Analysis of Ultrashort-Chain and Alternative PFAS: LC-MS/MS Method Development and Application to Water Samples

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Abstract & Introduction

LC-MS/MS methods for the analysis of legacy short-chain (C4, C5) and long-chain (>C5) per- and polyfluoroalkyl substances (PFAS) have been well-developed based on reversed-phase (RP) chromatography. With proper modification, these typical RP methods can be applied to the analysis of emerging PFAS alternatives such as GenX and ADONA, which are perfluoroalkyl ether carboxylic acids used as PFOA substitutes. F-53B is a China-produced PFOS alternative containing two polyfluoroalkyl ether sulfonate components, 9Cl-PF3ONS and 11Cl-PF3OUdS, which are included as analytes in the updated EPA 537.1 method. Current LC methods, however, may not be suitable for the analysis of newly trending ultrashort-chain (C2, C3) PFAS mainly due to their insufficient retention on typical RP columns. While the use of short-chain PFAS (PFBA and PFBS) is intentional, more and more studies have shown the ubiquitous occurrence of C2 and C3 PFAS in aqueous environmental samples. These include trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoroethane sulfonate (PFEtS), and perfluoropropane sulfonate (PFPrS). It was shown that PFPrA is the predominant PFAS (up to 45% of total detectable PFAS) in the rain and snow samples collected from USA, France, and Japan. To date, there are not many studies showing the contamination sources and levels for these ultrashort-chain PFAS. A recent study showed the detection of PFEtS and PFPrS in aqueous film-forming foams (AFFFs) and ground waters from 11 military sites in the US, indicating AFFF firefighting foam may be one of the sources of the ultrashort-chain PFAS. This presentation will discuss the LC-MS/MS method development for simultaneous quantification of C3, C4, C8, and alternative PFAS in a variety water samples.

Methods

Table 1: Analytical Conditions for Shimadzu Nexera X2 with Sciex 4500 MS/MS

Analytical Column	Raptor C18 100 mm x 3.0 mm, 2.7 µm (Restek Catalog # 9304A1E)	
Delay Column	PFAS Delay Colum (Restek Catalog # 27854)	
Mobile Phase A	5mM ammonium acetate in water	
Mobile Phase B	methanol	
Gradient	**Time (min)**	**%B**
	0.00	20
	7.00	95
	9.00	95
	9.01	20
	11.0	20
Flow Rate	0.25 mL/min	
Run Time	11 min	
Column Temp.	40°C	
Ion Mode	Negative ESI	
IonSpray Voltage	-2000	
Source Temp.	450°C	

Table 2: Analyte MS Transitions

Analyte	Precursor Ion	Product Ion	IS for Quantification
PFPrA	162.9	119.0	$^{13}C_2$-PFHxA
PFBA	212.8	169.0	$^{13}C_2$-PFOA
PFPrS	248.8	79.6	$^{13}C_2$-PFHxA
PFBS	298.8	79.9	$^{13}C_2$-PFHxA
HFPO-DA	285.0	168.9	$^{13}C_2$-PFOA
ADONA	376.9	250.7	$^{13}C_2$-PFOA
PFOA	413.1	368.9	$^{13}C_2$-PFOA
PFOS	498.8	80.0	$^{13}C_4$-PFOS
9Cl-PF3ONS	530.8	350.7	$^{13}C_4$-PFOS
11Cl-PF3OUdS	630.7	451.0	$^{13}C_4$-PFOS
$^{13}C_2$-PFHxA	314.9	270.0	-
$^{13}C_2$-PFOA	415.0	370.0	-
$^{13}C_4$-PFOS	503.0	80.0	-

Sample Preparation

In a polypropylene vial, mixed 250 µL of testing water sample with 250 µL of 40/60 reagent water/methanol and 5 µL of internal standard solution (5 ng/mL of $^{13}C_2$-PFHxA, $^{13}C_2$-PFOA, $^{13}C_4$-PFOS in methanol). The vial was capped with polyethylene cap for injection analysis.

Calibration Standards

Reagent water (Optima LC-MS water) was fortified with 10 analytes at a range of 5 – 400 ng/L. The calibration standard solutions were then prepared as described for sample preparation procedure.

Analysis of Fortified Water Samples

A tap water collected from Restek facility and 3 water samples (Chicago river water, groundwater, and POTW effluent water) supplied by United States Environmental Protection Agency (US EPA) were spiked at 10 (20 ppt for PFPrA) and 80 ppt. The unspiked and spiked waters were subjected to sample preparation procedure for chromatographic analysis and quantified with the calibration standards.

Chromatograms

Figure 1: Chromatograms of Standard and Fortified Water Sample

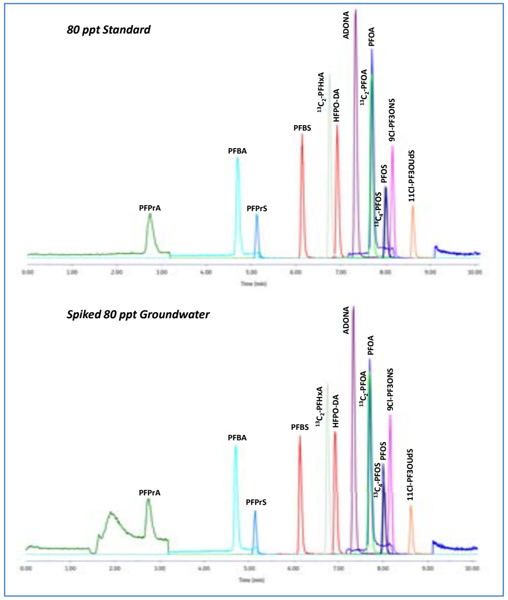
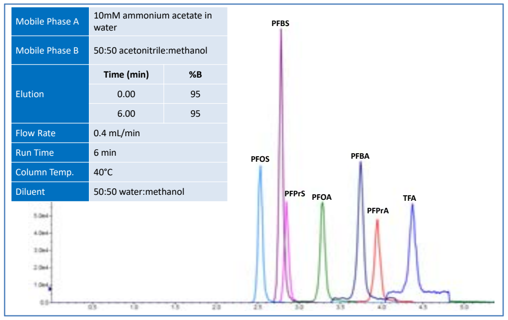


Figure 2: Analysis of C2 - C8 PFAS with a Hybrid HILIC/Ion Exchange Column



Mobile Phase A	10mM ammonium acetate in water	
Mobile Phase B	50:50 acetonitrile:methanol	
Elution	**Time (min)**	**%B**
	0.00	95
	6.00	95
Flow Rate	0.4 mL/min	
Run Time	6 min	
Column Temp.	40°C	
Diluent	50:50 water:methanol	

Results and Discussion

Chromatographic Performance: The analyte peak shapes, retention, and intensity were similar between reagent water and field water samples. There was a higher baseline and front noise for PFPrA signal in field water sample (Figure 1), which did not have negative impact on peak integration and quantification of PFPrA. No matrix interference was observed for all water samples upon 2-fold dilution.

Linearity: The calibration range is 10-400 ppt for PFPrA and 5-400 ppt for all other analytes. All compounds showed acceptable linearity with r value ≥ 0.999 and deviations <20%. 11Cl-PF3OUdS is the only analyte to be quantified with quadratic regression (1/x weighted) of standard curve. All other analytes are quantified with 1/x weighted linear regression.

Accuracy & Precision: The unspiked water samples showed various levels of C3, C4, and C8 PFAS with no detectable ADONA, HFPO-DA, 9Cl-PF3ONS, and 11Cl-PF3OUdS (Table 1). For accuracy determination, the analyte's measured amount in the spiked sample was adjusted to the unspiked concentration for recovery calculation. Water samples were spiked at low and high concentration in duplication for each batch of analysis. Total of 3 batches of analyses were performed on different days. Table 2 shows the accuracy and precision results calculated from the collection of all 3 batches of data. The method accuracy was demonstrated with recovery values of within 20% of the nominal concentration for both fortified levels and at LLOQ concentration in water samples. The %RSD was <15% indicating acceptable method precision.

Analysis of C2 (Trifluoroacetic Acid) with a Hybrid HILIC/Ion Exchange Column: The minimal retention of TFA on a typical reversed-phase column makes it difficult to analyze TFA together with other PFAS. A newly developed hybrid HILIC/ion exchange column was tested and showed versatile performance of simultaneous analysis of TFA, C3, C4, and C8 PFAS (Figure 2). This is accomplished with a fast and easy isocratic elution and therefore provides convenient set-up and high throughput analysis for the lab interested in adding ultrashort-chain compounds to PFAS assay.

Table 1. Analytes in Unspiked Water Samples

Samples	Detected Concentration (ng/L)									
	PFPrA	PFBA	PFPrS	PFBS	HFPO-DA	ADONA	PFOA	PFOS	9Cl-PF3ONS	11Cl-PF3OUdS
Tap Water	ND	1.1	ND*	ND	ND	ND	ND	ND	ND	ND
River Water	ND	1.6	ND	ND	ND	ND	ND	ND	ND	ND
Ground Water	9.0	3.4	ND	2.6	ND	ND	ND	ND	ND	ND
POTW Water	11.7	10.6	ND	3.1	ND	ND	15.0	6.0	ND	ND

*non-detected

Table 2: Accuracy and Precision

	Average Recovery, % (RSD, %)								
Matrices	Tap Water		River Water		Ground Water		POTW Water		Reagent Water
Conc. (ng/L)	10*	80	10*	80	10*	80	10*	80	5# (LLOQ)
PFPrA	96.9 (11.0)	105 (3.91)	105 (6.57)	95.4 (6.84)	92.0 (9.54)	99.4 (7.40)	94.2 (5.29)	87.2 (8.18)	103 (10.9)
PFBA	99.3 (9.19)	108 (1.81)	108 (5.20)	110 (1.70)	104 (8.21)	108 (6.68)	108 (8.12)	97.1 (8.17)	97.9 (12.0)
PFPrS	100 (4.24)	107 (3.14)	103 (6.71)	105 (2.64)	105 (8.48)	109 (6.68)	109 (5.65)	103 (9.28)	99.1 (8.59)
PFBS	101 (5.20)	106 (1.84)	99.7 (7.54)	105 (2.10)	100 (6.57)	106 (2.82)	103 (1.93)	97.8 (5.85)	96.0 (8.75)
HFPO-DA	96.2 (7.86)	102 (4.64)	96.2 (4.99)	105 (3.94)	95.0 (3.59)	101 (8.92)	92.9 (4.87)	90.3 (7.77)	99.3 (8.54)
ADONA	101 (6.23)	106 (3.82)	97.6 (6.36)	106 (2.32)	98.4 (2.68)	105 (4.08)	98.2 (7.09)	98.2 (7.09)	102 (10.3)
PFOA	105 (8.65)	105 (3.70)	108 (12.1)	107 (3.63)	108 (9.66)	105 (5.26)	99.9 (10.5)	94.5 (7.24)	100 (9.05)
PFOS	99.3 (2.10)	108 (4.24)	112 (1.87)	107 (4.93)	101 (2.96)	102 (2.31)	104 (4.46)	98.3 (5.82)	94.3 (8.85)
9Cl-PF3ONS	95.6 (4.60)	106 (5.93)	105 (5.37)	110 (8.20)	97.2 (4.52)	107 (7.41)	101 (6.52)	99.8 (4.89)	98.8 (5.47)
11Cl-PF3OUdS	114 (8.78)	112 (8.91)	102 (15.0)	91.5 (2.34)	96.7 (5.99)	105 (15.2)	115 (2.67)	103 (8.45)	105 (8.04)

*20 ng/L for PFPrA #10 ng/L for PFPrA

Conclusions

A simple dilute-and-shoot method was developed and validated for the simultaneous analysis of C3, C4, C8, and alternative PFAS in various water samples. Using a Raptor C18 (2.7µm) 100x3.0mm column, the analytical method was demonstrated to be fast, rugged, and sensitive with acceptable accuracy and precision. This method is suitable for the analytical labs wanting to include the C3 compounds for their existing PFAS analysis in drinking or non-portable water sources.