Comparing Methods for Testing PFAS in Solid Matrices: USEPA 1633, USFDA C-010.03, & European Union Reference Laboratory

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Introduction

Extraction protocols and analytical methods have been utilized and revised for detecting Per-and Polyfluoroalkyl Substances (PFAS) in aqueous matrices since 2013. The toxicity and persistence of PFAS drove the evolution of drinking water methods and initiated the process to regulate PFAS in solid matrices. The US Environmental Protection Agency (USEPA) published a single lab validation study in 2021 for Method 1633 and released 1633A in December 2024. This method monitors 40 PFAS targets in fish tissues, soils, biosolids, and wastewater. During that time, the US Food and Drug Administration (USFDA) released a single lab validation in April 2024. This study monitors 30 PFAS targets in different foodstuffs. In April 2023, the European Commission published Regulation (EU) 2023/915, which sets guidelines on maximum levels for 4 target PFAS analytes in food. In response to these guidelines, the European Union Reference Laboratory (EURL) developed 2 separate sample preparation methods for the determination of 33 PFAS analytes in food of animal and plant origin.

Through a simplified and automated workflow, the Biotage® Lysera, TurboVap® LV, and Extrahera™ HV-5000 provide efficient sample homogenization, liquid extraction, solid phase extraction, and solvent concentration that can be applied to all three techniques.

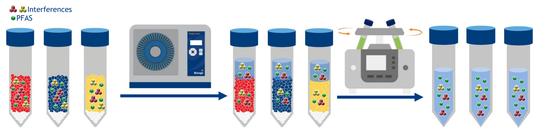
Procedure

Homogenization & Liquid Extraction

Biotage® Lysera bead mill homogenizer can be utilized for both sample homogenization & liquid extraction. Sample amounts, solvents, and extraction salts vary by both method and matrix type. Table 1 compares extraction method parameters for EPA 1633A, USFDA C-010-03, and EURL.

***Table 1**. Method Comparison for Homogenization & Liquid Extraction.*

Method	Matrix	Mass	Extraction Solvents	Extraction Salts
EPA 1633A	Tissue	1 - 2 g	10 mL 0.05M KOH in MeOH 10 mL MeCN 5 mL 0.05M KOH in MeOH	n/a
	Biosolids & Soils	0.5 g - 5 g	10 mL 0.3% NH_4OH in MeOH 15 mL 0.3% NH_4OH in MeOH 5 mL 0.3% NH_4OH in MeOH	
USFDA C-010.03	Food > 25% H_2O Food & Feed < 25% H_2O	5 g	5 mL - 25 mL H_2O 10 mL MeCN 0.15 mL Formic Acid	6 g $MgSO_4$ 1.5 g NaCl
	Food - Dry Powders	1 g		
EURL	Food & Feed	2 g	15 mL of 0.01M KOH in MeOH 5 mL of MeOH or MeCN 5 mL of MeOH or MeCN 2 mL H_2O 20 mL 0.1% NH_3 in MeCN 10 mL H_2O 2 mL 0.5M TBAHS	n/a

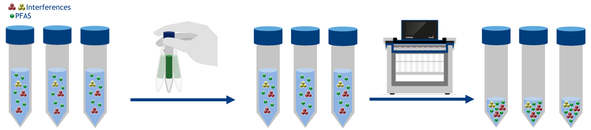


Dispersive SPE Cleanup & Solvent Evaporation

Table 2 outlines dispersive solid phase extraction (dSPE) media for matrix clean-up. After dSPE clean-up, all methods require the extract to be concentrated prior to further processing. The concentration temperatures for each method are listed below. This can be accomplished by utilizing the TurboVap® LV.

***Table 2**. Method Comparison for Dispersive SPE & Water Bath Temperatures.*

Method Parameter	EPA 1633A	USFDA C-010.03	EURL
Dispersive SPE (dSPE)	10 mg GCB	900 mg MgSO4 300 mg PSA 150 mg GCB	2.0 g MgSO4 0.5 g NaCl 0.1 g C18 0.1 g GCB
Evaporation Temperature	55˚C	60˚C	40˚C

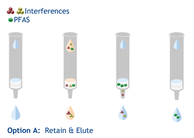


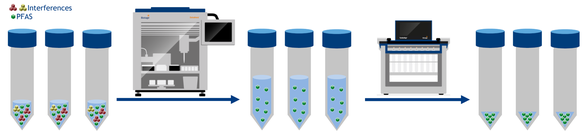
Column SPE & Final Concentration

The Extrahera™ HV-5000 is an automated workstation that can be utilized for column solid phase extraction (cSPE). Techniques such as retain & elute cSPE or pass-through cSPE can be utilized for further removal of matrix interferences. Table 3 outlines cSPE steps for both approaches.

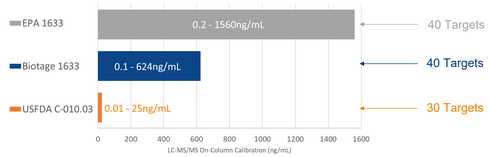
***Table 3**. Retain & Elute cSPE Steps Compared to Pass-Through cSPE.*

cSPE Technique	Retain & Elute	Pass-Through
cSPE Sorbent	WAX(GCB)	GCB
Sample Dilution	Yes	Optional
Dilution Factor	Up to 20X	Up to 8X
Conditioning	NH_4OH* in MeOH MeOH	NH_4OH* in MeOH MeOH
Equilibration	0.3M Formic Acid 25mM NaOAc in H2O Reagent Water	Reagent Water
Sample Load	≤ 50 mL	≤ 5 mL
Wash	Reagent Water 0.1M Formic Acid/MeOH 25mM NaOAc in H2O 1:1 H_2O/MeOH	n/a
Elute	NH_4OH* in MeOH MeOH	NH_4OH* in MeOH
Final Concentration	Optional	





Analytical Calibration Range Comparison

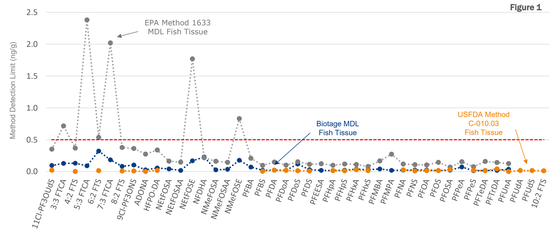


Method Performance

Detection Limits

Method detection limits (MDLs) for fish tissue were determined by processing seven replicate matrix blanks and seven laboratory fortified blanks (LFBs) spiked at concentrations ranging from 0.075 - 0.75ng/g. Figure 1 compares results to MDLs found in EPA 1633A and USFDA C-010.03. *Note: EURL does not publicly disclose test results.*

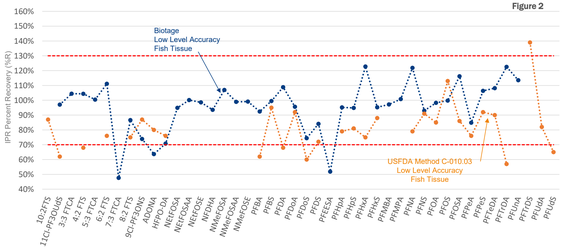
Figure 1



Accuracy: Low-Level Spike

Low-Level Spike Accuracy was determined by processing laboratory fortified matrix spikes with concentrations ranging from 0.075 - 2.5ng/g. Results are shown in figure 2. *Note: EPA 1633A does not provide accuracy results for low spike levels & EURL does not publicly disclose test results.*

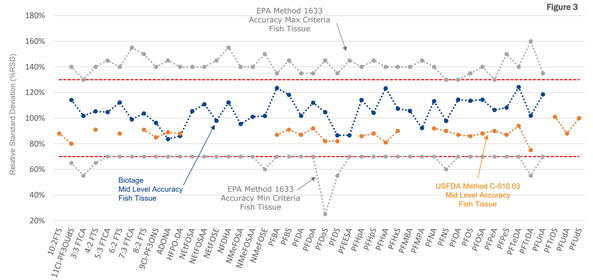
Figure 2



Accuracy: Mid-Level Spike

Mid-level spike accuracy was determined by processing laboratory fortified reference matrix spikes with concentrations ranging from 2.5 - 62.5ng/g. Results are shown in figure 3. *Note: EURL does not publicly disclose test results.*

Figure 3



Precision: Low & Mid Level Spikes

Low & mid level spike precision was determined by processing laboratory fortified matrix spikes. Results are shown in figures 4 & 5. *Note: EURL does not publicly disclose test results.*

Figure 4.

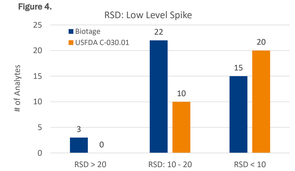
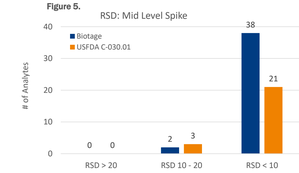


Figure 5.



Conclusion

This comparison study outlines techniques developed by the USEPA, USFDA, and EURL for testing PFAS in solid matrices. Additionally, this work outlines a simple sample preparation technique that enables high throughput processing of samples while maintaining exceptional data quality. Advantages of this workflow include elimination of manual transfer steps, complete preparation of up to 24 samples in less than four hours, and minimal cleaning required between sample batches. In conclusion, this automated workflow offers a highly efficient and reliable solution for PFAS analysis in solid samples for each technique described.

Biotage® Lysera | TurboVap® LV | Extrahera™ HV-5000 | Webinar 1633A