Application Note

EPA 1633 Automated Solid Phase Extraction of 40 Native PFAS Compounds in Wastewater Using Vacuum/Positive Pressure

Per- and polyfluoroalkyl substances (PFAS) constitute a group of compounds characterized by perfluorinated or polyfluorinated carbon chain moieties, typically denoted by structures such as F(CF2)n or F(CF2)n-(C2H4)n. Due to their unique properties, these substances have found extensive application in various industrial and consumer products.

Many industrial and consumer applications utilize perfluorooctane sulfonate (PFOS) and other PFAS compounds. These include but are not limited to, stain-resistant coatings for textiles, leather, and carpets; grease-proof coatings for food-contact paper products; firefighting foams; surfactants for mining and oil-well operations; floor polishes; and insecticide formulations. Their widespread usage has led to their ubiquitous presence in the environment.

In recent years, mounting concerns have emerged regarding the widespread distribution and potential adverse effects of PFAS, particularly notable compounds like PFOS and perfluorooctanoic acid (PFOA). These concerns have prompted intensified scrutiny of these substances' environmental occurrence, fate, and potential impacts.

Recent developments in the United States have led to the introduction of EPA method 1633, which addresses the need for robust methodologies to monitor and analyze PFAS. This method, unveiled in early 2024, enables comprehensive analysis across various matrices, including wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue. EPA Method 1633 represents a significant advancement in the analytical toolkit for assessing PFAS contamination and understanding their distribution and behavior in diverse environmental compartments.

Instrumentation

- FMS, Inc. TurboTrace® Parallel/Sequential PFAS SPE system (Solid Phase Extraction) is a modular expandable system designed for handling wastewater samples. The system can have from one to six modules. Each module can run 5 samples sequentially, the system can be expanded from one to 6 modules to run a total of 5 to 30 samples.

Consumables

- Agilent Bond Elut PFAS WAX 150 mg cartridges (# 5610-2150)
- Ultrapure DI water
- Methanol pesticide grade
- Ammonium hydroxide
- Formic acid
- Relevant PFAS spiking standards

Method

- Five synthetic wastewater samples (500 mL) spiked with native PFAS standards and relevant internals
- Load sample bottles onto system and install cartridges
- Rinse bottles are automatically filled during procedure
- Use positive pressure (nitrogen) for pumping solvents and mixes through the system and use vacuum to load the samples
- Condition cartridges with 15 mL 1% methanolic ammonium hydroxide followed by 5 mL of 0.3M formic acid.
- Load samples across the cartridges at 5-10 mL/min (~ 8-inch Hg)
- Sample bottles rinsed with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol and load rinses across the cartridges
- Dry 15 sec
- Rinse sample bottles with 5 mL 1% methanolic ammonium hydroxide
- Load rinses across cartridges and collect in polypropylene tubes
- Cleanup over 10 mg loose carbon
- As per the method no further concentration is carried out.

Further relevant standards were added prior to LC/MS analysis.

FMS
Fluid Management Systems

Analysis

- ■ Take aliquot from final 5 mL extract (Method 1633 does not require volume reduction of final extract)
- ■ Agilent 1290 Infinity II LC System
- ■ Agilent 6475 Triple quad LC/MS
- ■ Agilent Zorbax Eclipse Plus C18 column 3.0 x 50 mm, 1.8 um
- ■ Column temperature 40 °C
- ■ Injection 5.0 uL
- ■ Mobile phase 5 mM ammonium acetate in 95% water, 5% acetonitrile (A) and methanol (B)
- ■ Gradient
  - 0 min 98% A 2% B
  - 0.2 min 98% A 2% B
  - 10 min 5% a 95% B
- ■ Stop time 12.2 min
- ■ Dynamic MRM negative electrospray
- ■ T (gas) = 230 °C
- ■ T (sheath) = 355 °C

FMS Parallel-Sequential SPE System

Compound		SEQ-IDC-1	SEQ-IDC-2	SEQ-IDC-3	SEQ-IDC-4		
Name	**In percent**	**Final Conc.**	**Final Conc.**	**Final Conc.**	**Final Conc.**	**Window (%)**	**RSD (%)**
11Cl-PF3OUdS		86.0	88.0	83.5	89.5	50-150	3.0
3-3 FTCA		84.1	83.4	85.9	85.4	70-130	1.4
4-2 FTS		95.4	94.1	94.6	94.5	70-135	0.6
5-3 FTCA		96.9	90.6	88.8	89.0	70-130	4.2
6-2 FTS		101.7	113.1	99.7	99.7	70-135	6.2
7-3 FTCA		102.2	91.6	94.3	82.3	55-130	8.9
8-2 FTS		90.2	102.7	110.3	95.1	70-140	8.9
9Cl-PF3ONS		93.0	88.0	92.0	97.0	70-145	4.0
ADONA		103.0	97.7	84.0	85.7	70-135	10.0
EtFOSE		94.7	94.6	101.8	103.9	70-130	4.9
HFPO-DA		111.0	103.5	94.1	97.9	70-135	7.2
MeFOSE		95.2	91.7	87.0	93.2	70-135	3.8
N-EtFOSA		80.7	98.9	90.4	93.9	70-135	8.5
N-EtFOSAA		108.6	96.1	99.1	108.0	70-135	6.1
NFDHA		103.8	101.9	103.2	94.3	65-140	4.4
N-MeFOSA		86.5	87.6	100.0	93.7	70-135	6.8
N-MeFOSAA		120.3	124.7	105.0	104.6	65-140	9.2
PFBA		107.5	108.0	106.5	106.9	70-135	0.6
PFBS		114.6	124.9	118.3	120.0	70-140	3.6
PFDA		94.3	99.9	105.7	94.2	65-140	5.6
PFDoA		108.8	101.5	94.6	109.5	70-130	6.8
PFDoS		80.6	83.7	87.5	93.0	45-135	6.2
PFDS		85.5	86.5	92.0	98.0	70-135	6.4
PFEESA		103.0	95.9	98.3	99.4	70-135	3.0
PFHpA		106.0	103.3	108.1	98.4	70-135	4.0
PFHpS		81.8	84.9	97.2	88.1	70-140	7.6
PFHxA		120.2	121.9	126.2	126.5	70-135	2.5
PFHxS		91.4	97.9	93.3	93.2	70-135	3.0
PFMBA		106.8	103.3	105.2	98.6	65-145	3.4
PFMPA		85.3	89.1	91.9	85.4	60-140	3.6
PFNA		87.9	90.3	96.0	86.0	70-140	4.8
PFNS		83.5	96.0	95.0	86.6	70-135	6.8
PFOA		120.0	116.3	120.0	121.3	65-155	1.8
PFOS		96.1	103.0	114.8	109.4	70-140	7.7
PFOSA		105.4	101.5	88.3	107.5	70-135	8.5
PFPeA		113.2	116.6	114.3	109.4	70-135	2.7
PFPeS		106.8	101.5	100.1	114.2	70-135	6.0
PFTDA		94.2	86.7	93.5	91.9	70-145	3.7
PFTrDA		101.9	111.4	105.6	118.5	60-145	6.6
PFUnA		89.3	95.5	106.4	96.2	70-135	7.3

Table 1. Recoveries (%) and RSDs (%) for 40 native PFAS in synthetic wastewater (1633) using Parallel-Sequential SPE (spiked with 2-69 ng/L).

Compound		SEQ-IDC-1	SEQ-IDC-2	SEQ-IDC-3	SEQ-IDC-4	
Name	In percent	% Recoveries	% Recoveries	% Recoveries	% Recoveries	Window
13C2-4-2 FTSA		95.5	94.0	121.7	102.3	40-200
13C2-6-2 FTS		97.0	105.0	106.5	95.0	40-200
13C2-8-2 FTSA		95.5	99.4	96.5	105.3	40-300
13C2-PFDoDA		91.8	97.3	99.1	83.4	10-130
13C2-PFTDA		96.8	109.7	112.4	81.7	10-130
13C3-HFPO-DA		95.0	98.5	90.7	96.7	40-130
13C3-PFBS		98.0	110.7	94.0	110.7	40-135
13C3-PFHxS		92.8	93.7	89.2	88.4	40-130
13C4-PFBA		95.2	101.1	88.4	94.4	5-130
13C4-PFHpA		94.8	88.9	102.7	89.4	40-130
13C5-PFHxA		97.5	111.1	96.9	88.3	40-130
13C5-PFPeA		95.5	92.3	98.9	98.0	40-130
13C6-PFDA		95.1	85.5	81.4	80.9	40-130
13C7-PFUnA		85.6	83.6	81.2	79.6	30-130
13C8-PFOA		101.4	92.9	101.3	95.2	50-200
13C8-PFOS		84.0	91.5	92.5	82.2	50-200
13C8-PFOSA		105.1	120.8	120.7	117.3	40-130
13C9-PFNA		91.1	89.5	88.9	95.6	40-130
2H3-N-MeFOSA		95.9	103.5	85.0	94.6	10-130
2H3-N-MeFOSAA		82.5	80.5	84.5	80.5	40-170
2H5-N-EtFOSA		94.1	90.5	86.6	78.1	10-130
2H5-N-EtFOSAA		89.6	89.9	83.1	82.4	25-135
2H7-MeFOSE		93.0	90.6	90.6	83.7	10-130
2H9-EtFOSE		92.0	106.0	89.5	87.5	10-130

Table 2. Recoveries (%) and acceptance windows (%) for 24 surrogate PFAS in synthetic wastewater (1633) using Parallel-Sequential SPE.

Compound	SEQ-MDL-1	SEQ-MDL-2	SEQ-MDL-3	SEQ-MDL-4	SEQ-MDL-5	SEQ-MDL-6	SEQ-MDL-7		
Name In ng/L	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	STDEV	MDL
11Cl-PF3OUdS	0.71	0.68	0.65	0.56	0.59	0.59	0.68	0.06	0.18
3-3 FTCA	2.45	2.33	2.44	2.03	2.17	2.54	2.42	0.18	0.56
4-2 FTS	2.74	2.57	2.70	2.68	2.71	2.42	2.91	0.15	0.48
5-3 FTCA	13.98	13.93	13.91	13.81	13.62	13.56	14.72	0.38	1.20
6-2 FTS	2.55	2.68	2.55	2.70	2.71	2.15	2.61	0.19	0.61
7-3 FTCA	7.61	7.91	8.12	8.63	8.67	9.15	9.19	0.61	1.91
8-2 FTS	4.85	4.52	4.86	4.65	4.05	4.25	4.94	0.34	1.06
9Cl-PF3ONS	1.60	1.47	1.41	1.56	1.39	1.32	1.56	0.10	0.32
ADONA	1.95	2.01	1.88	1.88	2.08	2.03	2.10	0.09	0.28
EtFOSE	5.92	6.11	6.15	6.18	6.33	6.49	7.38	0.48	1.51
HFPO-DA	2.88	2.90	2.94	2.61	2.75	2.70	3.53	0.30	0.95
MeFOSE	5.12	5.31	5.33	5.57	5.96	5.99	6.83	0.59	1.84
N-EtFOSA	0.46		0.47	0.33	0.43	0.57	0.78	0.16	0.49
N-EtFOSAA	0.76	0.50	0.39	0.40	0.35	0.52	0.63	0.15	0.46
NFDHA	1.01	1.05	0.95	0.99	1.07	0.87	1.21	0.11	0.34
N-MeFOSA	0.71	0.57	0.49	0.56	0.55	0.52	0.58	0.07	0.22
N-MeFOSAA	0.70	0.61	0.53	0.59	0.64	0.30	0.62	0.13	0.41
PFBA	2.21	2.20	2.17	2.14	2.14	2.09	2.22	0.05	0.14
PFBS	0.53	0.59	0.64	0.69	0.57	0.57	0.59	0.05	0.17
PFDA	0.43	0.47	0.53	0.51	0.59	0.45	0.46	0.05	0.17
PFDoA	0.64	0.60	0.63	0.54	0.54	0.50	0.60	0.05	0.16
PFDoS	0.43	0.32	0.37	0.30	0.25	0.35	0.33	0.06	0.18
PFDS	0.44	0.50	0.37	0.41	0.37	0.38	0.43	0.05	0.16
PFEESA	1.13	1.14	1.12	1.18	1.19	1.20	1.21	0.03	0.11
PFHpA	0.53	0.57	0.59	0.66	0.57	0.56	0.51	0.05	0.15
PFHpS	0.63	0.67	0.72	0.69	0.57	0.69	0.60	0.05	0.17
PFHxA	0.53	0.58	0.58	0.52	0.58	0.53	0.56	0.03	0.08
PFHxS	0.58	0.50	0.59	0.47	0.39	0.62	0.60	0.08	0.26
PFMBA	0.85	0.87	0.86	0.85	0.86	0.85	0.88	0.01	0.04
PFMPA	0.94	0.91	0.86	0.88	0.86	0.86	0.88	0.03	0.10
PFNA	0.50	0.56	0.57	0.66	0.52	0.62	0.56	0.06	0.18
PFNS	0.59	0.52	0.50	0.52	0.45	0.57	0.52	0.05	0.14
PFOA	0.67	0.59	0.55	0.72	0.68	0.60	0.53	0.07	0.22
PFOS	0.68	0.63	0.55	0.64	0.58	0.69	0.68	0.05	0.17
PFOSA	0.53	0.47	0.63	0.56	0.56	0.51	0.60	0.05	0.17
PFPeA	1.04	1.10	1.10	1.03	1.04	1.08	1.09	0.03	0.10
PFPeS	0.63	0.58	0.56	0.53	0.50	0.59	0.50	0.05	0.15
PFTDA	0.60	0.70	0.57	0.46	0.58	0.59	0.65	0.08	0.24
PFTrDA	0.78	0.62	0.70	0.56	0.54	0.71	0.79	0.10	0.32
PFUnA	0.58	0.52	0.55	0.49	0.42	0.45	0.63	0.07	0.23

Table 3. Method Detection Limit values for 40 native PFAS in synthetic wastewater (1633) with Parallel-Sequential SPE (spiked with 0.5 - 13.5 ng/L).

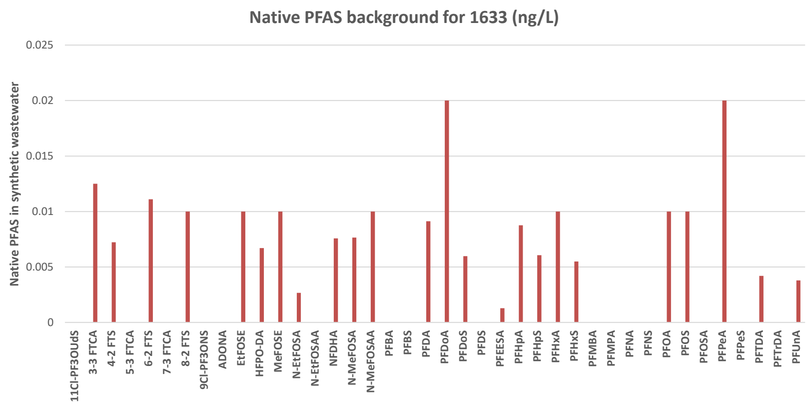

Table 4. Native PFAS background with Parallel-Sequential SPE for method 1633 (in ng/L).

Discussion and Conclusions

40 native PFAS compounds were analyzed using EPA method 1633 (Table 1) with the Parallel-Sequential SPE and synthetic wastewater. All recoveries were within the acceptance windows of the method with all RSDs < 11%. Run time of the automated system is 70 min. Note that with method 1633 no final concentration step is required. The Parallel-Sequential system produces very good recoveries with low standard deviations.

Surrogate PFAS recoveries (%) and acceptance windows (%) are shown in Table 2. Excellent data were obtained all well within those windows.

Table 3 shows the method detection limits for all 40 native PFAS using synthetic wastewater. Most MDL values are < 0.50 ng/L.

Note that the system has low, partially non-detect, native background values for PFAS and that the risk of cross-contamination is low (Table 4). Values are < 0.02 ng/L.

The Parallel-Sequential system produces data that is as good as other fully automated SPE systems while offering the possibility of running up to 30 samples in approximately 6 hours. The system is ideal for commercial laboratories that want to run extractions overnight with little personnel as it does samples completely unattended. Cleaning the system between runs is quick and easy.

An important problem with ground and wastewater extraction is the presence of particulate matter which can easily plug up cartridges. Use of plastic filtration wool in the barrel of the cartridges can eliminate this problem. In this work no clogging of cartridges was observed.

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