Application note | 002902

Environmental

Direct injection of drinking water for the analysis of 54 PFAS compounds by LC-MS/MS aligned with current and evolving global regulations

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Keywords

Direct injection, PFAS, drinking water, European regulation

Application benefits

- A simple, reproducible, and robust sample preparation based on the dilution of the sample with mobile phase B followed by direct injection for reducing labor, errors, and potential contamination issues compared to standard methods requiring pre-concentration with solid phase extraction.
- A complete PFAS workflow solution using direct injection of 100 µL of water samples that meets challenging regulatory detection and reporting limits within the EU and UK.
- Dedicated instrumental setup and appropriate sample handling procedures have been established to overcome the main challenges of this application which are sensitivity, contamination, and carryover handling.

Goal

To demonstrate the quantitative measurement of 54 per- and polyfluoroalkyl substances (PFAS) in water samples by direct injection with precision and accuracy that meets current European regulatory reporting requirements on the Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer.

Introduction

Per- and polyfluoroalkyl substances are a large, diverse group of synthetic compounds containing chains of linked carbon and fluorine atoms. Due to the ubiquitous use of PFAS, environmental exposure can come from numerous sources, including household dust, food, and drinking water,[1] and has been linked to a variety of health effects[2]. To evaluate the health risk, an expanding list of governmental regulatory agencies have established validated methods stating the quantitative levels for individual or collective PFAS. In Europe, the main law regulating the quality of drinking water is the drinking water directive (2020/2184/EU) that sets two thresholds for PFAS as follows: 0.1 µg/L for the sum of a group of 20 PFAS, and 0.5 µg/L for the PFAS total, which means the totality of per- and polyfluoroalkyl substances. In the case of natural waters, they are regulated under the Water Framework Directive (WFD, 2000/60/EC), the Environmental Quality Standards Directive (EQSD, 2008/105/EC), and the Groundwater Directive (GWD, 2006/118/EC). Table 1 summarizes some of the European[3] and national[4-7] regulations for analysis of PFAS in drinking water.

Table 1. PFAS compounds and thresholds set in European and national regulations

Compound	CAS number	Europe	France	Italy	UK	Belgium	In the method
Perfluorobutanoic acid (PFBA)	375-22-4	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluoropentanoic acid (PFPeA)	2706-90-3	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorohexanoic acid (PFHxA)	307-24-4	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluoroheptanoic acid (PFHpA)	375-85-9	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorooctanoic acid (PFOA)	335-67-1	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorononanoic acid (PFNA)	375-95-1	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorodecanoic acid (PFDA)	335-76-2	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluoroundecanoic acid (PFUdA)	2058-94-8	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorododecanoic acid (PFDoDA)	307-55-1	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorotridecanoic acid (PFTrDA)	72629-94-8	Sum 0.1 µg/L			< 0.01 µg/L	0.05 µg/L	*
Perfluorobutane sulfonic acid (PFBS)	375-73-5	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluoropentane sulfonic acid (PFPeS)	2706-91-4	Sum 0.1 µg/L			< 0.01 µg/L	0.01 µg/L	*
Perfluorohexane sulfonic acid (PFHxS)	355-46-4	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluoroheptane sulfonic acid (PFHpS)	375-92-8	Sum 0.1 µg/L		0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorooctane sulfonic acid (PFOS)	1763-23-1	Sum 0.1 µg/L	0.002 µg/L	0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
Perfluorononane sulfonic acid (PFNS)	68259-12-1	Sum 0.1 µg/L			< 0.01 µg/L	0.01 µg/L	*
Perfluorodecane sulfonic acid (PFDS)	335-77-3	Sum 0.1 µg/L	0.002 µg/L		< 0.01 µg/L	0.01 µg/L	*
Perfluoroundecane sulfonic acid (PFUnDS)	749786-16-1	Sum 0.1 µg/L			< 0.01 µg/L	0.05 µg/L	*
Perfluorododecane sulfonic acid (PFDoS)	79780-39-5	Sum 0.1 µg/L			< 0.01 µg/L	0.05 µg/L	*
Perfluorotridecane sulfonic acid (PFTrDS)	79156-89-8	Sum 0.1 µg/L				0.05 µg/L	*
4:2 Fluorotelomer sulfonate (4:2FTS)	757124-72-4			0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
6:2 Fluorotelomer sulfonate (6:2FTS)	27619-97-2			0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
8:2 Fluorotelomer sulfonate (8:2FTS)	39108-34-4			0.005 µg/L	< 0.01 µg/L	0.01 µg/L	*
10:2 Fluorotelomer sulfonate (10:2FTS)	120226-60-0					0.05 µg/L	*
Hexafluoropropylene oxide dimer acid (HFPO-DA or GenX)	13252-13-6			0.025 µg/L	< 0.01 µg/L	0.01 µg/L	*
Difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy} acetic acid (cC6O4)	1190931-41-9 1190931-27-1			0.04 µg/L			
Perfluorotetradecanoic acid (PFTeDA)	376-06-7				< 0.01 µg/L	0.01 µg/L	*
Perfluorohexadecanoic acid (PFHxDA)	67905-19-5				< 0.01 µg/L	0.01 µg/L	*
Perfluorooctadecanoic acid (PFOcDA)	16517-11-6				< 0.01 µg/L	0.05 µg/L	*
Hexafluoropropylene oxide trimer acid (HFPO-TA)	13252-14-7				< 0.01 µg/L		*
4,8-dioxa-3H-perfluorononanoic acid (ADONA)	919005-14-4				< 0.01 µg/L	0.01 µg/L	*

Table 1 (continued). PFAS compounds and thresholds set in European and national regulations

Compound	CAS number	Europe	France	Italy	UK	Belgium	In the method
Perfluoro-4-oxapentanoic acid (PFMPA or PF4OPeA)	377-73-1				< 0.01 µg/L		*
Perfluoro-3,6-dioxaheptanoic acid (NFDHA or 3,6-OPFHpA)	151772-58-6				< 0.01 µg/L		*
Perfluoro(4-methoxybutanoic) acid (PFMBA or PF5HxA)	863090-89-5				< 0.01 µg/L		*
Perfluoroethylcyclohexane Sulfonate (PFECHS)	133201-07-7				< 0.01 µg/L	0.01 µg/L	*
3:3 Fluorotelomer Carboxylic Acid (3:3 FTCA)	356-02-5				< 0.01 µg/L		*
5:3 Fluorotelomer Carboxylic Acid (5:3 FTCA)	914637-49-3				< 0.01 µg/L		*
7:3 Fluorotelomer carboxylic acid (7:3 FTCA)	812-70-4				< 0.01 µg/L		*
Perfluoro(2-ethoxyethane)sulphonic acid (PFEESA)	113507-82-7				< 0.01 µg/L		*
9-Chlorohexadecafluoro-3-oxanone-1-sulfonate (9CL-PF3ONS)	756426-58-1				< 0.01 µg/L		*
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	763051-92-9				< 0.01 µg/L		*
Perfluorobutanesulfonamide (FBSA)	30334-69-1				< 0.01 µg/L	0.01 µg/L	*
N-methylperfluoro-1-butanesulfonamide (N-MeFBSA)	68298-12-4					0.01 µg/L	*
N-methylperfluoro-1-butanesulfonamidoacetic acid (N-MeFBSAA)	159381-10-9					0.01 µg/L	*
Perfluorohexanesulfonamide (FHxSA)	41997-13-1				< 0.01 µg/L	0.01 µg/L	*
Perfluorooctanesulfonamide (FOSA)	754-91-6				< 0.01 µg/L	0.01 µg/L	*
N-Methylperfluoro-1-octanesulfonamide (N-MeFOSA)	31506-32-8				< 0.01 µg/L	0.01 µg/L	*
N-Ethylperfluoro-1-octanefulfonamide (N-EtFOSA)	4151-50-2				< 0.01 µg/L	0.01 µg/L	*
N-Methylperfluorooctanesulfonamidoethanol (MeFOSE)	24448-09-7				< 0.01 µg/L		*
N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE)	1691-99-2				< 0.01 µg/L		*
N-Methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA)	2355-31-9				< 0.01 µg/L	0.01 µg/L	*
N-Ethylperfluorooctanesulfonamidoacetic acid (N-EtFOSAA)	2991-50-6				< 0.01 µg/L	0.01 µg/L	*
bis(1H,1H,2H,2H-perfluorodecyl)phosphate (8:2diPAP)	678-41-1					0.01 µg/L	*
(1H,1H,2H,2H-perfluorooctyl-1H,1H,2H,2H-perfluorodecyl)phosphate (6:2/8:2diPAP)	943913-15-3					0.05 µg/L	*
bis(1H,1H,2H,2H-perfluorooctyl)phosphate (6:2diPAP)	57677-95-9					0.05 µg/L	*
Perfluorooctane sulfonamidoacetic acid (PFOSAA)	2806-24-8					optional 0.05 µg/L	
Mono[2-(perfluorohexyl)ethyl] phosphate (6:2 PAP)	57678-01-0					optional 0.05 µg/L	
Mono[2-(perfluorooctyl)ethyl] phosphate (8:2 PAP)	57678-03-2					optional 0.05 µg/L	
Total number of compounds in the regulation:		**20**	**7**	**18**	**47**	**45**	**54**

A common ground for all the cited regulations is that the sample preparation is not fixed and could be performed either by dilution, solid phase extraction, or any other sample preparation protocol. Direct injection of water samples for the analysis of PFAS has the advantage of reducing the risk of contamination during the sample handling and simplifies the workflow. An application note was published previously with a direct injection approach applied to U.S. EPA Method 8327 for ground, surface, and waste waters in which direct injection volumes (25 µL) were used following filtration[8]. To respond to the European regulations, a LC-MS/MS method has been developed for the analysis of 54 PFAS compounds in drinking waters using direct injection. The entire workflow, including a Standard Operation Procedure (SOP) that details hardware and consumables, chromatographic separation, and MS detection with full acquisition and data processing within the Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS), helps streamline routine PFAS analysis, and therefore accelerates lab throughput and productivity. Method performance was evaluated on its applicability to PFAS quantitation outlined in the diverse European regulations for drinking water (Table 1), based on Limit of Quantification (LOQ), linear dynamic range from LOQ (0.1 ng/L to 5 ng/L depending on the compounds) up to 100 ng/L, recovery, and precision.

Experimental

Instrumental method

A chromatographic method of 23 minutes was used for the analysis of 54 PFAS in drinking water samples using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system, coupled to a Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer equipped with a HESI ionization probe. To handle the 100 µL injection volume, a strong solvent loop was added to the fluidics between the autosampler and analytical column. To prevent the issues of solubility of long chain PFAS, a user-defined injection program was included in the method to shake the samples before each injection. Considering the low limits of detection required in the different European regulations, a Thermo Scientific™ Acclaim™ 120 C18, 50 x 2.1 mm, 2.2 µm column (P/N 068981) was used as a delay column to prevent potential contamination coming from solvent and system. The analytical separation was performed with an Acclaim 120 C18, 150 x 2.1 mm, 3 µm analytical column (P/N 059130) heated at 40 °C. Gradient elution was performed with water (phase A) and methanol containing 2 mM ammonium acetate and 0.1% of acetic acid (phase B) at a flow rate of 400 µL/min.

Acquisition was performed using selected reaction monitoring (SRM) in negative mode. The spray voltage was set at 500 V, sheath gas was set to 40 arb, auxiliary gas was set to 10 arb, and ion transfer tube and vaporizer temperatures were set to 200 °C and 300 °C, respectively. Table 2 summarizes the monitored SRM transitions.

Table 2. Monitored SRM transitions

Compound	RT	SRM quantitation	SRM confirmation
PFBA	6.46	212.98 / 168.97	168.97 / 168.97
$^{13}C_4$-PFBA	6.46	217.00 / 172.00	
PF4OPeA	6.97	229.00 / 85.00	229.00 / 185.00
PFPeA	7.82	262.98 / 219.04	
$^{13}C_5$-PFPeA	7.82	268.00 / 223.00	
PFBS	8	298.94 / 79.96	298.94 / 98.96
$^{13}C_3$-PFBS	8	302.00 / 80.00	
PF5HxA	8.2	279.00 / 85.00	279.00 / 235.00
PFEESA	8.51	314.95 / 135.00	314.95 / 83.00
3,6-OPFHpA	8.85	295.00 / 201.00	295.00 / 85.00
$^{13}C_2$-4:2FTS	8.95	329.00 / 309.00	
4:2FTS	8.95	327.00 / 307.00	327.00 / 81.00
PFHxA	9.06	312.97 / 268.97	312.97 / 119.04
$^{13}C_5$-PFHxA	9.06	318.00 / 273.00	
PFPeS	9.15	348.94 / 80.04	348.94 / 99.00
HFPO-DA	9.37	285.00 / 169.00	285.00 / 185.00
FBSA	9.53	298.00 / 78.00	298.00 / 63.80
3:3 FTCA	9.56	241.00 / 177.00	241.00 / 117.00
N-Me-FBSAA	10	369.97 / 282.97	369.97 / 218.97
PFHpA	10.13	362.97 / 319.04	362.97 / 168.97
$^{13}C_4$-PFHpA	10.13	367.00 / 322.00	
PFHxS	10.16	398.94 / 79.96	398.94 / 98.96
$^{13}C_3$-PFHxS	10.16	402.00 / 80.00	
ADONA	10.22	377.00 / 251.00	377.00 / 85.00
N-MeFBSA	10.82	311.88 / 218.88	311.88 / 64.80
PFECHS	10.92	460.93 / 99.00	460.93 / 381.00
6:2FTS	11.02	427.00 / 407.00	427.00 / 81.00
$^{13}C_2$-6:2FTS	11.02	429.00 / 409.00	
PFHpS	11.04	448.93 / 80.01	448.93 / 98.97
$^{13}C_8$-PFOA	11.05	421.00 / 376.00	
PFOA	11.05	412.97 / 369.04	412.97 / 169.00
FHxSA	11.6	398.24 / 77.80	398.24 / 377.88
5:3 FTCA	11.79	341.00 / 217.00	341.00 / 237.00
$^{13}C_8$-PFOS	11.81	507.00 / 80.00	
PFOS	11.81	498.93 / 79.96	498.93 / 98.96
PFNA	11.83	462.96 / 418.97	462.96 / 219.01
$^{13}C_9$-PFNA	11.83	472.00 / 427.00	
HFPO-TA	12	184.92 / 118.97	184.92 / 184.92

Table 2 (continued). Monitored SRM transitions

Compound	RT	SRM quantitation	SRM confirmation
9Cl-PF3ONS	12.18	530.90 / 350.95	532.90 / 352.95
PFNS	12.48	548.93 / 80.07	548.93 / 98.97
$^{13}C_6$-PFDA	12.51	519.00 / 474.00	
PFDA	12.51	512.96 / 469.04	512.96 / 269.04
8:2FTS	12.54	527.00 / 507.00	527.00 / 81.00
$^{13}C_2$-8:2FTS	12.54	529.00 / 509.00	
$^{13}C_8$-FOSA	13.05	506.00 / 78.00	
PFDS	13.05	598.92 / 80.04	598.92 / 98.93
FOSA	13.05	497.95 / 78.00	497.95 / 169.00
PFUdA	13.09	562.96 / 518.97	562.96 / 219.00
$^{13}C_7$-PFUdA	13.09	570.00 / 525.00	
d_3-N-MeFOSAA	13.17	573.00 / 419.00	
N-MeFOSAA	13.17	570.00 / 419.00	570.00 / 483.00
7:3 FTCA	13.27	441.00 / 317.00	441.00 / 337.00
11Cl-PF2OUdS	13.31	630.90 / 450.94	632.90 / 452.94
d_5-N-EtFOSAA	13.53	589.00 / 419.00	
N-EtFOSAA	13.53	584.00 / 419.00	584.00 / 526.00
PFUnDS	13.55	648.94 / 80.00	648.94 / 99.00
$^{13}C_2$-PFDoA	13.6	615.00 / 570.00	
PFDoA	13.6	612.95 / 569.00	612.95 / 169.03
10:2FTS	13.64	627.00 / 607.00	627.00 / 81.00
N-MeFOSE	13.85	616.00 / 59.00	
N-MeFOSA	13.86	511.96 / 169.00	511.96 / 219.00
PFDoDS	13.98	698.90 / 80.00	698.90 / 99.00
PFTrDA	14.04	662.95 / 619.04	662.95 / 168.97
N-EtFOSE	14.18	630.00 / 59.00	
N-EtFOSA	14.2	525.98 / 169.00	525.98 / 219.00
6:2diPAP	14.22	789.00 / 443.00	789.00 / 97.00
PFTrDS	14.37	748.92 / 80.00	748.92 / 98.80
PFTeDA	14.42	713.00 / 669.00	713.00 / 169.00
$^{13}C_2$-PFTeDA	14.42	715.00 / 670.00	
6:2/8:2diPAP	14.78	889.00 / 97.00	889.00 / 543.00
PFHxDA	15.07	812.91 / 768.88	812.91 / 168.97
8:2diPAP	15.24	989.00 / 543.00	989.00 / 97.00
PFOcDA	15.6	912.91 / 868.88	912.91 / 218.55

Sample preparation

To minimize PFAS contamination during method development and sample preparation, specific reagents, solvents, and consumables are specified to be used. These are listed in Table 3.

Target and isotopically labeled PFAS standard mixtures and individual standards, when not available in a mixture, in methanol at 1,000 µg/L were purchased from Wellington Laboratories. The mixture of internal standards used for this workflow was a 19-compound mix from Wellington Laboratories with the reference MPFAC-24ES. A stock solution of the 54 target PFAS compounds was prepared in methanol at a concentration of 5 µg/L. Spiking solutions were then prepared in methanol and further used to spike calibration solutions in 50:50 water:mobile phase B with concentrations of 0.1–100 ng/L. The water used for the calibrators was UHPLC-MS grade. Mobile phase B used for the calibrators and the sample preparation was spiked with internal standard mixture at a concentration of 20 ng/L.

Samples of tap water and bottled water were stored in the dark at 4 °C in Thermo Scientific™ Nunc™ 50 mL PP centrifuge tubes. For both water matrices, recovery was studied by spiking the matrix at three different concentration levels (5, 25, and 75 ng/L). For the analysis, 300 µL of sample were mixed with 300 µL of internal standard in mobile phase B solution and vortex mixed for 30 seconds. Vials were then placed in the autosampler for analysis.

Data analysis

All LC-MS/MS data were acquired and processed using the Chromeleon Chromatography Data System (CDS), version 7.3.2. A *.cmbx file is included with the workflow that contains all the optimized SRM transitions for the 54 native and associated labeled PFAS, with software view settings for easy data review and templates to allow laboratories to generate reports for a given regulation.

Table 3. Consumables and reagents used in the method

Pipette tips, sample vials, and caps	Thermo Scientific™ SureSTART™ 2 mL polypropylene screw top microvials for <2 mL samples, Level 1 everyday analysis	17303923
	9 mm Autosampler clear polypropylene vial screw thread caps	13246409
	Thermo Scientific™ Nunc™ 50 mL conical sterile polypropylene centrifuge tubes	339653
	Thermo Scientific™ Finntip™ extended length pipette tips 1,000	11759855
	Thermo Scientific™ Finntip™ Flex™ pipette tips 200	11803440
General reagents	Water, UHPLC-MS, 1 L, Thermo Scientific™	15339865
	Ammonium acetate, Optima™ LC/MS grade, 50 g, Fisher Chemical™	11317490
	Acetic acid, Optima™ LC/MS grade, 10 x 1 mL ampules, Fisher Chemical™	11377540
	ChromaCare™ LC-MS Instrument Flush Solution, Thermo Scientific™	15893187
	Methanol, UHPLC-MS, 1 L, Thermo Scientific™	15319865

Results and discussion

Method development

The method was developed to handle the wide variety of compounds currently in the European regulations. The compounds in this method include carbon chain lengths from 4 up to 20 carbons, and they are separated with reversed-phase chromatography across the gradient. Different sample compositions were tested to retain both short and long chained PFAS when injecting 100 µL of sample. In the final sample composition with 50% of mobile phase B, if samples or standards are allowed to sit in the LC vials, some long-chain PFAS compounds may settle, precipitate, or adsorb on the surface. To ensure a homogenous solution and optimum results, a User Defined Program was performed to shake the samples automatically before injection. This easy and flexible custom injection program expands the possibilities of liquid handling in the Vanquish autosampler. A strong solvent loop between the autosampler and the analytical column was included to obtain better retention and peak shape for the short-chained PFAS[9]. The resulting chromatogram for all the compounds is presented in Figure 1; some specific chromatograms from early, mid, and late eluting compounds among the different chemical classes are illustrated in Figure 2.

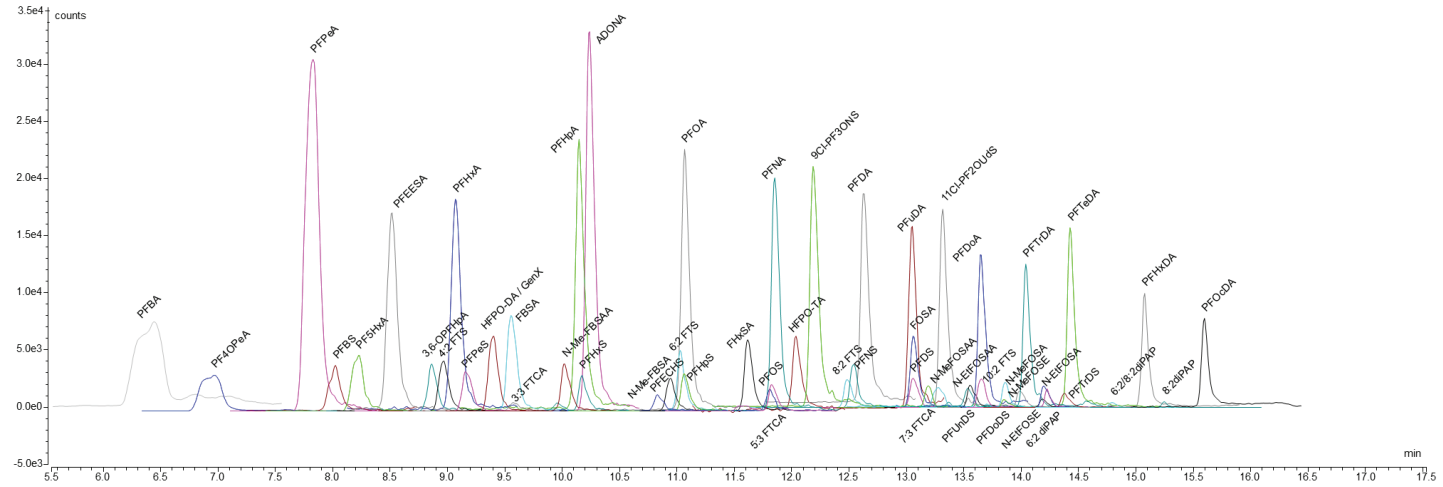

Figure 1. Chromatogram of the 54 compounds in a 5 ng/L calibration standard

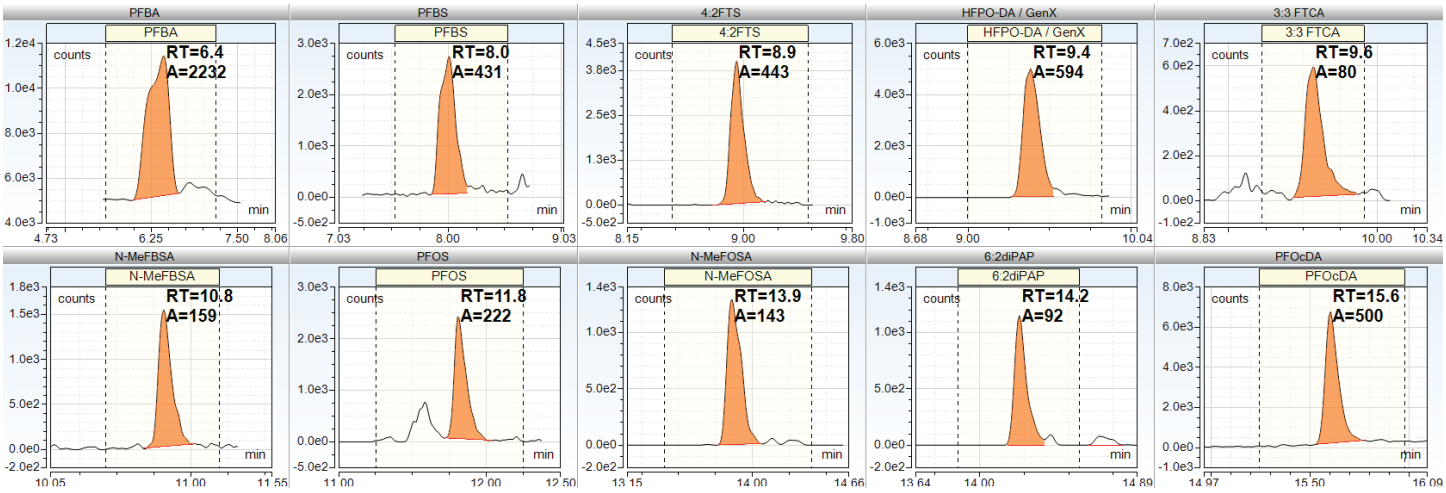

Figure 2. Example chromatograms for early, mid, and late eluting compounds in a 5 ng/L tap water sample

A delay column was used to handle any potential contamination coming from tubing and mobile phases, which is one of the main challenges faced during PFAS analysis. This column has a strong retention of the analyzed compounds and separates the analytes coming from the system away from the peaks coming from the sample. Also, the use of very simple sample preparation as well as the careful selection of the materials gives rise to considerably low levels of contamination in the blank samples. Considering both the low detection limits required for PFAS analysis and the high sensitivity of the TSQ Altis Plus mass spectrometer, having a low contamination level in the blank sample is essential. The resulting blank chromatograms for some regularly found contaminants as well as a low calibration level are presented in Figure 3.

In Figure 3, two types of blanks are presented: a system blank resulting from a run without any injection of sample, and a method blank that corresponds to the injection of a non-spiked water sample. System blank chromatograms show the separation of system peaks (coming from tubing, mobile phases, etc.) from analytical peaks, and we confirm the efficiency of the delay column to handle this contamination. As for the method blank, it has low levels of contamination for a few PFAS (e.g., PFBA, 6:2 FTS). The signal of the low calibrator is at least three times higher than the signal in the method blank. These results were considered for the calculation of LOQs that need to be at least three times the signal of the blank. With observed low levels of contamination, no background subtraction was needed, while accuracy and precision remained within the 30% limits. However, in the case of higher contamination levels, blank subtraction can be easily implemented within the software.

Linearity and sensitivity

Excellent linearity was obtained for the compounds from the lowest calibrator, corresponding to the LOQ, up to 100 ng/L, with correlation coefficients greater than 0.99 for all transitions and the respective residuals within 20% of the nominal values. A few exceptions can be noted for the fluorotelomer sulfonic acids (x:2FTS) that have a linear behavior up to 50 ng/L but quadratic after this point. Variability in the signal of PFOcDA was observed despite the use of vial rack shaking, which could be related to poor solubility of this long chain component. For this specific compound, the %RSD were higher but still below 30%. Examples of calibration curves are illustrated in Figure 4.

The LOQ values were obtained based on the concentration level for which both reproducibility and accuracy criteria were set as <30% based on 7 injections over 3 days. In practice, however, in terms of reproducibility, 81% of compounds were below 20%, while for accuracy, 93% of all PFAS were within the range of 80–120%. Then, the Method Detection Limits (MDL) were evaluated based on the injection of replicates (n=7) for the LOQ level. Table 4 presents the linearity and sensitivity results obtained for the 54 compounds of this method.

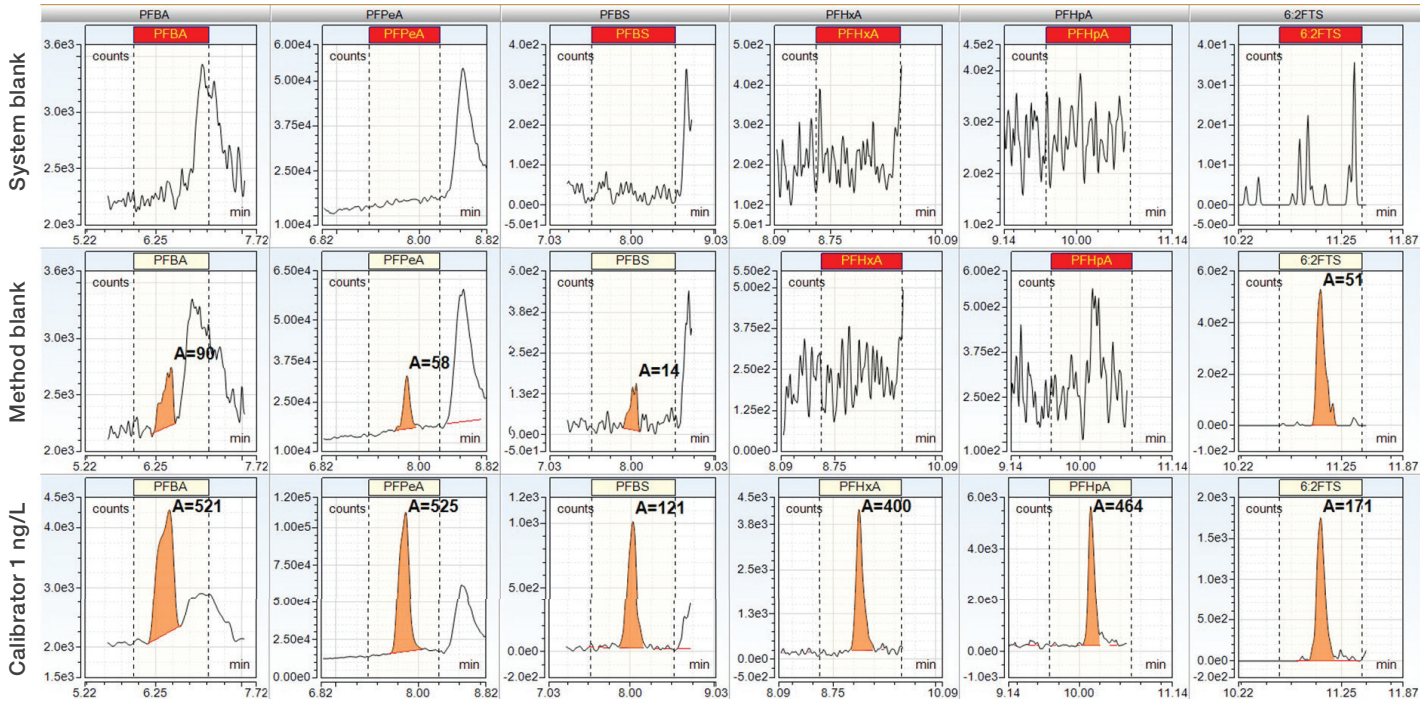

Figure 3. Selected chromatograms for a system blank, method blank, and a 1 ng/L calibration standard

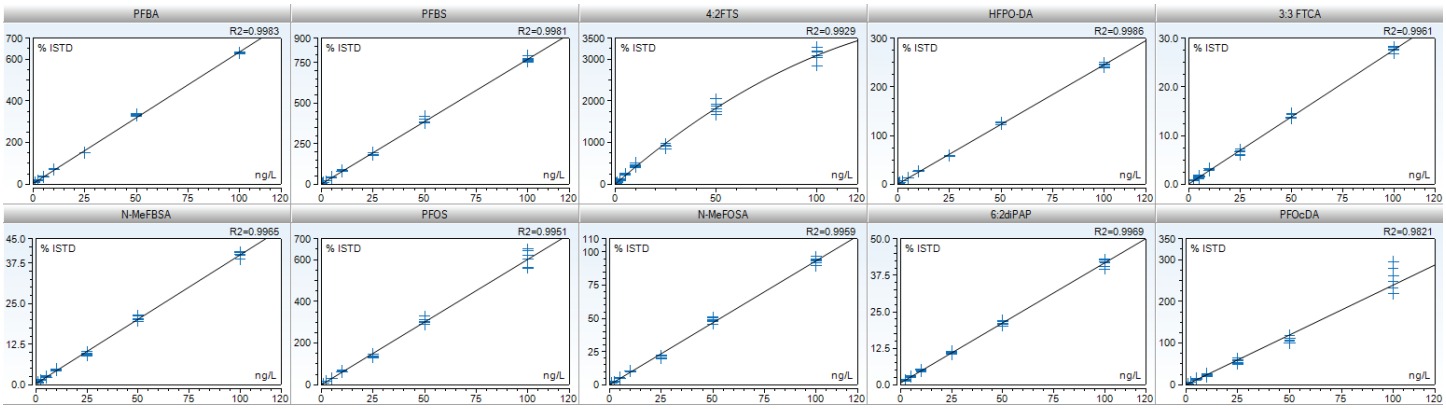

Figure 4. Calibration curves for some of the studied PFAS (for seven replicates)

Table 4. Linearity and sensitivity results obtained for the 54 compounds

Compound	Type	ISTD	MDL (ng/L)	LOQ (ng/L)	Accuracy LOQ (%)	RSD LOQ (%)	Linearity range (ng/L)	R^2
PFBA	Lin, 1/A	$^{13}C_4$-PFBA	0.21	1	99	7	1–100	0.9983
PF4OPeA	Lin, 1/A	$^{13}C_4$-PFBA	0.05	0.1	87	22	0.1–100	0.9973
PFPeA	Lin, 1/A	$^{13}C_5$-PFPeA	0.63	1	101	5	1–100	0.9990
PFBS	Lin, 1/A	$^{13}C_3$-PFBS	0.12	0.5	106	8	0.5–100	0.9981
PF5HxA	Lin, 1/A	$^{13}C_3$-PFBS	0.11	0.25	100	15	0.25–100	0.9950
PFEESA	Lin, 1/A	$^{13}C_3$-PFBS	0.19	0.25	116	22	0.25–100	0.9958
3,6-OPFHpA	Lin, 1/A	$^{13}C_3$-PFBS	0.26	1	94	10	1–100	0.9939
4:2FTS	Quad, 1/A	$^{13}C_2$-4:2FTS	0.16	0.5	103	11	0.5–100	0.9929
PFHxA	Lin, 1/A	$^{13}C_5$-PFHxA	0.11	0.25	112	13	0.25–100	0.9961
PFPeS	Lin, 1/A	$^{13}C_5$-PFHxA	0.1	0.5	108	7	0.5–100	0.9969
HFPO-DA	Lin, 1/A	$^{13}C_5$-PFHxA	0.23	0.5	90	16	0.5–100	0.9986
FBSA	Lin, 1/A	$^{13}C_4$-PFHpA	0.15	0.25	108	19	0.25–100	0.9961
3:3 FTCA	Lin, 1/A	$^{13}C_5$-PFHxA	1.11	2.5	100	15	2.5–100	0.9961
N-Me-FBSAA	Lin, 1/A	$^{13}C_5$-PFHxA	0.52	0.5	128	28	0.5–100	0.9962
PFHpA	Lin, 1/A	$^{13}C_4$-PFHpA	0.17	0.5	112	11	0.5–100	0.9957
PFHxS	Lin, 1/A	$^{13}C_3$-PFHxS	0.21	0.5	111	13	0.5–100	0.9956
ADONA	Lin, 1/A	$^{13}C_3$-PFHxS	0.03	0.1	118	10	0.1–100	0.9951
N-MeFBSA	Lin, 1/A	$^{13}C_4$-PFHpA	0.77	2.5	97	10	2.5–100	0.9965
PFECHS	Lin, 1/A	$^{13}C_3$-PFHxS	0.06	0.1	71	29	0.1–100	0.9956
6:2FTS	Quad, 1/A	$^{13}C_2$-6:2FTS	0.56	1	88	22	1–100	0.9908
PFHpS	Lin, 1/A	$^{13}C_3$-PFHxS	0.21	0.5	104	15	0.5–100	0.9958
PFOA	Lin, 1/A	$^{13}C_8$-PFOA	0.07	0.25	115	8	0.25–100	0.9956
FHxSA	Lin, 1/A	$^{13}C_8$-PFOA	0.08	0.25	105	11	0.25–100	0.9955
5:3 FTCA	Lin, 1/A	$^{13}C_8$-PFOA	0.29	0.5	106	18	0.5–100	0.9963
PFOS	Lin, 1/A	$^{13}C_8$-PFOS	0.16	0.5	94	13	0.5–100	0.9951
PFNA	Lin, 1/A	$^{13}C_9$-PFNA	0.13	0.25	103	17	0.25–100	0.9957
HFPO-TA	Lin, 1/A	d_3-N-MeFOSAA	0.39	0.5	103	24	0.5–100	0.9954
9Cl-PF3ONS	Lin, 1/A	$^{13}C_9$-PFNA	0.06	0.1	119	18	0.1–100	0.9968
PFNS	Lin, 1/A	$^{13}C_9$-PFNA	0.31	0.5	119	18	0.5–100	0.9943
PFDA	Lin, 1/A	$^{13}C_6$-PFDA	0.15	0.25	107	19	0.25–100	0.9952

Table 4 (continued). Linearity and sensitivity results obtained for the 54 compounds

Compound	Type	ISTD	MDL (ng/L)	LOQ (ng/L)	Accuracy LOQ (%)	RSD LOQ (%)	Linearity range (ng/L)	R^2
8:2FTS	Quad, 1/A	M2-8:2FTS	0.32	1	90	12	1–100	0.9951
PFDS	Lin, 1/A	$^{13}C_7$-PFUdA	0.6	1	106	20	1–100	0.9958
FOSA	Lin, 1/A	$^{13}C_8$-FOSA	0.17	0.25	91	26	0.25–100	0.9948
PFUdA	Lin, 1/A	$^{13}C_7$-PFUdA	0.12	0.25	106	15	0.25–100	0.9962
N-MeFOSAA	Lin, 1/A	d_3-N-MeFOSAA	0.44	0.5	124	26	0.5–100	0.9910
7:3 FTCA	Lin, 1/A	$^{13}C_2$-PFDoA	1.2	2.5	98	17	2.5–100	0.9923
11Cl-PF2OUdS	Lin, 1/A	$^{13}C_2$-PFDoA	0.07	0.1	113	20	0.1–100	0.9957
N-EtFOSAA	Lin, 1/A^2	d_5-N-EtFOSAA	1.02	2.5	97	14	2.5–100	0.9708
PFUnDS	Lin, 1/A	$^{13}C_2$-PFDoA	0.48	1	100	17	1–100	0.9948
PFDoA	Lin, 1/A	$^{13}C_2$-PFDoA	0.23	0.25	116	28	0.25–100	0.9948
10:2FTS	Lin, 1/A^2	d_5-N-EtFOSAA	0.55	1	98	19	1–100	0.9719
N-MeFOSE	Lin, 1/A	$^{13}C_2$-PFDoA	1.69	2.5	109	20	2.5–100	0.9907
N-MeFOSA	Lin, 1/A	$^{13}C_2$-PFDoA	0.36	1	103	11	1–100	0.9959
PFDoDS	Lin, 1/A	$^{13}C_2$-PFDoA	0.38	1	98	15	1–100	0.9905
PFTrDA	Lin, 1/A	$^{13}C_2$-PFDoA	0.09	0.25	102	12	0.25–100	0.9920
N-EtFOSE	Lin, 1/A	$^{13}C_2$-PFDoA	1.91	2.5	97	13	2.5–100	0.9904
N-EtFOSA	Lin, 1/A	$^{13}C_2$-PFDoA	0.91	2.5	97	13	2.5–100	0.9916
6:2diPAP	Lin, 1/A	$^{13}C_2$-PFTeDA	1.76	5	103	11	5–100	0.9969
PFTrDS	Lin, 1/A	$^{13}C_2$-PFTeDA	0.64	2.5	111	8	2.5–100	0.9918
PFTeDA	Lin, 1/A	$^{13}C_2$-PFTeDA	0.11	0.25	124	12	0.25–100	0.9949
6:2/8:2diPAP	Lin, 1/A	$^{13}C_2$-PFTeDA	2.19	5	107	2	5–100	0.9951
PFHxDA	Lin, 1/A	d_3-N-MeFOSAA	0.15	0.5	95	10	0.5–100	0.9955
8:2diPAP	Lin, 1/A	$^{13}C_2$-PFTeDA	3.26	5	118	18	5–100	0.9646
PFOcDA	Lin, 1/A	$^{13}C_2$-PFTeDA	0.31	0.5	82	24	0.5–100	0.9821

The sensitivity obtained for this direct injection method is consistent with the requirements from different European and local regulations.

Sample analysis

To evaluate the performance and robustness of this analytical method, tap water and bottled water samples were spiked at low, medium, and high concentration levels (5, 25, and 75 ng/L) for the recovery analysis of the developed analytical method. Results obtained in both tap water and bottled water were within 30% for both average recovery and %RSD (n=7). The corresponding data are presented in Figure 5 and Table 5.

Both water samples contained small amounts of PFAS, but even for the sum of all the positive compounds, the observed concentrations were below the regulated levels. As for the recovery and relative standard deviation for the three concentration levels, all compounds and levels were found within a limit of 30%. The use of 19 internal standards seems to be adequate for the studied matrices and compounds. This allows the use of calibrators in UHPLC-MS grade water for determination of PFAS in drinking water. For more complex matrices it would be recommended to include additional internal standards, especially in the case of phosphate compounds, and considering the presence of some particulate matter, long-chain compounds could experience stronger adsorption effects.

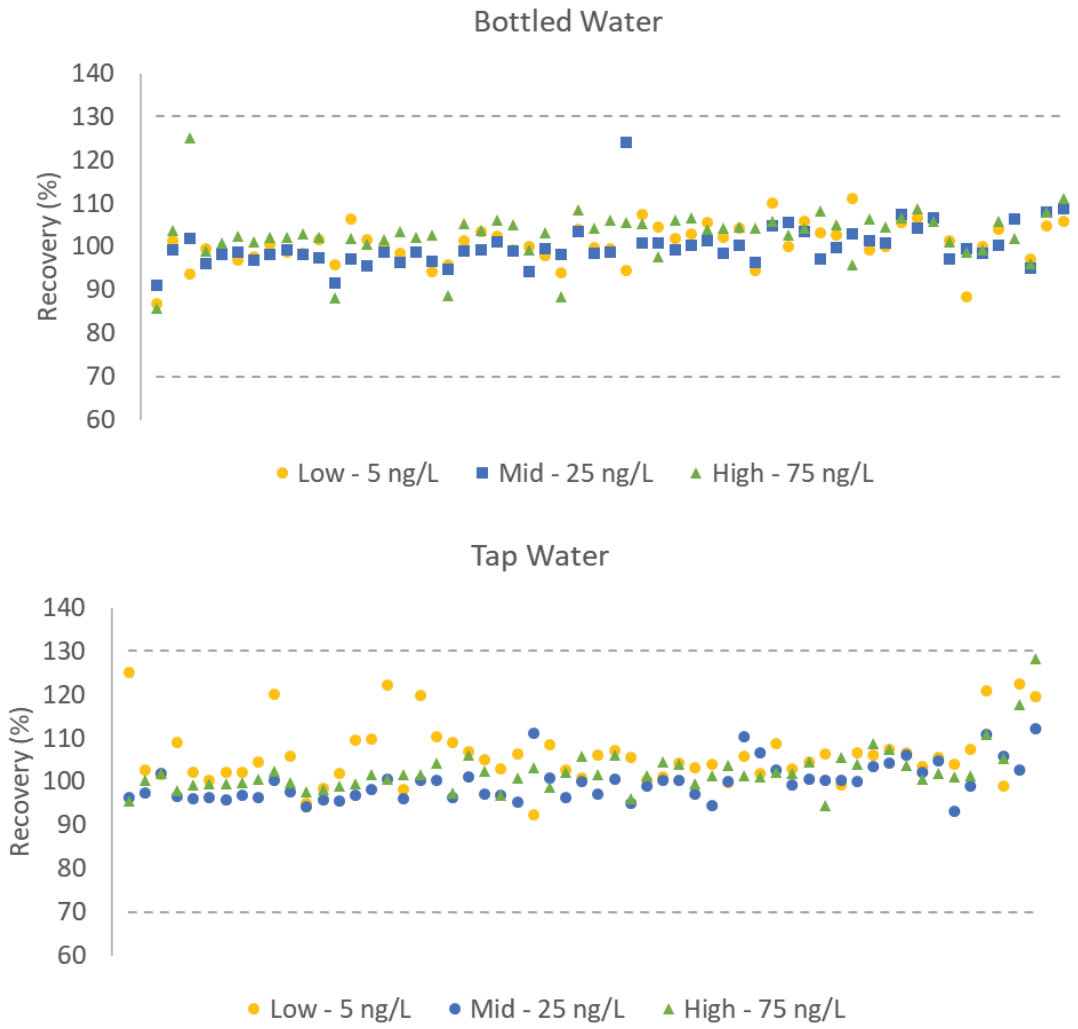

Figure 5. Mean recoveries (n=7) of all 54 analyzed PFAS in spiked bottled (A) and tap (B) water, at low (5 ng/L), mid (25 ng/L), and high (75 ng/L) levels

Table 5. Recovery data for tap water and bottled water samples spiked at low, medium, and high concentration levels with the developed analytical method

	Tap water						
	Non spiked	Low - 5 ng/L		Mid - 25 ng/L		High - 75 ng/L	
Compound	Concentration (ng/L)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)
PFBA	1.54	125	6.3	96	9.2	96	4.5
PF4OPeA	<LOQ	103	1.7	97	4.6	100	3.8
PFPeA	<LOQ	102	0.2	102	0.5	102	0.3
PFBS	<LOQ	109	2.5	97	6.0	98	3.8
PF5HxA	<LOQ	102	4.2	96	3.5	99	3.1
PFEESA	<LOQ	102	4.9	96	4.4	100	2.7
3,6-OPFHpA	<LOQ	102	5.2	97	5.9	100	4.0
4:2FTS	<LOQ	105	7.3	96	5.6	101	7.6
PFHxA	1.16	120	2.1	100	4.0	102	4.2
PFPeS	0.14	106	4.0	98	4.7	100	4.1
HFPO-DA	<LOQ	96	3.3	95	1.6	98	1.8
FBSA	<LOQ	102	3.2	95	4.2	99	4.6

Table 5 (continued). Recovery data for tap water and bottled water samples spiked at low, medium, and high concentration levels with the developed analytical method

	Tap water						
	Non spiked	Low - 5 ng/L		Mid - 25 ng/L		High - 75 ng/L	
Compound	Concentration (ng/L)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)
3:3 FTCA	<LOQ	99	10.8	96	6.6	98	4.5
N-Me-FBSAA	0.53	109	8.0	97	5.1	99	4.6
PFHpA	0.6	110	2.4	98	3.8	102	3.8
PFHxS	0.84	122	7.3	100	5.1	101	5.3
ADONA	<LOQ	98	3.7	96	5.0	102	4.2
N-MeFBSA	<LOQ	120	11.7	100	2.2	102	2.9
PFECHS	<LOQ	110	8.2	100	3.6	104	7.6
6:2FTS	<LOQ	109	3.8	96	6.9	97	5.1
PFHpS	<LOQ	107	4.5	101	4.3	106	4.2
PFOA	0.3	105	2.3	97	5.1	102	3.2
FHxSA	<LOQ	103	3.2	97	5.7	97	6.3
5:3 FTCA	<LOQ	106	5.1	95	7.3	101	4.8
PFOS	0.52	108	6.7	101	3.4	99	6.4
PFNA	<LOQ	103	2.8	96	5.6	102	3.7
HFPO-TA	<LOQ	92	11.3	109	6.2	104	5.5
9Cl-PF3ONS	<LOQ	101	4.1	100	4.7	106	3.1
PFNS	<LOQ	107	6.7	101	6.1	106	4.3
PFDA	<LOQ	100	3.9	99	4.4	101	3.8
8:2FTS	<LOQ	106	7.2	95	8.7	96	4.3
PFDS	<LOQ	104	6.5	100	6.6	104	3.1
FOSA	<LOQ	101	2.0	100	4.8	104	6.0
PFUdA	<LOQ	103	4.4	97	5.8	99	2.9
N-MeFOSAA	<LOQ	103	6.7	99	9.0	102	7.1
7:3 FTCA	<LOQ	104	14.4	94	7.7	101	5.8
11Cl-PF2OUdS	<LOQ	100	4.7	100	2.4	104	3.8
N-EtFOSAA	<LOQ	102	20.0	107	9.2	101	8.0
PFUnDS	<LOQ	109	11.3	103	3.7	102	8.0
PFDoA	<LOQ	105	4.9	101	3.4	105	6.6
10:2FTS	<LOQ	106	7.7	100	6.2	94	9.8
N-MeFOSE	0.88	106	15.6	110	7.4	101	5.3
N-MeFOSA	<LOQ	107	7.8	100	5.5	104	6.2
PFDoDS	<LOQ	106	9.0	104	7.9	109	7.5
PFTrDA	<LOQ	107	6.8	104	7.7	107	7.3
N-EtFOSE	<LOQ	107	8.8	106	5.5	104	6.1
N-EtFOSA	<LOQ	103	6.9	102	7.2	101	7.2
6:2diPAP	<LOQ	106	11.1	105	6.2	102	2.0
PFTrDS	<LOQ	104	7.9	93	10.2	101	4.8
PFTeDA	<LOQ	107	5.3	99	2.8	101	4.1
6:2/8:2diPAP	<LOQ	121	10.3	111	6.4	111	7.0
PFHxDA	<LOQ	99	7.4	106	5.6	105	4.2
8:2diPAP	<LOQ	120	8.3	112	9.8	128	4.3
PFOcDA	<LOQ	122	3.4	103	5.9	118	4.6

Table 5 (continued). Recovery data for tap water and bottled water samples spiked at low, medium, and high concentration levels with the developed analytical method

	Bottled water						
	Non Spiked	Low - 5 ng/L		Mid - 25 ng/L		High - 75 ng/L	
Compound	Concentration (ng/L)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)
PFBA	<LOQ	87	3.4	91	0.7	86	1.0
PF4OPeA	<LOQ	101	4.1	99	1.5	104	3.3
PFPeA	<LOQ	94	1.5	102	1.4	125	0.3
PFBS	<LOQ	99	3.8	96	3.7	99	5.9
PF5HxA	<LOQ	99	5.6	98	3.2	101	4.1
PFEESA	<LOQ	98	4.0	97	3.5	101	4.1
3,6-OPFHpA	<LOQ	101	8.9	98	4.0	102	5.1
4:2FTS	<LOQ	99	11.4	99	6.3	102	10.4
PFHxA	<LOQ	98	6.1	98	2.1	103	4.3
PFPeS	<LOQ	102	6.0	97	3.7	102	4.6
HFPO-DA	<LOQ	96	3.6	92	1.3	88	1.6
FBSA	0.29	102	7.4	96	3.6	101	6.5
3:3 FTCA	<LOQ	106	15.8	97	5.8	102	4.3
N-Me-FBSAA	<LOQ	100	7.6	99	3.6	102	4.6
PFHpA	<LOQ	98	7.2	96	2.3	103	4.9
PFHxS	<LOQ	99	7.4	99	3.2	102	4.5
ADONA	<LOQ	94	6.8	97	2.6	103	5.0
N-MeFBSA	<LOQ	96	10.2	95	6.4	89	2.0
PFECHS	<LOQ	101	6.6	99	2.8	105	6.2
6:2FTS	<LOQ	104	4.3	99	5.0	104	9.0
PFHpS	<LOQ	102	2.4	101	2.4	106	3.6
PFOA	<LOQ	99	5.0	99	2.8	105	4.6
FHxSA	<LOQ	100	6.2	94	7.5	99	9.9
5:3 FTCA	<LOQ	98	11.9	100	2.5	103	5.6
PFOS	<LOQ	104	4.6	103	4.6	109	12.3
PFNA	<LOQ	100	3.5	98	1.4	104	3.4
HFPO-TA	<LOQ	91	2.1	96	13.0	88	1.5
9Cl-PF3ONS	<LOQ	99	1.9	99	2.5	106	1.7
PFNS	<LOQ	107	3.8	101	6.3	105	2.0
PFDA	<LOQ	102	2.4	99	1.9	106	4.0
8:2FTS	<LOQ	104	8.8	101	7.5	98	6.8
PFDS	<LOQ	106	5.1	101	3.8	104	3.8
FOSA	<LOQ	103	2.8	100	2.6	107	5.3
PFUdA	<LOQ	102	1.9	98	2.2	104	4.5
N-MeFOSAA	<LOQ	103	5.2	97	6.6	108	6.1
7:3 FTCA	<LOQ	104	10.4	100	6.3	105	8.7
11Cl-PF2OUdS	<LOQ	95	3.4	96	3.3	104	6.3
N-EtFOSAA	<LOQ	100	15.1	106	11.7	103	9.9
PFUnDS	<LOQ	106	9.3	104	6.9	104	6.0
PFDoA	<LOQ	103	1.6	100	2.5	105	4.5
10:2FTS	<LOQ	111	10.6	103	6.4	96	7.6
N-MeFOSE	0.89	110	18.9	105	10.4	106	7.4
N-MeFOSA	<LOQ	100	6.8	101	6.3	105	5.4

Table 5 (continued). Recovery data for tap water and bottled water samples spiked at low, medium, and high concentration levels with the developed analytical method

	Bottled water						
	Non Spiked	Low - 5 ng/L		Mid - 25 ng/L		High - 75 ng/L	
Compound	Concentration (ng/L)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)	Recovery (%)	%RSD (%)
PFDoDS	<LOQ	105	6.1	107	10.7	107	4.7
PFTrDA	<LOQ	107	5.5	104	8.3	109	6.1
N-EtFOSE	<LOQ	107	14.6	107	4.7	106	5.2
N-EtFOSA	<LOQ	101	11.2	97	5.8	101	4.1
6:2diPAP	<LOQ	88	7.9	100	9.7	99	2.8
PFTrDS	<LOQ	100	10.6	99	4.9	99	5.0
PFTeDA	<LOQ	104	2.8	100	1.7	106	3.7
6:2/8:2diPAP	<LOQ	106	4.0	106	5.0	102	5.0
PFHxDA	<LOQ	97	2.1	95	8.9	96	5.8
8:2diPAP	<LOQ	106	4.9	109	15.7	111	5.8
PFOcDA	<LOQ	105	2.6	108	7.4	108	5.2

Conclusions

The method presented in this work achieves high-level sensitivity when performing direct injection analysis of 54 PFAS compounds in the low ng/L range in drinking water using the TSQ Altis Plus mass spectrometer. This workflow allows laboratories to overcome the challenges associated with this analysis, i.e., contamination and solubility, with the use of practical tools such as adapted consumables, a delay Acclaim short LC column, specific fluidics, and a defined injection program performed for each injected sample. The ease of use and robustness of the method are based on a fixed configuration including a SOP with detailed hardware and consumables, a complete acquisition and processing method with customized view settings and reports, and all data handling performed with Chromeleon CDS 7.3.2. Additionally, the benefit of direct injection allows laboratories to improve sample throughput in the lab due to significantly less sample preparation being required compared to traditional SPE clean-up workflows.

References

1. Jian, J.M. et al. A short review on human exposure to and tissue distribution of per- and polyfluoroalkyl substances (PFASs). *Sci Total Environ* **2018**, 636, 1058–1069. https://doi.org/10.1016/j.scitotenv.2018.04.380
2. Fenton, S.E. et al. Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research. E*nvironmental Toxicology and Chemistry* **2021**, 40 (3), 606-630. https://doi.org/10.1002/etc.4890
3. 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. https://eur-lex.europa.eu/eli/dir/2020/2184/oj
4. Avis relatif aux limites de quantification des couples « paramètre-matrice » de l'agrément des laboratoires effectuant des analyses dans le domaine de l'eau et des milieux aquatiques. https://www.legifrance.gouv.fr/jorf/id/JORFTEXT000039242459
5. Requirements for Poly and Perfluorinated Alkyl Substances (PFAS) monitoring by water companies in England and Wales. https://cdn.dwi.gov.uk/wp-content/uploads/2021/10/04203217/Information-Letter-PFAS-Monitoring.pdf
6. Bepaling van per- en polyfluoralkylverbindingen (PFAS) in water met LC-MS/MS. https://reflabos.vito.be/2023/WAC_IV_A_025.pdf
7. Metodi analitici di riferimento per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi chimici. https://www.salute.gov.it/imgs/C_17_pubblicazioni_2277_allegato.pdf
8. Thermo Fisher Scientific Application Note 65397: Direct analysis of selected per- and polyfluorinated alkyl substances (PFAS) in ground, surface, and wastewater by LC-MS/MS. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-65397-lc-ms-pfas-water-an65397-en.pdf
9. Thermo Fisher Scientific Technical Note 74138: Evaluation of custom injection programs and larger internal diameter capillary for strong solvent sample effects mitigation in liquid chromatography https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-74138-hplc-custom-injection-programs-tn74138-en.pdf

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