

Application Note

Extending PFAS Coverage and Sensitivity in a Direct Injection UPLC-MS/MS Method for Water Matrices Based on EU and UK Drinking Water Regulations

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Abstract

This application note describes a direct injection method to fulfil the requirements of a list of 47 per- and polyfluoroalkyl substances (PFAS) by the UK Drinking Water Inspectorate (DWI) and the 20 PFAS contained in the 2020 EU Drinking Water Directive (DWD) totaling 48 analytes. The work was carried out using an ACQUITY™ Premier UPLC System coupled to the XEVO™ TQ Absolute Tandem Quadrupole Mass Spectrometer using a UniSpray™ ion source. Sub-ng/L Method Detection Limits (MDLs) were achieved for all 48 analytes using a 50 µL injection volume. Method robustness was shown with validation batches analyzed over three days in drinking water, surface water and river water.

Benefits

Performing the direct injection on the XEVO TQ Absolute with UniSpray ion source allows:

- A direct injection method, suitable for the detection and quantification of a wide panel of PFAS covering both EU DWD and UK DWI regulations

- Improved productivity from reduced sample volume and preparation time
- Enhanced sensitivity allowing sub ng/L level limits of detection to exceed typical drinking water regulatory requirements for PFAS

Introduction

PFAS has been detected in the environment all around the world and are known colloquially as “forever chemicals”. They are derived from a wide range of consumer products and manufacturing processes. Some of their most common uses include water-resistant coatings on a variety of different products and firefighting foams. It is their widespread usage and toxicity, and potential to bioaccumulate that has led to their restriction.

For drinking water in the EU, a set of 20 PFAS are specified in Directive (EU) 2020/2184 on the quality of water intended for human consumption.¹ It imposes a quality-based parametric value (limit) of 0.1 µg/L for the sum of 20 specified PFAS which relates to a required limit of quantification for individual PFAS analytes of 5 ng/L and lower. For raw water sources that will ultimately be used for supplying drinking water, the Drinking Water Inspectorate (DWI) in England and Wales has outlined in a letter the requirements for the monitoring of 47 PFAS by water companies.² Maximum PFAS limits have yet to be determined in this case. The document refers to the DWI January 2021 published guidance for PFOS and PFOA in drinking water which states that water companies should use a tiered risk based approach, and test the analytes each to a level of 0.01 µg/L to ensure adequate quantification significantly below any trigger levels.³ The assumption is that raw water sources will have permissible levels similar to drinking water levels or higher.

Direct injection methods are increasingly desirable to laboratories carrying out routine analysis of PFAS where analyte preconcentration is not deemed necessary or required. They have a simple sample preparation method, which not only reduces the opportunity for contamination and error but speeds up analysis time and the requirements for large volumes of samples. This can provide labs with a higher throughput of samples and lower analysis costs. To achieve these low levels of detection without sample preconcentration, an ACQUITY Premier UPLC was coupled to the Xevo TQ Absolute with a UniSpray source. The UniSpray source gives additional sensitivity benefits, and more detail on how it achieves this can be found in previously published literature.⁴

UniSpray is an atmospheric ionization technique whereby the column effluent is nebulized as it exits a grounded capillary and then directed onto an impactor pin that is held at high voltage. This process creates smaller droplets compared to electrospray ionization (ESI), leading to an increased formation of gas phase ions and

improved ionization efficiency. Typically, PFAS have been seen to especially benefit from this type of ionization mechanism with improved response and signal to although the improvement amount varies between compounds. In order to comply with the current regulatory limits for drinking water in both the EU and England and Wales, and to ensure the method can cope with the likely lowering of those limits in the future, a target was set to achieve method detection limits (MDLs) for all the PFAS listed of 1 ng/L, in all water types.

Experimental

Background Contamination

Due to the widespread nature of PFAS in many products, background contamination is one of the biggest challenges when tackling the analysis of PFAS. It is vitally important that meticulous care is taken at every stage from sample collection and preparation, through to analysis. Installing the Waters PFAS kit reduces the background interference from the chromatographic system and solvents. The kit is comprised of PFAS-free components that replace items such as the conventional PTFE coated solvent lines with PEEK tubing. Included is an isolator column which, when installed, helps to delay any residual background interferences from co-eluting with the analytical peak. Installation of both the kit and column is straightforward and quick.⁵ A detailed account of the best practices for monitoring PFAS to avoid contamination can be found in the Waters™ White Paper.⁶

For the study samples of different types of drinking water (soft and hard) and two samples of raw water that is used to supply drinking water, were collected from sources around the UK and stored at 5 °C until analysis. Samples were prepared for analysis using the workflow found in Figure 1. Results were quantified using bracketed calibrations prepared in reagent water, with the use of internal standardized response. Internal standard concentrations in the final extract ranged from 5–50 ng/L, depending on the analyte. All the standards used were obtained from Wellington Laboratories. Information for 48 PFAS and 25 internal standards used in the application can be found in Appendix A.

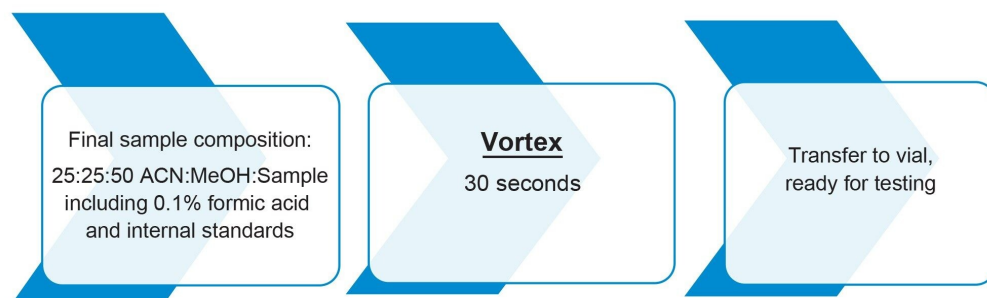


Figure 1. Preparation workflow for drinking water and environmental water samples.

LC Conditions

LC system:	ACQUITY Premier UPLC with BSM and FTN Sample Manager fitted with Waters PFAS kit and Atlantis™ Premier BEH™ C18 AX 5 µm, 2.1 x 50 mm isolator column (p/n: 186009407)
Vials:	Polypropylene 12 x 32 mm Screw Neck Vial, 700 µL (p/n: 186005219), with pre-slit PTFE/Silicone septa (p/n: 186000305)
Column:	ACQUITY Premier CSH™ C ₁₈ , 1.7 µm, 2.1 mm X 100 mm (p/n: 186009461)
Column temperature:	40 °C
Sample temperature:	15 °C
Injection volume:	50 µL
Flow rate:	0.350 mL

Mobile phase A: 2 mM ammonium acetate in water:methanol 95:5 (v/v)

Mobile phase B: 2 mM ammonium acetate in methanol

Purge solvent and seal wash: 50:50 methanol:water

Wash solvent: 90:10 methanol:water

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.35	100	0	Initial
1	0.35	80	20	6
6	0.35	55	45	6
13	0.35	20	80	6
14	0.35	5	95	6
17	0.35	5	95	6
18	0.35	100	0	1
22	0.35	100	0	1

MS Conditions

MS system: Xevo TQ Absolute

Ionization mode: UniSpray, negative ion mode

Acquisition range: MRM

Impactor voltage: 0.9 kV

Desolvation temperature: 400 °C

Desolvation gas flow: 900 L/hr

Cone gas flow: 150 L/hr

Source temperature: 110 °C

MRM Transitions

MRM parameters for each compound are listed in Appendix A.

Data Management

Informatics: waters_connect™ for quantitation

Results and Discussion

Method Optimization

When simultaneously analyzing PFAS with a wide range of functionality (including carbon chain length), a careful balance of parameters is required to obtain maximum performance for all analytes. Two factors that can make a significant impact on performance are sample composition and desolvation temperature. In the following section different aspects to optimize the methods are discussed.

Optimization of the Preparation of Samples for Analysis

The reduced solubility of long chain PFAS in water can result in precipitation of analytes or cause them to stick to the sides of vessels. To assess the recovery of analytes, samples were prepared using two different methods. For both methods the native analytes were spiked into water samples and homogenized. For method 1 an aliquot of the water was transferred into a centrifuge tube for sample preparation. For method 2 the water samples were not transferred and were prepared in the same container they were stored in. The mean recoveries, calculated using the concentration of six replicates of soft drinking water, for samples prepared using both methods are shown in Figure 2. The recovery of the longer chain analytes was found to be worse when samples were prepared using method 1. Whereas, by comparison, the recovery of the analytes from samples that were prepared using method 2, without any transferring, did not exhibit the same issues. It was determined that the

addition of organic solvent to the sample collection vessel assisted the solubility of analytes and washed any analytes from the sides of the container, resulting in improved recovery. When samples were prepared in this manner, they achieved recoveries between 83–124% for all 48 analytes.

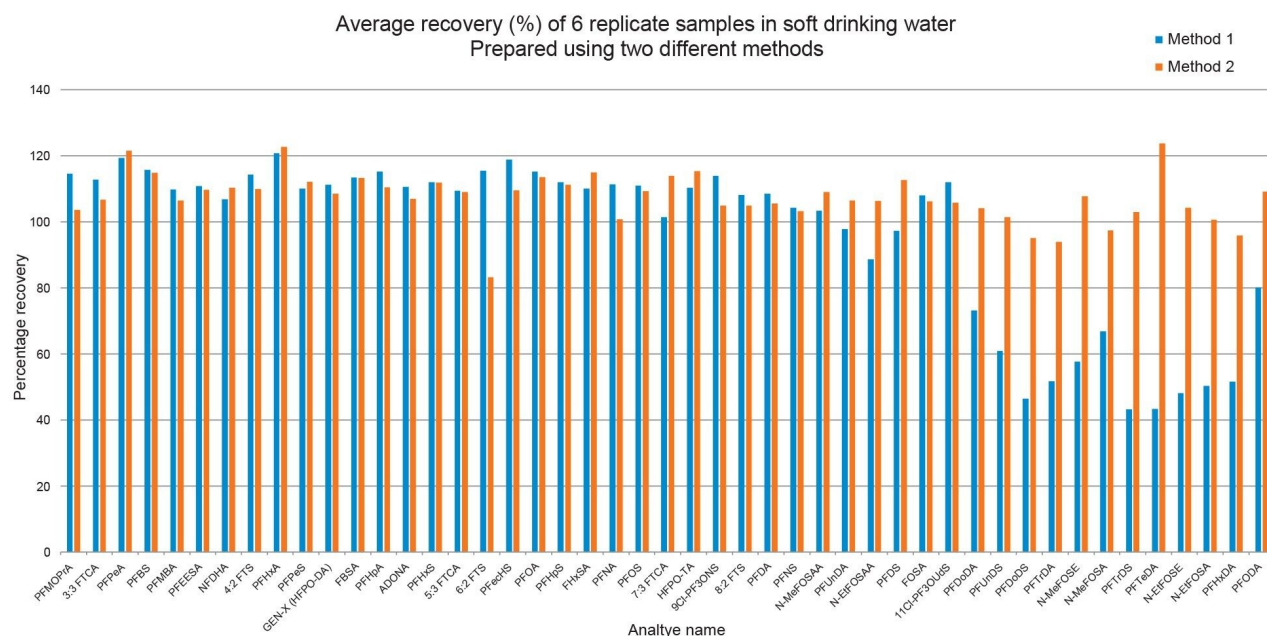


Figure 2. Average Mean recoveries (%) from six replicate samples prepared from soft drinking water by method 1 (blue) and method 2 (orange).

Effect of Sample Composition on Peak Shape

Sample composition and preparation can yield substantial improvements in both response and chromatography. The reduced solubility of long chain PFAS (carboxylic and sulfonic acids with C10 and higher) in water, is highlighted with PFHxDA and PFODA, C16 and C18 respectively. Improvements can be seen with these compounds by diluting to a higher final organic content. However, increasing the organic content above 50% showed peak shape deterioration for short chain PFAS, and adds a further dilution to the sample, making it difficult to achieve the required low levels of detection.

A compromise of 50% organic solvent sample composition, with a ratio of 25:25:50 (v/v/v) of acetonitrile:methanol:water was chosen as the optimum blend of organic solvents to yield the best solubility and therefore performance of all analytes. The addition of formic acid to the sample at a concentration of 0.1%, saw

improvements in the peak shape of early eluting analytes.

Adding sufficient time to vortex mix the sample is another key detail in sample preparation that is essential for reproducible results, specifically for long chain PFAS. The solubility data of PFAS, shows as the PFAS carbon chain increases the solubility in water decreases.⁶ Therefore, these longer chain analytes can and have a tendency to precipitate and adhere to the sides of vessels. Ensuring the analytes are dissolved through vortex mixing during sample preparation can significantly improve the reproducibility of the analytes.

Comparison of the Response for PFAS When Using Electrospray and UniSpray

A direct comparison of electrospray and UniSpray was made by the analysis of 20 repeat injections of a 10 ng/L reagent water sample, using both ionization techniques. All 48 analytes showed improved response using UniSpray, gaining on average 2 to 7-fold increase in signal. These performance gains are particularly relevant with compounds that typically struggle with sensitivity, for example GenX and PFHxDA. Figure 3 contains chromatograms of these analytes analyzed using both methods of ionization. It can be seen that GenX and PFHxDA demonstrate a 7-fold increase in response, with additional 4-fold gains in signal-to-noise (S/N), through the use of the UniSpray ion source. Additional information and background can be found in Waters Application Note [720007413](#).⁷

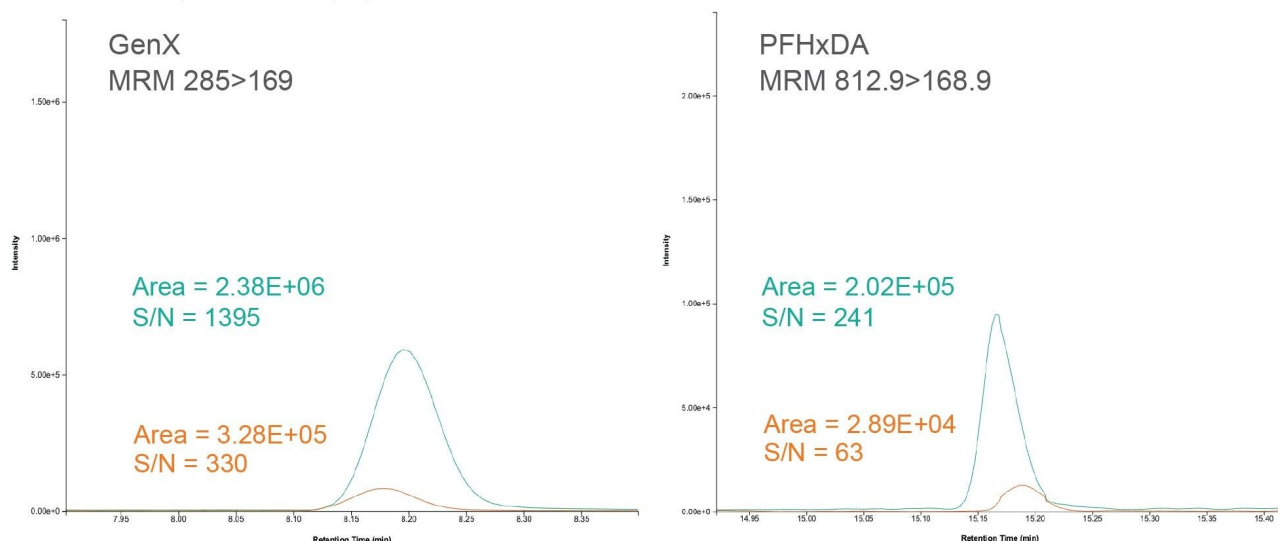


Figure 3. Signal to noise and peak response comparison of GenX and PFHxDA between electrospray (orange) and UniSpray (green) demonstrated at 10 ng/L in reagent water.

Improving the Sensitivity of Labile Compounds

MRM parameters for compounds were optimized using the waters_connect MRM optimization tool in System Control. This function automatically determines the fragment ions, cone voltage and collision energy for each compound through a direct infusion. This process is quick and simple and can significantly speed up time taken for method development.

Optimizing the desolvation temperature can yield to significant gains in response. Typically, labile PFAS favor low desolvation temperatures; this can be seen with GenX and HFPO-TA of which both are susceptible to in-source fragmentation. The overlaid chromatograms of different desolvation temperatures for four different analytes are shown in Figure 4. GenX and HFPO-TA have optimum desolvation temperatures of 350 °C, whereas the other more stable analytes favor higher temperatures. To accommodate all 48 PFAS, a compromise of 400 °C was chosen as the optimum temperature to gain the most method sensitivity overall.

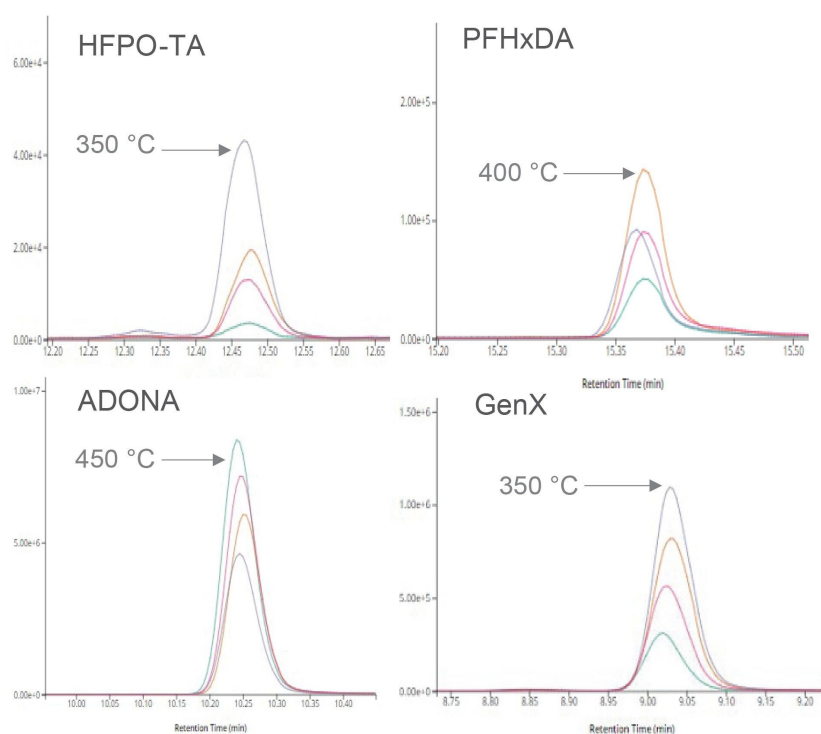


Figure 4. HFPO-TA, PFHxDA, ADONA and GenX at 350 °C (purple), 400 °C (orange), 450 °C (pink), and 500 °C (teal) Desolvation Temperatures.

Instrumental and Method Performance

Internally standardized reagent water bracketed calibrations were used throughout the whole study. Calibrations typically ranged from 0.5 ng/L to 50 ng/L, and full details can be found in Table 1. All analytes obtained R^2 values above 0.99 and residuals <20%. Example calibration lines are shown in Figure 5, where the calibrations were bracketed between 20 samples.

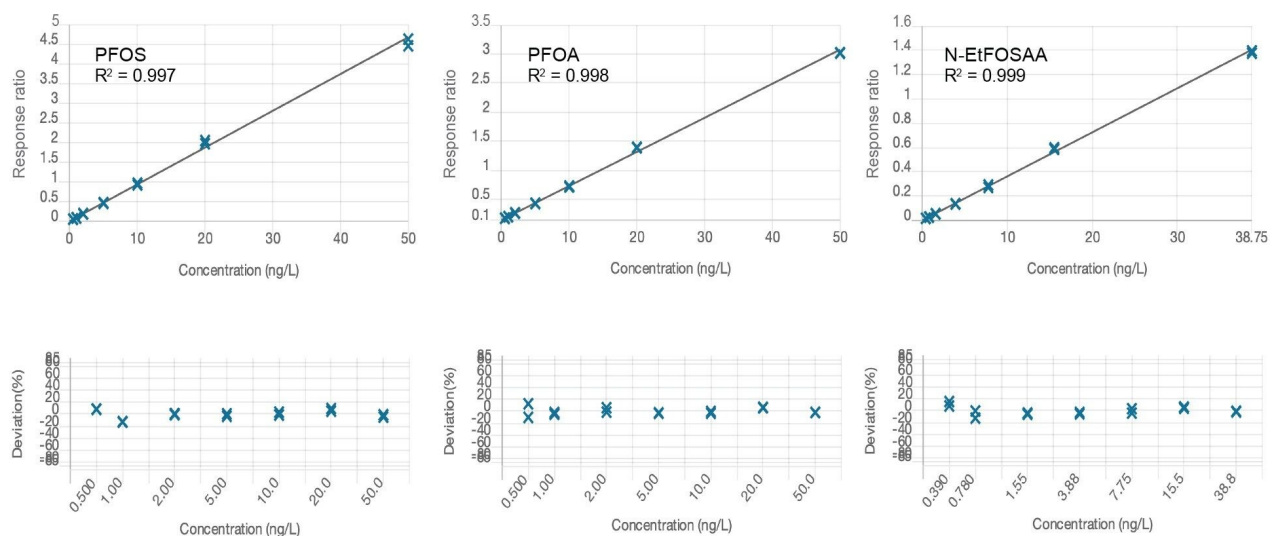


Figure 5. Bracketed calibration lines and residual deviation plots of PFOS, PFOA, and N-EtFOSAA.

Precision

To determine the precision of the method, in terms of retention time stability and repeatability of the response, an aliquot of each of the four water types was spiked at a concentration of 10 ng/L and homogenized and six replicate sub-samples prepared and analyzed each day for three days. The retention time for all the analytes shown to be stable, across the three days' of analyses ($SD \leq 0.03$ min). The repeatability from the mean concentrations from all the measurements shows all but two analytes had %RSDs of <15%, with PFOS and PFOA achieving <7% in all water types. The repeatability for PFBA in river water was worse (18%RSD), which was attributed to the increased levels of this analyte in the methanol used for sample preparation adding variation to the results as different bottles of methanol were used over the course of the three days. PFODA was an outlier for both drinking waters and the river water repeatability was >20%RSD. This is likely to be related to its limited solubility in water causing variation in the sub-samples. The values for the repeatability (%RSD) for all analytes across the three days can be found in Figure 6.

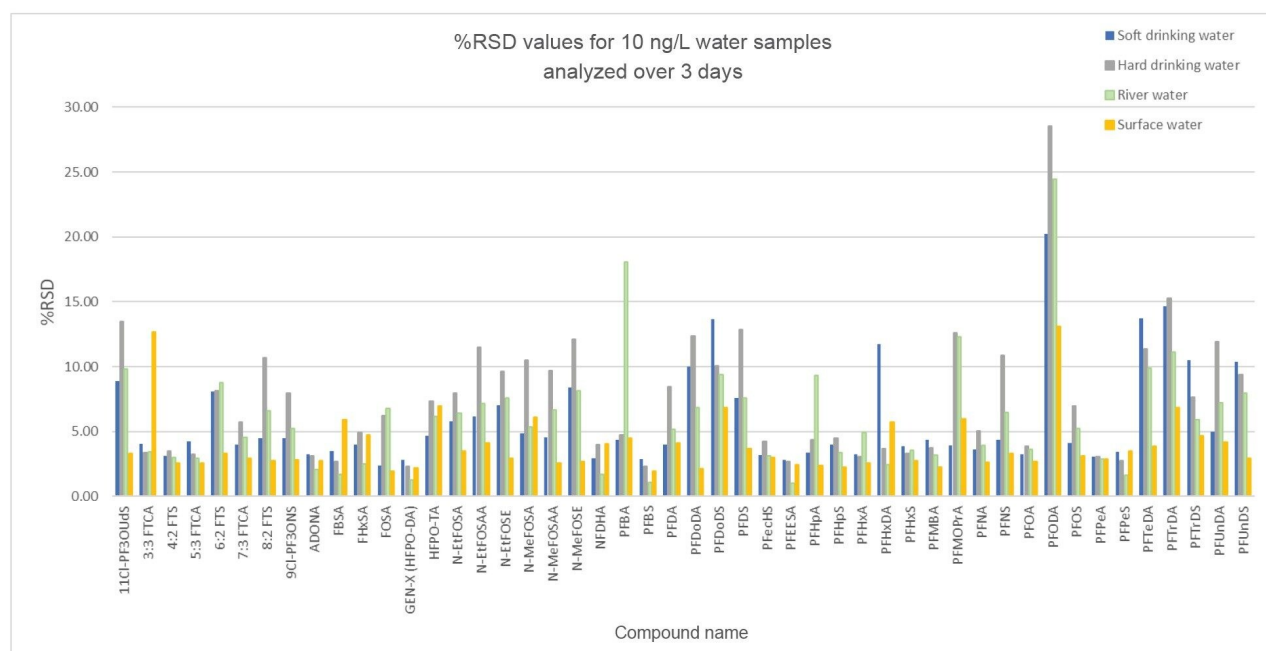


Figure 6. The repeatability (% RSD) for PFAS spiked into soft (blue) and hard (orange) drinking water, river water (green), and surface water (grey) at 10 ng/L (n=6) analyzed over three days.

Sensitivity

A Method Detection Limit (MDL) study was performed to assess the sensitivity of the method, using twenty replicate injections of a 2 ng/L spiked sample in reagent water. The MDL was calculated using the equation from EPA 821-R-16-006⁸, detailed below;

$$\text{MDL} = \text{SD} \times t_{n-1}$$

SD = standard deviation of replicates and t_{n-1} = student t value for n-1 samples

A minimum concentration of a tenth of the spiking level (0.2 ng/L) was applied to the dataset, all 48 compounds obtained sub ng/L MDLs.⁸ This surpasses the original target and showing the method is suitable to check compliance with current regulatory requirements set out in the 2020 EU DWD. The full list of MDL values can be found in Table 1. To verify these levels are achievable in real water samples, various water types were spiked and analyzed at a concentration of 0.2 ng/L. The chromatograms of FOSA, PFNA, PFUnDA, and PFTrDS are displayed in Figure 7, and confirm good chromatography and significant response above blank levels allowing for

quantification at these concentrations.

Analyte name	Calibration range (ng/L)	Spike (ng/L)	MDL (ng/L)	Analyte name	Calibration range (ng/L)	Spike (ng/L)	MDL (ng/L)
11CI-PF3OUdS	0.5–50	2	0.2	PFDODA	0.5–50	2	0.2
3:3 FTCA	0.1–10	0.4	0.2	PFDODS	0.5–50	2	0.2
4:2 FTS	0.5–50	2	0.2	PFDS	0.5–50	2	0.2
5:3 FTCA	0.5–50	2	0.2	PFecHS	0.5–50	2	0.2
6:2 FTS	0.5–50	2	0.2	PFEESA	0.5–50	2	0.2
7:3 FTCA	0.5–50	2	0.2	PFHpA	0.5–50	2	0.2
8:2 FTS	0.5–50	2	0.3	PFHpS	0.5–50	2	0.2
9CI-PF3ONS	0.5–50	2	0.2	PFHxA	0.5–50	2	0.2
ADONA	0.5–50	2	0.2	PFHxDA	0.5–50	2	0.2
FBSA	0.5–50	2	0.2	PFHxS	0.5–50	2	0.2
FHxSA	0.5–50	2	0.2	PFMBA	0.5–50	2	0.2
FOSA	0.5–50	2	0.2	PFMOPrA	0.5–50	2	0.2
GEN-X (HFPO-DA)	0.5–50	2	0.2	PFNA	0.5–50	2	0.2
HFPO-TA	0.5–50	5	0.8	PFNS	0.5–50	2	0.2
N-EtFOSA	0.05–5	0.2	0.2	PFOA	0.5–50	2	0.2
N-EtFOSAA	0.5–50	2	0.2	PFODA	0.5–50	2	0.2
N-EtFOSE	0.5–50	2	0.2	PFOS	0.5–50	2	0.2
N-MeFOSA	0.05–5	0.2	0.2	PFPeA	0.5–50	2	0.2
N-MeFOSAA	0.5–50	2	0.2	PFPeS	0.5–50	2	0.2
N-MeFOSE	0.5–50	2	0.2	PFTeDA	0.5–50	2	0.5
NFDHA	0.5–50	2	0.2	PFTTrDA	0.5–50	2	0.2
PFBA	0.5–50	2	0.3	PFTTrDS	0.5–50	2	0.2
PFBS	0.5–50	2	0.2	PFUnDA	0.5–50	2	0.2
PFDA	0.5–50	2	0.2	PFUnDS	0.5–50	2	0.2

Table 1. Method detection limits (MDLs) for all analytes calculated using the standard deviation of 20 replicate injections of a 2 ng/L standard in reagent water.

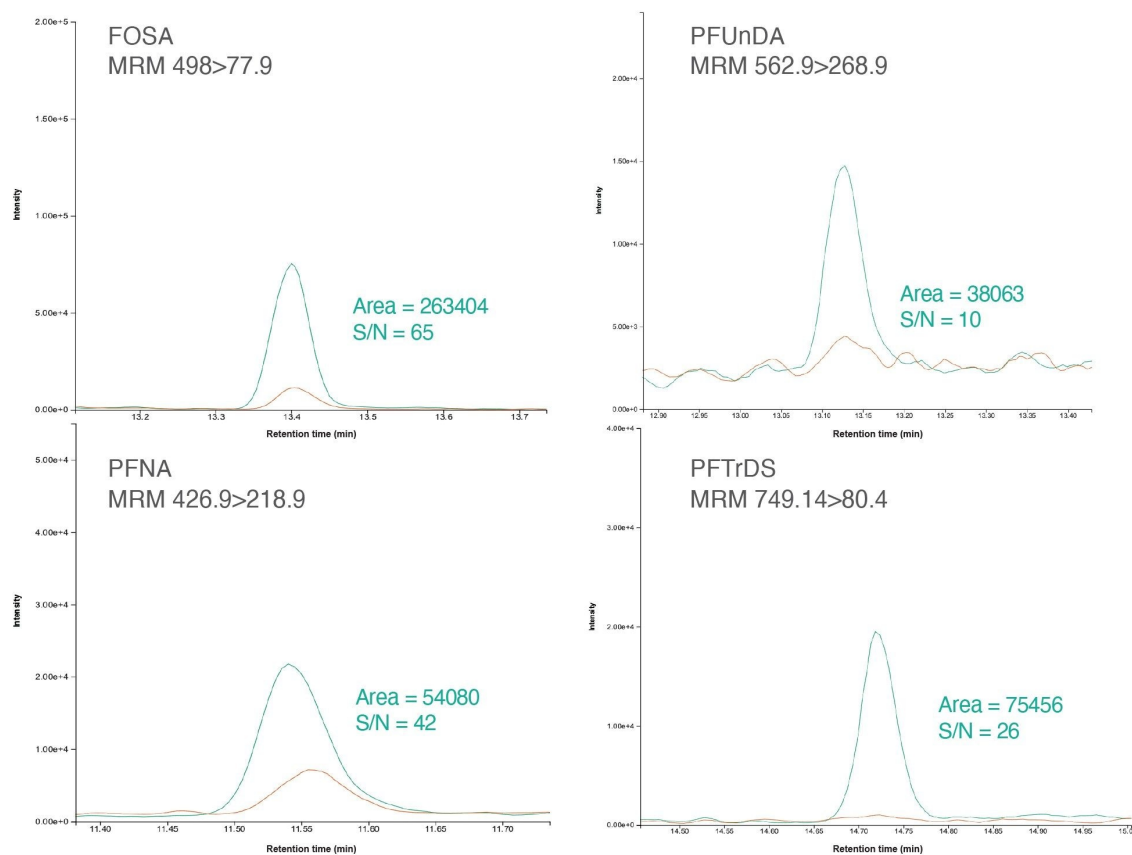


Figure 7. The overlaid chromatograms of 0.2 ng/L spiked samples (green) of FOSA and PFNA in river water and PFUnDA and PFTTrDS in surface water with their associated blank samples (orange).

The ability to resolve and quantify branched and linear forms of PFAS at low concentrations is shown in Figure 8, whereby a chromatogram of a reagent water sample containing 0.73 ng/L linear and 0.20 ng/L branched PFOS demonstrates that at sub ng/L concentrations, the linear and branched forms are quantifiable and clearly resolved from each other.

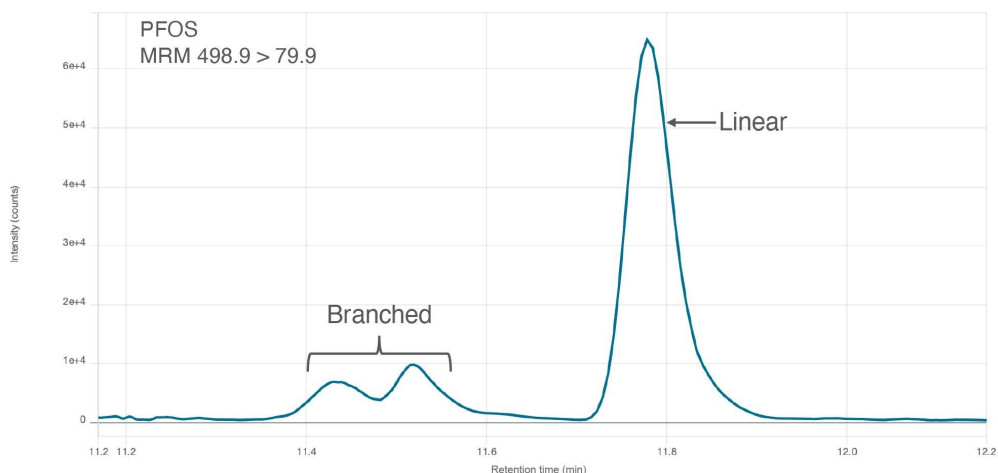


Figure 8. Detection of branched and linear forms of PFOS spiked into a reagent water sample at concentrations of 0.73 ng/L and 0.20 ng/L, respectively.

Conclusion

New regulations drive the demand for more sensitive methods with greater analytical scope to cope with extended lists of PFAS of interest. The work carried out here showed the required sensitivity gained by using Xevo TQ Absolute fitted with the UniSpray ion source can be used to check compliance with current regulations, using direct injection without the use of any preconcentration steps. The method performance study in various types of drinking and raw water from across the United Kingdom demonstrates a robust analytical method delivering repeatable results, with consistent retention of all analytes to meet and exceed regulatory requirement.

The optimized method concluded that;

- All 48 analytes achieved sub ng/L MDLs, showing that the method is suitable for checking compliance with current regulations including the EU Drinking Water Directive 2020/2184.
- Enhanced sensitivity was demonstrated through improved peak area response and S/N compared to ESI through using the UniSpray ion source.
- Reduction in overall systemic background with the use of the PFAS kit and special isolator column, allowing

trace level detection of PFAS.

Appendix A. MRM transitions of the analytes and respective isotopically labelled internal standards.

Analyte name	Parent ion	Product ion	Cone voltage	Collision energy	Retention time
PFBA	213.0	169.0	10	10	3.42
		19.0	10	14	
¹³ C ₄ -PFBA	217.0	172.0	7	8	3.42
PFMOPrA	228.9	84.9	13	10	4.25
3:3 FTCA	241.0	63.1	13	6	5.55
		177.0	13	8	
PFPeA	262.9	218.9	10	10	5.70
¹³ C ₅ -PFPeA	268.0	223.0	11	7	5.83
PFMBA	278.9	85.0	15	12	6.41
		234.9	15	6	
PFBS	299.1	80.1	15	27	6.44
		99.1	15	27	
¹³ C ₃ -PFBS	301.9	80.1	34	28	6.50
		99.1	34	24	
PFEESA	314.9	83.0	15	20	7.31
		135.0	15	20	
NFDHA	294.9	85.0	5	20	7.66
		201.0	5	10	
4:2 FTS	326.9	80.9	42	27	7.81
		306.9	42	18	
¹² C ₂ -4:2FTS	329.0	309.0	14	18	7.81
		80.9	14	21	
¹² C ₆ -PFHxA	318.0	273.0	10	6	7.99
		120.0	10	18	
PFHxA	312.9	118.9	5	22	7.99
		268.9	5	10	
PFPeS	348.9	79.9	32	31	8.35
		98.9	32	25	
¹³ C3-HFPO-DA	287.0	168.9	10	8	8.50
		184.9	10	18	
GEN-X (HFPO-DA)	285.0	119.0	12	35	8.50
		169.0	12	7	
FBSA	297.9	78.0	25	25	8.60
		118.9	25	15	
PFHpA	362.9	168.9	15	15	9.61
		318.9	15	10	
¹³ C ₄ -PFHpA	367.0	322.0	16	7	9.61
		172.0	16	15	
ADONA	376.9	84.9	12	22	9.75
		251.0	12	10	
PFHxS	398.9	80.1	10	35	9.81
		99.1	10	30	
¹³ C ₃ -PFHxS	402.0	80.1	13	38	9.81
		99.1	13	30	
5:3 FTCA	340.9	217.0	13	26	9.87
		237.0	13	13	
6:2 FTS	427.0	80.1	12	32	10.73
		406.9	12	22	
¹² C ₂ -6:2FTS	429.0	409.0	48	21	10.73
		80.9	48	27	
PFecHS	460.9	99.1	44	22	10.77
		380.9	44	22	
PFOA	412.9	168.9	10	18	10.81
		369.0	10	10	
¹² C ₈ -PFOA	421.0	376.0	6	8	10.81
		172.0	6	16	
PFHpS	448.9	79.9	15	35	10.92
		98.9	15	35	
FHxSA	398.0	78.1	30	25	11.70
		169.0	30	25	
¹³ C ₉ -PFNA	472.0	172.0	7	18	11.81
		223.0	7	18	
PFNA	462.9	218.9	10	15	11.81
		418.9	10	10	
¹³ C ₈ -PFOS	507.0	80.1	36	34	11.88
		99.1	36	34	
PFOS	498.9	79.9	30	40	11.88
		98.9	30	40	
7:3 FTCA	440.8	316.9	18	20	12.09
		336.9	18	13	

Analyte name	Parent ion	Product ion	Cone voltage	Collision energy	Retention time
HFPO-TA	494.7	119.1	40	42	12.09
		184.9	40	12	
9Cl-PF3ONS	531.0	82.9	14	25	12.39
		351.0	14	22	
¹³ C ₂ -8:2FTS	529.0	509.0	20	27	12.62
		80.9	20	37	
8:2 FTS	526.9	80.9	28	37	12.62
		506.9	28	26	
PFNS	548.9	80.1	20	40	12.66
		99.1	20	40	
¹³ C ₆ -PFDA	519.0	473.9	25	7	12.66
		219.0	25	13	
PFDA	512.9	218.9	15	18	12.66
		468.9	15	10	
N-MeFOSAA	569.9	168.9	36	30	12.95
		418.9	36	22	
d3-N-MeFOSAA	573.0	418.9	17	18	13.05
		515.0	17	18	
PFDS	598.9	80.1	25	50	13.35
		99.1	25	46	
PFUnDA	562.9	268.9	25	20	13.37
		518.9	25	10	
¹³ C ₇ -PFUnDA	569.9	524.9	9	8	13.37
		273.9	9	14	
N-EtFOSAA	584.0	418.9	34	15	13.37
		525.9	34	18	
d5-N-EtFOSAA	589.0	418.9	24	17	13.37
		482.9	24	13	
FOSA	498.0	77.9	40	30	13.60
		83.0	40	30	
¹³ C ₈ -FOSA	506.0	77.9	13	28	13.60
11Cl-PF3OUdS	631.0	82.9	30	30	13.72
		450.9	30	26	
¹³ C ₂ -PFDoDA	615.0	569.9	23	10	13.95
		168.9	23	22	
PFDoDA	612.9	168.9	30	25	13.97
		568.9	30	12	
PFUnDS	649.1	80.0	40	55	13.98
		99.0	40	55	
PFTrDA	662.9	168.9	5	30	14.50
		218.9	5	20	
PFDoDS	699.1	80.0	40	55	14.51
		99.0	40	55	
d7 N-MeFOSE	623.0	59.1	18	13	14.75
		168.9	18	30	
N-MeFOSE	615.9	59.1	23	14	14.81
N-MeFOSA	511.8	168.9	38	26	14.83
		219.0	38	26	
d-N-MeFOSA	515.0	168.9	34	21	14.83
		218.9	34	21	
PFTrDS	749.1	80.0	40	55	14.94
		99.0	40	55	
¹³ C ₂ -PFTeDA	715.0	168.9	18	25	14.96
		219.0	18	25	
PFTeDA	712.9	168.9	14	30	14.96
		668.8	14	14	
N-EtFOSA	525.8	169.0	38	28	15.05
		219.0	38	26	
d9 N-EtFOSE	639.0	59.1	18	13	15.12
		188.9	18	76	
N-EtFOSE	630.0	59.1	18	13	15.12
		605.0	18	13	
d-N-EtFOSA	531.0	168.9	15	25	15.17
		218.9	15	23	
PFHxDA	812.9	168.9	22	28	15.38
		218.9	22	22	
¹³ C ₂ -PFHxDA	814.8	769.9	23	15	15.38
		169.0	23	32	
PFODA	912.9	169.2	34	35	15.59
		868.9	34	15	

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