

Validation of EPA Method 1633 with Semi-Automated Solid Phase Extraction and LC/MS for Analysis of Per- and Polyfluoroalkyl Substances in Wastewater

Application Note

Introduction

Significant strides have been made in the past few years in the tools available to assess and address the PFAS challenge. A mere three years ago, the U. S. Environmental Protection Agency (EPA) had yet to publish a method for PFAS in environmental samples such as groundwater and soils. This lack of a standardized method led to a complex and confusing process, with each laboratory developing and implementing its own specialized PFAS analytical methods. The convention of naming these methods after the EPA's drinking water method 537 further added to the confusion. However, the development and validation of Method 1633 has significantly addressed these challenges, providing a standardized and reliable method for PFAS analysis, marking a crucial milestone in the field.

In January 2024 the EPA finalized the analytical method for environmental samples (also Method 1633). This application note is an overview of the validation of recent advancements in PFAS analytical methods, with a special focus on using the EZPFC Semi-Automated Solid Phase Extraction for Method 1633 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 produces the highest quality results, marking a new era in our understanding and management of PFAS contamination.

The EPA finally provided the long-awaited analytical method, [Method 1633](#), in draft form in August 2021. 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, and the 'draft' status was removed from Method 1633. Method 1633 is not just a tool, but a comprehensive solution designed to cover a wide range of PFAS sample matrices, including groundwater, surface water, wastewater, landfill leachate, soils, sediments, biosolids, and biological tissue. micrograms per kilogram ($\mu\text{g}/\text{kg}$) for solids, providing a high level of accuracy and reliability in our PFAS analysis.

Let's look at Method 1633 versus other U. S. EPA PFAS methods. Method 8327 ([finalized July 2021](#)) is intended for environmental aqueous matrices but is generally considered a screening-level method. Unlike Method 1633, 8327 utilizes direct injection rather than solid-phase extraction and does not include isotope dilution. The native analyte lists for EPA Methods 533, 537.1, and 8327 contain 25, 18, and 24 compounds respectively, significantly lower than the 40 native PFAS analytes covered by Method 1633. These differences highlight the comprehensive nature and low detection limits of Method 1633.

Instrumentation

- FMS EZPFC® System
- Vacuum pump
- Agilent 6475 TripleQuad LC/MS

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

- Twelve synthetic wastewater samples (500 mL) spiked with 50 ppt native PFAS standards and relevant internals
- Load sample bottles onto system and install cartridges
- Fill rinse bottles with 5 mL reagent water
- Turn on vacuum (stays on rest of procedure)

Stage 1

- 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 (using nitrogen)
- Rinses loaded across cartridges
- Dry 15 sec under vacuum

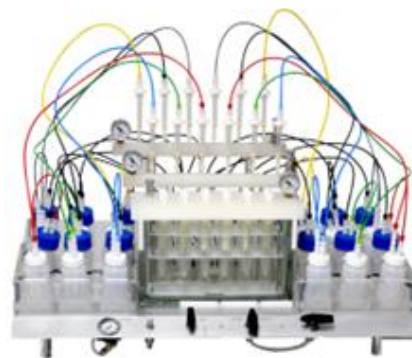
Stage 2

- Rinse sample bottles with 5 mL 1% methanolic ammonium hydroxide
- Load rinses across cartridges and collect in polypropylene tubes
- Cleanup carried out with 10 mg loose carbon
- As per the method no further concentration is carried out.

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

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 μ m
- Column temperature 40 °C
- Injection 5.0 μ L
- 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



EZPFC System used for Method 1633



Agilent LC/MS system



Compound							
Name	Percent	EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Window	RSD (%)
11Cl-PF3OUdS		92	93	100	97	50-150	3.62
3-3 FTCA		87	96	93	101	70-130	6.18
4-2 FTS		89	98	94	96	70-135	3.90
5-3 FTCA		96	96	93	98	70-130	2.14
6-2 FTS		92	95	95	96	70-135	1.89
7-3 FTCA		98	97	95	102	55-130	2.79
8-2 FTS		92	91	100	109	70-140	8.52
9Cl-PF3ONS		93	92	97	97	70-145	2.72
ADONA		87	90	94	89	70-135	3.24
EtFOSE		95	95	95	96	70-130	0.57
HFPO-DA		92	111	100	91	70-135	9.42
MeFOSE		89	96	89	94	70-135	4.02
N-EtFOSA		89	93	91	96	70-135	3.57
N-EtFOSAA		93	90	81	101	70-135	8.81
NFDHA		93	92	94	91	65-140	1.57
N-MeFOSA		94	101	100	106	70-135	5.12
N-MeFOSAA		86	95	105	96	65-140	8.21
PFBA		93	96	93	95	70-135	1.68
PFBS		109	98	100	99	70-140	4.88
PFDA		98	94	90	96	65-140	3.83
PFDoA		93	92	88	93	70-130	2.34
PFDoS		93	105	87	96	45-135	7.64
PFDS		85	91	97	86	70-135	6.07
PFEESA		95	99	96	96	70-135	1.99
PFHpA		99	101	94	116	70-135	9.44
PFHpS		91	108	101	99	70-140	7.22
PFHxA		96	97	93	100	70-135	3.07
PFHxS		90	91	77	95	70-135	9.03
PFMBA		91	92	96	95	65-145	2.55
PFMPA		106	107	115	113	60-140	3.83
PFNA		105	100	96	107	70-140	4.85
PFNS		96	100	95	99	70-135	2.44
PFOA		102	89	99	105	65-155	7.24
PFOS		97	109	120	104	70-140	8.89
PFOSA		107	102	105	107	70-135	2.16
PFPeA		93	98	96	96	70-135	2.36
PFPeS		93	91	87	103	70-135	7.17
PFTDA		91	93	94	89	70-145	2.50
PFTrDA		92	98	95	93	60-145	2.52
PFUnA		89	105	93	100	70-135	7.62

Table 1. Recoveries (%) and RSDs (%) for 40 native PFAS in synthetic wastewater (1633) using EZPFC (spiked with 1-38 ng/L).



Compound						
Name	Percent	EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Window
13C2-4-2 FTSA		95.1	72.1	105.3	86.3	40-200
13C2-6-2 FTS		96.9	75.4	106.7	96.0	40-200
13C2-8-2 FTSA		91.1	75.1	94.4	80.8	40-300
13C2-PFDoDA		81.6	87.7	98.4	89.6	10-130
13C2-PFTDA		90.3	83.5	99.3	94.4	10-130
13C3-HFPO-DA		103.0	85.0	106.0	99.3	40-130
13C3-PFBS		89.0	76.7	96.3	90.0	40-135
13C3-PFHxS		95.9	80.6	115.7	95.9	40-130
13C4-PFBA		92.0	78.5	100.7	93.4	5-130
13C4-PFHpA		95.9	76.8	107.0	88.5	40-130
13C5-PFHxA		93.4	75.7	103.2	91.3	40-130
13C5-PFPeA		94.2	76.5	97.2	87.6	40-130
13C6-PFDA		81.8	80.6	95.7	85.3	40-130
13C7-PFUnA		83.9	75.3	95.9	83.0	30-130
13C8-PFOA		82.0	81.6	99.6	96.1	50-200
13C8-PFOS		93.9	81.2	91.3	92.5	50-200
13C8-PFOSA		90.4	76.6	88.1	89.7	40-130
13C9-PFNA		85.0	66.8	82.6	80.1	40-130
2H3-N-MeFOSAA		87.6	91.0	76.8	74.2	10-130
2H3-N-MeFOSAA		95.5	79.6	87.7	84.5	40-170
2H5-N-EtFOSA		95.8	75.4	90.0	80.0	10-130
2H5-N-EtFOSAA		89.6	79.2	91.3	80.3	25-135
2H7-MeFOSE		93.3	120.6	90.1	87.3	10-130
2H9-EtFOSE		92.2	107.9	89.3	88.7	10-130

Table 2. Recoveries (%) and acceptance windows (%) for 24 surrogate PFAS in synthetic wastewater (1633) using EZPFC.

Compound		EZPFC-MDL-1	EZPFC-MDL-2	EZPFC-MDL-3	EZPFC-MDL-4	EZPFC-MDL-5	EZPFC-MDL-6	EZPFC-MDL-7		
Name	In ng/L	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	STDEV	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	8.39	0.57	1.81	
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	3.26	3.48	3.36	0.21	0.65	
N-EtFOSAA	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-MeFOSAA	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	
PFTDA	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	

Table 3. Method Detection Limit values for 40 native PFAS in synthetic wastewater (1633) using EZPFC (spiked with 0.2-9 ng/L).

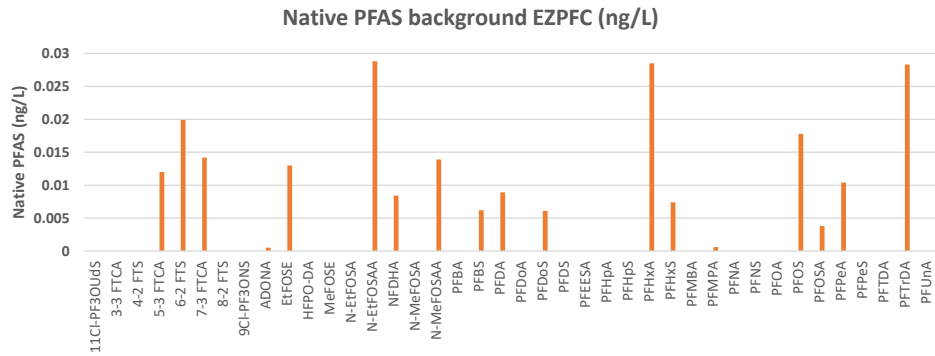


Table 4. Native PFAS background with EZPFC for method 1633 (in ng/L).

Compound	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
	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.
Name In ng/L									
11Cl-PF3OUds	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
3-3 FTCA	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
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	0.00	0.00	0.04	0.00	0.00	0.00	0.00
8-2 FTS	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9Cl-PF3ONS	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00
ADONA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ElFOSE	0.01	0.00	0.01	0.05	0.01	0.02	0.00	0.01	0.02
HFPO-DA	0.00	0.03	0.04	0.03	0.02	0.05	0.02	0.00	0.05
MeFOSE	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.06
N-EFOSA	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
N-EFOSAA	0.00	0.01	0.01	0.01	0.02	0.05	0.01	0.01	0.03
NFDHA	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00
N-MeFOSA	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.02
N-MeFOSAA	0.00	0.00	0.00	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	0.00
PFDoA	0.00	0.00	0.00	0.01	0.03	0.01	0.02	0.02	0.02
PFDoS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFDS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFEESA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
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	0.00	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFMPA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFNA	0.14	0.17	0.14	0.21	0.17	0.15	0.03	0.06	0.07
PFNS	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
PFOA	3.08	3.60	3.65	1.43	1.28	1.29	1.06	0.82	1.02
PFOA	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	0.00	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	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.02
PFTDA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFUnA	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00

Table 5. Native PFAS with EZPFC for method 1633 in river, well, and tap water (in ng/L).



Discussion and Conclusions

The EZPFC system demonstrates its efficiency by successfully analyzing all 40 native PFAS compounds using EPA method 1633 (Table 1) in twelve synthetic wastewater samples. The recoveries, well within the acceptance windows of the method with RSDs (%) all < 10%, further underline the system's reliability. Notably, the semi-automated system completes the process in under 70 minutes, without the need for a final concentration step, and consistently produces excellent recoveries with very good RSDs (%).

The EZPFC system's ability to produce excellent results is evident in the surrogate PFAS recoveries (%) and acceptance windows (%) shown in Table 2. Recoveries were consistently found well within those windows, further demonstrating the system's effectiveness and reliability.

Table 3 shows the method detection limits for all 40 native PFAS using synthetic wastewater. 32 out of 40 MDL values are < 0.50 ng/L.

The EZPFC system is designed with your analysis accuracy in mind. It boasts low native background values for PFAS, ensuring the precision of your results. Moreover, the system's low risk of cross-contamination, as demonstrated by Table 4, further enhances its cleanliness and precision. With about half of the native PFAS compounds being non-detectable and all values being < 0.03 ng/L, the system's reliability is underscored, giving you confidence in your results.

Table 5 analyzes three different water categories: river, well, and tap water. Good reproducibility was found between the replicates.

The EZPFC SPE system extracts 12 EPA 1633 samples in parallel delivering unprecedented throughput to your Sample Preparation Workflow.

The EZPFC system outperforms other, more expensive, fully automated SPE systems, providing you with superior data. Its user-friendly operation and simplified design minimize the risk of breakdown and contamination. Cleaning the system between runs is a breeze, further enhancing its practicality and efficiency.

A common challenge in ground- and wastewater extraction is the presence of particulate matter, which can easily lead to cartridge clogging. However, the EZPFC system has a solution. 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.

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