

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.

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.

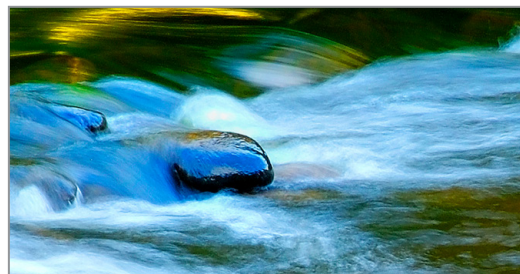
Experimental

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 μ m, 50 mm x 2.1 mm ID [cat.# 9311A52])	
Column temp.:	40 $^{\circ}$ C	
Injection volume:	10 μ L	
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.



Related Products

- Raptor Polar X, 2.7 μ m, 50 x 2.1 mm LC Column

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 ¹³C₂-PFHxA, ¹³C₂-PFOA, ¹³C₃-PFBS, ¹³C₄-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.

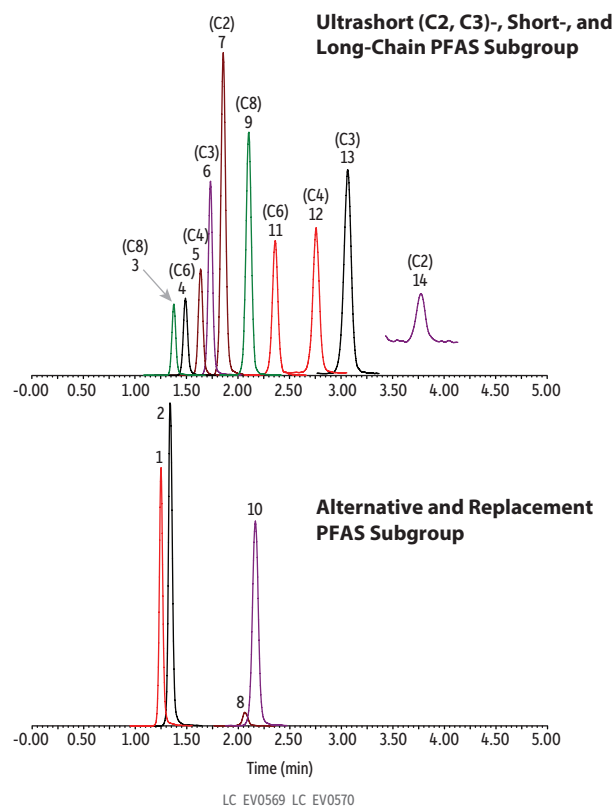
A tap water sample from the Restek facility and three water samples (Chicago river water, groundwater, and POTW effluent water) supplied by the United States Environmental Protection Agency (U.S. EPA) were fortified at 40 and 160 ppt. Blank and fortified water samples were diluted 1:1 in methanol as 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-PF30UdS)	1.25	400	630.78	450.80
2. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF30NS)	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

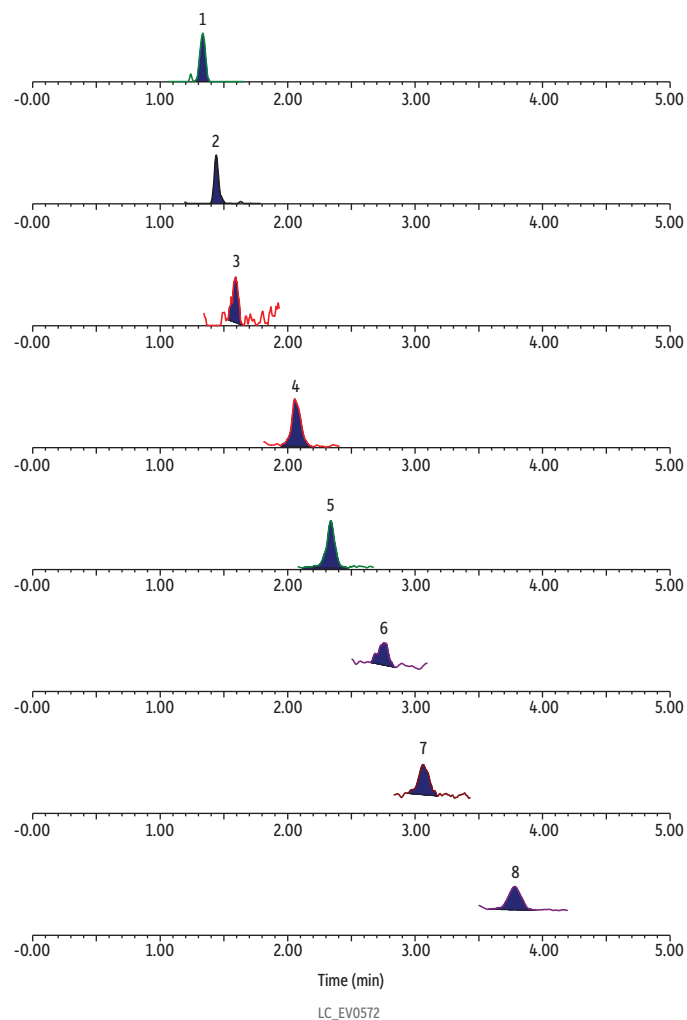
In our initial experiments, matrix interference for the TFA signal was observed for water sample analysis performed using a 5-minute isocratic run. Different analysis times were tested, and it was determined that an 8-minute run time was needed to avoid matrix interference for all analytes. The isocratic 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.

Samples	Detected Concentration (ng/L)													
	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 μ m) 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.

References

- [1] S. Taniyasu, K. Kannan, L.W.Y. Yeung, K.Y. Kwok, P.K.S. Lam, N. Yamashita, Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2-C4) in precipitation by liquid chromatography-tandem mass spectrometry: comparison to patterns of long-chain perfluorinated acids (C5-C18), *Anal. Chim. Acta.* 619 (2008) 221-230. <https://pubmed.ncbi.nlm.nih.gov/18558116/>
- [2] J. Janda, K. Nodler, H.-J. Brauch, C. Wiener, F.T. Lange, Robust trace analysis of polar (C2-C8) perfluorinated carboxylic acids by liquid chromatography-tandem mass spectrometry: method development and application to surface water, groundwater, and drinking water, *Environ. Sci. Pollut. R.* 26 (2018) 7326-7336. <https://pubmed.ncbi.nlm.nih.gov/29557039/>
- [3] K.Y. Kwok, S. Taniyasu, L.W.Y. Yeung, M.B. Murphy, P.K.S. Lam, Y. Horii, K. Kannan, G. Petrick, R.K. Sinha, N. Yamashita, Flux of perfluorinated chemicals through wet deposition in Japan, the United States, and other countries, *Environ. Sci. Technol.* 44 (2010) 7043-7049. <https://pubmed.ncbi.nlm.nih.gov/20795671/>

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.

Analyzing polar compounds using liquid chromatography has historically been a challenge due to poor retention, long equilibration times, low sensitivity, and the need to mitigate these problems with time-consuming sample derivatization or complex ion-pairing approaches. However, with the development of the Raptor Polar X column—a novel column that is specifically designed for the analysis of a broad range of polar compounds—scientists can avoid these problems. These new columns feature a unique phase chemistry that combines both HILIC and ion-exchange retention mechanisms on a single ligand. Because this ligand is bound to superficially porous particles, Raptor Polar X columns both reliably retain and efficiently separate a wide variety of polar analytes with simple mobile phase changes. By analyzing polar compounds on a column that provides purpose-built resolving power, you can avoid complex sample preparation procedures, save time and money, and reduce opportunities for error. Simplify the analysis of polar compounds with the resolving power of Raptor Polar X columns from Restek.



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



For information on Restek patents and trademarks, visit www.restek.com/patents-trademarks To unsubscribe from future Restek communications or to update your preferences, visit www.restek.com/subscribe To update your status with an authorized Restek distributor or instrument channel partner, please contact them directly.
© 2026 Restek Corporation. All rights reserved.

www.restek.com



Lit. Cat.# EVAN3220C-UNV