

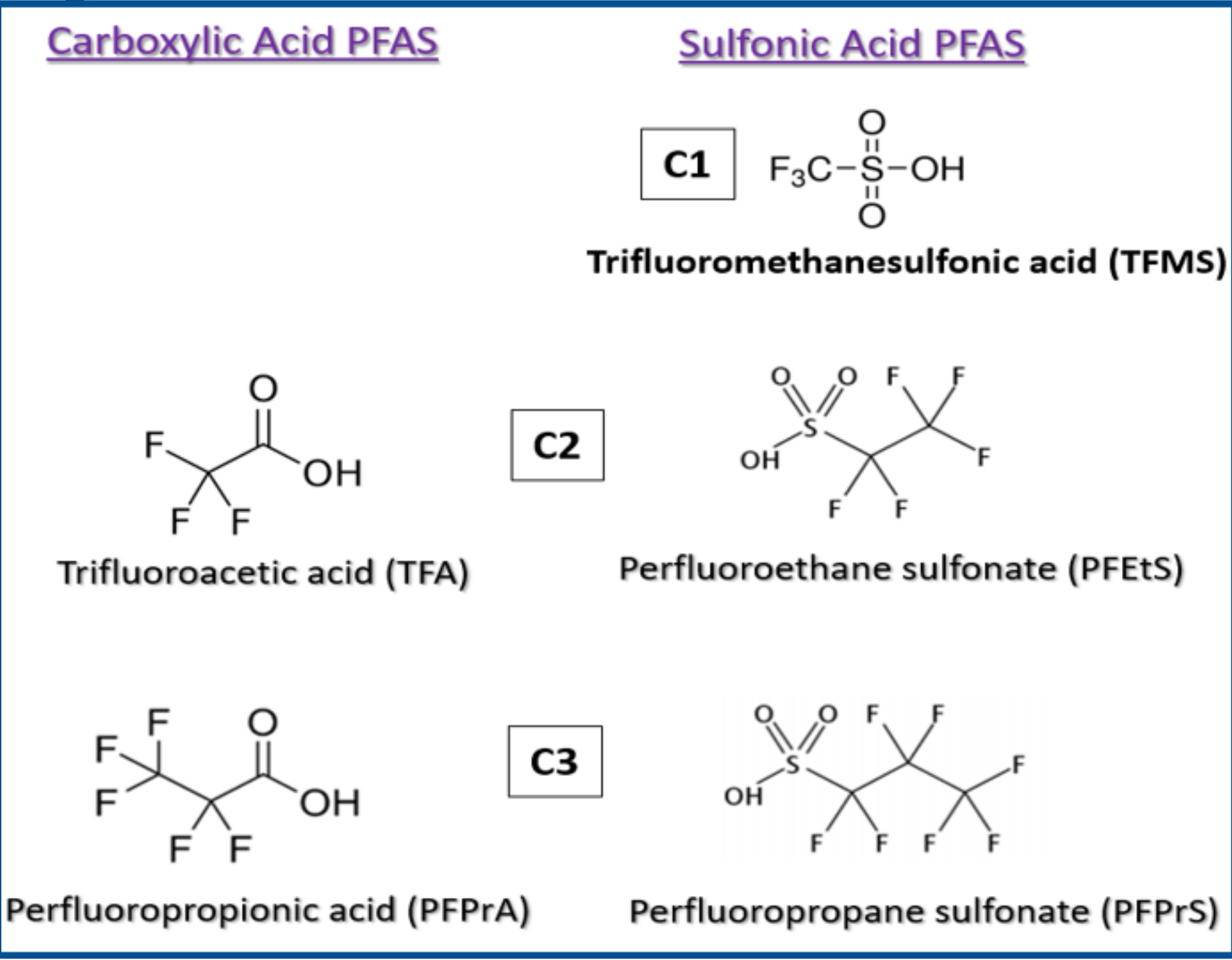
Incorporating Ultrashort-Chain Compounds into the Comprehensive Analysis of PFAS in Potable and Non-Potable Waters

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Introduction

Ultrashort-chain (USC) per- and polyfluoroalkyl substances (PFAS) are small and very polar compounds with carbon chain lengths shorter than C4. Their ubiquitous and high levels of occurrence 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 USC and long-chain PFAS together in water samples to comprehensively assess and address the full spectrum of PFAS contamination. The high polarity of USC PFAS poses a challenge for standard chromatographic practices in PFAS analysis, primarily due to insufficient chromatographic retention. In this study, 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, in both potable and non-potable waters. The chromatographic separation was conducted using a polar-embedded reversed-phase LC column with an inert coating on the hardware. A dilute-and-shoot workflow was evaluated by accuracy and precision analysis of fortified drinking waters and wastewaters. Additional potable and non-potable waters collected from various source waters were tested to further demonstrate that the established workflow is suitable for the accurate quantification of targeted PFAS in a wide range of water matrices.

Figure 1: Structures of C1 to C3 PFAS



Methods

Table 1: Analytical Conditions (Waters Xevo TQ-S with Acquity UPLC)

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

Conclusion

A simple and reliable dilute-and-shoot workflow was established in this study to provide a unique solution for incorporating ultrashort-chain compounds into a comprehensive measurement of PFAS in various water matrices. The reported method was sensitive, accurate, and precise by implementing an inert-coated polar-embedded alkyl phase column for chromatographic analysis. Most importantly, this solution can offer a valuable tool for monitoring these emergent PFAS in environmental water systems and assist in generating guidelines for future regulatory actions.

Standard and Sample Preparation

The calibration standard solutions (250 μL) were prepared in reverse osmosis water at the range of 1 to 1000 ng/L in the polypropylene HPLC vials. Five mass-labeled PFAS were implemented as quantitative internal standards (QIS) (see **Table 2**). A 2 μL aliquot of QIS working solution containing 40 ng/mL of ¹³C₃-PFBA, 20 ng/mL of ¹³C₂-PFHxA and ¹³C₄-PFOA, and 10 ng/mL of ¹³C₅-PFNA and ¹³C₂-PFDA, was added to each standard solution, followed by mixing with 250 μL of methanol containing 1% acetic acid.

Tap water (Restek Corp. diff households), spring bottled water and treated sewage wastewater effluent from a POTW (publicly owned treatment works, donated by GDIT (Falls Church, VA)) facility were used for the assessment of method accuracy and precision. Tap water and bottled water were used directly without filtration. POTW water (~ 10 mL) was filtered with polypropylene syringe filters and collected in 50-mL polypropylene tubes. These water samples (250 μL) were fortified at concentrations of 2, 4, 10, 50, and 250 ppt with native analytes and isotopically labeled ¹³C-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 (see **Table 2** for the list). A 250 μL aliquot of methanol containing 1% acetic acid was then added and mixed with fortified samples for LC-MS/MS analysis.

Results and Discussion

(1) **LC-MS/MS Method Development:** A chromatographic method was established (see **Table 1**) for the analysis of 45 PFAS compounds (**Figure 2**). The Ultra Inert IBD column exhibited satisfactory chromatographic performance under reverse-phased conditions. The inert-coated column showed a notable enhancement in detection sensitivity for most of the analytes. This improvement was evident by 3% to 75% increase in peak area and peak height. The MS/MS transition parameters for each analyte are provided in **Table 2**.

(2) **Linearity:** Employing quadratic regression (1/x weighted), all analytes exhibited acceptable linearities with r² >0.995 and deviations <30%. Table 2 shows differential linearity ranges of analytes, ranging from 1 ppt to 1000 ppt, with variation at the lowest calibration concentration.

(3) **Accuracy & Precision:** Three batches of analyses were conducted on different days, totaling nine repetitions at each fortified level. The average recovery and relative standard deviation (RSD) are presented in **Table 3**. All analytes exhibited recovery values within the range of 70 – 130% across all fortification levels. Satisfactory method precision was demonstrated with %RSD values within 20%. Additionally, the results indicated that all EIS had recovery values within 30% of the nominal concentration.

(4) **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 in a wide range of water matrices (see results in **Table 4**).

Figure 2: Chromatogram for the Analysis of a 500 pt Standard Solution

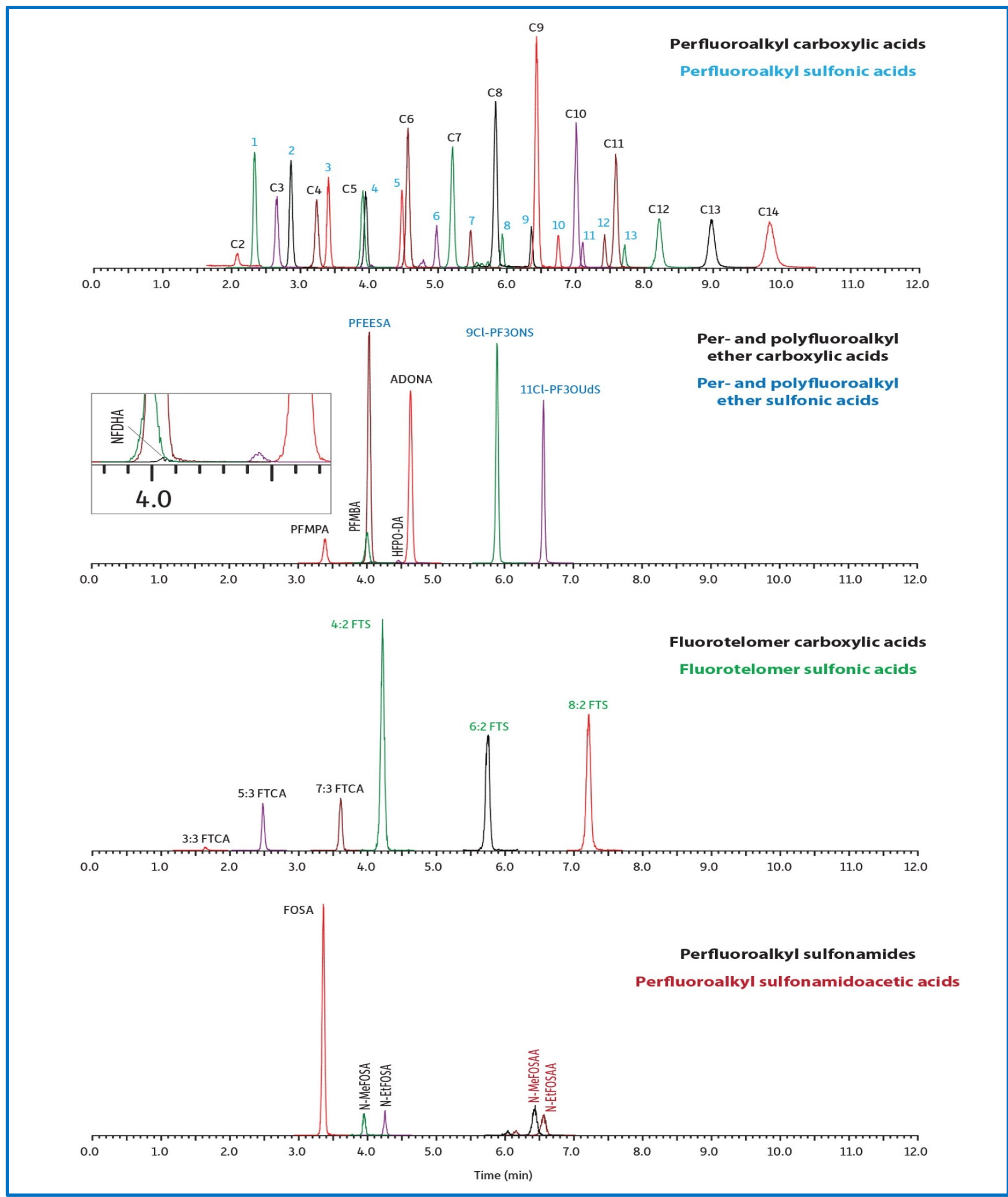


Table 3: Accuracy and Precision of Fortified Water Samples

Analytes	Average Recovery (RSD, %), n=9					Average Recovery (RSD, %), n=9					Average Recovery (RSD, %), n=9				
	Tap Water					Bottled Water					PFWH				
	Fortified Concentration (ng/L)					Fortified Concentration (ng/L)					Fortified Concentration (ng/L)				
	2	4	10	50	250	2	4	10	50	250	2	4	10	50	250
TC-TFA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PFBA	102 (5.88)	100 (7.72)	98.6 (6.71)	105 (8.98)	101 (6.85)	95 (7.90)	101 (6.64)	97.9 (6.08)	107 (5.13)	114 (7.77)	99 (7.96)	101 (11.9)	99 (7.18)	109 (13.90)	108 (8.57)
PFBA	108 (8.17)	107 (7.28)	104 (9.06)	104 (5.13)	103 (7.20)	96.4 (5.81)	94.7 (8.49)	96.2 (6.21)	103 (6.58)	112 (6.78)	100 (8.79)	98.0 (6.35)	99.1 (7.44)	104 (8.77)	106 (4.82)
PFPrA	109 (9.02)	115 (2.98)	105 (7.90)	111 (6.13)	107 (8.17)	99.3 (8.17)	99.3 (13.9)	95.5 (10.2)	105 (5.95)	111 (8.53)	104 (9.37)	110 (6.19)	101 (5.66)	109 (2.80)	116 (2.05)
PFHxA	88.2 (8.36)	99.8 (8.44)	96.2 (6.26)	105 (7.96)	103 (8.60)	100 (13.1)	96.5 (9.30)	91.0 (8.66)	99.5 (4.92)	108 (8.20)	91.1 (12.1)	98.3 (10.0)	92.9 (9.53)	101.0 (3.0)	103 (6.53)
PFHxA	88.7 (6.44)	101 (8.38)	96.2 (6.20)	105 (7.96)	103 (8.60)	100 (13.1)	96.5 (9.30)	91.0 (8.66)	99.5 (4.92)	108 (8.20)	91.1 (12.1)	98.3 (10.0)	92.9 (9.53)	101.0 (3.0)	103 (6.53)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
PFPrA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
PFPrA	103 (8.04)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
PFPrA	112 (9.36)	108 (10.4)	97.3 (8.10)	105 (6.42)	104 (7.25)	101 (9.04)	107 (6.85)	96.7 (4.30)	105 (2.88)	112 (7.17)	104 (15.6)	108 (7.24)	94.5 (5.45)	99.3 (3.30)	101 (6.28)
PFPrA	104 (5.33)	113 (3.79)	97.9 (6.84)	106 (7.28)	104 (9.30)	95.6 (10.3)	102 (5.73)	95.2 (3.80)	102 (2.79)	109 (6.05)	102 (13.3)	113 (8.62)	96.2 (4.13)	99.7 (7.46)	101 (6.28)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
PFPrA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
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PFPrA	112 (9.36)	108 (10.4)	97.3 (8.10)	105 (6.42)	104 (7.25)	101 (9.04)	107 (6.85)	96.7 (4.30)	105 (2.88)	112 (7.17)	104 (15.6)	108 (7.24)	94.5 (5.45)	99.3 (3.30)	101 (6.28)
PFPrA	104 (5.33)	113 (3.79)	97.9 (6.84)	106 (7.28)	104 (9.30)	95.6 (10.3)	102 (5.73)	95.2 (3.80)	102 (2.79)	109 (6.05)	102 (13.3)	113 (8.62)	96.2 (4.13)	99.7 (7.46)	101 (6.28)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
PFPrA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
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PFPrA	104 (5.33)	113 (3.79)	97.9 (6.84)	106 (7.28)	104 (9.30)	95.6 (10.3)	102 (5.73)	95.2 (3.80)	102 (2.79)	109 (6.05)	102 (13.3)	113 (8.62)	96.2 (4.13)	99.7 (7.46)	101 (6.28)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
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PFPrA	112 (9.36)	108 (10.4)	97.3 (8.10)	105 (6.42)	104 (7.25)	101 (9.04)	107 (6.85)	96.7 (4.30)	105 (2.88)	112 (7.17)	104 (15.6)	108 (7.24)	94.5 (5.45)	99.3 (3.30)	101 (6.28)
PFPrA	104 (5.33)	113 (3.79)	97.9 (6.84)	106 (7.28)	104 (9.30)	95.6 (10.3)	102 (5.73)	95.2 (3.80)	102 (2.79)	109 (6.05)	102 (13.3)	113 (8.62)	96.2 (4.13)	99.7 (7.46)	101 (6.28)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
PFPrA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
PFPrA	103 (8.04)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
PFPrA	112 (9.36)	108 (10.4)	97.3 (8.10)	105 (6.42)	104 (7.25)	101 (9.04)	107 (6.85)	96.7 (4.30)	105 (2.88)	112 (7.17)	104 (15.6)	108 (7.24)	94.5 (5.45)	99.3 (3.30)	101 (6.28)
PFPrA	104 (5.33)	113 (3.79)	97.9 (6.84)	106 (7.28)	104 (9.30)	95.6 (10.3)	102 (5.73)	95.2 (3.80)	102 (2.79)	109 (6.05)	102 (13.3)	113 (8.62)	96.2 (4.13)	99.7 (7.46)	101 (6.28)
PFPrA	111 (6.58)	117 (4.67)	103 (9.51)	104 (8.34)	101 (8.98)	94.4 (10.9)	100 (10.8)	101 (8.29)	105 (6.09)	112 (6.11)	108 (8.67)	105 (8.15)	101 (4.86)	105 (4.68)	109 (5.20)
PFOA	108 (7.53)	110 (5.46)	97.8 (3.83)	103 (2.33)	99.5 (8.40)	108 (9.34)	103 (8.32)	97.3 (3.99)	105 (5.46)	112 (7.09)	113 (8.50)	117 (5.50)	95.1 (5.73)	103 (6.97)	107 (4.67)
PFOA	99.4 (6.34)	104 (5.56)	95.1 (5.35)	98.5 (9.37)	95.8 (8.66)	101 (11.9)	96.4 (6.74)	92.2 (8.25)	100 (5.31)	109 (8.86)	108 (10.6)	111 (8.44)	91.8 (5.90)	97.6 (4.10)	101 (6.28)
PFUNA	110 (14.0)	109 (12.1)	108 (14.0)	107 (8.05)	95.5 (7.14)	109 (10.6)	104 (8.74)	96.8 (11.0)	99.7 (9.52)	105 (5.73)	106 (12.6)	102 (9.97)	91.8 (8.90)	97.1 (3.90)	101 (6.28)
PFOA	103 (8.31)	115 (5.52)	103 (3.25)	106 (4.64)	107 (5.60)	102 (7.45)	105 (4.66)	97.8 (3.00)	106 (2.03)	111 (2.50)	93.1 (15.3)	107 (1.71)	87.9 (6.50)	95.7 (4.51)	97.9 (2.12)
PFPrA	101 (13.3)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (9.41)	115 (9.94)	113 (13.2)	79.7 (9.70)	90.3 (7.13)
PFPrA	103 (8.04)	91.1 (11.0)	87.5 (7.09)	82.3 (5.23)	96.5 (3.05)	106 (9.03)	100 (8.97)	81.8 (13.1)	87.8 (18.6)	98.1 (6.33)	109 (				