

# An On-line SPE-UHPLC-HRMS Method for the Determination of 11 Classes of Per- and Polyfluoroalkyl Substances (PFAS) in Water

M. H. Belay<sup>1\*</sup>, E. Marengo<sup>1</sup>, F. Dondero<sup>1</sup>, E. Robotti<sup>1</sup>

<sup>1</sup> Department of Sciences and Technological Innovation, Università del Piemonte Orientale, Alessandria, Italy

\* Email correspondence: [masho.belay@uniupo.it](mailto:masho.belay@uniupo.it)

## INTRODUCTION

This study describes the development and validation of a UHPLC-HRMS method for the determination of 43 per- and polyfluoroalkyl substances (PFAS) in water. PFAS (*aka* 'forever chemicals') are an **emerging concern** due to their persistence in the environment and the human body, and they can cause health problems such as liver damage, thyroid disease, obesity, fertility issues and cancer [1]. The widespread presence of PFAS in a variety of environmental samples, occurrence of isomers for some compounds, and precursor transformations that may occur during preservation and storage of the samples present numerous **analytical challenges** [2]. Moreover, as more stringent PFAS regulations emerge, **sensitive analytical methods** with very low detection limits (<5 ng/L) are required.

The current method was developed based on-line solid phase extraction (SPE) coupled with UHPLC-HRMS. Low detection limits were achieved through a series of optimization experiments on the on-line SPE, UHPLC, and HRMS conditions. Background contamination was eliminated by using **PFAS-certified consumables** (i.e., polypropylene vials and caps, high-purity solvents and modifiers for mobile phases, replacing fluorinated solvent tubing with PEEK, removing PTFE filter frits, and installing a delay column between the pumps and the autosampler). A key drawback of conventional offline SPE in PFAS analysis is the possibility of sample contamination and loss of surface-active PFAS to container walls and other materials [3]. As a result, on-line SPE was used for simultaneous sample clean-up and enrichment, enabling quantitative transfer of analytes to the analysis system [4] and guaranteeing minimal contamination [5] by eliminating the use of perfluorinated materials such as SPE cartridges, tubing, and syringes.

## EXPERIMENTAL

**Delay column**

PFAS delay column (50x2.1 mm, 5 µm)


**On-line SPE columns**

1) Strata-X-AW (20x2.0 mm, 25 µm)  
2) Hypersil Gold C18 (20x2.1 mm, 12 µm)


**Separation columns**

1) Luna Omega PS C18 (2.1x100 mm, 1.6 µm)  
2) Acclaim RSLC 120 C18 (2.1x150 mm, 2.2 µm)  
3) Raptor C18 (50x2.1 mm, 2.7 µm)

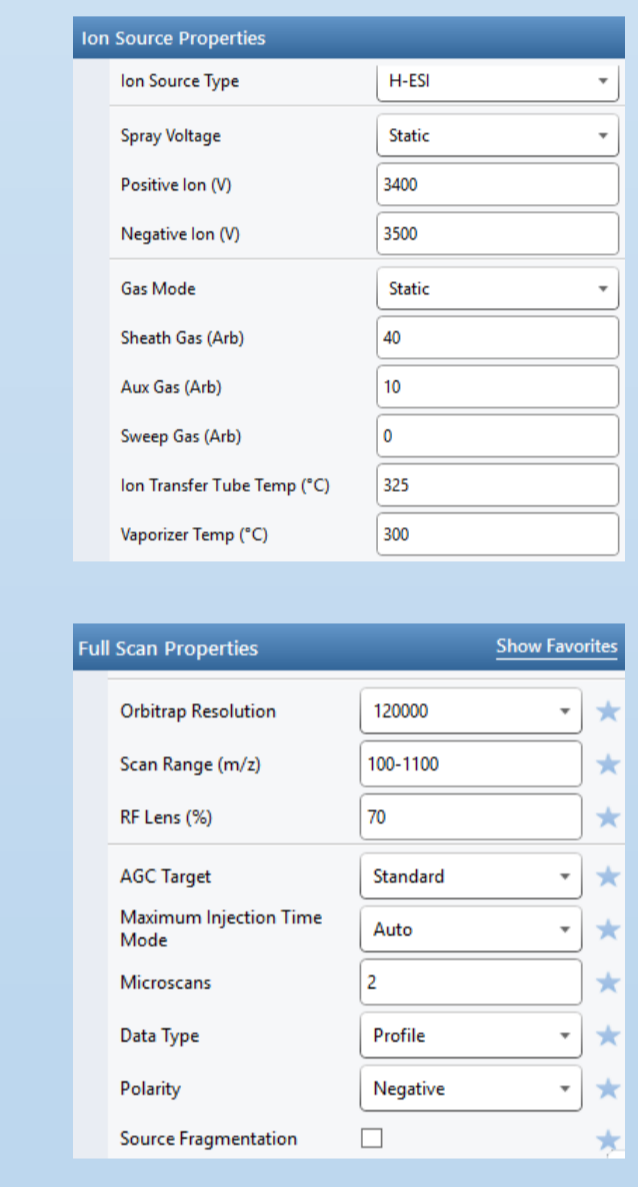
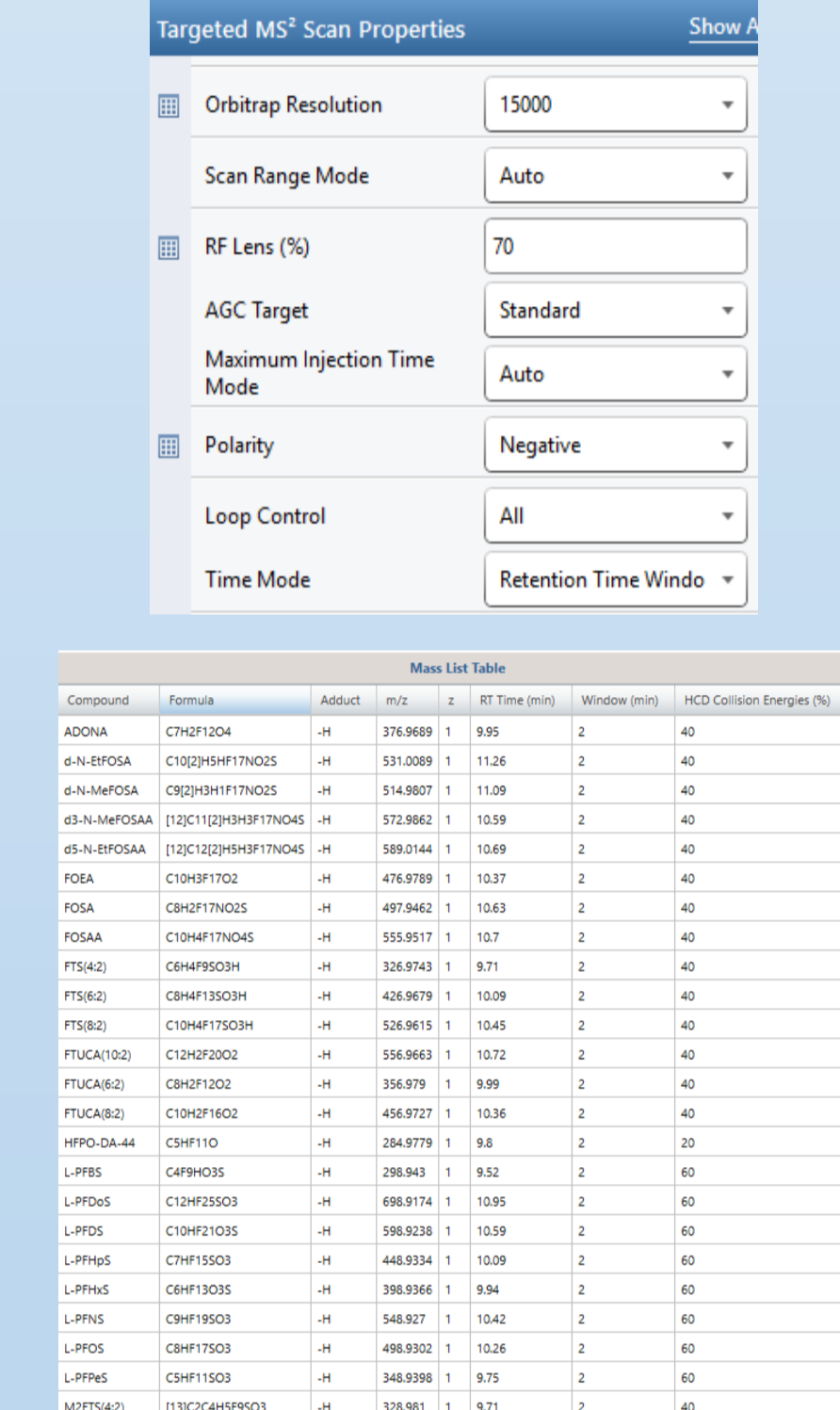
**Dionex 3000 UHPLC**



**Orbitrap Exploris 120 HRMS**



- Full Scan MS
- t-MS<sup>2</sup> Scan

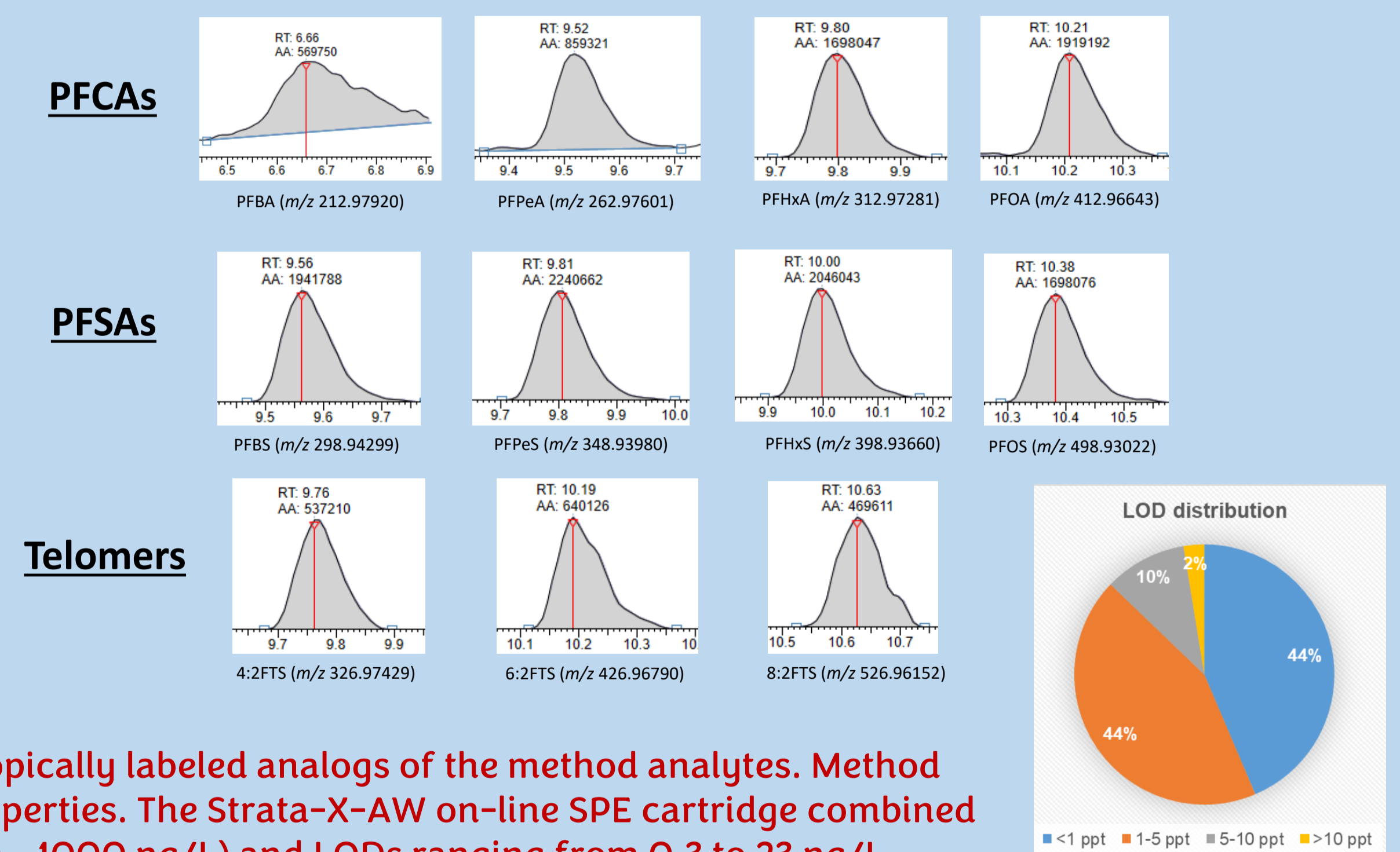
On-line SPE UHPLC-HRMS method developed and validated in-house for **41 PFAS in water** (drinking water, well water and wastewater)

## RESULTS

### Method validation

No.	PFAS Class	Target PFAS	Linear range (ng/L)	LOD (ng/L)	LOQ (ng/L)	% Recovery (±RSD)	Intraday precision (RSD)	Inter-day precision (RSD)
1	PFCAs	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUDA, PFDoA, PFTrDA, PFTeDA, PFHxDA, PFODA	1-500	0.4-18	1.4-27.9	78-113 (±1.3-8.8)	0.9-4.7	2.1-11.8
2	PFASs	PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS	0.5-500	0.3-8.1	0.9-13.2	92-118 (±2.1-5.5)	0.7-5.5	1.9-13.4
3	FOSAs	FOSA, MeFOSA, EtFOSA	5-500	1.3-5.8	1.8-13.9	98-116 (±2.4-6.5)	1.2-7.6	3.2-11.7
4	FOSAs	FOSAA, MeFOSAA, EtFOSAA	10-500	1.5-5.0	3.5-15.1	78-102 (±1.5-5.7)	2.3-9.1	2.7-14.1
5	PFESAs	HFPO-DA (GenX)	25-500	14.0	26.5	101-104 (±2.6-3.2)	1.2-3.9	2.2-7.1
6	PFECAs	ADONA, PF2EOESA, Nafion-BP2, PF4MOBA, PFOZDA, PFO3-3-6-9-DoA	1-500	0.5-23	1.1-37.7	94-117 (±3.0-3.3)	1.2-4.7	2.5-13.8
7	n:2FTUAs	FHUEA, FOUEA, FDUEA	10-500	1.5-6.4	2.3-9.8	90-107 (±3.6-9.1)	2.0-7.7	4.5-12.6
8	n:2FTSs	4:2FTS, 6:2FTS, 8:2FTS	1-500	0.5-3.2	0.8-5.1	98-104 (±2.1-4.3)	0.5-1.9	1.3-6.6
9	n:mPFPI	6:6PFPI, 6:8PFPI, 8:8PFPI	25-500	1.5-3.9	2.3-6.0	97-122 (±3.2-8.7)	3.1-7.7	6.3-14.4

### Some examples... (5 ng/L spiked tap water)



• Method validation using isotope dilution scheme with a mixture of 24 extracted internal standard (EIS) that are isotopically labeled analogs of the method analytes. Method analytes with no available isotopically labeled analogs were quantitated using the IS analogs of similar chemical properties. The Strata-X-AW on-line SPE cartridge combined with the Luna Omega PS C18 column provided the best results allowing for three orders of linear dynamic range (1.0 - 1000 ng/L) and LODs ranging from 0.3 to 23 ng/L.

## CONCLUSIONS

- The mixed-mode weak anion exchange on-line SPE cartridge provided better performance particularly for the short chain congeners and some telomers. Compared to the C18 stationary phases, the positive surface (PS) in the Luna Omega PS C18 column provided improved retention and selectivity.
- Sample pre-treatment using on-line SPE minimized the possible sample contamination and loss of surface-active PFAS to container walls and other materials used in the conventional offline SPE method. Moreover, the total sample handling and analysis time was significantly reduced.
- Recoveries were 78-123% and no significant matrix effect in tap water and well waters.

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**References:** (1) Emerging chemical risks in Europe — 'PFAS', EEA, <https://www.eea.europa.eu/publications/emerging-chemical-risks-in-europe/emerging-chemical-risks-in-europe>, 25 May 2023. (2) Rehman, A. U., et al., Trends Environ. Anal. Chem., 37 (2023), e00198. DOI:10.1016/j.teac.2023.e00198. (3) Mazzoni, M. et al., J. Anal. Methods Chem. 2015 (2015) 942016. DOI:10.1155/2015/942016. (4) Kourtchev, I. et al., Sci. Total Environ. 835 (2022) 155496. DOI:10.1016/j.scitotenv.2022.155496. (5) Sanan, T. et al., J. Chromatogr. A, 1626 (2020) 461324. DOI:10.1016/j.chroma.2020.461324.