ENVIRONMENTAL

APPLICATION AND METHOD COMPENDIUM

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

INTRODUCTION

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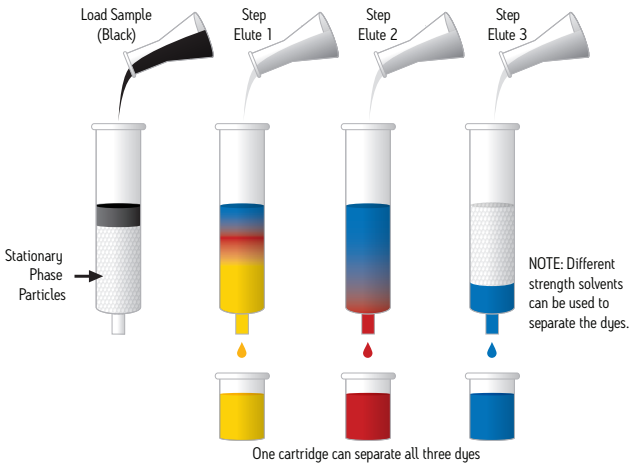
METHODS IN BRIEF

QUICK REFERENCE GUIDE

RETENTION-CLEANUP-ELUTION STRATEGY

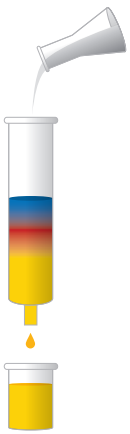
As the sample is loaded onto the cartridge, the analytes of interest are retained by the sorbent. If needed, an optimized series of washes are used to remove matrix interference from the cartridge. A strong solvent is used to elute the analytes from the cartridge. Sample enrichment results when the final elution volume is smaller than the load volume.



PASS-THROUGH CLEANUP STRATEGY

Pass-through cleanup methods optimize matrix retention while the analytes of interest pass-through the cartridge unretained. No sample enrichment occurs during the solid-phase extraction (SPE) step.

1. Sample is passed through sorbent and collected.
   No sample enrichment.
2. Matrix interferences are retained on sorbent.



Pass-through

SPE PROCEDURE STEPS

The following section describes the steps involved in a complete solid-phase extraction procedure:

1. PRETREATMENT

Solid samples (soil, tissue, etc.)

- Shake, sonicate, or use soxhlet extraction.
  - Extract sample with polar organic solvent (methanol, acetonitrile) for polar analytes.
  - Extract sample with organic solvent and drying agent (dichloromethane, acetone) for non-polar analytes and multi-residue extraction.

Non-aqueous liquid

- If the sample is soluble in water, dilute it with water for reversed-phase SPE.
- If the sample is soluble in hexane, dilute it with hexane for SPE.
- Alternatively, evaporate the solvent and exchange to hexane.

Wastewater

- Filter or centrifuge as necessary.

2. CONDITION

For reversed-phase sorbents, preconditioning of the sorbent with an organic solvent, such as methanol, acetonitrile, isopropanol, or tetrahydrofuran is usually necessary to obtain reproducible results. Without this step a highly aqueous solvent cannot penetrate the hydrophobic surface and wet the sorbent. Thus, only a small fraction of the sorbent surface area would be available for interaction with the analyte. For the same reason, it is important not to let silica-based SPE cartridges dry out between the solvation step and the addition of the sample.

A complete preconditioning of a reversed-phase cartridge includes the solvation step and an equilibration with a low-strength solvent, such as water or buffer.

3. LOAD

When the analytes of interest are not retained by the sorbent, this is called analyte breakthrough. For some methods, such as pass-through cleanup, analyte breakthrough is desirable and is maximized for those specific methods. However, in all other cases, analyte breakthrough is unwanted and contributes to poor recovery and method reproducibility. Breakthrough occurs when:

- There is too high an organic concentration in the load solution for very polar analytes. Dilute sample at least 1:1 with water or buffer prior to loading.
- The analytes are bound to proteins, they may pass through the sorbent. Ensure that analytes are not bound to proteins by acidifying or basifying the sample.
- Sorbent is overloaded by the matrix component. Therefore, it is important to choose the correct sorbent mass (see Tables 1 and 2).
- The flow rate of the load step is too fast. There is not enough contact time between the analytes and the sorbent. Look at the drops and adjust the vacuum so that you see discrete droplets, not a stream of liquid.

Table 1. Choice of Oasis® Cartridges Based on Sample Size

Sample Size	Oasis Cartridge
1 to 10 mL	1 cc/30 mg or 3 cc/60 mg
10 to 100 mL	3 cc/60 mg or 6 cc/200 mg
100 to 500 mL	6 cc/200 mg or 6 cc/500 mg (LP*)
500 to 1000 mL	6 cc/500 mg (LP) or 12 cc/1 g (LP)

** LP=large particules (60 µm)*

4. WASH

The wash steps are designed to remove unwanted matrix components that remain from the loading step. The ideal wash solvent removes only the matrix while keeping the analytes bound to the sorbent. For complex samples this is impossible, so the wash steps are optimized using pH, solvent strength, and solvent polarity to remove as much matrix as possible while maintaining acceptable analyte recovery.

5. ELUTE

Once the interferences are washed off the cartridge, a strong solvent is introduced to elute the analytes of interest. The volume and flow rate of the eluting solvents should be precisely controlled as in the load step to ensure reproducible results. Refer to Table 3 for guidelines on various types of separation mechanisms and recommended solvents.

Table 2. Choice of Sep-Pak® Cartridges Based on Sample Size

Sample Size	Sep-Pak Cartridge
10 to 100 mL	3 cc/200 mg or 6 cc/500 mg
100 to 500 mL	3 cc/200 mg or 6 cc/500 mg
500 to 1000 mL	6 cc/500 mg (LP) or 6 cc/1 g

Table 3. Guidelines on the Various Types of Separation Mechanisms

	Reversed Phase	Normal Phase	Ion Exchange
Analyte	Low to moderate polarity/ hydrophobic	Moderate to highly polar/uncharged	Charged or ionized
Matrix	Aqueous	Non-polar organic solvent	Aqueous/low ionic strength
Condition/Equilibrate	1. Solvate polar organic 2. Water	Non-polar organic	Low ionic strength buffer
Wash	Aqueous/buffer	Non-polar	Low ionic strength buffer
Elute	Increase polar organic content in steps	Increase moderate to high polarity organic content in steps	Stronger ionic strength buffers or pH to neutralize the charge

Sep-Pak®

Sample Extraction Products

- Traditional SPE phases
- Many product formats
- Many literature references and validated methods available
- Ultra low extractables from Certified Sep-Pak Cartridges
- Reduced interferences and increased sensitivity using Certified Sep-Pak Cartridges

The convenient format and features of Sep-Pak Cartridges overcome many of the procedural difficulties of traditional column liquid-solid extraction and allow the enormous benefits of solid-phase extraction to be realized. Adsorbent and packed bed quality, reproducibility, versatility, and ease-of-use are assured through intelligent design, production control, and quality testing.

Sep-Pak Cartridge Separation Guidelines

Chromatographic Mode	Normal Phase	Reversed Phase	Ion Exchange
Separation Characteristic	Silica, Florisil, Alumina, Diol, NH_2, CN	C_{18}, tC_{18}, C_8, Diol, PoraPak® RDX, NH_2, CN	Accell Plus QMA, Accell Plus CM, NH_2
Packing Surface Polarity	High	Low	High
Typical Solvent Polarity Range	Low to medium	High to medium	High
Typical Sample Loading Solvent	Hexane, toluene, dichloromethane	Water with low ionic strength	Water, buffers
Typical Elution Solvent	Ethyl acetate, acetone, acetonitrile	Methanol, acetonitrile, dichloromethane	Buffers, salt solutions with high ionic strength
Sample Elution Order	Least polar sample components first	Most polar sample components first	Most weakly ionized sample components first
Solvent Change Required to Elute Retained Compounds	Increase solvent polarity	Decrease solvent polarity	Increase ionic strength or increase pH (anion exchange) or decrease pH (cation exchange)

OASIS®
SAMPLE EXTRACTION PRODUCTS

- Waters premium brand for SPE
- Cartridges, plates, and µElution technology
- Co-polymer, water wettable, reproducible
- Outperforms C_{18} for polar bases

OASIS 2x4 METHOD—THE FASTEST, SIMPLEST, AND CLEANEST APPROACH TO SPE METHOD DEVELOPMENT

- Characterize your analyte (acid, base, p*K*a)
- Choose 1 of 5 Oasis Sorbents

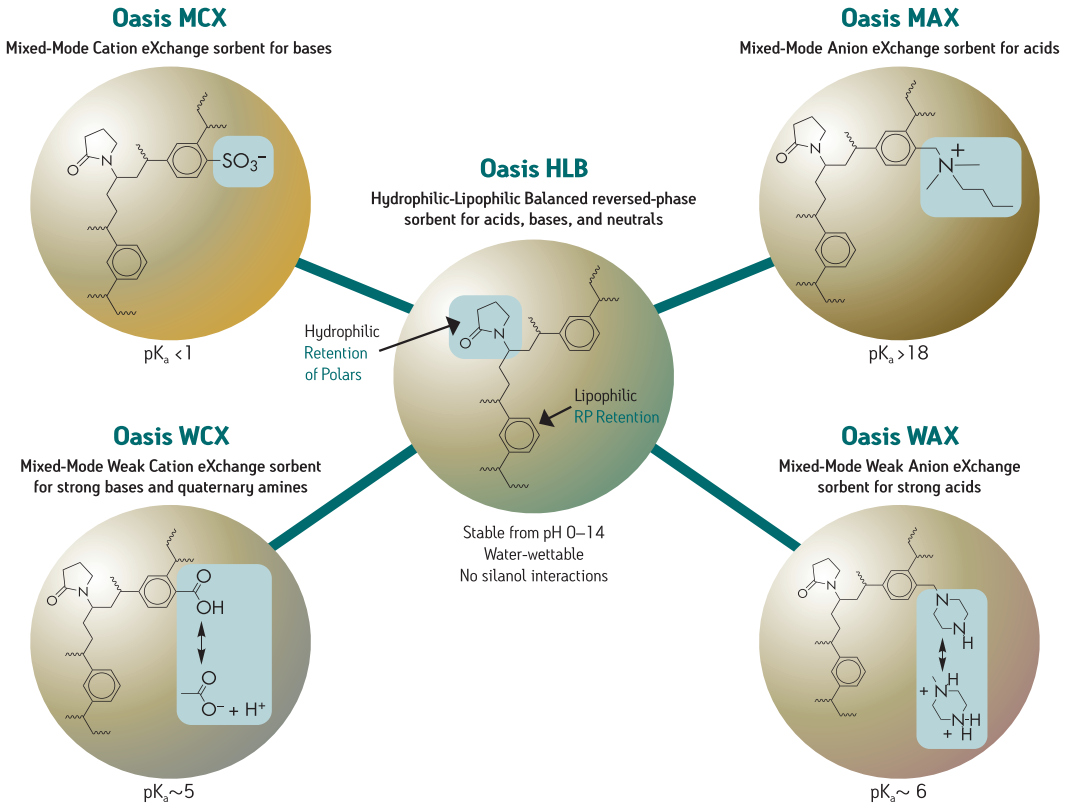
  HLB: Hydrophilic-Lipophilic-Balanced reversed-phase sorbent for acids, bases and neutrals

  MCX: Mixed-mode Cation eXchange sorbent for bases

  MAX: Mixed-mode Anion eXchange sorbent for acids

  WCX: Mixed-mode Weak Cation eXchange sorbent for strong bases and quaternary amines

  WAX: Mixed-mode Weak Anion eXchange sorbent for strong acids



DisQuE™
Dispersive Sample Preparation

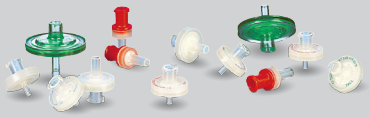
- Easy and straightforward method to implement, requiring little training
- Conforms to the AOAC and CEN official methods for determining pesticide residues in fruits in vegetables
- Cost effective
- Reliable, high quality product in a simple kit format

DisQuE DISPERSIVE SAMPLE PREPARATION KITS

Dispersive sample preparation, commonly referred to as "QuEChERS", is a simple and straightforward sample preparation technique suitable for multi-residue pesticide analysis in a wide variety of food and agricultural products. Waters DisQuE™ Dispersive Sample Preparation Kit contains conveniently packaged centrifuge tubes with pre-weighed sorbents and buffers designed for use with AOAC and European Committee for Standardization (CEN) official methods. DisQuE dispersive sample preparation is a well proven, high throughput sample preparation method for a wide array of pesticide in produce samples.

FILTERS

Filtration provides immediate protection for analytical system components and minimizes downtime. In partnership with Pall Life Sciences, Waters offers filtration products that are Certified for Compliance, which means they have been designed and developed to comply with regulatory and quality objectives.



CERTIFIED VIALS

Sample vials are a critical part to sample preparation. Ensure that the vials you use do not introduce unwanted contaminants and interferences. Waters provides a wide selection of certified vials, including the TruView™ LCMS Certified Vials, tested to maximize sensitivity and improve detection limits for LC-UV/MS and LC-MS analysis. Do not compromise your test results; avoid ghost peaks, dislodged septa, and damaged needles.

ANALYTICAL STANDARDS AND REAGENTS

Waters understands the importance of high quality analytical standards and reagents in ensuring continuous analytical instrumentation advancements and workflow success. That is why Waters now offers standards and reagents that are pure, precisely formulated, reproducible, and traceable to exact specifications. From system performance standards to application specific standards, you can rely on Waters.

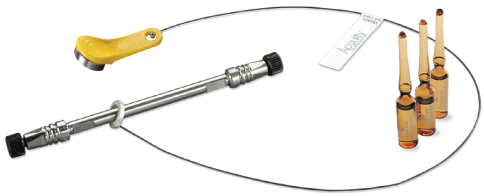
ENVIRONMENTAL SOLUTION KITS

Waters is committed to providing our customers with optimized solutions for their analytical challenges. In order to obtain highest quality results, analysts must optimize each step in their analytical process — sample preparation, sample handling, analytical methods, etc. Simplifying this task, Waters offers Total Solutions Kits for Environmental applications. These kits include a selection of components (such as Sample Preparation, LC Columns, Vials, and Standards) which have been verified to work together to provide the best results possible. For details on specific Total Solutions Kits see the listing below.

ACQUITY UPLC PFC COLUMN KIT

Optimized for trace level detection of Perfluorinated Compounds (PFCs) with the ACQUITY UPLC® System, this kit contains the ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm Column, the ACQUITY UPLC PFC Isolator Column, and PFC Reference Standards.

Part Number 176001692



EPA METHOD 1694 ANALYSIS KIT

Waters EPA Method 1694 Analysis Kit includes the XTerra® MS C_{18}, Atlantis® HILIC Columns, and Oasis HLB Cartridges; all of which are specified in the EPA Method 1694.

Part Number 176001634

ACQUITY UPLC PFC ANALYSIS KIT

The ACQUITY UPLC PFC Analysis Kit includes Oasis SPE Cartridges, PFC Calibration and Reference Standards, Certified Vials, ACQUITY UPLC Columns, and the necessary instrument components to optimize your instrument for trace level detection of PFCs.

Part Number 176001744

ACQUITY UPLC BISPHENOL A COLUMN AND METHOD KITS FOR ASTM METHOD D-7574-09

The ACQUITY UPLC Bisphenol A Column and Method Kits are fully compliant with ASTM Method D7574-09. Waters ACQUITY UPLC Solution provides optimum resolution and sensitivity for the analysis of Bisphenol A in water. The Column Kit includes the ACQUITY UPLC BEH C_{18} Column and ACQUITY UPLC Isolator Column. The Method Kit also includes Oasis HLB SPE Cartridges and LCMS Certified Vials.

Part Number 176001955 (Column Kit)
Part Number 186004932

CARBAMATE ANALYSIS KIT FOR ENVIRONMENTAL TESTING

Waters Carbamate Analysis Kit for environmental testing includes a Waters Carbamate Column, Oasis HLB Cartridges, Vials, and Reference Standards. Optimized for use with EPA Method 531.2, these products simplify your analysis while increasing your confidence in the results

Part Number 176001740

COLUMN SELECTION GUIDE

Waters is committed to material sciences and, with our ongoing research into HPLC and UPLC® Column Chemistries, we continue to develop ground-breaking column technologies. As scientific challenges evolve, Waters meets these changing needs with new column innovations.

XSELECT® Columns

XSelect®	
C_{18}	**Selectivity Features:** General purpose reversed-phase column that offers excellent pH stability and rapid mobile-phase re-equilibration for method development. Charged Surface Hybrid (CSH™) Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_{18} ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Phenyl-Hexyl	**Selectivity Features:** General purpose alternative selectivity ligand that provides pi-pi interactions with polyaromatic compounds, while maintianing excellent reproducibility at pH extremes. CSH Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_6 Phenyl ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Fluoro-Phenyl	**Selectivity Features:** General purpose column that provides a very high degree of analyte selectivity, especially when using low-pH mobile phases. CSH Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional propyl fluorophenyl ligand, non-endcapped, bonded to a CSH particle substrate.
HSS C_{18}	**Selectivity Features:** High performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH. Designed for UPLC separations where silica-based C_{18} selectivities are desired. **Bonding:** High coverage trifunctional C_{18}, fully endcapped, bonded to High Strength Silica (HSS) HPLC particle substrate.
HSS C_{18} SB	**Selectivity Features:** Unique, non-endcapped C_{18} chemistry designed specifically for method development scientists. Offers unique Selectivity for Bases (SB) when operating under low pH conditions and transferability between UPLC and HPLC separations. **Bonding:** Intermediate coverage trifunctionally bonded C_{18}, no endcapping, bonded to HSS HPLC particle substrate.
HSS T3	**Selectivity Features:** Aqueous mobile-phase compatible HPLC column designed for extreme retention. Combines polar compound retention with transferability between UPLC and HPLC separations. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to HSS HPLC particle substrate.
HSS PFP	**Selectivity Features:** General purpose column designed to maximize selectivity differences for Lewis bases through pi-pi interactions. The rigid aromatic ring provides additional selectivity based on shape, dipole moment and hydrogen bonding interactions. **Bonding:** Trifunctional pentafluorophenyl ligand, non-endcapped, bonded to a High Strength Silica (HSS) substrate.
HSS CN	**Selectivity Features:** A general purpose column that shows contrasting analyte selectivity when compared to C_{18} phases. This column can be used for both reversed-phase and normal phase separations. **Bonding:** Sterically hindered, mono-functional cyano-propyl ligand, non-endcapped, bonded to a High Strength Silica (HSS) substrate.

XBridge®

dC_{18}	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes. **Bonding:** Trifunctional C_{18}, fully endcapped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
Shield RP18	**Selectivity Features:** Alternate selectivity as compared to straight chain C_{18}, particularly with phenolic analytes. Compatible with 100% aqueous-phase composition. **Bonding:** Monofunctional embedded polar C_{18}, fully endcapped, bonded to substrate.
C_8	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compounds classes. **Bonding:** Trifunctional C_8, fully endcapped, bonded to BEH substrate.
Phenyl	**Selectivity Features:** Excellent method development column for alternate selectivity, particularly for polyaromatic compounds. Unique level of pH stability for a phenyl-bonded phase. **Bonding:** Trifunctional C_6 phenyl, fully endcapped, bonded to BEH substrate.
HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded BEH substrate.

Atlantis®

T3	**Selectivity Features:** Retention of polar compounds, compatible with 100% aqueous mobile phases, superior stability under low pH conditions. Specifically designed for enhanced retention of polar analytes. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to high purity silica substrate.
HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded high purity silica substrate.
dC_{18}	**Selectivity Features:** Retention of polar compounds. Designed for compatibility with 100% aqueous mobile phases. **Bonding:** Difunctional C_{18} bonding, fully endcapped, bonded to high purity silica substrate.

SunFire®

C_{18}	**Selectivity Features:** General purpose method development column. Very high loading capacity, particularly for basic analytes in low pH mobile phases. Ideally suited for purification and impurity profile assays. **Bonding:** Difunctional C_{18}, fully endcapped, bonded to high purity silica substrate.
C_8	**Selectivity Features:** General purpose method development column. Very high loading capacity, particularly for basic analytes in low pH mobile phases. Less hydrophobic, therefore, less retentive than C_{18} for most analytes. **Bonding:** Difunctional C_8, fully endcapped, bonded to high purity silica substrate.

Acquity UPLC®

ACQUITY UPLC®	
CSH C_{18}	**Selectivity Features:** General purpose reversed-phase column that offers excellent pH stability and rapid mobile-phase re-equilibration for method development. Charged Surface Hybrid (CSH™) Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_{18} ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Phenyl-Hexyl	**Selectivity Features**: General purpose alternative selectivity ligand that provides pi-pi interactions with polyaromatic compounds, while maintaining excellent reproducibility at pH extremes. CSH Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_6 phenyl ligand, fully end-capped, bonded to a CSH particle substrate.
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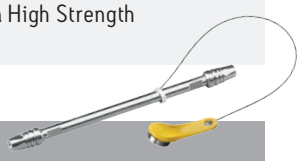
BEH C_{18}	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes. **Bonding:** Trifunctional C_{18}, fully endcapped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
BEH Shield RP18	**Selectivity Features:** Alternate selectivity as compared to straight chain C_{18}, particularly for phenolic analytes. Compatible with 100% aqueous-phase composition. **Bonding:** Monofunctional embedded polar C_{18}, fully endcapped, bonded to BEH substrate.
BEH Phenyl	**Selectivity Features:** Excellent method development column for alternate selectivity, particularly in regard to polyaromatic compounds. Unique level of pH stability for a phenyl-bonded phase. **Bonding:** Trifunctional C_6 phenyl, fully endcapped, bonded to BEH substrate.
BEH HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded BEH substrate.
BEH HSS C_{18}	**Selectivity Features:** Ultra performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH. Designed for UPLC separations where silica-based C_{18} selectivities are desired. **Bonding:** High coverage trifunctional C_{18}, fully endcapped, bonded to High Strength Silica (HSS) UPLC particle substrate.
BEH Amide	**Selectivity Features:** Rugged HILIC stationary phase designed to separate a wide range of very polar compounds. Especially good at separating carbohydrates (saccharides) using high concentrations of organic modifier, elevated temperature and high pH. Compatible with all modern detectors including MS, ELSD, UV and Fluorescence. **Bonding:** Trifunctional amide bonded to BEH substrate.
HSS C_{18}	**Selectivity Features:** Ultra performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH. Designed for UPLC separations where silica-based C_{18} selectivities are desired. **Bonding**: High coverage trifunctional C_{18}, fully endcapped, bonded to an HSS UPLC particle substrate.
HSS C_{18} SB	**Selectivity Features:** Unique, non-endcapped C_{18} chemistry designed specifically for method development scientists. Offers unique Selectivity for Bases (SB) when operating under low pH conditions. **Bonding:** Intermediate coverage tri-functionally bonded C_{18}, no endcapping, bonded to an HSS UPLC particle substrate.
HSS T3	**Selectivity Features:** Aqueous mobile-phase compatible UPLC column designed for extreme retention. Combines polar compound retention with UPLC efficiencies and performance. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to an HSS UPLC particle substrate.

ACQUITY UPLC continued	
HSS PFP	**Selectivity Features:** General purpose column designed to maximize selectivity differences for Lewis bases through pi-pi interactions. The rigid aromatic ring provides additional selectivity based on shape, dipole moment and hydrogen bonding interactions. **Bonding:** Trifunctional pentafluorophenyl ligand, non-endcapped, bonded to a High Strength Silica (HSS) substrate.
HSS CN	**Selectivity Features:** A general purpose column that shows contrasting analyte selectivity when compared to C_{18} phases. This column can be used for both reversed-phase and normal phase separations. **Bonding:** Sterically hindered, mono-functional cyano-propyl ligand, non-endcapped, bonded to a High Strength Silica (HSS) substrate.



CORTECS™ COLUMNS

CORTECS™	
C_{18}	**Selectivity Features:** High efficiency, general purpose, reversed-phase column. Balanced selective retention of acids, bases, and neutrals at low and mid-range pH. **Bonding:** Trifunctional C_{18} ligand, fully end-capped, bonded to a CSH particle substrate.
C_{18+}	**Selectivity Features**: High efficiency, general purpose, reversed-phase column. A positively charged surface delivers excellent peak shape for basic compounds. **Bonding:** Trifunctional C_{18}, encapped, bonded to sub-2-µm solid-core silica substrate.
HILIC	**Selectivity Features**: High efficiency column designed for retention of extremely polar analytes. Offers orthogonal selectivity versus C_{18} column. **Bonding:** Unbonded sub-2-µm solid-core silica.

ENVIRONMENTAL TESTING SPECIALITY COLUMNS

In addition to a complete selection of UPLC and HPLC Column Chemistries, Waters also provides columns optimized for specific environmental analysis. These columns are ideal for PAH analysis, carbamate analysis, and perflorinated compound analysis.

GUARD COLUMNS

VanGuard™ Pre-columns, Sentry™ Guard Columns, and Guard-Pak™ Inserts prolong column lifetime by removing contaminants from the sample, giving you enhanced reproducibility and performance. They are packed with the same high performance stationary phases used in Waters Analytical Columns.

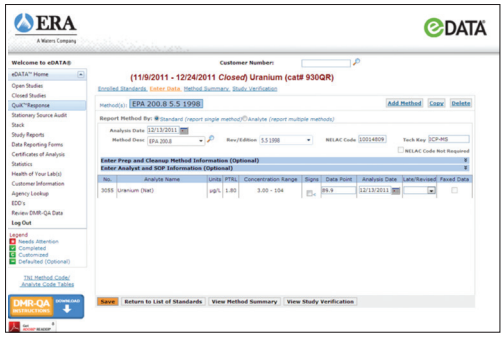
PROFICIENCY TESTING, CERTIFIED REFERENCE MATERIALS, AND QUALITY CONTROL STANDARDS FOR ANALYTICAL LABORATORIES

ERA is one of the most trusted providers of certified reference materials (CRMs) and proficiency testing (PT) products in the world. Since 1977, environmental laboratories have partnered with us to strengthen and simplify their quality assurance programs. With PT standards of impeccable quality, you can confidently make decisions based on ERA standards. Our industry-leading customer service, technical support, and PT data tools make it easier for you to find the products you need and solve any problems that you identify. And as an NELAC accredited PT provider, you can be certain of accurate and defensible data.

eDATA – ERA'S ONLINE DATA REPORTING TOOL

eDATA® gives you the ability to easily and accurately report laboratory proficiency testing study data, view preliminary results, track your historical performance, and identify opportunities to improve your laboratory. Review historical PT study results to identify any undesirable trends.

- Easily enter and review your data right up to the moment a study closes.
- Receive an email confirmation and report of the data you entered.
- Track the history and monitor your performance with the Health of Your Lab data tracking.



QuikResponse

Critical evaluations are just that—critical. ERA's Quik™Response PTs return results within minutes of entering your study data. No waiting. No wondering. No worries. Just results.

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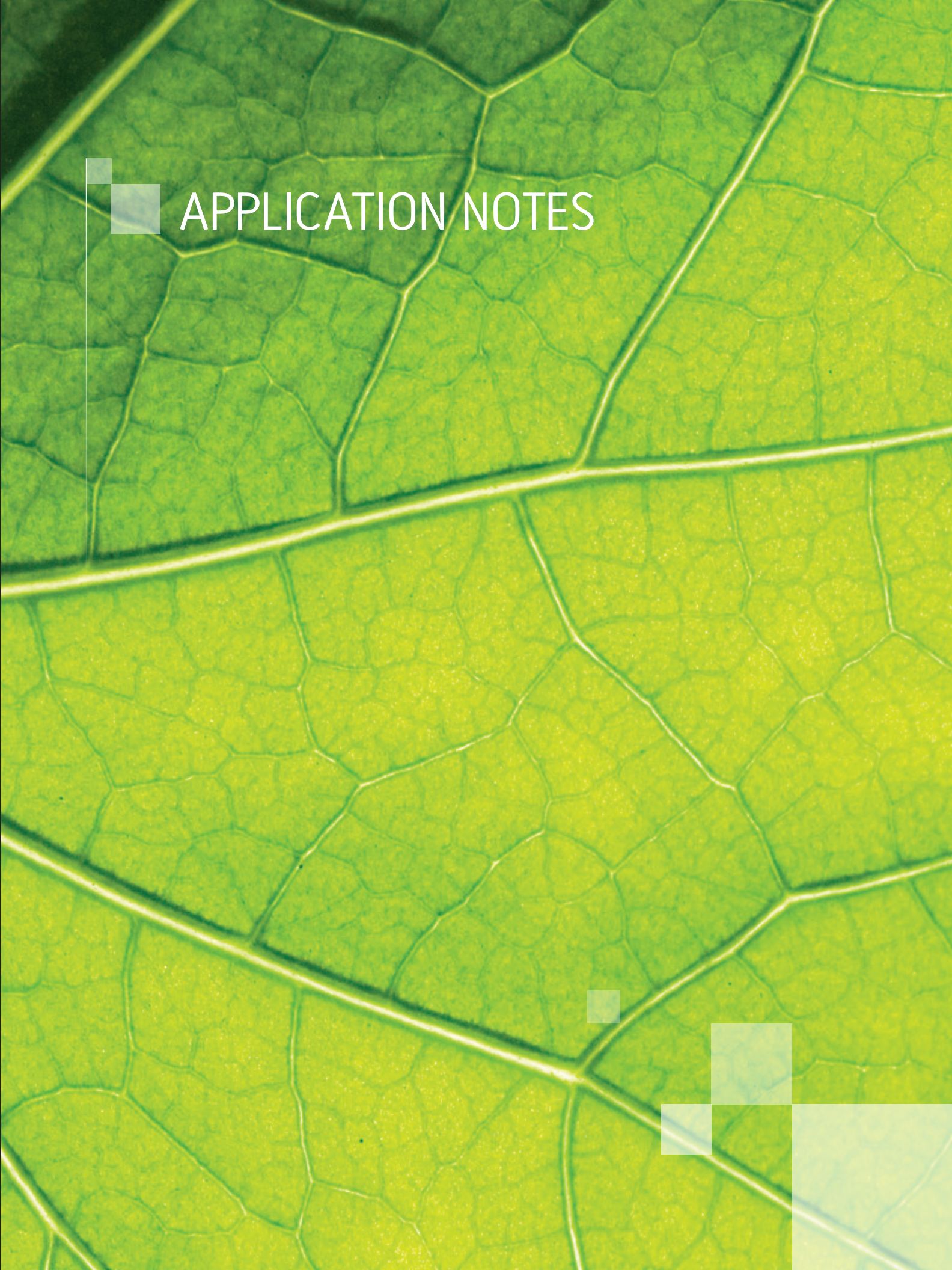
Why use QuikResponse?

- Comply with deadline-driven corrective action requirements.
- Swiftly expand your scope of accreditation.
- Document and validate the effectiveness of corrective actions.
- Complete the PT process in as fast as 2 business days of ordering your QuiKResponse PT samples.


APPLICATION NOTES

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Advancing Endocrine Disrupting Compound Analysis Through Integrated Technology and Workflow Solutions

Paul Silcock,[1] Alan Wainwright,[2] and Chris Hunter[2]
[1] Waters Corporation, Manchester, UK
[2] Environment Agency - National Laboratory Service, Leeds, UK

APPLICATION BENEFITS

- Using Solid Phase Extraction for isolation and enrichment of Endocrine Disruptors
- Use of LC-MS/MS with RADAR™ functionality to simultaneously acquire full scan data while maintaining the quality of MRM data
- Method development using IntelliStart™ Software
- Quantification of Endocrine Disruptors at low part-per-trillion concentrations

WATERS SOLUTIONS

ACQUITY UPLC® System

Xevo® TQ MS

ACQUITY UPLC BEH C_{18} Column

Oasis® Sample Extraction Products

IntelliStart Software

KEY WORDS

Environmental, water, endocrine disruptors, method development, SPE

INTRODUCTION

Protecting the environment for present and future generations is an important responsibility undertaken by organizations all over the globe. It allows for higher quality of life, both directly and indirectly by reduced exposure to pollutants, maintenance of ecosystems, and better health through safer food and water supplies. For companies using water for consumed products or supplying water, the efficiency of treatment processes and quality of final products is also of high importance. For regulators, a key part of protecting both the environment and consumers is through the monitoring and discovery of substances of concern. This becomes increasingly important due to the high publicity related to newer emerging contaminants.

Endocrine disrupting compounds (EDCs) have caused increased concern for organizations that monitor their occurrence in environmental and potable waters. These compounds often have physiological effects to humans and wildlife at very low concentrations.[1] One class of EDCs are the estrogenically active substances. These, of course include natural and synthetic estrogens as well as alkylphenol compounds that mimic at the estrogenic receptor.[2] There is a need to monitor these compounds reliably to low parts per trillion (ppt) concentrations in often complex samples such as environmental surface waters and treated sewage.

To achieve this, sophisticated sample preparation chemistries and powerful analytical systems are required in combination. Different approaches have been compared to analyze estrogenic substances and LC-MS/MS is highly applicable.[3] LC-MS/MS traditionally offers selective and sensitive analysis in a targeted fashion. While this is still the priority for this type of instrumentation, there are advantages in acquiring information simultaneously that are non-targeted and can offer intra-sample quality control or discovery of non-targeted components.

Development and setup of reliable, highly sensitive, multi-analyte methods using LC-MS/MS often requires a significant time and resource investment from organizations, in addition to their current responsibilities. This means that the speed at which a quality result can be produced is a key parameter. With this in mind the ability to quickly setup and run new high performance methodologies quickly is clearly desirable.

UPLC conditions

System:	ACQUITY UPLC
Runtime:	5.30 min
Column:	ACQUITY® BEH C_{18} 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Mobile phase A:	0.05% NH_4OH (aqueous)
Mobile phase B:	MeOH
Flow rate:	0.6 mL/min
Injection volume:	10 µL

Time (min)	Flow rate	%A	%B
1. Initial	0.60	65.0	35.0
2. 3.00	0.60	5.0	95.0
3. 4.20	0.60	5.0	95.0
4. 4.30	0.60	65.0	35.0

MS conditions

MS system:	Xevo TQ
Acquisition mode:	RADAR Dual Scan-MRM
Ionization mode:	ESI-
Capillary voltage:	2.0 kV
Source temp.:	150 °C
Desolvation temp.:	650 °C
Desolvation gas:	1100 L/hr
Cone gas flow:	20 mL/min
Collision gas flow:	0.18 mL/min

This application note describes the use of Waters® Oasis HLB sample preparation in combination with Xevo TQ MS for the analysis of endocrine disrupters to low ppt concentrations in groundwater, river water, and sewage effluent. It also describes the use of intelligent workflow tools for method development, as well as advanced LC-MS/MS analysis using Waters RADAR functionality to simultaneously acquire full scan data while maintaining the quality of MRM data.

Photo: Dave Gostisha/Ziptrivia llc

EXPERIMENTAL

Sample preparation

Spiked groundwater, river water, and sewage effluent extracts were prepared using Waters Oasis HLB glass 5-cc/200-mg SPE cartridges. The protocol employed is based on a method described in the Waters' Environmental Chromatography Methods Guide (p/n 720002543en) with final extract solvent composition mobile phase matched.

Xevo TQ MS setup (mass resolution, mass calibration, ion source optimization) was automated by IntelliStart Software. IntelliStart was also used to automatically develop fully optimized MRM acquisition methods for the endocrine disruptors targeted in this analysis. IntelliStart requires only the entry of basic compound information and automatically locates the precursor ion, optimizes cone voltage, locates product ions, and optimizes collision energy. Extracts from the IntelliStart method development report are shown in Figure 1.

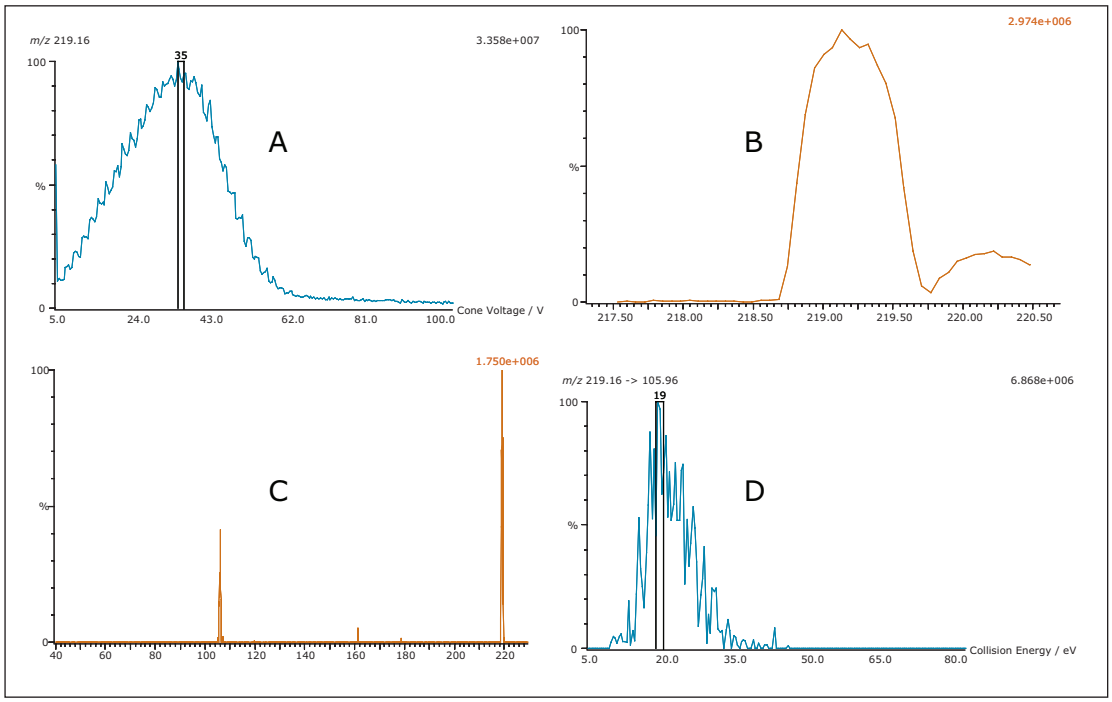


Figure 1. Extract from IntelliStart method development report showing optimization of cone voltage (A), optimized MS spectrum (B), location of product ions (C), and optimization of collision energy (D) for 4-nonylphenol.

The applied Xevo TQ MS method development workflow is shown in Figure 2. Multiple MRM transitions were produced by IntelliStart, and a selection of these were used based on selectivity and sensitivity in matrix. Table 1 provides a selection of the IntelliStart-produced MRM conditions for the endocrine disrupting compounds analyzed on Xevo TQ MS. Following IntelliStart method development, compound information along with key analytical parameters were exported to the QUANPEDIA™ compound database.

In addition to MRM data, full scan data were acquired using the RADAR Dual Scan-MRM mode of the Xevo TQ MS. This functionality allows real time qualitative information about the nature of the sample matrix to be acquired at the same time as routine quantitative analyses.

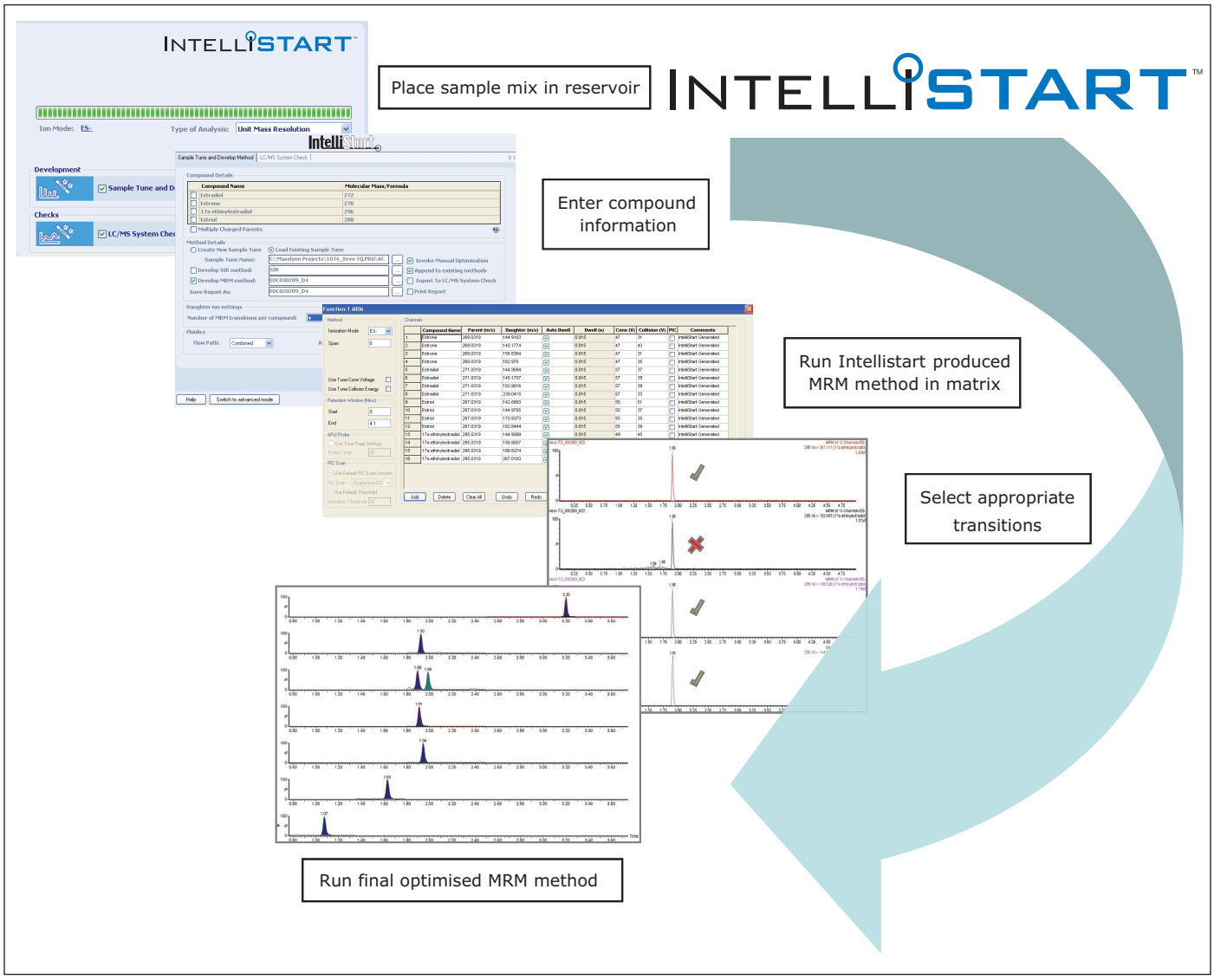


Figure 2. IntelliStart method development workflow on Xevo TQ MS.

Compound	Nominal Mass	Precursor Ion	Product Ion	Cone (V)	Collision (eV)
Estriol	288	287.2	145.0	53	39
Estriol	288	287.2	171.0	53	37
Bisphenol A	228	227.1	133.0	31	25
Bisphenol A	228	227.1	212.0	31	17
Diethylstilbestrol	268	267.1	237.1	35	29
Diethylstilbestrol	268	267.1	251.1	35	25
Estrone	270	269.2	145.1	47	36
Estrone	270	269.2	143.0	47	48
Estradiol	272	271.2	183.1	51	31
Estradiol	272	271.2	145.1	51	40
Estradiol D4	276	275.2	147.1	55	37
17α ethinylestradiol	296	295.2	145.1	45	37
17α ethinylestradiol	296	295.2	158.9	45	33
4-nonylphenol	220	219.2	106.0	35	19

Table 1. Selection of IntelliStart EDC MRM conditions.

Data acquisition and processing

MassLynx® Software v.4.1 was used for data acquisition and spectral processing. TargetLynx™ Software was used to process MRM data, and TrendPlot was used for over-batch comparisons of selected parameters.

RESULTS AND DISCUSSION

Spiked extracts of groundwater, river water, and sewage effluent showed high instrument selectivity and sensitivity to sub-ng/L levels with sample preconcentration using Oasis HLB solid-phase extraction. ACQUITY UPLC maintained good resolution between the critical pair 17α and 17β estradiol while eluting the last component at 3.2 min. This allowed a high sample throughput through the analytical system. Figure 3 shows groundwater, river water, and sewage effluent extracts spiked at 1 ng/mL (equivalent to 5 ng/L with Oasis HLB pre-concentration).

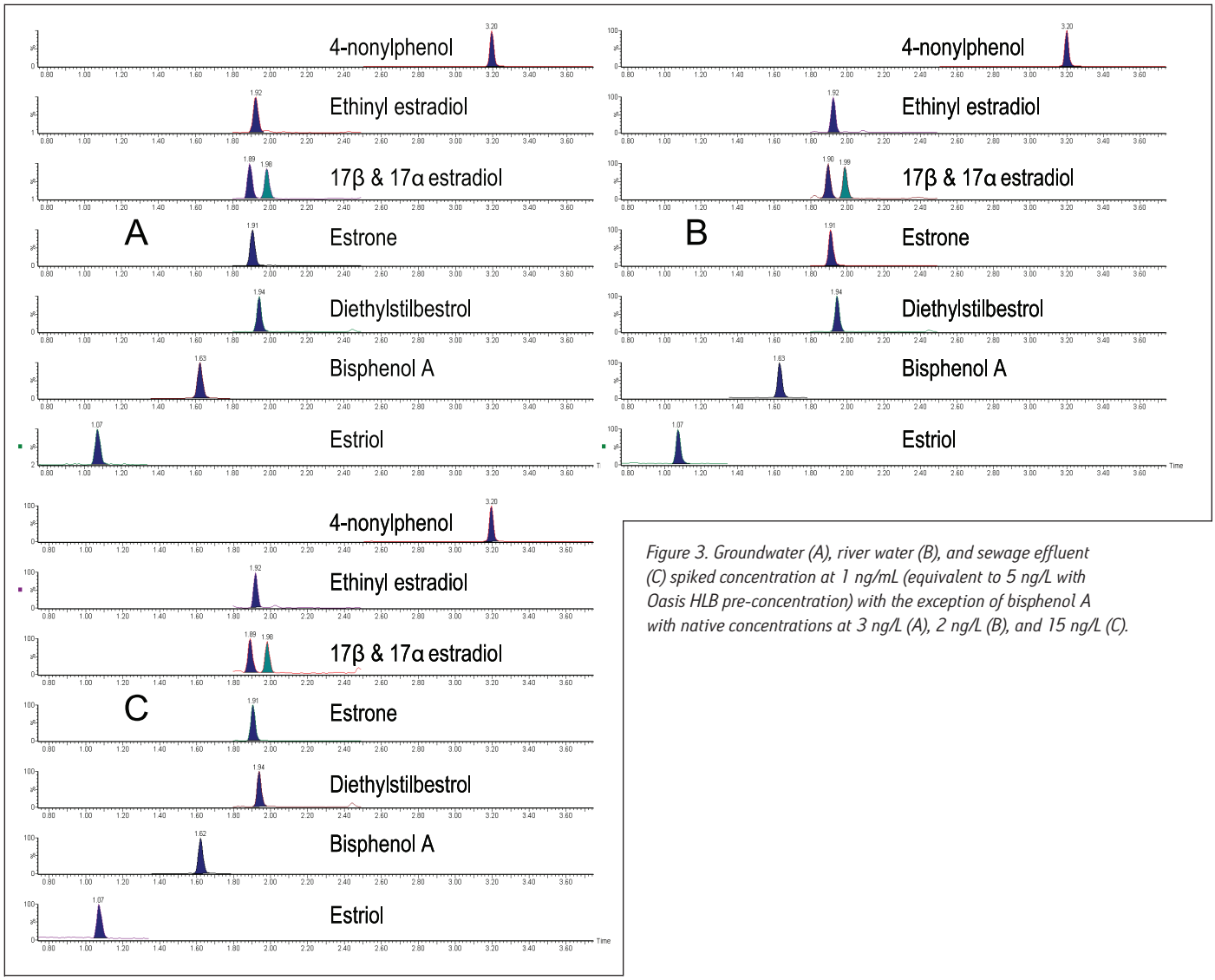


Figure 3. Groundwater (A), river water (B), and sewage effluent (C) spiked concentration at 1 ng/mL (equivalent to 5 ng/L with Oasis HLB pre-concentration) with the exception of bisphenol A with native concentrations at 3 ng/L (A), 2 ng/L (B), and 15 ng/L (C).

During data processing, the TrendPlot functionality of TargetLynx was used to trend some intra- batch parameters to monitor for any anomalies. Figure 4 shows internal standard D4 β-estradiol peak area plotted over one of the sample batches. This allows easy identification of possible spiking errors.

RADAR Dual Scan-MRM matrix monitoring

Full scan spectra were acquired alongside quantitative MRMs to monitor the background matrix in the sample. This allows acquisition of data that is often missed during routine quantitative analysis and can help to highlight areas where methodology can be improved, as well as provide information about non-targeted compounds.

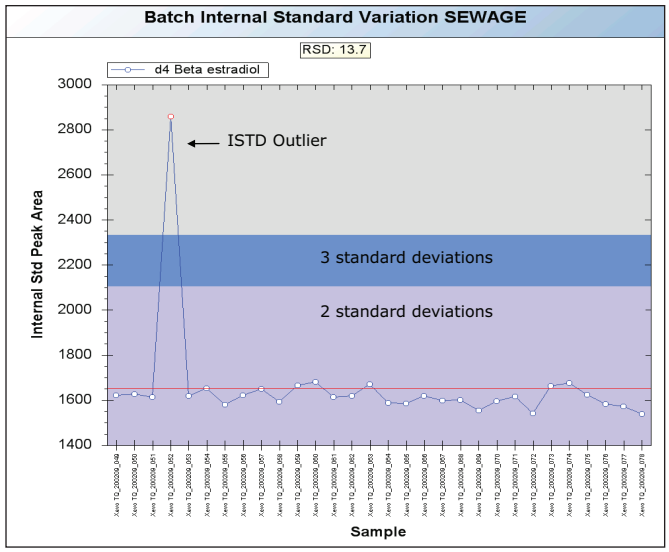


Figure 4. D4 β-estradiol peak area plotted over a batch of samples.

Matrix monitoring for method development and QC

Figure 5 shows matrix monitoring of a spiked sewage effluent using RADAR Dual Scan-MRM mode. Using this acquisition mode, it was possible to discover background matrix components that originated from the sample and/or laboratory processes. Humic and fulvic substances, which could potentially cause undesirable matrix effects, can be seen eluting prior to the first analyte peak (estriol) giving higher confidence in the quantitative performance of that targeted component. In addition, suspected anionic surfactant LAS (Linear alkylbenzene sulphonate) at high concentration can be observed in the chromatogram with spectra giving ions at 297, 311, 325, and 339 *m/z*. This was further confirmed using ScanWave™ product ion scanning revealing an intense characteristic 183 *m/z* product ion from each.

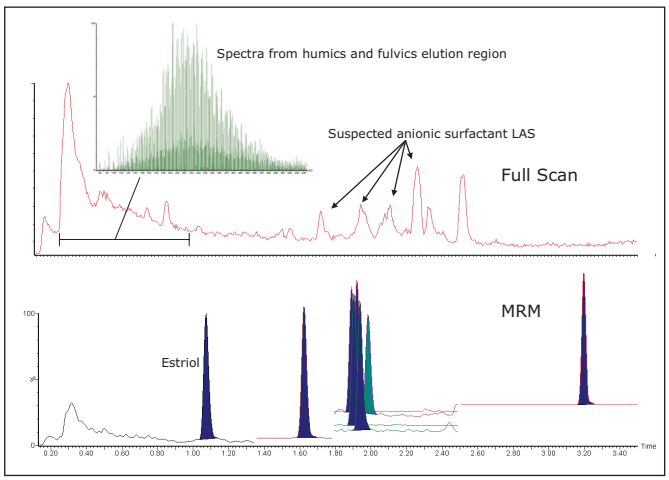


Figure 5. Matrix Monitoring of spiked sewage effluent using RADAR Dual Scan-MRM mode.

Matrix monitoring also allows observation of target ions that co-elute with matrix components. Figure 6 shows an expanded region of the same spiked sewage sample with co-elution of some matrix components including LAS. The specificity of the MRM acquisition allows quantitation of target analytes in the presence of matrix peaks, but the ability to investigate potential matrix effects for every sample can allow additional QC checks to be made and the continuous improvement of methodologies.

Matrix monitoring for non-MRM targeted compounds

Using RADAR Dual Scan-MRM in a routine quantitation also allows for a retrospective look at non-targeted data acquired from a sample in the light of new information about potential contaminants.

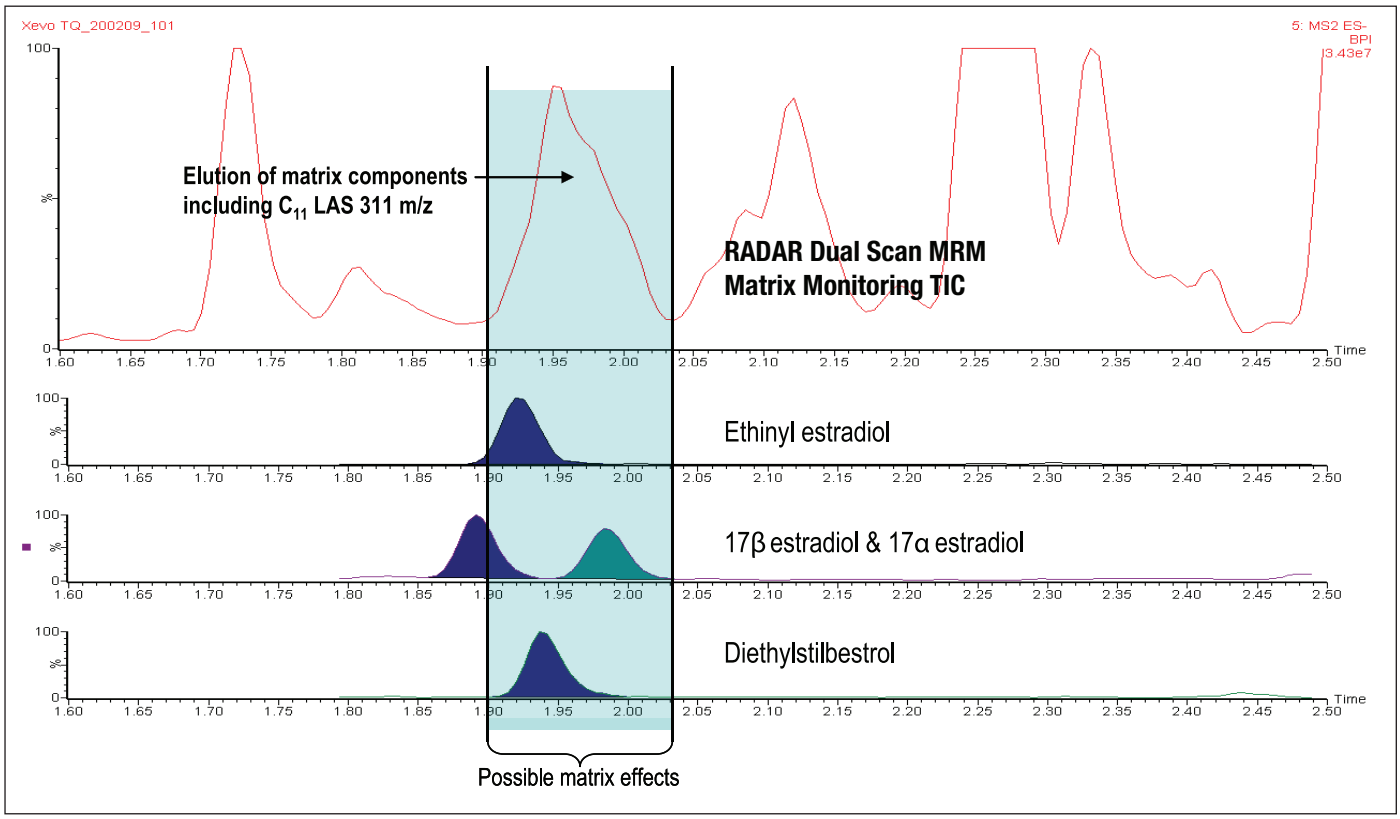


Figure 6. Expanded region of the same spiked sewage sample with some co-eluting of some matrix components including LAS, observed using RADAR Dual Scan-MRM matrix monitoring.

RADAR Dual Scan-MRM can be used to observe compounds in a sample that are not targeted in the original MRM experiment. To demonstrate this, another sewage extract was spiked to 20 ng/L equivalent with pentachlorophenol and analyzed alongside target MRMs for EDCs in RADAR Dual Scan-MRM mode. Figure 7 shows overlaid TIC and extracted 264.8 *m/z* for the pentachlorophenol spiked sewage effluent, as well as a combined mass spectrum taken from the peak observed in the TIC.

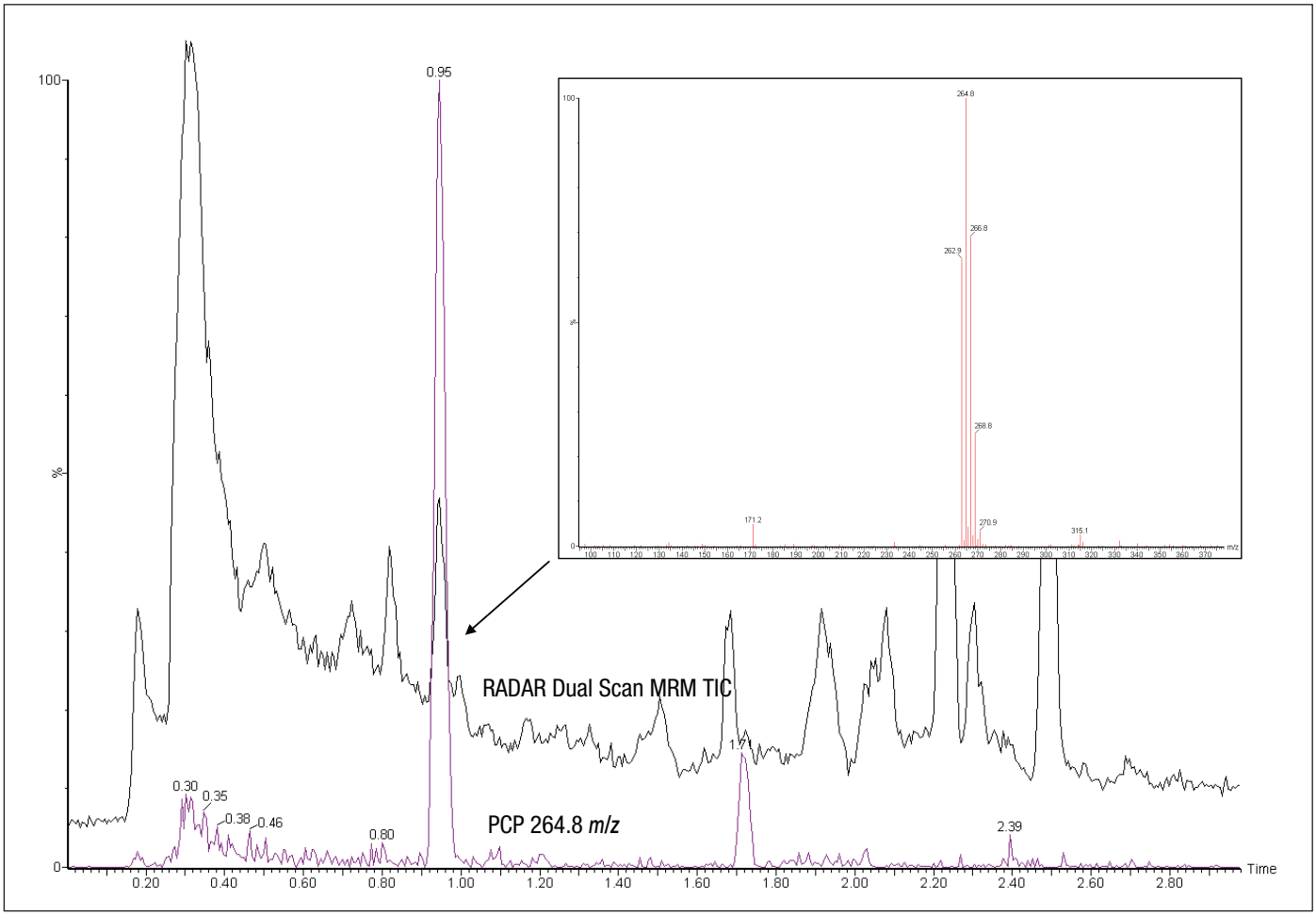


Figure 7. Overlay of TIC and extracted 264.8 m/z for the pentachlorophenol spiked sewage effluent, as well as a combined mass spectrum taken from the peak.

CONCLUSIONS

IntelliStart Technology on Xevo TQ MS can streamline the workflow process of developing highly sensitive MRM acquisition methods for endocrine disrupter analysis. This means that less time is taken to set up methods and results can be generated on real samples faster.

Xevo TQ MS gives high sensitivity and selectivity when applied to measurement of low levels of EDCs in groundwater, river water, and sewage effluent.

ACQUITY UPLC allows high sample throughput while maintaining resolution of the critical isomers 17α and 17β estradiol.

The TrendPlot tool allowed for intra-batch anomalies in data to be quickly discovered and helps ensure quality results are produced.

RADAR Dual Scan-MRM mode allows full scan data to be acquired simultaneously with MRM. This in turn allows matrix monitoring for method development, QC purposes, as well as discovery of non-targeted components.

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3. M Silvia Díaz-Cruz *et al, Journal of Mass Spectrometry.* 2003 38(9): 917-923.

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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

Claude Mallet, Gareth Cleland, and Jennifer A. Burgess
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 Column

Oasis® Sample Extraction Products

TargetLynx™ Application Manager

KEY WORDS

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.

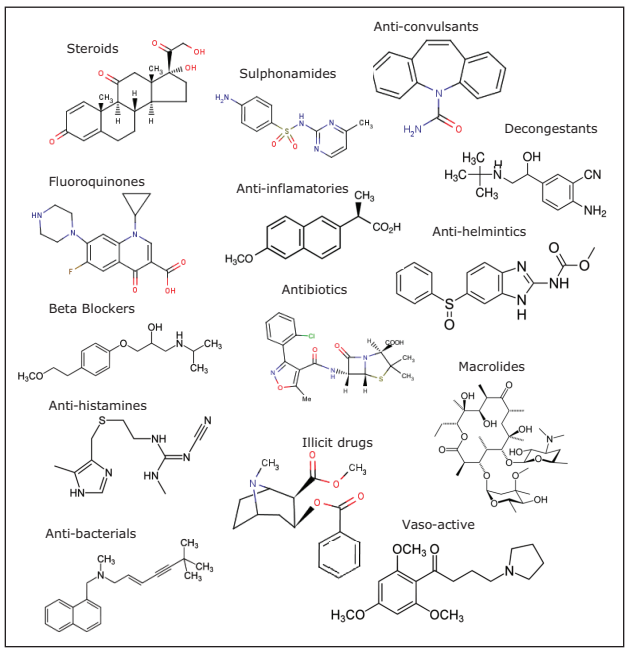

Figure 1. Example compounds from the range of pharmaceuticals and personal care products used in this work.

UPLC conditions

System:	ACQUITY UPLC H-Class
Runtime:	8.0 min
Column:	ACQUITY UPLC HSS T3 C_{18} 1.7 µm, 2.1 x 100 mm
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 and a 6-cc Oasis MCX 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 (2x 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 (2x 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.).

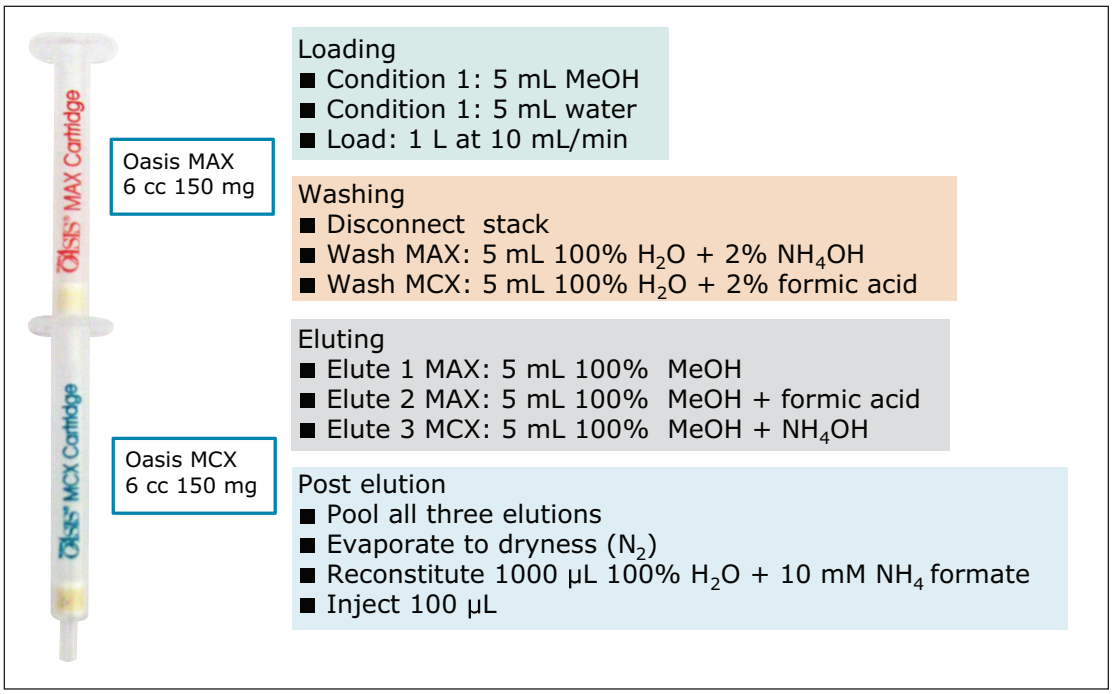

Figure 2. Schematic of solid phase extraction protocol for PPCPs in water.

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 a 2.1 x 100 mm ACQUITY UPLC HSS T3 analytical column (1.7 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.

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	

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

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.

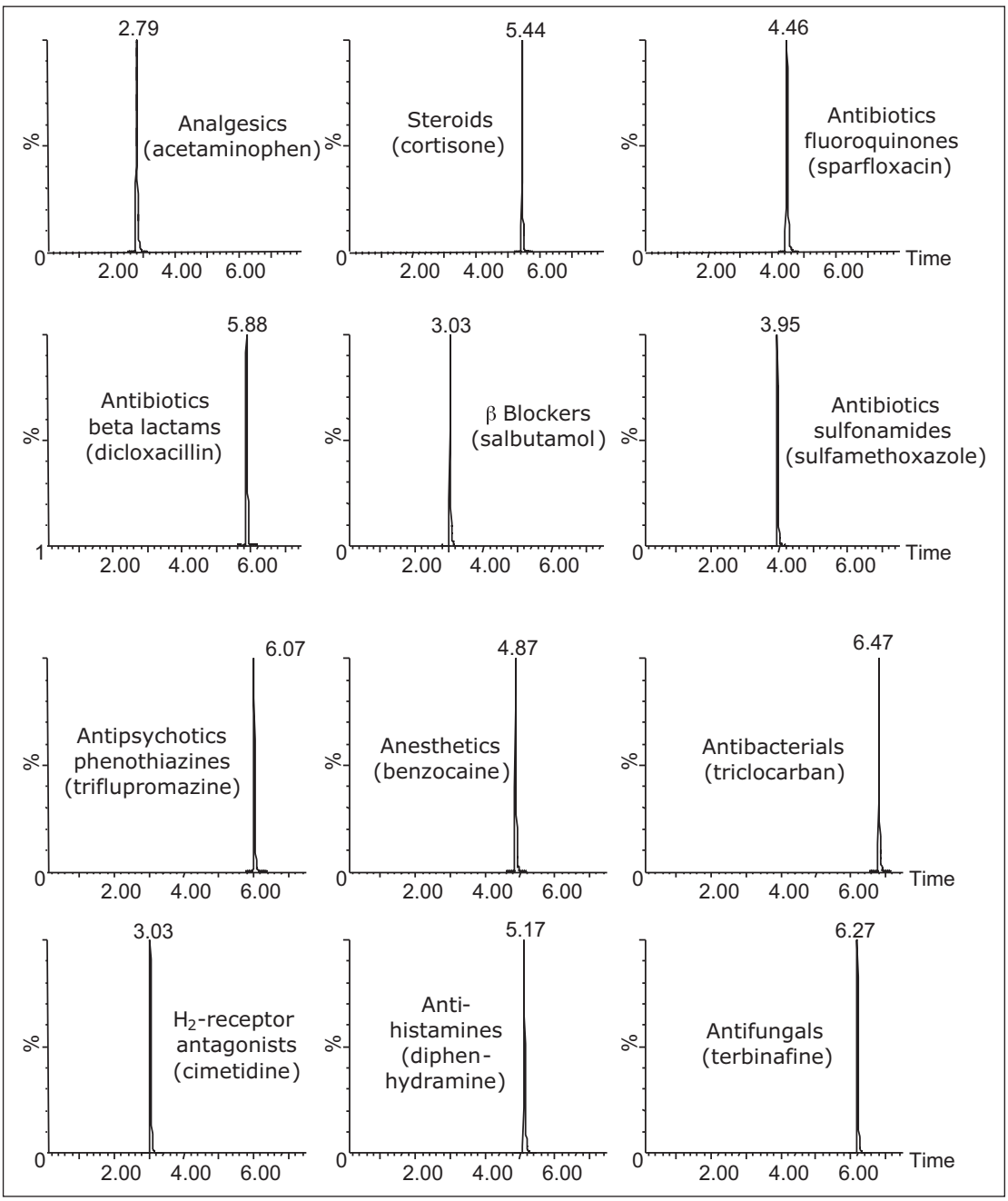

Figure 3. Example MRM chromatograms for compounds from the different classes of PPCPs represented in this work.

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.

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

*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).*

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.

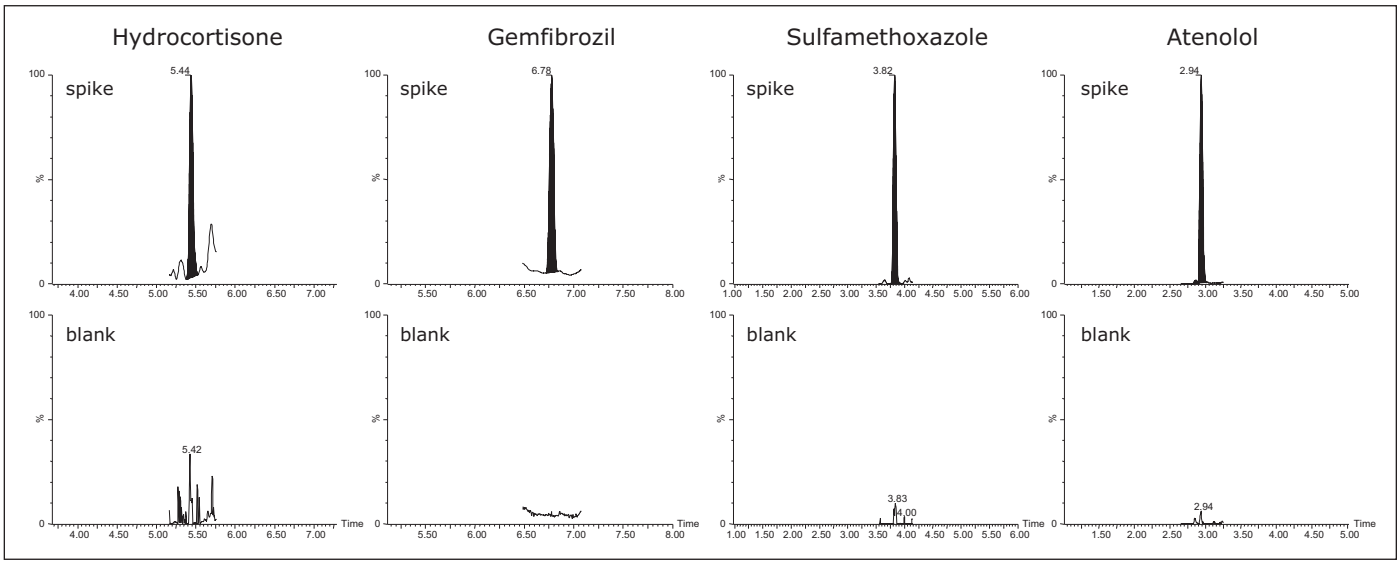

Figure 4. MRM chromatograms for example compounds that demonstrate blank responses in the extracted reagent water. The chromatograms in the top row demonstrate the expected response for the example compounds at the 0.1 ppt level (post-spiked into extracted reagent water). The bottom row shows the response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

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			

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

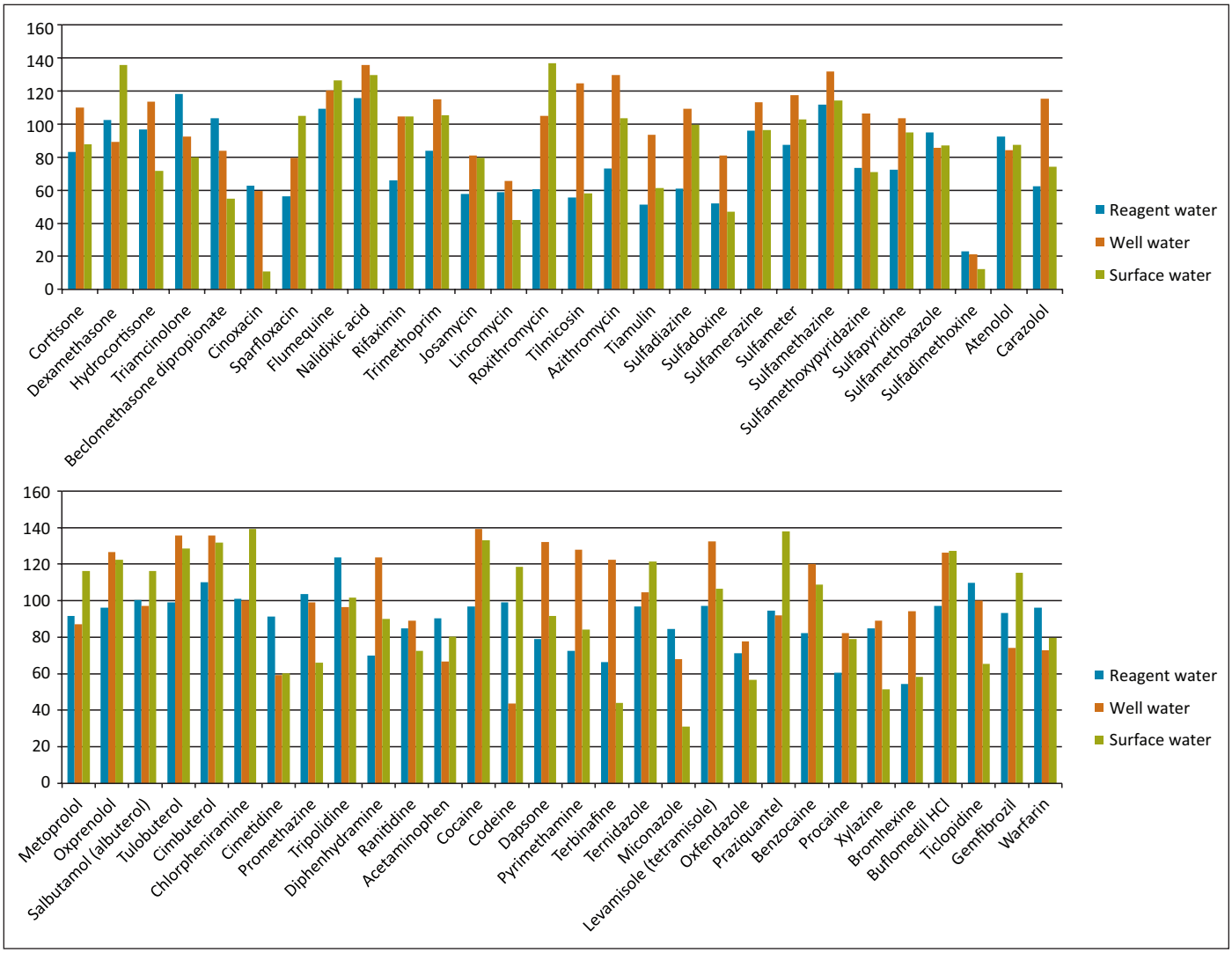

Figure 5. Column chart showing calculated recovery in different water matrices for a 1 ppt spike.

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.

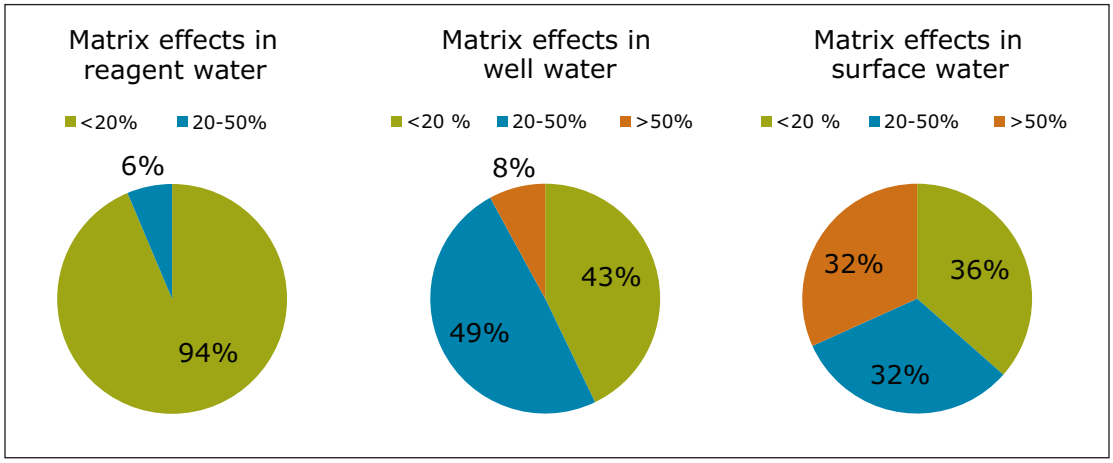

Figure 6. Pie charts showing the level of the matrix effects on the different PPCPs in three different water sample types. Low matrix effect (<20%) is shown in green; medium matrix effect (20% to 50%) is shaded blue; high matrix effect (>50%) is colored orange. The percentage of compounds showing the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

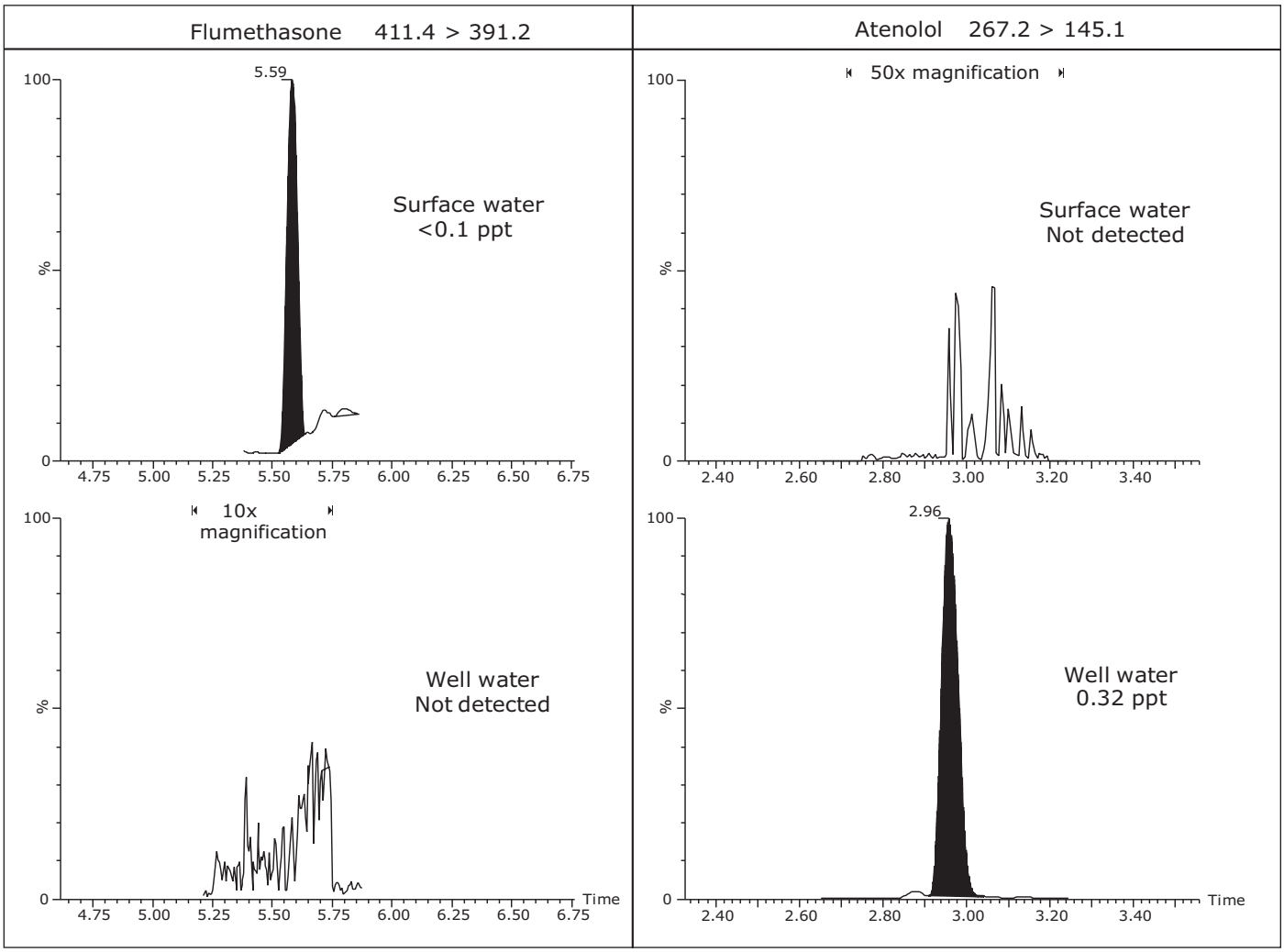

Figure 7. Example compounds that were detected as incurred residues in surface water (flumethasone) and well water (atenolol). To demonstrate a blank sample, the baseline of the sample that did not show the compound detection is shown with the noise level magnified.

CONCLUSIONS

- A method for the extraction, concentration, and quantification of diverse PPCPs including acidic, basic, and neutral compounds was 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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Identification of Potential Metabolites of Pharmaceutical Residues Detected in an Environmental Water Sample

Gareth Cleland, Mark Wrona, Lauren Mullin, and Jennifer Burgess
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

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- Incurred residue metabolite identification

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ACQUITY UPLC HSS 1.7 µm, C_{18} Column

KEY WORDS

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.

Component Summary — View: *Qualitative View

	Component na...	Formula	m/z	Retention Time Error (min)	Mass error (ppm)	Identified High Energy Fragments	Response	Adducts
1	Carbamazepine	C15H12N2O	237.1021	0.21	-0.62	3	10282	+H
2	Hexamine	C6H12N4	141.1136	0.38	0.92	3	40806	+H
3	Imidacloprid	C9H10ClN5O2	256.0597	0.18	0.66	1	7907	+H
4	Tramadol	C16H25NO2	264.1956	0.42	-0.68	1	16859	+H

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.

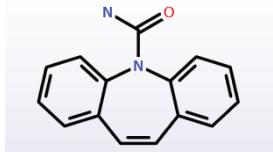
The inclusion of retention times and accurate mass fragment ions in the toxicology screening library allowed for confident matches to be made since they were based on more information other than accurate mass of the precursor ions alone. As indicated, this is critical for reducing false detection rates,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

Selected transformations

	Name	Delta Mass (Da):	Formula	Classifier
1	Ketone to alcohol	2.0157	+H2	Phase I
2	Oxidation	15.9949	+O	Phase I
3	Glucosylation	162.0528	+C6H10O5	Phase II
4	Methylation of alcohol	14.0157	+CH2	Phase II
5	Glucuronide conjugation of anything	176.0321	+C6H8O6	Phase II
6	Sulfate conjugation	79.9568	+SO3	Phase II



Carbamazepine
+H

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.

Component Summary — View: *Metabolite Summary

	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	5927	57.645	Identified
3	Carbamazepine+O	C15H12N2O2	253.0964	5.82	-2.80	6263	60.907	Identified
4	Carbamazepine+O	C15H12N2O2	253.0984	3.44	4.96	431	4.194	Identified

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

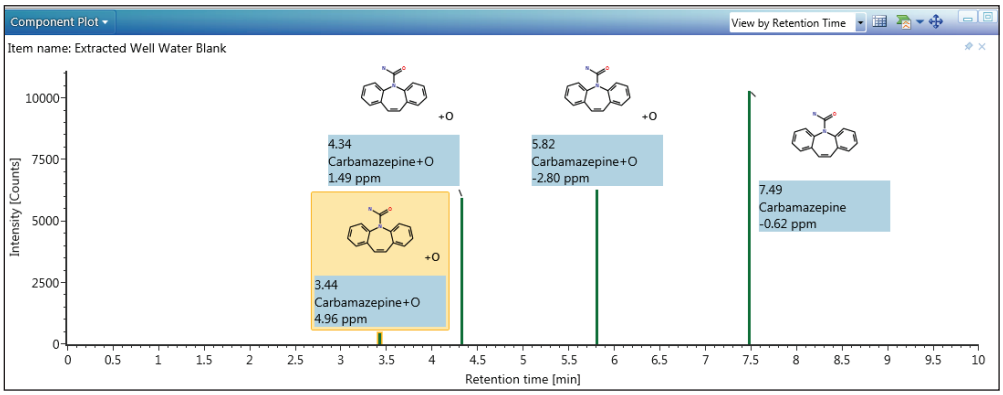


Figure 2. Component plot showing potential metabolites found for carbamazepine using the transformations and mol file shown in Figure 1.

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.

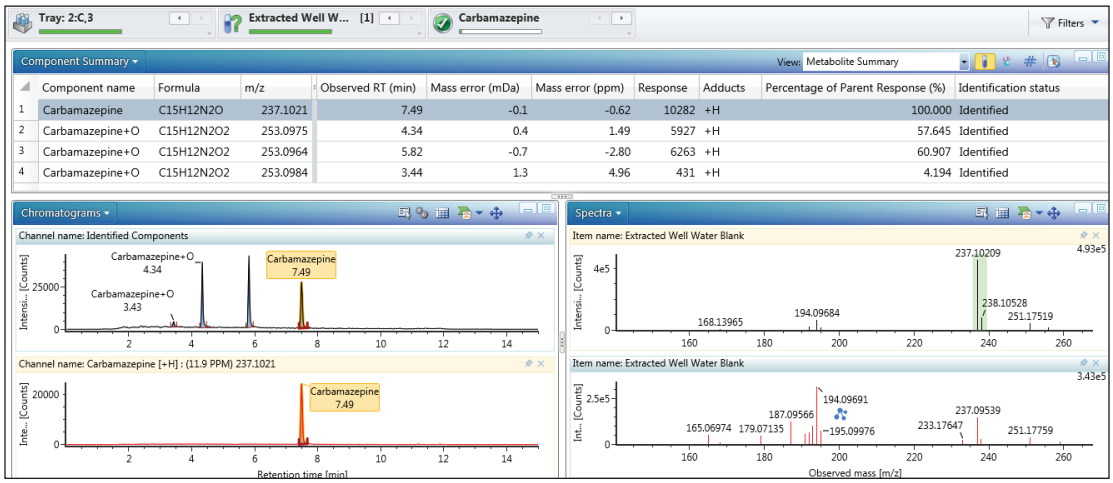


Figure 3. Full user interface (UI) information within UNIFI showing identification details of the carbamazepine parent. Component summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the component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

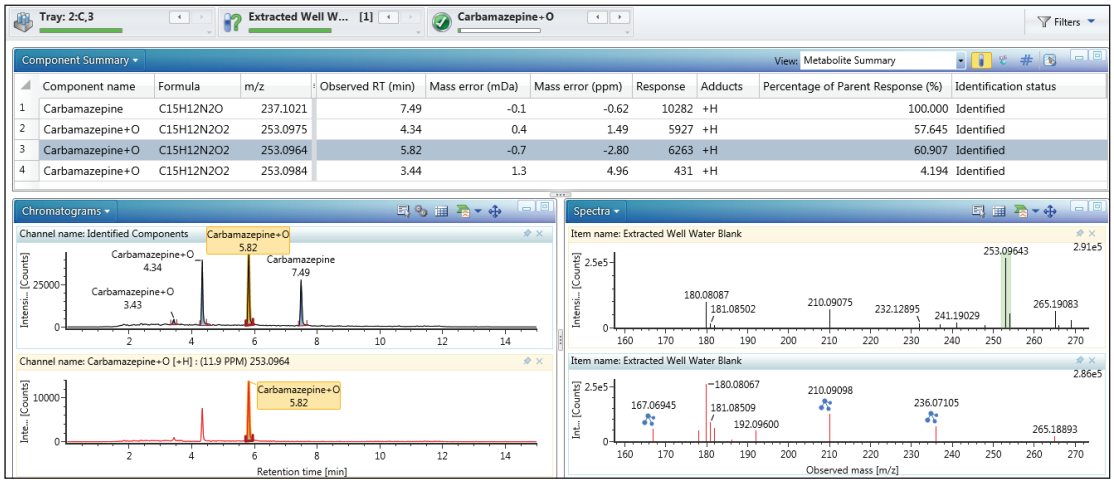


Figure 4. Full user interface (UI) information within UNIFI showing identification details of a proposed carbamazepine metabolite. Component summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the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

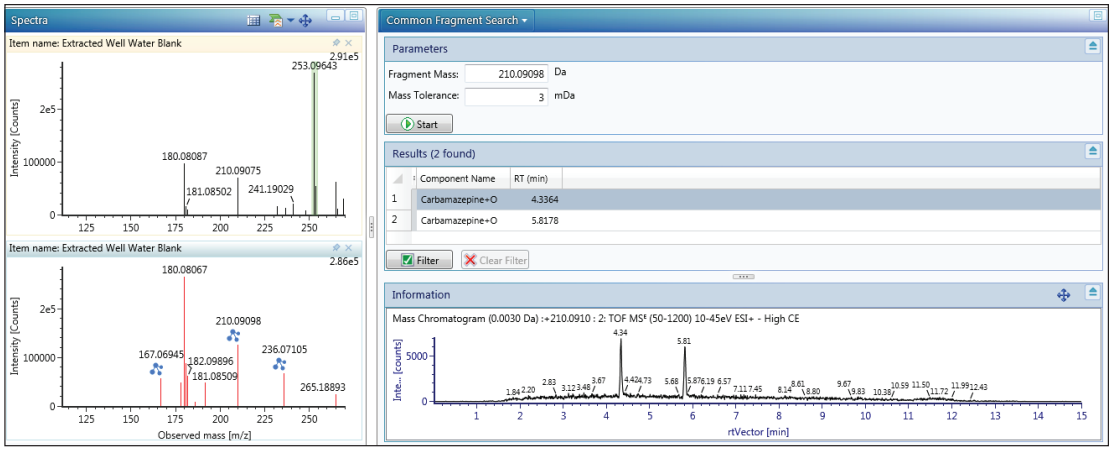


Figure 5. Results from a common fragment search of 210.09098 Da, performed within the elucidation toolset in UNIFI.

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.

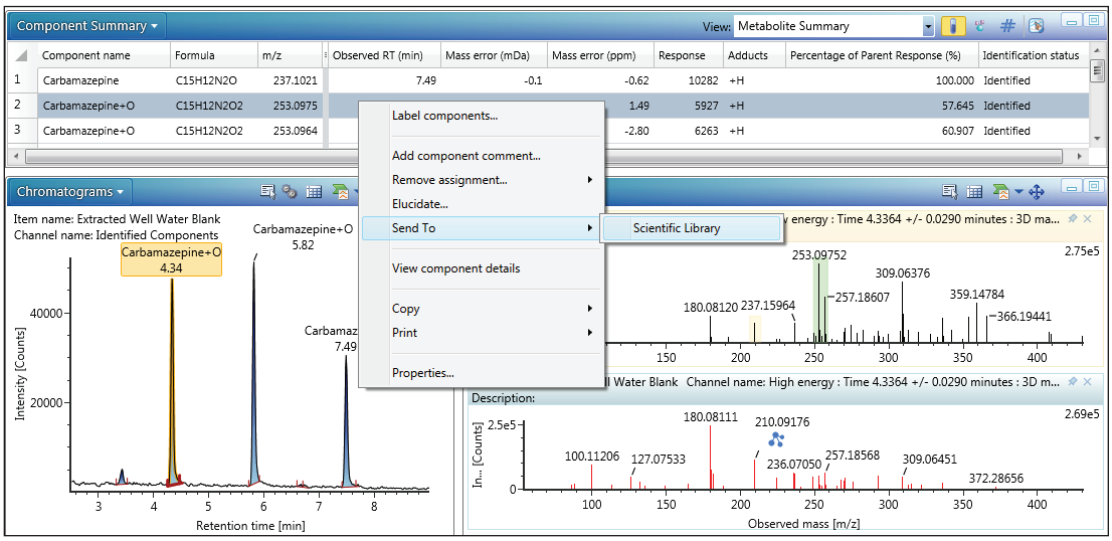


Figure 6. Sending reviewed metabolites to UNIFI's scientific library.

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.

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Ensuring Seafood Safety with Rapid Screening for Polyaromatic Hydrocarbons Using LC-Fluorescence

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- Selective measurement through the use of fluorescence detection

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KEY WORDS

Polyaromatic Hydrocarbons, PAHs, QuEChERS, fluorescence, food safety, environmental

GOAL

To demonstrate that the combination of the DisQuE Sample Preparation Kit with UPLC®-FLR provides a rapid screening tool for the detection of PAHs in seafood.

INTRODUCTION

Major oil spills, such as the Exxon Valdez in 1989 and the April 2010 Gulf of Mexico oil spill, have raised concerns over the quality of seafood harvested from these regions. Fish, crustaceans, and mollusks may come into contact with, or ingest the oil thereby introducing potential health risks to consumers.

Of the many compounds found in oil, an important subset is the Polyaromatic Hydrocarbons (PAHs). The US Environmental Protection Agency (US EPA) has defined these compounds as priority pollutants.[1] The US Food and Drug Administration (US FDA) has also established levels of concern ranging from 3.5×10^{-2} mg/kg benzo(a) pyrene in finfish, to 2.0×10^{3} mg/kg combined phenanthene and anthracene in oysters.[2] Confirmatory analysis is required if any PAHs are detected at half the level of concern.[2]

To prevent consumption of contaminated seafood and minimize the impact on the seafood industry, a fast screening method is required to analyze these compounds of concern at the stated levels. Here we demonstrate that, following a simple extraction method using Waters DisQuE Dispersive Sample Preparation Kit (QuEChERS), an analysis of PAHs can be achieved in less than 4 minutes using the ACQUITY UPLC H-Class System with Fluorescence Detection.

Figure 1. ACQUITY UPLC H-Class System with FLR.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC H-Class with Large Volume Flow Cell (LVFC)
Column:	PAH 4.6 x 50 mm, 3 µm
Column temp.:	35 °C
Injection volume:	10 µL
Sampling rate:	20 pts/sec
Detection:	Fluorescence using timed programmed wavelength changes
Software:	Empower 2
Mobile phase A:	Milli-Q water
Mobile phase B:	Methanol, Fisher Optima Grade
Mobile phase C:	Acetonitrile, Fisher Optima Grade
Standards:	PAH Certified Standard, AccuStandard M 8310
Flow rate:	2.0 mL/min

Gradient profile

Time (min)	Flow rate (mL/min)	%A	%B	%C	Curve
0.00	2.0	30	70	0	
2.25	2.0	0	70	30	6
3.50	2.0	0	0	100	6
3.60	2.0	30	70	0	6

Sample preparation

Individual samples of fish fillets (flounder), shelled shrimp, and shucked oysters with liquor were homogenized using a food processor per the method described by Ramalhosa *et. al.*[3] 15 grams of each homogenized tissue were added to individual centrifuge tubes and spiked at three different levels, 50 ng/g, 1 µg/g, and 10 µg/g for shrimp and oysters, 15 ng/g, 1 µg/g, and 10 µg/g for fish, with a spiking solution prepared from the certified PAH standard. 5 mL of water were added to the fish and shrimp samples to aid mixing. The oysters did not need extra liquid. The spiked samples were thoroughly mixed and allowed to sit at room temperature for an hour.

To each centrifuge tube was added the contents of a DisQuE tube (P/N 186004571), 6 g magnesium sulfate + 1.5 g sodium acetate, and 15 mL of acetonitrile. The centrifuge tube was shaken vigorously for at least one minute to produce an emulsion of seafood tissue, buffer salts and acetonitrile. Here also the procedure of Ramalhosa[3] was followed as no acetic acid was added to the acetonitrile, nor was a secondary PSA cleanup step carried out. Initial work in our laboratory confirmed that the PSA step was not required for LC-FLR analysis (data not shown). After centrifuging at 3000 rpm for 5 minutes, a portion of the clear acetonitrile supernatant layer was transferred to an autosampler tube for direct injection. The 1 µg/g and 10 µg/g spikes were diluted with acetonitrile 1:10 and 1:100 respectively. Samples were quantified using a six-point linear calibration curve. Standards were prepared by diluting the certified standard with acetonitrile.

RESULTS AND DISCUSSION

Dispersive sample preparation, often referred to as QuEChERS, is a well proven and fast sample preparation method for the analysis of pesticides in food commodities.[4] More recently, this method has been used to extract other contaminants from food matrices, including polyaromatic hydrocarbons.[3]

The separation of the 15 fluorescent PAHs that are listed as priority pollutants by the US EPA was achieved in only 3.5 minutes using the ACQUITY UPLC H-Class System. The separation of the analytes is shown in Figure 2, with the timed programmed wavelength changes indicated by arrows.

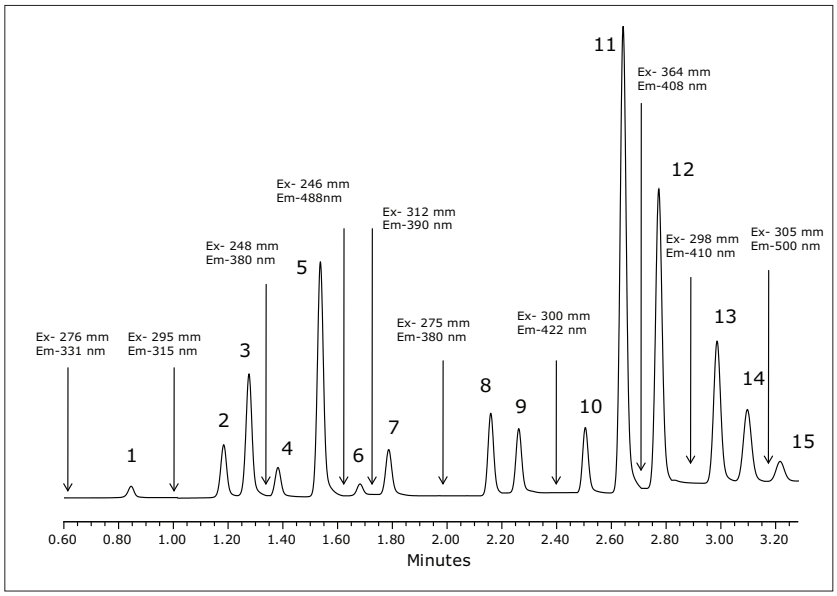

Figure 2. Separation of PAH analytes (0.1 mg/L) using timed programmed wavelength changes as indicated by arrows. PAH analytes are identified as follows: (1) naphthalene, (2) acenaphthene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) fluoranthene, (7) pyrene, (8) benzo(a)anthracene, (9) chrysene, (10) benzo(b)fluoranthene, (11) benzo(k)fluoranthene, (12) benzo(a)pyrene, (13) dibenzo(a,h)anthracene, (14) benzo(g,h,i)perylene, (15) indeno(1,2,3-cd)pyrene.

Example chromatograms of the shrimp, fish, and oyster matrices spiked at 10 µg/g are shown in Figure 3. Certain sections of the chromatograms have been magnified to more clearly show the peaks of interest. As shown in Figure 3D, the blank water sample that was also carried through the sample preparation procedure shows a very clean chromatogram.

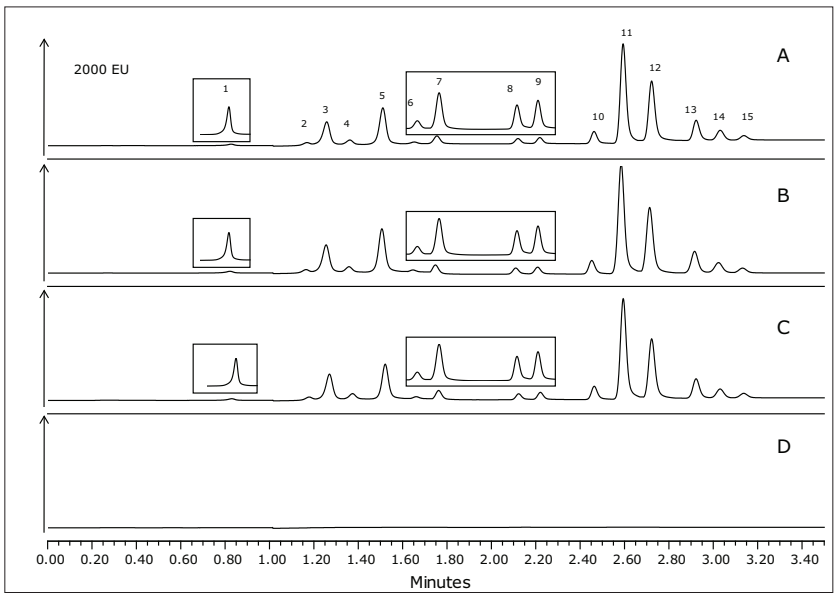

Figure 3. Chromatograms of PAHs spiked into (A) shrimp, (B) fish, and (C) oysters at 10.0 µg/g (diluted 1:100 with acetonitrile following extraction). Insets show zoomed peaks for (1) naphthalene, (6) fluoranthene, (7) pyrene, (8) benzo(a)anthracene, and (9) chrysene. The blank water sample (D) taken through the extraction procedure is also shown.

Samples of unspiked seafood matrices that were used in this sample preparation procedure also showed no matrix interference, as shown in Figure 4.

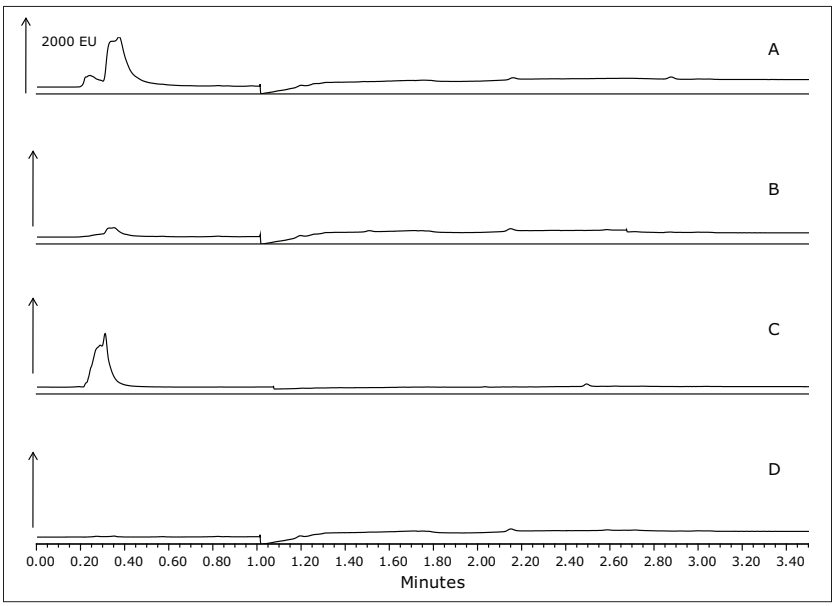

Figure 4. Chromatograms (A) unspiked shrimp, (B) fish, and (C) oysters carried through the QuEChERS procedure, along with a water blank (D). Changes in the baseline are a result of the programmed wavelength changes.

Samples were quantified against six point calibration curves of each of the analytes. An example calibration curve is shown for benzo(a)pyrene in Figure 5. Linearity (R^2) was > 0.995 for all analytes.

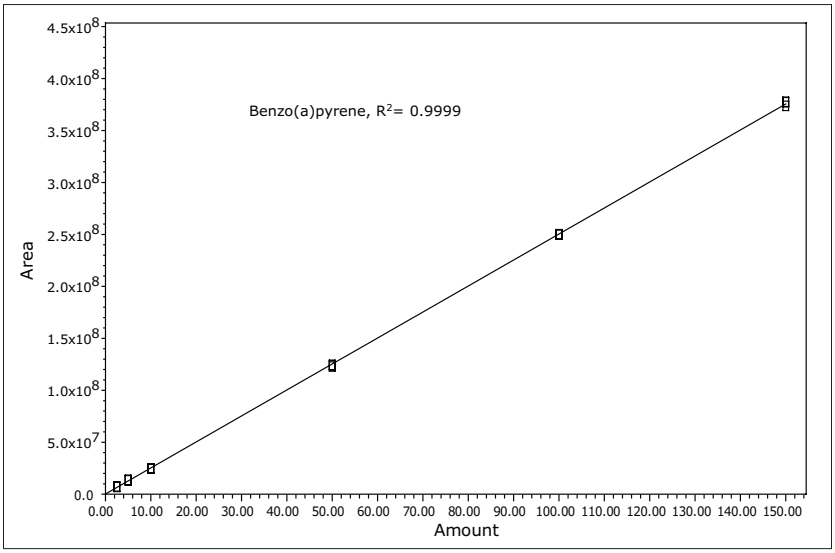

Figure 5. Calibration curve for benzo(a)pyrene (2.5 to 150.0 µg/L).

Using Waters DisQuE Dispersive Sample Preparation Kit, PAHs were extracted from three different seafood matrices. The recoveries and percentage RSDs for shrimp, fish, and oysters are shown in Tables 1 to 3. Recoveries were in the range of 68% to 149%. Table 4 lists the recoveries for a series of QC water spikes, fortified at the levels listed and carried through the sample prep procedure previously described.

Compound	Average RT N=9		10.0 µg/g spike N=3		1.0 µg/g spike N=3		50.0 ng/g spike N=3	
Reproducibility and Recovery data for Shrimp	RT	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Naphthalene	0.83	.07	93	4.3	85	12.0	94	3.2
Acenaphthene	1.17	.14	90	5.7	82	1.1	121	1.2
Fluorene	1.26	.11	89	6.4	76	1.4	84	2.4
Phenanthrene	1.36	.10	86	8.1	73	2.5	88	2.3
Anthracene	1.51	.09	89	6.7	75	1.8	78	3.2
Fluoranthene	1.65	.07	91	6.7	79	3.2	84	2.9
Pyrene	1.76	.06	86	8.4	75	2.1	78	4.5
Benzo(a)anthracene	2.12	.04	88	10.3	76	4.4	74	4.1
Chrysene	2.22	.04	85	12.1	77	3.1	76	3.0
Benzo(b)fluoranthene	2.46	.04	87	7.6	75	2.4	72	1.5
Benzo(k)fluoranthene	2.59	.03	84	10.4	77	3.1	72	2.7
Benza)pyrene	2.72	.03	84	9.4	75	2.6	72	2.6
Dibenzo(a,h)anthracene	2.92	.04	77	12.7	72	4.0	70	2.7
Benzo(g,h,i)perylene	3.03	.05	70	12.6	68	3.3	68	2.5
Indeno(1,2,3-cd)pyrene	3.14	.06	83	10.2	74	1.4	68	2.5

Table 1. Recovery and reproducibility data for spiked shrimp.

Compound	Average RT N=9		10.0 µg/g spike N=3		1.0 µg/g spike N=3		15.0 ng/g spike N=3	
Reproducibility and Recovery Data for Fish	RT	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Naphthalene	0.82	0.04	141	3.5	102	4.2	114	5.4
Acenaphthene	1.17	0.07	118	2.9	92	3.2	116	10.6
Fluorene	1.26	0.04	118	3.0	88	2.7	83	1.1
Phenanthrene	1.36	0.04	108	3.5	81	5.0	89	1.4
Anthracene	1.51	0.03	114	2.8	88	6.9	77	1.1
Fluoranthene	1.65	0.03	95	5.0	71	4.4	85	2.5
Pyrene	1.75	0.04	91	5.7	68	3.1	73	2.3
Benzo(a)anthracene	2.11	0.03	118	1.7	90	6.8	79	3.6
Chrysene	2.21	0.04	114	2.5	86	5.7	77	1.4
Benzo(b)fluoranthene	2.45	0.04	114	2.5	87	4.2	73	1.8
Benzo(k)fluoranthene	2.58	0.05	111	2.5	87	4.7	73	1.9
Benza)pyrene	2.71	0.06	105	1.8	81	5.1	68	1.0
Dibenzo(a,h)anthracene	2.91	0.06	101	3.5	78	6.0	69	2.6
Benzo(g,h,i)perylene	3.02	0.06	85	2.2	71	5.0	66	3.0
Indeno(1,2,3-cd)pyrene	3.13	0.06	99	1.1	75	6.2	61	9.2

Table 2. Recovery and reproducibility data for spiked fish.

Compound	Average RT N=3		10.0 µg/g spike N=3		1.0 µg/g spike N=3		50.0 ng/g spike N=3	
Reproducibility and Recovery Data for Oysters	RT	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Naphthalene	0.83	0.10	102	7.8	149	9.8	104	6.0
Acenaphthene	1.17	0.18	99	5.8	145	13.2	130	2.7
Fluorene	1.27	0.19	100	5.9	143	12.1	100	1.9
Phenanthrene	1.37	0.18	103	9.7	143	16.4	108	3.8
Anthracene	1.52	0.16	80	1.4	116	10.6	67	11.7
Fluoranthene	1.66	0.14	100	15.7	142	17.7	103	5.5
Pyrene	1.76	0.13	107	15.3	149	16.9	108	5.3
Benzo(a)anthracene	2.12	0.09	94	8.6	136	9.4	78	10.1
Chrysene	2.22	0.08	94	5.5	139	9.2	83	8.0
Benzo(b)fluoranthene	2.46	0.06	94	5.3	137	10.9	83	4.1
Benzo(k)fluoranthene	2.59	0.05	94	6.1	140	9.6	84	4.8
Benza)pyrene	2.72	0.04	86	5.2	125	11.2	75	7.5
Dibenzo(a,h)anthracene	2.92	0.04	78	5.4	124	7.8	81	6.0
Benzo(g,h,i)perylene	3.03	0.04	72	8.6	114	16.9	78	8.6
Indeno(1,2,3-cd)pyrene	3.14	0.03	86	4.8	126	9.0	82	5.8

Table 3. Recovery and reproducibility data for spiked oysters.

Compound	Average RT N=9		1000.0 µg/g QC3, A-C N=3		15.0 ng/g QC2, A-C N=3		5.0 ng/g QC1,A-C N=3	
Reproducibility and Recovery Data for QC Water Spikes	RT	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Naphthalene	0.83	0.09	82	3.0	89	4.6	100	13.3
Acenaphthene	1.17	0.19	77	0.7	95	3.4	32	27.4
Fluorene	1.26	0.04	76	1.0	76	1.8	80	7.4
Phenanthrene	1.36	0.04	78	1.9	74	3.6	70	7.5
Anthracene	1.51	0.03	78	2.5	67	2.5	66	8.3
Fluoranthene	1.66	0.04	84	3.5	73	12.5	78	11.9
Pyrene	1.76	0.04	83	1.0	72	9.9	86	4.7
Benzo(a)anthracene	2.12	0.03	93	1.9	76	0.6	72	5.0
Chrysene	2.22	0.04	95	1.8	79	0.4	78	5.5
Benzo(b)fluoranthene	2.46	0.04	95	0.8	76	2.9	70	6.7
Benzo(k)fluoranthene	2.60	0.04	95	1.6	81	0.9	72	5.0
Benza)pyrene	2.72	0.04	96	1.5	81	1.5	76	4.8
Dibenzo(a,h)anthracene	2.92	0.04	96	1.9	88	2.4	84	3.3
Benzo(g,h,i)perylene	3.03	0.04	96	2.1	87	3.8	86	3.0
Indeno(1,2,3-cd)pyrene	3.14	0.04	99	1.0	85	1.9	80	2.9

Table 4. Recovery and reproducibility data for QC water spikes.

The results were excellent for all of the compounds at each fortification level, except the lowest level for acenapthene in water (5 ng/g). At this low level, acenapthene showed more variation owing to the small peak area and a sloping baseline that was only noticeable at this level. Table 5 is an estimation of the Limit of Detection based on seven replicates of each seafood matrix spiked at a 5 ng/g level, and calculated per US EPA 40 CFR, Appendix B to part 136 Rev 1.15.

Compound	LOD Shrimp (ng/g)	LOD Fish (ng/g)	LOD Oysters(ng/g)
Naphthalene	1.21	5.00	2.06
Acenaphthene	2.35	2.78	2.15
Fluorene	0.78	0.72	1.91
Phenanthrene	0.60	0.62	2.96
Anthracene	0.62	0.33	1.43
Fluoranthene	1.29	1.27	2.75
Pyrene	0.65	0.91	3.17
Benzo(a)anthracene	0.39	0.38	2.28
Chrysene	0.49	0.48	1.70
Benzo(b)fluoranthene	0.37	0.24	1.93
Benzo(k)fluoranthene	0.41	0.26	1.77
Benzo(a)pyrene	0.34	0.63	1.62
Dibenzo(a,h)anthracene	0.39	0.19	1.73
Benzo(g,h,i)perylene	0.42	0.25	1.84
Indeno(1,2,3-cd)pyrene	0.51	0.64	1.79

Table 5. Limit of Detection (LOD) data for spiked shrimp, fish, and oysters, calculated per the standard deviation of seven individual spikes at the 5 ng/g level of each seafood matrix per US EPA 40 CFR, Appendix B to part 136 Rev 1.1.

This application note demonstrates that the combination of the DisQuE Sample Preparation Kit with LC-FLR provides a rapid screening tool for the detection of PAHs in seafood.

CONCLUSIONS

- Dispersive sample preparation provides a fast and effective method for extracting PAHs from different seafood matrices.
- This method demonstrates advantages over other sample preparation techniques as accurate results can be achieved with less sample preparation and in a shorter time.[3]
- With sample preparation times reduced, a rapid chromatographic separation is critical to manage the samples, standards, and QCs generated using this approach.
- The ACQUITY UPLC H-Class System's separation, which was achieved in less than 4 minutes, is able to address this demand.
- This solution allows laboratories to screen for PAHs in seafood, providing results in a timely and economical manner, so consumers can be confident that these products are safe.

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Advancing Perfluorinated Compound Analysis Using Simultaneous Matrix Monitoring

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APPLICATION BENEFITS

- Extraction and analysis of PFCs in water and biological samples
- Use of Triple Quadrupole Mass Spectrometry in Waters RADAR™ dual scan – multiple reaction monitoring (MRM) mode to quantify PFCs at low concentrations while monitoring matrix background simultaneously

WATERS SOLUTIONS

ACQUITY®

ACQUITY UPLC® System

Xevo® TQ MS

ACQUITY UPLC BEH C_{18} Column

PFC Analysis Kit

PFC Column Kit

Oasis® Sample Extraction Products

TargetLynx™ Application Manager

MassLynx® Software v.4.1

RADAR™ Duel Scan-MRM

KEY WORDS

Environmental, water, biological samples, PFCs, PFOS, SPE

GOAL

To demonstrate the importance of monitoring background sample matrix during high sensitivity perfluorinated compound analysis in environmental waters and biota.

INTRODUCTION

Understanding the occurrence, fate, and impact of persistent organic pollutants (POPs) is a global priority and consequently is undertaken by a diverse range of organizations. Perfluorinated compounds (PFCs) have become increasingly important and perfluorooctane sulphonic acid (PFOS) has been included in the Stockholm Convention on POPs.[1] The tracking of PFCs is critical to organizations whose activities might inadvertently facilitate exposure to populations through water, food supplies, and consumer products. This is also a priority for researchers and regulators and is especially true when taking into account the high publicity related to newer contaminants such as PFCs.

Over the past decade these compounds have been determined in an array of matrices by various techniques with liquid chromatography tandem quadrupole mass spectrometers (LC-MS/MS) featuring heavily.[2] The ability of laboratories to successfully measure PFCs in various matrices has improved greatly in recent times, largely due to improvements in labeled standard availability highlighted in recent inter laboratory studies.[3,4] These studies also attribute the continuous improvement in data quality to advances in instrumental technology. Advances in LC-MS/MS instrument performance have largely been focused on Multiple Reaction Monitoring (MRM) sensitivity to satisfy the need for increasingly lower detection limits. While this is clearly a priority for this type of instrumentation, there have been limitations previously in acquiring important qualitative information from a sample in a single injection with previous generation instruments.

This information can be of high value as a method development tool or intra-sample QC check when analyzing ultra-trace level contaminants in difficult sample matrices such as environmental waters and biological tissues. This ultimately leads to greater confidence in the analytical result.

EXPERIMENTAL

Sample preparation

Environmental water samples were obtained from Lake Mariestadssjön, River Svartån, and various drinking water sources in Sweden. Fish liver samples were from unknown locations in Norway.

Water samples were stored at 4 °C until analysis, and filtered through glass microfiber filters before extraction using Waters® Oasis WAX 6 cc/150-mg cartridges (substituting Oasis HLB in the PFC Analysis Kit[5]), according to standard method ISO 25101.[6] Both of these sorbents are described as suitable in ISO 25101. An outline is provided below.

Condition:	4 mL 0.1% NH_4OH/MeOH, 4 mL MeOH, 4 mL H_2O
Load sample:	Under vacuum between 3 mL/min and 6 mL/min
Dry:	Under vacuum
Wash:	Acetate buffer (4 mL, 0.025 M), 4 mL MeOH
Elute:	4 mL 0.1 % NH_4OH/MeOH

Eluant was evaporated and reconstituted to 40:60 MeOH:H_2O with 2-mM ammonium acetate. The final extracts were filtered or centrifuged if necessary.

For fish liver samples acetonitrile extraction was followed by cleanup using Oasis WAX and dispersive carbon. Detailed method description for fish liver extraction can be found elsewhere.[7] In brief, approximately 1-g liver was cut into pieces and homogenized using a probe homogenizer. Acetonitrile (10 mL) was added, and the mixture was repeatedly vortex-mixed and sonicated for 30 min. The supernatant acetonitrile phase was removed after centrifugation (10,000 xg, 30 min), and the extraction procedure was repeated. The acetonitrile fractions were combined and reduced to 10 mL after which 25 mL water was added. After mixing and centrifugation the solution was put through an Oasis WAX 6 cc/150 mg-cartridge, and the outline procedure is given below.

Condition:	4 mL MeOH, 4 mL H_2O
Load sample:	Under vacuum between 3 mL/min and 6 mL /min
Dry:	Under vacuum
Wash:	Acetate buffer (4 mL, 0.025 M), 4 mL 40% MeOH in water, 8 mL MeOH
Elute:	2 mL 2% NH_4OH in MeOH

Elution was placed into a tube with 50 mg graphitized non-porous carbon and 100-µL acetic acid. The carbon solution was vortex-mixed for 30 s, and then filtrated through 0.2-µm GHP membrane.

Sample extracts and standard solutions were prepared so that the solvent composition was 40:60 MeOH:H_2O with 2-mM ammonium acetate. The final extracts was filtered or centrifuged if necessary.

UPLC® conditions

System:	ACQUITY UPLC® with PFC Analysis Kit
Column:	ACQUITY BEH C_{18} Column 1.7 µm, 2.1 x 50 mm
Column temp.:	50 °C
Mobile phase A:	(98:2) 2 mM CH_3COONH_4 (aqueous): MeOH
Mobile phase B:	MeOH + 2 mM CH_3COONH_4
Flow rate:	0.65 mL/min
Injection volume:	10 µL

AQUITY UPLC gradient is detailed in Table 1.

Time (min)	Flow rate	%A	%B
Initial	0.65	75.00	25.00
0.50	0.65	75.00	25.00
5.00	0.65	15.00	85.00
5.10	0.65	0.00	100.00
6.60	0.65	0.00	100.00
6.70	0.65	75.00	25.00

Table 1. ACQUITY UPLC gradient.

MS conditions

MS system:	Xevo TQ MS
Acquisition mode:	RADAR Dual Scan-MRM
Ionization mode:	ESI negative
Capillary voltage:	0.44 kV
Source temp.:	150 °C
Desolvation temp.:	600 °C
Desolvation gas:	900 L/hr
Cone gas flow:	30 mL/min
Collision gas flow:	0.18 mL/min

Xevo TQ MS setup (mass resolution and mass calibration) was automated by IntelliStart Software. Table 2 shows MRM acquisition parameters and retentions time for each compound. RADAR Dual Scan-MRM full-scan spectra were acquired with mass range of 50 to 650 *m/z* at 3000 Da/s.

This application note describes advanced high-sensitivity Xevo TQ MS analysis of PFCs in environmental waters and biota, incorporating Waters RADAR dual scan-MRM functionality to understand the nature of the sample matrix, while simultaneously performing routine quantitation.

RT(min)	PFC	Precursor *m/z*	Product *m/z*	Cone (V)	Collision Energy (V)
1.67	PFBuS	299	80	40	30
		299	99	40	31
2.37	PFHxA	313	119	16	17
		313	269	16	10
3.05	PFHpA	363	119	16	17
		363	169	16	19
		363	319	16	10
3.13	PFHxS	399	80	45	33
		399	99	45	31
3.52	THPFOS	427	80	42	30
		427	407	42	19
3.55	PFOA	413	169	16	19
		413	219	16	17
		413	369	16	10
3.55	13CPFOA	417	372	16	10
		463	169	16	19
3.94	PFNA	463	219	16	17
3.94	13CPFNA	468	423	16	10
		499	80	60	39
3.97	PFOS	499	99	60	38
3.97	13CPFOS	503	80	60	39
		513	219	16	17
4.27	PFDA	513	469	16	10
		563	319	16	17
4.56	PFUnDA	563	519	16	10
4.80	PFDoDA	613	569	16	10

Table 2. Multiple Reaction Monitoring conditions and retention times for PFCs.

Data acquisition and processing

MassLynx Software v.4.1 was used for data acquisition and spectral processing. TargetLynx Application Manager was used to quantify PFC in samples.

RESULTS AND DISCUSSION

Rapid UPLC separations of PFCs were achieved with PFDoDA eluting at 4.8 min. This allowed for the analysis of eight samples per hour when taking into account column equilibration time. The flow rate used in the analysis was 0.65 mL/min, which is within the optimum range for UPLC. This helped reduce chromatographic band broadening and resulting in peak widths (at baseline) of approximately 3 s for all compounds. Source design improvements have enabled optimum UPLC efficiency at higher flow rates to be utilized without adversely affecting instrumental sensitivity. Figure 1 shows overlaid MRM chromatogram of each targeted PFC in a solvent standard with analyte concentrations ranging between 1 pg/µL and 2 pg/µL. The PFC Analysis Kit allowed solvent blanks with undetectable levels of all PFCs, meaning that system blank contribution was removed.

Environmental waters

Quantitation of PFCs in non-fortified tap water, surface water, and river water was performed to determine native concentrations. Comfortable detection of native PFCs was achieved in the range 0.23 to 1.50 ng/L for each sample. Positive detections in each sample included PFOA and PFOS. Calibration curves and extracted MRM chromatograms for each water sample are shown in Figure 2 for PFOA and PFOS.

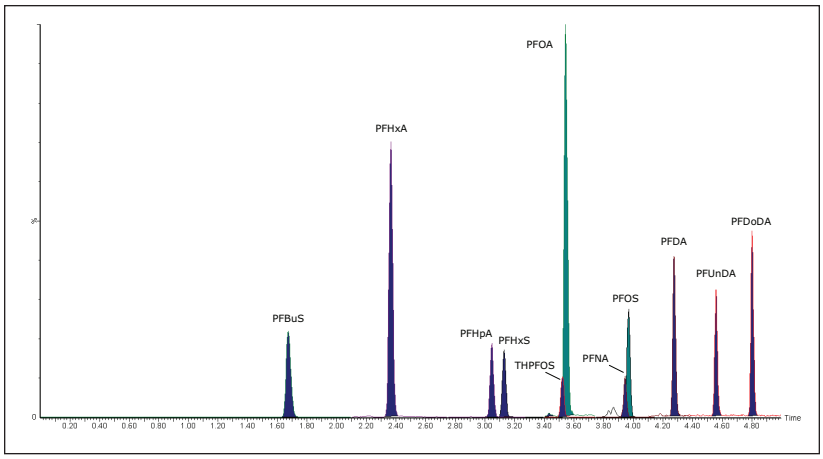

Figure 1. Overlaid MRM chromatograms of each target PFC (solvent standard; concentrations range between 1 pg/µL and 2 pg/µL).

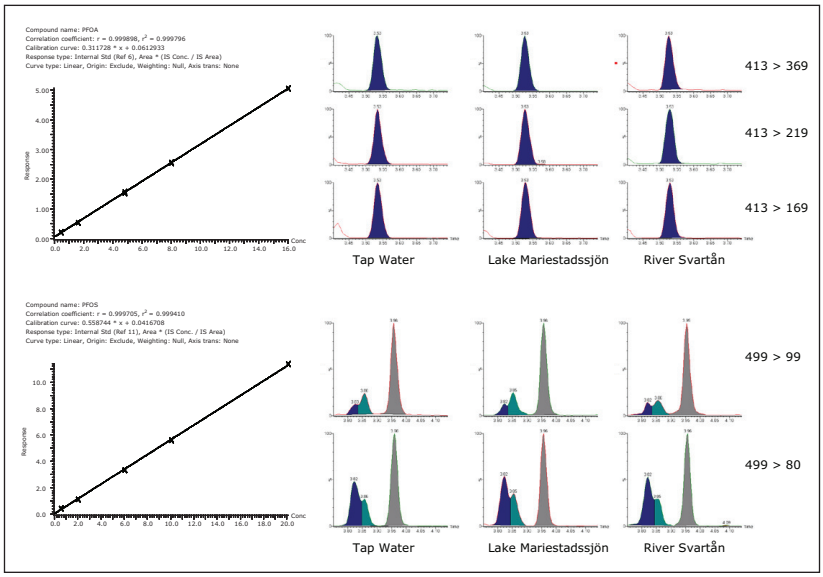

Figure 2. Quantitative data for PFOA (top) and PFOS (bottom), including calibration curves and extracted MRM chromatograms. Native concentrations in tap water (PFOA 0.42 ng/L, PFOS 1.50 ng/L), Lake Mariestadssjön (PFOA 1.30 ng/L, PFOS 1.30 ng/L), and River Svartån (PFOA 1.10 ng/L, PFOS 1.40 ng/L).

In addition to high sensitivity MRM detections of native PFCs, sample matrix effects were simultaneously monitored using RADAR dual scan-MRM functionality of the Xevo TQ MS. DS-MRM allows the simultaneous acquisition of full scan data while performing routine quantitative MRM analysis. High-value information about the background in each sample can be retained while maintaining good MRM performance. Figure 3 shows 413 > 369 MRM transition for PFOA at ~400 fg/µl acquired in RADAR dual scan-MRM mode (incorporating at 50-650 *m/z* scan at 3000 Da/s) and traditional MRM mode. Signal-to-noise sensitivity and peak area were not significantly affected when using RADAR dual scan-MRM.

Figure 4a shows overlaid and normalized RADAR dual scan-MRM chromatograms for detected native PFCs from a Lake Mariestadssjön sample. Dual scan-MRM acquisitions allowed enough sensitivity for low level detections (0.23 ng/L to 1.30 ng/L) of many of the native PFCs, as well as providing information about the complexity of each sample matrix. Figure 4b is a combined full scan spectrum taken from 0 to 1 min in the chromatogram. This spectrum is characteristic of humic and fulvic substances often found as the principal matrix component common in environmental samples. These substances contribute to the majority of the ion current in the sample and have been shown to cause matrix suppression in electrospray ionization. These humic and fulvic substances also appear to significantly increase the background noise level for PFBuS and could therefore effect detection limits.

The ability to observe the elution region for these substances in each sample at the same time as acquiring high sensitivity MRM transitions for target PFCs allows for greater confidence when good chromatographic separations are achieved and for appropriate action to be taken where problems occur.

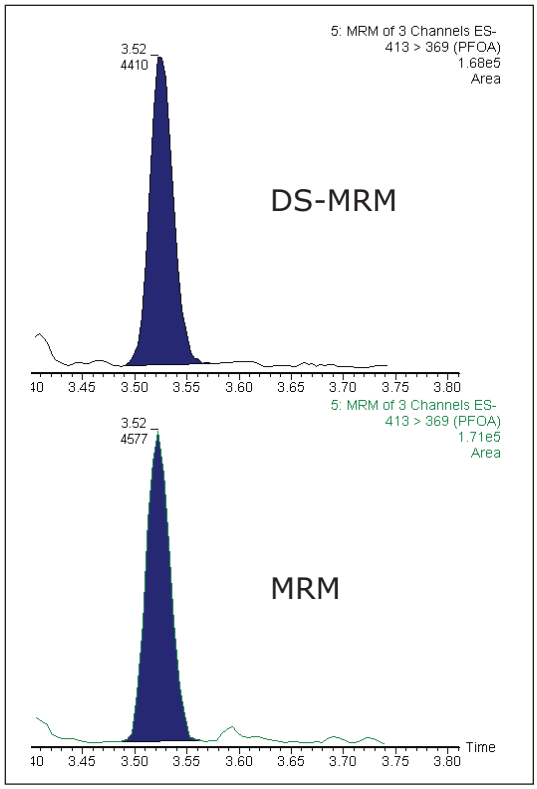

Figure 3. PFOA 413 > 369 acquired in RADAR dual scan-MRM and MRM only mode.

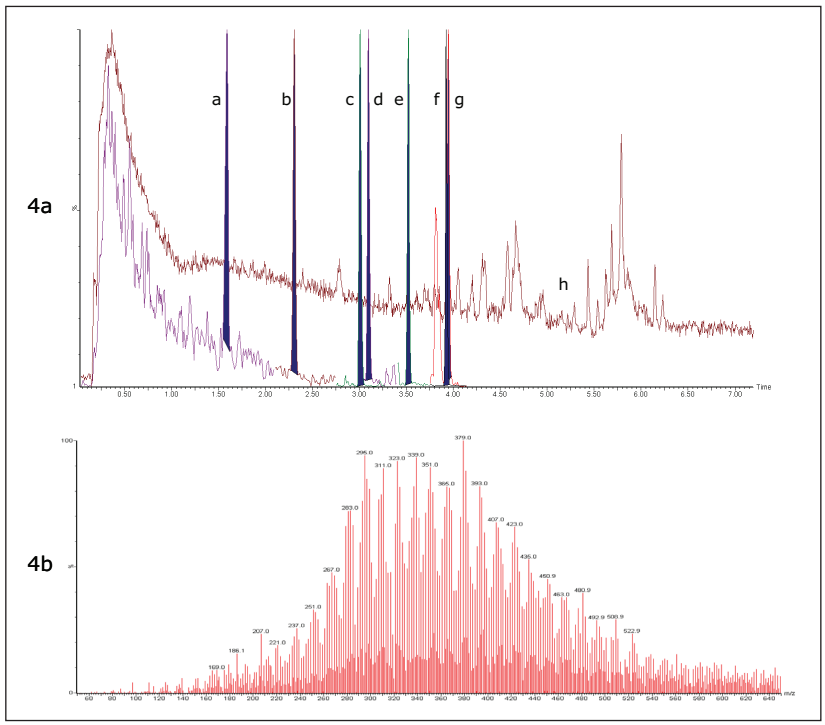

Figure 4a. RADAR Dual Scan-MRM chromatogram overlay of a non-fortified Lake Mariestadssjön sample. Detected PFC MRM chromatograms (normalized) (a) PFBuS 0.23 ng/L (b) PFHxA 0.41 ng/L (c) PFHpA 0.69 ng/L (d) PFHxS 0.42 ng/L (e) PFOA 1.30 ng/L (f and g) PFNA 0.45 ng/L and PFOS 1.30 ng/L, and (h) Full scan 50-650 m/z TIC. Figure (4b). Combined spectrum 0 to 1 min showing presence of humic and fulvic substances.

RADAR dual scan-MRM for biota samples

Salmon and cod liver samples were also analyzed for native PFCs using a RADAR dual scan-MRM approach. These samples are largely considered to be one of the most challenging matrices for low level PFC determination. Retention time shifts and some MRM interference were initially observed for both samples. As full scan data were available from RADAR dual scan-MRM acquisition, the cause of these problems were investigated.

Figure 5a shows overlaid RADAR dual scan-MRM chromatograms for PFOS present at 2.75 ng/g in salmon liver. Two high concentration matrix components taurocholate and deoxytaurocholate (peaks a and b), were observed eluting at a critical point in the chromatogram. These matrix components were identified as bile acids based upon product ion scanning (Figure 5b) and comparing data with existing references. Deoxytaurocholate co-eluted with PFOS and interfered with the 499>80 transition due to an isotope at 499 *m/z* fragmenting and sharing the 80 *m/z* product ion (SO3-). This component is a known interference for human serum analyses and using 499>99 transition allows for more accurate quantitation when using 13C labeled standards.

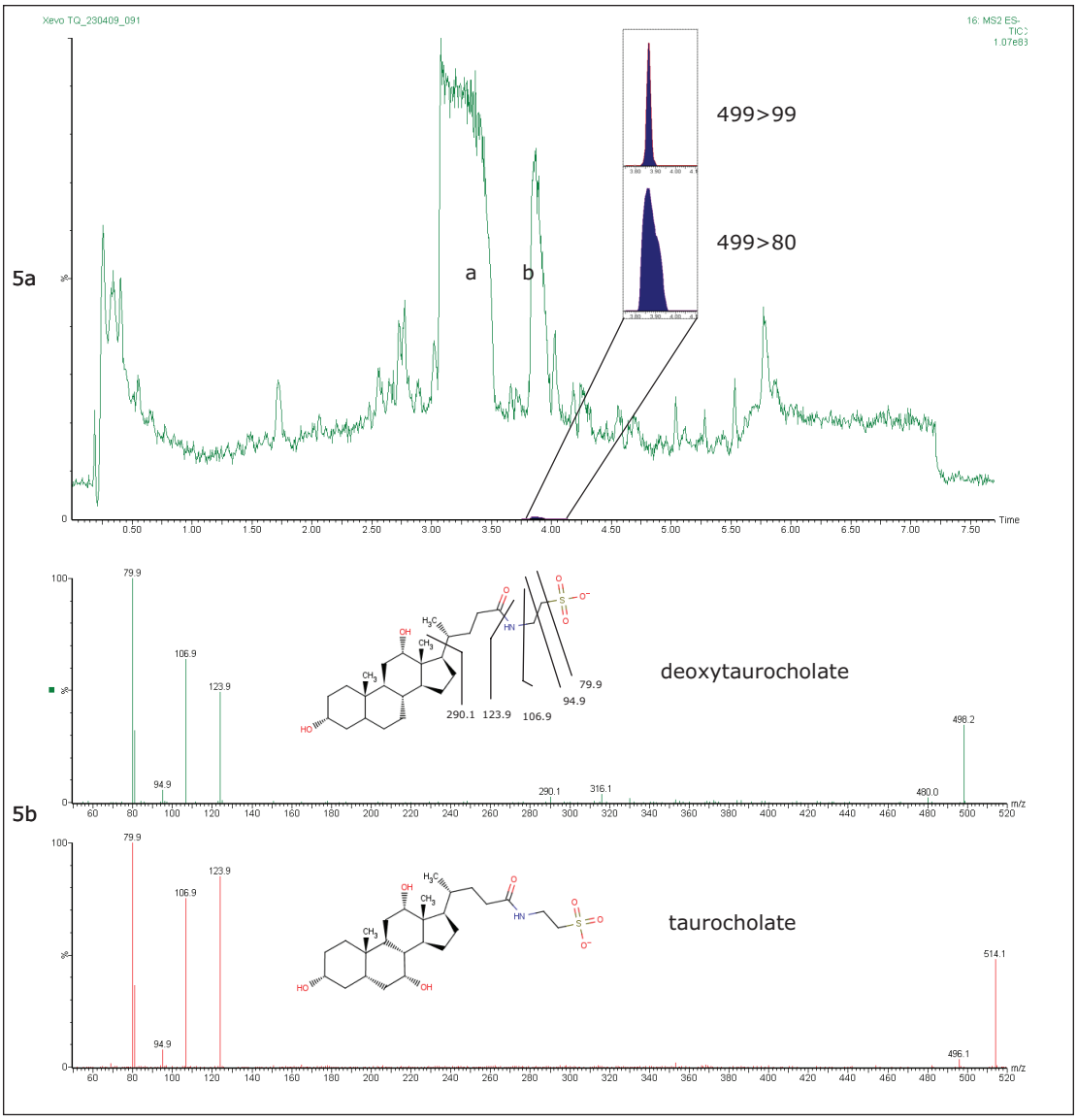

Figure 5. RADAR dual scan-MRM data for salmon liver (Figure 5a) with taurocholate (peak a) and PFOS interferent deoxytaurocholate (peak b) also shown is PFOS MRM transitions at 2.75 ng/g.

Figure 5b. Product ion scans used to aid identification of the two bile acids.

The high concentrations of taurocholate and deoxytaurocholate in the extracts lead to retention time shifting. This could be clearly observed in the RADAR dual scan-MRM data as the retention time shifts regions correlated with the elution region for these matrix components. This is likely due to stationary phase saturation caused by these two bile acids. The RADAR dual scan-MRM data allows a targeted approach to be taken to reduce matrix effects in future analysis with the development of an additional sample cleanup for this matrix.

It is clear that continuously monitoring sample background using a RADAR dual scan-MRM approach can lead to more information about the challenges of each individual sample. This is a novel intra-sample QC check that has the potential to help improve quality within PFC analysis and is a possibility brought by Xevo TQ MS.

CONCLUSIONS

- ACQUITY UPLC allows fast separations of PFCs to ensure high sample throughput.
- Xevo TQ MS used with the PFC Analysis Kit enables high sensitivity PFC analysis in a variety of environmental matrices.
- RADAR dual scan-MRM allows full scan data to be acquired alongside routine MRM data. This allows continuous monitoring of sample background and can lead to more information about the challenges of each individual sample.
- The ability to simultaneously acquire quantitative and qualitative information in a single run can lead to reduced method development time and ultimately offers a unique intra-sample QC check when used for routine analysis. This can help drive improvements in the quality of data produced by a laboratory.

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Direct Quantification of Diquat and Paraquat in Drinking Water Samples Using Ultra-Sensitive UPLC-MS/MS Analysis

Claude R Mallet
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Direct injection of clean water samples removes the need for sample extraction or concentration, saving valuable analyst time.
- Fast UPLC® analysis on an ACQUITY UPLC® BEH C_{18} Column decreases sample turn around time and improves lab productivity.
- The high sensitivity of Xevo TQ-S enables excellent trace-level quantification using a 100-µL direct injection, with no deterioration in performance apparent even after 250 sample injections.

WATERS SOLUTIONS

ACQUITY UPLC

Xevo® TQ-S
ACQUITY UPLC BEH C_{18} Column

TrendPlot™ MS Software

KEY WORDS

diquat, paraquat, drinking water, Xevo TQ-S, herbicide, bipyridyls

INTRODUCTION

Crop protection in countries around the globe is usually associated with the use of a wide range of pesticides, insecticides, or herbicides. These agricultural products can potentially have harmful effects on the environment and impact the health of both humans and animals. Despite the risk, they are a crucial part of the global economy[1] For example, the use of herbicides is important to control the growth of weeds, for if not suppressed weeds can reduce crop yields up to 80%.[2] In the herbicides family the bipyridyls are used extensively in agriculture to control broadleaf and aquatic weeds. The most common bipyridyls are diquat and paraquat. They constituted the largest share of the global market until recently overtaken by glyphosate.[3] Due to their high efficiency as pre-harvest desiccants and defoliants, diquat and paraquat are also classified as highly toxic.[4] The World Health Organization (WHO) has classified these compounds as moderately hazardous.[5] Even with a half-life in water of 48 hours, accidental or intentional ingestion can have serious health effects. For drinking water, the U.S. Environmental Protection Agency (U.S. EPA) has established a maximum contaminant level of 20 ppb for diquat and a desired goal of 3 ppb for paraquat[6] (not EPA regulated). The European Union (EU) has not regulated the levels of these compounds specifically in drinking water and continues to apply the value of 0.1 ppb.[7]

The analysis of bipyridylium herbicides can be difficult mainly because they are cationic molecules. Their inherent high polarity and positive charge, require the use of ion pairing additives when analyzing quaternary amines by reversed-phase chromatography. The U.S. EPA method 549.2 utilizes reversed-phase chromatography with ion pairing for the separation of diquat and paraquat using UV detection.[8] Ion pairing agents are typically avoided with ESI-MS applications owing to suppression of the ionization in the MS source. For MS applications, HILIC has provided suitable chromatography without the requirement of ion pairing agents.[9] However, recent advances in MS sensitivity have made the direct analysis of trace-level contaminants in water attainable and very attractive. The possibility of removing laborious and time-consuming solid phase extraction and sample concentration is highly desirable. Direct injection of an aqueous sample for RP chromatography is ideal as the sample matrix is similar to the initial mobile phase conditions. For HILIC, a water sample would first require dilution with the organic solvent.

EXPERIMENTAL

Diquat and paraquat standards were purchased from Sigma Alrich (St-Louis, MO, USA). HFBA (HPLC grade) was purchased from Thermo Scientific (Rockford, IL). MilliQ water was used to produce calibration standards. The water samples were collected from bottled and in-house tap water. The chemical structure and MRM conditions used for the quaternary herbicides are listed in Figure 1 and Table 1, respectively. MRM transitions stored in the Quanpedia™ database were selected for analysis. Chromatographic separation was performed on Waters® ACQUITY UPLC System equipped with an ACQUITY UPLC BEH C_{18} 2.1 x 30 mm Column. A one -minute linear water/methanol gradient with 10 mM HFBA was used. The detection was performed using a Xevo TQ-S.

UPLC conditions

System:	ACQUITY UPLC
Runtime:	3.0 min
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 30 mm, 1.7 µm
Column temp.:	25 °C
Mobile phase A:	10 mM HFBA in water
Mobile phase B:	10 mM HFBA in methanol
Elution:	1 minute linear gradient from 2% (B) to 95% (B)
Flow rate:	0.6 mL/min
Injection volume:	100 µL

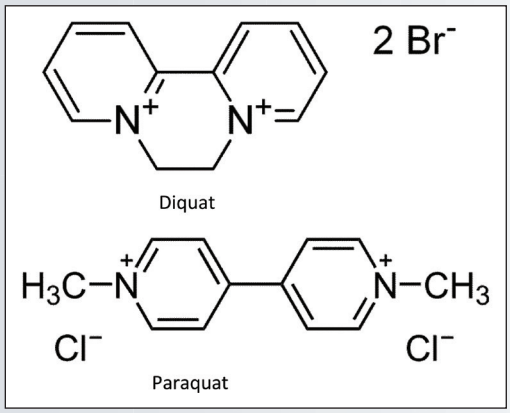

Figure 1. Chemical structure of diquat and paraquat.

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ESI positive
Capillary voltage:	3.0 kV
Cone voltage:	50.0 V
Source temp.:	140 °C
Desolvation temp.:	550 °C
Desolvation gas:	1100 L/hr
Cone gas:	50 L/hr

Herbicides	Precursor	Product	Cone	Collision
Diquat	183.0	157.0	50	20
	183.0	78.0	50	35
Paraquat	185.0	170.0	50	20
	185.0	107.0	50	30

Table 1. Diquat and paraquat MRM conditions.

This application note presents the analysis of diquat and paraquat herbicides in drinking water by direct injection using a volatile ion pairing reagent (heptafluorobutyric acid-HFBA), RP-UPLC, and the highly sensitive Xevo TQ-S.

RESULTS AND DISCUSSION

With the StepWave™ ion optics, Waters® Xevo TQ-S offers unsurpassed performance for trace-level analysis. The high sensitivity allows for the option to bypass the tedious sample concentration requirement associated with trace-level detection of contaminants in drinking water. With this high level of sensitivity, a clean water sample can be pre-concentrated directly on column by using a direct injection technique with the ACQUITY UPLC System. As shown in Figure 2, diquat and paraquat gave well-defined Gaussian peak shapes on the RP column. The vertical axes are linked in Figure 2 and show the difference in response of the two analytes. Even with the lower response seen for paraquat compared to diquat, the required levels of quantification for both compounds were achieved.

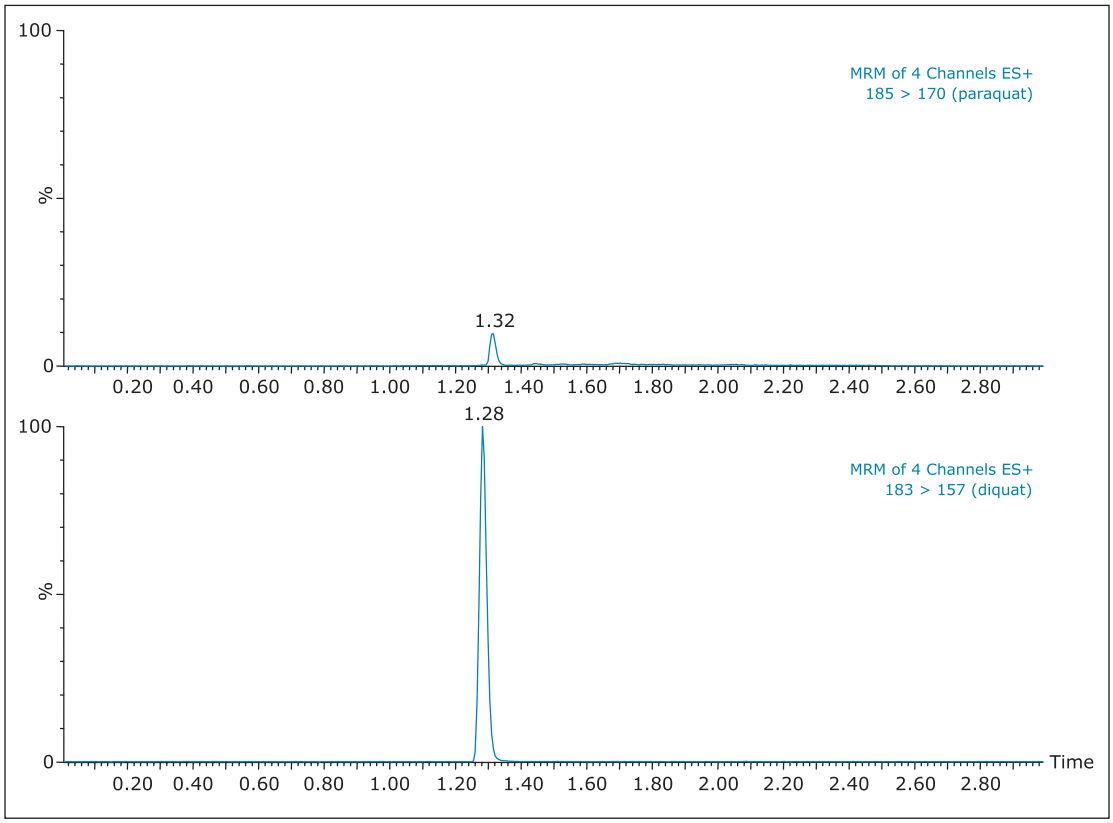

Figure 2. Reversed chromatograms of diquat and paraquat (1 ppb spike).

Quantification

Using the direct injection protocol, the quantification of bottled and tap water was measured against a calibration curve generated using standards made in MilliQ water. In this case, external calibration showed excellent results and an internal standard was not deemed necessary. As shown in Figure 3, the calibration curves for diquat and paraquat for tap water showed excellent linearity from 50 ppt to 100 ppb, with r^2 of 0.997 and 0.995 for diquat and paraquat, respectively. The recoveries for a 1 ppb spike are shown in Table 2, with recoveries in the range of 75% to 107%. The relative standard variation (RSD's) for diquat and paraquat was below 8% in both water samples.

Herbicides	Bottled water	Tap water
Diquat	107.0 (2.6)	75.1 (4.4)
Paraquat	99.0 (3.9)	76.5 (6.1)

Table 2. Recoveries and coefficient of variations at 1 ppb in bottle and tap water (n=3).

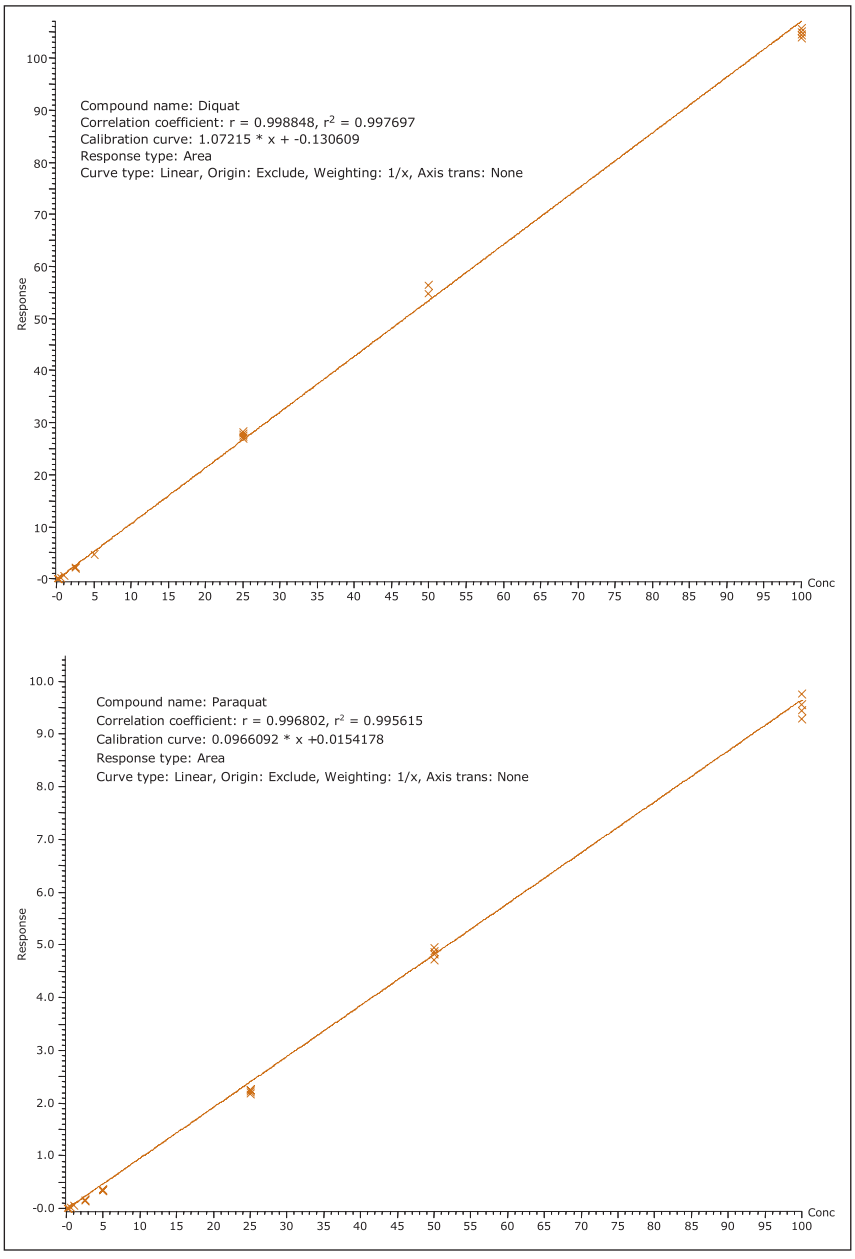

Figure 3A. Calibration curve for diquat from 50 ppt to 100 ppb.

Figure 3B. Calibration curve for paraquat from 50 ppt to 100 ppb.

In this application, since the ion pairing agent was added to both the mobile phases (aqueous and organic) and the sample, the purity of HFBA was crucial. During the development phase, the 185 → 170 *m/z* MRM transition for paraquat showed an interferent near the expected retention time of paraquat. It also showed high background levels which made it difficult to quantify paraquat below 500 ppt. This issue was attributed to the ion pair additive, most likely due to a lower purity grade that was employed. With a higher purity grade, the interferent was eliminated and the background noise was reduced to a satisfactory level. As a consequence, the limit of detection (LOD) of 50 ppt was achieved and the MRM chromatograms are presented in Figure 4 for bottled water. The ion ratios for both diquat and paraquat, calculated from the quantification and the confirmation MRM transitions (Figure 5) showed good correlation between the standard and spiked samples, further supporting the applicability of the direct injection method.

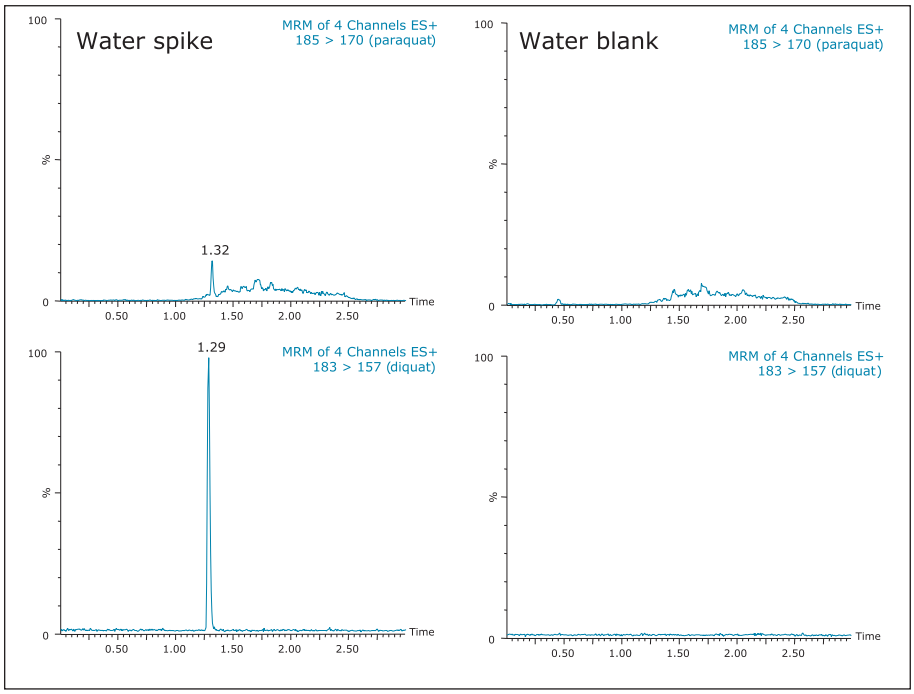

Figure 4. Chromatograms for paraquat and diquat at 50 ppt spike and blank (bottled water).

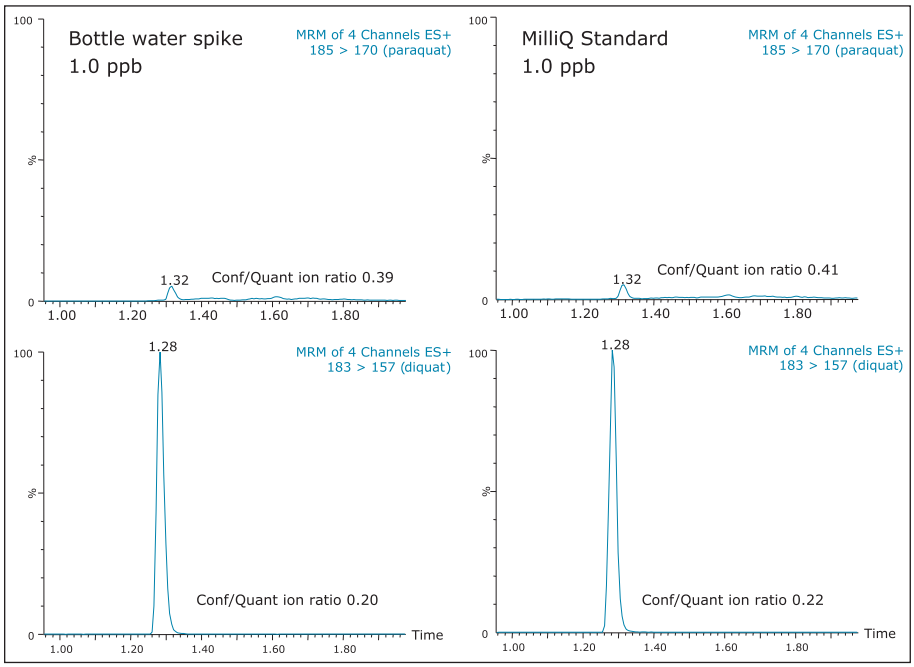

Figure 5. Ion ratio for diquat and paraquat using quantification and confirmation MRM transitions.

Lifetime and robustness

The direct injection approach is very efficient in term of speed and ease of use. However, the technique is not immune to potential situations which could affect the analytical performance over extended periods of time. The repeated injection and high injection volume of unfiltered and un-extracted samples could lead to peak distortion. During lifetime and robustness studies, the peak shape and column backpressure are excellent indicators of the column's overall performance. In this application, as seen in Figure 6, the peak shape of diquat and paraquat showed no noticeable distortion between the first and 250th injection. The initial column backpressure readout before injection of the first sample was recorded at 3500 psi. After 250 injections of tap water samples, the initial column backpressure shows a reading of 3900 psi, an increase of 400 psi. The key feature for quantification remains for the target analyte to elute with a Gaussian peak shape throughout the analysis.

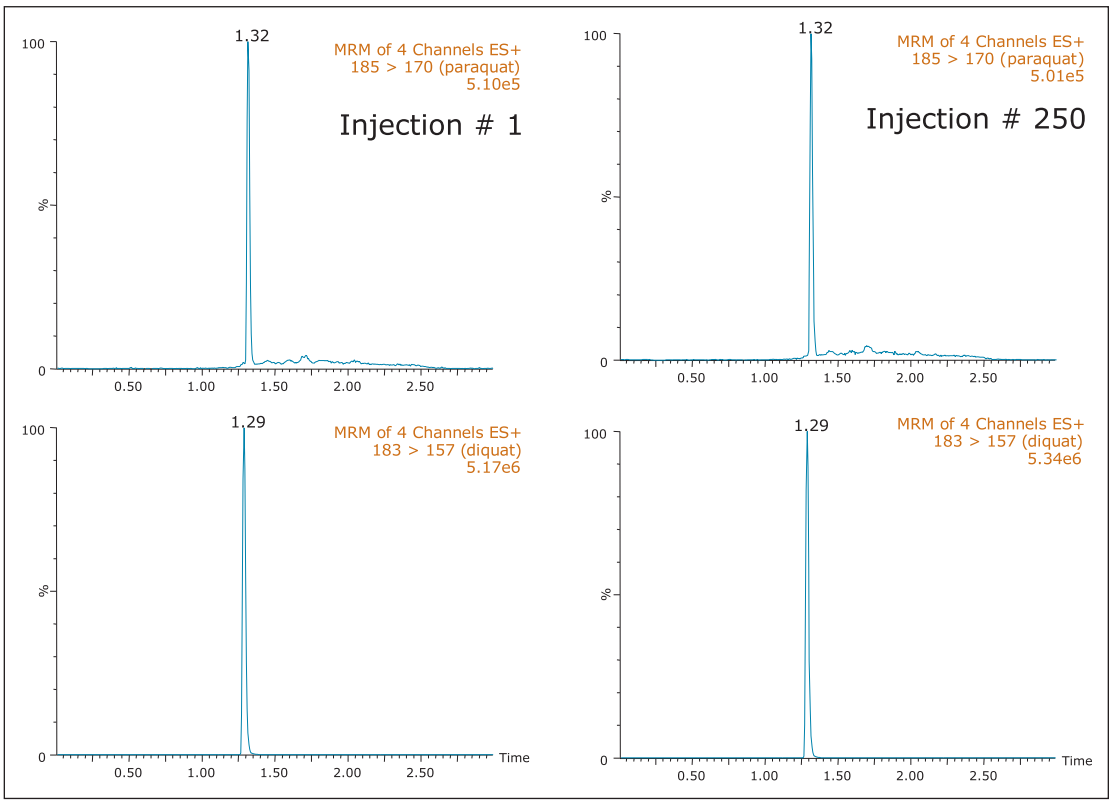

Figure 6. Example MRM chromatograms over the column lifetime study. Injections 1 and 250 are shown for diquat and paraquat in tap water.

In this application, the lifetime chromatograms for diquat and paraquat showed no signs of peak distortion and the RSDs on the quantification results were below 5%. Therefore, the small backpressure increase recorded for the tap water samples did not influence the overall analytical performance during this study. The TrendPlot™ profile report for diquat and paraquat are shown in Figure 7. As it can be seen in Figure 7, the TrendPlot shows excellent linearity for both compounds with RSDs at 4.7% and 7.5% for 100 injections, respectively.

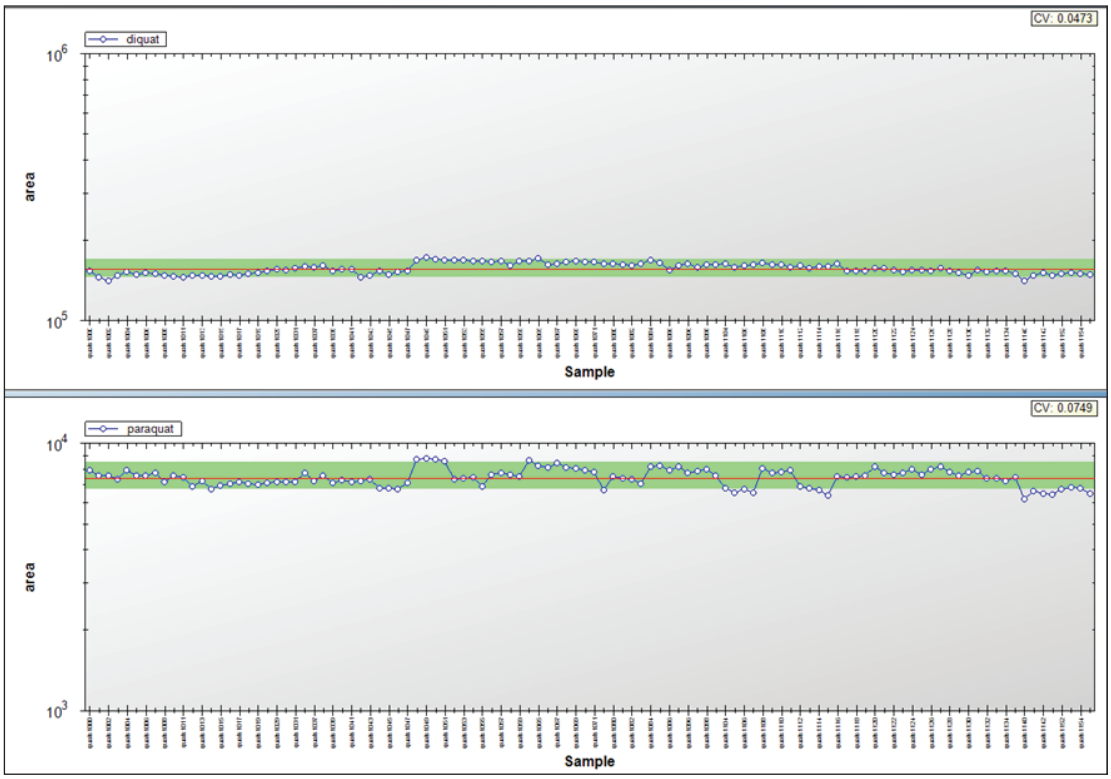

Figure 7. TrendPlot profiles of diquat and paraquat in tap water.

CONCLUSIONS

This application note has demonstrated the versatility of direct injection using the ACQUITY I-Class UPLC System with the Xevo TQ-S Mass Spectrometer for the analysis of diquat and paraquat in tap water and bottled water. The limit of detection in this study was 50 ppt, which is below the European Union Directive LOD of 100 ppt. The high sensitivity of Xevo TQ-S enabled excellent quantitation using a 100-µL injection without sample extraction or concentration prior injection. The recovery data showed good results with excellent RSD's below 8% for both water samples.

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INTRODUCTION

Microcystin-LR is a potent mammalian toxin which is known to have been responsible for the deaths of domesticated animals, livestock loss, and the potential presence in potable water supplies.

PRETREATMENT

1. Filter water sample through 0.45 µm membrane filter.
2. Add 100 µL of enkephalin (concentration 10 µg/L) to 10 mL filtered water sample and mix thoroughly.

SPE PROCEDURE

Oasis® HLB, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 3 mL methanol
B. 6 mL water

LOAD:
10 mL sample (1 mL/min)

WASH:
A. 3 mL water
B. 5 mL 20% methanol

Dry cartridge by vacuum for 1 minute

ELUTE:
5 mL methanol

Evaporate to dryness at 50 °C under nitrogen stream

Reconstitute residue with 1 mL 50% methanol

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	Symmetry300™ C_{18}, 3.5 µm, 4.6 x 75 mm
Flow rate:	0.2 mL/min
Mobile phase A:	0.2% formic acid in water
Mobile phase B:	0.2% formic acid in methanol

Gradient:

Time (min)	A%	B%
0.00	45	55
12.00	10	90
12.50	0	100
15.00	0	100
15.10	45	55
25.00	45	55

Injection volume:	10 µL
Column temp.:	30 °C

MS CONDITIONS

MS System:	Waters Quattro Ultima Pt™
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM	MW	$[M+H]^+$	$[M+H]^{2+}$	Characteristic Ion Fragment
Enkephalin	556.1→278.0	555.6	556.1	N.D	278.0
					397.1
MCYST-LR	519.9→135.0	994.5	995.7	498.4	135.0
					861.5
MCYST-RR	498.4→135.0	1037.6	1038.4	519.9	135.0
					620.0
MCYST-LW	1025.8→891.7	1024.5	1025.8	N.D	897.1
					583.2
MCYST-LF	986.8→852.5	985.5	986.8	N.D	852.5
					544.0

MRM method parameters.

RESULTS

Analyte	Concentration (µg/L)	Average Recovery (%)	RSD (%)
MCYST-RR	0.10	100.0	6.45
	0.20	95.2	4.02
	0.40	90.0	4.35
MCYST-LR	0.02	105.0	5.40
	0.05	96.0	4.53
	0.08	93.8	4.22
MCYST-LW	0.40	103.8	5.30
	1.00	102.7	5.87
	1.60	93.8	5.67
MCYST-LF	0.20	103.0	7.03
	0.50	109.8	5.69
	0.80	102.3	4.57

Recovery data for spiked samples at various concentrations.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 3 cc/60 mg, 30 µm, 100/box	WAT094226
Symmetry300 C_{18}, 3.5 µm, 4.6 x 75 mm	186000189
Nylon Filter 0.45 µm	WAT200524
LCMS Certified Vials	600000751CV

Ref: Determination of Microcystins in Natural Water by Liquid Chromatography Tandem Mass Spectrometry, Chen Qi, Huang Baifen, Zhang Jing, Ren Yiping; Zhejiang Provincial Center for Disease Prevention and Control

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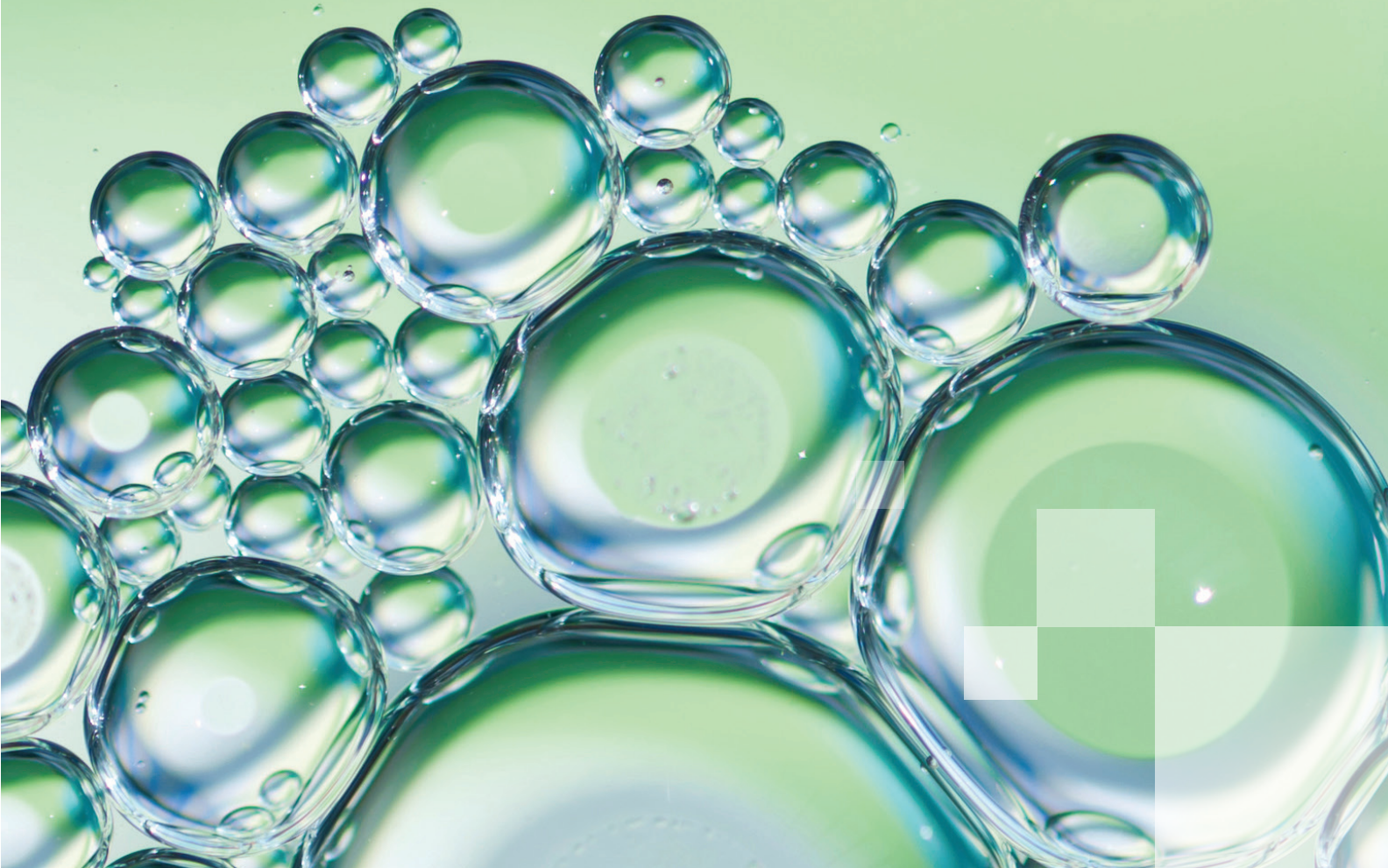

DETERMINATION AND CHARACTERIZATION OF PFOS IN ENVIRONMENTAL SAMPLES USING TRAVELLING WAVE ION MOBILITY MASS SPECTROMETRY

*Authors: Michael McCullagh[1], Kendon Graham[2], Dominic Roberts[1], **Kieran Neeson[1]**, Jeff Goshawk[1], Leonard Dillon[1], Antonietta Gledhill[2], Mike Hodgkinson[1], Ingrid Ericson Jogsten[3] and Bert van Bavel[3].*
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OVERVIEW

In this study we explore the use of ion mobility an important tool for identification of PFOS isomers in environmental samples. Ion mobility has been used to resolve isobaric matrix interferences from the PFOS isomers. Ion mobility is being used routinely has part of a non targeted screening workflow.

INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a class of man-made compounds that are frequently detected in biological and environmental samples. PFASs are used in a multitude of commercial/industrial processes and products. As with many anthropogenic compounds, the incidence of cancers resulting from exposure becomes a cause for concern.

Perfluorooctane sulfonate (PFOS) is frequently detected in biological and environmental samples. It is important that PFOS isomers are identified correctly because their physical, chemical and biological properties may be affected by perfluoromethyl branching. As a result there is increased scientific interest in relating toxicity, environmental transport, degradation and bioaccumulation to perfluoromethyl branching patterns. MRM based LC-MS/MS analyses have been used previously to investigate PFOS in marine animals and human serum. Benskin *et al*. reported common matrix interferences such as taurodeoxycholate (TDCA A) that can complicate PFOS quantification. These species share the same MRM transition (m/z 499 > m/z 80) and tend to co-elute with PFOS, leading to a positive quantitative bias[1,2].

Here we explore the use of high definition mass spectrometry (HDMS) as an important tool for unequivocal identification of PFOS isomers in environmental samples[3,4,5]. This technique offers some unique advantages to profiling complex matrices. It uses a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Compounds can be differentiated based on size, shape and charge. In addition, both precursor ion and fragment ion information can be acquired in a single injection in an HDMS experiment referred to as HDMS^E.

The study described here shows the use of HDMS^E using the SYNAPT HDMS platform (Figure 1). HDMS^E can provide a route to identification, enabling the distinction of PFOS isomers. For this work, and for research purposes only, the use of CO_2 enabled characteristic drift time separation for the PFOS isomers analysed.

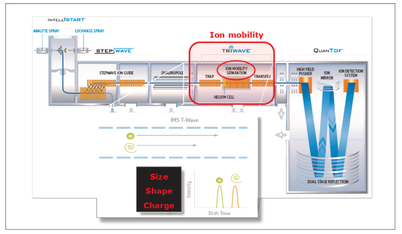

Figure 1. A schematic representation of the SYNAPT G2-S HDMS system and illustration of the mechanism of travelling wave ion mobility separations.

METHODS

Sample Preparation

The whole mink carcasses (juvenile and adult males) were provided by licensed hunters and kept frozen (-20ºC) before being autopsied at the Swedish University of Agricultural Sciences. Liver samples were prepared according to a method previously reported by Kärmann *et al.*, with minor modifications. In summary, liver samples were subject to repeated extraction in acetonitrile followed by SPE using Oasis® WAX. Final sample clean-up was performed using graphitized carbon black prior to filtration and dilution before analysis.

MS Conditions

MS System: Waters SYNAPT® G2-S HDMS
Ionization Mode: ESI-
Acquisition Mode: HDMS^E
Mass Range: *m/z* 50 – 600
Acquisition Rate: 10 spectra/sec
Capillary Voltage: 2.3 kV
Cone Voltage: 15 V
Source Temp: 120 °C
Desolvation Temp: 550 °C
Drift Gas: CO_2
MS^E High Energy: 35.0—75.0 V (ramped)
IMS Wave Velocity: 450 m/s
IMS Wave Height: 40V
Resolution: 20000 (FWHM)

UPLC Conditions

LC system: ACQUITY UPLC® System (equipped with PFC Kit)
Column: ACQUITY BEH C_{18}, 1.7 µ m, 2.1 x 100 mm, @ 45 °C
Mobile phase A: Water + 2mM Ammonium Acetate
Mobile phase B: 80% Methanol, 20% Acetonitrile

Gradient Table :

Time (min)	Flow Rate (mL/min)	%A	%B
Initial	0.30	100	0
0.5	0.30	100	0
16.00	0.30	65	35
22.00	0.30	65	35
27.00	0.30	10	90
27.10	0.30	0	100
28.00	0.30	0	100
28.10	0.30	100	0
34.00	0.30	100	0

Data Acquisition and Processing

Data were acquired using MassLynx and processed using prototype UNIFI software. PFOS standard mixtures were used to generate a scientific library containing retention times with elemental compositions of PFOS isomers and cholic acids. This permitted non-targeted data acquisition with a targeted screen to be performed using HDMS^E data generated from environmental extracts.

RESULTS & DISCUSSION

The use of travelling wave ion mobility offers an additional and orthogonal dimension of separation when compared to traditional LC-MS/MS experiments performed on QTof instrumentation. This can enable the detection and interpretation of spectral information of target molecules to be performed in the absence of interferences from co-eluting, nominal isobars.

As shown in Figure 2, without an additional dimension of separation, a number of PFOS isomers (upper) are not sufficiently chromatographically resolved to provide accurate quantitative analysis or to confirm their identity via examination of fragmentation spectra. Furthermore, commonly encountered co-extracted, nominally isobaric interferences (TDCA A & TCDCA B, Figure 2 lower), share common fragments with PFOS and also co-elute with a number of PFOS isomers.

Figure 3 shows ion mobility drift times plotted *vs* retention times for PFOS isomers and matrix-borne interferences TCDA and TCDCA. Separation between both the interferences and individual PFOS isomers is demonstrated using CO_2 as the drift gas. Table 1 shows the data in terms of drift time, retention time and accurate mass measurements for the identified PFOS isomers as well as TCDA A and TCDCA B. Structural elucidation and identification of each of the PFOS isomers detected in mink extracts was made possible by interpretation of the single component fragmentation spectra obtained via the use of an ion mobility separation in conjunction with HDMS^E. Interpretation of data acquired without this capability would have been extremely challenging due to the commonality of fragments observed for individual PFOS isomers and the nominally isobaric interferences present in the extracts.

Examples of single component precursor and HDMS^E fragmentation spectra for PFOS isomers and co-eluting matrix interferences are shown in Figures 4, 5, 6 & 7. Despite complete chromatographic co-elution of PFOS C (Figure 6) & PFOS J (Figure 4), their separation in the ion mobility dimension allows ions of both isomers and their fragmentation spectra to be investigated free from interference from one another.

In another example, Figure 5 shows precursor and fragmentation spectra for TCDA A. Comparing the fragmentation spectra to those collected for PFOS D (Figure 7) highlights the difficulty that co-elution of these species would cause in the correct identification of this PFOS isomer. The distribution and intensity of fragment ions generated by these two analytes is similar, and they share a common fragment at m/z 80. Once more, their differing drift times (Table 1) in the ion mobility separation allow single component spectra to be derived from the dataset to facilitate specificity of detection and identification.

The data shown here clearly demonstrate the utility of increased peak capacity, selectivity and specificity of detection that can be obtained by combining ion mobility separations with high resolution mass spectrometry. A total of 9 PFOS isomers have been observed in the mink liver extracts examined in this study. The use of drift times, dependent upon the collision cross section (CCS) of a molecule, offers an additional molecular characteristic that can be used to confirm the identity of chemical species and simplify the interpretation of their fragmentation spectra.

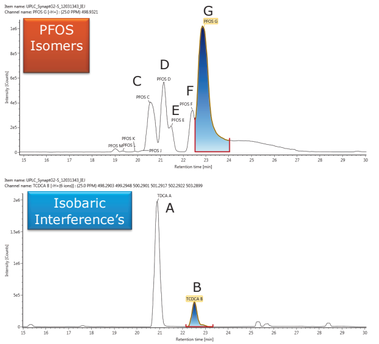

Figure 2. Accurate mass extracted ion chromatograms for the isobaric matrix interferences (Peaks A & B) and the PFOS isomers (Peaks C-G).

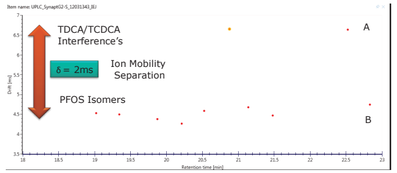

Figure 3. Component drift plot showing drift times vs retention time for nominally isobaric interferences (A) and PFOS isomers (B).

	PFOS ISOMER IDENTIFICATION				
PFOS Isomers	C 5mPFOS J 3mPFOS	D IsoPFOS	E 2,2-perfluoro-methyl PFOS (tentative)	F 1mPFOS	G nPFOS
Drift Time (ms)	4.59 4.27	4.68	4.47	4.43	4.75
Mass Measurement Error	3.4 ppm -0.23 ppm	3.66 ppm	3.12 ppm	3.72 ppm	-14.91 (2.68ppm HE)
Retention Time (mins)	20.55	21.14	21.48	22.40	22.80
TDCA Interferences	A TDCA	B TCDCA			
Drift Time (ms)	6.65	6.64			
Mass Measurement Error	3.59 ppm	1.64 ppm			
Retention Time (mins)	20.88	22.52			

Table 1. A summary of drift times, retention times and isomer assigments for major PFOS isomers and co-eluting matrix.

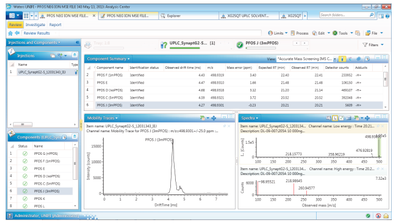

Figure 4. At 20.21 mins minor PFOS J (3mPFOS) isomer HDMS^E fragmentation spectra that are ion mobility resolved from co-eluting PFOS isomer C .

Figure 5. At 20.88 mins TDCA A HDMS^E fragmentation spectra that are ion mobility resolved from co-eluting PFOS isomers C .

Figure 6. PFOS C (5mPFOS) isomer HDMS^E precursor ion and fragmentation spectra that are ion mobility resolved from co-eluting PFOS isomer J at 20.21 mins.

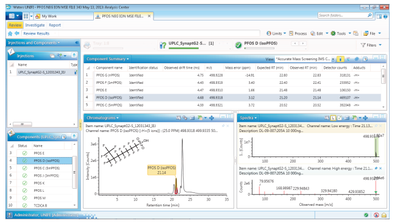

Figure 7. At 21.14 mins PFOS D (isoPFOS) isomer HDMS^E precursor ion and fragmentation spectra that are ion mobility resolved from co-eluting and isobaric TDCA .

A further benefit of the use of drift times is seen in the decreased dependence upon chromatographic retention times to correctly identify PFOS isomers. Traditionally, complex and lengthy chromatographic methods combined with extensive sample clean-up and highly specific MS experimental design have been used in the investigation of PFOS occurrence.

Sample matrices have been shown to cause shifts in chromatographic retention times which can complicate the identification and quantification of PFOS isomers. The approach described here reduces the reliance upon chromatographic retention times for the identification of PFOS isomers and acquires comprehensive mass spectral information as well as drift times to enable further characteristic profiling.

CONCLUSION

- **Co-eluting isobaric biological interferences TDCA and TCDCA been resolved from PFOS isomers using ion mobility with CO_2 as a drift gas .**
- **Increased confidence can be obtained from distinct drift times for the PFOS isomers and used as an additional identification point reducing the dependence upon chromatographic retention times.**
- **Using UPLC HDMS^E, single component precursor ion and fragmentation spectra have been generated for all PFOS isomers and TDCA/TCDCA isomers.**
- **UPLC HDMS^E offers an uncompromised, unique approach to the determination and characterization of PFOS within the environment by enabling isomer -specific analysis.**
- **UPLC HDMS^E in combination with targeted screening can provide an efficient route to specific identification of PFOS isomers.**

References

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METHODS IN BRIEF

EPA METHOD 8310.0

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic pollutants. PAHs are made up of fused aromatic rings and are formed during the combustion of carbon-based fuels (wood, coal, diesel), as well as being present in crude oil. The United States Environmental Protection Agency (US EPA) has classified seven PAH compounds as being potentially carcinogenic including benz[a]-anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k] fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd] pyrene.

HPLC CONDITIONS

System:	Alliance® HPLC system with PDA and Fluorescence detectors
Column:	Waters PAH, 4.6 x 250 mm at 30 °C
Eluent:	Water/acetonitrile
Injection:	20 µL of Supelco standard EPA 610 (#48743) diluted 1:50 in 40:60 water/acetonitrile
Flow rate:	1.2 mL/min
Detection:	UV at 254 nm and fluorescence using timed programmed wavelengths
Data:	Empower® Software

SAMPLE PREPARATION

$MeCl_2$ extraction.

ELUENT PREPARATION

Filter and degas through a 0.45-µm filter.

A: Water

B: Acetonitrile

Time	Flow	%A	%B	Curve
Initial	1.2	40	60	-
12.0	1.2	0	100	9
23.0	1.2	40	60	11

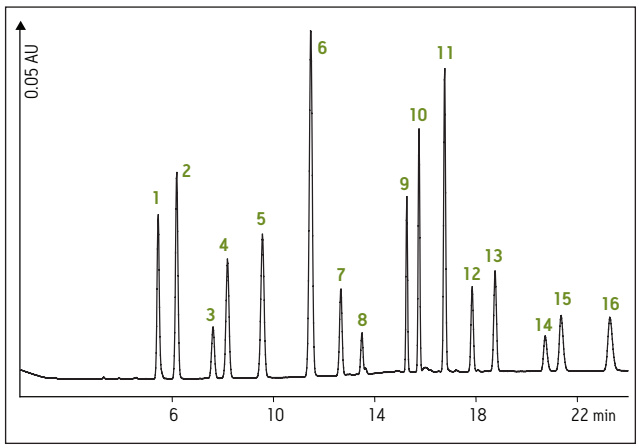
Eluent gradient.

	Analyte	UV max (nm)	EX (nm)	EM (nm)	Detection Limit (ppb)[1]
1	Naphthalene	220	277	330	0.14
2	Acenaphthylene	229	NA	NA	NA
3	Acenaphthene	227	270	323	0.01
4	Fluorene	261	265	310	0.03
5	Phenanthrene	251	252	365	0.02
6	Anthracene	252	250	402	0.01
7	Fluoranthene	236	284	467	0.02
8	Pyrene	240	332	378	0.01
9	Benzo(a)anthracene	287	284	390	0.01
10	Chrysene	267	270	367	0.04
11	Benzo(b)fluoranthene	256	298	436	0.09
12	Benzo(k)fluoranthene	307	303	432	0.01
13	Benzo(a)pyrene[2]	296	280	410	0.03
14	Dibenzo(a,h)anthracene	297	294	398	0.01
15	Benzo(g,h,I)perylene	299	290	420	0.03
16	Indeno(1,2,3-cd)pyrene	250	305	480	0.49

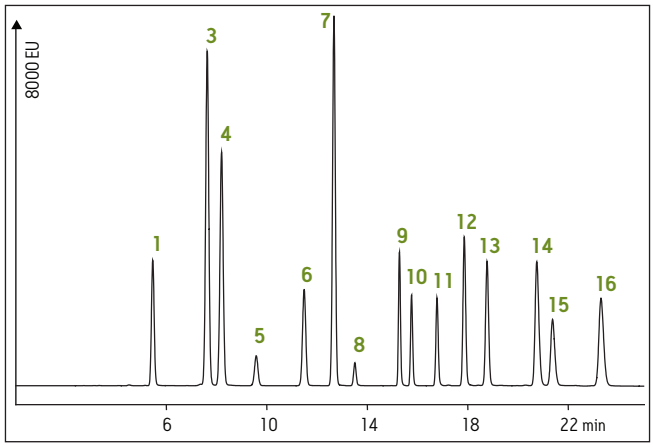
[1] *Fluorescence mode used for detection limit determination, no pre-concentration. Seven replicates per 40 CFR pt. 136 App. B.*

[2] *Regulated compound; action level 0.17 ppb.*

PAH target analytes.



Standard chromatogram, UV @ 254 nm, 1 to 20 ppm PAH analytes.



Standard chromatogram, fluorescence/programmed wavelengths, 1 to 20 ppm PAH analytes.

ORDERING INFORMATION

Description	Part Number
PAH Column, 4.6 x 250 mm	186001265
Semivolatiles #1 Standard	186004270

www.waters.com/order

Related Documents	Literature Code
The Determination of Biodegradation Products of PAH Using LC-MS/MS	WA20747
Waters PAH Columns Improve Analysis of PAH Compounds	720000382EN

www.waters.com/library

EPA METHOD 1694

Many hundreds of active compounds are used in both human and veterinary drug formulations. Due to the many different applications related to pharmaceuticals, their residues can reach the environment in multiple ways including excretion and manufacturing discharge. These compounds are not completely eliminated via sewage treatment plants, thus, they can reach surface and groundwater supplies. Recently, there has been increased interest in monitoring for the presence of pharmaceuticals in drinking water supplies and examining their long term effects on human health.

HPLC CONDITIONS

System: Waters 2690 HPLC or Waters 2795 HPLC, Quattro Ultima MS/MS
Column: XTerra® C_{18}, 3.5 µm, 2.1 x 100 mm
Ionization: ESI^+
Acquisition: MRM mode, unit resolution
Injection Volume: 15 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temp.	40 °C
0.0	95% Solvent A 5% Solvent B	0.150	1	**Flow Rate**	0.15-0.30 mL/min
4.0	95% Solvent A 5% Solvent B	0.250	6	**Max Pressure**	345 Bar
22.5	12% Solvent A 88% Solvent B	0.300	6	**Autosampler Tray Temp.**	4 °C
23.0	100% Solvent B	0.300	6	MS Conditions	
26.0	100% Solvent B	0.300	6	**Source Temp.**	140 °C
26.5	95% Solvent A 5% Solvent B	0.150	6	**Desolvation Temp.**	350 °C
33.0	95% Solvent A 5% Solvent B	0.150	6	**Cone/Desolvation Gas Rate**	80 L/hr /400 L/hr

[1] *Solvent A: 0.3% Formic Acid and 0.1% Ammonium Formate in HPLC water*
Solvent B: 1:1 Acetonitrile:Methanol
Group 1: Acidic extraction, positive electrospray ionization (ESI^+) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (µg/kg)		Extract (ng/µL)	
				MDL	ML	MDL	ML	MDL	ML
Group 1	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI+)**								
Native Compounds									
Sulfanilamide	2.5	190.0 - 155.8	$^{13}C_6$-Sulfamethazine	8.9	50	48	200	2.2	12.5
Cotinine	2.8	177.0 – 98.0	Cotinine-d_3	3.4	5	1.1	5	0.9	1.25
Acetaminophen	4.6	152.2 – 110.0	$^{13}C_2$-^{15}N-Acetaminophen	27	200	35	200	6.7	50
Sulfadiazine	6.0	251.2 – 156.1	$^{13}C_6$-Sulfamethazine	0.4	5	2.7	10	0.1	1.25
1,7-Dimethylxanthine	6.9	181.2 – 124.0	$^{13}C_3$-Caffeine	120	500	270	1000	30	125
Sulfathiazole	7.7	256.3 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.5	5	1.9	50	0.1	1.25
Codeine	8.3	300.0 – 152.0	$^{13}C_3$-Trimethoprim	1.5	10	3.4	10	0.4	2.5
Sulfamerazine	8.7	265.0 – 156.0	$^{13}C_6$-Sulfamethazine	0.3	2	1.4	5	0.1	0.5
Lincomycin	9.3	407.5 – 126.0	$^{13}C_3$-Trimethoprim	0.8	10	4.7	10	0.2	2.5
Caffeine	9.3	195.0 – 138.0	$^{13}C_3$-Caffeine	15	50	5.4	50	3.6	12.5
Sulfamethizole	10.0	271.0 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.4	2	0.88	5	0.1	0.5
Trimethoprim	10.0	291.0 – 230.0	$^{13}C_3$-Trimethoprim	1.1	5	3.3	10	0.3	1.25
Thiabendazole	10.0	202.1 – 175.1	Thiabendazole-d_6	0.7	5	2.1	10	0.2	1.25
Sulfamethazine	10.1	279.0 – 156.0	$^{13}C_6$-Sulfamethazine	0.6	2	0.83	5	0.2	0.5
Cefotaxime	10.2	456.4 – 396.1	$^{13}C_3$-Trimethoprim	10	20	18	50	2.5	5
Carbadox	10.5	263.2 – 231.2	$^{13}C_3$-Trimethoprim	2.3	5	2.1	10	0.6	1.25
Ormetoprim	10.5	275.3 – 259.1	$^{13}C_3$-Trimethoprim	0.3	2	0.50	2	0.1	0.5
Norfloxacin	10.7	320.0 – 302.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	28	50	15	50	7.0	12.5
Sulfachloropyridazine	10.8	285.0 – 156.0	$^{13}C_6$-Sulfamethazine	1.2	5	1.9	5	0.3	1.25
Ofloxacin	10.8	362.2 – 318.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	1.8	5	3.4	10	0.4	1.25
Ciprofloxacin	10.9	332.2 – 314.2	$^{13}C_3$ ^{15}N-Ciprofloxacin	5.1	20	8.1	20	1.3	5
Clinafloxacin	12.2	366.3 – 348.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	6.9	20	14	50	1.7	5
Digoxigenin	12.6	391.2 – 355.2	$^{13}C_3$-Trimethoprim	5.7	20	9.4	20	1.4	5
Oxolinic acid	13.1	261.8 – 243.8	$^{13}C_3$-Trimethoprim	0.6	2	0.62	2	0.2	0.5
Sulfadimethoxine	13.2	311.0 – 156.0	$^{13}C_6$-Sulfamethoxazole	0.1	1	0.55	2	0.03	0.25
Diphenhydramine	14.5	256.8 – 168.1	$^{13}C_3$-Trimethoprim	0.4	2	0.66	2	0.1	0.5
Penicillin G	14.6	367.5 – 160.2	$^{13}C_3$-Trimethoprim	2.4	10	13	50	0.6	2.5
Azithromycin	14.8	749.9 – 591.6	$^{13}C_3$-Trimethoprim	1.3	5	1.6	5	0.3	1.25
Flumeqine	15.2	262.0 – 173.7	$^{13}C_3$-Trimethoprim	2.7	5	1.4	5	0.7	1.25
Ampicillin	15.3	350.3 – 160.2	$^{13}C_3$-Trimethoprim	-	5	-	5	-	1.25
Diltiazem	15.3	415.5 – 178.0	$^{13}C_3$-Trimethoprim	0.6	2	0.30	2	0.2	0.25
Carbamazepine	15.3	237.4 – 194.2	$^{13}C_3$-Trimethoprim	1.4	5	1.6	5	0.4	1.25
Penicillin V	15.4	383.4 – 160.2	$^{13}C_3$-Trimethoprim	4.4	20	19	50	1.1	5
Erythromycin	15.9	734.4 – 158.0	$^{13}C_2$-Erythromycin	-	1	-	2	-	0.25
Tylosin	16.3	916.0 – 772.0	$^{13}C_2$-Erythromycin anhydrate	13	50	8.1	50	3.2	5
Oxacillin	16.4	434.3 – 160.1	$^{13}C_3$-Trimethoprim	3.3	10	9.4	20	0.8	2.5
Dehydronifedipine	16.5	345.5 – 284.1	$^{13}C_3$-Trimethoprim	0.6	2	0.41	2	0.2	0.5

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (µg/kg)		Extract (ng/µL)	
				MDL	ML	MDL	ML	MDL	ML
Group 1	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI^+)**								
Native Compounds									
Clarithromycin	17.5	748.9 – 158.2	13C2-Erythromycin anhydrate	1.0	5	1.2	5	0.3	1.25
Labeled compounds spiked into each sample									
Cotinine-d_3	2.8	180.0 – 79.9	$^{13}C_3$-Atrazine						
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2 – 111.0	$^{13}C_3$-Atrazine						
$^{13}C_3$ Caffeine	9.3	198.0 – 140.0	$^{13}C_3$-Atrazine						
Thiabendazole-d_6	9.8	208.1 – 180.1	$^{13}C_3$-Atrazine						
$^{13}C_3$-Trimethoprim	10.0	294.0 – 233.0	$^{13}C_3$-Atrazine						
$^{13}C_6$ Sulfamethazine	10.1	285.1 – 162.0	$^{13}C_3$-Atrazine						
$^{13}C_3$^{15}N-Ciprofloxacin	10.9	336.1 – 318.0	$^{13}C_3$-Atrazine						
$^{13}C_6$-Sulfamethoxazole	11.2	260.0 – 162.0	$^{13}C_3$-Atrazine						
$^{13}C_2$-Erythromycin	15.9	736.4 – 160.0	$^{13}C_3$-Atrazine						
Fluoxetine-d_5	16.8	315.3 – 153.0	$^{13}C_3$-Atrazine						
$^{13}C_2$-Erythromycin anhydrate	17.7	718.4 – 160.0	$^{13}C_3$-Atrazine						
Injection internal standard									
$^{13}C_3$ Atrazine	15.9	219.5 – 176.9 (134.0)	External standard						

Group 1: Acidic extraction, positive electrospray ionization (ESI^+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

HPLC CONDITIONS

System: Waters 2690 HPLC or 2795 HPLC, Quattro Ultima MS/MS
Column: XTerra C_{18}, 3.5 µm 2.1 x 100 mm
Ionization: ESI^+
Acquisition: MRM mode, unit resolution
Injection: 5 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temp.	40 °C
0.0	10% Solvent A 90% Solvent B	0.20	1	**Flow Rate**	0.20-0.23 mL/min
1.0	10% Solvent A 90% Solvent B	0.20	6	**Max Pressure**	345 Bar
18.0	40% Solvent A 60% Solvent B	0.23	6	**Autosampler Tray Temp.**	4 °C
20.0	90% Solvent A 10% Solvent B	0.23	6	MS Conditions	
24.0	90% Solvent A 10% Solvent B	0.23	6	**Source Temp.**	120 °C
24.3	10% Solvent A 90% Solvent B	0.20	6	**Desolvation Temp.**	400 °C
28	10% Solvent A 90% Solvent B	0.20	6	**Cone / Desolvation Gas Rate**	70 L/hr /450 L/hr

[1]*Solvent A: 1:1 acetonitrile:methanol, with 5 mM Oxalic Acid.*
Solvent B: HPLC H_2O, with 5 mM Oxalic Acid.
Group 2: Acidic extraction positive electrospray ionization (ESI^+) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/µL)	
				MDL	ML	MDL	ML	MDL	ML
Group 2	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (ESI^+)**								
Native Compounds									
Minocycline	5.1	458.0 – 441.0	Thiabendazole-d_6	51	200	-	200	13	50
Epitetracycline	8.1	445.2 – 410.2	Thiabendazole-d_6	3.6	20	8.6	20	0.9	5
Epioxytetracycline (EOTC)	8.6	461.2 – 426.2	Thiabendazole-d_6	4.1	20	18	50	1.0	5
Oxytetracycline (OTC)	9.4	461.2 – 426.2	Thiabendazole-d_6	2.1	20	2.2	20	0.5	5
Tetracycline (TC)	9.9	445.2 – 410.2	Thiabendazole-d_6	1.9	20	2.8	20	0.5	5
Demeclocycline	11.7	465.0 – 430.0	Thiabendazole-d_6	6.6	50	7.9	50	1.7	12.5
Isochlortetracycline (ICTC)[1]	11.9	479.0 – 462.2	Thiabendazole-d_6	1.7	20	3.5	20	0.4	5
Epichlortetracycline (ECTC)[1]	12.0	479.0 – 444.0	Thiabendazole-d_6	7.7	50	26	100	1.9	12.5
Chlortetracycline (CTC)	14.1	479.0 – 444.0	Thiabendazole-d_6	1.2	20	2.3	20	0.3	5
Doxycycline	16.7	445.2 – 428.2	Thiabendazole-d_6	2.8	20	2.3	20	0.7	5
Epianhydrotetracycline (EATC)	17.0	426.8 – 409.8	Thiabendazole-d_6	7.7	50	14	50	1.9	12.5
Anhydrotetracycline (ATC)	18.8	426.8 – 409.8	Thiabendazole-d_6	4.6	50	7.1	50	1.2	12.5
Epianhydrochlortetracycline (EACTC)	20.7	461.2 – 444.0	Thiabendazole-d_6	28	200	23	200	7.0	50
Anhdrochlortetracycline (ACTC)	22.1	461.2 – 444.0	Thiabendazole-d_6	5.2	50	11	50	1.3	12.5
Labeled Compound Spiked into Each Sample									
Thiabendazole-d_6	7.0	208.1 – 180.1	$^{13}C_3$-Atrazine						
Injection Internal Standard									
$^{13}C_3$-Atrazine	10.5	219.5 – 176.9 (134.0)	External standard						

[1] *Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.*

Group 2: Acidic extraction positive electrospray ionization (ESI^+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

HPLC CONDITIONS

System: Waters 2690 HPLC or 2795 HPLC, Quattro Ultima MS/MS

Column: XTerra C_{18}, 3.5 µm, 2.1 x 100 mm

Ionization: ESI^-

Acquisition: MRM mode, unit resolution

Injection: 5 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temp.	40 °C
0.0	60% Solvent A 40% Solvent B	0.2	1	**Flow Rate**	0.200 mL/min
0.5	60% Solvent A 40% Solvent B	0.2	6	**Max Pressure**	345 Bar
7.0	100% Solvent B	0.2	6	**Autosampler Tray Temp.**	4 C
12.5	100% Solvent B	0.2	6	**MS Conditions**	
12.7	60% Solvent A 40% Solvent B	0.2	6	**Source Temp.**	100 °C
16.0	60% Solvent A 40% Solvent B	0.2	1	**Desolvation Temp.**	350 °C
				Cone/Desolvation Gas Rate	50L/hr /300 L/hr

[1] *Solvent A: 0.1% Ammonium Acetate and 0.1% Acetic Acid in HPLC water.*
Solvent B: 1:1 MethanolAcetonitrile.
Group 3: Acidic extraction negative electrospray ionization (ESI^-) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/µL)	
				MDL	ML	MDL	ML	MDL	ML
Group 3	**Analytes Extracted Under Acidic Conditions and Analyzed Using Negative Electrospray Ionization (ESI^-)**								
Native Compounds									
Naproxen	6.7	228.9 – 168.6	^{13}C-Naproxen-d_3	3.9	10	6.1	20	1.0	2.5
Warfarin	7.1	307.0 – 117.0	Warfarin-d_5	0.9	5	1.6	5	0.2	1.25
Ibuprofen	8.4	205.1 – 161.1	$^{13}C_3$-Ibuprofen	6.0	50	11	50	1.5	12.5
Gemfibrozil	9.5	249.0 – 121.0	Gemfibrozil-d_6	0.8	5	1.2	5	0.2	1.25
Triclocarban	9.6	312.9 – 159.7	$^{13}C_6$-Triclocarban	2.1	10	2.7	10	0.5	2.5
Triclosan	9.7	286.8 – 35.0	$^{13}C_{12}$-Triclosan	92	200	56	200	23	50
Labeled Compounds									
^{13}C-Naproxen-d_3	6.6	232.9 – 168.6	$^{13}C_6$-TCPAA						
Warfarin-d_5	7.0	312.0 – 161.0	$^{13}C_6$-TCPAA						
$^{13}C_3$-Ibuprofen	8.5	208.2 – 163.1	$^{13}C_6$-TCPAA						
Gemfibrozil-d_6	9.5	255.0 – 121.0	$^{13}C_6$-TCPAA						
$^{13}C_6$-Triclocarban	9.6	318.9 – 159.7	$^{13}C_6$-TCPAA						
$^{13}C_{12}$-Triclosan	9.7	298.8 – 35.0	$^{13}C_6$-TCPAA						
Injection Internal Standards									
$^{13}C_6$-TCPAA	4.9	258.8 – 200.7	External standard						

Group 3: Acidic extraction negative electrospray ionization (ESI^-) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

HPLC CONDITIONS

System: Waters 2690 HPLC or 2795 HPLC, Quattro Ultima MS/MS
Column: Atlantis HILIC, 3.0 µm, 2.1 x 100 mm
Ionization: ESI^+
Acquisition: MRM mode, unit resolution
Purge Solvent: 100% CH_3CN (changed from H_2O)
Injection: 2.0 µL

LC Gradient Program		LC Flow Rate (mL/min)	Gradient	General LC Conditions	
Time (min)	Flow Mixture[1]			Column Temp.	40 °C
0.0	2% Solvent A 8% Solvent B	0.25	1	**Flow Rate**	0.25 mL/min
5.0	30% Solvent A 70% Solvent B	0.25	6	**Max Pressure**	345 Bar
12.0	30% Solvent A 70% Solvent B	0.25	6	**Autosampler Tray Temp.**	4 °C
12.5	2% Solvent A 98% Solvent B	0.25	6	**MS Conditions**	
16.0	2% Solvent A 98% Solvent B	0.25	6	**Source Temp.**	120 °C
				Desolvation Temp.	350 °C
				Cone/Desolvation Gas Rate	70L/hr /400 L/hr

[1] *Solvent A: 0.1% Acetic Acid/Ammonium Acetate Buffer.*
Solvent B: Acetonitrile.
Group 4: Basic extraction positive electrospray ionization (ESI^+) instrument conditions.

Analyte	RT (min)	Parent-Daughter M/ZS	Quantitation Reference	Detection Limits and Minimum Levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/µL)	
				MDL	ML	MDL	ML	MDL	ML
Group 4	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization ESI+**								
Native Compounds									
Cimetidine	6.9	253.1 – 159.0	Albuterol-d_3	0.6	2	0.78	2	0.2	0.5
Albuterol	9.4	240.0 – 148.0	Albuterol-d_3	0.9	2	0.39	2	0.2	0.5
Ranitidine	10.3	315.0 – 175.9	Albuterol-d_3	0.7	2	1.1	2	0.2	0.5
Metformin	11.0	131.1 – 60.1	Metformin-d_6	23	100	38	100	5.8	25
Labeled compounds spiked into samples									
Albuterol-d_3	9.4	243.0 – 151.0	Cotinine-d_3						
Metformin-d_6	11.0	285.1 – 163.0	Cotinine-d_3						
Injection Internal Standard									
Cotinine-d_3	5.9	180.0 – 79.9	External standard						
$^{13}C_3$-Atrazine	2.0	219.5 – 176.9 (134.0)	External Standard						

Group 4: Basic extraction positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

ORDERING INFORMATION

Description	Part Number
XTerra MS C_{18}, 3.5 µm, 2.1 x 100 mm	186000404
Atlantis HILIC, 3.0 µm, 2.1 x 100 mm	186002013
Oasis® HLB Extraction Cartridge, 20 cc, 1g, LP	186000117
EPA Method 1694 Analysis Kit	176001634
LCMS Certified Vials	see catalog

www.waters.com/order

Related Documents	Literature Code
Total Solutions for Environmental Applications	720002163EN
LC/MS Determination of Pharmaceutical Residues in Environmental Samples	720000421EN

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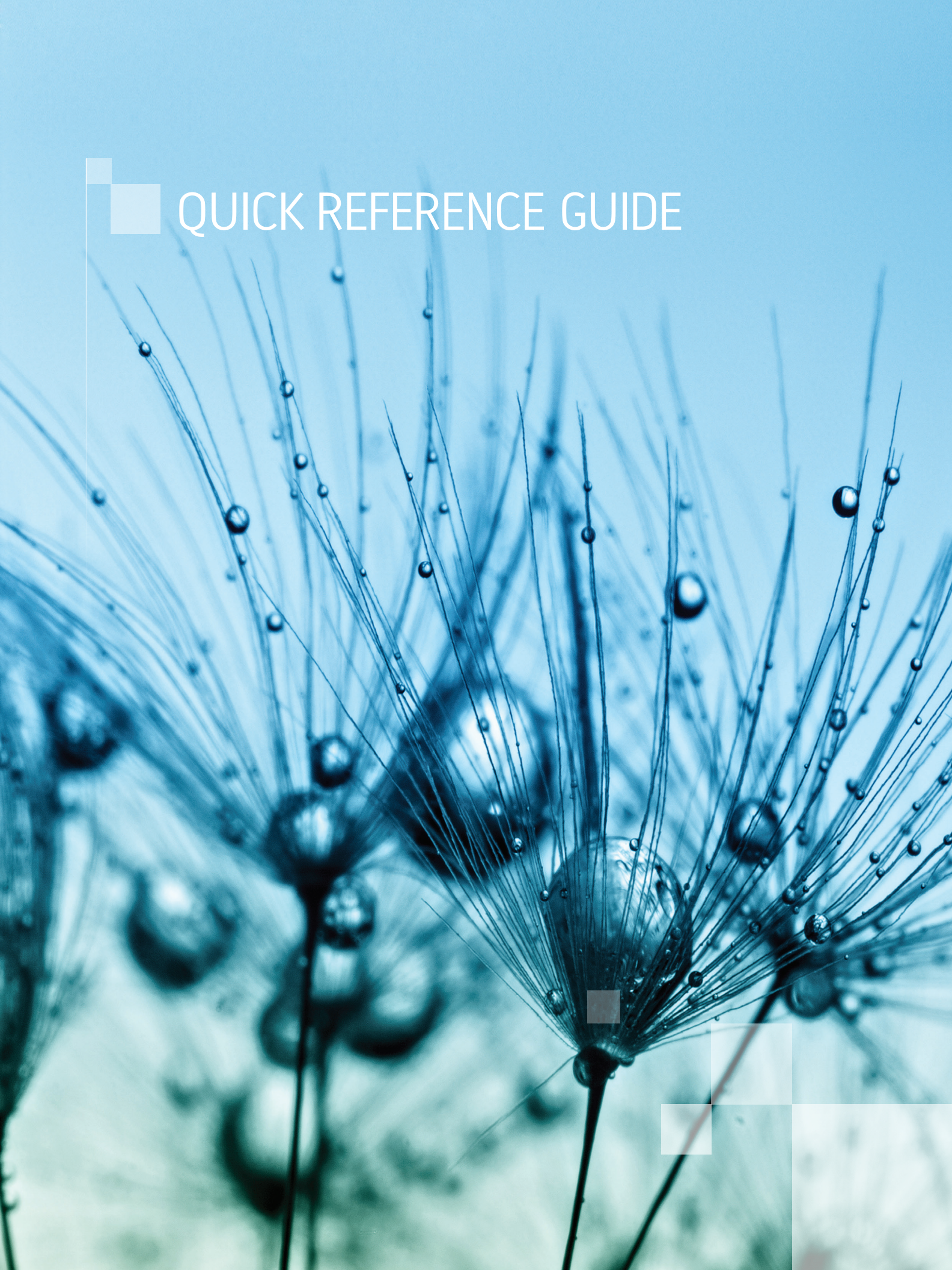
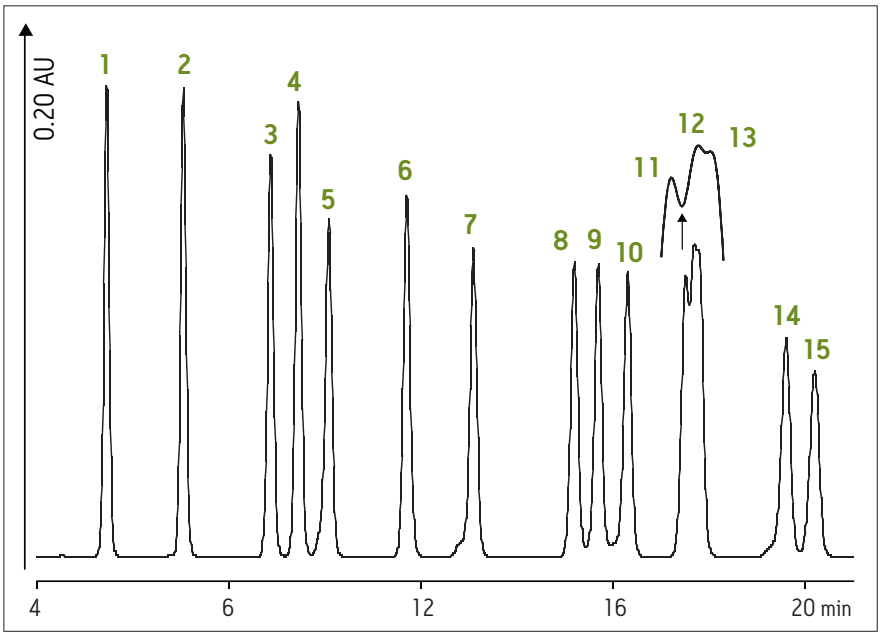
QUICK REFERENCE GUIDE



TO-11 – DETERMINATION OF FORMALDEHYDE IN AMBIENT AIR USING ADSORBANT CARTRIDGE FOLLOWED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Formaldehyde is an important industrial chemical used in the manufacturing of other chemicals, building materials, and household products. It is one of the large family of chemical compounds called volatile organic compounds or "VOCs". At normal room temperatures these compounds vaporize. When present in air at levels above 0.1 ppm it can cause watery eyes, burning sensations in the eyes and nasal passages, as well as coughing, wheezing, and allergic reactions. Formaldehyde has been classified as a potential carcinogen and, as such, is regulated in many countries: Japan, 0.08 ppm; World Health Organization Europe, 0.08 ppm; Sweden, 0.1 ppm; US Department of Housing and Urban Development, 0.4 ppm.



Peak	Analyte	Peak	Analyte
1	Formaldehyde	9	Isovaleraldehyde
2	Acetaldehyde	10	Pentanal
3	Acetone	11	o-Tolualdehyde
4	Acrolein	12	p-Tolualdehyde
5	Propanal	13	m-Tolualdehyde
6	Crotonaldehyde	14	Hexanal
7	Butanal	15	2-5 Dimenthylbenzaldehyde
8	Benzaldehyde		

EPA Method TO11 and 8315-02 analytes, 20 ppm as DNPH analytes.

ORDERING INFORMATION

Description	Part Number
XTerra Phenyl, 3.5 µm, 2.1 x 150 mm	186001181
Sep-Pak DNPH-Silica Cartridge	WAT037500

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Related Documents	Literature Code
Determination of Formaldehyde in Ambient Air	720001988EN
Analysis of DNPH Derivatives using XBridge Phenyl	WAT60186
Reducing Acetronitrile Usage for the HPLC Analysis of Aldehyde and Ketone Pollutants	720003012EN

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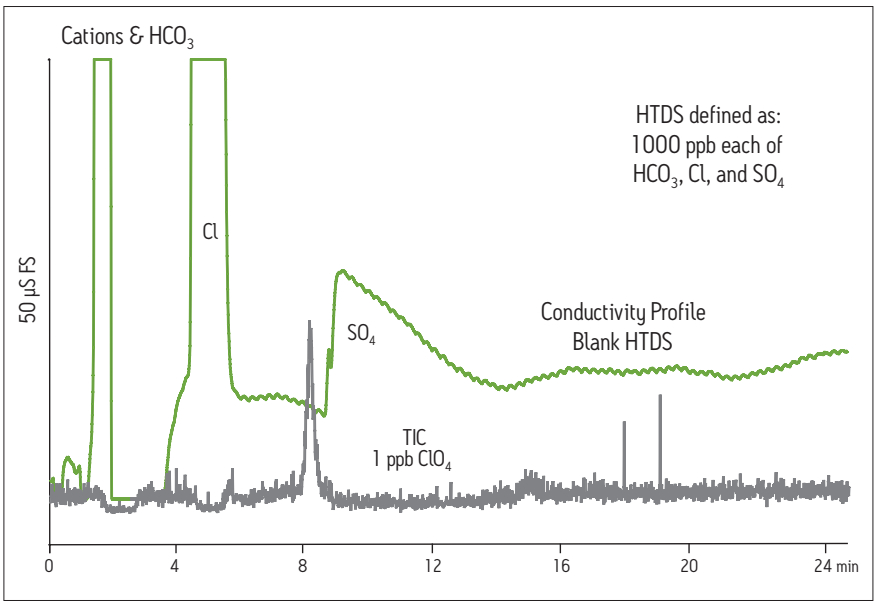
Perchlorate is both naturally occurring and man-made. In its natural form, perchlorate is a contaminent in fertilizers. Man-made perchlorate is used in a wide variety of industrial applications including the production of rubber and paint, in lubricants, and as a primary ingredient in solid rocket propellant. Perchlorate is highly water soluble and can migrate into groundwater and surface water, posing a concern to drinking water supplies. Thirty-five states have detected perchlorate in drinking water at higher levels than expected. The United States Environmental Protection Agency (U.S. EPA) has established an official reference dose of 0.0007 ppb per day of perchlorate. Maryland, Massachusetts, and New Mexico have established a one part per billion (ppb) action limit, while California and Texas have established 4 ppb limits.



1 ppb perchlorate in HTDS.

ORDERING INFORMATION

Description	Part Number
IC-Pak Anion HR, 7 µm, 4.6 x 150 mm	WAT26770
Perchlorate Standard	186004155
Perchlorate CRM	186004253
LCMS Certified Vials	see catalog

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Related Literature	Literature Code
Environmental System Solutions	720001601EN
The Determination of Perchlorate in Water Using LC-MS/MS	720000941EN
The Determination of Perchlorate in Drinking Water Using Single Quadrupole Mass Spectrometry	720001285EN

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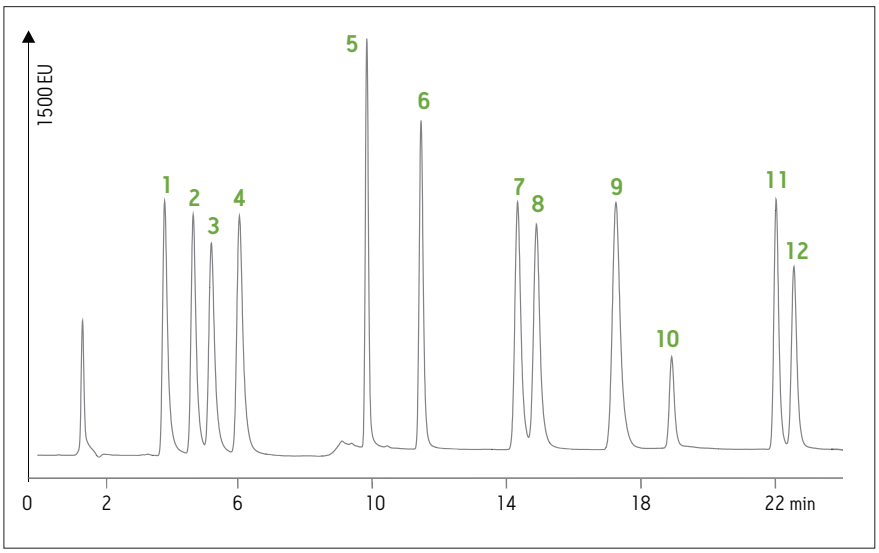
EPA 531.2 (ALTERNATIVE METHOD) – MEASUREMENT OF N-METHYLCARBAMOYLOXIME AND N-METHYLCARBAMATES IN WATER BY DIRECT AQUEOUS INJECTION HPLC

Carbamates are used worldwide as commercial pesticides for food crops. The resulting agricultural runoff can carry them into surface water, groundwater, and other drinking water resources. The U.S. EPA requires that drinking water and raw surface water be monitored for the presence of carbamate pesticides and related compounds using an established EPA Method 531.2. The European Union (EU) regulation regarding drinking water, provides a general rule for pesticides and metabolites. This regulation limits the maximum admissible concentration (MAC) at 0.1 µg/L (ppb) for each individual component, with the total concentration not to exceed 0.5 ppb. Detection of regulated compounds at ever decreasing levels is a challenge faced by many water testing laboratories. Because the concentration of these substances in real samples may be in the low parts per billion (ppb) or µg/L, optimal sensitivity is the ultimate goal.



Standard chromatogram of 25 ppb for each analyte.

Peak	Analyte	Retention Time (min)	Detection Limit (ppb)
1	Aldicarb Sulfoxide	3.77	0.019
2	Aldicarb Sulfone	4.66	0.041
3	Oxamyl	5.17	0.050
4	Methomyl	6.03	0.031
5	3-Hydroxy Carbofuran	9.83	0.022
6	Aldicarb	11.46	0.022
7	Propoxur	14.35	0.038
8	Carbofuran	14.94	0.028
9	Carbaryl	17.37	0.013
10	1-Naphthol	18.99	0.053
11	Methiocarb	22.02	0.022
12	BDMC*	22.56	0.031

** Internal Standard EPA Method 531.2 target analytes.*

ORDERING INFORMATION

Description	Part Number
Oasis HLB Cartridge, 6 cc, 200 mg	WAT106202
Carbamate Analysis Column, 3.9 x 150 mm	WAT35577
Carbamate/Carbamoxyloxime Pesticides Standard	186004278

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Related Documents	Literature Code
Waters Alliance Systems for Carbamate Analysis	720000126EN
Carbamates in Drinking Water	720000609EN
A Fully Automatic Multi-Analyte Quantification Protocol for Carbamates – A Comparison of LC/MS and LC-MS/MS	720000672EN
LC/MS of 52 Carbamates: A Fully Automated Protocol	WA20274

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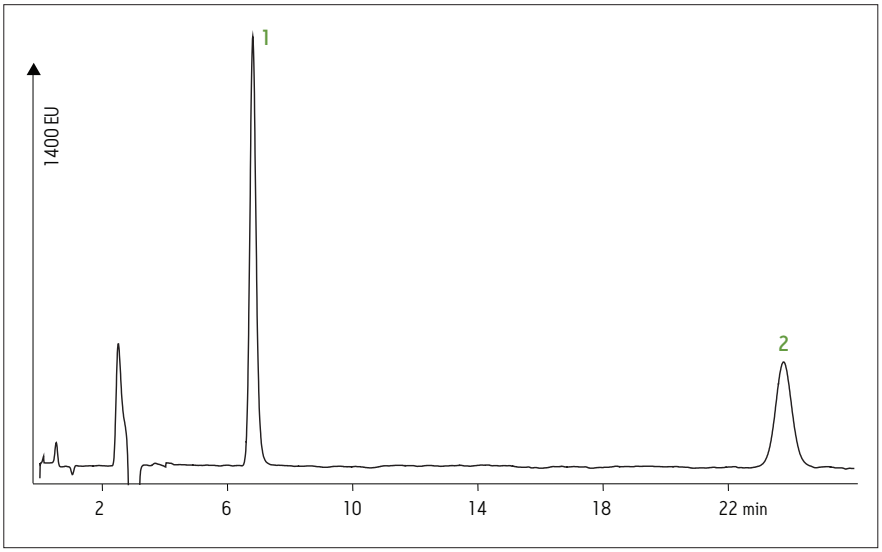
EPA 547 (ALTERNATIVE METHOD) – DETERMINATION OF GLYPHOSATE IN DRINKING WATER BY DIRECT AQUEOUS INJECTION HPLC, POST COLUMN DERIVITIZATION AND FLUORESCENCE DETECTION

Glyphosate is a non-selective herbicide which is adsorbed through leaves and was first sold by Monsanto® under the Roundup® trade name. This is one of the most widely used herbicides, regularly used for agriculture, horticulture, and silviculture applications. The U.S. EPA requires that drinking water and raw surface water be monitored for the presence of glyphosate and related compounds using EPA Method 547.0. The European Union (EU) regulation (EC Directive 2005/70/EU) provides guidance with regards to the presence of glyphosate in drinking water supplies.



Standard chromatogram, 100 ppb each analyte.

Peak	Analyte
1	Glyphosate
2	AMPA

ORDERING INFORMATION

Description	Part Number
IC-Pak™ Ion-Exclusion Column, 7.8 x 150 mm	WAT010295
Guard-Pak Holder	WAT88141
Semivolatiles #2 Herbicide Standard	186004271
Oasis MAX Cartridge, 6 cc, 150 mg	186000370
Oasis MAX Cartridge, 6 cc, 500 mg	186000865

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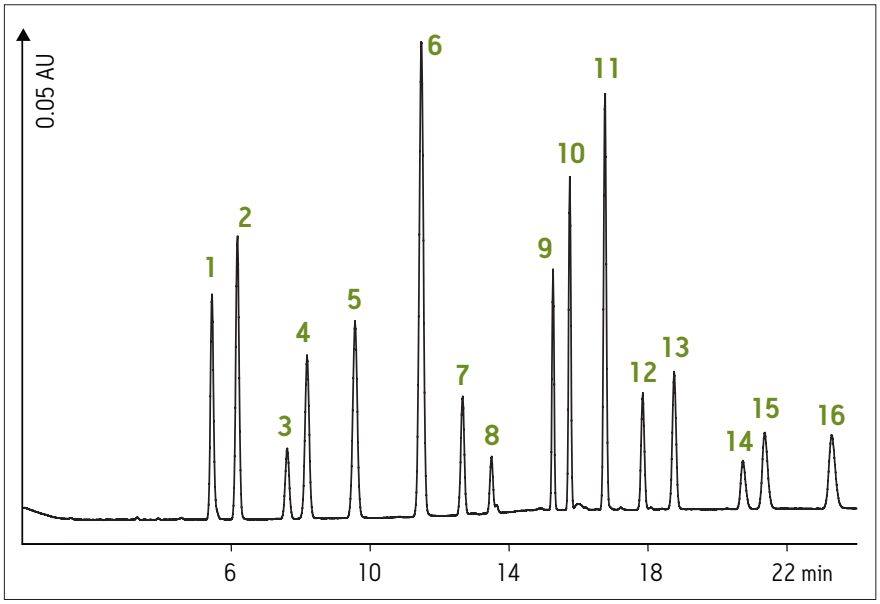
Related Documents	Literature Code
Environmental System Solutions	720001601EN
Glyphosate and AMPA in Drinking Water	WA31764.94
An LC-MS/MS Multi-Analyte Detection Method for Deleterious Organics in Drinking Water	720001090EN

www.waters.com/library

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic pollutants. PAHs are made up of fused aromatic rings and are formed during the combustion of carbon based fuels (wood, coal, diesel), as well as being present in crude oil. The U.S. EPA has classified seven PAH compounds as being potentially carcinogenic including benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno [1,2,3-cd]pyrene.



Standard chromatogram, UV at 254 nm, 1-20 ppm PAH analytes.

	Analyte	UV Max (nm)	EX (nm)	EM (nm)	Detection Limit (ppb)[1]
1	Naphthalene	220	277	330	0.14
2	Acenaphthylene	229	NA	NA	NA
3	Acenaphthene	227	270	323	0.01
4	Fluorene	261	265	310	0.03
5	Phenanthrene	251	252	365	0.02
6	Anthracene	252	250	402	0.01
7	Fluoranthene	236	284	467	0.02
8	Pyrene	240	332	378	0.01
9	Benzo(a)anthracene	287	284	390	0.01
10	Chrysene	267	270	367	0.04
11	Benzo(b)fluoranthene	256	298	436	0.09
12	Benzo(k)fluoranthene	307	303	432	0.01
13	Benzo(a)pyrene[2]	296	280	410	0.03
14	Dibenzo(a,h)anthracene	297	294	398	0.01
15	Benzo(g,h,I)perylene	299	290	420	0.03
16	Indeno(1,2,3-cd)pyrene	250	305	480	0.49

[1] Fluorescence mode used for detection limit determination, no pre-concentration. Seven replicates per 40 CFR pt. 136 App. B.
[2] Regulated compound; action level 0.17 ppb.
PAH target analytes.

ORDERING INFORMATION

Description	Part Number
PAH Column, 4.6 x 250 mm	186001265
Semivolatiles #1 Standard	186004270

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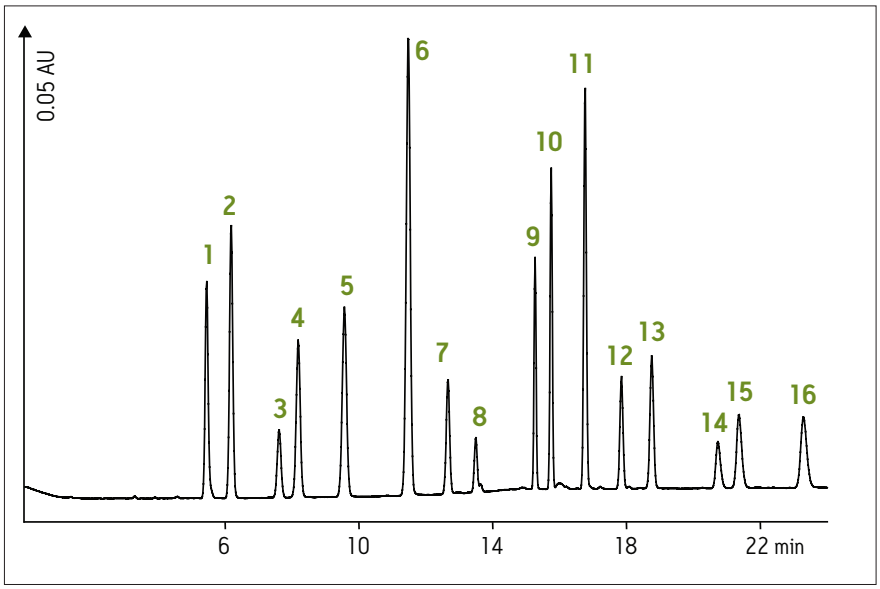
Related Documents	Literature Code
The Determination of Biodegradation Products of PAH Using LC-MS/MS	WA20747
PAHs in Drinking Water – Oasis Solution	WA31764.127
Waters PAH Columns Improve Analysis of PAH Compounds	720000382EN

www.waters.com/library

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic pollutants. PAHs are made up of fused aromatic rings and are formed during the combustion of carbon based fuels (wood, coal, diesel), as well as being present in crude oil. The U.S. EPA has classified seven PAH compounds as being potentially carcinogenic including benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno [1,2,3-cd]pyrene.



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9	Benzo(a)anthracene	287	284	390	0.01
10	Chrysene	267	270	367	0.04
11	Benzo(b)fluoranthene	256	298	436	0.09
12	Benzo(k)fluoranthene	307	303	432	0.01
13	Benzo(a)pyrene2	296	280	410	0.03
14	Dibenzo(a,h)anthracene	297	294	398	0.01
15	Benzo(g,h,I)perylene	299	290	420	0.03
16	Indeno(1,2,3-cd)pyrene	250	305	480	0.49

[1] Fluorescence mode used for detection limit determination, no pre-concentration. Seven replicates per 40 CFR pt. 136 App. B.
[2] Regulated compound; action level 0.17 ppb. PAH target analytes.

ORDERING INFORMATION

Description	Part Number
PAH Column, 4.6 x 250 mm	186001265
Semivolatiles #1 Standard	186004270

www.waters.com/order

Related Documents	Literature Code
The Determination of Biodegradation Products of PAH Using LC/MS/MS	WA20747
PAHs in Drinking Water – Oasis Solution	WA31764.127
Waters PAH Columns Improve Analysis of PAH Compounds	720000382EN

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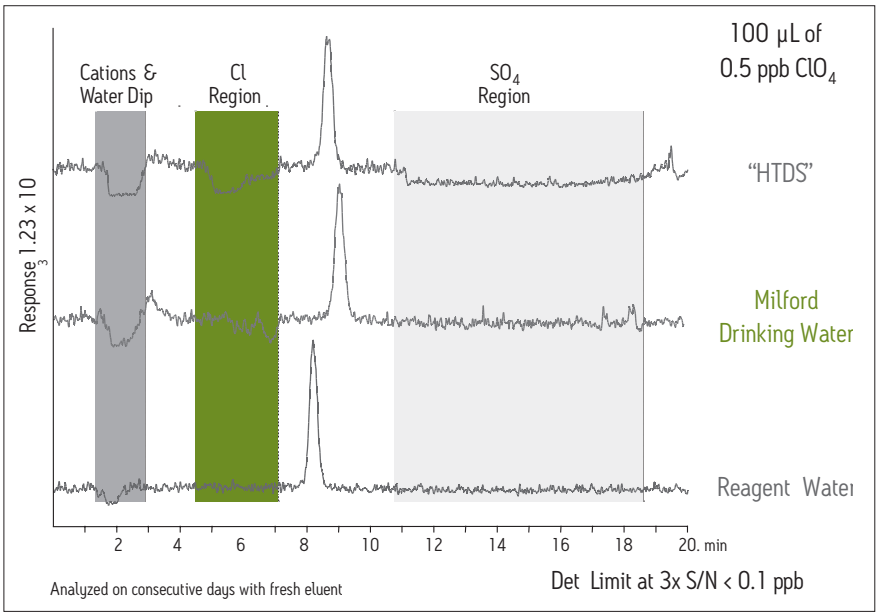
Perchlorate is both naturally occurring and man-made. In its natural form, perchlorate is a contaminent in fertilizers. Man-made perchlorate is used in a wide variety of industrial applications including the production of rubber and paint, in lubricants, and as a primary ingredient in solid rocket propellant. Perchlorate is highly water soluble and can migrate into groundwater and surface water, posing a concern to drinking water supplies. Thirty-five states have detected perchlorate in drinking water at higher levels than expected. The U.S. EPA has established an official reference dose of 0.0007 ppb per day of perchlorate. Maryland, Massachusetts, and New Mexico have established a one part per billion (ppb) action limit, while California and Texas have established 4 ppb limits.



0.5 ppb perchlorate detection in three different sample matrices by LC-MS/MS.

ORDERING INFORMATION

Description	Part Number
IC-Pak Anion HR, 6 µm, 4.6 x 75 mm	WAT026765
Perchlorate Standard	186004155
Perchlorate CRM	186004253
LCMS Certified Vials	see catalog

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Related Documents	Literature Code
Environmental System Solutions	720001601EN
The Determination of Perchlorate in Water Using LC-MS/MS	720000941EN
The Determination of Perchlorate in Drinking Water Using Single Quadrupole Mass Spectrometry	720001285EN

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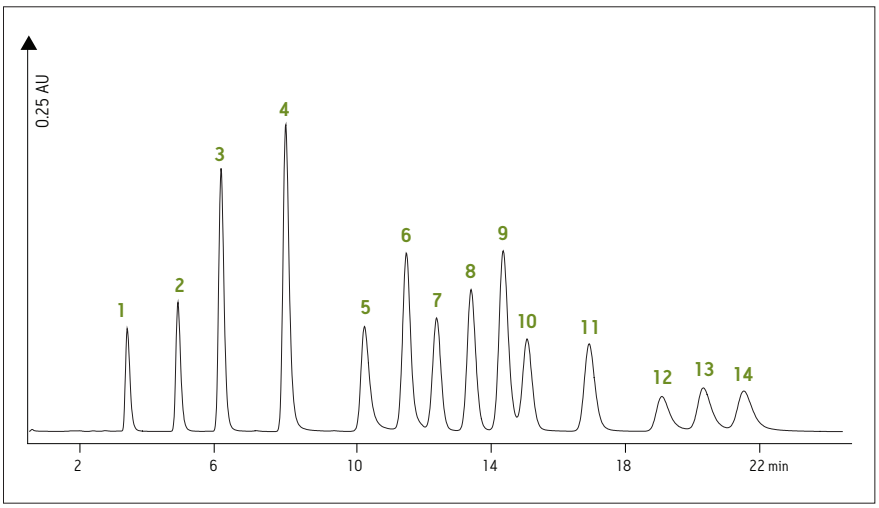
The presence of numerous military and defense sites around the world, both active and decommissioned, has resulted in the presence of explosives compounds in locations where they can enter the water supply. In the US, the evaluation of sites for potential contamination is carried out by the U.S. EPA, US Department of Defense, and US Department of Energy in support of Superfund, RCRA, and Base Closure environmental programs.



Peak	Analyte	Peak	Analyte
1	HMX	8	2 Amino-4,6 Dinitrotoluene
2	RDX	9	2,4 Dinitrotoluene
3	1,3,5-Trinitrobenzene	10	4 Amino-2,6 Dinitrotoluene
4	1,3 Dinitrobenzene	11	2,6 Dinitrotoluene
5	Nitrobenzene	12	4- Nitrotoluene
6	TNT	13	2- Nitrotoluene
7	Tetryl	14	3- Nitrotoluene

ORDERING INFORMATION

Description	Part Number
XTerra Phenyl, 3.5 µm, 2.1 x 150 mm	186001181
Porapak Reverse-Phase Sorbent (RDX)	WAT047220
UCMR2 Explosives in Water CRM	186004261

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Related Documents	Literature Code
The Science of ACQUITY UPLC Applied to Environmental Analyses of PAHs and Explosives in Water	720001398EN
Explosives in River Water – Oasis Solution	WA31764.82
An Improved Method for Determination of Nitroaromatic and Nitramine Explosives in Aqueous Samples	WA20717
High Speed Explosives Monitoring using UPLC	720000950EN

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Sweden 46 8 555 115 00
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