Supelco®
Analytical Products

Application Note

LC-MS Analysis of PFAS Compounds in EPA 533 after Supelclean™ ENVI-WAX™ SPE Cleanup

Introduction

Per- and polyfluoroalkyl substances (PFAS) have been in use since the 1940's. 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 negative health effects such as: low infant birth weights, effects on the immune system, cancer, and thyroid hormone disruption.

Multiple regulatory methods, such as EPA 537 and 533, detail the extraction of PFAS analytes from drinking water using solid phase extraction (SPE) cartridges followed by analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). For method EPA 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 the direct equivalent to the specified SPE cartridges in method EPA 533. This application note demonstrates the extraction of 25 analytes from water using Supelclean™ ENVI-WAX™ SPE.

Experimental

The procedure from method EPA 533 was followed for sample collection and sample preparation. Supelclean™ ENVI-WAX™ SPE 500 mg/6 mL cartridges (54057-U) were used with a vacuum manifold 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). Visiprep™ vacuum manifold (57030-U) was used for processing SPE samples. The Teflon guides in the original manifold were replaced with the stainless-steel solvent guides (57027). Analysis of the samples was done using an Agilent 6495C LC-MS/MS instrument. An Ascentis® Express PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm (Cat. No. 53560-U) was used as the analytical column. In addition, an Ascentis® Express PFAS Delay Column, 2.7 µm, 5 cm x 3 mm (Cat. No. 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 the 25 analytes in the EPA 533 method. The water was spiked at 10 or 40 pg/mL with 25 analytes to confirm the performance of Supelclean™ ENVI-WAX™ SPE cartridges for this method. 250 mL of water samples were loaded into 500 mg/6 mL SPE cartridges, eluted using methanol with 2% (v/v) ammonium hydroxide; 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.

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

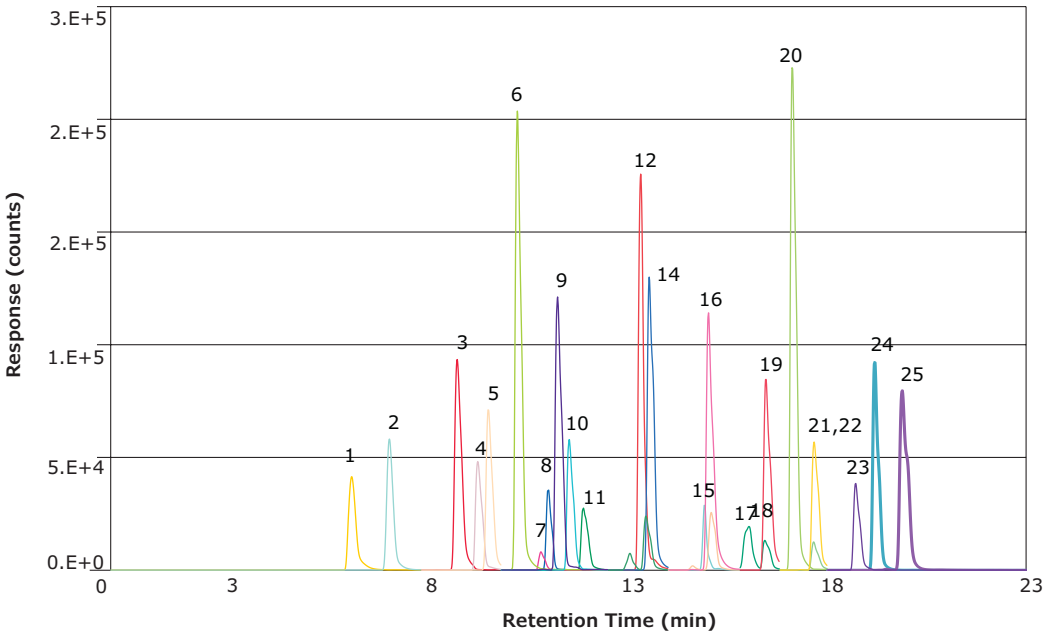

Table 1. LC-MS Conditions for PFAS analysis

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 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
Detector:	MS/MS, MRM (**Table 2**)
Injection Volume:	10 µL
Samples:	Water samples (spiked and unspiked) extracted by SPE

Table 2. Compound listing and MRM transitions

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	269.0->218.0
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->351.0
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.

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 %RSD for each of the 25 compounds indicating that less than 20% RSD requirement was met.

Drinking water samples were also analyzed using EPA 533 method. No analytes were detected in these samples above 0.5 ng/L concentrations, most were below LLOD. The samples of this drinking water were very clean.

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

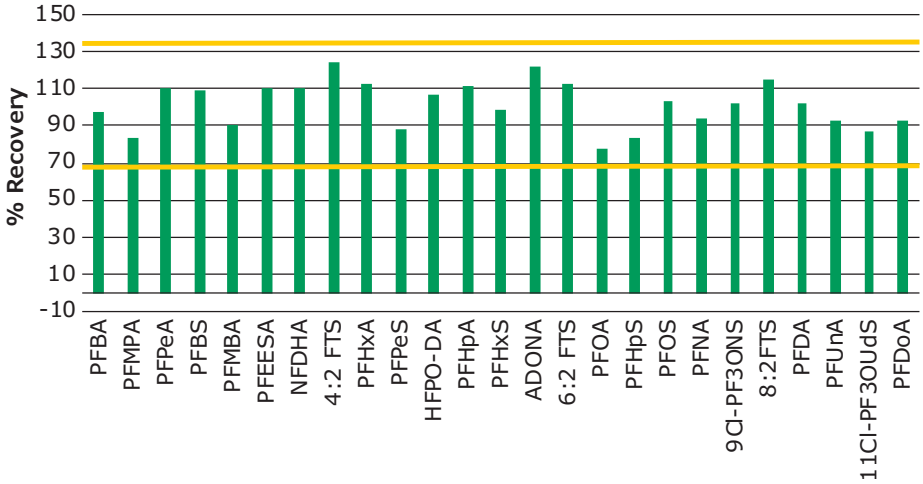

Figure 2. Recoveries of 25 analytes spiked into UHPLC-MS grade water samples. Most analytes were spiked at 10 ng/L perfluorosulfonic acids were spiked at 40 ng/L. Three replicate measurements were performed.

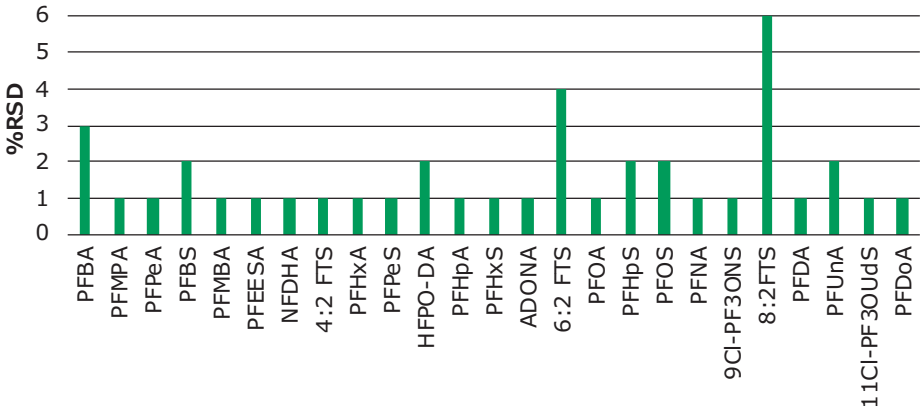

Figure 3. %RSD for recoveries of the 25 analytes spiked into UHPLC-MS water samples. Three replicate measurements were performed.

Conclusions

The workflow for EPA 533 method is presented in this application note. All 25 compounds were recovered with acceptable accuracy and precision using Supelclean™ ENVI-WAX™ 500 mg/6 mL 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.

Find more information on PFAS testing at **SigmaAldrich.com/PFAS**

Featured 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 mm	**53572-U**
Water UPLC suitable for mass spectrometry	**900682**
Methanol UPLC suitable for mass spectrometry	**900688**
Ammonium acetate, suitable for mass spectrometry	**73594**
Perfluorobutanoic acid	**68808-100 mg**
Perfluorononanoic acid	**91977-50mg**
Perflorodecanoic acid	**43929-50mg**

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