Application Note

Environmental

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Wastewater

Using EPA method 1633 with automated sequential parallel solid phase extraction

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Abstract

This study evaluates the performance of the Fluid Management Systems, Inc. (FMS) TurboTrace Sequential Parallel (SP) automated solid phase extraction system for implementing EPA Method 1633A in wastewater. Using Agilent Bond Elut PFAS WAX cartridges and LC/MS/MS analysis, the system achieved rapid, accurate, and precise sample processing. Spike recoveries in synthetic wastewater met all Method 1633 acceptance criteria across 40 PFAS targets, with relative standard deviations ≤ 10%. Method detection limits (MDLs) were determined for each analyte following 40 CFR Part 136 procedures, demonstrating sub-ng/L sensitivity. Process blanks showed PFAS levels well below MDLs, confirming minimal system-derived contamination. The TurboTrace SP provides a robust and contamination-resistant approach for high-throughput PFAS extraction under Method 1633A, supporting reliable monitoring for aqueous samples.

Introduction

Per- and polyfluoroalkyl substances (PFAS) constitute a group of compounds characterized by perfluorinated or polyfluorinated carbon chain moieties, typically denoted by structures such as $F(CF_2)_n$ or $F(CF_2)_n$-$(C_2H_4)_n$. Due to their unique properties, these substances have been found in extensive applications of various industrial and consumer products.

Many of these applications use 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; fire-fighting foams; surfactants for mining and oil-well operations; floor polishes; and insecticide formulations. Their widespread usage and strong C–F bond strength 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). As a result, the environmental occurrence and potential impact of PFAS on human health are under intense scrutiny.

Recent developments in the United States have led to the introduction of 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, ground water, soil, biosolids, sediment, landfill leachate, and fish tissues. Method 1633 represents a significant advancement in the analytical toolkit for assessing PFAS contamination, facilitating an increased understanding of their distribution and behavior in diverse environmental compartments.

This application note summarizes the validation of recent advancements in PFAS analytical methods, with a focus on the TurboTrace SP system for EPA Method 1633. The TurboTrace SP is a modular, expandable platform. Each module processes up to five samples sequentially, and up to six modules can be combined to operate in parallel, enabling scalable throughput from five to 30 samples in approximately six hours. The system uses controlled positive pressure for solvent delivery and precise sample loading and applies vacuum for high-speed loading of large-volume or particulate-rich samples, as well as efficient cartridge drying.

Experimental

Instrumentation

The TurboTrace SP system was used to extract five synthetic wastewater samples. For analysis of the final extracts, an Agilent 1290 Infinity II LC system was used with the Agilent 6475 triple quadrupole LC/MS. The LC system was modified for PFAS analysis using the Agilent InfinityLab PFAS Analysis HPLC Conversion kit (part number 5004-0006). The Agilent ZORBAX Eclipse Plus C18 3.0 × 50 mm, 1.8 µm HPLC (part number 959757-302), was used as the analytical column. Method parameters are listed in Table 1.

Consumables

Agilent Bond Elut PFAS WAX 150 mg/6 mL solid phase extraction (SPE) cartridges (part number 5610-2150) were used for sample extraction. The cartridges were packed with filtration wool to prevent clogging. Solutions were prepared using ultrapure DI water, pesticide grade methanol, ammonium hydroxide, and formic acid. Method 1633 PFAS standards including the native compounds, extracted internal standards (EIS), and nonextracted internal standards (NIS), were purchased from a standards supplier.

Table 1. Method parameters.

Parameter	Value
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phases	A) 5 mM ammonium acetate in 95:5 water:ACN B) ACN
Gradient	Time (min) %A %B 0.00 98.00 2.00 0.20 98.00 2.00 10.00 5.00 95.00 12.20 5.00 95.00
MS Polarity	Electrospray negative ion mode
Acquisition	Dynamic MRM
Source Gas Temperature	230 °C
Sheath Gas Temperature	355 °C

Method

Samples (500 mL) were spiked with native PFAS (if required) and EIS. The samples bottles were loaded onto the TurboTrace SP and the SPE cartridges were installed. Rinse bottles were automatically filled during the procedure. Positive pressure (nitrogen) was used for pumping solvents and solution mixes through the system and vacuum was used to load the samples. The sample processing stages, which follow the extraction procedure for aqueous samples in Method 1633[1], are listed in Figure 1.

Results and discussion

Initial demonstration of capability

Four replicate synthetic wastewater samples were spiked (1–38 ng/L concentration) and extracted using the TurboTrace SP. Table 2 lists the recovery accuracy and precision (RSD) for each of the 40 PFAS targets. The recovery accuracy and precision for each of the extracts were within the acceptance limits as listed in Method 1633 Table 5[1] for aqueous sample matrices.

Stage 1

1. Condition the cartridges with 15 mL of 1% methanolic ammonium hydroxide followed by 5 mL of 0.3 M formic acid.
2. Load the samples across the cartridges at 5 to 10 mL/min (~8 in Hg).
3. Rinse the sample bottles with two portions of 5 mL reagent water, followed by 5 mL of 1:1 0.1 M formic acid:methanol (using nitrogen).
4. Load sample bottle rinses onto the SPE cartridges.
5. Dry the SPE cartridges for 15 seconds under vacuum.

Stage 2

1. Rinse the sample bottles with 5 mL of 1% methanolic ammonium hydroxide.
2. Load sample bottle rinses onto the SPE cartridges.
3. Collect the 5 mL eluates in polypropylene tubes.

Stage 3

1. Add 25 µL of acetic acid and 10 mg of loose carbon to each tube. Shake for 5 minutes, vortex for 30 seconds, and then centrifuge for 10 minutes.
2. Add NIS to clean polypropylene tubes. Filter extracts into clean polpropylene tubes using a nylon syringe filter.
3. Analyze by LC/MS/MS.

Figure 1. Sample processing stages.

Table 2. Replicate synthetic wastewater target spike recoveries (continued on next page).

Native Compound	Recovery Accuracy (%)				Acceptance Window (%)	RSD (%)
	SEQ-IDC-1	SEQ-IDC-2	SEQ-IDC-3	SEQ-IDC-4		
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

The EIS recoveries for the same extraction set are listed in Table 3 along with the acceptance limits listed in Method 1633, Table 6[1] for aqueous sample matrices. For each of the 24 EIS, the recoveries were within the required limits.

Native Compound	Recovery Accuracy (%)				Acceptance Window (%)	RSD (%)
	SEQ-IDC-1	SEQ-IDC-2	SEQ-IDC-3	SEQ-IDC-4		
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	77.2	74.6	86.6	70–135	6.9
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	86.0	95.5	106.4	96.2	70–135	7.3

Table 3. Replicate synthetic wastewater EIS spike recoveries.

EIS Compound	Recovery Accuracy (%)				Acceptance Window (%)
	SEQ-IDC-1	SEQ-IDC-2	SEQ-IDC-3	SEQ-IDC-4	
$^{13}C_2$-4-2 FTSA	95.5	94.0	121.7	102.3	40–200
$^{13}C_2$-6-2 FTS	97.0	105.0	106.5	95.0	40–200
$^{13}C_2$-8-2 FTSA	95.5	99.4	96.5	105.3	40–300
$^{13}C_2$-PFDoDA	91.8	97.3	99.1	83.4	10–130
$^{13}C_2$-PFTDA	96.8	109.7	112.4	81.7	10–130
$^{13}C_3$-HFPO-DA	95.0	98.5	90.7	96.7	40–130
$^{13}C_3$-PFBS	98.0	110.7	94.0	110.7	40–135
$^{13}C_3$-PFHxS	92.8	93.7	89.2	88.4	40–130
$^{13}C_4$-PFBA	95.2	101.1	88.4	94.4	5–130
$^{13}C_4$-PFHpA	94.8	88.9	102.7	89.4	40–130
$^{13}C_5$-PFHxA	97.5	111.1	96.9	88.3	40–130
$^{13}C_5$-PFPeA	95.5	92.3	98.9	98.0	40–130
$^{13}C_6$-PFDA	95.1	85.5	81.4	80.9	40–130
$^{13}C_7$-PFUnA	85.6	83.6	81.2	79.6	30–130
$^{13}C_8$-PFOA	101.4	92.9	101.3	95.2	50–200
$^{13}C_8$-PFOS	84.0	91.5	92.5	82.2	50–200
$^{13}C_8$-PFOSA	105.1	120.8	120.7	117.3	40–130
$^{13}C_9$-PFNA	91.1	89.5	88.9	95.6	40–130
$^{2}H_3$-N-MeFOSA	95.9	103.5	85.0	94.6	10–130
$^{2}H_3$-N-MeFOSAA	82.5	80.5	84.5	80.5	40–170
$^{2}H_5$-N-EtFOSA	94.1	90.5	86.6	78.1	10–130
$^{2}H_5$-N-EtFOSAA	89.6	89.9	83.1	82.4	25–135
$^{2}H_7$-MeFOSE	93.0	90.6	90.6	83.7	10–130
$^{2}H_9$-EtFOSE	92.0	106.0	89.5	87.5	10–130

Method detection limits (MDLs)

A method detection limit study was conducted with seven replicate synthetic wastewater spikes with the 40 PFAS targets (0.2 to 9 ng/L). MDLs were calculated using the method described in 40 CFR Appendix B to Part 136.[2] The measured concentration for each of the spikes as well as the calculated standard deviations (STDs) and MDLs are listed in Table 4.

Table 4. MDLs in synthetic wastewater samples.

Native Compound	Concentration (ng/L)							STD	MDL
	SEQ-MDL-1	SEQ-MDL-2	SEQ-MDL-3	SEQ-MDL-4	SEQ-MDL-5	SEQ-MDL-6	SEQ-MDL-7		
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	14.73	13.62	13.69	13.42	14.26	13.56	15.15	0.67	2.09
6-2 FTS	2.55	2.68	2.55	2.70	2.71	2.15	2.61	0.19	0.61
7-3 FTCA	10.20	8.64	8.63	8.55	8.98	8.12	10.34	0.86	2.71
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	7.11	6.19	5.80	5.99	5.99	6.12	7.48	0.64	2.02
HFPO-DA	2.88	2.90	2.94	2.61	2.75	2.70	3.53	0.30	0.95
MeFOSE	6.83	5.34	6.09	5.90	5.00	5.13	6.61	0.72	2.26
N-EtFOSA	0.46	0.44	0.47	0.33	0.43	0.57	0.78	0.14	0.45
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

Blank analysis

A reagent water blank was processed to measure any residual PFAS contamination associated with the extraction process. Results of the analysis are shown in Figure 2. Residual PFAS were well below the MDLs indicating exceptional cleanliness of the TurboTrace SP system and associated consumables.

Conclusion

The FMS TurboTrace SP system demonstrates its efficiency by successfully analyzing all 40 native PFAS compounds using EPA Method 1633 in twelve synthetic wastewaters samples. Spike recoveries were well within the acceptance windows of the method with RSDs all ≤ 10%. MDLs were < 0.50 ng/L. Cartridge clogging was not observed with any of the matrix samples further underlining the system's reliability.

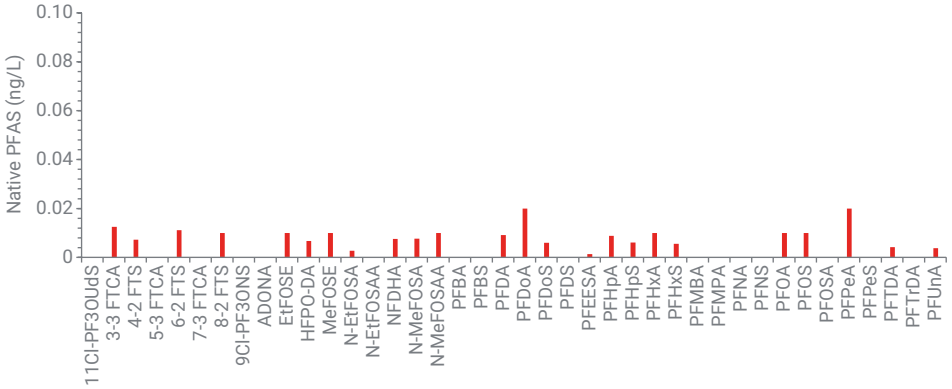

Figure 2. PFAS residue measure in process blank analysis.

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DE-012883

Printed in the USA, March 26, 2026
5994-9034EN

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