Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water Sources following EPA 533

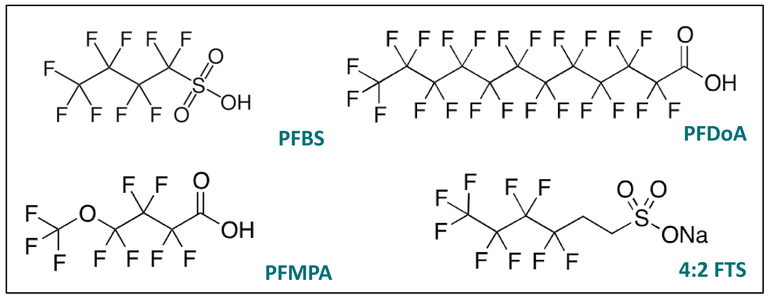
Amanda Belunis[1], Dr. William R. LaCourse[1]
[1]University of Maryland, Baltimore County, Baltimore, MD, 21250

1. Introduction

PFAS are a group of man-made chemicals that have been used in a wide variety of industries and consumer products since the 1940s, including nonstick cookware and food packaging. Originally considered biologically inactive, research has revealed their toxic nature to humans and wildlife. Growing health concerns regarding PFAS and their prevalence in consumer goods and the environment indicates a critical need to simply and reliably execute existing and upcoming regulatory methods on commercially avaliable instrumentation. The U.S. EPA has developed method 533, the determination of selected PFAS in drinking water utilizing isotope dilution, solid phase extraction, & liquid chromatography/mass spectrometry. **Here we present the validation of EPA 533 and the development of improved methodology for the detection of PFAS in drinking water sources.**

2. Experimental

Figure 1. *Structures of four PFAS showing two short chain (left), one long chain (top right) and one fluorotelomer (bottom right).*

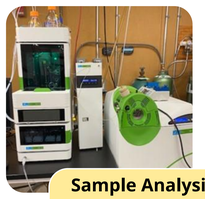


Sample Collection

Samples were collected in 250 mL bottles. Ammonium acetate (1 g/L) was added for preservation.

Sample Extraction

SPE was conducted using WAX cartridges. Extracts were concentrated to dryness under inert N_2 and 60°C, then resuspended in 1 mL 80/20% (v/v) MeOH/water.



Sample Analysis

Separation and detection were performed using PerkinElmer LX50 Ultra High-Performance Liquid Chromatography system and QSight 210 Mass Spectrometer.

Table 1. *Liquid Chromatography and Mass Spectrometry experimental parameters.*

LC Conditions					MS Conditions	
Analytical Column	Brownlee™ SPP C18 Column, 75 x 4.6mm, 2.7 µm	LC Gradient Program			Ion Source	Electrospray
Guard Column	Brownlee™ SPP C18 Column, 5mm x 4.6mm, 2.7 µm	Time (min)	A (%)	B (%)	Polarity	Negative
Delay Column	Brownlee™ SPP C18 Column, 50 x 3.0mm, 2.7 µm	0.00	95	5	Electrospray Voltage	-3500
Mobile Phase A	10 mM ammonium acetate in water	0.70	95	5	Drying Gas	60 L/hr
Mobile Phase B	Methanol	1.00	55	45	Nebulizer Gas	80 L/hr
Flow Rate	0.8 mL/min	7.00	2	98	Source Temp (°C)	350
Oven Temp (°C)	40	8.00	2	98	HSID Temp (°C)	280
Auto Sampler Temp (°C)	15	8.10	95	5	Detection Mode	MRM
Injection Volume	10	10.00	95	5		

3. Results

Figure 2. *Overlay of extracted ion chromatograms of all 25 analytes in the highest calibration point. Corresponding compounds to each peak number can be seen in table 2 below.*

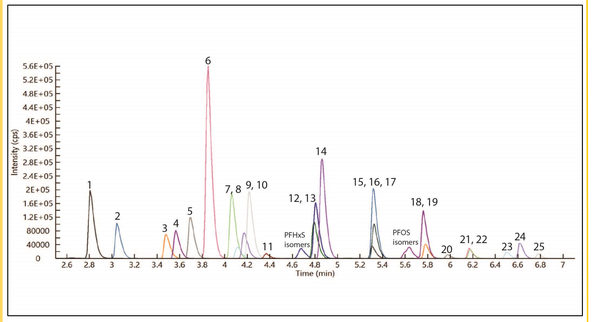


Table 2. *Peak identification, correlation coefficient, method detection limit, and experimental minimum reporting level for all 25 analytes of interest.*

Compound	Peak #	Correlation Coefficient R^2	Method Detection Limit (ng/L)	Experimental MRL (ng/L)
PFBA	1	0.9906	1.5	1
PFMPA	2	0.9980	1.3	1
PFPeA	3	0.9953	1.1	1
PFBS	4	0.9916	2.1	1
PFMBA	5	0.9950	1	1
PFEESA	6	0.9927	1.2	1
NFDHA	7	0.9964	1.9	4
4:2FTS	8	0.9935	0.8	1
PFHxA	9	0.9953	1.1	1
PFPeS	10	0.9954	1.3	1
HFPO-DA	11	0.9946	1.1	1
PFHpA	12	0.9927	1.2	1
PFHxS	13	0.9927	1.1	1
ADONA	14	0.9941	0.9	0.88
6:2FTS	15	0.9957	1.0	0.89
PFHpS	16	0.9936	2.0	0.94
PFOA	17	0.9931	1.0	0.94
PFOS	18	0.9962	1.1	0.91
PFNA	19	0.9951	0.6	0.95
9Cl-PF3ONS	20	0.9920	1.0	0.95
8:2FTS	21	0.9963	1.2	0.93
PFDA	22	0.9963	0.6	0.95
PFUnA	23	0.9942	1.2	3.74
11Cl-PF3OUdS	24	0.9933	1.6	0.96
PFDoA	25	0.9939	1.1	0.94

Calibration

Calibration standards were prepared at eight levels ranging from ~5 to 25,000 ng/L. Concentrations are 250 times higher than target concentration range to account for analyte enrichment from sample preparation. Standards were run in triplicate using isotope dilution for quantification and fit using a non-linear regression forcing through zero. All analytes demonstrated excellent linearity with $R^2 \geq 0.99$ as seen in **Table 2.**

Recovery

Recovery studies were carried out using seven replicate laboratory blanks at low (1 and 4 ng/L), midrange (10 and 20 ng/L) and high (80 ng/L) fortification levels. **Figure 3** summarizes the results from three of the fortification levels. Recoveries for all analytes at the five different fortification levels were within the range of 70% to 130% specified by EPA method 533. The low recovery levels (1 and 4 ng/L) were utilized to determine the method detection limit and the minimum reporting level (MRL). These determined values are summarized in **Table 2**.

Sample Collection

Drinking water samples were collected from three different sources in the Baltimore Metro Area. Four samples were collected from each area (two field duplicates and two LFSM). **Table 3** summaries the results of the levels found at each source. **Figure 4** shows the compounds found in sample 3 above the MRL.

Figure 3. *Average recovery of fortified blanks at three different fortification levels: low (4 ng/L), midrange (20 ng/L) and high (80 ng/L). The method 533 specified 70-130% recovery range can be seen in the red lines. Recoveries for all compounds at the three different fortification levels fall within this range.*

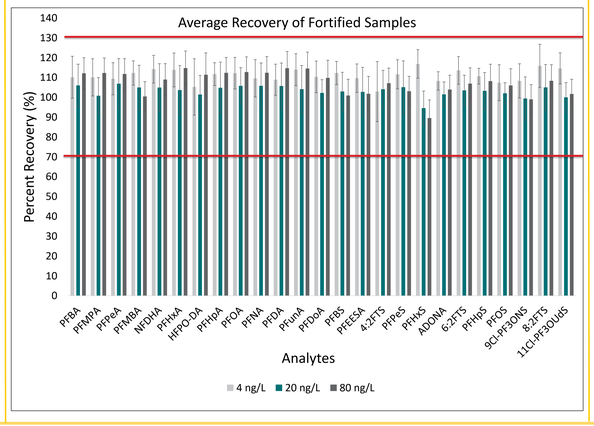


Figure 4. *Overlay of extracted ion chromatograms of the ten compounds found above the minimum reporting levels at drinking water site 3. Corresponding peak numbers can be seen in table 2.*

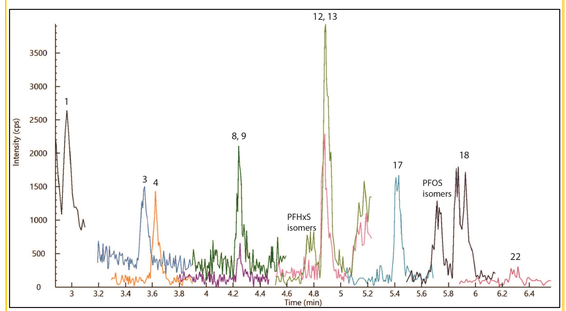


Table 3. *Average concentration of two field duplicates from three drinking water sites in the Baltimore Metro area. Only concentrations above the minimum reporting level (MRL) are reported.*

Analyte	Average Concentration (ng/L)		
	Drinking Water Source 1	Drinking Water Source 2	Drinking Water Source 3
PFBA	1.04	1.99	1.97
PFMPA	<MRL	<MRL	<MRL
PFPeA	1.19	2.01	2.40
PFBS	<MRL	2.11	2.30
PFMBA	<MRL	<MRL	<MRL
PFEESA	<MRL	<MRL	<MRL
NFDHA	<MRL	<MRL	<MRL
4:2FTS	1.47	1.70	1.10
PFHxA	1.41	3.10	3.53
PFPeS	<MRL	<MRL	<MRL
HFPO-DA	<MRL	<MRL	<MRL
PFHpA	<MRL	1.71	2.95
PFHxS	<MRL	1.80	2.32
ADONA	<MRL	<MRL	<MRL
PFOA	<MRL	2.15	2.23
6:2FTS	<MRL	<MRL	<MRL
PFHpS	<MRL	<MRL	<MRL
PFNA	<MRL	<MRL	<MRL
PFOS	<MRL	4.10	3.65
9Cl-PFONS	<MRL	<MRL	<MRL
PFDA	<MRL	<MRL	1.58
8:2FTS	<MRL	<MRL	<MRL
PFUnA	<MRL	<MRL	<MRL
11Cl-PF3OUdS	<MRL	<MRL	<MRL
PFDoA	<MRL	<MRL	<MRL

4. Conclusion

This poster reports the validation of an LC-MS/MS method for the determination of PFAS analytes and isotopically labelled standards in drinking water listed in US EPA Method 533. An improved chromatographic method was developed to decrease runtime from 35 minutes to 10 minutes; an over 71% decrease in LC-MS/MS runtime. An eight-point calibration curve (~5-25,000 ng/L) demonstrated that excellent linearity was achieved for all PFAS analytes, with R^2 values greater than 0.99. Recoveries of all analytes fell within the 70-130% requirements of the method for laboratory fortified blanks ranging from 1 to 80 ng/L. Drinking water samples from three sampling sites were quantified and recoveries were validated within method requirements. Although multiple analytes were detected above MRL at each location, the reportable levels are below any current action levels. Overall, an improved method was developed and validated showing the capabilities of the LX-50 UHPLC and QSight 210 MS for the analysis of 25 PFAS in drinking water.

5. References

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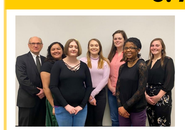
Rosenblum, L., Wendelken, S. C., "EPA Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry." *EPA Document # 815-B-19-020.* **December 2019**.

6. Acknowledgements



LaCourse Lab

This work was done in collaboration with PerkinElmer. The authors would like to extend a special thanks to Michael Costanzo, Jason Weisenseel, and Kathleen Young.

533 App Note