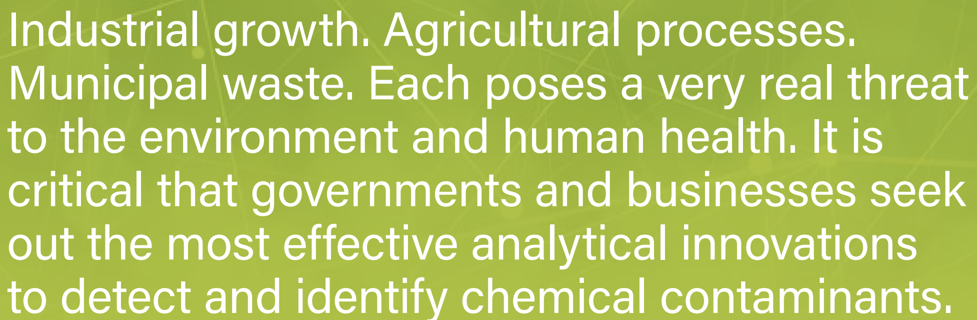
Waters Application Notes

Environmental



Industrial growth. Agricultural processes. Municipal waste. Each poses a very real threat to the environment and human health. It is critical that governments and businesses seek out the most effective analytical innovations to detect and identify chemical contaminants.

Waters thoroughly understands the specialized needs of environmental testing laboratories. Our system solutions include LC columns and sample preparation, environmental standards, chromatography, mass spectrometry and software. Whatever the challenge – sample throughput, trace detection, complex matrices, data management or regulatory compliance – Waters has a solution.

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[AFFFs]

[AFFFs]

[APPLICATION NOTE]

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Determination of Aqueous Film Forming Foam (AFFF) Composition Using a Multivariate Analysis Approach in UNIFI Scientific Information System

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[2]MTM Research Centre, Orebro University, Orebro, Sweden

APPLICATION BENEFITS

Reviewing complex high resolution, non-targeted MS^E datasets using workflows, filters, and views within an integrated scientific information system allows:

- Screening for a theoretical unlimited number of compounds in a single injection.
- Interrogation of data for the presence of unknown compounds of interest via filtering and statistical analysis.
- Structural elucidation of isolated unknown compounds of interest.
- Statistical tools to allow for isolation of unique markers.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

ACQUITY UPLC BEH C_{18} Column

Xevo® G2-XS QTof

UNIFI® Scientific Information System

KEYWORDS

AFFFs, foam, multivariate analysis, non-targeted screening, environmental forensics, MS^E, high resolution accurate mass (HRAM), high resolution mass spectrometry (HRMS), data independent analysis

AIM

Utilize streamlined multivariate analytical tools to determine compositional differences between AFFFs subject to environmental release.

INTRODUCTION

Aqueous film-forming foams (AFFFs) have been implemented in military and commercial fire-fighting activities to extinguish flammable liquid fuels. However, the use of these formulations has inadvertently resulted in the release of environmental contaminants due to migration from the site of application. The various formulations of AFFFs consist of numerous fluorocarbon and hydrocarbon compounds.[1] Characterizing the common as well as unique components of AFFFs that are used is the starting point to tracking these constituents through various environmental and biological compartments. In this work, seven AFFF mixtures were analyzed with a data independent acquisition approach (MS^E), using Waters® Xevo G2-XS QTof in order to obtain full spectral accurate mass data from which a multivariate analysis (MVA) approach could be taken to identify unique components within the mixtures.

The aim of these case studies is to identify the markers of interest in an easy workflow through the use of UNIFI software tools. Here, the use of built-in MVA functionality with EZ Info 3.0 software takes componentized data and enables rapid identification of markers associated with a particular sample. Markers are then elucidated using the Discovery Toolset and proposed identifications can be made in a streamlined and organized manner, using the approach described here.

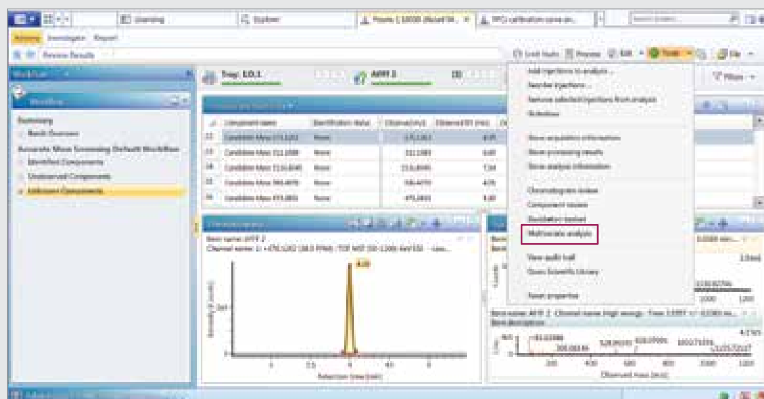

Figure 1. Easy access to MVA tools within the analysis tab enhances the data review process and provides the ability to perform complex differentiation analysis with information rich data.

EXPERIMENTAL

Sample analysis and data processing

Samples of seven industrial grade AFFFs provided were diluted 1:10,000 in methanol and chromatographic separation was performed using the ACQUITY UPLC I-Class System. Data were acquired using alternating high and low collision energy settings (MS^E) across the full analytical mass range, such that product ions were also generated on the Xevo G2-XS QTof. Instrumental performance with regards to mass accuracy (<5 ppm mass error), retention time conservation and repeatability of analyte response is particularly important in experiments involving non-targeted analysis, and the system was assessed using a solvent standard mixture of compounds. Electrospray positive (ESI+) and negative (ESI-) modes were acquired separately. Multiple injections of the seven AFFF mixtures were injected on the system, as well as composite sample. Injections were randomized to prevent bias due to carryover. Following analysis, data was subjected to principal component analysis (PCA). All data was acquired and processed using UNIFI Software with EZ Info 3.0.

LC conditions

LC System: ACQUITY I-Class

Column: ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 50 mm

Column temp.: 55 °C

Sample temp.: 4 °C

Mobile phase A: 98:2 water:MeOH 2mM ammonium acetate

Mobile phase B: MeOH 2 mM ammonium acetate

Gradient:

Min	Flow rate (mL/min)	%A	%B
Initial	0.65	90	10
0.5	0.65	90	10
5.1	0.65	0	100
6.6	0.65	0	100
6.7	0.65	90	10
8.5	0.65	90	10

MS conditions

MS system: Xevo G2-XS QTof

Full scan range: 50 to 1200 *m/z*

Source temp.: 120 °C

Capillary voltage: 1.0 µA

Cone voltage: 20 kV

Cone gas flow: 50 L/hr

Auxiliary gas flow: 1000 L/hr

Scan time: 0.2 min

Low energy CE: 4 eV

High energy CE ramp: 40 to 60 eV

Lock mass: Leucine enkephalin 556.2766 (positive ion) 554.2610 (negative ion)

RESULTS AND DISCUSSION

To ensure method quality control parameters were met, QC injections of previously characterized pesticide and perfluoroalkyl standards were interrogated at the beginning and end of the sample analysis. Pivot tables within UNIFI enabled rapid visualization of the required parameters for quality assessment including mass error, retention time, and response. Figure 2 summarizes the data for ESI+ QC injections of the pesticide standards at 10 ppb.

#	Item name	Replicate number	Sample position	Atrazine (ppm)	Atrazine-desethyl (ppm)	Chlorotoluron (ppm)	Diuron (ppm)	Linuron (ppm)	Metolachlor (ppm)	Monolinuron (ppm)	Sebuthylazine (ppm)
1	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
9	[illegible]	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10	[illegible]	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Figure 2. Example of a quality control assessment table for mass error. Values shown are mass error in ppm across 10 QC injections (5 prior to and 5 following the MVA experiment). An alternative parameter can easily be viewed using the pull-down menu.

The UNIFI componentized data was analyzed using principle component analysis (PCA). As can be seen in Figure 3A, a distinctive grouping was observed using positive ion MS for the AFFFs. Of the seven different AFFFs, three clustered very closely together, as seen in the top right quandrant of Figure 3A, AFFF1 and AFFF4 also fell on the right side of the scores plot, whereas AFFF3 was well separated from all other AFFFs. The composite samples were clustered appropriately towards the middle. The negative ion data scores plot is shown in Figure 3B. With the exception of AFFF3, the AFFFs grouped together. Both positive and negative ion datasets indicated that AFFF3 was quite different from the other AFFFs.

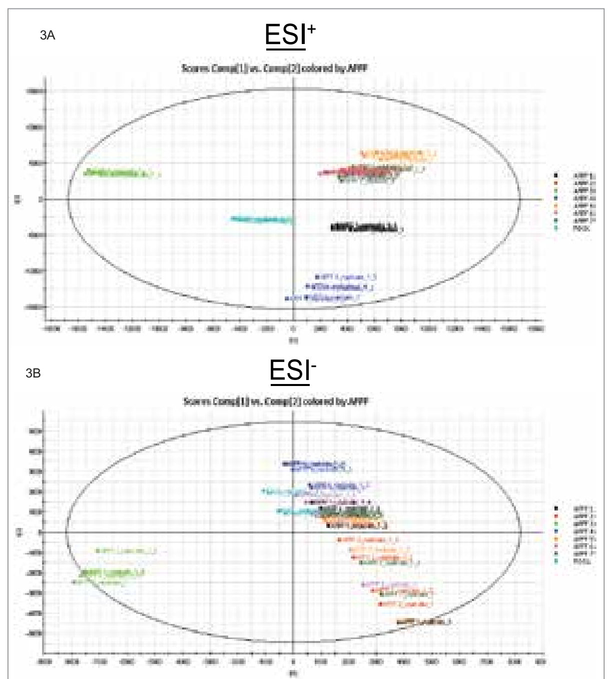

Figure 3. Principal component analysis (PCA) plots for the seven AFFFs analyzed in 3A. ESI+, and 3B. ESI. Both polarities were utilized in order to capture a comprehensive sampling of constituents in the samples.

Another means of visualizing the differentiation in the samples is the Loadings Plot, which shows the markers (exact mass retention time pairs) placed in the quadrants as they appear in the samples. Figure 4 shows the loadings plot of all markers and their spatial association with specific foams for positive ion data. Markers in the far left of the plot are those which occurred only or most intensely in AFFF3.

In order to identify markers of interest, group to group comparisons were carried out with two foams at a time, resulting in the generation of S-Plots of which an example is shown in Figure 5. Markers strongly correlated with individual AFFF formulations were tagged with a label indicating that they were more highly concentrated in that particular sample. Investigation of the labeled markers strongly associated with specific groupings using structural elucidation tools resulted in the identification of multiple sulfate, hydrocarbon, and fluorinated compounds.

Trend plots of these markers were used to assess the presence and abundance of these markers across all the injections of all AFFFs. Markers were either unique to specific formulations, or in some cases, common compounds across multiple AFFFs. For those constituents that had a proposed structure, product ion structures were assigned and used as a means to support identification. The aforementioned interrogation of markers of importance is carried out using the Discovery Toolset (Figure 6). Discovery Toolset, a feature within UNIFI Software, uses a combination of elemental composition proposals, theoretical isotopic distribution comparisons, ChemSpider searching, and fragment matching based on proposed structures. Markers were submitted as a batch and searched using this approach. Yellow highlighted hits (as well as the blue hit selected) have over 50% of their spectra explained by the proposed structure and associated fragments.

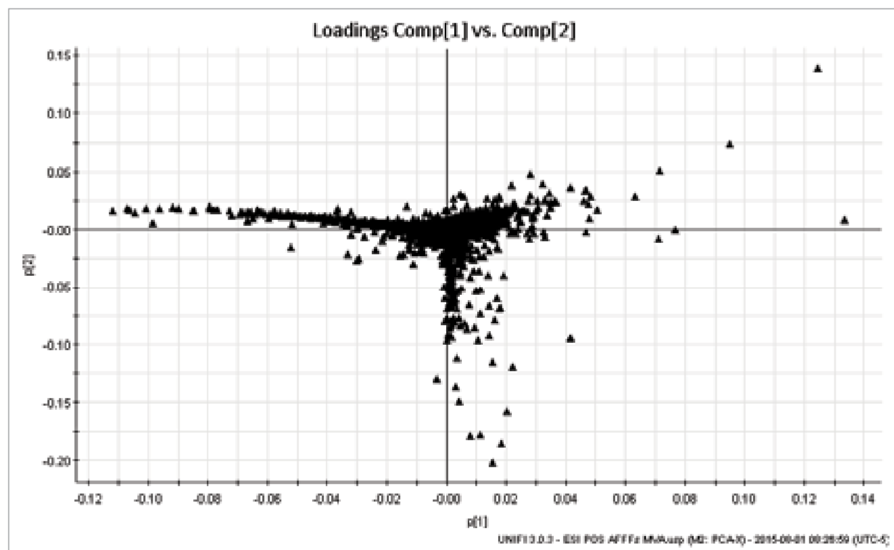

Figure 4. Loadings plot of all exact mass/retention time pairs (markers) identified in the samples, from ESI+ analysis. The position of the markers in the loadings plot is related to the position in the PCA plot of the samples from which they came. For example, markers on the far right are likely to be of higher abundance in AFFF3.

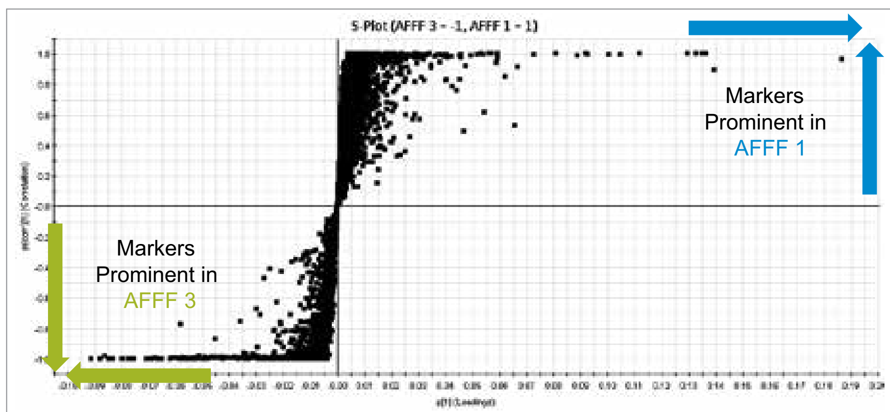

Figure 5. S-plot comparing markers associated with AFFF 1 and AFFF 3. The more strongly associated with the upper and lower corners of the plot, the higher the confidence in that marker being strongly associated with a particular sample.

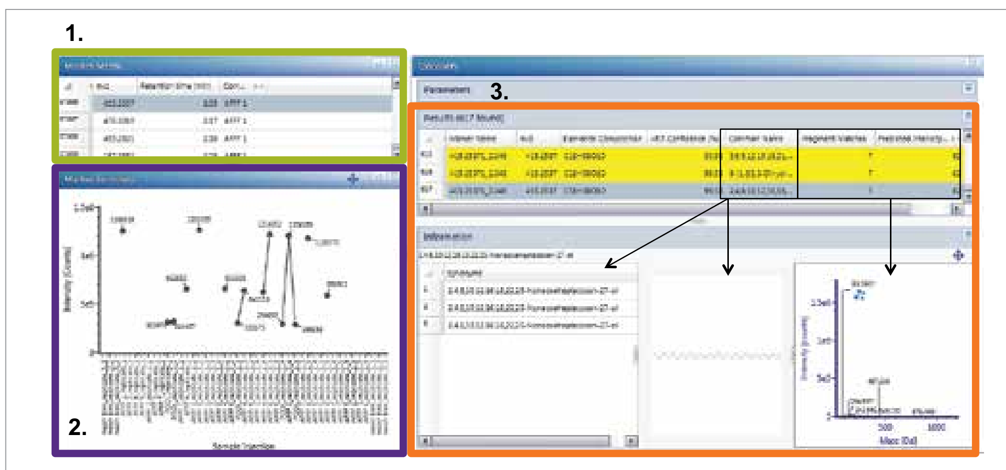

Figure 6. Proposed identification for a marker prominent in AFFF 1, and also present in AFFF 4. First, markers that have been identified as strongly correlated with one or more AFFF type are labeled in marker table (1). The markers are summarized by their intensity across the multiple injections in a trend plot (2). In this trend plot, the injections are shown in the order they were performed, so are randomized. However, upon inspection, it can be seen that the highlighted marker from (1) is present in consistent high proportions in AFFF 1 injections, consistent lower abundance in AFFF 4 injections, as well as in the composite (POOL) injections. Selected markers from (1) are then subjected to structural elucidation tools within UNIFI Software Discovery Toolset. The compound results of this structural elucidation are shown in (3). The compound name and resulting synonyms (arrow) are shown, as well as structure (arrow). The average high energy spectra (arrow) is used to arrive at fragment matches and scoring (Predicted Intensity) of theoretical against observed spectra.

CONCLUSIONS

- MVA software functionality affords facile differentiation between complex chemical mixtures and identification of potential environmental contaminants which comprise AFFFs.
- Exact mass measurements coupled with library searching, molecular formula calculations, and fragment ion proposals provide a means for identification of significant markers.
- The approach highlighted in this work offers potential for characterization of constituent migration from the point of use of various AFFFs.

Reference

1. Rotander A, Kärrman A, Toms L M, Kay M, Mueller J, Ramos M J G. *Environ Sci Technol Lett.* 49: 2434–2442, 2015.

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Analysis of Dioxins and Furans on a Xevo G2-XS QTof with APGC Using a QuEChERS Extraction Method

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[4]VUV Analytics Inc., Austin, TX, USA; [5]Waters Corporation, Milford, MA, USA

TECHNOLOGY BENEFITS

- Exceeds minimum performance limits for EPA method 1613
- >15X faster sample throughput over traditional techniques
- Less expensive using QuEChERS sample preparation

WATERS SOLUTIONS

Xevo® G2-XS QTof

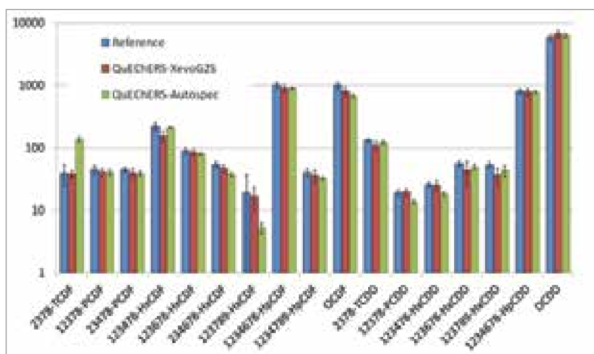
Atmospheric Pressure GC (APGC) Source

MassLynx® MS Software

KEYWORDS

Dioxins, furans, QuEChERS, QuanTof, charge transfer, APGC, persistent organic pollutants, POPs

QuEChERS extraction, combined with APGC and QTof allows dioxins analysis to be performed without the need for an expert operator, quicker and cheaper than traditional dioxin sample preparation and analysis.



Results from dioxins analysis using QuEChERS extraction followed by APGC-QTof MS are in good agreement with the NIST 1944 Standard Reference Material.

INTRODUCTION

The objective of this work was to develop a dioxin method that was faster and more cost effective than the traditional magnetic sector technique using APGC high resolution mass spectrometry (HRMS) analysis on a Waters® Xevo G2-XS QTof while exceeding the minimum performance limits required for EPA method 1613.

Dioxins and dioxin-like compounds are ubiquitous persistent organic pollutants (POPs) linked to various diseases including cancer.[1] They are restricted under the Stockholm Convention[2] and are monitored for their occurrence and toxicity by regulatory agencies worldwide.

The classical analytical method for testing dioxins in sediment using magnetic sector instruments is considered the "gold" reference standard. However it requires an expert operator and specialized instrumentation.[3] Traditional sample preparation times can exceed several days and use a large amount of costly and hazardous solvents.

Since sediment chemistry can vary spatially and temporally, it is necessary to analyze a large number of samples to properly characterize any site being evaluated for dioxin contamination.[4] This translates to an extreme expenditure of time for sample prep and massive solvent usage. Within the last decade, a single phase acetonitrile extraction known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) has been employed to prepare food samples for pesticide analysis in as little as 30 minutes.[5] This technique was modified and adapted as a rapid extraction and cleanup for the analysis of dioxins and furans in sediment samples and was used in the preparation of samples in this study. In this new approach, samples were investigated using the Xevo G2-XS QTof equipped with Atmospheric Pressure GC (APGC).

DISCUSSION

A modified QuEChERS sample preparation method for the screening of dioxins and furans in sediment was developed which reduced sample preparation time from 10 samples in four to five days, to as many as 30 samples in one day.[6] This study also exploits the use of an APGC source (Figure 1a) coupled to the Xevo G2-XS QTof (Figure 1b) as an alternative to a traditional magnetic sector instrument.

Wet sediment samples were fortified with ^{13}C-labeled standards and extracted using a modified QuEChERS method. The separated organic layer was solvent exchanged to hexane by liquid-liquid extraction. The extract was cleaned by a carbon column and then concentrated for instrumental analysis using a magnetic sector GC HRMS system and a Xevo G2-XS QTof equipped with an APGC source. The column used in this analysis was a Restek Rtx-Dioxin2 at 20 m, 30 m, and 40 m lengths.

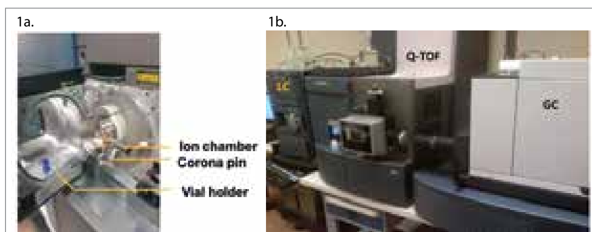


Figure 1a. Atmospheric Pressure Gas Chromatography (APGC) source on a 1b. Xevo G2-XS QTof Mass Spectrometer.

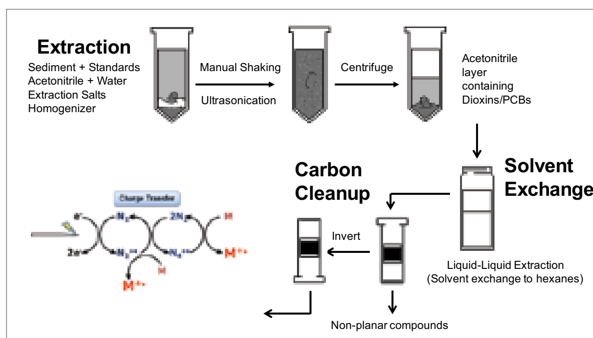


Figure 2. QuEChERS sample preparation schematic.

The capabilities and performance of the APGC-Xevo G2-XS QTof proved to be similar or better than the magnetic sector MS for the analysis of dioxins. Unlike conventional EI (electron ionization) systems, the APGC source allows for higher flow rates to improve analysis times. The effect of increased flow rate on the chromatographic resolution for four different congener classes are shown in Figure 3. Although chromatographic resolution decreases with increased column flow rates, adequate separation is maintained for quantitative analysis, in large part due to the selectivity of the stationary phase (Rtx-Dioxin2). Only the pair of HxCDD congeners appear to co-elute, but given their identical TEFs (toxic equivalency factors), the impact of the reduced chromatographic resolution on TEQ (toxic equivalency quantity) is expected to be negligible.

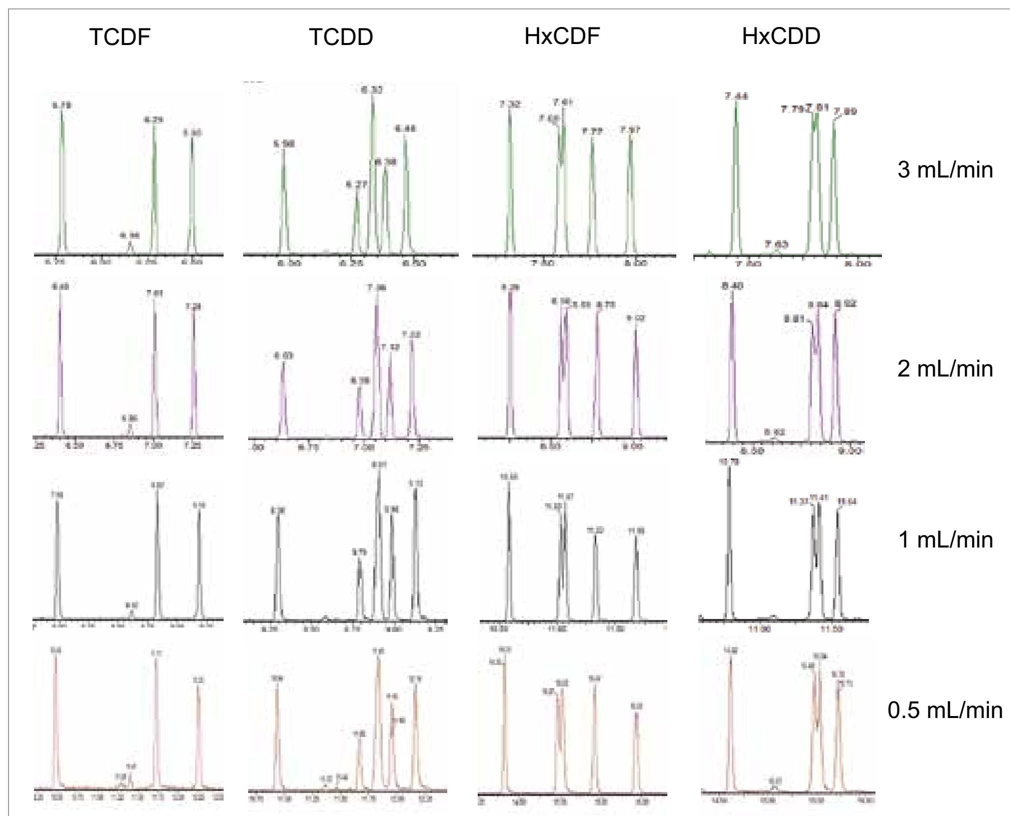


Figure 3. Chromatographic resolution as a function of flow using a mid-level calibration standard CS3WT. Closely eluting congeners were analyzed by APGC-QTof on an Rtx-Dioxin2 column. The higher flow rates possible with APGC will reduce run time while still maintaining adequate separation for quantitation.

Column length was also evaluated and the results are summarized in Figure 4. Shorter columns reduced backpressure, resulting in higher flow rates, and further reduced runtimes (<15 min/sample) with minimal loss in separation. APGC is sufficiently versatile to provide ultimate chromatographic performance (using a 40 m Rtx-Dioxin2 column at optimum flows) that satisfies the regulation, despite the method requirement for EI ionization and magnetic sector MS. When needed, high throughput and increased capacity is possible (using a 20 m Rtx-Dioxin2 column at flows >3 mL/min) while preserving separation of critical isomers.

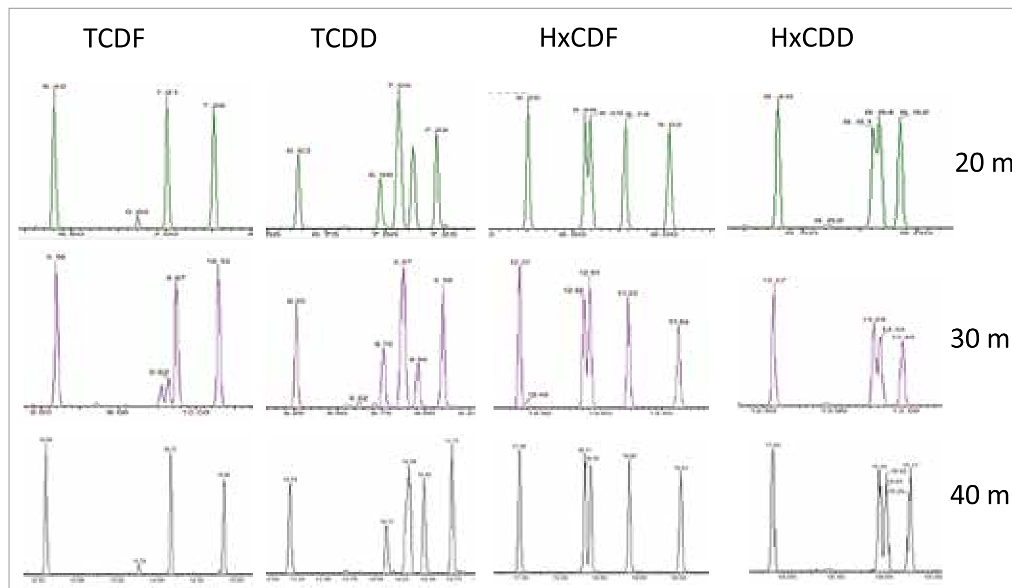


Figure 4. Chromatographic resolution as a function of column length in APGC-QTof on an Rtx-Dioxin2 column (flow rate 2 mL/min) using a mid-level calibration standard CS3WT. Shorter column lengths can reduce run time and provide less resistance to higher flows while maintaining separation.

Figure 5a shows results from a calibration curve of 2,3,7,8-TCDD from 0.5 to 200 pg with good linearity and R^2 of 0.9993 using APGC-Xevo G2-XS QTof.

Shown in Figure 5b are the results of the certified reference material compared to the APGC-Xevo G2-XS and magnetic sector. Results from the APGC-Xevo G2-XS compare favorably to the reference and the magnetic sector. It is worth noting, however, that the magnetic sector results for 2,3,7,8-TCDD and 1,2,3,7,8,9-HxCDF differ from the reference while the APGC results for those same congeners compare more favorably to the reference (Figure 5b).

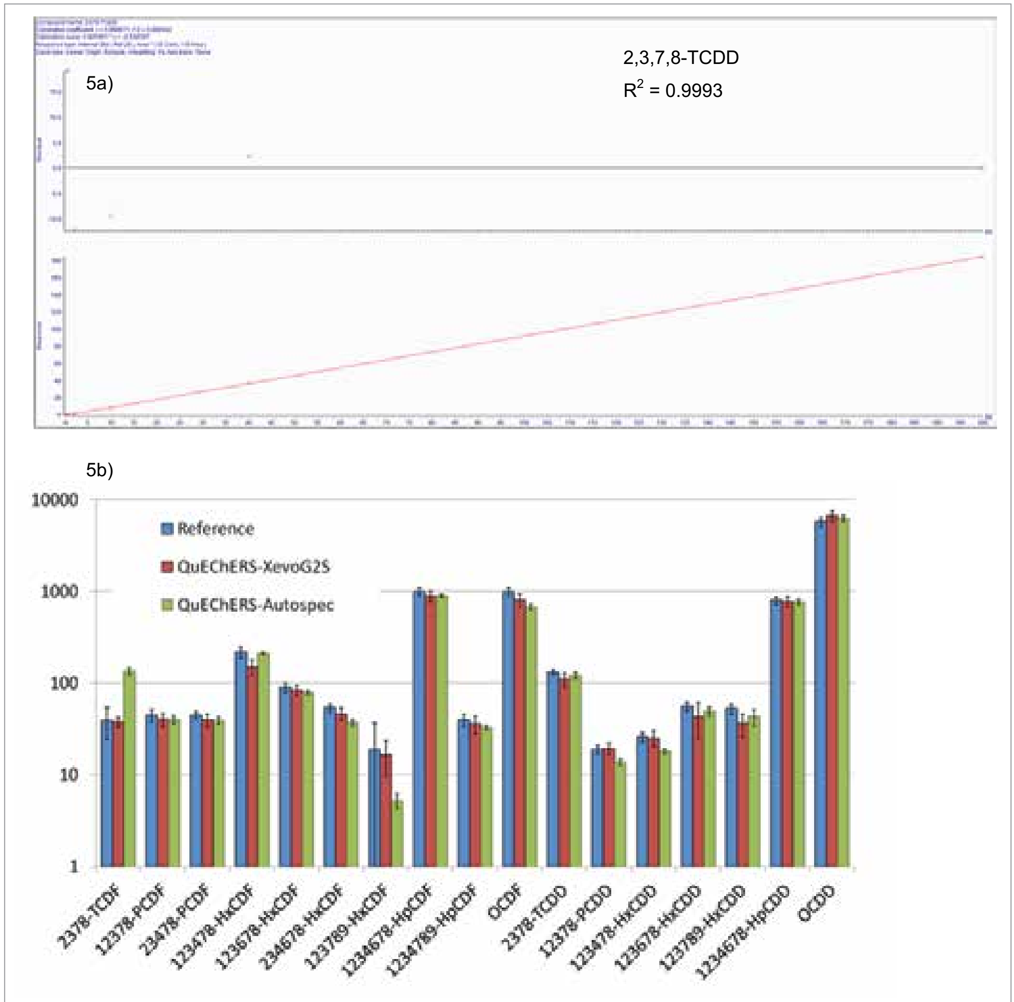


Figure 5a. Residuals plot and linear regression of a five point calibration of 2378-TCDD ranging from 0.5 pg to 200 pg run on APGC-QTof. 5b. Standard reference sediment NIST1944 (ng/kg dry-mass) extracted by the modified QuEChERS method and analyzed by GC-HRMS and with APGC-QTof. Comparison run on a 40 m Rtx-Dioxin2 column with a flow rate of 1 mL/min.

CONCLUSIONS

QuEChERS has been proven to be an effective sample extraction/clean-up method for the analysis of a large number of sediment samples from site remediation activities,[7] hence reducing the time and solvent as compared to the classic preparation. APGC along with the Xevo G2-XS QTof decreased instrumental run time due to its ability to handle higher flow rates than the GC-HRMS system. The combined method of QuEChERS extraction with APGC-QTof analysis provided a sample throughput increase of 15x over traditional techniques. The Xevo G2-XS QToF offers a flexible platform with inlet options including APGC, ESI, APCI, and UniSpray™ to name a few, thus permitting the instrument to perform other analysis when needed. It can operate in a non-targeted acquisition mode that can meet the limits of detection of dioxin regulatory method EPA1613, and can provide additional analytical information such as elemental composition on non-target analytes that can be encountered with both classical and generic sample preparation approaches.

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Confirmation of PCDDs and PCDFs at Sub-Femtogram Levels Using Atmospheric Pressure Gas Chromatography (APGC) with Xevo TQ-XS

Rhys Jones,[1] Keith Hall,[2] David Douce,[1] Jody Dunstan,[1] Ken Rosnack,[3] and Adam Ladak[3]
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GOAL

To determine the limits of detection for dioxins and furans in solvent standards, and to confirm their presence and accurate quantitation in a QC fly ash samples.

BACKGROUND

Polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a group of chemically related compounds that are toxic and persistent organic pollutants (POPs). These compounds are restricted internationally under the Stockholm Convention[1] and due to the bioaccumlative nature of these compounds, it is essential to monitor them at ultra trace levels in food and environmental samples. Traditionally these compounds have been analyzed using magnetic sectors with electron ionization sources which require expert users to obtain consistent results. As there is a growing concern for the analysis of these compounds, more user-friendly technology is essential to analyze potentially contaminated samples. Atmospheric Pressure Gas Chromatography (APGC), coupled with a highly sensitive tandem quadrupole mass spectrometer

Coupling APGC to Xevo TQ-XS takes sensitivity to the next level – Confirmdioxins in complex samples at concentrations that are unachievable by traditional magnetic sector GC systems.

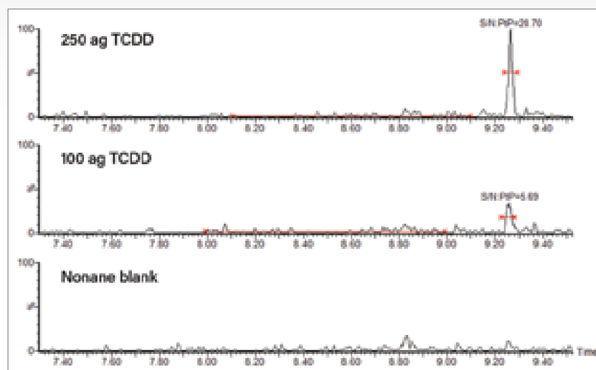

Figure 1. 2,3,7,8 TCDD at 100 ag on-column.

(Xevo® TQ-S), has already been demonstrated to be a sensitive and robust option for confirmatory analysis of PCDDs and PCDFs by GC-MS/MS in compliance with 589/2014/EU.[2] The recent introduction of the Xevo TQ-XS from Waters has allowed lower limits of detection to be reached. This can help reduce time spent on sample preparation/preconcentration, as well as reducing the cost of analysis as diluted standards can be utilized.

THE SOLUTION

GC Method for TCDD assessment

Agilent DB-5MS column, 30 m x 0.25 mm I.D. x 0.25 µm film, helium at 1 mL/min. 7890A GC Oven and Agilent autosampler, split/splitless injector at 290 °C operating in pulsed splitless mode (32 psi for 0.5 min) with a 1.0 µL injection volume. GC program, initial temp. of 130 °C, hold for 1.2 min, ramp at 20 °C/min to 320 °C, and hold for 3.3 min.

GC method for full PCDD and PCDF assessment

Zebron ZB-5MS column, 60 m x 0.25 mm I.D. x 0.25 µm film, helium at 1.4 mL/min. 7890A GC oven and Agilent autosampler, split/splitless injector at 290 °C, operating in pulsed splitless mode (50 psi for 1.8 min) with a 1.0 µL injection volume. GC program: initial temp. of 130 °C, hold for 1.8 min, ramp at 40 °C/min to 200 °C; ramp 2 at 2 °C/min to 235 °C; ramp 3 at 3 °C/minute to 305 °C; ramp 4 at 20 °C/min to 320 °C, and hold for 5 min. Total run time of 49.85 min.

MS parameters for both assessments

Corona pin at 2.0 µA, cone gas 260 L/hr, auxiliary gas 200 L/hr, makeup gas 300 mL/min, quad resolutions at 0.7 Da.

In order to assess the sensitivity of the APGC coupled with the Xevo TQ-XS, a standard of 2,3,7,8-TCDD was diluted in nonane giving a calibration range between 100 ag to 100 pg on column. In order to perform this test, two MRM transitions for TCDD were utilized. Figure 2 shows the linearity of 2,3,7,8 TCDD, which was excellent.

An on-column standard concentration of 100 fg was injected over 20 days in order to assess the reproducibility of the system. Figure 3 shows the outstanding reproducibility of the response, and Figure 4 shows the stability of the isotopic measurements over this series of injections.

Once the initial sensitivity of the system had been verified, a full suite of TCDDs and TCDFs was acquired on the system. A series of EPA 1613 standards were used from CSL to CS5, diluted 1 in 10 with nonane. Figure 5 shows that the isotope ratio assessment for each congener was consistent at all concentrations. This is essential for the confirmation of dioxins and furans in a sample. Legislation states that these ratios are required to be <15%.[3,4,5]

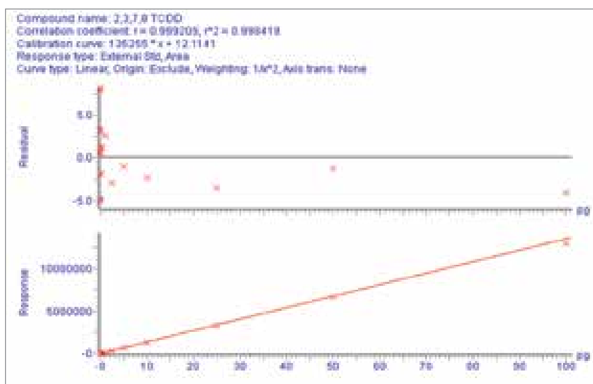

Figure 2. Linearity of 2,3,7,8 TCDD between 100 ag to 100 pg.

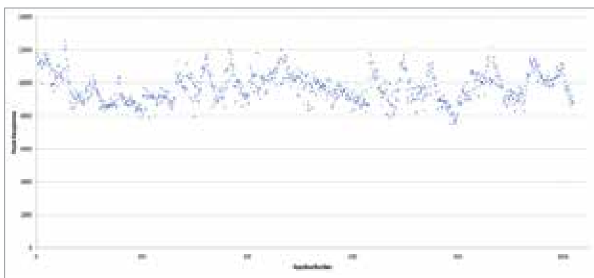

Figure 3. Stability of the response of 100 fg of 2,3,7,8 TCDD over 1000 injections with an RSD of 9.2% (no internal standard correction).

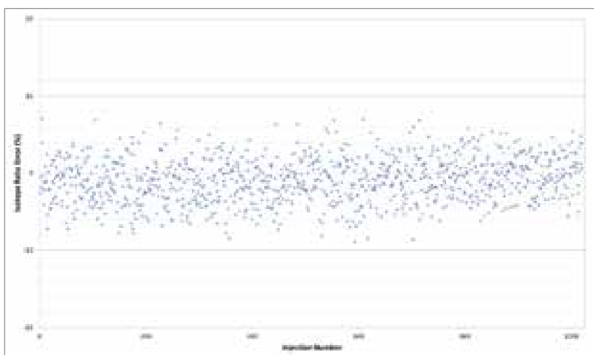

Figure 4. Stability of the isotope ratio of 100 fg of 2,3,7,8 TCDD over 1000 injections.

The final assessment was the analysis of a QC fly ash sample. These types of samples are very complex and often used as proficiency tests for dioxin labs in order to ensure that they are producing accurate results. Figure 6 shows the complexity of the samples and demonstrates the ability of APGC and Xevo TQ-XS to quantify the compound of interest, as highlighted in Figure 6. Figure 7 shows that the value obtained with APGC coupled with the Xevo TQ-XS were consistent with that of the QC sample.

SUMMARY

Utilizing APGC coupled with the Xevo TQ-XS allows sub-femtogram levels of dioxins to be analyzed in complex samples. The added sensitivity enables the dilution of expensive dioxin standards, reduces the need to pre-concentrate sample extracts (prior to analysis), and minimizes the amount of sample required for testing. Not only is this system exceptionally sensitive, it is also robust and produces consistent results over thousands of injections. The APGC coupled with the Xevo TQ-XS far surpasses the regulatory requirements for dioxin testing.

Figure 5. Consistency of the isotope ratio for 1 in 10 dilution of CSL to CS5.

Figure 6. Complex fly ash sample chromatogram showing the identification of 2,3,7,8 TCDD.

Figure 7. Results from fly ash QC sample.

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Quantitative Analysis of Natural and Synthetic Estrogens in Surface and Final Effluent Waters at Low ppq Levels Using UPLC-MS/MS

Euan Ross,[1] Benjamin Wuyts,[1] and Angela Boag[2]
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[2]Scottish Water, Edinburgh, UK

APPLICATION BENEFITS

- High sensitivity to achieve EU target LLOQs.
- Baseline separation and good peak shapes for target analytes.
- Suitable linearity and repeatability data in surface water and final effluent.
- Acceptable SPE spiked recoveries and repeatability.
- Quantitation of detected residues in final effluent using standard addition in the absence of isotopically labeled internal standard.

WATERS SOLUTIONS

Xevo® TQ-XS Triple Quadrupole Mass Spectrometry

ACQUITY® UPLC® H-Class System

MassLynx® MS Software

TargetLynx™ Application Manager

Oasis® HLB Cartridge

Certified Sep-Pak® Silica Cartridge

ACQUITY UPLC BEH C_{18} Columns

TruView™ LCMS Certified Vials

KEYWORDS

Estrone, 17α-ethinylestradiol, 17β-estradiol, synthetic estrogens, SPE, environmental analysis, large volume injection, trace analysis, surface water, final effluent, EDCs, endocrine disruptors

INTRODUCTION

Estrogens are routinely used either as contraceptive medicines or in hormone replacement therapy and they can enter aquatic environments via the discharge of final effluent waters.[1] Estrogens are believed to have a negative effect on aquatic environments by disrupting the hormonal systems of fish.[1] For the European Union, EU Directive 2013/39/EU[2] includes 15 additional priority substances to the water framework Directive 2000/60/EC.[3] In this update, 17α-ethinylestradiol and 17β-estradiol were not included in the priority substance list, but instead added to a watch list[2] in order to gather further data regarding the presence of these compounds in aquatic environments, and the risks they may pose.

This application note summarizes a method for the analysis of estrone, 17α-ethinylestradiol and 17β-estradiol in surface and final effluent waters. To achieve the challenging EU LLOQ parts per quadrillion (ppq) levels[4] required for these compounds, a combination of off-line solid phase extraction (SPE) clean-up and pre-concentration, combined with a large volume injection and triple quadrupole mass spectrometry were utilized.

Xevo-TQ-XS.

EXPERIMENTAL

Sample description

Extracted samples were prepared and supplied by Scottish Water. Spiked surface water and final effluent samples were first filtered, extracted, and concentrated using an off-line solid phase extraction (SPE) method[5-6] (Figure 1). After evaporation and reconstitution in LCMS grade water, the samples were then analyzed by UPLC-MS/MS using a large volume injection (100 µL).

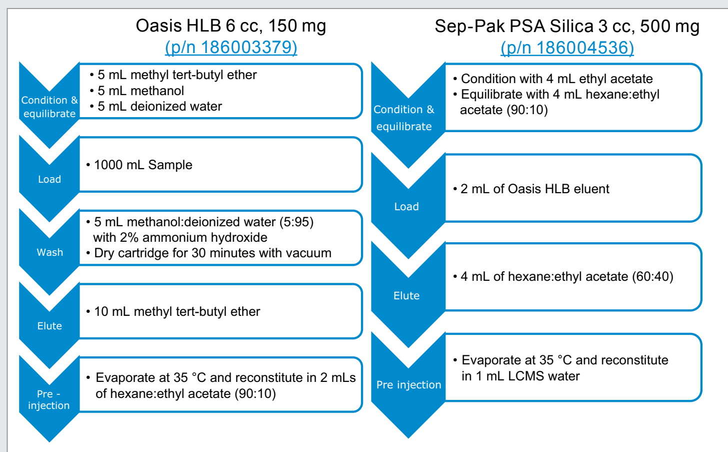

Figure 1. Solid phase extraction, clean-up, and concentration methodology, provided by Scottish Water.

LC conditions

LC system:	ACQUITY UPLC H-Class with extension loop, needle, and syringe for large volume injection
Vials:	TruView LCMS Certified
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 3.0 x 100 mm
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	100 µL
Flow rate:	0.6 mL/min
Mobile phase A:	LCMS grade water with 1 mM NH_4F (analytical grade)
Mobile phase B:	50:50 LCMS grade acetonitrile:methanol with 1 mM NH_4F (analytical grade)

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B
Initial	0.6	70	30
1.00	0.6	70	30
3.50	0.6	5	95
5.50	0.6	5	95
5.60	0.6	70	30
8.60	0.6	70	30

MS conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Acquisition mode:	MRM
Capillary voltage :	2.00 kV
Cone gas flow:	150 L/Hr
Desolvation temp.:	600 °C
Desolvation gas flow:	1200 L/Hr
Nebulizer:	7 bar

Compound	TRANSITions	Cone voltage (V)	Collision energy (eV)	Retention time (min)
17α-Ethinylestradiol	295.10 > 143.00	40	47	4.17
	295.10 > 145.00		36	
	295.10 > 159.00		34	
17β-Estradiol	271.20 > 143.00	40	45	4.11
	271.20 > 145.00		37	
	271.20 > 183.00		37	
Estrone	269.10 > 143.00	30	45	4.25
	269.10 > 145.00		35	
	269.10 > 159.00		35	

Table 1. Summary of optimized MS parameters and LC retention time for target analytes.

Data management

MS software: MassLynx v4.2

RESULTS AND DISCUSSION

Method optimization was accomplished by evaluating various columns, mobile phase compositions, gradients, and MS transitions. The conditions detailed in the Experimental section provided the best overall performance of those tested. Baseline separation of the target analytes was achieved, and a example of the chromatography from a 50 ng/L solvent standard is shown in Figure 2. Due to the hydrophobic nature of the analytes, they elute in the high organic part of the gradient. As a result, it is challenging to separate the analytes away from matrix components in the final effluent used for this analysis, as shown in the RADAR™ scan (full scan *m/z* 100 to 1000) in Figure 3. To minimize any contamination of the MS source, the integrated fluidics system on the Xevo TQ-XS was used to divert the chromatographic region to the MS system for analysis, and unwanted regions to waste.

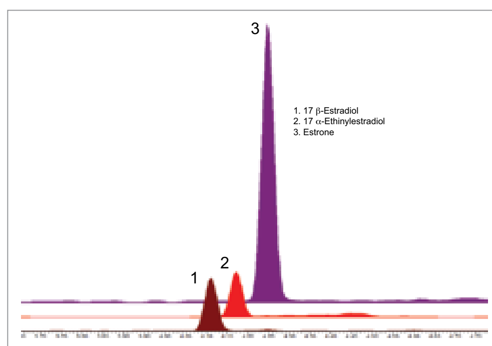

Figure 2. Example chromatography of a 50 ng/L solvent standard, separated on a 1.7 µm , 3.0 x 100 mm, ACQUITY, BEH C_{18} Column, 100 µL injection.

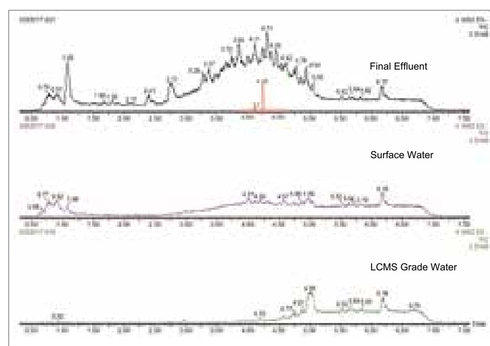

Figure 3. RADAR scan (full scan m/z 100 to 1000), on matrix samples after cleanup and concentration by SPE. Example peaks for the target compounds are shown against the final effluent RADAR scan indicate the area they elute.

Satisfactory linearity was achieved for all compounds in matrix matched (spiked post extraction) bracketed calibration curves, over the appropriate ranges. In surface water, a range of 10 to 320 ng/L for 17α-ethinylestradiol, and 62.5 to 2000 ng/L for 17β-estradiol and Estrone provided good linearity (R^2 >0.998, residuals <15%). An example of the calibration and associated residuals is shown for all three compounds in Figure 4. For final effluent, the matrix matched calibration curves ranged from 120 to 2000 ng/L for all three compounds, also giving acceptable linearity (R^2>0.997, residuals <10%). The robustness of the method was assessed using spiked water samples (n=8 for each matrix type) where %RSD values below 6% were obtained.

To evaluate the method performance in surface water, matrix samples were pre-spiked at suitable pg/L (ppq) levels prior to extraction, and prepared in accordance with the methodology detailed in Figure 1. An example of the chromatography and sensitivity observed for a sample pre-spiked at low ppq level in surface water, before clean-up and concentration, is shown in Figure 5.

Matrix effects were determined by quantifying post spiked surface water samples against a solvent calibration curve. For 17β-estradiol and estrone, matrix effects were calculated at ≤-22% (suppression), 17α-ethinylestradiol ≤16% (enhancement). Final effluent, even after the SPE clean-up remained a complex sample, as shown in the RADAR scan in Figure 3, this resulted in significant matrix suppression ≤-72% for all compounds. However even with this significant suppression, low levels of each compound are still detectable.

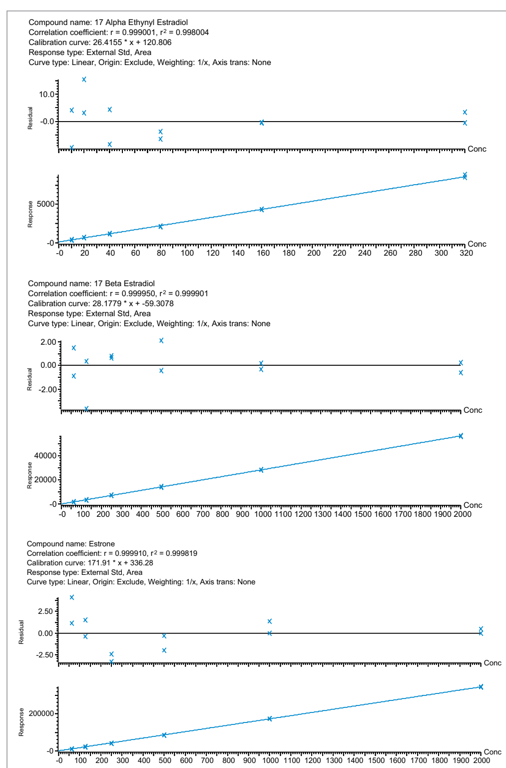

Figure 4. An example of the calibration and associated residuals (n=2) is shown for all compounds in surface water.

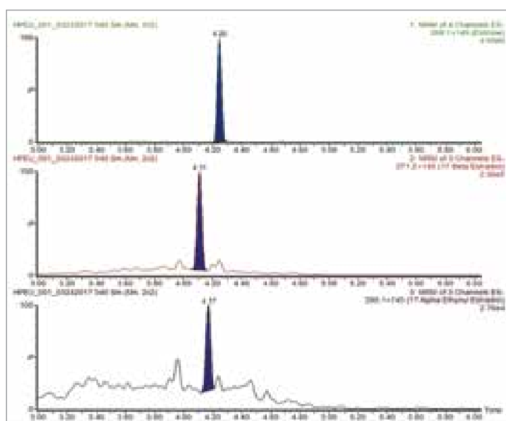

Figure 5. Sample pre-spiked (prior to extraction) with 17α-ethinylestradiol at 30pg/L , 17β-estradiol 120 pg/L and estrone 400 pg/L in surface water.

The recovery and repeatability data for the analytes, spiked at two levels in triplicate in surface water are detailed in Table 2.

The method showed high sensitivity, achieving the target European (2015/495/EU)[4] LLOQ (PtP s/n=10) levels for each compound in the surface water matrix. Low level concentrations of all compounds were detected in the final effluent matrix. A standard addition method was used to quantify the analytes present, where 17α-ethinylestradiol was measured at 16.9 pg/L, as shown in Figure 6.

Table 2. SPE method recovery and repeatability data for all analytes, spiked at two levels in triplicate into surface water matrix, pre-spiked levels are indicated in bold.

Compound	17α-Ethinylestradiol	17β-Estradiol	Estrone
Spike Level 1 (pg/L) (surface water)	**30.0**	**120.0**	**400.0**
% Recovery (n=3)	80.0	99.5	92.2
% RSD (n=3)	10.8	4.0	1.6
Spike Level 2 (pg/L) (surface water)	**60.0**	**300.0**	**1000**
% Recovery (n=3)	71.3	100.6	92.6
% RSD (n=3)	12.3	4.6	1.9

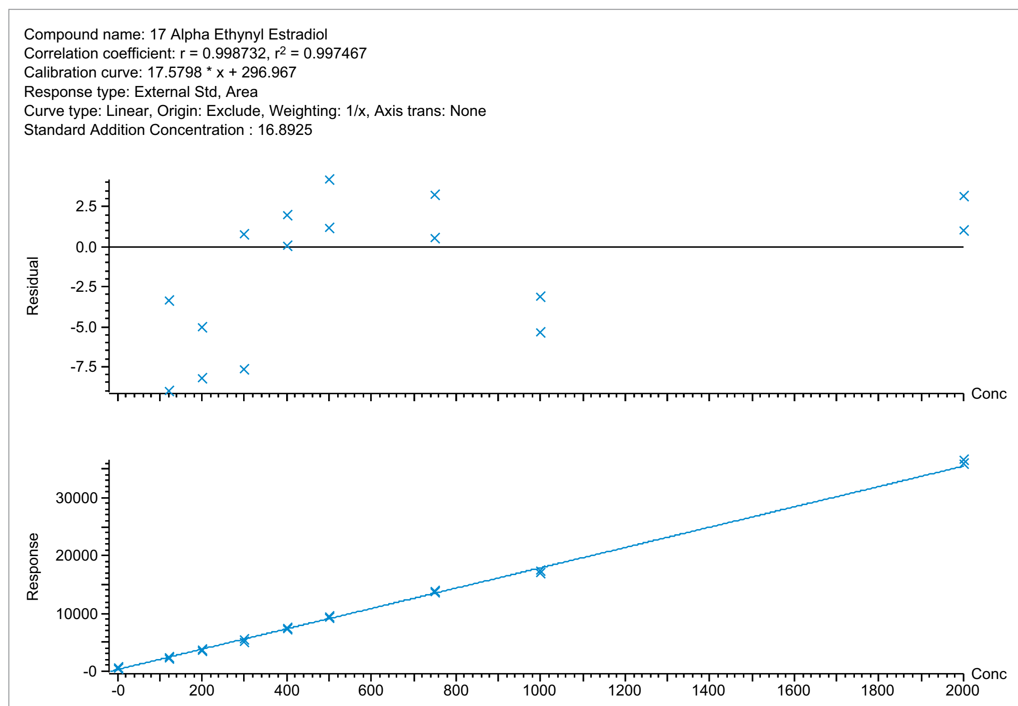

Figure 6. Standard addition method used to calculate low level concentration of 17α-ethinylestradiol in final effluent (n=2).

CONCLUSIONS

This method highlights the analysis of low ppq levels of synthetic estrogens in surface and final effluent waters. Utilizing Oasis SPE and Sep-Pak SPE sample preparation technologies, the optimized extraction method was found to provide the required concentration and clean-up, giving acceptable recoveries and repeatability in spiked surface water samples. The use of a large volume injection in combination with the ACQUITY UPLC H-Class and Xevo TQ-XS, allowed for the challenging detection requirements of this analysis to be achieved in surface water. The use of standard addition allowed for accurate quantitation of trace residues in final effluent samples.

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APGC-MS/MS Investigation of a Complex Mixture of Polyhalogenated Dioxins and Furans (PXDD/Fs) Generated in Fire Debris

Kari Organtini,[1] Adam Ladak,[1] Douglas Stevens,[1] Lauren Mullin,[1] Frank Dorman[2]
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[2]The Pennsylvania State University, University Park, PA, USA

APPLICATION BENEFITS

Using atmospheric pressure gas chromatography in combination with triple quadrupole mass spectrometry provides:

- Highly sensitive analysis for trace level components in a complex sample matrix.
- Increased ease-of-use and sensitivity over the more traditionally used magnetic sector instruments.
- Selectivity of the PXDD/F isomers from similar persistent organic compounds in the matrix.
- Understanding the level of exposure of first responders, especially fire fighters, to potentially toxic dioxins and furans that are currently unregulated.

WATERS SOLUTIONS

Atmospheric Pressure Gas Chromatography (APGC)

Xevo® TQ-S

MassLynx® MS Software

KEYWORDS

Dibenzo-p-dioxin, Dibenzofuran, PXDD, PXDF, mixed halogen, APGC, Atmospheric Pressure Gas Chromatography, TQ-S

INTRODUCTION

In the 15 years following the September 11th attacks on the World Trade Center, studies have revealed an overwhelming increase of 19% in the total cancer rates of firefighters exposed to the WTC debris.[1] Separate studies have also uncovered an overall increase in cancer rates of firefighters when compared to the general population.[2] First responders exposed to fire debris, either during an active fire or after the flames have been extinguished are exposed to a very complex mixture of compounds, including some potentially toxic compounds. Some of these compounds are combustion byproducts of flame retardants present in the materials burning in the fire.

Among some of the most used flame retardants are the brominated flame retardants (BFRs), specifically polybrominated diphenyl ethers (PBDEs). Although some PBDE formulations are being phased out of use, not all have been replaced and many consumer products in use still contain PBDEs. Studies have determined that when combusted, PBDEs create the dibenzo-p-dioxin and dibenzofuran backbones, substituted with halogens (Br and/or Cl) present in the combusted materials.[3-4]

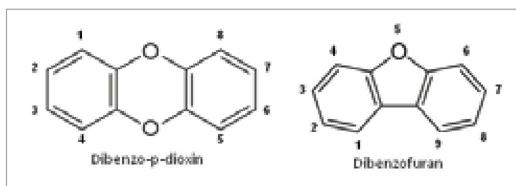

Figure 1. Structures of dibenzo-p-dioxin and dibenzofuran backbone. Halogen substitution can occur at any of the open numbered positions.

Currently, only 17 chlorinated dioxins and furans (PCDD/Fs) are regulated and routinely monitored. These monitoring methods exclude the polybrominated (PBDD/Fs) and mixed bromo-chloro (PXDD/Fs) congeners. Toxicities of the PBDD/Fs and PXDD/Fs may be equivalent or even exceed that of the most toxic PCDD/Fs, specifically 2,3,7,8-TCDD. Therefore, the potential toxicity of samples may not be fully represented using current regulations.

Traditionally, dioxin analysis is performed using a magnetic sector instrument that utilizes electron impact (EI) gas chromatography. However, in order to take advantage of the sensitivity the magnetic sector offers, only a limited number of compounds can be targeted in a single run. To be able to target the possible thousands of dioxin and furan congeners that can be formed, a tandem quadrupole mass spectrometer can be used. Waters® Xevo TQ-S is capable of maintaining the sensitivity and scanning speed needed to cover the wide range of MRM transitions required for this analysis.

Additionally, the emergence of softer ionization techniques, such as APGC, in combination with time-of-flight or tandem quadrupole mass spectrometry (APGC-MS/MS) has been shown to be selective and sensitive enough to enable routine analysis of these compounds.

In this application note, we focus on the combination of APGC coupled to a Xevo TQ-S tandem quadrupole instrument to better characterize the extent of dioxin and furan generation (polybromo- and mixed halogenated) in simulation fire debris in order to gain a better understanding of the levels of these compounds that first responders are exposed to.

EXPERIMENTAL

Sample preparation

Fire debris samples were generated at the Fire and Emergency Services Training Institute (FESTI) in Toronto, Ontario, Canada. Both a household fire (consisting of household furniture) and an electronics fire (consisting of electronics typically contained in an office) were simulated. Multiple samples from different locations in the fire debris were collected after the fires were extinguished. Samples collected included pieces of debris and ash, as well as wipes taken from the walls of the burn cell and firefighter equipment. Samples were extracted at The Pennsylvania State University using the Ontario Ministry of Environment and Climate Change (MOECC) method E3418.[5] Briefly, samples were extracted in hexane over a 24 hr period using a Soxhlet apparatus. Prior to extraction, the samples were fortified with a mixture of 13C labeled internal standards (Table 1). Following extraction, samples were concentrated and subjected to a two-stage column cleanup process: (a) acid-base silica (b) 5% carbon/silica (w/w). Prior to injection on the APGC-MS/MS, the extracts were concentrated to 100 µL using a rotary evaporator.

Table 1. List of the 13C labeled internal standards fortified into fire debris samples prior to extraction.

Dibenzofurans	Dibenzo-p-dioxins
$^{13}C_{12}$-2,3,7,8-tetrachloro	$^{13}C_{12}$-2,3,7,8-tetrachloro
$^{13}C_{12}$-3-bromo-2,7,8-trichloro	
$^{13}C_{12}$-2,3-dibromo-7,8-dichloro	^{13}C-2,3-dibromo-7,8-dichloro
	$^{13}C_{12}$-2,3,7,8-tetrabromo
$^{13}C_{12}$-1,2,3,7,8-pentachloro	$^{13}C_{12}$-1,2,3,7,8-pentachloro
$^{13}C_{12}$-2,3,4,7,8-pentachloro	
$^{13}C_{12}$-1,2,3,4,7,8-hexachloro	$^{13}C_{12}$-1,2,3,4,7,8-hexachloro
$^{13}C_{12}$-1,2,3,6,7,8-hexachloro	$^{13}C_{12}$-1,2,3,6,7,8-hexachloro
$^{13}C_{12}$-1,2,3,7,8,9-hexachloro	
$^{13}C_{12}$-2,3,4,6,7,8-hexachloro	
$^{13}C_{12}$-1,2,3,4,6,7,8-heptachloro	$^{13}C_{12}$-1,2,3,4,6,7,8-heptachloro
$^{13}C_{12}$-1,2,3,4,7,8,9-heptachloro	
	$^{13}C_{12}$-octachloro

A variety of MRM transitions were monitored for each PXDD, PXDF, PBDD, and PBDF congener class (3 to 6 transitions each). Two separate methods were created, with the PXDD and PXDF compounds in one method, and the PBDD and PBDF compounds in a separate method. The PXDD/F method contained approximately 150 total MRM transitions monitoring 40 different native and labeled groups of congeners, while the PBDD/F method had approximately 50 MRM transitions monitoring 17 different congener groups. Complete MRM information for all of the compounds is detailed in the Appendix. The method information can also be found in the APGC Quanpedia™ database.

APGC conditions

Column:	60 m x 0.18 mm x 0.10 µm Rtx Dioxin-2 1.0 m x 0.32 mm stainless steel Sulfinert tubing coupled to column exit through transfer line
Carrier gas:	Helium
Injection mode:	Splitless
Injector liner:	4.0 mm drilled hole Uniliner
Injector temp.:	290 °C
Injection volume:	0.5 µL
Flow rate:	1.1 mL/min
Oven program:	120 °C for 1 min
	35 °C/min to 200 °C
	4.5 °C/min to 280 °C, hold 8 min
	20 °C/min to 330 °C/min, hold 15 min

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	APGC positive ion mode
Ionization mechanism:	Charge transfer (dry)
Source temp.:	150 °C
Auxiliary gas:	400 L/h
Collision gas:	0.18 mL/min
Cone gas:	Off for first 8 min of run
	215 L/h for rest of run
Corona current:	20 µA for first 8 min of run
	4.0 µA for rest of run
Transfer line temp.:	360 °C

RESULTS AND DISCUSSION

MRM METHOD DEVELOPMENT

Due to only a handful of PXDD/F standards being commercially available, MRMs were developed for a wide range of PXDD/F congeners using a previously characterized fire debris sample. Dioxin and furan molecules are known to preferentially lose a –COX (X = Br or Cl) fragment, while –COBr occurs more readily than –COCl when a mix of halogens are present on the DD or DF backbone. Therefore, this fragment was chosen for all compounds using the most abundant mass in the molecular ion cluster (M+2 or M+4) as the parent ion. The second transition selected for all compounds utilized the second most abundant molecular ion losing the COBr group. A variety of other fragments were used to develop other MRMs as well, as described in the Appendix.

IDENTIFICATION OF MIXED HALOGEN DIOXINS AND FURANS IN FIRE DEBRIS SAMPLES

Due to the enhanced sensitivity of the Xevo TQ-S, a large variety of PXDF congener groups were identified in both the household and electronics fire debris samples. The fire debris generated in the electronics fire contained the largest variety and highest concentrations of PXDFs. This is expected due to the large quantity of the types of flame retardants typically present in electronics products. Within each group of congeners identified in the samples a large number of potential isomers were observed, as shown in Figure 2. Each peak in Figure 2 represents an isomer of the Br_2Cl substitution pattern and as expected, the peaks were not fully resolved due to the immense complexity resulting from the large number of possible isomers created in the fire. Individual PXDD/Fs were generated in the samples in the parts per trillion (ppt) to parts per billion (ppb) range, so the additional sensitivity of the Xevo TQ-S is required to detect most of the compounds. Figure 2 also demonstrates the calculated signal-to-noise values for a selection of the peaks, ranging from 12 to 89.

Using a set of 13C labeled internal standards (Table 1), semi quantification of the identified PXDFs was performed. Semi quantification was performed because internal standards for every congener are not commercially available. As a result of the complexity of the chromatograms collected, quantification was performed as the sum of all of the individual peak areas comprising one congener group. For example, all peaks in Figure 2 were summed together and treated as one peak area. Table 2 summarizes the concentration ranges calculated in the samples collected from each fire simulation. The concentrations varied greatly among the samples.

From the household fire, particulate debris scraped from the door of the burn cell contained the highest concentrations of PXDFs. In the electronics fire, the highest concentrations of PXDFs were actually collected from a firefighter's helmet. These results seem to suggest that the polyhalogenated furans are more likely to partition into airborne particulate matter than to remain in the debris itself. The electronics fire debris also contained PXDDs in some of the samples. The PXDDs were at much lower concentrations than the PXDFs, and fewer congeners were identified. Among the dioxins identified were BrCl2, BrCl3, Br2Cl, Br2Cl2, Br2Cl3, and Br3Cl substituted as well as Br through Br5 substituted.

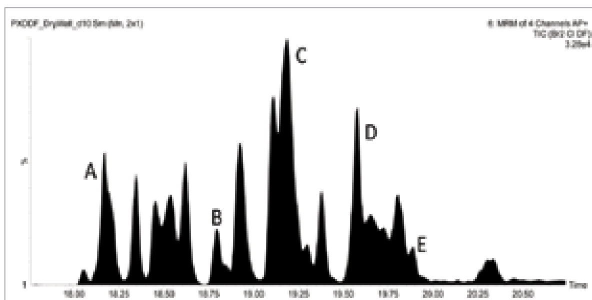

Figure 2. TIC of Br_2Cl dibenzofuran congener group in an electronics fire sample extract. The following S:N values were calculated for the labeled peaks: A. 46, B. 20, C. 89, D. 62, and E. 12.

Table 2. Range of concentrations (ng/g) of each PXDF identified in the various household and electronics fire debris samples. Results are semi-quantitative due to the complexity of each congener group and the commercial lack of internal standards. Concentrations are reported as the total concentration of all peaks present in the congener group. ND = not detected.

Compound	Household fire	Electronics fire
BrCl DF	0.01–1.42	0.10–21.48
$BrCl_2$ DF	0.01–0.76	0.26–10.30
$BrCl_3$ DF	0.01–0.58	0.48–10.05
Br_2Cl DF	ND–7.63	5.08–88.26
$BrCl_4$ DF	0.0008–0.07	0.09–5.05
Br_2Cl_2 DF	ND–5.11	3.53–103.56
Br_2Cl_3 DF	0.01–0.15	0.86–16.65
Br_3Cl DF	0.04–5.32	0.48–175.26
Br_3Cl_2 DF	ND–0.02	0.36–25.84
Br_4Cl DF	0.02–0.24	2.33–135.50
Br_4Cl_2 DF	ND–0.003	0.25–43.48
Br_5Cl DF	ND–0.01	0.77–56.62
Br DF	0.35–40.88	0.33–189.00
Br_2 DF	0.72–82.11	1.68–1468.09
Br_3 DF	0.64–50.40	4.60–6040.79
Br_4 DF	0.77–30.35	6.58–9254.41
Br_5 DF	0.39–7.40	2.88–2725.79
Br_6 DF	0.18–1.45	8.93–1560.32
Br_7 DF	ND	6.93–2349.78

Peak identifications were made based on retention time (R_t) match with a standard, if available. As the number of PXDD/Fs far outweighed the number of available standards, the remaining peaks were identified based on molecular ion isotope patterns, shown in Figure 3.

After the completion of the electronics fire, the firefighters' equipment was coated in black particulate debris. Wipes were taken from the helmets of the firefighters to determine what levels of polyhalogenated dioxins and furans were deposited on the firefighters' equipment. As mentioned previously, these samples turned out to contain the highest levels of PXDFs. These samples represent the fire debris and related particulate matter that first responders are directly exposed to. The tetra-halogenated congeners are typically considered the most toxic, especially when in the 2,3,7,8- substitution pattern. Figure 4 highlights the immense complexity of the helmet samples in each possible tetra-halogenated dibenzofuran group.

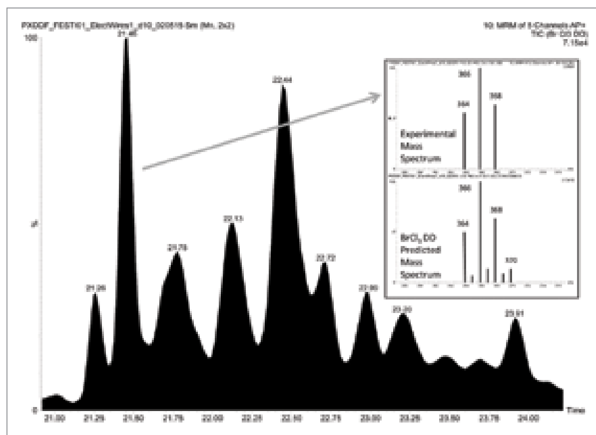

Figure 3. TIC of $BrCl_3$ dibenzo-p-dioxin congener group in an electronics fire sample extract. Inset shows the predicted and experimental isotope pattern match of the molecular ion.

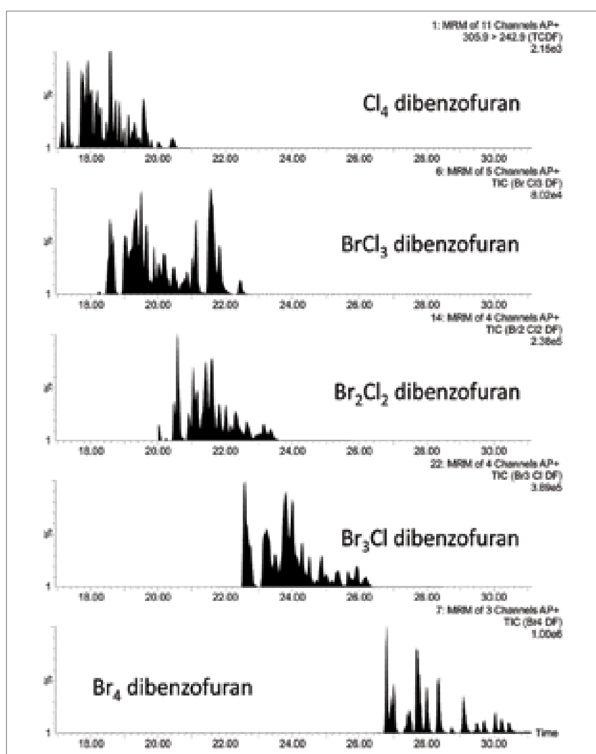

Figure 4. TIC traces of the tetra-halogen substituted dibenzofurans identified on a firefighter helmet.

CONCLUSIONS

The combination of APGC with the sensitivity of the Xevo TQ-S has allowed for the development of an extensive method for the analysis of polyhalogenated dioxins and furans in a complex sample matrix. Using this method, the analysis of simulated fire debris identified a large range of polybrominated and polyhalogenated dioxins and furans, ranging from mono- through hepta-substituted species. Semi-quantification revealed the total congener concentrations ranged from parts per trillion (ppt) to parts per million (ppm) levels, demonstrating the dynamic range of the analysis with a high level of sensitivity. The types of samples collected and evaluated provide valuable insights into the exposure of firefighters and first responders to these toxic compounds. The particulate samples, such as the samples collected off of the firefighter helmets, contained the highest levels of polyhalogenated dibenzofurans. Demonstrating the complexity of the type of debris first responders are directly exposed to holds implications that the current state of dioxin monitoring does not provide an accurate estimate of the toxicity of such samples. Analysis using APGC-MS/MS brings to light the complex nature of trace level mixed halogenated dioxins and furans present in these fire debris samples.

Acknowledgements

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APPENDIX

Appendix Table 1. MRM transitions and appropriate parameters for both PXDD/F and PBDD/F analysis.

Compound name	Start time (min)	End time (min)	Parent (*m/z*)	Daughter (*m/z*)	Cone voltage (V)	Collision energy (V)	Fragment
BrCl DF	12	16.3	279.9	172.9	30	40	-COBr
			281.9	137.9		50	-COBrCl
				172.9		40	-COBr
BrCl DD	13.75	18.75	295.9	188.9	30	40	-COBr
			297.9	125.9		50	-$(CO)_2BrCl$
				188.9		40	-COBr
				190.9		40	-COBr
$BrCl_2$ DF	15.25	19.75	313.9	206.9	30	40	-COBr
			315.9	171.9		55	-COBrCl
				206.9		40	-COBr
				208.9		40	-COBr
^{13}C-TCDF	18.5	20.25	315.9	251.9	30	35	-^{13}COCl
			317.9	253.9		35	-^{13}COCl
^{37}Cl-TCDD	18.5	20.25	327.9	262.9	30	35	-^{13}CO^{37}Cl
			329.87	264.87		35	-^{13}CO^{37}Cl
^{13}C-TCDD	18.5	20.25	331.9	267.9	30	35	-^{13}COCl
			333.9	269.9		35	-^{13}COCl
$BrCl_2$ DD	15.75	19.25	329.9	222.9	30	40	-COBr
			331.9	159.9		50	-$(CO)_2BrCl$
				161.9		50	-$(CO)_2BrCl$
				222.9		40	-COBr
				224.9		40	-COBr
$BrCl_3$ DF	18.2	22.75	347.8	240.8	30	40	-COBr
			349.8	170.8		55	-$COBrCl_2$
				205.8		50	-COBrCl
				240.8		40	-COBr
				242.8		40	-COBr
^{13}C-PeCDF	21.25	23.75	349.9	285.9	30	35	-^{13}COCl
			351.9	287.9		35	-^{13}COCl
^{13}C-PeCDD	21.25	23.75	365.9	301.9	30	35	-^{13}COCl
			367.9	303.9		35	-^{13}COCl
Br_2Cl DF	17.25	20.5	357.8	250.8	30	40	-COBr
			359.8	136.8		55	-$COBr_2Cl$
				215.8		50	-COBrCl
				250.8		40	-COBr
				252.8		40	-COBr
^{13}C-$BrCl_3$ DF	19.75	22.75	359.9	251.9	30	40	-^{13}COBr
			361.9	253.9		40	-^{13}COBr
$BrCl_3$ DD	18.75	24.25	363.8	256.8	30	40	-COBr
			365.8	193.8		50	-$(CO)_2BrCl$
				258.8		40	-COBr
			367.8	258.8		40	-COBr
				260.8		40	-COBr
Br_2Cl DD	17.75	23.75	373.8	203.8	30	50	-$(CO)_2BrCl$
				266.8		40	-COBr
			375.8	266.8		40	-COBr
				268.8		40	-COBr
$BrCl_4$ DF	20.75	27.75	381.8	274.8	30	40	-COBr
			383.8	239.8		50	-COBrCl
				276.8		40	-COBr
			385.8	278.8		40	-COBr

Compound name	Start time (min)	End time (min)	Parent (m/z)	Daughter (m/z)	Cone voltage (V)	Collision energy (V)	Fragment
^{13}C-HxCDF	25	28.75	385.8	321.8	30	35	$-^{13}$COCl
			387.8	323.8		35	$-^{13}$COCl
^{13}C-HxCDD	25	28.75	401.8	337.8	30	35	$-^{13}$COCl
			403.8	339.8		35	$-^{13}$COCl
Br_2Cl_2 DF	20	25.75	391.8	170.8	30	55	$-COBr_2Cl$
				284.8		40	-COBr
			393.8	286.8		40	-COBr
			395.8	288.8		40	-COBr
$BrCl_4$ DD	23.25	29.75	397.8	290.8	30	40	-COBr
			399.8	227.8		50	$-(CO)_2BrCl$
				290.8		40	-COBr
			401.8	292.8		40	-COBr
^{13}C-Br_2Cl_2 DF	22	24.5	405.8	297.8	30	40	$-^{13}$COBr
			407.8	297.8		40	$-^{13}$COBr
Br_2Cl_2 DD	21.75	26.75	407.8	300.8	30	40	-COBr
			409.8	239.8		50	$-(CO)_2BrCl$
				300.8		40	-COBr
			411.8	302.8		40	-COBr
				304.8		40	-COBr
^{13}C-HpCDF	29.75	32.75	419.8	355.8	30	35	$-^{13}$COCl
			421.8	357.8		35	$-^{13}$COCl
^{13}C-HpCDD	29.75	32.75	435.8	371.8	30	35	$-^{13}$COCl
			437.8	373.8		35	$-^{13}$COCl
^{13}C-Br_2Cl_2 DD	22.75	24.75	421.8	313.8	30	40	$-^{13}$COBr
			423.8	313.8		40	$-^{13}$COBr
Br_2Cl_3 DF	25	29.75	425.8	318.8	30	40	-COBr
			427.8	204.8		55	$-COBr_2Cl$
				320.8		40	-COBr
			429.8	285.8		50	-COBrCl
				322.8		40	-COBr
$BrCl_5$ DD	28.75	32.25	433.8	263.8	30	50	$-(CO)_2BrCl$
				324.8		40	-COBr
			435.8	324.8		40	-COBr
				326.8		40	-COBr
Br_3Cl DF	21.75	28.5	435.8	328.8	30	40	-COBr
			437.8	330.8		40	-COBr
			439.8	295.8		50	-COBrCl
				330.8		40	-COBr
Br_2Cl_3 DD	26.75	32.75	443.8	326.8	30	40	-BrCl
			445.8	273.8		50	$-(CO)_2BrCl$
				336.8		40	-COBr
			447.8	336.8		40	-COBr
				338.8		40	-COBr
Br_3Cl DD	22.75	27.75	453.7	346.7	30	40	-COBr
			455.7	283.7		50	$-(CO)_2BrCl$
				346.7		40	-COBr
			457.7	348.7		40	-COBr
$BrCl_6$ DD	30.25	35.25	467.7	297.7	30	50	$-(CO)_2BrCl$
				360.7		40	-COBr
			469.7	299.7		50	$-(CO)_2BrCl$
				362.7		40	-COBr
Br_3Cl_2 DF	28.25	32.25	469.7	362.7	30	40	-COBr
			471.7	283.7		45	$-COBr_2$
				364.7		40	-COBr
			473.7	366.7		40	-COBr
^{13}C-OCDD	33.75	35.5	469.7	405.7	30	35	$-^{13}$COCl
			471.7	407.7		35	$-^{13}$COCl

Compound name	Start time (min)	End time (min)	Parent (*m/z*)	Daughter (*m/z*)	Cone voltage (V)	Collision energy (V)	Fragment
Br_3Cl_2 DD	28	35.75	487.7	317.7	30	50	$-(CO)_2BrCl$
				380.7		40	-COBr
			489.7	380.7		40	-COBr
				382.7		40	-COBr
$BrCl_7$ DD	34.25	37.75	501.7	331.7	30	50	$-(CO)_2BrCl$
				394.7		40	-COBr
			503.7	333.7		50	$-(CO)_2BrCl$
				396.7		40	-COBr
			505.7	398.7		40	-COBr
^{13}C-TBDD	29.25	30.75	509.7	401.7	30	40	-COBr
			511.7	403.7		40	-COBr
Br_4Cl DF	30.75	34.25	515.7	408.7	30	40	-COBr
			517.7	329.7		50	$-COBr_2$
				408.7		40	-COBr
			519.7	412.7		40	-COBr
Br_4Cl DD	30.5	38	531.7	424.7	30	40	-COBr
			533.7	282.8		55	$-(CO)_2$Br2Cl
				284.8		55	$-(CO)_2Br_2Cl$
				363.8		50	$-(CO)_2BrCl$
				424.7		40	-COBr
			535.7	426.7		40	-COBr
Br_4Cl_2 DF	32.5	36.5	549.6	442.6	30	40	-COBr
			551.6	444.6		40	-COBr
			553.6	365.6		50	$-COBr_2$
				444.6		40	-COBr
Br_4Cl_2 DD	31.5	38	565.6	458.6	30	40	-COBr
			567.6	397.6		50	$-(CO)_2BrCl$
				458.6		40	-COBr
			569.6	316.6		55	$-(CO)_2Br_2Cl$
				460.6		40	-COBr
				462.6		40	-COBr
Br_5Cl DF	35.75	38.75	595.6	488.6	30	40	-COBr
			597.6	409.6		50	$-COBr_2$
				490.6		40	-COBr
			599.6	492.6		40	-COBr
Br_5Cl DD	35	42	611.6	504.6	30	40	-COBr
			613.6	360.6		55	$-(CO)_2Br_2Cl$
				441.6		50	$-(CO)_2BrCl$
				504.6		40	-COBr
			615.6	502.6		40	-BrCl
				506.6		40	-COBr
Br DF	10	12	245.9	138.9	30	40	-COBr
			247.9	138.9		40	-COBr
Br DD	10.5	12.5	261.9	154.9	30	40	-COBr
			263.9	126.9		50	$-(CO)_2Br_2$
				154.9		40	-COBr
Br_2 DF	14	17	323.9	216.9	30	40	-COBr
			325.9	137.9		50	$-COBr_2$
				218.9		40	-COBr
Br_2 DD	14	20	339.9	232.9	30	40	-COBr
			341.9	206.9		50	$-(CO)_2Br_2$
				234.9		40	-COBr
Br_3 DF	19	23	403.8	215.8	30	50	$-COBr_2$
				296.8		40	-COBr
			405.8	298.8		40	-COBr
Br_3 DD	20	27	419.8	312.8	30	40	-COBr
			421.8	286.8		50	$-(CO)_2Br_2$
				314.8		40	-COBr

Compound name	Start time (min)	End time (min)	Parent (*m/z*)	Daughter (*m/z*)	Cone voltage (V)	Collision energy (V)	Fragment
Br_4 DF	26.5	32.5	481.7	374.7	30	40	-COBr
			483.7	295.7		50	$-COBr_2$
				376.7		40	-COBr
Br_4 DD	26.5	33	497.7	390.7	30	40	-COBr
			499.7	364.7		50	$-(CO)_2Br_2$
				392.7		40	-COBr
Br_5 DF	30.5	38.5	561.6	373.6	30	50	$-COBr_2$
				454.6		40	-COBr
			563.6	456.6		40	-COBr
Br_5 DD	31	39	577.6	442.6	30	50	$-(CO)_2Br_2$
				470.6		40	-COBr
			579.6	472.6		40	-COBr
Br_6 DF	35	44.5	639.5	532.5	30	40	-COBr
			641.5	453.5		50	$-COBr_2$
				534.5		40	-COBr
Br_6 DD	35	44.5	655.5	548.5	30	40	-COBr
			657.5	522.5		50	$-(CO)_2Br_2$
				550.5		40	-COBr
Br_7 DF	43	61	719.4	531.4	30	50	$-COBr_2$
				612.4		40	-COBr
			721.4	614.4		40	-COBr
Br_7 DD	43	61	735.4	600.4	30	50	$-(CO)_2Br_2$
				628.4		40	-COBr
			737.4	630.4		40	-COBr
Br_8 DF	48	61.56	797.3	690.3	30	40	-COBr
			799.3	611.3		50	$-COBr_2$
				692.3		40	-COBr
Br_8 DD	50	61.56	813.3	706.3	30	40	-COBr
			815.3	680.3		50	$-(CO)_2Br_2$
				708.3		40	-COBr

An Untargeted Exposure Study of Small Isolated Populations Using Atmospheric Gas Chromatography Coupled with High Resolution Mass Spectrometry

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APPLICATION BENEFITS

- Generation of accurate mass measurements of low- and high-energy spectra allows targeted and untargeted data analysis in a single data set.
- "Soft" ionization using APGC results in preservation of the molecular ion. That combined with fragmentation after ionization produces comprehensive spectral details.
- Integrated MVA and elucidation tools aid in identification of markers of interest with automatic elemental composition, searching of online databases, and structural assignments.

WATERS SOLUTIONS

Xevo® G2-XS QTof Mass Spectrometry

Atmospheric Pressure Gas Chromatography (APGC)

Progenesis® QI Data Analysis Software

UNIFI® Scientific Information System

KEYWORDS

persistent organic pollutants, POPs, dioxins, PCBs, PAHs, MS^E, HRMS, APGC, universal source, MVA, exposomics

INTRODUCTION

Human exposure to environmental contaminants has been linked to various health problems. When analyzing known environmental contaminants of interest such as persistent organic pollutants (POPs) including dioxins, PCBs, and PAHs, targeted mass spectrometry methods are employed. Recently studies have been conducted using a metabolomic approach to determine differences of exposure between different populations. The term "exposomics" refers to studies that look at a wide array of contaminants in humans that may pose health risks.

In this study, pooled plasma samples from individuals living in various small isolated coastal communities were analyzed using an exposomics approach to determine whether differences exist between the communities with regard to families and concentrations of contaminants. Samples were analyzed using atmospheric pressure chemical ionization gas chromatography (APGC) coupled to high resolution mass spectrometry (HRMS), operated in data independent acquisition (DIA) mode, where precursor and fragment information were collected in a single run.

One of the major challenges of this type of study is interpreting the massive amounts of data generated. In order to facilitate data interpretation, Waters® Progenesis QI data analysis software was utilized. First, targeted analysis was performed against a defined contaminants database. Then, multi variant analysis (MVA) was carried out to determine any differences between the communities. Elucidation of unknown contaminants was also achieved using Progenesis QI Software, which involved searching online databases and matching structural information to the high energy data. Finally, confirmation of one of the findings was performed using a standard.

EXPERIMENTAL

Sample preparation

2 mL of plasma was taken and spiked with ^{13}C internal standard and mixed with ethanol and saturated ammonium sulphate solution (for denaturation). The samples were then extracted with hexane. The extracts were evaporated and purified on a florisil column (1 g). POPs were eluted with 25% dichloromethane in hexane. Purified extracts can be concentrated up to 20 µL of hexane prior to GC-MS analysis. For this study, this protocol was suitable, since the goal was to study POPs and POP-like compounds and this protocol intends to extract and purify contaminants related to the chemical property of POP's, such as non-polar lipophilic molecules.

GC conditions

GC system: A7890

Column: Rtx-5MS (Restek)
0.25 µm x 0.25 mm 0.25 µm

Injection mode: Splitless

Liner: Gooseneck splitless, deactivated (Restek)

Column pneumatics: Constant flow

Column flow: 2 mL/min

Injector temp.: 280 °C

GC oven temp. ramp:

Temp. (°C)	Temp. ramp (°C/min)	Hold time (min)
80		1.00
125	25	0.00
340	8	8.00

Total run time: 37.7 min

MS conditions

MS system: Xevo G2-XS QTof

Ionization mode: API+

Acquisition mode: MS^E

Acquisition range: 50 to 1000 *m/z*

Collision energy (LE): 6 eV

Collision energy (HE): 30 to 75 eV

Scan time: 0.15 sec

Source temp.: 150 °C

Interface temp.: 310 °C

Corona current: 3.0 µA

Cone voltage: 30 V

Cone gas: 200 L/hr

Auxiliary gas: 250 L/hr

Make-up gas: 300 L/hr

Lock mass: Polysiloxane (281.0512 *m/z*)

Data management

UNIFI Scientific Information System

Progenesis QI

RESULTS AND DISCUSSION

Before analysis of the samples, a standard GC mixture was run on the system. The mixture contained chlorinated pesticides that have similar physiochemical properties to the POPs of interest. As they were acquired with APGC and MS^E the spectra produced were specific and well conserved. The low energy spectra showed little fragmentation compared to traditional EI+ analysis due to the soft ionization produced by APGC. The high energy spectra yield fragmentation information that can be utilized to perform structural verification. Figure 1 shows the results for hexachlorobenzene analyzed in the standard and illustrates the intense precursor (low energy) with good fragmentation (high energy) from this soft ionization technique.

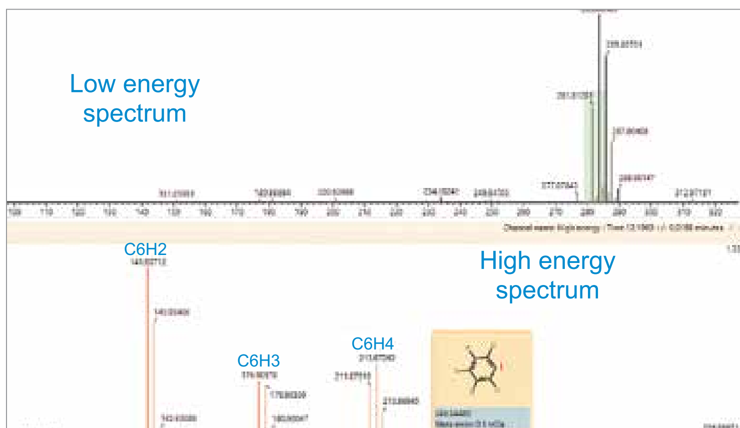

Figure 1. MS^E spectra for hexachlorobenzene showing low energy fragmentation of APGC.

Once the standard was run on the system and it was verified that the sensitivity and mass accuracy were as expected, the samples were analyzed. The samples were injected in triplicate. Normally in a metabolomics experiment the samples would be randomized to prevent any build-up of compounds by injecting the same sample and to account for any drop in instrument sensitivity over time. In this case the sample volumes were 20 µL in hexane which is volatile. If the samples were randomized after the first injection puncturing the vial septum the samples could have been concentrated due to solvent evaporation and bias the experimental results. For this reason it was decided to run the samples in series. Once the sample data was collected within UNIFI Software, it was transferred to Progenesis QI for data interpretation.

Upon import into Progenesis QI, the possible adducts that may have been present in the data set were selected. In this case the $M^{+\cdot}$ and the $(M+H)^+$ were selected due to the ionization mechanisms of APGC. The runs were then automatically aligned to account for any drift in retention time over long run periods such as in a metabolomics study. To ensure consistent peak picking and matching across all data files, an aggregate data set was created from the aligned runs. This contained peak information from all of the sample files, enabling detection of a single map of compound ions. This map was then applied to each sample, yielding 100% matching of peaks with no missing values aiding the multivariate statistical analysis.

TARGETED ANALYSIS

A MetaScope database containing precursor and fragment ion information for 98 expected compounds was searched for all samples present in the data set. The search parameters, shown in Figure 2, used a 5 ppm mass error, a 1-minute retention time window, and a 2 mDa fragment mass error to determine identifications. This yielded 24 positive results in the pooled plasma samples. Figure 3 shows the identifications and the sample in which the compound was found in the highest abundance. One of the things to note is that Community 1 seemed to have a high abundance of POP detections. This community may be of particular interest to investigate for the presence of other untargeted compounds.

Figure 2. MetaScope search parameters in Progenesis QI Software.

Table 1. Identification of 24 POPs from the manually created database showing the community that had the largest abundance.

Compound	Accepted ID	*m/z*	Retention time	Peak width	Identifications	Anova (p)	q Value	Max fold change	Highest mean
9.5_152.0626 *m/z*	Acenaphthylene	152.0626	9.50	0.33	1	0.002	0.004	1.836	Population 4
10.40_154.0771 *m/z*	Acenaphthylene	154.0771	10.40	0.09	1	0.658	0.666	11.849	Population 2
12.26_283.8095 *m/z*	Hexachlorobenzene	283.8095	12.26	0.11	1	6.39E-07	2.89E-06	2.502	Population 1
13.35_178.0775 *m/z*	Anthracene	178.0775	13.35	0.14	2	7.49E-05	1.99E-04	2.087	Population 5
16.67_202.0774 *m/z*	Florenthene	202.0774	16.67	0.08	1	3.00E-03	6.00E-03	2.073	Population 1
17.23_325.8795 *m/z*	PCB 99	325.8795	17.23	0.09	2	1.96E-04	4.64E-04	1.972	Population 1
17.28_202.0776 *m/z*	Florenthene	202.0776	17.28	0.09	1	1.60E-02	2.40E-02	1.989	Population 1
17.35_408.7827 *m/z*	trans-nonachlore	408.7827	17.35	0.14	1	4.86E-01	5.03E-01	1.757	Population 5
17.77_245.9996 *m/z*	o,p'-DDE	245.9996	17.77	0.13	1	4.84E-01	5.01E-01	2.183	Population 5
17.77_317.9344 *m/z*	p,p'-DDE	317.9344	17.77	0.15	1	4.75E-01	4.92E-01	2.313	Population 5
18.19_359.8417 *m/z*	PCB 138	359.8417	18.19	0.03	2	3.52-04	7.81E-04	Infinity	Population 1
18.56_325.8790 *m/z*	PCB 118	325.8790	18.56	0.09	2	2.50E-05	7.53E-05	2.602	Population 1
18.74_408.7820 *m/z*	cic-nonachlore	408.7820	18.74	0.07	1	2.10E-02	3.10E-02	2.212	Population 1
19.07_359.8402 *m/z*	PCB 153	359.8402	19.07	0.19	2	7.34E-07	3.25E-06	2.103	Population 5
19.58_235.0070 *m/z*	p,p'-DDT	235.0070	19.58	0.05	2	7.00E-02	8.90E-02	2.172	Population 1
19.61_359.8400 *m/z*	PCB 141	359.8400	19.61	0.2	3	4.07E-05	1.16E-04	1.869	Population 1
19.93_393.8011 *m/z*	PCB 187	393.8011	19.93	0.08	2	7.96E-04	2.00E-03	2.05	Population 1
20.79_393.8006 *m/z*	PCB 180	393.8006	20.79	0.07	4	4.15E-06	1.52E-05	2.875	Population 1
20.85_288.0929 *m/z*	Chrysene	288.0929	20.85	0.23	2	0.216	0.244	4.615	Population 1
21.07_393.8009 *m/z*	PCB 180	393.8009	21.07	0.11	2	2.61E-04	6.00E-04	1.888	Population 5
21.66_393.8006 *m/z*	PCB 170	393.8006	21.66	0.07	3	2.78E-04	6.34E-04	3.191	Population 1
23.21_563.6204 *m/z*	PBDE 99	563.6204	23.21	0.12	2	4.34E-06	1.58E-05	3.859	Population 1
26.97_276.0931 *m/z*	Benz(ghi)peryene	276.0931	26.97	0.15	1	8.70E-02	1.07E-01	30.756	Population 1
27.49_276.0926 *m/z*	Benz(ghi)peryene	276.0926	27.49	0.19	1	3.66E-01	3.98E-01	6.746	Population 1

p,p'-DDT, p,p'-DDE, and o,p'-DDE show a slight up regulation in Population 5; however the compounds were found to have no significant variation in concentration between the communities according to the p-values. Dichlorodiphenldichloroethylene (DDE) is formed by the dehyrdrohalogenation of dichlorodiphenyltrichloroethane (DDT). Due to DDT's historically wide use as an insecticide in agriculture, it is commonly seen in animal tissue as DDT is fat-soluble and bioaccumulative. It is also regularly found in fish that constitute a major part of the diet in these small communities.[2] DDT and DDE are endocrine disruptors and considered possible human carcinogens. DDE and DDT provide relevant POPs exposure markers in several populations, and therefore they are important to identify. This was possible using the targeted approach highlighted here.

The list of identified compounds was then subjected to a filter to show only the compounds that had a max fold change higher than 2, which highlighted the compounds that had significant differences between communities. This yielded a list of 11 compounds shown in Table 2. The up regulation of PCB 118 in Population 1 is shown in Figure 3. As Community 1 had the highest abundance of these target compounds, it was decided that further untargeted analysis should be performed on this community.

Table 2. List of identified compounds with a max fold change above 2.

Compound	Compound ID	Adducts	*m/z*	Retention time	Score	Fragment score	Mass error (ppm)	Anova (p)	q Value	Max fold change
12.26_283.8095 m/z	Hexachlorobenzene	MDot+	283.8095	12.26	29.10	0.0	-0.8	6.39E-07	2.89E-06	2.502
13.35_178.0775 m/z	Anthracene	MDot+	178.0775	13.35	35.60	0.0	0.2	7.49E-05	1.99E-04	2.087
16.67_202.0774 m/z	Florenthene	MDot+	202.0774	16.67	30.70	0.0	-0.7	3.00E-03	6.00E-03	2.073
18.19_359.8417 m/z	PCB 138	MDot+	359.8417	18.19	24.50	0.0	2.1	3.52-04	7.81E-04	Infinity
18.56_325.8790 m/z	PCB 118	MDot+	325.8790	18.56	46.00	65.1	-2.8	2.50E-05	7.53E-05	2.602
18.74_408.7820 m/z	cic-nonachlore	MDot+	408.7820	18.74	36.20	0.0	-3.8	2.10E-02	3.10E-02	2.212
19.07_359.8402 m/z	PCB 153	MDot+	359.8402	19.07	40.90	30.2	-2.3	7.34E-07	3.25E-06	2.103
19.93_393.8011 m/z	PCB 187	MDot+	393.8011	19.93	45.20	37.3	-2.3	7.96E-04	2.00E-03	2.05
20.79_393.8006 m/z	PCB 180	MDot+	393.8006	20.79	28.40	37.4	-3.6	4.15E-06	1.52E-05	2.875
21.07_393.8009 m/z	PCB 180	MDot+	393.8009	21.07	29.60	39.5	-3.7	2.61E-04	6.00E-04	1.888
23.21_563.6204 m/z	PBDE 99	MDot+	563.6204	23.21	44.80	91.4	-1.2	4.34E-06	1.58E-05	3.859

Figure 3. Detection of PCB 118 showing up regulated in Population 1.

UNTARGETED ANALYSIS

To investigate the data further all filters were removed. Progenesis QI software automatically generates a principle component analysis (PCA) plot that clearly depicts the separation of the communities (Figure 5). In order to perform further statistical tests the data was automatically exported to EZinfo. Community 1 was compared to all of the other communities using an orthogonal partial least squared discriminate analysis (OPLS-DA) model. This allowed an S-plot to be generated where significant compounds of interest could be identified at the extremes of the S-Plot. Figure 5 shows the S-plot generated from the OPLS-DA model. 17 significant markers were selected and imported directly into Progenesis QI.

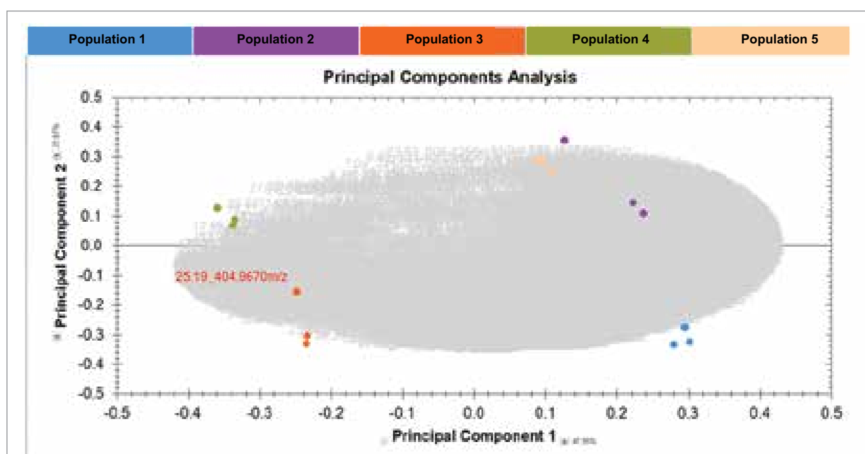

Figure 4. Principle component analysis (PCA) plot showing the separation of the three replicates of each community.

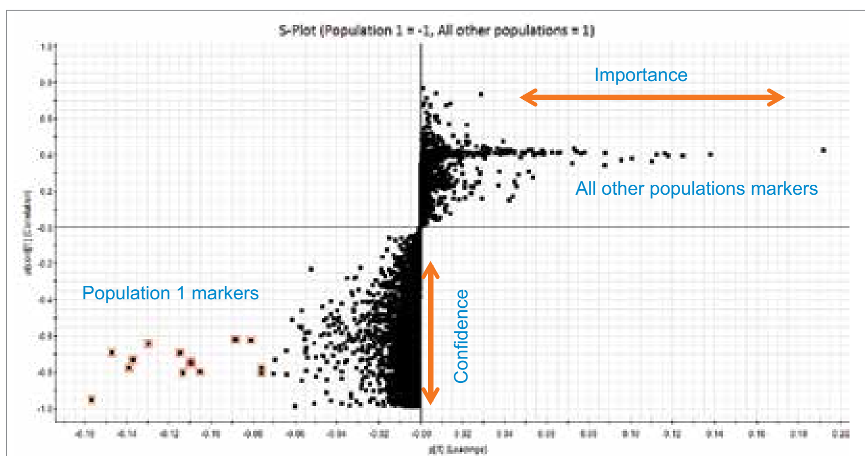

Figure 5. S-Plot showing significant markers of interest in Community 1.

The compounds of interest were then subjected to a database search. During this search the precursor accurate masses were searched against selected ChemSpider databases within a 5 ppm mass error. The structures of the possible compounds resulting from the ChemSpider search were then subjected to *in silico* fragmentation and compared to the experimental fragment peaks within the high energy spectra for the compound that was within a 10 ppm mass error. These results were then ranked using an accurate mass matching score and fragmentation score. This process was automatic and took less than 1 minute to complete. The search parameters are shown in Figure 6.

A number of interesting results were obtained from the ChemSpider database search, the first being tocopherol, which yielded a good fragmentation match where 37% of the fragments could be accounted for from the high energy spectrum. Tocopherols (TCPs) are a class of organic chemical compounds, many of which have Vitamin E activity. TCPs are found at high levels in vegetables and berries.[3] The isolated communities with a mostly vegetarian diet would explain the higher concentrations of TCPs in this population. The results from the database search are shown in Figure 7.

Edit ChemSpider Search Parameters

ChemSpider search parameters

Define a set of ChemSpider parameters that can be saved for later reuse. Learn more in the online reference.

Name:

Search

Required search parameters

Precursor tolerance: 5 ppm

Data sources: [1] KEGG, [2] NIST, [3] Human Metabolome Database, [4] Pesticide Common Names, [5] ChemSpiderman.

Select data sources...

Optional search parameters

Perform theoretical fragmentation

Fragment tolerance: 10 ppm

Filter by isotope similarity score

Isotope similarity: 90 %

Filter by elemental composition

Elemental composition: H: 0-500 C: 0-200 N: 0-10 O: 0-30 F: 0-2 S: 0-1 Cl: 0-10 Br: 0-10

Select elemental composition...

Figure 6. ChemSpider database search parameters.

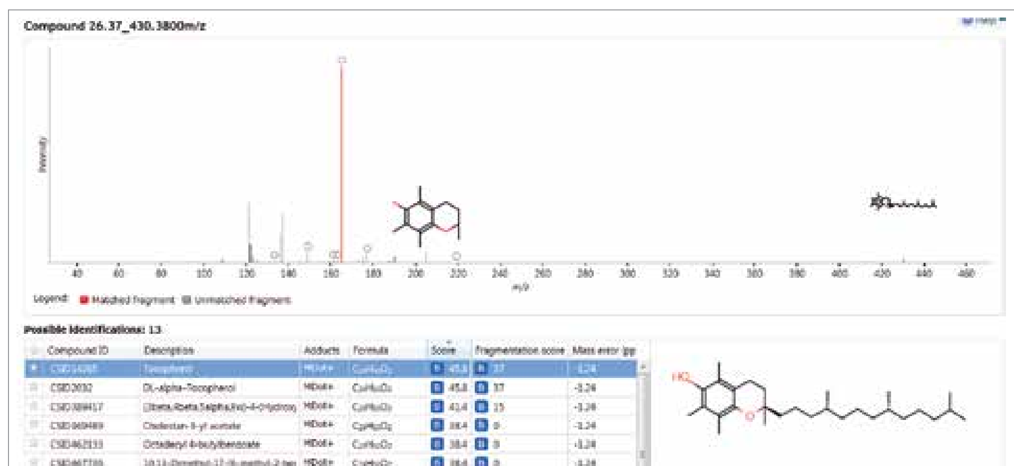

Figure 7. Possible identification for tocopherol in the database search results.

Another strong identification from the database search was 1,3-Benzothiazole. Benzothiazoles (BTHs) are a class of compounds that are produced in high volumes. They are used as corrosion inhibitors and found in rubber materials, herbicides, azo dyes, and food flavoring.[4] This finding is remarkable as these isolated communities would not directly be using materials containing BTH; hence it could be concluded that the exposure was due to environmental contamination. Another hypothesis is that Population 1 is the only population connected to modern food supply via a direct airport in the south. The presence of BTH may be a bio indicator of processed food consumption, as BTH is widely used as a freshness preservative in packaging. Both of these theories could be investigated further to verify the source of the BTH exposure. Figure 8 shows the possible identification results for 1,3-Benzothiazole.

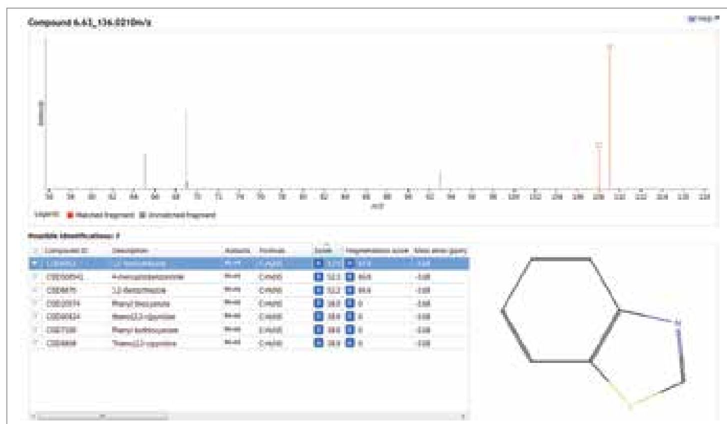

Figure 8. Possible identification for 1,3-Benzothiazole database search results.

As BTHs are a group of compounds, correlation analysis was performed using Progenesis QI. A dendrogram was automatically generated in the software showing the related compounds. A few compounds selected from the dendrogram were related to 1,3-Benzothiazole. The software visualization of these relationships is shown in Figure 9. These compounds were tagged and searched against the ChemSpider databases. This resulted in another possible identification of a thiazole compound, 4-phenyl-2-propyl-1,3-thiazole, (Figure 10).

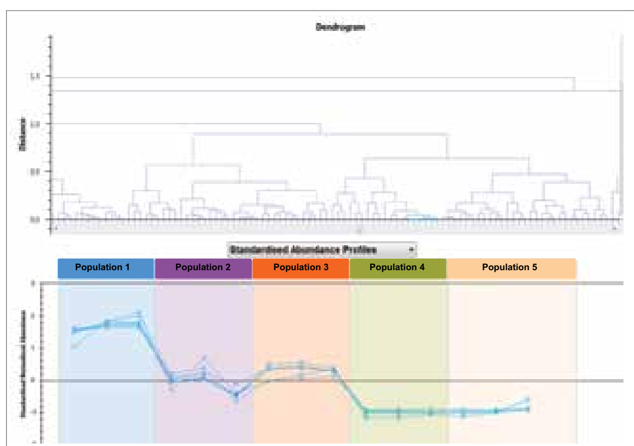

Figure 9. Dendrogram showing the relationship between compounds.

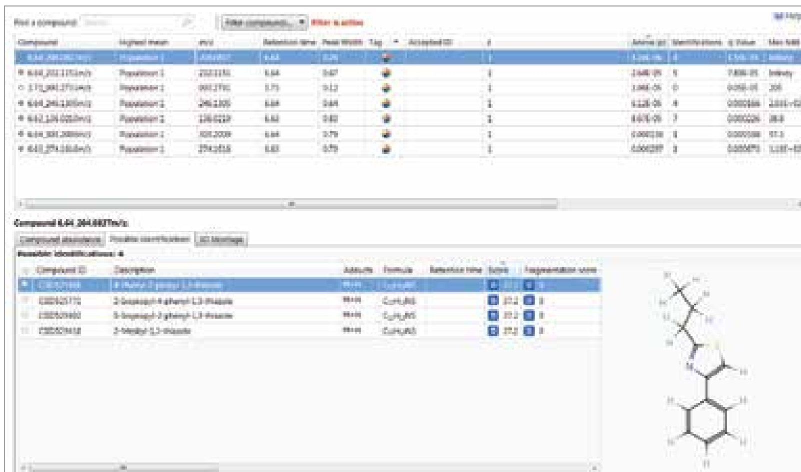

Figure 10. Possible identification of 4-phenyl-2-propyl-1,3-thiazole in the database search results.

CONFIMATION OF RESULTS

A standard of 1,3-Benzothiazole (BTH) was obtained in order to confirm the fragmentation pattern and the identification of this compound in the samples. A different GC method was employed for this analysis which was carried out at a later date than the initial analysis. The spectra from the standard matched that of the proposed identification of BTH from the ChemSpider search and the spectra from the sample. This allows the initial database to be updated to include BTH as a target exposure compound for further investigations of the population studies.

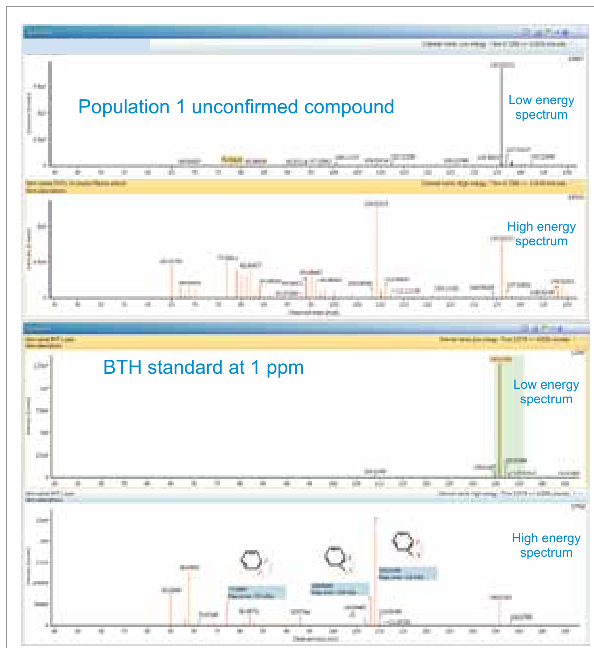

Figure 11. Comparison of the unknown compound spectra from Population 1 to a standard of BTH at 1 ppm.

CONCLUSIONS

Exposure studies involve complex data and subtle comparisons within the data sets. By utilizing the soft ionization of APGC and acquiring accurate mass data on both precursor and fragment ions in one method, a complete data set can be produced. This combined with the processing power of Progenesis QI Software allows complex statistical analysis to be performed quickly and easily. Progenesis Q1 also allows the searching of thousands of online databases and user generated libraries. This combination of hardware and software permits a simplified approach to exposomics workflows.

References

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Sensitive Analysis of Nodularin and Microcystins of Concern in Drinking Water Using Simplified Sample Preparation

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Increased sensitivity with reduced run time, minimized sample preparation and solvent consumption for microcystin analysis.

GOAL

To show nodularin and the major microcystins of concern in drinking water can be analyzed with minimized sample preparation and increased confidence in results.

BACKGROUND

There is an increased interest in the monitoring of microcystins that are generated by blue-green algae in drinking water in order to protect the public from exposure.[1] EPA Method 544, for instance, monitors for six microcystins and nodularin, and utilizes solid phase extraction (SPE) and LC-MS/MS to reach the minimum reporting level of 1 µg/L.[2]

One major challenge in using some current methods is they involve SPE extraction of 500 mL of water that is subsequently concentrated down to 1 mL. This process is time consuming as the loading and evaporation of the extract required to meet necessary detection levels can take hours. However, with less sensitive instrumentation, this is the only way that the challenging regulatory limits can be met.

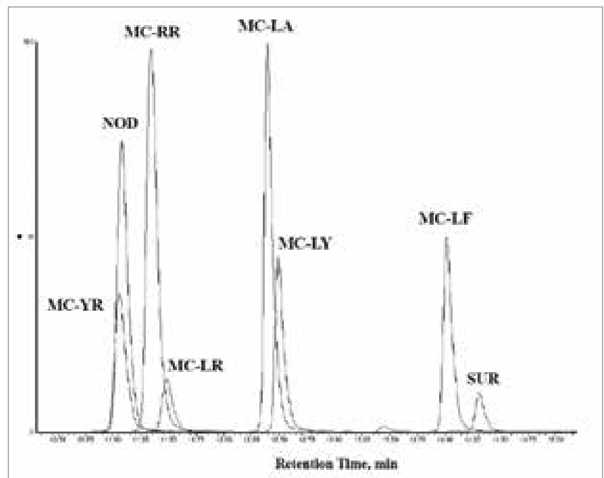


Figure 1. EPA Method 544 chromatographic separation example.[2]

Another challenge with the current method is the use of a single MRM transition for each analyte. This makes it difficult to confirm spurious results and can lead to re-analysis and delays in reporting results which are critical to ensure the public are not at risk from exposure. Having an analytical method that is more sensitive, with additional transitions and rapid run time provides multiple advantages in the targeted analysis of microcystins.

THE SOLUTION

In this work, the current EPA Method 544 was used as a starting point for method development. A Waters™ UHPLC column and the Waters Xevo™ TQ-S micro were used for this investigation. The CORTECS™ C_8 90Å, 2.7 µm, 2.1 mm x 100 mm Column (P/N 186008351) was used with a VanGuard™ C_8 90Å, 2.7 µm, 2.1 mm x 5 mm Cartridge (P/N 186008421) and holder (P/N 186007949) for the analysis. Chromatography was further optimized to improve separation between near eluting analytes. Table 1 shows the final chromatographic conditions utilized for this analysis. Figure 1 shows the separation defined in EPA Method 544 while Figure 2 shows the separation on the CORTECS™ Column. The method showed comparable separation to the current column used in EPA Method 544 and detection of the seven compounds of interest.

The seven compounds of interest were optimized on the Xevo TQ-S micro. An additional MRM transition was added for each compound. This allowed for further confirmation of the presence of the compound and verification of not only an additional transition but the ion ratios between the two transitions.

As the sensitivity of the Xevo TQ-S micro was excellent, no SPE or pre-concentration of drinking water was required for any of the work. While EPA Method 544 does not allow for the exclusion of SPE, this work does demonstrate that current generation tandem quads are able to meet the method's challenging detection requirements even without the enrichment provided by SPE sample preparation.

In order to assess the sensitivity of the method, a calibration curve was made of microcystin LR, YR, and RR compounds between 0.5 and 40 ppb in drinking water. The linearity and limit of detection were excellent as indicated by the R^2 values of >0.99 and %RSDs of less than 15%. Figure 3 shows the linearity of microcystin LR and Figure 4 shows the detection of microcystin LR 0.5 ppb.

Time	Flow (mL/min)	%A 20 mM Ammonium formate	%B Methanol	Curve
------	**0.3**	**90**	**10**	---
2	0.3	90	10	6
16	0.3	20	80	6
16.1	0.3	10	90	6
22	0.3	10	90	6
22.1	0.3	90	10	6
26	0.3	90	10	6

Table 1. LC gradient utilized for method. (as published in EPA Method 544).

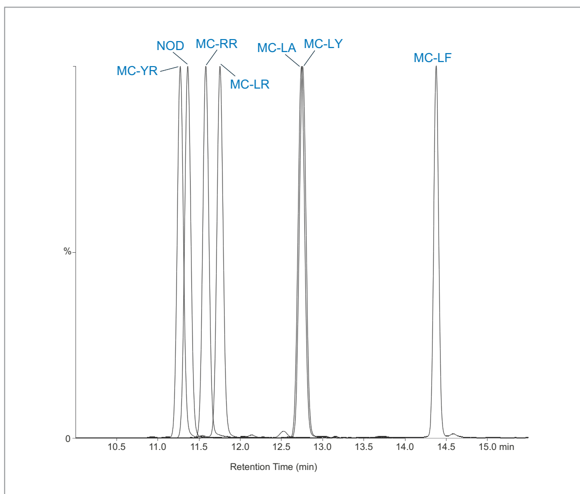


Figure 2. Standard between 40 and 60 µg/L showing separation of 6 microcystins and nodularin.

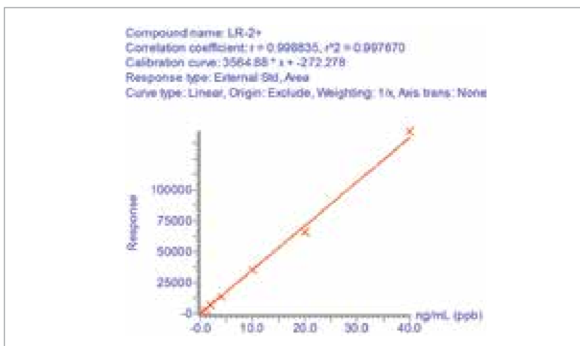


Figure 3. Linearity of microcystin LR between 0.5 µg/L and 40 µg/L .

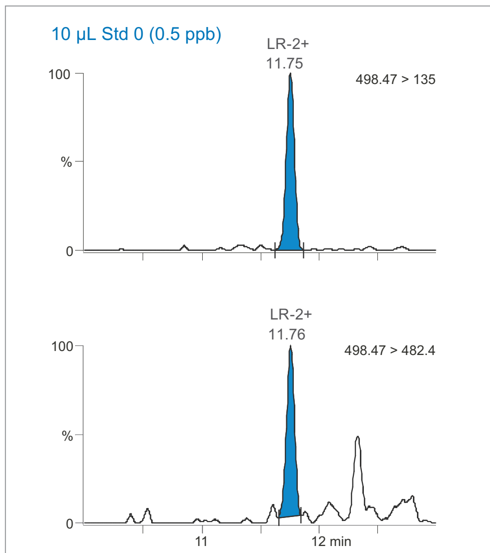
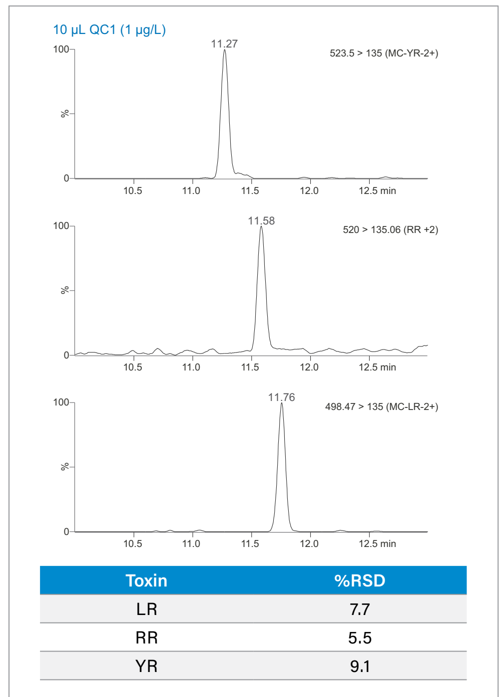


Figure 4. Detection of microcystin LR in drinking water at 0.5 µg/L with two transitions.

Finally, in order to ensure the method was reproducible, three example microcystins were spiked into a drinking water sample at 1 µg/L and injected 5 times. The % RSDs under 10% for the replicates fall within the requirements described in EPA Method 544.



Toxin	%RSD
LR	7.7
RR	5.5
YR	9.1

Figure 5. Reproducibility of microcystins at 1 ppb.

SUMMARY

The use of the CORTECS C_8 Column produces equivalent chromatographic separation within a shorter run time for the nodularin and the six microcystins investigated. Although EPA Method 544 does not allow for the exclusion of SPE, the increased sensitivity of the Xevo TQ-S micro allows the user to potentially eliminate SPE or use less water to concentrate while still meeting the challenging detection limit requirements for current analytical methods. The addition of a confirmatory MRM transition for each compound also ensures that the compound is accurately detected and reported.

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2. EPA Method 544. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=306953.

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Analysis of Microcystins RR, LY, and YR in Bottled, Tap, and Surface Water Using ACQUITY UPLC Systems with 2D-LC Technology

Claude Mallet
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Fast extraction protocol (15 min)
- Trace level detection (low ppt)
- 3D microextraction

WATERS SOLUTIONS

ACQUITY® UPLC® Systems with 2D-LC Technology

Xevo® TQD

Oasis® HLB SPE

KEYWORDS

Microcystin, water, microextraction, cyanobacteria, HABs

INTRODUCTION

Algae bloom is the result of a rapid accumulation of cyanobacteria in freshwater and other ecosystems. Their presence is predominantly linked to excess nutrients (fertilizers) from water runoff.[1] In some instances, harmful blooms can pose a serious health threat to humans and animals, and may also negatively impact several economic activities (fisheries, recreational parks, water treatment plants, etc). The health risk stems from the ability of cyanobacteria to produce neurotoxins, which through skin contact and water consumption can lead to several illnesses and even death.[2] Microcystins are the most detected of cyanotoxins and, in 1998, the World Health Organization (WHO set a guideline value of 1 ppb for total microcystin LR in drinking water.[3] While several analytical procedures can be found in the literature using various affinity techniques, liquid chromatography with mass spectrometry detection is the most common approach for the analysis of microcystins in water matrix. As seen in Figure 1, microcystin RR, LR, and YR share a common zwitterion backbone with a single R group. Their complex ring structure poses an additional level of difficulty because of a low abundance of fragment ions for MRM transitions. If a trace-level detection is required (sub ppb), it can be challenging to meet required guidelines in the analysis of microcystins in a water matrix.

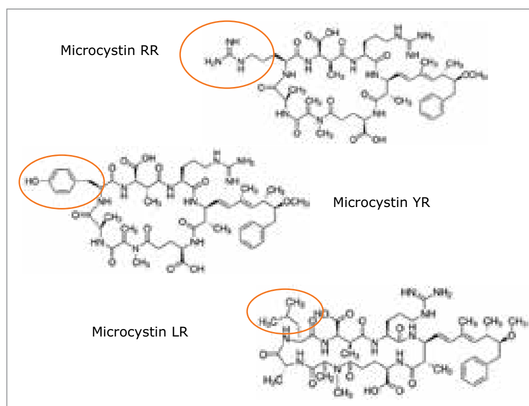

Figure 1. Chemical structures of microcystins RR, LR, and YR.

Water analysis brings a wide range of analytical challenges, especially during sample preparation. This is mainly due to its matrix complexity, from drinking water quality to waste water. As such, the removal of interferences and isolation of a target analyte usually requires extensive and laborious extraction protocols. If an extraction protocol fails to address the removal of interferences, it will ultimately lead to a high level of matrix co-elution in the final extract. As a consequence, the quantification will show poor recoveries, and detection will be affected by matrix effects. With trace-level requirements, an enrichment step is a necessity, thus creating a potential amplification effect.

Most extraction protocols designed for drinking water (low complexity) are ill equipped to produce acceptable results for surface water samples (high complexity). From this perspective, microextraction protocol can offer acceptable recoveries for a wide range of matrix diversity. ACQUITY UPLC Systems with 2D-LC Technology[4,5] offer the same analytical performance regarding recoveries, linearity, robustness, and lifetime, but at the microextraction level. The smaller sample volume allows faster loading time, by an average of less than 10 minutes. With the 2D's at-column dilution configuration, aqueous and organic extracts can be loaded and captured on a trap column with high efficiencies. The injection volume for this configuration is not a limitation, and gives the option to inject as much as needed to reach target detection limits.

In this application note, a sequential microextraction protocol was evaluated for the analysis of microcystin RR, LR, and YR in bottled, tap, and surface water. The entire extraction protocol was completed in less than 15 minutes.

EXPERIMENTAL

Two MRM transitions (quantification and confirmation) for all microcystins were selected and optimized. The MRM conditions are listed in Table 1. For this application, finding the optimum extraction and chromatographic condition for this multi-residue analysis poses a difficult challenge. As shown in Figure 1, the microcystins RR, LR, and YR have a zwitterionic structure (dipolar ion). The chromatographic conditions were tested on several trapping chemistries (Oasis® HLB, XBridge® C_{18}, and XBridge C_8) and separation chemistries (BEH C_{18} and HSS T3). The loading (low pH, high pH, and neutral pH) and eluting mobile phase (MeOH + 0.5% formic acid and ACN + 0.5 % formic acid) were also optimized using an automated process. The extraction process was performed using a reversed-phase sorbent with a 3-cc Oasis HLB SPE barrel using a sequential elution. The sorbent was conditioned by using 5 mL of methanol followed by 5 mL of water. The water samples (15 mL) were loaded at a flow rate of 10 mL/min. The cartridge was washed with 2 mL 10% acetonitrile with 1% formic acid. The microcystins were eluted with 1.5 mL of 50% acetonitrile with 1% formic acid. The internal standard was added at that step. From an acetonitrile stock solution of 1000 ppb, 15 µL of nodularin was added to the final extract (final IS concentration at 5 ppb).

Loading conditions

Column:	Oasis HLB 20 µm
Loading:	MilliQ Water (pH 7, no additives)
Flow rate:	2 mL/min
At-column dilution:	5% (0.1 mL/min pump A and 2 mL/min pump B)

UPLC conditions

UPLC system:	ACQUITY UPLC 2D-LC configured for "Trap and Elute" with AT-column dilution
Runtime:	10 min
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	60 °C
Mobile phase A:	Water + 0.5 % formic acid
Mobile phase B:	Acetonitrile + 0.5% formic acid
Elution:	5 min linear gradient from 5% (B) to 95% (B)
Flow rate:	0.500 mL/min (pump C)
Injection volume:	250 µL

MS conditions

MS system: Xevo TQD
Ionization mode: ESI+
Capillary voltage: 3.0 kV
Cone voltage: 90.0 V
Source temp.: 150 °C
Desolvation temp.: 550 °C
Desolvation gas: 1100 L/hr
Cone gas: 50 L/hr

Table 1. MRM transitions for microcystins RR, LR, and YR.

Microcystins	Ion mode	Precursor ion	Cone	Product ion	CE
Microcystin-RR	ESI +	520.0	50	135.2	50
				70.0	70
Microcystin-LR	ESI +	995.5	90	135.2	90
				86.0	100
Microcystin-YR	ESI +	1045.5	90	135.2	90
				70.0	100
Nodularin	ESI +	825.3	90	135.2	80
				70.0	90

RESULTS AND DISCUSSION

SEQUENTIAL MICROEXTRACTION PROTOCOL

The concept of sequential microextraction is designed to capture the retention behavior of a target analyte in response to various extraction parameters (sorbent strength, elution polarity, solubility, etc). By collating the results, optimized conditions can be selected to excise a region of interest during extraction. This approach is an added benefit when using a microextraction protocol. Within 60 minutes, several elution conditions (>20 cuts) can be performed, which is quite impractical to produce with a traditional large sample extraction protocol (too time-consuming). The sequential extraction begins with a water standard spiked of microcystin at 1 ppb. A 15-mL volume of water was loaded onto two Oasis HLB 3 cc SPE cartridges (See Figure 2).

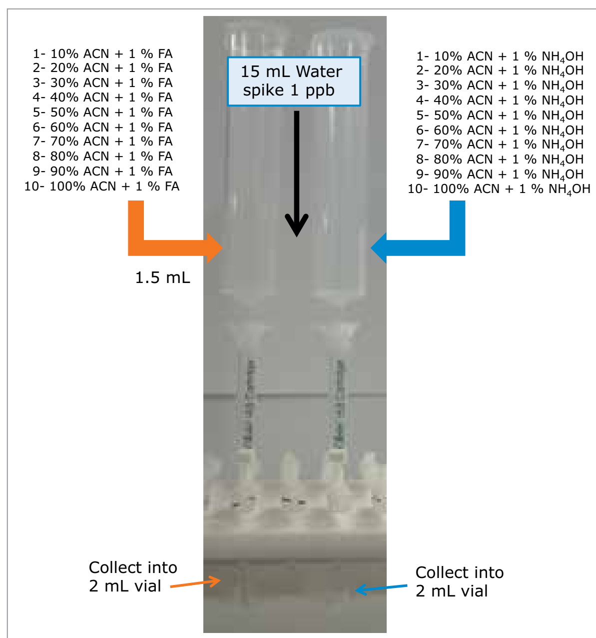

Figure 2. Sequential microextraction protocol with Oasis HLB.

Previously, a series of elution solutions were created by increasing the ratio of organic solvent-to-water. The incremental elution strength of these solutions reveals the chromatography profile of a target analyte. In this application, the elution solvent chosen was acetonitrile, with the incremental set from 10% up to 100% (increments of 10%). Since microcystins exhibit a zwitterionic structure (amine and carboxylic acid moities), two sets of elution solutions (first set added with 1% formic acid and second set added with 1% ammonium hydroxide) were created to evaluate the elution profile at pH 3 and pH 10. By neutralizing one functionality over the other, the sequential elution can display additional information as to which retention mechanism is used by the target analyte (weak vs strong or single vs dual). The sequential elution results for microcystin RR, LR, and YR are tabulated in Figure 3A, 3B, and 3C, respectively. The sequential elution for microcystin RR indicates a high polar nature due to the fact that the molecule is completely eluted off the HLB sorbent with only 20% acetonitrile (see Figure 3A).

When the elution profile for low pH and high pH are compared, microcystin RR was eluted in a single fraction (20% acetonitrile) under low pH conditions, but can be seen into the 20% and 30% fractions (50/50) under high-pH conditions. This elution behavior suggests that the acidic moities of the structure show a stronger retention on the polymer stationary phase. The retention profiles of microcystin LR and YR, however differ noticeably from microcystin RR. First, both LY and YR are eluted at higher organic fraction – in this instance about 95% was eluted at 40% acetonitrile under acidic conditions. This observation confirms the unique contribution of the R group for microcystin RR, LR, and YR (see Figure 1). With microcystin RR, the R group adds another amine functionality to the structure. As for microcystin LR and YR, their R groups are neutral moities, although the phenolic R group of microcystin YR could potentially create retention time or elution shift. Second, under basic elution, both LR and YR were eluted in lower organic fractions (20% acetonitrile at 95% recovery). No signals were measured in organic fractions higher than 40%. These results offer either a collective or fractionation elution option. In this application, the collective elution of all three microcystins was selected and the elution was performed by selecting the 10% acetonitrile with 1% formic acid for the minimum cut, and 50% acetonitrile with 1% formic acid for the maximum cut.

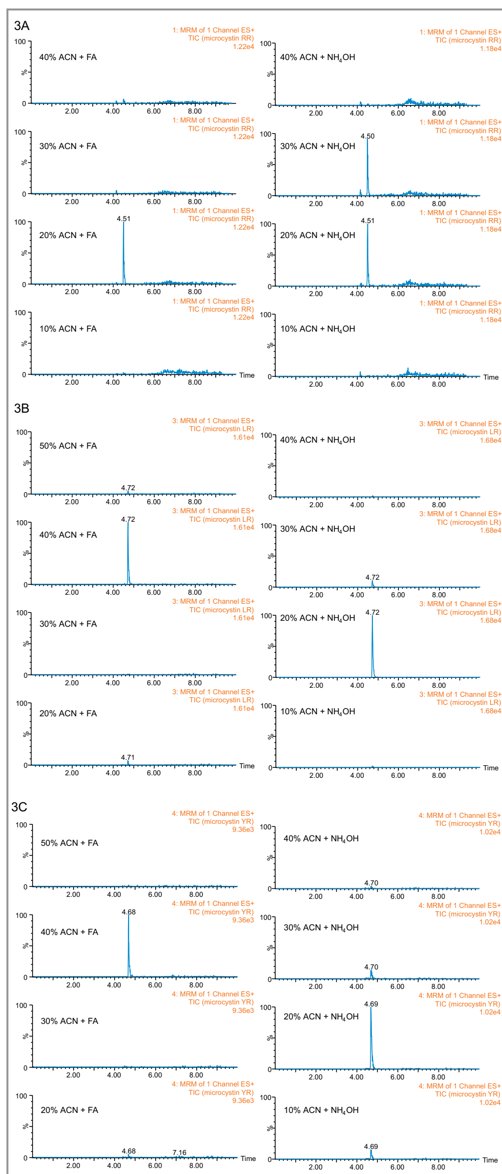

Figure 3. Sequential microextraction results for microcystins RR, LR, and YR.

LIMIT OF DETECTION, LINEARITY, AND QUANTIFICATION

With the extraction protocol optimized for all three microcystins, the next phase evaluate the detection limit, linearity, and recovery for bottled, tap, and surface water samples. Since microcystins RR, LR, and YR have a rigid ring structure, the optimization for a high abundance fragment ion for quantification is a difficult task. As seen in Figures 4a, 4b, and 4c, the MRM transitions show a common fragment ion at low mass with a weak intensity for all three microcystin. If trace-level detection is required, the extraction protocol will therefore be the main focal point of the analysis.

With a multi-dimensional chromatography configuration, a simple and effective enrichment process (10:1) was coupled to a high-volume injection (250 µL) and reached low ppt range, as seen in Figure 5. The chromatograms on the left show the response factor of microcystins RR, LR, and YR at 50 ppt in a water matrix (un-extracted) with a 250-µL injection volume. The 10x enrichment with the same injection volume shows a signal-to-noise ratio over 100:1 for microcystin YR, LR, and RR, thus indicating acceptable quantification performance (>10σ).

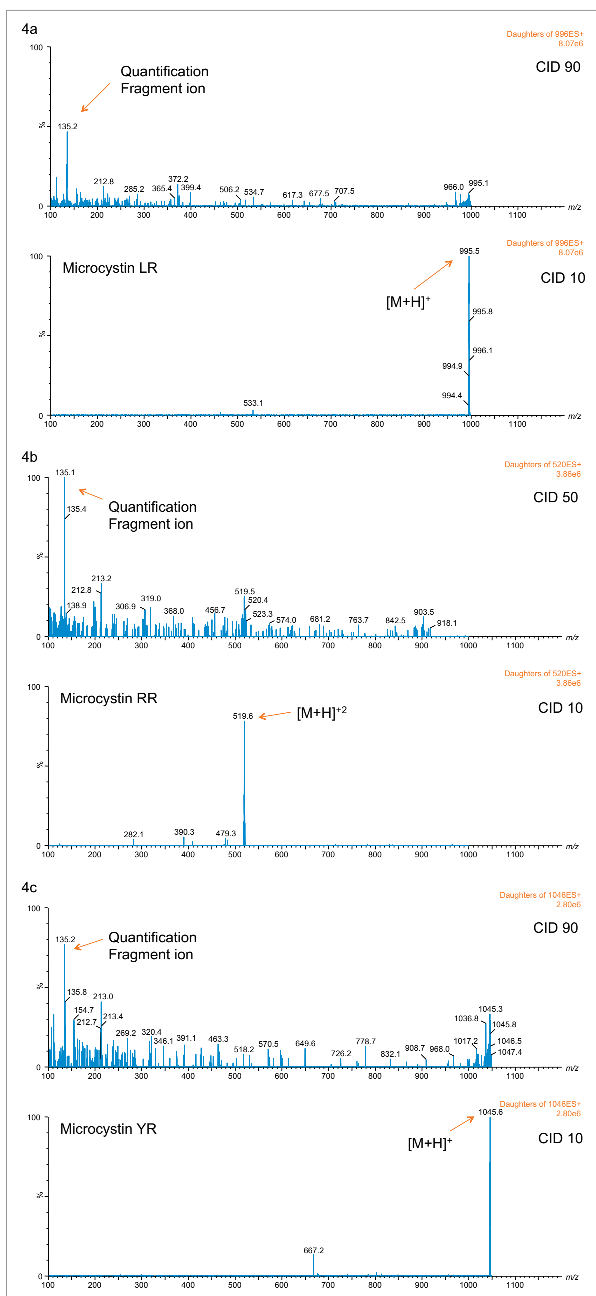

Figure 4. Daughter spectrums for microcystins RR, LR, and YR.

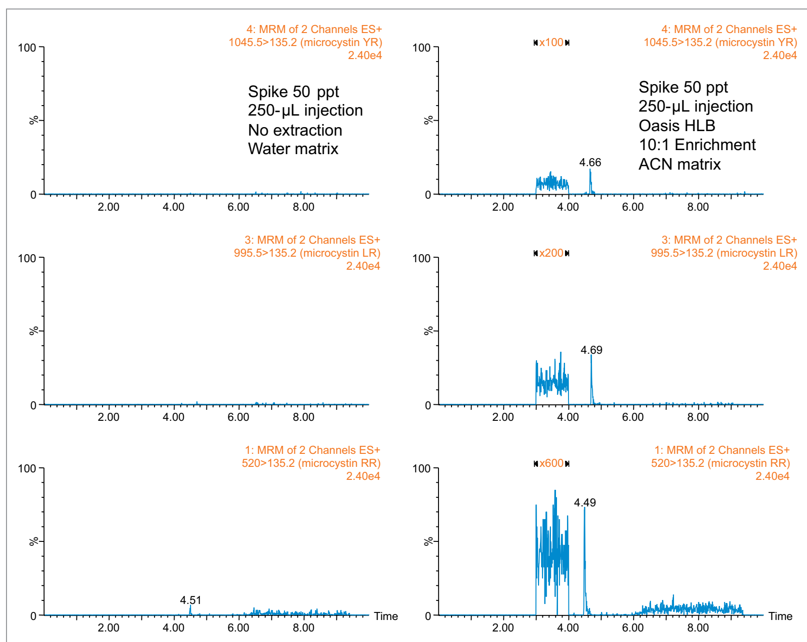

Figure 5. Extracted chromatograms at 50 ppt for microcystins RR, LR, and YR.

The linearity curve in Figure 6 shows a linear fitting with a 1/X weight for all three microcystins. Nodularin was used as internal standard. The r^2 values for microcystin RR, LR, and YR were calculated at 0.998, 0.995, and 0.997, respectively. The 1 ppb MRL requirement for microcystin in water from the WHO falls in the high end of the calibration curve.

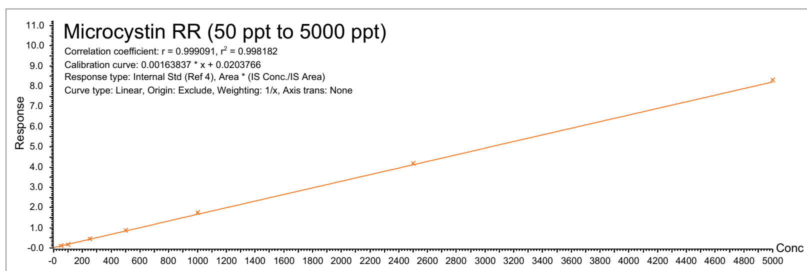

Figure 6. Calibration curve for microcystin RR from 50 ppt to 5000 ppt.

The level of interferences between all three samples type can have adverse effect on the overall performance of the optimized extraction protocol. Although the extraction method was optimized using high quality water, as the amount of interferences increases (from bottled to surface water sample), the analytical performance of the extraction protocol will ultimately decrease and yield poor extraction efficiencies. This is the case when dealing with trace-level extraction protocol with large-volume sample loading (1000:1 enrichment ratio). As the complexity increases, extra wash steps must also be added to keep recoveries within acceptable range, thus keeping potential matrix effect at negligible level.

With a reduced enrichment ratio (10:1) from the extraction protocol, the clean up step can be effective for a wider range of sample complexity (low, intermediate, and high). The recovery results are tabulated in Figure 7.

The recoveries in bottled, tap, and surface water samples were calculated against optima grade water standards (extracted calibration curve). The bottled water sample gave recovery values for all three microcystins in the 90% to 104% range, as to be expected with low complexity sample. The unexpected 75% to 85% recoveries for tap and surface water samples gives clear indication to the overall performance of the extraction protocol.

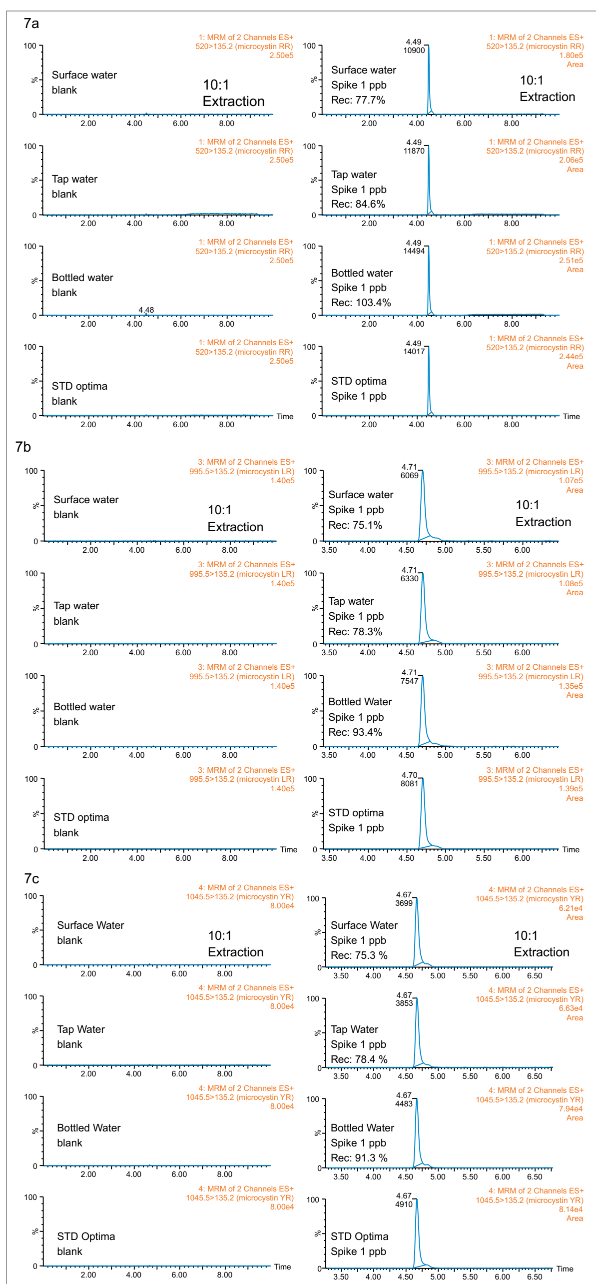

Figure 7. Extraction recoveries for microcystin RR, LR, and YR in bottled, tap, and surface water sample.

CONCLUSIONS

This application demonstrates the disruptive nature of the ACQUITY UPLC System with 2D-LC Technology with the Xevo TQD Mass Spectrometer. The application targeted the analysis of microcystin RR, LR, and YR in bottled, tap, and surface water. The limit of detection in this study was 50 ppt with a 10:1 enrichment from the extraction protocol (15 min total) and a 200:1 enrichment from the at-column dilution option, for a total of 2000:1. The recovery data for bottled, tap, and surface water samples using a microextraction protocol shows comparable results to applications with macroextraction protocols.

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Targeted and Untargeted Screening of Microcystins in Lake Water Samples Using High Resolution Mass Spectrometry

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APPLICATION BENEFITS

- Screening of water samples for targeted microcystins below regulatory limits.
- Perform qualitative and quantitative analysis using a single data set.
- Easily generate comprehensive HRMS library.
- Historical data review.

WATERS SOLUTIONS

Xevo® G2-XS QTof

ACQUITY UPLC® I-Class System

ACQUITY UPLC HSS T3 Column

MassLynx® MS Software

UNIFI® Scientific Information System

KEYWORDS

HRMS, MS^E, Pesticide screening, DIA, microcystin, algae, cyanobacteria, cyclic heptatpeptide hepatotoxins, CyanoHABS

INTRODUCTION

Lake closures due to harmful algae blooms have become a regular occurrence during the summer months. Recent data show that harmful algae blooms have been implicated in human and animal illnesses and death in at least 43 states in the U.S.[1] In August 2016, at least 19 U.S. states reported public health advisories due to CyanoHABS.[1] These algae blooms are fueled by phosphorus and nitrogen runoff from fertilizers, animal feedlots, and leaky septic systems. The algal population explosions occur due to higher summer temperatures believed to be caused by global warming. Blue-green algae generate microcystins which are cyclic heptatpeptide hepatotoxins produced by certain species of cyanobacteria found in freshwater environments. The structure of the most common microcystin, Microcystin-LR, is shown in Figure 1. These secondary metabolites are toxic to higher organisms, causing human sickness or even death in some cases.[2] As they are produced in fresh and brackish waters, they can contaminate drinking water supplies. These public health advisories can cause panic and negatively impact state and municipal economies due to lost income from tourism, as was the case in Toledo Ohio in 2014, and the beach closures in Florida in July of 2016.

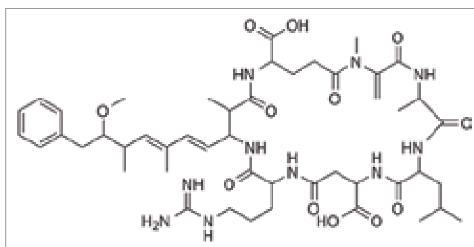

Figure 1. Chemical structure of microcystin-LR.

The WHO guideline limit for microcystin-LR is used in many countries. It includes a provisional value of 1 µg/L in drinking-water, and 10 µg/L for recreational exposure for total microcystin-LR (free plus cell-bound).[3] Some countries have set their own limits for microcystin-LR in drinking water (e.g. Australia and Canada have 1.3 µg/L and 1.5 µg/L respectively). There are only a handful of microcystin standards available on the market, while approximately 100 different microcystins variants have been reported in literature. These variants are produced by the substitution of the seven amino acids. Figure 2 shows the possible substitutions of a microcystin. As regulations are constantly changing and the fact that other microcystins may have a similar toxic effect as the regulated LR, it is important to develop targeted and untargeted methods for the analysis of these compounds.

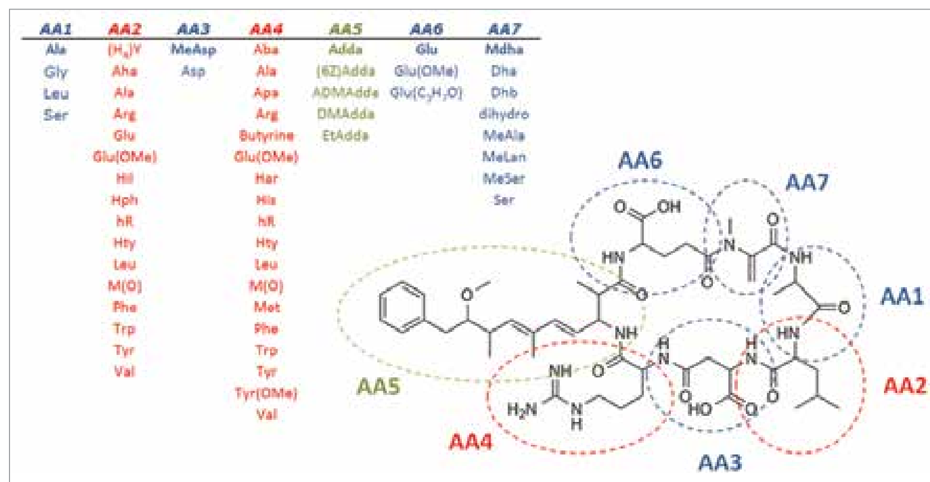

Figure 2. Illustration of the possible different combinations of seven amino acids that can produce over 100 different microcystin variants.

In this application note, we describe a method that utilizes a combination of LC and high resolution mass spectrometry (HRMS) to perform targeted screening of microcystins in lake water samples. A standard containing a mixture of 11 microcystins plus anatoxin A was used as a reference for positive identifications. Alongside the samples, a calibration curve of microcystin-LR was acquired to perform quantitation. The combination of accurate mass data for both precursor and fragment ions in a single analysis, combined with high quality UPLC® separation was used to identify targeted compounds. As the data were acquired using a data-independent approach, additional compounds that were not included at the time of the initial analysis could be investigated.

EXPERIMENTAL

Water samples from lakes in the U.S. were screened for 12 targeted compounds in the UNIFI Scientific Library. The library was generated by running a standard mix and includes structural information, molecular formula, and retention time for each of the targets. A calibration curve of microcystin-LR standard in HPLC water was also run between 0.1 to 50 µg/L in order to quantitate the amount in the samples. Data were acquired using full spectral acquisition and alternating high- and low-collision energy states (MS^E). This allowed us to use the structural information to confirm the presence of targeted compounds.

Sample description

Samples were obtained from U.S. lakes that reported harmful algae blooms in 2016. A lake water sample, a dock side sample, and scum layer sample were analyzed. The samples were lysed (freeze/thaw), filtered, and diluted before analysis. Prior to injection the samples were diluted 1 in 10 with water.

UPLC conditions*

UPLC system: ACQUITY UPLC I-Class

Column: ACQUITY UPLC HSS T3 1.8 µm, 2.1 x 100 mm

Column temp.: 35 °C

Sample temp.: 8 °C

Flow rate: 0.450 mL/min

Injection volume: 1, 5, and 10 µL

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Total run time: 12 min

Gradient:

Min.	Flow rate (mL/min)	%A	%B
Initial	0.45	98	2
0.80	0.45	98	2
9.00	0.45	30	70
9.05	0.45	10	90
9.90	0.45	10	90
9.91	0.45	98	2
11.50	0.45	98	2

**This UPLC method was established and previously published by Waters.[4]*

MS conditions

MS system: Xevo G2-XS QTof

Ionization mode: ESI+

Collision energy (LE): 4 eV

Collision energy (HE ramp): 25 to 80 eV

Scan time: 0.25 sec

Acquisition range: 50 to 1200 *m/z*

Capillary: 1.5 kV

Sampling cone: 36 V

Source temp.: 120 °C

Source offset: 50

Desolvation temp.: 500 °C

Cone gas flow: 150 L/Hr

Desolvation gas flow: 1000 L/Hr

Lockmass: Leucine enkephaline (556.2766 *m/z*)

Data management

MassLynx v4.1 MS Software and the UNIFI Scientific Information System

RESULTS AND DISCUSSION

IDENTIFICATION RESULTS IN U.S. LAKE WATER SAMPLES

A standard at 10 µg/L of all 12 standards in HPLC water was run in order to establish retention times for the UPLC method. These retention times were added along with molecular formula and available structural information to the UNIFI Scientific Library. The UNIFI library was used to interrogate the highly complex data set for the 12 target compounds. In order to ensure the system was performing as expected, the above standard mix was acquired along with the samples of interest. Figure 3A shows the results from the standard injections. Figure 3B overlay shows the extracted ion chromatograms for each of the 12 compounds found in the standard. The standard data shows that the retention time delta is very low and the mass error for each compound of interest is within 5 ppm.

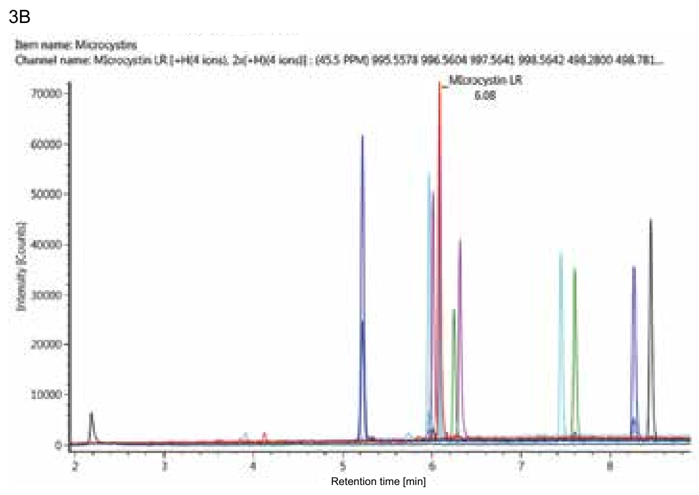

Figure 3A. Standard in HPLC water at 10 µg/L showing identification of all 12 Targets within 5 ppm; 3B. Overlaid chromatogram of microcystins standard at 10 µg/L.

Once it was established that the system was performing as expected, the four samples were run. A 1-µL aliquot from each sample was injected. The raw data was componentized and processed once by UNIFI Software.[6] In order to review the data of interest, a user-defined filter was applied (Figure 4). This filter was defined by the quality of the standard data and only showed the identified compounds that were within 5 ppm mass error, 0.1 minute retention time error, and above a minimal response. These user-defined filters can be combined with pieces of data the analyst wants to view, as well as previously saved data so that the same workflow can be followed for further data review. UNIFI's filters, views, and workflows allow analysts to follow their own protocol for interrogating data, and it can help standardize how the data is reviewed.

Enter the filter criteria

Match all groups Match any group

Match all of these expressions

Mass error (ppm)	is between	-5	: 5	ppm
Response	is greater than	100		
Retention Time Error (min)	is between	-0.1	: 0.1	min
Identification status	is	Identified		

Figure 4. Example of filter criteria used within UNIFI to show the data of interest. In this case only identified mass accurate, retention time consistent, and larger than 100 counts microcystins are displayed.

The results from each sample are shown in Figures 5a, 5b, and 5c. An example of one of the identifications depicted in these figures shows how an analyst can visualize the results. One of the advantages about using untargeted data acquisition is the ability to determine the presence different charge species of the target compounds. Microcystins can often form multiply charged species that can be potentially missed if the method is predefined by only one species. In this case both the single and double charged species were detected. By acquiring high and low energy data in one run the confirmation of the target compounds can be easily made. The high energy data is automatically used by the software to perform structural matching of the fragments to the compound of interest. Figure 6 shows the high and low energy spectra for the identification of microcystin-LR in the lake sample and *in silico* fragmentation using the compound's structure.

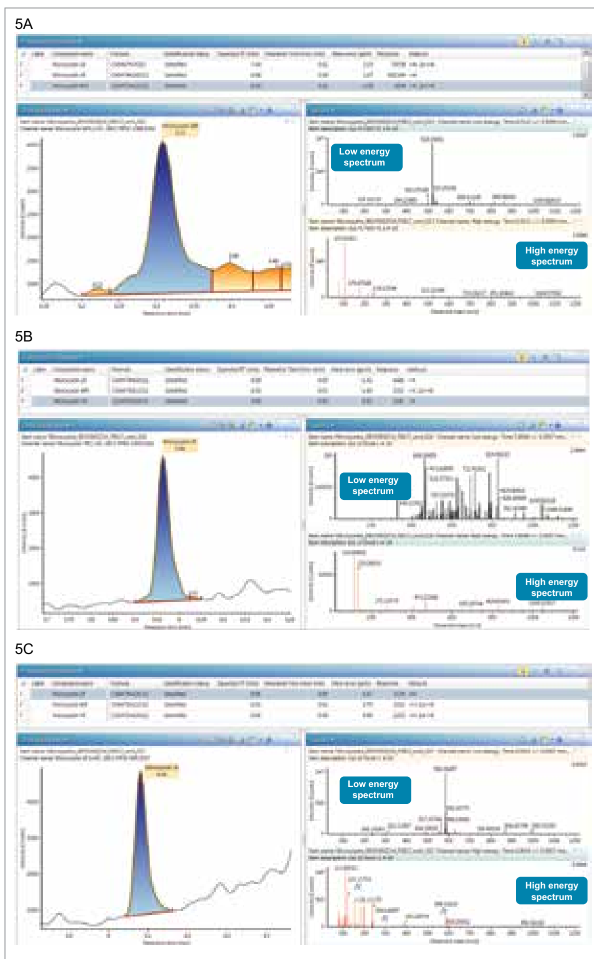

Figure 5A.Summary of results for the lake sample; 5B.Summary of results for the dock sample; 5C. Summary of results for the scum sample.

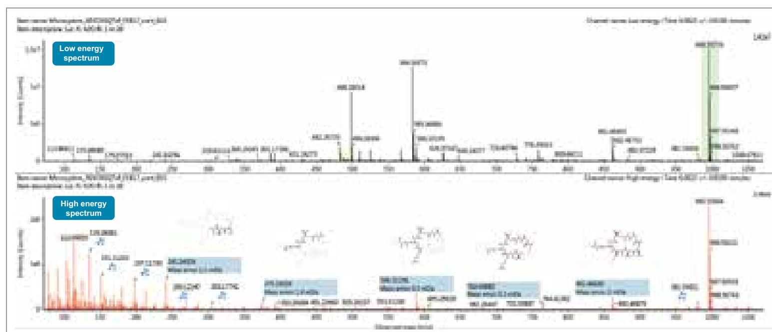

Figure 6. Example of high and low energy spectra for the identification of microcystin-LR in the lake sample and in silico fragmentation using the compound's structure.

QUANTIFICATION OF MICROCYSTIN-LR

Alongside the samples, a calibration curve for microcystin-LR was acquired in order to determine the concentration in the samples. Figure 7a shows the calibration curve for microcystin-LR between 0.1 and 50 µg/L, and Figure 7b shows the chromatogram for microcystin-LR at 0.1 ppb. The Xevo G2-XS QTof provided excellent mass accuracy across a wide dynamic range of detection (Table 1), demonstrating that this instrument is fit-for-purpose to achieve legislative limits in real samples. It also shows that even at low concentrations, the mass accuracy on the system is excellent and gives confidence in the quantitation data. The data obtained demonstrate that the Xevo G2-XS QTof is extremely sensitive and can be used to quantify low levels that meet regulatory requirements. When assessing the samples against the calibration curve and taking into consideration the dilution factors, the levels in the samples were 100 times higher than the action level for recreational water (Table 2). In drinking water, levels of contaminations will be a fraction of that at the source of the contamination. In this instance, to decrease detection limits even further, the use of 2D UPLC systems have been employed. These systems allow large volumes of water to be injected achieving lower detection limits.[5]

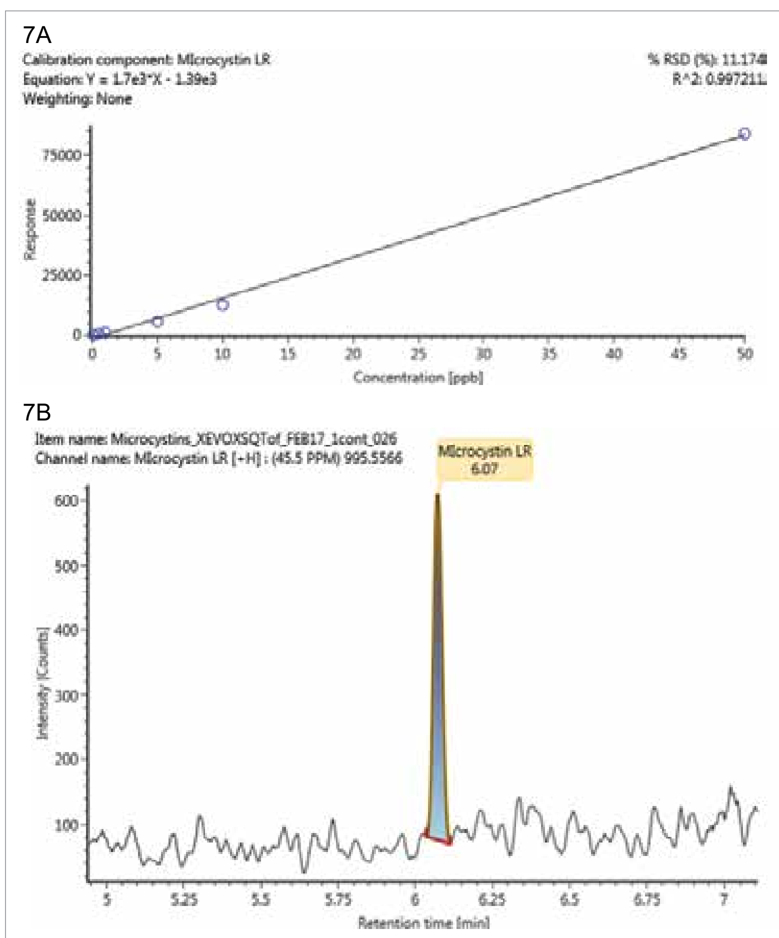

Figure 7A. Calibration curve for microcystin-LR between 0.1 and 50 µg/L; 7B. Microcystin-LR at 0.1 µg/L in HPLC water, with 10 µL injected.

Table 1. Results from the microcystin-LR calibration curve in HPLC water showing mass accuracy with 4 ppm for all levels.

Item name	Actual concentration	Component name	Expected RT (min)	Retention time Error (min)	Mass error (ppm)	Response
0.1 µg/L Microcystin std.	0.1	MIcrocystin-LR	6.08	-0.01	0.52	253
0.5 µg/L Microcystin std.	0.5	MIcrocystin-LR	6.08	-0.01	-3.86	675
1.0 µg/L Microcystin std.	1.0	MIcrocystin-LR	6.08	-0.01	-3.60	1199
5.0 µg/L Microcystin std.	5.0	MIcrocystin-LR	6.08	-0.01	-1.63	5728
10.0 µg/L Microcystin std.	10.0	MIcrocystin-LR	6.08	-0.01	-0.52	12,688
50.0 µg/L Microcystin std.	50.0	MIcrocystin-LR	6.08	-0.01	3.97	84,200
				Average mass error (ppm)	2.35	

Table 2. Calculated concentration in samples for microcystin-LR (the limit for recreational water is 10 µg/L).

Sample name	Component name	Formula	Expected RT (min)	Mass error (ppm)	Response	Calculated sample concentration (µg/L)
Lake water sample	Microcystin-LR	$C_49H74N10O12$	6.08	3.1	11312685	41222.2
Dock water sample	Microcystin-LR	$C_49H74N10O12$	6.08	-0.5	99719	403.5
Scum water sample	Microcystin-LR	$C_49H74N10O12$	6.08	-2.7	17348	103.6

Adapting to changing legislation

Microcystin-RR was not included in the UNIFI Scientific Library when the data was first acquired. In order to demonstrate that historical data could be reviewed on a QTof system, unlike traditional tandem quandrupole analysis, microcystin-RR was added to the scientific library by acquiring the standard using the same UPLC method. When working with a tandem quadrupole MS system, the MRM transitions must be defined up front and historical data review is not possible for compounds that were not included in the original method. It is not always possible to run the standard to add a compound to the library because they are not always available. If the standard is not available, the structure can be imported into the library and a literature search can be used to assess the relative retention time for that structure. Figure 8 shows the acquired standard spectra which displays a predominant doubly charged species. The sample data were then re-interrogated for the presence of microcystin-RR. In both the dock and scum samples, a significant level of microcystin-RR was found. Figure 9 shows the extracted ion chromatograms for both samples and the resulting spectra for microcystin-RR. The exact same data was used to detect and identify this additional compound. This approach allows historical occurrence studies to be performed on data in order to determine when emerging compounds were first present in recreational water.

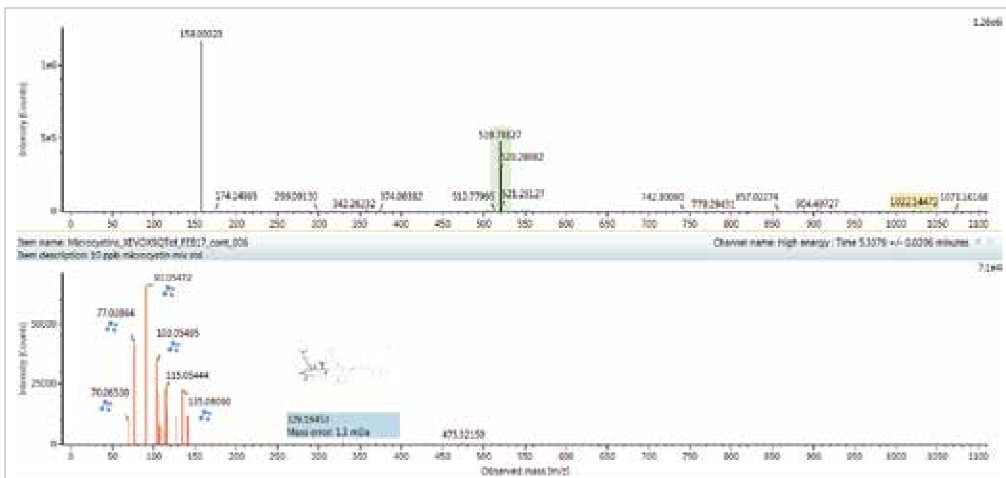

Figure 8. The acquired standard spectra which show a predominant doubly charged species for microcystin-RR.

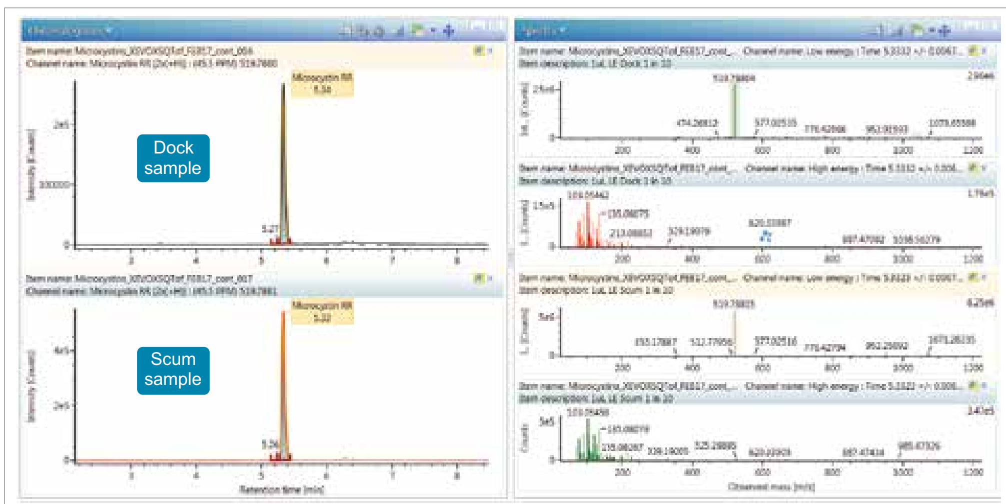

Figure 9. Extracted ion chromatogram and resulting spectra for microcystin-RR in the dock and scum samples.

CONCLUSIONS

- Three microcystins were confimed in lake water samples that were above legislative limits.
- Detection and quantification performed using HRMS demonstrated excellent sensitivity, even with a small volume injection and single dimensional chromatography.
- The use of the ACQUITY UPLC I-Class System and the Xevo G2-XS QTof with UNIFI Scientific Software successfully met the regulatory requirements for screening microcystins.
- Historical data review allowed for another identification to be made in the same data set which is an advantage of HRMS over tandem quadrupole analysis.

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[PCBs]

[PCBs]

[TECHNOLOGY BRIEF]

Increasing Sensitivity for Tof-MS Detection of Polychlorinated Biphenyls (PCBs) Using Tof MRM

Lauren Mullin and Adam Ladak,
Waters Corporation, Milford, MA, USA

GOAL

Tof MRM affords increased sensitivity while maintaining the ability to acquire accurate mass full scan data in the same injection.

BACKGROUND

Polychlorinated biphenyls (PCBs) are persistent organic pollutants which have been banned from production as a result of their observed accumulation in biota and the environment. Various levels of toxicity have also been associated with PCBs, in particular the 12 dioxin-like PCB congeners. Consequently, PCBs are monitored at sub-ppb levels in complex environmental matrices[1]. The use of product ions for identification is important and can be achieved using multiple reaction monitoring (MRM) on tandem quadrupole MS (MS/MS) systems. For this well accepted technique, only specific transitions of interest are monitored. While MRM provides excellent selectivity for those target compounds, unexpected yet highly abundant and significant components of the sample may go completely undetected. Conversely, time-of-flight (Tof) MS systems provide accurate mass measurement across a wide mass range, but historically have not met the same sensitivity levels achieved using tandem quadrupole MRMs. In this technology brief, we describe the application of a novel data acquisition mode for Waters® SYNAPT® G2-S*i* which utilizes a targeted enhancement of selected product ions. Full scan data was also collected in the same run time, providing comprehensive exact mass information for the samples.

Tof MRM affords increased sensitivity while maintaining the ability to acquire accurate mass full scan data in the same injection.

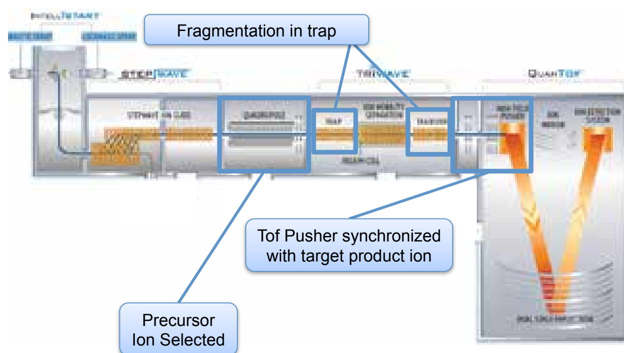

Figure 1. Instrument schematic of SYNAPT G2-Si. Tof MRM is achieved by the selection of a specified precursor ion in the quadrupole, followed by fragmentation induced in the trap, or transfer regions, and a pusher frequency synchronized with the specified product ion.

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THE SOLUTION

Tof MRM was achieved by first selecting a precursor ion in the quadrupole. Following CID in a T-Wave™ collision cell, the duty cycle of specified product ions were enhanced via timing of Tof pushes relative to the specified product ion (Figure 1). For PCBs, the 35Cl and 37Cl isotopes of the product ion were monitored, while targeted enhancement of the average mass was utilized. A full spectral acquisition channel from *m/z* 100 to 800 was also acquired. Solvent standards of seven routinely monitored PCBs (28, 52, 101, 118, 138, 153, and 180) were prepared at concentrations ranging from 0.1 to 100 ng/mL and analyzed by PGC-QTof MS. Linearity of response across three orders of magnitude was excellent for all congeners, with correlation coefficient values >0.995. Analysis of the standard at 10 ng/mL were repeated six times giving %RSD values <10%).

Increased sensitivity was evident for compounds monitored in this targeted experiment (Figure 2), as compared to a typical Tof MS full scan acquisition. Signal-to-noise (S/N) ratios were all above 7:1 for the 0.1 ng/mL standard injections.

In order to asses the method for the analysis of a complex biological matrix, whale blubber extracts were also analyzed using this method. PCB 118 was observed in all three extracts, as well as several other congeners. The use of Tof MRM in this analysis afforded the advantage of an improved S/N ratio in this complex matrix as compared to Tof MS acquisition (Figure 3). In addition to the targeted PCBs, the masses of selected polybrominated diphenyl eithers (PBDEs) were extracted from the full scan data, and positive identifications of congeners were made (Figure 4). Identifications were obtained using comparisons of accurate mass, isotope distribution patterns, and searching of online databases. Full spectral acquisition data affords the ability to mine the samples for a wide range of potentially unexpected contaminants, as well as facilitating historical data review. This feature will be useful for the identification of emerging contaminants and their occurance over time in samples.

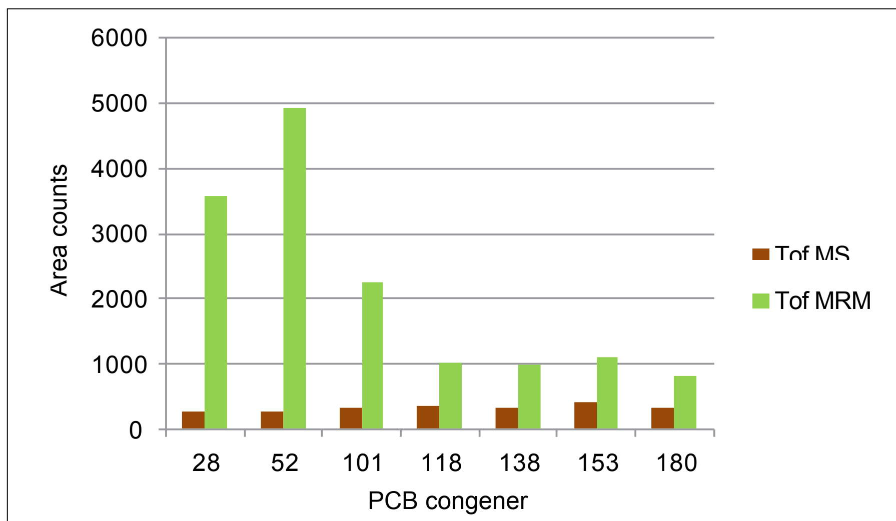

Figure 2. Comparison of peak area for PCB congeners using Tof MS (where peak was extracted ion chromatogram (EIC) from precursor mass) and Tof MRM (where peak was EIC from targeted product ion) acquisition modes. Sensitivity increases of at least 2x are a result of enhanced duty cycle for the specified product ions in Tof MRM mode.

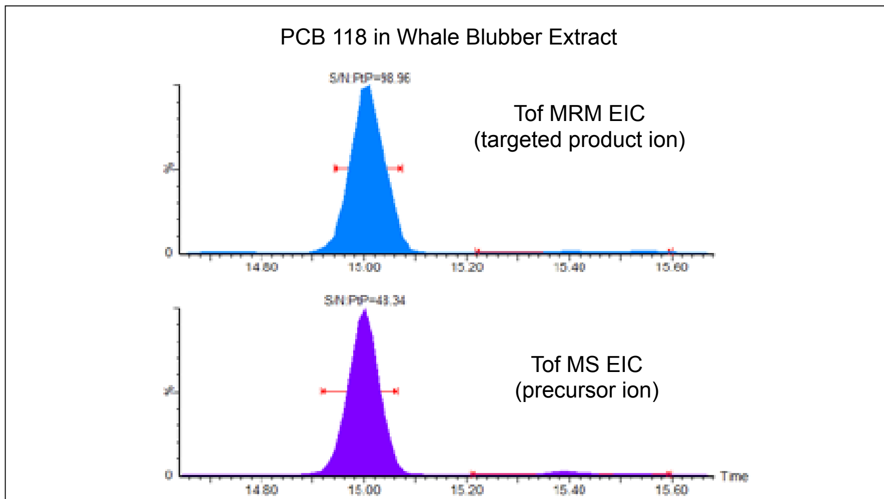

Figure 3. PCB 118 extracted ion chromatograms (EICs) for a Tof MRM (top) and Tof MS (bottom)analysis. The signal-to-noise ratio is almost doubled when using Tof MRM, which is the result of precursor ion selection in the quadrupole prior to CID and targeted enhancement of the production. This is advantageous in the analysis of complex matrices, such as the whale blubber shown here.

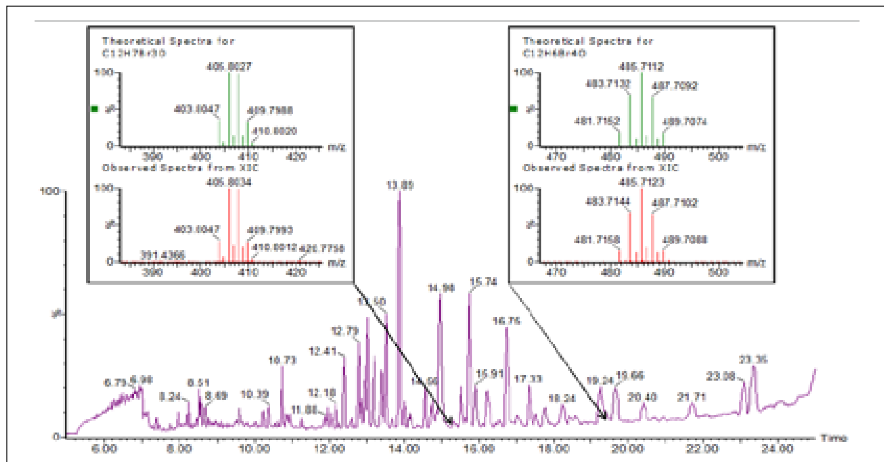

Figure 4. Full scan channel BPI from whale blubber extract. In addition to the targeted components in the Tof MRM method, the full spectral acquisition data can be searched for other contaminants such as PBDEs. Mass error for both identifications were <3ppm.

SUMMARY

Tof MRM enhances the analytical capabilities of high resolution mass spectrometry, affording lower limits of detection while maintaining the ability to collect information rich accurate mass full scan data. These benefits have been applied here to the analysis of PCBs, which requires instrumental sensitivity and selectivity for detection in complex environmental matrices.

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Acknowledgements

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Analysis of Glyphosate, AMPA, and Glufosinate in Water Using UPLC-MS/MS

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APPLICATION BENEFITS

Specific, targeted method for determination of glyphosate, AMPA, and glufosinate in water samples, suitable for monitoring both drinking water and ground/surface waters for compliance with European regulatory limits.

WATERS SOLUTIONS

ACQUITY™ UPLC™ I-Class System

ACQUITY UPLC BEH Phenyl Column

Xevo™ TQ-XS Triple Quadrupole Mass Spectrometry

UniSpray™ Ion Source

MassLynx™ MS Software

TargetLynx™ Application Manager

KEYWORDS

Glyphosate, AMPA, glufosinate, drinking water, surface water, herbicides, water analysis

INTRODUCTION

The widespread use of pesticides for agricultural and nonagricultural purposes has resulted in the presence of their residues in surface and ground water resources. Glyphosate is one of the most widely used broad-spectrum herbicides around the globe. Aminomethyl-phosphonic acid, commonly known as AMPA, is the major metabolite of glyphosate in the environment. Glufosinate-ammonium is another highly effective herbicide used to control weeds in many countries around the world and it has a similar chemical structure. The diverse and intensive use of such herbicides implies that residues have the potential to reach surface waters throughout the year from indirect routes of entry such as spray drift, runoff and drainage, as well as point source contamination. Numerous laboratory and field studies have been performed to investigate the transport of glyphosate and/or AMPA to the aquatic environment indicating some recognition and concern that these substances can move towards surface waters. At the same time, glyphosate and AMPA are only sporadically detected in deep groundwater systems and at low concentrations indicating that the leaching of these compounds is generally unlikely and probably negligible.[1]

The difficulties associated with determination of these compounds at trace levels in water samples are related to their high solubility in water, ionic nature, and chelation with metal ions. All three compounds can be derivatized to less polar compounds for improved retention and separation using solid phase extraction (SPE) and reversed-phase liquid-chromatography (LC). Fluorenylmethyloxycarbonyl (FMOC) chloride is the most common pre-column derivatization reagent used for this analysis, and it can be successfully used in combination with LC-MS/MS[2,3,4] for determination of all three compounds in one method as part of water monitoring programs.

Public water suppliers abstract raw water from a range of different sources depending on local availability. In some countries, supplies are taken almost entirely from groundwater, while in other countries surface waters (rivers, canals, lakes, or reservoirs) are the predominant source of drinking water. The presence of pesticides in water is regulated through different directives. Member States have the obligation to ensure that regular monitoring of the quality of water is carried out in order to check that the water available to consumers meets the requirements of the Drinking Water Directive.[5] This sets a maximum limit of 0.1 µg/L for individual pesticide residues present in a sample (0.5 µg/L for total pesticides). In general, the World Health Organization (WHO) guidelines for drinking water and the opinion of the Commission's Scientific Advisory Committee are used as the scientific basis for quality standards in drinking water. The Water Framework Directive (WFD),[6] which aims to improve the quality of water across Europe, deals with surface waters, coastal waters, and groundwater, and seeks to provide a good chemical status of water across Europe. Member States must identify River Basin Specific Pollutants and set their own national environmental quality standards (EQSs) for these substances. Specific Pollutants are substances that may have a harmful effect on biological quality and which have be identified as being discharged to the water environment in significant quantities in the Member States. Values for these EQS vary across Europe; for example, the long term mean EQS for glyphosate in the UK is 196 µg/L[7] but it is 28 µg/L in France and Germany.[8] Hence, there is a need for reliable analytical methods for monitoring these polar herbicides in drinking, surface, and ground waters.

This application note describes a method for the determination of glyphosate, AMPA, and glufosinate in water samples, without SPE, after derivatization with FMOC, by LC-MS/MS on Waters® ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS using a novel ionization technology, UniSpray.

EXPERIMENTAL

Sample preparation

The issue of complexation of glyphosate with various cations resulting in low recoveries has been well established in environmental water analysis.[3] All water samples (12 mL) were filtered (0.22 µm cellulose membrane filter); salts and metals removed using ion exchange (Dionex OnGuard II Na ion exchange syringe cartridge) and stored in polypropylene (PP) containers. A test portion from each filtered water sample was treated using the derivatization procedure shown in Figure 1. Solutions of standards were prepared in a sample of drinking water, internal standards were added and solutions derivatized using the same procedure.

The accuracy of the method was assessed by analyzing water samples spiked with the compounds of interest at various concentrations. Solutions of standards were prepared over the range 0.02 to 2.0 µg/L, in drinking water to determine the concentration of analytes in the recovery spikes and to evaluate linearity of response.

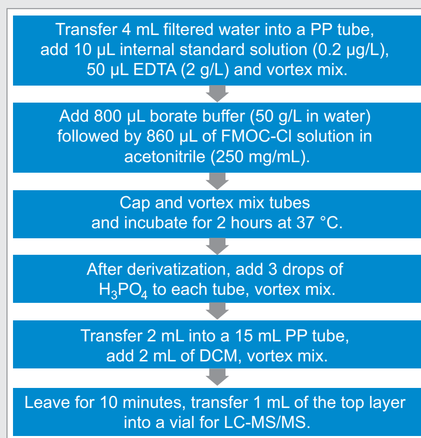

Figure 1. Schematic showing the procedure for FMOC derivatization.

UPLC conditions

UPLC system: ACQUITY UPLC I-Class with FTN Sample Manager equipped with a 50 µL extension loop and 250 µL sample syringe

Column: ACQUITY UPLC BEH Phenyl, 1.7 µm, 2.1 × 100 mm

Mobile phase A: 5 mM ammonium acetate (aq.), pH 9 (using 25–28% NH_4OH solution)

Mobile phase B: Methanol

Flow rate: 0.4 mL/min

Injection volume: 50 µL

Column temp.: 50 °C

Sample temp.: 10 °C

Run time: 16 min

Time (min)	%A	%B	Curve
0.00	90	10	–
5.00	54	46	6
7.00	54	46	6
8.00	0	100	6
9.50	0	100	6
11.0	90	10	1

MS conditions

MS system: Xevo TQ-XS

Source: UniSpray

Ionization mode: US

Capillary voltage: 3.0 kV

Desolvation temp.: 550 °C

Desolvation gas flow: 1000 L/Hr

Source temp.: 150 °C

Cone gas flow: 150 L/Hr

Cone voltage: 14 V

Collision gas flow: 0.14 mL/min

Nebulizer gas pressure: 7 Bar

Data acquisition and processing

Data were acquired using MassLynx MS Software (v4.2) and processed using TargetLynx XS Application Manager. The selection of MRM transitions and the optimization of critical parameters was performed by infusion of individual solutions of all the analytes and evaluation of the data by IntelliStart™ Software to automatically create acquisition and processing methods. Table 1 summarizes conditions for all MRM transitions including the retention times. The optimum dwell time was set automatically using the Autodwell function. For this work, stable isotope labeled AMPA was used as an internal standard for the determination of glufosinate.*

**Glufosinate-D3 is now available for a number of suppliers.*

Table 1. MRM parameters for glyphosate, glufosinate, AMPA, and stable isotope analogues (quantitative transitions in bold).

Compound	Retention time (min)	MRM	CE (eV)	Dwell time (s)
FMOC-Glyphosate	3.0	**392>179**	26	0.080
		392>88	16	0.080
		392>214	8	0.080
FMOC-Glufosinate	4.3	**404>136**	22	0.043
		404>179	28	0.043
		404>119	35	0.043
FMOC-AMPA	4.7	**334>156**	8	0.043
		334>179	22	0.043
		334>112	10	0.043
FMOC-Glyphosate-$^{13}C_2$,^{15}N	3.0	**394>179**	26	0.080
AMPA-^{13}C,^{15}N,D_2	4.7	**338>160**	8	0.043

RESULTS AND DISCUSSION

UniSpray is a novel, proprietary, ionization source that provides increased ionization efficiency.[9] The unique geometry of the UniSpray ion source generates several different mechanisms to produce smaller droplets and enhance desolvation. These effects combine together to generate a greater number of free ions from the same amount of sample compared to traditional ionization modes, such as electrospray, and typically result in an increase in response across a wide range of compounds.

Excellent sensitivity and selectivity was demonstrated by the response for each analyte detected from the analysis of drinking water spiked at 0.02 µg/L (see Figure 2), well below the limits required for drinking, surface, and ground waters. Laboratories are expected to provide methods with lower limits of quantification (LLOQ) of at least one third of the EQS. The sensitivity observed suggests that detection and quantification of all three compounds at much lower concentrations should be possible.

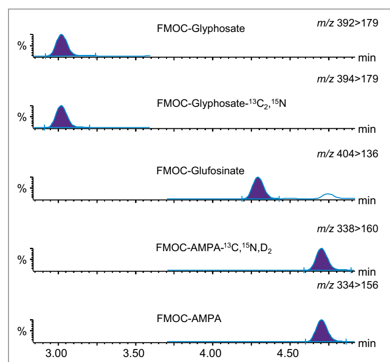

Figure 2. Chromatograms showing glyphosate, glufosinate, and AMPA from analysis of drinking water spiked at 0.02 µg/L.

Standard solutions, prepared in drinking water at seven concentrations (0.02, 0.05, 0.10, 0.50, 1.0, 1.5, and 2.0 µg/L), were used for calibration. The response for all three compounds was linear and the correlation coefficients (r^2) were >0.999 for all three compounds with residuals of <6% (see Figure 3).

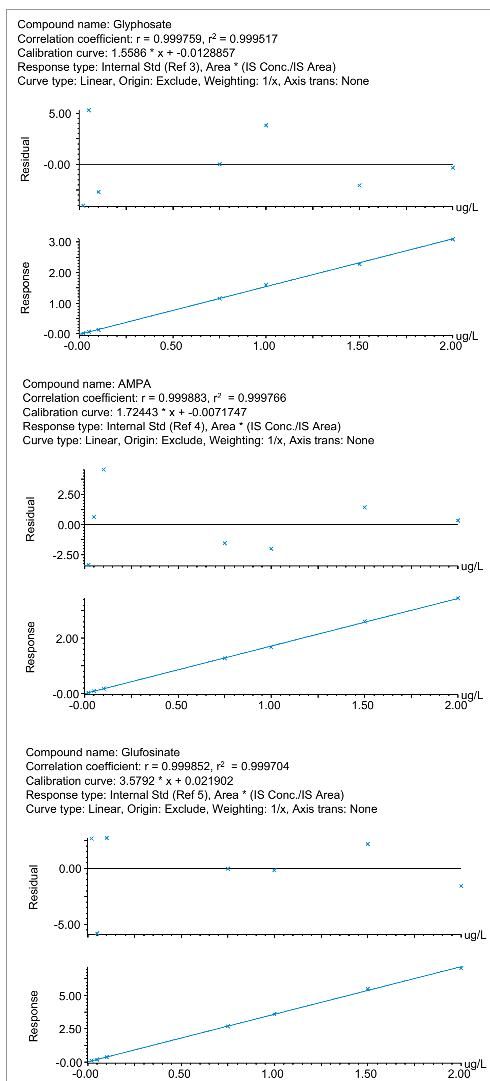

Figure 3. Calibration graphs for glyphosate, AMPA, and glufosinate prepared in drinking water.

The accuracy of the method was determined from the analysis of spiked water samples. The measured values were compared with the expected values from spiking and found to be within the range 85 to 110% (Table 2). Repeatability of the measurements was also good; e.g. <6% RSD in four different ground water samples spiked at 0.1 µg/L run in duplicate (n=8). Identification criteria, ion ratios, and retention times were all within acceptance tolerances.[10]

Table 2. Trueness from measurements of spiked water samples.

Sample	Measured concentration (µg/L) and trueness (%)		
	Glyphosate	AMPA	Glufosinate
Drinking water at 0.02 µg/L	0.021 (105)	0.020 (100)	0.022 (110)
Drinking water at 0.2 µg/L	0.196 (98)	0.196 (98)	0.213 (107)
Drinking water at 0.75 µg/L	0.821 (109)	0.734 (98)	0.721 (96)
Ground water Sample 1 at 0.10 µg/L	0.101 (101)	0.100 (100)	0.085 (85)
Ground water Sample 2 at 0.10 µg/L	0.100 (100)	0.099 (99)	0.087 (87)
Ground water Sample 3 at 0.10 µg/L	0.103 (103)	0.100 (100)	0.092 (92)
Ground water Sample 4 at 0.10 µg/L	0.104 (104	0.098 (98)	0.093 (93)
Ground water Sample 1 at 0.75 µg/L	0.812 (108)	0.717 (96)	0.735 (99)
Ground water Sample 2 at 0.75 µg/L	0.821 (109)	0.740 (99)	0.807(108)
Ground water Sample 3 at 0.75 µg/L	0.793 (106)	0.764 (102)	0.782 (104)

Glyphosate and AMPA were detected in the chromatograms from the analysis of the four water samples (Figure 4), but concentrations were found to be <LLOQ in all but one case; glyphosate (0.021 µg/L) in a sample of ground water.

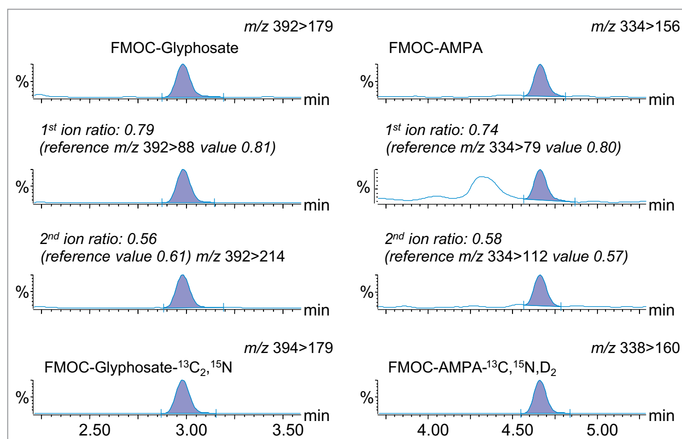

Figure 4. Chromatograms showing glyphosate (0.021 µg/L) and AMPA (<0.02 µg/L) detected in a sample of ground water.

CONCLUSIONS

This application note has demonstrated the performance of a method for the determination of glyphosate, AMPA, and glufosinate by UPLC-MS/MS, after derivatization with FMOC, on an ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS MS System. The method is simple, time-saving, and inexpensive, providing fast and reliable quantitation of glyphosate, AMPA, and glufosinate in various types of water samples. The results indicate that this method is suitable for the detection of glyphosate, AMPA, and glufosinate for monitoring purposes. Calibration characteristics, linearity, and residuals were excellent over the concentration range studied. The accuracy of the method was shown to be good, and the method was applied to the analysis of real water samples. Scientists must validate the method in their own laboratories and demonstrate that the performance is fit for purpose and meets the needs of the relevant analytical control assurance system.

Acknowledgements

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[PFASs]

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[APPLICATION NOTE]

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Analysis of Legacy and Emerging Perfluorinated Alkyl Substances (PFAS) in Environmental Water Samples Using Solid Phase Extraction (SPE) and LC-MS/MS

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APPLICATION BENEFITS

Performing SPE sample preparation of water samples using the ISO 25101 method for PFAS analysis provides:

- Highly sensitive analysis using the Xevo™ TQ-S micro
- Detection limits in the low to sub- ng/L range to meet regulatory requirements
- A robust and reliable solution for monitoring PFAS compounds in environmental water matrices

WATERS SOLUTIONS

ACQUITY™ UPLC™ I-Class PLUS System

ACQUITY UPLC BEH C_{18} Column

Oasis™ WAX SPE Cartridges

PFC Analysis Kit

Xevo TQ-S micro

MassLynx™ MS Software

KEYWORDS

Perfluorinated, polyfluorinated, PFAS, PFC, AFFF, PFOS, PFOA, WAX SPE cartridge, TQ-S micro

INTRODUCTION

Perfluoroalkyl substances (PFAS) are a group of persistent and bioaccumulative anthropogenic pollutants that are common to consumer and industrial processes. They are introduced to the environment through a variety of sources ranging from industrial manufacturing of non-stick coatings to their use in firefighting foams. While this group of compounds encompasses thousands of unique compounds, most advisories currently focus on the two most commonly known, PFOS and PFOA. While there currently are no legal requirements for monitoring of PFASs globally, many countries worldwide do recommend they be monitored at some level. In the United States, the U.S. EPA has set an advisory limit of 70 ng/L (ppt) of total PFOS and PFOA;[1] while in Europe, the European Water Framework Directive has singled out PFOS and its derivatives. The Water Framework Directive is an environmental quality standard and advises an annual average value of 0.65 ng/L for inland surface waters.[2]

To reach detection limits low enough to satisfy advisories, either a highly sensitive mass spectrometer is required, or sample preparation that allows for sample enrichment must be employed. The first option was discussed in a previous application note utilizing the ASTM 7979 procedure with the Xevo TQ-XS.[3] This application note will detail the second approach using SPE extraction to enrich water samples with analysis performed on Waters™ Xevo TQ-S micro. Methodology was adapted from ISO 25101 which was written for analysis of PFOS and PFOA in environmental water samples.[4] Both approaches are valid options and it depends on a laboratory's resources and testing needs as to which method should be considered.

EXPERIMENTAL

The ISO 25101 method was utilized as a guideline for the sample preparation methodology used for this analysis. Currently, ISO 25101 covers the extraction and analysis of only PFOA and PFOS. For this method, an extended list of PFAS compounds were considered and added. Appendix A contains information on all of the PFAS compounds analyzed in this method, together with a subset of emerging compounds being used to replace the legacy PFAS compounds, including GenX. All standards were obtained from Wellington Laboratories (Guelph, Ontario).

A Certified QC Standard (cat no.: 731) from ERA (Golden, CO), for use with ground and surface waters, was utilized as an instrumental QC check throughout the analysis. The standard contained a mix of 12 PFAS compounds. Certified values and QC Performance Acceptance Limits for each compound in the mix are provided with the standard, making instrumental QC evaluation quick and straightforward.

Due to widespread use of PFAS substances there are many common sources of potential contamination to the analysis. Since required detection limits are in the low- to sub-ng/L, care must be taken during sample collection, preparation, and analysis. Considering there are many common sources of PFAS contamination in the field and laboratory, it is recommended that any laboratory supplies to be used for this analysis be checked for PFAS contamination before use, as is practical. Contamination is also unavoidable from the chromatographic system. Therefore steps should be taken to minimize any system contribution, and as such, the Waters PFC Analysis Kit (p/n: 176001744) for the UPLC system was utilized. The kit is comprised of PFAS-free components (such as PEEK tubing to replace the conventional Teflon coated solvent lines) and an isolator column that helps to delay any residual background interferences from co-eluting with the analytical peak. Installation of the PFC Analysis Kit is straightforward and quick.[5] In addition, special mobile phase solvents from Honeywell (Muskegon, MI) were used that were bottled in a manner to reduce residual background PFAS levels.

Sample preparation

Standards were prepared as a mix in methanol and calibration standards were appropriately diluted into 1:1 water:methanol to match the final solvent composition of the samples.

Environmental water samples were collected from various sources including surface water, ground water, influent waste water, and effluent waste water. The surface water and ground water samples were collected locally. Waste water samples were provided by Dr. David Reckhow (University of Massachusetts, Amherst). Samples were collected into pre-washed 250 mL HDPE bottles. A blank of each sample was retained for extraction and the remaining samples were spiked with various levels of PFAS compounds and corresponding isotopically labeled standards. The isotope labeled internal standards were utilized to correct for matrix effects as well as any recovery losses from sample preparation.

Sample extraction was performed using ISO 25101 as a guideline with minor method adjustments to accommodate the extended list of PFAS compounds. Oasis WAX 6 cc, 150 mg SPE Cartridges (p/n: 186002493) were used for the sample extraction of 250 mL water samples. The full method for sample preparation is outlined in Figure 1. This method provides a sample enrichment factor of 250×.

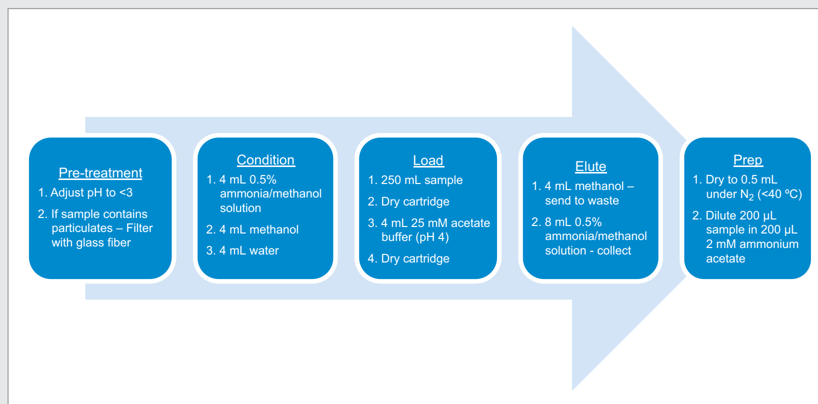

Figure 1. Full method details of SPE sample extraction for water samples.

LC conditions

LC system: ACQUITY UPLC I-Class PLUS fitted with the PFC Analysis Kit (p/n: 176001744)

Column: ACQUITY UPLC BEH C_{18} 2.1 × 100 mm, 1.7 µm (p/n: 186002352)

Column temp.: 35 °C

Sample temp.: 10 °C

Injection volume: 10 µL

Mobile phase A: 95:5 Water:methanol + 2 mM ammonium acetate

Mobile phase B: Methanol + 2 mM ammonium acetate

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B
0	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.3	20	80
14	0.4	5	95
17	0.4	5	95
18	0.3	100	0
22	0.3	100	0

MS conditions

MS system: Xevo TQ-S micro

Ionization mode: ESI-

Capillary voltage: 0.5 kV

Desolvation temp.: 350 °C

Desolvation gas flow: 900 L/hr

Cone gas flow: 100 L/hr

Source temp.: 100 °C

Method events: Divert flow to waste from 16 to 21 minutes

MRM parameters for each compound were optimized using the QuanOptimize™ tool in MassLynx Software and are listed in Appendix A.

RESULTS AND DISCUSSION

INSTRUMENT PERFORMANCE AND DETECTION LIMITS

The LC-MS/MS method utilized was fit for purpose for the determination of a range of PFAS compounds of interest. An overlay chromatogram showing the chromatography of all the compounds is shown in Figure 2. Peak shape of the early eluting compounds suffer from slight broadening due to the significant difference in solvent composition between the starting LC gradient and sample.

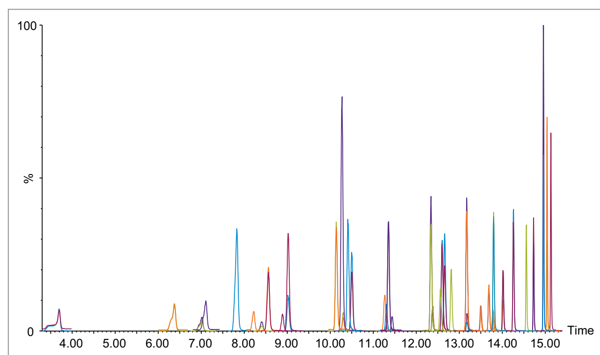

Figure 2. Overlay of all PFAS compounds analyzed in the method.

Detection limits can be seen for all compounds in Table 1. Due to the concentration enhancement provided from the sample preparation procedure, the detection limits are reported as both in-vial and in-sample (250-fold lower than vial concentration) limits. For the most part, in-sample detection limits were sub-ng/L (ppt), reaching to the pg/L (ppq) levels. A few of the less water soluble compounds had ng/L (ppt) detection limits. The detection limits detailed in Table 1 are suitable for current requirements for PFAS testing.

Calibration was very linear over several orders of magnitude for all compounds. An example of a typical solvent calibration curve can be seen in Figure 3, showing an example for PFOA, along with a chromatogram of PFOA at its detection limit.

During sample analysis, the ERA standard was used as a QC for instrument performance. The instrument performed within the designated Acceptance Limits for all compounds. The average error from the certified values was 15%, although many were below 10% error.

Table 1. Detection limits in vial and sample for all PFAS compounds.

Compound	LOD vial (ng/L)	LOD sample (ng/L)	R^2
PFBA	10	0.04	0.999
PFPeA	10	0.04	0.999
PFHxA	10	0.04	0.999
PFHpA	5	0.02	0.999
PFOA	<2	<0.01	0.999
PFNA	10	0.04	0.999
PFDA	10	0.04	0.999
PFUnDA	10	0.04	0.999
PFDoDA	10	0.04	0.999
PFTriDA	10	0.04	0.993
PFTreDA	10	0.04	0.999
PFHxDA	500	2.00	0.994
PFOcDA	2000	8.00	0.988
PFBS	4.4	0.02	0.999
PFPeS	4.7	0.02	0.999
PFHxS	3.7	0.01	0.999
PFHpS	9.5	0.04	0.999
PFOS	3.65	0.01	0.999
PFNS	4.8	0.02	0.999
PFDS	9.6	0.04	0.999
N-EtFOSAA	10	0.04	0.999
N-MeFOSAA	5	0.02	0.999
FHUEA	5	0.02	0.999
FOUEA	5	0.02	0.999
8:2 diPAP	500	2.00	0.997
4:2 FTS	23.4	0.09	0.999
6:2 FTS*	<95	<0.38	0.999
8:2 FTS	9.6	0.04	1.000
PFecHS	9.2	0.04	0.999
FHEA	20	0.08	0.999
FOEA	8	0.03	0.999
FDEA	20	0.08	0.999
FHpPA	5	0.02	0.999
GenX	20	0.08	0.999
ADONA	<2	<0.01	0.999
9Cl-PF3ONS	<1.9	<0.01	0.999
11Cl-PF3OUdS	9.42	0.04	0.996
NFHDA	5	0.02	0.999
PFEESA	<2	<0.01	0.999
PFMBA	<2	<0.01	0.999

**The true detection limit for 6:2 FTS cannot be determined due to contamination. The concentration listed here as the LOD signifies the approximate contamination level.*

SAMPLE PREPARATION PERFORMANCE

Overall performance of the sample preparation method can be summarized in the recovery values highlighted in Figure 4. A majority of the PFAS compounds fell within the recovery range of 75% to 130%. A few compounds had lower recoveries, including the C13 and C14 (PFTriDA and PFTreDA) carboxylates, as well as one of the emerging PFAS compounds, 11ClPF3OUdS. PFTriDA and PFTreDA are known to be less water soluble than the smaller chain PFCAs (perfluorinated carboxylic acids). Adjusting the final sample's solvent composition could be investigated to achieve better recoveries, but the impact to the remaining compounds must be evaluated. Also, a few compounds exhibited very high recovery rates, including PFBA, 6:2 FTS, and PFODA. PFBA and 6:2 FTS have been determined to be common contaminant compounds in the laboratory where the sample analysis was performed. Source(s) of the contamination was investigated but has not yet been able to be determined. PFODA appears to experience a matrix stabilization effect, and this was reported in a prior application note.[3] Use of the isotope labeled internal standards to correct for loss through sample prep improves the accuracy further, as demonstrated by the green bars in Figure 4.

Repeatability of the method was assessed from the analysis of six replicates of ground water spiked with the PFASs. The orange squares in Figure 4 represent the %RSD of the six replicates of ground water taken through the entire sample preparation method and analysis. All PFASs had a %RSD below 15%, with most being below 10%. This indicates the sample analysis method is reproducible.

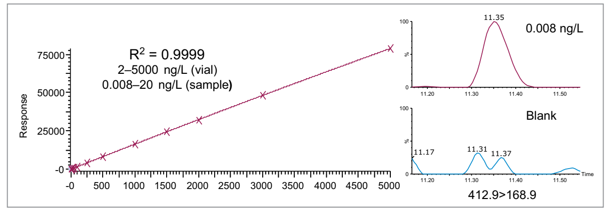

Figure 3. Demonstration of the linearity and sensitivity of PFOA showing calibration curve and peak at the detection limit of 0.008 ng/L compared to a blank.

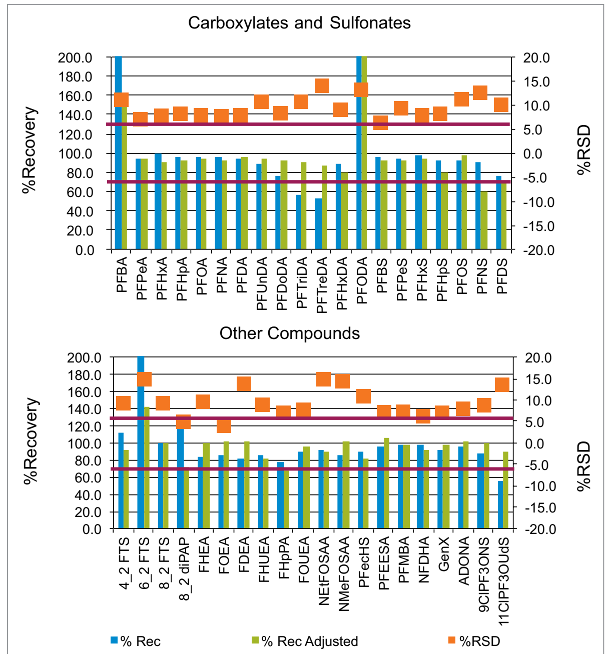

Figure 4. Method recovery (blue bars/left axis) and method reproducibility (orange squares/right axis) for all PFAS compounds covered in method. The adjusted recovery (green bars/left axis) represents the compound response corrected to its internal standard.

METHOD ROBUSTNESS

The robustness of the instrument over a series of matrix injections was evaluated using a spiked surface water extract. 20 replicate injections were performed to assess peak area, retention time, and ion ratio stability in a complex matrix. Stability of all three parameters over 20 injections are shown in Figure 5 for PFOA. Peak area is plotted in TrendPlot™ to determine the %RSD, a peak overlay is shown to represent the retention time is not shifting, and ion ratio data indicates the ion ratios are stable. In the example shown for PFOA, the %RSD of peak areas is approximately 3%. Overall, a %RSD of less than 10% was seen for all PFASs in the method.

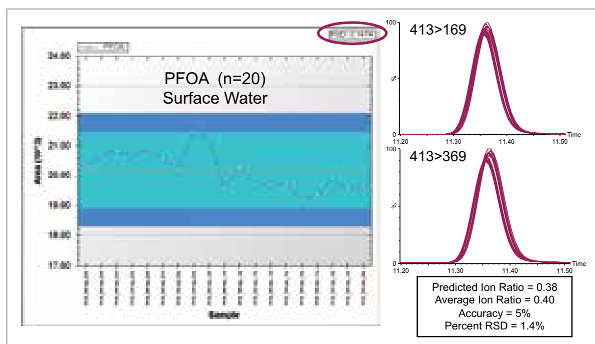

Figure 5. Repeatability assessed by 20 replicate injections of surface water. Peak area of PFOA for each injection is plotted in TrendPlot with an RSD of 3% (left) and the peak overlay of replicate injections with ion ratio information (right).

ANALYSIS OF ENVIRONMENTAL WATER SAMPLES

Four different types of environmental water samples were extracted and analyzed to test the described method including surface water, ground water, influent waste water, and final effluent waste water. A range of different PFASs were detected at varying concentrations in all samples. Figure 6 shows an example of a few PFASs identified in a surface water sample which include both legacy and emerging PFASs of interest. As shown in Figure 6, the identified PFASs were not present in the extraction blank and therefore can be confirmed as identified in the sample and not a from a source of background PFAS contamination.

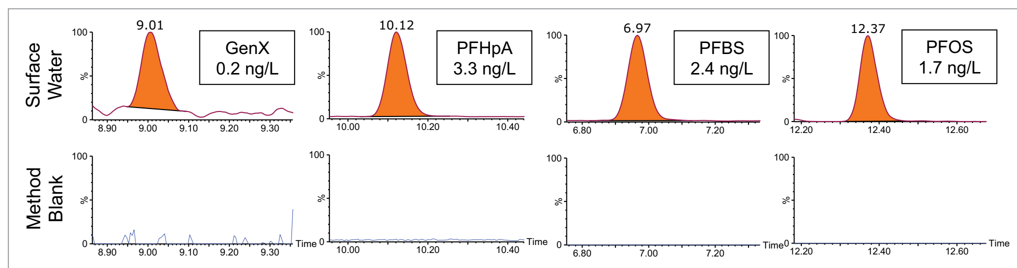

Figure 6. Identification of PFAS compounds in surface water sample extract (right) compared to the extraction blank (left). The blank is scaled to the surface water peak.

Figure 7 demonstrates the different patterns and concentrations of PFASs identified in the environmental water samples. From the list of 40 compounds screened, 27 were detected in the four samples. All samples contained both legacy and emerging PFAS compounds. Both waste water samples contained the highest levels and the largest numbers of different PFASs. Of the six PFASs detected in the ground water sample, half were emerging contaminants (PFEESA, PFMBA, and NFDHA).

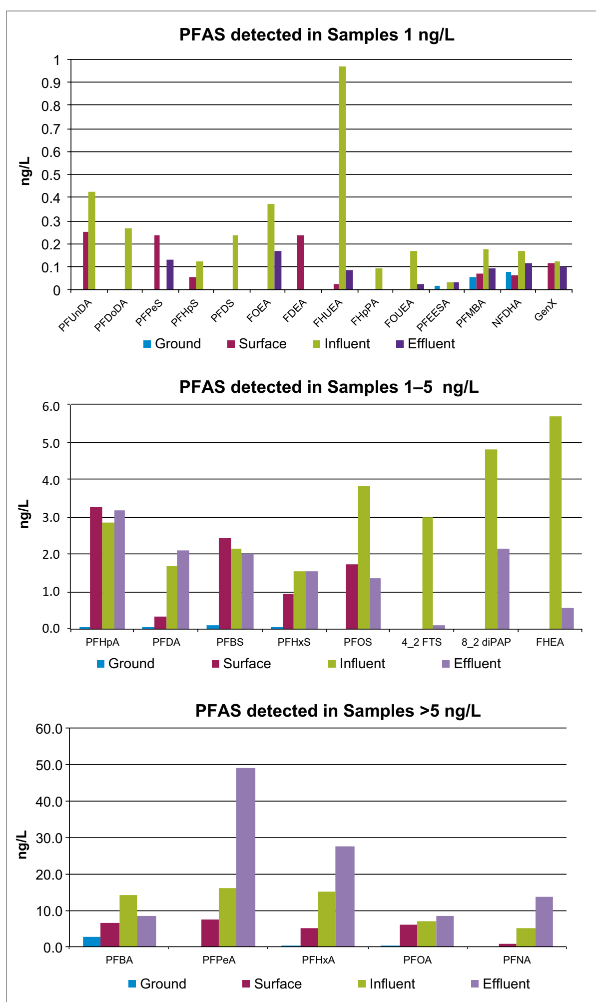

Figure 7. Patterns of PFASs detected in environmental water samples grouped by concentration level.

CONCLUSIONS

- Using SPE preparation of water samples provides a 250× enrichment of the sample allowing for analysis using the Xevo TQ-S micro.
- Achievable detection limits with this method on the Xevo TQ-S micro align with the necessary action levels set by the European Framework Directive and the U.S. EPA health advisory.
- Following the guidance of ISO 25101, analysis of environmental water samples can be accomplished for determination of both legacy and emerging PFASs.
- The method was verified by the use of the ERA certified QC standard, enhancing confidence in results.
- The method described is robust and has been applied to the analysis of a various range of environmental water samples including surface, ground, and waste waters.

Acknowledgements

The authors would like to acknowledge Honeywell for the productive conversations and considerations for providing the special mobile phase solvents for this work. The authors would also like to acknowledge Dr. David Reckhow and team at the University of Massachusetts, Amherst for providing waste water samples for this work.

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Appendix

Compound	CAS number	PFAS class	Type	Precursor	Product	CV	CE	RT
PFBA	375-22-4	carboxylate	legacy	212.9	169	10	10	3.6
PFPeA	2706-90-3	carboxylate	legacy	262.9	219	10	5	6.3
PFHxA	307-24-4	carboxylate	legacy	312.9	269 119	5	10 20	8.5
PFHpA	375-85-9	carboxylate	legacy	362.9	319 169	15	10 15	10.1
PFOA	335-67-1	carboxylate	legacy	412.9	369 169	10	10 15	11.3
PFNA	375-95-1	carboxylate	legacy	462.9	418.9 219	10	10 15	12.3
PFDA	335-76-2	carboxylate	legacy	512.9	468.9 219	15	10 15	13.1
PFUnDA	2058-94-8	carboxylate	legacy	562.9	518.9 269	25	10 20	13.8
PFDoDA	307-55-1	carboxylate	legacy	612.9	568.9 169	30	10 25	14.2
PFTriDA	72629-94-8	carboxylate	legacy	662.9	618.9 169	5	10 30	14.6
PFTreDA	376-06-7	carboxylate	legacy	712.9	668.9 169	10	15 25	14.7
PFHxDA	67905-19-5	carboxylate	legacy	812.9	768.8 169.2	40	10 40	15.0
PFODA	16517-11-6	carboxylate	legacy	912.9	868.9 169.2	35	15 35	15.1
PFBS	29420-49-3	sulfonate	legacy	298.9	80.1 99.1	15	30 30	7.0
PFPeS	2706-91-4	sulfonate	legacy	348.9	80.1 99.1	10	30 30	8.8
PFHxS	3871-99-6	sulfonate	legacy	398.9	80.1 99.1	10	35 30	10.3
PFHpS	375-92-8	sulfonate	legacy	448.9	80.2 99.1	15	35 35	11.4
PFOS	1763-23-1	sulfonate	legacy	498.9	80.2 99.1	15	40 40	12.3
PFNS	N/A	sulfonate	legacy	548.9	80.2 99.2	20	40 40	13.2
PFDS	335-77-3	sulfonate	legacy	598.9	80.2 99.1	25	40 40	13.8
N-MeFOSAA	2991-50-6	sulfonamidoacetic acid	legacy	569.9	418.9 219.1	35	20 25	13.5
N-EtFOSAA	2355-31-9	sulfonamidoacetic acid	legacy	584	418.8 525.9	15	20 20	13.8
FHUEA	70887-88-6	unsaturated telomer acid	legacy	356.9	292.9 243	10	10 35	10.4
FOUEA	70887-84-2	unsaturated telomer acid	legacy	456.9	393 343	10	10 40	12.6
8_2 diPAP	678-41-1	phosphate ester	legacy	989	97 542.5	10	40 20	15.0
4_2 FTS	757124-72-4	telomer sulfonate	legacy	326.9	307 81.1	15	15 35	8.4
6_2 FTS	29420-49-3	telomer sulfonate	legacy	426.9	407 344.9 81	15	20 10 35	11.3
8_2 FTS	39108-34-4	telomer sulfonate	legacy	526.9	506.8 444.6 81.2	15	25 10 40	13.1
PFecHS	67584-42-3	cyclic	legacy	460.9	380.9 99.1	40	30 30	11.2
FHEA	53826-12-3	telomer acid	legacy	376.9	292.9 313	5	15 5	10.5
FOEA	27854-31-5	telomer acid	legacy	476.9	393 413	5	10 5	12.6
FDEA	53826-13-4	telomer acid	legacy	576.9	492.9 512.9	15	15 5	14.0
FHpPA	812-70-4	other	legacy	440.9	336.9 317	15	10 20	12.5
ADONA	958445-44-8	other	emerging	376.9	251 85	10	10 25	10.2
9Cl-PF3ONS	73606-19-6	other	emerging	530.9	350.9 83	15	25 25	12.8

Compound	CAS number	PFAS class	Type	Precursor	Product	CV	CE	RT
11Cl-PF3OUdS	73606-19-6	other	emerging	630.9	450.8 83	30	30 30	14.0
GenX	13252-13-6	other	emerging	285	119 185	5	35 7	9.0
PFMBA	863090-89-5	other	emerging	278.9	85 235	10	10 5	7.0
NFDHA	151772-58-6	other	emerging	294.9	85 201	5	20 10	8.2
PFEESA	113507-82-7	other	emerging	314.9	83 135	15	20 20	7.8
13C-PFBA	–	–	–	216.9	172	10	10	3.6
13C5-PFPeA	–	–	–	267.9	223	10	5	6.3
13C5-PFHxA	–	–	–	317.9	272.9 119.9	10	5 20	8.5
13C4-PFHpA	–	–	–	366.9	321.9 169 172	15	10 15 15	10.1
13C8-PFOA	–	–	–	420.9	375.9 172	5	10 15	11.3
13C9-PFNA	–	–	–	471.9	426.9 223	10	10 15	12.3
13C6-PFDA	–	–	–	518.9	473.9 223	5	10 15	13.1
13C7-PFUnDA	–	–	–	569.9	524.9 274	5	10 15	13.8
13C-PFDoDA	–	–	–	614.9	569.9 169 269.1	10	10 25 20	14.2
13C2-PFTreDA	–	–	–	714.9	669.9 169	25	10 35	14.7
13C2-PFHxDA	–	–	–	815	769.9 169.3	30	15 35	15.0
13C3-PFBS	–	–	–	301.9	80 99	10	30 25	7.0
13C3-PFHxS	–	–	–	401.9	80.1 99.1	10	40 35	10.3
13C8-PFOS	–	–	–	506.9	80.1 99.1	15	40 40	12.3
D5-N-EtFOSAA	–	–	–	589	418.9 506.9	30	20 15	13.8
D3-N-MeFOSAA	–	–	–	572.9	418.9 482.7 514.7	35	20 15 20	13.5
13C-FOUEA	–	–	–	458.9	393.9 119.1	25	10 40	12.6
13C4-8:2 diPAP	–	–	–	993	97.3 544.8	30	40 25	15.0
13C2-4:2 FTS	–	–	–	328.9	308.9 81	40	15 25	8.4
13C2-6:2 FTS	–	–	–	428.9	367 408.8	10	10 20	11.3
13C2-8:2 FTS	–	–	–	528.9	508.9 81	10	20 35	13.1
13C-FHEA	–	–	–	378.9	293.9 64.1	5	10 5	10.5
13C-FOEA	–	–	–	478.9	393.9 64.1	10	15 10	12.6
13C-FDEA	–	–	–	578.9	493.9 64.2	25	5 5	14.0
13C3-GenX	–	–	–	287	169 119	5	12 12	9.0

Large Volume Direct Injection Method for the Analysis of Perfluorinated Alkyl Substances (PFASs) in Environmental Water Samples in Accordance with ASTM 7979-17

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APPLICATION BENEFITS

Performing the ASTM 7979-17 method on the Xevo™ TQ-XS allows:

- Limited sample preparation of small sample volumes to speed up analysis time and enhance sample throughput.
- Sensitive analysis of PFAS compounds in the low ng/L range to meet regulatory requirements.
- A robust and reliable solution for monitoring PFAS compounds in non-drinking water matrices.

WATERS SOLUTIONS

ACQUITY™ UPLC™ I-Class System

ACQUITY UPLC CSH Phenyl Hexyl Column

PFC Analysis Kit

Xevo TQ-XS

MassLynx™ MS Software

KEYWORDS

Perfluorinated, polyfluorinated, PFAS, PFC, AFFF

INTRODUCTION

Perfluoroalkyl substances (PFASs) are a class of anthropogenic compounds that are found in a range of consumer goods and industrial processes due to their chemical properties. Common uses include firefighting foams, insecticide formulations, water-resistant coating, floor polishes, and oil-resistant coatings for paper products approved for food contact. Due to their widespread use and subsequent leaching from materials, PFASs are so ubiquitous that they are frequently detected throughout the environment and in 2009, they were classified as persistent organic pollutants (POPs) within the Stockholm Convention.[1] Due to their persistent, ubiquitous nature, and possible toxicity, most regulatory agencies worldwide closely monitor the use, occurrence, and impact of both traditional/common and newer, replacement short-chain PFASs.

For monitoring and research purposes, ng/L, or part-per-trillion (ppt), detection of PFASs is often required. Within the U.S. drinking water is regulated under the Safe Drinking Water Act, while other environmental waters are regulated under the Clean Water Act. In the third Unregulated Contaminant Monitoring rule (UCMR3)[2] for drinking water, the U.S. EPA has required monitoring of six different PFAS compounds with a minimum reporting level in the range of 30 to 200 ng/L for each component. The U.S. EPA has also issued a health advisory[3] acute level at 70 ng/L based on the best available peer-reviewed studies of PFAS effects. Within the EU, drinking water is regulated under the Drinking Water Directive, 98/83/EC, while other environmental waters are regulated under the EC Water Framework Directive (WFD), 2013/39/EU.[4] In the WFD, PFOS is specifically identified as a "priority hazardous substance."

In this application note we describe the use of the recently developed ASTM 7979-17 method (EPA Region 5, Dr. Lawrence B. Zintek)[5] to analyze PFASs of interest in environmental waters, not only as described by U.S. legislation, but also those of interest elsewhere, including newer compounds (ADONA, 9Cl-PF3ONS, and 11Cl-PF3OUdS). Since many countries look to the U.S. EPA and other agencies for guidance, it was decided to include as many compounds in a single analysis as was feasible at relevant detection levels.

EXPERIMENTAL

The ASTM 7979-17 currently covers the analysis of 21 PFAS compounds, with 10 additional compounds listed for consideration in the appendix of the method. For this analysis, eight additional compounds were added to the method to bring the total number of PFAS analytes to 39. Three of the compounds added to the method are emerging PFAS compounds of interest including ADONA, 9Cl-PF3ONS (the main component of F-53B), and 11Cl-PF3OUdS (minor component of F-53B). Table 1 contains the compound information for all of the PFAS compounds included in this method. All standards were obtained from Wellington Laboratories (Guelph, Ontario).

Table 1. PFAS compounds included in the analysis.

Name	Abbreviation	CAS number	PFAS class	ASTM 7979-17 compound	Surrogate
perfluorobutanoic acid	PFBA	375-22-4	carboxylate	x	^{13}C-PFBA
perfluoropentanoic acid	PFPeA	2706-90-3	carboxylate	x	$^{13}C_5$-PFPeA
perfluorohexanoic acid	PFHxA	307-24-4	carboxylate	x	$^{13}C_5$-PFHxA
perfluoroheptanoic acid	PFHpA	375-85-9	carboxylate	x	$^{13}C_4$-PFHpA
perfluorooctanoic acid	PFOA	335-67-1	carboxylate	x	$^{13}C_8$-PFOA
perfluorononanoic acid	PFNA	375-95-1	carboxylate	x	$^{13}C_9$-PFNA
perfluorodecanoic acid	PFDA	335-76-2	carboxylate	x	$^{13}C_6$-PFDA
perfluoroundecanoic acid	PFUnDA	2058-94-8	carboxylate	x	$^{13}C_7$-PFUnDA
perfluorododecanoic acid	PFDoDA	307-55-1	carboxylate	x	^{13}C-PFDoDA
perfluorotridecanoic acid	PFTriDA	72629-94-8	carboxylate	x	
perfluorotetradecanoic acid	PFTreDA	376-06-7	carboxylate	x	$^{13}C_2$-PFTreDA
perfluorohexadecanoic acid	PFHxDA	67905-19-5	carboxylate		$^{13}C_2$-PFHxDA
perfluorooctadecanoic acid	PFOcDA	16517-11-6	carboxylate		–
perfluorobutyl sulfonate	PFBS	29420-49-3	sulfonate	x	$^{13}C_3$-PFBS
perfluoropentane sulfonate	PFPeS	2706-91-4	sulfonate	Additional	–
perfluorohexyl sulfonate	PFHxS	3871-99-6	sulfonate	x	$^{13}C_3$-PFHxS
perfluoroheptane sulfonate	PFHpS	375-92-8	sulfonate	Additional	–
perfluorooctyl sulfonate	PFOS	1763-23-1	sulfonate	x	$^{13}C_8$-PFOS
perfluorononane sulfonate	PFNS	N/A	sulfonate	Additional	–
perfluorodecane sulfonate	PFDS	335-77-3	sulfonate	Additional	–
perfluoro-1-octanesulfonamide	FOSA	754-91-6	sulfonamide	Additional	$^{13}C_8$-FOSA
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	sulfonamidoacetic acid	Additional	D_5-N-EtFOSAA
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	sulfonamidoacetic acid	Additional	D_3-N-MeFOSAA
N-methylperfluorooctane sulfonamide	N-MeFOSA	31506-32-8	sulfonamide		D-N-MeFOSA
N-ethylperfluorooctane sulfonamide	N-EtFOSA	4151-50-2	sulfonamide		D-N-EtFOSA
6:2 flurotelomer unsaturated carboxylic acid (n-2H-perfluoro-2-octenoic acid)	FHUEA	70887-88-6	unsaturated telomer acid	x	–
8:2 fluorotelomer unsaturated carboxylic acid (2H-perfluoro-2-decenoic acid)	FOUEA	70887-84-2	unsaturated telomer acid	x	^{13}C-FOUEA
8:2 fluorotelomer phosphate diester	8:2 diPAP	678-41-1	phosphate ester		$^{13}C_4$-8:2 diPAP
4:2 fluorotelomer sulfonate	4:2 FTS	757124-72-4	telomer sulfonate	Additional	$^{13}C_2$-4:2 FTS
6:2 fluorotelomer sulfonate	6:2 FTS	29420-49-3	telomer sulfonate	Additional	$^{13}C_2$-6:2 FTS
8:2 fluorotelomer sulfonate	8:2 FTS	39108-34-4	telomer sulfonate	Additional	$^{13}C_2$-8:2 FTS
n-decafluoro-4-(pentafluoroethyl) cyclohexanesulfonate	PFecHS	67584-42-3	cyclic	x	–
n-2-perfluorohexyl ethanoic acid	FHEA	53826-12-3	telomer acid	x	^{13}C-FHEA
n-2-perfluorooctyl ethanoic acid	FOEA	27854-31-5	telomer acid	x	^{13}C-FOEA
n-2-perfluorodecyl ethanoic acid	FDEA	53826-13-4	telomer acid	x	^{13}C-FDEA
n-3-perfluoroheptyl propanoic acid	FHpPA	812-70-4	other	x	–
dodecafluoro-3H-4,8-dioxanonanoate	ADONA	958445-44-8	other		–
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS	73606-19-6	other		–
11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	73606-19-6	other		–

A Certified QC Standard (Cat #731) from ERA (Golden, CO), for use with ground and surface waters, was used as an instrumental QC check throughout the analysis. The standard contained a mix of 12 PFAS compounds. Certified values and QC Performance Acceptance Limits for each compound in the mix are provided with the standard, making instrumental QC evaluation fast and straightforward.

Since required detection limits are in the low ng/L range and as a result of the widespread use of PFASs, specific challenges must be addressed for sample collection, preparation, and analysis. There are many common sources of PFAS contamination in the field and laboratory. In the field, caution should be taken to avoid Teflon containing materials (such as waterproof clothing/jackets), plastic clipboards, waterproof notebooks, and chemical ice packs. In the lab, items to avoid include sticky notes, certain glass disposable pipettes, aluminum foil, vial caps with Teflon seals, and LDPE containers, to name a few. In fact, it is recommended that all laboratory supplies be checked for PFAS contamination before use, as is practical. Contamination is unavoidable from the chromatographic system. Therefore steps should be taken to minimize any system contribution, and as such, the Waters™ PFC Analysis Kit (p/n: 176001744) for the UPLC system was utilized. The kit is comprised of PFAS-free components (such as PEEK tubing to replace the conventional Teflon coated solvent lines) and a PFC Isolator Column that helps to delay any residual background interferences from co-eluting with the analytical peak. Installation of the PFC Analysis Kit is straightforward and quick.[6]

Sample pretreatment

Samples were provided by the U.S. EPA Region 5 through a Cooperative Research and Development Agreement (EPA CRADA #884-16). Provided samples included reagent water, surface (river) water, ground water, influent waste water, and effluent waste water. Each water sample was spiked with a low and high level of a selection of PFAS compounds (three replicates of each concentration) prior to being received in the lab. Two blanks of each sample were also received.

The entirety of each 5-mL water sample was used to avoid any compound loss. Each sample was spiked with 160 ng/L of isotopically labeled surrogates (see Table 1). The surrogates are added to the sample prior to any preparation in order to determine method recoveries. 5 mL of methanol was then added to each water sample and vortexed for 2 min. The entire 10 mL sample was filtered using a disposable polypropylene syringe with a glass filter (25 mm diameter, 1.0 µm pore size) stacked on top of a polypropylene GHP filter (25 mm diameter, 0.2 µm pore size). Following filtration, 10 µL of acetic acid was added to each sample. An aliquot of each sample was transferred to a polypropylene autosampler vial and sealed with a Polyethylene Cap (p/n: 186005230).

LC conditions

LC system:	ACQUITY UPLC I-Class fitted with PFC Analysis Kit
Column:	ACQUITY UPLC CSH Phenyl Hexyl 1.7 µm, 2.1 × 100 mm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	30 µL
Mobile phase A:	95:5 Water:methanol + 2 mM ammonium acetate
Mobile phase B:	Methanol + 2 mM ammonium acetate
Gradient:	

Time (min)	Flow rate (mL/min)	%A	%B
0	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.3	20	80
14	0.4	5	95
17	0.4	5	95
18	0.3	100	0
22	0.3	100	0

MS conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Capillary voltage:	1.0 kV
Desolvation temp.:	500 °C
Desolvation gas flow:	1100 L/hr
Cone gas flow:	150 L/hr
Source temp.:	120 °C
Method events:	Divert flow to waste from 15 to 21 min

Method optimization using QuanOptimize

All MRM parameters for each compound were optimized using the QuanOptimize™ tool in MassLynx. QuanOptimize will automatically determine the parent ion, fragment ions, cone voltage, and collision energy required for each individual compound through injection. The MRMs generated from QuanOptimize for this method are detailed in Appendix Table A. By providing the mass or chemical formula in the MassLynx sample list, QuanOptimize will step through the cone voltages and collision energies designated in the QuanOptimize method. The software then automatically processes the results and generates a report with the MRM transition and corresponding cone voltage and collision energy (Figure 1). This tool also allows rapid and simple optimization of MRM method parameters for new compounds that may need to be added to the analysis method in the future.

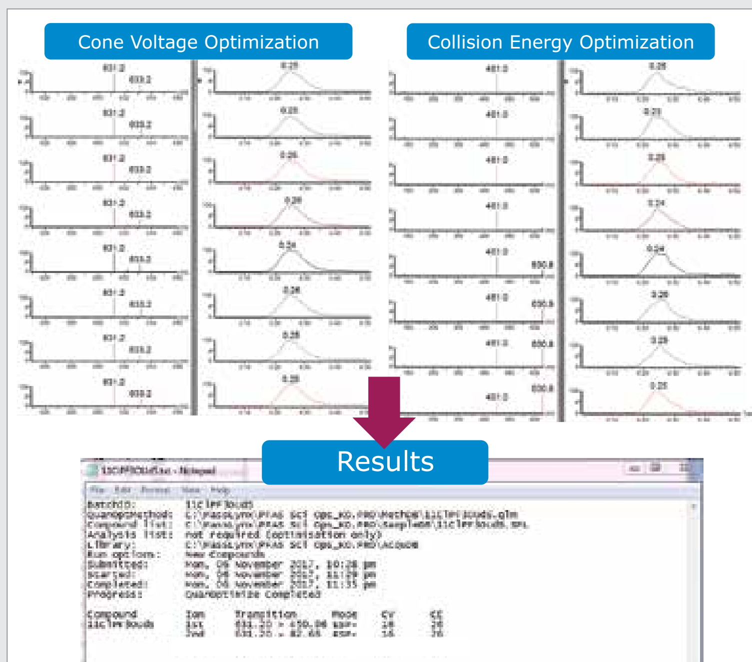

Figure 1. Example of the MassLynx QuanOptimize tool showing the process of cone voltage and collision energy optimization and the results from compound optimization.

RESULTS AND DISCUSSION

Sample analysis was performed as described in ASTM 7979-17. One minor change was made to the mobile phase composition. For this work, methanol was used in place of acetonitrile. Also, the concentration of ammonium acetate added to both mobile phases was reduced to 2 mM from the suggested 20 mM in the official method. Both changes were made due to solubility concerns of ammonium acetate in acetonitrile. These changes had no negative effects on method performance, such as peak resolution or response but made the LC method more robust. An overlaid chromatogram of all native compounds and isotope surrogates is demonstrated in Figure 2.

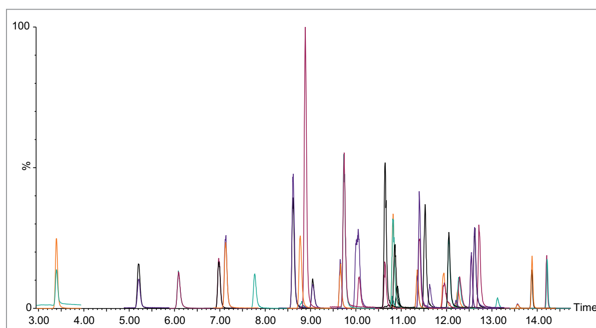

Figure 2. Overlay of all PFAS compounds analyzed in the method.

METHOD DETECTION LIMITS

A Method Detection Limit (MDL) study was performed to assess the sensitivity of the sample analysis method. Nine replicate samples were prepared by spiking reagent water with various concentrations of the PFAS analytes (Table 2) and 80 ng/L of the surrogate standard solution. All samples were taken through the sample pre-treatment procedure prior to analysis. MDL values were calculated using the equation:

MDL = SD x t_{n-1}, where SD = standard deviation of n replicates and t_{n-1} = 2.896 (student t value for n-1 samples)

The MDL values were all well below the required reporting limits stated in the ASTM 7979 method, indicating that this method is more than suitable for this analysis. An MDL value could not be calculated for telomer sulfonate isomer 6:2 FTS due to contamination of this compound in the solvents used for sample preparation. The remaining PFAS compounds experienced no background interference or contamination.

Calibration curve requirements in ASTM 7979 require an R^2 value of 0.98 or greater for linear regression fit. All compounds were within this requirement, as highlighted in Table 2. Example calibration curves are also shown in Figure 3 for PFOA and PFOS. Figure 3 also shows chromatograms for PFOA and PFOS injected at 2.5 ng/L. This demonstrates the sensitivity at half the required reporting limit for these two compounds.

Table 2. Method Detection Limit (MDL) results for all compounds in method.

Compound	Sample spike (ng/L)	MDL (ng/L)	Reporting range (ng/L)*	R^2
PFBA	100	25.20	50–2000	0.993
PFPeA	10	1.04	50–2000	0.999
PFHxA	10	1.33	10–400	0.999
PFHpA	10	0.91	10–400	0.999
PFOA	10	1.42	10–400	0.999
PFNA	10	1.32	10–400	0.999
PFDA	10	0.84	10–400	0.998
PFUnDA	10	2.52	10–400	0.996
PFDoDA	10	1.76	10–400	0.993
PFTriDA	10	2.34	10–400	0.991
PFTreDA	10	1.99	10–400	0.993
PFHxDA	200	25.41	–	0.984
PFOcDA	400	41.99	–	0.983
PFBS	10	1.21	10–400	0.999
PFPeS	10	1.07	10–400	0.999
PFHxS	10	1.41	10–400	0.999
PFHpS	10	1.57	10–400	0.999
PFOS	10	1.61	10–400	0.999
PFNS	10	1.67	10–400	0.999
PFDS	10	1.44	10–400	0.997
FOSA	10	1.29	10–400	0.999
N-Et-FOSAA	10	1.90	10–400	0.997
N-Me-FOSAA	10	1.59	10–400	0.999
N-Et-FOSA	10	1.45	–	0.997
N-Me-FOSA	10	1.19	–	0.999
FHUEA	10	1.53	10–400	0.999
FOUEA	10	1.36	–	0.999
8:2 diPAP	300	50.16	–	0.988
4:2 FTS	10	1.50	10–400	0.999
6:2 FTS	10	N/A	10–400	0.999
8:2 FTS	10	2.62	10–400	0.997
PFecHS	10	1.17	10–400	0.998
FHEA	200	42.19	300–8000	0.994
FOEA	200	50.38	200–8000	0.997
FDEA	200	79.48	200–8000	0.993
FHpPA	10	1.47	10–400	0.999
ADONA	10	0.82	–	0.999
9Cl-PF3ONS	10	1.06	–	0.999
11Cl-PF3OUdS	10	1.45	–	0.998

**Reporting ranges listed are as set in the ASTM 7979-17 method.[5]*

Figure 3. Example chromatograms and calibration curves for PFOA (top) and PFOS (bottom). Chromatograms are representative of an injection of 2.5 ng/L, which is half the required lower reporting limit.

CONTROL SAMPLES

The ASTM 7979-17 method requires control samples to be run with the criteria they must pass as outlined in Figure 4. All compounds passed the control criteria, with the exception of 6:2 FTS due to solvent contamination of that compound.

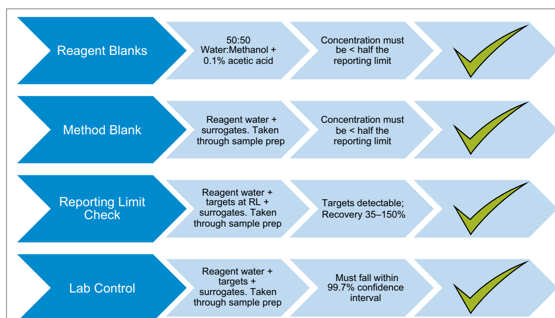

Figure 4. Criteria set by ASTM 7979-17 method for control samples.

SAMPLE ANALYSIS

Triplicates of each spiked matrix at both concentrations were prepared and analyzed using the method according to ASTM 7979-17. Only the compounds currently written into the ASTM method were spiked into the water samples. All PFAS compounds that were spiked into the various water samples were detected at both the high and low concentration spike. PFBA and PFPeA were spiked at 300 ng/L and 1000 ng/L in the low and high spike samples, respectively. 4:2, 6:2, and 8:2 FTS were spiked at 1200 ng/L and 4000 ng/L in the low and high spike, respectively. All other PFAS compounds were spiked at 60 ng/L in the low spike samples and 200 ng/L in the high spike samples. Figure 5 shows an example of all the PFAS compounds spiked at the low concentration level in the surface (river) water sample.

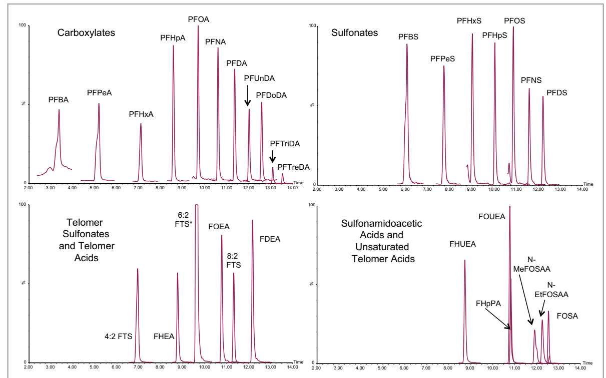

*Figure 5. All PFAS compounds detected in a low concentration spiked surface water sample. PFBA and PFPeA at 300 ng/L; 4:2, 6:2, and 8:2 FTS at 1200 ng/L; all other compounds at 60 ng/L. *Compound shown off scale.*

Recovery of the PFAS compounds was determined using isotope labelled surrogate standards that were spiked into the samples prior to sample pre-treatment and analysis. For compounds that did not have a surrogate available, a compound close in retention time and chemical structure was used as the surrogate. Table 3 demonstrates the percent recovery of all PFAS compounds spiked into the five water samples. ASTM 7979 requires percent recoveries to be in the range of 70% to 130%. All compounds included in the method were within this range with the exception of PFTreDA, PFTriDA, and FDEA. These compounds exhibited an enhancement effect when analyzed from a prepared sample compared to the response exhibited in solvent standards. The enhancement could be associated with co-elution of these compounds with matrix components in the sample. Correction of sample concentration can be performed if necessary based on the percent recovery exhibited by the surrogate standard or by quantification using matrix matched calibration curves.

The method proved to be repeatable as well, demonstrated by the %RSD values highlighted in Appendix Table B. All matrix samples were processed in triplicate, which is demonstrated by the n=3 RSD values. These values represent the full method reproducibility. A single sample of reagent water and ground water were also injected 20 times to produce instrument repeatability data (%RSD values in Appendix Table B). Again, due to solvent contamination of 6:2 FTS, an accurate %RSD value for the method replicates could not be calculated. For the most part, the %RSD values fell below 10%, with a majority of compounds exhibiting RSDs below 5%.

Table 3. Percent recoveries of all compounds spiked into water samples.

Compound	Average %recovery in matrix					Recovery compound
	Reagent water	Ground water	Surface water	Influent water	Effluent water	
PFBA	82.7	82.1	80.9	80.8	85.5	^{13}C-PFBA
PFPeA	89.1	87.7	90.2	88.1	91.4	$^{13}C_5$-PFPeA
PFHxA	89.7	90.1	91.7	91.3	93.3	$^{13}C_5$-PFHxA
PFHpA	90.6	89.8	92.6	91.3	91.9	$^{13}C_4$-PFHpA
PFOA	92.5	92.0	94.2	94.7	94.3	$^{13}C_8$-PFOA
PFNA	93.0	92.2	94.3	94.8	95.2	$^{13}C_9$-PFNA
PFDA	97.0	97.1	100.2	100.7	99.2	$^{13}C_6$-PFDA
PFUnDA	106.4	102.9	107.1	106.2	108.0	$^{13}C_7$-PFUnDA
PFDoDA	116.3	113.3	119.0	118.5	120.0	^{13}C-PFDoDA
PFTriDA	198.3	183.7	205.5	228.0	197.1	$^{13}C_2$-PFTreDA
PFTreDA	198.3	183.7	205.5	228.0	197.1	$^{13}C_2$-PFTreDA
PFBS	94.6	92.1	96.8	93.8	96.1	$^{13}C_3$-PFBS
PFPeS	94.6	92.1	96.8	93.8	96.1	$^{13}C_3$-PFBS
PFHxS	89.8	88.1	91.3	91.9	93.5	$^{13}C_3$-PFHxS
PFHpS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS
PFOS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS
PFNS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS
PFDS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS
FOSA	92.9	92.8	95.1	94.3	95.9	$^{13}C_8$-FOSA
N-Et-FOSAA	127.4	120.6	127.7	129.4	130.0	D_5-N-EtFOSAA
N-Me-FOSAA	122.7	122.7	123.2	127.3	126.3	D_3-N-MeFOSAA
FHUEA	98.2	96.3	100.3	102.2	100.8	^{13}C-FOUEA
FOUEA	98.2	96.3	100.3	102.2	100.8	^{13}C-FOUEA
4:2 FTS	108.0	97.5	99.1	104.0	110.6	$^{13}C_2$-4:2 FTS
6:2 FTS	108.3	96.4	117.9	107.6	100.0	$^{13}C_2$-6:2 FTS
8:2 FTS	107.9	116.3	103.5	117.8	121.2	$^{13}C_2$-8:2 FTS
FHEA	100.2	98.4	104.3	105.3	110.4	^{13}C-FHEA
FOEA	100.5	94.9	99.1	101.1	102.6	^{13}C-FOEA
FDEA	155.0	140.8	164.1	162.3	159.1	^{13}C-FDEA
FHpPA	97.0	97.1	100.2	100.7	99.2	$^{13}C_6$-PFDA

CONCLUSIONS

- The ASTM 7979-17 method allows for quick sample turnaround time due to minimal sample preparation.
- The results described here meet and exceed the ASTM 7979-17 method.
- The results described here meet and exceed the EPA health advisory acute levels of 70 ng/L PFOS.
- The large volume direct injection method used on the Xevo TQ-XS was extremely sensitive with method detection limits in the low ng/L range for many compounds.
- All targeted PFAS compounds were detected in the water samples analyzed at both low and high concentrations with excellent recovery and reproducibility.

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Appendix

Appendix Table A. MRM method details.

Compound	Parent	Fragment	CV	CE	RT
PFBA	213.0	169	8	5	3.01
PFPeA	262.9	218.9	5	5	4.78
PFHxA	312.9	268.9	16	6	6.68
		118.9	16	21	
PFHpA	362.9	318.9	14	8	8.18
		168.9	14	14	
PFOA	412.9	368.9	22	7	9.31
		168.9	22	15	
PFNA	462.9	418.9	18	9	10.23
		218.9	18	15	
PFDA	512.9	468.9	6	9	11.00
		218.9	6	15	
PFUnDA	562.9	518.9	8	8	11.66
		268.9	8	14	
PFDoDA	612.9	568.9	12	12	12.22
		168.9	12	21	
PFTriDA	662.9	168.9	14	22	12.73
		218.9	14	20	
PFTreDA	712.9	218.9	14	22	13.18
		168.9	14	20	
PFHxDA	812.9	168.9	22	28	13.86
		218.9	22	22	
PFODA	912.9	168.9	34	28	14.38
		218.9	34	28	
PFBS	289.9	80.1	7	27	5.62
		99.1	7	27	
PFPeS	348.9	79.9	32	31	7.31
		98.9	32	25	
PFHxS	398.9	80.1	38	35	8.59
		99.1	38	29	
PFHpS	448.9	79.9	16	34	9.62
		98.9	16	34	
PFOS	498.9	79.9	30	42	10.47
		98.9	30	40	
PFNS	548.9	80.1	24	40	11.20
		99.1	24	36	
PFDS	598.9	80.1	46	46	11.83
		99.1	46	46	
FOSA	498.0	77.9	40	29	12.25
N-EtFOSAA	584.0	418.9	34	15	11.89
		525.9	34	18	
N-MeFOSAA	569.9	418.9	36	15	11.55
		168.9	36	27	
N-EtFOSA	526.0	169	18	25	13.89
		219	18	21	
N-MeFOSA	511.9	168.9	16	25	13.56
		218.9	16	21	
FHUEA	356.9	292.9	20	12	8.34
		242.9	20	27	
FOUEA	456.9	392.9	20	11	10.41
		118.9	20	44	
8:2 diPAP	989.0	96.73	31	33	14.01
		542.9	31	25	
4:2 FTS	326.9	306.9	42	18	6.55
		80.9	42	27	
6:2 FTS	427.0	406.9	12	22	9.24
		80.1	12	32	
8:2 FTS	526.9	506.9	28	26	10.96
		80.9	28	37	
PFecHS	460.9	380.9	44	22	9.61
		99.1	44	22	
FHEA	376.9	292.9	13	11	8.37
		312.9	13	5	

Compound	Parent	Fragment	CV	CE	RT
FOEA	476.9	392.9	9	11	10.40
		242.9	9	22	
FDEA	576.9	492.9	5	24	11.83
		512.9	5	7	
FHpPA	440.9	336.9	26	10	10.48
		316.9	26	19	
ADONA	376.9	251	12	10	8.45
		84.9	12	22	
9Cl-PF3ONS	531.0	351	14	22	11.11
		82.9	14	20	
11Cl-PF3OUdS	631.0	450.9	16	26	12.31
		82.9	16	26	
^{13}C-PFBA	217	172	7	8	3.01
$^{13}C_5$-PFPeA	268	223	11	7	4.78
$^{13}C_5$-PFHxA	318	273	10	6	6.68
		120	10	18	
$^{13}C_4$-PFHpA	367	322	16	7	8.18
		172	16	15	
$^{13}C_8$-PFOA	421	376	6	8	9.31
		172	6	16	
$^{13}C_9$-PFNA	472	172	7	18	10.23
		223	7	18	
$^{13}C_6$-PFDA	519	473.9	25	7	11.00
		219	25	13	
$^{13}C_7$-PFUnDA	569.9	524.9	9	8	11.66
		273.9	9	14	
^{13}C-PFDoDA	615	569.9	23	10	12.22
		168.9	23	22	
$^{13}C_2$-PFTreDA	715	168.9	18	25	13.18
		219	18	25	
$^{13}C_2$-PFHxDA	815	169	14	31	13.86
		219	14	22	
$^{13}C_3$-PFBS	301.9	80.1	34	28	5.62
		99.1	34	24	
$^{13}C_3$-PFHxS	402	80.1	13	38	8.59
		99.1	13	30	
$^{13}C_8$-PFOS	507	80.1	36	34	10.47
		99.1	36	34	
$^{13}C_8$-FOSA	506	77.9	13	28	12.25
D_5-N-EtFOSAA	589	418.9	24	17	11.89
		482.9	24	13	
D_3-N-MeFOSAA	573	418.9	17	18	11.55
		515	17	18	
D-N-EtFOSA	531	168.9	15	25	13.89
		218.9	15	23	
D-N-MeFOSA	515	168.9	34	21	13.56
		218.9	34	21	
^{13}C-FOUEA	458.9	393.9	14	10	10.41
		395	14	18	
$^{13}C_4$-8:2 diPAP	993	97.1	38	33	14.01
$^{13}C_2$-4:2 FTS	329	309	14	18	6.55
		80.9	14	21	
$^{13}C_2$-6:2 FTS	429	409	48	21	9.24
		80.9	48	27	
$^{13}C_2$-8:2 FTS	529	509	20	27	10.96
		80.9	20	37	
^{13}C-FHEA	379	294	10	23	8.37
		63.9	10	5	
^{13}C-FOEA	478.9	393.9	14	14	10.40
		243.9	14	23	
^{13}C-FDEA	579	494	15	19	11.83
		244	15	40	

Appendix Table B. Reproducibility represented by %RSD of all compounds in each matrix. n=3 replicates represent the method reproducibility, n=20 replicates represent the instrument reproducibility.

	% RSD in matrix											
	Reagent water			Ground water			Surface water		Influent water		Effluent water	
	Low spike (n=3)	Low spike (n=20)	High spike (n=3)	Low spike (n=3)	Low spike (n=20)	High spike (n=3)	Low spike (n=3)	High spike (n=3)	Low spike (n=3)	High spike (n=3)	Low spike (n=3)	High spike (n=3)
PFBA	4.6	7.0	2.1	15.6	2.1	2.7	2.1	2.8	1.7	5.8	5.7	4.3
PFPeA	2.5	1.2	0.7	7.4	1.7	1.6	2.2	0.4	3.0	3.0	6.6	2.7
PFHxA	2.8	2.2	3.8	6.6	1.8	1.7	1.6	1.9	2.6	1.6	9.8	3.1
PFHpA	2.6	1.9	1.1	4.4	4.0	2.4	8.9	2.1	3.3	1.7	7.8	3.1
PFOA	8.8	5.6	1.0	5.9	5.1	1.5	9.5	2.5	4.5	1.5	5.1	2.3
PFNA	3.4	6.9	7.6	6.3	6.8	2.5	8.2	1.0	2.4	11.2	4.9	6.4
PFDA	1.4	4.2	2.7	8.7	5.1	1.3	5.2	1.9	2.2	4.3	8.8	5.1
PFUnDA	7.0	6.2	1.3	9.3	6.2	2.7	8.9	1.9	10.6	8.5	9.8	3.8
PFDoDA	1.0	5.8	2.4	2.0	6.5	11.5	8.1	0.5	4.8	8.2	7.0	4.2
PFTriDA	0.8	3.5	0.5	5.0	2.3	1.4	4.0	2.6	3.6	8.2	5.0	4.7
PFTreDA	4.0	5.5	3.1	8.4	6.0	2.2	2.3	1.5	1.5	14.4	8.2	2.5
PFBS	1.1	2.5	0.3	6.2	1.7	1.2	1.5	1.5	4.4	6.6	2.5	3.3
PFPeS	2.0	2.2	1.1	2.5	2.4	0.3	1.4	0.1	2.5	2.4	1.4	1.9
PFHxS	2.5	3.9	1.2	2.6	4.0	1.7	2.5	4.1	5.4	2.3	10.9	4.1
PFHpS	1.4	4.3	1.0	6.2	3.2	3.8	2.6	1.6	2.7	3.8	3.6	2.6
PFOS	9.0	4.4	1.8	7.9	5.7	3.1	7.2	1.5	4.9	1.5	9.5	3.0
PFNS	5.3	3.8	3.0	4.0	3.9	2.8	5.6	1.3	1.5	6.3	10.2	2.8
PFDS	5.7	6.6	1.6	3.0	5.5	3.7	3.0	1.8	3.8	4.0	0.3	3.3
FOSA	2.0	2.6	1.5	5.8	3.1	2.2	4.4	0.7	0.8	2.0	15.6	2.9
N-Et-FOSAA	4.4	5.0	3.5	2.8	4.9	3.0	2.3	4.1	6.9	7.5	1.0	4.2
N-Me-FOSAA	4.2	4.3	2.3	3.9	4.1	2.2	2.2	3.2	0.6	6.2	11.5	4.2
FHUEA	2.6	1.8	1.7	1.7	2.2	0.4	0.6	2.0	0.6	2.2	1.2	2.2
FOUEA	1.0	2.4	2.3	0.1	2.7	2.1	2.9	1.0	0.7	1.2	0.6	2.0
4:2 FTS	0.9	3.2	17.6	2.3	3.1	1.8	12.3	5.3	11.4	2.5	10.2	4.9
6:2 FTS	N/A	4.1	N/A	N/A	3.2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8:2 FTS	6.4	7.8	2.8	4.9	8.2	2.5	10.8	6.6	6.2	4.2	3.1	8.6
FHEA	4.8	5.3	6.3	3.2	7.6	1.5	8.2	0.6	3.6	4.5	9.1	3.5
FOEA	1.2	9.0	3.7	8.2	7.4	7.0	9.9	3.3	10.0	3.0	4.2	1.1
FDEA	6.2	6.8	4.4	3.6	7.4	4.6	7.8	5.1	8.6	11.2	9.5	7.8
FHpPA	2.1	2.9	0.9	1.5	3.2	2.6	2.1	1.8	0.9	1.3	0.3	2.4

[TECHNOLOGY BRIEF]

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Ultra Low-Level Detection of Perfluoroalkyl Substances (PFASs) Using the PFC Analysis Kit

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GOAL

To demonstrate the use of the PFC Analysis Kit on the ACQUITY UPLC® I-Class System and the limits of detection afforded for PFASs analyzed on the Xevo® TQ-S.

BACKGROUND

Perfluoroalkyl substances (PFASs) encompass a range of fully fluorinated alkyl compounds, typically with an anionic end group. Previously described as perfluoroalkyl compounds (PFCs), they have been implemented in a range of consumer goods and industrial processes due to their hydro- and lipophobic properties.
As a result of their widespread use and subsequent leaching from materials, they have been found in various environmental and biological samples. PFASs are also ubiquitous in the laboratory environment, namely in Teflon components used in analytical equipment. Waters® PFC Analysis Kit (part no. 176001744) specifically addresses this challenge. The kit is comprised of PFAS-free components to ensure a clean system, a sample preparation protocol, along with standards, columns, vials and caps.

The PFC Analysis Kit can be implemented on the ACQUITY UPLC I-Class System and Xevo TQ-S to achieve ultra-sensitive detection of routinely monitored PFASs.

Figure 1. Side panel removal for sample manager wash tubing removal (fitting circled in red).

Alterations to existing ACQUITY UPLC systems can easily be performed. Although minor variations exist in the solvent line configurations across the ACQUITY UPLC models, the PFC Analysis Kit can be easily implemented across all models. In this technology brief, we describe the detection of various routinely monitored PFASs that was performed on the ACQUITY UPLC I-Class System and Xevo TQ-S. Special considerations for the I-Class with flow through needle (FTN) hardware are also detailed.

SOLUTION

The LC analysis was performed on an ACQUITY UPLC I-Class System. Pre-cut PEEK tubing from the PFC Analysis Kit were used to replace all of the Teflon solvent lines, per the kit instructions. The side panel of the I-Class sample manager was removed in order to replace the sample manager wash line, as shown in Figure 1; all of the other solvent lines changed are affixed in the BSM. The use of the ACQUITY® BEH C_{18} Isolator Column and pre-cut stainless steel tubing provided in the kit further ensured that PFAS contamination was retained prior to injection of the sample. The LC method employed an ACQUITY BEH C_{18} (50 x 2.1 mm, 1.7 µm) Column, with the gradient described in Table 1a. Standards provided in the PFC Kit were used to create the dilution series, covering a range of three orders of magnitude. The Xevo TQ-S showed exceptional sensitivity for the analytes, with their respective MRMs and optimized instrumental parameters summarized in Table 1b.

Measured limits of detection (based on peak-to-peak, signal-to-noise measurements of 1:3) for the analytes were below 0.07 ng/mL in solvent standards. In the case of the most commonly monitored PFAS analyte, perfluorooctanesulfonate (PFOS), the limit of detection was 0.0125 ng/mL (Figure 2). These low limits of detection highlight the notable sensitivity of this platform for PFASs analysis.

Min	Flow rate (mL/min.)	%A	%B
Initial	0.65	90	10
0.5	0.65	90	10
5.1	0.65	0	100
6.6	0.65	0	100
6.7	0.65	90	10
8.5	0.65	90	10

Table 1a. ACQUITY UPLC I-Class gradient utilized for the analysis. Mobile phase A was 98:2 water:methanol, 2 mM ammonium acetate, and mobile phase B was methanol 2 mM ammonium acetate.

Compound	Formula	MRM*	RT (min)	CE (V)	Cone Voltage (V)
PFBuA	$C_4HF_7O_2$	**212.9 > 168.9**	0.76	15	30
		212.9 > 212.9		2	30
PFPA	$C_5HF_9O_2$	**262.9 > 262.9**	1.94	2	30
		262.9 > 218.9		10	30
PFBuS	$C_4HF_9O_3S$	**298.9 > 79.9**	2.25	35	20
		298.9 > 98.9		25	20
PFHxA	$C_6HF_{11}O_2$	**312.9 > 268.9**	2.84	5	30
		312.9 > 118.9		20	30
PFHpA	$C_7HF_{13}O_2$	**362.9 > 318.9**	3.42	10	30
		362.9 > 168.9		15	30
PFHxS	$C_6HF_{13}O_3S$	**398.9 > 79.9**	3.49	40	30
		398.9 > 98.9		40	30
		398.9 > 118.9		30	30
PFOA	$C_8HF_{15}O_2$	**412.9 > 368.9**	3.84	10	30
		412.9 > 168.9		20	30
		412.9 > 218.9		15	30
PFNA	$C_9HF_{17}O_2$	**462.9 > 418.9**	4.18	10	30
		462.9 > 218.9		10	30
PFOS	$C_8HF_{17}O_3S$	**498.9 > 79.9**	4.20	45	30
		498.9 > 98.9		45	30
		498.9 > 229.9		35	30
PFDA	$C_{10}HF_{19}O_2$	**512.9 > 218.9**	4.46	20	30
		512.9 > 268.9		15	30
		512.9 > 468.9		10	30
PFUnDA	$C_{11}HF_{21}O_2$	**562.9 > 518.9**	4.69	15	30
		562.9 > 268.9		20	30
		562.9 > 318.9		20	30
PFDoDA	$C_{12}HF_{23}O_2$	**612.9 > 568.9**	4.90	15	35
		612.9 > 168.9		30	35
PFTiA	$C_{13}HF_{25}O_2$	**662.9 > 618.9**	5.07	15	35
		662.9 > 318.9		20	35
		662.9 > 368.9		20	35
PFTeTA	$C_{14}HF_{27}O_2$	**712.9 > 668.9**	5.22	15	35
		712.9 > 218.9		25	35
13C PFOS		**502.9 > 79.9**	4.20	45	30
		502.9 > 98.9		35	30
13C PFOA		**416.9 > 168.9**	3.84	18	30
		416.9 > 171.9		16	30
13C PFDA		**514.9 > 269.9**	4.46	15	30
		514.9 > 219.9		20	30

Table 1b. PFASs surveyed with optimized MRMs, RT, collision energies (CE), and cone voltages. Bold MRM transitions indicate the quantitative trace. Italicized compounds are isotopically labeled standards provided in the PFC Analysis Kit.

Figure 2. MRM at limit of detection for PFOS, as linearity shown for calibration curve over three orders of magnitude.

SUMMARY

Accurate and highly sensitive PFASs analysis can be achieved using the PFC Analysis Kit, which is adaptable to any ACQUITY UPLC system. A complete analytical solution for the PFASs is presented, particularly when used in combination with the Xevo TQ-S for ultra low-level detection.

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Identification of Potential Metabolites of Pharmaceutical Residues Detected in an Environmental Water Sample

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APPLICATION BENEFITS

- HRMS Screening of a large target list, with adducts
- Fast UPLC® analysis with the ACQUITY® UPLC HSS C_{18} Column
- Incurred residue metabolite identification

WATERS SOLUTIONS

Screening Platform Solution with UNIFI®

ACQUITY UPLC I-Class System

Xevo® G2-S QTof Mass Spectrometer

ACQUITY UPLC HSS C_{18} Column

KEYWORDS

Pharmaceuticals, personal care products, PPCPs, pesticide, environmental water sample, UNIFI, screening, HRMS, metabolite identification, pesticide screening

INTRODUCTION

In recent years, there has been increasing concern regarding the presence of pesticides, pharmaceuticals, and personal care products (PPCPs) in water bodies throughout the world.[1] A greater demand is being placed on techniques not only used to screen for these compounds, but to screen for the presence of their metabolites.

Data obtained from a non-targeted acquisition on a high resolution mass spectrometer can be used to target a theoretical unlimited number of compounds. Moreover, information rich datasets collected using UPLC/MS^E can be used to reduce the large number of false detects that arise when targeting a large number of compounds verses accurate mass as a sole point of contaminant identification. MS^E provides accurate mass measurements for both precursor and fragment ion information in a single experiment by alternating scans between low and high collision energies. In combination with UNIFI, an integrated scientific information system, it is now possible to screen for the presence of PPCPs, their adducts, and potential metabolites in a routine laboratory environment.

Previous work presented described the use of the Waters Screening Platform Solution in combination with Waters' toxicology library to initially screen a local well water sample for the presence of a large number (>1000) of PPCPs, pesticides and drugs of abuse.[2] In this application note, we have processed the same dataset with the metabolite identification aspect of the integrated software system to isolate known and potential metabolites of the confident screening matches in the dataset. Once discovered, metabolites were made available for future screening experiments by adding the detection results (retention time and identified fragment ions) into a scientific library.

EXPERIMENTAL

A locally obtained well water sample was enriched one thousand times as previously described.[2,3] A comprehensive dataset, collected using UPLC/MS^E was obtained within UNIFI. The toxicology screening solution within UNIFI contains pre-defined LC-MS conditions and processing parameters. The toxicology library in UNIFI is comprised of over 1000 compounds including many PPCPs, such as drugs of abuse, veterinary medicines, and pharmaceuticals. Library entries also contain retention times and accurate theoretical fragment masses. Experimental conditions, sample preparation protocols, and data processing parameters are available in a previous application note by the same authors.[2]

RESULTS AND DISCUSSION

From a previous application note,[2] the screening of a local well water sample against the full toxicology library in UNIFI, with up to three adducts (H^+, Na^+, K^+), indicated the presence of the four compounds shown in Table 1.

Table 1. Component summary table in UNIFI showing details of confident matches made during a screening of the extracted well water sample against a library of over 1000 compounds.

Component name	Formula	m/z	Retention Time Error (min)	Mass error (ppm)	Identified High Energy Fragments	Response	Adducts
Carbamazepine	C15H12N2O	237.1021	0.23	-0.62	1	10262	+H
Hexamine	C6H12N4	141.1136	0.38	0.92	1	40806	+H
Imidacloprid	C9H10ClN5O2	256.0597	0.18	0.66	1	7907	+H
Tramadol	C16H25NO2	264.1956	0.43	-0.68	1	10893	+H

The inclusion of retention times and accurate mass fragment ions in the toxicology screening library allowed for confident matches to be made since they were based on more information other than accurate mass of the precursor ions alone. As indicated, this is critical for reducing false detection rates, enabling rapid data review for screening experiments.

Further investigation of the comprehensive dataset was possible using the metabolite identification functionality of UNIFI's screening solution software. This functionality requires a target molecule with mol file and a list of possible transformations, that are shown in Figure 1.

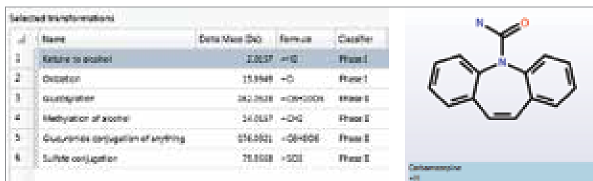

Figure 1. Transformations and an example mol file used to identify potential metabolites of compounds found in a screening experiment.

Primarily, using chemical intelligence,[4] the target mol file is systematically cleaved. This essentially increases the target list to include parent compounds and potential breakdown products in the metabolite search.Interrogation of the low energy function of the MS^E comprehensive dataset was performed, which automatically extracted the masses corresponding to the parent as well as the permutations of provided transformations, with and without systematic cleavages of the parent molecule. The list of possible metabolites for carbamazepine is shown in Table 2 and Figure 2.

No metabolites were observed for the other three compounds found in the screening experiment.

Table 2. Component summary of potential metabolites found for carbamazepine using the transformations and mol file shown in Figure 1.

#	Component name	Formula	m/z	Observed RT (min)	Mass error (ppm)	Response	Percentage of Parent Response (%)	Identification status
1	Carbamazepine	C15H12N2O	237.1021	7.49	-0.62	10282	100.000	Identified
2	Carbamazepine+O	C15H12N2O2	253.0975	4.34	1.49	3927	37.045	Identified
3	Carbamazepine+O	C15H12N2O2	253.0964	5.82	-2.80	6263	60.907	Identified
4	Carbamazepine+O	C15H12N2O2	253.0964	5.44	4.96	431	4.194	Identified

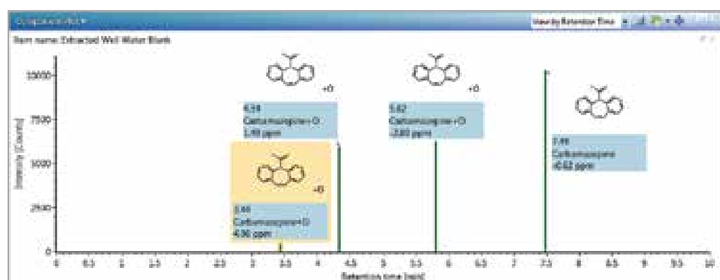

Figure 2. Component plot showing potential metabolites found for carbamazepine using the transformations and mol file shown in Figure 1.

Figures 3 and 4 show the full UI information details for the identification of carbamazapine and a carbamazepine oxidation respectively. Fragment match functionality within UNIFI uses similar intelligence as the cleavage algorithm above. It systematically dissects the mol file of the parent or proposed metabolite and assigns potential accurate mass fragment ions from the high energy function of the MS^E data. Identified fragment ions are annotated, as shown in Figure 3 for the mass 194.06691 Da, and in Figure 4 for the masses 210.09098 Da and 236.07105 Da.

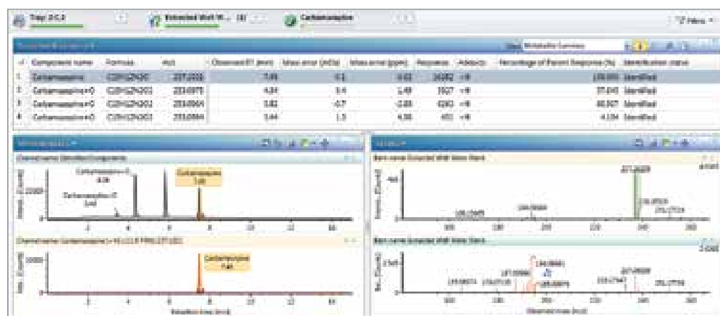

Figure 3. Full user interface (UI) information within UNIFI showing identification details of the carbamazepine parent. Component summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the component summary. The spectra section shows precursor and fragmentation spectra for the highlighted component.

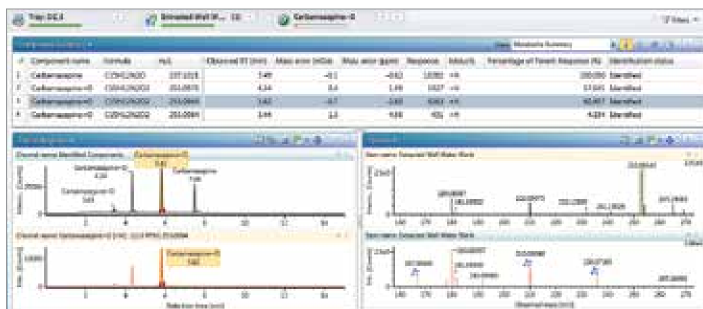

Figure 4. Full user interface (UI) information within UNIFI showing identification details of a proposed carbamazepine metabolite.Component Summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the component summary. The spectra section shows precursor and fragmentation spectra for the highlighted component.

Just as in screening experiments, the high energy fragment ions provided increased confidence that identified metabolites were correct. Common fragment and neutral loss discovery tools, readily available in UNIFI, can also be used to enhance the confidence in metabolite identification. Figure 5 shows the results of running a common fragment search. The two +O metabolites of carbamazepine at 4.3 and 5.8 minutes are shown to be related to each other by the fragment 210.0910 Da, which is the loss of 43.005 Da from the parent 253.0964 Da. This is the same neutral loss from the carbamazepine parent (237.1021 Da) to the primary fragment (194.0969 Da) thus giving further confidence in the metabolites identified.

Figure 5. Results from a common fragment search of 210.09098 Da, performed within the elucidation toolset in UNIFI.

Once the presence of a metabolite has been confirmed, the entry can be easily exported to an existing or new scientific library within UNIFI with the right click of the mouse, as shown in Figure 6. Details such as formula, retention time, theoretical accurate mass fragment ions, and spectra are made available for future users and analyses.

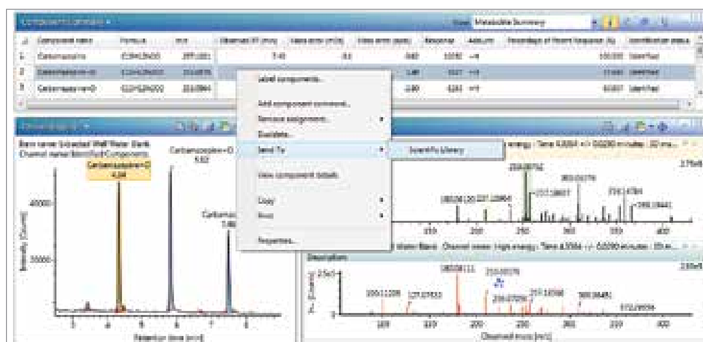

Figure 6. Sending reviewed metabolites to UNIFI's scientific library.

CONCLUSIONS

- Information rich MS^E acquisition and an integrated scientific information system make it possible to screen for the presence of compounds of interest, their adducts, and potential metabolites in a routine laboratory environment.
- The presence of retention times and accurate mass fragment ions in scientific libraries within UNIFI allowed identifications to be made on more information than accurate mass of the precursor ions alone. This proves critical for reducing false detection rates and enabling rapid data review for screening experiments.
- Using the metabolite identification functionality of UNIFI, three metabolites of carbamazepine were identified with confidence in an enriched local well water sample.
- Identified metabolites can easily be added to UNIFI's scientific library to expand the list of compounds targeted in future screening analyses.

References

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4. R J Mortishire-Smith, J M Castro-Perez, K Yu, J P Shockcor, J Goshawk, M J Hartshorn, A Hill. Generic dealkylation: a tool for increasing the hit-rate of metabolite rationalization, and automatic customization of mass defect filters. *Rapid Commun Mass Spectrom*. (2009) Apr 23, (7): 939–48. doi: 10.1002/rcm. 3951.

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[APPLICATION NOTE]

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Multi-Residue Analysis of Pharmaceuticals and Personal Care Products (PPCPs) in Water Using the ACQUITY UPLC H-Class System and the Xevo TQD Tandem Mass Spectrometer

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Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Extraction and concentration of low levels of compounds with a wide range of chemical diversity
- Use of a single LC-MS/MS method for separation and detection of PPCPs
- Quantification of PPCPs in the sub part-per-trillion range

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Xevo® TQD

ACQUITY UPLC HSS T3 Column

Oasis® Sample Extraction Products

TargetLynx™ Application Manager

KEYWORDS

environmental, personal care products, water, endocrine disruptors, PPCPs, PCPs

INTRODUCTION

In recent years, there has been increasing concern about the presence of pharmaceutical and personal care products (PPCPs)[1] in water bodies throughout the world. The effect of these emerging contaminants on human health and their potential impact on the environment is not yet fully understood. As concern continues to grow, many government agencies around the world are funding studies to assess if PPCPs can cause harmful ecological effects.

Many publications have shown that PPCPs are present at parts-per-trillion (PPT) levels in rivers and streams.[2-7] Methods therefore need to be able to detect compounds at these trace levels. In addition to the low level detection of PCPPs, a major analytical challenge for analysis lies in the wide chemical diversity of compound classes and structures, examples of which are shown in Figure 1. Furthermore, the complexity of the water samples requiring analysis can be very diverse. This application note demonstrates the extraction, separation, and detection of 78 PPCPs including acidic, basic, and neutral compounds in well and surface water samples.

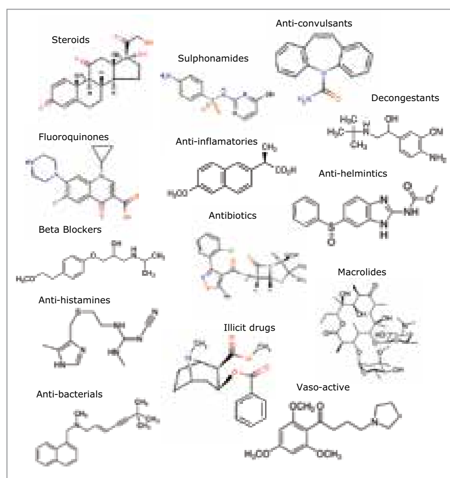

Figure 1. Example compounds from the range of pharmaceuticals and personal care products used in this work.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC H-Class
Runtime:	8.0 min
Column:	ACQUITY UPLC HSS T3 C_{18} 1.7 µm, 2.1 x 100 mm (p/n 186003539)
Column temp.:	60 °C
Mobile phase A:	10 mM NH_4formate pH 3.2 in water
Mobile phase B:	10 mM NH_4formate pH 3.2 in methanol
Elution:	5 min linear gradient from 5% (B) to 95% (B)
Flow rate:	0.450 mL/min
Injection volume:	100 µL

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI+/-
Capillary voltage:	3.0 kV
Cone voltage:	30.0 V
Source temp.:	150 °C
Desolvation temp.:	550 °C
Desolvation gas:	1100 L/hr
Cone gas:	50 L/hr

Samples

Two different water sample types were collected for analysis and stored at 4 °C prior to analysis. In addition, a reagent grade water sample with low levels of the PPCPs of interest was purchased for comparative analyses and to serve as a blank.

Reagent grade water: LC-MS grade water (Fisher Chemical, Optima brand)

Well-water sample: Sample collected from a local, private well-water source.

Surface water sample: Sample collected from a local water reservoir.

Sample preparation

The extraction process was performed using a tandem cartridge configuration with a Waters® 6-cc Oasis MAX (p/n 186000369) and a 6-cc Oasis MCX (p/n 186000256) SPE Cartridge. This configuration allows for a three-tiered extraction mechanism that uses reversed-phase, anion exchange, and cation exchange. The extraction protocol was designed to ensure retention of acidic, basic, and neutral PPCPs. The Oasis MCX Cartridge was connected below the Oasis MAX Cartridge, and both were conditioned by passing through 5 mL of methanol followed by 5 mL of water. The water samples (1 L) were loaded at 10 mL/min onto the dual stack by vacuum using a bottle-to-SPE adapter. Once the loading step was completed, the cartridge stack was disassembled and each cartridge followed specific wash and elution steps, as shown schematically in Figure 2. The Oasis MAX Cartridge was washed with 5 mL of 5% ammonium hydroxide in water. The elution was performed in two steps: first with 5 mL of methanol (neutral PPCPs), and second with 5 mL of methanol containing 5% formic acid (acidic PPCPs). Both elution fractions were collected in a 20-mL glass tube. The Oasis MCX Cartridge was washed with 5% formic acid and eluted with 5 mL methanol containing 5% ammonium hydroxide (basic PPCPs). The MCX and MAX elution fractions were pooled and evaporated to dryness at 60 °C under a gentle stream of nitrogen. The dried eluate was reconstituted with 900 µL (2 x 450 µL) 10 mM ammonium formate. The internal standard mix (100 µL) was then added to give a final concentration of 1.0 ppb. Matrix-matched calibration standards were prepared with the same protocol with the exception of the final eluate, which was reconstituted in 800 µL (2 x 400 µL) 10 mM ammonium formate, and 100 µL of the internal standard mix was added. The final 100 µL was utilized to post spike 100 µL of the PPCP mix at various concentrations in 10 mM ammonium formate. The standards for the majority of compounds were spiked at concentrations ranging from 0.1 to 5.0 ppb (0.1, 0.2, 0.25, 0.5, 1.0, 2.0, 2.5, and 5.0 ppb final concentration). This range equates to 0.1 to 5.0 ppt in the original sample. 13 compounds demonstrated higher limits of detection and were therefore analyzed from 1.0 to 50.0 ppb (equivalent to 1.0 to 50.0 ppt in the water samples). These compounds were cefalexin, cinoxacin, codeine, corticosterone, dicloxacillin, erythromycin, gemfibrozil, ibuprofen, ketoprofen, naproxen, tolfenamic acid, triamcinolone, and warfarin. The internal standard mix consisted of three isotopically labeled standards:

Cimetidine-d3-N-methyl-d3, Chlorpheniramine-d6-maleate-N,N dimethyl-d6, and Gemfibrozil-d6-2,2 dimethyl-d6 (purchased from C/D/N Isotopes Inc.).

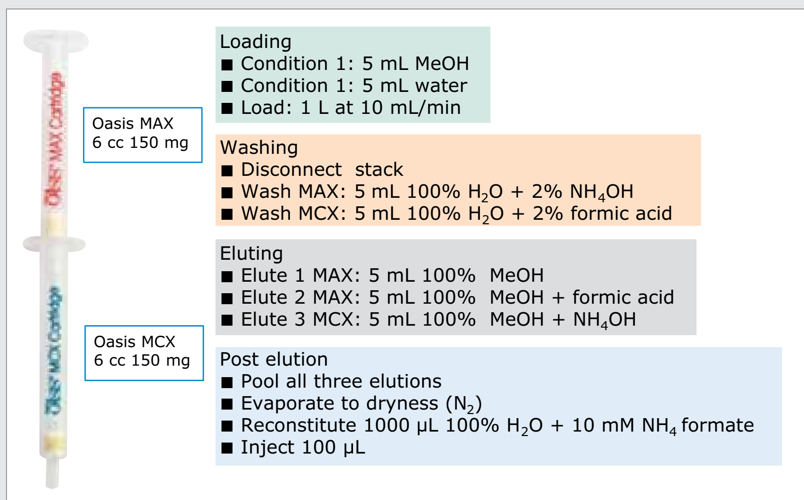

Figure 2. Schematic of solid phase extraction protocol for PPCPs in water.

LC-MS/MS

Two MRM transitions (quantification and confirmation) for the PPCPs were selected and optimized (Table 1). These results were added to the Quanpedia™ Database for future use in our own and other laboratories. For this application, finding the optimum chromatographic conditions for the multi-residue analysis posed a difficult challenge due to the chemical diversity of PPCPs. The best chromatographic separation was achieved with an ACQUITY UPLC HSS T3 2.1 x 100 mm analytical column (1.8 µm). The mobile phase that showed the best chromatography for the majority of compounds consisted of methanol/water with 10 mM ammonium formate (pH 3.2). Optima LC/MS grade methanol and water were purchased from Fisher Scientific.

Table 1. MRM tuning parameters and retention times for the PPCPs.

Compound	Ion mode	Precursor ion	Cone	Product ion	CE	RT (min)
6a-Methylprednisolone	ESI +	375.4	20	357.3	10	6.00
				339.3	10	
Acetaminophen	ESI +	152.1	35	110.0	15	2.58
				93.0	20	
Atenolol	ESI +	267.2	40	145.1	25	3.40
				190.1	20	
Azithromycin	ESI +	749.5	30	158.2	40	5.13
				591.5	30	
Beclomethasone dipropionate	ESI +	521.3	25	503.3	10	7.03
				319.2	15	
Benzocaine	ESI +	166.1	25	138.1	15	5.06
				77.0	25	
Bromhexine	ESI +	377.1	30	114.1	15	6.05
				263.9	30	
Buflomedil HCl	ESI +	308.3	30	140.1	15	4.46
				237.1	15	
Carazolol	ESI +	299.2	30	116.1	15	4.76
				221.1	20	
Cefalexin	ESI +	348.2	40	158.0	20	5.76
				139.9	35	
Chlorpheniramine	ESI +	275.2	25	230.1	15	5.14
				167.0	35	
Cimbuterol	ESI +	234.2	30	160.1	15	3.57
				143.1	25	
Cimetidine	ESI +	253.1	30	159.1	15	3.36
				117.1	15	
Cinoxacin	ESI +	263.2	35	245.1	15	4.79
				189.1	30	
Cocaine	ESI +	304.3	25	182.1	15	4.51
				82.0	25	
Codeine	ESI +	301.1	25	166.1	35	3.57
				216.1	25	
Corticosterone	ESI +	347.4	35	329.3	15	6.05
				311.2	15	
Cortisone	ESI +	361.3	40	163.1	25	5.61
				342.2	20	
Cotinine	ESI +	177.1	40	80.0	20	3.31
				98.0	20	
Dapsone	ESI +	249.2	40	156.0	15	3.88
				108.1	20	
Dexamethasone	ESI +	393.3	20	373.2	10	5.96
				355.2	10	
Dicloxacillin	ESI +	470.0	40	211.9	40	6.02
				254.0	25	
Diethylcarbamazine	ESI +	200.2	25	100.1	15	3.15
				72.0	25	
Difloxacin	ESI +	400.3	30	382.2	20	4.43
				356.2	20	
Digoxigenin	ESI +	391.5	30	355.3	15	5.00
				373.3	10	
Diltiazem	ESI +	415.2	30	178.1	20	5.51
				310.1	20	
Diphenhydramine	ESI +	256.1	20	167.1	5	5.30
				152.0	30	
Enrofloxacin	ESI +	360.3	25	342.3	20	4.28
				316.3	20	
Erythromycin	ESI +	734.50	30	158.1	30	5.89
				576.5	20	
Fleroxacin	ESI +	370.4	30	326.3	20	3.98
				269.3	25	
Flumequine	ESI +	262.1	35	244.0	15	5.50
				202.0	35	
Flumethasone	ESI +	411.4	25	391.2	5	5.85
				253.2	15	
Gemfibrozil	ESI -	249.1	30	121.0	10	7.06
				127.0	10	
Hydrocortisone	ESI +	363.4	35	121.1	25	5.73
				327.3	15	
Ibuprofen	ESI -	205.1	20	161.1	5	6.91
				NA		
Josamycin	ESI +	828.5	40	109	40	6.23
				174.2	35	
Ketoprofen	ESI -	253.1	20	209.1	5	6.02
				NA		
Levamisole (tetramisole)	ESI +	205.2	25	178.1	20	3.68
				91.1	30	
Lincomycin	ESI +	407.2	40	126.1	25	4.00
				359.3	20	
Metoprolol	ESI +	268.2	40	116.1	15	4.58
				74.1	20	
Miconazole	ESI +	417.1	40	161.1	30	7.12
				69.0	25	

Compound	Ion mode	Precursor ion	Cone	Product ion	CE	RT (min)
Nalidixic acid	ESI +	233.1	30	215.0	15	5.45
				187.0	25	
Naproxen	ESI -	229.0	20	170.1	15	6.12
				185.0	10	
Ofloxacin	ESI +	362.3	25	318.3	20	4.06
				261.3	30	
Oxfendazole	ESI +	316.1	40	159.0	30	5.29
				284.1	20	
Oxprenolol	ESI +	266.2	35	72.1	20	4.93
				116.1	15	
Pencillin G	ESI +	335.1	40	217.0	20	5.38
				317.0	20	
Praziquantel	ESI +	313.3	40	203.1	15	6.23
				83.1	25	
Procaine	ESI +	237.2	25	100.1	15	3.45
				120.0	25	
Promethazine	ESI +	285.2	25	86.1	15	5.59
				198.1	25	
Pyrimethamine	ESI +	249.2	40	177.1	30	4.95
				233.1	30	
Ranitidine	ESI +	315.2	25	176.1	15	3.38
				130.1	25	
Rifaximin	ESI +	786.5	40	151.1	45	6.61
				754.5	30	
Roxithromycin	ESI +	837.6	40	158.1	35	6.30
				679.5	20	
Salbutamol (albuterol)	ESI +	240.1	30	148.0	15	3.36
				222.1	10	
Sparfloxacin	ESI +	393.3	30	349.3	20	4.64
				292.3	25	
Sulfabenzamide	ESI +	277.1	30	156.0	15	4.45
				92.0	25	
Sulfadiazine	ESI +	251.1	30	156.0	15	3.42
				92.0	25	
Sulfadimethoxine	ESI +	311.1	40	156.0	15	4.78
				92.0	25	
Sulfadoxine	ESI +	311.3	40	156	15	4.40
				108.0	25	
Sulfamerazine	ESI +	265.1	35	92.0	25	3.72
				156.0	15	
Sulfameter	ESI +	281.1	35	92.0	25	3.93
				156.0	15	
Sulfamethazine	ESI +	279.1	35	186.0	15	4.13
				124.1	25	
Sulfamethizole	ESI +	271.1	30	156.0	15	3.93
				92.0	25	
Sulfamethoxazole	ESI +	254.1	30	92.0	25	4.18
				156.0	15	
Sulfamethoxypyridazine	ESI +	281.1	35	92.0	25	4.09
				156.0	15	
Sulfapyridine	ESI +	250.1	35	92.0	25	3.68
				156.0	15	
Terbinafine	ESI +	292.3	35	141	10	6.37
				93.0	15	
Ternidazole	ESI +	186.2	30	128.1	15	3.80
				82.0	25	
Tiamulin	ESI +	494.4	30	192.0	15	5.72
				119.0	30	
Ticlopidine	ESI +	264.1	30	125.0	25	5.32
				154.0	15	
Tilmicosin	ESI +	869.5	25	174.2	45	5.44
				696.5	40	
Tolbutamide	ESI +	271.1	30	91.0	30	5.77
				74.0	10	
Tolfenamic acid	ESI -	260.1	35	216.0	15	7.09
				180.0	15	
Triamcinolone	ESI +	395.4	30	375.0	10	4.80
				357.0	30	
Triamcinolone acetonide	ESI +	435.4	25	397.3	15	6.06
				415.3	5	
Triclocarban	ESI +	315.1	40	162.0	20	6.98
				128.0	30	
Trimethoprim	ESI +	291.3	40	123.0	30	3.95
				230.2	30	
Tripolidine	ESI +	279.1	25	208.2	15	5.26
				193.2	35	
Tulobuterol	ESI +	228.2	30	154.1	15	4.69
				118.0	25	
Warfarin	ESI -	307.1	40	161.0	20	6.22
				250.0	25	
Xylazine	ESI +	221.1	40	90.0	20	4.43
				164.0	25	

RESULTS AND DISCUSSION

Despite the chemical diversity of the compounds analyzed, excellent chromatographic profiles were obtained for all 82 compounds. Example chromatograms for the different classes of compounds are shown in Figure 3. Of the 82 PPCPs included in this work, 78 were found to be effectively extracted using the dual-cartridge SPE methodology. Five compounds (digoxigenin, fleroxacin, erythromycin, 6a-methylprednisolone, and tolbutamide) gave poor recoveries in the well water and surface water samples using this extraction protocol, although they were acceptable for the reagent water sample. Those compounds were therefore excluded from the quantitative analysis.

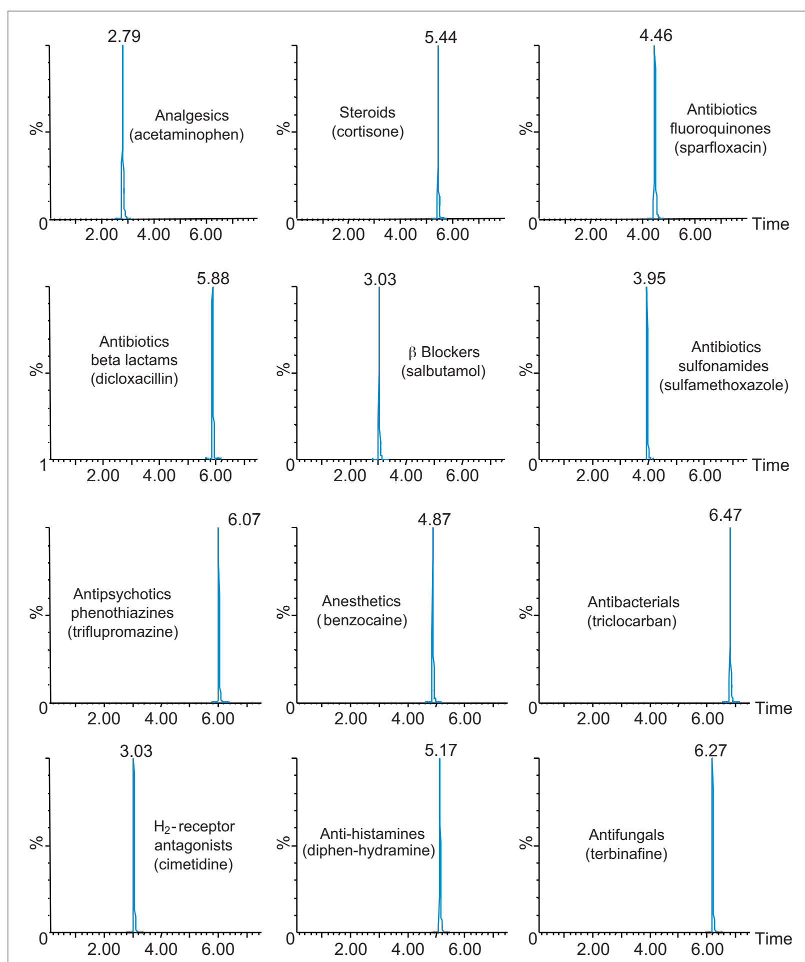

Figure 3. Example MRM chromatograms for compounds from the different classes of PPCPs represented in this work.

To ensure that the method did not result in carryover or false detections of PPCPs, blank reagent water samples were tested to find a clean water source that could be used as a blank sample and in order to create calibration standards. After screening several sources, Optima LC/MS grade water (Fisher Scientific) gave the best results. A blank sample of this reagent water was enriched using the SPE protocol. This extracted sample was analyzed and compared to post-spike samples of the same extract. From this work an estimation of the background level of the PPCPs in the reagent water could be made to determine whether it was sufficiently devoid of the target PPCPs. The results demonstrated that only four PPCPs were detected above the 100 ppq level in the reagent water sample (Table 2). Those compounds were enrofloxacin, fleroxacin, rifaximin, and diltiazem. These compounds were deemed to be present at levels between 100 ppq and 1 ppt in the reagent water. None of the compounds were found to have a response in the reagent water above 1 ppt. 46 compounds were detected below the lowest calibration point and 28 PPCPs were not detected at all in the reagent water blank.

Table 2. Results from the analysis of blank reagent water extract to determine levels of detected compounds. Any compounds that showed a response are indicated. Compounds that showed a response lower than the response of the post-spiked 0.1 ppt are labeled <0.1 ppt. Four compounds were detected above 0.1 ppt but below the 1.0 ppt level and are shown in ***bold*** *text. Compounds that did not show any response in the blank reagent water extract are labeled ND (not detected).*

Compound	Level detected	Compound	Level detected	Compound	Level detected
6a-Methylprednisolone	ND	**Enrofloxacin**	<1.0 ppt	Salbutamol (albuterol)	<0.1 ppt
Acetaminophen	<0.1 ppt	Erythromycin	ND	Sparfloxacin	<0.1 ppt
Atenolol	<0.1 ppt	**Fleroxacin**	<1.0 ppt	Sulfabenzamide	ND
Azithromycin	<0.1 ppt	Flumequine	<0.1 ppt	Sulfadiazine	ND
Beclomethasone dipropionate	ND	Flumethasone	ND	Sulfadimethoxine	<0.1 ppt
Benzocaine	<0.1 ppt	Gemfibrozil	ND	Sulfadoxine	ND
Bromhexine	<0.1 ppt	Hydrocortisone	ND	Sulfamerazine	<0.1 ppt
Buflomedil HCl	<0.1 ppt	Ibuprofen	ND	Sulfameter	ND
Carazolol	<0.1 ppt	Josamycin	<0.1 ppt	Sulfamethazine	ND
Cefalexin	ND	Ketoprofen	ND	Sulfamethoxazole	<0.1 ppt
Chlorpheniramine	<0.1 ppt	Levamisole (tetramisole)	<0.1 ppt	Sulfamethoxypyridazine	ND
Cimbuterol	<0.1 ppt	Lincomycin	<0.1 ppt	Sulfapyridine	ND
Cimetidine	<0.1 ppt	Metoprolol	<0.1 ppt	Terbinafine	<0.1 ppt
Cinoxacin	<0.1 ppt	Miconazole	<0.1 ppt	Ternidazole	<0.1 ppt
Cocaine	<0.1 ppt	Nalidixic acid	<0.1 ppt	Tiamulin	<0.1 ppt
Codeine	ND	Naproxen	ND	Ticlopidine	<0.1 ppt
Corticosterone	<0.1 ppt	Ofloxacin	<0.1 ppt	Tilmicosin	<0.1 ppt
Cortisone	ND	Oxfendazole	<0.1 ppt	Tolbutamide	ND
Cotinine	<0.1 ppt	Oxprenolol	<0.1 ppt	tolfenamic acid	ND
Dapsone	<0.1 ppt	Praziquantel	ND	Triamcinolone	ND
Dexamethasone	ND	Procaine	<0.1 ppt	Triamcinolone acetonide	ND
Dicloxacillin	ND	Promethazine	<0.1 ppt	Trimethoprim	<0.1 ppt
Difloxacin	<0.1 ppt	Pyrimethamine	<0.1 ppt	Tripolidine	<0.1 ppt
Digoxigenin	ND	Ranitidine	<0.1 ppt	Tulobuterol	<0.1 ppt
Diltiazem	<1.0 ppt	**Rifaximin**	<1.0 ppt	warfarin	ND
Diphenhydramine	<0.1 ppt	Roxithromycin	<0.1 ppt	Xylazine	<0.1 ppt

Figure 4 shows the MRM chromatograms (quantification transition) of four selected PPCPs that were not detected at all in the reagent water standard. The blank extracted reagent water and spiked extracted reagent water are shown together to demonstrate the response that would equate to 0.1 ppt (100 ppq) in the non-extracted sample.

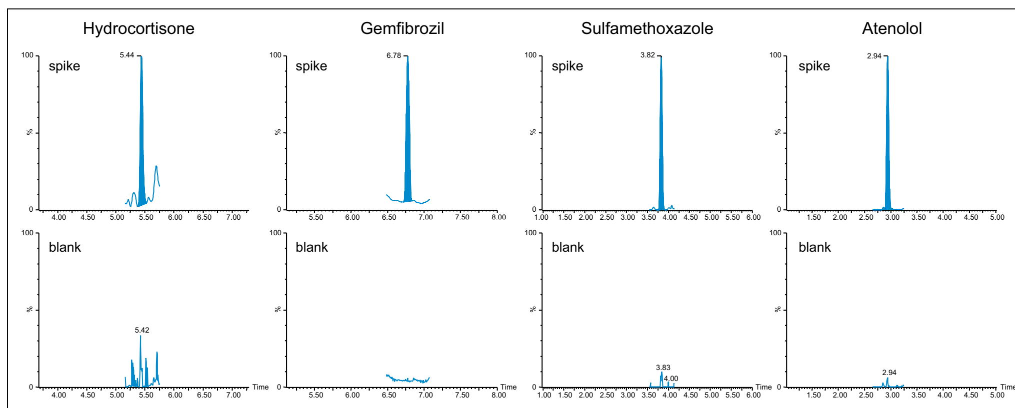

Figure 4. MRM chromatograms for example compounds that demonstrate blank responses in the extracted reagent water. The chromatograms in the top row demonstrate the expected response for the example compounds at the 0.1 ppt level (post-spiked into extracted reagent water). The bottom row shows the response in the blank extract of the reagent water.

In order to assess the quantitative capabilities of the method, three selected deuterated compounds were used as internal standards. Along with the reagent water, a well water sample, and surface water sample were used to demonstrate the method performance in different water matrices. From the 78 PPCPs applicable to this extraction protocol, excellent quantification results were obtained for 58 of the compounds with this initial work employing three of the selected deuterated compounds as internal standards. Further work with additional internal standards is required for the remaining compounds. Recoveries of those 58 compounds at the 1-ppt spike level are shown in Figure 5. For the PPCPs with appropriate internal standards, the R^2 value ranged from 0.991 to 0.997 (linear fit, 1/x weighting). The internal standard used and linear regression R^2 value for each of the compound are described in Table 3.

Table 3. Assignment of the most appropriate internal standard for compound quantification. The resulting R^2 value for the calibration curve is also reported.

Compound	Internal standard used	R^2	Compound	Internal standard used	R^2
Nalidixic acid	Cimetidine-d3	0.994	Tulobuterol	Cimetidine-d3	0.996
Rifaximin	Chlorpheniramine-d6	0.994	Cimbuterol	Cimetidine-d3	0.997
Trimethoprim	Cimetidine-d3	0.991	Chlorpheniramine	Chlorpheniramine-d6	0.993
Erythromycin	Chlorpheniramine-d6	0.995	Cimetidine	Cimetidine-d3	0.997
Josamycin	Cimetidine-d3	0.993	Promethazine	Chlorpheniramine-d6	0.993
Lincomycin	Cimetidine-d3	0.993	Tripolidine	Chlorpheniramine-d6	0.993
Roxithromycin	Chlorpheniramine-d6	0.994	Diphenhydramine	Chlorpheniramine-d6	0.995
Tilmicosin	Chlorpheniramine-d6	0.994	Ranitidine	Cimetidine-d3	0.994
Azithromycin	Chlorpheniramine-d6	0.994	Acetaminophen	Cimetidine-d3	0.995
Tiamulin	Cimetidine-d3	0.991	Cocaine	Cimetidine-d3	0.996
Sulfadiazine	Cimetidine-d3	0.996	Codeine	Cimetidine-d3	0.992
Sulfadoxine	Cimetidine-d3	0.995	Dapsone	Cimetidine-d3	0.993
Sulfamerazine	Cimetidine-d3	0.995	Pyrimethamine	Chlorpheniramine-d6	0.996
Sulfameter	Cimetidine-d3	0.995	Terbinafine	Chlorpheniramine-d6	0.993
Xylazine	Cimetidine-d3	0.993	Ternidazole	Cimetidine-d3	0.995
Bromhexine	Chlorpheniramine-d6	0.996	Miconazole	Chlorpheniramine-d6	0.991
Buflomedil HCl	Chlorpheniramine-d6	0.994	Levamisole (tetramisole)	Cimetidine-d3	0.993
Ticlopidine	Chlorpheniramine-d6	0.994	Oxfendazole	Cimetidine-d3	0.995
Gemfibrozil	Gemfibrozil-d6	0.994	Praziquantel	Cimetidine-d3	0.994
Warfarin	Gemfibrozil-d6	0.992	Benzocaine	Cimetidine-d3	0.995
Procaine	Cimetidine-d3	0.993			

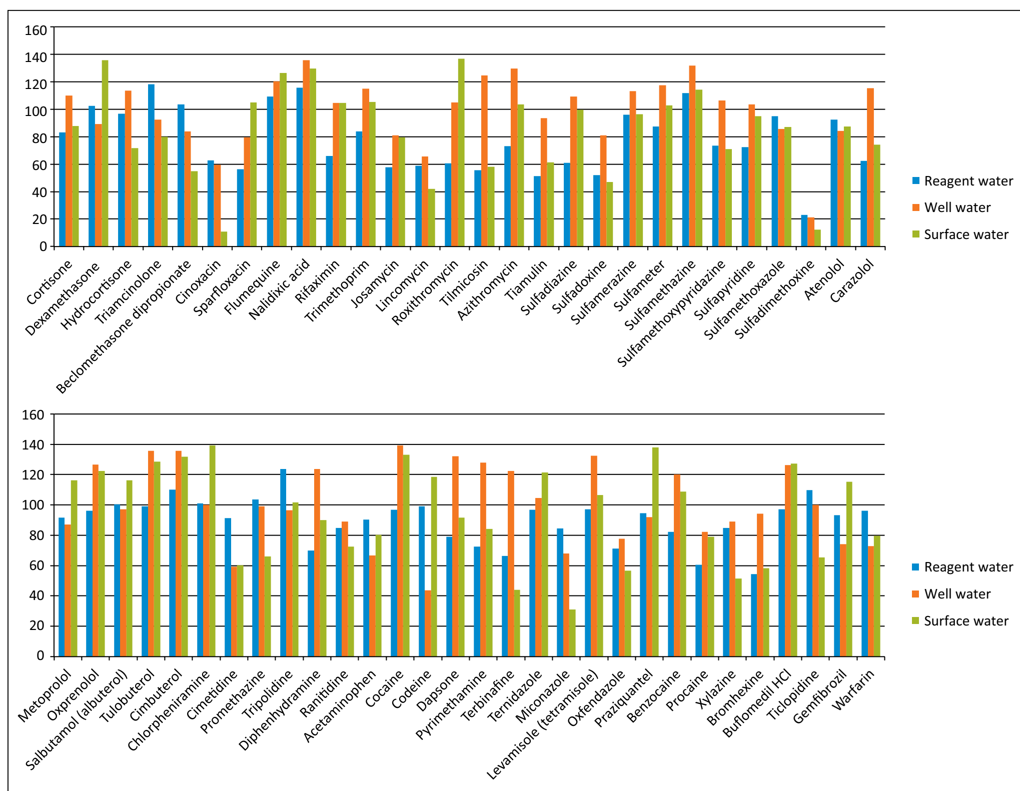

Figure 5. Column chart showing calculated recovery in different water matrices for a 1 ppt spike.

To assess the matrix effects in the three water samples, the response of a standard in non-extracted reagent water was compared to the post-spike extracted samples of the reagent water, the well water sample, and the surface water sample at the 1 ppt level, which are shown in Figure 6. The majority of PPCPs in the reagent water showed a matrix effect of <20%. This clearly indicates the cleanliness of this water sample. For the well and surface water samples, more than half of the PPCPs showed matrix effects of >20%. The surface water samples showed significantly higher complexity, with approximately one-third of the compounds showing a >50% matrix effect, shown in the orange pie sections of Figure 6. Since the extraction protocol was optimized for maximum trapping efficiency of a wide range of compound types, both extraction cartridges were subjected only to a mild wash protocol to ensure no compound breakthrough before final elution. With this mild wash, it is expected that complex water samples will still potentially show matrix effects compared to a clean sample, such as the reagent water. In order to contend with the high complexities, additional wash steps within the SPE protocol could be employed. Further investigation into the most appropriate internal standards could also help to account for heavy matrix loads. Other work,[2] has showed similar effects for two distinct surface water samples.

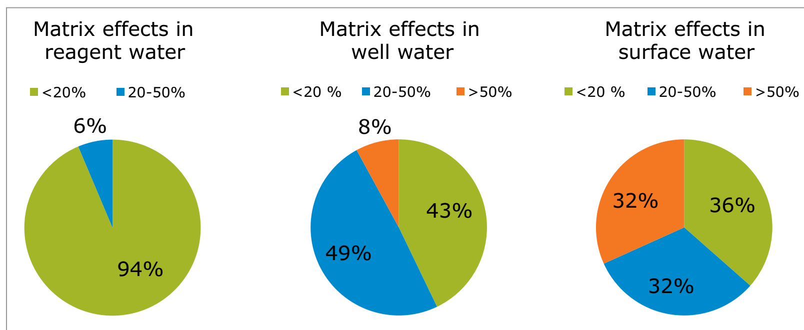

Figure 6. Pie charts showing the level of the matrix effects on the different PPCPs in three different water sample types. Low matrix effect (<20%) is shown in green; medium matrix effect (20% to 50%) is shaded blue; high matrix effect (>50%) is colored orange. The percentage of compounds showing the specified matrix effect are labeled on the pie segments.

The extraction method was used to evaluate the current PPCP level in the well and surface water samples. In well water, two PPCPs tested positive above the 100 ppq level: sulfamethoxazole at 0.97 ppt and atenolol at 0.32 ppt, and 14 PPCPs were detected below this level. For the surface water sample, 17 PPCPs were detected below 100 ppq. An example of a detected compound in each of the samples is shown in Figure 7. To demonstrate a blank sample, the equivalent compound trace for the other sample is also shown with the baseline magnified to show the noise level.

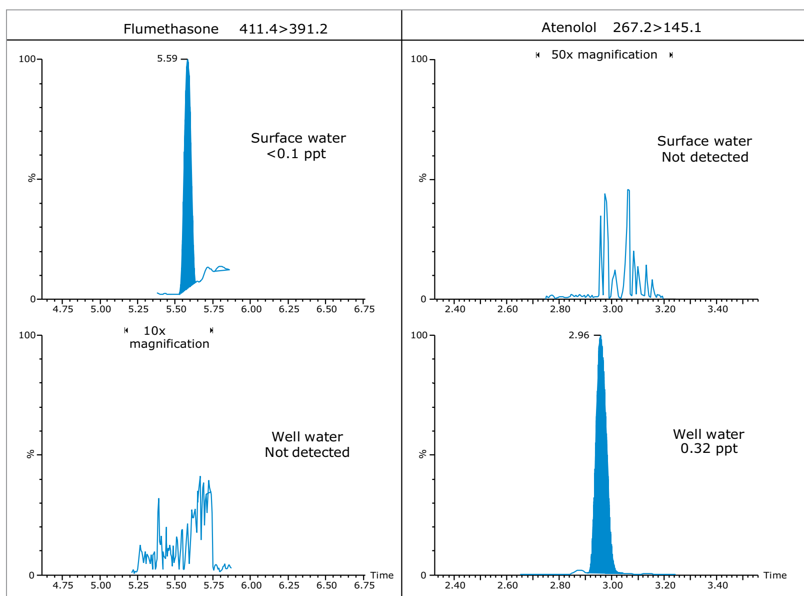

Figure 7. Example compounds that were detected as incurred residues in surface water (flumethasone) and well water (atenolol). To demonstrate a blank sample, the baseline of the sample that did not show the compound detection is shown with the noise level magnified.

CONCLUSIONS

- A method for the extraction, concentration, and quantification of diverse PPCPs including acidic, basic, and neutral compounds has been developed.
- Using the ACQUITY UPLC H-Class System with the small, benchtop Xevo TQD, it was possible to analyze all compounds in a single injection.
- Sensitive detection was achieved with limits of detection in the sub-parts per trillion range, and incurred residues were detected in both a surface water and a well water sample.

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