

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

Using Agilent Bond Elut PFAS WAX SPE Cartridges and Agilent Carbon S following EPA Draft 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) draft method 1633 (December 2022).¹ Results obtained in this study were comparable to results reported in the draft method for the single laboratory validation study. The overall average recovery accuracy of native PFAS and extracted internal standards from solid matrix was determined to be $98 \pm 2\%$ in this study compared to $94 \pm 4\%$ in the draft method (95% confidence level, 64 measurements). The overall precision was also comparable. For both data sets, the measurement precisions were well below 20%. For the draft method, the overall average RSD was $3.9 \pm 0.6\%$, and for this study, the overall average RSD was $3.8 \pm 0.6\%$ (95% confidence level, 64 measurements).

Introduction

US EPA draft method 1633 (December 2022) 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. To date, the draft method contains validation results for solids based on a single laboratory study for a total of 40 target PFAS across nine compound classes.

The draft method contains rigorous quality control procedures to ensure optimal data reliability. The requirements are described in Section 9 of the draft 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 draft 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 draft method 1633 for the single 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 draft 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, NetFOsAA	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 ₅ -PFOA, ¹³ C ₅ -PFOS, ¹³ C ₆ -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 draft method.¹

Table 3. PFAS suitable consumables and supplies.

Agilent Consumables and Supplies	Part Number
Bond Elut PFAS WAX 150 mg, 6 mL	5610-2152
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	5191-8150
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 draft method¹ 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.

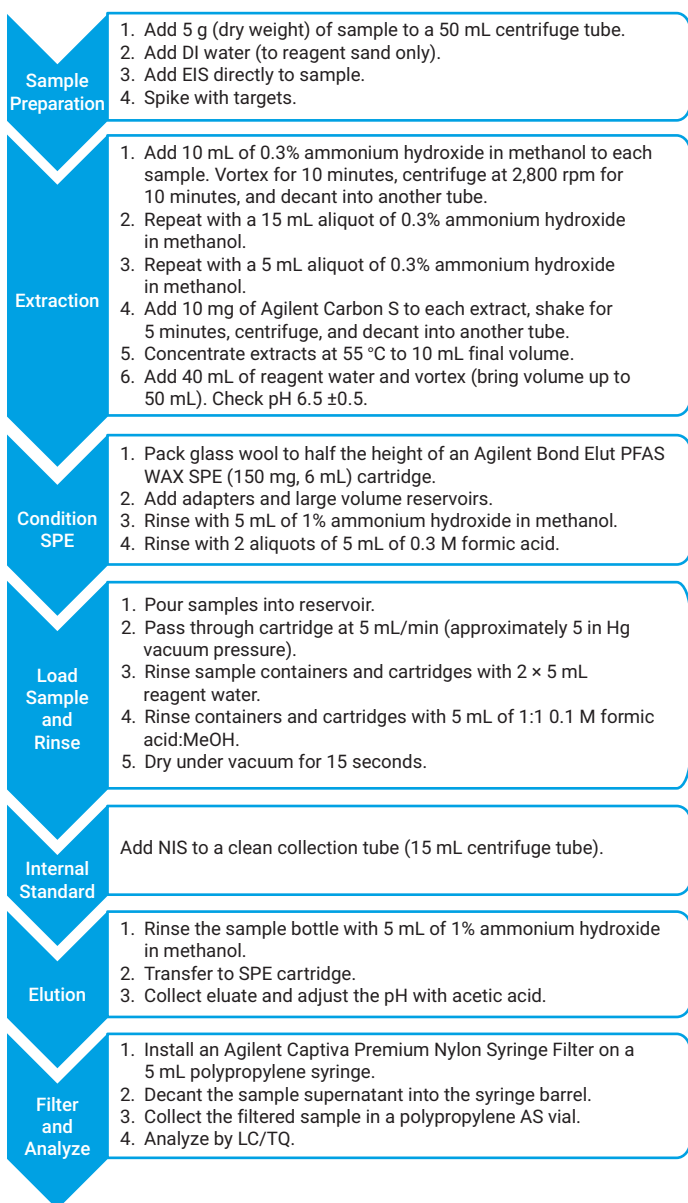


Figure 1. Sample preparation procedure.

Results and discussion

Initial precision and recovery

The first step in method validation was to demonstrate initial precision and recovery (IPR) for four replicate reagent sand spikes at a mid-level concentration as described in Section 9.2 of the draft method.¹ Figure 2 shows the average native PFAS and EIS recovery accuracies for the extractions carried out in this study and the average solid matrices IPR accuracies derived from Table 5 of the draft method. Table 5 in the draft method does not list average recoveries, but these can be back-calculated from the IPR recovery ranges by summing the high and low recoveries and dividing by 2. Acceptance limits have not yet been established for the draft method; however, recovery results are quite comparable. Most recoveries fall within the ranges in the draft method, with the greatest difference occurring for the labeled sulfonamide ethanols (D₇-MeFOSE, D₉-EtFOSE) in which recoveries measured in this study appeared significantly greater. For the draft method, the overall average recovery confidence interval was 94 ±4%, and for this study, the overall average recovery confidence interval was 98 ±2% (95% confidence level, 64 measurements).

Precision results were calculated from the same four replicate mid-level reagent sand spike extraction data. In Figure 3, the precision is represented by the percent relative standard deviation (RSD) for the extractions carried out in this study and the IPR results for solid matrices listed in Table 5 of the draft method.¹ Although acceptance limits have not yet been established for the draft method, Figure 2 includes a 20% RSD limit. For other common PFAS environmental methods, RSDs falling below the 20% limit are typically considered sufficiently precise.^{3,4,5} For both data sets, the measurement precisions were well below the 20% threshold. For the draft method, the overall average RSD was 3.9±0.6%, and for this study, the overall average RSD was 3.8 ±0.6% (95% confidence level, 64 measurements).

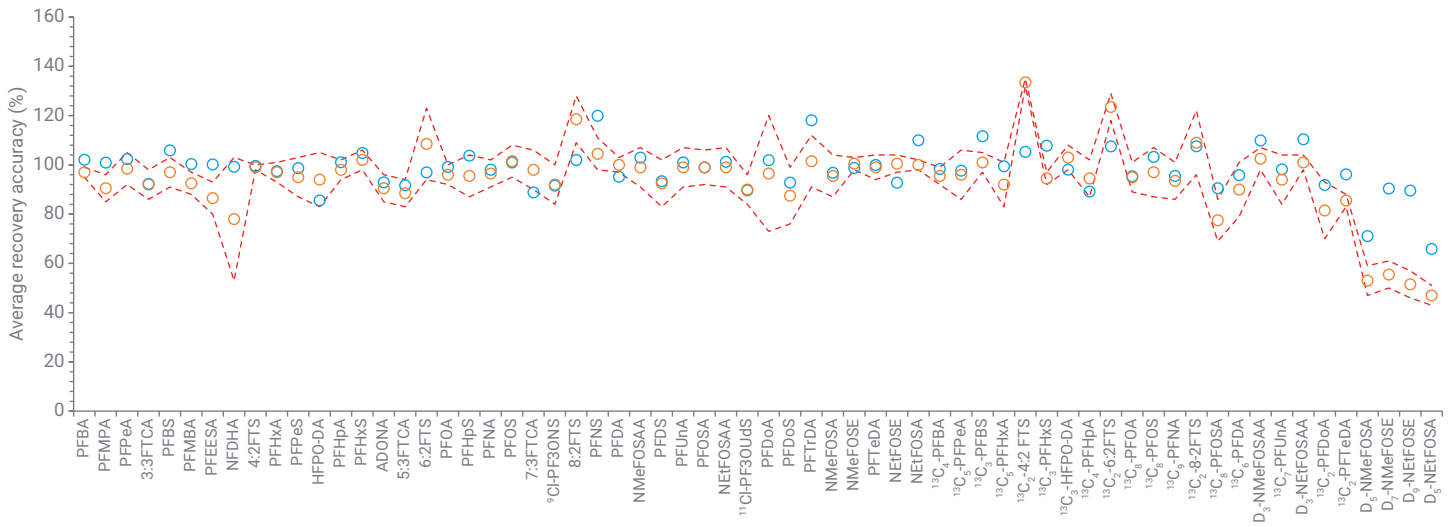


Figure 2. Average IPR accuracies for reagent sand spikes measured in this study (blue circles) and published draft method results (orange circles). The red hashed lines represent IPR recovery ranges published in the draft method.

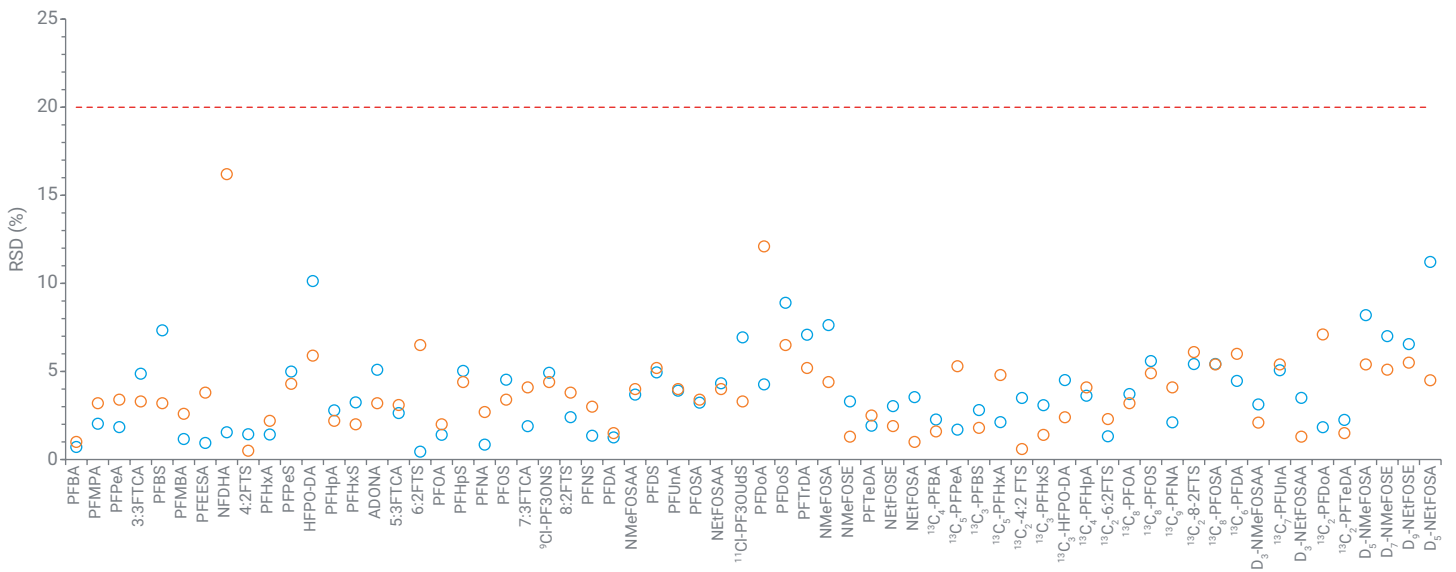


Figure 3. Relative standard deviations for reagent sand spikes (blue circles) and published draft method results (orange circles). The red hashed line represents the 20% precision threshold.

Method detection limits

According to the draft method¹ 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 results published for solid matrices in Table 6 in the draft method. Results between the two studies are comparable. Figure 4 plots the differences in the MDLs in the published method from the MDLs measured in this study. In Figure 4, compounds with lower MDLs measured in this study compared to the draft method have positive X-axis displacements, and compounds with greater MDLs measured in this study compared to the draft method have negative X-axis displacements. Interestingly, except for PFBA, the smallest differences between the two data sets occur for the straight chain alkyl sulfonic and carboxylic acids, while the fluorotelomer sulfonic acids, fluorotelomer carboxylic acids, ether sulfonic acids, and sulfonamide ethanols show greater differences. This may indicate wider method optimization ranges for the typical alkyl carboxylic and sulfonic acids and narrower optimization ranges for PFAS with more complex alkyl moieties and functional groups.

Table 4. Method detection limits.

Compound	Measured MDL (ng/g)	Draft Method MDL (ng/g)	Compound	Measured MDL (ng/g)	Draft Method MDL (ng/g)
PFBA	0.102	0.401	PFOS	0.043	0.067
PFMPA	0.035	0.033	7:3FTCA	0.110	0.308
PFPeA	0.026	0.021	9Cl-PF3ONS	0.107	0.038
3:3FTCA	0.154	0.060	8:2FTS	0.079	0.225
PFBS	0.031	0.014	PFNS	0.043	0.046
PFMBA	0.021	0.029	PFDA	0.023	0.031
PFEESA	0.028	0.018	NMeFOSAA	0.052	0.030
NFDHA	0.036	0.084	PFDS	0.059	0.040
4:2FTS	0.031	0.282	PFUnA	0.006	0.033
PFHxA	0.022	0.020	PFOSA	0.029	0.068
PFPeS	0.075	0.015	NEtFOSAA	0.043	0.044
HFPO-DA	0.120	0.136	11Cl-PF3OUdS	0.172	0.071
PFHpA	0.025	0.029	PFDoA	0.027	0.059
PFHxS	0.059	0.018	PFDoS	0.038	0.038
ADONA	0.139	0.057	PFTTrDA	0.029	0.038
5:3FTCA	0.195	0.363	NMeFOSA	0.080	0.049
6:2FTS	0.193*	0.116	NMeFOSE	0.109	0.203
PFOA	0.016	0.037	PFTeDA	0.025	0.032
PFHpS	0.042	0.057	NEtFOSE	0.172	0.247
PFNA	0.077	0.086	NEtFOSA	0.056	0.038

* Results based on five replicate spiked reagent sand extractions.

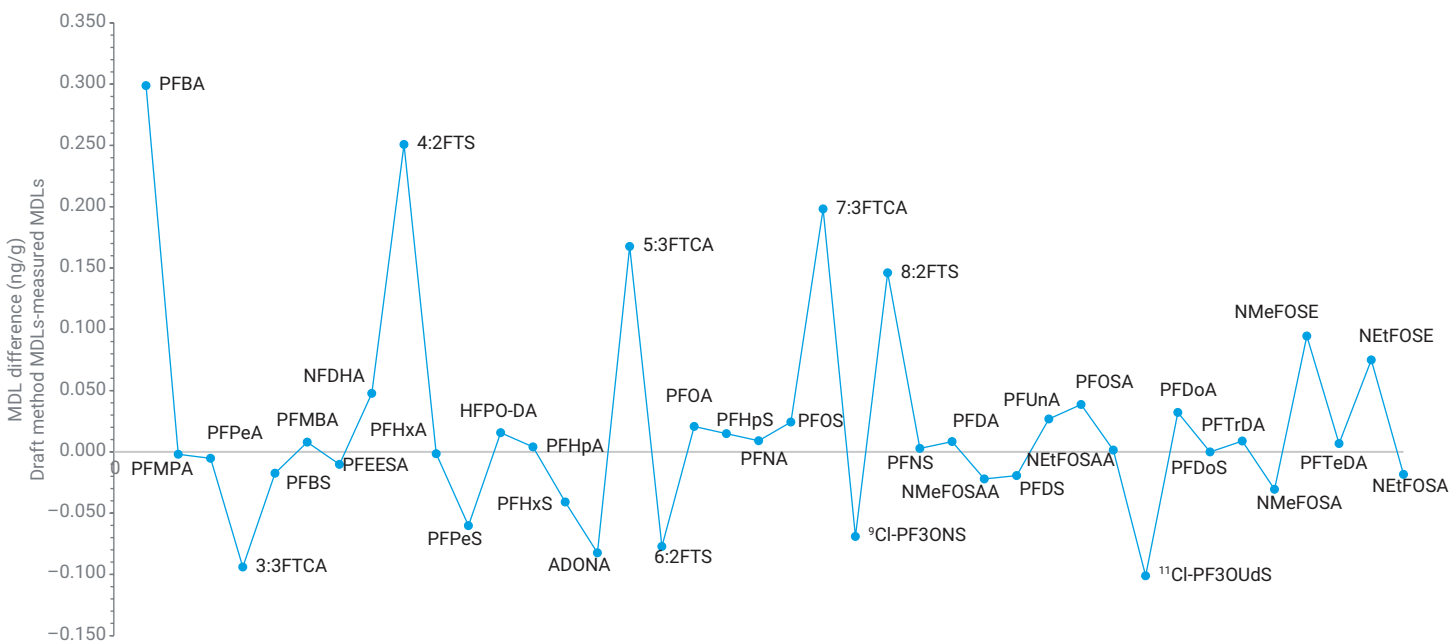


Figure 4. Calculated differences between MDLs in this study versus those in the published draft method.

Internal standard recovery

Calculation of EIS and NIS recoveries are required for all samples analyzed as specified in Section 9.3 of the draft method. EIS recoveries are used to assess method performance in sample matrix. Figure 5 plots the EIS recoveries from four topsoil extractions. Although the acceptance ranges have not yet been established for EIS recovery, Figure 5 includes the maximum and minimum solid matrix EIS recoveries listed in Table 9 of the draft method.

For most compounds, the EIS recoveries for the topsoil extracts are within the minimum and maximum recoveries listed in Table 9 of the draft method.¹ For reference, in EPA method 533, isotope dilution analog recoveries falling within 50 to 200% are considered acceptable.² The EIS recoveries in topsoil fall within these limits, with a minimum recovery of 71.3% for ¹³C₅-PFPeA and a maximum recovery of 163.9% for ¹³C₂-8:2FTS. The average EIS recovery across all extractions was 101 ±4% (95% confidence level, 96 measurements).

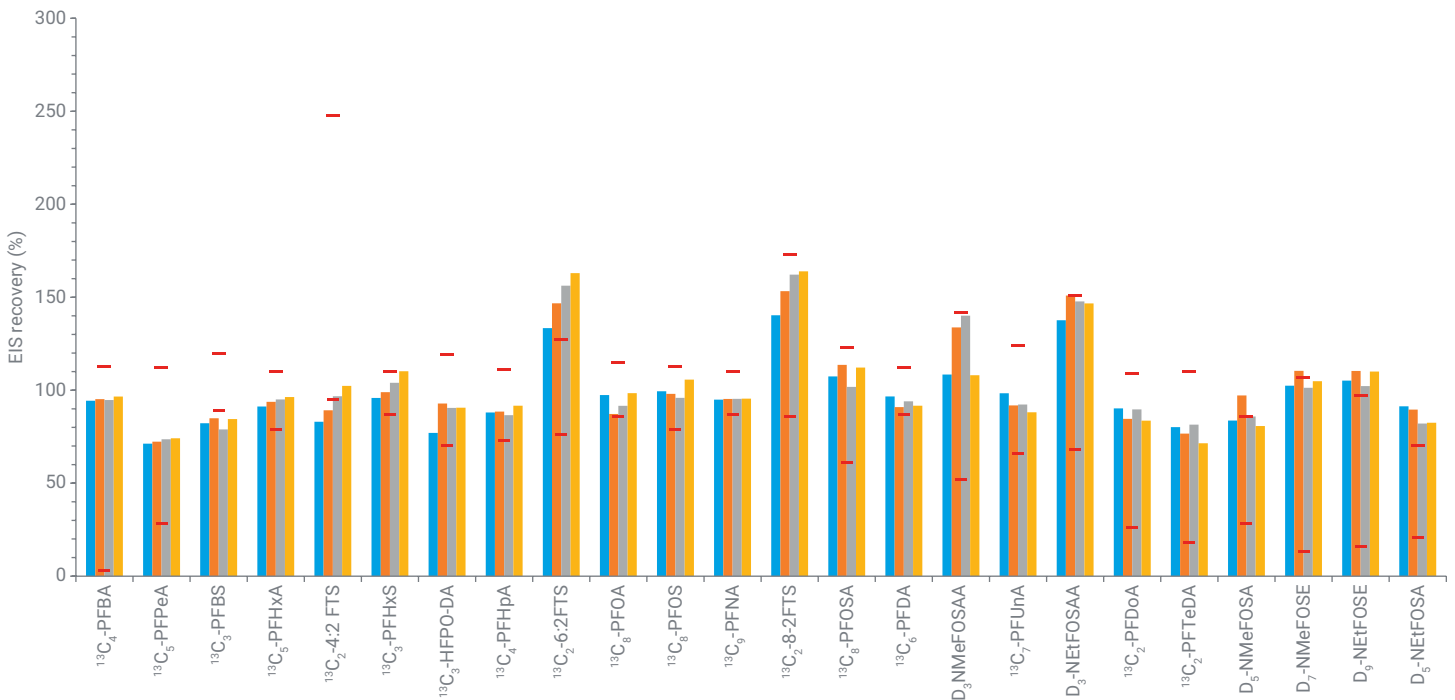


Figure 5. EIS extraction recoveries for four replicate topsoil spikes (blue, orange, gray, and yellow bars). The red hashmarks represent the EIS recovery results as listed in Table 9 of the draft method.

Calculation of NIS recoveries are 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 6 shows the average NIS recovery for four mid-level topsoil spike replicates. Included in the figure are the preliminary recovery limits from Table 10 in the draft method. The NIS recoveries determined in this study ranged from 87 to 104% and were

greater than the upper limits listed in Table 10 of the draft method, which ranged from 86 to 94%. The higher recoveries determined in this study could be a result of a lower final extract volume. In this study, the average volume recovered in the 5 mL extracts was around 4.5 mL. For other common PFAS environmental methods, non-extracted internal standard recoveries within 50 to 150% are typically considered acceptable.^{3,4} All NIS recoveries fell within this range.

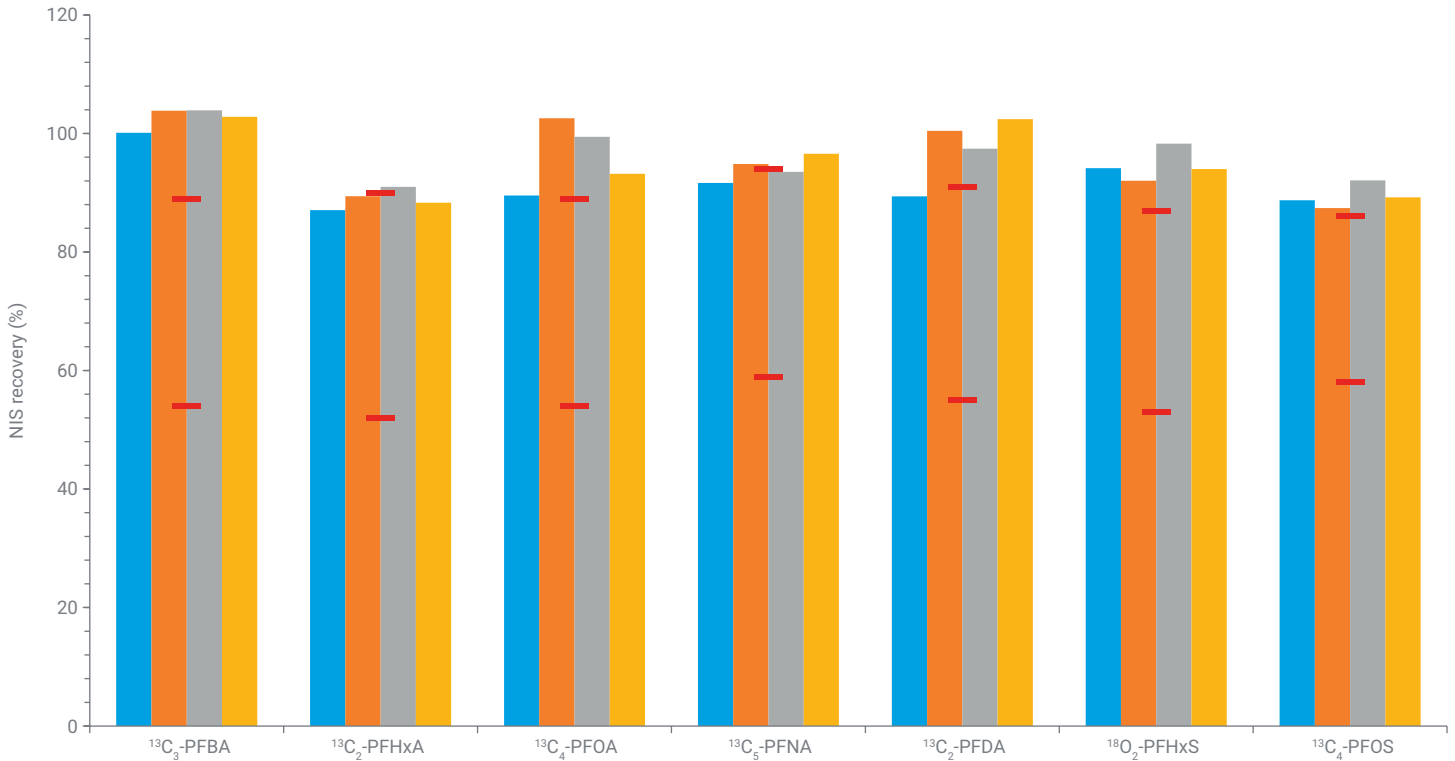


Figure 6. NIS recovery accuracies for four topsoil spikes (blue, orange, gray, and yellow bars). The red hashmarks represent the EIS recovery limits as specified in Table 10 of the draft method.

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 draft method.¹ Figure 7 shows the results of a blank reagent sand extraction. Included in Figure 7 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.

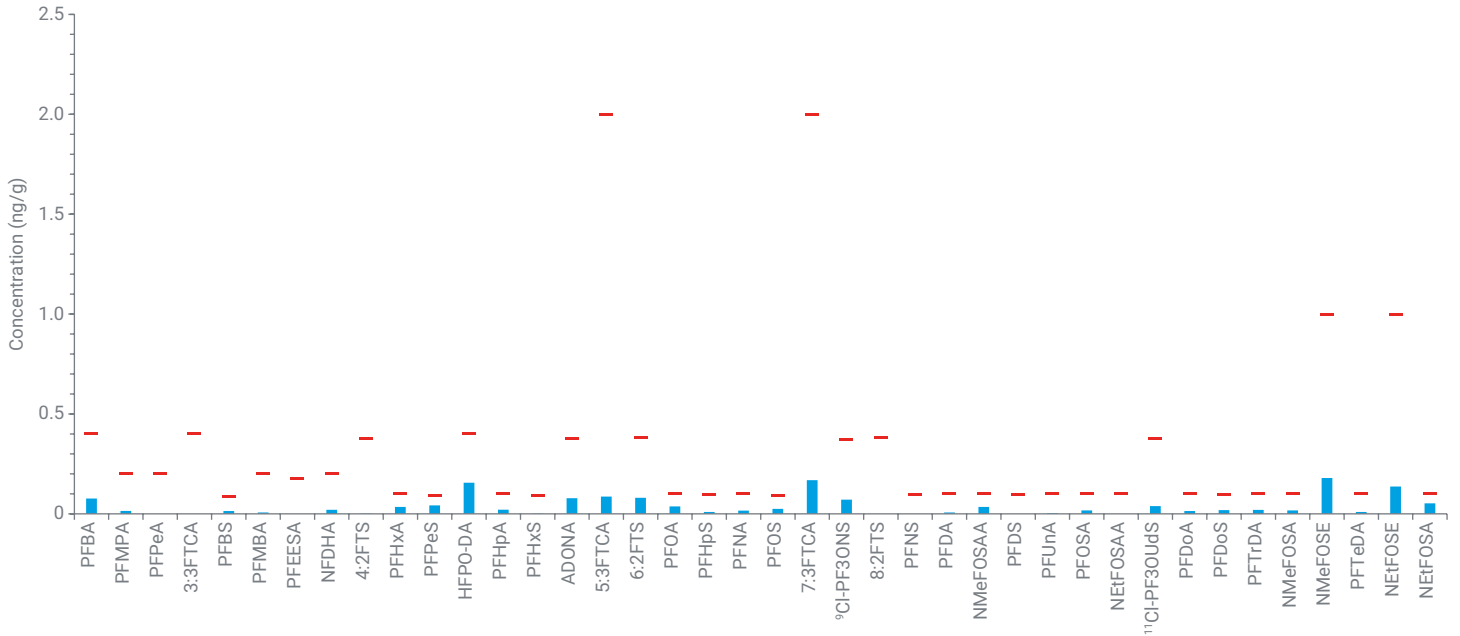


Figure 7. Method blank determination for reagent sand (blue bars). The red hashmarks represent the MLs as determined by the lowest level calibration standard.

Topsoil analysis

As described in Section 9.8 in the draft method¹, 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.

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	PFTTrDA	<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

Matrix spikes

Matrix spikes can be used as an additional assessment of matrix effects as described in Section 9.9 in the draft 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 ¹³C₃-PFHxS. Matrix spikes are also required as specified in Table B-24 of DoD/DoE QSM 5.4.⁵ Duplicate topsoil spikes were prepared and extracted with native PFAS at a mid-level concentration. Figure 8 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.

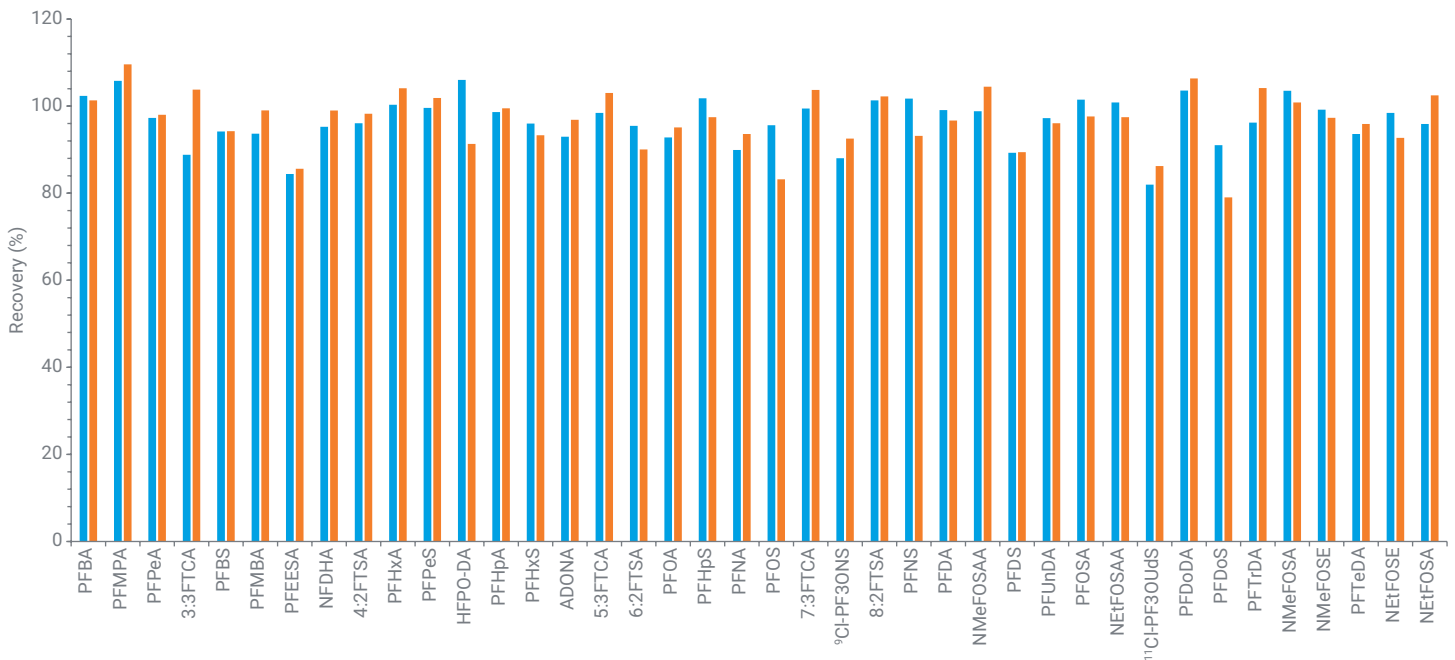


Figure 8. Matrix spike (blue) and matrix spike duplicate (orange) recoveries at mid-level spiking concentration.

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 draft method 1633 for the single lab validation study for solid matrices.

References

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