

PFAS

PFAS analysis strategy story – direct injection, DLLME, LC-MS/MS, LC- Orbitrap / GC-Orbitrap

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Abstract

Purpose: This work summarizes key components of PFAS analysis. The choice between direct injection and automated sample preparation depends on the instrument's dynamic range, detection limits, and matrix complexity. Complex matrices like waste/industrial water, soil, food, and biological samples require specific preparation techniques to mitigate matrix effects and ensure accurate quantification. Techniques such as Dispersive Liquid-Liquid Microextraction (DLLME) can significantly improve concentration factors and reduce matrix interference, enhancing overall method performance. Additionally, the choice of the analyzer is a critical consideration.

Methods: Direct injection of water sample was performed on Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer. For those sample prepared with automated dispersive liquid-liquid microextraction (DLLME), acquisition was performed on a Thermo Scientific™ Orbitrap Exploris™ MX high resolution mass spectrometer.

Results: The methods presented achieves high-level sensitivity for PFAS analysis in drinking water, detecting compounds in the low ng/L range. Using DLLME, 56 PFAS compounds were quantified to low part per trillion levels from just 15 mL of sample. With a FAPAS®-Drinking Water proficiency test, we validated the three strategies for PFAS analysis: direct injection on TSQ Altis Plus and the DLLME extract injected on both Orbitrap Exploris MX and Thermo Scientific™ Orbitrap Exploris™ GC.

Introduction

In Europe, the main laws regulating the quality of water are :

- **Drinking water directive (2020/2184/EU)** sets thresholds for PFAS:
 - 0.1 µg/L for the sum of a group of 20 PFAS
 - 0.5 µg/L for PFAS total (totality of per- and polyfluoroalkyl substances)
- **Natural waters regulation**, is governed by:
 - Water Framework Directive (WFD, 2000/60/EC)
 - Environmental Quality Standards Directive (EQSD, 2008/105/EC)
 - Groundwater Directive (GWD, 2006/118/EC)
- **Common ground for all regulations** : Sample preparation methods are flexible:
 - Can be performed by dilution
 - Solid phase extraction
 - Any other sample preparation protocol

Analytical methods

Table 1. Parameters for LC-MS/MS and LC-HRMS analytical methods

Method Parameter	Direct Injection LC/MSMS	DLLME LC-HRMS
Instrument tested	Thermo Scientific™ TSQ Altis™ Plus	Thermo Scientific™ Orbitrap Exploris™ MX
Analyser	Triple Quadrupole	Orbitrap
Injection volume	100 µL	10 µL
run time	23 minutes	17.5 minutes
Calibration range	from LOQ (0.1 ng/L to 5 ng/L depending on the compounds) up to 100 ng/L	
Acquisition mode	SRM	Full Scan



Thermo Scientific™ TSQ Altis™ Plus Thermo Scientific™ Orbitrap Exploris™ MX

Automated dispersive liquid-liquid microextraction (DLLME), acquisition was performed on an Orbitrap Exploris MX mass spectrometer.

The DLLME method development involved several essential steps with various challenges.

• pH Adjustment

- Enhances extraction efficiency of PFAS compounds with low pKa values by ensuring they are in their non-ionized state.

• Choice of solvents

- Water-immiscible organic solvent with higher density than the aqueous sample as extraction solvent.
- Lower density dispersing solvents chosen to reduce contamination and access the organic phase easily.
- Solvent volumes optimized to improve extraction efficiency and selectivity of PFAS compounds.

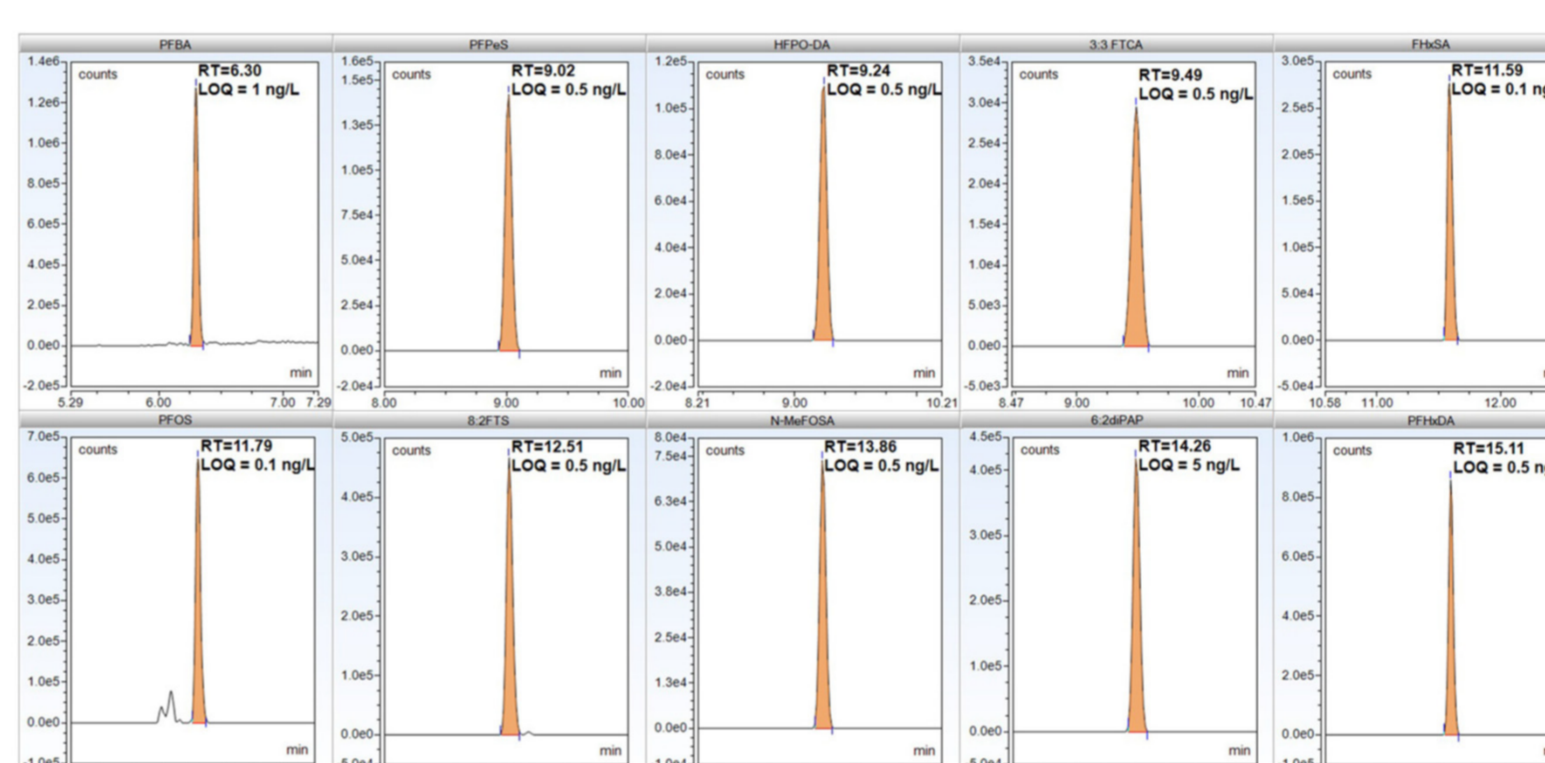
• Second extraction

- Increases pre-concentration factor for low concentration levels.
- Ensures compatibility with LC system.

Evaluation of limit of quantitation and blank

HPLC grade water was spiked with stock solutions in methanol at 7 concentration levels, keeping the spiking volume constant. Calibration curves were built in the range of 0.1 (or the LOQ) to 100 ng/L. LOQ values were determined based on concentration levels where intra-day precision and accuracy criteria were <30%, using 6 preparations and injections per level. The LOQ had to be at least three times the signal of the blank sample. Potential cross-contamination was also considered and had to be less than 20% of the LOQ signal. Chromatograms for PFAS from different classes at the LOQ level are shown in Figure 4.

Figure 4. Selected Chromatogram at LOQ levels

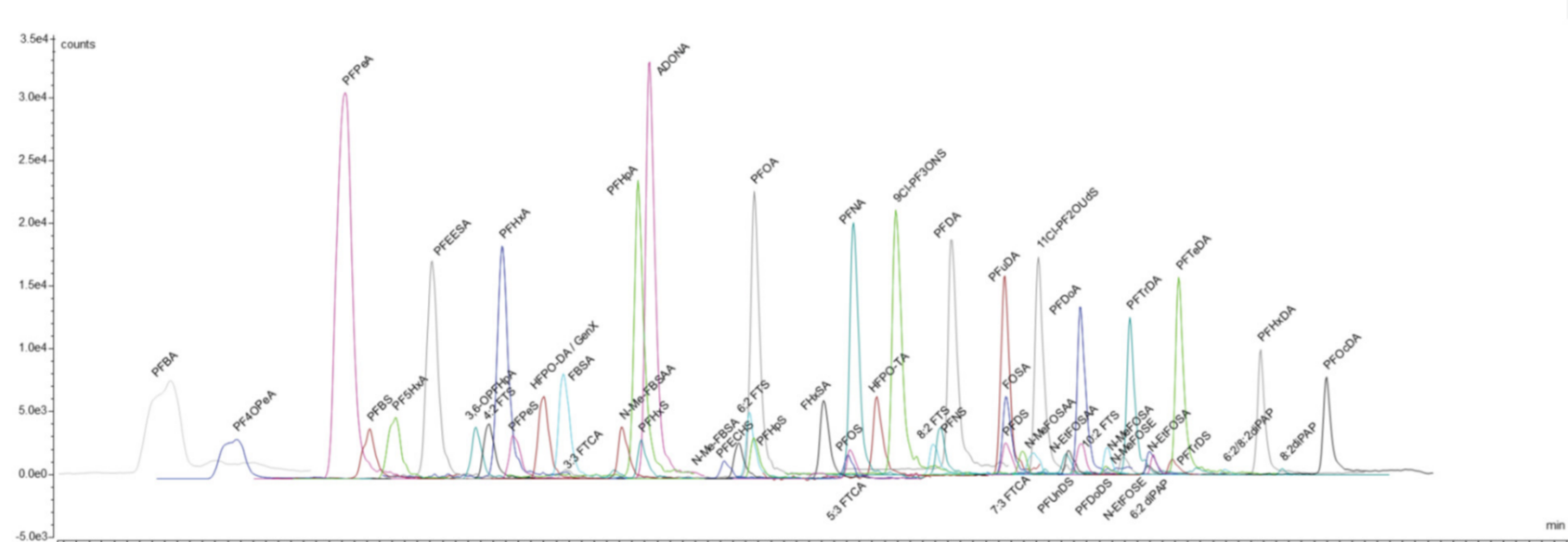


Results

Direct injection on the TSQ Altis Plus triple quadrupole mass spectrometer.

The method was developed to address a variety of compounds in European regulations, covering carbon chain lengths from 4 to 20 carbons, separated by reversed-phase chromatography. Different sample compositions were tested to retain both short and long-chained PFAS with 100 µL injections. In the final composition with 50% mobile phase B, long-chain PFAS may settle or adsorb on vial surfaces. To ensure homogeneity, a User Defined Program shakes samples automatically before injection, enhancing liquid handling in the Vanquish autosampler. A strong solvent loop improves retention and peak shape for short-chained PFAS. The resulting chromatogram is shown in Figure 2.

Figure 2. Chromatogram of the 54 compounds in a 5 ng/L calibration standard



Strategy with blank and contamination

Considering both the low detection limits required for PFAS analysis and the high sensitivity of the TSQ Altis Plus mass spectrometer, having a low contamination level is essential. The LOQs need to be at least three times the signal of the blank.

• Use of delay column

- Handles potential contamination from tubing and mobile phase
- Strong retention of analyzed compounds
- Separates system analytes from sample peaks

• Simple sample preparation

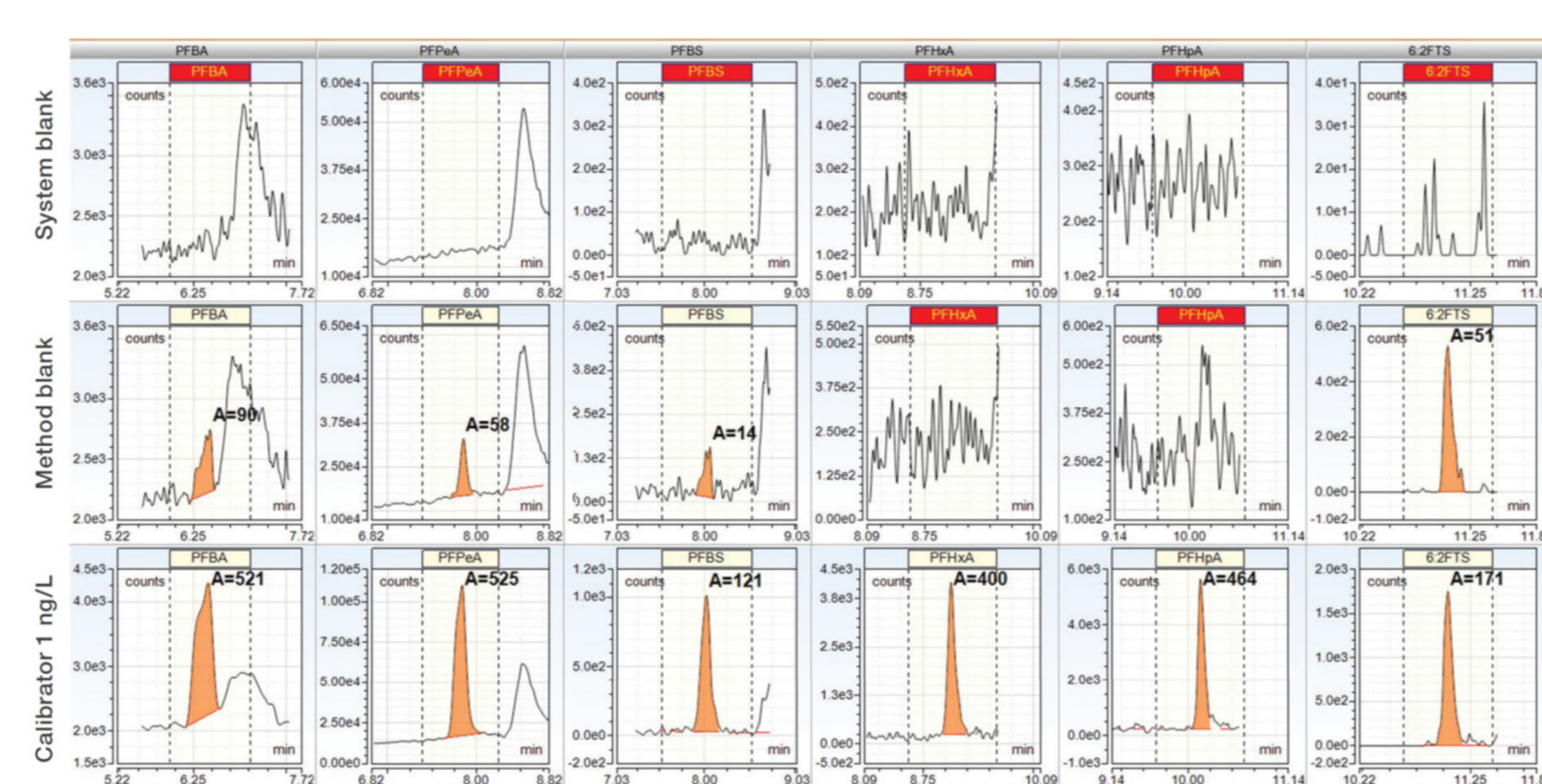
- Careful selection of materials
- Results in low contamination levels in blank samples

• Background subtraction

- Not needed here due to low contamination levels
- Can be implemented if higher contamination levels occur

The resulting blank chromatograms for some regularly found contaminants as well as a low calibration level are presented in Figure 3. System blank chromatograms show the separation of system peaks (coming from tubing, mobile phases, etc.) from analytical peaks, and we confirm the efficiency of the delay column to handle this contamination. As for the method blank, it has low levels of contamination for a few PFAS (e.g., PFBA, 6:2 FTS). The signal of the low calibrator is at least three times higher than the signal in the method blank.

Figure 3. Selected chromatograms for a system blank, method blank, and a 1 ng/L calibration standard



Discussion around strategy

When analyzing clean water, the choice between Direct Injection-TSQ and DLLME-HRAM depends on the required sensitivity for specific PFAS families. For instance, DLLME-HRAM offers better sensitivity for FASA and FOSE compounds (Figure 5), with LOQs more than 2.5 times better than Direct Injection-TSQ (Figure 6). For other matrices like wastewater or food, DLLME-HRAM is preferred for its versatility. Commission recommendation (EU) 2022/1431 mandates PFAS monitoring in food, with Table 3 showing the required LOQs. DLLME-HRAM was tested for extracting 56 PFAS from various matrices, including wastewater, apple, wheat, chicken, milk, and egg. Figure 7 shows that the chromatographic peaks are comparable in all tested matrices.

Figure 5. Number of PFAS per class showing better sensitivity on DI-TSQ, same, or better sensitivity on DLLME-HRAM

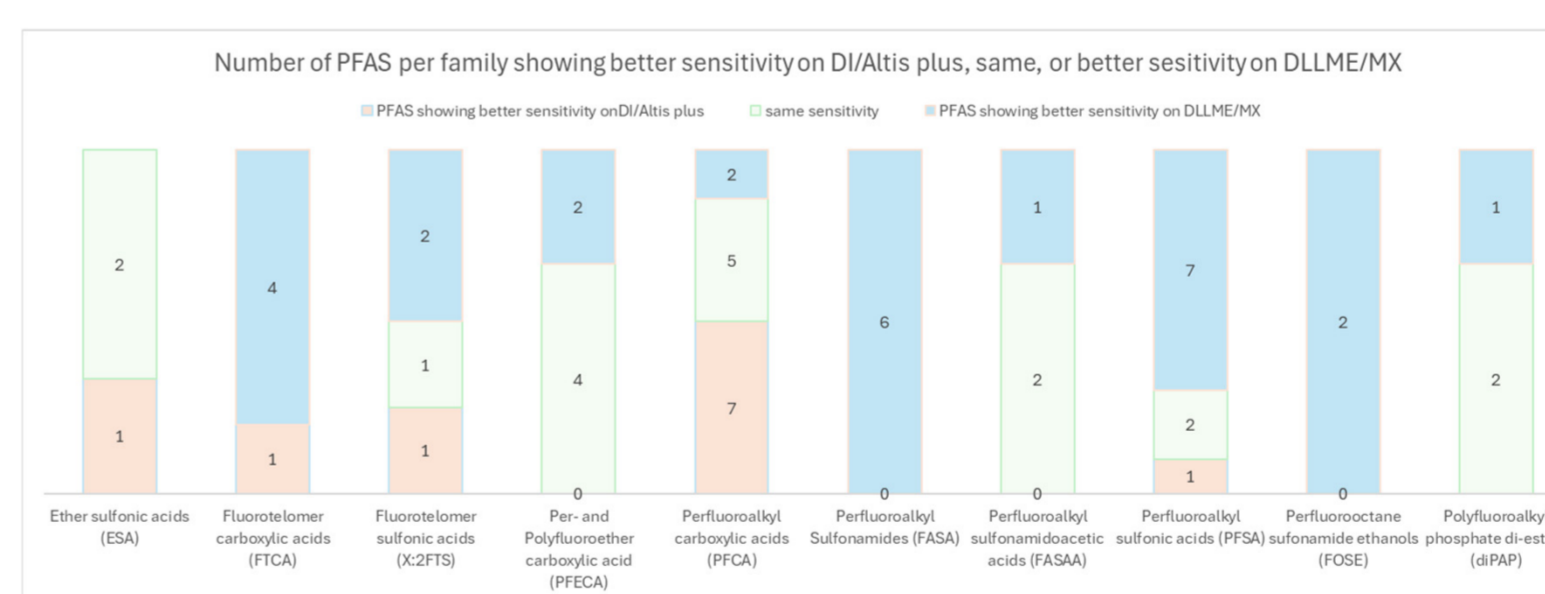


Figure 6. Mean value of sensitivity gain, factor of improvement of LOQ, when using the most sensitive strategy either DI-TSQ or DLLME-HRAM

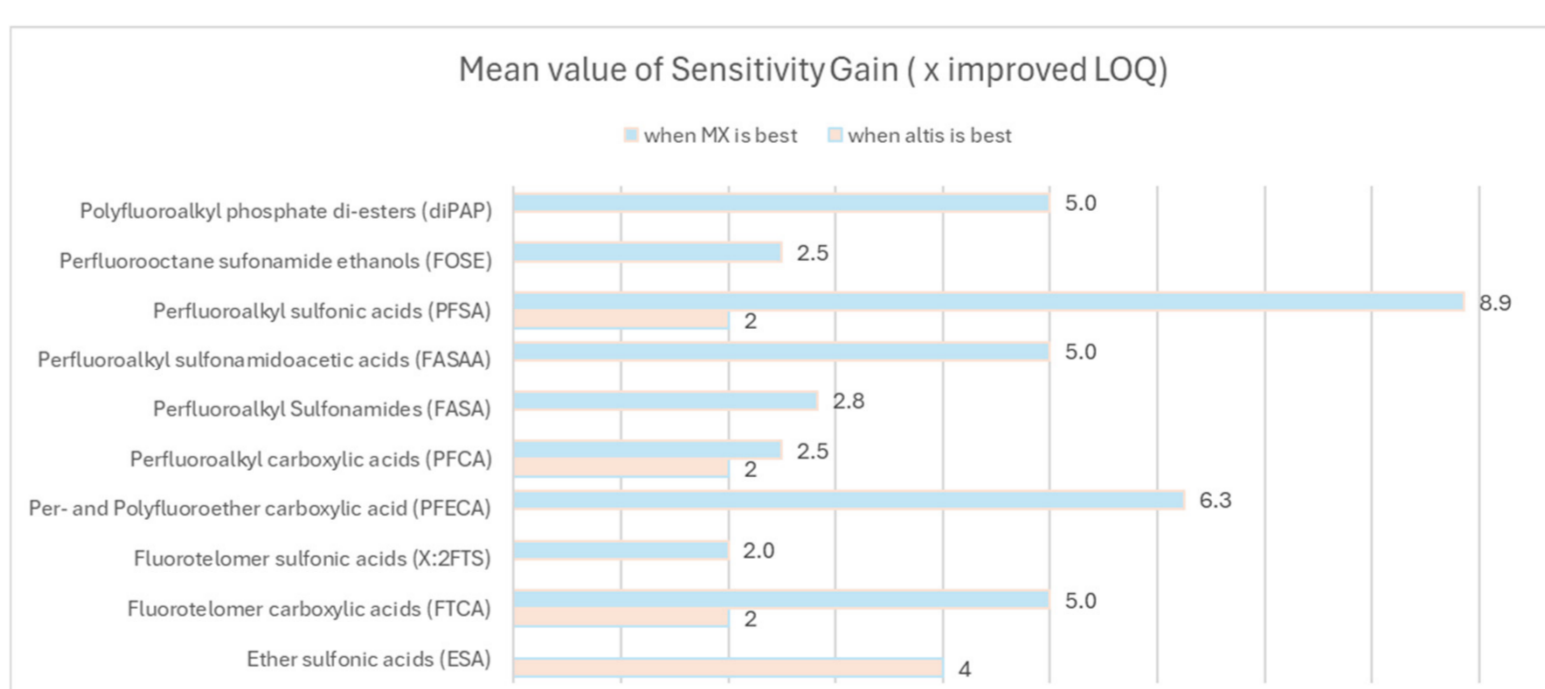


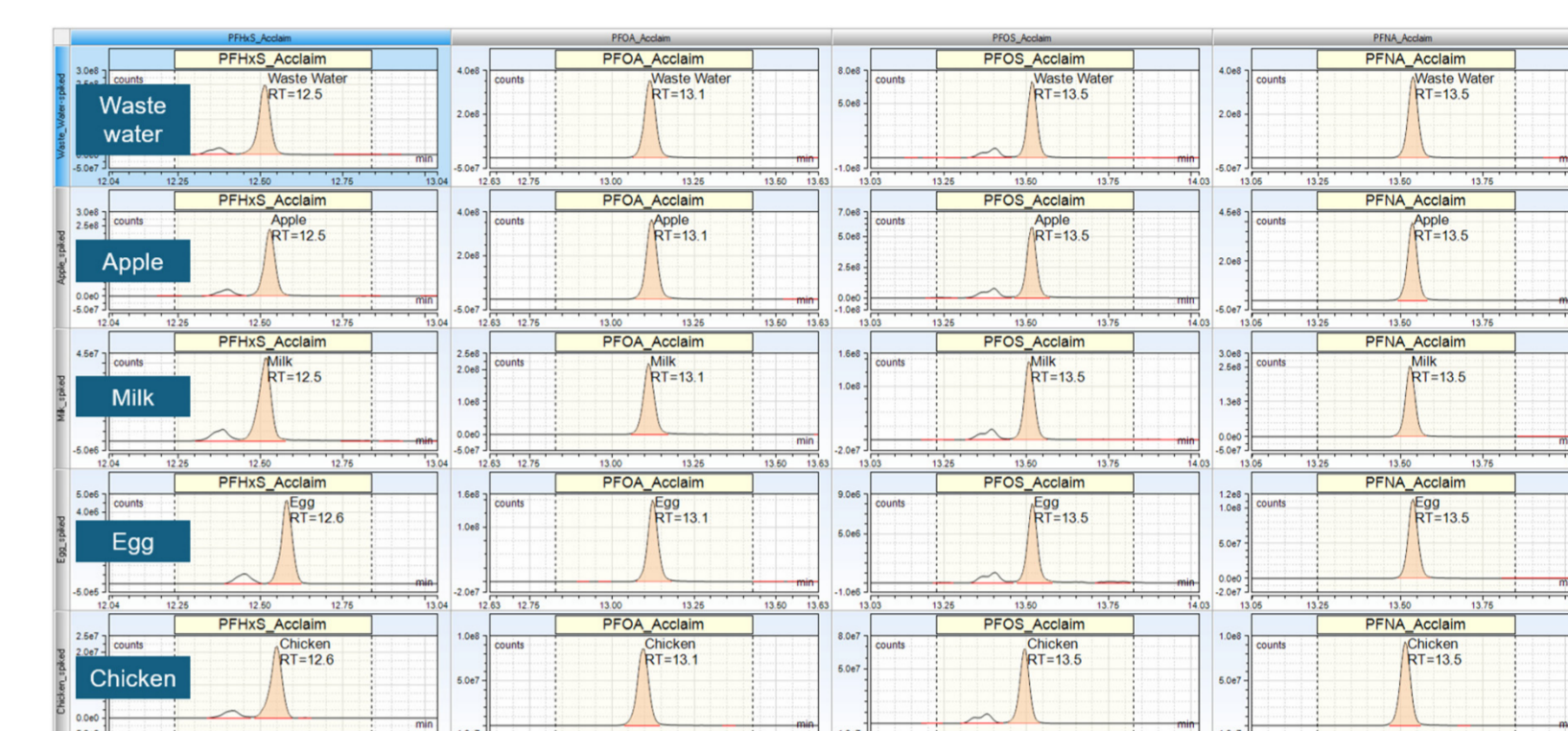
Table 2. Linearity and sensitivity results obtained for PFAS in EU regulation with both analytical strategy

FAMILY	NAME	DLLME + Thermo Scientific™ Orbitrap Exploris™ MX		Direct Injection + Thermo Scientific™ TSQ Altis™ Plus	
		LOQ (ng/L)	R ²	LOQ (ng/L)	R ²
PFCA	PFBA	1	0.9944	1	0.9983
	PFHxA	1	0.9936	1	0.9991
	PFHxMA	0.5	0.9941	0.25	0.9981
	PFHxA	0.5	0.9937	0.5	0.9957
	PFOA	0.5	0.9996	0.5	0.9956
	PFNA	0.5	0.9939	0.25	0.9957
	PFDA	0.5	0.9926	0.25	0.9952
	PFUNA or PFUNDA	0.5	0.9957	0.25	0.9962
	PFDoDA	0.5	0.9929	0.25	0.9948
	PFTfDA	0.1	0.9923	0.25	0.992
PFSA	PFBS	1	0.9917	0.5	0.9981
	PFNS	0.5	0.9909	0.5	0.9969
	PFHS	0.5	0.995	0.5	0.9956
	PFHS	0.1	0.9968	0.5	0.9958
	PFOS	0.1	0.9982	0.5	0.9951
	PFNS	0.1	0.9976	0.5	0.9943
	PFDS	0.1	0.9972	1	0.9958
	PFUnS PFUnDS	0.5	0.9937	1	0.9948
	PDFoDS	0.1	0.9971	1	0.9905
	PFTfDS	0.1	0.9908	2.5	0.9918

Table 3. Commission recommendation (EU) 2022/1431 requested limit of quantitation per matrices

Category	PFOS (µg/kg)	PFOA (µg/kg)	PFNA (µg/kg)	PFHxS (µg/kg)
Fruits, Vegetables, Starchy Roots, Tubers, Infant Food	0.002	0.001	0.001	0.004
Milk	0.010	0.010	0.020	0.040
Fish Meat and Terrestrial Animal Meat	0.100	0.100	0.100	0.100
Eggs, Crustaceans, Molluscs	0.300	0.300	0.300	0.300
Edible Offal of Terrestrial Animals, Fish Oil	0.500	0.500	0.500	0.500

Figure 7. Different spiked matrices applied to DLLME-HRAM



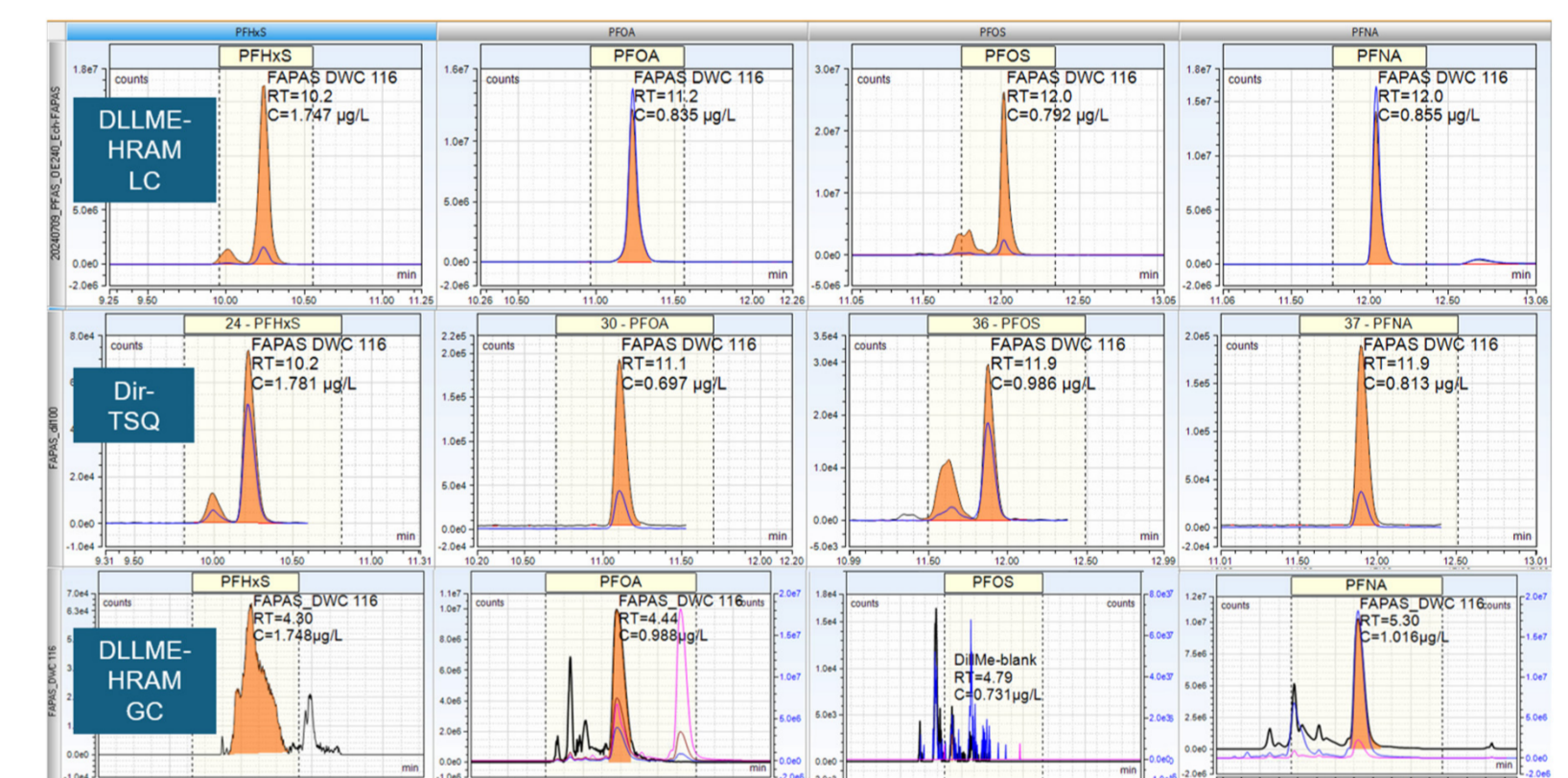
FAPAS proficiency test

A test material for the FAPAS®-Drinking Water proficiency test Distribution DW116 was dispatched in June 2024. We analyzed it using three strategic approaches: direct injection with the triple quadrupole, and DLLME with high-resolution accurate mass (HRAM) LC and GC. The results are presented in Table 4. While perfluoroalkyl sulfonic acids (PFSA) are not ideal for GC analysis due to poor peak shape, many can still be quantified at relevant concentration levels. In contrast, perfluoroalkyl carboxylic acids (PFCA) and hydroxylated fluorotelomers (X:2FTOH) are compatible with GC analysis. Figure 8 shows the compounds found in the FAPAS® sample, expected around 1 µg/L with the three strategies we have chosen.

Table 4. FAPAS®-proficiency test results, obtained and expected

Compound	DI-TSQ reported in µg/L	DLLME-HRAM reported in µg/L	DLLME-HRAM Exploris GC in µg/L	FAPAS Assigned value
PFHxS (L+B)	1.765	1.766	1.748	1.950
PFCA	0.683	0.840	0.988	0.963
PFOS (L+B)	0.921	0.865	0.935	0.905
PFNA	0.754	0.893	1.016	1.000

Figure 8. FAPAS®- sample, DLLME with Exploris™ MX (top), DI-TSQ Altis Plus™ (middle), DLLME with Exploris™ GC (bottom)



Conclusions

- Compared to traditional SPE workflows, direct injection improves sample throughput in laboratories by significantly reducing the required sample preparation.
- The DLLME method is a promising technique for extracting and pre-concentrating PFAS from drinking water and various other matrices. Its reduced sample volume facilitates easier handling, transportation, and storage.
- Both methods lower costs and environmental impact due to minimal solvent usage and the elimination of filters or SPE cartridges.
- The ease of use and robustness of both LCMS methods are based on fixed configuration including SOP with detailed hardware and consumables, a complete acquisition and processing method with customized view settings and reports, and all data handling performed with Chromeleon CDS 7.3.2.

References

1. Thermo Fisher Scientific Application Note 002902: Direct injection of drinking water for the analysis of 54 PFAS compounds by LC-MS/MS aligned with current and evolving global regulations. (thermofisher.com)
2. Thermo Fisher Scientific Application Brief 003164: Dispersive liquid-liquid micro-extraction for the automated sample preparation of PFAS in drinking water. (thermofisher.com)

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