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PFAS APPLICATION NOTEBOOK









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Our full library of PFAS resources contains dozens of app notes, articles, chromatograms, videos, and more across multiple markets including environmental, food, and clinical.

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Quick Tips

Leverage the experience of our PFAS team with this curated selection of FAQs for getting the most out of your PFAS analysis. Want to learn more? Visit **www.restek.com/pfas**

Why are system-related PFAS important to isolate from my samples?
If you are analyzing PFAS at very low levels, like parts per trillion (ppt), for accurate identification and quantitation, you need to make sure that the PFAS detected belong only to your sample. If background PFAS contamination from your LC coelutes with sample PFAS, they will interfere and cause inaccurate results.

What are the most common sources of lab or system-based PFAS?
The ubiquitous nature of PFAS in our environment means they can be found all over our lab, vials, or other sample prep products. They can elevate our results and make us waste numerous hours searching for contamination to get the analysis to pass. Where do they come from, and what are some of the most overlooked sources of contamination?

Sample Collecting
Avoid LDPE bottles, clothing containing polytetrafluoroethylene (such as water-resistant coats or stain repellent clothing), waterproof notebooks, plastic clipboards, stick it note pads, permanent markers, or reusable ice packs (blue ice).

Sample Preparation
Avoid glass transfer pipettes, vial caps with PTFE seals and even some glass HPLC vials (PFAS has been known to absorb to glass when it has been in contact for an extended period. Glass bottles or containers can be used if they are known to be PFAS-free). Avoid wearing sunscreens, insect repellents, fabric softener, moisturizers, and lab coats (stain-resistant). Avoid washing glassware with detergents that haven't been tested for PFAS presence.

Instrumentation
Avoid all PTFE tubing, PTFE frits, and sample valves with certain rotor seal or stator face. Be aware that when your LC-MS/MS system has been sitting overnight or extended time frame the first few injections will show a high level of PFAS due to the buildup of residual PFAS in the system.

Solvents
A part of the system that is often overlooked when searching for PFAS contamination is the mobile phase. Many HPLC and LC-MS/MS grade mobile phases (water, acetonitrile, and methanol) use PTFE filters before they are bottled by the company. Bottle caps could have PTFE lining.

For information on ways to mitigate PFAS contamination, visit: www.restek.com/pfas

What is a PFAS delay column?
A PFAS delay column is installed before the injector, so it traps system-related PFAS and delays their elution. This prevents them from coeluting and interfering with the PFAS in the sample. Functionally, as the gradient starts, the trapped PFAS are eluted from the delay column and then travel to the analytical column. They arrive at the analytical column after the PFAS from the injected samples, so they do not coelute with the sample PFAS.

PFAS in the sample will elute as a normal-shaped, symmetrical peak but the delayed system-related PFAS have been continuously moving throughout the system with the gradient. They were never focused on the analytical column, so when they elute, the "peak" they generate is just an elevated baseline. You know it belongs to one or more PFAS because the signal matches the MS/MS transition of the compounds you are monitoring. However, because it is after the retention time window of the same PFAS in your sample, it's not detected and quantitated as part of the sample.

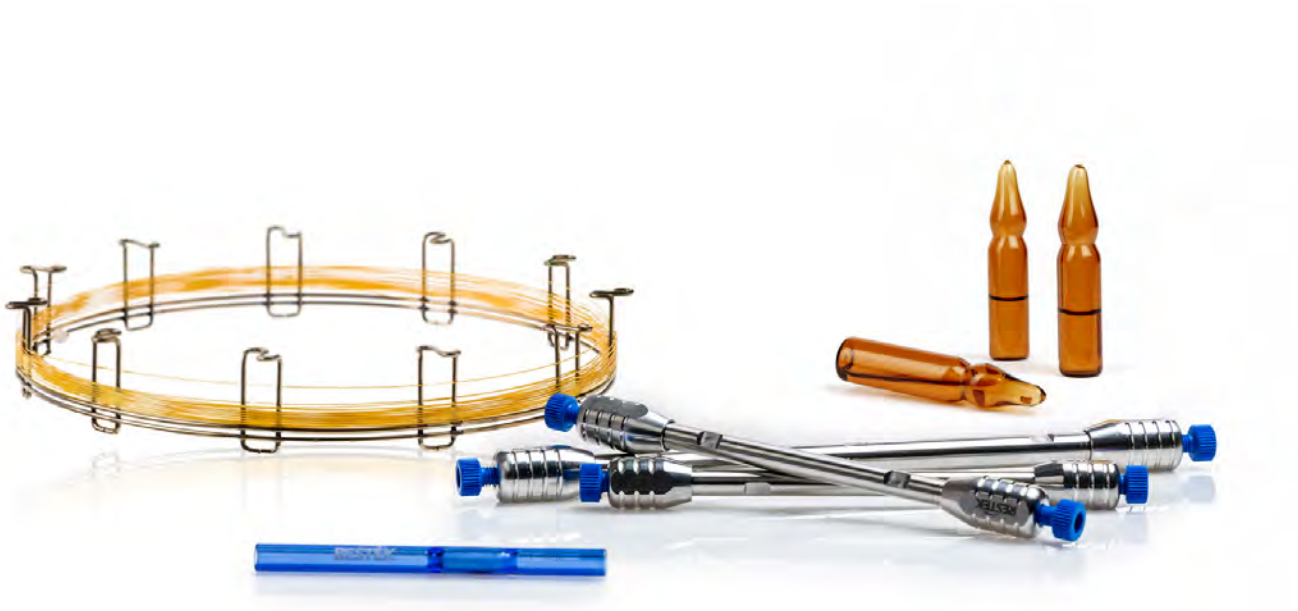
Learn more on page 92.

How can I compare PFAS testing methods?
Comparing PFAS methods starts with understanding your application, target compounds, and reporting needs. Our method guides help you review key differences and choose a method that fits your workflow. Access the guide at www.restek.com/pfas-methods

How do I choose the right column for my PFAS testing method?
Choosing the right column depends on your method, target compounds, and required separation. Our product guide helps you match column chemistry and performance to your specific PFAS workflow. Access the guide at www.restek.com/pfas-products

What kind of support can Restek provide?
Our teams understand the unique challenges with PFAS analysis and the problems labs experience. Whether you need assistance choosing or developing a method, finding the right products, or help with something else, our PFAS experts can help you navigate these challenges with personalized advice.

Let us know how we can help at explore.restek.com/PFAS



Incorporating Ultrashort-Chain Compounds into Comprehensive PFAS Analysis in Water

By Shun-Hsin Liang, PhD

Abstract

A simple and reliable workflow was established here for analyzing 45 PFAS compounds in both potable and non-potable waters. The target analytes included C2 to C14 perfluoroalkyl carboxylic acids (PFCA); C1 to C13 perfluoroalkyl sulfonic acids (PFSA); fluorotelomer carboxylic acids and sulfonic acids; perfluorooctane sulfonamides and sulfonamidoacetic acids; and per- and polyfluoroether carboxylic acids and sulfonic acids. Method suitability was evaluated in terms of linearity, accuracy, precision, and suitability for a range of water types. The method produced exceptional chromatographic performance, providing a tool for comprehensive PFAS analysis that overcomes the challenges associated with conventional reversed-phase liquid chromatography (RPLC).



Related Products

- *Ultra IBD Inert 3 µm, 100 mm x 2.1 mm (cat.# 9175312-T)*
- *PFAS 28 calibration standard (cat.# 30734)*
- *Norm-Ject syringe (cat.# 22775)*
- *Polypropylene syringe filter (cat.# 28936)*
- *Polypropylene centrifuge tubes (cat.# 25846)*

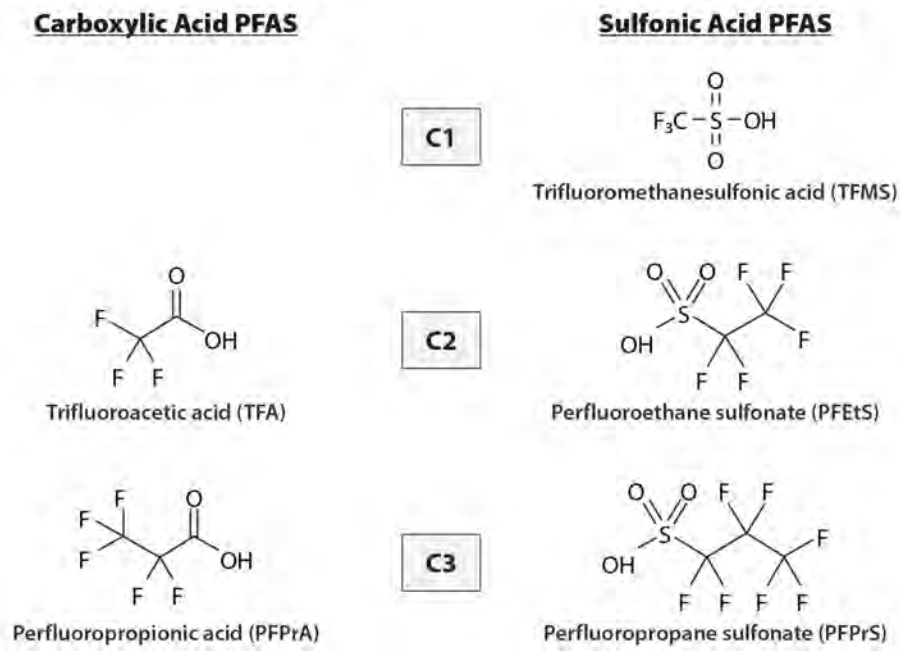
Introduction

Ultrashort-chain per- and polyfluoroalkyl substances (PFAS) are small, very polar compounds with carbon chain lengths shorter than C4 (Figure 1). Their ubiquitous presence and high levels in environmental aquatic systems are emerging as a significant concern, rivaling the well-established issues associated with long-chain PFAS contamination. Therefore, it is important to analyze both ultrashort-chain and longer chain PFAS together in water samples to comprehensively assess the full spectrum of PFAS contamination. Methods allowing concurrent analysis of C1-C14 PFAS will be important tools in PFAS testing because they will allow a better understanding of environmental fate and potential human exposure, which is critical to developing effective regulations and remediation strategies.

The development of comprehensive PFAS methods that include ultrashort-chain compounds is challenging because the high polarity of ultrashort-chain PFAS makes it difficult to obtain adequate retention with an RPLC method and C18 column, which is the typical approach for analyzing C4 and longer PFAS in water. Obtaining adequate retention and separation from matrix interferences is particularly problematic for early eluting compounds, such as TFA. GC-MS has been used for analyzing TFA and C4-C6 PFCA in water samples, but it requires an extra step for PFCA derivatization and does not allow simultaneous analysis of PFSA [1]. Ultrashort-chain PFAS have also been analyzed using anion-exchange LC, but method run times exceed 20 minutes and broad peaks were observed [2]. We have previously developed a rapid method for the analysis of ultrashort- and short-chain PFAS in water samples using a hybrid HILIC and ion-exchange LC column [3], but it did not incorporate long-chain PFAS. More recently, we developed a method to quantify C1 to C10 PFAS in human plasma and serum utilizing a polar-embedded alkyl phase LC column [4]. Based on the effectiveness of that method, we used it as a starting point for the current study, which aims to develop a new method for testing a broader range of PFAS in water matrices.

The method developed here uses an LC column with a polar-embedded alkyl stationary phase to ensure adequate retention of early eluting polar compounds. In addition, the column is made with hardware treated with an inert coating, which improves sensitivity by preventing any unwanted analyte interactions with the stainless-steel column. Using this Ultra IBD Inert column, a simple and reliable workflow was developed for the simultaneous analysis of C1 to C14 perfluoroalkyl carboxylic and sulfonic acids along with other groups of PFAS. Method performance was assessed for linearity, accuracy, precision, and suitability across a wide range of potable and non-potable water matrices.

Figure 1: Structures of C1 to C3 PFAS



Experimental

Water Samples

Wastewater samples were gifts from General Dynamics Information Technology (Falls Church, VA). These included effluents from a publicly owned treatment works (POTW); a hospital; a metal finisher; and a chemical manufacturer. Bottled waters were obtained from local grocery stores. Tap waters were collected from Restek Corporation (Bellefonte, PA) 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 regions in central Pennsylvania.

Standard and Sample Preparation

The working calibration standard solutions (250 µL each) were prepared in reverse osmosis water across a range of 1 to 1000 ng/L in polypropylene HPLC vials. Five mass-labeled PFAS were used as quantitative internal standards (QIS) (Table I). 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, bottled spring water, and treated sewage wastewater effluent from a POTW facility were used for the assessment of method accuracy and precision. Tap water and bottled water were used directly without filtration. The POTW water (~10 mL) was filtered with polypropylene syringe filters (cat.# 28936) and collected in 50-mL polypropylene tubes (cat.# 25846). These water samples (250 µL) were fortified at concentrations of 2, 4, 10, 50, and 250 ppt with native analytes and isotopically labeled ^{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-labeled PFAS as detailed in Table I. A 250 µL aliquot of methanol containing 1% acetic acid was then added and mixed with the fortified samples for LC-MS/MS analysis.

Analytical System

Analysis of C1-C14 PFAS in the water samples was performed by LC-MS/MS under the conditions shown below. A PFAS delay column was installed between the mixer and injector to prevent any potential PFAS contamination upstream of the injector from coeluting with PFAS in the samples. Although this delay column was used here and is typically adequate, subsequent work has shown that when analyzing some matrices on an Ultra IBD Inert column, better separation between target analytes and background contaminants from the instrument and mobile phases can be achieved using a long Ultra IBD column (150 × 2.1 mm, 3.0 µm, cat.# 9175362) as the delay column. The MS/MS transition parameters for each analyte are provided in Table I.

System: Waters ACQUITY UPLC and Xevo TQ-S triple quadrupole mass spectrometer
Columns:

- Analytical column: Ultra IBD Inert, 100 mm x 2.1 mm, 3 µm (cat.# 9175312-T)
- PFAS delay column (cat.# 27854)

Injection volume: 45 µL
Mobile phase A: 5 mM ammonium formate, 0.1% formic acid in water
Mobile phase B: Acetonitrile
Flow rate: 0.4 mL/min
Temperature: 40 °C

Gradient:	Time (min)	%B
	0.00	50
	7.00	95
	10.00	95
	10.01	50
	12.00	50

Ion mode: Negative ESI
Mode: Scheduled MRM

Table I: MS Transitions and Analyte Retention Times

Compounds	Time (min)	Precursor Ion	Product Ions*	Cone (V)	Collision (V)	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	$^{13}C_2$-PFHxA
Perfluorohexanoic acid (PFHxA)	4.59	313.10 [M-H]-	268.97/118.99	2	8/20	$^{13}C_2$-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	$^{13}C_2$-PFHxA
Perfluoropentanesulfonic acid (PFPeS)	4.50	349.10 [M-H]-	79.98/98.98	6	32/30	$^{13}C_2$-PFHxA
Perfluorohexanesulfonic acid (PFHxS)	5.01	398.90 [M-H]-	79.97/98.89	56	32/34	$^{13}C_2$-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

Table I: MS Transitions and Analyte Retention Times (cont.)

Compounds	Time (min)	Precursor Ion	Product Ions*	Cone (V)	Collision (V)	Internal Standard
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	$^{13}C_2$-PFHxA
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	5.75	427.17 [M-H]-	407.18/80.71	2	22/32	13C_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	$^{13}C_3$-PFBA
N-methyl perfluorooctanesulfonamide (NMeFOSA)	3.96	511.77 [M-H]-	168.95/218.91	2	26/24	$^{13}C_2$-PFHxA
N-ethyl perfluorooctanesulfonamide (NEtFOSA)	4.26	525.83 [M-H]-	168.96/218.92	10	26/24	$^{13}C_2$-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	$^{13}C_2$-PFHxA
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	4.06	294.93 [M-H]-	200.91/85.02	8	4/22	$^{13}C_2$-PFHxA
Hexafluoropropylene oxide dimer acid (HFPO-DA)	4.46	285.03 [M-COOH]-	169.02/185.02	2	6/16	$^{13}C_2$-PFHxA
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	4.63	376.90 [M-H]-	250.93/84.97	22	12/26	$^{13}C_2$-PFHxA
Ether sulfonic acids						
Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	4.04	314.83 [M-H]-	134.94/83.01	4	22/16	$^{13}C_2$-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	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.59	318.03 [M-H]-	272.93	2	7	$^{13}C_2$-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
$^{13}C_6$-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	$^{13}C_2$-PFHxA

Table I: MS Transitions and Analyte Retention Times (cont.)

Compounds	Time (min)	Precursor Ion	Product Ions*	Cone (V)	Collision (V)	Internal Standard
$^{13}C_3$-PFHxS	5.01	401.90 [M-H]-	79.97	2	36	$^{13}C_2$-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	$^{13}C_2$-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	$^{13}C_3$-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	
$^{13}C_2$-PFHxA	4.59	314.97 [M-H]-	269.93	8	8	
$^{13}C_4$-PFOA	5.86	416.87 [M-H]-	371.88	2	8	
$^{13}C_5$-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.

Results and Discussion

LC-MS/MS Method Development

An effective chromatographic method was established for the comprehensive analysis of 45 PFAS, including ultrashort-chain compounds, in potable and non-potable water samples (Figure 2). The Ultra IBD Inert column exhibited excellent chromatographic performance under reversed-phased conditions, with the embedded polar group providing good retention of TFA and other early eluting PFAS. In addition, as shown in Figure 3, the inert hardware resulted in a notable enhancement in detection sensitivity for most of the analytes (3-70% increase in peak area and 5-75% increase in peak height) compared to columns with uncoated hardware.

Figure 2: Analysis of a 500 ppt PFAS Standard

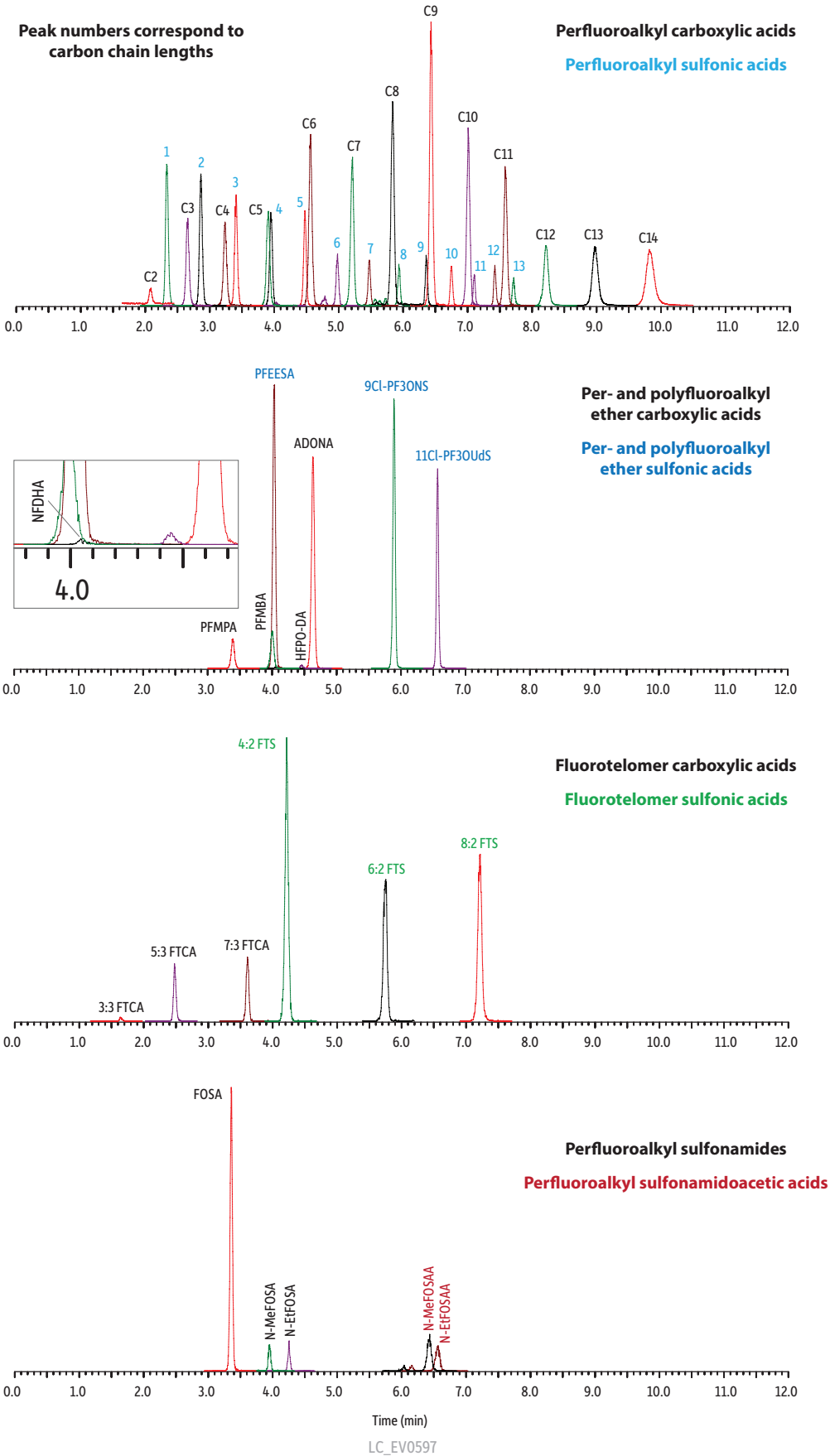
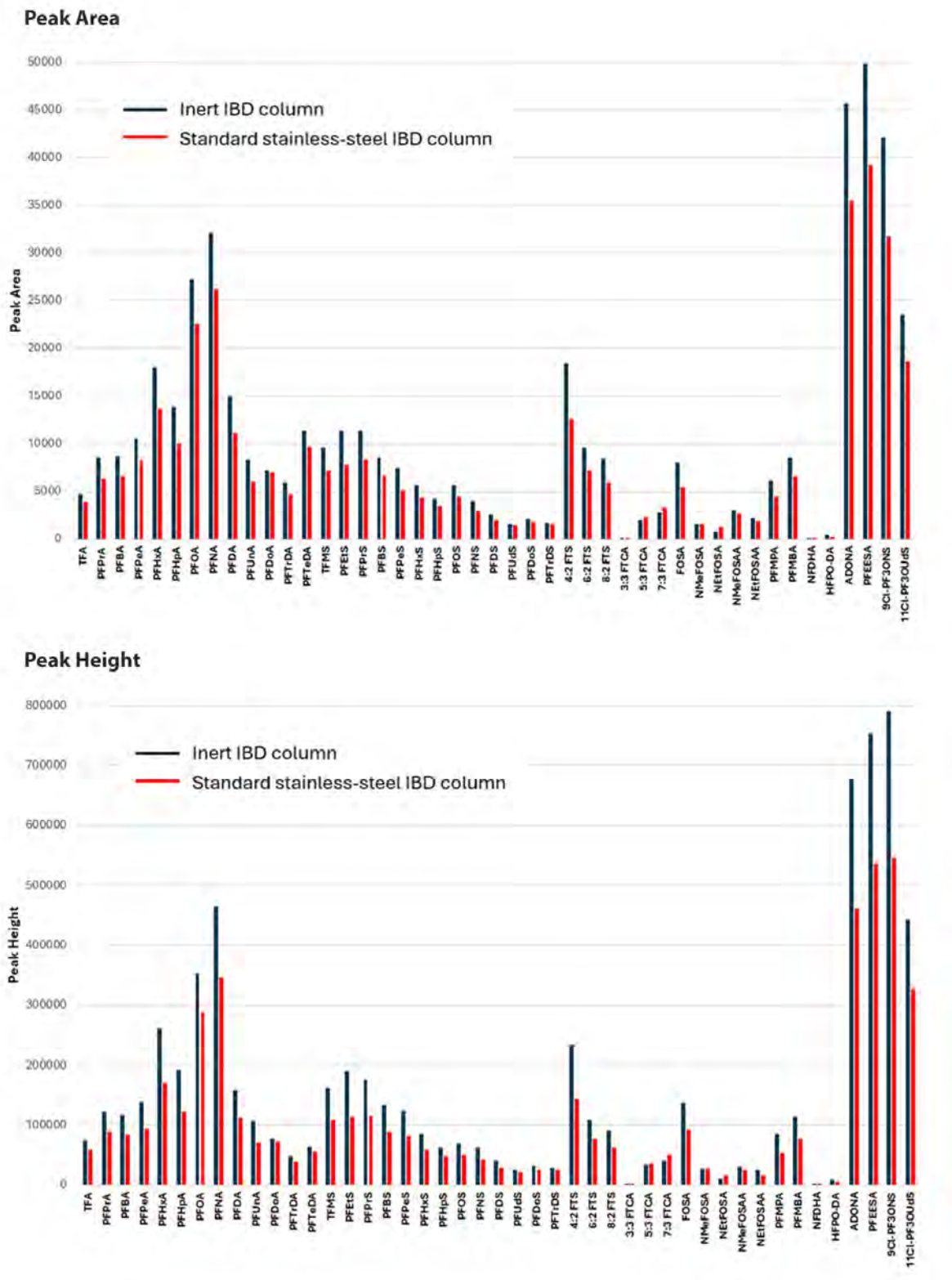


Figure 3: Inert LC column hardware increased peak area and height for most PFAS compared to columns made with traditional stainless-steel hardware.



Linearity

Employing quadratic regression (1/x weighted), all analytes exhibited acceptable linearities with r^2 >0.995 and deviations <30%. Table II shows the different linearity ranges for each target PFAS, which ranged from 1 ppt to 1000 ppt, with variation occurring at the lowest calibration concentration. LOQ and LOD values are also presented (LOQ values were defined as the concentration of the lowest calibration standard for each analyte).

Table II: LOQ* and LOD Values for PFAS in Tap, Bottled, and POTW Water

	Linearity Range	LOD (ng/L)		
Analytes	**(ng/L)**	**Tap Water**	**Bottled Water**	**POTW**
TFA	10–1000	1.7	1.4	2.1
PFPrA	1–1000	0.3	0.3	0.3
PFBA	2–1000	0.7	0.6	0.6
PFPeA	1–1000	0.5	0.4	0.3
PFHxA	1–1000	0.4	0.4	0.4
PFHpA	1–1000	0.4	0.4	0.5
PFOA	1–1000	0.4	0.4	0.4
PFNA	1–1000	0.3	0.3	0.3
PFDA	1–1000	0.4	0.3	0.5
PFUnA	1–1000	0.5	0.3	0.4
PFDoA	2–1000	0.7	0.4	0.5
PFTrDA	2–1000	0.8	0.6	0.6
PFTeDA	2–1000	0.8	0.7	0.6
TFMS	1–1000	0.2	0.2	0.2
PFEtS	1–1000	0.1	0.3	0.2
PFPrS	1–1000	0.2	0.2	0.4
PFBS	1–1000	0.2	0.2	0.5
PFPeS	1–1000	0.2	0.3	0.2
PFHxS	1–1000	0.3	0.6	0.5
PFHpS	2–1000	0.5	0.4	0.4
PFOS	2–1000	0.5	0.9	0.8
PFNS	2–1000	0.6	0.6	0.6
PFDS	2–1000	0.6	0.7	0.9
PFUdS	2–1000	0.5	0.7	0.9
PFDoS	2–1000	0.4	0.6	0.6
PFTrDS	2–1000	0.9	0.6	0.8
4:2 FTS	2–1000	0.4	0.6	0.5
6:2 FTS	2–1000	0.6	0.4	0.5
8:2 FTS	2–1000	0.4	0.4	0.5
3:3 FTCA	20–1000	8.3	7.4	-
5:3 FTCA	4–1000	1.2	1.1	1.5
7:3 FTCA	4–1000	1.6	1.3	1.2
FOSA	1–1000	0.2	0.2	0.2
NMeFOSA	10–1000	3.8	4.0	4.0
NEtFOSA	10–1000	4.6	4.0	4.0
NMeFOSAA	4–1000	1.2	1.2	1.9
NEtFOSAA	10–1000	4.3	2.5	3.3
PFMPA	1–1000	0.3	0.2	0.4
PFMBA	1–1000	0.4	0.3	0.4
NFDHA	20–1000	10.0	8.8	7.9

Table II: LOQ* and LOD Values for PFAS in Tap, Bottled, and POTW Water (cont.)

	Linearity Range	LOD (ng/L)		
Analytes	**(ng/L)**	**Tap Water**	**Bottled Water**	**POTW**
HFPO-DA	10–1000	3.8	3.3	5.2
ADONA	1–1000	0.1	0.1	0.2
PFEESA	1–1000	0.1	0.1	0.1
9Cl-PF3ONS	1–1000	0.2	0.2	0.2
11Cl-PF3OUdS	1–1000	0.2	0.1	0.3

*LOQ = concentration of the lowest calibration standard solution.

Accuracy and Precision

Three batches of samples were analyzed on different days, totaling nine replicates for each fortification level. The average recoveries and relative standard deviations (RSD) are presented in Tables III-V. All analytes exhibited good recovery values within the range of 70–130% across all fortification levels. Satisfactory method precision was demonstrated by %RSD values of <20%. Additionally, the results indicated that all extracted internal standards (EIS) had recovery values within 30% of the nominal concentration.

Table III: Accuracy and Precision Results for Tap Water Samples

Analytes	**Average Recovery (RSD, %), n=9**				
	Fortified Concentration (ng/L)				
	2	**4**	**10**	**50**	**250**
^{13}C-TFA	-	-	110 (9.61)	89.8 (5.10)	91.5 (2.04)
PFPrA	102 (6.88)	100 (3.72)	98.6 (6.71)	105 (8.98)	101 (6.85)
PFBA	108 (8.17)	107 (7.28)	104 (9.06)	104 (5.13)	103 (7.20)
PFPeA	109 (9.02)	115 (2.98)	105 (7.90)	111 (6.13)	107 (8.17)
PFHxA	88.2 (8.36)	99.8 (8.44)	92.0 (6.26)	105 (7.96)	103 (8.06)
PFHpA	88.7 (6.44)	101 (8.38)	86.2 (6.26)	95.1 (6.97)	91.2 (7.28)
PFOA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)
PFNA	108 (7.53)	110 (5.46)	97.8 (3.83)	104 (6.23)	99.5 (8.40)
PFDA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.6 (9.37)	95.8 (8.66)
PFUnA	110 (14.0)	109 (11.4)	108 (14.6)	107 (8.05)	95.5 (7.14)
PFDoA	103 (14.2)	104 (9.98)	96.5 (14.1)	101 (5.81)	102 (3.95)
PFTrDA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	86.5 (3.05)
PFTeDA	103 (6.04)	91.2 (10.6)	90.1 (8.18)	86.5 (10.9)	93.4 (4.21)
TFMS	112 (9.16)	108 (10.4)	97.3 (8.16)	105 (6.42)	104 (7.25)
PFEtS	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)
PFPrS	109 (9.22)	114 (7.23)	99.3 (8.52)	106 (7.76)	107 (7.32)
PFBS	109 (6.89)	109 (10.7)	94.3 (8.59)	101 (5.45)	102 (6.53)
PFPeS	104 (5.33)	113 (3.79)	97.9 (4.84)	106 (7.29)	104 (9.30)
PFHxS	84.1 (16.7)	105 (7.50)	90.8 (12.8)	104 (4.37)	102 (3.77)
PFHpS	111 (7.72)	108 (6.82)	99.7 (7.04)	108 (5.65)	105 (6.59)
PFOS	112 (7.55)	106 (7.10)	95.5 (10.2)	107 (7.39)	107 (6.83)
PFNS	117 (13.8)	104 (10.7)	91.9 (10.2)	104 (4.92)	99.1 (6.15)
PFDS	107 (15.0)	106 (8.32)	94.4 (17.2)	97.9 (11.1)	94.4 (3.40)
PFUdS	108 (13.5)	92.4 (15.5)	88.1 (1.58)	85.7 (2.32)	93.4 (3.38)
PFDoS	114 (13.4)	96.5 (11.9)	85.9 (5.01)	83.5 (11.6)	93.9 (2.71)
PFTrDS	91.0 (17.8)	72.5 (18.2)	72.0 (13.8)	72.9 (14.4)	84.6 (3.55)

Table III: Accuracy and Precision Results for Tap Water Samples (cont.)

Analytes	Average Recovery (RSD, %), n=9				
	Fortified Concentration (ng/L)				
	2	4	10	50	250
4:2 FTS	106 (8.72)	109 (8.83)	90.2 (13.0)	103 (9.27)	101 (10.2)
6:2 FTS	108 (8.94)	112 (6.81)	96.4 (17.3)	102 (4.45)	99.4 (7.60)
8:2 FTS	111 (13.4)	107 (12.1)	92.6 (11.3)	101 (5.28)	94.6 (5.86)
3:3 FTCA	-	-	-	83.7 (8.92)	82.6 (5.64)
5:3 FTCA	-	120 (6.07)	106 (12.0)	103 (8.99)	103 (8.78)
7:3 FTCA	-	107 (8.36)	91.4 (16.2)	105 (7.98)	106 (7.36)
FOSA	107 (9.41)	107 (5.20)	95.4 (8.59)	95.7 (4.63)	95.5 (6.05)
NMeFOSA	-	-	106 (12.9)	105 (6.50)	104 (9.06)
NEtFOSA	-	-	105 (14.7)	112 (4.89)	105 (7.49)
NMeFOSAA	-	123 (7.06)	88.5 (14.6)	102 (8.37)	99.5 (7.89)
NEtFOSAA	-	-	106 (10.9)	96.1 (11.1)	92.6 (5.99)
PFMPA	99.0 (15.9)	103 (10.6)	90.2 (6.11)	103 (8.22)	103 (8.21)
PFMBA	105 (7.67)	107 (8.82)	95.8 (7.63)	107 (6.27)	103 (7.01)
NFDHA	-	-	-	115 (9.78)	115 (8.61)
HFPO-DA	-	-	115 (13.1)	108 (5.70)	106 (7.76)
ADONA	94.4 (11.5)	109 (5.71)	98.4 (7.58)	106 (7.56)	104 (8.94)
PFEESA	102 (8.88)	113 (4.75)	98.8 (7.11)	108 (6.06)	105 (8.76)
9Cl-PF3ONS	100 (8.99)	107 (4.54)	95.7 (2.88)	105 (6.31)	104 (9.09)
11Cl-PF3OUdS	88.2 (12.3)	96.8 (7.36)	87.8 (8.81)	96.7 (4.65)	95.1 (4.17)

Table IV: Accuracy and Precision Results for Bottled Water Samples

Analytes	Average Recovery (RSD, %), n=9				
	Fortified Concentration (ng/L)				
	2	4	10	50	250
^{13}C-TFA	-	-	104 (15.6)	99.4 (8.09)	103 (7.45)
PFPrA	96.7 (5.90)	101 (6.64)	97.9 (6.08)	107 (3.13)	114 (7.77)
PFBA	96.4 (5.81)	94.7 (8.49)	90.2 (6.21)	103 (6.58)	112 (6.78)
PFPeA	93.2 (8.17)	99.1 (13.9)	95.5 (10.2)	105 (5.95)	111 (8.53)
PFHxA	100 (13.1)	96.5 (9.30)	91.0 (8.66)	99.5 (4.92)	108 (8.20)
PFHpA	96.9 (8.26)	92.3 (3.67)	87.3 (4.82)	97.9 (6.15)	104 (5.56)
PFOA	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)
PFNA	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)
PFDA	101 (11.9)	96.9 (6.73)	92.2 (8.25)	100 (5.31)	109 (8.86)
PFUnA	109 (10.6)	104 (8.74)	90.6 (11.0)	97.7 (9.52)	105 (5.72)
PFDoA	97.5 (5.77)	95.2 (4.93)	78.5 (1.90)	85.1 (5.14)	103 (5.68)
PFTrDA	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.2 (9.05)
PFTeDA	116 (3.97)	109 (6.74)	90.5 (8.94)	93.5 (18.2)	114 (5.74)
TFMS	101 (9.04)	107 (6.85)	96.7 (4.30)	106 (2.88)	112 (7.17)
PFEtS	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.01)	111 (7.50)
PFPrS	101 (10.4)	106 (7.28)	98.1 (6.13)	108 (1.98)	111 (6.44)
PFBS	103 (13.7)	101 (9.53)	85.8 (5.98)	101 (2.59)	108 (8.90)
PFPeS	95.6 (10.3)	102 (5.75)	92.3 (5.80)	102 (2.79)	109 (9.05)
PFHxS	110 (8.75)	99.4 (8.55)	90.9 (11.8)	96.5 (2.79)	103 (8.67)
PFHpS	99.8 (8.79)	94.2 (8.80)	90.9 (7.75)	105 (5.47)	110 (8.06)
PFOS	111 (12.9)	93.5 (8.65)	91.8 (9.70)	106 (6.02)	109 (6.08)
PFNS	102 (12.8)	90.8 (15.2)	86.8 (10.4)	101 (8.80)	112 (6.74)
PFDS	96.9 (19.1)	105 (12.1)	89.3 (11.4)	103 (10.2)	107 (5.82)
PFUdS	101 (18.5)	100 (16.3)	87.3 (10.5)	92.9 (14.5)	105 (5.71)
PFDoS	114 (7.52)	106 (10.7)	84.8 (14.6)	90.7 (14.5)	108 (7.49)
PFTrDS	126 (1.13)	109 (14.4)	86.2 (17.1)	88.4 (19.0)	112 (5.51)
4:2 FTS	103 (11.0)	98.1 (16.3)	88.6 (10.1)	94.3 (4.60)	108 (10.3)
6:2 FTS	108 (6.44)	95.2 (13.7)	88.0 (13.3)	97.7 (9.07)	106 (7.53)
8:2 FTS	112 (10.5)	109 (5.14)	95.0 (3.66)	103 (3.89)	109 (8.72)
3:3 FTCA	-	-	-	95.4 (10.4)	105 (6.94)
5:3 FTCA	-	103 (8.78)	94.3 (12.1)	105 (6.32)	109 (8.62)
7:3 FTCA	-	115 (5.16)	98.9 (9.92)	102 (8.75)	110 (7.09)
FOSA	111 (7.75)	103 (8.73)	91.6 (3.72)	104 (3.88)	112 (7.06)
NMeFOSA	-	-	105 (8.01)	101 (10.8)	115 (5.88)
NEtFOSA	-	-	121 (9.43)	100 (13.0)	110 (8.06)
NMeFOSAA	-	109 (12.1)	106 (10.1)	92.9 (13.8)	104 (9.53)
NEtFOSAA	-	-	101 (15.7)	100 (10.3)	105 (8.11)
PFMPA	99.5 (9.11)	99.6 (11.0)	92.2 (4.76)	103 (4.57)	112 (7.29)
PFMBA	92.3 (9.01)	102 (9.17)	90.1 (8.50)	100 (4.51)	109 (7.70)
NFDHA	-	-	-	111 (6.59)	111 (7.73)
HFPO-DA	-	-	106 (12.3)	101 (10.1)	112 (6.19)
ADONA	97.3 (11.4)	101 (6.41)	91.6 (3.35)	100 (4.26)	107 (10.3)
PFEESA	101 (8.99)	105 (8.33)	93.6 (5.67)	102 (3.03)	110 (9.16)
9Cl-PF3ONS	100 (8.53)	101 (7.16)	90.2 (4.28)	105 (4.22)	112 (8.47)
11Cl-PF3OUdS	91.0 (11.1)	96.8 (6.21)	85.0 (4.94)	100 (11.8)	108 (5.75)

Table V: Accuracy and Precision Results for POTW Water Samples

Analytes	Average Recovery (RSD, %), n=9				
	Fortified Concentration (ng/L)				
	2	4	10	50	250
^{13}C-TFA	-	-	-	104 (9.46)	108 (8.57)
PFPrA	99.7 (3.96)	101 (11.9)	99.7 (9.18)	109 (3.90)	113 (1.99)
PFBA	100 (8.79)	98.0 (6.35)	99.2 (7.44)	104 (8.77)	106 (4.82)
PFPeA	104 (9.17)	110 (6.19)	101 (5.66)	109 (2.80)	116 (2.05)
PFHxA	91.8 (12.1)	98.5 (10.0)	92.9 (9.53)	101(3.30)	103 (6.53)
PFHpA	115 (5.90)	111 (8.16)	97.6 (6.90)	97.1 (5.74)	99.5 (4.58)
PFOA	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFNA	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFDA	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (3.49)
PFUnA	106 (12.6)	102 (9.97)	95.1 (8.99)	97.7 (3.90)	101 (6.28)
PFDoA	98.4 (16.6)	107 (9.05)	93.0 (3.83)	111 (11.9)	112 (12.2)
PFTrDA	94.1 (15.9)	94.4 (13.2)	79.7 (9.70)	90.3 (11.3)	96.7 (13.5)
PFTeDA	113 (12.7)	97.5 (18.9)	82.9 (12.4)	95.2 (13.3)	104 (18.0)
TFMS	104 (15.6)	108 (7.24)	94.9 (5.45)	94.9 (5.32)	96.0 (1.88)
PFEtS	93.1 (15.3)	108 (7.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.89)
PFPrS	105 (9.36)	114 (4.26)	91.5 (4.65)	99.5 (3.97)	102 (4.11)
PFBS	93.4 (12.3)	107 (6.18)	91.8 (7.88)	100 (5.38)	105 (6.90)
PFPeS	102 (13.3)	113 (8.62)	90.2 (4.13)	99.7 (2.68)	103 (4.89)
PFHxS	89.3 (17.7)	95.2 (8.96)	100 (12.7)	98.6 (6.64)	105 (6.83)
PFHpS	85.1 (16.1)	109 (9.59)	96.0 (7.89)	106 (3.56)	111 (2.42)
PFOS	79.0 (18.5)	107 (6.22)	86.1 (8.60)	101 (7.45)	110 (4.62)
PFNS	104 (9.28)	92.5 (14.2)	85.0 (9.21)	98.9 (4.69)	108 (3.95)
PFDS	109 (11.1)	106 (11.0)	92.6 (13.1)	101 (7.80)	101 (4.53)
PFUdS	95.6 (18.8)	102 (8.93)	77.9 (7.72)	90.2 (8.96)	96.4 (7.42)
PFDoS	111 (9.06)	95.0 (10.7)	80.0 (13.7)	83.1 (8.45)	99.5 (12.4)
PFTrDS	94.1 (14.9)	107 (15.1)	79.8 (12.5)	85.2 (12.4)	96.4 (15.1)
4:2 FTS	122 (5.65)	113 (4.55)	91.5 (7.23)	100 (3.92)	104 (5.01)
6:2 FTS	110 (10.3)	109 (12.0)	93.1 (10.0)	95.2 (7.65)	102 (5.42)
8:2 FTS	116 (10.0)	102 (16.1)	89.3 (12.6)	101 (5.81)	106 (3.58)
3:3 FTCA	-	-	-	-	-
5:3 FTCA	-	115 (7.21)	104 (8.42)	108 (7.38)	108 (5.05)
7:3 FTCA	-	122 (6.51)	92.6 (18.9)	99.7 (9.76)	105 (5.88)
FOSA	110 (8.60)	101 (10.9)	79.1 (4.69)	89.4 (5.01)	96.6 (6.21)
NMeFOSA	-	-	110 (18.8)	92.1 (10.3)	116 (3.63)
NEtFOSA	-	-	116 (9.55)	103 (11.9)	104 (11.6)
NMeFOSAA	-	118 (10.3)	98.2 (12.1)	98.8 (9.01)	100 (6.96)
NEtFOSAA	-	-	106 (10.9)	96.1 (11.1)	92.6 (5.99)
PFMPA	94.3 (11.0)	105 (7.54)	88.4 (7.38)	98.1 (3.33)	102 (4.66)
PFMBA	85.9 (7.17)	99.9 (10.5)	87.0 (8.69)	101 (4.18)	109 (5.75)
NFDHA	-	-	-	90.5 (13.9)	114 (3.75)
HFPO-DA	-	-	111 (6.49)	92.5 (16.2)	95.6 (12.4)
ADONA	90.8 (12.6)	116 (5.19)	90.9 (4.78)	103 (3.45)	106 (5.37)
PFEESA	89.4 (12.7)	113 (8.74)	92.8 (1.95)	102 (1.80)	108 (3.07)
9Cl-PF3ONS	95.4 (10.3)	102 (9.40)	85.4 (6.64)	101 (4.97)	106 (4.67)
11Cl-PF3OUdS	95.1 (8.68)	105 (3.84)	82.7 (4.07)	97.5 (7.82)	101 (6.36)

Measurement of 45 Targeted PFAS in Potable and Non-Potable Waters

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, including ultrashort-chain PFAS, in a wide range of water matrices (Table VI).

Table VI: Measurement of 45 Targeted PFAS in Various Water Matrices (nd = non-detected)

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	8540	13.3	62.8	1.13	100	7.22	nd	12.0	20.8	nd	14.0	5709

Conclusions

A simple and reliable dilute-and-shoot workflow was established in this study to provide a unique solution that incorporates ultrashort-chain PFAS into a comprehensive analysis of PFAS in various water matrices. Use of an Ultra IBD Inert column, which is a polar-embedded alkyl phase column with an inert coating on the hardware, ensured that the method was sensitive, accurate, and precise. Most important, this method can serve as a valuable tool for monitoring these emergent PFAS in environmental water systems and assist in generating guidelines for future regulatory actions. Visit www.restek.com/PFAS for additional products, methods, and technical resources supporting PFAS analysis.

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Ultra IBD Inert HPLC Column

Catalog No.	Product Name	Units
9175312-T	Ultra IBD Inert HPLC Column, 3 µm, 100 mm x 2.1 mm	ea.



PFAS Delay Column

- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

Catalog No.	Product Name	Units
27854	PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column	ea.



Ultra IBD HPLC Column

Catalog No.	Product Name	Units
9175362	Ultra IBD HPLC Column, 3 µm, 150 mm x 2.1 mm	ea.

PFAS 28 Calibration Standard



Contains:
11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)(39108-34-4)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS) (757124-72-4)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS) (27619-97-2)
4,8-dioxa-3H-perfluorononanoic acid (ADONA) (919005-14-4)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)
2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6)
N-ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)* (2991-50-6)
N-methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)* (2355-31-9)
Perfluoro-1-decanesulfonic acid (PFDS) (335-77-3)
Perfluoro-1-nonanesulfonic acid (PFNS) (68259-12-1)
Perfluoro-1-octanesulfonamide (FOSA) (754-91-6)
Perfluoro-1-pentanesulfonic acid (PFPeS) (2706-91-4)
Perfluorobutanesulfonic acid (PFBS) (375-73-5)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFDOA) (307-55-1)
Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)
Perfluoroheptanoic acid (PFHpA) (375-85-9)
Perfluorohexanesulfonic acid (PFHxS)* (355-46-4)
Perfluorohexanoic acid (PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Heptadecafluorooctanesulfonic acid (PFOS)* (1763-23-1)
Perfluorooctanoic acid (PFOA)* (335-67-1)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluorotetradecanoic acid (PFTeDA) (376-06-7)
Perfluorotridecanoic acid (PFTrDA) (72629-94-8)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)

*Technical grade compound containing both branched and linear isomers; see certificate for details.

Catalog No.	Concentration	Solvent	Volume	Units
30734	1 µg/mL	Methanol (1 mM KOH)	1 mL/ampul	ea.

Norm-Ject Syringe



Catalog No.	Product Name	Units
22775	Norm-Ject Plastic Syringe, 10 mL Luer Lock Tip	100-pk.

Polypropylene Syringe Filter



Catalog No.	Product Name	Units
28936	25 mm Syringe Filter, 0.45 µm, Polypropylene, Black, Luer-Lock	100-pk.

Polypropylene Centrifuge Tubes



Catalog No.	Product Name	Units
25846	Empty Centrifuge Tubes, 50 mL, Polypropylene w/Cap	50-pk.

A Novel Approach for Ultrashort-Chain PFAS Analysis in Water Samples

Direct, Simultaneous Determination of Ultrashort-Chain, Alternative, and Legacy PFAS

By Dr. Shun-Hsin Liang

Abstract

As interest grows in monitoring a wider range of PFAS in both potable and non-potable waters, efficient methodology becomes more important. Here, we developed a unique approach that provides concurrent ultrashort-chain PFAS analysis along with alternative and legacy PFAS, allowing C2, C3, C4, C6, C8, and alternative compounds to be tested together instead of through separate methods. Results from verification experiments are presented.



Related Products

- *Raptor Polar X, 2.7 µm, 50 x 2.1 mm LC Column*

Introduction

Ultrashort-chain, or C2 and C3, per- and polyfluoroalkyl substances (PFAS) are small and very polar compounds that contribute to at least 40% of the total PFAS detected in environmental waters (e.g., rain, river, and groundwaters) [1, 2, 3]. Ultrashort-chain PFAS include trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoroethane sulfonate (PFEtS), and perfluoropropane sulfonate (PFPrS), with TFA being the most abundant and difficult to analyze chromatographically. Current practices for PFAS monitoring do not address the analysis of these newly trending ultrashort-chain compounds due to their insufficient retention on typical reversed-phase (RP) columns. On the other hand, analytical methods implementing anion-exchange chromatography often show too much retention and poor chromatographic performance for ultrashort-chain PFAS. The challenge becomes even greater for simultaneous monitoring of ultrashort-chain, alternative, and legacy PFAS in a single method.

To overcome this limitation, we used a unique hybrid HILIC/ion-exchange column (Raptor Polar X) to develop a fast and simple LC-MS/MS method for comprehensive analysis of C2, C3, C4, C6, C8, and alternative PFAS. Because the column employs balanced, multimode retention mechanisms, ultrashort-chain PFAS and long-chain PFAS can all be analyzed in a single isocratic run. This direct injection method was evaluated by precision and accuracy analysis of fortified water samples, including tap water, river water, groundwater, and water from publicly owned treatment works (POTW, sewage effluent). As demonstrated here, the method provides convenient setup and high-throughput conditions for water testing labs interested in adding ultrashort-chain PFAS analysis to the same workflow used to measure alternative and legacy PFAS.

Experimental

Chromatographic Method:

The chromatographic conditions were as follows. The transitions and internal standard used for each analyte are provided in Table I.

Column:	Raptor Polar X (2.7 µm, 50 mm x 2.1 mm ID [cat.# 9311A52])
Column temp.:	40 °C
Injection volume:	10 µL
Mobile phase A:	Water, 10 mM ammonium formate, 0.05% formic acid
Mobile phase B:	Acetonitrile:methanol (60:40), 0.05% formic acid

Time (min)	%B
0.00	85
8.00	85

Flow rate:	0.5 mL/min
Ion mode:	Negative ESI
Mode:	MRM

Update: To achieve more stable and robust performance with Polar X columns, the recommended LC conditions have changed from those that were used here. The new recommended conditions are an aqueous mobile phase (mobile phase A) consisting of 5 mM or 10 mM ammonium formate with 0.1% formic acid in water and an organic mobile phase (mobile phase B) of 100% acetonitrile. Higher ammonium formate concentrations will result in reduced analyte retention. Therefore, laboratories can optimize retention by adjusting both the ammonium formate concentration and the flow rate to best meet their analytical requirements. In addition, it is recommended that labs evaluate different isocratic conditions by varying the amount of mobile phase B between 70% and 85% acetonitrile. This approach can help labs identify the most suitable separation conditions for their specific target analytes and sample matrices of interest.

Table I: Analyte MS Transitions for Ultrashort-Chain PFAS Analysis Concurrent with Alternative and Legacy PFAS in Water Samples.

Analyte	Precursor Ion	Product Ion	IS for Quantification
TFA	113.03	69.01	$^{13}C_2$-PFHxA
PFPrA	163.03	119.01	$^{13}C_2$-PFHxA
PFBA	212.97	168.97	$^{13}C_2$-PFHxA
PFHxA	312.97	268.90	$^{13}C_2$-PFHxA
PFOA	412.90	368.91	$^{13}C_2$-PFOA
HFPO-DA	284.97	168.92	$^{13}C_2$-PFOA
ADONA	376.90	250.93	$^{13}C_2$-PFOA
PFEtS	198.98	79.92	$^{13}C_3$-PFBS
PFPrS	248.97	79.98	$^{13}C_3$-PFBS
PFBS	298.97	79.97	$^{13}C_3$-PFBS
PFHxS	398.90	79.97	$^{13}C_3$-PFBS
PFOS	498.84	79.97	$^{13}C_4$-PFOS
9Cl-PF3ONS	530.78	350.85	$^{13}C_4$-PFOS
11Cl-PF3OUdS	630.78	450.80	$^{13}C_4$-PFOS
$^{13}C_2$-PFHxA	314.97	269.93	—
$^{13}C_2$-PFOA	414.90	369.87	—
$^{13}C_3$-PFBS	301.90	79.97	—
$^{13}C_4$-PFOS	502.84	79.97	—

Sample Preparation

In a polypropylene vial (used to mitigate background contamination), 250 µL of each water sample was mixed with 250 µL of methanol and 5 µL of internal standard solution (10 ng/mL of $^{13}C_2$-PFHxA, $^{13}C_2$-PFOA, $^{13}C_3$-PFBS, $^{13}C_4$-PFOS in methanol). The vial was capped with a polyethylene cap (again, to reduce background contamination) for injection and analysis.

Calibration standards were prepared by using deionized water (generated by a Thermo Scientific Barnstead E-Pure system) and fortifying it with 14 analytes at a range of 10–800 ng/L. The calibration standard solutions were then diluted 1:1 in methanol following the sample preparation procedure above.

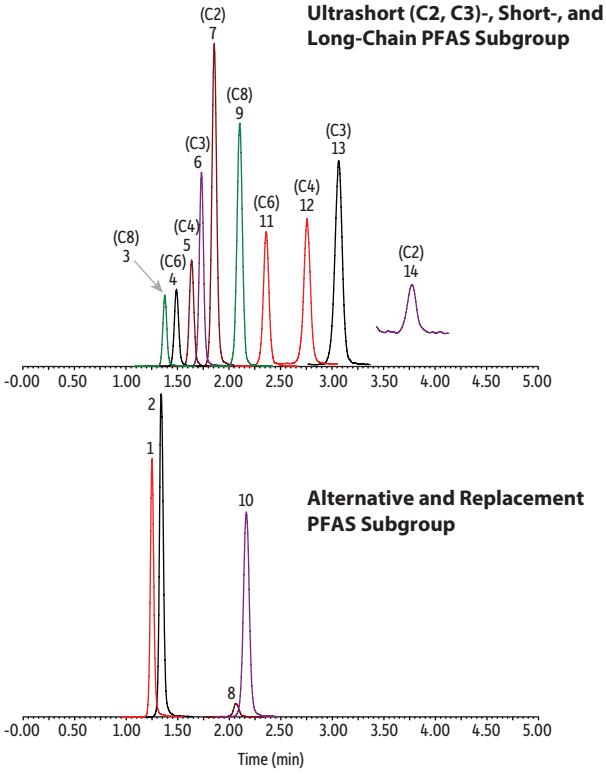
A tap water sample from the Restek facility and three water samples (Chicago river water, groundwater, and POTW effluent water) supplied by the United States Environmental Protection Agency (U.S. EPA) were fortified at 40 and 160 ppt. Blank and fortified water samples were diluted 1:1 in methanol as above for chromatographic analysis and quantified with the calibration standards. For TFA measurement in groundwater, the sample was diluted fivefold with deionized water before fortification at 40 and 160 ppt due to its high TFA concentration.

Results & Discussion

Chromatographic Performance

An isocratic elution was established that produced a fast, simple ultrashort-chain PFAS analysis along with alternative and legacy PFAS in water samples. All analytes eluted in 4 minutes with balanced retention and good peak shapes (Figure 1). No matrix interference was observed in any of the water samples using an 8-minute cycling time. As will be discussed, the approximately 4-minute hold after the last eluting compound was shown to be necessary to avoid possible matrix interferences.

Figure 1: Chromatogram of a 400 ng/L standard.



Peaks	t_R (min)	Conc. (ng/L)	Precursor Ion	Product Ion
1. 11-Chloroeicosafluoro-3-oxanonane-1-sulfonate (11CL-PF3OUdS)	1.25	400	630.78	450.80
2. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	1.34	400	530.78	350.85
3. Perfluorooctanesulfonic acid (PFOS)	1.38	400	498.84	79.97
4. Perfluorohexanesulfonic acid (PFHxS)	1.49	400	398.90	79.97
5. Perfluorobutanesulfonic acid (PFBS)	1.64	400	298.97	79.97
6. Perfluoropropanesulfonic acid (PFPrS)	1.73	400	248.97	79.98
7. Perfluoroethanesulfonic acid (PFEtS)	1.86	400	198.98	79.92
8. Hexafluoropropylene oxide dimer acid (HFPO-DA)	2.06	400	284.97	168.92
9. Perfluorooctanoic acid (PFOA)	2.11	400	412.90	368.91
10. Ammonium 4,8-dioxa-3H-perfluorononanoate (ADONA)	2.15	400	376.90	250.93
11. Perfluorohexanoic acid (PFHxA)	2.36	400	312.97	268.90
12. Perfluorobutanoic acid (PFBA)	2.76	400	212.97	168.97
13. Perfluoropropionic acid (PFPrA)	3.06	400	163.03	119.01
14. Trifluoroacetic acid (TFA)	3.77	400	113.03	69.01

Linearity

The calibration range is 20–800 ppt for TFA and 10–800 ppt for all other analytes. Four internal standards were evaluated to determine the best fitting standard curve for the different analytes. All compounds showed acceptable linearity with r^2 values >0.996, and deviations <20% using 1/x weighted quadratic regression.

Accuracy & Precision

In our initial experiments, matrix interference for the TFA signal was observed for water sample analysis performed using a 5-minute isocratic run. Different analysis times were tested, and it was determined that an 8-minute run time was needed to avoid matrix interference for all analytes. The isocratic hold time may need to be modified based on the specific instrumentation used and/or samples analyzed.

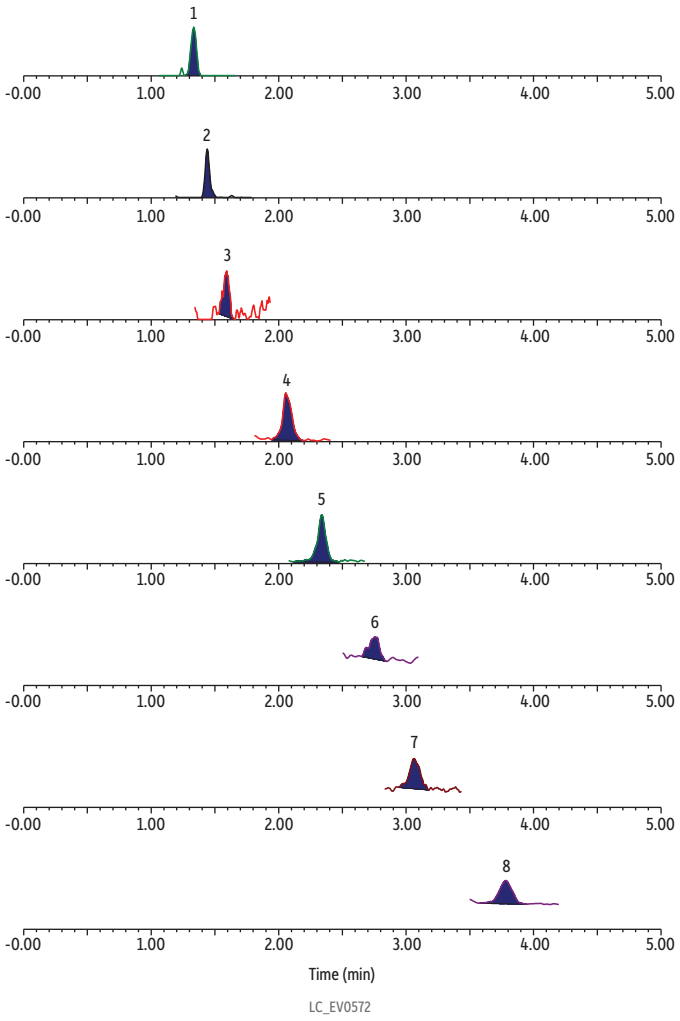
The blank water samples showed various levels of TFA, C3, C4, C6, and C8 PFAS with no detectable ADONA, HFPO-DA, 9Cl-PF3ONS, and 11Cl-PF3OUdS (Table II). An example chromatogram for ultrashort-chain PFAS analysis with concurrent determination of alternative and legacy PFAS in a blank POTW sample is shown in Figure 2.

Table II: Detectable analytes in blank water samples.

	Detected Concentration (ng/L)													
Samples	**TFA**	**PFPrA**	**PFBA**	**PFHxA**	**PFOA**	**HFPO-DA**	**ADONA**	**PFEtS**	**PFPrS**	**PFBS**	**PFHxS**	**PFOS**	**9Cl-PF3ONS**	**11Cl-PF3OUdS**
Tap Water	164.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
River Water	193.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Groundwater	1425	ND	ND	ND	5.4	ND	ND	ND	ND	6.7	3.9	ND	ND	ND
POTW Water	352.8	9.6	15.3	93.5	20.4	ND	ND	ND	ND	6.8	6.7	9.6	ND	ND

ND: non-detectable

Figure 2: Detectable PFAS in blank POTW water.



Peaks	t_R (min)	Precursor Ion	Product Ion
1. Perfluorooctanesulfonic acid (PFOS)	1.35	498.84	79.97
2. Perfluorohexanesulfonic acid (PFHxS)	1.45	398.90	79.97
3. Perfluorobutanesulfonic acid (PFBS)	1.58	298.97	79.97
4. Perfluorooctanoic acid (PFOA)	2.05	412.90	368.91
5. Perfluorohexanoic acid (PFHxA)	2.34	312.97	268.90
6. Perfluorobutanoic acid (PFBA)	2.76	212.97	168.97
7. Perfluoropropionic acid (PFPrA)	3.06	163.03	119.01
8. Trifluoroacetic acid (TFA)	3.78	113.03	69.01

For accuracy determination (percent recovery), the measured amounts in the fortified samples were adjusted to account for the concentration in blank samples. Water samples were fortified at low and high concentration in duplicate for each analytical batch. A total of three analytical batches were measured on different days. Table III shows the accuracy and precision results calculated from the collection of all three batches of data. The method accuracy was demonstrated by recovery values being within 30% of the nominal concentration for both fortified and LLOQ levels in water samples. The %RSD was <20%, indicating acceptable method precision for ultrashort-chain PFAS analysis concurrent with alternative and legacy compounds in water.

Table III: Method Accuracy and Precision

	Average %Accuracy (%RSD)								
Matrices	**Tap Water**		**River Water**		**Groundwater****		**POTW Water**		**Deionized Water**
Conc. (ng/L)	**40**	**160**	**40**	**160**	**40**	**160**	**40**	**160**	**10* (LLOQ)**
TFA	106 (16.9)	97.9 (7.10)	97.4 (10.8)	97.6 (6.12)	97.5 (14.5)	103 (8.87)	102 (17.1)	96.4 (7.33)	107 (3.55)
PFPrA	95.1 (4.08)	105 (3.48)	94.5 (6.85)	104 (2.36)	103 (9.37)	105 (8.34)	91.8 (4.90)	104 (7.09)	109 (1.61)
PFBA	106 (6.80)	117 (3.18)	105 (7.40)	114 (4.91)	111 (2.48)	120 (3.27)	106 (6.58)	114 (4.85)	104 (4.91)
PFHxA	93.3 (7.41)	111 (2.61)	91.8 (11.34)	103 (4.55)	102 (6.62)	109 (7.11)	103 (8.37)	108 (3.13)	115 (1.64)
PFOA	100 (4.24)	107 (3.14)	103 (6.71)	105 (2.64)	92.6 (3.85)	107 (3.09)	102 (4.57)	109 (3.64)	106 (3.28)
HFPO-DA	95.7 (11.9)	108 (9.05)	86.6 (8.97)	104 (5.45)	94.1 (18.6)	105 (9.35)	95.2 (8.49)	106 (9.23)	102 (16.8)
ADONA	106 (3.75)	116 (2.38)	100 (6.86)	110 (4.59)	104 (4.91)	113 (5.23)	111 (5.26)	115 (2.65)	105 (4.76)
PFEtS	94.8 (9.68)	110 (5.39)	89.4 (7.43)	102 (9.76)	96.5 (4.09)	108 (6.11)	104 (8.18)	109 (5.23)	99.8 (9.85)
PFPrS	104 (4.97)	115 (4.19)	95.0 (3.87)	107 (4.26)	106 (10.6)	114 (3.36)	111 (4.88)	114 (2.96)	108 (3.28)
PFBS	97.4 (10.1)	113 (3.97)	93.6 (5.24)	104 (4.19)	97.8 (4.47)	107 (4.23)	94.1 (10.7)	108 (4.48)	100 (11.0)
PFHxS	99.4 (15.7)	114 (3.56)	94.3 (9.79)	104 (5.28)	95.2 (5.63)	112 (3.20)	104 (8.19)	111 (4.07)	107 (11.7)
PFOS	104 (7.54)	107 (7.69)	103 (8.43)	105 (7.23)	97.3 (14.9)	110 (4.84)	109 (7.47)	108 (7.53)	102 (4.20)
9Cl-PF3ONS	98.7 (3.52)	105 (8.35)	91.8 (7.66)	103 (5.68)	94.7 (9.83)	105 (8.90)	105 (6.76)	107 (8.27)	107 (4.31)
11Cl-PF3OUdS	106 (10.1)	113 (3.54)	95.0 (3.52)	113 (8.15)	107 (6.61)	112 (4.54)	119 (4.25)	120 (9.10)	98.2 (11.3)

*20 ng/L LLOQ for TFA
**Groundwater was diluted fivefold for TFA only

Conclusion

A simplified isocratic method was developed and verified for ultrashort-chain PFAS analysis along with alternative and legacy compounds in water samples. Due to the balanced, multimode retention of these analytes on a Raptor Polar X (2.7 µm) 50 x 2.1 mm column, the analytical method was demonstrated to be fast, rugged, and sensitive with acceptable accuracy and precision. This method is suitable for analytical labs wanting to expand their existing PFAS assays for potable or non-potable water to include C2 and C3 compounds.

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Raptor Polar X LC Columns



- Reliably analyze a wide variety of polar analytes (acidic, basic, and neutral) without time-consuming derivatization or complex ion pairing.
- Switch between HILIC and ion-exchange retention modes with simple mobile phase changes and short equilibration times.
- 2.7 µm Raptor core-shell particles provide UHPLC-like speed and efficiency on all makes and models of LC systems.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Storage Conditions: When not in use, Raptor Polar X columns must be kept in 100% acetonitrile. If using a buffered mobile phase, first flush thoroughly with 50:50 water:acetonitrile, then fill with acetonitrile for storage.

Pore Size: 90 Å
Particle: 2.7 µm superficially porous particle (SPP or "core-shell" particle) silica
Surface Area: 130 m^2/g
End-Cap: Proprietary
Carbon Load: Proprietary
USP Phase Code: NA
Phase Category: Proprietary
Ligand Type: Proprietary

Recommended Usage:
pH Range: 2.0–8.0
Maximum Temperature: 60 °C
Maximum Pressure: 600 bar/8700 psi

Properties:

- Excellent resolution and separation of a wide variety of polar compounds.
- Combines HILIC and ion-exchange retention mechanisms together in a single ligand.
- Broadly applicable for polar compound analysis spanning different industries and methods.

Switch to a Raptor Polar X column when:

- You are analyzing neutral, acidic, basic, or permanently charged polar compounds.
- Performing LC-MS/MS analysis of polar compounds.
- You are struggling to retain or elute polar compounds and considering ion chromatography.

ID	Length	qty.	cat.#
2.1 mm	50 mm	ea.	9311A52

Analysis of Ultrashort-Chain and Short-Chain (C1 to C4) PFAS in Water Samples

By Dr. Shun-Hsin Liang; Justin Steimling; Mike Chang

Abstract

Due to their ubiquitous occurrence in aquatic environments, measuring ultra-short-chain per- and polyfluoroalkyl substances (PFAS) in various source waters to monitor their presence and the potential for human exposure has become very important. However, with carbon chain lengths of less than C4, these small, highly polar compounds are difficult to analyze using standard PFAS tests that are based on reversed-phased liquid chromatography (RPLC). In this study, an accurate, reliable analytical LC-MS/MS method for PFAS in water was developed to specifically quantify C1 to C4 PFAS in both potable and non-potable sources. A direct injection workflow was implemented to simplify the testing process and to avoid potential contamination originating from poor sample preparation procedures.



Related Products

- *Raptor Polar X column*
- *Norm-Ject syringes*
- *Nylon syringe filters*

Introduction

Short-chain PFAS, usually referred to as C4 to C6 compounds, are considered to be less bioaccumulative and thus less toxic than long-chain PFAS. But the practice of using short-chain PFAS as replacements for long-chain PFAS has resulted in their increased environmental accumulation. While the use of short-chain PFAS is intentional, additional studies have shown greater prevalence and higher levels of ultrashort-chain PFAS in environmental aquatic systems (e.g., rain, river, snow, groundwater, and wastewater). The ultrashort-chain PFAS are C1, C2, and C3 compounds, including trifluoroacetic acid (TFA); perfluoropropanoic acid (PFPrA); trifluoromethanesulfonic acid (TFMS); perfluoroethanesulfonic acid (PFEtS); and perfluoropropanesulfonic acid (PFPrS).

Other than TFA, the potential sources and the environmental fate of ultrashort-chain PFAS have not been well studied, partly because limited analytical methods are available to properly monitor their occurrence. GC-MS can be used for the analysis of TFA, but it requires derivatization and does not allow the simultaneous analysis of C1 to C4 perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Anion-exchange LC columns can be used for C1 to C4 PFAS analysis, but due to very strong retention, they produce long runtimes (>20 minutes) and broad peak shapes. Supercritical fluid chromatography (SFC) works well, but the instrument investment can be significant. Reversed-phased liquid chromatography (RPLC) is the predominant analytical approach used to measure PFAS with carbon chain lengths of C4 and up, but RPLC does not have sufficient retention for shorter PFAS. For example, when using RPLC, C4 perfluorobutanoic acid (PFBA) is the least retained short-chain compound, and it often elutes as a broad, asymmetrical peak. Poor peak shape leads to decreased detection sensitivity and less accurate quantification.

To overcome the limitations of RPLC, we previously developed an analytical method for the quantification of C2 to C8 PFAS in both potable and non-potable waters [1]. In that study, we determined that a Raptor Polar X column, with its hybrid HILIC/ion-exchange functionality, was well suited for the simultaneous analysis of legacy, alternative, and ultrashort-chain PFAS. The chromatographic performance of the Raptor Polar X column for C2 to C8 PFAS was characterized by PFSAs eluting earlier than PFCAs. In addition, the compounds with shorter chain lengths were more strongly retained than those with longer chain lengths. In that method, the aqueous mobile phase was 10 mM ammonium formate and 0.05% formic acid in water, and the organic mobile phase was 0.05% formic acid in 60:40 acetonitrile:methanol. The addition of methanol in the organic mobile phase was needed to increase the retention of long-chain PFAS. Furthermore, by using different ratios of acetonitrile and methanol, analyte retention and separation could be controlled in order to mitigate matrix interference. But the use of methanol in the organic mobile phase also had the negative effect of reducing MS sensitivity. In the current study, the original LC-MS/MS method for PFAS in water was reexamined with a focus on only the C1 to C4 PFAS with the goal of increasing detection sensitivity while still maintaining robust performance. Method suitability was evaluated in terms of linearity, accuracy, and precision.

Experimental

Standard and Sample Preparation

The calibration standard solutions (0.4 mL) were prepared in reverse-osmosis (RO) water across a range of 2.5 to 800 ng/L (ppt) in polypropylene HPLC vials. Isotopically-labeled PFBA and perfluorobutanesulfonic acid (PFBS) were used as the internal standards (IS), and an aliquot of 4 µL from a 10 ng/mL working solution was mixed into the standard solutions. Tap water and bottled water were used directly without filtration. Wastewater samples (~10 mL) were filtered with Norm-Ject syringes (10 mL Luer Lock tip; cat.# 22775) and nylon syringe filters (30 mm, 0.45 µm; cat.# 23981) and collected in 50 mL polypropylene tubes. For accuracy and precision experiments, 1 mL 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 LC-MS/MS analysis.

LC-MS/MS Method for PFAS in Water

Analysis of PFAS in water samples was performed on a Waters ACQUITY I-class UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer. Compound tuning was conducted using negative mode electrospray ionization to determine the precursor and product ions. The product ion with the strongest intensity was used as the quantifier for concentration determination. A secondary product ion (qualifier) was used for confirmation of the targeted PFAS. There was no qualifier for TFA, PFPrA, and PFBA because only one product ion could be identified. The retention times and MS transition parameters for each analyte are provided in Table I.

Note that while a PFAS delay column is necessary to reduce background contamination of long-chain PFAS from the LC system in gradient elution RPLC methods, it is not needed for this isocratic analysis of the C1 to C4 compounds on a HILIC/ion-exchange Raptor Polar X column.

Column:	Raptor Polar X (2.7 µm, 50 mm x 2.1 mm ID [cat.# 9311A52])	
Column temp.:	40 °C	
Injection volume:	10 µL	
Mobile phase A:	Water, 10 mM ammonium formate, 0.1% formic acid	
Mobile phase B:	Acetonitrile:isopropanol (95:5), 0.1% formic acid	
	Time (min)	%B
	0.00	85
	7.00	85
Flow rate:	0.3 mL/min	
Ion mode:	Negative ESI	
Mode:	MRM	

Update: To achieve more stable and robust performance with Polar X columns, the recommended LC conditions have changed from those that were used here. The new recommended conditions are an aqueous mobile phase (mobile phase A) consisting of 5 mM or 10 mM ammonium formate with 0.1% formic acid in water and an organic mobile phase (mobile phase B) of 100% acetonitrile. Higher ammonium formate concentrations will result in reduced analyte retention. Therefore, laboratories can optimize retention by adjusting both the ammonium formate concentration and the flow rate to best meet their analytical requirements. In addition, it is recommended that labs evaluate different isocratic conditions by varying the amount of mobile phase B between 70% and 85% acetonitrile. This approach can help labs identify the most suitable separation conditions for their specific target analytes and sample matrices of interest.

Table I: MS Ion Transitions, Parameters, and Chromatographic Retention Time of Analytes.

Compounds	Retention Time (min)	Precursor Ion	Product Ions*	Cone (V)	Collision (V)
PFBS	1.01	298.97 [M-H]-	79.97/98.89	2	26/26
PFPrS	1.06	248.97 [M-H]-	79.91/98.91	2	24/24
PFEtS	1.12	198.90 [M-H]-	79.92/98.91	38	22/22
TFMS	1.25	148.97 [M-H]-	79.93/98.92	62	18/18
PFBA	1.93	213.03 [M-H]-	168.98	14	8
PFPrA	2.23	162.97 [M-H]-	119.02	22	10
TFA	3.05	113.03 [M-H]-	69.01	10	10
$^{13}C_3$-PFBS	1.01	301.97 [M-H]-	79.97	2	28
$^{13}C_4$-PFBA	1.93	217.03 [M-H]-	171.98	2	8

** Quantifier ion/qualifier ion*

Results and Discussion

Cleanliness of Solvents and Materials for Ultrashort-Chain PFAS Contamination

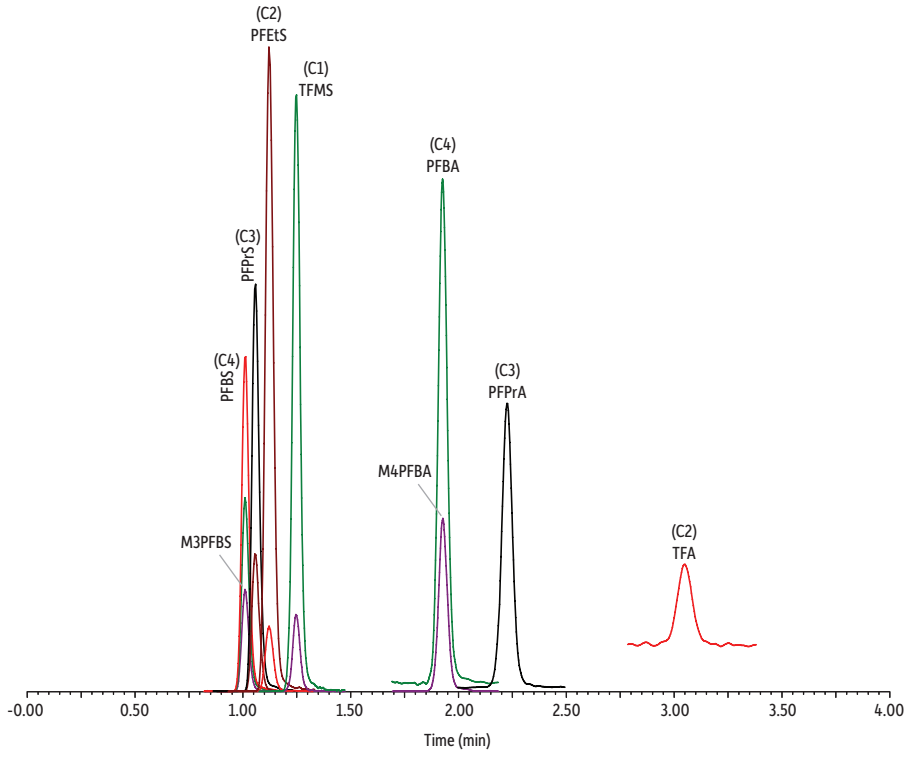
As ultrashort-chain PFAS are ubiquitous, it is particularly critical to examine the cleanliness of laboratory reagents and materials to ensure they are not contributing background contamination of ultrashort-chain PFAS. Upon testing several brands of LC/MS or HPLC grade water, acetonitrile, isopropanol, and methanol, it was shown that all these solvents had trace or higher amounts of TFA. There were variable amounts of detectable PFPrA and PFBA in the solvents as well. In the end, the commercial solvents with the lowest detectable levels of TFA, PFPrA, and PFBA were selected for the preparation of mobile phases in this study. Deionized water and reverse-osmosis (RO) purified water (generated at Restek) were also tested for cleanliness with regard to the target analytes. The results indicated that the RO water was free of PFPrA and PFBA and contained barely detectable levels of TFA. Therefore, the RO water was used for the preparation of mobile phases, working and final calibration standards, and sample dilutions.

Chromatographic Method Development

When testing a 100% acetonitrile organic mobile phase during method development, the detection signal for C1 to C4 PFAS increased significantly, but this was accompanied by low retention of all analytes. Proper retention was achieved by adjusting the flow rate and the formic acid concentration in the mobile phases. In addition, mixing a small portion of isopropanol with acetonitrile resulted in a stable chromatographic baseline for TFA. For the final method, an aqueous mobile phase of 10 mM ammonium formate plus 0.1% formic acid in RO water (mobile phase A) and an organic mobile phase of 0.1% formic acid in 95:5 acetonitrile:isopropanol (mobile phase B) were used with an isocratic elution under 85% B and flow rate of 0.3 mL/min.

Two isotopically labeled C4 PFAS standards, $^{13}C_3$-PFBS (M3PFBS) and $^{13}C_4$-PFBA (M4PFBA), were examined to determine if their use as internal standards was appropriate to compensate for matrix effects. The results showed that the accuracy of quantification was greatly improved by implementing M4PFBA and M3PFBS as the internal standard for the PFCA and PFSA compound classes, respectively. A 5-minute cycle time was appropriate for the standard solution prepared in RO water. However, a 7-minute cycle time was necessary for the analysis of tap water, bottled water, and wastewater to avoid matrix suppression upon repeat injections. Figure 1 shows a chromatogram obtained with the established LC method.

Figure 1: Chromatogram of a C1-C4 PFAS standard (400 ng/L) analyzed under the final LC-MS/MS method conditions.



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Perfluorobutanesulfonic acid (PFBS)	1.01	400	298.97	79.97	98.89
2. $^{13}C_3$-Perfluorobutanesulfonic acid (M3PFBS)	1.01	100	301.97	79.97	-
3. Perfluoropropanesulfonic acid (PFPrS)	1.06	400	248.97	79.91	98.91
4. Perfluoroethanesulfonic acid (PFEtS)	1.12	400	198.90	79.92	98.91
5. Trifluoromethanesulfonic acid (TFMS)	1.25	400	148.97	79.93	98.92
6. Perfluorobutanoic acid (PFBA)	1.93	400	213.03	168.98	-
7. $^{13}C_4$-Perfluorobutanoic acid (M4PFBA)	1.93	100	217.03	171.98	-
8. Perfluoropropanoic acid (PFPrA)	2.23	400	162.97	119.02	-
9. Trifluoroacetic acid (TFA)	3.05	400	113.03	69.01	-

Column Raptor Polar X (cat.# 9311A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 40 °C
Standard/Sample
Diluent: Reverse osmosis water
Conc.: 100 - 400 ng/L (ppt)
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 10 mM ammonium formate, 0.1% formic acid
B: 95:5 Acetonitrile:isopropanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	15	85
7.00	0.3	15	85

Detector Waters Xevo TQ-S
Ion Mode: ESI-
Mode: MRM
Instrument Waters ACQUITY UPLC I-Class
Sample Preparation The standard solution was prepared in reverse osmosis water at 400 ppt. An aliquot of 0.4 mL was transferred to a polypropylene HPLC vial and mixed with 4 µL of internal standard solution (10 ng/mL) containing M3PFBS and M4PFBA.
Notes To achieve more stable and robust performance with Polar X columns, the recommended LC conditions have changed from those that were used here. The new recommended conditions are an aqueous mobile phase (mobile phase A) consisting of 5 mM or 10 mM ammonium formate with 0.1% formic acid in water and an organic mobile phase (mobile phase B) of 100% acetonitrile. Higher ammonium formate concentrations will result in reduced analyte retention. Therefore, laboratories can optimize retention by adjusting both the ammonium formate concentration and the flow rate to best meet their analytical requirements. In addition, it is recommended that labs evaluate different isocratic conditions by varying the amount of mobile phase B between 70% and 85% acetonitrile. This approach can help labs identify the most suitable separation conditions for their specific target analytes and sample matrices of interest.

Linearity

With linear regression (1/x weighted), all analytes showed acceptable linearities with r^2 values >0.995 and deviations of <20% across calibration ranges of 2.5–800 ppt for the C1 to C4 PFSA compounds; 5.0–800 ppt for PFBA and PFPrA; and 20–800 ppt for TFA.

Method Accuracy and Precision

Tap water, bottled spring water, and publicly owned treatment works (POTW) wastewater (sewage treatment plant effluent) samples were fortified at 25, 50, and 175 ppt for all analytes. Three batches were analyzed on three different days giving a total of nine replicates at each fortification level for each water sample.

Different incurred amounts of TFA were present in all three water types. The tap water sample contained TFMS as well. In addition to TFA and TFMS, the POTW water sample contained PFBS and PFPrA. These incurred concentrations were subtracted from the calculated concentrations of fortified samples to determine the recovery. Due to a much higher TFA concentration in the POTW water sample, it was diluted fivefold in RO water prior to fortification for determining TFA accuracy and precision. Data were not collected for TFA from 25 ppt fortified samples as accurate quantification by concentration subtraction could not be obtained due to the high levels of incurred TFA. The average recovery and relative standard deviation (RSD) data are shown in Table II. All analytes had recovery values of 86.6–107% across three fortification levels and three different types of waters. Satisfactory method precision was demonstrated by RSD values being within 1.62–10.7%.

Measurement of C1 to C4 PFAS in Potable and Non-Potable Waters

Following the method verification experiments, this direct injection workflow was applied to the determination of C1 to C4 PFAS in a variety of source waters, including tap waters, bottled waters, natural spring water, well water, and wastewaters. Three preparations of blank and fortified (50 ppt) samples were injected for analysis. It was shown that the average recoveries of fortified QC samples were all within 75 to 120%. This demonstrated that the established LC-MS/MS method for PFAS in water was suitable for accurate measurement of C1 to C4 PFAS in both potable and non-potable sources.

Test results for incurred levels are shown in Table III. The data indicated that TFA was ubiquitously present in tap waters at a range of ~120 to 500 ppt. TFMS was present and quantifiable in most tap waters, and PFPrA was detectable in several tap water samples. The bottled spring water samples contained TFA as well. RO purified bottled water and RO purified tap water did not contain detectable levels of C1 to C4 PFAS. The well water sample had a relatively high amount of TFMS compared to the other samples. The wastewater samples, which originated from a POTW facility, a hospital, a metal finisher, and a chemical manufacturer, all had higher levels of TFA and PFPrA. The wastewater effluent collected from the chemical manufacturer was notably elevated for TFA, PFPrA, and PFBA contamination.

Conclusion

An LC-MS/MS method for PFAS in water was established in this study to provide a unique solution for the determination of ultra-short-chain PFAS and short-chain PFAS in various water matrices. The reported method was demonstrated to be rugged, accurate, and precise. In addition, the use of direct injection and the seven-minute chromatographic analysis time make it easy and fast to perform, allowing higher sample throughput. Most important, this solution offers an effective tool for monitoring the emergent C1-C4 PFAS contaminants in environmental water systems, which can assist scientists in generating guidelines for future regulatory actions.

References

[1] S.H. Liang, A novel approach for ultrashort-chain PFAS analysis in water samples: direct, simultaneous determination of ultrashort-chain, alternative, and legacy PFAS, Application note EVA-N3220A-UNV, Restek Corporation, 2021. https://www.restek.com/articles/a-novel-approach-for-ultrashort-chain-pfas-analysis-in-water-samples

Table II: Method accuracy and precision of fortified water samples.

	Average Recovery (%RSD)								
	Tap Water			Bottled Spring Water			POTW Water		
Concentration (ng/L)	25	50	175	25	50	175	25	50	175
TFA	-	98.2 (7.63)	97.4 (6.68)	-	107 (5.92)	97.1 (4.27)	-	96.7 (10.7)	106 (4.02)
PFPrA	106 (3.49)	107 (2.26)	103 (2.19)	96.6 (4.10)	107 (4.29)	102 (2.19)	102 (3.08)	102 (3.02)	101 (1.71)
PFBA	99.5 (4.61)	100 (5.09)	101 (1.72)	94.4 (9.17)	101 (5.08)	99.6 (3.12)	100 (6.36)	95.2 (5.25)	97.4 (1.62)
TFMS	87.5 (1.62)	95.8 (5.66)	96.4 (3.02)	86.6 (5.99)	95.5 (5.74)	94.6 (3.99)	92.6 (7.42)	94.5 (7.94)	93.8 (5.25)
PFEtS	96.2 (5.68)	100 (7.62)	96.9 (3.93)	92.0 (6.18)	101 (6.24)	95.1 (6.77)	93.8 (6.54)	97.2 (7.75)	95.7 (7.48)
PFPrS	94.2 (4.80)	99.8 (5.38)	97.3 (3.60)	92.5 (7.94)	99.4 (6.31)	96.1 (4.50)	97.6 (4.47)	97.6 (6.52)	96.8 (5.78)
PFBS	98.7 (4.02)	102 (4.92)	101 (3.79)	95.5 (8.10)	104 (7.03)	98.6 (5.09)	99.8 (6.97)	103 (5.99)	100 (3.58)

Table III: The quantification of C1 to C4 PFAS in various potable and non-potable waters.

Water Samples	Average Concentration (ng/L; ppt)						
	TFA	PFPrA	PFBA	TFMS	PFEtS	PFPrS	PFBS
Potable Waters							
Tap water #1	230	nd	nd	5.58	nd	nd	nd
Tap water #2	520	nd	nd	6.88	nd	nd	nd
Tap water #3	450	<5.00	nd	3.20	nd	nd	nd
Tap water #4 (filtered well water)	267	nd	nd	nd	nd	nd	nd
Tap water #5	297	<5.00	nd	4.68	nd	nd	nd
Tap water #6	428	<5.00	nd	<2.5	nd	nd	nd
Tap water #7 (RO filtered tap water #6)	nd	nd	nd	nd	nd	nd	nd
Tap water #8	400	<5.00	nd	nd	nd	nd	nd
Tap water #9	228	nd	nd	5.22	nd	nd	nd
Tap water #10	117	nd	nd	nd	nd	nd	nd
Bottled water #1 (RO purified)	nd	nd	nd	nd	nd	nd	nd
Bottled water #2 (spring water)	102	nd	nd	nd	nd	nd	nd
Bottled water #3 (spring water)	368	nd	nd	<2.5	nd	nd	nd
Natural spring water	527	<5.00	nd	<2.5	nd	nd	nd
Well water	342	nd	nd	15.6	nd	nd	nd
Non-Potable Waters							
POTW water (treated sewage wastewater, effluent)	1113	36.6	<5.00	8.53	nd	nd	4.35
Hospital effluent	1363	24.6	<5.00	4.67	nd	nd	nd
Metal finisher	741	11.4	<5.00	5.16	nd	nd	2.77
Chemical manufacturer effluent	131,200	11,084	52.0	4.02	nd	nd	nd

nd = not detected

Raptor Polar X LC Columns



- Reliably analyze a wide variety of polar analytes (acidic, basic, and neutral) without time-consuming derivatization or complex ion pairing.
- Switch between HILIC and ion-exchange retention modes with simple mobile phase changes and short equilibration times.
- 2.7 µm Raptor core-shell particles provide UHPLC-like speed and efficiency on all makes and models of LC systems.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Storage Conditions: When not in use, Raptor Polar X columns must be kept in 100% acetonitrile. If using a buffered mobile phase, first flush thoroughly with 50:50 water:acetonitrile, then fill with acetonitrile for storage.

Pore Size: 90 Å
Particle: 2.7 µm superficially porous particle (SPP or "core-shell" particle) silica
Surface Area: 130 m^2/g
End-Cap: Proprietary
Carbon Load: Proprietary
USP Phase Code: NA
Phase Category: Proprietary
Ligand Type: Proprietary
Recommended Usage:
pH Range: 2.0–8.0
Maximum Temperature: 60 °C
Maximum Pressure: 600 bar/8700 psi

Properties:

- Excellent resolution and separation of a wide variety of polar compounds.
- Combines HILIC and ion-exchange retention mechanisms together in a single ligand.
- Broadly applicable for polar compound analysis spanning different industries and methods.

Switch to a Raptor Polar X column when:

- You are analyzing neutral, acidic, basic, or permanently charged polar compounds.
- Performing LC-MS/MS analysis of polar compounds.
- You are struggling to retain or elute polar compounds and considering ion chromatography.

ID	Length	qty.	cat.#
2.1 mm	50 mm	ea.	9311A52





NORM-JECT and HENKE-JECT Plastic Syringes

NORM-JECT and HENKE-JECT Plastic Syringe Specifications

Luer Lock						
Restek cat.#	**Volume (mL)**	**Total Length (mm)**	**Length of Cylinder (mm)**	**Outside Diameter (mm)**	**Inside Diameter (mm)**	**Nozzle Configuration**
22775	10	98.5	85.3	17.30	15.90	Centric

Volume	Modification	Type	qty.	Similar to Part #	cat.#
10 mL	graduations to 12 mL	Luer Lock Tip	100-pk.	Agilent UCB310; Air-Tite AL10	22775



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Nylon					
Syringe Filter	Pink	30 mm	0.45 µm	100-pk.	23981

Cellulose Acetate, Nylon, PES, PP, PVDF—hydrophilic applications
Syringe filters are for laboratory use only.

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Analyze EPA Method 533 PFAS Reliably with Resprep WAX SPE

By Jason Hoisington

Abstract

Low-level analysis of per-and polyfluoroalkyl substances (PFAS) requires high sensitivity and low background levels throughout the entire workflow from sample collection, transportation, and storage to analytical instrumentation and sample preparation. Resprep polymeric WAX SPE cartridges allow for accurate low-level quantitation of PFAS compounds in drinking water while meeting the requirements of EPA Method 533.



Introduction

Due to the low levels required by many regulatory agencies, the analysis of PFAS in drinking water often employs solid phase extraction (SPE) coupled with LC-MS/MS to reach low detection limits. EPA Method 533 [1] uses weak anion exchange (WAX) SPE to better retain short-chain acid compounds and obtain part-per-trillion (ppt) detection limits. However, due to the ubiquitous nature of PFAS compounds, the selection and screening of all materials and consumables that contact samples, including SPE cartridges, is critical for maintaining a clean background and allowing continued low-level analysis. In this assessment, Resprep WAX cartridges were used with a Resprep QR-12 vacuum manifold to extract samples for EPA Method 533 PFAS analysis using a Shimadzu Nexera LC and 8045 MS/MS.

Related Products

- *PFAS delay column (cat. # 27854)*
- *Force C18 1.8 µm, 50 mm x 2.1 mm (cat.# 9634252)*
- *PFAS EPA Method 533 Analyte Primary Dilution Standard (PDS) (cat.# 30840)*
- *Resprep polymeric WAX SPE cartridges (cat. # 28268)*
- *Resprep QR-12 vacuum manifold (cat. # 28298-VM)*
- *Quick replace liners (cat. # 28310-VM)*
- *Resprep sample delivery system (cat. # 26250)*
- *Polypropylene vials (cat.# 23246)*
- *Polyethylene caps (cat.# 23247)*
- *Chemker vacuum pump (cat.# 27427)*

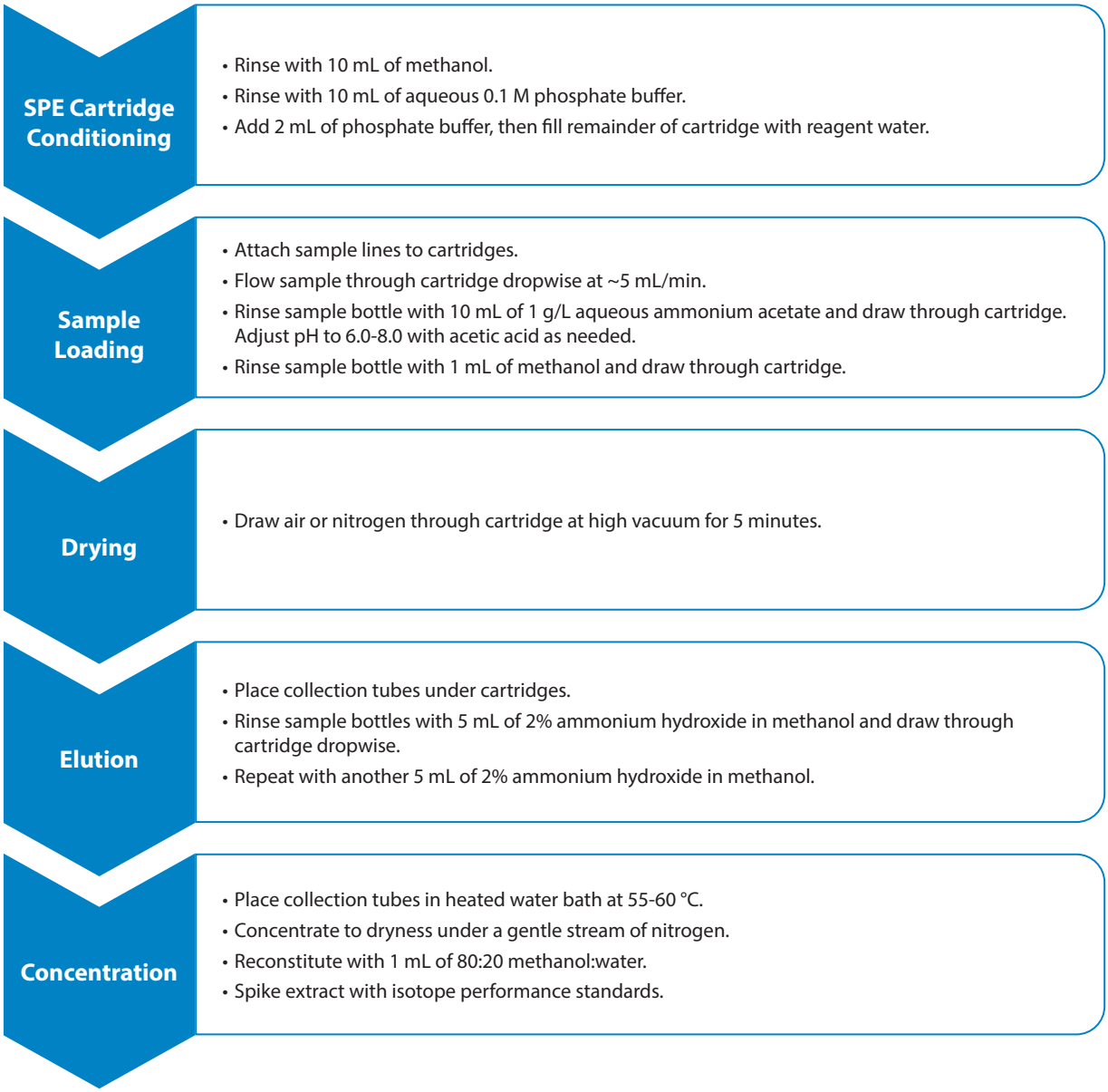
Experimental

Sample Preparation

Spikes and blanks were prepared in polypropylene bottles using 250 mL deionized water spiked with isotope dilution standards as per EPA Method 533, Section 7.16.1. The 6 mL SPE cartridges, which contained 500 mg of 30 µm WAX (cat.# 28291*), were placed on a Resprep QR-12 vacuum manifold (cat.# 28298-VM) that was fitted with quick-replace liners (cat.# 28310-VM). Resprep sample delivery system lines (cat.# 26250) were used to transfer the samples to the SPE cartridges. While both the quick-replace liners and sample delivery lines contain PTFE, which can be a potential source of PFAS contamination, investigation of blanks taken using all lines and all ports on the manifold showed no detectable PFAS leaching. Thorough and regular blank checking of all SPE components and solvents, especially when using new lots of materials, is recommended to ensure that background contamination is below acceptable levels.

After preparation of the samples and setup of the SPE system, the samples were extracted following the instructions in EPA Method 533, Section 11.4, which is summarized in Figure 1.

Figure 1: Sample Preparation Procedure for EPA Method 533 PFAS Analysis



Analytical System

After extraction, the samples were analyzed by LC-MS/MS under the EPA Method 533 PFAS analysis conditions shown below. The use of a PFAS delay column is important to prevent any PFAS contamination upstream of the injector from coeluting with the samples. Thorough blank checking of the analytical system was performed and showed no detectable PFAS contamination.

Instrument Conditions for EPA Method 533 PFAS Analysis

System: Shimadzu Nexera X2/Shimadzu LCMS-8045

Columns:

- PFAS delay column (cat.# 27854)
- Analytical column: Force C18, 1.8 µm x 50 mm x 2.1 mm (cat.# 9634252)

Injection volume: 3 µL

Mobile phase A: Water, 5 mM ammonium acetate

Mobile phase B: Methanol

Flow rate: 0.4 mL/min

Temperature: 40 °C

Gradient:

Time (min)	%B
0	20
6	95
6.6	95
6.61	20
7.5	20

Ion source: electrospray

Ion mode: ESI-

Mode: MRM

Method Detection Limits (MDL)

The method detection limit was calculated from the analysis of seven blank replicates and seven low-level spikes, as outlined in EPA's Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 [2]. The spikes were made at 0.5 ng/L using a 2 µg/mL stock solution of native PFAS compounds (EPA 533 PFAS calibration standard, cat.# 30736). The standard deviations of the spike and blank results were multiplied by the Student's *t*-value of 3.143 to determine the MDL, and the higher of the results between the spikes and blanks was selected as the MDL.

Accuracy and Precision

Accuracy and precision were determined by analyzing five replicate 10 ng/L spikes. The accuracy of the spikes was calculated and compared to the recovery limits of 70-130% from EPA Method 533. The relative standard deviations of the spike results were also determined.

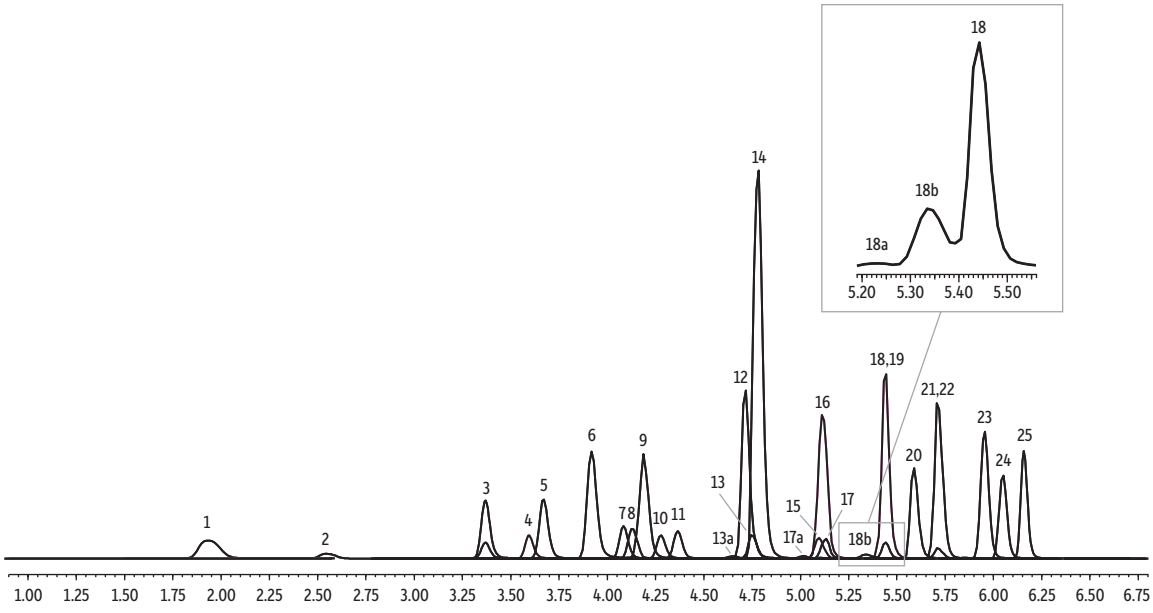
The recovery of the isotope dilution standards was calculated from the spike replicates and compared to the recovery limits of 50-200% from EPA Method 533 for PFAS analysis.

Results and Discussion

Good chromatographic results were obtained for all compounds, as shown in Figure 2. The MDL, accuracy, and precision results for native PFAS analytes are shown in Table I. The calculated MDLs were below the reporting limits shown in Table 7 in EPA Method 533, and the accuracy of the 10 ng/mL spikes ranged from 84 to 119% of the spiked value, well within the 70-130% recovery required by the method. The spikes showed good precision as well, with the results being ≤20% RSD.

Similarly, the recoveries for the isotope dilution standards were also within 20% of the true value and had precision ≤20% RSD. The results are shown in Table II.

Figure 2: Analysis of 25 ng/mL Standard for EPA Method 533



LC_EV0584

Peaks	tR (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Perfluoro-*n*-butanoic acid (PFBA)	1.933	25	213	169
2. Perfluoro-3-methoxypropanoic acid (PFMPA)	2.544	25	229	85
3. Perfluoro-*n*-pentanoic acid (PFPeA)	3.369	25	263	219
4. Perfluorobutanesulfonic acid (PFBS)	3.594	25	299	80
5. Perfluoro-4-methoxybutanoic acid (PFMBA)	3.669	25	279	85
6. Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	3.919	25	315	135
7. Perfluoro-3,6-dioxaheptanoic acid (NFDHA)	4.084	25	295	201
8. 1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	4.129	25	327	307
9. Perfluorohexanoic acid (PFHxA)	4.189	25	313	269
10. Perfluoro-1-pentanesulfonic acid (PFPeS)	4.278	25	349	80
11. Perfluoro(2-methyl-3-oxahexanoic) acid (HFPO-DA)	4.365	25	285	169
12. Perfluoroheptanoic acid (PFHpA)	4.715	25	363	319
13. Perfluoro-1-hexanesulfonic acid (PFHxS)	4.750	25	399	80
14. 4,8-dioxa-3H-perfluorononanoic acid (ADONA)	4.78	25	277	251
15. 1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	5.096	25	427	407
16. Perfluoro-1-heptanesulfonic acid (PFHpS)	5.132	25	449	80
17. Perfluorooctanoic acid (PFOA)	5.115	25	413	369
18. Perfluorooctanesulfonic acid (PFOS)	5.441	25	499	80
19. Perfluorononanoic acid (PFNA)	5.439	25	463	419
20. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	5.588	25	531	351
21. 1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	5.712	25	527	507
22. Perfluorodecanoic acid (PFDA)	5.712	25	513	469
23. Perfluoroundecanoic acid (PFUnA)	5.954	25	563	519
24. 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	6.049	25	631	451
25. Perfluorododecanoic acid (PFDoA)	6.158	25	613	569

Column
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 1.8 µm
Pore Size: 100 Å
Temp.: 40 °C
Standard/Sample
Diluent: EPA 533 PFAS calibration standard
Conc.: 80:20 Methanol:water
Inj. Vol.: 25 ng/mL
Mobile Phase 3 µL
A: Water, 5 mM ammonium acetate
B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.60	0.4	5	95
6.61	0.4	80	20
7.50	0.4	80	20

Detector Shimadzu 8045
Ion Source: Electrospray
Ion Mode: ESI-
Mode: MRM
Instrument Shimadzu Nexera X2
Notes Branched isomers for PFOA, PFOS, and PFHxS labeled as peak number "a" and "b."
PFAS delay column used (cat.# 27854).

Standard cat.# 30736 was used to produce this chromatogram, but it has since been discontinued. We recommend cat.# 30840 as an alternative. For assistance choosing a replacement for this application, contact Restek Technical Service or your local Restek representative.

Table I: Results from MDL, Precision, and Accuracy Experiments for Native PFAS

Compound	Abbreviation	MDL (ng/L)	Accuracy (%)	%RSD
Perfluorobutanoic acid	PFBA	8.5	95	20
Perfluoro-3-methoxypropanoic acid	PFMPA	0.2	119	20
Perfluoropentanoic acid	PFPeA	0.2	114	10
Perfluorobutane sulfonate	PFBS	0.3	94	14
Perfluoro-4-methoxybutanoic acid	PFMBA	1.1	88	8
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	0.2	84	12
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	0.2	103	15
1H, 1H, 2H,2H-perfluorohexane sulfonate	4:2 FTS	0.3	97	19
Perfluorohexanoic acid	PFHxA	0.1	98	11
Perfluoropentane sulfonate	PFPeS	0.2	96	13
Hexafluoropropylene oxide dimer acid	HFPO-DA	1.0	89	13
Perfluoroheptanoic acid	PFHpA	0.4	101	17
Perfluorohexane sulfonate	PFHxS	0.3	110	15
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	1.1	100	7
1H, 1H, 2H,2H-perfluorooctane sulfonate	6:2 FTS	0.7	105	8
Perfluoroheptane sulfonate	PFHpS	0.5	101	12
Perfluorooctanoic acid	PFOA	0.2	112	9
Perfluorooctane sulfonate	PFOS	0.3	109	5
Perfluorononanoic acid	PFNA	0.5	101	10
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	0.2	93	7
Perfluorodecanoic acid	PFDA	0.4	111	6
1H, 1H, 2H,2H-perfluorodecane sulfonate	8:2 FTS	0.6	109	5
Perfluoroundecanoic acid	PFUnA	0.8	110	6
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	0.3	95	11
Perfluorododecanoic acid	PFDoA	1.1	94	7

Table II: Results from Precision and Accuracy Experiments for Isotope Dilution Standards

Compound	Abbreviation	Accuracy (%)	%RSD
Perfluoro-*n*-[1,2,3,4- $^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	111	13
Perfluoro-*n*-[1,2,3,4,5- $^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	118	14
Sodium perfluoro-1-[2,3,4- $^{13}C_3$]butanesulfonate	$^{13}C_3$-PFBS	108	16
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- $^{13}C_2$]hexane sulfonate	$^{13}C_2$-4:2FTS	97	12
Perfluoro-*n*-[1,2,3,4,6- $^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	115	14
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	89	14
Perfluoro-*n*-[1,2,3,4- $^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	110	14
Sodium perfluoro-1-[1,2,3- $^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	114	14
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	$^{13}C_2$-6:2FTS	92	7
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	113	14
Sodium perfluoro-[$^{13}C_8$]octanesulfonate	$^{13}C_8$-PFOS	102	7
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	101	9
Perfluoro-*n*-[1,2,3,4,5,6- $^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	111	12
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- $^{13}C_2$]-decane sulfonate	$^{13}C_2$-8:2FTS	118	14
Perfluoro-*n*-[1,2,3,4,5,6,7- $^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	114	13
Perfluoro-*n*-[1,2- $^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	112	13

Conclusions

EPA Method 533 PFAS analysis in drinking water can be challenging with low-level analysis complicated by background contamination. Resprep WAX cartridges have been shown to provide performance that meets or exceeds the requirements of EPA Method 533, allowing for analysis of PFAS at ng/L levels and lower. Visit www.restek.com/PFAS for additional products, methods, and technical resources.

References

1. U.S. Environmental Protection Agency, 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, November 2019. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf
2. U.S. Environmental Protection Agency, Definition and procedure for the determination of the method detection limit, revision 2, December 2016. https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

* This Resprep SPE cartridge (cat.# 28291) has been discontinued. The direct replacement is cat.# 28268.



PFAS Delay Column

- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

Catalog No.	Product Name	Units
27854	PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column	ea.

Force C18



- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- High carbon load (20%) offers high hydrophobic retention.

Catalog No.	Product Name	Units
9634252	Force C18, 1.8 µm, 50 x 2.1 mm LC Column	ea.

PFAS EPA Method 533 Analyte Primary Dilution Standard (PDS)

(25 components)



Hexafluoropropylene oxide dimer acid (HFPO-DA) (13252-13-6)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA) (151772-58-6)
Perfluoro-3-methoxypropanoic acid (PFMPA) (377-73-1)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFDoA) (307-55-1)
Perfluoroheptanoic acid (PFHpA) (375-85-9)
Perfluorohexanoic acid (PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Perfluorooctanoic acid (PFOA) (335-67-1)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONSK) (73606-19-6)
Potassium Perfluorobutanesulfonate (PFBSK) (29420-49-3)
Potassium Perfluorohexanesulfonate (br-PFHxSK) (3871-99-6)
Sodium 1H,1H,2H,2H-Perfluorodecane sulfonate (8:2 FTSNa) (27619-96-1)
Sodium 1H,1H,2H,2H-Perfluorohexane sulfonate (4:2 FTSNa) (27619-93-8)
Sodium 1H,1H,2H,2H-Perfluorooctane sulfonate (6:2 FTSNa) (27619-94-9)
Sodium 4,8-dioxa-3H-perfluorononanoate (NaDONA) (2250081-67-3)
Sodium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdSNa) (136176-45-9)
Sodium perfluoro(4-methoxybutanoate) (PFMBANa) (863008-55-3)
Sodium Perfluoro(2-ethoxyethane)sulfonate (PFEESANa) (113507-87-2)
Sodium Perfluoroheptanesulfonate (PFHpSNa) (21934-50-9)
Sodium Perfluorooctanesulfonate (br-PFOSNa) (4021-47-0)
Sodium Perfluoropentanesulfonate (PFPeSNa) (630402-22-1)

Conc. in Solvent	CRM?	Min Shelf Life on Ship Date	Max Shelf Life on Ship Date	Shipping Conditions	Storage Temp.	qty.	cat.#
2 µg/mL, Methanol (1 mM NaOH)/2-Propanol (98:2), 1 mL/ampul	Yes	6 months	36 months	Ambient	10 °C or colder	ea.	30840

Resprep Polymeric SPE Cartridge, WAX



- Silica-free, bonded polymeric material—no unwanted secondary silica interactions, even with basic compounds.
- High surface area—higher loading capacity compared to silica-based sorbents.
- Stable over a wide pH range (0–14)—won't hydrolyze under extreme conditions.
- Water-wettable—streamlined conditioning and equilibration steps drastically reduce solvent usage and sample prep time.
- No flow-rate dependence—maintains retention and capacity after conditioning, even if dried out from vacuum or positive pressure flows.
- Choose cartridges for high loading capacity; 96-well plates for high throughput and automation.

Catalog No.	Product Name	Units
28268	Resprep Polymeric SPE Cartridge, WAX, 6 mL/500 mg, 30 µm	30-pk.



Resprep QR-12 Vacuum Manifold

Catalog No.	Product Name	Units
28298-VM	Resprep QR-12 Vacuum Manifold, includes cover with flow control valves & gasket (cat.# 28316-VM); collection rack (cat.# 28318-VM); plate for 16 mm test tubes (cat.# 28319-VM); 100-pk. Quick Replace liners, PTFE (cat.# 28310-VM); 12-pk. liner guide (cat.# 28312-VM); 12-pk. test tubes (cat.# 28315-VM).	kit

Quick Replace Liners

Catalog No.	Product Name	Units
28310-VM	Quick Replace Liners, PTFE, for Resprep QR Vacuum Manifolds	100-pk.



Resprep Sample Delivery System

- Compatible with Resprep 1, 3, 6, and 15 mL SPE cartridges.
- Six PTFE transfer lines (⅛" OD x 1⁄16" ID x 36" long); each is banded with a different color for easy sample identification.
- Specified in EPA drinking water methods.
- Tested to pH of 1 to ensure no contaminants leach from system.

Catalog No.	Product Name	Units
26250	Resprep Sample Delivery System	6-pk.



Polypropylene Vials

Catalog No.	Product Name	Units
23246	Limited-Volume Screw-Thread Polypropylene Vials, 9 mm, 700 µL, 12 x 32 mm	1000-pk.

Polyethylene Caps

Catalog No.	Product Name	Units
23247	2.0 mL, 9 mm Solid-Top Polyethylene Caps, Screw-Thread, 10 mil thick membrane, Clear	1000-pk.



Chemker Vacuum Pump

Catalog No.	Product Name	Units
27427	Chemker 300 PTFE Vacuum Pump, 18 L/min, AC220 V, 50 Hz	ea.

Incorporating Ultrashort-Chain PFAS into Comprehensive PFAS Analysis in Ready-to-Drink Teas and Fruit Juices

By Shun-Hsin Liang, PhD

Key Highlights

- A dilute-and-shoot LC-MS/MS workflow on the Ultra IBD Inert column simultaneously quantifies 43 PFAS—from ultrashort-chain through long-chain (C1–C14) and alternative PFAS—in a single, 12-minute run.
- The mixed-mode stationary phase packed in inert-coated hardware enables sufficient retention for highly polar compounds, such as TFA and TFMS, while maintaining optimal retention of long-chain PFAS, resulting in comprehensive PFAS coverage with a short analytical run time.
- Simple dilute-and-shoot sample preparation limits lab-introduced PFAS contamination and supports high sample throughput.
- Evaluated across five diverse ready-to-drink teas and juices, the method delivered recoveries of 71–122% and %RSD ≤12% with LOQs as low as 2 ng/L for most analytes.
- Applied to 23 commercial beverages, the method revealed that ultrashort-chain PFAS—particularly TFA and TFMS—are the dominant PFAS contaminants in teas and juices, a class largely missed by conventional C18-based methods.



Related Products

- *Ultra IBD Inert column, 3.0 µm, 100 x 2.1 mm (cat.# 9175312-T)*
- *Ultra IBD column, 3.0 µm, 150 x 2.1 mm (cat.# 9175362)*
- *PFAS 28 calibration standard (cat.# 30734)*
- *Polypropylene vials (cat.# 23243)*
- *Polyethylene caps (cat.# 23244)*

Abstract

Ultrashort-chain (USC) PFAS are a highly polar subset of PFAS with carbon chain lengths shorter than C4 that are difficult to retain using conventional reversed-phase LC methods, yet their detection is increasingly important in monitoring dietary and environmental PFAS exposure. This application note describes a straightforward, dilute-and-shoot LC-MS/MS workflow for the simultaneous determination of C1 to C14 perfluoroalkyl carboxylic and sulfonic acids, along with other PFAS classes, in diverse beverage matrices. Chromatographic separation used the Ultra IBD Inert column, a highly inert mixed-mode column that provides enhanced retention of highly polar USC compounds compared to traditional C18 columns while maintaining excellent performance for long-chain PFAS. The method was evaluated across five representative teas and juices for the quantification of 43 PFAS compounds and subsequently applied to the analysis of 23 commercially available beverages..

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large family of synthetic chemicals valued for their chemical stability and resistance to water, oil, and heat [1]. Their widespread use and persistence have led to global dissemination in soil, water, and the food supply, raising concerns about chronic dietary exposure [2–4]. While PFAS studies have historically emphasized short- and long-chain compounds, emerging evidence indicates that ultrashort-chain (USC) PFAS, which contain one to three perfluorinated carbon atoms, may contribute substantially to overall contamination because of their exceptional mobility, environmental persistence, and relevance to dietary exposure [5]. These C1–C3 compounds include trifluoroacetic acid (TFA); perfluoropropanoic acid (PFPrA); trifluoromethanesulfonic acid (TFMS); perfluoroethanesulfonic acid (PFEtS); and perfluoropropanesulfonic acid (PFPrS), which are increasingly recognized as major contributors to overall PFAS burdens in aquatic environments [6].

Due to analytical challenges, current methods for PFAS analysis in foods often exclude USC compounds from target lists, which underestimates the level and impact of dietary PFAS exposure [7]. Beverages are an important exposure route because they are consumed extensively across all age groups and, as predominantly water-based products, may contain elevated levels of polar USC PFAS. Although comprehensive PFAS analysis incorporating USC compounds has been reported for bottled water and ready-to-drink (RTD) milk products, PFAS surveillance data remains limited for other beverages, such as teas and fruit juices. These products are manufactured with ingredients and water sources that may already contain trace PFAS, with additional potential contamination through processing equipment, filtration systems, storage tanks, and packaging materials. Therefore, robust workflows capable of detecting both USC and long-chain compounds are essential for comprehensive monitoring of PFAS contamination in beverages.

Chromatographic analysis of USC PFAS is inherently challenging because of their high polarity, minimal retention on traditional reversed-phase LC columns, and susceptibility to loss during multistep sample preparation. Conventional LC-MS/MS methods using C18 columns are generally optimized for PFAS with chain lengths of C4 and longer and provide insufficient retention for USC compounds. Anion-exchange columns have been explored as an alternative but often suffer from long retention times and broad peak shapes that reduce sensitivity and sample throughput. In contrast, the Ultra IBD Inert column has previously demonstrated balanced retention of USC and long-chain PFAS in water and milk matrices [8,9] and is a promising approach for the development of new methods that can effectively cover the full range of PFAS chain lengths in beverage matrices.

Building on that chromatographic strategy, this study establishes a streamlined workflow for comprehensive PFAS determination—targeting 43 analytes encompassing C2–C14 perfluoroalkyl carboxylic acids, C1–C13 perfluoroalkyl sulfonic acids, fluorotelomer acids, perfluorooctane sulfonamides and sulfonamidoacetic acids, and per- and polyfluoroether acids—across a variety of RTD teas and fruit juices. The objectives were to (1) develop a simplified sample preparation procedure that minimizes contamination while enabling accurate quantification; (2) optimize chromatographic conditions to achieve efficient analysis of USC through long-chain PFAS within a short run time; and (3) apply the finalized workflow to assess PFAS contamination across a broader range of commercial beverages..

Experimental

Standard and Sample Preparation

Method performance was assessed using commercially purchased RTD green tea, black tea, apple juice, blended berry juice, and orange juice. Since matrix effect can vary significantly across sample types, isotopically labeled internal standards were used to correct for matrix interferences and ensure accurate, reliable quantification across the chemically diverse beverages analyzed in this study.

Calibration standards (500 µL) covering 1–2000 ng/L were prepared from Restek's PFAS 28 calibration standard (cat.# 30734) and standards obtained separately in a 1:1 mixture of ultrapure water and acetonitrile, fortified with 2.5 µL of a quantitative internal standard (QIS) working solution (10 ng/mL for each of the labeled analytes).

For teas and juices, 0.5 mL of sample was aliquoted into a 15-mL polypropylene centrifuge tube, spiked with 5.0 µL of QIS working solution, and mixed with 0.5 mL of acetonitrile by vortexing for 30 seconds—a simple, time-saving dilute-and-shoot approach with no extraction, evaporation, or cleanup steps. The tube was centrifuged at 4000 rpm, and the resulting supernatant was transferred directly to clean polypropylene vials for LC-MS/MS analysis.

Because orange juice samples exhibited stronger matrix effects than other beverages, they required an initial twofold dilution with water prior to the acetonitrile dilute-and-shoot step to maintain acceptable peak shape for early-eluting compounds. An in-depth discussion of matrix effects and method development is available in a separate publication detailing the complete study [10].

Instrumentation and Chromatographic Method

LC-MS/MS analysis was performed on a Waters ACQUITY I-Class UPLC coupled to a Xevo TQ-S triple-quadrupole mass spectrometer under negative electrospray ionization. Chromatographic separation of 43 target analytes and 13 quantitative internal standards was achieved using the Ultra IBD Inert column (100 × 2.1 mm, 3.0 µm; cat.# 9175312-T). An Ultra IBD column (150 × 2.1 mm, 3.0 µm; cat.# 9175362) was installed as a delay column to effectively sequester background PFAS contamination originating from the LC system and mobile phases.

Mobile phase A consisted of ultrapure water with 2 mM ammonium formate and 0.1% formic acid; mobile phase B was acetonitrile:water (95:5, v/v) with the same additives. The gradient began at 50% B, increased linearly to 100% B over 0–7 min, held at 100% B until 10 min, and then returned to 50% B for re-equilibration through 12 min. The column was held at 40 °C with a flow rate of 0.4 mL/min, and 20 µL of sample was injected per run.

Retention times, MRM transitions, and cone and collision cell voltages are provided in Appendix I for all analytes, the surrogate, and internal standards.

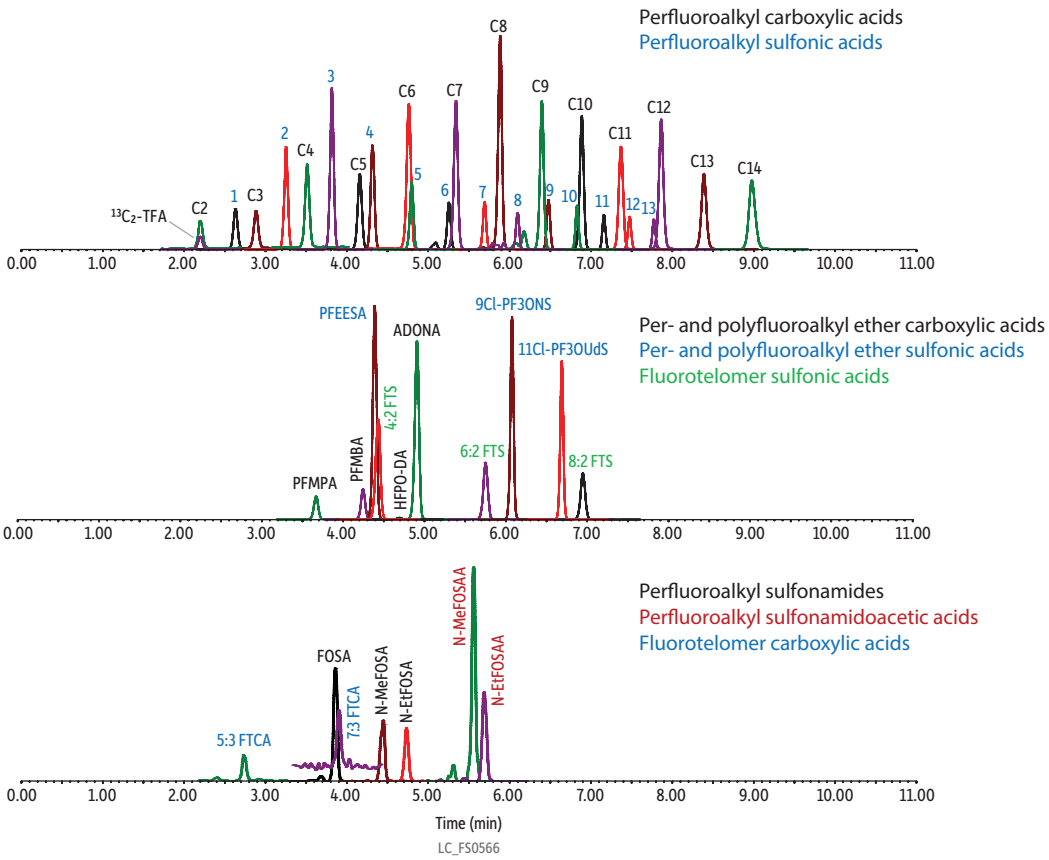
Results and Discussion

Chromatographic Performance

Building on the chromatographic strategies previously established for USC-inclusive PFAS analysis in water and milk matrices, this study applied the Ultra IBD Inert column to RTD beverages with diverse compositions. The optimized method conditions improved retention and separation of early-eluting USC compounds while also promoting faster elution of long-chain PFAS. The fast 12-minute total run time produced good chromatography for all analytes and did not sacrifice resolution for retention, which allows increased sample throughput—a meaningful advantage for laboratories running large numbers of samples.

Background contamination is a persistent challenge for PFAS analysis as it can originate from reagents, system components, and common lab materials. Use of the delay column and consumables that were previously shown to have low background levels were important factors in minimizing contamination. All labs performing PFAS analysis should prescreen solvents, reagents, and consumables for background PFAS contamination. The implementation of a delay column is highly recommended to help distinguish system-derived PFAS signals from those originating in the sample.

Figure 1: The Ultra IBD Inert column provided good retention and separation of both ultrashort chain and longer chain PFAS.



Column	Ultra IBD Inert (cat.# 9175312-T)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Temp.:	40 °C
Standard/Sample	
	PFAS 28 calibration standard (cat.# 30734) Other standards were obtained externally.
Diluent:	50:50 Water:acetonitrile
Conc.:	The blend berry juice was fortified at 500 ng/L and diluted two-fold with acetonitrile.
Inj. Vol.:	20 µL
Mobile Phase	
A:	Water, 2mM ammonium formate, 0.1% formic acid
B:	95:5 Acetonitrile:water, 2mM ammonium formate, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	50	50
7.00	0.4	0	100
10.00	0.4	0	100
10.01	0.4	50	50
12.00	0.4	50	50

Max Pressure:	540 bar
Detector	Waters Xevo TQ-S
Ion Source:	Waters Zspray ESI
Ion Mode:	ESI-
Mode:	MRM
Instrument	Waters ACQUITY UPLC I-Class
Sample Preparation	The blend berry juice (0.5 mL) was transferred into a 15 mL polypropylene centrifuge tube, fortified at 500 ng/L, and mixed with 0.5 mL of acetonitrile by vortexing for 30 seconds. The mixture was then centrifuged at 4000 rpm, and the resulting supernatant was transferred to polypropylene HPLC vials for LC-MS/MS analysis.
Notes	The confirmation ion peaks were not shown in the chromatogram.
	An Ultra IBD column (150 x 2.1 mm, 3.0 µm; cat # 9175362) was used as the delay column.
	For the chromatograms of perfluoroalkyl carboxylic and sulfonic acids, the peak numbers correspond to the carbon chain lengths of the PFAS compounds.

Evaluation of Linearity and LOD

Using quadratic regression with 1/x weighting, all analytes demonstrated strong linearity, with r^2 values greater than 0.995 and deviations below 30%. Calibration ranges were tailored by analyte: 1–1000 or 2–1000 ng/L for most compounds; 10–1000 ng/L for HFPO-DA; 10–2000 ng/L for TFA; and 4–1000 ng/L for fluorotelomer carboxylic acids and sulfonamidoacetic acids.

Limits of detection (LOD) were estimated from a signal-to-noise ratio of 3 in fortified beverage samples. A summary of linearity and LOD performance by matrix is given in Table I below; individual results for each analyte in each matrix are provided in Appendix II (Table IV).

Table I: Summary of LOD, Calibration Range, and Linearity Results by Beverage Type Across All 43 Target PFAS

Beverage Matrix	LOD Range (ng/L)	Calibration Range (ng/L)*	Analytes with $r^2 > 0.995$
Green tea	0.31 – 15.0	1–1000	43/43
Black tea	0.25 – 12.0	1–1000	43/43
Apple juice	0.18 – 12.7	1–1000	43/43
Blended berry juice	0.16 – 15.0	1–1000	43/43
Orange juice	0.30 – 12.5	1–1000	43/43

*The calibration range for most compounds was 1–1000 ng/L, although the lowest calibrator for some analytes was slightly above 1 ng/L. TFA was a notable exception and had a calibration range of 10–2000 ng/L.

Evaluation of Method Accuracy, Precision, and LOQ

Method accuracy and precision were evaluated by fortifying the five representative beverages with native analytes at 2, 4, 10, 20, 100, and 500 ng/L. Isotopically labeled $^{13}C_2$-TFA was used as a surrogate for determining TFA recovery. Three analytical batches were conducted on separate days, with three replicates per fortified concentration in each batch, resulting in a total of nine replicates for each fortification level. Background (incurred) PFAS concentrations were subtracted from fortified sample results to calculate recovery.

Recovery and %RSD results, summarized in Table II, demonstrate that the method meets or exceeds typical regulatory expectations [11] for PFAS accuracy and precision across all five beverage types. Complete accuracy and precision data for all 43 analytes at each of the six fortification levels, in each of the five beverage matrices, are provided in Appendix III (Tables V–IX).

Limits of quantitation (LOQ) for each analyte were defined as the lowest fortified concentration that could be quantified with acceptable accuracy [11]. Overall, the majority of the 43 targeted PFAS were accurately quantifiable at LOQs of 2 or 4 ng/L, with orange juice (which required additional dilution during preparation) requiring somewhat higher LOQs. A few compounds with lower MS sensitivity or stronger matrix interference (TFA, TFMS, HFPO-DA; fluorotelomer carboxylic acids; perfluorooctane sulfonamides; and sulfonamidoacetic acids) exhibited LOQs of 10–20 ng/L depending on beverage type. Individual LOQs for each analyte in each matrix are the lowest fortification levels shown in Appendix III (Tables V–IX).

Table II: Summary of Accuracy and Precision for Fortified Beverage Samples Across All Spike Levels and Days

Beverage Matrix	Average Recovery Range (%)	Max %RSD	Typical LOQ (ng/L)
Green tea	75.3-115	≤12	2 – 20
Black tea	71.1-118	≤12	2 – 20
Apple juice	83.6-117	≤12	2 – 20
Blended berry juice	78.5-121	≤12	2 – 20
Orange juice	83.9-122	≤12	4 – 20

Screening for PFAS in More Commercial Beverage Matrices

The method was used to profile PFAS in 23 commercial beverages purchased from local grocery stores, including unsweetened and sweetened green and black teas; several fruit-flavored black teas; and an expanded variety of fruit juices (apple, grape, cranberry, pineapple, orange, and multiple mixed-fruit blends).

Ultrashort- and short-chain PFAS were the primary contributors to PFAS contamination across the expanded beverage panel. Every beverage tested contained TFA at substantially higher concentrations than any other PFAS. In several samples, TFA levels were so high that they exceeded the upper calibration limit and required additional dilution for accurate quantification. TFMS, PFPrA, and PFPrS were also commonly detected, but long-chain PFAS were rarely found, with PFOA and PFOS appearing in only a few samples. Detailed methodology and quantitative results are available in the full study [10].

These results reinforce the central finding of this work: ultrashort-chain PFAS, not conventional long-chain PFAS, are the predominant contaminants in tea and juice beverages, and they will likely go undetected if labs use traditional workflows that cannot retain and quantify them.

Conclusion

This study establishes a simple, reliable, and broadly applicable LC-MS/MS method for the comprehensive determination of ultrashort-chain, long-chain, and alternative PFAS in diverse RTD tea and fruit juice beverages. The Ultra IBD Inert column provides increased retention of USC PFAS, resolving the fundamental analytical gap that leaves these compounds undetected by conventional C18 methods. In addition, an Ultra IBD delay column keeps background contamination from compromising low-level quantification. Combined with a minimal-handling dilute-and-shoot sample preparation procedure, the workflow minimizes contamination risk and turnaround time, delivering accurate, precise results (71–122% recovery, %RSD ≤ 12%) across five chemically distinct beverage matrices at LOQs as low as 2 ng/L.

Application of the qualified method to 23 commercial beverages revealed that ultrashort-chain PFAS, particularly TFA and TFMS, are the predominant contaminants in RTD teas and fruit juices, with long-chain PFAS only sporadically detected. This finding underscores why comprehensive food and beverage safety testing must include USC PFAS: methods that target only C4-and-longer compounds risk missing the majority of PFAS burden in these products [12].

The workflow described here provides laboratories, CROs, and food safety programs with a practical, single-injection solution for complete PFAS characterization in beverages, supporting ongoing surveillance and regulatory efforts as USC PFAS gain increasing scrutiny.

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Appendix I: MS/MS Ion Transitions and Parameters

Table III: MS/MS Ion Transitions, Parameters, and Chromatographic Retention Times of Target Analytes, Surrogate Standards, and Internal Standards

Compound	RT (min)	Precursor Ion	Product Ions (Quantifier/Qualifier)	Cone (V)	Collision (V)	Quant. IS
Perfluoroalkyl Carboxylic Acids						
Trifluoroacetic acid (TFA)	2.20	113.03 [M-H]-	69.01	10	10	$^{13}C_3$-PFPrA
Perfluoropropanoic acid (PFPrA)	2.88	162.97 [M-H]-	119.02	10	8	$^{13}C_3$-PFPrA
Perfluorobutanoic acid (PFBA)	3.51	213.03 [M-H]-	168.98	14	8	$^{13}C_4$-PFBA
Perfluoropentanoic acid (PFPeA)	4.16	262.97 [M-H]-	218.97	2	6	$^{13}C_5$-PFPeA
Perfluorohexanoic acid (PFHxA)	4.76	313.10 [M-H]-	268.97/118.99	2	8/20	$^{13}C_5$-PFHxA
Perfluoroheptanoic acid (PFHpA)	5.34	363.16 [M-H]-	319.09/169.06	8	10/18	$^{13}C_4$-PFHpA
Perfluorooctanoic acid (PFOA)	5.88	413.10 [M-H]-	368.96/168.90	2	10/16	$^{13}C_8$-PFOA
Perfluorononanoic acid (PFNA)	6.40	463.10 [M-H]-	419.01/219.02	4	10/16	$^{13}C_9$-PFNA
Perfluorodecanoic acid (PFDA)	6.89	513.17 [M-H]-	469.16/219.06	4	12/16	$^{13}C_6$-PFDA
Perfluoroundecanoic acid (PFUnA)	7.37	563.23 [M-H]-	519.24/269.07	6	12/18	$^{13}C_2$-PFTeDA
Perfluorododecanoic acid (PFDoA)	7.86	613.23 [M-H]-	569.19/169.06	8	12/26	$^{13}C_2$-PFTeDA
Perfluorotridecanoic acid (PFTrDA)	8.38	663.23 [M-H]-	619.21/169.06	8	14/28	$^{13}C_2$-PFTeDA
Perfluorotetradecanoic acid (PFTeDA)	8.98	712.67 [M-H]-	668.69/168.94	10	12/26	$^{13}C_2$-PFTeDA
Perfluoroalkyl Sulfonic Acids						
Trifluoromethanesulfonic acid (TFMS)	2.63	148.97 [M-H]-	79.93/98.92	62	13/18	$^{13}C_3$-PFBS
Perfluoroethanesulfonic acid (PFEtS)	3.25	198.90 [M-H]-	79.92/98.91	38	22/22	$^{13}C_3$-PFHxS
Perfluoropropanesulfonic acid (PFPrS)	3.81	248.97 [M-H]-	79.92/98.91	2	24/24	$^{13}C_5$-PFPeA
Perfluorobutanesulfonic acid (PFBS)	4.32	298.97 [M-H]-	79.97/98.89	2	26/26	$^{13}C_3$-PFBS
Perfluoropentanesulfonic acid (PFPeS)	4.79	349.10 [M-H]-	79.98/98.98	6	32/30	$^{13}C_3$-PFHxS
Perfluorohexanesulfonic acid (PFHxS)	5.25	398.90 [M-H]-	79.97/98.89	56	32/34	$^{13}C_3$-PFHxS
Perfluoroheptanesulfonic acid (PFHpS)	5.69	449.17 [M-H]-	79.98/98.97	4	42/38	$^{13}C_3$-PFHxS
Perfluorooctanesulfonic acid (PFOS)	6.11	499.03 [M-H]-	79.92/98.90	8	40/40	$^{13}C_8$-PFOS
Perfluorononanesulfonic acid (PFNS)	6.48	549.10 [M-H]-	79.92/98.83	12	42/40	$^{13}C_8$-PFOS
Perfluorodecanesulfonic acid (PFDS)	6.83	599.17 [M-H]-	79.98/98.83	8	44/46	$^{13}C_8$-PFOS
Perfluoroundecanesulfonic acid (PFUdS)	7.16	648.73 [M-H]-	79.94/98.94	38	50/44	$^{13}C_8$-PFOS
Perfluorododecanesulfonic acid (PFDoS)	7.47	698.77 [M-H]-	79.95/98.94	10	60/44	$^{13}C_8$-PFOS
Perfluorotridecanesulfonic acid (PFTrDS)	7.77	748.73 [M-H]-	79.94/98.94	8	76/52	$^{13}C_8$-PFOS
Fluorotelomer Sulfonic Acids						
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	4.44	327.10 [M-H]-	307.08/80.83	50	18/24	$^{13}C_3$-PFHxS
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	5.75	427.17 [M-H]-	407.18/80.71	2	22/32	$^{13}C_8$-PFOA
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	6.94	527.17 [M-H]-	507.16/80.83	66	26/32	$^{13}C_3$-PFHxS
Fluorotelomer Carboxylic Acids						
3-perfluoropentyl propanoic acid (5:3 FTCA)	2.73	340.93 [M-H]-	216.96/236.93	2	24/14	$^{13}C_3$-PFPrA
3-perfluoroheptyl propanoic acid (7:3 FTCA)	3.92	440.90 [M-H]-	336.88/316.91	20	12/22	$^{13}C_4$-PFBA
Perfluoroalkyl Sulfonamides						
Perfluorooctanesulfonamide (FOSA)	3.87	498.17 [M-H]-	77.97/477.76	8	28/26	$^{13}C_8$-FOSA
N-methyl perfluorooctanesulfonamide (NMeFOSA)	4.45	511.77 [M-H]-	168.95/218.91	2	26/24	$^{13}C_5$-PFPeA
N-ethyl perfluorooctanesulfonamide (NEtFOSA)	4.73	525.83 [M-H]-	168.96/218.92	10	26/24	$^{13}C_5$-PFHxA

(Continued)

Table III *(continued)*

Compound	RT (min)	Precursor Ion	Product Ions (Quantifier/Qualifier)	Cone (V)	Collision (V)	Quant. IS
Perfluoroalkyl Sulfonamidoacetic Acids						
N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	5.55	570.20 [M-H]-	419.17/483.16	46	20/14	$^{13}C_3$-PFPrA
N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	5.68	584.20 [M-H]-	419.18/483.11	6	20/16	$^{13}C_3$-PFPrA
Per- and Polyfluoroether Carboxylic Acids						
Perfluoro-3-methoxypropanoic acid (PFMPA)	3.68	228.93 [M-H]-	84.97/198.94	10	10/14	$^{13}C_4$-PFBA
Perfluoro-4-methoxybutanoic acid (PFMBA)	4.24	278.87 [M-H]-	84.96/234.93	8	10/6	$^{13}C_5$-PFHxA
Hexafluoropropylene oxide dimer acid (HFPO-DA)	4.68	285.03 [M-COOH]-	169.02/185.02	2	6/16	$^{13}C_3$-PFHxS
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	4.90	376.90 [M-H]-	250.93/84.97	22	12/26	$^{13}C_4$-PFHpA
Per- and Polyfluoroether Sulfonic Acids						
Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	4.09	314.83 [M-H]-	134.94/83.01	4	22/16	$^{13}C_3$-PFHxS
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	6.08	530.78 [M-H]-	350.85/82.96	12	26/24	$^{13}C_2$-PFTeDA
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	6.69	630.78 [M-H]-	450.80/82.95	8	26/32	$^{13}C_2$-PFTeDA
Surrogate						
$^{13}C_2$-TFA (surrogate)	2.20	114.90 [M-H]-	69.95	14	8	$^{13}C_3$-PFPrA
Quantification Internal Standards (QIS)						
$^{13}C_3$-PFPrA	2.88	165.97 [M-H]-	120.96	10	11	–
$^{13}C_4$-PFBA	3.51	217.03 [M-H]-	171.98	2	8	–
$^{13}C_5$-PFPeA	4.16	267.97 [M-H]-	222.99	2	6	–
$^{13}C_5$-PFHxA	4.76	318.03 [M-H]-	272.93	2	7	–
$^{13}C_4$-PFHpA	5.34	366.90 [M-H]-	321.93	2	10	–
$^{13}C_8$-PFOA	5.88	420.97 [M-H]-	375.94	2	10	–
$^{13}C_9$-PFNA	6.40	471.97 [M-H]-	426.87	4	12	–
$^{13}C_6$-PFDA	6.89	518.90 [M-H]-	473.87	4	13	–
$^{13}C_2$-PFTeDA	8.98	714.78 [M-H]-	669.80	8	14	–
$^{13}C_3$-PFBS	4.32	301.97 [M-H]-	79.97	2	28	–
$^{13}C_3$-PFHxS	5.25	401.90 [M-H]-	79.97	2	36	–
$^{13}C_8$-PFOS	6.11	506.84 [M-H]-	79.97	4	42	–
$^{13}C_8$-FOSA	3.87	505.91 [M-H]-	77.95	4	32	–

Appendix II: Linearity and Limit of Detection by Beverage Matrix

Table IV: Linearity Range and Matrix-Specific LOD in Matrix for Target Analytes

Analyte	Linear Calibration Range (ng/L)	LOD Green Tea	LOD Black Tea	LOD Apple Juice	LOD Berry Juice	LOD Orange Juice
TFA (via $^{13}C_2$-TFA)1	10 – 2000	1.11	1.85	1.76	1.71	1.76
PFPrA	1 – 1000	1.2	2.40	1.00	0.86	2.20
PFBA	1 – 1000	1.81	1.90	0.92	1.00	1.09
PFPeA	1 – 1000	1.36	1.50	1.35	0.83	1.44
PFHxA	1 – 1000	1.09	1.42	0.83	0.86	1.30
PFHpA	1 – 1000	0.52	0.81	0.43	0.36	0.72
PFOA	1 – 1000	1.03	1.10	0.52	0.55	1.25
PFNA	1 – 1000	1.25	1.33	0.48	0.51	1.35
PFDA	1 – 1000	0.71	1.00	0.44	0.45	0.71
PFUnA	1 – 1000	0.74	1.21	0.41	0.40	1.14
PFDoA	1 – 1000	0.68	0.73	0.26	0.34	0.87
PFTrDA	1 – 1000	0.57	0.82	0.27	0.28	0.71
PFTeDA	1 – 1000	0.41	0.52	0.27	0.26	0.44
TFMS	1 – 1000	0.48	0.67	0.42	0.32	0.60
PFEtS	1 – 1000	0.38	0.50	0.33	0.35	0.53
PFPrS	1 – 1000	0.39	0.73	0.27	0.27	0.79
PFBS	1 – 1000	0.62	0.65	0.39	0.30	0.69
PFPeS	1 – 1000	0.33	0.63	0.31	0.23	0.55
PFHxS	1 – 1000	0.78	0.59	0.90	0.79	0.97
PFHpS	1 – 1000	0.81	0.92	0.58	0.57	0.53
PFOS	1 – 1000	1.12	1.83	1.15	1.28	1.80
PFNS	1 – 1000	0.95	1.12	0.46	0.51	1.23
PFDS	1 – 1000	0.91	1.11	0.47	0.96	1.13
PFUdS	1 – 1000	0.50	0.55	0.71	0.78	0.77
PFDoS	1 – 1000	0.45	0.43	0.54	0.56	0.85
PFTrDS	1 – 1000	0.80	0.71	0.70	0.71	0.80
4:2 FTS	1 – 1000	0.54	0.41	0.38	0.44	0.63
6:2 FTS	1 – 1000	0.75	1.00	1.49	1.57	1.60
8:2 FTS	1 – 1000	0.46	0.63	0.5	0.49	0.85
5:3 FTCA	4 – 1000	6.67	7.50	11.72	7.50	10.0
7:3 FTCA	4 – 1000	15.0	12.0	12.71	15.0	8.33
FOSA	2 – 1000	2.00	2.11	1.58	1.12	2.31
NMeFOSA	2 – 1000	5.00	5.25	3.11	3.13	4.29
NEtFOSA	2 – 1000	7.10	8.25	2.66	4.50	6.00
NMeFOSAA	4 – 1000	8.21	6.64	5.77	7.50	10.0
NEtFOSAA	4 – 1000	6.98	5.24	3.00	7.73	6.67
PFMPA	1 – 1000	1.09	1.33	1.00	1.36	1.42
PFMBA	1 – 1000	0.78	0.91	0.73	0.65	1.20
HFPO-DA	10 – 1000	4.29	11.2	4.62	4.29	12.5
ADONA	1 – 1000	0.45	0.42	0.48	0.41	0.91
PFEESA	1 – 1000	0.31	0.31	0.18	0.16	0.34
9Cl-PF3ONS	1 – 1000	0.50	0.57	0.29	0.39	0.55
11Cl-PF3OUdS	1 – 1000	0.43	0.25	0.20	0.21	0.30

1Due to background levels of TFA, $^{13}C_2$-TFA is used as a surrogate for TFA to generate the calibration curve.

Appendix III: Full Accuracy and Precision Data by Beverage Matrix

The following tables report average recovery (%RSD, n=9) at each fortification level (2, 4, 10, 20, 100, and 500 ng/L) for all 43 target analytes in each of the five representative beverage matrices. A dash (–) indicates the fortification level was below the analyte's LOQ in that matrix.

Table V: Accuracy and Precision for Green Tea

Analyte	2 ng/L	4 ng/L	10 ng/L	20 ng/L	100 ng/L	500 ng/L
$^{13}C_2$-TFA	–	–	–	101 (6.01)	97.8 (8.72)	113 (3.76)
PFPrA	–	105 (6.54)	105 (5.99)	108 (3.90)	107 (2.95)	111 (4.51)
PFBA	–	107 (5.66)	98.0 (5.67)	102 (7.19)	107 (4.64)	110 (2.45)
PFPeA	–	98.2 (7.18)	97.6 (8.27)	94.9 (8.82)	104 (7.73)	97.8 (4.89)
PFHxA	–	106 (5.53)	108 (3.28)	105 (7.67)	113 (3.28)	112 (5.54)
PFHpA	95.3 (8.89)	93.4 (8.32)	94.8 (7.10)	98.5 (3.49)	106 (2.38)	90.2 (2.60)
PFOA	–	109 (8.46)	107 (7.81)	109 (7.91)	104 (8.73)	109 (6.47)
PFNA	–	106 (7.63)	110 (5.85)	111 (6.41)	105 (6.69)	109 (3.72)
PFDA	109 (3.55)	94.5 (7.33)	89.5 (7.12)	99.1 (4.35)	100 (4.29)	90.1 (5.56)
PFUnA	110 (6.51)	106 (8.12)	98.8 (7.02)	107 (5.46)	114 (4.04)	109 (5.62)
PFDoA	109 (5.62)	110 (6.66)	103 (6.74)	109 (4.25)	104 (5.74)	101 (8.09)
PFTrDA	99.0 (8.03)	103 (8.56)	107 (8.41)	114 (4.09)	107 (7.30)	100 (8.00)
PFTeDA	113 (7.51)	105 (3.06)	115 (3.26)	106 (7.68)	108 (7.45)	109 (5.41)
TFMS	–	–	105 (4.55)	108 (3.03)	106 (3.74)	111 (3.47)
PFEtS	104 (5.11)	98.2 (8.56)	99.9 (5.89)	97.4 (6.70)	108 (6.50)	107 (6.73)
PFPrS	102 (6.95)	100 (3.67)	107 (5.77)	99.6 (7.01)	104 (6.67)	98.0 (10.1)
PFBS	113 (3.63)	108 (5.07)	110 (5.95)	102 (8.78)	113 (2.56)	104 (4.80)
PFPeS	103 (8.22)	103 (4.62)	102 (2.89)	106 (3.80)	108 (3.27)	112 (8.41)
PFHxS	112 (8.41)	103 (9.71)	98.5 (4.13)	99.5 (7.79)	105 (3.03)	104 (4.54)
PFHpS	111 (5.52)	97.7 (6.31)	99.0 (8.59)	98.3 (9.38)	105 (5.27)	88.7 (6.48)
PFOS	–	100 (8.90)	104 (5.40)	100 (9.13)	111 (4.76)	104 (5.18)
PFNS	–	103 (8.62)	107 (5.07)	113 (5.69)	111 (5.45)	105 (4.31)
PFDS	–	103 (10.2)	100 (9.92)	109 (6.48)	112 (4.47)	100 (6.20)
PFUdS	109 (9.79)	108 (6.41)	103 (7.23)	109 (4.61)	104 (5.29)	100 (2.91)
PFDoS	100 (9.44)	106 (8.64)	107 (7.05)	109 (6.32)	108 (7.18)	107 (3.77)
PFTrDS	–	99.7 (10.0)	98.4 (10.3)	103 (4.11)	110 (7.00)	108 (5.14)
4:2 FTS	102 (10.7)	105 (6.42)	97.0 (8.35)	97.2 (6.37)	110 (5.97)	107 (6.41)
6:2 FTS	113 (3.95)	107 (6.25)	105 (9.47)	100 (9.47)	106 (5.97)	98.5 (7.66)
8:2 FTS	111 (6.32)	105 (10.6)	97.6 (10.7)	101 (8.33)	103 (4.80)	89.4 (9.41)
FOSA	–	–	104 (9.57)	91.8 (10.6)	101 (10.0)	93.4 (7.50)
NMeFOSA	–	–	109 (8.85)	102 (9.02)	96.4 (9.18)	89.0 (6.37)
NEtFOSA	–	–	–	104 (8.60)	107 (6.61)	95.6 (8.79)
5:3 FTCA	–	–	–	104 (8.59)	99.8 (8.23)	86.6 (9.52)
7:3 FTCA	–	–	–	95.1 (4.81)	98.8 (5.82)	86.0 (6.81)
NMeFOSAA	–	–	–	92.9 (11.0)	87.9 (11.5)	75.3 (10.4)
NEtFOSAA	–	–	–	92.2 (10.4)	95.9 (8.36)	83.7 (10.0)
PFMPA	–	95.7 (5.69)	96.3 (8.57)	94.8 (4.06)	107 (4.69)	104 (5.62)
PFMBA	103 (7.98)	101 (8.80)	106 (6.65)	109 (7.77)	109 (5.97)	113 (3.08)
HFPO-DA	–	–	–	105 (5.97)	100 (9.22)	103 (4.26)
ADONA	109 (7.76)	104 (9.97)	104 (6.68)	102 (9.77)	112 (3.25)	105 (2.95)
PFEESA	105 (6.83)	100 (8.27)	101 (8.66)	101 (8.66)	110 (4.99)	106 (6.19)
9Cl-PF3ONS	110 (5.62)	107 (7.20)	101 (6.31)	112 (5.55)	111 (5.64)	93.0 (2.54)
11Cl-PF3OUdS	112 (6.11)	111 (4.29)	105 (5.28)	108 (6.78)	110 (4.00)	94.6 (1.68)

Table VI: Accuracy and Precision for Black Tea

Analyte	2 ng/L	4 ng/L	10 ng/L	20 ng/L	100 ng/L	500 ng/L
$^{13}C_2$-TFA	–	–	–	99.4 (5.47)	91.8 (3.33)	95.0 (4.11)
PFPrA	–	93.0 (7.52)	103 (9.72)	99.1 (7.80)	115 (5.92)	110 (2.92)
PFBA	–	98.6 (7.36)	100 (9.20)	95.4 (7.10)	110 (6.93)	106 (2.97)
PFPeA	–	106 (9.50)	92.0 (7.30)	95.1 (9.03)	108 (4.32)	90.4 (3.90)
PFHxA	–	107 (8.90)	101 (9.22)	100 (6.33)	112 (3.85)	103 (4.62)
PFHpA	103 (7.55)	99.6 (8.09)	91.5 (9.38)	92.8 (5.09)	99.8 (3.77)	85.7 (5.22)
PFOA	–	105 (8.15)	102 (9.20)	93.4 (8.04)	96.0 (9.55)	94.0 (9.17)
PFNA	–	104 (9.44)	105 (8.42)	104 (8.33)	102 (9.58)	96.6 (9.69)
PFDA	107 (4.85)	96.2 (6.76)	93.2 (9.95)	91.8 (7.02)	97.4 (8.89)	88.6 (7.49)
PFUnA	–	98.4 (9.41)	91.2 (7.66)	102 (9.44)	100 (10.1)	100 (8.96)
PFDoA	–	109 (5.39)	101 (9.69)	108 (3.90)	109 (6.28)	111 (5.41)
PFTrDA	93.2 (9.53)	105 (8.03)	101 (9.83)	103 (8.29)	110 (8.85)	103 (5.66)
PFTeDA	115 (5.91)	113 (6.22)	114 (3.43)	109 (6.73)	113 (5.98)	113 (2.38)
TFMS	–	–	104 (6.51)	110 (4.55)	108 (7.52)	110 (8.31)
PFEtS	112 (8.35)	100 (10.0)	102 (7.37)	98.4 (9.90)	103 (8.86)	100 (10.3)
PFPrS	101 (8.73)	99.1 (7.37)	103 (5.91)	101 (3.42)	105 (3.07)	108 (4.04)
PFBS	110 (9.58)	100 (9.93)	99.2 (9.05)	102 (7.77)	111 (7.82)	103 (2.68)
PFPeS	–	–	104 (6.34)	100 (5.59)	107 (7.03)	102 (3.90)
PFHxS	105 (9.74)	97.1 (5.50)	97.2 (5.88)	97.9 (8.12)	106 (6.39)	102 (5.89)
PFHpS	103 (8.93)	93.1 (9.22)	98.3 (8.71)	91.0 (6.18)	103 (5.98)	86.7 (8.88)
PFOS	–	108 (8.98)	100 (9.15)	98.3 (5.19)	108 (5.13)	109 (5.53)
PFNS	–	118 (7.54)	99.8 (8.86)	101 (8.79)	115 (6.06)	110 (5.99)
PFDS	–	110 (8.97)	100 (8.63)	105 (9.99)	107 (7.70)	103 (7.84)
PFUdS	109 (11.8)	108 (9.72)	101 (9.71)	105 (7.29)	106 (6.34)	105 (4.41)
PFDoS	–	114 (8.33)	104 (6.72)	106 (7.27)	109 (7.51)	111 (4.69)
PFTrDS	106 (9.37)	111 (4.99)	108 (9.24)	101 (7.75)	108 (7.26)	109 (6.00)
4:2 FTS	95.0 (7.72)	94.6 (10.0)	90.7 (5.21)	94.6 (9.67)	109 (7.20)	94.8 (5.73)
6:2 FTS	97.0 (6.81)	93.8 (9.89)	93.6 (10.6)	88.6 (9.93)	105 (8.29)	89.9 (8.03)
8:2 FTS	94.7 (7.66)	110 (5.56)	102 (8.86)	106 (7.60)	112 (4.14)	102 (9.42)
FOSA	–	–	86.4 (5.30)	100 (8.73)	106 (8.86)	109 (9.15)
NMeFOSA	–	–	–	101 (5.95)	107 (9.34)	88.8 (8.29)
NEtFOSA	–	–	–	99.1 (7.83)	109 (2.97)	90.8 (6.63)
5:3 FTCA	–	–	–	100 (7.89)	95.9 (7.54)	81.3 (4.23)
7:3 FTCA	–	–	–	94.8 (10.6)	89.4 (9.19)	86.9 (6.49)
NMeFOSAA	–	–	–	–	76.4 (8.76)	71.1 (4.74)
NEtFOSAA	–	–	–	–	75.0 (8.01)	74.1 (11.0)
PFMPA	108 (7.29)	98.8 (9.85)	94.5 (7.70)	92.2 (7.52)	100 (6.58)	85.4 (3.52)
PFMBA	106 (9.72)	102 (9.42)	104 (9.19)	105 (8.71)	111 (5.86)	111 (6.33)
HFPO-DA	–	–	–	102 (9.32)	111 (8.12)	111 (6.47)
ADONA	107 (9.56)	104 (9.56)	98.6 (8.03)	102 (9.91)	111 (4.21)	113 (7.52)
PFEESA	101 (9.34)	100 (7.72)	105 (8.36)	105 (5.85)	112 (5.12)	106 (4.67)
9Cl-PF3ONS	111 (9.82)	106 (9.47)	94.7 (7.67)	91.3 (8.82)	96.9 (5.88)	95.9 (6.71)
11Cl-PF3OUdS	111 (5.78)	112 (5.65)	86.4 (5.54)	101 (9.05)	100 (6.18)	98.7 (5.68)

Table VII: Accuracy and Precision for Apple Juice

Analyte	2 ng/L	4 ng/L	10 ng/L	20 ng/L	100 ng/L	500 ng/L
$^{13}C_2$-TFA	–	–	–	100 (8.34)	95.6 (7.39)	116 (4.89)
PFPrA	–	103 (5.33)	109 (6.40)	104 (5.05)	107 (4.58)	115 (4.46)
PFBA	–	100 (9.92)	105 (9.86)	101 (9.06)	101 (5.41)	112 (8.78)
PFPeA	–	103 (7.73)	105 (7.77)	110 (6.49)	98.0 (4.20)	108 (5.33)
PFHxA	105 (8.21)	100 (8.48)	107 (9.32)	105 (5.56)	101 (8.88)	111 (9.21)
PFHpA	103 (8.42)	104 (8.64)	109 (7.15)	108 (8.42)	108 (3.76)	112 (8.68)
PFOA	105 (10.5)	104 (7.28)	111 (8.64)	113 (7.04)	102 (7.24)	110 (9.30)
PFNA	99.5 (8.21)	103 (9.73)	107 (7.87)	108 (5.42)	98.2 (9.41)	100 (6.70)
PFDA	100 (9.26)	101 (6.55)	106 (9.75)	111 (3.84)	92.8 (8.63)	102 (4.88)
PFUnA	105 (9.15)	107 (9.54)	110 (9.40)	114 (4.80)	98.6 (4.51)	105 (5.11)
PFDoA	100 (9.71)	109 (6.91)	114 (4.47)	114 (6.60)	103 (6.14)	110 (2.07)
PFTrDA	105 (4.88)	109 (3.75)	109 (7.75)	111 (4.93)	106 (5.96)	108 (8.77)
PFTeDA	106 (7.72)	109 (5.79)	108 (7.63)	114 (5.80)	109 (6.67)	110 (7.47)
TFMS	–	–	110 (3.57)	110 (6.49)	107 (3.28)	112 (4.25)
PFEtS	111 (6.55)	104 (9.68)	112 (7.92)	113 (4.56)	96.2 (9.75)	105 (7.20)
PFPrS	102 (9.57)	113 (4.99)	113 (5.94)	113 (6.68)	110 (7.61)	112 (6.55)
PFBS	111 (7.77)	103 (9.12)	111 (8.36)	113 (5.16)	102 (9.78)	108 (4.99)
PFPeS	107 (7.80)	109 (6.29)	115 (6.03)	114 (4.66)	101 (8.29)	111 (4.08)
PFHxS	115 (5.37)	100 (9.99)	107 (9.81)	105 (7.65)	100 (6.73)	109 (2.40)
PFHpS	103 (8.96)	103 (8.83)	110 (7.81)	114 (4.11)	103 (8.55)	107 (8.21)
PFOS	100 (9.28)	105 (7.20)	108 (7.07)	110 (7.20)	109 (7.24)	112 (6.52)
PFNS	94.3 (9.44)	95.7 (8.01)	108 (8.01)	108 (5.01)	103 (8.24)	106 (3.87)
PFDS	114 (9.37)	108 (7.91)	106 (10.1)	114 (3.49)	100 (5.96)	108 (4.84)
PFUdS	108 (9.03)	106 (9.85)	106 (8.80)	112 (4.96)	103 (7.50)	111 (5.14)
PFDoS	117 (7.01)	112 (4.44)	111 (7.11)	110 (7.82)	106 (7.68)	110 (8.99)
PFTrDS	98.1 (7.20)	104 (10.2)	102 (7.95)	107 (5.62)	106 (4.43)	106 (4.68)
4:2 FTS	113 (2.78)	107 (8.03)	113 (4.82)	111 (4.07)	106 (7.23)	108 (7.46)
6:2 FTS	–	103 (5.75)	108 (8.96)	106 (6.80)	113 (2.87)	111 (3.83)
8:2 FTS	98.1 (9.26)	109 (5.66)	108 (8.65)	112 (2.95)	103 (6.38)	110 (8.67)
FOSA	–	113 (7.22)	93.1 (8.26)	108 (6.88)	101 (8.20)	112 (4.83)
NMeFOSA	–	–	98.9 (8.53)	111 (7.32)	100 (10.8)	104 (7.25)
NEtFOSA	–	–	102 (7.18)	109 (6.10)	83.6 (3.59)	98.5 (6.59)
5:3 FTCA	–	–	–	104 (8.88)	104 (8.63)	107 (9.66)
7:3 FTCA	–	–	–	107 (8.80)	97.8 (9.49)	91.5 (9.88)
NMeFOSAA	–	–	94.9 (9.09)	109 (9.19)	105 (9.36)	112 (4.56)
NEtFOSAA	–	–	108 (9.13)	106 (8.94)	93.7 (10.6)	111 (5.95)
PFMPA	–	92.4 (8.56)	98.4 (9.49)	103 (7.70)	95.5 (4.59)	105 (8.86)
PFMBA	97.6 (5.16)	102 (5.63)	109 (9.29)	110 (8.37)	94.5 (7.43)	104 (5.55)
HFPO-DA	–	–	117 (3.35)	112 (4.40)	110 (7.84)	113 (3.93)
ADONA	93.9 (7.70)	98.7 (9.16)	106 (8.27)	106 (9.42)	97.2 (4.43)	103 (9.28)
PFEESA	109 (7.55)	104 (4.63)	110 (8.58)	108 (6.43)	95.9 (9.48)	104 (3.72)
9Cl-PF3ONS	102 (7.89)	110 (10.0)	112 (6.40)	110 (6.53)	94.9 (7.97)	100 (3.36)
11Cl-PF3OUdS	109 (5.77)	108 (4.73)	110 (7.77)	109 (6.35)	99.0 (5.55)	105 (3.19)

Table VIII: Accuracy and Precision for Blended Berry Juice

Analyte	2 ng/L	4 ng/L	10 ng/L	20 ng/L	100 ng/L	500 ng/L
$^{13}C_2$-TFA	–	–	–	94.3 (5.65)	104 (1.94)	114 (0.88)
PFPrA	–	110 (9.34)	110 (5.05)	107 (7.17)	111 (4.92)	105 (3.80)
PFBA	–	109 (8.06)	110 (8.65)	108 (6.62)	104 (9.48)	94.1 (6.35)
PFPeA	–	102 (8.13)	106 (8.92)	94.8 (9.61)	105 (4.38)	101 (8.78)
PFHxA	106 (8.34)	99.1 (9.69)	97.6 (9.07)	95.6 (5.25)	104 (2.97)	99.6 (7.18)
PFHpA	106 (9.74)	104 (8.49)	110 (7.86)	107 (9.03)	107 (8.88)	103 (7.69)
PFOA	106 (6.78)	107 (8.53)	105 (7.54)	108 (5.82)	102 (5.41)	101 (9.17)
PFNA	106 (9.47)	106 (6.45)	103 (4.80)	108 (6.15)	97.2 (8.85)	96.3 (5.25)
PFDA	100 (8.41)	109 (8.62)	112 (4.56)	114 (4.42)	104 (9.84)	108 (5.66)
PFUnA	104 (8.55)	106 (5.85)	103 (8.94)	110 (5.94)	96.5 (6.33)	101 (7.31)
PFDoA	111 (6.98)	109 (7.06)	108 (6.30)	115 (3.95)	97.9 (6.15)	104 (7.28)
PFTrDA	102 (4.85)	108 (6.37)	97.2 (9.78)	111 (6.43)	99.6 (6.35)	108 (9.52)
PFTeDA	106 (4.73)	105 (4.56)	96.6 (7.63)	114 (3.89)	102 (3.29)	109 (8.41)
TFMS	–	–	105 (4.65)	109 (3.53)	111 (5.55)	114 (4.54)
PFEtS	113 (5.75)	109 (6.75)	111 (6.78)	112 (6.59)	109 (7.19)	112 (4.10)
PFPrS	104 (9.93)	105 (7.84)	110 (8.21)	106 (6.13)	110 (4.55)	110 (9.02)
PFBS	110 (9.59)	101 (9.58)	106 (5.83)	98.5 (6.93)	108 (5.87)	96.8 (2.27)
PFPeS	102 (8.01)	103 (8.47)	100 (4.04)	104 (7.94)	111 (8.67)	100 (4.41)
PFHxS	121 (2.63)	97.9 (7.91)	102 (7.27)	97.3 (7.45)	101 (5.40)	98.7 (7.35)
PFHpS	104 (9.71)	107 (7.04)	110 (6.22)	112 (4.28)	111 (4.77)	99.3 (5.76)
PFOS	109 (8.60)	104 (9.08)	99.4 (9.43)	107 (4.43)	105 (6.60)	109 (9.20)
PFNS	106 (10.0)	106 (9.05)	103 (9.53)	113 (3.87)	99.4 (5.39)	102 (8.75)
PFDS	117 (5.26)	115 (7.64)	104 (10.1)	110 (6.35)	99.2 (7.59)	106 (8.96)
PFUdS	110 (9.55)	99.4 (9.91)	102 (8.31)	114 (3.77)	97.7 (5.54)	105 (9.64)
PFDoS	112 (8.38)	114 (7.34)	106 (7.36)	111 (6.22)	98.5 (4.55)	109 (7.70)
PFTrDS	118 (5.45)	105 (8.22)	107 (9.63)	111 (7.64)	98.6 (5.14)	109 (9.31)
4:2 FTS	113 (4.84)	96.7 (9.18)	100 (9.62)	93.7 (9.99)	92.5 (9.98)	95.4 (9.41)
6:2 FTS	–	97.9 (9.49)	103 (9.92)	95.8 (5.54)	95.3 (7.85)	101 (9.59)
8:2 FTS	97.9 (8.92)	94.9 (9.01)	108 (4.02)	99.4 (7.84)	92.5 (6.18)	93.1 (5.47)
FOSA	–	112 (9.69)	95.8 (10.3)	108 (9.65)	100 (9.18)	96.5 (7.23)
NMeFOSA	–	–	107 (6.32)	108 (9.18)	105 (9.20)	107 (5.20)
NEtFOSA	–	–	97.5 (9.70)	114 (4.09)	99.2 (9.06)	104 (8.33)
5:3 FTCA	–	–	–	107 (9.06)	110 (5.34)	101 (6.95)
7:3 FTCA	–	–	–	103 (8.73)	100 (6.43)	87.6 (9.60)
NMeFOSAA	–	111 (5.76)	106 (3.40)	102 (3.10)	78.5 (7.91)	111 (2.71)
NEtFOSAA	–	–	102 (9.91)	108 (8.12)	95.2 (9.76)	94.2 (9.68)
PFMPA	103 (9.19)	98.1 (9.01)	98.2 (8.02)	90.9 (8.82)	100 (9.17)	91.6 (4.13)
PFMBA	106 (3.93)	95.0 (6.39)	106 (9.20)	92.8 (8.93)	104 (3.69)	96.8 (4.67)
HFPO-DA	–	–	–	108 (9.00)	99.1 (10.3)	113 (4.02)
ADONA	111 (9.21)	103 (9.02)	95.7 (9.26)	92.8 (8.61)	99.0 (7.31)	92.0 (4.84)
PFEESA	101 (9.28)	95.8 (9.76)	101 (6.99)	100 (7.70)	107 (3.21)	95.1 (3.23)
9Cl-PF3ONS	102 (8.98)	101 (9.11)	97.8 (7.91)	109 (5.06)	99.3 (4.23)	101 (7.61)
11Cl-PF3OUdS	109 (6.95)	117 (3.67)	109 (6.38)	115 (5.11)	97.3 (2.80)	103 (7.69)

Table IX: Accuracy and Precision for Orange Juice

Analyte	2 ng/L	4 ng/L	10 ng/L	20 ng/L	100 ng/L	500 ng/L
$^{13}C_2$-TFA	–	–	–	109 (4.78)	97.0 (7.06)	114 (3.48)
PFPrA	–	–	112 (9.01)	101 (8.91)	111 (4.88)	113 (2.79)
PFBA	–	104 (6.26)	102 (6.19)	106 (5.90)	109 (6.11)	105 (2.32)
PFPeA	–	112 (8.29)	107 (8.34)	97.6 (8.75)	103 (5.43)	104 (3.63)
PFHxA	–	108 (8.53)	102 (7.96)	98.7 (9.41)	106 (7.22)	102 (5.65)
PFHpA	–	107 (9.56)	104 (8.25)	109 (4.34)	113 (9.31)	114 (2.85)
PFOA	–	112 (5.83)	101 (9.36)	93.8 (8.96)	99.2 (7.07)	90.8 (3.00)
PFNA	–	107 (7.92)	97.1 (9.17)	95.0 (7.20)	99.3 (8.94)	95.3 (4.97)
PFDA	–	105 (9.58)	97.1 (8.98)	89.6 (7.06)	103 (4.58)	95.3 (5.14)
PFUnA	–	103 (8.83)	102 (7.80)	98.7 (9.25)	104 (7.57)	92.3 (2.80)
PFDoA	–	118 (4.35)	104 (9.54)	103 (9.09)	111 (5.50)	97.7 (2.60)
PFTrDA	–	111 (4.93)	101 (8.79)	95.7 (8.87)	96.6 (4.50)	86.5 (2.11)
PFTeDA	–	115 (6.24)	106 (5.81)	101 (6.93)	111 (6.56)	104 (1.60)
TFMS	–	–	–	105 (7.35)	108 (5.60)	102 (3.51)
PFEtS	–	110 (6.57)	108 (5.56)	109 (5.22)	108 (2.56)	112 (4.06)
PFPrS	–	109 (5.62)	110 (6.21)	105 (9.07)	107 (7.16)	109 (5.84)
PFBS	–	107 (9.79)	102 (7.49)	99.6 (9.76)	105 (6.19)	102 (3.54)
PFPeS	–	106 (9.30)	105 (7.37)	108 (8.49)	107 (6.11)	112 (7.53)
PFHxS	–	111 (7.18)	99.0 (7.59)	96.0 (6.94)	95.9 (9.08)	101 (6.24)
PFHpS	–	111 (5.92)	98.3 (9.71)	102 (8.34)	100 (6.60)	102 (2.52)
PFOS	–	114 (8.86)	103 (9.19)	94.3 (8.99)	96.8 (6.13)	98.9 (4.38)
PFNS	–	120 (7.66)	86.8 (5.72)	102 (10.1)	95.2 (8.09)	92.4 (3.12)
PFDS	–	111 (7.72)	97.2 (8.59)	95.7 (10.7)	95.1 (6.71)	83.9 (3.69)
PFUdS	–	112 (6.21)	99.7 (8.65)	96.1 (9.44)	100 (6.93)	90.4 (3.81)
PFDoS	–	109 (6.06)	98.3 (9.53)	98.8 (9.43)	106 (6.57)	93.6 (3.54)
PFTrDS	–	115 (5.44)	109 (6.27)	95.9 (10.6)	104 (7.01)	95.0 (5.06)
4:2 FTS	–	102 (8.22)	104 (8.92)	93.0 (6.36)	98.4 (9.32)	93.4 (6.29)
6:2 FTS	–	104 (3.08)	97.7 (7.27)	94.4 (9.75)	110 (7.26)	109 (5.80)
8:2 FTS	–	108 (8.43)	96.5 (8.58)	103 (8.41)	108 (8.02)	115 (3.39)
FOSA	–	116 (8.38)	101 (7.95)	102 (9.79)	101 (8.26)	107 (8.98)
NMeFOSA	–	–	103 (9.25)	107 (9.74)	109 (7.03)	115 (3.60)
NEtFOSA	–	–	107 (4.13)	106 (7.54)	104 (9.22)	100 (9.16)
5:3 FTCA	–	–	–	94.5 (9.06)	100 (8.37)	100 (6.81)
7:3 FTCA	–	–	–	99.3 (9.08)	109 (6.13)	111 (3.62)
NMeFOSAA	–	–	122 (2.96)	99.4 (8.85)	106 (8.90)	109 (6.56)
NEtFOSAA	–	–	–	94.7 (9.55)	102 (9.15)	102 (9.44)
PFMPA	–	100 (7.26)	95.7 (7.44)	93.5 (7.34)	93.1 (7.00)	87.3 (4.14)
PFMBA	–	111 (4.98)	107 (8.50)	91.7 (6.34)	110 (6.47)	103 (5.88)
HFPO-DA	–	–	–	91.9 (8.61)	104 (9.74)	88.5 (8.47)
ADONA	–	102 (9.87)	99.4 (8.22)	94.4 (6.41)	109 (5.66)	107 (4.46)
PFEESA	–	110 (6.86)	96.9 (9.85)	91.4 (9.96)	102 (7.64)	98.9 (1.32)
9Cl-PF3ONS	–	111 (3.64)	97.8 (8.46)	104 (9.83)	107 (6.47)	103 (2.06)
11Cl-PF3OUdS	–	108 (5.31)	91.8 (7.72)	93.0 (7.28)	98.4 (3.61)	87.3 (1.49)

Featured Products



Ultra IBD Inert, 3 µm, 100 x 2.1 mm HPLC Column

- Inert LC column technology reduces nonspecific binding of chelating analytes, enabling sensitive analysis and smooth integration of peaks.
- Increased response and analyte recovery, allowing lower detection limits.
- Improved peak shape without passivation or mobile phase additives.
- Specialized columns for mixed polar and nonpolar compounds.
- Part of Restek's Ultra LC column line, our broadest selection of stationary phases on a single silica support.

Catalog No.	Product Name	Units
9175312-T	Ultra IBD Inert, 3 µm, 100 x 2.1 mm HPLC Column	ea.



Ultra IBD HPLC Column

Catalog No.	Product Name	Units
9175362	Ultra IBD, 3 µm, 150 x 2.1 mm HPLC Column	ea.



PFAS 28 Calibration Standard

Catalog No.	Product Name	Units
30734	PFAS 28 Calibration Standard, 1 µg/mL, Methanol (1 mM KOH), 1 mL/ampul	ea.



Analysis of PFAS in Milk by LC-MS/MS

By Jamie York

Abstract

The ubiquitous use of per- and polyfluoroalkyl substances (PFAS) in everyday products has resulted in the accumulation of these compounds in the environment. Due to the prevalence of these compounds in environmental sources, such as water and soil, they are finding avenues into food commodities meant for human consumption. Dairy milk is one such commodity that can be impacted by environmental contaminants, such as PFAS, so it is important to implement extensive, robust, and accurate testing. In this work, a sensitive and reliable method was developed for the analysis of PFAS in milk by LC-MS/MS at levels as low as 0.01 µg/kg.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured organic compounds that are used in a wide array of applications and products. The carbon-fluorine bond is very stable resulting in compounds that do not readily degrade in the environment. As such, these compounds have been detected in drinking water, soil, wastewater, food commodities, and biological matrices. There are several potential adverse health effects associated with exposure to PFAS. These include increased cholesterol levels, decreased birth weight, lower antibody response to vaccines, kidney and testicular cancer, pregnancy-induced hypertension (preeclampsia), and changes in liver enzymes [1]. Since food sources have been identified as one of the avenues of exposure to PFAS, it is important to have robust, reliable, and sensitive methods to monitor these compounds in foods intended for human consumption.

Related Products

- *Force C18 3 µm, 50 mm x 2.1 mm (cat.# 9634352)*
- *Force C18 EXP guard cartridge, 5 mm x 2.1 mm (963450252)*
- *PFAS delay column (cat. # 27854)*
- *PFAS 28 calibration standard (cat.# 30734)*
- *50 mL centrifuge tube (cat.# 25846)*
- *Q-sep QuEChERS extraction salt packet (cat.# 25847)*
- *Q-sep QuEChERS dSPE 15 mL centrifuge tube (cat.# 26126)*
- *Polypropylene vial (cat.# 23243)*
- *Polyethylene screw cap (cat.# 23244)*

More than six billion people around the world consume dairy milk and milk products [2] so it is imperative to monitor PFAS contamination in these products. The European Food Safety Authority (EFSA) has assessed four PFAS that make up half of the lower bound exposure: perfluorooctane sulfonic acid (PFOS); perfluorooctanoic acid (PFOA); perfluorohexane sulfonic acid (PFHxS); and perfluorononaoic acid (PFNA). Toddlers and children were found to have two-fold higher exposure [3], and this could potentially be from their consumption of milk and other related food items. The EFSA recommends limiting exposure to these four PFAS to no more than 4.4 nanograms per kilogram of body weight per week [3]. In the United States, Maine Department of Agriculture, Conservation, and Forestry (DACF) established an action threshold for PFOS in milk at 210 parts per trillion [4].

In this work, a method was developed for the analysis of PFAS in milk by LC-MS/MS, including the four PFAS assessed by the EFSA and 24 other compounds included in the European Union Reference Laboratory for Halogenated POPs (persistent organic pollutants) in Feed and Food guidance document. This guidance document outlines required limits of quantitation for the four PFAS analytes identified by EFSA in milk to be as low as ≤0.01 µg/kg [5]. This target LOQ was used in these experiments and applied to all 28 PFAS. One potential complication of this analysis is the presence of coextractables, such as bile acids. Bile acids tauroursodeoxycholic acid (TUDCA); taurochenodeoxycholic acid (TCDCA); and taurodeoxycholic acid (TDCA) can be present in high concentrations in samples of biological origin and share the same mass transition as PFOS. To accurately quantify PFOS, the bile acids need to either be removed from the sample during sample preparation or separated from PFOS chromatographically. In this application, a workflow was developed for the analysis of 28 PFAS in milk. To prepare samples, a QuEChERS approach coupled with dSPE was implemented. This workflow returned exceptional results for analysis of the four PFAS assessed by the EFSA and showed excellent method accuracy and precision for most target compounds.

Experimental

Chromatographic Method

LC-MS/MS analysis of PFAS in milk was performed on a Waters ACQUITY Premier system coupled with a Waters XEVO TQ Absolute triple quadrupole mass spectrometer. Note that a PFAS delay column was installed between the mixer and injector to prevent any potential PFAS contamination upstream of the injector from coeluting with PFAS in the samples. The method conditions are as follows:

Columns:

- Analytical column: Force C18, 50 mm x 2.1 mm, 3 µm (cat.# 9634352)
- Guard column: Force C18 EXP, 5 mm x 2.1 mm (cat.# 963450252)
- PFAS delay column, 50 mm x 2.1 mm, 5 µm (cat.# 27854)

Injection volume: 5 µL
Mobile phase A: Water, 5 mM ammonium acetate
Mobile phase B: Methanol:acetonitrile (50:50)
Flow rate: 0.6 mL/min
Temperature: 50 °C

Gradient:

Time (min)	%B
0.00	5
3.00	40
9.00	70
9.50	95
10.50	100
11.00	100
11.01	5
13.00	5

Ion mode: Negative ESI
Mode: Scheduled MRM

Sample and Standard Preparation

All products and solvents were tested for PFAS contamination prior to use. Milk samples were obtained locally and included reduced-fat milk (Sample 1); fat-free milk (Sample 2); and low-fat milk (Sample 3). Ten gram samples of milk were weighed into 50 mL centrifuge tubes (cat.# 25846) and fortified at 0.01 or 0.05 µg/kg. Isotopically labeled internal standards were spiked into the sample at 0.1 µg/kg and briefly vortexed. Acetonitrile (10 mL), formic acid (150 µL), and a Q-sep QuEChERS extraction salt packet (cat.# 25847) were added to the sample and vortexed for approximately 30 seconds. The samples were transferred to a shaker table and shaken for 10 minutes. The samples were then centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a Q-sep QuEChERS dSPE 15 mL centrifuge tube containing 900 mg $MgSO_4$, 300 mg PSA, 150 mg GCB (cat.# 26126) and vortexed for approximately 30 seconds followed by centrifugation for 5 minutes at 4200 rpm. The supernatant was aliquoted (6 mL) into clean 15 mL centrifuge tubes and dried down at 35 °C for 90 minutes using a blowdown evaporator. The samples were reconstituted in methanol:water (60:40) (400 µL), vortexed for approximately 30 seconds, and centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a polypropylene vial (cat.# 23243) and capped with a polyethylene screw cap (cat.# 23244) and 5 µL was injected for analysis.

The working calibration standards were prepared using Restek's PFAS 28 calibration standard (cat.# 30734), and the standards for other PFAS were obtained separately. The standards were aliquoted into polypropylene LC vials and diluted in 60:40 methanol:water to a concentration range of 0.03–50 ng/mL. Each calibration curve included seven or eight concentration levels depending on analyte sensitivity. Internal standards were spiked in the calibration standards at 1.5 ng/mL. Solvent calibration using isotope dilution was performed for quantitation. The mass transitions and the isotopically labeled internal standards that were used for each analyte are shown in Table I.

Table I: Precursor Ions, Product Ions, and Isotopically Labeled Internal Standards Used for the Analysis of PFAS in Milk by LC-MS/MS

Compound	Precursor 1	Product 1	Precursor 2	Product 2	Internal Standard
PFBA	212.94	168.89	NA	NA	M4PFBA
PFPeA	262.93	218.89	NA	NA	M5PFPeA
PFHxA	313.10	119.03	313.10	268.88	M5PFHxA
PFHpA	363.16	169.06	363.16	319.09	M4PFHpA
PFOA	413.10	168.90	413.10	368.96	M8PFOA
PFNA	463.10	219.02	463.10	419.01	M9PFNA
PFDA	513.17	219.06	513.17	469.16	M6PFDA
PFUnDA	562.78	268.82	562.78	518.80	M7-PFUnDA
PFDoA	612.52	318.90	612.52	568.80	M2-PFDoA
PFTrDA	662.78	168.87	662.78	618.74	M2-PFTeDA
PFTeDA	712.78	168.87	712.78	668.74	M2-PFTeDA
PFBS	298.97	79.97	298.97	98.89	M3PFBS
PFPeS	349.10	79.98	349.10	98.98	M5PFHxA
PFHxS	398.80	79.82	398.80	98.82	M3PFHxS
PFHpS	448.78	79.82	448.78	98.82	M9PFNA
PFOS	498.78	79.89	498.78	98.82	M8PFOS
PFNS	548.65	79.89	548.65	98.82	M7-PFUnDA
PFDS	598.78	98.82	598.79	79.89	M2-PFDoA
PFUnDS	648.65	79.88	648.65	98.82	M2-PFDoA
PFTrDS	748.59	79.88	748.90	98.88	M2-PFTeDA
PFDoS	698.71	79.89	698.71	98.88	M2-PFTeDA
FOSA	497.75	77.87	497.75	477.76	M7-PFUnDA
ADONA	376.90	84.97	376.90	250.93	M4PFHpA
HFPO-Da	284.87	168.89	284.87	184.83	M5PFHxA
9Cl-PF3ONS	530.78	350.85	532.84	352.78	M7-PFUnDA
11Cl-PF3OUdS	630.71	450.81	632.71	452.81	M2-PFDoA
Capstone A	526.90	180.96	526.90	506.91	M9PFNA
Capstone B	568.81	548.85	568.81	548.90	M9PFNA

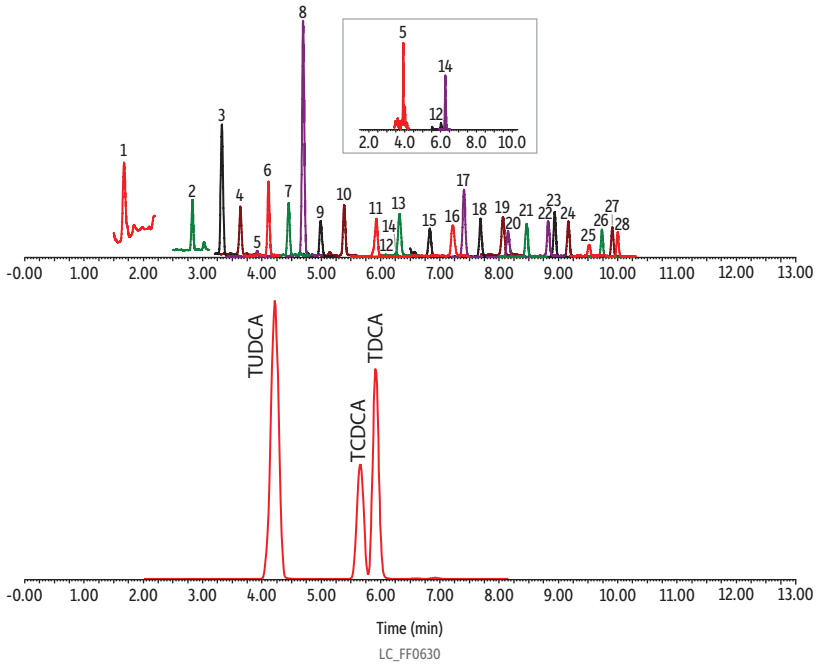
Results & Discussion

Chromatographic Performance

Bile acids are often a concern when analyzing tissue samples for PFAS. Taurodeoxycholic acid (TDCA) is an endogenous bile acid that is formed in the liver and can be detected at high concentrations in biological samples. This compound, along with tauroursodeoxycholic acid (TUDCA) and taurochenodeoxycholic acid (TCDCA), all share the same mass transition (499→80) as PFOS and can also potentially interfere with FOSA. As a result, bile acids either need to be removed from the sample or be chromatographically resolved from the impacted compounds. It can be challenging to selectively remove bile acids from the sample without also removing analytes of interest; therefore, the chromatographic approach was taken here for the analysis of PFAS in milk samples.

Methanol and acetonitrile were both tested as organic mobile phases. Using a scouting gradient, methanol was tested first, and the results indicated that the use of this organic mobile phase would require significantly more method development to be able to potentially resolve the interferences. When using acetonitrile, all three bile acids were easily resolved from PFOS using the scouting conditions, but sensitivity for some of the PFAS was reduced compared to when using methanol. Therefore, a 50:50 mixture of the two organic modifiers was used, and it provided good sensitivity and resolution of the bile acids from PFOS. The final LC-MS/MS method for the analysis of PFAS in milk used a 50:50 mixture of methanol and acetonitrile and can be seen in Figure 1.

Figure 1: Top: Chromatogram of 28 PFAS and Internal Standards (The PFAS that can potentially be affected by bile acid coelution are PFOS and FOSA.) Bottom: Chromatogram of Three Bile Acids Analyzed Using the Same Chromatographic Conditions



	Peaks	Precursor Ion 1	Product Ion
1.	Perfluorobutanoic acid (PFBA)	212.94	168.89
2.	Perfluoropentanoic acid (PFPeA)	262.93	218.89
3.	Perfluorobutane sulfonic acid (PFBS)	298.96	79.97
4.	Perfluorohexanoic acid (PFHxA)	313.09	119.03
5.	Hexafluoropropylene oxide dimer acid (HFPO-DA)	284.86	168.88
6.	Perfluoropentane sulfonic acid (PFPeS)	349.10	79.98
7.	Perfluoroheptanoic acid (PFHpA)	363.15	169.06
8.	Ammonium 4,8-dioxa-3*H*-perfluorononanoate (ADONA)	376.90	84.97
9.	Perfluorohexane sulfonic acid (PFHxS)	398.80	79.82
10.	Perfluorooctanoic acid (PFOA)	413.09	168.90
11.	Perfluoroheptane sulfonic acid (PFHpS)	448.78	79.82
12.	1-Propanaminium, *N*-(carboxymethyl)-*N*,*N*-dimethyl-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide (Capstone B)	568.81	548.85
13.	Perfluorononanoic acid (PFNA)	463.09	219.01
14.	1-Propanaminium, *N*,*N*-dimethyl-*N*-oxide-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide (Capstone A)	526.90	180.96
15.	Perfluorooctane sulfonic acid (PFOS)	498.78	79.89
16.	Perfluorodecanoic acid (PFDA)	513.16	219.06
17.	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	530.77	350.85
18.	Perfluorononane sulfonic acid (PFNS)	548.65	79.89
19.	Perfluoroundecanoic acid (PFUnA)	562.78	268.82
20.	Perfluorooctane sulfonic acid (FOSA)	497.75	77.87
21.	Perfluorodecane sulfonic acid (PFDS)	598.78	98.82
22.	Perfluorododecanoic acid (PFDoA)	612.52	318.90
23.	11-Chloroeicosafluoro-3-oxanonane-1-sulfonate (11Cl-PF3OUdS)	630.71	450.81
24.	Perfluoroundecane sulfonic acid (PFUnDS)	648.65	79.88
25.	Perfluorotridecanoic acid (PFTrDA)	662.78	168.87
26.	Perfluorododecane sulfonic acid (PFDoS)	698.71	79.89
27.	Perfluorotetradecanoic acid (PFTeDA)	712.78	168.87
28.	Perfluorotridecane sulfonic acid (PFTrDS)	748.59	79.88

Column Force C18 (cat.# 9634352)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Guard Column: Force EXP C18 guard cartridge 5 mm, 2.1 mm ID (cat.# 963450252)
Temp.: 50 °C
Standard/Sample PFAS 28 calibration standard (cat.# 30734)
Other standards obtained externally.
Diluent: Methanol:water (60:40)
Conc.: Analytes spiked at 0.01 µg/kg in milk
Inj. Vol.: 5 µL
Mobile Phase
A: Water, 5 mM ammonium acetate
B: Methanol:acetonitrile (50:50)

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	95	5
3.00	0.6	60	40
9.00	0.6	30	70
9.50	0.6	5	95
10.50	0.6	0	100
11.00	0.6	0	100
11.01	0.6	95	5
13.00	0.6	95	5

Max Pressure: **360 bar**
Detector Waters Xevo TQ Absolute
Ion Mode: ESI-
Mode: MRM
Instrument Waters ACQUITY Premier
Sample Preparation Ten grams of milk were weighed into a 50 mL centrifuge tube (cat.# 25846) and fortified at 0.01 µg/kg. Isotopically labeled internal standards (50 µL) were spiked into the sample and briefly vortexed. Acetonitrile (10 mL); formic acid (150 µL); and a Q-sep QuEChERS extraction salt packet (cat.# 25847) were added to the sample and vortexed for ~30 seconds. The samples were transferred to a shaker table and shaken for 10 minutes. The samples were then centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a Q-sep QuEChERS dSPE 15 mL centrifuge tube containing 900 mg $MgSO_4$; 300 mg PSA; and 150 mg GCB (cat.# 26126) and then vortexed for ~30 seconds followed by centrifuging for 5 minutes at 4200 rpm. The supernatant was aliquoted (6 mL) into a clean 15 mL centrifuge tube and dried down at 35 °C for 90 minutes using a Biotage TurboVap. The samples were reconstituted in methanol:water 60:40 (400 µL); vortexed for ~30 seconds; and centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a polypropylene vial (cat.# 23243) and capped with a polyethylene screw cap (cat.# 23244) and 5 µL was injected for analysis.
Notes A PFAS delay column (cat.# 27854) was installed before the injector.

Linearity, Accuracy, & Precision

For this LC-MS/MS method for the analysis of PFAS in milk, calibration was performed across a range of 0.03/0.1–50 ng/mL at seven or eight different concentrations. To assess linearity, 1/x weighting was used. Good linearity was demonstrated by R^2 values ≥ 0.991 for all compounds (Table III).

Three different milk samples were obtained and fortified at 1X target LOQ and 5X target LOQ levels (0.01 µg/kg and 0.05 µg/kg, respectively). Blank samples were prepared in duplicate and analyzed in triplicate. Milk samples were prepared in triplicate and analyzed in triplicate. Analytes detected in the blank samples are identified by an asterisk in Tables II and III, and incurred levels were subtracted from the fortified samples. Excellent recoveries were obtained at the LOQ spiking level for all four of the PFAS assessed by the EFSA. These recoveries were 101–112% at 0.01 µg/kg, and excellent precision was demonstrated by %RSDs of 3.81–16.6% (Table II).

Table II: Percent Recoveries and %RSD at 0.01 µg/kg for the Four PFAS Assessed by EFSA

	Average Percent Recovery (%RSD) n=9		
	Sample 1	Sample 2	Sample 3
PFOA*	108 (5.09)	102 (6.21)	105 (7.74)
PFNA	112 (7.97)	106 (8.19)	101 (16.6)
PFHxS	112 (5.93)	106 (7.25)	106 (12.8)
PFOS*	109 (7.25)	109 (3.81)	102 (6.72)

**Incurred levels were present in the blank samples for these analytes and were subtracted from the fortified samples.*

Most of the 28 PFAS monitored in these experiments had acceptable recoveries within the 80–120% range. Some long-chain PFAS showed low percent recoveries, namely PFTrDA, PFTeDA, PFUnDS, PFTrDS, PFDoS, FOSA, and Capstone B, which contain carbon chains of eight to thirteen carbons. Low recoveries were not observed in Sample 2, the fat-free milk, indicating that the presence of milk fat in the other samples could potentially be hindering the recoveries for these compounds. A summary of the linear dynamic range, R^2 values, percent recoveries, and percent RSD values can be seen in Table III for all 28 PFAS.

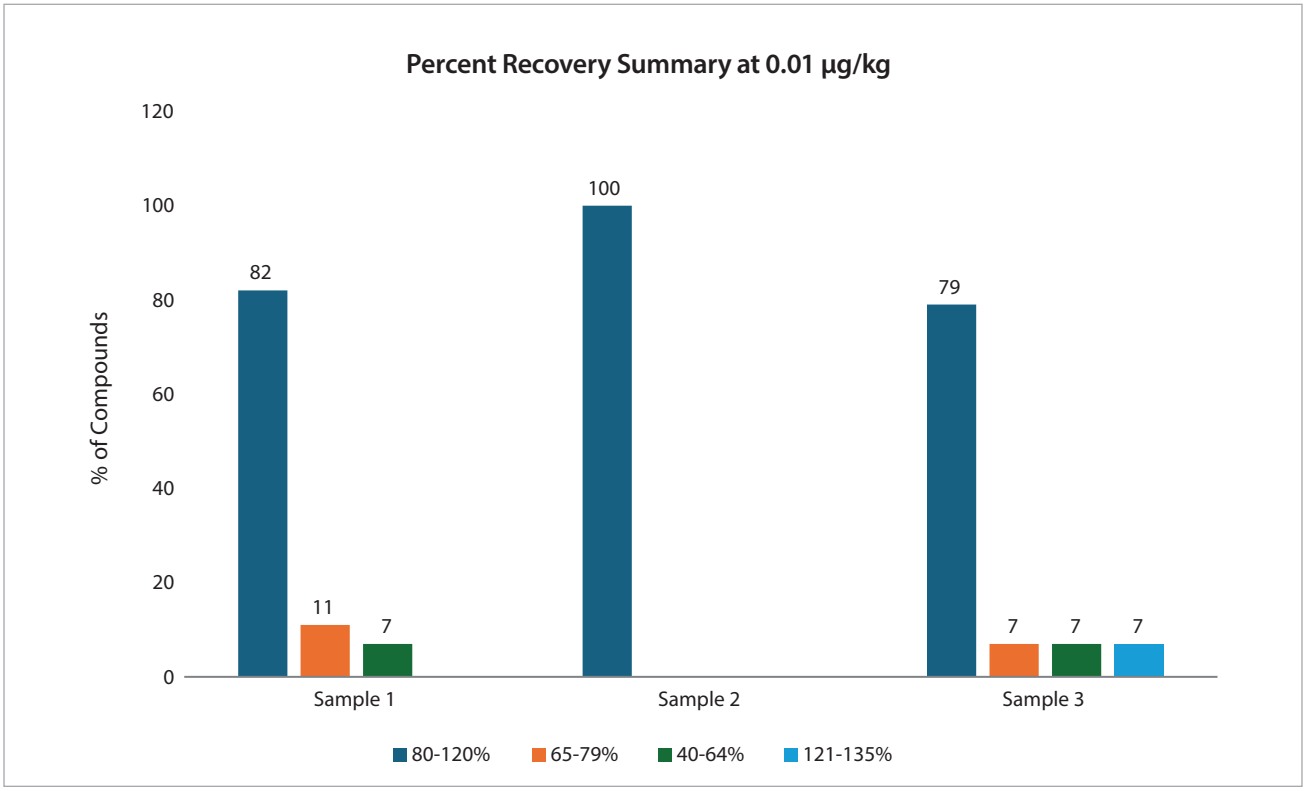
Table III: Linearity, Accuracy, and Precision in Milk

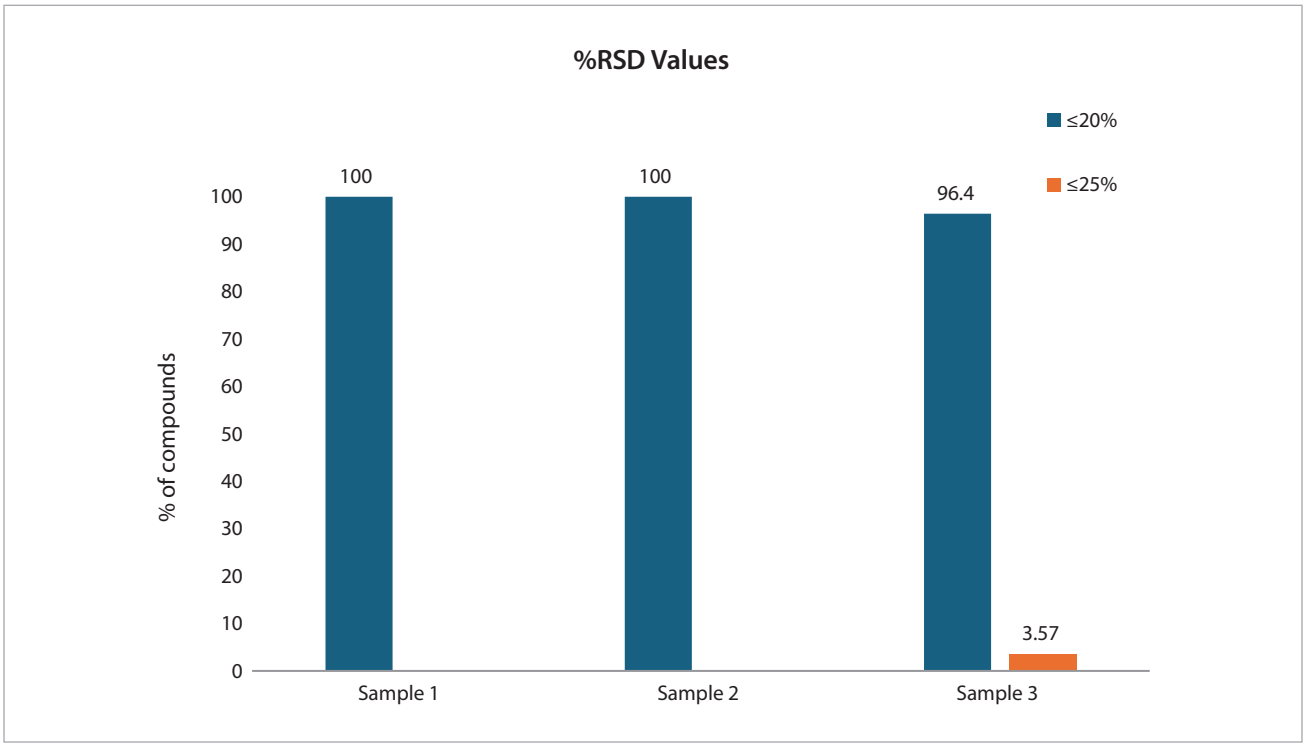
Compound	Linear Range (ng/mL)			Percent Recovery (Percent RSD) n=9					
	Low	High	R^2	Sample 1		Sample 2		Sample 3	
				0.01 µg/kg	0.05 µg/kg	0.01 µg/kg	0.05 µg/kg	0.01 µg/kg	0.05 µg/kg
PFBA*	0.1	50	0.996	90.1 (4.81)	105 (7.06)	103 (8.61)	116 (2.91)	115 (11.5)	116 (4.95)
PFPeA	0.1	50	0.996	105 (7.89)	110 (5.45)	102 (6.72)	111 (2.55)	107 (10.8)	114 (2.80)
PFHxA	0.1	50	0.996	114 (10.6)	113 (5.13)	104 (8.11)	112 (5.49)	118 (11.3)	113 (6.11)
PFHpA	0.03	50	0.997	117 (5.09)	112 (7.31)	108 (7.14)	115 (2.69)	111 (16.2)	118 (4.21)
PFOA*	0.1	50	0.995	108 (5.09)	109 (4.62)	102 (6.21)	113 (2.56)	105 (7.74)	113 (3.28)
PFNA	0.03	50	0.996	112 (7.97)	109 (2.63)	106 (8.19)	110 (3.81)	101 (16.6)	101 (10.4)
PFDA	0.03	50	0.997	111 (9.02)	113 (5.55)	107 (6.08)	112 (4.13)	112 (7.38)	115 (3.26)
PFUnDA	0.03	50	0.992	104 (7.52)	109 (11.6)	103 (10.9)	107 (7.57)	109 (8.90)	110 (4.54)
PFDoA	0.03	50	0.997	110 (7.57)	109 (4.34)	102 (6.72)	112 (2.76)	108 (9.77)	114 (2.45)
PFTrDA	0.03	50	0.999	75.9 (12.5)	65.8 (7.68)	86.7 (8.95)	73.3 (8.64)	71.8 (11.0)	68.8 (12.2)
PFTeDA	0.03	50	0.997	83.2 (14.5)	83.8 (12.4)	103 (6.91)	109 (8.95)	74.1 (17.2)	78.4 (11.3)
PFBS*	0.1	50	0.995	106 (11.9)	109 (11.4)	110 (11.2)	112 (5.21)	103 (7.18)	109 (5.32)
PFPeS	0.1	50	0.996	113 (6.32)	119 (5.78)	105 (6.19)	113 (4.87)	114 (8.03)	115 (5.08)
PFHxS	0.03	50	0.996	112 (5.93)	114 (5.29)	106 (7.25)	111 (3.59)	106 (12.8)	113 (3.92)
PFHpS	0.03	50	0.995	117 (5.14)	121 (9.15)	112 (5.57)	116 (3.81)	121 (5.04)	119 (5.32)
PFOS*	0.1	50	0.995	109 (7.25)	110 (7.21)	109 (3.81)	113(4.03)	102 (6.72)	107 (4.62)
PFNS	0.03	50	0.999	114 (17.1)	88.5 (18.1)	110 (11.5)	90.1 (15.4)	103 (15.5)	84.3 (19.9)
PFDS	0.03	50	0.997	92.6 (11.7)	95.8 (6.01)	101 (7.31)	100 (9.33)	99.2 (12.3)	102 (11.8)
PFUnDS	0.03	50	0.997	74.2 (9.48)	95.5 (9.11)	100 (9.94)	113 (9.25)	81.4 (20.7)	104 (12.7)
PFTrDS	0.03	50	0.994	67.9 (15.6)	46.2 (9.53)	88.1 (14.1)	95.5 (10.6)	43.2 (15.1)	55.5 (6.04)
PFDoS	0.03	50	0.997	55.6 (9.8)	59.6 (8.22)	96.3 (10.0)	103 (9.33)	62.1 (11.5)	73.9 (7.29)
FOSA	0.1	50	0.992	60.8 (12.6)	59.4 (10.2)	92.6 (8.16)	95.5 (9.41)	87.1 (15.3)	84.0 (12.5)
ADONA	0.03	50	0.996	111 (7.17)	109 (7.91)	93.8 (11.3)	98.9 (4.52)	93.8 (9.54)	104 (6.00)
HFPO-Da	0.1	50	0.996	106 (13.7)	110 (7.69)	111 (15.4)	114 (12.7)	125 (9.00)	124 (4.31)
9Cl-PF3ONS	0.03	50	0.992	94.5 (3.37)	99.3 (8.09)	96.6 (10.3)	98.9 (7.64)	102 (12.8)	106 (6.08)
11Cl-PF3OUdS	0.03	50	0.997	93.5 (6.74)	97.8 (6.78)	103 (12.2)	114 (10.0)	97.7 (11.2)	102 (13.1)
Capstone A	0.1	50	0.991	120 (12.1)	126 (9.27)	102 (16.5)	108 (7.34)	119 (6.44)	114 (5.06)
Capstone B	0.1	50	0.992	92.1 (11.6)	68.3 (10.9)	85.1 (15.2)	71.7 (8.24)	84.6 (15.3)	71.4 (10.6)

**Incurred levels were present in the blank samples for these analytes and were subtracted from the fortified samples.*

Figure 2 outlines the results for the analysis of all 28 PFAS in milk samples using this LC-MS/MS method. At 0.01 µg/kg, 82% of analytes meet the 80–120% recovery range for Sample 1; 100% did for Sample 2; and 79% did for Sample 3. All but one analyte, PFUnDS, returned a %RSD value of ≤20%.

Figure 2: Percentage of Analytes in Each Sample Meeting the Acceptable Accuracy and Precision Criteria at 0.01 µg/kg for the Analysis of PFAS in Milk





Conclusion

This workflow provides straightforward and reliable methodology for the analysis of 28 PFAS in milk by LC-MS/MS. The method effectively separates target analytes from bile acid interferences that share the same mass transition. With a fast, simple sample preparation using QuEChERS extraction and dSPE cleanup, this workflow is ideal for high-throughput PFAS testing labs. The results showed exceptional results for the four PFAS assessed by the EFSA, and acceptable results for the majority of the other analytes. The recoveries of a few of the long-chain PFAS returned low values, which may be related to the presence of fat in milk samples. Although the recoveries for these compounds were low, the precision of the method was still within the acceptable range. This method can easily be implemented in labs to confidently monitor PFAS contamination in dairy milk.

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Force C18 LC Column

- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- High carbon load (20%) offers high hydrophobic retention.

Catalog No.	Product Name	Units
9634352	Force C18 LC Column, 3 µm, 50 mm x 2.1 mm	ea.



Force C18 Guard Cartridge

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.
- For use with 3 or 5 µm Force LC columns. For 1.8 µm Force columns, use a 0.2 µm UltraShield filter.

Catalog No.	Product Name	Units
963450252	Force C18 Guard Cartridge, 5 x 2.1 mm EXP	3-pk.

PFAS Delay Column



- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

Catalog No.	Product Name	Units
27854	PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column	ea.

PFAS 28 Calibration Standard



Contains:
11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)
1*H*,1*H*,2*H*,2*H*-Perfluorodecane sulfonic acid (8:2 FTS) (39108-34-4)
1*H*,1*H*,2*H*,2*H*-Perfluorohexane sulfonic acid (4:2 FTS) (757124-72-4)
1*H*,1*H*,2*H*,2*H*-Perfluorooctane sulfonic acid (6:2 FTS) (27619-97-2)
4,8-dioxa-3*H*-perfluorononanoic acid (ADONA) (919005-14-4)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)
2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6)
N-ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)* (2991-50-6)
N-methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)* (2355-31-9)
Perfluoro-1-decanesulfonic acid (PFDS) (335-77-3)
Perfluoro-1-nonanesulfonic acid (PFNS) (68259-12-1)
Perfluoro-1-octanesulfonamide (FOSA) (754-91-6)
Perfluoro-1-pentanesulfonic acid (PFPeS) (2706-91-4)
Perfluorobutanesulfonic acid (PFBS) (375-73-5)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFDOA) (307-55-1)
Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)
Perfluoroheptanoic acid ((PFHpA) (375-85-9)
Perfluorohexanesulfonic acid (PFHxS)* (355-46-4)
Perfluorohexanoic acid ((PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Heptadecafluorooctanesulfonic acid (PFOS)* (1763-23-1)
Perfluorooctanoic acid (PFOA)* (335-67-1)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluorotetradecanoic acid (PFTeDA) (376-06-7)
Perfluorotridecanoic acid (PFTrDA) (72629-94-8)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)
*Technical grade compound containing both branched and linear isomers; see certificate for details.

Catalog No.	Concentration	Solvent	Volume	Units
30734	1 µg/mL	Methanol (1 mM KOH)/2-Propanol (98:2)	1 mL/ampul	ea.



Q-sep QuEChERS Extraction Salt Packets

- Free-flowing salts transfer easily and completely.
- Easy-open packets eliminate the need for a second empty tube for salt transfer.
- Convenient slim packets fit perfectly into tubes to prevent spills.
- Ready-to-use tubes, no glassware required.
- Pre-weighed, ultra-pure extraction salts.
- Ideal for original unbuffered, AOAC (2007.01), and European (EN 15662) QuEChERS methods.

Catalog No.	Product Name	Units
25847	Q-sep QuEChERS Extraction Salt Packets Only (Original), 4 g $MgSO_4$, 1 g NaCl	50-pk.



Q-sep QuEChERS dSPE

- Packaged in foil subpacks of 10 for enhanced protection and storage stability.
- Ready-to-use tubes, no glassware required.
- Pre-weighed, ultra-pure sorbents.
- Support original unbuffered, AOAC (2007.01), European (EN 15662), and mini-multiresidue QuEChERS methods.

Catalog No.	Product Name	Units
26126	Q-sep QuEChERS dSPE 15 mL Centrifuge Tube, Contains 900 mg $MgSO_4$, 300 mg PSA, 150 mg GCB	50-pk.



Empty Centrifuge Tubes

Catalog No.	Product Name	Units
25846	Empty Centrifuge Tubes, 50 mL, Polypropylene w/Cap	50-pk.



Limited-Volume Screw-Thread Polypropylene Vials

Catalog No.	Product Name	Units
23243	Limited-Volume Screw-Thread Polypropylene Vials, 9 mm, 700 µL, 12 x 32 mm	100-pk.



Solid-Top Polyethylene Caps, Screw-Thread

Catalog No.	Product Name	Units
23244	2.0 mL, 9 mm Solid-Top Polyethylene Caps, Screw-Thread, 10 mil thick membrane, Clear	100-pk.

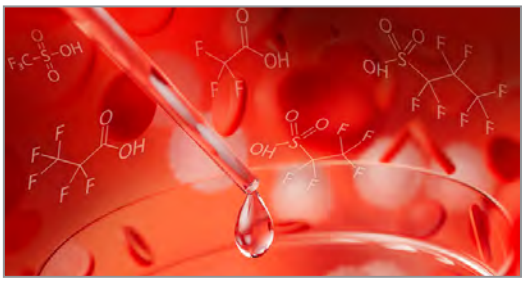
C1-C10 PFAS Analysis in Human Plasma and Serum

Integrating Ultrashort-Chain Compounds into PFAS Biomonitoring

By Shun-Hsin Liang and Justin Steimling

Abstract

Human exposure to ultrashort-chain (C1 to C3) PFAS and their potential adverse health effects are poorly understood, partly due to a lack of analytical methods to properly monitor their occurrence. This study introduces an innovative methodology for the simultaneous analysis of C1 to C10 carboxylic and sulfonic acid PFAS, along with four alternative compounds, in human plasma and serum samples. The chromatographic analysis was conducted using a polar-embedded, reversed-phase LC column for accurate and robust quantification.



Related Products

- *PFAS delay column (cat. # 27854)*
- *Ultra IBD 3 µm, 100 mm x 2.1 mm (cat.# 9175312)*
- *Ultra IBD Inert 3 µm, 100 mm x 2.1 mm (cat.# 9175312-T)*
- *PFAS 28 calibration standard (cat.# 30734)*
- *Big Mouth Step screw-thread vials (cat.# 24626)*
- *Polypropylene vial inserts (cat.# 24518)*
- *Survival kit for HPLC, stainless steel (cat.# 25097)*

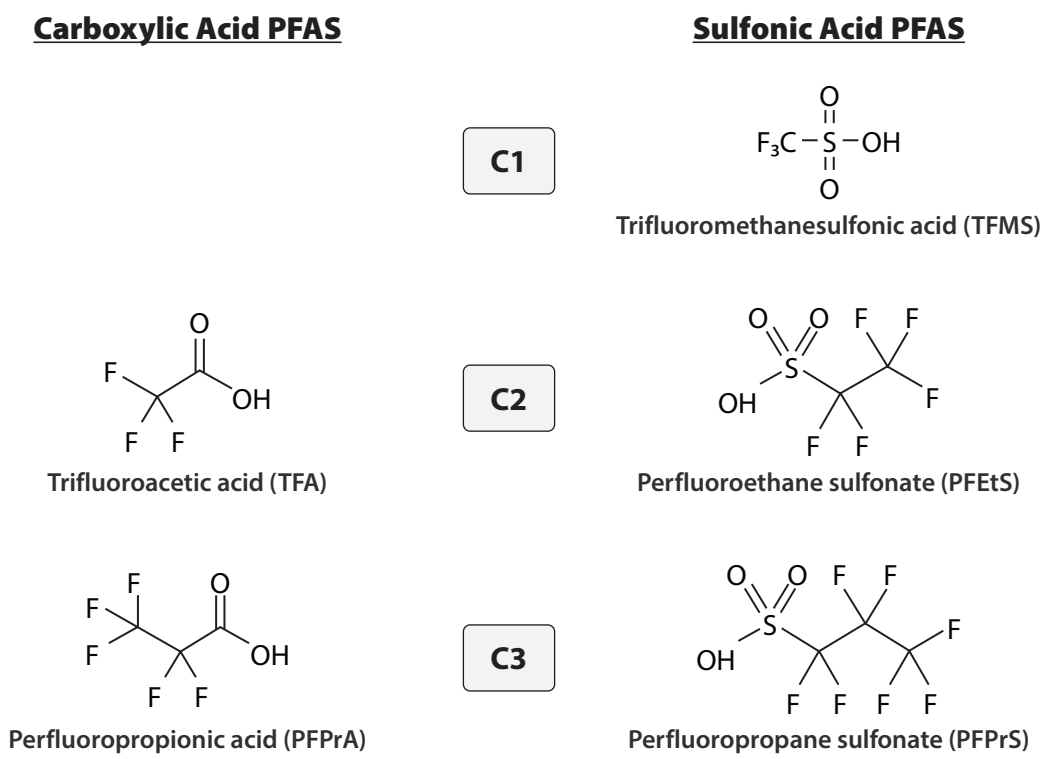
Introduction

While concerns about the adverse effects of long-chain per- and polyfluoroalkyl substances (PFAS) on human health have led to increased biomonitoring, less is known about the potential toxicity of ultrashort-chain PFAS. Ultrashort-chain PFAS are ubiquitous in environmental aquatic systems and can occur at very high concentrations, so determining exposure levels is important, particularly for vulnerable populations. Since both human plasma and serum are widely used for biomonitoring longer-chain PFAS, developing methods that include shorter chain PFAS is critical to gaining a more comprehensive understanding of the relationship between PFAS exposure and adverse health outcomes.

Ultrashort-chain PFAS are small, highly polar compounds with carbon chain lengths shorter than C4 (Figure 1). Their high polarity creates a challenge for analytical practices that are based on reversed-phase liquid chromatography, primarily due to insufficient chromatographic retention. To improve analytical performance, the following method was developed on an Ultra IBD column because its polar-embedded stationary phase provides stronger retention of polar compounds, even in the presence of the various salts, electrolytes, and buffers that are inherently found in human plasma and serum.

In this study, a simple and reliable workflow was developed for the simultaneous analysis of C1 to C10 perfluoroalkyl carboxylic and sulfonic acids, along with four alternative PFAS, in human plasma and serum. The samples underwent a simple, single-step protein precipitation procedure and were analyzed with a user-friendly LC-MS/MS method. Method accuracy and precision were evaluated with fortified fetal bovine serum. In addition, the method's suitability for real-world samples was confirmed by accurately measuring targeted PFAS with known concentrations in NIST standard reference human plasma (NIST SRM 1950) and serum (NIST SRM 1957).

Figure 1: Structures of C1 to C3 PFAS



Experimental

Plasma and Serum Samples

Since PFAS-free human plasma and serum were not found during the screening of commercially obtained samples, charcoal-stripped fetal bovine serum (FBS) obtained from MilliporeSigma (Burlington, MA) was used as the blank matrix for method verification because it did not contain any of the analytes of interest except for TFA. To compensate for this, a TFA isotope, ^{13}C-TFA, was employed as a surrogate to assess method accuracy for TFA in FBS. NIST SRM 1950 (metabolites in human plasma) and NIST SRM 1957 (organic contaminants in non-fortified human serum) were purchased from NIST Store (Gaithersburg, MD). These SRMs are characterized by known concentrations of six or seven short-chain and long-chain PFAS.

Standard and Sample Preparation

TFA is commonly present in laboratory materials, such as pipette tips, glass and polypropylene HPLC vials, and polypropylene tubes. Following a comprehensive examination, the materials with the lowest TFA contamination levels were chosen for standard and sample preparation. Additionally, deionized water and reverse osmosis (RO) water generated at Restek were assessed to determine if they contained PFAS. The results revealed that RO water was free of PFAS contamination, with the exception of TFA, which was found to be present at very low levels. Consequently, RO water was used for the preparation of mobile phase and calibration standards.

The calibration standard solutions (100 µL) were prepared in RO water, supplemented with 1x phosphate-buffered saline (PBS), across a range of 0.05 to 40 ng/mL (ppb) in polypropylene HPLC vials. Initial testing revealed that early eluting compounds displayed slightly broader peak shapes in the human plasma and serum samples compared to the neat standard solutions prepared in 100% water. Therefore, PBS was incorporated into the standard solutions to mitigate this variability and ensure uniform chromatographic performance between standard and sample solutions.

Five isotopically labeled PFAS were used as quantitative internal standards (QIS) (Table I). A 5 µL aliquot of QIS working solution (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 the standard solutions, which were then mixed with 200 µL of methanol containing 1.5% formic acid.

For the assessment of method accuracy and precision, 100 µL aliquots of FBS were fortified at 0.4, 2, 10, and 30 ppb with non-labeled PFAS and isotopically labeled ^{13}C-TFA, which served as a surrogate for the determination of TFA recovery. The fortified FBS samples were mixed with 5 µL of QIS working solution and 2 µL of extracted internal standard (EIS) working solution (50 ng/mL of $^{13}C_3$-PFPrA, $^{13}C_4$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_3$-PFHxS, and $^{13}C_8$-PFOS). A 200 µL aliquot of methanol containing 1.5% formic acid was then added to the fortified FBS samples followed by vortexing for 30 seconds at 3000 rpm. After centrifugation at 4200 rpm for 10 minutes, approximately 150 µL of supernatant was transferred to a polypropylene vial insert (cat.# 24518), placed in a glass HPLC vial (cat.# 24626), and subsequently injected for LC-MS/MS analysis. This single-step protein precipitation in clean polypropylene HPLC vials was used to minimize background PFAS contamination and to ensure accurate measurement of target analytes. Non-fortified human plasma and serum samples were also prepared using the same procedure that was used for the fortified FBS samples.

Analytical System

PFAS analysis was performed by LC-MS/MS under the conditions shown below. A PFAS delay column was installed between the mixer and injector to prevent any potential PFAS contamination upstream of the injector from coeluting with PFAS in the samples.

System: Waters ACQUITY UPLC I-Class and Xevo TQ-S triple quadrupole mass spectrometer

Columns:

- Analytical column: Ultra IBD, 100 mm x 2.1 mm, 3 µm (cat.# 9175312)
- PFAS delay column (cat.# 27854)

Injection volume: 5 µL

Mobile phase A: 5 mM ammonium formate, 0.1% formic acid in water

Mobile phase B: Acetonitrile

Flow rate: 0.3 mL/min

Temperature: 40 °C

Gradient:

Time (min)	%B
0.00	20
7.00	95
9.00	95
9.01	20
11.00	20

Ion mode: Negative ESI

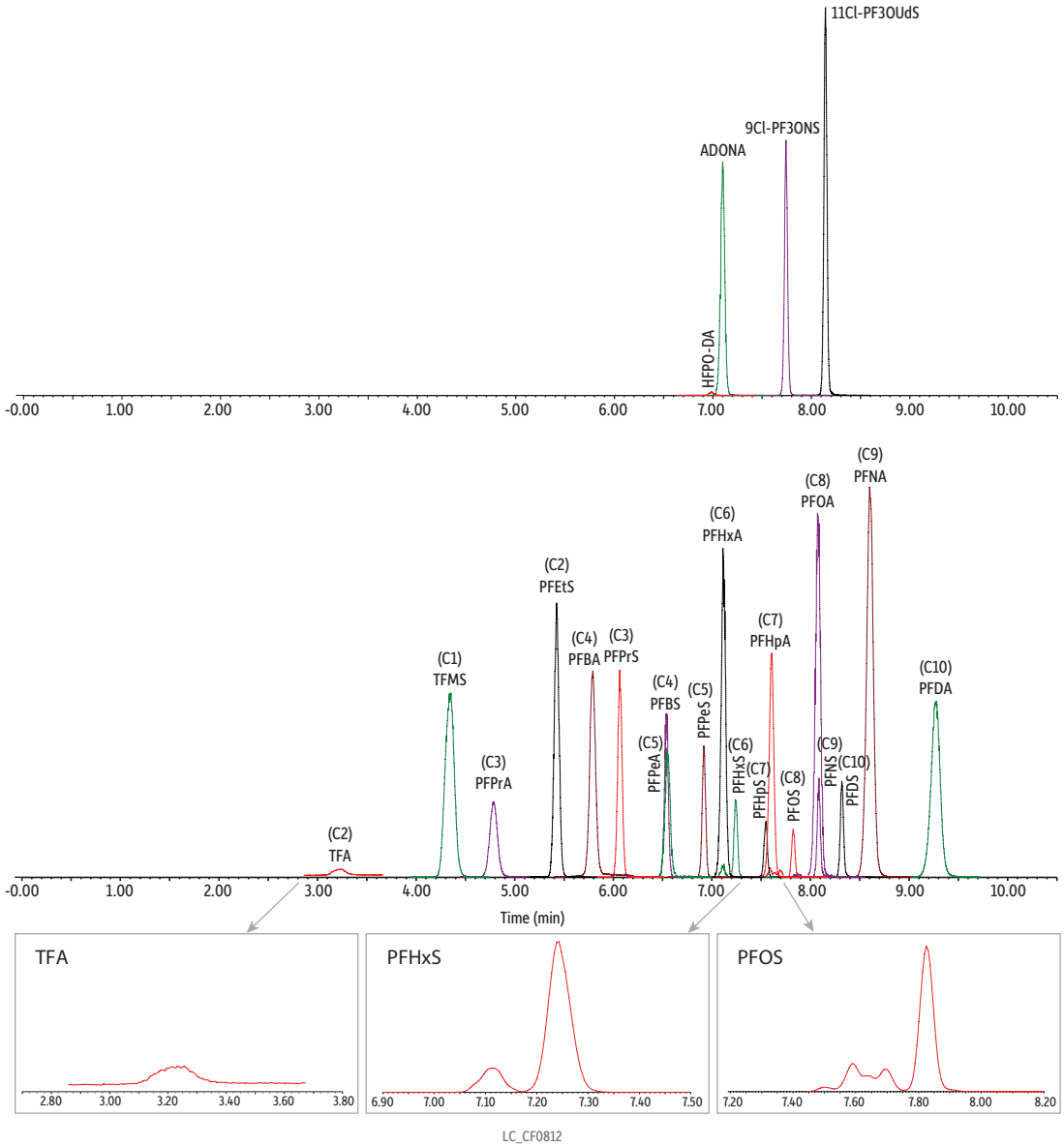
Mode: Scheduled MRM

Results and Discussion

LC-MS/MS Method Development

After exploring various mobile phases and chromatographic conditions, a final method (Figure 2) was established for the analysis of C1 to C10 PFAS in human plasma and serum. The polar embedded Ultra IBD column provided strong retention and good separation of early eluting, ultrashort-chain PFAS analyzed under reversed-phase conditions. More important, the extensive retention of ultrashort-chain PFAS, especially for the first-eluted analyte (TFA), led to reduced matrix interferences. Matrix impact was further minimized by using stable isotopes as internal standards to correct for any matrix interference during quantification. The MS/MS transition parameters and internal standards used for each analyte are provided in Table I.

Figure 2: Analysis of a 10 ppb PFAS Standard



LC_CF0812

Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Temp.: 40 °C

Standard/Sample PFAS 28 calibration standard (cat.# 30734)
Diluent: 1:2 water:methanol
Conc.: 10 ng/mL
Inj. Vol.: 5 µL

Mobile Phase
A: Water, 5 mM ammonium formate, 0.1% formic acid
B: Acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	80	20
7.00	0.3	5	95
9.00	0.3	5	95
9.01	0.3	80	20
11.00	0.3	80	20

Max Pressure: 275 bar

Detector Waters Xevo TQ-S
Ion Mode: ESI-
Mode: MRM
Instrument Waters ACQUITY UPLC I-Class
Sample Preparation The standard solution (100 µL) was prepared in reverse osmosis water supplemented with 1x phosphate-buffered saline at 10 ng/mL (ppb) in a polypropylene HPLC vial followed by mixing with 200 µL of methanol containing 1.5% formic acid.
Notes A PFAS delay column (cat.# 27854) was installed before the injector. The peaks of confirmation ions are not displayed in this chromatogram.

Table I: MS Transitions and Analytes Retention Times

Compounds	Retention Time (min)	Precursor Ion	Product Ions*	Cone (V)	Collision (V)	Quantification Internal Standard
Target Analytes						
TFA	3.25	113.03 [M-H]-	69.01	10	10	$^{13}C_3$-PFBA
PFPrA	4.81	162.97 [M-H]-	119.02	10	8	$^{13}C_3$-PFBA
PFBA	5.80	213.03 [M-H]-	168.98	14	8	$^{13}C_3$-PFBA
PFPeA	6.56	262.97 [M-H]-	218.97	2	6	$^{13}C_2$-PFHxA
PFHxA	7.13	313.10 [M-H]-	268.97/118.99	2	8/20	$^{13}C_2$-PFHxA
PFHpA	7.62	363.16 [M-H]-	319.09/169.06	8	10/18	$^{13}C_4$-PFOA
PFOA	8.10	413.10 [M-H]-	368.96/168.90	2	10/16	$^{13}C_4$-PFOA
PFNA	8.62	463.10 [M-H]-	419.01/219.02	4	10/16	$^{13}C_5$-PFNA
PFDA	9.29	513.17 [M-H]-	469.16/219.06	4	12/16	$^{13}C_2$-PFDA
TFMS	4.37	148.97 [M-H]-	79.93/98.92	62	18/18	$^{13}C_3$-PFBA
PFEtS	5.44	198.90 [M-H]-	79.92/98.91	38	22/22	$^{13}C_3$-PFBA
PFPrS	6.08	248.97 [M-H]-	79.92/98.91	2	24/24	$^{13}C_3$-PFBA
PFBS	6.55	298.97 [M-H]-	79.97/98.89	2	26/26	$^{13}C_2$-PFHxA
PFPeS	6.93	349.10 [M-H]-	79.98/98.98	6	32/30	$^{13}C_2$-PFHxA
PFHxS	7.24	398.90 [M-H]-	79.97/98.89	56	32/34	$^{13}C_2$-PFHxA
PFHpS	7.56	449.17 [M-H]-	79.98/98.97	4	42/38	$^{13}C_2$-PFHxA
PFOS	7.82	499.03 [M-H]-	79.92/98.90	8	40/40	$^{13}C_4$-PFOA
PFNS	8.09	549.10 [M-H]-	79.92/98.83	12	42/40	$^{13}C_4$-PFOA
PFDS	8.32	599.17 [M-H]-	79.98/98.83	8	44/46	$^{13}C_5$-PFNA
HFPO-DA	7.01	285.03 [M-COOH]-	169.02/185.02	2	6/16	$^{13}C_2$-PFHxA
ADONA	7.11	376.90 [M-H]-	250.93/84.97	22	12/26	$^{13}C_2$-PFHxA
9Cl-PF3ONS	7.75	530.78 [M-H]-	350.85/82.96	12	26/24	$^{13}C_2$-PFHxA
11Cl-PF3OUdS	8.15	630.78 [M-H]-	450.80/82.95	8	26/32	$^{13}C_5$-PFNA
Extracted Internal Standards						
$^{13}C_3$-PFPrA	4.81	165.97 [M-H]-	120.96	10	11	$^{13}C_3$-PFBA
$^{13}C_4$-PFBA	5.80	217.03 [M-H]-	171.98	2	8	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	6.56	267.97 [M-H]-	222.99	2	6	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	7.13	318.03 [M-H]-	272.93	2	7	$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	7.62	366.90 [M-H]-	321.93	2	10	$^{13}C_4$-PFOA
$^{13}C_8$-PFOA	8.10	420.97 [M-H]-	375.94	2	10	$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	8.62	471.97 [M-H]-	426.87	4	8	$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	9.29	518.90 [M-H]-	473.87	4	13	$^{13}C_2$-PFDA
$^{13}C_3$-PFHxS	7.24	401.90 [M-H]-	79.97	2	32	$^{13}C_2$-PFHxA
$^{13}C_8$-PFOS	7.82	506.84 [M-H]-	79.97	4	48	$^{13}C_4$-PFOA
Quantitative Internal Standards						
$^{13}C_3$-PFBA	5.80	215.97 [M-H]-	171.97	10	8	
$^{13}C_2$-PFHxA	7.13	314.97 [M-H]-	269.93	8	8	
$^{13}C_4$-PFOA	8.10	416.87 [M-H]-	371.88	2	8	
$^{13}C_5$-PFNA	8.62	467.87 [M-H]-	422.89	16	10	
$^{13}C_2$-PFDA	9.29	514.87 [M-H]-	469.84	8	10	

*Quantifier ion/qualifier ion. No qualifier ion was used for TFA, PFPrA, PFBA, and PFPeA because only one product ion could be identified for each.

Linearity

With quadratic regression (1/x weighted), all analytes showed acceptable linearities with r2 >0.995 and deviations <20%. Table II shows the specific linear range for each analyte, ranging from 0.05 ppb to 40 ppb, with variation occurring at the lowest calibration concentration.

Accuracy and Precision

Accuracy and precision for most analytes were assessed at 0.4, 2, and 10 ppb. However, for HFPO-DA and TFA, the evaluation was conducted at 2, 10, and 30 ppb because 0.4 ppb was below the linear range of these compounds. Three batches of samples were prepared and analyzed on different days, totaling nine replicates at each fortification level. The average recoveries and relative standard deviations (%RSD) are presented in Table II. All analytes exhibited recovery values within the range of 82.3–115% across the three fortification levels. Satisfactory method precision was demonstrated with %RSD values ranging from 0.965 to 11.3%. Additionally, the results indicated that all EIS had recovery values within 20% of the nominal concentration. The use of EIS as an additional quality control measure enhanced the reliability and robustness of the method.

Based on the results of the accuracy and precision experiments, the method LOQ was set as the lowest concentration of the standard calibration curve for each analyte. The method LOD was estimated from the analysis of fortified FBS and was defined by a peak signal-to-noise ratio of 3:1.

Measurement of C1–C10 and Alternative PFAS in NIST 1950 and 1957 SRMs

Six preparations of each SRM were analyzed with the developed LC-MS/MS method. The results indicated that all measured EIS concentrations fell within 20% of the nominal concentration. Moreover, Table III illustrates that the average experimental concentrations of most PFAS closely matched the reference concentrations, with deviations within 20%. Although the measured PFDA concentration in NIST SRM 1957 exhibited a slightly higher deviation of 26%, it remained within the deviation range of the reference concentration. These results demonstrated that the established method was suitable for accurate measurement of PFAS—including ultrashort-chain PFAS—in both human plasma and serum samples. In addition to the reference PFAS, other measurable PFAS were also reported in Table III.

Table II: Linearity, LOD, Accuracy, and Precision Results for Fortified Fetal Bovine Serum Samples

			Average Recovery (%RSD)			
			Fortified Concentration (ng/mL)			
Analytes	Linearity Range (ng/mL)	LOD (ng/mL)	0.4	2	10	30
^{13}C-TFA	0.50 - 40	0.1250*	—	90.6 (9.28)	98.0 (2.99)	100 (10.7)
PFPrA	0.25 - 40	0.0102	108 (2.53)	115 (0.965)	105 (6.99)	—
PFBA	0.10 - 40	0.0222	104 (5.57)	109 (1.75)	104 (1.51)	—
PFPeA	0.10 - 40	0.0125	97.4 (3.25)	93.3 (4.19)	89.1 (5.57)	—
PFHxA	0.10 - 40	0.0098	99.2 (2.46)	109 (4.65)	102 (6.31)	—
PFHpA	0.10 - 40	0.0050	86.7 (5.37)	99.2 (2.04)	89.2 (1.80)	—
PFOA	0.10 - 40	0.0051	94.8 (8.24)	107 (5.30)	95.6 (3.35)	—
PFNA	0.10 - 40	0.0012	96.2 (1.44)	111 (1.87)	99.1 (2.62)	—
PFDA	0.10 - 40	0.0083	93.6 (1.93)	102 (2.03)	95.5 (3.64)	—
TFMS	0.05 - 40	0.0070	89.4 (7.33)	88.8 (3.80)	91.9 (3.19)	—
PFEtS	0.05 - 40	0.0020	98.0 (2.62)	103 (1.47)	99.3 (2.26)	—
PFPrS	0.05 - 40	0.0030	98.1 (8.18)	108 (4.23)	98.9 (4.15)	—
PFBS	0.05 - 40	0.0124	88.0 (8.84)	94.1 (4.95)	86.5 (6.17)	—
PFPeS	0.10 - 40	0.0031	94.8 (4.57)	100 (8.05)	94.3 (5.43)	—
PFHxS	0.10 - 40	0.0115	85.8 (7.88)	96.0 (10.0)	92.2 (8.98)	—
PFHpS	0.10 - 40	0.0088	92.5 (6.75)	99.8 (6.45)	93.4 (5.74)	—
PFOS	0.10 - 40	0.0200	97.8 (8.66)	97.9 (7.01)	95.9 (3.37)	—
PFNS	0.10 - 40	0.0129	92.1 (7.98)	94.2 (4.43)	91.9 (2.78)	—
PFDS	0.10 - 40	0.0111	92.6 (8.20)	82.3 (3.48)	87.6 (3.55)	—
HFPO-DA	0.50 - 40	0.1875	—	99.9 (11.3)	91.1 (8.95)	90.4 (6.13)
ADONA	0.10 - 40	0.0035	90.4 (7.14)	106 (4.67)	95.7 (4.25)	—
9Cl-PF3ONS	0.10 - 40	0.0031	95.8 (4.20)	93.7 (5.12)	93.8 (6.71)	—
11Cl-PF3OUdS	0.10 - 40	0.0023	106 (5.10)	84.5 (4.20)	97.2 (4.86)	—

*For non-labeled TFA

Table III: Quantification of PFAS in NIST 1950 and 1957 SRMs

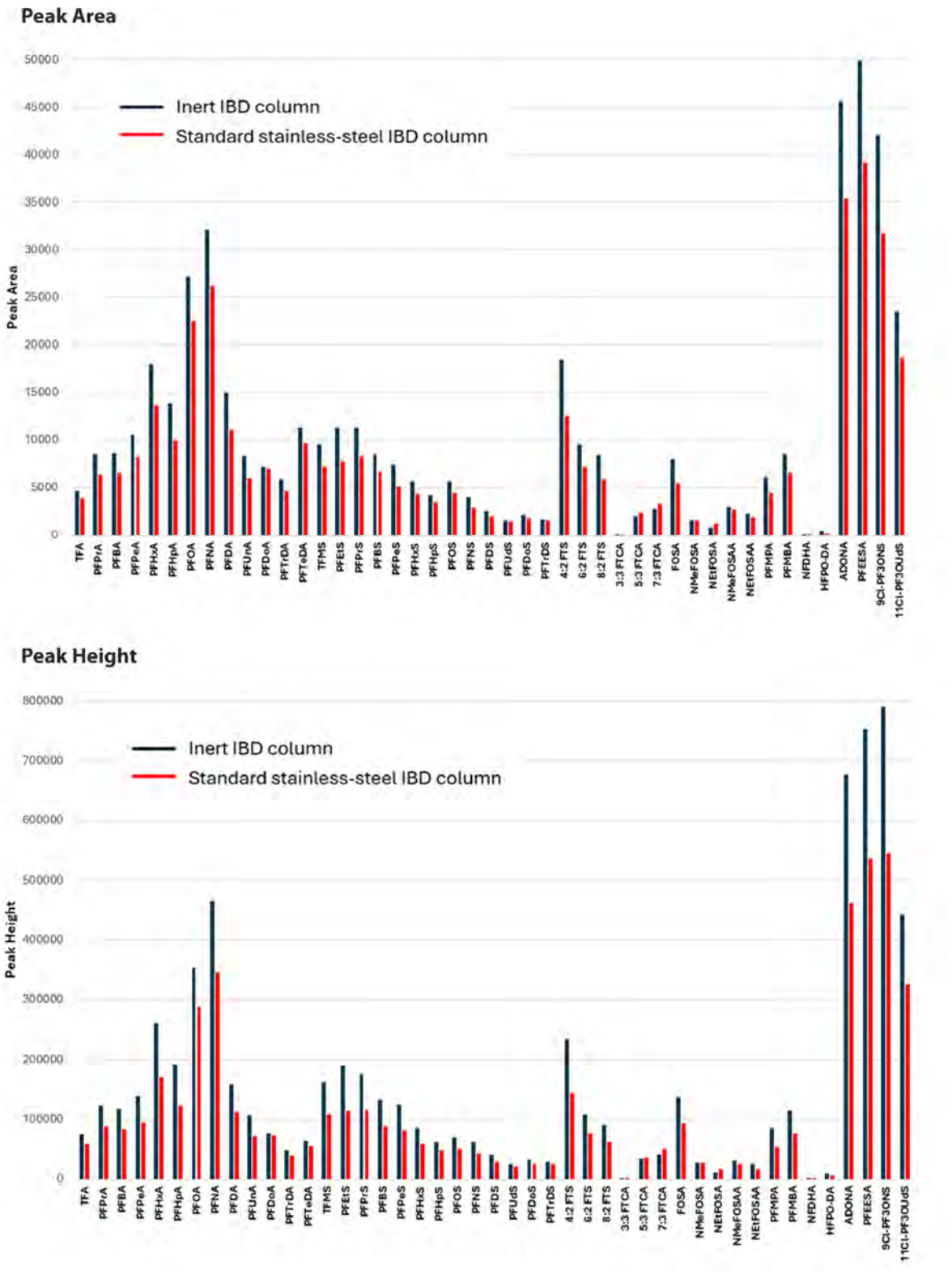
Analytes	Reference Conc. (ng/mL)	Experimental Avg. Conc. (ng/mL)	Experimental Precision (%RSD)	Relative Concentration (%)**
NIST SRM 1950				
PFOA	3.27 ± 0.06	3.12	3.70	95.4
PFNA	0.720 ± 0.028	0.85	0.74	117
PFDA	0.322 ± 0.007	0.30	3.47	91.6
PFHxS	3.25 ± 0.08	2.91	6.26	89.5
PFOS	10.64 ± 0.13	12.57	2.92	118
TFA	—	5.74	4.45	—
PFPrA	—	0.26	6.90	—
PFHpA	—	0.23	3.04	—
TFMS	—	0.08	3.95	—
PFPeS	—	0.15	4.31	—
PFHpS	—	0.36	4.90	—
PFDS	—	0.10	4.64	—
NIST SRM 1957*				
PFHpA	0.305 ± 0.051	0.28	2.16	92.1
PFOA	5.00 ± 0.44	4.21	1.91	84.2
PFNA	0.878 ± 0.077	0.77	1.38	87.9
PFDA	0.39 ± 0.12	0.29	2.69	74.4
PFHxS	4.00 ± 0.83	3.35	9.88	83.8
PFOS	21.1 ± 1.3	20.46	3.81	97.0
TFA	—	3.22	3.79	—
TFMS	—	0.07	0.10	—
PFPeS	—	0.10	7.97	—
PFHpS	—	0.48	6.20	—
PFDS	—	0.10	4.23	—

*The reference concentration for NIST SRM 1957 is presented as mass fraction (µg/kg).
**Relative concentration = (experimental concentration/reference concentration) x 100

Future Directions

While the data reported here were generated using a standard, stainless-steel Ultra IBD column (cat.# 9175312), the same column (stationary phase and dimensions) is now available in an inert hardware format (Ultra IBD Inert, cat.# 9175312-T). The hardware used to make the Ultra IBD Inert column is treated with an inert coating, which improves sensitivity by preventing unwanted analyte interactions with the stainless-steel surface. As shown in Figure 3, the inert hardware results in a notable enhancement in detection sensitivity for most of the PFAS tested (3-70% increase in peak area and 5-75% increase in peak height) compared to columns made with uncoated hardware. Future applications of the method developed here may benefit from using an Ultra IBD Inert column, particularly for samples containing PFAS at trace levels.

Figure 3: Inert LC column hardware increased peak area and height for most PFAS compared to columns made with traditional stainless-steel hardware.



Conclusions

A simple, reliable workflow was established in this study to incorporate ultrashort-chain PFAS analysis into a method that also included longer-chain PFAS that are commonly monitored in human plasma and serum. The method utilized a polar-embedded Ultra IBD column to increase chromatographic retention of these small polar analytes, and the results demonstrated that the method was rugged, accurate, and precise. Most important, this method can offer a valuable tool for gaining insights into human exposure to these emergent ultrashort-chain PFAS. Visit www.restek.com/PFAS for additional products, methods, and technical resources.

PFAS Delay Column



- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

Catalog No.	Product Name	Units
27854	PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column	ea.

Ultra IBD HPLC Column

The Restek IBD is a polar-embedded column that acts as a strong hydrogen bonder and may be the most versatile column available today. With a unique polar group, this column is very retentive and selective for acids. It also provides symmetrical peak shape for strong bases. Restek's IBD is compatible with 100% aqueous mobile phases and can be used under reversed-phase or HILIC conditions to retain very polar, ionic compounds in highly organic mobile phases.



Catalog No.	Product Name	Units
9175312	Ultra IBD HPLC Column, 3 µm, 100 mm x 2.1 mm	ea.

Ultra IBD Inert HPLC Column



- Inert LC column technology reduces nonspecific binding of chelating analytes, enabling sensitive analysis and smooth integration of peaks.
- Ideal for the analysis of metal-sensitive compounds.
- Increased response and analyte recovery, allowing lower detection limits.
- Improved peak shape without passivation or mobile phase additives.
- Specialized columns for mixed polar and nonpolar compounds.

Catalog No.	Product Name	Units
9175312-T	Ultra IBD Inert HPLC Column, 3 µm, 100 mm x 2.1 mm	ea.



PFAS 28 Calibration Standard

Contains:
11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS) (39108-34-4)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS) (757124-72-4)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS) (27619-97-2)
4,8-dioxa-3H-perfluorononanoic acid (ADONA) (919005-14-4)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)
2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6)
N-ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)* (2991-50-6)
N-methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)* (2355-31-9)
Perfluoro-1-decanesulfonic acid (PFDS) (335-77-3)
Perfluoro-1-nonanesulfonic acid (PFNS) (68259-12-1)
Perfluoro-1-octanesulfonamide (FOSA) (754-91-6)
Perfluoro-1-pentanesulfonic acid (PFPeS) (2706-91-4)
Perfluorobutanesulfonic acid (PFBS) (375-73-5)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFD0A) (307-55-1)
Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)
Perfluoroheptanoic acid ((PFHpA) (375-85-9)
Perfluorohexanesulfonic acid (PFHxS)* (355-46-4)
Perfluorohexanoic acid ((PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Heptadecafluorooctanesulfonic acid (PFOS)* (1763-23-1)
Perfluorooctanoic acid (PFOA)* (335-67-1)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluorotetradecanoic acid (PFTeDA) (376-06-7)
Perfluorotridecanoic acid (PFTrDA) (72629-94-8)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)

*Technical grade compound containing both branched and linear isomers; see certificate for details.

Catalog No.	Concentration	Solvent	Volume	Units
30734	1 µg/mL	Methanol (1 mM KOH)	1 mL/ampul	ea.



Big Mouth Step Screw-Thread Vials

Catalog No.	Product Name	Units
24626	Big Mouth Step Screw-Thread Vials with Grad Marking Spot, 2.0 mL, 12 x 32 mm, 10 mm/425 Thread (Vial Only), Clear	100-pk.



Vial Inserts, Polypropylene

Catalog No.	Product Name	Units
24518	Vial Inserts, Polypropylene, Big Mouth w/Bottom Spring, 250 µL	100-pk.



Survival Kit for HPLC, Stainless Steel

For start-up and maintenance in all HPLC systems.

The stainless-steel survival kit contains a wide range of tubing, fittings, and tools necessary to set up and maintain your HPLC system: a selection of lengths and IDs of 1/16-inch tubing, nuts, ferrules, a ValvTool wrench, and a zero-dead-volume union.

Catalog No.	Product Name	Units
25097	Survival Kit for HPLC	kit

Resprep

Resprep PFAS Cartridges

A Two-in-One Solution for PFAS SPE Sample Preparation

- Single cartridge with both WAX and carbon beds simplifies workflows.
- Factory-packed filter aid saves time and prevents clogs.
- Ultra-clean sorbents ensure MDLs are met.



Prepacked filter aid only from Restek!

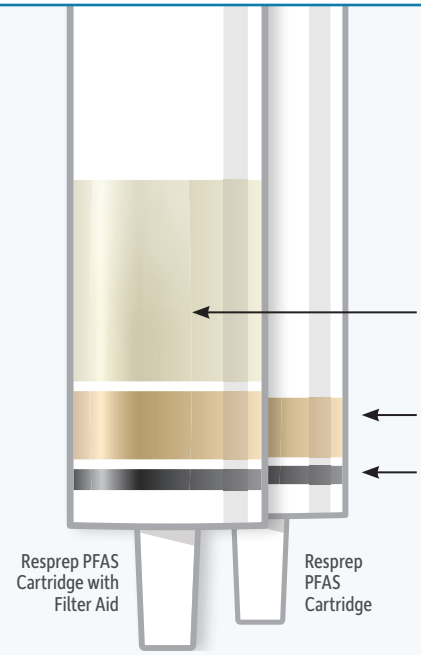
RESTEK

Streamline PFAS Sample Preparation with Dual-Bed Resprep PFAS Cartridges

Effective sample preparation is essential for accurate trace-level PFAS analysis in complex sample matrices, such as non-potable waters, solids, biosolids, and tissues. Solid phase extraction (SPE) is typically used, but good results are difficult to achieve if background PFAS are present or if sample components clog the SPE cartridges. EPA Method 1633, which has been validated in multiple laboratories, uses a wool-packed weak anion exchange (WAX) cartridge for matrix extraction, followed by a dispersive graphitized carbon black (dGCB) cleanup step, with many labs adopting a carbon cartridge instead of using dGCB. While effective, this two-cartridge approach with manually packed wool adds extra time, complexity, and risk of introducing contaminants to the sample preparation process.

Resprep PFAS Cartridges Are a Simpler Solution

PFAS SPE sample preparation can be streamlined significantly with dual-bed Resprep PFAS cartridges, which combine SPE extraction and carbon cleanup into a single cartridge. In this fast, simple workflow, the ultra-clean WAX and CarboPrep Plus sorbents used in Resprep PFAS cartridges ensure that method performance requirements will be met. In addition, an optional, factory-packed filter aid eliminates the time and variability associated with manually packing glass wool. This ready-to-use solution prevents clogging so that even dirty matrices can be processed quickly with a single extraction.



Get the Dual-Bed Advantage!

One cartridge with both WAX and carbon beds simplifies and speeds up workflows.

Only available from Restek!
Factory-packed filter aid prevents clogs for faster, more consistent sample preparation.

Polymeric WAX is tested for Method 1633 PFAS to prevent background contamination during extraction.

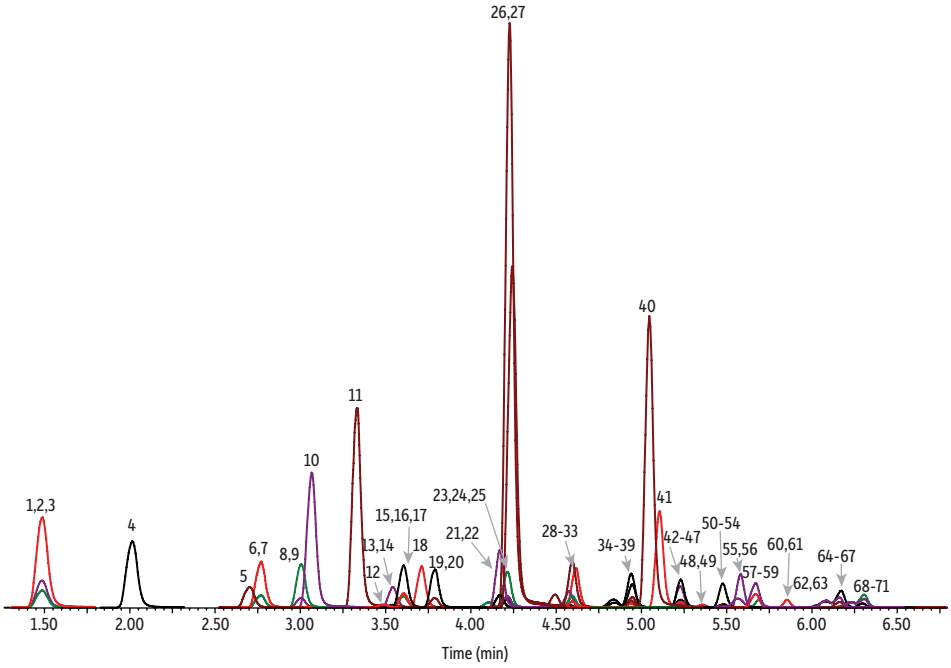
Ultra-clean CarboPrep Plus carbon bed ensures effective clean up.



Meet Performance Requirements Quickly and Consistently

Developed specifically to help labs meet PFAS performance requirements, dual-bed Resprep PFAS cartridges contain ultra-clean, high-quality WAX and carbon sorbent layers that provide effective extraction and cleanup. Every lot of our WAX sorbent is tested against Method 1633 PFAS analytes to demonstrate cleanliness and to ensure it does not contribute background PFAS. In addition, our high-performance CarboPrep Plus sorbent provides more effective and consistent cleanup than other carbon materials. The combination of both sorbents in a single cartridge makes sample preparation much faster and results in clean extracts and excellent analytical performance (Figure 1 and Table I).

Figure 1: Resprep PFAS cartridges provide effective SPE sample preparation and are faster and easier to use than separate extraction and cleanup procedures.



Column	Force C18 (cat.# 9634252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	100 Å
Temp.:	40 °C
Standard/Sample	
Inj. Vol.:	3 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6	0.4	5	95
6.6	0.4	5	95
6.61	0.4	80	20
7.5	0.4	80	20

Max Pressure:	~400 bar
Detector	Waters Xevo TQ-S
Ion Mode:	ESI-
Mode:	MRM
Instrument	Waters ACQUITY Premier
Sample Preparation	Resprep PFAS cartridges (cat.# 28930) and a Thermo AutoTrace PFAS instrument were used for the following sample preparation procedure. 1. Spike 500 mL DI water with 200 µL of Wellington EPA-1633STK and 25 µL of Wellington MPFAC-HIF-ES. 2. Rinse cartridge with 15 mL of 1% ammonium acetate in methanol. 3. Rinse with 5 mL of 0.3 M formic acid in water. 4. Load sample onto SPE cartridge dropwise at 5 mL/min. 5. Rinse bottle with 5 mL DI water, load on to SPE cartridge dropwise at 5 mL/min, repeat. 6. Rinse bottle with 5 mL of 1:1 0.1 M formic acid:methanol and load onto SPE cartridge dropwise at 5 mL/min. 7. Flow nitrogen through cartridge for 15 minutes. 8. Rinse bottles with 5 mL of 1% ammonium hydroxide in methanol, elute into collection vessel. 9. Add 25 µL of concentrated acetic acid and 25 µL of Wellington MPFAC-HIF-IS. 10. Transfer aliquot to autosampler vial (cat. # 23243) and cap (cat. # 23244).
Notes	A PFAS delay column (cat.# 27854) was installed before the injector.

Figure 1 continued on next page.

Figure 1 *(continued)*

Peaks	tR (min)	Conc. (ng/L)	Precursor 1	Product 1	Product 2	Precursor 2	Product 1
1. Perfluoro-*n*-[1,2,3,4- 13C3]butanoic acid (13C3-PFBA)	1.485	5	216	172	-	-	-
2. Perfluoro-*n*-[1,2,3,4- 13C4]butanoic acid (13C4-PFBA)	1.487	10	217	172	-	-	-
3. Perfluorobutanoic acid (PFBA)	1.489	10	213	169	-	-	-
4. Perfluoro-3-methoxypropanoic acid (PFMPA)	2.007	5	229	85	-	-	-
5. 3-Perfluoropropyl propanoic acid (3:3FTCA)	2.7	12.5	241	177	117	-	-
6. Perfluoro-*n*-[1,2,3,4,5- 13C5]pentanoic acid (13C5-PFPeA)	2.765	5	268	223	-	-	-
7. Perfluoropentanoic acid (PFPeA)	2.768	5	263	219	69	-	-
8. Perfluorobutane sulfonate (PFBS)	3.006	2.5	299	80	99	-	-
9. Sodium perfluoro-1-[2,3,4- 13C3]butanesulfonate (13C3-PFBS)	3.007	2.5	302	80	99	-	-
10. Perfluoro-4-methoxybutanoic acid (PFMBA)	3.071	5	279	85	-	-	-
11. Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	3.333	5	315	135	83	-	-
12. Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	3.493	5	295	201	85	-	-
13. 1*H*, 1*H*,2*H*,2*H*-perfluorohexane sulfonate (4:2 FTS)	3.545	10	327	307	81	-	-
14. Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2- 13C2]hexane sulfonate (13C2-4:2FTS)	3.55	5	329	81	309	-	-
15. Perfluorohexanoic acid (PFHxA)	3.608	2.5	313	269	119	-	-
16. Perfluoro-*n*-[1,2,3,4,6- 13C2]hexanoic acid (13C2-PFHxA)	3.609	2.5	315	270	119	-	-
17. Perfluoro-*n*-[1,2,3,4,6- 13C5]hexanoic acid (13C5-PFHxA)	3.61	2.5	318	273	120	-	-
18. Perfluoropentane sulfonate (PFPeS)	3.718	2.5	349	80	99	-	-
19. 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy13C3-propanoic acid (13C3-HFPO-DA)	3.79	10	287	169	185	-	-
20. Hexafluoropropylene oxide (HFPO-DA)	3.795	10	285	169	185	-	-
21. Perfluoroheptanoic acid (PFHpA)	4.174	2.5	363	319	169	-	-
22. Perfluoro-*n*-[1,2,3,4- 13C4]heptanoic acid (13C4-PFHpA)	4.174	2.5	367	322	-	-	-
23. Perfluoro-1-hexane[18O2]sulfonic acid (18O2-PFHxS)	4.218	2.5	403	84	-	-	-
24. Sodium perfluoro-1-[1,2,3- 13C3]hexanesulfonate (13C3-PFHxS)	4.218	2.5	402	80	99	-	-
25. Perfluorohexane sulfonate (PFHxS)	4.22	2.5	399	80	99	-	-
26. 4,8-Dioxa-3*H*-perfluorononanoic acid (ADONA)	4.232	10	377	251	85	-	-
27. 2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid (5:3FTCA)	4.247	62.5	341	237	217	-	-
28. 1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate (6:2 FTS)	4.58	10	427	407	81	-	-
29. Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-13C2]-octane sulfonate (13C2-6:2FTS)	4.581	5	429	81	409	-	-
30. Perfluoro-*n*-[13C8]octanoic acid (13C8-PFOA)	4.598	2.5	421	376	-	-	-
31. Perfluorooctanoic acid (PFOA)	4.601	2.5	413	369	169	-	-
32. Perfluoro-*n*-[13C4]octanoic acid (13C4-PFOA)	4.601	2.5	417	172	-	-	-
33. Perfluoroheptane sulfonate (PFHpS)	4.621	2.5	449	80	99	-	-
34. Perfluorononanoic acid (PFNA)	4.944	2.5	463	419	219	-	-
35. Perfluoro-*n*-[13C5]nonanoic acid (13C5-PFNA)	4.944	1.25	468	423	-	-	-
36. Perfluoro-*n*-[13C9]nonanoic acid (13C9-PFNA)	4.944	1.25	472	427	-	-	-
37. Perfluorooctane sulfonate (PFOS)	4.949	2.5	499	80	99	-	-
38. Sodium perfluoro-[13C4]octanesulfonate (13C4-PFOS)	4.95	2.5	503	80	99	-	-
39. Sodium perfluoro-[13C8]octanesulfonate (13C8-PFOS)	4.95	2.5	507	80	99	-	-
40. 3-Perfluoroheptyl propanoic acid (7:3FTCA)	5.046	62.5	441	317	337	-	-
41. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	5.114	10	531	351	-	533	353
42. Perfluorodecanoic acid (PFDA)	5.237	2.5	513	469	219	-	-
43. Perfluoro-*n*-[1,2,3,4,5,6- 13C2]decanoic acid (13C2-PFDA)	5.237	1.25	515	470	-	-	-
44. Perfluoro-*n*-[1,2,3,4,5,6- 13C6]decanoic acid (13C6-PFDA)	5.237	1.25	519	474	-	-	-
45. 1*H*,1*H*,2*H*,2*H*-perfluorodecane sulfonate (8:2 FTS)	5.238	10	527	507	81	-	-
46. Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2- 13C2]-decane sulfonate (13C2-8:2FTS)	5.238	5	529	81	509	-	-
47. Perfluorononanesulfonic acid (PFNS)	5.239	2.5	549	80	99	-	-
48. *N*-methyl-d 3-perfluoro-1-octanesulfonamidoacetic acid (D3-NMeFOSAA)	5.361	5	573	419	-	-	-
49. *N*-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	5.366	2.5	570	419	483	-	-
50. Perfluorodecanesulfonic acid (PFDS)	5.475	2.5	599	80	99	-	-
51. Perfluoroundecanoic acid (PFUnA)	5.486	2.5	563	519	269	-	-
52. Perfluoro-*n*-[1,2,3,4,5,6,7- 13C7]undecanoic acid (13C7-PFUnA)	5.486	1.25	570	525	-	-	-
53. *N*-ethyl-d 5-perfluoro-1-octanesulfonamidoacetic acid (D5-NEtFOSAA)	5.487	5	589	419	-	-	-
54. *N*-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	5.487	2.5	584	419	526	-	-
55. 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	5.59	10	631	451	-	633	453
56. Perfluorooctanesulfonamide (PFOSA)	5.669	2.5	498	78	478	-	-
57. Perfluoro-1-[13C8]octanesulfonamide (13C8-PFOSA)	5.669	2.5	506	78	-	-	-
58. Perfluorododecanoic acid (PFDoA)	5.691	2.5	613	569	319	-	-
59. Perfluoro-*n*-[1,2- 13C2]dodecanoic acid (13C2-PFDoA)	5.692	1.25	615	570	-	-	-
60. Perfluorododecanesulfonic acid (PFDoS)	5.861	2.5	699	80	99	-	-
61. Perfluorotridecanoic acid (PFTrDA)	5.876	2.5	663	619	169	-	-
62. Perfluorotetradecanoic acid (PFTeDA)	6.031	2.5	713	669	169	-	-
63. Perfluoro-*n*-[1,2- 13C2]tetradecanoic acid (13C2-PFTeDA)	6.031	1.25	715	670	-	-	-
64. *N*-methyl perfluorooctanesulfonamide (NMeFOSA)	6.158	2.5	512	219	169	-	-
65. *N*-methyl-D 3-perfluoro-1-octanesulfonamide (D3-NMeFOSA)	6.159	2.5	515	219	-	-	-
66. *N*-methyl perfluorooctanesulfonamidoethanol (NMeFOSE)	6.166	25	616	59	-	-	-
67. *N*-methyl-D 7-perfluorooctanesulfonamidoethanol (D7-NMeFOSE)	6.167	25	623	59	-	-	-
68. *N*-ethyl-D 5-perfluoro-1-octanesulfonamide (D5-NEtFOSA)	6.295	2.5	531	219	-	-	-
69. *N*-ethyl perfluorooctanesulfonamide (NEtFOSA)	6.296	2.5	526	219	169	-	-
70. *N*-ethyl perfluorooctanesulfonamidoethanol (NEtFOSE)	6.298	25	630	59	-	-	-
71. *N*-ethyl-D 9-perfluorooctanesulfonamidoethanol (D9-NEtFOSE)	6.298	25	639	59	-	-	-

Table I: Target recoveries (70-135%) were met for all PFAS in reagent water samples prepared using Resprep PFAS cartridges.

Compound	Abbreviation	MDL (ng/L)	Blank (ng/L)*	Accuracy (%Recovery)	Precision (%RSD)
Perfluorobutanoic acid	PFBA	0.34	ND	112	5
Perfluoro-3-methoxypropanoic acid	PFMPA	0.20	ND	109	6
3-Perfluoropropyl propanoic acid	3:3FTCA	0.31	ND	95	6
Perfluoropentanoic acid	PFPeA	0.26	ND	111	6
Perfluorobutane sulfonate	PFBS	0.19	ND	101	5
Perfluoro-4-methoxybutanoic acid	PFMBA	0.18	ND	111	6
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	0.15	ND	94	5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	0.46	ND	121	5
1*H*,1*H*,2*H*,2*H*-perfluorohexane sulfonate	4:2 FTS	0.36	ND	104	5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	1.48	ND	102	6
Perfluorohexanoic acid	PFHxA	0.08	ND	113	5
Perfluoropentane sulfonate	PFPeS	0.07	ND	122	4
Hexafluoropropylene oxide dimer acid	HFPO-DA	0.40	ND	114	12
Perfluoroheptanoic acid	PFHpA	0.08	ND	109	5
Perfluorohexane sulfonate	PFHxS	0.07	ND	104	7
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	0.39	ND	99	9
1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate	6:2 FTS	0.37	ND	107	4
3-Perfluoroheptyl propanoic acid	7:3FTCA	2.32	ND	100	6
Perfluoroheptane sulfonate	PFHpS	0.76	ND	121	8
Perfluorooctanoic acid	PFOA	0.32	ND	104	2
Perfluorooctane sulfonate	PFOS	0.20	ND	112	7
Perfluorooctanesulfonamide	PFOSA	0.21	ND	81	11
Perfluorononanoic acid	PFNA	0.08	ND	114	5
N-methyl perfluorooctanesulfonamide	NMeFOSA	0.07	ND	104	13
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	0.61	ND	85	11
Perfluorononanesulfonic acid	PFNS	0.25	ND	79	7
Perfluorodecanoic acid	PFDA	0.18	ND	117	8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	0.14	ND	112	15
1*H*,1*H*,2*H*,2*H*-perfluorodecane sulfonate	8:2 FTS	0.91	ND	113	11
Perfluoroundecanoic acid	PFUnA	0.26	ND	125	12
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	0.15	ND	95	12
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	0.23	ND	93	12
Perfluorodecanesulfonic acid	PFDS	0.25	ND	94	23
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	0.56	ND	77	25
Perfluorododecanoic acid	PFDoA	0.27	ND	118	15
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	1.11	ND	106	9
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1.10	ND	111	9
Perfluorotridecanoic acid	PFTrDA	0.33	ND	108	15
Perfluorododecanesulfonic acid	PFDoS	0.13	ND	89	12
Perfluorotetradecanoic acid	PFTeDA	0.32	ND	102	23

*ND = Not detected above MDL.

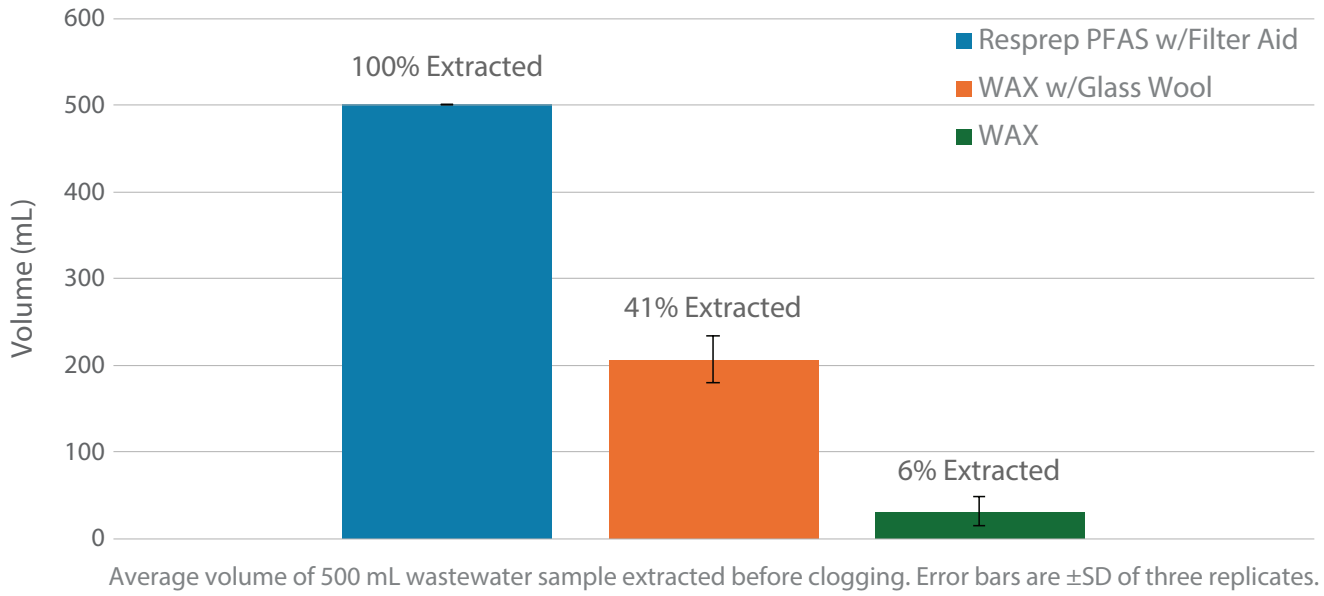
Factory-Packed Filter Aid Option Ensures Faster, More Effective Sample Prep

Exclusive to Restek, Resprep PFAS cartridges are also available with a factory-packed filter aid that prevents clogs and keeps samples flowing better than manually packed glass wool. As shown in Table II, when technicians follow Method 1633 instructions to pack glass wool to half the height of the cartridge barrel for all sample matrices (Section 12.1.1), the amount of wool and packing density can vary significantly, which means sample flow and clogging can also be affected. The filter aid used in Resprep PFAS cartridges is uniform and provides effective, consistent filtering and protection. In an experiment comparing the flow of diluted ASTM wastewater matrix through Resprep PFAS cartridges with filter aid, WAX cartridges with manually packed wool, and WAX cartridges alone, only the Resprep PFAS cartridges with the factory-packed filter aid did not clog and were able to extract the full sample volume (Figure 2).

Table II: When cartridges are packed manually following the height-based instructions in Method 1633, the amount of glass wool and packing density can vary significantly between cartridges and technicians, which can affect method performance.

	Technician 1	Technician 2	Technician 3	Technician 4
Average mg wool (n = 3)	39	150	51	116
SD	2	13	7	49
%RSD	5%	8%	14%	42%

Figure 2: Only Resprep PFAS cartridges with a factory-packed filter aid successfully extracted all 500 mL of 2.5x diluted ASTM wastewater matrix (total suspended solids = 100 mg/L).



Average volume of 500 mL wastewater sample extracted before clogging. Error bars are ±SD of three replicates.

Simplify your sample prep—save time, reduce variation, and ensure performance with Resprep PFAS cartridges with a factory-packed filter aid!

Resprep PFAS Cartridges

- Optional factory-packed filter aid prevents clogging so samples are processed completely, in less time, and more consistently than with manually packed glass wool.
- Streamlined sample prep: WAX and CarboPrep Plus carbon sorbents are factory packed in a single dual-bed SPE cartridge for a faster, simpler workflow.
- Ultra-clean polymeric and carbon sorbents—every WAX lot is tested for Method 1633 PFAS to ensure method detection limits can be met.
- Compatible with both vacuum manifolds and automated systems.



582051

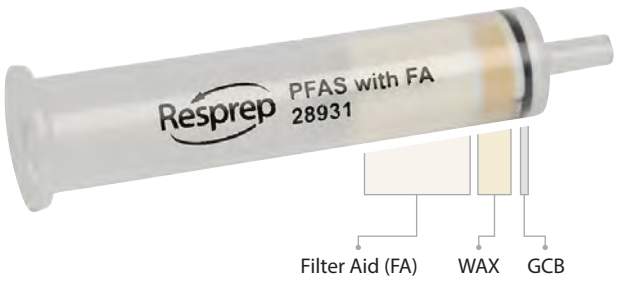
Carbon on top for solid samples.

Catalog No.	Product Name	Sorbent Phase	Volume (mL)	Units
28930	Resprep PFAS Cartridge	WAX 150 mg/GCB 50 mg	6	30-pk.
28931	Resprep PFAS Cartridge with Filter Aid	Filter aid 2000 mg/ WAX 150 mg/GCB 50 mg	6	30-pk.
582051*	Resprep PFAS Cartridge	GCB 50 mg/WAX 150 mg	6	30-pk.

*Please contact Restek Customer Service to order custom catalog no. 582051.

WAX on top for aqueous samples.





Automating SPE for PFAS Extraction

By Jason Hoisington and Diego López

Key Highlights

- Simplify your workflow with this method for automating SPE for PFAS on a PromoChrom SPE-03 system.
- Generate accurate, reliable results with the proven performance of Resprep PFAS SPE cartridges with filter aid.
- Factory-packed filter aid prevents clogs, reducing the need for time-consuming re-extractions.



Related Products

- *Resprep PFAS SPE Cartridge (cat.# 28931)*
- *Resprep Polymeric SPE Cartridge (cat.# 28268)*
- *PFAS 28 Calibration Standard (cat.# 30734*
- *Force C18 LC Column (cat.# 9634252)*
- *PFAS Delay Column (cat.# 27854)*

Introduction

For many environmental labs, solid phase extraction (SPE) is a key tool in the analysis of per- and polyfluoroalkyl substances (PFAS) because it is required by most EPA methods and many ASTM methods. Usually based on styrene divinyl benzene (SDVB) or weak anion exchange (WAX) cartridges, SPE can allow labs to effectively extract PFAS compounds from aqueous matrices without the use of solvent-heavy techniques, like separatory funnels or continuous liquid-liquid extraction. Even solid matrices can be prepared using SPE with an initial solvent extraction and water exchange. SPE has traditionally been performed on vacuum manifolds, but automated systems offer significant efficiency advantages.

Move Beyond the Manifold: Automating SPE for PFAS

Most methods written for SPE primarily address the use of vacuum manifolds, providing detailed steps for using them in manual extractions. Automated SPE is often mentioned as being appropriate to use, but specific details on how to implement it are not provided due to potential differences among instruments. This means labs must determine for themselves how to implement automated SPE and whether certain cartridges will work for the methods they are using.

In a previous study, it was demonstrated that dual-bed Resprep PFAS SPE cartridges with filter aid (cat.# 28931) worked well with a Thermo AutoTrace 280 PFAS system [1]. Resprep PFAS SPE cartridges with filter aid were employed because the dual-bed carbon and WAX format provides a faster, simpler workflow compared to using separate WAX and carbon SPE cartridges. In addition, the factory-packed filter aid prevents clogging, so even dirty samples can be successfully prepared in a single extraction.

In the current work, we explored using a different automated system and compared the performance of Resprep PFAS SPE cartridges to dual-bed cartridges from several competitors and to a single-bed, WAX-only cartridge. Here, an SPE-03 system from PromoChrom was used because it features several conveniences, such as an integrated programable interface, bottle rinsing capabilities, and a smaller footprint. Our goal was two-fold: (1) to establish instrument parameters and demonstrate cartridge compatibility with the SPE-03 instrument, and (2) to compare the relative performance of the different cartridges.

Simplifying Method Transfer

The first hurdle with automating SPE for PFAS analysis is translating the vacuum manifold steps listed in a method to the SPE instrument. Due to the interface and detailed step-by-step listing of the SPE process required by most instruments, this can make the automated method seem more complicated than using a vacuum manifold. However, once you understand the detailed breakdown of the method, transferring the method becomes a fairly straightforward process. For convenience, Table I shows the PromoChrom method used for this study.

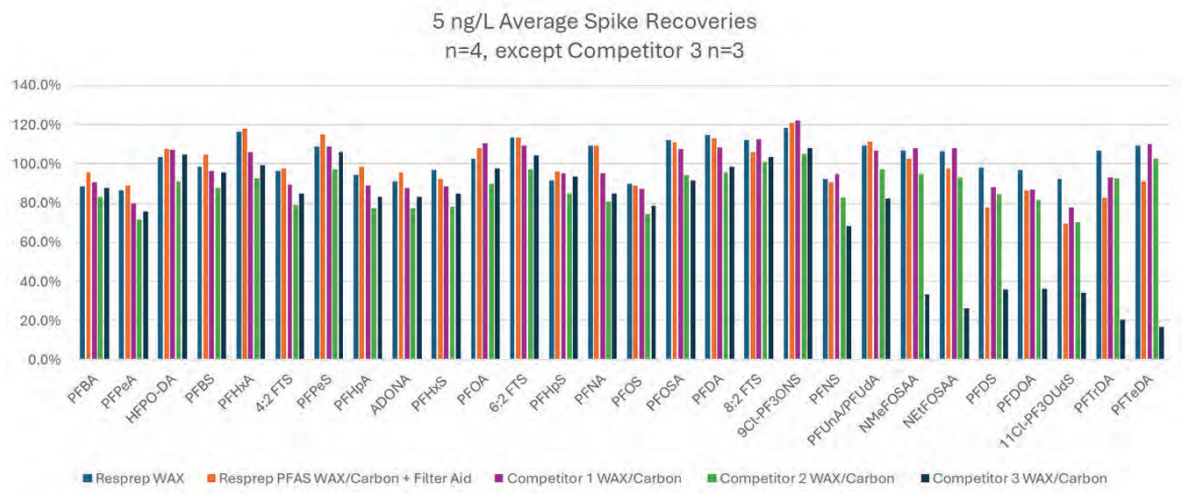
Table I: PromoChrom SPE-03 Parameters for Automating SPE for PFAS (Based on EPA Method 1633)

Action	Inlet 1 (solvent/sample)	Inlet 2 (air ratio)	Flow (mL/min)	Volume (mL)	Time
Elute to Waste 2	1% Ammonium Hydroxide in Methanol		8	15	
Elute to Waste 1	0.3 M Formic Acid in Water		8	5	
Add Sample to Waste 1	Sample		5	560	
Rinse	Water	20%	70	2.5	
Add Sample to Waste 1	Sample		5	5	
Rinse	Water	20%	70	5	
Add Sample to Waste 1	Sample		5	5	
Rinse	Water	20%	70	5	
Add Sample to Waste 1	Sample		5	5	
Shake					20 sec
Rinse	1:1 0.1 M Formic Acid:Methanol	20%	70	1.3	
Add Sample to Waste 1	Sample		5	3	
Rinse	1:1 0.1 M Formic Acid:Methanol	20%	70	5	
Add Sample to Waste 2	Sample		5	5	
Shake					20 sec
Air Purge to Waste 2	Air		5	3	
Add Sample to Waste 2	Sample		5	5	
Blow N2					1 min
Rinse	1% Ammonium Hydroxide in Methanol	20%	70	1.3	
Collect 1	Sample		5	3	
Rinse	1% Ammonium Hydroxide in Methanol	20%	70	5	
Collect 1	Sample		5	5	
Shake					10 sec
Collect 1	Sample		5	5	
Wait					1 min

Assessing Accuracy and Precision

To evaluate cartridge performance for automating SPE for PFAS with the PromoChrom system, we spiked deionized water samples at 5 ng/L using Restek's PFAS 28 calibration standard (cat.# 30734). Extracted internal standards were not used so that true recoveries from the SPE process could be measured. We evaluated Resprep polymeric (WAX only) cartridges (cat.# 28268); dual-bed, WAX/carbon Resprep PFAS SPE with filter aid cartridges (cat.# 28931); and dual-bed WAX/carbon cartridges from three competitors. Figure 1 shows the recovery results from the cartridges.

Figure 1: Average Recoveries for 5 ng/L Spikes from PromoChrom SPE-03 Extraction



The recoveries are relatively similar for most compounds until the later eluting PFAS. For the last few eluting PFAS, we see that the WAX-only cartridge outperforms all the WAX/carbon dual-bed cartridges, indicating that the carbon is likely more retentive for these longer chain PFAS. Competitor 3 is especially affected by this, showing recoveries of less than 40% for some compounds.

Figure 2 shows the precision from the extractions. Overall, the Restek cartridges showed better precision than the competitors, with most results being much less than 15% RSD. There is a slight trend of increasing %RSD for later eluting PFAS, again likely due to increased retention due to the carbon layer. Competitor 3 is again an outlier, showing high variability for later eluting PFAS in addition to the poor recovery shown in Figure 1. Average accuracy and precision data across all PFAS are presented in Table II.

Figure 2: Precision for 5 ng/L Spikes from PromoChrom SPE-03 Extraction

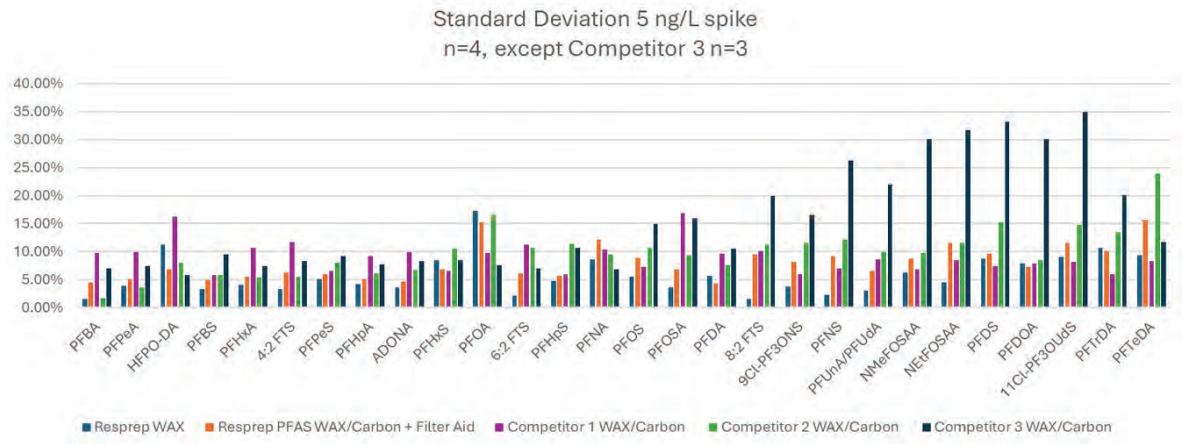


Table II: Average Recovery and Precision Across All PFAS Tested

SPE Cartridge	Average %Recovery	Average %RSD
Resprep Polymeric (WAX only)	102.2%	5.8%
Resprep PFAS + Filter Aid (WAX/Carbon)	99.6%	7.9%
Competitor 1 (WAX/Carbon)	98.7%	9.0%
Competitor 2 (WAX/Carbon)	87.7%	10.0%
Competitor 3 (WAX/Carbon)	75.7%	15.3%

Conclusion

Resprep WAX and WAX/carbon SPE cartridges have been shown to be compatible with common automated SPE systems, allowing labs interested in automating SPE for PFAS analysis to easily streamline their sample preparation. In addition, Resprep PFAS SPE cartridges with filter aid provided comparable or better accuracy and precision when evaluated against dual-bed WAX/carbon SPE cartridges from other vendors, ensuring that labs using them can generate accurate, reliable data. The factory-packed filter aid provides an additional advantage in that it prevents clogging, reducing the need for time-consuming re-extraction.

Acknowledgements

Restek thanks Maryland Spectral Services Inc. (https://www.mdspectral.com/) for use of their SPE-03 PromoChrom system for this study on automating SPE for PFAS testing.

References

1. J. Hoisington, Simplified SPE for PFAS analysis of non-potable waters, Application note, EVAN4369-UNV, Restek Corporation, 2024. https://discover.restek.com/en/application-notes/evan4369/simplified-spe-for-pfas-analysis-of-non-potable-waters

Resprep PFAS SPE Cartridge with Filter Aid

Sorbent Phase	Volume	Units	Cat.#
2000 mg/WAX 150 mg/GCB 50 mg	6 mL	30-pk.	28931



Resprep Polymeric SPE Cartridge

Sorbent Phase	Particle Size	Volume	Units	Cat.#
Polymeric WAX	30 µm	6 mL, 500 mg	30-pk.	28268



PFAS 28 Calibration Standard

(28 components)

11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS) (39108-34-4)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS) (757124-72-4)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS) (27619-97-2)
4,8-dioxa-3H-perfluorononanoic acid (ADONA) (919005-14-4)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)
2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6)
N-ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)* (2991-50-6)
N-methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)* (2355-31-9)
Perfluoro-1-decanesulfonic acid (PFDS) (335-77-3)
Perfluoro-1-nonanesulfonic acid (PFNS) (68259-12-1)
Perfluoro-1-octanesulfonamide (FOSA) (754-91-6)
Perfluoro-1-pentanesulfonic acid (PFPeS) (2706-91-4)
Perfluorobutanesulfonic acid (PFBS) (375-73-5)
Perfluorobutanoic acid (PFBA) (375-22-4)
Perfluorodecanoic acid (PFDA) (335-76-2)
Perfluorododecanoic acid (PFDOA) (307-55-1)
Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)
Perfluoroheptanoic acid (PFHpA) (375-85-9)
Perfluorohexanesulfonic acid (PFHxS)* (355-46-4)
Perfluorohexanoic acid (PFHxA) (307-24-4)
Perfluorononanoic acid (PFNA) (375-95-1)
Heptadecafluorooctanesulfonic acid (PFOS)* (1763-23-1)
Perfluorooctanoic acid (PFOA)* (335-67-1)
Perfluoropentanoic acid (PFPeA) (2706-90-3)
Perfluorotetradecanoic acid (PFTeDA) (376-06-7)
Perfluorotridecanoic acid (PFTrDA) (72629-94-8)
Perfluoroundecanoic acid (PFUnA) (2058-94-8)



*Technical grade compound containing both branched and linear isomers; see certificate for details.

Conc. in Solvent	CRM?	Min Shelf Life on Ship Date	Max Shelf Life on Ship Date	Shipping Conditions	Storage Temp.	qty.	cat.#
1 µg/mL, Methanol (1 mM KOH), 1 mL/ampul	Yes	6 months	60 months	Ambient	0 °C or colder	ea.	30734

Force C18 LC Columns



- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- High carbon load (20%) offers high hydrophobic retention.

ID	Length	Particle Size	Units	Cat.#
2.1 mm	50 mm	1.8 µm	ea.	9634252

PFAS Delay Column



- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

ID	Length	Particle Size	Units	Cat.#
2.1 mm	50 mm	5 µm	ea.	27854

Limited-Volume Screw-Thread Polypropylene Vials



Size	Type	Volume	Units	Cat.#
12 x 32 mm	9 mm Screw-Thread	700 µL	1000-pk.	23246

Solid-Top Polyethylene Caps, Clear



Cap Size	Type	Material	Units	Cat.#
9 mm	Screw-Thread	Polyethylene	1000-pk.	23247



Eliminate the Impact of Instrument-Related PFAS Interferences by Using a Delay Column

- Traps and "delays" system-related PFAS, preventing interference.
- Allows accurate identification and quantitation of trace-level PFAS in samples.
- Highly retentive—no breakthrough even with extended equilibration times.

Poly- and perfluoroalkyl substances, or PFAS, are rapidly emerging as some of the most important environmental contaminants to monitor around the world. Their widespread use and environmental persistence make them truly a global issue. Concerns over possible health risks are driving environmental scientists to look for these compounds everywhere from penguins [1] in the south to polar bears [2] in the north, and many points in between, and they are discovering the extent of PFAS contamination is literally pole to pole [3,4]. However, one other concerning place where at least some of these compounds are present is inside the very instruments used for PFAS analysis of environmental samples. Background PFAS contamination can interfere with low-level quantification requirements, which are usually in the range of parts per trillion (ppt) or below. Instrument-related background contamination can stem from LC components that contain fluoropolymers (e.g., PTFE), which can leach into mobile phase solutions. Using tubing and containers without fluorochemicals and switching instrument components to PEEK or stainless steel mitigates some of the risk of background contamination, but it does not eliminate the impact of interferences on trace-level analysis, so another solution is required.

In a standard setup, instrument-related PFAS interferences move through the system with the mobile phase and collect at the head of the analytical column between sample injections. However, adding a second column, called a "delay column," into the mobile phase flow path immediately before the injector is an effective way catch the background PFAS before they reach the analytical column (Figure 1). In this scenario, when the sample is injected, only PFAS in the sample will focus at the head of the analytical column. Then, during the course of a gradient analysis, PFAS from the sample will begin to move through the analytical column like normal. The background PFAS trapped in the delay column during equilibration will be eluted and pass through the analytical column as well. However, the background PFAS will elute later than the sample PFAS because they entered the analytical column after the injected sample due to additional retention by the delay column prior to reaching the injector. In addition, the background PFAS never focus on the head of the analytical column because they are present in the flow path in continuum rather than as a discrete injection. The net effect is that by using a delay column you can differentiate instrument-related PFAS background contamination from target PFAS in a sample.

Figure 1: Installation Position of the PFAS Delay Column.

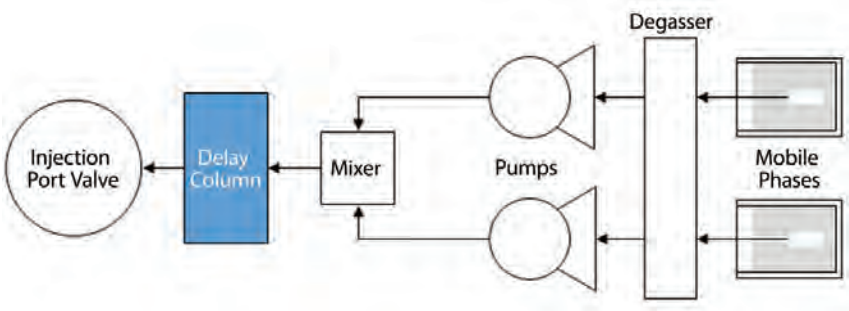
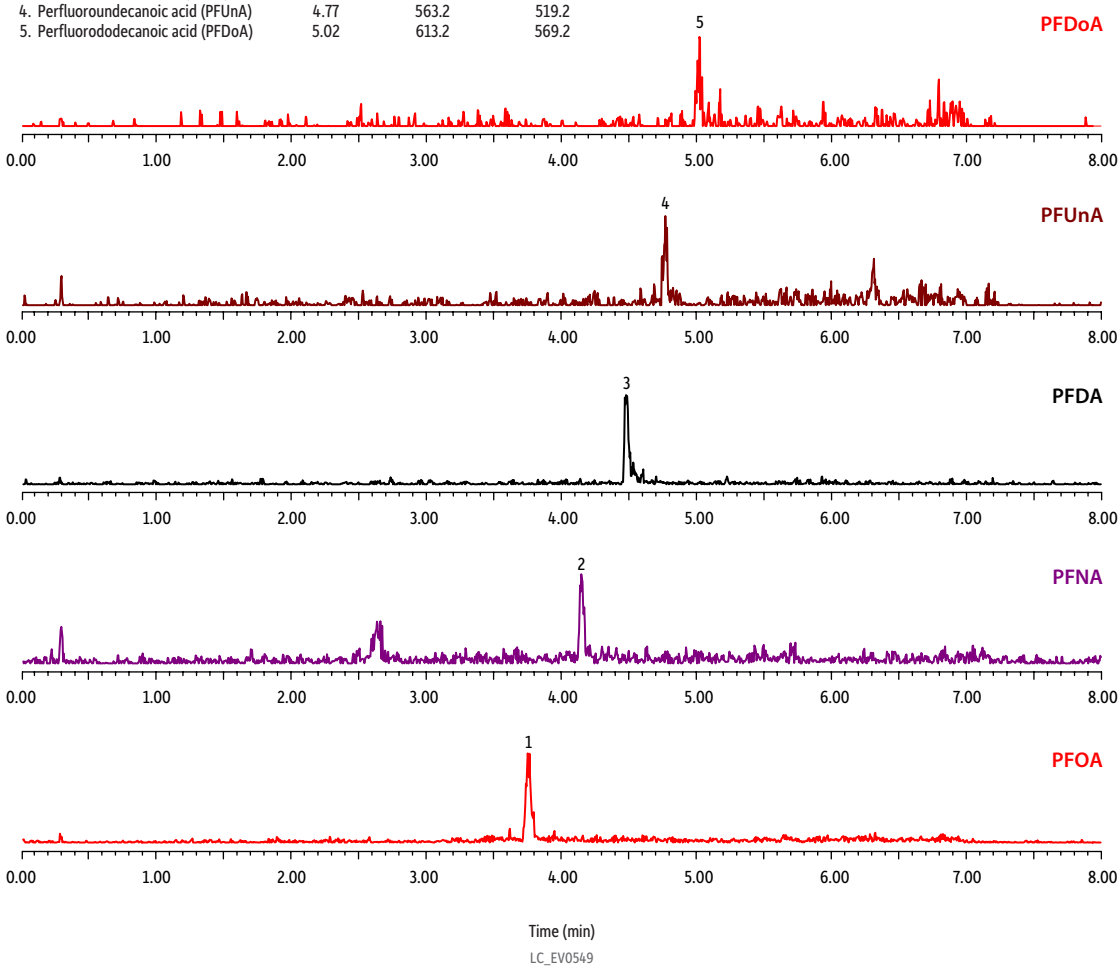


Figure 2 is an example of the kind of background contamination that interferes with accurate PFAS analysis. The system was equilibrated for 10 minutes without a delay column to allow instrument-related PFAS interferences to accumulate at the head of the analytical column before a blank injection. In this case, five potential target compounds were observed in the instrument blank. Which compounds are present and to what degree they are observed ultimately depends on the specific analytical setup. System-related PFAS contamination can be affected by factors such as instrument make and model, tubing and fitting materials, mobile phase solvent grade, and analytical method conditions.

Figure 2: System-related PFAS contamination is present in a blank sample analyzed without a delay column installed.

Peaks	t_R (min)	Precursor Ion	Product Ion
1. Perfluorooctanoic acid (PFOA)	3.75	413.2	369.1
2. Perfluorononanoic acid (PFNA)	4.15	463.2	419.2
3. Perfluorodecanoic acid (PFDA)	4.48	513.2	469.2
4. Perfluoroundecanoic acid (PFUnA)	4.77	563.2	519.2
5. Perfluorododecanoic acid (PFDoA)	5.02	613.2	569.2



Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	Blank injection without delay column after 10 minutes of equilibration.
Diluent:	80:20 Water:methanol
Inj. Vol.:	5 µL

Mobile Phase
A: Water, 5mM ammonium acetate
B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

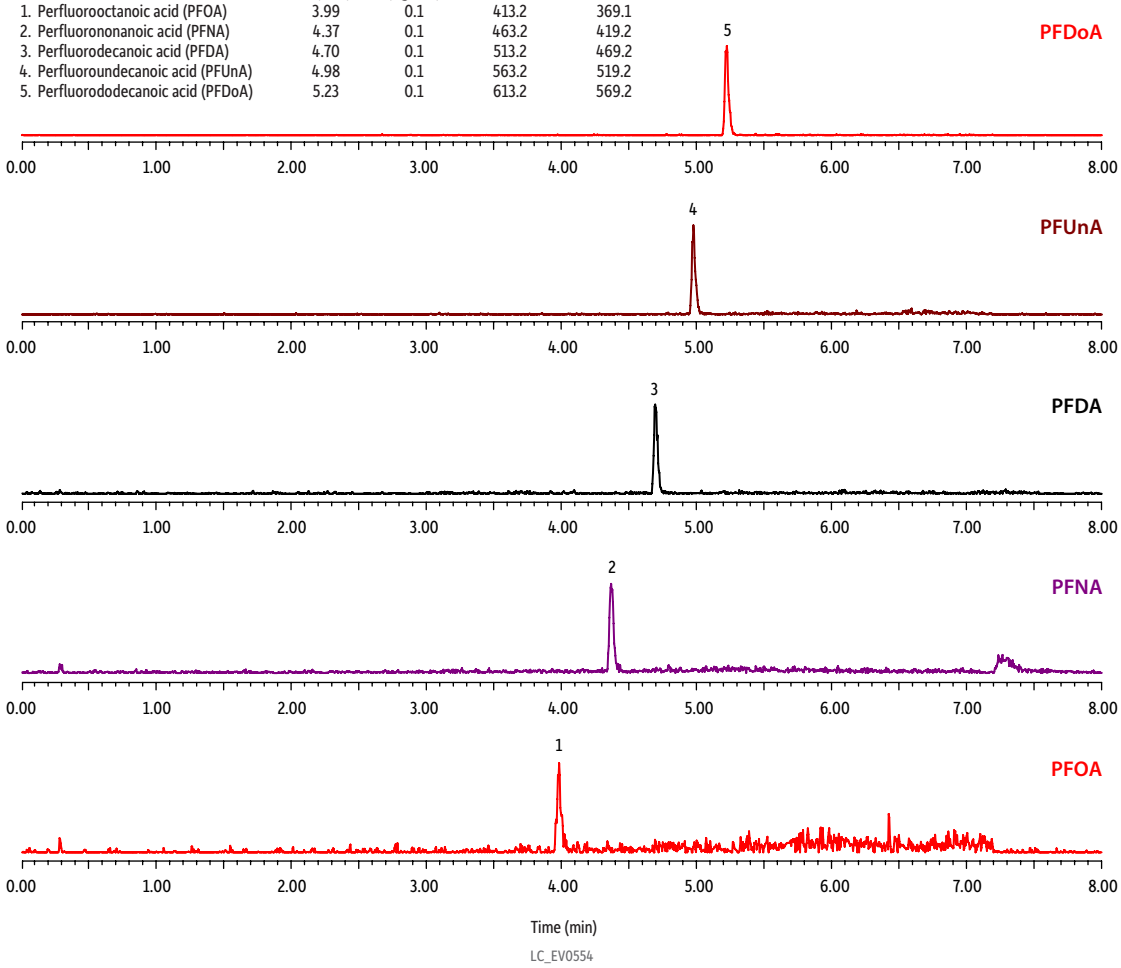
Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

Adding a delay column to the flow path is an effective way to retain background-related PFAS interferences so they do not coelute with PFAS from the sample during analysis. When the experiment in Figure 2 was repeated with a delay column installed, there were no observable peaks in the blank injection at the retention time for each PFAS previously found, even after relatively long equilibration times. Instead, the background PFAS interferences from the instrument eluted in a wide band after the target retention time. Because band broadening occurs while the background PFAS compounds are being retained by the delay column during equilibration, the signal for background PFAS does not appear as a well-defined peak once the background PFAS elute. Instead, the background signal is observed as a region of slightly elevated baseline (Figure 4).

Since the delay column adds additional length to the flow path between the mixer and the injector, target analyte retention times also shift slightly because of the longer time it takes for mobile phase changes to reach the analytical column during a gradient analysis (Figure 3). The net effect of a PFAS delay column is demonstrated in Figure 4: target analyte retention times are distinct from the instrument-related PFAS elution region, so there is no interference from the instrument background during sample analysis. Note that the elution time regions for instrument-related PFAS shown in Figure 4 were determined by lengthening the equilibration times and observing the baseline deflection caused by a broad band of delayed instrument-related PFAS eluting from the analytical column.

Figure 3: The expected retention times for PFAS in samples with a delay column installed were established using a prepared standard.

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Perfluorooctanoic acid (PFOA)	3.99	0.1	413.2	369.1
2. Perfluorononanoic acid (PFNA)	4.37	0.1	463.2	419.2
3. Perfluorodecanoic acid (PFDA)	4.70	0.1	513.2	469.2
4. Perfluoroundecanoic acid (PFUnA)	4.98	0.1	563.2	519.2
5. Perfluorododecanoic acid (PFDoA)	5.23	0.1	613.2	569.2



Column
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 1.8 µm
Pore Size: 90 Å
Temp.: 40 °C

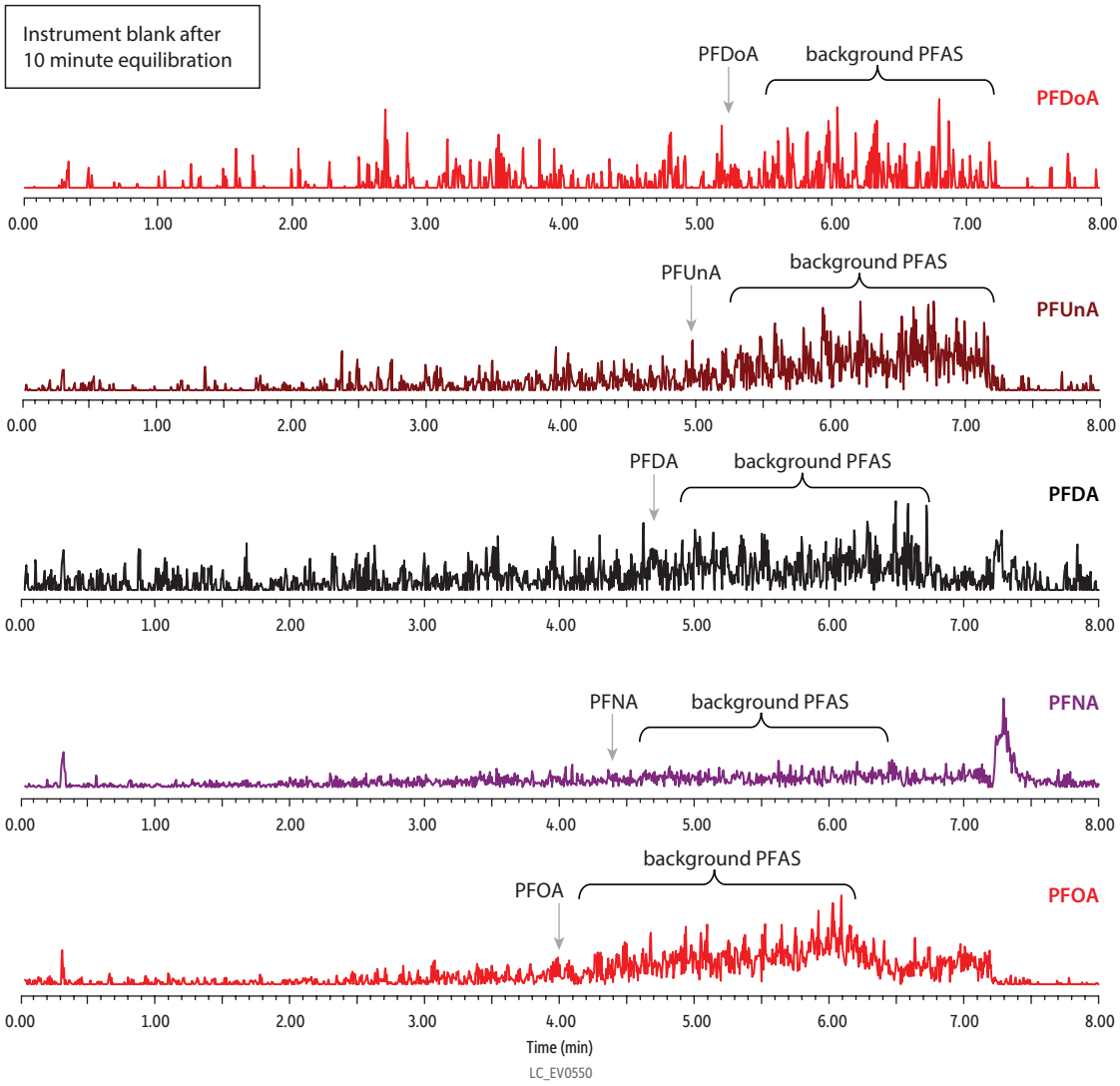
Sample
Diluent: 80:20 Water:methanol
Conc.: 0.1 ng/mL
Inj. Vol.: 5 µL

Mobile Phase
A: Water, 5 mM ammonium acetate
B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

Detector MS/MS
Ion Mode: ESI-
Mode: MRM
Instrument UHPLC
Notes A PFAS delay column (cat.# 27854) was installed before the injector.

Figure 4: Using a delay column eliminates the impact of instrument-related PFAS interferences by delaying their elution until after PFAS from the sample have eluted. No sample PFAS were detected. Arrows indicate expected retention time.



Peaks	Precursor Ion	Product Ion
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4. Perfluoroundecanoic acid (PFUnA)	563.2	519.2
5. Perfluorododecanoic acid (PFDoA)	613.2	569.2

Column	Raptor C18 (cat.# 9304252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	Blank injection with delay column (cat.# 27854) after 10 minutes of equilibration.
Diluent:	80:20 Water:methanol
Inj. Vol.:	5 µL

Mobile Phase

A:	Water, 5mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.50	0.4	5	95
6.51	0.4	80	20
8.00	0.4	80	20

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

In summary, using a PFAS delay column can eliminate the detrimental impact of background PFAS interferences from instrument-related sources by retaining contaminants prior to the analytical column and eluting them only after the sample has been injected and the gradient elution has started. The elution delay between the target PFAS in the sample and the background PFAS from the LC system is enough to separate them sufficiently to allow for accurate quantification of the target compound in the sample.

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50 mm, 2.1 mm ID	9304252

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Description	cat.#
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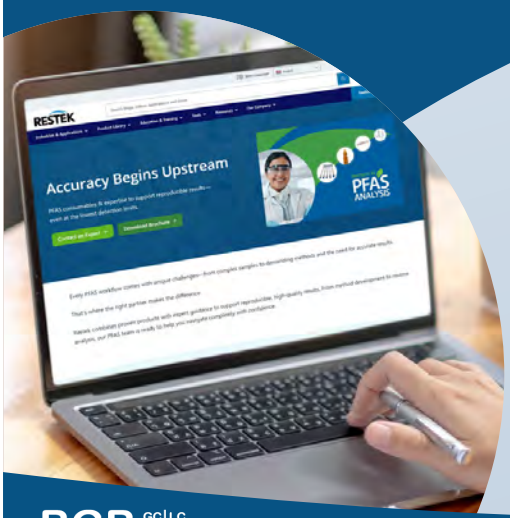

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