

ANALYSIS OF PFAS IN DRINKING WATER USING AUTOMATED SOLID PHASE EXTRACTION AND LC/MS/MS FOLLOWING EPA METHOD 533

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are a group of nearly 5000 synthetic compounds that have been used in firefighting foam, chrome-plating, waterproof textiles and other Teflon products. They have attracted particular attention due to their toxicity, persistence and prevalence in the environment. Alpha Analytical, a leading environmental lab, has been utilizing PromoChrom's fully automated 8-Channel solid phase extraction (SPE) system, SPE-03, to analyze PFAS in drinking water and other matrices. This application note presents the results of using the SPE-03 and draws comparison with manual extraction.

INTRODUCTION

EPA Method 533 was announced in December 2019 as a complement to EPA Method 537.1 for analyzing short-chain and more polar PFAS compounds in drinking water. It covers a list of 25 PFAS compounds using

isotope dilution and anion-exchange SPE¹. The extraction procedure requires meticulous handling, proper control of flow rates and many different conditioning, elution and rinsing steps.

This Application Note demonstrates the full automation of EPA Method 533 cartridge extraction using SPE-03 and uses manual extraction results as performance reference. It includes data on background, detection limit, accuracy, reproducibility and matrix effects. Results were taken from field sample extractions performed by Alpha Analytical.



MATERIALS

- PromoChrom SPE-03 system with MOD-004 (sample bottle rinsing) and MOD-005 (minimal-Teflon option)
- Phenomenex Strata™-X-AW 33 µm Polymeric Weak Anion, 500 mg/6 mL SPE cartridge (Cat. No.: 8B-S038-HCH)
- Reagents and standards following EPA Method 533
- SCIEX 4500 LC/MS/MS using ExionLC UHPLC

METHOD SUMMARY

The workflow is based on EPA Method 533. Samples preserved with ammonium acetate are extracted using the following method programmed on the SPE-03.

Solvent 1 = MeOH, **Solvent 2** = H₂O, **Solvent 3** = 0.1 M phosphate buffer, **Solvent 4** = 1 g/L ammonium acetate, **Solvent 5** = MeOH with 2% ammonium hydroxide (Basic MeOH)

W1 = Aqueous waste, **W2** = Organic waste

Table 1 - EPA Method 533 Steps Programmed on SPE-03

Action	Inlet 1	Flow	Volume	Description
Elute W2	Solvent 1	10 mL/min	10 mL	Condition cartridges with 10 mL MeOH
Elute W1	Solvent 3	10 mL/min	10 mL	Condition with 10 mL 0.1 M phosphate buffer
Elute W1	Solvent 3	10 mL/min	3 mL	Add 3 mL of 0.1 M phosphate buffer to cartridges
Elute W1	Solvent 2	10 mL/min	3 mL	Fill cartridges with 3 mL water
Add Samp W1	Sample	5 mL/min	270 mL	Load samples at 5 mL/min, using 270 mL to ensure full bottles are loaded
Rinse W1	Solvent 4	5 mL/min	10 mL	Rinse bottles with 10 mL of 1 g/L ammonium acetate and pass rinsate through cartridges
Shake	Time based		30 s	Shake bottles to collect any residual droplets
Clean	Solvent 1	5 mL/min	1 mL	Add 1 mL MeOH to sample bottles

Add Samp W2	Sample	5 mL/min	9 mL	<i>Draw MeOH through sample lines and deliver through SPE cartridges</i>
Blow N2	Time based		5 min	<i>Dry cartridges with N₂ for 5 mins (2.5 L/min @ 30 psi)</i>
Rinse 1	Solvent 5	2 mL/min	5 mL	<i>Rinse bottles with 5 mL of Basic MeOH and elute into fraction 1 at 2 mL/min</i>
Rinse 1	Solvent 5	2 mL/min	5 mL	<i>Rinse bottles with 5 mL of Basic MeOH and elute into fraction 1 at 2 mL/min</i>
Shake	Time based		10 s	<i>Shake bottles to collect any residual droplets</i>
Collect 1	Sample	2 mL/min	4.5 mL	<i>Ensure remaining Basic MeOH in lines and cartridges are delivered into fractions</i>

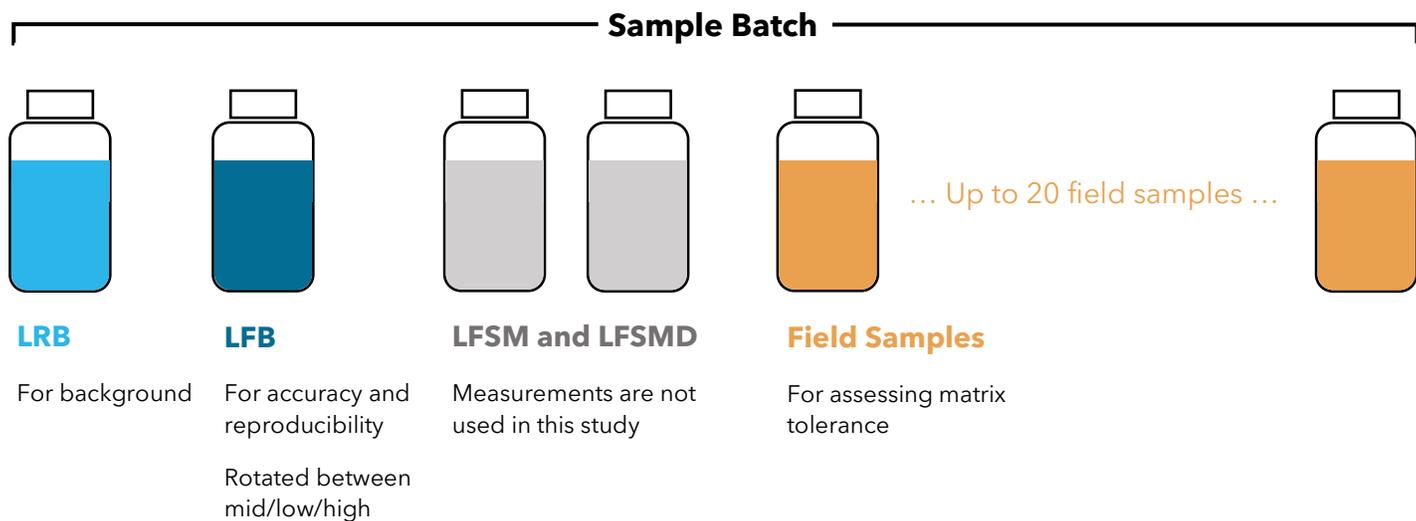
Up to 8 samples can be extracted simultaneously in just under 2 hours using the method above, following the 5 mL/min sample loading speed in the EPA method. The collected fractions are concentrated to dryness and reconstituted with 1.0 mL of 20% reagent water in methanol. Internal standards are then added and vortexed before injecting into LC/MS/MS.

After each extraction, the sample bottles are replaced with bottles set aside for cleaning purposes and a default cleaning method is run. The used SPE cartridges do not need to be removed during this process.

DATA

Data was collected from field sample extraction batches between March and April 2021. As required by EPA Method 533, each field sample batch must be extracted alongside QC samples consisting of a Lab Reagent Blank (LRB), Lab Fortified Blank (LFB), Laboratory Fortified Sample Matrix (LFSM), Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD). All QC samples are rotated between different channels of the SPE-03 and vacuum manifold for each extraction.

The diagram below shows a typical sample batch and how the QC and field samples are used for this study.



RESULTS

Detection Limit and Background

The minimal-Teflon option of the SPE-03 has all PTFE lines replaced and remaining wetted materials have been validated to be clean. This allows for reporting limits well below regulatory levels. Detection limits were determined by an extraction of 8 LFBs at 1 ng/L. Background is assessed using LRBs from 7 separate field sample extraction batches.

Detection Limit

A set of 1 ng/L spikes (0.25 ng/mL in 1 mL reconstituted fraction) were extracted on the SPE-03. The Minimum Detection Limit (MDL) is calculated by multiplying the stand deviation by 2.998 and then multiplying by 4 to convert from fraction (ng/mL) to sample concentration (ng/L). The resulting MDLs for each compound are shown on the right-most column. All calculated MDLs were below 1 ng/L, with most compounds under 0.5 ng/L. Alpha Analytical has set their Minimum Reporting Level (MRL) at 2 ng/L.

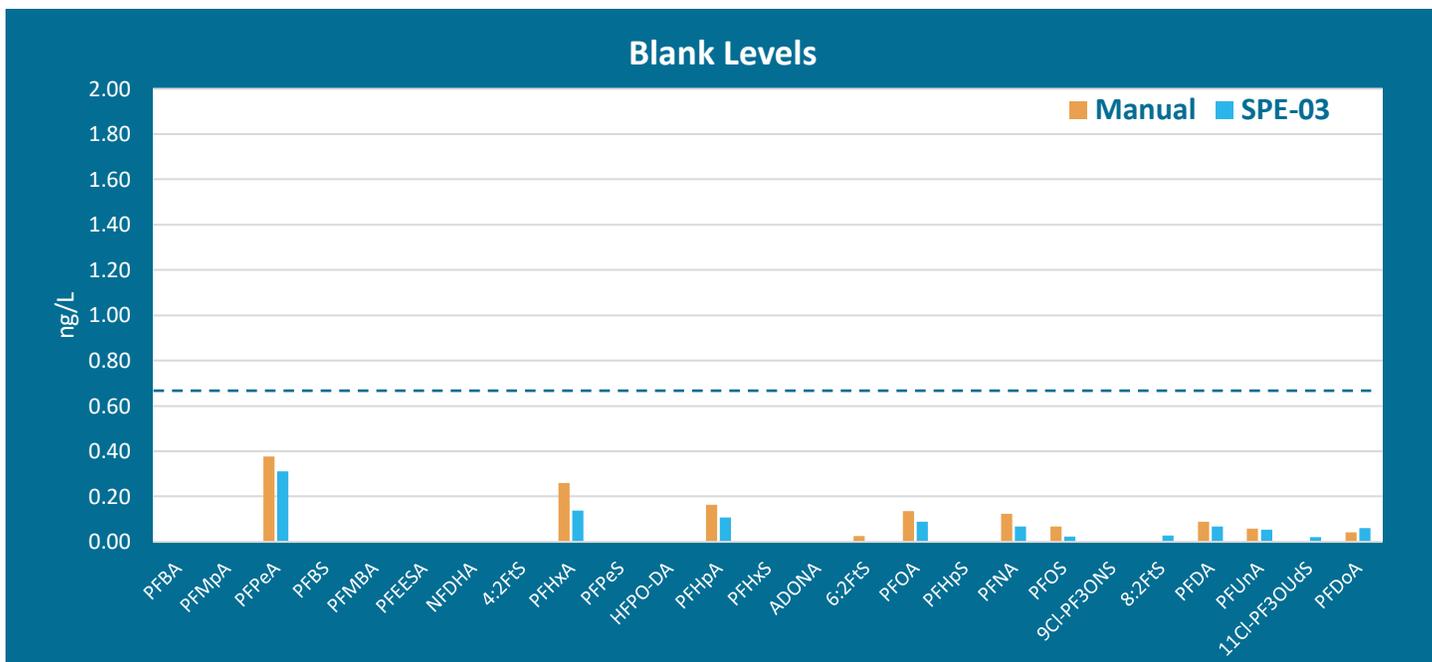
Table 3 - MDL recoveries at 1.0 ng/L (0.25 ng/mL)

Compound	Concentration of analytes in 1 mL fraction [ng/mL]								Std Dev	MDL [ng/L]
	#1	#2	#3	#4	#5	#6	#7	#8		
PFBA	0.25	0.24	0.29	0.25	0.25	0.21	0.25	0.22	0.024	0.29
PFMpA	0.26	0.25	0.28	0.28	0.26	0.25	0.24	0.24	0.016	0.20
PFPeA	0.36	0.34	0.36	0.33	0.32	0.35	0.34	0.32	0.017	0.20
PFBS	0.24	0.23	0.33	0.23	0.24	0.21	0.23	0.20	0.040	0.48
PFMBA	0.27	0.25	0.28	0.25	0.22	0.26	0.29	0.25	0.023	0.27
PFEESA	0.23	0.21	0.22	0.24	0.22	0.20	0.22	0.23	0.012	0.14
NFDHA	0.28	0.27	0.30	0.26	0.23	0.24	0.27	0.27	0.022	0.26
4:2FtS	0.24	0.21	0.24	0.25	0.19	0.26	0.24	0.28	0.028	0.34
PFHxA	0.26	0.24	0.32	0.27	0.30	0.25	0.26	0.22	0.033	0.39
PFPeS	0.18	0.25	0.23	0.32	0.24	0.20	0.27	0.18	0.047	0.56
HFPO-DA	0.29	0.26	0.22	0.22	0.18	0.24	0.28	0.23	0.036	0.44
PFHpA	0.26	0.27	0.28	0.22	0.28	0.24	0.22	0.22	0.029	0.34
PFHxS	0.26	0.19	0.24	0.25	0.21	0.18	0.23	0.16	0.037	0.44
ADONA	0.26	0.28	0.28	0.29	0.21	0.23	0.24	0.26	0.027	0.33
6:2FtS	0.20	0.47	0.39	0.28	0.32	0.36	0.30	0.36	0.080	0.95
PFOA	0.25	0.29	0.28	0.24	0.25	0.22	0.32	0.27	0.031	0.37
PFHpS	0.26	0.21	0.19	0.27	0.24	0.20	0.19	0.19	0.034	0.41
PFNA	0.28	0.28	0.20	0.27	0.26	0.26	0.31	0.32	0.036	0.43
PFOS	0.24	0.21	0.26	0.28	0.23	0.23	0.18	0.27	0.035	0.42
9CI-PF3ONS	0.24	0.21	0.20	0.21	0.20	0.25	0.20	0.23	0.019	0.23
8:2FtS	0.25	0.22	0.27	0.33	0.29	0.24	0.25	0.30	0.037	0.44
PFDA	0.30	0.26	0.28	0.27	0.26	0.24	0.28	0.22	0.025	0.30
PFUnA	0.27	0.28	0.30	0.29	0.25	0.26	0.26	0.27	0.019	0.22
11CI-PF3OUdS	0.19	0.18	0.19	0.25	0.19	0.20	0.18	0.20	0.021	0.26
PFDoA	0.30	0.27	0.28	0.28	0.26	0.25	0.27	0.25	0.018	0.22

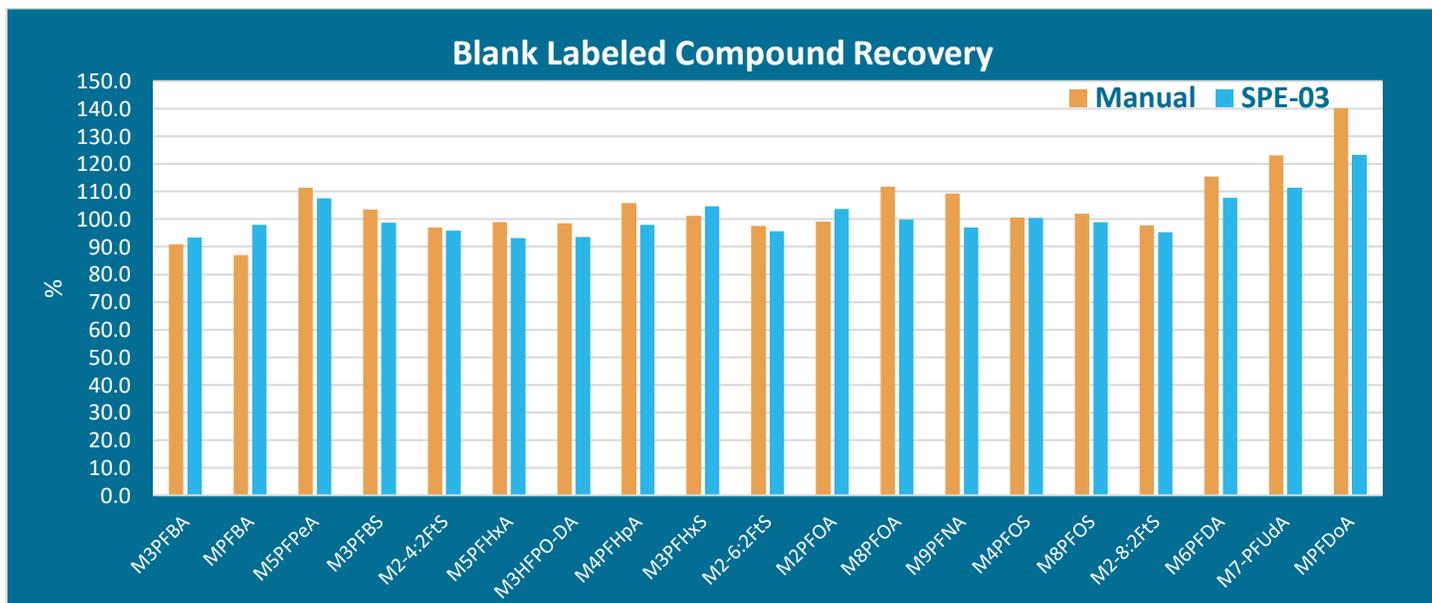
Background

EPA Method 533 requires background to be $<1/3$ MRL, which corresponds to 0.67 ng/L for Alpha Analytical. Analyzing the LRBs from 7 separate extraction batches, both manual and SPE-03 setup demonstrated clean background. Any detected compounds >0.1 ng/L were seen on both setups, suggesting contamination from other sources.

Figure 1 - Background levels of Manual vs SPE-03 Extraction



Below are the corresponding isotope dilution analog (labeled compound) recoveries of the blanks showing good extraction performance. The higher MPFDoA recoveries is attributed to the method quantifying the 12-carbon compound with an 8-carbon M2PFOA performance standard. A more similar internal standard should produce better accuracy.



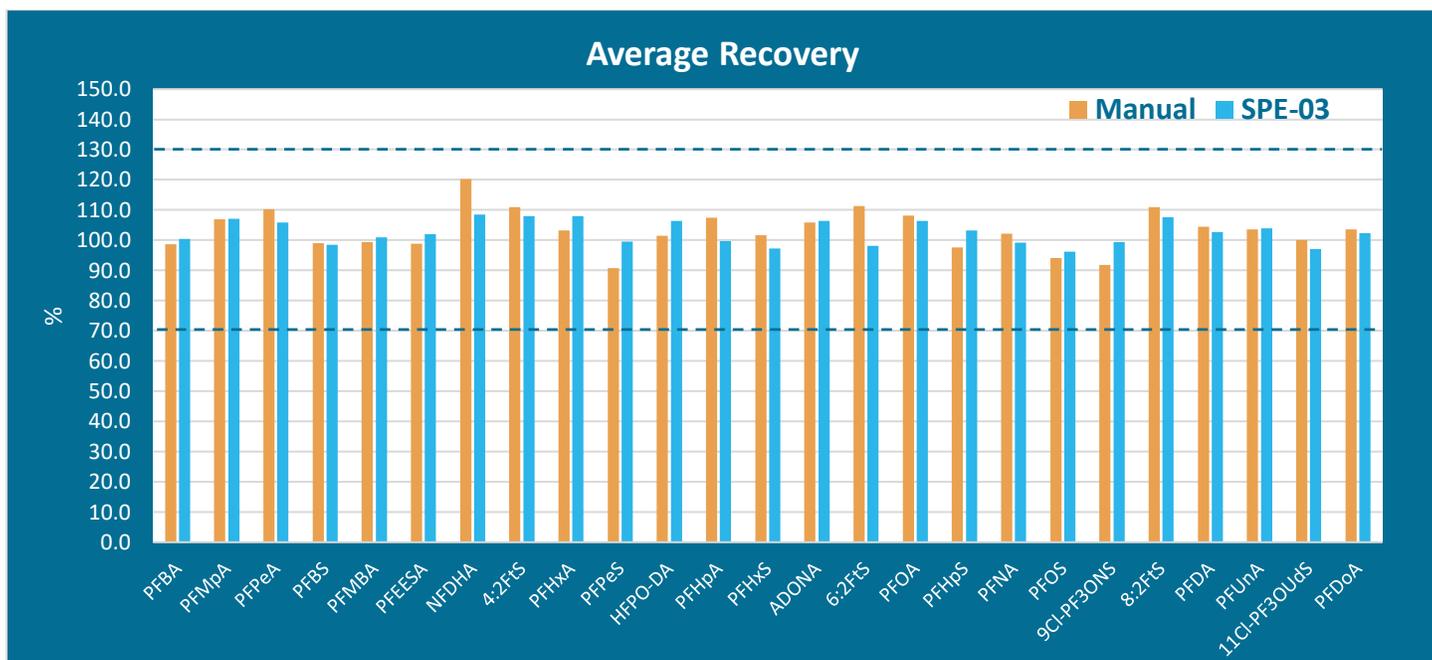
Accuracy and Reproducibility

LFBs from 8 extraction batches, consisting of 2 low (2 ng/L), 4 mid (40 ng/L) and 2 high (160 ng/L) concentration spikes are used to assess accuracy and reproducibility of the extractions.

Accuracy

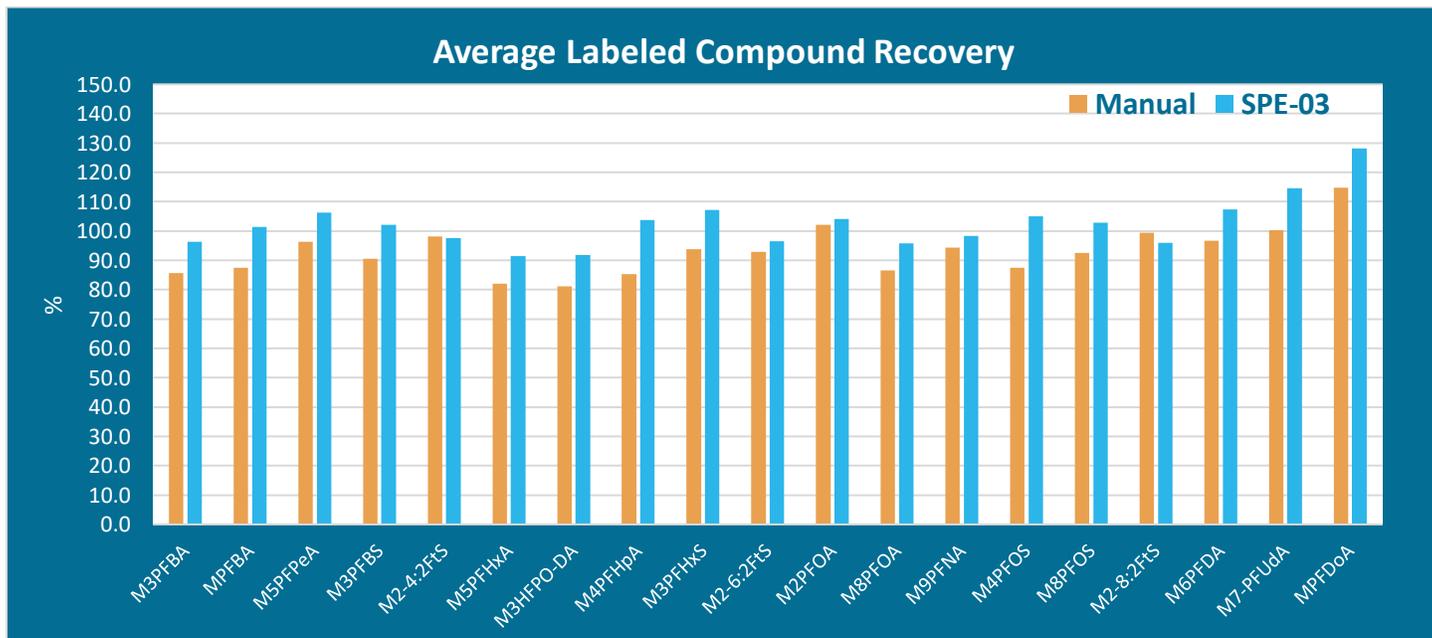
The SPE-03 demonstrated average recoveries between 95% to 110%, compared to manual extraction recoveries between 90% to 120%. Method requirements are 70% to 130%. NFDHA is known to behave differently than its corresponding M5PFHxA isotope, but was seen to have better accuracy on the SPE-03.

Figure 2 - Average Recoveries of Manual vs SPE-03 Extraction



Since the analyte recoveries are corrected through isotope dilution, the actual extraction performance can be determined by looking at the recoveries of the labeled compounds. All labeled compounds recovered above 90% on the SPE-03 and follow a similar trend as manual extraction. Method limits are 50% to 200%.

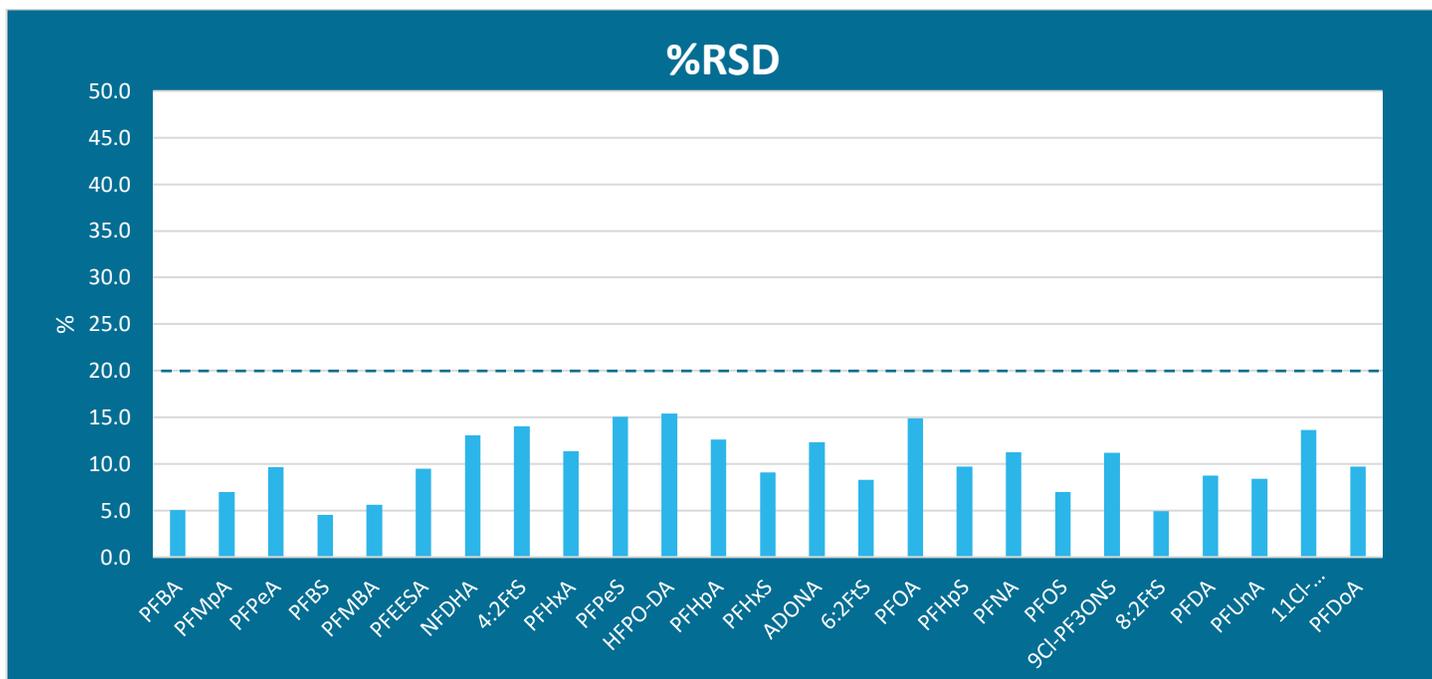
Figure 3 - Average Labeled Compound Recoveries of Manual vs SPE-03 Extraction



Reproducibility

EPA Method 533 requires %RSD to be <20% on the same batch of spikes during the Initial Demonstration of Capability (IDC). By analyzing the LFBs from 8 separate extractions, the data encompasses reproducibility across different concentrations, sample batches, lab personnel and ports on the system. With these conditions, the %RSD remained below 20% on the SPE-03.

Figure 4 - %RSD on SPE-03 Extraction



Tolerance to Field Sample Matrices

The positive pressure syringe pumps on the SPE-03 are resistant to clogging and provide uniform flow rates. Despite minimal inconsistencies in the extraction procedure, it is still observed that field samples which are discolored or have particulates tend to enhance or suppress certain compounds. The labeled compound recoveries of 20 field samples are used to discuss matrix effects.

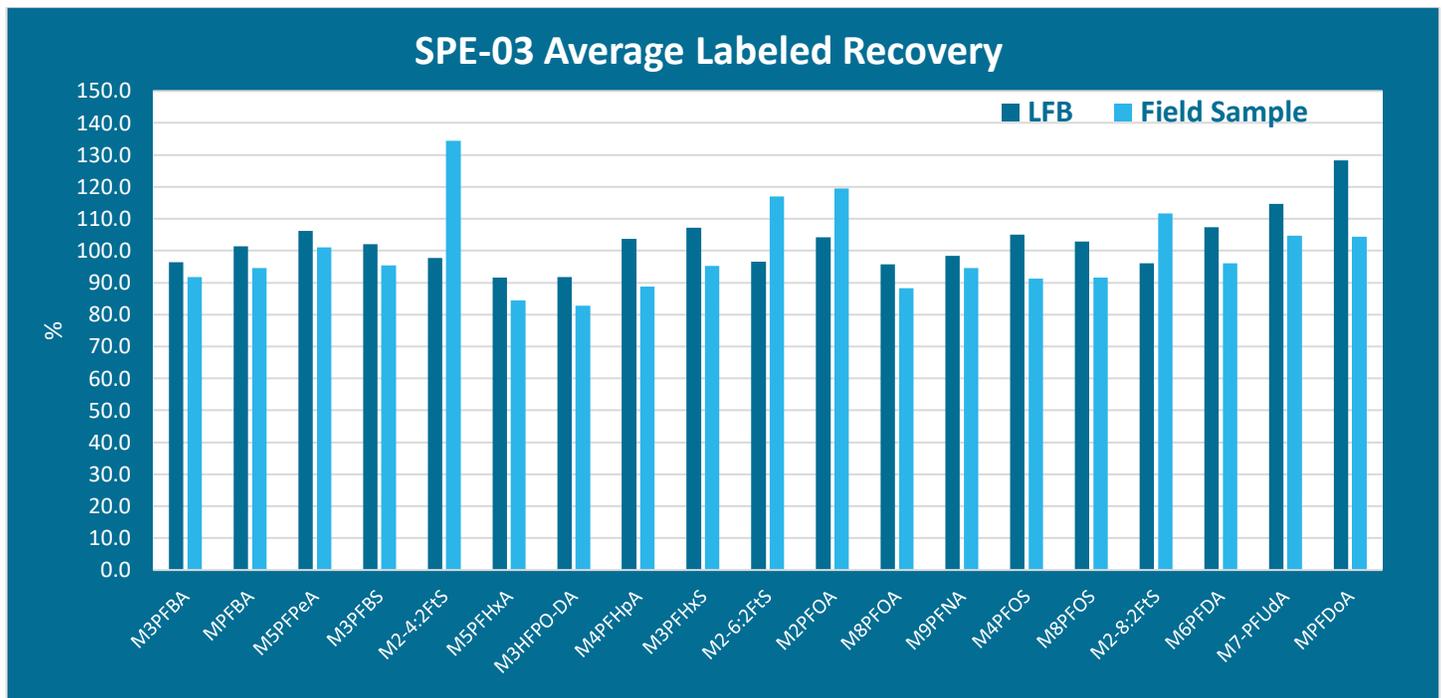
Accuracy

Figure 5 below compares average labeled compound recoveries between the 8 LFBs and 20 field samples extracted on the SPE-03.

Most notably, the response of the 3 fluorotelomers were enhanced, with M2-4:2FtS having a significant increase of about 30%. This is quite commonly seen in field samples, especially for non-potable water applications. In Wellington Labs' reference guide², it mentions that matrix effects can have a considerable impact on the ionization of fluorotelomer sulfonic acids. This phenomenon is speculated to be independent of the extraction. The only other compound that recovered higher was M2PFOA, and is interesting to note that these are the only 4 isotopes that have 2 C13 atoms.

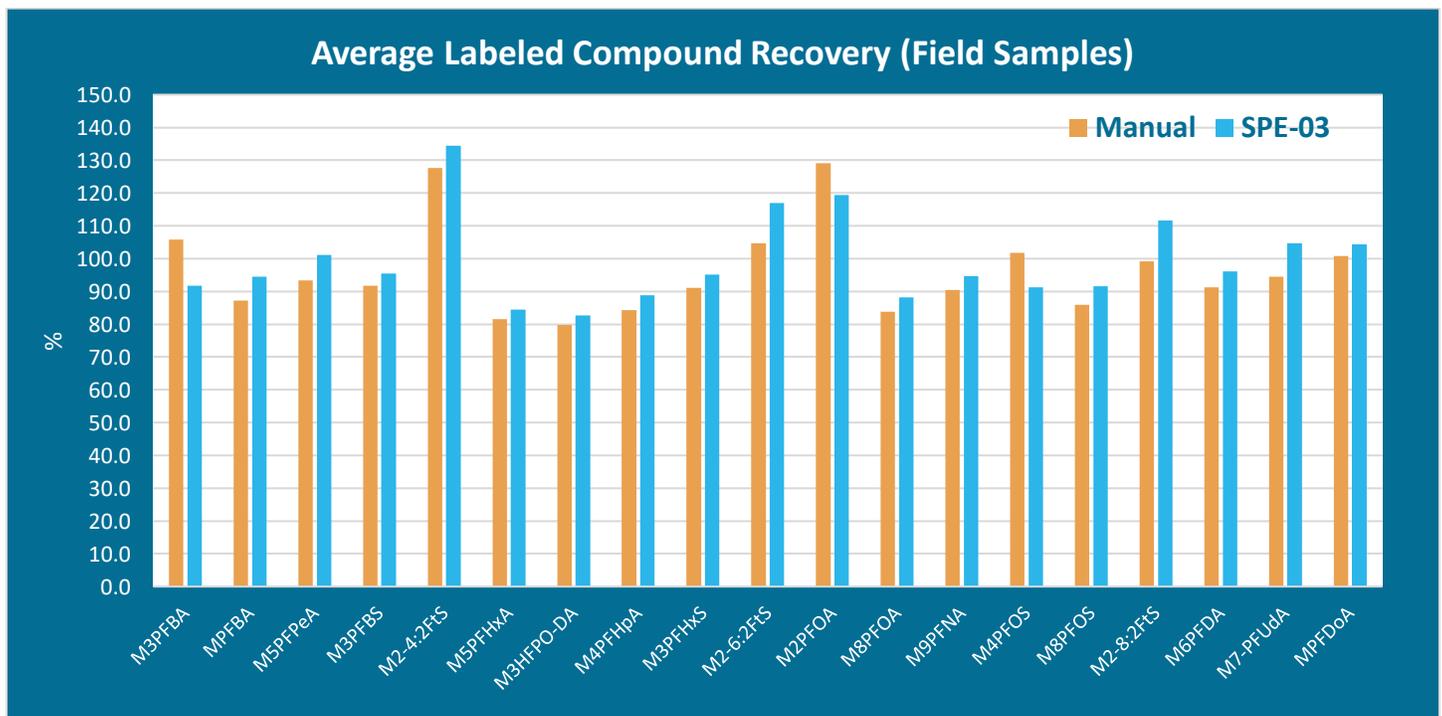
Later eluting compounds like MPFDoA can stick to the sorbent and particles in dirtier samples, resulting in lower recoveries. The effects again are more significant on non-drinking water applications, but the corresponding methods have the flexibility to increase elution volume.

Figure 5 - Average Labeled Compound recovery of LFB vs Field Samples on the SPE-03



To confirm whether the matrix effects are independent of the SPE-03, Figure 6 compares the labeled recoveries of 20 fields samples extracted on the manifold vs SPE-03. Both systems had similar trends with higher perceived recoveries for the fluorotelomers and M2PFOA, as well as reduced recoveries for MPFDoA.

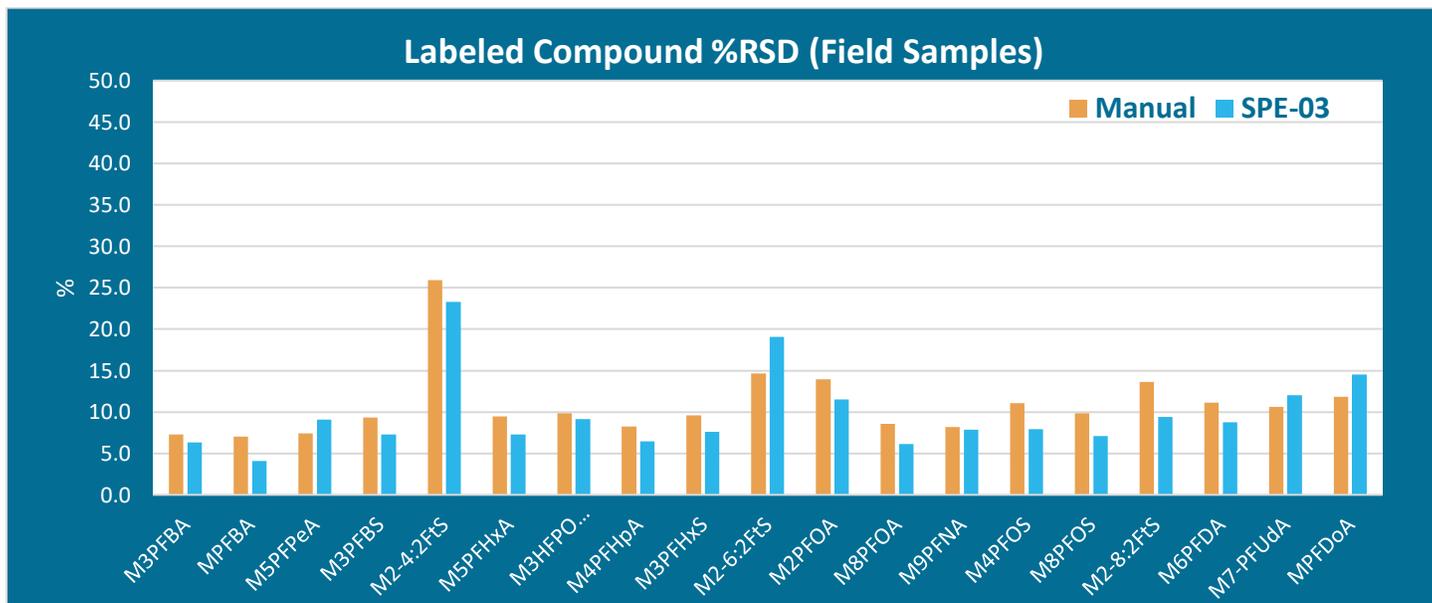
Figure 6 - Average Labeled Compound Recoveries of Manual vs SPE-03 Extraction



Reproducibility

The %RSD of labeled compounds in field samples also show strong agreement between both setups. As discussed, fluorotelomers are more susceptible to matrix enhancement which explains the higher variation.

Figure 7 -Labeled Compound %RSD of Manual vs SPE-03 Extraction



CONCLUSIONS

The SPE-03 system provides a fully automated solution that exceeds all EPA Method 533 requirements, with results comparable to manual extractions performed by one of the leading PFAS labs. It has demonstrated clean background, excellent recovery and high reproducibility across analyte concentrations and extraction batches.

Once the samples, cartridges and fraction tubes are attached to the SPE-03, it takes less than 2 hours of unattended operation to complete a batch of 8 samples. System cleaning involves changing out the sample bottles and running an automated cycle for 15 minutes.

The SPE-03 can be used for a wide range of PFAS applications. For drinking and non-potable water, Alpha Analytical performs EPA Method 537.1, EPA Method 533, modified methods, DoD and a proprietary method that covers 40 PFAS compounds. The same system is utilized for clean up of soil, solid and tissue after solvent extraction.

Phenomenex WAX cartridges (Cat. 8B-S038-HCH) were used for the development of EPA Method 533 and by default meets all sorbent requirements of the method. They are seen here to produce good results on both the manual and automated setups.



References

1. EPA Method 533 <https://www.epa.gov/sites/production/files/2019-12/documents/method-533-815b19020.pdf>
2. Wellington Laboratories https://well-labs.com/docs/pfc_reference_handling_guide.pdf

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