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Considerations for Improved EPA Method 533 Accuracy and Precision for the Analysis of PFAS in a Production Laboratory Environment

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Introduction

The first official EPA method for Per- and Polyfluoroalkyl Substances (PFAS) was EPA 537 developed in 2009, in part to support the UCMR3 study for drinking water systems in the US. This method included 14 PFAS compounds, including both PFOS and PFOA, which were then considered to have potential impact on human health. In 2018, EPA 537.1 was introduced to include 4 of the "replacement" PFAS compounds which had replaced PFOA and PFOS in many manufacturing processes in the interim. In 2019, EPA released their PFAS Action Plan, which outlined the steps that the EPA proposed to take to identify and regulate PFAS in the environment.

The PFAS Action Plan called for the development and promulgation of new analytical methods that would allow scientists to effectively measure more PFAS compounds, with greater accuracy and precision. Published at the end of 2019, EPA 533 is the first of these new PFAS analytical methods. EPA 533 is complementary to EPA 537.1 and it analyzes 14 of the 18 compounds from EPA 537.1, plus an additional 11 "short chain" (C4-C12) PFAS compounds. Of the original EPA 537 and EPA 537.1 compounds, 4 were not included in EPA 533, since they had been shown not to be present in drinking water during the previous UCMR study. Of the new EPA 533 compounds, PFBA and PFPeA had been intentionally excluded from EPA 537.1 because they were too polar to be extracted by a styrene divinylbenzene (SDVB) solid phase extraction (SPE) sorbent from the sample preparation step.

However, EPA 533 was able to include these 2 compounds, along with the other short chain analytes because this new method employs a polymeric weak anion-exchange (WAX) sorbent in the SPE sample preparation step, which is selective for the more polar/acidic PFAS analytes. An additional distinction of EPA 533 is that it uses the isotope dilution technique to enhance method accuracy and robustness.

Table 1. EPA Method Comparison.

EPA 537.1	EPA 533
18 analytes	25 analytes (including 14 from 537.1 and 11 new short-chain compounds)
SDVB SPE sorbent	WAX SPE sorbent
Isotopic Internal Standards	Isotopic Internal Standards plus Isotope Dilution standards for each analyte

Materials and Methods

Sample Preparation

Step	Description
Sample Pre-treatment:	100-250 µL of sample was fortified with isotopically labeled analogues of the method analytes.
Condition:	Strata™-X-AW, 500 mg / 6 mL cartridge (8B-S038-HCH) with 10 mL of aqueous 0.1 M Phosphate buffer without allowing the water to drop below the top edge of the packing. Close the valve and add 2-3 mL of Phosphate buffer to the cartridge reservoir and fill the remaining voume with reagent water.
Load:	Pre-treated sample and pass through the cartridge.
Wash 1:	With aqueous Ammonium Acetate followed by Methanol. Rinse each cartridge with 10 mL of Methanol.
Wash 2:	With Methanol.
Elute:	With Ammonium Hydroxide in Methanol.
Dry Down:	Under a gentle stream of Nitrogen in a heated water bath.
Reconstitute:	To a final volume of 1 mL in 20 % Water in Methanol before analyzing by LC-MS/MS.

LC Conditions

Column: Gemini™ 3.0 µm C18
Dimension: 50 x 2.0 mm
Part No.: 00B-4439-B0
Mobile Phase: A: 20 mM Ammonium Acetate
B: Methanol

Gradient:

Time (min)	%B
0	5
0.5	5
3	40
16	80
18	80
20	95
22	95
25	5
35	5

Flow Rate: 0.25 mL/min
Injection Volume: 2 µL
Detection: ESI-MS/MS

Results

Table 2. Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References.

Isotopically Labeled Analyte	Retention Time (min)	Suggested Isotope Performance Standard
$^{13}C_4$-PFBA	4.14	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	6.13	$^{13}C_3$-PFBA
$^{13}C_3$-PFBS	6.62	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	8.12	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	8.35	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	9.06	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	10.34	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	10.61	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	12.05	$^{13}C_4$-PFOS
$^{13}C_8$-PFOA	12.19	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	13.70	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	13.73	$^{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	14.94	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	15.00	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	16.14	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	17.13	$^{13}C_2$-PFOA

Figure 1. Chromatogram from EPA Method 533.

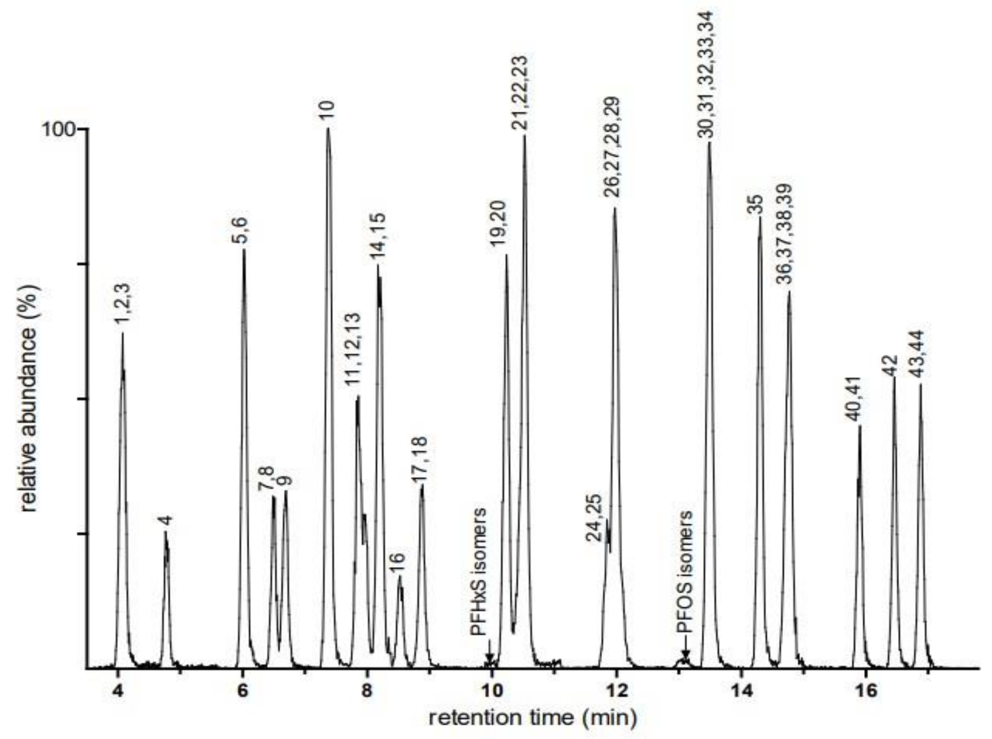

Table 3. Method Analytes, Retention Times, and Suggested Isotope Dilution Analogue References.

Analyte	Peak No.	Retention Time (min)	Isotope Dilution Analogue
PFBA	3	4.15	$^{13}C_4$-PFBA
PFMPA	4	4.84	$^{13}C_4$-PFBA
PFPeA	6	6.13	$^{13}C_5$-PFPeA
PFBS	8	6.62	$^{13}C_3$-PFBS
PFMBA	9	6.81	$^{13}C_5$-PFPeA
PFEESA	10	7.53	$^{13}C_3$-PFBS
NFDHA	11	8.01	$^{13}C_5$-PFHxA
4:2FTS	13	8.12	$^{13}C_2$-4:2FTS
PFHxA	15	8.36	$^{13}C_5$-PFHxA
PFPeS	16	8.69	$^{13}C_3$-PFHxS
HFPO-DA	18	9.06	$^{13}C_3$-HFPO-DA
PFHpA	20	10.42	$^{13}C_4$-PFHpA
PFHxS	22	10.62	$^{13}C_3$-PFHxS
ADONA	23	10.73	$^{13}C_4$-PFHpA
6:2FTS	25	12.04	$^{13}C_2$-6:2FTS
PFOA	28	12.19	$^{13}C_8$-PFOA
PFHpS	29	12.28	$^{13}C_8$-PFOS
PFNA	31	13.70	$^{13}C_9$-PFNA
PFOS	34	13.74	$^{13}C_8$-PFOS
9Cl-PF3OUdS	35	14.53	$^{13}C_8$-PFOS
8:2FTS	37	14.94	$^{13}C_2$-8:2FTS
PFDA	39	15.00	$^{13}C_6$-PFDA
PFUnA	41	16.14	$^{13}C_7$-PFUnA
11Cl-PF3OUdS	42	16.70	$^{13}C_8$-PFOS
PFDoA	44	17.13	$^{13}C_2$-PFDoA

Results

Table 4. Precision and Accuracy Data for Reagent Water.

Analyte	Low Fortification (ng/L)	Mean % R_a (N=7)	%RSD_a	High Fortification (ng/L)	Mean % R (N=5)	%RSD
PFBA	10	128	8.6	80	98.4	2.4
PFMPA	10	108	4.5	80	98.1	2.2
PFPeA	10	107	4.9	80	99.6	3.6
PFBS	10	102	9.1	80	96.2	2.9
PFMBA	10	111	6.8	80	101	3.4
PFEESA	10	107	10	80	98.8	4.0
NFDHA	10	110	15	80	98.5	5.4
4:2FTS	10	94.4	14	80	100	5.7
PFHxA	10	102	8.0	80	97	7.7
PFPeS	10	99.5	19	80	101	7.8
HFPO-DA	10	102	9.7	80	102	4.7
PFHpA	10	108	7.0	80	104	4.1
PFHxS	10	103	9.0	80	97.7	5.5
ADONA	10	96.3	3.1	80	96.8	5.6
6:2FTS	10	109	15	80	111	11.0
PFOA	10	108	7.4	80	98.5	6.9
PFHpS	10	98.8	8.9	80	102	7.0
PFNA	10	109	6.2	80	99.6	5.6
PFOS	10	104	8.7	80	98.0	4.3
9Cl-PF3OUdS	10	99.7	4.6	80	103	6.8
8:2FTS	10	100	17	80	100	13.0
PFDA	10	100	4.2	80	100	1.8
PFUnA	10	102	10	80	97.3	8.1
11Cl-PF3OUdS	10	106	5.3	80	102	6.1
PFDoA	10	101	6.2	80	96.3	5.1

Table 5. Precision and Accuracy Data from a Commercial Laboratory.

Analyte	MS %	MSD %	BS %	BSD %
PFBA	102	116	89	95
PFMPA	99	100	108	117
PFPeA	97	96	92	88
PFBS	117	106	97	105
PFMBA	106	101	111	118
PFEESA	116	109	119	115
NFDHA	117	126	117	114
4:2FTS	113	104	109	100
PFHxA	113	107	91	95
PFPeS	86	99	103	104
HFPO-DA	100	97	110	101
PFHpA	112	115	94	97
PFHxS	96	101	108	110
ADONA	118	116	111	99
6:2FTS	94	96	108	102
PFOA	101	104	101	100
PFHpS	119	117	119	114
PFNA	107	104	105	110
PFOS	117	115	108	108
9Cl-PF3OUdS	101	107	99	119
8:2FTS	97	100	89	101
PFDA	102	99	112	104
PFUnA	105	103	115	113
11Cl-PF3OUdS	85	84	95	86
PFDoA	104	107	108	109

Discussion and Conclusion

In **Table 1**, the EPA methods are compared to show where they differ. **Tables 2** and **3** outline the specifics for the analytes in EPA Method 533 and then the suggested isotopes in relation to each. Specified retention times (RT) are also mentioned for each of the analytes. In **Table 4**, the acceptable precision and accuracy data is presented. In **Table 5** the data is displayed from an actual laboratory example that displays the results of how a laboratory implements EPA 533 and in **Figure 1** all necessary peaks from the specified method are shown in the example chromatogram. These data demonstrate that EPA Method 533 using Strata-X-AW SPE for clean-up and a Gemini C18 column for analysis provide accurate and sufficient results for a commercial laboratory running this method.

EPA 533 is a significant improvement over EPA 537.1 for the analysis of PFAS in drinking water. This new method eliminates the 4 compounds from the EPA 537.1 analyte list that were not detected over the 10-year period that EPA 537.1 was being used to monitor these compounds. The 11 compounds that were not included in EPA 537.1 include many of the "replacement" compounds that are currently being used in the manufacturing of products that utilize PFAS chemistry. This makes EPA 533 a much more relevant environmental method. Furthermore, EPA 533 is a more robust analytical method owing to the use of the isotope dilution technique which provides a means to correct for the loss of analytes during sample preparation step, as well as to offset the potential effects of ion suppression or enhancement arising from matrix variation.

However, there are a few specific requirements in this method that the analyst must carefully follow. The SPE sorbent mass (in mg) must be at least 2x the sample volume (in mL) to prevent potential overloading of the sorbent. In addition, the SPE media must meet the following specifications listed in the method:

- Approximately 33 µm particle size
- Employ a mixed-mode polymeric sorbent mechanism (polymeric backbone and a diamino ligand functional group)
- Display a pKa above 8 so that the SPE media remains positively charged during extraction

Strata-X-AW meets all these requirements and was found to show excellent performance in EPA 533 during routine laboratory operation as demonstrated by the performance data presented above. In addition, both the Strata-X-AW SPE sorbent and the Gemini 3 µm C18 HPLC column were used in the development of EPA 533 and its validation.

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