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EPA Method 533: PFAS in Drinking Water

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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. 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 very 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.

Materials and Methods

The following is a summary of the prescribed experimental conditions taken from EPA 533. It should be noted that Strata™-X-AW and Gemini™ 3 µm C18 were the respective SPE sorbent and the LC column used in the development of EPA 533 and in its subsequent multi-laboratory validation.

Sample Preparation Protocol

Pre-treatment: 100-250 mL sample is fortified with isotopically labeled analogues of the method analytes
Cartridge: Strata-X-AW 500 mg/6 mL
Part No.: 8B-S038-HCH
Load: Pass pre-treated sample through the cartridge
Wash 1: Aqueous Ammonium acetate followed by Methanol
Wash 2: Methanol
Elute: Ammonium hydroxide in Methanol
Dry Down: Under a gentle stream of Nitrogen in a heated water bath
Reconstitute: Adjust the final volume to 1 mL with 20 % Water in Methanol (v/v) before analyzing by LC-MS

LC Conditions

Column: Gemini 3 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

Injection Volume: 2 µL
Flow Rate: 0.25 mL/min
MS Detection: Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)

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

Results

Table 2.
Isotopically Labeled Isotope Performance Standards and Retention Times

Isotopes Analytes	RT (min)
$^{13}C_3$-PFBA	4.14
$^{13}C_2$-PFOA	12.19
$^{13}C_4$-PFOS	13.73

Table 3.
Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References

Isotopically Labeled Analyte	RT (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

Table 4.
Method Analytes, Retention Times, and Suggested Isotope Dilution Analogue References

Analyte	Peak No. (Figure 1)	RT (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-PF3ONS	35	14.53	$^{13}C_8$-PFOS
8:2 FTS	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

Table 5.
Precision and Accuracy Data for Reagent Water

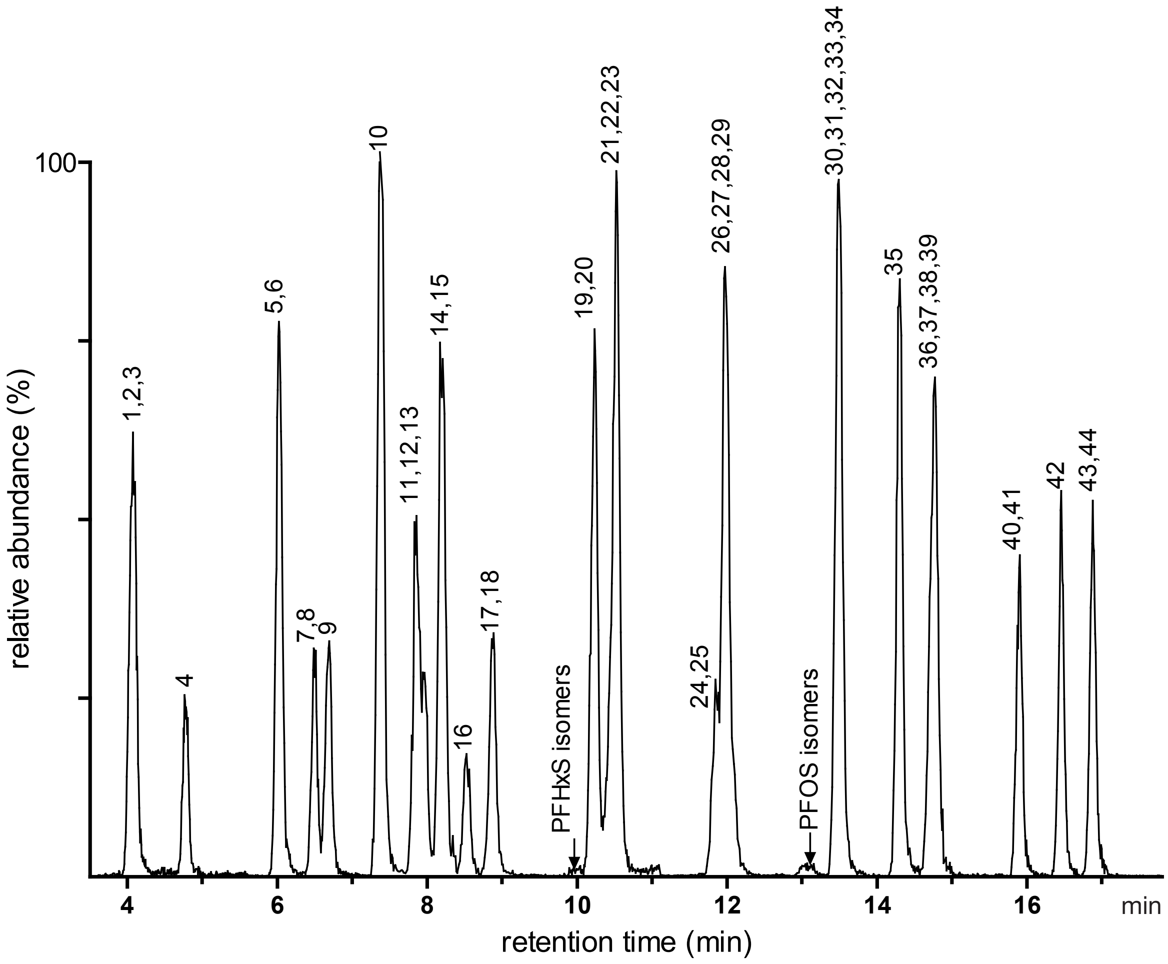
Analyte	Low Fortification (ng/L)	Mean % R[a] (n=7)	% RSD[a]	High Fortification (ng/L)	Mean % R (n=5)	% RSD
PFBA	10	128	8.6	80	98.4	2.4
PFMPA	10	108	4.5	80	98.1	2.2
PFPeA	10	107	4.9	80	99.6	3.6
PFBS	10	102	9.1	80	96.2	2.9
PFMBA	10	111	6.8	80	101	3.4
PFEESA	10	107	10	80	98.8	4.0
NFDHA	10	110	15	80	98.5	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
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-PF3ONS	10	99.7	4.6	80	103	6.8
8:2FTS	10	100	17	80	100	13
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 6.
EPA 533 Precision and Accuracy Data from a Commercial Laboratory

Analyte	MS	MSD	BS	BSD
11Cl-PF3OUdS	85 %	84 %	95 %	86 %
4-2FTS	113 %	104 %	109 %	100 %
6-2 FTS	94 %	96 %	108 %	102 %
8-2 FTS	97 %	100 %	89 %	101 %
9Cl-PF3OUdS	101 %	107 %	99 %	119 %
ADONA	118 %	116 %	111 %	99 %
HFPO-DA	100 %	97 %	110 %	101 %
NFDHA	117 %	126 %	117 %	114 %
PFBA	102 %	116 %	89 %	95 %
PFBS	117 %	106 %	97 %	105 %
PFDA	102 %	99 %	112 %	104 %
PFDoA	104 %	107 %	108 %	109 %
PFEESA	116 %	109 %	119 %	115 %

Analyte	MS	MSD	BS	BSD
PFHpA	112 %	115 %	94 %	97 %
PFHpS	119 %	117 %	119 %	114 %
PFHxA	113 %	107 %	91 %	95 %
PFHxS	96 %	101 %	108 %	110 %
PFMBA	106 %	101 %	111 %	118 %
PFMPA	99 %	100 %	108 %	117 %
PFNA	107 %	104 %	105 %	110 %
PFOA	101 %	104 %	101 %	100 %
PFOS	117 %	115 %	108 %	108 %
PFPeA	97 %	96 %	92 %	88 %
PFPeS	86 %	99 %	103 %	104 %
PFUnA	105 %	103 %	115 %	113 %

Figure 1.
Chromatogram from EPA Method 533



Discussion

In this application, the method is outlined for both the SPE method and the HPLC conditions. In **Table 1**, the EPA methods are compared to show where they differ. **Tables 2-4** 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 5**, the acceptable precision and accuracy data is presented and then in **Table 6** the data is displayed from an actual laboratory example that is 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. This data provided proves that EPA Method 533 using Strata™-X-AW SPE for clean-up and a Gemini™ C18 column for analysis provides accurate and sufficient results for a commercial laboratory running this method.

Conclusion

EPA 533 is a significant improvement over EPA 537.1 for the analysis of PFAS in Drinking Water. It 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. However, it also includes the addition of 11 new PFAS compounds that were not included in EPA 537.1 which are believed to be of greater environmental significance. These 11 compounds 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 much 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 offsetting the potential effects of ion suppression or enhancement arising from matrix variation. Consequently, EPA 533 will play a critical role in the UCMR5 cycle beginning in 2021 to assess the safety of US public drinking water systems. In this way, EPA 533 will play an essential role in the EPA PFAS Action Plan, potentially leading to official PFAS drinking water regulations.

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. Therefore, a 100 mL sample must be extracted with an SPE mass of at least 200 mg, a 250 mL sample must use an SPE sorbent mass of at least 500 mg and so forth. 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 pK_a 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, as has also been noted, 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. Understandably, both products are now widely employed in environmental laboratories for the routine analysis of PFAS by EPA 533.

Acknowledgement

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Reference

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’ (2019) https://www.epa.gov/sites/production/files/2019-12/documents/method-533-815b19020.pdf

Ordering Information

Gemini™ LC Columns

3 µm Microbore, Minibore and MidBore™ Columns (mm)									SecurityGuard™ Cartridges (mm)
Phases	**50 x 1.0**	**20 x 2.0**	**30 x 2.0**	**50 x 2.0**	**100 x 2.0**	**150 x 2.0**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
										/10pk
Gemini C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

for ID: 2.0-3.0 mm

Strata™-X-AW Solid Phase Extraction

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S038-TAK**	1 mL (100/box)
	30 mg	8B-S038-TBJ	3 mL (50/box)
	60 mg	8B-S038-UBJ	3 mL (50/box)
	100 mg	8B-S038-EBJ	3 mL (50/box)
	100 mg	8B-S038-ECH	6 mL (30/box)
	200 mg	8B-S038-FBJ	3 mL (50/box)
	200 mg	8B-S038-FCH	6 mL (30/box)
	500 mg	8B-S038-HBJ	3 mL (50/box)
	500 mg	8B-S038-HCH	6 mL (30/box)

*Tab-less tubes available. Contact Phenomenex for details.

PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

Product	PN	Qty.	Conc.
EPA 533 mix	AL0-101838	1 mL	2 µg / mL in methanol
EPA 537.1 mix	AL0-101839	1 mL	2 µg / mL in methanol
EPA 533 + 537.1 mix	AL0-101840	1 mL	2 µg / mL in methanol

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Description	Part No.
Luna™ Omega Column 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm	00D-4633- AN
Strata™ PFAS (WAX/GCB) SPE 200 mg, /50 mg, /6 mL tubes, 30/pk	CS0-9207
Strata SDB-L SPE 500 mg/6 mL tubes, 30/pk	8B-S014-HCH
Verex™ Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk	AR0-39P0-13
Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk	AR0-39P2-13
Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk	AR0-39P1-13
Vial Cap Verex™ Cert+ Cap (one-piece), 9 mm, PE w/ Starburst pre-Slit, 2 mL, 1000/pk	AR0-89P6-13-C

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Strata-X is patented by Phenomenex. U.S. Patent No. 7,119,145

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Have questions or want more details on implementing this method? We would love to help!
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