Methodologies for Ultrashort-Chain and Comprehensive PFAS Analysis in Water Samples

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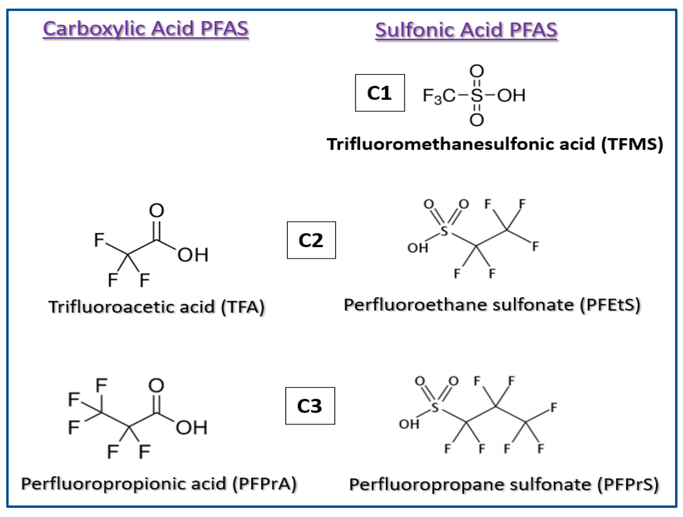
Introduction

Ultrashort-chain (USC) per- and polyfluoroalkyl substances (PFAS) are small and very polar compounds with carbon chain lengths shorter than C4 (**Figure 1**). Their ubiquitous nature and elevated concentrations in environmental aquatic systems are emerging as a significant concern, rivalling the well-established issues associated with long-chain PFAS contamination. USC PFAS contribute to at least 40% of total PFAS detected in environmental waters, with trifluoroacetic acid (TFA) being both the most abundant, and most difficult to analyse by conventional reversed-phase liquid chromatography due to both its size and polarity. These qualities mean established standardised analytical workflows are not sufficient, and alternative LC methodologies are necessary for USC PFAS analysis.

This poster introduces two chromatographic approaches designed to enable either targeted analysis of C1-C4 PFAS or comprehensive analysis encompassing USC and long-chain PFAS. A direct injection workflow was developed for C1-C4 PFAS analysis implementing a unique hybrid HILIC/ion exchange column. Additionally, a dilute-and-shoot workflow was developed for the simultaneous analysis of C1 to C14 perfluoroalkyl carboxylic and sulfonic acids, along with other PFAS classes, using an inert-coated polar-embedded alkyl phase LC column. Both workflows were evaluated for accuracy and precision by analysis of fortified drinking waters and wastewaters. Additional potable and non-potable waters collected from various water sources were tested to further demonstrate that the established workflows are suitable for the accurate quantification of targeted PFAS in a wide range of water matrices.

Figure 1: Structures of C1 to C3 PFAS



Water Samples

Wastewater samples were gifts from GDIT (Falls Church, VA). These include a publicly owned treatment works (POTW), a hospital, a metal finisher, and a chemical manufacturer wastewater effluents. Bottled waters were obtained from local grocery stores. Tap waters were collected from the facility of Restek Corp. and local households served by different borough water authorities. In addition, a natural spring water, two well waters, and three creek waters were collected from central Pennsylvania regions.

Methods

Table 1: Polar X Method for C1 – C4 PFAS Analysis

Analytical Column	Raptor Polar X 50 mm x 2.1 mm, 2.7µm (Restek Cat.# 9311A52)	
Mobile Phase A	10 mM ammonium formate, 0.1% formic acid in water	
Mobile Phase B	0.1% formic acid in 95:5 acetonitrile:isopropanol	
Gradient	**Time (min)**	**%B**
	0.0	85
	7.0	85
Flow Rate	0.3 mL/min	
Injection Volume	10 µL	
Column Temp.	40°C	
Ion Mode	Scheduled MRM with negative ESI	

Table 2: Ultra Inert IBD Method for Comprehensive PFAS Analysis

Analytical Column	Ultra Inert IBD 100 mm x 2.1 mm, 3 µm (Restek Cat.# 9175312-T)	
Delay Column	PFAS Delay Column (Restek Cat.# 27854)	
Mobile Phase A	5 mM ammonium formate, 0.1% formic acid in water	
Mobile Phase B	Acetonitrile	
Gradient	**Time (min)**	**%B**
	0.00	50
	7.00	95
	10.00	95
	10.01	50
	12.00	50
Flow Rate	0.4 mL/min	
Injection Volume	45 µL	
Column Temp.	40°C	
Ion Mode	Scheduled MRM with negative ESI	

Standard and Sample Preparation

(1) Polar X Method :

Seven C1 – C4 PFAS analytes were analysed using the Polar X column. The calibration standard solutions (0.4 mL) were prepared in the reverse osmosis (RO) water at the range of 2.5 to 800 ng/L (ppt) in the polypropylene HPLC vials. The isotopically-labelled PFBS and PFBA were implemented as the internal standard (IS) and an aliquot of 4 µL from a 10 ng/mL working solution was added and mixed with the standard solution. Tap water and bottled water were used directly without filtration. Wastewater samples (~ 10 mL) were filtered with nylon syringe filters and collected in 50-mL polypropylene tubes. For accuracy and precision analysis, 1 mL of water samples were fortified at 25, 50, and 175 ppt and then a 0.4 mL aliquot was transferred to a polypropylene vial and mixed with 4 µL of the IS working solution before injecting for the LC-MS/MS analysis. For the regular determination of C1 – C4 PFAS concentration in various potable and non-potable water samples, a fortified sample at 50 ppt was prepared to act as the QC sample.

(2) Ultra Inert IBD Method :

A total of 45 PFAS including ultrashort-chain compounds were analysed using the Ultra Inert IBD column (**Table 3**). The calibration standard solutions (250 µL) were prepared in reverse osmosis water at the range of 1 to 1000 ng/L in polypropylene HPLC vials. Five mass-labelled PFAS were implemented as quantitative internal standards (QIS). A 2 µL aliquot of QIS working solution containing 40 ng/mL of $^{13}C_3$-PFBA, 20 ng/mL of $^{13}C_2$-PFHxA and $^{13}C_4$-PFOA, and 10 ng/mL of $^{13}C_5$-PFNA and $^{13}C_2$-PFDA, was added to each standard solution, followed by mixing with 250 µL of methanol containing 1% acetic acid. Tap water, spring bottled water, and treated sewage wastewater effluent from a POTW (publicly owned treatment works) facility were used for the assessment of method accuracy and precision. Tap water and bottled water were used directly without filtration. POTW water (~ 10 mL) was filtered with polypropylene syringe filters and collected in 50-mL polypropylene tubes. These water samples (250 µL) were fortified at concentrations of 2, 4, 10, 50, and 250 ppt with native analytes and isotopically labelled ^{13}C-TFA, which served as a surrogate for the determination of TFA recovery. Each fortified sample was mixed with 2 µL of QIS working solution and 2.5 µL of extracted internal standards (EIS) working solution containing 10 ng/mL of mass-labelled PFAS. A 250 µL aliquot of methanol containing 1% acetic acid was then added and mixed with fortified samples for LC-MS/MS analysis.

Results & Discussion

(1) LC-MS/MS Analysis **: Table 1** and **Table 2** present chromatographic methods for the Polar X and Ultra Inert IBD columns, respectively. **Figure 2** and **Figure 3** display the corresponding chromatographic results obtained using these methods.

(2) Linearity : For Polar X method, all analytes showed acceptable linearities with r^2 >0.995 and deviations <20% at the range of 2.5 – 800 ppt for C1 – C4 sulfonic acid PFAS, 5.0 – 800 ppt for PFBA and PFPrA, and 20 – 800 ppt for TFA. For Ultra Inert IBD method, all analytes exhibited acceptable linearities with r^2 >0.995 and deviations <30% at the range of 1 ppt to 1000 ppt, with variation at the lowest calibration concentration.

(3) Accuracy & Precision : Three batches of analyses were performed on different days for a total of nine repetitions at each fortified level. For the Polar X method, all analytes had recovery values of 86.6 - 107% across three fortification levels among three different types of waters. Satisfactory method precision was demonstrated with %RSD values between 1.62 - 10.7%. For the Ultra Inert IBD method, all analytes exhibited recovery values within the range of 70 – 130% across all fortification levels. Satisfactory method precision was demonstrated with %RSD values below 20%.

(4) Measurement of 45 Targeted PFAS in Water Samples (Ultra IBD Method) **:** Each sample was prepared in triplicate with the addition of EIS. Consistent with the accuracy and precision analysis, the recoveries of EIS were within 30% of the nominal concentration (100 ppt) across all source waters. This demonstrated that the established method was suitable for accurate measurement of targeted PFAS in a wide range of water matrices (see results in **Table 4**).

Figure 2: C1-C4 PFAS Analysed Using the Polar X Column

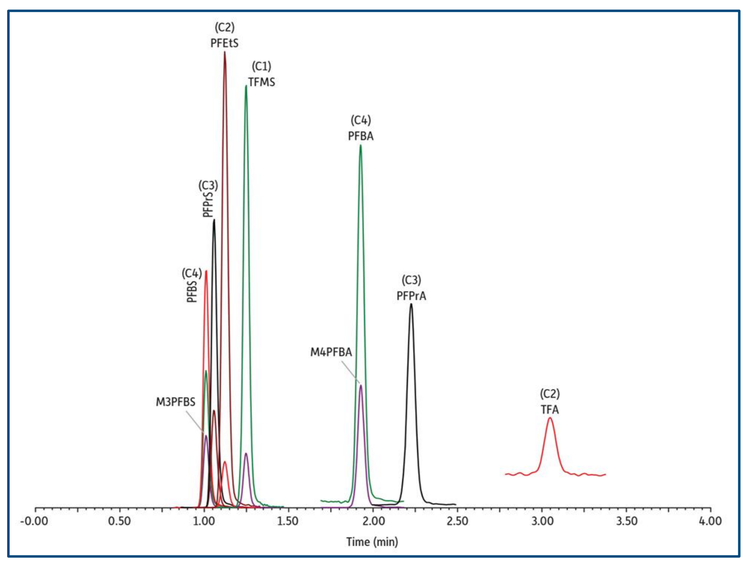


Figure 3: Comprehensive PFAS Analysis Using the Ultra Inert IBD Column

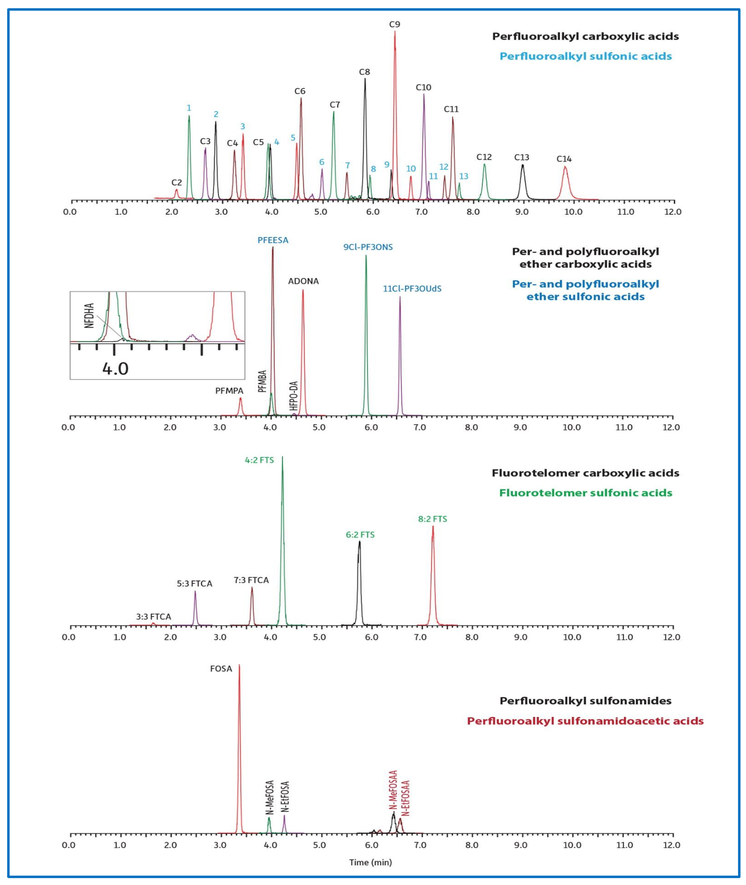


Table 3: Targeted PFAS for Ultra Inert IBD Method

Compounds	Retention Time (min)	Precursor Ion	Product ions*	Cone (V)	Collision (V)	Quantification Internal Standard
Target Analytes						
Perfluoroalkyl carboxylic acids						
Trifluoroacetic acid (TFA)	2.12	113.03 [M-H]-	69.01	10	10	$^{13}C_3$-PFBA
Perfluoropropanoic acid (PFPrA)	2.69	162.97 [M-H]-	119.02	10	8	$^{13}C_3$-PFBA
Perfluorobutanoic acid (PFBA)	3.27	213.03 [M-H]-	168.98	14	8	$^{13}C_3$-PFBA
Perfluoropentanoic acid (PFPeA)	3.94	262.97 [M-H]-	218.97	2	6	$^{13}C_3$-PFHxA
Perfluorohexanoic acid (PFHxA)	4.59	313.10 [M-H]-	268.97/118.99	2	8/20	$^{13}C_3$-PFHxA
Perfluoroheptanoic acid (PFHpA)	5.24	363.16 [M-H]-	319.09/169.06	8	10/18	$^{13}C_4$-PFOA
Perfluorooctanoic acid (PFOA)	5.86	413.10 [M-H]-	368.96/168.90	2	10/16	$^{13}C_4$-PFOA
Perfluorononanoic acid (PFNA)	6.45	463.10 [M-H]-	419.01/219.02	4	10/16	$^{13}C_5$-PFNA
Perfluorodecanoic acid (PFDA)	7.03	513.17 [M-H]-	469.16/219.06	4	12/16	$^{13}C_2$-PFDA
Perfluoroundecanoic acid (PFUnA)	7.60	563.23 [M-H]-	519.24/269.07	6	12/18	$^{13}C_2$-PFDA
Perfluorododecanoic acid (PFDoA)	8.23	613.23 [M-H]-	569.19/169.06	8	12/26	$^{13}C_2$-PFDA
Perfluorotridecanoic acid (PFTrDA)	8.99	663.23 [M-H]-	619.21/169.06	8	14/28	$^{13}C_2$-PFDA
Perfluorotetradecanoic acid (PFTeDA)	9.83	712.67 [M-H]-	668.69/168.94	10	12/26	$^{13}C_2$-PFDA
Perfluoroalkyl sulfonic acids						
Trifluoromethanesulfonic acid (TFMS)	2.36	148.97 [M-H]-	79.93/98.92	62	18/18	$^{13}C_3$-PFBA
Perfluoroethanesulfonic acid (PFEtS)	2.89	198.90 [M-H]-	79.92/98.91	38	22/22	$^{13}C_3$-PFBA
Perfluoropropanesulfonic acid (PFPrS)	3.44	248.97 [M-H]-	79.92/98.91	2	24/24	$^{13}C_3$-PFBA
Perfluorobutanesulfonic acid (PFBS)	3.97	298.97 [M-H]-	79.97/98.89	2	26/26	$^{13}C_3$-PFHxA
Perfluoropentanesulfonic acid (PFPeS)	4.50	349.10 [M-H]-	79.98/98.98	6	32/30	$^{13}C_3$-PFHxA
Perfluorohexanesulfonic acid (PFHxS)	5.01	398.90 [M-H]-	79.97/98.89	56	32/34	$^{13}C_3$-PFHxA
Perfluoroheptanesulfonic acid (PFHpS)	5.50	449.17 [M-H]-	79.98/98.97	4	42/38	$^{13}C_4$-PFOA
Perfluorooctanesulfonic acid (PFOS)	5.96	499.03 [M-H]-	79.92/98.90	8	40/40	$^{13}C_4$-PFOA
Perfluorononanesulfonic acid (PFNS)	6.38	549.10 [M-H]-	79.92/98.83	12	42/40	$^{13}C_5$-PFNA
Perfluorodecanesulfonic acid (PFDS)	6.77	599.17 [M-H]-	79.98/98.83	8	44/46	$^{13}C_2$-PFDA
Perfluoroundecanesulfonic acid (PFUdS)	7.12	648.73 [M-H]-	79.94/98.94	38	50/44	$^{13}C_2$-PFDA
Perfluorododecanesulfonic acid (PFDoS)	7.44	698.77 [M-H]-	79.95/98.94	10	60/44	$^{13}C_2$-PFDA
Perfluorotridecanesulfonic acid (PFTrDS)	7.73	748.73 [M-H]-	79.94/98.94	8	76/52	$^{13}C_2$-PFDA
Fluorotelomer sulfonic acids						
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	4.22	327.10 [M-H]-	307.08/80.83	50	18/24	$^{13}C_3$-PFHxA
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	5.75	427.17 [M-H]-	407.18/80.71	2	22/32	$^{13}C_4$-PFOA
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	7.22	527.17 [M-H]-	507.16/80.83	66	26/32	$^{13}C_2$-PFDA
Fluorotelomer carboxylic acids						
3-perfluoropropyl propanoic acid (3:3 FTCA)	1.64	241.00 [M-H]-	177.00/117.00	2	6/6	$^{13}C_3$-PFBA
3-perfluoropentyl propanoic acid (5:3 FTCA)	2.49	340.93 [M-H]-	216.96/236.93	2	24/14	$^{13}C_3$-PFBA
3-perfluoroheptyl propanoic acid (7:3 FTCA)	3.61	440.90 [M-H]-	336.88/316.91	20	12/22	$^{13}C_3$-PFBA
Perfluorooctane sulfonamides						
Perfluorooctanesulfonamide (FOSA)	3.36	498.17 [M-H]-	77.97/477.76	8	28/26	$^{13}C_8$-PFBA
N-methyl perfluorooctanesulfonamide (NMeFOSA)	3.96	511.77 [M-H]-	168.95/218.91	2	26/24	$^{13}C_8$-PFHxA
N-ethyl perfluorooctanesulfonamide (NEtFOSA)	4.26	525.83 [M-H]-	168.96/218.92	10	26/24	$^{13}C_8$-PFHxA
Perfluorooctane sulfonamidoacetic acids						
N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	6.44	570.20 [M-H]-	419.17/483.16	46	20/14	$^{13}C_5$-PFNA
N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	6.56	584.20 [M-H]-	419.18/483.11	6	20/16	$^{13}C_5$-PFNA
Per- and Polyfluoroether carboxylic acids						
Perfluoro-3-methoxypropanoic acid (PFMPA)	3.40	228.93 [M-H]-	84.97/198.94	10	10/14	$^{13}C_3$-PFBA
Perfluoro-4-methoxybutanoic acid (PFMBA)	4.00	278.87 [M-H]-	84.96/234.93	8	10/6	$^{13}C_3$-PFHxA
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	4.06	294.93 [M-H]-	200.91/85.02	8	4/22	$^{13}C_3$-PFHxA
Hexafluoropropylene oxide dimer acid (HFPO-DA)	4.46	285.03 [M-COOH]-	169.02/185.02	2	6/16	$^{13}C_3$-PFHxA
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	4.63	376.90 [M-H]-	250.93/84.97	22	12/26	$^{13}C_3$-PFHxA
Ether sulfonic acids						
Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	4.04	314.83 [M-H]-	134.94/83.01	4	22/16	$^{13}C_3$-PFHxA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	5.88	530.78 [M-H]-	350.85/82.96	12	26/24	$^{13}C_4$-PFOA
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	6.56	630.78 [M-H]-	450.80/82.95	8	26/32	$^{13}C_5$-PFNA
Extracted Internal Standards						
$^{13}C_3$-PFPrA	2.69	165.97 [M-H]-	120.96	10	11	$^{13}C_3$-PFBA
$^{13}C_4$-PFBA	3.27	217.03 [M-H]-	171.98	2	8	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	3.94	267.97 [M-H]-	222.99	2	6	$^{13}C_3$-PFHxA
$^{13}C_5$-PFHxA	4.59	318.03 [M-H]-	272.93	2	7	$^{13}C_3$-PFHxA
$^{13}C_4$-PFHpA	5.24	366.90 [M-H]-	321.93	2	10	$^{13}C_4$-PFOA
$^{13}C_8$-PFOA	5.86	420.97 [M-H]-	375.94	2	10	$^{13}C_4$-PFOA
$^{13}C_8$-PFDA	7.03	518.90 [M-H]-	473.87	4	13	$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	7.60	569.90 [M-H]-	524.87	2	12	$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	8.23	614.84 [M-H]-	569.87	2	12	$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	9.83	714.78 [M-H]-	669.80	8	14	$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	3.97	301.97 [M-H]-	79.97	2	28	$^{13}C_3$-PFHxA
$^{13}C_3$-PFHxS	5.01	401.90 [M-H]-	79.97	2	36	$^{13}C_3$-PFHxA
$^{13}C_8$-PFOS	5.96	506.84 [M-H]-	79.97	4	42	$^{13}C_4$-PFOA
$^{13}C_2$-4:2 FTS	4.22	328.97 [M-H]-	308.96	2	18	$^{13}C_3$-PFHxA
$^{13}C_2$-8:2 FTS	7.22	528.90 [M-H]-	508.90	2	24	$^{13}C_2$-PFDA
$^{13}C_8$-FOSA	3.36	505.91 [M-H]-	77.95	4	32	$^{13}C_8$-PFBA
d3-NMeFOSAA	6.44	572.90 [M-H]-	418.91	50	18	$^{13}C_5$-PFNA
d5-NEtFOSAA	6.56	588.97 [M-H]-	418.86	48	20	$^{13}C_5$-PFNA
Quantification Internal Standards						
$^{13}C_3$-PFBA	3.27	215.97 [M-H]-	171.97	10	8	
$^{13}C_5$-PFHxA	4.59	314.97 [M-H]-	269.93	8	8	
$^{13}C_8$-PFOA	5.86	416.87 [M-H]-	371.88	2	8	
$^{13}C_9$-PFNA	6.45	467.87 [M-H]-	422.89	16	10	
$^{13}C_2$-PFDA	7.03	514.87 [M-H]-	469.84	8	10	

* Quantifier Ion/Qualifier Ion

Table 4: Measurement of 45 Targeted PFAS in Various Water Matrices

Water Samples	Averaged Concentration (ng/L)											
	Ultrashort-Chain			Short-Chain						Long-Chain		Alternatives	
	TFA	PFPrA	TFMS	PFBA	PFBS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFMPA	HFPO-DA
Potable Waters													
Tap Water #1	176	2.66	8.40	nd*	nd	< 1.00	2.13	nd	nd	< 1.00	nd	nd	nd
Tap Water #2	317	1.54	7.14	nd	2.25	nd	nd	nd	nd	nd	< 2.00	nd	nd
Tap Water #3	166	3.15	10.9	nd	2.14	2.51	1.24	nd	nd	< 1.00	4.76	nd	nd
Tap Water #4	330	5.48	12.8	nd	1.68	1.97	nd	nd	nd	nd	nd	nd	nd
Tap Water #5	1186	29.0	25.4	13.2	8.58	13.7	17.7	nd	5.78	18.0	5.63	nd	nd
Bottled Water #1 (spring water)	107	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Bottled Water #2 (spring water)	257	1.66	< 1.00	< 2.00	1.21	nd	nd	nd	nd	nd	< 2.00	nd	nd
Bottled Water #3 (RO purified)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Natural Spring Water	486	2.91	4.81	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Well Water #1	557	1.82	1.74	nd	nd	nd	nd	nd	nd	< 1.00	nd	nd	nd
Well Water #2	171	1.16	< 1.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Non-Potable Waters													
Creek Water #1	605	4.88	6.75	5.82	19.5	3.50	1.52	nd	< 1.00	< 1.00	6.06	4.40	nd
Creek Water #2	626	3.07	2.26	nd	2.68	nd	nd	nd	nd	nd	< 2.00	nd	nd
Creek Water #3	627	10.9	8.28	< 2.00	2.19	4.94	2.35	nd	nd	3.08	5.97	nd	nd
POTW Water Effluent	1156	24.2	9.66	nd	2.84	6.50	24.0	2.28	< 1.00	12.9	3.88	nd	nd
Hospital Effluent	487	3.08	1.45	nd	nd	2.15	1.50	nd	nd	nd	nd	nd	nd
Metal Finisher Effluent	678	2.78	8.89	2.87	3.11	2.52	2.09	nd	nd	< 1.00	112	nd	nd
Chemical Manufacture Effluent	90,473	8,540	13.3	62.8	1.13	100	7.22	nd	12.0	20.8	nd	14.0	5,709

*non-detected

Conclusions

Two workflows were developed and verified to provide unique solutions for measuring ultrashort-chain PFAS in various water matrices. The reported methods, utilizing the Polar X and Ultra Inert IBD columns, demonstrated robustness, accuracy, and precision. Most importantly, these approaches serve as valuable tools for monitoring these emergent PFAS in environmental water systems and assist in generating a guideline for future regulatory action.