Application Note

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

Using EPA method 1633 with the FMS TurboTrace PFC automated solid phase extraction

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Abstract

This study evaluates the performance of the Fluid Management Systems, Inc. (FMS) TurboTrace PFC automated solid phase extraction (SPE) system for implementing EPA Method 1633A in aqueous samples. 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 low ng/L sensitivity. Process blanks showed PFAS levels well below MDLs, confirming minimal system-derived contamination. The TurboTrace PFC 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; firefighting 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 particular emphasis on the TurboTrace PFC SPE workflow for EPA Method 1633. The TurboTrace PFC system is a fully automated SPE platform designed to meet the requirements of all PFAS SPE methods. Its modular architecture supports up to four modules, each capable of processing two samples simultaneously, allowing throughput of up to eight samples at once. The system uses controlled positive pressure for consistent solvent delivery and precise sample loading, while applying vacuum to enable high-speed processing of large-volume or particulate-rich samples and to ensure efficient cartridge drying.

Experimental

Instrumentation

The TurboTrace PFC system was used to extract PFAS from synthetic wastewater and two well water samples. For analysis of the final extracts, an Agilent 1290 Infinity II LC system was used with an Agilent 6475 triple quadrupole LC/MS. The LC system was modified for PFAS analysis using an Agilent InfinityLab PFAS analysis HPLC conversion kit (part number 5004-0006). An 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.

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

Consumables

Agilent Bond Elut PFAS WAX 150 mg/6 mL 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.

Method

Samples (500 mL) were spiked with native PFAS (if required) and EIS. The sample bottles were loaded onto the TurboTrace PFC 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.

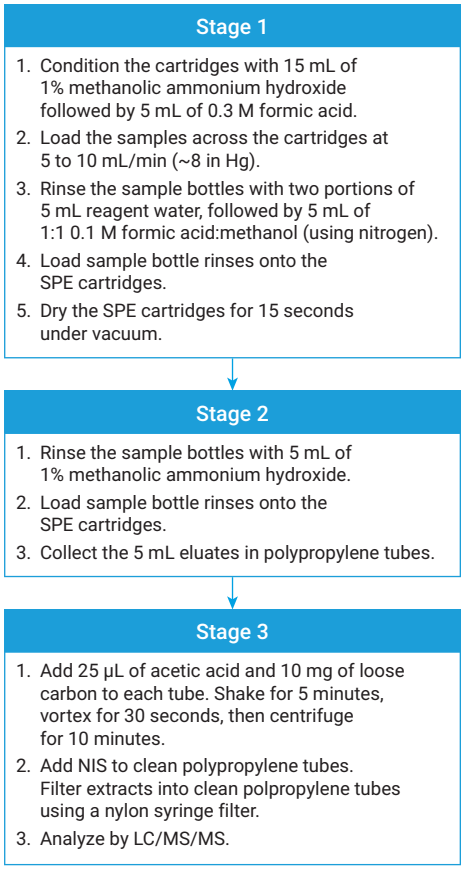

Figure 1. Sample processing stages.

Results and discussion

Initial demonstration of capability

Four replicate synthetic wastewater samples were spiked (1 to 38 ng/L concentration) and extracted using the TurboTrace PFC. 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 51 for aqueous sample matrices.

Table 2. Replicate synthetic wastewater target spike recoveries.

Native Compound	Recovery Accuracy (%)				Acceptance Window (%)	RSD (%)
	Turbo IDC-1	Turbo IDC-2	Turbo IDC-3	Turbo IDC-4		
11Cl-PF3OUdS	89.7	89.4	83.2	82.8	50–150	4.4
3-3 FTCA	98.0	98.7	99.5	98.0	70–130	0.7
4-2 FTS	95.9	100.3	96.3	93.3	70–135	3.0
5-3 FTCA	99.7	92.8	100.1	100.2	70–130	3.7
6-2 FTS	104.3	111.0	107.3	108.7	70–135	2.6
7-3 FTCA	93.2	98.2	95.5	96.7	55–130	2.2
8-2 FTS	94.8	95.8	96.6	97.7	70–140	1.3
9Cl-PF3ONS	103.0	101.8	91.4	99.7	70–145	5.3
ADONA	107.4	104.1	96.5	99.3	70–135	4.8
EtFOSE	102.0	96.3	104.9	104.0	70–130	3.8
HFPO-DA	102.4	98.0	93.1	100.6	70–135	4.1
MeFOSE	99.5	103.9	95.3	100.8	70–135	3.6
N-EtFOSA	103.2	89.5	98.6	108.7	70–135	8.1
N-EtFOSAA	101.3	93.6	98.9	100.2	70–135	3.5
NFDHA	97.6	95.0	98.1	99.6	65–140	2.0
N-MeFOSA	106.3	95.2	90.7	97.0	70–135	6.7
N-MeFOSAA	98.8	97.4	96.4	90.6	65–140	3.8
PFBA	99.6	99.2	100.9	99.9	70–135	0.7
PFBS	100.9	100.6	95.5	89.3	70–140	5.6
PFDA	107.8	103.0	107.4	93.8	65–140	6.3
PFDoA	101.8	94.0	99.3	91.9	70–130	4.7
PFDoS	86.7	88.2	98.0	91.0	45–135	5.5
PFDS	94.7	98.3	106.2	96.5	70–135	5.1
PFEESA	95.8	93.1	97.9	94.6	70–135	2.1
PFHpA	96.1	99.3	91.1	103.2	70–135	5.3
PFHpS	118.9	112.7	121.0	103.8	70–140	6.7
PFHxA	98.6	96.2	99.4	98.1	70–135	1.4
PFHxS	116.6	113.1	106.6	113.1	70–135	3.7
PFMBA	101.1	100.7	99.8	98.7	65–145	1.0
PFMPA	98.0	100.6	100.6	98.7	60–140	1.3
PFNA	92.3	103.8	99.3	97.3	70–140	4.8
PFNS	100.4	96.3	110.7	93.4	70–135	7.6
PFOA	115.7	117.0	104.9	102.9	65–155	6.6
PFOS	93.5	94.7	98.7	102.2	70–140	4.1
PFOSA	110.8	105.5	94.3	101.1	70–135	6.8
PFPeA	100.9	101.0	101.2	99.5	70–135	0.8
PFPeS	103.7	102.8	99.3	94.1	70–135	4.3
PFTDA	105.5	106.4	108.1	106.0	70–145	1.1
PFTrDA	96.8	101.9	99.9	88.0	60–145	6.3
PFUnA	115.8	99.9	101.4	95.6	70–135	8.5

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.

Table 3. Replicate synthetic wastewater EIS spike recoveries.

EIS Compound	Recovery Accuracy (%)				Acceptance Window (%)
	Turbo IDC-1	Turbo IDC-2	Turbo IDC-3	Turbo IDC-4	
$^{13}C_2$-4-2 FTSA	95.3	102.2	105.9	91.6	40–200
$^{13}C_2$-6-2 FTS	92.4	99.7	93.0	82.5	40–200
$^{13}C_2$-8-2 FTSA	87.6	106.4	106.7	87.8	40–300
$^{13}C_2$-PFDoDA	95.7	82.2	94.2	81.4	10–130
$^{13}C_2$-PFTDA	82.3	88.1	86.4	87.1	10–130
$^{13}C_3$-HFPO-DA	97.1	109.2	102.6	102.9	40–130
$^{13}C_3$-PFBS	98.0	108.8	105.3	95.8	40–135
$^{13}C_3$-PFHxS	86.7	93.6	104.4	96.3	40–130
$^{13}C_4$-PFBA	96.6	104.3	99.5	97.4	5–130
$^{13}C_4$-PFHpA	98.3	99.3	101.2	99.3	40–130
$^{13}C_5$-PFHxA	99.2	101.2	99.7	103.3	40–130
$^{13}C_5$-PFPeA	98.9	104.5	99.1	103.2	40–130
$^{13}C_6$-PFDA	80.9	89.9	82.5	93.8	40–130
$^{13}C_7$-PFUnA	96.0	92.7	103.8	86.8	30–130
$^{13}C_8$-PFOA	110.9	101.9	106.8	101.9	50–200
$^{13}C_8$-PFOS	102.0	83.6	84.8	88.5	50–200
$^{13}C_8$-PFOSA	84.6	97.7	106.0	92.9	40–130
$^{13}C_9$-PFNA	97.8	93.2	106.3	88.9	40–130
$^{2}H_3$-N-MeFOSA	80.6	80.3	87.1	80.3	10–130
$^{2}H_3$-N-MeFOSAA	105.4	95.5	91.0	114.6	40–170
$^{2}H_5$-N-EtFOSA	81.9	79.3	76.4	78.0	10–130
$^{2}H_5$-N-EtFOSAA	86.2	97.6	95.6	88.8	25–135
$^{2}H_7$-MeFOSE	95.8	85.7	102.2	83.0	10–130
$^{2}H_9$-EtFOSE	87.1	81.6	87.5	84.7	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	0.79	0.77	0.94	0.68	0.74	0.73	0.75	0.08	0.25
3-3 FTCA	1.82	1.81	1.83	1.77	1.86	1.78	1.88	0.04	0.13
4-2 FTS	3.05	3.12	3.17	3.16	3.18	3.11	3.08	0.05	0.16
5-3 FTCA	2.34	2.47	2.29	2.42	2.45	2.38	2.49	0.07	0.22
6-2 FTS	2.96	3.20	3.20	3.02	3.17	2.87	3.19	0.13	0.42
7-3 FTCA	1.92	1.91	1.96	1.93	2.09	1.95	2.09	0.08	0.24
8-2 FTS	4.19	4.15	4.57	4.37	4.43	4.54	4.61	0.18	0.57
9Cl-PF3ONS	1.03	0.85	1.05	0.92	0.88	0.87	0.93	0.08	0.24
ADONA	0.96	0.82	1.69	0.83	0.87	0.83	0.93	0.32	0.99
EtFOSE	2.90	2.88	2.76	2.88	2.91	2.93	3.08	0.10	0.30
HFPO-DA	5.97	6.48	6.09	6.07	6.86	6.62	7.00	0.41	1.28
MeFOSE	2.58	2.67	2.61	2.67	2.73	2.77	2.86	0.10	0.30
N-EtFOSA	2.78	2.75	2.87	2.86	2.72	2.88	2.82	0.06	0.19
N-EtFOSAA	2.41	2.39	2.23	2.33	2.36	2.31	2.32	0.06	0.18
NFDHA	3.17	3.10	3.01	3.25	3.25	3.67	2.61	0.32	1.00
N-MeFOSA	2.18	2.79	2.22	2.32	2.51	2.34	2.36	0.21	0.65
N-MeFOSAA	2.92	2.88	2.92	2.67	2.91	2.92	2.80	0.09	0.29
PFBA	2.76	2.97	2.67	2.96	2.92	2.95	2.99	0.12	0.38
PFBS	3.16	3.29	3.16	3.27	3.09	3.15	3.24	0.07	0.23
PFDA	2.38	2.26	2.25	2.38	2.34	2.47	2.32	0.08	0.24
PFDoA	2.77	2.65	2.63	2.71	2.65	2.77	2.64	0.06	0.20
PFDoS	3.10	3.00	2.87	2.87	2.90	2.95	2.94	0.08	0.26
PFDS	2.89	2.83	2.83	2.77	2.87	2.87	2.87	0.04	0.13
PFEESA	2.91	3.55	3.20	3.62	3.70	3.66	3.86	0.33	1.03
PFHpA	2.80	2.50	2.25	2.58	2.61	2.70	2.61	0.17	0.54
PFHpS	2.85	2.74	3.18	3.10	3.04	3.16	2.94	0.16	0.51
PFHxA	2.53	2.67	2.41	2.48	2.65	2.54	2.70	0.11	0.34
PFHxS	2.92	3.36	3.59	3.50	3.22	3.57	3.56	0.24	0.77
PFMBA	2.50	2.63	2.76	2.64	2.74	2.64	2.70	0.09	0.27
PFMPA	2.31	2.32	2.22	2.25	2.30	2.30	2.40	0.06	0.18
PFNA	2.35	2.30	2.15	2.40	2.36	2.37	2.21	0.09	0.29
PFNS	2.73	2.77	2.74	2.72	2.64	2.70	2.68	0.04	0.13
PFOA	2.39	2.41	2.45	2.48	2.44	2.41	2.43	0.03	0.09
PFOS	2.95	2.98	2.75	2.83	2.83	2.78	2.92	0.09	0.28
PFOSA	2.33	2.49	2.24	2.31	2.48	2.49	2.57	0.12	0.38
PFPeA	2.63	2.68	2.77	2.76	2.76	2.74	2.66	0.06	0.18
PFPeS	2.52	2.44	2.50	2.28	2.57	2.75	2.74	0.17	0.52
PFTDA	2.86	2.84	2.99	2.81	2.91	2.80	3.00	0.08	0.25
PFTrDA	2.32	2.28	2.47	2.28	2.33	2.24	2.44	0.09	0.28
PFUnA	2.35	2.49	2.48	2.67	2.55	2.74	2.38	0.14	0.45

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 PFC system and associated consumables.

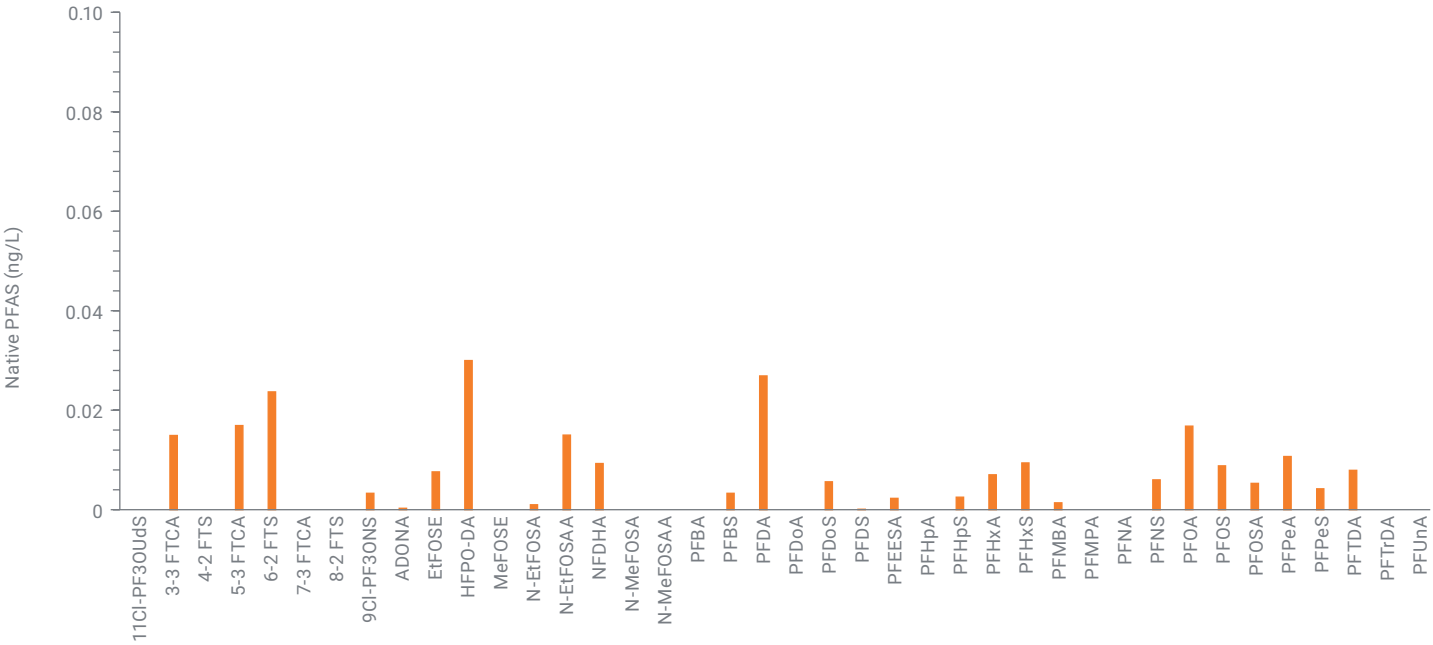

Figure 2. PFAS residue measure in process blank analysis.

Aqueous matrix samples

Well water from two sources were analyzed in duplicate. Results are listed in Table 5. Good reproducibility was found between the replicates. The presence of particulate matter is a common challenge with water samples because it can easily lead to cartridge clogging. By incorporating a stainless-steel sample bottle filter and plastic filtration wool in the barrel of the cartridges, this issue is effectively eliminated. In our work, we observed no instances of cartridge clogging, underscoring the system's reliability and robustness.

Table 5. PFAS measurements in well, river, and tap water samples.

	Concentration (ng/L)			
Native Compound	Well Water A1	Well Water A2	Well Water B1	River Water B2
11Cl-PF3OUdS	ND	ND	ND	0.02
3-3 FTCA	ND	ND	ND	ND
4-2 FTS	0.12	0.06	0.09	0.01
5-3 FTCA	0.02	0.03	0.05	0.04
6-2 FTS	0.05	0.03	0.05	0.09
7-3 FTCA	0.03	0.02	ND	ND
8-2 FTS	ND	0.01	ND	ND
9Cl-PF3ONS	0.01	0.01	ND	ND
ADONA	ND	ND	ND	ND
EtFOSE	0.01	ND	0.01	0.05
HFPO-DA	ND	0.03	0.04	0.03
MeFOSE	0.01	0.01	ND	ND
N-EtFOSA	0.01	ND	ND	0.01
N-EtFOSAA	ND	0.01	0.01	0.01
NFDHA	0.01	ND	ND	0.01
N-MeFOSA	ND	ND	ND	0.01
N-MeFOSAA	ND	ND	ND	0.01
PFBA	1.46	1.40	1.52	0.77
PFBS	1.47	1.54	1.52	1.41
PFDA	0.09	0.04	0.07	0.06
PFDoA	ND	ND	ND	0.01
PFDoS	ND	ND	ND	ND
PFDS	ND	ND	ND	ND
PFEESA	ND	ND	ND	ND
PFHpA	0.68	0.89	0.84	0.59
PFHpS	0.02	0.08	0.03	0.06
PFHxA	1.84	1.92	1.85	0.99
PFHxS	0.81	0.93	0.89	0.83
PFMBA	ND	ND	ND	ND
PFMPA	ND	ND	ND	ND
PFNA	0.14	0.17	0.14	0.21
PFNS	ND	0.01	ND	ND
PFOA	3.08	3.60	3.65	1.43
PFOS	0.44	0.38	0.41	1.30
PFOSA	0.02	0.01	0.01	0.01
PFPeA	1.58	1.65	1.74	0.94
PFPeS	0.09	0.14	0.09	0.12
PFTDA	0.01	ND	ND	ND
PFTrDA	ND	ND	ND	ND
PFUnA	ND	ND	ND	ND

Conclusion

The FMS TurboTrace PFC system demonstrates its efficiency by successfully analyzing all 40 native PFAS compounds using EPA Method 1633 in 12 synthetic wastewaters samples. Spike recoveries were well within the acceptance windows of the method with RSDs all ≤ 10%. MDLs were in the low-to-sub ng/L range. Cartridge clogging was not observed with any of the matrix samples, further demonstrating the reliability of the system.

References

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