Application note | 003992

Environmental

Novel, rapid, and flexible tandem dispersive liquid-liquid microextraction for the determination of PFAS in environmental samples

Using full-scan high-resolution with the Orbitrap Exploris EFOX mass detector or a selected ion monitoring approach with the TSQ Altis Plus EFOX Edition MS

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Keywords

PFAS, groundwater, wastewater, soil, sludge, industrial effluent, European regulation, workflow, extraction, DLLME, EFOX

Application benefits

The automated tandem dispersive liquid-liquid microextraction (DLLME) method for per- and polyfluoroalkyl substances (PFAS) analysis offers significant benefits for environmental testing:

- **Efficiency and speed:** Enhanced accuracy and reproducibility due to automation; 54 samples in 8 hours with unattended operation, equivalent to 9 minutes per sample.
- **Full automation of sample preparation:** The 3-in-1 method allows for the extraction, concentration, and clean-up of target analytes from each matrix, starting from raw sample to ready-to-inject extract, enhancing efficiency and consistency.
- **Flexibility and reactivity:** The ability to alternate any type of sample, as the protocol is identical regardless of the matrix type, increases lab productivity and reactivity.
- **Walkaway automation:** The system's ability to operate with minimal human intervention allows users to focus on other tasks, increasing overall lab productivity.
- **Ease of use:** User-friendly software; with the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.3.2, the same software and user interface are used for managing sample preparation and sample analysis.

- **Robust and reliable:** Provides reliable PFAS quantitation across diverse matrices, supporting diverse environmental testing needs. This automated sample preparation ensures injection of clean and enriched extracts into the LC-MS system, minimizing overall sample handling and reducing the chances of PFAS cross-contamination with the lab environment.

- **Regulatory compliance:** Both LC-MS analytical methodologies presented here, in combination with DLLME, meet the performance sensitivity requirements set by regulatory agencies across Europe and in the U.S. for PFAS in drinking water.

- **Cost and environmental benefits:** Reduced solvent consumption (up to 2 mL per sample) and elimination of filters or cartridges lower the environmental impact when compared to methods based on solid-phase extraction (SPE) for clean-up purposes.

- **Reduction in user's exposure to solvent vapors:** The system minimizes the exposure of users to solvent vapors, promoting a safer and healthier working environment.

Goal

This study aims to demonstrate the benefits of a simplified and automated method for analyzing PFAS in wastewater, drinking water, soil, and industrial effluent. The method can be used with high resolution or triple quadrupole mass spectrometry for detection of target PFAS, with reduced solvent consumption and reliable quantification.

Introduction

The global demand for testing of PFAS compounds is increasing, driven by growing awareness of their environmental and health impacts. Efficient and reliable testing methods are essential to maintain sample throughput and laboratory productivity, especially as regulatory requirements become more stringent. As an example, the U.S. EPA Method 1633A outlines for a variety of matrices, including non-potable water, soil, bio solids, and tissue samples, a labor-intensive, multi-step workflow involving sonication and centrifugation, followed by evaporation, reconstitution, and solid-phase extraction for final clean-up of the enriched extracts.

Our study aims to simplify PFAS quantitation by employing automated tandem DLLME. This approach streamlines the extraction, enrichment, and clean-up processes. By reducing solvent consumption and automating the extraction process, we aim to support environmental sustainability and cost-efficiency in PFAS analysis.

Experimental

The PFAS analysis method involves automated dispersive liquid-liquid microextraction (DLLME), followed by chromatography and mass spectrometry (Figure 1). The Thermo Scientific™ Vanquish™ Duo UHPLC System is equipped with one unique column or with two different columns. Detection is performed in one of two ways: (1) using triple quadrupole MS in SRM mode with the Thermo Scientific™ TSQ Altis™ Plus EFOX (Environmental Food Organic Xenobiotics) Edition Mass Spectrometer or (2) using full scan HRMS with the Thermo Scientific™ Orbitrap Exploris™ EFOX Mass Detector. In both cases, data analysis is conducted with Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS).

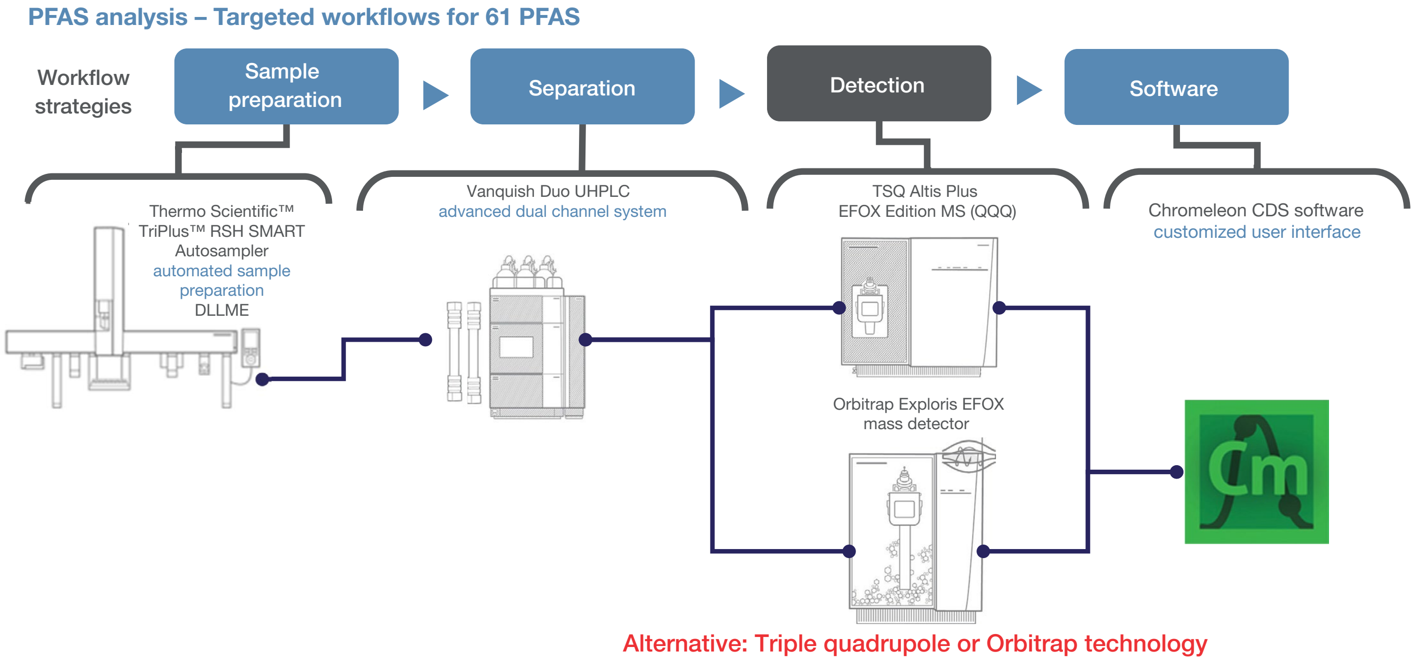


Figure 1. PFAS workflow includes sample preparation, separation, and software.

Sample preparation

Sample preparation was conducted for drinking water, wastewater, soil, and industrial effluents using automated DLLME (Figure 2). This method yields high concentration factors with minimal solvent use. The total solvent usage is about 2 mL per sample, including the initial extraction mix and the second clean-up/injection solvent. This automated method is highly efficient, capable of running 54 samples unattended for each batch. Due to the centrifuge needing to be balanced by centrifuging two samples at a time, two samples need to be fully prepared in parallel. After 18 minutes, two samples are ready to be injected. The process is driven by Chromeleon software.

Chromatography

Chromatography was performed using the Vanquish Duo UHPLC system with one or two columns and methanol and water as mobile phases.

Detection

Detection was carried out using the Orbitrap Exploris EFOX mass detector operating in full scan mode at a resolution of 60,000 or the TSQ Altis Plus EFOX Edition MS operating in SRM mode.

Software

Data was processed using Chromeleon CDS version 7.3.2.

Results and discussion

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 monitored in this method, several key compounds are emphasized:

- **PFOA (Perfluorooctanoic acid, CAS Number: 335-67-1):** Used in the manufacture of Teflon™ and other fluoropolymers, PFOA 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, PFOS 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)**: Used in firefighting foams and other industrial applications, PFHxS is bioaccumulative and persistent with potential health effects.
- **PFNA (Perfluorononanoic acid, CAS Number: 375-95-1):** Found in non-stick coatings and other products, PFNA is persistent in the environment and associated with liver toxicity and developmental effects.
- **PFDA (Perfluorodecanoic acid, CAS Number: 335-76-2):** Used in various industrial applications, PFDA is bioaccumulative and persistent, with potential health impacts.

The 61 PFAS used in this work were selected based on European and U.S. regulations and include the 40 PFAS that are in EPA Methods 533, 537.1, and 1633A. Twenty of these compounds are regulated under the Drinking Water Directive (2020/2184/EU), with additional PFAS compounds monitored by individual EU member states or flagged as emerging contaminants. This ensures that the method remains adaptable and compliant with evolving environmental and food safety legislation.

Demonstration of workflow

To demonstrate the workflow performance, a simplified matrix-extracted calibration curve was first prepared by spiking distilled water, followed by DLLME extraction. The extracted calibration curves, using internal standard (ITSD) and ranging from 0.1 ng/L to 100 ng/L, are shown in Figure 4. In the same batch, samples of dirty groundwater containing colored sediment (undiluted), soil (diluted with distilled water), and industrial effluent (diluted with distilled water) were also extracted using DLLME (Figure 3).

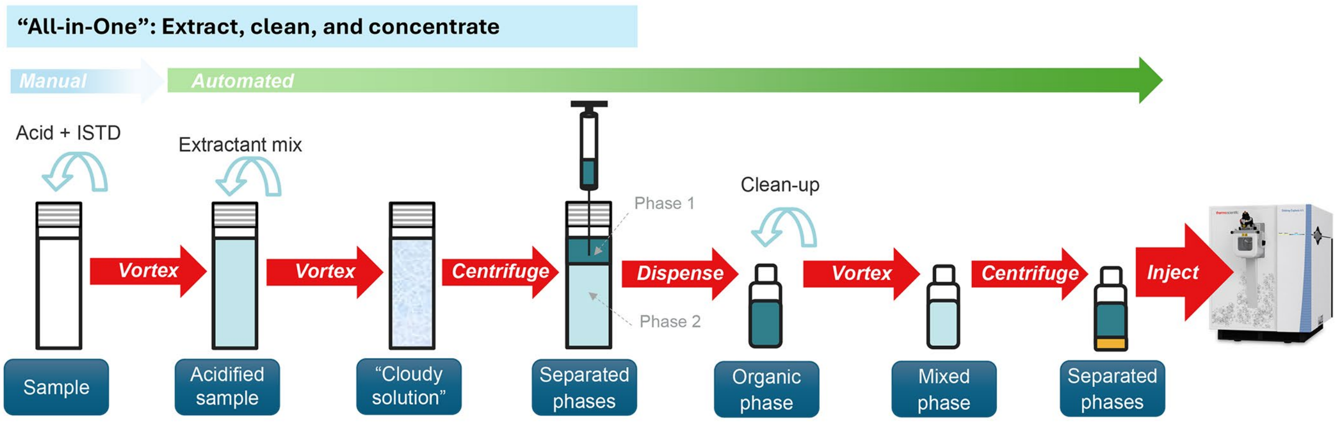
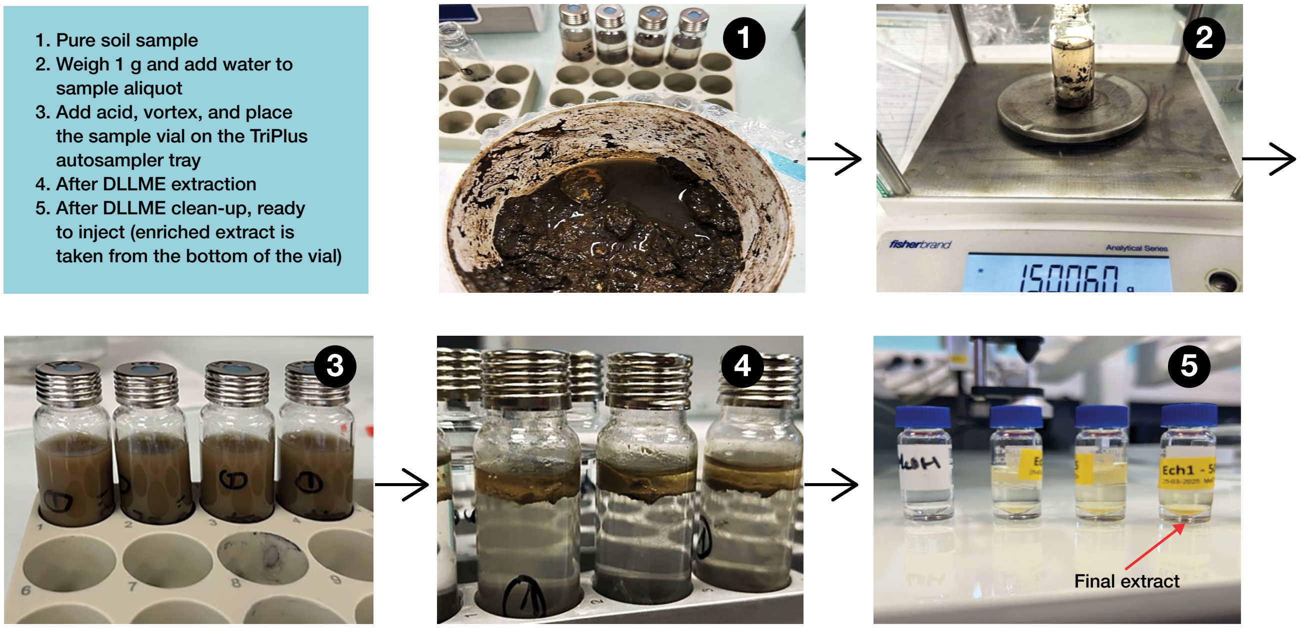


Figure 2. Step-by-step DLLME procedure from raw sample to clean, concentrated extract.

To verify matrix effects and confirm that the simplified matrix-extracted calibration curve provides the same quantification accuracy as the standard addition method, which is known to be more accurate but also more time-consuming, all samples were also quantified using the standard addition method. Additionally, the groundwater and industrial effluent samples were analyzed by independent accredited laboratories. Results are shown as comparative data with the DLLME approach.

All samples were extracted and analyzed in the same batch. However, for clarity, the results are presented in separate paragraphs and tables. Data were acquired with the Orbitrap Exploris EFOX mass detector operating in full scan mode at a resolution of 60,000 and the *m/z* were extracted with a 5 ppm accuracy extraction window.



1. Pure soil sample
2. Weigh 1 g and add water to sample aliquot
3. Add acid, vortex, and place the sample vial on the TriPlus autosampler tray
4. After DLLME extraction
5. After DLLME clean-up, ready to inject (enriched extract is taken from the bottom of the vial)

Figure 3. DLLME step-by-step process for soil samples.

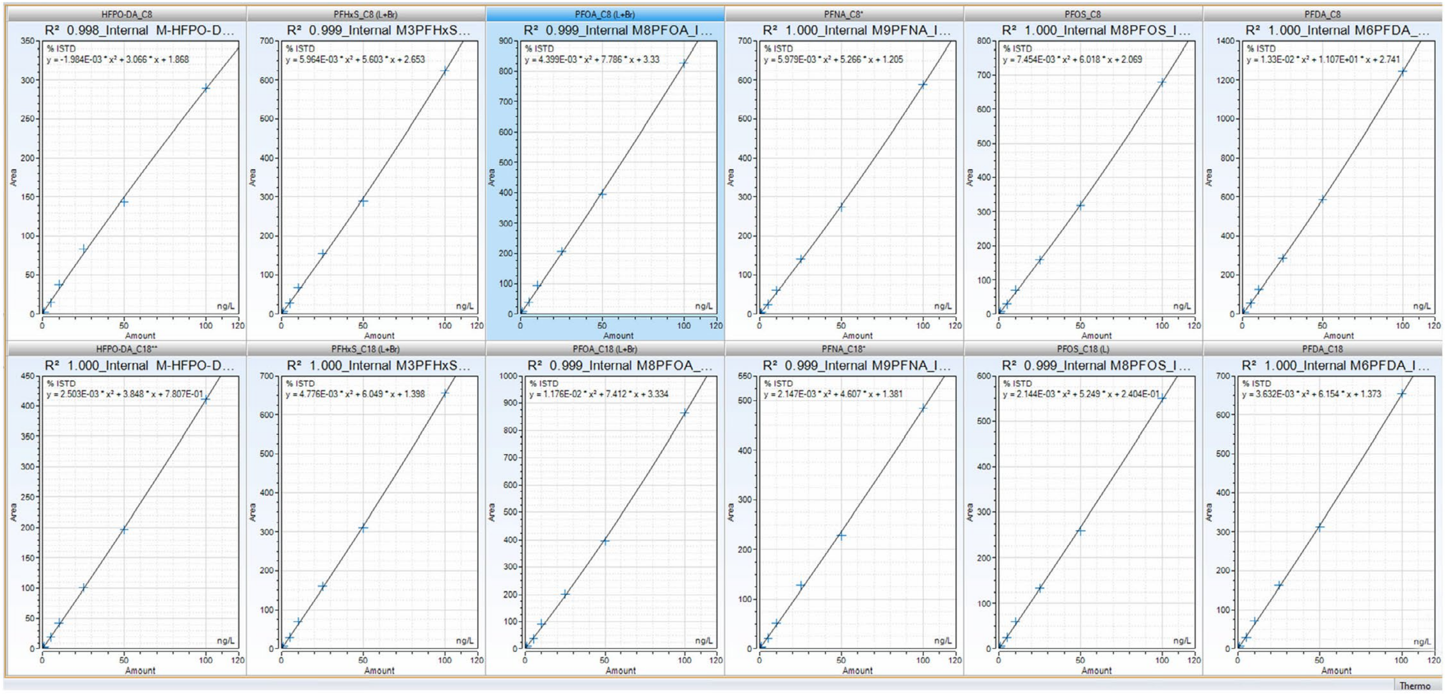


Figure 4. Simplified matrix-DLLME extracted calibration curve: distilled water spiked from 0.1 ng/L (ppt) to 100 ng/L (ppt), internal standard added before extraction, processed with DLLME, and LC-MS analyzed with the Orbitrap Exploris EFOX mass detector operating in full scan mode.

Groundwater sample

Groundwater samples containing colored sediment were shaken prior to transferring 15 mL to the DLLME extraction vial. Figure 5 presents the chromatogram results for one groundwater sample, with the overlaid *m/z* measurements at 60K resolution and 5 ppm accuracy extraction window, demonstrating separation and quantitation on a C18 selectivity column and confirmation on a C8 column. The overlays correspond to the extracted ion chromatograms of the peak from the full-scan data and the corresponding confirmation ion, which can be an adduct ion, a fragment ion, or an isotope depending on the specific structural and chemical characteristics of the PFAS. PFHxS, PFOS, and PFOA show the expected resolved peaks for linear "L" and branched "Br" isomers.

Table 1 presents the quantitation of specific PFAS of interest for this groundwater sample, along with the recoveries when spiked at 50 ng/L. For comparison, sample analysis by traditional SPE and triple quadrupole MS are included. SPE results were provided by an independent accredited laboratory. This comparison demonstrates the accuracy of the DLLME Orbitrap Exploris EFOX mass detector workflow.

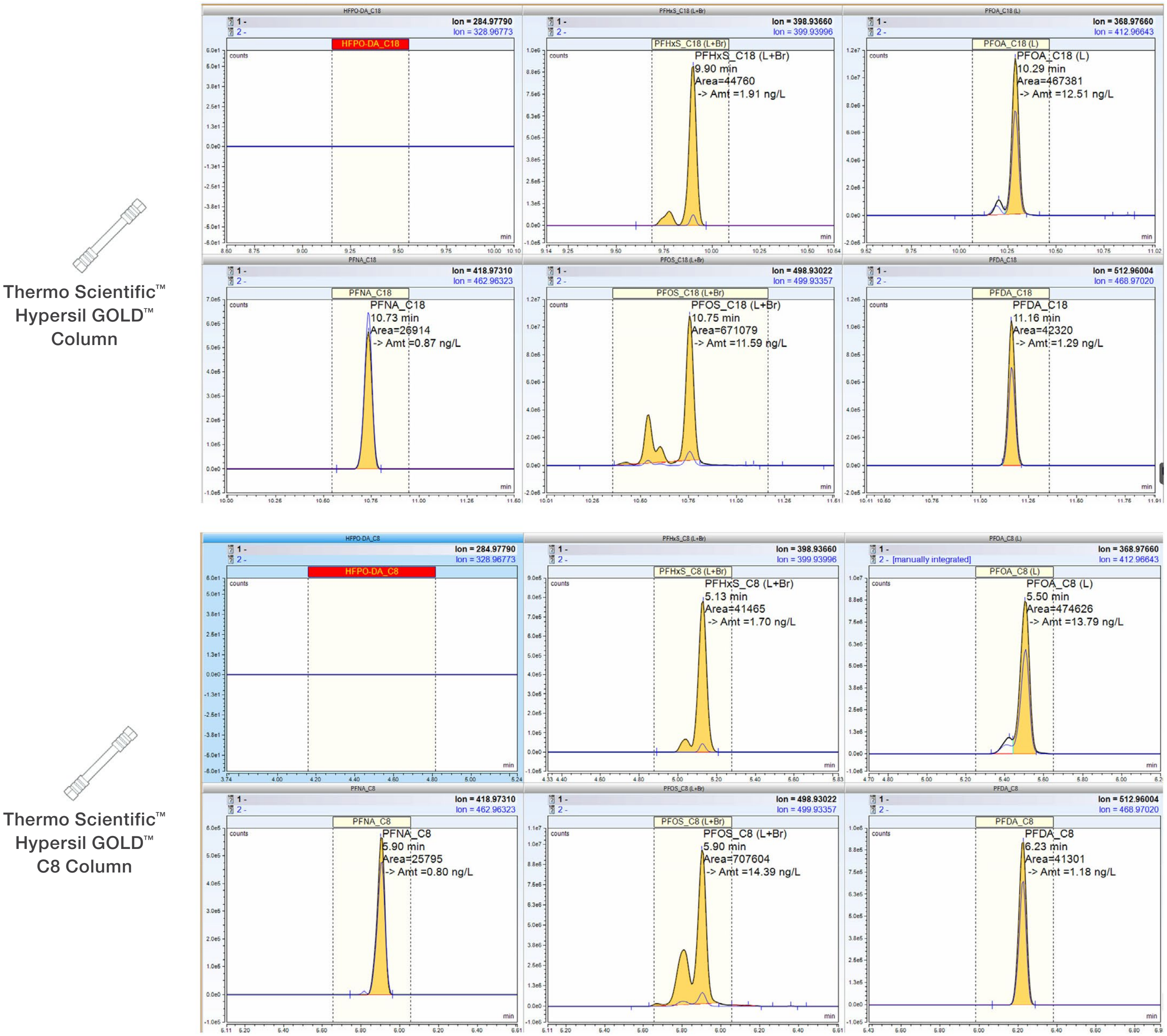


Figure 5. Chromatograms for DLLME extracted groundwater samples, demonstrating separation and quantification on both C18 and C8-selective columns. Dual-channel confirmation is efficient because the second-column injection is performed while the first column is equilibrating.

Table 1. Groundwater quantitation results and comparison for the six PFAS of primary interest.

Peak name	CAS	Column type	DLLME extracted groundwater-4 (amount, ng/L)	DLLME extracted groundwater spiked at 50 ng/L (recovery, %)	Third-party lab comparative data SPE extracted groundwater-4 (amount, ng/L
HFPO-DA	13252-13-6	Thermo Scientific Hypersil GOLD	< LOD	112	< LOD
HFPO-DA_C8	13252-13-6	Thermo Scientific Hypersil GOLD C8	Confirmed		
PFDA	335-76-2	Thermo Scientific Hypersil GOLD	1.29	109	1.04
PFDA_C8	335-76-2	Thermo Scientific Hypersil GOLD C8	Confirmed		
PFHxS (L+Br)	355-46-4	Thermo Scientific Hypersil GOLD	1.91	109	1.47
PFHxS_C8 (L+Br)	355-46-4	Thermo Scientific Hypersil GOL C8	Confirmed		
PFNA	375-95-1	Thermo Scientific Hypersil GOLD	0.87	114	< LOD
PFNA_C8	375-95-1	Thermo Scientific Hypersil GOLD C8	Confirmed		
PFOA (L)	335-67-1	Thermo Scientific Hypersil GOLD	12.51	109	8.2
PFOA_C8 (L)	335-67-1	Thermo Scientific Hypersil GOLD C8	Confirmed		
PFOS (L+Br)	1763-23-1	Thermo Scientific Hypersil GOLD	11.59	106	12.3
PFOS_C8 (L+Br)	1763-23-1	Thermo Scientific Hypersil GOLD C8	Confirmed		

Soil sample

One gram of soil sample was weighed in a DLLME extraction vial and diluted with water (Figure 3).

Figure 6 shows the results for one soil sample, with the overlaid *m/z* measurements at 60K resolution and a 5 ppm accuracy extraction window, demonstrating separation and quantitation on a C18 selective column and confirmation on a C8 column. Table 2 presents the quantitation of specific PFAS of interest for this soil sample, along with the recoveries when spiked at 750 ng/kg.

Table 2. Soil quantitation results for the 6 PFAS of primary interest.

Peak name	CAS	Column type	Amount measured in soil (ng/kg)	DLLME extracted soil spiked at 750 ng/kg (recovery, %)
HFPO-DA	13252-13-6	Thermo Scientific Hypersil GOLD	< LOD	106
HFPO-DA_C8	13252-13-6	Thermo Scientific Hypersil GOLD C8	confirmed	
PFDA	335-76-2	Thermo Scientific Hypersil GOLD	4.05	124
PFDA_C8	335-76-2	Thermo Scientific Hypersil GOLD C8	confirmed	
PFHxS (L+Br)	355-46-4	Thermo Scientific Hypersil GOLD	111.08	118
PFHxS_C8 (L+Br)	355-46-4	Thermo Scientific Hypersil GOLD C8	confirmed	
PFNA	375-95-1	Thermo Scientific Hypersil GOLD	6.79	105
PFNA_C8	375-95-1	Thermo Scientific Hypersil GOLD C8	confirmed	
PFOA (L)	335-67-1	Thermo Scientific Hypersil GOLD	96.73	95
PFOA_C8 (L)	335-67-1	Thermo Scientific Hypersil GOLD C8	confirmed	
PFOS (L+Br)	1763-23-1	Thermo Scientific Hypersil GOLD	531.01	127
PFOS_C8 (L+Br)	1763-23-1	Thermo Scientific Hypersil GOLD C8	confirmed	

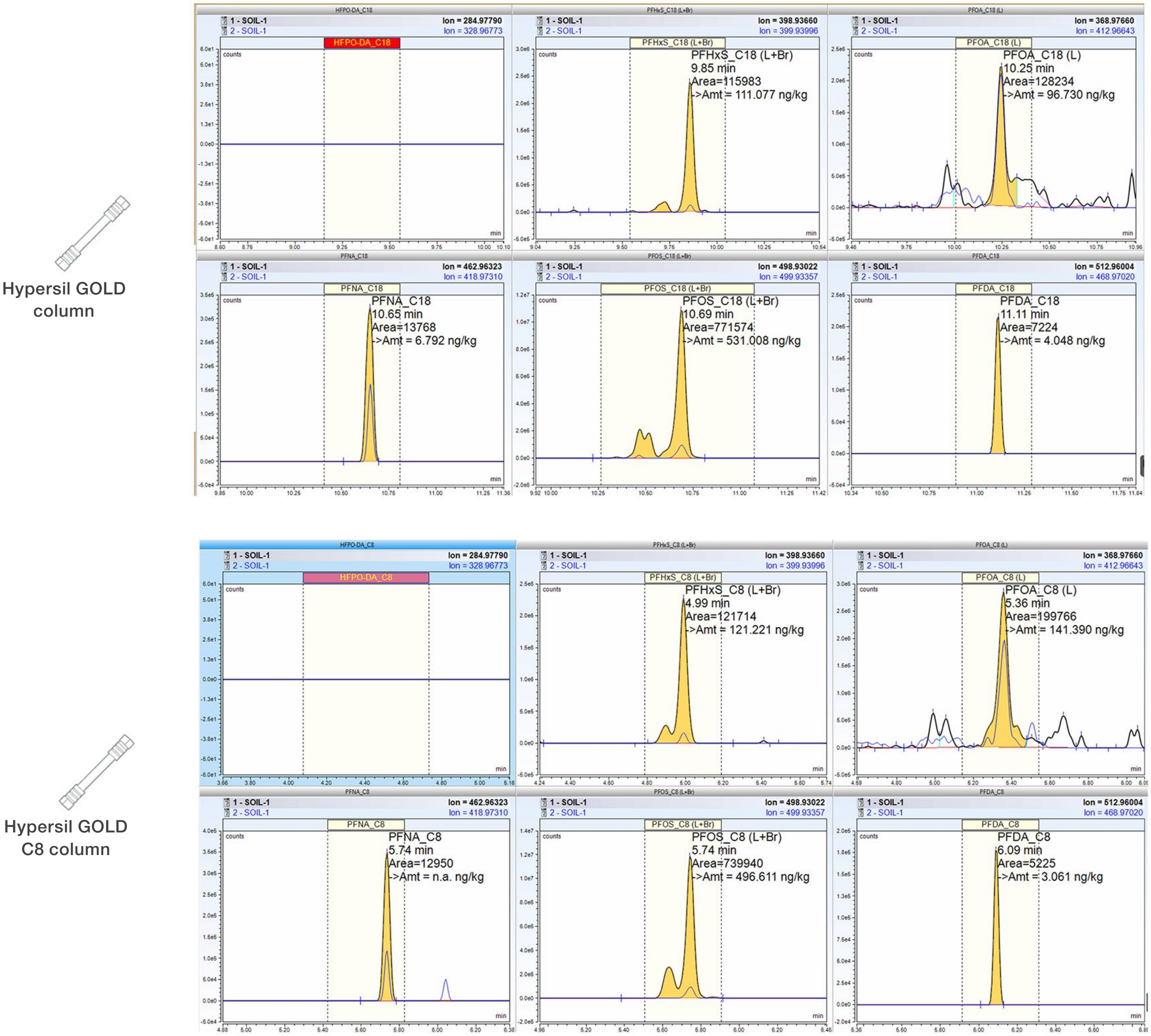


Figure 6. DLLME extracted soil sample, demonstrating separation and quantitation on a C18 selectivity column and ongoing confirmation on a C8 column.

Industrial effluent samples

Two types of industrial effluent samples were analyzed using the DLLME technique. The first type is a calcium chloride brine prepared by reacting hydrochloric acid with calcium hydroxide, containing trace metals like molybdenum, nickel, lead, and zinc. The second type is a calcium carbonate-based effluent containing Fe_3 chloride and sodium hydroxide. Samples were diluted with water in the DLLME vial prior to extraction.

Considering the potential challenge of effluent sample extraction, and for performance demonstration, the quantitation was carried out with different approaches:

1. Direct calibration curve (no recovery factor applied): Shows the amount of PFAS calculated using the calibration curve without any recovery adjustments.
2. Direct calibration curve (recovery factor applied): Shows the amount of PFAS calculated using the calibration curve and adjusted for recovery.
3. Standard addition method: Shows the amount of PFAS calculated using the standard addition method.
4. Recovery value: The recovery percentage calculated for the specific sample.
5. Third-party lab results: Results from an accredited lab using a well-accepted extraction technique (SPE). This serves as an additional reference to validate the method.

The recoveries were computed with effluents spiked at 1,500 ng/L (within the calibration range considering a 30-fold dilution factor). For comparison, traditional SPE results were provided by an independent accredited laboratory. This comparison attests to the accuracy of the DLLME Orbitrap Exploris EFOX mass detector workflow.

Figure 7 shows the chromatographic peaks obtained from both samples. Tables 3 and 4 provide a comparison between the quantitative approaches listed above, along with a comparison of the DLLME approach to an independent accredited laboratory using SPE extraction.

The amounts found using all three methods (direct calibration, recovery applied, and standard addition) are very similar. This consistency demonstrates the accuracy and reliability of the DLLME extraction technique. The third-party lab results further validate the DLLME method, showing that DLLME results are comparable to those obtained by an accredited lab.

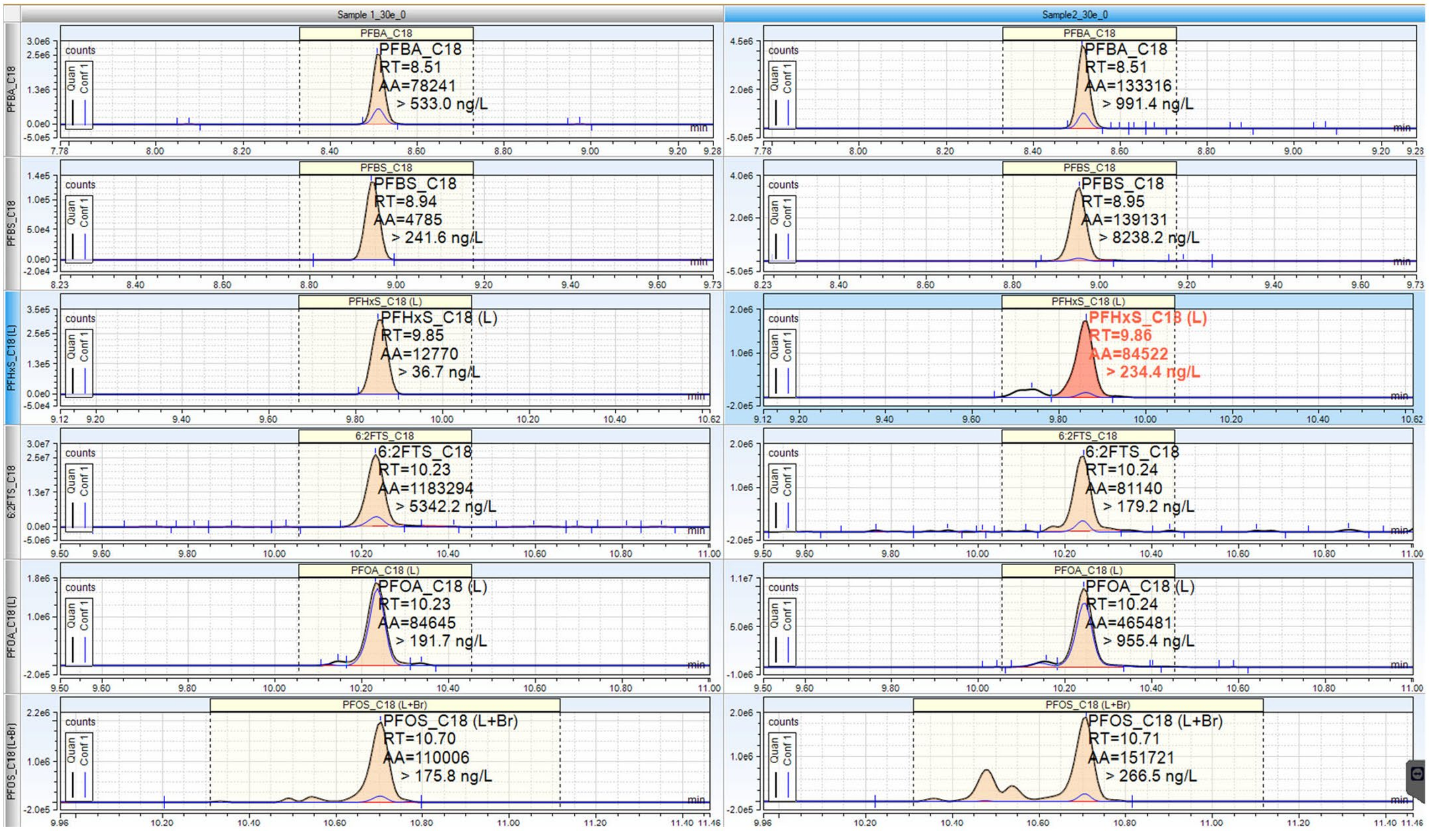


Figure 7. DLLME extracted industrial effluent samples (left, calcium chloride brine; right, calcium carbonate-based).

Table 3. Calcium chloride brine DLLME extract quantitation results and comparison. PFAS quantification of calcium chloride brine by simplified matrix-matched extracted calibration curve without and with correction for recovery, standard addition and third-party lab SPE extract, showing consistent results between the DLLME workflow and third-party results. Standard addition was compared to DLLME, and results demonstrate consistent accuracy between standard addition and the external calibration curve.

DLLME extracted calcium chloride brine effluent						
Peak name	CAS	By direct calibration curve method (amount, ng/L)	By calibration curve with recovery factor applied (amount, ng/L)	By standard addition method (amount, ng/L)	Spiked at 1,500 ng/L, calibration curve (recovery, %)	Third-party lab comparative data for SPE extraction (amount, ng/L)
PFDS	335-77-3	1.1	0.8	0.9	131	<20
PFUdA	2058-94-8	3	2.4	0.8	126	<20
PFHpS (L)	375-92-8	6.2	5.6	5.3	111	<10
6:2FTS	27619-97-2	5342.2		5356.8	outside cal range	5350
PFHxA	307-24-4	457	648.4	663.5	70	476
PFBA	375-22-4	533	411.4	471	130	397
PFBS	375-73-5	241.6	216.6	242.3	112	290
PFHpA	375-85-9	399.4	374	378.4	107	191
PFOA (L)	335-67-1	191.7	178.4	180.9	107	129
PFPeA8	2706-90-3	90.4	73	143.9	107	116
PFOS (L+Br)	1763-23-1	175.8	123.7	121	142	110
PFHxS (L)	355-46-4	36.7	31.7	31.1	116	15.4
PFNA	375-95-1	7.6	7	5.5	109	11.7

Table 4. Calcium carbonate based DLLME extract quantitation results and comparison. PFAS quantification of calcium chloride brine by simplified matrix-matched extracted calibration curve without and with correction for recovery, standard addition and third-party lab SPE extract, showing consistent results between the DLLME workflow and third-party results. Standard addition was compared to DLLME, and results demonstrate consistent accuracy between standard addition and the external calibration curve.

DLLME extracted calcium carbonate-based effluent						
Peak name	CAS	By direct calibration curve method (amount, ng/L)	By calibration curve with recovery factor applied (amount, ng/L)	By standard addition method (amount, ng/L)	Spiked at 1,500 ng/L, calibration curve (recovery, %)	Third-party lab comparative data for SPE extraction (amount, ng/L)
PFDS	335-77-3	0.7	0.7	0.5	104	<20
PFUdA	2058-94-8	_		_	134	<20
PFHpS (L)	375-92-8	4.7	3.9	3.7	120	<10
6:2FTS	27619-97-2	179.2	213.6	352.2	84	370
PFHxA8	307-24-4	1410.8	1048.9	1180.1	135	1160
PFBA	375-22-4	991.4	677.3	755.4	146	850
PFBS	375-73-5	8238.2	3503.5	4069.6	235	5250
PFHpA	375-85-9	327	254.6	261.2	128	284
PFOA (L)	335-67-1	955.4	802.2	864.8	119	719
PFPeA8	2706-90-3	651.4	504.2	566.2	112	621
PFOS (L+Br)	1763-23-1	266.5	178.5	174.5	149	115
PFHxS (L)	355-46-4	234.4	179.2	181.5	131	143
PFNA	375-95-1	54.8	41.8	41.3	131	21.6

Comparison of the two workflows

We optimized two analytical approaches for targeted PFAS analysis. The first approach, using the TSQ Altis Plus EFOX Edition MS, known as the "gold standard" for targeted methods, is highly regarded for its ability to handle hundreds of target compounds. It is considered a workhorse for high-throughput laboratories and offers out-of-the-box methods. This approach relies on the identification of compounds using one retention time (RT) and two selected reaction monitoring (SRM) transitions, with specific ion ratio, along with high-speed MS/MS fragmentation.

The second approach, using an Orbitrap Exploris EFOX mass detector, is a streamlined method for targeted analysis, serving as a stepping stone for chromatographers in high-resolution mass spectrometry (HRMS). This approach simplifies MS method development by using full scan HRMS only. It supports a theoretically unlimited number of targets, allows for retrospective analysis, and does not require RT alignment. Identification and confirmation are achieved using one or two RTs (if the dual column approach is chosen) and several accurate mass ions, with ion source fragmentation, where an ion ratio check is not necessary according to SANTE or EURL guidelines.

Both techniques comply with regulatory performance standards. In some laboratories, the triple quadrupole MS is a trusted method that provides reassurance. However, HRMS is not exclusively for mass spectrometry experts, it may offer less noise in chromatograms, making automatic integration easier and eliminating the need for manual reintegration. The full scan approach also eliminates the need to recalibrate retention time making HRMS easy to use.

Figure 8 shows chromatograms obtained for the analyses of a tap water sample after DLLME extraction and using the workflow designed on the TSQ Altis Plus EFOX Edition MS, with a single C18 selectivity column. Seven PFAS were detected and quantified between 0.2 and 11 ng/L. The retention time and the alignment of the two ions and their ratio, shown on top of the peak, are used for confirmation.

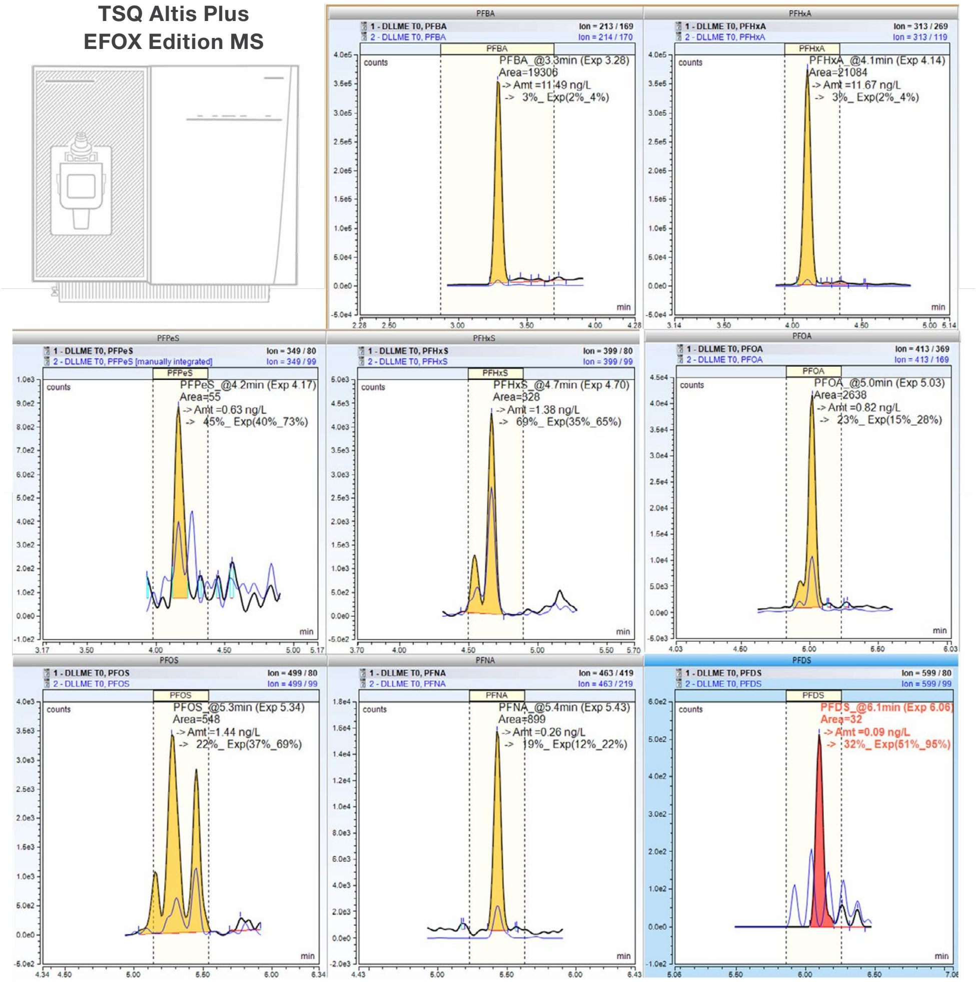


Figure 8. DLLME extracted tap water sample, C18-selective column, using TSQ Altis Plus EFOX Edition MS detection. The TSQ Altis Plus EFOX Edition MS confirms the absence of PFDS based on the ion ratio range and is automatically flagged by the Chromeleon 7.3.2 software. This is due to matrix interference and not true PFDS signal.

Figure 9 shows chromatograms obtained for the analyses of the same tap water sample after DLLME extraction and using the workflow designed on the Orbitrap Exploris EFOX mass detector, with a dual column configuration using C8 and C18 selectivity columns. Seven PFAS were detected and quantified between 0.3 and 13 ng/L. The retention time and the two ions shown on top of the peak for the two columns are used for confirmation.

Similar quantitation values are reported for both workflows in Table 5.

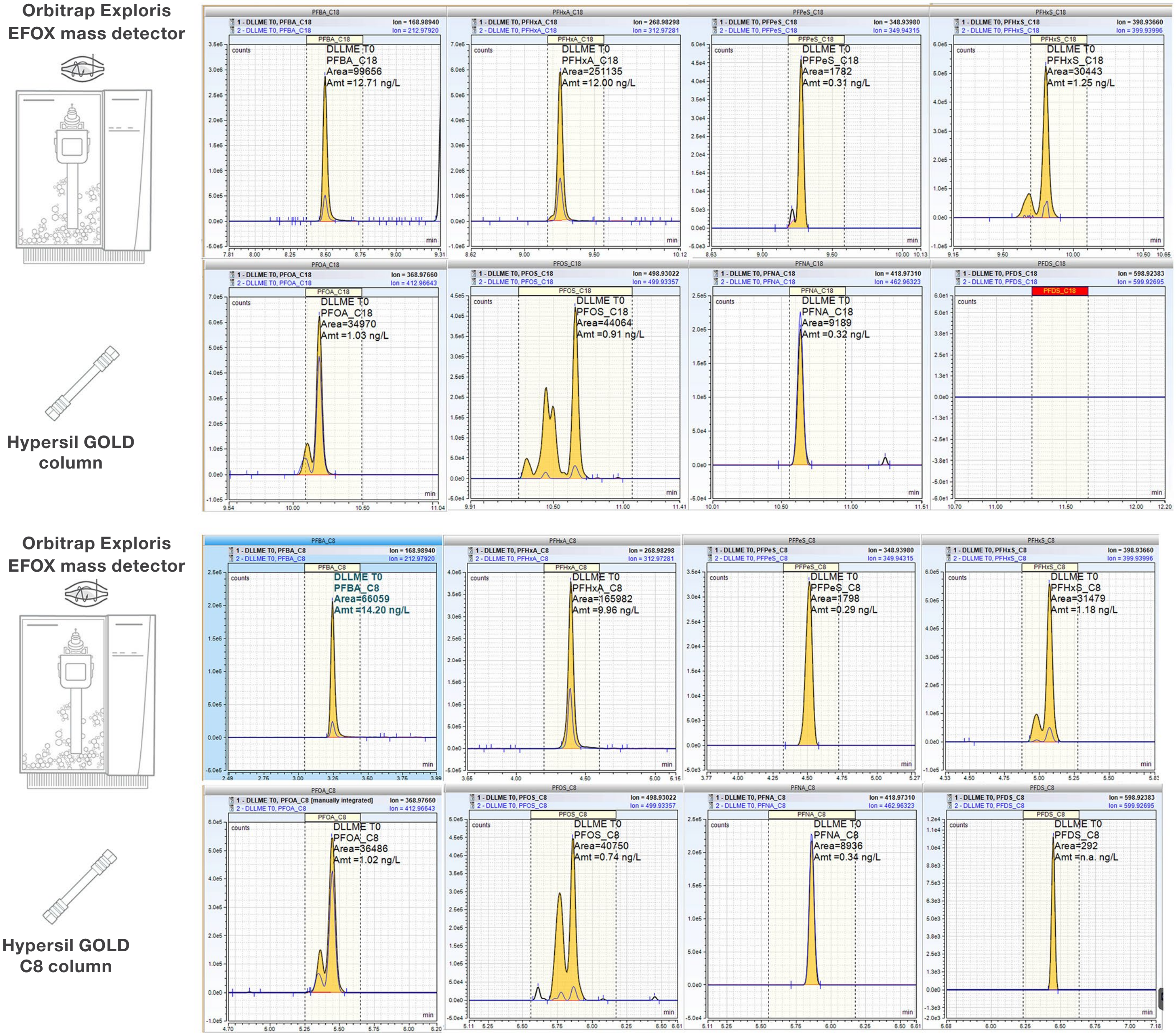


Figure 9. DLLME extracted of same tap water sample using the Orbitrap Exploris EFOX mass detector. The Orbitrap Exploris EFOX mass detector immediately confirms there is not any PFDS in the sample (no signal in high resolution), highlighting the benefit of the high resolution to clearly distinguish true signal from interference.

Table 5. Tap water DLLME extract, quantitation results with simplified matrix-matched extracted calibration curve, comparison between the TSQ Altis Plus EFOX Edition MS and the Orbitrap Exploris EFOX mass detector, showing consistency across the two instrument platforms in both sensitivity and quantification.

		DLLME extracted tap water analyzed and quatified with simplified matrix-extracted calibration curve method	
Peak name	CAS	TSQ Altis Plus EFOX Edition MS (amount, ng/L)	Orbitrap Exploris EFOX mass detector (amount, ng/L)
PFBA	375-22-4	11.5	12.7
PFHxA	307-24-4	11.7	12.0
PFPeS	2706-91-4	0.6	0.3
PFHxS (L)	355-46-4	1.4	1.3
PFOA (L)	335-67-1	0.8	1.0
PFOS (L+Br)	1763-23-1	1.4	0.9
PFNA	375-95-1	0.3	0.3
PFDS	335-77-3	<LOQ	<LOD

Conclusions

The automated DLLME method effectively quantifies PFAS with either triple quadrupole or high-resolution accurate mass detectors, meeting EURL PFAS standards, and sensitivity requirements in the U.S. This method supports diverse environmental testing needs and demonstrates performance comparable to solid-phase extraction (SPE) followed by LC/MS analysis using triple quadrupole instrumentation, as validated through independent laboratory studies on complex matrices.

Key advantages

The key advantages of the automated DLLME method are efficiency and speed, cost and environmental benefits, ease of use, regulatory compliance, robustness and reliability, sample versatility, and minimal manual preparation. The DLLME protocol is exactly the same regardless of the type of sample to be extracted, allowing different types of samples to be processed within the same batch. Overall, the automated DLLME method is efficient, environmentally friendly, and provides robust and reliable PFAS quantitation across diverse matrices (Figure 10).

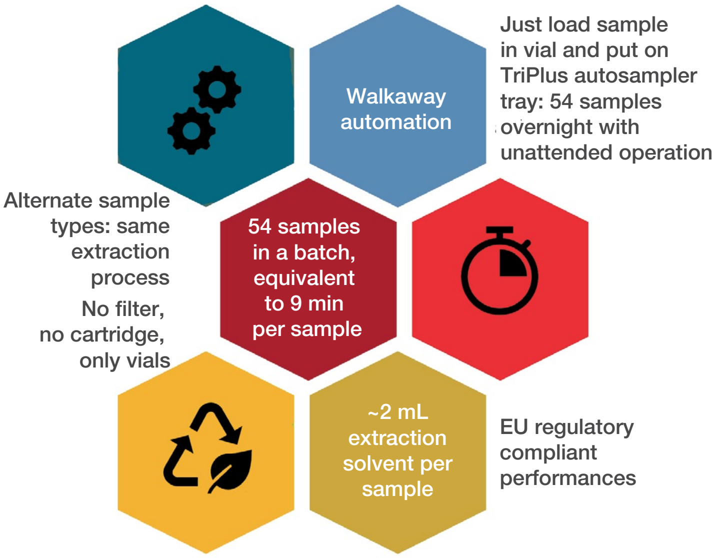


Figure 10. Key advantages of automated DLLME method for PFAS analysis.

Acknowledgements

We would like to thank Nicole Murray from Envirolab Ltd UK for providing raw samples and results.

References

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2. Thermo Fisher Scientific Poster: PFAS analysis strategy story – direct injection, DLLME, LC-MS/MS, LC-Orbitrap / GC-Orbitrap

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