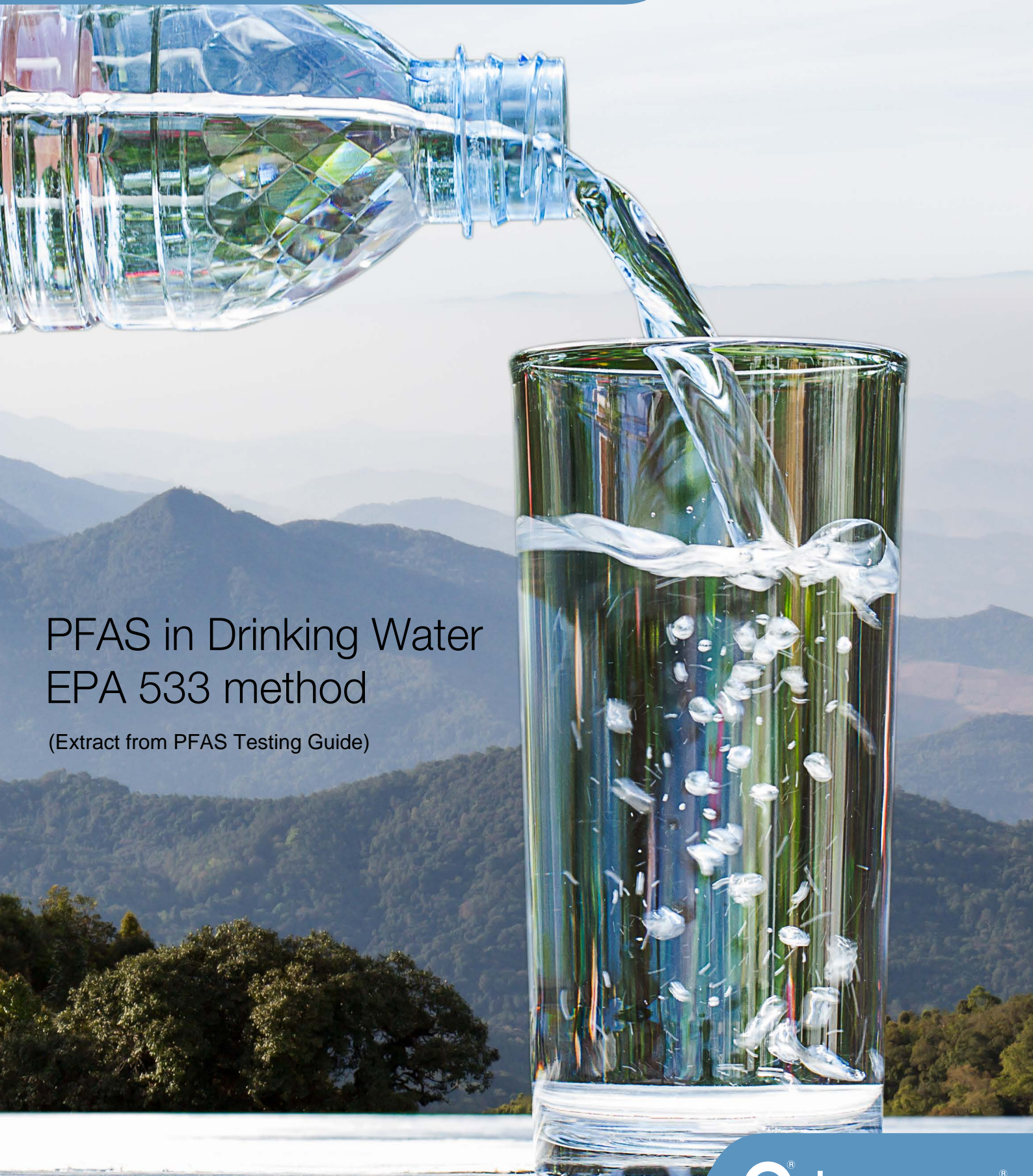


# Perfluoroalkyl Substances (PFAS) Application



## PFAS in Drinking Water EPA 533 method

(Extract from PFAS Testing Guide)

# 3. 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 during 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 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

**Flow Rate:** 0.25 mL/min  
**Injection Volume:** 2 µL  
**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

# 3. EPA Method 533 (continued)

## Results

**Table 2.**  
Isotopically Labeled Isotope Performance Standards and Retention Times

Isotopes Analytes	RT (min)
<sup>13</sup> C <sub>3</sub> -PFBA	4.14
<sup>13</sup> C <sub>2</sub> -PFOA	12.19
<sup>13</sup> C <sub>4</sub> -PFOS	13.73

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

Isotopically Labeled Analyte	RT (min)	Suggested Isotope Performance Standard
<sup>13</sup> C <sub>4</sub> -PFBA	4.14	<sup>13</sup> C <sub>3</sub> -PFBA
<sup>13</sup> C <sub>5</sub> -PFPeA	6.13	<sup>13</sup> C <sub>3</sub> -PFBA
<sup>13</sup> C <sub>3</sub> -PFBS	6.62	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>2</sub> -4:2FTS	8.12	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>5</sub> -PFHxA	8.35	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>3</sub> -HFPO-DA	9.06	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>4</sub> -PFHpA	10.34	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>3</sub> -PFHxS	10.61	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>2</sub> -6:2FTS	12.05	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>6</sub> -PFOA	12.19	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>9</sub> -PFNA	13.70	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>8</sub> -PFOS	13.73	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>2</sub> -8:2FTS	14.94	<sup>13</sup> C <sub>4</sub> -PFOS
<sup>13</sup> C <sub>6</sub> -PFDA	15.00	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>7</sub> -PFUnA	16.14	<sup>13</sup> C <sub>2</sub> -PFOA
<sup>13</sup> C <sub>2</sub> -PFDoA	17.13	<sup>13</sup> C <sub>2</sub> -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	<sup>13</sup> C <sub>4</sub> -PFBA
PFMPA	4	4.84	<sup>13</sup> C <sub>4</sub> -PFBA
PFPeA	6	6.13	<sup>13</sup> C <sub>5</sub> -PFPeA
PFBS	8	6.62	<sup>13</sup> C <sub>3</sub> -PFBS
PFMBA	9	6.81	<sup>13</sup> C <sub>5</sub> -PFPeA
PFEESA	10	7.53	<sup>13</sup> C <sub>3</sub> -PFBS
NFDHA	11	8.01	<sup>13</sup> C <sub>5</sub> -PFHxA
4:2FTS	13	8.12	<sup>13</sup> C <sub>2</sub> -4:2FTS
PFHxA	15	8.36	<sup>13</sup> C <sub>5</sub> -PFHxA
PFPeS	16	8.69	<sup>13</sup> C <sub>3</sub> -PFHxS
HFPO-DA	18	9.06	<sup>13</sup> C <sub>3</sub> -HFPO-DA
PFHpA	20	10.42	<sup>13</sup> C <sub>4</sub> -PFHpA
PFHxS	22	10.62	<sup>13</sup> C <sub>3</sub> -PFHxS
ADONA	23	10.73	<sup>13</sup> C <sub>4</sub> -PFHpA
6:2FTS	25	12.04	<sup>13</sup> C <sub>2</sub> -6:2FTS
PFOA	28	12.19	<sup>13</sup> C <sub>6</sub> -PFOA
PFHpS	29	12.28	<sup>13</sup> C <sub>8</sub> -PFOS
PFNA	31	13.70	<sup>13</sup> C <sub>9</sub> -PFNA
PFOS	34	13.74	<sup>13</sup> C <sub>8</sub> -PFOS
9CI-PF3ONS	35	14.53	<sup>13</sup> C <sub>6</sub> -PFOS
8:2 FTS	37	14.94	<sup>13</sup> C <sub>2</sub> -8:2FTS
PFDA	39	15.00	<sup>13</sup> C <sub>6</sub> -PFDA
PFUnA	41	16.14	<sup>13</sup> C <sub>7</sub> -PFUnA
11CI-PF3OUdS	42	16.70	<sup>13</sup> C <sub>6</sub> -PFOS
PFDoA	44	17.13	<sup>13</sup> C <sub>2</sub> -PFDoA

### 3. EPA Method 533 (continued)

**Table 5.**  
Precision and Accuracy Data for Reagent Water

Analyte	Low Fortification (ng/L)	Mean % R <sub>a</sub> (n=7)	% RSD <sub>a</sub>	High Fortification (ng/L)	Mean % R <sub>a</sub> (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
PFEEESA	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

# 3. EPA Method 533 (continued)

**Table 6.**  
EPA 533 Precision and Accuracy Data from a Commercial Laboratory

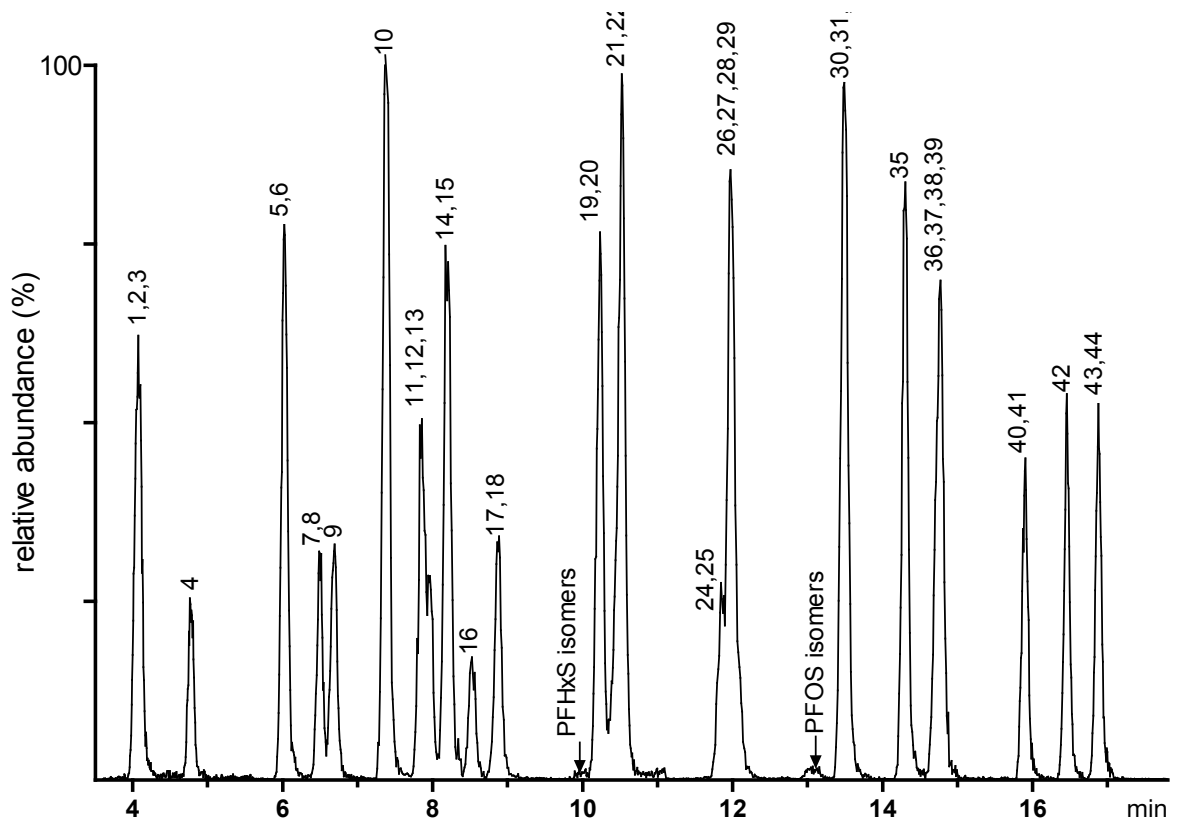
Analyte	MS %	MSD %	BS %	BSD %
11Cl-PF30UdS	85	84	95	86
4-2FTS	113	104	109	100
6-2 FTS	94	96	108	102
8-2 FTS	97	100	89	101
9Cl-PF30UdS	101	107	99	119
ADONA	118	116	111	99
HFPO-DA	100	97	110	101
NFDHA	117	126	117	114
PFBA	102	116	89	95
PFBS	117	106	97	105
PFDA	102	99	112	104
PFDoA	104	107	108	109
PFEESA	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%

Continued in next column

**Figure 1.**  
Chromatogram from EPA Method 533



## 3. EPA Method 533 (continued)

### 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. In **Table 6** 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<sup>®</sup>-X-AW SPE for clean-up and a Gemini<sup>®</sup> C18 column for analysis provide 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. 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. However, it also i.e. features 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 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. 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. To illustrate, 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>