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Question: How can ultrashort-chain and branched PFAS in water sources be detected and quantified?

Problem: Per- and polyfluoroalkyl substances (PFAS) are short to long chains of alkyl molecules that have their carbons fully or partially saturated with fluorine bonds. These PFAS are not made naturally, and their carbon-fluorine bonds are too strong for them to be naturally broken down.¹ PFAS have industrial applications in non-stick cookware and some water-repellent clothing.¹ Consequently, PFAS are leaching into the environment and entering bodies of water where they eventually get consumed. As PFAS can cause health problems such as altered immune and thyroid function, liver disease, kidney disease, and cancer, there is a need for an accurate quantitative analysis method of PFAS in bodies of water and drinking water.²

Proposed Solution: A common method used for the quantification of PFAS in water samples is high resolution mass spectrometry (HRMS).³ HRMS is a great technique for analytical chemistry, including quantifying PFAS, as it can very accurately distinguish small changes in mass/charge ratios and provides the ability to discover unknown PFAS by non-targeted screening.³ However, there are some issues with this technique. HRMS instruments are much more expensive than low resolution mass spectrometers, are much less common in laboratories, and require specially trained analysts.⁴ As an alternative method for PFAS quantification, Huerta et al. propose a method using high performance liquid chromatography (HPLC) in tandem with mass spectrometry (MS).⁵ Using HPLC with a triple quadrupole mass spectrometer provides a more cost-effective and accessible analytical approach to this problem, providing better sensitivity compared to HRMS and still yielding great results.⁴ Additionally, as only specific PFAS were of interest in this experiment, the non-targeting ability of HRMS was not needed.

Technique: Huerta et al. present a method applying high performance liquid chromatography (HPLC) to quantify 15 perfluorinated substances.⁵ This was done by collecting water samples from different sources around Ireland including river water, drinking water, and sea water. Each sample was then further processed in laboratory where they were adjusted to a pH of 3 and cooled to 4 °C. Samples were then loaded into solid-phase extraction (SPE) cartridges that have been conditioned with 5mL 5% NH₃/MeOH then 5mL EtOH and 5mL ultra pure water. Samples were then concentrated by eluting, and 5 µL of 1 ppb standard compound solution were added to the extracts. The samples were then injected into HPLC coupled to a triple quadrupole mass spectrometer equipped with electrospray ionization source in negative mode. Concentrations were calculated by comparing samples' peak area to that of the internal standards on a calibration curve generated from linear regression analysis (0.01-1000 ng/mL, $R^2 > 0.99$).⁵

Requirements: Time requirements were not explicitly stated in the article for most steps, but the requirements for the method are as follows: sample collection, sample preservation in fridge or freezer (depending on time), conditioning SPE cartridges, drying the sample extracts (30 mins), and running the samples on LC-MS/MS.⁵ In total, this process would likely take 4 hours.

Instruments and Reagents: This method used an Agilent 1260 series Infinity binary pump HPLC coupled to an Agilent 6470 triple quadrupole mass spectrometer, using an Agilent Zorbax

Eclipse Plus C18 column, 3.0 x 50 mm, 1.8 μ L column and guard column. Mobile phases of 2 mM formic acid/ammonium formate at pH 3.2 and methanol were used, both at 0.4 mL/min flow rate.⁵

Precision and Limits: The reported regression analysis was $R^2 > 0.99$ over a concentration range of 0.01 to 1000 ppb.⁵ Of the various matrices analyzed the limits of detection of all PFAS for drinking, river, and sea water ranged from 0.46-12.62 ng/L, 0.58-15.15 ng/L, and 0.65-17.7 ng/L respectively. Additionally, the reported limits of quantification for the different matrices ranged from 1.54-15.15 ng/L, 1.85-50.48 ng/L, and 2.16-58.90 ng/L. Also, the reported extraction efficiency for all but 4 PFAS was above 50%.

Precautions: To prevent contamination in the laboratory, non-PFAS lab equipment should be used whenever possible. Additionally, polypropylene should be used whenever possible instead of glass as PFAS won't be retained on the polypropylene.⁵

Practical Applications: The method proposed by this study offers great practical applications. This technique provides greater accessibility to PFAS quantification as it is more cost-effective and utilizes more common instruments in comparison to alternative methods.⁶ Additionally, although HRMS can provide greater accuracy and flexibility due to non-targeted screening, HPLC-MS/MS gives greater sensitivity and still provides great results.⁴ Furthermore, the technique designed for this procedure can easily be manipulated for the analysis of other compounds in similar matrices. However, another slight disadvantage that this method has in comparison to HRMS is that it requires individual standard solutions of all PFAS used. Although this would increase the solution preparation time and budget, this method still appears to be more practical than alternatives.

Search Technique: A general search engine was used to assess what analytical methods were commonly used to quantify substances in water samples. An environmentally concerning chemical that is not often talked about, such as PFAS, was chosen to be the analyte of interest. Multiple articles and methods were consulted from verified journals and were assessed for their relevance, practicality, and method validation. When an article was chosen to be the basis for this scientific paper, the same method was applied to find other techniques also aiming to quantify PFAS so they could be compared.

Articles Surveyed:

Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environmental Toxicology and Chemistry*

Rapid and Simultaneous Determination of Ultrashort-, Short- and Long- Chain Perfluoroalkyl Substances by a Novel Liquid Chromatography Mass Spectrometry Method. *Journal of Chromatography A*

High-Resolution Mass Spectrometry (HRMS) Methods for Nontarget Discovery and Characterization of Poly- and Per-Fluoroalkyl Substances (PFASs) in Environmental and Human Samples. *TrAC Trends in Analytical Chemistry*

References:

- (1) Zou, J.; Zhao, M.; Chan, S.-A.; Song, Y.; Yan, S.; Song, W. Rapid and Simultaneous Determination of Ultrashort-, Short- and Long- Chain Perfluoroalkyl Substances by a Novel Liquid Chromatography Mass Spectrometry Method. *Journal of Chromatography A* **2024**, *1734*, 465324–465324. <https://doi.org/10.1016/j.chroma.2024.465324>.
- (2) Fenton, S. E.; Ducatman, A.; Boobis, A.; DeWitt, J. C.; Lau, C.; Ng, C.; Smith, J. S.; Roberts, S. M. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environmental Toxicology and Chemistry* **2020**, *40* (3), 606–630. <https://doi.org/10.1002/etc.4890>.
- (3) Liu, Y.; D'Agostino, L. A.; Qu, G.; Jiang, G.; Martin, J. W. High-Resolution Mass Spectrometry (HRMS) Methods for Nontarget Discovery and Characterization of Poly- and Per-Fluoroalkyl Substances (PFASs) in Environmental and Human Samples. *TrAC Trends in Analytical Chemistry* **2019**, *121*, 115420. <https://doi.org/10.1016/j.trac.2019.02.021>.
- (4) Lazofsky, A.; Brinker, A.; Zorimar Rivera-Núñez; Buckley, B. A Comparison of Four Liquid Chromatography–Mass Spectrometry Platforms for the Analysis of Zeranols in Urine. *Analytical and Bioanalytical Chemistry* **2023**, *415* (20), 4885–4899. <https://doi.org/10.1007/s00216-023-04791-8>.
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- (6) Wilson, C. L.; Wilson, D. W.; G Svehla; Weber, S. G.; Damià Barceló; J Paulik; F Paulik; Jespersen, N. D.; V Balek; Juraj Tölgyessy; Jaroslav Sesták; Lúdivít Kubicár; Slavoj Hála; Mecislav Kuras; Popl, M.; M Marhol; L Mázor; H -H Emons; H Kenne; H -H Seyfarth. *Comprehensive Analytical Chemistry. Vol. 58 : TOF-MS within Food and Environmental Analysis*; 2012.

Abstract

Poly- and perfluoroalkyl substances (PFASs) are a group of synthetic organic surfactants that have become a global concern because of their toxicity and widespread presence in the aquatic environment and organisms globally. In this study, a new analytical method has been developed and validated for the analysis of 15 perfluorinated compounds in different water matrices: river water, drinking water and seawater. Water extraction was performed in anion exchange solid phase extraction cartridges, and extracts were analysed by liquid chromatography in tandem with mass spectrometry. Recoveries for target analytes were between 35 and 120%, depending on the water matrix. Method detection limits were in the range of 0.5–17 ng L⁻¹. The validated method was applied to the determination of perfluorinated compounds in water samples around Ireland. Eight compounds out of fifteen were detected at least in one sample. Measured concentrations were higher in river water than seawater, and drinking water had the lowest levels, although still detectable for a considerable amount of compounds. The most prevalent compounds were PFPeA, PFOA and PFHxA, present in all types of water, and they had the highest concentrations.

Table 3 Mean percent recoveries ($n = 3$) of 15 PFASs in the three water matrices, and method detection and quantification limits (MDL, MQL) in each water matrix (ng L⁻¹)

	Recoveries ($\pm$ RSD)			MDL (ng L ⁻¹)			MLQ (ng L ⁻¹)		
	TW	SW	RW	TW	SW	RW	TW	SW	RW
PFPeA	120 (13)	98 (12)	101 (10)	1.40	1.96	1.68	4.67	6.54	5.61
PFBS	103 (4)	96 (9)	99 (12)	0.48	0.67	0.58	1.60	2.24	1.92
PFHxA	61 (12)	57 (9)	59 (3)	1.78	2.49	2.13	5.92	8.29	7.11
PFPeS	104 (7)	105 (12)	102 (4)	0.75	1.05	0.90	2.49	3.48	2.99
GenX	109 (5)	103 (2)	105 (2)	1.45	2.03	1.74	4.82	6.75	5.79
PFHxS	71 (10)	60 (13)	67 (3)	0.46	0.65	0.55	1.54	2.16	1.85
PFOA	59 (7)	51 (5)	55 (7)	1.27	1.78	1.53	4.24	5.93	5.09
PFOS	55 (13)	59 (9)	55 (12)	0.91	1.28	1.10	3.04	4.26	3.65
PFNA	65 (2)	67 (12)	63 (14)	0.81	1.14	0.98	2.71	3.80	3.26
PFNS	62 (9)	58 (3)	61 (12)	0.66	0.93	0.80	2.21	3.09	2.65
PFDA	57 (12)	55 (9)	59 (10)	0.48	0.67	0.58	1.61	2.25	1.93
PFDS	39 (5)	35 (3)	41 (3)	2.43	3.40	2.91	8.09	11.32	9.70
FOSA	35 (7)	39 (4)	41 (6)	12.62	17.67	15.15	42.07	58.90	50.48
PFUdA	48 (4)	45 (7)	51 (6)	0.91	1.27	1.09	3.02	4.23	3.62
PFDoA	37 (6)	35 (8)	39 (7)	4.11	5.75	4.93	13.69	19.17	16.43