ENVIRONMENTAL

LC-MS Analysis of PFAS Compounds in EPA Method 533 using Supelclean™ ENVI-WAX™ SPE

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Introduction

Per- and polyfluoroalkyl substances (PFAS) have been in use since the 1940s. Consisting of over 4700 different compounds, PFAS substances are used in almost every facet of modern life. The utility of these compounds resulted in their rapid adoption in consumer goods manufacturing. PFAS compounds can now be found in food packaging, cookware, cosmetics, stain and water repellants, firefighting foams, and are commonly used in many manufacturing processes. While incredibly useful, these compounds also carry a risk to health that we have only recently started to understand clearly.

PFAS compounds are also commonly known as “forever chemicals” which means they do not break down in the environment like other chemicals. This persistence can result in the concentration of these compounds growing to levels that are unsafe for human exposure and that can cause negative health effects such as low infant birth weights, effects on the immune system, cancer, and thyroid hormone disruption.

PFAS detection plays therfore a crucial role in safeguarding public health and the environment. PFAS detection in water is essential for assessing water quality and to identifying potential health risks. To achieve accurate measurements and quantification of these contaminants in water samples, various PFAS analysis methods are employed.

Multiple regulatory methods, such as EPA 537 and 533, detail the extraction of PFAS analytes from drinking water using SPE cartridges followed by LC-MS/MS analysis. For EPA method 533, weak anion exchange (WAX) cartridges are specified and should contain 500 mg of the mixed-mode polymeric adsorbent. Supelclean™ ENVI-WAX™ SPE cartridges are direct equivalent to the specified SPE in EPA method 533. This application note demonstrates the extraction of 25 analytes from water using Supelclean™ ENVI-WAX™ SPE.

Experimental

The procedure from EPA method 533 was followed for sample collection and sample preparation. Supelclean™ ENVI-WAX™ SPE 500 mg/6 mL cartridges (**54057-U**) were used with a Visiprep™ vacuum manifold (**57030-U**) for processing the samples. The large volume sampling kit (57275) was also used but the Teflon tubing was replaced with silicone tubing (1/8" diameter). The Teflon guides in the original manifold were replaced with stainless-steel solvent guides (**57027**). Analysis of the samples was done using an Agilent 6495C LC-MS/MS instrument. Ascentis® Express PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm (**53560-U**) was used as an analytical column. In addition, an Ascentis® Express PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm (**53572-U**) was used (**Table 1**). The chromatogram of 25 compounds in a calibration standard is shown in **Figure 1**.

UHPLC-MS grade water samples were tested for PFAS contamination and found to be free of 25 analytes as per the EPA method 533. The water was spiked at 10 or 40 ng/L with 25 analytes to demonstrate the performance of Supelclean™ ENVI-WAX™ SPE cartridges for this method. 250 mL of water samples were loaded onto 500 mg/6 mL SPE cartridges, and eluted using methanol with 2% (v/v) ammonium hydroxide; the resulting eluate was evaporated to dryness and reconstituted into 1.0 mL of 4% (v/v) methanol in water for LC-MS/MS detection.

Following the performance assessment of the method using Supelclean™ ENVI-WAX™ SPE, a tap water sample was analyzed using the same methodology for the presence of 25 PFAS compounds.

Filters Suitable for PFAS Analysis

Read more about recommended Millex® syringe filter and cut disc membrane filters at **SigmaAldrich.com/pfassamplefiltration**

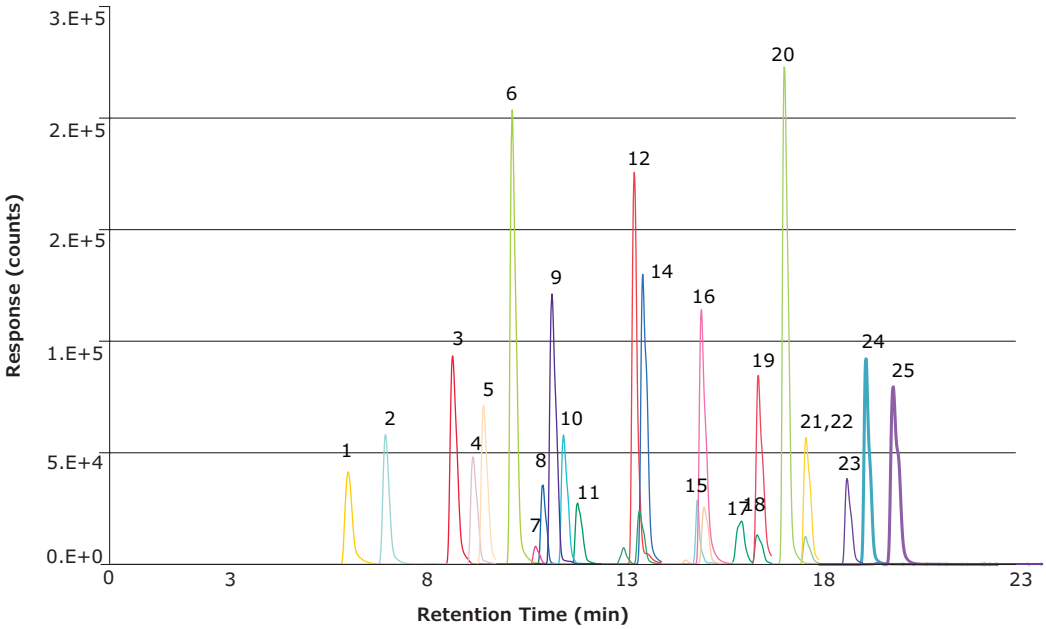

Figure 1. 25 PFAS compounds at 50 ppb in 96:4 water:methanol (v/v) solvent.

Table 1. LC-Conditions for analysis of 25 PFAS compounds

Chromatography Conditions			
Column:	Ascentis® Express PFAS, 2.7 µm, 15 cm x 2.1 mm (**53560-U**)		
Delay column:	Ascentis® Express PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm (**53572-U**)		
Mobile Phase:	[A] 20 mM Ammonium acetate; [B] Methanol		
Gradient:	**Time (min)**	**%A**	**%B**
	Inital	95.0	5.0
	0.5	95.0	5.0
	3.0	60.0	40.0
	16.0	20.0	80.0
	18.0	20.0	80.0
	20.0	5.0	95.0
	22.0	5.0	95.0
	25.0	95.0	5.0
	35.0	95.0	5.0
Flow Rate:	0.25 mL/min		
Injection:	10 µL		
Detector:	MS, MRM (see **Table 2**)		
Samples:	Water samples (spiked and unspiked) extracted by SPE		

Table 2. MRM trasition used for 25 PFAS compounds in EPA method 533

Peak	Compound		MRM
1	PFBA	Perfluorobutanoic acid	213.0->169.0
2	PFMPA	Perfluoro-3-methoxypropanoic acid	229.0->85.0
3	PFPeA	Perfluoropentanoic acid	263.0->219.0
4	PFBS	Perfluorobutanesulfonic acid	298.9->80.0
5	PFMBA	Perfluoro-4-methoxybutanoic acid	279.0->85.1
6	PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	314.5->135.0
7	NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	295.0->201.0
8	4:2FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid	327.0->307.0
9	PFHxA	Perfluorohexanoic acid	313.0->269.0
10	PFPeS	Perfluoropentanesulfonic acid	348.9->80.0
11	HFPO-DA	Hexafluoropropylene oxide dimer acid	285.0->169.0
12	PFHpA	Perfluoroheptanoic acid	363.0->319.0
13	PFHxS	Perfluorohexanesulfonic acid	389.9->80.0
14	ADONA	4,8-Dioxa-3H-perfluorononanoic acid	377.0->251.0
15	6:2 FTS	1H,1H,2H,2H-Perfluorooctane sulfonic acid	427.0->406.9
16	PFOA	Perfluorootanoic acid	413.0->369.0
17	PFHpS	Perfluoroheptanesulfonic acid	448.9->80.0
18	PFOS	Perfluorooctanesulfonic acid	498.9->80.0
19	PFNA	Perfluorononanoic acid	463.0->419.0
20	9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1- sulfonic acid	530.9->350.9
21	8:2FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid	527.0->507.0
22	PFDA	Perfluorodecanoic acid	513.0->469.0
23	PFUnA	Perfluoroundecanoic acid	563.0->519.1
24	11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	631.0->451.0
25	PFDoA	Perfluorododecanoic acid	613.0->569.0

Results and Discussion

The background evaluation of the method using all SPE consumables and accessories resulted in excellent low background values (shown in **Table 3**). The result for screening all compounds in the UHPLC-MS solvent was at or below the lower limit of detection (LLOD) of the LC-MS/MS instrument.

Table 3. Results of background testing for the evaluation

Compound	Background in UHPLC-MS water (ng/L)*
PFBA	Below LLOD*
PFMPA	2[1]
PFPeA	Below LLOD
PFBS	Below LLOD
PFMBA	Below LLOD
PFEESA	Below LLOD
NFDHA	Below LLOD
4:2FTS	Below LLOD
PFHxA	3[2]
PFPeS	Below LLOD
HFPO-DA	Below LLOD
PFHpA	Below LLOD
PFHxS	Below LLOD
ADONA	Below LLOD
6:2 FTS	Below LLOD
PFOA	Below LLOD
PFHpS	Below LLOD
PFOS	Below LLOD
PFNA	Below LLOD
9Cl-PF3ONS	Below LLOD
8:2FTS	Below LLOD
PFDA	Below LLOD
PFUnA	Below LLOD
11Cl-PF3OUdS	Below LLOD
PFDoA	Below LLOD

[1] LCMRL (Lowest Concentration Minimum Reporting Level) is 5.3 ng/L per EPA method 533

[2] LCMRL is 3.8 ng/L per EPA method 533

*LLOD were 2-6 ppt for all compounds

Per EPA method 533 the recovery of the laboratory spiked blank water samples should fall in the range 70-130% with reproducibility of better than 20%. **Figure 2** demonstrates the recoveries from laboratory spiked UHPLC-MS water blanks, where the recoveries for 25 compounds met the EPA method requirements. **Figure 3** presents the %RSD for each of the 25 compounds indicating that the less than 20% RSD requirement was met.

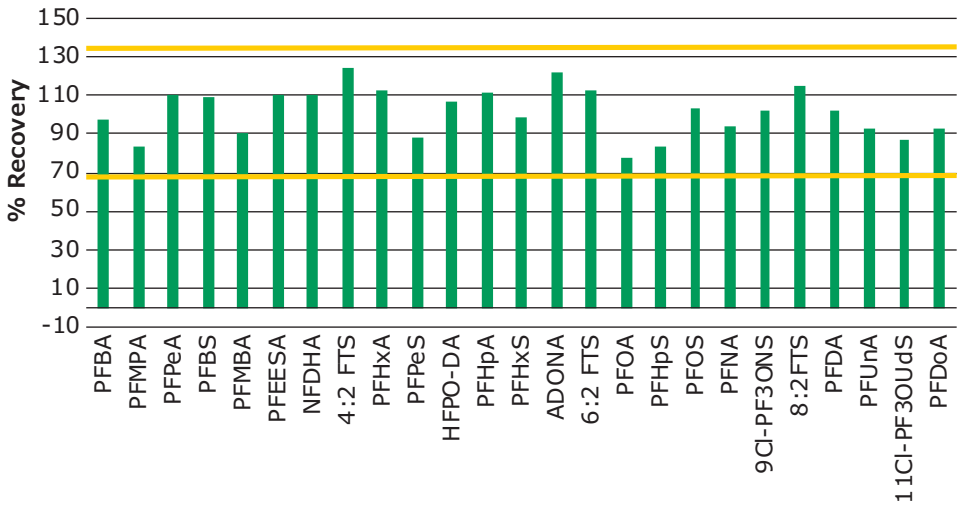

Figure 2. Recoveries of 25 analytes spiked into UHPLC-MS grade water samples. Most analytes were spiked at 10 ng/L, perfluorosulfonic acids were spiked at 40 ng/L. 3 replicate measurements were performed.

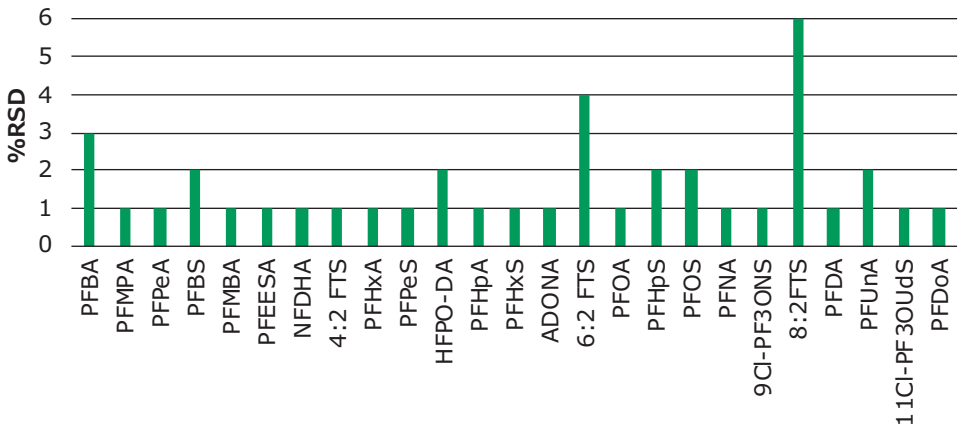

Figure 3. %RSD for recoveries of the 25 analytes spiked into UHPLC-MS water samples. 3 replicate measurements were performed.

A drinking water sample was also analyzed using EPA 533 method. No analytes were detected in the sample above 0.5 ng/L concentrations, and most were below LLOD.

Conclusions

The workflow for EPA method 533 is presented in this article. All 25 compounds were recovered with acceptable accuracy and precision using Supelclean™ ENVI-WAX™ SPE cartridges, Visiprep™ vacuum manifold, Ascentis® Express PFAS columns and UHPLC-MS grade solvents. The background from all consumables and LC system was low and acceptable for detecting low levels of PFAS analytes.

Featured & Related Products

Description	Cat. No
Supelclean™ ENVI-WAX™ SPE 500 mg/6 mL cartridges, pk of 30	**54057-U**
Supelclean™ ENVI-WAX™ SPE 200 mg/6 mL cartridges, pk of 30	**54056-U**
Visiprep™ vacuum manifold	**57030-U**
Stainless steel solvent guides for vacuum manifold, pk of 12	**57027**
Ascentis® Express PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm	**53560-U**
Ascentis® Express PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm	**53572-U**
Water UHPLC suitable for mass spectrometry	**900682**
Methanol UHPLC suitable for mass spectrometry	**900688**
Standards	
Perfluoro-3,6-dioxaheptanoic acid, analytical standard, 100 mg	**94712**
Perfluorobutanoic acid, analytical standard, 25 mg	**68808**
Perfluorodecanoic acid, analytical standard, 25 mg	**43929**
Perfluorododecanoic acid, analytical standard, 50 mg	**92291**
1,1,2,2-Tetrafluoro-2-(1,1,2,2,2-pentafluoroethoxy) ethanesulfonic acid, analytical standard, 100 mg	**93896**
Perfluoroheptanoic acid, analytical standard, 25 mg	**43996**
Perfluorohexanoic acid, analytical standard, 25 mg	**43809**
Heptadecafluorooctanesulfonic acid potassium salt, analytical standard, 100 mg	**89374**
Heptadecafluorooctanesulfonic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33607**
Perfluorooctanoic acid, analytical standard, 100 mg	**33824**
Pentadecafluorooctanoic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33603**
Perfluoropentanoic acid, analytical standard, 25 mg	**68542**
Perfluoroundecanoic acid, analytical standard, 50 mg	**80444**
Perfluorotetradecanoic acid, analytical standard, 50 mg	**80312**

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