Environmental and Food Safety

Simplified PFAS in groundwater analysis through automation, retention time confirmation, and high-resolution full scan data

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Abstract

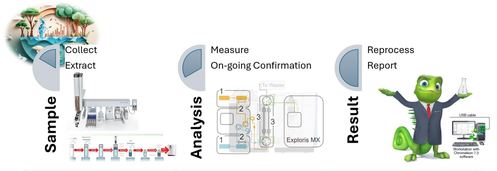
Purpose: To demonstrate a simplified, automated PFAS analysis method in wastewater (also applicable to clean water and soil) using high-resolution mass spectrometry, reducing solvent consumption and ensuring reliable quantitation.

Methods: Automated DLLME extraction, followed by chromatography on a Thermo Scientific™ Vanquish™ Duo system using two different columns and detection using a Thermo Scientific™ Orbitrap Exploris™ MX mass detector. Data analysis was performed with Thermo Scientific™ Chromeleon™ CDS 7.3.2.

Results: The method achieved high precision, accuracy, sensitivity, and on-going confirmation with low solvent consumption. It supports environmental sustainability and cost-efficiency while providing reliable accreditation-grade data across different matrices.

Introduction

As PFAS testing demand increases globally, efficient and reliable testing methods are essential for maintaining sample throughput and laboratory productivity whilst regulations become more demanding. The US EPA Method 1633 highlights the manual SPE-based approach for non-drinking waters. Our study aims to simplify and enhance PFAS detection using automated DLLME and high-resolution mass spectrometry.



Materials and Methods

Sample preparation: conducted for clean water, wastewater, and soil using automated DLLME extraction, which yields high concentration factors with minimal solvent use. The total solvent usage is less than 2 mL per sample, including the initial dispersant/extractant and the second extraction/injection solvent. This automated method is highly efficient, with sample preparation taking less than 10 minutes per sample.

Figure 2. Schematic of the Automated DLLME Extraction Procedure

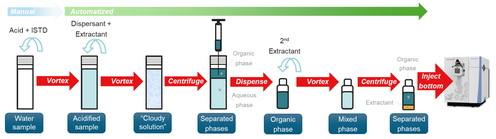
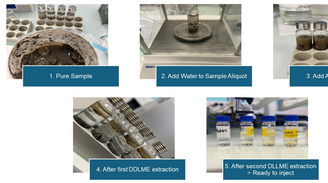


Figure 3. Step by step sample appearance



Method:

- **Chromatography:** Performed using the Thermo Scientific™ Vanquish™ Duo system with two columns and methanol and water as mobile phases.
- **Detection:** Utilized the Thermo Scientific™ Orbitrap Exploris™ MX, operating in full scan mode at 60,000 resolution.
- **Software:** Data processed with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.3.2.

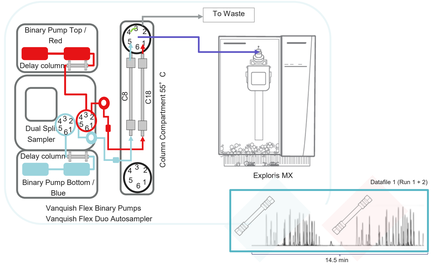
Table 1. Method parameters

PARAMETERS	
Column 1	Hypersil GOLD C8™ 100x2.1mm, 1.9 µm
Column 2	Hypersil GOLD™ 100x2.1mm, 1.9 µm
Delay Column 1+2	Hypersil GOLD™ 50x2.1mm, 1.9 µm
Mobile Phase A	Water + 0.1M Ammonium Fluoride
Mobile Phase B	Methanol + 0.1M Ammonium Fluoride
Total Run Time	14.5 min
Injection Volume	5 µL
Acquisition type	Full Scan (+2 in-source fragmentation FS)
Scan range (m/z)	70-1000
Resolution	60,000
Source Type	HESI
Polarity mode	Negative

Table 2. Recognition criteria

Recognition Criteria (EURL)	
1	Retention Time on C18-Column
2	Accurate mass measurements with full scan
3	Monitoring 2 ions with m/z accuracy ≤ 5 ppm
4	Matching ion ratios are not necessary
PFAS Specific Considerations	
Some PFAS compounds may have only one ion or one MRM (quantifier only, no qualifier) : EURL recommends a second chromatographic separation for confirmation	
During first LC-Column stabilization, sample is re-injected on a second LC-column, for on-going confirmation as per EURL recommendation.	
5	Additional Retention Time on C8-Column

Figure 2. Instrument configuration with confirmation concept



Results

PFAS included in workflow

Soil can act as a reservoir for PFAS, leading to potential contamination of groundwater and uptake by plants, which can then enter the food chain. Among the 61 PFAS we monitor in this method (Table 3 and 4), we emphasize several key compounds:

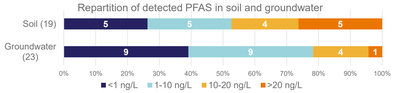
PFOA (Perfluorooctanoic Acid, CAS Number: 335-67-1) is used in the manufacture of Teflon and other fluoropolymers and is persistent and bioaccumulative, linked to various health issues. **PFOS (Perfluorooctane Sulfonate, CAS Number: 1763-23-1),** previously used in products like Scotchgard and firefighting foams, is also persistent in the environment and poses significant health concerns. **GenX (Hexafluoropropylene Oxide Dimer Acid, CAS Number: 13252-13-6)** serves as a replacement for PFOA in fluoropolymer production but has emerging concerns about its environmental persistence and toxicity. **PFHxS (Perfluorohexane Sulfonate, CAS Number: 355-46-4)** is used in firefighting foams and other industrial applications and is bioaccumulative and persistent with potential health effects. **PFNA (Perfluorononanoic Acid, CAS Number: 375-95-1),** found in non-stick coatings and other products, is persistent in the environment and associated with liver toxicity and developmental effects. **PFDA (Perfluorodecanoic Acid, CAS Number: 335-76-2)** is used in various industrial applications and is bioaccumulative and persistent, with potential health impacts.

Table 3. Workflow analyte list

CAS	Name	EPA Method
763051-92-9	11Cl-PF2OUdS	533/537.1/1633
151772-58-6	3,6-OPFHpA (NFDHA)	533/1633
356-02-5	3:3 FTCA	1633
757124-72-4	4:2FTS	533/1633/8327
914637-49-3	5:3 FTCA	1633
27619-97-2	6:2FTS	533/1633/8327
812-70-4	7:3 FTCA	1633
39108-34-4	8:2FTS	533/1633/8327
756426-58-1	9Cl-PF3ONS	533/537.1/1633
919005-14-4	ADONA (DONA)	533/537.1/1633
754-91-6	FOSA (PFOSA)	1633/8327
13252-13-6	HFPO-DA (Gen X)	533/537.1/1633
4151-50-2	N-EtFOSA	1633
2991-50-6	N-EtFOSAA	537.1/1633/8327
1691-99-2	N-EtFOSE	1633
31506-32-8	N-MeFOSA	1633
2355-31-9	N-MeFOSAA	537.1/1633/8327
24448-09-7	N-MeFOSE	1633
377-73-1	PF4OPeA (PFMPA)	533/1633
863090-89-5	PF5HxA (PFMBA)	533/1633
375-22-4	PFBA	533/1633/8327
375-73-5	PFBS	533/537.1/1633/8327
335-76-2	PFDA	533/537.1/1633/8327
307-55-1	PFDoA (PFDoDA)	533/537.1/1633
79780-39-5	PFDoS (PFDoDS)	1633
335-77-3	PFDS	1633/8327
113507-82-7	PFEESA	533/1633
375-85-9	PFHpA	533/537.1/1633/8327
375-92-8	PFHpS	533/1633/8327
307-24-4	PFHxA	533/537.1/1633/8327
355-46-4	PFHxS	533/537.1/1633/8327
375-95-1	PFNA	537.1/1633/8327
68259-12-1	PFNS	1633/8327
335-67-1	PFOA	533/537.1/1633/8327
1763-23-1	PFOS	533/537.1/1633/8327
2706-90-3	PFPeA	533/1633/8327
2706-91-4	PFPeS	533/1633/8327
376-06-7	PFTeDA (PFTeA)	1633/8327
72629-94-8	PFTrDA (PFTeA)	537.1/1633/8327
2058-94-8	PFUdA (PFUnDA; PFUnA)	533/537.1/1633/8327
120226-60-0	10:2FTS	
34455-29-3	6:2 FTAB (Capstone B)	
647-42-7	6:2 FTOH (FHET)	
943913-15-3	6:2/8:2diPAP	
57677-95-9	6:2diPAP	
1546-95-8	7HPFHpA	
678-39-7	8:2 FTOH (FOET)	
70887-84-2	8:2 FTUCA (FOUEA)	
678-41-1	8:2diPAP	
34598-33-9	8:3FTCA	
30334-69-1	FBSA	
41997-13-1	FHxSA	
27854-31-5	FOEA (8:2 FTCA)	
13252-14-7	HFPO-TA	
68298-12-4	N-MeFBSA	
159381-10-9	N-MeFBSAA	
646-83-3	PFECHS	
67906-19-5	PFHxDA	
16517-11-6	PFOcDA (PFODA)	
791563-89-8	PFTrDS	
749786-16-1	PFUnDS	

Groundwater and Soil Samples: Demonstration of Workflow

To demonstrate the workflow, 15 mL of a dirty groundwater containing colored sediment and 1 g of soil (mixed with 14 mL of distilled water) were extracted using DLLME. The calibration curve was prepared by spiking 15 mL of distilled water, followed by DLLME extraction. Groundwater and soil samples were also quantified using the standard addition method to verify matrix effects. Additionally, the groundwater sample was analyzed by an independent accredited laboratory.



The extracted calibration curves (using internal standards) ranging from 0.1 ng/L to 100 ng/L are shown in Figure 5. Figure 6 presents the groundwater sample results, with the overlaid m/z measurements at 60K resolution and 5 ppm accuracy, demonstrating separation and quantitation on a C18 column (second row) and ongoing confirmation on a C8 column (first row). The blue-shadowed dotted line represents the overlay of the 5 ng/L extracted standard as a trace reference.

Figure 7 shows the soil sample results, with the overlaid m/z measurements at 60K resolution and 5 ppm accuracy, demonstrating separation and quantitation on a C18 column (second row) and ongoing confirmation on a C8 column (first row). The blue-shadowed dotted line represents the overlay of the 0.5 ng/L extracted standard as a trace reference.

Figure 5. DLLME extracted calibration curves from 0.1 ng/L to 100 ng/L

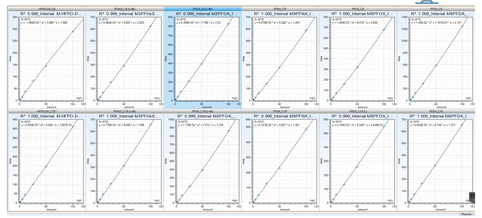


Figure 6. DLLME extracted groundwater sample

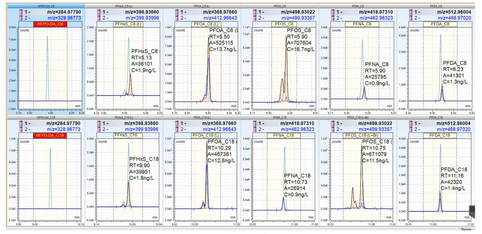


Figure 7. DLLME extracted soil sample

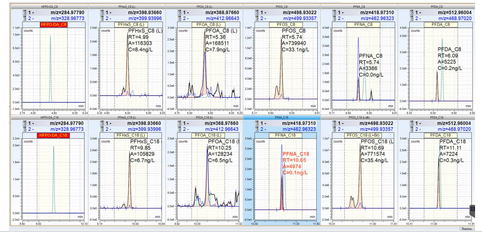


Table 4 presents the quantitation of specific PFAS of interest for the groundwater and soil samples, along with the 0.5 ng/L and 1 ng/L extracted standards. For comparison, sample analysis by traditional SPE and triple quadrupole MS are included. This comparison attests the accuracy of our DLLME Exploris MX workflow.

Table 4. Quantitation results and comparison for the 6 PFAS of primary interest

Peak Name	CAS	Column Type	Groundwater			Soil	
			DDLME % recovery at 1 ng/L	DLLME (ng/L)	SPE (ng/L)	DDLME % recovery at 750 ng/kg	SPE (ng/kg)
HFPO-DA_C18	13252-13-6	Hypersil GOLD™	93	ND	ND	107	ND
HFPO-DA_C8	13252-13-6	Hypersil GOLD C8™	✓	ND	ND	✓	ND
PFDA_C18	335-76-2	Hypersil GOLD™	90	1.4	1.04	124	3.25
PFDA_C8	335-76-2	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFHxS_C18 (L+Br)	355-46-4	Hypersil GOLD™	83	1.9	1.47	98	113.63
PFHxS_C8 (L)	355-46-4	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFNA_C18	375-95-1	Hypersil GOLD™	97	0.9	ND	72	1.07
PFNA_C8	375-95-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFOA_C18 (L+Br)	335-67-1	Hypersil GOLD™	85	13.8	8.20	95	101.32
PFOA_C8 (L)	335-67-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFOS_C18 (L+Br)	1763-23-1	Hypersil GOLD™	100	11.5	12.30	117	416.72
PFOS_C8	1763-23-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed

Conclusions

The automated DLLME method effectively quantifies PFAS with high resolution and on-going confirmation using dual-column injections, meeting EURL PFAS standards. This method supports diverse environmental testing needs and shows comparable results to SPE-Triple Quadrupole analyses of dirty groundwater by independent laboratories.
For soil samples, ongoing recovery evaluation by running samples twice (pure and spiked) is recommended to address matrix variability.

Key advantages include:
- **Efficiency and Speed**: This method provides a high sample concentration while only using less than 2 mL of solvent per sample and less than ten minutes to prepare each sample.
- **Cost and Environmental Benefits**: Reduced solvent use, no filters or cartridges needed, and lower environmental impact than traditional SPE methods.
- **Ease of Use**: User-friendly LCMS setup managed with Chromeleon CDS 7.3.2.
- **Regulatory Compliance**: Meets European PFAS standards in drinking water.
- **Accurate Quantitation**: Precise PFAS measurement with high-resolution scans and dual-column analysis.
- **Configuration Advantage**: The use of accurate mass and orthogonal on-going confirmation enhances confidence in results, eliminates the need for MS method development, and enables retrospective analysis.

Overall, the automated DLLME method is efficient, environmentally friendly, and provides robust and reliable PFAS quantitation across diverse matrices.

References

1. Thermo Fisher Scientific Application Brief 003164: Dispersive liquid-liquid micro-extraction for the automated sample preparation of PFAS in drinking water. (thermofisher.com)
2. Thermo Fisher Scientific Poster: PFAS analysis strategy story – direct injection, DLLME, LC-MS/MS, LC- Orbitrap / GC-Orbitrap

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All authors are employees of Thermo Fisher Scientific.

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