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Determination of Pharmaceuticals in Water by SPE and LC/MS/MS in Both Positive and Negative Ion Modes

Application Note

Environmental

Author

Chin-Kai Meng
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS), 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability from six injections of analytes at 5 pg on column showed RSDs below 15%, for all target compounds except for fluoxetine at 23%.

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Introduction

Many articles in leading medical journals and newspapers reported sexual development and reproductive problems in animals and humans, for example, low sperm counts, genital deformities, male fish making eggs, and others. Scientists suggested that man-made chemicals (for example, pesticides and pharmaceuticals) are disrupting the endocrine system.

Compounds like antibiotics, over-the-counter medicines, and caffeine drain through the sewage system largely unaltered into rivers and streams, and even get into the drinking water supply in very small amounts. In order to monitor the trace pharmaceuticals in surface and ground water, an effective sample preparation and analysis method is required.

In 1999, the U.S. Geological Survey National Water Quality Laboratory (NWQL) developed and implemented an Oasis HLB, solid-phase extraction (SPE), and a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) method to analyze pharmaceuticals.

Using the Multiple Reaction Monitoring (MRM) technique, any interference and matrix signal from organic matters in the water can be minimized from the target compound signals for better confirmation and quantitation. In this application note, SPE and LC/MS/MS methods are described to analyze 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode.

Experimental

Sample Preparation Procedure

See Reference 1 for more information.

1. Filter water samples in the field or laboratory using 0.7-µm glass fiber filters.
2. Pump 1 L of the filtered water sample, at a flow rate of 10 mL/min, through an Oasis HLB (SPE) cartridge containing 0.5 g of sorbent.
3. Elute the HLB column with 6 mL of methanol followed by 4 mL of 0.1% trifluoroacetic acid (TFA) in methanol.

Instrumentation

Positive Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	40 °C
Mobile phases:	A: 0.1% formic acid in water, add NH_4OH buffer to pH 5.5 B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	%B
0	0
15	100
20	100
21.5	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI–(+)
Mass range:	125 to 800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	350 °C
Skimmer:	35 V

Negative Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, 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

Gradient:

Time	%B
0	0
1	40
2	52
3	70
6	100
13	100
14	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120–800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

The MRM parameters for positive ion mode and negative ion mode are listed in Tables 1 and 2, respectively.

Table 1. Positive Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Collision V	Dwell	Segment
Metformin HCl	0.856	129	130.4	71.5	15	300	1
Acetaminophen	4.591	151	152.3	110.3	18	30	2
Salbutamol	4.717	239	240.4	148.4	15	30	2
Cimetidine	4.815	252	253.4	94.9	17	30	2
1,7,-Dimethylxanthine	4.89	180	181.3	123.9	20	30	2
Cotinine	5.24	176	177.3	118.3	29	30	2
Codeine	5.321	299	300.4	164.9	30	30	2
Caffeine	5.493	194	195.3	137.9	22	30	2
Trimethoprim	5.935	290	291.4	122.8	25	30	2
Thiabendazole	7.194	201	202.3	131.3	35	100	3
Sulfamethoxazole	7.309	253	254.3	156.0	15	100	3
Azithromycin	7.326	749	375.5	157.9	16	100	3
Diphenhydramine	8.446	255	256.5	167.1	5	100	4
Diltiazem HCl	8.693	414	415.4	177.6	18	100	4
Carbamazepine	8.912	236	237.4	194.0	20	100	4
Fluoxetine HCl	9.71	309	310.4	148.5	0	100	5
Dehydronifedipine	10.635	344	345.4	283.9	27	100	5
Warfarin	11.152	308	309.4	163.3	15	100	5
Miconazole nitrate salt	12.865	416	417.2	159.3	30	300	6

Table 2. Negative Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Frag. V	Collision V	Dwell	Segment
Hydrochlorothiazide	3.42	297	296	269	140	20	70	1
Aspirin	3.49	180	179	122	120	15	70	1
Enalaprilat	3.71	348	347	114	120	10	70	1
Furosemide	4.51	330	329	285	140	15	70	1
Ketoprofen	5.17	254	253	209	80	5	70	2
Clofibric acid	5.20	214	213	127	80	10	70	2
Napoxen	5.20	230	229	170	80	10	70	2
Diclofenac sodium salt	5.84	294	294	250	100	10	100	3
Ibuprofen	6.03	206	205	161	80	0	100	3
Ibuprofen-d3	6.03	209	208	164	80	0	100	3
Gemfibrozil	6.49	250	249	121	120	25	150	4
Triclocarban	6.66	314	313	160	140	15	150	4

Results and Discussion

The total ion chromatogram (TIC) in negative ion mode is shown in Figure 1. The analysis time in negative ion mode is less than 7 minutes for the 11 analytes. Their peak widths are about 0.1 minute, using a 1.8-µm particle size column. The narrower peak width gives a higher signal-to-noise (s/n) ratio compared to a 3.5-µm or larger particle size column.

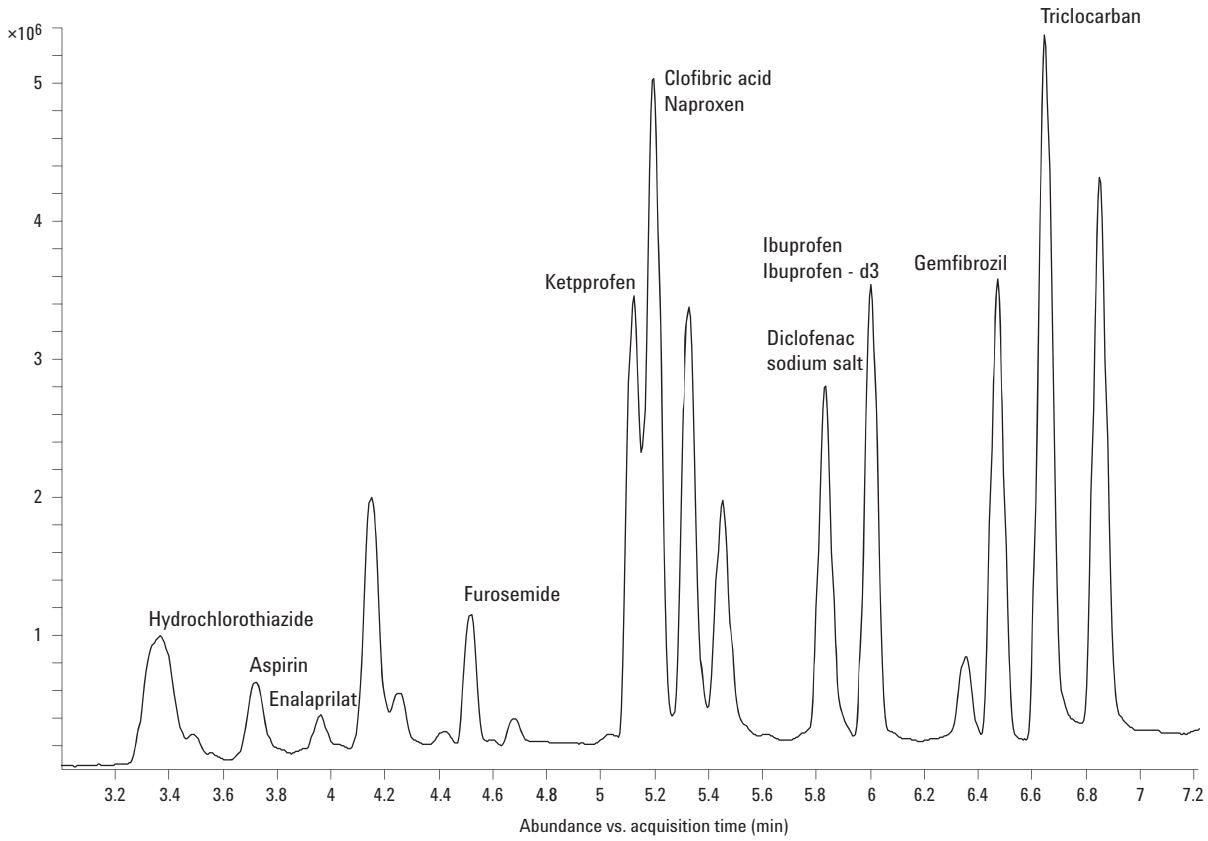

Figure 1. Negative ion mode TIC of 11 pharmaceuticals.

A few compounds, for example, ketoprofen (Figure 2), are sensitive to heat from the drying gas. Higher drying gas temperature (350 °C) lowers the intensity of the precursor ions. Therefore, in the negative ion mode, the drying gas temperature was set to 200 °C.

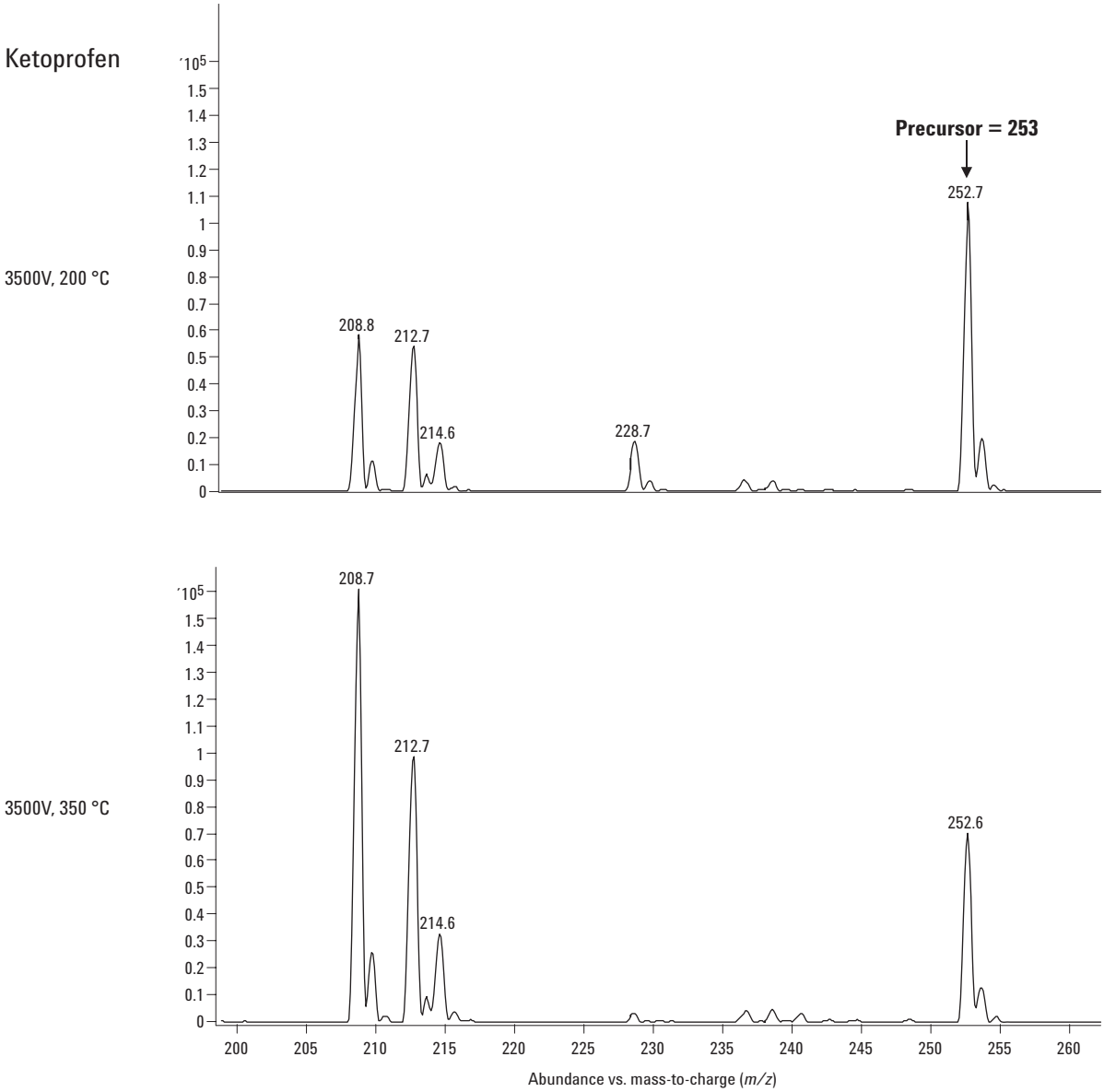

Figure 2. Higher drying gas temperature lowers precursor intensity for certain compounds.

In Figure 3, it was interesting to see that the fragment ion actually had a higher *m/z* value than the precursor ion. For azithromycin, the doubly charged ion showed higher intensity than the singly charged ion and was chosen as the precursor. Therefore, depending on the precursor chosen, it is sometimes necessary to set the upper mass of the product ion scan to be higher than the precursor ion.

Figure 4 shows the overlaid chromatograms of 19 pharmaceuticals, each at 5 pg on column, from the positive ion mode MRM analysis.

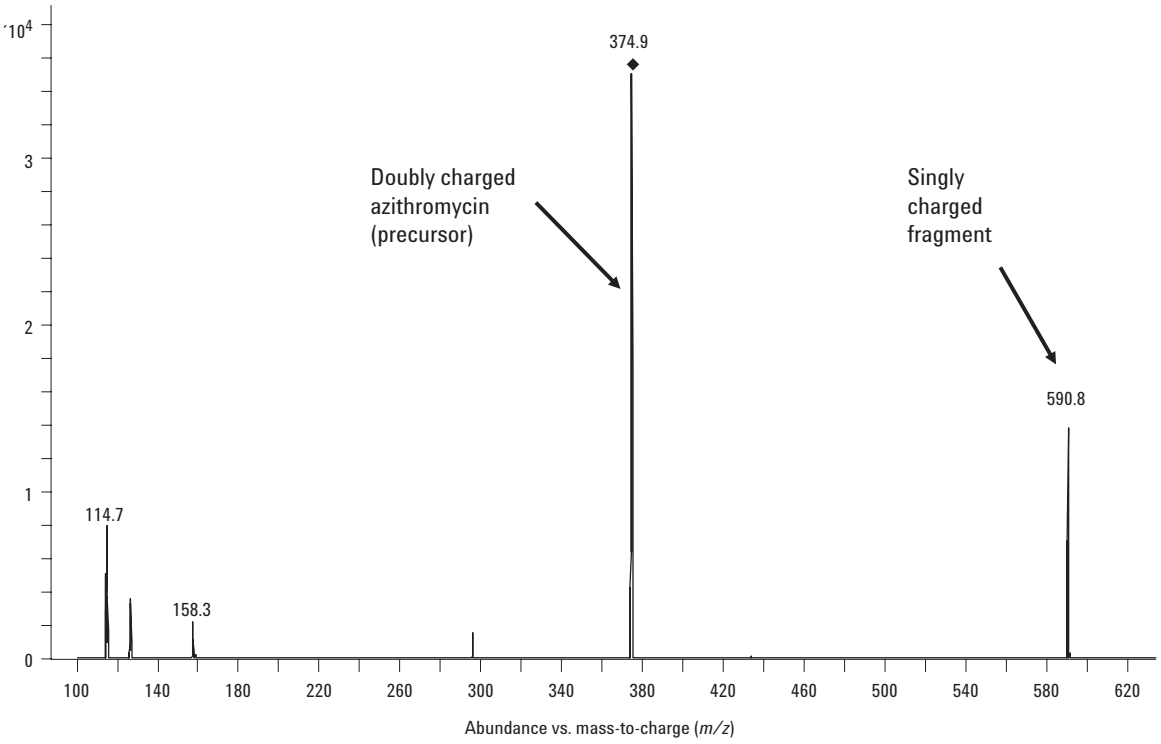

Figure 3. Doubly charged precursor results in a fragment at higher m/z.

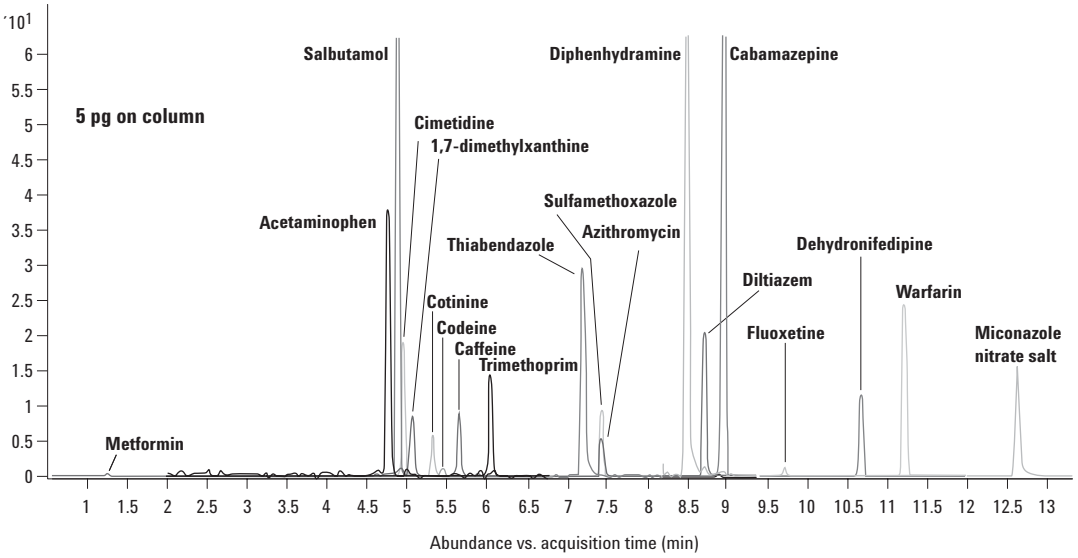

Figure 4. Overlaid MRM chromatograms of the 19 pharmaceuticals in positive ion mode.

Figure 5 shows the overlaid chromatograms of 10 pharmaceuticals, each at 10 pg on column, from the negative ion mode MRM analysis. In both Figures 4 and 5, the analysis times were relatively short and s/n ratios were high.

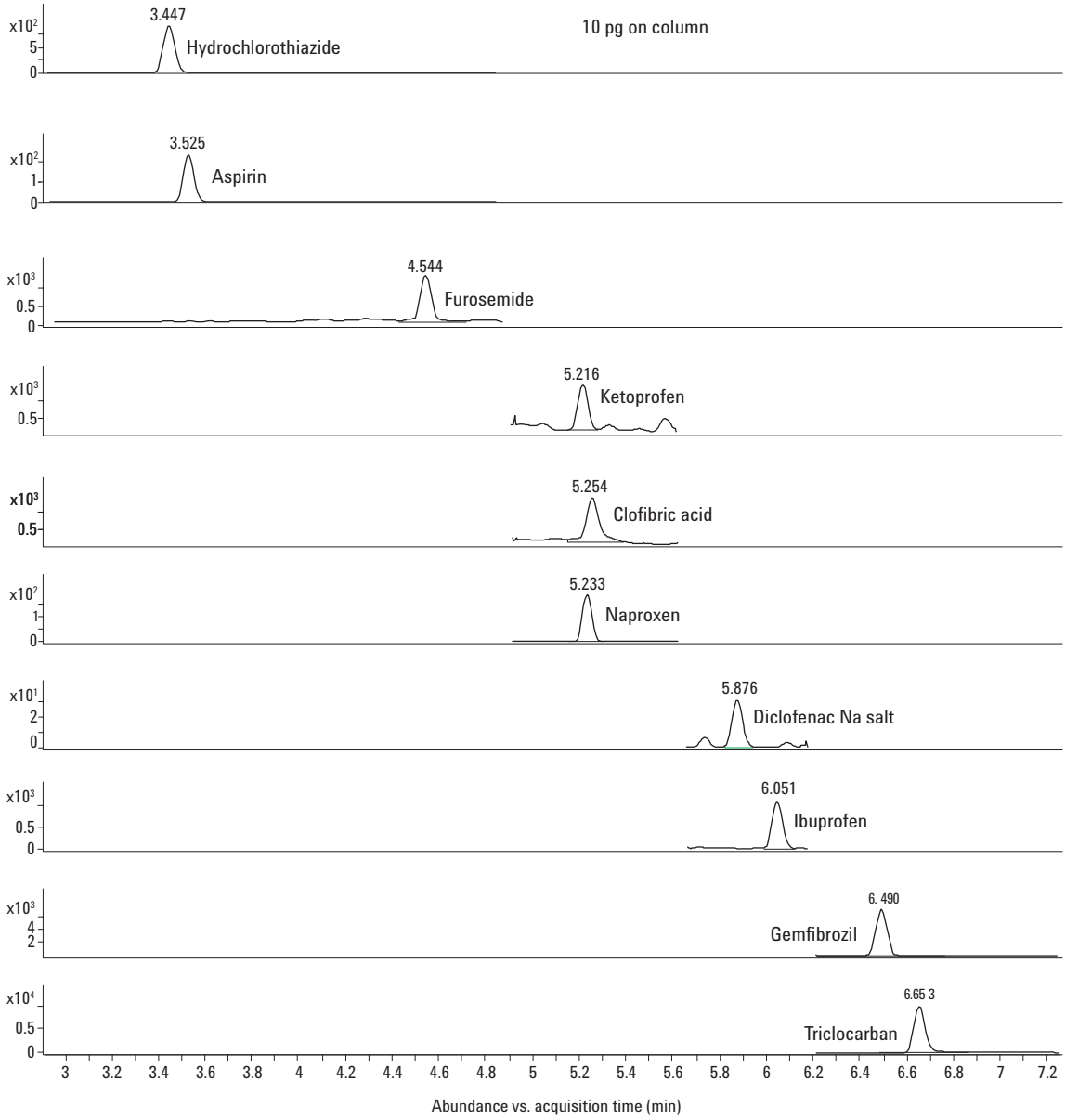

Figure 5. Overlay of MRM results from the 10 pharmaceuticals in negative ion mode.

Table 3 shows the linearity results of all 19 pharmaceuticals (ESI+) over the range of 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on column. Two calibration models were used: a linear model and a quadratic model that both included origin with no weighting. Some of the compounds showed significant fitting improvement from the linear model to the quadratic model. This is the nature of these compounds.

Table 3. Linearity: 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on Column (ESI+), Origin Included, No Weighting

Compound	R^2 (linear fit)	R^2 (quadratic fit)
Metformin HCl	0.9975	0.9999
1,7,-Dimethylxanthine	0.9998	0.9998
Acetaminophen	0.9852	0.9999
Caffeine	0.9992	0.9997
Cimetidine	0.9968	0.9998
Codeine	0.9989	0.9997
Cotinine	0.9971	0.9998
Salbutamol	0.9850	0.9994
Trimethoprim	0.9980	0.9999
Azithromycin	0.9633	0.9998
Sulfamethoxazole	0.9998	0.9999
Thiabendazole	0.9997	0.9998
Carbamazepine	0.9926	0.9999
Diltiazem HCl	0.9997	0.9997
Diphenhydramine	0.9975	0.9998
Dehydronifedipine	0.9985	0.9993
Fluoxetine HCl	0.9984	0.9997
Warfarin	0.9989	0.9997
Miconazole nitrate salt	0.9989	0.9995

Table 4 shows the repeatability results from six injections of 5 pg of each analyte on column. In general, the RSDs are below 15%, except for fluoxetine, which was at 23%.

Table 4. Repeatability from Six Injections at 5 pg/µL (5 pg on column), ESI(+)

Compound	%RSD
Metformin HCl	12.4
1,7,-Dimethylxanthine	8.6
Acetaminophen	6.1
Caffeine	5.7
Cimetidine	4.1
Codeine	16.2
Cotinine	10.5
Salbutamol	3.7
Trimethoprim	3.6
Azithromycin	9.4
Sulfamethoxazole	10.7
Thiabendazole	5.3
Carbamazepine	2.8
Diltiazem HCl	4.7
Diphenhydramine	3.7
Dehydronifedipine	5.4
Fluoxetine HCl	23.4
Warfarin	4.4
Miconazole nitrate salt	2.9

Table 5 shows the linearity results of all 11 pharmaceuticals (ESI–) over the range of 10, 20, 40, 80, 400, and 800 pg on column. All the R^2 values were above 0.99, except triclocarban, which was about 0.97.

Table 5. Linearity: 10, 20, 40, 80, 400, and 800 pg on Column (ESI–), Origin Included, No Weighting

Compound	R^2 (linear fit)
Hydrochlorothiazide	0.9999
Aspirin	0.9977
Enalaprilat	0.9981
Furosemide	0.9997
Ketoprofen	0.9988
Clofibric acid	0.9997
Naproxen	0.9994
Diclofenac Na salt	0.9993
Ibuprofen	0.9997
Ibuprofen-d3	0.9998
Gemfibrozil	0.9993
Triclocarban	0.9655

Once the method is established, one can screen and quantitate target analytes in water. Figures 6, 7, and 8 are MRM analyses of actual water sample extracts in positive and negative ion modes.

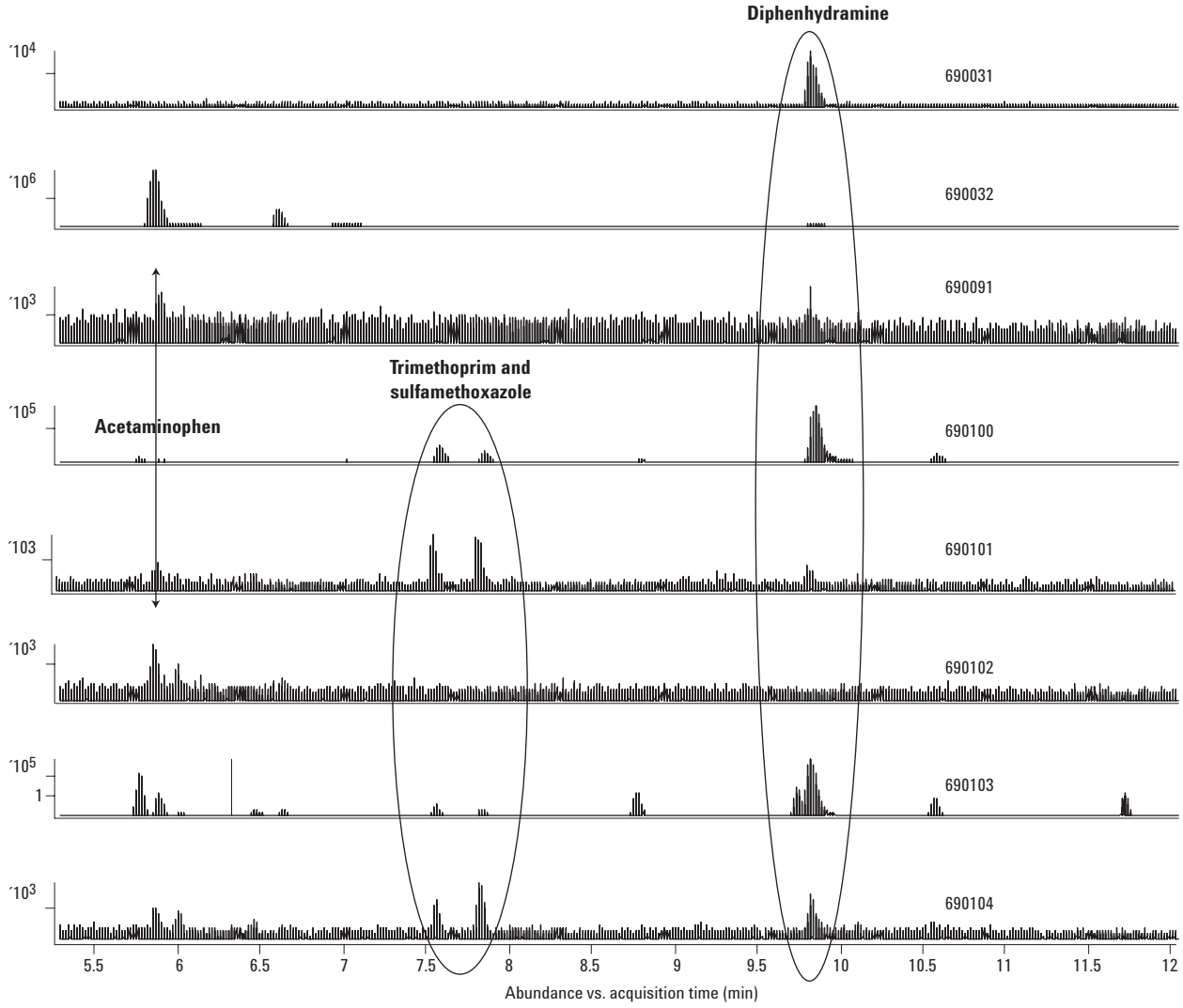

Figure 6. Pharmaceuticals screening in positive ion mode.

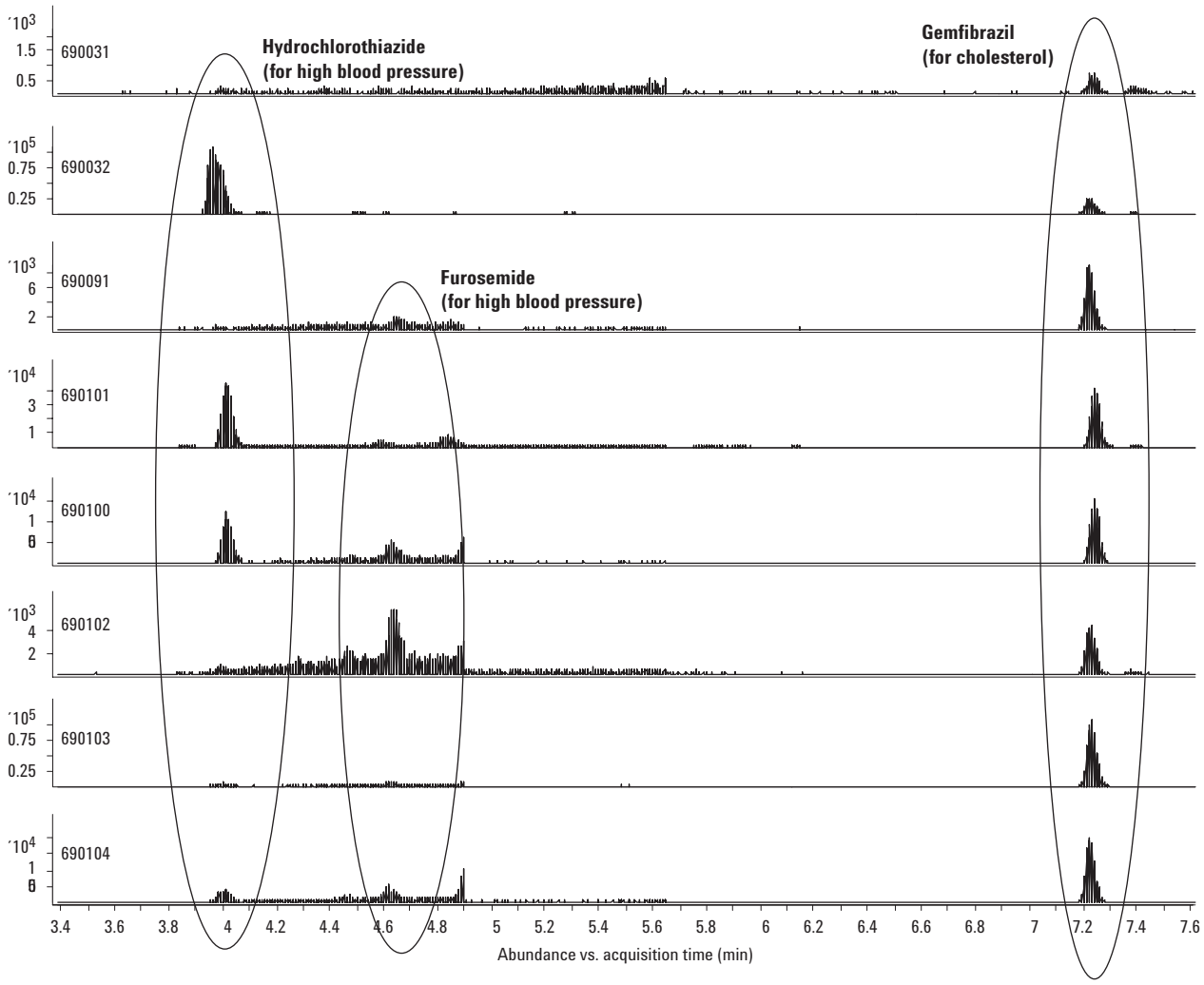

Figure 7. Pharmaceuticals screening in negative ion mode.

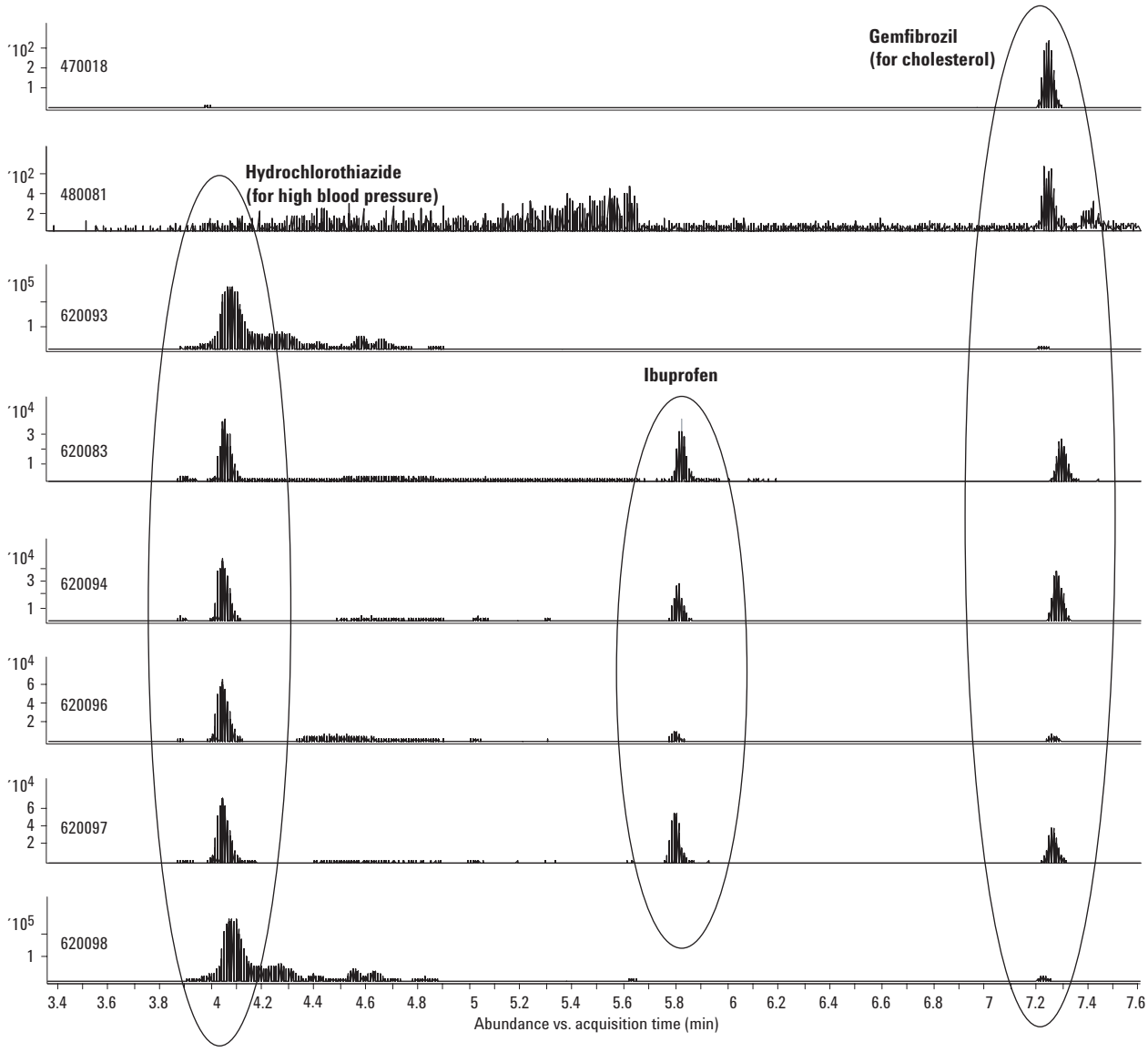

Figure 8. Pharmaceuticals screening in negative ion mode.

Figure 6 shows several of the pharmaceuticals, for example, diphenhydramine and acetaminophen, that were common to several of the water samples. Some of the antibiotics were also found in the samples. Interestingly enough, in Figures 7 and 8, the most common pharmaceuticals in the water samples were related to high blood pressure and cholesterol medications.

Conclusions

Using SPE and LC/MS/MS, 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability study from six injections of target analytes at 5 pg on column showed RSDs below 15%, except for fluoxetine at 23%.

This method was applied to water sample extracts, finding that several target pharmaceutical drugs were commonly found among the analyzed samples.

Reference

1. USGS SOP: Instrumental Analysis for Determination of Human Health Pharmaceuticals in Water by Chemically Modified Styrene-Divinylbenzene Resin-Based Solid-Phase Extraction and High-Performance Liguid Chromatography/Mass Spectrometry, by Steve Werner, 2006.

Acknowledgments

The author gratefully acknowledges the assistance of Stephen Werner and Ed Furlong of the National Water Quality Lab – United States Geological Survey (Lakewood, CO) for providing the sample preparation procedures and extracts used in this work.

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Agilent Technologies

The Use of Accurate Mass, Isotope Ratios, and MS/MS for the PPCPs in Water

Application

Environmental

Authors

Michael Zumwalt
Agilent Technologies, Inc.
9780 S Meridian Blvd.
Englewood, CO 80112-5910
USA

David Weil
Agilent Technologies, Inc.
10 N. Martingale Road, Suite 550
Schaumburg, Il 60173-2292
USA

Chin-Kai Meng
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

An Agilent 6510 Quadrupole Time-of-Flight Mass Spectrometer (QTOF) is used to analyze several surface water samples for the presence of pharmaceutical compounds. A simple gradient elution is carried out on a Rapid Resolution High Throughput Extend C18 column (particle size 1.8 µm). Of 54 potential compounds, as many as 11 are identified in one of the samples using an algorithm known as the Agilent Molecular Feature Extractor (MFE). To make comparisons among several samples, another algorithm, known as Mass Profiler, is applied to the data processed by the MFE. Since the MFE may generate thousands of potential compounds known as features, Mass Profiler makes statistical comparisons of the features between two different samples to determine what is unique and what is common. All of this work is done with the full-scan mass spectral data. When compounds of interest are determined, accurate mass full-scan MS/MS can be invoked for structural elucidation. The results of full-scan MS/MS applied to caffeine are included as an example and are relevant because many medications include caffeine as an ingredient.

Introduction

During the three decades prior to the year 2000, the study of chemical pollution was confined primarily to pesticides. Following a seminal article by C. Daughton [1], this focus began to shift to the emerging environmental concern for pharmaceuticals and personal care products (PPCPs). Many of these pharmaceuticals, including estrogen, have been known as endocrine disruptors, or chemicals that disrupt the physiological function of hormones in organisms. In 2004 a report from the United States Geological Survey [2] was made as a result of discovering a high preponderance of intersex (male fish exhibiting female characteristics) in smallmouth bass of the Potomac River.

The USGS has found pesticides, flame retardants, and personal-care products containing known or suspected endocrine-disrupting compounds in the Potomac River. Many of these compounds continue to be known as emerging contaminants because they are still being discovered and don't exist on any currently regulated target lists. As such, it is important to use adequate techniques to help identify these compounds and possible metabolites.

Using accurate mass in full-scan (mass range) mass spectrometry (MS), compound empirical formulas can be determined for purposes of identification. Furthermore, the high degree of spectral resolution allows for selective identification among co-eluting compounds. Isotope ratios are an additional tool because they help identify com-

pounds with high carbon numbers as well as those that contain elements like chlorine and sulfur. Although these tools do a lot to confirm chemical formula, it may still be left to the user to decide which of the possible structures of isobaric compounds apply.

To assist in the analytical need for structural elucidation, selective MS/MS by using the quadrupole time-of-flight mass spectrometer (QTOF) is implemented. Because the Agilent QTOF also has very accurate mass at the MS/MS level, it is easier to determine the structures of the product ions, which correspond as substructures of the precursor ion and thereby reduce the number of possible structures pertaining to the derived empirical formulas from several to one.

The list of pharmaceuticals to look for in the environment is ever-increasing and many of them are metabolites with unknown structures. Identifying these compounds requires the technology of the QTOF. Furthermore, the fast scanning capability is necessary for identifying 10s to 100s of these compounds in samples with relatively short run times. The Agilent QTOF is capable of acquiring full-scan MS data at the rate of 20 spectra/sec. The resulting large amount of data representing a possibly large number of compounds needs to be converted into useful information. The Agilent Molecular Feature Extractor (MFE), which is a standard part of the MassHunter Qualitative Analysis software, carries out the following steps:

- Persistent chemical background removed
- Co-eluting interferences resolved
- Isotopic clusters recognized and grouped
- 2D/3D data visualization
- Chemical identification (accurate mass, isotope matching)
- Database searching (NIST, ChemIDPlus)

In addition to applying the algorithm Mass Feature Extractor to pull out the features from the chromatographic data, which could be compounds, another algorithm, known as Mass Profiler, is applied to the list of features among different samples to determine differences and commonalities. Each sample is injected three times, or multiple samples from the same source could be used to determine what is statistically consistent in terms of the features derived for the sample by MFE. The result is called a group. Two groups representing two different sample sources can then be compared to see what features differ, are unique, or are common, and, if common, whether they differ in abundance.

A batch of water samples is filtered and extracted using solid-phase extraction, which resulted in an approximate 1,000-fold increase in concentration. Samples analyzed in this work are believed to contain compounds at the 10 to 100 ppb level, which corresponds to the 10 to 100 ppt range in the original water sample. The compounds that may be in these samples are included with their exact neutral masses in Table 1.

Table 1. List of Compounds with Corresponding Neutral Masses That May Be in a Given Sample

Compound	Neut. mass	Compound	Neut. mass	Compound	Neut. mass
Acetaminophen	151.06333	Diphenhydramine	255.16231	Paroxetine	329.14272
Albuterol	239.15214	Duloxetine	297.11873	Ranitidine	314.14126
Aspirin	180.04226	Enalaprilat	348.16852	Sertraline	305.07380
Buproprion	239.10769	Erythromycin	573.51210	Simvastatil	418.27192
Caffeine	194.08038	Fluoxetine	309.13405	Sulfachloropyridazine	284.01347
Carbamazepine	236.09496	Fluvoxamine	318.15551	Sulfadimethoxine	310.07358
Cimetidine	252.11572	Furosemide	330.00772	Sulfamethazine	278.08375
Clofibric acid	214.03967	Gemifrozil	250.15698	Sulfamethizole	270.02452
Citalopram	324.16379	HCTZ	296.96447	Sulfamethoxazole	253.05211
Codeine	299.15215	Ketoprofen	254.09429	Thiabendazole	201.03607
Cotinine	176.09496	Miconazole	413.98602	Triclocarban	313.97805
Dehydronifedipine	344.10084	Naproxen	230.09429	Triclosan	287.95116
Diclofenac	295.01668	Norfluoxetine	295.11840	Trimethoprim	274.14298
Diltiazem	414.16133	Norsertraline	293.05000	Venlafaxine	267.12593
		1,7-dimethylxanthine	180.06473	Warfarin	308.10486

Experimental

Sample Preparation

Prepared samples are provided by the United States Geological Service National Water Quality Laboratory (USGS/NWQL) in Denver, Colorado. The details of the procedure used are not included in this application, but are available upon request. Pharmaceuticals are typically extracted from surface water by using disposable polypropylene syringe cartridges that contain 0.5 g of polymeric sorbent. One liter of sample is pumped through the solid-phase extraction (SPE) cartridge. The analyte material is later eluted into 1 mL of methanol, resulting in a concentration increase of three orders of magnitude.

LC/MS Method Details

LC Conditions

Agilent 1100 Series binary pump, degasser, wellplate sampler, and thermostatted column compartment

Column	Agilent ZORBAX RRHT Extend C18 2.1 mm × 50 mm, 1.8 µm Agilent p/n: 727700-902
Column temperature	40 °C
Mobile phases	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Flow rate	0.3 mL/min
Injection volume	5 µL
Gradient	

Time (min)	%B	
0.0	0	
10.0	67	Stop time: 15 min
11.0	100	Post run: 10 min

MS Conditions

Mode	Positive electrospray ionization using the Agilent G3251A Dual ESI source
Nebulizer pressure	40 psig
Drying gas flow	9 L/min
Drying gas temp.	350 °C
V_{cap}	3500 V
Scan range	*m/z* 50–1000
Scan speed	1 scan/sec

MS/MS Conditions

Collision energy	30 V
Scan range	*m/z* 50–1000
Scan speed	1 scan/sec

Results and Discussion

Of the several samples analyzed, results for Samples 4 and 10 will be considered here. To get an idea of the task at hand, an overlay of the total ion and base peak chromatograms for the first injection of Sample 4 is shown in Figure 1. The base peak chromatogram is generated to help the analyst identify peaks in the chromatogram corresponding to real compounds. Figure 2 shows the spectrum at the apex of one such peak. Note the complexity of this spectrum and the difficulty involved in not only determining which spectral peaks are of value, as they may pertain to co-eluting compounds, but then having to apply this reasoning to several peaks in the chromatogram.

Applying the algorithm of the Molecular Feature Extractor program to this data file results in the display of the processed chromatogram and the corresponding contour plot shown in Figure 3. The upper left-hand chromatogram is the unprocessed TIC, same as shown in Figure 1. On the right is the processed chromatogram after applying the steps listed in the Introduction. Random background noise has been removed. Below each of these chromatograms are shown the corresponding contour plots, which are the presentations of spectral data points in an *m/z* versus chromatographic retention time plots. The contour plot at the lower left-hand corner of the display shows a very dense distribution of data points, most of which correspond to random noise.

The contour plot at the lower right-hand corner is the result of processing the data so that a significant amount of molecular features are derived for closer examination. In fact, using the following settings for filtering the data, some 5,431 features are derived for this sample in the first injection:

- Spectral S/N > 2
- Mass range: *m/z* 150 to 800
- $[M + Na]^+$ and $[M + NH_4]^+$ adducts considered
- Relative intensity in the spectrum > 0.1%
- Each feature must contain at least 2 ions

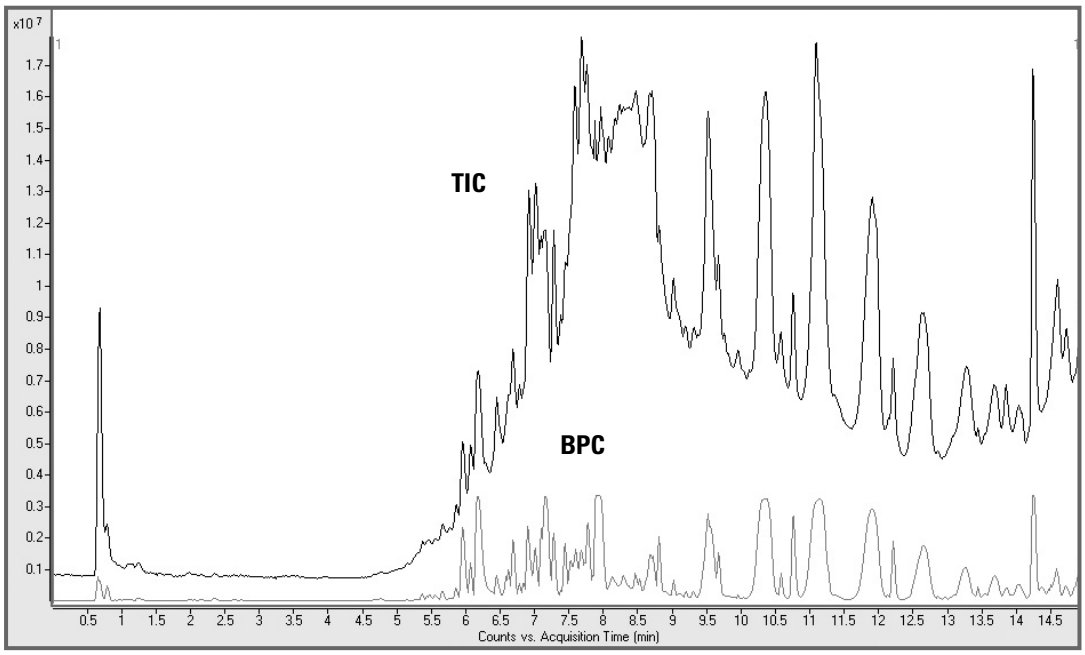


Figure 1. Overlay of total ion chromatogram (TIC) and base peak chromatogram (BPC) for Sample 4.

If we now investigate some of the features that have been found we can begin with the peak apex spectrum examined in Figure 2. The retention time is 6.445 minutes and MFE has derived features at 6.448 minutes as shown in Figure 4. The unprocessed spectrum at the top of the figure matches that of Figure 2. However, removing random noise and using the filtering rules above a processed spectrum containing 12 features is derived and shown at the bottom. A subset of the list of features is shown at right.

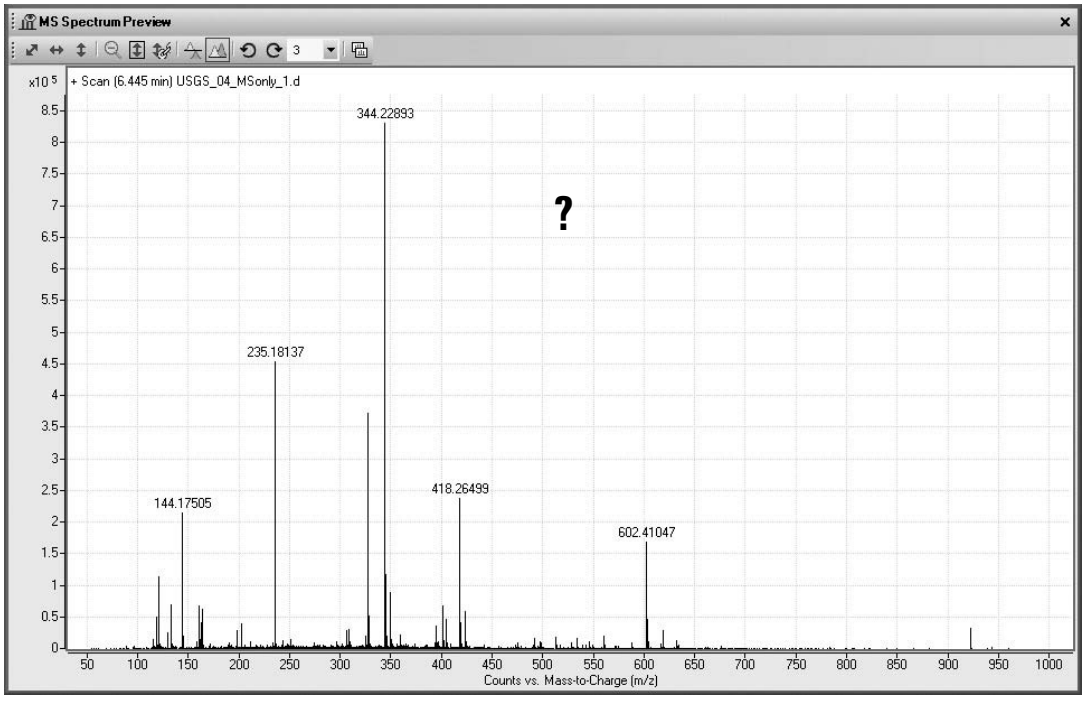


Figure 2. Spectrum at apex of base peak with retention time of 6.445 minute.

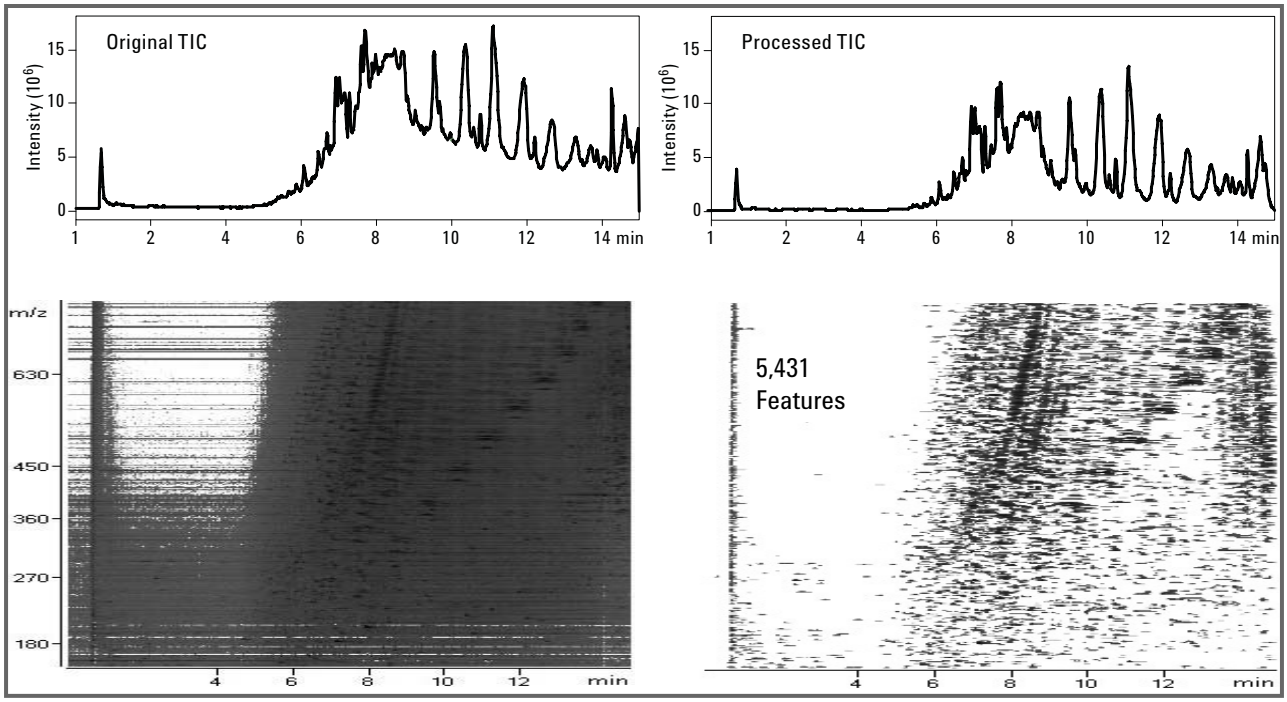
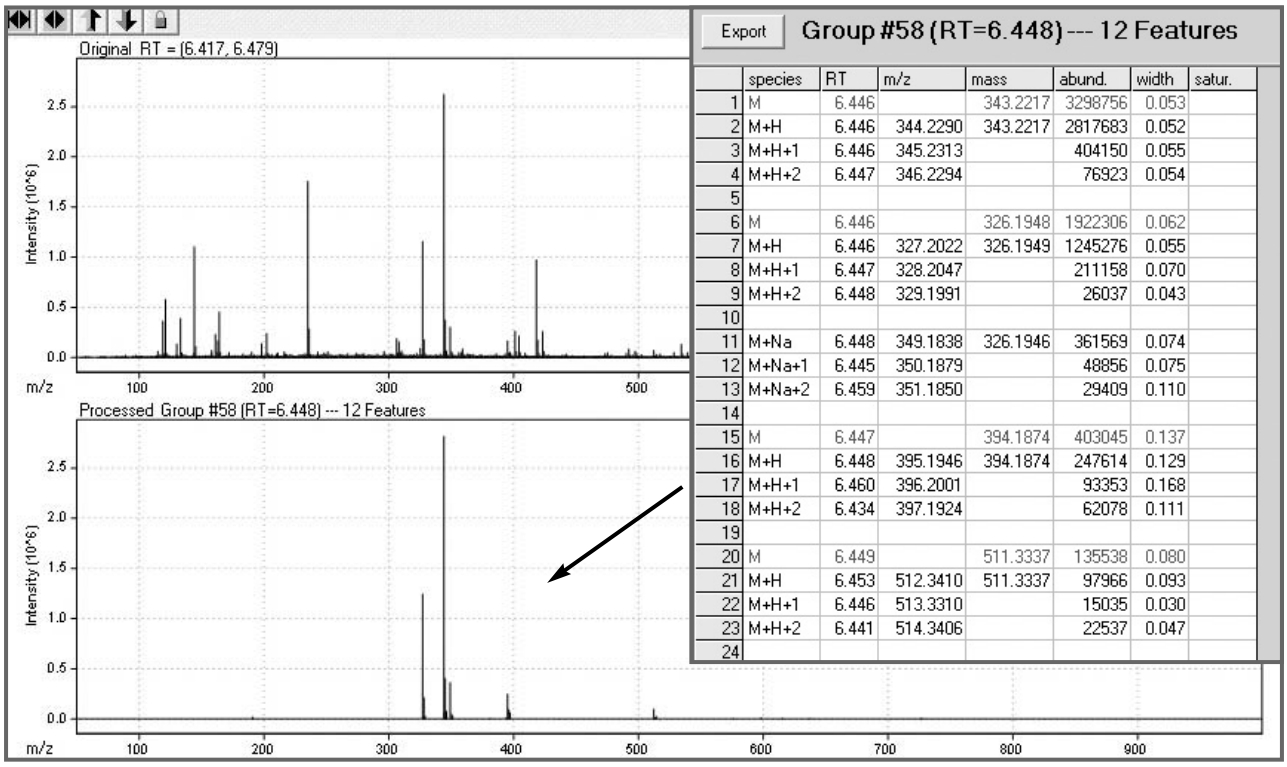


Figure 3. Both unprocessed and processed data of Sample 4 using MFE.



	species	RT	m/z	mass	abund.	width	satur.
1	M	6.446		343.2217	3298756	0.053	
2	M+H	6.446	344.2290	343.2217	2817683	0.052	
3	M+H+1	6.446	345.2313		404150	0.055	
4	M+H+2	6.447	346.2294		76923	0.054	
5							
6	M	6.446		326.1948	1922306	0.062	
7	M+H	6.446	327.2022	326.1949	1245276	0.055	
8	M+H+1	6.447	328.2047		211158	0.070	
9	M+H+2	6.448	329.1991		26037	0.043	
10							
11	M+Na	6.448	349.1838	326.1946	361569	0.074	
12	M+Na+1	6.445	350.1879		48856	0.075	
13	M+Na+2	6.459	351.1850		29409	0.110	
14							
15	M	6.447		394.1874	403045	0.137	
16	M+H	6.448	395.1946	394.1874	247614	0.129	
17	M+H+1	6.460	396.2001		93353	0.168	
18	M+H+2	6.434	397.1924		62078	0.111	
19							
20	M	6.449		511.3337	135538	0.080	
21	M+H	6.453	512.3410	511.3337	97966	0.093	
22	M+H+1	6.446	513.3310		15035	0.030	
23	M+H+2	6.441	514.3406		22537	0.047	
24							

Figure 4. Twelve features shown at derived retention time of 6.448 minutes.

If we want to filter the data to only show compounds corresponding to the list at the beginning of this article, we can place the neutral masses into an inclusion list of MFE as shown in Figure 5. We also assume that the compounds of interest do not elute until after 4 minutes and the mass range of interest is 150 to 600, which corresponds to the compounds of Table 1.

After applying the filtering of data with the compound list shown in Figure 5, eight features appear to be found in Sample 4 as shown in Figure 6. The corresponding chromatogram containing these eight features is also shown.

Before looking more closely at any one of these compounds, the data of Sample 4 is now going to be compared with data from another sample, Sample 10. The comparison will be carried out using an algorithm known as Mass Profiler. In order to use Mass Profiler, at least three injections of each sample must be made to determine what is consistently there and what is random and should be disregarded. In this work each sample is injected three times. The data is first processed by MFE to generate features. Mass Profiler filters out features that are inconsistent among the three injections for each sample. The resulting data is called a Group. Therefore, in comparing Samples 4 and 10 Mass Profiler will be referring to them as Group 4 and Group 10.

In Figure 7, Mass Profiler shows a plot of features common to both Groups 4 and 10 and displays them as mass versus retention time. By clicking on any one of the feature points in the display, one can see the common feature for both Groups along with possible empirical formulas for the derived neutral mass.

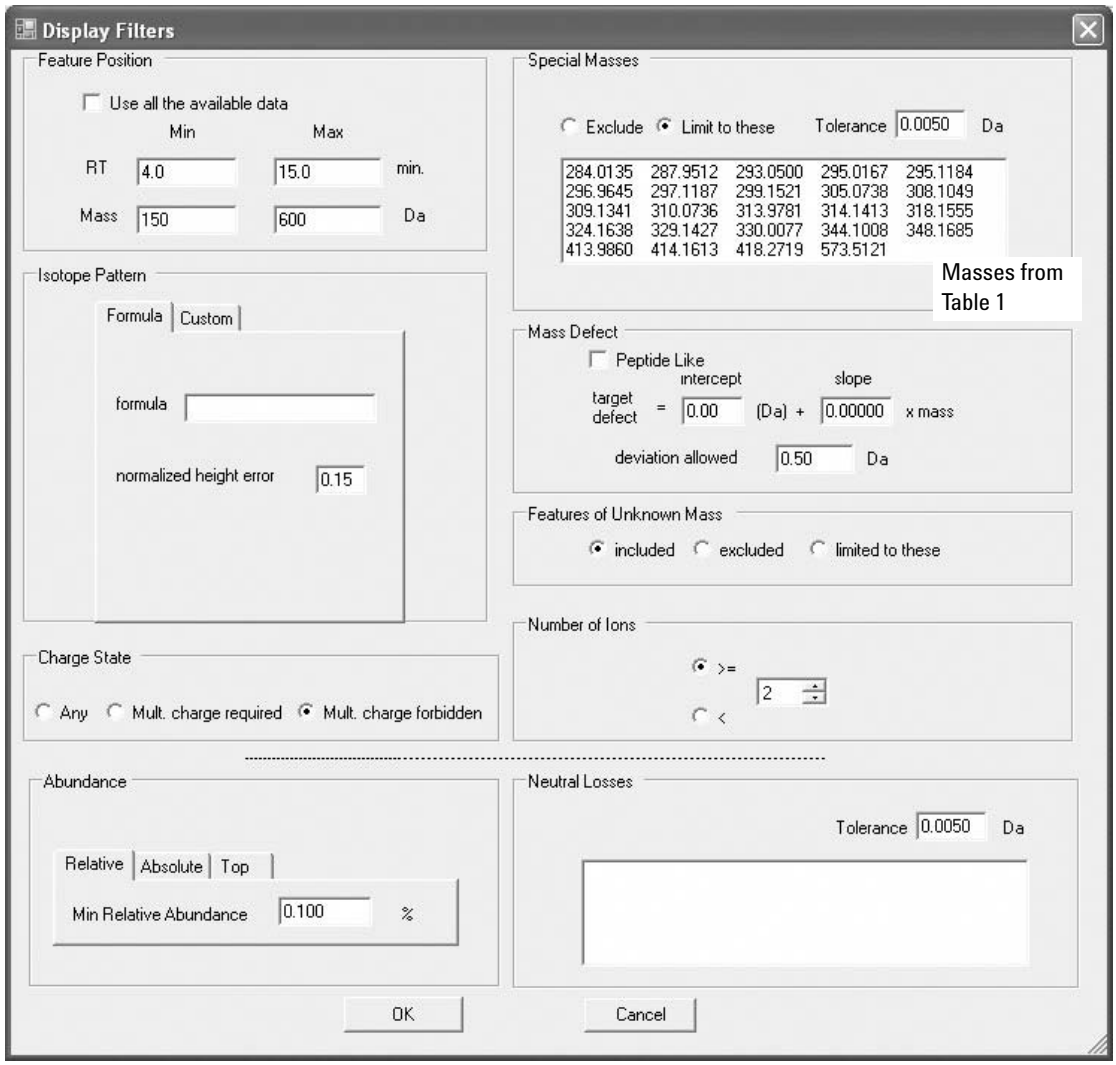


Figure 5. Display filter settings for finding features that match compound list of Table 1.

Export features:8 / groups:8

	Group	RT	mass	mass SD	abund.	satur.	height	#ions	minZ	maxZ	#z	width	#features
1	1	9.629	236.0961		915731		267469	2	1	1	1	0.045	1
2	2	5.678	299.1536		431858		91085	2	1	1	1	0.083	1
3	3	8.095	236.0961		382835		96590	2	1	1	1	0.052	1
4	4	8.671	299.1527		229538		23883	3	1	1	1	0.121	1
5	5	6.064	299.1525		146722		26146	2	1	1	1	0.076	1
6	6	11.598	250.1607		134252		19366	2	1	1	1	0.128	1
7	7	8.262	252.1204		63528		12743	2	1	1	1	0.058	1
8	8	5.307	296.9660	0.0019	28147		3522	4	1	1	1	0.072	1

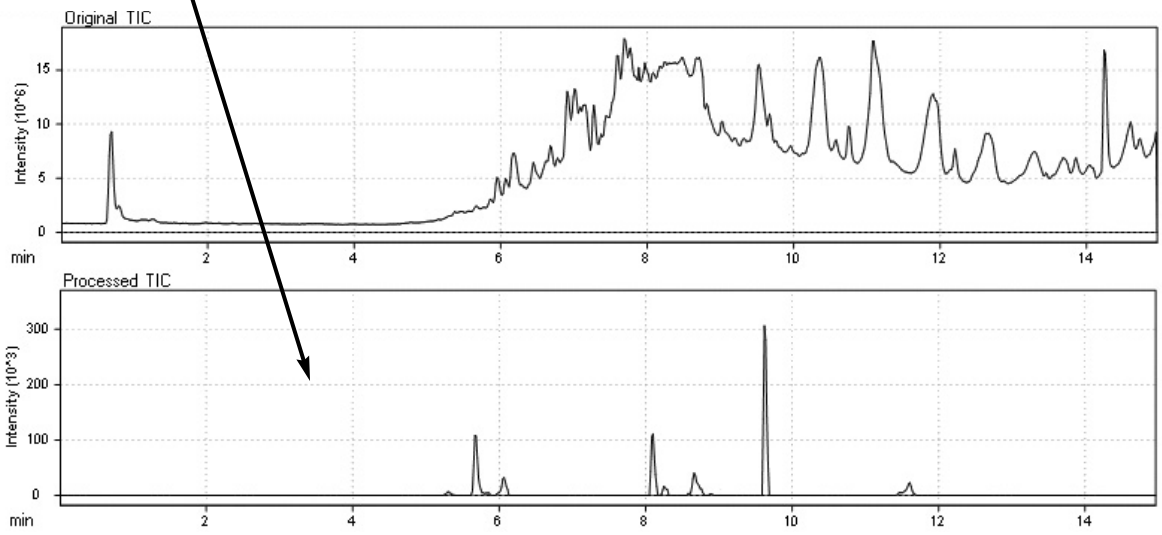


Figure 6. Eight features found corresponding to the neutral masses of Table 1. Corresponding processed chromatogram also shown.

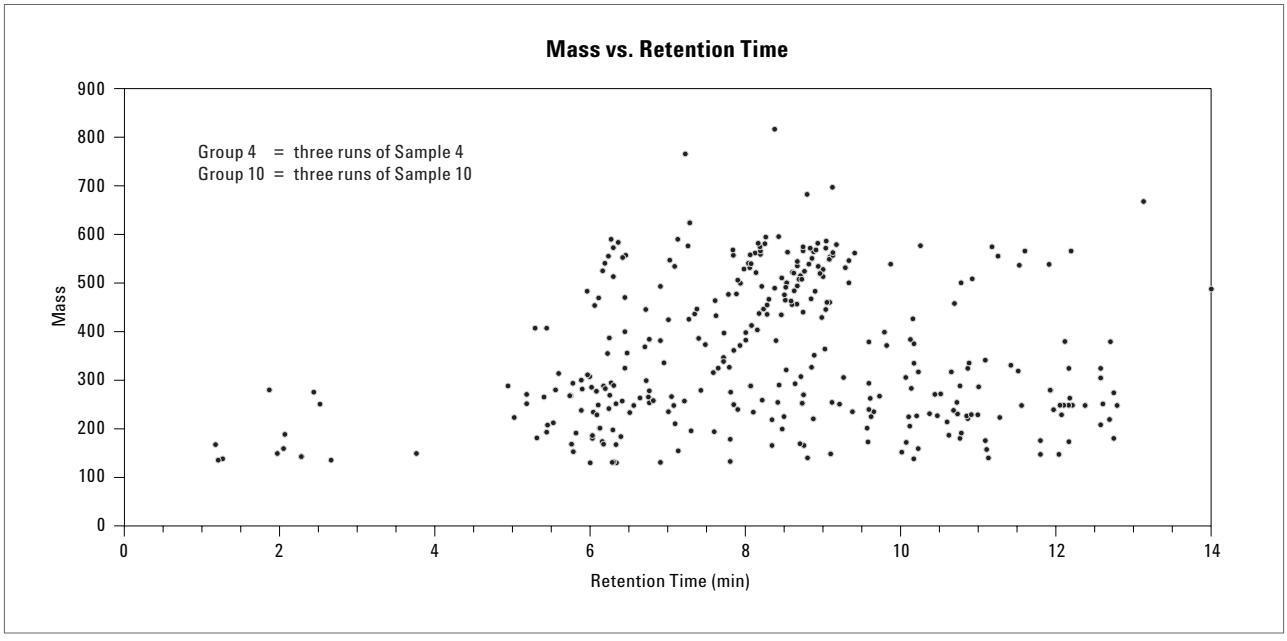


Figure 7. Features present in both Groups 4 and 10 total 346.

For example, in comparing features between the two sample groups a differential analysis plot can be generated as shown in Figure 8. In this plot, the features of Group 10 that are more or less abundant than the corresponding features in Group 4 are represented. More specifically, at a retention time of 8.495 minutes there is a data point in Figure 8 that corresponds to a feature in Group 10 that is approximately 4× intensity over the corresponding feature in Group 4, which corresponds to a log 2 ratio of 2. By clicking on this data point in the display of Figure 8 one can see that this feature is identified as diphenhydramine, with a chemical formula of $C_{17}H_{21}NO$ and accurate mass of 0.7 ppm. See Figure 9.

With Mass Profiler it is also possible to compare two samples in terms of what features are in one sample that are not in the other. In Figure 10 we see such a comparison.

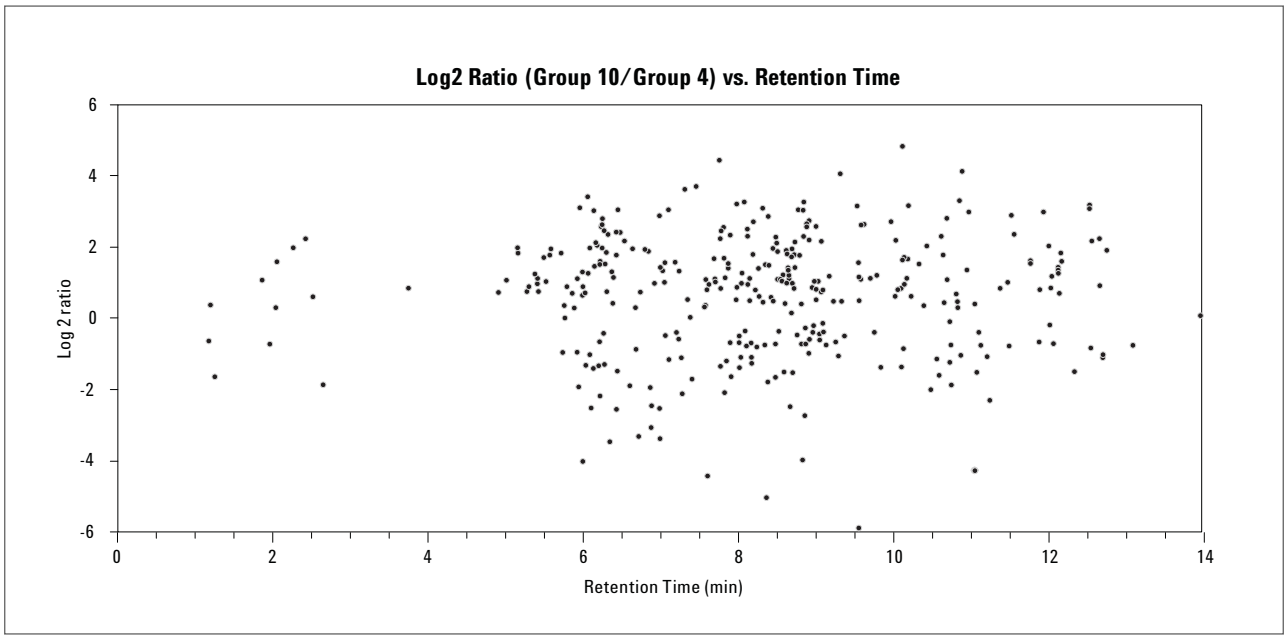


Figure 8. Features common to Groups 4 and 10 but differing in magnitude.

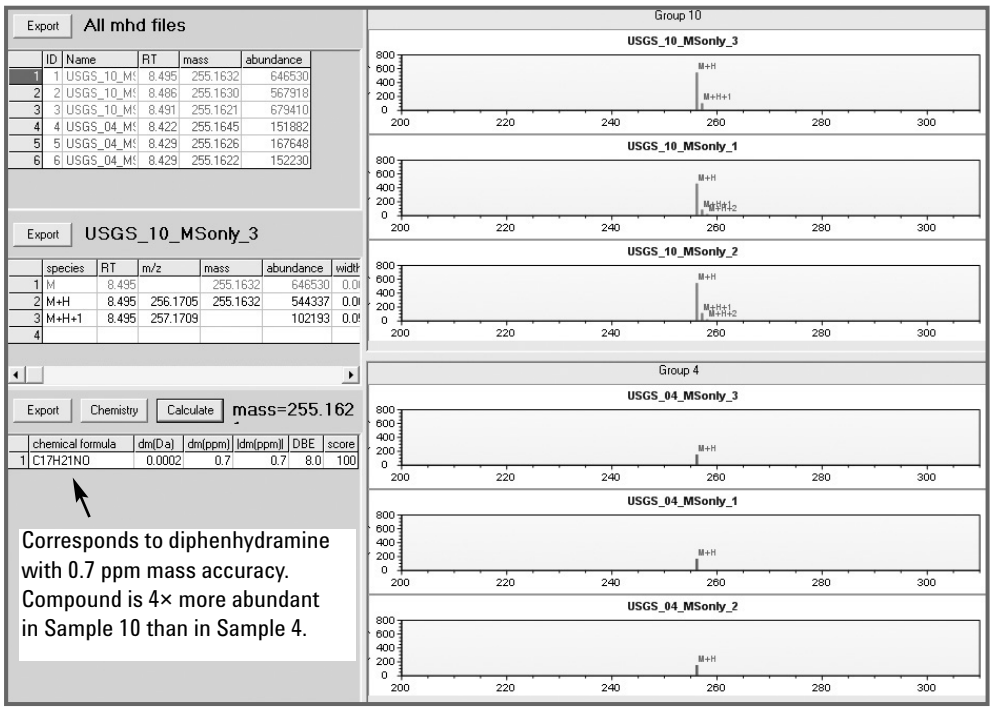


Figure 9. Feature at 8.495 minutes retention time corresponds to diphenhydramine.

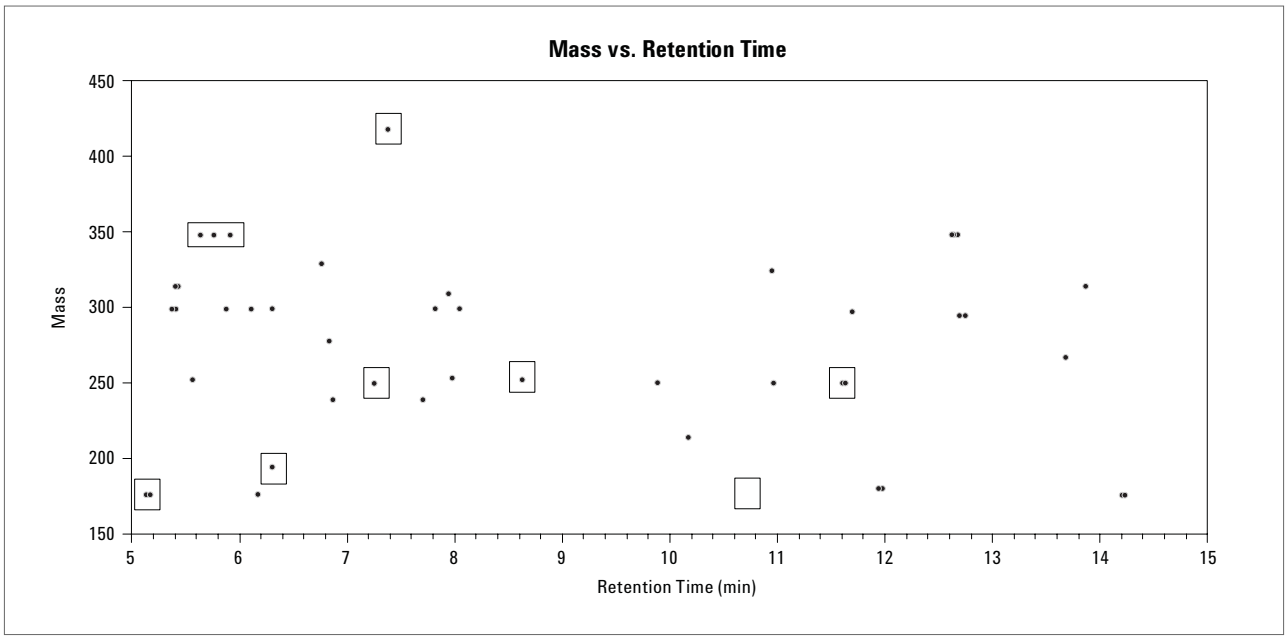


Figure 10. Features only in Group 4 (highlighted with boxes) or in Group 10.

Mass Profiler has determined that there are 33 features only in Group 4 or in Group 10 and are not common to the two samples. Since the display in Mass Profiler is in color the features exclusive to Group 4 are blue and the features exclusive to Group 10 are red. Since Agilent applications are normally published in black and white, boxes have been placed around the blue features for Group 4 for viewing convenience.

So far, all of the data have been collected in full-scan MS mode. Once features are identified as compounds needing more structural information, or it is of interest to perform some quantitation, a targeted MS/MS analysis can be performed in which the ion mass of the feature is considered as precursor ion and fragmented to form accurate mass product ions. The accurate mass of these product ions can determine their chemical formula and possible structures. Because the QTOF also has a high degree of spectral resolution in MS/MS mode, very narrow extracted ion chromatograms may be generated for each ion and then summed together for quantitation signal.

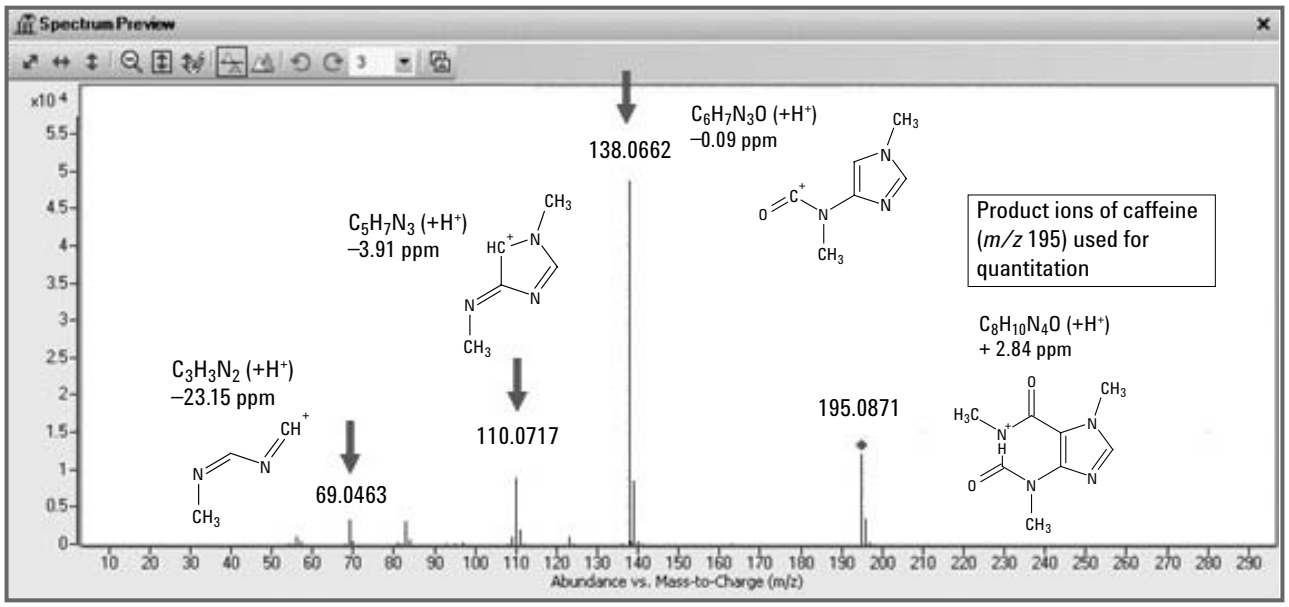
In Figure 11 we see the accurate mass MS/MS fragmentation of caffeine using the MS/MS settings noted in the LC/MS Method Details. Caffeine is of environmental interest because many medications contain it as an ingredient. Chemical formulas for each product ion is derived based on the possible arrangements of C, H, N, and O. Knowing the structure of caffeine, structures of the fragment ions can be proposed using their corresponding chemical formula. The fragment structures are generated using ACD/MS Fragmentor (ACD Labs Release v. 10, Advanced Chemistry Development, Inc., Toronto, ON, Canada).

Conclusions

The QTOF is an excellent instrument for identifying compounds using accurate mass in full-scan MS and MS/MS. Accurate mass leads to chemical formula, which can also give structural information when forming product ions in MS/MS. As a lot of data is acquired by this type of instrument to look at samples that may contain large amounts of known and unknown compounds, it is important to have algorithms like Molecular Feature Extractor that can filter usable features out of the chemical background. These features are generated from spectra as a result of removing random background signal and finding clusters of isotopes that make sense.

While this analysis is useful for one sample it may also be important to make comparisons among multiple samples as well. Another algorithm known as Mass Profiler makes such comparisons. More specifically, comparisons such as what is common to two samples and how they differ in amount. Or, what features are in one sample that aren't in the other. Once the feature is considered for more investigation, targeted MS/MS may be carried out on that feature to get structural information based on the generation of product ions.

Calculate chemical formula given accurate mass measurement and using elements C, H, N, and O



Proposed Structures

Figure 11. Targeted MS/MS mode for caffeine-producing product ions that may be used for structural elucidation as well as quantitation.

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1. C. G. Daughton and T. A Ternes, "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?," *Environmental Health Perspectives*, 107, Suppl. 6, Dec 1999.
2. D. B. Chambers and T. J. Leiker, "A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April–October 2004," Open File Report 2006-1393, United States Geological Survey, http://pubs.usgs.gov/of/2006/1393/.

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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

Authors

Imma Ferrer and E. Michael Thurman
Center for Environmental Mass Spectrometry
University of Colorado
Civil, Environmental, and Architectural Engineering
ECOT 441, 428 UCB
Boulder, CO 80309
USA

Jerry Zweigenbaum
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

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.

Agilent Technologies

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.

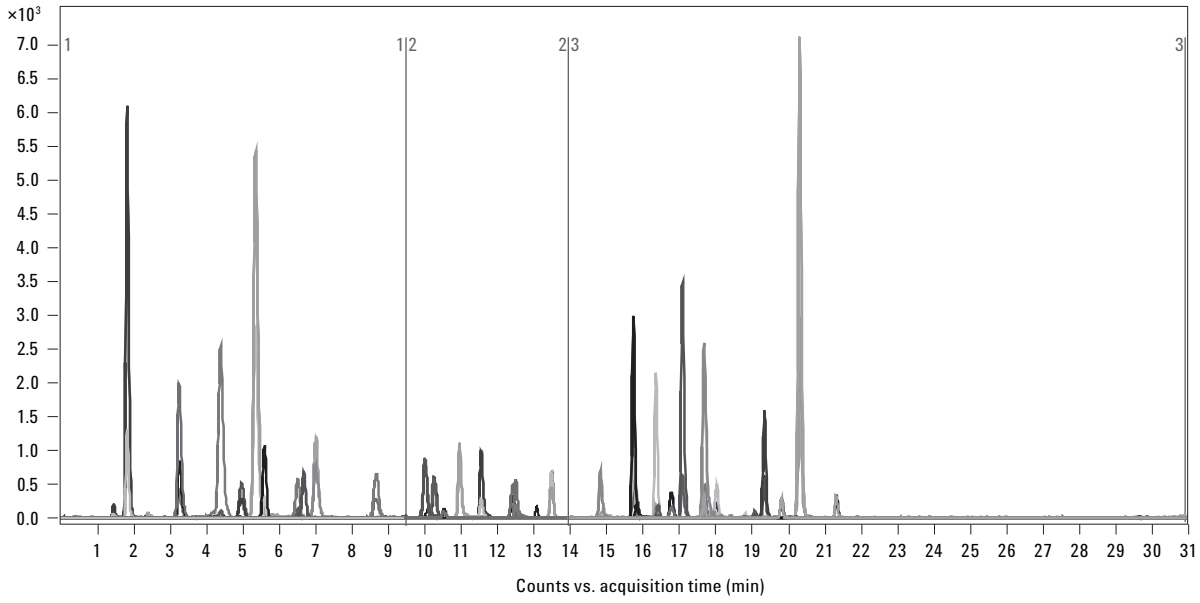

Figure 1A. MRM extracted chromatogram for pharmaceuticals in Group 1. Three time segments were used in this chromatographic separation.

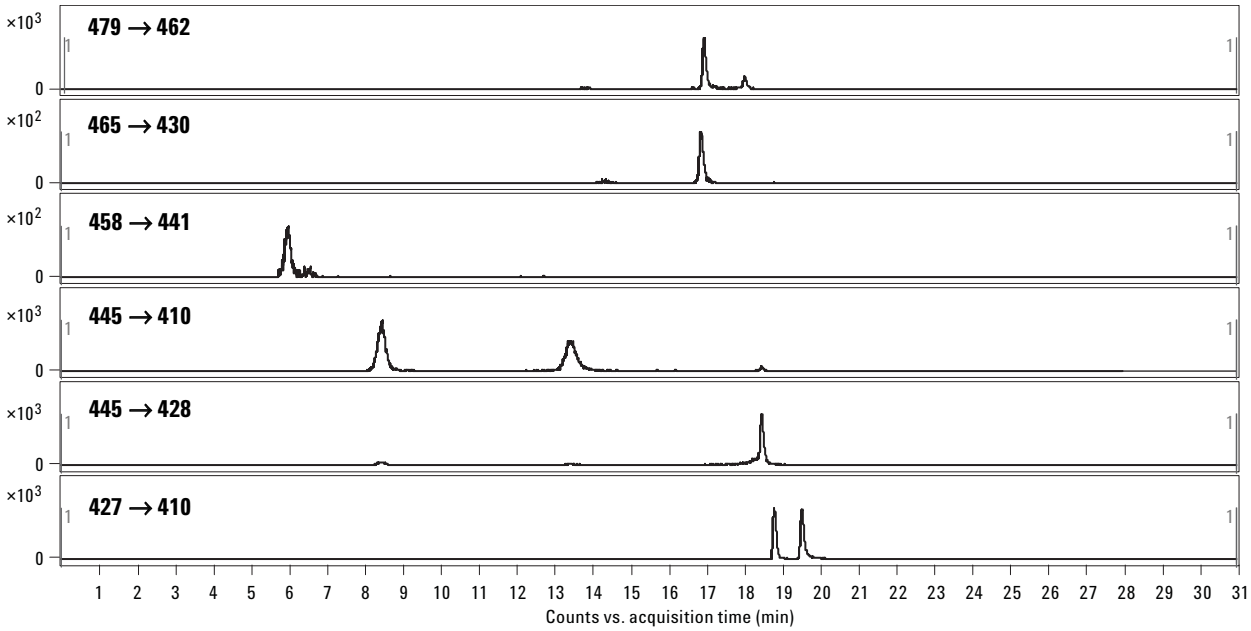

Figure 1B. MRM extracted chromatogram for pharmaceuticals in Group 2. Only one transition shown. See Table 2B for compound identification.

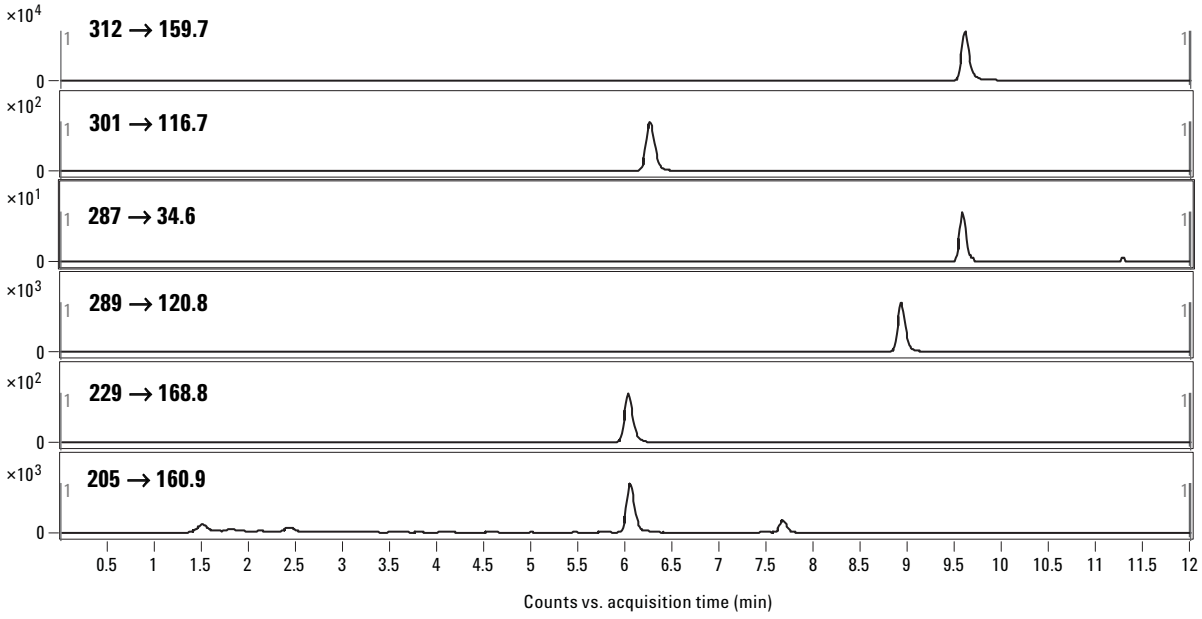

Figure 1C. MRM extracted chromatogram for pharmaceuticals in Group 3. Only one transition shown. See Table 2C for compound identification.

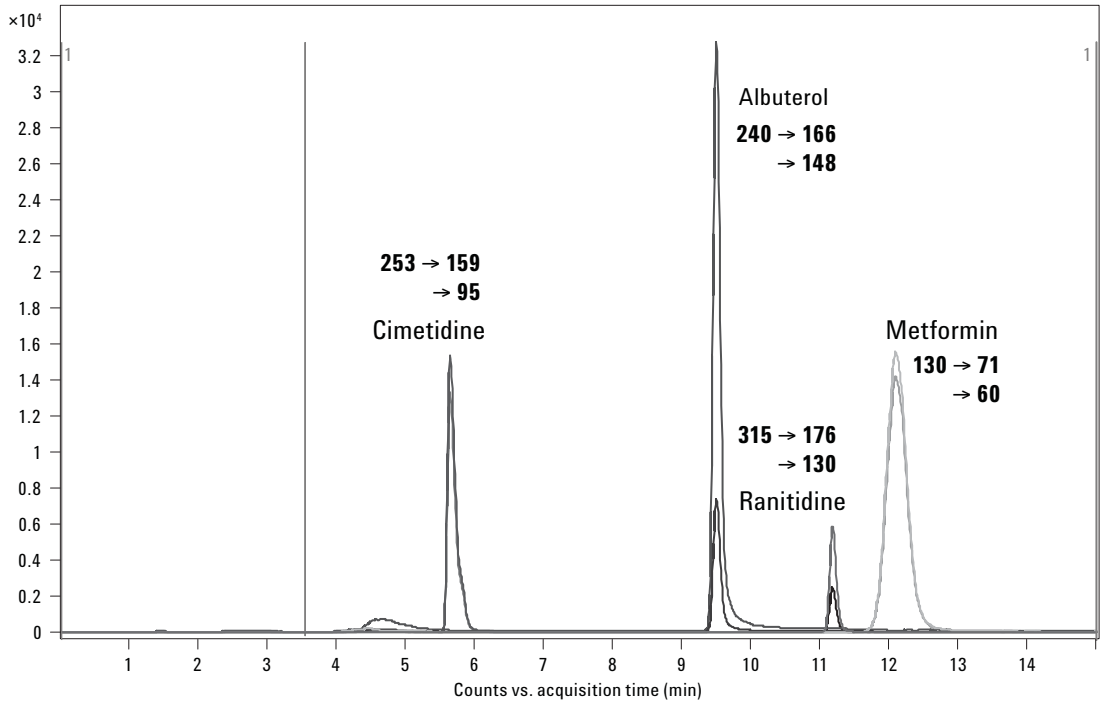

Figure 1D. MRM extracted chromatogram for pharmaceuticals in Group 4.

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.

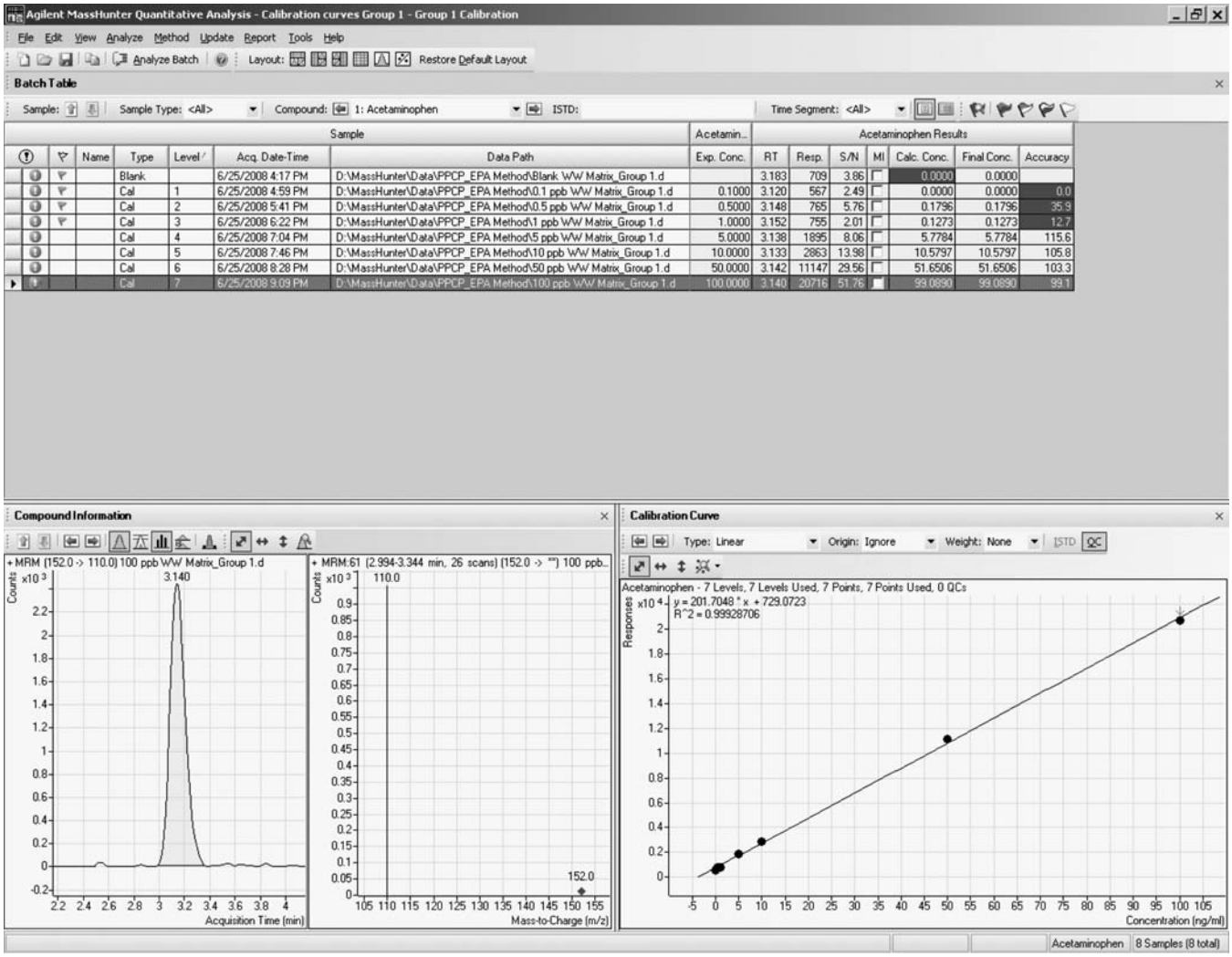

Figure 2. Calibration curve for acetaminophen in a wastewater matrix using a seven-point curve from 0.1 to 100 ng/mL (ppb) using a linear fit with no origin treatment.

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.

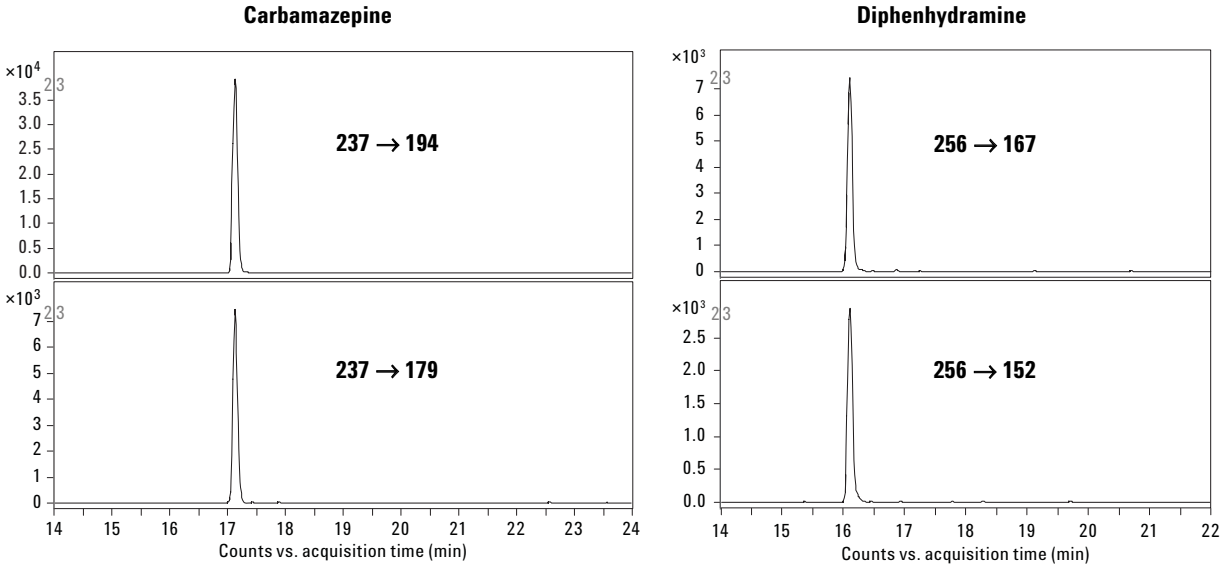

Figure 3. MRM chromatograms of a wastewater sample for carbamazepine and diphenhydramine using two transitions.

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.

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PFOA/PFOS Applications

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Quantitative Analysis of Perfluorooctanoic Acid by LC/MS/MS

Application Brief

Environmental

Authors

Sheher Mohsin
Agilent Technologies, Inc.
10 N. Martingale Rd., Suite 550
Schaumburg, IL 60173-2292
USA

Michael Zumwalt
Agilent Technologies, Inc.
9780 S Meridian Blvd., MS 1-1E
Englewood, CO 80112-5910
USA

Abstract

An Agilent 6410 Triple Quadrupole Mass Spectrometer (QQQ) is used to analyze perfluorooctanoic acid (PFOA). A simple isocratic elution is carried out on a Rapid Resolution High Throughput C18 column (particle size 1.8 µm) with only water and methanol solvents containing 10 mM ammonium acetate. Elution time for standard dilutions of PFOA is only 2.3 minutes. Good linearity over more than 4 orders of magnitude, from 9 fg/µL to 150 pg/µL, is demonstrated with excellent peak area reproducibility of 5.5 % RSD at the 9 fg/µL level. The average peak-to-peak signal-to-noise (S/N) ratio at this level is 7.2. Sensitivity of surface water extracts is expected to be similar.

Introduction

Perfluorooctanoic acid (PFOA) is a synthetic chemical that does not occur naturally in the environment. Companies use PFOA to make fluoropolymers, which are substances with valuable properties for applications such as fire resistance and oil, stain, and grease repellence. Toxicological studies have shown that exposure to PFOA can result in developmental/reproductive toxicity, liver damage, and possibly cancer. PFOA is highly persistent in the environment and has been found at very low levels in both the environment and in the blood of the general U.S. population. Recent studies by the EPA have indicated the need for additional testing and monitoring. Detection at levels < 100 ppt in drinking water is required by the European Union.

In this work, dilutions of the PFOA standard are run at levels ranging from 9 fg/µL to 150 pg/µL, with a correlation coefficient of linearity of $R^2 > 0.997$. Nonlinearity is seen with the 1,500 pg/µL level. During the worklist the 9 fg/µL level is injected six times in a row to determine reproducibility. Based on peak areas the reproducibility at this lowest level of investigation is 5.5% RSD. Since the injection volume is 10 µL, the on-column injection amount at this lowest level is only 90 fg.

The structure of PFOA is shown below.

$CF_3-(CF_2)_6-COOH$

This molecule is a carboxylic acid, which is expected to show good sensitivity in negative ion mode using electrospray ionization (ESI).

Experimental

Sample Preparation

The PFOA standard is obtained at a concentration of 1,500 ng/µL. Dilutions in methanol are made up at 0.009, 0.015, 0.15, 0.45, 0.75, 1.5, 15.0, 150, and 1,500 pg/µL concentrations.

LC/MS Method Details

LC Conditions

Agilent 1100 Series binary pump, degasser, wellplate sampler, and thermostatted column compartment

Column:	Agilent ZORBAX Eclipse Plus RRHT C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)
Column temperature:	40 °C
Mobile phase:	A = 10 mM ammonium acetate in water B = 10 mM ammonium acetate in 80:20 methanol/water
Flow rate:	0.3 mL/min
Injection volume:	10 µL
Isocratic:	85% B
Stop time:	3 minutes
Needle wash:	75:25 methanol/water; flush port 10 seconds

MS Conditions

Mode:	Negative ESI using the Agilent G1948B ionization source
Nebulizer:	35 psig
Drying gas flow:	10 L/min
Drying gas temp:	300 °C
V_{cap}:	4,000 V
Resolution (FWHM):	Q1 = 0.7 amu; Q2 = 0.7 amu
MRM transition:	*m/z* 413.0 > 369.0
Fragmentor:	67 V
Collision energy:	3 V
Dwell time:	200 msec

Results and Discussion

The calibration curve for this work is shown in Figure 1. At the lowest level of investigation (9 fg/µL) nine replicate injections are made. Nine replicate injections are also made at the 15 fg/µL level. As seen in Figure 2, the peak area reproducibility is 5.5% RSD. The average S/N ratio at this level is 7.2. Noise is calculated from the 1– to 1.5–minute region.

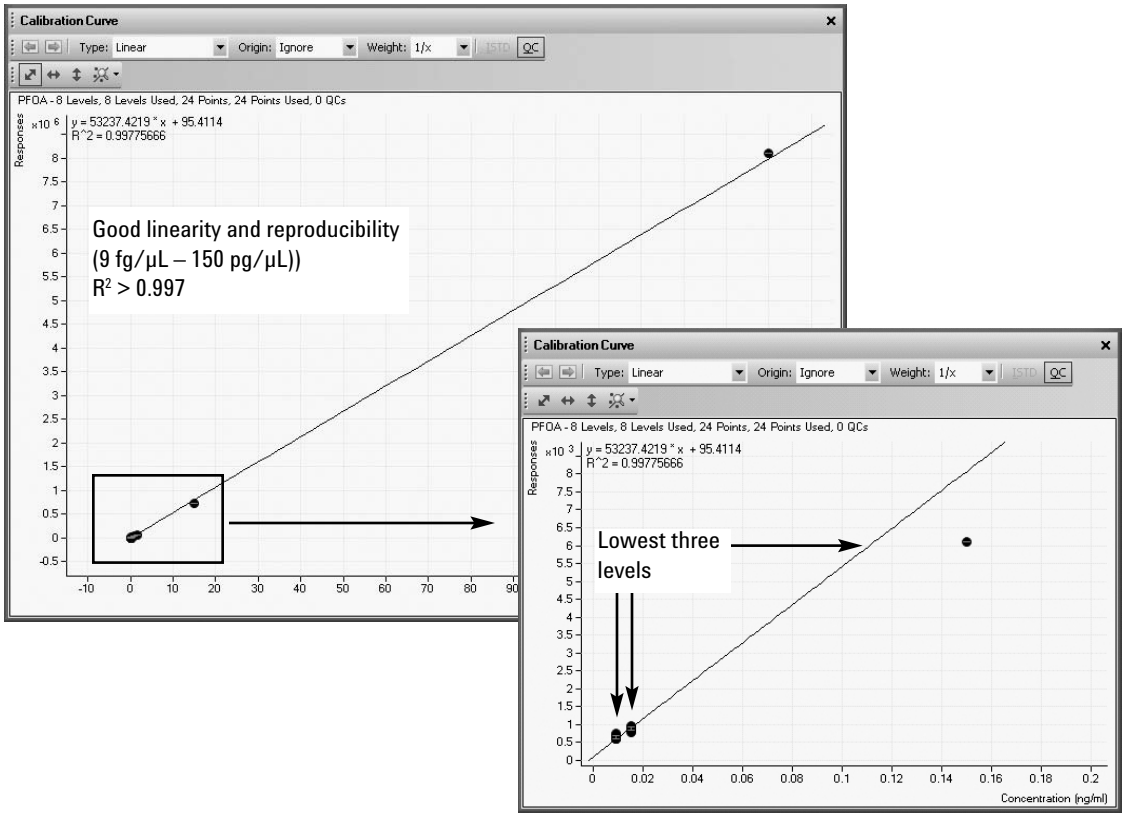

Figure 1. Calibration curve for PFOA. Lowest three levels consist of nine replicate injections at 9 fg/µL and 15 fg/µL each, and one injection at 150 fg/µL.

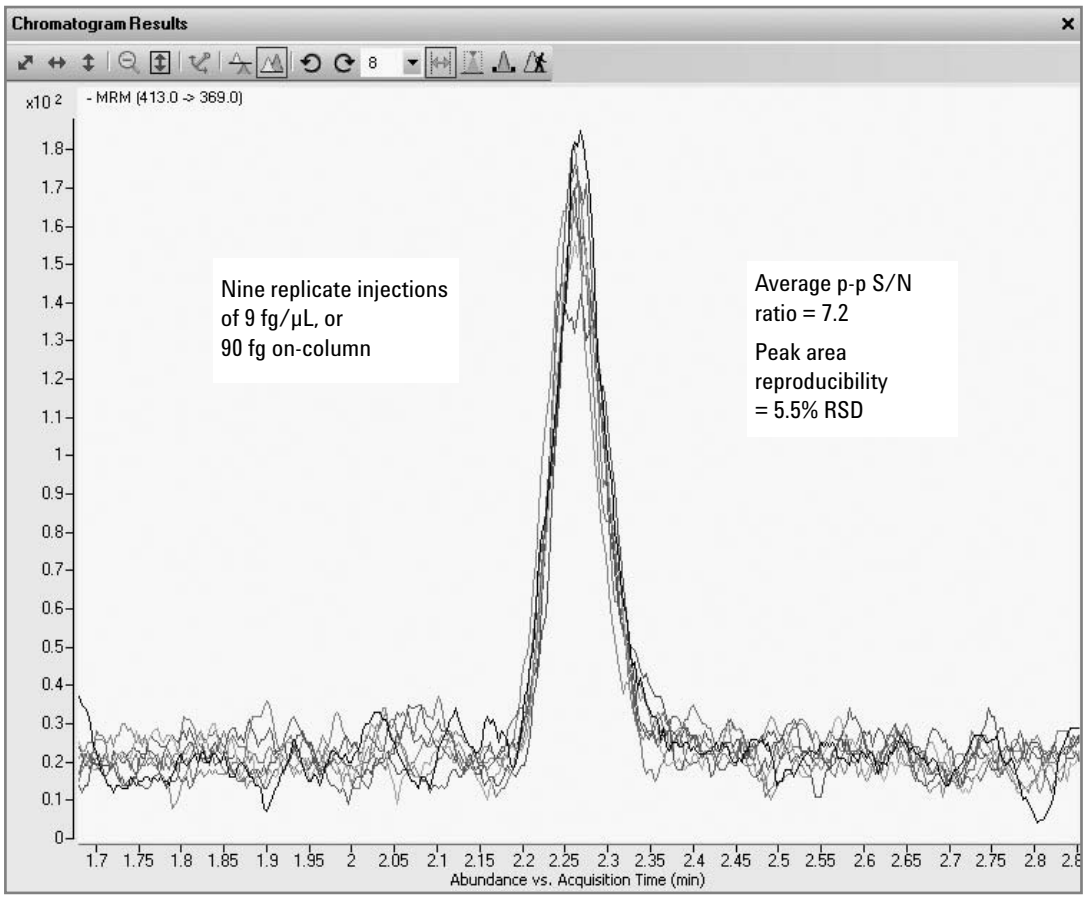

Figure 2. Excellent sensitivity and reproducibility at the lowest level investigated.

If an additional dilution level of 1,500 pg/µL is added to the calibration curve, then we see the result shown in Figure 3, in which a quadratic curve fit of the data is required. However, note the excellent correlation coefficient of $R^2 > 0.99999$.

Conclusions

The perfluorooctanoic acid compound is very sensitive to negative electrospray ionization mode. Very good linearity is demonstrated with the standard at dilutions ranging from 9 fg/uL to 150 pg/µL, which corresponds to an on-column injection amount ranging from 90 fg to 1.5 ng. Peak area reproducibility at the lowest level investigated of 9 fg/µL is excellent at 5.5% RSD, and the average signal-to-noise ratio of the replicate injections at this level is 7.2. Signal saturation is seen at 1,500 pg/µL, or 15 ng on-column. As drinking water is a relatively clean matrix, it is expected that similar sensitivity will be achievable with extracted samples.

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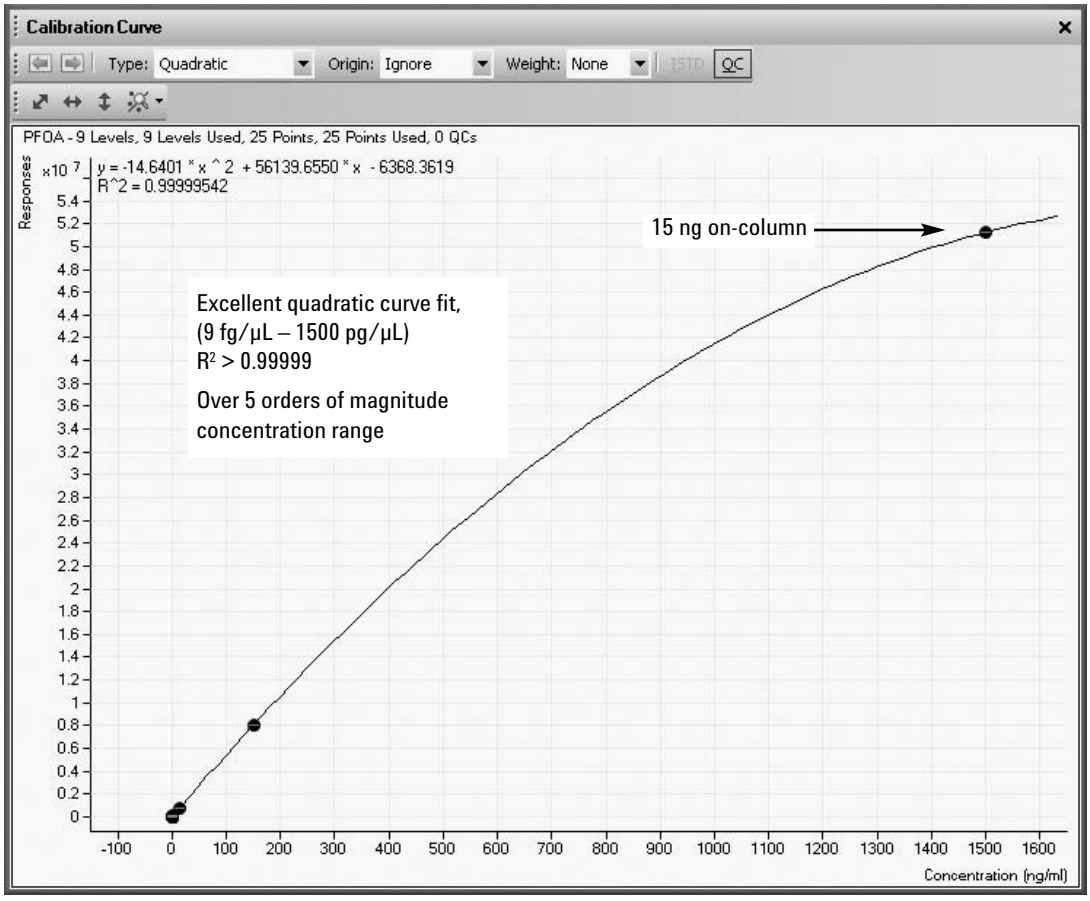

Figure 3. Saturation seen with addition of 1500 pg/µL level, or 15 ng on-column.

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

Application

Food, Environmental

Authors

Robert D. Voyksner
LCMS Limited
PO Box 27228
Raleigh, NC 27611-7228
USA

Chin-Kai Meng
Agilent Technologies
2850 Centerville Road
Wilmington, DE 19808
USA

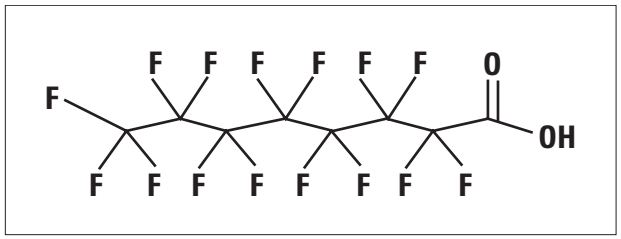
Abstract

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%.

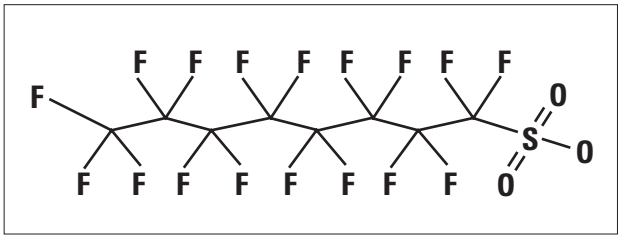
Introduction

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.

Quantitative Analysis of Perfluorooctanoic Acid by LC/MS/MS

Application Brief

Environmental

Authors

Sheher Mohsin
Agilent Technologies, Inc.
10 N. Martingale Rd., Suite 550
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Michael Zumwalt
Agilent Technologies, Inc.
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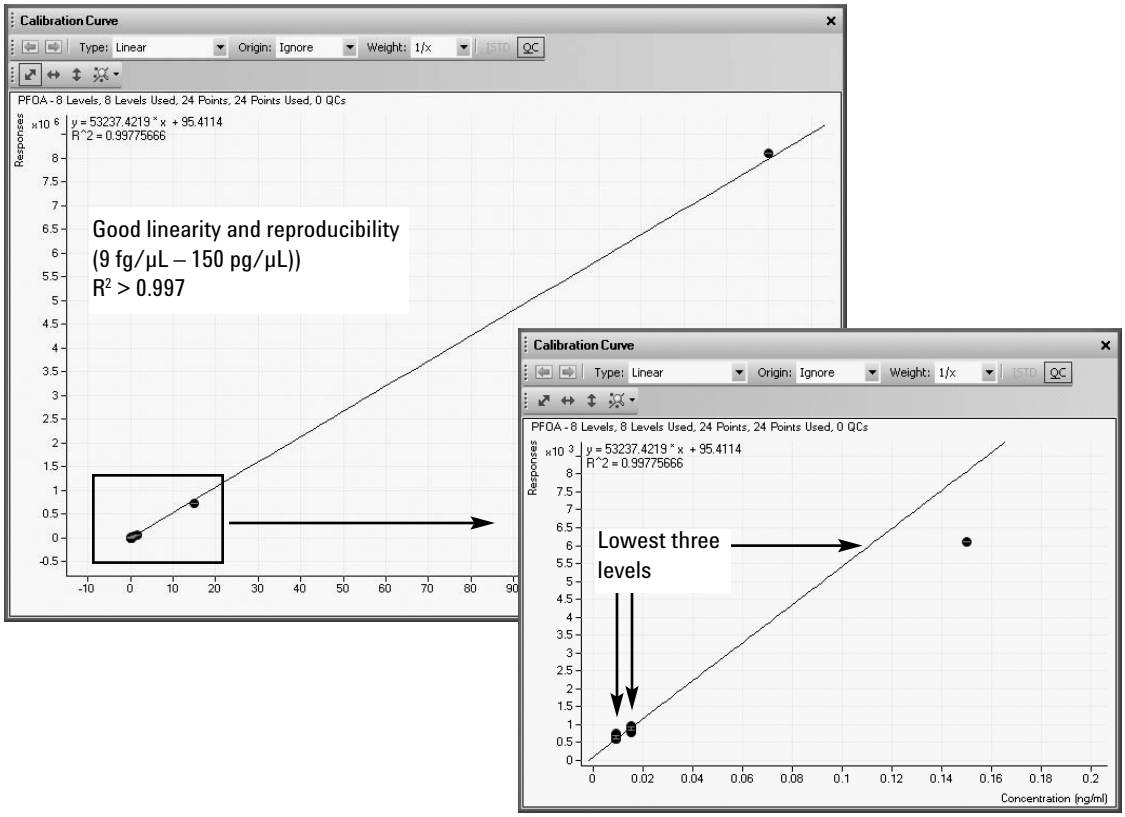

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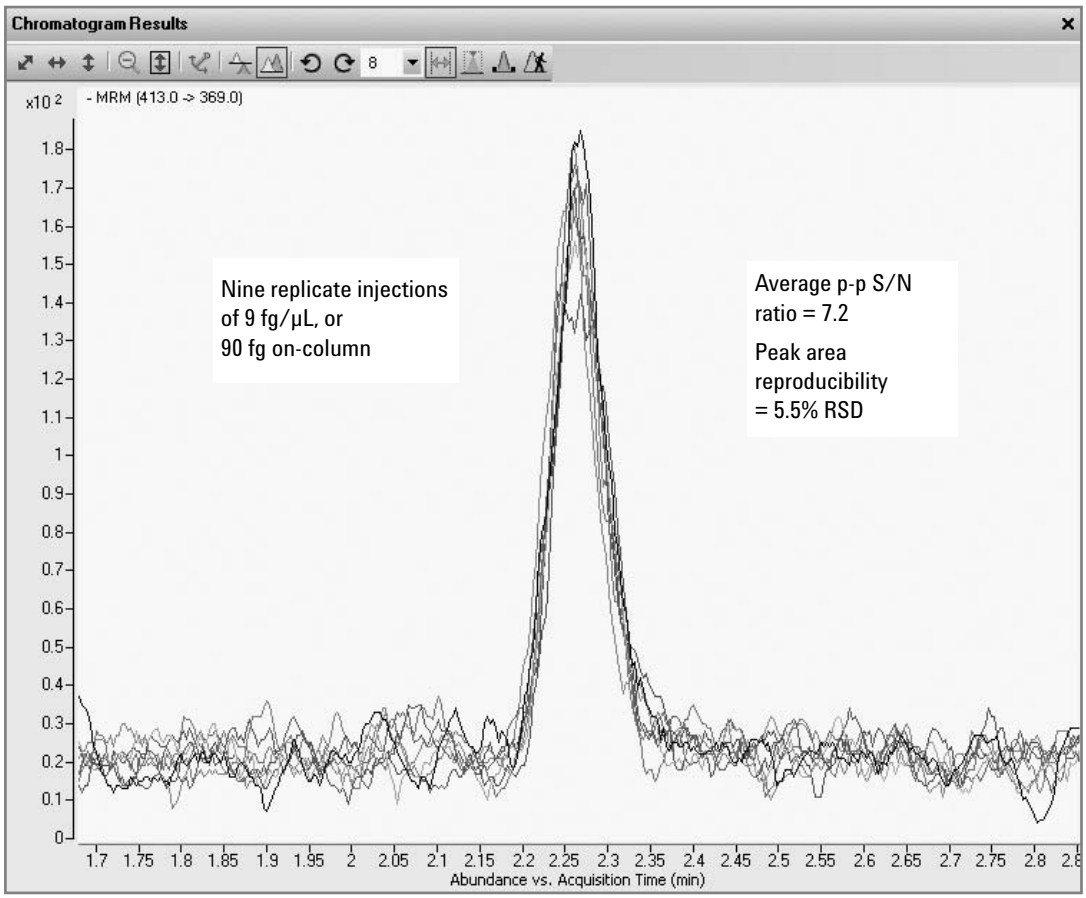

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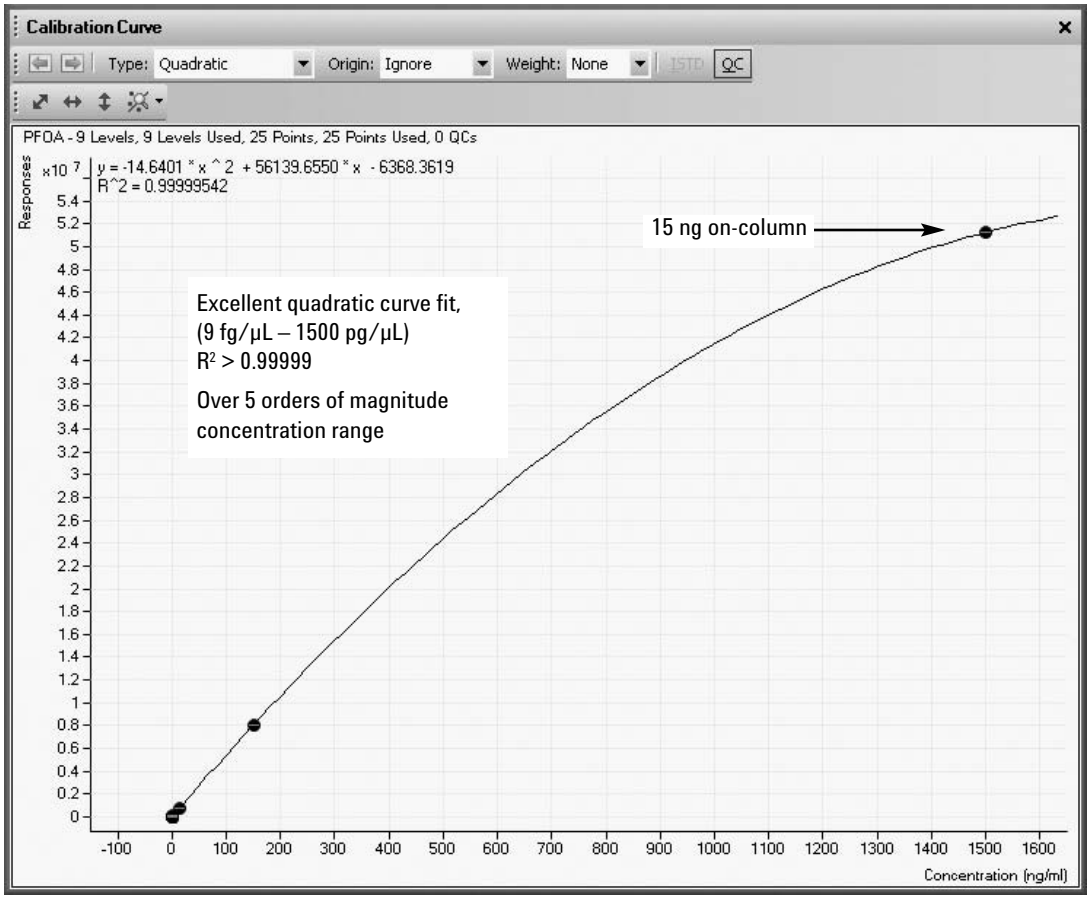

Figure 3. Saturation seen with addition of 1500 pg/µL level, or 15 ng on-column.

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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

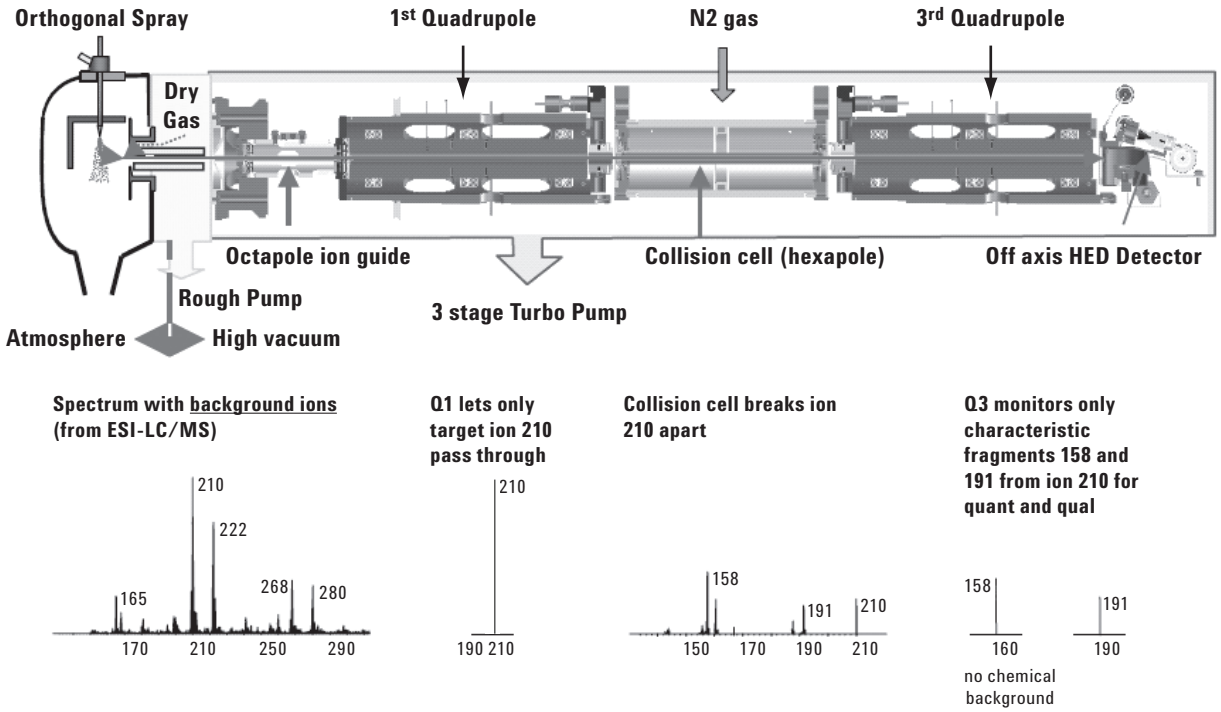


Figure 2. A cross-section of the Agilent 6410 QQQ above a sequence of spectra characteristic of ion transitions within the instrument for a hypothetical sample (*not* PFOA or PFOS). Note that the final spectrum is very clean, containing only the desired target ions. (HED = high-energy dynode electron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

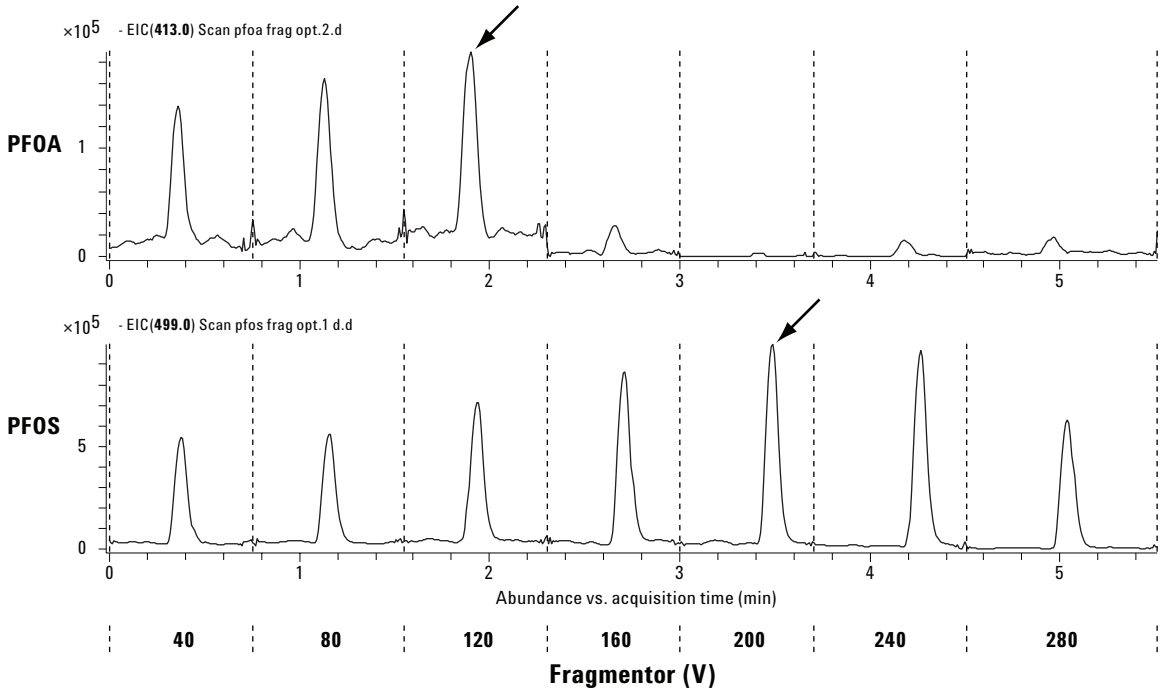


Figure 3. Determination of optimal fragmentor voltage using sequential plots of signal intensity versus applied voltage.

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.

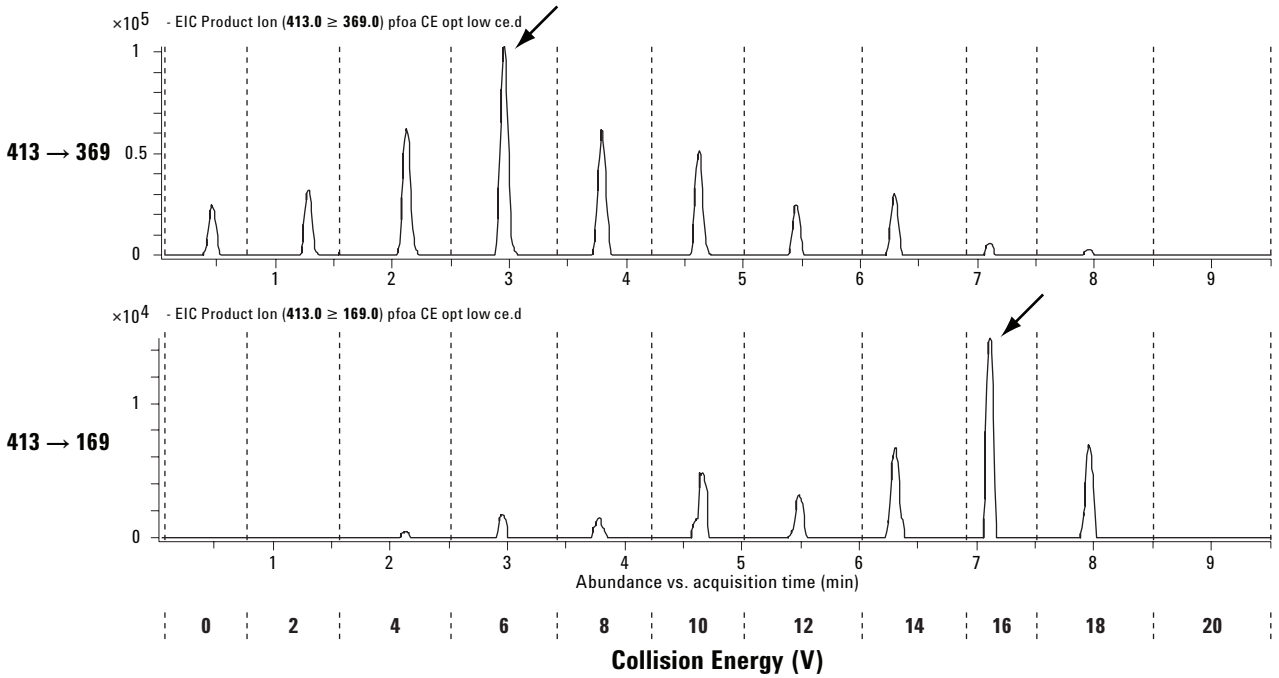


Figure 4. Signal intensity as a function of collision energy for PFOA product ions *m/z* 369 $[M\text{-}CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$.

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).

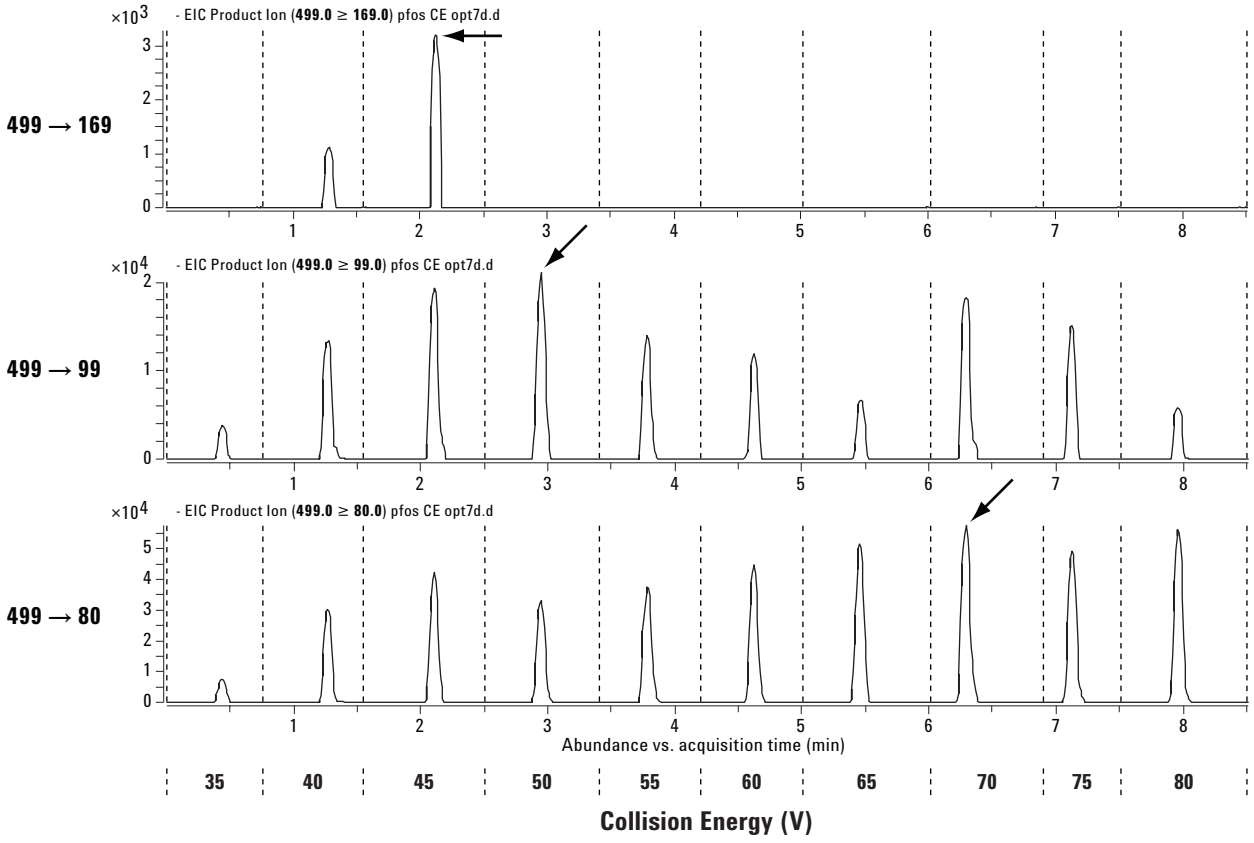
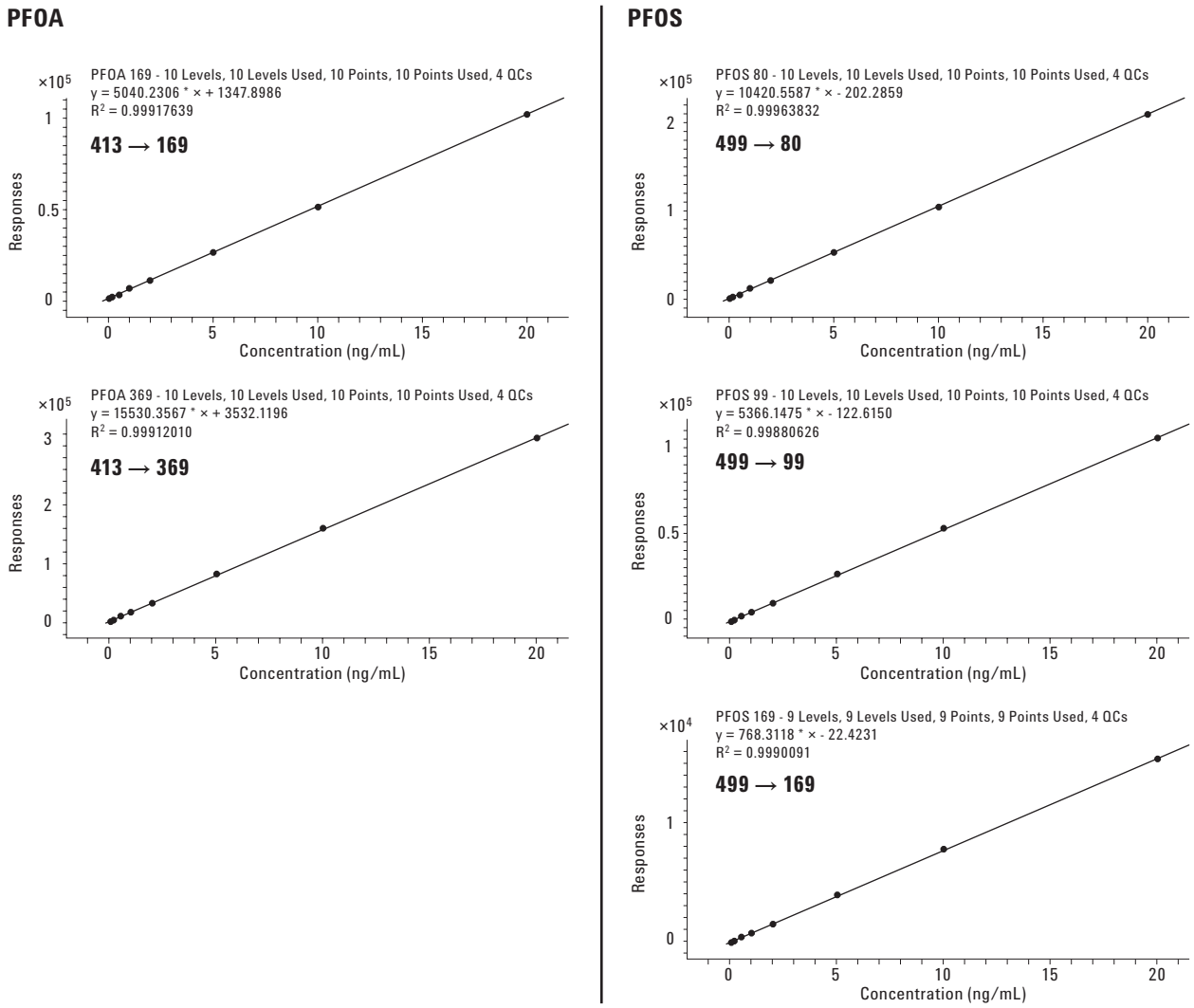


Figure 5. Signal intensity as a function of collision energy for PFOS product ions at *m/z* 169, 99, and 80.



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.

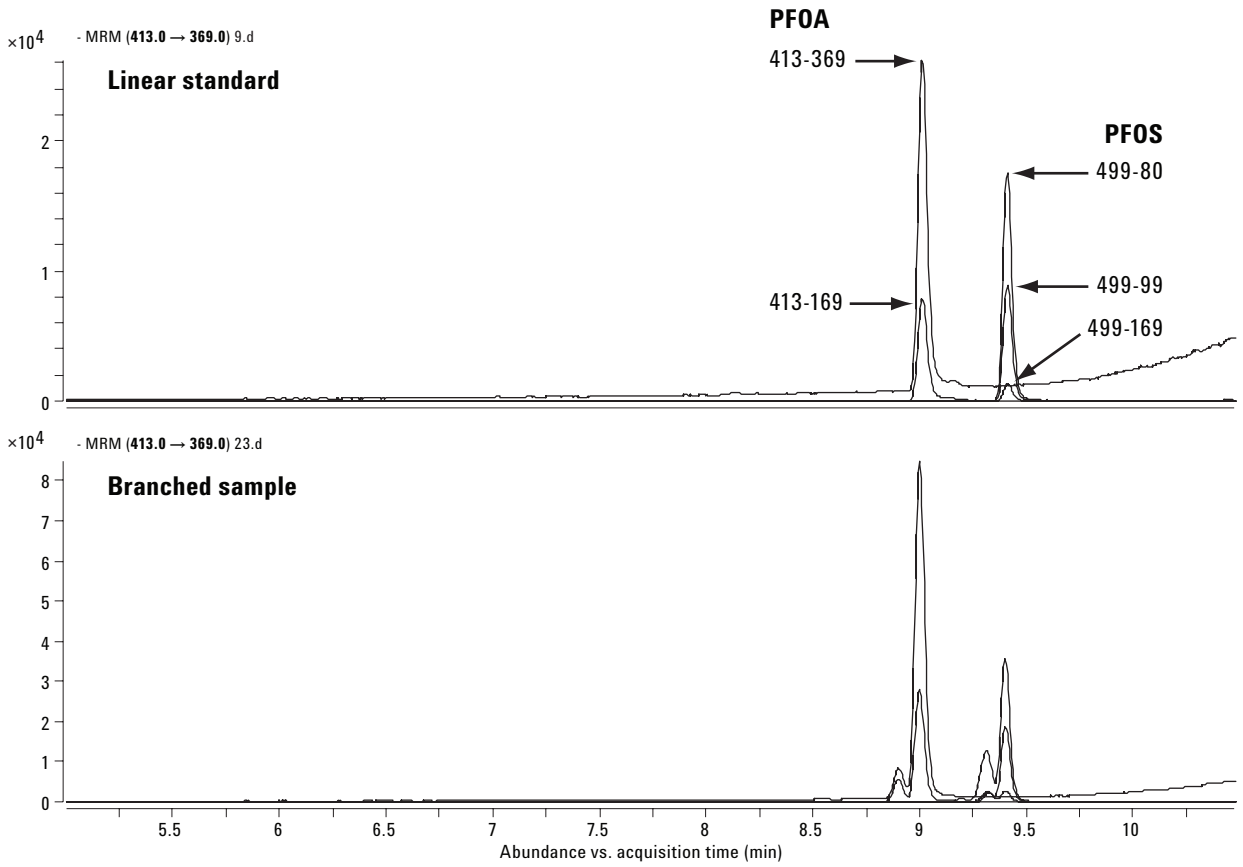


Figure 7. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

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.

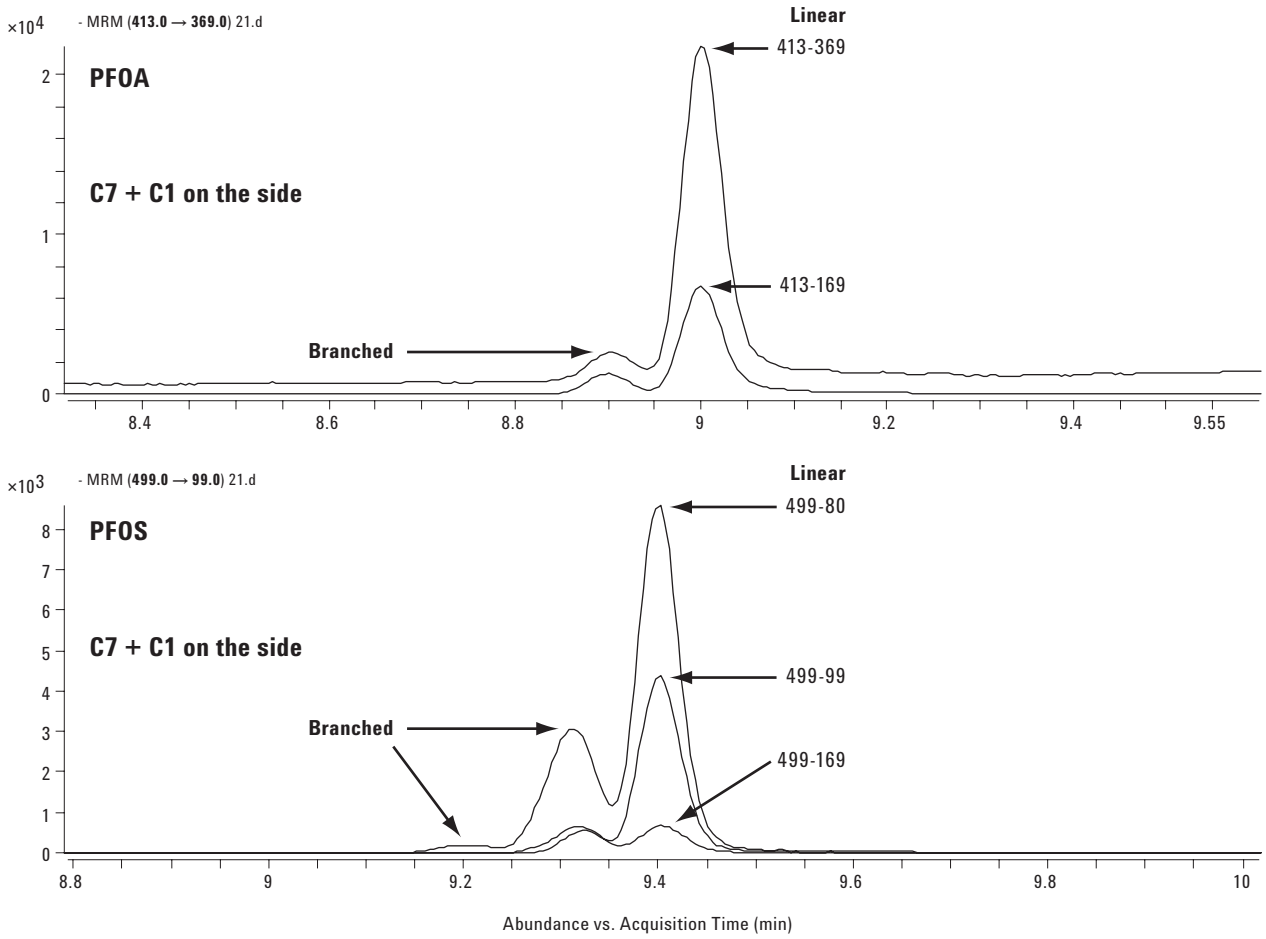


Figure 8. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

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.

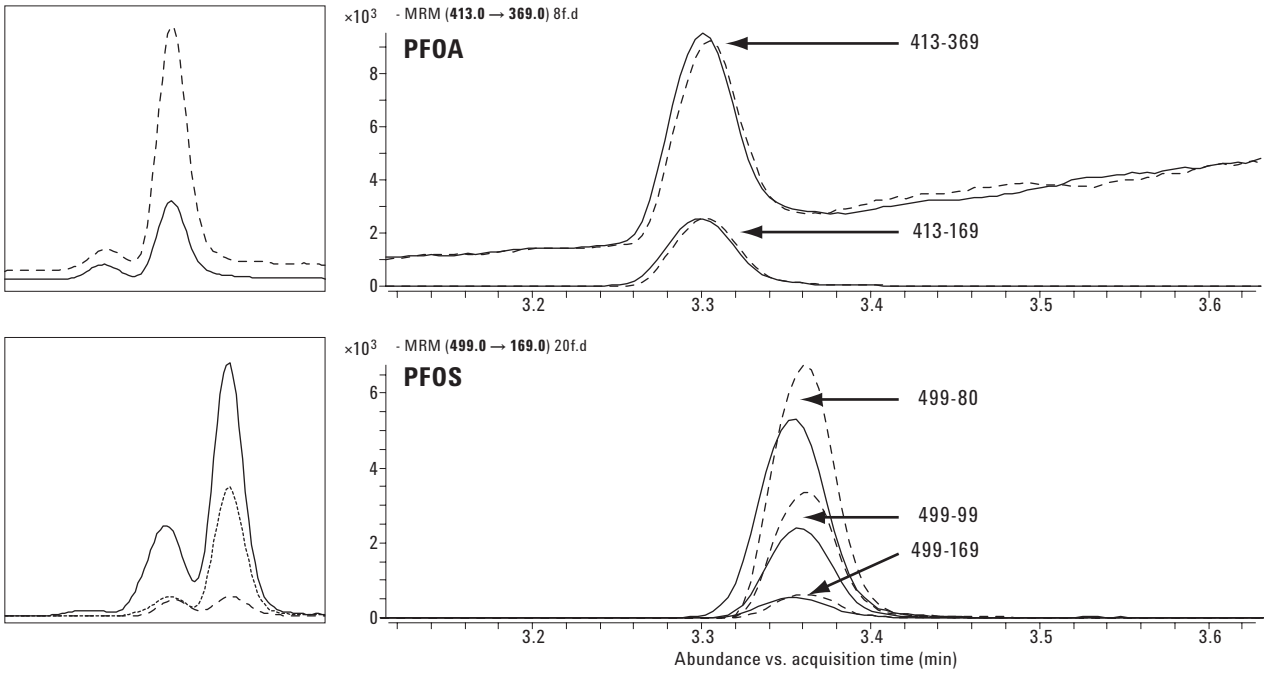


Figure 9. Comparison of PFOA and PFOS MRM chromatograms produced using both 10- and 3-minute gradients. The 3-minute gradient chromatograms are on the right.

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.

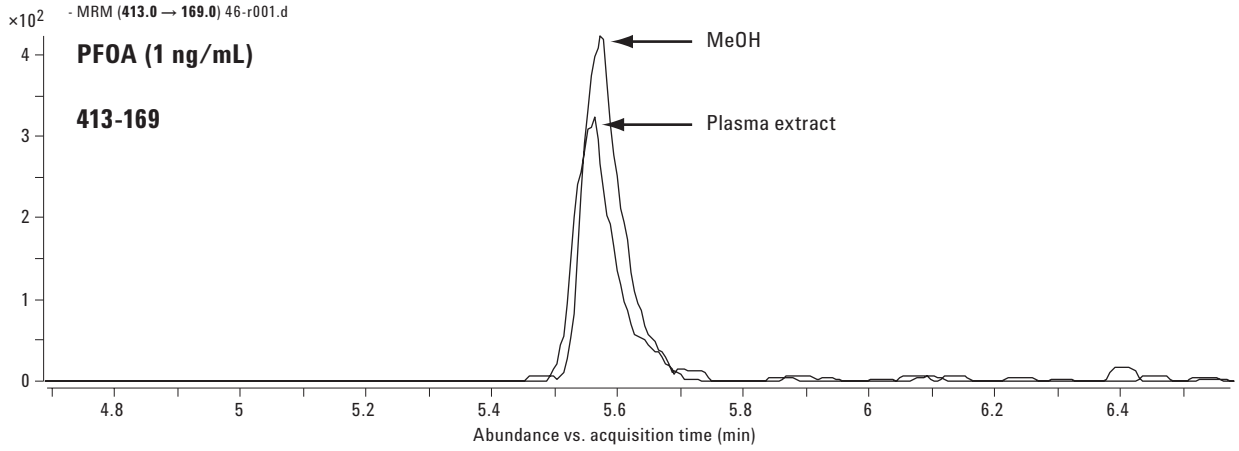
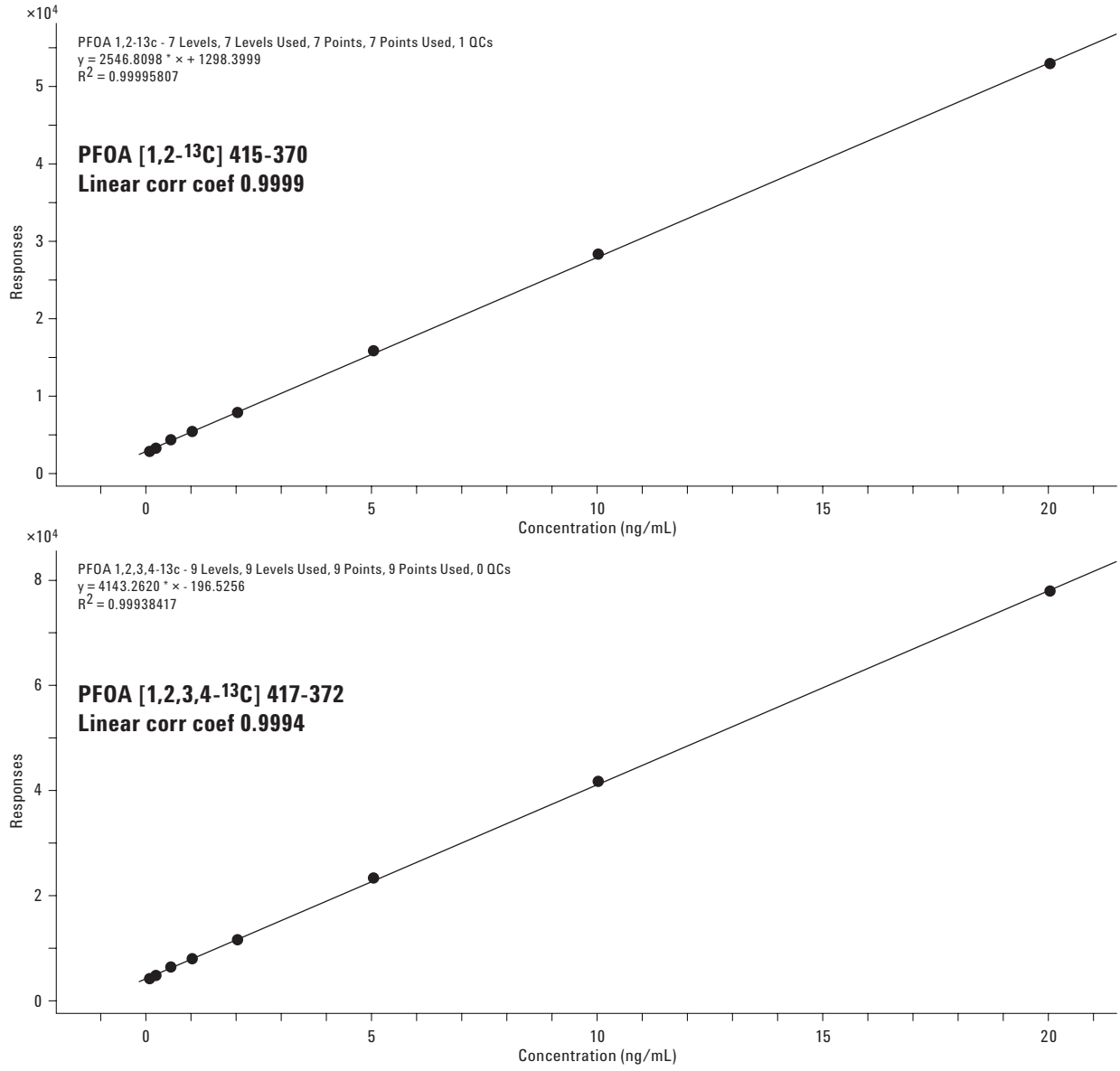


Figure 10. PFOA responses in MeOH and plasma extract at the same concentrations.



Calibration in plasma from 0.02 to 20 ng/mL

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

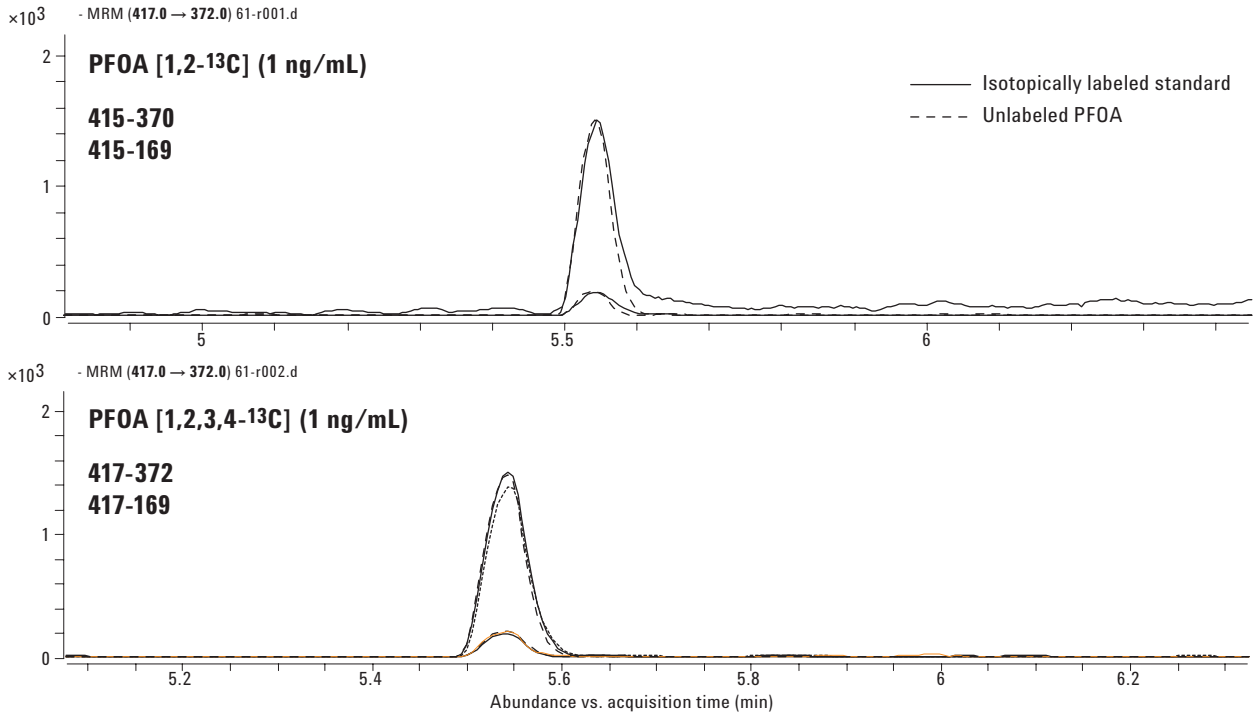


Figure 12. Both isotopically labeled PFOA compounds show good correlation to the unlabeled PFOA. The same transitions for the labeled and native forms of the PFOA were used.

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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Analyzing Compounds of Environmental Interest Using an LC/Q-TOF Part 2: Fluorotelomer Unsaturated Acids

Application

Environmental

Authors

Jim Lau, Chin-Kai Meng, and Jennifer Gushue
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Robert J. Letcher and Shaogang Chu
Wildlife and Landscape Science Directorate
Science and Technology Branch
Environment Canada
National Wildlife Research Centre
Carleton University
Ottawa, Ontario, K1A 0H3
Canada

Abstract

Perfluoroalkyl substances (PFASs) have been widely used in a variety of products due to their chemical inertness, resistance to heat, and ability to repel water and oils. PFASs exhibit a high propensity for persistence and bioaccumulation in wildlife, which is causing concern. Due to different manufacturing and degradation processes, different chain lengths and functional groups of PFAS exist in the environment. An LC/Q-TOF is best suited to screen, identify, and quantify many perfluoroalkyl compounds in different matrices.

This LC/Q-TOF application shows good mass accuracy (well below 3 ppm) and good resolution (> 13,500). Excellent quantification results for selected PFASs were obtained from extracts derived from blind liver samples.

Introduction

Environment Canada is tasked with risk assessment and the evaluation of impact of a variety of compounds in environmental matrices, including wildlife tissues, water, sediment, and air [1, 2]. A number of compounds classified as perfluoroalkyl substances (PFASs) have been widely used in a variety of products due to their chemical inertness, resistance to heat, and ability to repel water and oils. Some of the commercial PFAS products include lubricants, adhesives, stain and soil repellents, paper coatings, and fire-fighting foams. Due to their unique chemical and biological stability, some PFASs exhibit a high propensity for persistence and bioaccumulation in wildlife. In recent years, particular environmental concern has arisen as a number of PFASs have been reported in tissues of marine mammal, seabird, and fish species inhabiting various regions of the Arctic. More specifically, some PFASs, such as perfluorooctane sulfonate (PFOS) [$CF_3(CF_2)_7SO_3H$] and C8 to C15 chain length perfluorinated carboxylates (PFCAs), have been reported present at similar or higher concentrations than persistent organochlorines (OCs) in polar bears [3–5].

Many of the PFASs originate from the two manufacturing processes of electrochemical fluorination (perfluoroalkyl sulfonamido alcohols degrade to PFOS) and telomerization (fluorotelomer alcohols [FTOHs] are transformed to PFOA). The degradation pathway for telomers is:

From 8:2 fluorotelomer alcohol (8:2 FTOH) to:

- Major products: 8:2 fluorotelomer aldehyde (8:2 FTAL), 8:2 fluorotelomer carboxylate (8:2 FTCA), and perfluorooctanoic acid (PFOA) [$CF_3(CF_2)_6CO_2H$]
- Minor products: 8:2 fluorotelomer unsaturated carboxylate (8:2 FTUCA, $CF_3(CF_2)_6CF$=COOH) and perfluorononanoic acid (PFNA)

Then from 8:2 FTCA and 8:2 FTUCA (degradation products from above) to:

- Major product: PFOA
- Minor product: PFNA

This application uses fluorotelomer unsaturated carboxylates (FTUCAs – metabolites of telomer alcohols) to demonstrate the mass accuracy, resolution, and the quantification capability of the LC/Q-TOF.

Experimental

Samples

This study analyzed three standard solutions of the following three target compounds at 1, 100, and 250 ppb. Three internal standards (ISTDs) at 50 ppb each were also added to each of the three solutions. A fourth sample was a blind liver extract with an undisclosed concentration of the three target compounds.

Target Compounds:

F**H**UEA (C8, **6**:2 FTUCA)	2H-Perfluoro-2-octenoic acid $CF_3(CF_2)_4CF$=CH-COOH
F**O**UEA (C10, **8**:2 FTUCA)	2H-Perfluoro-2-decenoic acid $CF_3(CF_2)_6CF$=CH-COOH
F**D**UEA (C12, **10**:2 FTUCA)	2H-Perfluoro-2-dodecenoic acid $CF_3(CF_2)_8CF$=CH-COOH

ISTDs:

MFHUEA	2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoic acid $CF_3(CF_2)_4CF$=C^{13}H-C^{13}OOH
MFOUEA	2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid $CF_3(CF_2)_6CF$=C^{13}H-C^{13}OOH
MFDUEA	2H-Perfluoro-[1,2-$^{13}C_2$]-2-dodecenoic acid $CF_3(CF_2)_8CF$=C^{13}H-C^{13}OOH

Instrument Parameters

All sample analyses were performed on an Agilent 1200 SL Rapid Resolution LC coupled to an Agilent 6520 Q-TOF.

All sample analyses were performed under LC/Q-TOF autotune conditions. Mass accuracy, sensitivity, and resolution for all samples were measured without any changes to 6520 Q-TOF instrument parameters, except ion source conditions appropriate for the spray chamber type, LC flow, and sample thermal stability.

Mobile A	3 mM NH_4OAc
Mobile B	MeOH
LC column	ZORBAX XDB 2.1 mm × 50 mm, C-18, 3.5-µm particle size
Flow rate	0.5 mL/min
Injection volume	10 µL
MS	Scanned at 2.5 scans/sec, 50 to 1,100 *m/z*
Negative ref. ions	*m/z* 113, 1034
Q-TOF parameters	Set by autotune
Drying gas	12 L/min N_2 at 300 °C
Nebulizer pressure	50 psi
ESI (–)	3 KV
Fragmentor	275 V

Results and Discussion

Table 1 shows the mass accuracy and resolution of the six compounds analyzed in this study. The accuracy for each compound was under 3 ppm and the resolution was > 13,500. Table 2 shows the quantitation ions and results. The quantitation ion was not the molecular ion but a fragment ion from the collision-induced dissociation (CID). The fragment ion is a lost fragment of "FCOOH" ($[M–H]^-$ – 64) from the deprotonated molecular ion. The calibration range was 1 to 250 ppb (that is, 10 pg to 2.5 ng on column). Good signal-to-noise ratios (un-smoothed signal) were observed for the three FTUCA standards at 1 ppb (see Figure 1).

A blind mixture of the three standards in liver extract was quantified by Q-TOF. Excellent quantification results were obtained for all three targets:

Compound	Measured	Actual
FOUEA	4.0 ppb	5 ppb
FHUEA	2.7 ppb	5 ppb
FDUEA	6.7 ppb	5 ppb

By comparing the ISTD responses, ion suppression (matrix effect) was observed in analyzing the liver extract. It has been shown using an LC/QQQ system with atmospheric pressure photoionization (APPI) is a more effective technique than electrospray ionization (ESI) to analyze FTOHs and perfluorinated sulfonamides [6].

Table 1. Mass Accuracy and Resolution of the Six FTUCA Analyzed in This Study

	Formula	Mass accuracy (ppm)	Resolution
FHUEA	$C_8H_2F_{12}O_2$	– 0.98	13692
MFHUEA	$[^{13}C]_2C_6H_2F_{12}O_2$	– 0.39	13789
FOUEA	$C_{10}H_2F_{16}O_2$	– 0.19	14018
MFOUEA	$[^{13}C]_2C_8H_2F_{16}O_2$	– 2.08	13564
FDUEA	$C_{12}H_2F_{20}O_2$	– 0.62	15399
MFDUEA	$[^{13}C]_2C_{10}H_2F_{20}O_2$	– 1.33	14322

Table 2. Quantification Ions and Linearity of the FTUCA (The quantitation ion is a lost fragment of "FCOOH" from the molecular ion.)

	Formula	$[M–H]^-$	Quant Ion ($[M–H]^- – 64$)	R^2 (1–250 ppb)
FHUEA	$C_8H_2F_{12}O_2$	357	293	0.9992
MFHUEA	$[^{13}C]_2C_6H_2F_{12}O_2$	359	294	–
FOUEA	$C_{10}H_2F_{16}O_2$	457	393	0.9998
MFOUEA	$[^{13}C]_2C_8H_2F_{16}O_2$	459	394	–
FDUEA	$C_{12}H_2F_{20}O_2$	557	493	0.9972
MFDUEA	$[^{13}C]_2C_{10}H_2F_{20}O_2$	559	494	–

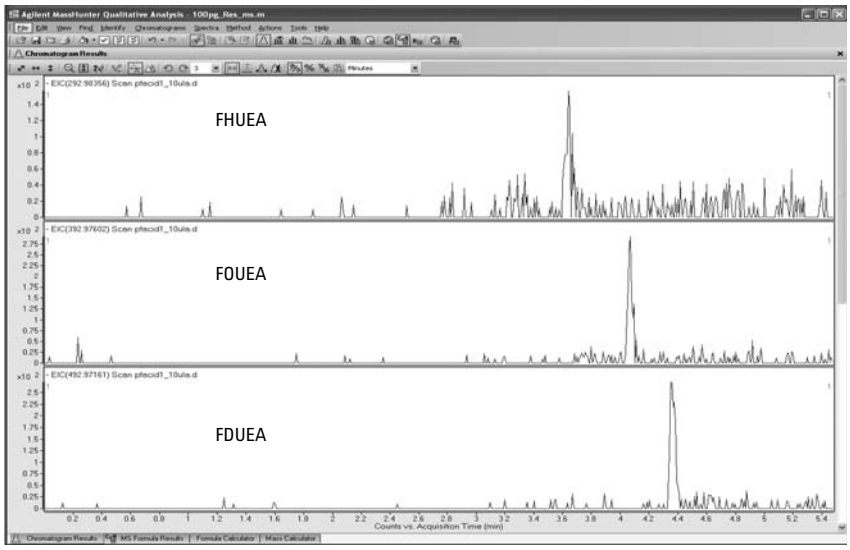

Figure 1. Expansion of EICs for quant ions of 1 ppb FTUCA standards (S/N > 5, unsmoothed signal).

Conclusions

Some of the most impressive aspects about the LC/Q-TOF were the mass accuracy, the linear dynamic range, and the signal-to-noise ratios. For environmental applications, using Q-TOF can greatly reduce interference from complex matrices and improve the accuracy of the results. This application of Q-TOF shows good mass accuracy (well below 3 ppm) and good resolution (> 13,500). Excellent quantification results were obtained from a blind liver extract.

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GC Analysis of Polybrominated Flame Retardants

Application

Environmental

Authors

Eberhardt Kuhn, Jason Ellis
Agilent Technologies, Inc.
91 Blue Ravine Rd,
Folsom, CA 95630
USA

Steve Wilbur
Agilent Technologies, Inc.
3380 146th Place SE
Suite 300
Bellevue, WA 98007
USA

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.

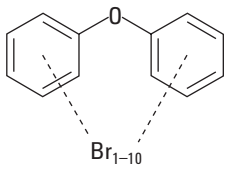


Figure 1. Structure of PBDE.

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)
3-MonoBDE (#2)
4-MonoBDE (#3)

2,6-DiBDE (#10)
2,4-DiBDE (#7)
3,3′-DiBDE (#11)
2,4′-DiBDE (#8)
3,4-DiBDE (#12)
3,4′-DiBDE (#13)
4,4'-DiBDE (#15)

2,4′,6-TriBDE (#32)
2,4,6-TriBDE (#30)
2,2',4-TriBDE (#17)
2,3′,4-TriBDE (#25)
2',3,4-TriBDE (#33)
2,4,4'-TriBDE (#28)
3,3′,4-TriBDE (#35)
3,4,4'-TriBDE (#37)

2,4,4′,6-TetraBDE (#75)
2,2',4,5'-TetraBDE (#49)
2,3′,4′,6-TetraBDE (#71)
2,2',4,4'-TetraBDE (#47)
2,3′,4,4′-TetraBDE (#66)
3,3',4,4'-TetraBDE (#77)

2,2',4,4′,6-PentaBDE (#100)
2,3′,4,4′,6-PentaBDE (#119)
2,2',4,4',5-PentaBDE (#99)
2,3,4,5,6-PentaBDE (#116)
2,3′,4,4′,5-PentaBDE (#118)

2,2',4,4',6,6'-HexaBDE(#155)

2,2′,3,4,4′-PentaBDE (#85)
3,3',4,4',5-PentaBDE (#126)

2,2',4,4',5,6′HexaBDE(#154)
2,2',4,4',5,5'-HexaBDE(#153)
2,2',3,4,4',5'-HexaBDE(#138)
2,3,4,4',5,6-HexaBDE (#166)

2,2',3,4,4',5',6-HeptaBDE (#183)
2,2',3,4,4',5,6-HeptaBDE(#181)
2,3,3',4,4',5,6-HeptaBDE (#190)

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

3-MonoBDE (#2)
2-MonoBDE (#1)
4-MonoBDE (#3)

2,6-DiBDE (#10)
2,4-DiBDE (#7)
3,3′-DiBDE (#11)
2,4′-DiBDE (#8)
3,4-DiBDE (#12)
3,4′-DiBDE (#13)
4,4'-DiBDE (#15)

2,4′,6-TriBDE (#32)
2,4,6-TriBDE (#30)
2,3′,4-TriBDE (#25)
2,2',4-TriBDE (#17)
2,4,4'-TriBDE (#28)
2',3,4-TriBDE (#33)
3,3′,4-TriBDE (#35)
3,4,4'-TriBDE (#37)

2,2',4,5'-TetraBDE (#49)
2,4,4′,6-TetraBDE (#75)
2,3′,4′,6-TetraBDE (#71)
2,2',4,4'-TetraBDE (#47)
2,3′,4,4′-TetraBDE (#66)
3,3',4,4'-TetraBDE (#77)

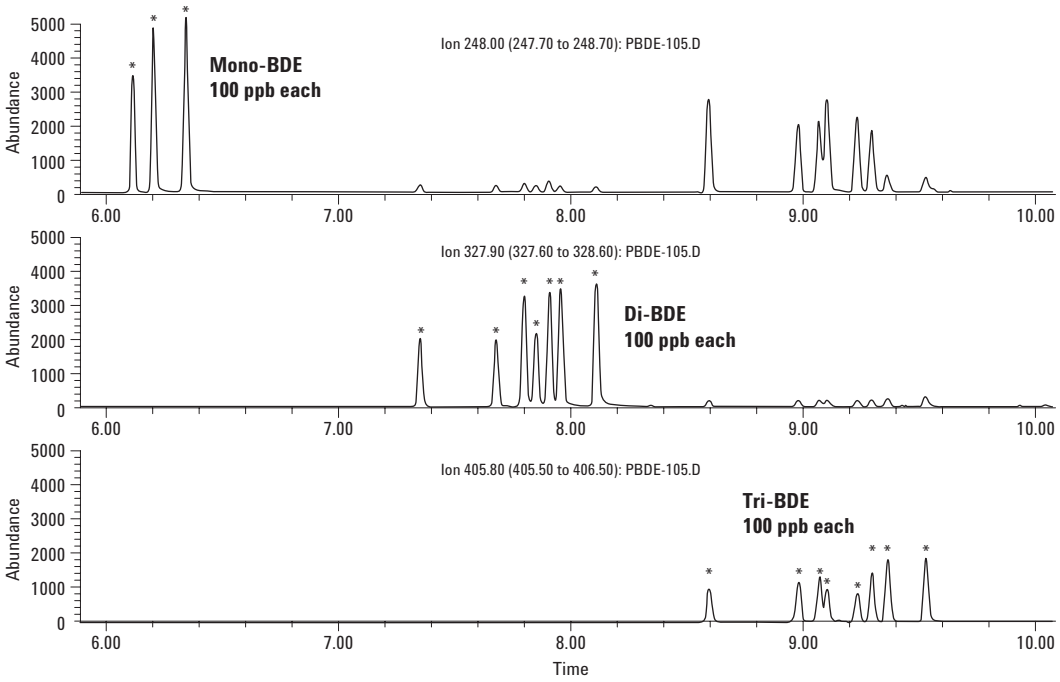
2,2',4,4′,6-PentaBDE (#100)
2,3′,4,4′,6-PentaBDE (#119)
2,2',4,4',5-PentaBDE (#99)
2,3′,4,4′,5-PentaBDE (#118)
2,3,4,5,6-PentaBDE (#116)

2,2',4,4',6,6'-HexaBDE(#155)

3,3',4,4',5-PentaBDE (#126)
2,2′,3,4,4′-PentaBDE (#85)

2,2',4,4',5,6′HexaBDE(#154)
2,2',4,4',5,5'-HexaBDE(#153)
2,2',3,4,4',5'-HexaBDE(#138)
2,3,4,4',5,6-HexaBDE (#166)

2,2',3,4,4',5',6-HeptaBDE (#183)
2,2',3,4,4',5,6-HeptaBDE(#181)
2,3,3',4,4',5,6-HeptaBDE (#190)

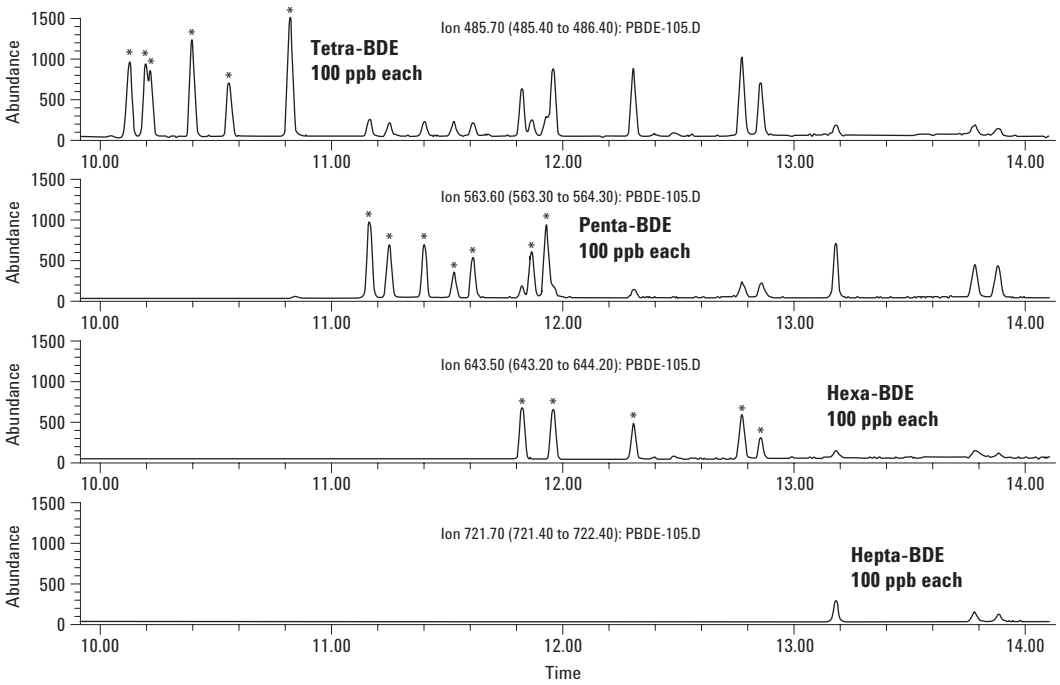


Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas:	Helium at 38 cm/s at 100 °C (1.2 mL/min), constant flow mode
Oven:	100 °C for 1 min; 100 °C to 340 °C at 20 °C/min , 340 °C 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).



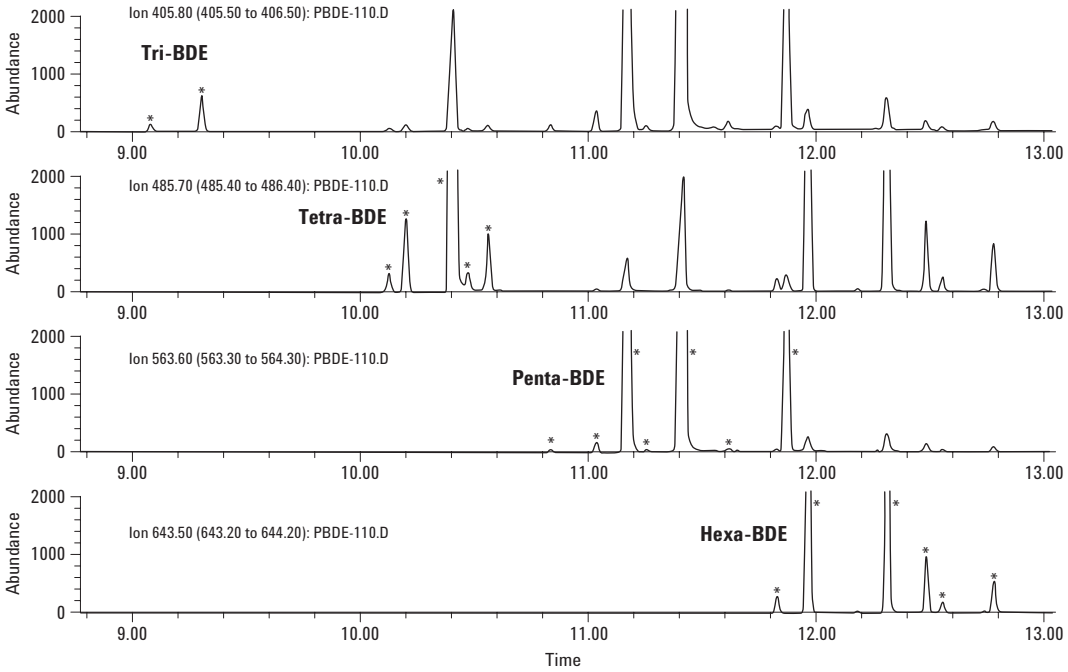
Instrument:	Agilent 6890 Gas Chromatograph with ALS and ChemStation Software
Column:	DB-XLB, 30 m × 0.25 mm id × 0.1 µm (Agilent Technologies, part number 122-1231)
Carrier gas:	Helium at 38 cm/s at 100 °C (1.2 mL/min), constant flow mode
Oven:	100 °C for 1 min; 100 °C to 340 °C at 20 °C/min, 340 °C 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).

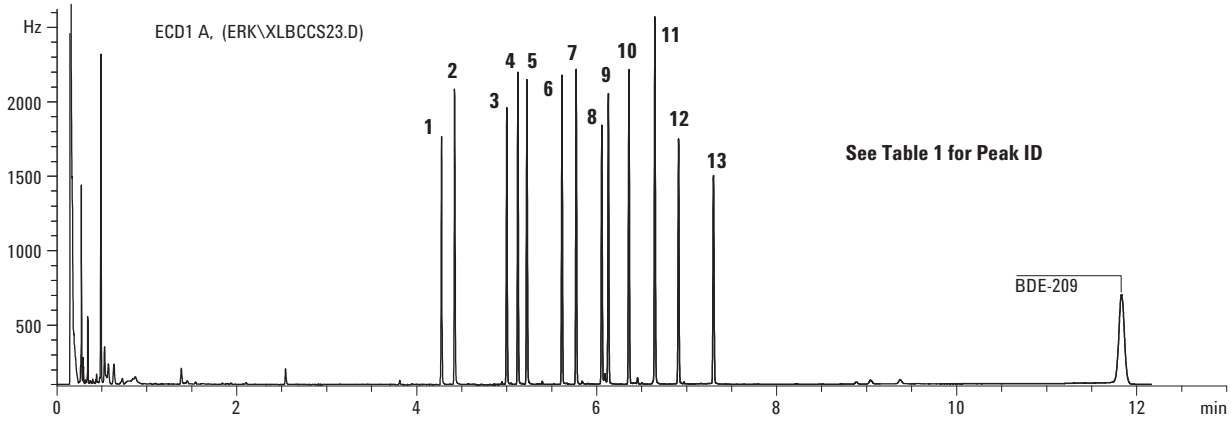
Figure 3 shows a chromatogram of a commercial flame retardant mixture. While commercial samples are typically classified as “penta”, “octa”, or “deca”, they contain other congeners as well. Again, the congeners in this mixture are well resolved, and the run time is very short (13 minutes).



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)

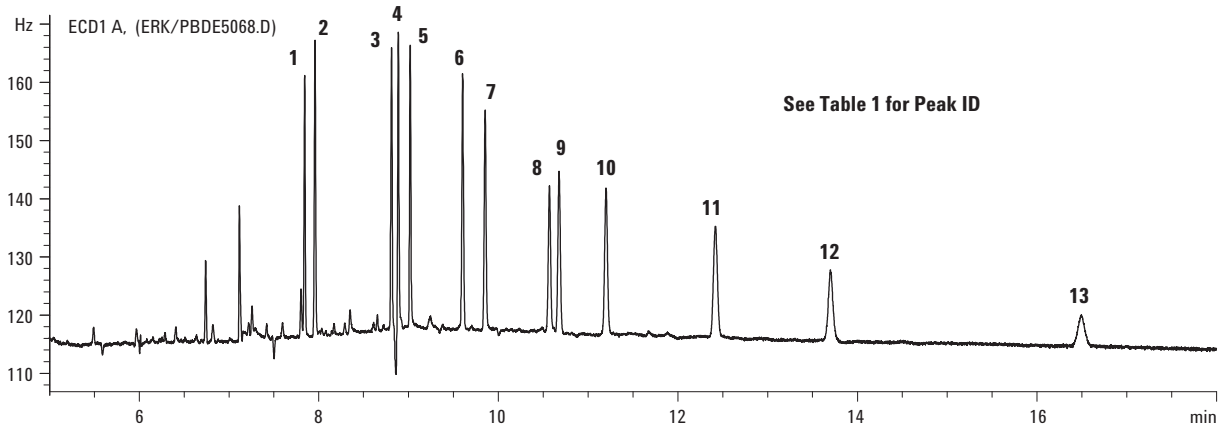
Figure 3. Commercial flame retardant penta DE71-R.

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

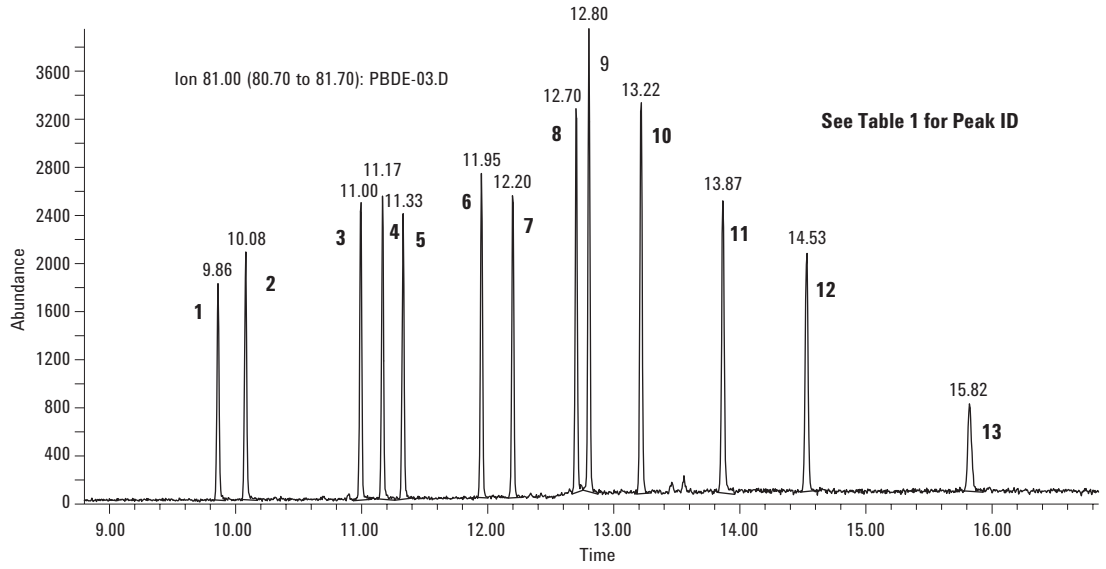
Figure 4. GC-µECD of PBDE congener mixture (EO-5103).



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

Application

Environmental

Authors

Sonia Wanner
Centre d'Analyse et de Recherche (CAR)
76 route du Rhin
F-67441 Illkirch
France

Isabelle Chanel
Agilent Technologies France
Parc Club du Moulin à Vent
33 rue du Docteur Levy
F-69693 Venissieux
France

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.

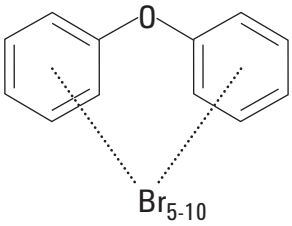

Figure 1. Structure of PBDEs.

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.

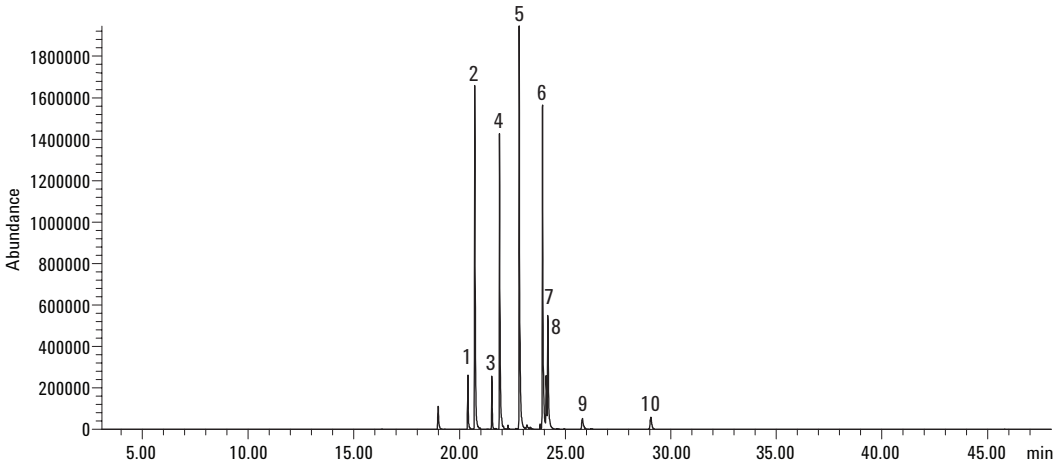

Figure 2. Total Ion Chromatogram (TIC) of a standard mixture at 2–20 ng/µL
1–2: pentaBDE
3–9: octaBDE
10: decaBDE

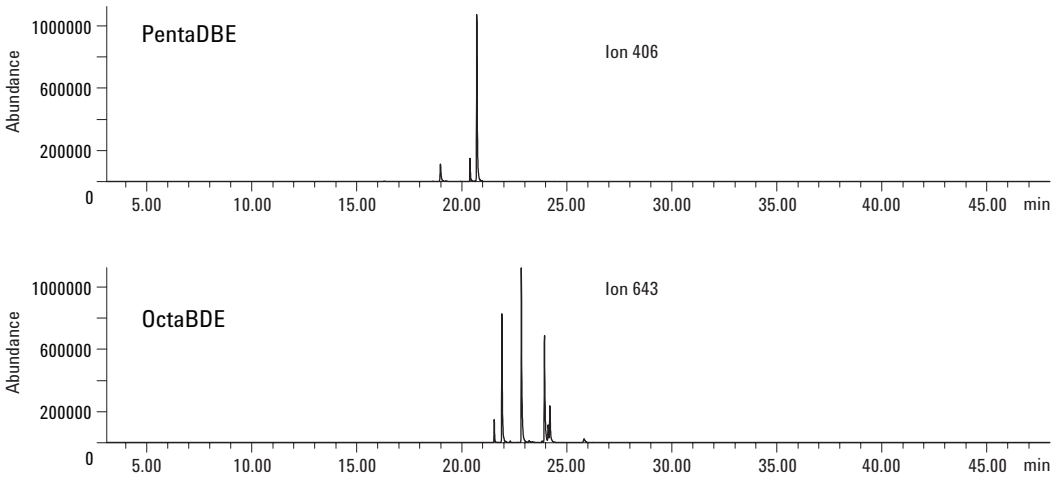

Figure 3. Selected Extracted Ion Chromatograms (EICs) of a standard mixture at 0.5–5 ng/µL.

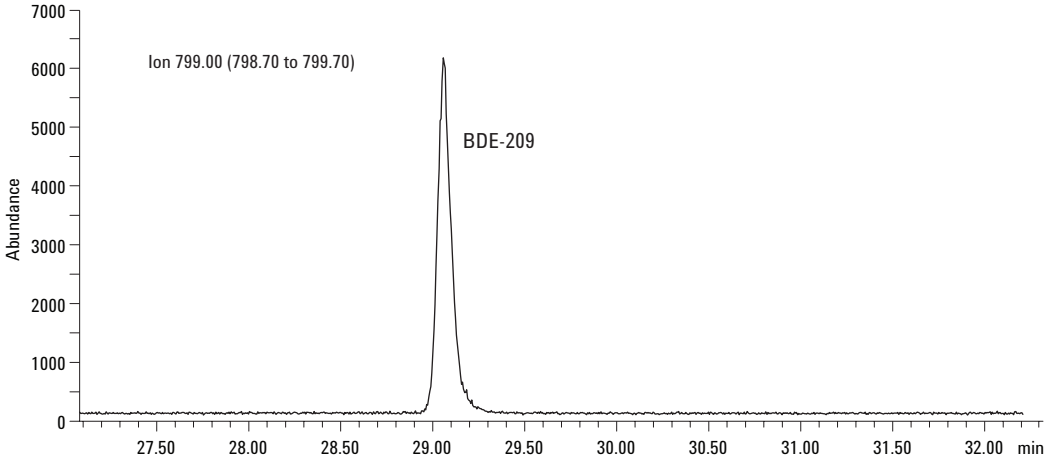

Figure 4. EIC of BDE-209 in a standard mixture at 5 ng/µL.

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

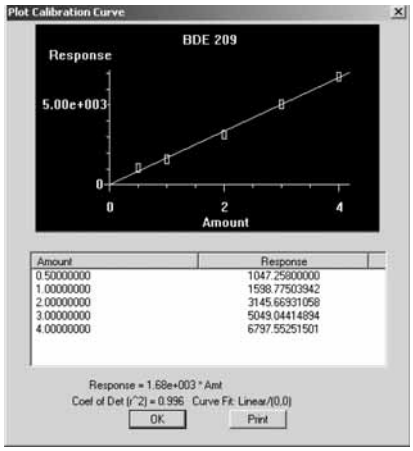

Figure 5. Calibration curve for decaBDE (BDE-209) by GC/MS.

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.

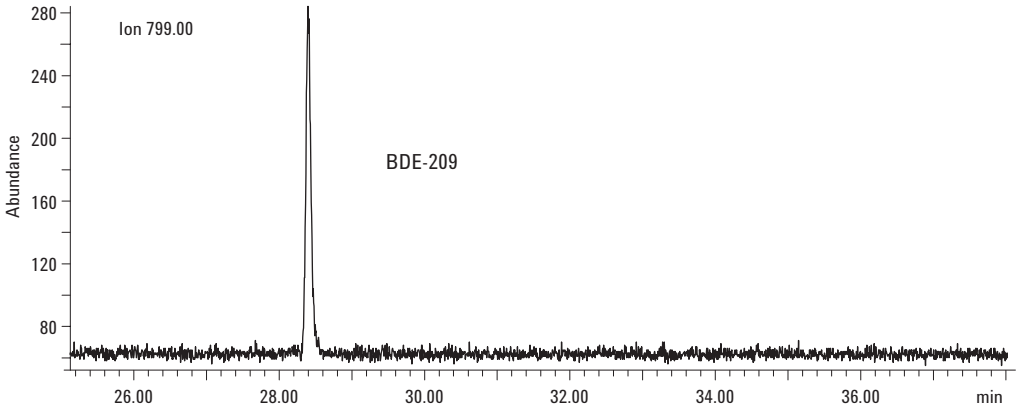

Figure 6. EIC of BDE-209 in a standard mixture at 0.5 ng/µL, which is the required LOD 50 µg/kg of sediment.

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.

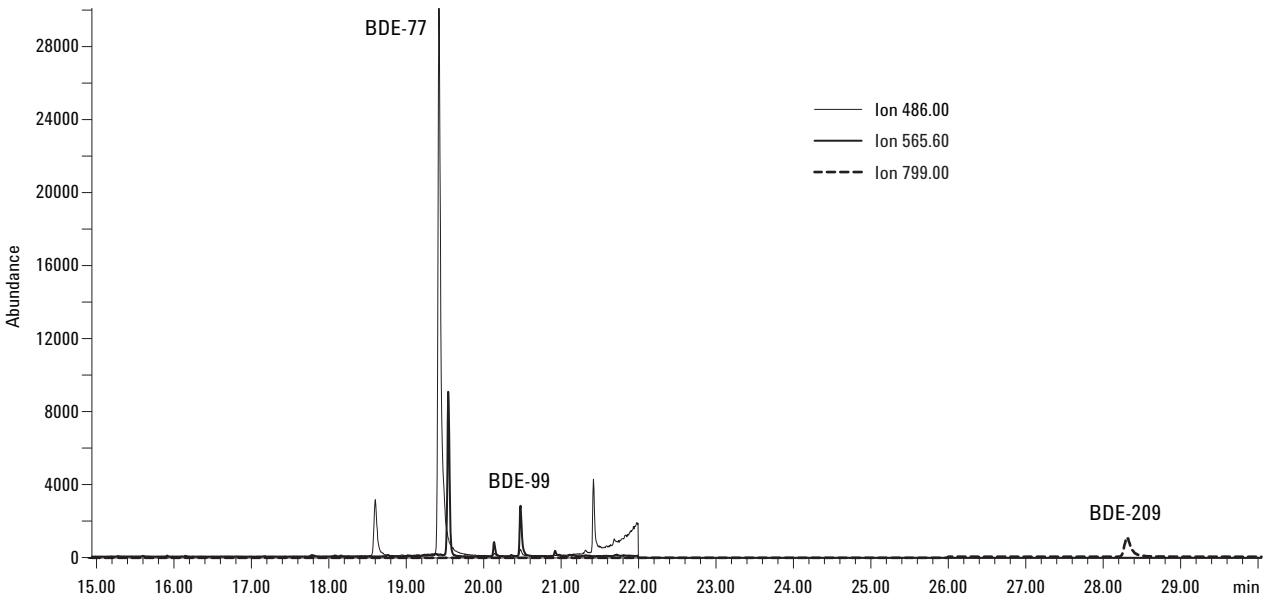

Figure 7. Overlaid EICs for BDE-77, 99, and 209 from sewage sludge.

Table 4. Quantitation Report of Real Sewage Sludge

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

Application

Environmental, Component Testing

Authors

Chuanhong Tu
Agilent Technologies, PuDong
Shanghai CHINA

Harry F. Prest
Agilent Technologies, Inc.
Santa Clara, CA
USA

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]

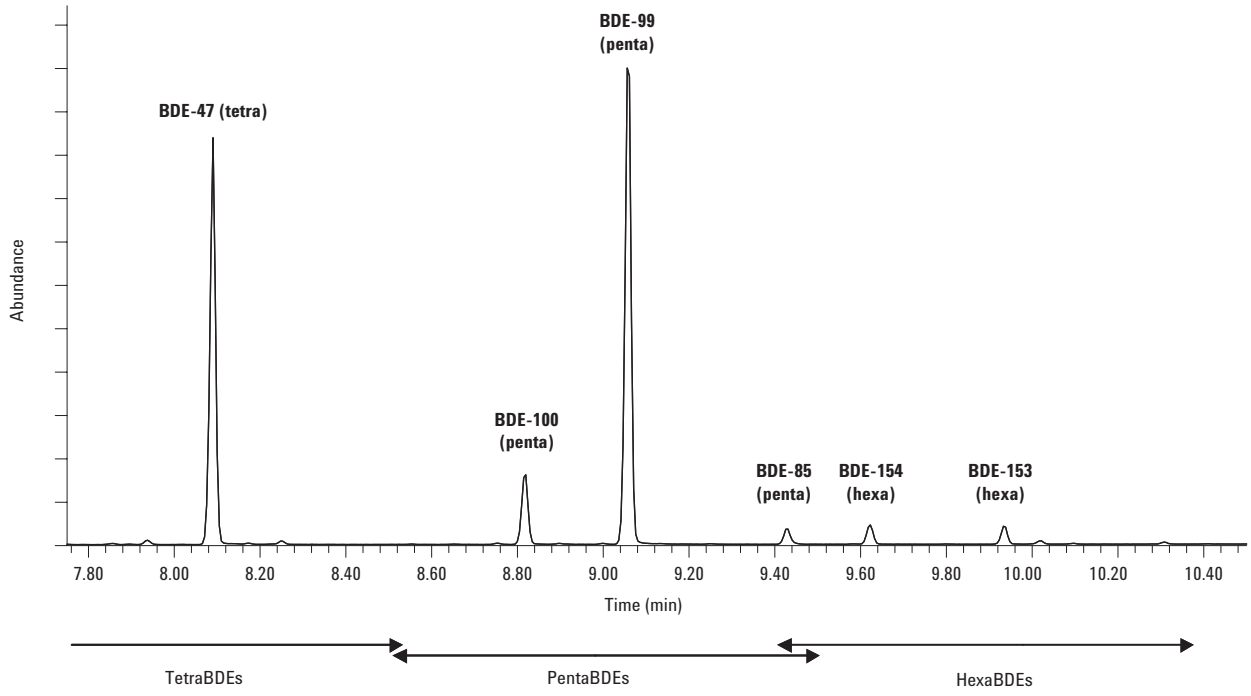

Figure 1 Reconstructed ion chromatogram (RIC) for the GC/MS EI-SIM acquisition of a PentaBDE technical mixture (Cambridge Isotope Laboratories).

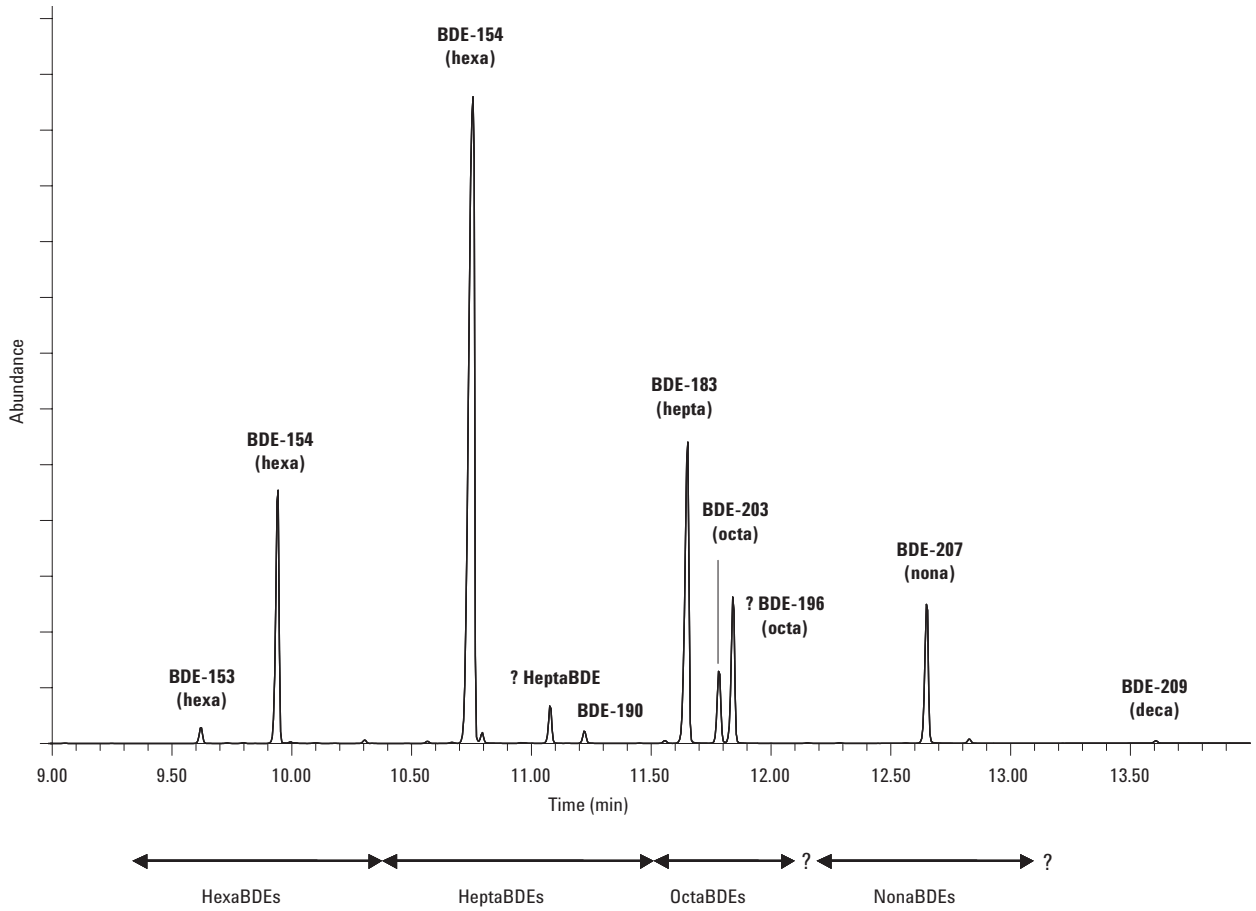

Figure 2 RIC for the GC/MS EI-SIM acquisition of a OctaBDE technical mixture (Cambridge Isotope Laboratories)

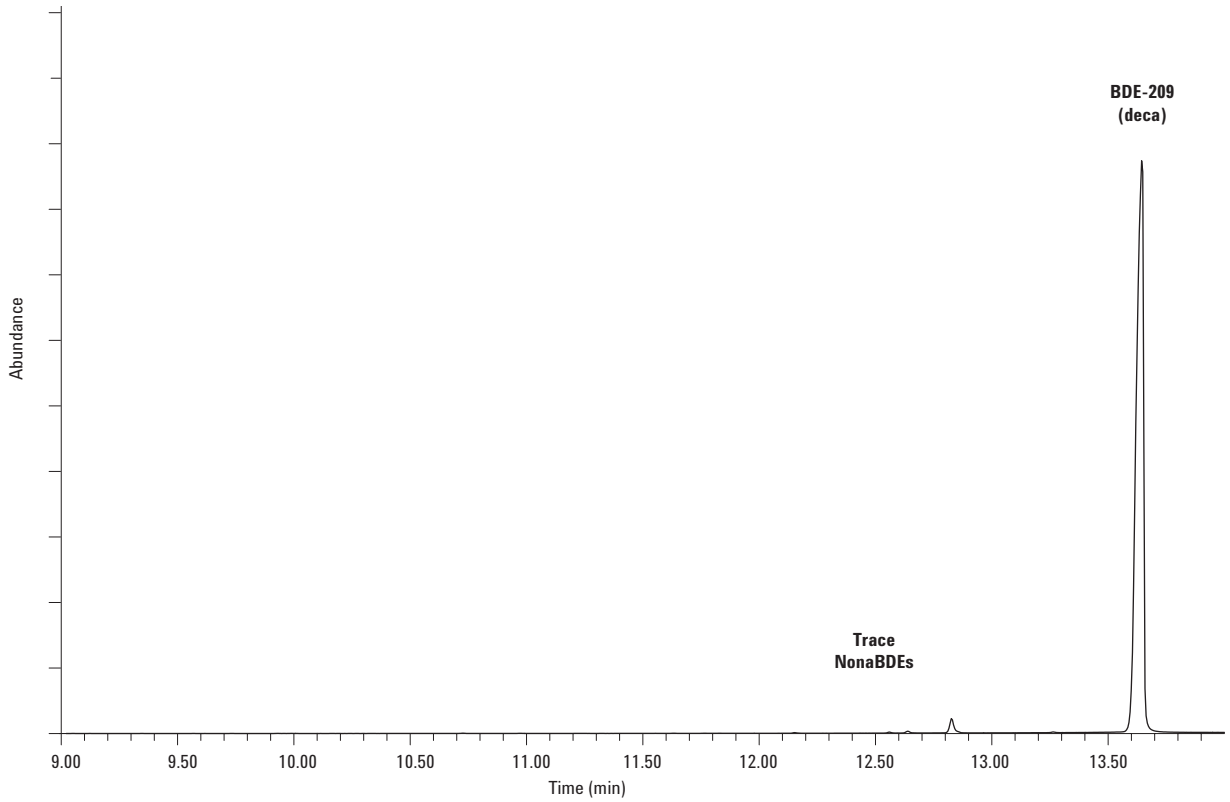

Figure 3 RIC for the GC/MS EI-SIM acquisition of a DecaBDE technical mixture (Cambridge Isotope Laboratories).

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.

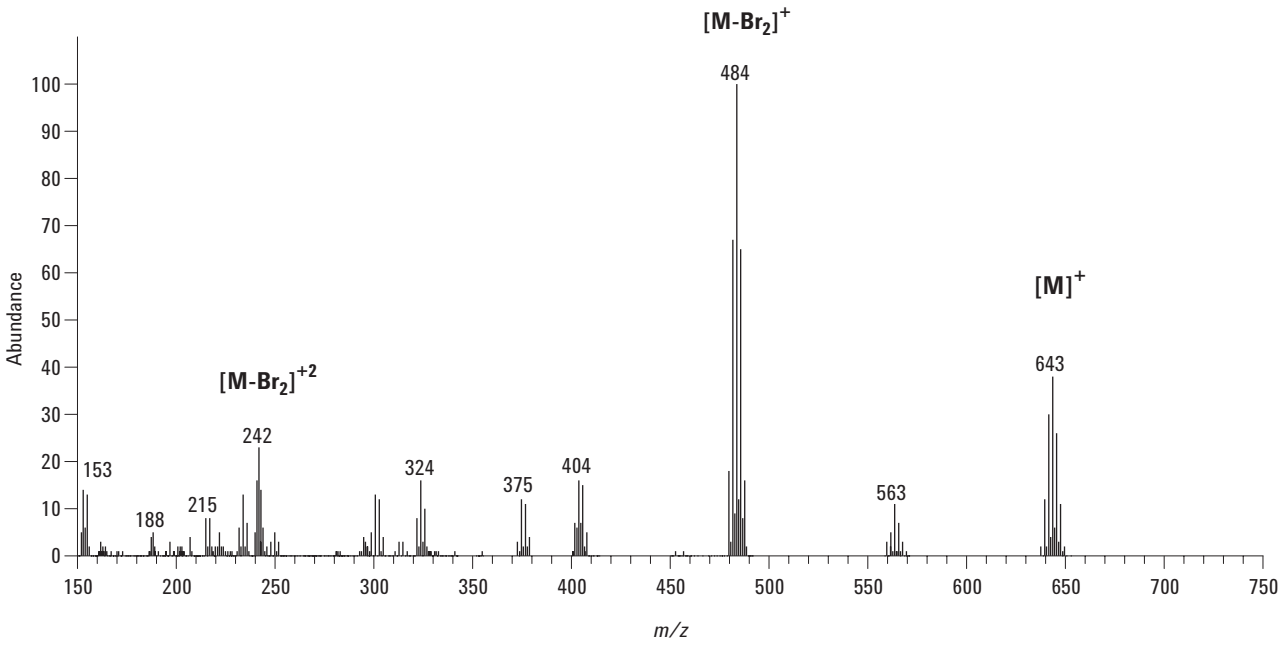

Figure 4 Normalized EI mass spectrum of a hexabrominated-DE, PBDE-138, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

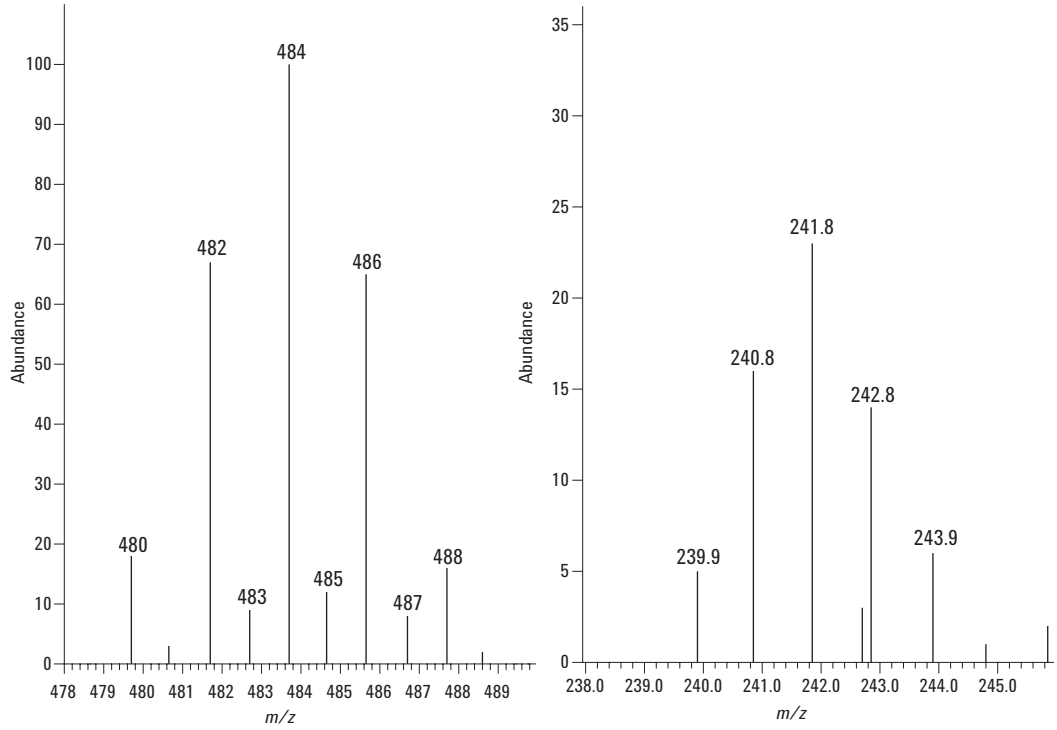

Figure 5 **Sections of the normalized EI mass spectrum of the hexabrominated-DE, PBDE-138, for the $[M\text{-}Br_2]^+$ and proposed $[M\text{-}Br_2]^{+2}$ clusters.**

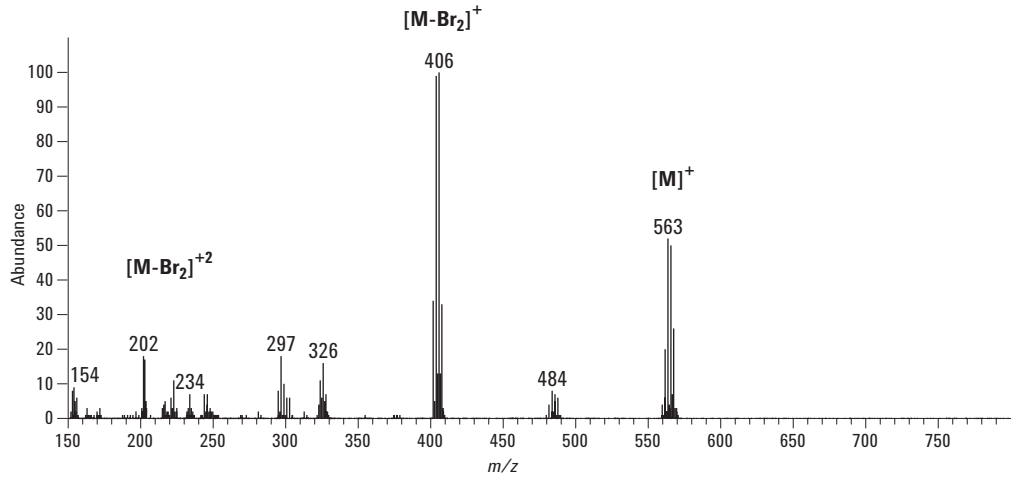

Figure 6 **Normalized EI mass spectrum of a pentabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.**

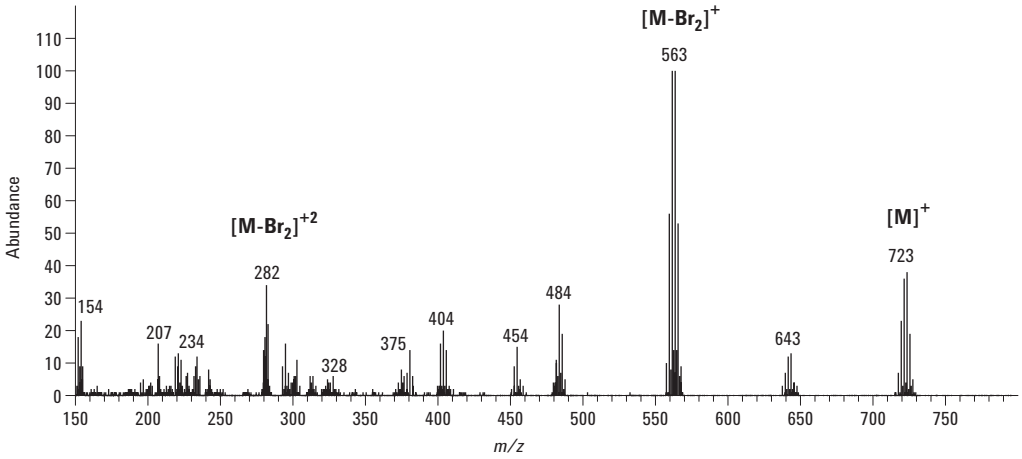

Figure 7 Normalized EI mass spectrum of a heptabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

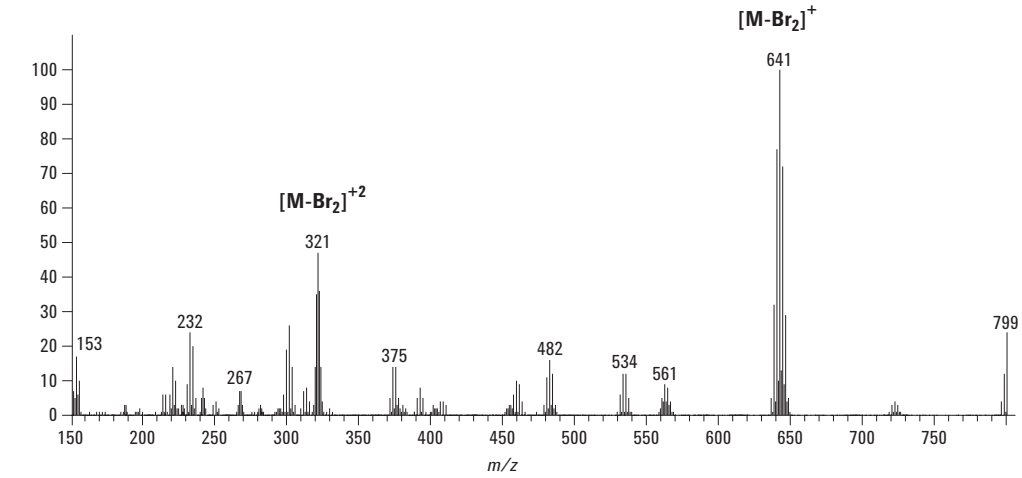

Figure 8 Normalized EI mass spectrum of a octabrominated-DE, PDBE-203, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

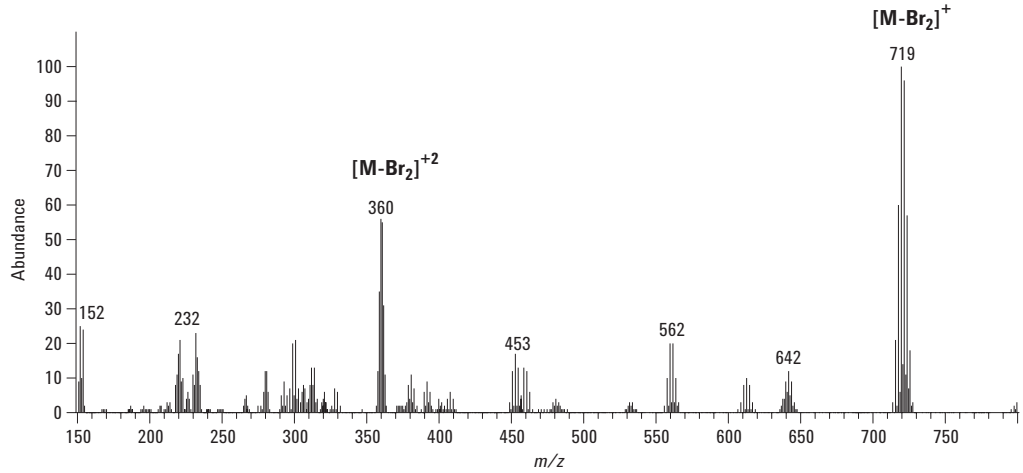

Figure 9 Normalized EI mass spectrum of a nonabrominated-DE, PDBE-208, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

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.

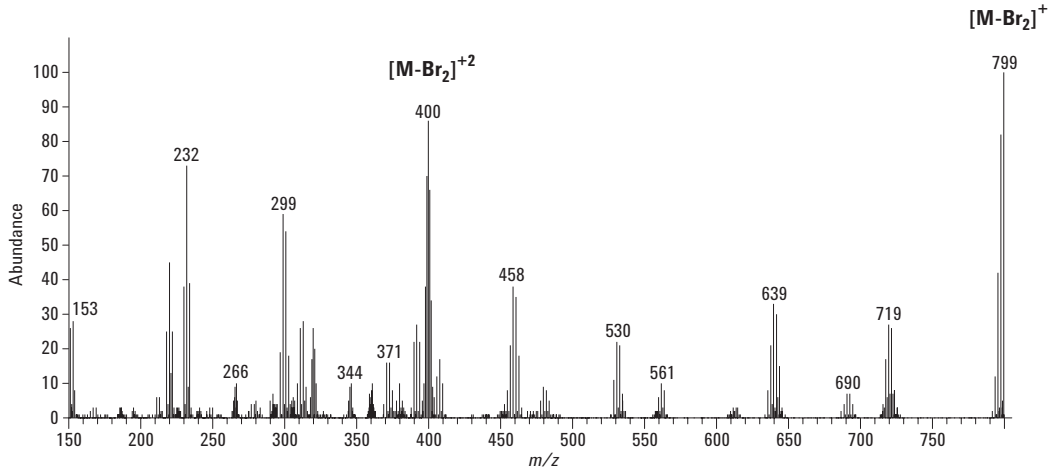

Figure 10 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

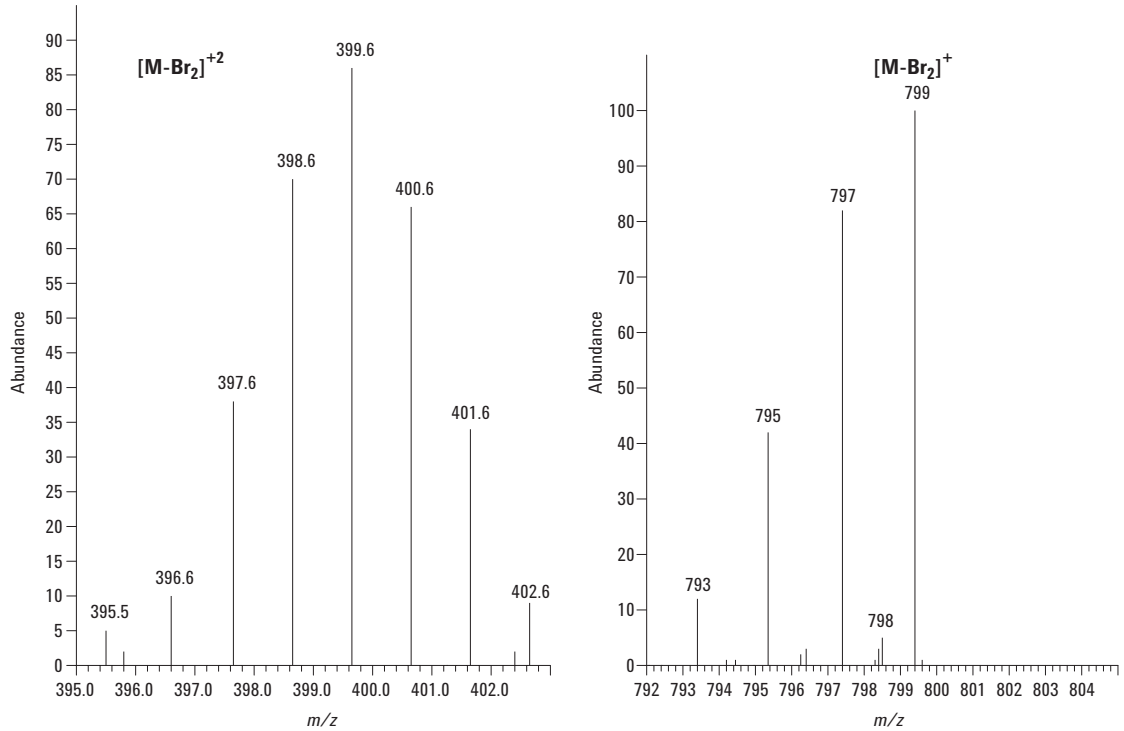

Figure 11 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

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.

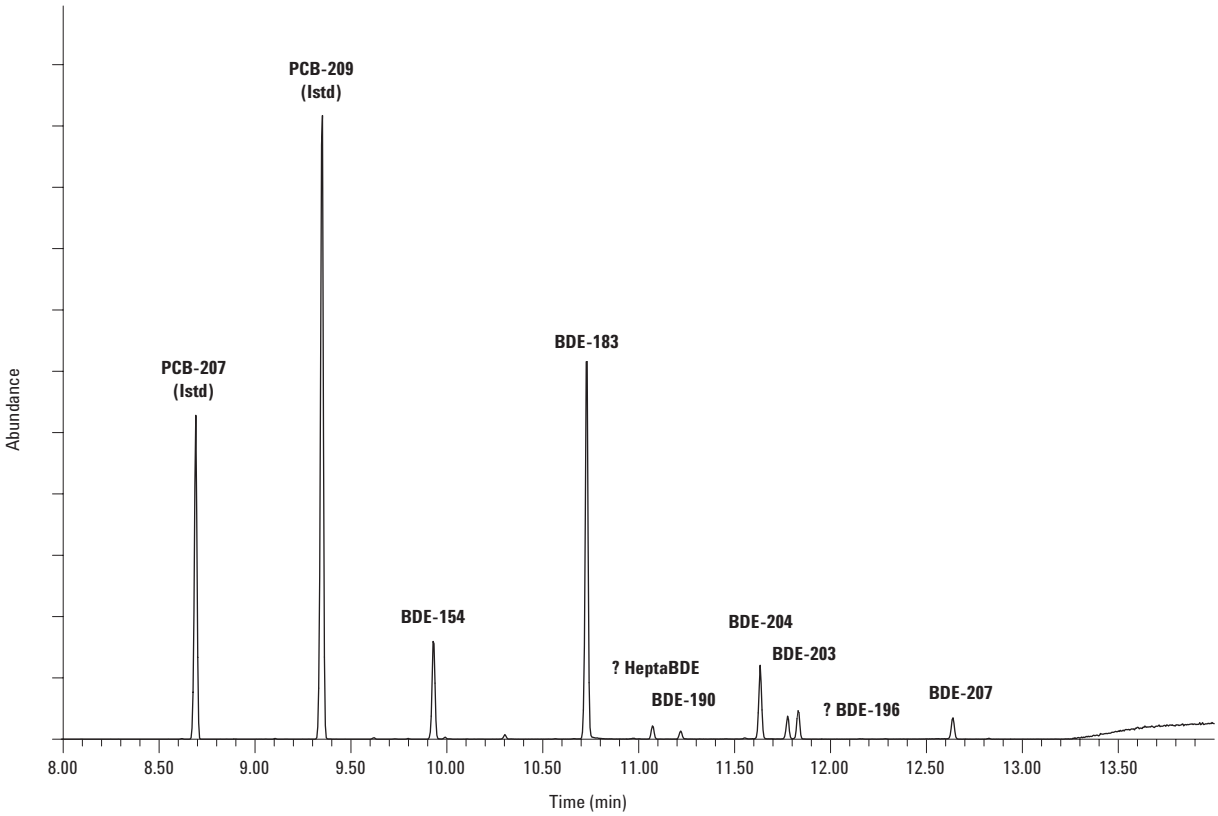

Figure 12 RIC of the GC/MS SIM acquisition of an extracted HIPS polymer sample.

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.

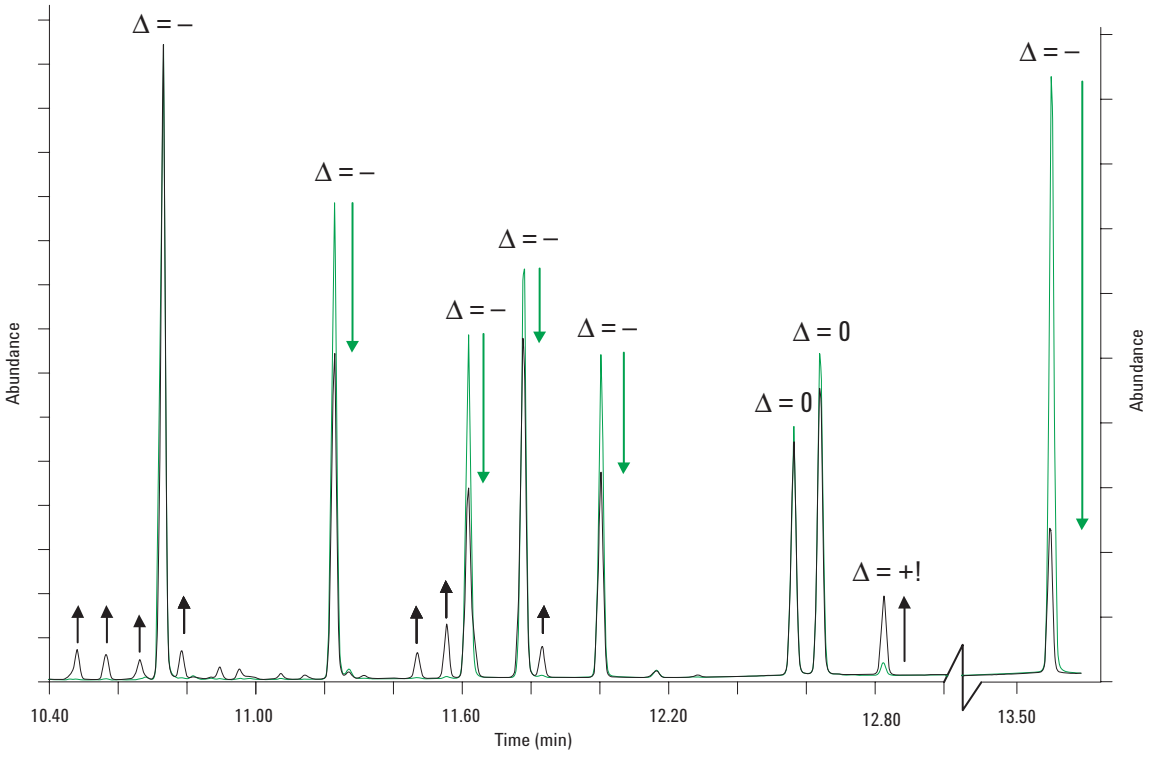

Figure 13. PBDE standard unexposed (green) and exposed to laboratory light. Delta (Δ) indicates change in response as Exposed-Unexposed (with negative signs indicating loss in response and positive an increased response).

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Agilent Technologies

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

Application

Environmental, Component Testing

Author

Harry F. Prest
Agilent Technologies, Inc.
Santa Clara, CA
USA

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.

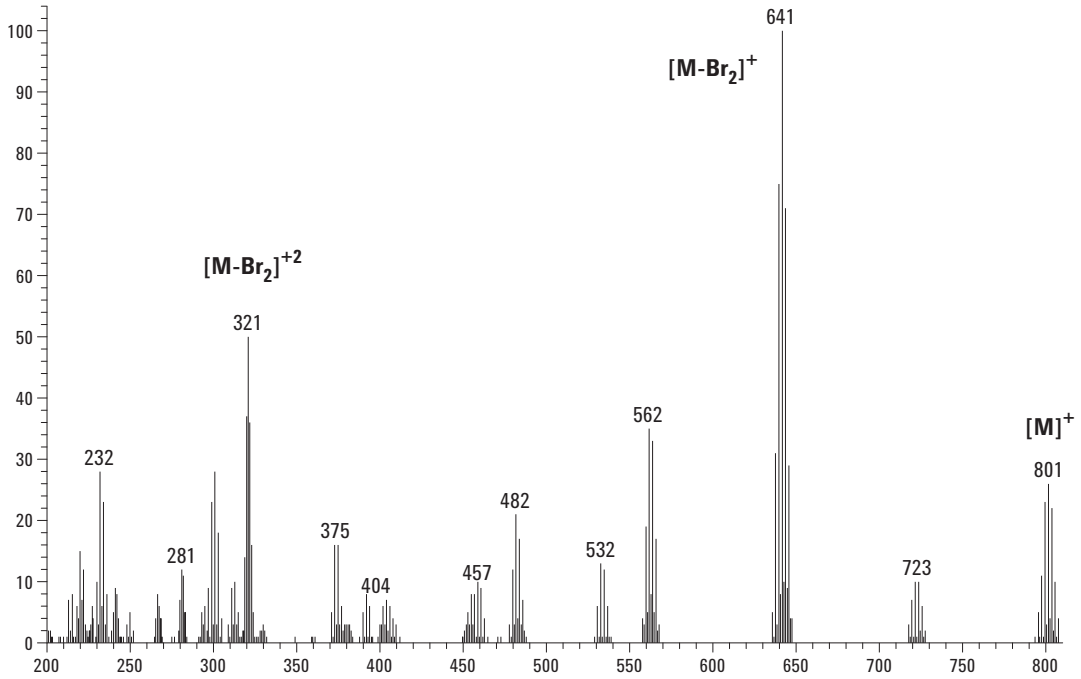

Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 *m/z*.

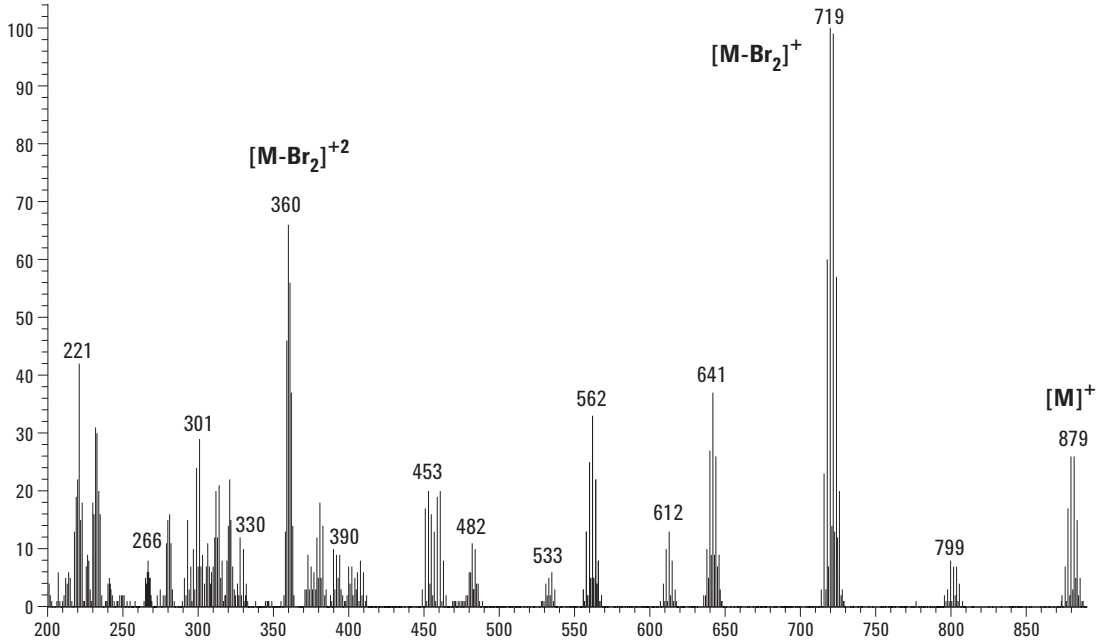

Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 *m/z*.

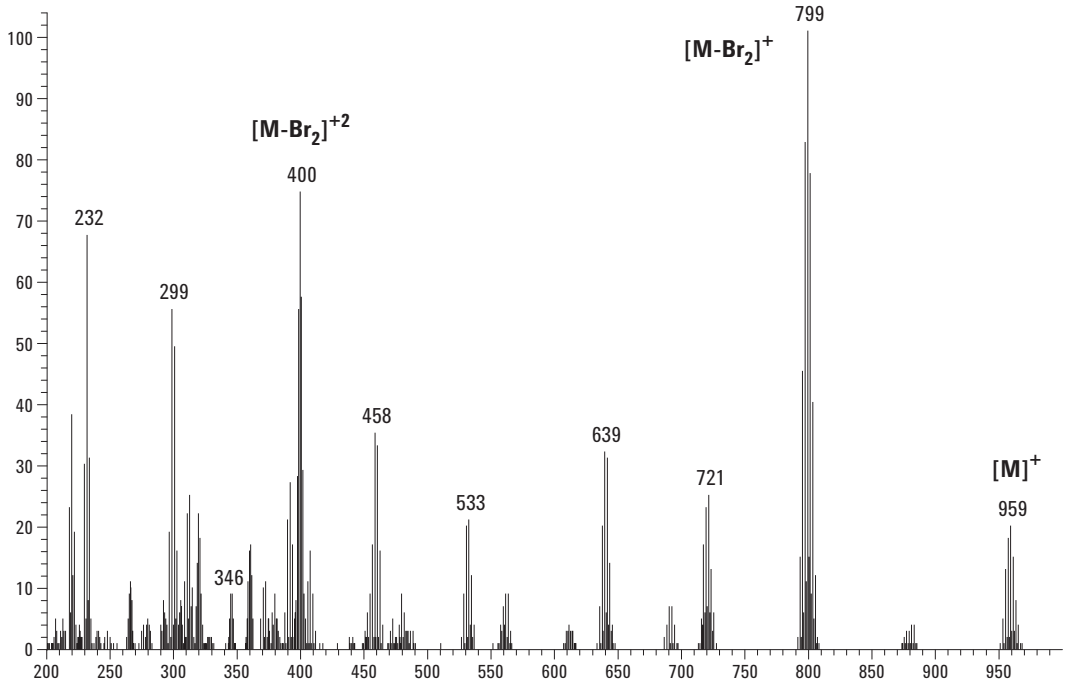

Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 *m/z*.

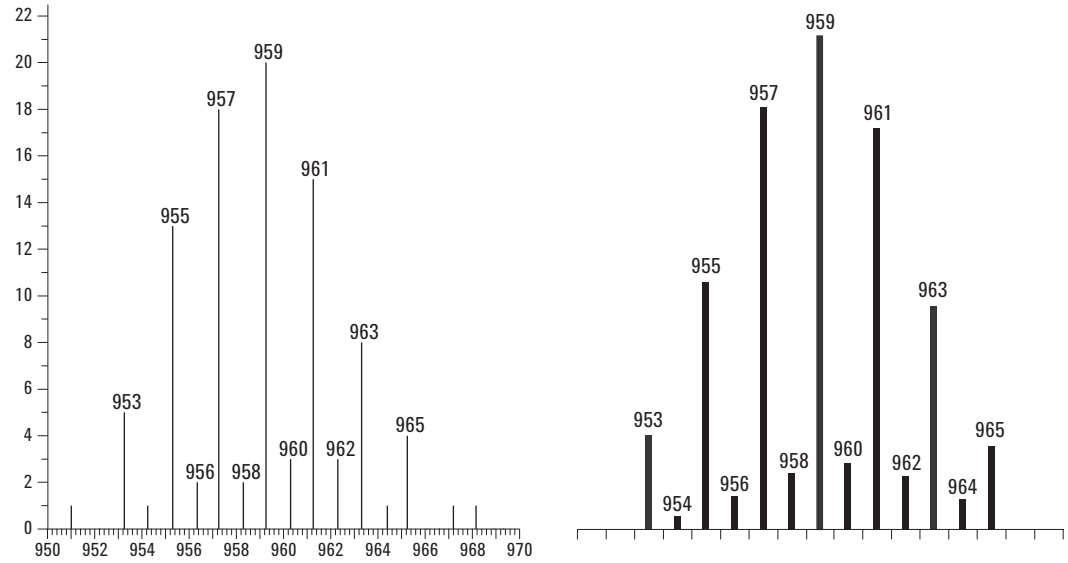

Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

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.

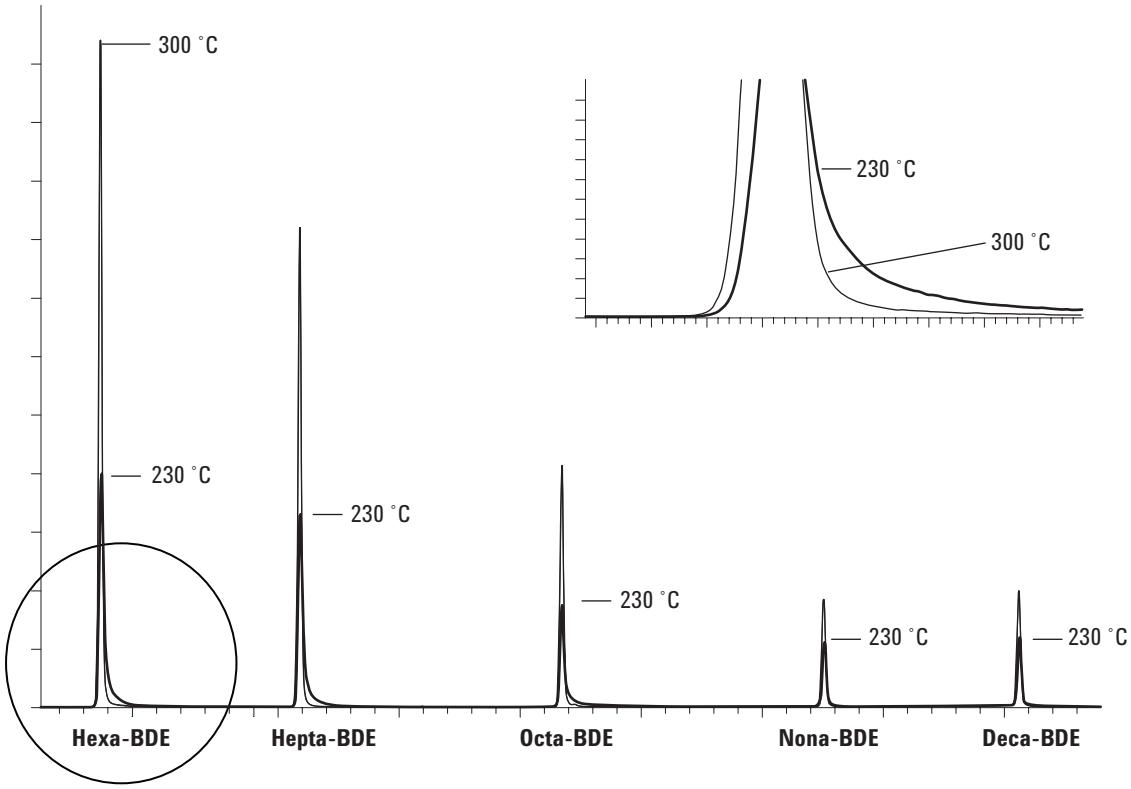

Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

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

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

Application

Environmental

Authors

Ken Lynam and Doris Smith
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

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.

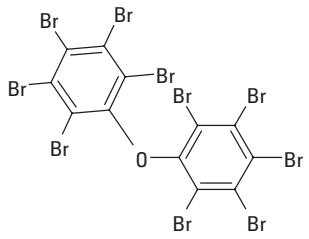
Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate column inertness and quality. These extremely active probes, including 1-propionic 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.

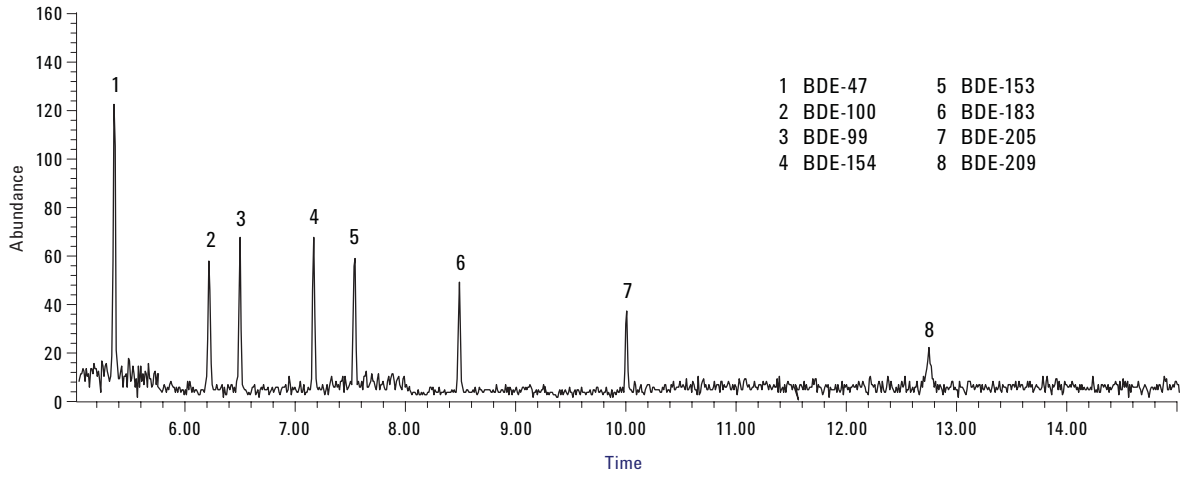


Figure 1. Total ion chromatogram (SIM mode) of a 0.005-ng (BDEs -47, -100, -99, -154, -153, -183, -205, and -209) and 0.025-ng (BDE-209) on-column loading on an Agilent J&W DB-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5512UI).

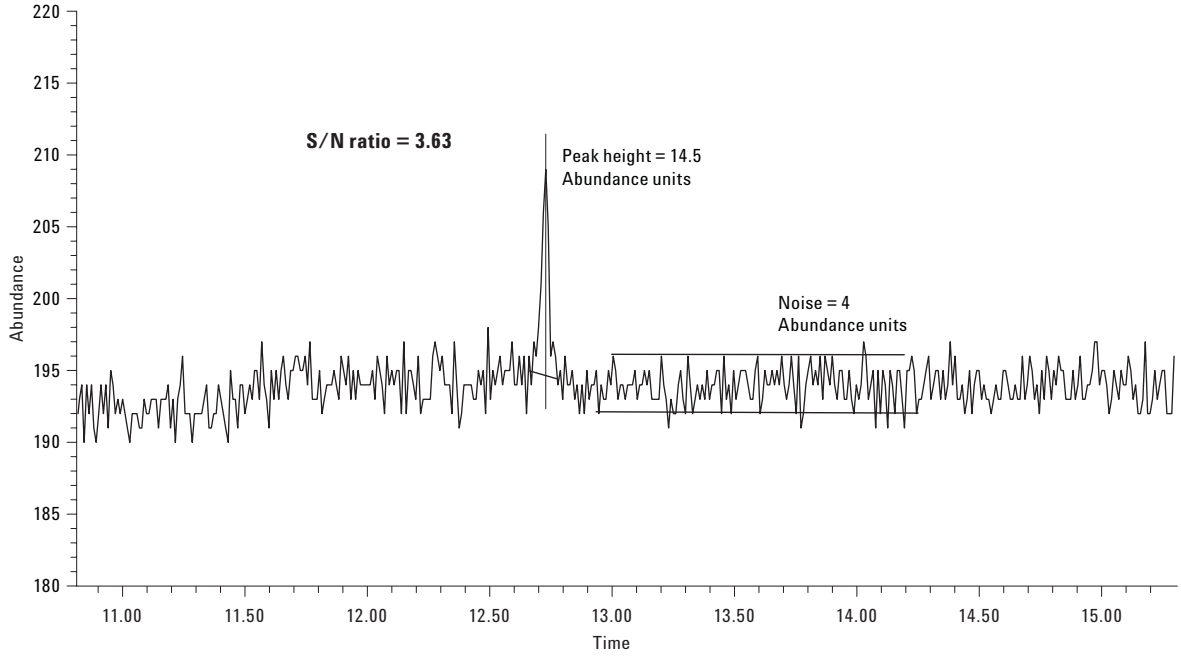


Figure 2. Enlarged section of the total ion chromatogram (SIM mode) of a 0.025-ng BDE-209 on-column loading. The large peak in the figure is BDE-209, a particularly challenging BDE due to its long retention and thermal instability.

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

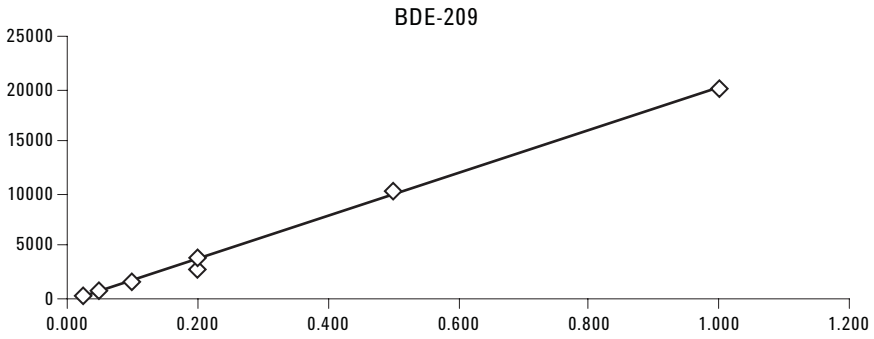


Figure 3. Correlation coefficients for the eight components over the 0.5 ng/mL to 1,000 ng/mL concentration range (BDE-209 2.5 to 1,000 ng/mL) used in this study. An example linear regression plot of particularly challenging BDE-209 is also shown.

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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Emerging Contaminants

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Analyzing Compounds of Environmental Interest Using an LC/Q-TOF Part 3: Imidacloprid and Manool

Application

Environmental

Authors

Jim Lau, Chin-Kai Meng, and Jennifer Gushue
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

L. Mark Hewitt and Suzanne Batchelor
Aquatic Ecosystem Protection Research Division
Water Science and Technology Directorate
Environment Canada
867 Lakeshore Road
Burlington, ON, L7R 4A6
Canada

Abstract

Effluent discharges from pulp mill, sewage, or pesticide run-off are released into aquatic environments as complex mixtures. This study uses two types of discharge compounds (an insecticide and a pulp mill condensate) to illustrate the LC/Q-TOF sensitivity, linear range, quantitative, and qualitative analysis functionalities.

Introduction

Environment Canada is tasked with risk assessment and the evaluation of environmental impact of a variety of compounds [1, 2]. Effluent discharges from pulp mill, sewage, or pesticide run-off are released into aquatic environments as complex mixtures. Solid-phase extraction (SPE) or gel permeation chromatography (GPC) and GC/MS are typically used to characterize substances from the discharge. LC/MS has been used to identify difficult-to-analyze polar compounds. However, the potential for LC/MS to identify unknown polar compounds has yet to be fully realized.

This study uses two types of discharge compounds to illustrate the LC/Q-TOF sensitivity, linear range, quantitative, and qualitative analysis functionalities. Imidacloprid (an insecticide) is tricky to analyze by GC/MS. It has been used in Atlantic Canada (run-off from potato fields) and there appear to be some nontargeted toxicological effects by this insecticide. Accurate mass and quantitation capability from a Q-TOF are critical for the routine analysis of target compounds.

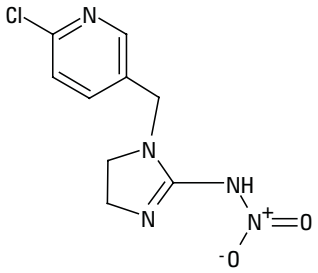
The other sample is a pulp mill condensate. Pulp mills are the largest users of fresh water in Canada. Bleached kraft pulp and paper mill final effluents are known sources of compounds that affect reproductive endocrine homeostasis in fish [3, 4]. Environment Canada is working closely with a pulp mill that has developed a reverse osmosis process to help meet its effluent regulatory requirements for toxicity. Manool, a terpenoid present in trees, is one of the compounds removed by reverse osmosis and is related to compounds suspected of causing the reproductive problems in fish. An analysis is needed to confirm its presence in the condensates treated by reverse osmosis. A Q-TOF is capable of screening and identifying impurities and degradation products.

Experimental

Samples

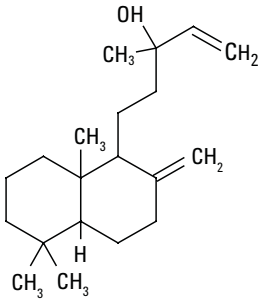
Figures 1 and 2 show the two compounds used in this study. Calibration solutions (10, 20, 50, 100, and 200 ppb) of imidacloprid were made from a 10.11 ppm stock solution in methanol. An additional sample was a blind with an undisclosed concentration of imidacloprid to evaluate the quantitation ability of the Q-TOF.

Samples of manool included a standard solution in methanol and a pulp extract treated with reverse osmosis process.



Formula: $C_9H_{10}ClN_5O_2$
CAS: 13826-41-3
MW: 255.05230

Figure 1. Imidacloprid.



Formula: $C_{20}H_{34}O$
CAS: 596-85-0
MW: 290.26095

Figure 2. Manool.

Instrument Parameters

All sample analyses were performed on an Agilent 1200 SL Rapid Resolution LC coupled to an Agilent 6520 Q-TOF.

All sample analyses were performed under Q-TOF autotune conditions. Mass accuracy, sensitivity, and resolution for all samples were measured without any changes to 6520 Q-TOF instrument parameters, except ion source conditions appropriate for the spray chamber type, LC flow, and sample thermal stability.

Mobile A	5 mM NH_4OAc, pH 4
Mobile B	MeOH
LC column	ZORBAX XDB 2.1 × 50 mm, C-18, 3.5-µm particle size
Flow rate	0.5 mL/min
Injection volume	5 µL
MS	Scanned at 2 scans/sec, 50 to 1,100 *m/z*
Positive reference ions	*m/z* 121, 922
AutoMS/MS	2 scans/sec MS and 2 scans/sec MS/MS
Q-TOF parameters	Set by autotune
Drying gas	12 L/min N_2
Drying gas temperature	300 °C (imidacloprid), 150 °C (manool)
Nebulizer pressure	50 psi
ESI (+)	3 KV
Fragmentor	120 V

Results and Discussion

Imidacloprid

Figure 3 is a "Batch-at-a-Glance" screen from the MassHunter software. All the samples analyzed are listed on the top half of the screen. The calibration curve and the corresponding quantitation ion for each sample are displayed on the bottom half of the screen. All calibration standards (10, 20, 50, 100, and 200 ppb) and samples were analyzed in triplicate during the batch to check precision and accuracy.

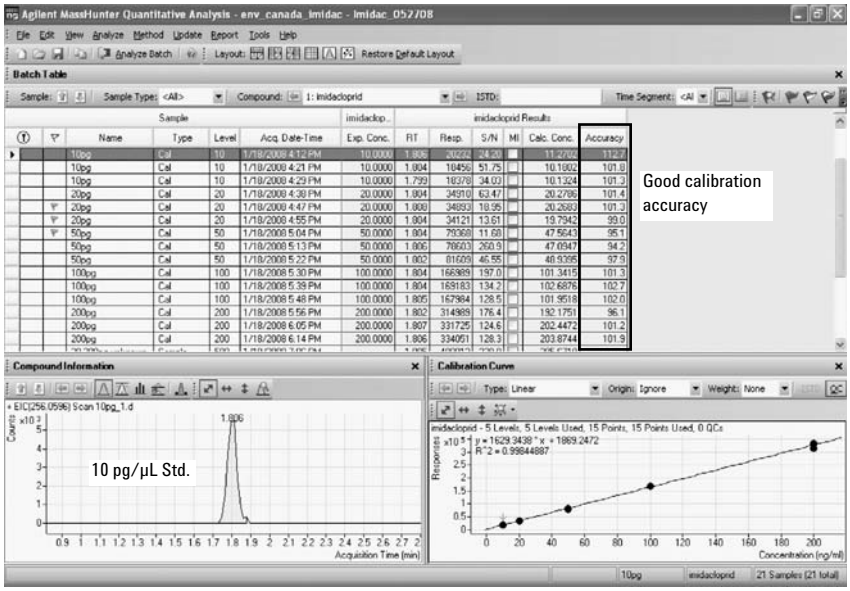


Figure 3. "Batch-at-a-Glance" screen from the Mass Hunter software showing the quantitation and calibration results of imidacloprid.

The R^2 for the calibration curve was 0.9984 and the accuracy for each standard was between 94 and 113%. The quantitation results (triplicate) of a sample with unknown concentration of imidacloprid were 305, 287, and 296 pg/µL. The results were very close to the actual amount of 303.3 pg/µL. The largest difference from the actual was less than 6% (287 versus 303.3). Table 1 shows the precision results for each calibration standard (three injections each). This demonstrates the good precision and accuracy of the LC/Q-TOF system.

Table 1. Instrument Precision for Each Calibration Standard and the Unknown Sample (three injections for each level).

Level	%RSD
10	5.5
20	1.3
50	2.0
100	0.7
200	3.2
(303.3)	3.1

Table 2 shows that the mass accuracy for the 10-ppb imidacloprid standard was 1.76 ppm and 1.03 ppm for the 200-ppb standard. This illustrates that there is no trade-off between mass accuracy and dynamic range under the same autotune settings.

Manool

A standard solution of manool in methanol was analyzed by LC/Q-TOF. Multiple peaks were observed as shown in Figure 4. Several peaks are manool-related impurities (diterpenes). It is interesting to note that MH^+ of manool was not observed in the Q-TOF spectrum (Figure 5). The most significant ion was $MH^+ - H_2O$. Other ions included $MNH_4^+ - H_2O$, MNH_4^+, and MNa^+. The thermal neutral loss ions were confirmed by MS/MS analyses. Figures 6 and 7 show the MS1 and MS/MS spectra of ion *m/z* 308 and 290, respectively. The MS/MS spectra of both ions are very similar, suggesting similar precursor ions that differ by a loss of H_20 (*m/z* 18).

Table 2. Mass Accuracy of Measuring Different Concentrations of Imidacloprid ($C_9H_{10}ClN_5O_2$)

	Exact mass	Calculated MH^+	Measured MH^+	Accuracy
10 ppb	255.052299	256.05958	256.06003	1.76 ppm
200 ppb	255.052299	256.05958	256.05984	1.03 ppm

A pulp mill condensate extract was also analyzed by LC/Q-TOF. Figure 8 is the overlay of the TIC and the largest 15 compounds (within *m/z* 200 to 600) found by molecular feature extractor. The major compounds were identified as:

- $C_{20}H_{30}O_4$ (dihexyl phthalate)
- $C_{20}H_{34}O$ (Manool)
- $C_{22}H_{34}O_4$ (diheptyl phthalate)
- $C_{24}H_{38}O_4$ (dioctyl phthalate)

There were also unknowns with molecular formulae $C_{20}H_{30}O$, $C_{20}H_{30}O_3$, and $C_{20}H_{34}O_2$ that are C_{20} suspected diterpene analogues of manool. These data show that the reverse osmosis system removes these natural products from the condensates.

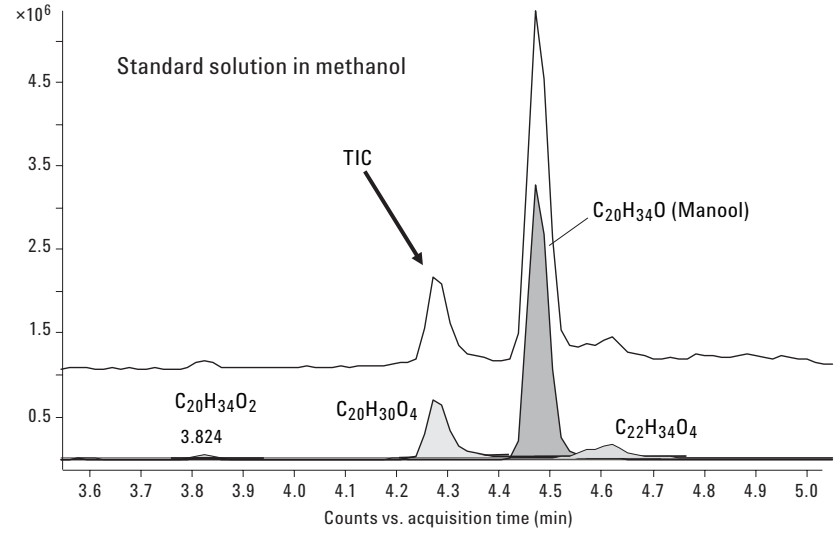


Figure 4. Characterization of manool and impurities.

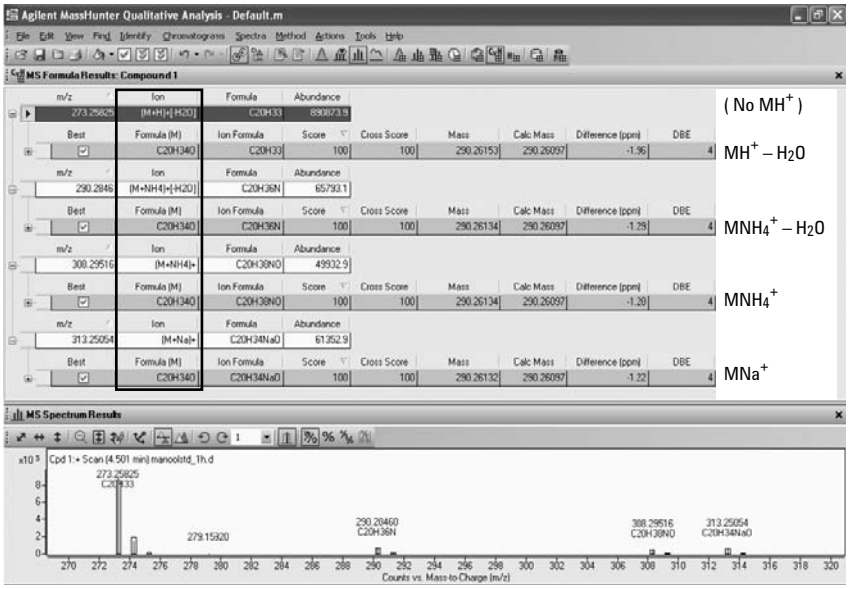


Figure 5. Characteristic ions of manool. This spectrum shows all of the different possibilities (for example, loss of water, adducts).

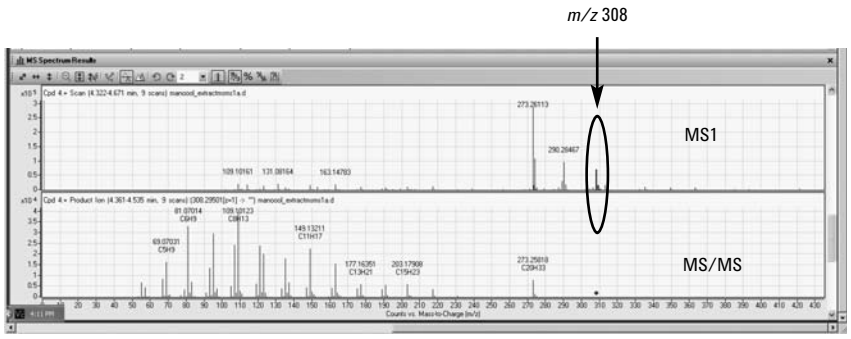


Figure 6. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for *m/z* 308 (manool + NH_4)$^+$.

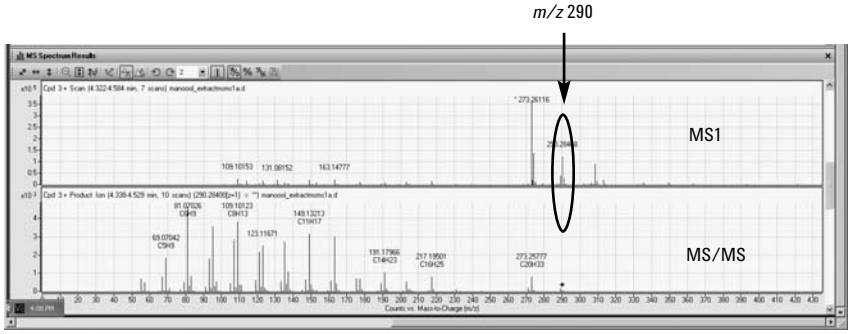


Figure 7. MS/MS of pulp sample confirming origin of thermal neutral loss ions in MS spectrum for *m/z* 290 (manool -H_2O + NH_4)$^+$.

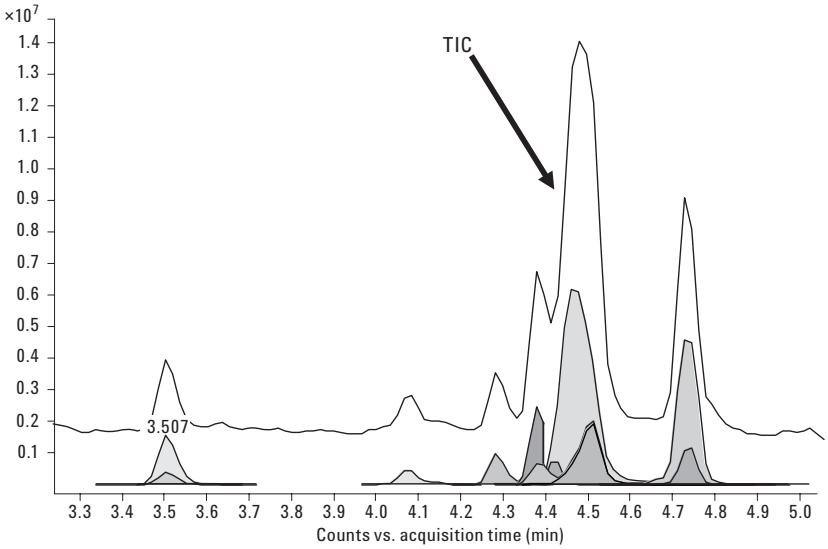


Figure 8. Molecular feature extractor extraction of the 15 largest *m/z* 200 to 600 compounds in a pulp extract.

Conclusions

Excellent mass accuracy (< 2 ppm), instrument precision (%RSD < 6%), and quantitation results (quant accuracy < 6%) were obtained from the imidacloprid analysis.

Many manool-related compounds (terpenes) were identified by Q-TOF using formula search or molecular feature extractor followed by exact mass database search. The lower sprayer temperature and adding NH_4OAc in mobile phase were critical to get molecular ions and MS/MS ions.

References

1. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Mehran Alaee, and Vimal Balakrishnan, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 1: Dyes and Pigments," Agilent Technologies publication 5989-9336EN, August 2008.
2. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Robert J. Letcher, and Shaogang Chu, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 2: Fluorotelomer Unsaturated Acids," Agilent Technologies publication 5989-9132EN, August 2008.
3. L. Mark Hewitt et al., "Isolation of Compounds from Bleached Kraft Mill Recovery Condensates Associated with Reduced Levels of Testosterone in Mummichog (*Fundulus heteroclitus*)," *Environmental Toxicology and Chemistry*, Vol. 21, No. 7, pp. 1359–1367, 2002.
4. L. Mark Hewitt et al., "Altered Reproduction in Fish Exposed to Pulp and Paper Mill Effluents: Roles of Individual Compounds and Mill Operating Conditions," *Environmental Toxicology and Chemistry*, Vol. 27, No. 3, pp. 682–697, 2008.

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Analyzing Compounds of Environmental Interest Using an LC/Q-TOF Part 1: Dyes and Pigments

Application

Environmental

Authors

Jim Lau, Chin-Kai Meng, and Jennifer Gushue
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Vimal Balakrishnan and Mehran Alaee
Environment Canada
Aquatic Ecosystem Protection Research Division
867 Lakeshore Road
Burlington, ON, L7R 4A6
Canada

Abstract

Dyes/pigments are produced worldwide and it is estimated that 10,000 tons are produced each year. About 10 percent of this is released into the environment in some form (such as the original compounds and degradants). Dyes and pigments have been identified as priority substances on the Chemical Management List by Environment Canada. There are many dyes/pigments in existence in the environment. An LC/Q-TOF is best suited to screen and identify many compounds in a single analysis.

Good mass accuracy (< 3 ppm) and MS/MS on an LC/Q-TOF provide powerful capability and high confidence to confirm ion identity and structure, useful for QA or unknown confirmation.

Introduction

In the past, environmental applications were carried out with GC, GC/MS, and other types of instruments. LC/MS did not have the sensitivity nor the robustness required for this field. However, in recent years, LC/MS technology has improved significantly and is now routinely used for environmental applications to monitor a list of hazardous compounds, for example, using LC/QQQ for screening and quantification of target compounds.

With the advent of new LC/MS techniques and improved performance, more compounds are being identified (new and emerging compounds). The compounds that are of interest for environmental analysis can be characterized by three categories:

1. Known knowns: the targets are known (for example, QQQ targeted analysis)
2. Known unknowns: it is known that compounds of interest are in a sample, but it is not clear what the compounds are (for example, metabolites, degradation products, or characteristic patterns/losses)
3. Unknown unknowns: it is not known what the compounds are and it is not know if they are present

A QQQ can be used to screen for category 1. However, a Q-TOF will be required for categories 2 and 3, where the compound must be identified. The routine accurate mass measurement allows a Q-TOF to find compounds via exact mass database search. The compounds found can be further confirmed by MS/MS on the Q-TOF.

Environment Canada is tasked with risk assessment and the evaluation of environmental impact of a variety of compounds [1, 2]. Dyes and pigments have been identified as priority substances on the Chemical Management List. Dyes/pigments are produced worldwide and it is estimated that 10,000 tons are produced each year. About 10 percent of this is released into the environment in some form (such as the original compounds and degradants). There are many dyes/pigments in existence in the environment; however, there is a limited number of standards available for analytical work. Standards can be produced, but the compound of interest must first be identified and characterized.

This study is to demonstrate the mass accuracy and Q-TOF's capability to generate useful formulas from accurate masses. In addition, MS/MS combined with accurate mass can be used to confirm ion identity and structure.

Experimental

Samples

The two classes of dyes/pigments studied for the application were azo dyes and anthracenediones.

The compounds that were evaluated were: Acid Blue 80, Acid Blue 129, Sudan Green 3, Toluidine Red, and Sudan III. These are the primary targets to be characterized; however, more sample categories will be added later for evaluation. The immediate concern is screening, which requires high mass accuracy for confirmation; the secondary concern is degradation products.

Instrument Parameters

All sample analyses were performed on an Agilent 1200 SL Rapid Resolution LC coupled to an Agilent 6520 Q-TOF.

All sample analyses were performed under Q-TOF autotune conditions. Mass accuracy, sensitivity, and resolution for all samples were measured without any changes to 6520 Q-TOF instrument parameters, except ion source conditions appropriate for the spray chamber type, LC flow, and sample thermal stability.

Mobile A	5 mM NH_4OAc, pH 4
Mobile B	MeOH
LC column	ZORBAX XDB 2.1 x 50 mm, C-18, 3.5 µm particle size
Flow rate	0.5 mL/min
MS	Scanned at 2 scans/sec, 50–1100 *m/z*
Positive ref. ions	*m/z* 121, 922
Negative ref. ions	*m/z* 113, 1034
AutoMS/MS	2 scans/sec MS and 2 scans/sec MS/MS
Q-TOF parameters	Set by autotune
Drying gas	13 L/min N_2 at 300 °C
Nebulizer pressure	50 psi
ESI (+)	3 KV
Fragmentor	140 V

Results and Discussion

One of the many ways to find compounds in a Q-TOF data file is by entering compound formulas to search. The formulas can be entered individually or as a group in a database in comma-separated value (CSV) format (see Figure 1). The database entry can include compound name, formula, and exact mass. Compound retention time is optional, but is very useful for getting results you can have confidence in. Figure 2 shows the spectra of the five matches using a mass tolerance of ± 5 ppm. The protonated ion is automatically labeled with the corresponding formula. Table 1 shows the mass accuracy (found by formula) of all five target compounds used in this study. All MH^+ and MNa^+ ions are within 3 ppm accuracy.

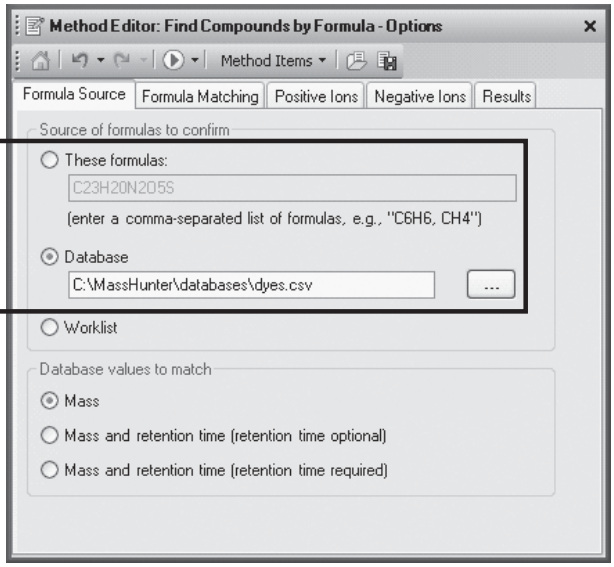

Figure 1. Find compounds in a Q-TOF data file by searching a manually entered formula or a formula database.

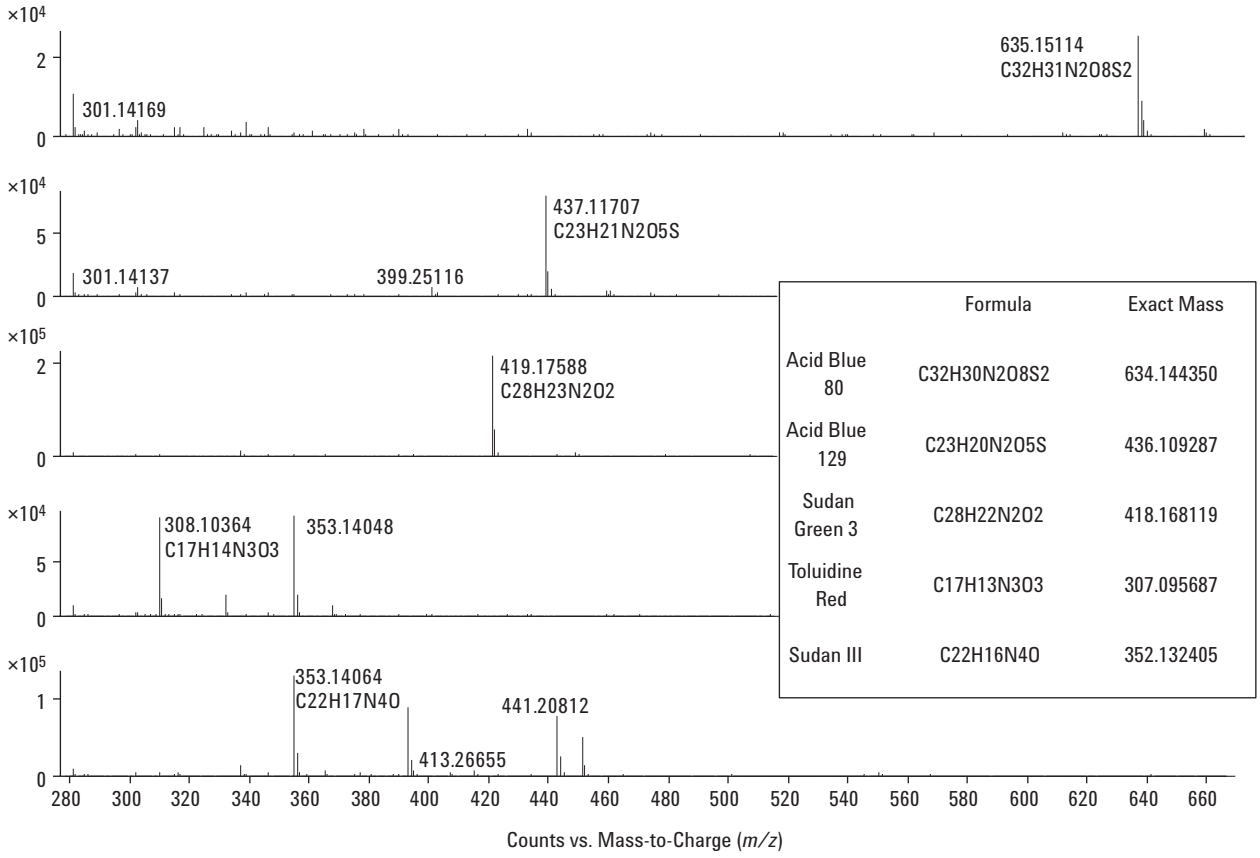

Figure 2. The five dye compounds found by formula database search.

Table 1. The Mass Accuracy of the Five Compounds Found by Formula Match

	Formula	Exact Mass	Conc. (in MeOH)	MH^+	MNa^+
Acid Blue 80	$C_{32}H_{30}N_2O_8S_2$	634.144350	10 mg/L	0.33 ppm	0.40 ppm
Acid Blue 129	$C_{23}H_{20}N_2O_5S$	436.109287	10 mg/L	–1.13 ppm	–0.73 ppm
Sudan Green 3	$C_{28}H_{22}N_2O_2$	418.168119	10 mg/L	–1.65 ppm	–0.97 ppm
Toluidine Red	$C_{17}H_{13}N_3O_3$	307.095687	10 mg/L	–2.39 ppm	–1.69 ppm
Sudan III	$C_{22}H_{16}N_4O$	352.132405	10 mg/L	–2.89 ppm	–1.28 ppm

Another way to find compounds in a data file is by using Molecular Feature Extractor (MFE). This software program looks at ion characteristics and pulls out compounds from the total ion chromatogram (TIC). Figure 3 is an overlay of TIC and the 18 compounds (signal > 100 counts) found by MFE. To confirm the identity of each found compound, all compounds are searched against an exact mass database. Figure 4 shows the database search results where five out of 18 compounds had a match. Figure 5 is a screen capture of the Mass Hunter Software showing the five compounds identified from the exact mass database search. Two of the five compounds (both identified as Sudan III) at different retention times showed very similar spectra. By clicking the hot link built into the Mass Hunter software, a compound formula can be searched against several online databases for further confirmation. Figure 6 is the online ChemID database search results of Sudan III that showed two positional isomers that would likely give similar MS/MS information and four isomers that differ by more than position and would give dissimilar MS/MS information. Figure 7 shows the spectra from data-dependent MS/MS (auto MS/MS) that give automatic generation of MS/MS product ion formulas and loss formulas correlated with precursor ion formulas. The results show identical fragmentation of the two peaks, confirming that the two compounds were isomers of Sudan III.

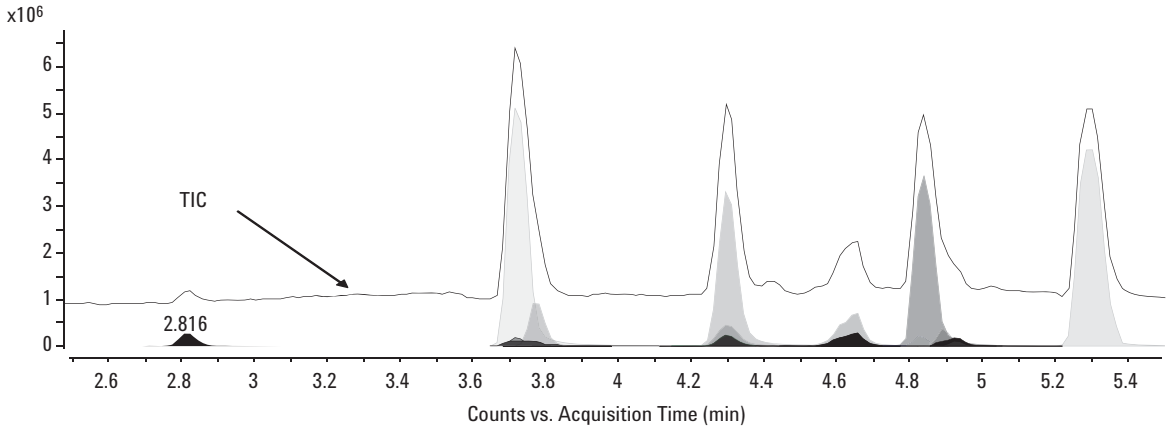

Figure 3. Overlay of total ion chromatogram (TIC) and Molecular Feature Extractor (MFE) chromatograms of dye mixture. Eighteen compounds were found using the criterion of signal > 100 counts.

Compound List

Name	RT	Mass	DB Formula	DB Diff (ppm)	Height
Cpd 1: Acid Blue 129	2.816	436.10981	C23H20N2O5S	-1.19	175661
Compound 2	3.72	307.25877			178040
Cpd 3: Toluidine Red	3.722	307.0967	C17H13N3O3	-3.27	3385650
Compound 4	3.733	329.07803			96154
Cpd 5: Sudan III	3.775	352.13362	C22H16N4O	-3.44	739956
Compound 6	4.299	232.1104			204562
Compound 7	4.3	334.2155			2067458
Compound 8	4.3	148.01592			191203
Compound 9	4.301	392.26843			364637
Compound 10	4.622	329.10603			141919
Compound 11	4.641	420.29968			210481
Compound 12	4.642	362.24654			471542
Compound 13	4.83	390.27811			156716
Cpd 14: Sudan III	4.84	352.13377	C22H16N4O	-3.86	2430832
Compound 15	4.893	440.2012			281429
Compound 16	4.921	390.27759			128443
Compound 17	4.928	157.05266			100077
Cpd 18: Sudan Green	5.296	418.16943	C28H22N2O2	-3.12	2842571

Figure 4. Five compounds had hits from searching the Exact Mass Database.

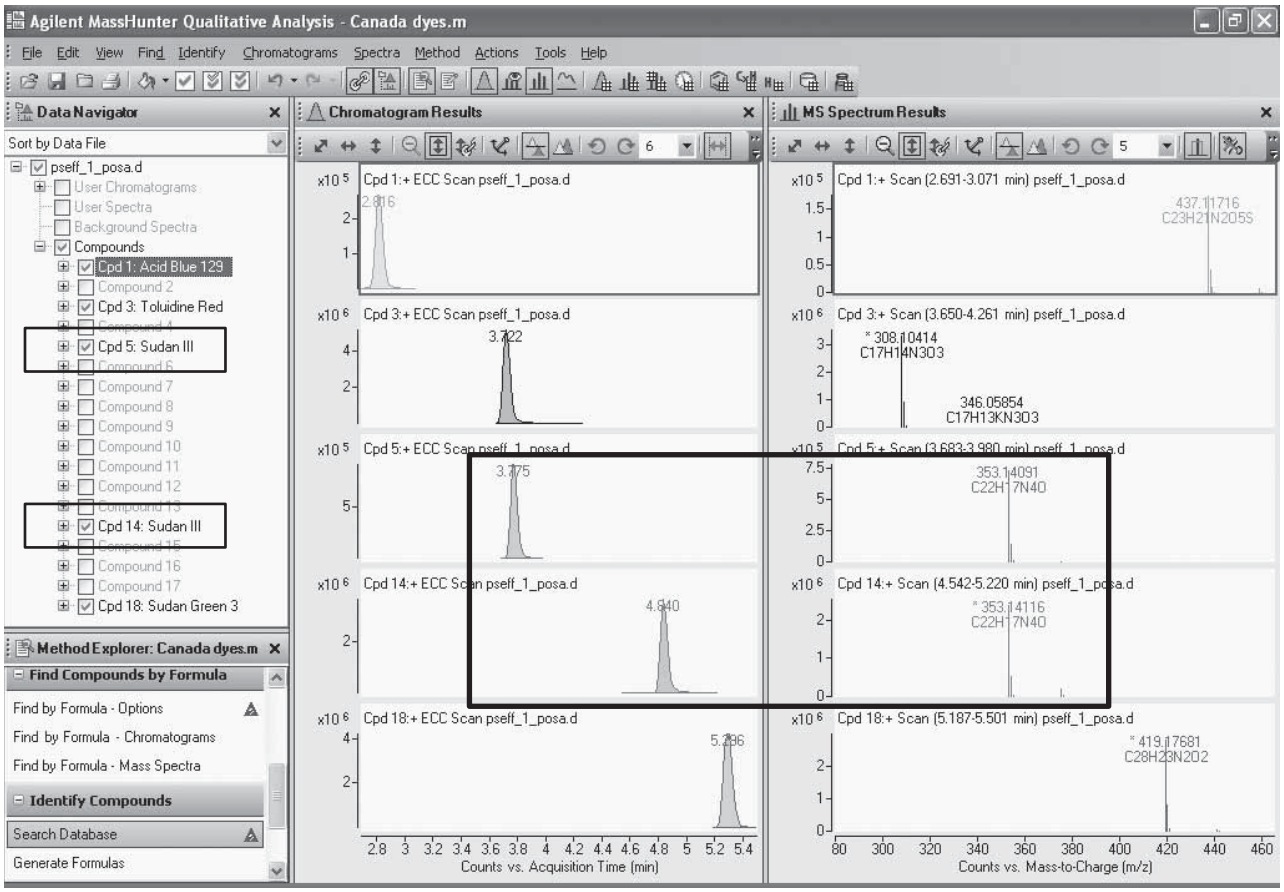

Figure 5. Mass Hunter software showing the five hits from the Exact Mass Database search.

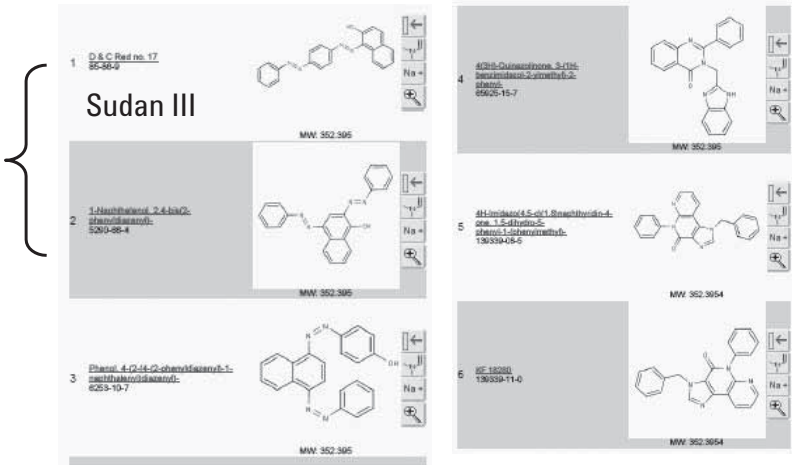

Figure 6. Online ChemID database search results from the Sudan III formula hot link.

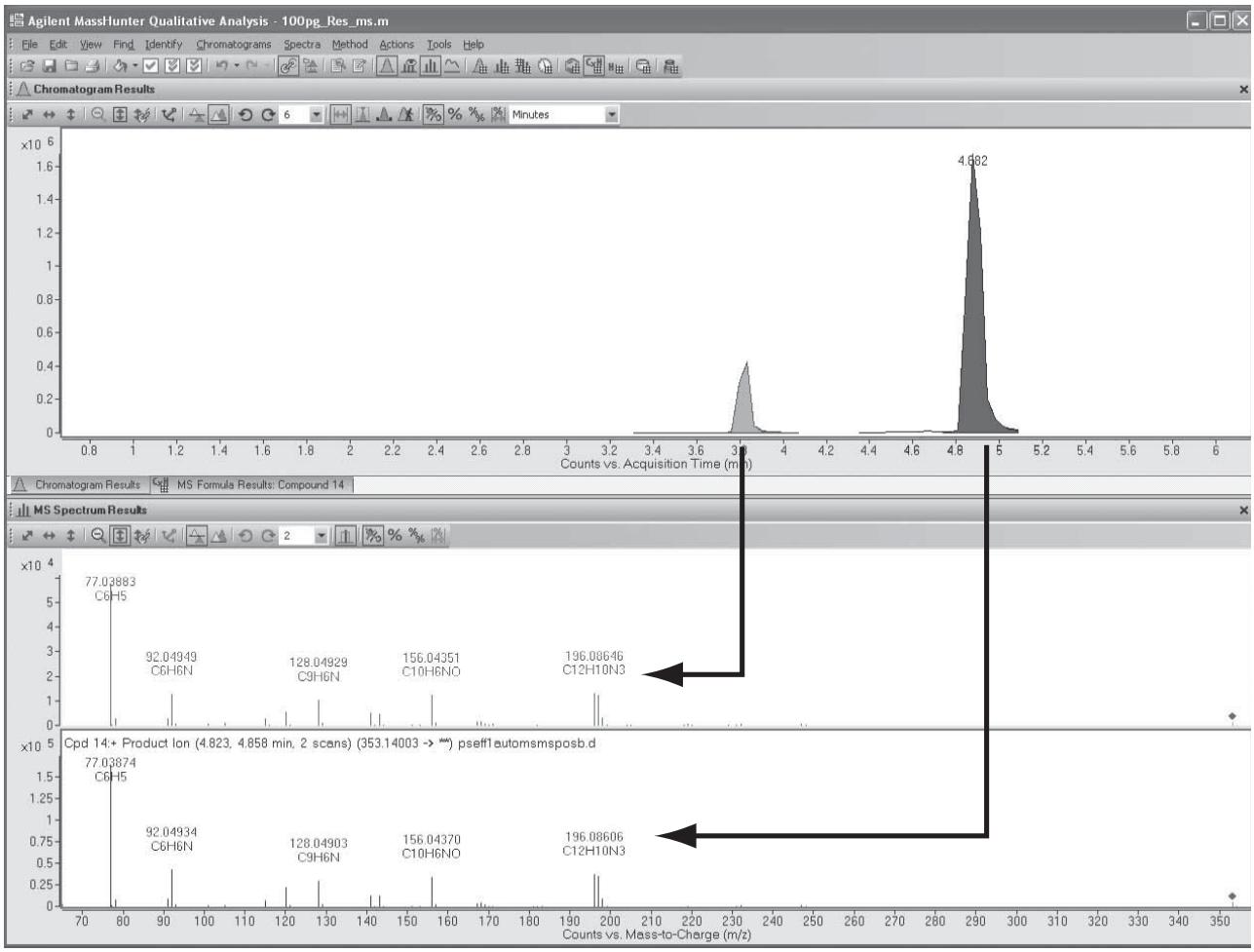

Figure 7. Examination of data-dependent MS/MS shows identical fragmentation from the two peaks, isomers of Sudan III.

Conclusions

- Compounds were found by either formula searching or by MFE and exact mass database searching, allowing a large number of compounds to be screened in a single analysis.
- Good mass accuracy (< 3 ppm) was achieved for five dye compounds, providing high confidence in results (formulas generated and compounds confirmed).
- MS/MS on Q-TOF provides powerful capability to confirm ion identity and structure, useful for QA or unknown confirmation.
- Hot link in Mass Hunter software allows quick formula searching against several online databases for compound identification and ease of use.

References

1. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Robert J. Letcher, and Shaogang Chu, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 2: Fluorotelomer Unsaturated Acids," Agilent Technologies publication 5989-9132, August 2008.
2. Jim Lau, Chin-Kai Meng, Jennifer Gushue, Mark Hewitt, and Suzanne Batchelor, "Analyzing Compounds of Environmental Interest Using an LC/Q-TOF – Part 3: Imidacloprid and Manool," Agilent Technologies publication 5989-9129, August 2008.

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