Waters™

Application Note

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

Kari L. Organtini, Henry Foddy, Nicola Dreolin, Stuart Adams, Kenneth J. Rosnack, Peter Hancock

Waters Corporation

Abstract

Per- and Polyfluoroalkyl Substances (PFAS) are synthetic chemicals numbering in the thousands that are toxic and highly persistent in humans, animals, and the environment. They have negative impact on human health and are a threat to ecosystems since they bioaccumulate and biomagnify. Yet, due to their extreme stability, physical, and chemical properties, they are heavily used in industrial and consumer products, such as nonstick coatings or waterproofing materials.

To reach detection limits low enough to satisfy exposure regulations, advisories, or tolerances, a carefully designed and executed workflow must be implemented including an analyte enrichment step, (e.g. solid phase extraction), Ultra Performance Liquid Chromatography (UPLC™), and a highly sensitive mass spectrometer. This application note details the approach using SPE extraction with Oasis™ WAX for PFAS to enrich water samples with analysis performed on a Waters™ ACQUITY™ Premier UPLC and Xevo™ TQ Absolute Mass Spectrometer. Limit of quantitation (LOQ) in drinking water for the analysis of PFOA, PFOS, and PFBS were each 0.001 ng/L while GenX (HFPO-DA) was 0.004 ng/L. All of which outperform the US EPA updated 2022 interim Health Advisory

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

1

Levels (HALs).

Benefits

- Ultra-trace analysis meeting new US EPA Health Advisory Levels (HALs) for detection of PFAS as low as 0.001 ng/L in drinking water

- Confidence in results by reducing and separating possible PFAS contamination from solvents and LC components from analytes in sample by utilizing the PFAS Installation Kit

- Comprehensive analytical workflow solution that includes standards, consumables, hardware, software, training, and proficiency testing is available from Waters

Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with a variety of commercial and consumer uses. They are characterized by their extreme chemical stability and structural diversity. PFAS usage ranges from industrial manufacturing of non-stick or waterproof coatings, to cosmetics, to their use in firefighting foams. The original chemicals, as well as their degradation products, are highly persistent and toxic contaminants that accumulate in humans, animals, and the environment. PFAS persist in the environment for many decades, hence, are sometimes referred to as 'forever chemicals'.

Human exposure to PFAS can occur in many ways, including being exposed through consumer products, industrial or house dust, and ingestion through contaminated drinking water. The negative impacts on human health are extensive and continue to be investigated. In addition, PFAS pollution affects ecosystems and generates costs through the need for remediation of polluted soil and water. Some countries impose regulatory or advisory limits on the concentration of various PFAS in drinking, ground, and surface waters. Although this application note focuses on water, it is worthwhile mentioning that there are plans to set regulatory levels for PFAS in other matrices, such as food for example.

Detection requirements for PFAS have been getting more challenging as advisory and regulatory limits, to protect public health and the environment, continue to be created and updated. For example, on June 15, 2022, the US EPA tightened its lifetime health advisory levels (HALs) in drinking water for perfluorooctanoic acid (PFOA) and

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

2

perfluorooctanesulfonic acid (PFOS).[2] The recommendation is 0.004 ng/L (part per trillion, ppt) for PFOA and 0.02 ng/L for PFOS, both of which are categorized as interim levels as of writing this document. These levels are dramatically more stringent than the 70 ng/L (ppt) total that the EPA recommended in 2016.[3] As a second example, in early 2021, the EU began enforcing the revised Drinking Water Directive (EU) 2020/2184 whereby a select group of 20 PFAS have a recommended summed limit of 0.1 µg/L (100 ng/L) with a limit of 0.5 µg/L (500 ng/L) for total PFAS observed.[4] This directive requires individual PFAS to be detected in the single digit ng/L range.

This application note will focus on reaching the more stringent limits of the EPA HALs, detailing a PFAS analytical workflow approach using SPE extraction with Oasis WAX for PFAS to enrich water samples with analysis performed on a Waters ACQUITY Premier UPLC and Xevo TQ Absolute MS with data analysis using waters_connect™ for Quantitation software.

Experimental

Sample Preparation

When extremely low quantitation limits are required, such as the EPA 2022 Interim HALs for PFOA and PFOS, best practices in sample handling, preparation, and analysis are critical to reduce sample contamination that can result in inaccurate sample reporting for these ubiquitous compounds. During sample preparation and analysis, extreme care was taken to screen all consumables and reagents for PFAS contamination before use. It is advised that the same process should be employed in each individual laboratory running this analysis to assess and address PFAS contamination levels before working with samples. All samples were prepared in a typical shared laboratory environment without major laboratory modifications.

During sample analysis, contamination levels were evaluated and consistently monitored in both solvent blank injections (1:1 water:methanol containing 2 mM ammonium acetate) and in extraction blank samples (250 mL sample of reagent water processed through all sample preparation steps).

All standards were obtained from Wellington Laboratories. An extraction internal standard (EIS) was spiked into the samples prior to sample extraction. This EIS contained $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_3$-PFBS, and $^{13}C_3$-HFPODA. An injection internal standard (IIS) was spiked into the samples during the reconstitution step following sample

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

3

extraction. The IIS contained $^{13}C_2$-PFOA and ^{13}C-PFOS.

Water samples representative of drinking water were spiked with 0.004 and 0.02 ng/L PFAS standards to test the interim PFOA and PFOS levels. PFBS and HFPO-DA (GenX) were also evaluated at these levels to determine quantitation limits for all compounds covered by the 2022 HAL update. Since it has been shown previously that the PFBS and GenX HALs (2000 and 10 ng/L respectively) are not a challenge to reach, these specific concentrations were not tested in this experiment.[5,6] Samples were extracted using the Oasis WAX SPE method described in Figure 1. Using the provided method, samples were prepared with an enrichment factor of 500x.
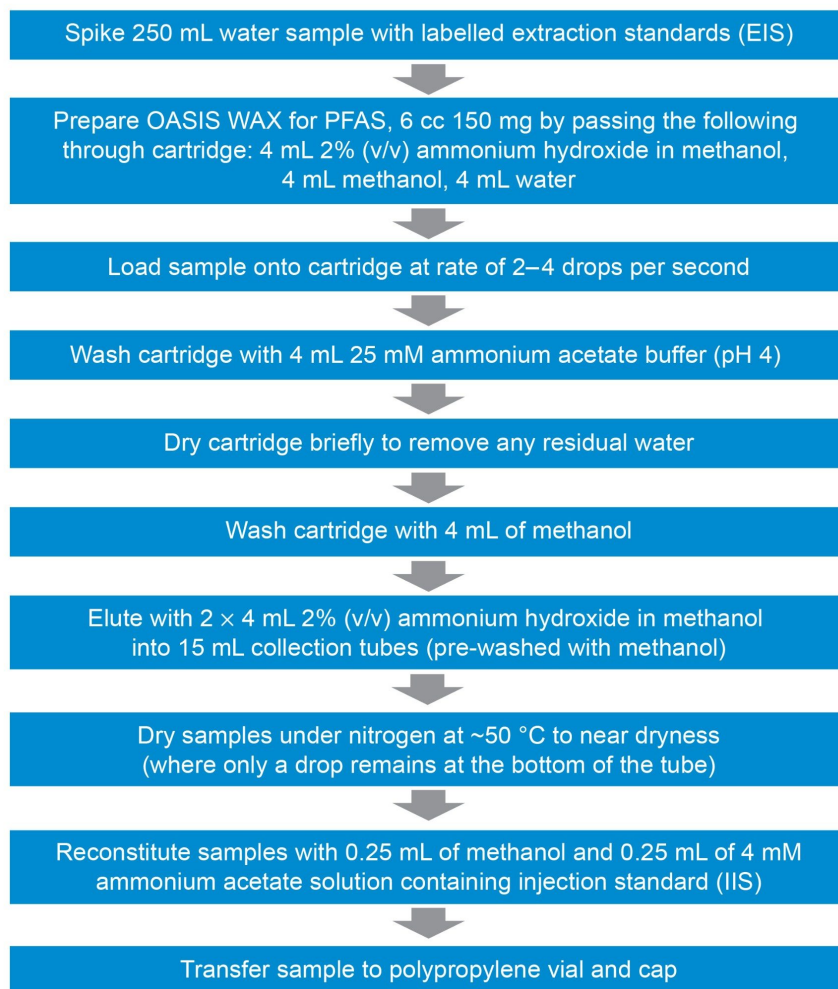
Figure 1. Full SPE method used for extracting drinking water samples using Oasis WAX for PFAS cartridges.

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

4

LC Conditions

LC system:	ACQUITY Premier BSM with FTN
Vials:	700 µL Polypropylene Screw Cap Vials
Column(s):	ACQUITY Premier BEH C$_{18}$ 2.1 x 100 mm, 1.7 µm (p/n: 186009453)
Column temp.:	45 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Mobile phase A:	2 mM ammonium acetate in water
Mobile phase B:	2 mM ammonium acetate in methanol

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

5

Gradient Table

Time (min)	%A	%B	Curve
0	95	5	initial
1	75	25	6
6	50	50	6
13	15	85	6
14	5	95	6
17	5	95	6
18	95	5	6
22	95	5	6

MS Conditions

MS system: Xevo TQ Absolute

Ionization mode: ESI-

Capillary voltage: 0.5 kV

Source temp.:   100 °C

Desolvation temp.:   350 °C

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

Desolvation flow:	900 L/hr
Cone flow:	150 L/hr
MRM method:	See Appendix for Full MRM Method details

Data Management

Chromatography software:	waters_connect for Quantitation
MS software:	waters_connect for Quantitation
Informatics:	waters_connect for Quantitation

Results and Discussion

Instrument sensitivity of the Xevo TQ Absolute was evaluated on two different instruments located in two different laboratories. Laboratory 1 was located in Wilmslow, United Kingdom and Laboratory 2 was located in Milford, Massachusetts, United States. The Limit of Quantitation (LOQ) for each laboratory as well as the signal:noise (S:N) at the LOQ is listed in Table 1. The LOQ was determined to be the calibration level at which the S:N value was ≥10 and is reported in terms of sample concentration considering the 500x enrichment factor from sample preparation. As seen in Table 1, the LOQ for each compound was well below the minimum reporting level suggested by the EPA guidelines, as well as below the HAL levels (both interim and final). These results indicate that the TQ Absolute has the sensitivity required for this challenging analysis.

Calibration samples were acquired in the sample range of 0.0005 to 0.08 ng/L (0.25 to 40 ng/L in vial equivalent) with all curves linear over this range, with a correlation coefficient of ≥0.992 and residuals within 30%. Figure 2 depicts the calibration curves for all four PFAS across this range.

Care was taken during sample preparation to minimize and control contamination as much as possible. All four

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

7

PFAS compounds were absent from all solvent blank injections, indicating the LC-MS/MS system itself, as well as sample vials, were free from PFAS contamination. PFOA, PFOS, and PFBS were detected in extraction blanks at varying levels indicating introduction of these compounds during the sample preparation step. PFOA, PFOS, and PFBS were detected in extraction blanks at varying levels indicating introduction of these compounds during the sample preparation step. The PFOA contamination of the extraction blank represented approximately 21% of the 0.004 ng/L sample spike, while PFOS was approximately 4% of the 0.02 ng/L sample spike. PFBS contamination was also compared to the 0.02 ng/L sample spike, and was 8% of that response. Since the HAL for PFBS is orders of magnitude higher than this sample spike, this extraction blank contamination can be considered negligible. An overlay of the chromatograms for all four compounds in the solvent blank, extraction blank, and extracted sample can be seen in Figure 3. Additionally, in the absence of a final ruling, the EPA has suggested the Minimum Reporting Levels listed in Table 1 as guidance for the interim. Therefore, according to data quality guidelines in EPA methods, such as those found in EPA 537.1 and 533, the contamination levels are significantly below the 1/3 MRL requirement.[7,8]

Sample preparation method performance was evaluated in the 0.004 and 0.02 ng/L sample spikes by their calculated concentrations and recovery values, with results listed in Table 2. The percent recovery is an average of three replicate extracts performed for each concentration. The average recovery and %RSD values demonstrate excellent method accuracy and repeatability. Recovery for each compound at both concentration levels ranged from 90 to 111%, and RSD was within the range of 2 to 13%. This lends high confidence in reporting results for drinking water samples at these challenging trace levels.

Compound	Laboratory 1 LOQ ng/L	Laboratory 1 S:N of LOQ	Laboratory 2 LOQ ng/L	Laboratory 2 S:N of LOQ	EPA HAL ng/L	Interim minimum reporting level ng/L
PFOA	0.001	10	0.001	10	0.004 (interim)	4
PFOS	0.001	12	0.001	27	0.02 (interim)	4
PFBS	0.0009	24	0.002	27	2,000	3
HFPO-DA (GenX)	0.004	21	0.004	10	10	5

Table 1. Limit of quantitation (LOQ) and signal:noise (S:N) for each PFAS determined in both laboratories using two different TQ Absolute systems.

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels
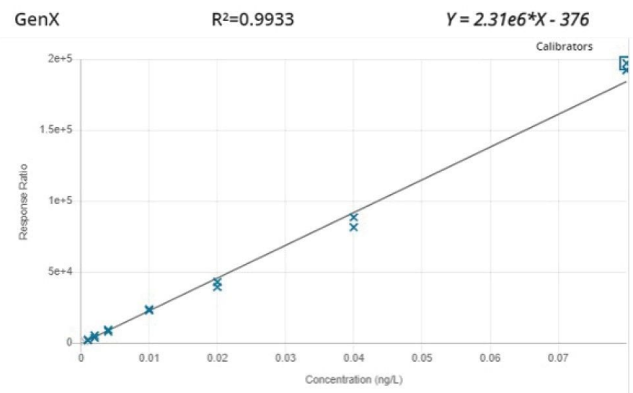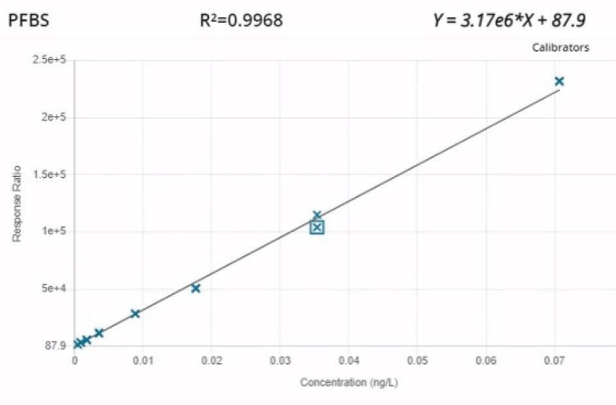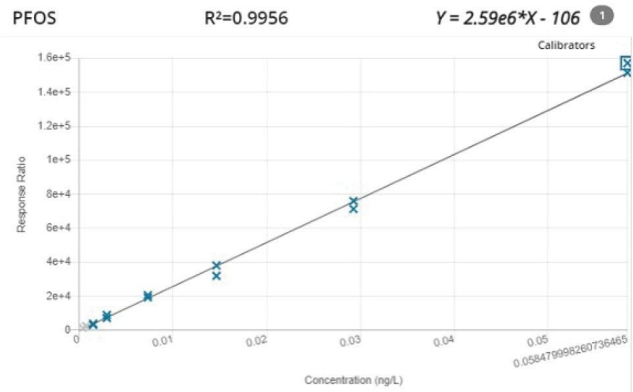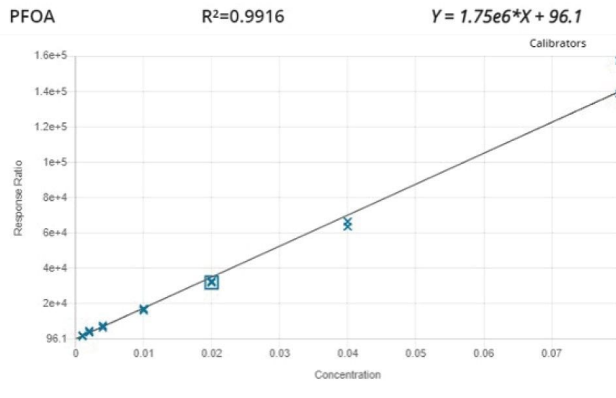
8

Figure 2. Calibration curves for PFOA, PFOS, PFBS, and GenX on the Xevo TQ Absolute.

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels
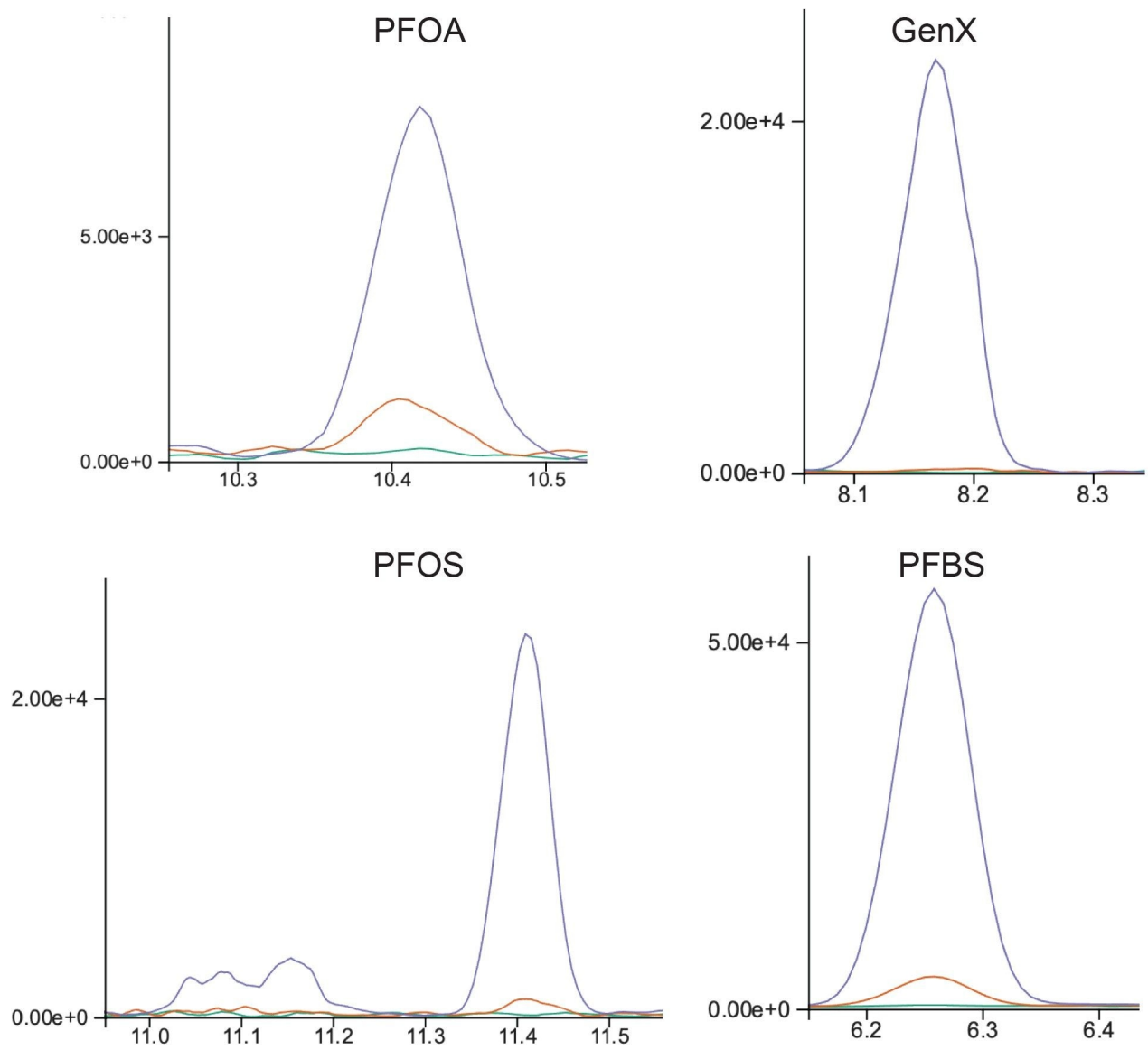
9

Figure 3. Overlaid chromatograms of PFOA (0.004 ng/L), PFOS (0.02 ng/L), PFBS (0.02 ng/L), and GenX (0.02 ng/L) demonstrating spiked sample extracts (blue) to extraction blanks (orange) and solvent blanks (green).

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

10

0.004 ng/L spike				
	Expected concentration (ng/L)	Average calculated concentration (ng/L)	% RSD	Average % recovery
PFOA	0.0040	0.0042	5	105
PFOS	0.0029	0.0030	5	103
PFBS	0.0035	0.0032	2	90
GenX	0.0040	0.0037	13	91
0.02 ng/L spike				
	Expected concentration (ng/L)	Average calculated concentration (ng/L)	% RSD	Average % recovery
PFOA	0.020	0.022	9	111
PFOS	0.015	0.014	6	94
PFBS	0.018	0.017	3	96
GenX	0.020	0.021	10	107

Table 2. Average calculated concentration, %RSD and average percent recovery (n=3) of each PFAS in water samples spiked at 0.004 and 0.02 ng/L.

Conclusion

With PFAS regulations and guidelines continuing to trend to lower detection requirements, as seen with the 2022 EPA Health Advisory Levels, it is critical to continue to advance PFAS workflows in order reach such levels. With the use of the enhanced negative ion mode sensitivity of the Xevo TQ Absolute along with sample enrichment gained by sample extraction using Oasis WAX SPE cartridges, this work has demonstrated that a typical laboratory can reach the HALs requirements from the EPA. This includes the interim sub-ppt levels for PFOA and PFOS, which were reached without additional or special resources outside the normal range of an analytical prep shared-space laboratory. The challenging factor in this analysis was demonstrated to be cleanliness during

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

11

sample preparation to limit the amount of PFAS contamination during this step. As demonstrated in this work, it may not be possible, even with the best practice and care in any laboratory, to fully eliminate all contamination, but with focus on laboratory practices and sample handling, it can be kept to a minimum that does not significantly impact the reported results. Of the four PFAS with assigned HALs, PFOA proved to be the compound that had the most background contamination, but was still within typical data quality guidelines. The SPE method using Oasis WAX cartridges was shown to be accurate and repeatable with excellent recoveries and %RSD of replicates. The full workflow presented in this study demonstrates it is possible to reliably detect challenging trace levels of PFAS, in the ppq range, in drinking water samples.

References

1. De Silva AO, Armitage JM, Bruton TA, Dassuncao C, Heiger-Bernays W, Hu XC, Kärrman A, Kelly B, Ng C, Anna Robuck, Sun M, Webster TF, Sunderland EM. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Env. Tox. & Chem.*; 2021 Mar; 40(3): 631–657.

2. United States Environmental Protection Agency, Drinking Water Health Advisories for PFOA and PFOS, epa.gov, [Internet] Accessed 14 December 2022: https://www.epa.gov/sdwa/drinking-water-health-advisories-pfoa-and-pfos.

3. United States Environmental Protection Agency, FACT SHEET PFOA & PFOS Drinking Water Health Advisories, epa.gov, [Internet] Accessed 14 December 2022: https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf.

4. European Union, EUR-Lex, Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast), [Internet] Accessed 14 December 2022: https://eur-lex.europa.eu/eli/dir/2020/2184/oj.

5. K Organtini, K Rosnack, D Stevens, E Ross. Analysis of Legacy and Emerging Perfluorinated Alkyl Substances (PFAS) in Environmental Waters Samples Using Solid Phase Extraction (SPE) and LC-MS/MS. Waters Application Note 720006471, 2019.

6. K Organtini, S Adams. Improved Sensitivity for the Detection of Per- and Polyfluoro Alkyl Substances in

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

12

Environmental Water Samples Using a Direct Injection Approach on Xevo TQ Absolute. Waters Application Note 720007559, 2022.

7. Shoemaker, J and Tettenhorst, D. Method 537.1: Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, 2018.

8. Rosenblum, L and Wendelken, S. Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry. U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water, Standards and Risk Management Division, 2019.

Appendix

Compound	Parent	Fragment	Quan	CV	CE	Internal standard	Type of internal standard
PFOA	412.9	369	x	10	10	$^{13}C_8$-PFOA	
		169		10	15		
PFBS	298.9	80.1	x	15	30	$^{13}C_3$-PFBS	
		99.1		15	30		
PFOS	498.9	80.1	x	15	40	$^{13}C_8$-PFOS	
		99.1		15	40		
GenX (HFPO-DA)	285	169	x	5	7	$^{13}C_3$-GenX	
		119		5	35		
$^{13}C_8$-PFOA	420.9	375.9	x	5	15	$^{13}C_2$-PFOA	Extraction (EIS)
		172		5	10		
$^{13}C_3$-PFBS	301.9	80.1	x	10	30	^{13}C-PFOS	Extraction (EIS)
		99.1		10	25		
$^{13}C_8$-PFOS	506.9	80.1	x	15	40	^{13}C-PFOS	Extraction (EIS)
		99.1		15	40		
$^{13}C_3$-GenX	287	169	x	5	12	$^{13}C_2$-PFOA	Extraction (EIS)
		119		5	12		
$^{13}C_2$-PFOA	415	370	x	10	10	–	Injection (IIS)
		169		10	15		
^{13}C-PFOS	503	80.1	x	5	40	–	Injection (IIS)
		99.1		5	40		

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

13

Featured Products

[ACQUITY Premier System </nextgen/br/pt/products/chromatography/chromatography-systems/acquity-premier-system.html>](</nextgen/br/pt/products/chromatography/chromatography-systems/acquity-premier-system.html>)

[Xevo TQ Absolute Triple Quadrupole Mass Spectrometry </nextgen/br/pt/products/mass-spectrometry-systems/xevo-tq-absolute.html>](</nextgen/br/pt/products/mass-spectrometry-systems/xevo-tq-absolute.html>)

[waters_connect for Quantitation </nextgen/br/pt/products/informatics-and-software/waters_connect-for-quantitation.html>](</nextgen/br/pt/products/informatics-and-software/waters_connect-for-quantitation.html>)

720007855, February 2023

Ultra-Trace Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water to Meet New US EPA Interim Health Advisory Levels

14