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EPA 533: Method Performance for the Analysis of Per-and Polyfluoroalkyl Substances (PFAS) by LC-MS/MS.

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1. Introduction

Method EPA 533 is the latest method published by the US Environmental Protection Agency for the analysis of per- and polyfluoroalkyl substances (PFAS) in drinking water. The list of targets in this method includes short-chain compounds that were not part of EPA 537 or EPA 537.1. Method 533 measures 25 PFAS by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS). Shimadzu Scientific Instruments was one of eight laboratories that participated in the external laboratory validation of the method for its publication by EPA. This poster includes Shimadzu's data from the validation study.

2. Sample preparation

Samples (laboratory reagent water and tap water) were processed exactly as outlined in EPA method 533 (section 6.8.1); sample preconcentration was performed with weak anionic exchange Solid Phase Extraction cartridges. Extraction for Precision & Accuracy 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.

3. 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 (Shimadzu LCMS-8045) (Figure 1). The chromatographic parameters are based on the chromatographic method described in EPA Method 533. A Shim-pack XR-ODS 50 x 3.0 mm column was used as a delay column (to minimize background PFAS contamination), 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 optimized MRMs and isotopic dilution. Instrumental conditions are included in Table 1 and MRM transitions are included in Table 2.

4. Calibration

Standards available from Wellington Laboratories were used for this study (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.

Figure 1. LCMS-8045 triple quadrupole mass spectrometer.

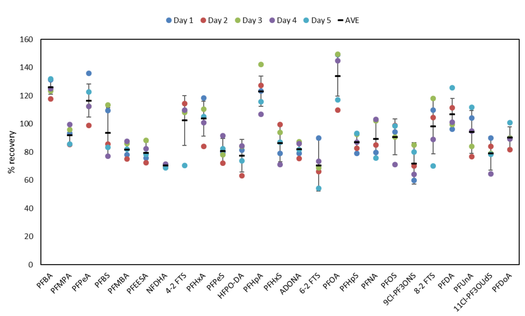
Table 1. Instrumental Conditions.

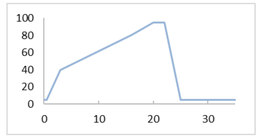
LCMS Instrument	Shimadzu LCMS-8045
Injection Volume	10 µL
LC Flow Rate	0.25 mL/min
Mobile Phase A	20 mM Ammonium Acetate in LCMS-grade Water
Mobile Phase B	Methanol
Run / Acquisition Cycle Time	35 minutes (all 44 PFAS compounds are eluted in 20 minutes)
Interface	ESI, Negative Mode
Interface Temperature	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

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 PFDA and example MRL 0.1 ng/L chromatogram. The chromatogram shown in Figure 4 is a standard with concentration 6 ng/L.

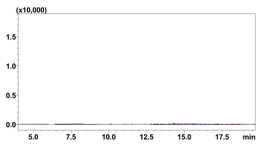
4. Results

A good chromatographic separation for all compounds including branched and linear isomers was achieved. All calibration curves (aggregate curve and 5 individual curves analyzed I 5 consecutive days) demonstrated r^2 values greater than 0.99. All RSD results for the aggregate curve were less than 20%. All MRL level accuracies were between 50 – 150%.





UHPLC gradient



Clean instrument blank (80:20 MeOH:H_2O)

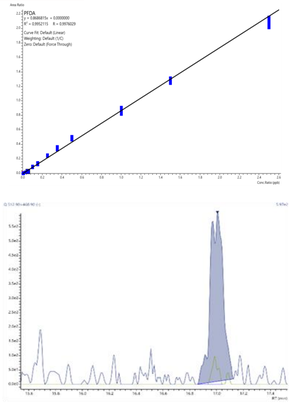


Figure 3. Aggregate calibration curve for PFDA (top); example MRL 0.1 ng/L chromatogram (bottom).

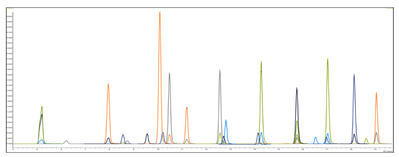


Figure 4. TIC of all 44 compounds at Level 7, 6 ng/L.

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 5.

Figure 5. %recovery (individual injections from five consecutive days and average) at MRL.

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

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

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 6.

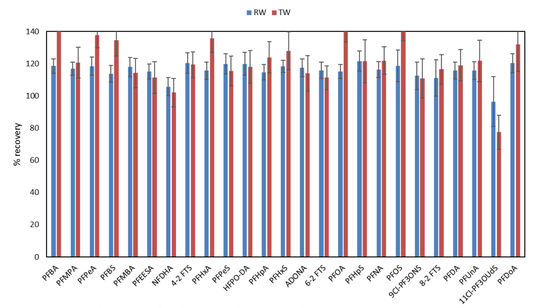


Figure 6. Precision and accuracy results.

5. Conclusions

This study demonstrates the performance of Shimadzu LCMS-8045 to meet the criteria outlined in method EPA 533 for the analysis of PFAS in drinking water. This data was generated as part of the EPA method 533 second laboratory validation organized by EPA.