Application Note
Environmental

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

Using EPA method 1633 with semi-automated solid phase extraction

Authors

Ruud Addink, Rashid Juma, and Tom Hall
Fluid Management Systems, Inc.

Matthew Giardina
Agilent Technologies, Inc.

Abstract

This study evaluates the performance of the Fluid Management Systems, Inc. (FMS) EZPFC Semi-Automated Solid Phase Extraction System for implementing EPA Method 1633A in wastewater and other aqueous samples. Using Agilent Bond Elut PFAS WAX cartridges and LC/MS/MS analysis, the system achieved rapid sample processing (< 70 minutes for 6 to 12 samples). Across 40 PFAS targets, spike recoveries in synthetic wastewater met all Method 1633 acceptance criteria, with relative standard deviations below 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. Triplicate analyses of well, river, and tap water samples exhibited strong reproducibility and no cartridge clogging, even in particulate-rich matrices. Overall, the EZPFC workflow provides a robust, low-cost, and contamination-resistant approach for high-throughput PFAS extraction under Method 1633A, supporting reliable monitoring across diverse environmental waters.

Introduction

In recent years, new tools have significantly improved efforts to assess and address the PFAS challenge. In 2021, the U.S. Environmental Protection Agency (EPA) released Method 1633, their first version of an analytical method addressing complex sample matrices. Since then, the EPA has published several updates as Method 1633 progressed through the evaluation/validation process. In January 2024, the final multi-laboratory validation steps were completed, removing and the "draft" status. The latest revision of the method was released by the EPA as not just a tool, but as a comprehensive solution designed to cover a wide range of PFAS sample matrices. These include groundwater, surface water, wastewater, landfill leachate, soils, sediments, biosolids, and biological tissue, providing a high level of accuracy and reliability in PFAS analysis.

This application note is an overview of the validation of recent advancements in PFAS analytical methods, with a special focus on using the FMS EZPFC system for EPA Method 1633A in multiple water matrices. This development is crucial for all environmental scientists and professionals involved in PFAS analysis and environmental monitoring; the EZPFC provides a low-cost, high-throughput sample preparation workflow that provides the highest quality results while minimizing PFAS contamination. The semi-automated system completes sample processing in under 70 minutes for 6 or 12 samples, without the need for a final concentration step, and consistently produces excellent recovery accuracy and precision. The EZPFC system is user-friendly with a simplified design that minimizes the risk of target breakdown and contamination. Cleaning the system between runs is easy, further enhancing its practicality and efficiency.

Experimental

Instrumentation

The EZPFC system and vacuum pump were used for sample extraction. An Agilent 1290 Infinity II LC System with the Agilent 6475 triple quadrupole LC/MS were used to analyze final extracts, and the LC system was modified for PFAS analysis using the Agilent InfinityLab PFAS Analysis HPLC Conversion kit. The Agilent ZORBAX Eclipse Plus C18 3.0 × 50 mm, 1.8 µm HPLC, was used as the analytical column. Method parameters are listed in Table 1.

Table 1. Method parameters.

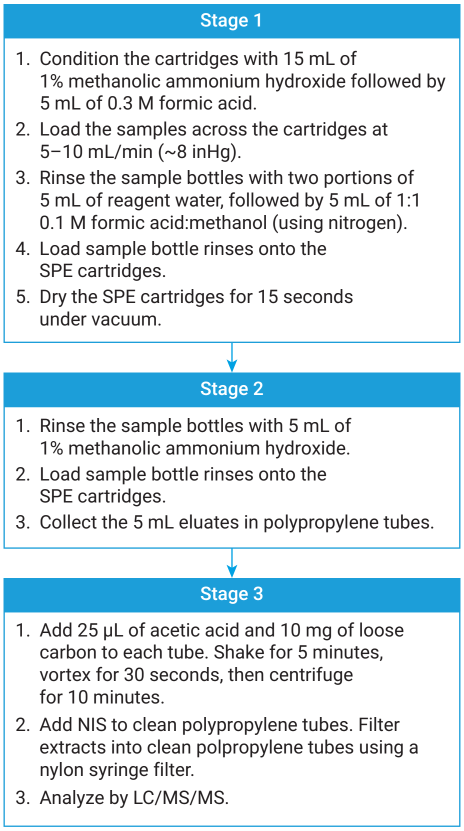
Parameter	Value
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phases	A) 5 mM ammonium acetate in 95:5 water:ACN B) ACN
Gradient	Time (min) %A %B 0.00 98.00 2.00 0.20 98.00 2.00 10.00 5.00 95.00 12.20 5.00 95.00
MS Polarity	Electrospray negative ion mode
Acquisition	Dynamic MRM
Source Gas Temperature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

Consumables

Agilent Bond Elut PFAS WAX 150 mg/6 mL solid phase extraction (SPE) cartridges (part number 5610-2150) were used for sample extraction. Solutions were prepared using ultrapure DI water, pesticide-grade methanol, ammonium hydroxide, and formic acid. EPA 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 samples bottles were loaded onto the EZPFC and the SPE cartridges were installed. The rinse bottles were filled with 5 mL of reagent water, and the vacuum pump was turned on and remained on for the duration of the extractions. The sample processing stages, which follow the extraction procedure for aqueous samples in EPA Method 1633, are listed in Figure 1.



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 5–10 mL/min (~8 inHg).
3. Rinse the sample bottles with two portions of 5 mL of 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, 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.

Results and discussion

Initial demonstration of capability

Four replicate synthetic wastewater samples were spiked (1 to 38 ng/L concentration) and extracted using the EZPFC. 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 EPA Method 1633 Table 5[1] for aqueous sample matrices.

The EIS recoveries for the same extraction set are listed in Table 3 along with the acceptance limits listed in EPA 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.

	Recovery Accuracy (%)				
EIS Compound	EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Acceptance Window (%)
$^{13}\text{C}_2$-4-2 FTSA	95.1	93.7	105.3	86.3	40–200
$^{13}\text{C}_2$-6-2 FTS	96.9	104.7	106.7	96.0	40–200
$^{13}\text{C}_2$-8-2 FTSA	91.1	98.3	94.4	80.8	40–300
$^{13}\text{C}_2$-PFDoDA	81.6	97.7	98.4	89.6	10–130
$^{13}\text{C}_2$-PFTDA	90.3	96.9	99.3	94.4	10–130
$^{13}\text{C}_3$-HFPO-DA	103.0	110.3	106.0	99.3	40–130
$^{13}\text{C}_3$-PFBS	89.0	96.9	96.3	90.0	40–135
$^{13}\text{C}_3$-PFHxS	95.9	108.0	115.7	95.9	40–130
$^{13}\text{C}_4$-PFBA	92.0	102.6	100.7	93.4	5–130
$^{13}\text{C}_4$-PFHpA	95.9	112.5	107.0	100.0	40–130
$^{13}\text{C}_5$-PFHxA	93.4	102.0	103.2	91.3	40–130
$^{13}\text{C}_5$-PFPeA	94.2	98.9	97.2	87.6	40–130
$^{13}\text{C}_6$-PFDA	81.8	93.0	95.7	85.3	40–130
$^{13}\text{C}_7$-PFUnA	83.9	86.4	95.9	83.0	30–130
$^{13}\text{C}_8$-PFOA	89.0	106.0	99.6	96.1	50–200
$^{13}\text{C}_8$-PFOS	93.9	110.4	91.3	92.5	50–200
$^{13}\text{C}_8$-PFOSA	90.4	90.0	88.1	89.7	40–130
$^{13}\text{C}_9$-PFNA	85.0	89.0	82.6	80.1	40–130
$^{2}\text{H}_3$-N-MeFOSA	87.6	91.0	76.8	74.2	10–130
$^{2}\text{H}_3$-N-MeFOSAA	95.5	93.0	87.7	84.5	40–170
$^{2}\text{H}_5$-N-EtFOSA	95.8	96.6	90.0	80.0	10–130
$^{2}\text{H}_5$-N-EtFOSAA	89.6	79.0	91.3	80.3	25–135
$^{2}\text{H}_7$-MeFOSE	93.3	89.0	90.1	87.3	10–130
$^{2}\text{H}_9$-EtFOSE	92.2	105.8	89.3	88.7	10–130

Table 2. Replicate synthetic wastewater target spike recoveries.

	Recovery Accuracy (%)					
Native Compound	EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Acceptance Window (%)	RSD (%)
11Cl-PF3OUdS	92.4	93.2	99.9	96.9	50–150	3.6
3-3 FTCA	87.3	96.1	92.6	101.2	70–130	6.2
4-2 FTS	89.1	97.6	93.5	95.8	70–135	3.9
5-3 FTCA	96.2	96.3	92.9	97.7	70–130	2.1
6-2 FTS	91.8	95.1	95.1	95.7	70–135	1.9
7-3 FTCA	98.0	97.0	95.4	101.8	55–130	2.8
8-2 FTS	92.2	91.1	99.5	109.2	70–140	8.5
9Cl-PF3ONS	93.0	91.7	96.8	96.6	70–145	2.7
ADONA	87.3	89.9	94.1	88.7	70–135	3.2
EtFOSE	95.2	94.8	94.7	95.9	70–130	0.6
HFPO-DA	92.2	111.0	100.1	90.8	70–135	9.4
MeFOSE	88.6	95.8	88.8	94.2	70–135	4.0
N-EtFOSA	88.6	93.1	90.6	96.2	70–135	3.6
N-EtFOSAA	93.0	90.3	81.5	101.0	70–135	8.8
NFDHA	92.6	92.3	94.2	90.7	65–140	1.6
N-MeFOSA	93.9	101.4	100.1	106.4	70–135	5.1
N-MeFOSAA	85.9	95.1	105.1	95.9	65–140	8.2
PFBA	93.3	96.4	93.1	95.1	70–135	1.7
PFBS	108.8	98.4	99.6	98.9	70–140	4.9
PFDA	98.2	94.3	89.7	96.1	65–140	3.8
PFDoA	93.0	92.0	88.4	92.7	70–130	2.3
PFDoS	93.2	104.7	87.2	95.7	45–135	7.6
PFDS	84.6	91.2	96.8	86.5	70–135	6.1
PFEESA	95.3	99.4	95.5	96.0	70–135	2.0
PFHpA	99.4	100.8	93.9	116.4	70–135	9.4
PFHpS	90.8	108.3	101.4	99.0	70–140	7.2
PFHxA	95.6	97.5	93.3	100.3	70–135	3.1
PFHxS	89.9	90.6	87.0	95.4	70–135	3.9
PFMBA	90.9	92.2	95.9	95.1	65–145	2.5
PFMPA	106.2	107.4	115.0	112.7	60–140	3.8
PFNA	104.6	100.3	95.9	107.1	70–140	4.9
PFNS	96.1	100.4	95.4	99.1	70–135	2.4
PFOA	101.7	88.7	98.8	105.3	65–155	7.2
PFOS	97.0	109.5	119.6	103.7	70–140	8.9
PFOSA	106.8	102.1	105.1	107.1	70–135	2.2
PFPeA	92.8	98.2	96.5	96.1	70–135	2.4
PFPeS	92.9	90.5	87.3	102.8	70–135	7.2
PFTDA	90.6	92.5	94.3	89.0	70–145	2.5
PFTrDA	92.5	97.8	94.5	93.1	60–145	2.5
PFUnA	88.8	105.5	93.4	100.4	70–135	7.6

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.

	Concentration (ng/L)								
Native Compound	EZPFC MDL-1	EZPFC MDL-2	EZPFC MDL-3	EZPFC MDL-4	EZPFC MDL-5	EZPFC MDL-6	EZPFC MDL-7	STD	MDL
11Cl-PF3OUdS	1.24	1.30	1.47	1.00	1.19	1.30	0.93	0.19	0.58
3-3 FTCA	1.08	1.53	1.71	1.07	1.39	1.29	1.26	0.23	0.73
4-2 FTS	1.46	1.43	1.48	1.44	1.21	1.62	1.50	0.12	0.39
5-3 FTCA	8.16	8.96	10.10	7.16	8.24	9.73	6.98	1.19	3.75
6-2 FTS	1.54	1.61	1.58	1.51	1.30	1.74	1.28	0.17	0.53
7-3 FTCA	8.73	9.36	9.52	8.02	8.94	9.47	6.94	0.94	2.96
8-2 FTS	1.26	1.32	1.51	1.76	1.32	1.56	1.43	0.17	0.55
9Cl-PF3ONS	1.02	1.31	1.26	0.98	1.13	1.17	0.95	0.14	0.44
ADONA	0.95	1.07	1.27	0.95	0.97	1.18	0.92	0.13	0.42
EtFOSE	3.21	3.20	3.79	3.46	3.04	3.80	3.24	0.30	0.95
HFPO-DA	0.98	1.22	1.75	1.43	1.34	1.46	1.16	0.25	0.79
MeFOSE	3.41	3.23	3.83	3.28	2.77	3.48	3.36	0.32	1.01
N-EtFOSA	0.24	0.23	0.25	0.28	0.34	0.34	0.37	0.05	0.17
N-EtFOSAA	0.30	0.34	0.35	0.33	0.38	0.21	0.32	0.05	0.17
NFDHA	0.60	0.64	0.63	0.55	0.55	0.64	0.52	0.05	0.16
N-MeFOSA	0.41	0.28	0.27	0.31	0.24	0.35	0.21	0.07	0.21
N-MeFOSAA	0.31	0.33	0.28	0.31	0.25	0.34	0.36	0.04	0.12
PFBA	1.12	1.19	1.35	1.21	1.05	1.30	1.16	0.10	0.33
PFBS	0.26	0.34	0.33	0.33	0.22	0.41	0.31	0.06	0.19
PFDA	0.27	0.31	0.29	0.32	0.22	0.36	0.28	0.04	0.14
PFDoA	0.33	0.31	0.43	0.29	0.30	0.31	0.30	0.05	0.15
PFDoS	0.36	0.25	0.35	0.29	0.28	0.33	0.32	0.04	0.12
PFDS	0.25	0.32	0.49	0.26	0.26	0.31	0.28	0.08	0.26
PFEESA	0.72	0.70	0.86	0.67	0.53	0.90	0.66	0.13	0.39
PFHpA	0.33	0.31	0.30	0.28	0.26	0.29	0.30	0.02	0.07
PFHpS	0.34	0.28	0.49	0.39	0.26	0.33	0.20	0.09	0.29
PFHxA	0.30	0.30	0.35	0.30	0.27	0.32	0.29	0.02	0.08
PFHxS	0.40	0.28	0.32	0.27	0.27	0.34	0.38	0.05	0.17
PFMBA	0.51	0.58	0.61	0.54	0.45	0.61	0.56	0.06	0.18
PFMPA	0.50	0.54	0.58	0.38	0.41	0.49	0.46	0.07	0.22
PFNA	0.29	0.25	0.33	0.31	0.29	0.35	0.23	0.04	0.13
PFNS	0.23	0.21	0.25	0.34	0.22	0.07	0.23	0.08	0.25
PFOA	0.46	0.32	0.28	0.23	0.26	0.30	0.32	0.08	0.24
PFOS	0.40	0.28	0.32	0.52	0.33	0.35	0.30	0.08	0.26
PFOSA	0.30	0.32	0.32	0.28	0.30	0.32	0.26	0.02	0.07
PFPeA	0.55	0.60	0.70	0.63	0.50	0.63	0.57	0.06	0.20
PFPeS	0.27	0.31	0.35	0.23	0.29	0.35	0.40	0.06	0.18
PFTDA	0.21	0.26	0.39	0.23	0.23	0.22	0.31	0.06	0.20
PFTrDA	0.29	0.24	0.39	0.29	0.28	0.30	0.30	0.04	0.14
PFUnA	0.29	0.28	0.29	0.32	0.30	0.31	0.25	0.02	0.08

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 EZPFC system and associated consumables.

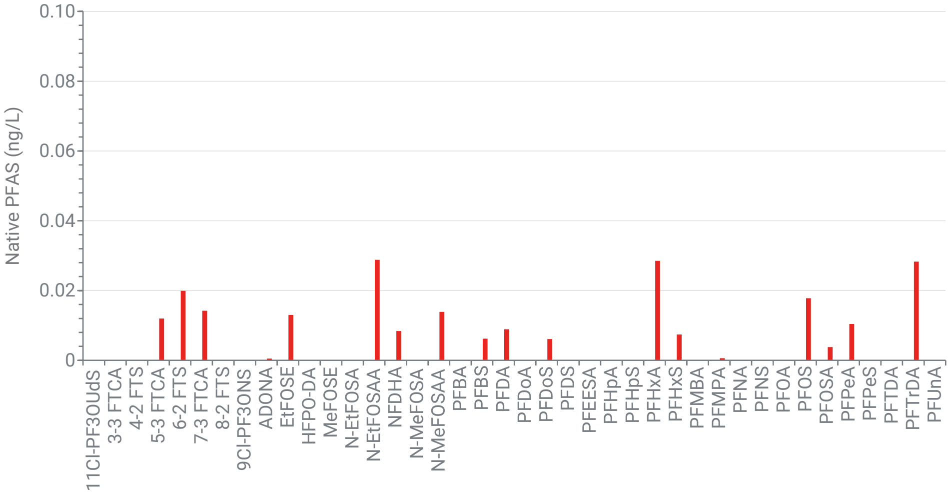


Figure 2. PFAS residue measure in process blank analysis.

Aqueous matrix samples

Three aqueous sample types including well water, river water, and tap water were analyzed in triplicate. Results are listed in Table 5. Good reproducibility was found between the replicates. A common challenge in ground water and wastewater extraction is the presence of particulate matter, which can easily lead to cartridge clogging. By incorporating 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.

Native Compound	Concentration (ng/L)								
	Well Water-1	Well Water-2	Well Water-3	River Water-1	River Water-2	River Water-3	Tap Water-1	Tap Water-2	Tap Water-3
11Cl-PF3OUdS	ND	ND	ND	0.02	ND	ND	ND	ND	ND
3-3 FTCA	ND	ND	ND	ND	0.01	ND	ND	ND	ND
4-2 FTS	0.12	0.06	0.09	0.01	0.01	0.03	0.02	0.01	0.01
5-3 FTCA	0.02	0.03	0.05	0.04	0.12	0.02	0.03	0.02	0.02
6-2 FTS	0.05	0.03	0.05	0.09	0.03	0.03	0.04	0.05	0.04
7-3 FTCA	0.03	0.02	ND	ND	0.04	ND	ND	ND	ND
8-2 FTS	ND	0.01	ND	ND	ND	ND	ND	ND	ND
9Cl-PF3ONS	0.01	0.01	ND	ND	ND	ND	0.01	ND	ND
ADONA	ND	ND	ND	ND	ND	ND	ND	ND	ND
EtFOSE	0.01	ND	0.01	0.05	0.01	0.02	ND	0.01	0.02
HFPO-DA	ND	0.03	0.04	0.03	0.02	0.05	0.02	ND	0.05
MeFOSE	0.01	0.01	ND	ND	ND	ND	0.01	0.01	0.06
N-EtFOSA	0.01	ND	ND	0.01	ND	ND	ND	ND	ND
N-EtFOSAA	ND	0.01	0.01	0.01	0.02	0.05	0.01	0.01	0.03
NFDHA	0.01	ND	ND	0.01	ND	0.01	ND	0.01	ND
N-MeFOSA	ND	ND	ND	0.01	0.01	ND	ND	ND	0.02
N-MeFOSAA	ND	ND	ND	0.01	0.02	0.01	0.01	0.01	0.01
PFBA	1.46	1.40	1.52	0.77	0.56	0.73	0.60	0.57	0.65
PFBS	1.47	1.54	1.52	1.41	1.04	1.39	0.75	0.80	0.86
PFDA	0.09	0.04	0.07	0.06	0.03	0.06	0.02	0.01	ND
PFDoA	ND	ND	ND	0.01	0.03	0.01	0.02	0.02	0.02
PFDoS	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFDS	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFEESA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFHpA	0.68	0.89	0.84	0.59	0.55	0.62	0.36	0.39	0.42
PFHpS	0.02	0.08	0.03	0.06	0.03	0.03	0.04	ND	0.07
PFHxA	1.84	1.92	1.85	0.99	0.90	1.03	0.54	0.57	0.61
PFHxS	0.81	0.93	0.89	0.83	0.49	0.74	0.66	0.55	0.59
PFMBA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFNA	0.14	0.17	0.14	0.21	0.17	0.15	ND	0.06	0.07
PFNS	ND	0.01	ND	ND	0.01	ND	ND	ND	ND
PFOA	3.08	3.60	3.65	1.43	1.28	1.29	1.06	0.82	1.02
PFOS	0.44	0.38	0.41	1.30	1.38	1.70	0.64	0.55	0.81
PFOSA	0.02	0.01	0.01	0.01	0.01	ND	0.02	0.01	0.01
PFPeA	1.58	1.65	1.74	0.94	0.80	0.89	0.66	0.63	0.70
PFPeS	0.09	0.14	0.09	0.12	0.10	0.05	0.11	0.11	0.12
PFTDA	0.01	ND	ND	ND	ND	0.05	ND	ND	0.02
PFTrDA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFUnA	ND	ND	ND	ND	ND	0.01	ND	0.01	ND

Conclusion

The FMS EZPFC system demonstrates its efficiency by successfully analyzing all 40 native PFAS compounds using EPA Method 1633 in 12 synthetic wastewater samples and three aqueous matrix samples. Spike recoveries were well within the acceptance windows of the method with RSDs all < 10%. Cartridge clogging was not observed with any of the matrix samples, further underlining the system's reliability.

References

1. U.S. Environmental Protection Agency. *Method 1633A: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS*; EPA 820-R-24-007; Office of Water, Engineering and Analysis Division: Washington, DC, December 2024. https://www.epa.gov/system/files/documents/2024-12/method-1633a-december-5-2024-508-compliant.pdf.
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