Application Note

Environmental

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Solid Samples

Using Agilent Bond Elut PFAS WAX SPE Cartridges and Agilent Carbon S following EPA Method 1633

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Abstract

This application note presents an evaluation of Agilent PFAS Bond Elut WAX and Agilent Carbon S for the extraction and matrix cleanup of per- and polyfluoroalkyl substances (PFAS) in solid matrices following the protocols specified in United States Environmental Protection Agency (US EPA) method 1633.[1] Results obtained in this study were comparable to the initial precision and accuracy (IPR) results reported in the validated EPA method for solid matrices. The overall average recovery accuracy of native PFAS and extracted internal standards from solid matrix was determined to be 98 ± 2%, and the overall average relative standard deviation (RSD) was 3.8 ± 0.6% (95% confidence level, 64 measurements).

Introduction

US EPA method 1633 was developed to consolidate procedures for the extraction and quantitation of PFAS in aqueous (nonpotable water), solids (soil, biosolids, and sediment) and tissue samples.[1] Principally, the method utilizes polymeric weak anion exchange (WAX) solid phase extraction (SPE) for the selective extraction of target analytes in addition to matrix removal using graphitized carbon black (GCB). The target analytes are extracted along with isotopically labeled standards followed by separation and detection using liquid chromatography/tandem quadrupole (LC/TQ) mass spectrometry. The method contains validated results for solids based on a multi-laboratory study for a total of 40 target PFAS across nine compound classes.

The EPA method contains rigorous quality control procedures to ensure optimal data reliability. The requirements are described in Section 9 of the method and include: the initial demonstration of precision, accuracy, and method detection limits (Section 9.2); the recovery of extracted internal standards and non-extracted internal standards (Section 9.3, 9.4); method blank determination (Section 9.5); instrument calibration verification and maintenance (Section 9.6); laboratory duplicates (Section 9.7); analysis of field replicates when necessary (Section 9.8); and analysis of matrix spikes when necessary (Section 9.9).[1]

In this application note, the performance of the extraction and analysis procedures for solid matrices was verified following the EPA method quality control protocols using Bond Elut PFAS WAX SPE cartridges, Carbon S as a replacement for GCB, and the Agilent Infinity II 1290 LC and Agilent 6470B triple quadrupole LC/MS. The results were compared to the US EPA method 1633 for the multi-lab validation study.

Experimental

Chemicals and reagents

Native PFAS standards and isotopically labeled analogues were purchased as kits from Wellington Laboratories, Inc. (Guelph, ON, Canada). HPLC-grade methanol (MeOH) was from Honeywell (Muskegon, MI, USA). Reagent-grade acetic acid, ammonium acetate, formic acid, and ammonium hydroxide were from Sigma-Aldrich (St Louis, MO, USA). Reagent water was prepared using a Milli-Q Integral 3 purification system from Millipore Sigma (Burlington, MA, USA). Ottawa sand (20–30 mesh) was obtained from Spectrum Chemicals and Laboratory Products (New Brunswick, NJ, USA) and used as reagent sand. Topsoil was purchased from a local home gardening retailer (Wilmington, DE, USA).

Solutions and standards

All solutions required for the standard preparation and sample extraction followed the protocols listed in the method.[1] Table 1 lists the nominal calibration concentrations levels for the native PFAS, extracted internal standards (EIS), and non-extracted internal standards (NIS).

Table 1. Calibration level concentrations.

Compounds	Level Concentration (ng/mL)						
	1	2	3	4	5	6	7
Native PFAS							
PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSAA, NEtFOSSA	0.1	0.2	0.52	1.2	2.4	5.2	12
PFPeA, PFMPA, NFDHA, PFMBA, PFEESA	0.2	0.4	1.0	2.4	4.8	10.4	24
PFBA, 4:2FTS, 6:2FTS, 8:2FTS, HFPO-DA, ADONA, 9Cl-PF3ONS, 11CL-PF3OUdS, 3:3FTCA	0.4	0.8	2.1	4.8	9.6	21	48
NMeFOSE, NEtFOSE	1	2	5.2	12	24	52	120
5:3FTCA, 7:3FTCA	2	4	10.4	24	48	104	240
EIS							
$^{13}C_2$-PFDoA, $^{13}C_2$-PFTeDA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, $^{13}C_9$-PFNA	1	1	1	1	1	1	1
$^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, $^{13}C_4$-PFHpA, $^{13}C_5$-PFHxA, $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_8$-PFOSA, D_3-NMeFOSA, D_5-NEtFOSA	2	2	2	2	2	2	2
$^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, $^{13}C_5$-PFPeA, D_3-NMeFOSAA, D_5-NEtFOSAA	4	4	4	4	4	4	4
$^{13}C_3$-HFPO-DA, $^{13}C_4$-PFBA	8	8	8	8	8	8	8
D_7-MeFOSE, D_9-EtFOSE	20	20	20	20	20	20	20
NIS							
$^{13}C_5$-PFNA, $^{13}C_2$-PFDA	1	1	1	1	1	1	1
$^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS	2	2	2	2	2	2	2

For extraction performance evaluation, both low and mid-level matrix spikes were used. Low-level spikes were used for the determination of method detection limit (MDL) in reagent sand. Mid-level spikes were used for recovery precision and accuracy measurements in reagent sand and in topsoil matrix. Table 2 lists the final concentrations of the native PFAS in low and mid-level spikes based on a 5 g sample. The spiking concentrations of the EIS and NIS were selected to match the concentrations in the calibration standards (Table 1).

Table 2. Low and mid-level spiking concentrations of native PFAS.

Compounds	Spike Concentration (ng/g)	
	Low-Level	Mid-Level
PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSAA, NEtFOSSA	0.2	2
PFPeA, PFMPA, NFDHA, PFMBA, PFEESA	0.4	4
PFBA, 4:2FTS, 6:2FTS, 8:2FTS, HFPO-DA, ADONA, 9Cl-PF3ONS, 11CL-PF3OUdS, 3:3FTCA	0.8	8
NMeFOSE, NEtFOSE	2	20
5:3FTCA, 7:3FTCA	4	40

Equipment and materials

Sample analysis was performed using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II high-speed pump (G7120A), an Agilent 1290 Infinity II multisampler (G7167B), and an Agilent 1290 Infinity II multicolumn thermostat (G7167B). The LC system was modified for PFAS analysis using the Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006). The LC system was coupled to an Agilent 6470B triple quadrupole LC/MS equipped with an Agilent Jet Stream electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis. The Agilent PFAS MRM database (G1736AA) was used for optimized MRM settings. The optimized instrument conditions are specified in a previously published application note.[2]

The PFAS-suitable consumables and supplies used for the PFAS extraction and analysis are listed in Table 3. The consumables were used as specified by the method.[1]

Table 3. PFAS suitable consumables and supplies.

Agilent Consumables and Supplies	Part Number
Bond Elut PFAS WAX 150 mg, 6 mL	5610-2150
Carbon S SPE Bulk Sorbent, 25 g Bottle	5610-2093
Centrifuge Tubes and Caps, 50 mL	5610-2049
Centrifuge Tubes and Caps, 15 mL	5610-2039
Bond Elut Empty SPE Cartridges, 60 mL	12131012
Bond Elut Adapter Cap for 1, 3, and 6 mL Bond Elut Cartridges	12131001
Glass Wool, Silane-Treated, 50 g, for Gas Chromatograph	8500-1572
Captiva Disposable Syringe, 5 mL	9301-6476
Captiva Premium Syringe Filter, Polypropylene Housing, Nylon Membrane, 25 mm Diameter, 0.2 µm Pore Size	5190-5092
Vac Elut SPS 24 Manifold with Collection Rack for 10 × 75 mm Test Tubes	12234003
Collection Rack and Funnel Set for 12 or 15 mL Conical Tubes, for Vac Elut SPS 24 Manifold	12234027
Vac Elut 20 Manifold Long Valve Stopcock	12234520
2 mL Polypropylene Screw Style Vials	5191-8121
9 mm Screw Style Cap with Polypropylene/Silicone Screw Septa	5191-8151
InfinityLab PFC Delay Column, 4.6 × 30 mm	5062-8100
ZORBAX RRHD Eclipse Plus C18 Column, 2.1 × 100 mm, 1.8 µm	959758-902
InfinityLab PFC-Free HPLC Conversion Kit	5004-0006

Calibration and quantitation

Stable-isotope dilution methodology was used for quantitation where the responses and concentrations of the native PFAS are measured relative to the responses and concentrations of EIS. The responses and concentrations of the EIS are measured relative to the responses and concentrations of the NIS. Response curves were fitted including the origin (0,0) using 1/x weighted linear least squares regression model for all compounds except for 4:2FTS, 6:2FTS, and 8:2FTS which used a 1/x weighted quadratic least squares regression model. The PFAS standards supplied as salts were corrected to the acid concentrations.

Sample preparation

The sample preparation closely followed the extraction procedure specified in the method[1] for solid matrices with a few modifications as listed in Figure 1. For topsoil samples, the percent moisture content was determined to be approximately 37%, therefore in order to achieve a 5 g sample dry weight, an 8 g sample size was used.

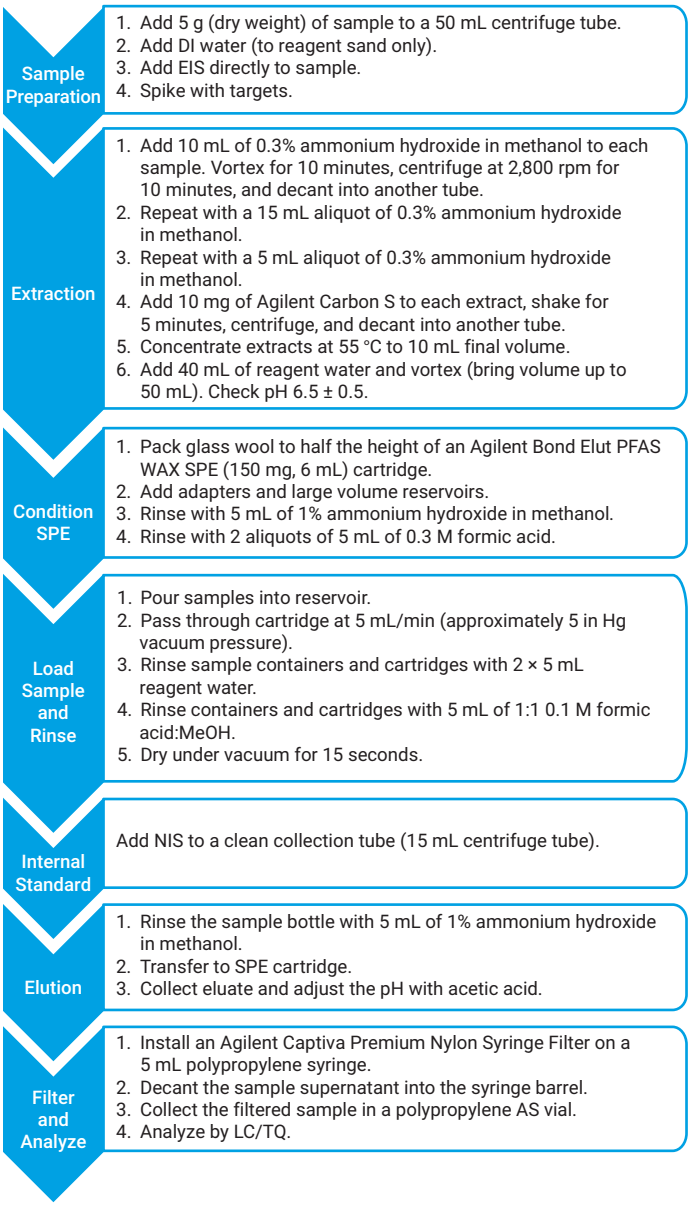

Figure 1. Sample preparation procedure.

Results and discussion

Initial recovery and precision

The first step in method validation was to demonstrate that the IPR requirements could be achieved for four replicate reagents and spikes at mid-level concentration as described in Section 9.2 of the method. Figures 2A and 2B show the average native PFAS and EIS recovery accuracies achieved for the IPR study. The hashed lines in Figure 2 are the IPR acceptance limits for the PFAS targets and EIS for solids as listed in Tables 7 and 8 (respectively) of the method.[1] As indicated in Figure 2, all recoveries were well within the acceptance limits.

Precision results were calculated from the same four replicate mid-level reagents and spikes used for the accuracy measurements. Figure 3 shows the RSD for the extractions carried out in this study along with the RSD acceptance levels listed in Table 7 of the method.[1] All RSDs were well below the acceptance level threshold.

Method detection limits

According to the method[1] Section 9.2.2, each lab must also establish MDLs for each native PFAS at the 99% confidence level following the procedure in 40 CFR Part 136, Appendix B. Table 4 lists the MDLs for seven replicate reagent sand spike extractions performed in this study and the pooled MDLs for solid matrices as listed in Table 9 of the method. As expected, the MDLs measured in this study were below the pooled MDLs listed in the method.

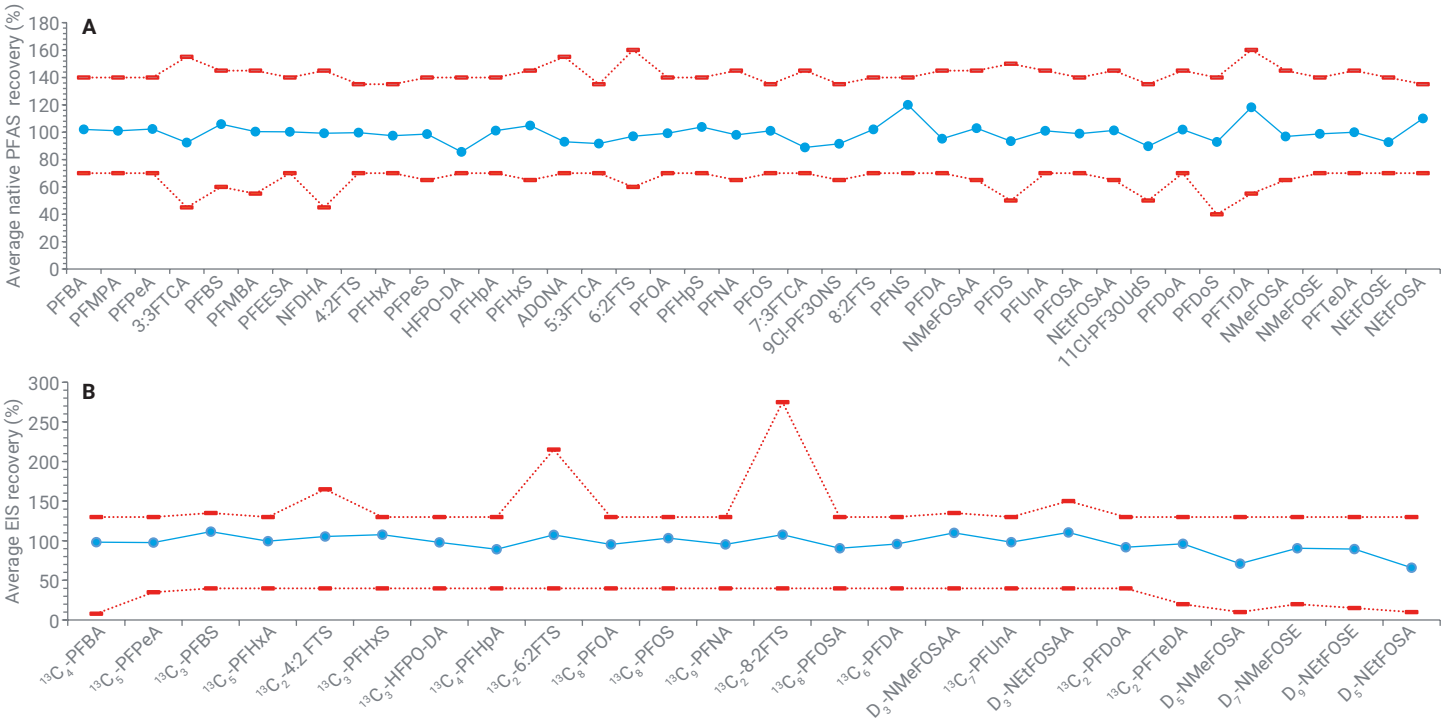

Figure 2. Average native PFAS recoveries (blue circles) and EPA 1633 acceptance limits (red hashed lines) (A) and average EIS recoveries (blue circles) and EPA acceptance limits (red hashed lines) (B).

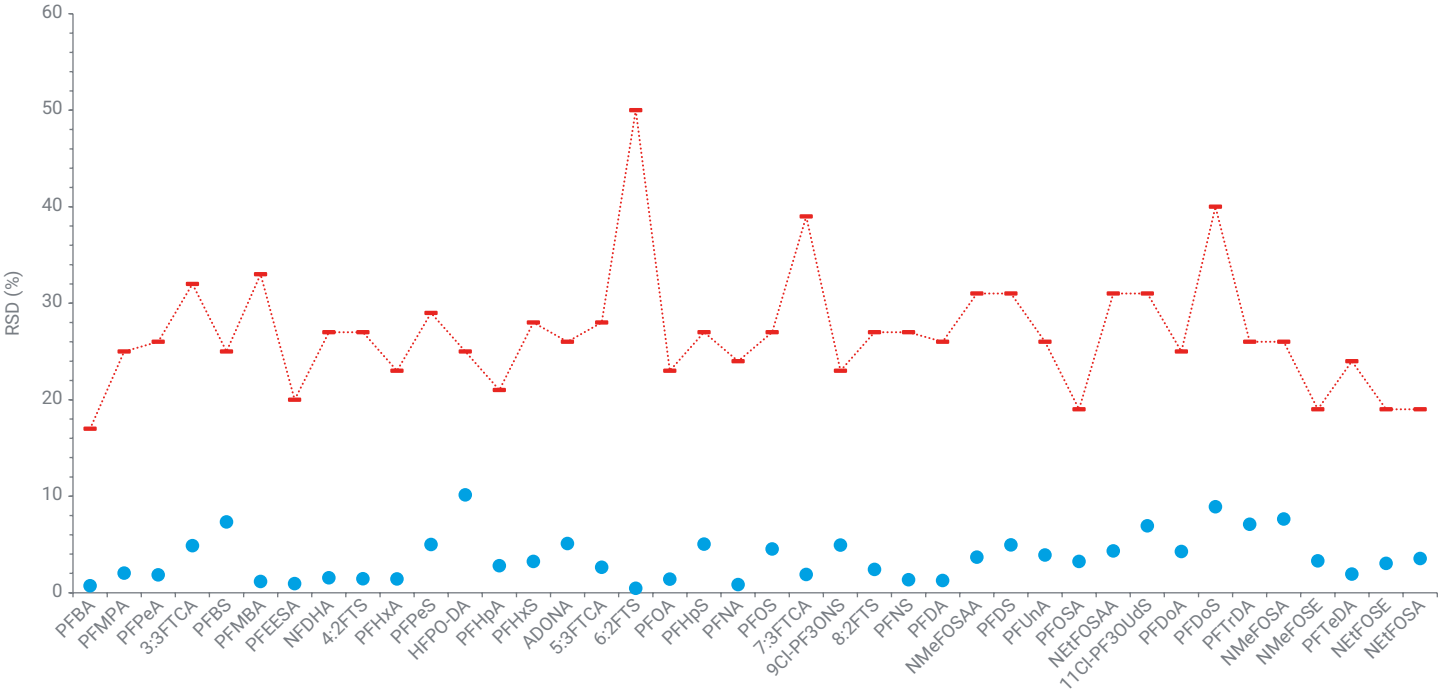

Figure 3. Precision of native PFAS recoveries (blue circles) and EPA 1633 acceptance limits (red hashed lines).

Table 4. Method detection limits.

Analyte	Bond Elut PFAS WAX Initial MDL (ng/g)	EPA 1633 Aq. MDL (ng/g)
PFBA	0.111	0.15
PFPeA	0.022	0.07
PFHxA	0.037	0.06
PFHpA	0.039	0.05
PFOA	0.017	0.07
PFNA	0.021	0.14
PFDA	0.029	0.06
PFUnA	0.023	0.12
PFDoA	0.027	0.06
PFTrDA	0.031	0.07
PFTeDA	0.030	0.05
PFBS	0.034	0.05
PFPeS	0.051	0.08
PFHxS	0.030	0.08

Analyte	Bond Elut PFAS WAX Initial MDL (ng/g)	EPA 1633 Aq. MDL (ng/g)
PFHpS	0.050	0.07
PFOS	0.033	0.07
PFNS	0.043	0.07
PFDS	0.040	0.08
PFDoS	0.064	0.06
4:2FTS	0.087	0.20
6:2FTS	0.239*	0.39
8:2FTS	0.122	0.31
PFOSA	0.061	0.04
NMeFOSA	0.045	0.07
NEtFOSA	0.099	0.07
NMeFOSAA	0.039	0.08
NEtFOSAA	0.029	0.08
NMeFOSE	0.267	0.36

Analyte	Bond Elut PFAS WAX Initial MDL (ng/g)	EPA 1633 Aq. MDL (ng/g)
NEtFOSE	0.255	0.35
HFPO-DA	0.194	0.25
ADONA	0.084	0.23
PFMPA	0.040	0.07
PFMBA	0.033	0.05
NFDHA	0.067	0.20
9Cl-PF3ONS	0.020	0.22
11Cl-PF3OUdS	0.058	0.18
PFEESA	0.026	0.08
3:3 FTCA	0.066	0.23
5:3 FTCA	0.101	0.86
7:3 FTCA	0.283	0.87

* Results based on five replicate spiked reagent sand extractions.

Internal standard recovery

Calculation of EIS and NIS recoveries are required for all samples analyzed as specified in Sections 9.3 and 9.4 of the method. EIS recoveries are used to assess method performance in sample matrix. Figure 4 plots the EIS recoveries from four topsoil extractions including the maximum and minimum acceptance limits for solid matrix as listed in Table 8 of the method. All EIS recoveries from topsoil were within the required acceptance limits.

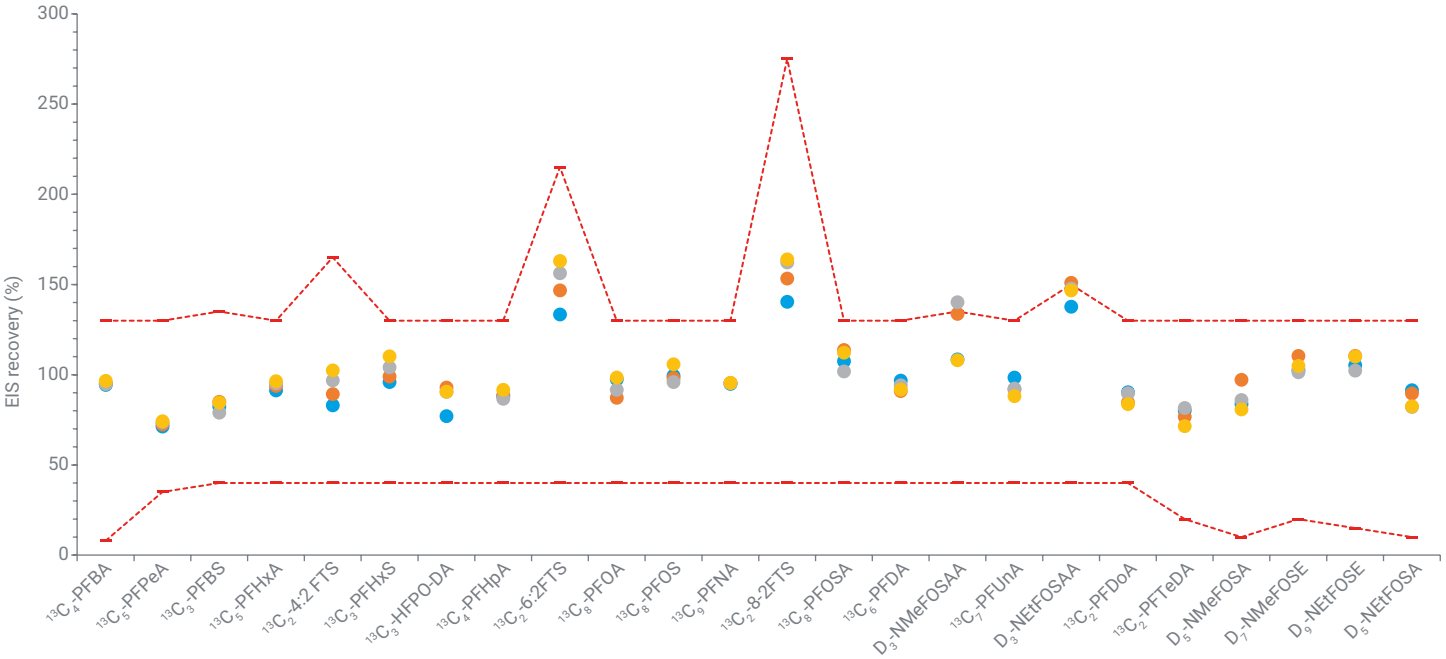

Figure 4. EIS extraction recoveries for four replicate topsoil spikes (blue, orange, gray, and yellow circles). The red hashmarks represent the EIS recovery results as listed in Table 8 of the method.

Calculation of NIS recoveries is also required by the method.[1] Since these compounds are added to the final extracts just before analysis, their main purpose is to ensure data quality during the sample analysis. Figure 5 shows the average NIS recovery for four mid-level topsoil spike replicates. Included in the figure are the acceptance limits from Table 8 of the method. The NIS recoveries determined in this study ranged from 87 to 104% and were well within the acceptance limits of the method.

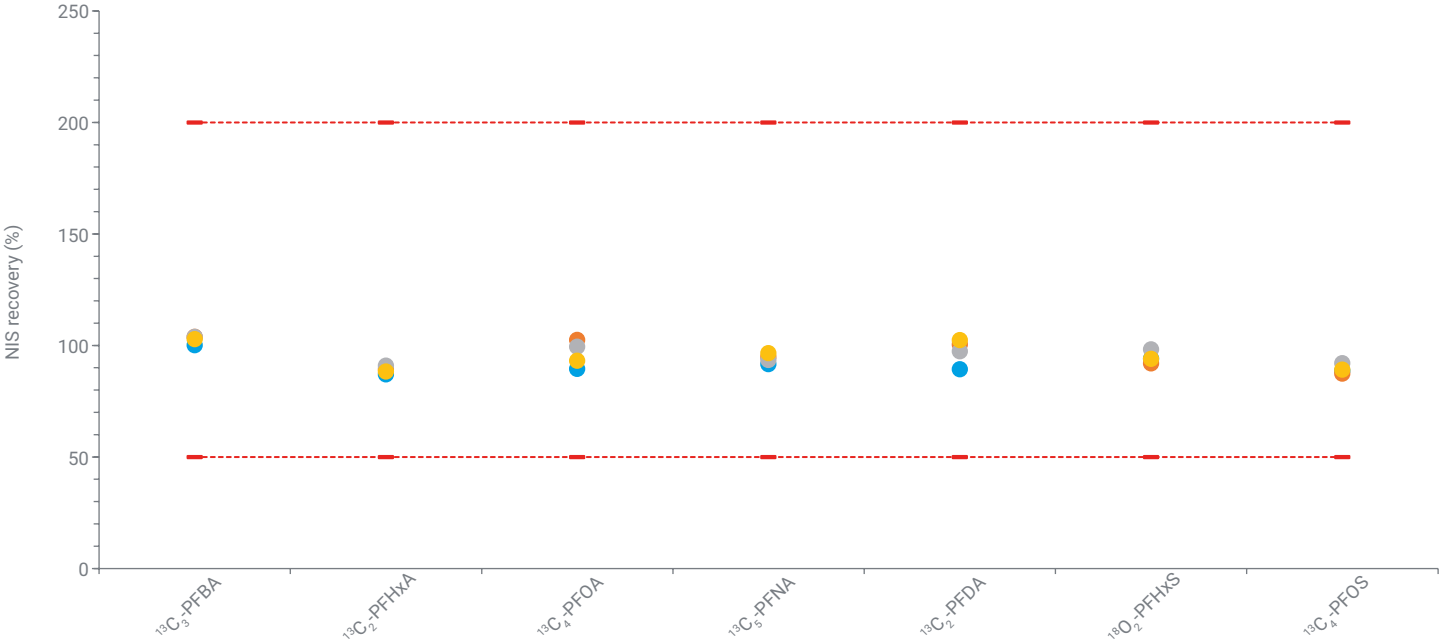

Figure 5. NIS recovery accuracies for four topsoil spikes (blue, orange, gray, and yellow circles). The red hashmarks represent the NIS recovery limits as specified in Table 8 of the method.

Method blanks

Analysis of method blanks are required for each sample batch. Corrective action must be taken if the blank concentration exceeds the requirements listed in Section 9.5.2 of the method.[1] Figure 6 shows the results of a blank reagent sand extraction. Included in Figure 6 are the minimum levels of quantitation (MLs), which were defined as the lowest level calibration standard in this study. For all compounds, the blank PFAS levels are well below the MLs. Most of the nonzero concentrations measured in the blank were a result of noise integration within the MRM windows. For these compounds, the measured concentrations in the blank were on average a factor of 16 below the MLs.

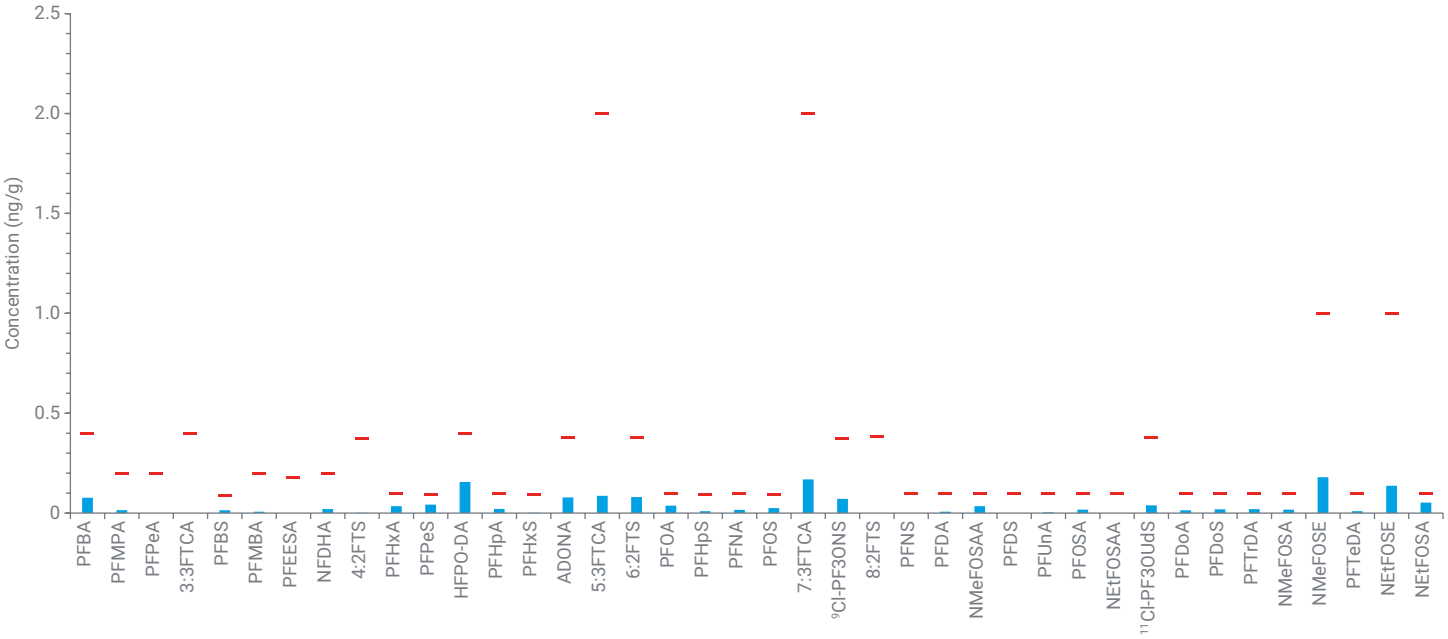

Figure 6. Method blank determination for reagent sand (blue bars). The red hashmarks represent the MLs as determined by the lowest level calibration standard.

Topsoil analysis

As described in Section 9.8 in the method[1], replicate topsoil samples were analyzed to determine the precision of the sampling technique. Results are listed in Table 5. Four compounds, 6:2FTS, PFOA, PFNA, and PFOS, were found at concentrations greater than the ML. The percent difference in values ranged from 9.5% for PFNA to 19.6% for PFOS. The greatest contributors to variability were attributed to sample inhomogeneity and sample mass differences. The topsoil contained pieces of twigs and small rocks that were difficult to remove, and samples masses were approximate, with recoveries scaled to a nominal 5 g dry mass.

Matrix spikes

Matrix spikes can be used as an additional assessment of matrix effects as described in Section 9.9 in the method.[1] They can be used to assess matrix effects for native PFAS in which there are no isotope analogues, such as PFPeS quantified by $^{13}C_3$-PFHxS. Matrix spikes are also required as specified in Table B-24 of DoD/DoE QSM 5.4.[3] Duplicate topsoil spikes were prepared and extracted with native PFAS at a mid-level concentration. Figure 7 plots the percent recovery for the 40 target compounds spiked and extracted from the topsoil. The concentrations of the four target compounds determined to be above the ML were subtracted from the spiked concentration. For both sample spikes, recoveries ranged from 79.0 to 109.6% with an average recovery of 97 ± 1% (95% confidence level, 80 measurements), indicating outstanding method performance in matrix.

Table 5. Topsoil extraction results.

Compound	First Replicate (ng/g)	Second Replicate (ng/g)	Compound	First Replicate (ng/g)	Second Replicate (ng/g)
PFBA	< ML	< ML	PFOS	0.395	0.325
PFMPA	< ML	< ML	7:3FTCA	< ML	< ML
PFPeA	< ML	< ML	9Cl-PF3ONS	< ML	< ML
3:3FTCA	< ML	< ML	8:2FTS	< ML	< ML
PFBS	< ML	< ML	PFNS	< ML	< ML
PFMBA	< ML	< ML	PFDA	< ML	< ML
PFEESA	< ML	< ML	NMeFOSAA	< ML	< ML
NFDHA	< ML	< ML	PFDS	< ML	< ML
4:2FTS	< ML	< ML	PFUnA	< ML	< ML
PFHxA	< ML	< ML	PFOSA	< ML	< ML
PFPeS	< ML	< ML	NEtFOSAA	< ML	< ML
HFPO-DA	< ML	< ML	11Cl-PF3OUdS	< ML	< ML
PFHpA	< ML	< ML	PFDoA	< ML	< ML
PFHxS	< ML	< ML	PFDoS	< ML	< ML
ADONA	< ML	< ML	PFTrDA	< ML	< ML
5:3FTCA	< ML	< ML	NMeFOSA	< ML	< ML
6:2FTS	0.914	0.802	NMeFOSE	< ML	< ML
PFOA	0.227	0.203	PFTeDA	< ML	< ML
PFHpS	< ML	< ML	NEtFOSE	< ML	< ML
PFNA	0.235	0.214	NEtFOSA	< ML	< ML

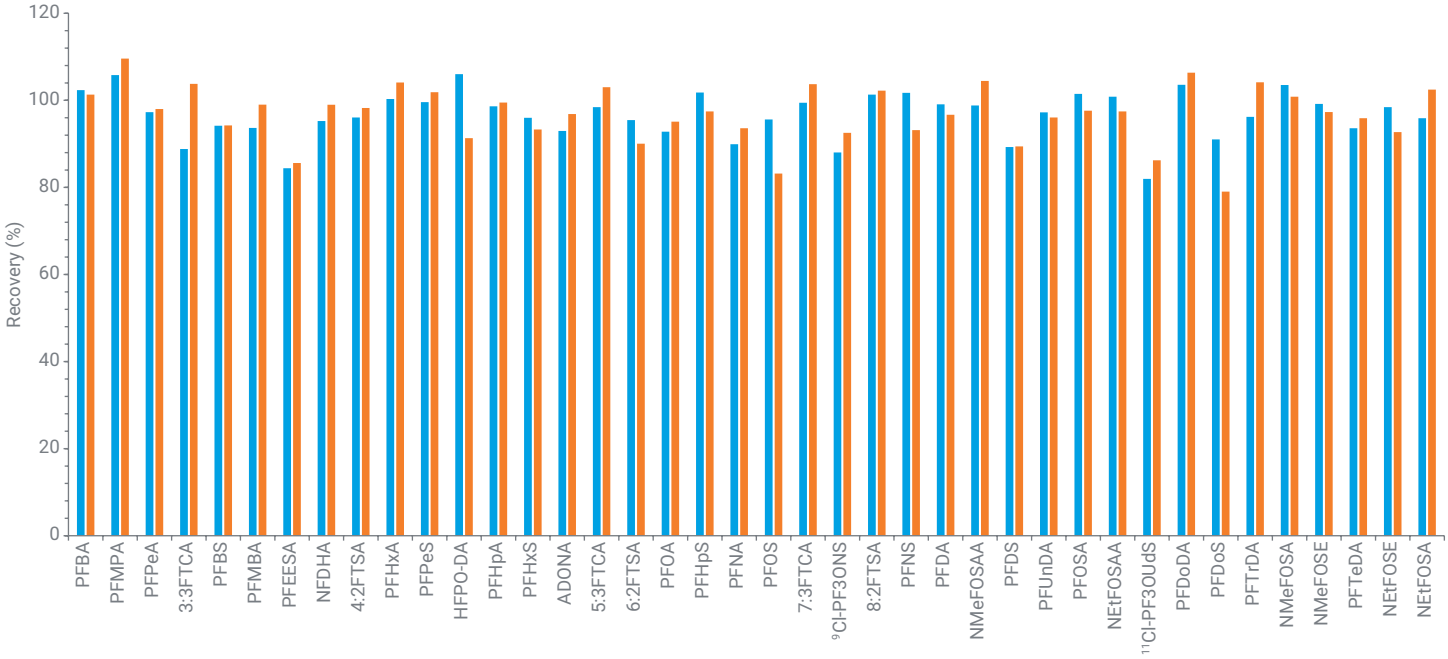

Figure 7. Matrix spike (blue) and matrix spike duplicate (orange) recoveries at mid-level spiking concentration.

Conclusion

The results of this application note demonstrate that the use of Agilent Bond Elut PFAS WAX SPE and Agilent Carbon S provide comparative results to the US EPA method 1633 for the multi-lab validation study for solid matrices.

References

1. Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. *United States Environmental Protection Agency*, **2024**. https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf
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DE59054271

Printed in the USA, August 29, 2025
5994-5667EN

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