

# PFAS Determination in Water According to EPA 1633

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## Abstract

Per- and Polyfluoroalkyl Substances (PFAS), known for their environmental persistence and potential health impacts, are a significant concern in water quality management. This study demonstrates the utilization of the EPA Method 1633 to analyze and quantify 40 PFAS chemicals in water samples. The method utilizes Liquid Chromatography combined with Tandem Mass Spectrometry (LC-MS/MS) after a Solid-Phase Extraction (SPE) using Supelclean™ ENVI-WAX™ SPE cartridges and cleanup by loose Supelclean™ ENVI-Carb™ adsorbent. The procedure allows for highly sensitive detection and quantitation of PFAS analytes in various matrices. Performance evaluation demonstrated that recoveries for all 40 PFAS compounds and 24 isotopically labeled standards (EIS) were within the typical EPA acceptable range of 70-130% with low corresponding standard deviations, indicating high precision and robustness. The method's suitability for comprehensive PFAS analysis in environmental samples underscores its value for regulatory compliance and environmental monitoring.

## Introduction

Per- and Polyfluoroalkyl Substances (PFAS), also referred to as "forever chemicals," are a broad category of more than 4700 synthetic fluorinated aliphatic compounds that have been applied in consumer goods since the 1950s. Known for their resistance to lipids and water, PFAS are highly stable due to the strong carbon-fluorine bond. They are commonly used as surface-active agents in products like stain repellents and firefighting foams. However, they persist in the environment due to their slow degradation rate.

With their widespread use in various consumer and commercial applications, PFAS have drawn significant attention regarding the contamination of water, soil,

and even the bloodstreams of humans and animals. This emphasizes their persistence and impact on environmental and human health. The United States Environmental Protection Agency (US EPA) and the European Union (EU) played key roles in developing stringent regulatory guidelines for PFAS testing, which are essential for protecting ecosystems and human populations.<sup>1,2,3</sup>

The US EPA adopted a comprehensive strategy, as demonstrated by the development of established analytical methods such as EPA Methods 533 and 537.1 for drinking water, to thoroughly evaluate PFAS levels in various environmental samples. At the same time, the EU has implemented strict regulations under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) framework.<sup>4</sup> These regulations use advanced analytical tools such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) to monitor PFAS in different chemical substances, showing a dedication to improving analytical capabilities and safeguarding the environment and human health.

The EPA Method 1633<sup>1</sup> is a laboratory-validated approach that employs LC-MS/MS to analyze aqueous, solid, biosolid, and tissue samples for 40 PFAS across nine compound classes. This "performance-based" method allows for condition adjustments to improve performance if all requirements are met, providing a stable platform for precise calibration and quantification of PFAS analytes using isotopically labeled standards. With the inclusion of analytes from EPA drinking water Methods 533 and 537.1, EPA Method 1633 tackles emerging PFAS classes, filling important voids in testing consistency, scope, sensitivity, and applicability across different sample types, helping to address the complex challenges posed by PFAS contaminations and ensuring public health and environmental protection.

The objective of this application note is to demonstrate the determination of 40 PFAS analytes from PFAS-spiked water samples by Solid-Phase Extraction (SPE) using Supelclean™ ENVI-WAX™ SPE cartridges on a PTFE-free Visiprep™ SPE vacuum manifold, followed by extract cleanup with loose Supelclean™ ENVI-Carb™ adsorbent and LC/MS-MS analysis employing Fused-Core® Ascentis® Express PFAS columns (analytical and delay). Obtained method performance characteristics are compared to the criteria stated in EPA 1633.

## Experimental

### Solutions and Standards Preparation

The sample collection and preparation followed the EPA Method 1633 procedure.

Native (40) and isotopically (31) labeled PFAS standards were used as methanolic 50 µg/mL stock solutions. The labeled compounds are either used as extracted internal standards (EIS, 24 compounds) or non-extracted internal standards (NIS, 7 compounds). Following the recommendations of the EPA 1633 method, these stock solutions were used to prepare seven calibration solutions containing the native PFAS compounds in various concentrations (CS1-CS7; compound specific concentrations ranging from 0.2-5 ng/mL for CS1 to 62.5-1560 ng/mL for CS7 as indicated in **Table 4** of the EPA 1633 method) to cover the working range of the MS instrument.

### Sample Preparation

#### Method Performance Assessment

In accordance with the EPA 1633 method, the method performance was investigated for water samples. 500 mL of water (tap fresh water from a Milli-Q® system) was collected in HDPE bottles with liner-less polypropylene caps. The water sample was fortified at three different levels (2 x CS1, 12.5 x CS1, 40 x CS1) with 40 native PFAS and spiked with 24 isotopically (<sup>13</sup>C or D) labeled standards (extracted internal standards – EIS) according to EPA 1633 (**Table 3**). For the extraction by SPE, Supelclean™ ENVI-WAX™ SPE tubes (500 mg/6 mL, **54057-U**) were equipped with large volume SPE reservoirs (25 mL, **54258-U**) and placed on a PTFE free Visiprep™ vacuum manifold (**57030-U**). The tubes were conditioned with 15 mL of 1.0% NH<sub>4</sub>OH in MeOH and equilibrated with 5 mL of aqueous 0.3 M formic acid. After the water sample (500 mL) was loaded and passed through the cartridge, 2 x 5 mL of water and 5 mL of 0.1 M formic acid/methanol (1:1 (v/v)) were added as a washing step. The cartridge was subsequently dried for 1 min before 5 mL 1.0% NH<sub>4</sub>OH in MeOH was used to elute the analytes. For further clean-up 25 µL concentrated acetic acid and approximately 10 mg loose/bulk Supelclean™ ENVI-Carb™ adsorbent (**57210-U**) were added to the eluate and mixed for less than 5 minutes using a vortex shaker, followed by centrifugation for 10 minutes at 4000 g. Subsequently, the supernatants were filtered using Millex® Nylon 0.2 µm syringe filters (**SLGNX13**)

into collection tubes containing additional 7 isotopically (<sup>13</sup>C or <sup>18</sup>O) labeled standards (non-extracted internal standards – NIS) prior to LC-MS/MS analysis. The sample preparation represents an enrichment of 1:100 from the original water sample to the final extract.

### Instrumental Analysis

LC-MS/MS analysis was performed using an Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer (**Table 1**). Chromatographic separation was achieved using an Ascentis® Express PFAS 90 Å (2.7 µm, 5 cm x 2.1 mm, **53557-U**) analytical column. In addition, an Ascentis® Express PFAS Delay 90 Å column (2.7 µm, 5 cm x 3.0 mm, **53572-U**) was installed after the mixing valve and before the autosampler to offset PFAS contamination potentially originating from the LC system (e.g. pump, tubings, fittings, filters). Polypropylene snap cap vials were used instead of standard glass vials to avoid possible PFAS adsorption to the glass surface.

For the quantification of the native PFAS based on stable-isotope dilution, the MRM transitions shown in **Table 2** were used. The EIS compounds were allocated in accordance with what is stated in EPA 1633.

**Table 1. LC Conditions used for analysis of 40 PFAS compounds**

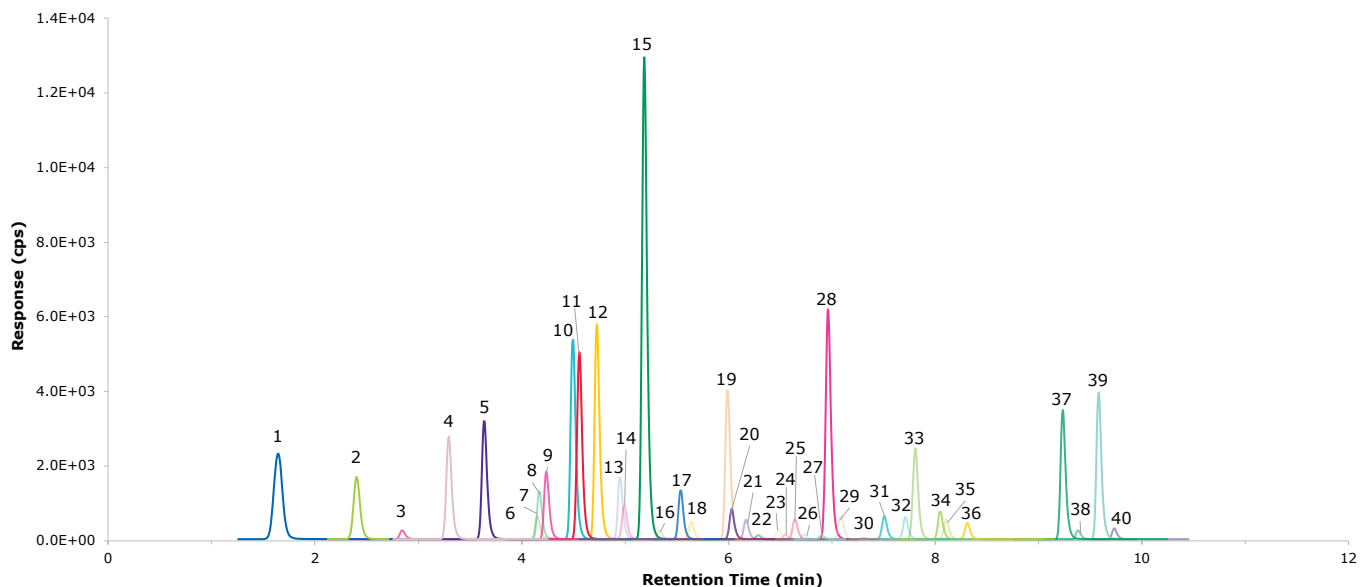
LC Conditions				
<b>Instrument:</b>	Agilent 1290 Infinity II LC system and Agilent 6495C triple quadrupole mass spectrometer			
<b>Columns:</b>	Ascentis® Express PFAS 90 Å, 2.7 µm, 5 cm x 2.1 mm ( <b>53557-U</b> ) Delay Column: Ascentis® Express PFAS Delay 90 Å, 2.7 µm, 5 cm x 3.0 mm ( <b>53572-U</b> )			
<b>Mobile phase:</b>	[A] 2 mM Ammonium acetate in 95:5 water/ acetonitrile (v/v); [B] Acetonitrile			
<b>Gradient:</b>	<b>Time (min)</b>	<b>A%</b>	<b>B%</b>	<b>Flow rate (mL/min)</b>
	0.0	98.0	2.0	0.35
	0.2	98.0	2.0	0.35
	4.0	70.0	30.0	0.40
	7.0	45.0	55.0	0.40
	9.0	25.0	75.0	0.40
	10.0	5.0	95.0	0.40
	10.4	98.0	2.0	0.40
	11.8	98.0	2.0	0.40
	12.0	98.0	2.0	0.35
<b>Flowrate:</b>	See gradient table			
<b>Pressure:</b>	195 bar			
<b>Column temp.:</b>	40 °C			
<b>Detector:</b>	MS (ESI-), MRM (see <b>Table 2</b> for details)			
<b>Injection:</b>	2 µL			
<b>Sample(s):</b>	See text			

**Table 2. MRM (ESI-) chromatographic and linearity (R<sup>2</sup>, 1/x weighting, 6 calibrants for FTS compounds and 7 for all other) data for the 40 native PFAS analytes**

Peak	Acronym	Compound	MRM Transition (Quantifier)	Collision energy (eV)	RT (min)	R <sup>2</sup>
1	PFBA	Perfluorobutanoic acid	213.0 -> 169.0	8	1.6	0.9998
2	PFMPA	Perfluoro-3-methoxypropanoic acid	229.0 -> 85.0	12	2.4	0.9997
3	3:3FTCA	3-Perfluoropropyl propanoic acid	241.0 -> 177.0	3	2.8	0.9992
4	PFPeA	Perfluoropentanoic acid	263.0 -> 219.0	4	3.3	0.9999
5	PFMBA	Perfluoro-4-methoxybutanoic acid	279.0 -> 85.0	8	3.6	0.9999
6	4:2FTS	1 <i>H</i> ,1 <i>H</i> ,2 <i>H</i> ,2 <i>H</i> -Perfluorohexane sulfonic acid	327.0 -> 307.0	20	4.0	0.9962
7	NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	295.0 -> 201.0	4	4.1	0.9990
8	PFBS	Perfluorobutanesulfonic acid	299.0 -> 80.0	48	4.2	0.9982
9	PFHxA	Perfluorohexanoic acid	313.0 -> 269.0	4	4.2	0.9989
10	HFPO-DA	Hexafluoropropylene oxide dimer acid	285.0 -> 169.0	4	4.5	0.9998
11	PFEESA	Perfluoro (2-ethoxyethane) sulfonic acid	315.0 -> 135.0	28	4.6	0.9978
12	5:3FTCA	2 <i>H</i> ,2 <i>H</i> ,3 <i>H</i> ,3 <i>H</i> -Perfluorooctanoic acid	341.0 -> 237.0	11	4.7	0.9990
13	PFHpA	Perfluoroheptanoic acid	363.0 -> 319.0	8	5.0	0.9996
14	PFPeS	Perfluoropentanesulfonic acid	349.0 -> 80.0	44	5.0	0.9988
15	ADONA	4,8-Dioxa-3 <i>H</i> -perfluorononanoic acid	377.0 -> 251.0	8	5.2	0.9998
16	6:2FTS	1 <i>H</i> ,1 <i>H</i> ,2 <i>H</i> ,2 <i>H</i> -Perfluorooctane sulfonic acid	427.0 -> 407.0	24	5.3	0.9926
17	PFOA	Perfluorooctanoic acid	413.0 -> 369.0	8	5.5	0.9999
18	PFHxS	Perfluoropentanesulfonic acid	399.0 -> 80.0	52	5.6	0.9991
19	7:3FTCA	3-Perfluoroheptyl propanoic acid	441.0 -> 337.0	11	6.0	0.9990
20	PFNA	Perfluoronanoic acid	463.0 -> 419.0	8	6.0	0.9999
21	PFHpS	Perfluoroheptanesulfonic acid	449.0 -> 80.0	68	6.2	0.9981
22	8:2FTS	1 <i>H</i> ,1 <i>H</i> ,2 <i>H</i> ,2 <i>H</i> -Perfluorodecane sulfonic acid	527.0 -> 507.0	28	6.3	0.9982
23	PFDA	Perfluorodecanoic acid	513.0 -> 469.0	8	6.5	0.9998
24	NMeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	570.0 -> 419.0	19	6.6	0.9998
25	PFOS	Perfluorooctanesulfonic acid	499.0 -> 80.0	72	6.6	0.9976
26	NEtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	584.0 -> 419.0	19	6.7	0.9995
27	PFUnA	Perfluoroundecanoic acid	563.0 -> 519.0	8	6.9	0.9992
28	9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	531.0 -> 351.0	28	7.0	0.9975
29	PFNS	Perfluorononanesulfonic acid	549.0 -> 80.0	79	7.1	0.9980
30	PFDoA	Perfluorododecanoic acid	613.0 -> 569.0	8	7.3	0.9985
31	PFDS	Perfluorodecanesulfonic acid	599.0 -> 80.0	79	7.5	0.9985
32	PFTTrDA	Perfluorotridecanoic acid	663.0 -> 619.0	11	7.7	0.9986
33	11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	630.9 -> 451.0	32	7.8	0.9994
34	PFOSA	Perfluorooctanesulfonamide	498.0 -> 78.0	35	8.1	0.9999
35	PFTeDA	Perfluorotetradecanoic acid	713.0 -> 669.0	11	8.1	0.9996
36	PFDoS	Perfluorododecanesulfonic acid	699.0 -> 80.0	80	8.3	0.9973
37	NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	616.0 -> 59.0	11	9.2	0.9999
38	NMeFOSA	N-methyl perfluorooctanesulfonamide	512.0 -> 169.0	27	9.4	0.9996
39	NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	630.0 -> 59.0	15	9.6	0.9998
40	NEtFOSA	N-ethyl perfluorooctanesulfonamide	526.0 -> 169.0	27	9.7	0.9988

## Results And Discussion

A chromatogram of the calibration solution 5 (CS5) containing the 40 native compounds is shown in **Figure 1**; as evident from **Table 3**, linear calibration curves with  $R^2 \geq 0.99$  were obtained for all PFAS analytes.



**Figure 1.** 40 PFAS compounds at CS5 concentration in methanol with 4% water, 1% ammonium hydroxide and 0.6% acetic acid (Peak IDs see **Table 2**).

The targeted native PFAS analytes were quantified based on stable-isotope dilution using Extracted Internal Standards (EIS) which were added to the sample before the SPE extraction. The recovery of the EIS surrogates was determined using the Non-Extracted Internal Standards (NIS) which were spiked to the concentrated extract after the clean-up step; IS allocation was in accordance with the EPA 1633 method. **Tables 3** and **4** display the recoveries and RSDs from the experimental study where 40 native PFAS compounds (**Table 2**) were spiked to the

water samples at three different concentration levels and 24 EIS surrogates (**Table 4**) were added prior to extraction. All recoveries were between 84.0% and 110.7% and RSDs ranged from 0.2% to 18.1%. All were well within the acceptance limits for recovery from aqueous matrices as listed in Tables 5 and 6 of EPA Method 1633.

**Table 3. Recovery and precision ( $n = 3$ ) of native PFAS compounds at 3 spike levels (2 x CS1, 12.5 x CS1, 40 x CS1) listed by compound class**

Native PFAS compounds	Recovery in %			
		2 x CS1	12.5 x CS1	40 x CS1
Perfluoroalkyl carboxylic acids	<b>Spike levels*</b>	<b>0.4 ng/mL, except PFBA 1.6 ng/mL &amp; PFPeA 0.8 ng/mL</b>	<b>2.5 ng/mL, except PFBA 10 ng/mL &amp; PFPeA 5 ng/mL</b>	<b>8 ng/mL, except PFBA 32 ng/mL &amp; PFPeA 16 ng/mL</b>
	PFBA	101.6 ± 6.0	99.6 ± 2.2	98.6 ± 1.3
	PFPeA	100.1 ± 4.6	100.8 ± 1.7	98.3 ± 0.5
	PFHxA	105.1 ± 6.5	97.9 ± 3.7	97.7 ± 2.5
	PFHpA	101.1 ± 5.3	99.2 ± 2.6	99.9 ± 2.5
	PFOA	97.6 ± 3.0	96.7 ± 1.1	96.1 ± 1.8
	PFNA	98.8 ± 12.9	99.1 ± 6.9	96.1 ± 2.3
	PFDA	92.5 ± 13.5	94.9 ± 2.4	95.5 ± 1.7
	PFUnA	90.5 ± 3.3	100.8 ± 4.2	97.0 ± 5.2
	PFDoA	107.1 ± 12.6	99.0 ± 6.1	100.6 ± 2.2
	PFTTrDA	93.6 ± 7.7	107.9 ± 1.6	104.1 ± 0.9
	PFTeDA	94.8 ± 6.2	103.5 ± 2.2	101.9 ± 1.6
Perfluoroalkyl sulfonic acids	<b>Spike levels*</b>	<b>0.4 ng/mL</b>	<b>2.5 ng/mL</b>	<b>8 ng/mL</b>
	PFBS	109.8 ± 3.7	94.9 ± 5.9	91.5 ± 0.8
	PFPeS	103.1 ± 5.7	103.0 ± 4.5	98.7 ± 2.8
	PFHxS	97.0 ± 8.0	94.0 ± 3.3	99.9 ± 1.9
	PFHpS	90.7 ± 12.8	88.8 ± 3.7	91.6 ± 5.8
	PFOS	101.3 ± 10.6	94.2 ± 6.2	93.8 ± 5.0
	PFNS	107.1 ± 11.0	97.2 ± 4.7	94.7 ± 4.6
	PFDS	110.7 ± 7.5	100.4 ± 5.2	93.8 ± 6.3
	PFDoS	88.6 ± 8.8	87.1 ± 4.0	84.0 ± 5.6
Fluorotelomer sulfonic acids	<b>Spike levels*</b>	<b>1.6 ng/mL</b>	<b>10 ng/mL</b>	<b>32 ng/mL</b>
	4:2FTS	97.9 ± 2.9	108.1 ± 1.3	109.6 ± 4.9
	6:2FTS	101.7 ± 5.9	114.9 ± 5.2	109.2 ± 1.3
	8:2FTS	103.8 ± 4.3	113.7 ± 3.7	99.8 ± 2.0
Perfluorooctane sulfonamides	<b>Spike levels*</b>	<b>0.4 ng/mL</b>	<b>2.5 ng/mL</b>	<b>8 ng/mL</b>
	PFOSA	95.1 ± 10.8	94.1 ± 2.9	98.3 ± 1.8
	NMeFOSA	91.1 ± 28.5	93.7 ± 6.3	100.6 ± 0.2
	NEtFOSA	88.7 ± 18.1	98.5 ± 2.5	98.3 ± 2.0
Perfluorooctane sulfonamidoacetic acids	<b>Spike levels*</b>	<b>0.4 ng/mL</b>	<b>2.5 ng/mL</b>	<b>8 ng/mL</b>
	NMeFOSAA	84.1 ± 9.9	96.7 ± 5.3	100.5 ± 1.3
	NEtFOSAA	97.7 ± 6.7	102.3 ± 0.4	95.3 ± 3.5
Perfluorooctane sulfonamide ethanols	<b>Spike levels*</b>	<b>4 ng/mL</b>	<b>25 ng/mL</b>	<b>80 ng/mL</b>
	NMeFOSE	96.2 ± 3.0	100.3 ± 0.2	100.9 ± 2.0
	NEtFOSE	96.7 ± 8.0	101.0 ± 1.7	100.6 ± 1.0
Per- and polyfluoroether carboxylic acids	<b>Spike levels*</b>	<b>0.8 ng/mL, except HFPO-DA &amp; ADONA 1.6 ng/mL</b>	<b>5 ng/mL, except HFPO-DA &amp; ADONA 10 ng/mL</b>	<b>16 ng/mL, except HFPO-DA &amp; ADONA 32 ng/mL</b>
	HFPO-DA	95.3 ± 10.6	101.6 ± 2.9	102.2 ± 2.0
	ADONA	100.0 ± 9.7	108.4 ± 0.5	109.6 ± 1.9
	PFMPA	98.5 ± 6.4	99.6 ± 1.1	96.5 ± 0.6
	PFMBA	98.7 ± 7.0	100.8 ± 2.6	98.3 ± 0.5
	NFDHA	102.8 ± 9.6	106.2 ± 3.4	102.0 ± 3.4
Ether sulfonic acids	<b>Spike levels*</b>	<b>1.6 ng/mL, except PFEESA 0.8 ng/mL</b>	<b>10 ng/mL, except PFEESA 5 ng/mL</b>	<b>32 ng/mL, except PFEESA 16 ng/mL</b>
	9Cl-PF3ONS	93.9 ± 9.4	103.1 ± 1.5	102.3 ± 1.4
	11Cl-PF3OUdS	91.2 ± 3.3	99.2 ± 2.0	100.4 ± 1.4
	PFEESA	95.1 ± 13.1	106.6 ± 2.5	107.6 ± 1.2
Fluorotelomer carboxylic acids	<b>Spike levels*</b>	<b>10 ng/mL, except 3:3 FTCA 2 ng/mL</b>	<b>62.5 ng/mL, except 3:3 FTCA 12.5 ng/mL</b>	<b>200 ng/mL, except 3:3 FTCA 40 ng/mL</b>
	3:3FTCA	102.6 ± 8.9	98.4 ± 2.5	98.9 ± 2.6
	5:3FTCA	103.0 ± 6.0	100.9 ± 1.2	103.0 ± 1.7
	7:3FTCA	90.7 ± 8.5	96.9 ± 3.2	105.8 ± 3.0

\*The concentration of the spikes, which correspond to the lowest calibration standard, CS1, refer to the concentration in the final extract. The spiked sample concentration in the actual water samples is 100 times lower due to the 100-fold enrichment resulting from the sample preparation.

**Table 4. Recovery and precision ( $n = 3$ ) of Extracted Internal Standards (EIS) calculated for the water samples spiked with the native PFAS at three different concentration levels (2 x CS1, 12.5 x CS1, 40 x CS1)**

EIS compounds	Recovery in %		
	2 x CS1*	12.5 x CS1*	40 x CS1*
<sup>13</sup> C <sub>4</sub> -PFBA	95.1 ± 2.7	96.6 ± 1.6	97.4 ± 1.1
<sup>13</sup> C <sub>5</sub> -PFPeA	98.2 ± 2.2	97.8 ± 1.0	98.0 ± 0.7
<sup>13</sup> C <sub>5</sub> -PFHxA	97.2 ± 1.4	97.6 ± 1.9	98.0 ± 2.3
<sup>13</sup> C <sub>4</sub> -PFHpA	102.0 ± 2.1	102.2 ± 3.0	102.0 ± 3.5
<sup>13</sup> C <sub>8</sub> -PFOA	95.8 ± 4.7	102.4 ± 1.7	99.0 ± 2.8
<sup>13</sup> C <sub>9</sub> -PFNA	98.0 ± 2.8	97.3 ± 5.2	99.1 ± 4.5
<sup>13</sup> C <sub>6</sub> -PFDA	98.1 ± 2.8	100.0 ± 1.1	100.9 ± 2.3
<sup>13</sup> C <sub>7</sub> -PFUnA	95.2 ± 0.7	97.3 ± 3.8	97.1 ± 1.5
<sup>13</sup> C <sub>2</sub> -PFDoA	92.5 ± 4.9	93.4 ± 2.7	92.5 ± 1.1
<sup>13</sup> C <sub>2</sub> -PFTeDA	89.3 ± 4.1	89.8 ± 3.9	87.9 ± 3.3
<sup>13</sup> C <sub>3</sub> -PFBS	95.0 ± 7.2	100.3 ± 2.1	97.4 ± 1.1
<sup>13</sup> C <sub>3</sub> -PFHxS	92.4 ± 6.1	102.7 ± 1.8	100.0 ± 3.7
<sup>13</sup> C <sub>8</sub> -PFOS	98.4 ± 2.3	101.4 ± 2.6	106.2 ± 5.4
<sup>13</sup> C <sub>2</sub> -6:2FTS	82.9 ± 5.2	91.9 ± 5.2	95.5 ± 0.4
<sup>13</sup> C <sub>2</sub> -8:2FTS	78.0 ± 5.6	83.0 ± 5.9	91.0 ± 2.1
<sup>13</sup> C <sub>8</sub> -PFOSA	89.8 ± 7.6	95.4 ± 2.7	95.3 ± 3.1
D <sub>5</sub> -NEtFOSA	77.9 ± 11.1	74.9 ± 1.5	81.1 ± 2.5
D <sub>3</sub> -NMeFOSAA	87.2 ± 3.2	90.9 ± 1.8	93.0 ± 1.4
D <sub>5</sub> -NEtFOSAA	84.3 ± 5.0	87.6 ± 1.9	94.8 ± 0.5
D <sub>7</sub> -NMeFOSE	87.5 ± 5.6	89.3 ± 1.5	92.6 ± 1.9
D <sub>9</sub> -NEtFOSE	89.0 ± 6.6	90.7 ± 0.1	95.5 ± 1.3
<sup>13</sup> C <sub>3</sub> -HFPO-DA	101.0 ± 2.5	100.2 ± 0.5	100.1 ± 2.8

\*The concentration of the native PFAS varied between the spiking levels; however, the spiking levels of the EIS standards was the same in each.

## Conclusions

This application note presents the workflow for EPA method 1633 to analyze 40 PFAS compounds in water samples by applying SPE extraction using a Supelclean™ ENVI-WAX™ cartridge, followed by additional dispersive clean-up with loose/bulk Supelclean™ ENVI-Carb™ adsorbents and quantification by LC-MS/MS employing Ascentis® Express PFAS analytical and delay columns. The method demonstrated robust performance with excellent recoveries for all 40 PFAS compounds and 24 Extracted Internal Standards (EIS) well within acceptance limits for aqueous matrices listed in the method at the three

recommended fortification levels. The calculated RSDs were below 20%, further indicating a satisfactory precision. This proves the suitability of the PTFE free Visiprep™ SPE Vacuum Manifold with Supelclean™ ENVI-WAX™ SPE cartridges for the extraction of PFAS compounds from water samples and of the Supelclean™ ENVI-Carb™ adsorbent for clean-up prior to subsequent LC-MS/MS analysis according to the EPA 1633 method.

## Related Products

Description	Cat. No.
<b>Sample Prep</b>	
Supelclean™ ENVI-WAX™ SPE Tube, 500 mg, volume 6 mL, Pk.30	54057-U
Visiprep™ SPE Manifold standard, 12-port model – PTFE Free	57030-U
Large Volume SPE Reservoir, volume 25 mL, Pk. 30	54258-U
Supelclean™ ENVI-Carb™, 50 g	57210-U
Millex™ Syringe Filter, Nylon, Non-sterile, 0.20 µm pore size, 13 mm diameter	SLGNX13
<b>HPLC</b>	
Ascentis® Express PFAS 90 Å, 2.7 µm, 5 cm x 2.1 mm	53557-U
Ascentis® Express PFAS Delay 90 Å, 2.7 µm, 5 cm x 3.0 mm	53572-U
<b>Solvents &amp; Reagents</b>	
Acetonitrile tested for EPA 533 and EPA 537.1 PFAS Methods LiChrosolv®	1.04726
Methanol tested for EPA 533 and EPA 537.1 PFAS Methods LiChrosolv®	1.04732
Water tested for EPA 533 and EPA 537.1 PFAS Methods LiChrosolv®	1.04735
Ammonium acetate LiChropur™, eluent additive for LC-MS	73594
Formic acid for LC-MS LiChropur™	5.33002
Ammonium hydroxide for HPLC LiChropur™	5.43830
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO, Reag. Ph Eur	1.00063
Milli-Q® IQ 7000 Ultrapure Water Purification System (or similar)	ZIQ7000T0C
Millipak® 0.22 µm Filter	MPGP002A1
<b>Accessories</b>	
BRAND® PP graduated centrifuge tube, screw cap, volume 50 mL, without base, non-sterile, Pk. 300	BR114820
BRAND® PP graduated centrifuge tube, screw cap, volume 15 mL, without base, non-sterile, Pk. 750	BR114817
Syringe PP/PE without needle, 10 mL	Z683590
<b>Certified Reference Materials - TraceCERT®</b>	
Perfluorobutanoic acid (PFBA), 10 mg	75930
Perfluoropentanoic acid (PFPeA), 10 mg	73551
Perfluorohexanoic acid (PFHxA), 25 mg	93899
Perfluoroheptanoic acid (PFHpA), 25 mg	93983
Perfluorooctanoic acid (PFOA), 25 mg	93973
Perfluorononanoic acid (PFNA), 10 mg	05167
Perfluorodecanoic acid (PFDA), 10 mg	91367
Perfluoroundecanoic acid (PFUnA), 10 mg	89988
Perfluorododecanoic acid (PFDoA), 10 mg	76467
Perfluorotridecanoic acid (PFTrDA), 10 mg	76705
Perfluorotetradecanoic acid (PFTeDA), 10 mg	38400
Perfluorobutane sulfonic acid (PFBS), 25 mg	93634
Perfluoroheptane sulfonic acid (PFHpS), 10 mg	78049
Perfluorooctane sulfonic acid (PFOS), 25 mg	95181
Hexafluoropropylene oxide dimer acid (HFPO-DA), 25 mg	94275
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2FTS), 25 mg	93497
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2FTS), 25 mg	93587
Perfluorooctane sulphonamide (PFOSA/FOSA), 25 mg	95179
N-Methylperfluorooctanesulfonamide (NMeFOSA), 10 mg	89868
N-Ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA), 25 mg	94707
N-Methylperfluorooctanesulfonamidoethanol (NMeFOSE), 10 mg	89348
Perfluoro-3-methoxypropanoic acid (PFMPA), 25 mg	73014
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA), 50 mg	04292
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), 25 mg	04453
Perfluoro (2-ethoxyethane) sulfonic acid (PFEEESA), 25 mg	95201
2H,2H,3H,3H-Perfluorooctanoic acid, 10 mg	94743

See more on PFAS testing at [SigmaAldrich.com/PFAS](https://www.sigmaaldrich.com/PFAS)

## References

1. EPA Method 1633, Revision A, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. U.S. EPA. 2024. [www.epa.gov/system/files/documents/2024-12/method-1633a-december-5-2024-508-compliant.pdf](http://www.epa.gov/system/files/documents/2024-12/method-1633a-december-5-2024-508-compliant.pdf)
2. US EPA. CWA Analytical Methods for Per- and Polyfluorinated Alkyl Substances (PFAS). [accessed 2024 Oct 2]. <https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas>
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4. Cockcroft L-J, Lloyd J, Orell A, Persich T. EU REACH restrictions: How to prepare for the proposed PFAS restriction. ERM.com. 2023. [accessed 2024 Oct 2]. <https://www.erm.com/insights/eu-reach-restrictions-how-to-prepare-for-the-proposed-pfas-restriction/>

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