

Automated targeted and non-targeted LC-Orbitrap MS workflow for analysis of more than 40,000 PFAS compounds

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Abstract

Purpose: Demonstrate the analytical performance of a comprehensive workflow for PFAS in water samples based on a fully automated novel extraction technique followed by targeted quantitation and non-targeted analysis through two separate injections.

Methods: Acquisition was performed on a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer for both targeted and untargeted analyses. Sample preparation was based on an automated dispersive liquid-liquid microextraction (DLLME), providing reduced solvent consumption, cost-per-sample, and sample contamination.

Results: A panel of 56 PFAS compounds was quantified to low part per trillion levels for most analytes in drinking water from an initial volume of only 15 mL of sample. Untargeted data generated from this experiment includes a comparison with the targeted data to determine true positive and true negative rates of the workflow.

Introduction

Per- and polyfluoroalkyl substances (PFAS) have gained attention in recent years due to their widespread occurrence and potential adverse effects on human health and the environment. To ensure accurate measurements of PFAS in environmental samples, efficient, robust and sensitive extraction techniques are crucial. DLLME has emerged as a promising technique for the extraction of PFAS due to its simplicity, speed, and low sample and consumable consumption. Extracts can be measured by targeted quantitative methods to meet very low regulatory limits, while screening methods utilizing the high mass resolution and mass accuracy of the Orbitrap™ analyzer can explore potential unknown PFAS with confidence within the same sample extract.

Materials and methods

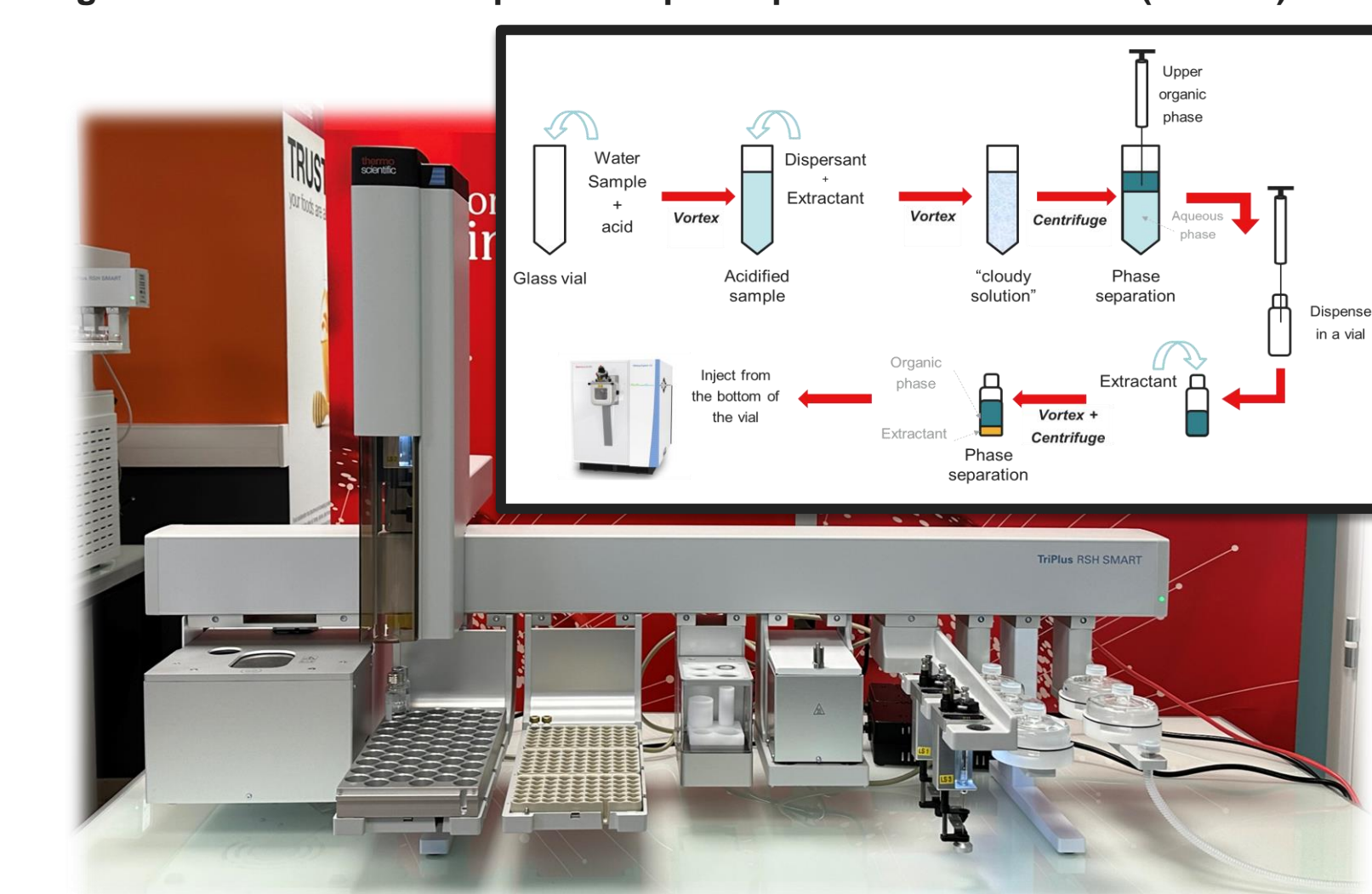
Sample Preparation

Drinking water samples analyzed included tap water and bottled water from French brands. Figure 1 illustrates the developed sample extraction workflow using the automated Thermo Scientific™ TriPlus™ RSH SMART liquid handling station, which is fully integrated in Thermo Scientific™ Chromeleon™ CDS software. DLLME involves the dispersion of a small volume of an extraction solvent into a larger volume of a sample solution, followed by the formation of fine droplets and subsequent phase separation.

Liquid Chromatography Method

Both the delay and analytical column used were Thermo Scientific™ Acclaim™ 120 C18 columns with a flow rate of 0.4 mL/min for a total run time of 23 min on a Vanquish™ Flex UHPLC system.

Figure 1. Schematic of dispersive liquid-liquid micro extraction (DLLME)



Targeted Quantitation Results

Targeted Quantitation MS Method

For quantitative analysis, a combination of full scan (60k), SIM (60k), and AIF (15k) scan modes were used to target 56 PFAS (Table 1). MS1 data from the full scan were used for quantitation for most compounds. Compounds requiring extra sensitivity were quantitated by SIM scans. MS2 confirmation was obtained through AIF to provide confirmation ions across the entire chromatographic peak quickly. Data was acquired and analyzed using Chromeleon CDS version 7.3.2. LOQ values (Figure 2) were obtained based on the concentration level for which both reproducibility (<30%) and accuracy (70-130%) criteria were met on 6 injections over 3 days in neat solution. Reproducibility and accuracy data in the bottled and tap water is shown in Figure 3. Blank matrix correction was conducted for compounds positively quantified in both types of water.

Table 1. List of the 56 target compounds divided into their chemical classes, *denotes compounds with specific labelled internal standard

Perfluoroalkyl carboxylic acids (PFCA)	Perfluoroalkyl sulfonic acids (PFSA)	Ether sulfonic acids (ESA)
PFBA*	PFBS*	11C-PP3OUdS
PFPeA*	PFPeS	9CI-PF3ONS
PFHxA*	PFHxS*	PFESA
PFHpA*	PFHpS	Perfluorooctane sulfonamidoacetic acid (FOSAA)
7HPFHpA	PFOS*	N-MeFOSAA*
PFOA*	PFNS	N-EtFOSAA*
PFNA*	PFDS	Perfluorooctane sulfonamide ethanol (FOSSE)
PFDA*	PFUnS PFUnDS	NMeFOSE
PFUnA or PFUnDA*	PFDoS	NEiFOSE
PFDoDA*	PFTdS	Per- and Polyfluoroether carboxylic acid (PFCEA)
PFTdA	Fluorotelomer carboxylic acids (FTCA)	NFDHA, 3,6-OPFHpA
PFTdA*	3:3 FTCA, FPpPA	DNNA, ADONA
PFHxDA	5:3 FTCA, FPpPA	PFMPA, PF4QPeA
PFODA, PFOcDA	7:3 FTCA, FHpPA	PFMBA, PF5HxA
Perfluoroalkyl sulfonamides (FASA)	8:3 FTCA	HFPO-DA (Gen X)
FBSA	FEOA	HFPO-TA
N-MeFBSA	Fluorotelomer sulfonic acids (X:2FTS)	Polyfluoroalkyl phosphate di-esters (diPAP)Other
FhxA	4:2FTS*	6:2diPAP
FOSA*	6:2FTS*	6:2/8:2diPAP
N-EtFOSA	8:2FTS*	8:2diPAP
N-MeFOSA	10:2FTS	PFECBS

Figure 2. Distribution of targeted PFAS LOQ (ng/L) values

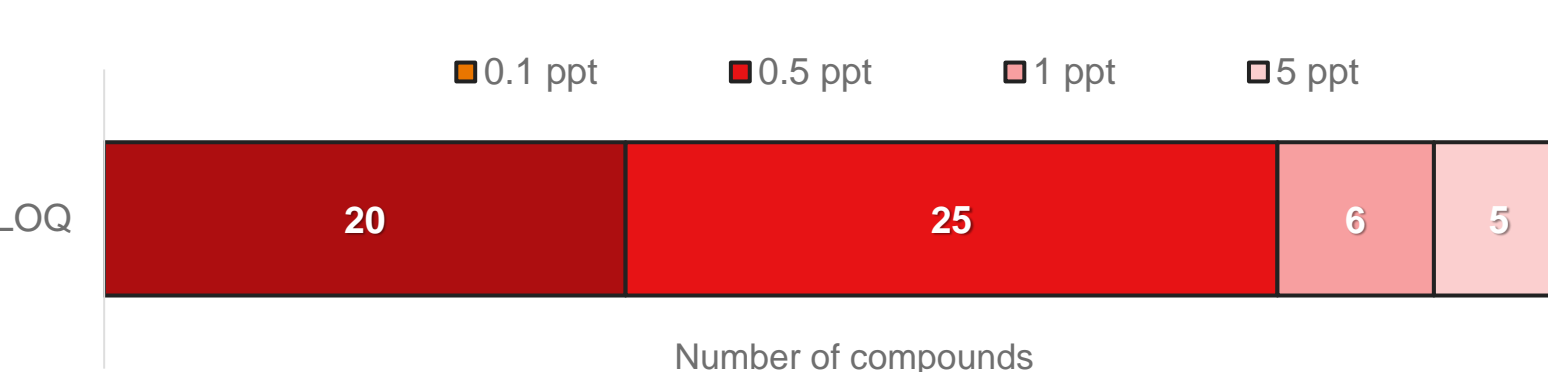
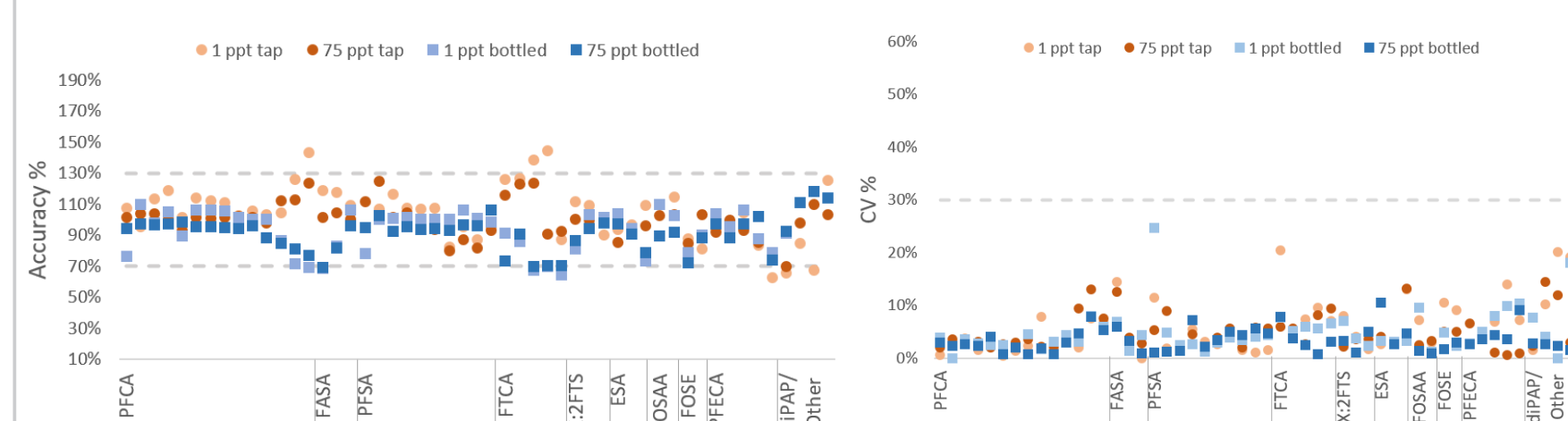


Figure 3. Mean accuracy% (n=7) and CV% of all 56 analyzed PFAS (grouped by class) in spiked bottled and tap water, at low (1 ng/L) and high (75 ng/L) levels – matrix blank corrected

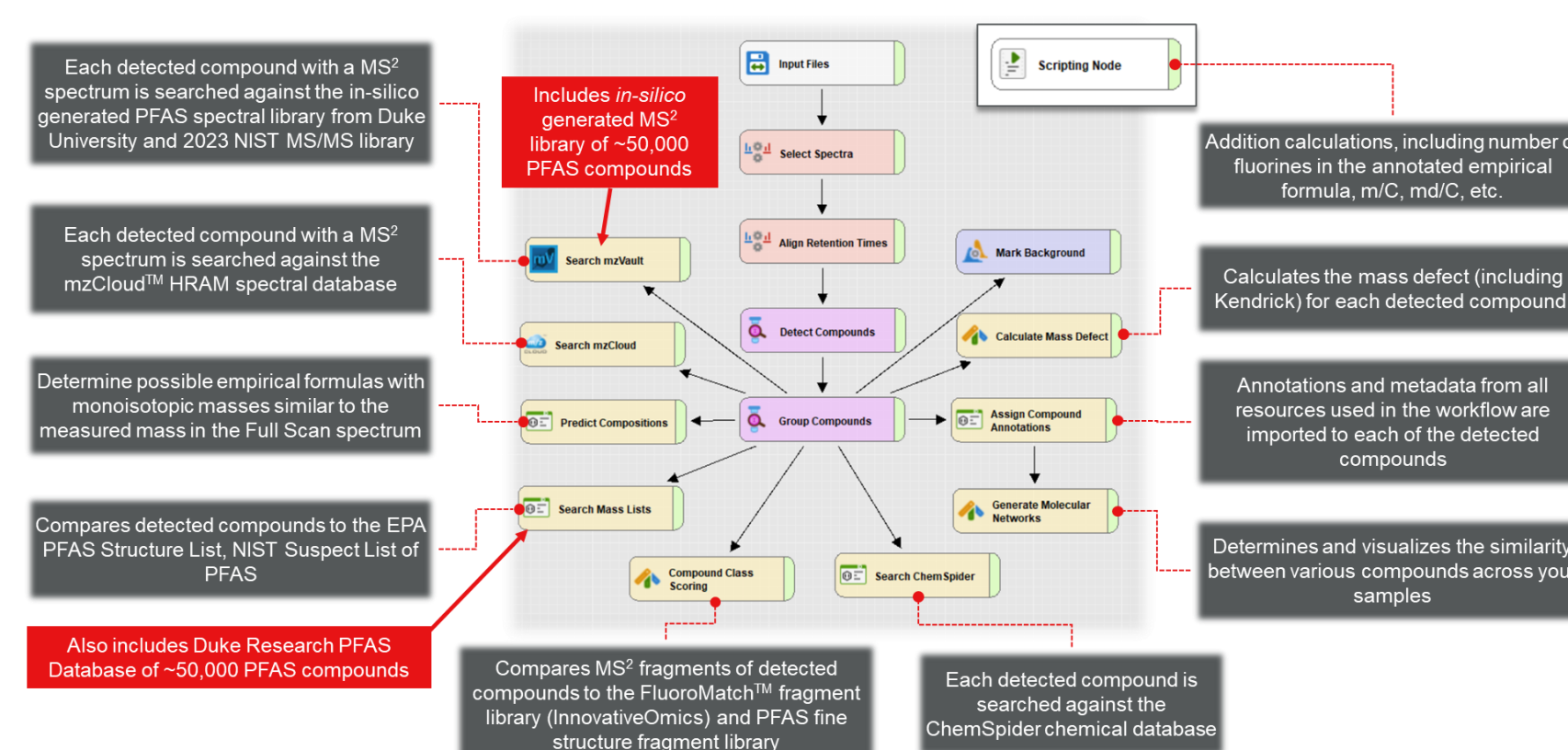


Non-Targeted Analysis Overview

Predefined Template for Non-Targeted PFAS Analysis

Compound Discoverer 3.3 SP3 comes with a predefined template tailored specifically to maximize annotation confidence (as defined by Charbonnet et al. 2022) in the detection and annotation of unknown PFAS compounds (see workflow in Figure 4). This is accomplished by combining a multitude of the best resources available within the PFAS community, including EPA and NIST PFAS chemical databases, the FluoroMatch™ PFAS fragmentation databases, the Duke University *in-silico* generated PFAS spectral library, and both the 2023 NIST HRMS MS/MS and mzCloud™ spectral libraries.

Figure 4. Compound Discoverer node-based workflow



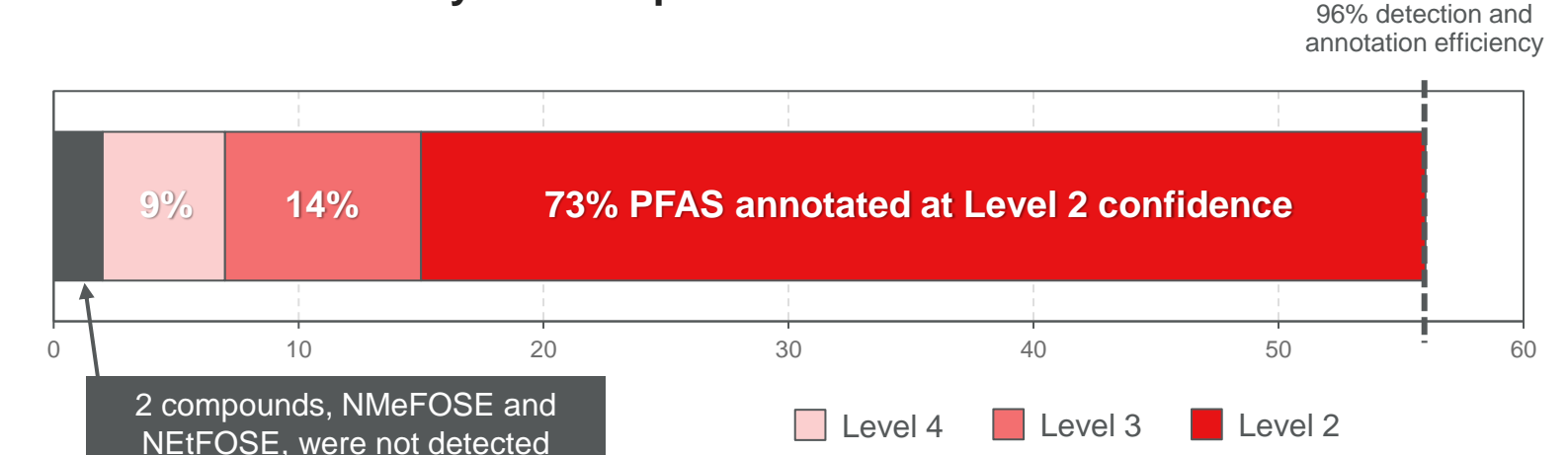
Confidence Levels in Compound Annotations

Following the confidence scale described by Charbonnet et al., 2022¹, the level of confidence in the annotation of each detected PFAS compounds was assigned based on the matches to each of the resources used within the workflow. Table 2 provides an overview of the criteria that needs to be met in order to achieve each confidence level. In this work we evaluate the efficiency of Compound Discoverer™ detecting the spiked PFAS compounds and annotating them at confidence levels between 2–4.

Table 2. Criteria for confidence levels in PFAS annotation

Level	Using Full Scan (MS1) data			Using MS2 data			Retention Time match to Reference Standard
	Measured mass within ± 2 ppm of Mass List	Standard Mass Defect (-0.11–0.12)	Isotopic Pattern Match	±1 Fragment with match to FluoroMatch™ database	>50% similarity match to <i>in-silico</i> PFAS spectral libraries	>50% similarity match to mzCloud™ or 2023 NIST MS/MS spectral libraries	
1	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓

Figure 5. Number of spiked compounds successfully detected and annotated across Levels 2–4 by the Compound Discoverer™ workflow.



Confidence in Annotating Unknown PFAS

Bringing together multiple resources to confidently annotate unknown detected compounds

By combining multiple mass list databases and spectral libraries, we can increase the capability of achieving Level 2 annotations for more unknown PFAS compounds (see example in Figure 6).

Figure 6. Example of utilizing multiple spectral libraries and databases to annotate a PFAS compounds at Level 2 confidence.

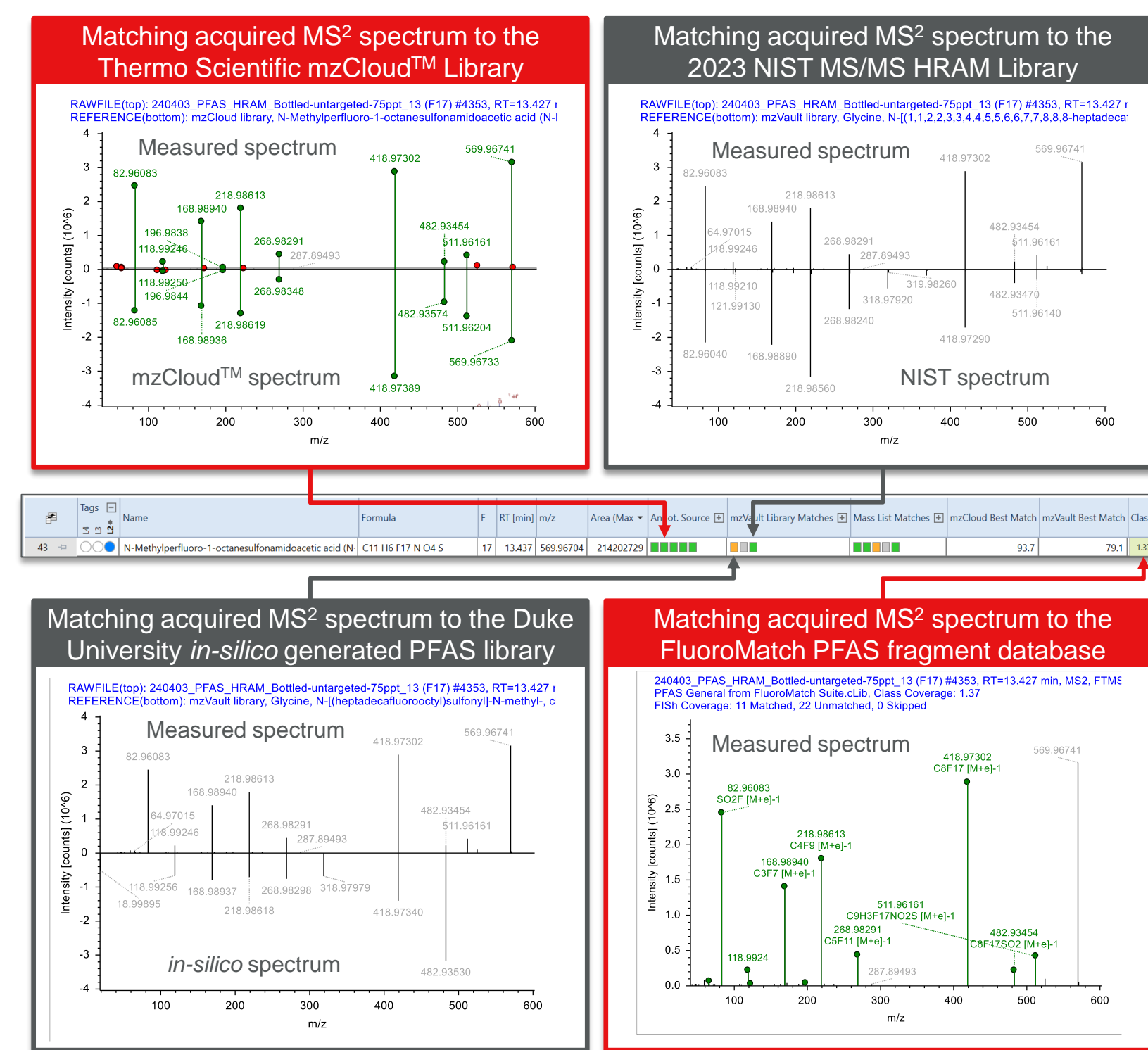
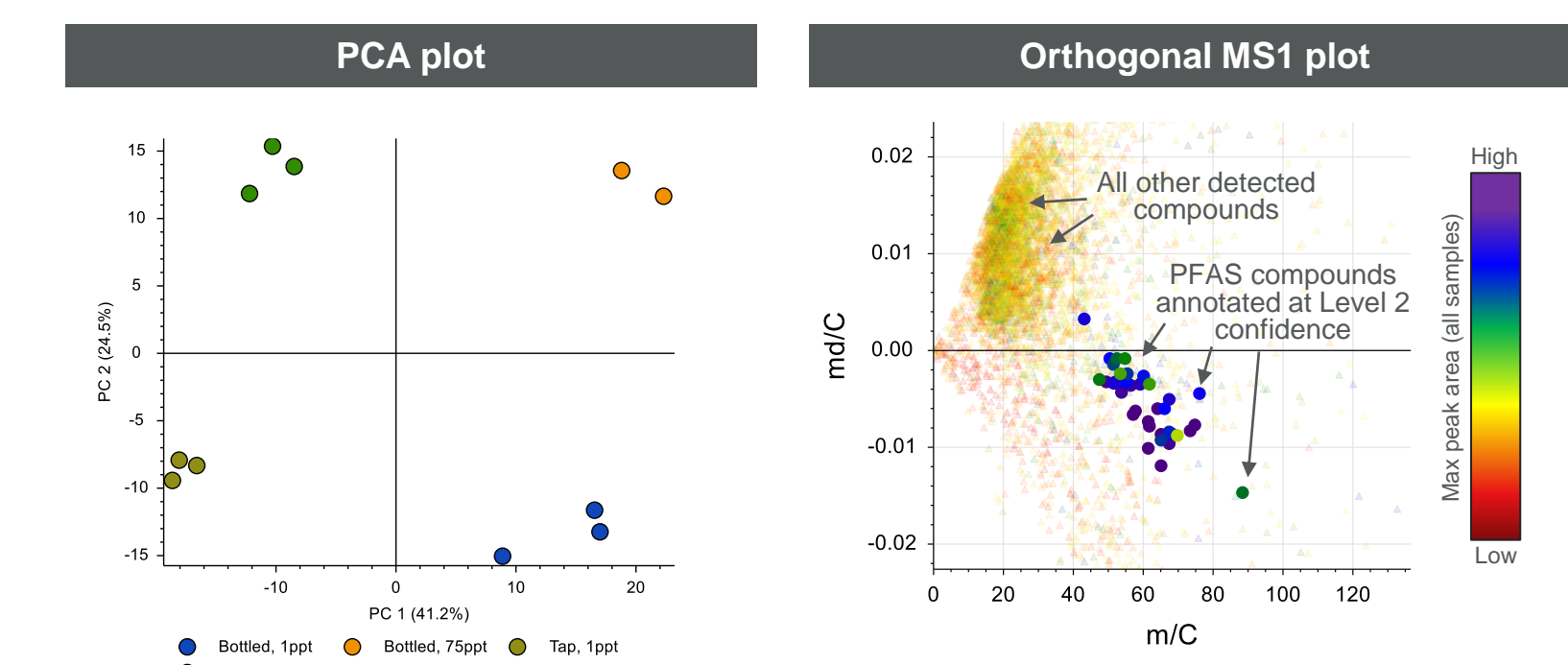


Figure 7. Finalized list of PFAS compounds detected across the Tap and Bottled water samples and then annotated with Levels 2–4 confidence.

Evaluations of Sample PFAS Composition

Compound Discoverer includes multiple plotting tools that can be used to visually investigate differences in the PFAS composition across samples, both from the perspective of compounds detected and compound concentration (see Figure 7). This includes statistical plots (e.g., PCA plots) and orthogonal MS1 plots, as developed by Kaufman et al. 2022.²

Figure 7. Various plotting tools within Compound Discoverer to evaluate overall PFAS composition across samples.



Conclusions

- Automated dispersive liquid-liquid microextraction is a promising technique to extract and concentrate PFAS from drinking water samples. The resulting extract from a single sample preparation can then be measured by both quantitative targeted and non-targeted analysis in separate runs.
- Quantitative targeted analysis showed good accuracies at low (1 ng/L) and high (75 ng/L) spiking level, as well as reproducibility (<30%, n=7) over several days. The use of internal standards for selected PFAS classes can additionally improve obtained results in terms of accuracy.
- The non-targeted analysis workflow in Compound Discoverer 3.3 SP3 provides a comprehensive package enabling confident annotation of unknown PFAS compounds as well as understanding differences in PFAS composition across samples.

References

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