Environmental Compendium

Volume 2

SCIEX

The Power of Precision

Contents

SCIEX Environmental Compendium Volume 2

Contents

Unknown Screening

Perfluoroalkyl Acids

Naphthenic Acids

Haloacetic Acids

Explosives

Flame Retardants

Microcystins

Contents

Micro LC

Software

Support

Advantages of Using Triple Quadrupole over Single Quadrupole Mass Spectrometry to Quantify and Identify the Presence of Pesticides in Water and Soil Samples

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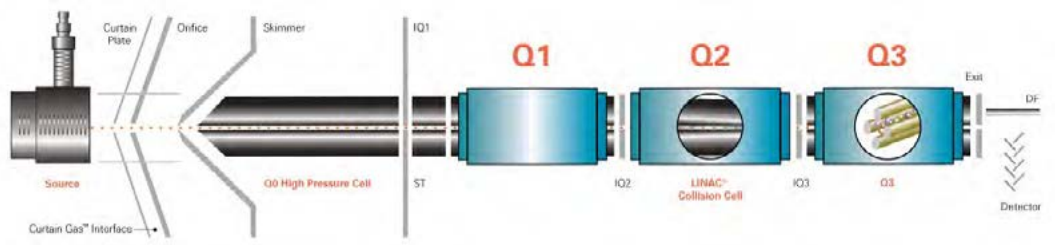

Figure 1. Schematic of Triple Quadrupole Configuration

Triple quadrupole (MS/MS) systems provide in comparison to single quadrupole (MS) systems:

- Higher selectivity resulting in less interference of co-eluting compounds and matrix, thus less HPLC separation is required
- Better Signal-to-Noise (S/N) allowing quantitation with lower limits of quantitation
- More reliable identification of detected analytes using Multiple Reaction Monitoring (MRM) in comparison to Selected Ion Monitoring (SIM)
- Wider linear range of quantitation
- Better accuracy and reproducibility especially at low concentrations

Overview

This paper describes the use of single quadrupole and triple quadrupole Mass Spectrometry coupled to Liquid Chromatography for the analysis of 17 pesticides in drinking water and soil samples. Both detection technologies are compared with respect to selectivity, sensitivity, identification, linear range, accuracy, and reproducibility for quantitative analysis.

Introduction

The coupling of Liquid Chromatography (LC) and tandem Mass Spectrometry (MS/MS) is a widely used analytical technique for quantitative and qualitative analysis. Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), or Photo Ionization (APPI) allow the ionization of various semi-volatile, thermally labile, and polar to nonpolar compounds, such as pharmaceuticals, pesticides, personal care products, steroids, explosives, drugs of abuse etc., in trace levels. Generated ions will be transferred after ionization through a vacuum interface into the mass analyzer.

Quadrupoles are mass analyzers which consist of four rods with DC and RF voltages applied. An ion of a specific mass-to-charge ratio (m/z) will be stable and can pass through the quadrupole only when a specific DC/RF voltage combination is applied. Quadrupoles are therefore called mass filters.

Contents

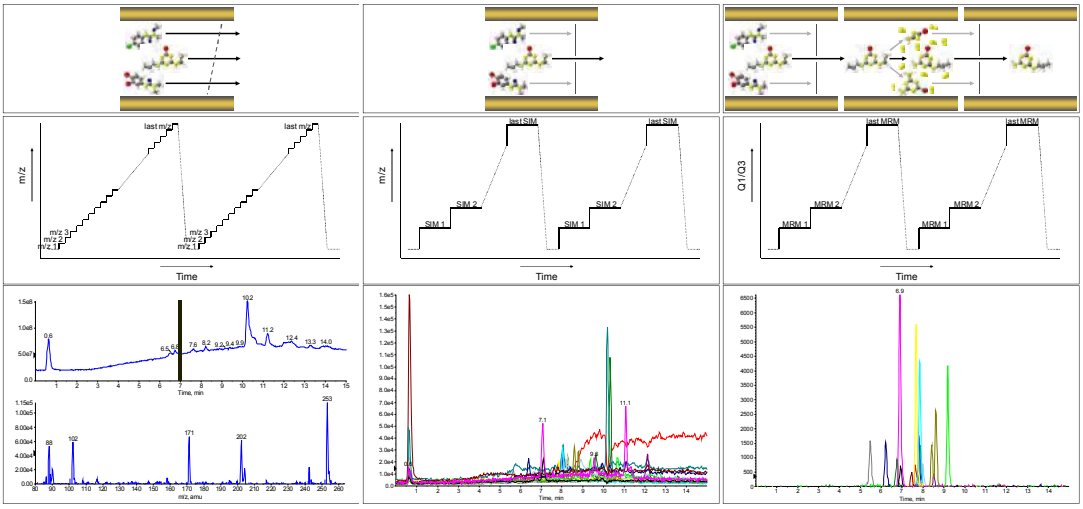

Figure 2. Pesticide in a soil extract detected in different single and triple quadrupole scan modes (left to right): Full scan with MS spectrum (50 µg/kg), Selected Ion Monitoring (5 µg/kg), and Multiple Reaction Monitoring (5 µg/kg)

Single quadrupole systems contain only one mass filtering quadrupole while triple quadrupole systems consist of three quadrupoles. Q1 and Q3 are working as mass filters while Q2 is acting as collision cell.

Quadrupoles can be used in scanning or filtering mode. During a mass scan, DC and RF voltages are ramped resulting in the acquisition of full scan mass spectra. Such spectra are typically used for qualitative data analysis. However, scanning a quadrupole suffers from low sensitivity and slow scan speed. Thus, quantitative studies are performed with quadrupoles working in filtering mode.

The most selective mode to use a single quadrupole MS is called Selected Ion Monitoring (SIM). Hereby, a fixed set of DC and RF voltages is applied to the quadrupole and thus only a single m/z can pass. Ions with different m/z are filtered out.

Multiple Reaction Monitoring (MRM) is the most common mode of using a triple quadrupole MS/MS for quantitative analysis, allowing enhanced sensitivity and selectivity. The first quadrupole filters a specific precursor ion of interest. Ions generated in the ion source having a different m/z can not pass Q1. The collision cell is optimized to produce a characteristic product ion by collision of the precursor ion with a neutral collision gas, such as nitrogen. This process is called Collision Induced Dissociation (CID). Generated product ions are transferred into the third quadrupole where only a specific m/z is allowed to pass. All other product ions are filtered out in Q3.

Thus MRM mode works like a double mass filter which drastically reduces noise and increases selectivity. The principle and resulting chromatograms of various scan modes analyzing a mix of pesticides spiked into soil is given in Figure 2. It illustrates how increasing selectivity of the mass spectrometric experiment reduces the noise of eluting background and matrix components.

Single quadrupole and triple quadrupole systems allow the detection of many SIM and MRM transitions, respectively. This enables quantitation of many targeted analytes in a single experiment. Typically, additional SIM and MRM transitions have to be detected to perform identification of quantified compounds. Hereby, the most intense ion is called the ‘quantifier’ and all additional ions are called ‘qualifiers’. The EU Commission Decision 2002/657/EC defined performance criteria for confirmatory methods, such as MS and MS/MS, by introducing the concept of identification points. The required number of 4 identification points can be achieved by detecting 4 SIM on a single quadrupole MS or 2 MRM transitions on a triple quadrupole MS/MS.

Contents

Table 1. Pesticides detected in Multiple Reaction Monitoring (MRM) and Selected Ion Monitoring (SIM)

Pesticide	CAS	MRM 1	MRM 2	SIM 1	SIM 2	SIM 3	SIM 4	t_R (min)
Atrazine	1912-24-9	216/174	216/104	216	174	146	104	7.8
Chlortoluron	15545-48-9	213/72	213/46	213	72	140	168	7.6
Cyanazine	21725-46-2	241/214	241/104	241	214	104	132	6.7
Desethylatrazine	6190-65-4	188/146	188/104	188	146	104	110	5.5
Diuron	330-54-1	233/72	233/46	233	72	235	160	8.0
Hexazinone	51235-04-2	253/171	253/71	253	171	71	85	6.9
Isoproturon	34123-59-6	207/72	207/46	207	72	165*	134*	7.9
Linuron	330-55-2	249/160	249/182	249	160	251	182	8.5
Metazachlor	67129-08-2	278/134	278/210	278	134*	210	105	7.7
Methabenzthiazuron	18691-97-9	222/165	222150	222	165*	150	124	7.8
Metobromuron	3060-89-7	259/170	259/148	259	261	148	170	7.7
Metolachlor	51218-45-2	284/252	284/176	284	252	176	134	9.2
Metoxuron	19937-59-8	229/72	229/46	229	72	156	106	6.2
Monolinuron	1746-81-2	215/126	215/99	215	126	99	148	7.4
Sebuthylazine	7286-69-3	230/174	230/104	230	174	104	146	8.4
Simazine	122-34-9	202/132	202/124	202	132	104	174	6.9
Terbuthylazine	5915-41-3	230/174	230/104	230	174	104	146	8.6

* Not enough HPLC separation to differentiate these in-source fragment ions

Experimental

HPLC

An Agilent 1200 standard HPLC system with binary pump, well plate autosampler, and column oven was used. Chromatographic separation was performed on a Phenomenex Synergi 4u Fusion RP-80 (50x2 mm) column. A gradient of eluent A (water with 5 mM ammonium formate) and eluent B (methanol with 5 mM ammonium formate) from 80/20 to 10/90 (A/B) over 8min was used. The column temperature was set to 25°C. A sample volume of 10 µL was injected.

MS/MS

MS and MS/MS detection was performed using an API 3200™ LC-MS/MS system with Turbo V™ source and an Electrospray ionization probe. All pesticides were detected in positive polarity using an ionization voltage of 5000V. The ion source temperature was set to 500°C. The detected quantifier and qualifier ions are listed in Table 1. A dwell time of 25 ms was used to detect 34 MRM transitions and 15ms to detect 68 SIM.

Results and Discussion

Selectivity

Detection on a triple quadrupole MS/MS results in higher selectivity due to the double mass filtering (Figure 3). In MRM typically a single signal per analyte is detected while SIM can result in multiple signals per compound. The reason for this is the generation of identical in-source fragment ions of pesticides of the same compound class. Thus more time consuming HPLC is required to separate such compounds to allow quantitation and identification. Needed HPLC development and analysis times are then comparable to traditional UV detection.

Sensitivity

Detection on a MS/MS system also results in better sensitivity. Detected Signal-to-Noise (S/N) is higher resulting in lower Limits of Quantitation (LOQ). The typical difference in sensitivity observed is at least one order of magnitude as illustrated in Figure 4.

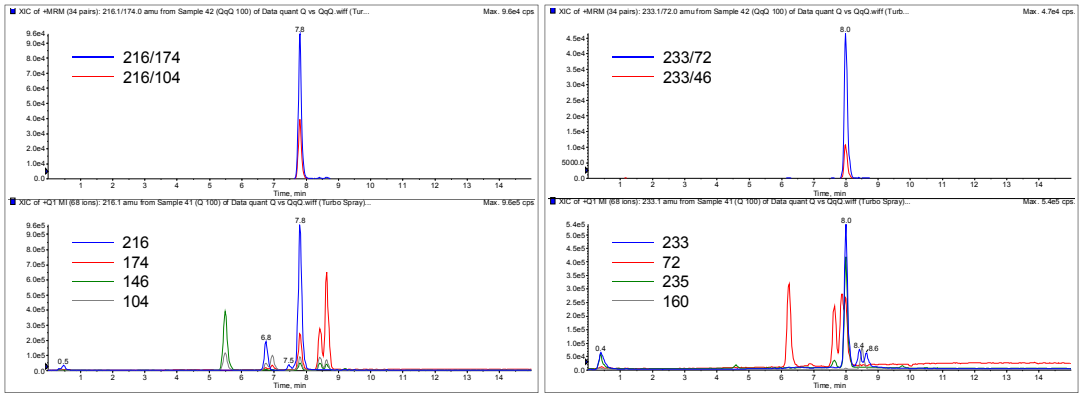

Figure 3. Comparison of selectivity detecting Atrazine (left) and Diuron (right) in MRM and SIM mode at 100 µg/L in drinking water

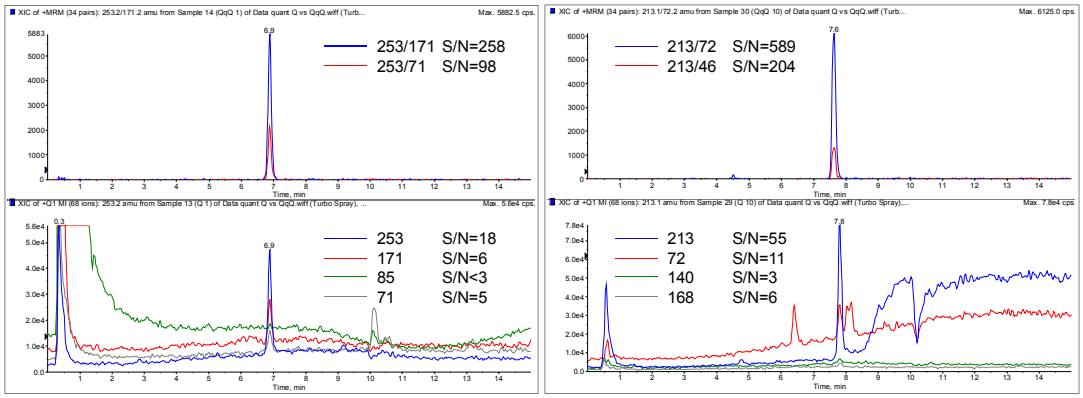

Figure 4. Comparison of sensitivity detecting 1 µg/L Hexazinone (left) and 10 µg/L Chlortoluron (right) in MRM and SIM mode in drinking water

If identification with 4 identification points is required and more than a single ion has to be detected the difference in LOQ based on quantifier MRM and SIM is significantly higher (Table 2).

Linearity and Reproducibility

Generally a wider linear range can be used for quantitation if a triple quadrupole MS/MS is used because of increased selectivity allowing detection at lower LOQ (Figure 5). The upper limit of quantitation of SIM and MRM is typically comparable due to saturation of the ion source or detector. The typical linear range in MRM using Electrospray Ionization is 3 to 4 orders of magnitude while SIM provides only 2 to 3 orders of magnitude.

Additionally, MS/MS allows more accurate and reproducible quantitation. Table 3 compares Coefficients of Variation (%CV) of detected pesticides at different concentrations. Triple quadrupole MS/MS provides %CV below 10 at LOQ and below 5 at higher concentrations. The reproducibility on single quadrupole MS is significantly lower.

Contents

Table 2. Limits of Quantitation (LOQ) and Limit of Identification (LOI) of detected pesticides*

Pesticide	LOQ (MRM 1)	LOI (MRM 2)	LOQ (SIM 1)	LOI (SIM 4)	Enhanced Quantifier	Enhanced Qualifier
Atrazine	0.02	0.05	0.2	1	10x	20x
Chlortoluron	0.1	0.5	1	10	10x	20x
Cyanazine	0.1	0.2	0.5	2	5x	10x
Desethylatrazine	0.1	0.1	0.5	2	5x	20x
Diuron	0.2	0.5	1	10	5x	20x
Hexazinone	0.02	0.05	0.2	1	10x	20x
Isoproturon	0.05	0.1	0.5	N/A	10x	N/A
Linuron	0.1	0.2	2	10	20x	50x
Metazachlor	0.02	0.02	0.5	5	25x	250x
Methabenzthiazuron	0.02	0.05	0.5	2	25x	40x
Metobromuron	0.2	0.5	2	5	10x	10x
Metolachlor	0.02	0.1	0.5	50	25x	500x
Metoxuron	0.1	0.5	0.5	20	5x	40x
Monolinuron	0.1	0.2	2	5	20x	25x
Sebuthylazine	0.1	0.1	0.5	2	5x	20x
Simazine	0.1	0.2	0.5	1	5x	5x
Terbuthylazine	0.02	0.1	0.5	2	25x	20x

*using Multiple Reaction Monitoring (MRM) and Selected Ion Monitoring (SIM) in drinking water samples based on S/N=6

Table 3. Reproducibility (%CV) of detected pesticides in MRM and SIM at different concentrations

Pesticide	%CV at 1µg/L (MRM)	%CV at 10µg/L (MRM)	%CV at 100µg/L (MRM)	%CV at 1µg/L (SIM)	%CV at 10µg/L (SIM)	%CV at 100µg/L (SIM)
Atrazine	4.4	3.7	0.9	16.2	1.8	0.6
Chlortoluron	4.2	3.4	1.1	20.3	2.9	1.7
Cyanazine	9.0	2.1	1.8	16.6	4.6	0.9
Desethyl-atrazine	3.8	2.0	1.5	5.8	0.8	1.4
Diuron	4.4	2.5	1.2	23.7	2.9	1.6
Hexazinone	1.7	1.3	1.2	3.0	1.5	1.5
Isoproturon	8.7	0.9	1.3	17.2	3.0	1.3
Linuron	9.6	7.0	0.9	<LOQ	3.9	2.4
Metazachlor	3.6	1.9	0.9	5.4	1.5	1.1
Methabenzthiazuron	3.0	0.4	0.9	9.9	1.4	0.5
Metobromuron	4.0	3.9	1.4	<LOQ	8.5	2.5
Metolachlor	4.7	1.0	0.7	7.8	1.6	1.6
Metoxuron	5.9	2.3	0.9	13.5	2.7	1.5
Monolinuron	6.2	6.0	1.3	<LOQ	5.5	2.9
Sebuthylazine	7.9	2.3	1.0	7.5	1.3	2.5
Simazine	7.0	2.8	1.8	8.7	1.2	1.5
Terbuthylazine	6.3	3.4	1.2	8.5	3.9	2.4

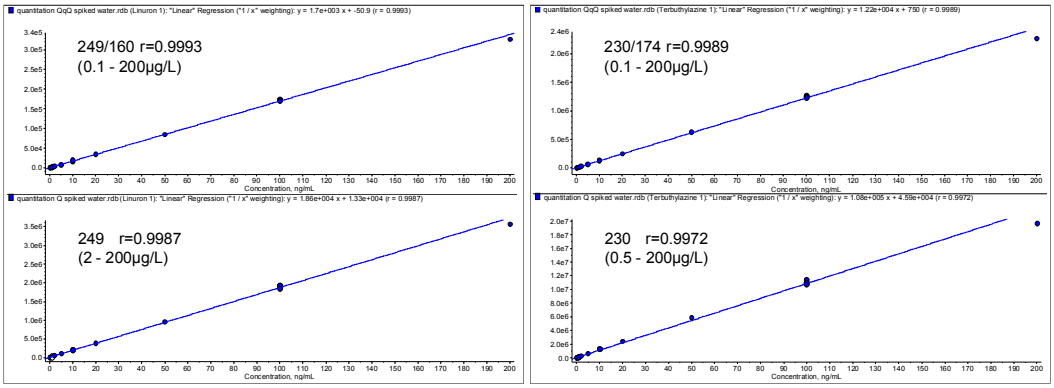

Figure 5. Comparison of linearity detecting Linuron (left) and Terbuthylazine (right) in MRM (top) and SIM (bottom) mode in spiked drinking water samples

Summary

The detection in Multiple Reaction Monitoring (MRM) using a triple quadrupole MS/MS has a number of advantages in comparison to Selected Ion Monitoring (SIM) of a single quadrupole MS.

Due to double mass filtering MS/MS detection provides much higher selectivity with less interference of co-eluting compounds and matrix components, resulting in less time consuming method development and faster analysis times. Better Signal-to-Noise allows quantitation with lower Limits of Quantitation (LOQ).

Fewer ions have to be detected per compound on MS/MS in comparison to MS for confirmatory analysis. The concept of identification points introduced by the EU Commission Decision requires the detection of 2 MRM transitions and 4 SIM, respectively. If identification is required the detected qualifier signals have to be compared to specify LOQ. The difference between both technologies can be then at least two orders of magnitude. Finally, a wider linear range, higher accuracy, and reproducibility can be obtained on triple quadrupole MS/MS.

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Contents

The Detection of Acidic Herbicides and Phenyl Ureas by LC-MS/MS with Large Volume Injection and Automated Column Switching

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Introduction

Acidic herbicides like Dicamba are used to kill broadleaf weeds before and after sprout. They control annual and perennial weeds in grain crops and highlands, are used to control brush and bracken in pastures, and in combination they are also used in pastures, range land, and noncrop areas (fence rows, roadways, and wastage) to control weeds. Phenyl urea pesticides such as Linuron are used as selective herbicides for pre- and post-emergence weed control in vegetables including potatoes, peas, carrots and beans; also on wheat, celery, parsnip and parsley. Both classes of pesticides are toxic to wildlife and some are suspected hormone-disrupting substances.

The provision of clean, uncontaminated drinking water is of paramount importance to the water industry. In recent times the requested limits of detection for such pesticides have been decreasing as methodologies improve. Typically water companies need to be able to have limits of quantitation for pesticides between 0.1 – 1 µg/L (100 – 1000 part-per-trillion, ppt) which often means that methods should have limits of detection for certain pesticides in the range of 10 – 50 µg/L.

These low levels have often meant that water samples have to be prepared either by liquid/liquid or solid phase extraction in order to concentrate these contaminants to such a level where they can be detected using traditional techniques such as GC-MS or HPLC with UV detection. Where GC-MC is used an additional derivatization step is often required before sample analysis. This sample pre-treatment used for traditional techniques can often be time consuming and add additional cost to the analyses. Therefore in this work the direct injection of filtered samples was used for sample analysis, to reduce both cost and speed up the sample throughput.

Experimental

Sample Preparation

River and ground water samples (10 mL) were filtered through a Chromfil PET 20/25, 0.2 µm 25 mm filter. The filter was washed by acetonitrile (0.85 mL) with the filter wash added directly into the sample. This filtered sample was directly injected onto the LC-MS/MS system.

Chromatography

Samples (200 µL) were directly injected and separated by reversed-phase HPLC using a Dionex Ultimate 3000 system. A Gemini 3 µm, 150 x 2.0 mm C18 and a LUNA 3 µm C18 (2), 150 x 3 mm column from Phenomenex were used to analyze acid herbicides and phenyl ureas respectively. Both columns were kept at 40ºC and gradients from water containing 0.1% acetic acid to acetonitrile containing 0.1% acetic acid were used to separate analytes. Automated column switching, involving a 10 port Valco switching valve, was used to switch between the column for acidic herbicide and the one for phenyl urea analysis (the gradient profiles are shown in Table 1).

Contents

Table 1. Gradient profiles used for the separation of acidic herbicides and phenyl ureas

Acidic herbicides			Phenyl ureas		
Time (min)	Flow (mL/min)	% B	Time (min)	Flow (mL/min)	% B
0.0	10	0.4	0.0	10	0.2
1.5	10	0.4	5.0	10	0.2
10.0	95	0.7	9.0	100	0.3
18.0	95	0.7	16	100	0.3
18.5	10	0.4	17	10	0.2
18.6	10	0.4			

Mass Spectrometry

Analysis was performed on an SCIEX API 4000™ LC-LC-MS/MS system with Turbo V™ source electrospray ionisation (ESI) probe in negative polarity (acidic herbicides) and positive polarity (phenyl ureas). The MRM transitions for acidic herbicides and phenyl ureas are shown in Table 2.

Results and Discussion

Examples of calibrations for both acidic herbicides and phenyl ureas are shown in Figures 1, 2 and 3. For both classes of pesticides linear responses were obtained over the range tested with 'r' values never less than 0.998 (Table 3).

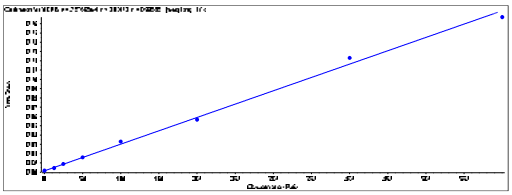

Figure 1. Calibration for MCPB from 12.5 – 600 ng/L

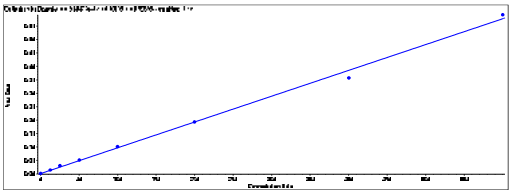

Figure 2. Calibration for Dicamba from 12.5 – 600 ng/L

Table 2. MRM transitions to detect acidic herbicides and phenyl ureas using the SCIEX API 4000™ system

Pesticide	Q1	Q3	DP	CE
MCPA	199.0	141.1	-55	-20
Clopyralid A	189.9	146.0	-20	-12
Clopyralid B	191.9	148.0	-20	-12
2,4-D	218.9	161.1	-20	-20
Dicamba	218.9	175.1	-20	-8
2,4-DB	246.9	161.0	-20	-18
Dichlorprop	232.9	161.1	-25	-18
Bromoxynil	275.8	81.0	-50	-45
Ioxynil	369.7	127.0	-55	-50
Bentazone	239.0	132.0	-50	-36
MCPB	227.1	141.1	-35	-25
MCPP	213.0	141.1	-30	-22
Triclopyr	253.9	196.0	-20	-16
Fluroxypyr	253.0	195.0	-35	-20
Benazolin	242.0	170.1	-25	-20
Aminopyralid	204.8	160.8	-55	-14
2,4-DPA (S)	203.1	159.1	-35	-12
4-CAA (IS)	169.0	125.0	-20	-12
2,B-4,C-phenol (IS)	195.9	78.9	-45	-32
Isoproturon A	207.1	134.2	45	35
Isoproturon B	207.0	72.0	56	35
Diuron	233.0	72.0	71	35
Isoproturon	207.0	72.0	56	35
Monolinuron	215.0	126.0	56	25
Chlorotoluron A	215.0	182.9	51	11
Chlorotoluron B	213.0	72.0	51	15
Metoxuron	229.0	72.0	106	35
Fenuron	165.2	72.0	86	29
Pencycuron	329.0	124.8	90	39
Linuron	249.0	159.9	51	27
Isoproturon	207.1	134.2	45	35

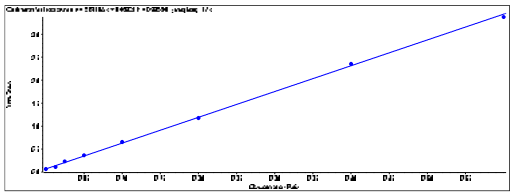

Figure 3. Calibration for Isoproturon from 12.5 – 600 ng/L

Table 3. Signal-to-noise (S/N)* of the lowest calibration standard and r' values taken from calibration lines 12.5 – 600 ng/L

Pesticide	S/N at 12.5 ng/L	'r' value
MCPA	86.7	0.99967
Clopyralid[#]	25.1	0.99769
2,4-D	51.3	0.99963
Dicamba	25.5	0.99856
2,4-DB	25.8	0.99936
Dichlorprop	76.5	0.99934
Bromoxynil	50.3	0.99956
Ioxynil	148.5	0.99932
Bentazone	368.1	0.99888
MCPB	15.3	0.99868
MCPP	102.1	0.99968
Triclopyr	27.6	0.99871
Fluroxypyr	22.3	0.99846
Benazolin	26	0.99876
Aminopyralid	100.7	0.99955
Diuron	41.2	0.99816
Isoproturon	39.5	0.99864
Monolinuron	32	0.99904
Metoxuron	54.9	0.99882
Fenuron	53.1	0.99913
Pencycuron	167.9	0.99982
Linuron	26.2	0.9993
Chlorotoluron	50.5	0.99921

* S/N was calculated in MultiQuant™ software version 2.0.1

[#] S/N of Chlopyralid at 25 ng/L

It can also be seen that every compound with the exception of Clopyralid gave a good signal-to-noise (> 15:1) from the lowest standard 12.5 ng/L (Table 3). Clopyralid, the least sensitive of all the compounds, gave a signal to noise of 25:1 at its lowest standard level of 25 ng/L. There was no carryover observed for either method.

This method has been validated and used routinely for testing water samples as part of surveillance exercises. Normally such tests produce negative results but in certain instances positive results can be observed which normally result from the illegal disposure of pesticides.

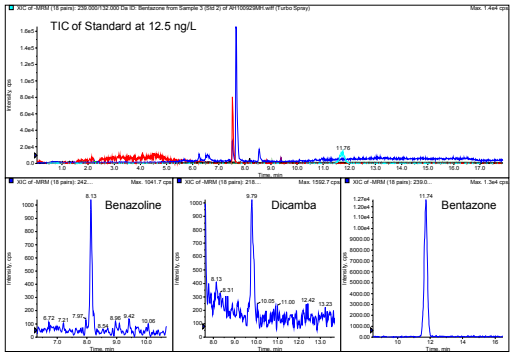

Figure 4a. 12.5 ng/L standard in negative polarity

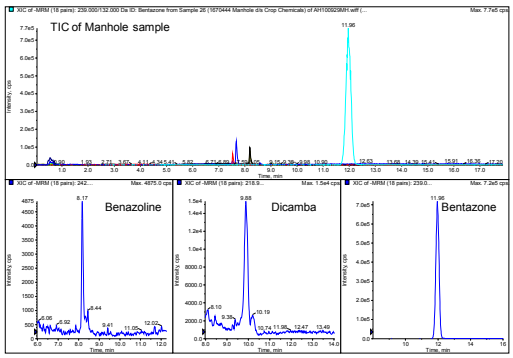

Figure 4b. Manhole sample in negative polarity

Figures 4 and 5 show examples of where this method has detected both the presence of certain acidic herbicides and phenyl ureas in samples of water from manholes. In each example the amount of pesticide detected varies with analyte and is in the parts per trillion range but exceeds the lowest calibration standard.

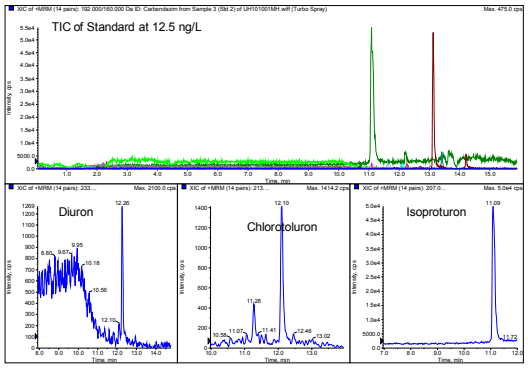

Figure 5a. 12.5 ng/L standard in positive polarity

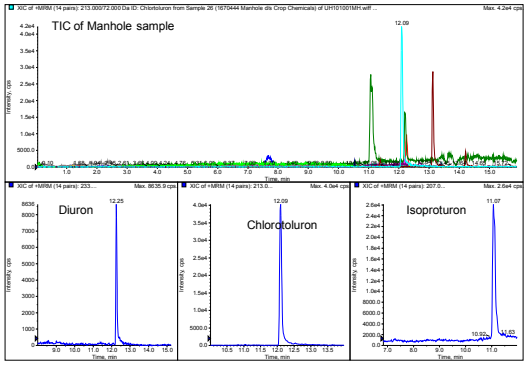

Figure 5b. Manhole sample in positive polarity

Conclusion

The results show that both acidic herbicides and phenyl ureas can be detected at the required limits set by the water industry in the UK. The sample preparation used involved a simple filtration step which removed the cost and time associated with solid phase extraction and/or liquid liquid extraction traditionally used for GC-MS analysis. Acidic herbicides and phenyl urea pesticides ionise under different polarities and require different HPLC conditions to obtain their best sensitivity. Using conventional LC and a timed switching valve samples can be run under the optimised LC conditions for either class of compounds, without supervision. The automated column switching enables researchers to optimise the pH of the mobile phase and column chemistry to produce the best sensitivity for both compound classes.

Such a method has been shown to be robust and sensitive enough to be applied to surveillance work, in the UK, needed to maintain a safe water supply.

Publication number: 3370611-01

Contents

Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in River Water

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Overview

Endocrine disrupting compounds (EDC) encompass a wide range of pollutants, including pharmaceuticals and personal care products (PPCP), pesticides, and steroids to name a few. EDC are thought to disrupt the endocrine function of mammals and fish, and as a result their biological effects are a growing concern. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. A method is presented for analyzing up to 100 EDC and PPCP compounds using LC-MS/MS. This method is a straight forward approach for the quantitation and identification of these compounds with excellent sensitivity and ruggedness.

Introduction

A wide range of endocrine disrupting compounds were determined in river water sampled near a water treatment plant. Compound levels upstream and downstream from the plant were quantified and compared. A combination of Solid Phase Extraction (SPE) and LC-MS/MS analysis in Multiple Reaction Monitoring (MRM) mode achieved low parts per trillion detection limits across multiple compound classes with a linear range of 3-4 orders of magnitude for all compounds.

Both positive and negative ionization modes were utilized. APCI and ESI ionization techniques were investigated using the DuoSpray™ ionization source. Electrospray ionization with polarity switching on the Turbo V™ source yielded the broadest coverage across compound classes. Two MRM transitions were monitored for each compound to achieve sensitive and specific quantitation as well as ion ratio identification. A total of 160 MRM transitions were monitored on a chromatographic time scale.

Two sets of river water samples were collected from a rural river (River 1) and an urban city river (River 2) both upstream and downstream of a sewage treatment plant in North America. The upstream and downstream samples for these two areas were then compared to determine environmental impact

Experimental

A SCIEX API 4000™ LC-MS/MS System equipped with a Shimadzu Prominence autosampler and binary LC pump was used. Ionization was achieved by Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) using the DuoSpray™ and Turbo V™ ionization sources. All compounds were monitored using two Multiple Reaction Monitoring (MRM) transitions per compound. Each MRM transition had a dwell time of 5ms/sec. The most sensitive, first MRM transition was used for quantitation while the second MRM transition was used for qualitative identification using ion ratio determination. See Figure 3 and 4 for examples. The total cycle time for the method with polarity switching was approximately 3 seconds. Instrument conditions were as follows: CUR 20, CAD 7, GS1 75, GS2 65, IS 5000, and TEM 600. Chromatography was performed on a Phenomenex Ultracarb (20) C18 250 X 4.5 mm 5 µm reverse phase column at 30°C. The total flow rate was 600 µL/min and used a gradient starting at 95% A and held for 1 minute before ramping to 50% over 24 minutes. At a run time of 25 minutes the gradient was then ramped to 4% A over 10 minutes and held for

an additional 10 minutes. Re-equilibration time was 10 minutes for a total run time of 55 minutes. Eluent A was 0.01% formic acid in water and eluent B was 0.01% formic acid in acetonitrile.

Laboratory control samples and matrix spike samples were prepared to monitor extraction efficiency. After conditioning with 20 mL of methanol followed by 40 mL of water, 1.0 L of sample was loaded onto the cartridge at a flow rate of 25.0 mL/min. After loading, nitrogen was then pulled through the cartridge for 15 minutes to allow for sample drying. Then 5.0 mL of acetonitrile was added to the SPE bed and allowed to stand for 15 minutes. The SPE cartridges were then eluted at gravity flow into a 12 mL amber vial. Finally, water was added to the extract to a final volume of 10.0 mL. Samples were kept at 4 °C ± 1 °C until analysis. Figure 1 shows a schematic of the sample preparation procedure.

Table 1. Compound list including MRM transitions (positive polarity)

		Quantifier		Qualifier				Quantifier		Qualifier	
Compound	**Type**	**Q1**	**Q3**	**Q1**	**Q3**	**Compound**	**Type**	**Q1**	**Q3**	**Q1**	**Q3**
Acetaminophen	Analgesic	152	110	152	65	*Estradiol*	Estrogen	255	159		
Ketoprofen	Analgesic	255	105	255	77	*Ethinylestradiol*	Estrogen	271	133		
Codeine	Analgesic	300	215	300	165	*17α-Hydroxy-progesterone*	Estrogen	331	97		
Hydrocodone	Analgesic	300	199	300	171	*Progesterone*	Estrogen	315	109	315	97.
Androstenedione	Androgen	287	97	287	97	*Equilin*	Estrogen replacement	269	211	269	157
Testosterone	Androgen	289.5	97	289	109	*Diethylstilbestrol*	Estrogen replacement	269	135	269	107
Dilantin	Anti-convulsant	253	182			*TCEP*	Flame retardant	285	223	285	239
Meprobamate	Anti-anxiety	219	158	219	115	*Simazine*	Herbicide	202	132	202	124
Sulfadiazine	Antibiotic	251	92	251	65	*Isoproturon*	Herbicide	207	72		
Sulfamethoxazole	Antibiotic	254	92	254	108	*Chlorotoluron*	Herbicide	213	72	213	140
Sulfathiazole	Antibiotic	256	156	256	92	*Atrazine*	Herbicide	216	174	216	68
Sulfamerazine	Antibiotic	265	92	265	108	*Chloridazon*	Herbicide	222	104	222	92
Sulfamethizole	Antibiotic	271	156	271	92	*Propazine*	Herbicide	230	146	230	188
Sulfamethazine	Antibiotic	279	92	279	124	*Diuron*	Herbicide	233	72	233	46
Sulfachlorop-yridazine	Antibiotic	285	92	285	65	*Hexazinone*	Herbicide	253	171	253	85
Trimethoprim	Antibiotic	291	230	291	123	*Bromacil*	Herbicide	261	205	261	188
Sulfadimethoxine	Antibiotic	311	156	311	92	*Metazachlor*	Herbicide	278	134	278	210
Ciprofloxacin	Antibiotic	332	288			*Metolachlor*	Herbicide	284	252	284	175
Penicillin G	Antibiotic	335	176	335	217	*DEET*	Insect repellant	192	119		
Amoxicillin	Antibiotic	366	114	366	208	*Bezafibrate*	Lipid regulator	362	139	362	121
Lincomycin	Antibiotic	407	126	407	359	*Diazepam*	Muscle-relaxant	285	154	285	193
Doxycycline	Antibiotic	445	428	445	339	*Norethisterone*	Ovulation Inhibitor	299	109	299	91
Tetracycline	Antibiotic	445	410	445	154	*Theophylline*	Stimulant	181	124	181	96
Oxytetracycline	Antibiotic	461	426	461	443	*Theobromine*	Stimulant	181	138	181	110
Chlortetracycline	Antibiotic	479	462	479	154	*Caffeine*	Stimulant	195	138	195	110

Compound	Type	Q1	Q3	Q1	Q3	Compound	Type	Q1	Q3	Q1	Q3
Virginiamycin	Antibiotic	526	109	526	67	*Oxybenzone*	Sunscreen	229	151	229	105
Monensin	Antibiotic	694	461	694	479	*Sildenafil*	Virility regulator	475	100	475	283
Erythromycin	Antibiotic	735	158	735	576	*Vardenafil*	Virility regulator	490	72	490	114
Roxithromycin	Antibiotic	838	679	838	158	*Salicylic Acid*	Skin care, acne	139	61	139	79
Tylosin	Antibiotic	917	174	917	772	*Cotinine*	Nicotine metabolite	177	80	177	98
Meclocycline Sulfosalinicyclate	Antibiotic	477	460			*4-Aminoantipyrine*	Aminopyrine metabolite	204	56		
Sulfadimethoxine	Antibiotic	311	156			*Ketorolac*	Anti-inflammatory	256	105	256	77
Sulfachloro-Pyridazine	Antibiotic	285	156			*Fenoprop*	Herbicide	269	181	269	85
Norifloxacin	Antibiotic	320	276			*Meclofenamic acid*	Anti-inflammatory	296	278	296	243
Enroflofacin	Antibiotic	360	316			*Piroxicam*		332	95	332	121
Fluoxetine	Antidepressant	310	148			*Nifedipine*	Dihydropyridine calcium channel blocker	347	315		
Carbamazepine	Anti-seizure	237	194	237	193	*Indomethacin*	Anti-inflammatory	358	139	358	75
Pentoxifylline	Blood viscosity reducing agent	279	181	279	138	*Diatrizoate*	Radiocontrasting agent	615	361		

Table 1 (continued). Compound list including MRM transitions (negative polarity)

		Quantifier		Qualifier				Quantifier		Qualifier	
Compound	Type	Q1	Q3	Q1	Q3	Compound	Type	Q1	Q3	Q1	Q3
Acetylsalicylic acid	Analgesic	179	137	179	93	*Estrone*	Estrogen	269			
Ibuprofen	Analgesic	205	161	205	159	*Estradiol*	Estrogen	271			
Naproxen	Analgesic	229	183	229	155	*Estriol*	Estrogen	287			
Warfarin	Anti-coagulant	307	161	307	250	*Ethinylestradiol*	Estrogen	295			
Diclofenac	Anti-arthritic	294	250	294	214	*Tetrabromo-bisphenol A*	Flame retardant	443	103	443	239
Carbadox	Antibiotic	261	122			*2,4-D*	Herbicide	219	161	219	125
Triclosan (Irgasan)	Antibiotic	287	35			*Clofibric acid*	Metabolite of lipid regulator	213	127	213	85
Chloramphenicol	Antibiotic	321	257	321	152	*Iopromide*	X-ray contrast agent	790	127		
Gemfibrozil	Anti-cholesterol	249	121			*2,4-Dichloro-benzoic acid*		189	101	189	145

Results and Discussion

Quantitative optimization in Analyst® Software was utilized to streamline method development for this large list of compounds. The final method contains the analytes and MRM transitions listed in Table 1.

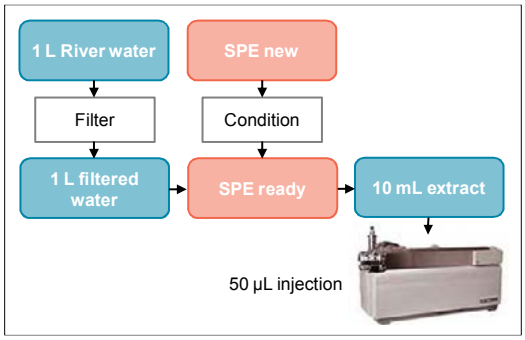

Figure 1. Sample preparation procedure for solid phase extraction

A calibration curve was prepared in water/acetonitrile (1/1) at the following concentrations, 0.2, 0.4, 1.6, 3.1, 6.3, 25, and 100 ng/mL. Linearity was achieved for all monitored compounds. Examples of linearity are shown in Figure 4.

Samples were collected and extracted using the procedure described above. To monitor the extraction efficiency of the sample preparation a laboratory control sample (LCS) was prepared. This sample consisted of tap water being free of all target compounds. This water was then spiked with all of the target analytes. The final concentration of all analytes in the LCS was 20 ng/L.

Recoveries in the LCS ranged from 30 to 115% across all compounds. Based on these results, it was determined that the sample preparation procedure used is adequate for a full screen of the compounds reported. For future work, once the final sample list is determined, surrogate compounds will be selected for each compound class to closely monitor the sample preparation procedure. If possible, a deuterated surrogate will be chosen for each compound class and will only be used to monitor sample preparation efficiency and not instrument variability. It has been shown in previous work that an internal standard, used to monitor instrument variability, may introduce more error in the quantitation results of this large list of compounds.

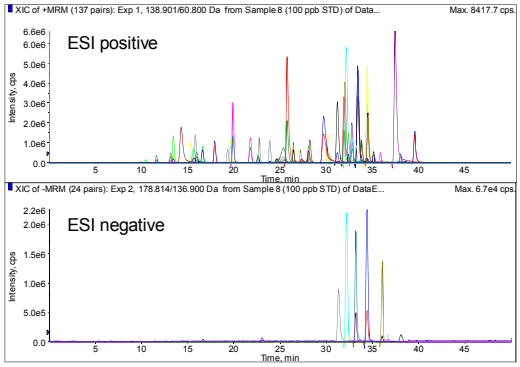

Figure 2. Polarity switching is utilized to encompass a large list of analytes – 100 ng/mL standard injection

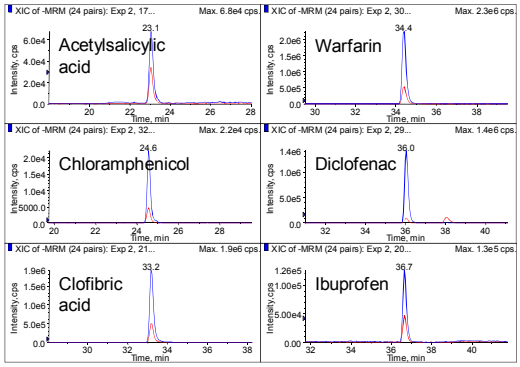

Figure 3. Overlay of two MRM transitions used for six selected analytes. The most sensitive transition in blue for each analyte is used for quantitation. The area ratio of the second MRM in red is used for identification

Table 2. Lower Limits of Quantitation (LLOQ) of selected analytes

Analyte	LLOQ (ng/L) ppt	Analyte	LLOQ (ng/L) ppt
DEET	11.6	*Propazine*	0.46
Ketoprofen	3.3	*Progesterone*	3.9
Sulfadiazine	13.0	*Trimethoprim*	6.4
Fluoxetine	280	*Androstenedione*	4.7
2,4-D	2.3	*Erythromycin*	14.0

Result of both River 1 and River 2 showed detection of several compound classed. As expected, a significantly larger number of compound classes were detected in the urban river (River 2). Lower limit of quantitation (LLOQ) was determined to be the level at which a peak is detected with a signal to noise of at least 10:1. This level was theoretically determined using the standards and assuming linearity down to zero concentration. Table 2 shows a selected list of compounds and their LLOQ. All compounds had LLOQ in the sub part per billion (ppb) range.

Detection of each analyte was identified using the area ratio of two MRM's collected. For River 2, Erythromycin, Ketorolac, and Meprobamate along with 20 other compounds were detected in either the upstream and downstream samples. Ion ratios on the samples were compared to the ion ratios measure on the standards for compound identification. See Figure 5. Final results of River 1 and River 2 are shown in Table 3.

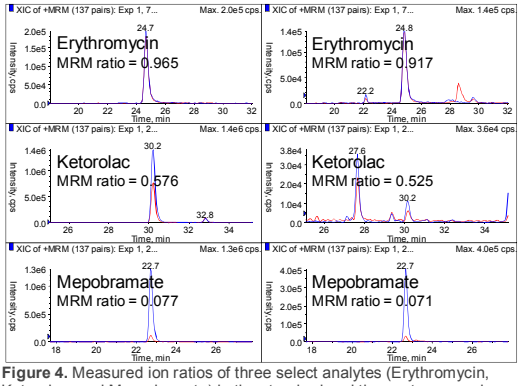

Figure 4. Measured ion ratios of three select analytes (Erythromycin, Ketorolac, and Meprobamate) in the standard and the upstream and downstream sample of river 2, respectively. Despite low level detection like that seen for Ketorolac in the River 2 sample, the ion ratios of the two MRM transitions still confirm with the standard. MRM ratio calculation was done automatically using the Analyst® Reporter software

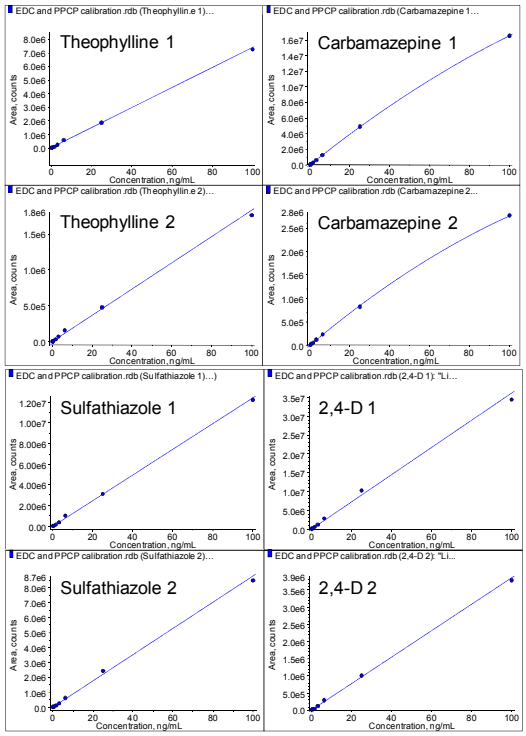

Figure 5. Example calibrations for selected analytes

Table 3. Eight EDC and PPCP compounds were detected in the samples of river 1. Despite the rural nature of this location, low level of these widely used herbicides and pharmaceuticals are still detected. As expected a larger list of compounds were detected in the river 2 samples because of it urban origin. In total 23 EDC and PPCP compounds were founds at low to mid part per trillion (ppt) levels. These results show that it is possible to scan for a functionally diverse set of compounds in one analysis and achieve high sensitivity and accurate quantitation

Analytes in River 1	Concentration (ng/L) upstream	Concentration (ng/L) downstream	Analytes in River 2	Concentration (ng/L) upstream	Concentration (ng/L) downstream
Erythromycin	3.08	53.5	*Oxybenzone*	ND	6.25
Carbamazepine	65.5	152	*Bromacil*	ND	7.40
2,4-D	ND	9.35	*Diazepam*	ND	0.388
DEET	1.49	7.67	*Warfarin*	ND	0.930
Sulfamethoxazole	13.2	13.3	*Triclosan (Irgasan)*	5.90	31.4
Caffeine	41.0	23.5	*Codeine*	17.1	77.5
Ciprofloxacin	3.81	ND	*Diuron*	1.38	4.35
Cotinine	2.05	ND	*Trimethoprim*	58.5	123
			Lincomycin	1.53	3.02
			Carbamazepine	870	1305
			DEET	24.0	29.9
			Ketorolac	2.49	3.06
			Meprobramate	85.5	97.5
			Atrazine	1.08	0.88
			Sulfamethoxazole	95.5	74.5
			Pentoxifylline	6.60	3.39
			Caffeine	57.0	13.5
			Cotinine	14.4	ND
			Simazine	1.01	ND
ND not detected			*Norethisterone*	1.15	ND
Increases by more than 2x			*Erythromycin*	135	ND
Within ± 2x			*Tylosone Tartrate*	4.28	ND
Decreases by more than 2x			*2,4-D*	3.24	ND

Summary

LC-MS/MS analysis has been shown to be a highly feasible approach for the monitoring of a large set of endocrine disrupting compounds spanning multiple categories and chemical classes. MRM mode allows for the determination of these compounds in river water matrix with low detection limits and high selectivity.

Additional compound identification was achieved by the simultaneous monitoring of a second MRM transition and calculation of the corresponding ion ratio, which was done automatically by Analyst Reporter™ software. Electrospray ionization with polarity switching was found to be the most suitable approach.

Contents

References

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Contents

Rapid and Comprehensive Screening for Pharmaceuticals and Personal Care Products using LC-MS/MS with Fast Polarity Switching

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Introduction

There is an emerging environmental concern that pharmaceuticals and personal care products (PPCP) are entering and contaminating the drinking water supply. Chemicals like hormones and antibiotics are especially of interest because of proven endocrine disrupting effects and a possible development of bacterial resistance. Powerful screening methods are required to detect and quantify the presence of these compounds in our environment.

LC-MS/MS is the technology of choice to monitor numerous compounds in environmental samples. Multiple Reaction Monitoring mode (MRM) is typically used because of its excellent sensitivity, selectivity, and speed. Because PPCP span such a wide variety of compound classes and chemical properties, it is necessary to employ both positive and negative Electrospray Ionization (ESI) for complete analysis. It is also desirable to obtain the maximum amount of information in the shortest amount of time.

The novel SCIEX QTRAP® 5500 Systems incorporates the proven technology of the Turbo V™ source and the Curtain Gas™ interface for ultimate sensitivity and robustness. The advanced eQ™ electronics and the new Qurved LINAC® collision cell was designed for unparalleled speed of MRM detection and fast polarity switching for multi-component analysis. In addition, the new Linear Accelerator™ Trap technology allows the acquisition of fast and highly sensitive Enhanced Product Ion (EPI) spectra for compound confirmation with highest confidence.

The ability of the QTRAP® 5500 System to detect a large panel of PPCP while performing fast positive/negative switching, resulting in the maximum amount of information from a single LC-MS/MS injection is demonstrated.

Method Details

- Ultra High Pressure Liquid Chromatography using a Shimadzu $UFLC_{XR}$ system with a Zorbax SB-C18 column (1.8 µm) and a gradient of water and methanol with 0.1% formic acid
- SCIEX QTRAP® 5500 System with Turbo V™ Source and ESI probe
- Experiment 1: dedicated positive mode to monitor 78 MRM transitions (5 ms dwell time and 3 ms pause time)
- Experiment 2: dedicated negative mode to monitor 33 MRM transitions (5 ms dwell time and 3 ms pause time)
- Experiment 3: positive/negative switching to monitor all 111 MRM transitions (3 ms dwell time, 3 ms pause time, and settling time of 50 ms)
- Experiment 4: positive/negative switching to monitor all 111 MRM transitions with Information Dependent Acquisition (IDA) of EPI spectra with CE = +/- 35V and CES = 15V

Contents

Table 1. Details comparing parameters of the various experiments: Chromatographic peak widths were on the order of 8-10 seconds base-to-base. Cycle times were adjusted to allow a minimum of 10 scans across the chromatographic peak.

Experiment	MRM Transitions	Dwell Time (ms)	Cycle Time (ms)
Dedicated positive	78	5	624
Dedicated negative	33	5	264
Polarity switching	111	3	766

Results

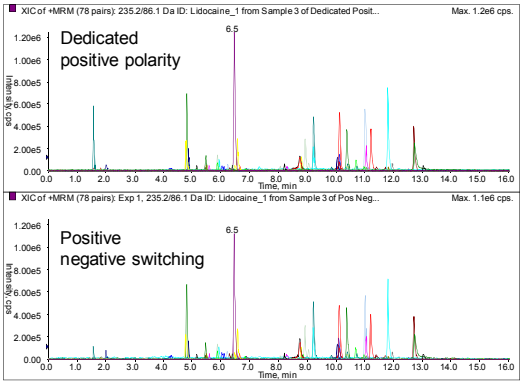


Figure 1. LC-MS/MS analysis in positive polarity: Comparison of a data acquired using a dedicated positive mode acquisition method (top) versus a method that utilized positive/negative switching (bottom)

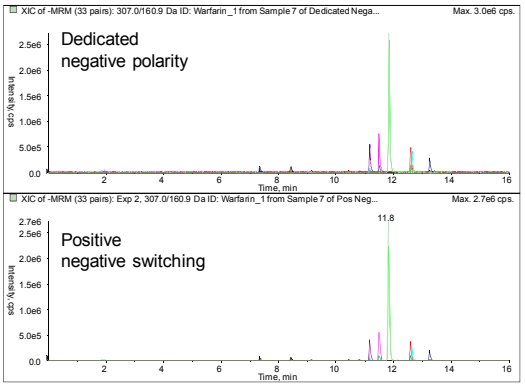


Figure 2. LC-MS/MS analysis in negative polarity: Comparison of a data acquired using a dedicated negative mode acquisition method (top) versus a method that utilized positive/ negative switching (bottom)

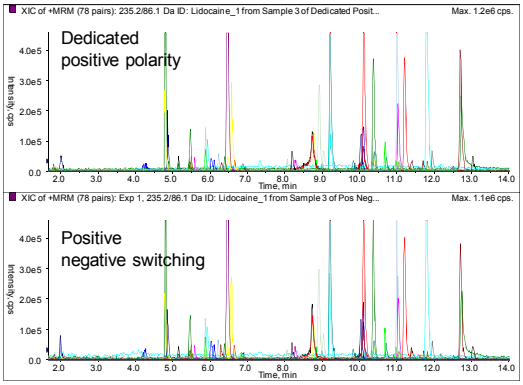


Figure 1a. Zoomed in view of Figure 1

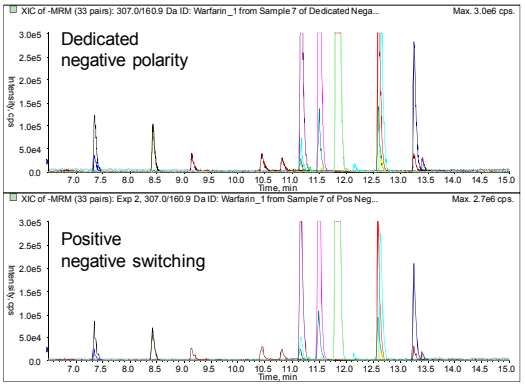


Figure 2a. Zoomed in view of Figure 2

Contents

Data from the dedicated positive and negative experiment were compared to data from the polarity switching experiment. Peak areas and signal-to-noise (S/N) were compared, as well as %CV of the peak areas.

Variations between the sets of data were minimal, demonstrating that no significant loss of data quality was observed when a positive/negative switching method was used versus a dedicated method (see Figure 1-4).

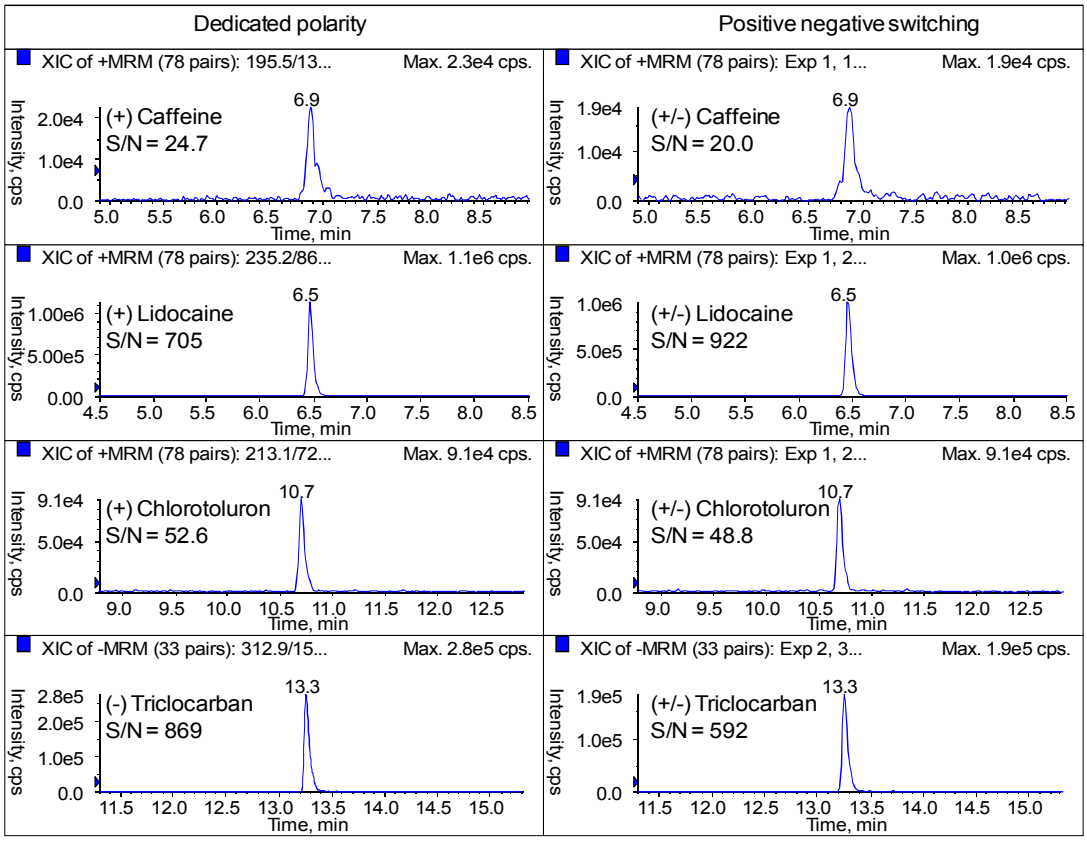
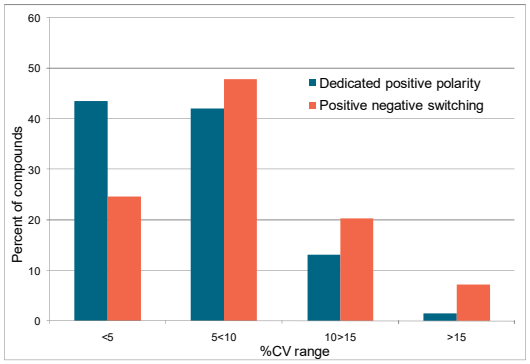


Figure 3. Comparison of S/N for representative analytes: Data from the dedicated positive or negative experiment is shown on the left and data acquired using a positive/negative switching experiment is shown on the right. No significant degradation of data quality was observed when using the polarity switching experiment



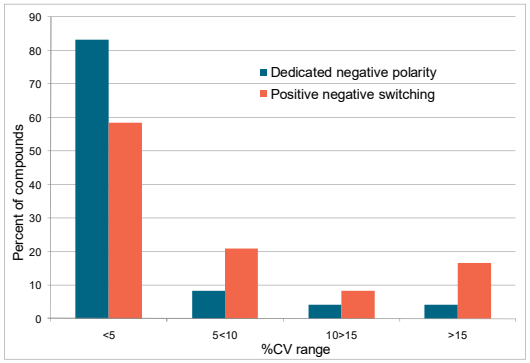


Figure 4. Comparison of reproducibility: Peak areas of all analytes monitored across five replicate injections and the %CV for each analyte were determined. The charts compare the percent of compounds having coefficients of variation in the various ranges. %CV from the dedicated positive or negative experiments were compared with the %CV from a positive/negative switching experiment. Even with increased acquisition cycle times in the switching experiment, %CV of <15% were maintained for the majority of the analytes.

Summary

It has been shown that the new QTRAP® 5500 LC/MS/MS System has sufficient speed to screen samples for the presence of over 100 analytes spanning a wide variety of compound classes. When a polarity switching experiment was utilized to obtain the maximum amount of information from a single injection, overall data quality was comparable to dedicated positive or negative experiments when intensity, signal-to-noise and reproducibility were compared. In addition Information Dependent Acquisition can be utilized to acquire library searchable MS/MS spectra for identification (Figure 5). A screening method for PPCP in environmental samples was developed to highlight key features of this approach.

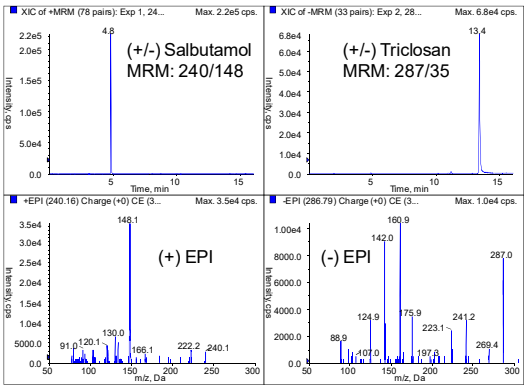


Figure 5. Information Dependent Acquisition of positive and negative polarity EPI spectra: The chromatograms and spectra above are examples for using an MRM survey scan in both polarities to automatically acquire EPI spectra. These spectra were generated using Collision Energy Spread (CES) settings for maximum fragment ion information and can be searched against mass spectral libraries for confirmation of detected compounds

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Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) in Water Samples

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Overview

A wide range of Pharmaceuticals and Personal Care Products (PPCP) were determined in river water samples using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Different water samples were injected directly into the LC-MS/MS to quantify PPCP at low parts-per-trillion levels (ng/L). Multiple Reaction Monitoring was used for detection on an SCIEX QTRAP® 5500 system for highest sensitivity and selectivity with the *Scheduled* MRM™ algorithm activated for best accuracy and reproducibility.

Introduction

PPCP encompass a wide range of pollutants, including Endocrine Disrupting Compounds (EDC), pesticides, hormones, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products to name a few. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. The diversity of chemical properties of these compounds makes method development challenging. LC-MS/MS is able to analyze polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range. In addition, state-of the-art LC-MS/MS instruments operated in selective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivity and sensitivity to quantify PPCP reproducibly at trace levels without time consuming and extensive sample preparation.[1-4]

A method is presented for analyzing 80 EDC and PPCP compounds using LC-MS/MS. This method is a straight forward approach for the analysis and identification of these compounds with excellent sensitivity and ruggedness.[5]

Experimental

Sampling and Sample Preparation

More than 70 water samples in different cities and countries from different type of waters, including drinking water, creeks, rivers, lakes, sea etc were collected by different scientist. Samples were kept frozen until analysis.

Water samples were injected directly after filtration without additional cleanup.

LC Separation

A Dionex Ultimate 3000 Rapid Separation LC system was used with a Phenomenex LUNA 2.5u C18(2)-HST 100 x3 mm column and fast gradients of water and acetonitrile with 0.1% formic acid at a flow rate of 0.8 mL/min. An injection volume of 100 µL was used.

MS/MS Detection

The SCIEX QTRAP® 5500 LC/LC-MS/MStem with Turbo V™ source and Electrospray Ionization (ESI) probe was used. The mass spectrometer was operated in MRM mode using the *Scheduled* MRM™ algorithm. MRM mode allowed screening and quantifying targeted compounds with highest selectivity and sensitivity by monitoring the transition from the precursor ion (filtered in Q1) to a product ion (generated in a collision cell Q2 and filtered in Q3). The *Scheduled* MRM™ algorithm monitors transitions automatically during a short retention time window only. This allows many more MRM transitions to be monitored in a single LC run, while still maintaining maximized dwell time and optimized cycle time.

Results and Discussion

The combination of a small particle size column (2.5 µm), high flow rate (0.8 mL/min), and large injection volume (100 µL), with high sensitivity MS/MS detection on a QTRAP® 5500 equipped with Turbo V™ source allowed the direct injection of water samples and detection of PPCP with Limits of Detection (LOD) in the low parts per trillion range. Two MRM transitions were monitored for each of the 80 analytes to quantify and identify using MRM ratio calculation. The *Scheduled* MRM™ algorithm automatically adjusts dwell times for best Signal-to-Noise (S/N) based on retention time and targeted cycle time input.

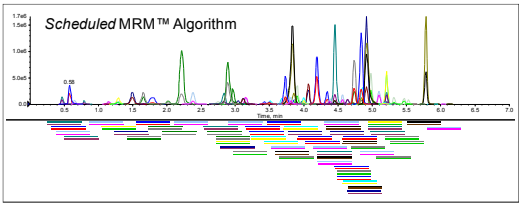


Figure. 1 The *Scheduled* MRM™ Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

An LC-MS/MS example chromatogram of 80 PPCP at a concentration of 1 µg/L is shown in Figure 2.

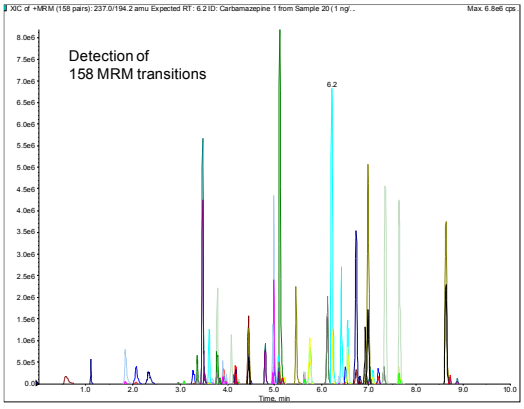


Figure 2. LC-MS/MS Detection of 80 PPCP at 1 µg/L

Two MRM transitions were monitored for each analyte, the most sensitive, first MRM transition was used for quantitation while the second MRM transition was used for qualitative identification based on ion ratio calculation.

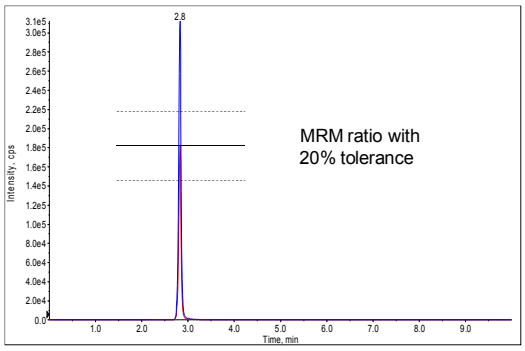


Figure 3. Identification based on MRM ratio calculation with tolerance levels of 20%

Example chromatograms of 15 selected analytes at a concentration of 10 ng/L (10 ppt) are presented in Figure 4. The superior sensitivity of the new QTRAP® 5500 system is highlighted by S/N values between 10 and 1500 (calculated with an algorithm using 3 times the standard deviation of the noise). Such low LOD allow the detection of PPCP in water samples by direct injection without additional cleanup or time consuming and extensive concentration.

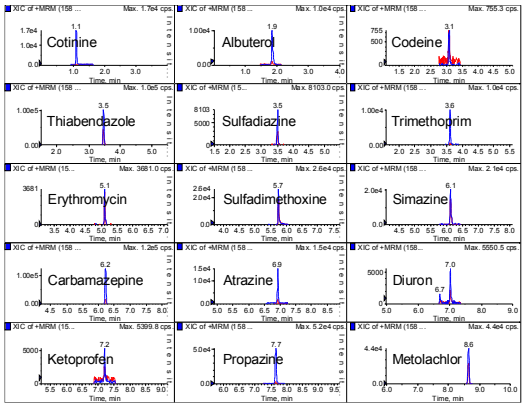


Figure 4. Example chromatograms of 15 selected PPCP at a concentration of 10 ng/L

Contents

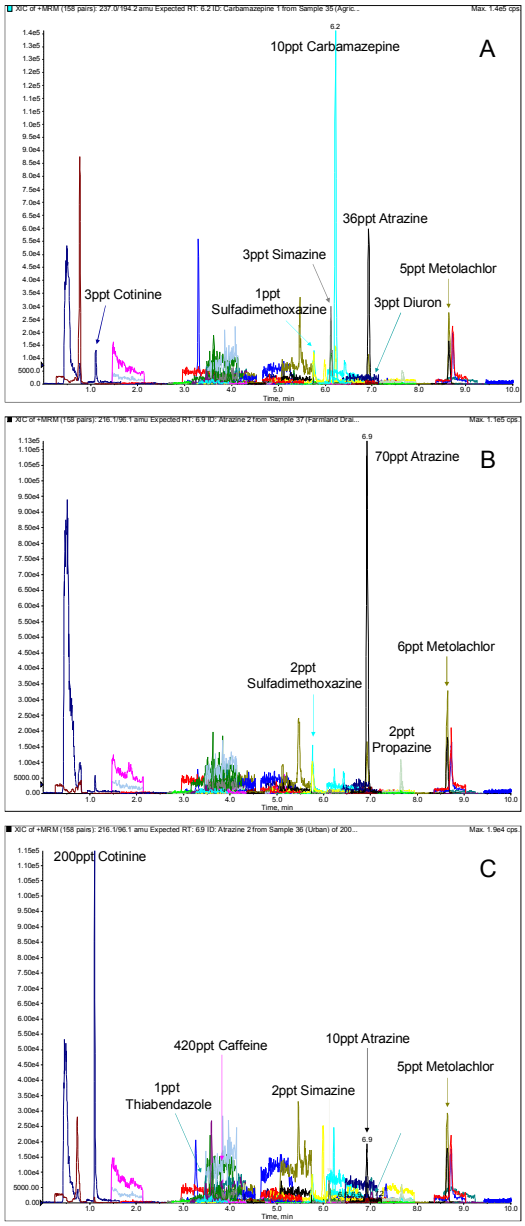
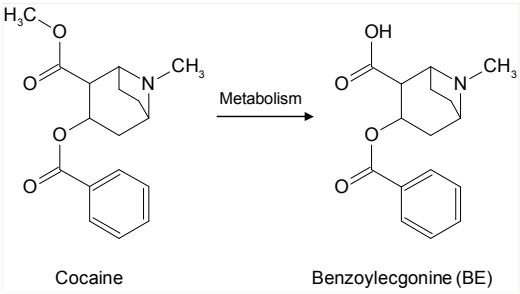


Figure 5. Quantitation of PPCP in different water samples, A: sample from an agricultural area, B: sample from a farmland drain, C: sample from an urban area

The developed LC-MS/MS method was used to screen collected water samples. Results of quantified PPCP are shown in Figure 3 A-C. All findings were identified by comparing the MRM ratio of the unknown sample with the average MRM ratio of standard injections.

Figure 6 shows the quantitative results of Benzoylecgonine, a metabolite of Cocaine, in the studied water samples. All drinking water samples had a concentration of benzoylecgonine below 5 ng/L. As expected, Benzoylecgonine was found in rivers running through major cities at concentrations of up to 200 ng/L indicating the abuse of cocaine. The concentration of Benzoylecgonine in water samples collected in less urban and wilderness areas was much lower with the exception of one creek and one lake in popular vacation destinations. The concentration of benzoylecgonine reflects the amount collectively excreted in urine and can be used the estimate drug consumption. Also when analyzed over time drug consumption habits can be investigated.[6]



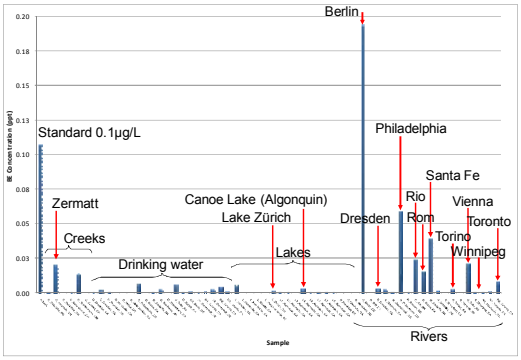


Figure 6. Findings of Benzoylecgonine, a metabolite of cocaine and indicative for cocaine abuse, in various water samples

Figure 7 shows the quantitative results of Atrazine in the studied water samples. The herbicide was found in two river samples collected in Canada at a concentration above 100 ng/L. Atrazine was detected in several water samples, including drinking water, in samples, collected throughout the world at concentrations above 10 ng/L. Atrazine was also found in a river water sample from Italy at a concentration of 12 ng/L although Atrazine is banned in the European Union.

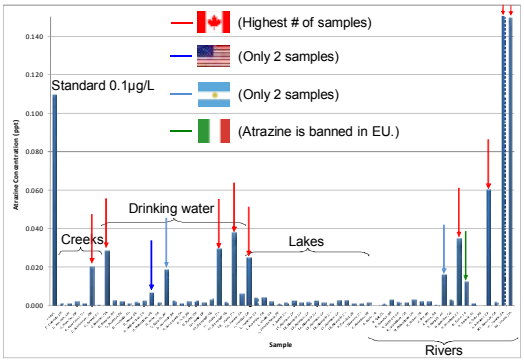


Figure 7. Findings of the herbicide Atrazine in various water samples

Summary

A method using fast LC coupled MS/MS using the *Scheduled* MRM™ algorithm for the quantitation of 80 PPCP in environmental water samples was developed and successfully applied to real samples.

The SCIEX QTRAP® 5500 system operated in MRM mode offers superior selectivity and sensitivity allowing the direct injection of water to quantify PPCP with Limits of Detection in the low ppt range. Quantified compounds were further identified using a quantifier and qualifier ratio.

As an example, results of findings of benzoylecgonine, a cocaine metabolite, and Atrazine were discussed.

Acknowledgement

The authors would like to thank colleagues of SCIEX for collecting water samples all over the world during their vacation or while attending conferences.

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3 Susan D. Richardson and Thomas Ternes: 'Water Analysis: Emerging Contaminants and Current Issues' Anal. Chem. 77 (2005) 3807-3838

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Publication number: 1790210-01

Contents

Analysis of Personal Care Products (PPCP) in Water Samples by Way of Large Volume Sample Injections

Lower Detection Limits With Large Injection Volumes

Adam Latawiec and André Schreiber
SCIEX Concord, Ontario (Canada)

Overview

A series of Pharmaceuticals and Personal Care Products (PPCP) were determined in surface waters using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Water samples were injected directly into the LC-MS/MS to quantify PPCP at parts-per-trillion levels (ng/L). Multiple Reaction Monitoring (MRM) was used on an SCIEX QTRAP® 4500 system equipped with a 2000 µL sample loop to obtain the maximum sample loading and sensitivity. Accuracy and reproducibility was increased by employing the *Scheduled* MRM™ algorithm to maximize dwell times for each analyte.

Introduction

PPCP have become important emerging contaminants, due to their presence in environmental waters (following incomplete removal in wastewater treatment or diffuse-source contamination), threat to drinking water, and concern about possible estrogenic and other adverse effects, both to wildlife and humans. It is estimated that approximately 3000 different substances are used as pharmaceutical ingredients, including painkillers, antibiotics, antidiabetics, betablockers, contraceptives, lipid regulators, antidepressants, and impotence drugs. However, only a small subset of these compounds has been investigated in environmental studies so far.[1]

The diversity of chemical properties make method development a challenge. LC-MS/MS is able to analyze polar, non-polar, and thermally labile compounds without time consuming and extensive sample preparation. When coupled to an LC system capable of injecting large sample volumes, MRM offers the selectivity and sensitivity to quantify PPCP reproducibly at trace levels.

A method is outlined showing the analysis of 40 PPCP compounds using LC-MS/MS. The method employs a large sample injection technique and high flow rates to provide identification of PPCP with excellent sensitivity.

Experimental

Sampling and Sample Preparation

More than 20 water samples from different types of waters, including drinking water, ponds, creeks, rivers, and lakes were collected and kept refrigerated until analysis. Water samples were acidified with formic acid at a level of 0.1% and injected directly after filtration without additional cleanup.

LC Preparation

An Eksigent ekspert™ ultraLC 110 system was equipped with a 1000 µL sample syringe, a 2000 µL buffer tubing line, and a 2000 µL sample loop. A Supelco core-shell PFP column (Ascentis Express F5, 10 x 4.6 mm, 2.7µm) and a fast gradients of water and methanol with 0.1% formic acid at a nominal flow rate of 1.2 mL/min was used. The flow rate was varied from 200 µL/min. during injection to 1500 µL/min. during elution to improve peak shape. Injection volumes of 100-1000 µL were used without evidence of breakthrough.

Contents

MS/MS Detection

An SCIEX QTRAP® 4500 LC/LC-MS/MS tem with Turbo V™ source and Electrospray Ionization (ESI) probe was used. The mass spectrometer was operated in MRM mode using the *Scheduled* MRM™ algorithm. The *Scheduled* MRM™ algorithm monitors transitions automatically during a short retention time window only. This allows many more transitions to be monitored in a single LC run, while maintaining maximized dwell time and optimized cycle time.

Data Processing

Data was processed in PeakView® software version 2.1 and MultiQuant™ software version 3.0.

Results and Discussion

The combination of a small fused core particle size (2.6 µm), high flow rate (1.5 mL/min), and a large injection volume (1000 µL) with high sensitivity MS/MS detection on a QTRAP® 4500 instrument allowed the direct injection of waters samples and detection of PPCP with Limits of Detection (LOD) in the low parts per trillion range. Two MRM transitions were monitored for each of the 40 analytes to quantify and identify using the ratio of quantifier and qualifier MRM.

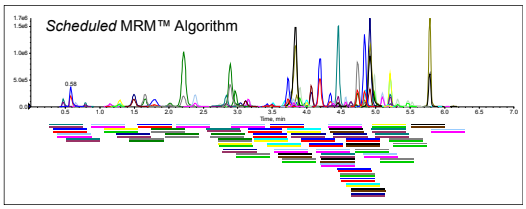

Figure 1. The *Scheduled* MRM™ algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

An LC-MS/MS example chromatogram of 40 PPCP at a concentration of 0.1 µg/L (100 ppt) is shown in Figure 2.

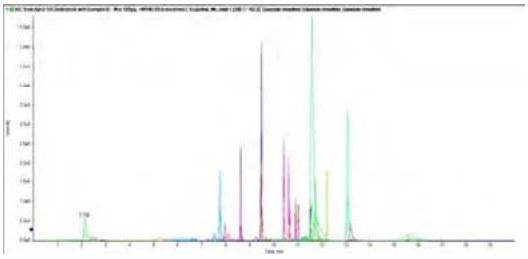

Figure 2. LC-MS/MS Detection of 40 PPCP at 0.1 µg/L (100 ppt) using an injection volume of 1 mL

Two MRM transitions were monitored for each analyte, the most sensitive MRM transition was used for quantitation while the second MRM transition was used for qualitative identification based on the automatic ion ratio calculation in MultiQuant™ software.

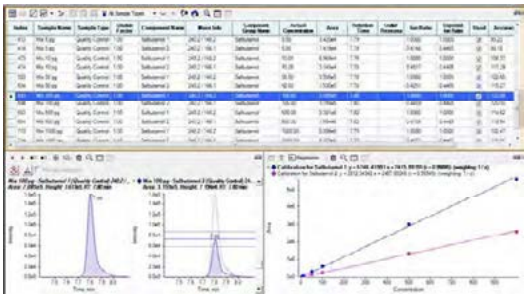

Figure 3. Identification of 100 ppt Salbutamol based on MRM ratio calculation with a tolerance levels of 20%

The sensitivity and signal-to-noise (S/N) gain using a large volume sample injection on the SCIEX QTRAP® 4500 system is highlighted in Figure 5.

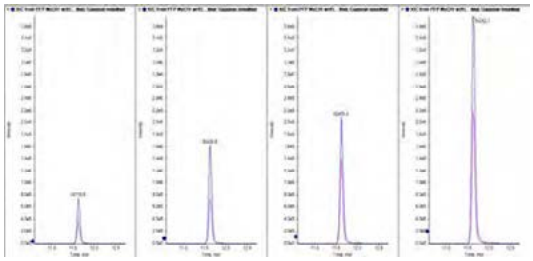

Figure 4. Sensitivity and S/N gain for Salbutamol when injecting 100, 250, 500, and 1000 µL (The S/N was automatically calculated in PeakView® software using 3 times the standard deviation of the adjacent noise.)

Example chromatograms of 12 selected analytes at a concentration of 5 ng/L (5 ppt) are presented in Figure 5

Such low Limits of Detection (LODs) allows for the use of less sensitive mass spectrometers for the determination of PPCP in water samples by simples changes to the syringe and sample loop injection volumes of the ultraLC 110 system.

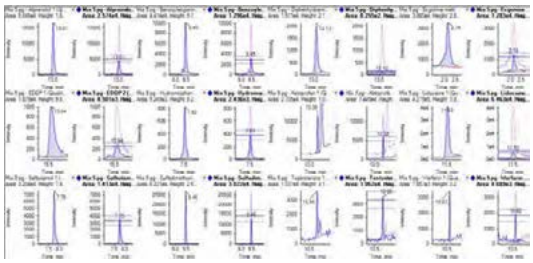

Figure 5. Example chromatograms of 12 selected PPCP at a concentration of 5 ng/L (top left to bottom right: Alprenolol, Benzoylecgonine, Diphenhydramine, Ecgonine methyl ester, EDDP, Hydromorphone, Ketoprofen, Lidocaine, Salbutamol, Sulfadimethoxine, Testosterone, Warfarin)

The detection of PPCP at low ppt levels can now be accomplished without additional cleanup or time consuming and extensive sample concentration.

Summary

Large sample injection using 1000 µL or larger sample loops provides an easy and effective way to expand the analytical capabilities of the Eksigent ekspert™ ultraLC 110 system with a mid-range mass spectrometers such as the SCIEX QTRAP® 4500. In this example we are able to achieve very low detection limits of many common pharmaceuticals and personal care products. Even in the absence of any extensive sample preparation, LOD in the ppt range (ng/L) were routinely achieved. The large sample injection procedure provides an effective mechanism to screen drinking and surface waters for PPCP.

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Publication number: 10160814-01

Contents

Determination of Selected Illegal Drugs and its Important Metabolites in Waste Water by Large Volume Direct Injection HPLC-MS/MS

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[2] *SCIEX Concord, Ontario (Canada)*

Overview

The present application note explores the capability of determining priority drugs such as cocaine, its main metabolite benzoylecgonine, and morphine in the complex matrix waste water using the highly sensitive API 5000™ LC-MS/MS system with Electrospray Ionization (ESI) operated in Multiple Reaction Monitoring (MRM). Sewage treatment plant influents and effluent samples were injected directly into the HPLC-MS/MS system.

Introduction

It is well known that the use of illicit drugs is increasing worldwide, and millions of individuals have been reported to consume cocaine, heroin, amphetamine-like stimulants, Marijuana as well as other drugs with sometimes dramatic consequences for human health and social behavior. Particularly, according to the World Drug Report 2009 cocaine consumption is still an important issue.[1]

So far, several methods have been established to determine illicit drug consumption in clinical and forensic toxicology. Online and off-line Solid Phase Extraction (SPE) techniques have been used to enrich residues and metabolites of illicit drugs from biological fluids (blood, urine etc.). For separation and quantification high resolution Gas Chromatography Mass Spectrometry (GC-MS) and High Pressure Liquid Chromatography Mass Spectrometry (HPLC-MS) have been shown to be the methods of choice. For GC-MS however, derivatization reactions are necessary to get the compounds into the gas phase, making the sample preparation time-consuming. According to the most recent literature HPLC-MS/MS using specific transitions of the different compounds in the so-called Multiple Reaction Monitoring mode (MRM) has evolved as a promising method to detect traces of illicit drugs.[2-5]

Large volume injection (LVI) is a rather little-known technique that involves the direct injection sample volumes from 100 – 5000 µL versus the more conventionally injected volumes of 5 - 20 µL. LVI offers significant advantages such as an increase in sensitivity and accuracy due to minimal sample manipulation. Moreover, for LVI the sample volume required is smaller compared to SPE techniques since the entire sample can be injected.

Experimental

Standards and Internal Standards (IS)

Target analytes and corresponding IS are listed in Figure 1 and Table 3. Standards and IS were obtained as solution in methanol or acetonitrile at a concentration of 100 µg/mL or 1 mg/mL. Working standard solutions were prepared by appropriate dilution with methanol.

Contents

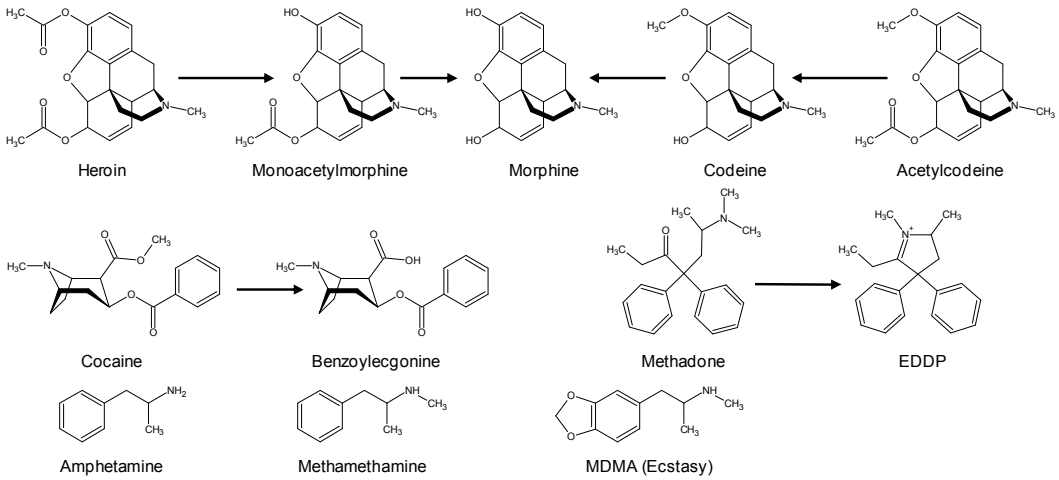

Figure 1. Studied illicit drugs and metabolites

Sample Preparation

24 h composite influent/effluent waste water samples were immediately acidified to pH 2 using HCl, filtered and stored in the dark at 4°C until analysis. For the analysis 1 mL of waste water sample was transferred to an HPLC vial, 10 µL of deuterated IS was added and the vial capped. The final concentration of IS was 500 ng/L.

High Performance Liquid Chromatography (HPLC)

- Agilent 1200 system, including degasser, binary pump, column oven
- Gerstel MPS 3C injection system with needle washing station
- Phenomenex Synergi Hydro-RP (100 x 2.1 mm) 2.5 µm column
- Gradient of water/methanol + 2 mM ammonium formate + 0.2% of formic acid, details in Table 1
- Flow rate 0.3 mL/min at 40°C
- Injection volume 100 µL

Tandem Mass Spectrometry (MS/MS)

- API 5000™ LC-MS/MS system
- Turbo V™ source with ESI probe in positive polarity
- Monitoring of two characteristic MRM transitions per compound (Table 3) with Collision Gas set to 10
- Gas and ion source parameters (Table 2)

Table 1. HPLC gradient

Step	Time (min)	% A	% B
0	0.0	98	2
1	1.0	98	2
2	11.0	5	95
3	13.0	5	95
4	14.0	98	2
5	18.0	98	2

Table 2. Ion source parameters using Electrospray Ionization (ESI)

Parameter	Value
Curtain Gas (CUR)	40 psi
IonSpray Voltage (IS)	3000 V
Temperature (TEM)	650°C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	50 psi

Contents

Table 3. MRM transitions of target analytes and internal standards, MRM ratios and tolerance intervals according to guideline 2002/657/EC[6]

Compound	MW	Q1	Q3	MRM Ratio	Tolerance	Tolerance Interval
Morphine-d3	288.1	289.2	152.1	-	-	-
Morphine	285.1	286.2	152.0 165.2	0.48	25	0.37 - 0.63
Amphetamine-d3	138.1	139.1	122.1	-	-	-
Amphetamine	135.1	136.1	90.0 119.2	0.43	25	0.36 - 0.59
Codeine-d3	302.1	303.2	215.1	-	-	-
Codeine	299.2	300.1	215.1 165.0	0.91	20	0.77 - 1.11
Methamphetamine-d5	154.1	155.1	92.0	-	-	-
Methamphetamine	149.1	150.1	91.0 119.2	0.27	25	0.22 - 0.36
MDMA-d5	198.1	199.1	165.3	-	-	-
MDMA	193.1	194.1	163.0 105.1	0.27	25	0.22 - 0.36
Monoacetylmorphine-d3	330.1	331.1	165.2	-	-	-
6-Monoacetylmorphine	327.1	328.1	165.2 211.1	0.91	20	0.77 - 1.11
Benzoylecgonine-d3	292.1	293.1	171.0	-	-	-
Benzoylecgonine	289.1	290.1	168.0 105.0	0.40	25	0.32 - 0.55
Acetylcodeine-d3	344.1	345.2	225.1	-	-	-
Acetylcodeine	341.1	342.2	225.2 164.9	0.45	25	0.36 - 0.59
Cocaine-d3	306.4	307.3	185.0	-	-	-
Cocaine	303.1	304.2	182.3 104.9	0.16	30	0.13 - 0.24
EDDP-d3	281.2	281.2	234.1	-	-	-
EDDP	278.2	278.2	234.1 249.1	0.38	25	0.30 - 0.50
Methadone-d3	312.2	313.2	268.2	-	-	-
Methadone	309.2	310.2	265.2 105.0	0.45	25	0.36 - 0.59

Figure 1: SCIEX Triple Quad™ 3500

Method validation data

- Recovery: matrix spike (c = 200 ng/L) 79-118% for all analytes
- Blank analysis: laboratory blanks did not contain any traces of drug residues (< 10% of the lowest calibration standard)
- Linearity: 5 point calibration, working range: 20-1000 ng/L, r^2: 0.999-1.000
- Limits of Quantitation (LOQ): 20 ng/L; S/N > 10 for all compounds at 20 ng/L (except for amphetamine: S/N 5)
- Precision: t_R deviations ≤ 0.15% for most compounds, area deviation: ≤ 6% for most compounds, n = 6

Contents

Results and Discussion

The compound optimization feature implemented in the Analyst® software (version 1.5) was used to optimize compound dependent parameters and source dependent parameters. The final method was built using two MRM transitions per compound, the more sensitive one for quantitation, the second one for identification. MRM ratios, tolerances and tolerance intervals were calculated according to the European guideline (Table 3).[6] Due to the complexity of the matrix and the direct injection mode the ion source temperature was set to 650°C to keep the source as clean as possible.

The separation of the drug mixture was performed on a 100 mm Synergi Hydro-RP column which contains a polar endcapping (Figure 2). Superior separation and peak shape of the rather polar molecule morphine could be observed in comparison to separation on a traditional C18 column.

The corresponding separation of a real waste water influent sample under the same chromatographic conditions is presented in Figure 3. Note the excellent retention time comparability between the chromatogram of the standards and the real extract.

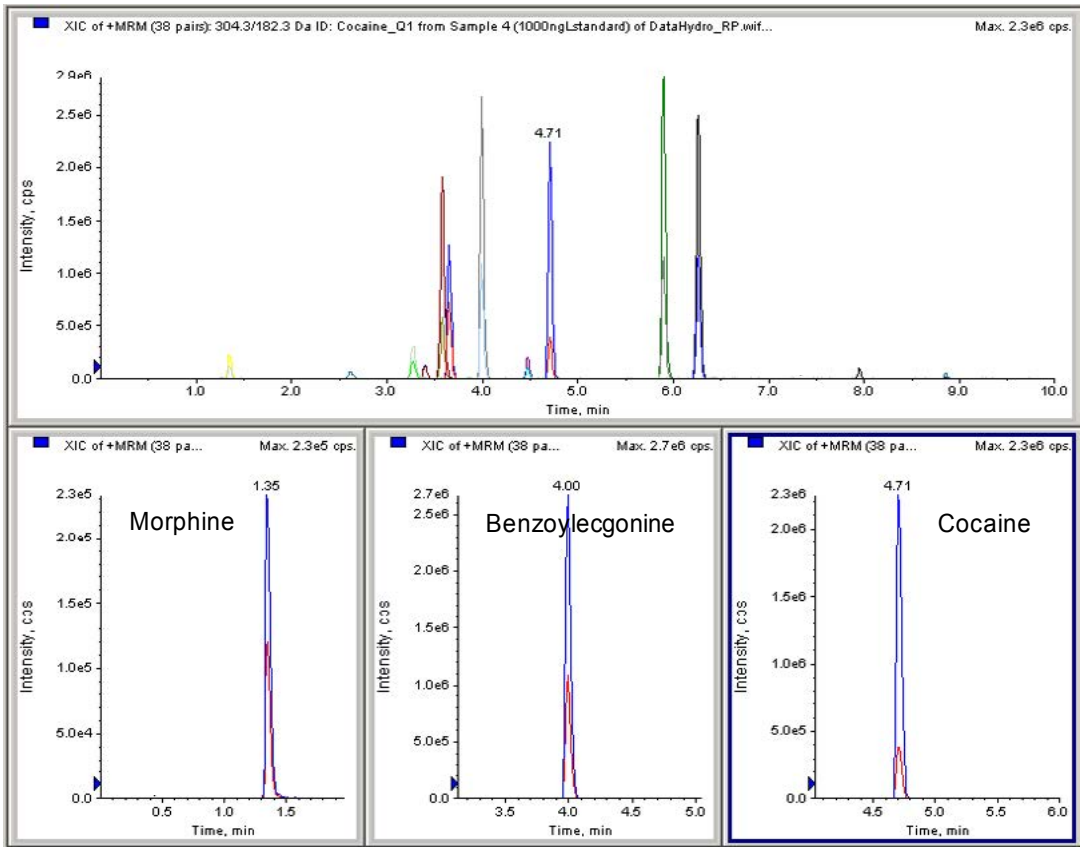

Figure 2. Total ion chromatogram (TIC) of a 1000 ng/L standard and extracted ion chromatograms (XIC) of morphine, benzoylecgonine, and cocaine

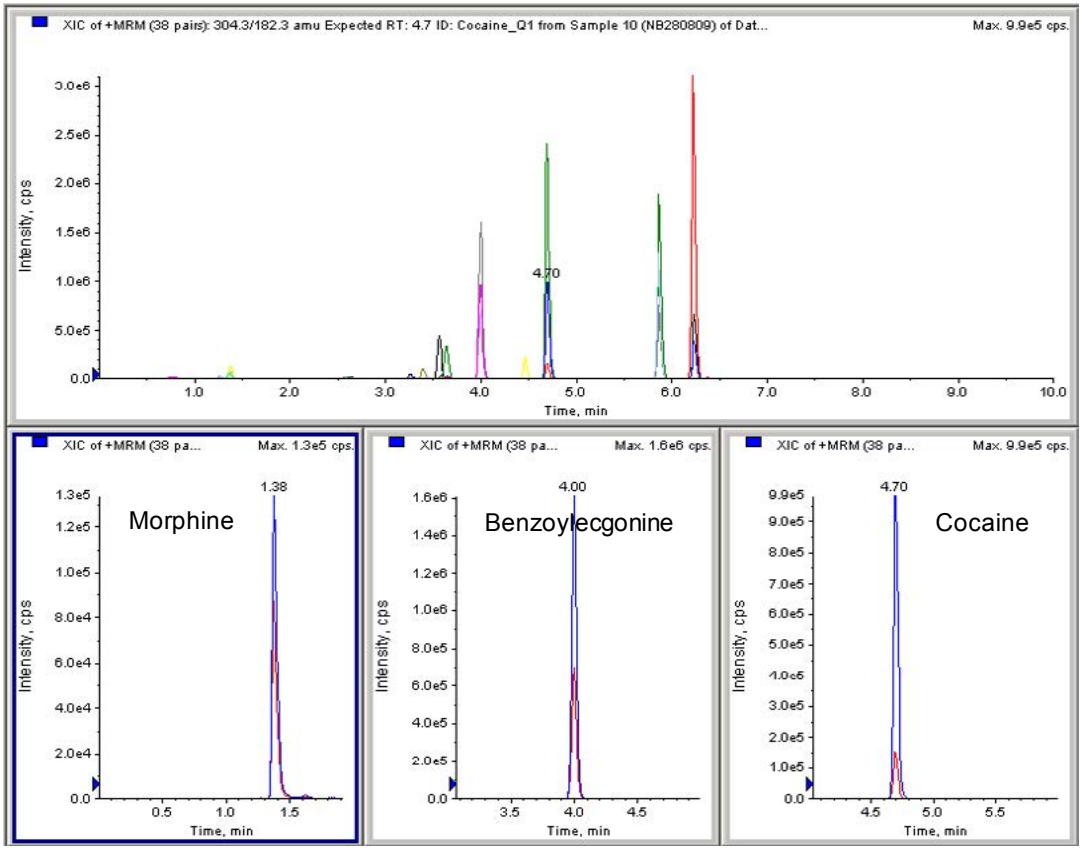

Figure 3. Total ion chromatogram (TIC) of a waste water influent sample and XIC of morphine, benzoylecgonine, and cocaine

During method development different injection volumes (20 – 100 µL) were investigated. As for the 100 µL injection no real difference in peak shape and signal saturation was observed this injection volume was maintained throughout the study.

During method validation standard addition experiments were performed in order to study the matrix influence on quantitation. Results are exemplified with the two most important compounds cocaine and benzoylecgonine. In a first attempt these two drugs were quantified in a waste water sample using standards dissolved in milliQ water (c = 168.4 and 453.2 ng/L respectively, n = 5, Figure 4). Then the waste water sample was spiked with predefined amounts of the target compounds and a regression line was obtained after quantification of the spiked extracts (c = 168.2 and 453.4 ng/L, n = 5). As can be seen from Figure 4 comparable results were obtained for the quantitation in the matrix.

Furthermore, the matrix effect was studied using post-column infusion of target analytes using a T-piece and simultaneous injection of a waste water samples (Figure 5). As for other water matrices the typical strong ion suppression can be observed at the beginning of the chromatographic run between 0.5-1.5 min whereas no ion suppression can be observed in later part of the chromatogram. Therefore, the only compound which might be influenced by the matrix will be morphine which elutes at 1.35 min. However, the use of morphine-d3 as an internal standard will compensate for possible ion suppression.

Contents

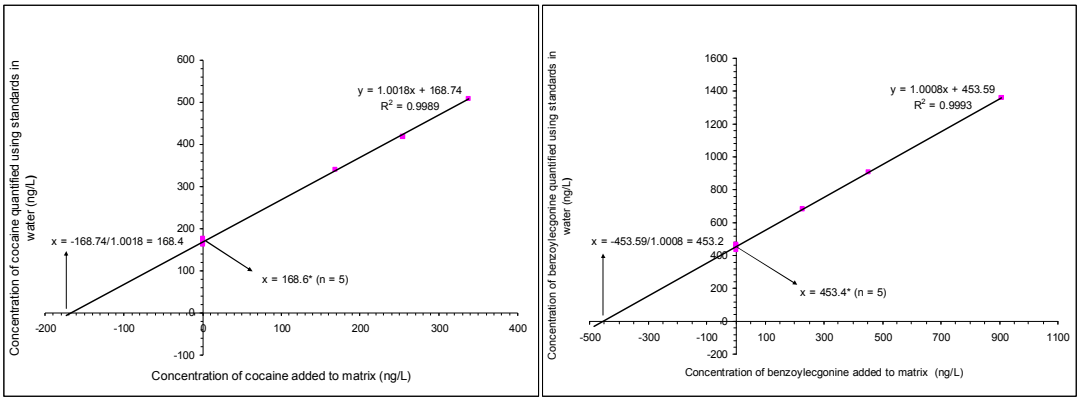

Figure 4. Standard addition of cocaine and benzoylecgonine to quantify both analytes in a waste water matrix

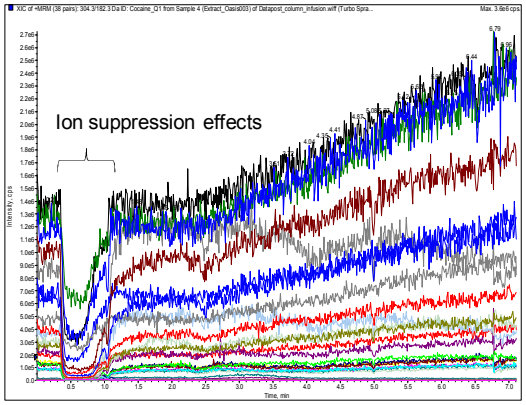

Figure 5. Matrix effect study using post column infusion of analytes

After validation the method was applied to determine the concentrations of these drugs in different waste water influent samples of sewage treatment plants (STP).

In Figure 6 results of three different STP are shown, one STP being a small, rural STP and two being urban STP.

Benzoylecgonine, the main metabolite of cocaine, was detected in all three STP, however at different levels. The same is true for morphine, which is however not exclusively originated form heroin but also heavily applied as analgesic medication (painkiller) in hospitals. 6-Monoacetylmorphine, a specific marker for heroin use, was detected in concentrations up to 30 ng/L. Methadone, a synthetic opioid, used medically as an analgesic and antitussive (cough suppressant) and a maintenance anti-addictive for use in patients on opioid drugs, and its main metabolite EDDP were detected in similar concentrations in all STP, EDDP showing higher levels. The amphetamine, methamphetamine, and MDMA (ecstasy) were present in trace amounts (< 50 ng/L).

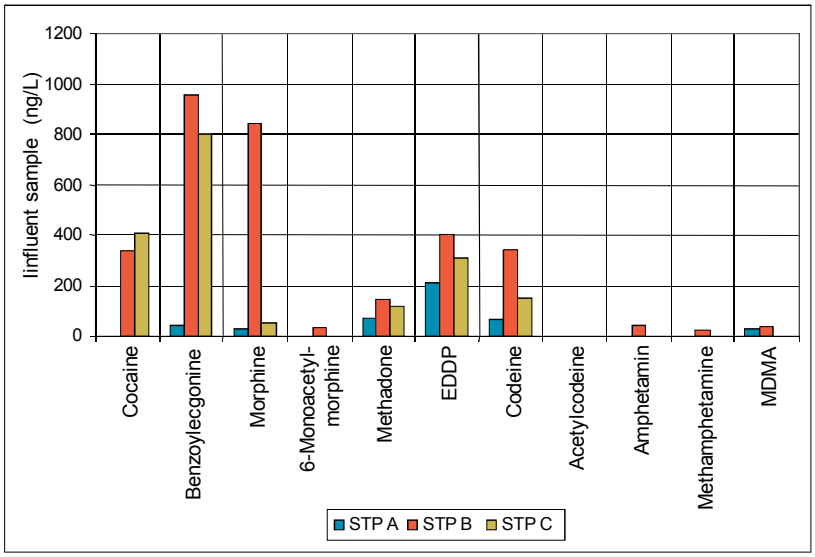

Figure 6. Drug concentrations in 3 different sewage treatment plants (STP) in ng/L

Summary

The highly sensitive API 5000™ LC-MS/MS system turned out to be an ideal instrument for the detection of important illegal drugs such as cocaine in waste water samples. Two characteristic MRM transitions were monitored per compound for the specific detection of the different drugs of abuse and their metabolites. Direct injection of large volumes (100 µL) of water samples allowed the detection of these compounds down to 20 ng/L. A dominant matrix effect was only noticed in the void volume of the column at the beginning of the chromatographic run. The method was used to determine drug levels in waste water samples of different sewage treatment plants. The dominant compounds were cocaine and it main metabolite benzoylecgonine, morphine, methadone and its metabolite EDDP.

Acknowledgements

The authors wish to thank Prof. Dr. R. Brenneisen, and Chr. Mathieu (Department of Clinical Research, University of Bern) for providing standards of illegal drugs and for waste water sampling and preparation.

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6 European Guideline: 2002/657/EC

Publication number: 0990310-01

Contents

Quantitation and Identification of Legal and Illicit Drugs in Wastewater in the low Nanogram per Liter Range using Large Volume Direct Injection and SCIEX QTRAP® Technology

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Overview

The present application note describes the optimization of the front-end HPLC methodology by improving the separation of legal and illicit drugs such as cocaine, MDMA and methamphetamine and its important metabolites such as benzoylecgonine and monoacetylmorphine using an unusual 5 µm particle core-shell column with 4.6 mm ID. The SCIEX QTRAP® 5500 system was used to detect target compounds in Multiple Reaction Monitoring (MRM) mode. To overcome saturation effects observed for high sensitive MRM transitions the collision energies (CE) were detuned for some compounds. Different acquisition modes such as *Scheduled* MRM™ and *Scheduled* MRM™ Pro with Information Dependent Acquisition (IDA) of MS/MS full scan spectra were explored. Thus, the analytes could be quantified in a traditional way using two MRM transitions in MultiQuant™ 3.0 software, and additionally, QTRAP® MS/MS spectra could be used for identification at trace levels in MasterView™ 1.1 software. The optimized method was successfully applied to the measurement of drugs in influent wastewater samples collected during a party event (Street Parade Zürich).

Introduction

Drug abuse is a global problem with major negative impacts on human health and social welfare. Illicit drugs are substances for which nonmedical use is prohibited by national or international laws. Important groups of illicit drugs are opioids, cocaine, cannabis, amphetamines and ecstasy (MDMA). Among those, amphetamines and MDMA currently demand the most attention by law enforcement agencies.[1] In Europe, the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) is the reference point on drugs and drug addiction.[2]

For drug consumption, questionnaire-based surveys have traditionally been performed to estimate drug use. However, it is recognized that this method is not sufficient to monitor trends in drug use quickly and adequately and therefore complementary data from other sources are needed.[3]

Since several years, the chemical analysis of influent-wastewater for the combined excretions products of illicit drugs has become a potent approach for monitoring patterns and trends of drugs consumed in a community.[3, 4] Meanwhile, the study of spatial differences and temporal changes in illicit drug use through the method of wastewater analysis, also called sewage epidemiology, is becoming an important tool to estimate drug consumption in Europe.

Nowadays, LC-MS/MS has become the method of choice for the quantitative determination of illicit drugs in aqueous matrices.[6] The *Scheduled* MRM™ algorithm using unique fragment ions and specific retention times of the molecules has evolved as a promising method for the reliable quantitation of compounds in water matrices.[7] Large volume direct injection (LVDI) techniques together with the exceptional sensitivity of the SCIEX QTRAP® 5500 system allow limits of quantitation (LOQ) in the low ng/L range.[8, 9] Finally, the acquisition of MS/MS spectra using Enhanced Product Ion scanning (EPI) in the Linear Ion Trap of the QTRAP® mass spectrometer provides additional confidence of the presence of the analytes under investigation.

Contents

The Street Parade Zürich is an interesting event to study such trends because the wastewater influent is strongly affected due to the relationship between number of inhabitants (approx. 300000) and visitors (approx. 950000).

Experimental

Standards and Internal Standards (IS)

Target analytes (morphine, monoacetylmorphine, amphetamine, methamphetamine, codeine, monoacetylcodeine, MDMA, cocaine, benzoylecgonine, methadone, EDDP, and mephedrone) as well as their corresponding deuterated IS were obtained as solutions in methanol or acetonitrile from Lipomed, Arlesheim, Switzerland. Working standard and calibration solutions were freshly prepared by appropriate dilution with methanol and water (purified using a water purification system from ELGA, Villmergen, Switzerland).

Sampling and sample preparation

Wastewater samples were obtained from the Zürich-Werdhölzli sewage treatment plant (STP) and immediately acidified to pH 2 using HCl, filtered and stored in the dark at 4°C until analysis. For the analysis 1 mL of wastewater sample was transferred to an HPLC vial, diluted 1:1 and 1:10 respectively, with ELGA water, and 10 µL of deuterated IS added. The final concentration of the IS was 500 ng/L. For ion suppression studies samples were also diluted 1:1 or 1:10.

Samples were collected as 24 h composite influent wastewater samples over 7 days between Wednesday July 30th (SP1) and Tuesday August 5th, 2014 (SP7). Sample SP 4 corresponds to the day of the Street Parade event and SP 5 to the day after.

LC Separation

A Dionex Ultimate 3000 HPLC system with a binary gradient pump, autosampler and column oven (30°C) was used for the chromatographic separation.

The LC method was completely redesigned. In the previous method a Phenomenex Synergi Hydro-RP column 100 x 2.1 mm 2.5 µm was used.[6] In this study, a core-shell LC column, Phenomenex Kinetex C18, 100 x 4.6 mm, 5 µm, was applied. Mobile phase A was water + 0.1% formic acid + 2 mM ammonium formate and mobile phase B acetonitrile. A flow rate 900 µL/min was used. The gradient with a total run time of 12 minutes is listed in Table 1. The injection volume was set to 100 µL.

Table 1. LC gradient

Step	Time (min)	A (%)	B (%)
0	0.0	98	2
1	1.0	98	2
2	7.0	35	65
3	7.1	0	100
4	9.0	0	100
5	9.1	98	2
6	12.0	98	2

MS/MS Detection

A SCIEX QTRAP® 5500 system with Turbo V™ source with ESI probe was used. The target compounds were detected in positive polarity. The ion source parameters were optimized for the new LC conditions using the Compound Optimization (FIA) function in Analyst® software.

Table 2. Ion source parameters

Parameter	Value
Curtain Gas (CUR)	30 psi
IonSpray voltage (IS)	3000 V
Temperature (TEM)	650°C
Nebulizer Gas (GS1)	70 psi
Heater Gas (GS2)	70 psi

Two characteristic MRM transitions were monitored for each analyte, and 1 MRM transition for each internal standard (Table 3). The MRM transitions were taken over from the existing method[6] and MRM transitions of mephedrone and the corresponding IS were added.

The *Scheduled* MRM™ algorithm was activated to monitor compounds only around the expected retention time to maximize dwell times and optimize the cycle time of the methods.

Table 3. MRM transitions and retention time (RT)

Compound	RT (min)	Q1	Q3
Morphine (MOR)	3.4	286	152
	3.4	286	165
Codeine (COD)	4.2	300	215
	4.2	300	165
Amphetamine (AMP)	4.3	136	91
	4.3	136	119
Monoacetylmorphine (MAM)	4.5	328	165
	4.5	328	211
Methamphetamine (MAMP)	4.5	150	91
	4.5	150	119
MDMA	4.6	194	163
	4.6	194	105
Mephedrone (MEP)	4.7	178	160
	4.7	178	145
Benzoylecgonine (BEC)	4.9	290	168
	4.9	290	105
Monoacetylcodeine (MAC)	5.2	342	225
	5.2	342	165
Cocaine (COC)	5.4	304	182
	5.4	304	105
EDDP	6.4	278	234
	6.4	278	249
Methadone (MET)	6.7	310	265
	6.7	310	105
IS Morphine	3.4	289	152
IS Codeine	4.2	303	215
IS Amphetamine	4.3	139	122
IS Monoacetylmorphine	4.5	331	165
IS Methamphetamine	4.5	155	92
IS MDMA	4.6	199	165
IS Mephedrone	4.7	181	163
IS Benzoylecgonine	4.9	293	171
IS Monoacetylcodeine	5.2	345	225
IS Cocaine	5.4	307	185
IS EDDP	6.4	281	234
IS Methadone	6.7	313	268

The *Scheduled* MRM™ pro algorithm in Analyst® software (version 1.6.2) was used as an alternative survey scan for information dependent acquisition of MS/MS full scan spectra for identification. Two MRM transitions were monitored and at the same time EPI spectra were recorded when a signal exceeds a compound specific threshold. The setup of this methods is described in a separate note.[10] The thresholds of the internal standards were set very high to exclude them from MS/MS acquisition.

Results and Discussion

Optimization of LC Conditions

The column dimension 100 x 4.6 mm allowed large volume direct injection without the breakthrough of polar compounds like morphine. The void time of the column was approximately 1 minute, and the elution window of the analytes was between 3.4 and 6.8 minutes.

The 5 µm core-shell material resulted in very sharp chromatographic peaks of approximately 4 seconds width (Figure 1) while the column pressure was very low, ~100 bar, at 900 µL/min and 30°C.

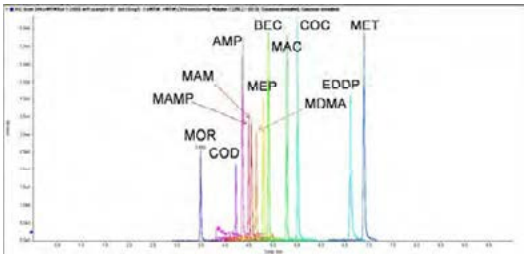

Figure 1. Example chromatogram of a 10 ng/L standard

The optimization of the LC conditions lead to intensity gains up to a factor of 10 for early eluting compounds like morphine and a factor of 2 for medium and late eluting compounds like MDMA (Figure 2). Signal-to-Noise (S/N) values were increased by a factor of 2 to 10.

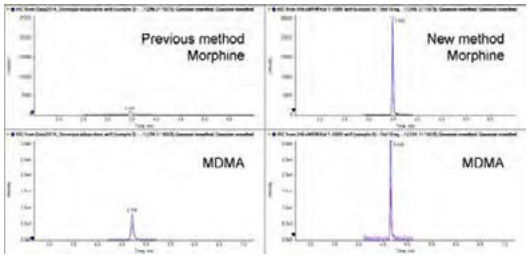

Figure 2. Sensitivity gains for morphine and MDMA by optimizing LC conditions, previous method (left) and newly optimized method (right)

Detuning of Collision Energies (CE)

With sharper and higher LC signals some compounds (e.g. MDMA, benzoylecgonine, cocaine) could cause detector saturation when present at higher concentration in water samples. Some high sensitivity transitions were detuned to minimize this effect and maintain linear dynamic range for quantitation.

The example shown in Figure 3 shows the detuning of CE for benzoylecgonine.

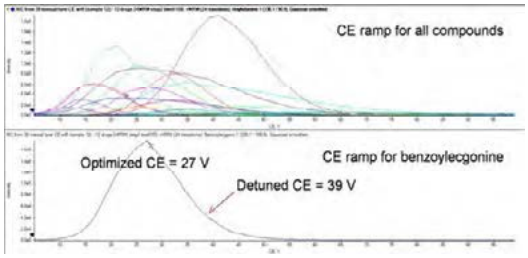

Figure 3. Detuning of CE to minimize detector saturation for benzoylecgonine

Method Data

Method data are listed in Table 4 and summarized below.

Table 4. Sensitivity (S/N calculated using 3x standard deviation in PeakView® software), repeatability (coefficient of variation, %CV) and linearity from 1 to 1000 ng/L (linear fit with 1/x weighting, coefficient of regression, r, using the *Scheduled* MRM™ and the *Scheduled* MRM™-IDA-MS/MS method)

Compound	S/N at 1 ng/L	%CV at 10 ng/L	r (MRM)	r (IDA)
Morphine 1	35	2.5	0.999	0.994
Morphine 2	22	1.4	0.999	0.991
Codeine 1	33	4.4	0.999	0.998
Codeine 2	14	7.1	0.999	0.993
Amphetamine 1	4	4.2	0.997	0.996
Amphetamine 2	8	5.5	0.999	0.997
Monoacetylmorphine 1	14	11.4	0.996	0.992
Monoacetylmorphine 2	15	9.3	0.999	0.995
Methamphetamine 1	3	6.4	0.995	0.994
Methamphetamine 2	17	4.8	0.995	0.993
MDMA 1	24	4.3	0.999	0.993
MDMA 2	2.5	7.7	0.997	0.998
Mephedrone 1	28	6.6	1.000	0.997
Mephedrone 2	14	4.4	0.999	0.994
Benzoylecgonine 1	37	5.9	0.999	0.996
Benzoylecgonine 2	18	5.9	0.999	0.997
Monoacetylcodeine 1	62	4.9	0.998	0.997
Monoacetylcodeine 2	7	4.2	0.998	0.996
Cocaine 1	72	1.9	0.999	0.996
Cocaine 2	15	3.1	1.000	0.998
EDDP 1	47	2.7	0.998	0.998
EDDP 2	43	2.7	0.999	0.998
Methadone 1	43	3.5	0.996	0.985
Methadone 2	22	2.7	0.996	0.989

- Linearity and working range: 1 ng/L to 1000 ng/L for all compounds (except amphetamine and methamphetamine). This corresponds to 2-2000 ng/L for 1:1 diluted samples, and 10-10000 ng/L for 1:10 diluted samples.

Contents

- Limits of quantitation (LOQ): 1 ng/L; S/N > 10 for all compounds (except amphetamine.1 S/N=4, methamphetamine.1 S/N=3)
- Linearity: r > 0.994
- Accuracy of the standards from 1 to 1000 ng/L: between 80 and 120%
- Precision: RSD% typically between 2.5 and 6% for 6 consecutive injections of a standard at 10 ng/L (except acetylmorphine)

Ion suppression

Matrix effects have been investigated by T-infusion experiments. Matrix load of wastewater samples can strongly differ from sample to sample. Generally dilution is necessary to minimize ion suppression when large volume direct injection is used. Figure 4 shows that some suppression effects can still be observed in the elution window of the analytes with a 1:1 dilution. But with dilution 1:10 nearly no ion suppression was observed.

It can also be seen that strong matrix effects are present in the range of the void time up to a retention time of 2.0 min. However, the earliest eluting compound morphine has a retention time of 3.4 min and is not affected by ion suppression.

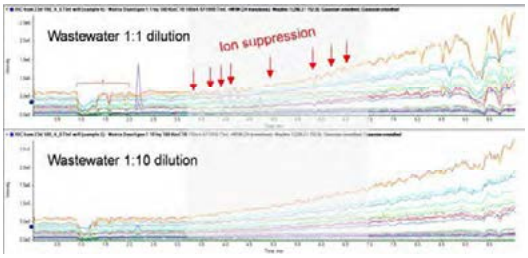

Figure 4. Investigation of matrix effects

Street Parade Results

The gain in sensitivity and the lower detection limits help to detect low levels of illegal drugs. Figure 5 shows the day 5 sample of the Street Parade 2014, diluted 1:10.

Figure 6 shows the profile of MDMA (ecstasy), benzoylecgonine (metabolite of cocaine) and monoacetylmorphine (metabolite of heroin) over the time period of the Street Parade. These profiles indicate different consumption amounts during the event resulting in different wastewater profiles.

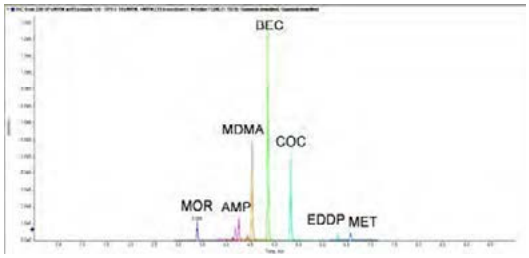

Figure 5. Day 5 sample of the Street Parade Zürich, injected directly after 1:10 dilution

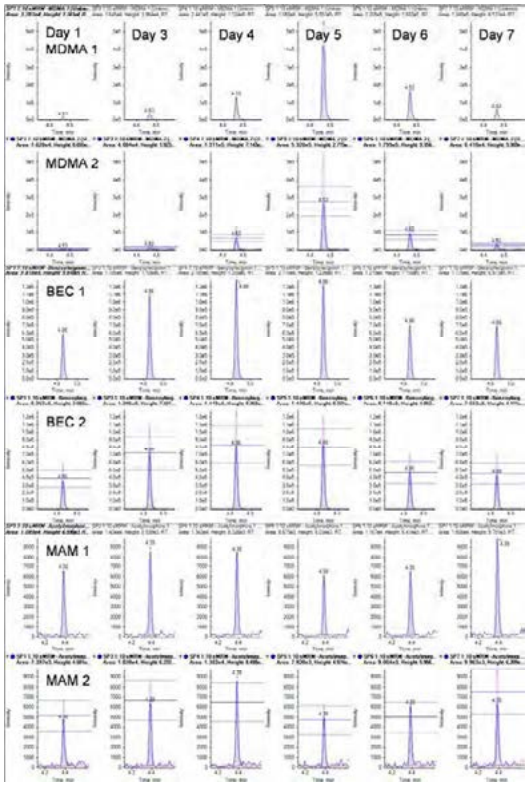

Figure 6. Profile of MDMA (ecstasy), benzoylecgonine (metabolite of cocaine) and monoacetylmorphine (metabolite of heroin) over the time period of the Street Parade. Data indicate high consumption of MDMA and increased consumption of cocaine during the event. The concentration of MAM in wastewater was relatively constant. The peak review in MultiQuant™ software allows reviewing MRM ratios and tolerance levels for compound identification.

Compound Identification

Commonly at least two MRM transitions are monitored per compound and the ratio of quantifier and qualifier ion is used for compound identification. Guidelines define identification criteria and tolerance levels for ion ratios.[11] However, MRM ratios are not always unambiguous. Matrix interferences might disturb one of the two transitions, and thus, ion ratio identification fails. In addition, ion ratios are often falsified at the upper end of the linear dynamic range because of detector saturation. On the other hand, the qualifier MRM can be too weak to be used for identification at the lower end of the dynamic range.

With the *Scheduled* MRM™ pro-IDA-MS/MS workflow it is possible to monitor two transitions for each compound and use the ratio for identification. In addition, QTRAP® MS/MS full scan spectra are collected automatically. These spectra can be searched against mass spectral libraries in MasterView™ software for increased confidence in identification.

The IDA triggering works effectively due to individual thresholds for each compound. Figure 7 shows that chromatograms acquired in IDA mode have slightly less data points across the LC peak at the time where the MS/MS spectrum was acquired, but still enough data points for accurate and reproducible quantitation. Results presented in Table 4 also show that linearity was not compromised when using the IDA method.

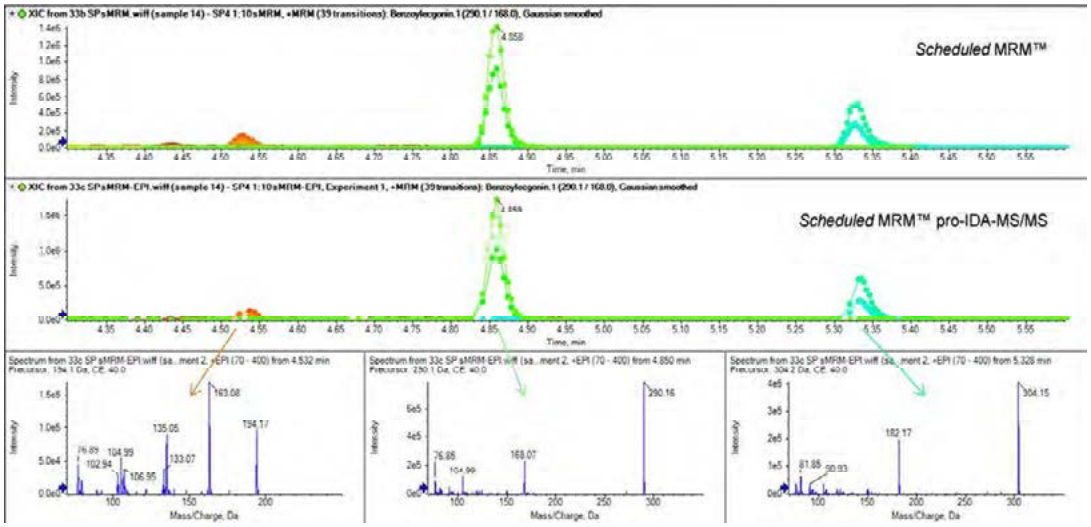

Figure 7. Day 4 sample of the Street Parade Zürich, quantitative data quality is not compromised when using *Scheduled* MRM™ or *Scheduled* MRM™ pro-IDA-MS/MS since sufficient number of data points is acquired using both workflows (top and middle), the IDA methods provides additional information for compound identification (bottom left to right: MS/MS of MDMA, benzoylecgonine, and cocaine)

Standard samples were injected to find out at what concentration compounds can be identified using retention time matching and MS/MS library searching. At 1 ng/L, 9 of the 12 drugs could be clearly identified (no MS/MS spectra were acquired for codeine, amphetamine and monoacetylmorphine) at 5 ng/L, all of the 12 compounds were identified with high confidence (Figure 8).

Figure 9 shows the day 7 sample, diluted 1:10 prior LC-MS/MS analysis. MDMA was identified with high confidence, although the concentration in the injected sample was only 7 ng/L, which corresponds to 70 ng/L in the undiluted sample.

Contents

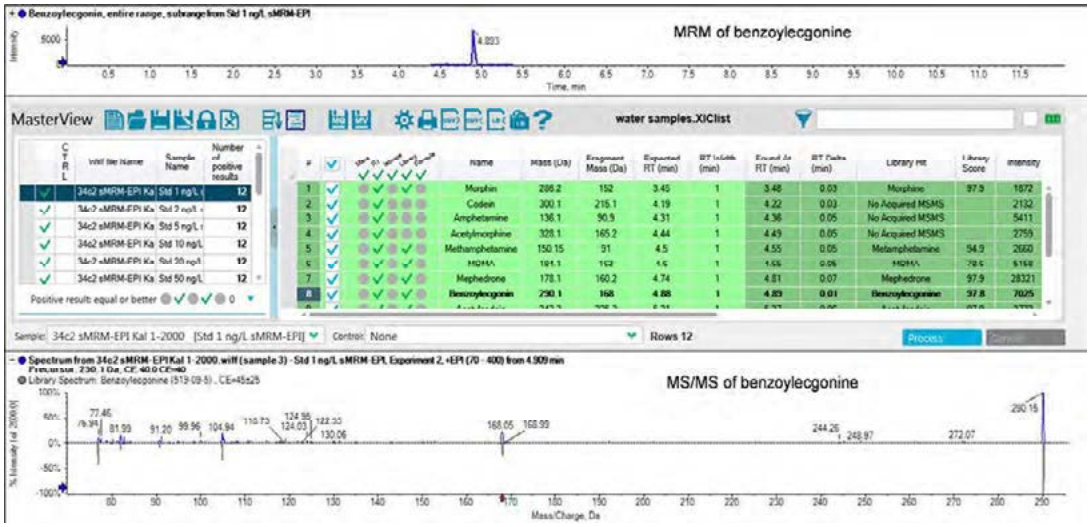

Figure 8. Identification based on retention time matching and MS/MS library searching in MasterView™ software, 9 out of 12 compounds were identified at 1 ng/L, all 12 compounds were identified at 5 ng/L, the example shows the MRM (retention time error = 0.01 min) and MS/MS spectrum (FIT = 97.8%) of benzoylecgonine at a concentration of 1 ng/L

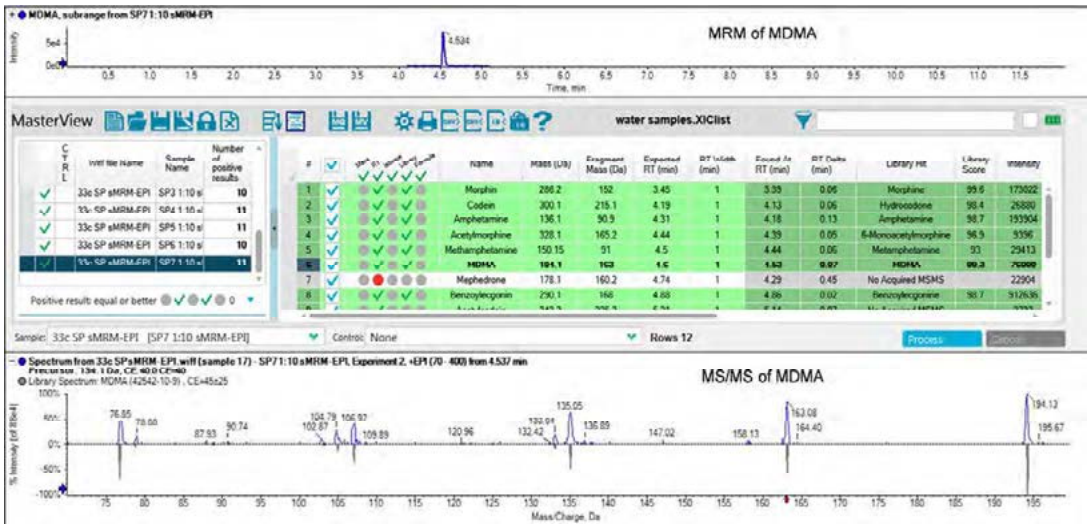

Figure 9. Identification based on retention time matching and MS/MS library searching in MasterView™ software, MDMA was identified with a retention time error of 0.07 min and a library FIT of 99.3%

Contents

Summary

The existing method for the determination of drugs of abuse in complex matrices like wastewater by large volume direct injection was significantly improved. Sensitivity gains and S/N gains by a factor of up to 10 were obtained by optimizing the LC and ion source conditions. This was achieved by using a Phenomenex Kinetex core-shell column with 4.6 mm ID and a high-flow method design. The column backpressure was only ~100 bar due to the use of 5 µm particles and therefore, the method can be run on a traditional LC systems.

The collision energies of high abundant MRM transitions were detuned to avoid detector saturation. Two method workflows were developed using *Scheduled* MRM™ and *Scheduled* MRM™ pro-IDA-MS/MS. Both method allow accurate and reproducible quantitation down to low ng/L range and identification based on ion ratios. The IDA method offers the additional benefit of identifying target analytes based on MS/MS library searching resulting in increased confidence in results.

The method was successfully applied to the determination of drugs of abuse wastewater during a party event (Street Parade Zürich 2014). Different profiles were observed for different drugs indicating different consumption during the time period of the event.

Acknowledgement

The authors wish to thank the people of the Sewage Treatment Plant Werdhölzli, Zurich for the daily wastewater sampling during the Street Parade.

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11 European Guideline 2002/657/EC

Publication number: 11130615-01

Contents

Detection of Estrogens in Aqueous and Solid Environmental Matrices by Direct Injection LC-MS/MS

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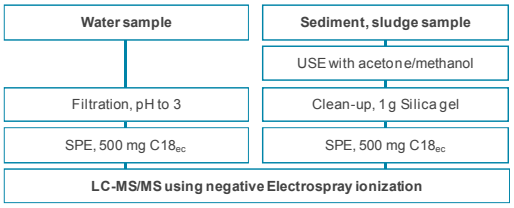
Introduction

Various analyses of water have shown ubiquitary presence of pharmaceutical residues in the aqueous environment.[1] Due to their ecotoxic effects native and synthetic estrogens, Estrone (E1), 17β-Estradiol (E2), Estriol (E3) and 17α-Ethinylestradiol (EE2), are of special relevance even at very low concentrations. A significant feminization could be observed at a concentration of approximately 1 pg/mL reflecting the strong endocrine potential of these compounds.[2] As a result of these very low concentrations a powerful analytical set-up is essential for their reliable detection and quantification. Residues of estrogens in aqueous and solid environmental samples are commonly analyzed by GC-MS^n, however the necessary derivatization steps are time consuming and laborious. This study investigates the power of LC-MS/MS for the analysis of estrogens, and compares a traditional Solid Phase Extraction (SPE) approach to direct injections of filtered wastewater, sediment and sludge samples.

Experimental

Sample Preparation

Direct injections of environmental samples were compared to samples prepared using the following procedure. Wastewater (250-500 mL), sediment (5 g), and sludge samples (0.5 g) were prepared according to the following scheme:



Liquid Chromatography

- Clean-up column: Phenomenex MercuryMS Luna C18(2) 20x2 mm, 3 µm
- Analytical column: Phenomenex Gemini 50x2 mm, 5 µm
- Eluent A: water, eluent B: acetonitrile
- Eluent C (post column): water + 2.5% NH_3

Contents

- Injection volume: 20 µL of extracts and 100 µL of wastewater without clean-up

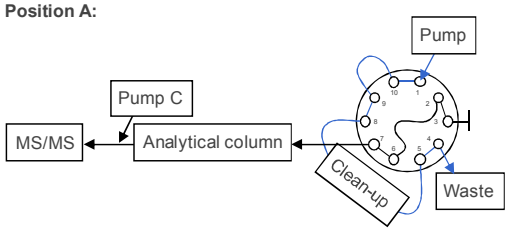
Table 1. LC Flow and Gradient

Time (min)	Flow (µl/min)	A/B	C Flow (µl/min)
0.0	1000	90/10	0
4.0	1000	90/10	0
4.5	250	90/10	10
15.0	250	34/66	10
16.0	250	0/100	10
20.0	250	0/100	10
4.0	Re-equilibration to 1000 µL/min and 90/10		

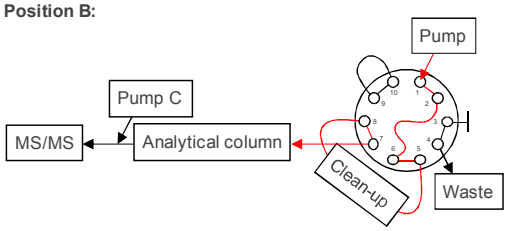
Switching Valve

Switching valve: 0.0 min position A, 4.6 min position B, 25.0 min position A For valve connections see the following schematics:

Position A:



Position B:



Mass Spectrometric Detection

An SCIEX API 5000™ LC-MS/MS system with Turbo V™ source with Electrospray Ionization (ESI) probe in negative polarity was used. Gas and source parameters:

CUR: 20 psi; GS1: 45 psi; GS2: 65 psi; TEM: 360°C (optimized for Ethinylestradiol); and CAD value: 7; IonSpray voltage (IS) was set to 0 V between 0.0-4.7 min and 20.0-21.0 min, while IS was set to -4500 V between 4.7- 20.0 min, respectively.

The following Multiple Reaction Monitoring (MRM) transitions were detected with a dwell time of 80 ms:

E1: 269/145, E2: 271/145, E3: 287/171, EE2: 295/145, Internal Standards E1-D4: 273/147, E2-13C2: 273/147and EE2-D2: 297/145.

Results and Discussion

Ecotoxic effects down to sub-ng/L levels in combination with the limited sensitivity of older LC-MS/MS systems are the reasons to use time consuming sample preparation steps. A typical sample preparation step is Solid Phase Extraction (SPE) of wastewater or Ultrasonic Extraction (USE) followed by SPE of sediment and sludge samples.[3-4] However, simultaneously matrix components are enriched as well, leading to an increased background which in the worst case leads to false positive results.

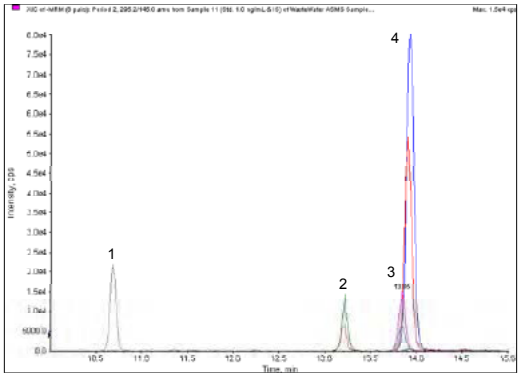


Figure 1. Chromatogram of E3 (1), E2 (2), EE3 (3), E1 (4) and their internal standards E1-D_4 and E2-$^{13}C_2$

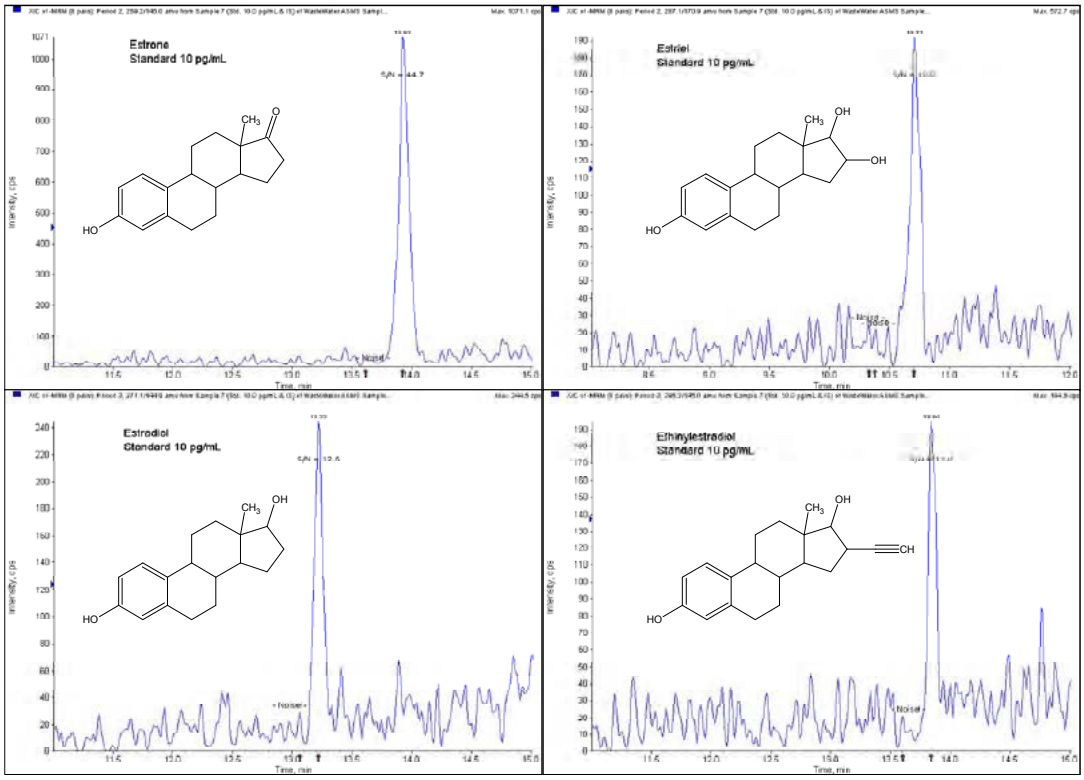


Figure 2. Injection of a 10 pg/mL standard of Estrone, Estradiol, Estriol, and Ethinylestradiol

An example chromatogram of all analytes and internal standards is presented in Figure 1. The ionization conditions were optimized for Ethinylestradiol, the least sensitive analyte. The use of APCI or APPI sources results in thermal degradation of Ethinylestradiol to Estradiol. Electrospray Ionization (ESI) in negative polarity with post column infusion of ammonia shows highest intensity. The sensitivity of these conditions using the API 5000™ LC-MS/MS system is presented in Figure 2. All investigated estrogens have a Signal-to-Noise ratio (S/N) higher than 10 at a concentration of 10 pg/mL.

It is known that Estriol can be entirely eliminated during clean-up. However, the direct injection of 100 µL of a filtered wastewater sample results in an Estriol response of 3.4e4 counts per second (Figure 3).

Extraction of sediment and sludge samples followed by a traditional SPE clean-up leads to high background as well as many interfering signals. Figure 4 shows chromatograms of Estrone, Estradiol, and Ethinylestradiol after a traditional clean-up procedure and non- SPE treated sediment samples which were diluted by a factor of 100. The analysis of non-SPE treated 100 times diluted samples delivers results with comparable S/N ratios to those samples analyzed after complete clean-up procedure.

Estriol could not be detected in sludge samples.

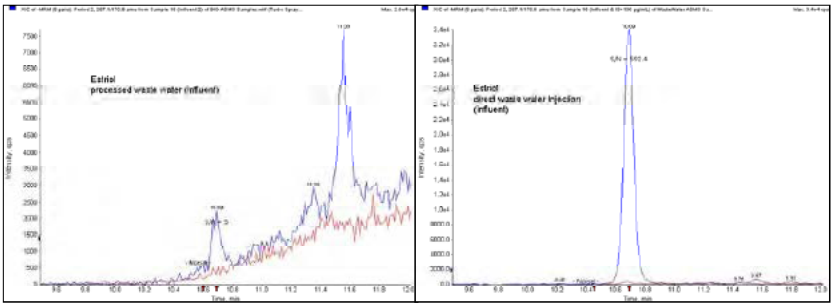


Figure 3. Comparison of Signal-to-Noise ratios of Estriol in extracted (left) and directly injected wastewater (right), red trace: internal standard

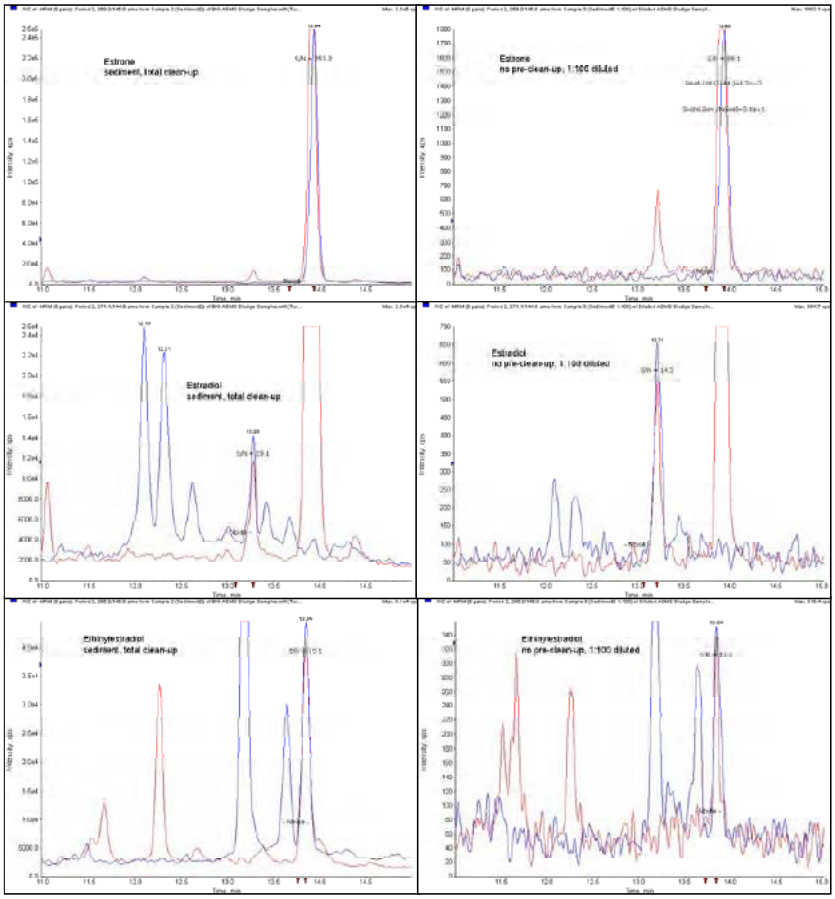


Figure 4. Comparison of Signal-to-Noise ratios of Estrone, Estradiol, and Ethinylestradiol in extracted sediments (left) and directly injected wastewater (right), red trace: internal standard

Summary

Limits of quantitation of all detected estrogens in a mix including Estrone, 17β-Estradiol, Estriol and 17α-Ethinylestradiol were found below 10 pg/mL. In this study highest sensitivity was achieved using Turbo V™ source in negative polarity on an API 5000™ LC-MS/MS with post column infusion of ammonia.

The developed direct injection method provides enough sensitivity to analyze estrogens in filtered wastewater samples with minimum sample preparation. This approach reduces time consuming sample preparation and avoids disturbing matrix signals. Furthermore it eliminates the loss of Estriol during traditional Solid Phase Extraction.

The improvement in sensitivity allows similar Signal-to-Noise ratios analyzing sediment and sludge samples to those prepared with traditional clean-up or a simplified procedure. Dilution of crude samples reduces the background noise and the presence of interfering signals while reducing time of sample treatment significantly.

References

1 Baronti et al.: Environ. Sci. & Technol. Vol. 34 (2000) 5059-50662

2 Routledgeet al.: Environ. Sci. & Technol. Vol. 32 (1998) 1559-1565

3 Ternes: Trends Anal. Chem. 20 (2001) 419-434

4 Ternes, et al.: Anal. Chem. 74 (2002) 3498-3504

Publication number: 1280910-01

EPA Method 539: Hormones in Drinking Water Using the SCIEX QTRAP® 6500 LC-MS/MS System

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Overview

This application note highlights the sensitivity and precision of the SCIEX QTRAP® 6500 system for the analysis of hormones in drinking water. The hormones analyzed are specified in EPA Method 539 (Determination of Hormones in Drinking Water by Solid Phase Extraction [SPE] and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry [LC-ESI-MS/MS]).[1] The goal was to meet the Unregulated Contaminant Monitoring Rule 3 Assessment Monitoring list (UCMR3) reporting limits.[2] Statistically validated method detection limits range from 0.014 to 0.50 ng/L, surpassing the UCMR3 reporting limits.

Introduction

Endocrine disrupting hormones in waste, surface and drinking waters have been studied extensively in the last decade. These compounds (and their metabolites) enter the environment through a variety of anthropogenic activities, with typical concentrations found in different water sources in the ng/L range.[3]

Within the scope of EPA Method 539 there are seven hormones monitored in finished drinking water (Table 1).

Table 1. Hormones in EPA Method 539 and their UCMR3 reporting limits

Compound	CASRN	UCMR3 MRL (ng/L)
16α-Hydroxyestradiol (Estriol)	50-27-1	0.8
17β-Estradiol	50-28-2	0.4
Equilin	474-86-2	4
Estrone	53-16-7	2
17α-Ethynylestradiol	57-63-6	0.9
Testosterone	58-22-0	0.1
4-Androstene-3,17-dione	63-05-8	0.3

This paper describes the performance of the SCIEX QTRAP® 6500 system for the evaluation of these hormones, with the aim of meeting the UCMR3 reporting limits.

Experimental

Sample preparation and data processing were carried out according to EPA Method 539 (EPA 539 sections 10, 11 and section 12), unless otherwise noted. All quality control requirements (EPA 539 section 9.3) were met or exceeded for each batch of calibrators and/or samples analyzed. Quantitation and signal-to-noise (s/n) calculations were performed using MultiQuant™ 3.0 software. The internal standards (ISTD) used were: Estriol-D_2 in the first experiment; β-Estradiol-$^{13}C_6$ and 17α-Ethinylestradiol-$^{13}C_2$ in the second experiment; and Testosterone-D3 in the third experiment. The surrogate used was 17α-Ethinylestradiol-D4 and it was fortified into samples at 70 ng/L.

Analyses were carried out using the SCIEX QTRAP® 6500 system coupled with an Agilent 1260 HPLC (binary pump, degasser and column oven) with an Eksigent ULC 100 HTC-xt autosampler. The mobile phases consisted of 0.02% NH_4OH and 0.02% NH_4OH in methanol. Gradient parameters are provided in Table 2. Samples were analyzed with a 10 µL injection (vs. 50 µL in EPA 539) onto a Phenomenex Kinetex C18 column (100 x 2.1 mm, 5 µm) heated to 40°C.

Contents

Table 2. LC gradient conditions

Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0.0	300	90	10
0.5	300	90	10
12.5	300	2	98
12.6	300	90	10
15.0	300	90	10

The QTRAP® 6500 system was operated in Multiple Reaction Monitoring (MRM) mode using three separate experiments to facilitate polarity switching. The IonDrive™ Turbo V ion source was used with the electrospray ionization (ESI) probe. The ESI and MRM parameters are outlined in Tables 3 and 4.

Table 3. ESI source parameters

Parameter	Value
Polarity	positive / negative
Curtain Gas	20 psi
Collision Gas	12 psi
IonSpray Voltage	5500 / -4500V
Temperature	600˚C
GS1	50 psi
GS2	70 psi

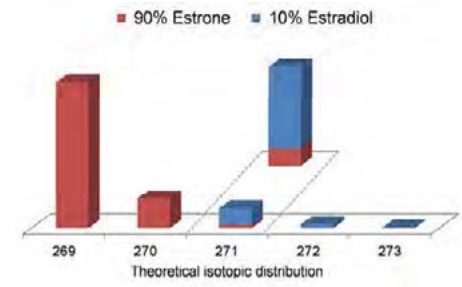

Figure 1. Example showing the importance of resolving critical pairs of isotopes in EPA 539. The M+2 ion of Estrone (red) at *m/z* 271 has the potential to interfere significantly with the molecular ion of β-Estradiol (blue), depending on the relative concentration of the two analytes. In this example (Estrone/Estradiol = 90/10) ~20% of the β-Estradiol signal is due to the M+2 ion of Estrone.

Table 4. Experiment number, polarity, MRM transitions, dwell time, Declustering Potential (DP), and Collision Energy (CE) for target hormones, ISTDs (*) and surrogates (^)

Compound	Experiment (Polarity)	Q1	Q3	Dwell (ms)	DP (V)	CE (V)
Estriol 1	1 (-)	287.0	170.9	200	-115	-46
Estriol 2	1 (-)	287.0	144.9	200	-115	-50
Equilin 1	2 (-)	267.0	142.8	20	-100	-42
Equilin 2	2 (-)	267.0	223.0	20	-100	-44
Estrone 1	2 (-)	269.0	145.0	20	-130	-48
Estrone 2	2 (-)	269.0	143.0	20	-130	-64
Estradiol 1	2 (-)	271.0	145.1	20	-140	-50
Estradiol 2	2 (-)	271.0	143.0	20	-140	-66
Ethynylestradiol 1	2 (-)	295.0	143.0	20	-130	-70
Ethynnylestradiol 2	2 (-)	295.0	159.0	20	-130	-44
Androstenedione 1	3 (+)	287.0	97.0	35	116	27
Androstenedione 2	3 (+)	287.0	109.0	35	116	29
Testosterone 1	3 (+)	289.0	97.0	40	96	27
Testosterone 2	3 (+)	289.0	108.9	40	96	31
*Estriol-D_2**	1 (-)	289.0	146.9	200	-165	-54
*Estradiol-$^{13}C_6$**	1 (-)	277.0	144.9	20	-145	-50
*Ethynylestradiol-$^{13}C_2$**	1 (-)	297.0	145.0	20	-155	-54
*Testosterone-D_3**	3 (+)	292.0	97.1	35	106	27
*Ethynylestradiol-D_4^*	2 (-)	299.0	145.0	20	-145	-72

Results and Discussion

During method development, three critical pairs of isotopes (Estrone/Estradiol, Equilin/Estrone, and Testosterone/Androstenedione) were monitored to ensure sufficient chromatographic resolution was maintained. Since each of these pairs share product ions, their resolution was required to avoid potential interference from the M+2 ion of the lower *m/z* precursor in each pair (Figure 1).

Chromatography was carried out using a Phenomenex Kinetex C18 column and a gradient that was designed to increase method throughput relative EPA 539. This method deviation falls under EPA 539 Section 9.1.1, which permits alternate chromatographic conditions provided adequate method performance is demonstrated. The resulting chromatography demonstrated acceptable resolution for all critical pairs (Figure 2).

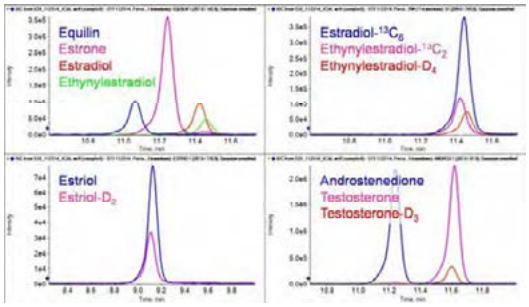

Figure 2. Final chromatography using0.02% NH_4OH and 0.02% NH_4OH in methanol mobile phases. Chromatograms are color coded in each pane, with ISTDs and surrogates overlaid. All critical pairs were adequately resolved.

Initial Calibration

The Initial Calibration range (EPA 539 section 10.2) varied by compound. All targets covered 0.5 to 15 ng/L, while Estradiol, Testosterone, and Androstenedione were analyzed at levels below 0.5 ng/L, corresponding to their UCMR3 reporting limits (Table 1).

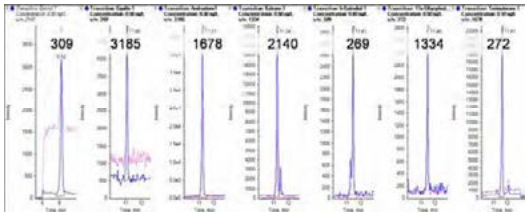

Figure 3. Signal-to-noise values (S/N) for the lowest calibration level assessed for each target compound (left to right: Estriol, Equilin, Androstenedione, Estrone, Estradiol, Ethynylestradiol, and Testosterone). The quantifier transition is displayed in blue and the qualifier transition is overlaid in pink for each chromatogram.

Due to the sensitivity of the QTRAP® 6500 system, low ng/L detection levels were obtained for all compounds. Signal-to-noise values (S/N) of 270 to 3200 after 2-point Gaussian smoothing were observed for the lowest calibration level of each target compound (Figure 3).

The correlation (r) value for all calibration curves were > 0.999 (Figure 4).

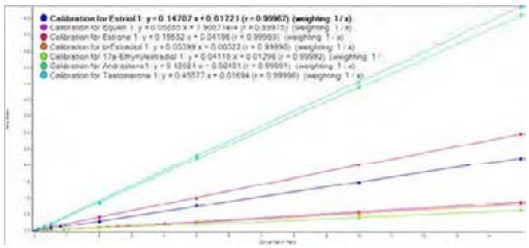

Figure 4. Calibration lines and regression equations for all hormones

These results demonstrate the suitability of this method for the analysis of hormones in drinking water, especially given the excellent S/N of the low ng/L calibration samples. All calibration acceptance criteria specified in EPA 539 section 10.2 were met.

Initial Demonstration of Capability

To demonstrate method suitability for EPA 539 it is necessary to perform an Initial Demonstration of Capability (IDC) following the Initial Calibration. In addition to the ongoing QC criteria specified in EPA 539 section 9.3, adhering to the IDC requires:

1. Extraction of four Laboratory Fortified Blanks (LFB) to assess Accuracy (±30%) and Precision (RSD <20%). Fortification should correspond to a mid-level calibrator.
2. Extraction of seven LFBs that must meet a Prediction Interval of Results (PIR) of 50 to 150% to define the Method Reporting Limits (MRL).
3. Determination of Method Detection Limits (MDL). This is an optional part of the IDC that requires seven replicates prepared over three days. In this study the MRL replicates were used to obtain an approximation of the MDL.
4. All target compounds in a Laboratory Reagent Blank (LRB) and Sample Matrix Blank (SMB) after the Initial Calibration must quantify to <1/3 of MRL. As well, a Reagent Water Blank (RWB) after the high calibrator should be <1/3 of MRL.

Contents

5. Evaluate method accuracy (±30%) using a Quality Control Sample (QCS) that is sourced from a vendor other than the one that provided the calibration samples.

Each of these criteria are discussed below.

Accuracy and Precision

Fortification for the evaluation of Accuracy and Precision was done at 5 ng/L. This corresponded to calibration level four of six. For the four replicate extracts analyzed the relative standard deviations (RSD) ranged from 1.3 to 6.5%, while the recoveries ranged from 85 to 97% (Table 5). All of these values were within the ranges specified in the EPA 539 sections 9.2.2 and 9.2.3 of (< 20% RSD and ±30% recoveries).

Table 5. Method performance

Compound	Accuracy (%)	Precision (%)	QCS (%)	RPD (%)
Estriol	93%	3.0%	94 ± 8.2	5.2
Equilin	85%	6.5%	94 ± 2.4	2.9
Androstenedione	94%	1.3%	102 ± 0.19	2.1
Estrone	95%	2.7%	97 ± 1.8	3.0
Estradiol	94%	2.1%	98 ± 0.21	2.3
Testosterone	96%	1.9%	99 ± 3.0	1.1
Ethynylestradiol	97%	2.4%	100 ± 0.81	11

Method Reporting Limits

The concentrations used to fortify the seven extractions required for the calculation of the Method Reporting Limit (MRL) correspond to the UCMR3 reporting limits. To be a valid MRL the results of the seven replicate extractions must meet a set of statistical criteria, which are outlined in detail in section 9.2.4 of EPA 539. Briefly, the calculations are:

$$HR_{PIR} = 3.963s$$

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100\%$$

HR_{PIR} = Half Range for the prediction interval of results
s = the standard deviation of replicate analyses
3.963 = a constant value for seven replicates

The Upper PIR Limit must be ≤ 150 percent recovery and the Lower PIR Limit must be ≥ 50 percent recovery.

The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above.

Using the above equations on samples that had been fortified at the UCMR3 reporting limits yielded acceptable PIR values (Table 6). This validated the UCMR3 concentrations as MRLs for all compounds in this method.

Table 6. MRL and MDL determination and statistical verification

Compound	Fortification Level (ng/L)	Lower PIR (%)	Upper PIR (%)	MDL (ng/L)
Estriol	0.8	77%	98%	0.068
Equilin	4.0	61%	93%	0.50
Androstenedione	0.3	82%	101%	0.023
Estrone	2.0	79%	100%	0.17
Estradiol	0.4	81%	126%	0.071
Testosterone	0.1	68%	104%	0.014
Ethynylestradiol	0.9	62%	112%	0.18

Method Detection Limits

The Method Detection Limit (MDL) was calculated using the following equation:

$$MDL = s \times t_{(n-1,1-\alpha=0.99)}$$

s = the standard deviation of replicate analyses
$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom
n = number of replicates

Using the MRL extracts, the calculated MDLs ranged from 0.014 to 0.50 ng/L (Table 6). It is conceivable that the QTRAP® 6500 could detect lower concentrations with this method based on the S/N for the low calibrators (Figure 3).

Laboratory Reagent and Reagent Water Blanks

A Laboratory Reagent Blank (LRB) is a system blank that has been taken through the entire extraction procedure to assess for background contamination. Following the Initial Calibration a LRB was assessed to evaluate systemic background. Once established, the LRB was evaluated relative to the MRLs (Figure 5).

In the present method, all target compounds were observed under 1/3 of their respective MRLs.

A Reagent Water Blank (RWB) analyzed immediately following the high calibrator during the Initial Calibration demonstrated negligible carry-over (Figure 5).

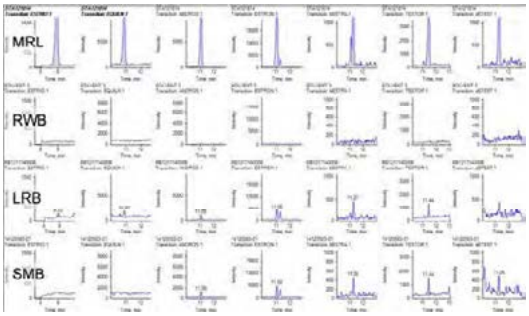

Figure 5. RWB (50% methanol), LRB and SMB results. All results showed background levels that were < 1/3 of the calculated MRLs. For each compound (left to right: Estriol, Equilin, Androstenedione, Estrone, Estradiol, Testosterone, and Ethynylestradiol), the y-axis is scaled to ~1/3 of the MRL response for each compound (column). The LRB/FRB matrix was finished tap water.

Quality Control Sample and Ongoing QC Results

Two separate Quality Control Samples (QCS) were evaluated at 5 ng/L. The ±30% accuracy specification set in EPA 539 section 9.3.8 was met for all compounds, validating the accuracy of the Initial Calibration (Table 5; shown ± 1 RSD).

Two components of the ongoing QC requirements specified in EPA 539 section 9.3, the LRB and QCS, have already been discussed as they are also specified components of the IDC. In addition, the following ongoing QC criteria were required:

1. Laboratory fortified blank (LFB) should be analyzed with each batch. Acceptance criteria will depend on the fortified concentration, which should change from batch-to-batch.
2. Continuing Calibration Check (CCC) samples should be run at the beginning and end of each batch and after every 10 samples. Acceptance criteria vary with concentration relative to the MRLs (EPA 539 section 10.3).
3. Internal standard (ISTD) responses should not deviate more than 50% from the average ISTD response in the Initial Calibration.
4. Surrogate recovery should be 70 to 130% of the expected value.
5. Laboratory fortified sample matrix (LFSM) and a duplicate (LFSMD) should yield accuracies within ±30% of expected values and the relative percent difference (RPD) between the LFSM and LFSMD must be < 50% for MRL levels and < 30% for mid-level fortifications.

The first four of these criteria were met or exceeded in all samples discussed herein. The RPD results from duplicate 5 ng/L fortifications with all compounds in LFSM samples were 1.1 to 11.0, well within the ±30% RPD permitted in EPA 539 (Table 5). The LRB/SMB matrix in this study was finished tap water. Figure 5 demonstrates that all compounds were < 1/3 of the MRLs, which meets EPA 539 criteria and further validates the RPD results since there was negligible hormone contamination in the sample matrix.

There is also criteria for CCC accuracy 70-130%; surrogate accuracy 70-130% that were met for all samples analyzed.

Conclusion

The QTRAP® 6500 LC-MS/MS system is a sensitive and robust platform for the analysis of hormones in drinking water. The demonstrated MRLs meet the UCMR3 reporting limits and the MDLs exceed the UCMR3 reporting limits by 5-10x.

References

1 EPA Method 539 'Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry LC-ESI-MS/MS)' version 1.0 (2010) http://water.epa.gov/scitech/drinkingwater/labcert/upload/met539.pdf

2 Unregulated Contaminant Monitoring Rule 3 (UCMR3) http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3

3 S.D. Richardson and Th. A. Ternes: 'Water Analysis: Emerging Contaminants and Current Issues' Anal. Chem. 86 (2014) 2813-2848

Abbreviations

A_s – asymmetry factor
CASRN – chemical abstracts registration number
CCC – continuing calibration check
CE – collision energy
DP – declustering potential
EPA – environmental protection agency
ESI – electrospray ionization
HR_{PIR} – half range prediction interval of results
IDC – initial demonstration of capability
ISTD – internal standard
LFB – laboratory fortified blank
LFSM – laboratory fortified sample matrix
LFSMD – laboratory fortified sample matrix duplicate
LRB – laboratory reagent blank
MDL – method detection limit
MRL – method reporting limit
MRM – multiple reaction monitoring
PFAAs – perfluoroalkyl acids
PIR – prediction interval of results
QCS – quality control sample
RPD – relative percent difference
RSD – relative standard deviation
RT – retention time
RWB – reagent water blank
SMB – sample matrix blank
S/N – signal-to-noise
UCMR3 – unregulated contaminant monitoring rule 3 assessment monitoring list

Publication number: 15-01

Screening and Identification of Unknown Contaminants in Untreated Tap Water Using a Hybrid Triple Quadrupole Linear Ion Trap LC-MS/MS System

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Introduction

Protection of our drinking water resources from contaminants is a major responsibility for both government and water producing bodies. The response taken to a potential drinking water emergency will depend upon both the composition and the nature of the identified contaminant(s). Furthermore it is essential that there is a high degree of confidence in the correct and rapid identification of the problem before remedial action is taken. To date it has been a necessity to employ a combination of multiple analytical techniques to meet this end.

Screening Using Accurate Mass Measurements and MS/MS

One method of detecting contaminants is the use of accurate mass as a way to predict the formula and identity of a contaminant. In this approach the mass spectrometer has to be accurately calibrated because the greater the error the more potential contaminants would be a match for the detected peak, as <2ppm mass error is ideal.

In this example two structural related but different pesticides (Prometryn and Terbutryn) produce the same molecular ion because they have identical molecular formulae. In the environment there are hundreds of compound with the same mass (Figure 2). Thus, a complete identification of unknown contaminants by accurate mass alone may not yield to a complete answer as this does not provide any structural information. In the example above separation of these two pesticides by HPLC was not clear-cut as they eluted with very similar retention times (Figure 3). However, Prometryn and Terbutryn have different MS/MS fragmentation patterns (Figure 2). Therefore product ion spectra are essential for confident identification of unknown contaminants.

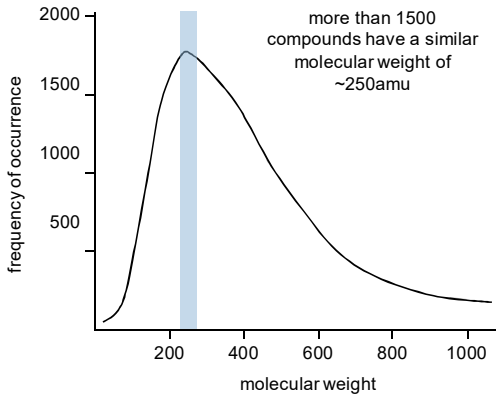

Figure 1. Abundance of compounds over molecular weight range of 100-1000 amu

Table 1. Comparison of sensitivities between the General Unknown Screening (GUS) and Multi Target Screening (MTS) approaches

			Multi Target Screening			General Unknown Screening		
Compound Name	**Compound Class**	**Polarity**	**MRM**	**Intensity at 1 µg/mL**	**~LOD (µg/mL)**	**Q3 Mass**	**Intensity at 10 µg/mL**	**~LOD (µg/mL)**
Brodifacoum	Rat poison	Negative	521.0/79.0	5.80E+04	0.05	521.0	7.70E+05	5.00
Chlorophacinone	Rat poison	Negative	373.0/201.1	1.23E+04	0.20	373.0	3.40E+05	15.0
Difenacoum	Rat poison	Negative	443.1/135.0	1.40E+04	0.25	443.1	1.80E+06	1.25
Difethialone	Rat poison	Negative	537.0/79.0	6.00E+04	0.07	537.0	1.40E+06	5.00
Flocoumafen	Rat poison	Negative	541.1/161.0	1.30E+04	0.12	541.1	1.40E+06	2.00
Warfarin	Rat poison	Negative	307.0/161.1	1.80E+04	0.20	307.0	1.80E+05	40.0
Endothal	Rat poison	Negative	185.0/141.0	6.00E+03	2.0	185.0	-	100
DNOC	Cresol	Negative	197.0/137.1	5.00E+04	0.10	197.0	2.00E+06	1.25
Azinphos-ethyl	Organo-phosphorus	Positive	346.0/160.1	5.13E+03	1.00	346.0	6.50E+04	200
Demeton-S-methyl	Organo-phosphorus	Positive	231.0/89.0	1.00E+04	0.50	231.0	2.30E+05	20.0
Dichlorvos	Organo-phosphorus	Positive	221.0/127.0	9.33E+02	10.0	221.0	4.00E+04	200
Disulfoton	Organo-phosphorus	Positive	275.1/89.0	2.00E+03	5.00	275.1	2.00E+04	2000
Propetamphos	Organo-phosphorus	Positive	282.1/156.0	2.20E+03	2.50	282.1	5.20E+04	200
Tebupirimfos	Organo-phosphorus	Positive	319.0/153.1	1.90E+04	0.50	319.0	2.90E+05	20.0
Parathion-ethyl	Organo-phosphorus	Positive	292.1/236.0	4.73E+03	2.00	292.1	1.00E+04	500
Parathion-methyl	Organo-phosphorus	Positive	281.1/264.3	5.00E+02	10.0	264.1	2.00E+04	400

General Unknown Screening and Multi Target Screening

There are two possible approaches of screening methods. The first would to screen for a complete unknown. This General Unknown Screening (GUS) would use a single 'universal' survey scan over a defined mass range and could either be a Time-of-Flight (TOF), quadrupole or ion trap scan. This survey scan can be used to trigger automatically the acquisition of a product ion spectrum if a signal of a detected compound is above a defined threshold. Finally, this spectrum can be searched against a mass spectral library for identification. Comparison of Total Ion Chromatograms (TIC) of unknown samples to that of the control reveal compounds that are either unique to the sample or those that are present at significantly higher concentrations than in the control.

The other approach is often called Multi Target Screening (MTS). In this approach a predefined list of compounds is looked for in a Single Ion Monitoring (SIM) or Multiple Reaction Monitoring (MRM) experiment. MRM mode is generally preferred because of higher selectivity and sensitivity. Once a compound is detected above a defined threshold a product ion scan is collected and compared against a library. Dynamic exclusion of compounds where MS/MS spectra are already acquired allows the data collection of co-eluting compounds (Figure 4).

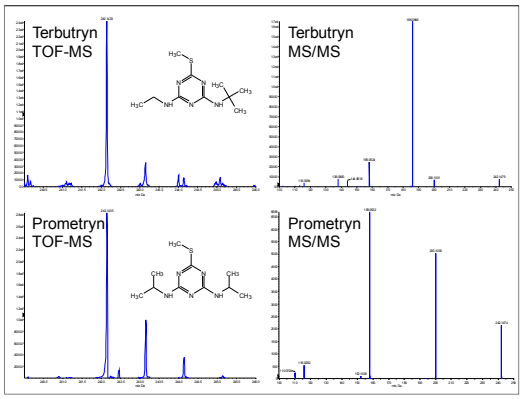

Figure 1. Accurate mass measurement of Prometryn (top) and Terbutryn (bottom) using a Quadrupole quadrupole-Time-of-Flight system in MS mode and MS/MS spectra of both pesticides

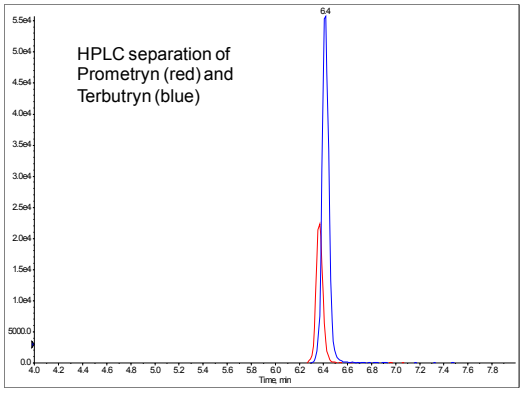

Figure 3. HPLC analysis of Prometryn and Terbutryn on a standard C18 reverse phase column, both compounds elute with a retention time difference of less than 6s

The technology that lends itself to this application is the hybrid triple quadrupole linear ion trap technology (QTRAP® LC-MS/MS systems). It allows the use of any triple quadrupole scan, including MRM, to trigger the acquisition of Linear Ion Trap MS/MS spectra by Enhanced Product Ion scanning. Enhanced Product Ion scan spectra give maximum sensitivity for library searching with a complete pattern characteristic for Collision Induced Dissociation (CID).

Experimental

In order to maximize sensitivity two injections, one in positive and the other in negative polarity, for both the GUS and MTS approach, were done. Additionally, this allowed the mobile phase to be optimum for either polarity.

HPLC

A Shimadzu HPLC system with binary LC10ADvp binary gradient pump and SIL-HT autosampler was used for all HPLC separations. The mobile phase used in positive mode was:

A: H_2O + 2 mM NH_4CH_3COO

B: CH_3OH + 0.1% HCOOH

The mobile phase used in negative mode was:

A: H_2O

B: CH_3OH + 0.1% NH_4OH

HPLC separation for Multi Target Screening was performed on a C18 monolithic column (Merck). Samples were analyzed using a rapid gradient over 1.5 minutes at a flow rate of 1200 µL/min (without splitting of the flow prior to the mass spectrometer). Injection volumes of 50 or 100 µL were used for analysis.

An ACE C18 (50 mm 5 µm HICHROM) column was used for HPLC separation for General Unknown Screening. The HPLC flow was set at 1200 µL/min with a gradient used from 25% B to 100% B over 16 minutes. An injection volume of 50 µL was used.

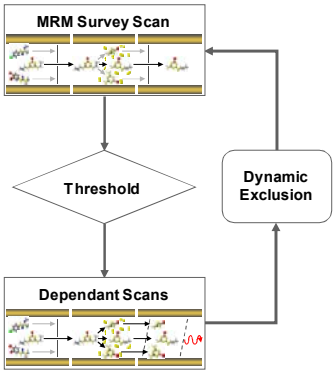

Figure 4. Experimental setup of a Multi Target Screening (MTS) approach

MS/MS

A 4000 QTRAP® LC-MS/MS system was used for both MTS and GUS experiments which triggered dependant Enhanced Product Ion scanning (mass range of 50 to 750 amu at 4000 amu/s) with a Collision Energy (CE) of 35 V and Collision Energy Spread (CES) of 20 V. The MTS survey scan used MRM transitions which have been optimized for each targeted analyte while the GUS screen used a Q3 scan with a mass range of 90 to 750 amu and a Declustering Potential (DP) of 60 V.

The source and gas settings for both MTS and GUS experiments were the same (Table 2)

Table 2. Ion source and gas parameters

Parameter	Value
Curtain gas	25 psi
Gas 1	50 psi
Gas 2	60 psi
CAD	10
Temperature	650°C
IonSpray™ source voltage	-4500 V
	+5500 V

Results and Discussion

Figure 5 and 6 present data obtained for an injection of 100 ng/mL Terbuthylazine and MCPP in both mineral and tap water, using the MRM to EPI MTS approach. The LINAC® collision cell of the 4000 QTRAP® system allows the simultaneous monitoring of up to hundreds of MRM transitions (contaminants) in a single sample injection. These MRM transitions triggered Enhanced Product Ion scan spectra in a cycle time of approximately 2.5 s without loss in sensitivity and full spectral quality.

Mineral water typically contains high levels of sodium, which may affect sensitivity due to adduct formation. However, Figure 5 and 6 indicate that there is nearly no effect on S/N to detect Terbuthylazine and MCPP in these water samples.

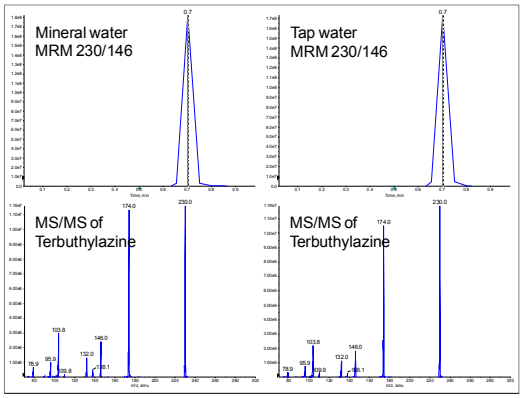

Figure 5. 100 ng/mL Terbuthylazine spiked into mineral and tap water analyzed in positive polarity MRM and EPI

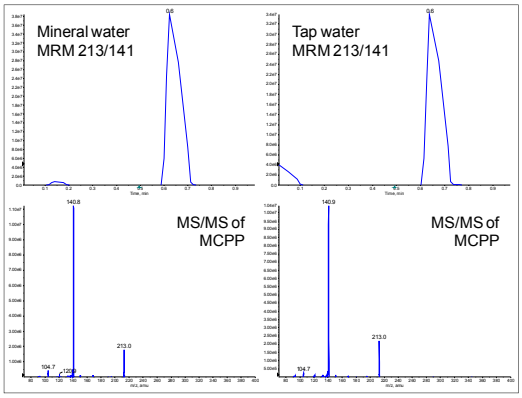

Figure 6. 100 ng/mL MCPP spiked into mineral and tap water analyzed in negative polarity MRM and EPI

The GUS approach shows the comparison of a blank control sample to a sample that has been spiked with 0.1 µg/L of a compound to be identified (Figure 7). The presence of the compound with m/z=350 amu is detected in the sample by comparing the two Q3 scan chromatograms. Acquisition of an Enhanced Product Ion scan spectrum followed by library searching allows to identification of Chlorpyrifos.

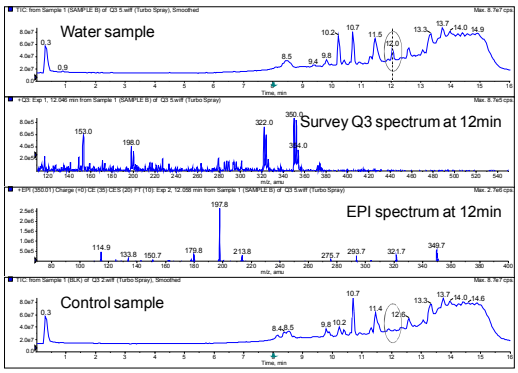

Figure 7. Comparison of a water sample to a blank control water with resulting Q3 scan and EPI spectrum of Chlorpyrifos detected and identified by library searching

In order to compare the relative sensitivities of both approaches, GUS and MTS, over 70 compounds were tested including compounds such as organophosphorus pesticides and rat poisons. Limits of Detection (LOD) were determined to be the triggering threshold of both approaches. In the GUS method the LOD was set at 500,000 cps of the parent ion in Q3 scan (background noise was generally lower than 500,000 cps). For the MTS approach LOD was 5000 cps in MRM which was determined as 2-3 times the background level of the most intense MRM trace. The chromatographic conditions of MTS were applied for this comparison work. Examples of results for 16 different compounds are given in Table 1 highlighting the higher sensitivity of the MTS approach. An average of 2 orders of magnitude comparing LOD of both approaches was found.

Summary

The 4000 QTRAP® LC-MS/MS system allows Multi Target Screening (MTS) and General Unknown Screening (GUS) of water samples to identify emerging contaminants. The MTS approach is the most rapid and sensitive method to screen for and detect the presence of targeted organic contaminants in water. More than 2000 targeted compounds can be screened in less than 20 minutes at low and sub µg/L level using the described procedure and multiple sample injections. The GUS method is an alternative to identify unknown compounds as it does not rely on any knowledge of the analytes. Here, a sample control comparison will detect unknown contaminants. In both approaches automatically generated Enhanced Product Ion spectra can be searched against a comprehensive mass spectral library and the fragmentation information can be used for identification and identification. However, the GUS approach is lower in sensitive and requires significantly longer run times.

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Contents

Analysis of Perfluoroalkyl Acids Specified Under the UCMR3 Using the QTRAP® 6500 LC/MS/MS System

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Overview

This application note highlights the sensitivity and precision of the QTRAP® 6500 LC/MS/MS system for the analysis of perfluoroalkyl acids (PFAAs) in drinking water. The PFAAs analyzed are a subset of EPA Method 537 (Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry [LC/MS/MS])[1], comprising the PFAAs outlined in the Unregulated Contaminant Monitoring Rule 3 Assessment Monitoring list (UCMR3).[2] Statistically validated method detection limits range from 1.4 – 35.9 ng/L.

Introduction

PFAAs are ubiquitous chemicals that are used in a variety of industrial and consumer products including carpets, cookware, paints, shampoos, food packaging, etc.[3] PFAAs have high thermal and chemical stability and are highly resistant to degradation in aquatic environments. Typical concentrations of PFAAs found in various water sources range from pg/L to µg/L levels.

Within the scope of EPA 537 there are 14 PFAAs (Table 1). Of these 14, six are specified in the UCMR3 Assessment Monitoring list: PFBS, PFHpA, PFHxS, PFOA, PFOS and PFNA.

This paper describes the performance of the QTRAP® 6500 system for the evaluation of the PFAAs in the UCMR3 using the guidelines laid out in EPA 537.

Table 1. PFAAs in EPA Method 537. Those compounds in bold type face are included in the UCMR3 Assessment Monitoring list.

Compound	Abbreviation	CASRN	UCMR3 MRL (ng/L)
Perfluorohexanoic acid	PFHxA	307-24-4	-
Perfluoroheptanoic acid	**PFHpA**	**375-85-9**	**10**
Perfluorooctanoic acid	**PFOA**	**335-67-1**	**20**
Perfluorononanoic acid	**PFNA**	**375-95-1**	**20**
Perfluorodecanoic acid	PFDA	335-76-2	-
Perfluoroundecanoic acid	PFUnA	2058-94-8	-
Perfluorododecanoic acid	PFDoA	307-55-1	-
Perfluorotridecanoic acid	PFTrDA	72629-94-8	-
Perfluorotetradecanoic acid	PFTA	376-06-7	-
Perfluorobutanesulfonic acid	**PFBS**	**375-73-5**	**90**
Perfluorohexanesulfonic acid	**PFHxS**	**355-46-4**	**30**
Perfluorooctanesulfonic acid	**PFOS**	**1763-23-1**	**40**
N-methyl perfluorooctane-sulfonamidoacetic acid	NMeFOSAA	-	-
N-ethyl perfluorooctane-sulfonamidoacetic acid	NEtFOSAA	-	-

Contents

Experimental

Sample preparation and data processing were carried out according to EPA Method 537 without deviation (EPA 537 sections 10, 11 and section 12), unless specifically noted. All required quality control parameters (EPA 537 section 9.3) were met or exceeded for each batch of calibrators and/or samples analyzed. Quantitation was performed using MultiQuant™ 3.0 software. All calibration curves had a 1/x concentration weighting and were forced through the intercept as specified in EPA 537 section 10.2.6. For carboxylic acids $^{13}C_2$-PFOA was used as the internal standard (ISTD), while all sulfonic acids used $^{13}C_4$-PFOS as the ISTD. The surrogates used were $^{13}C_2$-PFHxA and $^{13}C_2$-PFDA, both of which were fortified into samples at 40 ng/L.

Analyses were carried out using the SCIEX QTRAP® 6500 system coupled with an Agilent 1260 HPLC (degasser, binary pump and column oven) with an Eksigent ULC 100 HTC-xt autosampler. The mobile phase consisted of 20mM ammonium acetate with methanol. Gradient parameters are provided in Table 2. All samples were analyzed with a 5 µL injection (vs. 10 µL in EPA 537) onto an Atlantis T3 analytical column (150 x 2.1 mm, 5 µm) heated to 35˚C. An Atlantis T3 column (50 x 2.1mm, 5 µm) was also used as a delay column.

Table 2. LC gradient conditions

Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0.0	450	60	40
1.0	450	60	40
6.0	450	35	65
6.1	350	35	65
14.0	350	10	90
15.0	350	10	90
15.1	350	60	40
16.0	450	60	40
18.0	450	60	40

The QTRAP® 6500 system was operated in negative polarity Electrospray Ionization (ESI) using Multiple Reaction Monitoring (MRM) and the *Scheduled* MRM™ algorithm. ESI source and MRM parameters are outlined in Tables 3 and 4.

Table 3. ESI source parameters

Parameter	Value
Polarity	negative
Curtain Gas	30 psi
Collision Gas	12 psi
IonSpray Voltage	-4500 V
Temperature	400˚C
GS1	30 psi
GS2	30 psi

Table 4. MRM transitions, retention time (RT), Declustering Potential (DP), and Collision Energy (CE) for target PFAAs, ISTDs (*) and surrogates (^)

Compound	Q1	Q3	RT	DP (V)	CE (V)
PFBS 1	298.8	79.8	6.8	-60	-68
PFBS 2	298.8	98.9	6.8	-60	-36
PFHpA 1	362.8	318.8	10.7	-5	-12
PFHpA 2	362.8	168.8	10.7	-5	-22
PFHxS 1	398.9	79.7	10.7	-70	-86
PFHxS 2	398.9	98.7	10.7	-70	-74
PFOA 1	412.8	368.9	12.1	-5	-14
PFOA 2	412.8	168.7	12.1	-5	-24
PFOS 1	498.9	79.8	13.2	-60	-122
PFOS 2	498.8	98.9	13.2	-60	-98
PFNA 1	462.9	418.9	13.3	-30	-14
PFNA 2	462.9	218.9	13.3	-30	-24
*$^{13}C_2$-PFOA**	414.9	369.8	12.1	-20	-14
*$^{13}C_4$-PFOS**	502.9	79.8	13.3	-10	-102
*$^{13}C_2$-PFHxA^*	314.8	269.8	8.9	-15	-12
*$^{13}C_2$-PFDA^*	514.9	469.9	14.3	-25	-16

Results and Discussion

EPA 537 permits deviation from the LC conditions provided in the method. To that end, the method presented here used an Atlantis T3 column (5 µm) and a gradient that was designed to increase method throughput, while still providing sufficient chromatographic resolution (Figure 1).

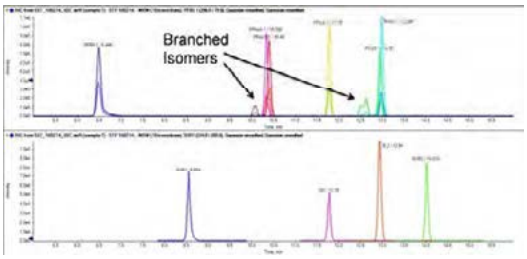

Figure 1. Final chromatography using a 20mM ammonium acetate / methanol mobile phase. Targets are shown on top with branched isomers of PFHxS and PFOS indicated. ISTDs ($^{13}C_2$-PFOA and $^{13}C_4$-PFOS) and surrogates are shown on the bottom (SUR1 = $^{13}C_2$-PFHxA and SUR2 = $^{13}C_2$-PFDA)

For PFHxS and PFOS the presence of additional small peaks points to the presence of branched isomers, which are known contaminants in the technical PFAAs suggested for purchase in EPA 537. When present, these isomers were summed into a combined value for the branched and linear isomers. This adheres to section 12.4 of EPA 537.

Initial Calibration

The Initial Calibration (EPA 537 section 10.2) was carried out using the UCRM3 Assessment Monitoring list as a guide, with the lowest calibration level for each target compound corresponding to ½ of the UCMR3 reporting limit (Table 1). Owing to the high sensitivity of the QTRAP® 6500 system these low ng/L levels were easily obtained for all compounds, with Signal-to-noise values (S/N) of 50 to 1700 after 1-point Gaussian smoothing using a peak-to-peak algorithm (Figure 2). All calibration acceptance criteria specified in EPA 537 section 10.2 were met.

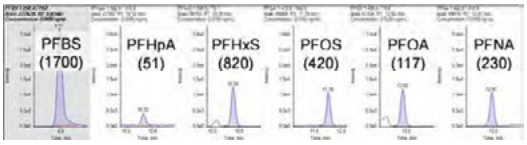

Figure 2. Signal-to-noise values (S/N) for the low calibrators. Low calibration levels for each compound are ½ of the UCMR3 reporting limits

The correlation (r) value for all calibration curves were > 0.99 (Figure 3).

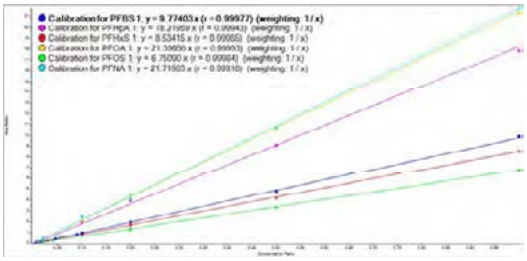

Figure 3. Calibration lines and regression equations for all six PFAAs

Initial Demonstration of Capability

To demonstrate method suitability for EPA 537 it is necessary to perform an Initial Demonstration of Capability (IDC) following the Initial Calibration. In addition to the ongoing QC criteria specified in EPA 537 section 9.3, adhering to the IDC necessitates the following:

1. Extraction of four Laboratory Fortified Blanks (LFB) to assess Accuracy (±30%) and Precision (RSD <20%). Fortification should correspond to a mid-level calibrator.
2. PFBS and $^{13}C_2$-PFHxA (surrogate) must have peaks Asymmetry Factors between 0.8 to 1.5.
3. Extraction of seven LFBs that must meet a Prediction Interval of Results (PIR) of 50 to 150% to define the Method Reporting Limits (MRL).
4. Determination of Method Detection Limits (MDL). This is an optional part of the IDC that requires seven replicates prepared over three days. In this study the MRL replicates were used.
5. All targets compounds in a Laboratory Reagent Blank (LRB) and Field Reagent Blank (FRB) after the Initial Calibration must quantify to <1/3 of MRL.
6. Evaluate method accuracy (±30%) using a Quality Control Sample (QCS) that is sourced from a vendor other than the one that provided the calibration samples.

Each of these criteria are discussed below.

Accuracy and Precision

Fortification for evaluation of Accuracy and Precision was done at 200 ng/L. This corresponded to calibration level four of six. For the four replicates extractions analyzed the relative standard deviations (RSD) ranged from 3.1 to 9.8%, while the recoveries ranged from 89 to 96% (Table 5). All of these values were within the EPA 537 specified ranges of < 20% RSD and ±30% recoveries.

Table 5. Method performance

Compound	Precision (%)	Accuracy (%)	QCS (%)		RPD (%)
			Batch 1	Batch 2	
PFBS	3.5	91	71.2	87.6	5.65
PFHpA	6.1	89	86.0	109.0	0.20
PFHxS	3.3	93	95.3	116.0	4.81
PFOA	4.7	96	96.8	101.4	3.84
PFOS	3.1	92	91.9	111.5	5.11
PFNA	9.8	91	72.8	103.6	9.21

Asymmetry Factor

To ensure acceptable chromatography of the two earliest eluting peaks in the method, the user is required to calculate the Asymmetry Factor (A_S) for every batch of samples analyzed. In the present method this corresponded to PFBS and $^{13}C_2$-PFHxA. The A_S was calculated from a mid-level calibrator of 200 ng/L. Figure 4 demonstrates that the A_S for PFBS (1.31) and $^{13}C_2$-PFHxA (1.37) meet the EPA 537 acceptance criteria of: A_S must fall in the range of 0.8 to 1.5. The A_S values were calculated automatically using MultiQuant™ software version 3.0.

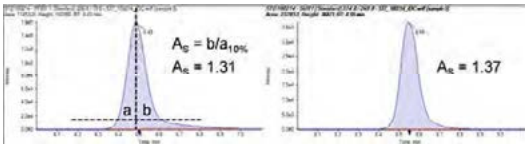

Figure 4. Asymmetry Factor for PFBS (left) and $^{13}C_2$-PFHxA (right). The example on the left demonstrates how MultiQuant™ software 3.0 calculates A_S.

Method Reporting Limits

As the current method was designed to meet the UCMR3 reporting limits, the levels used to fortify the seven extractions required for the calculation of the Method Reporting Limit (MRL) correspond to the UCMR3 reporting limits. To be a valid MRL the results of the seven replicate extractions must meet a set of statistical criteria, which are outlined in detail in section 9.2.5 of EPA 537. Briefly, the calculations are:

$$HR_{PIR} = 3.963s$$

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100\%$$

HR_{PIR} = Half Range for the prediction interval of results
s = the standard deviation of replicate analyses
3.963 = a constant value for seven replicates

The PIR must be within 50 and 150% to be a validated MRL. Using the above equations on samples that had been fortified at the UCMR3 reporting limits yielded acceptable PIR values (Table 6). Based on these calculations and the UCMR3 reporting limits that were used as sample fortification guidelines, all compounds in the current method were validated.

Table 6. MRL and MDL determination and statistical verification

Compound	Fortification Level (ng/L)	Lower PIR (%)	Upper PIR (%)	MDL (ng/L)
PFBS	90	81	99	8.3
PFHpA	10	75	114	1.4
PFHxS	30	86	99	1.6
PFOA	20	77	109	3.1
PFOS	40	56	144	35.9
PFNA	20	75	98	7.0

Method Detection Limits

The Method Detection Limit (MDL) was calculated using the following equation:

$$MDL = s \times t_{(n-1,1-\alpha=0.99)}$$

s = the standard deviation of replicate analyses
$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom
n = number of replicates

Using the MRL extracts, the calculated MDLs ranged from 1.4 to 35.9 ng/L. It is conceivable that the QTRAP® 6500 could detect lower concentrations based on the S/N for the low calibrators (Figure 2).

Laboratory Reagent Blank

A Laboratory Reagent Blank (LRB) is a system blank that has been taken through the entire extraction procedure to assess for background contamination. Following the Initial Calibration a LRB was assessed. Once MRLs were established, the LRB was evaluated with regards to the background levels relative to the calculated MRLs (Figure 5).

In the present method, all target compounds were observed well under 1/3 of their respective MRLs.

Quality Control Sample and Ongoing QC Results

The Quality Control Sample (QCS) was evaluated at 200 ng/L for all compounds to verify the validity of the Initial Calibration. All compounds met the ±30% accuracy criterium for the QCS samples (Table 5).

Three components of the ongoing QC requirements specified in EPA 537, the LRB, Asymmetry Factor and QCS, have already been discussed as they are also specified components of the IDC. In addition, the following ongoing QC criteria were required:

1. Laboratory fortified blank (LFB) should be analyzed with each batch. Acceptance criteria will depend on the fortified concentration, which should change from batch-to-batch.
2. Internal standard (ISTD) responses should not deviate more than 50% from the average ISTD response in the initial calibration and the ISTD in all samples should be 70-140% of the response in the latest continuing calibration check (CCC).
3. Surrogate recovery should be ±30% of the expected value.
4. Laboratory fortified sample matrix (LFSM) and a duplicate (LFSMD) should yield accuracies within ±30% of expected values and the relative percent difference (RPD) between the LFSM and LFSMD must be < 50%.
5. A field reagent blank (FRB) should not contain residue levels > 1/3 of the calculated MRLs.

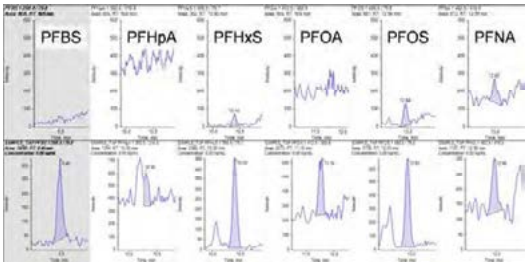

Figure 5. LRB (top) and FRB (bottom) results. Both LRB and FRB results showed background levels that were all < 1/3 of the calculated MRLs. The FRB matrix was finished tap water.

Table 6. LRB and FRB background levels in comparison to the MRL

(ng/L)	PFBS	PFHpA	PFHxS	PFOA	PFOS	PFNA
1/3 MRL	30	3.3	10	6.7	13.3	6.7
LRB	-	-	0.06	-	0.2	0.2
FRB	0.3	0.3	0.4	0.8	0.3	0.2

The first four of these criteria were all met or exceeded in all samples discussed herein. The RPD results ranged from 0.2 to 9.2, well within the ±30% RPD permitted in EPA 537 (Table 5). The FRB matrix in this study was finished tap water. Figure 5 demonstrates that all compounds were < 1/3 of the calculated MRLs, which meets EPA 537 criteria and further validates the RPD results since there was negligible background PFAA contamination in the sample matrix.

There is also criteria for CCCs (low CCC accuracy 50-150%; mid/high CCC accuracy 70-130%; surrogate accuracy 70-130%) that were met for all samples analyzed.

Conclusion

The QTRAP® 6500 LC-MS/MS system is a sensitive and robust platform for the analysis of PFAAs in drinking water. The demonstrated MRLs easily meet the UCMR3 reporting limits.

References

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Abbreviations

A_s – asymmetry factor
CASRN – chemical abstracts registration number
CCC – continuing calibration check
CE – collision energy
DP – declustering potential
EPA – environmental protection agency
ESI – electrospray ionization
FRB – field reagent blank
HR_{PIR} – half range prediction interval of results
IDC – initial demonstration of capability
ISTD – internal standard
LFB – laboratory fortified blank
LFSM – laboratory fortified sample matrix
LFSMD – laboratory fortified sample matrix duplicate
LRB – laboratory reagent blank
MDL – method detection limit
MRL – method reporting limit
MRM – multiple reaction monitoring
PFAAs – perfluoroalkyl acids
PIR – prediction interval of results
QCS – quality control sample
RPD – relative percent difference
RSD – relative standard deviation
RT – retention time
S/N – signal-to-noise
UCMR3 – unregulated contaminant monitoring rule 3 assessment monitoring list

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Contents

Quantitation of PFASs in Water Samples using LC-MS/MS Large-Volume Direct Injection and Solid Phase Extraction

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Overview

This application note presents two methods for the quantitation of per- and polyfluorinated alkyl substances (PFASs) in water samples. While the MS/MS detection method using the SCIEX Triple Quad™ 5500 is similar between the two methods, the sample preparation and injection volume differ significantly. The first method presented here utilizes a weak-anion exchange solid phase extraction (SPE) method to concentrate water samples for analysis using a 7.5 minute HPLC gradient. The second method utilizes dilution of a water sample in methanol and direct injection of 950 µL of the diluted sample using a 17.5 minute HPLC gradient. Special modifications to the pumps and autosampler are described to mitigate laboratory-based contamination of PFASs. Both methods achieved accurate quantitation at levels of approximately 1-10 ng/L for more than 17 PFASs.

Introduction

PFASs are unique chemicals whose physicochemical properties make them important for use in a variety of industrial and consumer products including carpets, cookware, food packaging, fire suppressants, and others[1]. Chemically, PFASs are aliphatic structures containing one or more C atoms on which H substituents have been replaced by F atoms. Classification and naming is typically by the particular functional group present, such as carboxylic acids, sulfonates, phosphonic acids, etc., as well as the length of the carbon chain. Desirable in various industrial applications for their chemical stability and low reactivity, these properties also make PFASs highly resistant to degradation in aquatic environments. Typical concentrations of PFASs found in various environmental water sources range from pg/L to µg/L levels[2].

Human exposure to PFAS residues has been implicated in the incidence of cancer, obesity, endocrine system disruption, and other adverse health effects[3-4]. In recognition of these potential risks, sources of human exposure to these chemicals (*e.g.*, via drinking water) are receiving public and scientific attention. PFASs exhibit relatively high aqueous solubility and can be transported and bioaccumulated from contaminated water sources. The US EPA maintains health advisory limits for select PFASs (*e.g.*, perfluorooctanoic acid (PFOA) at a limit of 70 ng/L)

in water, but these levels have been exceeded in some areas experiencing extreme point source inputs of these chemicals[5].

Given the tremendous persistence of PFASs in the environment and their known presence in human populations exposed via drinking water and other environmental routes, demonstration of the capability for accurate and precise low-level quantitation is paramount for research and testing laboratories. Robust quantitative analytical methods utilize the specificity and sensitivity of LC-MS/MS with MRM monitoring. However, a primary analytical challenge to this assay is the prevention and reduction of background PFASs originating from the LC system and contamination during sample collection and preparation. The two analytical methods described here employ strategies to address PFAS contamination. These include the use of a delay column for separation of a contamination PFAS peak from the analytical peak, and a large volume injection of an aqueous sample intended to achieve method sensitivity while reducing accumulated background during sample concentration steps.

Experimental

HPLC System

Shimadzu LC-20ADXR binary pumps with a Shimadzu DGU-20A5 degasser provided the gradient chromatographic conditions. All fluoroethylene polymer (FEP) tubing on the Shimadzu pumps and degasser was replaced with PEEK tubing with similar internal and external dimensions. A Phenomenex Luna C18(2) column (dimensions shown in **Table 1**) was installed between the pump mixing chamber and the column,

Contents

outside of a Shimadzu CTO-20AC column oven. This column served as a delay or hold-up column to isolate PFAS contamination originating from the pumps and eluents. A longer and/or larger diameter Luna C18(2) column must be installed on heavily contaminated systems to prevent breakthrough of contamination.

Chromatographic separation was performed using a Phenomenex Gemini C18 HPLC column at 0.6 mL/min (**Table 1**). The Gemini C18 column was heated to 40°C in the column oven. A PAL-HTC-xt autosampler with dynamic load-wash (DLW) was modified by replacing all FEP tubing from the rinse solvent lines, the needle seal, and the sample holding loop with PEEK or stainless steel. The autosampler syringe and sample holding loop was rinsed with methanol and 1:1 methanol:acetonitrile between samples.

Table 1. LC columns for Methods 1 and 2

	Column	Dimensions
Delay Column	*Phenomenex Luna C18(2)*	5 µm; 30 x 2 mm
Method 1 HPLC Column	*Phenomenex Gemini C18*	3 µm; 50 x 2 mm
Method 2 HPLC Column	*Phenomenex Gemini C18*	3 µm; 100 x 3 mm

Standards and Internal Standards (IS)

The PFAS standards and internal standards were obtained from Wellington Laboratories (Guelph, Ontario) and were prepared in Baker HPLC-grade methanol. Standard stock solutions were prepared by dilution with 96% methanol and 4% water (purified using a Millipore water purification system).

Sampling and sample preparation

Water samples were obtained anonymously from various sources in the United States. Samples were stored in the dark at 4°C in 250 mL high density polyethlyene bottles until analysis.

Method 1: Solid Phase Extraction and 10 µL Injection

A mixture of surrogate standards (25 ng) was added to 250 mL water samples in the sampling bottle, and the entire volume was extracted using weak anion exchange SPE as recommended by ISO standard 25101[6]. The empty sample container was rinsed with 10 mL of methanol with 0.3% NH_4OH, which was then added to the SPE tube to elute the PFASs. The extract was

evaporated to dryness, reconstituted in 500 µL of 80% methanol/20% water, and transferred to a polypropylene vial for analysis. All standards and blanks were also prepared at a final methanol concentration of 80%.

For Method 1, 10 µL injections of the standards and samples were analyzed using a 6.5 min gradient method (**Table 2**) with a 7.5 min total runtime, including the 1 min autosampler injection cycle. Water with 20 mM ammonium acetate was used as the "A" solvent and methanol was the "B" solvent.

Table 2. LC gradient for Method 1 at a flow rate of 0.6 mL/min.

Step	Time (min)	A (%)	B (%)
0	0.00	90	10
1	0.10	45	55
2	4.50	1	99
3	4.95	1	99
4	5.00	90	10
End	6.50		

Method 2: Dilution and Large Volume Injection

A 1 mL aliquot of a water sample was added to a 2 mL clear glass autosampler vial with a polyethylene septum cap containing 0.65 mL of methanol and a mix of surrogate standards at a final concentration of 50 ng/L. The final concentration of methanol in the diluted sample was 40%, and standards, blanks, and quality control samples were all prepared at the same concentration. A PAL HTC-xt autosampler was modified to inject 950 µL of the diluted samples and standards.

For Method 2, samples were analyzed using an extended 15.5 min gradient method (**Table 3**) with a 17.5 min total runtime, including the 2 min autosampler injection cycle. Water with 20 mM ammonium acetate was used as the "A" solvent, and methanol was the "B" solvent.

Table 3. LC gradient for Method 2 at a flow rate of 0.6 mL/min.

Step	Time (min)	A (%)	B (%)
0	0.00	90	10
1	1.50	35	65
2	8.00	5	95
3	8.10	1	99
4	12.00	1	99
5	12.50	90	10
End	15.50		

MS/MS Detection

A SCIEX Triple Quad® 5500 system with a Turbo V™ source and ESI probe was used for analysis in negative polarity. The ion source parameters were optimized for the LC conditions using the Compound Optimization (FIA) function in Analyst® software (**Table 4**).

Table 4. Ion source parameters for Methods 1 and 2

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	-4500 V
Temperature (TEM)	600°C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	50 psi

One characteristic MRM transition was monitored for each analyte and internal standard (**Appendix Table 1**). The *Scheduled* MRM™ algorithm was activated to monitor compounds only during a 60 second expected retention time window to maximize dwell times and optimize the cycle time of the method. As a result, all of the peaks in the calibration contained >12 points per peak.

Calibration was performed using a 7-point curve at concentrations of 25, 50, 250, 1000, 2500, 10000, and 20000 ng/L for Method 1 and 1, 2, 5, 20, 50, 100, and 200 ng/L for Method 2. Quantitation was performed using MultiQuant™ 3.0.2 using 1.0 Gaussian smoothing and $1/x^2$ weighted linear regression. PFASs with matched isotopically labelled surrogate standards were quantified using isotope dilution, while PFASs without matched surrogate standards were quantified using internal standard calibration with structurally similar isotopically labeled standards (full analyte and internal standard list shown in Appendix Figure 1). A concentration factor of 500 was applied to samples analyzed using Method 1, and a dilution factor of 1.65 was applied to samples analyzed using Method 2.

Results and Discussion

Method 1 Chromatography

The Gemini C18 column was selected for both methods based on its strong retention and predictable resolution of PFASs. All of the other columns tested exhibited breakthrough of the short chain acids in the column dead volume during optimization of the 950 µL injection method. The Luna C18(2) column was selected as the delay column for both methods after initial testing indicated that it provided better separation of PFAS contamination than other columns. For PFASs, blank contamination is a major concern for analysis due to potential contamination during sample preparation or contamination originating from analytical instrumentation. **Figure 1** shows a small carryover peak at 2.5 min for PFHxS in a blank analyzed immediately following the injection of the highest calibration standard of 20,000 ng/L. The area of the carryover peak was only 0.078% of the highest standard and 21% of the lowest calibration standard for Method 1 (25 ng/L). The second peak at 3.2 min in **Figure 1** is attributed to delayed PFHxS contamination originating from the HPLC pumps. Without the delay column, this contamination would instead focus on the analytical column and elute at 2.5 min along with the standard and sample peak.

A 50 mm x 2 mm, 3 um Gemini C18 column was selected for Method 1, which utilized a 10 µL injection volume. The chromatographic separation of 25 PFASs is shown in **Figure 2**.

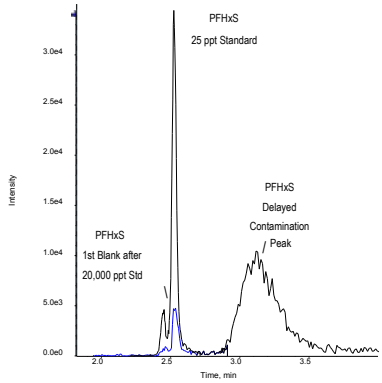

Figure 1. Overlaid MRM traces for PFHxS in the lowest calibration standard (25 ng/L) and a blank injection that followed the highest concentration standard (20 µg/L). The delayed peak in the calibration standard trace represents the ambient LC system contamination retained by the delay column.

The average peak asymmetry factor for the first 2 eluting peaks (PFBA and PFBS) in the initial calibration standards was calculated to be 1.3 in Method 1 using MultiQuant™ 3.0.2. This is within the acceptance criteria set by EPA 537 of 0.8-1.5 [7].

Partial resolution of the branched and linear isotopes is necessary for PFAS analysis to distinguish between samples containing only linear isotopes or isotope mixtures. As shown in **Figure 2**, the earlier eluting branched isotopes are clearly distinguishable from the major peak corresponding to the linear isotopes for the 2 compounds that contained both branched and linear isotopes in the standards (PFHxS and PFOS). Most methods recommend that these two peaks are summed for quantitation, which was performed in this application note using MultiQuant™ 3.0.2.

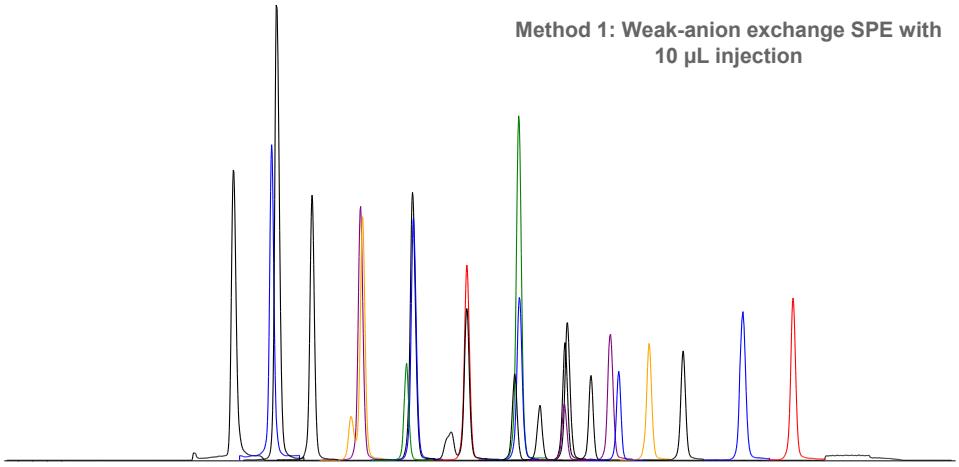

Figure 2. Overlaid chromatograms of a 1 µg/L standard injected using Method 1.

Method 1 Calibration

The initial 7-point calibration for Method 1 exhibited good accuracy within +/- 30% of the expected values for all points, accuracy within +/- 10% for the lowest calibrator, and R^2 coefficients of >0.990, as shown in **Table 5**. Based on the S:N ratio of the low calibrator and the linearity of the curve, the calibration range could be extended on both the high and low levels to improve the dynamic range. A water sample analyzed using Method 1 exhibited concentrations of several PFASs ranging from 0.974 to 53.3 ng/L, as shown in **Figure 3**.

Method 2 Chromatography

Method 2 is a large-volume, direct aqueous injection method designed for drinking, surface, and ground water samples. After the addition of surrogate standards and a simple dilution with

methanol, 950 µL of the sample was injected directly onto the Gemini C18 column. In contrast to Method 1, a longer and larger diameter column was used to improve retention of the analytes in the large volume injection. This resulted in a longer total runtime (17.5 minutes compared with 7.5 minutes), but provided robust results for the large volume injection and minimal retention time shift (**Figure 4**). The only compound that exhibited deteriorated peak shape due to the large injection volume was PFBA. However, the broadened peak shape of PFBA did not affect quantitation accuracy or precision.

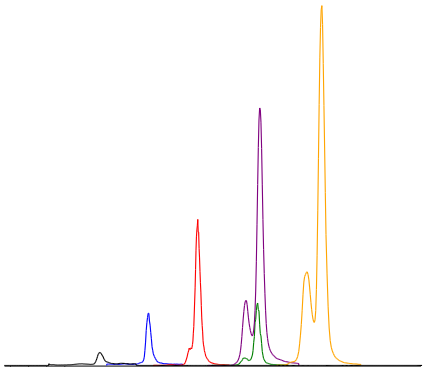

Figure 3. Overlaid chromatograms of PFASs quantified in a water sample using Method 1 (solid-phase extraction and 10 µL injection).

Table 5. Sensitivity (S/N calculated using Multiquant 3.0.2™) and linearity from 25 to 20,000 ng/L and 1 to 200 ng/L (coefficient of regression, R^2) using Method 1 and Method 2, respectively.

	Method 1				Method 2			
Compound	Calibration Range (ng/L)	Linear Correlation (R^2)	S:N of 25 ng/L Standard	Accuracy of 25 ng/L Standard	Calibration Range (ng/L)	Linear Correlation (R^2)	S:N of 1 ng/L Standard	Accuracy of 1 ng/L Standard
PFCAs								
PFBA	25-20,000	0.997	108	104%	1-200	0.997	328	97%
PFPeA	25-20,000	0.998	88	103%	1-200	0.999	137	101%
PFHxA	25-20,000	0.998	104	93%	1-200	0.999	284	101%
PFHpA	50-20,000	0.999	116	101%	1-200	0.993	267	96%
PFOA	25-20,000	0.999	117	106%	1-200	0.999	113	99%
PFNA	25-20,000	0.990	91	109%	1-200	0.999	137	101%
PFDA	25-20,000	0.998	103	105%	1-200	0.997	176	96%
PFUdA	25-20,000	0.995	84	101%	1-200	0.998	168	99%
PFDoA	25-20,000	0.998	60	101%	1-200	0.994	127	94%
PFTrDA	25-20,000	0.998	32	104%	1-200	0.995	125	95%
PFTeDA	25-20,000	0.994	15	107%	1-200	0.998	56	98%
PFHxDA	25-20,000	0.999	21	103%				
PFODA	25-20,000	0.999	33	102%				
PFSAs								
PFBS	25-20,000	0.995	31	92%	2-200	0.994	1178	100%
PFHxS	25-20,000	0.999	604	103%	1-200	0.998	229	96%
PFHpS	25-20,000	0.997	103	105%	1-200	0.999	327	99%
PFOS	25-20,000	0.995	312	105%	1-200	0.999	251	99%
PFDS	25-20,000	0.998	88	102%	1-200	0.999	516	98%
Other PFASs								
6:2 FTS	25-20,000	0.991	100	98%				
8:2 FTS	25-20,000	0.992	113	97%				
PFOSA	25-20,000	0.997	118	104%	1-100	0.997	1012	96%
MeFOSA	25-20,000	0.996	96	103%				
EtFOSA	25-20,000	0.994	90	101%				
N-MeFOSAA	25-20,000	0.996	109	100%				
N-EtFOSAA	25-20,000	0.994	61	103%				

Similar to Method 1, blank contamination from the instrument was minimized by using a delay column in Method 2. Blank contamination from sample preparation was also minimized in Method 2 by reducing the number of pipetting steps and testing all new batches of solvents prior to use. The integrated areas of the first blank after the highest concentration sample (200 ng/L) were less than 50% of the lowest calibrator. For example, the area of the first blank analyzed after the 200 ng/L calibration standard was 22% of the area of the 1 ng/L standard for PFOA as shown in **Figure 5**. The other blanks shown in **Figure 5** exhibited even lower response for PFOA, which could be contributed to laboratory contamination for the method blank and solvent contamination for the instrument blank.

To be compatible with common sampling practices, the Method 2 was not optimized for recovery of the longest chain PFASs, PFHxDA and PFODA, from the sample container or from the autosampler vial. Due to the stronger hydrophobicity of these compounds compared with the shorter chain PFAS, PFHxDA and PFODA appeared to bind to polypropylene containers when the methanol concentration was <40%. Modifications to this method to improve the recovery and precision of PFHxDA and PFODA analysis may include collecting smaller samples (10-20 mL), diluting the entire sample with methanol in the sampling

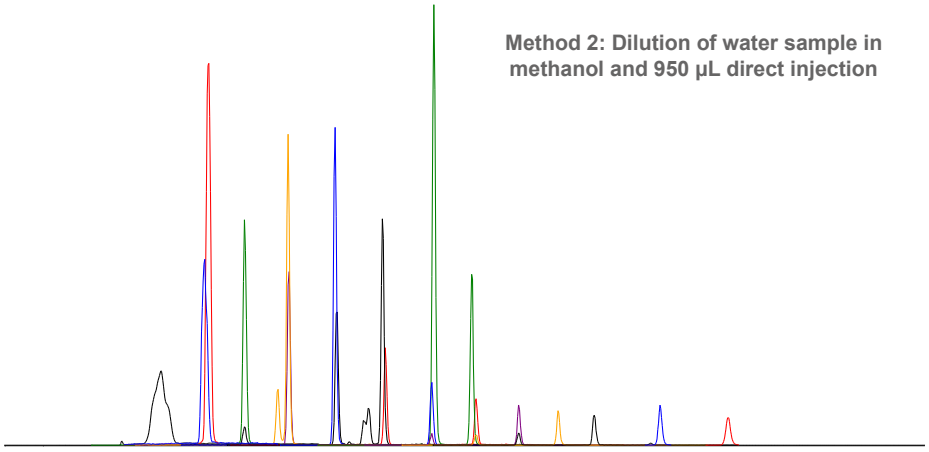

Figure 4. Overlaid chromatograms of a 10 ng/L spike into groundwater matrix that was diluted with methanol and injected according to Method 2

container, and adding surrogate standard directly to the sampling container.

Direct analysis of water samples is impaired by the presence of 5 g/L Trizma in samples, which is added to drinking water samples as a requirement by EPA method 537. Trizma suppresses ionization of the PFASs and elutes slowly from the column for minutes after the injection. Therefore, Trizma should not be added to samples that will be analyzed using direct aqueous injection, but is fully compatible with SPE as performed in Method 1.

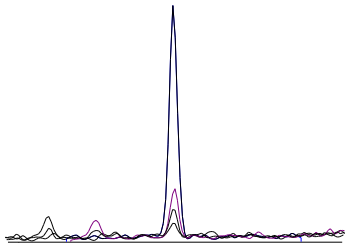

Figure 5. Overlaid PFOA traces in a 1 ng/L calibration standard and a series of blank injections analyzed using Method 2: a blank injection following a high concentration standard, a method blank, and an instrument blank analyzed before the calibration standards.

Method 2 Calibration

Similar to Method 1, the initial calibration results for Method 2 exhibited good accuracy within +/- 30% of the expected values for all points, accuracy within +/- 10% for the lowest calibrator, and R^2 coefficients >0.990, as shown in **Table 5**. In the development of Method 2, calibration standards for 6:2 and 8:2 FTS, MeFOSA, EtFOSA, MeFOSAA, and EtFOSAA were not analyzed in the full calibration curve.

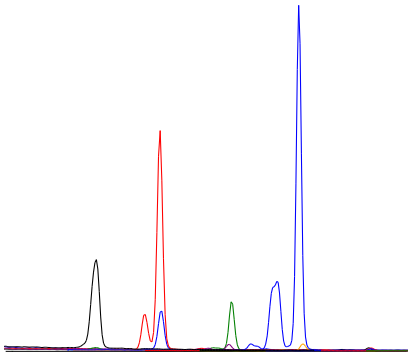

Figure 6. Overlaid MRM traces of PFASs detected in a groundwater sample with the calculated concentrations of each PFAS. The sample was prepared and analyzed using Method 2.

Method 2 Performance

Because large-volume injection methods are less common for PFASs compared with offline extraction methods, this application note reports the recovery and precision of continuing calibration standards over 1 week of continuous water sample analysis to demonstrate the robustness and accuracy of Method 2. The chromatogram and quantitated values for several PFASs in one of these water samples are shown **Figure 6**.

As shown in **Table 6**, a continuing calibration standard at 20 ng/L analyzed 1 week after the initial calibration exhibited quantitative accuracy of 92-99% for all compounds with the exception of PFTrDA (81%) and PFBS (84%). Due to limited availability of surrogate standards, PFBS was analyzed using $^{18}O_2$ PFHxS as an internal standard, and PFTrDA was analyzed using $13C_2$ PFDoA. The absence of an exact isotope-labelled surrogate for these two compounds likely contributed to the decreased accuracy of the ongoing calibration standard.

During the 1 week period of full-time water sample analysis, 9 replicates of the 20 ng/L continuing calibration verification (CCV) were analyzed as shown in **Table 6**. The precision (%CV) for all of the PFASs was <5%, which indicates excellent precision for the large volume injections. The surrogate recovery, calculated as the response of the surrogate standard in the 20 ng/L ongoing

calibration standard divided by the response of the surrogate standard during the initial calibration, was within 73-120% for all of the PFASs analyzed.

Summary

The 2 methods reported in this application note were designed for optimum robustness using the SCIEX Triple Quad® 5500 system as the analytical platform. Both methods may be expanded to include soil, sediment, and biological extracts. Minimum and maximum reporting limits of approximately 1 ng/L to 400 µg/L could be achieved using both methods. These ranges could be expanded by increasing the extracted volume in Method 1 or by further dilutions in Method 2. The example chromatograms shown in this application note also demonstrate that the lower calibration levels than the levels analyzed here could be included in initial calibration curves to further improve the sensitivity of the method.

Method 1 has the advantage of compatibility with EPA Method 537 and allows sample concentration using solid phase extraction. Method 2 has the advantages of minimal sample preparation and fewer steps to introduce lab-based PFAS contamination. With the growing need for PFAS analysis of environmental samples, these versatile methods will be useful for labs aiming to evaluate growing lists of PFASs.

Table 6. Accuracy of a 20 ng/L CCV analyzed 1 week after the initial calibration and precision of 9 replicates of a 20 ng/L CCV analyzed between 5 and 7 days after the initial calibration using Method 2.

Compound	Calculated Conc. of 20 ng/L CCV	Accuracy of 20 ng/L CCV	Surrogate Standard Recovery	Precision of 9 20 ng/L CCVs (%CV)
PFCAs				
PFBA	19.4	96%	107%	1.50%
PFPeA	19.7	98%	107%	1.40%
PFHxA	19.7	99%	108%	2.26%
PFHpA*	18.5	92%	103%	3.11%
PFOA	19.2	96%	105%	2.07%
PFNA	19.3	97%	107%	1.11%
PFDA	19.4	97%	107%	2.62%
PFUdA	18.8	94%	109%	2.90%
PFDoA	18.7	94%	99%	1.90%
PFTrDA	16.3	81%	99%	4.77%
PFTeDA	18.9	95%	73%	1.43%
PFSAs				
PFBS	16.8	84%	112%	2.65%
PFHxS	19.2	96%	112%	1.94%
PFHpS	19.4	97%	112%	3.85%
PFOS	18.8	94%	120%	2.62%
PFDS	18.6	93%	120%	2.69%
Other PFASs				
PFOSA	19.0	95%	112%	0.98%

Acknowledgements

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Appendix Table 1. MRM masses for Methods 1 and 2. Analytes are shown in **bold** font, and internal standards are shown in *italic* font.

Compound	Q1	Q3	DP	CE
PFBA	212.9	169	-25	-12
PFPeA	262.9	219	-20	-12
PFHxA	313	269	-25	-12
PFHpA	363	319	-25	-12
PFOA	413	369	-25	-14
PFNA	463	419	-25	-14
PFDA	513	469	-25	-16
PFUdA	563	519	-25	-18
PFDoA	613	569	-25	-18
PFTrDA	663	619	-25	-20
PFTeDA	713	669	-25	-22
PFHxDA	813	769	-25	-24
PFODA	913	869	-25	-26
PFBS	298.9	80	-55	-58
PFHxS	399	80	-60	-74
PFHpS	449	80	-65	-88
PFOS	499	80	-65	-108
PFDS	599	80	-85	-118
6:2 FTS	427	407	-50	-32
8:2 FTS	527	507	-50	-40
PFOSA	498	78	-60	-85
MeFOSA	512	169	-75	-37
EtFOSA	526	169	-75	-37
N-MeFOSAA	570	419	-40	-36
N-EtFOSAA	584	419	-50	-36
13C4_PFBA	217	172	-25	-12
13C5_PFPeA	268	223	-20	-12
13C2_PFHxA	315	270	-25	-12
13C4_PFHpA	367	322	-25	-12
13C2_PFOA	415	370	-25	-14
13C4_PFOA	417	372	-25	-14
13C5_PFNA	468	423	-25	-14
13C2_PFDA	515	470	-25	-16
13C2_PFUdA	565	520	-25	-18
13C2_PFDoA	615	570	-25	-18
13C2_PFTeDA	715	670	-25	-22
13C2_PFHxDA	815	770	-25	-24
18O2_PFHxS	403	84	-60	-74
13C4_PFOS	503	80	-65	-108
13C8_PFOSA	506	78	-60	-85
M2-6:2FTS	429	409	-50	-32
M2-8:2FTS	529	509	-50	-40
d3MeFOSA	515	169	-75	-37
d5EtFOSA	531	169	-75	-37
d3-MeFOSAA	573	419	-40	-36
d3-EtFOSAA	589	419	-50	-36

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Contents

Rapid Characterization of Naphthenic Acids Using High Resolution Accurate Mass MS and MS/MS with SelexION™ Differential Mobility Separation

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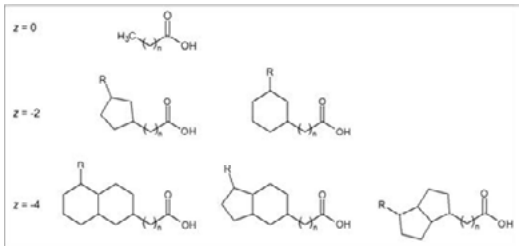
Overview

This study demonstrates the rapid gas-phase analysis of naphthenic acids using the SCIEX TripleTOF® 5600+ system with SelexION™ differential mobility separation. The assets of this workflow include:

1. The rapid characterization (< 2 min) of naphthenic acids in complex samples.
2. Utilizing SelexION™ to isolate individual naphthenic acids on-demand (including isomers and isobars) for in-depth structural analysis.
3. Accounting for background ions using their SelexION™ mobility.

Introduction

Naphthenic acids (NA) from oil sands process-affected water (OSPW) have been the subject of numerous mass spectrometry-based environmental studies.[1, 2]



The classical definition of these compounds ($C_nH_{2n+z}O_2$, where z is an even negative integer representing hydrogen deficiency) has recently been expanded to the naphthenic acid fraction component (NAFC), which includes unsaturated and aromatic NA derivatives, increased oxygen content and compounds containing nitrogen and/or sulfur.[1-3]

NAFCs are of particular concern in northern Alberta, Canada, where the caustic extraction of bitumen from surface mineable oil sands produces large volumes of OSPW.[4]

A common workflow for NAFC analysis involves direct infusion into an ultrahigh resolution mass spectrometer. A resolving power >100,000 is critical to obtaining useful NAFC profiles with this approach. While such instruments can provide elemental compositions, additional steps are required to interrogate molecular structure. To overcome this, chromatography is often utilized. Unfortunately, the high complexity of OSPW extracts may necessitate relatively long run times, multiple sample handling steps, or multi-dimensional chromatography. Moreover, traditional chromatography is an inherently serial process, with limited time available to perform deeper structural interrogation of individual analytes.

Contents

The SCIEX TripleTOF® 5600+ high resolution accurate mass system coupled with SelexION™ differential mobility spectrometry presents a unique workflow for NAFC analysis.[5]

The combination of gas-phase separation and the ability to access analytes on-demand via direct-infusion resolves NAFCs from OSPW extracts rapidly (< 2 min), generating complex and insightful datasets. Such rapid, information rich methods will become important as regulatory guidelines for NAFC analyses are implemented and testing laboratories have to accommodate increased demand.

Experimental

A technical NA mix was obtained from the Merichem Company (Houston, TX) and an OSPW extract was obtained by extracting a sample from an industrial location in the Athabasca River Basin, Alberta, Canada.[6]

Analyses were conducted using the SCIEX TripleTOF® 5600+ system with SelexION™ or the SCIEX QTRAP® 5500 system with SelexION™. The operating parameters are listed in Table 1.

Table 1. Typical SelexION™ and TripleTOF® operating parameters

Parameter	Value
Infusion rate	15 µL/min
IonSpray voltage	-4500 V
Source temperature	100°C
Gas 1	20 psi
Gas 2	10 psi
Modifier composition	1.5% (v/v)
SelexION™ temperature	150°C
Separation Voltage (SV)	4000 V_{pp}
Compensation Voltage (CoV)	-17 to +20 V (0.25 V steps)
TOF m/z range	50 - 2000
TOF accumulation time	250 msec
Declustering Potential (DP)	-100 V
MS/MS Collision Energy (CE)	-35 to -50 V

For the SelexION™ experiments two operational modes were employed:

1. Separation voltage was held at an optimum value while the compensation voltage was scanned; and
2. At each CoV increment, MS or MS/MS spectra were recorded.

Data were plotted as ionograms (Signal Intensity versus CoV).

Data acquisition was carried out using Analyst® TF software and data was interrogated using PeakView® software version 2.0 and an accurate mass database (8,127 entries) with the following parameters: $C_{5\text{-}20}$, z0-16, $O_{2\text{-}8}$, $N_{0\text{-}2}$, $S_{0\text{-}2}$. Results were confined to ±10 ppm of the database masses and were compared to suitable blanks.

Results and Discussion

SelexION™ mobility functions by transmitting ions between two planar electrodes, across which a high-voltage rf asymmetric waveform is applied (Figure 1).

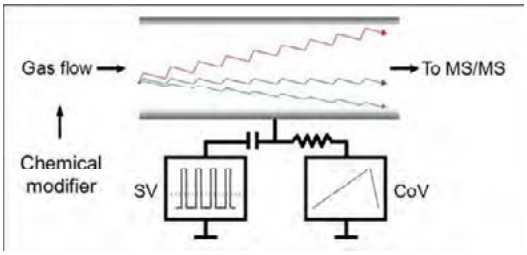


Figure 1. Schematic of the SelexION™ mobility cell

The difference between the ion's mobility during the high- and low-field portions of the waveform determines their SelexION™ mobility. Chemical effects are also critical to ion separation using SelexION™, as the addition of volatile modifiers like methanol to the transport gas can alter ion mobility. To mitigate the complexity of NAFC analysis, a series of chemical modifiers were added to the transport gas and the resulting SelexION™ peak capacities were evaluated. Since each ion was transmitted with a full-width half maximum of ~2.5 V, the greater the spread in total CoV space covered by all of the NAFC ions, the greater the peak capacity of the SelexION™ separation. Among the modifiers examined, methanol yielded the greatest total spread in CoV (Figure 2).

Contents

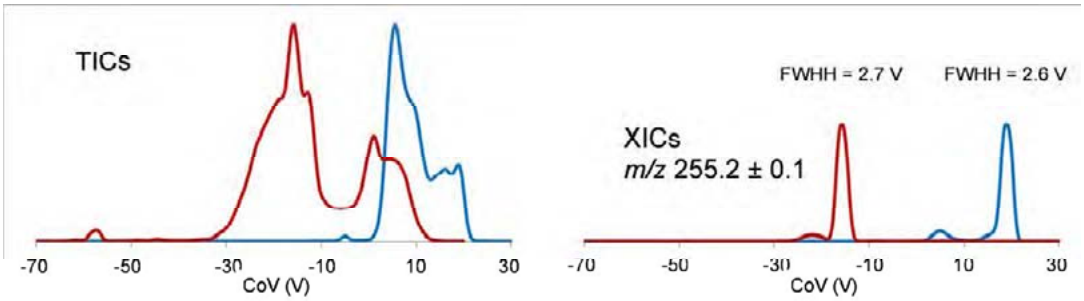


Figure 2. SelexION™ behavior of an OSPW extract with (red) and without (blue) methanol added in the N_2 carrier gas. Extracted ion chromatograms are shown as an example of typical peak widths (right). Signal intensity was normalized for ease of data presentation. FWHM – full width at half maximum height.

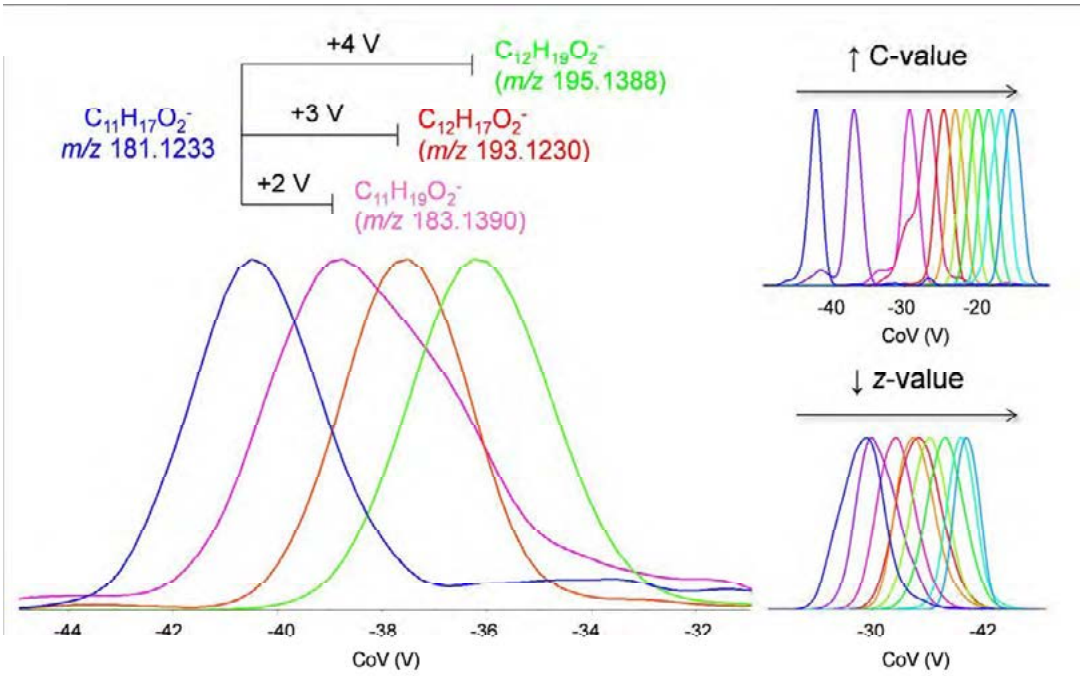


Figure 3. Thorough data analysis demonstrated that SelexION™ behavior was not simply correlated to *m/z* (left). Trends in CoV shifts for carbon number (top right) and z-series (bottom right) are consistent with reported LC behavior of similar samples. Data was normalized to 100% within each ionogram and is shown for an OSPW extract.

The SelexION™ separation of individual NAFC ions suggested a correlation between ion mobility and structure (Figure 3).

For example, *m/z* 181.1233 ($C_{11}H_{17}O_2^-$) was separated from the more saturated analogue at *m/z* 183.1390 ($C_{11}H_{19}O_2^-$, only two hydrogens added) by +2 V. However, this $C_{11}H_{17}O_2^-$ ion was only separated by +3 V from $C_{12}H_{17}O_2^-$ (an acid one carbon atom heavier) and by +4 V separated from $C_{12}H_{19}O_2^-$ (one CH_2 unit heavier).

Clearly, the more unsaturated analogues exhibited more negative CoVs, and the ring/double bond analogues displayed different mobility behavior than the linear chain extended analogues. The fact that such subtle structural differences result in the separation of closely related ions demonstrates the analytical potential of SelexION™-based workflows. In addition to the above observations, correlations between CoV and homologous carbon series and *z*-series emerged (Figure 3).

A Kendrick mass plot (plot of Kendrick mass defect as function of Kendrick mass) provides a high level means of assessing the composition of complex mixtures like OSPW extracts. In the Kendrick mass plot (Figure 4) all relevant mass peaks in a spectrum are normalized against methylene, such that a horizontal line represents a homologous carbon series for a particular compound class. Moreover, as the degree of unsaturation increases, so does the Kendrick mass defect. This translates to easy to assess trends. For instance, in Figure 4 the red lines indicate a series of simple acids (i.e., only O_2 compounds) with increasing *z*-values, while the green lines represent a homologous series of O_3 compounds at differing degrees of unsaturation. Finally, the blue lines demonstrate the observable trends in unsaturation for a given carbon number.

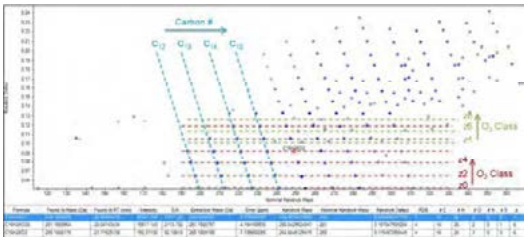


Figure 4. Kendrick mass plot of the OSPW extract generated in an add-in of PeakView™ software

Another interesting attribute of the SelexION™ separation of NAFCs was the gas-phase resolution of isobaric and isomeric ions. For example, in the OSPW extract *m/z* 143.1080, corresponding to $C_8H_{15}O_2^-$ (1.7 ppm), was transmitted though the SelexION™ cell at two CoVs (Figure 5).

The separation of these two potential isomers was rapid (~3 sec) and due to the infusion-based sample analysis, MS/MS analysis of each *m/z* 143.1080 ion was easily performed by fixing the SelexION™ cell at the appropriate CoV value. The resulting MS/MS spectra yielded fragmentation patterns that were consistent with the presence of distinct isomeric species. Analysis of three authentic $C_8H_{16}O_2$ isomers revealed that the OSPW extract contained 2-ethylhexanoic acid and n-octanoic acid (Figure 5). Separation of such isomers by GC or LC generally requires minutes of elution time. Using SelexION™, these isomers were separated in seconds and could be analyzed on-demand.

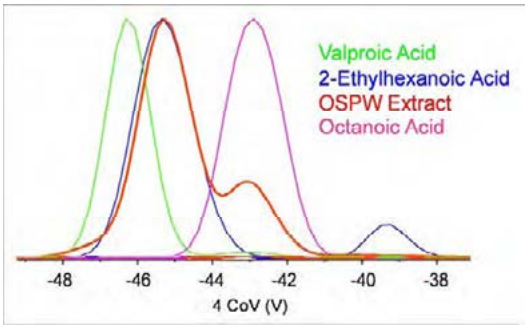


Figure 5. Apparent structural isomers of $C_8H_{16}O_2$ were identified in the OSPW extract (red). Subsequent analysis of valproic acid (green), 2-ethylhexanoic acid (blue) and octanoic acid (pink) standards showed that 2-ethylhexanoic acid and octanoic acid were present in the OSPW extract and partially resolved using SelexION™.

There were also numerous examples where SelexION™ separated isobaric species. In one case, palmitic acid ($C_{16}H_{31}O_2^-$), which is a known contaminant in laboratory environments, was observed at *m/z* 255.2329 (Figure 6).

Alongside the ionized palmitic acid was an isobar at *m/z* 255.1405. TOF-MS can easily resolve these ions, but further interrogation by MS/MS would be complicated without SelexION™ since both ion populations would be sampled by the quadrupole mass filter and a heavily convolved MS/MS spectrum would result. Like the $C_8H_{16}O_2$ isomer separation, selectively tuning the CoV facilitated the interrogation (MS and MS/MS) of each isobar in real-time.

The anion at *m/z* 255.2329 (CoV = -21 V) fragmented via loss of water (*m/z* 237.2234, 4.3 ppm) as the dominant product ion, which is consistent with palmitic acid. The MS/MS of *m/z* 255.1405 (CoV = -26 V) showed carbon dioxide loss (*m/z* 211.1520, 2.3 ppm) as the dominant product ion, suggesting a carboxylic acid (Figure 6). The elemental composition $C_{17}H_{19}O_2^-$ correlated with *m/z* 255.1405 (5.7 ppm).

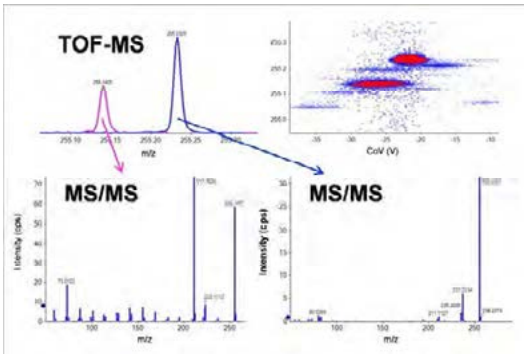


Figure 6. SelexION™ is capable of rapidly resolving isobars from an OSPW extract via mass resolution (top left) and ion mobility (top right). MS/MS analysis of *m/z* 255.2329 is consistent with palmitic acid (bottom right), while the *m/z* 255.1405 is consistent with another carboxylic acid (bottom left).

In LC-MS analyses of NAFCs, interferences like palmitic acid, stearic acid, or dodecyl sulfate, often present as systematic contaminants, are ionized continuously throughout the chromatographic run. These ever-present background ions may deteriorate mass spectrometer performance. SelexION™ efficiently removes background ions by sequestering them in discrete CoV ranges, outside of which they will not appear in the analytical data. In this study, it was critical to obtain an accurate assessment of the potential interfering species present in the analytical samples to ensure that only NAFCs were reported from the database search.

The combination of SelexION™ differential mobility separation and detection using the SCIEX TripleTOF® 5600+ revealed 12 background compounds that were present, including saturated and monounsaturated fatty acids, several of which are known background ions in laboratory solvents and analytical instrumentation. One series of interest belonged to the O_3S compound class, with *z* = -6. The series ranged from C_{16} to C_{19} with mass accuracies of 0.5 to 3.0 ppm. MS/MS of these ions after isolation with SelexION™ verified the presence of alkyl-substituted benzylsulfonates (Figure 7).

These detailed structural analyses were quickly and easily facilitated using SelexION™ by tuning the CoV to the appropriate value for each background ion. While this series exhibited relatively low responses, their inclusion in the Merichem and OSPW data would present a false indication of the compound classes present.

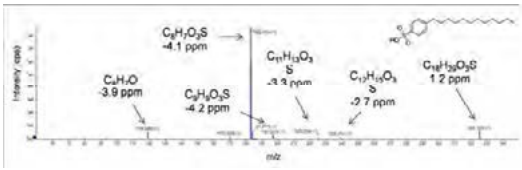


Figure 7. Example MS/MS analysis for the O_3S components identified in the ACN Blank, Merichem standard and OSPW extract. Proposed structure and detailed MS/MS assignments are shown for *m/z* = 325.1839, which corresponds to $C_{18}H_{30}O_3S$ within 1.2 ppm at the MS level and within 3.7 ppm at the MS/MS level. Values shown for product ion assignments are given with calculated mass errors. The product ions at *m/z* 183.0114 and *m/z* 119.0498 are characteristic of alkyl-substituted benzylsulfonates.[7]

All Merichem standard and OSPW extract database matches were compiled as a function of compound class and *z*-value. Results were broken down further based on total response and the number of homologues identified (Figure 8).

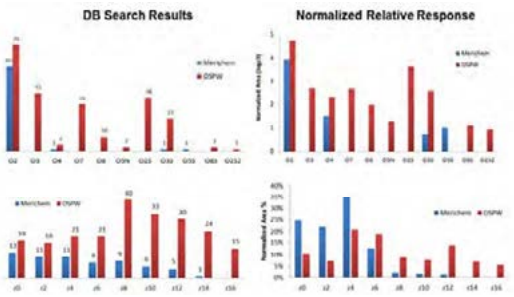


Figure 8. Evaluation of Merichem and OSPW samples as a function of the number of database-identified compounds grouped by class (left) and area response (right). Results are further broken down by compounds class (top) and *z*-value (bottom). Values shown above the homologue plots are the number of homologues identified during database (DB) searching. Due to the large differences in absolute response between compound classes this data was converted to a log10 scale.

Exact agreement between the distribution of ion classes observed in the current study and literature values was not expected given the heterogeneity of NAFCs.

By area response (99%) and the number of identified homologues (97%), the Merichem standard consisted primarily of O_2 species. Analysis of the Merichem results by *z*-value showed a significant number (42%) of homologues $z \leq -6$ (i.e., -8, -10, etc.). This result seemed contradictory to reported compositions, which state that the Merichem standard consists predominantly of compounds $z \geq -4$.[1, 3] However, these compositions were all response comparisons, not an identification of the number of homologues present. The response data in the current study showed that those compounds $z \geq -4$ accounted for 82% of the total area response, consistent with literature reports. The results by compound class (log10 scale) supported this comparison, with the Merichem standard containing almost exclusively O_2 species and a small amount of higher oxygen content and heteroatom-containing species.

The OSPW extract showed a more widely distributed number of database matches across the compound classes and *z*-values evaluated (Figure 8). Interestingly, despite comprising 49% of the positive DB matches, O_3 to O_8 compounds only accounted for 2.8% of the total area response. Similar to the Merichem results, this suggests that such compounds either have low response factors and/or are present at low levels. Conversely, the *z*-value results for the OSPW extract showed a relatively even distribution across the number of database matches and the relative area responses of the different *z*-classes. These results are consistent with the demonstrated composition of OSPW extracts, which are known to contain higher oxygen content and increased unsaturation/polycyclic compounds due to natural weathering and metabolic processes.[1, 3]

A detailed breakdown of the compound classes (log10 scale) by area response showed that the OSPW extract was composed of mostly O_2-species (86%), with significant amounts of O_2S (6.6%), O_3 (0.8%) and O_3S (0.6%) compounds (Figure 7). In addition, several potentially interesting compound classes (e.g., O_2NS, O_5NS, and $O_4N_2S_2$) were observed at very low abundances (<0.08% of total area).

Summary

The SCIEX TripleTOF® 5600+ system with SelexION™ differential mobility separation generates complex and insightful datasets very quickly (< 2 min), including the resolution of isomers and isobars. The ability to scan the SelexION™ cell to target specific analytes provides on-demand access to deeper structural interrogation. With its speed and lower resource cost compared to chromatographic alternatives, SelexION™ represents a greener analytical technique that requires much less organic solvent or gas consumption.

Analysis of a technical Merichem standard and an OSPW extract demonstrated results that are consistent with literature values for similar samples. Owing to the unique separation mechanism of SelexION™, structural isomers can be resolved and rapidly interrogated in real-time. Finally, compared to standard infusion-based NAFC analyses, SelexION™ coupled to the TripleTOF® 5600+ should provide more accurate qualitative and quantitative results owing to the mitigation of background ions and deleterious space charge effects possible when directly infusing complex mixtures.

Acknowledgement

The authors thank Michael McDonell and Lyle Burton (SCIEX) for helpful discussions, as well as Carmai Seto and Ashish Pradhan (SCIEX) for invaluable assistance with these experiments.

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Publication number: 10930214-01

EPA Method 557:

Determination of Haloacetic Acids, Bromate, and Dalapon in Drinking Water by IC-MS/MS

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SCIEX Foster City, California

Overview

All drinking water plants in the United States must determine the concentration of disinfection by products in drinking water prior to release. This IC-MS/MS method follows the current guidelines outlined in EPA Method 557, and was found to be sensitive and reproducible for the quantitation of low-level haloacetic acids, bromate, and dalapon in drinking and surface waters.[1-2]

Introduction

We rely on municipalities to clean drinking water for the prevention of bacterial illness. However, when by-products from the disinfection process are formed in our drinking water, we may experience unexpected health risks. For instance, when chlorine used to disinfect water reacts with organic decaying vegetation, haloacetic acids (HAAs) form. Bromate is formed when disinfecting ozone reacts with naturally occurring bromide. Long term ingestion of bromate or haloacetic acids may cause cancer.[3]

Another mechanism for the contamination of drinking water is introduction through runoff. Dalapon, an herbicide used to control grasses in a wide variety of crops, can be introduced to waterways from runoff when used on rights of way. People who drink water containing dalapon in excess of the maximum contaminant level (MCL) for many years could experience minor kidney changes.[4]

Haloacetic acids are a family of organic compounds based on the acetic acid molecule (CH_3COOH), where one or more hydrogen atoms attached to carbon atoms are replaced by a halogen (chlorine or bromine). There are nine species of HAAs: monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), dibromoacetic acid (DBAA), trichloroacetic acid (TCAA), bromodichloroacetic acid (BDCAA), chlorodibromoacetic acid (CDBAA), and tribromoacetic acid (TBAA), however only five acids (MCAA, MBAA, DCAA, DBAA, and TCAA) are regulated with a cumulative legal limit of 60 µg/L in drinking water. The MCL for bromate is 10 µg/L, while dalapon is 0.2 mg/L.[2-3]

Typical approaches to the detection of these compounds require derivatization and multiple extraction steps followed by gas chromatography with electron capture detection. Using ion chromatography as a separation technique prior to detection with tandem mass spectrometry (IC-MS/MS) with the API 3200™ system, we achieve a sensitive direct injection method for the detection and quantitation of nine haloacetic acids, dalapon, and bromide without time consuming derivatization steps.

Experimental

Chemicals

Deionized water (18MΩ) was produced in house.

Acetonitrile was purchased from JT Baker.

Haloacetic acid and dalapon standards were purchased from Sigma-Aldrich.

Bromate and internal standards of: MCAA-2-^{13}C, MBAA-2-^{13}C, DCAA-2-^{13}C, and TCAA-2-^{13}C were purchased from Dionex.

Contents

IC

A Dionex ICS3000 system equipped with a Dionex IonPac AG24 2.1 x 50 mm and AS24 2.1 x 250 mm performed the separation with a column temperature of 15ºC. The potassium hydroxide gradient was made online via online eluent generation; a one hour gradient (Table 1) separated the nine haloacetic acids, bromate, and dalapon from typical matrix ions such as chloride, sulfate, nitrate, and carbonate.

Table 1. Ion chromatography gradient

Time	[KOH]
0	7
16.8	7
34.2	18
34.4	60
51.2	60
51.4	7

The potassium hydroxide mobile phase was passed through a suppressor prior to its introduction into the mass spectrometer. The suppressor exchanged the potassium counter ion of the mobile phase with hydronium, making the IC effluent into the mass spectrometer predominantly water. Acetonitrile was added post-column at 200 µL/min to assist in desolvation, and to provide a makeup flow during periods of diversion.

Internal standards of monochloroacetic acid 2-^{13}C, monobromoacetic acid 2-^{13}C, and trichloroacetic acid 2-^{13}C were added for a final concentration of 4 ng/mL.

The injection volume was 100 µL.

MS/MS

An SCIEX API 3200™ system with Turbo V™ source operated in negative Electrospray Ionization (ESI) mode was used. During periods of matrix elution, the switching valve on the API 3200™ system diverted column effluent from the source.

The Turbo V™ source is equipped with a static grounding union. Traditionally this union is installed with a two-port (in/out flow through) piece. In order to introduce acetonitrile to the sample stream, this two-port piece was replaced with the three-port (tee) piece. Operating in this fashion, the eluent composition entering the source is 60% aqueous / 40% organic, which assists in desolvation. During periods of matrix diversion, the acetonitrile flow through the tee acts as a make-up flow to the Turbo V™ source.

The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode. Analyte detection was separated into four periods, with temperature and gas settings optimized for compound detection (Table 2 and 3).

Table 2. MRM Transitions used for analysis

	Analyte	Q1	Q3
Period 1	*MCAA*	93	35
	MBAA	137	79
	Bromate	127	111
	MCAA-ISTD	94	35
	MBAA-ISTD	138	79
Period 2	*DCAA*	127	83
	Dalapon	141	97
	BCAA	173	129
	DBAA	217	173
	DCAA-ISTD	128	84
Period 3	*TCAA 1*	161	117
	TCAA 2	163	119
	TCAA-ISTD	162	118
Period 4	*TBAA*	251	79
	BDCAA	163	81
	CBDAA	207	79

Table 3. Temperature and ion spray voltage by period

	IS (V)	TEM (°C)
Period 1	-4500	400
Period 2	-3500	550
Period 3	-4500	250
Period 4	-4500	350

Results and Discussion

A method for quantitation and identification of nine haloacetic acids, bromate, and dalapon was examined to screen drinking and surface water samples. The method followed the guidelines and met the requirements of EPA method 557 (Figure 1).

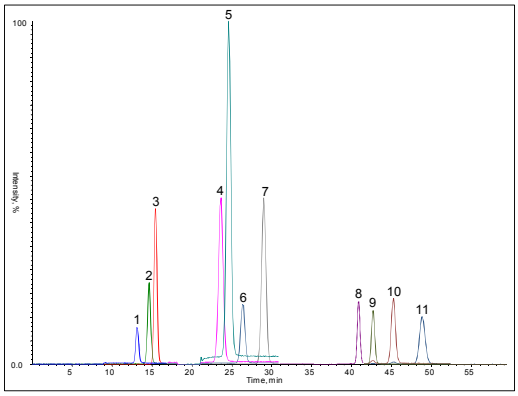

Figure 1. Chromatogram of a 10 µg/L standard, with the following order of elution: (1) MCAA (2) MBAA (3) bromate (4) dalapon (5) DCAA (6) BCAA (7) DBAA (8) TCAA (9) BDCAA (10) CDBAA (11) TBAA

The periods of detection had to be optimized for maximum sensitivity of the analytes, as the response of haloacetic acids in the mass spectrometer is very sensitive to temperature and ion spray voltage. For instance, a third period had to be defined specifically for temperature sensitive TCAA, whereas other analytes in the method had much better performance at higher temperatures (Table 3).

All analytes showed good linearity with r > 0.998 over two and a half orders of magnitude in reagent water (Table 5). The detection limit (DL) was calculated from the formula:

$$DL = S \times t_{(n-1,\ 1-\alpha = 0.99)}$$

where $t_{(n-1,\ 1-\alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom (for seven replicate injections, the Student's t value is 3.143 at a 99% confidence level)

n = number of replicates, and
S = standard deviation of replicate analyses.[1]

DLs were found between 0.025 µg/L and 0.25 µg/L (Table 5). These detection levels are well below the EPA MCL of 60 µg/L and the maximum contaminant level goals (MCLG) established in the National Primary Drinking Water Regulations Stage 1 and Stage 2 for Disinfectants and Disinfection Byproducts Rule. The low DLs allow dilution of real water samples before analysis to reduce possible matrix effects and interferences (i.e. retention time shifts).

Precision, accuracy and robustness were demonstrated by injecting all analytes at 1 µg/L in reagent water and fortified into the EPA defined laboratory synthetic sample matrix of 20 mg/L nitrate, 150 mg/L bicarbonate, 250 mg/L chloride, 250 mg/L sulfate and 100 mg/L ammonium chloride.

Relative standard deviation (RSD) and recovery (REC) were calculated from the formulae:

$$\%RSD = \frac{\text{Standard deviation of measured conc.}}{\text{Average concentration}} \times 100$$

$$\%REC = \frac{\text{Average measured concentration}}{\text{Fortified concentration}} \times 100^{1}$$

Method robustness in reagent water was examined for 24 hours; the RSD was less than 0.06% for all analytes.

Samples were fortified into the synthetic sample matrix. During periods of the matrix elution, column effluent was diverted from the mass spectrometer (Figure 2). The peak shape of the haloacetic acids was altered in the presence of the matrix ions. However, recoveries of all nine haloacetic acids, bromate, and dalapon were within 15% of the expected concentration at 1 µg/L (Table 4).

Contents

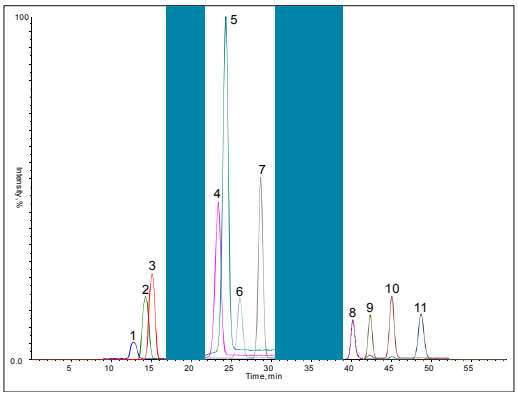

Figure 2. 5 µg/L standard fortified into a synthetic sample matrix of 20 mg/L nitrate, 150 mg/L bicarbonate, 250 mg/L chloride, 250 mg/L sulfate and 100 mg/L ammonium chloride. Periods shaded blue were those of matrix elution, and diverted from the mass spectrometer source. Order of elution: (1) MCAA (2) MBAA (3) bromate (4) dalapon (5) DCAA (6) BCAA (7) DBAA (8) TCAA (9) BDCAA (10) CDBAA (11) TBAA

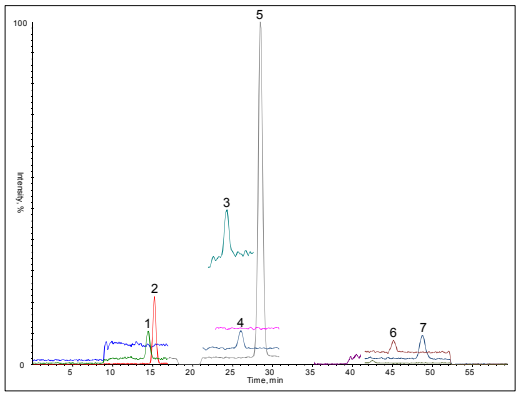

Figure 3. Sample of urban drinking water shows the presence of: (1) MBAA, (2) bromate, (3) DCAA, (4) BCAA, (5) DBAA, (6) CDBAA, and (7) TBAA. The concentrations reflected in this sample do not exceed the regulatory requirement of 60 µg/L

Table 4. Reproducibility of a 1 µg/L sample in synthetic sample matrix over 24 hours of injections. The table shows relative standard deviation for retention time and peak area (RSD), and recovery (REC).

Analyte	RSD RT (%)	RSD Area (%)	REC (%)
MCAA	0.36	1.5	102
MBAA	0.40	4.6	104
Bromate	0.28	2.7	105
Dalapon	0.00	2.5	90
DCAA	0.00	6.1	100
DBAA	0.00	2.8	101
BCAA	0.00	3.7	95
TCAA	0.13	2.7	102
BDCAA	0.05	7.7	114
CDBAA	0.05	7.0	113
TBAA	0.09	6.8	106

The developed method was used to evaluate two types of drinking water samples: urban drinking water that has been processed through traditional drinking water treatment, and well water samples, where the samples were not treated to disinfectants. The results (Figures 3 and 4) show that in the urban water sample, haloacetic acids and bromate are present, although at concentrations lower than the action levels required by the US EPA. No dalapon was detected in this sample.

With the sample obtained from a rural well, neither haloacetic acids nor bromide were detected. However, there was a small amount of dalapon detected. Most likely this herbicide entered the sample from runoff.

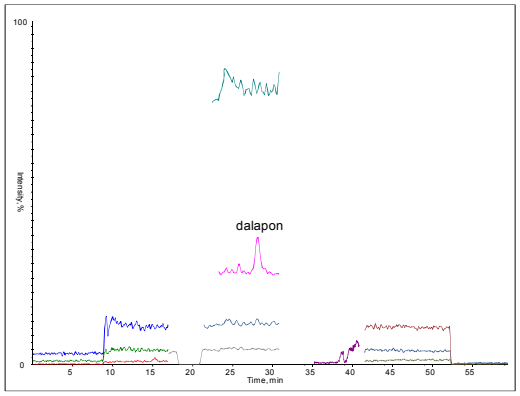

Figure 4. Drinking water sample from a rural well does not have any detectable haloacetic acids. However, dalapon is detected, with a concentration less that the action level of 0.2 mg/L

Contents

Table 5. Obtained method performance values

Analyte	Retention Time (min)	Internal Standard	Method 557 DL (µg/L)	DL (µg/L)	Calibration Range (µg/L)	r value
MCAA	13.1	MCAA-2-^{13}C	0.20	0.25	0.25-50	0.9990
MBAA	14.6	MBAA-2-^{13}C	0.06	0.03	0.25-50	0.9991
Bromate	15.4	MBAA-2-^{13}C	0.02	0.04	0.25-50	0.9989
Dalapon	23.5	DCAA-2-^{13}C	0.04	0.03	0.025-50	0.9992
DCAA	24.5	DCAA-2-^{13}C	0.06	0.03	0.025-50	0.9997
BCAA	26.3	DCAA-2-^{13}C	0.11	0.05	0.05-50	0.9994
DBAA	28.9	DCAA-2-^{13}C	0.02	0.04	0.05-50	0.9996
TCAA	40.9	TCAA-2-^{13}C	0.09	0.03	0.05-50	0.9998
BDCAA	42.6	TCAA-2-^{13}C	0.05	0.06	0.05-50	0.9997
CDBAA	45.1	TCAA-2-^{13}C	0.04	0.06	0.05-50	0.9996
TBAA	48.7	TCAA-2-^{13}C	0.07	0.05	0.05-50	0.9996

Summary

When disinfecting agents react with naturally organic or inorganic matter in the water, harmful byproducts may form. A robust and reliable analysis for the detection of haloacetic acids, bromate, and dalapon in drinking water was performed. Following the guidelines of EPA Method 557, a direct injection of drinking water into an ion chromatography system coupled to an API 3200™ system allowed for the quantitation of nine haloacetic acids, bromate, and dalapon. Running the analysis by this methodology spares the analyst from derivatization required by gas chromatography methodology. Method robustness over the course of several hours is a key component to this method, and this method was found to be robust over 24 hours.

References

1 US Environmental Protection Agency Method 557: 'Determination of Haloacetic Acids, Bromate, and Dalapon in Drinking Water by Ion Chromatography Electrospray Ionization Tandem Mass Spectrometry' (September 2009)

2 http://water.epa.gov/scitech/drinkingwater/labcert/upload/met557.pdf

3 http://water.epa.gov/drink/contaminants/basicinformation/disinfectionbyproducts.cfm

4 http://water.epa.gov/drink/contaminants/basicinformation/dalapon.cfm

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Quantitative Analysis of Explosives in Surface Water Comparing Off-Line Solid Phase Extraction and Direct Injection LC-MS/MS

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Overview

Presented is an efficient method for measuring selected explosives in lake water at the sub-ng/L level applying either off-line Solid Phase Extraction (SPE) with LC-MS/MS detection and comparing it to direct injection LC-MS/MS.

Introduction

Between 1918 and 1967 some 8200 tons of ammunition, Trinitrotoluene (TNT) being the main explosive, was dumped to the lakes of Thun, Brienz and Lucerne in Switzerland.[1]

The amount of ecologically harmful compounds was considered to be negligible. In order for explosives to leak to the environment the casing must have rusted.[2-3] This corrosion process very much depends on environmental water conditions such as: temperature, oxygen content and pH value. Meanwhile a sediment layer of 20-30 cm covers the ammunition at the lakes' bottom and represents a natural barrier preventing the compounds to enter the aqueous phase.

Nevertheless water quality of the lakes should be monitored as lake water is frequently used as a source for drinking water.

Due to the very low concentrations of explosive residues expected in the lakes a powerful analytical set-up is important for a reliable detection and quantitation. LC-MS/MS analysis with Electrospray Ionization (ESI) is the method of choice to analyze polar and thermally labile compounds, such as explosives and their degradation products.

Experimental

The following explosives and degradation products were investigated:

- 2,4,6-Trinitrotoluene (TNT)
- 2,4-Diamino-6-nitrotoluene (2,4-DA-6-NT)
- 2,6-Diamino-4-nitrotoluene (2,6-DA-4-NT)
- 2-Amino-4,6-dinitrotoluene (2-A-4,6-DNT
- 4-Amino-2,6-dinitrotoluene (4-A-2,6-DNT
- Hexogen (RDX)
- Nitroglycerin (NG)
- Octogen (HMX)
- Pentaerythritol tetranitrate (PETN)
- Tetryl

Sample Preparation

50 mL of water samples were extracted on Phenomenex StrataX SPE cartridges. These extracts were analyzed by LC-MS/MS and compared to direct injections of filtered water samples.

Contents

Samples from different depths were analyzed within 48 hours after sampling. If water had to be stored for a longer period of time it was stabilized by acidifying to pH 3.5 with acetic acid and adding 2% of acetonitrile.

Liquid Chromatography

- HPLC column: Xbridge Phenyl (2.1x150 mm), 3.5 µm
- Eluent A: water + 2.5 mM ammonium acetate
- Eluent B: methanol + 2.5 mM ammonium acetate
- Gradient (A/B): 55/45 to 30/70 within 13 min and re-equilibration
- Flow: 200 µL/min
- Injection volume: 100 µL
- Oven temperature: 40°C

Mass Spectrometry

- API 5000™ LC-MS/MS System
- Turbo V™ source with ESI probe
- Gas and source parameters: CUR: 20 psi, GS1: 40 psi, GS2: 40 psi, TEM: 350°C, CAD: 7, IonSpray voltage (IS): 5500 V (positive) and -4500 V (negative)
- Two periods with detection in positive and negative polarity using Multiple Reaction Monitoring (MRM) were programmed: 0 to 4.5 min (positive) and 4.5 to 15 min (negative). MRM transitions of detected explosives and MRM ratios are listed in Table 1.

Calibration

Standards were prepared in MilliQ water and blank matrix water (matrix matched standards) over a range of 1-100 ng/L for off-line SPE and 0-1 ng/L for direct injection LC-MS/MS. Serial dilutions were obtained starting with a 10 ng/mL standard. All standards were prepared in water and kept at 4 °C in the dark. Under these conditions standards were stable for at least three months – with the exception of TNT and Tetryl, which degrade rapidly and thus must be prepared freshly.

Method validation data

- Recoveries (SPE): between 89% and 110% for all analytes
- Blank analysis: field blanks, travel blanks and laboratory blanks did not contain any traces of explosives (< 10% of lowest calibration standard)
- Linearity: 7 point equidistant calibration, statistical tests (Mandel, sensitivity plots and residual analysis) proved linearity of regression lines, residual analysis with normal distribution of the calibration points around the zero line
- Limit of Quantification (LOQ) with S/N=10 and Limit of Detection (LOD) S/N=3
- LOQ: 1 ng/L for DANT, NG and TNT, 0.03 ng/L for HMX, RDX, PETN and ADNT

Table 1. Retention times, MRM transitions of explosives with detected MRM ratio and tolerance intervals regarding the guideline 2002/657/EC[b]

Compound	t_R (min)	MW	Precursor Ion	MRM Transition	MRM Ratio	Tolerance (%)	Tolerance Interval
2,4-DA-6-NT	3.7	167	168 $[M+H]^+$	168/121 168/77	0.43	25	0.32-0.54
2,6-DA-4-NT	4.1	167	168 $[M+H]^+$	168/121 168/77	0.37	25	0.28-0.46
HMX	5.0	296	355 $[M+CH_3COO]^-$	355/46 355/147	0.4	25	0.30-0.50
RDX	6.5	222	281 $[M+CH_3COO]^-$	281/46 281/93	0.04	50	0.02-0.06
NG	9.6	227	286 $[M+CH_3COO]^-$	286/62 286/46	0.83	20	0.67-1.00
4-A-2,6-DNT	9.9	197	196 $[M-H]^-$	196/46 196/136	0.06	50	0.03-0.09
2-A-4,6-DNT	10.2	197	196 $[M-H]^-$	196/46 196/136	0.26	25	0.20-0.33
Tetryl	11.9	287	286 $[M-H]^-$	286/240 286/206	0.83	20	0.67/1.00
TNT	12.0	227	226 $[M-H]^-$	226/46 226/196	0.49	25	0.37-0.61
PETN	13.1	316	375 $[M+CH_3COO]^-$	375/62 375/46	0.44	25	0.33-0.56

Contents

Results and Discussion

Clearly, Electrospray Ionization turned out to be the method of choice for detecting traces of explosives in water samples.[4] Tests using either APCI or APPI were generally less sensitive (results not shown). As shown in Table 1 precursor ions of explosives were either detected as $[M+H]^+$ or $[M-H]^-$ for the DANT, ADNT, Tetryl and TNT, as $[M+CH_3COO]^-$ for HMX, RDX, NG and PETN.

Selective detection was performed in MRM mode using two characteristic transitions for each compound. The ratio of both transitions was used to identify the presence of explosives in lake water regarding the guideline 2002/657/EC.[5]

Optimization of the compound dependent parameters was obtained by automatic Quantitative Optimization in Analyst® Software. The ion source temperature was a crucial parameter during source optimization. TNT, Nitroglycerine and above all Tetryl, known as being very labile, could only be detected using a rather low temperature of 350°C. As Nitroglycerine and Tetryl are not expected to persist for a longer time in the environment they were not included in the final target method.

The separation of the different isomers of the diaminonitrotoluenes and aminodinitrotoluenes became difficult on traditional C_{18} stationary phases. Figure 1 presents a total ion chromatogram (TIC) with baseline separated analytes on the selected phenyl type phase.

Concentrations of explosive residues in lake water were assumed to be very low if present at all. Therefore, in a first attempt an off-line SPE enrichment procedure of the water samples was performed using an enrichment factor of 100. Using this procedure a typical TIC as shown in Figure 2 was obtained. Quantitation of the compounds revealed concentrations between 0.1-0.4 ng/L. Concentrations at different depths were very similar assuming a homogeneous distribution of the explosives in the water body.

In a second step, direct injection of 100 µL of water samples was performed. A representative chromatogram of HMX is shown in Figure 3. The calibration curve (working range 0-1 ng/L) is presented in Figure 4. Quantitation of the sample resulted in a concentration of 0.21 ng/L. The calculated MRM ratio of 0.42 was well within the limits of the ratio obtained from the calibration line (0.40). Note the excellent agreement between the intensity (cps) of HMX in the concentrated sample (2.0 x 10^4 cps; enrichment factor 100) and the directly injected sample (200 cps).

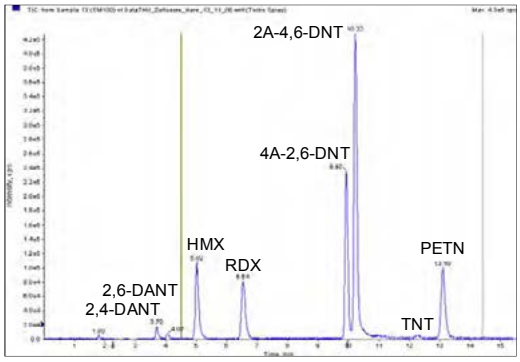

Figure 1. Total ion chromatogram of a 100 ng/L standard: 0 to 4.5 min in positive polarity 4.5 to 15 min in negative polarity

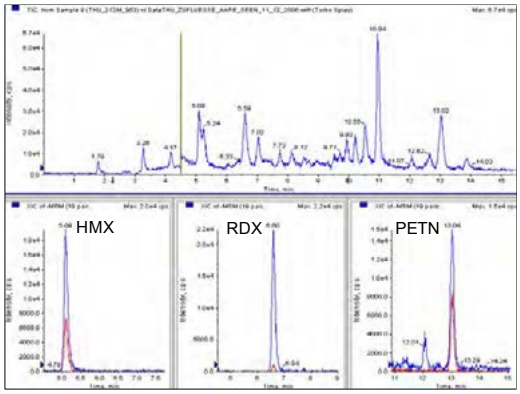

Figure 2. TIC of a lake water sample taken at a depth of 212 m showing the presence of HMX, RDX and PETN using off-line SPE

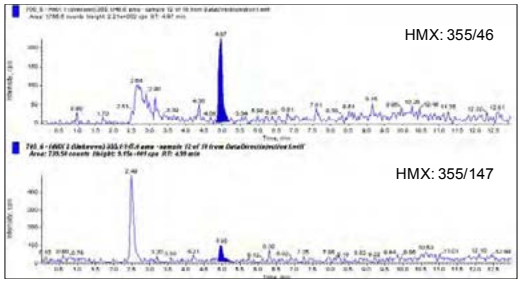

Figure 3. Direct injection of a lake water sample taken at a depth of 212 m showing the two transitions of HMX: 355/46 (upper trace), 355/147 (lower trace)

Contents

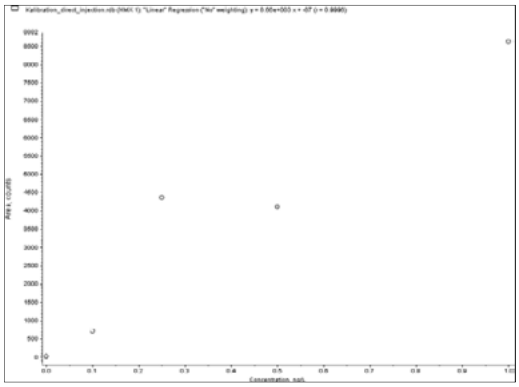

Figure 4. Calibration curve of HMX with a working range of 0-1 ng/L (r = 0.9996) used for direct injection analysis

A comparison of the concentrations of direct injection and SPE enriched samples from different depths of the lake for HMX, RDX and PETN is shown in Figure 5. Concentrations of direct injection do not significantly deviate from the SPE samples. The lower concentrations detected after SPE can be explained by a recovery less than 100% and/or stronger ion suppression due to increased matrix concentration after extraction. However, uncertainty of measurement can drastically be reduced using direct injection LC-MS/MS.

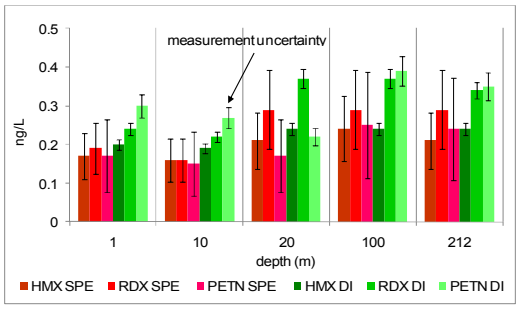

Figure 5. Comparison of concentrations between direct injection and off-line SPE for HMX, RDX and PETN with error bars for uncertainty of measurement

Summary

A highly sensitive LC-MS/MS method for the analysis of sub-ng/L levels of selected explosives such as TNT and the corresponding monoamino and diamino metabolites, HMX, RDX, and PETN has been presented. Specificity was obtained using Multiple Reaction Monitoring with identification based on ion ratio calculation using two transitions for each analyte. Sensitivity turned out to be optimal using Electrospray Ionization (ESI) with positive or negative polarity on an API 5000™ LC-MS/MS System equipped with a Turbo V™ source. Using direct injection analysis of water samples comparable results were obtained as from SPE enriched samples for the three main explosives HMX, RDX and PETN. In addition reproducibility was found to be much better using direct injection LC-MS/MS analysis.

Acknowledgements

The authors would like to thank Dr. M. Zeh for his help with lake water sampling from different depths.

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3 H. Stucki: Chimia 58, 409-413, 2004

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Publication number: 1281710-01

Determination of Polybrominated Diphenyl Ethers (PBDEs) and Hexabromocyclododecanes (HBCDs) in indoor dust and biological material using APPI-LC-MS/MS

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Overview

This note describes the application of LC-MS/MS with Atmospheric Pressure Photo Ionization (APPI) for the determination of PBDE target congeners and HBCD stereoisomers in indoor dust samples and biological material.

Instrumental detection limits (IDL) are included and range from 0.07 ppb to 0.24 ppb for selected PBDE congeners and from 0.12 to 0.32 ppb for HBCD stereoisomers.

Introduction

In recent years polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have emerged as a subject of great concern because of their increasing levels in the human body, causing disturbance of the thyroid hormone homeostasis and chronic neurotoxicity (Alaee, 2003), and because of their ubiquity in the environment, especially indoors. Indoor dust and biological material have become a repository for PBDEs and HBCDs, resulting in developments of sampling strategies and analytical methodology for determination of these chemicals (Covaci, 2003). Traditionally, GC-MS has been employed for the analysis of PBDEs and HBCDs in environmental samples, but this technique causes thermal degradation of higher brominated PBDE congeners and interconversion of HBCDs. Hence, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has more recently been used for the determination of PBDEs and HBCDs (Lagalante, 2008; Vilaplana, 2008; Abdallah, 2009; Zhou, 2010).

Experimental

The SCIEX 4000 QTRAP® system was coupled with an Agilent 1200 series LC system for the determination of PBDE target congeners (BDE-47, 85, 99, 100, 138, 153, 154, 183, 190, 196, 206, 209) and α-, β-, γ-HBCD stereoisomers.

A Phenomenex Kinetex C18 (150x4.6mm) column was used for chromatographic separation using H_2O (A) and methanol (B) mobile phases with a gradient from 90% B increasing at 4 min to 100% and holding for 9 min, with a 4 minute equilibration between runs. The mobile phase flow was set to 400 µL/min, and 10µL of standards and extracts were injected for analysis.

All experiments were performed on an SCIEX 4000 QTRAP® system with PhotoSpray® ion source operated in negative polarity. Nebulizer gas (GS1) and lamp gas (GS2) were supplied with nitrogen, resulting in a 3 fold increase in signal compared to the atmospheric air (Figure 1).

Contents

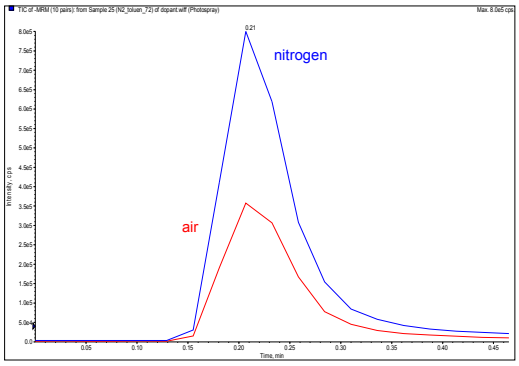

Figure 1. Sensitivity gain for PBDE congeners when using nitrogen as GS1 and GS2 in comparison to air

Toluene was used as a dopant at a flow rate of 72 µL/min which was equal to 18% of the total mobile phase flow (Figure 2).

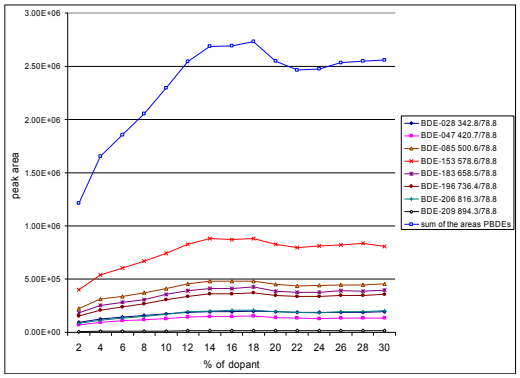

Figure 2. A comparison of peak area to the dopant flow rate (in % of total mobile phase flow)

All source parameters were optimized using automatic flow injection analysis using the Analyst® software and set for optimal intensity of MRM transitions for all analytes: CUR 12; CAD 12; TEMP 300°C; GS1 30 psi; GS2 30 psi; IS -700 V.

Analytes were monitored in Multiple Reaction Monitoring (MRM) using the *Scheduled* MRM™ algorithm with two transitions for each target compound. MRM conditions are listed in Tables 1 and 2.

Table 1. MRM transitions and optimized parameters for HBCDs

HBCD	Q1	Q3	DP (V)	CE (V)
α-HBCD	640.6	78.8	-35	-40
		80.9	-35	-40
β-HBCD	640.6	78.8	-35	-40
		80.9	-35	-40
γ-HBCD	640.6	78.8	-35	-40
		80.9	-35	-40

Table 2. MRM transitions and optimized parameters for PBDEs

PBDE	Q1	Q3	DP (V)	CE (V)
BDE-047	420.8	78.8	-36	-38
		80.9	-36	-38
BDE-085	500.7	78.8	-60	-90
		80.9	-60	-90
BDE-099	500.7	78.8	-60	-90
		80.9	-60	-90
BDE-100	500.7	78.8	-60	-90
		80.9	-60	-90
BDE-138	578.6	78.8	-50	-100
		80.9	-50	-100
BDE-153	578.6	78.8	-50	-100
		80.9	-50	-100
BDE-154	578.6	78.8	-50	-100
		80.9	-50	-100
BDE-183	658.5	78.8	-50	-110
		80.9	-50	-110
BDE-190	658.5	78.8	-50	-110
		80.9	-50	-110
BDE-196	736.4	78.8	-70	-90
		80.9	-70	-90
BDE-206	816.3	78.8	-60	-100
		80.9	-60	-100
BDE-209	894.3	78.8	-60	-100
		80.9	-60	-100

Contents

Results and Discussion

A standard chromatogram is shown in Figure 3. Two MRM transitions were monitored for each target analyte. The *Scheduled* MRM™ algorithm was used to maximize signal-to-noise and to collect enough data points across the LC peak for best accuracy and reproducibility.

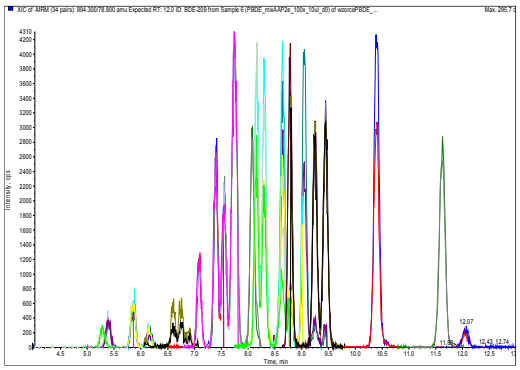

Figure 3. Chromatogram of a standard mix using the *Scheduled* MRM™ algorithm (the standard solution contained additional non-examined congeners)

Standards for calibration curves were prepared in a mixture of methanol/toluene (4/6) ratio in a concentration which depends on the PBDE congener. Example calibration curves for selected PBDE congeners are shown on the Figure 4. All studied PBDEs had excellent linearity with R values between 0.9994 and 0.999.

Based on these calibration lines instrument detection limits (IDL) and limits of quantitation (LOQ) were determined for individual congeners (Table 3).

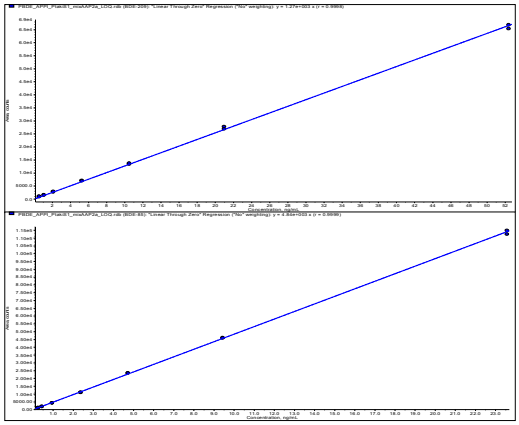

Figure 4. Calibration lines of selected PBDE congeners with an R value of greater than 0.999

Table 3. IDL, LOQ, and linear dynamic range for HBCDs stereoisomers and PBDE congeners

Compound	IDL (ppb)	LOQ (ppb)	Range (ppb)*	IDL (pg on column)
α-HBCD	0.12	0.58	0.58 - 5.24	1.22
β-HBCD	0.14	0.49	0.49 - 2.09	1.39
γ-HBCD	0.32	1.53	1.53 - 5.24	3.19
BDE-047	0.31	1.48	0.71 - 3.14	1.51
BDE-085	0.15	0.71	0.41 - 4.71	0.74
BDE-099	0.07	0.41	0.41 - 4.71	0.74
BDE-100	0.07	0.41	0.39 - 2.36	0.80
BDE-138	0.13	0.69	0.69 - 6.28	1.25
BDE-153	0.10	0.57	0.57 - 4.71	1.04
BDE-154	0.12	0.66	0.66 - 6.28	1.21
BDE-183	0.17	0.96	0.96 - 7.85	1.74
BDE-190	0.13	0.71	0.71 - 7.85	1.29
BDE-196	0.16	0.91	0.91 - 10.50	1.65
BDE-206	0.16	0.87	0.87 - 10.50	1.57
BDE-209	0.24	1.33	1.33 - 10.50	2.42

* Range used to determine the parameters IDL and LOQ

The above described analytical method was used for the analysis of indoor dust samples and biological material which were extracted using toluene in a Soxhlet apparatus for 8 hours in a dark room. Extracts were concentrated using a rotary evaporator and purified by gel permeation chromatography (GPC) (Brezee 1525). The eluent, dissolved in methylene chloride, was evaporated to exchange the final sample solvent to methanol/toluene (4/6).

Figure 5 and Table 4 show the results from an analysis of the dust reference material NIST SRM 2585 (µg/kg dry weight).

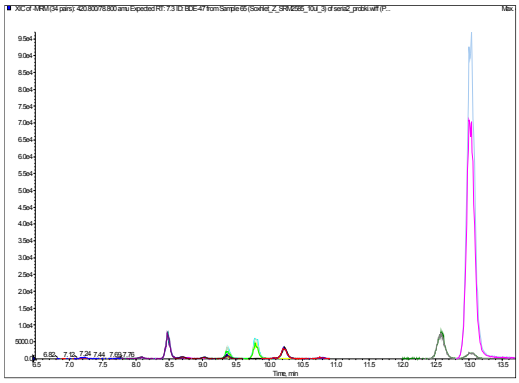

Figure 5. Chromatogram of the NIST dust reference material SRM 2585

Summary

The developed LC-MS/MS method was used for the determination of PBDE congeners and HBCD stereoisomers in indoor dust and biological material after sample extraction. Obtained detection limits are acceptable and the influence of the matrix was not observed. The disadvantage of the described method is the lack of signal for one to two substituted PBDE congeners, however results for a NIST standard reference material showed acceptable results for 10 of 11 PBDE compounds, showing that this method is accurate and suitable for detection of PBDE congeners and HBCD stereoisomers in indoor dust and biological material.

Table 4. Quantitative results of analyzing the reference material NIST SRM 2585

PBDE	NIST certified concentration (µg/kg)	Found concentration (µg/kg)	Recovery
BDE-209	2510	2613	104.1
BDE-206	271	298	109.9
BDE-190	5.1	<LOQ	<LOQ
BDE-183	43.0	39.7	92.4
BDE-154	83.5	95.1	113.9
BDE-153	119	125	105.4
BDE-138	15.2	16.7	110.0
BDE-100	145	157	108.1
BDE-099	892	888	99.6
BDE-085	43.8	41.4	94.6
BDE-047	497	522	104.9

Acknowledgements

The author wishes to thank Amelia Staszowska and Jacek Czerwinski.

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Contents

Quantitation of Microcystins and Nodularins in Water Samples Using LC-MS/MS

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Overview

This application note describes the quantitation of 8 individual microcystin (MC) isoforms and Nodularin-R using the SCIEX QTRAP® 4500 system with Turbo V™ source in positive mode electrospray ionization (ESI). Chromatography was performed using a Phenomenex Kinetex® C8 column with an 11.0 min gradient run. Excellent sensitivity, accuracy and precision was shown with LOQ values ranging from 5.5 to 43.8 ng/L, varying by compound. The calculated lowest concentration minimal reporting levels (LCMRL) for the standards ranged from 4.8 ng/L for MC-RR to 91.8 ng/L for MC-YR, suggesting that the direct analysis of ambient water samples is possible. However, EPA Method 544 advises a 500-fold concentration factor which equates to LCMRL values of 0.010 to 0.184 ng/L in the water sample.

Introduction

Microcystins (MC) and nodularins (NOD) are toxins produced by cyanobacteria in saline and freshwaters. MC and NOD are released during cell death and are potential drinking water contaminants. Therefore, accurate and sensitive methods for quantifying MC and NOD in water samples are needed.

MC and NOD both share the common amino acid ADDA, but MC are cyclic heptapeptides whereas NOD are cyclic pentapeptides. Over 130 MC and 10 NOD isoforms have been identified primarily based on variations of two L-amino acids in their cyclic peptide structure [1,2].

MC and NOD are primarily liver toxicants and toxicity varies by isoform with the Microcystin-LR (leucine/arginine variant) thought to be the most harmful. Therefore, the quantification of individual isoforms in necessary. MC and NOD contamination from harmful algal blooms is widespread in surface and drinking water, resulting in occasional consumption advisories [3,4]. The US EPA 10-day drinking water health advisory for microcystins is 0.3 µg/L for infants and children up to 6 years old, and 1.6 µg/L for adults [5]. In addition, Health Canada has set a maximum acceptable concentration (MAC) of MC-LR of 1.5 µg/L [6] and the World Health Organization (WHO) MC-LR provisional guideline is 1 µg/L [7]. Drinking water guidelines for NOD do not exist.

Previous analysis techniques for MC and NOD in water include LC-MS, LC-UV and enzyme linked immunosorbent assay (ELISA). However, liquid chromatography tandem mass spectrometry methods are superior analytical techniques due to the high selectivity, high dynamic linear range and ability to quantify many MC and NOD isoforms in a single analysis run.

Experimental

Standards

Neat standards were obtained from Enzo Life Sciences (Farmingdale, NY) and reconstituted in 1 ml of methanol. An intermediate mixed stock was prepared by diluting the standards in methanol to yield 500 ng/ml for MC-RR and Nodularin-R, and 2000 ng/ml for MC-LA, MC-LF, MC-LR, MC-LY, MC-LW, MC-YR, MC-WR. Calibration standards were prepared with 5% acetonitrile in water to match the initial LC conditions. Standards were prepared in glass vials to reduce sorption to plastic surfaces. All standards were kept at -20 °C until analysis.

HPLC System

A SCIEX ExionLC™ AC was used as the LC system. Chromatographic separation was achieved under gradient conditions using a Phenomenex Kinetex® C8 column (2.6 µm particle size, 100 x 2.1 mm) and flow rate of 0.500 mL/min **(Table 1)**. The mobile phases were water ("A") and acetonitrile ("B"), both

Contents

modified with 0.1% formic acid. The column oven was set to 40°C and injection volume was 20 µL. To reduce sample carryover the autosampler rinse solvent was 60:20:20 isopropyl alcohol: methanol: acetonitrile using a rinse volume of 2 mL and dip time of 8 s.

MS/MS Detection

Analysis was performed on a SCIEX QTRAP® 4500 system with a Turbo V™ source using an electrospray ionization (ESI) probe in positive mode. Compound-specific and ion source parameters were manually optimized (Tables 2 & 3) and two MRMs per compound were monitored except for MC-LY which showed only 1 product ion. The *Scheduled* MRM™ (sMRM) algorithm was used to maximize dwell times and optimize the number of points across the chromatographic peaks. The MRM detection window was set to 45 s and target scan time was 0.25 s.

Data Analysis and Calculations

The standard batch was run 7 times to generate method performance statistics (i.e. accuracy and precision of LOQ standard) as well as to calculate the LCMRL values. Quantification was performed with MultiQuant™ 3.0.2 using 1.0 Gaussian smoothing and 1/x or $1/x^2$ weighted linear regression. The signal/noise ratio was calculated using the peak-to-peak S/N algorithm in PeakView® 2.2 on unsmoothed chromatograms. The LOD was determined as S/N>3. The LOQ was determined using the following criteria: S/N>8, at least 8 points across the peak and accuracy between 80-120%. LOQ and LOD concentrations were calculated using the first MRM transition, per compound, described in Table 3.

Table 1. LC gradient program at a flow rate of 0.5 mL/min, injection volume = 20 µL.

Step	Time (min)	A (%)	B (%)
0	0.0	95	5
1	0.5	95	5
2	6.0	40	60
3	7.0	5	95
4	9.0	5	95
5	9.1	95	5
End	11.0		

Table 2. Ion source parameters.

Parameter	Value
Curtain Gas (CUR)	30 psi
Collision Gas (CAD)	high
IonSpray voltage (IS)	3500 V
Temperature (TEM)	650°C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	60 psi

The lowest concentration minimum reporting level (LCMRL) was calculated as described by Winslow et al. [8] using Excel 2016. The LCMRL values were calculated using the LOD standard and subsequent three standard levels. Briefly, the measured versus actual concentrations were plotted and linear regression calculated. The 99% prediction intervals and data quality objective bounds (50% and 150% sample recovery) were calculated and plotted on the original graph. The LCMRL was defined as the intersection of the upper and lower prediction interval lines with the data quality objective (DQO) bounds, using the higher calculated concentration.

Results and Discussion

Using the developed gradient program, baseline separation was achieved for all compounds with excellent peak shape **(Figure 1)**. The gradient is 15 min shorter than the program described in EPA Method 544, resulting in considerable time savings but still maintaining baseline separation.

The LOD concentrations varied by compound and ranged from 2.7 to 21.9 ng/L **(Table 4)**. Specifically, MC-LA, MC-RR and Nodularin showed the lowest LOD values, whereas MC-LR and MC-YR showed the highest. The LOQ concentrations also varied by compound (5.5-43.8 ng/L) and showed similar trends as the LOD values. MRM chromatograms for the LOQ standard (43.8 ng/L) of MC-LR, following 1 Gaussian smooth, are shown in **Figure 2**. The reported LOQ concentrations are significantly below the US EPA drinking water advisory level for children of 300 ng/L.

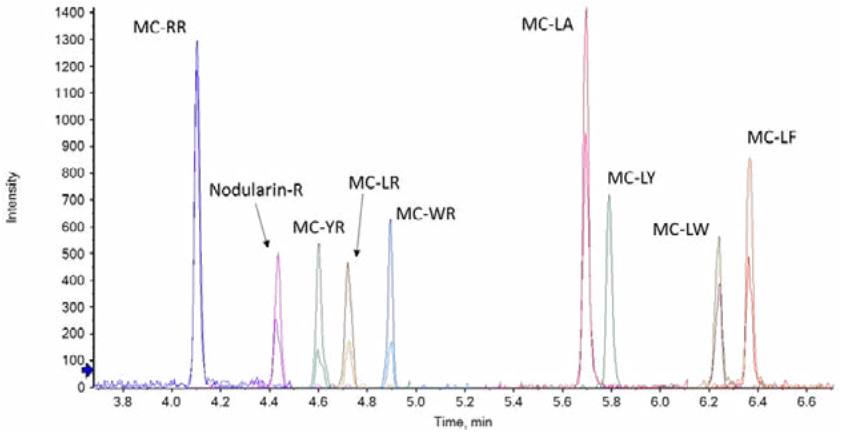

Figure 1. Overlaid chromatogram of 21.9 ng/L standard for MC-RR and Nodularin-R, 87.5 ng/L standard for MC-YR, MC-LR, MC-WR, MC-LA, MC-LY, MC-LW and MC-LF using the SCIEX QTRAP® 4500. 1 Gaussian smooth performed.

Table 3. MRM masses and compound-specific MS parameters for QTRAP® 4500 system.

Compound	Q1	Q3	EP (V)	DP (V)	CE (V)	CXP (V)
MC-LA 1	910.5	776.1	10	70	26	15.0
MC-LA 2	910.5	135.0	10	70	91	8.0
MC-LF 1	986.5	852.5	10	70	30	17.0
MC-LF 2	986.5	134.7	10	70	100	9.0
MC-LR 1	995.6	102.8	10	70	165	6.0
MC-LR 2	995.6	135.2	10	70	139	11.0
MC-LW 1	1025.5	107.1	10	70	146	15.0
MC-LW 2	1025.5	135.2	10	70	106	14.0
MC-LY 1	1002.5	135.3	10	70	119	12.0
MC-RR 1	519.9	135.1	10	70	35	9.3
MC-RR 2	519.9	103.1	10	70	96	9.5
MC-WR 1	1068.6	103.0	10	70	165	9.0
MC-WR 2	1068.6	134.9	10	70	150	11.0
MC-YR 1	1045.5	103.1	10	70	160	6.0
MC-YR 2	1045.5	135.4	10	70	139	10.0
Nodularin-R 1	825.5	103.0	10	70	160	5.8
Nodularin-R 2	825.5	135.3	10	70	110	12.0

Table 4. Method performance parameters (sensitivity, linear range, LOQ accuracy and precision, signal-to-noise). Peak-to-peak S/N was calculated using PeakView® 2.2 with unsmoothed chromatograms.

Analyte	Calibration Range (ng/L)	LOD (ng/L)	LOQ (ng/L)	Linear Correlation (r^2)	Accuracy of LOQ Std. (%)	Precision of LOQ Std. (%)	Peak-to-Peak S/N at LOQ
MC-LA	10.9 – 100,000	5.5	10.9	1.000	113.0	4.6	13.3
MC-LF	21.9 – 100,000	10.9	21.9	0.991	102.0	4.8	10.7
MC-LR	43.8 – 10,000[1]	21.9	43.8	0.998	104.0	18.0	10.9
MC-LW	21.9 – 100,000	10.9	21.9	0.983	99.7	15.2	11.2
MC-LY	21.9 – 100,000	10.9	21.9	0.987	98.9	12.8	18.3
MC-RR	5.47 – 25,000	2.7	5.5	0.996	98.1	8.8	10.0
MC-WR	21.9 – 10,000	10.9	21.9	0.999	105.0	14.1	10.8
MC-YR	87.5 – 10,000[1]	21.9	43.8	0.998	106.0	20.8	10.0
Nodularin-R	10.9 – 25,000	5.5	10.9	0.999	101.0	16.3	10.8

[1] MC-LR and MC-YR have been shown to be linear up to 40,000 ng/L in previous data.

The LOQ standard showed excellent accuracy, with the mean accuracy ranging from 98.1% to 113% (n=7). Further, the precision of the LOQ standard was very good and was generally <20% (n=7). Finally, the LOQ standard signal-to-noise ratio was >10 for all compounds.

The method showed approximately 3 orders of linear dynamic range for all compounds with linearity maintained up to 25,000 ng/L for MC-RR and Nodularin-R, and up to 100,000 ng/L for MC-LA, MC-LF, MC-LW and MC-LY. Previous analysis showed that MC-LR and MC-YR were linear up to 40,000 ng/L.

LCMRL values were calculated using the results of the standards analysis **(Table 5)**. For all compounds, the LCMRL graphs met the required criteria of seven replicate samples at four concentration levels, and at least one standard level below the calculated LCMRL [8]. An example LCMRL graph is shown in **Figure 3** for MC-LR. The LCMRL values – calculated as "in vial" concentrations – ranged from 4.8 ng/L for MC-RR to 91.8 ng/L for MC-YR. However, EPA Method 544 uses solid phase extraction techniques to clean and concentrate the water samples with a suggested concentration factor of 500-fold. Therefore, the LCMRL values – calculated on "sample" basis – range from 0.10 ng/L to 0.184 ng/mL

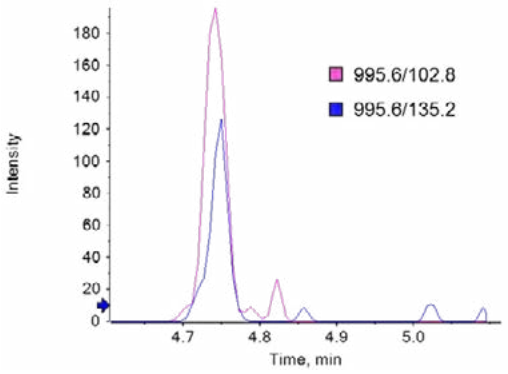

Figure 2. MRM chromatogram for the LOQ standard (43.8 ng/mL) of MC-LR. 1 Gaussian smooth performed.

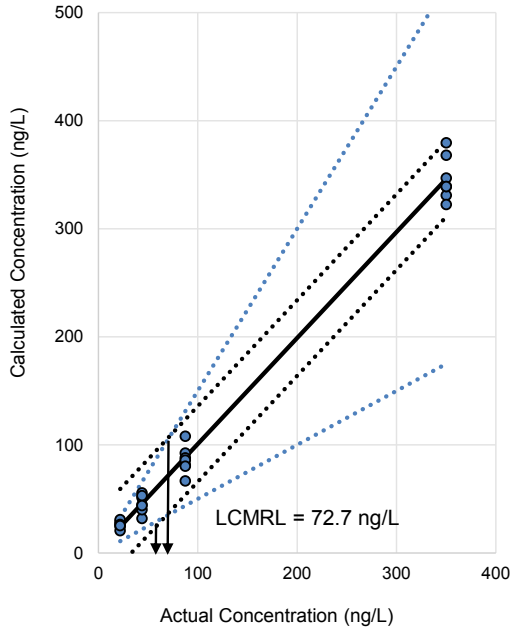

Figure 3. LCMRL graph for MC-LR.

Table 5. Lowest concentration minimum report level (LCMRL) concentrations.

Analyte	LCMRL (ng/L)
MC-LA	13.9
MC-LF	24.7
MC-LR	72.7
MC-LW	31.2
MC-LY	31.4
MC-RR	4.8
MC-WR	49.8
MC-YR	91.8
Nodularin-R	12.9

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Document number: RUO-MKT-02-5272-A

LC-MS/MS Analysis of Water using the Eksigent ekspert™ microLC 200 and SCIEX QTRAP® 4500

Maximizing performance, reducing costs

Jason Causon
SCIEX, Warrington, UK

Introduction

Water testing is typically performed using large volume injections combined with UHPLC-MS/MS. This utilizes high flow rates and small particle size UPLC columns. This is used to yield higher resolution and greater sensitivity, but at the sacrifice of higher column backpressures. The other drawback to UHPLC is the high consumption of solvents especially methanol and acetonitrile. This is an ever growing cost in both purchasing and disposal due to their environmental impact.

Micro flow chromatography with column diameters ≤1mm is an exciting approach for sensitive, high-throughput LC-MS/MS for environmental testing. It has been demonstrated that reducing the LC flow rate and using micro flow LC-MS/MS can result in an increase in sampling efficiency and sensitivity compared to conventional HPLC flow rates of 0.2 mL/min or greater.[1] The other benefits of micro flow chromatography include reduced solvent consumption, smaller injection volumes and reduced contamination of the mass spectrometer.

The Eksigent ekspert™ microLC 200 system is a dedicated micro flow UHPLC system that has been designed for optimal performance in the micro flow regime. It includes a new autosampler injection system with modifications for very small volume sample handling, minimal sample waste and very low carryover.

Here we present a new approach using low volume sample injections on the Eksigent ekspert™ microLC200 system on an SCIEX QTRAP® 4500 to quantify pesticides in environmental water samples.

Key Features of the Eksigent ekspert™ microLC 200 System

- High performance pumping system
  - Microfluidic Flow Control™ (MFC) for accurate rapid gradients with exceptional accuracy and reproducibility
  - Robust UHPLC performance with operating pressures up to 10,000 psi
- Fast reproducible sample injections
  - Small volume injections with minimal sample waste
  - Very low carry-over
  - Lowest delay volumes enable ultrafast gradient separations for LC-MS applications
- Green LC with smaller ID columns to reduce mobile phase consumption by over 95%, providing significant cost savings
- Robust integration with SCIEX hardware and software

Contents

Experimental

Liquid Chromatography:

All experimental data was acquired with the Eksigent ekspert™ microLC 200 system equipped with a HTC-xt Dynamic Load and Wash (DLW) autosampler. The ekspert™ microLC 200 system's solvent delivery system is based on binary gradient pumps that use patented Microfluidic Flow Control™ (MFC) pump technology

The column used was an Eksigent ChromXP™ Halo Fused-Core 2.7 µm, Phenyl Hexyl 0.5 x 150 mm. The column temperature was maintained at 30°C for all experiments. The mobile phases were ammonium formate in water and methanol. The flow rate was set at 15 µL/min with a 4 µL injection volume.

For the conventional HPLC experiments a Kinetex Fused-Core column was used with a flow rate of 600 µL/min and a 4 µL injection volume. The following gradient profile was used for both micro flow and conventional flow (Table 1).

Table 1. Analytical gradient profile used at both flow rates

Time (min)	A (%)	B (%)
0.0	95	5
0.5	95	5
5.0	25	75
7.0	5	95
8.5	5	95
8.75	95	5
15.0	95	5

Mass Spectrometry:

The SCIEX QTRAP® 4500 system was used for all data acquisition. Analyses were performed using multiple reaction monitoring (MRM) with simultaneous positive/negative switching electrospray ionization. The Eksigent hybrid 25 µm ID electrospray electrode was used for all micro flow analyses. This hybrid electrode is designed to minimize post column peak dispersion to maintain excellent peak shapes.[2] The source settings were setup for micro flow rates with the curtain, nebulizer and heater gas set to 20 psi with a temperature of 350°C. For the conventional flow rates the source conditions were scaled to the appropriate values.

Results and Discussion

The aim of this work was to determine the quantitative performance using low volume injections with micro flow rates. To establish the performance a number of pesticides were spiked into water and serially diluted down to the limits of detection. The dilutions were analyzed at both flow rates; Figure 1 shows a comparison for Desphenyl-chloridazon at 1ng/mL.

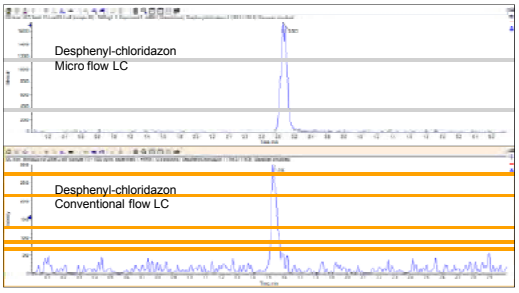

Figure 1. Desphenyl-chloridazon analyzed using micro vs. conventional flow LC-MS/MS (1 ng/mL)

At 1 ng/mL when comparing the peak areas at both flow rates the micro flow showed a 6.5 fold increase in peak area and a 10 fold increase in signal to noise. The parent Chloridazon was also analyzed and the comparison is shown in Figure 2.

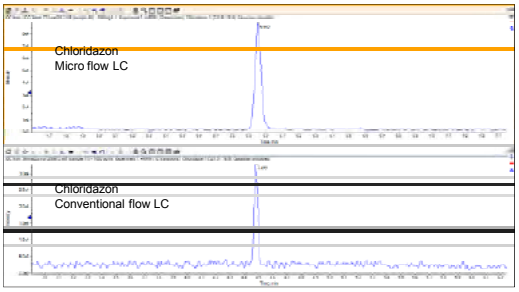

Figure 2. Chloridazon analyzed using micro vs. conventional flow LC-MS/MS (1 ng/mL)

At 1 ng/mL when comparing at both flow rates, micro flow showed a 4 fold increase in both peak area and signal to noise. Both compounds showed when using the ekspert™ microLC 200 system significant sensitivity gains. Table 2 shows the lower limits of quantitation (LLOQ, where signal to noise is 10) for both micro and conventional flow.

Table 2. Comparison of lower limits of quantitation (LLOQ)

Compound Name	LLOQ (ng/L) Micro Flow LC	LLOQ (ng/L) Conventional Flow LC
Chloridazon	50	500
Desphenyl-chloridazon	50	500
Atrazine	10	25
Atrazine-desethyl	10	50
2,4-D	50	250

Table 2 shows the ekspert™ micro LC200 system gave a 2.5 to 10 fold lower limit of quantitation compared to conventional flow HPLC. The accuracy and precision of the micro flow methodology was tested at and around the limits of quantitation. As shown in Table 3 all five pesticides gave very good precision and accuracies of less than 10% and +/- 3% respectively.

Table 3. Comparison of lower limits of quantitation (LLOQ)

Compound Name	CV (%)	Accuracy (%)
Chloridazon	6.6	102
Desphenyl-chloridazon	4.2	101
Atrazine	4.6	99
Atrazine-desethyl	8.9	103
2,4-D	3.9	103

The linearity of response for atrazine and atrazine-desethyl were analyzed from the LLOQ to 1 µg/L. shown in Figure 3. Both pesticides gave an 'r' value of 0.999 or better.

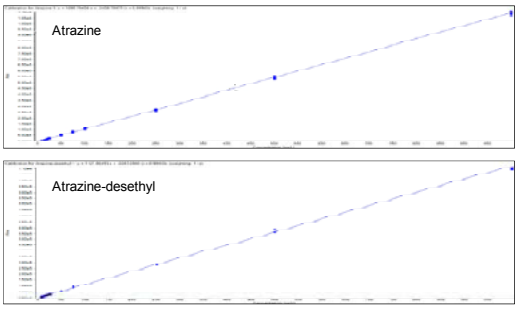

Figure 3. Calibration lines for Atrazine (top) and Atrazine-desethyl (bottom) with 'r' values of 0.999 or better

Conclusions

We have presented here a new approach for the analysis of pesticides in water. In order to use low volume injections, micro flow LC has been considered as a way to enhance performance and maximize sensitivity. The use of reduced diameter columns using the HALO fused-core particle allowed for significant reduction in the flow rates, injection volumes and sample consumption. This combines to give sensitivity gains reducing the lower limits of quantitation by 2.5 to 10 fold.

The other benefits of using micro flow technology is less mass spectrometer down time and reduced cost of ownership. For a typical overnight batch the conventional flow rates would use over 0.5 L of solvent. Whereas the Eksigent ekspert™ microLC 200 system ran at 15 µL/minute used just under 13 mL of solvent. Therefore over the course of a year the micro flow LC would use approximately 1/40 of the solvent.

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2 K. Mrizig et al.: 'Higher Sensitivity and Improved Resolution Microflow UHPLC with Small Diameter Turbo V™ Source electrodes and Hardware for use with the expressHT™-Ultra System' Technical Note Eksigent (2011) # 4590211-01

Publication number: 6440212-01

Contents

MultiQuant™ Software Version 3.0

Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples

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Key Features of MultiQuant™ 3.0

For laboratories analyzing food, water, or environmental samples for residues, contaminants and, pollutants data processing can be laborious and time consuming. The new MultiQuant™ software version 3.0 addresses some of the common bottlenecks laboratories face in data processing in order to improve quality and throughput:

- Full support of Windows XP, Windows 7 (32 and 64 bit) operating systems
- Processing of SCIEX triple quadrupole, QTRAP®, and TripleTOF® system data, including data generated using the *Scheduled* MRM™ Pro algorithm and *Scheduled* MRM^{HR}
- Processing of UV, DAD, and ACD data
- Built-in queries for the calculation and flagging of:
  - Outliers in accuracy
  - Analytes below or above a target concentration
  - Ion ratios and ion ratio tolerances
- Easy result review using the display of ion ratios
- Side-by-side peak review to quickly compare the response of samples
- Peak review magnifier for easy review and adjustment of (manual) peak integration
- Additional result table columns to assess peak quality, including Asymmetry Factor, Tailing Factor, Slope of Baseline, Peak Width, Points Across Peak

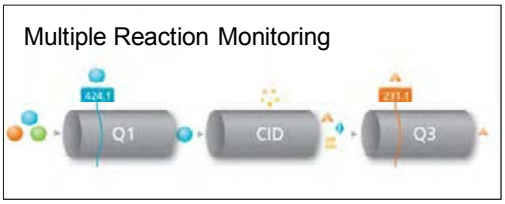

Figure 1a. Highly selective and sensitive quantitation using Multiple Reaction Monitoring (MRM) with triple quadrupole systems

Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the quantitation and identification of chemicals in food samples and environmental samples. Triple quadrupole-based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results (Figure 1a). Advancements in TripleTOF® technology also provide the ability to perform targeted quantitation with triple quadrupole-like performance (Figure 1b) and, at the same time, high confidence

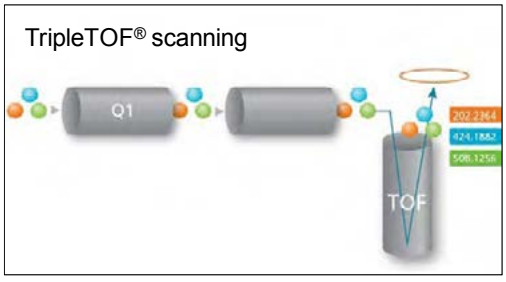

Figure 1b. Highly selective and sensitive quantitation using narrow extracted chromatograms (XIC) of accurate mass TOF-MS ions generated using TripleTOF® systems

Contents

in compound identification based on accurate mass MS and MS/MS information, making accurate mass LC-MS/MS an interesting alternative for modern food and environmental laboratories.

The increase in throughput using simplified and automated sample preparation techniques and the ability to screen for hundreds of target compounds in a single analysis using the *Scheduled* MRM™ Pro algorithm and full scan accurate mass techniques has placed greater demand for faster data processing and review, which has remained a significant bottleneck. Peak integration, review of quantitative and qualitative results, and reporting are time consuming and labor intensive tasks.

MultiQuant™ software version 3.0 was designed for laboratories with the goal of improving data processing efficiency. Integrated within Analyst® software, the user has the ability to quantify and identify chemicals of interest in complex samples in data files generated on SCIEX triple quadrupole, QTRAP®, and TripleTOF® systems.

Here, innovative new features in MultiQuant™ software version 3.0 are highlighted which significantly improve the data analysis workflow for quantitation and identification of compounds of interest in food and environmental laboratories.

Experimental

Pesticide Residues in Fruits and Vegetables

Pesticides were quantified and identified in food samples after QuEChERS extraction with automated DPX cleanup automated cleanup using a GERSTEL MultiPurpose Sampler (MPS) 2XL. The SCIEX QTRAP® 4500 system was used with Turbo V™ source and Electrospray Ionization (ESI) probe. The *Scheduled* MRM™ algorithm was used to achieve best data quality while monitoring over 200 pesticides using two MRM transitions per analyte to allow simultaneous quantitation and identification based on the ion ratio.[1]

Glyphosate, Glufosinate and AMPA in Drinking Water

These pesticides were analyzed using automated FMOC-derivatization and LC-MS/MS using a GERSTEL MPS 2XL coupled to an SCIEX QTRAP® 4500 system. Water samples were injected directly into the LC-MS/MS system providing sufficient sensitivity to identify and quantify targets at sub 100 µg/L concentrations.[2]

PAH in Food and Water Samples

Polycyclic Aromatic Hydrocarbons (PAH) were detected by LC-FLD-MS/MS. A Shimadzu NEXERA UHPLC system with fluorescence detector followed by MS/MS confirmation with an SCIEX QTRAP® 5500 system was used for analysis.[3]

PPCP in Environmental Samples

Pharmaceuticals and Personal Care Products (PPCP) were quantified and identified using direct injection of water samples and TOF-MS and MRM^{HR} scanning techniques utilizing an SCIEX TripleTOF® 4600 system.

Results

Built-in Queries to Calculate and Flag Outliers

Built-in queries of MultiQuant™ software can be used to calculate and flag outliers in standard and quality control samples, as defined in the settings tab of the quantitation method editor (Figure 2).

An example of highlighted outliers is shown in Figure 3. This software feature enables easy data review and quick adjustments of integration parameters and calibration lines.

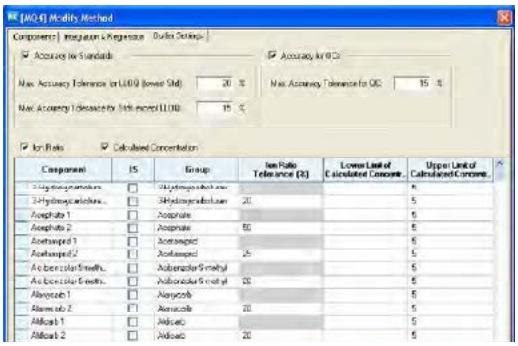

Figure 2. Query settings in the quantitation method editor of MultiQuant™ software version 3.0 to calculate and flag outliers, target concentrations, and ion ratios

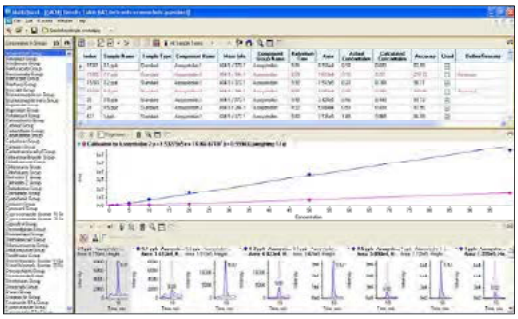

Figure 3. Azoxystrobin detected in a pesticide screening method using a QTRAP® 4500 system with highlighted outliers for the qualifier MRM transition at 0.1 and 0.2 ng/mL (calibration line of 0.1 to 100 ng/mL with r2 = 0.999 and %CV = 3.72% at 1 ng/mL, n = 10)

Built-in Queries to Highlight Analytes Below and Above a Target Concentration

Built-in queries of MultiQuant™ software can be used to highlight concentrations below or above a user specified value. The lower limit and upper limit of the calculated concentration can be defined in the method editor (Figure 2).

Examples of Spinosyn A detected in different food samples at a concentration higher than 5 µg/kg are shown in Figure 4.

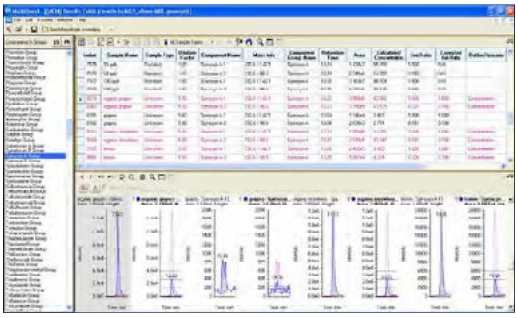

Figure 4. Spinosyn A quantified in different fruit and vegetable samples at a concentration higher than 5 µg/kg with positive identification using the MRM ratio

Built-in Queries to Calculate Ion Ratios for Compound Identification

Despite the high selectivity of MRM detection, there is always a risk of false positive or negative findings due to interfering matrix signals. Accordingly, quantitative results have to be confirmed using additional qualitative criteria. Often a second MRM or accurate mass fragment ion is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the ion ratio of standards. Various guidelines such as the European Commission Decision 2002/657/EC and SANCO/12495/2011 define MRM ratio tolerance levels for compound identification.

Built-in queries of MultiQuant™ software can be used to calculate ion ratios and flag outliers. Ion ratio tolerances for each analyte can be defined in the quantitation method editor (Figure 2).

Examples of Thiabendazole identified in different fruit and vegetable samples with MRM ratios inside of defined tolerance levels are shown in Figure 5a. The ion ratio is also visualized using tolerance bars in the Peak Review pane. The calculated ion ratio and expected ratio can be found in result table columns.

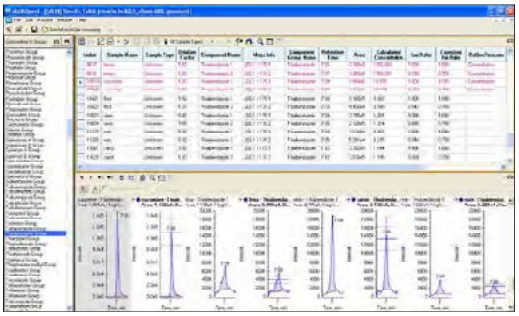

Figure 5a. Thiabendazole identified in different fruit and vegetable using the MRM ratio and compound dependent tolerance criteria, the ion ratio is also visualized using tolerance bars in the Peak Review

Figure 5b shows an example of reproducibility of ion ratios for identification detecting glyphosate, glufosinate, and AMPA in drinking water after automated FMOC-Cl derivatization using a GERSTEL MPS 2XL and LC-MS/MS.

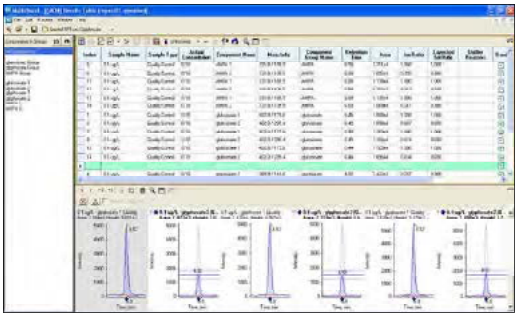

Figure 5b. Quantitation of glyphosate, glufosinate, and AMPA in drinking water at 0.1 µg/L with automatic ion ratio calculation for identification

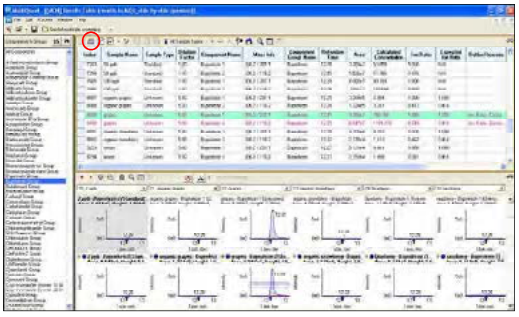

Figure 6b. Buprofezin detected in a grape sample above the target concentration, the side-by-side peak review allows comparison of the response compound in different samples at a glance, the automatic calculation and visualization of ion ratios helped to identify a matrix interference causing the positive detection of Buprofezin

Side-by-Side Peak Review

MultiQuant™ software allows a side-by-side peak review of chromatograms to compare the response of a selected compound in different samples at a glance.

Figures 6a and b show examples of detection of Boscalid and Buprofezin in different fruit samples. The side-by-side review with linked intensity axis of standard and samples allowed to quickly identify compounds above the target concentration of 10 µg/kg in the 5x diluted food extract.

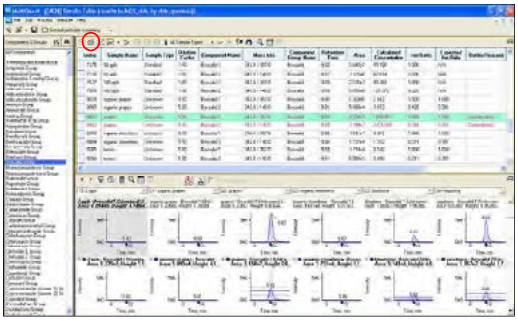

Figure 6a. Boscalid detected in different fruit samples above the target concentration with positive identification using the MRM ratio, the side-by-side peak review allows comparison of the response compound in different samples at a glance, helping to quickly find samples of interest

Processing of UV, DAD, ADC Data

MultiQuant™ software enables the use of UV, DAD, and other detectors via ADC channel for the quantitation. Method details are defined in the quantitation method editor (Figure 7).

An example of quantifying Benzo(a)pyrene using fluorescence detection (FLD) and MRM mode is shown in Figure 8.

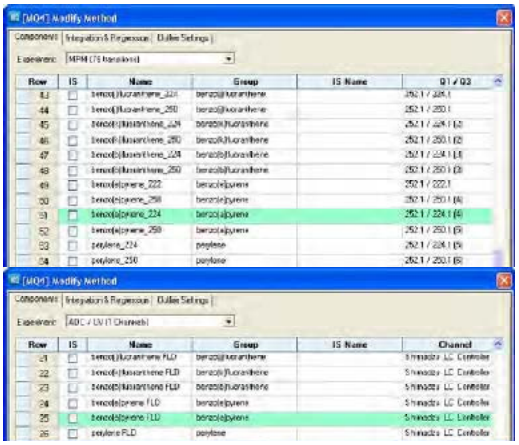

Figure 7. Method settings to process MRM and FLD data

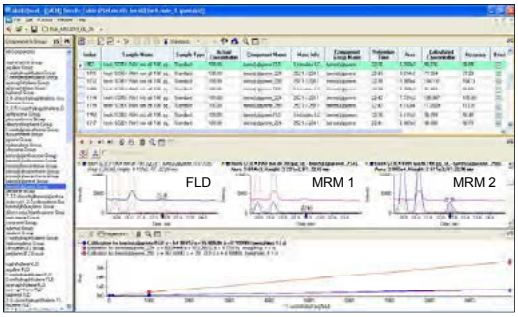

Figure 8. Quantitation of Benzo(a)pyrene using FLD (left) and MRM mode (middle and right)

Easy Peak Review and Adjustments of Peak Integration

MultiQuant™ software offers a number of features to allow an easy peak review to correct peak integration of necessary. This includes the new 'Peak Magnifier' 'Peak Demagnifier'.

The 'Peak Magnifier' allows increasing the size of the peak review for selected chromatogram to the entire window. This enables better peak review to verify and adjust integration parameters, manual peak integration, or using the 'Set peak not found' feature. The example chromatogram shown in Figure 9 is a magnified display of Boscalid detected in a blueberry sample (compare Figure 6a).

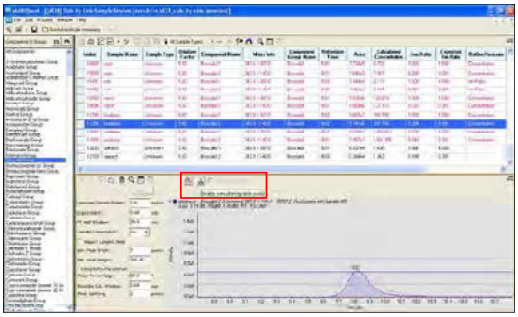

Figure 9. 'Peak Magnifier' to review a chromatogram of Boscalid detected in a blueberry sample, the peak integration can be adjusted quickly by changing integration parameters, manual peak integration, or using the 'Set peak not found' feature

Processing of TripleTOF® TOF-MS and MRM^{HR} Data

MultiQuant™ software can be used to process data generated on any SCIEX LC-MS/MS systems. TripleTOF® systems gain popularity for quantitative applications because of it triple quadrupole-like performance.

Figure 10 shows an example of quantifying Carbamazepine in a water sample spiked at 100 ng/L. The comparison of TOF-MS and MRM^{HR} data reveals the increased selectivity of the MRM^{HR} workflow. The different product ions detected in high resolution mode can also be used to calculate ion ratios automatically.

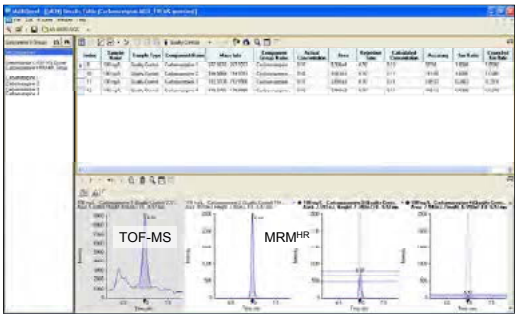

Figure 10. Carbamazepine at 100 ng/L quantified using TOF-MS and MRM^{HR} and identified based on ion ratio calculation

Summary

New features in MultiQuant™ software version 3.0, such as build-in queries, automatic ion ratio calculation, and side-by-side peak review with peak magnifier, significantly improve the data analysis workflow for quantitation and identification of food residues, contaminants, and environmental pollutants.

Contents

References

1 André Schreiber et al.: 'Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screening in Food Samples using DPX-QuEChERS with LC-MS/MS' Application Note SCIEX (2013) 8013613-01

2 André Schreiber and Oscar Cabrices: 'Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides' Application Note SCIEX (2013) 8013813-01

3 Takeo Sakuma et al.: 'Analysis of Polycyclic Aromatic Hydrocarbons (PAH), Alkylated Derivatives, and Photo-degradation Products in Environmental and Food Samples using LC-FLD-MS/MS with Q TRAP® Technology' Application Note SCIEX (2011) 4520411-01

4 André Schreiber et al.: 'Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) in Environmental Samples using Advanced TripleTOF® MS/MS Technology' Application Note SCIEX (2013) 7200213-02

Publication number: 8160213-01

Contents

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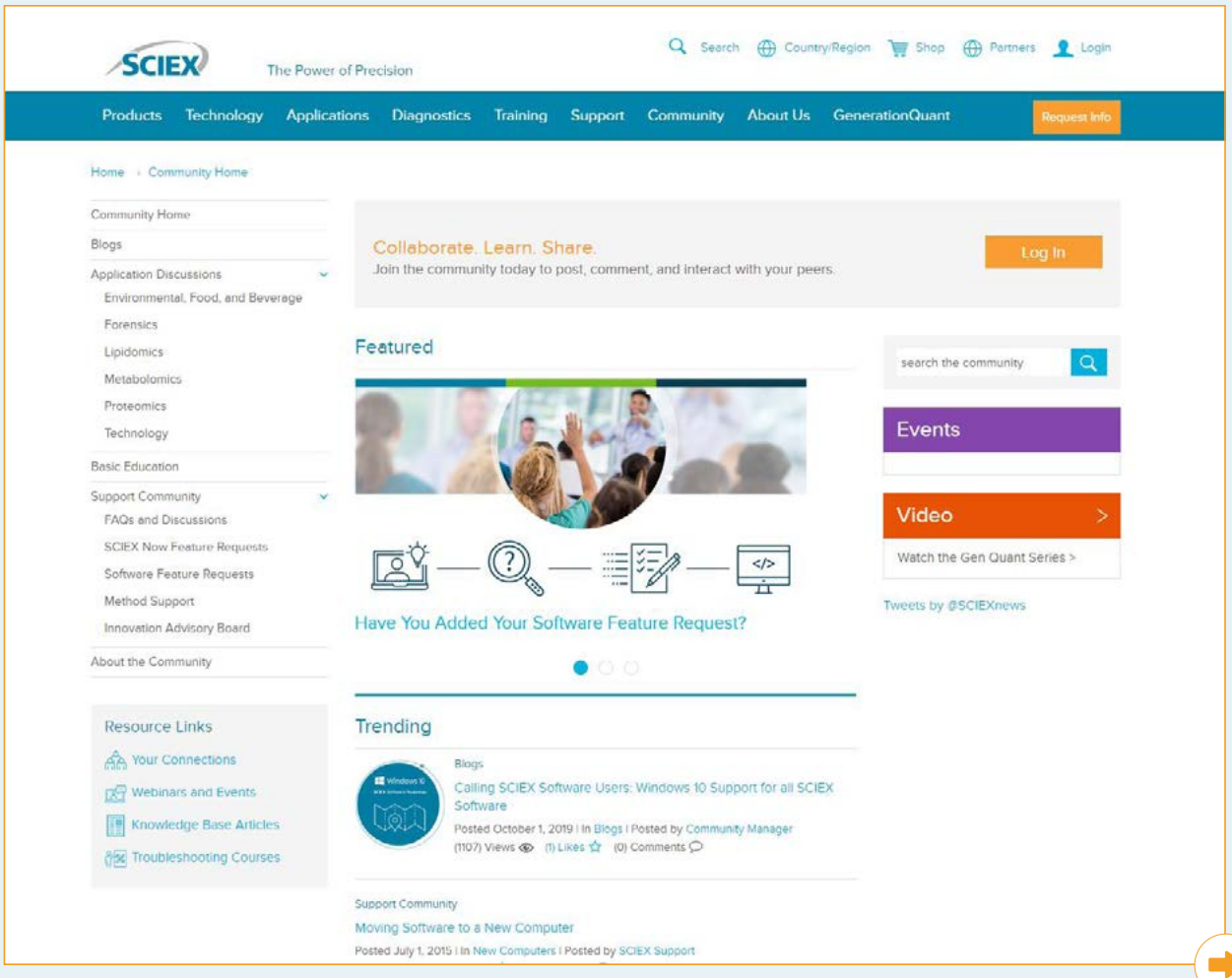

Contents

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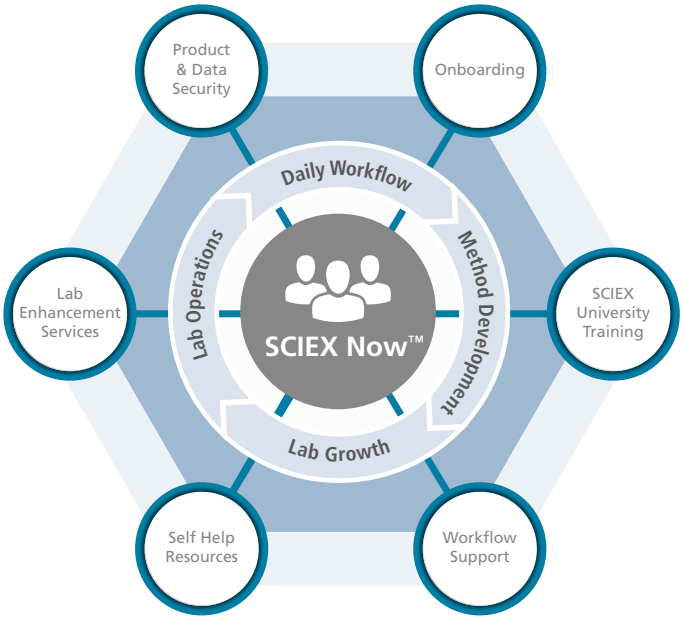

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Contents

Environmental Compendium

Volume 2

Contents

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