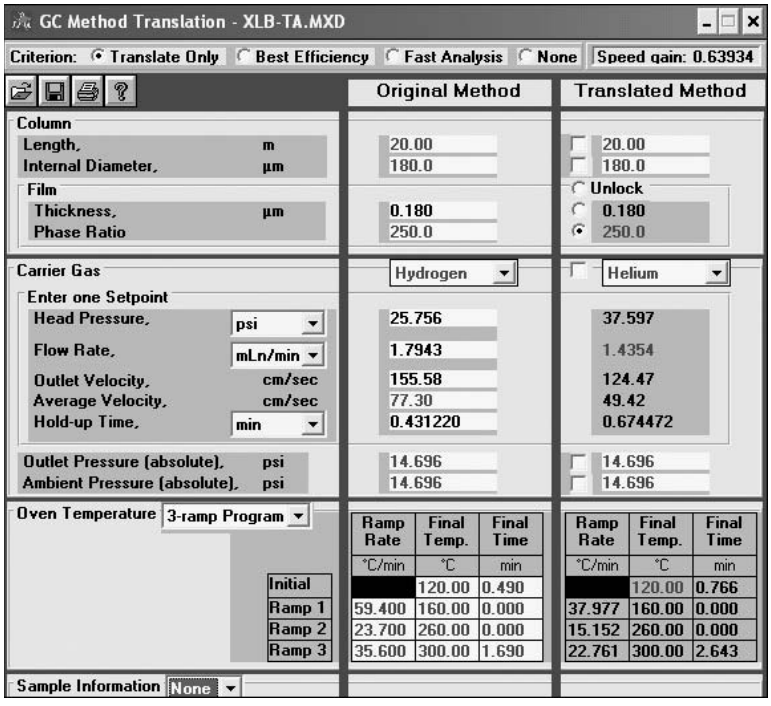

Figure 1. Method translation using translate-only mode.

Figure 2 shows the resulting CLP pesticide separations produced using translate-only mode in the method translation software on the DB-17ms column. Note that all 22 species are baseline resolved on the DB-17ms column where there is a partially separated triplet consisting of gamma chlordane, alpha chlordane, and endosulfan 1 on the DB-XLB column (Figure 3). This partially separated triplet was also observed in the original DB-XLB separation using hydrogen carrier.

Table 3 is a standard compound key for the numbered peaks in the chromatograms. Separation characteristics such as degree of separation and elution order were maintained exactly as they were in the original method using the new translated method with helium carrier. The original method was successfully translated with no additional method development.

Unfortunately, the unresolved triplet on DB-XLB observed in the original method remained unresolved in the translated method. Additional method development attempts focused on resolving the partially separated triplet on the DB-XLB confirmation column and reduction of analysis time. Some success was achieved; however, the trailing two peaks in the triplet remained partially resolved on the DB-XLB confirmation column while analysis time was reduced to 11 minutes. The DB-17ms column resolved all of the species in the protocol throughout these experiments (Figure 4). Triplet resolution on the DB-XLB (Figure 5), though not ideal, is adequate for the purpose of peak confirmation of well-resolved species on the DB-17ms.

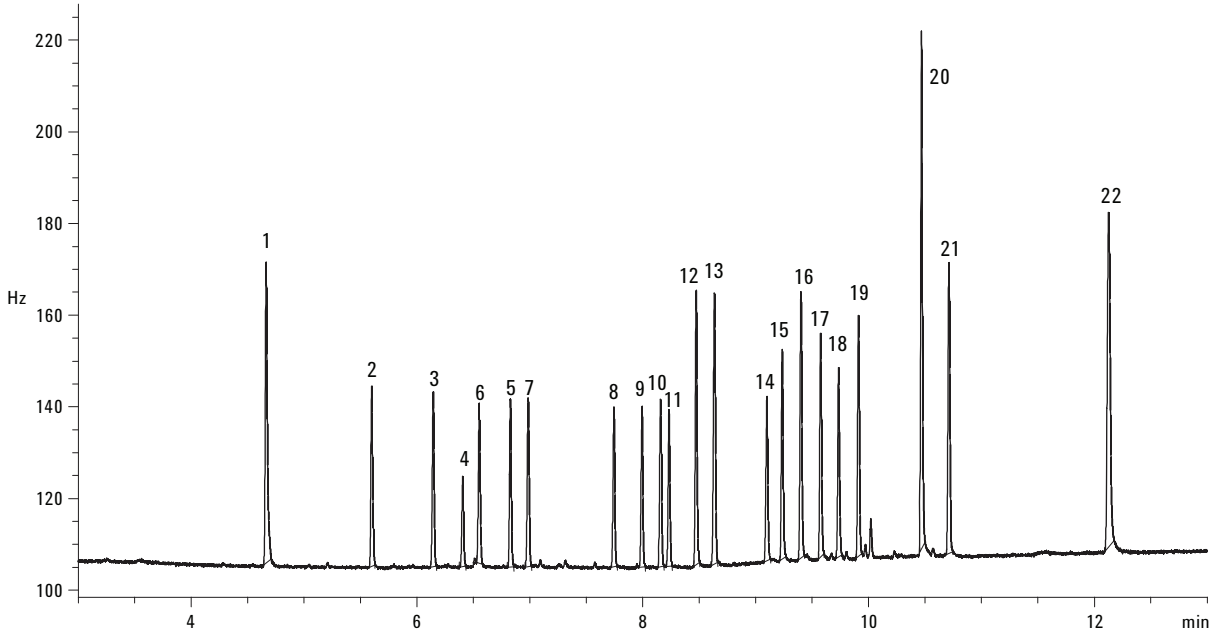

Figure 2. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene
2. Alpha BHC
3. Gamma BHC
4. Beta BHC
5. Delta BHC
6. Heptachlor
7. Aldrin
8. Heptachlor epoxide
9. Gamma chlordane
10. Alpha chlordane
11. Endosulfan I
12. 4,4′ DDE
13. Dieldrin
14. Endrin
15. 4,4′ DDD
16. Endosulfan II
17. 4,4′ DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl

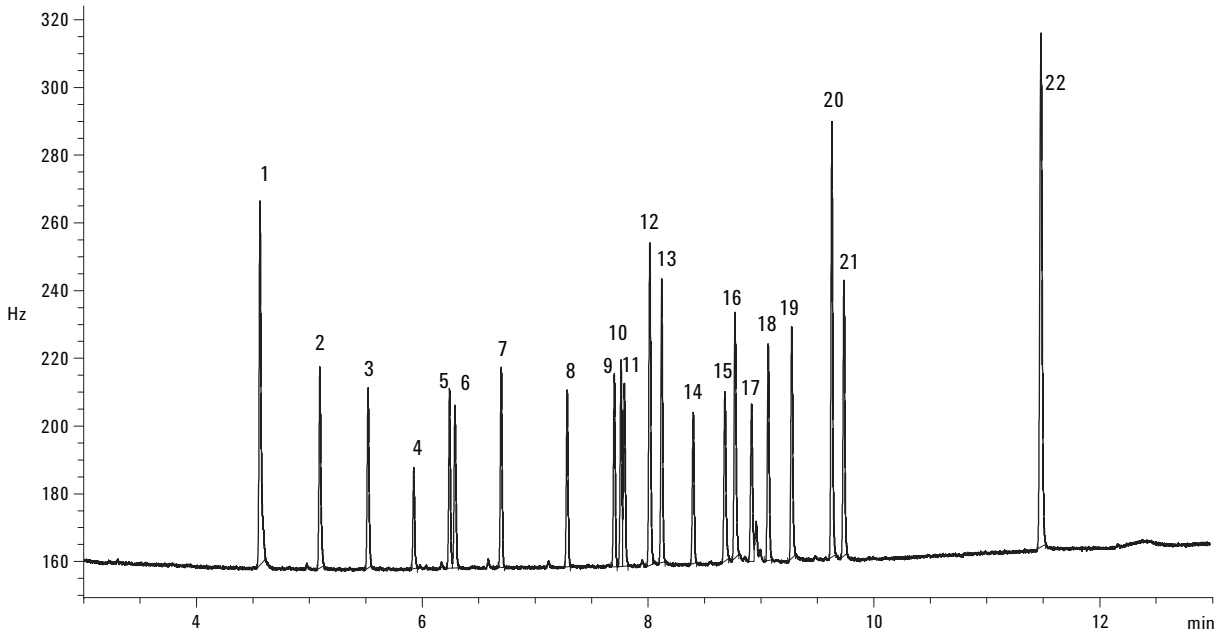

Figure 3. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

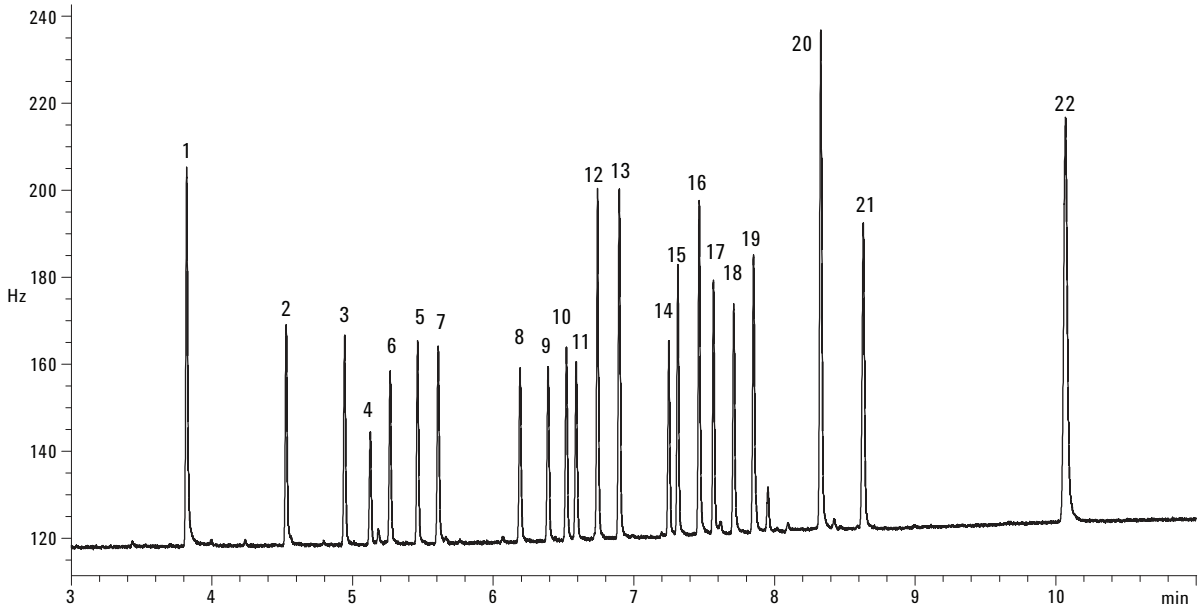

Figure 4. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

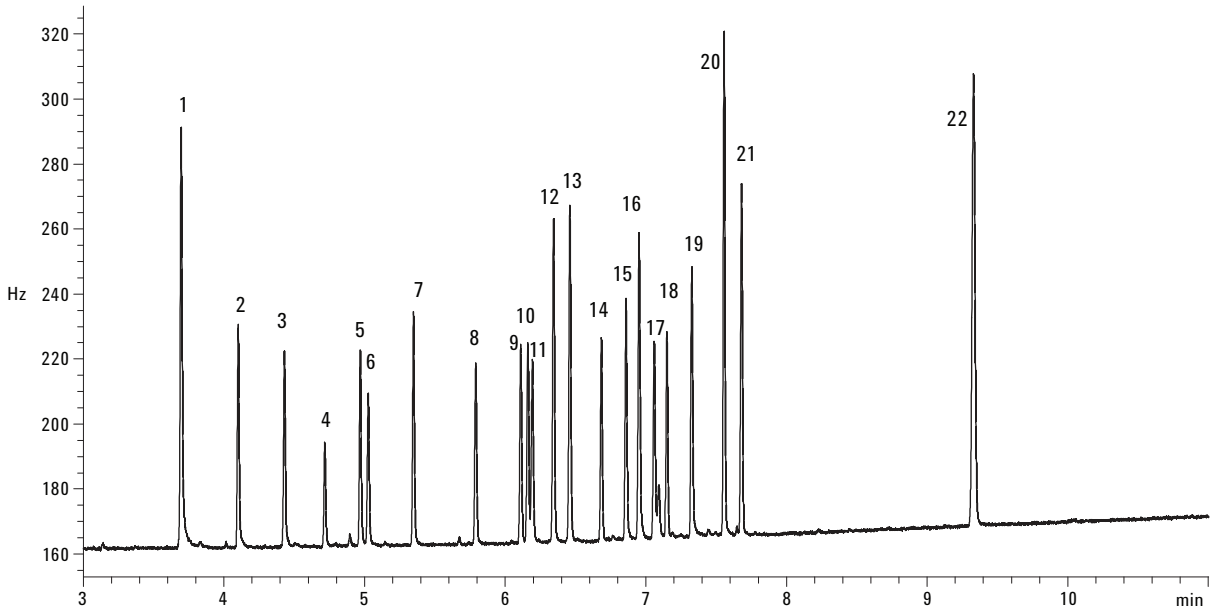

Figure 5. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

Detector Sensitivity and Linearity

The 0.5-µL injections were split between two columns for an on-column loading of 0.4 pg per component of the low-level target compounds. The data suggest that detection limits of at least an order of magnitude lower are possible. Sensitivity and linearity measurements conducted with these chemical species using µECD detection support this assertion [4]. Analyte concentration range investigated here was from 1.6 – 40 ng/mL. This range meets the 16-fold low- to high-check standard criteria for the protocol and appears to cover only the middle of the dynamic range the detector is capable of fielding. Figure 3 shows the DB-17ms separation where low-level component loading was 0.4 pg. Figure 6 shows the same separation with a 10-pg loading for the same components.

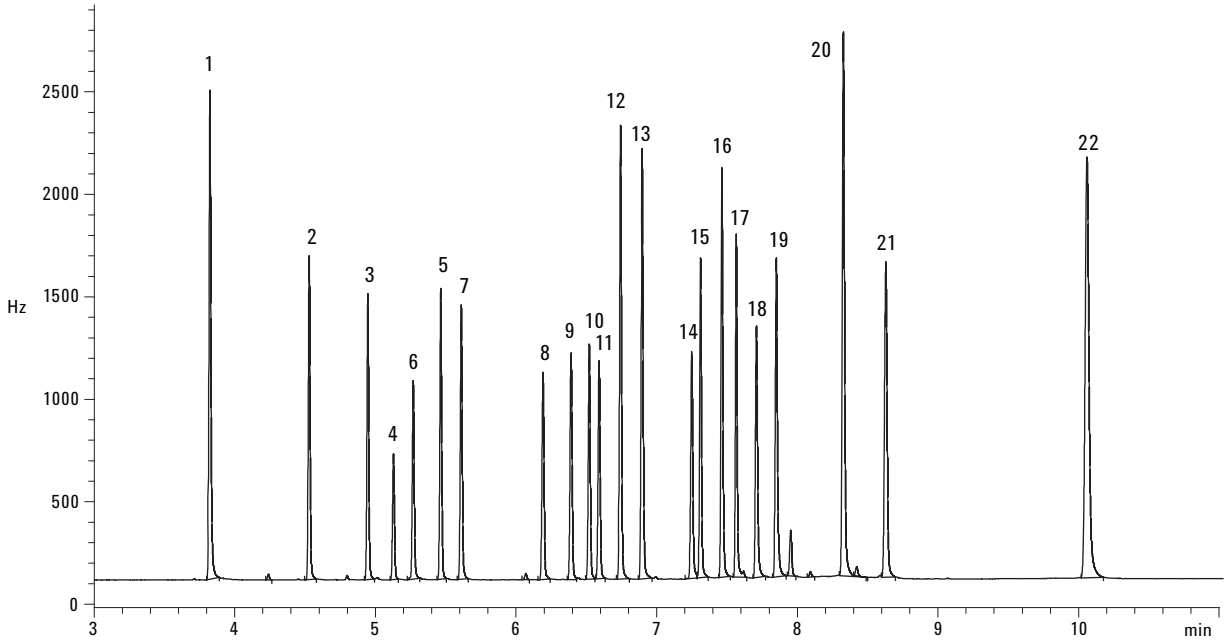

Figure 6. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 10-pg/component loading for low-level target compounds.

Conclusions

Complete separation and confirmation of all 22 species in the CLP pesticide protocol were accomplished in an 11-minute analysis with helium carrier gas. These results demonstrate the utility of these 0.18-mm id high-efficiency GC columns for CLP pesticide analysis. Using a 0.5-µL injection of pesticide standard solutions over a concentration range of 1.6 – 40 ng/mL gave excellent results. These results easily meet the 16x high/low dynamic range requirement for the protocol and suggest that expanding the range to both lower and higher concentrations is certainly possible with these 0.18-mm columns.

Full compatibility for use of these columns with standard GC equipment and helium carrier was also established by this successful separation. Operating pressure for use of these columns at the high point of the temperature program (300 °C) was 50 psi, well within the operation pressure range for standard GC equipment.

Method translation software successfully translated the original method using hydrogen carrier to the new method using helium carrier. Separation characteristics from the original method, such as elution order and degree of separation, were matched exactly in the translated method. This exercise served once again to validate the simplicity of method translation using the software. Method development beyond the translated method setpoint only became necessary when improvements to the original separation were attempted.

References

1. To download Agilent Method Translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.
2. C. George, “Rapid Analysis of CLP Pesticides Using High-Temperature DB-35ms and DB-XLB Columns,” Application Note 5988-4973EN, December 18, 2001
3. L. Wool and D. Decker, “Practical Fast Gas Chromatography for Contract Laboratory Program Pesticide Analysis,” *Journal of Chromatographic Science,* Vol. 40 September 2002
4. I. L. Chang, M. S. Klee, and J. Murphy, “Validation Analysis of EPA CLP Target Organochlorine Pesticides with the Agilent 6890 Series GC and Micro-ECD,” Application Note 5966-3742E, February 1998

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Agilent Technologies

Determination of Chlorinated Acid Herbicides in Soil by LC/MS/MS

Application Note

Environmental

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Abstract

Chlorinated acid herbicides were analyzed at the picogram level on column without any derivatization using liquid chromatography and tandem mass spectrometry (LC/MS/MS). Good linearity was observed for all the selected analytes, from low pg to low ng levels on column.

Introduction

Chlorinated acid herbicides are a popular class of broad-leaf weed killer in lawn and grain crops. Due to their widespread use, environmental contamination in water and soil from run-off is a serious concern.

Traditional analytical methods based on gas chromatography (GC) and/or mass spectrometry (MS) require derivatization of the analytes. Combining LC and electrospray ionization (ESI) in negative ion mode, these herbicides can be analyzed without derivatization. The multiple reaction monitoring (MRM) mode in MS/MS operation provides low pg detection limits.

This application note is based on standards and sample preparation procedures from the Montana Department of Agriculture in Bozeman, Montana.

Experimental

Standard and Sample Preparation

A stock solution of each analyte at 200 ppm is prepared in methanol. Intermediate mixed solutions for fortifying soil samples and making calibration standards are made by accurately mixing aliquots of each standard stock solution. The concentrations of each analyte in the intermediate solution used in this study are listed in Table 1.

Table 1. Acid Herbicide Mixed Intermediate Standards in Methanol

Clopyralid	5930 (pg/µL)	3,6-dichloro-2-pyridinecarboxylic acid
Picloram	1800	4-amino-3,5,6-trichloropicolinic acid
Dicamba	8200	3,6-dichloro-2-methoxybenzoic acid
2,4-D	1740	2,4-dichlorophenoxyacetic acid
MCPA	5480	2-methyl-4-chlorophenoxyacetic acid
Triclopyr	1240	[(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid
2,4-DP	1410	2,4-dichlorophenoxypropionic acid or dichloroprop
MCPP	2710	2-(2-methyl-4-chlorophenoxy) propionic acid
2,4-DB	6900	2,4-dichlorophenoxybutyric acid

Sample extraction and cleanup procedures are shown below.

Sample Extraction Procedure

1. Weigh 20 ± 0.1 g of soil.
2. Add 50 mL of 0.5N KOH in 10% KCl extracting solution to each sample. Mix thoroughly by shaking.
3. Place samples in boiling water bath for 15 minutes.
4. Place samples on horizontal shaker for 15 minutes.
5. Centrifuge samples at 1200 to 1500 rpm for 15 minutes.
6. Transfer a 3.0-mL aliquot into a 13-mL conical centrifuge tube and add 150 µL of 12 N sulfuric aicd.
7. Vortex and confirm the pH is <1.5. If not, add additional acid solution.

Sample Cleanup Procedure

1. Add 2 mL of chloroform to the acidified extract.
2. Vortex 30 seconds and centrifuge at 3000 rpm for 2 minutes.
3. Remove the lower chloroform layer into another centrifuge tube. Repeat these three steps two more times.
4. Evaporate the chloroform extract to just dryness.
5. Immediately add 4.0 mL HPLC-grade water, vortex briefly, sonicate 5 minutes, and briefly vortex again. Fill autosampler vial.

See Reference 1 for more detailed information on sample preparation.

Instrumentation

LC:	1200 LC
Column:	ZORBAX Extend-C18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	60 °C
Mobile phases:	A: 0.04% Glacial acetic acid in water
	B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min
Injection volume:	1.0 µL

Gradient:

Time, min.	%B
0	0
1	40
2	52
3	60
4	100
8	100
9	0

MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120 to 400 amu
Capillary:	3500 V
Nebulizer pressure:	40 psi
Drying gas flow:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

MRM parameters are listed in Table 2.

Table 2. Method Parameters for MRM

Name	RT	MW	Quant	Qual	Frag V	Col cell	Dwell	Segment
Clopyralid	3.47	191	190 > 146	192 > 148	80	5	70	1
Picloram	3.69	240	239 > 195	241 > 197	80	5	70	1
Dicamba	4.31	220	219 > 175	219 > 145	60	0	150	2
2,4-D	5.02	220	219 > 161	221 > 163	80	15	25	3
MCPA	5.09	200	199 > 141	201 > 143	100	10	25	3
Triclopyr	5.26	255	254 > 196	256 > 198	80	10	25	3
2,4-DP	5.42	234	233 > 161	235 > 163	80	5	25	3
MCPP	5.46	214	213 > 141	215 > 143	100	10	25	3
2,4-DB	5.66	248	247 > 161	249 > 163	80	10	25	3

Due to the concentration differences for these analytes in the intermediate solution (Table 1), Dicamba's concentration was used as the "concentration level" shown in Table 3.

Table 3. Concentration Levels (8000 to 10 pg/µL) Used in This Study

Solution concentration level	8000	800	400	200	80	40	20	10
Clopyralid	5930	593	296.5	148.2	59.3	29.7	14.8	7.4
Picloram	1800	180	90	45	18	9.0	4.5	2.3
Dicamba	8200	820	410	205	82	41.0	20.5	10.3
2,4-D	1740	174	87	43.5	17.4	8.7	4.4	2.2
MCPA	5480	548	274	137	54.8	27.4	13.7	6.9
Triclopyr	1240	124	62	31	12.4	6.2	3.1	1.6
2,4-DP	1410	141	70.5	35.2	14.1	7.1	3.5	1.8
MCPP	2710	271	135.5	67.7	27.1	13.6	6.8	3.4
2,4-DB	6900	690	345	172.5	69	34.5	17.3	8.6

For example, concentration level 8000 is the intermediate solution, and concentration level 20 is a 1:400 dilution of the intermediate solution.

Results and Discussion

Figure 1 shows the overlaid chromatograms of all nine herbicides from the MRM analysis. The run time was less than 6 minutes. Using a 1.8-µm particle size column, the peak widths of these analytes are in the range of 0.1 to 0.2 min. The narrower peak width helps to achieve a higher signal-to-noise ratio.

The MRM results of all nine herbicides at 1.6 to 10.3 pg on column are shown in Figure 2.

As shown in Table 3, the linearity range, incorporating 10, 20, 40, 80, 200, 400, 800, and 8000 pg on column, mimics the concentration of Dicamba.

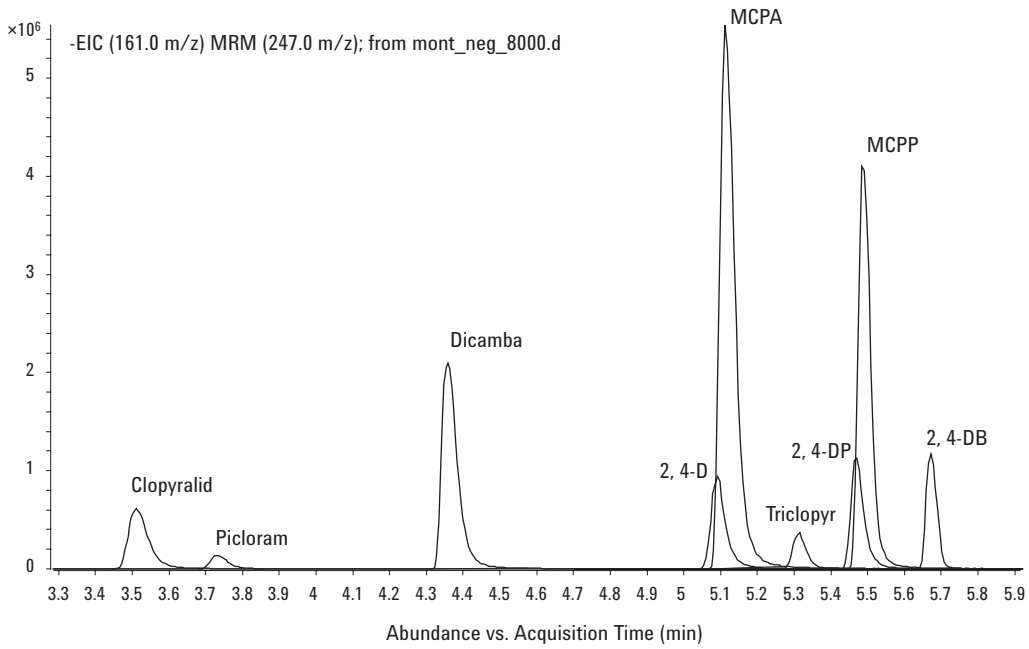

Figure 1. Overlaid MRM results from the nine selected herbicides.

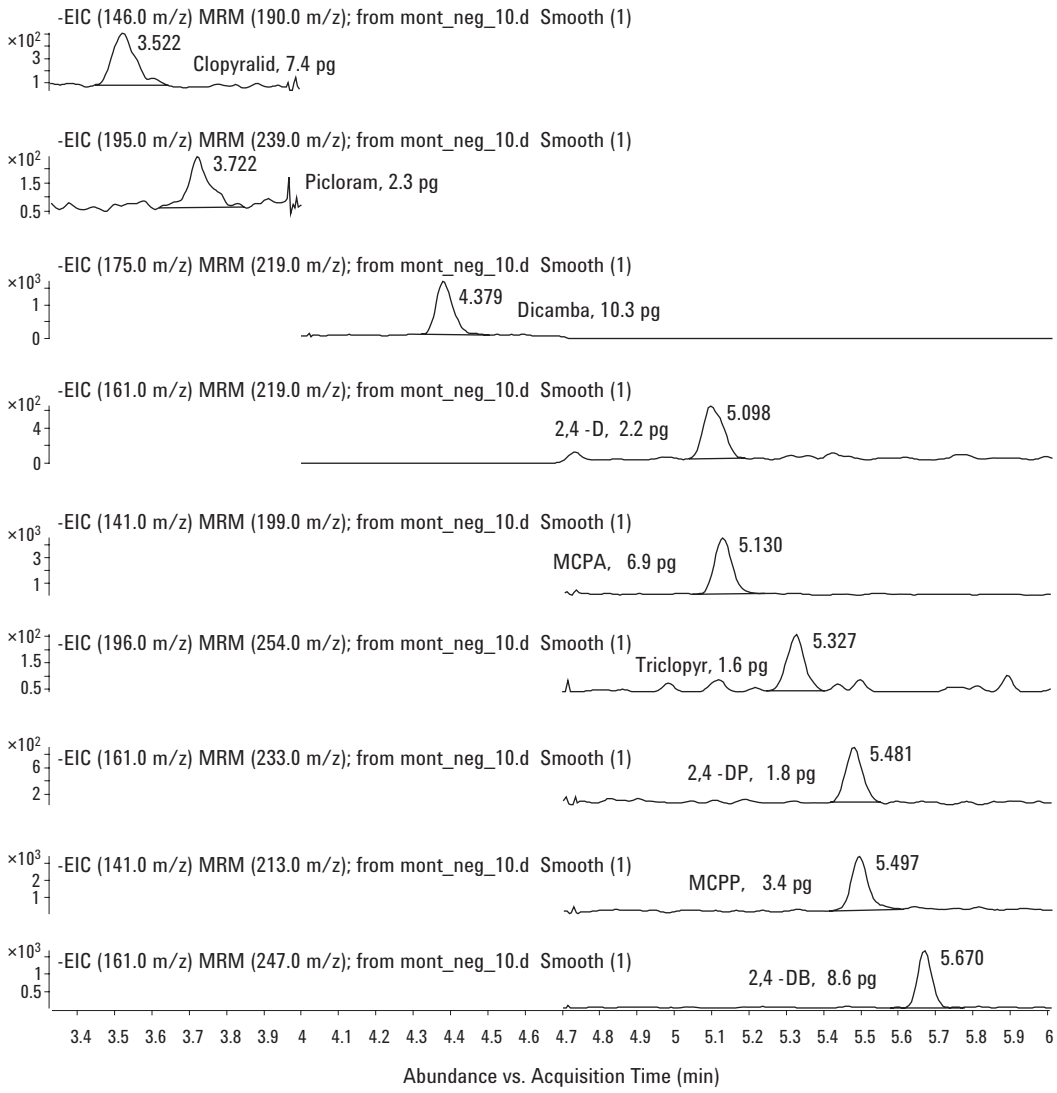

Figure 2. MRM results.

Therefore, the corresponding on column amounts for Triclopyr are: 1.6, 3.1, 6.2, 12.4, 31, 62, 124, and 1240 pg. The calibration model used was a linear model that included origin with no weighting. All analytes showed excellent linearity.

In the repeatability study, seven 1-µL injections of the level 40 solution (Table 4) were analyzed to calculate the RSD. At this low concentration, all RSDs were <15%, with the majority in the single digits.

The matrix effect from three different matrices was evaluated. Water and soil extracts were spiked with the herbicide standards (50 µL of the level 400 standard were added to 950 µL of water, silt, clay, or sandy extracts). The resulting concentration of the analytes is equivalent to the level 20 solution (Table 3).

Table 5 shows the RSD of eight 1-µL injections of the level 20 solutions, that is, <20 pg of each ana-

Table 4. Linearity (10 to 8000 pg/µL) and Repeatability

Compound	R^2 (linear fit, include origin)	Repeatability, %RSD (n=7)	Amount on column
Clopyralid	0.9995	3.5	29.7 pg
Picloram	0.9991	13.1	9.0
Dicamba	0.9999	2.4	41.0
2,4-D	0.9998	6.5	8.7
MCPA	0.9999	2.2	27.4
Triclopyr	0.9993	11.9	6.2
2,4-DP	0.9998	4.5	7.1
MCPP	0.9999	5.0	13.6
2,4-DB	0.9973	8.4	34.5

lyte on column, in three different matrices. As expected, analytes with lower absolute responses showed higher RSD value.

Table 5. RSDs from Eight Injections of <20 pg of Each Analyte in Three Matrices

	Clay	Sandy	Silt	On column
Triclopyr	27	22	22	3.1 pg
MCPP	6	5	7	6.8
MCPA	2	6	5	13.7
Clopyralid	14	15	13	14.8
2,4-DP	9	8	13	3.5
2,4-DB	9	3	9	17.3
2,4-D	11	14	13	4.4

The repeatabilities of responses in three matrices for all analytes were <15% except for Triclopyr, which was >20%.

The responses of ≤20 pg analytes among water and matrices are compared in Figure 3. The listed response for each matrix is the average of responses from eight injections. The RSDs for water and the three matrices are shown in Figure 3 and are comparable. In general, the variation of the responses among water and different matrices is less than 5% for all analytes except for 2,4-D, which has an RSD close to 10% due to the higher responses from the silt matrix. This shows that the method described in this application note is free from matrix interferences from clay, sand, and silt.

Conclusion

Using LC/MS/MS, chlorinated acid herbicides were analyzed at pg levels on column without any derivatization. At <40 pg on column, the repeatability of seven 1-µL injections showed RSD <15%. Good linearity was observed for all analytes from low pg to low ng on column.

The RSDs of <20 pg analytes in the selected matrices were comparable to that in water (RSDs ~5%), except for 2,4-D, due to the higher response from the silt.

Acknowledgement

The author would like to thank Ms. Heidi Hickes and Ms. Angela Schaner from the Montana Department of Agriculture for valuable discussions, sample preparation procedures, and the samples used in this study.

Reference

1. "Determination of Chlorinated Acid Herbicides in Soils by Liquid Chromatography-Electrospray/Mass Spectrometry/Mass Spectrometry," by Angela Schaner and Laura Luckey, Revision 2, April 2, 2004. Montana Department of Agriculture, Laboratory Bureau, McCall Hall, Montana State University, Bozeman, MT 59717.

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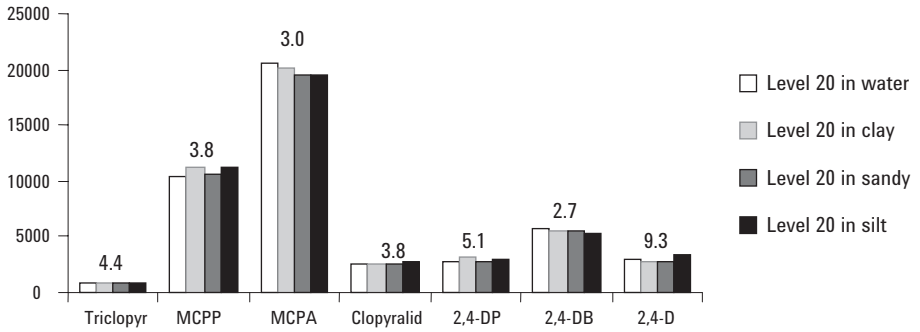

Figure 3. Analyte RSDs from water and soil extracts are comparable.

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Agilent Technologies

Screening for 926 Pesticides and Endocrine Disruptors by GC/MS with Deconvolution Reporting Software and a New Pesticide Library

Application Note

Food and Environmental

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Abstract

An updated and greatly expanded collection of mass spectral libraries has been introduced, replacing Agilent's RTL Pesticide Library and DRS pesticide solution. The new library contains 926 pesticides, endocrine disruptors, and related compounds – 359 more than the original library. Included are all compounds specified for GC/MS analysis in the new Japanese "Positive List" regulations. All compounds have locked retention times that can be accurately reproduced using an Agilent GC/MS system with the ChemStation's Retention Time Locking software. The new Database can be used as a standard GC/MS library for compound identification or with Agilent's Screener software for identifications based upon retention time and mass spectral matching. The greatest benefit accrues when these libraries are used with Agilent's new version of Deconvolution Reporting Software (part number G1716AA version A.03.00). This solution allows one to screen GC/MS files for all 926 pesticides and endocrine disrupters in about two minutes per sample. Deconvolution helps identify pesticides that are buried in the chromatogram by co-extracted materials. The new database was compared to the smaller one for the DRS analysis of 17 surface water samples. With the new database, DRS found 99 pesticides, metabolites, fire retardants, and related contaminants that were not contained in the original RTL Pesticide and Endocrine Disruptor Library.

Introduction

Several years ago Agilent Technologies introduced Retention Time Locking (RTL) for gas chromatography (GC) and GC with mass spectral detection (GC/MS). RTL software makes it possible to reproduce retention times from run-to-run on any Agilent GC or GC/MS, in any laboratory in the world, so long as the same nominal method and GC column are used (1). Since any laboratory can reproduce retention times generated in another, it is possible to create mass spectral libraries that contain locked retention times. By locking their method to the published database, users can screen GC/MS files for all of the library's compounds. "Hits" are required to have the correct retention time as well as the correct spectrum, which eliminates many false positives and gives more confidence in compound identifications (2).

More recently, Agilent introduced Deconvolution Reporting Software (DRS) that incorporates mass spectral deconvolution with conventional library searching and quantification. DRS results from a marriage of three different GC/MS software packages:

1) The Agilent GC/MS ChemStation,
2) The National Institute of Standards and Technology (NIST) Mass Spectral Search Program with the NIST '05 MS Library, and
3) The Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, also from NIST.

The original DRS software was intended to be a comprehensive solution for pesticide analysis and, therefore, included the mass spectra (in AMDIS format) and locked retention times for 567 pesticides and suspected endocrine disrupters (3).

Recently, Agilent introduced an updated and greatly expanded Pesticide and Endocrine Disruptor Database (part number G1672AA) that now contains 926 entries. This represents the addition of 359 new compounds to the original library. At the same time, Agilent introduced a new version of the DRS software (part number G1716AA version A.03.00) that can be used with any Agilent-provided or user-developed DRS library.

Pesticide and Endocrine Disruptor Database Contents

The G1672AA Pesticide and Endocrine Disruptor Database contains virtually all GC-able pesticides, including those introduced very recently. In addition, the database includes numerous metabolites, more endocrine disruptors, important PCBs and PAHs, certain dyes (for example, Sudan Red), synthetic musk compounds, and several organophosphorus fire retardants.

This new database includes:

- A conventional mass spectral library for use with Agilent GC/MS ChemStations
- A screener database for use with Agilent's powerful screener software that is integrated into the GC/MS ChemStation
- Locked Retention Times for all 926 compounds that any Agilent 5975 or 5973 GC/MS user can reproduce in their laboratory
- Files for use with Agilent's G1716AA (A.03.00) Deconvolution Reporting Software
- An e-method that can be loaded into Agilent's G1701DA (version D.02.00 SP1 or higher) with instrument parameters for acquiring GC/MS files and analyzing the data with DRS. These parameters are listed in Table 1.
- Example files
- Application notes

On November 29, 2005, the Japanese Government published a "Positive List" system for the regulation of pesticides, feed additives, and veterinary drugs. Maximum Residue Limits (MRL) have been set for 758 chemicals while 65 others have been exempted from regulation. Fifteen substances must have no detectable residues. Other agricultural chemicals not mentioned have a uniform MRL of 0.01 ppm (4). This new regulation is scheduled to take effect on May 29, 2006.

Of the pesticides in the Japanese Positive List, 265 are to be analyzed by GC/MS. The new G1672AA Pesticide library contains mass spectra and locked retention times for all of these compounds. Thus, a laboratory could screen for all 265 "positive list" compounds and several hundred more pesticides in just 1–3 minutes after the GC/MS run.

Experimental

Table 1 lists the instrumentation, software, and analytical parameters used by Agilent for pesticide analysis. Depending upon the desired injection volume, a PTV inlet or split/splitless inlet can be used.

Table 1. Instrumentation and Conditions of Analysis

Gas Chromatograph	**Agilent 6890N**
Automatic Sampler	Agilent 7683 Injector and AutoSampler
Inlet	Agilent PTV operated in the solvent vent mode or Split/Splitless
Column	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MSi (part number 19091S-433i)
Carrier gas	Helium in the constant pressure mode
Retention time locking	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 17.1 psi)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C /min to 200 °C (0 min), 8 °C /min to 280 °C (10–15 min)
PTV inlet parameters	Temp program: 40 °C (0.25 min), 1600 °C/min to 250 °C (2 min); Vent time: 0.2 min; Vent flow: 200 mL/min; Vent pressure: 0.0 psi; Purge flow: 60.0 mL/min; Purge time: 2.00 min
Injection volume	15 µL (using a 50-µL syringe)
Mass Selective Detector	**Agilent 5975 inert**
Tune file	Atune.u
Mode	Scan (or SIM with SIM DRS library)
Scan range	50–550 u
Source, quad, transfer line temperatures	230, 150, and 280 °C, respectively
Solvent delay	4.00 min
Multiplier voltage	Autotune voltage
Software	
GC/MSD ChemStation	Agilent part number G1701DA (version D02.00 sp1 or higher)
Deconvolution Reporting Software	Agilent part number G1716AA (version A.03.00) Deconvolution Reporting Software
Library searching software	NIST MS Search (version 2.0d or greater) (comes with NIST '05 mass spectral library – Agilent part number G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.62 or greater; comes with NIST '05 mass spectral library – Agilent part number G1033A)
MS Libraries	NIST '05 mass spectral library (Agilent part number G1033A) Agilent RTL Pesticide and Endocrine Disruptor Libraries in Agilent and NIST formats (part number G1672AA)

Results and Discussion

DRS, which has been described in preceding papers (3,5,6), can be summarized as follows:

Three separate, but complimentary, data analysis steps are combined into the DRS. First, the GC/MS ChemStation software performs a normal quantitative analysis for target pesticides using a target ion and up to three qualifiers. An amount is reported for all calibrated compounds that are detected. For other compounds in the database, an estimate of their concentration can be reported based upon an average pesticide response factor that is supplied with the DRS software. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the Agilent RTL Pesticide Library using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's retention time to fall within a user-specified time window. Because RTL is used to reproduce the RTL database retention times with high precision, this window can be quite small – typically 10–20 seconds. Finally, the deconvoluted spectra for all of the targets found by AMDIS are searched against the 147,000-compound NIST mass spectral library for confirmation; for this step, there is no retention time requirement.

This approach was rapidly adopted by many laboratories because of its ability to identify pesticides in complex chromatograms containing high levels of co-extracted interferences. Indeed, the solution proved to be so useful that users began to create their own DRS libraries (7). Therefore, the DRS was unbundled from the pesticide database so that it could be used with any agilent-provided or user-created database.

The original 567-compound RTL Pesticide Library (G1049A) included pesticides, a few metabolites, and most of the GC-amenable endocrine disruptors that were known at the time. The new version of the library includes many more pesticides, endocrine disruptors, and metabolites. This update also contains important compounds from other classes of contaminants that have been found in food and water supplies. Included are eighteen polychlorinated biphenyls (PCBs), four polybrominated biphenyls (PBBs), several polynuclear aromatic hydrocarbons (PAHs), several organophosphorus fire retardants, three important toxaphene congeners, and three Sudan dyes.

Advantages of Deconvolution

Figure 1 shows a screen from AMDIS that illustrates the power of this deconvolution software. The white trace in Figure 1A is the total ion chromatogram while the other three are extracted ions of a deconvoluted peak (a "component" in AMDIS terminology). Note that the TIC and extracted ions are not scaled to each other and this component is actually obscured by co-eluting compounds. Figure 1B juxtaposes the deconvoluted component spectrum (white) with the complete "undeconvoluted" spectrum (black). Clearly, this component is buried under co-eluting peaks that would ordinarily obscure the analyte. Figure 1C shows that the deconvoluted peak (white spectrum) is a good library match for norflurazon (black spectrum). The locked retention time for norflurazon in the RTL Pesticide Database is 26.933 min, which is just 2.3 seconds away from its observed RT in this chromatogram. Confidence in peak identifications is greatly enhanced by the combination of spectral deconvolution and locked retention time filtering.

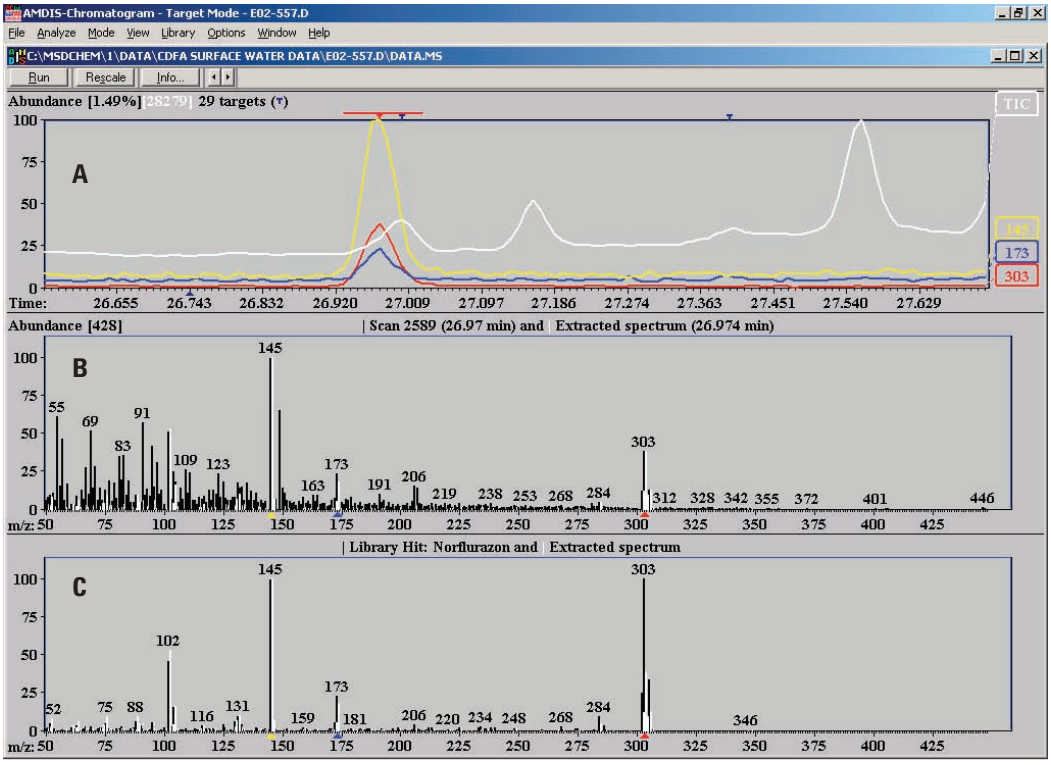

Figure 1. AMDIS screen showing the identification of norflurazon.
A) The total ion and extracted ion chromatograms where norflurazon elutes.
B) The deconvoluted component spectrum (white) juxtaposed with the spectrum at 26.972 min (black).
C) The deconvoluted component matched to the library spectrum of norflurazon.

Surface Water Analysis - Revisiting an Earlier Study

In an earlier study, a comparison was made between Agilent's DRS and conventional pesticide analysis (3). The California Department of Food and Agriculture (CDFA) provided data files for 17 surface water extracts that had been analyzed in their laboratory. Since the GC/MS chromatograms were locked to the Agilent pesticide method, it was possible to analyze these data files using DRS without having to re-run the samples. The original DRS analysis was made using the 567-compound RTL Pesticide Database. For comparison, these same data files were re-analyzed using the new 926-compound RTL Pesticide Database. The chromatogram (Figure 2) and the DRS report (Figure 3) from one of these samples are shown below.

Excluding phthalates, seven new compounds (shown with bold type in Figure 3) were identified using the 926-compound database: 4-chlorophenyl isocyanate (a phenylurea herbicide metabolite); 3,4-dichlorophenyl isocyanate (diuron metabolite); tris(2-chloroethyl) phosphate (a fire retardant); caffeine (a stimulant); Cyprodinil (a fungicide); desmethyl-norflurazon (a metabolite of norflurazon, an herbicide); and tris(2-butoxyethyl) phosphate (a fire retardant). Although caffeine is not generally considered to be dangerous, it is included in the database because it has been found frequently in sewage effluent and in numerous waterways together with a various pharmaceuticals and pesticides (8).

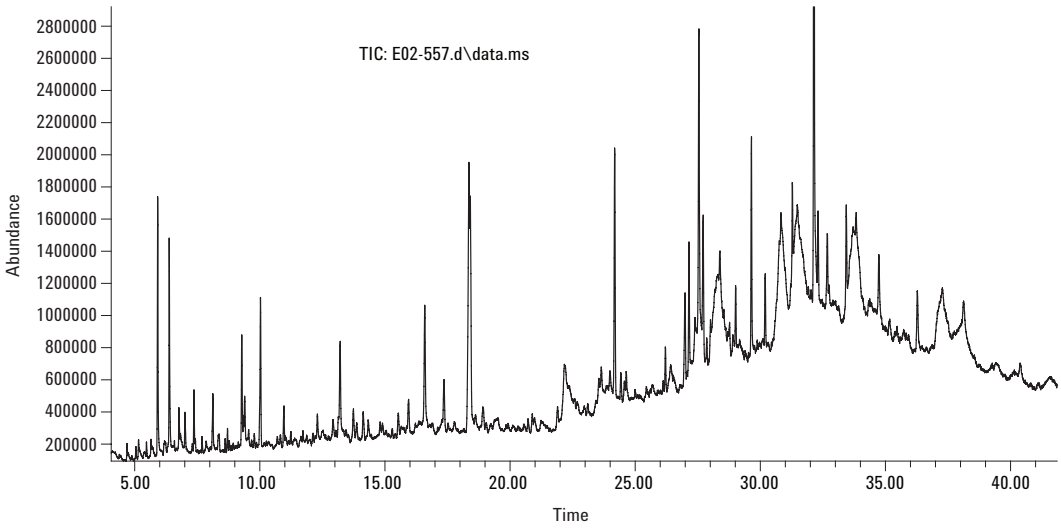

Figure 2. Chromatogram of a surface water extract that was analyzed by DRS using the new RTL Pesticide and Endocrine Disrupter Database. The results of this analysis are shown in Figure 3.

MSD Deconvolution Report
Sample Name: **E02-557**
Data File: **C:\MSDChem\1\DATA\CDFA surface water data\E02-557.d**
Date/Time: **11:24 AM Tuesday, Apr 4 2006**

The NIST library was searched for the components that were found in the AMDIS target library.

RT	Cas #	Compound name	Agilent ChemStation amount (ng)	AMDIS match	RT Diff (sec.)	NIST reverse match	Hit number
4.4689	106445	4-Methylphenol		62	3.2		
4.4689	0000	3-Carbobenzyloxy-4-ketoproline				48	1
4.8840	104121	**4-Chlorophenyl isocyanate**		84	–1.8	86	2
6.3879	102363	**Diuron Metabolite [3,4-Dichlorophenyl isocyanate]**		99	3.1	95	1
6.8357	759944	EPTC		84	2.0	85	1
7.6988	95761	3,4-Dichloroaniline		93	2.1	89	2
7.9342	131113	Dimethylphthalate		67	1.7	84	2
8.1112	25013165	Butylated hydroxyanisole		63	–7.7		
8.1112	0000	7-Methoxy-2,2,4,8-tetramethyltricyclo [5.3.1.0(4,11)]undecane				62	1
8.941	29878317	Tolyltriazole [1H-Benzotriazole, 4-meth-]	1.29				
9.7903	134623	N,N-Diethyl-m-toluamide		85	2.2	84	2
10.0019	84662	Diethyl phthalate		98	2.6	92	1
10.7109	119619	Benzophenone		86	2.6	88	2
10.9684	126738	Tributyl phosphate		96	3.0	90	1
11.6491	1582098	Trifluralin		83	0.7	74	1
12.9326	122349	Simazine		88	1.4	86	2
13.4309	115968	**Tris(2-chloroethyl) phosphate**		79	1.0	78	1
13.7478	1517222	Phenanthrene-d10		95	1.3	83	1
15.4048	58082	**Caffeine**		80	1.6	74	1
15.9474	84695	Diisobutyl phthalate		90	3.2	88	4
16.5988	5598130	Chlorpyrifos Methyl		97	0.4	90	1
17.3653	7287196	Prometryn		90	1.5	84	1
18.4213	84742	Di-n-butylphthalate		99	0.4	94	1
18.9214	51218452	Metolachlor		90	0.7	87	1
20.5633	121552612	**Cyprodinil**		69	–0.1		
20.5633	76470252	9,9-Dimethoxy-9-sila-9, 10-dihydroanthracene				70	1
26.4247	23576241	**Norflurazon, Desmethyl-**		87	–4.5	69	2
26.9700	27314132	Norflurazon		87	1.5	79	1
26.9992	85687	Butyl benzyl phthalate		94	–0.5	94	1
27.3984	51235042	Hexazinone		89	0.8	83	1
28.0127	78513	**Tris(2-butoxyethyl) phosphate**		75	3.3	83	1
29.6537	117817	Bis(2-ethylhexyl)phthalate		98	0.3	90	3
33.9298	84764	Di-n-nonyl phthalate		65	–1.9		
33.9298	0000	Phthalic acid, 3,4-dichlorophenyl propyl ester				71	1
13.739		Phenanthrene-d10	10				

Figure 3. **DRS report from the analysis of a surface water sample.** The compounds shown in bold type were found by the new RTL Pesticide Database but not the original one because these compounds were not included.

For this sample, the ChemStation identified only tolyltriazole at 8.941 min, but AMDIS did not confirm this assignment, nor could it be confirmed manually. Butylated hydroxyanisole was tentatively identified by AMDIS with a low match value, but the retention time is off by –7.7 seconds which is considerably more than most other hits. This compound is not in the NIST library so it could not be confirmed. The ChemStation method used for this analysis required that all three qualifier ions fall within ±20% (relative) which is a rigorous requirement for such a complex sample. This explains why so few compounds were found by the ChemStation.

Cyprodinil (20.563 min) was identified by AMDIS but the NIST library search failed to confirm its presence. The next line shows that the best NIST library match is an anthracene derivative that is nothing like cyprodinil. This result was obtained when AMDIS was configured to "use uncertain peaks" as shown in Figure 4. When this feature is turned off in DRS Compound Identification Configuration, the best NIST library hit for this spectrum is, indeed, cyprodinil. When a compound's identity is ambiguous, as with cyprodinil, it may be useful to perform the DRS search both ways and compare the results.

In the comparison described earlier (3), DRS was able to identify all 37 pesticides found by the CDFA chemist. However, DRS completed the task for all 17 samples in about 20 minutes compared to ~8 hours for the manual procedure (Table 2). Moreover, DRS identified one false positive in the CDFA report and found 34 additional pesticides and related compounds.

Using the new 926-compound Database, it took 32 minutes to analyze all of the samples and DRS was able to find an additional 99 pesticides, metabolites, fire retardants, and related compounds (Table 2).

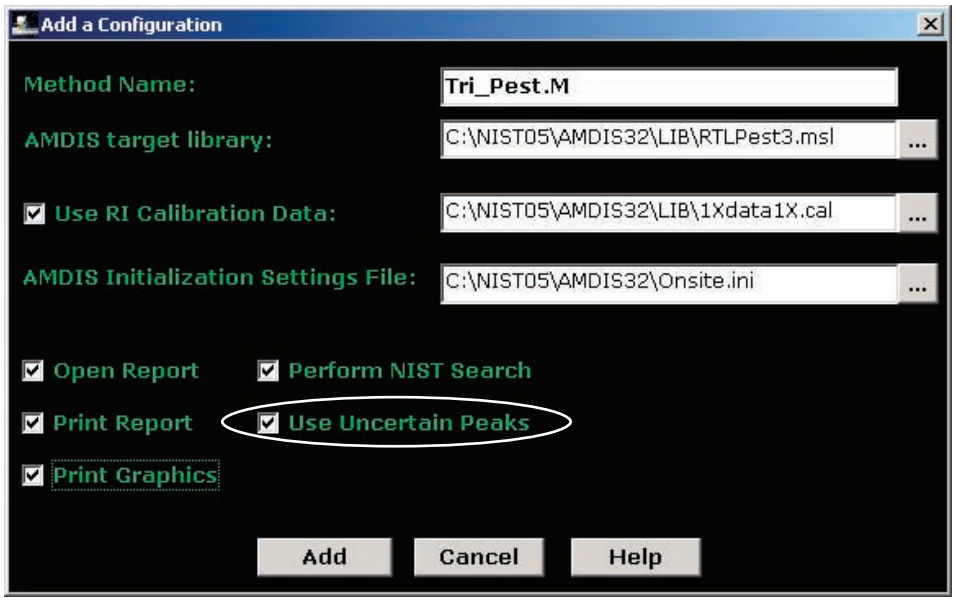

Figure 4. DRS configuration screen for the method called Tri_Pest. When the box labeled "Use Uncertain Peaks" is checked, AMDIS will use uncertain peaks for library searches. When unchecked, AMDIS ignores uncertain mass spectral peaks. Sometimes, this can affect the quality of a library match.

Table 2. Comparison of the Results Obtained by Screening 17 Surface Water Extracts Using Traditional Methods (CDFA) and Using DRS With Two Different Databases – the G1049A With 567 Compounds and the G1672AA With 926 Entries

	CDFA	Agilent DRS (Original G1049A database)	Agilent DRS (G1672 AA database)
Targets found (not counting ISTD)	37	Same 37 +34 more	Same 37 +99 more
False positives	1	0	0
Processing time	~8 hrs (ChemStation only)	20 minutes	32 min

Handling Stereoisomers

Many pesticides have multiple stereoisomers with virtually identical mass spectra. For example, cyfluthrin has four diastereomers arising from its three chiral centers. It is very difficult and sometimes impossible to determine the elution order of these isomers and most analysts report them as a sum of the isomer amounts. Agilent's G1049A RTL Pesticide database arbitrarily assigned each isomer a Roman numeral with I for the earliest eluting isomer, II for the next, and so on. The same Chemical Abstracts Service number (CAS #) was assigned to all of the isomers. Generally, it was a CAS # for the compound with "unstated stereochemistry." This caused some incompatibility with AMDIS as explained below.

AMDIS software differentiates among compounds using a "chemical identification number." The easiest and most consistent approach is to use each compound's CAS #. The default setting for AMDIS is to allow each CAS # to be used only once when analyzing a GC/MS data file. While this seems logical, it requires that each database entry have a different CAS #. It is possible to allow multiple hits per compound by checking the box in AMDIS found in the drop down menu under Analyze/Settings/Identif. However, this allows multiple peaks to be assigned the same compound name.

In the new RTL Pesticide Database (G1672AA), the Roman numeral designations remain and the first isomer in the series is given its genuine CAS #. Subsequent isomers in the series are given unique, but fictitious "CAS #s" generated by Agilent. The compound's real CAS # appears in braces after the compound name. For example, the cyfluthrin isomers are entered into the database as shown in Table 3.

Table 3. Method for Listing Compounds with Multiple Stereoisomers in the New G1672AA RTL Pesticide Database

RT	Compound name*		CAS #**
32.218	Cyfluthrin I		68359-37-5
32.359	Cyfluthrin II	{CAS # 68359-37-5}	999028-03-4
32.477	Cyfluthrin III	{CAS # 68359-37-5}	999029-03-7
32.536	Cyfluthrin IV	{CAS # 68359-37-5}	999030-03-4

* In a series, the earliest eluting isomer is identified with "I" and is assigned its legitimate CAS #. Subsequent isomers are assigned unique, but fictitious CAS #s (see footnote **). Their actual CAS # is put in braces behind the compound name.

** Cyfluthrin I has been given it's genuine CAS #. Cyfluthrin II-IV have been given unique numbers that can be distinguished from actual CAS numbers because they all have six digits before the first hyphen (9 total) and all begin with the series 999.

Figure 5 shows how permethrin was identified in a spinach sample using both databases with AMDIS configured to allow one hit per compound. Using the older 567-compound database (G1049A) only one permethrin isomer was identified because its CAS # could be used only once. With the new format used in the 926-compound RTL Pesticide Database (G1672AA), both isomers of permethrin were identified. Not surprisingly, the NIST library search found no hits with the same fictitious CAS # assigned to permethrin II. So, the software printed the best match on the following line. This compound, a cyclopropanecarboxylic acid derivative, is a permethrin isomer.

So long as the NIST library search is turned on in DRS, it will always print another line after reporting a compound with a fictitious CAS #. Note that these fictitious CAS #s always contain 9 digits and begin with 999.

A)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.6158	52645531	Permethrin II		88	3.9	91	3

B)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.4127	52645531	Permethrin I		78	2.6	81	3
31.6088	999046036	Permethrin II {CAS # 52645-53-1}		65	3.5		
31.6088	51877748	Cyclopropanecarboxylic acid, 3-(2,2-dichlorovinyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, (1R-trans)-				95	1

Figure 5. A) A single isomer of permethrin was identified by DRS using the G1049A 567-compound database when AMDIS was not allowed to use multiple hits per compound.
B) Two permethrin isomers are identified by DRS with the G1672AA 926-compound database under the same circumstances.

Conclusions

The new G1672AA RTL Pesticide and Endocrine Disruptor library contains substantially more target analytes than its predecessor. With the addition of 359 new compounds, it is the most comprehensive library of its type available today. Many new pesticides, metabolites, and endocrine disruptors were added along with important PCBs, PBBs, PAHs, synthetic musk compounds, Sudan dyes, and organophosphorus fire retardants. The database contains all of the analytes specified for GC/MS analysis in the new Japanese "Positive List" regulations.

When combined with the complete DRS solution, one can screen GC/MS data files for all 926 compounds in about two minutes per sample. This is the fastest, most comprehensive, most accurate, and least tedious method for screening food and environmental samples for these compounds.

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Appendix A

Lists of Compounds in Databases

1,2,4-Trichlorobenzene
1,2-Dibromo-3-chloropropane
1,3,5-Tribromobenzene
1,3-Dichlorbenzene
17a-Ethynylestradiol
1-naphthalenol
2-(1-naphthyl)acetamide
2-(2-Butoxyethoxy)ethyl thiocyanate
2-(Octylthio)ethanol
2,3,4,5-Tertrachloronitrobenzene
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
2,3,5,6-Tetrachlorophenol
2,3,5,6-Tetrachloro-p-terphenyl
2,3,5-Trichlorophenol
2,3,5-Trimethacarb
2,3,6-Trichloroanisole
2,3,7,8-Tetrachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,4,5,6-Tetrachloro-m-xylene
2,4,5-T methyl ester
2,4,5-Trichloroaniline
2,4,5-Trichlorophenol
2,4,5-Trichloro-p-terphenyl
2,4,5-Trimethylaniline
2,4,6-Tribromoanisole
2,4,6-Tribromophenol
2,4,6-Trichloroanisole
2,4,6-Trichlorophenol
2,4-D methyl ester
2,4-D sec-butyl ester
2,4-DB methyl ester
2,4'-Dichlorobenzophenone (2,4'-Dicofol decomposition product)
2,4-Dichlorophenol
2,4-Dichlorophenyl benzenesulfonate
2,4-Dimethylaniline
2,4-Dimethylphenol
2,6-Dichlorobenzamide
2,6-Dichlorobenzonitrile
2,6-Dimethylaniline
2-[3-Chlorophenoxy]propionamide
2-Chlorophenol
2-Ethyl-1,3-hexanediol
2-ethyl-6-methylaniline
2-Hydroxyestradiol
2-Methyl-4,6-dinitrophenol
2-Methylphenol
2-Nitrophenol
2-Phenoxypropionic acid
3,4,5-Trimethacarb
3,4-Dichloroaniline
3,5-Dichloroaniline
3-Aminophenol
3-Chloro-4-fluoroaniline
3-Chloro-4-methoxyaniline
3-Chloroaniline
3-Hydroxycarbofuran
3-Indolylacetonitrile
3-Trifluormethylaniline
4,4'-Dichlorobenzophenone
4,4'-Oxydianiline
4,6-Dinitro-o-cresol (DNOC)
4-Aminodiphenyl
4-Bromoaniline
4-Chloro-2-methylaniline
4-Chloro-3-methylphenol
4-Chloroaniline
4-Chlorophenyl isocyanate
4-Isopropylaniline
4-Methylphenol
4-Nitrophenol
4-Nonylphenol
5,7-Dihydroxy-4'-methoxyisoflavone
9,10-Anthraquinone
Acenaphthene
Acenaphthylene
Acephate
Acequinocyl
acetamiprid
Acetochlor
Acifluorfen methyl ester
Aclonifen
Acrinathrin
Alachlor
Aldrin
Allidochlor
Ametryn
Amidithion
Aminocarb
Amitraz
Amitraz metabolite [Methanimidamide, N-(2,4-dimethylphenyl)-N'-methyl-]
Ancymidol
Anilazine
Aniline
Anilofos
Anthracene
Aramite I
Aramite II {CAS # 140-57-8}
Atraton
Atrazine
Atrazine-desethyl
Azaconazole
Azamethiphos
Azibenzolar-S-methyl
Azinphos-ethyl
Azinphos-methyl
Aziprotryn metabolite [2-Amino-4-isopropylamino-6-methylthio-1,3,5-triazine]
Aziprotryne
Azobenzene
Azoxybenzene
Azoxystrobin
Barban
Beflubutamid
Benalaxyl
Benazolin-ethyl
Bendiocarb
Benfluralin

Benfuracarb
Benfuresate
Benodanil
Benoxacor
Bentazone
Bentazone methyl derivative
Benthiocarb
Benzene, 1,3-*bis*(bromomethyl)-
Benzenesulfonamide
Benzidine
Benzo(a)anthracene
Benzo(a)pyrene
Benzo[b]fluoranthene
Benzo[g,h,i]perylene
Benzo[k]fluoranthene
Benzophenone
Benzoximate metabolite
Benzoylprop ethyl
Benzyl benzoate
b-Estradiol
BHC alpha isomer
BHC beta isomer
BHC delta isomer
BHC epsilon isomer
Bifenazate metabolite
(5-Phenyl-o-anisidine)
Bifenox
Bifenthrin
Binapacryl
Bioallethrin
Bioallethrin S-cyclopentenyl isomer
Bioresmethrin
Biphenyl
Bis(2,3,3,3-tetrachloropropyl) ether
Bis(2-butoxyethyl) phthalate
Bis(2-ethylhexyl)phthalate
Bisphenol A
Bitertanol I
Bitertanol II {CAS # 55179-31-2}
Boscalid (Nicobifen)
Bromacil
Bromfenvinphos-(E)
Bromfenvinphos-(Z)
Bromobutide
Bromocyclen
Bromophos
Bromophos-ethyl
Bromopropylate
Bromoxynil
Bromoxynil octanoic acid ester
Bromuconazole I
Bromuconazole II {CAS # 116255-48-2}
Bufencarb
Bupirimate
Buprofezin
Butachlor
Butafenacil
Butamifos
Butoxycarboxim
Butralin
Butyl benzyl phthalate
Butylate
Butylated hydroxyanisole
Cadusafos
Cafenstrole
Caffeine
Captafol
Captan
Carbaryl
Carbetamide
Carbofuran
Carbofuran-3-keto
Carbofuran-7-phenol
Carbophenothion
Carbosulfan
Carboxin
Carfentrazone-ethyl
Carpropamid
Carvone
Cashmeran
Cekafix
Celestolide
Chinomethionat
Chloramben methyl ester
Chloranocryl
Chlorbenside
Chlorbenside sulfone
Chlorbicyclen
Chlorbromuron
Chlorbufam
Chlordecone
Chlordene, *trans*-
Chlordimeform
Chlorethoxyfos
Chlorfenapyr
Chlorfenethol
Chlorfenprop-methyl
Chlorfenson
Chlorfenvinphos
Chlorfenvinphos, *cis*-
Chlorfenvinphos, *trans*-
Chlorflurecol-methyl ester
Chlormefos
Chlornitrofen
Chlorobenzilate
Chloroneb
Chloropropylate
Chlorothalonil
Chlorotoluron
Chlorpropham
Chlorpyrifos
Chlorpyrifos Methyl
Chlorthal-dimethyl
Chlorthiamid
Chlorthion
Chlorthiophos
Chlorthiophos sulfone
Chlorthiophos sulfoxide
Chlozolinate
Chrysene
Cinerin I
Cinerin II
Cinidon-ethyl
cis-Chlordane
Clodinafop-propargyl
Clomazone
Cloquintocet-mexyl
Coumaphos
Crimidine
Crotoxyphos
Crufomate
Cyanazine
Cyanofenphos
Cyanophos
Cyclafuramid
Cycloate
Cyclopentadecanone
Cycluron

Cyflufenamid
Cyfluthrin I
Cyfluthrin II {CAS # 68359-37-5}
Cyfluthrin III {CAS # 68359-37-5}
Cyfluthrin IV {CAS # 68359-37-5}
Cyhalofop-butyl
Cyhalothrin I (lambda)
Cyhalothrin (Gamma)
Cymiazole
Cymoxanil
Cypermethrin I
Cypermethrin II {CAS # 52315-07-8}
Cypermethrin III {CAS # 52315-07-8}
Cypermethrin IV {CAS # 52315-07-8}
Cyphenothrin *cis*-
Cyphenothrin *trans*- {CAS # 39515-40-7}
Cyprazine
Cyproconazole
Cyprodinil
Cyprofuram
Cyromazine
d-(*cis-trans*)-Phenothrin-I
d-(*cis-trans*)-Phenothrin-II {CAS # 260002-80-2}
Dazomet
DDMU [1-Chloro-2,2-*bis*(4'-chlorophenyl)]
Decachlorobiphenyl
Deltamethrin
Demephion
Demeton-S
Demeton-S-methylsulfon
Desbromo-bromobutide
Desmedipham
Desmetryn
Dialifos
Di-allate I
Di-allate II {CAS # 2303-16-4}
Diamyl phthalate
Diazinon
Diazinon-oxon
Dibenz[a,h]anthracene
Dicamba
Dicamba methyl ester
Dicapthon
Dichlofenthion
Dichlofluanid
Dichlofluanid metabolite (DMSA)
Dichlone
Dichlormid
Dichlorophen
Dichlorprop
Dichlorprop methyl ester
Dichlorvos
Diclobutrazol
Diclocymet I
Diclocymet II {CAS # 139920-32-4}
Diclofop methyl
Dicloran
Dicrotophos
Dicyclohexyl phthalate
Dicyclopentadiene
Dieldrin
Diethatyl ethyl
Diethofencarb
Diethyl dithiobis(thionoformate) (EXD)
Diethyl phthalate
Diethylene glycol
Diethylstilbestrol
Difenoconazol I
Difenoconazol II {CAS # 119446-68-3}
Difenoxuron
Diflufenican
Diisobutyl phthalate
Dimefox
Dimepiperate
Dimethachlor
Dimethametryn
Dimethenamid
Dimethipin
Dimethoate
Dimethomorph-(E)
Dimethomorph-(Z) {CAS # 110488-70-5}
Dimethylphthalate
Dimethylvinphos(z)
Dimetilan
Dimoxystrobin
Di-n-butylphthalate
Di-n-hexyl phthalate
Diniconazole
Dinitramine
Di-n-nonyl phthalate
Dinobuton
Dinocap I
Dinocap II {CAS # 39300-45-3}
Dinocap III {CAS # 39300-45-3}
Dinocap IV {CAS # 39300-45-3}
Di-n-octyl phthalate
Dinoseb
Dinoseb acetate
Dinoseb methyl ether
Dinoterb
Dinoterb acetate
Di-n-propyl phthalate
Diofenolan I
Diofenolan II {CAS # 63837-33-2}
Dioxabenzofos
Dioxacarb
Dioxathion
Diphacinone
Diphenamid
Diphenyl phthalate
Diphenylamine
Dipropetryn
Dipropyl isocinchomeronate
Disulfoton
Disulfoton sulfone
Ditalimfos
Dithiopyr
Diuron
Diuron Metabolite [3,4-Dichlorophenyl isocyanate]
Dodemorph I
Dodemorph II {CAS # 1593-77-7}
Drazoxolon
Edifenphos
Empenthrin I
Empenthrin II {CAS # 54406-48-3}
Empenthrin III {CAS # 54406-48-3}
Empenthrin IV {CAS # 54406-48-3}
Empenthrin V {CAS # 54406-48-3}
Endosulfan (alpha isomer)
Endosulfan (beta isomer)
Endosulfan ether
Endosulfan lactone
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone

EPN
Epoxiconazole
EPTC
Erbon
Esfenvalerate
Esprocarb
Etaconazole
Ethalfluralin
Ethidimuron
Ethiofencarb
Ethiolate
Ethion
Ethofenprox
Ethofumesate
Ethofumesate, 2-Keto
Ethoprophos
Ethoxyfen-ethyl
Ethoxyquin
Ethylenethiourea
Etoxazole
Etridiazole
Etridiazole, deschloro- (5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole)
Etrimfos
Eugenol
Exaltolide [15-Pentadecanolide]
Famoxadon
Famphur
Fenamidone
Fenamiphos sulfoxide
Fenamiphos-sulfone
Fenarimol
Fenazaflor
Fenazaflor metabolite
Fenazaquin
Fenbuconazole
Fenchlorazole-ethyl
Fenchlorphos
Fenchlorphos-oxon
Fenclorim
Fenfuram
Fenhexamid
Fenitrothion
Fenitrothion-oxon
Fenobucarb
Fenoprop
Fenoprop methyl ester
Fenothiocarb
Fenoxanil
Fenoxaprop-ethyl
Fenoxycarb
Fenpiclonil
Fenpropathrin
Fenpropidin
Fenson
Fensulfothion
Fensulfothion-oxon
Fensulfothion-oxon -sulfone
fensulfothion-sulfone
Fenthion
Fenthion sulfoxide
Fenthion-sulfone
Fenuron
Fenvalerate I
Fenvalerate II {CAS # 51630-58-1}
Fepropimorph
Fipronil
Fipronil, desulfinyl-
Fipronil-sulfide
Fipronil-sulfone
Flamprop-isopropyl
Flamprop-methyl
Fluacrypyrim
Fluazifop-p-butyl
Fluazinam
Fluazolate
Flubenzimine
Fluchloralin
Flucythrinate I
Flucythrinate II {CAS # 70124-77-5}
Fludioxonil
Flufenacet
Flumetralin
Flumiclorac-pentyl
Flumioxazin
Fluometuron
Fluoranthene
Fluorene
Fluorodifen
Fluoroglycofen-ethyl
Fluoroimide
Fluotrimazole
Fluoxastrobin *cis*-
Fluquinconazole
Flurenol-butyl ester
Flurenol-methylester
Fluridone
Flurochloridone I
Flurochloridone II {CAS # 61213-25-0}
Flurochloridone, deschloro-
Fluroxypyr-1-methylheptyl ester
Flurprimidol
Flurtamone
Flusilazole
Fluthiacet-methyl
Flutolanil
Flutriafol
Fluvalinate-tau-I
Fluvalinate-tau-II {CAS # 102851-06-9}
Folpet
Fonofos
Formothion
Fosthiazate I
Fosthiazate II {CAS # 98886-44-3}
Fuberidazole
Furalaxyl
Furathiocarb
Furilazole
Furmecyclox
Halfenprox
Haloxyfop-methyl
Heptachlor
Heptachlor epoxide isomer A
Heptachlor exo-epoxide isomer B
Heptenophos
Hexabromobenzene
Hexachlorobenzene
Hexachlorophene
Hexaconazole
Hexazinone
Hexestrol
Hydroprene
Imazalil
Imazamethabenz-methyl I
Imazamethabenz-methyl II {CAS # 81405-85-8}
Imibenconazole
Imibenconazole-desbenzyl

Indeno[1,2,3-cd]pyrene

Indoxacarb and Dioxacarb decomposition product [Phenol, 2-(1,3-dioxolan-2-yl)-]

Ioxynil

Ioxynil octanoate

Ipconazole

Iprobenfos

Iprodione

Iprovalicarb I

Iprovalicarb II {CAS # 140923-25-7}

Irgarol

Isazophos

Isobenzan

Isobornyl thiocyanoacetate

Isocarbamide

Isocarbophos

Isodrin

Isofenphos

Isofenphos-oxon

Isomethiozin

Isoprocarb

Isopropalin

Isoprothiolane

Isoproturon

Isoxaben

Isoxadifen-ethyl

Isoxaflutole

Isoxathion

Jasmolin I

Jasmolin II

Jodfenphos

Kinoprene

Kresoxim-methyl

Lactofen

Lenacil

Leptophos

Leptophos oxon

Lindane

Linuron

Malathion

Malathion-o-analog

MCPA methyl ester

MCPA-butoxyethyl ester

MCPB methyl ester

m-Cresol

Mecarbam

Mecoprop methyl ester

Mefenacet

Mefenpyr-diethyl

Mefluidide

Menazon

Mepanipyrim

Mephosfolan

Mepronil

Metalaxyl

Metamitron

Metasystox thiol

Metazachlor

Metconazole I

Metconazole II {CAS # 125116-23-6}

Methabenzthiazuron [decomposition product]

Methacrifos

Methamidophos

Methfuroxam

Methidathion

Methiocarb

Methiocarb sulfone

Methiocarb sulfoxide

Methomyl

Methoprene I

Methoprene II {CAS # 40596-69-8}

Methoprotryne

Methoxychlor

Methoxychlor olefin

Methyl (2-naphthoxy)acetate

Methyl paraoxon

Methyl parathion

Methyl-1-naphthalene acetate

Methyldymron

Metobromuron

Metolachlor

Metolcarb

Metominostrobin (E)

Metominostrobin (Z) {CAS # 133408-50-1}

Metrafenone

Metribuzin

Mevinphos

Mirex

Molinate

Monalide

Monocrotophos

Monolinuron

Musk amberette

Musk Ketone

Musk Moskene

Musk Tibetene (Moschustibeten)

Musk xylene

Myclobutanil

N,N-Diethyl-m-toluamide

N-1-Naphthylacetamide

Naled

Naphthalene

Naphthalic anhydride

Naproanilide

Napropamide

Nicotine

Nitralin

Nitrapyrin

Nitrofen

Nitrothal-isopropyl

N-Methyl-N-1-naphthyl acetamide

Nonachlor, *cis*-

Nonachlor, *trans*-

Norflurazon

Norflurazon, desmethyl-

Nuarimol

o,p'-DDD

o,p'-DDE

o,p'-DDT

Octachlorostyrene

o-Dianisidine

o-Dichlorobenzene

Ofurace

Omethoate

o-Phenylphenol

Orbencarb

ortho-Aminoazotoluene

Oryzalin

Oxabetrinil

Oxadiazon

Oxadixyl

Oxamyl

Oxycarboxin

Oxychlordane

Oxydemeton-methyl

Oxyfluorfen

p,p'-DDD
p,p'-DDE
p,p'-DDM [*bis*(4-chlorophenyl)methane]
p,p'-DDT
p,p'-Dibromobenzophenone
p,p'-Dicofol
Paclobutrazol
Paraoxon
Parathion
PBB 52 Tetrabrombiphenyl
PBB 101
PBB 15
PBB 169 Hexabrombiphenyl
PCB 101
PCB 105
PCB 110
PCB 118
PCB 126
PCB 127
PCB 131
PCB 136
PCB 138
PCB 153
PCB 169
PCB 170
PCB 180
PCB 30
PCB 31
PCB 49
PCB 77
PCB 81
p-Dichlorobenzene
Pebulate
Penconazole
Pendimethalin
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol
Pentanochlor
Permethrin I
Permethrin II {CAS # 52645-53-1}
Perthane
Phantolide
Phenamiphos
Phenanthrene
Phenanthrene-d10
Phenkapton
Phenol
Phenothiazine
Phenothrin I
Phenothrin II
Phenoxyacetic acid
Phenthoate
Phorate
Phorate sulfone
Phorate sulfoxide
Phorate-oxon
Phosalone
Phosfolan
Phosmet
Phosphamidon I
Phosphamidon II {CAS # 13171-21-6}
Phthalide
Phthalimide
Picloram methyl ester
Picolinafen
Picoxystrobin
Pindone
Piperalin
Piperonyl butoxide
Piperophos
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Plifenat
p-Nitrotoluene
Potasan
Prallethrin, *cis*-
Prallethrin, *trans*- {CAS # 23031-36-9}
Pretilachlor
Probenazole
Prochloraz
Procymidone
Prodiamine
Profenofos
Profenofos metabolite (4-Bromo-2-chlorophenol)
Profluralin
Prohydrojasmon I
Prohydrojasmon II {CAS # 158474-72-7}
Promecarb
Promecarb artifact [5-isopropyl-3-methylphenol]
Prometon
Prometryn
Propachlor
Propamocarb
Propanil
Propaphos
Propargite
Propargite metabolite [Cyclohexanol, 2-(4-tert-butylphenoxy)]
Propazine
Propetamphos
Propham
Propiconazole-I
Propiconazole-II {CAS # 60207-90-1}
Propisochlor
Propoxur
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Prothiofos
Prothoate
Pyracarbolid
Pyraclofos
Pyraflufen-ethyl
Pyrazon
Pyrazophos
Pyrazoxyfen
Pyrene
Pyrethrin I
Pyrethrin II
Pyributicarb
Pyridaben
Pyridaphenthion
Pyridate
Pyridinitril
Pyrifenox I
Pyrifenox II {CAS # 88283-41-4}
Pyriftalid
Pyrimethanil
Pyrimidifen
Pyriminobac-methyl (E)
Pyriminobac-methyl (Z) {CAS # 136191-64-5}

Pyriproxyfen
Pyroquilon
Quinalphos
Quinoclamine
Quinoxyfen
Quintozene metabolite (pentachlorophenyl methyl sulfide)
Quizalofop-ethyl
Rabenzazole
Resmethrin
Resmethrine I
Resmethrine II {CAS # 10453-86-8}
Rotenone
S,S,S-Tributylphosphorotrithioate
Schradan
Sebuthylazine
Sebuthylazine-desethyl
Secbumeton
Silafluofen
Silthiopham
Simazine
Simeconazole
Simetryn
Spirodiclofen
Spiromesifen
Spiroxamine I
Spiroxamine II {CAS # 118134-30-8}
Spiroxamine metabolite (4-tert-butylcyclo-hexanone)
Sudan I
Sudan II
Sudan Red
Sulfallate
Sulfanilamide
Sulfentrazone
Sulfotep
Sulfur (S8)
Sulprofos
Swep
Tamoxifen
TCMTB
Tebuconazole
Tebufenpyrad
Tebupirimifos
Tebutam
Tebuthiuron
Tecnazene
Tefluthrin, *cis*-
Temephos
Terbacil
Terbucarb
Terbufos
Terbufos-oxon-sulfone
Terbufos-sulfone
Terbumeton
Terbuthylazine
Terbuthylazine-desethyl
Terbutryne
Tetrachlorvinphos
Tetraconazole
Tetradifon
Tetraethylpyrophosphate (TEPP)
Tetrahydrophthalimide, *cis*-1,2,3,6-
Tetramethrin I
Tetramethrin II {CAS # 7696-12-0}
Tetrapropyl thiodiphosphate
Tetrasul
Thenylchlor
Theobromine
Thiabendazole
Thiazopyr
Thifluzamide
Thiofanox
Thiometon
Thionazin
Thymol
Tiocarbazil I
Tiocarbazil II {CAS # 36756-79-3}
Tolclofos-methyl
Tolfenpyrad
Tolylfluanid
Tolylfluanid metabolite (DMST)
Tolyltriazole [1H-Benzotriazole, 4-methyl-]
Tolyltriazole [1H-Benzotriazole, 5-methyl-]
Tonalide
Toxaphene Parlar 26
Toxaphene Parlar 50
Toxaphene Parlar 62
trans-Chlordane
Transfluthrin
Traseolide
Triadimefon
Triadimenol
Tri-allate
Triamiphos
Triapenthenol
Triazamate
Triazophos
Tributyl phosphate
Tributyl phosphorotrithioite
Trichlamide
Trichlorfon
Trichloronate
Triclopyr methyl ester
Triclosan
Triclosan-methyl
Tricresylphosphate, *meta*-
Tricresylphosphate, *ortho*-
Tricresylphosphate, *para*
Tricyclazole
Tridemorph, 4-tridecyl-
Tridiphane
Trietazine
Triethylphosphate
Trifenmorph
Trifloxystrobin
Triflumizole
Trifluralin
Triphenyl phosphate
Tris(2-butoxyethyl) phosphate
Tris(2-chloroethyl) phosphate
Tris(2-ethylhexyl) posphate
Triticonazole
Tryclopyrbutoxyethyl
Tycor (SMY 1500)
Uniconizole-P
Vamidothion
Vernolate
Vinclozolin
XMC (3,4-Dimethylphenyl N-methylcarbama
XMC (3,5-Dimethylphenyl N-methylcarbama
Zoxamide
Zoxamide decomposition product

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Identifying Pesticides with Full Scan, SIM, µECD, and FPD from a Single Injection

Application

Food Safety, Environmental

Authors

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Abstract

In this application note, a gas chromatography/mass spectrometry (GC/MS) system capable of providing up to four signals from a single injection is described. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from GC detectors can be acquired together with the MS data from a single injection. This multi-signal configuration provides: full-scan data for library searching, selective ion monitoring (SIM) data for trace analysis, micro-electron capture detector and flame photometric detector data for excellent selectivity and sensitivity from complex matrices. A combination of element selective detectors, SIM/Scan, and deconvolution reporting software makes a very powerful pesticide analysis system. Examples for trace-level compound quantitation/confirmation or for screening are discussed.

Introduction

Many laboratories in the world are analyzing pesticide residue levels in both foods and the environment to protect human health. The process usually involves homogenizing the sample, extracting the pesticides, and analyzing the target compounds with a Gas Chromatograph (GC) or a Liquid Chromatograph (LC) depending on the nature of the compounds. For GC amenable compounds, the traditional detectors are NPD (Nitrogen Phosphorus Detector), µECD (micro-Electron Capture Detector), and FPD (Flame Photometric Detector) for their excellent sensitivity and selectivity. However, even with dual-column confirmation analysis, these GC detectors cannot be used to verify the identity of the compounds with high confidence.

Full scan mass spectral data and library searching are typically used for final compound verification. However, full-scan analysis has a worse (higher) detection limit (DL) compared to selective detectors on a GC. To improve the DL, the technique selective ion monitoring (SIM) is often used. With SIM, the MS monitors only a few characteristic ions for each target compound within the retention time (RT) range that the target elutes from the column. By monitoring only a few specific ions, the signal-to-noise ratio (S/N) improves significantly. The ions monitored are time programmed in groups corresponding to the RTs of the targets. SIM analyses with closely eluting targets require precise alignment of chromatographic RTs with the time programming of SIM groups. The Retention Time Locking (RTL) technique can be applied to eliminate the need to adjust SIM group time-windows after column maintenance or replacement.

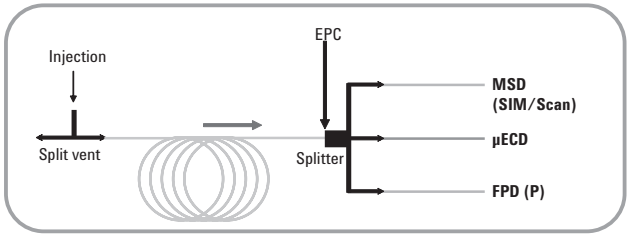
In this application note, a GC/MS system capable of providing up to four signals from a single injection is described. The benefits of the multi-signal detection include:

- Confirmatory information – Full-scan data for library search capability

- Maximum sensitivity – SIM data enables trace analysis
- Excellent selectivity – µECD and FPD detect trace-level hetero-compounds from complex matrices

Experimental

A recent technical note describes "Synchronous SIM/Scan", which takes advantages of the Performance Electronics in the 5975 inert MSD to get both SIM and full-scan signals in a single run without sacrificing performance [1]. The SIM method can be easily developed automatically using the ChemStation's AutoSIM tool [2]. By simply selecting a checkbox in the method, the SIM and full-scan data can be acquired together. The trade-off is giving up some cycles per second but gaining an additional signal (full-scan data or SIM data) for the whole analysis. With properly chosen acquisition parameters, for example, increasing the scan speed, the decrease of cycles per second is usually not significant and does not affect peak quantitation or the quality of results (for example, S/N).



At the end of the column, effluent flow is split three ways according to the length and diameter of the capillary tubing (restrictor) used.

Figure 1. A schematic of the multi-signal configuration. Note: the EPC flow adds to the column flow into the splitter.

Besides the SIM/Scan data, the ChemStation software can simultaneously acquire up to two additional GC detector signals, for example, FPD (in phosphorus- or sulfur- mode) and NPD (nitrogen-phosphorus detector) signals or both P- and S- signals from a dual-wavelength FPD (DFPD). See Figure 1.

Figure 1 is a schematic for multi-signal detection. At the end of the column, a three-way micro-fluidic splitter was used to split the column effluent to different detectors [3]. For this study, an FPD and a µECD were installed. Notice on the figure that an Auxiliary Electronic Pneumatics Control (Aux EPC) gas channel was connected to the splitter to maintain the pressure at the end of the column so that the split ratios/flows are kept constant throughout a run. Figure 2 shows a close-up view of the micro-fluidic splitter installed in the GC oven.

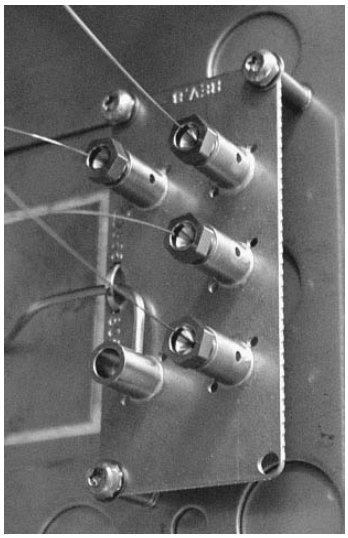


Figure 2. A close-up view of the micro-fluidic three-way splitter in the 6890 GC oven.

The size of the micro-fluidic plate is 1.25 inches (3.2 cm) wide and 2.5 inches (6.4 cm) tall. The device was designed to eliminate the common problems of large thermal mass, excess dead volume, and leaky connection due to oven temperature cycling etc. The splitter's flow paths and connection points are laid out and etched onto a thin, stainless steel plate using photolithography and chem-milling technologies. The plate is diffusion bonded, mounted with column connectors, and surface deactivated, resulting in an integrated and compact micro-fluidic splitter. Metal ferrules are used at the connectors that are leak-free after temperature cycling and will not absorb solvents or sample matrix, improving sensitivity for trace analysis applications.

Deactivated capillary tubing between the splitter and each detector was used as a flow restrictor. Aux EPC pressure and the restrictor dimensions were determined using a spreadsheet-like calculator program to achieve the proper split ratio among all detectors. The three-way splitter can easily turn into a two-way splitter when a connector is capped.

Other advantages of a splitter include back-flushing [3] and quick-swapping. The Aux EPC flow can be run-time programmed to a higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the column flow to reverse direction, back-flushing the less volatile materials out of the split vent of the inlet. The Aux EPC on the splitter also allows column changing and inlet maintenance without cooling and venting the MSD. The splitter's flow paths and connection points were designed in such a way

that when the column fitting is removed, the helium gas from the Aux EPC purges the fitting, preventing air from entering the splitter/MSD. See Table 1 for hardware details and settings.

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Splitless, 1.0 µL injected (7683 ALS)		
Inlet temp	280 °C		
Pressure	~27 psi (chlorpyrifos methyl RT locked to 16.596 min)		
Purge flow	50.0 mL/min		
Purge time	0.75 min		
Total flow	55.3 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Siltek Cyclosplitter, 4-mm id, Restek p/n 20706-214.1		
Oven			
Oven ramp	°C/min	Final (°C)	Hold (min)
Initial		70	2.00
Ramp 1	25	150	0.00
Ramp 2	3	200	0.00
Ramp 3	8	280	15
Total run time		46.87 min (last standard elutes around 35 min)	
Equilibration time		0.5 min	
Oven max temp		325 °C	
Column		Agilent Technologies HP 5-ms, p/n 19091S-433	
Length		30.0 m	
Diameter		0.25 mm	
Film thickness		0.25 µm	
Mode		Constant pressure	
Nominal initial flow		2.5 mL/min	
Outlet		Unspecified	
Outlet pressure		3.8 psi (Aux EPC pressure to splitter)	
Front detector (FPD)			
Phosphorus mode		**Sulfur mode**	
Hydrogen flow:	75.0 mL/min	Hydrogen flow:	50.0 mL/min
Oxidizer flow:	100.0 mL/min	Oxidizer flow:	60.0 mL/min
Temperature:	250 °C		
Oxidizer gas type:	Air		
Mode:	Constant makeup flow		
Makeup flow:	60.0 mL/min		
Makeup gas type:	Nitrogen		
Lit offset:	2.00		
Data rate:	5 Hz		

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters (Continued)

Back detector (μECD)	
Temperature:	300 °C
Mode:	Constant makeup flow
Makeup flow:	60.0 mL/min
Makeup gas type:	Nitrogen
Date rate:	5 Hz
Thermal AUX 2	
Use:	MSD Transfer line heater
Initial temp:	280 °C
Pressure AUX 5	
Gas type:	Helium
Initial pressure:	3.80 psi
Initial time:	0.00 min (this value will follow oven ramp)
MSD	Agilent Technologies 5975 inert MSD
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45 amu
High mass	555 amu
Threshold	100
Sampling	2
A/D Samples	4
Scans/s	2.89
Quad temp	150 °C
Source temp	230 °C
Three-way splitter	Agilent 6890N Option 890, when installed on the GC during factory assembly
Split ratio	10:10:1 MSD:FPD:μECD
MSD restrictor	1.444 m × 0.18-mm id Deactivated fused silica tubing
FPD restrictor	0.532 m × 0.18-mm id Deactivated fused silica tubing
μECD restrictor	0.507 m × 0.10-mm id Deactivated fused silica tubing
Flow to MSD (at 280 °C)	1.53 mL/min
Flow to FPD (at 280 °C)	1.53 mL/min
Flow to μECD (at 280 °C)	0.153 mL/min
Makeup flow (at 280 °C)	1.38 mL/min

Software Used in this Application Note

GC/MSD ChemStation	G1701DA
Deconvolution Reporting Software (DRS)	G1716AA
NIST Library	G1033A
AMDIS (included for free with the NIST library CD)	

Results and Discussion

Figure 3 shows four signals that were simultaneously acquired from a single injection of a pesticide mixture. Due to the high sensitivity of the µECD, the split ratios for the three detectors was set to MSD:FPD:µECD = 10:10:1. This split ratio distributes the sample of a 1-µL splitless injection of a 1-ppm (1000 pg/µL) sample to the different detectors as labeled in Figure 3.

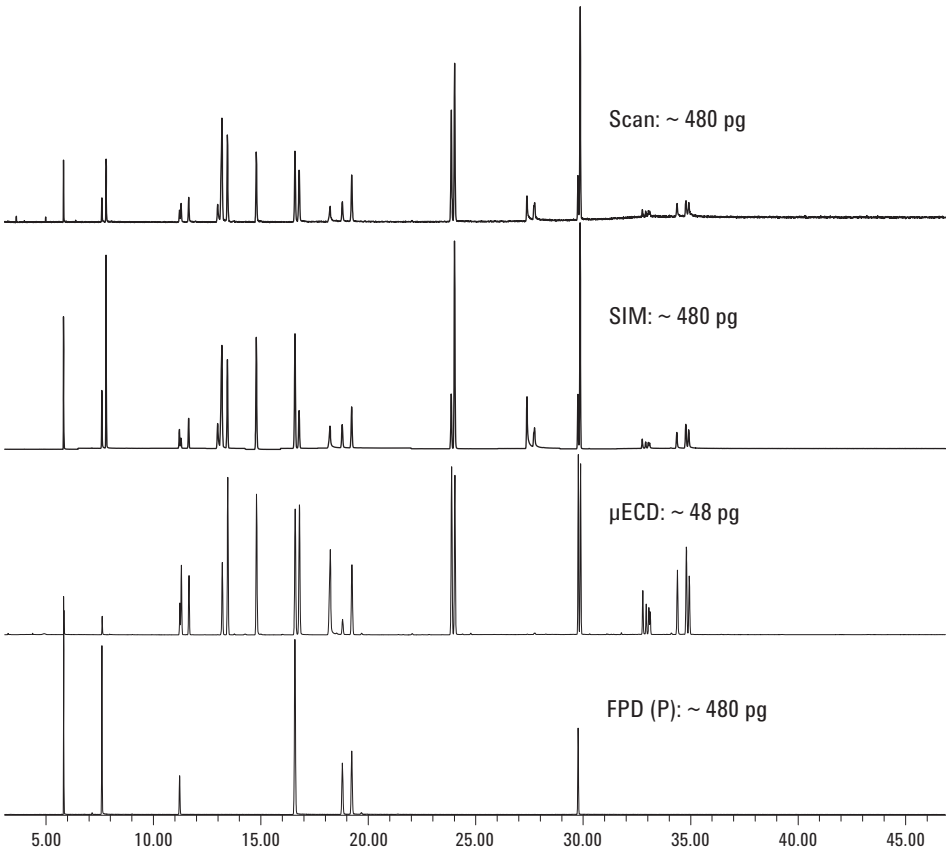


Figure 3. Signals acquired simultaneously from a 1-µL splitless injection of 1-ppm standard. The split ratios were MSD:FPD:µECD = 10:10:1.

Figure 4 shows the signals when the pesticide standard was diluted 100-fold in a produce matrix. The total ion chromatogram (TIC) from full scan was not shown due to the lack of sensitivity. The FPD(P) and µECD were able to detect all the pesticides spiked in this extract. For trace-level target compound analysis, the SIM signal can be used for quantitation and the GC signals used for further confirmation.

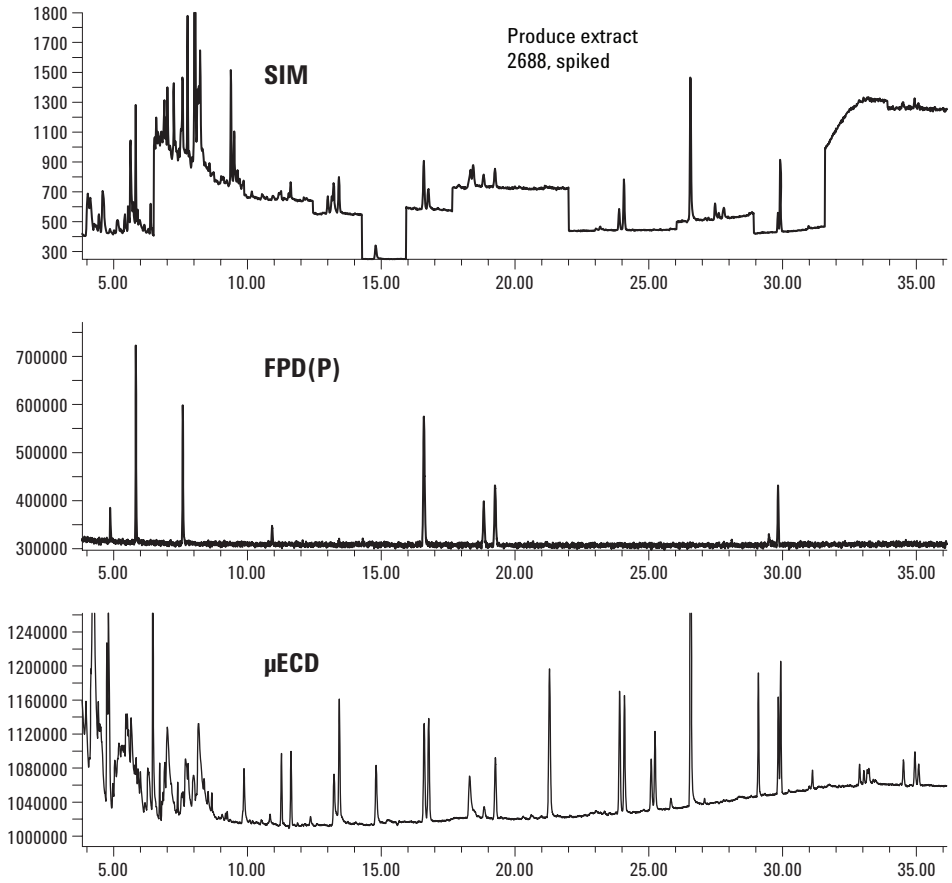


Figure 4. Data of a produce extract spiked at 10 ppb. FPD and µECD were able to detect the respective standards spiked into the extract.

Another application for this multi-signal system is for screening. In screening, no target list is available for the analysis; therefore, SIM acquisition or MS/MS is not possible. Figure 5 shows three signals (no SIM) from a produce extract.

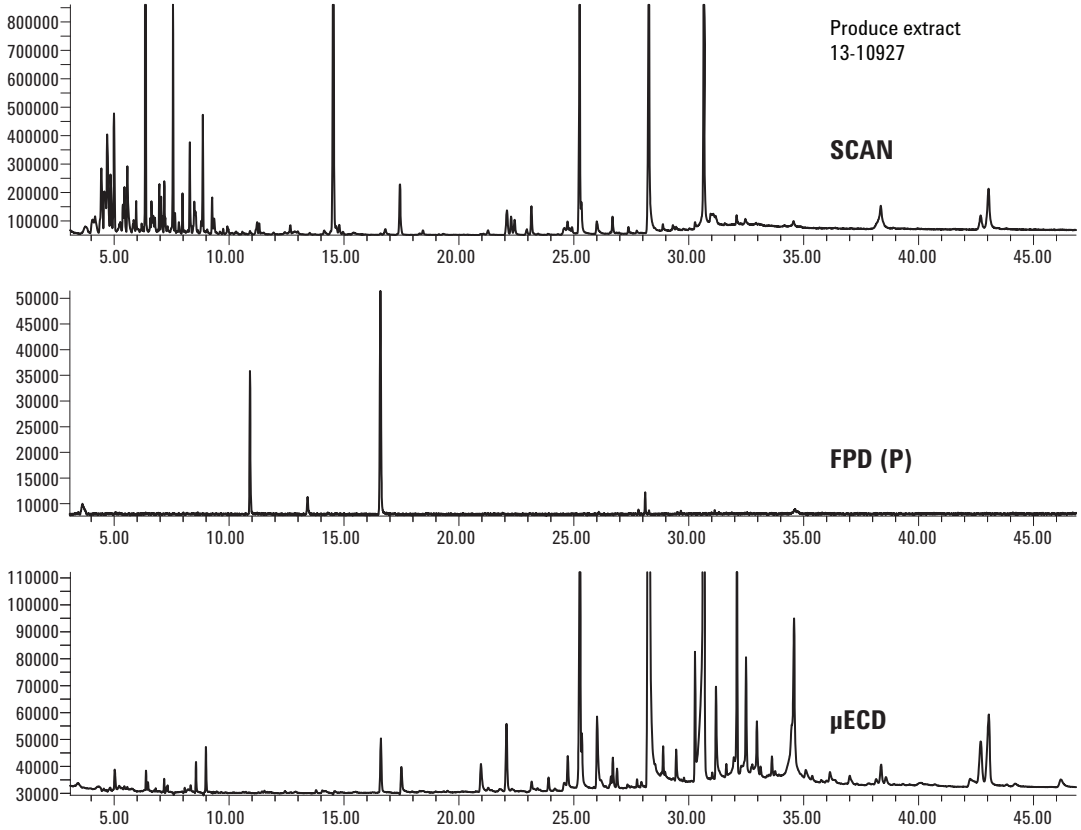
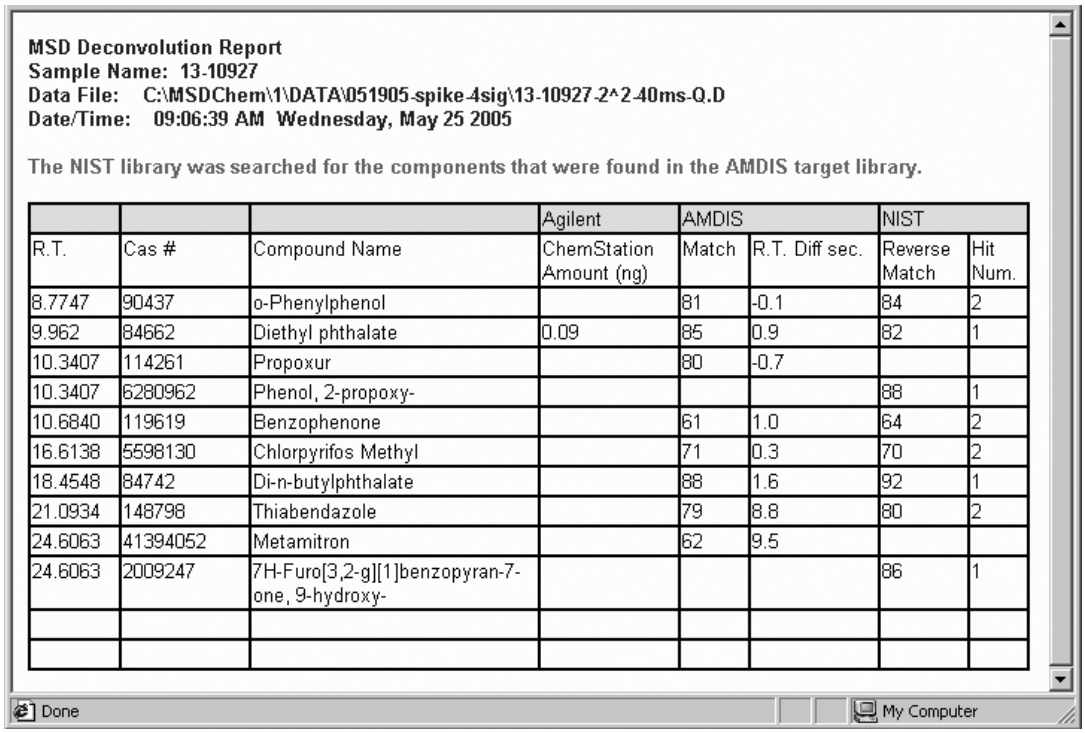


Figure 5. Full-scan, FPD(P), and µECD data for extract 13-10927.

The Deconvolution Reporting Software (DRS) [3, 4] found several pesticides in the TIC as shown in Figure 6.



MSD Deconvolution Report
Sample Name: 13-10927
Data File: C:\MSDChem\1\DATA\051905-spike-4sig\13-10927-2^2-40ms-Q.D
Date/Time: 09:06:39 AM Wednesday, May 25 2005

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
8.7747	90437	o-Phenylphenol		81	-0.1	84	2
9.962	84662	Diethyl phthalate	0.09	85	0.9	82	1
10.3407	114261	Propoxur		80	-0.7		
10.3407	6280962	Phenol, 2-propoxy-				88	1
10.6840	119619	Benzophenone		61	1.0	64	2
16.6138	5598130	Chlorpyrifos Methyl		71	0.3	70	2
18.4548	84742	Di-n-butylphthalate		88	1.6	92	1
21.0934	148798	Thiabendazole		79	8.8	80	2
24.6063	41394052	Metamitron		62	9.5		
24.6063	2009247	7H-Furo[3,2-g][1]benzopyran-7-one, 9-hydroxy-				86	1

Done My Computer

Figure 6. Report for extract 13-10927 generated from DRS.

The possible pesticides in the sample were benzophenone, chlorpyrifos methyl, and thiabendazole. Propoxur and metamitron were not confirmed by both AMDIS and NIST; therefore, they were most likely false positives.

Due to the complexity of the sample matrix and other interferences, it is sometimes difficult to get a high library match factor from peaks in the TIC, even after background subtraction. Therefore, element selective detectors would be very useful in providing the supporting information for compound confirmation. The multi-signal system was retention time locked, therefore, from the RT and the aligned peaks from the FPD(P) and the µECD responses, chlorpyrifos methyl ($C_7H_7Cl_3NO_3PS$) was confirmed.

It usually takes less than 3 minutes to turn off the FPD photomultiplier, swap the P-filter with the S-filter, and turn the photomultiplier back on. After the swap, adjust the detector gas flows to optimize the response in either P- or S- mode. A new injection of the same extract was made in FPD(S) mode. The FPD(S) result is shown with previously acquired signals in Figure 7. Two major peaks were seen on the FPD(S) chromatogram. From the peak RTs, they supported the presence of chlorpyrifos methyl and thiabendazole ($C_{10}H_7N_3S$) respectively. Note that the full-scan TIC barely showed a peak for either compound, which made it impossible for traditional data analysis to identify both compounds. The FPD(S) mode is very selective, but it is not as sensitive as the FPD(P) mode. Although the µECD is very sensitive, it is not as selective as the FPD. A combination of GC detectors, SIM/Scan, and DRS makes a very powerful pesticide analysis system.

Conclusion

The Synchronous SIM/Scan provides users with library searchable full-scan spectra as well as trace level SIM data in a single analysis. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from element selective detectors can be acquired together with the MS data from a single injection. This configuration makes it very attractive for the analysis of trace-level pesticide residues in foods or environmental samples.

This multi-signal configuration provides: full-scan data for library searching, SIM data for trace analysis, µECD and FPD data for excellent selectivity and sensitivity from complex matrices. In this application note, examples of µECD signal and FPD signal (P- or S- mode) were acquired together with the SIM/Scan data from a single injection for trace-level compound quantitation/confirmation, or for screening.

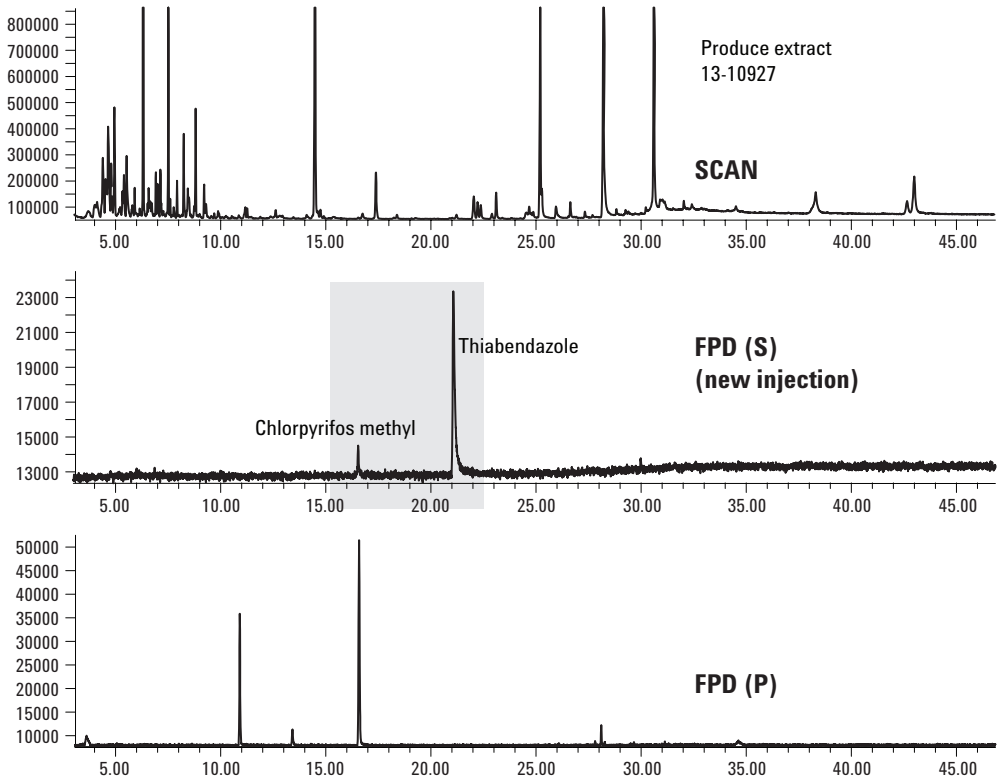


Figure 7. Full-scan, FPD(S), and FPD(P) data for extract 13-10927.

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Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software

Application

Food Safety

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Introduction

According to The Pesticide Manual, more than 700 pesticides are currently approved for use around the world [1]. About 600 more were used in the past, but are either banned or no longer marketed. In spite of their discontinuance, some of these still persist in the environment where they may bioaccumulate in the flora and fauna. Many pesticides or their degradation products can be found at trace levels in food and beverages; in soil, water, and air; in aquatic and terrestrial flora and fauna; and in human blood, adipose tissue, and breast milk. The World Health Organization has classified pesticides into five groups based upon their acute toxicity to humans [2]. The categories range from "Acutely Hazardous" to those that are "Unlikely to Present Acute Hazard in Normal Use." Certain pesticides are classified as persistent organic pollutants (POPs), carcinogens, teratogens, or endocrine disrupters. It is now common to analyze for pesticides in food and environmental samples to track their distribution in the environment and to ensure a safe food supply.

Current analytical methods target only a subset of the possible compounds. Whether for food or environmental samples, analyses are often complicated by the presence of co-extracted natural products. Food or tissue extracts can be exceedingly complex matrices that require several stages of sample cleanup prior to analysis [3]. Even then, it can be difficult to detect trace levels of contaminants in the presence of the remaining matrix.

For efficiency, multiresidue methods (MRMs) must be used to analyze for most pesticides. Traditionally, these methods have relied upon gas chromatography (GC) with a constellation of element-selective detectors to locate pesticides in the midst of a variable matrix [4, 5, 6]. GC with mass spectral detection (GC/MS) has been widely used for confirmation of hits. Liquid chromatography (LC) has been used for those compounds that are not amenable to GC [2]. Today, more and more pesticide laboratories are relying upon LC with mass spectral detection (LC/MS) and GC/MS as their primary analytical tools [7, 8]. Still, most MRMs are target compound methods that look for a small subset of the possible pesticides. Any compound not on the target list is likely to be missed by these MRMs.

Using the techniques of retention time locking (RTL) [9, 10, 11] and spectral deconvolution [12], a method has been developed to screen for 567 pesticides and suspected endocrine disrupters in a single GC/MS analysis. Spectral deconvolution

helps to identify pesticides even when they are buried under co-eluting matrix compounds. RTL helps to eliminate false positives and gives greater confidence in the results. Users can easily add compounds to the method if they wish.

Experimental

Table 1 lists the instrumentation, software, and analytical parameters used by Agilent for pesticide analysis. Depending upon the desired injection volume, a PTV inlet or split/splitless inlet can be used.

Samples

Vegetable extracts were obtained from Dr. Mark Lee and Stephen Siegel at The California Department of Food and Agriculture (CDFA; Sacramento, CA USA) and from Dr. J.G.J. Mol at TNO Nutrition and Food Research (Zeist, The Netherlands). Seventeen data files from the GC/MS analysis of surface water samples were also contributed by CDFA and were processed in this laboratory using the Deconvolution Reporting Software (DRS). GC/MS data files (locked to the Agilent Pesticide Method) for 17 crop extracts were supplied by NRM Laboratories, Berkshire, UK.

Table 1. Instrumentation and Conditions of Analysis

Gas chromatograph	**Agilent 6890N**
Automatic sampler	Agilent 7683
Inlet	Agilent PTV operated in the solvent vent mode
Column	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MS (p/n 19091S-433)
Carrier gas	Helium in the constant pressure mode
RTL	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 17.1 psi)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C /min to 200 °C (0 min), 8 °C /min to 280 °C (10–15 min)
PTV inlet parameters	Temp program: 40 °C (0.25 min), 1600 °C/min to 250 °C (2 min); Vent time: 0.2 min; Vent flow: 200 mL/min; Vent pressure: 0.0 psi; Purge flow: 60.0 mL/min; Purge time: 2.00 min
Injection volume	15 µL (using a 50-µL syringe)
Mass Selective Detector (MSD)	**Agilent 5973 inert**
Scan range	50–550 amu
Source, quad, transfer line temperatures	230, 150, and 280 °C, respectively
Solvent delay	4.00 min
Multiplier voltage	Autotune voltage
Software	
GC/MSD ChemStation	Agilent p/n G1701DA (Version D01.00 sp1)
Deconvolution Reporting Software (DRS)	Agilent p/n G1716AA
Library searching software	NIST MS Search (version 2.0) (included with NIST '02 mass spectral library, Agilent p/n G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS) (included with NIST '02 mass spectral library, Agilent p/n G1033A)
MS Libraries	NIST '02 mass spectral library (Agilent p/n G1033A); Agilent RTL Pesticide Library (p/n G1049A)

Results and Discussion

RTL and RTL Databases

RTL is a technique developed by Agilent that allows users to match analyte retention times (RTs) on any Agilent 6890 GC, in any laboratory in the world, so long as the same nominal GC method and capillary column are used [13]. Using RTL, Agilent has developed several retention-time-locked databases for GC and GC/MS that include the locked retention time, compound name, CAS number, molecular formula, molecular weight, and mass spectrum (GC/MS databases only) for each entry [14]. The Agilent RTL Pesticide Library contains this information for almost all GC-amenable pesticides, as well as several endocrine disrupters - 567 compounds in all. For use with the DRS discussed below, this library was converted into the NIST format [15]. Separate Automated Mass Spectral Deconvolution and Identification Software (AMDIS) libraries for the RTs and compound information were created from the original RTL Pesticide Library. Users can easily augment these libraries with newer pesticides or other compounds of interest [15].

Basics of Deconvolution

In GC/MS, deconvolution is a mathematical technique that "separates" overlapping mass spectra into "cleaned" spectra of the individual components. Figure 1 is a simplified illustration of this process. Here, the total ion chromatogram (TIC) and apex spectrum are shown. As is often the case, the peak is composed of multiple overlapping components and the apex spectrum is actually a composite of these constituents. A mass spectral library search would give a poor match, at best, and certainly would not identify all of the individual components that make up the composite "spectrum."

The deconvolution process finds ions whose individual abundances rise and fall together within the spectrum. In this case, it first corrects for the spectral skew that is inherent in quadrupole mass spectra and determines a more accurate apex RT of each chromatographic peak. As illustrated in Figure 1, deconvolution produces "clean" spectra for each overlapping component. These individual spectra can be library searched with a high expectation for a good match.

The AMDIS that is incorporated into the Agilent DRS is supplied by the National Institute of Science and Technology (NIST) [12].

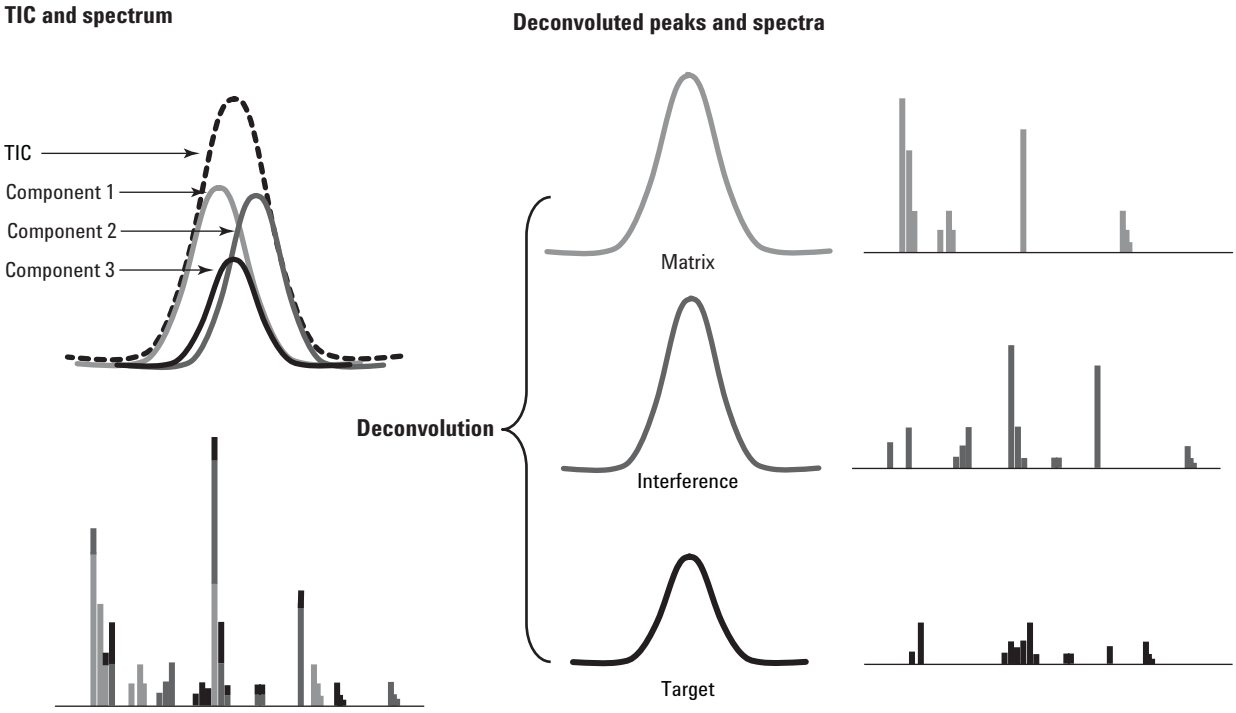

Figure 1. An illustration of mass spectral deconvolution process.

DRS

Agilent's DRS results from the combination of three different GC/MS software packages: 1) the Agilent GC/MS ChemStation, 2) the NIST Mass Spectral Search Program with the NIST '02 MS Library, and 3) the AMDIS software, also from NIST. Included in the DRS, are mass spectral and locked RT libraries for 567 pesticides and suspected endocrine disrupters.

Three separate, but complimentary, data analysis steps are combined into the DRS. First, the GC/MS ChemStation software performs a normal quantitative analysis for target pesticides using a target ion and up to three qualifiers. An amount is reported for all calibrated compounds that are detected. For other compounds in the database, an estimate of their concentration can be reported based upon an average pesticide response factor (RF) that is supplied with the DRS software. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the Agilent RTL Pesticide Library (in AMDIS format) using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's RT to fall within a user-specified time window. Because RTL is used to reproduce the RTL database RTs with high precision, this window can be quite small - typically 20 seconds or less. Finally, the deconvoluted spectra for all of the targets found by AMDIS are searched against the 147,000-compound NIST mass spectral library for confirmation; for this step, there is no RT requirement.

Once the appropriate method is loaded, the DRS report can be generated with a single mouse click as shown in Figure 2. The software can run automatically after each analysis or at a later time on a single file or a batch of files.

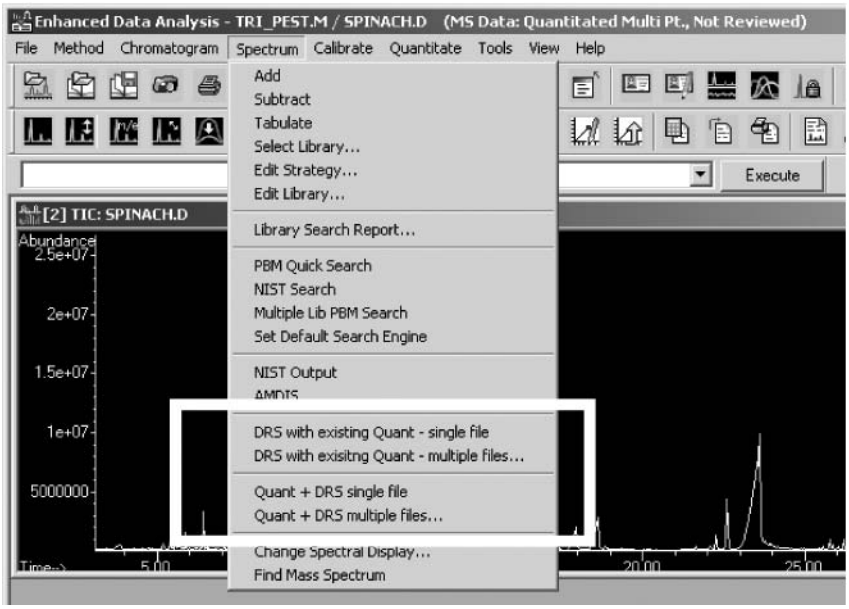

Figure 2. ChemStation pull down menu showing options for running the DRS on single or multiple files.

Pesticides in an Herbal Mix

Figure 3 shows a TIC from the extract of an herbal mix. Figure 4 shows the MSD Deconvolution Report for this sample, which is produced in html format so it can easily be emailed or copied into a spreadsheet. This sample was chosen because herbs are among the most difficult vegetable products to analyze. Their extracts contain a large number of natural products that interfere with pesticide analysis.

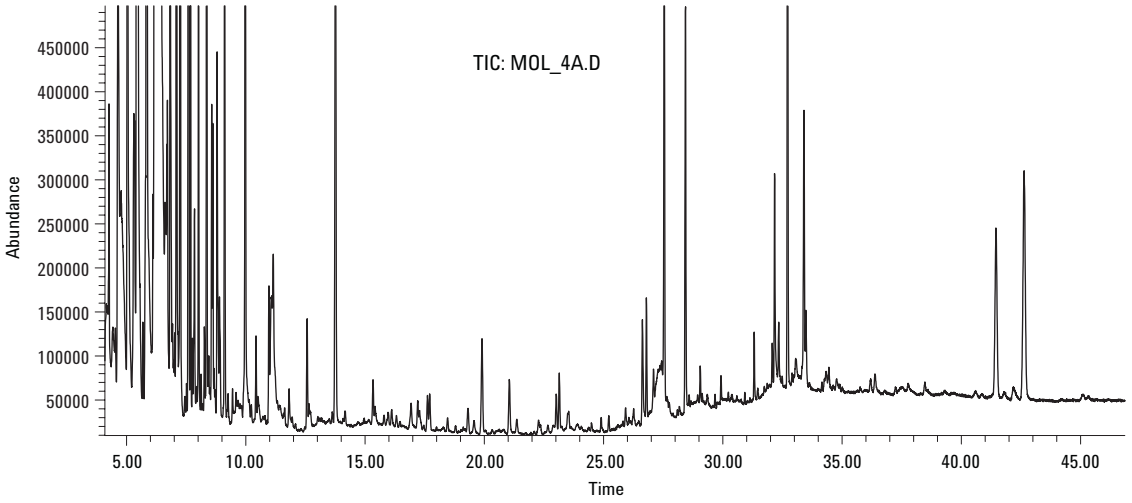

Figure 3. TIC of an herbal mix.

Document - Microsoft Internet Explorer provided by Agilent Technologies, Inc.

File Edit View Favorites Tools Help

Address C:\MSDChem\1\DATA\Hans Mol Data Feb 04 samples\Mar03_X4\MOL_4A.D\MOL_4A.htm

MSD Deconvolution Report
Sample Name: Herbal Mix Control
Data File: C:\MSDChem\1\DATA\Hans Mol Data Feb 04 samples\Mar03_X4\MOL_4A.D
Date/Time: 02:03:11 PM Tuesday, Apr 27 2004

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
13.038	1610180	Prometon		84	2.5	71	1
18.468	84742	Di-n-butylphthalate	1.7	90	2.5	94	1
23.654	38727558	Diethatyl ethyl		69	3.2	73	1
24.079	72559	p,p'-DDE		64	3.3	55	1
27.436	51235042	Hexazinone		61	3.3	80	1
29.681	117817	Bis(2-ethylhexyl)phthalate	0.62	92	2.7	88	3
29.770	21609905	Leptophos		87	3.0	71	1
29.864	2385855	Mirex	0.06	63	2.4	66	2
34.344	51630581	Fenvalerate I		70	5.3	83	2
34.779	102851069	Fluvalinate-tau-I		63	4.6		
34.779	69409945	Fluvalinate				71	1
13.766		Phenanthrene-d10	10				

Done My Computer

Figure 4. MSD Deconvolution Report generated for the herbal mix extract shown in Figure 3.

The DRS report in Figure 4 lists the RT, CAS number, and compound name for each hit. Phenanthrene-d_{10}, listed at the bottom of the report, is the internal standard (ISTD) used by the ChemStation to estimate the quantity of each compound that it found. Since an average pesticide response factor was used for all 567 target compounds, the amounts listed in column 4 are only estimates. Experience has shown that most estimates reported using an average pesticide response factor fall within a factor of 10 of their actual values. True quantitation requires calibration with pesticide standards in the normal way, but this is not practical for all of the pesticides in the database. A laboratory would normally generate calibration curves for their target set of pesticides and use the average RF for the remaining compounds in the database. In this way, when a new compound is detected, the lab can immediately get a rough estimate of its concentration and decide if it should be added to the calibration list.

Column 5 in the report shows the match factor obtained through AMDIS deconvolution and RTL Pesticide Library searching using the deconvoluted full spectra. In this case, several more targets were identified by AMDIS than were found by the ChemStation software (for example, Prometon and p,p'-DDE), which is typical for complex samples. When locked RTs are available, it is a significant advantage to set a RT requirement in the AMDIS software. In this case, hits that did not fall within ±10 seconds of the database RT were eliminated. Column 6 shows the RT difference (in seconds) between the compound's library RT and its actual value in the chromatogram.

Figure 4 shows that the software identified two phthalates (suspected endocrine disrupters) in addition to the pesticides. Phthalates are ubiquitous in the environment and are extremely difficult to remove from the background. In this case, no attempt was made to determine if the phthalates were actually extracted from the sample or were introduced in the laboratory.

The last two columns in the DRS report show the results from searching all of the AMDIS hits against the NIST 147,000-compound mass spectral library. When the NIST library search finds a compound in the top 100 matches (a user-settable value) that agrees with the AMDIS results, its match factor is listed in column seven. The hit number is shown in the last column, with "1" being the best match (highest match factor) in the NIST database. Occasionally, the NIST library search does not find the AMDIS hit among the top 100 spectral matches. In this case, the next line in the report shows the best library match for that spectrum. This is evident for fluvalinate-tau-I (Figure 4), which eluted at 34.779 min. The next line shows the best NIST library match for that spectrum - fluvalinate. In this case, no compound with the same CAS number as fluvalinate-tau-I is contained in the NIST mass spectral library. In fact, fluvalinate-tau-I is the D isomer, while fluvalinate is the DL isomer mixture.

Blind Comparison Between DRS and Traditional Data Review

Many comparisons have shown that the DRS is much better than conventional methods at identifying target compounds in complex samples, such as food and environmental extracts. Two such studies are described here. In the first case, 17 unspiked crop samples were analyzed by NRM Laboratories in Berkshire, UK using Agilent's RT-locked pesticide method. The data files, but not their list of pesticide hits, were sent to Agilent for analysis using the new DRS. Table 2 shows a comparison of the results from the two laboratories. Using manual data review, NRM identified 28 pesticides in the 17 samples, four of which were below their lowest calibration level. Using the same data files, the DRS identified 33 pesticides.

Agilent's automated method did not identify azoxystrobin in the spring onion sample because it is not included in the RTL pesticide library. While it can be found in the NIST library, it has a molecular ion at 403 amu and method used at NRM only scanned to 400 amu. The DRS method confirmed all four pesticides that were below the NRM calibration range and found five more (terbacil, pyrimethanil, methiocarb, pyridaben, and propamocarb) that were not included in their method.

The agreement between the manual and automated methods was excellent. However, the DRS looks for many more pesticides and was able to find several that were missed by the manual method. In addition, manual data review took a chemist about 7 hours for the 17 samples while the DRS finished the task in 50 minutes of unattended computer time.

Table 2. A Comparison of the Pesticides Found in 17 Unspiked Crop Samples Using Conventional Data Review and Agilent's DRS. Pesticides that Were Found by Only One Method Are Underlined

Sample	Agilent DRS results*	NRM Manual Analysis**
Coriander	Propyzamide Chlorthal-dimethyl p,p'-DDE	Propyzamide Chlorthal-dimethyl p,p'-DDE
Rosemary	Terbacil Pirimicarb Chlorthal-dimethyl	Not found*** Pirimicarb Chlorthal-dimethyl
Spring Onion	Propyzamide Pyrimethanil Pirimicarb Metalaxyl Iprodione Not in DRS library†	Propyzamide Not found*** Pirimicarb Metalaxyl Iprodione Azoxystrobin
Chives	Methiocarb Iprodione	Not found*** Iprodione
Cherry Tomato	Procymidone Pyridaben	Procymidone Not found***
Courgette	Propamocarb	Not found***
Aubergine	Procymidone Buprofezin Endosulfan sulfate Iprodione	Procymidone Buprofezin Endosulfan sulfate Iprodione
Flat Leaf Parsley	Chlorthal-dimethyl	Chlorthal-dimethyl
Lambs Lettuce	Iprodione	Iprodione†††
Cos Lettuce	Dimethoate Metalaxyl Procymidone Terbuconazole Omethoate††	Dimethoate Metalaxyl Procymidone Terbuconazole††† Omethoate
Fine Endive	Procymidone lamda-Cyhalothrin	Procymidone lamda-Cyhalothrin
Red Potato	Chloropropham Pirimicarb	Chloropropham Pirimicarb†††
Fine Endive	Pirimicarb	Pirimicarb†††

* Pesticides found by re-analyzing NRM datafiles using Agilent's DRS software.

** Pesticides found by NRM using target compound analysis and manual verification.

*** This compound was not in the NRM target compound list.

† This compound is not included in the Agilent RTL Pesticide Library or the DRS software.

†† Found by the Agilent ChemStation but not found by AMDIS or NIST library searching after deconvolution. After careful review of this hit, omethoate was judged not to be in the sample.

††† Compound was detected but was below the calibration range.

Analysis of Surface Water Samples: In another study, the CDFA analyzed 17 surface water extracts for pesticides. TICs for two typical samples are shown in Figure 5. The CDFA used RTL and RTL database searching but without the benefit of spectral deconvolution. The same data files were then analyzed using the DRS for comparison.

Table 3 shows the results from the CDFA manual analysis of the 17 samples compared to results using the DRS. The CDFA found 38 pesticide hits in the 17 samples, some of which were for the same pesticide in multiple samples. It took a skilled analyst about 8 hours to review the results, eliminate false positives, and verify all of the hits. The DRS found 37 of the compounds seen by the CDFA and identified one CDFA hit as a false positive. In addition, 34 more pesticides were found for a total of 71 hits in the 17 samples. The process was fully automated and took about 20 minutes of unattended computer time to process all of the data files.

Table 3. A Comparison of Results from the Analysis of 17 Surface Water Samples by GC/MS. The CDFA Used RTL and RTL Database Searching, but No Deconvolution. Agilent's DRS Was Used to Analyze the Same Data Files

	CDFA	DRS
Number of pesticide hits	37	Same 37 + 34 additional
Number of false positives	1	0
Time required for analysis	~ 8 hours	20 minutes

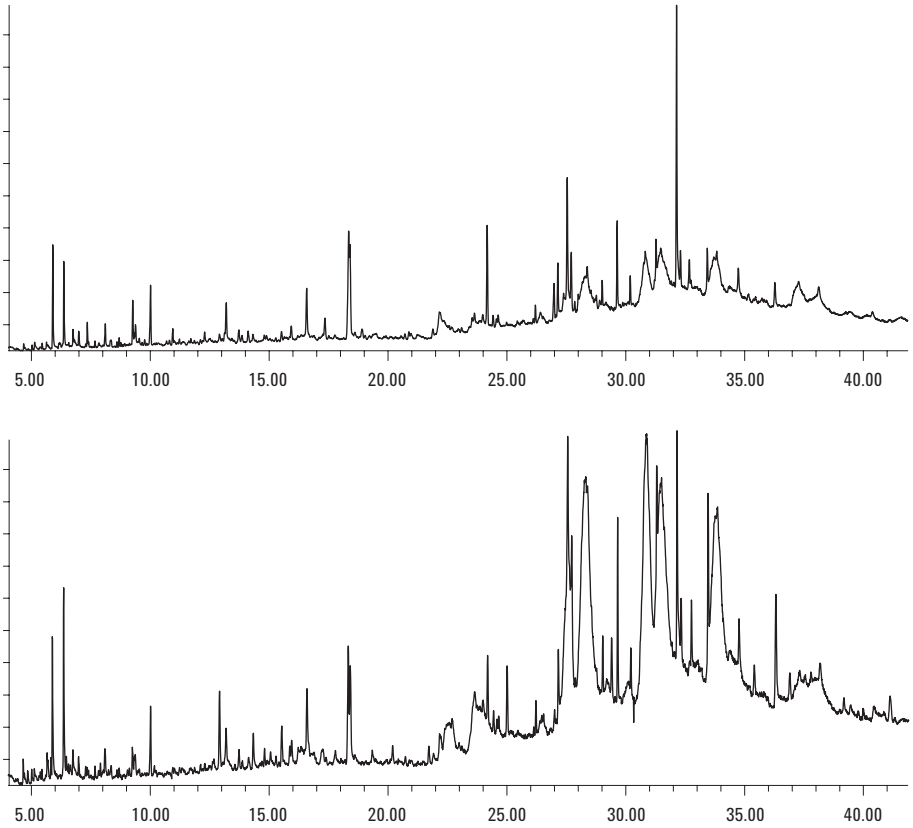

Figure 5. TICs of typical surface water extracts provided by the CDFA.

Conclusions

Agilent's new DRS solution for pesticide analysis offers laboratories a number of real benefits.

- **Ease of use**: This software solution is very simple to use and takes no more skill than is needed to operate the 6890N/5973 inert GC/MS system. There is no need for the user to learn about the intricacies of deconvolution or to master a new software package.
- **Automation**: The deconvolution report can be generated automatically after each run or a batch of samples can be processed all at once.
- **Time savings**: Data review is reduced from hours to minutes.
- **Quality**: It produces results with the fewest false positives and false negatives.
- **Reproducibility**: Results are not dependent upon the skill or experience of the operator.
- **Accuracy**: Comparisons such as those discussed in this application note show that the DRS finds pesticides with greater accuracy than manual methods of data analysis. It is particularly useful for relatively complex samples where co-eluting matrix components might obscure traces of target pesticides.
- **Comprehensive**: This method screens for almost all GC-amenable pesticides as well as several suspected endocrine disrupters in a single GC/MS run. With 567 compounds in the method, it is the most comprehensive pesticide-screening tool available. Users can add more compounds to the method as needed.
- **Produces quantitative, semi-quantitative, and qualitative results**: All calibrated compounds can be quantified. The concentrations of any other compounds can be estimated using an average pesticide response factor provided with the software.

Use of the DRS is not limited to pesticide analysis. Other target compound mass spectral libraries can be converted into the AMDIS format and used with this software. For example, one could use existing libraries for forensic drugs, flavors and fragrances, organic pollutants, etc. Users can even generate their own libraries and use them with the DRS. While not required, it is a big advantage to have an RTL library with locked RTs for each entry, as this will give the fewest false positives.

Acknowledgements

The authors wish to thank Dr. Mark Lee and Stephen Siegel of the California Department of Food and Agriculture, Dr. J.G.J. Mol of TNO Research, The Netherlands, and the management and staff at NRM Laboratories, UK, for their contribution of samples and data.

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Printed in the USA
May 19, 2004
5989-1157EN

Agilent Technologies

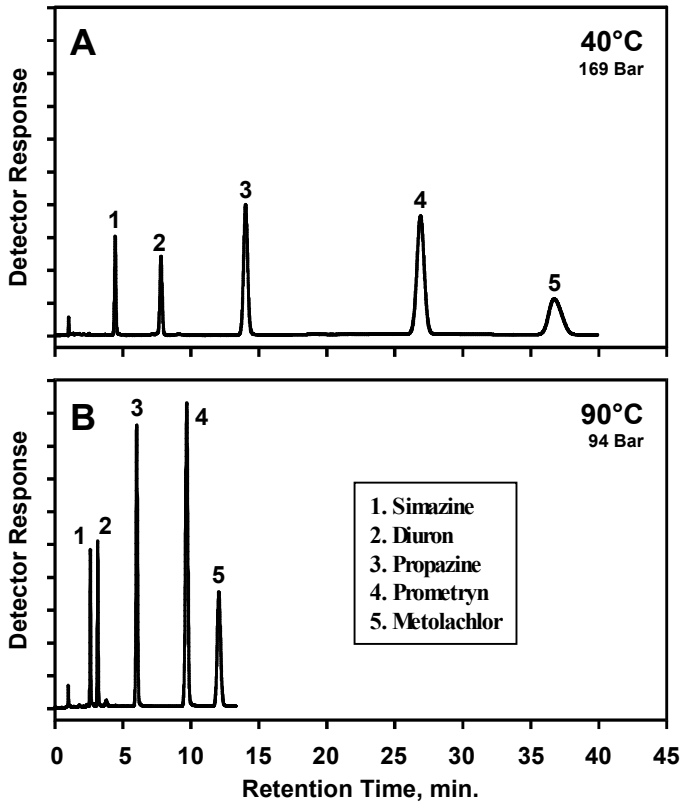
Effect of Column Temperature on Separation of Herbicides

Application

Agrichemical

Robert D. Ricker

Herbicides are a group of molecules that selectively control plant growth through a variety of mechanisms. The large differences in molecular structure of herbicides result in a wide range of retention times and, therefore, long runtimes when performing HPLC separations in isocratic mode. HPLC analysis of these molecules may be used as a means of determining their presence as contaminants in soil, groundwater, and wastewater and may also be used as a means of monitoring their uptake and metabolism in target and non-target plant species.



Conditions:
Column: ZORBAX SB-C18, 4.6 x 150 mm, Agilent Part No. 883975-902
Mobile Phase: MeOH : pH 6.0 NaOAc buffer (48:52)
Flow: 1.5 mL / min.

Highlights

- *Higher temperatures provide capability for rapid, high efficiency reversed-phase separations.*
- *Agilent ZORBAX SB-C18 columns are uniquely stable at temperatures of even 90°C.*
- *Higher temperatures reduce mobile-phase viscosity and decrease column back pressure.*

Agilent Technologies

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www.agilent.com/chem

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5988-6284EN

Agilent Technologies

Rapid Analysis of CLP Pesticides Using High-Temperature DB-35ms and DB-XLB Columns

Application

Environmental

Author

Cameron George
Agilent Technologies, Inc.
91 Blue Ravine Road
Folsom, CA 95630-4714
USA

Abstract

Arylene-phase column pairs (primary and confirmation) permit high oven temperature for rapid analysis of CLP chlorinated pesticides. The columns are also suitable for phenoxy acids, haloacetic acids, polychlorinated biphenyls and Environmental Protection Agency Method 508.1 pesticides.

Introduction

Accurate identification and confirmation of trace level chlorinated pesticides are difficult tasks facing environmental laboratories. The chromatographic system, including the analytical columns, must be optimized. The gas chromatography (GC) columns must possess the selectivity, inertness and thermal stability needed to achieve optimum resolution and sensitivity in the shortest possible time. These needs are realized with Agilent Technologies' J&W Brand DB-35ms (primary) and DB-XLB (confirmation) columns.

The excellent selectivity of high phenyl content phases for chlorinated pesticides is well documented. However, these phases typically suffer from poor thermal stability resulting in high bleed and excessively long analysis times.

DB-35ms uses arylene-phase technology to provide improved thermal stability through a "stiffening" of the polymer backbone. The result is increased sensitivity and an upper temperature limit of 360°C. The column bleed contribution to background noise is reduced, giving a much improved signal-to-noise ratio and increased usable sensitivity compared to standard 35%-phenyl phases. The high thermal limit translates into shorter analysis times, increased column lifetime and the ability to periodically bake the column at a high temperature to remove semivolatile contaminants.

DB-XLB uses a proprietary second-generation arylene technology giving it the same 360°C upper temperature limit and the lowest bleed of any phase available.

Experimental

The columns and related inlet parts are described in Table 1.

Table 1. Columns and Related Parts

Phase/Description	id (mm)	Film (µm)	Length (m)	Part Number
DB-35ms	0.32	0.25	30	123-3832
DB-XLB	0.32	0.50	30	123-1236
Quartz deactivated splitter	-	-	-	5181-3398
Deactivated fused silica guard column	0.53	-	5	160-2535-5

This is a small sampling of the many DB columns and dimensions available.

Results and Discussion

Simple window diagramming identified the exact film thickness necessary to allow DB-XLB to give optimum confirmation power when run using the primary column temperature program. Figure 1 shows the optimized primary and confirmation chromatograms for the DB-35ms/DB-XLB column pair.

Because these columns are designed for enhanced thermal resistance, it is not necessary to bake them excessively upon installation to reduce bleed to acceptable levels. A simple 1 to 2 hour conditioning period is typically more than adequate. Conditioning columns overnight is a common requirement with cyanopropyl- and trifluoropropyl-containing CLP pesticide columns. This practice can result in increased column activity and decreased column life time, but is not required with DB-35ms/DB-XLB. In short, you are ready sooner after column installation.

Environmental laboratories are also interested in other gas chromatograpy/electron capture detector (GC/ECD) methods with the same dual column pair used for the chlorinated pesticides. These methods include phenoxy acid herbicides (EPA Method 8151A), haloacetic acids (EPA Method 552.2), PCBs (EPA Method 8082) and EPA Method 508.1 pesticides. This column pair provides baseline resolution of all 8151A analytes on both columns in just over 16 minutes. In addition, DB-35ms and DB-XLB provide the best confirmation and the fewest coelutions of any dual column pair commercially available for 508.1 pesticides.

Conclusions

The DB-35ms and DB-XLB column pair perform rapid, high-resolution separations of CLP pesticides. The high temperature limit and low column bleed make these columns attractive for analyses of similar semivolatile sample mixtures.

For More Information

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

Column: **DB-35ms**
30 m × 0.32 mm id, 0.25 µm
P/N: 123-3832

Column: **DB-XLB**
30 m × 0.32 mm id, 0.50 µm
P/N: 123-1236

Carrier: Helium at 45 cm/sec
(EPC in constant flow mode)

Oven: 110 °C for 0.5 min
110-320 °C at 15 °/min
320 °C for 2 min

Injector: Splitless, 250 °C
30 sec purge activation time
50 pg per component

Detector: Micro ECD, 350 °C
Nitrogen makeup gas
(column + makeup flow =
30 mL/min constant flow)

1. Tetrachloro-m-xylene (SS)
2. α-BHC
3. γ-BHC
4. β-BHC
5. Heptachlor
6. δ-BHC
7. Aldrin
8. Heptachlor epoxide
9. γ-Chlordane
10. α-Chlordane
11. Endosulfan I
12. 4,4´-DDE
13. Dieldrin
14. Endrin
15. 4,4´-DDD
16. Endosulfan II
17. 4,4´-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (SS)

SS-Surrogate Standard

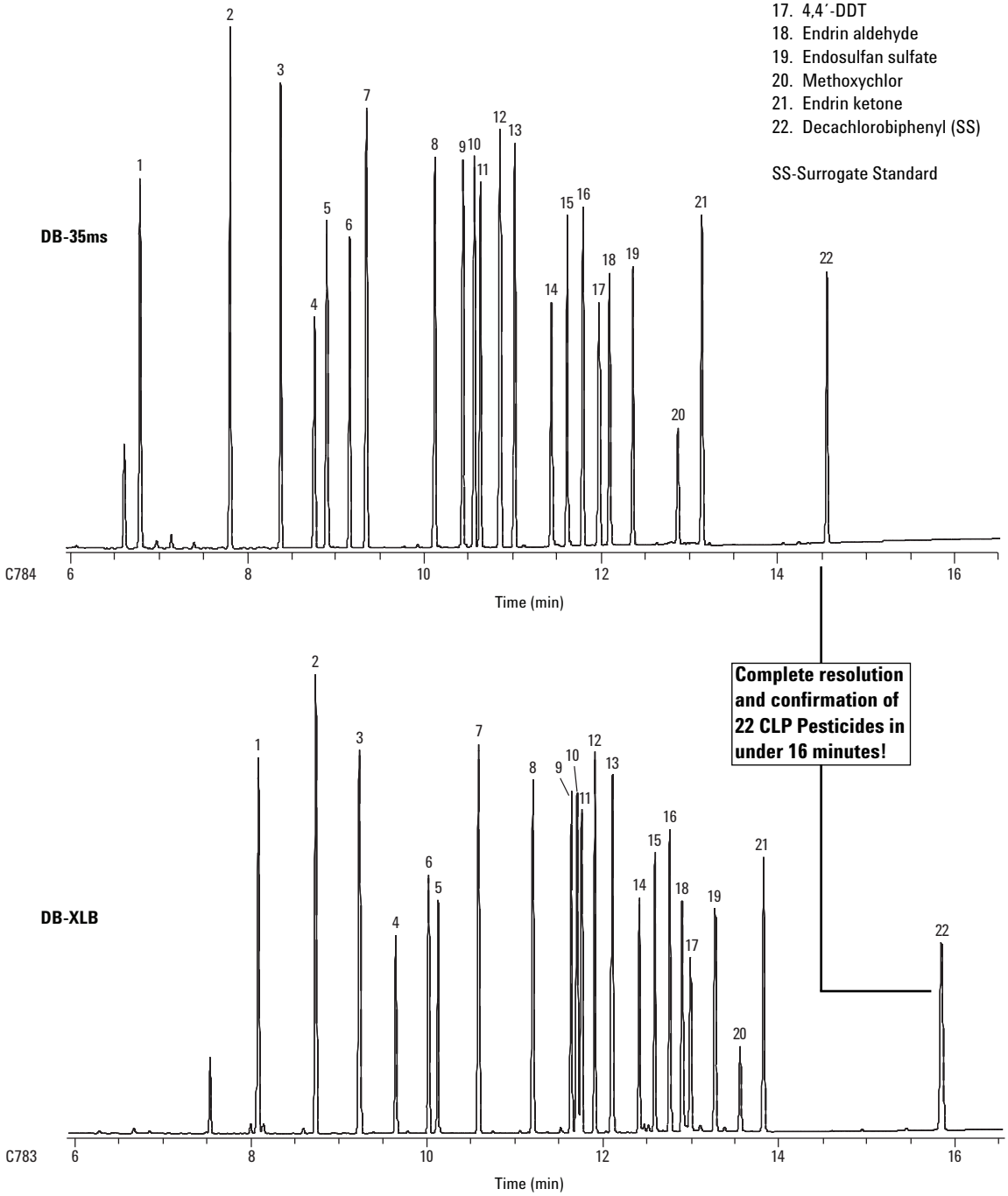

Figure 1. CLP Pesticides

www.agilent.com/chem

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December 18 2001
5988-4973EN

Agilent Technologies

A Complete Solution for Chlorinated Pesticides and Herbicides Using DB-35ms and DB-XLB Columns

Application

Environmental

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Abstract

DB-35ms (primary) and DB-XLB (confirmation) columns, used with inert inlet components and hydrogen carrier gas, perform CLP pesticide analyses in less than 15 minutes total cycle time. Phenoxy acids can be analyzed with the same configuration.

Introduction

Obtaining high quality data in the shortest possible time is a desire of all analytical testing laboratories. To achieve this goal, all aspects of the chromatographic system must be optimized. The GC columns must possess the selectivity, inertness and thermal stability needed to achieve optimum resolution and sensitivity in the least amount of time. For the analysis of CLP pesticides and phenoxy acid herbicides, these needs are met with DB-35ms (primary) and DB-XLB (confirmation) columns.

In another Application Note[1], DB-35ms and DB-XLB show excellent selectivity and inertness for CLP pesticides, achieving one hundred percent confirmation in less than 16 minutes. However, there is always the desire for a faster analysis. This note reports the result of changing to hydrogen carrier gas and increasing the oven ramp rate.

Experimental

Table 1 describes the columns and related inlet parts.

Table 1. Columns and Related Parts

Phase/Description	id (mm)	Film (µm)	Length (m)	Part number
DB-35ms	0.32	0.25	30	123-3832
DB-XLB	0.32	0.50	30	123-1236
Quartz deactivated splitter	-	-	-	5181-3398
Deactivated fused silica guard column	0.53	-	5	160-2535-5

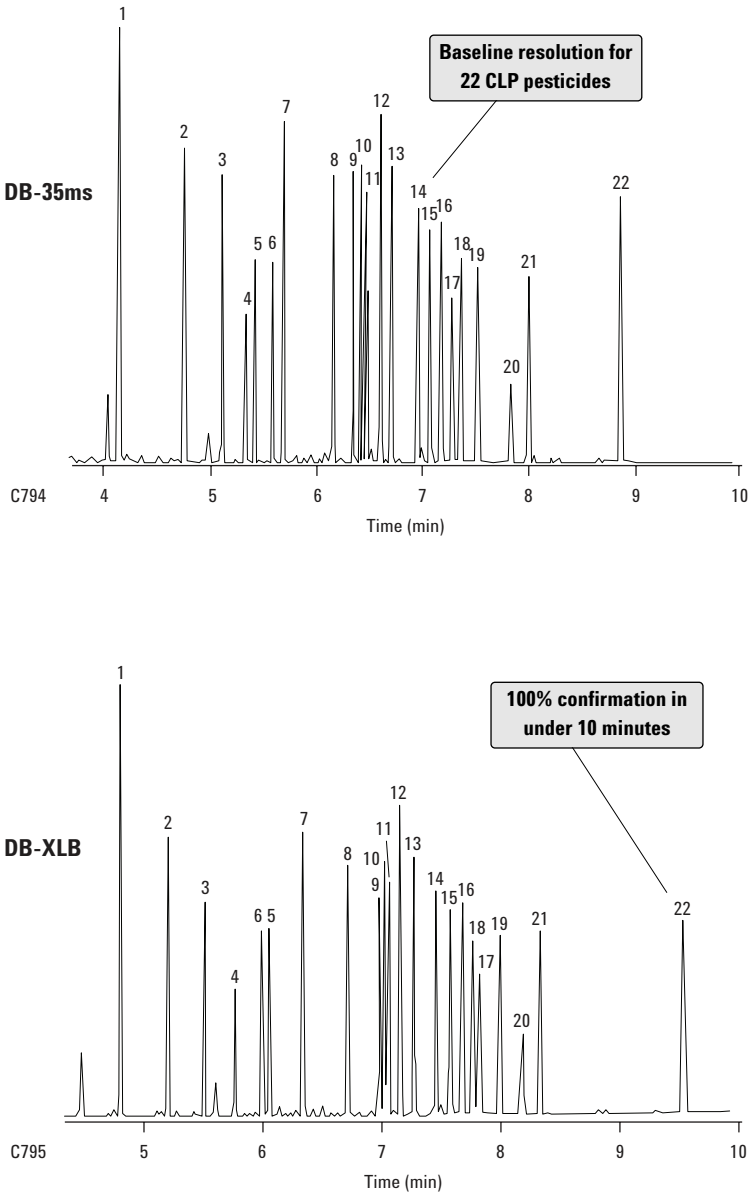
This is a small sampling of the many DB columns and dimensions available.

Results and Discussion

To reduce analysis time without a significant loss in resolution, the carrier gas was changed to hydrogen. Using hydrogen carrier gas with a linear velocity of 65 cm/sec, and increasing the oven ramp rate from 15 °C/min to 25 °C/min, reduces analysis time to less than 10 minutes. Considering a typical cool-down time of 4 to 5 minutes, the total instrument cycle-time is now less than 15 minutes.

Results and Discussion

Figure 1 shows the excellent resolution and confirmation available for CLP pesticides using DB-35ms/DB-XLB with hydrogen carrier gas and a properly scaled temperature program.



Columns: DB-35ms
30 m × 0.32 mm I.D., 0.25 µm
Part No.: 123-3832
DB-XLB
30 m × 0.32 mm I.D., 0.50 µm
Part No.: 123-1236
Carrier: Hydrogen at 65 cm/sec
(EPC in constant flow mode)
Oven: 110 °C for 0.5 min
110-320 °C at 25 °C/min
320 °C for 2 min
Injector: Splitless, 250 °C
30 sec purge activation time
50 pg per component
Detector: µECD, 350 °C
Nitrogen makeup gas (column + makeup flow = 30 mL/min constant flow)

1. Tetrachlorom-xylene (SS)
2. α-BHC
3. γ-BHC
4. β-BHC
5. Heptachlor
6. δ-BHC
7. Aldrin
8. Heptachlor epoxide
9. γ-Chlordane
10. α-Chlordane
11. Endosulfan I
12. 4,4′-DDE
13. Dieldrin
14. Endrin
15. 4,4′-DDD
16. Endosulfan II
17. 4,4′-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl

Figure 1 Analysis of CLP pesticides using DB-35ms and DB-XLB columns.

DB-XLB and DB-35ms have flexibility for a range of GC/ECD methods, a result of their ideal selectivity, inertness and the robustness of low bleed phases. Phenoxy acid herbicides (EPA Method 8151A) are nicely resolved with these columns. All twenty common herbicides are resolved in slightly over 16 minutes, as shown in Figure 2. The analysis can be optimized for faster analysis. To obtain chromatograms and analysis conditions for additional GC/ECD methods, go to Agilent's Technical Support at www.agilent.com/chem

Columns: DB-35ms
30m × 0.32 mm I.D., 0.25 µm
Part No.: 123-3832
DB-XLB
30m × 0.32 mm I.D., 0.50 µm
Part No.: 123-1236
Carrier: Helium at 45 cm/sec
(EPC in constant flow mode)
Oven: 50 °C for 0.5 min
50-100 °C at 25 °C/min
100-320 °C at 12 °C/min
320 °C for 2 min

Injector: Splitless, 250 °C
30 sec purge activation time
50 pg per component
Detector: µECD, 350 °C
Nitrogen makeup gas
(column + makeup flow =
30 mL/min constant flow)

1. Dalapon
2. 3,5-Dichlorobenzoic acid
3. 4-Nitrophenol
4. Methyl-2,4-dichlorophenyl-acetate (SS)
5. Dicamba
6. MCPP
7. MCPA
8. 4,4´, Dibromooctafluorobi-phenyl (IS)
9. Dichloroprop
10. 2,4-D
11. Pentachlorophenol
12. 2,4,5-T,P
13. 2,4,5-T
14. Chloramben
15. Dinoseb
16. 2,4-DB
17. Bentazone
18. DCPA
19. Picloram
20. Acifluorofen

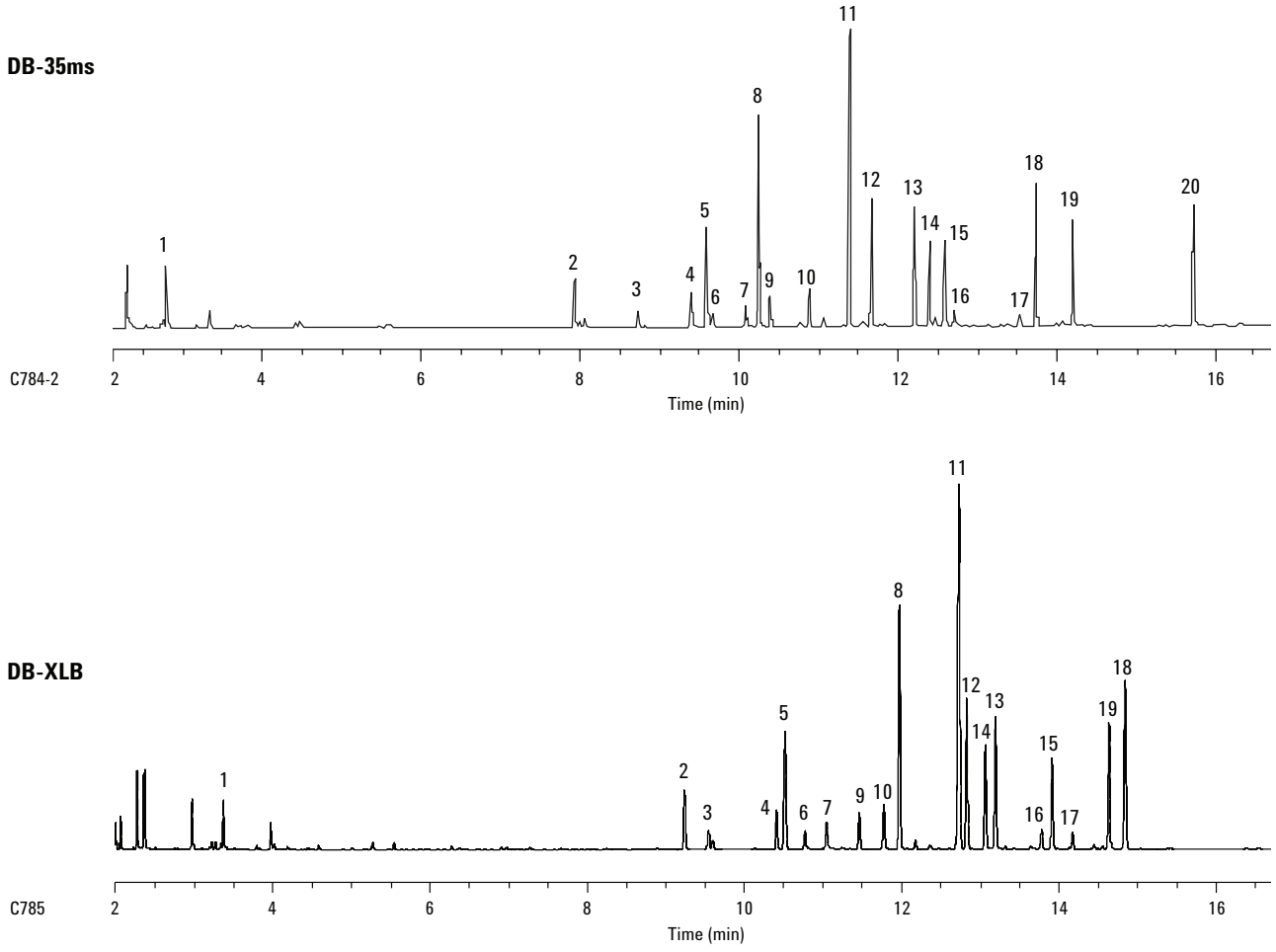


Figure 2. EPA 8151A phenoxy acid herbicides.

Conclusions

DB-XLB and DB-35ms columns, when used with inert inlet components, hydrogen carrier gas and an appropriate carrier velocity, yield these benefits:

- Short analysis times for better productivity
- Excellent thermal stability with 360 °C upper temperature limit
- Confirmation for CLP pesticides and phenoxy acid herbicide

Reference

1. “Rapid Analysis of CLP Pesticides Using High-Temperature DB-35ms and DB-XLB Columns,” Application Note 5988-4973EN, Nov 26, 2001.

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Agilent Technologies

Identification and Quantitation of Pesticides in the Parts-per-Trillion Range Using Retention Time Locking and GC/MS

Application

Environmental, Food

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Abstract

The typical pesticide quantitation limit for a mass spectrometer in the Scan mode is in the sub-ppm range. By using a selected ion monitoring method, a lab can lower the target compound quantitation limit to the low parts-per-billion (pg/µL) range using a retention time locked gas chromatography/mass spectrometry method. By adding large volume injection capability to the method, target compounds at parts-per-trillion can be quantified.

A specially developed 567-compound retention time locking pesticide mass spectral library can automatically screen an acquired sample's data file for all 567 compounds in seconds. The library can also be applied for rapid screening of samples acquired in selected ion monitoring method. Using the compound library information, a selected ion monitoring method for 80 target compounds was created in less than 2 hours without running any analyses.

Introduction

Most pesticides are typically analyzed on a gas chromatograph (GC) with element-selective detectors (ESDs). Although these ESDs provide low ppb detection limits and are easy to operate, the data do not provide sufficient information to confirm a compound's presence with confidence. Due to the universal nature of mass spectrometric detection, a mass spectrometer (MS) provides additional information and increased confidence in the assignment of compound identity. With recent advances in GC/MS hardware and software and the decrease in cost of ownership, more and more laboratories are routinely analyzing pesticide residue samples with MS detection.

To match the GC/ESD detection limits and/or to eliminate sample concentration steps, a user must lower the MS detection limit by 2 to 3 orders of magnitude. This application note, discusses the following approaches.

- Run the MS in single ion monitoring (SIM) mode
- Make large volume injections (LVIs)
- Use higher electron multiplier voltage (EMV)

For compound identification, a specially developed 567-compound retention time locking (RTL) [1] pesticide library could perform the entire 567-compound screening in seconds using Scan data. A subset of the library could be screened in seconds from SIM data.

Experimental

A pesticide standard mixture was used to compare the lowest detection limits of splitless injection and LVI under Scan and SIM modes.

System Configuration for Screening and Quantitation:

- 6890 GC with a programmable temperature vaporizer (PTV) [2,3] inlet
- 5973 Mass Selective Detector (MSD)
- 7683 Automatic Liquid Sampler (ALS) tray and autoinjector
- HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), P/N 19091S-433
- G1701BA version B.00.00 MSD ChemStation software or higher
- G1049A MSD RTL Pesticide Database/Library

Table 1. GC Method Parameters

Oven	70(2)/25/150(0)/3/200(0)/8/280(10) = 41.87 min
Inlet	PTV
Inlet pressure	17.30 psi (locked to methyl chlorpyrifos at 16.593 min), constant pressure mode

Table 2. Injection Parameters

Injection mode	Solvent vent	Splitless
Injection volume (syringe)	25 µL (50-µL syringe, P/N 5183-0318)	1 µL (10-µL syringe, P/N 9301-0713)
Injection speed	Inject @ 100 µL/min Draw @ 300 µL/min Dispense @ 4500 µL/min	Fast
Inlet temp	40(0.35)/600/320 (3)/50/200 (Hold until end)	280 °C
Vent	Vent time = 0.29 min Vent flow = 150 mL/min Vent pressure = 0.00 psi	
Purge	60 mL/min @ 2 min	60 mL/min @ 2 min
Liner	Deactivated, Multi Baffled (P/N 5183-2037)	Deactivated, Multi Baffled (P/N 5183-2037)
Inlet cooling	Liquid CO_2	None

Table 3. MS Method Parameters

Solvent delay	3 min
Tune file	Atune.u
Transfer line	280 °C
MS Quad	150 °C
MS source	230 °C
Threshold	150
Sample #	2
Scan range	35 to 500 amu (in Scan mode)
Forty (40) SIM groups (in SIM mode)	

Table 4. Pesticide Screening Parameters for the SIM Method

Extraction window	±0.100 minute
Qualifier mode	Absolute
Qualifier %	30
Zero qualifiers	Included
Subtraction mode	Average start/stop
Screen database	Rtlpest.SCD

Results and Discussion

RTL [1] was used to:

1. Expedite data comparison in overlay format
2. Achieve lower target compound detection limit
3. Allow rapid pesticide screening using the RTL pesticide database/library
4. Help to differentiate isomers by their retention time (RT) differences
5. Eliminate the tedious SIM method RT updating process after column maintenance
6. Simplify the editing of the SIM ion groups

A mixture from the California Department of Food and Agriculture (CDFA) of 80 pesticides at 5000 pg/µL each was used as the stock solution for this study. The mixture contained carbamate, organochlorine, organophosphorus, and organonitrogen pesticides. Figure 1 is an offset overlay of three total ion chromatograms (TIC) with 50, 100, and 500 pg of each of the pesticides injected. These TICs were obtained in the Scan mode from 1-µL spiltless injections. For many of these pesticides the quantitation limit in the Scan mode is about 500 pg on column.

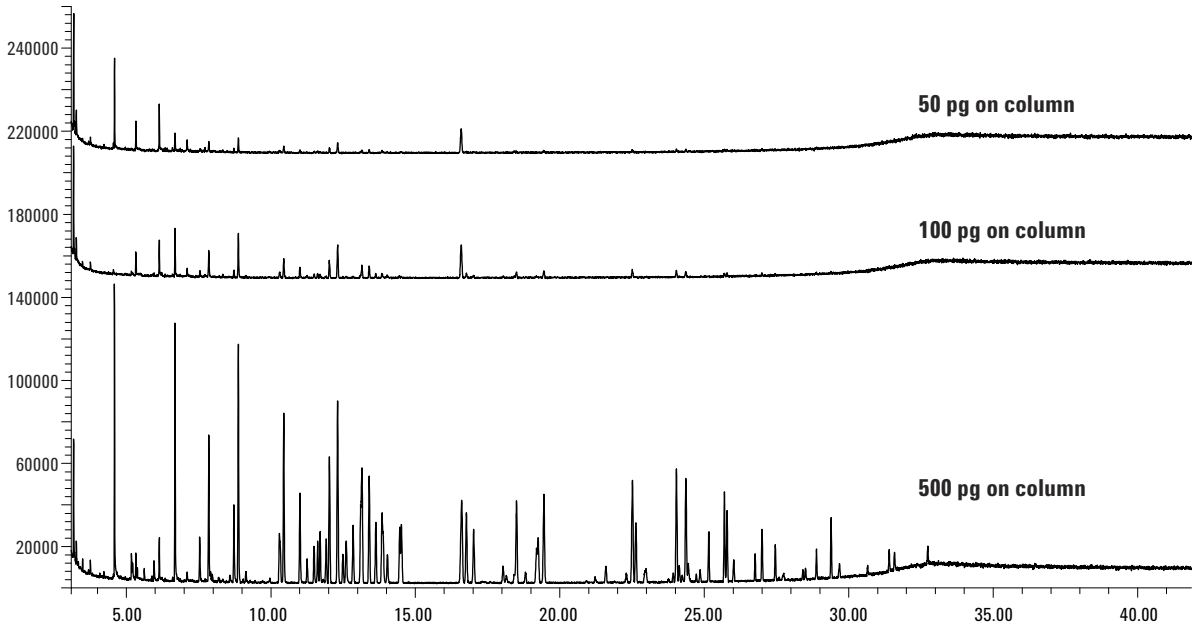


Figure 1. Total ion chromatograms from 1-µL splitless injections of 80 pesticides with 50, 100, and 500 pg of each compound injected.

SIM Mode

To lower the detection limit, a SIM method was created. Instead of the traditional way of making a SIM method, a user can use the information in the RTL Pesticide Database to build a SIM method without running an analysis. Here are the steps for editing SIM ion group parameters:

1. List the MSD RTL Pesticide Database from the ChemStation (Figure 2 is a partial listing) and paste the complete listing into a spreadsheet.

Scdlist.txt - Notepad

File Edit Search Help

SCD Compound List Report

Screen Database : C:\DATABASE\RTLPEST.SCD
Total SCD Cpnds : 567

Cpd#	Compound Name	TIon	Exp_RT	Q1	Q2	Q3
1	Diethylene glycol	45	3.39	75	76	44
2	Aniline	93	3.55	66	65	92
3	p-Dichlorobenzene	146	3.88	148	111	75
4	Dicyclopentadiene	66	4.00	132	67	65
5	Dimefox	44	4.01	110	154	42
6	o-Dichlorobenzene	146	4.09	148	111	75
7	2-Methylphenol	108	4.25	107	77	79
8	4-Methylphenol	107	4.42	108	77	79
9	m-Cresol	108	4.42	107	79	77
10	1,2-Dibromo-3-chloropropane	157	4.53	155	75	159
11	2,4-Dimethylaniline	121	5.19	120	106	77
12	2,6-Dimethylaniline	121	5.20	106	120	77
13	2,4-Dichlorophenol	162	5.19	164	63	98
14	1,2,4-Trichlorobenzene	180	5.29	182	184	145
15	Ethiolate	100	5.41	72	161	44
16	3-Chloroaniline	127	5.45	129	65	92
17	4-Chloroaniline	127	5.48	129	65	92
18	2-Ethyl-1,3-hexanediol	56	5.51	55	73	57
19	p-Nitrotoluene	91	5.57	137	65	107
20	Methamidophos	94	5.66	95	141	47
21	Dichlorvos	109	5.83	185	79	187
22	Allidochlor	41	6.18	56	138	132
23	2,3,5-Trichlorophenol	196	6.63	198	200	160
24	2,6-Dichlorobenzonitrile	171	6.75	173	136	100

Figure 2. A partial listing of the pesticide screener database. The listing includes the compound number, compound name, target ion, expected retention time, and three qualifier ions.

2. In the spreadsheet, delete the rows of the compounds not needed in the method.
3. Separate target compounds into groups (see the added "Group #" column on Figure 3) using these criteria:
   - One to three compounds in each group, and
   - The RTs of the adjacent compounds in adjacent groups are at least 0.2 minute apart. For example, compounds 42 and 51 are more than 0.2 minute apart, so they are in different groups. Compounds 51 and 55 are less than 0.2 minute apart, so they are in the same group.
4. Use the average RT of the adjacent compounds in adjacent groups as the SIM group RT (see the added "Group RT" column on Figure 3). For example, the average retention time of compound 42 (7.91 min, in group 2) and compound 51 (8.78 min, in group 3) is 8.35 minute which is used as the starting retention time of group 3. When all the group numbers and respective starting retention times are determined, make a hardcopy of the spreadsheet for easy entry into the "MS SIM/Scan Parameters" in the next step.
5. Enter the target ion and qualifier ion(s) (Q1, Q2, and/or Q3) of all compounds into the respective ChemStation SIM group (Figure 4). Notice that all the information for building the SIM groups came from Figure 3.

#	Compound Name	MSD_RT	T	Q1	Group #	Group RT
24	2,6-Dichlorobenzonitrile	**6.75**	171	173	**1**	**3.00**
35	Mevinphos	**7.60**	127	192		
42	Propham	**7.91**	93	179	**2**	**7.75**
51	o-Phenylphenol	**8.78**	170	169	**3**	**8.35**
55	Pentachlorobenzene	**8.95**	250	252		
76	Propoxur	**10.35**	110	152	**4**	**9.60**
82	Diphenylamine	**10.52**	169	168		
92	Chlorpropham	**11.05**	127	213	**5**	**10.76**
98	Ethalfluralin	**11.28**	276	316		
102	Bendiocarb	**11.54**	151	126	**6**	**11.41**
103	Trifluralin	**11.64**	306	264		
104	Benfluralin	**11.73**	292	264		
111	Phorate	**11.96**	75	121	**7**	
113	BHC alpha isomer	**12.09**	181	219		
117	Hexachlorobenzene	**12.38**	284	286		
120	Dicloran	**12.56**	206	176		
122	Demeton-S	**12.63**	88	60		
124	Dimethoate	**12.68**	87	93		
129	Simazine	**12.91**	201	186		

Figure 3. A spreadsheet of target compounds separated into different SIM groups with RTs of the adjacent compounds in adjacent groups at least 0.2 minute apart. The starting retention time of each group was determined by calculating the average RT of the adjacent compounds in adjacent groups.

The number of qualifier ions used in a SIM method depends on the number of analytes of interest. For a method monitoring 20 to 30 compounds, all three qualifier ions should be used in the SIM method. As the list of target compounds grows, fewer qualifier ions should be used in the method to maintain a reasonable and comparable ion dwell time and sampling rate.

In general, 10 scans (cycles) per peak are recommended for quantitation purposes. For example, if an analyte peak is 6 seconds wide, about 1.7 cycles per second should be maintained for that SIM ion group. Once the number of cycles per second is determined, the dwell time of the ions can be varied to meet that. As the dwell time is entered for each ion, the ChemStation automatically shows the number of cycles per second. In Figure 4, Group 6 has 3.03 cycles per second.

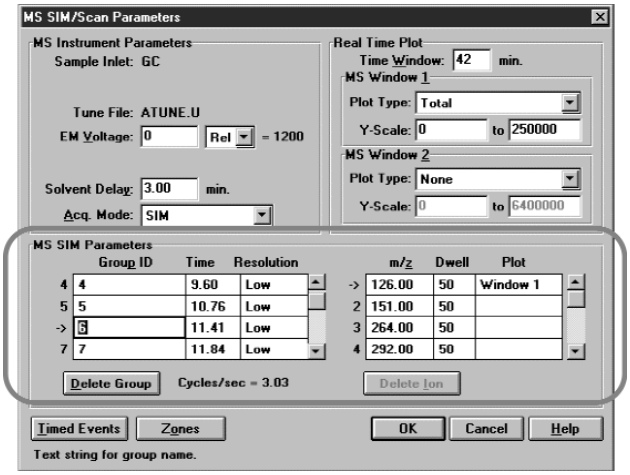


Figure 4. A screen capture of the MSD ChemStation showing the MS and SIM parameters. The SIM parameters (group ID, group retention time, and ions) were all derived from Figure 3.

Figure 5 shows two chromatograms obtained from 1-µL splitless injections at 50 pg/µL using both Scan and SIM modes. The Scan mode has significantly higher baseline noise than the SIM mode. Some of the compounds, especially the late eluters, were not detected in the Scan mode. When the Scan method was changed to a SIM method at this concentration, the signal-to-noise ratio (S/N) increased by a factor of 100. It is worth pointing out that a SIM method does not record background ions from the sample matrix, therefore minimizing the baseline noise and improving the S/N.

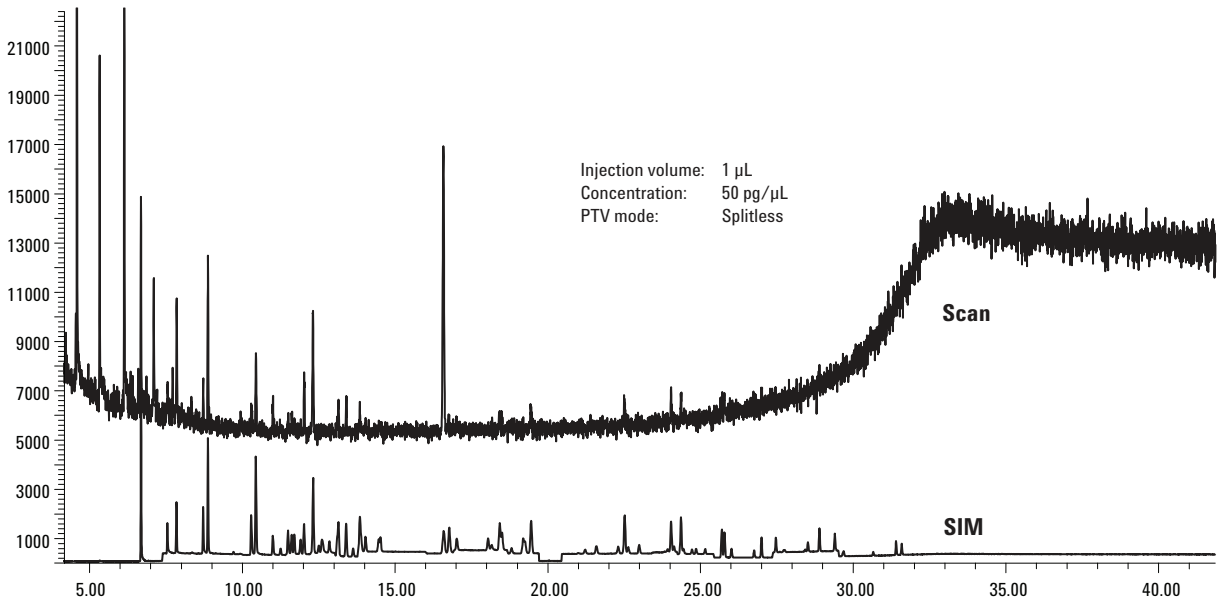


Figure 5. Chromatograms of 1-µL splitless injections at 50 pg/µL from Scan and SIM modes.

In a SIM method, the retention times of the ion groups normally need updating after column maintenance. By using RTL, a user can not only eliminate the tedious RT updating process [4] but also decrease the detection limit. With reproducible known RTs of target compounds, the start and end time of each ion group can be determined optimally. By narrowing the time windows of an ion group to monitor only one or two compounds at a time, the MS can monitor fewer ions in each window, allowing more sampling time for the target ions.

Ideally, a SIM method will have the maximum number of ion groups and the minimum number of ions in each group. In this way, each ion group can get more scans per unit time resulting in better peak shape and more accurate quantitation.

LVIs

To decrease the detection limit further, a user can put more sample on column using the LVI technique. The typical “solvent-vent” approach is to inject the sample slowly into a PTV inlet at a temperature just below the solvent boiling point and let solvent evaporate before ramping up the inlet temperature to move the compounds onto the capillary column. Figure 6 compares a 1-µL splitless injection with a 25-µL solvent-vent injection. Both injections resulted in 50 pg per compound on column. Note that the solvent-vent ion chromatogram is plotted upside down for ease of comparison with the splitless ion chromatogram. It is obvious from the figure that the two techniques provide very similar results. This demonstrates that the solvent-vent technique is a viable approach for sample introduction.

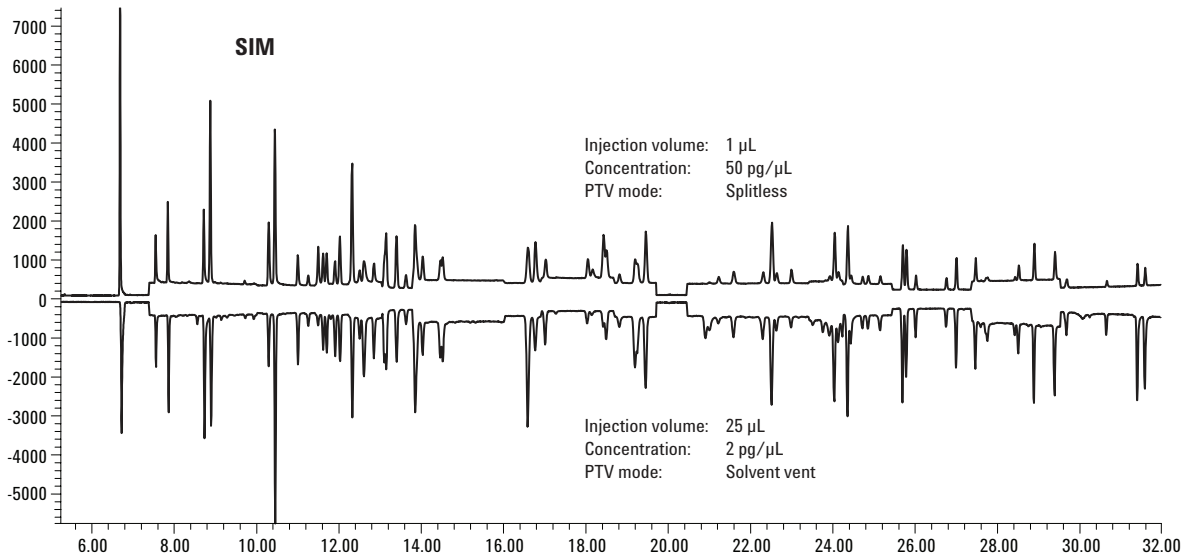


Figure 6. SIM results of 50 pg on column using either a 1-µL splitless or a 25-µL solvent-vent injection.

Higher EMV

It is known that the signal increases with higher EMV on the MS. In Figure 7, the upper signal, after 10-fold magnification, is a 25-µL LVI of 0.5 pg/µL at tune voltage. The bottom signal is the same injection with the electron multiplier set to tune +400 V. Adding 400 V to the EMV increases the signal by 10X, which makes the integration more accurate. However, the baseline noise also increases by 10X, so the S/N stays the same.

Although increasing the EMV does help to bring small peaks over the detection threshold, it shortens the life of the multiplier. In general, the EMV should be kept at the tune voltage.

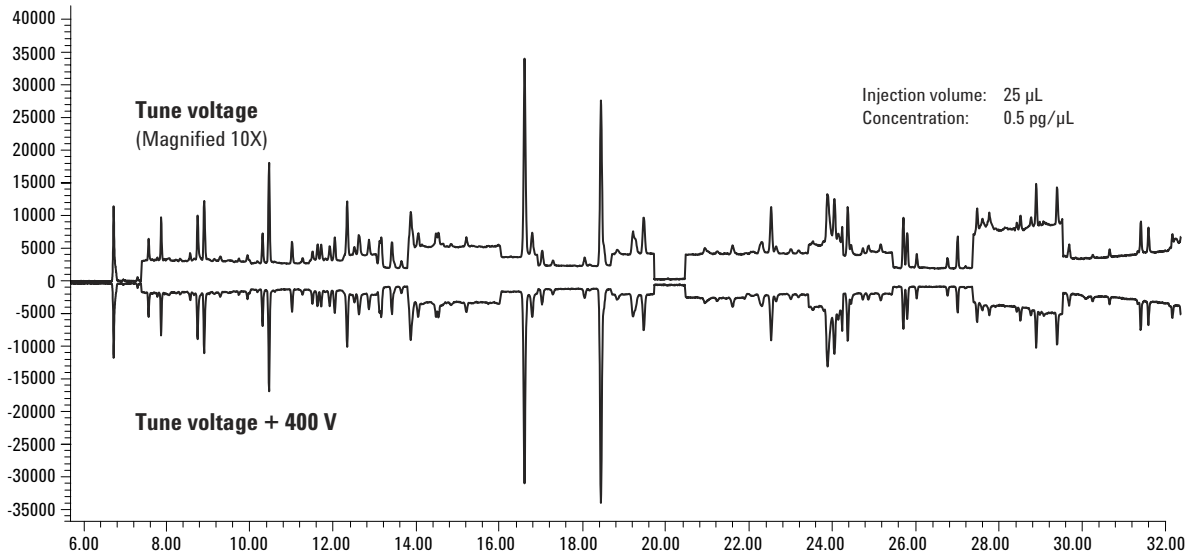


Figure 7. SIM results of 12.5 pg on column using either EMV at Tune voltage or Tune +400 V.

LVIs in Combination with SIM Methods

Combining LVI and SIM, Figures 8 and 9 show quantifiable peaks of three compounds at as low as 5 pg on column. In Figure 8, ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL are shown. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method. By using LVI and SIM, it is interesting to see that similar S/N ratios were achieved even with a 2500-fold decrease (from 500 to 0.2 pg/µL) in sample concentration.

By increasing the injection volume to 100 µL, samples at concentration as low as 0.05 pg/µL can also be quantified as shown in Figure 9. The top portion shows the chlorthal-dimethyl extracted ion chromatograms (EIC) of mass 299 and 301 from a 100-µL full Scan run. The bottom portion shows the same ions from a 100-µL SIM run. The SIM method shows better peak shape and lower baseline noise.

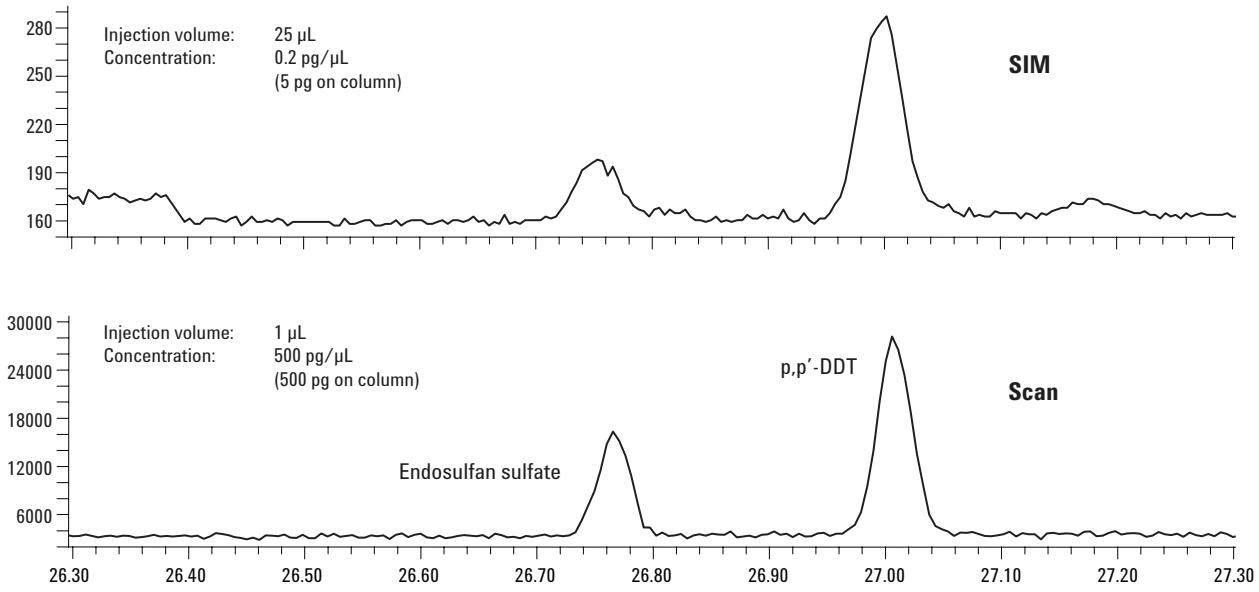


Figure 8. Ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method.

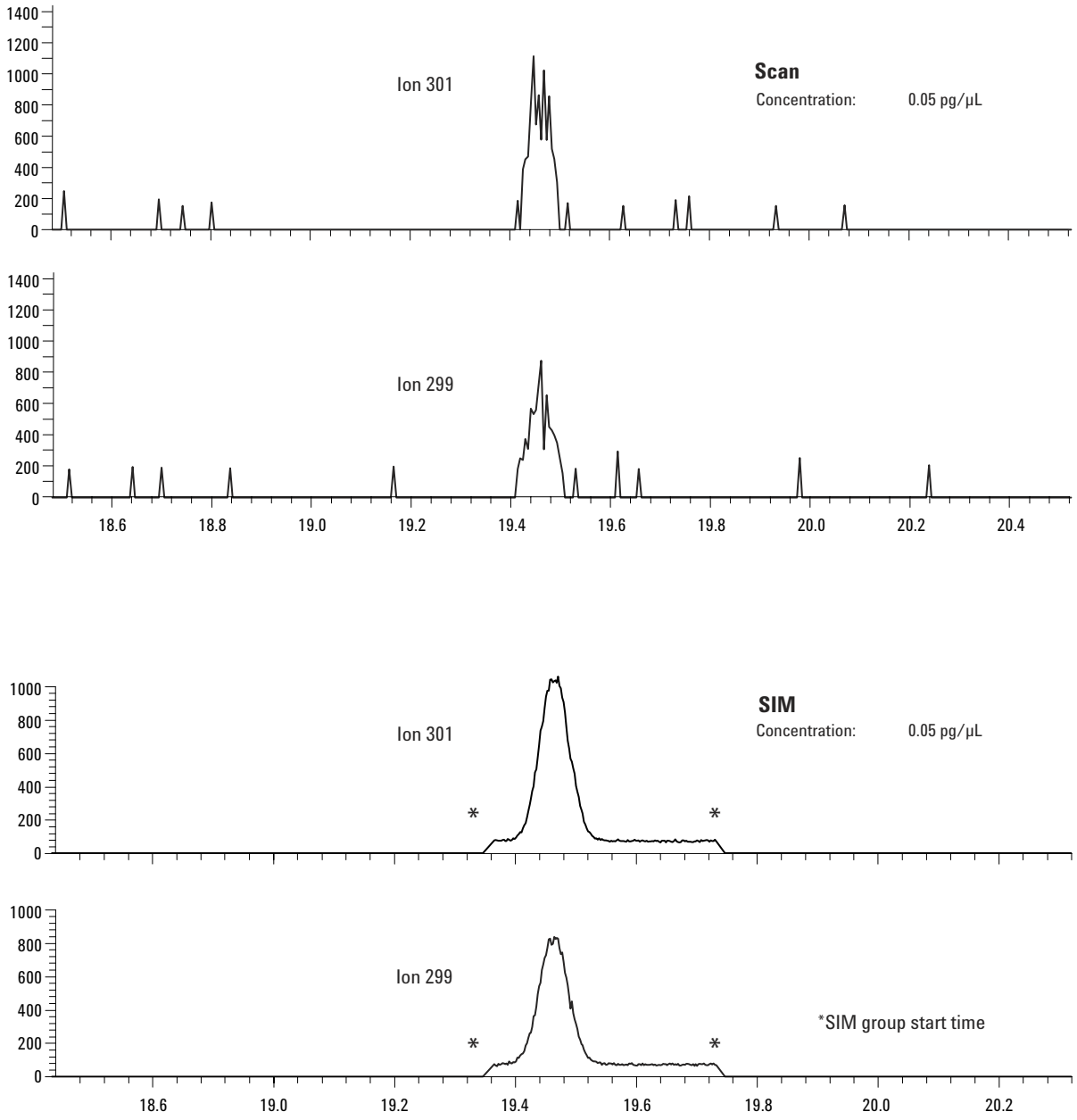
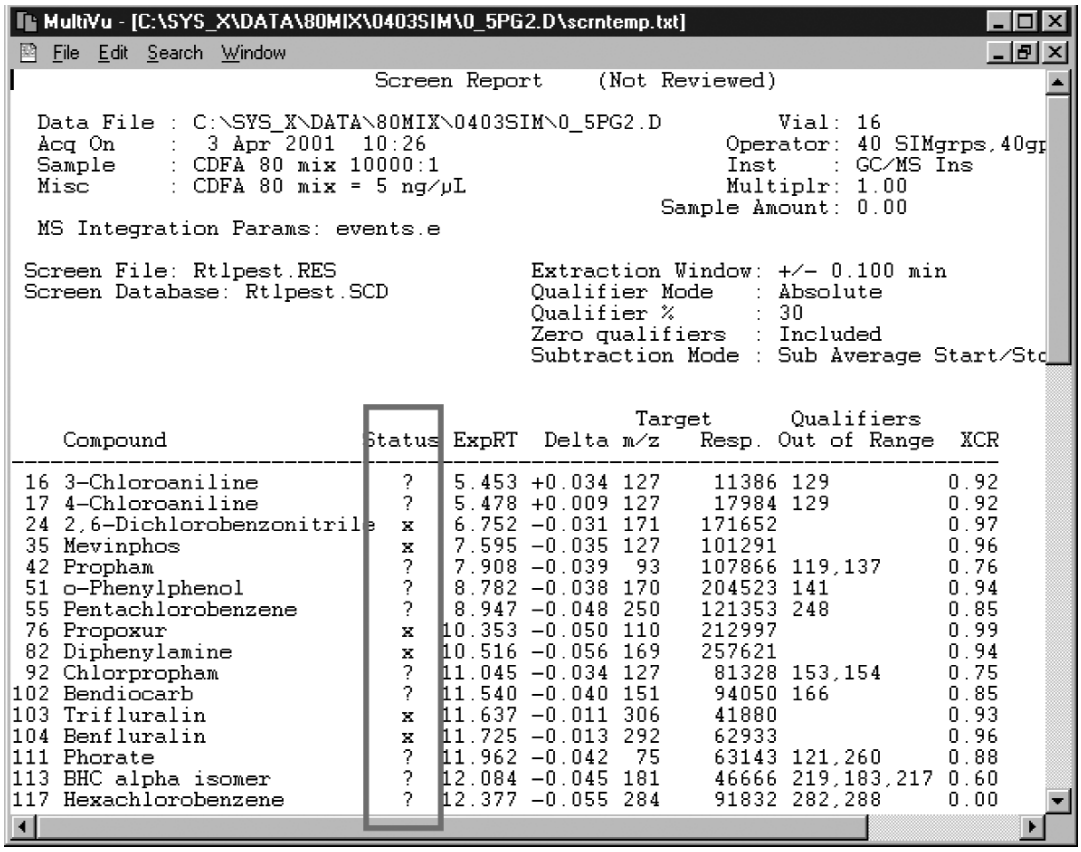


Figure 9. Ion chromatograms of 100-µL chlorthal-dimethyl injected at 0.05 pg/µL. The top portion was from a full Scan run and the bottom portion was from a SIM run.

Target Compound Screening

Combing RTL and the G1049A MSD RTL Pesticide Database/Library, a user can screen for 567 pesticides and suspected endocrine disrupters from any Scan run [5]. A user can screen a subset of the library with improved sensitivity using a SIM method. The MSD ChemStation can generate a 567-compound screening report automatically in less than 30 seconds. Figure 10 is a report of the 0.5 pg/µL sample (25 µL injected in SIM mode) that lists the "probable hits" (marked with an x) and "possible hits" (marked with a ?). All target compounds at this 12.5 pg on column level were found by the software.



MultiVu - [C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D\scrntemp.txt]

File Edit Search Window

Screen Report (Not Reviewed)

Data File : C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D Vial: 16
Acq On : 3 Apr 2001 10:26 Operator: 40 SIMgrps,40gp
Sample : CDFA 80 mix 10000:1 Inst : GC/MS Ins
Misc : CDFA 80 mix = 5 ng/µL Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: events.e

Screen File: Rtlpest.RES Extraction Window: +/- 0.100 min
Screen Database: Rtlpest.SCD Qualifier Mode : Absolute
Qualifier % : 30
Zero qualifiers : Included
Subtraction Mode : Sub Average Start/Sto

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
16	3-Chloroaniline	?	5.453	+0.034	127	11386	129	0.92
17	4-Chloroaniline	?	5.478	+0.009	127	17984	129	0.92
24	2,6-Dichlorobenzonitrile	x	6.752	-0.031	171	171652		0.97
35	Mevinphos	x	7.595	-0.035	127	101291		0.96
42	Propham	?	7.908	-0.039	93	107866	119,137	0.76
51	o-Phenylphenol	?	8.782	-0.038	170	204523	141	0.94
55	Pentachlorobenzene	?	8.947	-0.048	250	121353	248	0.85
76	Propoxur	x	10.353	-0.050	110	212997		0.99
82	Diphenylamine	x	10.516	-0.056	169	257621		0.94
92	Chlorpropham	?	11.045	-0.034	127	81328	153,154	0.75
102	Bendiocarb	?	11.540	-0.040	151	94050	166	0.85
103	Trifluralin	x	11.637	-0.011	306	41880		0.93
104	Benfluralin	x	11.725	-0.013	292	62933		0.96
111	Phorate	?	11.962	-0.042	75	63143	121,260	0.88
113	BHC alpha isomer	?	12.084	-0.045	181	46666	219,183,217	0.60
117	Hexachlorobenzene	?	12.377	-0.055	284	91832	282,288	0.00

Figure 10. Typical report from the GC/MS pesticide screener showing probable "hits" (marked with an x) and possible hits (marked with a ?). Other information includes the library retention time followed by the RT difference in this chromatogram, the target ion, its abundance, out of range qualifier(s), and a cross correlation value with the library spectrum.

Conclusions

Using the information (compound names, retention times, and ion masses) in the RTL pesticide database, a SIM method of 80 target compounds can be created in less than 2 hours without running any analyses. The examples show that both LVI and SIM are effective techniques to decrease the quantitation limit of target compounds from sub-ppm to ppt.

Any lab can decrease the quantitation limit by a factor of 100 without any hardware modification. Lowering the quantitation limit from 500 pg down to 5 pg on column can be done using a SIM method and RTL. By adding LVI to the system, target compounds in femtogram/µL can be quantified.

Acknowledgement

The author would like to acknowledge Alex Chung and Mark Lee at CDFA for providing the pesticide mixture used in this study.

References

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2. Bill Wilson, Philip L. Wylie, and Matthew S. Klee, *Large Volume Injection for Gas Chromatography Using a PTV Inlet*, Agilent Technologies Application note, 5965-7770E, printed February 2000, www.agilent.com/chem

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5. Harry Prest, Philip L. Wylie, Ken Weiner, and Doug Agnew, *Efficient Screening for Pesticides and Endocrine Disrupters Using the 6890/5973 GC/MSD System*, Agilent Technologies Application note, 5968-4884E, printed December 1999, www.agilent.com/chem

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Agilent Technologies

Analysis of Simazine, Thiobencarb, and Thiuram by Liquid Chromatography/Mass Spectrometry

Application

Environmental

Author

Hiroki Kumagai

Abstract

A liquid chromatography/mass spectrometry method using electrospray ionization in positive ion mode was successfully applied to the sensitive and simultaneous determination of the pesticides Simazine, Thiobencarb, and Thiuram.

Background

In recent years, the potential contamination of water supplies by runoff of many kinds of pesticides from golf courses and agricultural fields has become a societal problem. Many governments have established guidelines for pesticide use and water quality standards to limit such contamination. In Japan, the concentration limits in drinking water for the pesticides Simazine, Thiobencarb, and Thiuram are 3, 20, and 6 ppb, respectively.

Typically, gas chromatography-mass spectrometry (GC/MS) is used to determine Simazine and Thiobencarb in drinking water, while Thiuram is determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. However, the Thiuram method used to date has problems with both selectivity and sensitivity. A better method of analysis is needed for this chemical. Such a method is described below.

Method

- Instrument: Agilent 1100 Liquid Chromatograph/Mass Spectrometer (LC/MS) with electrospray ionization (ESI) positive ion mode
  Drying gas: N_2 (8 L/min, 350 °C)
  Nebulizer: N_2 40 psi
  Fragmentor: 40 V (Thiuram), 70 V
  Mass range: 100–500 amu
- LC Conditions:
  Mobile phase A: CH_3OH/30 mM CH_3COONH_4 (50/50)
  Mobile phase B: CH_3OH
  Gradient: 0 % to100 % B in 20 min
  Flow rate: 0.2 mL/min
  Oven temperature: 40 °C
  Injection volume: 50 µL
- Column: Inertsil ODS3, 3.1 mm id × 250 mm long × 5 µm

Sample Analysis

All three pesticides were determined simultaneously using the Agilent 1100 LC/MS. The following figures illustrate both the sensitivity and applicability of this method.

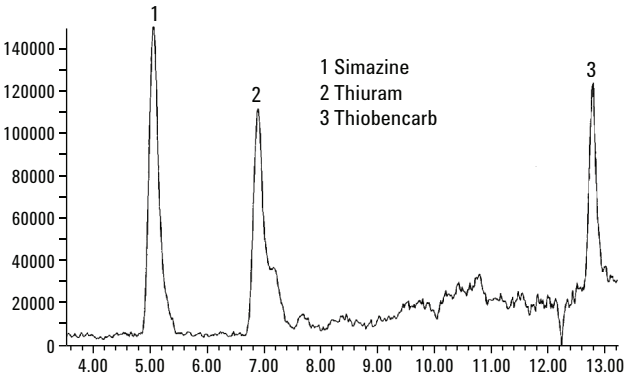

Figure 1. Total ion chromatogram of target pesticides, each at 5 ng.

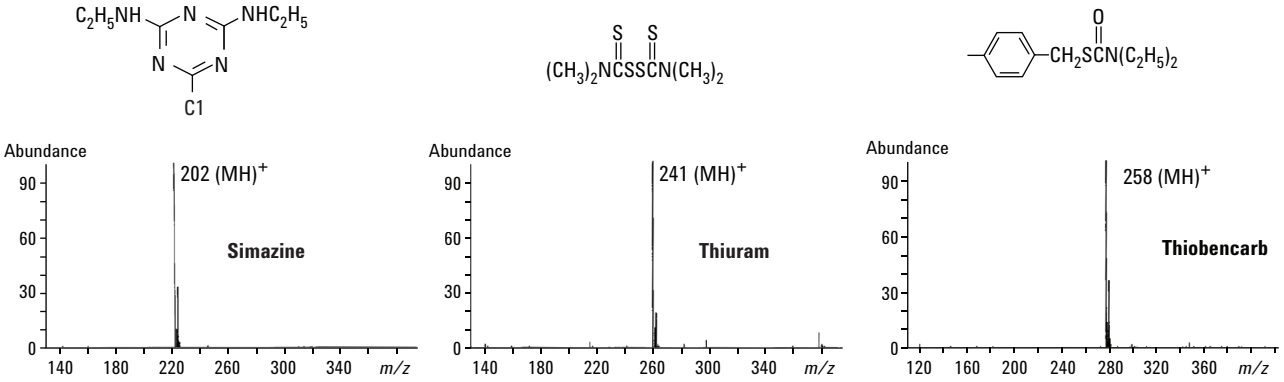

Figure 2. Mass spectra of target pesticides.

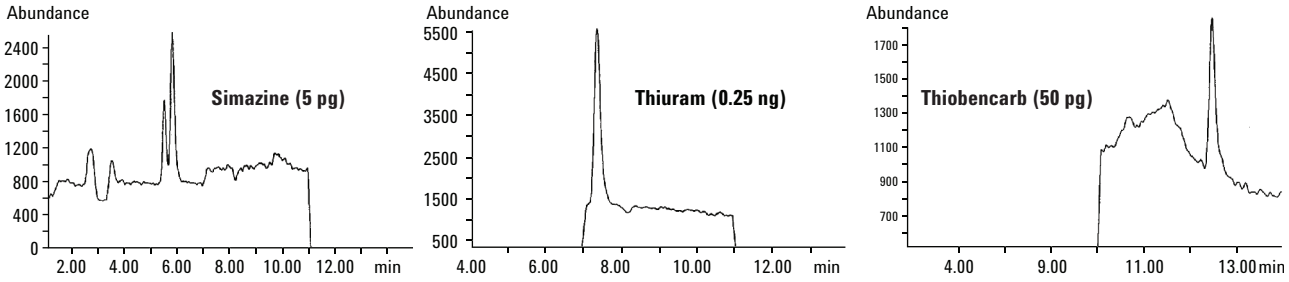

Figure 3. SIM chromatograms of target pesticides.

Conclusion

The LC/MS method described above is suitable for the simultaneous determination of the pesticides Simazine, Thiuram, and Thiobencarb. The peaks are well separated with detection limits of 0.02, 2.5, and 1 ppb, respectively, approximately 1/10 of the Japanese concentration limits.

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www.agilent.com/chem

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The Analysis of Organophosphate Pesticides by LC/MS

Application

LC-MS

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Abstract

Organophosphate pesticides were readily analyzed using liquid chromatography-mass spectrometry with electrospray ion source. Sensitivity and selectivity were significantly better than using a diode-array UV detector.

Overview

Liquid chromatography-mass spectrometry (LC-MS) is rapidly becoming a routine technique for efficient trace analysis of polar pesticides in various types of samples. In comparison to existing methodologies, such as gas chromatography-mass spectrometry (GC-MS) and ultraviolet (UV) detection, LC-MS considerably simplifies cleanup procedures, reducing both time of analysis and method development time.[1]

LC-MS with an electrospray ion (ESI) source avoids the thermal degradation of labile pesticides encountered with GC and eliminates the need for preliminary derivatization to increase compound volatility.

Additionally, LC-MS provides unequivocal identification of each pesticide, even if the pesticide was not completely resolved from neighboring eluants. Traditional UV detection cannot provide the required specificity because many of the pesticides within the same class exhibit similar UV spectra.

Sample case

A mixture of organophosphate pesticides and an internal standard were analyzed using an Agilent 1100 LC/MS with an ESI source (Table 1).

Table 1. Mixture of Organophosphate Pesticides

Elution order	Compound	$[M+H]^+$	Concentration µg/mL
1	Mevinphos isomer 1	225	0.2
2	Dimethoate	230	0.5
3	Mevinphos isomer 2	225	0.5
4	Dichlorvos	221	0.5
5	Azinphos methyl	318	0.05
6	Parathion methyl	264	0.2
7	Malathion	331	0.5
8	Diazinon	305	0.2
9	Triphenyl orthophosphate*	327	1.0
10	Parathion ethyl	292	0.1
11	Phorate	261	0.1
12	Reldan	322	0.5
13	Ronnel	321	0.1
14	Terbuphos	289	0.2
15	Dursban	350	0.1
16	Ethion	385	0.2
17	Temephos	467	0.1

* Internal standard

Method summary

- Column 2.1 mm id × 5 cm long, filled with 3.5 µm particles, C18 chemistry
- 20 mM ammonium acetate vs. acetonitrile mobile phase gradient
  - 5 % to 95 % acetonitrile in 4 minutes
  - Hold 2 minutes
- Splitless 400 µL/min flow
- 3 µL injection volume
- Scan data 120 to 600 m/z
- SIM data as per Table 1. 95 msec dwell/ion in two groups

Results

Simultaneous UV (220 nm) and MS detector outputs are compared in Figure 1. The MS plot is a composite of all the individual extracted ion chromatograms. Each was obtained at the $[M+H]^+$ value given in Table 1, and are separated and stacked in Figure 2 for easy comparison.

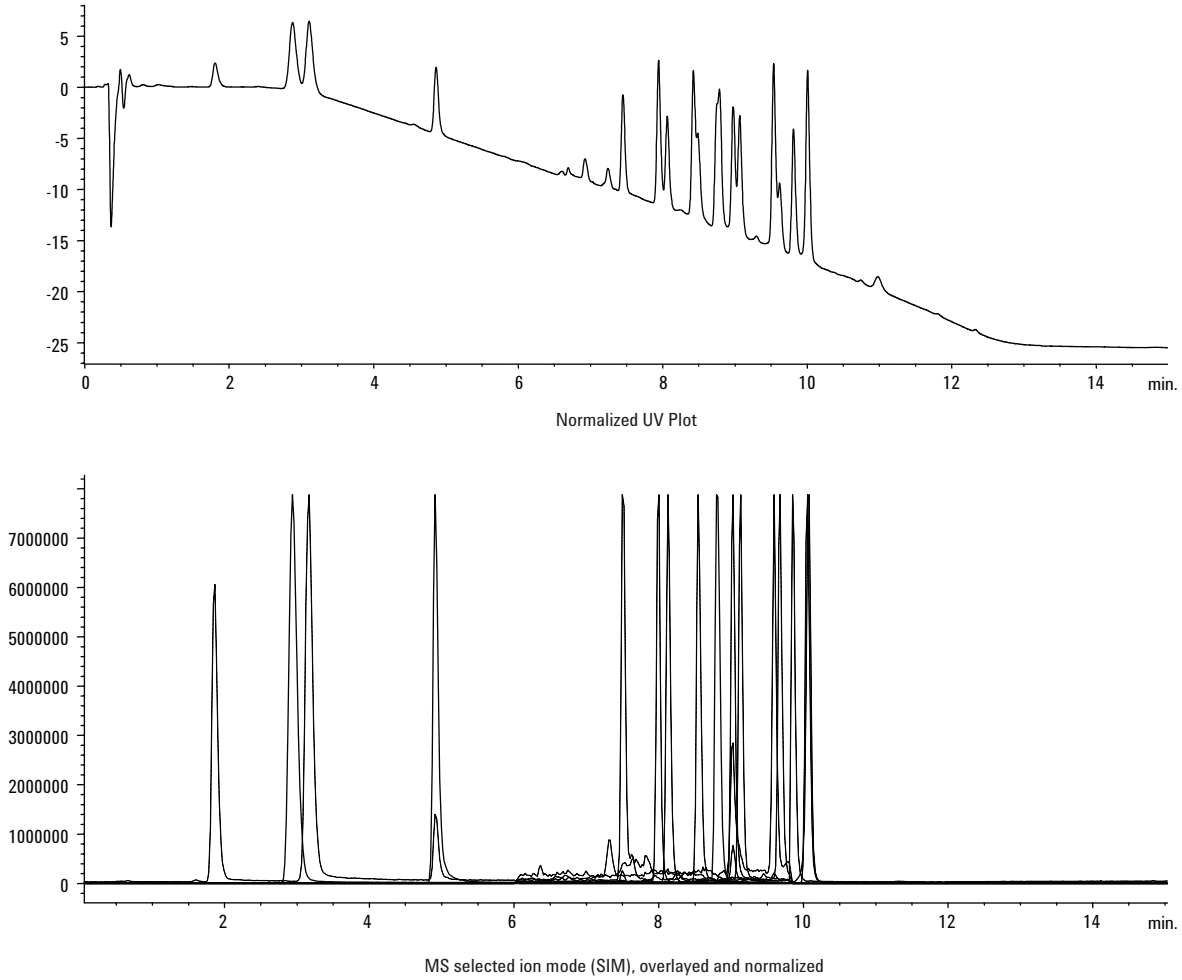

Figure 1. Comparison of UV and MS chromatograms.

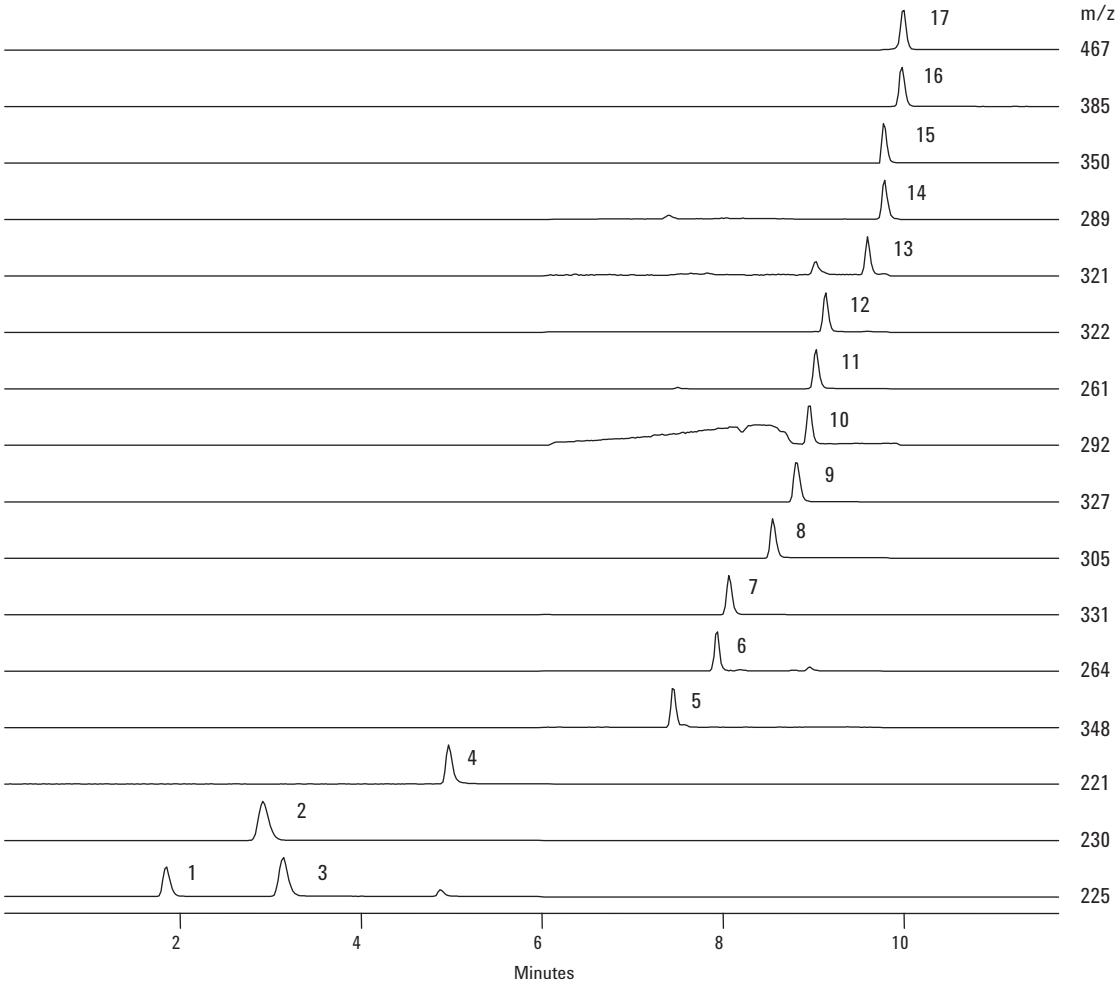

Figure 2. Stacked normalized extracted ion chromatograms for compounds 1 through 17.

Figures 3 through 6 show the resulting normalized mass ion spectra for each compound included in Table 1.

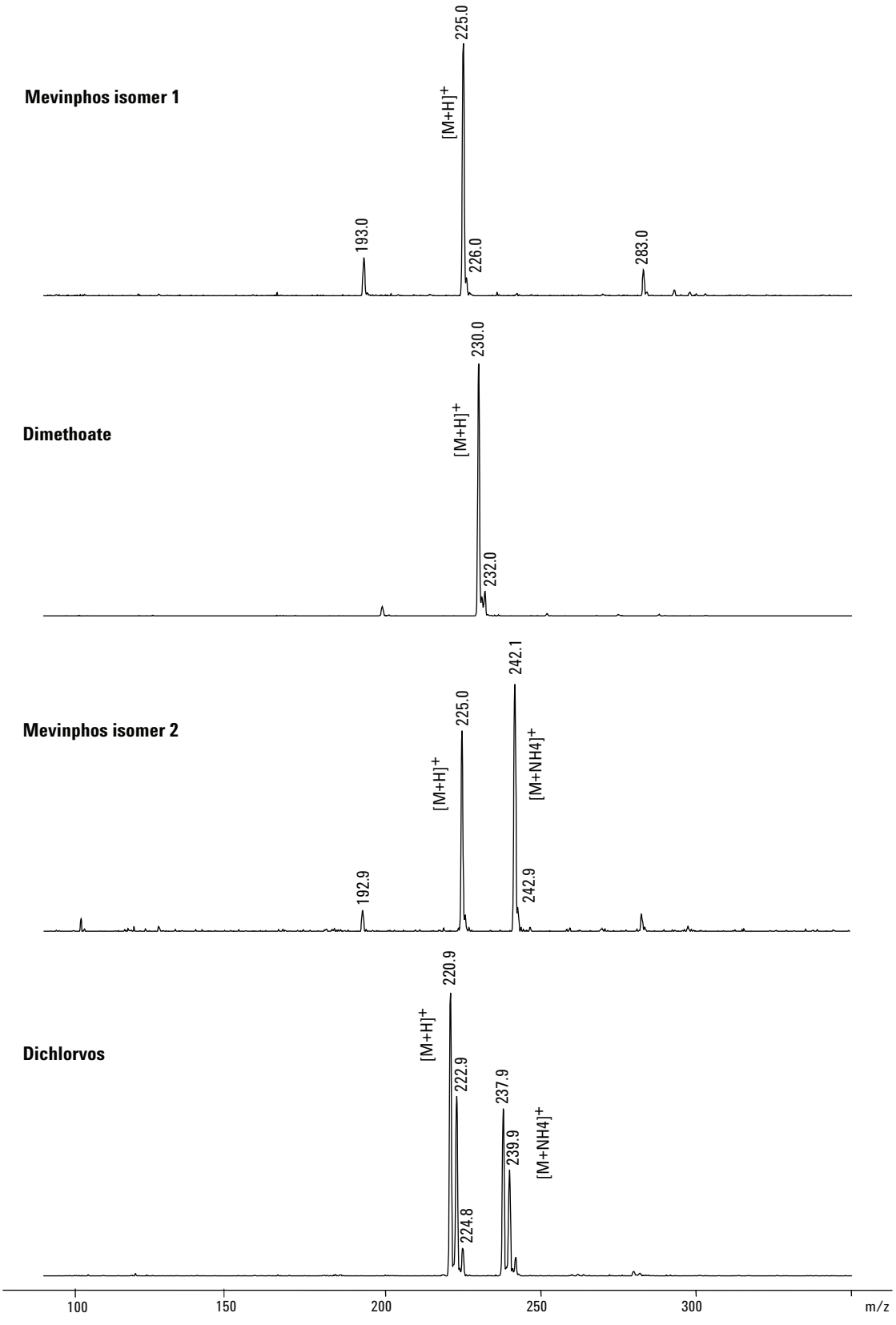

Figure 3. Stacked normalized ion mass spectra for compounds 1 through 4.

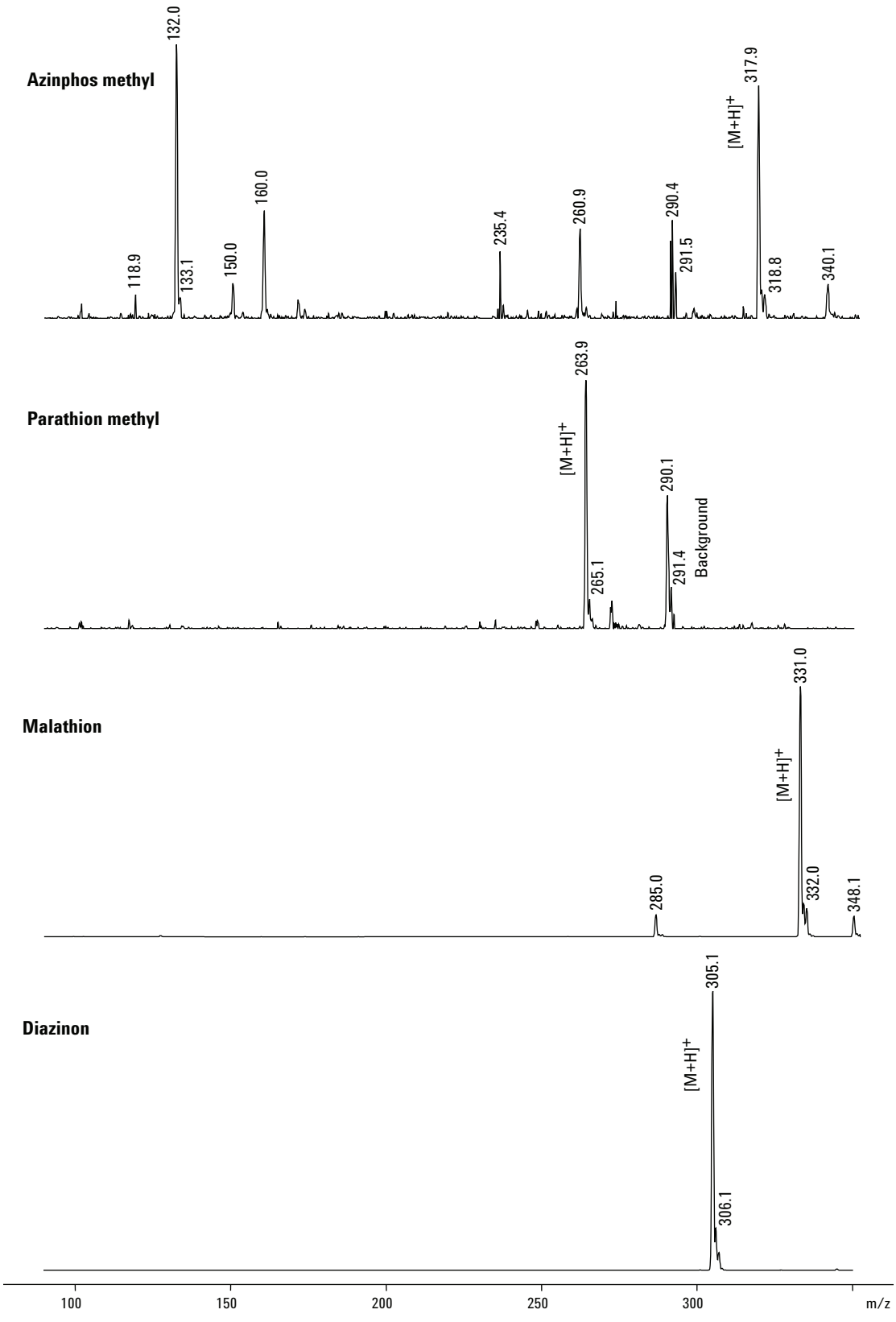

Figure 4. Stacked normalized ion mass spectra for compounds 5 through 8.

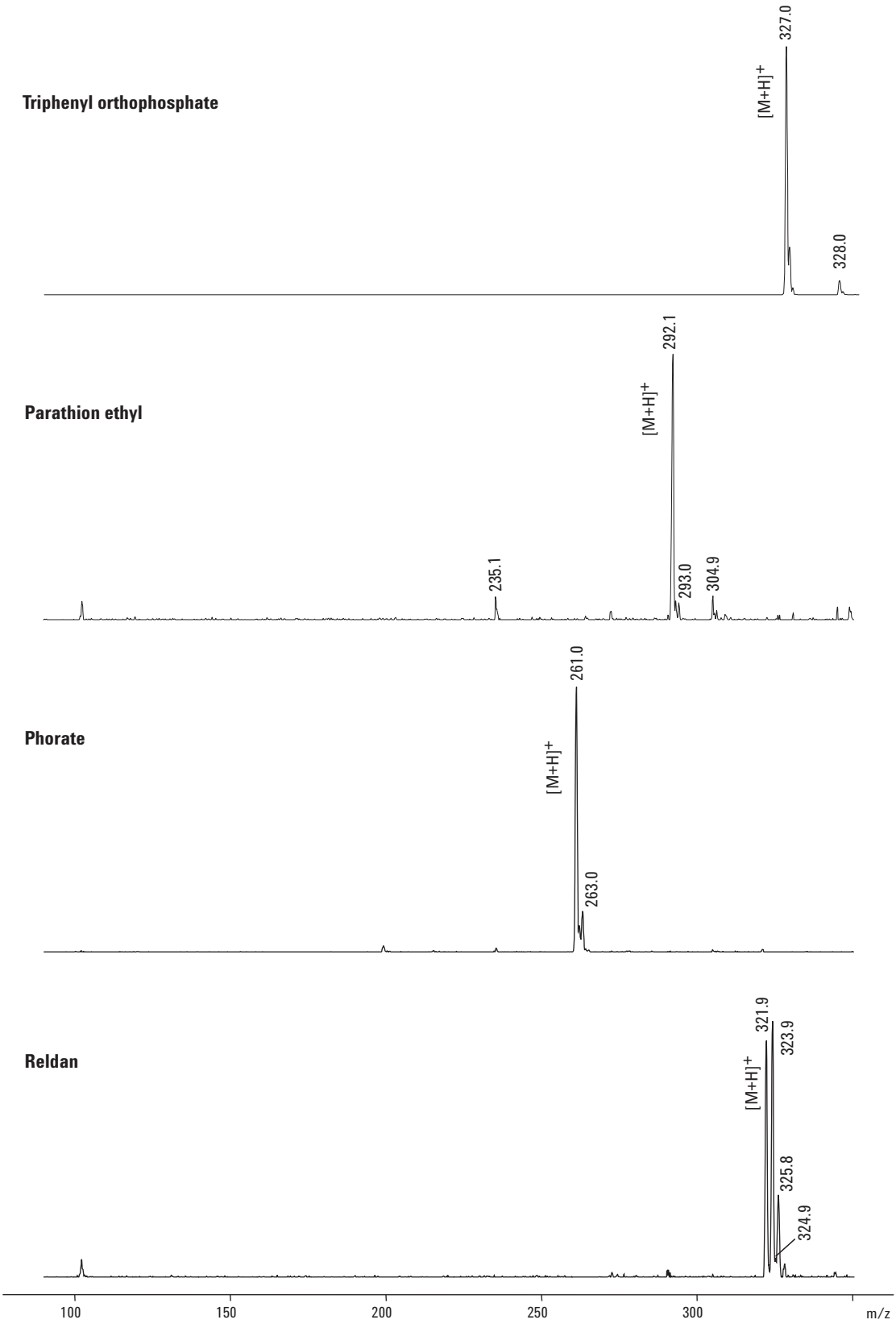

Figure 5. Stacked normalized ion mass spectra for compounds 9 through 12.

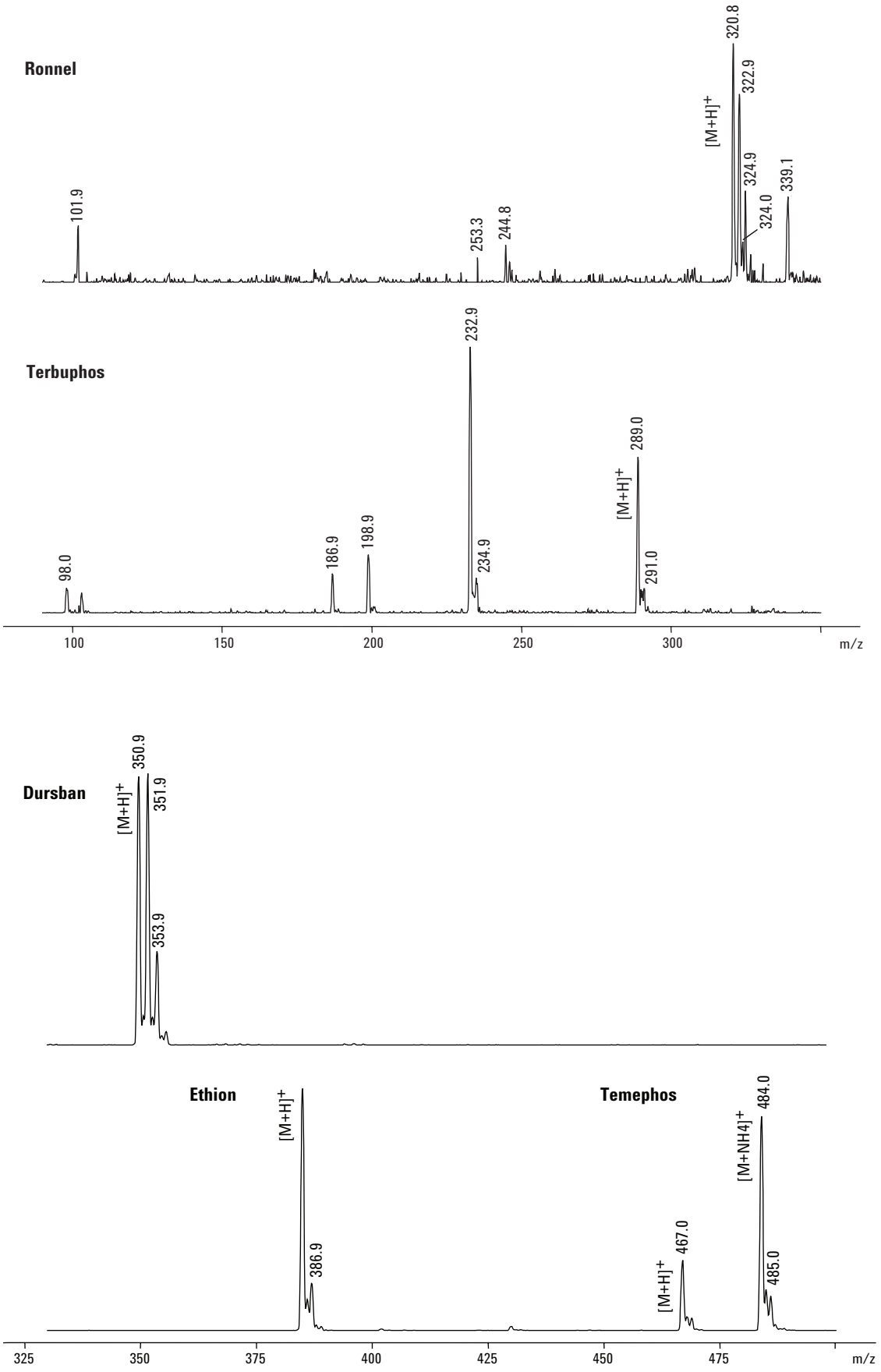

Figure 6. Stacked normalized ion mass spectra for compounds 13 through 17.

Conclusions

When determining organophosphate pesticides using LC-MS with an ESI source:

- All the tested organophosphate pesticides ionized well and gave definite $[M+H]^+$ ions
- Sensitivity and selectivity are significantly better than using diode-array UV detector
- Overall chromatography and analysis is simple and straightforward
- Positive identification and quantification are performed using integrated software

For more information, contact your local Agilent sales representative, or visit www.agilent.com.

Reference

1. Elbert Hogendoorn and Peit van Zoonen, *Journal of Chromatography A*, 892 (2000) 435-453.

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Agilent Technologies

Fast Screening of Pesticides and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part II

Application

Gas Chromatography
May 2000

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Abstract

Agilent Technologies' new, fast GC/MSD method can significantly speed up the screening of pesticides. Agilent's GC Method Translation software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on the standard 42-min method. A 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column was used to increase analysis speed up to fourfold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the effect of scaling on the signal-to-noise ratio.

Key Words

RTL, pesticide, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Analysts want faster analyses to improve laboratory productivity. Often, when speeding up GC methods, an analyst will trade resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD).

Agilent Technologies has developed new techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) and a new mass spectral library that contains the locked retention times and characteristic ions for 567 of the most common pesticides and endocrine disrupters of concern worldwide. A GC/MSD method was developed based on the standard 42-min method[1] to screen for all 567 of the most common analytes. A specific combination of column stationary phase, carrier gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when analyzing extracts containing significant sample matrix content because of overlapping peaks and noisy baselines.

The new screening tool, integrated within Agilent's ChemStation for MSD, searches for all 567 compounds. It first checks and integrates four characteristic ions within the expected time window and then prints a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In Part I of the MSD fast screening application brief[3], a 10 m × 0.1 mm × 0.1 µm Agilent HP-5 column was used to increase analysis speed up to fourfold. In this application brief, a 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column was used. The faster methods were scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software. Because scaling was exact, these faster methods can be used with precisely-scaled pesticide libraries, making the screening process even more powerful and adaptable to individual needs.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of MTL software data entry showing the original conditions and the new chromatographic conditions for a fourfold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[4], also is found in the table. In this study, a turbo pump was used, which could handle the 3.8 mL/min carrier flow. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

General chromatographic conditions are listed in table 1. The standard used was a mixture of 26 pesticides at 10 ppm. A 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column (part number 19091S-431) was used. The head pressure determined by the method translation software (18 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by 4) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD.

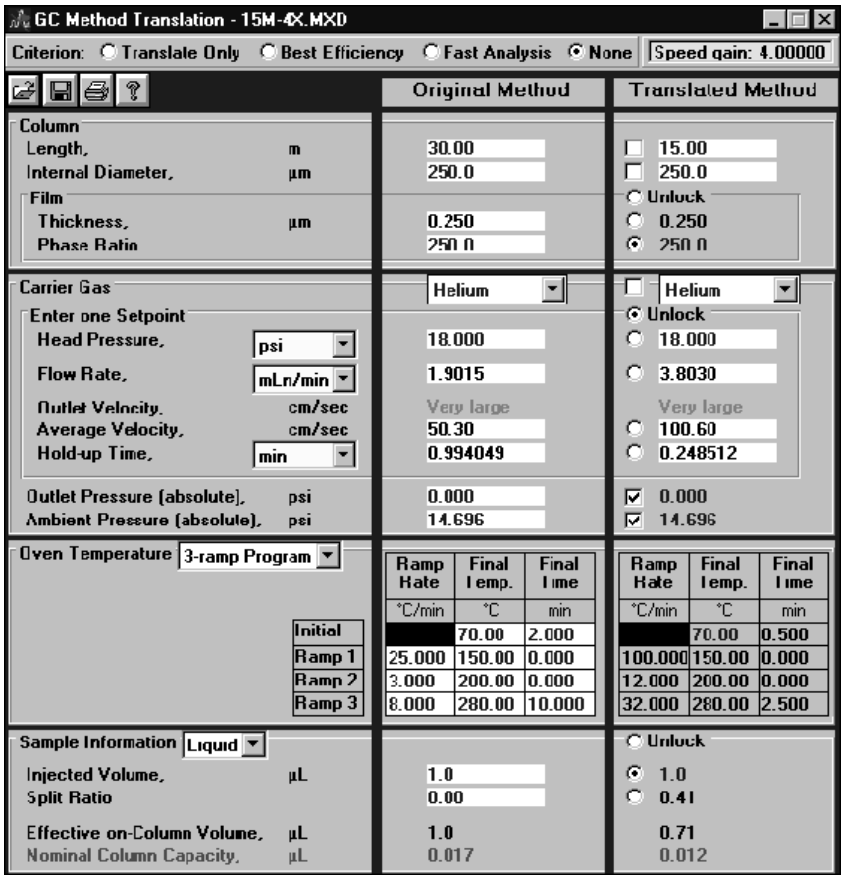

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a 4X speed gain translation.

This process (first translate the method then lock the retention times) was repeated for the 2.5X time reductions.

Figure 2 shows the results of the shortened analysis times. The three chromatograms look extremely similar, except that the time axis is scaled proportionally. Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer). Using the same injection method (1-µL splitless), the peak heights of the faster runs were twice those from the original

Table 1 Chromatographic Conditions

Speed	**Onefold**	**Two and a half fold**	**Fourfold**
GC	110 V	220/240 V	
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (P/N 19091S-433)	15 m × 0.25 mm × 0.25 µm HP-5MS (P/N 19091S-431)	
Injection mode	Splitless	Splitless	
Column head pressure	18.0 psi	5.74 psi	18.0 psi
Column flow (mL/min)	1.9	1.49	3.8
Inlet control mode	Constant pressure	Constant pressure	
Carrier gas	Helium	Helium	
Injector Temp.	250 °C	250 °C	
Oven Temp.	70 (2 min)	70 (0.8 min)	70 (0.5 min)
Ramp 1	25 °C/min	62.5	100
	150 (0 min)	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	7.5	12
	200 (0 min)	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	20	32
	280 (10 min)	280 (4 min)	280 (2.5 min)
Oven equilibration	2 min	2 min	
Injection volume	1 µL	1 µL	
Liner	5183-4647	5183-4647	

MS Conditions (Turbo pump)			
Solvent delay	3 min	1.44 min	0.9 min
Tune file	Atune.u	Atune.u	
Low mass	35 amu	35 amu	
High mass	500 amu	450 amu	
Threshold	150	250	
Sampling	2	2	1
Scans/sec	3.15	3.50	6.54
Quad Temp.	150 °C	150 °C	
Source Temp.	230 °C	230 °C	
Transfer line Temp.	280 °C	280 °C	
Acquisition mode	Scan (EI)	Scan (EI)	

analysis. A faster oven ramp and the shorter column made the peaks narrower and higher, so an improvement in the signal-to-noise ratio is realized with the faster methods.

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of the standard 42-min GC/MSD pesticide method. Run time was shortened to 10.5 minutes using a fast oven ramp rate and a 15-meter, 250-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can be scaled accurately to correspond to the faster analyses.

References

1. 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," Application Note 228-401, Agilent publication number 5967-5820E, May 1998.
2. H. Prest, P. L. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the HP 6890/5973 GC/MSD System," Agilent publication number 5968-4884E, April 1999.
3. C. K. Meng and M. Szelewski, "Fast Screening of Pesticide and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System", Agilent publication number 5968-9220, January 2000.
4. H. Prest, "GC Column Selection and Pumping Considerations for Electron and Chemical Ionization MSD operation," Agilent publication number 5968-7958E, November 1999.

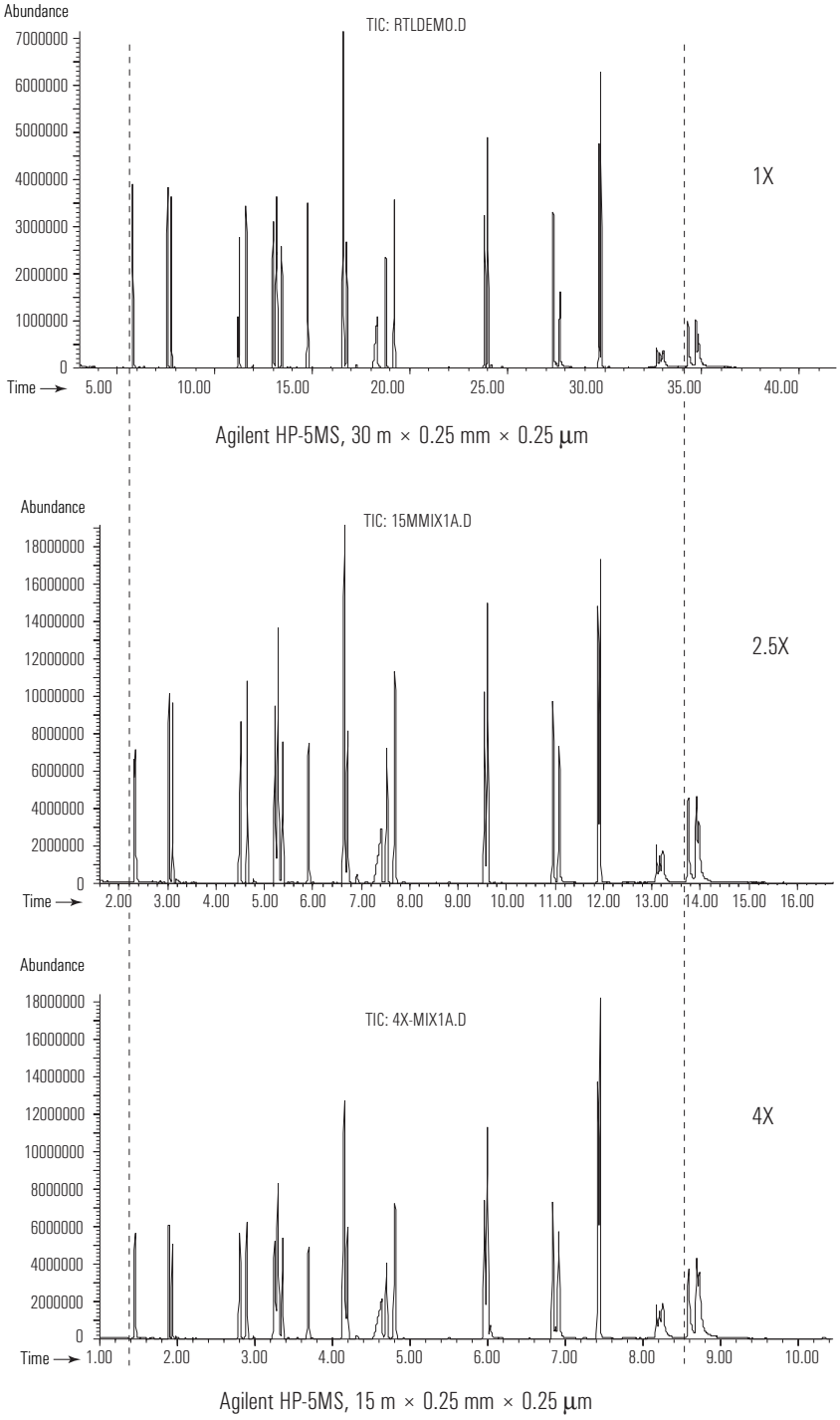

Figure 2. The TICs of the 2.5X and 4X speedups. The standard analysis (1X) was 42 minutes long.

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Agilent Technologies
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Fast Screening of PCB Congeners Using the Agilent 6890/5973N GC/MSD System

Application

Gas Chromatography

May 2000

Authors

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Abstract

Agilent Technologies' fast GC/MSD method can significantly speed up the screening of PCB congeners. Agilent's GC method translation software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on a standard 42-min method. A 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column was used to increase analysis speed up to four-fold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the affect of scaling on the signal-to-noise ratio.

Key Words

RTL, PCB, polychlorinated biphenyls, congeners, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Polychlorinated biphenyls (PCBs) are a group of 209 individual compounds (known as congeners) with varying harmful effects. Chronic (long term) exposure to some PCB formulations by inhalation in humans results in respiratory tract symptoms, gastrointestinal effects, mild liver effects, and effects on the skin and eyes such as chloracne, skin rashes, and eye irritation.

PCBs are no longer produced in the United States and are no longer used in the manufacture of new products. Smaller amounts of PCBs may be released to the air from disposal sites containing transformers, capacitors, and other PCB wastes, incineration of PCB-containing wastes, and improper disposal of the compounds to open areas. Today, PCBs are still detected in water and soil due to the environmental recycling of the compound. PCBs have been detected in foods and they bio-accumulate through the food chain, with some of the highest concentrations found in fish.

The analysis of PCBs normally is accomplished using GC with an electron-capture detector (ECD). Because of the drastically different toxicity of the different congeners, it is of great interest to identify the individual congeners using a mass spectrometer (MS).

Agilent Technologies has developed techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) and a mass spectral library that contains the locked retention times and characteristic ions for all 209 PCB congeners. A GC/MSD method was developed based on a standard 42-min method[1] to screen for all congeners. A specific combination of column stationary phase, carrier-gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when the isomers have the same mass spectra.

The screening tool, integrated within Agilent's ChemStation for MSD software, searches for all 209 congeners by first checking and integrating the

expected target ion within the expected time window. If the target ion is found, the software will then search and integrate the three qualifier ions within the expected time window. Last, the software will print out a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In order to improve laboratory productivity, we scaled the method for four-fold speed-up. While a 30-m × 0.25-mm × 0.25-µm Agilent HP-5MS column is used for standard speed, a 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column is used for the four-fold speed. These faster methods were able to be scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software.

Often, when speeding up GC methods, an analyst trades resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD). However, because scaling was exact, the faster methods can be used with precisely scaled congener libraries, making the screening process even more powerful and adaptable to individual needs.

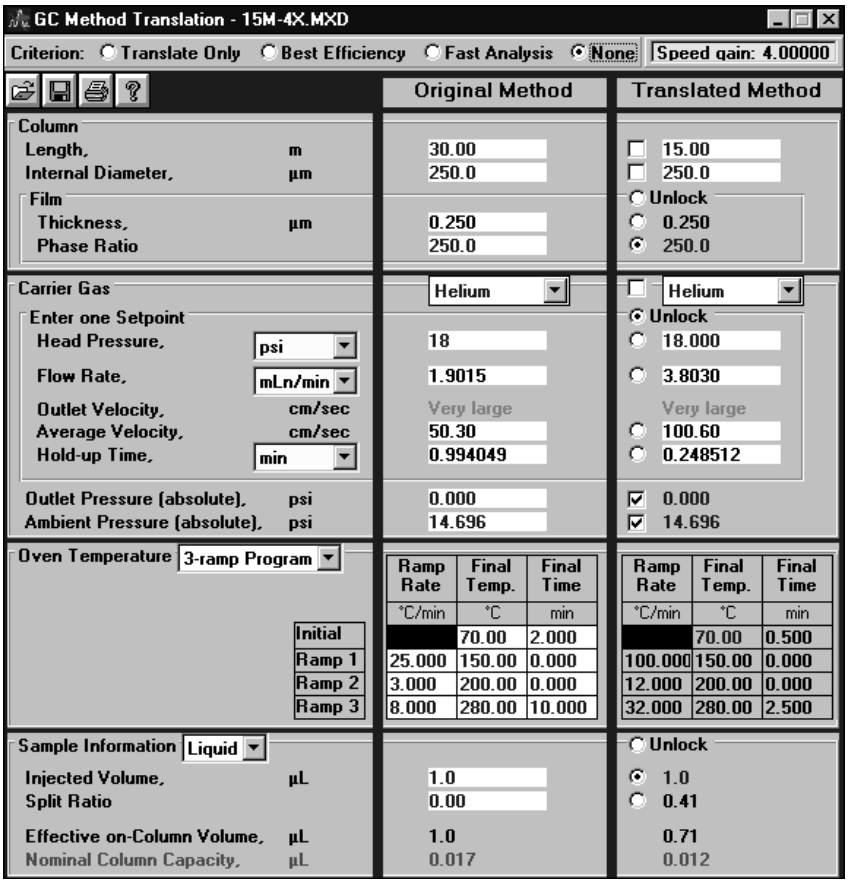

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a 4X speed-gain translation.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of the MTL software data entry showing the original conditions and the new chromatographic conditions for a four-fold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[3], also is found in the table. In this study, a turbo pump that could handle the 3.8 mL/min carrier flow was used. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

General chromatographic conditions are listed in Table 1. The RTL standard used was a mixture of pesticides and PCB congeners at 10 ppm. A 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column (part number 19091S-431) was used. The head pressure determined by the method translation software (18 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by four) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD.

A very important modification to the MS method is changing the default values of "Use mass range from" to **–0.50** to **+0.50** amu (the default values are –0.3 to +0.7). The changes can be made from the "Extracted Ion Chromatograms…" dialog box selected from the "Chromatogram" on the menu bar.

Figure 2 shows the results of the shortened analysis times. The two chromatograms look extremely similar, except that the time axis is scaled proportionally. It is interesting to note that the last peak in the 4X analysis came out *before the first peak of the 1X analysis.* Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time

reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer).

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of a standard 42-min GC/MSD method. The run time was shortened to 10.5 minutes using a fast oven ramp rate and a 15-meter 250-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can be scaled accurately to correspond to the faster analyses. The GC/MSD conditions used are the same as the fast pesticide method[4], which allows for screening pesticides and PCB congeners in a single analysis.

References

1. 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," Application Note 228-401, Agilent publication number 5967-5820E, May 1998.
2. H. Prest, P. L. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the HP 6890/5973 GC/MSD System," Agilent publication number 5968-4884E, April 1999.
3. H. Prest, "GC Column Selection and Pumping Considerations for Electron and Chemical Ionization MSD operation," Agilent publication number 5968-7958E, November 1999.
4. C. Kai Meng and Michael Szelewski, "Fast Screening of Pesticides and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part II", Agilent publication number 5980-1057E, May 2000.

Table 1. Chromatographic Conditions

Speed	Standard	Four-fold
GC	110 V	220/240 V
Column	30-m × 0.25-mm × 0.25-µm Agilent HP-5MS (part number 19091S-433)	15-m × 0.25-mm × 0.25-µm Agilent HP-5MS (part number 19091S-431)
Injection mode	Splitless	Splitless
Column head pressure	18.0 psi	18.0 psi
Column flow (mL/min)	1.9	3.8
Inlet control mode	Constant pressure	Constant pressure
Carrier gas	Helium	Helium
Injector Temperature	250 °C	250 °C
Oven Temperature	70 (2 min)	70 (0.5 min)
Ramp 1	25 °C/min	100
	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	12
	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	32
	280 (10 min)	280 (2.5 min)
Oven equilibration	2 min	2 min
Injection volume	1 µL	1 µL
Liner	5183-4647	5183-4647
MS Conditions (Turbo pump)		
Solvent delay	3 min	0.9 min
Tune file	Atune.u	Atune.u
Low mass	50 amu	50 amu
High mass	550 amu	550 amu
Threshold	200	200
Sampling	3	1
Scans/sec	1.52	5.56
Quad Temperature	150 °C	150 °C
Source Temperature	230 °C	230 °C
Transfer line Temperature	280 °C	280 °C
Acquisition mode	Scan (EI)	Scan (EI)

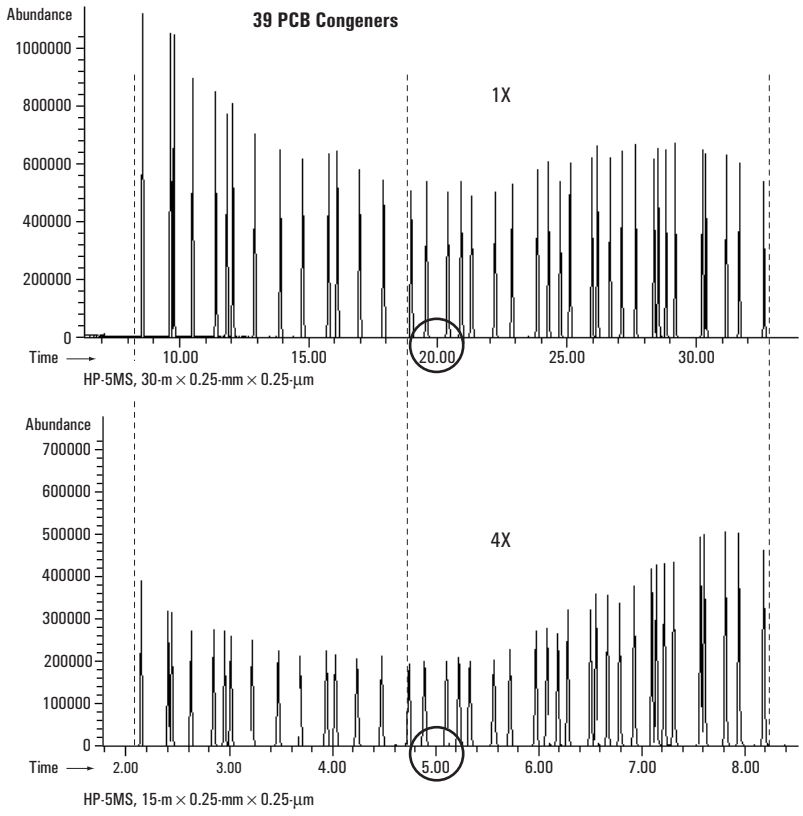

Figure 2. The TICs of the standard speed and fast (4X) analyses. The standard analysis (1X) was 42 minutes long.

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Agilent Technologies
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Validation Analysis of EPA CLP Target Organochlorine Pesticides with the Agilent 6890 Series GC and Micro-ECD

Application

Gas Chromatography

February 1998

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Abstract

Generating environmental data for organochlorine pesticides in various matrices can be time-consuming for laboratories and engineering firms. To keep a gas chromatograph/electron capture detector (GC/ECD) system operating within control limits, precious analytical time must be spent on tasks such as recalibration, reinjection of samples, detector cleaning, and reintegration of chromatographic peaks. These tasks take time away from running billable samples and adversely affect laboratory throughput.

The Agilent 6890 Series Micro-ECD used in this study shows improved performance in several key areas: increased linear working range (greater than 4 orders of magnitude for some components), increased sensitivity (organochlorine pesticides at sub-ppb levels), increased stability, and increased resistance to contamination.

Introduction

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are found worldwide in the environment. Because many of these pesticides are suspected to be carcinogenic and/or endocrine hormone disrupters[1,2], determination of their presence in water, air, soil, and food is required by governmental agencies such as the U.S. EPA, the FDA, and the World Health Organization.

The U.S. EPA provides several comprehensive guidelines[3,4] and regulations[5,6] for analysis of OCPs and PCBs by gas chromatography with electron capture detectors (GC/ECD). These include EPA method 8081 for wastewater/solid wastes, EPA methods 505 and 508 for drinking water/water supplies, EPA method 608 for municipal and industrial discharges, and the Contract Laboratory Program (CLP) method for waste/clean-up sites. Most contract laboratories competing for the large number of potential CLP samples find that competition is strong and profit margins very low compared with other environmental methods.

CLP methods have very specific performance criteria that can be very time-consuming for laboratories to meet consistently. To keep a GC/ECD system operating within control limits, precious analytical time must be spent on tasks such as recalibration, reinjection of samples, detector cleaning, reintegration of chromatographic peaks, etc. Spending too much time with any of these tasks takes time away from running billable samples, and adversely affects the throughput and profitability of the laboratory.

In this study, the 6890 Series Micro-ECD greatly reduced the time required to meet CLP quality control criteria for CLP analysis of OCPs and PCBs. Validated results show four key improvements: increased linear working range (greater than 4 orders of magnitude), increased sensitivity (detecting OCPs at sub-ppb level), more stable calibration, and increased resistance to contamination (more robust, fast detector recovery and reduced maintenance).

Experimental

Water and soil samples were extracted after spiking with surrogates tetrachloro-m-xylene (TCX) and decachlorobiphenyl (DBC). Extracts of OCPs were analyzed in accordance with EPA CLP method OLM03.1[6]. Typically, a 1-L volume of water sample was extracted with methylene chloride by liquid-liquid extraction or a 30-g aliquot of soil/sediment sample extracted with 1:1 acetone/methylene chloride by sonication. These extracts were concentrated and solvent-exchanged into a 10-mL volume of hexane.

Working standards for checking linearity and CLP QA/QC criteria were prepared from certified standards (available commercially) in hexane, as described in the CLP method[5].

All analyses were performed using a 6890 Series GC with an automatic liquid sampler, a single split/splitless inlet, a pair of primary and confirmatory columns, and two 6890 Micro-ECDs. Instrument conditions are listed in table 1.

A sample extract or working standard (1 µL) was injected into the 6890 Series GC in the splitless mode. A guard column (equivalent to a 5-m retention gap, part no. 19095-60610) was used. It was connected to a "Y" glass butt connector that split the sample equally between the pair of columns.

Column A (an equivalent of the Agilent HP-608 column) was used as the primary analytical column, and column B (an equivalent of the Agilent HP-1701 column) was used as the confirmatory column, in accordance with the CLP method.

In the case of poor chromatography or a failing control limit for inlet degradation, routine maintenance was performed. This involved changing the inlet septum, installing a new inlet liner, and clipping a short piece of the retention gap. Columns were routinely conditioned to remove late-eluting column contaminants. When CLP criteria could not be met after routine maintenance, columns were replaced with new columns of the same type.

Table 1. Experimental Conditions

Sampler	Agilent 7673, 10-µL syringe, 1-µL injection
Inlet	Split/splitless; 200 °C, pulsed splitless mode (28 psi for 1 min)
Carrier	Helium, 16.8 psi (150 °C); 3.5 mL/min constant flow (each column)
Column	(1) 30 m, 0.53 mm id, 0.8-µm film DB-608, an equivalent of Agilent HP-608 (part no. 19095S-023)
	(2) 30 m, 0.53 mm id, 1.0-µm film RTX-1701, an equivalent of Agilent HP PAS-1701 (part no. 19095S-123)
Oven	150 °C (0.5 min); 5 °C/min to 280 °C (5–15 min).
Detector	330 °C; makeup gas: nitrogen, constant column and makeup flow (60 mL/min)

Results and Discussion

Sensitivity

Figures 1 and 2 show chromatograms of CLP target organochlorine pesticides on column A using the GC conditions listed in table 1. All 20 OCPs in the midpoint calibration standards (mix A and mix B) were baseline resolved with both the primary analytical column (column A) and the confirmation column (column B, shown in figure 3). The amount of individual OCPs in the midpoint calibration standard was 20–40 pg on-column (methoxychlor was 200 pg). Table 2 lists the concentration of midpoint calibration standards, peak identification, and the Contract Required Quantitation Limits (CRQLs)[6] for all CLP target OCPs.

Figures 1 and 2 also show good responses for dilute OCPs (0.25–0.5 pg on-column, 1/20th of the concentration of those for CRQLs). Quantitation at this level was easy with the micro-ECD; most OCPs exhibited a signal-to-noise ratio greater than 10 (see the lower chromatograms in figures 1 and 2). These results, confirmed by column B and the second micro-ECD (see figure 3), show that the 6890 Series Micro-ECD can easily detect low levels of OCPs (lower than 1/20 of those required by CLP). This is in good agreement with Channel and Chang[7], who reported detection of OCPs as low as 0.050 pg on-column. However, detection of this low level is not necessary because the CRQLs[5] range from 5 to 10 pg (methoxychlor at 50 pg) on-column (see table 2).

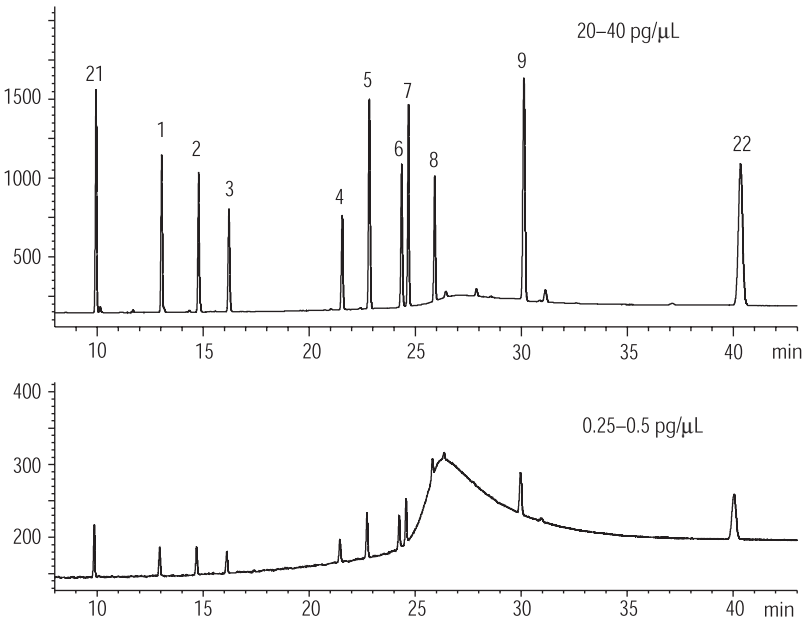

Figure 1. Pesticides in CLP calibration mix A on the primary column (A). 20 pg/µL (upper chromatogram) and 0.25 ng/µL (lower chromatogram) for methoxychlor (peak 9).

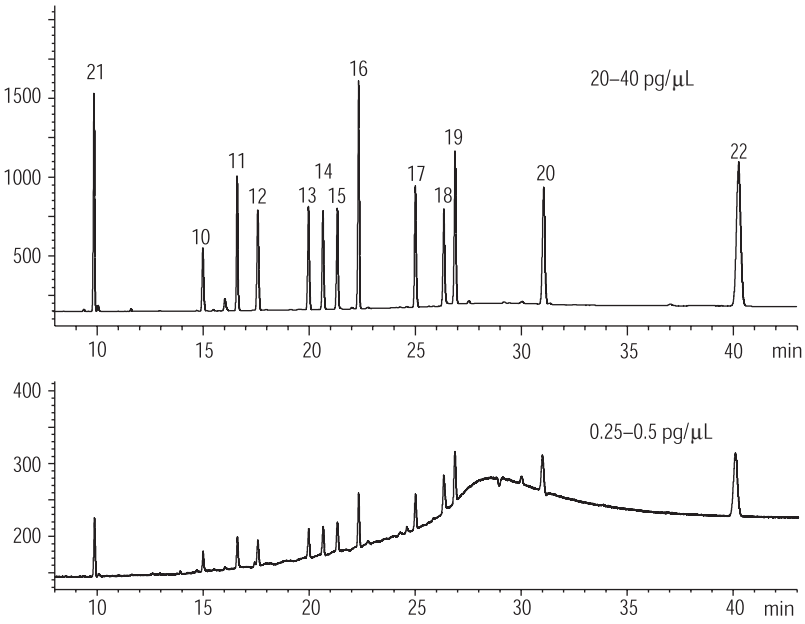

Figure 2. Pesticides in CLP calibration mix B on the primary column (A).

To ensure reliable results, three-point initial calibrations were routinely performed in accordance with CLP requirements using standards of 5, 20, and 80 pg/µL for lindane. Table 2 lists typical response factors and percent relative standard deviation (% RSD) for all CLP target OCPs. Typical % RSDs ranged from 2 percent for beta-BHC to 14 percent for endrin aldehyde, easily meeting the CLP criterion of 20.0 percent or less over the CLP calibration range.

Micro-ECD Linearity

Although classical electron capture detectors can provide sensitive detection, they are notorious for nonlinear response toward OCPs. For example, linearity is problematic for isomers of BHCs, particularly at the high concentration level. On the other hand, linearity as well as low response is problematic for methoxychlor, particularly at the low concentration level. These problems were not encountered using the 6890 Series GC system with micro-ECDs.

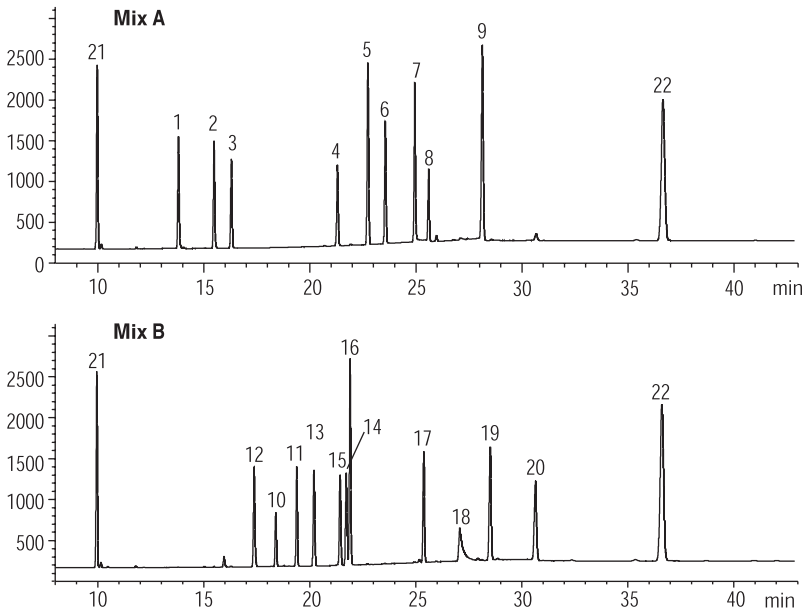

Figure 3. Pesticides on the confirmatory column (B). 20 to 40 pg/µL each, 200 pg/µL for methoxychlor.

Table 2. CLP Target Organochlorine Pesticides and Responses

	Peak	Pesticides	Mid-Level Standard pg/µL (4X)	CRQLs (on column) pg	Response Factors*	
					Average (peak height)	% Relative Standard Deviation
Mix A	1	alpha-BHC	20	5	23052	10.90
	2	gamma-BHC(lindane)	20	5	21729	6.51
	3	Heptachlor	20	5	17661	3.40
	4	Endosulfan I	20	5	15536	2.68
	5	Dieldrin	40	10	16204	4.83
	6	Endrin	40	10	10515	4.54
	7	4,4'-DDD	40	10	14334	5.06
	8	4,4'-DDT	40	10	12418	7.65
	9	Methoxychlor	200	50	4652	5.55
	21	TCX	20	50	16567	2.13
	22	DCB	40	10	5752	14.73
Mix B	10	beta-BHC	20	5	16190	2.40
	11	delta-BHC	20	5	10586	5.40
	12	Aldrin	20	5	20609	11.58
	13	Heptachlor epoxide	20	5	16482	7.01
	14	alpha-Chlordane	20	5	15929	5.20
	15	gamma-Chlordane	20	5	16527	5.69
	16	4,4'-DDE	40	10	15913	5.29
	17	Endosulfan II	40	10	16791	9.52
	18	Endrin aldehyde	40	10	8453	14.20
	19	Endosulfan sulfate	40	10	8926	5.59
	20	Endrin ketone	40	10	2144	3.39
	21	TCX	20	5	10114	3.01
	22	DCB	40	10	5667	14.97

* Typical three-point calibration from column A (concentrations: 1X, 4X, and 16X)

Linearity of the micro-ECD was determined by analyzing a series of dilutions of OCPs at concentrations ranging from 0.1 pg/µL to 3.2 ng/µL for lindane (see the 15-level calibration in table 3). For most OCPs, correlation coefficients were better than 0.99 over a concentration range greater than 5 orders of magnitude (0.1 to 3.2 pg/µL for lindane).

Table 3. Linearity Study

Pesticides		15-Point Calibration		10-Point Calibration			
		Concentration pg/µL	Correlation Coefficients	Concentration pg/µL	Response Factors		Correlation Coefficients
					Average	% Relative Standard Deviation	
Mix A	alpha-BHC	0.1 to 32,000	0.995	1 to 1,600	52,557	19.3	0.998
	Lindane	0.1 to 32,000	0.997	1 to 1,600	46,635	17.3	0.997
	Heptachlor	0.1 to 32,000	0.997	1 to 1,600	35,712	18.0	0.997
	Endosulfanl	0.1 to 32,000	0.997	1 to 1,600	31,858	13.9	0.998
	Dieldrin	0.2 to 64,000	0.995	2 to 3,200	35,718	19.0	0.995
	Endrin	0.2 to 64,000	0.992	2 to 3,200	24,849	19.5	0.996
	4,4'-DDD	0.2 to 64,000	0.995	2 to 3,200	33,903	17.3	0.996
	4,4'-DDT	0.2 to 64,000	0.992	2 to 1,600	20,618	18.2	0.993
	Methoxychlor	1 to 320,000	0.990	10 to 4,000	8,199	16.1	0.998
	TCX	0.1 to 32,000	0.997	1 to 1,600	72,423	10.8	0.998
	DCB	0.2 to 64,000	0.996	2 to 3,200	23,956	17.2	0.998
Mix B	beta-BHC	0.1 to 32,000	0.995	1 to 1,600	21,388	11.6	0.998
	delta-BHC	0.1 to 32,000	0.993	1 to 1,600	47,532	17.0	0.997
	Aldrin	0.1 to 32,000	0.994	1 to 1,600	35,851	14.3	0.997
	Heptachlor epoxide	0.1 to 32,000	0.994	1 to 1,600	36,234	11.9	0.998
	alpha-Chlordane	0.1 to 32,000	0.995	1 to 1,600	34,958	12.2	0.997
	gamma-Chlordane	0.1 to 32,000	0.995	1 to 1,600	35,250	11.3	0.997
	4,4'-DDE	0.2 to 64,000	0.989	2 to 3,200	40,065	18.6	0.996
	Endosulfan II	0.2 to 64,000	0.991	2 to 1,600	24,212	16.4	0.997
	Endrin aldehyde	0.2 to 64,000	0.990	2 to 3,200	18,628	16.6	0.995
	Endosulfan sulfate	0.2 to 64,000	0.992	2 to 3,200	27,644	14.7	0.996
	Endrin ketone	0.2 to 64,000	0.990	2 to 3,200	20,803	13.6	0.996

For a smaller concentration range (3 orders of magnitude), correlation improved and % RSDs of calibration factors for most OCPs were within 20 percent as required by CLP (see the 10-point calibration in table 3). Figure 4 shows a linear curve for lindane (1 to 1,600 pg/µL), typical of most OCPs in this concentration range. Figure 4 also shows the linear curve for methoxychlor (10 to 4000 pg/µL), a pesticide that typically responds poorly to classical ECD. This concentration range, typically from 1 to 1,600 or from 2 to 3,200 pg/µL for most OCPs, represents a 100-fold improvement over that required by CLP (CLP specifies 5 to 80 pg/µL for lindane). This wider linearity range allows more analyses for samples without requiring rework (dilution/concentration and re-analysis). If dilution of samples is required, the higher linearity of the detector results in more accurate estimations of correct dilution factors to bring sample concentrations within the CLP range.

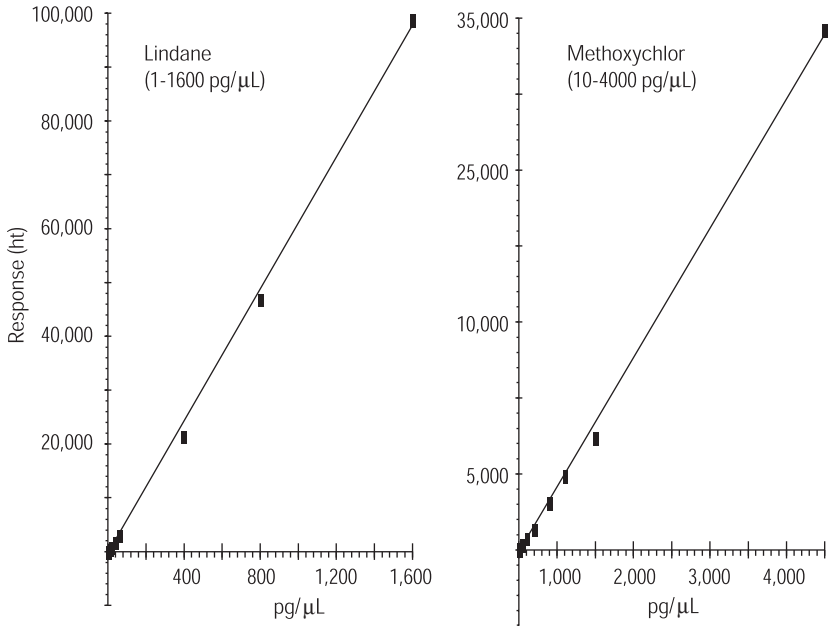

Figure 4. Linear calibration curves for lindane and methoxychlor over extended ranges.

Calibration Stability and System Robustness

The 6890 Series GC system with 6890 Micro-ECDs was regularly calibrated in accordance with CLP requirements. Analyses of blanks, continuous calibration using the midlevel standards, and performance evaluation mix were performed for each 12 hours of operation or every 10 to 20 samples. If results of these analyses failed to meet CLP breakdown, retention time, and response criteria, routine maintenance (such as changing inlet septum and liner or clipping a few inches off the guard column) was performed. If necessary, the instrument was recalibrated (using a three-point initial calibration). No cleaning or baking of the micro-ECD was required, even though a wide variety of samples was analyzed, including some dirty soil extracts[8].

At a minimum, CLP requires that system stability be monitored by analyzing midpoint calibration standards every 12 hours. In this study, system (or calibration) stability was based on verification of the calibration factors and retention times of target OCPs to match those from the initial calibration run within specific limits. The difference in calibration response (RPD—relative percent difference) between the later midpoint calibration run and the initial calibration run must be less than ± 25 percent (upper and lower RPD control limits).

Figure 5 is a continuous calibration verification (CCV) control chart of RPD for lindane and methoxychlor on column A over a 6 month period, typical of most OCPs on both column A and column B.

Throughout this study, the system was within RPD control limits and other calibration verification criteria for several days at a time without performing any re-calibration. When any OCP failed to meet CLP calibration verification criteria (that is, when an OCP was outside the RPD control limits of the CCV), nonintrusive system maintenance was conducted and a new initial calibration was performed. These steps were also done when the instrument was switched for 1 or 2 weeks to analyze a different type of sample, requiring a different GC method. When the instrument was switched back to the original CLP analysis of OCPs and PCBs, the instrument still met calibration verification criteria (within the RPD control limits). This represents a significant improvement over previous designs that usually required full recalibration after switching between methods and indicates that using the micro-ECD saved time and improved laboratory productivity.

Over a period of 6 months, the 6890 Series GC/dual micro-ECD system was in continuous operation

and performed several different methods. For example, the system was used for 2 to 3 weeks to analyze pesticides and aroclors by the CLP method and solid waste method (EPA method 8081). The system was then switched to a drinking water method[8] for a few weeks and later returned to the CLP method for OCPs. In other instances, the system was switched to analyze herbicides (EPA method 8150), then to drinking water (EPA method 504), and back again to the CLP method or method 8081 for OCPs and aroclors. In each case, the stabilization of the micro-ECDs was fast, requiring only a few injections of hexane blanks prior to running the CCV calibration standards.

Throughout this study (which included continuous operations over 6 months), even though routine column and inlet maintenance was needed (columns were replaced once during the course of the study), no micro-ECD maintenance was needed.

Conclusion

The improved performance of the Agilent 6890 Series GC/dual micro-ECD system met all CLP criteria for the analysis of OCPs over a period of 6 months. System validation was performed throughout this period for a wide variety of samples and analyses of different EPA methods. The 6890 Series GC with micro-ECDs easily met and maintained CLP criteria during the study. In addition, the micro-ECD showed improved sensitivity, greater dynamic and linear operating ranges, and more stable response. Moreover, it required minimal maintenance, and showed rapid recovery after switching between methods. Use of the Agilent 6890 Micro-ECD has a high potential to save time, improve quality of data, and increase laboratory productivity.

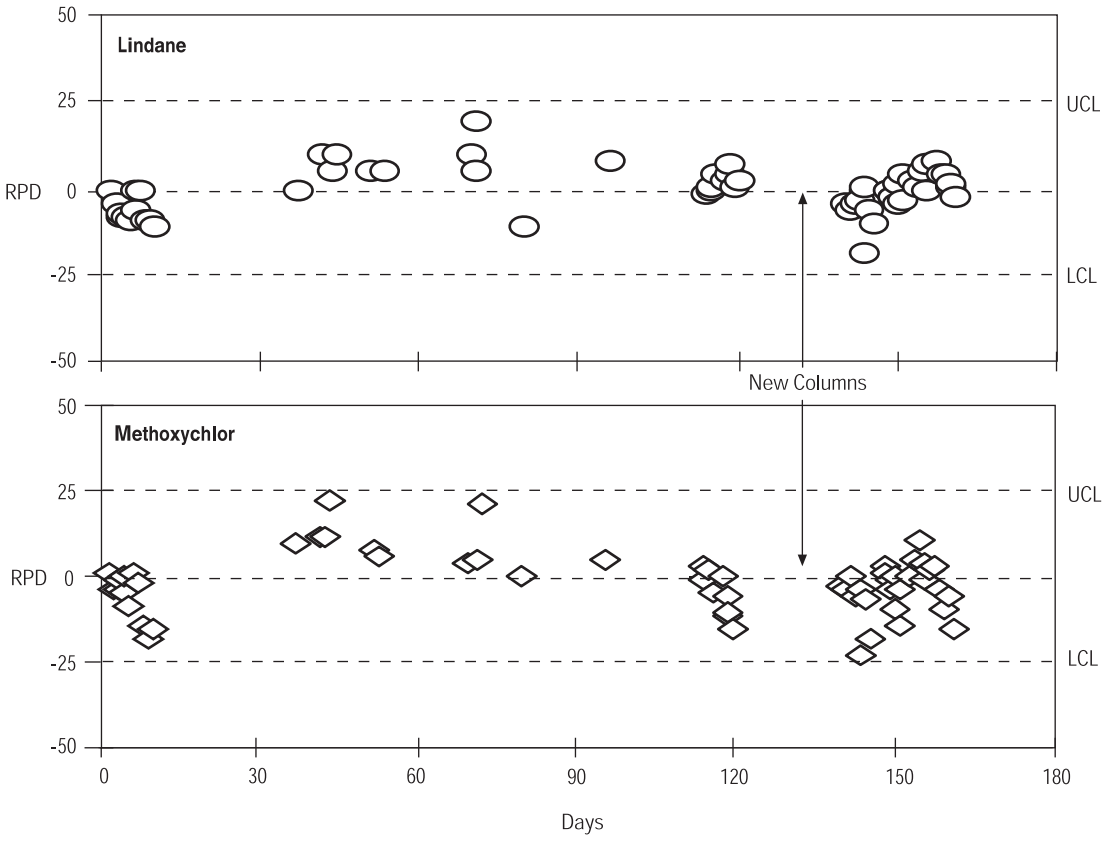

Figure 5. CCV control chart demonstrating stability of response and performance during the study.

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Fast Dual-Column GC/ECD Analysis of Chlorinated Pesticides—EPA Methods 608 and 8080

Application Note
228-305

Author

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Abstract

Dual-column analysis with HP-35 and HP PAS-1701 columns was used to analyze chlorinated pesticides targeted in EPA Methods 608 and 8080 for wastewater and solid wastes. GC parameters were optimized using the Agilent 5890 Series II gas chromatograph (GC) with electronic pressure control (EPC), a dual injector, and a dual electron capture detector (ECD) system. The analysis of 18 pesticides was completed in 12 minutes.

Introduction

Currently, many testing laboratories use dual-column/dual-ECD GC systems to analyze the chlorinated pesticides specified in EPA Methods 608 and 8080[1,2]. For this application, EPC was used with an HP-35 column (35% phenyl, 65% methyl polysiloxane phase) as the primary column and the HP PAS-1701 column for confirmation.

The unique selectivity of the HP-35 column for this set of chlorinated pesticides permitted focus on the optimization of oven temperature for the HP PAS-1701 column. Individual EPC ports for each injector permitted individual regulation of column flow for both the HP-35 and the HP PAS-1701.

Experimental

EPA Method 608 and 8080 targeted pesticides were separated using 30 m x 0.53 mm x 1.0 µm HP-35 and HP PAS-1701 columns (part no. 19095G-123 and 19094U-023, respectively). Analyses were performed on an HP 5890 Series II GC with EPC, dual split/splitless inlets, and dual ECDs. An Agilent 7673 automatic liquid sampler was used to process the simultaneous splitless injections. A deactivated single-tapered glass liner with a small plug of glass wool (part no. 5181-3316) and a Merlin Microseal septum (part no. 5181-8816) were used with each split/splitless inlet. Instrumentation and GC conditions are listed in **Table 1**.

A test mix containing 18 pesticides (50 ppb per component) and two surrogates was prepared from the dilution of certified standard mixes with pesticide-grade hexane (Burdick & Jackson). Pesticides in the test mix are listed in **Table 2**.

Table 1. Experimental Conditions

Instrument Requirement	
Gas Chromatograph	Agilent Technologies 5890 Series II with EPC
Injection Ports	Dual split/splitless inlets
Column	HP-35, 30 m x 0.53 mm x 1.0 µm (Part no. 19095G-123)
	HP PAS-1701, 30 m x 0.53 mm x 1.0 µm (Part no. 19095S-123)
Detector	Dual ECD
Sample Introduction	7673 automatic sampler with dual injectors
Data Collection	3365 ChemStation and HP Vectra 486/33T PC
Experimental Conditions	
Injection	Splitless 1 µl, purge delay, 0.75 min, inlet temperature of 250°C
Carrier gas	(A) HP-35, pressure program: 8.6 psi (1 min) at 0.5 psi/min to 12 psi and at 3.0 psi/min to 25 psi (0 min)
	(B) HP-1701, helium, 10 ml/min constant flow
Oven	160°C (1 min) to 280°C at 10°C/min and to 300°C (2 min) at 25°C/min
Detector	ECD (300°C), 120 ml/min N_2 makeup, 6 ml/min anode purge

Results and Discussion

In a dual-column/dual-ECD system, samples introduced in a single injection can be split between two columns using a Y-connector and detected by different ECDs. However, when using a Y-connector without EPC, the split sample flow to each column cannot be optimized, and equal and consistent sample splits cannot be presumed. The only variable that can be optimized, in dual-column ECD analysis using a Y-connector is the oven temperature program, which can be optimally balanced for the two dissimilar columns. Using dual-column GC/ECD without EPC, it would typically require 45 to 60 minutes to obtain baseline separations for EPA Method 608 and 8080 targeted pesticides (see **Figure 1**).

A typical run from an environmental testing laboratory for a test mix containing 18 targeted pesticides and two surrogates is shown in **Figure 1**. A Yconnector was used to split samples for both columns, DB-608 and DB-1701, and good baseline separations were obtained for most analytes. This dual-column run was completed in 45 to 53 minutes using the following oven temperature program: 150°C (1 minute) to 260°C (18.34 minute) at 3°C/minute, then to 275°C (5 minutes) at 25°C/minute. Clearly this oven temperature program was optimized to separate critical pairs, such as DDE/dieldrin, DDD/endosulfan II, endosulfan sulfate/mehtoxychlor, and methosychlor/endrin ketone for both columns.

Table 2. Chlorinated Pesticides.

Peak No.	Pesticides
1	Tatrachloro-m-xylene (SS1)
2	alpha-BHC
3	Lindane
4	beta-BHC
5	Heptachlor
6	delta-BHC
7	Aldrin
8	Heptachlor epoxide
9	Endosulfan I
10	4,4′-DDE
11	Dieldrin
12	Endrin
13	4,4′-DDD
14	Endosulfan II
15	4,4′-DDT
16	Endrin aldehyde
17	Endosulfan sulfate
18	Methoxychlor
19	Endrin ketone
20	Decachlorobiphenyl (SS2)

Figure 2 shows chromatograms of the same pesticide test mix using the HP-35 and HP PAS-1701 columns and EPC. The oven program, 160°C (1 minute) to 280°C at 10°C/minute and to 300°C (2 minutes) at 25°C/minute, was optimized to separate the critical pairs, endosulfan

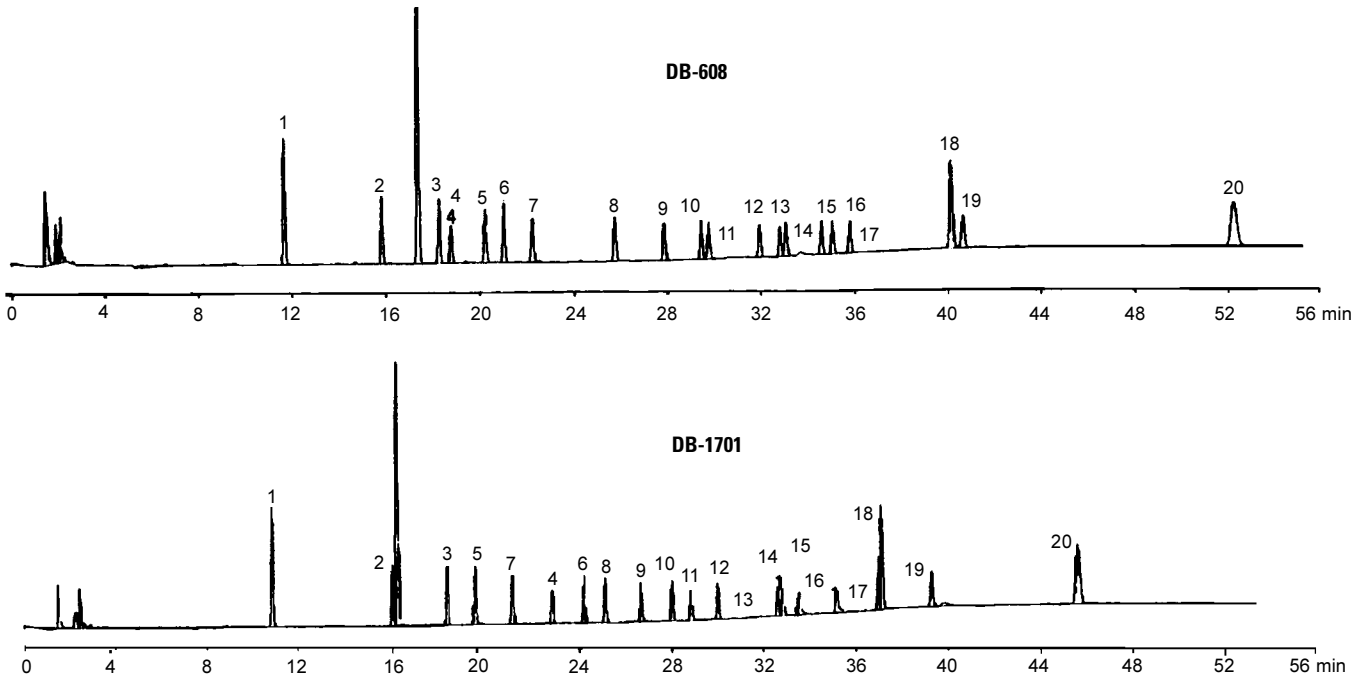

Figure 1. Typical chromatograms of a pesticides standard mix using DB-608 and DB-1701 columns under GC conditions used in environmental testing laboratories. (See Table 2 for peak identification.)

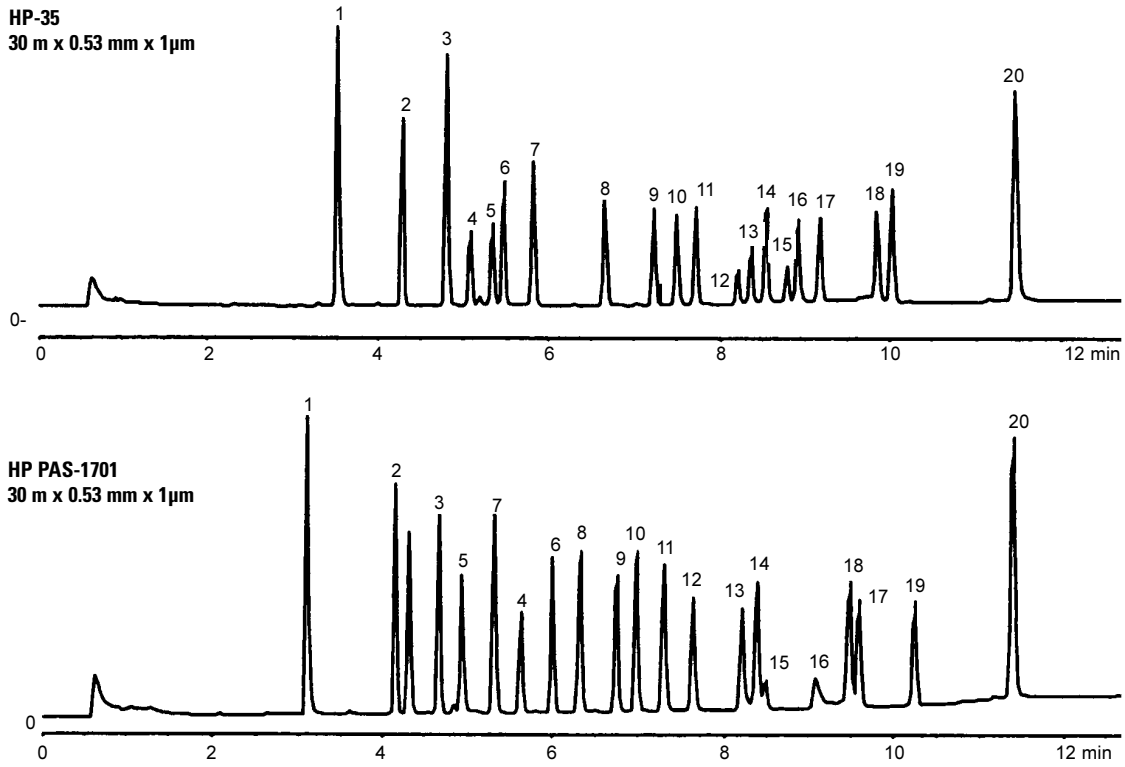

Figure 2. Chromatograms of a pesticides standard mix using HP-35 and HP PAS-1701 columns under the GC conditions listed in Table 1. (See Table 2 for peak identification.)

II/DDT and methoxychlor/endosulfan sulfate, for the HP PAS-1701 column. In this run, EPC provided a constant 10 ml/minute helium flow to the HP PAS-1701 column throughout the entire run.

For the HP-35 column, the following pressure program was used: 8.6 psi (hold 1 minute) at 0.5 psi/minute to 12 psi and at 3.0 psi/minute to 25 psi (hold for constant flow for the remaineder of the run). This pressure program actually provided a 10 ml/minute constant flow to elute most of the pesticides and an increased flow (up to 20 ml/minute) near the end of the run to elute the last analyte, surrogate decachlorobiphenyl and other high-boiling materials from the column.

GC parameters optimized for dual-column/dual-injector/dual-ECD analysis of chlorinated pesticides reduced analysis time to less than 12 minutes. In addition to speed, all EPA Methods 608 and 8080 targeted pesticides and surrogates were well resolved with good sharp peaks for accurate quantitation.

Conclusion

The use of EPC permitted individual column flow control to each ECD. The unique selectivity of the HP-35 column for chlorinated pesticides permitted focus on the optimization of oven temperature for the HP PAS-1701 column. Run time was 11.5 minutes with good baseline separations for all 20 target pesticides and surrogates. The result was a reduction in sample turnaround time from 54 to 11.5 minutes for a 400% increase in productivity. This is more than a twofold improvement in productivity when compared with conventional methods currently used at many environmental testing laboratories with DB-608 and DB-1701 columns.

Acknowledgement

The author wishes to thank Ms. Joann Faulkner and her colleagues at the Pace Laboratory in Petaluma, California, for providing chromatograms and pesticide standards.

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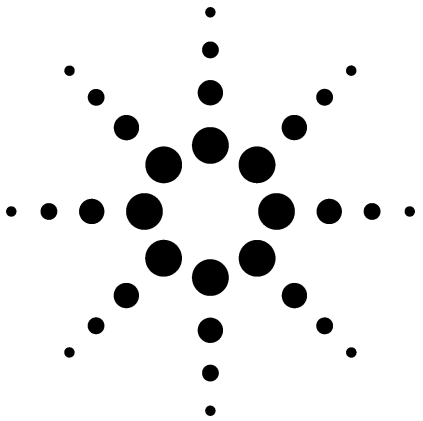
Trace Level Pesticide Analysis by GC/MS Using Large-Volume Injection



Application

Gas Chromatography

September 1997

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Abstract

Large-volume injection (LVI) using the Agilent programmable temperature vaporizing (PTV) inlet can improve gas chromatography system detection limits by one to two orders of magnitude over standard methods that call for 1- or 2-µL injections. An Agilent 6890 Series gas chromatograph (GC), configured with a PTV inlet, a 6890 Series automatic liquid sampler (ALS), and an Agilent 5973 mass selective detector (MSD), was used for the analysis of pesticides in standards and several food extracts. By making 100-µL injections, several pesticides could be identified by scanning gas chromatography/mass spectrometry (GC/MS) at the 100 ppt (100 ng/L) level. The PTV inlet tolerated dirty food extracts very well; more than 1,500 µL of such samples were injected into a single PTV liner. This application note includes recommendations for doing LVI using the PTV/6890/5973 GC/MSD system.

Introduction

More than 700 pesticides are registered for use in the world[1] , and many more continue to persist in the environment, even though they are no longer being applied. For the protection of human health and the environment, pesticide residues are routinely monitored in food, water, soil, and tissue samples. "Acceptable" residue limits have been set for various foods and environmental samples by agencies such as the United States Environmental Protection Agency (U.S. EPA), the Codex Alimentarius Commission[2] , and many other governmental organizations around the world. A great many methods have been developed to screen for pesticides in food[3-7] and the environment[8-10] to ensure that risks associated with pesticide use are minimized.

Recently, concern has increased that certain pesticides and other synthetic chemicals may be acting as pseudo hormones which disrupt the normal function of the endocrine system in wildlife and humans. Birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence are among the many disorders that have been blamed on these "endocrine disrupting" compounds, though much research must be done to verify these assertions. In 1996, Colborn, Domanoski, and Myers[11] brought these issues into the public spotlight with the publication of their book *Our Stolen Future*. Recently, the United States Congress passed legislation calling for increased testing of suspected endocrine disrupters and monitoring their levels in food[12] and water[13] supplies. Because the endocrine system can be exquisitely sensitive to extremely low hormone concentrations, there is a need to measure concentrations of suspected endocrine disrupters (many of which are pesticides) at very low levels. Initiatives such as the Pesticide Data Program, developed by the United States Department of Agriculture[14] , seek to

determine the lowest measurable pesticide levels in various foods to develop a total exposure model. Clearly, there is pressure to push pesticide detection limits to even lower levels than are routinely achieved today. Most residue measurements are made by gas chromatography using a variety of element-selective or mass spectral detectors (GC/MS). Therefore, to achieve lower detection limits, it is necessary to improve the detection limits of these GC methods.

In GC, there are primarily four ways to improve method detection limits: 1) increase the concentration of analytes in a sample, usually by reducing the volume of an extract; 2) increase the sensitivity of the detector; 3) increase the selectivity of the detector to reduce chemical background "noise" or 4) increase the volume of sample injected. Because GC/ MS can be highly selective and extremely sensitive, it is often the method of choice for pesticide analysis and/or confirmation. However, for the reasons discussed above, there are occasions when even greater sensitivity is required. This application note describes a method for increasing GC/MS system detection limits by making large-volume injections (LVI) using Agilent's new programmable temperature vaporizing (PTV) inlet. Because this LVI technique is detector-independent, it is applicable to other GC configurations that may be used for pesticide residue analysis.

Experimental

Pesticide Standard Solution

Stock solutions of 14 pesticides were prepared at 1 mg/mL by adding 10 mg each of trifluralin, hexachlorobenzene, pentachloronitrobenzene, dichloran, chlorothalonil, chlorpyrifosmethyl, chlorpyrifos, endosulfan I, p,p'-DDE, propargite, iprodione, methoxychlor, and fenvalerate (mix of isomers I and II) to individual 20mL vials and diluting with 10.0 mL of acetone. Permethrin was obtained as a mixture of permethrin I and permethrin II comprising 32 percent and 27 percent of the sample, respectively, so 16.95 mg of this mixture was diluted with 10 mL of acetone giving a solution in which the combined permethrins represented 1 mg/mL. A stock mixture was prepared by adding 4 mL of the permethrin and fenvalerate solutions and 1 mL of each of the other stock solutions to a 100-mL volumetric flask and diluting to volume with acetone. The resultant solution contained 40 ng/µL each of the combined permethrin and fenvalerate isomers and 10 ng/µL each of the other 12. This sample was diluted further with acetone to prepare standards that were analyzed by LVI. All these pesticides were obtained in neat form from Chem Service (West Chester, PA USA).

Extracts

Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee, FL USA). Commodities were extracted using a version of the Luke procedure[15-17] that gave a final sample representing 1.75 g of the commodity per mL of extract.

Table 1. Instrumentation and Conditions Used for Pesticide Samples

GC/MS System	
Gas chromatograph	6890 Series GC
Automatic liquid sampler	6890 Series ALS
Mass spectral detector	5973 Series MSD
Programmable temperature vaporizing inlet	PTV with CO_2 cooling
Computer for data acquisition and analysis	HP Vectra XU 6/200
Software	G1701AA Version A.03.00 running Microsoft® Windows™ 95
Column	30 m x 0.25 mm x 0.25 µm Agilent HP-5MS
Instrumental Conditions	
GC Parameters	
Carrier gas	Helium
Inlet liner	Prototype deactivated borosilicate with fritted glass on interior walls (part no. 5183-2041)
Syringe size	50 µL
Injection volume	100 µL (Inject 10 µL 10 times)
Injection delay	12 sec
Inlet temperature program	40 °C (4.2 min), 200 °C/min to 320 °C (2 min)
Vent flow	400 mL/min Vent pressure 0.0 psi for 4.00 min
Purge flow to split vent	50.0 mL/min at 6.50 min
Column head pressure	0 psi (4 min) then 17.3 psi (constant pressure)
Oven temperature program	50 °C (6.13 min), 30 °C/min to 150 °C (2 min), 3 °C/min to 205 °C (0 min), 10 °C/min to 250 °C (20 min)
MSD Parameters	
Acquisition mode	Scan (35-550 amu)
Temperatures	Transfer line = 280 °C, MS quad = 150 °C, MS source = 230 °C

Instrumentation

Table 1 lists the instrumentation and chromatographic conditions used for LVI and GC/MS analysis of pesticide samples.

Brief PTV Tutorial

Before focusing on the PTV/GC/ MS analysis of pesticides, it is important to understand how the PTV inlet operates in the solvent vent mode for large-volume injections.

The PTV Inlet

The PTV inlet has the same basic functions as the split/ splitless inlet except that it is temperature programmable from -60 °C (using CO_2 cooling) or -160 °C (using liquid N_2 cooling) to 450 °C at rates up to 720 °C/min. However, the PTV's design has been optimized for its main uses-LVI and cold split/splitless injection. Although hot split and splitless injections may be made with or without a pressure pulse, care must be taken not to exceed the small internal volume of the PTV inlet. In practice, it is best to choose the Agilent split/splitless inlet for hot injections and the PTV inlet for LVI and cold split/ splitless techniques.

Most GC pesticide methods call for injecting 1-2 µL; splitless injection is used because it is compatible with dirty extracts of food, soil, or water. Pulsed splitless injection allows one to make injections of up to 5 µL using standard equipment[18]. Enormous gains in system sensitivity can be realized by using the PTV inlet in the "solvent vent" mode, which is compatible with injections of 5-1,000 µL. These large injections may be made manually or automatically using either a standard 6890 Series ALS in the multiple injection mode or by using a controlled speed injector available from Gerstel[19]. Because the injection process may take several minutes, manual injections are usually impractical and good precision may be hard to achieve.

The 6890 Series ALS is designed to make one or more injections of up to 25 µL into the PTV inlet. After the desired number of injections has been made, the inlet is heated and the chromatography begins. Though the system controls allow up to 99 injections, a reasonable upper limit is about 10, making 250 µL the typical injection volume limit for this system. For even larger injections, the controlled speed injector[19] should be used. For all of the analyses described below, 100 µL were injected by making 10 sequential injections of 10 µL each.

How the PTV Works in the Solvent Vent Mode

Figure 1 shows a diagram of the PTV inlet. For large-volume injections, three steps are required. These are: 1) injection and solvent elimination; 2) splitless sample transfer to the GC column; and 3) chromatographic separation and, if desired, a simultaneous inlet bake-out step. The steps are described more completely below.

Injection and Solvent Elimination (Step 1)

During injection, the column head pressure is set to 0 psi to eliminate or, in the case of GC/MS, reduce the flow through the column. When mass spectral detection is used, there is still

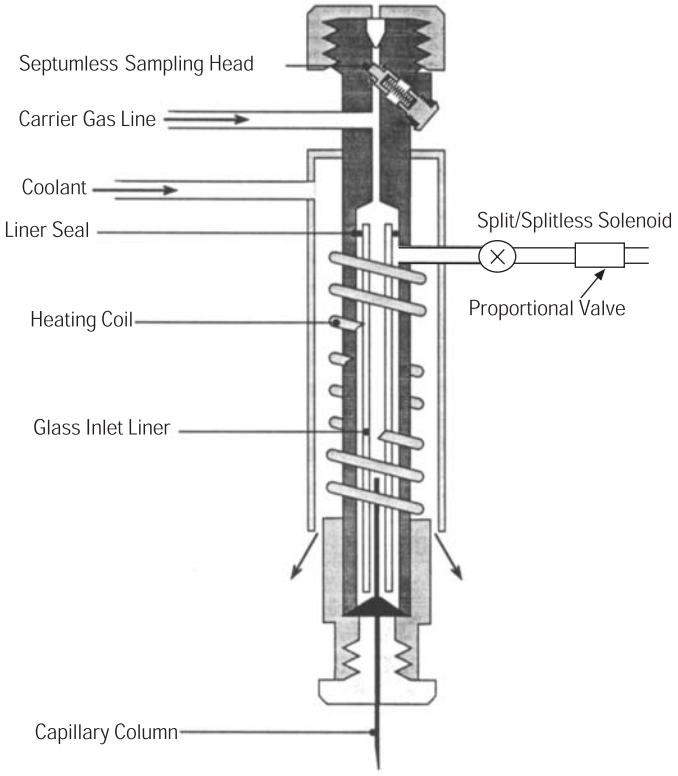


Figure 1. The PTV inlet shown with the septumless head. The inlet is also available with a septum head that may be equipped with a standard septum or a Merlin Microseal. (Figure reproduced with permission of Gerstel GMBH.)

some flow because the column outlet is under vacuum. At the same time, a steady stream of carrier gas passes through the inlet and out through the split vent. This flow is typically between 100 and 500 mL/min. The sample is injected into the cool liner where it remains as a liquid, dispersed over the liner walls or any packing material that may be in the liner. The steady flow of carrier gas through the liner causes the solvent (and any volatile fraction of the sample) to evaporate and be swept with the carrier gas out through the split vent. This is analogous to "blowing down" a sample with a stream of inert gas, except that this takes place inside the PTV inlet. When most of the solvent has evaporated, the next injection is made and the evaporation process repeats, accumulating more sample in the inlet. To recover an analyte completely, its boiling point should be at least 100 °C greater than that of the solvent; most pesticides fall into this category.

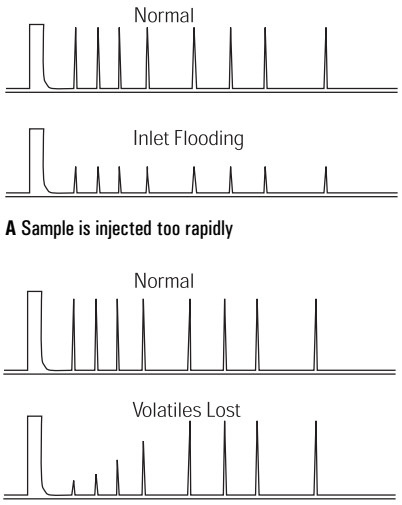


Figure 2. Chromatograms A and B illustrate the result of poor timing of multiple injections.

The timing of these multiple injections can be important. If the sample is introduced too rapidly, the liner may become flooded and liquid will be forced out through the split vent. Chromatographically, this shows up as reduced area counts for all analytes (see figure 2A). If there is too much time between injections, all of the solvent may evaporate and more of the volatile analyte fraction may be lost too. This results in poor recovery of volatiles but 100 percent recovery of the less volatile compounds (see figure 2B). Set-points such as inlet temperature, vent flow, and injection delay times can affect recovery of volatiles. Note that for 100 percent recovery, an analyte should have a boiling point at least 100 °C greater than the solvent. One can adjust the delay between injections by entering the desired value in the ChemStation software. Some experimentation is usually necessary when setting this delay for a new method. It will be dependent upon such factors as the solvent type, injection volume, vent flow, and inlet temperature.

Splitless Sample Transfer to the GC Column (Step 2)

Once the desired number of injections has been made, the column head pressure is restored and the vent flow is tur ned off. At this point, the inlet temperature is programmed up to a value that is sufficient to transfer all of the desired analytes to the GC column. This step is similar to a splitless injection, except that instead of flash vaporization, the sample is transferred as the inlet temperature is programmed up. For the most gentle treatment of labile analytes, slow ramp rates may be used. This allows analytes to be flushed into the column at the minimum temperature needed for volatilization. When sample decomposition is not a problem, the inlet may be heated as fast as 720 °C/min.

Chromatographic Separation (Step 3)

During sample transfer, the oven temperature is usually held between 30 °C below and 20 °C above the solvent's atmospheric boiling point, depending on whether the solvent effect is needed to focus the more volatile fraction of the analytes. Again, some experimentation is necessary to optimize peak shapes. After the sample has been transferred in step 2, the oven temperature is programmed up and chromatography begins.

After the inlet has reached its maximum temperature and sufficient time has elapsed to transfer the sample to the column, a purge flow of 30-50 mL/min is restored to the split vent. If desired, one can set a very large split flow for a few minutes and bake out the inlet at a higher temperature to remove nonvolatile impurities. To conserve carrier gas, gas saver should be turned on at the end of this bake-out step.

Entering PTV Inlet Parameters into the Agilent ChemStation

When preparing the PTV portion of a GC method, one should first decide on the sample size and how many injections are required. In this work, ten 10-µL injections were made for a total of 100 µL. When entering parameters into the ChemStation screen, the Injector icon is first selected (figure 3) under the "GC edit parameters" menu. Next, the Configure button is pressed to enter the syringe size and enable multiple injections. From the main injector screen, the injection volume (10 µL) and number of injections are entered[10] . For this work, a 12-second delay was chosen between injections to allow for solvent evaporation.

The estimated total injection time is listed on the Inlets screen (figure 4). This is helpful when setting the inlet and oven parameters. First, the vent flow rate (400 mL/min for these analyses) is chosen, which sets the vent pressure to 0 psi until the injection sequence is done and solvent from the last injection has largely evaporated (4.00 min in figure 4). This is done by entering these values in the following fields:

Vent Flow 400 mL/min
Vent pressure 0.0 psi until 4.00 min

Next, the purge flow and elapsed time are set by entering values in the following field:

Purge Flow to Split Vent
50.0 mL/min @ 6.50 min

Note that as an aid in setting up the method, the "estimated total injection time" is shown just above the previous data entry fields.

In this example, the normal column head pressure was restored and the vent flow was turned off at 4.00 min. This prepares the inlet for the splitless transfer of the sample to the column. The vent flow remained off until it was set to 50 mL/min at 6.5 min. Thus, there is a 2.5-min period for inlet temperature programming and splitless sample transfer to the column. In this example, the inlet was held at 40 ° C for 4.2 min, enough time to make 10 injections, turn off the purge flow, and restore the column head pressure; the PTV was then programmed to 320 ° C at 200 ° C/min (figure 4).

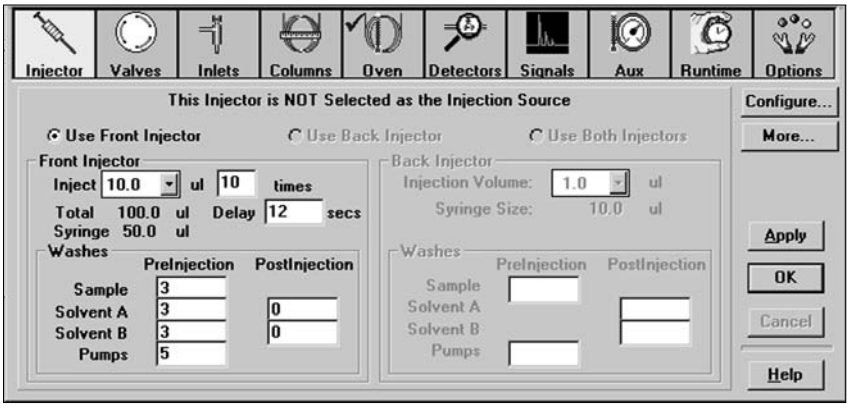


Figure 3. The injector screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for multiple injections. To configure the sampler for multiple injections, set the syringe size, and choose slow injection, click on the Configure button.

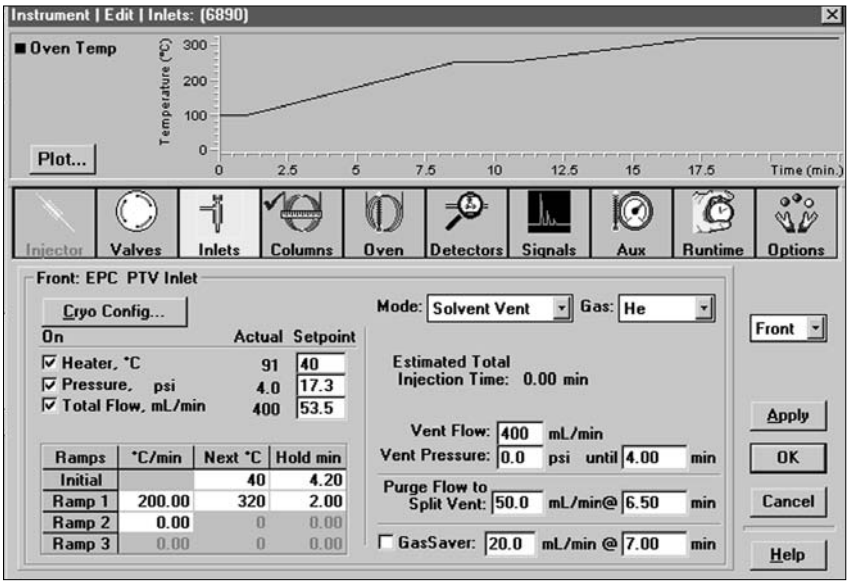


Figure 4. The inlets screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for operation of the PTV inlet in the solvent vent mode.

Although not done for these analyses, the inlet could be baked out by setting the "purge flow to split vent" to a large value (perhaps 500 mL/min) at the end of the splitless time (6.50 min) and at the same time, program the inlet to a higher temperature. After the bake-out period, the inlet temperature is programmed downward and gas saver is turned on.

Normally, the GC oven is held at its starting temperature until the splitless injection is complete (6.50 min in this case) at which time oven temperature programming is begun. For this work, the oven temperature program was begun at 6.13 min so that the pesticide retention times would match a retention time data base that was in use. Figure 5 diagrams the PTV and GC oven setpoints used for this work.

PTV Inlet Liner Considerations

The correct liner choice is critical to the success of any pesticide analysis by PTV injection. The liner must be thoroughly deactivated or many labile pesticides may decompose or adsorb in the inlet. In general, any liner containing glass wool will be unsatisfactory for the analysis of labile pesticides, whether or not the glass wool is deactivated. At this time, two PTV liners are suggested for pesticide analysis:

- Part no. 5183-2037 is a deactivated, open multibaffled liner with no internal packing that may be used for single or multiple injections of 5 µL or less. This liner gives very good recovery for pesticides, even extremely difficult ones such as acephate and methamidophos.
- Part no. 5183-2041 is a deactivated liner with an internal coating of sintered glass to give it more surface area and is, therefore, suitable for single or multiple 25-µL injections. This liner gives better than 70 percent recovery for most pesticides, although tests have shown that acephate and methamidophos cannot be analyzed using this liner, and that recoveries of guthion are often less than 50 percent. A prototype version of this liner was used for all of the work described in this application note.

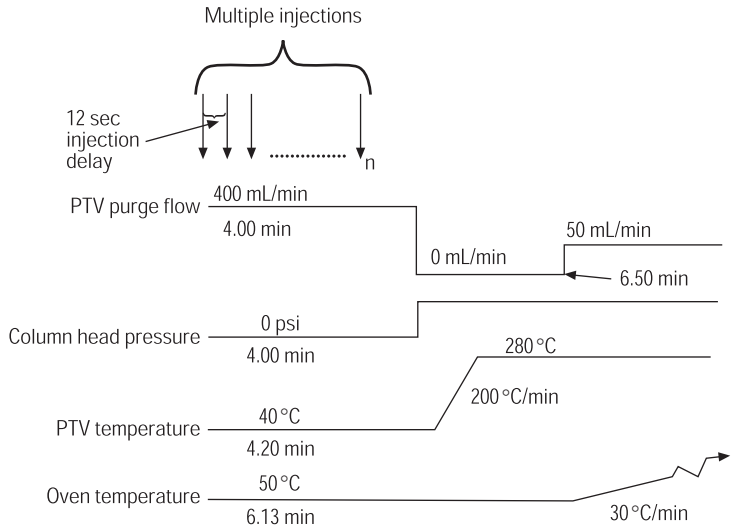
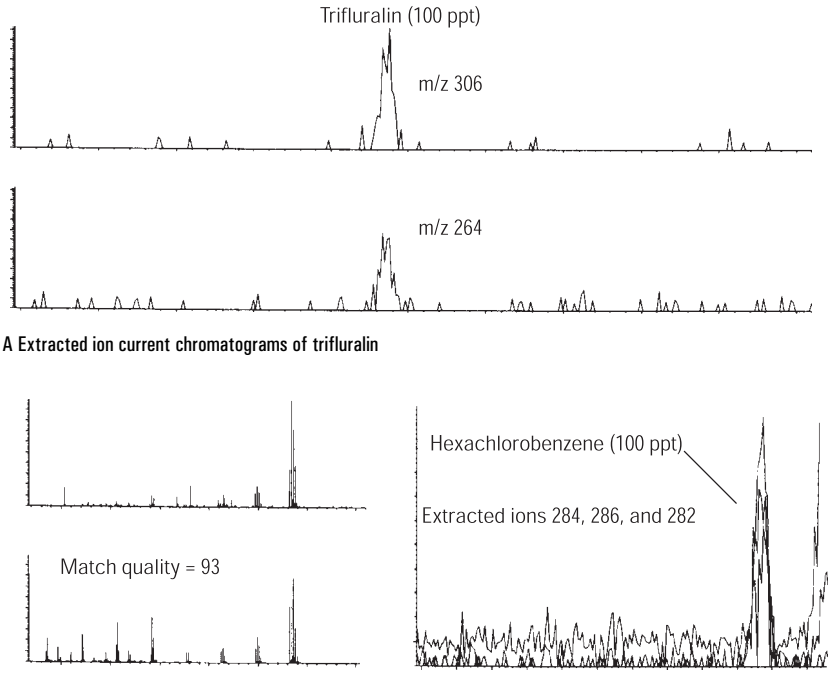


Figure 5. Illustration of the GC and sampler setpoints used for 100-µL injections of pesticide samples. Note that normally, the GC oven hold period would have been at least 6.5 min for this method. A value of 6.13 min pesticide retention times to a data base.

Results and Discussion

When compared to a typical 2-µL splitless injection, 100-µL PTV injections can often result in a 50-fold improvement in system detection limits. Selective detectors such as the MSD can help the analyst to realize the full measure of this sensitivity improvement by excluding background that may be introduced from solvent impurities, vial cap extract, and indigenous compounds coextracted with the analytes. In this application, it was possible to see most of the pesticides in the 14-component mixture at 100 ppt in the scan mode (400 ppt for the isomer mixes of permethrin and fenvalerate). Figure 6 shows extracted ion chromatograms for trifluralin and hexachlorobenzene (HCB) at 100 ppt. Library searching gave a match quality of 93 for the HCB peak. Fenvalerate isomers I and II were found in the solution in a ratio of about 78:22. Figure 7 shows extracted ion chromatograms for fenvalerate I at a concentration of 311 ppt.



A Extracted ion current chromatograms of trifluralin

B Extracted ion current chromatogram of HCB with its mass spectrum and library match

Figure 6. Scanning GC/MS results for a pesticide standard containing Trifluralin and Hexachlorobenzene at 100 ppt. (Ten 10-µL injections were made using the PTV inlet.)

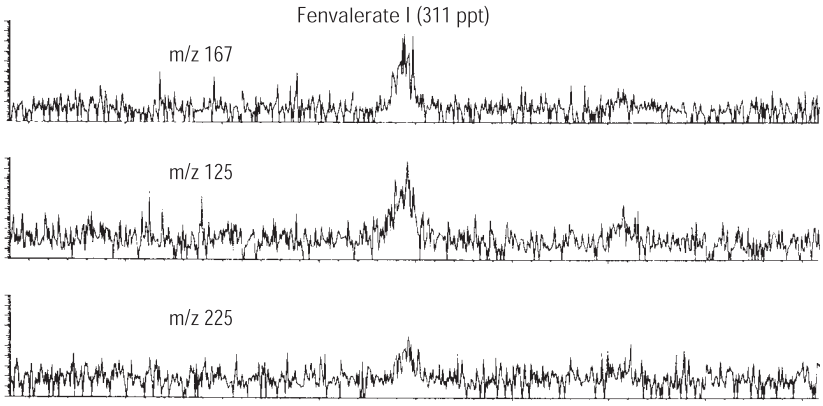


Figure 7. Extracted ion current chromatograms of Fenvalerate I at a concentration of 311 ppt in a pesticide standard. (Ten 10-µL injections were made using the PTV inlet.)

Analysis of a bell pepper extract revealed several pesticide residues. As seen in figure 8, chlorpyrifos and the endosulfans were easily detected. The Florida Department of Agriculture determined the concentration of chlorpyrifos, alpha-endosulfan, betaendosulfan, and endosulfansulfate to be 0.210, 0.011, 0.018, and 0.013 ppm, respectively. It is important to note that these compounds could be detected with very high selectivity by extracting high mass ions that are characteristic of these pesticides but not of the matrix. Using LVI, there is ample signal from these less abundant ions for good quantitation. With normal injection volumes, selectivity may have to be compromised and the most abundant ions extracted in a pesticide spectrum to gain sensitivity.

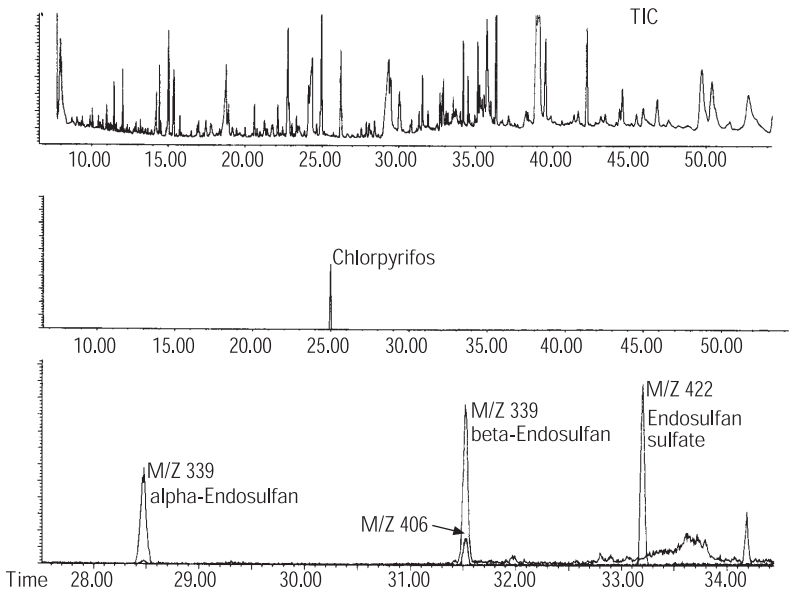


Figure 8. GC/MS Analysis of a bell pepper extract. (Ten 10-µL injections were made using the PTV inlet.) Using LVI, there was sufficient signal to use high mass ions with smaller abundances to achieve greater selectivity.

Phosmet, captan, and propoxur were all easily detected in a pear sample. The total ion current chromatogram (TIC) is shown in figure 9 along with spectrum obtained for captan juxtaposed with the library spectrum. Figure 10 shows the propoxur peak along with 2,4,6-tribromoanisole and 2,4,6-tribromophenol, two other compounds that were surprising to find in a pear sample. Though the origin of these brominated compounds is not known, a recent paper by Hoffmann and Sponholz 20 suggests that tribromophenol is used to treat storage palettes for the prevention of fire and mold growth, and that the anisole is formed from the phenol microbiologically. Perhaps these pears were shipped in containers that had been similarly treated.

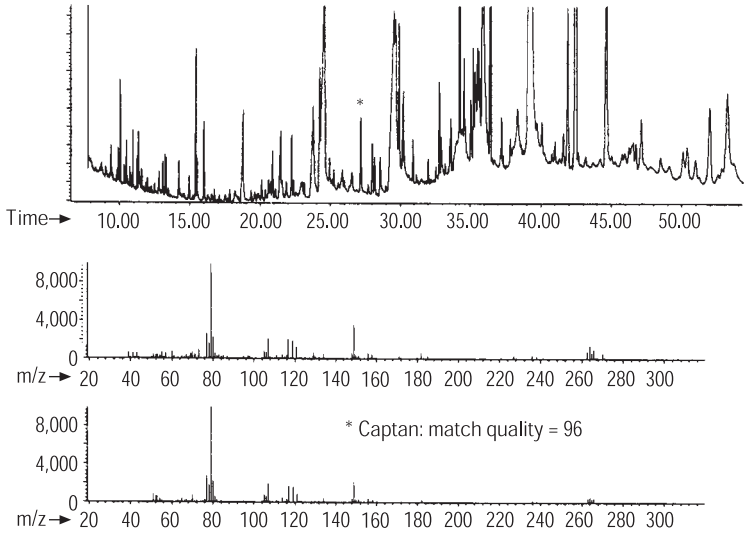


Figure 9. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Captan was easily detected, and its spectrum gave a library match quality of 96.

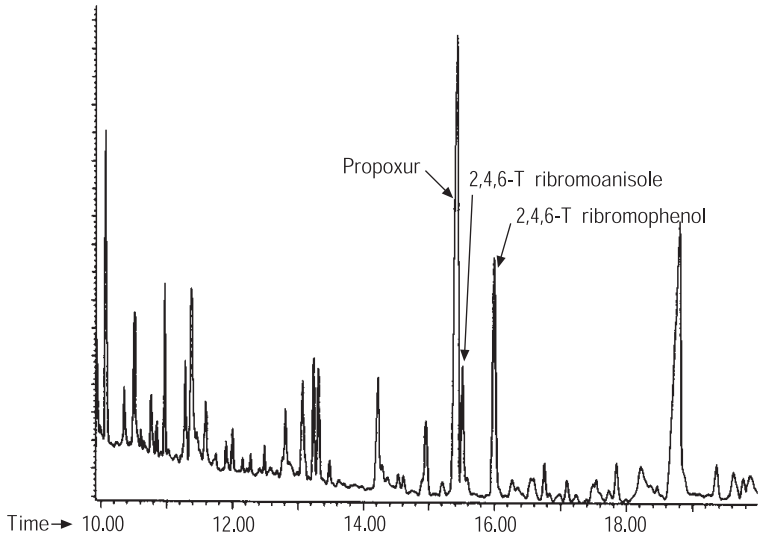


Figure 10. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Propoxur and two brominated phenolics were easily identified.

A single sintered glass coated liner of the type described above (part no. 5183-2041) was used for about ten 50- and ten 100-µL injections (ca. 1,500 µL total) of vegetable extracts before it was replaced. All of the extracts were rather dirty, and an inlet bake-out step was not used. Although the liner looked somewhat discolored for about 2 cm where injections were made, it still performed well at the time it was replaced.

Conclusion

Using the PTV inlet in the solvent vent mode, it is relatively simple to increase system detection limits by one or two orders of magnitude. When combined with the Agilent 6890 Series automatic liquid sampler, multiple injections of up to 25 µL each into the inlet can be made, allowing the solvent to vent while pesticides and other less volatile analytes accumulate. After the desired sample volume has been introduced (typically 5-250 µL), the solvent vent is closed and the sample is transferred to the column in a temperature-programmed splitless injection. By making 100-µL injections into a PTV-equipped Agilent 6890 Series GC coupled to the Agilent 5973 MSD, it was possible to see several pesticides at the 100 ng/L level (100 ppt) in the scan mode. With such low detection limits, less abundant ions can be used to identify and quantitate pesticides at low ppb levels, thereby gaining in selectivity as well.

When performing LVI, there are several parameters to adjust and some method development time is usually required. However, the method described herein worked well and can be duplicated for the PTV/GC/MS analysis of pesticides in food.

Acknowledgment

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Agilent Technologies
Innovating the HP Way

A Method Used to Screen for 567 Pesticides and Suspected Endocrine Disrupters

Application

Gas Chromatography

May 1998

Authors

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Abstract

A gas chromatographic (GC) method has been developed that can be used to screen for 567 pesticides and suspected endocrine disrupters. In principle, it can be used to screen for any GC-amenable pesticide, metabolite, or endocrine disrupter. The method relies on a technique called retention time locking (RTL). RTL is a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with the same stationary phase, nominal phase ratio, and diameter are used. Because RTL increases retention time precision and predictability, raw retention times can be used as a more reliable indicator of compound identity. The chromatographer first locks the GC method so that all retention times match those listed in a 567-compound pesticide and endocrine disrupter retention time table. After analyzing a sample by GC with atomic emission detection (GC-AED), the analyst enters a peak's retention time and known elemental content (presence or absence of heteroatoms) into a dialog box. If element-selective detectors are used, detector response can be entered in addition to or in place of GC-AED data. The software then searches the pesticide table for those compounds that elute at the correct retention time and have the right elemental content or detector response. Most often, the software finds just one compound that meets these criteria, and rarely does it find more than three. Confirmation is performed by GC with mass spectral detection (GC-MS) or by calculation of elemental ratios using GC-AED data. With retention time locking, pesticides have the same retention time on all GC systems; this makes GC-MS confirmation much easier because the analyte's retention time is already known.

Key Words

Pesticides, endocrine disrupters, gas chromatography, retention time locking, RTL

Introduction

The Pesticide Manual[1] lists 759 compounds and biological agents that are used currently as active ingredients in various pesticide formulations. Many compounds, though no longer used, still persist in the environment. For the protection of human health and the environment, acceptable limits in food and water have been set by governmental bureaus such as the United States Environmental Protection Agency (USEPA) and the Codex Alimentarius Commission.[2] Numerous methods have been developed to screen for pesticide contamination in food[3–7] and the environment[8–10] to ensure that these standards are met.

Certain pesticides and other synthetic chemicals have been suspected of behaving as pseudo hormones, disrupting normal functions of the endocrine system in wildlife and humans. Maladies such as birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence have been blamed on exposure to endocrine disrupters.[11] The 1996 publication of *Our Stolen Future*, a book by Colborn,

Dumanoski, and Myers,[11] brought these concerns to the attention of the public. Recently passed legislation in the U.S. calls for more testing of suspected endocrine disrupters and monitoring of them in food[12] and water[13] supplies. To facilitate more research into the endocrine disrupter issue, methods are needed to detect suspected compounds at trace levels.

Because so many pesticides are in use, it is usually impractical to screen for large numbers of them individually and, therefore, multiresidue methods are preferred. Most laboratories that analyze for pesticides in food or the environment screen for only a few dozen compounds because it is often very difficult to screen for more. Recently however, methods have been developed using gas chromatography with mass spectral detection (GC-MS), that can screen for more than 200[5] or even 300[6] pesticide residues.

Still, there is no universal method to analyze for all GC-amenable pesticides. While GC-MS methods are gaining in popularity, there are still some limitations. When methods employ selected ion monitoring (SIM) or tandem mass spectrometry (MS-MS), method development is more tedious and any shift in GC retention times requires that individual analyte retention time windows be shifted accordingly. These methods are only capable of detecting compounds on the target list; there are still hundreds of pesticides, metabolites, and suspected endocrine disrupters that could be missed. On the other hand, methods based on scanning GC-MS alone may require more sample cleanup to avoid interferences from co-extracted indigenous compounds. Typically, these methods do not screen for many pesticide metabolites, endocrine disrupters, or other environmental contaminants. A method that could be used to screen for endocrine disrupters and almost all of the volatile pesticides and metabolites would offer a better means of monitoring the food supply and the environment.

This paper describes a universal method that, in principle, could be used to screen for any pesticide, metabolite, or endocrine disrupter that can elute from a gas chromatograph. The screening procedure relies on a new gas chromatographic technique called retention time locking (RTL)[14–16] with database searching based on retention time and elemental content or detector response. This technique is used to narrow an analyte's identity to a few possibilities. Confirmation is performed by GC-MS or by calculation of a compound's elemental ratio using GC with atomic emission detection (GC-AED).

Experimental

Standards and Extracts

Pesticide standards used to develop the retention time table were obtained from Chem Service (West Chester, PA, USA), Promochem Ltd (Welwyn Garden City, Hertfordshire, England), Dr. Ehrenstorfer (Augsburg, Germany), Hayashi Pure Chemical Industries, Ltd (Osaka, Japan), Wako Pure Chemical Industries, Ltd (Osaka, Japan), and GL Sciences Inc (Tokyo, Japan).

Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee, FL, USA). Samples were extracted with acetonitrile followed by solid-phase extraction (SPE) using a C-18 cartridge. Extracts intended for analysis by halogen-selective detectors were also subjected to floracil SPE.

Pesticide Retention Time Table

The table containing GC and GC-MS retention times for 567 pesticides, metabolites, and suspected endocrine disrupters was obtained from Agilent Technologies, Wilmington, DE, USA (G2081AA).

Instrumentation

Table 1 lists the instrumentation and chromatographic conditions used for GC-AED screening and GC-MS confirmation.

Software for Method Translation

Software for use in translating the normal GC method to one that runs three times faster was obtained from Agilent Technologies,Wilmington, DE, USA.[17]

Results and Discussion

Retention Time Locking

Key to the development of this method is a new concept in gas chromatography called retention time locking (RTL).[14–16] Agilent RTL software allows the chromatographer to match analyte retention times from run to run, independent of the GC system, detector, or manufacturing variations in column dimensions. The only requirement is that the columns used have the same stationary phase and the same nominal diameter and phase ratio. For example, with RTL it is possible to match analyte retention times on a GC-AED and a GC-MS even though the MS operates under vacuum and the AED operates at 1.5 psi above ambient pressure. The

procedure also compensates for differences in GC column length resulting from variations in manufacturing or from column cutting required during routine maintenance.

RTL is accomplished by adjusting the GC column head pressure until a given analyte, such as an internal standard, has the desired retention time. When this is done, all other analytes in the chromatogram will have the correct retention times as well. Software has been developed that can be used to determine the column head pressure that will lock the retention times correctly after one or two "scouting" runs.

With RTL, it is possible to measure pesticide retention times using a given GC method, and then reproduce those retention times in subsequent runs on the same or different instruments. With this increased retention time precision and predictability, retention times become a far more useful indicator of analyte identity. For many years, relative retention times[3,6] or retention indices[18,19] have been used to identify compounds. These techniques were developed to compensate for the fact that retention times were not predictable from day to day, column to column, or instrument to instrument. With the increased retention time precision of the Agilent 6890 GC and RTL, it seemed that raw retention times could be used for compound identification instead of retention indices. The chromatographer could simply scan a table of pesticide retention times, eliminating all possibilities but those with close elution times under the same locked GC conditions.

Table 1. Instrumentation and Conditions of Analysis

Agilent GC-AED System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Atomic emission detector	G2350A atomic emission detector
Computer for data acquisition and analysis	HP Vectra XM Series 4 5/150
Software	G2360AA GC-AED software running on Microsoft® Windows™ 3.11
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
GC inlet	Split/splitless, 250 °C or 260 °C
Inlet liner	Single-tapered deactivated (part no. 5181-3316) with 2-cm deactivated glass wool plug centered ~3 cm from the top
Injection volumes	3–5 µL splitless when running method at 3× speed; 2–3 µL splitless at 1× speed
Inlet pressure (splitless)*	87.5 psi constant pressure for method at 3× speed; 27.6 psi constant pressure for 1× speed
Inlet pressure program (pulsed splitless)*	60 psi (2.01 min), 10 psi/min to 27.9 psi (hold)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C (0 min), 8 °C/min to 280 °C (10 min)
AED transfer line temperature	290 °C
AED cavity temperature	320 °C
AED elements and wavelengths (nm)	Group 1: Cl 479, Br 478 Group 2: C 193, S 181, N 174 Group 3: P 178 Group 4: F 690 (optional)
Agilent GC-MS System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Mass selective detector	5973 MSD
Computer for data acquisition and analysis	HP Vectra XU 6/200
Software	G1701AA Version A.03.00 running on Microsoft® Windows® 95
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
Inlet	Split/splitless, 250 °C
Inlet liner	Single-tapered deactivated with small amount of glass wool at the bottom (part no. 5062-3587)
Injection volume	2 µL
Inlet pressure*	15.5 psi (constant pressure)
Oven temperature program	Same as GC-AED
MSD parameters	
Acquisition mode	Scan (35–550 amu)
EM voltage	200 rel
Solvent delay	3.20 min
Threshold	150
Scans/sec	2.86
Temperatures	Transfer line = 280 °C, MS quad = 150 °C, MS source = 230 °C

*The column head pressures shown are typical values. Exact values were determined as part of the retention time locking procedure.

Pesticides almost always contain heteroatoms and often have several in a single molecule. The most frequently encountered heteroatoms are O, P, S, N, Cl, Br, and F. GC with atomic emission detection (GC-AED) has been shown to be a useful tool for pesticide screening because it is selective for all of the elements found in these compounds.[20–22] Thus, GC-AED screening provides valuable information about the elemental content of an unknown molecule. By including this elemental information along with the retention time, it should be possible to narrow pesticide "hits" to just a few possibilities.

To implement this screening procedure, a table of pesticide and endocrine disrupters retention times had to be created using a suitable method under locked conditions.

GC Method for Pesticide Screening

First, a GC method was needed that could elute hundreds of pesticides and endocrine disrupters in a reasonable time with adequate separation. However, the goal was not to separate every possible analyte in a single GC run. Because the intention was to build a table of locked retention times using this method, it had to reproduce these retention times under a variety of conditions. For example, the method needed to accommodate a variety of injection techniques including splitless, pulsed splitless,[23,24] cold splitless using a PTV inlet, and on-column injection which is occasionally used for the more labile pesticides.

The method also needed to perform well with samples dissolved in common solvents such as acetone and methylene chloride. Because a retention gap (or guard column) is sometimes added to protect the analytical column, the method had to be tested to see if it could still be locked with a retention gap installed.

The column chosen for the method was a 30 m × 0.25 mm × 0.25 µm HP-5MS because the same column could be used with any GC-detector combination. In particular, this column was chosen for its low bleed at high temperatures and because its optimum column flow is compatible with GC-MS. The 5% phenyl methyl silicone phase in this column has been widely used for pesticides.

Method translation software[17,25,26] can be used to increase the speed of a method while retaining the same relative retention times. This can be done by translating the method to a column having the same phase ratio but a smaller id or by increasing the flow rate and oven temperature program while using the same column. The final goal was to design a method that could run at three times the normal speed on the 30-m × 0.25-mm × 0.25-µm HP-5MS column or be translated to a 100-µm id column.

After several weeks of method development, the GC oven temperature program shown in figure 1a was chosen because it met all of the development criteria. Chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) was chosen as the locking standard. It is an ideal choice because chlorpyrifos-methyl elutes near the middle of the chromatogram (16.596 minutes), has good peak shape, and can be seen by most element-selective detectors. Because GC-AED requires three runs to generate element-selective chromatograms for C, Br, Cl, N, S, and P, the method was translated to run three times faster using software for method translation.[17,25,26] The faster oven temperature program used by this method requires 6890 GC systems that are configured for fast oven temperature ramping. The method translation software can be used to speed up the method by any desired factor; even 120-V 6890 GCs can run the method two times faster. However, the original method must be used for GC-MS because of the restriction in flow rates into the MSD. Figure 1b lists the threefold (3×) faster GC method.

Pesticide Retention Time Table

Once developed, this method was employed to create a table of locked retention times for the 567 pesticides, metabolites, and suspected endocrine disrupters. Increasing international food trade requires the analysis of pesticides that may be used in the supplying country but not in the recipient country. The goal was to create a table that included pesticides used around the world so pesticide standards were obtained from sources in Europe, Japan, and the USA.

A list of suspected endocrine disrupters was compiled from various lists published on the World Wide Web.[27–31] Many of these compounds are, in fact, pesticides. Most of the GC-amenable endocrine disrupters were analyzed and their retention times appear in the table. However, the 209 polychlorinated biphenyl congeners were not included because their inclusion might actually complicate the identification of organochlorine pesticides.

Standards, diluted to 10 ppm in acetone, were first analyzed by GC-MS using the oven temperature program shown in figure 1a and instrumental conditions listed in table 1. Compound identities were verified by matching their spectra to library entries,[32] by comparison with a published spectral compendium,[33] or by matching spectra to a list of charac-

teristic ions.[6] When reference spectral information was not available, the pesticides were verified by spectral interpretation. Samples were then analyzed on two different 6890 GC-FID instruments under the same locked conditions (chlorpyrifos-methyl retention time = 16.596 minutes). The GC-MS retention time and the average of the two GC-FID retention times were tabulated for each compound along with its molecular formula, molecular weight, and CAS number. In addition to these fields, there are four user-definable columns in table 2 that can be used to add such things as mass spectral information, internal catalog numbers, or comments. Table 2 lists a small portion of the database. It must be noted that all retention time values were created using constant column head pressure. This is because GC-MS retention times are very close to those obtained with other detectors when constant pressure is used. In this mode, GC-MS and GC-FID retention times match within ± 0.1 minute except for three compounds that elute at the very end of the chromatogram. Even in this case, the differences are no more than 0.2 minute. The discrepancy between GC-MS and GC-FID retention times is larger in the constant flow mode.

Pesticide Screening Method

Figure 2 diagrams the pesticide screening method. First, RTL was used to match GC-AED and GC-MS analyte retention times to those listed in the pesticide table. Software for RTL[14–16] was used to determine the

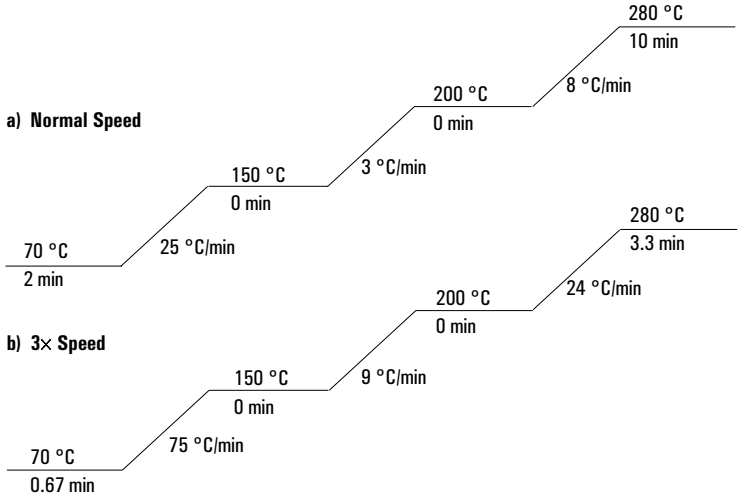

Figure 1. a) GC oven temperature program for the Agilent pesticide method at normal speed. When using this method, chlorpyrifos-methyl must be locked to 16.596 minutes. This method is used by GC-MS and can be used by any other GC system. b) GC oven temperature program for the Agilent pesticide method translated to run three times faster. This method may be used with 6890 GCs configured with any detector except an MSD so long as the GC is configured for fast oven temperature ramping. Chlorpyrifos-methyl must be locked to 5.532 minutes.

Table 2. Small Portion of the Pesticide and Endocrine Disrupter Retention Time Table That Contains 567 Entries. The retention times shown here are for the pesticide method run at normal speed as shown in figure 1a. Chlorpyrifos-methyl was locked to 16.596 minutes (± 0.015 minute for the collection of the tabulated retention time values. The table includes four additional columns for user-defined information.

FID RT	Name	CAS No.	Molecular Formula	MW	MSD RT
16.542	Acetochlor	34256-82-1	C:14,H:20,Cl:1,N:1,O:2,	269.77	16.542
16.549	Fuberidazole	3878-19-1	C:12,H:8,N:2,O:1,	196.21	16.549
16.583	Methyl parathion	298-00-0	C:8,H:10,N:1,O:5,P:1,S:1,	263.20	16.594
16.596	Chlorpyrifos methyl	5598-13-0	C:7,H:7,Cl:3,N:1,O:3,P:1,S:1,	322.53	16.593
16.637	Vinclozolin	50471-44-8	C:12,H:9,Cl:2,N:1,O:3,	286.11	16.630
16.650	Plifenat	21757-82-4	C:10,H:7,Cl:5,O:2,	336.43	16.641
16.689	Terbucarb	001918-11-2	C:17,H:27,N:1,O:2,	277.41	16.686
16.730	Chloranocryl	2164-09-2	C:10,H:9,Cl:2,N:1,O:1,	230.09	16.736
16.752	3-Hydroxycarbofuran	16655-82-6	C:12,H:15,N:1,O:4,	237.26	16.741
16.773	Heptachlor	76-44-8	C:10,H:5,Cl:7,	373.32	16.796
16.800	Carbaryl	63-25-2	C:12,H:11,N:1,O:2,	201.22	16.806

column head pressure needed to produce a retention time of 16.596 minutes for chlorpyrifos-methyl. When analyzing samples by GC-AED, the method was usually run at 3× speed and chlorpyrifos-methyl was locked to 5.532 minutes.

Figure 3 shows the RTL software screen that is used to develop the retention time calibration. To accomplish this for the pesticide method, one should install the 30 m × 0.25 mm × 0.25 µm HP-5MS column (part no. 19091S-433) and set the column head pressure to one of the appropriate nominal values as shown below, making sure to use the constant pressure mode.

- 26 psi for atmospheric pressure detectors run at normal speed (eg, NPD, FPD)
- 16 psi for GC-MSD operated at normal speed
- 27.5 psi for GC-AED operated at normal speed
- 88 psi for GC-AED operated at 3× speed

To prepare a calibration table similar to the one shown in figure 3, the chromatographer must make five analyses of chlorpyrifos-methyl at the following column head pressures: the nominal pressure, the nominal pressure + 20%, the nominal pressure + 10%, the nominal pressure – 10%, and the nominal pressure – 20%. Because of the first run affect, it is usually wise to make one or two blank runs before performing the five calibration runs. The five pressures and the chlorpyrifos-methyl retention times are entered into the table provided by the RTL software. This calibration table stays with the method and can be used to lock, or re-lock, the GC

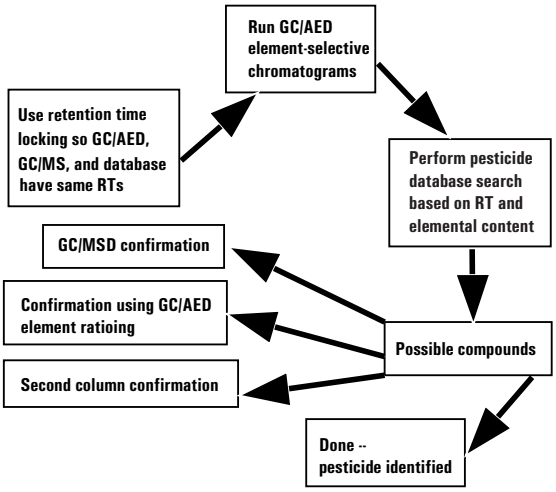

Figure 2. Diagram of the screening method that uses retention time locking and retention time table searching to identify pesticides and suspected endocrine disrupters.

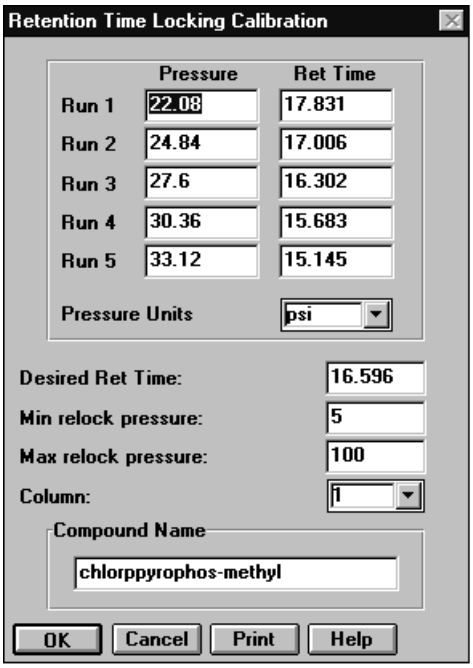

Figure 3. RTL software screen showing typical retention time locking calibration data for the pesticide method run at normal speed using a GC detector that operates at atmospheric pressure.

method as long as that method is used. That is, the five calibration runs only need to be made once for a given method.

The software screen for locking the GC method is shown in figure 4. To lock the method, one enters the retention time of chlorpyrifos-methyl and clicks on the "Calc new pressure" button. The RTL software calculates the pressure needed to lock the chlorpyrifos-methyl peak at the desired retention time. By clicking on the "Update current 6890 Method" button, this value is entered automatically into the method.

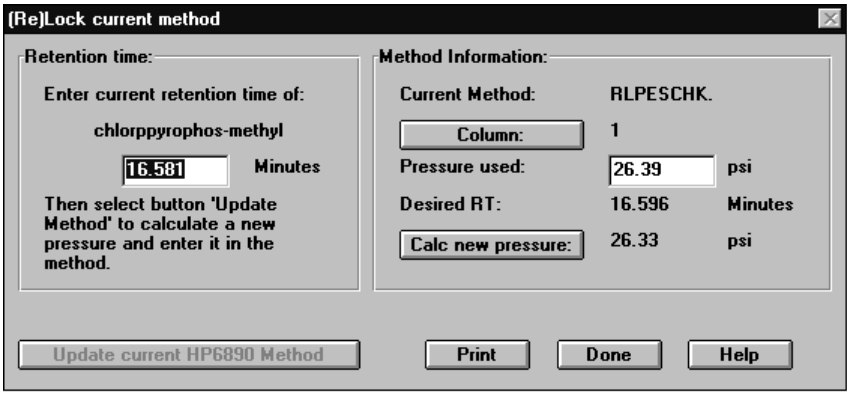

Figure 4. RTL software screen used to calculate the column head pressure needed to lock or re-lock a method. In this case, the chlorpyrifos-methyl retention time was 16.581 minutes and the pressure needed to re-lock the method was calculated to be 26.33 psi. By clicking on the "Update current 6890 Method," button, the new pressure is entered automatically into the GC method.

One can use Agilent's software for method translation[17] to convert the method to other speeds (eg, 1.9×) and determine the nominal column head pressure required. If this is done, the pesticide table must be exported to a spreadsheet program where the analyte retention times can be divided by the appropriate factor (1.9 in this case). This new table can then be imported back into the ChemStation for use with the new method.

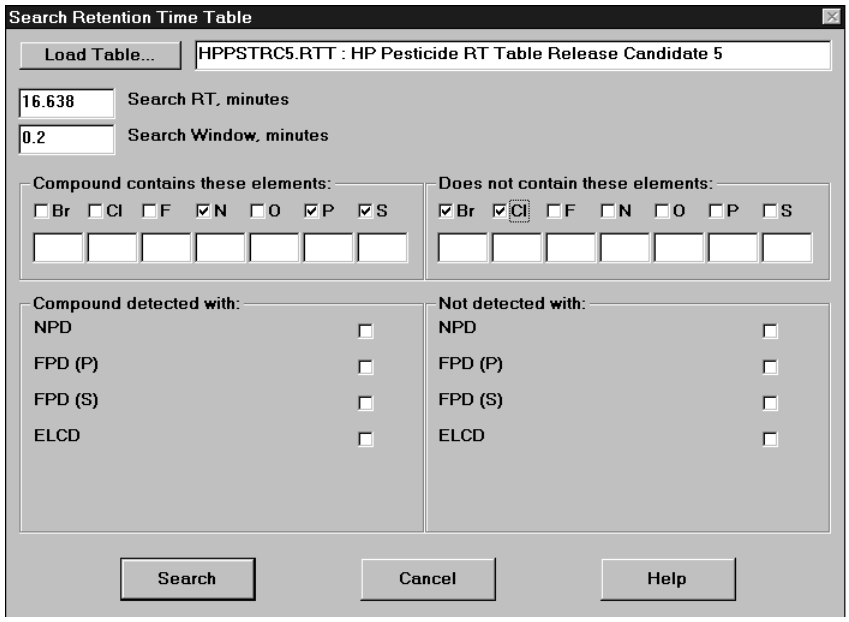

Figure 5. RTL software screen used to search a retention time table on the basis of retention time and known elemental content. In this case, the software will search the Agilent pesticide table at 16.638 ± 0.1 minutes for compounds that contain N, P, and S but do not contain Br or Cl. If element-selective detectors (such as the NPD) are used, this information can be provided to the search routine. Up to six different element-selective detectors can be configured as shown for NPD, FPD (P), FPD (S), and ELCD.

After locking the method to the table, GC-AED element-selective chromatograms were obtained for C, Cl, Br, N, S, P, and sometimes F. From the GC-AED chromatograms, it was usually possible to determine which heteroatoms were present or absent in the suspected pesticide peak. RTL software was then used to search the database by retention time and elemental content. Figure 5 shows the RTL software screen used for retention time table searching. One can enter the elements known to be present or not present in the GC-AED peak of interest. Up to six other element-selective detectors can be configured for use in the search algorithm. When the presence or absence of a heteroatom is uncertain, nothing is added to the search routine for that element.

One must choose a search time window wide enough to include the correct analyte, but narrow enough to eliminate as many extraneous "hits" as possible. Experience has shown that the normal speed method requires a search window of 0.2 to 0.3 minute. The 3× speed method can use a search window of 0.1 minute. If the heteroatom content is known for a peak, retention time table searching

with these search windows most often finds just one pesticide and rarely finds more than three possibilities.

Confirmation is usually done by GC-MS under locked conditions so that all GC-MS retention times match the values listed in the pesticide retention time table. This was found to be of enormous benefit. Prior to GC-MS confirmation, the analyst already knows which pesticides to look for and their expected retention times. Alternatively, when there is adequate signal to quantitate the analyte in multiple AED element-selective chromatograms, it is often possible to confirm a pesticide's identity simply by calculating its heteroatom ratio. GC-AED software for element ratioing facilitates this procedure.

Analysis of a Green Onion Extract

Numerous samples of fruit and vegetable extracts have been analyzed using this methodology. The results for a green onion extract illustrate the versatility and potential of this method.

Green onion extracts are usually very dirty and contain a large number of co-extracted sulfur compounds that can obscure sulfur-containing pesticides. The onion chromatograms shown in figure 6 were run under locked conditions at 2× speed in Tallahassee, Florida, by the Department of Food and Agriculture using a 5890 SERIES II/5921A GC-AED system. Retention time searching indicated that folpet was present in the sample, but it could not be confirmed at the time. The same sample was sent to the Agilent Technologies Little Falls Site in Wilmington, DE, where it was analyzed by scanning GC-MS using an 6890/5973 system. As shown in figure 7, folpet was easily confirmed at the expected retention time. In addition, the pesticides trichlorophenol, chlorothalonil, propoxur, and prochloraz were identified. Searching the Cl peak at about 6 minutes gave no pesticide hits. However, GC-MS suggested the presence of a trichloronaphthalene isomer at the corresponding retention time in the GC-MS chromatogram (about 12 minutes because the GC-MS was operated at normal speed). Though not a pesticide, trichloronaphthalene is considered to be a hazardous compound that should not be in food.

The same green onion sample was then analyzed by the newer model GC-AED system (6890/ G2350A) at 3× speed (figure 8). Several more pesticides were identified by searching the pesticide/ endocrine disrupter table using a 0.1-minute retention time window. Table 3 lists the pesticide hits that were obtained for each retention time search using the available GC-AED data. Sulfur was not included in any of the searches because onion extracts have such a high sulfur background.

Confirmation by GC-MS was much easier because the GC-MS retention time for each pesticide hit was printed out with the RT search report. Thus, the retention times and probable identities of each pesticide were already known before the GC-MS analysis was run. As is shown in figure 7 for folpet, one can simply extract the ions characteristic for each pesticide hit and look in the extracted ion chromatogram at the expected retention time.

Quantitative Analysis

The Agilent pesticide screening method is a qualitative tool to identify any of the 567 pesticides and endocrine disrupters listed in the retention time table. This, of course, is the first step in any pesticide screening method. Quantitative analysis can be performed in one of two ways.

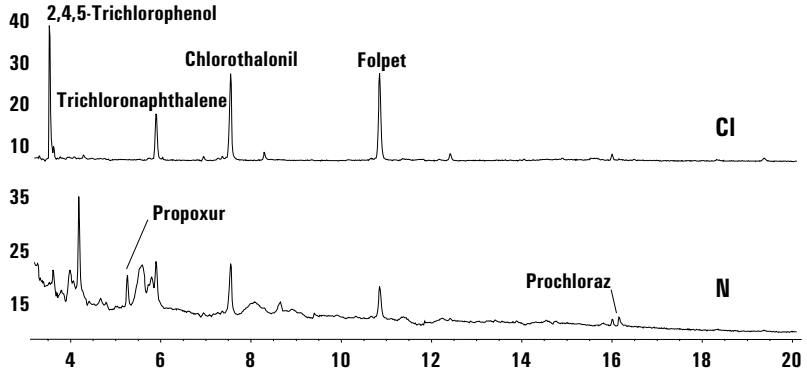

Figure 6. Cl- and N-selective chromatograms of a green onion extract from an 5890/5921A GC-AED system. The analysis was performed at 2× speed under locked conditions in Tallahassee, Florida, by the Department of Agriculture and Consumer Services. In addition to folpet, trichlorophenol, propoxur, and prochloraz were identified by retention time table searching and confirmed by GC-MS at their expected retention times. There were no hits for the Cl peak at about 6 minutes, which was identified by GC-MS as a trichloronaphthalene isomer.

The traditional method is to inject standards into the GC, GC-AED, or GC-MS system to determine response factors from which quantitative results are calculated by the ChemStation software. However, because the GC-AED elemental response is almost independent of molecular structure, compound-independent calibration (CIC) can be used to quantitate all of the pesticides and endocrine disrupters that are found. For example, one could spike chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) at a known concentration into each pesticide extract and obtain element-specific calibration curves for Cl, N, P, and S. These curves could then be used to calibrate for any other compound containing one or more of these elements. Because the GC-AED is quite stable, external standard CIC often works just as well. The GC-AED software facilitates CIC. Unfortunately, this procedure determines the amount of a compound that reaches the AED and does not compensate for losses due to decomposition or adsorption in the inlet or column.

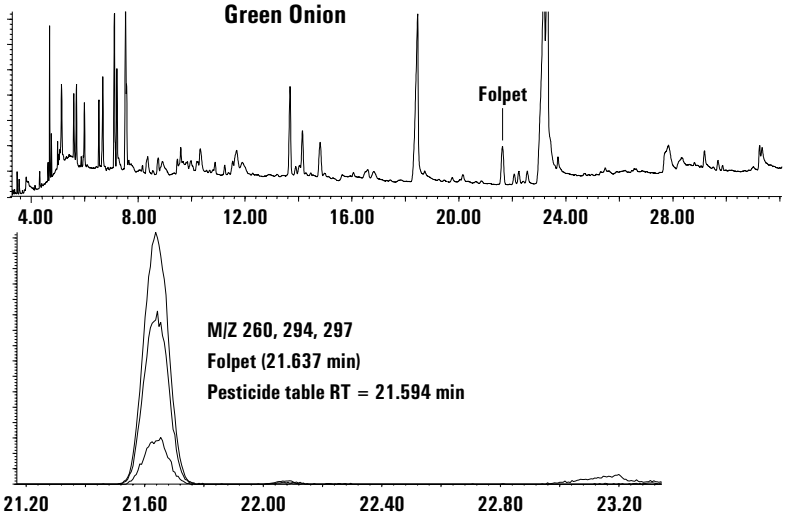

Figure 7. Confirmation of folpet in a green onion extract. The tabulated GC-MS retention time is 21.594 minutes, and folpet was detected in this sample at 21.637 minutes by simply extracting its characteristic ions.

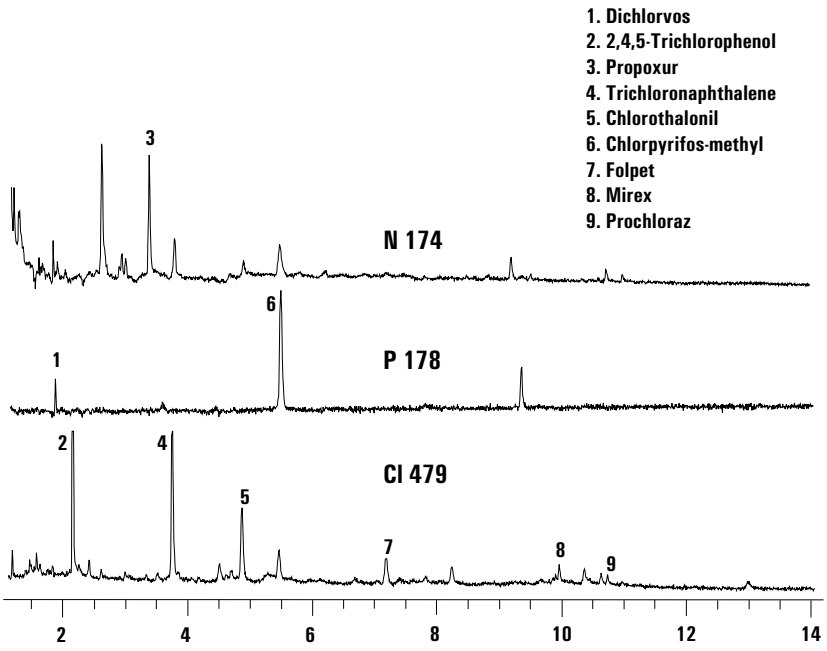

Figure 8. Element-selective chromatograms obtained for the same green onion extract shown in figure 6. These chromatograms were obtained at 3× speed using an 6890/G2350A GC-AED system.

Conclusions

Most screening procedures in use today are capable of finding only a fraction of the pesticides that are registered around the world. This new method has the capability of screening for virtually any volatile pesticide, metabolite, or endocrine disrupter. Although confirmation is usually required, GC-MS analysis is made much easier and more reliable because the pesticide's retention time and probable identity are already known.

While GC-AED is an ideal tool for element-selective pesticide screening,[20–22] many laboratories rely on a combination of other selective detectors. It is still possible to apply this method if each GC system runs the Agilent pesticide method under the same locked conditions. Any combination of GC-AED and/or element-selective detector response data can be entered into the RTL searching software.

When combined with RTL and retention time searching, GC-AED and GC-MS provide the most comprehensive and reliable screening method available for pesticides, metabolites, and suspected endocrine disrupters. Unlike most target compound methods in use today, this procedure has a good chance of finding and identifying unexpected or unknown pesticides, even in complex food extracts. RTL software makes it easy to add more compounds to the method, simply by determining their retention times under the same locked conditions.

Retention time locking with database searching could easily be applied to similar types of analyses. For example, one might use the procedure to identify polychlorinated biphenyls, polynuclear aromatics, drugs of abuse, or flavor and fragrance compounds.

Table 3. Green onion pesticide "hits" obtained by searching the 567-compound pesticide/endocrine disrupter RT table using a 0.1-minute RT window and element-selective GC-AED data. Compounds confirmed by GC-MS are shown for comparison. The GC/MS (figure 7) and GC-AED (figure 8) chromatograms were obtained at normal and 3X speeds, respectively. Sulfur peaks were not used to narrow the search because of the high background of sulfur-containing compounds in the onion extract.

GC-AED RT	RT Search Hits	Confirmed by GC-MS
1.933	Dichlorvos	Dichlorvos
2.281	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol	2,4,5-Trichlorophenol
3.440	Fenobucarb Propoxur 4,6-Dinitro-o-cresol	Propoxur
3.854	No pesticide hits	Trichloronaphthalene isomer
4.955	Terbacil Chlorothalonil	Chlorothalonil
5.538	Chlorpyrifos-methyl	Chlorpyrifos-methyl
7.232	Folpet Chlorbenside	Folpet
9.965	Mirex	Mirex
10.588	Prochloraz	Prochloraz

Acknowledgments

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Agilent Technologies
Innovating the HP Way

Analysis of Sulfur and Phosphorus Compounds with a Flame Photometric Detector on the Agilent 6890 Series Gas Chromatograph

Application

Gas Chromatography

February 1997

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Abstract

A gas chromatograph (GC) with a flame photometric detector (FPD) is frequently employed in analyzing complex samples for specific compounds. The wavelength filter of the FPD can be set to select for many elements, but it is most commonly used to detect sulfur and phosphorus. This application note discusses the uses of the FPD in gas chromatography, demonstrates the linearity and method detection limits (MDL) of the 6890 Series GC with an FPD, and gives examples of analyses of organophosphorus pesticides using the 6890 GC with an FPD.

Key Words

Gas chromatography, flame photometric detector, FPD, sulfur analysis, phosphate analysis, pesticides, organophosphorus pesticides, EPA method 1618, EPA method 622.

Introduction

The flame photometric detector is one of the most widely used selective detectors in gas chromatography. The FPD consists of a reducing flame that produces chemiluminescent species. These species emit characteristic light that is optically filtered for the desired wavelength; the wavelength selection determines which compound is detected. The filtered light is measured by a photomultiplier and transduced into a signal. A second photomultiplier can be added, which allows simultaneous detection of a second signal.

FPD filters can be selected for many different compounds, but the most common uses are for the selective detection of sulfur and phosphorus compounds in complex mixtures. The selectivity of classical FPDs is typically (as a ratio by weight to carbon) 10^5 for sulfur and 10^6 for phosphorus. The FPD operates over a dynamic range of 1×10^3 for sulfur and 1×10^4 for phosphorus.[1]

Gas chromatography with an FPD can be used to detect sulfur compounds in crude oil and sulfur contaminants in natural gas.

In food analysis it is used to detect off-flavors resulting from the liberation of volatile sulfur compounds. It is also used to simultaneously detect sulfur and phosphorus in chemical warfare agents. In the environmental area, the FPD is used for detection of organophosphorus pesticides and herbicides. Several EPA methods for pesticide detection, including EPA methods 1618[2] and 622[3], specify the use of an FPD.

A schematic of a single FPD for the 6890 Series GC is shown in figure 1. A dual wavelength version is available that has a second photomultiplier mounted perpendicular to the first for simultaneous detection of a second wavelength. The 6890 GC is available with either a single or dual FPD.

The sensitivity of any FPD is affected by detector temperature, flame chemistry, and filter wavelength.

- **Detector temperature**. To protect the photomultiplier, the maximum temperature limit for the 6890 FPD is 250 °C. Photomultiplier tube (PMT) noise increases with setpoint temperature, so the detector temperature should be as low as possible. Generally, the temperature should be set about 25 °C above the highest temperature reached in the oven program. To prevent water condensation and clouding of the window, the minimum operating temperature is 120 °C.[4]

- **Flame chemistry**. FPD sensitivity is highly dependent on detector gas flows. On the 6890 GC, the gas flows are electronically controlled. This allows rapid and precise optimization of flow rates. Sulfur and phosphorus modes have different optimum flow requirements, so the ability to easily set and reset flows increases the quality of results and saves time.
- **Filter wavelength**. For the FPD, filters of specific wavelength are physically installed in the detector. A 394-nm filter is used for sulfur detection, and 526-nm filter for phosphorus detection.

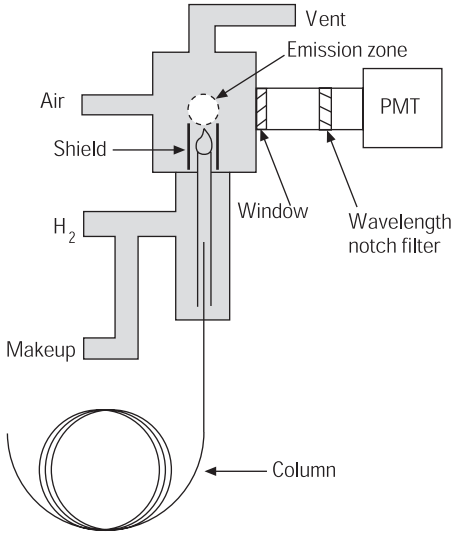


Figure 1. Single photomultiplier tube FPD for 6890 Series GC

Experimental

All experiments were performed on a 6890 Series GC with electronic pneumatics control (EPC) and an Agilent 7673 automatic liquid sampler (ALS). An Agilent 1707A ChemStation was used for instrument control and data acquisition. Chromatography conditions are shown with the individual chromatograms in figures 2, 3, and 4.

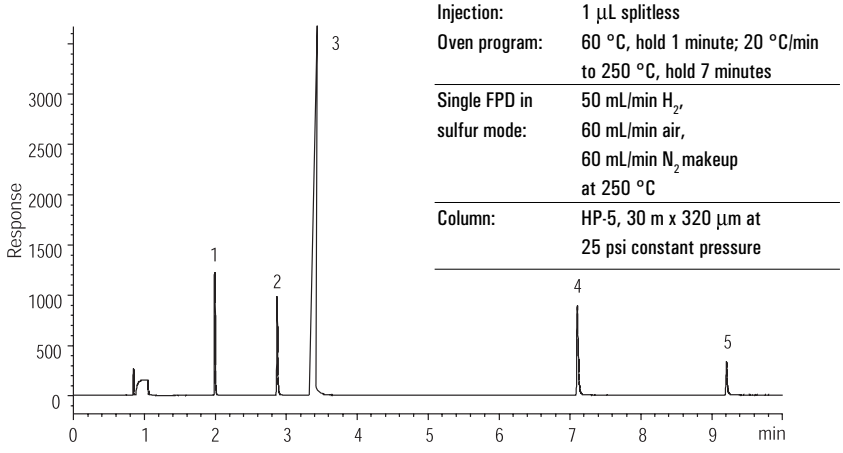
Table 1. MDL and Linearity over 10^2 Range Sulfur Mix on the FPD

Peak Number	Compound Name	MDL pgS/sec n = 11	Linearity r^2 n = 15
1	2,5-dimethylthiophene	26.22	0.9986
2	sec-butylsulfide	20.10	0.9983
3	1,4-butanedithiol	22.27	0.9972
4	dodecanethiol	16.90	0.9985
5	octyl sulfide	16.14	0.9979

Results and Discussion

Linearity and MDL

In sulfur mode, the response of the FPD is proportional to analyte concentration squared. The calculated MDL and r^2 values from linearity experiments for a single photomultiplier in sulfur mode are listed in table 1, and the chromatogram for a 20–40 ppb sample from the experiment is shown in figure 2. The square of the concentration was used to calculate regression statistics. When using a ChemStation for data analysis, a quadratic calibration fit is used for sulfur.



Conditions

Injection:	1 µL splitless
Oven program:	60 °C, hold 1 minute; 20 °C/min to 250 °C, hold 7 minutes
Single FPD in sulfur mode:	50 mL/min H_2, 60 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	HP-5, 30 m x 320 µm at 25 psi constant pressure

Figure 2. 1 µL of 1.5 ppm sulfur standard, FPD in sulfur mode (The peaks are identified in table 1.)

The response of the FPD is linear in phosphorus mode. Table 2 shows the r^2 values for an organo-phosphorus pesticide mixture and the MDL calculated from the study. Figure 3 shows the chromatogram. A standard linear curve fit is used for phosphorus when using a ChemStation for data analysis.

Analysis of EPA Method 1618

Figure 4 shows the chromatogram obtained from the analysis of organophosphorus pesticides according to EPA method 1618. The injected concentration of each compound was 1–2 ppm.

Table 2. MDL and Linearity over 10^3 Range for Organophosphorus Pesticides on the FPD

Peak Number	Compound Name	MDL pgPesticide/sec n = 11	Linearity r^2 n = 15
1	phorate	1.85	0.9996
2	demeton	1.13	> 0.9998
3	disulfoton	1.31	> 0.9999
4	diazinon	1.74	> 0.9999
5	malathion	1.74	> 0.9999
6	fenthion	1.75	> 0.9999
7	parathion	1.84	> 0.9999
8	trichloronate	2.27	> 0.9999
9	tokuthion	2.51	> 0.9999
10	fensulfothion	—	> 0.9999
11	ethion	1.29	> 0.9999
12	sulprofos	2.36	> 0.9999
13	guthion	1.24	> 0.9999
14	coumaphos	2.08	> 0.9999

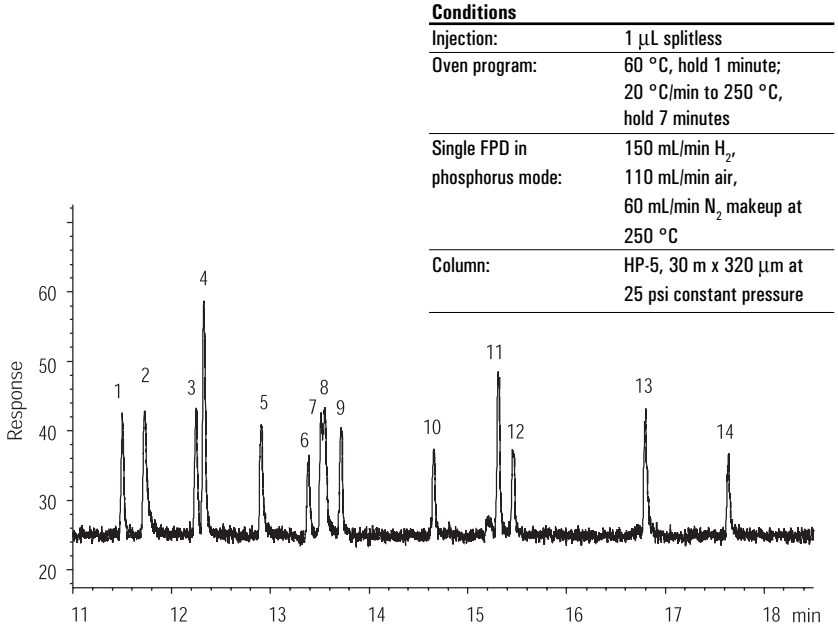


Figure 3. 1 µL Splitless injection of 20–40 ppb organophosphorus pesticide standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conditions	
Injection:	1 µL cool on-column
Oven program:	60 °C, hold 0.5 minute; 25 °C/min to 110 °C, 3 °C/min to 250 °C, hold 10 minutes
Single FPD in phosphorus mode:	75 mL/min H_2, 100 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	1.5 µm HP-1, 30 m x 530 µm, 7 mL/min He constant flow

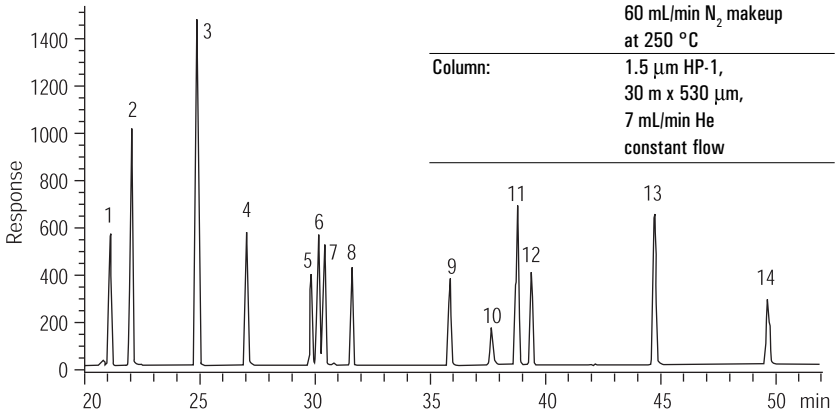


Figure 4. Analysis of organophosphorus pesticides according to EPA method 1618, 1 µL on-column injection of 1–2 ppm standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conclusions

The Agilent 6890 Series GC with an FPD can be used for the sensitive, and selective measurement of sulfur- and phosphorus-containing compounds in complex mixtures. The electronic pneumatics control on the Agilent 6890 GC ensures rapid and accurate gas flow control, provides for easier method setup and documentation, and simplifies optimization.

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Analysis of Organochlorine Pesticides and PCB Congeners with the Agilent 6890 Micro-ECD

Application

Gas Chromatography

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Abstract

A new electron capture detector (ECD) for the Agilent 6890 Series gas chromatograph (GC) was used to analyze polychlorinated biphenyl congeners and organochlorine pesticides. The linearity of the 6890 Micro-ECD in the calibration range of 2 to 400 ppb was evaluated. The micro-ECD easily meets the linearity requirements of U.S. EPA contract laboratory programs for pesticides. Its limit of detection for these compounds goes down to less than 50 ppt. The micro-ECD also exhibits good reproducibility.

Key Words

Organochlorine pesticides, PCB congeners, 6890 GC, micro-ECD; pesticide analysis, ECD.

Introduction

The electron capture detector (ECD) is the detector of choice in many Contract Laboratory Programs (CLP)[1] and EPA methods for pesticide analysis because of its sensitivity and selectivity for halogenated compounds. However, there are drawbacks to the ECD design. The ECD is inherently nonlinear[2], with a limited linear range. The limited linear range means that dilution and reanalysis are frequently required for samples that are outside the calibration range.

Also, the typical ECD is designed to be compatible with both packed and capillary columns. This results in a flow cell that is larger than that required for capillary columns alone, which reduces detector sensitivity.

To address these problems, a new ECD was developed for the 6890 Series gas chromatograph (GC). The 6890 Micro-ECD has a smaller flow cell optimized for capillary columns and was redesigned to improve the linear operating range.

This application note examines the linearity, reproducibility, and limit of detection of the new ECD with mixtures of polychlorobiphenyl (PCB) congeners and organochlorine pesticides (OCPs).

Experimental

All experiments were performed on an 6890 Series GC with electronic pneumatics control (EPC) and the 6890 Micro-ECD. Table 1 shows the experimental conditions for PCB congeners and OCPs.

Table 1. Experimental Conditions for PCB Congener and OCP Analysis.

System Conditions	PCB Congener Analysis	OCP Analysis
Oven	80 °C (2 min); 30 °C/min to 200 °C; 10 °C/min to 320 °C (5 min).	80 °C (2 min); 25 °C/min to 190 °C; 5 °C/min to 280 °C; 25 °C/min to 300 °C (2 min).
Inlet	Split/splitless; 300 °C	Split/splitless; 250 °C
Carrier	Helium, 16.8 psi (80 °C); 1.3-mL/min constant flow	Helium, 23.9 psi (80 °C); 2.2-mL/min constant flow
Sampler	Agilent 7673, 10-µL syringe, 1-µL splitless injection	7673, 10-µL syringe, 1-µL splitless injection
Column	30-m, 250-µm id, 0.25-µm film HP-5MS (part no. 19091S-433)	30-m, 250-µm id, 0.25-µm film HP-5MS (part no. 19091S-433)
Detector	330 °C; makeup gas: nitrogen, constant column and makeup flow	330 °C; makeup gas: nitrogen, constant column and makeup flow

The solutions were prepared by making appropriate dilutions of a stock solution with isooctane. For PCB congeners, the stock solution was an EPA PCB congener calibration check solution (from Ultra Scientific Company, part number RPC-EPA-1). For OCPs, the solution was an OCP calibration check solution (part number 8500-5876).

Results and Discussion

Linearity and Response Factors

A series of dilutions of the PCB mixture from 2 ppb to 200 ppb and of the OCP mixture from 2 ppb to 400 ppb was injected into the 6890 Micro-ECD system. The linearity was determined by calculating the correlation coefficient from the resulting calibration curve.

Figures 1 and 2 present typical chromatograms of OCPs and PCBs at 20 or 40 ppb and 50 ppb, respectively. Figure 3 is a calibration curve of decachlorobiphenyl, typical of other PCB congeners. Figure 4 shows the calibration curve of 4, 4' DDE, typical of OCPs. The correlation coefficient,

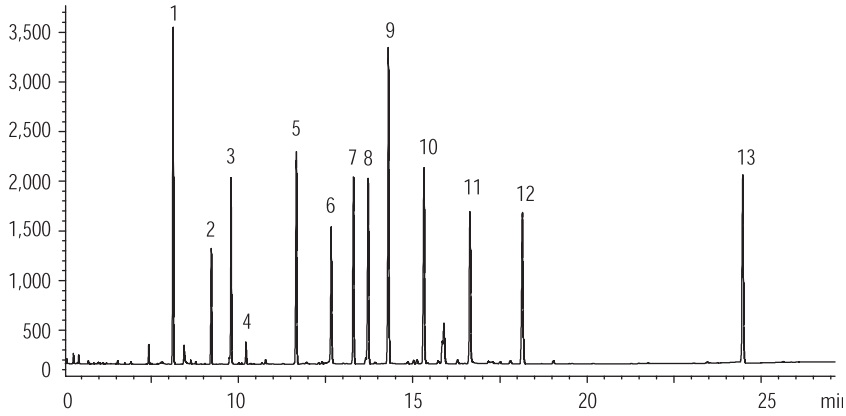

Figure 1. Typical chromatogram of OCPs at 20 or 40 ppb.
See table 1 for conditions. See table 5 for peak identification.

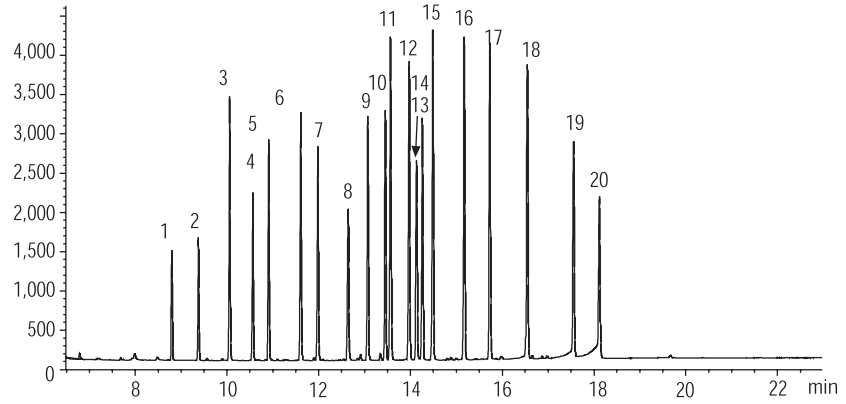

Figure 2. Typical chromatogram of PCB congeners at 50 ppb.
See table 1 for conditions. See table 4 for peak identification.

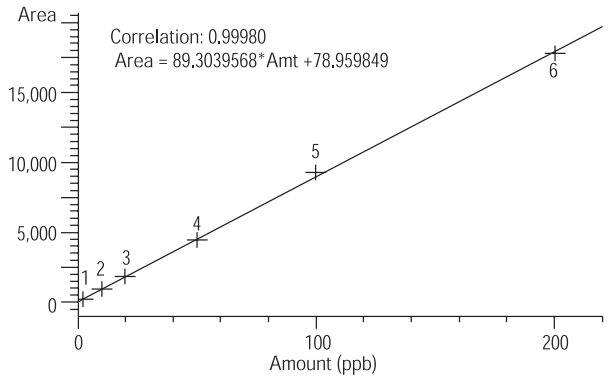

Figure 3. Typical linearity of PCB congener analysis: decachlorobiphenyl from 2-200 ppb.

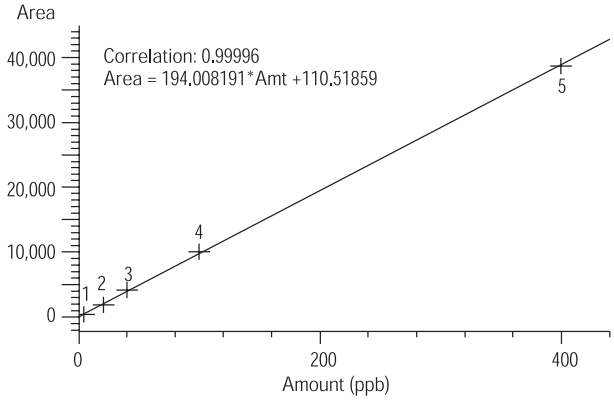

Figure 4. Typical linearity of OCP analysis: 4,4' DDE from 4 to 400 ppb.

average response factors, and percent relative standard deviation (%RSD) for the response factors for each analyte are shown in tables 2 and 3.

All correlation coefficients were at least 0.9996. In these experiments, the 6890 Micro-ECD is linear over this range. The typical range required by CLP methods is 5-80 ppb[1], so the 6890 Micro-ECD exceeds the range by almost twofold.

In addition, the CLP method requires the percent RSD of the response factors for most components to be less than 20 percent for a three-point calibration curve (5 to 80 ppb). As shown in tables 2 and 3, the percent RSD of the response factors ranged from 0.55 percent to 12. 5 percent for the PCB congeners and from 2.8 percent to 10 percent for the OCPs over a concentration range of two orders of magnitude (2 to 400 ppb). Furthermore, the average response factor of each analyte was so consistent and reproducible that the internal standard technique can be used to quantitate all OCPs and PCB congeners.

Table 2. PCB Congener Analysis: Linearity of the 6890 Micro-ECD 2 ppb to 200 ppb.
See table 1 for conditions.

Peak	Name	Average Response Factor	%RSD of Response Factor	Correlation (%)
1	2,4-Dichlorobiphenyl	2e-2	12.5	99.97
2	2,2′,5-Trichlorobiphenyl	2e-2	11.1	99.97
3	2,4,4′-Trichlorobiphenyl	8.5e-3	7.5	99.99
4	2,2′,5,5′-Tetrachlorobiphenyl	1.3e-2	10.2	99.97
5	2,2′,3,5-Tetrachlorobiphenyl	1e-2	9.4	99.98
6	2,3,4,4′-Tetrachlorobiphenyl	8e-3	6.7	99.99
7	2,2′,4,5,5′-Pentachlorobiphenyl	9e-3	8.8	99.98
8	3,3′,4,4′-Tetrachlorobiphenyl	1.2e-2	12.6	99.97
9	2,3,4,4′,5-Pentachlorobiphenyl	8e-3	5.5	99.99
10	2,2′,4,4′,5,5′-Hexachlorobiphenyl	8e-3	8.1	99.98
11	2,3,3′,4,4′-Pentachlorobiphenyl	6e-3	1.9	99.99
12	2,2′,3,4,4′,5-Hexachlorobiphenyl	6.5e-3	3.8	99.99
13	3,3′,4,4′,5-Pentachlorobiphenyl	9e-3	6.5	99.99
14	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	8e-3	5.7	99.99
15	2,2′,3,3′,4,4′-Hexachlorobiphenyl	5.6e-3	1.8	99.99
16	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	5.8e-3	1.0	99.99
17	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	5.8e-3	0.57	99.99
18	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	6e-3	0.78	99.99
19	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl	8e-3	3.1	99.96
20	Decachlorobiphenyl	1e-2	9.5	99.98

Table 3. OCP Analysis: Linearity of the 6890 Micro-ECD *2 or 4* ppb to *200 or 400* ppb.
See table 1 for conditions.

Peak	Name	Average Response Factor	% RSD of Response Factor	Correlation (%)
1	2,4,5,6-Tetra-m-xylene	4.2e-3	5.3	99.97
2	beta-BHC	1.1e-2	7.1	99.99
3	delta-BHC	6.4e-3	4.7	99.99
4	Aldrin	4.7e-3	9.5	99.97
5	Heptachlor epoxide	4.7e-3	5.4	99.99
6	gamma-Chlordane	6.6e-3	6.6	99.99
7	alpha-Chlordane	5e-3	4.3	99.98
8	4,4' DDE	5e-3	2.8	99.99
9	Endosulfan II	2.9e-3	4.4	99.98
10	Endrin aldehyde	4.5e-3	5.9	99.94
11	Endosulfan sulfate	5.1e-3	5.3	99.97
12	Endrin ketone	4.7e-3	9.0	99.89
13	Decachlorobiphenyl	3.7e-3	9.9	99.96

Reproducibility

The reproducibility of the 6890 Micro-ECD was established by analyzing each mixture using identical conditions five times. Each analyte in the PCB congener mixture was injected at a concentration of 50 ppb, and the analytes in the OCP mixture were 20 or 40 ppb. The results are shown in tables 4 and 5. The highest %RSD for any analyte is 3.69 percent for aldrin, which is well below the CLP maximum allowable RSD of 15 percent.[1]

Table 4. PCB Congener Analysis: Reproducibility of the 6890 Micro-ECD 50 ppb; N=5.
See table 1 for conditions.

Peak	Name	Average Area	RSD (%)
1	2,4-Dichlorobiphenyl	2229	1.26
2	2,2′,5-Trichlorobiphenyl	2547	1.29
3	2,4,4′-Trichlorobiphenyl	5687	1.41
4	2,2′,5,5′-Tetrachlorobiphenyl	3721	1.43
5	2,2′,3,5-Tetrachlorobiphenyl	4941	1.46
6	2,3,4,4′-Tetrachlorobiphenyl	5943	1.40
7	2,2′,4,5,5′-Pentachlorobiphenyl	5089	1.47
8	3,3′,4,4′-Tetrachlorobiphenyl	3822	1.72
9	2,3,4,4′,5-Pentachlorobiphenyl	6203	1.62
10	2,2′,4,4′,5,5′-Hexachlorobiphenyl	6189	1.44
11	2,3,3′,4,4′-Pentachlorobiphenyl	8375	1.68
12	2,2′,3,4,4′,5-Hexachlorobiphenyl	7538	1.56
13	3,3′,4,4′,5-Pentachlorobiphenyl	5092	2.02
14	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	6224	1.69
15	2,2′,3,3′,4,4′-Hexachlorobiphenyl	8921	1.67
16	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	8527	1.82
17	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	8625	1.91
18	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	8338	2.13
19	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl	6097	2.55
20	Decachlorobiphenyl	4622	2.85

Table 5. OCP Analysis: Reproducibility of the 6890 Micro-ECD; N=5.
See table 1 for conditions.

Peak	Name	Concentration (ppb)	Average Area	RSD (%)
1	2,4,5,6-Tetra-m-xylene	20	4785	0.7
2	beta-BHC	20	1802	0.81
3	delta-BHC	20	3251	1.50
4	Aldrin	20	402	3.69
5	Heptachlor epoxide	20	4316	1.58
6	gamma-Chlordane	20	2958	1.23
7	alpha-Chlordane	20	4219	1.06
8	4,4' DDE	40	4103	1.76
9	Endosulfan II	40	7176	1.27
10	Endrin aldehyde	40	4719	0.85
11	Endosulfan sulfate	40	4040	3.04
12	Endrin ketone	40	4386	2.52
13	Decachlorobiphenyl	40	5369	0.85

Detection Limit

To establish the lower limit of detection for the 6890 Micro-ECD with PCBs and OCPs, 1-µL injections were made at gradually decreasing concentrations. Figures 5 and 6 show chromatograms with analyte concentrations of 50 to 100 ppt.

All the analyte peaks for both the PCB congener and OCP mixtures are still easy to quantitate, and in fact smaller concentrations can be reliably analyzed. Aldrin, which has the lowest response of the OCPs, still exhibits an adequate signal-to-noise ratio at the 50 ppt level under these analysis conditions.

Conclusion

The Agilent 6890 Micro-ECD response was linear over the concentration range of 2 to 200 ppb, produced reproducible results, and exhibited excellent sensitivity for mixtures of PCB congeners and OCPs.

References

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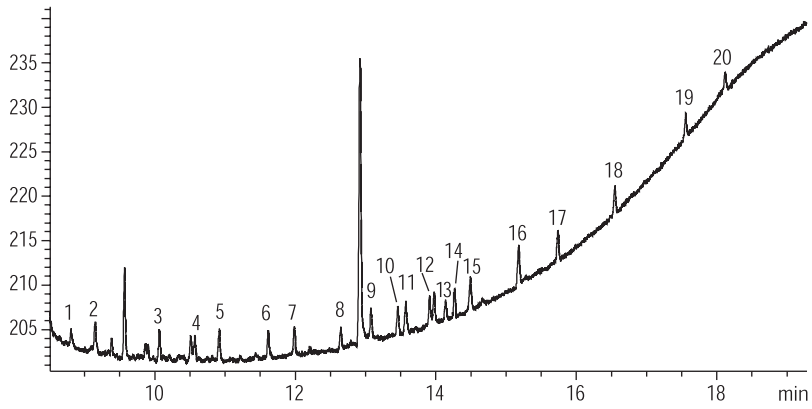

Figure 5. PCB congener mixture at 50 ppt each.
See table 1 for conditions. See table 4 for peak identification.

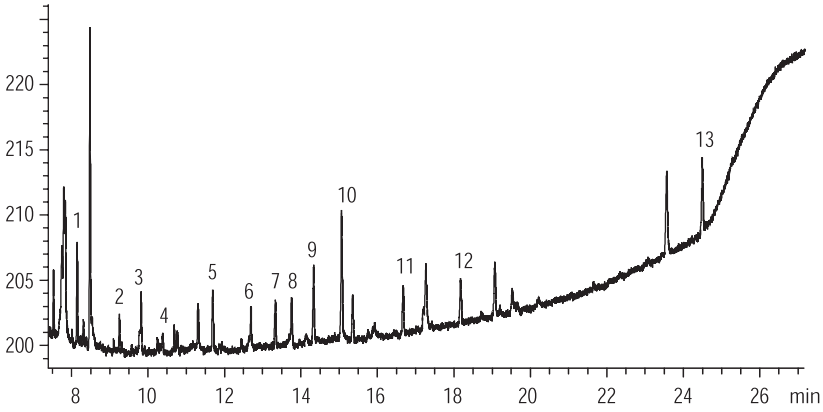

Figure 6. OCP Mixture at 50 to 100 ppt.
See table 1 for conditions. See table 5 for peak identification.

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Fast Screening of Pesticide and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part I

Application

Gas Chromatography
January 2000

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Abstract

Agilent Technologies' new, fast GC/MSD method can significantly speed up the screening of pesticides. Agilent's GC method translation software (available free from the Agilent Technologies Web site, http://www. chem. agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on the standard 42-min method. A 10 m x 0.1 mm x 0.1 µm HP-5 column was used to increase analysis speed up to fourfold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the effect of scaling on the signal-to-noise ratio.

Key Words

RTL, pesticide, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Analysts want faster analyses to improve laboratory productivity. Often, when speeding up GC methods, an analyst will trade resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD).

Agilent Technologies has developed new techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) software and a new mass spectral library that contains the locked retention times and characteristic ions for 567 of the most common pesticides and endocrine disrupters of concern worldwide. A GC/MSD method was developed based on the standard 42-min method[1] to screen for all 567 of the most common analytes. A specific combination of column stationary phase, carrier gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when analyzing extracts containing significant sample matrix content because of overlapping peaks and noisy baselines.

The new screening tool, integrated within Agilent's ChemStation for MSD, searches for all 567 compounds by first checking and integrating four characteristic ions within the expected time window, and second by printing out a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In one application, the analysis time of the standard pesticide method was reduced by one half, two-thirds, and three-fourths. The faster methods were scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software. Because scaling was exact, these faster methods can be used with precisely-scaled pesticide libraries, making the screening process even more powerful and adaptable to individual needs.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of MTL software data entry showing the original conditions and the new chromatographic conditions for a twofold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[3], is also found in the table. A 16:1 split ratio was suggested in the table as a proportional scaling from the original column to the smaller i.d. column with corresponding lower capacity. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

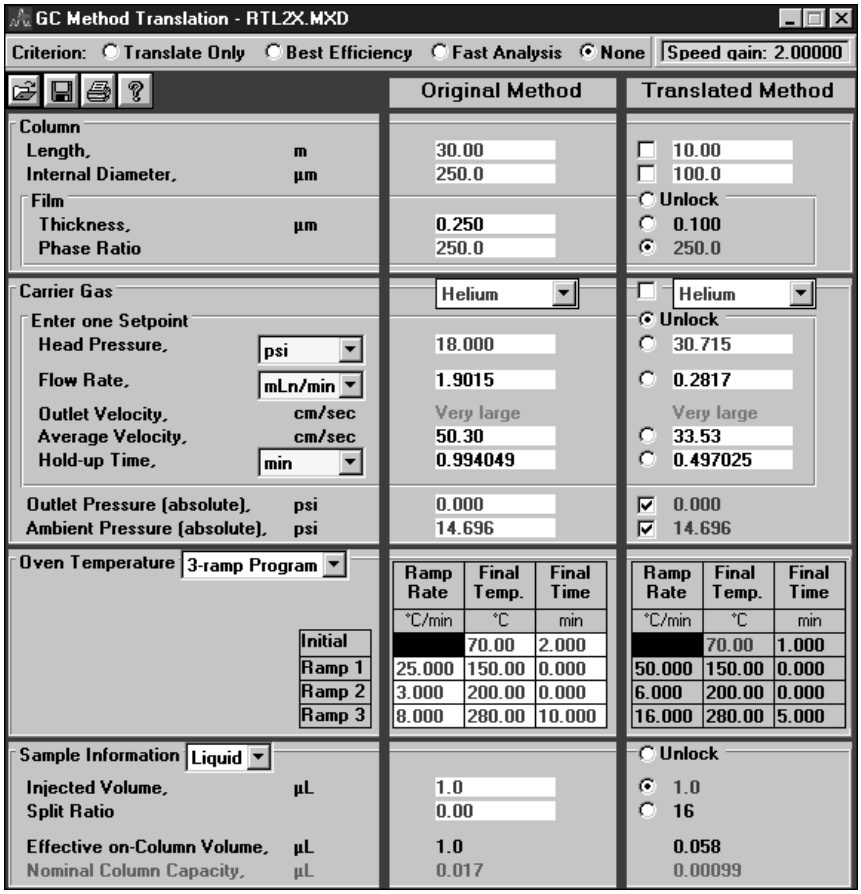

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a twofold speed gain translation.

General chromatographic conditions are listed in table 1. The standard used was a mixture of 26 pesticides at 10 ppm. A 10 m x 0.1 mm x 0.1 µm HP-5 column (part number 19091J-141) was used. The head pressure determined by the method translation software (30.72 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by 2) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD. This process (first translate the method then lock the retention times) was repeated for the threefold and fourfold time reductions.

Table 1. Chromatographic Conditions

Speed	Onefold (1X)	Twofold (2X)	Threefold (3X)	Fourfold (4X)
GC	110 V	220/240 V		
Column	30 m x 0.25 mm x 0.25 µm HP5-MS (P/N 19091S-433)	10 m x 0.1 mm x 0.1 µm HP-5 (P/N 19091J-141)		
Injection mode	Splitless	16:1 split		
Column head pressure	18.0 psi	36.55 psi	63.17 psi	90.0 psi
Column flow (mL/min)	1.5	0.4	0.8	1.5
Inlet control mode	Constant pressure	Constant pressure		
Carrier gas	Helium	Helium		
Injector temperature	250 °C	250 °C		
Oven temperature	70 (2 min)	70 (1 min)	70 (0.67 min)	70 (0.5 min)
Ramp 1	25 °C/min	50	75	100
	150 (0 min)	150 (0 min)	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	6	9	12
	200 (0 min)	200 (0 min)	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	16	24	32
	280 (10 min)	280 (5 min)	280 (3.33 min)	280 (2.5 min)
Oven equilibration	2 min	2 min		
Injection volume	1 µL	1 µL		
Liner	5183-4647	5183-4647		
MS Conditions				
Solvent delay	3 min	1.8 min	1.2 min	0.9 min
Tune file	Atune.u	Atune.u		
Low mass	35 amu	35 amu		
High mass	500 amu	450 amu		
Threshold	150	250		
Sampling	2	2	1	1
Scans/sec	3.15	3.50	6.54	6.54
Quad temperature	150 °C	150 °C		
Source temperature	230 °C	230 °C		
Transfer line temperature	280 °C	280 °C		
Acquisition mode	Scan (EI)	Scan (EI)		

Figure 2 shows the results of the shortened analysis times. The three chromatograms look extremely similar, except that the time axis is scaled proportionally. Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer). The peak heights from all the methods are very similar. Although the sample was split 16:1 for the smaller column, the small column i.d. and faster oven ramp combination made the peaks narrower and higher, so there was minimal loss in the signal to noise ratio.

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of the standard 42-min GC/MSD pesticide method. Run time was shortened to 10.5 minutes using a fast oven ramp rate and a 10-meter 100-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can accurately be scaled to correspond to the faster analyses.

References

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2. H. Prest, P. L. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the HP 6890/ 5973 GC/MSD System," Agilent publication number 5968-4884E, April 1999.
3. H. Prest, "GC Column Selection and Pumping Considerations for Electron and Chemical Ionization MSD operation," Agilent publication Number 5968-7958E, November 1999.

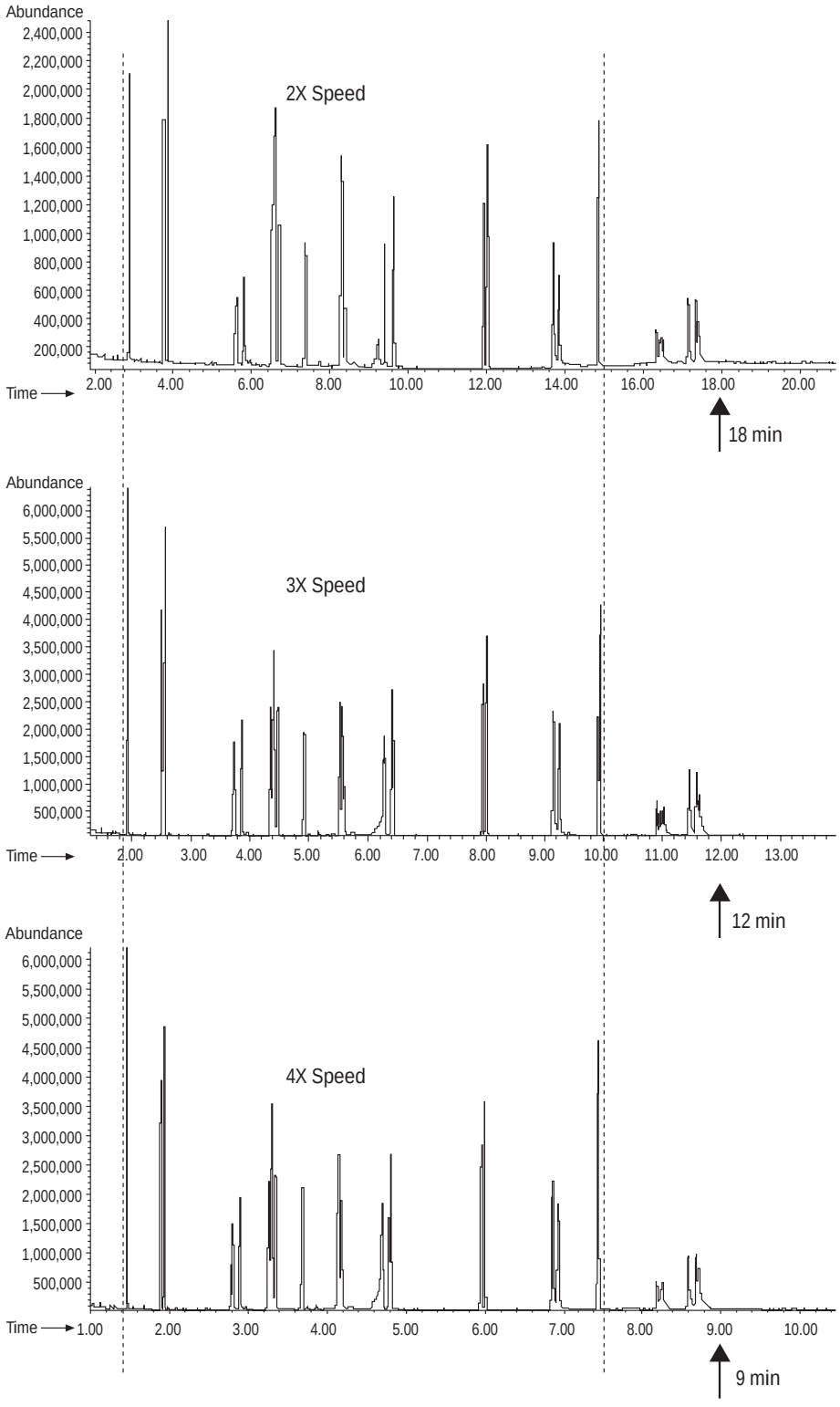

Figure 2. Three TICs of the 2X, 3X, and 4X speedups. The standard analysis (1X) was 42 minutes long. The two vertical lines on the figure are used as references to show the similarity of the TICs.

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LC/DAD/MS Analysis of Carbonyl (2,4-Dinitrophenyl)hydrazones

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Abstract

A simple and sensitive LC/MS method has been developed for the analysis of carbonyl compounds derivatized with (2,4-dinitrophenyl) hydrazine (DNPH) using the Agilent 1100 LC/MSD system. Detection is carried out simultaneously with the diode-array detector and the LC/MSD using negative ion atmospheric pressure chemical ionization (APCI).[1] The method was applied to 78 carbonyls including 1-alkanals (from formaldehyde to octadecanal), saturated and unsaturated aliphatic aldehydes and ketones, aromatic carbonyls (including hydroxy- and/or methoxy-substituted compounds), aliphatic dicarbonyls, and aliphatic carbonyl esters.

Introduction

The ability to identify carbonyls and to measure their concentrations at levels of parts per billion (ppb) or lower in complex mixtures is important in many areas, including biomedical research and environmental chemistry—especially air pollution. A well-established method utilizing UV detection of the DNPHs of both simple and multifunctional carbonyl compounds[2, 3] has been extended to include simultaneous MS detection using APCI in negative ion mode. Concentration of the compounds of interest from ambient air samples and subsequent derivatization is simplified by the use of C18 SPE cartridges impregnated with the derivatizing reagent. The combined methodology has been applied to several studies involving air pollution phenomena.[1]

Experimental

The system included an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector, and an LC/MSD. The LC/MSD was used with the APCI source. Complete system control and data evaluation were carried out using the Agilent ChemStation for LC/MS.

Sample Collection and Preparation

Carbonyl-DNPH standards were synthesized in our laboratory as described previously.[2, 3] Carbonyls were purchased from commercial suppliers (Aldrich Chemical Co., Lancaster Synthesis, Wiley Organics, Fluka Chemical Corp.) or were prepared as described in previous work.[2, 3]

Air samples were collected by drawing air at 1 liter/minute through C18 Sep-Pak cartridges (Waters Corporation) impregnated with (2,4-dinitrophenyl)hydrazine/phosphoric acid.[4] Collected carbonyl compounds were derivatized to (2,4-dinitrophenyl)hydrazones on the cartridge, and were then eluted with 2 mL of acetonitrile. The eluate was analyzed directly by LC/MS. The sample can be concentrated for the analysis of the higher molecular weight carbonyls, which are present at lower levels in ambient air.

Results and Discussion

DNPH derivatives are used to analyze carbonyl compounds by liquid chromatography to maximize detection of small, polar molecules, many of which cannot be analyzed using gas chromatography. The original LC/UV method for the analysis of DNPH derivatives of carbonyls was first improved by the use of a diode-array detector and HP particle beam LC/MS interface to provide positive identification of about 40 carbonyls at ppb levels in laboratory studies of air pollution chemistry[5] and in urban air.[6] The mass spectrometer provided extra dimensions of information to the already-rich data of the diode-array LC method, allowing the quantitation of coeluting analytes and the identification of unknowns for which standards were not initially available. However, the particle beam interface could not provide the detection limits necessary for measurement of carbonyls in ambient air, due to the significant percentage of water in the LC gradient required for the separation of the more complex mixtures.

To overcome this limitation, API-LC/MS was evaluated for this application. Both electrospray (ESI) and APCI in positive ion and negative ion modes were evaluated. APCI negative ion detection was found to provide the most sensitive and specific information about these compounds, giving 1–2 orders of magnitude better sensitivity than either ESI positive or negative ion or APCI positive ion detection.

Parameters for the acquisition of mass spectral data were automatically optimized by carrying out multiple injections of a standard mixture of 13 carbonyl-DNPH derivatives, using the system's Flow Injection Analysis Series capability. A fragmentor setting was chosen to obtain maximum $[M–H]^-$ for all 13 compounds present in the test mixture. Further optimization of the fragmentor voltage for specific compounds could be carried out to obtain distinct fragments, as the fragmentor voltage is time-programmable during acquisition. Those compounds which required a high percentage of acetonitrile for elution (eluting after 33 minutes) were found to have much better response with a corona current of 10 µA versus 4 µA for the smaller, early-eluting analytes. The scan range can be lowered to 50 amu if significant fragment ions below 125 amu are generated by in-source collision-induced dissociation (CID); the chemical noise, especially in the TIC, is lower when starting the scan at 125 amu.

Mobile phases containing acetonitrile often do not give optimal response in APCI compared to methanol/water eluents, and acetonitrile seems to form carbon on the corona needle more quickly than methanol. However, for this analysis, adequate separation of carbonyls in a reasonable analysis time could not be achieved using methanol/water instead of acetonitrile/water, even trying a variety of columns. Nonetheless, maintenance of the APCI spray chamber after extended use with high flow rates of acetonitrile/water only required cleaning of the corona needle and spray shield with mild abrasive cloth and solvent.

Early work with this method was carried out using a similar column but with dimensions of 4.6 mm i.d. × 150 cm at a flow rate of 1.4 mL/min, with results comparable to those obtained on the 3mm i.d. column. An additional gradient has also been developed utilizing THF as a mobile phase modifier. This gradient method is capable of better separation of C3 and C4 carbonyl compounds which co-elute using the acetonitrile/water gradient.

Tables 1–6 (shown on pages 9–14) list the first 78 carbonyl compounds that have been analyzed with this method, along with chromatographic and spectral details. The method has been used for more than 140 carbonyl compounds, including several with molecular weights of approximately 650 Da.

Figure 1 shows the 360 nm and MS total ion chromatograms of a mixture of the DNPH derivatives of 13 carbonyls. The amount injected per component is 60 ng (as carbonyl). The UV chromatogram is labeled with the identity of the peaks and the MS chromatogram with the mass of the base peak in the spectrum ($[M–H]^-$ anion).

Figure 2 shows extracted ion chromatograms from the data in Figure 1, illustrating how the specificity of the MS detector can help with coelution, sometimes even allowing quantitation of coeluting peaks.

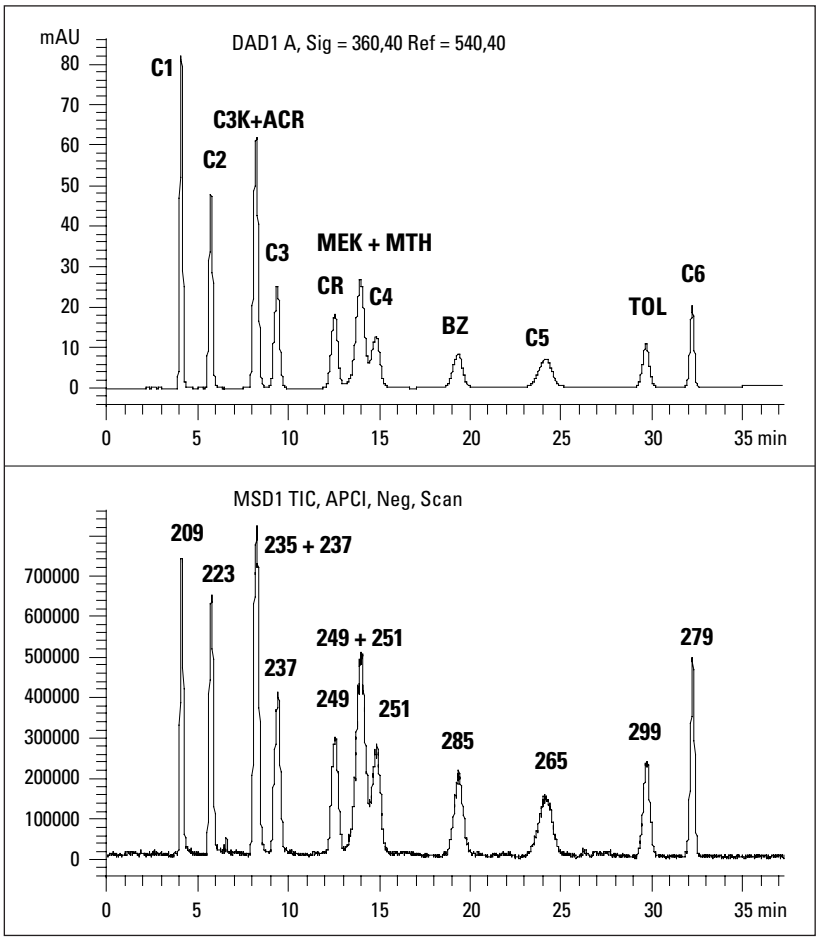

Figure 1. Liquid chromatography analysis of a mixture of the DNPH derivatives of 13 carbonyls by ultraviolet absorption at 360 nm (diode array detector, top) and by atmospheric pressure negative chemical ionization mass spectrometry (total ion current, bottom): C1, formaldehyde; C2, acetaldehyde; C3K, acetone; ACR, acrolein; C3, propanal; CR, crotonaldehyde; MEK, 2-butanone; MTH, methacrolein; C4, butanal; BZ, benzaldehyde; C5, pentanal; TOL, *m*-tolualdehyde; C6, hexanal.

Chromatographic Conditions

Column:	Nucleosil 100-5 C18 HD 5 µm, 3 × 250 mm
Guard column:	Phenomenex Security Guard C18, 3 mm i.d. × 4 mm
Mobile phase:	A = water B = acetonitrile
Gradient:	Start with 49% B at 26 min 49% B at 40 min 100% B
Post-time:	5 minutes
Flow rate:	1.0 ml/min
Column temp:	38°C
Injection vol:	20 µl
Diode-array detector:	Signal: 360, 40; 385, 40; 430, 40 nm Reference: 540, 40 nm

MS Conditions

Source:	APCI
Ionization mode:	Negative
Vcap:	1500 V
Corona current:	10 µA
Nebulizer:	60 psig
Drying gas flow:	4 l/min
Drying gas temp:	350°C
Vaporizer temp:	500°C
Scan:	125–600 amu,
Threshold:	150 counts
Gain:	5
Step size:	0.1 amu
Peak width:	0.1 min
Time filter:	On
Fragmentor:	50 V

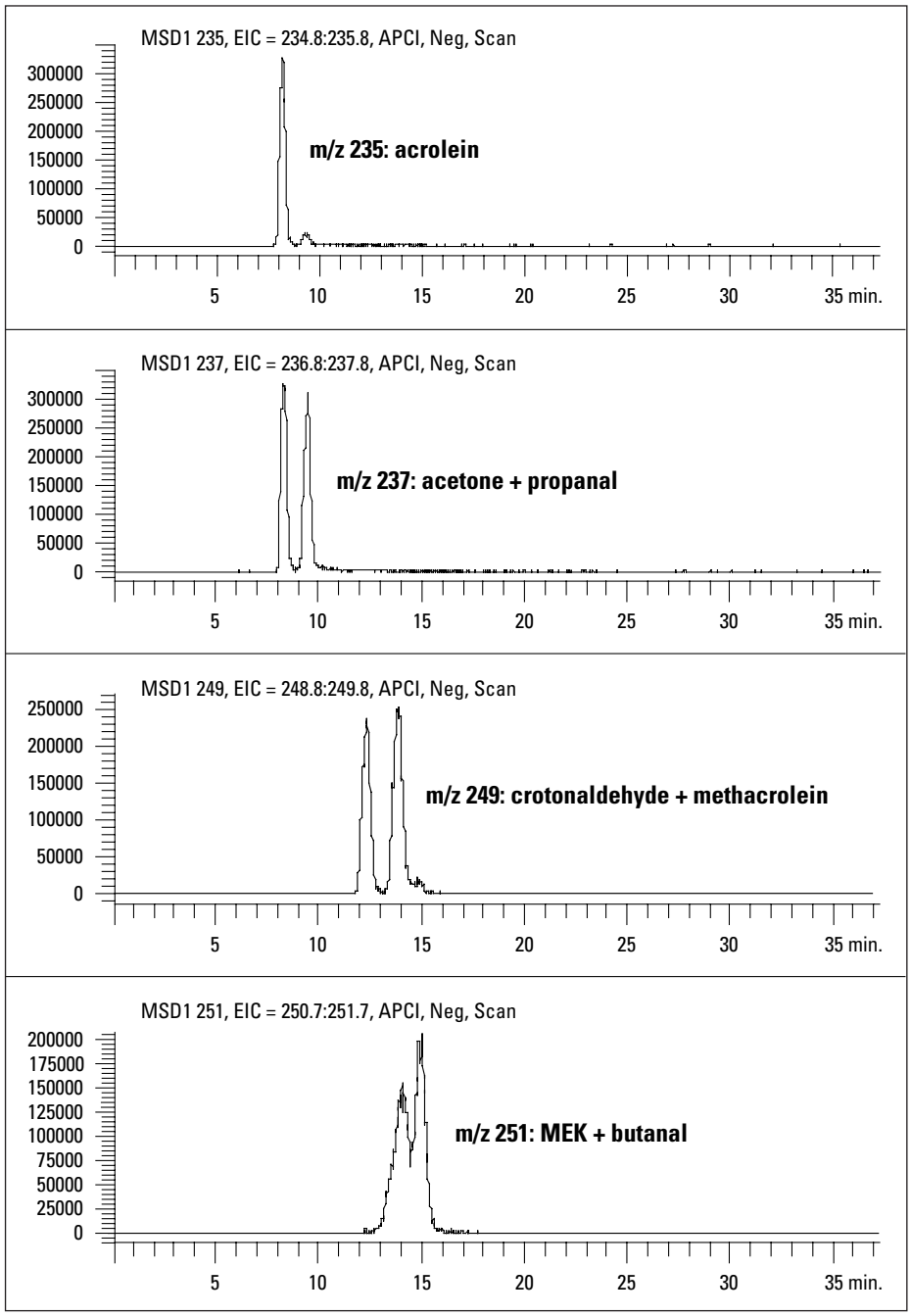

Figure 2. Extracted ion chromatograms for the region of Figure 1 containing acrolein (*m/z* 235), acetone and propanal (*m/z* 237), crotonaldehyde and methacrolein (*m/z* 249), and MEK (2-butanone) and butanal (*m/z* 251).

Figure 3 shows mass spectra of two carbonyl DNPHs from the data in Figure 1: formaldehyde DNPH and hexanal DNPH. Using conditions optimized for best detection of the $[M–H]^-$ ion, these spectra show little fragmentation even with the high vaporizer temperature (500°C) found to be optimal for the method.

Figure 4a shows the MS total ion chromatogram of a sample taken from a study of the reaction of the unsaturated ketone 4-hexen-3-one with ozone in a laboratory smog chamber. The LC/MS analysis allows the identification of unreacted 4-hexen-3-one, and of the carbonyl reaction products acetaldehyde, 2-oxobutanal, formaldehyde, glyoxal, and cyclohexanone (the latter a product of oxidation of cyclohexane, added to scavenge any OH radical which may form as a side product of the ozone-unsaturated ketone reaction). Figure 4b shows the mass spectra of the DNPH derivatives of cyclohexanone and of the dicarbonyl compound 2-oxobutanal. The spectra contain the ion *m/z* 182, which is characteristic of many carbonyl DNPHs and can be used to help locate and identify carbonyl DNPHs in complex mixtures.

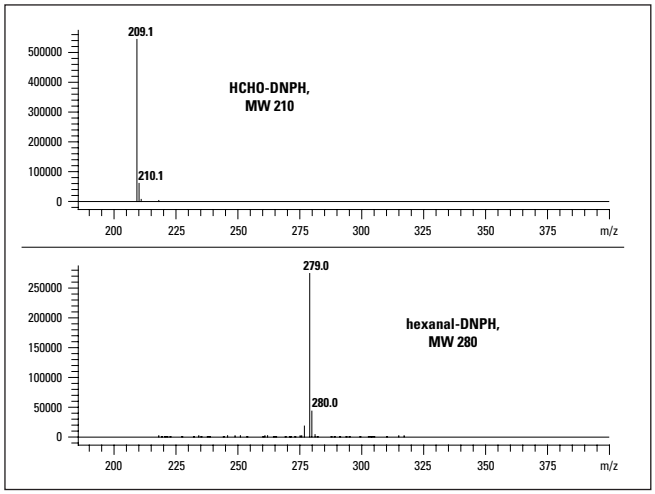

Figure 3. Atmospheric pressure negative chemical ionization mass spectra of analytes in Figure 1: (top) formaldehyde DNPH, (bottom) hexanal DNPH.

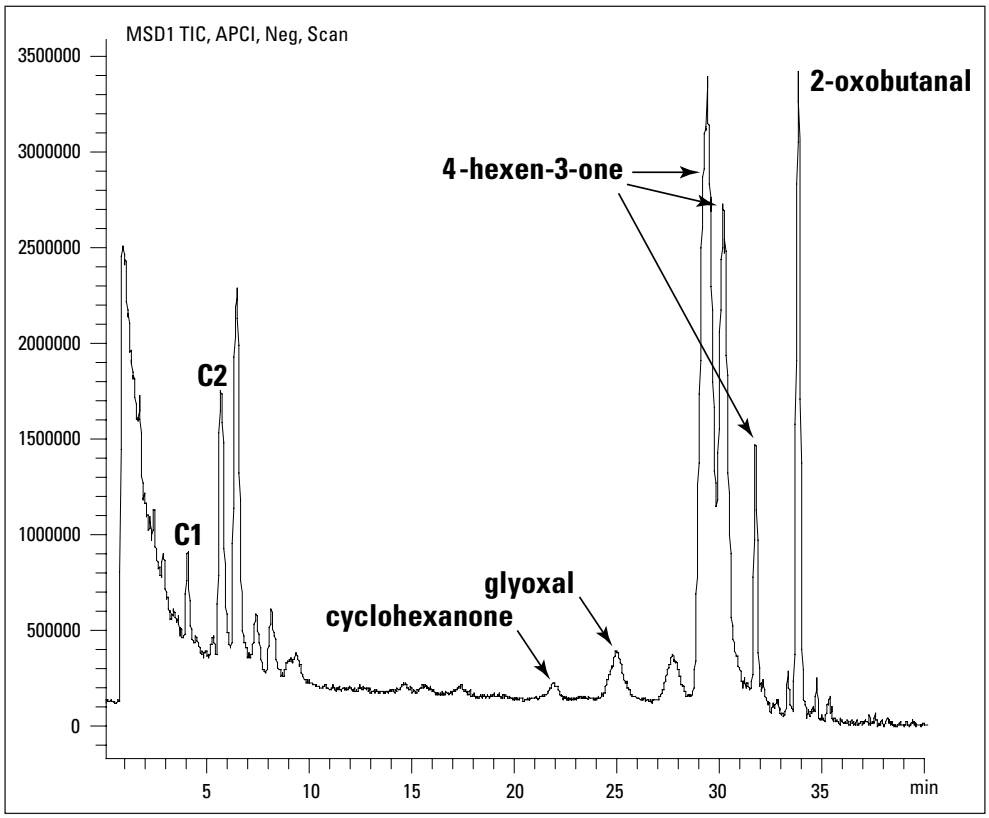

Figure 4a. Atmospheric pressure negative chemical ionization mass spectrometry analysis of the carbonyl products of the reaction of ppb levels of ozone with 4-hexen-3-one in the presence of cyclohexane: (a) total ion current chromatogram with DNPH derivatives of unreacted 4-hexen-3-one (three peaks due to syn/anti isomers of DNPH) and of the reaction products formaldehyde (C1), acetaldehyde (C2), cyclohexanone, glyoxal, and 2-oxobutanal.

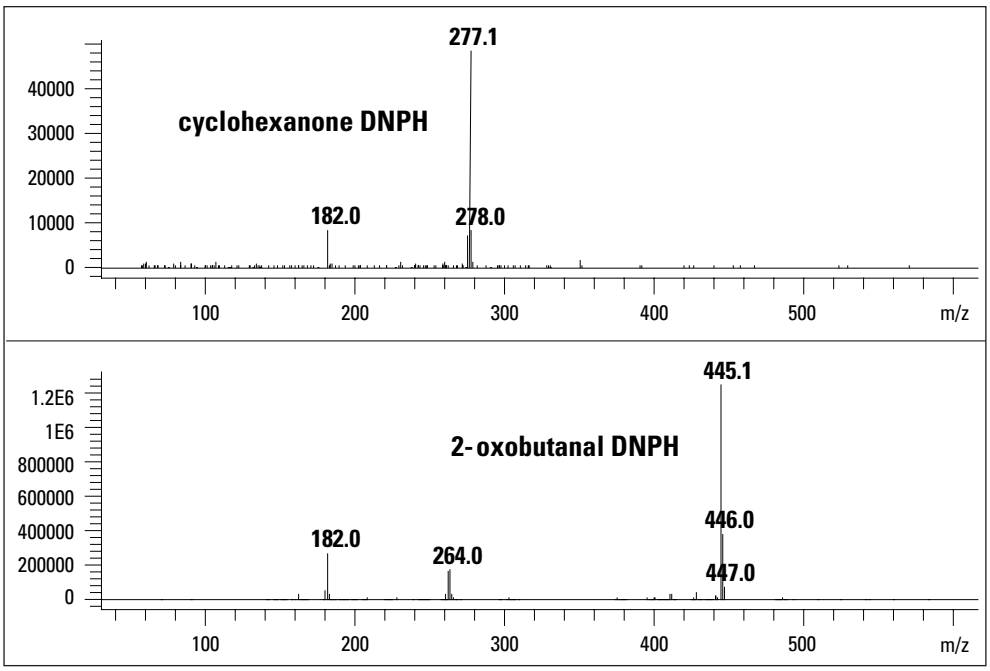

Figure 4b. Atmospheric pressure negative chemical ionization mass spectra of DNPHs of cyclohexanone and 2-oxobutanal.

Figure 5a shows the UV and MS chromatograms from the LC/MS analysis of an ambient air sample collected during early morning peak traffic in Porto Alegre, Brazil, where the mixture of vehicle fuels is unique in the world.[7] The MS data is shown using the base peak chromatogram (BPC), a very useful tool for helping to filter noise from the MS data. The BPC reconstructs an MS chromatogram using only the most intense ion (the base peak) from each spectrum, rather than adding up the abundances of all ions in each spectrum as does the total ion chromatogram (TIC).

Figure 5b shows an expanded view of the region of the UV and MS chromatograms, in which the C6 to C18 straight-chain alkanals elute. In Figure 5c, the extracted ion chromatograms for specific compounds show the distinctive masses of the $[M-H]^-$ ions, which confirm and/or identify the peaks detected with the UV detector.

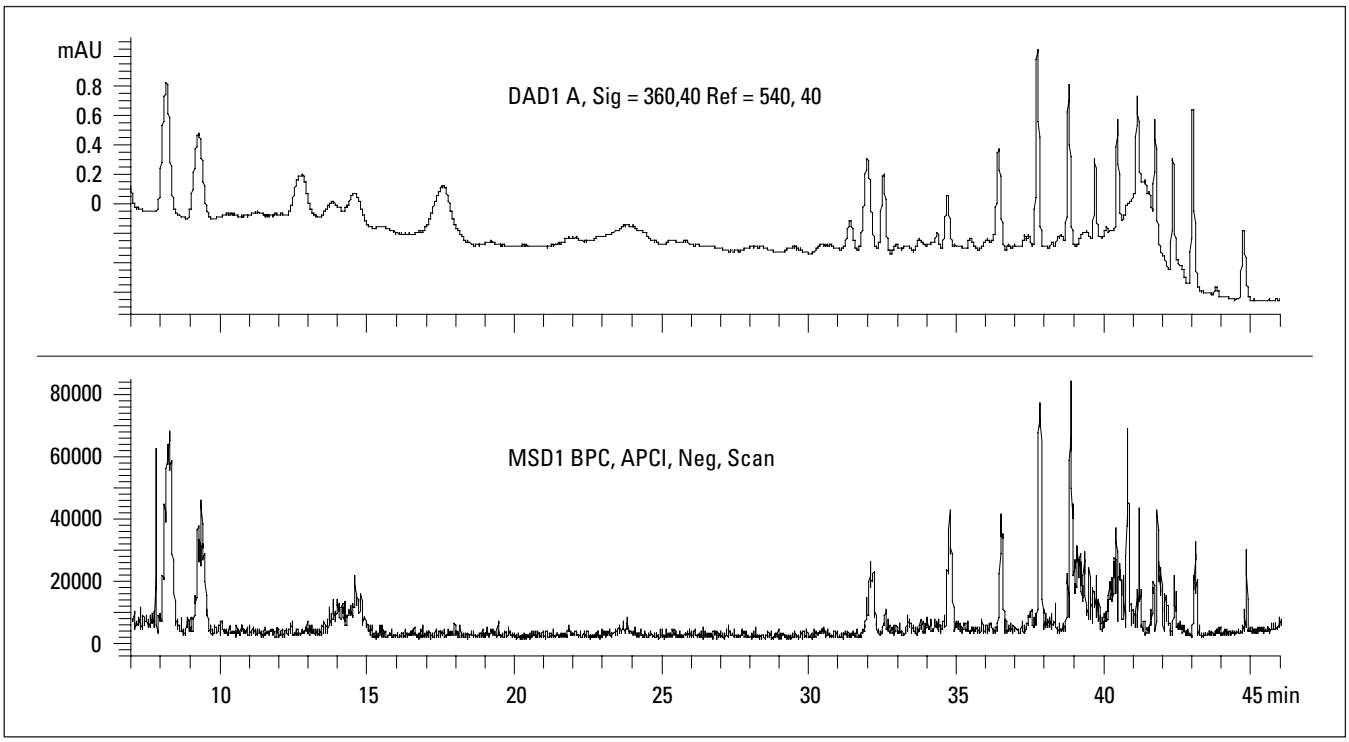

Figure 5a. LC/MS analysis of an ambient air sample collected in Porto Alegre, Brazil, during early morning peak traffic: (top) UV 360 nm chromatogram; (bottom) APCI negative ion base peak chromatogram (BPC).

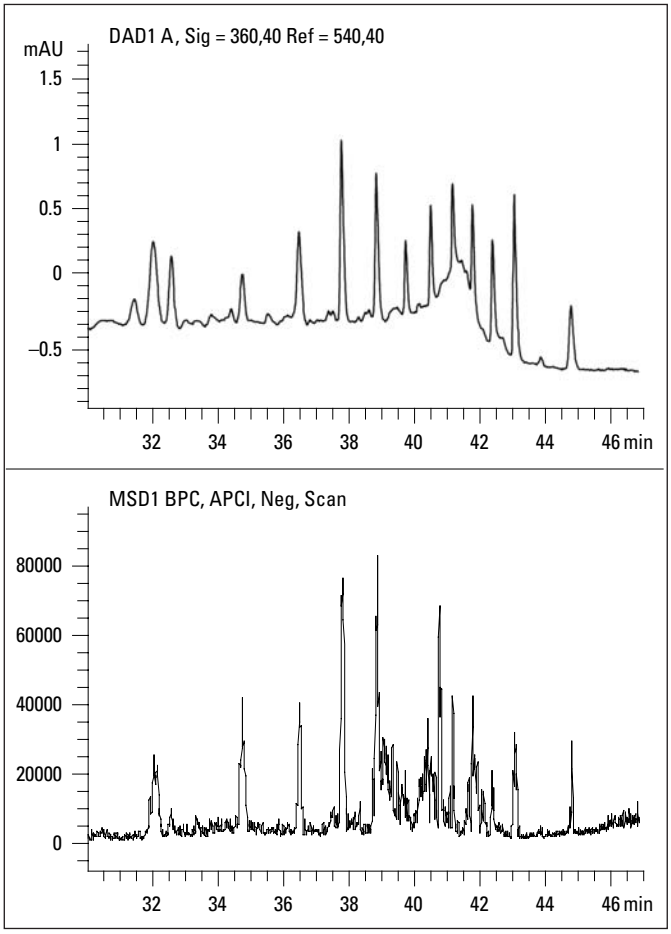

Figure 5b. Expanded region from C6 to C18 alkanals of the analysis in Figure 5b: (top) UV 360 nm chromatogram; (bottom) APCI negative ion base peak chromatogram (BPC).

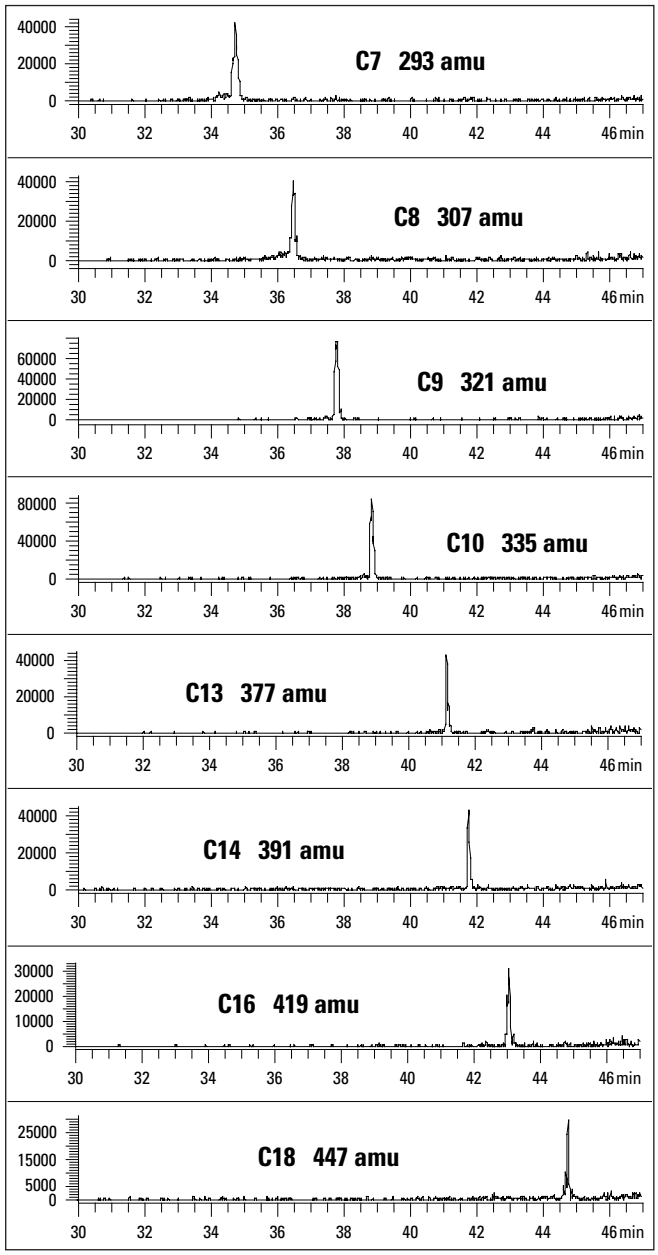

Figure 5c. Extracted ion chromatograms of the $[M–H]^-$ ion for 1-alkanal DNPH derivatives in Brazil air sample. Each EIC is labeled with the carbon number of the 1-alkanal DNPH derivative and the observed mass of the M–H ion.

Summary and Conclusions

This note describes the straightforward addition of API mass spectrometry to a well-established LC method for carbonyl analysis. The resulting APCI-LC/MS method is robust and sensitive, with application not only to simple aldehydes and ketones, but also to hydroxy carbonyls, dicarbonyls, carbonyl esters and keto acids as well. This development has improved a long-standing technique in environmental research, and is applicable to many other fields in which carbonyl-containing compounds are important but difficult to analyze with adequate selectivity, sensitivity, and/or confidence in identification.

Acknowledgments

The authors would like to thank ***Christine Miller*** of Agilent Technologies for review and helpful comments.

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Table 1. Summary of Data for the DNPH Derivatives of 1-Alkanals.

Carbonyl	Carbonyl-DNPH			
	RRT[a]	UVmax[b]	MW[c]	BP[de]
formaldehyde	1.00	355	210	209
acetaldehyde	1.40	364	224	223
propanal	2.30	365	238	237
butanal	3.65	366	252	251
pentanal	5.98	367	266	265
hexanal	7.92	366	280	279
heptanal	8.54	366	294	293
octanal	8.96	365	308	307
nonanal	9.28	363	322	321
decanal	9.52	362	336	335
undecanal	9.74	362	350	349
dodecanal	9.92	361	364	363
tridecanal	10.09	361	378	377
tetradecanal	10.24	361	392	391
pentadecanal	10.43	361	406	405
hexadecanal	10.62	361	420	419
heptadecanal	10.85	360	434	433
octadecanal	11.12	360	448	447

[a] RRT = retention time of carbonyl-DNPH relative to that of formaldehyde-DNPH (4.08 ± 0.02 min).

[b] UV max = wavelength of maximum absorption, nm, from 200–600 nm absorption spectrum recorded with diode array detector.

[c] MW = molecular weight of carbonyl-DNPH.

[d] BP = base peak (most abundant ion), *m/z,* in atmospheric pressure negative chemical ionization mass spectrum.

[e] No ions other than BP and ^{13}C contribution to BP (see text) were present in the spectra of the DNPH derivatives of 1-alkanals.

Table 2. Summary of Data for the DNPH Derivatives of Other Saturated Aliphatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
ALDEHYDES					
2-methylpropanal	3.69	363	252	251	none
3-methylbutanal	5.51	363	266	265	none
2-methylbutanal	5.70	363	266	265	263 (1)
2,2-dimethylpropanal	5.66	364	266	265	none
cyclohexylmethanal	8.09	366	292	291	none
KETONES					
acetone	2.01	368	238	237	none
acetone-d_6	1.98	367	244	243	237–242[c]
2-butanone	3.44	369	252	251	none
2-pentanone	5.51	371	266	265	none
3-pentanone	5.51	370	266	265	263 (2)
3-methyl-2-butanone	5.52	370	266	265	263 (2)
3,3-dimethyl-2-butanone	7.78	370	280	279	263 (1)
2,4-dimethyl-3-pentanone	8.27	370	294	293	277 (8)
cyclohexanone	5.36	373	278	277	275 (23)
2-methylcyclohexanone	7.94	371	292	291	289 (25)
nopinone[d]	8.30	372	318	317	315 (5)

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* Not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Abundances relative to that of BP = 3% (*m/z* = 237), 4% (238), 6% (239), 13% (240), 24% (241), and 44% (242).

[d] 6,6-Dimethylbicyclo [3.1.1] heptan-2-one.

Table 3. Summary of Data for the DNPH Derivatives of Unsaturated Aliphatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
ALDEHYDES					
acrolein	2.01	380	236	235	none
crotonaldehyde[c]	3.08	382	250	249	none
methacrolein	3.44	381	250	249	none
2-ethylacrolein	5.55	379	264	263	none
trans-2-hexenal	7.60	382	278	277	275 (7)
2-methyl-2-pentenal	7.67	384	278	277	275 (3)
cis-4-heptenal	7.78[d]	365	292	291	289 (7)
	7.95[e] (2%)	366	292	291	289 (7)
trans-2-decenal	9.41	381	334	333	331 (6)
trans-2-undecenal	9.64	380	348	347	345 (5)
KETONES					
methyl vinyl ketone	2.68[e] (13%)	368	250	249	none
	2.87[e] (3%)	372	250	249	none
	3.06[d]	379	250	249	none
1-penten-3-one	4.76[d]	378	264	263	247 (8)
	4.99[e] (12%)	376	264	263	247 (9)
3-penten-2-one	4.43[e] (3%)	382	264	263	none
	4.83[d]	384	264	263	none
4-methyl-3-penten-2-one	6.94	386	278	277	263 (3)
4-hexen-3-one[c]	7.12[e] (4%)	385	278	277	none
	7.38[e] (35%)	357	278	277	none
	7.72[d]	357	278	277	none
6-methyl-5-hepten-2-one[f]	8.27	368	306	305	289 (4)
4-acetyl-1-methylcyclohexene	8.64	369	318	317	301 (4)

[a] RRT, UV max, MW, and BP are defined in footnotes *a*–*d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to base peak; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Predominantly the trans isomer.

[d] Largest peak.

[e] Smaller peak; percent of largest peak (peak height basis at 360 nm) given in parentheses.

[f] Two coeluting peaks.

Table 4. Summary of Data for the DNPH Derivatives of Aromatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
benzaldehyde	4.75	384	286	285	none
o-tolualdehyde	7.13	386	300	299	none
m-tolualdehyde	7.29	385	300	299	none
p-tolualdehyde	7.35	388	300	299	none
acetophenone	6.77	382	300	299	none
2,5-dimethylbenzaldehyde	8.15	389	314	313	none
2-hydroxybenzaldehyde (salicylaldehyde)	2.97	391	302	301	none
4-methoxybenzaldehyde (*p*-anisaldehyde)	4.98	398	316	315	none
3,4-dimethoxybenzaldehyde	3.07	398	346	345	none
4-hydroxy-3-methoxybenzaldehyde (vanillin)	1.75	402	332	331	329 (2), 315 (1)
4-hydroxy-3-methoxyacetophenone (acetovanillone)	2.37	393	346	345	343 (5), 329 (45), 313 (4), 298 (2)
3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde)	1.54	436	362	361	360 (40), 359 (1), 345 (1)
4-hydroxy-3-methoxycinnamaldehyde (coniferyl aldehyde)	2.67	415	358	357	356 (12), 355 (22), 325 (5), 310 (10)

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

Table 5. Summary of Data for the DNPH Derivatives of Dicarbonyls.[a]

Carbonyl	Carbonyl-DNPH					
	RRT	UV max	MW (mono)	MW (di)	BP	Other Ions[b]
glyoxal	6.09	415	238	418	417	237 (14), 238 (16)
methylglyoxal	7.90	432	252	432	431	251 (14), 249 (17)
2-oxobutanal[c]	8.31	410	266	446	445	263 (12)
2,3-butanedione	1.50[e] (1%)	362	266		265	none
	1.79[e] (2%)	369	266		265	none
	8.31[d]	403		446	445	265 (7), 263 (48)
succinic dialdehyde	0.81[e] (5%)	360	266		265	
	1.55[d f]	338[f]			247[f]	
	6.42[e] (12%)	368		446	445	263 (80)
glutaraldehyde	7.34	368	280	460	459	279 (10)
2,3-pentanedione	8.72	402	280	460	459	443 (8), 279 (15)
2,4-pentanedione	1.03	310	280	460	262	302 (14), 232 (6), 360 (7), 279 (0.1), 288 (5)
3,4-hexanedione	8.89	400	294	474	473	293 (5), 291 (12)
pinonaldehyde[g]	3.73[e] (9%)	368	348		347	none
	9.07[d]	368		528	527	345 (16)

[a] RRT, UV max, and BP are defined in footnotes *a–d* of Table 1. MW (mono) and MW (di) are the molecular weights of the mono-DNPH derivative and di-DNPH derivative, respectively.

[b] *m/z;* not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Prepared by reaction of ozone with 1-penten-3-one, 2-ethylacrolein, and 4-hexen-3-one.

[d] Largest peak.

[e] Smaller peak; percent of largest peak (peak height basis at 360 nm) is given in parentheses.

[f] This compound is not the mono-DNPH derivative; see text.

[g] (2,2-Dimethyl-3-acetylcyclobutyl) ethanal, prepared by reaction of ozone with pinene.

Table 6. Summary of Data for the DNPH Derivatives of Other Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
methyl glyoxylate[c]	0.89[f] (60%)	355	268	267	none
	1.72[e]	357	268	267	none
ethyl glyoxylate	1.24[f] (65%)	356	282	281	none
	2.71[e]	359	282	281	none
2-oxoethyl acetate[d]	1.14[e]	360	282	281	249 (18)
	1.22[f] (13%)	356	282	281	249 (16)
methoxyacetone	1.59[e]	363	268	267	none
	2.25[f] (30%)	370	268	267	none
2-furaldehyde	2.14[e]	392	276	275	none
	3.00[f] (25%)	383	276	275	none

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to base peak; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Prepared by reaction of ozone with methyl acrylate and with methyl *trans*-3-methoxyacrylate (MTMA).

[d] Prepared by reaction of ozone with MTMA and with *trans*-2-hexenyl acetate.

[e] Largest peak.

[f] Smaller peak; percent of largest peak (peak height basis at 360 nm) is given in parentheses.

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Analysis of Bendiocarb and Metabolite by HPLC

Rainer Schuster

Environmental

Abstract

The bendiocarb insecticide can be extracted from soil either with Soxhlet equipment or by ultrasonic treatment in solution and from water by either a liquid–solid or a liquid–liquid technique.

Separation

Figure 1 shows the separation on a 2.1 mm internal diameter Hypersil ODS column. A constant oven temperature of 40 °C is important here.

- UV-visible detection
- Diode-array detection—for simultaneous multiple wave-lengths and peak identity confirmation by spectra.

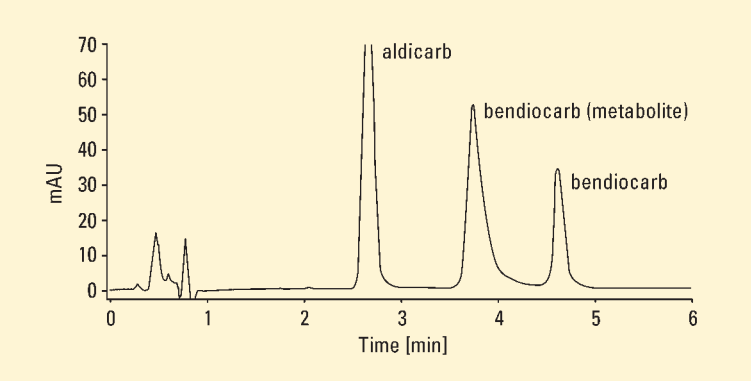

Figure 1
Separation of a 20 µl injection containing aldicarb, bendiocarb and metabolite monitored at 212 nm

Conditions

Column
100 x 2.1-mm Hypersil ODS C18, 5 µm
Mobile phase
Water–acetonitrile
(65:35 isocratic mixture)
Flow rate
0.36 ml/min
Temperature
40 °C
Detection
212 nm (16 nm bandwidth)
reference 450 nm (100 nm bandwidth)

Diode array detector performance
Detection limit 4 µg/l
(without sample enrichment

Agilent Technologies
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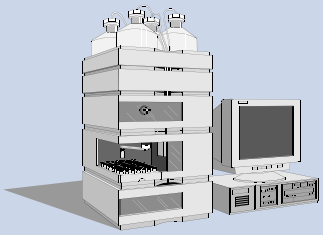
Sample preparation

Narrow-bore technology for
lowest solvent consumption and highest sensitivity.

Equipment

Agilent 1100 Series
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Analysis of Paraquat and Diquat by HPLC

Rainer Schuster

Environmental

Abstract

The paraquat and diquat herbicides can be extracted from soil either with Soxhlet equipment or by ultrasonic treatment in solution and from water by either a liquid–solid or a liquid–liquid technique.

Separation

Figure 1 shows the separation on a 2.1-mm internal diameter Hypersil ODS column.

- UV-visible detection
- Diode-array detection—for simultaneous multiple wavelengths and peak identity confirmation by spectra.

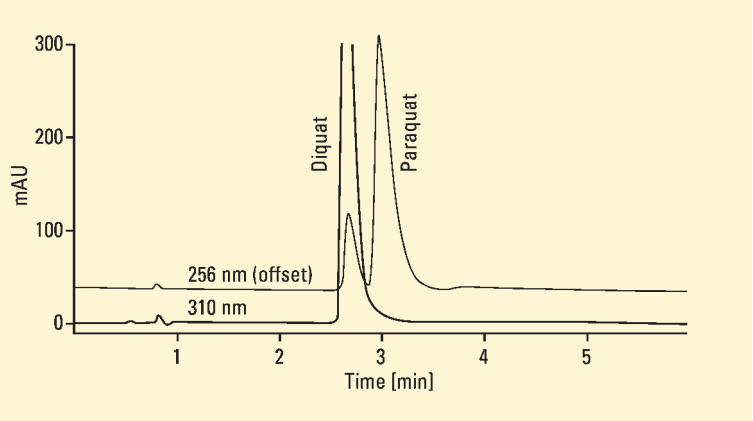

Figure 1
Separation of 10 µl injection of a paraquat and diquat standard

Conditions

Column
100 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
Hexane sulfonic acid
0.35 % triethylamine
pH 2.5 (H_3PO_4)
Flow rate
0.4 ml/min
Detection
256 nm (10 nm bandwidth),
310 nm (10 nm bandwidth)
reference 450 nm (100 nm bandwidth)

Diode array detector performance
Detection limit 4 µg/l
1 ng (absolute) with enrichment factor of 100

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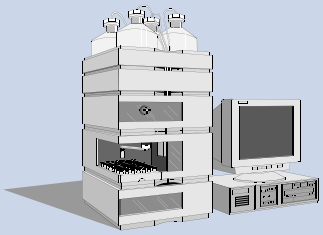
Sample preparation

Narrow-bore technology for lowest solvent consumption and highest sensitivity.

Equipment

Agilent 1100 Series
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Soil & Sediment

Inorganics Applications

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Direct Analysis of Undiluted Soil Digests Using the Agilent High Matrix Introduction Accessory with the 7500cx ICP-MS

Application

Environmental

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Abstract

Agilent has developed the High Matrix Introduction (HMI) accessory for ICP-MS as an alternative to conventional dilution. The HMI modifies the sample introduction system of the Agilent 7500 Octopole Reaction System (ORS) ICP-MS, making it possible to directly measure sample solutions with total dissolved solids (TDS) exceeding 1%. In collaboration with the Eurofins Analytico laboratory in the Netherlands, a 7500cx/HMI was used to directly measure high TDS soil extracts in compliance with Dutch regulatory guidelines. The results show that the HMI is a suitable replacement for conventional autodilution, allowing Analytico to use a single 7500cx/HMI ICP-MS in place of several instruments, including conventional ICP-MS, ICP-OES and a dedicated mercury analyzer.

Introduction

The determination of trace elements in high-matrix samples has always been a difficult analytical challenge. While inductively coupled plasma mass spectrometry (ICP-MS) has unsurpassed detection capability for trace metals, the potential for salt accumulation on the MS interface has always required that dissolved solids levels be limited. Furthermore, extremely corrosive or acidic samples can damage conventional ICP-MS interface components, including the sampler and skimmer cones, requiring the use of platinum or other expensive components. Compared to ICP-Optical Emission Spectroscopy (ICP-OES) or other non-MS-based techniques, this has been considered an inherent limitation of ICP-MS. To compensate for this limitation, samples with total dissolved solids (TDS) levels higher than 0.1 to 0.2%, depending on the matrix, typically require dilution before measurement by ICP-MS, relying on ICP-MS's high sensitivity to compensate for the sensitivity loss due to dilution. However, conventional sample dilution has a number of other disadvantages, including reduced productivity, introduction of contaminants, dilution factor errors, and increased waste volume. As a superior alternative to conventional dilution, Agilent has developed a simple, novel modification to the sample introduction system of the Agilent 7500 ICP-MS with Octopole Reaction System (ORS) that can significantly improve the

tolerance to high-matrix samples. The Agilent High Matrix Introduction (HMI)[1] accessory reduces sample matrix load on the plasma, making it possible to directly measure sample solutions with TDS exceeding 1%. As a result, samples previously measurable only by ICP-OES can now be measured directly by ICP-MS, using a 7500 ORS fitted with HMI. In this work, an Agilent 7500cx/HMI was used to test the system's ability to directly measure high TDS soil extracts in aqua regia and meet Dutch regulatory guidelines for reporting limits and data quality.

Direct Analysis of Aqua Regia Digests of Soils Using HMI-ICP-MS

This work was done in collaboration with the Eurofins Analytico laboratory (Analytico Milieu) in the Netherlands. Analytico has been part of the Eurofins group since 2001. Eurofins is a bio-analytical company with approximately 50 laboratories distributed over France, Germany, the UK, Denmark, Norway, Switzerland, and the United States. One of three divisions within Analytico, Analytico Milieu's expertise extends to the analysis of soil, sludge, groundwater, wastewater, air, building materials and residual matter, and, additionally, method development, validation, logistics, project management, and data management.

In this collaboration, an Agilent 7500cx equipped with the HMI was used to generate performance data to be used for compliance with the Dutch regulation pertaining to contaminated soils (AS3000). Due to the high TDS levels and high acid concentrations of digested soils, dilution is necessary prior to analysis by ICP-MS. Analytico currently uses the Agilent Integrated Sample Introduction System (ISIS) to perform online dilutions to meet these requirements. However, the limitations of conventional dilutions prompted Analytico to evaluate the potential of HMI as a faster, simpler, less expensive replacement for conventional autodilution that would also eliminate the maintenance issues associated with a conventional autodilutor.

Instrumentation

An Agilent 7500cx ICP-MS with the second peripump option for high sample throughput was equipped with the HMI. A Burgener MiraMist nebulizer was used. Instrument tune parameters, including HMI settings, are listed in Table 1. The plasma was optimized in ultra robust mode with 1/12 aerosol dilution. This is the maximum dilution factor that can be set with HMI and is approximately equivalent to diluting the sample x12 conventionally. All analytes except selenium were acquired in helium collision mode, thus eliminating the need for no-gas mode and the associated time required for mode switching. Selenium was acquired in hydrogen reaction mode due to the low detection limits required.

Table 1. Instrument Tune Conditions (Values relating to HMI are shaded.)

RF power (W)	1600
Carrier gas (L/min)	0.28
HMI dilution gas (L/min)	0.67
Aerosol dilution factor	1/12
Sample uptake rate (mL/min)	0.17
ISTD uptake rate (mL/min)	0.17
Total nebulizer flow (mL/min)	0.34
Extract 1 (V)	0
Extract 2 (V)	–160
He flow (He mode)	4.0 mL/min
KED (He mode)	2 volts
H_2 flow (H_2 mode)	4.0 mL/min
KED (H_2 mode)	2 volts

Sample Preparation

Actual soil samples received from Analytico's customers were prepared by adding 1 gram of soil to 8 mL of aqua regia prior to microwave digestion. Digested samples were then diluted to 50 mL final volume in ultra pure (18.2 MΩ) water. The final acid concentration is 4% HNO_3 and 12% HCl.

Calculation of Method Detection Limits (MDLs)

Since the HMI is effectively applying a dilution, the effects on detection limits are of critical importance. While ICP-MS possesses high sensitivity, the requirement for ultra trace detection limits in high TDS samples can still be challenging. Analytico has required MDLs for most analytes (based on a 1–g soil sample diluted to a final volume of 50 mL; see Table 2), which must be met in order for them to meet their reporting limits and satisfy Dutch regulatory requirements.

[1] HMI theory and performance are discussed in detail in Agilent Product Overview: Performance Characteristics of the Agilent High Matrix Sample Introduction (HMI) Accessory for 7500 Series ICP-MS, 5989-7737EN.

Table 2. Analytico 3-Sigma Required Detection Limits for Soils

Analyte	Soil dry wt (mg/kg)	After 50x dilution (µg/L)
Cd	0.17	3.4
Cr	15	300
Cu	5	100
Ni	3	60
Pb	13	260
Zn	17	340
Hg	0.05	1
As	4	80
Ag	1	20
Se	10	200
Sb	1	20
Sn	6	120
Ba	15	300
Co	1	20
Mo	1.5	30
V	1	20
Be	0.1	2
Te	10	200
Tl	3	60

MDLs were calculated as 3-sigma of 10 replicates of a low-level (between one to three times the required MDL) spiked sand sample measured consecutively and also measured on 10 different days over a 30-day period[2] (Table 3). For all regulated elements, the calculated MDLs exceeded the Dutch regulatory requirements by nearly an order of magnitude or better. So with the HMI operating at maximum effective dilution, the system has ample sensitivity for the application.

Analysis of Certified Reference Materials

Two certified reference materials (BCR-144R Domestic Sewage Sludge, IRMM, Belgium, and

Table 3. Calculated Method Detection Limits (mg/kg) According to the Requirements of Dutch Regulation AS3000
MDL(1) = 3σ of 10 replicates taken on the same day
MDL(2) = 3σ of 10 replicates taken on different days[1]

Analyte - isotope	ORS mode	MDL (1) (mg/kg)	MDL (2) (mg/kg)	Dutch required MDL (mg/kg)
Be 9	He	0.042	0.046	0.1
V 51	He	0.255	0.481	1
Cr 52	He	2.300	4.517	15
Co 59	He	0.147	0.348	1
Ni 60	He	0.770	0.922	3
Cu 63	He	0.502	1.303	5
Zn 66	He	1.704	3.104	17
As 75	He	0.549	1.079	4
Se 78	H_2	0.832	2.041	10
Se 78	He	1.064	1.991	10
Mo 95	He	0.195	0.413	1.5
Ag 107	He	0.278	0.701	1
Cd 114	He	0.058	0.066	0.17
Sn 118	He	0.589	1.353	6
Sb 121	He	0.333	0.401	1
Te 125	He	1.217	2.112	10
Ba 135	He	3.041	6.227	15
Hg 201	He	0.014	0.025	0.05
Tl 203	He	0.285	0.546	3
Pb 208	He	1.197	2.844	13

FeNeLab River Clay, FeNeLab, Netherlands) were analyzed in replicate as part of the validation procedure. The CRMs were prepared in the same manner as standard soil samples and measured on 10 different days during a 30-day time period. Table 4 shows the results of replicate (n = 10) analyses of both CRMs in mg/kg. Recoveries ranged from 87 to 108%, well within the regulatory requirement of 80 to 110%.

[2] MDLs calculated from 10 different days are for information only.

Table 4. Results of Replicate (n = 10) Analyses of Two Certified Reference Soil Samples (FeNeLab and BCR-144R)

		FeNeLab River Clay			BCR-144R Sewage Sludge		
Analyte	ORS Mode	Measured mg/kg (ave, n = 10)	Certified mg/kg	Rec. % (ave)	Measured mg/kg (ave, n = 10)	Certified mg/kg	Rec. % (ave)
Be 9	He	1.6			0.2		
V 51	He	59.6			13.9		
Cr 52	He	191.9	187	103	88.8	90	99
Co 59	He	19.8	18.7	106	13.6	13.3	102
Ni 60	He	55.7	52.9	105	40.7	44.9	91
Cu 63	He	153.9	156	99	270.0	300	90
Zn 66	He	1031.6	970	106	825.1	919	90
As 75	He	44. 7	44	102	3.2		
Se 78	H_2	2.0			1.7		
Se 78	He	2.4			1.5		
Mo 95	He	1.3			6.9		
Ag 107	He	2.9			8.2		
Cd 114	He	8.5	8.07	105	1.7	1.84	90
Sn 118	He	0.02			36.0	40.8	88
Sb 121	He	1.6			2.8	3.05	92
Te 125	He	0.3			0.1		
Ba 135	He	828.3	817	101	319.2	367	87
Hg 201	He	4.1	3.83	107	3.2	3.11	102
Tl 203	He	1.1			0.1	0.14	
Pb 208	He	297.0	274	108	94.9	96	99

Determination of Precision and Accuracy at High and Low Concentrations

In addition to analysis of replicate CRMs, both low-level and high-level spiked samples were analyzed in replicate (n = 10; 10 different days during 30-day time period) in order to determine both accuracy and precision over a wide range of concentrations (Table 5).

Table 5. Results of Replicate (n = 10) Measurements Taken on 10 Nonconsecutive Days During a 30-Day Period of Both Low-Level and High-Level Spikes of Soil Samples

Analyte	ORS mode	Low-level spike conc. mg/kg	Measured mg/kg (ave, n = 10)	RSD (%)	Rec. % (ave)	High-level spike conc. mg/kg	Measured mg/kg (ave, n = 10)	RSD (%)	Rec. % (ave)
Be 9	He	1	0.8	3.5	81	800	773.9	3.6	97
V 51	He	50	50.9	4.0	102	800	771.4	2.5	96
Cr 52	He	150	149.6	4.0	100	1100	1062.7	2.5	97
Co 59	He	10	10.4	4.1	104	800	766.3	2.0	96
Ni 60	He	25	25.3	5.3	101	1100	1074.1	3.1	98
Cu 63	He	40	40.0	6.2	100	1100	1058.5	2.5	96
Zn 66	He	150	151.6	4.2	101	1100	1094.3	4.7	99
As 75	He	40	39.0	5.0	97	400	395.5	2.8	99
Se 78	H_2	100	102.7	3.7	103	1300	1336.0	2.2	103
Mo 95	He	13	12.2	5.0	93	1300	1235.3	3.3	95
Ag 107	He	8	8.3	4.6	103	40	40.8	4.2	102
Cd 114	He	1.2	1.1	7.1	94	1100	1062.8	2.9	97
Sn 118	He	40	41.7	4.0	104	1300	1262.6	3.2	97
Sb 121	He	10	9.7	5.9	97	1300	1188.0	2.4	91
Te 125	He	80	82.1	4.2	103	750	807.4	2.4	108
Ba 135	He	120	121.1	4.5	101	1100	1102.2	5.6	100
Hg 201	He	0.4	0.4	4.4	106	13	13.5	4.3	104
Tl 203	He	23	24.0	4.6	104	40	41.3	3.4	103
Pb 208	He	100	101.9	4.7	102	800	778.1	4.0	97

Sample Analysis

In order to test the long-term robustness of the HMI-equipped system, a 23-hour sequence consisting of an initial calibration and 235 soil samples was analyzed. Absolute drift was measured by monitoring the recovery of the five internal standards (^{6}Li, Ge, Rh, In, Ir) in both gas modes (He and H_2) over the course of the sequence. Normalized recoveries (relative to the method blank) are shown in Figure 1. Overall downward drift over 23 hours was approximately 20%, which is easily corrected by internal standards and not sufficient to have a detrimental effect on method accuracy or sensitivity. By comparison, a similar system without HMI, running these samples directly would suffer severe loss of sensitivity (> 80%) due to cone clogging before the sequence was completed.

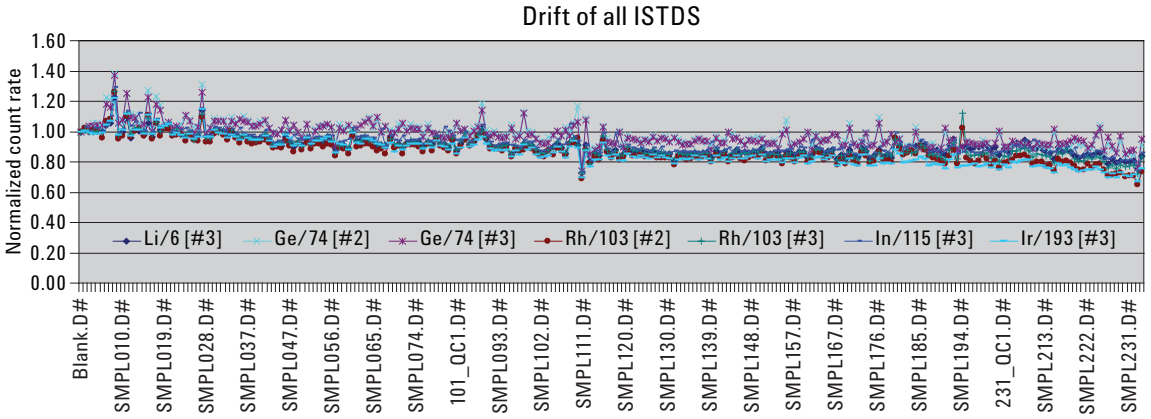

Figure 1. Normalized internal standard recovery in digested soil samples for the duration of the 23-hour sequence. Numbers next to each ISTD in the caption denote the ORS gas mode (#2 = H_2, #3 = He).

AS3000, like most methods used for regulatory compliance, requires ongoing calibration accuracy checks. In this case continuing calibration verification (CCV) samples (1 mg/kg As, 50 µg/kg Hg, remaining elements 2 mg/kg) were analyzed after each 12 soil samples. Results are shown in Figure 2. Acceptable recoveries must be within ± 10%. Ten percent control limits are shown in red, indicating that all CCV recoveries were well within the prescribed limits for the entire sequence. This excellent calibration stability not only ensures the most accurate sample results, but also eliminates time wasted in unnecessary recalibrations should a CCV fail during the sequence.

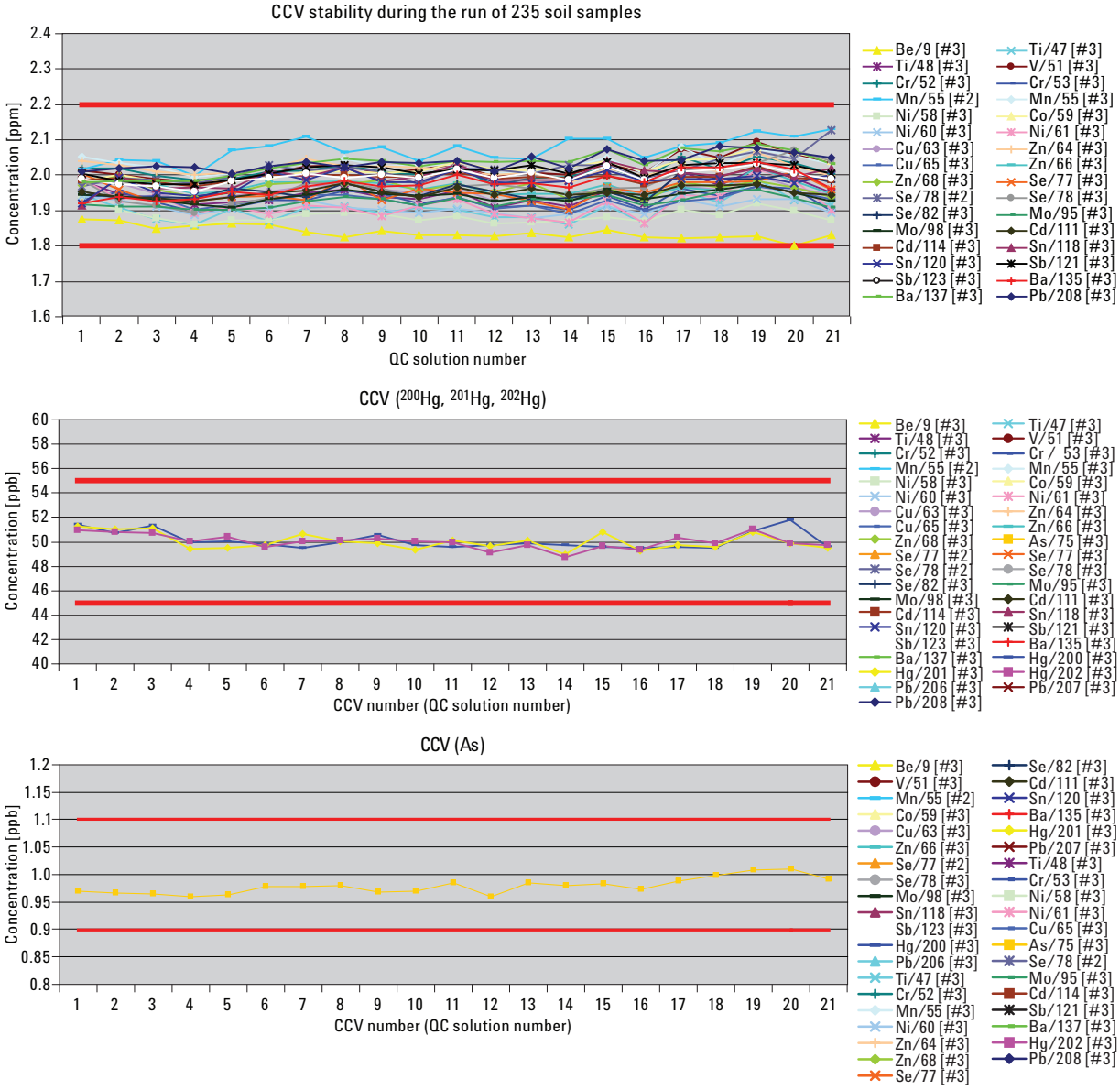

Figure 2. Continuing calibration check (CCV) recoveries (n-21) for all analyte elements over the 235-sample sequence of soils and sludges. Analytes at 2 mg/kg are shown on top, Hg, at 50 µg/kg in the middle, and As at 1 mg/kg at the bottom. Method required control limits (90 to 110%) are shown in red. Numbers after element names indicate ORS mode, #2 – H_2 mode, #3 – He mode.

Productivity

The operation of the HMI accessory does not adversely affect productivity in any way, since the HMI conditions are constant throughout operation and do not require any additional execution or stabilization time. Therefore, a given method run with HMI would take the same amount of time per sample as one run without HMI. HMI is also fully compatible with Agilent's time-saving pre-emptive and intelligent rinse functions, which minimize time wasted during both sample uptake and rinse-out. Furthermore, since HMI permits the direct analysis of undiluted samples of many types, the extra time associated with either manual or autodilution is saved, which considerably shortens the total time (prep plus analysis) required per sample. The improved stability as a result of HMI use can also minimize the need for recalibrations and sample reruns, further reducing the average run-to-run time. In this work, the average run time for a sample in a 23-hour, 235-sample sequence of undiluted soil digestates was 5.9 minutes, including acquisition in both H_2 and He modes.

Conclusions

Based on Analytico's evaluation, when compared with conventional autodilution for high TDS, high acid digests of soil and sludge samples, Agilent's HMI interface provided a number of significant advantages.

- Speed – HMI does not require liquid dilution of sample and stabilization of diluted sample. It also permits the use of Agilent's pre-emptive rinse function, which allows rinsing of the sample tubing to begin before acquisition has finished.
- Low maintenance – There is no tubing to replace and no moving parts to maintain.
- Simple – There are no critical timing issues or plumbing common to continuous flow autodilutors.
- Flexibility – Since hardware changes or reconfigurations are not required after installation of HMI, the system can be switched between conventional mode and HMI mode on the fly.

These advantages have allowed Analytico to use a single 7500cx ICP-MS fitted with HMI to replace several instruments required for the analysis of these sample types, including conventional ICP-MS, ICP-OES, and a dedicated mercury analyzer.

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Agilent Technologies

High-Speed Environmental Analysis Using the Agilent 7500cx with Integrated Sample Introduction System – Discrete Sampling (ISIS–DS)

Application Note

Environmental

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Abstract

Agilent has further improved the sample throughput capabilities of its 7500cx ICP-MS with Octopole Reaction System (ORS) using a newly configured Integrated Sample Introduction System–Discrete Sampling (ISIS-DS) accessory, and helium collision mode. Employing this new methodology, a complete suite of 30 or more elements can be analyzed in compliance with USEPA criteria (spectrum mode, three replicates, and sub-ppb MDLs) in approximately 75 seconds, sample to sample, with excellent removal of polyatomic interferences. Performance data showing stability, interference control, accuracy, precision, and washout are presented. The new system is applicable to labs requiring extremely high sample throughput and with its low sample consumption of ~2.2 mL/sample, for applications where sample volume is limited.

Agilent Technologies

Introduction

The Agilent Integrated Sample Introduction System (ISIS) has always permitted the highest sample throughput, using either high-speed uptake with constant flow nebulization or discrete sampling using a six-port valve and sample loop with time-resolved acquisition. In keeping with Agilent's continual focus on product enhancement, ISIS Discrete Sampling (ISIS-DS) has been reconfigured to further improve productivity. The resulting enhanced ISIS-DS sampling mode takes advantage of the ability of the 7500cx ICP-MS to analyze environmental samples using a single collision cell mode (He mode). This new mode of operation permits USEPA-compliant analysis (spectrum mode, three replicates, and sub-ppb MDLs) of a complete suite of 30 or more elements in approximately 75 seconds, sample to sample.

ISIS Configuration

Figure 1 shows the ISIS configuration used. It is a typical discrete sampling configuration with a couple of important modifications. Pump 1 (P1) is the large ISIS sample uptake peristaltic pump. Pump 2 (P2) is the standard 7500 nebulizer pump.

The ISIS uptake pump (P1), which is located downstream of the valve, draws the sample from the autosampler into the sample loop. As a result, the sample loaded in the sample loop is never exposed to peristaltic pump tubing, thereby eliminating a common source of contamination and carryover. This high-speed, high-capacity peristaltic pump is capable of rinsing and filling the sample loop in approximately 10 seconds when using the Cetac ASX-520 autosampler with the wide-bore 0.8 mm id probe. The other modification is the addition of the tee joint between the valve and nebulizer to allow the use of online internal standard addition. By minimizing both the length and diameter of the tubing between the valve and nebulizer, the time from rotation of the valve (sample injection) to the realization of a constant analyte signal is less than 15 seconds. A 300-µL loop is sufficient to allow more than 30 seconds of continuous spectrum mode acquisition. Larger loop sizes can be used to achieve any duration of acquisition required. After acquisition has completed, the valve returns to the load position, flushing any remaining sample to waste and rinsing the nebulizer and spray chamber with clean rinse solution. At this point, approximately 15 seconds is required for the signal to return to baseline in preparation for the next analysis (Figure 2).

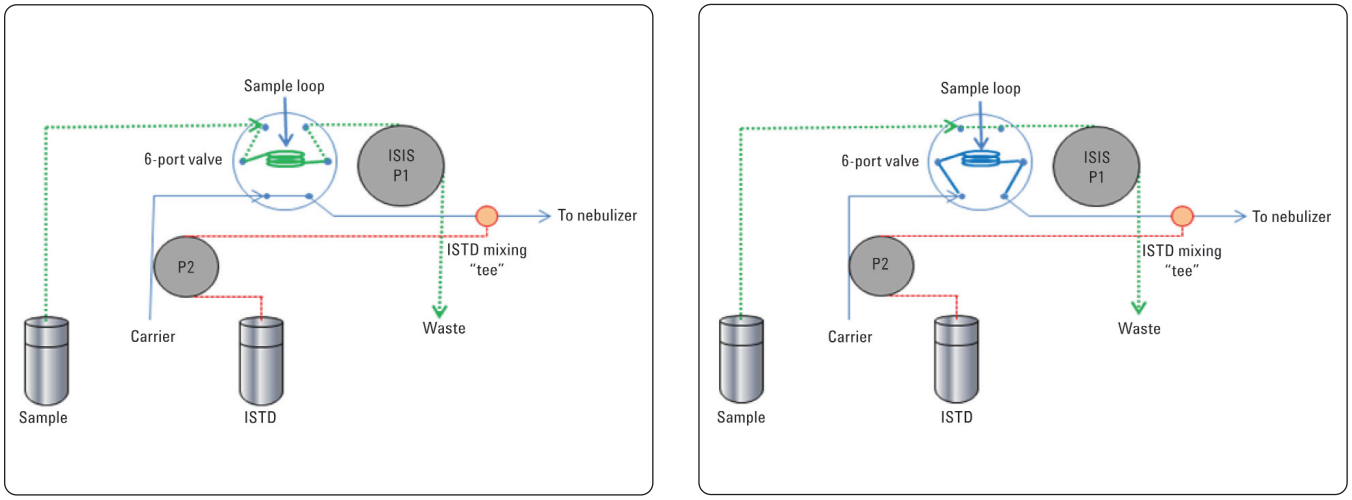

Figure 1. ISIS-DS sampling with online internal standard configuration. Valve in "load" position on left and in "inject" position on right.

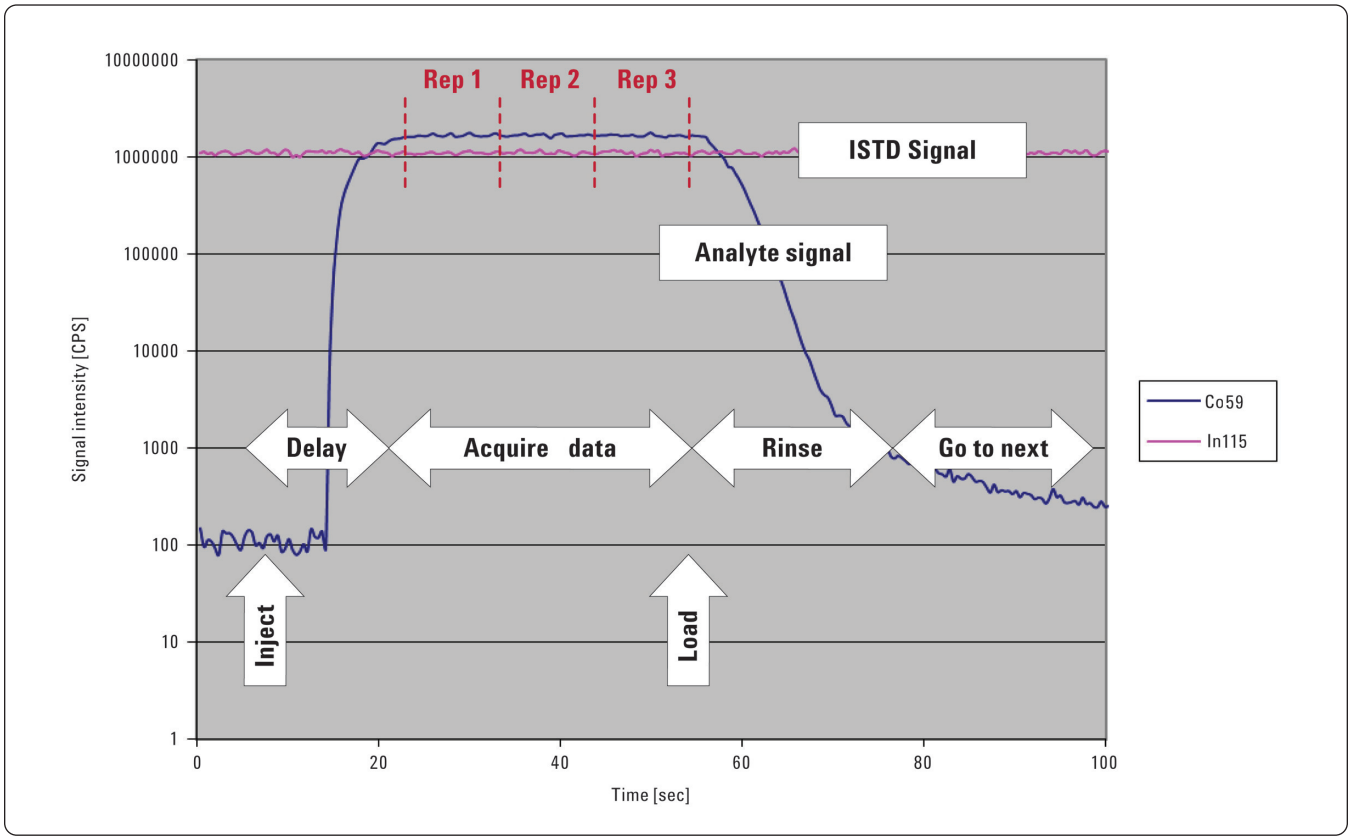

Figure 2. Analyte and internal standard profile during discrete sampling analysis (log scale for signal intensity). Time 0 – autosampler probe enters the sample and loop is loaded. Inject at 10 seconds, 15 seconds acquisition delay, 30 seconds acquisition, 10 seconds rinse, 10 seconds overhead. Total time is approximately 75 seconds.

Experimental

A sequence of 216 samples was analyzed in 4 hours 29 minutes using the new ISIS-DS sampling configuration depicted in Figure 1 and the acquisition conditions listed in Table 1. The 7500cx was operated in a single cell mode (He collision mode) resulting in both excellent removal of polyatomic interferences and very fast acquisitions since no cell gas switching or stabilization was required[1]. The sequence consisted of a single initial calibration at 0.1, 1, 10, and 100 ppb for all elements, followed by repeated (n = 26) analyses of a block of samples consisting of:

- 50 ppb calibration check (CCV)
- NIST 1643e water
- CCB (blank)
- USEPA Interference Check Solution – A (ICS-A)
- Blank
- USEPA ICS-AB (spiked with all analytes at 100 ppb to monitor carryover)
- Blank
- Blank

[1] This is a key benefit over reaction cell ICP-MS instruments that have to operate in multiple cell modes to cover all analytes. While it is possible to use multiple cell modes with discrete sampling, the resulting acquisition time is significantly lengthened, minimizing the benefits in terms of both run time and matrix exposure. If multiple cell modes are employed using the Agilent Octopole Reaction System, the small cell volume and very rapid gas switching reduce the cost in time and matrix exposure.

Table 1. ISIS/7500cx ICP-MS Acquisition Conditions for Spectrum Mode Discrete Sampling Analysis

Plasma	Robust mode – 1550 watts
Nebulizer	Glass concentric (standard)
Number of elements (including internal standards)	31
ORS mode	Helium - 4 mL/min (single mode)
Integration time per point	0.1 seconds (all elements)
Points per peak	1
Replicates	3
Total acquisition time (3 replicates)	29 seconds
Loop volume	300 µL
Loop rinse and fill time	10 seconds
Acquisition delay (after valve rotation to inject)	15 seconds
Steady state signal time (before valve rotation to fill again)	30 seconds

Results

Total Run Time and Sample Consumption

The resulting run-to-run time was measured at approximately 75 seconds per sample. Total sample consumption was determined by weighing each sample before and after analysis and was calculated to be 2.2 mL per sample per analysis. The method thus lends itself to samples in which the volume available for analysis is limited, and because small amounts are used, waste disposal costs are reduced. The small sample consumption also permits samples to be automatically reanalyzed by intelligent sequencing if needed from a 10-mL autosampler vial while allowing the ASX-520 to be configured for the maximum possible number of samples.

Stability

Long-term stability was monitored using internal standards. The abstracted internal standard data are illustrated in Figure 3, and show no downward drift, even after repeated (52 total) injections of ICS-A and ICS-AB. Only ^{6}Li demonstrated matrix suppression greater than 10% in the highest matrix samples, otherwise internal standard recoveries were within ~ ± 10% for the entire sequence. Calibration stability was monitored by measuring a 50-ppb CCV once in each 8-sample block (Figure 4). USEPA limits for CCV recovery are ± 10%. No CCV failures occurred; in fact, nearly all CCV recoveries were within ± 5% for the entire sequence.

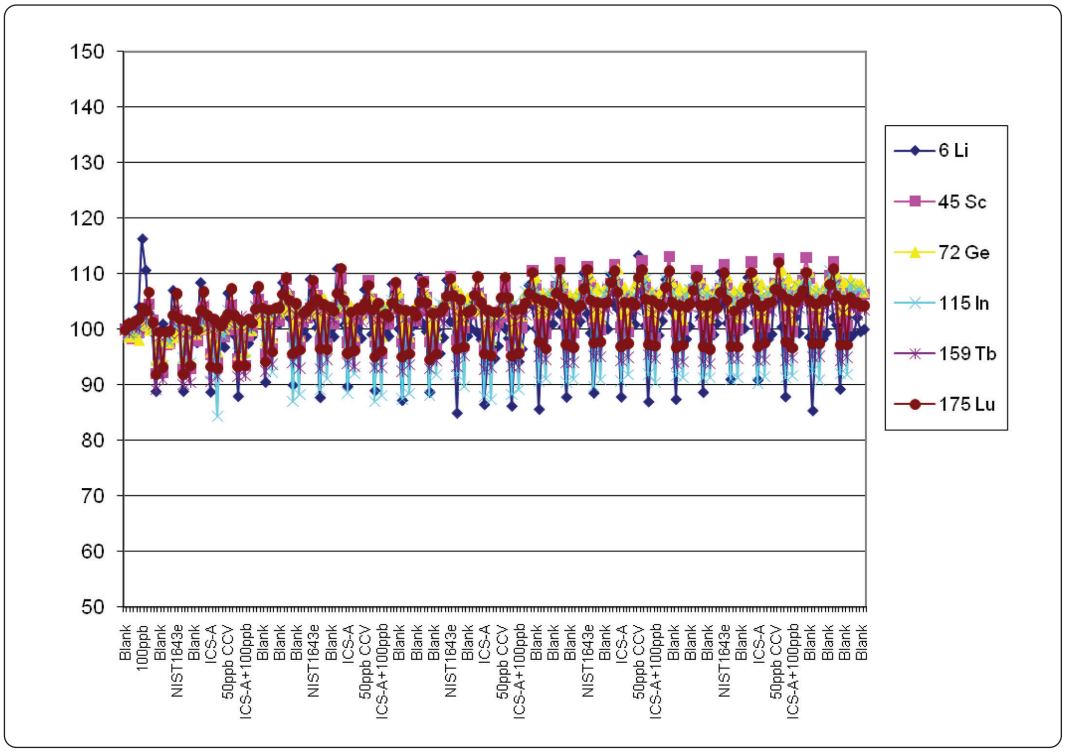

Figure 3. Internal standard recoveries compared to calibration blank for all 216 samples.

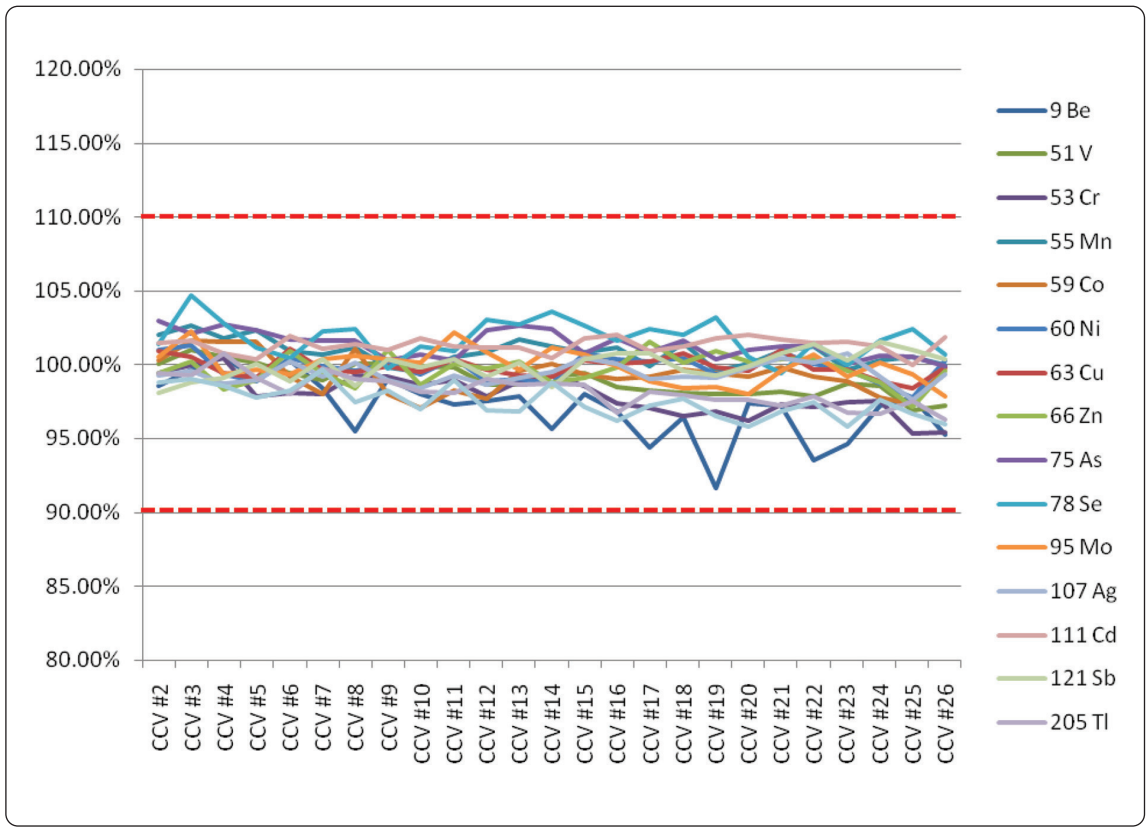

Figure 4. CCV recoveries (50 ppb) for entire sequence. USEPA limits for CCV recoveries in both Method 200.8 and 6020 are ± 10% (shown in red).

Accuracy

Long-term accuracy and precision were also determined through repeated analysis of NIST 1643e Certified Reference Water (n = 26). Results are tabulated in Table 2, showing recoveries within ± 10% or better of certified values and relative standard deviations near 1% for most elements. Be and Se had higher %RSDs due to the short integration times used and slightly lower count rates for these elements in He mode. Longer integration times can be used if higher counts or better precision are required for these elements. When ultimate DLs for Se are required (low ppt), H_2 mode is recommended. Due to the fast switching time of the ORS, Se can be measured in H_2 mode with only ~30 seconds added to the sample to sample run time.

Table 2. Precision (%RSD) and Mean Recovery of NIST 1643e Water for 26 Separate Analyses

Mass/ element	Mean measured value (µg/L)	RSD (%)	Certified value (µg/L)	Mean recovery (%)
9 Be	13.8	2.5	14.0	101.0
23 Na	22689.2	2.0	20740.0	109.4
24 Mg	7300.3	2.1	8037.0	90.8
27 Al	142.3	3.3	141.8	100.4
39 K	1837.8	1.1	2034.0	90.4
43 Ca	32170.1	0.7	32300.0	99.6
51 V	37.8	1.1	37.9	99.8
53 Cr	19.2	1.7	20.4	93.9
55 Mn	38.0	0.9	39.0	97.6
56 Fe	98.1	3.9	98.1	100.0
59 Co	28.8	0.7	27.1	106.4
60 Ni	59.2	0.8	62.4	94.9
63 Cu	23.2	0.8	22.8	101.9
66 Zn	70.0	0.5	78.5	89.2
75 As	54.3	0.9	60.5	89.8
78 Se	10.0	3.4	12.0	83.2
95 Mo	121.7	1.1	121.4	100.3
107 Ag	1.1	1.4	1.1	101.1
111 Cd	6.2	0.8	6.6	94.3
121 Sb	59.5	0.9	58.3	102.0
205 Tl	7.4	0.8	7.4	100.0
208 Pb	19.6	0.9	19.6	99.7

Table 3. Washout Performance (Mean value of 26 ICS-AB spikes [100 ppb], each immediately followed by two consecutive blanks. Percent reduction calculated as 1-([mean Blank]/[mean ICS-AB]) in percentage.)

Mass/ element	ICS-AB spike Mean	Blank 1 Mean	Percent reduction Mean	Blank 2 Mean	Percent reduction Mean
9 Be	94.9315	0.0199	99.979	0.0097	99.990
23 Na	96707.6923	19.6032	99.980	13.5090	99.986
24 Mg	79238.8462	14.2332	99.982	9.8046	99.988
27 Al	75758.0769	11.7913	99.984	7.8004	99.990
39 K	82694.2308	17.6441	99.979	13.2657	99.984
43 Ca	9092.8462	1.4105	99.984	0.9697	99.989
53 Cr	95.7327	0.0419	99.956	0.0441	99.954
55 Mn	94.8977	0.0132	99.986	0.0069	99.993
56 Fe	77021.9231	12.5122	99.984	8.0837	99.990
57 Fe	75266.5385	12.0863	99.984	7.7304	99.990
59 Co	106.8577	0.0140	99.987	0.0092	99.991
60 Ni	101.3692	–0.0161	100.016	–0.0129	100.013
63 Cu	98.5700	0.0163	99.984	0.0043	99.996
66 Zn	99.9350	0.0055	99.994	0.0011	99.999
75 As	95.8615	0.0290	99.970	0.0171	99.982
78 Se	94.0162	0.0841	99.911	0.0428	99.955
95 Mo	1862.3077	1.4281	99.923	0.6278	99.966
107 Ag	96.8769	0.0181	99.981	0.0098	99.990
111 Cd	104.0538	0.0134	99.987	0.0084	99.992
121 Sb	109.1346	0.2629	99.759	0.1077	99.901
205 Tl	93.4731	0.0339	99.964	0.0131	99.986
208 Pb	92.4704	–0.0175	100.019	–0.0241	100.026

Washout

Washout is always a concern in high sample throughput applications, particularly when analyzing high-matrix, variable samples. In order to evaluate the washout for each element, two sequential blank samples were measured immediately after each spiked ICS-AB sample. The spiked ICS-AB contained 100 ppb of all calibrated elements, plus very high concentrations of Na, Mg, Al, K, and Fe. Memory effects were determined by measuring the blank immediately following the ICS-AB. Any subsequent carryover was measured in the second blank (Table 2). In all cases, greater than 3 orders of magnitude reduction (> 99.9%) was achieved before the first blank, even for the high-concentration matrix elements. The second blank showed nearly no additional reduction, indicating that essentially complete washout was achieved during the configured sample uptake and rinse-out steps of the analysis. Even "sticky" elements, such as Mo, Sb, and Tl, demonstrated the same high degree of washout. This level of washout is comparable to or better than standard peristaltic pumped systems using much longer rinse times.

Conclusions

The results of this simple experiment illustrate that discrete sampling in spectrum mode (as opposed to time-resolved mode) using the Agilent Integrated Sample Introduction System can achieve extremely high sample throughput for typical environmental analyses using USEPA criteria. These data highlight that this novel method, using ISIS, easily exceeds the demanding USEPA requirements for stability, interference control, accuracy, precision, and washout.

The ISIS-DS system offers several advantages over other discrete sampling systems: Full integration into the ICP-MS mainframe, fully integrated software, compatibility with the industry standard ASX 520 autosampler, no vacuum pump and associated pump valve to wear and replace, very low sample consumption (~2.2 mL/sample), and the flexibility to use the ISIS for other supported sample-introduction tasks, such as constant-flow nebulization, autodilution, or hydride generation.

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Agilent Technologies

Direct Analysis of Undiluted Soil Digests Using the Agilent High Matrix Introduction Accessory with the 7500cx ICP-MS

Application

Environmental

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Abstract

Agilent has developed the High Matrix Introduction (HMI) accessory for ICP-MS as an alternative to conventional dilution. The HMI modifies the sample introduction system of the Agilent 7500 Octopole Reaction System (ORS) ICP-MS, making it possible to directly measure sample solutions with total dissolved solids (TDS) exceeding 1%. In collaboration with the Eurofins Analytico laboratory in the Netherlands, a 7500cx/HMI was used to directly measure high TDS soil extracts in compliance with Dutch regulatory guidelines. The results show that the HMI is a suitable replacement for conventional autodilution, allowing Analytico to use a single 7500cx/HMI ICP-MS in place of several instruments, including conventional ICP-MS, ICP-OES and a dedicated mercury analyzer.

Introduction

The determination of trace elements in high-matrix samples has always been a difficult analytical challenge. While inductively coupled plasma mass spectrometry (ICP-MS) has unsurpassed detection capability for trace metals, the potential for salt accumulation on the MS interface has always required that dissolved solids levels be limited. Furthermore, extremely corrosive or acidic samples can damage conventional ICP-MS interface components, including the sampler and skimmer cones, requiring the use of platinum or other expensive components. Compared to ICP-Optical Emission Spectroscopy (ICP-OES) or other non-MS-based techniques, this has been considered an inherent limitation of ICP-MS. To compensate for this limitation, samples with total dissolved solids (TDS) levels higher than 0.1 to 0.2%, depending on the matrix, typically require dilution before measurement by ICP-MS, relying on ICP-MS's high sensitivity to compensate for the sensitivity loss due to dilution. However, conventional sample dilution has a number of other disadvantages, including reduced productivity, introduction of contaminants, dilution factor errors, and increased waste volume. As a superior alternative to conventional dilution, Agilent has developed a simple, novel modification to the sample introduction system of the Agilent 7500 ICP-MS with Octopole Reaction System (ORS) that can significantly improve the

tolerance to high-matrix samples. The Agilent High Matrix Introduction (HMI)[1] accessory reduces sample matrix load on the plasma, making it possible to directly measure sample solutions with TDS exceeding 1%. As a result, samples previously measurable only by ICP-OES can now be measured directly by ICP-MS, using a 7500 ORS fitted with HMI. In this work, an Agilent 7500cx/HMI was used to test the system's ability to directly measure high TDS soil extracts in aqua regia and meet Dutch regulatory guidelines for reporting limits and data quality.

Direct Analysis of Aqua Regia Digests of Soils Using HMI-ICP-MS

This work was done in collaboration with the Eurofins Analytico laboratory (Analytico Milieu) in the Netherlands. Analytico has been part of the Eurofins group since 2001. Eurofins is a bio-analytical company with approximately 50 laboratories distributed over France, Germany, the UK, Denmark, Norway, Switzerland, and the United States. One of three divisions within Analytico, Analytico Milieu's expertise extends to the analysis of soil, sludge, groundwater, wastewater, air, building materials and residual matter, and, additionally, method development, validation, logistics, project management, and data management.

In this collaboration, an Agilent 7500cx equipped with the HMI was used to generate performance data to be used for compliance with the Dutch regulation pertaining to contaminated soils (AS3000). Due to the high TDS levels and high acid concentrations of digested soils, dilution is necessary prior to analysis by ICP-MS. Analytico currently uses the Agilent Integrated Sample Introduction System (ISIS) to perform online dilutions to meet these requirements. However, the limitations of conventional dilutions prompted Analytico to evaluate the potential of HMI as a faster, simpler, less expensive replacement for conventional autodilution that would also eliminate the maintenance issues associated with a conventional autodilutor.

Instrumentation

An Agilent 7500cx ICP-MS with the second peripump option for high sample throughput was equipped with the HMI. A Burgener MiraMist nebulizer was used. Instrument tune parameters, including HMI settings, are listed in Table 1. The plasma was optimized in ultra robust mode with 1/12 aerosol dilution. This is the maximum dilution factor that can be set with HMI and is approximately equivalent to diluting the sample x12 conventionally. All analytes except selenium were acquired in helium collision mode, thus eliminating the need for no-gas mode and the associated time required for mode switching. Selenium was acquired in hydrogen reaction mode due to the low detection limits required.

Table 1. Instrument Tune Conditions (Values relating to HMI are shaded.)

RF power (W)	1600
Carrier gas (L/min)	0.28
HMI dilution gas (L/min)	0.67
Aerosol dilution factor	1/12
Sample uptake rate (mL/min)	0.17
ISTD uptake rate (mL/min)	0.17
Total nebulizer flow (mL/min)	0.34
Extract 1 (V)	0
Extract 2 (V)	–160
He flow (He mode)	4.0 mL/min
KED (He mode)	2 volts
H_2 flow (H_2 mode)	4.0 mL/min
KED (H_2 mode)	2 volts

Sample Preparation

Actual soil samples received from Analytico's customers were prepared by adding 1 gram of soil to 8 mL of aqua regia prior to microwave digestion. Digested samples were then diluted to 50 mL final volume in ultra pure (18.2 MΩ) water. The final acid concentration is 4% HNO_3 and 12% HCl.

Calculation of Method Detection Limits (MDLs)

Since the HMI is effectively applying a dilution, the effects on detection limits are of critical importance. While ICP-MS possesses high sensitivity, the requirement for ultra trace detection limits in high TDS samples can still be challenging. Analytico has required MDLs for most analytes (based on a 1–g soil sample diluted to a final volume of 50 mL; see Table 2), which must be met in order for them to meet their reporting limits and satisfy Dutch regulatory requirements.

[1] HMI theory and performance are discussed in detail in Agilent Product Overview: Performance Characteristics of the Agilent High Matrix Sample Introduction (HMI) Accessory for 7500 Series ICP-MS, 5989-7737EN.

Table 2. Analytico 3-Sigma Required Detection Limits for Soils

Analyte	Soil dry wt (mg/kg)	After 50x dilution (µg/L)
Cd	0.17	3.4
Cr	15	300
Cu	5	100
Ni	3	60
Pb	13	260
Zn	17	340
Hg	0.05	1
As	4	80
Ag	1	20
Se	10	200
Sb	1	20
Sn	6	120
Ba	15	300
Co	1	20
Mo	1.5	30
V	1	20
Be	0.1	2
Te	10	200
Tl	3	60

MDLs were calculated as 3-sigma of 10 replicates of a low-level (between one to three times the required MDL) spiked sand sample measured consecutively and also measured on 10 different days over a 30-day period[2] (Table 3). For all regulated elements, the calculated MDLs exceeded the Dutch regulatory requirements by nearly an order of magnitude or better. So with the HMI operating at maximum effective dilution, the system has ample sensitivity for the application.

Analysis of Certified Reference Materials

Two certified reference materials (BCR-144R Domestic Sewage Sludge, IRMM, Belgium, and

Table 3. Calculated Method Detection Limits (mg/kg) According to the Requirements of Dutch Regulation AS3000
MDL(1) = 3σ of 10 replicates taken on the same day
MDL(2) = 3σ of 10 replicates taken on different days[1]

Analyte - isotope	ORS mode	MDL (1) (mg/kg)	MDL (2) (mg/kg)	Dutch required MDL (mg/kg)
Be 9	He	0.042	0.046	0.1
V 51	He	0.255	0.481	1
Cr 52	He	2.300	4.517	15
Co 59	He	0.147	0.348	1
Ni 60	He	0.770	0.922	3
Cu 63	He	0.502	1.303	5
Zn 66	He	1.704	3.104	17
As 75	He	0.549	1.079	4
Se 78	H_2	0.832	2.041	10
Se 78	He	1.064	1.991	10
Mo 95	He	0.195	0.413	1.5
Ag 107	He	0.278	0.701	1
Cd 114	He	0.058	0.066	0.17
Sn 118	He	0.589	1.353	6
Sb 121	He	0.333	0.401	1
Te 125	He	1.217	2.112	10
Ba 135	He	3.041	6.227	15
Hg 201	He	0.014	0.025	0.05
Tl 203	He	0.285	0.546	3
Pb 208	He	1.197	2.844	13

FeNeLab River Clay, FeNeLab, Netherlands) were analyzed in replicate as part of the validation procedure. The CRMs were prepared in the same manner as standard soil samples and measured on 10 different days during a 30-day time period. Table 4 shows the results of replicate (n = 10) analyses of both CRMs in mg/kg. Recoveries ranged from 87 to 108%, well within the regulatory requirement of 80 to 110%.

[2] MDLs calculated from 10 different days are for information only.

Table 4. Results of Replicate (n = 10) Analyses of Two Certified Reference Soil Samples (FeNeLab and BCR-144R)

		FeNeLab River Clay			BCR-144R Sewage Sludge		
Analyte	ORS Mode	Measured mg/kg (ave, n = 10)	Certified mg/kg	Rec. % (ave)	Measured mg/kg (ave, n = 10)	Certified mg/kg	Rec. % (ave)
Be 9	He	1.6			0.2		
V 51	He	59.6			13.9		
Cr 52	He	191.9	187	103	88.8	90	99
Co 59	He	19.8	18.7	106	13.6	13.3	102
Ni 60	He	55.7	52.9	105	40.7	44.9	91
Cu 63	He	153.9	156	99	270.0	300	90
Zn 66	He	1031.6	970	106	825.1	919	90
As 75	He	44. 7	44	102	3.2		
Se 78	H_2	2.0			1.7		
Se 78	He	2.4			1.5		
Mo 95	He	1.3			6.9		
Ag 107	He	2.9			8.2		
Cd 114	He	8.5	8.07	105	1.7	1.84	90
Sn 118	He	0.02			36.0	40.8	88
Sb 121	He	1.6			2.8	3.05	92
Te 125	He	0.3			0.1		
Ba 135	He	828.3	817	101	319.2	367	87
Hg 201	He	4.1	3.83	107	3.2	3.11	102
Tl 203	He	1.1			0.1	0.14	
Pb 208	He	297.0	274	108	94.9	96	99

Determination of Precision and Accuracy at High and Low Concentrations

In addition to analysis of replicate CRMs, both low-level and high-level spiked samples were analyzed in replicate (n = 10; 10 different days during 30-day time period) in order to determine both accuracy and precision over a wide range of concentrations (Table 5).

Table 5. Results of Replicate (n = 10) Measurements Taken on 10 Nonconsecutive Days During a 30-Day Period of Both Low-Level and High-Level Spikes of Soil Samples

Analyte	ORS mode	Low-level spike conc. mg/kg	Measured mg/kg (ave, n = 10)	RSD (%)	Rec. % (ave)	High-level spike conc. mg/kg	Measured mg/kg (ave, n = 10)	RSD (%)	Rec. % (ave)
Be 9	He	1	0.8	3.5	81	800	773.9	3.6	97
V 51	He	50	50.9	4.0	102	800	771.4	2.5	96
Cr 52	He	150	149.6	4.0	100	1100	1062.7	2.5	97
Co 59	He	10	10.4	4.1	104	800	766.3	2.0	96
Ni 60	He	25	25.3	5.3	101	1100	1074.1	3.1	98
Cu 63	He	40	40.0	6.2	100	1100	1058.5	2.5	96
Zn 66	He	150	151.6	4.2	101	1100	1094.3	4.7	99
As 75	He	40	39.0	5.0	97	400	395.5	2.8	99
Se 78	H_2	100	102.7	3.7	103	1300	1336.0	2.2	103
Mo 95	He	13	12.2	5.0	93	1300	1235.3	3.3	95
Ag 107	He	8	8.3	4.6	103	40	40.8	4.2	102
Cd 114	He	1.2	1.1	7.1	94	1100	1062.8	2.9	97
Sn 118	He	40	41.7	4.0	104	1300	1262.6	3.2	97
Sb 121	He	10	9.7	5.9	97	1300	1188.0	2.4	91
Te 125	He	80	82.1	4.2	103	750	807.4	2.4	108
Ba 135	He	120	121.1	4.5	101	1100	1102.2	5.6	100
Hg 201	He	0.4	0.4	4.4	106	13	13.5	4.3	104
Tl 203	He	23	24.0	4.6	104	40	41.3	3.4	103
Pb 208	He	100	101.9	4.7	102	800	778.1	4.0	97

Sample Analysis

In order to test the long-term robustness of the HMI-equipped system, a 23-hour sequence consisting of an initial calibration and 235 soil samples was analyzed. Absolute drift was measured by monitoring the recovery of the five internal standards (^{6}Li, Ge, Rh, In, Ir) in both gas modes (He and H_2) over the course of the sequence. Normalized recoveries (relative to the method blank) are shown in Figure 1. Overall downward drift over 23 hours was approximately 20%, which is easily corrected by internal standards and not sufficient to have a detrimental effect on method accuracy or sensitivity. By comparison, a similar system without HMI, running these samples directly would suffer severe loss of sensitivity (> 80%) due to cone clogging before the sequence was completed.

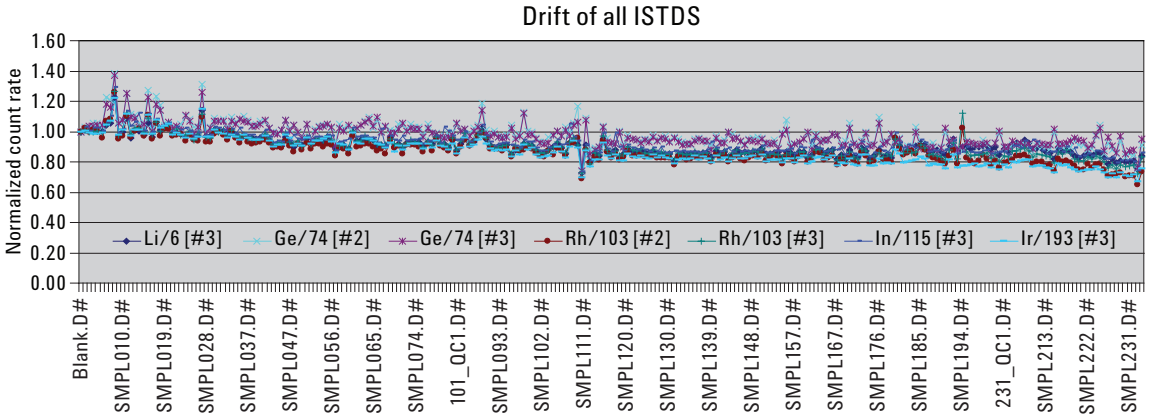

Figure 1. Normalized internal standard recovery in digested soil samples for the duration of the 23-hour sequence. Numbers next to each ISTD in the caption denote the ORS gas mode (#2 = H_2, #3 = He).

AS3000, like most methods used for regulatory compliance, requires ongoing calibration accuracy checks. In this case continuing calibration verification (CCV) samples (1 mg/kg As, 50 µg/kg Hg, remaining elements 2 mg/kg) were analyzed after each 12 soil samples. Results are shown in Figure 2. Acceptable recoveries must be within ± 10%. Ten percent control limits are shown in red, indicating that all CCV recoveries were well within the prescribed limits for the entire sequence. This excellent calibration stability not only ensures the most accurate sample results, but also eliminates time wasted in unnecessary recalibrations should a CCV fail during the sequence.

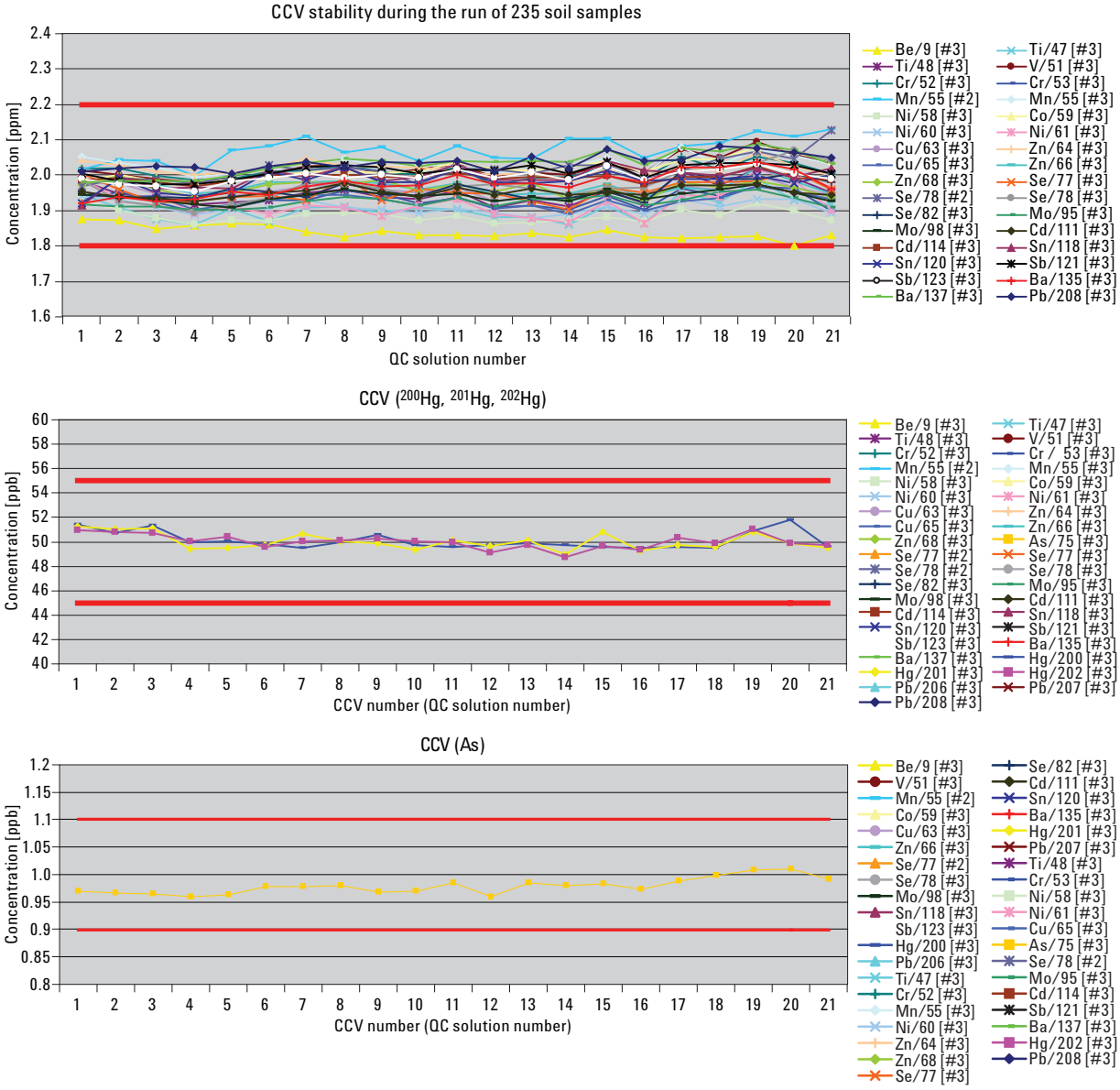

Figure 2. Continuing calibration check (CCV) recoveries (n-21) for all analyte elements over the 235-sample sequence of soils and sludges. Analytes at 2 mg/kg are shown on top, Hg, at 50 µg/kg in the middle, and As at 1 mg/kg at the bottom. Method required control limits (90 to 110%) are shown in red. Numbers after element names indicate ORS mode, #2 – H_2 mode, #3 – He mode.

Productivity

The operation of the HMI accessory does not adversely affect productivity in any way, since the HMI conditions are constant throughout operation and do not require any additional execution or stabilization time. Therefore, a given method run with HMI would take the same amount of time per sample as one run without HMI. HMI is also fully compatible with Agilent's time-saving pre-emptive and intelligent rinse functions, which minimize time wasted during both sample uptake and rinse-out. Furthermore, since HMI permits the direct analysis of undiluted samples of many types, the extra time associated with either manual or autodilution is saved, which considerably shortens the total time (prep plus analysis) required per sample. The improved stability as a result of HMI use can also minimize the need for recalibrations and sample reruns, further reducing the average run-to-run time. In this work, the average run time for a sample in a 23-hour, 235-sample sequence of undiluted soil digestates was 5.9 minutes, including acquisition in both H_2 and He modes.

Conclusions

Based on Analytico's evaluation, when compared with conventional autodilution for high TDS, high acid digests of soil and sludge samples, Agilent's HMI interface provided a number of significant advantages.

- Speed – HMI does not require liquid dilution of sample and stabilization of diluted sample. It also permits the use of Agilent's pre-emptive rinse function, which allows rinsing of the sample tubing to begin before acquisition has finished.
- Low maintenance – There is no tubing to replace and no moving parts to maintain.
- Simple – There are no critical timing issues or plumbing common to continuous flow autodilutors.
- Flexibility – Since hardware changes or reconfigurations are not required after installation of HMI, the system can be switched between conventional mode and HMI mode on the fly.

These advantages have allowed Analytico to use a single 7500cx ICP-MS fitted with HMI to replace several instruments required for the analysis of these sample types, including conventional ICP-MS, ICP-OES, and a dedicated mercury analyzer.

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Agilent Technologies

Rapid Analysis of High-Matrix Environmental Samples Using the Agilent 7500cx ICP-MS

Application

Environmental

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Abstract

The new Agilent 7500cx with Octopole Reaction System (ORS) is capable of analyzing most typical environmental samples using only one mode of analysis: helium mode. For the first time, it is possible to analyze an entire environmental suite of elements, including Hg and the major elements such as Na, K, Ca, Mg, Al, and Fe, in less than 2.5 minutes per sample, under conditions that remove or reduce practically all matrix-based interferences.

Introduction

Contract analytical laboratories, particularly those focused on the analysis of environmental samples, face challenges that are significantly different from those of research institutes in government and academia. The samples are typically more numerous, unknown and highly variable in composition, and generally require rapid turnaround. Despite these challenges, the environmental laboratory must ensure that the data produced is of high quality and is supported by extensive analytical quality control (AQC) in order to remain productive and profitable. The recent great improvement in productivity (and, consequently, profitability) of the metals section in contract environmental labs is largely due to the increasing use of ICP-MS, with its rapid multi-element capability, wide elemental coverage and dynamic range, low detection limits, and ease of use. With the advent of collision/reaction cell (CRC) ICP-MS, the ability of the technique to eliminate or significantly reduce the effects of polyatomic interferences in complex matrices has further improved its usability for many applications. However, until recently, the improved accuracy delivered by CRC ICP-MS came at a significant cost to productivity. Typical CRC ICP-MS systems must use reactive cell gases to specifically target known interferences, which requires time-consuming, matrix-specific method development. Furthermore, multiple cell conditions are necessary depending on the matrix and analyte list, which can add minutes to each sample analysis.

Agilent pioneered the use of helium (collision) mode coupled with kinetic energy discrimination (KED) on the 7500c instrument, allowing most polyatomic interferences to be removed using a single set of cell conditions. Subsequent advances in instrument design and in the understanding of the collision mechanisms involved have resulted in the 7500cx, an ICP-MS capable of analyzing typical environmental samples using only helium mode. By eliminating the need for both hydrogen (reaction) mode[1] and no-gas mode, sample throughput is significantly improved and routine operation is greatly simplified. Coupled with improvements in uptake and rinse-out speed through various hardware and software innovations, it is now possible to analyze an entire environmental suite of elements, including Hg and the major elements such as Na, K, Ca, Mg, Al, and Fe, in less than 2.5 min-

[1] Trace level selenium analysis (i.e., below 0.2 ng/mL) requires the use of hydrogen mode to eliminate the Ar_2^+ interferences on the preferred isotopes at mass 78 and 80.

utes per sample, under conditions that remove or reduce all matrix-based interferences. This application documents the performance of the 7500cx for the high-throughput analysis of long sequences of typical high-matrix environmental samples.

Instrumentation

A standard Agilent 7500cx ICP-MS with a glass concentric nebulizer was used for all analyses. The instrument was tuned for standard robust plasma conditions (Table 1) and the ORS was operated in helium mode only. This means that all elements were measured under identical helium mode collision conditions and no mode switching was necessary. Furthermore, the helium mode conditions used are generic and do not have to be set up or modified for specific sample matrices. Method parameters are shown in Table 1.

Table 1. Instrument Tune and Acquisition Conditions Used

Instrument	**7500cx**
Sampler	Ni (standard)
Skimmer	Ni (standard)
Nebulizer	MicroMist (standard)
Plasma torch	Quartz, 2.5 mm (standard)
Integration Time	
Li, Be, As, ^{78}Se, ^{111}Cd	0.3 sec x 1 point
All other	0.1 sec x 1 point
Tune Parameters	
RF power	1550 W
Sample depth	8.5 mm
Carrier gas	0.80 L/min
Makeup gas	0.23 L/min
Extract 1	0V
Extract 2	–120 V
Energy discrimination	2 V
Reaction gas	He 5.0 mL/min
CeO/Ce	0.52%
Ce^{++}/Ce	2.06%

Sensitivity in Helium Mode

Real sensitivity, as determined by practical limits of detection (LOD), is a function of signal to background (high signal, low background) and the precision of the background measurement. The greatest analytical benefit in using helium mode will be realized for analytes that suffer from polyatomic ion overlaps (essentially every isotope of every element from mass 45 to 82). However, it is important to assess the possible degradation in performance for elements that do not suffer from polyatomic interference where helium mode is used for all analytes. Poorer signal to noise for noninterfered elements is a possibility, as any ICP-MS operating with the cell pressurized (in collision or reaction mode) will suffer some loss of signal for low-mass elements when compared with no-gas mode. This signal loss occurs as a result of collisions between analyte ions and gas molecules in the cell. However, in most cases, the reduction in background more than compensates for the loss of signal, so real detection limits for noninterfered elements are not significantly impacted. In order to measure actual sensitivity under helium conditions, signal-to-background ratios and 3 sigma instrument detection limits (IDLs) were determined in helium mode for all commonly measured elements[2]. For nearly all elements, IDLs are in the low- to sub-ppt range. More important for environmental applications are the background equivalent concentrations (BECs) and IDLs for those elements that typically suffer from interferences in high-matrix samples. Table 2 compares the BECs, IDLs, and equivalent concentration of interferences for several critical elements in no-gas and helium mode in USEPA Interference Check Solution (ICS-A[3] - see Table 3 for composition). Note the significant reduction in all three measurements for all isotopes, showing that helium mode is capable of simultaneously removing interferences on multiple elements (and even multiple isotopes) in complex matrices.

Interference Removal in Helium Mode

USEPA Method 6020 specifies an interference check sample (ICS-A) designed specifically to monitor the effect of polyatomic interferences resulting from high concentrations of common matrix components. Traditionally, these interferences have been compensated for through the use of mathematical correction equations. However, experienced ICP-MS users know that in the case of multiple interferences on a single analyte or interferences from uncommon matrix components, mathematical correction is unreliable. Additionally, many polyatomic interferences cannot be corrected mathematically because of the lack of a free mass at which to monitor the interferent. A common example is the interference from $^{40}Ar^{23}Na$ on ^{63}Cu. This is a significant interference in saline matrices, but because Na is monoisotopic (at mass 23), it is not possible to derive a mathematical cor-

[2] Performance characteristics of the Agilent 7500cx ICP-MS. Agilent application note 5989-6663EN.

[3] ICS-A is the USEPA-specified "Interference Check Solution" designed to alert the user to the possibility of isobaric, doubly charged, polyatomic and memory interferences in high-matrix samples. ICS-AB is the same high-matrix solution spiked with 100 to 200 ppb of each analyte element in order to measure the effects of high matrix on analyte recovery. In this work, the target analytes were spiked much lower (20 ppb, ICS-AB Modified) in order to test the effectiveness of interference removal at trace analyte levels.

Table 2. Comparison of No-Gas Mode and Helium Mode on BEC, IDL, and Measured Concentration in ICS-A Solution (Note the much higher measured concentration values obtained in no-gas mode due to polyatomic interferences. Se 77 and 78 values do not agree in no-gas mode, and V gives a negative concentration reading.)

	No Gas Mode			Helium Mode		
	BEC (ppt)	IDL (ppt)	Measured conc (ppb)	BEC (ppt)	IDL (ppt)	Measured conc (ppb)
^{51}V	1461	143	–1.35	107	45	0.13
^{75}As	1945	186	3.23	120	149	0.70
^{77}Se	9973	540	12.31	401	204	0.50
^{78}Se	9738	313	3.84	342	162	0.43

rection based on the abundance of a second ArNa polyatomic ion. This has typically led ICP-MS users to select the alternative (and much lower abundance) Cu isotope at mass 65. However, ^{65}Cu suffers from a much higher level of S-based interferences (S_2 and SO_2) than ^{63}Cu as well as a significant $^{25}Mg^{40}Ar$ interference, so switching to ^{65}Cu to avoid the ArNa overlap can result in compromised data quality in many sample types.

Cr is another example of an element that commonly suffers from polyatomic interferences ($^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{36}Ar^{16}O$, and $^{38}Ar^{14}N$ on ^{52}Cr, and $^{37}Cl^{16}O$, $^{40}Ar^{13}C$, and $^{36}Ar^{16}OH$ on ^{53}Cr), which cannot be reliably corrected mathematically due to the lack of a free reference mass. For these reasons, helium mode, with its ability to remove all polyatomic interferences regardless of sample matrix composition, is vastly more reliable and more widely applicable than the use of mathematical corrections[4].

Figure 1 shows overlaid spectra for USEPA ICS-A, measured from mass 73 to 82 in no-gas, helium, and hydrogen modes. The spectra have been normalized on the bromine peak at *m/z* 79 to compensate for differences in sensitivity between modes. The differences in spectral complexity are clear, with almost every mass showing some level of interference in no-gas mode, while helium mode has reduced all of these interferences to background levels.

Table 3. Composition of ICS-A and ICS-AB (modified)[3] (ICS-AB was prepared by spiking ICS-A with a 20-ppb standard containing all analyte elements of interest.)

Component	ICS-A concentration (mg/L)	ICS-AB concentration (mg/L)
Al	100.0	100.0
Ca	300.0	300.0
Fe	250.0	250.0
Mg	100.0	100.0
Na	250.0	250.0
P	100.0	100.0
K	100.0	100.0
S	100.0	100.0
C	200.0	200.0
Cl	2000.0	2000.0
Mo	2.0	2.0
Ti	2.0	2.0
As	0.0	0.02
Cd	0.0	0.02
Cr	0.0	0.02
Co	0.0	0.02
Cu	0.0	0.02
Mn	0.0	0.02
Hg	0.0	0.02
Ni	0.0	0.02
Se	0.0	0.02
Ag	0.0	0.02
V	0.0	0.02
Zn	0.0	0.02

[4] Note that because helium mode works only on polyatomic interferences, it is not capable of removing elemental isobaric interferences (e.g., ^{40}Ar on ^{40}Ca) or doubly charged interferences. Fortunately, these types of interferences are rare, and simple methods are available to avoid them, such as choosing an alternative analyte isotope.

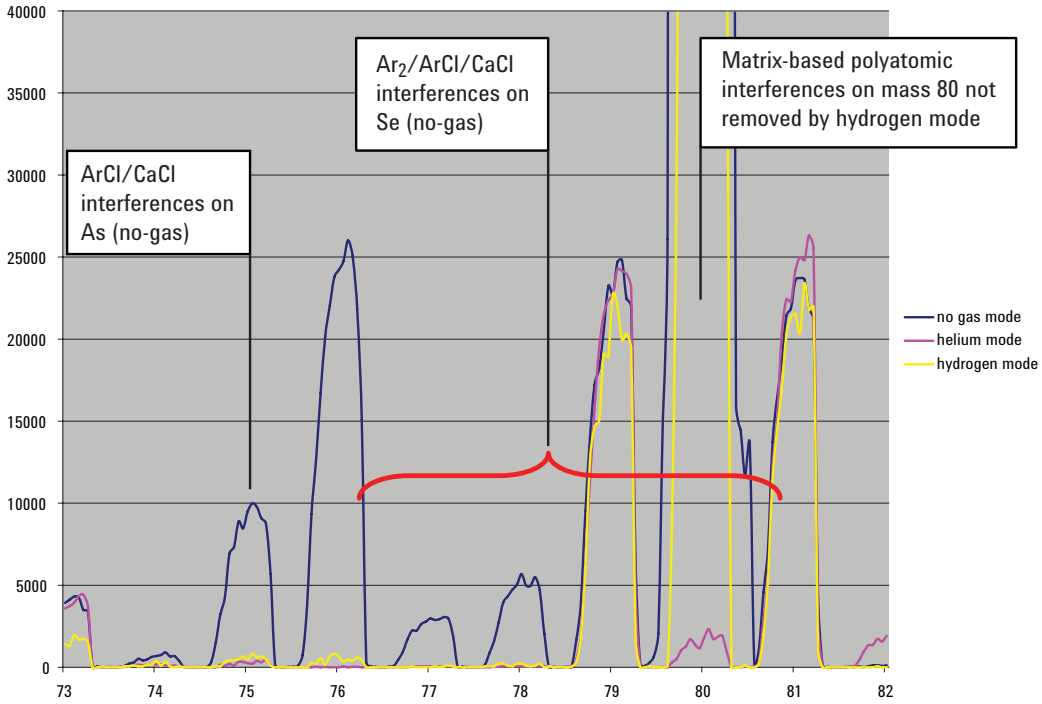

Figure 1. Overlaid spectra of ICS-A obtained in no-gas, hydrogen, and helium modes over the range from mass 73 to 82 to show interferences on As and Se. Spectra normalized on Br signal at *m/z* 79. Note that while H_2 mode is effective for the removal of the Ar_2^+ overlap at mass 80 (main isotope of Se) in simple matrices, it is not effective for several other interferences at this mass in ICS-A (ArCa, Ca_2, S_2O, SO_3, etc.). Ar_2^+ is completely removed by H_2 mode at *m/z* 78, which is therefore the preferred isotope.

Experimental

A 12-hour, 300-sample sequence, representing a typical environmental batch, was analyzed after a single initial calibration consisting of a blank and standards at 1, 10, 50, and 100 ppb (Figure 2). The sequence consisted of repeated blocks of 10 samples, including NIST 1640 standard reference water, ICS-A, ICS-AB, and two commercially available high total dissolved solids (TDS) mineral water samples. After each block, blank check and calibration check samples (USEPA sample types continuing calibration blank [CCB] and continuing calibration verification [CCV]) were automatically inserted to check for memory effects and calibration accuracy. No recalibrations were performed during the 12-hour run.

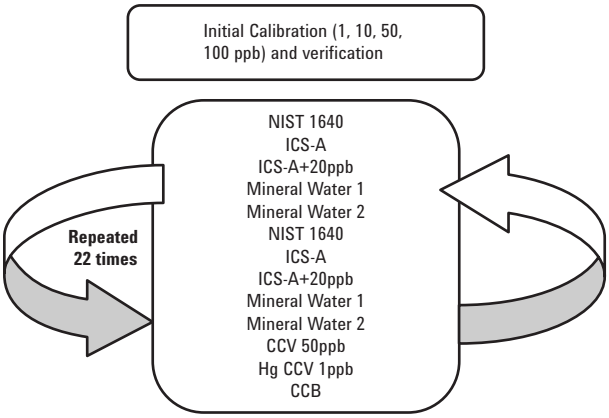

Figure 2. Schematic of analytical sequence. 300 sample analyses were performed, including an initial calibration and 22 repeated analyses of a block of samples containing 10 samples followed by 2 CCV samples and a CCB.

Long-Term Stability

Analysis of CCV Samples

As a check on calibration stability for all analyte elements, a CCV standard (50 ppb for all analytes except Hg – 1 ppb) was analyzed repeatedly throughout the sequence. USEPA Methods 200.8 and 6020 require that the measured CCV values fall within ± 10% of the true value in order to report samples. Figure 3 shows the results of 25 measurements of the CCV sample over the 12-hour sequence, indicating no failures throughout the run, despite the fact that no recalibrations were performed after the initial calibration.

Analysis of High-Matrix Samples

In order to simulate difficult, high-matrix sample types, ICS-A and ICS-AB were each analyzed twice in each 10-sample block (giving a total of 48 replicate analyses of each), in addition to the two high-TDS mineral water samples. ICS-A and ICS-AB were selected because they are well characterized and were specifically designed by the USEPA to challenge the ICP-MS's ability to handle high-matrix samples in terms of controlling interferences, managing ionization suppression, eliminating memory effects, and maintaining long-term stability. Long-term precision and accuracy for trace-level measurement in high-matrix samples can be determined by examining the results of repeated analysis of ICS-AB. Recoveries ranged from 97 to 104% with %RSDs ranging from less than 1% to approximately 5% over the 12-hour sequence (Figure 4).

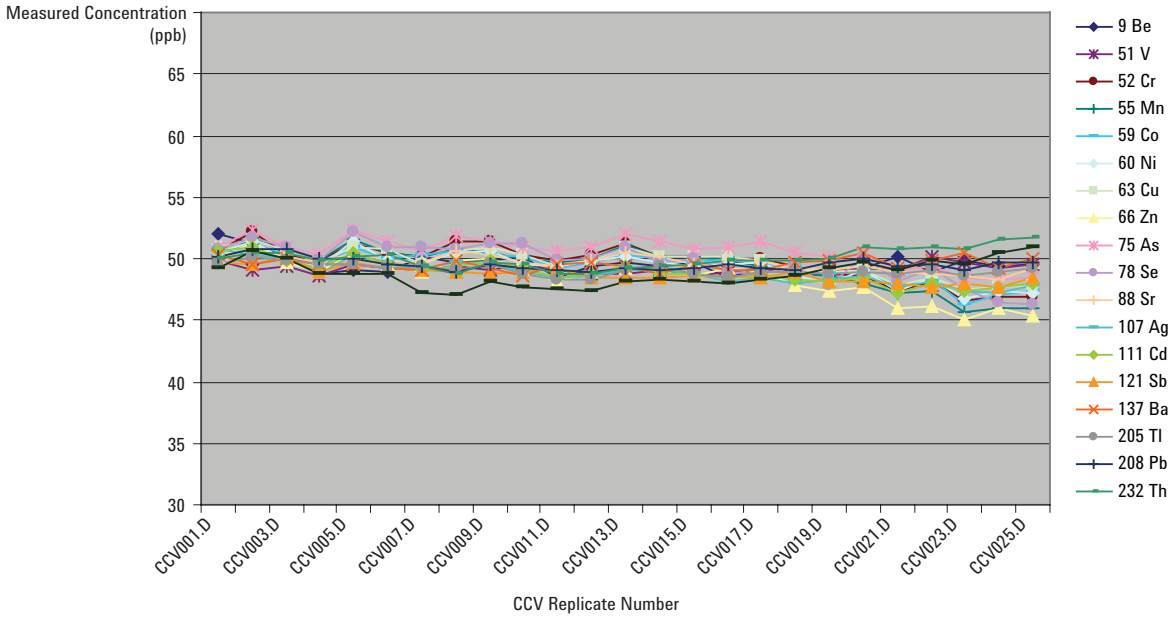

Figure 3. Measured values of 50 ppb CCV samples (n = 25) over the course of the sequence. USEPA criteria are ± 10% (i.e., 45 to 55 ppb).

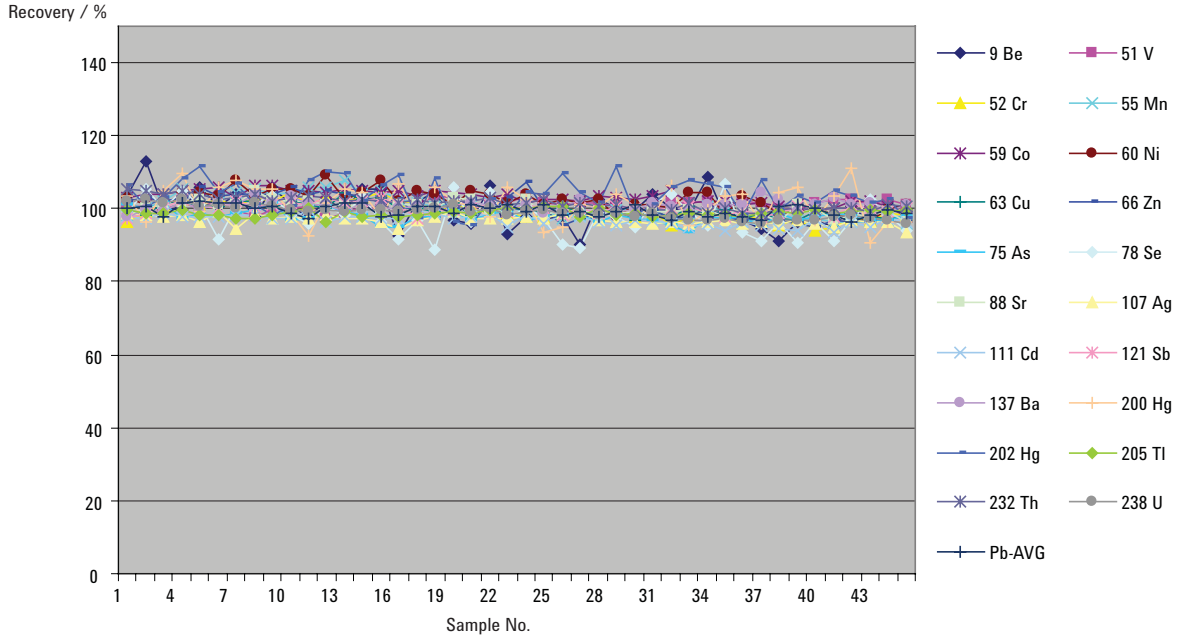

Figure 4. Spike recovery (20 ppb, 1 ppb Hg) for ICS-AB over 12 hours (n = 44).

Analysis of Certified Reference Material

NIST 1640 certified reference water was analyzed repeatedly (n = 44) as part of the sequence. Results are shown in Table 4.

Table 4. Results of Repeated Analysis of NIST 1640 (n = 44) Over a 12-Hour Sequence

Element	Mean (n = 44)	RSD (%)	Certified value (µg/L)	Recovery (%)
^{9}Be	32.36	4.72	34.94	92.6
^{27}Al	48.62	3.90	52.00	93.5
^{42}Ca	6652.25	2.59	7045	94.4
^{51}V	12.66	1.40	12.99	97.4
^{52}Cr	36.14	3.19	38.60	93.6
^{55}Mn	114.96	3.87	121.50	94.6
^{59}Co	19.64	2.27	20.28	96.8
^{60}Ni	26.76	2.86	27.40	97.7
^{63}Cu	84.95	2.16	85.20	99.7
^{66}Zn	52.64	2.66	53.20	99.0
^{75}As	25.28	1.52	26.67	94.8
^{78}Se	20.69	4.61	21.96	94.2
^{88}Sr	118.03	1.31	124.20	95.0
^{107}Ag	7.15	1.67	7.62	93.8
^{111}Cd	21.31	1.26	22.79	93.5
^{121}Sb	13.48	1.68	13.79	97.7
^{137}Ba	140.78	1.03	148.00	95.1
^{200}Hg	0.10	12.23	—	—
^{202}Hg	0.10	9.83	—	—
^{204}Pb	26.98	3.62	27.86	96.9
^{205}Tl	0.01	54.91	—	—
^{206}Pb	25.04	1.06	27.86	89.9
^{207}Pb	26.94	1.11	27.86	96.7
^{208}Pb	26.17	0.86	27.86	94.0
^{232}Th	0.05	45.36	—	—
^{238}U	0.73	2.90	—	—

Average Analysis Time

One of the major goals of using a single ORS mode is to improve productivity. To ensure that this end was met:

- Integration times were kept short, typically 0.1 second per point.
- A single point per mass was used.
- Intelligent and pre-emptive rinse functions were employed (minimizes wasted time in uptake and rinseout and ensures that carryover could not occur).

Figure 5 graphically shows the time savings possible. In a conventional CRC system, after sample uptake and initial stabilization, acquisition occurs in the first of several CRC modes, followed by cell evacuation, repressurization, and restabilization (top). The process continues until all necessary modes have been completed (typically 3). In the 7500cx helium mode (bottom), initial uptake and stabilization are the same. After that, helium mode acquisition can begin immediately, since no cell evacuation or repressurization is necessary, followed by rinse. Pre-emptive rinsing begins up to 60 seconds before acquisition has finished, and intelligent rinse monitors rinseout, ensuring complete washout without any wasted time. The total acquisition time for all analytes and internal standards was 9.7 seconds per replicate. Three replicates were acquired according to USEPA methods,

resulting in a total acquisition time of 29.2 seconds. Overall, the average run-to-run time based on 300 runs beginning at 4:44 p.m. and ending at 5:04 a.m. the following morning was 2.46 minutes per run. As the data in Table 4 illustrate, despite the short acquisition time, precision was not compromised and all data returned excellent %RSDs over the 12-hour period.

Conclusions

Since helium mode is universal, all interferences are removed without prior sample knowledge. Tuning is simplified and problems associated with reactive cell processes such as the creation of new interferences or loss of analyte or internal standard are avoided. Stability is not compromised since cell conditions are static and run times are markedly improved through the elimination of multiple cell conditions along with the associated stabilization times.

For many applications, particularly commercial analysis of high-matrix environmental samples, the use of helium mode offers significant benefits in productivity, data reliability, and ease of use.

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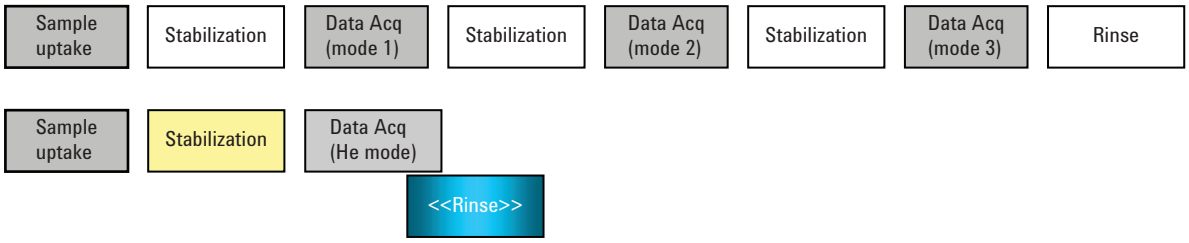

Figure 5. Typical multimode CRC operation (top), and 7500cx using helium mode and pre-emptive rinse software (bottom).

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Agilent Technologies

Performance Characteristics of the Agilent 7500cx

Evaluating Helium Collision Mode for Simpler, Faster, More Accurate ICP-MS

Application

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Abstract

The new Agilent 7500cx collision/reaction cell (CRC) ICP-MS was designed to improve the speed and accuracy of multielement analyses in even the most complex, unknown sample matrices. While most CRC instruments require the use of reactive cell gases or gas mixes to remove interfering species, the 7500cx employs an Octopole Reaction System (ORS) with inert helium gas (helium mode). Being nonreactive, helium forms no new interferences in the cell and no analytes are lost by reaction. This application outlines the performance benefits of the Agilent 7500cx ICP-MS using helium collision mode for trace level multielement analysis in different matrices.

Introduction

Collision/reaction cell (CRC) technology revolutionized ICP-MS by virtually eliminating the problems associated with polyatomic interferences for most elements in most matrices. However, it became apparent when using reaction-based systems, that in the majority of cases, the conditions required to eliminate a specific interference in a specific matrix were, in fact, specific. Different interferences, different matrices—or both—typically require different CRC conditions.

This requirement for multiple conditions compromises the multielement capability and productivity of CRC ICP-MS. Most CRC ICP-MS systems require at least two or more distinct acquisition steps for a typical multielement suite. Techniques devised to overcome the disadvantages associated with multiple CRC conditions include the use of mixed gases, compromised cell conditions, and automated mode switching. However, these compromised CRC conditions cannot achieve optimum interference removal or the throughput of a non-cell instrument. The interference removal of a CRC instrument combined with the productivity of a non-cell instrument can only be achieved through the use of a single cell mode. Because reactive CRC processes work only for specific analytes in specific matrices, only nonreactive mechanisms can be used reliably with unknown samples.

A CRC process using a nonreactive collision gas, helium (helium mode), with kinetic energy discrimination (KED) is capable of universally removing all polyatomic interferences, regardless of the matrix. In addition, helium mode does not produce new interferences due to reaction with matrix components or cause specific loss of analyte or internal standard due to reaction processes. The purpose of this application is to demonstrate the

performance benefits in both speed and accuracy of the Agilent 7500cx ICP-MS using *only* helium mode with KED for trace level multielement analysis.

Experimental

All work was performed using a standard Agilent 7500cx ICP-MS fitted with a glass concentric nebulizer and standard autosampler. The Agilent 7500cx ICP-MS is the successor to the highly successful 7500ce. The 7500cx was designed for the high-throughput commercial laboratory that demands absolute confidence in results in the most demanding of matrices with the simplest possible operation and highest possible throughput.

With these goals in mind, the 7500cx has been optimized to operate efficiently using only helium mode. It can also be operated in no-gas mode, which will give slightly improved DLs for low-mass, uninterfered elements, such as Li, Be, and B. In special cases where the measurement of selenium at less than 100 ppt is required, the optional hydrogen cell gas kit can be installed, which enables reaction mode using hydrogen. Hydrogen mode also offers improved LODs for some other elements, such as Si and Ca, by allowing access to their most abundant isotopes, but this is not typically required for most sample types. A comparison of the performance of the instrument in hydrogen, helium, and no-gas modes has shown that for routine labs, the productivity gains through the use of a single mode (helium mode), significantly outweigh the small DL improvements for a few elements that can be achieved by the use of multiple gas modes. Like the 7500ce, the 7500cx can also take advantage of additional hardware and software features such as a second peripump option as well as intelligent and pre-emptive rinse to maximize throughput [1].

Table 1. Instrument Conditions Used to Measure IDL Values for All Masses Between 6 and 238 (Only helium mode was used for all elements)

Acquisition Parameters	
Instrument	**Agilent 7500cx**
Cones	Ni
Nebulizer	Glass concentric
Integration Time (total - 3 points)	
Cl(35,37), Ca(43,44), As(75), Se(78,82), Hg(200,201,202)	3.0 sec
All other	1.0 sec
Tune Parameters	
RF power	1,550 W
Sample depth	8.5 mm
Carrier gas	0.90 L/min
Makeup gas	0.23 L/min
Energy discrimination	2 V
Cell gas	He 5.0 mL/min

Results and Discussion

Measured Instrument Detection Limits in Helium Mode

Full scan acquisitions using a single set of helium mode conditions were performed for all elemental masses between 6 and 238. The conditions listed in Table 1 were selected for both optimum performance and throughput.

Three sigma instrument detection limits (IDLs) in parts per trillion in ultrapure water are shown in Table 2.

Table 2. Three Sigma IDLs in Ultrapure Water Using Helium Mode

Mass	Element	3σ IDL (ppt)	Mass	Element	3σ IDL (ppt)
7	Li	18.0	114	Cd	0.27
9	Be	8.8	115	In	0.35
11	B	88.0	118	Sn	0.87
23	Na	490	121	Sb	1.0
24	Mg	1.6	126	Te	5.2
27	Al	26.0	127	I	20.0
28	Si	360	133	Cs	0.50
31	P	560	137	Ba	0.85
34	S	19,600	139	La	0.13
35	Cl	4,040	140	Ce	0.10
39	K	400	141	Pr	0.07
44	Ca	21.0	146	Nd	0.35
45	Sc	1.3	147	Sm	0.43
47	Ti	3.7	153	Eu	0.19
51	V	0.28	157	Gd	0.23
52	Cr	0.53	159	Tb	0.07
55	Mn	0.79	163	Dy	0.20
56	Fe	9.4	165	Ho	0.06
59	Co	0.50	166	Er	0.13
60	Ni	1.7	169	Tm	0.04
63	Cu	2.0	172	Yb	0.33
68	Zn	3.1	175	Lu	0.11
69	Ga	0.47	178	Hf	0.83
72	Ge	1.3	181	Ta	0.09
75	As	1.4	182	W	1.1
78	Se	35.0	185	Re	0.24
79	Br	130	189	Os	2.7
82	Se	26.0	193	Ir	0.53
85	Rb	0.87	195	Pt	1.1
88	Sr	0.35	197	Au	0.97
89	Y	0.09	202	Hg	0.56
90	Zr	0.17	205	Tl	0.71
93	Nb	0.25	208	Pb	0.29
95	Mo	1.1	209	Bi	0.33
103	Rh	0.10	232	Th	0.77
105	Pd	3.3	238	U	0.16
107	Ag	0.72			

Because helium is a light, inert gas, and KED has little effect on monatomic ions, IDLs are excellent across the entire mass range. Even low-mass, high-ionization-potential elements like beryllium yield single-digit ppt IDLs. Overall, of the 73 elements measured, 57% show IDLs less than 1 ppt and 80% less than 10 ppt. Only sulfur and chlorine had IDLs higher than 1 ppb. If needed, sulfur can be analyzed at ppt levels using the optional xenon cell gas option.

Accuracy of Helium Collision Mode

To test the accuracy of helium mode, a certified reference water standard (NIST 1640) was analyzed using standard, high-throughput conditions and helium collision mode for all elements. The results are displayed in Table 3. No interference correction equations were used, since all polyatomic interferences are removed, and no analytes are lost to reactions within the cell. Even elements that are normally run in no-gas mode, such as Be, and Se, which is normally run in hydrogen mode, showed excellent recoveries.

Table 3. Results of Analysis of NIST 1640 in Helium Collision Mode (with ISTD)

Element	Certified (ppb)	Measured (ppb)	Recovery (%)
9 Be	34.94	34.48	98.7%
11 B	301.1	300.3	99.7%
23 Na	29.35	30.42	103.6%
24 Mg	5.819	5.60	96.2%
27 Al	52.0	50.97	98.0%
39 K	994.0	1,016.0	102.2%
42 Ca	7,045.0	7,018.0	99.6%
51 V	12.99	12.95	99.7%
52 Cr	38.6	37.17	96.3%
55 Mn	121.5	125.0	102.9%
56 Fe	34.3	33.88	98.8%
59 Co	20.28	20.38	100.5%
60 Ni	27.4	27.39	100.0%
63 Cu	85.2	85.88	100.8%
66 Zn	53.2	53.96	101.4%
75 As	26.67	27.20	102.0%
78 Se	21.96	22.98	104.6%
88 Sr	124.2	125.9	101.4%
95 Mo	46.75	47.56	101.7%
107 Ag	7.62	7.13	93.6%
111 Cd	22.79	22.59	99.1%
121 Sb	13.79	13.67	99.1%
137 Ba	148.0	147.3	99.5%
208 Pb	27.89	25.98	93.2%

Comparing the Effectiveness of Helium Mode for Selenium, Arsenic, and Vanadium in Variable Matrices

Of all the elements typically measured in environmental or other high-matrix samples, only selenium benefits from the use of hydrogen mode compared to either no-gas mode or helium mode. Because selenium is subject to common spectroscopic interferences on all of its six isotopes, it is difficult to measure in no-gas mode. While hydrogen reaction mode is very effective at removing the Ar_2^+ polyatomic at masses 78 and 80, resulting in low-ppt IDLs in most matrices, helium collision mode is also very efficient, resulting in an IDL between 35 and 150 ppt at mass 78, depending on the matrix. Helium collision mode is also effective at removing the $ArCl^+$ and $CaCl^+$ interferences at mass 77 even in high-chloride matrices, freeing up a second isotope with sub-ppb IDL. Helium mode also provides superior detection limits for both arsenic and vanadium, which also suffer from chloride-based interferences in high-chloride matrices (Table 4).

Table 4. Results of Analysis of 1/50 Diluted Aquaregia (0.5 vol% HNO_3 + 1.5 vol% HCl) and EPA 6020 Interference Check Solution A (ICS-A) to Determine the Background Equivalent Concentration (BEC) and Instrument Detection Limit (IDL) in Each Matrix

BEC (ppt)

	1/50 aquaregia		ICS-A	
Element	No-gas mode	He mode	No-gas mode	He mode
77 Se	26,700	630	10,000	400
78 Se	5,700	130	9,700	340
51 V	11,300	330	1,500	110
75 As	7,500	130	1,900	120

3σ IDL (ppt)

	1/50 aquaregia		ICS-A	
Element	No-gas mode	He mode	No-gas mode	He mode
77 Se	1,300	270	540	200
78 Se	270	150	310	160
51 V	830	91	140	45
75 As	600	84	190	150

Performance Advantages in Real-World Samples

In order to test the expected advantages in simplicity, speed, and accuracy, a sequence composed of typical environmental samples was analyzed for 12 hours after a single initial calibration. Acquisition parameters are shown in Table 5. In all, 300 analyses were performed, including replicate ICS-A samples, commercial mineral waters, and replicates of NIST 1640. NIST 1640 was analyzed 48 times over the course of the sequence.

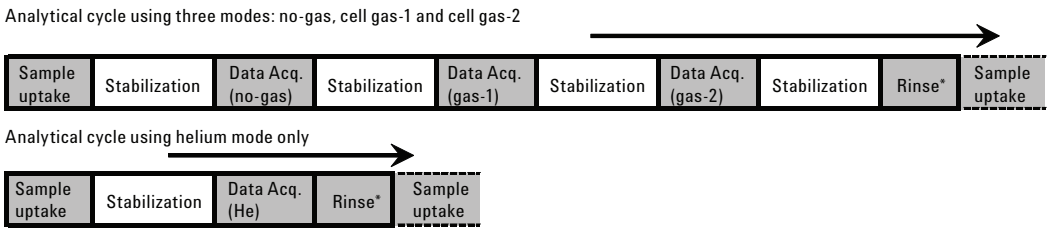
The primary advantages of using only helium collision mode over multiple modes are speed and simplicity. Tuning is reduced to a single set of standardized conditions that work well for any analyte in any matrix. No special optimizations are required, and the need to generate and store tune conditions for multiple modes is eliminated. During acquisition, a single set of instrument conditions is used, eliminating the gas changeover and stabilization time required when switching between modes (Figure 1). The result is reduced setup time and significantly reduced acquisition times, making the 7500cx the most productive ICP-MS available.

An additional benefit from the use of a single mode is improved long-term stability. There are several reasons for this. First, since the sample analysis time is shorter than multimode analysis, the interface is exposed to less sample matrix, which reduces drift due to sample cone deposition when high-matrix samples are analyzed. Additionally, maintaining static gas and pressure conditions within the cell eliminates a common source of instability associated with gas changes. Figure 2 is a normalized plot showing the long-term stability of NIST 1640 recoveries over the 12-hour, 300-sample sequence, which also included high-TDS mineral water samples (n = 96) and EPA interference check solutions A and AB (n = 48 each).

Table 5. Method Parameters Used for the 12-Hour Sequence (Average run time 2.46 minutes.)

Average sample-to-sample time	**2.46 minutes**
Number of isotopes	29
Integration time	
Li, Be, As, Se(78), Cd(111)	0.3 sec
All others	0.1 sec
Points per peak	1
Replicates per sample	3
Total acquisition time	9.72 seconds
Uptake time and flow rate	20 sec at 0.3 rps
Total rinse time and rinse flow rate	30 seconds at 0.3 rps
Preemptive* rinse	On, time = 28 seconds

*Preemptive rinse begins rinsing before acquisition has finished, using the sample remaining in the sample and peripump tubing to complete the acquisition, thereby reducing the total time by as much as 30 to 60 seconds per run.



*In both cases, rinse time can be shortened by using preemptive rinse.

Figure 1. A comparison of acquisition time and complexity between a system using three cell modes and the Agilent 7500cx using helium collision mode.

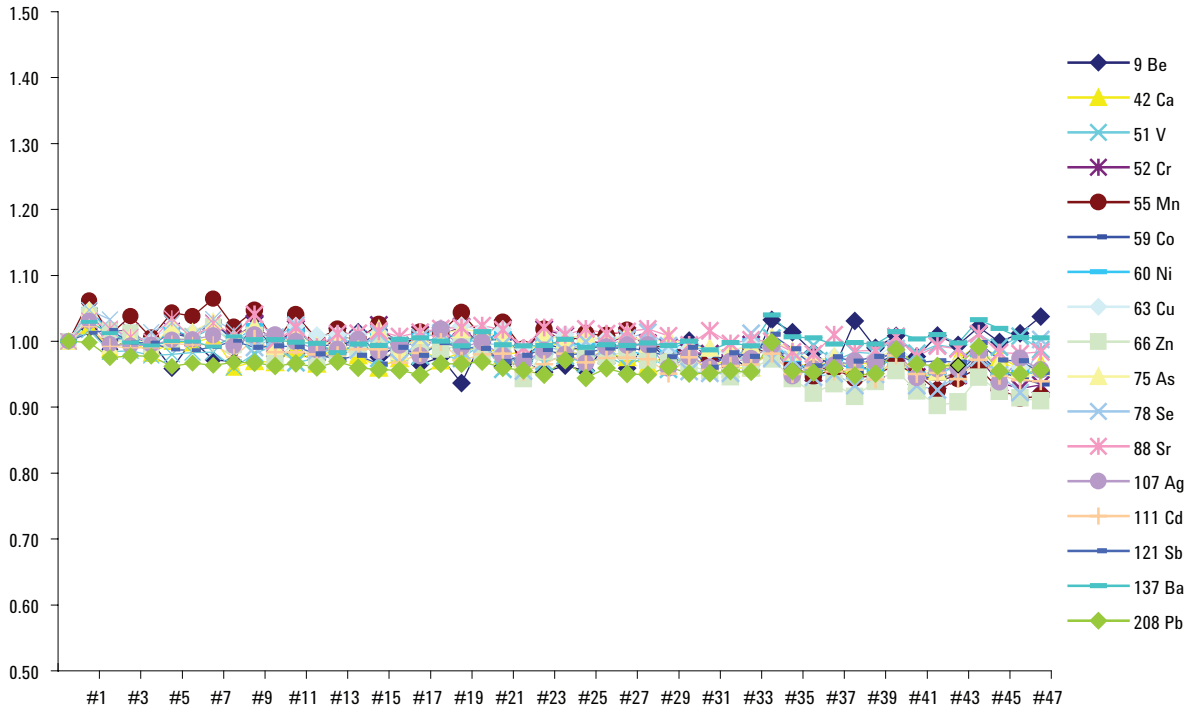


Figure 2. Normalized recovery of NIST 1640 components (n = 48) over a 12-hour 300-sample sequence in helium mode.

Conclusions

Helium mode with KED as implemented on the Agilent 7500cx ICP-MS has been shown to be a simple, fast, and effective solution to the problems associated with polyatomic interferences in ICP-MS. Instrument detection limits, measured in helium mode, across the mass range are sub-ppb for all elements except sulfur and chlorine. The addition of the optional xenon flow controller kit can be used if sub-ppb IDLs are required for sulfur. Most other elements, including selenium, exhibit IDLs in the low- to sub-ppt range. The optional hydrogen kit can provide single-digit-ppt DLs for selenium if needed. By eliminating the requirement for multiple collision cell modes, the 7500cx operating only in helium collision mode significantly reduces the run time and complexity of CRC ICP-MS. A single, universal tune is utilized for all analytes in all matrices. No time is spent acquiring data, such as internal standards, in more than one mode, and stabilization time after mode changes is completely eliminated. The result is a significant reduction in run time. Coupled with software enhancements such as preemptive and intelligent rinse, a full suite of environmental metals can be analyzed in less than 2.5 minutes per sample. Furthermore, data integrity in unknown or complex matrices is also significantly improved compared with systems that depend on either the use of mathematical corrections or reactive cell gases.

Reference

1. Achieving Optimum Throughput in ICP-MS Analysis of Environmental Samples with the Agilent 7500ce ICP-MS. Agilent Application Note 5989-5001EN, 2006.

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Agilent Technologies

Faster, Simpler, More Accurate Semiquantitative Analysis Using the Agilent 7500cx ICP-MS

Application

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Abstract

The new Agilent 7500cx allows the user to achieve the full potential of ICP-MS for semiquantitative elemental screening of a wide range of sample types. Complex, unknown samples can be analyzed with better speed, accuracy, and data integrity than ever before, since all matrix interferences are removed in the Octopole Reaction System (ORS) using helium collision mode. Results are presented for three different certified reference materials.

Introduction

Semiquantitative elemental analysis (semiquant) by ICP-MS is a powerful tool for quick screening of unknown samples for a wide range of trace elements. The ability to perform accurate semiquant is a strength of ICP-MS that is not shared by other elemental analysis techniques. It is based on the fact that the relative response of any element can be estimated from the response of any other element under a given set of conditions. These relative responses are determined by the unique properties of each element as well as the instrument and operating conditions, and can be stored in a semiquant response factor database. The use of internal standards or other calibration elements allows the database to be updated as needed to reflect the specific acquisition and matrix conditions. In practice, however, spectral interferences have limited the usefulness of semiquant for a number of elements in many common matrices.

Collision/Reaction Cell ICP-MS and Semiquant

In most collision/reaction cell (CRC) instruments, specific information about the matrix and target analytes is required in order to set up the correct collision/reaction chemistry to eliminate the interferences. Additionally, the conditions required to eliminate one interference in one matrix are generally not effective for all analytes in all matrices. For this reason, multiple sets of collision/reaction conditions are typically used. However, accurate semiquant response factors cannot be determined for elements acquired under different CRC conditions. As a result, it has not previously been possible to use CRC technology to reduce interferences in semiquant in the same way as in full quantification. However, the unique ability of Agilent's Octopole Reaction System (ORS) to eliminate polyatomic interferences using carefully controlled kinetic energy discrimination (KED) in helium collision mode permits all elements to be acquired under a single, universal set of CRC conditions.

KED eliminates the transmission of the larger polyatomic ions from the collision cell to the quadrupole by placing an energy barrier between the collision cell and quadrupole. Since polyatomic ions are always larger than atomic (analyte) ions of the same mass (Figure 1), they undergo more energy-reducing collisions with the helium cell gas than do the smaller atomic ions. As a result, the polyatomic ions have insufficient residual energy to cross the energy barrier at the cell exit, and so are excluded from the ion beam. Figure 2 depicts the effects of KED on ion energy. Only the high-energy atomic ions exceed the stopping potential

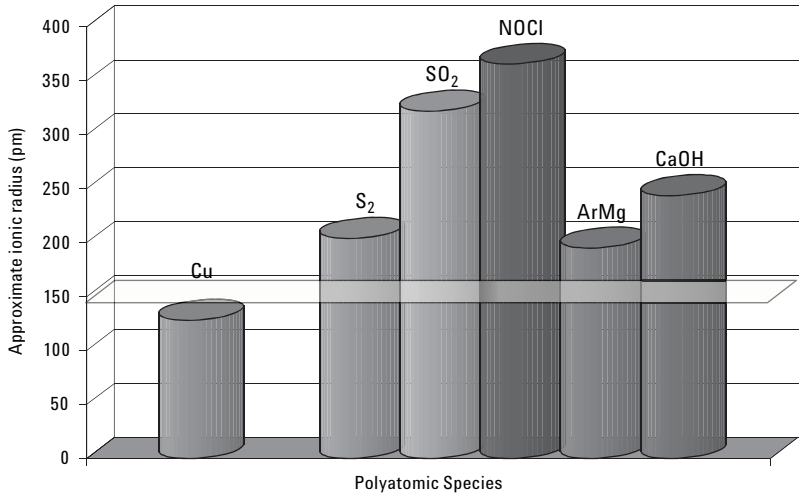

Figure 1. Graphic representation of the relative diameter of an atomic ion (Cu) compared with the polyatomic ions that can interfere. Most elemental ions are smaller than 150-picometer radius, while most polyatomic ions are larger.

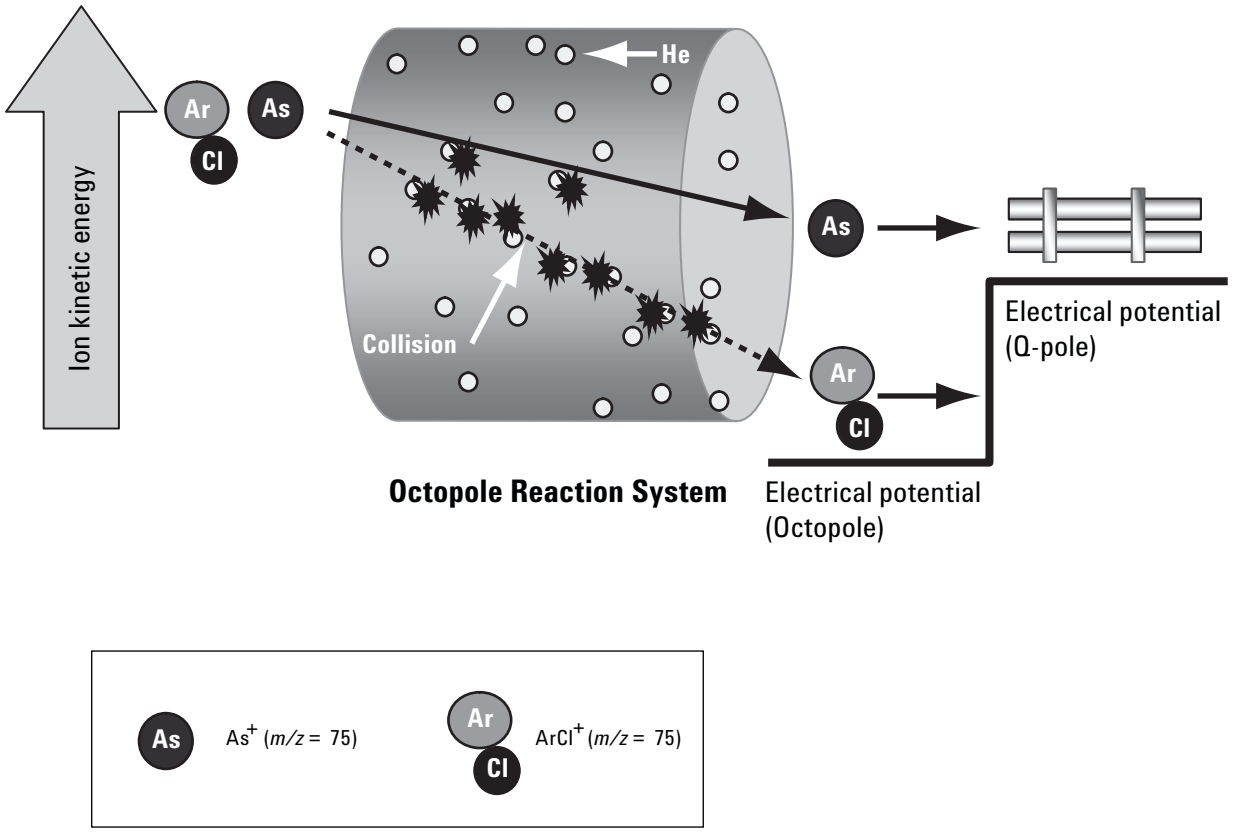

Figure 2. Diagrammatic representation of Kinetic Energy Discrimination after energy-reducing collisions within the Octopole Reaction System cell.

and are transmitted. Since helium is a nonreactive gas, no new interferences are formed in the cell and no analyte signal is lost by reaction, as occurs with any reactive cell gas.

The use of helium collision mode with semiquant conveys all the advantages normally associated with the use of CRC technology in full quant. It also solves the previously critical limitations of semiquant due to unresolved interferences. The advantages include:

- Semiquant is simple, fast, accurate, and interference-free for all analytes in any matrix.
- Helium collision mode allows the use of HCl, H_2SO_4, or other acids in digestion without danger of chlorine- or sulfur-based interferences on elements such as As, Cr, Se, V, Zn, etc.
- Improved stability for elements like Ag, Hg, Sb, Sn, and the Pt group due to the ability to add HCl to samples and standards.
- Ability to select the most abundant isotope for the best sensitivity, or multiple isotopes for absolute data confidence.
- Freedom to use any internal standards.

Table 1. Tune Conditions Used for NIST 1640 Semiquant Analysis in Helium Collision Mode

RF power	1550 W
Sample depth	8.0 mm
Carrier gas flow rate	0.90 L/min
Makeup gas flow rate	0.23 L/min
Sample flow rate	0.4 mL/min
Spray chamber temperature	2 °C
Helium flow rate	5 mL/min
KED	2V

Table 2. Semiquant Acquisition Parameters for NIST 1640

Total run time	**170 seconds**
Acquisition mode	Spectrum - peak hopping
Number of masses	250
Integration time[sec] masses 2 - 260	0.1 sec/point
Number of points per mass	1
Acquisition time	50.9 [sec]
Number of replicates	1
Uptake time	20 sec
Stabilization time	60 sec
Post acquisition rinse	30 sec
Preemptive rinse	On (time = 30 sec)

Experimental

The 7500cx ICP-MS was tuned for the same typical robust plasma conditions that are used in routine quantitative analysis (Table 1). No special tuning is required. Semiquant acquisition parameters are listed in Table 2.

A single calibration standard containing 200 ppb of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th, Tl, U, V, and Zn made up in 1% HNO_3/0.5% HCl was used to update the semiquant response factor database for a range of elements across the mass range. Non-calibrated elements are updated by interpolating between calibrated isotopes, which the ChemStation does automatically. Any number of calibration elements may be used, but increasing the number of calibration elements will improve semiquantitative accuracy. Internal standardization was applied using a typical suite of internal standard elements distributed across the mass range.

Results and Discussion

Tables 3 and 4 show the results of a semiquantitative screen of three standard reference materials, NIST 1640 water, LGC 6010 hard drinking water,

Table 3. Results of Helium Collision Mode Semiquant Anaysis NIST 1640 Standard Reference Water

Element	**NIST 1640 certified value**	**SQ conc.**	**Unit**	**Recovery (%)**
9 Be	34.94	33.42	µg/L	95.6
11 B	301.1	335.83	µg/L	111.5
23 Na	29.35	22.25	**mg/L**	75.8
24 Mg	5.819	4.24	**mg/L**	72.9
27 Al	52	48.92	µg/L	94.1
39 K	994	919.17	µg/L	92.5
42 Ca	7.045	5.81	µg/L	82.4
51 V	12.99	12.83	µg/L	98.8
52 Cr	38.6	36.58	µg/L	94.8
55 Mn	121.5	121.67	µg/L	100.1
56 Fe	34.3	30.92	µg/L	90.1
59 Co	20.28	19.75	µg/L	97.4
60 Ni	27.4	25.83	µg/L	94.3
63 Cu	85.2	81.17	µg/L	95.3
66 Zn	53.2	51.83	µg/L	97.4
75 As	26.67	27.75	µg/L	104.0
78 Se	21.96	24.08	µg/L	109.7
88 Sr	124.2	122.50	µg/L	98.6
95 Mo	46.75	46.17	µg/L	98.8
107 Ag	7.62	7.31	µg/L	95.9
111 Cd	22.79	21.50	µg/L	94.3
121 Sb	13.79	12.83	µg/L	93.1
137 Ba	148	139.17	µg/L	94.0
208 Pb	27.89	23.5	µg/L	84.3

Table 3 has been simplified to show only those elements with some reference values, although many other elements were determined in each reference material.

Table 4. Results of Helium Collision Mode Semiquant Analysis of LGC 6010 Hard Drinking Water and LGC 6177 Landfill Leachate Standard Reference Materials

	LGC 6010 hard drinking water			LGC 6177 landfill leachate		
Element	LGC certified value (µg/L)	SQ conc. (µg/L)	Recovery (%)	LGC certified value (µg/L)	SQ conc. (µg/L)	Recovery (%)
10 B	N/A	83	N/A	9,800	6,700	68.4
23 Na	21,900	20,000	91.3	1,750,000	1,500,000	85.7
24 Mg	4,200	3,700	88.1	73,500	62,000	84.4
27 Al	208	160	76.9	N/A	110	N/A
31 P	N/A	670	N/A	11,500	12,000	104.3
39 K	5,100	5,100	100.0	780,000	810,000	103.8
44 Ca	83,200	73,000	87.7	74,800	77,000	102.9
52 Cr	48	51	106.3	180	160	88.9
55 Mn	48	45	93.8	140	130	92.9
56 Fe	236	240	101.7	3,800	3,300	86.8
60 Ni	48	42	87.5	210	170	81.0
66 Zn	542	540	99.6	260	250	96.2
75 As	55	49	89.1	N/A	86	N/A
78 Se	9.5	13	136.8	N/A	< 16.00	N/A
107 Ag	6.2	4.3	69.4	N/A	1.8	N/A
121 Sb	11.9	13	109.2	N/A	5	N/A
137 Ba	116	110	94.8	N/A	770	N/A
208 Pb	95	92	96.8	N/A	17	N/A

Table 4 has been simplified to show only those elements with some reference values, although many other elements were determined in each reference material.

and LGC 6177 landfill leachate. No attempt was made to matrix-match; tune conditions used were as shown in Table 1; and all elements were acquired in helium collision mode. In all cases, for every certified element, the semiquantitative result was within ± 40% of the certified concentration, from as low as 7 ppb for Ag in NIST 1640 to over 1700 ppm for Na in the LGC 6177 landfill leachate.

Conclusions

Semiquant has always been a powerful tool available to the ICP-MS analyst for quickly estimating the concentration of unknown, uncalibrated elements in a variety of simple matrices. However, in complex matrices, polyatomic interferences could render the results for many elements useless. Collision/reaction cell technology, which requires more than one set of conditions for all masses, cannot be used since it would result in deviation from the standard relative response tables upon which semiquant is based. Helium collision mode coupled with kinetic energy discrimination in the Agilent 7500cx can overcome these limitations. By effectively removing polyatomic interferences, rapid, accurate, semiquantitative screening of a wide range of sample types for most analyte elements is possible. In this work, a full mass range, 250 isotope semiquant screen was performed in less than 3 minutes total sample-to-sample time with accuracy comparable to full quantification, for most elements, when measuring three different certified reference materials.

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Unmatched Removal of Spectral Interferences in ICP-MS Using the Agilent Octopole Reaction System with Helium Collision Mode

Application

Metals Analysis

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Abstract

Many routine laboratories have adopted ICP-MS as their primary technique for metals analysis due to its simple operation as a multi-element analyzer. However, despite its higher performance for the targeted removal of specific interferences, collision/reaction cell (CRC) ICP-MS remains relatively understudied in terms of its multi-element capability. This work demonstrates that the Agilent 7500ce ICP-MS can be operated with a single set of He cell gas conditions, to provide effective interference removal for a range of elements in a challenging and complex sample matrix.

Introduction

ICP-MS is an immensely powerful multi-element analytical technique, but it does suffer from some well-documented spectral interferences, which can be especially problematic when complex and variable samples are analyzed. Most interferences in ICP-MS arise due to an overlap from a molecular (or polyatomic) ion at the same nominal mass as the analyte of interest. Commonly reported interferences can be broadly divided into two groups: those derived from the plasma and aqueous solution (plasma-based), such as ^{40}Ar, $^{40}Ar^{16}O$, and $^{40}Ar^{38}Ar$, and those derived from sample matrix components (matrix-based), such as $^{35}Cl^{16}O$, and $^{32}S^{34}S$. Plasma-based polyatomic ions are both predictable and reasonably constant, regardless of sample matrix, whereas matrix-based polyatomic ions are less predictable and vary with sample matrix components and their relative concentrations.

Recent advances in CRC technology have led to dramatic improvements in the analysis of interfered elements which previously proved difficult or impossible to measure at required levels in certain sample matrices. In a CRC ICP-MS, the cell is typically pressurized with a reactive gas that reacts with the interference (referred to as reaction mode). Attenuation of the interfering species occurs by one of several different processes depending on the gas and the interference. However, in practice, “reaction mode-only” CRCs limit the system to the removal of single interfering ions from single analytes [1–8], using highly reactive gases and specific measurement conditions. Some instruments use “simpler” or less reactive cell gas such as H_2, but its use is limited mainly to plasma-based interferences, as it reacts slowly or not at all with matrix-based interferences which are much more difficult to remove.

Helium (He) Collision Mode

The development of the Agilent Octopole Reaction System (ORS) introduced a new and much more powerful mode of CRC operation – He collision mode – which uses an inert collision gas to remove all polyatomic species based on their size rather

than their relative reactivity with a reaction gas. Since all polyatomics are larger than analyte ions of the same mass, their larger cross-section means that they suffer more collisions with the cell gas and so lose more energy as they progress through the pressurized region. On arrival at the cell exit, the large cross section polyatomic species all have distinctly lower ion energy (due to collisions with the He cell gas) than the analyte ions and so can be prevented from leaving the cell using a stopping voltage, allowing only the analytes to pass through to the analyzer. This separation process is known as kinetic energy discrimination (KED), and this simple yet extremely effective approach offers a number of significant analytical advantages over reaction mode.

Advantages of He Collision Mode:

- In contrast with a reactive cell gas, He is inert - so does not react with the sample matrix - no new interferences are formed in the cell
- As He is inert, it does not react with and cause signal loss for analyte or internal standard ions
- ALL interferences (plasma-based AND matrix-based) are removed or attenuated so multi-element screening or semiquant analysis can be combined with effective interference removal
- Since He collision mode is not interference-specific, multiple interferences can be removed from the same analyte (or different analytes) simultaneously [9, 10]
- No prior knowledge of the sample matrix is required, and no method development is required, in contrast to the extensive, analyte- and matrix-specific method development which is required for any reactive mode of interference removal [11]
- He collision mode can be applied to every sample, every matrix, and the same setup (gas flow rate) is used for every application
- No cell voltages to set up or optimize
- NO interference correction equations are used

Why Can't Other CRC-ICP-MS Use He Collision Mode?

To work properly, He collision mode requires efficient analyte/interference separation by KED, which requires two conditions to be met: first, the energy of all the ions entering the cell must be very tightly controlled. Agilent's unique ShieldTorch interface insures a very narrow ion energy spread of 1 eV: its physically grounded shield plate provides better control of initial ion energy than electrically grounded plasma designs (such as balanced, center-tapped or interlaced coils). Second, in the cell, polyatomic species must experience a sufficiently high number of collisions to differentiate them from the analyte ions at the cell exit. In the Agilent ORS this is achieved by the use of an octopole ion guide – the only implementation of an octopole cell in ICP-MS. There are two key benefits to the use of an octopole cell:

- Octopoles have a small internal diameter. As a result, the cell entrance and exit apertures are small – so the cell operates at relatively higher pressure compared to quadrupole or hexapole cells which increases ion/gas collisions.
- Octopoles also have better focusing efficiency than hexapole and quadrupole ion guides. The ion beam is tightly focused, which insures good ion transmission and high sensitivity at its higher cell operating pressure.

Only the Agilent ORS combines the ShieldTorch interface with an octopole cell and so only the Agilent ORS can effectively use He collision mode.

Testing He Collision Mode – a Worst Case Scenario

A synthetic sample matrix was prepared to give rise to multiple interferences across a range of common analytes and test the ability of He collision mode to remove all overlapping polyatomic species. A standard solution was prepared, containing 1% HNO_3, 1% HCl and 1% H_2SO_4 (all UpA UltraPure Reagents, Romil, Cambridge, UK), 1% Butan-1-ol (SpS Super Purity, Romil, Cambridge, UK) and 100 mg/L (ppm) each of Na and Ca (both prepared from 10,000 mg/L Spex CertiPrep Assurance single element standards), to simulate a very complex natural sample matrix. Table 1 summarizes the potential polyatomic species in this sample matrix, illustrating that practically every element in the mid-mass region (from 50 to 80 amu) suffers from multiple interferences. This makes the accurate determination of these elements in complex sample matrices extremely challenging for conventional ICP-MS, as the complex nature of the multiple interferences means mathematical corrections will be unreliable. This also illustrates why reactive cell gases are unsuitable for the multi-element analysis of complex samples; no single reaction gas can be effective for a range of

polyatomic ions, each of which will have different reactivity with any given reactive cell gas. However, every interference shown in Table 1 is a polyatomic ion and can therefore be attenuated effectively using a single set of He collision mode conditions. Two sets of spectra were acquired to show the ability of the He collision mode to remove multiple interferences; one in no-gas mode and the second with He added to the cell. No data correction or background subtraction was applied. Finally, a 5-ppb multi-element spike was added to the matrix and spectra acquired to confirm the recovery of all analytes and check for correct isotopic fit.

Table 1. Principal Polyatomic Interferences from an Aqueous Matrix Containing N, S, Cl, C, Na, and Ca

Isotope	Principal interfering species
^{51}V	$^{35}Cl^{16}O$, $^{37}Cl^{14}N$
^{52}Cr	$^{36}Ar^{16}O$, $^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{37}Cl^{14}NH$
^{53}Cr	$^{36}Ar^{16}OH$, $^{40}Ar^{13}C$, $^{37}Cl^{16}O$, $^{35}Cl^{18}O$, $^{40}Ar^{12}CH$
^{54}Fe	$^{40}Ar^{14}N$, $^{40}Ca^{14}N$
^{55}Mn	$^{37}Cl^{18}O$, $^{23}Na^{32}S$
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$
^{57}Fe	$^{40}Ar^{16}OH$, $^{40}Ca^{16}OH$
^{58}Ni	$^{40}Ar^{18}O$, $^{40}Ca^{18}O$, $^{23}Na^{35}Cl$
^{59}Co	$^{40}Ar^{18}OH$, $^{43}Ca^{16}O$
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$
^{61}Ni	$^{44}Ca^{16}OH$, $^{38}Ar^{23}Na$, $^{23}Na^{37}ClH$
^{63}Cu	$^{40}Ar^{23}Na$, $^{12}C^{16}O^{35}Cl$, $^{12}C^{14}N^{37}Cl$
^{64}Zn	$^{32}S^{16}O_2$, $^{32}S_2$, $^{36}Ar^{12}C^{16}O$, $^{38}Ar^{12}C^{14}N$, $^{48}Ca^{16}O$
^{65}Cu	$^{32}S^{16}O_2H$, $^{32}S_2H$, $^{14}N^{16}O^{35}Cl$, $^{48}Ca^{16}OH$
^{66}Zn	$^{34}S^{16}O_2$, $^{32}S^{34}S$, $^{33}S_2$, $^{48}Ca^{18}O$
^{67}Zn	$^{32}S^{34}SH$, $^{33}S_2H$, $^{48}Ca^{18}OH$, $^{14}N^{16}O^{37}Cl$, $^{16}O_2{}^{35}Cl$
^{68}Zn	$^{32}S^{18}O_2$, $^{34}S_2$
^{69}Ga	$^{32}S^{18}O_2H$, $^{34}S_2H$, $^{16}O_2{}^{37}Cl$
^{70}Zn	$^{34}S^{18}O_2$, $^{35}Cl_2$
^{71}Ga	$^{34}S^{18}O_2H$
^{72}Ge	$^{40}Ar^{32}S$, $^{35}Cl^{37}Cl$, $^{40}Ar^{16}O_2$
^{73}Ge	$^{40}Ar^{33}S$, $^{35}Cl^{37}ClH$, $^{40}Ar^{16}O_2H$
^{74}Ge	$^{40}Ar^{34}S$, $^{37}Cl_2$
^{75}As	$^{40}Ar^{34}SH$, $^{40}Ar^{35}Cl$,$^{40}Ca^{35}Cl$
^{77}Se	$^{40}Ar^{37}Cl$, $^{40}Ca^{37}Cl$
^{78}Se	$^{40}Ar^{38}Ar$
^{80}Se	$^{40}Ar_2$, $^{40}Ca_2$, $^{40}Ar^{40}Ca$

Instrumentation

An Agilent 7500ce ICP-MS was optimized using the typical tuning conditions for high and variable sample matrices (plasma conditions optimized as usual for ~0.8% CeO/Ce). No attempt was made to optimize any parameter for the targeted removal of any specific interference. 5.5 mL/min He gas (only) was added to the cell for the collision mode measurements.

Comparison of Spectra

The background spectrum obtained in no-gas mode is shown in Figure 1a, together with the same spectrum (same mass range and intensity scale) under He collision mode conditions, in Figure 1b. From Figure 1a, it is clear that the normal background components of the argon plasma gas and aqueous sample solution (Ar, O, H), together with the additional components of the synthetic sample matrix (HNO_3, HCl, H_2SO_4, butanol, Ca and Na), lead to the formation of several high intensity background peaks in the no-gas mode spectrum, notably $^{40}Ar^{16}O^+$ and $^{40}Ar_2{}^+$ from the plasma, but also $^{40}Ar^{12}C^+$, $^{32}S_2{}^+$, $^{35}Cl^{16}O^+$, etc, from the matrix. These high intensity background peaks show why several interfered elements (^{56}Fe, ^{78}Se and ^{80}Se, ^{52}Cr in a carbon matrix, ^{64}Zn in a sulfur matrix) have traditionally been considered as difficult elements for ICP-MS.

When helium is added to the cell (He collision mode conditions) all of these high intensity background peaks are removed from the spectrum, (Figure 1b – same sample, same intensity scale as Figure 1a) demonstrating the effectiveness and the universal applicability of He collision mode. Figures 2a and 2b are the same two spectra as in Figure 1, but with the vertical scale expanded 100x. Many more, lower intensity, matrix-derived polyatomic species are now observed. These interferences, though present at lower levels than the plasma-based polyatomic ions, have the potential to cause more serious errors in routine sample analysis, as their presence and intensity is dependent on matrix composition, which, in routine laboratories, may be variable and unknown. At this expanded scale, it is clear that the use of He collision mode has reduced the background

species to very low levels, including the high intensity plasma-based species ArO^+ and Ar_2^+. The only peaks clearly visible in He collision mode (Figure 2b) on this scale are Fe and Zn (the peak template confirms the Zn isotopic pattern at *m/z* 64, 66, and 68), due to trace level contamination present in the matrix components. By contrast, in no-gas mode (Figure 2a), almost every isotope of every element in this mass region has an overlap from at least one matrix-derived polyatomic interference.

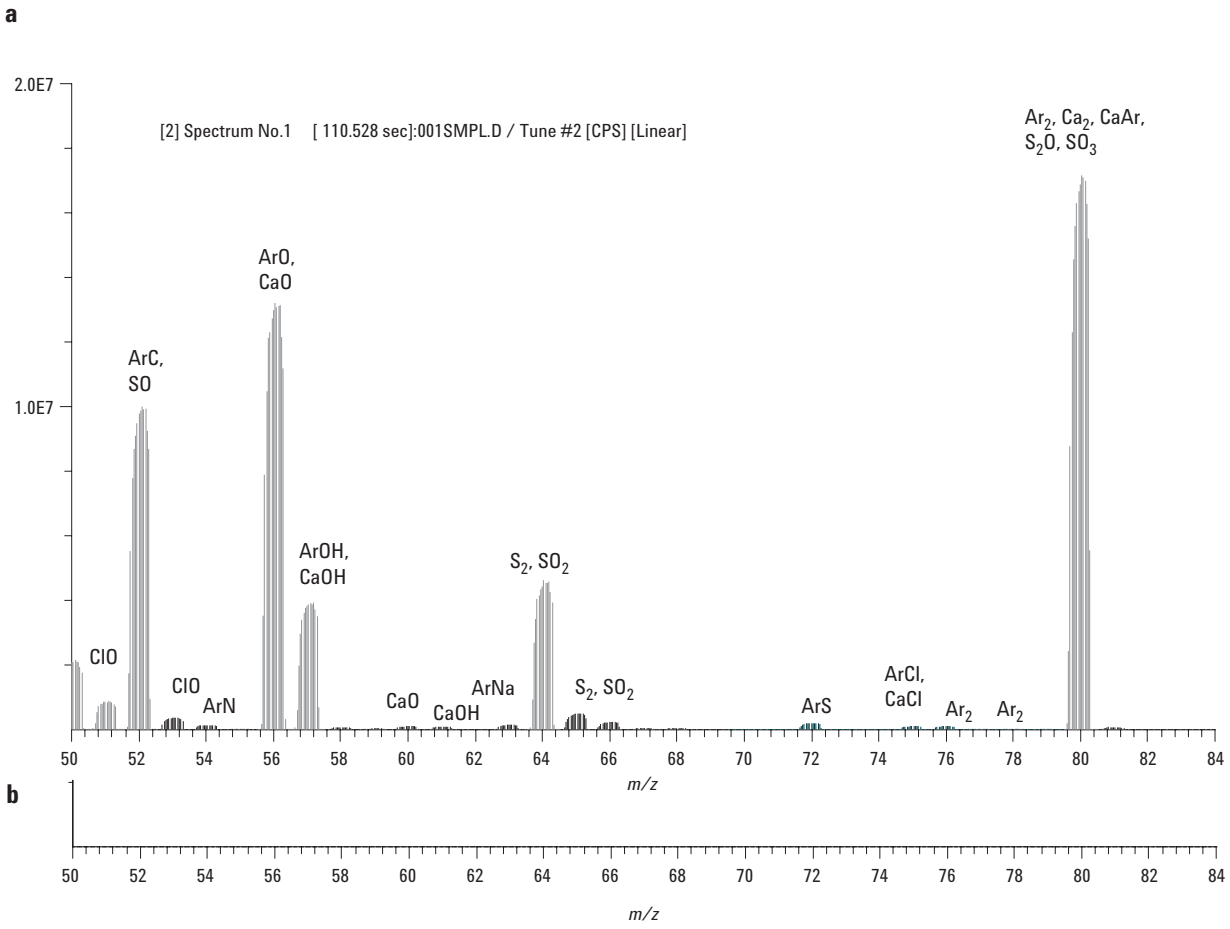

Figure 1. High intensity interfering polyatomic ions from complex matrix sample (see text for composition) in (a) no-gas mode and (b) He collision gas mode, on same intensity scale (2.0E7).

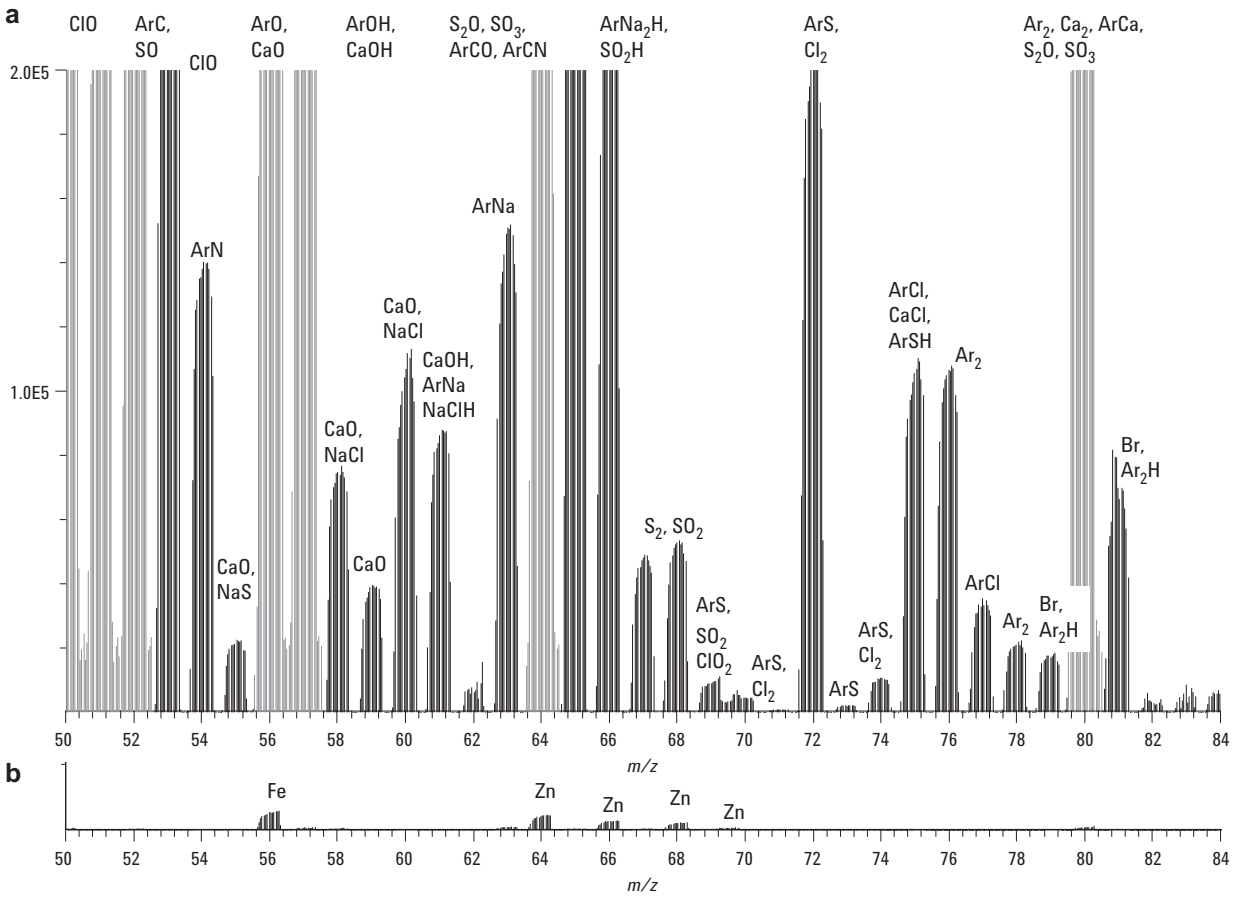

Figure 2. Low intensity interfering polyatomic ions from complex matrix sample in (a) no-gas mode and (b) He collision gas mode on same intensity scale (2.0E5), which is expanded 100x compared to Figure 1.

Measurement of Analytes in the Presence of the Sample Matrix

Having demonstrated the effective reduction of both plasma-based and matrix-based polyatomic ions using a single set of He collision mode cell conditions (Figures 1b and 2b), a second sample was analyzed. This time the sample consisted of the same multi-component matrix, but was spiked with a 5-ppb multi-element standard. Data was acquired in He collision mode to ensure that the same cell conditions used for interference removal also gave sufficient analyte sensitivity to permit the measurement of the previously interfered trace elements in this mass range. The spike consisted of 5 ppb each of V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, As and Se, all of which had at least one analytically useful isotope which suffered a polyatomic overlap in no-gas mode in this matrix.

Spectra obtained in He collision mode for the blank (unspiked) matrix and the spiked matrix are compared in Figures 3a and 3b respectively. Note that these spectra are shown on an intensity scale that is a further 4x lower than that used for Figures 2a and 2b, allowing the presence of the contaminant elements (Fe, Ni, Cu, Zn) to be confirmed from their isotopic templates (Figure 3b). The spectrum shown in Figure 3a clearly illustrates the capability of He collision mode to perform multi-element measurements at the low ppb level in this most complex and challenging sample matrix. Good isotopic fit is shown for every analyte. The only residual interferences observed were the plasma-based species ArOH and Ar_2 at mass 57 and 80 respectively. The Ar_2 signal at mass 80 is equivalent to ~5 µg/L Se. However, the polyatomic interferences on the other Se isotopes at *m/z* 77, 78, and 82 were removed completely, allowing Se determination at any of these isotopes (^{76}Se would also be available, but is overlapped by ^{76}Ge which was in the spike mix).

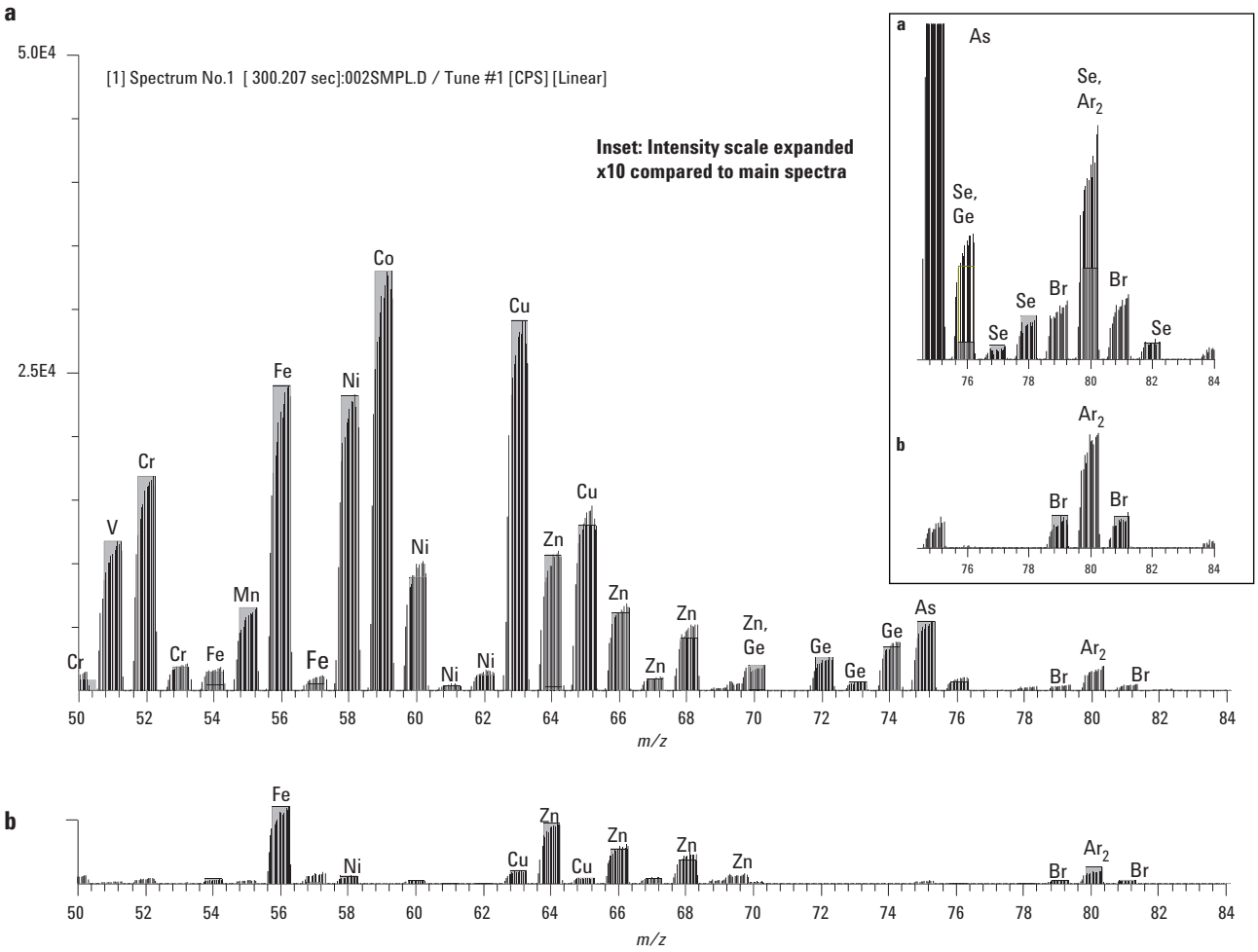

Figure 3. Complex matrix sample in He collision mode, (a) spiked at 5 ppb with V, Cr, Fe, Mn, Ni, Co, Cu, Zn, Ge, As, and Se and (b) unspiked. Intensity scale is 5.0E4 (5.0E3 for inset spectra).

Conclusions

The ability to remove ALL polyatomic interferences under a single set of conditions means that He mode is effectively universal – being suitable for any isotope of any element in any sample matrix. The use of He collision mode provides a unique new mode of operation, in which ALL the isotopes of each analyte become accessible. This, in turn, means that major isotopes that could not previously be used due to interferences (for example: ^{52}Cr in a carbon matrix, ^{56}Fe in any aqueous sample, ^{63}Cu in a sodium matrix, and ^{64}Zn in a sulfate matrix) - now become available. This is a great advantage to the analyst since, if desired, results can be verified by measuring many elements at both the preferred isotope AND at a second, "qualifier" isotope. Since both isotopes are free from polyatomic interference when measured using He collision mode, the use of two independent measurements gives a valuable confirmation of the reported result.

A further benefit of this powerful mode of analysis concerns sample preparation. In normal (non-CRC) ICP-MS, the choice of dilution media was limited mostly to nitric acid. Hydrochloric and sulfuric acid could not be used because of the problems of chloride or sulfur-based matrix interferences. Analysts can now choose the most appropriate digestion technique for the sample, secure in the knowledge that any new polyatomic interferences will be removed under the existing, standard He mode conditions.

The use of He collision mode on the 7500ce was demonstrated to provide effective removal of all polyatomic interferences under a single set of conditions, thereby enabling accurate multi-element analysis in complex and unknown samples. The use of an inert cell gas insures that there is no loss of analyte signal by reaction and that no new interfering species are generated, in contrast to the use of a reactive cell gas.

Since no analytes are lost by reaction and no new interferences are formed, uninterfered elements (and internal standards) can be measured under the same conditions as potentially interfered elements, and the use of a single set of cell conditions for all analytes allows multi-element analysis of transient signals (such as those derived from chromatography or laser ablation sample introduction), as well as semiquantitative screening analysis.

He collision mode is suitable for all analytes that suffer from polyatomic ion interferences and the cell conditions do not need to be set up specifically for each analyte, so the same cell conditions can be applied to new analyte suites, without requiring method development. Furthermore, since the He mode conditions are not set up specifically for the removal of individual interferences, identical cell conditions can be used for highly variable or completely unknown sample matrices, which greatly simplifies operation in a routine laboratory. The ORS enables ICP-MS to be used for the trace multi-element measurement of the most complex, real world sample matrices with no method development and with complete confidence.

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Agilent Technologies

A Comparison of the Relative Cost and Productivity of Traditional Metals Analysis Techniques Versus ICP-MS in High Throughput Commercial Laboratories

Application

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Abstract

A financial model was developed to help the metals laboratory using graphite furnace atomic absorption and inductively coupled plasma optical emission spectroscopy calculate the potential savings by switching to inductively coupled plasma mass spectrometry. Results based on several typical laboratory examples are presented.

Introduction

The past 5 years have seen significant growth in the use of inductively coupled plasma mass spectrometry (ICP-MS) for the analysis of trace metals in many applications in the environmental, semiconductor, geological, and health sciences industries. This growth is driven by three factors. First is the need for increasingly lower limits of detection for many metals in many applications. Second is the significantly improved performance, reliability, and ease of use of modern ICP-MS instruments. And third is economics.

Traditionally, most elemental analysis has been performed by either atomic absorption (AA) or optical emission spectroscopy (OES). Generally, the ultratrace (sub-ppb) elements were measured by graphite furnace atomic absorption (GFAA), a highly sensitive single-element technique. The trace and minor (ppb to ppm) elements were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), which is less sensitive but capable of simultaneous multi-element analysis.

As the need for sub-ppb detection limits extends to more elements in more samples, ICP-OES becomes less useful and the reliance on GFAA increases. However, GFAA, while sensitive, is slow, expensive to operate, and has limited dynamic range. Because GFAA is much slower than ICP-OES, many routine labs have a dedicated GFAA instrument for each analyte that is required to be measured by GFAA - multiple GFAAs working with one ICP-OES. Furthermore, the analysis of mercury will add the need for a third technique, either cold vapor AA or atomic fluorescence. However, in the interest of simplicity, a separate mercury analyzer was not considered in the examples used. Each of these techniques may require separate sample handling and preparation, as well as separate analysis, data processing and archival, significantly increasing the cost per sample.

The subject of this application note is to evaluate the productivity and cost effectiveness of ICP-MS as a routine, highly sensitive, multi-element technique where a single ICP-MS instrument has the potential to replace an ICP-OES, multiple GFAAs, and a mercury analyzer for most routine elemental analyses. The analytical applicability of ICP-MS to many types of samples is already well established. More recently, the introduction of the Octopole Reaction System on the 7500 Series ICP-MS instruments from Agilent has removed the final performance barriers that have prevented ICP-MS being proposed as a complete replacement for GFAA and ICP-OES.

Methods

To facilitate this study, a spreadsheet-based sample cost comparison model was developed in Excel. This tool allows the user to provide detailed parameters related to numbers and types of samples, as well as associated costs of sample preparation, instrumentation, and analysis. Output is simply cost of analysis per sample. Also reported are the total time required for sample analysis per month, the number of analysts required, and the number of instruments. The model compares the results for GFAA, ICP-OES, and ICP-MS. While it will allow almost any values to be entered for most parameters, the results presented here are based on values obtained from several commercial laboratories doing these analyses. No model can exactly predict the results for all situations and still be simple enough to be useful. Therefore, in the interest of simplicity, a number of assumptions were made in the design of the model and in the example data entered. We feel that the assumptions are realistic and do not impart significant bias on the results. The tool is easy to use and can allow a laboratory to quickly and simply evaluate the cost effectiveness of the three techniques based on laboratory-specific information.

Assumptions

- GFAA system costs US$30K
- ICP-OES system costs US$100K
- ICP-MS system costs US$180K
- Cost of funds (finance) is 6%
- General facilities costs, such as laboratory space, utilities etc., are ignored since they are difficult to estimate and do not significantly affect the results in most cases.
- An instrument operator can keep a modern, automated GFAA, ICP-OES, or ICP-MS running for two shifts (16 hours) per day. When analysis times exceed 16 hours per day for any technique, additional instrumentation and operators will be required. Instruments are added in increments of one; operators are added in fractions since it is assumed that they can be shared with other tasks in the laboratory and cost calculations are based only on the portion of time the operator spends on the specific analysis.
- GFAA is a single element technique. Instruments with multiple lamps still perform a single analysis at a time. Typical analysis time is 90 seconds per element and each element requires two replicate analyses (burns).
- ICP-OES and ICP-MS are multi-element techniques and the number of elements does not significantly effect the analysis time. This is not strictly true, but the assumption is reasonable for the sake of simplicity.
- GFAA will use pressurized argon and the consumption is 40 hours of use per cylinder ($100).
- GFAA graphite tubes and platforms cost $50 per set and last for 100 burns.
- ICP-MS and ICP-OES will use liquid argon and the typical consumption is 3 weeks of use per dewar ($250).
- ICP-MS detectors last typically for 3 years and the cost per year is amortized based on 3-year lifetime.

Results

Several typical laboratory scenarios were evaluated by varying the current instrument complement of the laboratory, and by varying the current and anticipated number of samples to be analyzed per month. Also examined was the effect of the number of elements that must be analyzed by GFAA (in the case of laboratories without ICP-MS) to meet required DLs.

Scenario 1

Laboratory currently has one GFAA plus one ICP-OES, which are paid for. ICP-MS must be purchased and amortized over 3 years. See Table 1.

Table 1. Scenario 1

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$41	1	$30	$4,536
1000	8	2	$33	1	$15	$18,196
5000	8	9	$31	2	$9	$112,968

Scenario 2

Laboratory currently has two GFAA plus one ICP-OES, which are paid for. ICP-MS must be purchased and amortized over 3 years. See Table 2.

Table 2. Scenario 2

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$41	1	$30	$4,536
1000	8	2	$32	1	$15	$17,283
5000	8	9	$31	2	$9	$112,055

Scenario 3

Laboratory currently has no instrumentation and must decide on purchasing GFAA plus ICP-OES versus ICP-MS. See Table 3.

Table 3. Scenario 3

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$51	1	$30	$8,491
1000	8	2	$37	1	$15	$22,151
5000	8	9	$32	2	$9	$116,923

Scenario 4

Comparison of costs per sample as a function of number of GFAA elements. (All instruments must be purchased.) See Table 4.

Table 4. Scenario 4

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
1000	2	1	$24	1	$14	$9,601
1000	4	1	$28	1	$14	$12,751
1000	8	2	$38	1	$14	$22,151
1000	10	3	$42	1	$14	$27,490

Discussion

In all cases, even when the laboratory already owns two graphite furnaces and one ICP-OES (a common configuration) and must purchase the ICP-MS, the cost per sample is lower for ICP-MS. This is mainly due to the high cost of consumables for GFAA plus the fact that GFAA and ICP-OES requires two separate sample prep steps. Additionally, as the number of samples increases from a conservative number of 400 per month to 1000 and 5000 per month, the differential becomes much greater. This is caused by rapidly increasing labor costs for GFAA, as well as the much higher sample capacity of ICP-MS, lower consumables costs, and requirements for only a single sample prep.

Return on Investment for ICP-MS

A simple return on investment (ROI) can be calculated from the above tables. In this case, the cost per month of the new ICP-MS system is approximately US $5500.00 (assuming purchase price of US$180K financed for 3 years at 6%). Figure 1 shows the payback times for a laboratory that already owns two GFAAs and one ICP-OES as a function of the sample load. The y-axis represents the accumulated monthly savings of using ICP-MS versus GFAA + ICP-OES for three different sample loads compared to the unpaid balance on the ICP-MS instrument. As can be seen, the accumulated savings of ICP-MS is equal to the payoff amount after just 4 months when analyzing 2000 samples per month. Even when analyzing as few as 400 samples per month, the accumulated savings is sufficient to pay off the ICP-MS instrument in around 20 months. In this case, eight furnace elements are assumed. Other assumptions are as above.

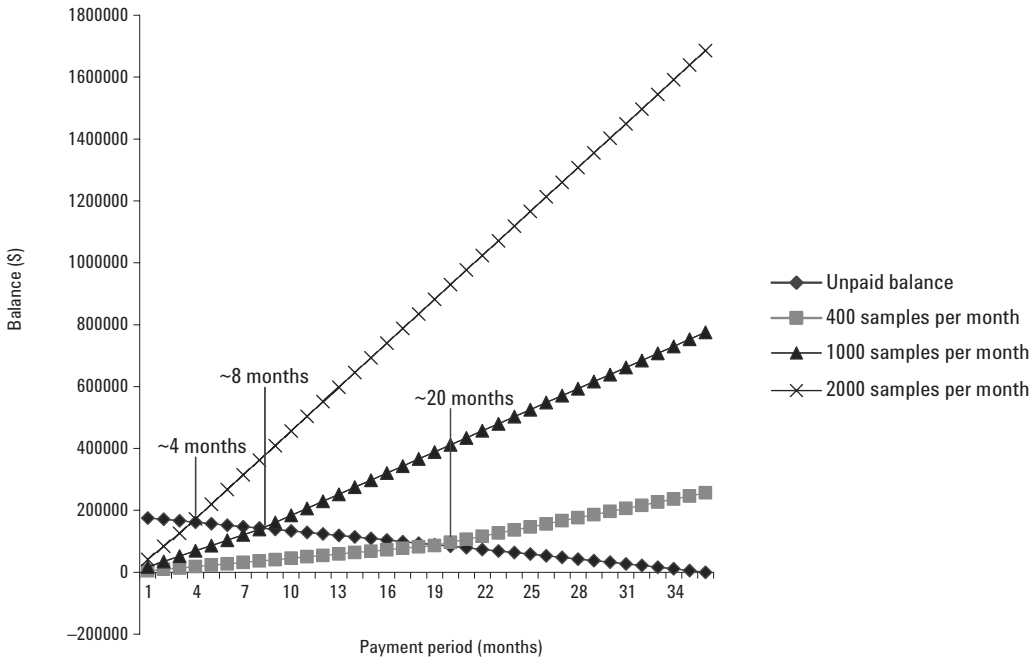

Figure 1. Cumulative return on investment of ICP-MS purchase for three sample levels plotted against the monthly unpaid balance on the ICP-MS. In this case, it is assumed that the accumulated revenue will be used to pay off the loan when the balance equals the residual loan amount. At that point, the net monthly revenue is increased by the loan amount. In this example, laboratories running 2000 samples per month will be able to pay off the ICP-MS in about 4 months, 1000 sample laboratories in about 8 months, and 400 sample laboratories in about 20 months. At the end of 36 months (the original loan period), net revenue exceeds $200K for the 400 sample lab, $750K for the 1000 sample lab, and $1.7 million for the 2000 sample lab.

Conclusions

For almost any metals laboratory, analyzing at least 100 samples per week (400 per month) and using a combination of GFAA and ICP-OES for the analysis, converting to ICP-MS will save money. Depending on the number of samples, the payback for the ICP-MS can be as short as a few months. The cost advantages are not reduced significantly, even if the laboratory already owns its GFAA and ICP-OES instruments. They are also not significantly affected by the number of GFAA elements. As Scenario 4 shows, for the laboratory analyzing at least 1000 samples per month with only two elements by GFAA, the cost savings of switching to ICP-MS is approximately $10,000 per month. Add to this the increased confidence in results obtained by ICP-MS, the ability to analyze all analyte elements at GFAA (or better) DLs, and the robustness and simplicity of operation of modern ICP-MS instruments, and the choice becomes simple. The productivity of ICP-MS in a high-volume laboratory can quickly pay off the purchase price and increase laboratory profitability significantly.

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Analysis of High Matrix Environmental Samples with the Agilent 7500ce ICP-MS with Enhanced ORS Technology

Part 3 of a 3 part series on Environmental Analysis

Application

Environmental

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Abstract

The Agilent 7500ce ICP-MS was designed and optimized specifically to analyze unknown, high matrix samples. The 7500ce uses enhanced Octopole Reaction System technology for removal of interferences and improved ion optics for greater sensitivity than previous ORS instruments. This application note describes the performance of the instrument when analyzing various, high-matrix samples.

Introduction

This application note represents *Part Three* of the three part series of environmental application notes based on the Agilent 7500ce ICP-MS (inductively coupled plasma mass spectrometer). It examines its suitability for the routine analysis of trace metals in unknown high-matrix samples.

- Part one of this series details the theory of operation of the 7500ce ORS ICP-MS system and the related hardware and software [1].
- Part two is a drinking water application note demonstrating the ability of the Agilent 7500ce ICP-MS system to measure trace elements in drinking water substantially below regulated levels under challenging real-world conditions [2].

The experimental setup, instrument conditions, and sample sequence are described in *Part Two* [2]. The data for both application notes was acquired in a single 15.5 h sequence of samples including drinking waters, ground waters, synthetic seawaters, soil digests, and EPA interference check samples (ICS-A, ICS-AB). A single optimization, calibration and method were used for all samples as described in *Part Two*. Calibrations were not matrix-matched, and octopole reaction system (ORS) conditions were not optimized for a particular analyte or matrix. No mathematical interference correction equations were used. No re-optimizations, recalibrations or maintenance were performed during the sequence of samples. A graphic representation of the analytical sequence is displayed in Figure 1.

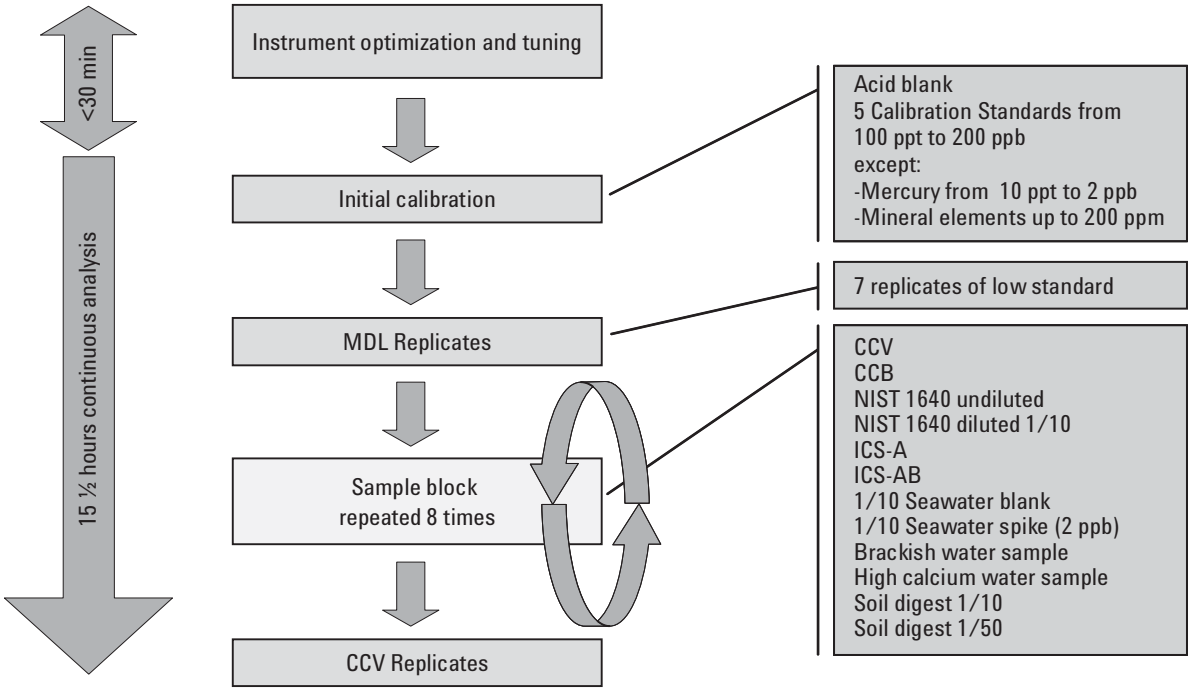

Figure 1. Analytical sequence.

Analytical Challenges

Since the inception of ICP-MS, numerous difficult challenges have slowed its complete adoption over the more traditional techniques of graphite furnace atomic absorption (GFAA) and ICP optical emission spectroscopy (ICP-OES) in the environmental monitoring industry. In the analysis of high matrix samples including soils, sludges, industrial wastes, and even food samples by ICP-MS, the principal obstacles have been overcoming interferences and improving stability. Numerous approaches* have had incomplete success at resolving these problems. More recently, the use of collision/reaction cells (CRCs) to remove interferences has had good success [3]. However, CRCs alone cannot completely eliminate the detrimental effects of high matrix samples on the ICP-MS instrument. This is because in addition to the formation of polyatomic interferences, high matrix samples can have other negative effects on the plasma, interface, and mass spectrometer of the ICP-MS. These include ionization suppression, reduced ion transport efficiency, and matrix deposition in the interface, ion optics, and mass spectrometer that can affect sensitivity, and stability. In order to overcome these obstacles, the ideal environmental ICP-MS must have excellent matrix tolerance, the ability to remove interferences, high sensitivity, and wide dynamic range. It must possess these attributes for a variety of unknown and varied matrices, for all analytes using a simple, universal set of conditions. The Agilent 7500ce was designed specifically to address these challenges. A new ion optic and a highly efficient on-axis ORS easily and effectively eliminate polyatomic interferences. Robust plasma conditions due to the use of high RF power (1500-1600 W), efficient RF coupling, and a cooled, low-flow sample introduction system minimize the effects of matrix on the ICP-MS interface. Hardware and software details are covered in *Part One* of this series.

*Techniques used to control the effects of sample matrix on the ICP-MS have included the use of mathematical interference equations, aerosol desolvation, high efficiency nebulizers, various means of controlling plasma temperature and secondary ionization in the interface and even high-resolution mass spectrometry. None were completely successful at eliminating interferences and other matrix effects.

In summary, improvements in ion optic and octopole design, created specifically for the environmental laboratory have resulted in an ICP-MS instrument with unprecedented sensitivity, matrix tolerance and stability [1]. By using a highly efficient octopole reaction cell and careful control of ion energy, most polyatomic interferences can be removed under a single set of generic conditions using helium-only collision mode with kinetic energy discrimination. A few argon-based polyatomics are more efficiently removed using pure hydrogen in reaction mode.

Experimental

Detailed experimental conditions are discussed elsewhere [2]. Instrumental conditions are outlined in Table 1. This work was designed to replicate the workload in a typical environmental laboratory where sample matrices vary widely and are frequently unknown. Under these conditions, it is not practical to matrix-match calibrations to multiple sample matrices. It is also not practical to depend on matrix-specific or analyte-specific reaction cell conditions. The data shown in this note were all generated using a single set of calibration standards in 1% HNO_3/0.5% HCl. Calibration was performed once only at the beginning of the sequence and not repeated or updated during the sequence. No attempt at matrix matching either the calibration standards or CRC conditions was made. No mathematical interference corrections were employed and all analytes were measured at their elemental masses.** The instrument was tuned for robust plasma conditions*** resulting in sensitivity of approximately 50 million cps/ppm at mid-mass with background less than 5 cps, CeO^+/Ce^+ less than 1% and Ce^{++}/Ce^+ less than 1.5%. The samples included a natural water certified reference material (CRM), NIST 1640, a 1/10 diluted synthetic seawater and low-level spike, as well as various ground waters and soil samples. In addition, the US Environmental Protection Agency (EPA) ICS-A and ICS-AB were used to simulate a challenging high matrix reference material. This was done due to the lack of suitable, prepared CRMs for high matrix samples. Rather than introduce extraction efficiency into recovery calculations of nonprepared samples, it was decided to use a well-characterized sample designed to simulate a difficult waste sample digestate. ICS-A contains high concentrations of elements known to cause interferences in ICP-MS. It is intended to test the ability of the ICP-MS system to compensate for both spectral and nonspectral interferences. ICS-A also contains sufficient total dissolved solids (TDS) to test the robustness of the ICP-MS interface and ion optics to salt buildup. ICS-AB is a spiked ICS-A sample intended to test the ability of the system to accurately detect low-level analyte elements in this challenging matrix. The composition of ICS-A and ICS-AB are listed in Table 2. Table 3 depicts the ORS mode each element was acquired in. Details of hardware and reagents are described elsewhere [1]. All are typical of a routine commercial environmental laboratory. The accuracy and precision of the repeat analyses of each sample type over the entire sequence were monitored.

**Some CRC ICP-MS systems depend on the use of reactive gases to deliberately form polyatomic species of certain analyte elements. In this way the element is "shifted away" from the interference to another mass. However, the rate of formation of the polyatomic species can be concentration and matrix dependent resulting in potentially inaccurate results in variable or unknown matrices.

***Robust plasma conditions are defined as those promoting the most complete atomization and ionization of analyte and matrix components, minimizing polyatomic interferences and the deposition of salts on the interface and mass spectrometer. The generally accepted measure of plasma robustness is the ratio of CeO^+/Ce^+ when Ce is introduced. The ratio should be as low as possible, ideally less than 1%, indicating excellent breakdown of metal oxides (and therefore, other matrix interferences) in the plasma.

Table 1. Instrument Conditions Used for All Samples for Maximum Plasma Robustness and Polyatomic Interference Removal. No Analyte-Specific Settings Were Required.

Instrument parameter	Normal mode	Hydrogen mode	Helium mode
RF Power	1500 W	<Same	<Same as H_2
Sample depth	8 mm	<Same	<Same as H_2
Carrier gas	0.85 L/min	<Same	<Same as H_2
Makeup gas	0.2 L/min	<Same	<Same as H_2
Spray chamber temp	2 °C	<Same	<Same as H_2
Extract 1	0 V	<Same	<Same as H_2
Extract 2	–160 V	<Same	<Same as H_2
Omega bias	–24 V	<Same	<Same as H_2
Omega lens	–0.6 V	<Same	<Same as H_2
Cell entrance	–30 V	<Same	<Same as H_2
QP focus	3 V	–11 V	<Same as H_2
Cell exit	–30 V	–44 V	<Same as H_2
Octopole bias	–7 V	–18 V	<Same as H_2
QP bias	–3.5 V	–14.5 V	<Same as H_2
Cell gas flow	0	3.0 mL/min H_2	4.5 mL/min He

Table 2. Composition of EPA Interference Check Samples, ICS-A and ICS-AB

Solution component	Comment	Solution A concentration mg/L	Solution AB concentration mg/L
Al	Possible interference with Ni as AlCl	100	100
Ca	Interferes with Fe as CaO	300	300
Fe	Can interfere with Zn and Se as FeN and FeOH	250	250
Mg	Interferes with Ca, Ni, and Cu as MgCl	100	100
Na	Interferes with Cu as ArNa	250	250
P	Interferes with Cu and Ti as PO2 and PO	100	100
K	Easily ionized, suppresses Hg, As, Se, Zn, Cd, etc.	100	100
S	Interferes with Ti as SO, SOH	100	100
C	Interferes with Cr as ArC	200	200
Cl	Interferes with As, Se, Cr, Co, Cu, Ba, etc. as various chlorides	2000	2000
Mo	Interferes with Cd as MO	2	2
Ti		2	2
As		0	0.02
Cd		0	0.02
Cr		0	0.02
Co		0	0.02
Cu		0	0.02
Mn		0	0.02
Hg		0	0.02
Ni		0	0.02
Se		0	0.02
Ag		0	0.02
V		0	0.02
Zn		0	0.02

Table 3. Summary of Analyte Masses, Analytical Conditions and Method Detection Limits in Both Screening Mode and Full Quantitative Mode for Regulated Elements

Analyte	Isotope	ORS mode (typical)*	Integration time (s)	Calibration range (ppb)	MDL screening (ppt)**	MDL Tri-Mode (ppt)†
Calcium (Ca)	40	H2	0.3	50–200,000	-	16.2
Iron (Fe)	56	H2	0.3	50–200,000	31.6	19.9
Selenium (Se)	78	H2	1.5	0.5–100	117.2	16.3
Sodium (Na)	23	He	0.3	50–200,000	55.2	55.2
Magnesium (Mg)	24	He	0.3	50–200,000	24.6	24.6
Potassium (K)	39	He	0.3	50–200,000	785.8	785.8
Vanadium (V)	51	He	1.5	0.5–100	32.6	32.6
Chromium (Cr)	52	He	1.5	0.5–100	27.1	27.1
Nickel (Ni)	60	He	1.5	0.5–100	25.6	25.6
Copper (Cu)	63	He	1.5	0.5–100	12.7	12.7
Arsenic (As)	75	He	1.5	0.5–100	45.2	45.2
Beryllium (Be)	9	Norm	0.3	0.5–100	113.2	26.5
Boron (B)	10	Norm	0.3	0.5–100	125.7	35.1
Aluminum (Al)	27	Norm	0.3	0.5–100	131.4	23.7
Manganese (Mn)	55	Norm	0.3	0.5–100	26.8	16.2
Cobalt (Co)	59	Norm	0.3	0.5–100	28.1	18.0
Zinc (Zn)	66	Norm	0.3	0.5–100	33.7	24.3
Molybdenum(Mo)	95	Norm	0.3	0.5–100	22.4	20.4
Silver (Ag)	107	Norm	0.3	0.5–100	18.2	15.4
Cadmium (Cd)	111	Norm	0.3	0.5–100	45.3	27.9
Tin (Sn)	118	Norm	0.3	0.5–100	51.2	14.0
Antimony (Sb)	121	Norm	0.3	0.5–100	51.2	13.7
Barium (Ba)	137	Norm	0.3	0.5–100	32.6	15.7
Mercury (Hg)	202	Norm	3.0	0.01–2.0	13.6	7.3
Thallium (Tl)	205	Norm	0.3	0.5–100	29.7	13.0
Lead (Pb)	208††	Norm	0.3	0.5–100	30.8	10.4
Thorium (Th)	232	Norm	0.3	0.5–100	27.5	12.0
Uranium (U)	238	Norm	0.3	0.5–100	29.3	10.2
Useful ISTDs						
^{6}Lithium (Li)	6	Norm	0.3	50 ppb		
Scandium (Sc)	45	All	0.3	50 ppb		
Germanium (Ge)	70,74	All	0.3	50 ppb		
Indium (In)	115	Norm	0.3	50 ppb		
Terbium (Tb)	159	Norm	0.3	50 ppb		
Platinum (Pt)	195	Norm	0.3	50 ppb		
Bismuth (Bi)	209	Norm	0.3	50 ppb		

*Typical ORS mode selected for best overall performance for most common matrices.

**Screening protocol uses He collision mode only for rapid screening where optimum sensitivity is not required for all elements, MDLs calculated according to EPA 200.8 requirements

†Method detection limits calculated according to EPA 200.8 requirements. Three sigma of seven replicate analyses of a fortified blank at 3-5 times the estimated MDL. MDLs are reported in ng/L (ppt) for ease of presentation

††Lead is measured as the sum of isotopes 206, 207, and 208 to eliminate error due to variable isotope ratios.

Results and Discussion

Analysis of Spiked Sea Water

In addition to the water CRM described in detail in *Part Two* [2], and the high TDS ground water samples, the sequence included replicate analyses of spiked synthetic seawater samples. The synthetic seawater consisted of 0.3% high purity sodium chloride solution (SPEX Certiprep) to simulate 1/10 diluted seawater. The synthetic seawater was spiked with 2 ppb of the trace elements and 200 ppb of Mg. Spike recoveries were calculated for all elements and are shown in Figure 2 and Table 5. Saline waters are a particularly challenging matrix due to potential Ar, Na, and Cl-based interferences on Cu, As, Se, V, and Ni (Table 4). Significant suppression of high ionization potential elements such as Zn, Cd, and Hg can also limit the sensitivity for these elements. The maximization of plasma temperature and use of well-matched internal standards (ISTD) is necessary to avoid this suppression. Typical recoveries (Table 5) are 90% or greater for most elements with the exception of Ag, which has limited solubility in chloride solutions. Long-term stability as measured by %RSD of eight replicate analyses over the 15.5-hour sequence is excellent, indicating no cumulative effects of long-term exposure to high TDS samples on the analytical accuracy, even at low (2 ppb) concentrations.

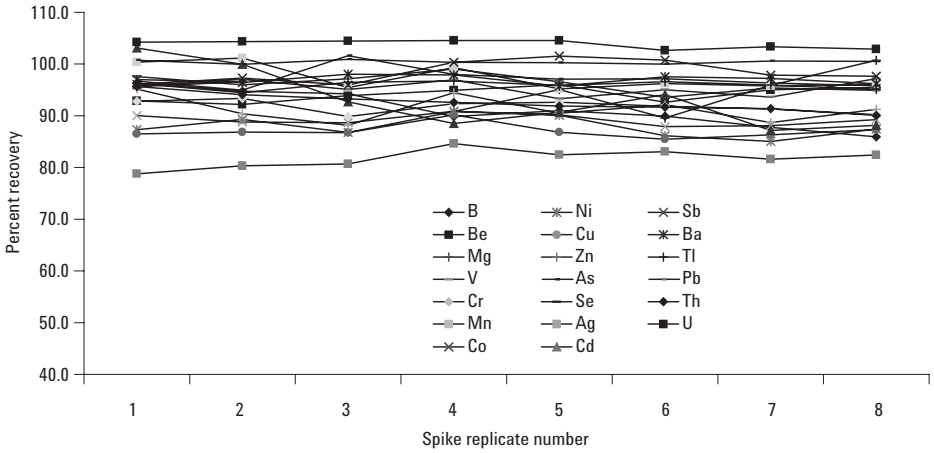

Figure 2. Spike recoveries in 1/10 synthetic seawater for eight replicate spikes at 2 ppb for trace elements and 200 ppb for magnesium measured over 15.5-hour sequence.

Table 4. Possible Polyatomic Interferences in Typical Environmental Samples and the ORS Mode Used to Eliminate Them

Analyte Isotope	Principal Interferences	Corrective ORS Mode
^{24}Mg	$^{12}C^{12}C$	He
^{27}Al	$^{12}C^{14}N^{1}H$	He
^{40}Ca	^{40}Ar	H_2
^{51}V	$^{35}Cl^{16}O$	He
^{52}Cr	$^{40}Ar^{12}C$, $^{35}Cl^{16}O^{1}H$, $^{36}Ar^{16}O$	He
^{55}Mn	$^{40}Ar^{14}N^{1}H$, $^{38}Ar^{17}O$	He
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$	H_2 or He
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{43}Ca^{16}O^{1}H$, ArS	He
$^{(63,65)}Cu$	$^{40}Ar^{23}Na$, SO_2	He
$^{(64,66,68)}Zn$	SO_2, ArS	
^{75}As	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}Cl$	He
$^{(78,80)}Se$	$^{40}Ar^{38}Ar$, SO_3	H_2

Table 5. Spike Recoveries and %RSDs for Eight Replicate Analyses of 1/10 Synthetic Seawater over a 15.5-Hour Period

Element	B	Be	Mg	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Ag	Cd	Sb	Ba	Tl	Pb	Th	U
Recovery % (mean)	91	94	95	96	92	97	89	88	87	91	97	95	82	93	98	97	96	100	93	104
%RSD	4.0	1.5	3.9	1.8	1.4	2.5	1.2	2.3	1.6	2.7	2.1	1.6	2.2	6.2	2.2	0.8	0.8	0.3	1.9	0.8

Analysis of EPA ICS-A and ICS-AB

Of the samples analyzed, the ICS-A and ICS-AB samples were the most demanding. A total of 16 analyses of these samples was performed over the course of the sequence. Under routine conditions, a laboratory in the US analyzing waste samples would be required by EPA method 6020 to analyze a single ICS pair with each sequence or every 12 hours of sample analysis. Because of the difficulty of this analysis, no control limits are specified for recovery of analytes in the ICS-AB spiked solution, corrective action being left to the judgment of the laboratory QA manager [4]. In this work, all elements showed excellent recovery, most between 90%–105%, over the entire sequence (Figure 3). No mathematical interference correction equations were used and all analytes were measured at their elemental masses. No reslope or recalibration was performed by the ChemStation. There is no evidence of drift from the beginning to the end of the sequence. Examination of the results of ICS-A in Figure 4 shows very low levels of analytes (<1 ppb), even though no interference correction equations were used and most elements were acquired in the generic He collision mode. Previous determinations of this standard using multiple isotopes per element have shown that most of the "interferences" are actually low-level contaminants.

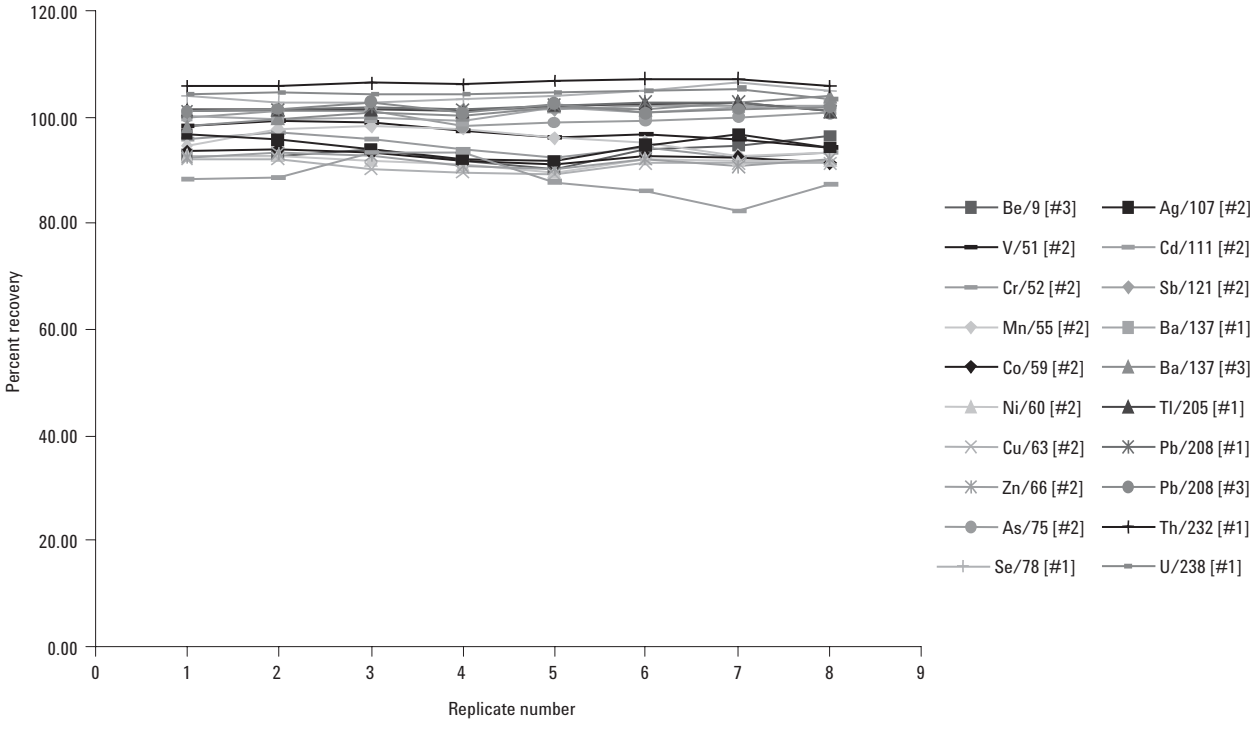

Figure 3. Recovery of analytes in EPA ICS-AB mix. Analytes (B) are spiked into ICS-A at 20-ppb each. Eight replicate analyses distributed over 15.5 hours.

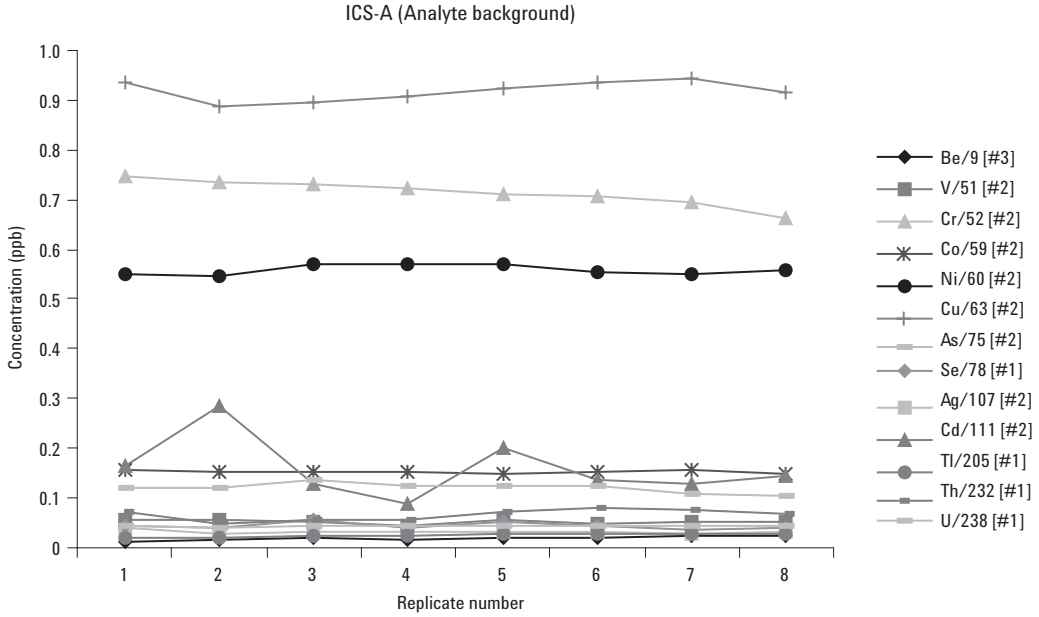

Figure 4. Measurement of apparent analyte concentrations in ICS-A replicates in ppb. No interference correction equations were used. Inspection of multiple isotopes indicates that apparent interferences were due to contaminants rather than interferences.

ISTD Response

All environmental ICP-MS methods recommend or require the use of ISTDs to correct for both mass-dependent long-term drift of instrument response as well as suppression or enhancement within a particular sample. These nonspectroscopic matrix effects are common in ICP-MS, particularly in older design instruments and when analyzing high matrix samples. EPA methods use ISTD responses in samples and the ICS solutions to monitor these effects. By setting control limits on ISTD response, the sensitivity of the instrument can be monitored and controlled. If the ISTD response falls outside the recommended control limits, the sample must be diluted to reduce matrix effects and reanalyzed. The control limits vary with the method and sample type. Figure 5 provides ISTD recoveries relative to the calibration blank for all ISTD elements in all samples of the sequence. EPA method 200.8, a drinking water method, mandates the strictest limits (60%–125%) over the course of the samples run. The EPA method for waste analysis, EPA 6020, in its most recent version, 6020a, specifies only a lower recovery limit of >30%. This is based on the knowledge that waste samples will typically display more severe nonspectroscopic interferences than clean drinking-water samples. The interference check solutions are an excellent indicator of the instruments ability to tolerate such interferences. Figure 5 illustrates the ISTD recoveries for the ISTDs, ^{6}Li, Sc, Ge, In, Tb and Bi in all three ORS modes for all samples of the sequence. The cyclic appearance is due to the repeated nature of the samples. (eight replicate analyses of sample group). The lowest recoveries in each block are for ICS-A and ICS-AB (approximately 80%) which are well within even the acceptable range for drinking water (60%–125%) and do not approach the lower EPA limit for waste samples. It is important to note that the ISTD response recovers immediately after each ICS sample indicating an absence of residual matrix effects. The small amount of gradual drift seen near the end of the 15.5-hour sequence would be corrected automatically by the ChemStation via periodic recalibration if necessary, though most analytical sequences do not approach the duration or difficulty of this one. The plots in Figure 5 show that the average sensitivity of the instrument has not changed from the beginning of the sequence to the end. The relatively minor divergence in ISTD responses is due to a slight shift in mass response toward greater high mass sensitivity at the cost of low mass sensitivity as a result of conditioning the interface with high TDS samples. Since high mass sensitivity is generally more critical, this shift is usually acceptable, even desirable. However, a simple adjustment in the extraction lens voltage is all that is required to return the system to the original condition if necessary.

Calibration Stability

Good laboratory practices require the monitoring of calibration accuracy for all analytes over the course of the sequence. This is normally accomplished by periodically analyzing a midpoint calibration standard as an unknown and comparing the result with the known value. Typically control limits of ±10 percent are set for acceptance of the continuing calibration verification (CCV) result. If the CCV sample results fall outside the 10 percent limit for any element, then sample results for that element will be inaccurate. If this occurs, the system must be recalibrated and any samples analyzed under the out-of-control conditions must be reanalyzed. CCV recoveries for all analytes over 13 replicate analyses are shown in Figure 6. In no case did any analyte recovery fall outside the ±10 percent limit. Had this occurred, the ChemStation would have automatically determined the degree of the failure, resloped or recalibrated the method as needed and rerun any out-of-control samples. This was, however, unnecessary, as stated earlier.

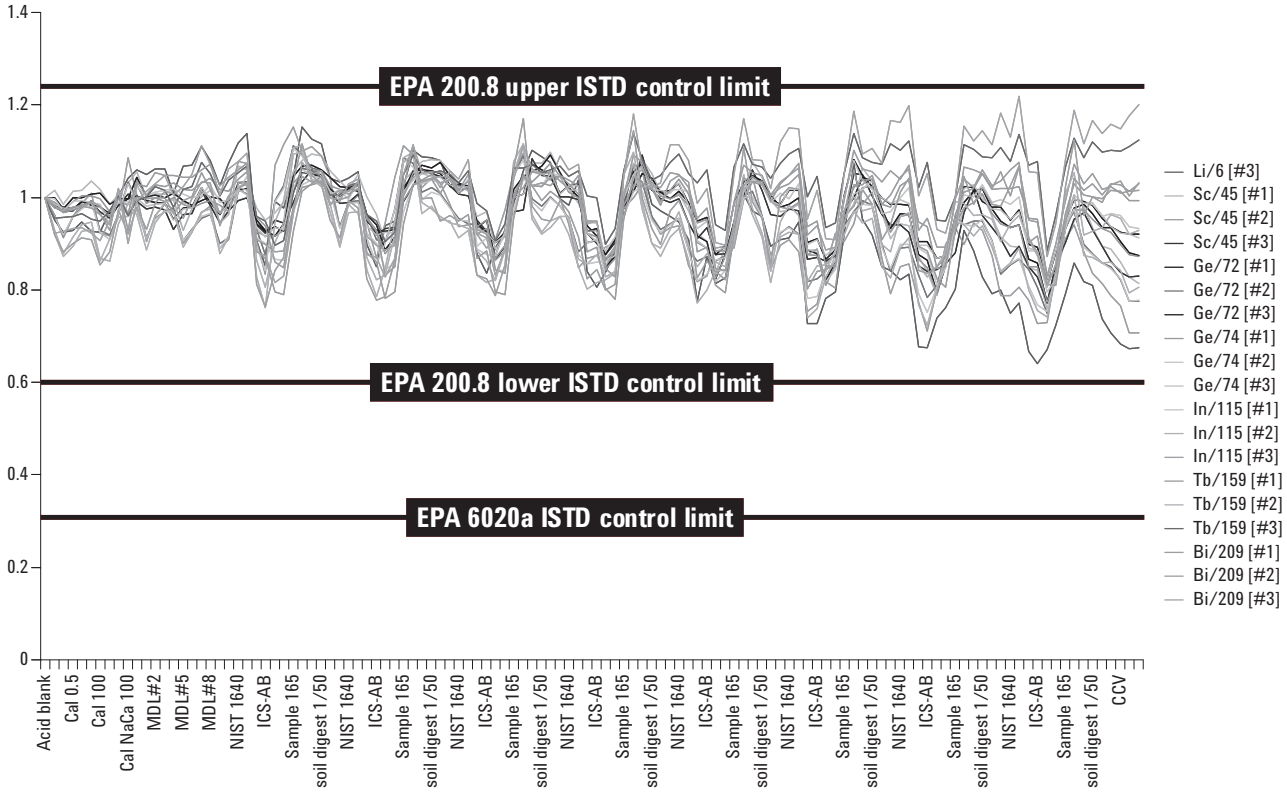

Figure 5. ISTD recoveries for all ISTD used in three ORS modes (mode indicated by number to the right of IS mass, 1 = hydrogen, 2 = helium, 3 = normal). Control limits for EPA methods 200.8 (Drinking Waters) are 60%–125% relative to the calibration blank, EPA 6020a (Wastes) has only a lower control limit at 30% relative to the calibration blank.

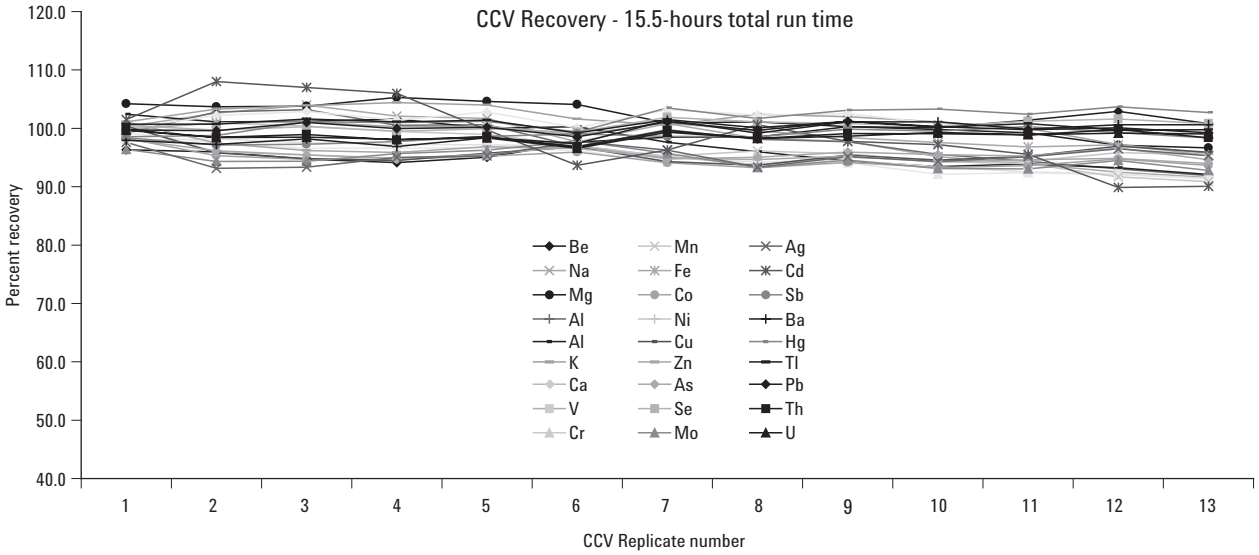

Figure 6. Results of 13 separate analyses of the CCV sample over the 15.5-hour sample sequence. All analyte elements are reported. Acceptable control limits according to US EPA method 6020 ±10%. At no time did any element fall outside the 10% control limits.

Conclusions

The Agilent 7500ce ICP-MS was designed specifically to meet the demanding requirements of environmental laboratories worldwide that must adhere to rigorous regulatory requirements while analyzing a wide range of difficult and unknown sample types with the highest sample throughput. Using ORS technology operating predominantly in He-only collision mode, the 7500ce is easy to set up and operate and delivers unprecedented performance in a wide range of unknown sample types.

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Speciation of Organic Compounds, Using a Newly Developed, Experimental GC-ICP-MS Interface

Application Note

ICP-MS

Environmental

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Introduction

Tin has been an important metal for thousands of years, having been used in the formulation of alloys such as bronze, in mirrors and in the production of glass. More recently, organotin compounds have been used as industrial materials such as stabilizers in polymers. The trialkylated forms are efficient biocides and fungicides and their properties have been used in many applications. For example, triphenyltin (TPhT) has been used as a pesticide and tributyltin (TBT) was used extensively both as a wood preservative and as the active component in marine anti-fouling paints, applied to the hulls of sailing vessels. While organotin compounds degrade rapidly under photolytic conditions, some trialkyltin compounds are persistent once introduced in the environment (e.g. TBT). Despite of the fact that TBT has been banned from use on small boats for over a decade, it is still commonly used on the hulls of large ships, to prevent the growth of marine organisms. In 1989, TBT was banned in all states of the USA on vessels of 25 meters or less in length. Despite a general reduction in the use of organotin compounds, they can accumulate in sediments over many years and can be ingested and absorbed by marine organisms, leading to accumulation in the marine food chain and ultimately presenting a potential threat both to the environment and later to human consumption.

Recent studies provide strong evidence that many organotin compounds can act as endocrine disruptors, even at very low concentrations. Endocrine disruptors interfere with the action of many hormones, and can be very damaging to the development of animal embryos. As a consequence, there is an increasing demand for a new analytical method for these compounds, which is fast, sensitive and offers high chromatographic resolution.

Capillary Gas Chromatography (GC) offers fast and high-resolution speciation, and is well suited for organotin compounds. Measurement limits using currently available GC detectors (FPD, MS and AED) are good, but the need for determination of organotins at ever-lower levels of concentration has fuelled the investigation of alternative detection systems. Further the presence of sulfur compounds in many of the samples requires a highly selective and sensitive detector.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) offers ultra trace detection limits and high selectivity for most elements. The principles of ICP-MS are summarized in Figure 1. Samples are introduced into a high temperature argon plasma, where they are decomposed, atomized and ionized. The resultant ions are transported, through a sampling interface, into a mass spectrometer for measurement. The high temperature in the ICP source means that all forms of an element are decomposed into individual atoms, so ICP-MS results represent total element levels. However, in combination with an on-line separation technique, such as Liquid Chromatography (LC), Ion Chromatography (IC) or Capillary Electrophoresis (CE), ICP-MS is increasingly being used as a sensitive and highly specific detector in a wide variety of speciation applications.

Combining the separation capabilities of a GC with the selectivity and sensitivity of ICP-MS could offer benefits in the measurement of ultra-trace levels of organically bound metals. In this paper, we describe some initial investigations into the coupling of GC to ICP-MS, for the analysis of organotin compounds.

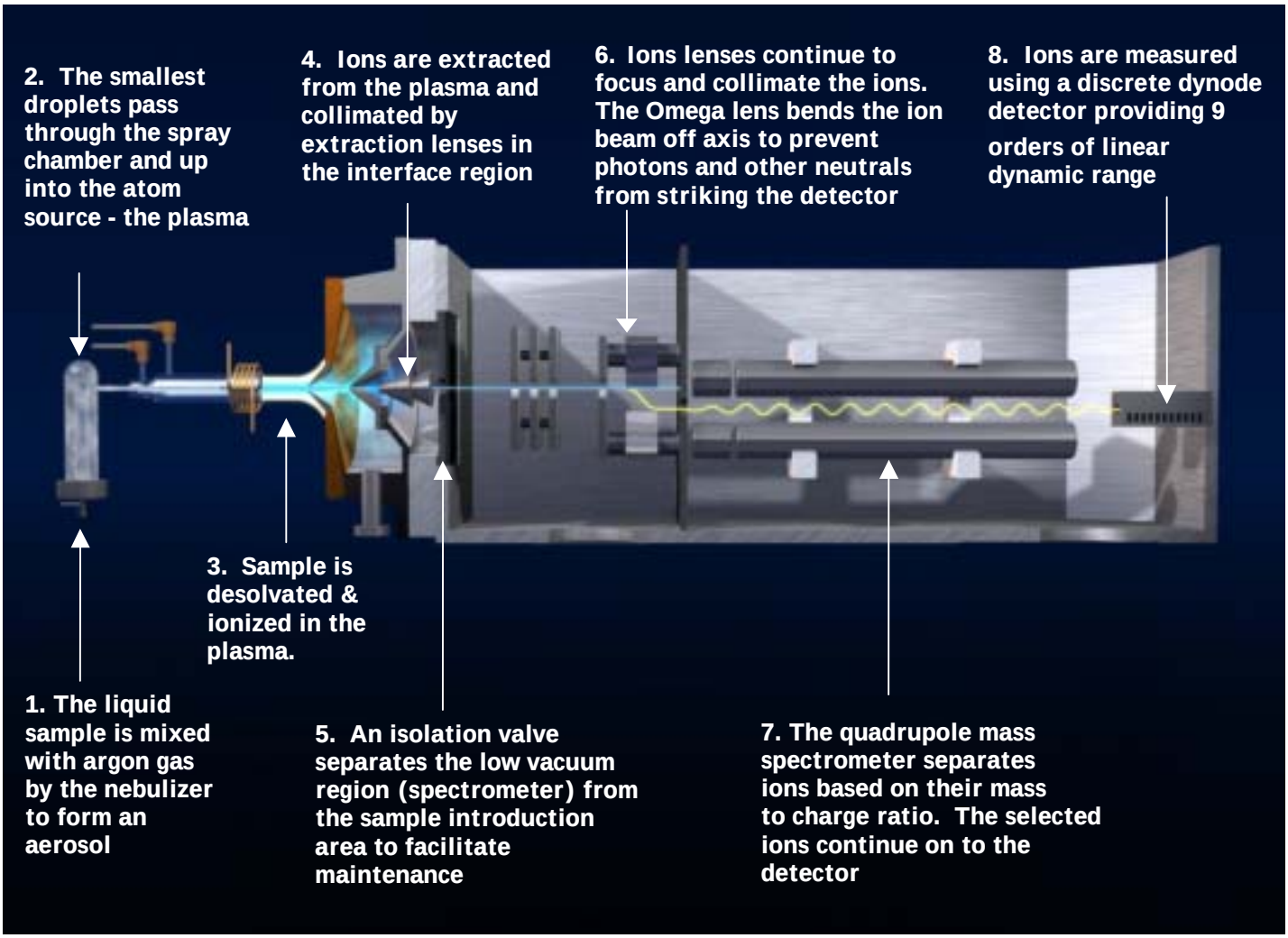

Figure 1. Schematic of an ICP-MS

The interface is not yet commercially available, but it shows some promise. We have used the GC-ICP-MS device to separate and quantify organotin compounds – monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPhT), diphenyltin (DPhT), and triphenyltin (TPhT) - in marine environmental samples of oyster tissue and sediment, collected from the same locality.

Instrumentation

The GC used in these studies was a model 5890, and the ICP-MS was a 4500, both from Agilent Technologies.

The general principal of combining GC with ICP-MS is simple. The end of the capillary GC column is fastened to the base of the ICP torch, so that separated species are carried directly into the plasma by a heated Ar flow.

Using a heated transfer line to connect the GC to the ICP-MS prevents material condensing within the interface and so enables the analysis of high boiling point compounds. Figure 2 is a schematic that describes the interface used during these experiments. Xenon was added to the argon make-up gas as a means of optimizing the ICP-MS operating conditions. The Xe:Ar gas mixture was preheated by passing it through a stainless steel coil mounted within the GC oven.

Initial evaluations of the interface were undertaken at the laboratory of O.F.X. Donard at the University of Pau in France. Figure 3 is a chromatogram of a GC-ICP-MS separation of a 1µL injection of a standard, which contained a mixture of organotin species. Each peak in Figure 3 represents the equivalent of 5 pg tin.

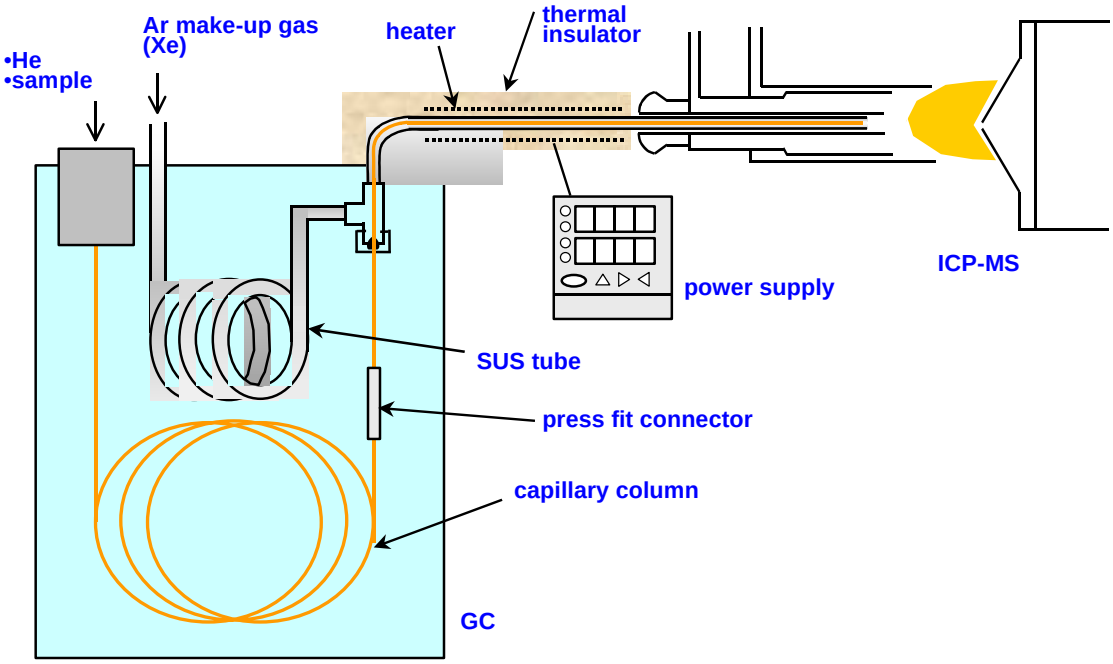

Figure 2. Schematic of the GC-ICP-MS and Interface

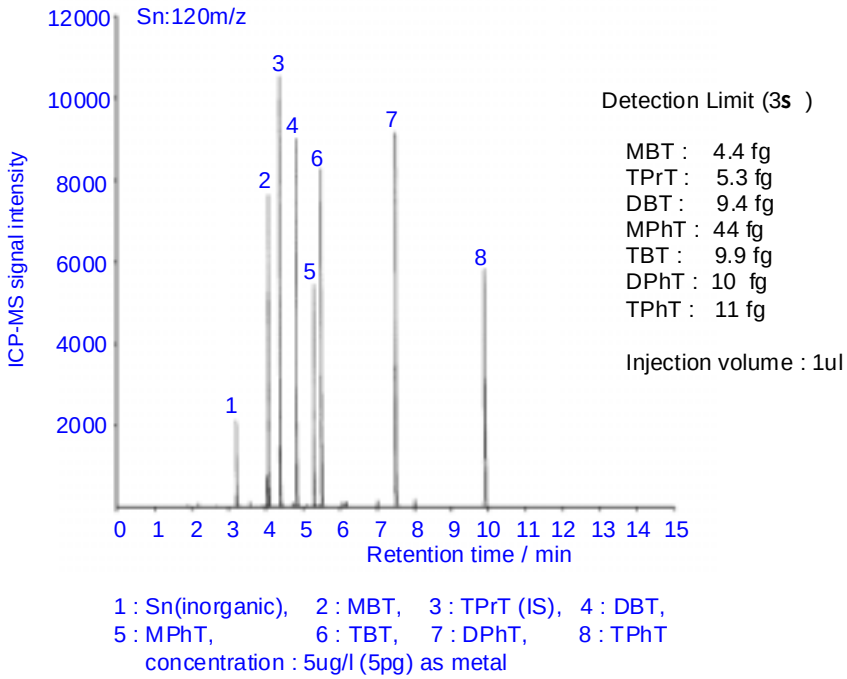

Figure 3. GC-ICP-MS Chromatogram of a 1uL Injection of a 5ppb Organotin Mixed Standard

Table 1 GC-ICP-MS Acquisition Conditions

GC	
Injection mode	Splitless
Injection volume	1uL
Inlet temp	290° C
Column	Non-polar capillary – HP-1 (30m,0.32mm, 0.25um)
Carrier gas	He – 1.0mL/min
Oven program	70°C (1 min):ramp to 190°C (30°C/min): ramp to 270°C (15°C/min)
Interface temperature	250° C
ICP-MS	
RF power	1300W
Sampling depth	8mm
Carrier gas flow	0.8L/min
Oxygen gas flow	0.02L/min (added to auxiliary gas)

Ethylation using sodium tetraethylborate ($NaBEt_4$) was chosen as the derivatization method for this work. The reaction between the organotin compound and the $NaBEt_4$ is in aqueous conditions, which makes it much more suitable for environmental and biological samples than a normal Grignard reagent.

As the chromatogram illustrates, the peak shapes obtained are excellent, suggesting little or no broadening caused by the interface.

Initial results

Preliminary studies on organotin content of oysters and sediments were done in collaboration with the University of Pau . We have taken here samples from the Bay of Arcachon since it is one of the most productive areas for oyster farming. Despite of the fact that organotin concentrations have declined in the water and the sediment of the bay, organotin compounds can be founds in oysters, due to bioaccumulation. In general there is an increasing concern about the occurrence of organotin in shellfish worldwide, particularly as many species are used for human consumption. Samples of oysters and sediment were collected and analysed using the GC with ICP-MS detection.

Some care had to be exercised in the preparation of the oyster tissue, to prevent any potential decomposition of the analyte. The sample preparation method is summarized in Figure 4. Tripropyl tin (TPrT) was added to each sample as an internal standard.

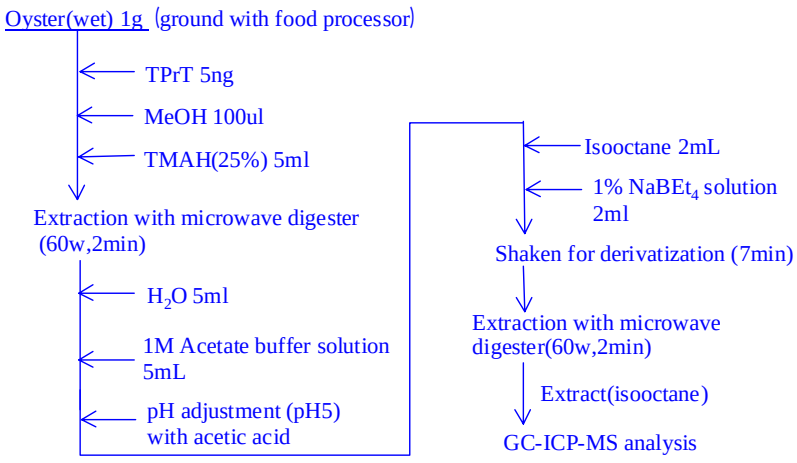

Figure 4. Sample Preparation Steps for the Oyster Tissue

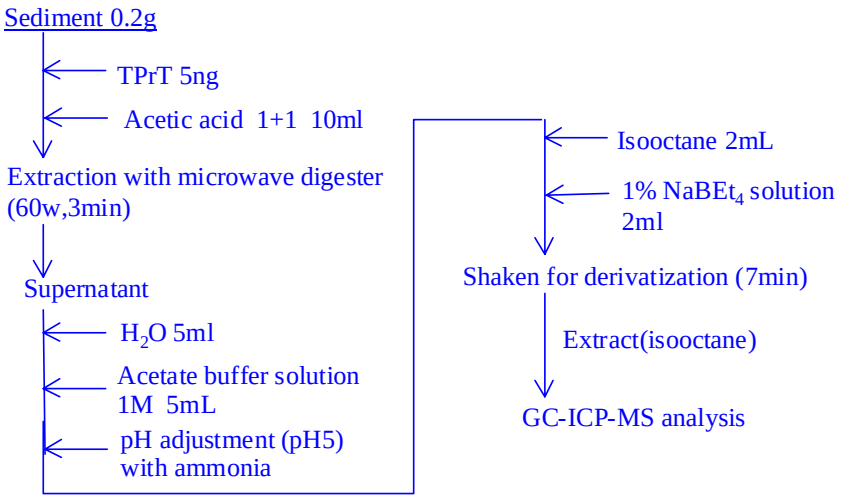

Figure 5. Sample Preparation Steps for the Sediment

A similar procedure was used for the preparation of sediment samples (Figure 5), except that acetic acid was used instead of Tetramethyl ammonium hydroxide (TMAH).

Figure 6 is a typical GC-ICP-MS chromatogram from one of the oyster extracts, illustrating excellent separation and peak shape. As the data show, there are substantial and measurable amounts of a variety of organotin compounds in the sample.

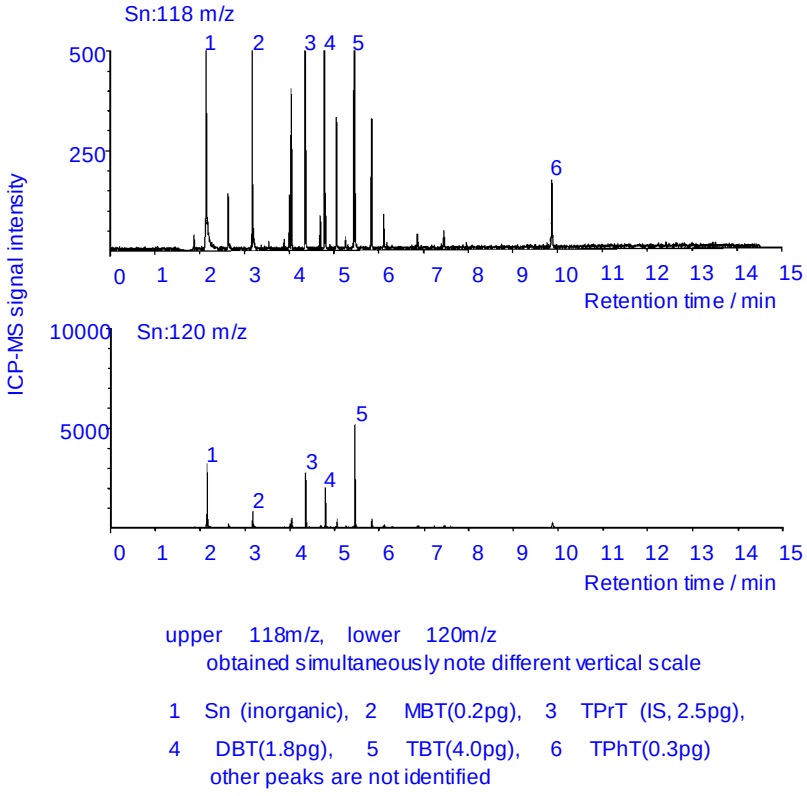

Figure 6. GC-ICP-MS Chromatogram from the Analysis of One of the Oyster Tissue Samples

Table 2. Results Summary from the Analysis of Oyster Samples across Arachon Bay, Plus Analysis of a Sediment Sample

Area	water(%)	concentration / ng/g (Dry)					
		MBT	DBT	TBT	MPhT	DPhT	TPhT
Area1	85	8.3	13	28	0.3	*N.D.*	*N.D.*
Area2	81	0.8	9.5	21	*N.D.*	*N.D.*	1.6
Area3	85	3.7	15	46	1.3	*N.D.*	*N.D.*
Area4	81	1.1	12	42	*N.D.*	*N.D.*	*N.D.*
Area5	86	0.7	11	51	*N.D.*	*N.D.*	2.1
Area6	83	1.8	13	39	*N.D.*	*N.D.*	*N.D.*
Area7	81	0.5	5.3	16	*N.D.*	*N.D.*	1.6
Area8	82	1.7	18	54	*N.D.*	*N.D.*	*N.D.*
Area9	83	2.4	20	141	*N.D.*	*N.D.*	*N.D.*
sediment	36	6.0	5	7	3.0	*N.D.*	2.0

Table 2 summarizes data from oysters sampled in and around Arachon Bay, as well as a sediment sample. Tributyltin (TBT) is the single largest component in each case, although there are several other species present at significant levels. Monobutyltin (MBT) and Dibutyltin (DBT) are breakdown products of TBT. Although the use of TBT in marine antifouling paints has been discontinued in France, the organotin compound still exists in the sediment where the oysters develop. Of particular interest is Area 9, which is a part of the Bay where oyster production has been poor. This also coincides with the largest level of TBT.

The exceptional resolution of the chromatographic separation allows an anticipation of the formation of metabolite products from organotin compounds (e.g. methylation of butyltin compounds) opening the way to new understanding of environmental and biometabolic pathways for these contaminants after further identification.

Summary

These preliminary results suggest that GC-ICP-MS offers a highly sensitive and selective method for the determination of organometallic compounds in environmental matrices The exceptional chromatographic separation capability of the CGC, coupled to the sensitivity, selectivity and multielemental capability of the ICP-MS detector, certainly makes this combination a very promising tool for environmental studies. The interface used in these studies is not yet commercially available and will require some further refinement and characterization. The robustness of the heating system will require some refinement to ensure long-term reliability. Future applications work is under way evaluating the potential of this interface for the simultaneous determination of other organometals such as tin, lead and mercury compounds.

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Separation and Analysis of Toxic Arsenic Species, Using LC-ICP-MS

Application Note

ICP-MS

Environmental

Tetsushi Sakai

Introduction

Arsenic is a common element in the natural environment and in biological materials. It is used for industrial purposes such as in agricultural chemicals, semiconductor materials, industrial gases and so on. Arsenic exists as arsenopyrite in nature. Water from some volcanic hot springs can contain large amounts of arsenic.

Arsenic in environmental water is generally assumed to exist primarily as its anionic forms, such as As(III) or As(V).

Arsenic takes various chemical forms and is known to be "bio-active", which means that it is easily converted from one form to another by biological processes. Of the various forms of As, some are essentially harmless to human life (such as arsenobetaine and arsenocholine), while others, notably the inorganic forms, are not only specified as toxic, but have also been shown to be carcinogenic. Issues relating to As toxicity are of interest all over the world and several million people are affected by arsenic pollution, which has been highlighted in West Bengal in India, Bangladesh and Inner Mongolia.

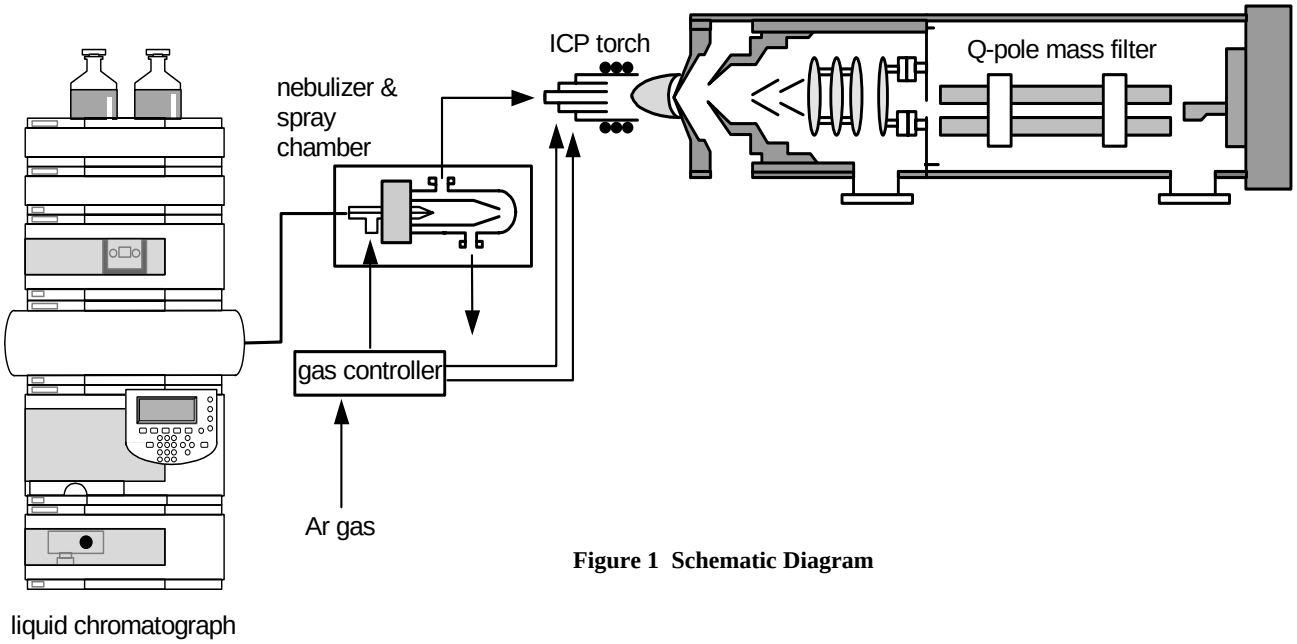

Figure 1 Schematic Diagram

Nowadays, arsenic analysis is required under various laws. According to the World Health Organisation (WHO) drinking water guidelines, Japan's drinking and environmental water quality standards strictly require that the concentration of arsenic should be less than 10 ug/L(ppb) . Ensuring a low total As level will automatically mean that the toxic forms of the element are also low, but separate identification and quantification of the individual forms of As would be of much greater use in assessing the potential toxicity.

Since the toxicity and the metabolism of As alters depending upon its form, evaluation of each chemical form is essential, to correctly measure the potential impact of the arsenic content of environmental, nutritional and other inputs to the human body.

In this application note, a system combining liquid chromatography (LC) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to separate and analyze several arsenic species. This LC-ICP-MS system allows highly sensitive and selective analysis of arsenic species contained in drinking water, river water and many other sample types.

Mechanism and Configuration of LC-ICP-MS System

Generally, there are no cationic organo-arsenic compounds present in either environmental water or drinking water, therefore the target analytes selected for this study were As(III) (which is both toxic and carcinogenic), As(V), dimethylarsinic acid (DMAA) (which functions as a carcinogen promoter) and methylarsonic acid (MMAA).

As only total arsenic concentration is specified in many existing regulations, AAS, ICP-OES or ICP-MS (either alone or in combination with hydride generation) can be used to analyze arsenic in many cases. However, with improved understanding of the potential for toxic effects at very low concentrations, it is becoming increasingly important to acquire information on each different chemical form, to fully understand the potential toxicity.

Separation technique such as LC is generally effective analytical method to separate the individual chemical forms of an element. However, there are some problems in anion exchange chromatography when it is applied to the arsenic speciation analysis in environmental water.

It is not easy to perform rapid, simultaneous analysis of different arsenic compounds because the ionicity varies with each form. For example, As(III) has weak ionicity, and it is difficult to separate from the cationic arsenic compounds, also some species such as As(V) can react with other metallic species forming a precipitate. Due to its reactivity, some arsenic containing samples can react with metallic elements on the column, and the correct results cannot be obtained. Moreover, analysis at ug/L(ppb) level is almost impossible with LC's inadequate sensitivity.

On the other hand, ICP-MS has very high sensitivity and selectivity, making it ideally suited to the analysis of trace elements, albeit with the limitation that it is an elemental analyser and so provides no information on the different forms of an element. Virtually the only potential difficulties in the determination of As by ICP-MS relate to its high first ionisation potential (which reduces the proportion of ions formed and therefore the sensitivity) and the potential overlap on As from $^{40}Ar^{35}Cl$ at mass 75. Both of these potential problems can be reduced or virtually eliminated, through optimisation of the plasma and sample introduction parameters. The potential for suppression effects and interface clogging due to the presence of highly ionic eluent and buffer solutions is also reduced, as the Agilent 7500 is designed to operate under sample introduction conditions which ensure complete matrix decomposition.

The combination of LC and ICP-MS makes use of the best features of each technique, to give efficient separation of the various forms of an element, followed by sensitive and selective detection. The commercial availability of arsenic speciation kits, which contain all the required columns and the LC connection kit, makes this application a routine possibility in high-throughput and commercial laboratories.

One reported limitation of coupled chromatographic techniques relates to the speed of the ICP-MS detector, which is typically operated in "dual-mode", meaning signals are recorded simultaneously in high sensitivity pulse-count and low gain analog modes. In conventional ICP-MS detectors, the response time of the Analog mode is much slower than the pulse-count mode, so measurement speed is compromised when using dual mode. The Agilent 7500, by contrast, features a true simultaneous dual mode detector with a new, high-speed log amplifier, which allows the system to acquire data at the same high speed, whether analyzing in pulse counting mode, analog mode or both. This new detector also covers a linear range of 9 orders of magnitude, making the Agilent 7500 the ultimate tool for time resolved measurements, such as those required for chromatographic analysis.

Method developed with the LC-ICP-MS column and eluent configuration and operating conditions allowed separation and analysis of four arsenic species, As(III), DMAA, MMAA and As(V), in only 10 minutes. On-column loss of As was prevented by the addition of a complexing agent (EDTA) to the eluent. The high sensitivity of the ICP-MS allowed these As species to be determined easily at the ug/L(ppb) levels required under current legislation.

Analysis Example

The operating conditions used for this experiment are shown in Table 1. The data were processed using the ICP-MS Chromatographic Software, which integrates the LC and ICP-MS modules to allow completely automatic acquisition and data calculation from chromatographic measurements, in conjunction with the standard ICP-MS ChemStation.

Figure 2a shows the chromatogram from the measurement of a standard solution which contained 20 ug/L(ppb) of each arsenic species, illustrating the complete separation of the four As species, As(III), DMAA, MMAA, As(V) in only 10 minutes. Figure 2b shows a comparable chromatogram from the analysis of a drinking water sample.

Due to the fact that ozonation or other oxidative methods are frequently used during the treatment of drinking water supplies, the various forms of As present in the source water may be converted to As(V) following treatment, so only this form is found in the final water. Table 2 shows the results of reproducibility tests (n=6) of these four species under the same column and analytical conditions. All species showed excellent limits of detection (3 sigma) in the region of 0.1 ug/L(ppb) and the reproducibility (RSD%) at 10 ug/L(ppb) was less than 2%.

Figure 3 shows 5-point calibration curves within the concentration range from 1 and 100 ug/L(ppb) for each of the four As species studied, As(III), DMAA, MMAA and As(V). The results demonstrate exceptional linearity with correlation coefficients (R2) better than 0.9997 for all four species.

Table 1 Operating Conditions

LC

LC	Agilent 1100 Series
Column	Anion exchange columns (G3154A/101, G3154A/102))
Mobile phase	2.0 mM PBS/0.2 mM EDTA solution
Flow rate	1.0 mL/min
Column temperature	Ambient
Injection volume	0.05 mL
Run time	10 min (600 sec)
Number of injection	1

ICP-MS

ICP-MS	Agilent 7500
RF power	1.4 kW
Plasma gas	15 L/min
Aux. gas	1.0 L/min
Carrier gas	1.1 L/min
Sampling depth	7.5 mm
Acquired mass	75
Points/mass	1
Dwell time	0.5 sec/mass

Conclusion

Recently, anionic arsenic compounds in environmental water have received widespread attention due to their potential toxicity to humans. They can be analyzed quickly and precisely at the low concentrations required under current legislation, using an optimized coupled technique consisting of the Agilent 1100 LC, coupled to the Agilent 7500 ICP-MS system. The compatibility and automation of this coupled system means that LC-ICP-MS can be considered a routine, high sample throughput method for monitoring levels of potentially toxic arsenic species in environmental and nutritional samples.

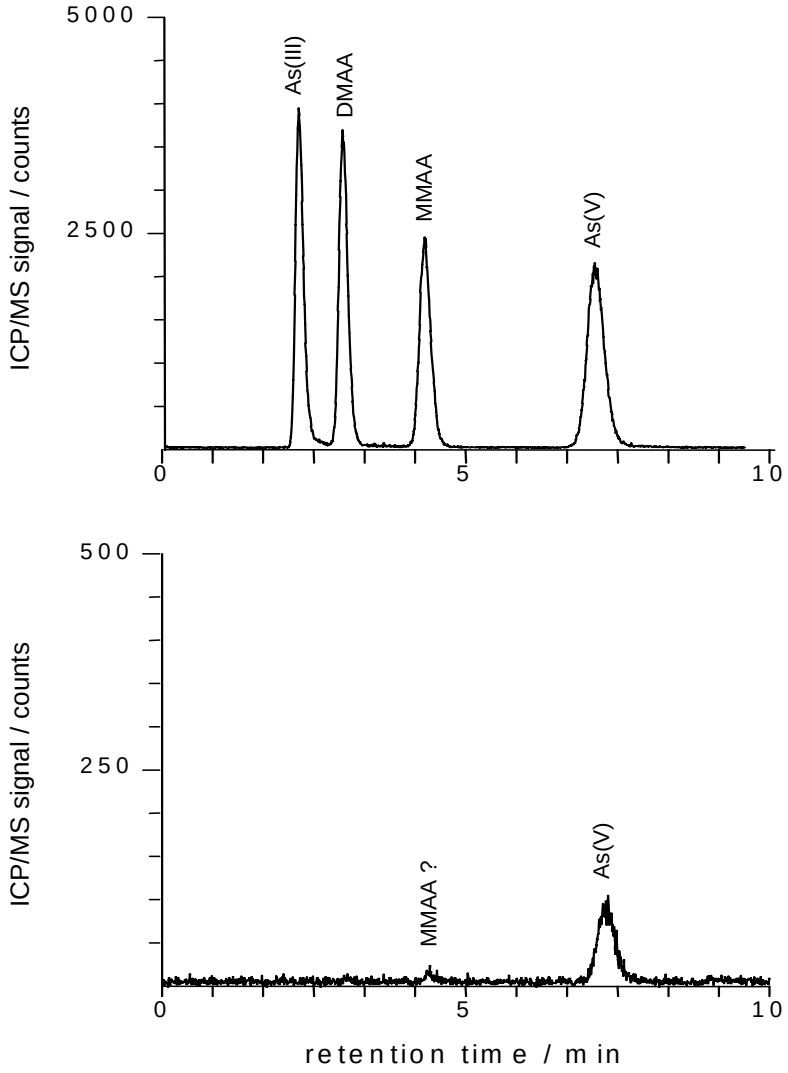

Figure 2 Chromatograms of As Species
a) 10 ug/L(ppb) As species mixed standard solution
b) drinking water

Table 2 Repeatability and Detection Limits

	Repeatability (n=6)	DL (ug/L)
As(III)	2.0%	0.1
DMAA	1.5%	0.1
MMAA	1.3%	0.1
As(V)	1.6%	0.2

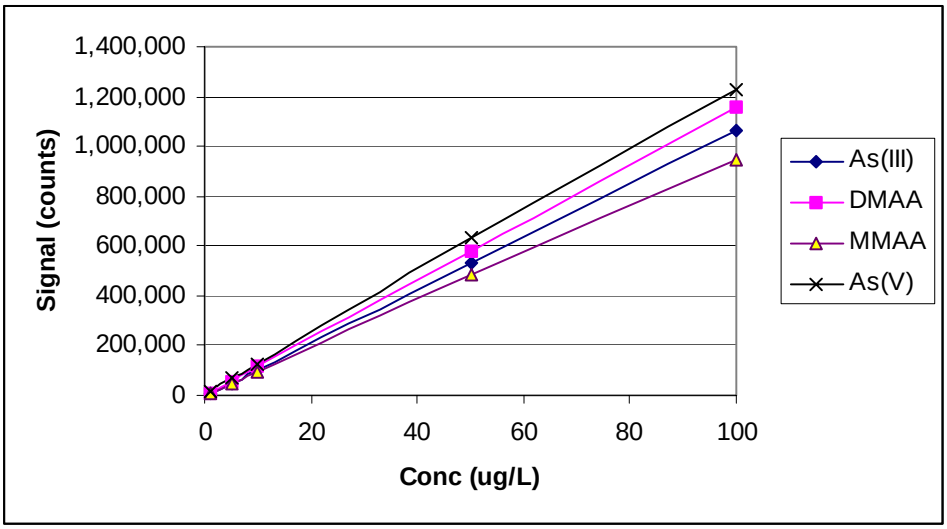

Figure 3 Calibration Curves for As Species

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Improving the Analysis of Organotin Compounds Using Retention Time Locked Methods and Retention Time Databases

Application

Environmental

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Abstract

The analysis of organotin compounds is becoming increasingly important in both environmental analysis and in food and consumer product analysis. This application note describes a retention time locked (RTL) gas chromatography/mass spectrometry (GC/MS) method for the analysis of derivatized organotin compounds. Three retention time locked libraries are made available, corresponding to three different derivatization methods. The retention time databases allow easy peak location and identification of the target solutes based on mass spectra and retention times.

Introduction

For many years, organometal speciation has been an important topic in environmental analysis, primarily due to increasing awareness of the toxicological effects of many organometal compounds. Within the class of organometalics, organotin compounds are probably the most widely spread in the environment due to their use as additives in polymers and in antifouling paints. Organotin compounds degrade in the environment into more polar metabolites [1]. Tributyltin, one of the most frequently used organotin additives (as tributyltinchloride or tributyltinoxide), for instance, degrades into dibutyltin and monobutyltin species. Consequently, a large diversity of organotin compounds can be detected in various environmental samples [2]. More recently, organotin contamination of diapers and printed T-shirts was reported and numerous analyses were performed on different consumer products, including all types of absorbent hygiene products.

Different methods were used for the extraction and analysis of organotin compounds in environmental, food, and consumer product matrices. Since the organotin compounds with less than four alkyl groups are very polar, they cannot be analyzed directly by GC and must be derivatized into tetraalkyltin compounds prior to analysis. Initially, most methods were based on extraction with

tropolone (a complexing agent) and n-hexane, followed by Grignard derivatization and determination with GC-flame photometric detection (FPD) [3–9]. Recently, in-situ ethylation with sodium tetraethylborate ($NaBEt_4$) [10–13] has largely replaced Grignard derivatization. At the same time, mass selective detectors (MSD) and atomic emission detectors (AED) have replaced the FPD as the preferred GC detector for organotin compounds [11,13].

A few years ago, solid phase micro extraction (SPME) in combination with capillary gas chromatography-inductively coupled plasma mass spectrometry (CGC-ICP-MS) was used for the determination of volatile and semi-volatile organometal compounds, resulting in excellent sensitivity and selectivity [14,15]. SPME was performed in the headspace or directly in the aqueous sample using a 100 mm polydimethylsiloxane (PDMS) coated fiber. Using $NaBEt_4$, organotin compounds could be derivatized in-situ and simultaneously extracted into the PDMS phase.

More recently, stir bar sorptive extraction (SBSE) using a magnetic stir bar coated with a 0.5–1 mm PDMS layer was developed [16]. After extraction, the solutes were thermally desorbed online to GC/MS, GC-AED or GC-ICP-MS. SBSE in combination with CGC-ICP-MS was applied for the determination of organotins in environmental samples after in-situ derivatization with $NaBEt_4$, resulting in unsurpassed sensitivity with detection limits reaching the ppq (pg/L) level [17].

For standard applications such as the determination of organotin compounds in sediments, or soils, and in extracts or leachates of consumer products, these extremely high sensitivities are not required. For these applications, sufficient sensitivity is obtained using mass spectrometric detection. In comparison to AED or ICP-MS, where specific tin-chromatograms are obtained, the chromatograms obtained by mass spectroscopy are far more complex, even when using the selected ion monitoring (SIM) mode. Several ions per solute need to be monitored, and the derivatized sample extracts often contain many co-extracted solutes or by-products of the derivatization reaction. Therefore, data interpretation is more demanding requiring the use of extracted ion chromatograms, retention time matching, and calculation of the relative abundances of target and qualifier ions. In this respect, the use of retention time locked methods offers several advantages. If a selected ion method is used, the switching times between groups of monitored ions are fixed and do not need to be adjusted after column maintenance or column change, since the retention times of all solutes can be relocked. Moreover, quantification databases do not need to be updated for variations in retention times. Finally, a retention time locked database can be used, allowing easy peak allocation. Solute detection and confirmation are far more reliable using the results screener option [18,19], which combines the power of spectral matching with locked retention time matching.

In this application note, a GC/MS method is described for the analysis of organotin compounds in environmental, food, or consumer product extracts. Since derivatization by Grignard reaction and derivatization using $NaBEt_4$ are both easy and convenient, three types of derivatives are considered: methyl-derivatives using methylmagnesium bromide, pentyl- derivatives using pentylmagnesium bromide (both Grignard reagents), and ethyl-derivatives using $NaBEt_4$. The most important organotin compounds are listed in Table 1 together with typical ions for the mass spectra of all three derivatives. Tin has several isotopes and the mass spectra are characterized by typical isotope clusters. The relative abundances of the tin isotopes are Sn-116 (14.24%), Sn-117 (7.57%), Sn-118 (24.01%), Sn-119 (8.59%), Sn-120 (32.97%), Sn-122 (4.71%), and Sn-124 (5.98%). For the organotin compounds listed in Table 1, mass spectral libraries and retention-time-locked screener libraries were created for all three types of derivatives. After selecting the appropriate derivitization method, a library and screener database can be selected, allowing fast data interpretation. Sample extraction and clean-up are beyond the scope of this application note.

Experimental

Samples

The organotin compounds listed in Table 1 were purchased from Dr Ehrenstorfer, Augsburg, Germany (http://www.analytical-standards.com). For analysis, the standards were dissolved in methanol at a 1000 ppm (1mg/mL) concentration. These solutions were further diluted, depending on the derivatization method used. For creation of the databases, approximately 10 µg of compound was derivatized, resulting in a final concentration of 10 ppm.

Derivatization method 1: The sample extract is concentrated to 1 mL in an apolar solvent (typically hexane) in a reaction tube. To this solution, 0.5 mL methylmagnesiumbromide Grignard reagent (1.4 M in 75/25 toluene/THF, Sigma-Aldrich cat no 28,223-5) is added. The solution is vortexed for 10 s and allowed to stand at room temperature for 15 min. This procedure should be performed in a fume hood, since toxic vapors evolving from the reaction and the solvents are flammable. The reaction is stopped and the excess reagent is removed by adding 2 mL of a saturated ammoniumchloride solution in water or 2 mL 0.25 mol/L aqueous sulphuric acid. The mixture is vortexed for 10 s and the two phases are allowed to separate. The clear upper layer (apolar hexane phase) is transferred to an autosampler vial for analysis. The resulting organotin compounds are the methyl-derivatives.

Derivatization method 2: The sample extract is concentrated to 1 mL in an apolar solvent (typically hexane) in a reaction tube. To this solution, 0.5 mL pentylmagnesiumbromide Grignard reagent (2 M in diethylether, Sigma-Aldrich cat no 29,099-8) is added. The remaining steps in this procedure are identical to those used in derivitization method 1. The resulting organotin compounds are the pentyl-derivatives.

Derivatization method 3: The sample extract is concentrated to 1 mL in a polar solvent (typically ethanol) in a reaction tube. To this solution, 1 mL acetate buffer (82 g/L sodium acetate in water, adjusted to pH 4.5 with acetic acid) and 50 µL

Table 1: Organotin Compounds and Characteristic Ions for the Three Derivatization Products

Organotin solute Reagent Derivatives	Abbreviation	Derivatization 1 Methyl- magnesium bromide Methyl-	Derivatization 2 Pentyl- magnesium bromide Pentyl-	Derivatization 1 Sodium tetraethylborate Ethyl-
Triethyltin	TET	193, 191, 165, 163	179, 177, 249, 247	207, 205, 179, 177
Tetraethyltin	TeET	207, 205, 179, 177	207, 205, 179, 177	207, 205, 179, 177
Tripropyltin	TPT	179, 177, 221, 219	277, 275, 165, 163	235, 2331, 249, 247
Tetrapropyltin	TePT	249, 247, 207, 205	249, 247, 207, 205	249, 247, 207, 205
Monobutyltin	MBT	165, 163, 151, 149	319, 317, 193, 191	235, 233, 179, 177
Dibutyltin	DBT	151, 149, 207, 205	319, 317, 179, 177	263, 261, 207, 205
Tributyltin	TBT	193, 191, 249, 247	305, 303, 179, 177	291, 289, 207, 205
Tetrabutyltin	TeBT	291, 289, 179, 177	291, 289, 179, 177	291, 289, 179, 177
Monophenyltin	MPhT	227, 225, 223, 197	339, 337, 197, 195	255, 253, 197, 195
Diphenyltin	DPhT	289, 287, 285, 197	345, 343, 197, 195	303, 301, 197, 195
Triphenyltin	TPhT	351, 349, 347, 197	351, 349, 347, 197	351, 349, 347, 197
Tetraphenyltin	TePhT	351, 349, 347, 197	351, 349, 347, 197	351, 349, 347, 197
Tricyclohexyltin (Cyhexatin)	TCT	301, 299, 219, 217	357, 355, 205, 203	315, 313, 233, 231
Monooctyltin	MOT	165, 163, 263, 261	375, 373, 193, 191	291, 289, 179, 177
Dioctyltin	DOT	263, 261, 151, 149	417, 415, 375, 373	375, 373, 263, 261

derivatization reagent are added. The derivatization reagent is prepared by dissolving 2 g $NaBEt_4$ (Sigma-Aldrich cat no 48,148-3) in 10 mL ethanol. This solution should be freshly prepared. The sample is shaken and allowed to react for 30 min. After addition of 5 mL water, the derivatized compounds are extracted in 1 mL hexane. The mixture is vortexed for 10 s and the two phases are allowed to separate. The clear upper layer (apolar hexane phase) is transferred to an autosampler vial for analysis. The resulting organotin compounds are the ethyl-derivatives.

These derivatization methods can be adapted to the type of sample analyzed. For example, derivatization method 3 is often applied to aqueous samples directly, combining *in-situ* derivatization and simultaneous extraction. This method is also used for sediment samples. Typically 1 g sample (dry weight) is extracted with 10 mL acetate buffer, 7 mL methanol and 10 mL hexane. Four mL of a 5% $NaBEt_4$ solution is added while stirring. The derivatized organotin compounds are simultaneously extracted into the hexane layer.

Analytical Conditions

All analyses were performed on an Agilent 6890-5973N GC-MSD system. Automated splitless injection was performed using an Agilent 7683 automatic liquid sampler. The instrumental configuration and analytical conditions are summarized in Table 2. The retention time of tetrabutyltin (used as the locking standard) was locked at 16.000 min. To duplicate this method, the initial column head pressure can be set to the pressures indicated in Table 2 (nominal pressure). Then the retention time locking (RTL) calibration runs can be performed automatically (at –20%, –10%, +10% and +20% of the nominal pressure) [18]. The retention time versus head pressure curve is then calculated and stored in the method. Agilent's RTL software uses this curve to set the column head pressure so that retention time of the locking standard (tetrabutyltin) is 16.000 min.

Table 2. Instrumentation and Conditions of Analysis

Instrumentation	
Chromatographic system	Agilent 6890 GC
Inlet	Split/Splitless
Detector	Agilent 5973 N MSD
Automatic sampler	Agilent 7683
Liner	Splitless liner (part number 5062-3587)
Column	30 m × 0.25 mm id × 0.25 µm HP-5MS (Agilent part number 19091S-433)
Experimental conditions	
Inlet temperature	280 °C
Injection volume	1 µL
Injection mode	Splitless, purge time: 1 min, purge flow: 50 mL/min.
Carrier gas	Helium
Head pressure	Tetrabutyltin is retention time locked at 16.000 min (pressure around 45 kPa at 50 °C, 34 cm/s at 50 °C)
Oven temperature	50 °C, 1 min, 10 °C/min to 300 °C, 4 min.
Transfer line temperature	300 °C
Detector	Scan (40–550 amu), threshold 100, MS quad 150 °C, MS source 230 °C. Solvent delay: 4 min SIM mode: 50 ms dwell time per ion, ions listed in Table 3

Results and Discussion

A typical chromatogram, for an organotin standard mixture, derivatized using method 3 (ethyl-derivatives with $NaBEt_4$), is shown in Figure 1. The compounds elute according to their boiling point, and the elution sequence can be predicted by calculating the total number of carbon atoms after derivatization. With this derivatization, the elution sequence of the butyltin compounds is MBT (10 C atoms) < DBT (12 C atoms) < TBT (14 C atoms) < TeBT (16 C atoms). The spectrum obtained for tributyltin (as tributylethyltin) is shown in Figure 2. The typical ion clusters, resulting from the different tin isotopes, are clearly detected.

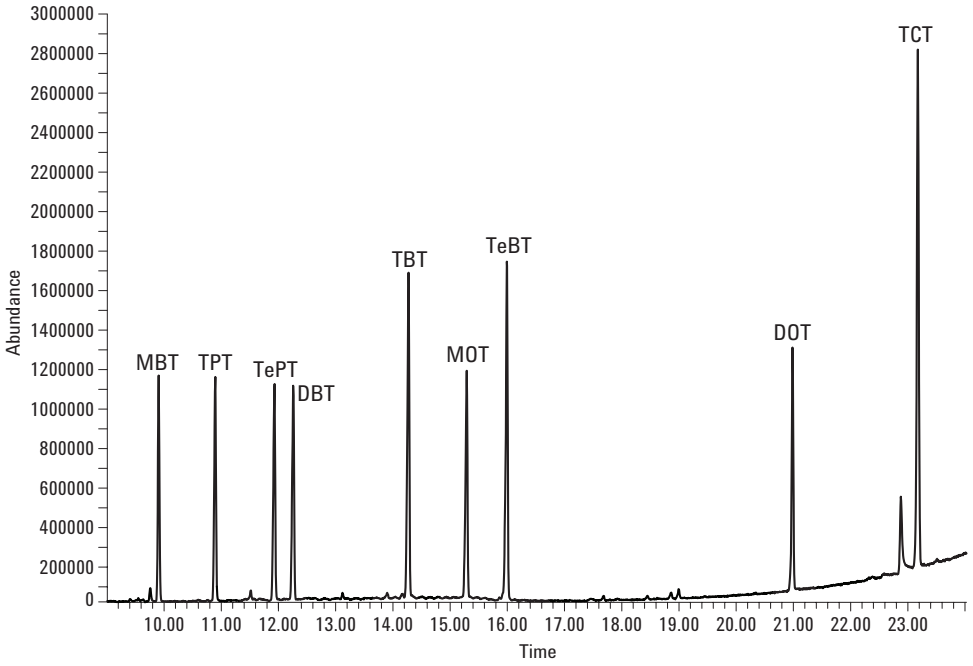


Figure 1. GC/MS chromatogram for the analysis of an organotin standard mixture after derivatization with $NaBEt_4$ (ethyl-derivatives).

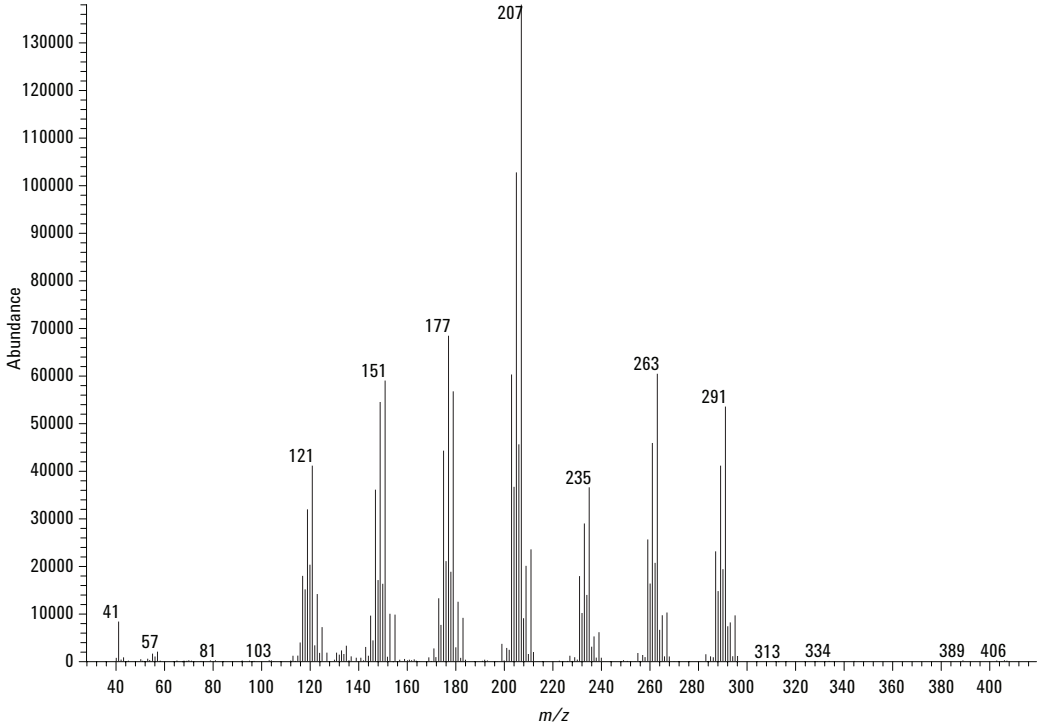


Figure 2. Mass spectrum of tributyltin after derivatization with $NaBEt_4$ (ethyl-derivative).

The analysis of a coastal sediment sample is shown in Figure 3. In this case, derivatization method 2 (Grignard reaction with pentylmagnesium bromide) was applied and a complex chromatogram was obtained. Using the extracted ion chromatogram at *m/e* 179 the butyltin compounds were easily detected (Figure 4). Tetrabutyltin, eluting at 16.000 min, was added as internal standard. In this case, pentyl- derivatives are analyzed. Therefore the elution order is reversed since the derivatization adds a C5-group for every free valency. The elution sequence is now TeBT (16 C atoms = unchanged) < TBT (17 C atoms) <DBT (18 C atoms) < MBT (19 C atoms). The mass spectrum obtained for the pentyl derivative of tributyltin is shown in Figure 5.

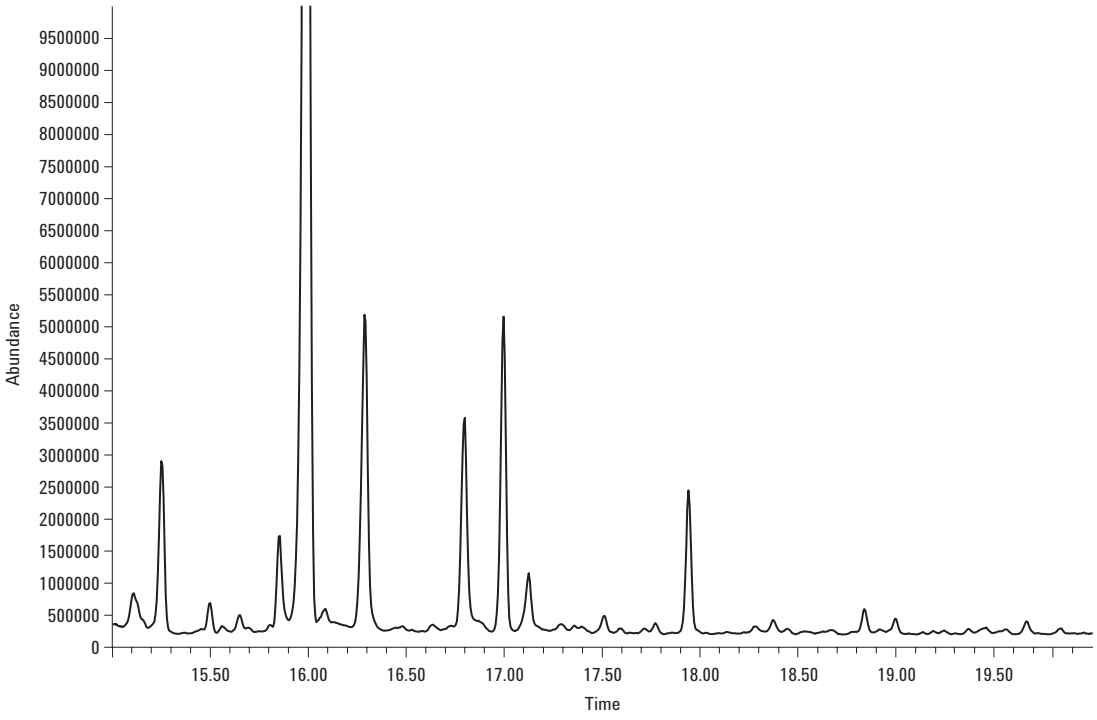


Figure 3. GC/MS chromatogram for the analysis of a coastal sediment sample after derivatization with pentylmagnesium bromide (pentyl-derivatives).

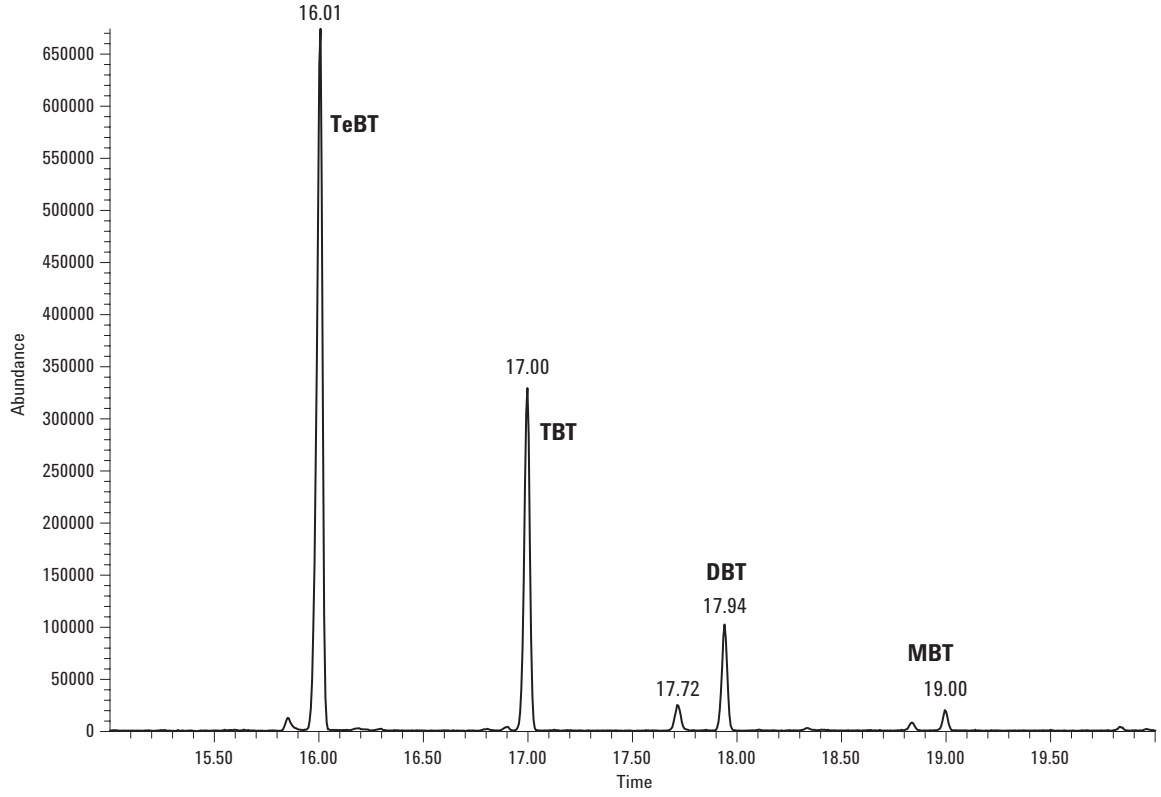


Figure 4. Extracted ion chromatogram showing the presence of butyltin compounds in the coastal sediment sample extract(shown in Figure 3) after derivatization with pentylmagnesium bromide (pentyl-derivatives).

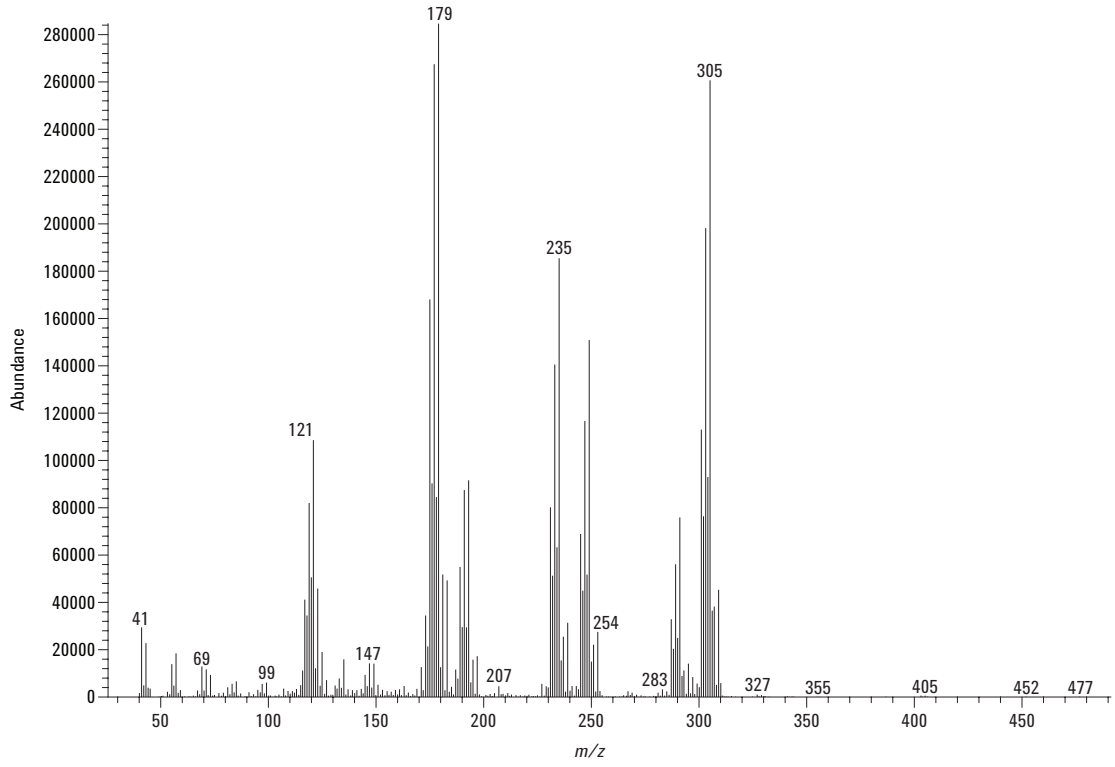
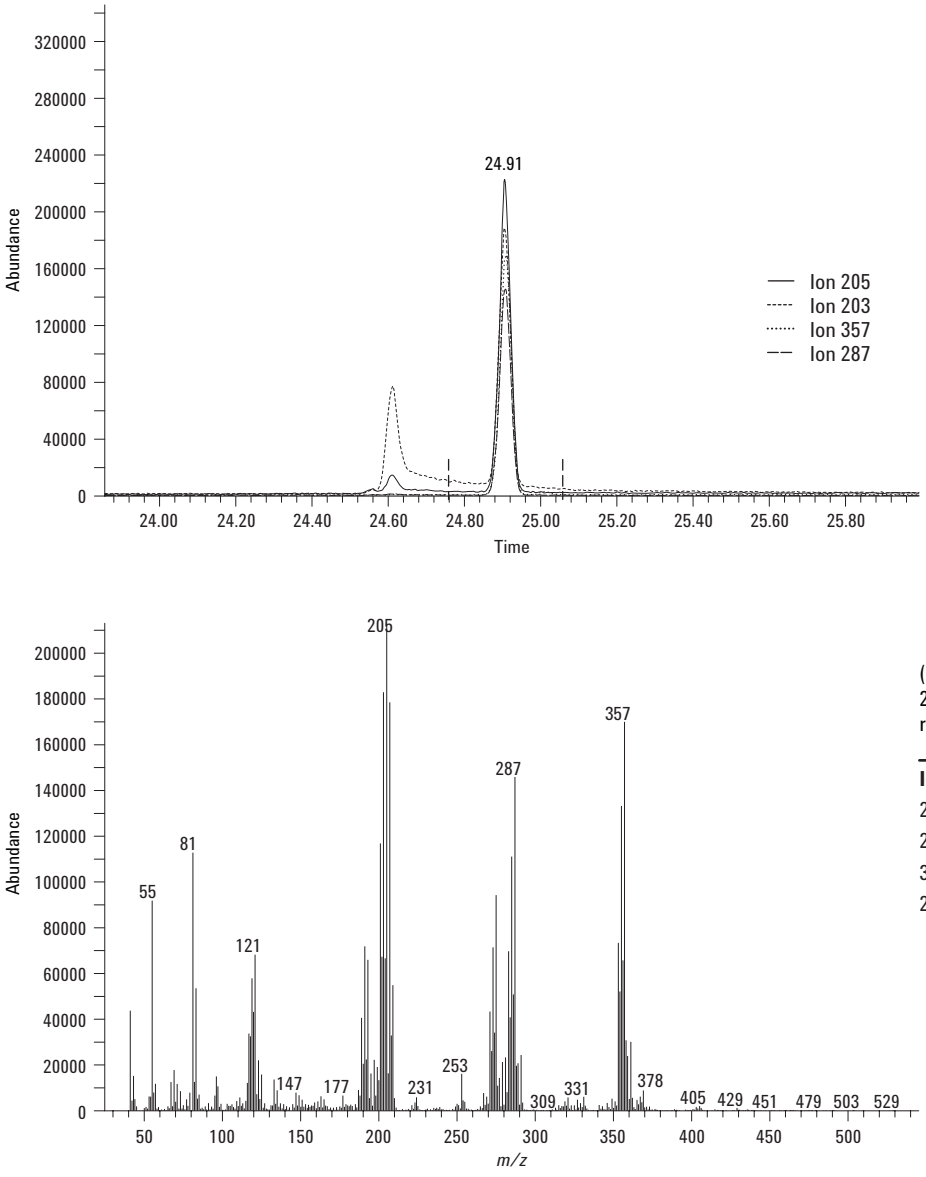


Figure 5. Mass spectrum of tributyltin after derivatization with pentylmagnesium bromide (pentyl-derivative).

Using the Agilent results screener and the appropriate screener library, the files can be screened for the presence of all compounds listed in the screener database. Figure 6 shows a typical result, with the identification of pentyltricyclohexyltin at 24.908 min. The target ions for this compound are extracted and overlaid in the top window. For easy comparison, the apex mass spectrum is displayed. Though not shown in Figure 6, the Agilent RTL Screener Software can display the library and apex spectra together for easy spectral comparison. In addition, the relative abundances of the target ion and qualifiers are measured and compared to the library data. What distinguishes the Agilent screener methods from conventional GC/MS techniques is the comparison of a peak's locked retention time to values stored with the RTL database. In this case, the locked retention time of pentyltributyltin is within 0.002 min (0.12 s) of the database value. The Agilent results screener compares locked retention times and spectral information for fast peak allocation and more reliable identification.



(13) Pentyltricyclohexyltin
24.908 min (-0.002)
response 4581312

Ion	Exp %	Act %
205.00	100	100
203.00	82.90	83.87
357.00	77.20	80.36
287.00	66.70	68.98

Figure 6. Screener result for the detection of tricyclohexyl tin in a sample extract after derivatization with pentylmagnesium bromide (pentyl-derivative).

For added specificity and sensitivity, SIM methods were developed for all three alkyl derivatives of the target tin compounds. Table 3 lists the SIM ions and target compounds in each group. Note that the start time for each SIM group is also listed. Normally, this timing could not be published with confidence, because of retention time differences between instruments. However, RTL allows analysts to duplicate locked methods directly and reproduce all analyte retention times within a few thousandths of a minute. Thus, it is possible to apply this method directly, including the SIM group timing, after locking tetrabutyltin to the method-specified retention time of 16.000 minutes.

Table 3. SIM Groups and Timing for Methyl, Pentyl, and Ethyl Derivatives of the Target Tin Compounds Listed in Table 1. The GC/MS Method Shown in Table 2 was used with the Retention Time of Tetrabutyltin Locked to 16.000 Minutes.

	Start time (min)	Solutes	Ions
Derivatization 1			
1	5.00	TET, MBT	193, 191, 165, 163, 151, 149
2	6.50	TeET	207, 205, 179, 177
3	8.00	MPhT, DBT, TPT	227, 225, 223, 151, 149, 207, 205, 179, 177, 221, 219
4	10.50	MOT, TePT	165, 163, 263, 261, 249, 247, 207, 205
5	12.50	TBT, TeBT	193, 191, 249, 247, 291, 289, 179, 177
6	16.40	DPhT, DOT	289, 287, 285, 197, 263, 261, 151, 149
7	21.00	TCT, TPhT	301, 299, 219, 217, 351, 349, 347, 197
8	25.00	TePhT	351, 349, 347, 197
Derivatization 2			
1	5.00	TeET	207, 205, 179, 177
2	9.00	TET, TePT	179, 177, 249, 247, 207, 205
3	13.50	TPT, TeBT	277, 275, 165, 163, 291, 289, 179, 177
4	16.50	TBT, DBT, MBT	305, 303, 179, 177, 319, 317, 193, 191
5	20.00	MPhT, MOT, DPhT	339, 337, 197, 195, 375, 373, 193, 191
6	22.80	DPhT, TCT	345, 343, 275, 273, 357, 355, 205, 203
7	24.00	DOT, TPhT	417, 415, 375, 373, 351, 349, 347, 197
8	26.00	TePhT	351, 349, 347, 197
Derivatization 3			
1	5.00	TeET (=TET)	207, 205, 179, 177
2	8.50	MBT, TPT	235, 233, 179, 177, 249, 247
3	11.40	TePT, DBT	249, 247, 207, 205, 263, 261, 207, 205
4	13.00	MPhT, TBT	255, 253, 197, 195, 291, 289
5	14.80	MOT, TeBT	291, 289, 179, 177, 291, 289
6	17.00	DPhT, DOT	303, 301, 197, 195, 375, 373, 263, 261
7	22.00	TPhT, TCT	351, 349, 347, 197, 315, 313, 233, 231
8	25.00	TePhT	351, 349, 347, 197

Conclusion

A GC/MS method is presented for the analysis of organotin compounds in extracts of environmental, food, or consumer product samples. Three different derivatization methods are described. For each derivatization method, mass spectral and retention time-locked screener databases were created. By itself, RTL is a valuable tool for maintaining GC and GC/MS methods and for comparing results among different laboratories. It also allows analysts to duplicate methods exactly, including SIM group timing and peak timing in quantitative methods.

When combining RTL with locked mass spectral database searching, peak identifications become far more convenient and reliable. While many compounds can have similar spectra, they usually do not have similar spectra and identical retention times. Agilent's ability to reproduce retention times for a given method on any 6890 GC makes it possible to differentiate closely-related compounds and to screen for large numbers of analytes in a matter of seconds. This rapid GC/MS screening technique is now available for a wide variety of important tin compounds.

The three organotin databases are available for free from the Life Sciences and Chemical Analysis portion of the Agilent web site (www.agilent.com).

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Meeting Worldwide Regulatory Requirements for the Analysis of Trace Metals in Drinking Water Using the Agilent 7500c ICP-MS

Application

Environmental

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Abstract

The Agilent 7500c ICP-MS can be used to meet the regulatory requirements for trace metals in drinking water around the world. Elements previously relegated to other techniques, such as GFAA or ICP-OES due to very high or low concentrations or the presence of interferences, can now be measured in a single analysis.

Introduction

Virtually all developed countries have adopted programs and regulations to monitor and maintain the quality of public water systems. In the US, water quality is regulated by the United States Environmental Protection Agency (USEPA) as mandated by the *Safe Drinking Water Act of 1974*. In the European Union, drinking water is regulated by the *Council Directive 98/83/EC of 3, November, 1998 on the Quality of Water Intended for Human Consumption*. In Japan, quality of drinking water is regulated by the *Japan Water Supply Act*, dating from 1957, and most recently updated in 2001. Most of the rest of the world's developed countries have adopted drinking water quality standards based on World Health Organization (WHO) Standards, *Guidelines for Drinking Water Quality, 1996, 1998*, or on the USEPA standards. While these guidelines, as they pertain to trace metals, vary somewhat in their lists of regulated metals and concentrations, they are fundamentally similar. They all require accurate, precise measurement of multiple toxic metals in drinking waters at the lowest practical limits of quantification. This application note will demonstrate that the sensitivity, accuracy, and precision requirements for the analysis of trace metals in drinking water worldwide can be met by a single, robust technique using the Agilent 7500c ICP-MS system with Octopole Reaction System (ORS) technology.

US Regulations

In the US, the quality of public drinking water is safeguarded by the provisions of the Safe Drinking Water Act of 1974.

The Safe Drinking Water Act (SDWA) was originally passed by Congress to protect public health by regulating the nation's public drinking water supply. The law, amended in 1986 and 1996, requires many actions to protect drinking water and its sources in rivers, lakes, reservoirs, springs, and ground water wells (SDWA does not regulate private wells, which serve fewer than 25 individuals). SDWA authorizes the USEPA to set primary national health-based standards for drinking water to protect against both naturally-occurring and man-made contaminants that may be found in drinking water. These primary national drinking water standards include maximum contaminant level goals (MCLGs), levels below which there is no known or expected health risk. From these MCLG values, EPA determines maximum contaminant levels (MCLs), which are

enforceable levels that may not be exceeded. The MCLs are set as closely as possible to the MCLGs and are based on best available current technology and economic feasibility. These limits are reviewed and updated periodically as new information becomes available and technology improves.

Japanese Regulations

Drinking water quality in Japan is regulated by the Japan Water Supply Act, which was first promulgated in 1957 with the Quality Standard for Drinking Water set the following year. This standard currently regulates the drinking water quality of more than 97% of the population. The Quality Standard sets maximum allowable concentrations (MAC) for 17 metals. It also requires that quantification limits be set at 1/10 of the MAC to assure accurate measurements at trace levels. Because of this, in 2001 the Drinking Water Test Method was revised and expanded to include the use of ICP-MS for 14 of the 17 metals. The approval of the use of ICP-MS has eliminated the need for costly and time consuming preconcentration, which was required to meet the required detection limits using ICP-OES.

European Union Regulations

Currently, water quality in the European Union (EU) is regulated by Council Directive 80/778/EEC. This directive applies to all waters intended for human consumption, except natural mineral waters or waters which are medicinal products. As of December 2003, Directive 80/778/EEC will be repealed and replaced by Council Directive 98/83/EC *Directive on the Quality of Water Intended for Human Consumption,* which came into force on December 25, 1998. The standards are based largely on recommendations by the WHO[1]. Member states of the European Community, while they must comply fully, are permitted to implement regulation and enforcement locally. As a result, no single regulation exists for the analysis of trace metals in water throughout Europe.

[1]World Health Organization Guidelines and International Standards for Drinking-Water Quality, 1998

Table 1. Drinking Water Standards for Trace Metal Content from WHO Recommendations, EU Regulations, Japan Drinking Water Regulations and USEPA.

Analyte	WHO Standard (mg/L)	EC Directive 98/83/EC (mg/L)	Japan Drinking Water Standard (mg/L)	USEPA Primary MCL (mg/L)	Agilent 7500c MDLs*** (mg/L)
Aluminum (Al)		0.2	0.2	0.02-0.2*	0.000054
Antimony (Sb)	0.005**	.005	0.002	0.006	0.000035
Arsenic (As)	0.01**	.01	0.01	0.01	0.000052
Barium (Ba)	0.7			2	0.000027
Beryllium (Be)				0.004	0.000028
Boron (B)	0.5**	1	1.0		
Cadmium (Cd)	0.003	0.005	0.01	0.005	0.000025
Chromium (Cr)	0.05**	0.05		0.1	0.000019
Copper (Cu)	2**	2	1.0	1.3	0.000023
Iron (Fe)		0.2	0.3	0.3*	0.00125
Lead (Pb)	0.01	.01	0.05	0.015	0.000017
Manganese (Mn)	0.5**	.05	0.05	0.05*	0.000020
Mercury (Hg)	0.001	0.001	0.0005	0.002	0.000005
Molybdenum(Mo)	0.07				0.000030
Nickel (Ni)	0.02**	0.02	0.01		0.000024
Selenium (Se)	0.01	0.01	0.01	0.05	0.000047
Silver (Ag)				0.01*	0.000027
Sodium (Na)		200	200		0.0276
Thallium (Tl)				0.002	0.000021
Uranium (U)	0.002**		0.002	0.030	0.000015
Zinc (Zn)				5.0*	0.000101

*Secondary Standard, **Provisional Guideline Value, ***MDLs Calculated as Three Sigma of 10 Replicates of Low Standard, as Described in this Work. MDLs Reported in mg/L to Match Regulatory Requirements.

Table 1 includes the trace metals that are regulated by various worldwide regulatory and advisory agencies. ICP-MS is the only analytical technique capable of meeting all the required detection limits for all the regulated trace metals. Therefore, while not mandated as the only acceptable technique for most regulations, ICP-MS is becoming the instrument of choice for trace metals analysis in water worldwide.

While the details of QA/QC criteria and reporting requirements vary significantly from jurisdiction to jurisdiction, Table 1 shows that the actual detection limit requirements are very similar. In addition, the fundamental goals of the QA/QC requirements in all jurisdictions are the same. This is to insure that the reported values for all samples meet commonly accepted guidelines for accuracy and precision. Typically, these guidelines are met through the analysis of periodic QC samples inserted into the sample queue. Such QC samples should include: a check on the accuracy of the initial instrument calibration; a control sample of known concentration similar to that of the analytes in a similar matrix; a sample designed to test the ability of the system to eliminate interferences as false positives; a sample designed to detect sample carryover or memory problems; and periodic calibration check samples to check for instrument drift. If samples are to be analyzed outside the calibration range of the analytical method, then a linear range check sample must also be analyzed. It is outside the scope of this application note to detail the specific QA/QC requirements for each regulation where they exist at all. Instead, a general QA/QC protocol will be outlined which will demonstrate the ability of the Agilent 7500c to meet generally accepted guidelines while easily meeting the required reporting limits for drinking water monitoring worldwide. Simple modifications to this procedure can be implemented to insure strict compliance with detailed local requirements.

Advantages to the Use of the ORS for Drinking Water Analysis

Generally, drinking water is not considered a particularly difficult matrix for analysis by ICP-MS. There are, however, a few significant challenges. These challenges are due to the very low desired reporting limits for several elements (Table 1), as well as the possibly high concentrations for others, such as Ca and Na. This combination of very low and very high analyte concentrations presents a challenge that no other analytical technique can overcome. In order to measure all elements simultaneously, the ICP-MS must be able to accurately measure mercury at 0.05 ppb or less and Na or Ca as high as 1000s of ppm. In addition, the ICP-MS must be able to eliminate common interferences on Fe, As, Se, Cu, V, and other elements which originate in the plasma and interface region. If unmanaged, these interferences make trace level analysis of the above elements difficult or impossible in many water samples.

The ORS serves two purposes. First, it uses collision/reaction cell technology to virtually eliminate polyatomic interferences on most elements. This allows the analyst to select the most abundant isotope of each analyte for analysis and avoid the use of mathematical correction factors. The result is sub-ppb detection limits for virtually all elements of interest. Second, it allows the analyst to use passive collisions in the ORS to reduce the ion current for high concentration, low-mass elements such as Na and Ca. In this way, the dynamic range for these elements is shifted to allow accurate, linear measurement at levels previously impossible by ICP-MS. It is this ability to simultaneously improve the sensitivity for ultra-trace analytes and extend the dynamic range upward for matrix analytes that is unique to the ORS system.

Instrument Conditions

Table 2 shows the instrument conditions used for typical water analysis. Listed are the preferred isotope, the tune mode (normal, hydrogen reaction, or helium collision), integration time, calibration range, and approximate detection limit based on normal commercial laboratory conditions. RF power is typically set high, 1400–1500 W, to maximize decomposition of the matrix. Other tune conditions such as ion optics, quadrupole, and detector parameters are set according to standard instrument tune guidelines. No special tuning is required.

Table 2. Elements of Interest with Appropriate Isotopes, ORS Acquisition Mode, Integration Time, Calibration Range and Measured MDLs for Each Isotope

Analyte	Isotope	ORS mode	Integration time (s)	Calibration range (ppb)	~MDL (ppb)
Aluminum (Al)	27	Normal	0.1	0.5–100	0.054
Antimony (Sb)	121	Normal	0.1	0.5–100	0.035
Arsenic (As)	75	Helium	0.5	0.5–100	0.052
Barium (Ba)	137	Normal	0.1	0.5–100	0.027
Beryllium (Be)	9	Normal	0.1	0.5–100	0.028
Boron (B)	10	Normal	0.1	0.5–100	
Cadmium (Cd)	111	Normal	0.1	0.5–100	0.025
Calcium (Ca)	40	Hydrogen	0.1	50–200,000	2.02
Chromium (Cr)	52	Helium	0.5	0.5–100	0.019
Copper (Cu)	63	Helium	0.5	0.5–100	0.023
Iron (Fe)	56	Hydrogen	0.1	50–200,000	1.25
Lead (Pb)	Sum of isotopes 206, 207, 208	Normal	0.1	0.5–100	0.017
Manganese (Mn)	55	Normal	0.1	0.5–100	0.020
Mercury (Hg)	202	Normal	1.0	0.05–1.0	0.005
Molybdenum(Mo)	95	Normal	0.1	0.5–100	0.030
Nickel (Ni)	60	Helium	0.5	0.5–100	0.024
Potassium (K)	39	Helium	0.5	50–200,000	3.02
Selenium (Se)	78	Hydrogen	0.5	0.5–100	0.047
Silver (Ag)	107	Normal	0.1	0.5–100	0.027
Sodium (Na)	23	Hydrogen	0.1	50–200,000	27.6
Thallium (Tl)	205	Normal	0.1	0.5–100	0.021
Uranium (U)	238	Normal	0.1	0.5–100	0.015
Vanadium (V)	51	Helium	0.5	0.5–100	0.034
Zinc (Zn)	66	Normal	0.1	0.5–100	0.101
Useful Internal Standards					
6Li	6	Normal	0.1	50	
Sc	45	All	0.1	50	
Ge	70,72,74	All	0.1	50	
Y	89	Normal	0.1	50	
In	115	Normal	0.1	50	
Tb	159	Normal	0.1	50	
Pt	195	Normal	0.1	50	
Bi	209	Normal	0.1	50	

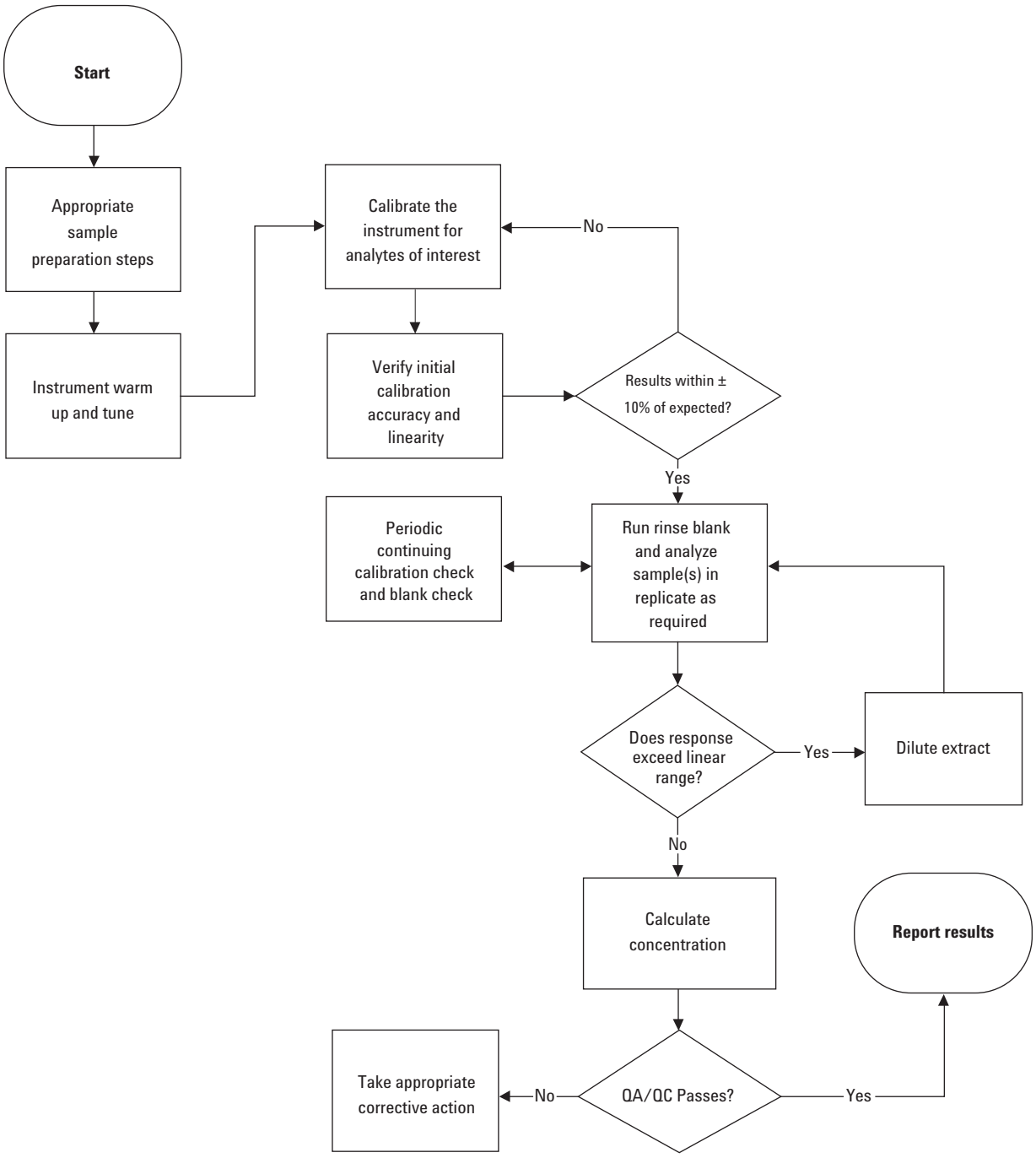

Figure 1. Summary of general water analysis protocol.

Figure 1 depicts the general flow of sample analysis and QA/QC that would be performed to meet the daily requirements of most drinking water regulations. Specific QA/QC details vary from jurisdiction to jurisdiction and are not outlined here. In addition to the daily requirements, less frequent, periodic QA/QC documentation must be performed to ensure ongoing accuracy and precision. Such periodic requirements include: verification of method detection limits, dynamic range, management of interferences (both isobaric and memory effects), as well as general instrument condition and performance. Specific examples of these requirements are found in USEPA Method 200.8 and the UK Drinking Water Inspectorate publication, "NS-30."

Interference Correction

Because the ORS is capable of efficiently removing polyatomic interferences and most isobaric

elemental interferences in unknown, complex matrices, the use of mathematical interference correction is all but eliminated. The elements which typically require interference correction in water, Ca, V, Fe, As, Se, Mo, and Cd can all be analyzed without the need for mathematical correction. This simplifies the analysis and improves confidence in the results. In this work, only Li-6, In-115, and Pb are corrected (see Table 3). The Li-6 correction is used to correct the abundance of the Li-6 internal standard in the presence of high concentrations of Li-7 in some samples. The In-115 correction is used to correct an internal standard, In, in the presence of high concentrations of tin. Neither of these cases is common and normally these can be ignored. The Pb correction is used to normalize the lead response in the case of varying lead isotope ratios and is not an interference correction.

Table 3. Typical Mathematical Corrections Used for Water Matrices with the Agilent 7500c ORS System

Mass	Equation
6	(6)*1 - (7)*0.082
115	(115)*1 - (118)*0.014
208	(208)*1 + (206)*1 + (207)*1

Experiment

The following data and results were all obtained from a single sequence of 44 analyses of standards, blanks, QC samples, unknown groundwater samples, and seawater samples. All calibrations are based on a single set of standards prepared in 1% nitric acid/0.5% hydrochloric acid. No attempt at matrix matching beyond simple acidification was made. The instrument and conditions were like those of a typical commercial environmental laboratory. “Clean room” conditions or ultra-high purity reagents were not employed. The Agilent 7500c ICP-MS with ORS and Integrated Sample Introduction System (ISIS), configured for autodilution, was used.

Quality Control

Quality control in this experiment consisted of four components:

- Verification of tune performance for each ORS mode
- Initial Calibration linearity check
- Verification of accuracy of initial calibration using NIST 1640 standard reference water
- Periodic verification of calibration accuracy through measurement of continuing calibration verification (CCV) samples

Autodilution

The Agilent 7500c was configured with an ISIS for rapid sample uptake and autodilution. ISIS uses flowing stream autodilution rather than discrete sample dilution. This greatly enhances the throughput and minimizes the possibility of contamination compared with other types of autodiluters. In the ISIS autodiluter, the sample stream is mixed with a flowing stream of diluent in an entirely closed system. Dilution factor is controlled by high precision peristaltic pumps that are automatically and periodically monitored for accuracy throughout the run. Autodilution is invoked automatically by the intelligent sequencing software whenever the system encounters a user-definable out-of-range condition, such as an analyte outside the calibration range or an internal standard outside predefined bounds. Autodilution was invoked in a number of the samples in this work. An excellent check on both the linearity of the instrument and the accuracy of the autodilution can be obtained by comparing the results for diluted and undiluted samples. If the results match well, both the instrument linearity and autodilution accuracy are in control. Tables 5 and 7 show excellent examples of this.

Results

QC results are depicted in Tables 4 (CCV results) and 5 (NIST 1640 results). Examples of calibration linearity are depicted in Figures 2, 3, and 4, which are representative. Calibration “R” values of .9998 or greater are considered linear.

Table 4. Recovery of Periodic Calibration Check Standard in a Sequence of Water Samples Including Drinking Waters, Ground Waters, and Seawaters. Calibration Checks were Run After 30 and 43 Real Samples in this Experiment

	CCV Actual value	CCV 50/5000/0.5	% Recovery	CCV 50/5000/0.5	% Recovery
Total DF:		**1**		**1**	
File:		**031_CCV.D#**		**044_CCV.D#**	
Be/9 [#1]	50	50.62	101.2	50.01	100.0
Na/23 [#2]	5000	4933.00	98.7	4838.00	96.8
Mg/24 [#1]	5000	4700.00	94.0	4802.00	96.0
Al/27 [#1]	50	47.09	94.2	46.84	93.7
K/39 [#3]	5000	5260.00	105.2	5076.00	101.5
Ca/40 [#2]	5000	5053.00	101.1	5063.00	101.3
V/51 [#3]	50	51.52	103.0	50.84	101.7
Cr/52 [#3]	50	51.43	102.9	50.78	101.6
Mn/55 [#1]	50	49.92	99.8	50.89	101.8
Fe/56 [#2]	5000	5067.00	101.3	5068.00	101.4
Co/59 [#1]	50	49.88	99.8	50.16	100.3
Ni/60 [#3]	50	51.99	104.0	51.36	102.7
Cu/63 [#3]	50	52.64	105.3	51.74	103.5
Zn/66 [#1]	50	49.27	98.5	49.44	98.9
As/75 [#3]	50	51.63	103.3	51.58	103.2
Se/78 [#2]	50	50.90	101.8	50.61	101.2
Se/80 [#2]	50	51.45	102.9	51.10	102.2
Mo/95 [#1]	50	49.44	98.9	48.11	96.2
Ag/107 [#1]	50	48.73	97.5	47.02	94.0
Cd/111 [#1]	50	49.34	98.7	48.40	96.8
Sb/121 [#1]	50	47.71	95.4	47.03	94.1
Ba/137 [#1]	50	50.35	100.7	49.19	98.4
Hg/202 [#1]	0.5	0.49	98.3	0.47	94.8
Tl/205 [#1]	50	49.68	99.4	50.46	100.9
Pb/208 [#1]	50	49.41	98.8	49.25	98.5
Th/232 [#1]	50	48.54	97.1	49.09	98.2
U/238 [#1]	50	49.46	98.9	49.84	99.7

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 5. Analysis of Certified Reference Water NIST 1640 as a Calibration Check. Sample was Measured Neat and Autodiluted 1/20 (actual measured DF = 21.72), since Na Value Exceeded Upper Calibration Limit. Note that Even in the Undiluted Sample, the Recovery for Na is 101.2%

	Certified value (ppb)	NIST 1640	% Recovery undiluted	NIST 1640	% Recovery diluted
Total DF:		**1**		**21.72**	
Be/9 [#1]	34.94	35.750	102.3	34.860	99.77
Na/23 [#2]	29350	29690.000	101.2	29140.000	99.28
Mg/24 [#1]	5819	5893.000	101.3	6154.000	105.76
Al/27 [#1]	52	49.180	94.6	69.290	133.25
K/39 [#3]	994	947.900	95.4	858.800	86.40
Ca/40 [#2]	7045	7328.000	104.0	7488.000	106.29
V/51 [#3]	12.99	13.030	100.3	12.930	99.54
Cr/52 [#3]	38.6	37.470	97.1	38.540	99.84
Mn/55 [#1]	121.5	119.500	98.4	120.100	98.85
Fe/56 [#2]	34.3	35.840	104.5	31.820	92.77
Co/59 [#1]	20.28	19.400	95.7	20.010	98.67
Ni/60 [#3]	27.4	26.920	98.2	28.000	102.19
Cu/63 [#3]	85.2	86.450	101.5	92.350	108.39
Cu/65 [#3]	85.2	86.350	101.3	91.340	107.21
Zn/66 [#1]	53.2	55.380	104.1	55.560	104.44
As/75 [#3]	26.67	26.910	100.9	28.080	105.29
Se/78 [#2]	21.96	21.990	100.1	20.930	95.31
Mo/95 [#1]	46.75	45.310	96.9	43.280	92.58
Ag/107 [#1]	7.62	7.210	94.6	7.497	98.39
Cd/111 [#1]	22.79	22.560	99.0	22.420	98.38
Sb/121 [#1]	13.79	13.090	94.9	12.590	91.30
Ba/137 [#1]	148	143.900	97.2	142.100	96.01
Hg/202 [#1]		0.017		0.019	
Tl/205 [#1]		0.009		-0.042	
Pb/208 [#1]	27.89	26.690	95.7	26.370	94.55
Th/232 [#1]		0.011		-0.429	
U/238 [#1]		0.725		0.698	

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 6. Replicate Analyses of Low Standard After Sequence of 33 High Level Samples, Standards, and Blanks for MDL Calculations. Three Sigma MDL are Calculated in ppb

	MDL rep 01	MDL rep 02	MDL rep 03	MDL rep 04	MDL rep 05	MDL rep 06	MDL rep 07	MDL rep 08	MDL rep 09	MDL rep 10	3 Σ MDL
Be/9 [#1]	0.50	0.50	0.49	0.49	0.50	0.47	0.49	0.49	0.50	0.49	**0.028**
Na/23 [#2]	53.45	47.78	43.96	39.85	40.52	36.48	34.69	30.58	30.17	22.08	**27.617**
Mg/24 [#1]	49.82	49.13	49.75	48.94	48.83	48.92	49.32	48.84	48.24	48.41	**1.530**
Al/27 [#1]	0.30	0.26	0.25	0.25	0.25	0.23	0.24	0.24	0.25	0.26	**0.054**
K/39 [#3]	56.28	55.34	55.09	53.35	55.02	55.15	53.73	53.25	54.17	53.70	**3.023**
Ca/40 [#2]	52.33	51.76	51.55	51.81	52.32	51.86	51.28	51.33	53.42	51.15	**2.023**
V/51 [#3]	0.51	0.53	0.53	0.53	0.54	0.51	0.53	0.50	0.52	0.52	**0.034**
Cr/52 [#3]	0.52	0.52	0.51	0.51	0.50	0.51	0.52	0.51	0.51	0.51	**0.019**
Mn/55 [#1]	0.49	0.49	0.49	0.47	0.48	0.48	0.49	0.48	0.48	0.47	**0.020**
Fe/56 [#2]	53.84	53.69	53.43	53.46	53.97	53.18	53.10	52.91	53.17	52.65	**1.251**
Co/59 [#1]	0.48	0.48	0.49	0.48	0.48	0.48	0.49	0.49	0.48	0.47	**0.016**
Ni/60 [#3]	0.50	0.49	0.49	0.48	0.50	0.48	0.48	0.49	0.48	0.49	**0.024**
Cu/63 [#3]	0.48	0.48	0.46	0.47	0.46	0.48	0.48	0.47	0.46	0.47	**0.023**
Zn/66 [#1]	0.50	0.45	0.43	0.43	0.43	0.42	0.42	0.46	0.44	0.42	**0.074**
As/75 [#3]	0.50	0.53	0.53	0.49	0.54	0.54	0.54	0.53	0.52	0.52	**0.052**
Se/78 [#2]	0.52	0.51	0.53	0.52	0.49	0.52	0.52	0.51	0.48	0.51	**0.047**
Se/80 [#2]	0.58	0.62	0.56	0.56	0.55	0.57	0.58	0.55	0.54	0.57	**0.066**
Mo /95 [#1]	0.47	0.46	0.46	0.46	0.45	0.46	0.48	0.44	0.47	0.45	**0.030**
Ag/107 [#1]	0.45	0.47	0.46	0.44	0.46	0.46	0.46	0.45	0.44	0.45	**0.027**
Cd/111 [#1]	0.45	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.45	0.43	**0.025**
Sb/121 [#1]	0.46	0.45	0.44	0.45	0.43	0.44	0.46	0.45	0.43	0.45	**0.035**
Ba/137 [#1]	0.49	0.47	0.47	0.49	0.48	0.47	0.47	0.47	0.46	0.48	**0.027**
Hg/202 [#1]	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	**0.005**
Tl/205 [#1]	0.40	0.42	0.42	0.43	0.42	0.42	0.42	0.42	0.41	0.41	**0.021**
Pb/208 [#1]	0.46	0.46	0.46	0.45	0.46	0.45	0.45	0.45	0.45	0.45	**0.017**
Th/232 [#1]	0.29	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	**0.050**
U/238 [#1]	0.43	0.44	0.44	0.43	0.43	0.43	0.44	0.44	0.43	0.43	**0.015**

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Detection Limits

The method detection limits reported in Table 6 were generated at the end of a sequence of 33 real world samples, standards, and blanks. Column one lists the isotope and ORS acquisition mode, #1 = Normal Mode, #2 = Hydrogen Mode, #3 = Helium Mode. Actual method detection limits will vary depending on instrument and laboratory conditions. These detection limits should be achievable with normal levels of laboratory cleanliness, using trace metal grade acids and ASTM type 1 water. The instrument used for this work was equipped with the ISIS, which typically improves DLs somewhat by increasing sample introduction precision and minimizing carryover.

Dynamic Range

One of the advantages of using the ORS is its ability to reduce interferences on certain trace level analytes and simultaneously attenuate the signal on high concentration or matrix elements. In this work, calibrations were generated from a low of 50 ppt for Hg to a high of 200 ppm for the mineral elements, Na, K, Ca, Mg, and Fe. Sample calibration curves follow. Additionally, while Na was calibrated only as high as 200 ppm, which is the highest regulated concentration in any of the elements in the worldwide drinking water regulations (see Table 1), it yields linear response at much higher concentrations.

Table 7. A Series on Analyses on Three High Dissolved Solids Ground Water Samples. Each Sample was Analyzed Undiluted and Automatically Autodiluted. Elements which were Undetected were Removed for Simplicity.

	Water 1	Water 1	Water 2	Water 2	Water 3	Water 3
Total DF:	**1**	**21.72**	**1**	**21.72**	**1**	**21.72**
File:	**014SMPL.D**	**015SMPL.D**	**016SMPL.D**	**017SMPL.D**	**018SMPL.D**	**019SMPL.D**
Na/23 [#2]	489100.000	492500.000	330500.000	324100.000	563700.000	554000.000
Na/23 [#3]	480300.000	505800.000	337200.000	342800.000	563000.000	571800.000
Mg 24 [#1]	559.000	599.900	511.700	534.800	3099.000	3407.000
K/39 [#3]	1564.000	1365.000	794.000	721.400	2513.000	2333.000
Ca/40 [#2]	8708.000	8760.000	2337.000	2255.000	13350.000	13400.000
Mo/95 [#1]	0.776	0.773	1.482	1.535	49.070	49.180
Ba/137 [#1]	17.070	16.990	29.250	28.800	5.263	5.154
U/238 [#1]	0.043	0.037	0.036	0.034	0.115	0.103

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 7 shows the results of the analysis of three brackish ground water samples. Each sample was analyzed directly and then autodiluted. Both sets of results show both the dynamic range of the Agilent 7500c and the accuracy of the autodilution. The autodilution factor of 21.72 is the result of the system automatically calibrating the dilution factor at the beginning of the sequence and periodically, as needed. Note that for the uranium result, where the undiluted concentration is only 30–40 ppt, the autodiluted result agrees very well. This translates to accurate measurement of uranium in the diluted samples of ~35/21.7 = 1.6 ppt.

Table 8. Results of Analysis of a 1/10 "Synthetic Seawater" Blank (High Purity 0.3% NaCl) Plus a Spike at 5 ppb for Trace Elements and 500 ppb for Matrix Elements.

	1/10 Synth Sea H_20	Spike 1/10 Synth Sea H_20 + 5 ppb	% Recovery 5/500 ppb spike
File:	**020SMPL.D#**	**021SMPL.D#**	
Be/9 [#1]	0.000	4.591	91.8
Na/23 [#1]	over range	over range	N/A
Na/23 [#2]	1233000.000	1215000.000	N/A
Na/23 [#3]	1193000.000	1193000.000	N/A
Mg/24 [#1]	2.382	477.000	94.9
I/27 [#1]	-0.409	4.250	93.2
K/39 [#1]	13.730	491.500	95.6
K/39 [#2]	8.195	548.600	108.1
K/39 [#3]	16.510	597.400	116.2
Ca/40 [#2]	6.740	532.600	105.2
V/51 [#3]	0.031	5.426	107.9
Cr/52 [#3]	0.045	5.287	104.8
Mn/55 [#1]	-0.003	4.497	90.0
Fe/56 [#2]	-0.258	508.600	101.8
Co/59 [#1]	0.122	4.569	89.0
Ni/60 [#1]	0.024	4.318	85.9
Ni/60 [#3]	-0.040	4.801	96.8
Cu/63 [#3]	-0.117	4.691	96.2
Cu/65 [#3]	-0.117	4.564	93.6
Zn/66 [#1]	0.025	4.520	89.9
Zn/67 [#1]	0.007	4.714	94.1
As/75 [#3]	0.011	5.027	100.3
Se/78 [#2]	0.006	4.366	87.2
Se/80 [#2]	0.143	4.620	89.5
Mo/95 [#1]	0.043	5.040	99.9
Ag/107 [#1]	-0.010	4.254	85.3
Cd/111 [#1]	0.033	4.545	90.2
Sb/121 [#1]	0.034	4.598	91.3
Ba/137 [#1]	0.010	4.789	95.6
Hg/202 [#1]	0.017	0.020	N/A
Tl/205 [#1]	-0.003	4.883	97.7
Pb/208 [#1]	0.175	5.066	97.8
U/238 [#1]	0.000	4.968	99.4

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 8 shows the results of the analysis of a 0.3% 3000 ppm NaCl or 1180.5 ppm Na, both unspiked and spiked with trace elements and other matrix elements. Recoveries are reported in column 4. Note that in this case, for demonstration purposes, Na was acquired in all three ORS modes (normal, hydrogen, and helium). As expected, in the normal mode, the sodium signal was over range and the detector was protected from excessive signal. However, sodium was measurable in both hydrogen and helium modes at 1233 and 1193 ppm respectively, yielding recoveries of 104% and 101% respectively without further dilution or any other manipulation of instrument conditions. Under identical conditions, in the same run at the same time, Arsenic in the spike was also measured using He collision mode at 5.03 ppb to give 100.3% recovery.

1180.5 ppm sodium

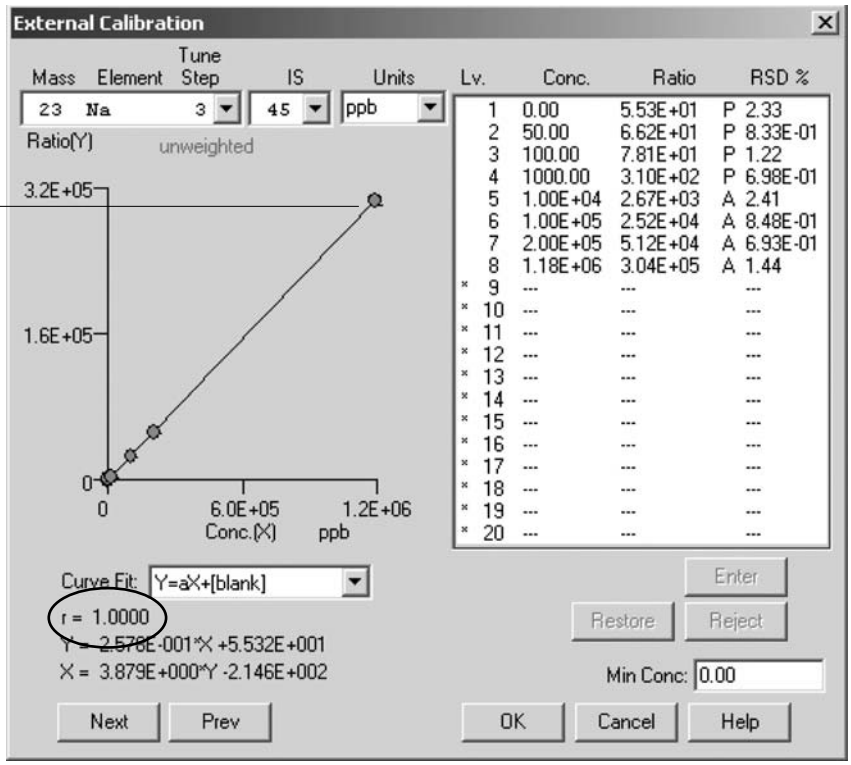

Figure 2. Calibration curve for Na in Helium collision mode showing linearity from 50 ppb to 1180 ppm (0.3% NaCl).

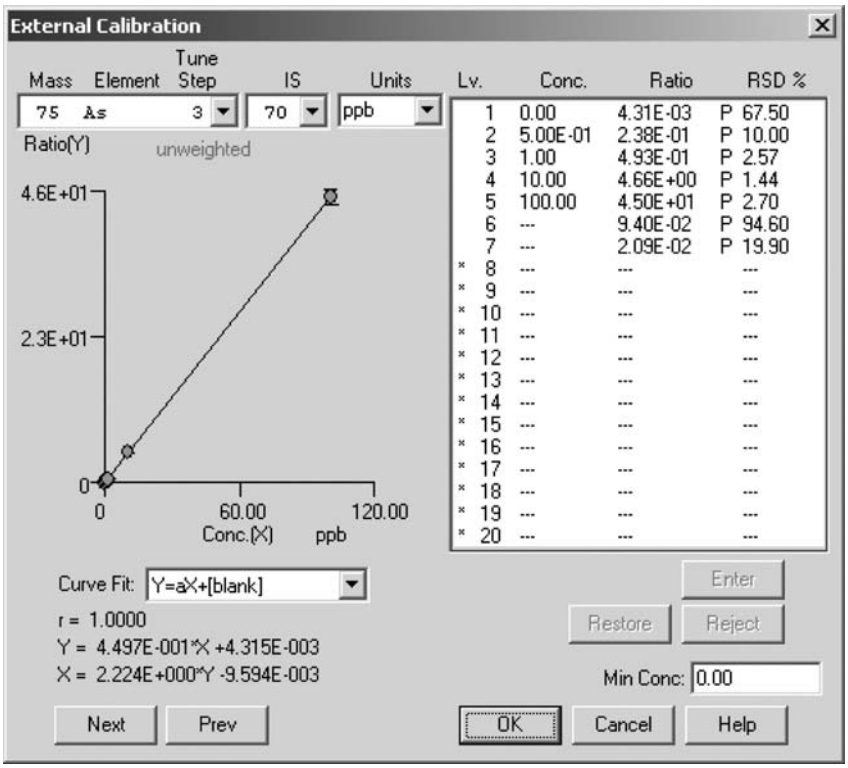

Figure 3. Arsenic calibration acquired in helium collision mode (same as Na in Figure 2) from 0.5 to 100 ppb.

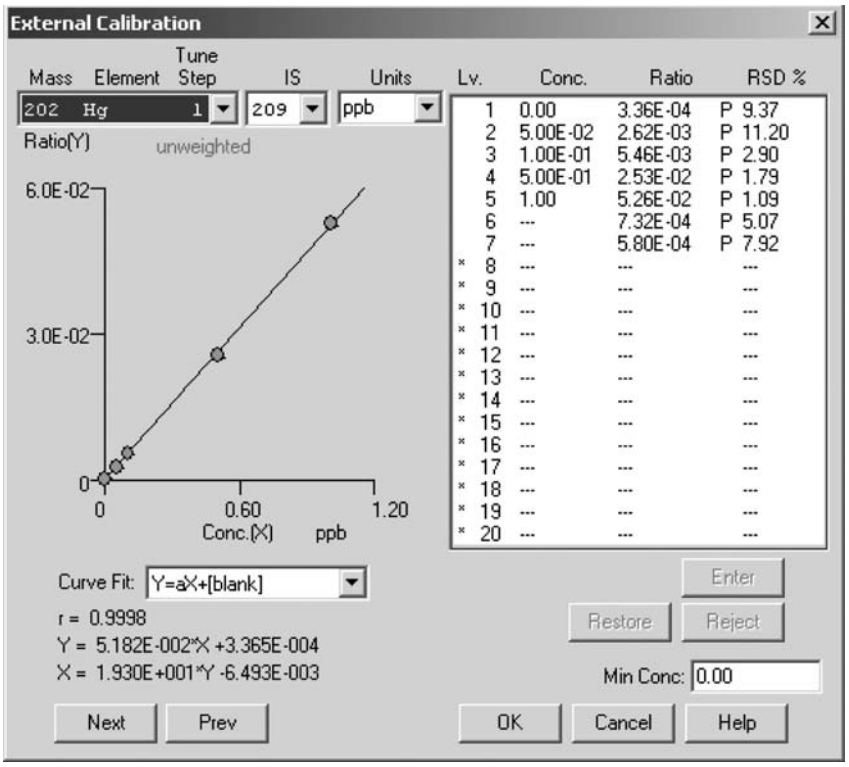

Figure 4. Mercury calibration acquired in normal (no gas) mode from 0.05–1 ppb.

The calibration curves in Figures 2–4 were all acquired from the same mixes of standard elements in dilute nitric/hydrochloric acid. That means that the low standard contained 50 ppt of mercury, 500 ppt of the other trace elements and 50 ppb of the mineral elements (Na, K, Ca, Mg, and Fe), and so on through the levels. In the sodium curve, the actual calibration was performed up to 200 ppm (level 7 in Figure 2); the 1180.5 ppm level was the 1/10 "synthetic seawater" NaCl solution.

Conclusions

While the specific details for drinking water monitoring vary from country to country around the world, the overall requirements, both from a reporting limit and quality control standpoint, are very similar. Currently, of the many available techniques for monitoring trace metals in water, only ICP-MS has the sensitivity and elemental coverage to meet all worldwide requirements. In addition, the use of collision/reaction cell technology in the form of the Agilent 7500c ORS allows the user both to easily meet the strictest ultra-trace reporting limits and to measure mineral or matrix elements at 1000s of ppm simultaneously, without fear of false positives from polyatomic interferences or out-of-range elements.

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A Comparison of GC-ICP-MS and HPLC-ICP-MS for the Analysis of Organotin Compounds

Application

Environmental

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Abstract

An inductively coupled plasma mass spectrometer (ICP-MS) was used as a detector for gas chromatography (GC) and high performance liquid chromatography (HPLC) analysis of organotin compounds. ICP-MS is a highly sensitive detector with detection limits in the pg–ng range, as well as enabling calibration by isotope dilution mass spectrometry (IDMS). Calibrating using isotopically labeled organotin species reduces measurement uncertainties and leads to greater precision compared to external calibration methods. This application note details the relative merits of the two techniques for the analysis of organotin compounds.

Introduction

The toxic effects of organotin compounds in the environment have been well documented [1] and have led to extensive research into analytical methodologies for their determination in a variety of matrices. The widespread use of organotin compounds has resulted in their detection in most marine and fresh-water sediments as well as in open-ocean waters [2]. In recent years, the focus of research in organotin analysis has begun to include matrices with human health implications, such as seafood [3], manufactured products (PVC pipes used for drinking water distribution [4]), and human blood samples [5].

Organotin analysis has traditionally been performed by chromatographic separation (gas chromatography (GC) or high performance liquid chromatography (HPLC)) coupled to a variety of detectors. GC separations enable the analysis of many different groups of organotin compounds (for example, butyl-, phenyl-, octyl-, and propyl) in a single analysis after derivatization [6]. However, derivatization is time-consuming and yields may vary between species and in terms of efficiency depending on matrix components. GC-ICP-MS has the potential to facilitate simultaneous multi-elemental speciation analysis, because species of Se [7], Pb [8], Hg [9], and Sn [10] have volatile forms and could be analyzed in a single analysis. Organotin separations by HPLC offer the advantage that derivatization is not required, which eliminates a potential source of uncertainty in the final result and can reduce analysis time significantly. However, the range of compounds that can be analyzed in a single run are limited compared to GC. The use of ICP-MS as a detector enables calibration by isotope dilution mass spectrometry as well as providing very low limits of detection (pg–ng range). In conjunction with isotopically labeled organotin species, this approach offers many advantages from an analytical point of view including reduced measurement uncertainties and greater precision compared to external calibration methods.

Experimental

Reagents and Standards

Acetonitrile (UpS™ ultra-purity solvent grade) was obtained from Romil (Cambridge, UK). Glacial

acetic acid (TraceSelect) and anhydrous sodium acetate (Microselect ≥ 99.5% NT) were obtained from Fluka (Gillingham, Dorset, UK). Triethylamine, methanol and hexane were used as HPLC grade. Deionized water was obtained from a water purification unit at >18MΩ (Elga, Marlow, UK). Sodium tetra-ethylborate ($NaBEt_4$) was obtained from Aldrich (Gillingham, Dorset, UK).

Tributyltinchloride (TBTCl), Dibutyltinchloride ($DBTCl_2$), Triphenyltinchloride (TPhTCl) and Diphenyltinchloride ($DPhTCl_2$) were obtained from Aldrich and purified according to the procedure described by Sutton et al [11]. The ^{117}Sn isotopically enriched TBTCl was synthesized according to the procedure described in the same paper. Monobutyltinchloride ($MBTCl_3$) and Tetrabutyltinchloride (TeBTCl) were obtained from Aldrich, and Dioctyltin (DOT), Tripropyltin (TPrT), and Tetrapropyltin (TePrT) were obtained from Alfa Aesar (Johnson Matthey, Karlsruhe, Germany).

Instrumentation

Accelerated solvent extraction was carried out using a Dionex ASE 200 system. An Agilent 7500i ICP-MS was used for time-resolved analysis of ^{120}Sn, ^{118}Sn, and ^{117}Sn. The ShieldTorch system was used, and a second roughing pump was added in-line to increase sensitivity.

An Agilent Technologies (Palo Alto, California, USA) 1100 HPLC system was used for HPLC separations. All stainless steel parts of the HPLC system that come into contact with the sample were replaced by polyether ether ketone (PEEK) components. A 100-cm length piece of PEEK tubing was used to connect the analytical column to the 100-µL min^{-1} PFA MicroFlow nebulizer of the ICP-MS. Optimization of the ICP-MS conditions was achieved prior to HPLC analysis by adjusting the torch position and tuning for reduced oxide and doubly charged ion formation with a standard tuning solution containing 10 ng g^{-1} of ^{7}Li, ^{89}Y, ^{140}Ce, and ^{205}Tl in 2% HNO_3. After this preliminary optimization, the HPLC system was coupled to the nebulizer and a final optimization was carried out using ^{103}Rh added to the HPLC mobile phase. To reduce the solvent loading on the plasma, the double-pass spray-chamber was Peltier-cooled to -5 °C. Oxygen (0.1 L min^{-1}) was mixed into the make-up gas and added post-nebulization to convert organic carbon to CO_2 in the plasma and avoid a carbon build-up on the cones. The final optimization was important because the nebulizer gas and make-up gas flows had to be adjusted to ensure plasma stability with the organic mobile phase conditions. HPLC separations were performed using a C-18 ACE column (3-µm particle size, 2.1 mm × 15 cm) with a mobile phase of 65: 23: 12: 0.05 % v/v/v/v acetonitrile/ water/ acetic acid/TEA. The flow rate was 0.2 mL min^{-1}, and 20 µL of sample blends and mass-bias blends were injected. See Table 1.

GC separations were performed on an Agilent 6890 GC. The Agilent G3158A GC interface [12] was used to couple the GC to the ICP-MS. The GC method was used as described by Rajendran et al [6]. The analytical column was connected to a length of deactivated fused silica, which was inserted along the ICP transfer line and injector. After installation of the interface, the torch position and the ion lenses were tuned using a 100-ppm xenon in oxygen mixture, which was added to the ICP-MS carrier gas at 5% volume via a T-piece. The isotope monitored for this adjustment was ^{131}Xe.

Table 1. ICP-MS Parameters Used

	HPLC-ICP-MS	GC-ICP-MS
Interface cones	**Platinum**	**Platinum**
Plasma gas flow	14.5–14.9 L min^{-1}	14.5–14.9 L min^{-1}
Carrier gas flow	0.65–0.75 L min^{-1}	0.80–0.85 L min^{-1}
Make-up gas flow	0.15–0.25 L min^{-1}	Not used
RF power	1350–1550 W	1100–1200 W
Sampling depth	4–7 mm	6.5–7.5 mm
Integration time per mass	300 ms	100 ms
Isotopes monitored	^{120}Sn ^{117}Sn ^{103}Rh	^{120}Sn ^{118}Sn ^{117}Sn
Other parameters	ICP torch injector diameter: 1.5 mm Peltier cooled spray chamber at -5 °C 5% O_2 added post-nebulization ShieldTorch fitted	5% N_2 or O_2 added to enhance sensitivity ShieldTorch fitted

Extraction of Organotin Compounds

The ASE extraction cells were fitted with PTFE liners and filter papers and filled with dispersing agent. The sediment and the isotopically enriched spike were added and left to equilibrate overnight. Each cell was extracted using five 5-minute cycles at 100 °C and 1500 psi after a 2-minute preheat and 5-minute heat cycle. 0.5 M sodium acetate/ 1.0 M acetic acid in methanol was used as the extraction solvent [13]. A calibrated solution (mass-bias blend) was prepared by adding the appropriate amounts of both ^{120}Sn TBTCl and ^{117}Sn TBTCl into an ASE cell filled and extracting under the same conditions as the samples. Digestion blanks were prepared by extracting ASE cells filled with hydromatrix and PTFE liners. After the extraction, each cell was flushed for 100 seconds with 60% of the volume and purged with N_2. Prior to analysis, the extracts were diluted two- to five-fold in ultrapure water for HPLC-ICP-MS analysis. For GC-ICP-MS analysis, 5 mL of sample-, blank-, and mass-bias blend solutions were derivatized with 1 mL of 5% $NaBEt_4$ and shaken for 10 minutes with 2 mL of hexane. An aliquot of the hexane fraction was then injected for analysis.

Isotope Dilution Mass Spectrometry (IDMS) Methodology

The method used for IDMS consisted of analyzing a blend of the sample together with a mass-bias calibration blend. Each sample blend was injected four times and bracketed by injections of the mass-bias calibration blend. The mass-bias calibration blend was prepared to match the concentration and isotope amount ratio in the sample by mixing the same amount of spike added to the sample with a primary standard of the analyte of interest [14], [15]. The estimation of the standard uncertainties for the measured isotope amount ratios was different to the one described in [14] as they were calculated as peak area ratios and not spectral measurement intensities. The chromatographic peaks were integrated manually using the RTE integrator of the Agilent ICP-MS chromatographic software. The mass fraction obtained from the measurement of each sample blend injection was then calculated according to:

$$w'_X = w_Z \bullet \frac{m_Y}{m_X} \bullet \frac{m_{Zc}}{m_{Yc}} \bullet \frac{R_Y - R'_B \bullet \frac{R_{Bc}}{R'_{Bc}}}{R'_B \bullet \frac{R_{Bc}}{R'_{Bc}} - R_Z} \bullet \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

R'_B	Measured isotope amount ratio of sample blend (X+Y)
R'_{Bc}	Measured isotope amount ratio of calibration blend (Bc=Z+Y)
R_{Bc}	Gravimetric value of the isotope amount ratio of calibration blend (Bc=Z+Y)
R_Z	Isotope amount ratio of Primary standard Z (IUPAC value)
R_Y	Isotope amount ratio of spike Y (value from certificate)
w'_X	Mass fraction of Sn in sample X obtained from the measurement of one aliquot
w_Z	Mass fraction of Sn in primary standard Z
m_Y	Mass of spike Y added to the sample X to prepare the blend B (=X+Y)
m_X	Mass of sample X added to the spike Y to prepare the blend B (=X+Y)
m_{Zc}	Mass of primary standard solution Z added to the spike Y to make calibration blend Bc (=Y+ Z)
m_{Yc}	Mass of spike Y added to the spike Y primary standard solution Z to make calibration blend Bc (=Y+ Z)

The representative isotopic composition of Sn taken from IUPAC was used to calculate the isotope amount ratios of the primary standard. For the spike TBTCl, the isotopic composition was obtained from the certificate supplied with the ^{117}Sn enriched material from AEA Technology plc (UK). For the measured isotope amount ratio of the calibration blend (R'_{Bc}), the average of the two ratios measured before and after each sample blend isotope amount ratio (R'_B) were taken. A mass fraction was calculated for each sample blend injection and the average of the bracketing mass-bias calibration blend injections. The average of the four mass fractions was then reported as the mass fraction obtained for the blend analyzed. The final mass fraction was recalculated back to the original sample and corrected for moisture content.

Results and Discussion

General Comparison

Analysis of mixed organotin standard solutions showed that the GC method could separate a greater number (10–12) of compounds in a single run compared to HPLC-ICP-MS (5–6). The injection-to-injection time was ~40% shorter for HPLC-ICP-MS, due to the temperature profile used for GC separations. Because of the cost of the derivatizing agent, the reagent cost per sample is approximately double for GC sample preparation.

Sensitivity Enhancement of GC-ICP-MS by Using Additional Gases

Figure 1 and Table 2 illustrate the effect of adding different additional gases on the signal response for a range of organotin compounds. Adding 5% O_2 results in an increase in the measured peak area ranging from 9-fold (DBT and MPhT) to 12-fold (MBT). The addition of N_2 results in a further increase compared to analysis without addition of an optional gas. Response factors range from 105 (DBT and TPhT) to 136 for MBT and 150 for TeBT. This translates to a reduction of the method detection limit (3s) for TBT from 0.4 ng mL^{-1} (no gas) to 0.03 ng mL^{-1} (with 5% O_2 added) to 0.006 ng mL^{-1} (with 5% N_2 added). The table below summarizes detection limits based on analysis of a calibration standard for MBT, DBT, and TBT.

Detection limits (ng mL^{-1} as Sn) by GC-ICP-MS

	No gas added	5% N_2 added
MBT	0.7	0.01
DBT	0.5	0.008
TBT	0.4	0.006

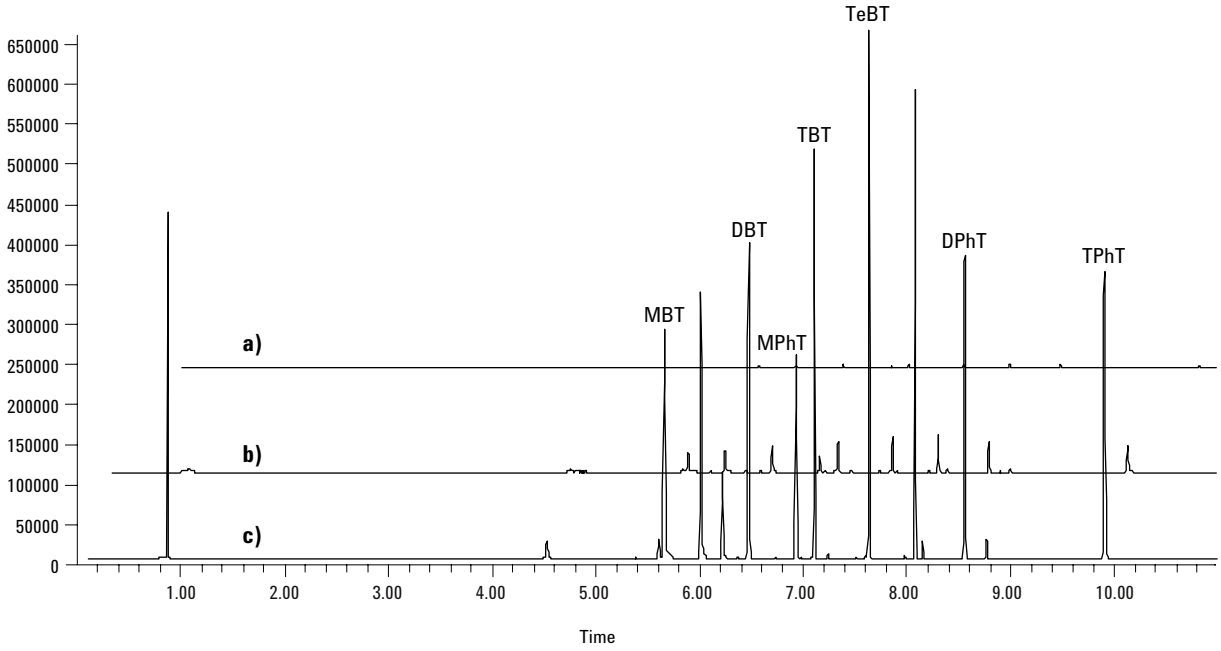

Figure 1. Sensitivity increase on a 20 ng mL^{-1} mixed standard by using a) no additional gas, b) 5% O_2, and c) 5% N_2.

Table 2. Effect of Different Additional Gases on Sensitivity of Organotin Compounds by GC-ICP-MS

Compound	Retention time (min)	a) No gas added (peak area)	b) 5% O_2 added (peak area)	Response factor compared to a)	c) 5% N_2 added (peak area)	Response factor compared to a)	Response factor compared to b)
MBT	5.57	2274	27029	12	309702	136	12
DBT	6.38	3247	29238	9	340436	105	12
MPhT	6.84	2026	18173	9	215182	106	12
TBT	7.02	3490	33132	10	399868	115	12
TeBT	7.54	3717	34225	9	558916	150	16
DPhT	8.46	3181	29665	9	338057	106	11
TPhT	9.81	4287	41119	10	450803	105	11

Comparison of HPLC-ICP-MS and GC-ICP-MS for Analysis of TBT in Sediment

Table 3 shows the comparative data obtained by analysis of the same sediment extracts by both methodologies. There is no statistically significant difference between the two data sets. This confirms that the chromatographic separation and the different sample pretreatment (dilution/derivatization) used has no influence on the analytical result obtained. The chromatography for both methods appears in Figure 2 and Figure 3. The isotope amount ratio measurement precision, measured for 15 injections over a 6–8 hour period, is good for both methods (1.6% for HPLC-ICP-MS and 1.7% for GC-ICP-MS). The uncertainty estimates provided by HPLC-ICP-MS tend to be larger than for GC separations. This is a result of broader peaks (50–60s by HPLC, compared to 4–6s by GC) and greater baseline noise.

Detection limits for sediment analysis are estimated by peak height measurements (3s) as 3 pg TBT as Sn for HPLC-ICP-MS and 0.03 pg TBT as Sn for GC-ICP-MS with 5% O_2 addition. This demonstrates the superior sensitivity of GC-ICP-MS even without sample preconcentration.

The accuracy of the analytical procedure was evaluated by measuring extractions of the certified reference sediment PACS-2 (NRC, Canada). The mean mass fraction obtained by the HPLC-ICP-MS analysis of four extracts was 864 ±35 ng g^{-1} TBT as Sn compared to a certified value of 980 ±130 ng g^{-1} TBT as Sn.

Table 3. TBT Data for Sediment Extracts

Sample	HPLC-ICP-MS (ng/g as Sn) n = 4	Standard uncertainty k = 1 (ng/g as Sn)	GC-ICP-MS (ng/g as Sn) n = 4	Standard uncertainty k = 1 (ng/g as Sn)
1	827	19	853	12
2	805	38	846	13
3	845	9	838	8
Mean	**826**	**22**	**846**	**11**
Expanded uncertainty (k = 2)	**±87**		**±39**	

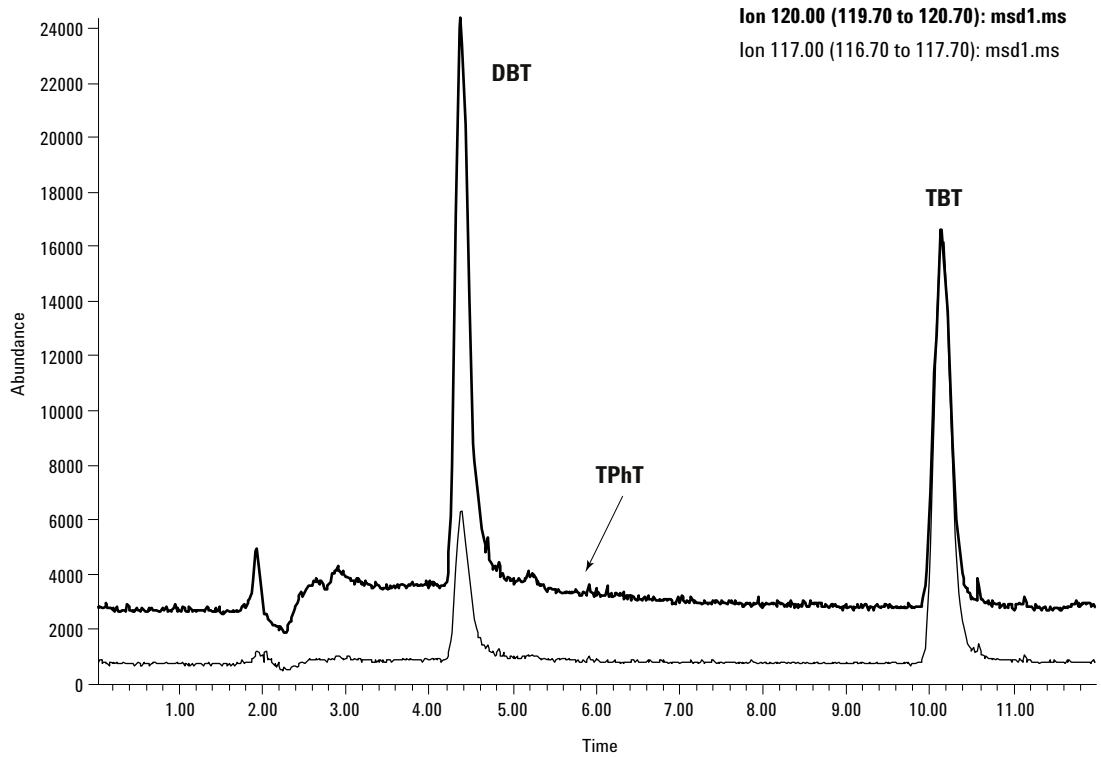

Figure 2. HPLC-ICP-MS chromatogram.

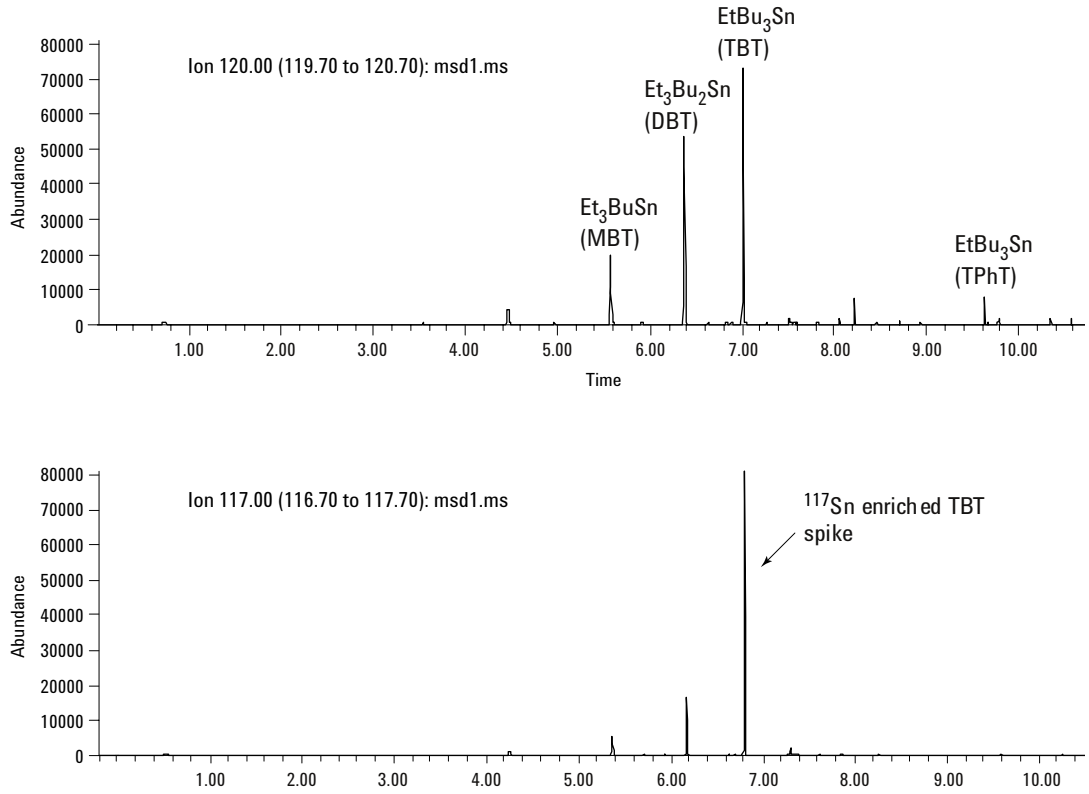

Figure 3. GC-ICP-MS chromatogram.

Conclusions

Both HPLC-ICP-MS and GC-ICP-MS offer advantages for organotin speciation analysis. While there is no statistical difference in the results obtained, HPLC-ICP-MS can be used for cheaper and faster determinations of large sample batches, while the superior sensitivity and the greater number of analytes separated make GC-ICP-MS an ideal tool for monitoring studies at the ultratrace level.

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Analysis of Rare Earth Elements in Geological Samples by Laser Ablation - Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

Geological

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Abstract

The unique, coherent chemical properties of the rare earth elements (REE) make them highly suitable for geological studies into the history of rocks. Prior to the advent of reliable laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) methods, the steps required to prepare the rock samples for REE analysis were both time consuming and costly. Now, the same fusion beads prepared for X-ray-fluorescence (XRF) analysis, used for the determination of major and certain trace elements, can also be used for LA-ICP-MS analysis, without additional sample preparation. Data published in the literature [1] for geostandards: BE-N (Basalt), MRG-1 (Gabbro) and MAG-1 (Marine Mud), compares well to the results obtained by LA-ICP-MS.

Introduction

The rare earth elements (REE) belong to the inner transition metals and play an important part in understanding the geological history of rocks. In most cases, the REE content of a geological sample reflects the pristine composition of the rock formation and can, therefore, yield reliable information concerning magmatic processes and the reconstruction of palaeo-tectonic settings. This is because REE are amongst the most immobile elements during diagenesis, metamorphism, hydrothermal alteration and weathering.

In the past, the determination of REE in geological samples was a time consuming and expensive task: sample digestion and separation of the REE via ion exchange was required prior to analysis by ICP-AES or ICP-MS. More recently, the methodology has developed whereby REE can be determined in the same whole-rock fusion glass beads which are used for X-ray fluorescence (XRF) analyses, by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), with two key advantages:

1. Only one chemical sample preparation procedure is necessary, thereby reducing the use of expensive supra pure chemicals.
2. The bulk chemistry and certain trace elements of a rock sample can be determined by XRF prior to analysis of the same fusion bead by LA-ICP-MS.

Sample preparation

0.6 g very finely (<40µm) ground geological sample (international geostandards BE-N, MRG-1, MAG-1) was added to 3.6 g Spectromelt A12 (66:34 mixture of di-lithiumtetraborate and lithiummetaborate, Merck). Just before melting, 1.1 g NH_4NO_3 was added as an oxidant and merged once again. Fusing was carried out in a Pt-crucible at 1100°C. A different preparation method is described by Becker & Dietze [2]. The bulk chemistry and certain trace elements, as well as the homogeneity of the glass targets, were determined using X-ray-fluorescence (PHILIPS PW1480; Brätz & Klemd, in prep). No further sample preparation is required for additional analysis of the REE in the whole-rock glass target by LA-ICP-MS. At most, the surface of the glass targets can be wiped with alcohol. The measurements were undertaken at the Mineralogisches Institut of the Universität Würzburg by means of a 266 nm Nd:YAG Laser (New Wave Research Inc., Merchantek Products) connected to an Agilent 7500i ICP-MS. Table 1 displays the instrument operating parameters of the LA-ICP-MS, which was tuned for maximum sensitivity.

Table 1. Laser ablation and ICP-MS operating parameters

Laser ablation adjustment	**ICP-MS adjustment**
Merchantek 266 LUV	Agilent 7500i
Wavelength: 266 nm	Plasma power: 1390 W
Pulse duration: 6 ns	Carrier Gas (Ar): 1.27 L/min.
Repetition frequency: 20 Hz	Plasma Gas (Ar): 14.9 L/min.
Laser energy: 70% (=1.9 mJ)	Auxiliary Gas (Ar): 0.9 L/min.
Ablation pattern: line, 50 µm spot ∅	Acquisition Mode: Time Resolved Analysis
Scan speed: 20 µm/sec.	Integration time: Si 0.01 sec.; REE 0.15 sec.
Laser warm-up: 10 sec.	Background: 10 sec.
On delay: 0 sec.	Acquisition time: 40 sec.

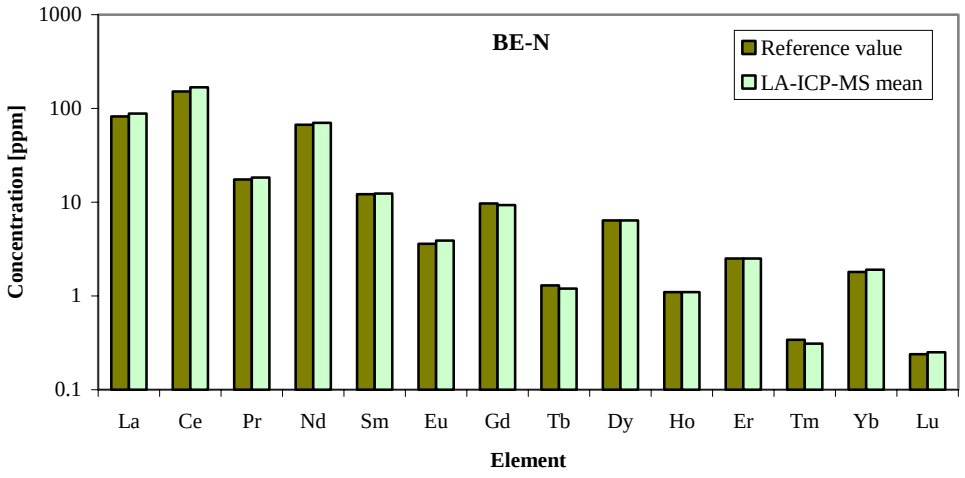
Results

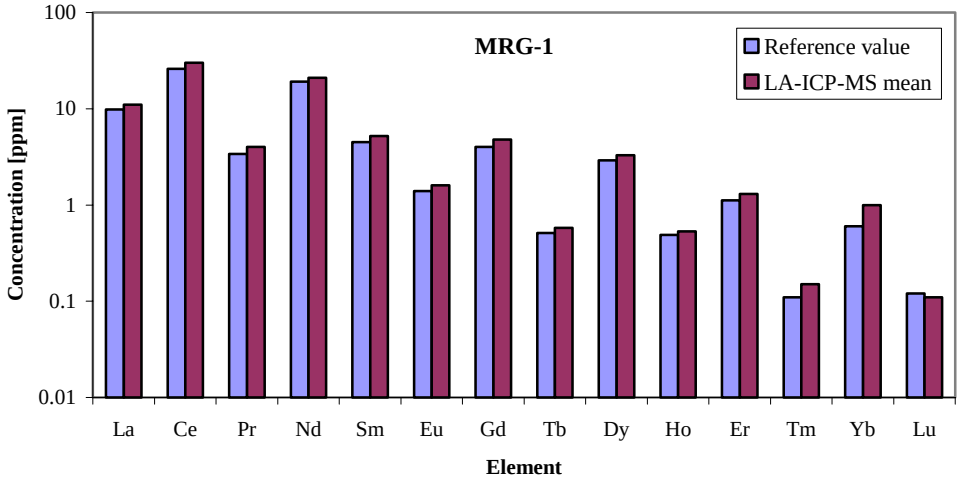
Quantification of the REE was undertaken using the GLITTER software [3, 4]. The REE in the glass standard reference material NIST612 were used as calibration standards, and ^{29}Si as the internal standard. Each prepared geostandard (BE-N, MRG-1, MAG-1) was analysed several times on different days, the relative standard deviation differs from 2 to 14% (the majority from 3 to 8%), and, in agreement with the XRF-measurements of major and certain trace elements, the LA-ICP-MS data highlight an almost homogeneous REE distribution in the glass targets. The mean concentration values for the geostandards, see Table 2, generally show excellent agreement when compared to data from the literature [1]. A graphical representation of the LA-ICP-MS results in comparison to the reference values is shown in Figure 1.

It is important to note that the data presented in this application note has been corrected solely by subtracting the instrument background counts from the counts for each of the elements investigated. While there is the potential for oxide overlaps on the REE - although at much lower levels than would be seen with solution nebulization - the results were in good agreement with the reference values, even when no interference correction was applied. The low level of oxide interferences on the Agilent 7500i is due to the high efficiency of the plasma, which decomposes matrix components efficiently, and thus permits difficult applications to be run without over reliance on correction equations [5].

Table 2. Results for REE concentrations [ppm] in lithium borate glasses from this study and comparison data from the literature [1] (underlined data are proposed values, other values are recommended, except those in parentheses which are informative values). LA-ICP-MS results for BE-N are the mean values of at large 53 measured patterns at six days, for MRG-1 the mean values of 44 measured patterns at five days and for MAG-1 the mean values of 20 measured patterns at two days.

Element	BE-N (Basalt)	LA-ICP-MS		MRG-1 (Gabbro)	LA-ICP-MS		MAG-1 (Marine Mud)	LA-ICP-MS	
	[1]	mean	%RSD	[1]	mean	%RSD	[1]	Mean	%RSD
^{139}La	82	88	3.3	9.8	11	7.1	43	44	5.7
^{140}Ce	152	168	3.1	26	30	9.1	88	81	11.1
^{141}Pr	17.5	18.3	3.1	3.4	4.0	6.4	9.3	9.0	8.9
^{146}Nd	67	70	3.4	19.2	21	5.1	38	40	3.8
^{147}Sm	12.2	12.4	3.7	4.5	5.2	8.0	7.5	8.1	3.1
^{153}Eu	3.6	3.9	3.9	1.39	1.60	8.1	1.55	1.45	10.3
^{157}Gd	9.7	9.3	2.7	4	4.8	11.6	5.8	6.7	4.5
^{159}Tb	1.3	1.2	6.4	0.51	0.58	6.8	0.96	0.86	9.9
^{163}Dy	6.4	6.4	4.0	2.9	3.3	6.6	5.2	5.4	3.7
^{165}Ho	1.1	1.1	3.4	0.49	0.53	6.2	1.02	0.89	7.3
^{166}Er	2.5	2.5	4.9	1.12	1.3	11.3	3	3.1	3.2
^{169}Tm	0.34	0.31	3.5	0.11	0.15	7.9	0.43	0.37	6.8
^{172}Yb	1.8	1.9	3.6	(0.6)	1.0	14.2	2.6	2.9	1.7
^{175}Lu	0.24	0.25	3.8	0.12	0.11	8.9	0.40	0.33	9.1





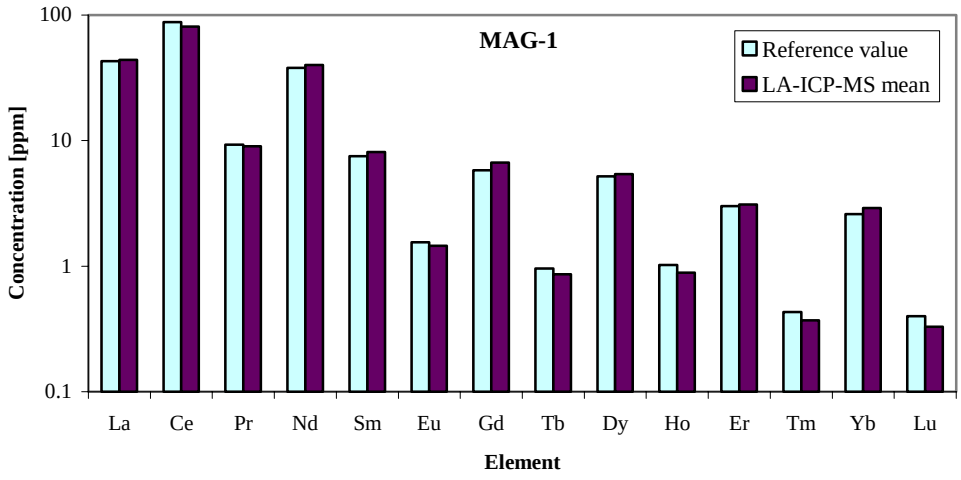


Figure 1. LA-ICP-MS results for REE in comparison to reference values of the international geostandards BE-N, MRG-1 and MAG-1 [1].

Conclusions

The samples prepared for XRF-analysis for the determination of major and certain trace elements (Brätz & Klemd, in prep.) can be used readily for LA-ICP-MS analysis, without additional sample preparation. This way of analysing the REE is fast and relatively inexpensive in comparison to conventional REE-analysis methods. The measured values for the REE in international geostandards are in good agreement with data available in the literature [1]. A disadvantage of the Li borate fusion method of sample preparation is the very high Li-content in the glasses [2], which causes Li contamination of the glassware as well as interface and requires careful cleaning of both the laser ablation and ICP-MS systems in order to permit the subsequent measurement of these elements at trace levels.

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Measurement of Macro and Trace Elements in Plant Digests Using the 7500c ICP-MS System

Application

Food

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Abstract

Inductively coupled plasma mass spectrometry is a powerful tool for the investigation of many materials. The Agilent 7500c with Octopole Reaction System was used to analyze major, minor and trace elements in two standard reference plant materials. The data obtained using the 7500c is compared to the certificate reference values and to results that were generated using inductively coupled plasma optical emission spectroscopy. Results for all elements obtained using the 7500c agree with the certified values.

Introduction

The reliable measurement of trace elements in food is becoming more important as information is revealing that over-dependence on processed grains such as wheat and rice is resulting in a nutritionally poor diet. Micronutrient [1] malnutrition is an identified problem that has coincided with the rapid adoption of modern cereal cropping systems. Profitable and sustainable agriculture depends on the understanding of the nutrients required and available for plant growth, as well as the nutrients for a balanced human diet.

"World food production will need to double over the next 30 years to keep pace with increasing demands from both industrialized and rapidly developing countries. As well as the need to increase production, there will be an increase in demand for higher quality and healthier food products as developing countries become more affluent."

Taken from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) website: http://www.csiro.gov.au (select: Agribusiness/Field Crops/Field Crops & Australia)

Human dietary micronutrients are required by humans in very small amounts. They include at least 14 trace elements (As, B, Cr, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, V, Zn) as well as 13 vitamins (thiamin, riboflavin, niacin, pantothenic acid, biotin, folic acid, vitamins B6, B12, C, A, D, E, K)

The recommended daily intake of the micronutrient trace elements is of the order of:

- mg per day for B, Cu, F, Fe, Mn, Zn
- µg per day for As, Cr, I, Mo, Ni, Se, Si, V

Accurate determination of these trace elements in food materials is useful in ensuring that dietary intake is providing adequate levels of micronutrient elements. Due to the very low concentrations that must be measured and, in many cases, the high and variable sample matrix in which the measurements must be made, this analysis has proved challenging for elemental analysis instrumentation. Traditionally, a combination of techniques was required for a complete analysis of the plant digest—typically Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Hydride-Atomic Absorption Spectroscopy (HG-AAS) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Such is the performance and elemental coverage of modern inductively coupled plasma mass spectrometry (ICP-MS) instrumentation, in many cases (metals analysis in drinking water, for example) a single ICP-MS has replaced all of the above mentioned techniques, enabling all analytes to be determined in a single measurement. The analysis of plant and food digests for nutritional studies is more challenging. In ICP-MS, isobaric interferences arise from the argon used to sustain the plasma and from the reagents used for sample preparation. Table 1 summarizes some well-known interfering species. In biological sample analysis, there are well-documented interferences for ICP-MS that can bias the measurement of Fe, Cr, V, As and Se at trace levels, with the result that ICP-MS has not yet been widely adopted by the foods industry.

Table 1. Examples of Potential Interferences in Biological/Clinical Matrices

Element	Mass	Molecular interference
Cr	52; 53	$^{40}Ar^{12}C$, $^{36}Ar^{16}O$, $^{35}Cl^{16}O^{1}H$; $^{37}Cl^{16}O$
V	51	$^{35}Cl^{16}O$
Fe	56	$^{40}Ar^{16}O$
Cu	63	$^{40}Ar^{23}Na$
As	75	$^{40}Ar^{35}Cl$
Se	77; 78; 80	$^{40}Ar^{37}Cl$; $^{40}Ar^{38}Ar$; $^{40}Ar^{40}Ar$;

One obvious way to remove interferences is to eliminate the source of the interfering species. Traditionally plant materials are digested on a hot plate using a mixture of nitric and perchloric acids. Chloride-based mass spectral interferences are introduced by this method. An alternative sample preparation method is available using microwave digestion with hydrogen peroxide and nitric acid. This digestion media does not generate additional interferences for ICP-MS and is a complete digest. However, for high sample numbers, the traditional hot plate digest offers higher sample throughput than closed vessel microwave digestion [2].

Recently, the advent of collision/reaction cells has improved the detection capability of quadrupole ICP-MS (ICP-QMS) by removing spectral interferences on analytes such as Fe, Cr, V, As and Se. The Agilent 7500c ICP-MS features an Octopole Reaction System (ORS) for highly efficient removal of multiple interferences arising from complex sample matrices. The ORS removes interferences by either reacting a gas with the interference or by preventing the interfering species from entering the analyzer stage using a process called energy discrimination. The 7500c exhibits highly efficient interference removal. The Ar_2 overlap on Se at mass 80 is virtually eliminated, reducing the background equivalent concentration from 100's of ppb to <10 ppt. Moreover, the 7500c was designed specifically to handle complex matrices such as plant and food digests.

The key to the successful multi-element determination of trace elements in complex samples is a combination of matrix tolerance and efficient interference removal. Matrix tolerance is mainly determined by the "plasma efficiency", which must be optimized to ensure efficient sample decomposition, and is monitored by the CeO/Ce ratio. An efficient plasma minimizes the formation of plasma- and matrix-based interferences, while maximizing the conversion of analyte atoms into ions.

The importance of matrix tolerance of any ICP-MS system should not be underestimated, as this leads to improved analytical accuracy, better tolerance to matrix changes and reduced requirements to carry out routine maintenance of the vacuum, ion lens and pump components.

All of these aspects contribute to the usability of the analytical instrument, as routine maintenance contributes far more to the down-time of a modern, reliable ICP-MS instrument than hardware breakdowns. The unique capability of the Agilent 7500 Series lies in the mode of operation of the plasma source, which decomposes sample matrices five to 10 times more efficiently than is typical for other ICP-MS instruments.

The 7500c was designed specifically to handle complex, high matrix samples. A robust 27.12-MHz plasma, low sample uptake rate, cooled spray chamber and proven small orifice interface protect the ORS from contamination by undissociated sample matrix. A novel ion optic, mounted outside the high vacuum region for easy access, further protects the reaction cell, which features an octopole for optimum ion transmission. The octopole is mounted off-axis to minimize random background levels. A schematic of the 7500c is shown in Figure 1.

Some of the important instrument parameters that contribute to good matrix decomposition are:

- The standard low sample flow rate (100 to 400 µL/min) and Peltier-cooled spray chamber reduce the sample and water vapor loading on the plasma, which leads to a hotter plasma central channel.
- The 7500 Series uses a high efficiency, solid state 27.12-MHz plasma RF generator, ensuring good energy transfer into the plasma central channel.
- The unique wide internal diameter plasma torch design ensures that the sample aerosol is resident in the plasma for sufficient time to ensure complete matrix decomposition, leading to exceptionally good matrix decomposition (low CeO/Ce ratio).

The optimized interface design, which uses the smallest skimmer cone orifice of any commercial ICP-MS instrument, ensures that minimal sample matrix is passed into the high-vacuum part of the instrument, dramatically reducing the requirement for routine maintenance of the interface cones, the ion lenses and the collision cell.

In summary, as the complexity of the sample matrix increases, the benefit of minimized interference levels becomes more significant. Because modern analytical laboratories rarely have the luxury of pre-analyzing samples to identify the matrix, it is impractical to rely on matrix matching of the samples or data correction using complicated interference equations.

Sample Preparation and Analysis

About 800 mg of sample was accurately weighed and carefully heated with 10 mL nitric acid (70%), followed by gentle heating with the addition of 8 mL perchloric acid (70%) until colorless. After cooling, 30 mL water was added and heating resumed for 10 min. Finally, the solutions were cooled, then made to 100 mL volume with water.

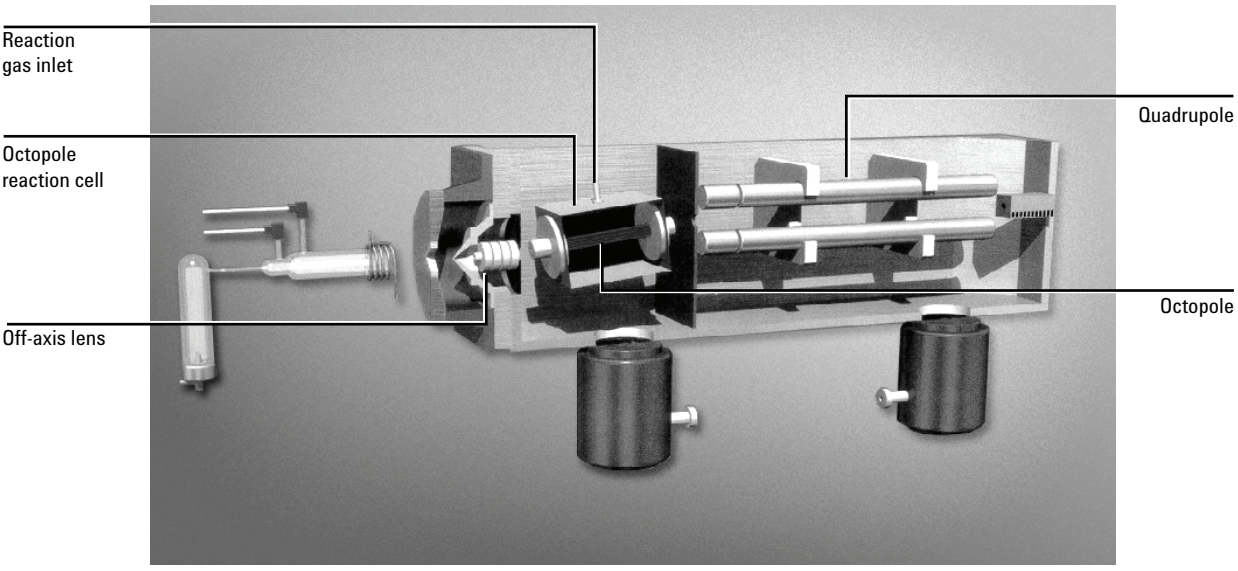

Figure 1: Schematic diagram of the Agilent 7500c Octopole Reaction System.

The instrument was tuned and optimized as detailed in Table 2. Calibrations were performed using external standards prepared from 1000 ppm single element stock, made up as appropriate with 2% nitric acid.

Table 2. Agilent 7500c Operating Conditions

Plasma RF power	1500 W
Sample depth	9.5 mm from load coil
Carrier gas flow	1.1 L/min
Spray chamber temperature	2 °C
Sample flow rate	240 µL/min
Nebulizer	Agilent microflow (PFA)
Interface	Nickel sample and skimmer cones

The external calibrations were run in the same analytical sequence as the samples. Sample concentration was calculated using the internal standard method. Table 3 summarizes the element and relevant internal standard information.

Table 3. Reaction Gases and Internal Standards Used

Measured element	Reaction gas	Internal standard
Potassium	Helium	Scandium
Calcium	Helium	Scandium
Chromium	Helium	Gallium
Iron	Helium	Gallium
Copper	Helium	Cobalt
Zinc	Helium	Cobalt
Arsenic	Helium	Yttrium
Selenium	Hydrogen	Indium (115)
Cadmium	Hydrogen	Indium (115)

Results and Discussion

The practical effect of the 7500c's unique combination of matrix tolerance and interference removal is that complex and variable samples can be measured with a simple quantification procedure using external standard calibration and internal standard correction for all masses. As and Se were accurately quantified at sub-ppb levels, even in a matrix containing 8% perchloric acid. Tables 4 and 5 summarize the results obtained in a blind analysis of plant digests using the 7500c, comparing the results with both the certified values and data obtained from analysis by ICP-OES.

Table 4. NIST 1573a (Tomato Leaves, Blank Corrected)

Name	Certified (mg/kg)	ICPOES (mg/kg)	7500c (mg/kg)
43 Ca	5.05%	5.00%	5.08%
39 K	2.70%	2.72%	2.62%
52 Cr	1.99	1.7, 1.8	1.60
53 Cr	1.99	1.7, 1.8	1.63
54 Fe	368	342, 347	368
56 Fe	368	342, 347	368
63 Cu	4.7	2.49, 2.40	4.43
65 Cu	4.7	2.49, 2.40	4.47
75 As	0.112	5.7, 6.6	0.175
78 Se	0.054	0.1, 0.8	0.061
111 Cd	1.52	5.5, 5.9	1.32

Table 5. NIST 1570a (Spinach, Blank Corrected)

Name	Certified (mg/kg)	Reference 2: (mg/kg)	7500c (mg/kg)
39 K	2.90%	2.63%	2.56%
43 Ca	1.53%	1.32%	1.39%
52 Cr	-	-	1.24
53 Cr		-	1.29
54 Fe	-	252	248
56 Fe		252	250
63 Cu	12.20	11.6	10.48
65 Cu	12.20	11.6	10.51
75 As	0.07	-	0.062
78 Se	0.12	-	0.09
111 Cd	2.89	-	2.33
54 Fe	-	252	248
56 Fe		252	250
63 Cu	12.20	11.6	10.48
65 Cu	12.20	11.6	10.51
75 As	0.07	-	0.062
78 Se	0.12	-	0.09
111 Cd	2.89	-	2.33

Measurements of Cr, Fe and Cu were made on two separate isotopes for each element. Because molecular interferences will, in many cases, only affect one of the analyte isotopes, the presence of an interference can cause a large discrepancy between results for different isotopes of the same element. An example of this is the measurement of Cu in a high Na matrix, where $^{40}Ar^{23}$ Na gives an overlap on ^{63}Cu, but no interference on ^{65}Cu. As the results indicate, the 7500c obtained excellent agreement for all the pairs of isotopes, highlighting the capabilities of the ORS in reducing interfering molecular species that, until now, have prevented the accurate trace analysis of transition metals in complex matrices by ICP-QMS.

Values for major and trace element concentrations agreed both with the expected value and the results obtained from ICP-OES. In the cases where the trace values for some elements were below the detection limit of the ICP-OES, the 7500c returned results in excellent agreement with the certified value. This data illustrates the wide dynamic range of the system and demonstrates its advantages as a replacement for traditional techniques such as ICP-OES.

The quantitative analysis of the NIST SRM samples also demonstrates that both the 7500c and the operating conditions are robust and tolerant of the changing matrix composition found in plant digests.

Conclusions

The trace analysis of plant digests is an application that can be suitably addressed by the 7500c. Advances in technology now allow the determination of multiple elements in complex sample matrices, with efficient interference removal and, in the case of the 7500c, with the excellent matrix tolerance for which the 7500 Series is renowned. Accurate quantification of As and Se at low and even sub-ppb levels in plant digests is possible, even where high concentrations of perchloric acid have been added during the sample preparation stage.

Acknowledgement

The ICP-OES measurements and NIST sample preparation were performed at the University of Queensland, School of Land and Food Sciences, Australia.

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Agilent Technologies

Automated Real-Time Determination of Bromate in Drinking Water Using LC-ICP-MS and EPA Method 321.8

Application

ICP-MS

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Abstract

The suitability of coupling an HPLC to an ICP-MS for the fully automated, routine analysis of bromate in drinking water as per the proposed EPA Method 321.8 was investigated. The necessity to monitor the carcinogen bromate in ozonated drinking waters at single ppb levels has led the USEPA to investigate HPLC-ICP-MS as an alternative technique to the ion chromatography with conductivity detection method currently specified. During this investigation, a series of rigorous performance checks were used to assess the implementation of the proposed method including the determination of bromate in a series of EPA disinfection byproduct (DBP) standards.

Introduction

Ozonation is a common method used for the disinfection of drinking waters. In waters containing bromide (Br^-), such as those found in coastal regions subject to salt-water intrusion, a disinfection byproduct (DPB) of the ozonation process is the bromate ion (BrO_3^-). The bromate ion, produced by the oxidation of bromide, is very carcinogenic, with an estimated lifetime cancer risk of 1:10,000 for a concentration of 5 ppb.[1] The current method specified by the USEPA for the determination of bromate in drinking water uses ion chromatography (IC) with conductivity detection. One disadvantage of this method is the need for a tedious and time consuming sample pretreatment step.

The need for sample pretreatment arises from the potential for co-elution of chloride and bromide ions present in the sample, potentially resulting in false positive results. In order to prevent this from occurring, chloride present in the sample is precipitated out of solution using silver cartridges with subsequent pre-concentration of the bromate ions. This time consuming and lengthy clean-up procedure and pre-concentration step can result in pre-concentration of sulfate ions present in the water. Sulfate can subsequently displace the bromate ions on the resonating column resulting in false negatives.

For these reasons, ICP-MS has been investigated as an alternative, ion selective detector for this analysis. ICP-MS provides the resolution necessary to separate the bromate and chloride ion, thereby eliminating the need for a matrix elimination step. Furthermore, ICP-MS has been used successfully for the analysis of bromate in water samples containing concentrations of chloride in excess of 5000 ppb - much higher than the typical content of ozonated drinking water - without the need for sample pretreatment.[2]

This study will investigate the suitability of ion chromatography coupled to ICP mass spectrometry (ICP-MS) as an automated, real-time measurement approach, to determine low levels of bromate in ozonated drinking water samples, using the proposed EPA Method 321.8.[3]

Instrumentation

The Agilent Technologies 1100 Series HPLC system, coupled to a 7500 Series ICP mass spectrometer using the real-time Plasma Chromatographic software was used for this study. This system was specifically designed for the rigors of automated trace element speciation work, mainly in response to laboratory demands, particularly in the environmental, clinical and food application areas, that need to carry out routine elemental speciation. Its design takes advantage of Agilent's expertise in chromatography and its recognized leadership position in ICP-MS.

During the past few years, the potential of ICP-MS as a detector for elemental speciation studies has been realized.[4] When coupled to a chromatographic separation device, ICP-MS offers unmatched detection capability for laboratories interested in quantifying different species, forms, oxidation states or biomolecules associated with trace elements.[2, 5] Traditional approaches of coupling ICP-MS to chromatography devices are cumbersome, labor intensive and not readily automated. In fact, the majority of ICP-MS chromatography data handling software packages were designed specifically for liquid and gas chromatography (LC, GC) applications and required modification for use with ICP-MS. Some approaches even analyzed the chromatographic spectral peaks "post-run", meaning the data had to be imported into another software package after the analysis was completed, for quantitation purposes. It was clear that there was a real demand for a fully automated system, designed specifically for trace element speciation analysis. Agilent Technologies answered that demand with a fully integrated package for trace element speciation, comprising an 1100 Series HPLC system, coupled to a 7500 Series ICP mass spectrometer, using the Agilent ChemStation and real-time Plasma Chromatographic software.[6]

Methodology

ICP-MS Conditions

The ICP-MS instrumental conditions were optimized to give maximum signal at *m/z* 79, the most sensitive mass for Br. Because bromine is not completely ionized in argon ICP, sampling depth, nebulizer flow, RF power and ion lens voltages have to be optimized very carefully to guarantee the most efficient sampling of bromide ions. Operating conditions for the 7500 are shown in Table 1. These conditions gave an instrument response of 110,000 cps for a 100 ppb bromate standard, with a background of 1,800 cps (partially due to trace levels of bromide in the 18 MΩ deionized water).

Table 1: Optimized Operating Conditions for ^{79}Br Using the Agilent 7500 ICP-MS

Parameter	Optimized conditions
Nebulizer	Meinhard concentric - glass
Nebulizer flow rate	1.05 L/min
Spray chamber	Scott double pass - glass
Spray chamber temperature	2 °C
Sample flow rate	1 mL/min
RF power	1200 W
Sampling depth	Optimized for max signal at ^{79}Br
Ion lens voltages	Optimized for max signal at ^{79}Br

Chromatographic Conditions

See Table 2 for the chromatographic conditions for the separation. The column eluent was passed via a short length of PEEK tubing to a six-port Rheodyne injector equipped with a 100 µL (or 500 µL depending on the measurement) PEEK loop. A post column injection was performed at the beginning of each run (for internal standard purposes, specified in the proposed EPA Method) at the exact time the data acquisition began on the ICP-MS. See Figure 1 for a schematic of the HPLC instrumentation coupled to the ICP-MS.

Table 2: Chromatographic Conditions for the Bromate Study

Parameter	Specification
Eluent mobile phase	25 mM Ammonium nitrate, 5 mM Nitric acid (~pH 2.7) in 18 MΩ Deionized water
Injection volumes	100 µL, 500 µL loops
Post-column injector	Used for internal standardization
Pump flow rate	1 mL/min
Column	Dionex CarboPac PA-100 (94 × 250 mm) - with guard

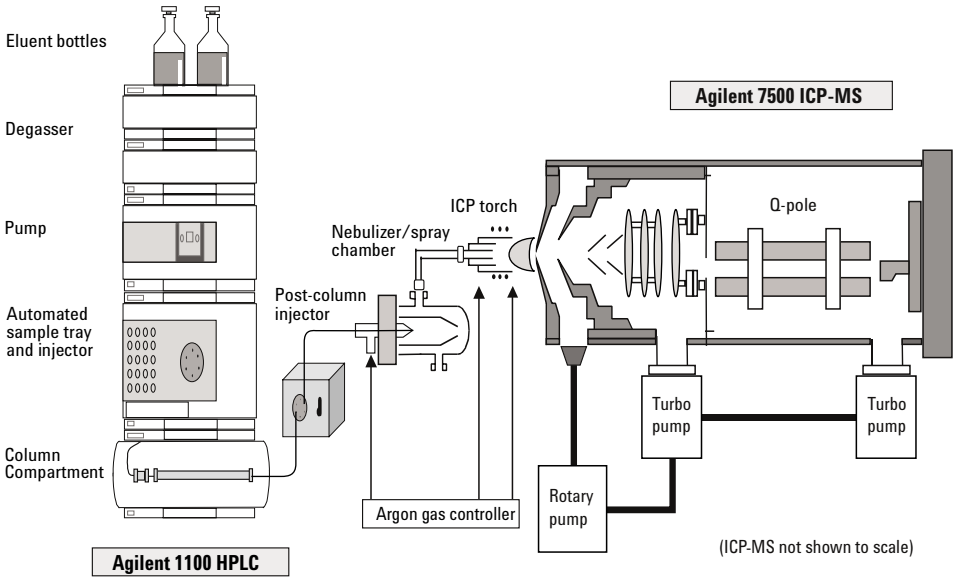

Figure 1: A schematic of the 1100 HPLC instrumentation coupled to the 7500 ICP-MS used for the bromate study.

Sample Preparation

The blank was 18 MΩ deionized water adjusted to pH 10 with NaOH. Standards were prepared daily from a USEPA 1 mg/mL bromate stock solution.

Demonstration of Instrument and Method Performance

As a way of maintaining data quality, the EPA uses performance checks to monitor the instrument and also ensure that the methodology is working correctly. Some of the more important performance checks for this proposed EPA method 321.8 include the measurement of:

- Abundance Sensitivity of ICP Mass Spectrometer
- Method Detection Limit
- Chromatographic Interferences
- Laboratory Fortified Blank
- Laboratory Fortified Matrix
- DBP Performance Sample

These measurements were used to assess the performance of the integrated system used for this study.

Abundance Sensitivity

A large argon dimer, $^{40}Ar^{40}Ar^{+}$ at mass 80 adjacent to the bromate ion $^{79}Br^{+}$ at mass 79, has the potential to bias results in the determination of bromate by ICP-MS. It is therefore critical that the abundance sensitivity, which is a measure of the instrument's ability to separate a trace peak from a major one,[7] is optimized to allow for maximum rejection of the ions at mass 80. The very high operating vacuum of the 7500, and the high frequency of its quadrupole, combined with optimization of the rod-bias voltages, ensures that it achieves clean separation of both peaks, even at a mass of 79.5 amu, where the tail of the $^{40}Ar^{40}Ar^{+}$ might interfere with the Br^{+} at mass 79. The excellent abundance sensitivity of the quadrupole's hyperbolic rods is demonstrated in Figure 2, which shows a spectral scan of 2% HNO_3. The effect of the large signal at mass 80 is shown to have minimal affect on the small bromine signal at mass 79.

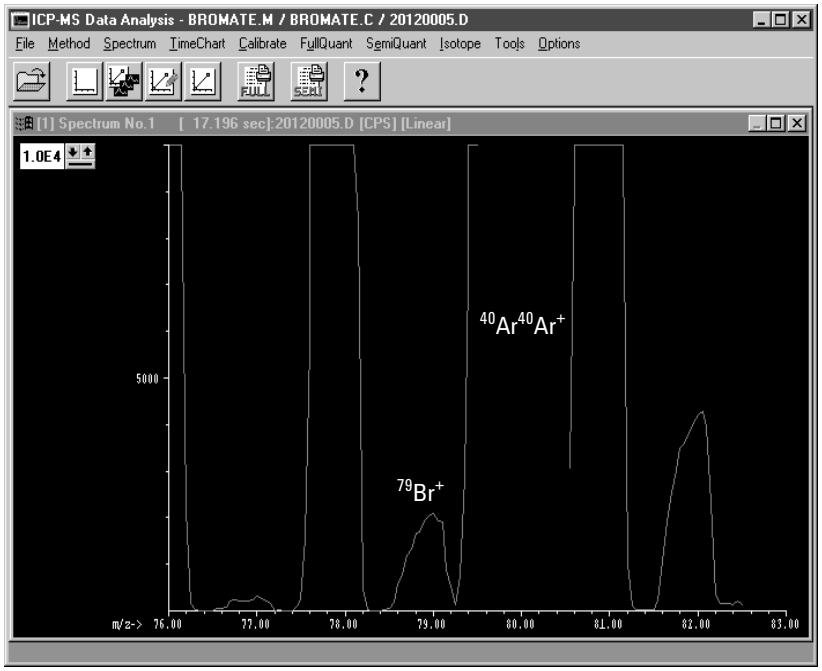

Figure 2: Mass spectrum showing clean separation of $^{79}Br^{+}$ from the argon dimer $^{40}Ar^{40}Ar^{+}$.

Method Detection Limit (MDL)

Two method detection limits were performed - one using a 500 µL loop, as specified in the method, and another using a 100 µL loop. A blank and three calibration standards (1, 5, and 25 ppb bromate) were used for both method detection limit tests.

Seven individually prepared bromate standards of 1 ppb (for the 100 µL loop) and 0.5 ppb (for the 500 µL loop) were then analyzed to determine the method detection limit (MDL). From this, an MDL was calculated for each loop by multiplying the standard deviation of the seven replicate results by 3.14, as indicated in the EPA method. Individual MDL replicate concentrations and statistics for both loops are shown in Table 3.

Table 3: Method Detection Limit Data for a 100 µL and 500 µL Loops

Replicate #	100µL Loop Concentration (ppb)	500 µL Loop Concentration (ppb)
MDL-1	1.1	0.46
MDL-2	0.98	0.39
MDL-3	0.77	0.35
MDL-4	0.77	0.46
MDL-5	0.97	0.45
MDL-6	0.83	0.48
MDL-7	0.90	0.41
Mean	0.90	0.42
SD	0.131	0.044
RSD (%)	14.6	10.5
MDL	0.41	0.14

Chromatographic Interferences

To show that other halogenated compounds do not elute at similar retention times as bromate, a haloacetic acid standard (HAA) standard solution, provided by the EPA, was analyzed. The stock solution was diluted 1:100 yielding final concentrations of six different halogenated compounds reported in Table 4.

Table 4: Concentrations of Six Haloacetic Acid Compounds that Could Potentially Interfere with the Determination of Bromate

Compound	Concentration (ppb)
Monochloroacetic acid	15
Dichloroacetic acid	15
Trichloroacetic acid	5
Monobromoacetic acid	10
Dibromoacetic acid	5
Bromochloroacetic acid	10

A chromatogram containing the haloacetic acid mixture and a 10 ppb bromate standard is shown in Figure 3. The retention time for bromate is 3.5 minutes. The bromine-containing HAA standards elute at 2.5 minutes, 5.9 minutes and 7.1 minutes indicating no chromatographic interference with bromate. Average bromate recovery (n = 2) for this standard spiked with 10 ppb bromate was 102%.

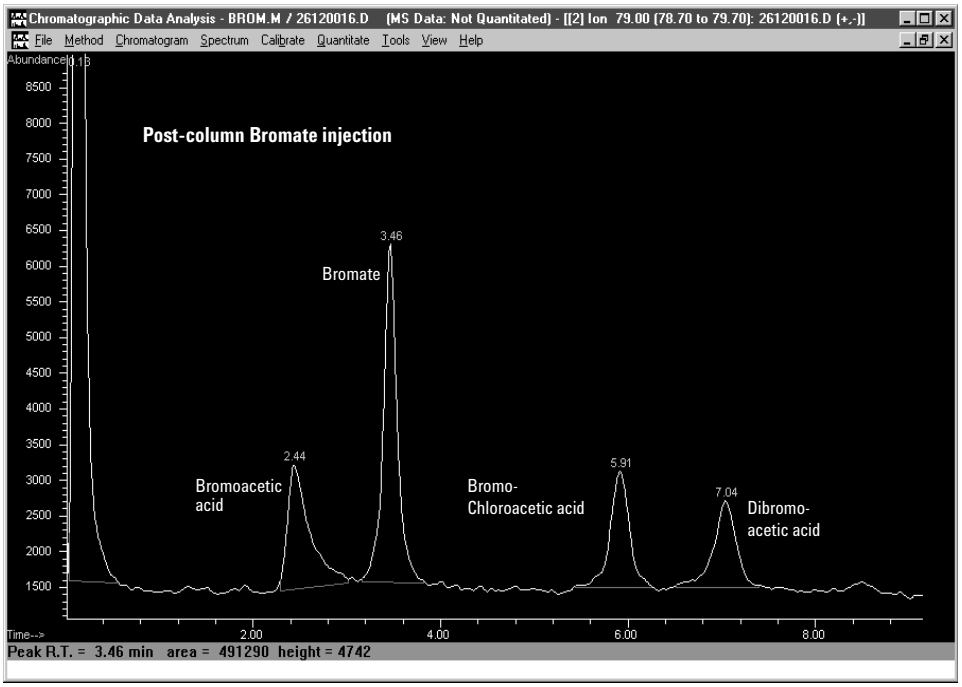

Figure 3: A chromatogram containing haloacetic acid mixture and a 10 ppb bromate standard.

Laboratory Fortified Blank

Ten replicates of a laboratory-fortified blank (LFB) were analyzed at a concentration of 5 ppb, which was approximately ten times the MDL. The LFB samples consisted of 18 MΩ deionized water adjusted to pH 10 with NaOH and spiked with 5 ppb bromate standard. The average for the replicates was 4.7 ppb (8.9% RSD) with a 93% recovery.

Laboratory Fortified Matrices

Four fresh samples supplied by the EPA, taken from ozonation utilities in the U.S., were analyzed using this methodology. Each sample was adjusted to pH 10 with NaOH, and analyzed twice, unfortified and fortified with 10 ppb bromate. The results for all four samples are shown in Table 5. The recovery results for these matrices are all within the EPA guidelines of 70-130% for this method.

Table 5: Bromate Results from Ozonation Utilities

Sample ID	Concentration of bromate in unfortified sample (ppb)	Concentration of bromate in fortified sample (ppb)	% Recovery
A	2.0	12	102%
B	2.7	12	89%
C	4.0	16	118%
D	8.9	18	100%

USEPA DBP Performance Evaluation Check

An EPA check ampule (USEPA ICR PE ampule for inorganic DBPs - Study 9), whose concentration was not known at the time of analysis, was also analyzed as a blind check sample. The ampule was prepared in duplicate by diluting 1:100 and analyzing immediately. Results are shown in Table 6. Once again, the recoveries are both within the recommended guidelines.

Table 6: Recovery of Inorganic DBPs in EPA Check Ampules

Sample	Concentration in original ampule (ppb)	% Recovery (917 ppb true value)
Ampule 1	1120	120
Ampule 2	1040	113

Conclusion

The ability to measure bromate in ozonated drinking waters at sub-ppb levels is essential to understanding its risk assessment as a carcinogen. Once USEPA Method 321.8 is validated for use, ICP-MS detection coupled to HPLC will become an approved method for achieving this. It has been shown that the instrumentation used in this study surpasses all the performance criteria specified in

the methodology, achieving a method detection limit of 0.14 ppb, with a 500 µL loop and 0.41 ppb with a much smaller injection volume (100 µL). Furthermore, this has been implemented in an automated fashion with real time data analysis using the Agilent 1100 LC and 7500 Series ICP-MS demonstrating that the technique is well suited for use as a routine analytical tool.

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The Determination of As in Samples Containing High Concentrations of Chloride by ICP-MS

Technical Note

Michiko Yamanaka

Introduction

One of the main advantages of ICP-MS over ICP-AES is its relative freedom from spectral interferences. There are, however, a few cases where spectral overlap is a problem. One example is the determination of As in samples containing high (%) levels of chloride. The polyatomic ion $^{40}Ar^{35}Cl$ interferes with ^{75}As, and in addition $^{40}Ar^{37}$ Cl interferes with ^{77}Se. The interference of ArCl on Se is not a problem since a different Se isotope can be selected. As, however is monoisotopic, so no alternate isotope is available. One method to overcome such polyatomic overlaps is to resolve the interference using high resolution. In this case, however, a resolving power of >7500 is required to effectively separate As and ArCl. At this resolution, ion transmission is only ~1% of the transmission at unit mass resolution and so detection limits are compromised.

The 4500 ICP-MS offers the precise, routine determination of As even in very high concentrations of chloride by the use of mathematical correction. Elemental (interference correction) equations resident in the 4500 ICP-MS ChemStation software correct for the interferences on both As and Se simultaneously. Most importantly, the inherent ion signal stability of the 4500 ICP-MS allows for very precise correction, making it possible for the 4500 ICP-MS to determine As at the ppb and sub-ppb level even in 5% HCl. This technical note examines the effect of increasing Cl concentration on the observed As signal, and determines the ability of the 4500 ICP-

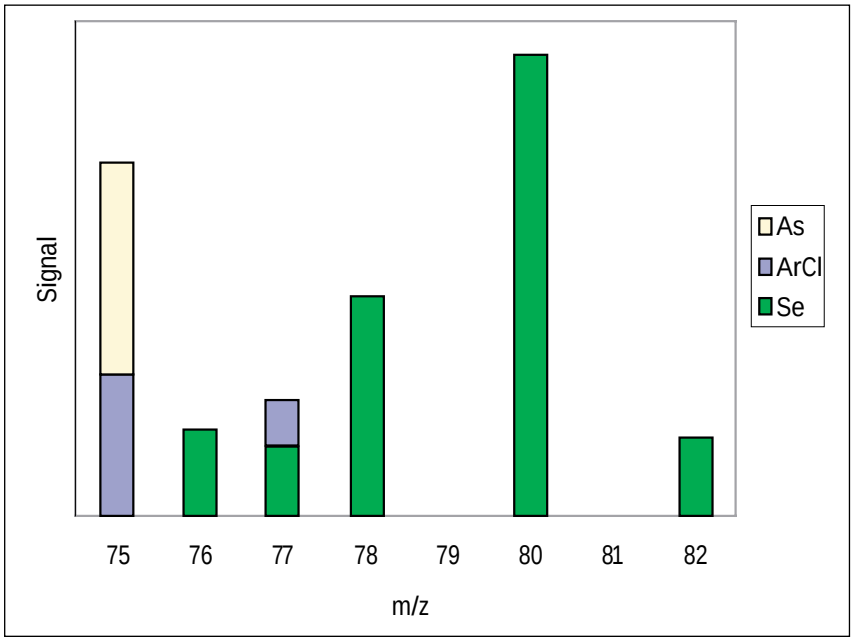


Fig. 1
Isotopic patterns of As, Se and ArCl

MS to measure As in HCl. The derivation and use of elemental equations is also explained.

Elemental equations

In samples containing chloride, the ion signal measured at mass 75 is the sum of ^{75}As and $^{40}Ar^{35}Cl$. This is shown in Fig. 1, which depicts the elemental ratios of As, Se and ArCl. This diagram is for graphical representation only; the intensities of the bars are arbitrary and do not relate to concentration values of each individual species. Also, Ar_2 species have been omitted for clarity. The ratio of the ArCl species at masses 75 and 77 is the same as the ratio of the Cl isotopes at masses 35 and 37. Therefore, the signal intensity of ArCl at mass 75 can be derived from the ArCl signal at mass 77. However, Se also has an isotope at mass 77, so the presence of Se will increase the observed signal at mass 77. Thus the contribution of Se to the total signal intensity at mass 77 must also be calculated using an alternate Se isotope. For Se correction, mass 82 is normally chosen, since the 78 and 80 isotopes suffer interference from Ar_2. In practice, this correction is simply and automatically performed by the ChemStation software using elemental equations. In this example, the equation given in EPA method 200.8 (trace metals in drinking water and wastewater by ICP-MS) was used and is shown in Fig. 2.

Influence of Cl Concentration on As Signal

To study the effectiveness of using elemental equations to correct for Cl interference, a series of 1 µg/l (ppb) As solutions were spiked with Cl at 0, 100, 200, 500 and 1000 mg/l (ppm). The solutions were measured for As, both with and without the use interference correction. A plot of observed As concentration vs. added Cl is shown in Fig. 3. Without correction, the observed As signal increased with added Cl, as expected. At 1000 mg/l Cl, the apparent increase in As was approx. 1 µg/l. Using interference correction, however, no increase in As concentration was reported, demonstrating the effectiveness of interference correction. The 4500 ICP-MS generates a very stable ion signal from both elemental and polyatomic ions, allowing precise, reproducible correction for ArCl. Ion signal stability (typically <1%RSD over 2 hours) is due mainly to the mechanical and electronics design of the 4500 ICP-MS, but also partly due to the precise temperature control of the spray chamber (+/-0.1 °C), enabling a very stable sample aerosol to be generated.

As signal intensity (mass 75)

$= 1.000 * (^{75}C) - 3.127[(^{77}C) - (0.815) * (^{82}C)]$

$= 1.000 * (^{75}C) - 3.127 * (^{77}C) + 2.549 * (^{82}C)$

(^{M}C): ICP-MS signal (counts) at mass M

Fig. 2
Elemental equation for As

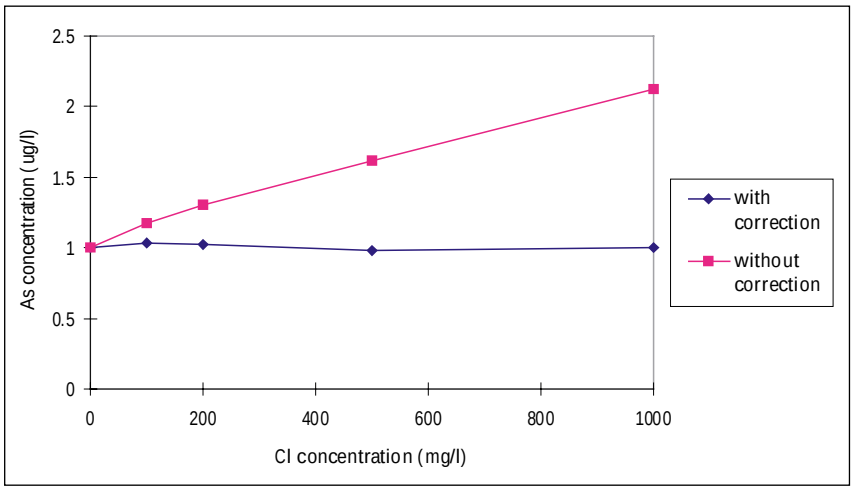
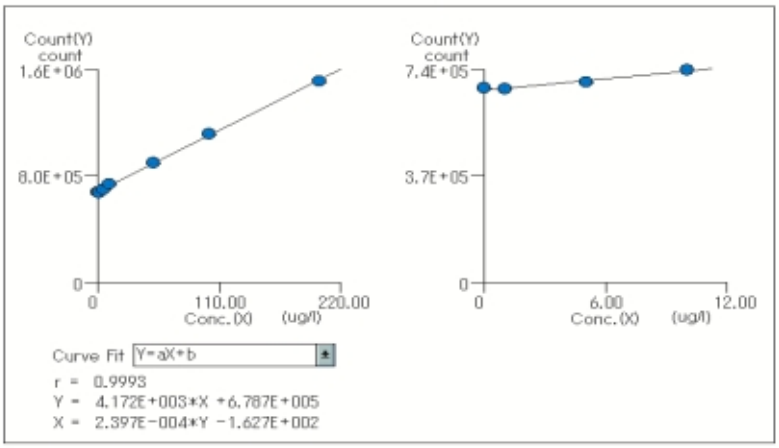


Fig. 3
Influence of Cl on As signal

Determination of As in Cl Matrix

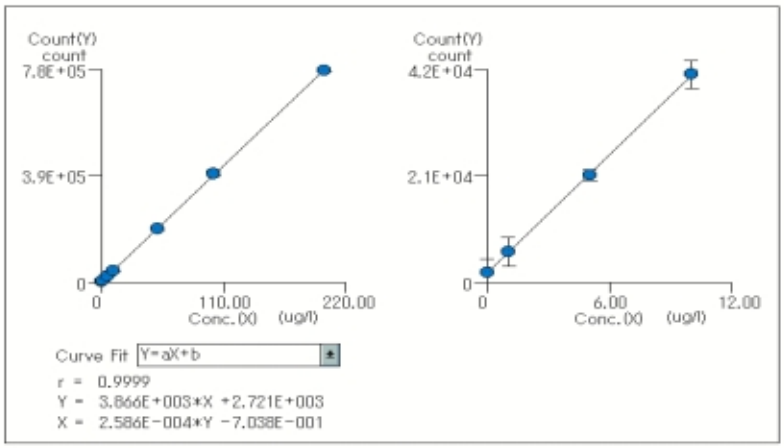
To study the effectiveness of applying interference correction to the quantitative determination of As in a chloride matrix, a series of standard solutions in a 5% HCl matrix were prepared. The As concentrations were 0, 1, 5, 10, 50, 100 and 200 µg/l. The standards were measured using the 4500 ICP-MS - the operating parameters used are given at the bottom of this page. No internal standards were used. Calibration plots for As were constructed, both with and without interference correction selected. The data was



Concentration range:

0-200 µg/l **0-10 µg/l**

Fig. 4-1
As calibration plots in 5% HCl without interference correction



Concentration range:

0-200 µg/l **0-10 µg/l**

Fig. 4-1
As calibration plots in 5% HCl with interference correction

not blank subtracted. The plots shown in Fig. 4-1 were generated without applying interference correction. Although the corration was good, the high background due to ArCl can be seen. The scale is expanded on the right to show the low concentration points. Below 10 µg/l, the calibration plot becomes essentially flat. In contrast, when interference correction is applied (Fig. 4-2), excellent correlation and linearity were obtained, even at the 1 µg/l level. Clearly, the combination of interference correction and good ion signal stability (elemental and polyatomic) allow the 4500 ICP-MS to precisely determine As even in a chloride matrix. The ability to detect As at sub-ppb levels in the presence of chloride is particularly important to the study of toxic metals in foods, biomedical, environmental and clinical applications. In addition, the demonstrated ability of interference correction to compensate for polyatomic overlap at low ion concentrations can be applied to other classic ICP-MS interferences. Other interferences will be studied in future Agilent Technical Notes.

Operating conditions

RF power	: 1.3 kW
Sampling depth	: 8 mm
Plasma gas	: 16 l/min.
Auxiliary gas	: 1.0 l/min.
Carrier gas	: 1.15 l/min.
Nebulizer	: Concentric type

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Agilent Technologies
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Determination of Mercury in Drinking Water Samples by ICP-MS Using EPA Method 200.8

Application Note

Environmental

Abstract

The quantitative determination of mercury in drinking water samples, simultaneously with 20 other elements (as described in the EPA Method 200.8) is presented. To avoid the Hg memory effects normally experienced with conventional nebulizer/spray chamber sample introduction systems, gold was added off-line to all standards and samples to act as a complexing agent. The addition of gold and the design of the 4500 ICP-MS assure fast washout time and allow the determination of all elements, including mercury, in drinking water by a single ICP-MS run.

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Introduction

EPA Method 200.8[1] describes the multi-element determination of trace metals waters and wastes by inductively coupled plasma-mass spectrometry (ICP-MS). This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for the determination of total recoverable element concentrations in these waters as well as waste waters, sludges and solid waste samples. EPA Method 200.8 is applicable to 21 elements, including mercury, enabling all required elements to be analyzed by a single technique - ICP-MS. This application note describes the additional sample preparation necessary for the successful determination of mercury.

As stated in the method, samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as *direct analysis* (section 1.4 - EPA Method 200.8).

For the *direct analysis* of water samples which do not require digestion/extraction prior to analysis, and for which turbidity is < 1 NTU, the combined concentrations of inorganic and organo-mercury species in solution can be determined provided gold is added off-line to both samples and standards alike (section 1.6 - EPA Method 200.8)[1].

System Design

The 4500 ICP-MS was developed with the routine user in mind: innovative hardware and software design has resulted in the automation of routine tasks such as the optimization of ion lenses, plasma conditions, and adjustment of the ICP torch position. By making optimization independent of the operator, performance becomes more consistent, even in a multi-user environment.

A programmable computer-controlled peristaltic pump system allows for a selection of the optimum rinse time to accommodate both high sample throughput and effective elimination of memory effects. An example of a peristaltic pump program is shown in Figure 1.

The pump speed and time both before acquisition (sample uptake) and after acquisition (rinse) can be set by the user. Pumping the sample into the system at high speed reduces sample uptake time, and a stabilization time allows the system to stabilize at the normal acquisition uptake rate prior to the commencement of data acquisition. The system can also be programmed to rinse longer after standards than samples, minimizing total rinse time. An second optional rinse following acquisition is also available, enabling the use of two different rinse solutions for special applications.

Memory Interferences

Memory interferences, commonly referred to as *memory effects* arise when analyte signal is enhanced due to contribution

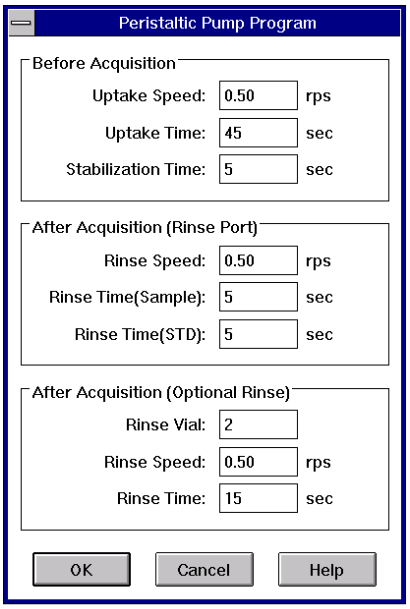

Figure 1.
Peristaltic Pump Program

from a previous high concentration sample. Memory effects can result from the adsorption/desorption of the analyte anywhere in the sample introduction system: Peristaltic pump tubing, nebulizer, spray chamber, torch or interface.

In acidic solution, mercury has a tendency to be retained on the glassware, particularly on the injector tip of the torch and in the spray chamber. As a result, the analyst has to program long washout times, and the aspiration of a sample containing very high levels of mercury will require the sample introduction system to be dismantled and thoroughly cleaned. The off-line addition of gold to the sample solution dramatically reduces washout times, since gold complexes with mercury presumably forming an amalgam, allowing it to be washed effectively from the system. The addition of gold to both standards and samples enables determination of mercury in the same analysis as for the other 20

elements in the EPA Method 200.8, allowing all elements to be measured in a single run by ICP-MS alone.

Instrumentation

The instrument used for this work was a 4500 ICP-MS fitted with a Babington-type nebulizer, glass spray chamber and quartz one-piece torch. An ASX-500 autosampler (CETAC Technologies Inc., Omaha, NE), was also fitted.

Reagents, Standards and Labware

The importance of good quality of reagents used was discussed in the Agilent Application Note (publication No. 5964-4277E)[2]. For this work, a Milli-Q SP point-of-use deionized water system (Millipore, Bedford, MA) was used to prepare all standards.

Plasma gas flow rate	15.0 L/min	
Aux. gas flow rate	1.0 L/min	
Carrier gas flow rate	1.17 L/min	
RF Power	1300 W	
Nebulizer	PEEK, Babington - type	
Spray chamber	Glass, double pass	
Spray chamber temp	1 deg C	
ICP torch injector	Quartz, 2.5 mm	
Sample uptake rate	0.4 mL/min	
Sampler cone	Nickel	
Skimmer cone	Nickel	
Sampling depth	6.4 mm	
Acquisition parameters	**Quantitative**	**Monitoring**
Points/mass	3	6
Integration time/mass	0.99 sec	0.6 sec
Total acquisition time/replicate	36 sec	139 sec
Replicates	3	1
Total acquisition time/sample	109 sec	139 sec

Table 1.
4500 ICP-MS Operating Parameters

Fresh mercury standards were prepared daily from a 10 mg/L (ppm) stock solution. Gold was added off-line to all standards and samples at the level of 100 mg/L (ppb), along with the internal standard (Tb at 50 µg/L). Both standards were prepared from

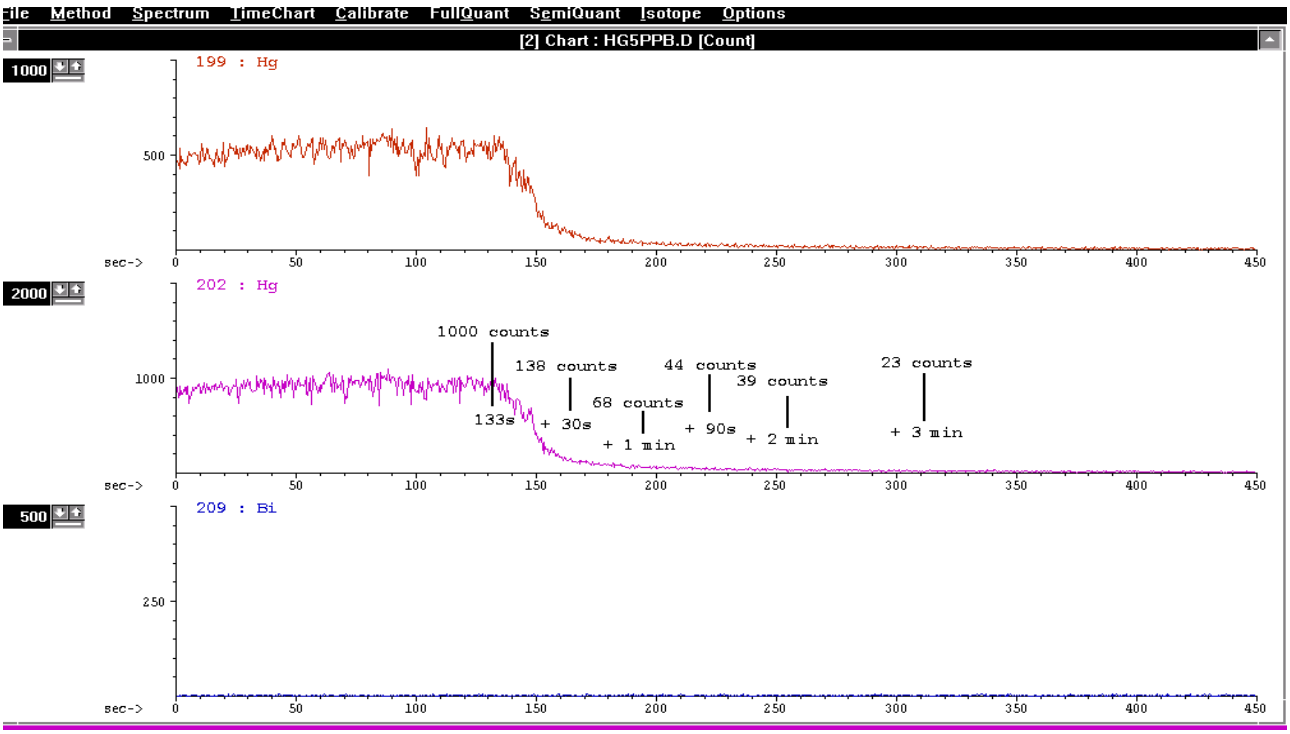

Figure 2.
Monitoring of the Mercury Washout Time

1,000 mg/L stock solutions (Inorganic Ventures, Lakewood, NJ). All standards and samples were acidified with 1% (v/v) ultrapure nitric acid (Optima Grade - Fisher Scientific, Pittsburgh, PA)

Experimental

To study the effect of gold addition on mercury washout, a 5 µg/L Hg solution was spiked with 100µg/L Au and aspirated by the 4500 ICP-MS. The washout of the mercury signal with a gold wash solution was measured by monitoring the signal in time resolved mode. Two mercury isotopes were monitored, plus bismuth for the purpose of background monitoring. Figure 2 shows the washout characteristics observed for a ^{5} ppb mercury standard solution. For ^{202}Hg, the signal counts measured at readings signal counts 30 seconds apart are shown. Two orders of magnitude washout for mercury was achieved in less than one minute, demonstrating much better washout than in acidic solution without the addition of gold. Operating parameters for both the washout study and routine quantitative analysis are given in Table 1.

A graphical representation of the acquisition method printed from the Agilent ChemStation software is shown in Figure 3. This method was applied for all 21 elements listed in EPA Method 200.8, including Mercury. A mercury calibration containing standards at 0, 2 and 5 ppb Hg was constructed and is shown in Figure 4. Terbium (mass 159) was used as the internal standard (IS). As can be seen, an excellent fit was obtained, and from the slope of the curve, detection limits in the low ng/L range can be estimated.

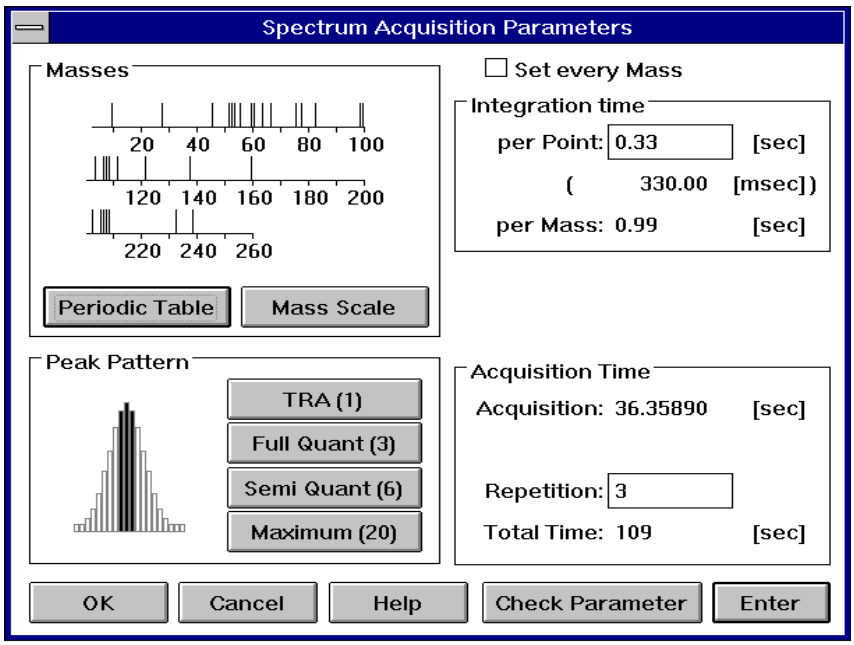

Figure 3.
Acquisition Parameters for Quantitative Determination of 21 Elements (including Mercury) by ICP-MS According to EPA Method 200.8

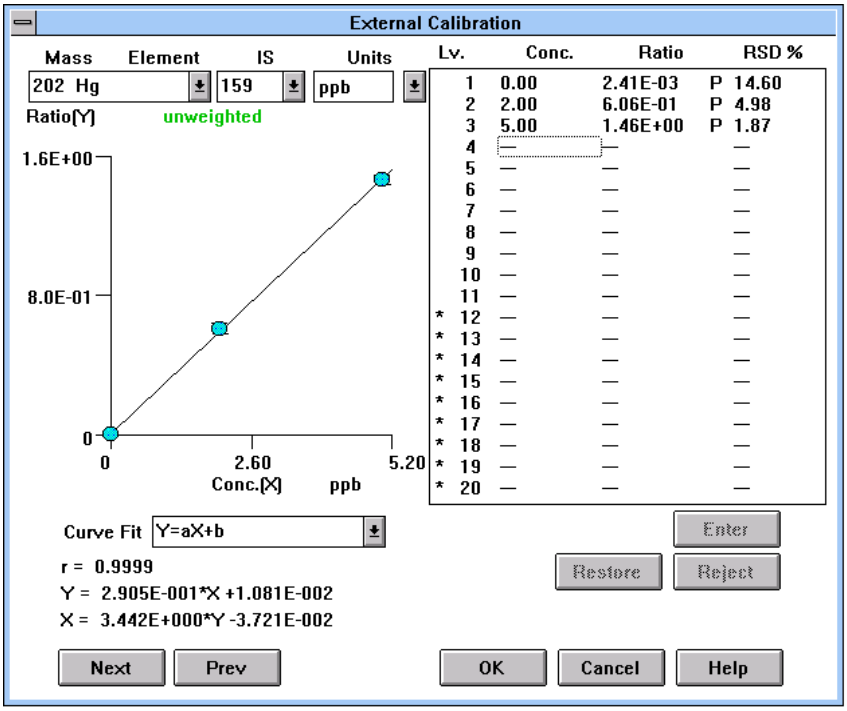

Figure 4.
Mercury Calibration Curve (Tb used as internal standard)

EPA Method 200.8 (section 7.4.1) specifies the maximum concentration of the calibration standard to be 5 ppb.

Conclusions

The determination of mercury has been shown to be easily i ncorporated into the standard multi-elemental analysis protocol of water samples using ICP-MS. Mercury carry-over was readily eliminated by the off-line addition of gold. This procedure allowed the analysis of mercury in the same run as the other analytes, enabling the measurement of all required elements by a single instrument. The addition of gold to the samples at the time of collection will minimize losses of mercury in sampling vessels.

References

[1] EPA Method 200.8 EMMC version. Revision 5.4. EMSL Cincinnati OH 45268 - May 1994

[2] Analysis of Drinking Water and Waste Water by ICP-MS Using EPA Method 200.8. Agilent Application Note (publication No5964-4277E), December 1997

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Publication number 5965-5383E

Agilent Technologies
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Meeting UK Drinking Water Inspectorate Requirements for Trace Metals Analysis Using the 4500 ICP-MS

Application Note

Environmental

Glenn D. Woods
Ed McCurdy

Abstract

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has gained wide acceptance for the determination of many trace elements, but is less frequently used for the determination of higher levels of elements, due to its perceived limitations in dynamic range and matrix tolerance.

Several applications require the measurement of trace and minor elements in the same sample, which generally means that laboratories must employ multiple techniques to perform a complete analysis. One such application is the measurement of inorganic components in drinking water, where the analyte concentrations that must be measured range from sub-ug/L (ppb) to 100's of mg/L (ppm). Traditionally, this analysis would have been carried out using a combination of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and hydride and fluorescence techniques specifically for As, Se, Sb and Hg.

This paper discusses the validation of the 4500 ICP-MS ICP-MS for this analysis, allowing all of the controlled elements to be measured in a single run, using a single technique. Results are presented from the performance testing of the 4500 ICP-MS for the analysis of 26 trace and minor elements in Drinking Water, using the validation and quality control criteria defined in the NS-30 "A Manual on Analytical Quality Control for The Water Industry" by the UK Water Authority.

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Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) has been used for the determination of many trace and minor elements in various samples from diverse fields including environmental, geological, metallurgical, semiconductor, petrochemical and biomedical. One of the principal benefits which has led to the widespread use of ICP-MS has been its wide elemental coverage and excellent detection limits for a range of elements which are impossible to measure by a single alternative technique. In particular, for the measurement of trace elements at ug/L and ng/L levels, ICP-MS has often been able to replace Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), hydride generation and fluorescence techniques, allowing all important toxic trace elements to be measured in a single analysis.

Typically, however, ICP-MS has been perceived as inappropriate for the determination of higher levels of analytes, due to limited matrix tolerance and excessive sensitivity, which limits the upper calibration level. Although most ICP-MS instruments have the capability to measure over a wide dynamic range (8 orders of magnitude), this usually requires regular and time consuming cross calibration across two detector modes and still allows measurement only up to a few 10's mg/L.

(1) Drinking Water Quality

In the United Kingdom, drinking water quality is monitored by the Drinking Water Inspectorate (DWI), using the Water Supply (Water Quality) Regulations of 1989. These regulations list a total of 56 parameters, including colour, turbidity, organic compounds, anions and inorganic elements, which must be monitored in drinking water supplies. The list includes 21 inorganic elements, ranging from Ca (maximum concentration or Prescribed Concentration Value (PCV) of 250 mg/l) to Hg (PCV of 1 ug/L). The regulation requires that the detection limit (calculated from 4.65 x the standard deviation of the blank) for each determinand must be less than one tenth of the PCV, so the detection limit of Hg, for example, must be less than 0.1 ug/L.

Under the Drinking Water Regulations, each laboratory is required to performance test the analytical systems for each parameter before that analytical system can be used for routine analysis of compliance samples. The design of the performance testing and calculation of the performance characteristics should be in accordance with the guidance given in the publication "NS-30", a manual on analytical quality control in the Water Industry.

(2) Requirements for Acquisition of Performance Testing Data

Taking into account the DWI requirements and NS-30 guidelines, the following protocol for performance testing is typical for metals analysis:

1) The calibration range should be such that all results fall within the range.

2) The calibration must have at least 3 points plus blank, to demonstrate a straight line.

3) Samples and standards must be prepared fresh, before each batch.

4) A maximum of 2 batches can be analysed on any one day, provided the instrument is switched to overnight conditions between batches.

5) Samples must be analyzed in random order.

6) Samples must be analysed in replicate, in at least 5 batches. In practice, analysis of duplicate samples in 11 batches satisfies the DWI condition on degrees of freedom.

7) A batch of samples must consist of the following:
Blank, Standards (typically at concentrations appropriate to the PCV and the levels found in representative samples) and Samples of the type to be measured routinely.

(3) Requirements for Statistical Validation

After acquiring the concentration data, the results must satisfy the following QC criteria:

1) The maximum tolerable error of individual results should not exceed 1/10 of the PCV or 20% of the result, whichever is the greater.

2) The maximum tolerable standard deviation of individual results should not exceed 1/40 of the PCV or 5% of the result, whichever is the greater.

3) The maximum tolerable systematic error (or bias) of individual results should not exceed 1/20 of the PCV or 10% of the result, whichever is the greater.

4) The estimates of total standard deviation must not be significantly greater at the 95% confidence level than the specified maximum tolerable total standard deviation at the relevant concentration.

5) The recovery of an added spike should not be significantly less than 95%, or significantly greater than 105%.

6) The limit of detection must be lower than 1/10 of the PCV.

The performance testing protocol described in NS-30 validates not only an analytical instrument, but also the entire laboratory protocol. If any aspect of sample or calibration standard preparation is not reproducible, then the error will be observed in the between batch variation. For this reason, it is essential that sample and standard preparation techniques are well developed and carried out reproducibly.

In a large analyte suite, there may be several issues that must be addressed regarding element stability, compatibility and cross-contamination, in addition to straightforward issues of the selection of appropriate glass/plasticware to avoid element leaching or adsorption. As Hg was one of the required analytes, Au was added to the standards and samples at 100 ug/L final concentration to stabilize Hg. In the absence of Au, the Hg signal is found to be unstable and exhibits extended washout times. The internal standard (IS) mixture, which contained Be, Sc, Y, In, Tb and Tl, was added to the standards and samples automatically by means of the on-line IS addition system.

The 4500 ICP-MS was validated for the 21 controlled elements (B, Na, Mg, Al, P, K, Ca, Cr, Fe, Mn, Ni, Cu, Zn, As, Se, Ag, Cd, Sb, Ba, Hg and Pb) in drinking water, according to the NS-30 protocol. At the same time, the 4500 ICP-MS was also validated for a further 5 elements (Li, V, Co, Sr and Sn). Whilst NS-30 lists 21 inorganic components which must be monitored, additional elements may also be measured, provided that the NS-30 protocol has been followed and validation requirements have been met. Thus, each lab can extend the validated elemental range of the technique to meet their own needs and also their customer's specific requirements.

Instrumentation

The ICP-MS instrument used was a standard Hewlett-Packard 4500 ICP-MS, in conjunction with a Cetac ASX-500 random access autosampler. The 4500 ICP-MS was configured with the standard sample introduction system, which consists of a Agilent High Solids nebuliser, quartz spray chamber, quartz one-piece torch and Ni sample and skimmer cones. The standard 4500 ICP-MS sample introduction system is ideally suited to the analysis of high-matrix environmental samples, as discussed below:

• Low Sample Uptake Rate

The sample uptake rate of the 4500 ICP-MS is only 0.1 to 0.4mL/min, compared to between 0.8mL/min and 2.5mL/min, which is typical for conventional ICP-MS or ICP-OES instrumentation. This lower solution flow rate means that matrix loading on the plasma is minimised. In turn, this means that the plasma can dry, decompose, dissociate, atomise and ionise the sample analytes and matrix more efficiently, resulting in reduced spectral interferences and reduced sample matrix effects.

The matrix tolerance capabilities of the Babington-type nebuliser are well known, but commercially available versions of these nebulisers tend to require very high solution flow rates and can be prone to pulsing and poor washout. The Agilent High Solids nebuliser is a modified Babington-type design, manufactured for the 4500 ICP-MS. It features a wide, square section groove cut into an angled front face and optimised to produce a stable aerosol at low sample uptake rates. The design of the groove ensures that no sample solution is trapped on the nebuliser face, which in turn prevents spiking during washout due to sample re-nebulisation.

• Low Polyatomic Ion Formation

Some of the most troublesome interferences in ICP-MS are caused by the overlap of polyatomic ions formed from combinations of oxygen with the argon carrier gas or matrix ions. If the sample introduction area, in particular the spray chamber, is maintained at a constant low temperature (0-2 °C), the water vapour loading in the sample aerosol can be reduced and so the cooling effect of the aerosol on the plasma is reduced. This results in a higher plasma temperature and gives more efficient breakdown of oxide species.

The normal method for monitoring the likely impact of oxide overlaps in the ICP-MS spectrum is by measuring cerium. Of all elements,

cerium has one of the highest metal-oxide (MO) bond strengths. The CeO/Ce ratio can therefore be used as a "worst-case" indicator of the likely interference problems an ICP-MS instrument will suffer in real sample analyses, where high levels of elements such as S, Cl, Al, Mg, Ca, etc. (all of which will form oxide species at much lower levels than Ce) might be encountered.

On the 4500 ICP-MS, the typical CeO/Ce ratio is <0.5%, partly due to the use of a cooled spray chamber, but also as a result of the use of a wide bore injector (2.5mm diameter) in the plasma torch. The wide injector diameter ensures that the sample aerosol is relatively diffuse in the central channel of the torch and so the plasma energy can decompose the sample matrix more efficiently, breaking up any refractory oxide species. Furthermore, the wide torch injector reduces gas velocity through the central channel giving a longer sample residence time in the plasma, which also assists matrix oxide decomposition.

• Linear Calibration

In order to determine high and low level elements in the same run, an ICP-MS instrument should have a wide dynamic range. Most ICP-MS instruments achieve this using a detector that can be operated in both pulse-count and analog mode for the measurement of low intensity and high intensity signals respectively. The 4500 ICP-MS has the capability to construct a single linear calibration line for all elements, from ng/L to 100's mg/L levels, without regular adjustment of detector or tuning parameters. The cross-calibration of the 2 detector modes is achieved using a single solution, analysed once, and the calibration is stable over long periods of analysis.

Standard and Sample Preparation

Calibration stock standards were obtained from BDH and a series of working stock solutions was prepared, each stock containing compatible groups of the analytes. Ca, Na, Mg, K, Al, Fe, Cu and Zn were prepared from 10,000 mg/L stock solutions while the other elements were prepared from 1,000 mg/L single element stocks. Calibration standard solutions were prepared at concentrations appropriate to the levels normally found in the sample types to be tested. Hg and Se were prepared in a separate stock from the other elements. In order to analyze Ag, all samples and calibration standards were prepared in 1% v/v HNO3 and 0.5% v/v HCl solution.

Two tap waters, one river derived and the other borehole derived, were analyzed as samples and two Blanks, two Analytical Quality Control (AQC) solutions and two Spiked solutions were prepared. Two vials were prepared for each blank, sample, spike and AQC. All the standard and sample solutions were freshly prepared for each batch.

The Internal Standard solution, which contained Be, Sc, Y, In, Tb and Tl, was added to the samples and standards by means of the on-line IS addition system of the 4500 ICP-MS. After on-line dilution in the sample stream, the final concentration of the internal standards was approximately 0.1mg/L, with the exception of Be, which was 10x higher to compensate for its low degree of ionisation.

Automatic setup of the pulse count/analog (P/A) factor of the detector was carried out using a tuning solution which contained Ca, B, P, Fe, Ba, Na, Mg, Al, K, Cu Zn and Sr at concentrations between 0.1mg/L and 100mg/L. The appropriate concentration for each element was selected, to give an acceptable count-rate in both detector modes.

Experimental

(1) Instrumental Conditions

After turning on the plasma and allowing 15 minutes for the system to warm up, the instrument was tuned by using 3 of the elements present in the internal standard solution (Be, Sc and Tl).
The instrument was tuned by monitoring mass 9, 45 and 205, to give a sensitivity of around 200,000, 200,000 and 300,000 counts per second, respectively. This represents at least a factor of 10 lower sensitivity than can be achieved when the instrument is tuned for maximum sensitivity. i.e. the system was "detuned". With a system tuned for maximum sensitivity, the higher level analytes such as Na and K would be "over-range", i.e. above the maximum measurable concentration of the instrument.

For the successful analysis of environmental samples by ICP-MS, several sample introduction and plasma parameters must be considered, namely RF power, carrier gas flow, sample uptake rate and sampling depth. Higher plasma temperature and longer residence time of the analytes are critical parameters in order to decompose heavy matrices effectively and to minimize oxide formation. Table 1 shows the parameters used for routine

Parameter	Setting
Forward power	1350W
Peri-pump speed (analysis)	0.1 rps
Peri-pump speed (uptake/rinse)	0.3 rps
Sampling depth	8.5 mm
Carrier gas flow	1.25 L/min
Rinse time	30 sec
Acquisition Time	73 sec
Number of repeats	3

Table 1
4500 ICP-MS Operating Conditions for the Analysis of High Matrix Samples

1. Blank 1	13. Tap water A + Spike (L) 1
2. Blank 2	14. Tap water A + Spike (L) Hg/Se 1
3. AQC (L) 1	15. Tap water A + Spike (L) 2
4. AQC (L) Hg/Se 1	16. Tap water A + Spike (L) Hg/Se 2
5. AQC (L) 2	17. Tap water B 1
6. AQC (L) Hg/Se 2	18. Tap water B 2
7. AQC (H) 1	19. Tap water B + Spike (H) 1
8. AQC (H) Hg/Se 1	20. Tap water B + Spike (H) Hg/Se 1
9. AQC (H) 2	21. Tap water B + Spike (H) 2
10. AQC (H) Hg/Se 2	22. Tap water B + Spike (H) Hg/Se 2
11. Tap water A 1	23. Drift
12. Tap water A 2	24. Drift Hg/Se

analysis of environmental samples using the 4500 ICP-MS.

(2) Sample Analysis

Each analytical batch consisted of the blanks, samples, spikes and AQC's shown below. Following analysis of the calibration standards, the sample batch was analysed in random order. A different random order was used for each batch, ensuring that no bias was introduced by running the test solutions in a constant order. Drift check solutions, which do not form part of the validation sequence, were analyzed at the end of each batch.

The analysis of Hg and Se was separated from the other elements for two reasons:

1) Following successful validation of the method, the mixed calibration standards should be stable for a week. However, Hg would not be expected to be stable over this period, so the Hg standards would need to be prepared fresh daily.

2) The Ca standard solution contained a small amount of Se. This contamination introduced a bias into the Se calibration at low level, so Se was calibrated using a standard mix that contained no Ca.

With regard to Fe, As and V, which are considered difficult elements to analyse by ICP-MS due to polyatomic overlaps from ArN, ArCl and ClO respectively, an interference correction equation was used to correct for background contributions from these polyatomics. Although HNO3 and HCl were intentionally added to all samples in order to stabilise the solutions and allow the analysis of Ag, interference correction worked well and excellent results were obtained for all of these elements.

Results and Discussion

(1) Calibration Curves

Figures 1 shows the calibration curves of some of the major elements, determined at concentrations up to 300mg/L. Figure 2 shows calibrations for some of the trace elements, calibrated at low ug/L levels. Linear calibrations were obtained in all cases.

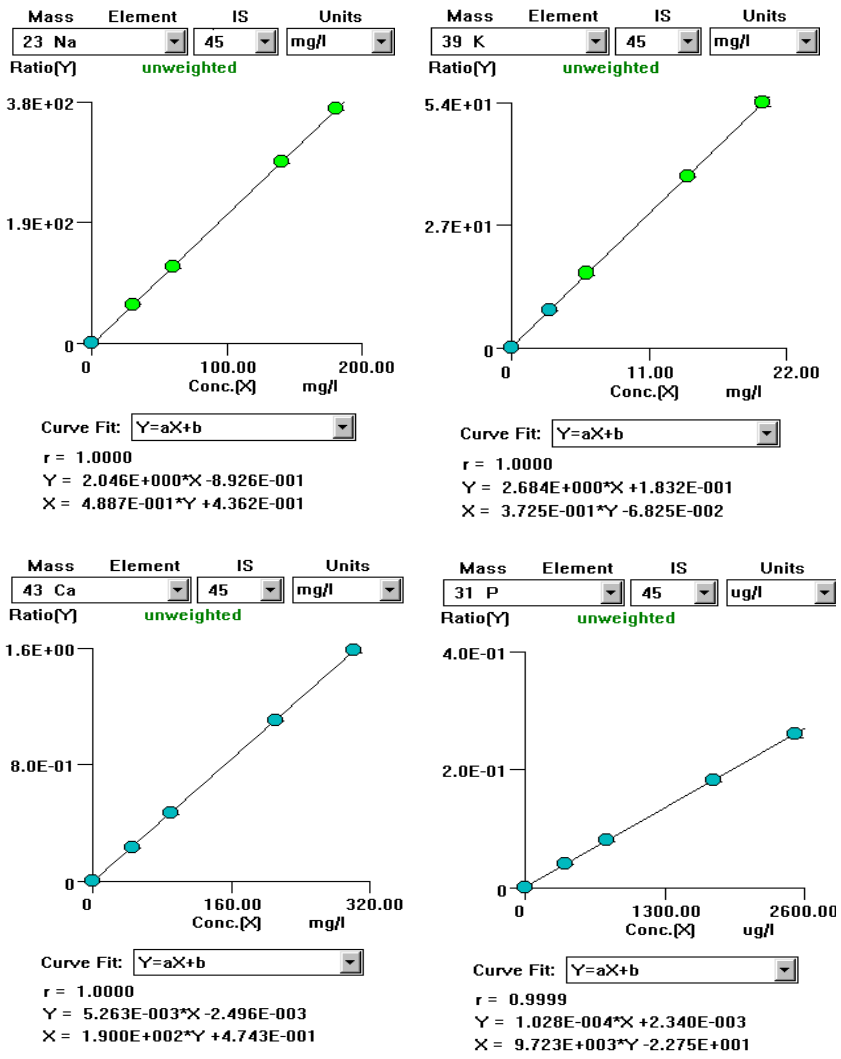

Figure 1
Calibrations for Selected Major Elements

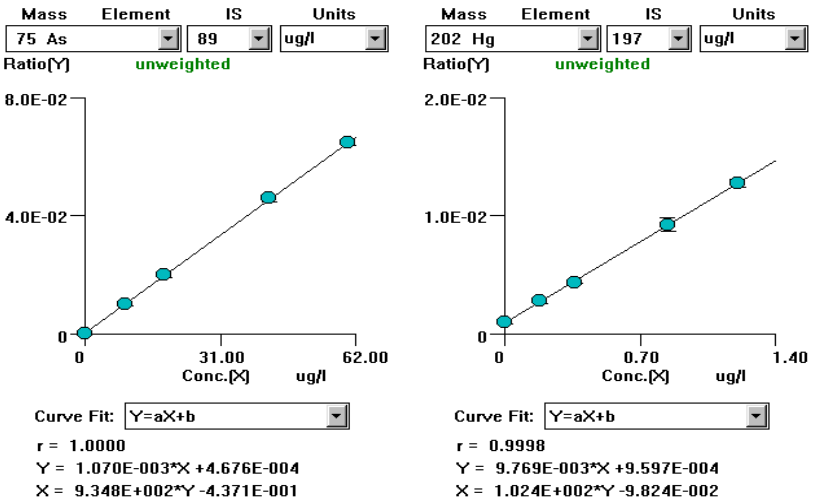

Figure 2
Calibrations for Selected Trace Elements

(2) NS-30 Performance Testing Results

Once quantitative results had been obtained from all of the batches, the statistical processing defined in NS-30 was carried out. Tables 2 and 3 show the calculated results of one high and one low concentration element found in the tap water samples (Na and Hg respectively)

Na (23) IS – Sc	Blank	AQC(L)	AQC(H)	Sample 1	Spike 1	Sample 2	Spike 2
Mean (mg/L)	-0.7005	53.0727	123.1818	34.0273	155.954	44.0409	95.8818
M1	6.0556	16.3264	32.9273	8.1404	67.2455	9.9548	19.7033
M0	0.0002	2.6527	13.0909	0.7782	10.5909	1.4223	5.8182
F value	26122.086	6.1546	2.5153	10.4607	6.3494	6.9992	3.3865
Significant	p=0.001	p=0.01	NS	p=0.001	p=0.01	p=0.01	p=0.05
Sw	0.0152	1.6287	3.6181	0.8821	3.2544	1.1926	2.4121
Sb	1.7400	2.6147	3.1493	1.9186	5.3223	2.0655	2.6349
St	1.7401	3.0805	4.7968	2.1117	6.2384	2.3851	3.5722
F 0.05	1.8307	1.7202	1.6228	1.7522	1.7202	1.7202	1.6435
Calc. F	0.2153	0.6748	0.6065	0.3171	0.6401	0.4045	0.5552
Degree F	10	13	17	12	13	13	16
Bias OK?		*Pass*	*Pass*				
SD OK ?		*Pass*	*Pass*	*Pass*	*Pass*	*Pass*	*Pass*
Recovery		107.55	103.24		101.61		103.68
95% Conf. Limits		2.83	1.55		2.48		2.79
Recovery OK ?					*Pass*		Pass
Limit of Detection	0.077 mg/L						
LOD OK?	*Pass*						

Table 2
NS-30 Performance Test Results for Na

Hg (202) IS – Au	Blank	AQC(L)	AQC(H)	Sample 1	Spike 1	Sample 2	Spike 2
Mean (mg/L)	-0.0011	0.2121	1.0650	0.0105	1.0623	0.0138	0.2295
M1	0.0001	0.0004	0.0011	0.0001	0.0012	0.0002	0.0004
M0	0.0002	0.0003	0.0006	0.0001	0.0009	0.0002	0.0007
F value	1.2233	1.4911	1.8473	2.3750	1.4631	1.2303	1.8405
Significant	NS	NS	NS	NS	NS	NS	NS
Sw	0.0127	0.0163	0.0244	0.0077	0.0292	0.0134	0.0260
Sb	0.0000	0.0081	0.0159	0.0064	0.0140	0.0045	0.0000
St	0.0127	0.0182	0.0291	0.0100	0.0324	0.0141	0.0260
F 0.05	1.5558	1.5705	1.5865	1.6228	1.5705	1.5558	1.5705
Calc. F	0.2562	0.5278	0.2990	0.1589	0.3711	0.3194	1.0840
Degree F	21	20	19	17	20	21	20
Bias OK?		*Pass*	*Pass*				
SD OK ?		*Pass*	*Pass*	*Pass*	*Pass*	*Pass*	*Pass*
Recovery		106.61	106.61		105.18		107.841
95% Conf. Limits		3.57	1.23		1.07		3.4205
Recovery OK ?					*Pass*		Pass
Limit of Detection	0.064 ug/L						
LOD OK?	*Pass*						

Table 3
NS-30 Performance Test Results for Hg

Tables 4 and 5 show the summary results of all elements. In every case, all of the statistical analysis indicated that the 4500 ICP-MS gave acceptable results under the requirements of the protocol defined in NS-30.

Elements	PCV	Units	LOD	AQC	Recovery	Spike	Recovery	95% SD
Li		ug/L	0.328	40	102.52	40	100.00	2.48
B	2000	ug/L	99.453	400	104.41	400	91.34	4.50
Na	150	mg/L	0.077	50	106.15	50	103.68	2.79
Mg	50	mg/L	0.064	10	102.77	10	105.42	2.43
Al	200	ug/L	1.562	200	102.34	200	100.68	2.06
P	2200	ug/L	4.524	500	97.07	500	98.34	3.44
K	12	mg/L	0.021	6	104.97	6	98.91	1.56
Ca	250	mg/L	0.338	100	103.32	100	97.73	2.70
V		ug/L	0.606	10	101.09	10	100.10	2.74
Cr	50	ug/L	1.332	10	104.09	10	98.46	3.64
Fe	200	ug/L	14.692	200	98.84	200	98.94	2.90
Mn	50	ug/L	0.900	50	100.45	50	101.69	2.40
Co		ug/L	0.052	10	102.36	10	105.24	1.02
Ni	50	ug/L	0.869	10	108.91	10	105.58	2.05
Cu	3000	ug/L	1.173	500	101.78	500	100.60	0.71
Zn	5000	ug/L	2.632	500	107.12	500	102.08	1.34
As	50	ug/L	1.067	10	93.13	10	98.51	2.49
Se	10	ug/L	0.837	2	102.77	2	108.95	5.96
Sr		ug/L	1.062	120	104.47	120	90.34	3.29
Ag	10	ug/L	0.055	3	102.47	3	100.17	2.09
Cd	5	ug/L	0.130	1	105.91	1	104.59	3.29
Sn		ug/L	0.107	10	102.86	10	103.53	1.88
Sb	10	ug/L	0.032	2	100.73	2	101.46	1.20
Ba	1000	ug/L	1.029	200	101.93	200	101.03	1.15
Hg	1	ug/L	0.064	0.2	106.05	0.2	107.84	3.42
Pb	50	ug/L	0.130	50	100.52	50	100.00	1.23

Table 4
NS-30 Performance Test Summary Results For All Elements Low AQC & Spike

Elements	PCV	Units	AQC	Recovery	Spike	Recovery	95% SD
Li		ug/L	180	101.14	180	100.15	2.02
B	2000	ug/L	2000	97.77	2000	97.40	1.85
Na	150	mg/L	120	102.65	120	101.61	2.48
Mg	50	mg/L	50	103.95	50	103.80	2.38
Al	200	ug/L	1800	99.81	1800	100.53	1.41
P	2200	ug/L	2200	98.74	2200	103.53	1.70
K	12	mg/L	12	103.97	12	101.31	1.26
Ca	250	mg/L	250	100.96	250	93.62	1.20
V		ug/L	50	98.84	50	97.70	2.26
Cr	50	ug/L	50	99.78	50	96.95	2.36
Fe	200	ug/L	1600	97.78	1600	95.99	2.94
Mn	50	ug/L	120	99.13	120	99.78	1.88
Co		ug/L	50	98.87	50	98.52	0.92
Ni	50	ug/L	50	101.14	50	98.76	1.45
Cu	3000	ug/L	3000	99.73	3000	98.17	0.90
Zn	5000	ug/L	5000	100.72	5000	98.47	1.36
As	50	ug/L	50	93.36	50	96.84	2.38
Se	10	ug/L	10	100.73	10	105.16	3.71
Sr		ug/L	600	100.48	600	94.38	0.76
Ag	10	ug/L	10	103.91	10	99.38	1.63
Cd	5	ug/L	5	103.58	5	102.52	1.71
Sn		ug/L	50	102.88	50	103.04	2.11
Sb	10	ug/L	10	100.50	10	100.65	0.61
Ba	1000	ug/L	1000	100.23	1000	98.48	1.08
Hg	1	ug/L	1	106.50	1	105.18	1.07
Pb	50	ug/L	90	99.70	90	100.26	1.12

Table 4
NS-30 Performance Test Summary Results For All Elements High AQC & Spike

Conclusions

The 4500 ICP-MS was applied to the analysis of drinking water, following the methodology defined in NS-30. Linear calibrations were obtained for trace elements at low and sub-ug/L levels, in the same acquisition as the major elements at 100's mg/L.

All QC criteria were met, and the system was validated for 21 NS-30 elements plus an additional 5 elements. Operating in a reduced sensitivity mode allowed for the measurement of high concentration elements, such as Na and Ca, while the wide dynamic range of the instrument still allowed detection limit criteria for trace elements such as Hg to be met.

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The Determination of Selenium by ICP-MS, Using the ShieldTorch

Application Note

Ed McCurdy
Glenn Woods

Selenium is an element of considerable interest in environmental and biomedical applications. Several instances of selenium deficiency and toxicity in agricultural livestock have been reported, whilst there is increasing interest in Se as it relates to human physiology.

Se is an essential trace element in humans, but there is a narrow range between Se deficiency and Se toxicity. An excess of Se leads to seleniosis, whilst deficiency has been implicated in coronary heart disease, arthritis, cirrhosis and cancer. Se is available in several forms as a dietary supplement, many of which are derived from yeasts.

The determination of Se by ICP-MS has been one of the most enduring challenges for the technique, due to a combination of factors.

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Firstly, Se is typically present at relatively low levels in natural (uncontaminated) biological materials and the total concentrations present may comprise several different forms of Se, which can have an impact on certain methods of analysis.

Secondly, whilst the Ar plasma is probably the most capable source yet devised for atomic spectrometry, it does suffer from certain limitations with respect to the ionisation environment and the background spectrum. In the case of Se, these two factors conspire to give a low analyte signal level and a relatively high blank signal.

Se has a high first ionisation potential, which means that a smaller proportion of the Se atoms in the plasma are converted into ions. The signal for Se is around 10% of the signal which would be obtained for a fully ionised element, so the signal to noise is about 10x poorer than it could be. Furthermore, all of the Se isotopes are potentially overlapped by polyatomic interferences from plasma or matrix-based peaks, which restricts the choice of Se isotopes which can be used for quantitation. The available Se isotopes and their respective potential overlaps are shown in Table 1.

Se Isotopic Mass	Isotopic Abundance (%)	Potential Overlap(s)
74	0.89	$^{36}Ar^{38}Ar$
76	9.36	$^{38}Ar^{38}Ar$ and $^{36}Ar^{40}Ar$
77	7.63	$^{40}Ar^{37}Cl$
78	23.78	$^{38}Ar^{40}Ar$
80	49.61	$^{40}Ar^{40}Ar$
82	8.73	^{82}Kr and $^{81}Br^{1}H$

Table 1.
Selenium Isotopic Abundances and Potential Interferences

Clearly, many of these potential interferences are Ar-based, which makes them difficult to avoid in an Ar plasma. Several techniques have been suggested to alleviate the problems associated with these overlaps, each of which has its own advantages and limitations.

The various techniques which have been used are discussed in the following paper, together with a novel approach which relies on the selective ionisation of the analyte in a plasma environment which is optimised for the reduction of the Ar-based polyatomic ions.

Hydride generation can be used for Se measurements, either utilising a stand-alone hydride generation instrument, or using a hydride generation accessory as the sample introduction method for ICP-MS. The transport efficiency of hydride generation is certainly improved over the use of a conventional spraychamber, but there are several chemical limitations to the technique, most notably that all of the Se must be converted to a form which will form hydrides. In addition, with hydride generation-ICP-MS, the main problem of Ar_2 overlaps on 4 of the Se isotopes is not addressed.

Alternative plasmas have been used, particularly He-based microwave induced plasmas (MIP). This solution offers the dual benefit of a more highly ionising plasma environment (as He has a higher ionisation potential than Ar), together with the removal of the Ar-based polyatomic species. However, these systems are expensive to run, and are not widely available or established as commercial instrumentation.

In common with most polyatomic interferences, the Ar-based overlaps on the Se isotopes can be separated from the analyte peak through the use of a high-resolution magnetic sector mass spectrometer. In addition to offering a very expensive and non-routine solution to the problem, high-resolution mass spectrometers do not address the fundamental problem of the presence of the interfering peak. In order to separate a polyatomic from an adjacent analyte peak, a theoretical resolution is normally calculated, based on equal heights for the 2 peaks and separation only to the 10% valley definition. However, in the case of a trace analyte (such as Se) adjacent to a major interference (such as Ar_2), these calculations are not appropriate. A much higher resolution setting will typically be required and the transmission and sensitivity will therefore be severely compromised. Typically, operation at resolutions of several thousand (as needed even for the separation of equal height peaks) will result in a reduction in transmission of around 95%, i.e. only around 5% of the original signal remains.

Techniques have been investigated for the removal of various polyatomic species using selective collisions with a gas which is introduced into the mass

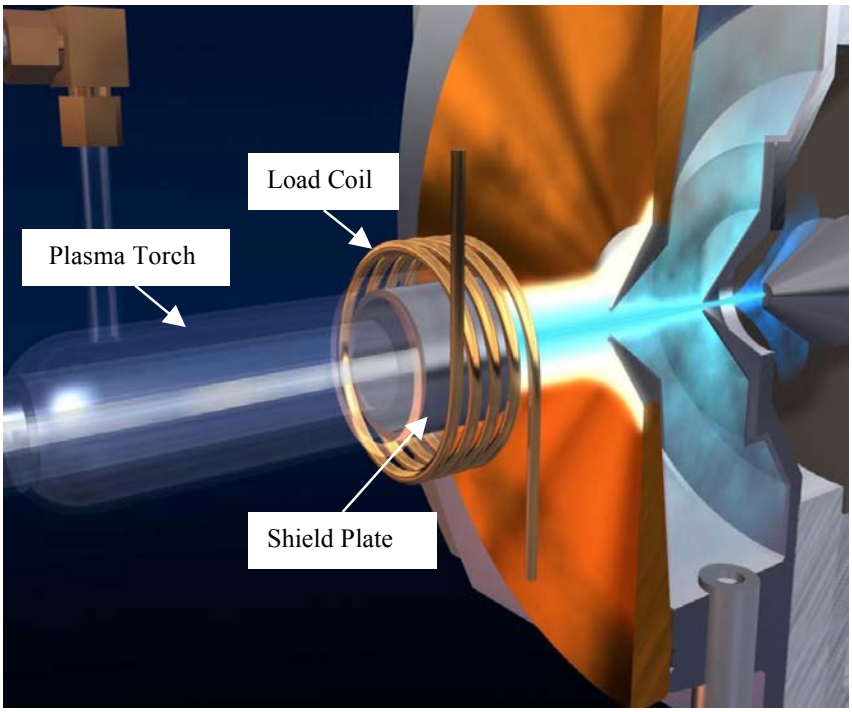

Figure 1.
Schematic of Shield Torch System on the 4500 ICP-MS Series

Forward Power	1000 W
Sampling Depth	13 mm
Carrier Gas Flow	1.2 L/min
Blend Gas Flow	0.8 L/min
Ion Lens Tuning	Typical Cool Plasma Settings
Acquisition Time	21 seconds/repeat
Number of Repeats	3 (10 for LOD blank

Table 2.
4500 ICP-MS Operating Conditions for Se Analysis

spectrometer vacuum chamber. These techniques have not, as yet, proved robust in their ability to decompose specific polyatomics without the introduction of numerous additional clusters ions, which can result in an increase in the overall complexity of the spectrum. Furthermore, collision cells have been shown to be highly susceptible to contamination from matrix components, resulting in poor tolerance to natural samples. Whilst an individual poly-atomic ion can be attenuated, albeit usually with severe attenuation of the analyte signal, it has been demonstrated that different optimum conditions are required for each poly-atomic ion, so the technique is appropriate only on a single-element batch processing basis.

A simple and elegant solution to the problem of Se analysis has been developed by Agilent Technologies application staff, working with the ShieldTorch System on the 4500 ICP-MS.

The ShieldTorch system comprises a grounded metal plate which lies between the plasma RF load coil and the plasma torch, as shown in Figure 1. This has the effect of removing the capacitive coupling between the coil and the plasma, so the plasma is held at the same potential (ground) as the mass spectrometer interface.

Combined with changes to the operating parameters of the plasma (gas flows and sampling depth), which reduce the temperature of the central channel of the plasma, this leads to a background spectrum which is virtually free from Ar-based peaks. Since Se has a relatively high first ionisation potential, it might be expected that reducing the plasma temperature would dramatically reduce the Se signal. However, it is straightforward to optimise the 4500 ICP-MS to give minimal Ar_2 signal whilst retaining good sensitivity for Se. The principal operating parameters are outlined in Table 2.

Under these conditions, the Ar_2 background is much reduced, as shown in Figure 2, whilst Se can be measured at the low ppb level, also shown in the same Figure. From these spectra, it is clear that the Se isotopic pattern matched the theoretical isotopic abundance, indicating that the Ar_2 polyatomics have been effectively removed. This analytical methodology was applied to the measurement of Se in HCl. With the robustness of the higher-power Cool Plasma of the 4500 ICP-MS, this change in matrix did not require any further optimisation or re-tuning of the plasma parameters or ion lenses.

For the quantitative measurement of low concentrations of analytes,

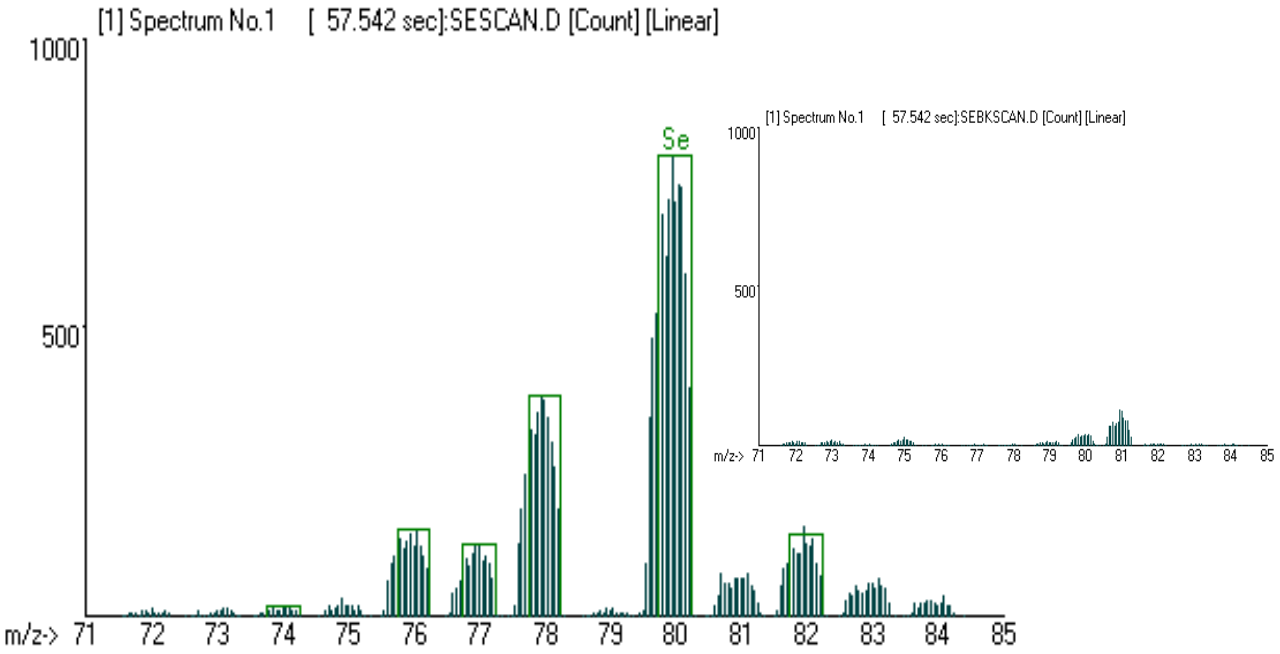

Figure 2.
Selenium Isotopic Pattern in 10ppb Standard and Blank (inset)

Isotope	Dwell time/point (s)	Dwell time/mass (s)
76	2.0	6.0
77	2.0	6.0
78	0.4	1.2
80	0.2	0.6
82	2.0	6.0

Table 3.
Integration Times Used for Selenium Analysis

it is normal to concentrate the integration time on the peaks of interest. For this reason, a peak jumping acquisition was used, where the quadrupole settles only at the top of each set mass. This ensures that the best signal to noise is achieved, although the spectral information is much more limited. The integration times used for the Se isotopes in this study are shown in Table 3. Different integration times were used, in approximately inverse proportion to the isotopic abundance of the individual isotopes, so approximately equal counts (in raw counts) were collected for each.

The calibrations obtained for the Se isotopes at mass 76, 78, 80 and 82, measured in a matrix of 4% HCl, are shown in Figures 3 to 6. In each case, the calibration was in the range from 0ppb to 5ppb. The linearity for each calibration was 1.000.

No blank subtraction was used, no interference correction was required and no internal standard was added.

In HCl, a further possible polyatomic peak might be encountered, due to the formation of $^{40}Ar^{37}Cl$, which would overlap the Se isotope at mass 77. Whilst there are 4 Se isotopes which are free from interference (as shown in Figures 3 to 6), it would be useful if a further isotope could be measured as well. A greater number of available isotopes increases the possibility of carrying out stable isotope tracer analyses in biological systems, as well as extending the possibility of conducting isotope dilution analysis to improve the accuracy of measurements at ultra-trace levels.

The fit of the Se isotopic template to the spectrum for 10ppb Se in 4% HCl is shown in Figure 7. Inset in this spectrum is the spectrum for the blank 4% HCl, on the same intensity scale. The residual ArCl peak at mass 77 is equivalent to about 3ppb, suggesting that the ^{77}Se isotope might be analytically useful at the sub-10ppb level, in addition to the other 4 Se isotopes shown in Figures 3 to 6.

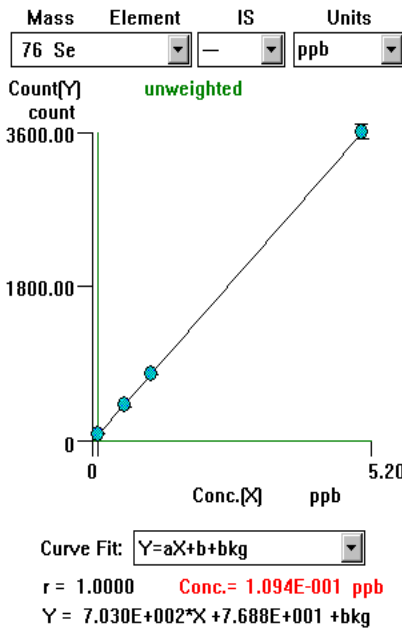

Figure 3.
Calibration for Selenium 76

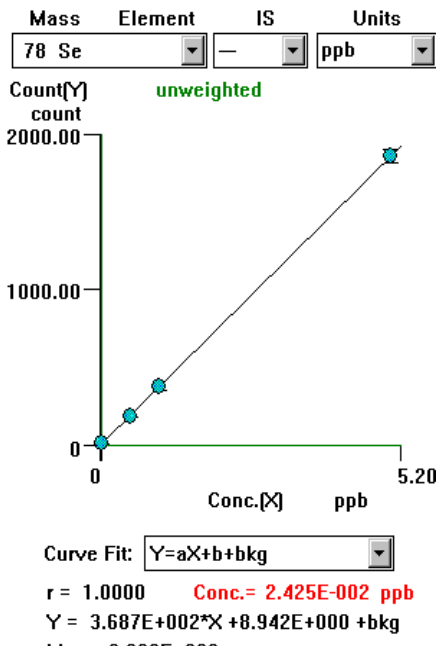

Figure 4.
Calibration for Selenium 78

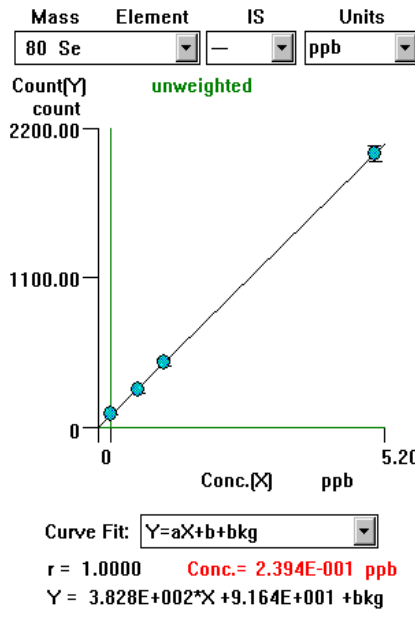

Figure 5.
Calibration for Selenium 80

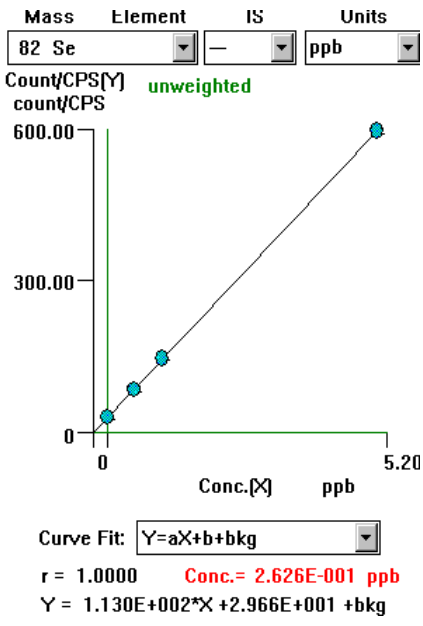

Figure 6.
Calibration for Selenium 82

The calibration for Se at mass 77 is shown in Figure 8. As with all of the other calibrations shown in Figures 3 to 6, this calibration was generated using the method of standard additions, so the intercept on the y-axis indicates the contribution from the ArCl background. Even in the presence of this background, an acceptable calibration was obtained at the sub-5ppb level.

The 4% HCl Blank was repeated 10 times and the detection limit for each Se isotope was calculated based on the multi-point calibration in 4% HCl. The 3 sigma detection limits calculated are shown in Table 4. Note that these are conservative detection limits, as they are based on the analysis of a matrix blank (4% HCl) analysed as a real sample, immediately following the analysis of the calibration standards. Detection limits a factor of 2-5 lower than these values have been obtained under optimum analytical conditions. Also shown in Table 4 are the background equivalent concentrations for each of the Se isotopes, indicating that backgrounds well below 100ppt were achieved for the 3 Se isotopes.

To be useful in real analysis, it must be demonstrated that good precision can be obtained at analytically useful concentrations. The calibration graphs show the good precision obtained on each calibration standard and Table 4 shows the actual precision obtained at the 1ppb level.

The analysis of Se is of increasing interest in human nutrition and toxicology. The ability to measure trace Se concentrations without resorting to separate analytical techniques is beneficial. The

Shield Torch System of the 4500 ICP-MS allows operation of the ICP-MS under optimum conditions for Se analysis, without the limitations in matrix tolerance associated with collision or reaction cells. The possibility of mass spectrometric determination of multiple Se isotopes, allowing stable isotope tracers to be used, is an exciting development in the growing applications for higher power cool plasma analysis using the ShieldTorch System on the 4500 ICP-MS.

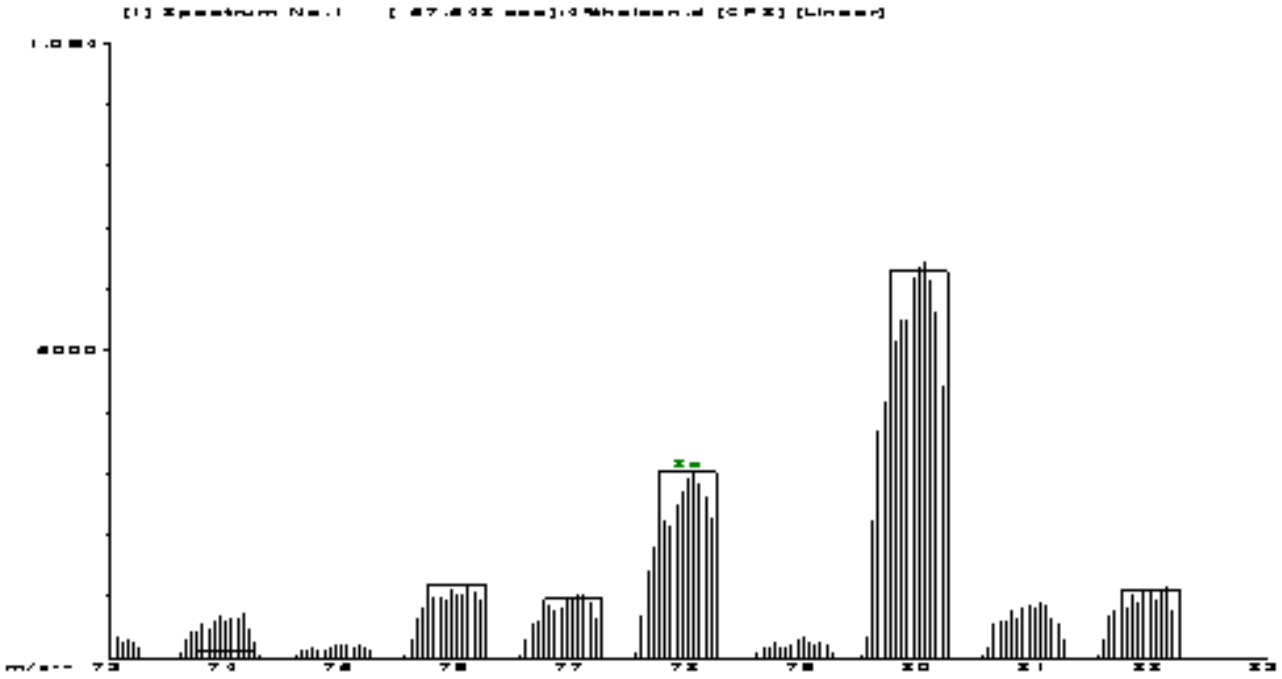

Figure 7.
Selenium Isotopic Pattern in 4% HCl Blank (inset) and 10ppb Standard

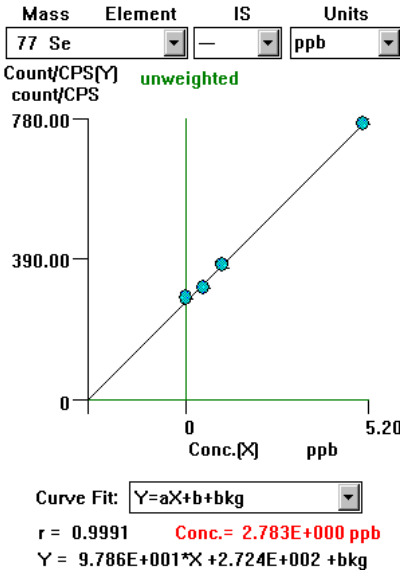

Figure 8.
Calibration for ^{77}Se in 4% HCl

Se Isotope	BEC (ppt)	3s LOD (ppt)	Precision at 1ppb (%)
76 Se	172.8	38.04	2.61
77 Se	2880	81.12	2.47
78 Se	89.5	49.14	5.24
80 Se	29.6	59.82	4.97
82 Se	31.1	59.7	2.35

Table 4.
Summary of Detection Limit Data for Selected Se Isotopes

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Specific Determination of Bromate and Iodate in Ozonized Water by Ion Chromatography with Two Detection Methods : Postcolumn Derivatization and ICP-MS Detection

Submitted to : Journal of Chromatography A, 789, 259-265 (1997)

Application Note

Environmental

Michiko Yamanaka

Abstract

A specific determination for bromate, iodate and other halogen anions in drinking water by direct injection using ion chromatography (IC) with either inductively coupled plasma mass spectrometry (ICP/MS), or the postcolumn derivatization is described. The advantages of ICP/MS as an element selective detector was evaluated for bromate and iodate by considering the comparison with the postcolumn derivatization. Samples were directly injected into the IC column, and halogen anions were separated. The eluates were directly introduced into ICP/MS and detected at 79 and 127 amu. The detection limit (*S/N* = 3) for bromate and iodate with injection of 0.5 mL were 0.45 µg Br/L and 0.034 µg I/L, respectively. The IC combined with ICP/MS was applied to the simultaneous determination of bromate, bromide and other halogen anions in raw and ozonized water. Good agreement was obtained for the determined values by IC-ICP/MS and postcolumn derivatization. Furthermore, several bromine species different from bromate or bromide were detected by IC-ICP/MS.

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Introduction

Bromate can be formed by the oxidation of bromide ions during ozonation and possibly by other oxidants in water treatment [1-4]. Bromate has been estimated as a potential carcinogen, and has been classified in Group 2B by the International Agency of Research on Cancer (IARC). The concentration of bromate in drinking water associated with an excess lifetime cancer risk of 10^{-5} corresponds to 3 µg/L [5]. The World Health Organization (WHO) recommended the provisional guideline value of 25 µg/L which is associated with an excess lifetime cancer risk of 7×10^{-5}, because of limitation in available analytical and treatment methods [5].

Ion chromatography (IC) with a pretreatment method [6] or an on-line preconcentration method [7-8] has been reported for the determination of trace bromate. However, the peak of bromate at the detection limit level will often vanish in that of chloride which is always present in water at a level of three orders of magnitude higher. The authors have developed a sensitive and selective ion chromatographic determination method of bromate with postcolumn conversion into tribromide by hydrobromic acid [9]. Sub-µg/L of bromate in water was determined by using the developed postcolumn derivatization. Furthermore, other disinfectant by-products such as chlorite and iodate were also detected with similar detection limits.

On the other hand, inductively coupled plasma mass spectrometry (ICP/MS) combined with liquid chromatography or IC (LC-ICP/MS or IC-ICP/MS) is an effective technique for the speciation study of metallic and organometallic species because of its element selectivity and sensitivity. The combined technique has been also applied to the determination of halogen species, especially, iodine that can be sensitively detected by ICP/MS [10-14].

In the present work, the specific determination of bromate, iodate and other halogen species in drinking water by direct injection using IC with ICP/MS and the postcolumn derivatization is described. The advantages of ICP/MS as an element selective detector was evaluated for bromate and iodate by considering the comparison with the postcolumn derivatization. Furthermore, the IC-ICP/MS system was applied to the simultaneous determination of halogen anions in raw and ozonized water.

Experimental

Reagents

All reagents used were purchased from Wako Pure Chemical Industries (Osaka, Japan). Stock solutions (1000 mg/L as elements) for each anion were prepared by dissolving with pure water and stored in refrigerator. Analytical solutions were prepared by diluting the stock solution to the required concentration just before use. Pure water was obtained from Milli-Q system (Nihon Millipore, Tokyo, Japan).

Instrument

Ion chromatograph used in this experiment was Model IC7000S (Yokogawa Analytical Systems Inc., Japan) equipped with a UV/VIS detector, and ICP/MS was Model 4500 (Agilent Technologies, Inc. USA). Excelpak ICS-A23 and ICS-A13 (7.6 mm x 4.6 mm i.d. each, Yokogawa Analytical Systems Inc.) were chosen as separation columns. ICS-A23 and ICS-A13 were packed with hydrophilic and semi-hydrophilic anion exchange resin with 0.05 mequiv./g of dry, respectively.

IC-ICP/MS

Ion chromatograph and ICP/MS were connected by 500 mm x 0.3 mm i.d. of ETFE tube. Ammonium carbonate was chosen as a mobile phase. Ammonium salt was used to prevent a salt deposition and clogging at sampling orifice of ICP/MS caused by sodium in a mobile phase. The operating conditions of ICP/MS are described in Table 1.

Postcolumn derivatization

Two Excelpak ICS-A13 columns in series were chosen to separate the halogen species according to the previous paper [9]. The operating conditions of the postcolumn derivatization are described in Table 2.

Results and Discussion

Separation of halogen anion

First of all, the separation of halogen anions using ICS-A13 as the separation column according to previous paper [9] was examined to establish appropriate separation conditions. The chromatography behaviour of iodide on anion-exchange resins has been described [15]. In this experiment, however, the peak of iodide showed a broad and tailing shape, while bromate, bromide and iodate showed good peak shapes. It was also noted that the retention time was long (more than 30 min) and depended on its concentration. It was not drastically improved in spite of a series of change of mobile phase.

RF power	1300 W
RF reflected power	<1 W
Plasma gas	16.0 L/min
Auxiliary gas	1.00 L/min
Carrier gas flow	1.06 L/min
Sampling depth	7.5 mm
Mass	79 amu (Br), 127 amu (I)
Integration time	0.5 sec
Number of scans	1

Table 1
Operational conditions of ICP-MS.

Ion Chromatography	
column	Excelpak ICS-A13 x 2
mobile phase	5 x 10^{-3} mol/L Na_2CO_3/1 x 10^{-3} mol/L $NaHCO_3$, 1.0 mL/min
column temp.	40 °C
injection volume	0.1 mL
Reagent preparation	
reagent	5 mg/L $NaNO_2$ in 0.5 mol/L NaBr, 1.0 mL/min
preparation reagent	0.75 mol/L H_2SO_4, 1.0 mL/min
cation hollow fiber	5 m
Postcolumn derivatization	
reaction coil	3 m x 0.5 mm i.d.
reaction temp.	40 °C
detection	UV-268 nm

Table 2
Operating conditions of postcolumn derivatization

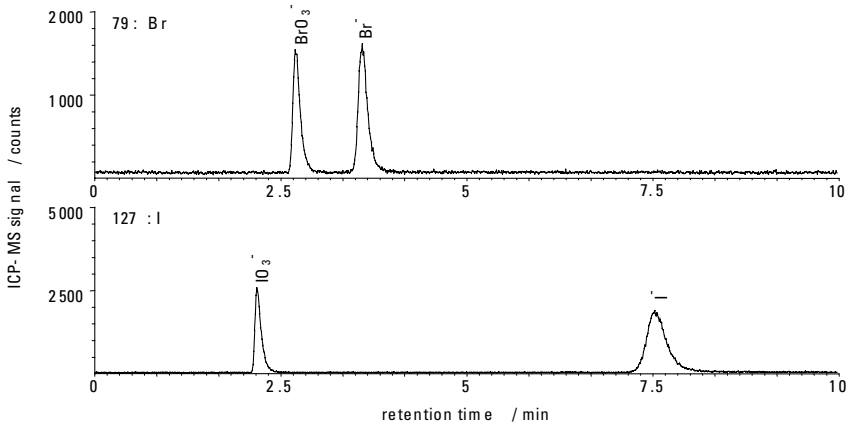


Fig. 1
Chromatograms of halogen anion standards by IC-ICP/MS. Peaks: BO_3 (10 µg/L), Br (10 µg/L), IO_3 (1 µg/L), and I (2 µg/L).
Experimental conditions: column, Excelpak ICS-A23; mobile phase, 0.03 mol/L $(NH_4)_2CO_3$; flow rate, 1.0 mL/min; column temperature, 40 °C; injection volume, 0.5 mL.

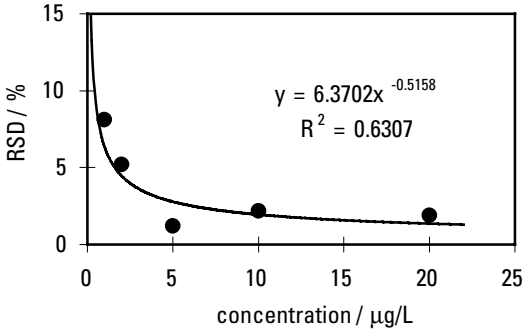
Therefore, ICS-A23 was used on behalf of ICS-A13 because of increase of hydrophilicity of packing materials. The use of the ICS-A23 with 0.03 mol/L ammonium carbonate solution (pH 9.2) made it possible to improve peak shape and retention time of iodide. Fig. 1 shows the chromatograms of halogen anion standards by direct injection with a 0.5 mL sample. Four halogen anions were completely separated within 8 minutes. The analytical time will be reduced by increasing the concentration or pH of mobile phase. For the purpose of this work, that is the simultaneous separation of many halogen species, these separation conditions were a compromise between the number of determinants and analytical time.

Evaluation of IC-ICP/MS

The linearity, detection limits and repeatability for bromate and iodate were determined. The linear range of bromate and iodate was more than 3 orders of magnitude, from 0.5 x 10^{-3} to 1 mg/L and from 0.1 x 10^{-3} to 1 mg/L, respectively. Equally, good linearity for bromide and iodide was also obtained. The detection limits (n = 3) for bromate, bromide, iodate and iodide were 0.45 µg/L, 0.44 µg/L 0.034 µg/L and 0.051 µg/L, respectively. The repeatability (n = 6) for 1.0 µg/L of bromate, 1.0 µg/L of bromide, 0.1 µg/L of iodate and 0.2 µg/L of iodide was 8.1 %, 8.0 %, 6.2 % and 6.8 %, respectively.

In the standard method for water quality, the quantitative limit is determined by the sample concentration which gives 10 % of relative standard deviation (RSD) [16]. The quantitative limits of this method were obtained from the

a) BrO_3^-



b) IO_3^-

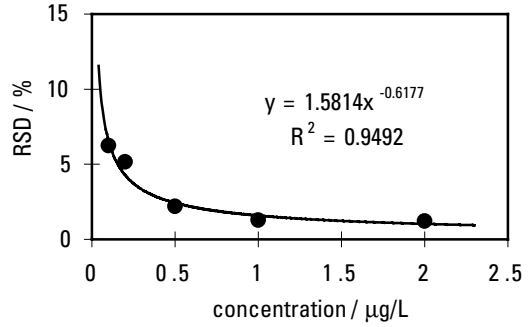


Fig. 2
Relationships between the RSD and sample concentration for bromate a) and iodate b). Experimental conditions are same as those given in Fig. 1.

RSD values which were calculated for each set of 10 measurements of bromate and iodate solutions at various concentrations.
Fig. 2 shows the relationships between the RSD and the sample concentration. The concentrations of bromate and iodate at 10 % of RSD were 0.42 µg/L and 0.051 µg/L, respectively. Fig. 2 also shows the signal stability in the concentrations which give sufficient sensitivity for bromate and iodate.
In both species, RSDs were saturated around 1% even in high concentrations. The saturated RSD is considered to be affected by ICP-MS stability.

Interference by coexistent substance
The interference from coexistent substances such as chloride, sulfate and nitrate has been reported by Creed and others[14]. They reported that bromate can be determined in a chloride matrix with 5-6 orders of magnitude higher. However, a retention time shift for bromate in 1000 mg/L of chloride matrix was observed. So, the interference from coexistent substances such fluoride, chloride, nitrite, phosphate and sulfate was examined. Mixed anion standard solutions ranging in concentration from 5 to 50 mg/L of anions were injected. Peaks of these anions were not observed on the chromatogram. The retention time shift for halogen anions in the concentration below 50 mg/L of anions matrix was not observed. However, one peak was observed at void volume in chromatogram of 79 amu. This peak was recognized to be a polyatomic ion ($^{40}Ar^{39}K+$) formed by combination of potassium in sample solution with argon as the plasma gas, because it appeared at the retention time of potassium that was observed in 39 amu. Conclusively, this peak due to potassium will be neglected on the determination of bromate because it is eluted at the void volume of the anion exchange column and completely separated from that of bromate under these separation conditions.

Application to the determination of halogen anions in the water
The presented method was applied to the determination of halogen anions in several water samples. The chromatograms of the ozonized water by using IC-ICP/MS and the postcolumn derivatization were shown in Fig. 3. The determined concentrations of halogen anions in raw (river) and ozonized water are listed in Table 3. The concentrations of halogen anions determined by both method were relatively in agreement. However, some iodate values obtained using ICP-MS were slightly higher than that of postcolumn derivatization. The disagreement could be due to lack of precision in such low concentration. Furthermore, there could be interference from other iodine-containing species coeluting with iodate, because ICP-MS would detect any species containing iodine, and it would give positive error in the iodate values. Bromate values by ICP-MS were a little lower than that of postcolumn method. The reason is

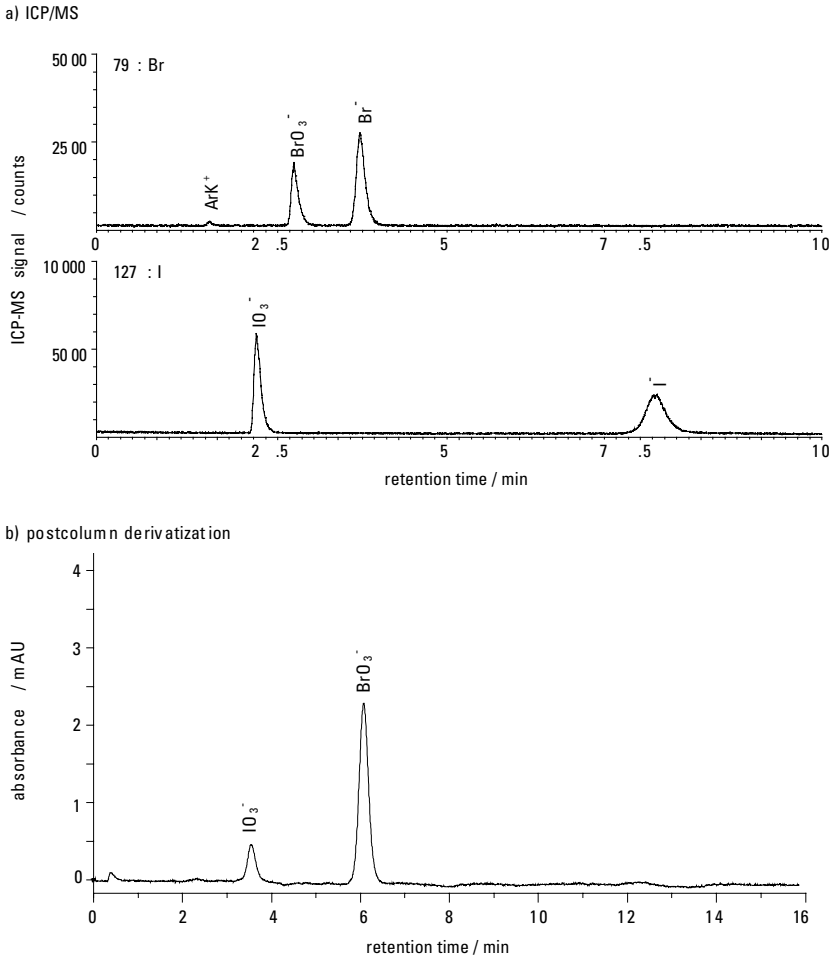


Fig. 3
Chromatograms of ozonized water (sample B) using IC-ICP/MS a) and postcolumn derivatization b). Peaks: a) BO_3 (13.0 µg/L), Br (17.8 µg/L), IO_3 (3.57 µg/L), and I (3.56 µg/L) b) IO_3 (4.13 µg/L), BO_3 (15.7 µg/L) Experimental conditions are same as those given in Fig. 1 and Table 2. Unit of the concentrations is µg/L as species.

	determined concentrations [µg/L as species]					
	IC–ICP–MS				postcolumn	
samples	BrO_3^-	Br^-	IO_3^-	I^-	BrO_3^-	IO_3^-
A. raw water	0.26	28.9	0.44	0.63	0.29	0.09
B. ozonized sample A	13.0	17.8	3.57	3.56	15.7	4.13
C. raw water	1.64	59.1	1.26	2.97	1.65	0.60
D. ozonized sample C	1.88	38.5	5.66	0.14	2.31	4.98
E. ozonized water	1.87	5.73	5.45	0.05	1.85	4.77

Table 3
Comparison of determined concentrations of halogen anions in raw and ozonized water. Experimental conditions are same as those given in Fig. 1 and Table 2.

not clear because bromate values in this postcolumn reaction procedure doesn't suffer from interference from other oxidants [9].

Bromate and iodate with the concentration of µg/L level were detected even in the raw water. Probably, the contamination of river water with trace bromate was caused by a waste water. On the other hand, the existence of iodate in mineral water has been also reported [17]. The concentrations of bromate and iodate in the ozonized water were rather increased than those in the corresponding raw water, while that of bromide was decreased by ozonation. Apparently, bromate and iodate will be formed during ozonation for the water treatment. However, the material balances of bromine and iodine were absolutely incompatible. These results suggest the halo-oxyacids are produced by oxidation of the corresponding halides, but that they are not always produced by the same mechanism.

Sample E gave a distinctive chromatogram at 79 amu (Fig. 4). Several unidentified species different from bromate or bromide were detected. These species are estimated as bromine compounds because no interferences from other elements are observed at 79 amu. The existence of other bromine species suggests that these species could lead to bromate during ozonation. It can also explain that the sum of bromate and bromide was not constant for ozonized water and its raw water (sample D and C) in Table 3. Furthermore, a large unidentified peak with a broad peak shape was also detected at the retention time of about 40 minutes in the chromatograms of ICP/MS at 127 amu. Heumann

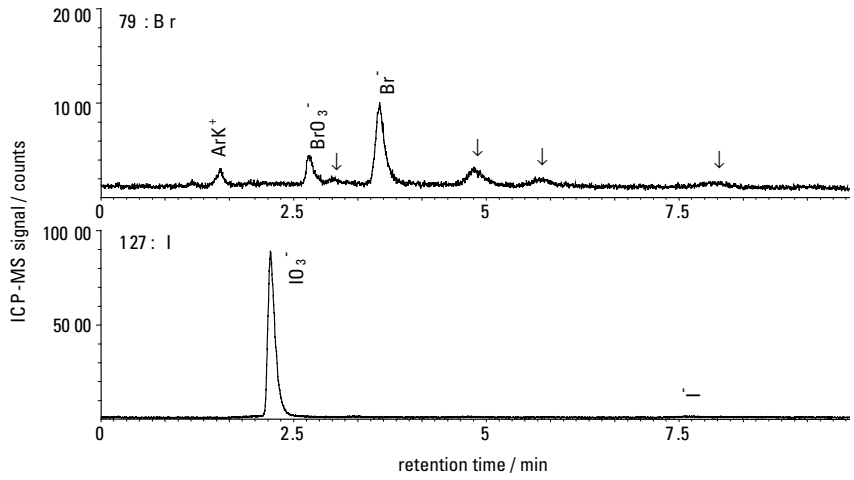


Fig. 4
Chromatograms of ozonized water (sample E). Peaks: BO_3 (1.87 µg/L), Br (5.73 µg/L), IO_3 (5.45 µg/L), and I (0.05 µg/L).
Experimental conditions are same as those given in Fig. 1. Unit of the concentrations is µg/L as species.

et al. reported that organoiodide exists in river water, because the peaks with exactly the same retention time were obtained in both chromatograms of ICP/MS and UV detector at 254 nm [13].

Therefore, the detection of these unidentified peaks by a simultaneous detection using ICP/MS and UV detector was examined. No peaks in the chromatogram of UV at 254 nm were observed at the retention times of these unidentified peaks in the chromatogram of ICP/MS. Furthermore, the retention behaviors of the unidentified peaks were evaluated by adding ethanol to the mobile phase. The retention times were drastically decreased as the concentration of ethanol increased. Clearly, these species were retained by their hydrophobicity, not ionicity. The elucidation of the unidentified peaks detected at 79 amu will be very difficult because of their lower amounts. The unidentified peak detected at 127 amu might be based on inorganic iodine rather than organoiodine but its chemical structure is not still determined. These unidentified peaks might be concerned in the production mechanism of the halo-oxyacids by the ozonation. A further detailed examination would be necessary to elucidate these unidentified peaks.

Conclusions

A specific determination for bromate, iodate and other halogen anions in drinking water by direct injection using IC with ICP/MS and the postcolumn derivatization is presented. Bromate and iodate in ozonized water were determined at the µg/L level without any interference from other anions. The sensitivity of the ICP/MS detector for halogens was also very high similar to that of metals and greater than that of other detectors for halogens. The proposed method will be effective for the simultaneous determination of halogen anions.

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The Determination of Trace Elements in Soils and Sediments by ICP-MS

Application Note

Environmental

Concerns regarding “safe” levels of contaminants in the environment, particularly heavy metals, continue to grow. The requirement for analysis of more elements at ever decreasing concentrations is exposing the limitations of currently used analytical techniques. Further improvements in sensitivity and elemental coverage are required. While GFAAS (Graphite Furnace Atomic Absorption Spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) are still the most commonly used techniques in environmental elemental analysis, ICP-MS is the only technique that offers the improvements in sensitivity that will be demanded in the near future. The 4500 ICP-MS benchtop ICP-MS offers high throughput multielement analysis at the sub ug/ml (ppb) level with the robustness and ease of operation required for true routine use. In this application brief, the analysis of two typical environmental solids - lake sediment and soil - is described. The samples analyzed were standard reference materials - IAEA (International Atomic Energy Agency) SL-1 (lake sediment), and IAEA SOIL-7 (soil).

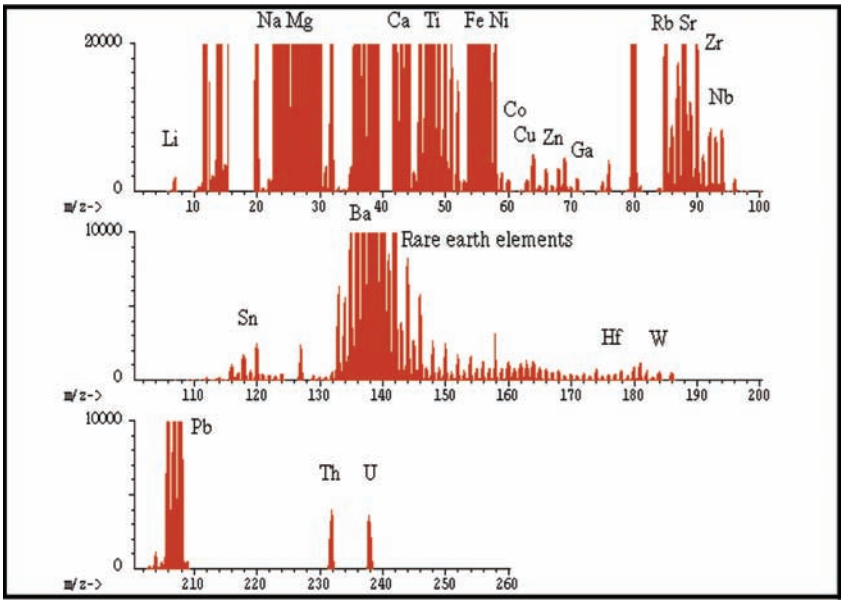

Fig. 1.
Qualitative spectrum of SL-1 (Lake sediment)

Sample Preparation

0.1g of each sample was digested with 1ml of pure water, 0.3ml of hydrofluoric acid (38%) and 0.7ml of nitric acid (68%) using microwave digester for 1 hour. After digestion the sample was diluted to 100ml with deionized water.

Procedure

An internal standard mix containing Be, In and Bi was added to each sample. The sample solutions were quantified by external standardization, by measuring them against multi-element standards.

Operating conditions

RF power	: 1.3 kW
Sampling depth	: 8 mm
Plasma gas	: 16 l/min.
Auxiliary gas	: 1.0 l/min.
Carrier gas	: 1.15 l/min.
Nebulizer	: Babington type

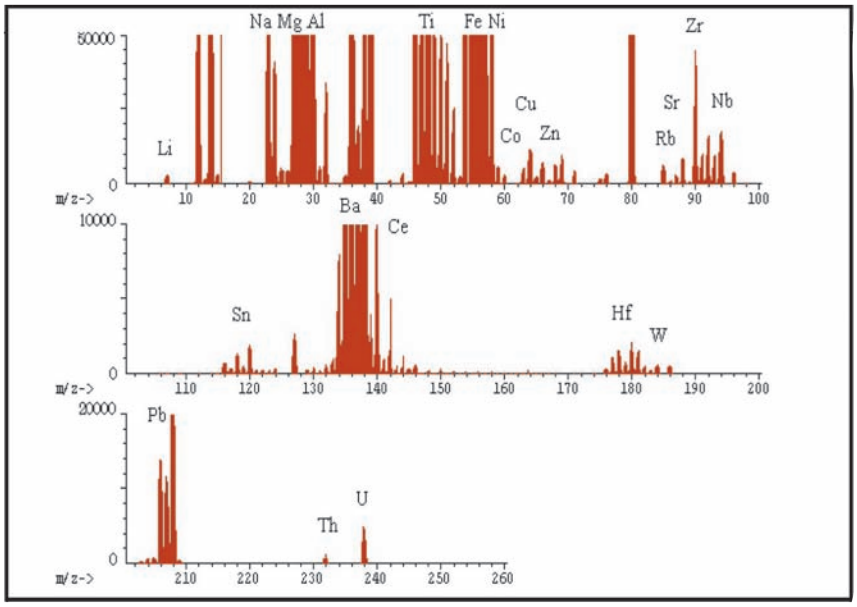

Fig. 2.
Qualitative spectrum of SOIL-7

Results

Lake sediment

Fig.1 demonstrates the qualitative spectrum of SL-1. A large number of elements, ranging from Li at low mass to U at high mass can be clearly observed, even though the total analysis time was only 100 sec.

The quantitative results and the certified values are given in Table 1. The major constituents in this sample are shown in Table 2. After digestion, Fe, Mn, Mg, K and Al were present in solution at levels ranging from a few mg/l(ppm) to 100s of mg/l(ppm), giving rise to the possibility of interference due to spectral overlap. The 4500 ICP-MS's excellent abundance sensitivity and low levels of polyatomic species ensured that the analyte values obtained were in good agreement with certified values.

Element	m/z	Certified	Measurement
V	51	170±15	169
Cr	52	104±9	115
Mn	55	3400±160	3520
Co	59	19.8±1.5	20.2
Ni	60	44.9±8.0	49.6
Cu	65	30±5.0	32.0
Zn	66	223±10	222
As	75	27.5±2.9	29.6
Se	82	*2.9	1.10
Cd	114	0.26±0.05	0.38
Sb	121	1.31±0.12	1.20
Pb	208	37.7±7.4	40.7
Units: mg/kg			

Table 1.
Quantitative values: SL-1
*** Not certified - information only**

Element	Content
Fe	6.7
Mn	0.34
Ti	0.52
Na	0.17
Al	8.9
Ca	0.25
K	1.5
Mg	2.9
S	1.2
Units: %	

Table 2.
Main constituents of SL-1

Soil

Fig.2 shows the qualitative spectrum obtained from SOIL-7. The presence of over 20 elements can be clearly observed, although the elemental composition is quite different to SL-1, even from visual inspection of the qualitative spectra.

Quantitative results and the certified values are given in Table 3 and again very good agreement was obtained. The main components of this sample are also given in Table 4. As can be seen, the sample matrix contains Ca at over 20%, which can affect the determination of Co and Ni, due to interference from CaO. Nevertheless, the 4500 ICP-MS values for Co and Ni agree well with the values supplied, which demonstrates the applicability of this technique to real life sample matrices, even where analytes are present at trace levels in the sample digest.

Element	m/z	Certified Conc.	Certified Confidence interval	Measurement
V	51	66	59-73	67.3
Cr	52	60	49-74	60.7
Mn	55	631	604-650	629
Co	59	8.9	8.4-10.1	8.60
Ni	60	*26	*21-37	24.1
Cu	65	11	9-13	9.85
Zn	66	104	101-113	104.4
As	75	13.4	12.5-14.2	13.8
Se	82	*0.4	*0.2-0.8	3.11
Cd	114	*1.3	*1.1-2.7	1.20
Sb	121	1.7	1.4-1.8	1.60
Pb	208	60	55-71	61.7
Units: mg/kg				

Table 3.
Results of SOIL-7
*** Not certified - information only**

Component	Content
Al^2O^3	8.9
Ca^0	22.9
Fe^2O^3	3.7
K^2O	2.9
MgO	1.9
Na^2O	0.6
SO^3	0.3
SiO^2	38.5
TiO^2	0.5
Loss on ignition (900°C)	20.5
Units: %	

Table 4.
Matrix components of SOIL-7

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Analysis of Arsenic, Selenium and Antimony in Seawater by Continuous-Flow Hydride ICP-MS with ISIS

Application Note

ICP-MS

Environmental

Steve Wilbur

Analysis of arsenic and selenium in seawater at trace levels presents a number of challenges. While ICP-MS is generally considered to be a highly sensitive, interference free technique for analysis of trace metals in environmental samples, matrix effects can result in unacceptably high detection limits for these two elements. These matrix effects are based on two phenomena; 1) ionization suppression in the plasma of high ionization energy elements such as As (9.81 EV) and Se (9.75 EV) in the presence of a significant excess of easily ionizable elements such as Na (5.14 EV) and 2) spectral interferences by argon based polyatomic species such as ArCl and ArAr. For example, ArCl interferes with the only isotope of arsenic and all the significant selenium isotopes suffer from polyatomic interferences of Ar, Cl, or Br. Optimum sensitivity therefore requires some mechanism for separating the analyte from the matrix and reducing or eliminating the argon based polyatomic species. Since arsenic, selenium and a number of other elements (Sb, Te, Bi, Ge, Sn, Pb) are known to form gaseous hydrides under specific reducing conditions, these elements can be removed from the matrix (for example the Na and Ca) and analyzed as gasses in a flowing stream of argon. Reduction or elimination of argon polyatomics can be achieved in the Agilent 4500 or 7500 ICP-MS systems through the use of the ShieldTorch™ and cooler plasma conditions. As a result, by combining hydride generation with cool plasma/ShieldTorch, it is possible to lower the background equivalent concentrations for all As and Se isotopes to low ppb to mid ppt levels in seawater samples.

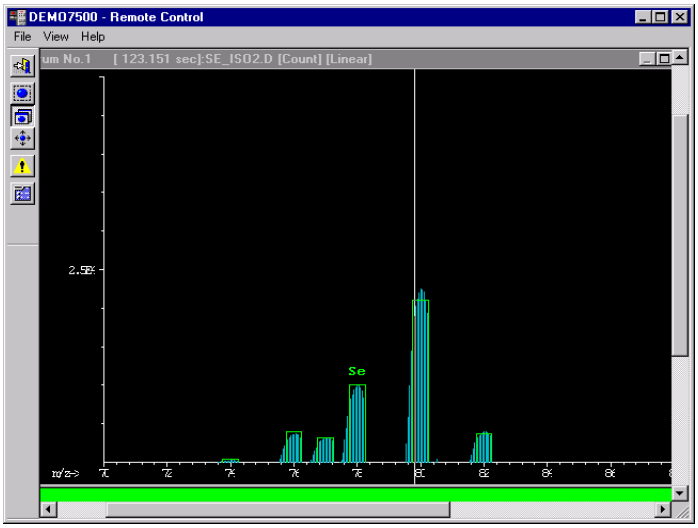

Figure 1. Full-scan Mass Spectrum of Selenium (20 ppb) Showing Excellent Agreement with Expected Isotope Ratios.

Table 1. Common Interferences on As and Se by Normal Plasma, Direct Nebulization ICP-MS

Selenium Isotopes	% Abundance	Major Interferent(s)	% Abundance of Interferent Mass(s)
74	0.89	Ge	35.94
76	9.36	ArAr	0.671
77	7.63	ArCl	24.13
78	23.78	ArAr	0.125
80	49.61	ArAr, BrH	99.202, 50.682
82	8.73	Kr, BrH	11.6, 49.303
Arsenic Isotope			
75	100	ArCl, CaCl	75.48, 43.45

Reaction Chemistry:

For optimum sensitivity, accuracy and precision, both As and Se must be pre-reduced to the most efficient oxidation state for hydride formation. This is achieved through the use of a pre-reduction step. In the case of Se, pre-reduction to the +IV state can be achieved by the use of HCl plus heat. Arsenic requires a stronger reducing environment, in this case a solution of KI plus ascorbic acid is used to reduce As to the desired +III state.

Standards and Reagents:

Tune solution:

20 ppb solution of Se or As pre-reduced as follows.

Pre-reductant Stock for As and Sb (KI + ascorbic acid)

Dissolve 5 grams each KI and ascorbic acid in 100 mL DI water in a polyethylene bottle. Cap and shake to dissolve solids.

Reductant ($NaBH_4$ solution)

Weigh 0.5 g high-purity NaBH4 and 0.125 g NaOH into a 250 mL polyethylene bottle. Bring to volume (250 mL), cap and shake to dissolve solids. Prepare fresh daily.

Calibration standards:

While plasma matrix effects are all but eliminated by using hydride generation, the efficiency of the pre-reduction and reduction steps can be affected by matrix. Therefore, best results will be obtained using matrix-matched standards. In the case of seawater, calibration by method of standard additions gives good results. The standard addition calibration can then be converted to an external standard calibration for analysis of subsequent seawater samples. Replicate 10 mL aliquots of CASS 3 or NASS 5 [1]were spiked with a multi-element calibration stock containing selenium and pre-reduced as described below. Spike levels were 0, 0.01, 0.05, 0.1, 0.5, 1.0 and 5 ppb.

Pre-reduction of samples and standards:

- Arsenic (Antimony and Bismuth)

10 mL of sample (seawater) is added to a 50 ml polypropylene centrifuge tube. 1 ml of the KI/ascorbic acid pre-reduction reagent is added with swirling. 3 mL of concentrated tracemetal grade HCl is added with swirling. The tube is capped loosely and allowed to set for 15 minutes after which it is brought to a final volume of 25 ml with 18 MOhm deionized water.

- Selenium (and Telurium)

10 mL of sample is added to a 50 mL polypropylene centrifuge tube. 10 mL of concentrated, tracemetal grade HCl is slowly added with swirling. The tube is loosely capped and heated in a heat block or boiling water bath at 100 degrees C. for 10 minutes. After allowing to cool, the sample is brought to 25 mL final volume with 18 Mohm DI water.

[1] *National Research Council, Canada*

Tuning

Optimized tuning involves maximizing the analyte signal(s) while minimizing the interferences. Since the interferences are primarily due to argon-based polyatomics, the use of ShieldTorch with slightly reduced forward power to minimize the ionization of Ar is quite effective. The following conditions were set to allow the measurement of Se at m/z 78 or 80 and also work quite well for As.

Basically, a combination of forward power, sample gas flow (carrier plus makeup), and sample depth which minimizes m/z 78 and 80 in the blank and maximizes those masses in the tune solution (20 ppb Se, pre-reduced as described) is desired.

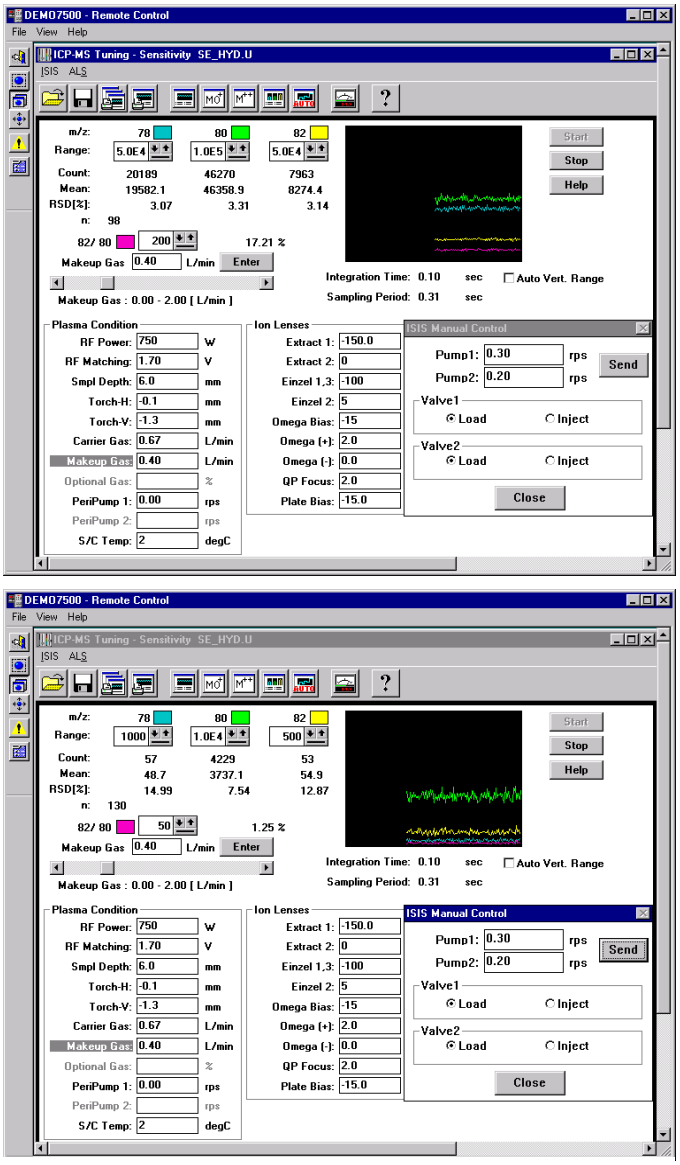

Figure 2. Tune Screens, 20 ppb Se Standard and Prep Blank

As a first step, while aspirating a prep blank under hydride generating conditions, try to reduce the background at m/z = 80 to 10-20,000 counts per 0.1 sec. This is accomplished by cooling the plasma using a combination of RF power and carrier/makeup gas. See figure 2 for sample tune condiitions. Some of the background may be due to trace Se in the reagents used for pre-reduction or hydride formation. Now aspirate the 20 ppb Se tuning solution. Set the acquisition masses at 78, 80 and 82. Set the displayed ion ratio to calculate the ratio of 82 to 80. The natural isotope ratio of Se82 to Se80 is 8.73/49.61 or 17.59%. Therefore as the displayed ratio approaches 17.6%, the background at m/z 80 due to ArAr is minimized. Try to maximize the signal at 78 while maintaining as close to 17.6% for 82/80 as possible.[2]

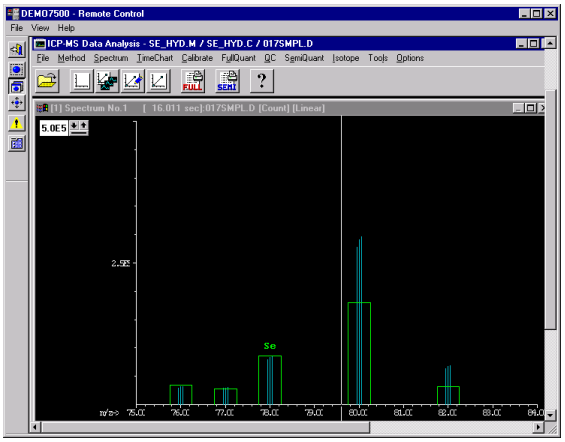

Figure 3. Se spike in CASS 3 Showing Interferences at m/z 80 and 82 from BrH

[2] *Note: This must be done on a clean Tune solution, not a sea water spike since BrH from the sea water can cause high background at both 80 and 82. Occasionally, krypton (m/z 82) in the argon supply can be sufficiently high to adversely affect the ratio as well.*

ISIS Program

The ISIS program builder was used to create the ISIS program below. Typical values for ISIS parameters for hydride generation are shown. As a rule, sensitivity increases with sample flow at the expense of sample consumption. The ratio of sample to reductant is important and should be optimized as well. Normal rinseout times are very fast due to the high sample flows utilized.

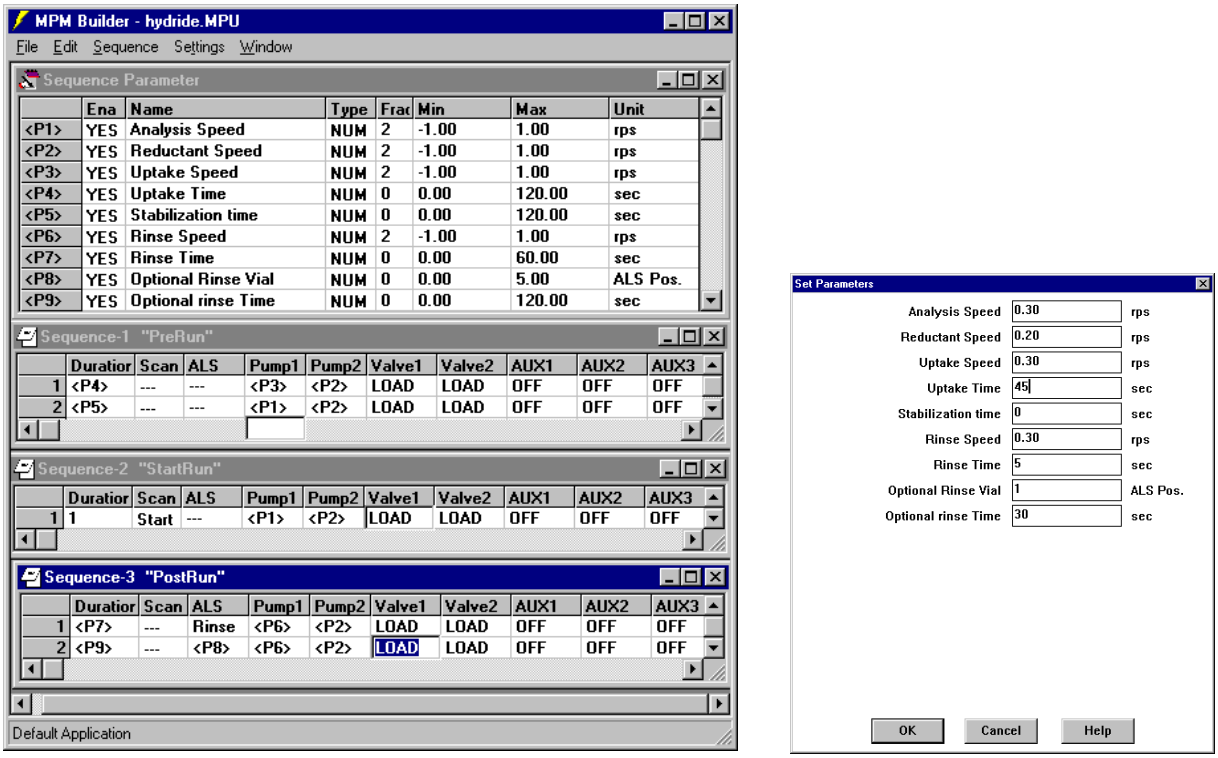

Figure 4. ISIS Program Builder and Method ISIS Parameters Showing Prerun, Startrun and Postrun Programs and Setpoints for ISIS Pumps

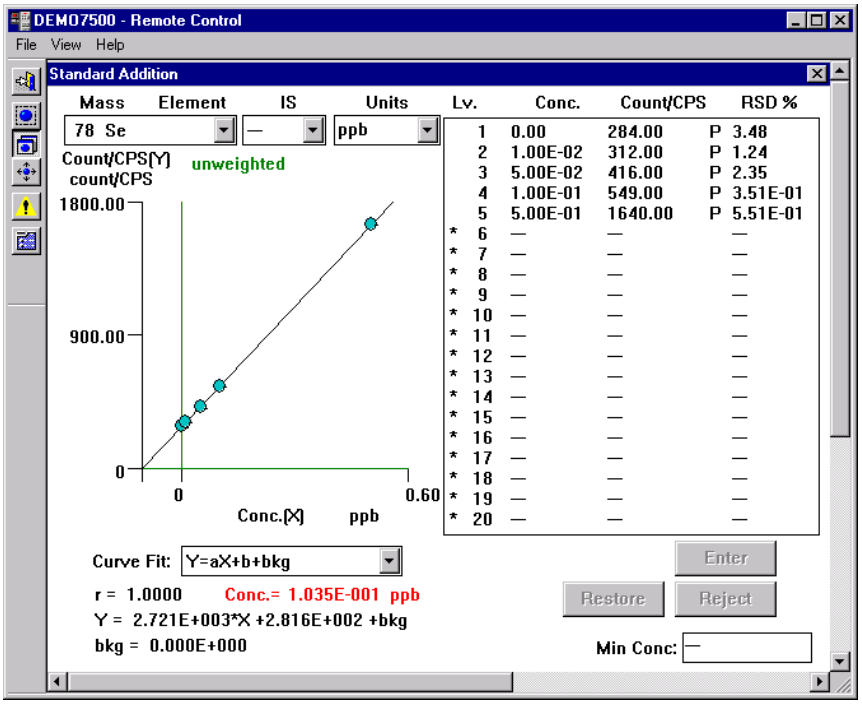

Figure 5. Selenium Standard Addition Calibration in DI Water at 0, 10, 50, 100 and 500 ppt.

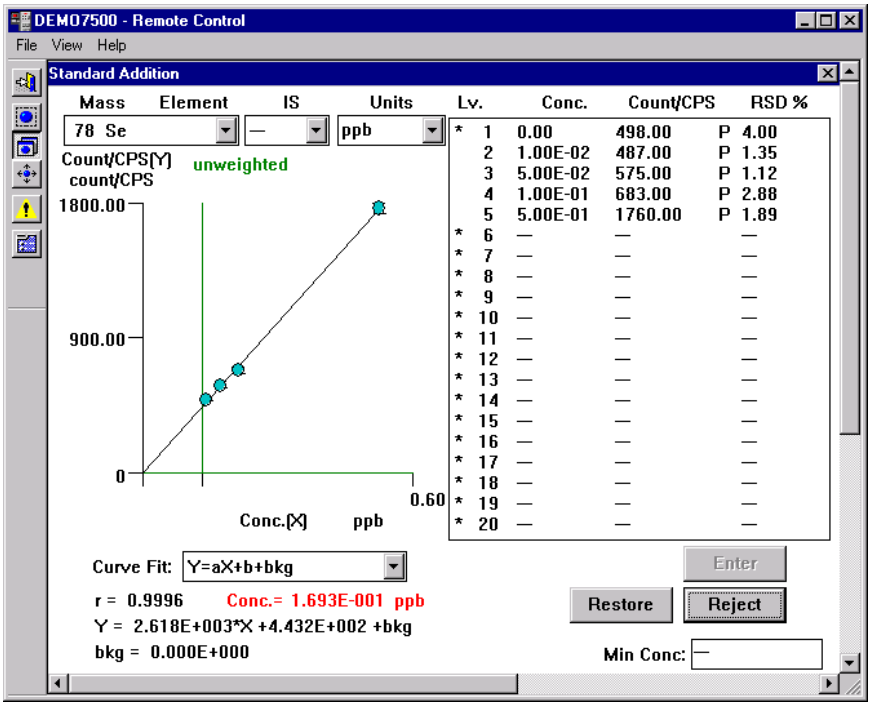

Figure 6. Standard Addition Calibration in CASS 3 Standard Reference Seawater. Calibration Levels; 0, 10, 50, 100, and 500 ppt.

Analysis of Certified Reference Materials

Seawater certified reference materials CASS 3 and NASS 5 were analyzed for As, Se and Sb. Standard addition calibrations were prepared by spiking 10 aliquots of sample with a mixed calibration solution containing the elements of interest. Standard addition calibrations were prepared and then converted to external calibrations for subsequent sample analysis. 3 sigma MDLs were calculated from seven replicate analyses of the unspiked seawater samples using the converted external calibrations. Spike recoveries were also calculated for samples spiked at 0.05 and 2.5 ppb for both elements.

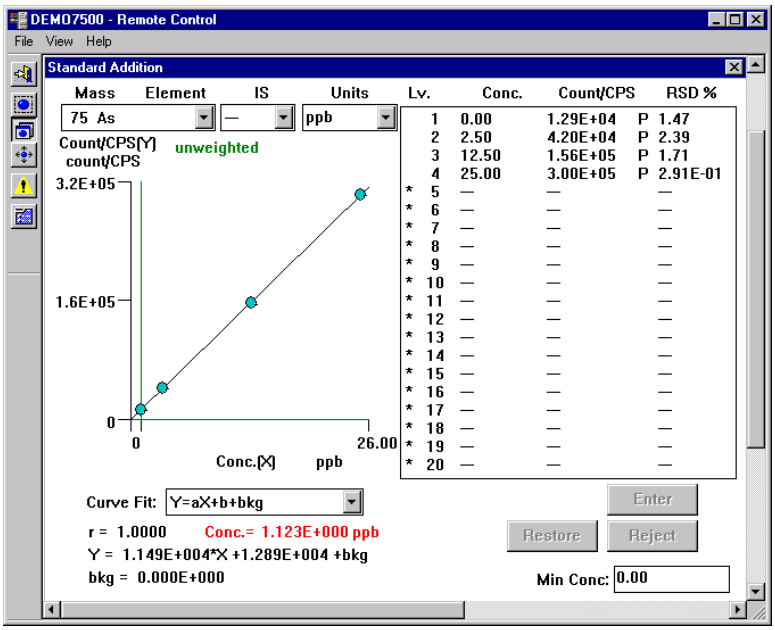

Figure 7. Arsenic in CASS 3 by Standard Addition

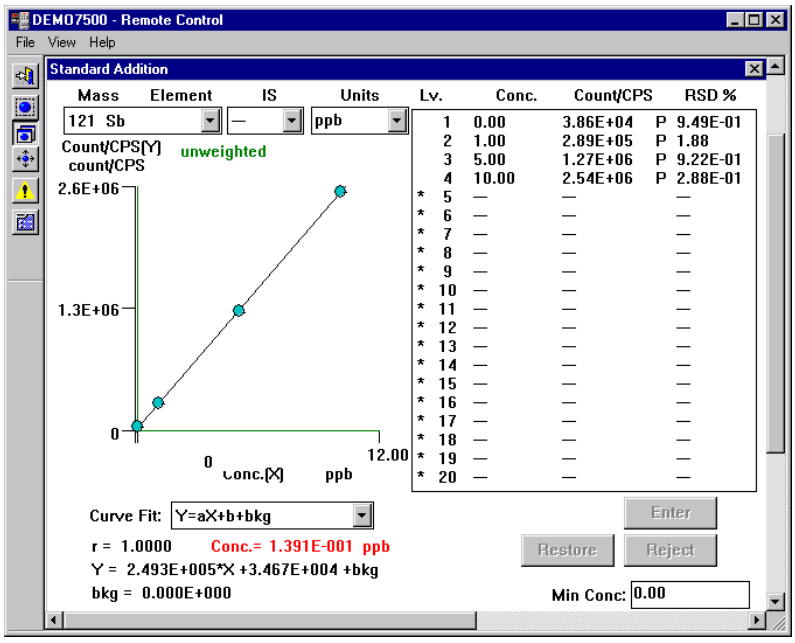

Figure 8. Antimony in CASS 3 by Standard Addition

Summary

The use of online, continuous-flow hydride generation coupled to the Agilent 4500 or 7500 ICP-MS offers a fast, sensitive, routine analytical technique for the analysis of the hydride forming elements such as As, Se and Sb in difficult matrices such seawater. The process can be fully automated for multiple samples using the Cetac ASX-500 autosampler and the Agilent Integrated Sample Introduction system (ISIS). 3-sigma detection limits are typically 10 – 30 ppt for these elements, which is below ambient levels for all three. However, slightly elevated background equivalent concentration for Se can make ambient-level Se analysis borderline at best.

The use of purified reagents may help to reduce the BEC for Se to levels closer to the calculated detection limit. When compared to direct nebulization ICP-MS analysis of 10X diluted seawater, detection limits are improved from 10 to 50 times with no long-term matrix effects on the ICP-MS interface or ion lenses.

Table 2. Results of Analysis of Certified Reference Seawater Materials

Sample	Element /Isotope	Measured Value	Certified Value	Spike Amount	Blank Measurement	% Recovery(3)	MDL (4)
CASS 3	As/75	1.12 ppb	1.09	N/A	-	102	0.03
CASS 3	Se/78	0.682(1)	0.042(2)	0.5	0.193	97.6	0.01
CASS 3	Sb/121	0.34	not certified	0	-	-	0.02
NASS 5	Sb/121	2.87	not certified	2.5	0.34	101	0.02
NASS 5	As/75	1.21 ppb	1.27	N/A	-	95	0.03

(1) raw measured concentration, not corrected for prep(reagent) blank

(2) total selenium is listed but not certified in CASS 3

(3) recovery calculated against certified value where available and against matrix spike recovery where certified value is not available.

(4) 3-sigma using seven replicates

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5980-0243E

New ASTM Standard:

Recommended operating conditions for the Agilent Capillary Electrophoresis system

Maria Serwe

Environmental

Abstract

ASTM Subcommittee D19.05 on Inorganic Constituents in Water approved a new standard test method for determination of dissolved inorganic anions in aqueous matrices using capillary ion electrophoresis and chromate electrolyte[1]. The Agilent Capillary Electrophoresis system provided equivalent performance during the inter-laboratory study preceding approval (c/w sect. 17.6 in test method). This document (reference B1.16 in test method) describes equivalent method parameters specific for the Agilent system equipped with DAD detection and computer control through Agilent ChemStation.

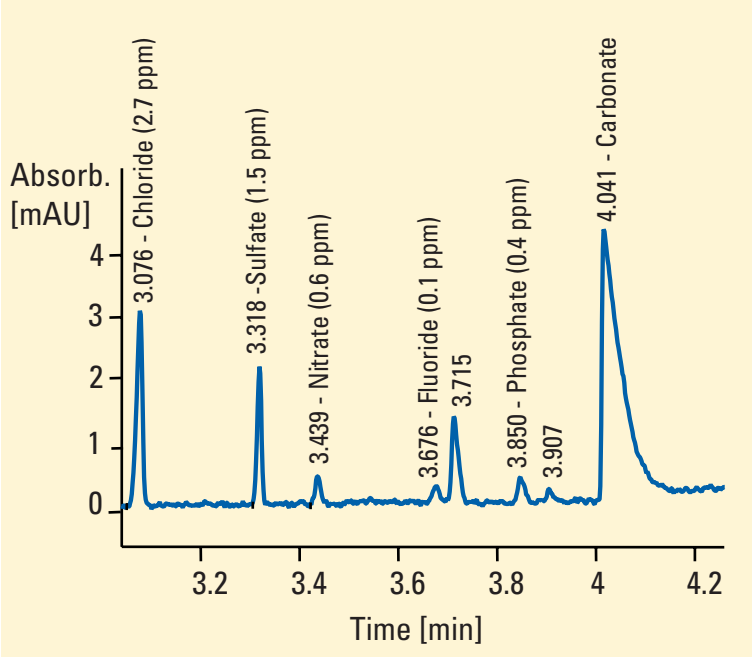

Figure 1
Analysis of waste water from a municipal waste treatment plant

Method Entries

Lift offset	4 mm
Cassette temp.	25° C
Preconditioning	
flush 1.1 min from flush buffer vial into waste vial	
Electric	on
Polarity	negative
Voltage	system limit
Current	0.00 µA
Power	system limit
Low current limit	0.00 µA
Time table	
0.3 min, current = 14.00 µA	
Injection	
by pressure, 50 mbar x 6.2 sec (37 nl)	
UV-detection	
Signal = 470/50 nm, reference = 275/10 nm, response time = 0.2 sec (PW > 0.01 min)	
Integration	
peak top type = center of gravity	
Calibration	
calculate with corrected areas	

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Method parameters
The parameters described here are supplementary to the test method (see also reference 2).

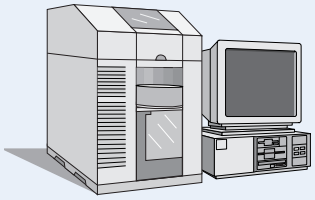
Capillary
Standard bare fused silica capillary (L = 64.5 cm, l = 56 cm, 75 µm id), fitted with a blue alignment interface. A new capillary is prepared by flushing 0.5 N NaOH for 5 min, water for 1 min and run buffer for 3 min (at 1 bar). If the current on a new capillary must be tested (c/w sect. 11.4), a voltage of 18.5 kV should be applied. If the system is idle overnight, leave the capillary in buffer. For long-term storage flush the capillary with water followed by air.

Vials
2-mL glass vials with polyurethane caps are used as buffer or waste container. 1-mL capped polypropylene vials are used as sample container. The buffer vials (inlet, outlet and flush buffer vial) are filled to 1 mL, the waste vial is filled with 0.6 mL buffer. For best migration time stability the run buffer vials should be replaced after 10 runs. It is not recommended to use the replenishment system with the Waters IonSelect™ High Mobility Anion Electrolyte.

Sample preparation
The waste water samples were diluted (1:20) and filtered through a 0.45 µm filter prior to injection.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation



References

1. D6508-00 (2000)

2. M. Serwe and J. Krol, "Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte" Poster presentation at HPCE 2000 in Saarbrücken, Germany.

Maria Serwe is an application chemist at Agilent Technologies, Waldbronn, Germany.

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Elemental Characterization of River Water using the Agilent 7500a ICP-MS

Application Note

ICP-MS

Environmental

Tetushi Sakai and Chris Tye

Abstract

The quality of river water is often used as a measurement of the overall "environmental health" of a given region. Rivers provide a means of disposal of waste in industrialized countries, yet can also be a source of potable water for domestic use. Monitoring the levels of toxic elements in river water is therefore of utmost importance.

The analysis of river water requires the measurement of trace and minor elements in the same sample, which generally means that laboratories must employ multiple techniques to perform a complete analysis. Inorganic components in river water samples range from sub-μg/L (ppb) to 100's of mg/L (ppm). A combination of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) is usually used for this type of analysis.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has gained wide acceptance for the determination of many trace elements, but perceived limitations in dynamic range and matrix tolerance mean that it is less frequently used for the determination of higher levels of elements. This Application Note summarizes the validation of the Agilent 7500a ICP-MS for river water analysis, allowing the measurement of all elements in a single run, using a single technique.

Introduction

Ambient concentrations of elements in river water can span from ultra-trace levels, for most heavy metals, through to tens or even hundreds of ppm for elements such as sodium, magnesium, potassium and calcium. River water itself can vary in major element composition depending on the underlying geology. Instruments used to characterize the elemental composition of river water should ideally:

- offer the ability to measure many elements in a single acquisition.
- be capable of quantifying species over the complete anticipated concentration range.
- be tolerant to gross changes in major species.

ICP-MS is well suited to environmental samples such as river water analysis because it is a multielement technique,

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offers excellent detection limits and large linear dynamic range.

The Agilent 7500a ICP-MS offers all of the features expected of a fourth generation commercial instrument, but also has excellent tolerance to changes in total dissolved solids. The design features that make the Agilent 7500a particularly suitable for this type of analysis include:

- a nebulizer that operates at low sample flow rates (typically 0.4 mL/min), reducing the sample load on the plasma.
- a thermoelectrically cooled spray chamber that removes much of the water vapor, increasing the plasma temperature.
- an ICP torch that ensures that the sample aerosol is resident in the plasma for sufficient time to ensure complete matrix decomposition. This is typically monitored using the accepted "plasma robustness" indicator of the CeO/Ce ratio. The Agilent 7500a typically has a CeO/Ce ratio of 0.4-0.5%.
- an optimized interface design that ensures minimal sample matrix is passed into the high-vacuum part of the instrument, dramatically reducing the requirement for routine maintenance of the interface cones, the ion lenses and the interface pump oil.
- a simultaneous Dual Mode detector with an exclusive high speed amplifier providing 9 orders of linear dynamic range.

The Agilent 7500a is designed for maximum flexibility and routine ease of use. With its rugged sampling interface, Omega ion lens system, true hyperbolic quadrupole mass analyzer and simultaneous detector, the 7500a offers the performance and flexibility to handle the widest range of sample types and applications.

Table 1 Agilent 7500 Operating Parameters

Plasma gas flow rate	15.0 L/min
Aux. gas flow rate	1.0 L/min
Carrier gas flow rate	1.22 L/min
RF Power	1600 W
Nebulizer	PEEK, Babington - type
Spray chamber	Glass, double pass
Spray chamber temp	2°C
ICP torch injector	Quartz, 2.5 mm
Sample uptake rate	0.4 mL/min
Sampler cone	Nickel
Skimmer cone	Nickel
Sampling depth	6 mm
Points/mass	3
Integration time/mass	3 sec for Be, Cr, As, Se, Hg and U 1 sec for others
Replicates	3

Results

The operating conditions used for this study are shown in Table 1.

Table 2 summarizes the results from an analysis of two Japanese river water standards, JAC 0031 and JAC 0032, using the Agilent 7500a. These standards are useful for understanding the accuracy of a given measurement device; JAC 0031 consists of neat river water, while JAC 0032 is the same water spiked at known levels with different elements. Spike values range from 1 to 50 ppb depending on the element.

The Agilent 7500a demonstrates, by default, low levels of polyatomic species and good matrix tolerance. Consequently, all elements could be determined in the river water standards, without the need for extensive interference correction equations.

The results agree very well with the expected value for all elements. Of particular note is that the recoveries were good across a wide range of concentrations. For instance calcium was measured at over 12 ppm in the same acquisition cycle that mercury was quantified at less than 10 ppt.

ArO at well known polyatomic species can influence the Fe data at low levels, and although the spike recoveries in JAC 0031 are good, agreement with the expected value is affected. If Fe is a regulatory requirement, then the 7500 optional T-mode interface can be used to reduce Ar based polyatomic species even further to improve accuracy and consistency for Fe data at low ppb levels.

The spike recoveries from the analysis of JAC 0032 again return very good agreement with the certified values.

Table 2 Analysis of Two Certified River Standards using the Agilent 7500a

			JAC 0031 (unspiked)		JAC 0032 (spiked)		
Element	m/z	ISTD	Certified	Measured	Certified	Measured	Unit
Be	9	7	---------	<0.001	---------	<0.001	ppb
B	11	7	9.1±0.5	10.2	59±2	60.7	ppb
Na	23	7	4.2±0.1	4.28	4.5±0.1	4.52	ppm
Mg	24	7	2.83±0.06	2.75	2.86±0.04	2.77	ppm
Al	27	7	13.4±0.7	13.5	61±2	62.4	ppb
K	39	7	0.68±0.02	0.65	0.67±0.01	0.64	ppm
Ca	43	89	12.5±0.2	12.3	12.5±0.2	12.3	ppm
V	51	89	---------	7.17	---------	6.96	ppb
Cr	52	89	0.14±0.02	0.15	10.1±0.2	9.70	ppb
Cr	53	89	0.14±0.02	0.17	10.1±0.2	9.76	ppb
Mn	55	89	0.46±0.02	0.49	5.4±0.1	5.35	ppb
Fe	56	89	6.9±0.5	4.30	57±2	51.0	ppb
Co	59	89	---------	0.018	---------	0.019	ppb
Ni	60	89	---------	0.10	10.2±0.3	9.52	ppb
Cu	65	89	0.88±0.03	0.98	10.5±0.2	10.8	ppb
Zn	66	89	0.79±0.05	0.77	11.3±0.4	11.2	ppb
As	75	89	0.28±0.04	0.26	5.5±0.3	5.22	ppb
Se	82	89	(0.1)	<0.1	5.2±0.3	4.82	ppb
Sr	88	89	---------	20.0	---------	19.9	ppb
Mo	95	89	---------	0.53	---------	0.55	ppb
Cd	111	115	(0.003)	<0.02	1.00±0.02	0.98	ppb
Sb	121	115	---------	0.074	---------	0.16	ppb
Ba	137	115	---------	0.87	---------	0.90	ppb
Hg	202	205	---------	<0.007	---------	<0.007	ppb
Pb	208	205	0.026±0.003	0.037	9.9±0.2	10.0	ppb
U	238	205	---------	<0.002	---------	<0.002	ppb

Another well known river water reference material is SLRS-3 from the National Research Council in Canada. SLRS-3 has very low certified levels of many elements and provides a good test of the trace level measurement capabilities of an instrument. The results are summarized in Table 3. Again the results confirm excellent agreement with the expected values of all elements from single figure ppt amounts (beryllium) through to ppm (sodium, magnesium and calcium). The data highlights one of the major strengths of the Agilent 7500a, the accurate quantification of elements from ultra trace to major concentrations under a single set of tuning conditions.

To illustrate the robustness of the sample introduction system over extended measurement periods, a sample of SLRS-3 was analyzed repeatedly over a period of 6 hours. Figures 1a, 1b and 1c summarize the results grouped according to concentration for clarity. Figure 1a is the data from those elements at 500 ppb and above, Figure 1b 5 ppb to 500 ppb, and Figure 1c everything at a concentration less than 5 ppb.

As all of the graphs show, the Agilent 7500a offers good stability over the six-hour period, for all elements, without any systematic change in measured value. Obviously, precision is a function of signal and there is slightly more variation at low concentration values when compared to high, however, the data highlights the excellent long-term stability of the instrument. This stability is derived from a fusion of design features within the instrument. The robust sample introduction system, mass flow controlled plasma gas and rugged interface combine to provide highly

Table 3 Analysis of SLRS-3 River Water Standard using the Agilent 7500a

			SLRS-3		
Element	m/z	ISTD	Certified	Measured	Unit
Be	9	7	0.005±0.001	0.004	ppb
B	11	7	---------	7.14	ppb
Na	23	7	2.300±0.200	2.37	ppm
Mg	24	7	1.600±0.200	1.50	ppm
Al	27	7	31±3	30.0	ppb
K	39	7	0.700±0.100	0.61	ppm
Ca	43	89	6.000±0.400	5.53	ppm
V	51	89	0.3±0.02	0.30	ppb
Cr	52	89	0.3±0.04	0.31	ppb
Cr	53	89	0.3±0.04	0.30	ppb
Mn	55	89	3.9±0.3	3.74	ppb
Fe	56	89	100±2	88.6	ppb
Co	59	89	0.027±0.003	0.025	ppb
Ni	60	89	0.83±0.08	0.76	ppb
Cu	65	89	1.35±0.07	1.40	ppb
Zn	66	89	1.04±0.09	1.02	ppb
As	75	89	0.72±0.05	0.71	ppb
Se	82	89	---------	<0.1	ppb
Sr	88	89	(28.1)	30.1	ppb
Mo	95	89	0.19±0.01	0.29	ppb
Cd	111	115	0.013±0.002	<0.02	ppb
Sb	121	115	0.12±0.01	0.14	ppb
Ba	137	115	13.4±0.6	12.8	ppb
Hg	202	205	---------	<0.007	ppb
Pb	208	205	0.068±0.007	0.078	ppb
U	238	205	(0.045)	0.038	ppb

stable source of sample ions into the mass spectrometer.

Three sigma detection limits were calculated from the standard deviation of ten replicate measurements of a blank solution. These values are shown in Table 4 and illustrate the potential quantification limit for the matrix.

Discussion

The Agilent 7500a ICP-MS is a full-featured high-performance benchtop instrument for the routine determination of elements in a variety of sample types.

With the optimized sample introduction system, 27.12MHz RF generator and robust sample introduction system, the instrument offers very low levels of polyatomic species and extremely good stability. The combination of optimized components yields a high ion transmission at low sample flow rates; therefore preventing contamination of the mass spectrometer components. The off-axis lens system and true hyperbolic quadrupole provide extremely high efficiency ion transmission resulting in excellent signal to noise, and thus low detection limits. The wide dynamic range detector allows measurement of signals over a wide dynamic range allowing all elements to be quantified in a single acquisition and improving throughput.

The results indicate that the Agilent 7500a meets or exceeds all of the criteria required for the analysis of river water.

Table 4 Three Sigma Detection Limits

Element	m/z	DL(3sigma)	Unit
Be	9	0.001	ppb
B	11	0.1	ppb
Na	23	0.0002	ppm
Mg	24	0.00005	ppm
Al	27	0.06	ppb
K	39	0.003	ppm
Ca	43	0.01	ppm
V	51	0.003	ppb
Cr	52	0.01	ppb
Cr	53	0.02	ppb
Fe	54	2	ppb
Mn	55	0.03	ppb
Fe	56	0.3	ppb
Co	59	0.003	ppb
Ni	60	0.02	ppb
Cu	65	0.01	ppb
Zn	66	0.01	ppb
As	75	0.007	ppb
Se	82	0.1	ppb
Sr	88	0.0008	ppb
Mo	95	0.006	ppb
Cd	111	0.02	ppb
Sb	121	0.0007	ppb
Ba	137	0.003	ppb
Hg	202	0.007	ppb
Pb	208	0.001	ppb
U	238	0.002	ppb

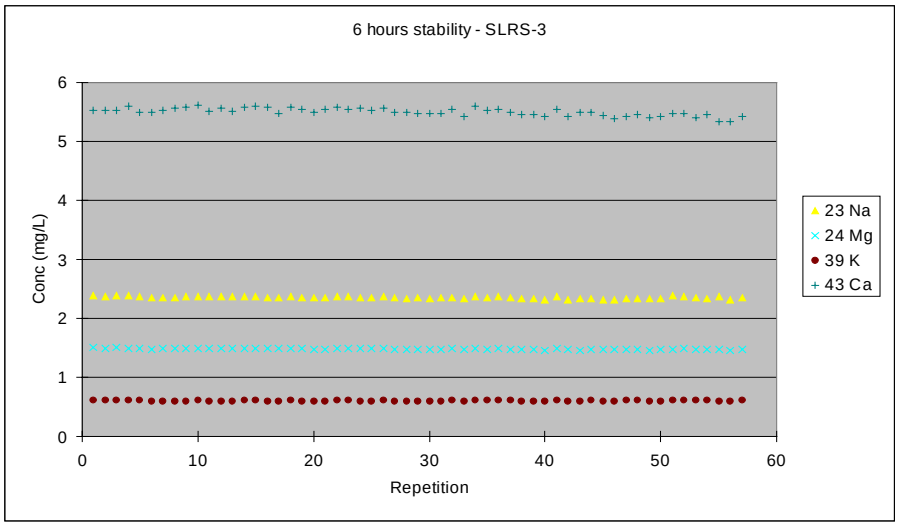

Fig. 1a Agilent 7500a Long Term Stability of Major Elements in SLRS-3

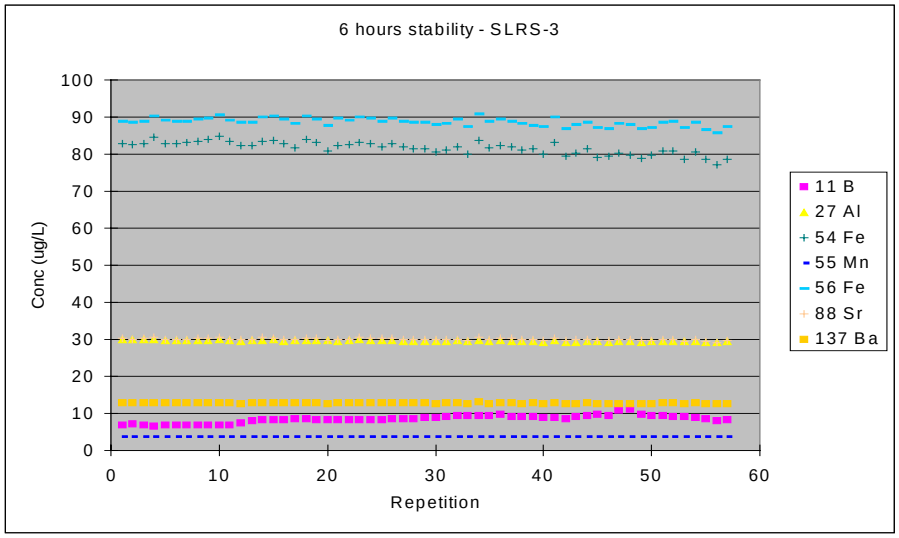

Fig. 1b Agilent 7500a Long Term Stability of Minor Elements in SLRS-3

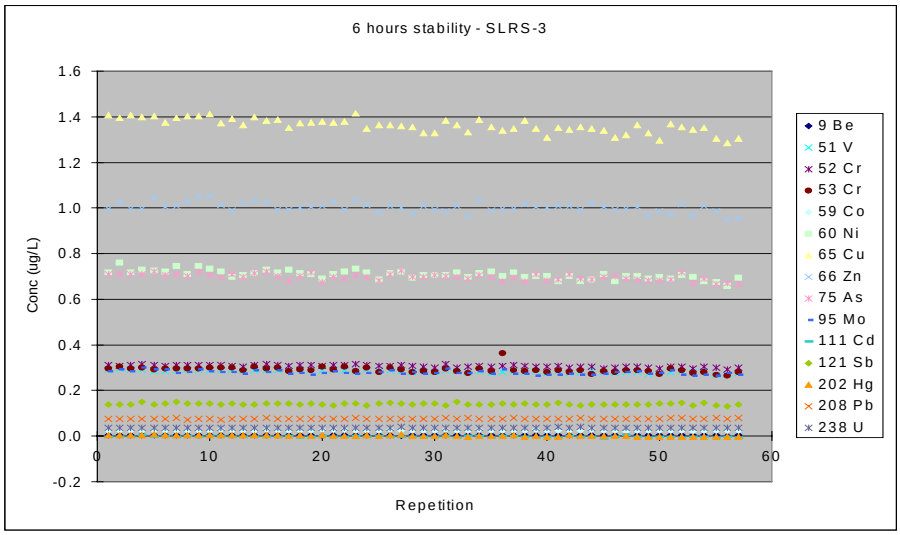

Fig. 1c Agilent 7500a Long Term Stability of Trace Elements in SLRS-3

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5980-0242E

Indirect Determination of Fluoride Traces in Natural Waters by Ion Chromatography and ICP-MS Detection

Application Note

ICP-MS

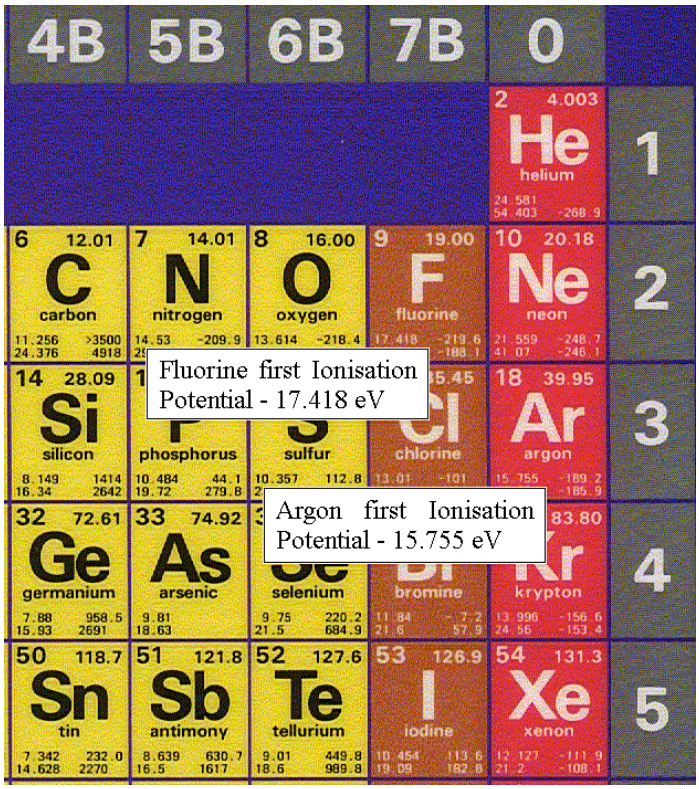
Environmental

Maria Montes Bayon, University of Oviedo, Spain

Abstract

ICP-MS has been widely accepted as a powerful analytical technique for trace element determination in a wide variety of sample types. The technique is rapid, measures virtually all elements in a single acquisition and has limits of detection typically at or below the ng/L (ppt) level. Even initially problematic elements, such as K, Ca, Fe, As and Se, are now routinely measured using the power and flexibility of the Ar ICP to preferentially remove troublesome spectral overlaps.

However, there are some analytical challenges which cannot be overcome by plasma optimisation, most notably the analysis of elements which are not ionised in the Ar plasma. This Application Note presents a novel method for the indirect determination of one such element, fluorine, where the preliminary data indicates that the ICP-MS measurement is not only possible, but offers significant advantages over traditional analytical methods.



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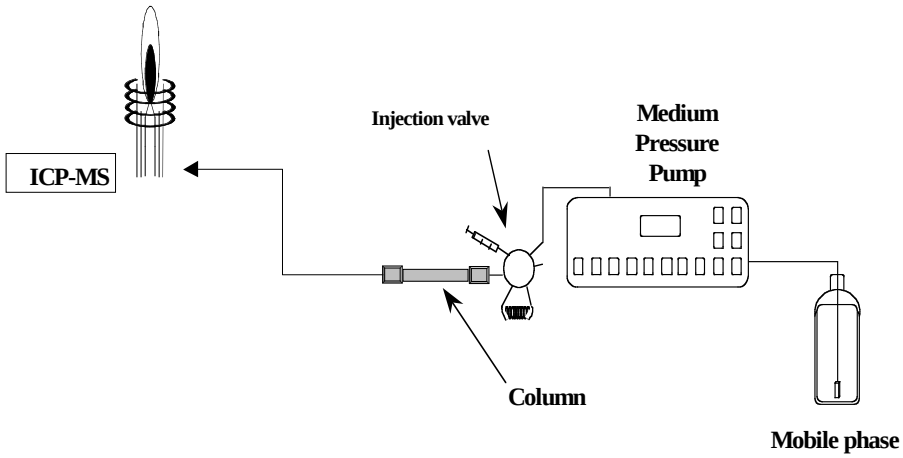

Figure 1 Experimental Set-Up for Fluoride Determination

Table 1 Typical Operating Conditions

Instrument	HP 4500
Rf Power	1300 Watts
Nebuliser	Meinhard
Spray Chamber	Scott type, double pass, room temperature
Sampling depth	5.7 mm
Gas Flow Rates:	
Cool	15 $L.min^{-1}$
Auxiliary	1 $L.min^{-1}$
Carrier	1.17 $L.min^{-1}$
Oxide level (CeO^+/Ce^+)	<0.5 %
Doubly charged level (Ce^{2+}/Ce^+)	<1%

Measurement of Fluoride

During the last decade, the majority of fluoride determinations have been performed using techniques such as potentiometry with fluoride Ion Selective Electrodes (ISE) , ion-chromatography with conductivity detection and, most recently, capillary ISEs has been the preferred technique, but is limited to determinations in the limited to determinations in the mg/L (ppm) range.

The chromatographic separation of Al-fluoride species was first described by Bertsch and Anderson, who determined the stability constants of several AlFx species. In acid aqueous solution, aluminium ions are present as [Al (H2O)6]3+ which can react with F- to form the AlF2+ complex.

Optimum PH for the complex formation seems to be between 2-4, therefore in the present work pH 2.6-3 was selected, where the complex AlF2+ proved to be stable.

Samples and standard solutions were adjusted to pH=3 with nitric acid and spiked with Al3+ requiring at least a 5-fold weight excess of Al to fluoride to assure that only AlF2+ was formed. The samples were diluted by weight, transferred to 10ml polypropylene test tubes and immersed in a water bath at 50ºC for 60 minutes, to ensure quantitative formation of the AlF2+ complex. Under these conditions, several parameters were evaluated to obtain selective separation of the complex AlF2+ in a 5 cm long ion exchange Dionex Ion Pac Column HPIC-CG2. HNO3 was found to be an effective eluent for the separation of AlF2+ from the excess of Al3+. Different molarities of nitric acid, from 0.15 M to 0.75 M, were tested and the conditions chosen for future studies were 0.45 M nitric acid at a flow rate of 0.5 mL/min. The AlF2+ complex was measured indirectly by ICP-MS by collecting data for Al at mass 27.

Figure 1 shows the instrumental set-up of the IC-ICP-MS system. The exit tube from the column was connected directly to the concentric nebulizer of the ICP-MS, which can accept flow rates anywhere from 20uL/min to over 2mL/min.

ICP-MS operating conditions are summarised in Table 1.

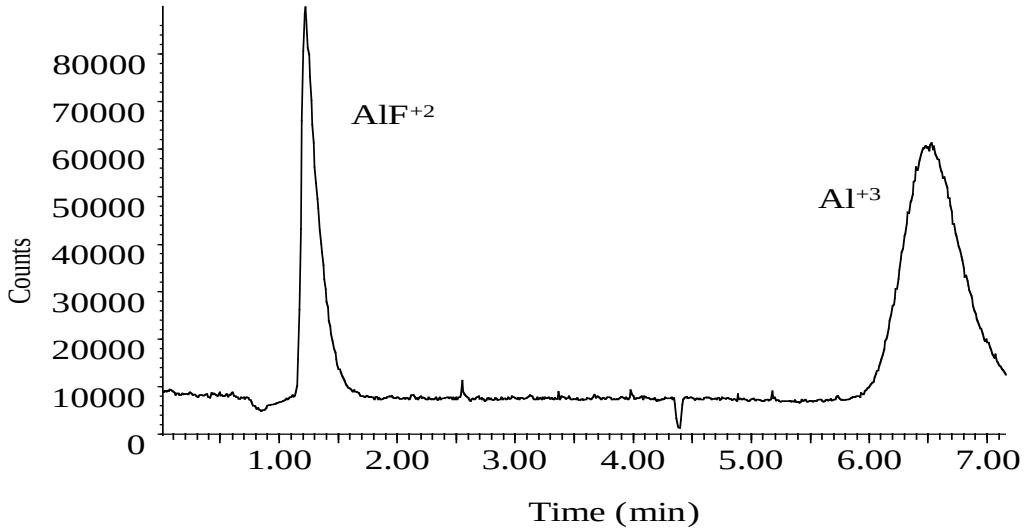

Figure 2 Chromatogram Corresponding to 20 ng.g^{-1} F^{-}

Table 2 Analytical Characteristics

Analytical Characteristics	ICP-MS Detection
Detection Limit	0.1 ng.g^{-1}
Precision	4 % (1)
Linear Range	up to 100(2) ng.g^{-1}
Regression coefficient (r) (n=7 points)	0.9993

(1) on 5 injections of 20 ng.g^{-1} fluoride **(2) using 500 ng.g^{-1} aluminium**

The chromatogram obtained under these conditions for 20 ng/g F^- in the presence of 100 ng/g of Al is shown in Figure 2.

As can be observed, two aluminium containing peaks are detected. The first peak could be ascribed to the AlF^{2+}complex as its peak height/area was found to be proportional to the concentration of fluoride in the sample.

Analytical Performance Characteristis

Analytical performance characteristics are summarised in Table 2. The linear dynamic range for fluoride determination depends on the aluminium excess added to the sample. It was observed that, for a given aluminium concentration, the upper linear limit for fluoride determinations was about one fifth of the total aluminium concentration. Aluminium concentrations higher than 500 ng/g were not tested, to avoid contamination effects in the ICP-MS. In practice, it should be straightforward to dilute samples to a level of fluoride where Al addition would be at an acceptable level, as shown in this study. Alternatively, ISE could be used as a screening tool, after which the IC-ICP-MS method could be used to determine those fluoride levels which were found to be below the limit of detection for ISE.

The detection limit obtained by the IC-ICP-MS method was 0.1 ng/g, calculated as three times the standard deviation of the blank, divided by the slope of a linear calibration between 0-5 ng/g. As can be observed, the detection limit using ICP-MS detection is one of the lowest ever reported for the determination of fluoride.

Figure 3 shows a typical calibration curve obtained from 5 to 50 ng/g (ppb)

of fluoride and containing 200 ng/g aluminium in each standard solution The linear calibration demonstrates that the peak area of the complex is proportional to the fluoride concentration. Determination of Fluoride in Fresh and Sea Water Samples.

Under the optimum separation conditions using HNO_3 for elution, the retention time for Al^{3+} is under 7 minutes, allowing a sampling rate of 6-7 samples per hour. To evaluate the use of the proposed ICP-MS method in routine operation, it was applied to the determination of fluoride in natural and drinking waters from a variety of sources and with different saline concentration.

At this time, no stable aqueous fluoride reference material was available, so it was decided to compare the proposed methodology with the fluoride ion selective electrode (FISE). Since many natural water samples contain fluoride levels below the limit of detection of FISE, a spike-recovery exercise was also undertaken.

In order to minimise aluminium addition to the samples and contamination of the ICP-MS, up to 200 fold dilution of some drinking and sea-water samples was necessary. The results obtained are summarised in Table 3.

Some of the water samples contained fluoride levels too low to be measured by FISE (at around 150 ng/g), so comparison between the FISE method and the IC-ICP-MS method was not possible. However, where FISE was able to measure the levels present, good agreement with the IC-ICP-MS results was obtained. In the other cases tested, the spike recovery exercise indicated that the IC-ICP-MS method gave good recoveries (within 100±10%), showing the applicability of the proposed methodology to perform fluoride determination at extremely low levels in natural water samples.

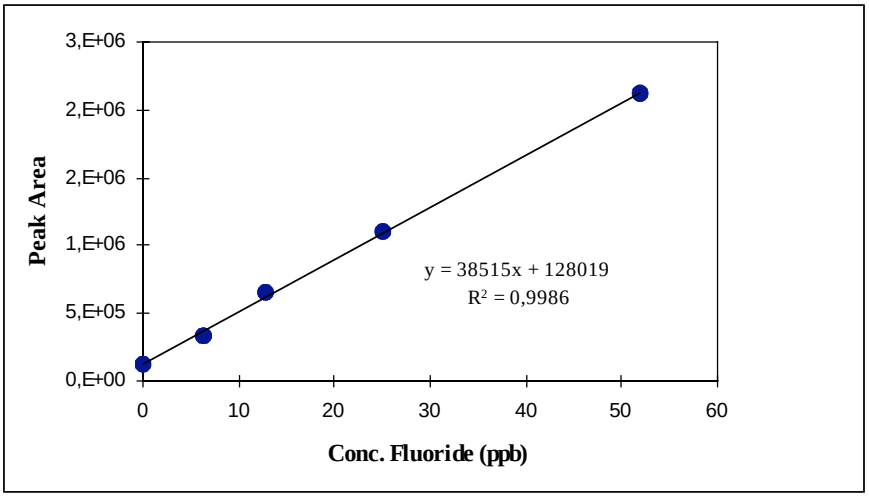

Figure 3 Typical Calibration Curve of Fluoride

Table 3 Results Obtained for Fluoride in Water Samples Using ICP-MS

Water Sample (dilution factor)	Conc. found(n=3) ICP-MS($ng.g^{-1}$)	Conc. found FISE ($ng.g^{-1}$)	Spiked amount ($ng.g^{-1}$)	Recovery (%)
Fontecelta (200)	8050±80	7700	4300	104
Font-Vella (10)	182±2	-	205	97.8
Tap-water (10)	161±1	-	210	90
*Sea-water(100)	1030±60	1080	1080	97.5

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Analysis of Mercury in Wastewater by ICP-MS using the Agilent 7500i

Application Note

ICP-MS

Environmental

Steven Wilbur

The analysis of wastewater for mercury by ICP-MS can present a number of challenges. First, mercury has a relatively low response factor since it is only about 40 percent ionized in a typical argon plasma. It is also subject to ionization suppression in the presence of easily ionized matrix elements that can reduce the response even further. Secondly, because of its high vapor pressure, it can be subject to severe memory effects. Finally, the most abundant Hg isotope available for quantitation is 202Hg, which is only 29.9% abundant.

In order to analyze mercury efficiently in high matrix samples like wastewater, the ICP-MS must be able to maximize the transfer of energy to the analyte atoms. This is achieved in the Agilent 7500i ICP-MS by minimizing the matrix load on the plasma, so ensuring a high and stable plasma temperature. The high plasma temperature also ensures good matrix decomposition, which reduces the impact of the matrix on the interface, ion lenses, vacuum pumps and mass analyser. The use of constant-flow nebulization with the Agilent Micro Flow 100 nebulizer significantly reduces memory effects for Hg, by reducing the total sample flow to the nebulizer and spray chamber. A low sample flow rate, removal of water vapour and use of a wide-bore injector in the plasma torch all contribute to a reduced total matrix load on the plasma, increasing the available energy for analyte ionization. The Agilent 7500's good stability and low random background also allow accurate and precise measurements to be made at very low concentrations.

Acquisition Parameters

Tune conditions are displayed in Figure 1 and Figure 2.

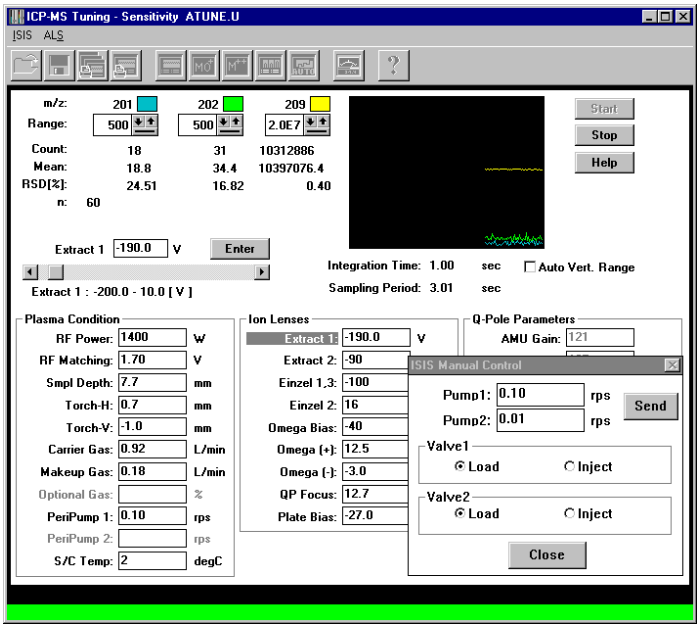

Figure 1 Tune Screen Showing Calibration Blank: counts at m/z 201 and 202 are due to very low background of Hg present in the blank, m/z 209 is bismuth internal standard, (1 sec. integrations)

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Hg measurements were made using 3 sec. integrations per peak. Acquired masses 199, 200, 201, 202 and 209. All 4 mercury masses were summed (at m/z 202) using a correction equation, in order to improve counting statistics.

ISIS parameters

30 seconds uptake at 0.5 rps, analysis speed = 0.1 rps. Constant flow nebulization at ~100 uL/min, using Agilent Micro Flow 100 nebulizer. Rinse 30 seconds at 1 rps.

Calibration

Calibration standards were prepared in DI water acidified to 2% with nitric acid and containing 100 ppb Au to stabilize the mercury at low concentrations in solution. Standards were prepared at 10, 20, 50, 100, 200 and 500ppt Hg. The calibration curve obtained is shown in Figure 3.

Method Detection Limits

3 sigma detection limits were calculated from 7 replicate analyses of the Standard Reference Wastewater. It is shown in Table 2.

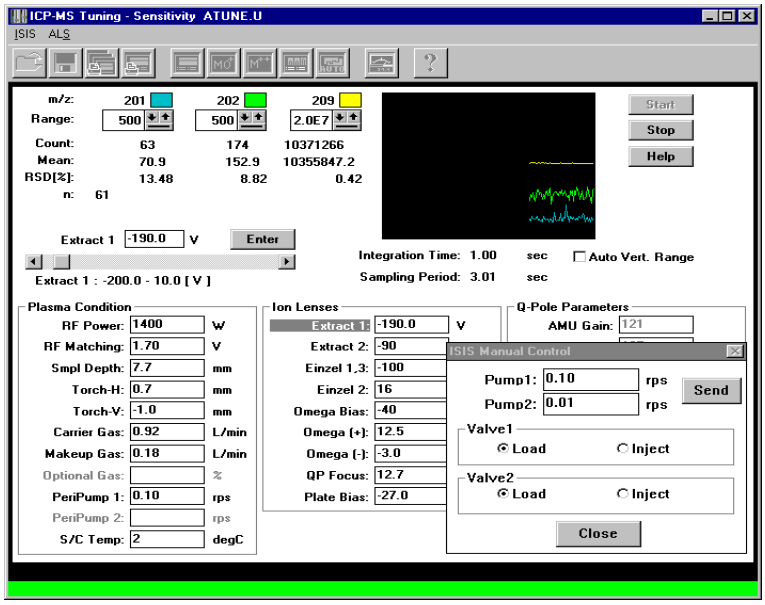

Figure 2 Tune Screen Showing High Purity Standards Certified Wastewater Spiked with 50 ppt Hg (1 sec. integrations). Note signal increase at m/z 201 and 202

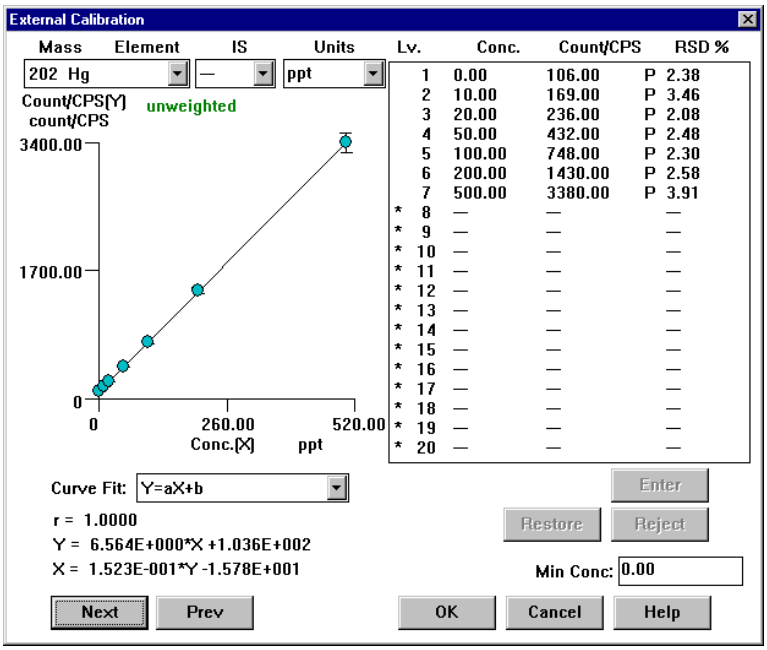

Figure 3 External Calibration, Hg in 2% HNO_3, 10 - 500 ppt

Table 1 Spike Recoveries from Undiluted Wastewater Standard [1]

[Unspiked Sample]	Spike Amount	[Spiked Sample]	% Recovery
14.11 ppt	50 ppt	65.9 ppt	103.6 %
14.11 ppt	100 ppt	118.8 ppt	104.6 %

[1]) High Purity Standards Certified Wastewater – Trace Metals, Lot # 590209

Uptake and Rinseout

By using ISIS in the Rapid Sample Uptake mode, it is possible to transport the sample from the autosampler to the nebulizer input tee very rapidly and at high flow. Since the nebulizer is operating at constant flow, the excess sample or rinse flow is split to the drain line and does not overload the spray chamber or plasma. Rapid uptake and constant-flow nebulization both serve to reduce mercury memory effects. The data in Figure 4 were acquired with the ISIS sample pump at uptake speed using the Agilent 7500 time resolved mode of acquisition. The nebulizer pump was operated at constant flow of ~100 uL/minute. Acquisition was begun with the sample probe in the blank solution containing 2% nitric acid and 100 ppb Au. After 60 seconds, the probe was moved to a 1000 ppt Hg standard solution containing 100 ppb Au. Following an additional 120 seconds, the probe was returned to the blank solution.

Summary

The high sensitivity, low random background and excellent matrix tolerance of the Agilent 7500i ICP-MS, coupled with the low flow and high efficiency of the Agilent Micro Flow nebulizer, permit the analysis of the very difficult element mercury in wastewaters. The 7500i system is capable of excellent linearity, precision and accuracy at sub-ppb concentrations in waters and wastewaters. In this range of concentrations, mercury can be analyzed simultaneously with the other important trace elements, without impacting uptake and rinseout times to an unacceptable degree, thus eliminating a separate analysis for mercury.

Table 2 3 Sigma Detection Limits (ppt) for Mercury Isotopes m/z 199, 200, 201 and Sum of 199, 200, 201 and 202, Calculated from 7 Replicate Analyses of HPS Wastewater Certified Reference Material

File:	Date/Time:	Mercury /199	Mercury /200	Mercury /201	Sum (199,200,201,202)
009SMPL.D#	2/17/2000 11:50	11.19	10.78	12.68	16.53
010SMPL.D#	2/17/2000 11:55	10.84	10.16	10.3	15.54
011SMPL.D#	2/17/2000 11:59	10.34	10.9	12.48	16.23
012SMPL.D#	2/17/2000 12:04	11.82	11.09	12.93	16.41
013SMPL.D#	2/17/2000 12:09	10.54	10.68	11.53	15.88
014SMPL.D#	2/17/2000 12:13	10.62	9.245	10.43	15.72
015SMPL.D#	2/17/2000 12:18	10.3	11.13	11.42	16.09
	3 Sigma MDL (ppt)	**1.676**	**2.077**	**3.040**	**0.938**

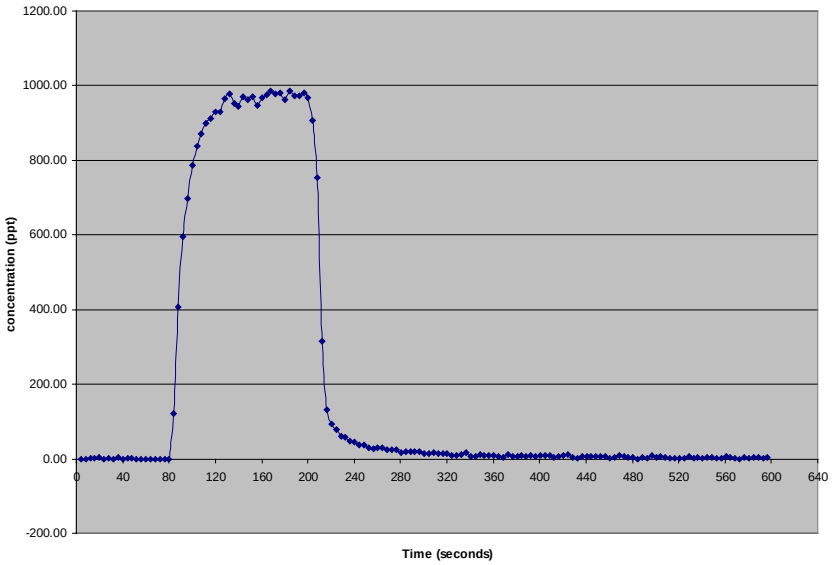

Figure 4 Uptake and Rinseout Profile
blank, 60 sec -> 1000 ppt standard, 120 sec -> blank

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Soil & Sediment

Other Applications

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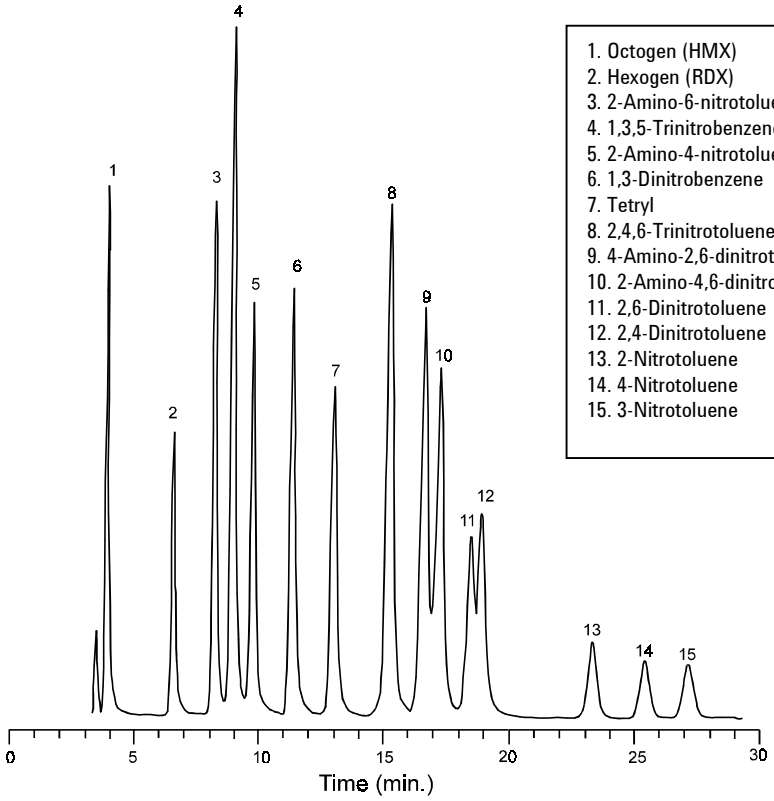
Reversed-Phase Separation
15 Explosives from Soil Extract

Application

Environmental

Robert Ricker



1. Octogen (HMX)
2. Hexogen (RDX)
3. 2-Amino-6-nitrotoluene
4. 1,3,5-Trinitrobenzene
5. 2-Amino-4-nitrotoluene
6. 1,3-Dinitrobenzene
7. Tetryl
8. 2,4,6-Trinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2-Nitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene

Courtesy of Ursula Hechler, Lab Dr. Wessling, Altenberge, Germany, Terra. Tech. **1**, 27-28 (1995)

Highlights

- *Medium-bore columns reduce both the amount of sample required and generation of mobile-phase waste. This medium-sized bore does not require a special low-volume chromatograph but does require that the system contain minimal dead volume.*
- *Isocratic operation has the advantage of quick turn-around between injections since re-equilibration is not required.*

Conditions:
ZORBAX SB-C18 (3 x 250 mm) (Agilent P/N: 880975-302)
Mobile Phase: Methanol:Water (50:50) (v/v)
Injection volume 10µl, 0.3 mL/min, Ambient , Detect. UV (230 nm)

Agilent Technologies

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April 25, 2002
5988-6342EN

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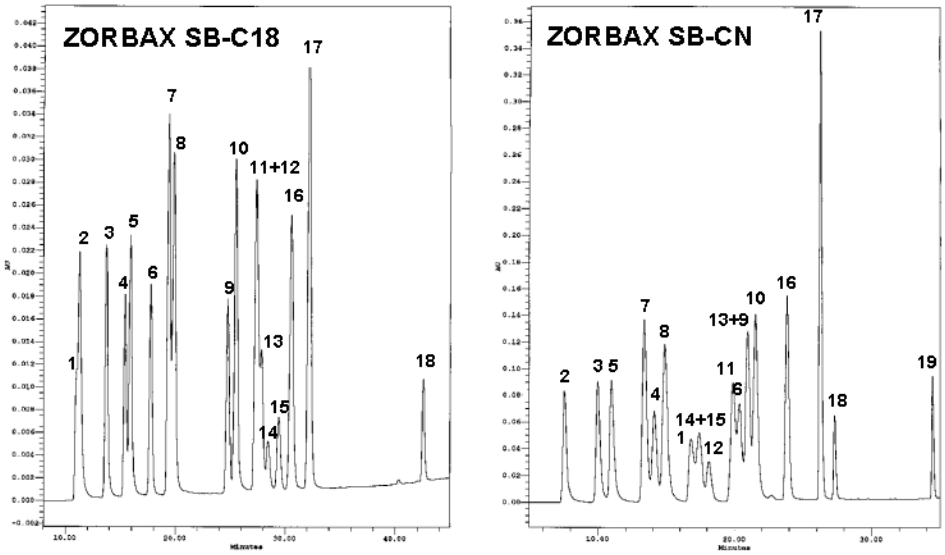
Qualitative and Quantitative Analysis of Explosives and Related Compounds Using Polar and Nonpolar HPLC Columns

Application

Environmental

Robert Ricker



Courtesy of Th. Renner, Hess. Landesanstalt f. Umwelt, Dez. III/2, P.O. Box 3209, 65022 Wiesbaden, Germany

Conditions:
ZORBAX® SB-C18, SB-CN (2.1 x 150 mm) (Agilent P/N: 883700.905, 883700.922)
Mobile Phase: A: ACN + 5% H_2O + 5 mM CF_3COONH_4
B: H_2O + 5% ACN + 5 mM CF_3COONH_4
pH 2.7 (CF_3COOH)
Inject: 10 µL of 19 nitromethanes in ACN:H_2O (20:80), 5mM CF_3COONH_4; 0.23mL/min, 18°C, Detect. UV(210, 240, 360 nm)

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Gradient Shapes

ZORBAX® SB-C18 (2.1x150 mm) (P/N:883700.922)
Sample: 0.5ng / µL

Time [min]	Flow [mL/min]	% A [%]	% B [%]
0	0.23	20	80
2	0.23	20	80
10	0.23	30	70
20	0.23	35	65
25	0.23	40	60
35	0.23	70	30
40	0.23	70	30
42	0.23	20	80
45	0.23	20	80

ZORBAX® SB-CN (2.1x150 mm) (P/N:883700.905)
Sample: 5ng / µL

Time [min]	Flow [mL/min]	% A [%]	% B [%]
0	0.23	20	80
1	0.23	20	80
15	0.23	30	70
30	0.23	80	20
35	0.23	80	20
37	0.23	20	80
40	0.23	20	80

Table 1 - List of Analytes

	Compound	Rt [min] on Zorbax SB-C18	Rt [min] on Zorbax SB-CN	Quantitation on Zorbax SB
1	picric acid	11.02	16.82	CN (360 nm)
2	4-amino-2-nitrotoluene	11.31	7.57	C18 (240 nm)
3	2-amino-6-nitrotoluene	13.72	10.00	C18 (240 nm)
4	RDX	15.42	14.13	C18 (210 nm)
5	2-amino-4-nitrotoluene	15.92	11.02	C18 (240 nm)
6	HMX	17.76	20.37	C18 (210 nm)
7	1,3-dinitrobenzene	19.41	13.40	CN (240 nm)
8	1,3,5-trinitrobenzene	19.87	14.90	CN (240 nm)
9	2-amino-4,6-dinitrotoluene	24.79	20.98	C18 (240 nm)
10	2,4-dinitrotoluene	25.52	21.55	C18 (240 nm)
11	4-amino-2,6-dinitrotoluene	27.37	19.90	CN (240 nm)
12	2-nitrotoluene	27.41	18.13	CN (240 nm)
13	2,6-dinitrotoluene	27.84	20.87	C18 (240 nm)
14	4-nitrotoluene	28.47	17.39	C18 (240 nm)
15	3-nitrotoluene	29.46	17.43	C18 (240 nm)
16	2,4,6-trinitrotoluene	30.59	23.85	CN (240 nm)
17	tetryl	32.22	26.30	CN (240 nm)
18	diphenylamine	42.59	27.32	CN (210 nm)
19	hexyl	45.78	34.45	CN (360 nm)

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Agilent Technologies

Analysis of ionic tenside surfactants in wastewater by HPLC

Rainer Schuster

Environmental

Abstract

The detergents used for cleaning floors, worktop surfaces and laundry in the home and hygene industry are the main source of the ionic species of surfactants known as tensides.

Sample preparation

Tensides can be extracted from either surface water or waste water by a liquid–solid technique. Narrow bore technology for lowest solvent consumption and highest sensitivity, with automated diode-array detection for evaluating peak purity and identity.

Separation

Figure 1 shows a normal flow rate elution on a 10 cm Hypersil ODS column with 2.1 mm internal diameter, 5 µm particles. A simple linear gradient and a constant oven temperature of 40 °C achieve good resolution.

- UV absorbance detection or
- Diode-array detection—for peak purity check and peak identity confirmation using UV absorbance spectra.

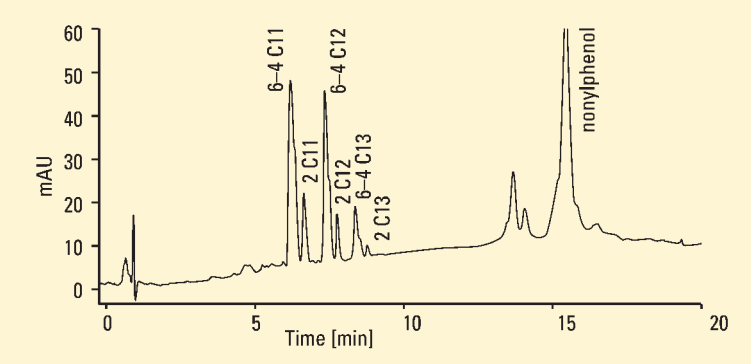

Figure 1
Separation of 10 µl injection of a Marlon A ionic tenside surfactant standard

Conditions

Column
250 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
A: 0.005 M KH_2PO_4
B: acetonitrile
Gradient
0 min 26% B
20 min 100% B
Flow rate
0.25 ml/min
Temperature
40 °C
Detection
222 nm (20 nm bandwidth)
reference 450 nm (100 nm bandwidth)

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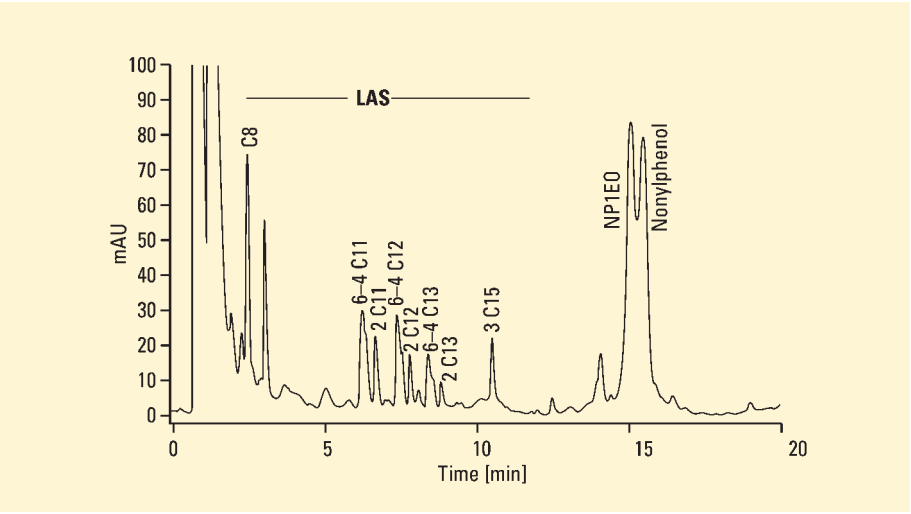

Figure 2
Analysis of linear alkylbenzenesulfonates (LAS), alkylphenol polyethoxylates (APEO) and nonylphenol in waste water

References

1.
Rainer Schuster, "Analysis of ionic tenside surfactants in wastewater by HPLC and diode-array detection," *Hewlett-Packard application note*, publication number 12-5091-1818E,

Conditions

Column
250 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
A: 0.005 M KH_2PO_4
B: acetonitrile
Gradient
0 min 26% B
20 min 100% B
Flow rate
0.25 ml/min
Temperature
40 °C
Detection
222 nm (20 nm bandwidth)
reference 450 nm
(100 nm bandwidth)

Equipment

Agilent 1100 Series
- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector
- fluorescence detector

Agilent ChemStation + software

Rainer Schuster is application chemist at Agilent Technologies, Waldbronn, Germany.

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Released 10/97
Publication Number 5966-1877E

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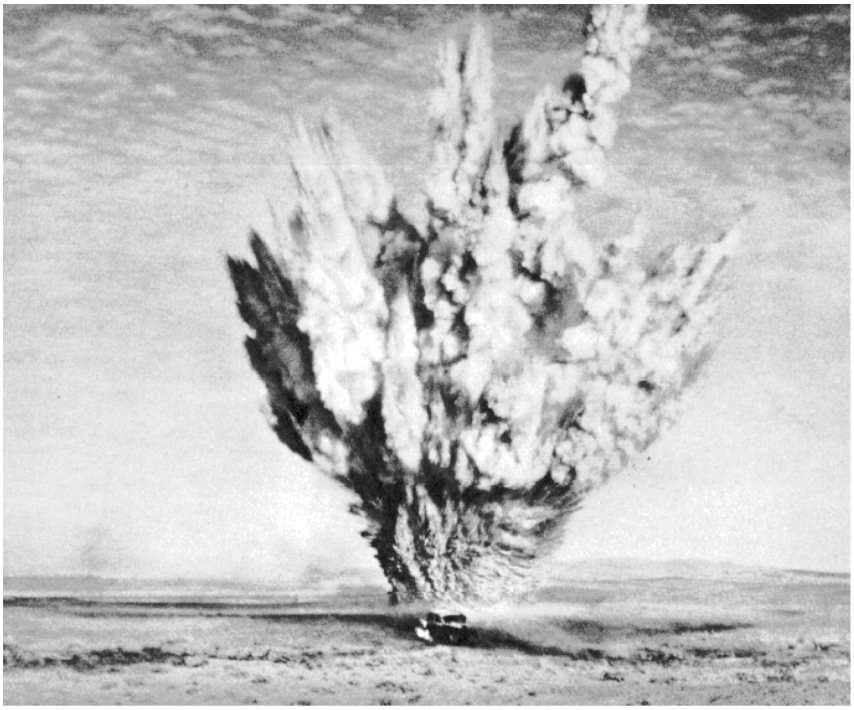
HPLC Analysis of Explosive Constituents in Soil Samples

Application Note

Environmental

Rainer Schuster and
Angelika Gratzfeld-Huesgen



An HPLC method for detecting traces of explosive constituents at low nanogram levels in soil samples is described. The analytes were extracted from soil with acetonitrile in an ultrasonic bath. Analysis was performed using a newly-designed base-deactivated Hypersil column. Compounds were identified and confirmed by spectral library search and by retention time tagging. The detection limit from a 2-g sample in 10 ml ACN extracted was 8 to 20 mg/kg at an injection volume of 10 µl. The retention time precision was better than 0.5 % RSD and the quantitative precision was between 0.5 and 2.5 %. Linearity was better than 2.5 % over the range from 2 to 500 ng/µl.

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Introduction

Armed forces all over the world have been disposing of expired munitions in appreciable quantities for the last century. Most of this disposal involves direct combustion, such as detonation or incineration, which partially removes some of the toxic constituents. However, a significant proportion of dumped explosives contaminates soil and/or groundwater.

The most widely found explosives are trinitrotoluene (TNT), its corresponding metabolites and hexogen. The most commonly used explosives are listed in table 1. Over time, explosive residues and their transformation products have accumulated in large areas of soil formerly occupied by settling lagoons. Concern about the environmental fate of these residues is now intensified because recent studies of the toxicity of TNT have shown liver damage and anaemia among chronically exposed munitions workers.[1] In addition, TNT has been found to be mutagenic.[2]

The metabolism of TNT in bacteria, animal and plant systems has been examined in a number of investigations. According to McCormick,[1] TNT is reduced by bacteria to 2-amino-4,6-dinitrotoluene (2-AMDNT) and 4-amino-2, 6-dinitro- toluene (4-AMDNT); a metabolism that occurs also in plants and animals. This universal metabolism of TNT by bacteria, animals and plants to the AMDNT isomers is significant, as both metabolites show severe toxicity and mutagenicity. Other metabolites found are dinitrotoluenes, and both di- and trinitrobenzenes.

Accurate assessment of environmental and health risks demands analytical methods that provide for an acceptable mass balance. Due to the limited thermal stability of explosives residues, like octogen, hexogen, tetryl, nitropenta and aminometabolites, a method utilizing high-performance liquid chromatography (HPLC) has been developed. The US Environmental Protection Agency (EPA) has published a draft method for the analysis of 12 explosives in soil using HPLC and UV-Visible detection.[3]

In this Application Note we describe the analysis of the 12 EPA-listed explosives plus 7 other commonly used explosives and metabolites (table 1) using a newly-developed base-deactivated Hypersil reversed phase column. For peak confirmation we used a diode-array detector on-line coupled to the HPLC column.

A special software program for the Agilent Technologies HPLC 3DChemStation (DOS Series) quantified the explosives residues and automatically identified them as present when retention times and spectral identification matched the specified match threshold values.

		Listed in EPA 8330[3]
2,4,6-Trinitrophenol	2,4,6-TNP	
Octogen (RMX)		x
Hexogen (HMX)		x
1,3,5-Trinitrobenzene	1,3,5-TNB	x
2-Amino-6-Nitrotoluene	2-A-6-NT	
1,2-Dinitrobenzene	1,2-DNB	
1,3-Dinitrobenzene	1,3-DNB	x
2-Amino-4-Nitrotoluene	2-A-4-NT	
Nitrobenzene	NB	x
Tetryl		x
2,4,6-Trinitrotoluene	TNT	x
2-Amino-4,6-Dinitrotoluene	2-A-4,6-DNT	
4-Amino-2,6-Dinitrotoluene	4-A-2,6-DNT	
2,4-Dinitrotoluene	2,4-DNT	x
2,6-Dinitrotoluene	2,6-DNT	x
2-Nitrotoluene	2-NT	x
4-Nitrotoluene	4-NT	x
3-Nitrotoluene	3-NT	x
Nitropenta		

Table 1
19 commonly-used explosives analyzed in this study together with the 12 explosives listed in EPA draft method 8303[3]

Experimental

Soil samples were prepared according to EPA Draft Method No. 8330.[3] A 2-g sample was placed in a 15-ml glass vial and 10 ml of acetonitrile was added. The vial was capped with a Teflon-lined cap, vortex swirled for one minute, and then placed in an ultrasonic bath for 18 hours. When tetryl was being analyzed, the ultrasonic bath was kept at, or below, room temperature.

After sonication, the sample was allowed to settle for 30 minutes; 5 ml of supernatant was removed and combined with 5 ml of calcium chloride solution (5 g/l). The mixture was shaken and then left to stand for 15 minutes. The super- natant was placed in a syringe and filtered through a 0.5-µm filter. The first 2 to 3 ml were discarded and the remainder was retained for analysis.

Chromatography

For the chromatographic separation we used an HP 1090 Series M liquid chromatograph with DR 5 binary solvent delivery system, variable-volume autoinjector, temperature-controlled column compartment and solvent-preheating device.

For instrument control and data evaluation we used an HPLC ^{3D}ChemStation (DOS Series) from Agilent Technologies. A Hypersil BDS column from Agilent Technologies was used for all experiments. The column contained 3-µm particles, and had a 4-mm inner diameter and a length of 100 mm. The mobile phase was a gradient system water-methanol (HPLC) from Baker (Gross-Gerau, Germany) at a flow rate of 0.72 ml per min. The column compartment temperature was 38 °C.

A UV-Visible diode-array detector was used for dual wavelength detection and on-line spectral acquisition. The detection wavelengths were 214 nm with a bandwidth of 10 nm, and 235 nm with a 40-nm bandwidth. The complete chromatographic conditions are listed with figure 1.

Detection and compound confirmation

Due to possible interferences from the soil matrix it is mandatory to identity compounds of interest not only by retention time, but also by a further confirmation step.

With diode-array based detection, several wavelengths can be selected and peak spectra can be acquired automatically. This enables an individual library to be compiled for each class of compound. The confirmation used the dedicated spectral library for explosives with retention-time tagging. A software routine developed for this task searched every peak within each chromatogram for the best match to library spectra within a given retention time range, expressed as a percentage.

In addition, for each peak, a purity check — an overlaid baseline corrected for up- and downslope spectra — was performed. After each search a chromatogram is plotted and a customized report is printed. The report contains all the data: retention times, analysis, library and calibration table, amounts, library purity data and peak names.

The method is fully automatic. Data acquisition and data evaluation, including quantification and qualitative identification are performed automatically.

Method validation

For method validation such as precision, linearity, and sensitivity, we used the system suitability software employed by the HPLC ^{3D}ChemStation.

Results and discussion

We were able to separate the common explosives and metabolites. Figure 1 shows a chromatogram of a standard at about 10 to 20 ng/injected amount.

Several soil samples were analyzed for these explosive constituents. One soil sample was analyzed for tear gas.

Gradient	26 % B, to 40 % B at 10 min, to 55 % B at 20 min, to 70 % B at 30 min, to 26 % B at 31 min
Oven temperature	38°C
Detection wavelengths	214 nm for nitropenta 235 nm for other nitrocompounds

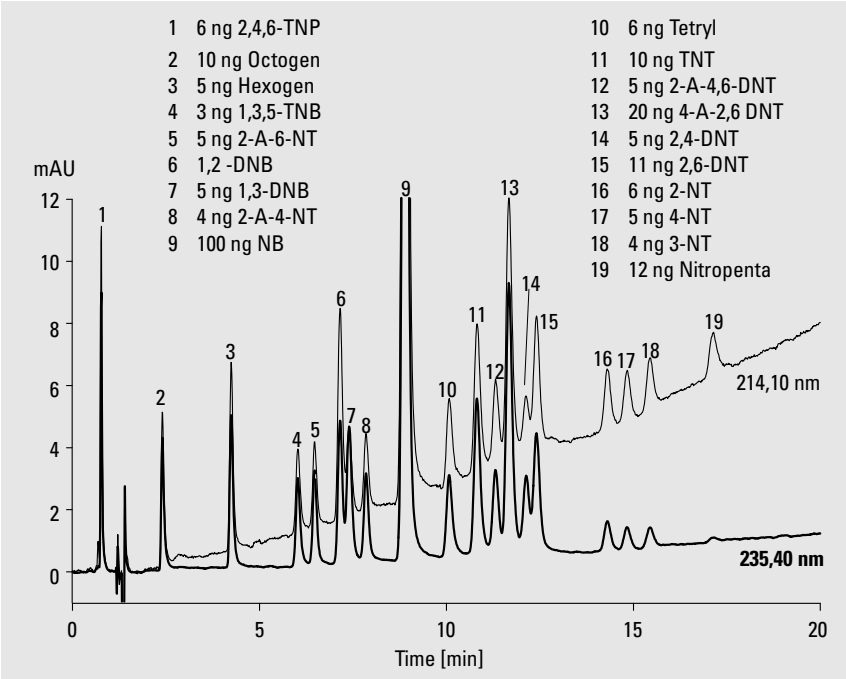


Figure 1
Chromatogram of standard sample

Figure 2 shows the chromatogram of a soil sample. The major explosive constituent 2,2',4,4',6,6'-hexanitrodiphenylamine (2,2',4,4',6,6'-HNDA) at 20.2 min was identified by spectral library search and retention time comparison with a standard.

After spectral search without any retention time-window (throughout the whole library) the strong tailing peak at 5.7 min was indicated to be a metabolite of the major constituent 2,2',4,4',6,6'-HNDA.

In addition, large amounts of 2,4,6-TNT and the corresponding metabolites 2,4-DNT, 3,4-DNT and 1,3,5-DNB could be identified. Amounts were in the µg to mg/g-range (for example, 3.2 mg TNT/g soil, 1.9 mg 2,4-DNT/g soil), figure 2.

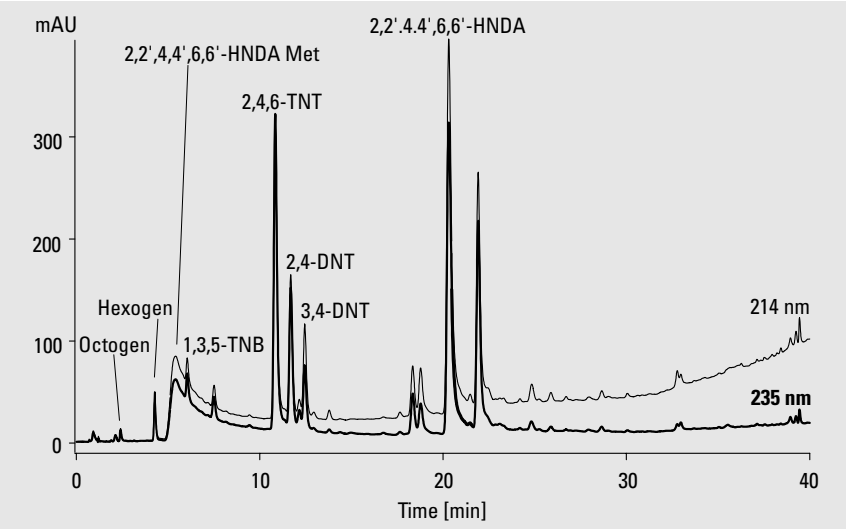


Figure 2
Soil sample after extraction with acetonitrile and ultrasonic extraction

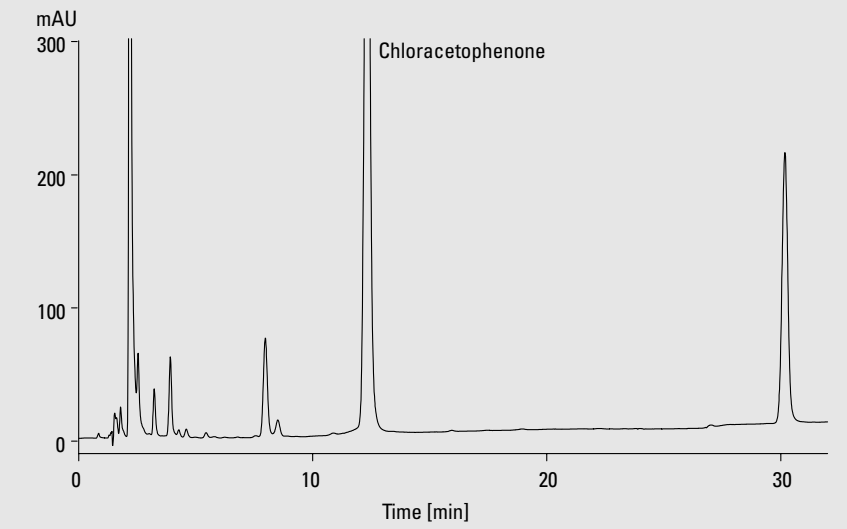


Figure 3a
Soil sample from Huenxe (N. Germany) extracted according to EPA Draft Method No. 8330[3]

In figure 3 a soil analysis with rather high levels for 1,3-dinitrobenzene and TNT, 240 mg/kg of soil for 1,3-DNT, and 14 mg/kg soil for TNT, is shown. After the chromatographic run a report was generated with compound names, retention time of sample and standards, and with purity and identity match factors. TNT and 1.3-DNT have been positively confirmed by retention times and UV spectra.

The following peaks at 12.07, 18.9 and 21.1 min all show similar spectra. They are not found in the library, however, the spectral characteristics indicated them to be nitrometabolites.

Report

Quantification method:	ESTD	calibrated by	Area response
Sample Info:	PROJECT: EXPLOSIVES		
Misc. Info:	SAMPLE: HUENXE 40 mg/CH_3CN .2 ml		
Method File Name:	EXPLO.M	Wavelength from:	205 to 400 nm
Library File Name:	UMW:EXPLO.L	Library Threshold:	950
Reference Spectrum:	Apex	Peak Purity Threshold:	950
Time Window from:	8.0 & to 5.0 %	Smooth Factor:	5
Dilution Factor: 1.0	Sample amount: 0.0	Resp.Fact.uncal.peaks:	None

Name	Amount [mg/kg]	Peak-Ret. [min]	Cal.-Ret. [min]	Lib.-Ret [min]	Purity Matchfactor	Library	Res.
1,3 Dinitrobenzene	239.52	A 7.372	7.402	7.377	1000	1000	0.9
2,4,6-Trinitrotoluene	13.65	A 10.789	10.829	10.782	1000	1000	4.7
...?-Amino-4.6-dinti	-----	A 10.789	-----	11.211	1000	961	4.7
	=====						
	253.17						
	=====						
Not identified peaks							
Hexogen ?	1.21	A 4.520	4.249	4.288	965	496	3.1
2-nitrotoluene ?	5.08	A 14.055	14.308	14.483	987	821	1.2

Figure 3b
Automatically-printed report accompanying chromatogram in figure 3a

A critical "non-explosive" compound — often carelessly disposed of — is chloracetophenone, the major constituent of tear gas. The analysis of this compound in soil can be performed under the same conditions as for explosives, figure 4.

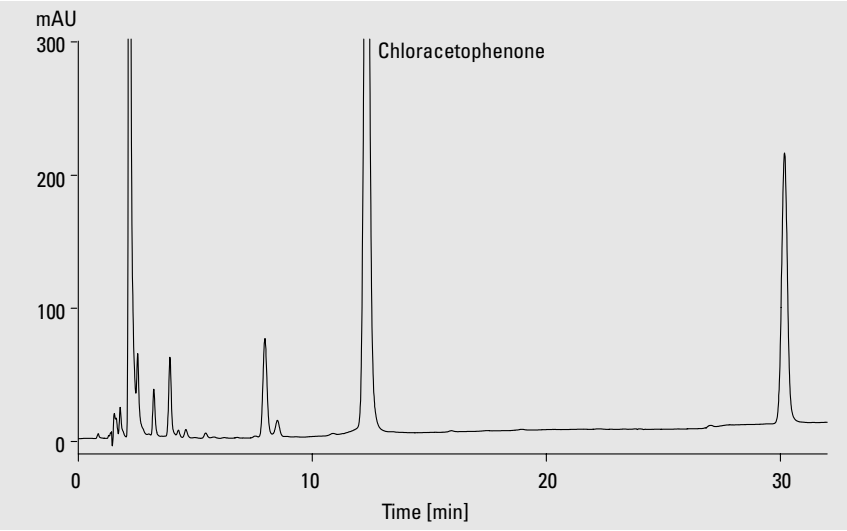


Figure 4
Analysis of chloracetophenone in soil

Figure 5 shows the separation of explosive constituents in a soil sample that are often described as critical: 2,4,6-TNT, 2A-2,6-DNT and 4-A-2-6-DNT.

Concentrations were in the low ng-range for each compound: 50 ng TNT; 15 ng 2-A-4,6-DNT; and 15 ng 4-A-2,6-DNT.

All three compounds were identified by retention times and UV spectra. in figure 5 the sample spectrum of 2,4,6-TNT is overlaid with the library spectrum.

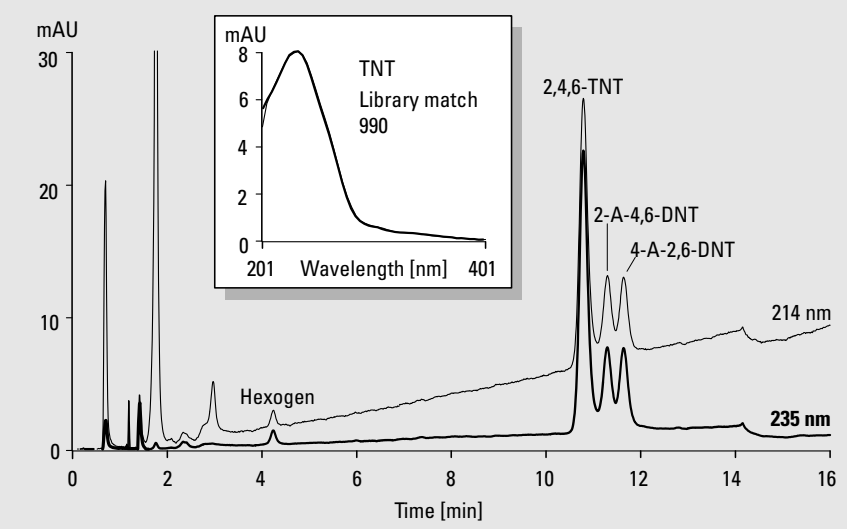


Figure 5
Shows the determination of TNT and the aminometabolites 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT) and 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT) in soil samples.

Selectivity and recovery

Surprisingly, most of the analyzed real life soil samples did not show many interferences from other compounds; most of them showed a purity factor of 1000 and identification via library search was close to 1000, indicating efficient sample preparation.

Recovery rates for a spiked soil sample (10 mg/kg) for these compounds have been found to be between 85 and 100 % according to an interlaboratory comparison[4].

Linearity and repeatability

The linearity for 2,4,6-TNT was checked and found to be better than 2.5 % within the range from 2 to 500 ng/µl.

The repeatability for all compounds was better than 0.2 % for retention time and between 1 and 3.5 % for peak area, except for nitropenta, which was 7 %, due to its instability. The calculation was based on 8 runs with injected amounts of 8 to 20 ng absolute. Figure 6 shows parts of print-outs from a system suitability report as generated by the HPLC ChemStation.

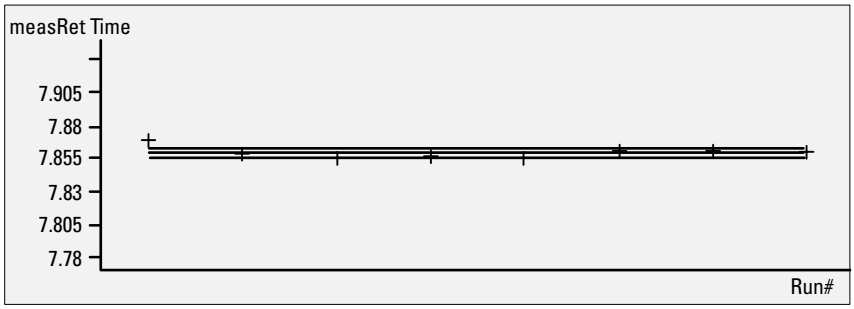
	%RSD	
	RT	**Peak area**
2,4,6-Trinitrophenol	0.37	2.18
Octogen (RMX)	0.04	2.38
Hexogen (HMX)	0.09	1.14
1,3,5-Trinitrobenzene	0.09	1.86
2-Amino-6-Nitrotoluene	0.12	1.99
1,2-Dinitrobenzene	0.13	1.23
1,3-Dinitrobenzene	0.10	1.00
2-Amino-4-Nitrotoluene	0.13	2.74
Nitrobenzene	0.11	0.77
Tetryl	0.14	3.36*
2,4,6-Trinitrotoluene	0.11	1.52
2-Amino-4,6-Dinitrotoluene	0.14	2.68
4-Amino-2,6-Dinitrotoluene	0.14	1.46
2,4-Dinitrotoluene	0.11	3.69
2,6-Dinitrotoluene	0.10	2.15
2-Nitrotoluene	0.08	2.13
4-Nitrotoluene	0.08	2.44
3-Nitrotoluene	0.09	2.44
Nitropenta	0.06	6.91*

* due to instability of these compounds

Table 2
Precision of retention times and peak areas

Compound (signal No. 1): 1,3-DNB

Run #	measRet Time [min]
1	7.8670
2	7.8573
3	7.8539
4	7.8552
5	7.8540
6	7.8586
7	7.8586
8	7.8567
Mean:	7.8577
S.D.:	0.0042
RSD [%]:	0.0537



Run #	Area [ ]
1	57.8521
2	58.2713
3	58.2464
4	58.4260
5	58.7530
6	58.8206
7	58.5031
8	57.9159
Mean:	58.2841
S.D.:	0.4798
RSD [%]:	0.8232

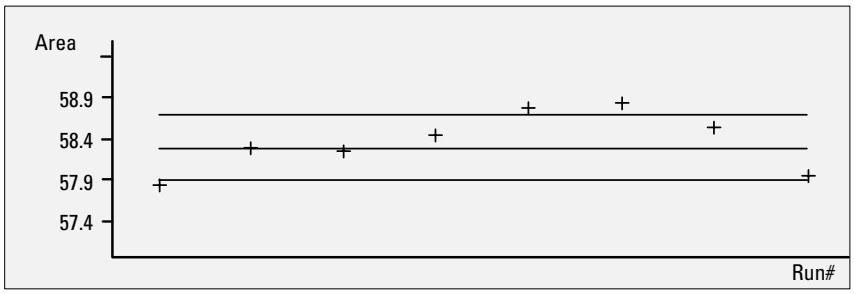


Figure 6
Repeatability for 1,3-DNB for retention time and peak area

Sensitivity

The detection limit for all explosive constituents and metabolites was 1 ng to 3 ng/µl except for nitropenta, which was 8 ng/µl. Peak spectra acquired at these low levels still matched standard spectra at higher concentrations.

Conclusion

We have shown that the commonly used explosive constituents and their metabolites (nitroaromatics and nitramines) can be covered by one comprehensive method including sample preparation and HPLC. An automated spectral library search can positively confirm explosive constituents at levels as low as 1 to 3 ng/µl.

References

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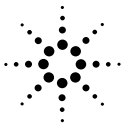

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