Analysis of Per- and Polyfluoroalkyl Substances (PFAS) Specified in EPA M533 Using the Triple Quadrupole LCMSMS

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Novel Aspect

The Quantitation of short chain per- and polyfluoroalkyl substances (PFAS) in drinking water[1] by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Introduction

Recently, EPA announced a new method for testing short chain per- and polyfluoroalkyl substances (PFAS) in drinking water. Structures for select short chain and long chain PFAS[2] are included in Figure 1. Method 533[3] measures PFAS by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS). The lowest concentration minimum reporting levels (LCMRLs) for the method analytes range from 1.4 to 16 ng/L. Shimadzu Scientific Instruments was one of eight laboratories that participated in providing EPA with outside laboratory validation data along with a review of the method draft. This poster includes Shimadzu Scientific Instruments data from the validation study.

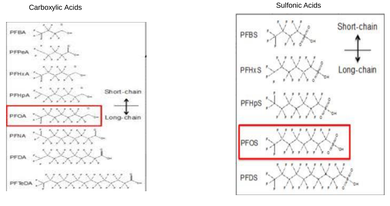


Figure 1. Structure of Long and Short Chain PFAS compounds.

SPE Method

Solid Phase Extraction (SPE) with a WAX sorbent (500 mg) was used for the extraction, as outlined in EPA method 533 (section 6.8.1). Each cartridge was cleaned and conditioned first, following EPA 533 (section 11.4.1). A vacuum manifold with a high-volume sampling kit outfitted with large bore PEEK tubing was used to reduce potential contamination.

All sample bottles were rinsed with the elution solvent prior to use. Each water sample (250 mL) is adjusted to pH 6-8 and fortified with PFAS analyte and Isotope dilution analytes, mixed, and loaded onto the conditioned cartridge. Compounds were eluted at a high pH from the solid phase with two 5 mL aliquots of methanol containing 2% ammonium hydroxide (v/v) and evaporated to dryness using nitrogen. Extracted samples were reconstituted to a final volume of 1 mL in 80:20 Methanol: H2O with internal standards added.

Extraction for P & A study was performed by fortifying five replicates of reagent water and tap water samples at 10 ng/L. For LCMRL calculations (results not shown in this poster) samples were extracted at eight concentration levels ranging from 0.2 ppt and 14 ppt. Four replicates were prepared at each concentration level and a minimum of four laboratory reagent blanks (LRB) were also included in the extraction batches

Figure 2. LCMS-8045 triple quadrupole mass spectrometer

Instrumental Method

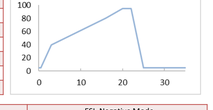
The analysis of 25 PFAS compounds, with16 Isotope Dilution Analogues and 3 post extraction internal standards was performed using a UHPLC system coupled with a triple quadrupole mass spectrometer LC/MS/MS. MRM transitions were optimized using Flow Injection Analysis (FiA) for all compounds[4]. Source parameters were optimized to reduce fragmentation and increase sensitivity. Fluorotelomer acids, observed as [M-H]- and [M-HF-H]- can result in an ion with the same m/z as the unsaturated fluorotelomer acid. Even under optimized chromatography, these compounds have near identical retention times. The lower ESI heater temperature reduces HF loss and minimizes false identification of fluorotelomer acids. The chromatographic parameters are based on the chromatographic method used in EPA Method 533. A Shim-pack XR-ODS 50 x 3.0 mm column was used as a delay column, and a Phenomenex Gemini™ C18, 2.0 mm ID × 50 mm, 3.0 µm particle size column was used as the analytical column. Quantitation was performed using MRM on tandem mass spectrometer (LC-MS/MS). LCMS system and instrumental conditions are included in Table 1 and MRM transitions are included in Table 2.

Table 1. LCMS Method conditions

LCMS Instrument	Shimadzu LCMS-8045
Analytical Column	Gemini 3µm C18 110A LC Column 50 x 2mm
Solvent Delay Column	Shim-pack XR-ODS 2.2-micron, 3.0 x 50mm
Injection Volume	10 µL
LC Flow Rate	0.25 mL/min
Mobile Phase A	20 mM Ammonium Acetate in LCMS-grade Water
Mobile Phase B	Methanol
Run / Acquisition Cycle Time	35 minutes (all 44 PFAS compounds are eluted in 20 minutes)

Gradient Conditions

Time (min)	% MPA	%MPB
0	95	5
3	60	40
16	20	80
20	5	95
22	5	95
25	95	5



Interface	ESI, Negative Mode
Interface Temperature	100 °C
Desolvation Line Temperature	160 °C
Heat Block Temperature	200 °C
Heating Gas Flow	15 L/min
Drying Gas Flow	5 L/min
Nebulizing Gas Flow	3 L/min
Total MRMs	66
Minimum Dwell Time	19 msec
Maximum Dwell Time	124 msec

Calibration

Standards available from Wellington Laboratories were used for these studies (EPA method analyte stock 2 mL volume in methanol at 1 ug/L, Internal standard in methanol Wellington Catalog No. 533-IS and Isotope Dilution Analogue PDS in Methanol Wellington Catalog No. 533-ES). These standards were then diluted to working standards as outlined in Section 7.17.5 of EPA Method 533 using 20% water in methanol as diluent to match the extract solvent composition. The working standards were used to create a calibration curve ranging from 1 ng/L to 1000 ng/L for NFDHA, and from 0.1 ng/L to 100 ng/L for all other analytes. During this study Initial Calibration curve was ran 5 consecutive days. Figure 3 shows aggregate calibration curve for PFMPA and PFPeA and Figure 4 shows aggregate calibration curve for PFDA and example MRL 0.1 ng/L chromatogram. The chromatogram shown in Figure 5 is from a level 7, 6 ng/L calibrator. Figure 6 shows a clean instrument blank for 80% MeOH:20% H2O, indicating that the system is free from PFAS contamination as no PFAS was detected.

Table 2. Target and labelled PFAS m/z, retention times, and correlation coefficients from the aggregate curve (Days 1-5)

ID#	Compound	MRL in vial (ng/mL)	MRL in sample (ng/L)	Type	ISTD Group#	m/z	RT	Collision	R2
1	M3PFBA	----	----	ISTD	3	216.00>172.00	5	10	----
2	MPFBA	----	----	Surrogate	1	217.00>172.00	5	10	----
3	PFBA	0.05	0.2	Target	1	212.90>168.90	5.18	10	0.9945
4	PFMPA	0.025	0.1	Target	1	229.00>85.00	6.2	10	0.9947
5	PFPeA	0.05	0.2	Target	1	263.00>219.00	7.95	8	0.9947
6	M5PFPeA	----	----	Surrogate	1	268.00>223.00	7.94	8	----
7	M3PFBS	----	----	Surrogate	1	302.00>80.00	8.54	34	----
8	PFBS	0.1	4	Target	2	298.90>80.10	8.55	30	0.9949
9	PFMBA	0.025	0.1	Target	1	279.00>85.00	8.72	20	0.994
10	PFEESA	0.025	0.1	Target	1	314.90>134.85	9.54	25	0.9958
11	NFDHA	5	20	Target	1	295.00>201.15	10.08	8	0.9982
12	M2-4-2 FTS	----	----	Surrogate	2	329.00>309.00	10.22	20	----
13	4-2 FTS	1	4	Target	2	327.00>307.00	10.2	18	0.9938
14	PFHxA	0.05	0.2	Target	1	312.90>269.00	10.48	8	0.9947
15	PFPeS	0.1	0.4	Target	2	349.00>80.00	10.82	9	0.9949
16	HFPO-DA	0.025	0.1	Target	1	285.00>169.00	11.21	42	0.9953
17	13C-HFPO-DA	----	----	Surrogate	1	287.00>169.20	11.21	8	----
18	PFHpA	0.025	0.1	Target	1	362.90>319.00	12.57	9	0.9942
19	M4PFHpA	----	----	Surrogate	1	367.00>322.00	12.57	10	----
20	M3PFHxS	----	----	Surrogate	2	402.00>80.00	12.75	9	----
21	PFHxS	0.1	0.4	Target	2	398.90>80.10	12.08	49	0.9965
22	ADONA	0.025	0.1	Target	1	377.00>250.90	12.8	43	0.9948
23	6-2 FTS	0.5	2	Target	2	427.00>407.00	14.12	11	0.9955
24	M2-6-2 FTS	----	----	Surrogate	2	429.00>409.00	14.14	22	----
25	M8PFOA	----	----	Surrogate	1	421.00>376.00	14.27	23	----
26	PFOA	0.1	0.4	Target	1	412.90>369.00	14.25	10	0.9944
27	M2PFOA	----	----	ISTD	1	415.00>370.00	14.28	10	----
28	PFHpS	0.1	0.4	Target	2	449.00>80.00	14.33	10	0.9952
29	PFNA	0.05	0.2	Target	1	462.90>418.90	15.76	51	0.9942
30	M8PFOS	----	----	Surrogate	3	507.00>80.00	15.75	12	----
31	M9PFNA	----	----	Surrogate	1	472.00>427.00	15.73	11	----
32	PFOS	0.05	0.2	Target	2	498.90>80.10	15.23	45	0.9952
33	M4PFOS	----	----	ISTD	2	503.00>80.00	15.76	45	----
34	9Cl-PF3ONS	0.025	0.1	Target	1	530.90>351.00	16.5	54	0.9954
35	8-2 FTS	1	4	Target	2	527.00>507.00	16.97	27	0.997
36	M2-8-2 FTS	----	----	Surrogate	2	529.00>509.00	16.98	26	----
37	PFDA	0.025	0.1	Target	1	512.90>468.90	17.04	26	0.9952
38	MPFHxA	----	----	Surrogate	1	318.00>273.00	10.48	12	----
39	PFUnA	0.025	0.1	Target	1	562.90>519.00	18.14	11	0.9948
40	M7PFUnA	----	----	Surrogate	3	570.00>525.00	18.11	12	----
41	11Cl-PF3OUdS	0.025	0.1	Target	1	630.70>451.00	18.63	12	0.9953
42	PFDoA	0.025	0.1	Target	1	612.90>568.90	19.06	30	0.9951
43	M2PFDoA	----	----	Surrogate	3	615.00>570.00	19.06	10	----
44	MPFDA	----	----	Surrogate	1	519.00>474.10	17.04	12	----

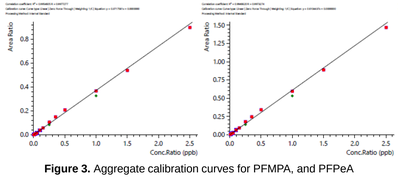


Figure 3. Aggregate calibration curves for PFMPA, and PFPeA

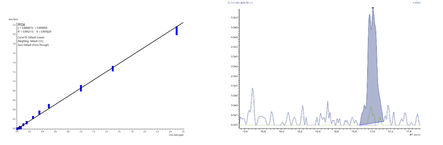


Figure 4. Aggregate calibration curve for PFDA and example MRL 0.1 ng/L chromatogram

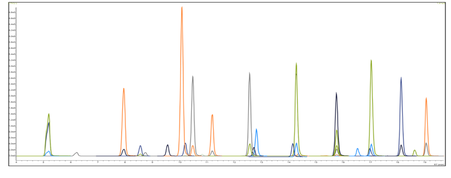


Figure 5. TIC of all 44 compounds at Level 7, 6 ng/L.

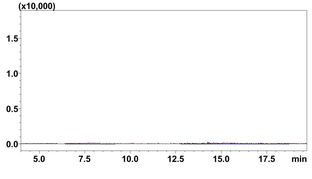


Figure 6. Blank: 80:20 MeOH: H2O.

Results

Method development for PFAS

The use of a Phenomenex Gemini™ C18, 2.0 mm ID × 50 mm, 3.0 µm particle size analytical column and a Shim-Pack XR-ODS 50 x 3.0 mm column as a delay column provided a good chromatographic separation for all compounds including branched and linear isomers. Calibration curves for PFAS analytes were prepared in the range of 0.025 – 25 ng/mL, representing pre-SPE sample concentrations of 0.1 – 100 ng/L (except for NFDHA which was analyzed from 0.25 – 250 ng/L). All calibration curves (aggregate curve and Day 1-5 individual curves) demonstrated r2 values greater than 0.99. All RSD results for the aggregate curve were less than 20%. All MRL level accuracies were between 50 – 150%. Accuracies at the MRL for each day (against the aggregate curve), and %RSDs are shown in Figure 7. Precision and accuracy studies in reagent water (RW) and tap water (TW) were performed at 10 ng/L and recoveries of majority of analytes were within 70-130% with %RSDs below 20% for all method analytes. The P & A study results were within EPA method 533 requirements; the data is included in Figure 8.

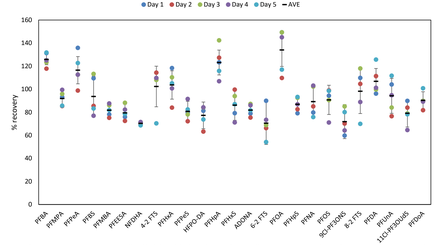


Figure 7. %recovery (individual injections from five consecutive days and average) at MRL.

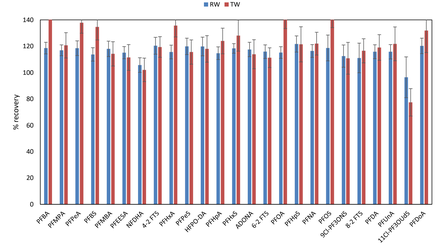


Figure 8. Precision and accuracy results/

Conclusions

This study showed good chromatographic separation for all compounds listed in the method using the delay and analytical columns recommended by EPA. Recoveries for most target compounds and precision and accuracy data for all target analytes in reagent water and tap water were within EPA requirements of 70 -130%, with %RSD below 20% for all method analytes. This data was generated as part of the EPA method 533 second laboratory validation organized by EPA. Shimadzu participated in this validation, as acknowledged in the final method.

References

(1) EPA Method 537 rev1.1, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (U.S. Environmental Protection Agency, Washington, D.C., Sept. 2009).

(2) Evoqua Water Technologies, Webinar, March 6, 2018

(3) 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(U.S. Environmental Protection Agency, Washington, D.C., December 2019).

(4) Shimadzu Application News No. C184, "Analysis of PFAS Specified in EPA Method 537 and Beyond Using Shimadzu UFMS", 2019