Application brief | 003164

Environmental

Dispersive liquid-liquid micro-extraction for the automated sample preparation of PFAS in drinking water

Authors

Aristide Ganci, Valérie Thibert, Bénédicte Gauriat, Jean-François Garnier

Thermo Fisher Scientific
Customer Solution Center,
Les Ulis, France

Keywords

DLLME, PFAS, sample preparation, automation, liquid handling, drinking water

Application benefits

- Improved sensitivity: DLLME provides high enrichment factors, which can improve the detection sensitivity of PFAS in drinking water samples, down to challenging regulatory detection and reporting limits.
- Cost-effective: DLLME uses a small volume of solvent, which not only reduces the cost but also makes the method more environmentally friendly.
- Versatility: DLLME can be employed for the analysis of a wide range of PFAS classes, followed by either high performance liquid chromatography or gas chromatography as a separation technique, coupled to tandem mass spectrometry or high-resolution accurate mass as detection method.

Goal

To demonstrate a comprehensive method for the analysis of per- and polyfluoroalkyl substances (PFAS) by harnessing the potential of automated sample preparation on the Thermo Scientific™ TriPlus™ RSH SMART liquid handling station, with dispersive liquid-liquid micro extraction (DLLME) as a simple, cost-effective, and versatile extraction and pre-concentration technique. This was performed on LC-MS, but the same workflow can be also applied to GC-MS.

Introduction

Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been in use since the mid-20th century in various industries due to their resistance to heat, water, and oil. However, their environmental persistence and potential adverse health effects have led to a growing concern about PFAS contamination monitoring in the environment. Therefore, the analysis of PFAS in different matrices, drinking water amongst others, has become increasingly important.

PFAS include several chemical classes, which adds to the complexity of extracting and analyzing them in a single workflow. Further, regulations (national and international) and different matrixes (i.e., drinking and wastewater) are based on discrete lists of PFAS compounds. The aim of this work was to cover a wide range of PFAS from different chemical classes. Table 1 shows the list of the 56 target compounds.

Sample preparation approaches

In previous work, we demonstrated the ability to achieve the regulatory needs for the analysis of PFAS with a large volume injection and using high end LC-triple quadrupole technology[1]. For other types of instruments, and to handle a larger range of matrices, sample extraction and pre-concentration procedures can be implemented.

The most common approach for PFAS extraction and pre-concentration is based on solid phase extraction (SPE) with a mixed mode weak anion exchange support. The extraction can be performed offline either with manual or automated loading of cartridges[2,3], as well as online with an extraction column[4]. SPE has the advantage of efficiently extracting all kinds of PFAS compounds based on the different chemical interactions obtained with the extraction phase. On the downside, it needs important care regarding potential sources of contamination that could come from the cartridges, the solvents, and the tubing when using an automated or manual approach. Further, SPE has a high cost of analysis due to the use of a single cartridge required per sample, as well as being a more labor-intensive procedure with relatively high organic solvent consumption.

Dispersive liquid-liquid microextraction (DLLME) is a highly effective sample preparation technique that has gained significant attention in analytical chemistry, since its first publication in 2006[5], due to its simplicity, low cost, robustness, and high enrichment factors. DLLME is a miniaturized form of liquid-liquid extraction that uses minimal amounts of extraction solvent, making it a greener and environmentally friendly alternative. This technique has been widely used for the simultaneous extraction and preconcentration of various organic and inorganic contaminants from different matrices, thereby reducing the overall analysis time. Furthermore, it can be easily automated, improving reproducibility and reducing potential for human error.

Its working principle is based on a ternary component solvent system and involves the use of a dispersing solvent and an extraction solvent which are rapidly injected into an aqueous sample resulting in a cloudy solution[6]. The extraction solvent is typically a high-density organic solvent that forms fine droplets when dispersed in a sample by the dispersing solvent. The compounds of interest in the sample are then extracted into a smaller volume of these droplets. After extraction, the droplets are collected by centrifugation. This results in a pre-concentrated extract, due to a reduction of the volume of the extraction phase, which is then analyzed using suitable analytical methods.

Table 1. List of the 56 target compounds divided into their chemical classes. *Denotes compounds with specific labeled internal standard

Perfluoroalkyl carboxylic acids (PFCA)
PFBA*
PFPeA*
PFHxA*
PFHpA*
7HPFHpA
PFOA*
PFNA*
PFDA*
PFUnA or PFUnDA*
PFDoDA*
PFTrDA*
PFTeDA*
PFHxDA
PFODA, PFOcDA
Perfluoroalkyl Sulfonamides (FASA)
FBSA
N-MeFBSA
FHxSA
FOSA*
N-EtFOSA*
N-MeFOSA*

Perfluoroalkyl sulfonic acids (PFSA)
PFBS*
PFPeS*
PFHxS*
PFHpS*
PFOS*
PFNS
PFDS
PFUnS PFUnDS*
PDFoDS
PFTrDS
Fluorotelomer carboxylic acids (FTCA)
3:3 FTCA, FPrPA
5:3 FTCA, FPePA
7:3 FTCA, FHpPA
8:3 FTCA
FOEA
Fluorotelomer sulfonic acids (X:2FTS)
4:2FTS
8:2FTS*
10:2FTS

Ether sulfonic acids (ESA)
11Cl-PF3OUdS
9Cl-PF3ONS
PFEESA
Perfluoroalkyl sulfonamidoacetic acids (FASAA)
N-MeFOSAA*
N-EtFOSAA*
N-MeFBSAA
Perfluorooctane sufonamide ethanols (FOSE)
NMeFOSE
NEtFOSE
Per- and Polyfluoroether carboxylic acid (PFECA)
NFDHA, 3,6 OPFHpA
DONA; ADONA
PFMPA, PF4OPeA
PFMBA, PF5HxA
HFPO-DA (Gen X)*
HFPO-TA
Polyfluoroalkyl phosphate di-esters (diPAP)/Other
6:2diPAP
6:2/8:2diPAP
8:2diPAP*
PFECHS

Automated liquid handling

Automatization of DLLME sample preparation was implemented on the TriPlus RSH SMART autosampler. This system offers several advantages for sample handling, while meeting users' challenges:

- High sample throughput for efficient and automated sample handling, thereby increasing productivity and reducing manual labor.
- Versatility and compatibility with a wide range of sample vials, including various sizes and types. It can handle different sample matrices, making it suitable for applications including environmental, as well as food and beverage analysis.
- Precise and accurate sample injections to ensure reproducible results, minimize carryover, and improve sample integrity.
- User-friendly software interface offering intuitive navigation, simplified operation, and reduced learning curve for users.
- Automation and ease of use for a seamless integration with various analytical instruments, such as gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS) systems.

This application brief will delve into the use of DLLME as a sample preparation technique for PFAS analysis in drinking water samples, its advantages over traditional manual extraction methods like SPE, as well as discuss its challenges which were overcome during method development.

Experimental

Instrument configuration

The automated sample preparation was performed with a standalone TriPlus RSH SMART autosampler. A picture and schematic of the instrument used are presented in Figure 1.

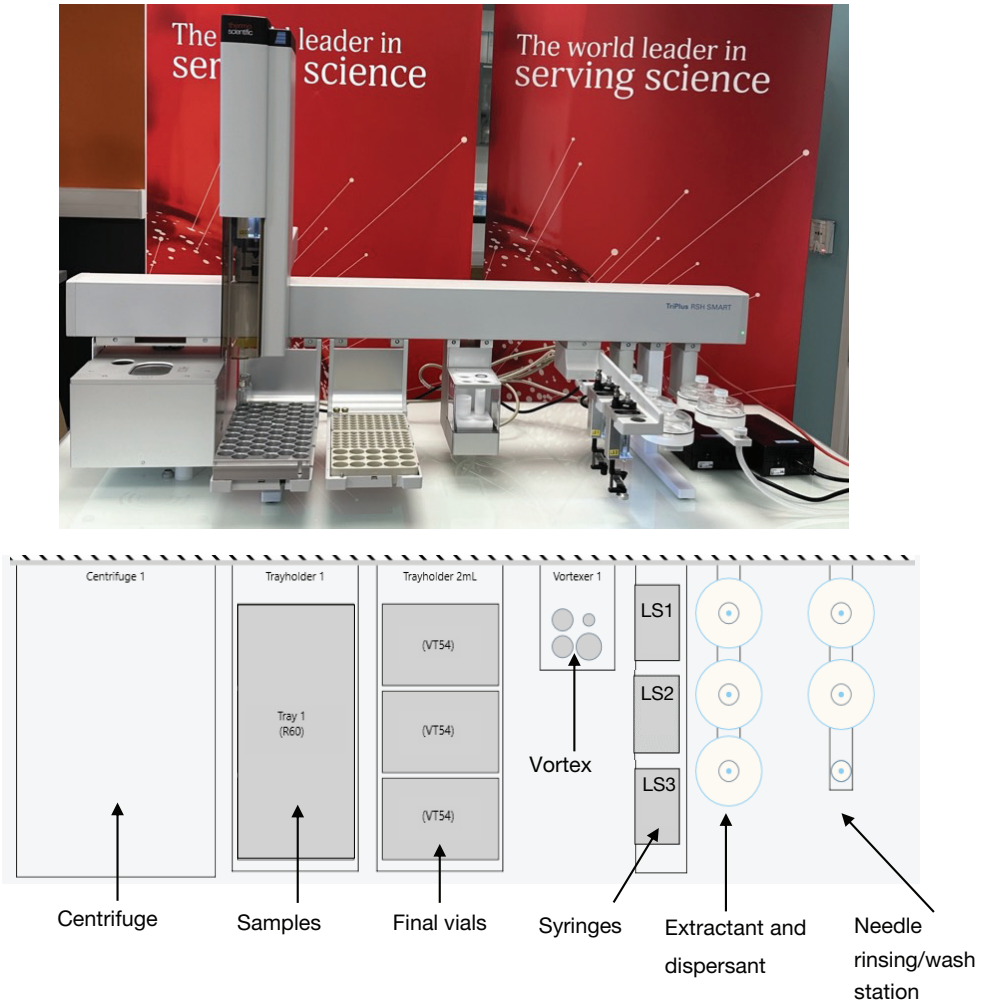


Figure 1. Picture (top) and schematic (bottom) of standalone TriPlus RSH SMART autosampler used for automated PFAS extraction and pre-concentration workflow. Including (left to right) a centrifuge, a sample tray holder, a tray holder for the extracted and pre-concentrated samples, a vortex unit, a syringe tool holder, solvent vessels for extractant and dispersant, as well as a needle rinsing/wash station holding multiple washing solvents.

Table 2 lists the TriPlus RSH SMART hardware configuration for running the DLLME sample extraction and pre-concentration workflow, while Table 3 lists the necessary consumables. The entire workflow, including a Standard Operating Procedure (SOP) that details hardware and all required consumables, chromatographic separation, and MS detection with full acquisition and data processing within the Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS), streamlines the implementation of the DLLME PFAS analysis, and therefore accelerates lab throughput and productivity.

Table 2. Hardware parts list

Hardware	Part number
TriPlus RSH SMART configured for liquid injection on extended X-axis length, including one Liquid syringe tool (for 0.5–100 µL syringes, 57 mm), one trayholder, three 54 x 2 mL vials trays	1R77010-2004
Automatic Tool Change Station (ATC) Station	1R77010-1019
Centrifuge Combi	1R77010-1193
Solvent Station for 3x 100 mL solvent bottles	1R77010-1031
Vortexer Module	1R77010-1033
Large Wash Station	1R77010-1030
Tray Holder	1R77010-1021
Sample aluminum tray for 10/20 mL vials	1R77010-1025
Large Volume Liquid syringe tool for 57 mm syringe needle – 250-1000 µL	1R77010-1009
1 mL FN GT LC, 22 G, 57 mm, PTFE-tipped Plunger	365K2811-SM
100 µL FN GT, 23 G, Side Hole, 57 mm, PTFE-tipped Plunger	365H2181-SM
Standard support for bench installation	1R77010-1111

Table 3. Consumables

Consumable	Part number
Thermo Scientific™ SureSTART™ 20 mL Glass Screw Top Headspace Vials	6ASV20-1
Thermo Scientific™ SureSTART™ 18 mm Precision Magnetic Screw Caps	6PMSC18-ST2
Thermo Scientific™ SureSTART™ Total Recovery Glass Screw Top Microvials for <2 mL Samples	6PSV9-TR1
9 mm Open Top Short Screw Cap, 6mm hole, magnetic	6PMSC9ST1
Thermo Scientific™ SureSTART™ 9 mm Screw Caps	6ASC9ST1G

Sample preparation

Figure 2 illustrates the developed DLLME workflow. Manually 15 mL of drinking water are added into a 20 mL capacity screw cap vial. 1 mL of strong acid is added to lower the pH. A mix of 22 labeled internal standards dissolved in methanol is added to the sample at a final concentration of 15 ng/L before ensuring a proper homogenization through a vortex step. The internal standards are added to the samples to correct for any possible extraction and matrix effects, while an asterisk in Table 1 signals which compounds have a corresponding isotopically labeled internal standard.

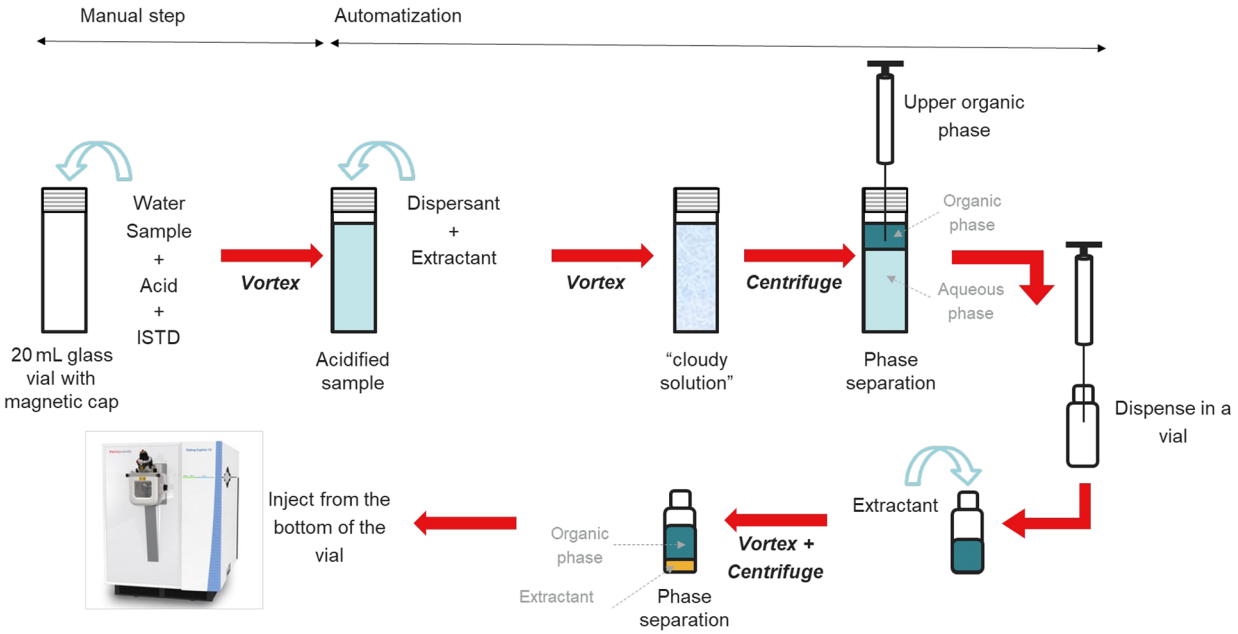


Figure 2. Sample preparation workflow and automated DLLME steps

The acidified sample vial is then placed onto the tray holder (Figure 1, Tray 1), from where the automated sample preparation and pre-concentration workflow can be launched via Chromeleon CDS, version 7.3.2. This includes the following steps and takes only 9 minutes per water sample:

- Add the extractant and dispersing solvent (low density)
- Vortex to form the afore mentioned cloudy solution
- Centrifuge to ensure complete phase separation
- Transfer of the organic lower density upper solution to a 2 mL high recovery vial on the adjacent tray holder
- Add an additional extractant (high density)
- Vortex and centrifuge

HPLC-HRAM method

The analysis of the DLLME PFAS extract was performed on a Thermo Scientific™ Vanquish™ Flex HPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer. The HPLC part was based on the method published in the direct injection application note[1]. Briefly, the HPLC method consisted of the injection of 10 µL of sample extract, then separation by gradient elution with water and methanol with 2 mM ammonium acetate and 0.1% acetic acid.

For quantitative analysis, a combination of full scan (resolution of 60k), SIM (resolution of 60k), and AIF (resolution of 15k) scan modes were used to target the 56 PFAS in the current study and eventually allow the addition of more compounds.

Data analysis

All data were acquired and processed using Chromeleon CDS, version 7.3.2. The workflow was written using the TriPlus RSH SMART Sampling Workflow Editor version 1.5 and can be fully integrated, modified and launched within Chromeleon CDS.

Results and discussion

Method development

The DLLME method development presented in this work involved several essential steps with various challenges faced at each stage:

1. Sample preparation: Prior to DLLME, the drinking water sample needs to be properly prepared. pH adjustment is required to enhance the extraction efficiency of specific PFAS compounds, especially those with a low pkA value, to ensure that they are present in their non-ionized state.

2. Selection of extraction solvent: The choice of an appropriate extraction solvent is crucial for DLLME. Typically, a water-immiscible organic solvent with a higher density than the aqueous sample is used as the extraction solvent for PFAS compounds. To reduce potential contamination and easily access the organic phase in the first extraction step, lower density extractant and dispersing solvents were chosen. This transforms the presented DLLME into a low-density solvent type DLLME (LDS-DLLME)[7]. In addition to the selection of the extraction solvent, the type of dispersing solvent and their volumes were optimized. These parameters can significantly affect the extraction efficiency and selectivity of PFAS compounds.

3. Second liquid-liquid extraction step: For the second extraction step (as shown in the bottom part of Figure 2), a higher density solvent was chosen, thus programming the injection from the bottom of the vial during the analysis of the final extract. This step significantly increases the pre-concentration factor to achieve low concentration levels in the sample and ensures compatibility with an LC system.

4. DLLME procedure: This stage of method development consisted of the optimization of the speed and duration of each of the steps to generate an efficient automated sample preparation program with a capacity of 54 unattended samples with the current instrument configuration.

The final method starts with a volume of 15 mL water and ends with an extract of 30 µL, giving a potential preconcentration factor of 500 to deal with the low sensitivity requirements in drinking water.

Results

Method validation tests and acceptance criteria results, which are presented in this section, can be found in Table 4.

Linearity and LOQ

Calibration curves were measured in the range of 0.1 (or the LOQ) up to 100 ng/L by spiking HPLC grade water with stock solutions in methanol at 7 concentration levels, thereby ensuring that the spiking volume was constant for each calibration level.

The LOQ values were obtained based on the concentration level for which both intra-day and accuracy criteria were set as <30% based on 6 preparations and injections at each calibration level. For LOQ determination, the signal in the blank sample was also considered, where the LOQ needs to have at least 3 times the signal obtained in the blank. Also, potential cross-contamination was considered and needs to be less than 20% of the signal of the LOQ. Figure 3 shows the calibration curves overlay of 3 different measurement days, with at least 2 repetitions per day.

Table 4. Summary of the method validation tests and acceptance criteria

	Tested	Acceptance criteria
Sample throughput	54 samples with ~9 min per sample	-
Linearity	0.1 (LOQ) – 100 ng/L Day 1 = 6 replicates Day 2 = 2 replicates Day 3 = 2 replicates	$R^2 > 0.99$ Overlay of 3 days
LOQ	6 replicates at 0.1, 0.5, 1 and 5 ng/L in HPLC grade water	Intra-day precision and accuracy < 30% LOQ > 3 x blank signal
Accuracy **Precision intra-day** **Precision inter-day**	Tap and bottled water at 5 and 75 ng/L Day 1 – 6 replicates Day 2 – 2 replicates Day 3 – 2 replicates	Amount (5 and 75 ng/L) ± 30% day 1, 2 and 3 – 10 repeats Precision intra-day <30 RSD% (6 repeats) Precision inter-day <30 RSD% (10 repeats)
Cross-contamination	TriPlus RSH SMART autosampler 3 x 100 ng/mL followed each by a blank	Cross-contamination < 20% LOQ signal
Stability	TriPlus RSH SMART autosampler – acidified sample at 5 and 75 ng/L at room temperature (~20 °C) for 12 h Vanquish Flex autosampler – sample at 5 and 75 ng/L in final injection solution at 25 °C for 24 h	Amount (5 and 75 ng/L) ± 30%

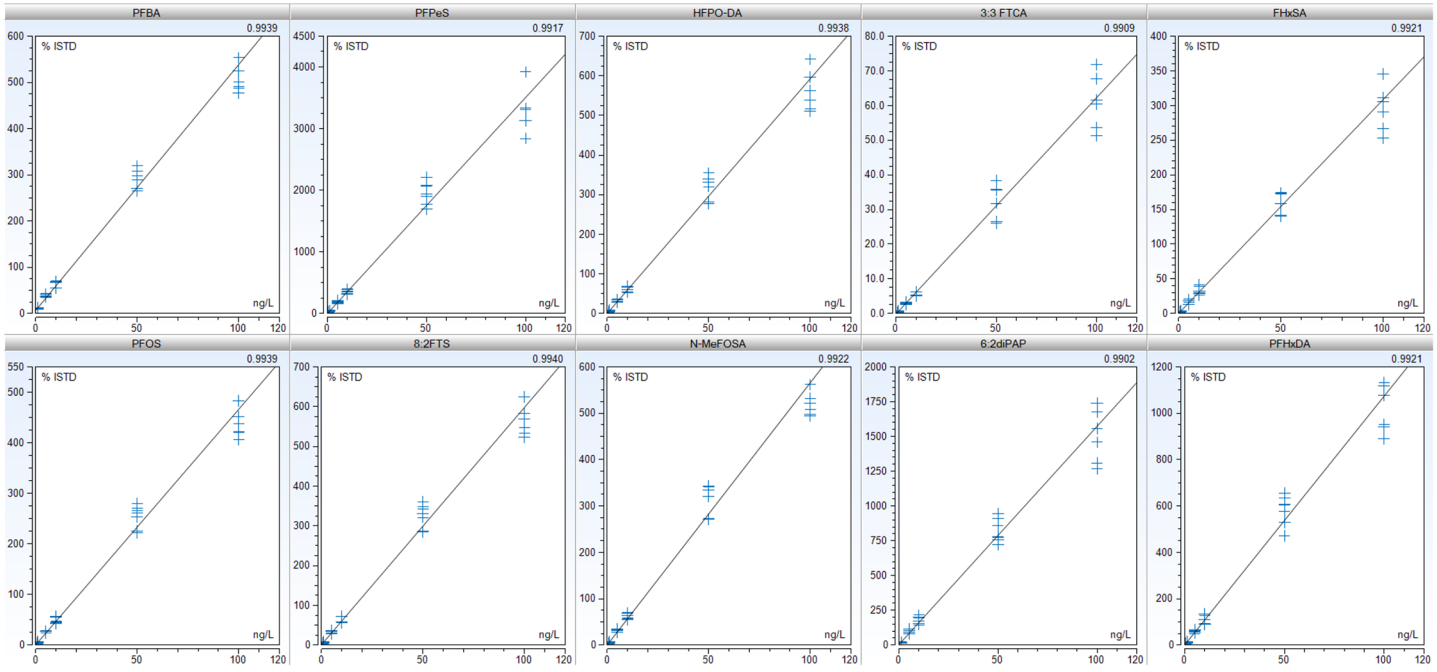


Figure 3. Examples of calibration curves (3 different days with at least two replicates per day) for PFAS compounds from different classes

From the presented data, we can deduce that the extraction protocol is both repeatable on the same day and reproducible between days. Some chromatograms for PFAS from different classes at the LOQ level are presented in Figure 4 with a full overview of all target compounds being displayed in Appendix A, B, C, and D. A numerical summary of all 56 PFAS with additional information on the general results for linearity (R^2, linear range and curve weighting), LOQ (highest bias % and %RSD (n=6) at the LOQ) and use of internal standards (assigned compounds) is presented in Table 5.

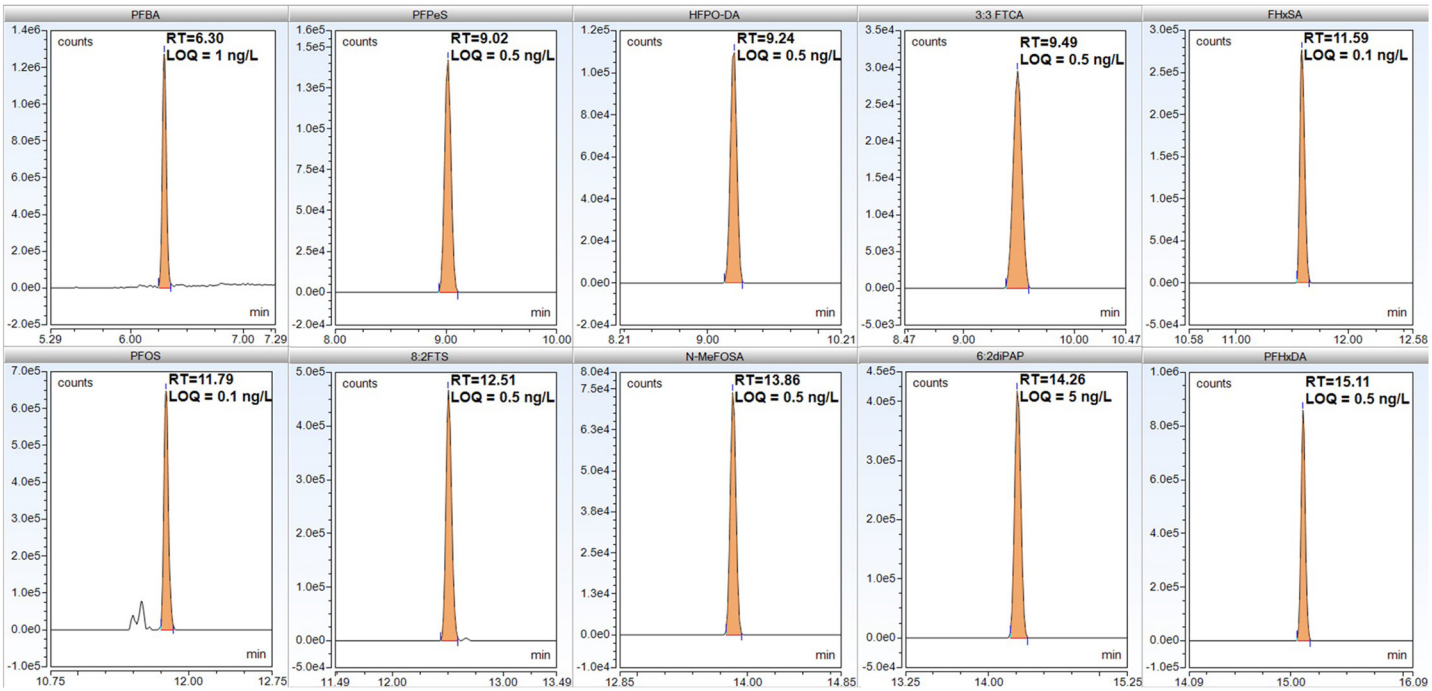


Figure 4. Selected chromatograms at LOQ levels

Table 5 (part 1). A numerical summary of all 56 PFAS

Compound	Type	ISTD	LOQ (ng/L)	Highest bias at LOQ (%)	RSD (n=6) LOQ (%)	Linearity range (ng/L)	R^2
PFBA	Lin, 1/A	13C4-PFBA	1.0	14.8	9.1	1–100	0.9944
PF4OPeA	Lin, 1/A	13C5-PFPeA	0.1	25.9	22.8	0.1–100	0.9962
PFPeA	Lin, 1/A	13C5-PFPeA	1.0	25.3	17.1	1–100	0.9936
PFBS	Lin, 1/A	13C3-PFBS	1.0	27.9	20.0	1–100	0.9917
PF5HxA	Lin, 1/A	13C5-PFHxA	0.1	27.2	11.3	0.1–100	0.9979
7HPFHpA	Lin, 1/A	13C3-PFHxS	0.5	23.7	12.0	0.5–100	0.9869
PFEESA	Lin, 1/A	13C3-PFBS	1.0	25.2	14.4	1–100	0.9915
3,6-OPFHpA	Lin, 1/A	13C5-PFPeA	0.1	28.2	19.6	0.1–100	0.9972
4:2FTS	Lin, 1/A	13C3-PFBS	0.5	21.4	20.6	0.5–100	0.9948
PFHxA	Lin, 1/A	13C5-PFHxA	0.5	23.0	8.7	0.5–100	0.9941
PFPeS	Lin, 1/A	13C3-PFBS	0.5	21.9	14.9	0.5–100	0.9909
HFPO-DA	Lin, 1/A	13C3-HFPO-DA	0.5	23.3	15.6	0.5–100	0.9953
FBSA	Lin, 1/A	13C5-PFHxA	0.1	29.4	12.1	0.1–100	0.9951
3:3 FTCA	Lin, 1/A	13C5-PFHxA	0.5	23.2	10.2	0.5–100	0.9956
N-MeFBSAA	Lin, 1/A	13C4-PFHpA	0.5	23.8	15.6	0.5–100	0.9914
PFHpA	Lin, 1/A	13C4-PFHpA	0.5	22.2	8.7	0.5–100	0.9937
PFHxS	Lin, 1/A	13C3-PFHxS	0.5	16.8	8.0	0.5–100	0.9950
ADONA	Lin, 1/A	13C2-6:2FTS	0.1	14.9	11.1	0.1–100	0.9956
N-MeFBSA	Lin	13C2-6:2FTS	1.0	23.5	10.8	1–100	0.9958
PFECHS	Lin, 1/A	13C3-PFHxS	0.1	13.5	4.3	0.1–100	0.9928

Table 5 (part 2). A numerical summary of all 56 PFAS

Compound	Type	ISTD	LOQ (ng/L)	Highest bias at LOQ (%)	RSD (n=6) LOQ (%)	Linearity range (ng/L)	R^2
PFOA	Lin, 1/A	13C8-PFOA	0.5	9.5	5.3	0.5–100	0.9996
PFHpS	Lin, 1/A	13C2-6:2FTS	0.1	20.2	3.9	0.1–100	0.9968
FHxSA	Lin, 1/A	13C8-PFOS	0.1	15.0	20.4	0.1–100	0.9966
5:3 FTCA	Lin, 1/A	13C8-PFOA	1.0	15.7	7.1	1–100	0.9945
PFOS	Lin, 1/A	13C8-PFOS	0.1	26.3	22.2	0.1–100	0.9982
PFNA	Lin, 1/A	13C9-PFNA	0.5	24.4	7.9	0.5–100	0.9939
HFPO-TA	Lin, 1/A	13C3-HFPO-DA	0.5	15.8	7.4	0.5–100	0.9951
9Cl-PF3ONS	Lin, $1/A^2$	13C8-PFOS	0.1	6.6	2.0	0.1–100	0.9948
PFNS	Lin, 1/A	13C2-8:2FTS	0.1	19.2	12.2	0.1–100	0.9975
PFDA	Lin, 1/A	13C6-PFDA	0.5	26.7	14.4	0.5–100	0.9926
8:2FTS	Lin, 1/A	13C2-8:2FTS	0.5	24.7	11.0	0.5–100	0.9946
FOEA	Lin, 1/A	13C6-PFDA	5.0	26.0	10.9	5–100	0.9884
FOSA	Lin, 1/A	13C8-FOSA	0.1	28.3	19.2	0.1–100	0.9982
PFDS	Lin, 1/A	13C2-8:2FTS	0.1	23.2	9.8	0.1–100	0.9972
PFUdA	Lin, 1/A	13C7-PFUdA	0.5	13.0	7.0	0.5–100	0.9957
N-MeFOSAA	Lin, 1/A	d3-N-MeFOSAA	0.5	26.1	20.1	0.5–100	0.9913
7:3 FTCA	Lin, 1/A	d3-N-MeFOSAA	0.5	22.5	10.1	0.5–100	0.9895
11Cl-PF3OUdS	Lin, 1/A	d3-N-MeFOSAA	0.1	27.3	8.3	0.1–100	0.9932
N-EtFOSAA	Lin, 1/A	d5-N-EtFOSAA	0.5	29.4	20.4	0.5–100	0.9935
PFUnDS	Lin, 1/A	13C7-PFUdA	0.5	12.9	11.0	0.5–100	0.9937
PFDoA	Lin, 1/A	13C2-PFDoA	0.5	21.1	3.9	0.5–100	0.9929
10:2FTS	Lin, 1/A	d3-N-MeFOSA	0.5	28.1	10.8	0.5–100	0.9889
8:3FTCA	Lin, 1/A	d5-N-EtFOSAA	5.0	23.3	11.7	5–100	0.9910
NMeFOSE	Lin, 1/A	d3-N-MeFOSA	1.0	22.0	9.9	1–100	0.9959
N-MeFOSA	Lin, 1/A	d3-N-MeFOSA	0.5	16.6	8.1	0.5–100	0.9942
PFDoS	Lin, 1/A	13C7-PFUdA	0.1	9.3	4.9	0.1–100	0.9971
PFTrDA	Lin, $1/A^2$	13C7-PFUdA	0.1	11.0	6.5	0.1–100	0.9923
NEtFOSE	Lin, $1/A^2$	13C7-PFUdA	1.0	25.0	16.9	1–100	0.9899
N-EtFOSA	Lin, 1/A	d5-N-EtFOSA	0.5	28.5	14.5	0.5–100	0.9942
6:2diPAP	Lin, 1/A	13C4-8:2diPAP	5.0	17.2	11.5	5–100	0.9946
PFTrDS	Lin, 1/A	13C4-8:2diPAP	0.1	26.9	13.8	0.1–100	0.9908
PFTeDA	Lin, 1/A	13C2-PFTeDA	0.5	19.6	10.5	0.5–100	0.9963
6:2/8:2diPAP	Lin, 1/A	13C4-8:2diPAP	5.0	14.4	7.0	5–100	0.9928
PFHxDA	Lin, 1/A	13C2-PFTeDA	0.5	23.3	17.6	0.5–100	0.9940
8:2diPAP	Lin, 1/A	13C4-8:2diPAP	1.0	28.4	14.1	1–100	0.9920
PFOcDA	Lin, 1/A	13C2-PFTeDA	1.0	17.9	8.8	1–100	0.9943

Accuracy and precision (inter- and intra-day)

To further evaluate the accuracy and precision of the method two different water samples were tested, tap and bottled water, at two spiking levels, 5 ng/L and 75 ng/L. To further increase the variability, bottled water samples were taken each day from a new bottle and tap water was freshly sampled each day. Samples were prepared and analyzed 6 times on day 1 for intra-day study, while the inter-day variation was based on day 1 plus 2 injections on days 2 and 3. Accuracy percentage was calculated as the average of all the ten measured concentrations obtained from spiked tap and bottled water on day 1, 2, and 3, as compared to the theoretical 5 and 75 ng/L. Most of the compounds were found with values lower than 30% both in terms of accuracy (Figure 5) and precision (Figure 6) for (A) tap and (B) bottled water, respectively.

Cross-contamination and stability

To further evaluate the robustness of the extraction protocol, the cross-contamination and the stability of the samples were tested. To study the cross-contamination on the TriPlus RSH SMART autosampler, a series of blank HPLC water samples were run just after the extraction of 100 ng/L spiked samples. This was repeated three times. For all the internal standards, there was less than 5% signal in the blank as compared to spiked samples. In the case of target compounds, the area observed in the blank was lower than 20% of the signal obtained at the LOQ except for PFOcDA. Some carryover was observed for this long chain PFAS at 0.1 ng/L and 0.5 ng/L, but no issues were observed for 1 ng/L standard, therefore the LOQ was set at this value. These results confirmed that the workflow efficiently manages potential cross-contamination.

Spiked tap water stability was evaluated on both TriPlus RSH SMART and Vanquish Flex autosampler platforms offering the possibility to run up to 54 samples unattended. On the TriPlus RSH SMART autosampler, samples were either prepared in the instrument immediately after addition of the acid or run 12 hours later while being kept at room temperature on the sample tray, considering that running 54 samples takes about 8 hours (Figure 7A). On the Vanquish Flex autosampler, the final extraction solution from the same sample set was either injected immediately or injected 24 hours later while being maintained at 25 °C, bearing in mind that analyzing 54 samples takes an estimated 18 hours (Figure 7B).

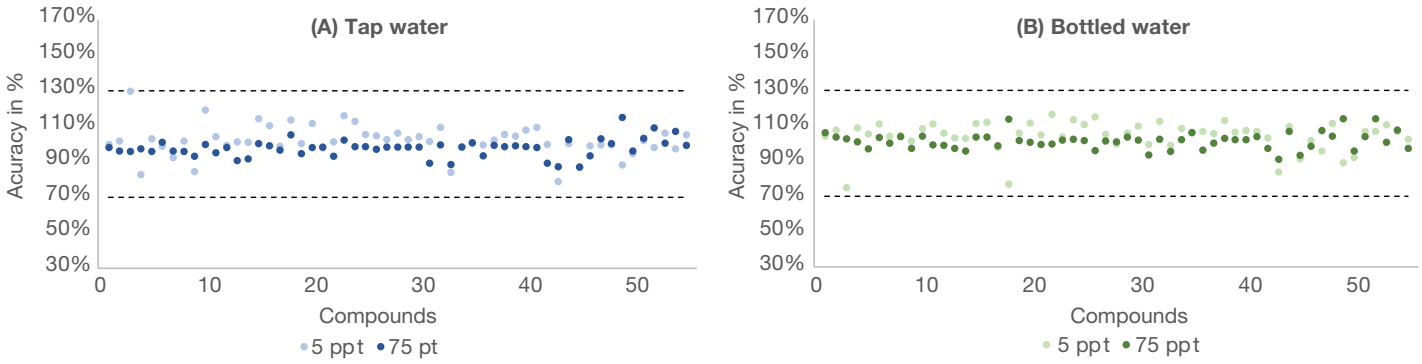


Figure 5. Accuracy of spiked (n=10) (A) tap and (B) bottled water samples at 5 and 75 ng/L together with the acceptance criteria of 70–130%

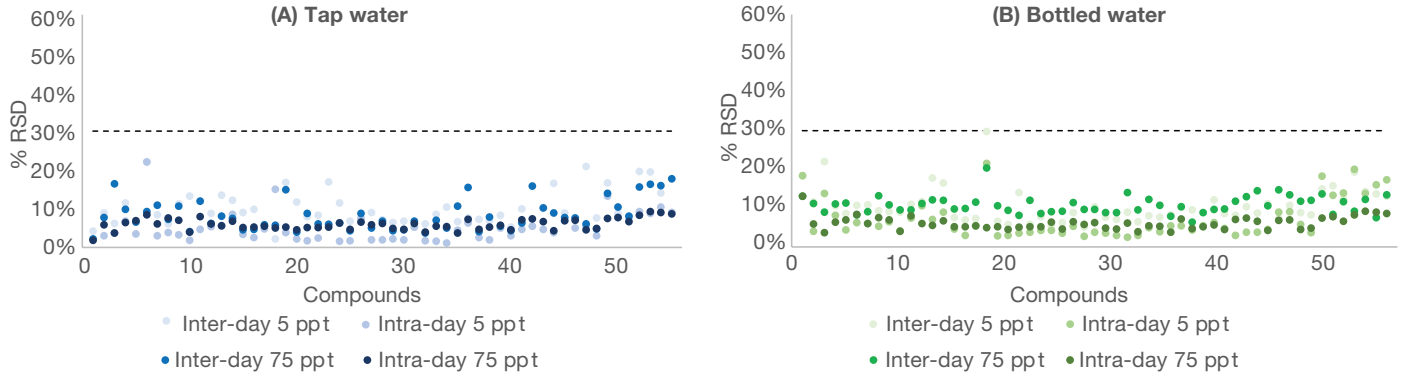


Figure 6. Inter-day (3 days, n=10) and intra-day (n=6) precision of spiked (A) tap and (B) bottled water samples at 5 and 75 ng/L together with acceptance limit of RSD <30%

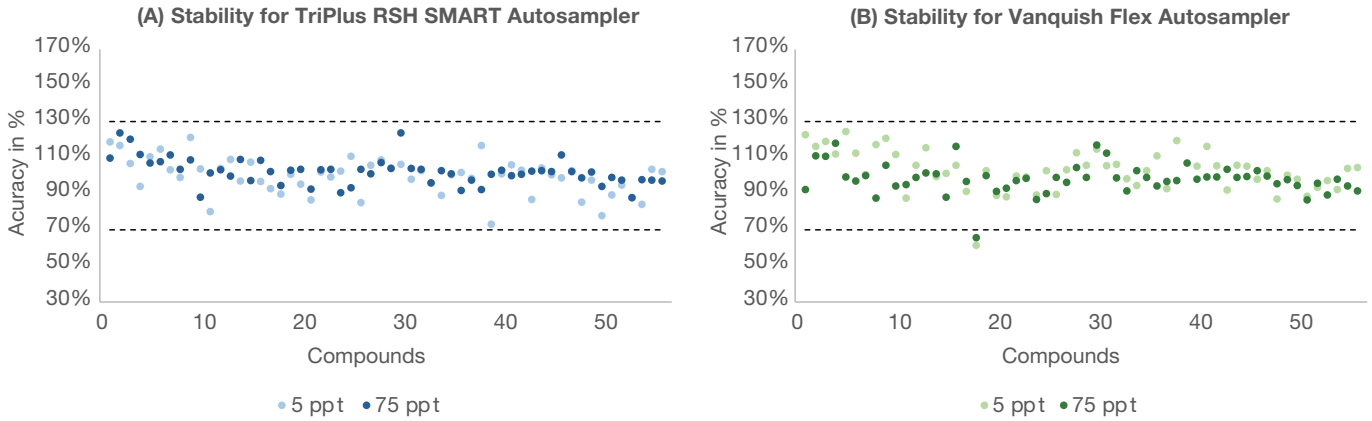


Figure 7. Stability of the (A) acidified samples for the TriPlus RSH SMART autosampler (after 12 h) and (B) for extracted samples in the final injection solution for the Vanquish Flex autosampler (after 24 h) at 5 and 75 ng/L with the acceptance criteria of ±30%

As can be seen in both graphs in Figure 7, most of the compounds are found within a 30% limit, the range corresponding to the accuracy of the calculated concentration in the sample. In the case of autosampler stability, fluorotelomer carboxylic acid (FOEA) presents a bias that is higher than 30%, both at 5 ng/L and 75 ng/L. For this compound class, no specific internal standard was used, and this probably explains some of the higher LOQ observed as well as the accuracy issue observed in the stability study. A mix of only 22 internal standards was used to correct for any possible extraction and matrix effects, while additional internal standards specific to the targeted compounds could have been included to improve the results.

Conclusions

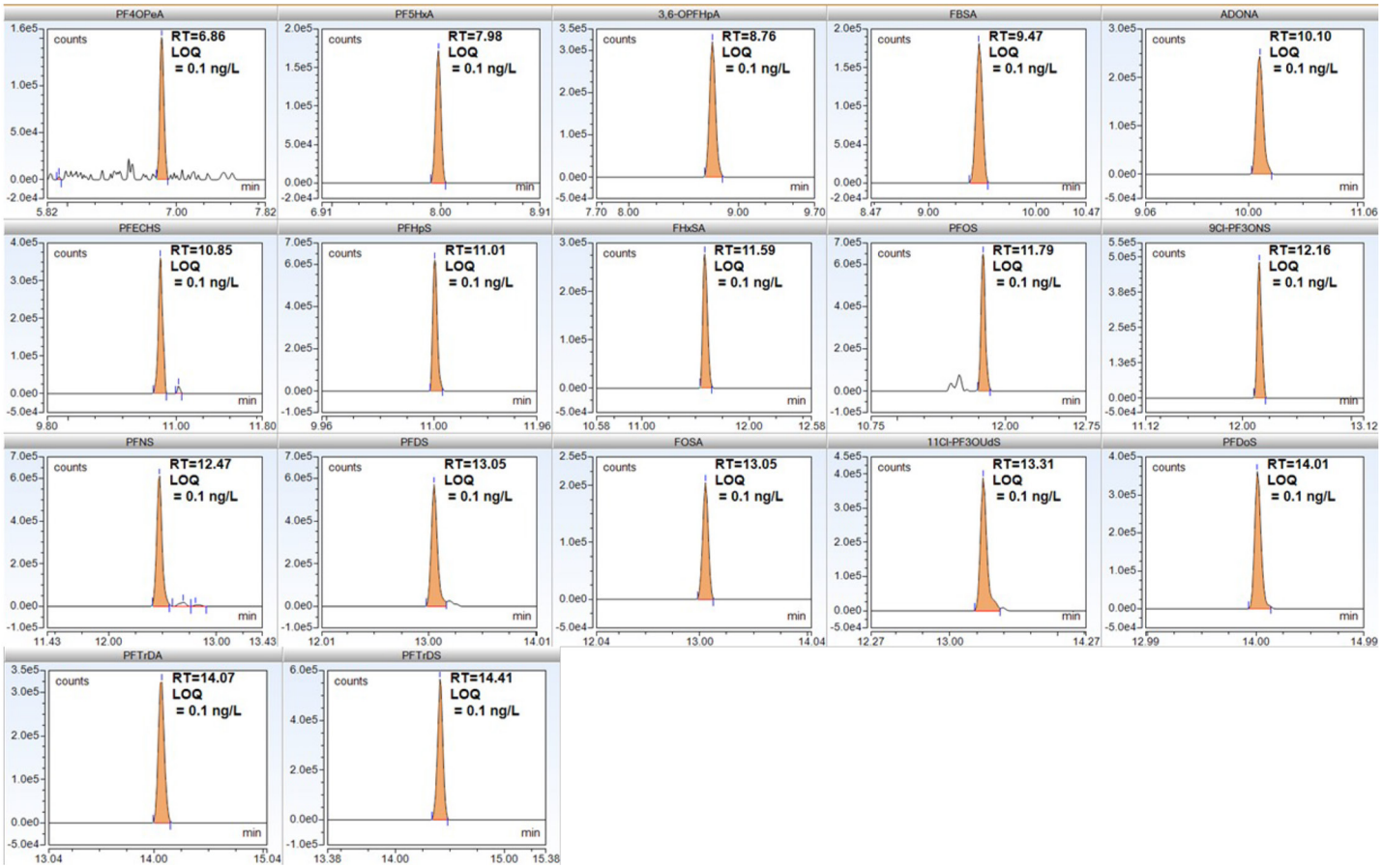
The DLLME method presented in this work is a promising technique for the extraction and pre-concentration of PFAS from drinking water samples. Thanks to the described careful optimization and validation of the method, it provides an automated, fast, efficient, and green alternative to traditional and manual extraction methods. As compared to SPE procedures, the only manual steps consist in adding internal standards and an acidic solution to the sample, and then the TriPlus RSH SMART instrument will perform the extraction automatically, while for SPE procedures, the rest of the steps are mostly manual. This workflow allows laboratories to overcome the challenges associated with this analysis, providing high enrichment factors for all the different compound classes studied, as well as good reproducibility and robustness of the extraction process. In summary, the presented automated DLLME sample preparation has the following advantages:

- Reduced sample volume (15 mL) for easy sample handling, transportation, and storage.
- Cost reduced by low solvent usage and no need for filters or SPE cartridges.
- Automation enables reproducible and accurate results, with low inner- and cross-contamination.
- Short runtime and automated process managed by Chromeleon software saves time and allows staff to perform more profitable activities.
- The short runtime of the TriPlus RSH SMART instrument opens the possibility to feed one or more analytical instruments, including HPLC-MS or GC-MS technologies.
- The extraction protocol without using filters and based on the use of low-density solvent for extraction can open the way for the extraction of other matrices such as wastewater.

References

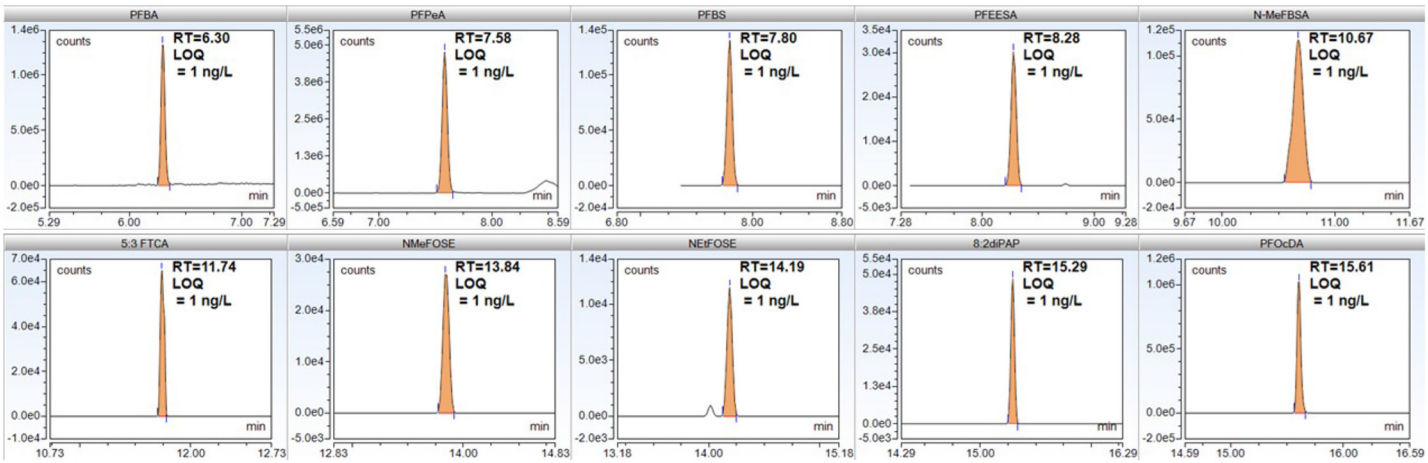
1. Thermo Fisher Scientific Application Note 002902: Direct injection of drinking water for the analysis of 54 PFAS compounds by LC-MS/MS aligned with current and evolving global regulations. Direct injection of drinking water for the analysis of 54 PFAS compounds by LC-MS/MS aligned with current and evolving global regulations (thermofisher.com)
2. Thermo Fisher Scientific Application Note 73883: Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water - Using automated solid phase extraction and LC-MS/MS for U.S. EPA Method 533. Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water (thermofisher.com)https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-73883-lc-ms-spe-per-pfas-drinking-water-an73883-en.pdf
3. Thermo Fisher Scientific Application Note 73346 Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water using automated solid-phase extraction and LC-MS/MS. Determination of Per- and Polyfluorinated Alkyl Substances (PFAS) in Drinking Water using Automated Solid-Phase Extraction and LC-MS/MS (thermofisher.com)
4. Sanan, T. et al., Analysis of per- and polyfluorinated alkyl substances in sub-sampled water matrices with online solid phase extraction/isotope dilution tandem mass spectrometry. *Journal of Chromatography A* **2020,** 461324, 1626. https://doi.org/10.1016/j.chroma.2020.461324
5. Rezaee, M. et al. Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A* **2006,** 1–9, 1116. https://doi.org/10.1016/j.chroma.2006.03.007
6. Herrera-Herrera, A. V. et al. Dispersive liquid-liquid microextraction for determination of organic analytes. Trends in *Analytical Chemistry* **2010,** Vol. 29, No. 7. https://doi.org/10.1016/j.trac.2010.03.016
7. Mansour, F. R. and Danielson, N. D. Solidification of floating organic droplet in dispersive liquid-liquid microextraction as a green analytical tool. *Talanta* **2017,** 170, 22–35. https://doi.org/10.1016/j.talanta.2017.03.084

Appendix

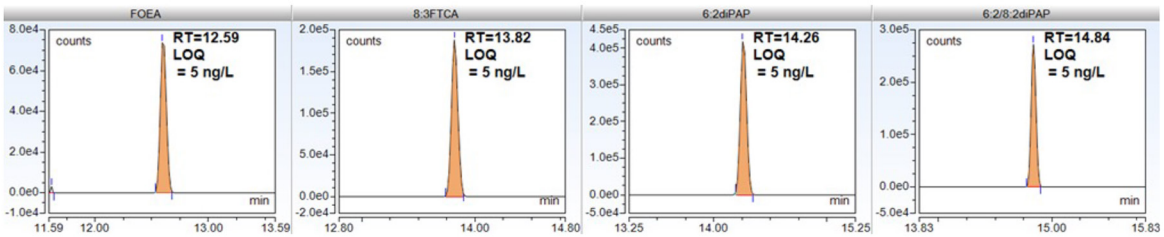


Appendix A. Chromatograms at the LOQ level for LOQ = 0.1 ng/L

Appendix B. Chromatograms at the LOQ level for LOQ = 0.5 ng/L



Appendix C. Chromatograms at the LOQ level for LOQ = 1 ng/L



Appendix D. Chromatograms at the LOQ level for LOQ = 5 ng/L

Learn more at **thermofisher.com/triplusrsh**

AB003164-EN 0824S