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Article

New LC-MS/MS method for the determination of unconventional organic pollutants: perfluoroalkyl sulfonic acids in wastewater, surface water, and drinking water

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Abstract

The increasing prevalence of perfluoroalkyl sulfonic acids (PFSAs) in aquatic environments has raised significant concerns regarding their environmental and health implications, particularly due to their endocrine-disrupting properties. This study aims to develop and validate a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection and quantification of nine PFSAs in drinking water, surface water, and wastewater. The method comprises comprehensive steps, including the optimization of chromatographic separation conditions, enhancement of detection limits, and selection of effective extraction techniques tailored for diverse water matrices. The results reveal that the developed method achieves quantification limits ranging from 0.11 to 0.50 ng/L for drinking water, 0.22 to 0.59 ng/L for surface water, and 0.50 to 1.43 ng/L for wastewater, with extraction recoveries consistently exceeding 75%. The validated LC-MS/MS method demonstrates high sensitivity and robustness, enabling the monitoring of PFSAs in various water environments. This advancement not only contributes to the ongoing monitoring efforts of chemical contaminants at both national and international levels but also supports public health initiatives aimed at mitigating the risks associated with these hazardous substances. The implementation of such methods is crucial for ensuring water quality and protecting ecosystems from the adverse effects of PFSAs, ultimately fostering a safer environment for human health and biodiversity.

Keywords: *PFSAs, LC-MS/MS, environmental monitoring, water quality, organic pollutants*

INTRODUCTION

Emerging contaminants, particularly per- and poly-fluoroalkyl substances (PFAS), have become a global concern due to their potential environmental harm and threat to human health. PFAS are a broad class of fluorinated compounds known for their prolonged persistence, water-repellent, oil-repellent, and heat-resistant properties. Their strong C-F bonds in their molecular composition have earned them the nickname "forever chemicals" [1]. These substances find extensive application in various household items, including non-stick surfaces on cookware [2] and food packaging materials [3, 4]. They are also prevalent in industrial uses, such as aqueous film-forming foams for firefighting [5, 6], metal plating [1], detergents, inks, semiconductors, lubricants [7], coating additives [8], surfactants, agricultural products [9], textiles, and clothing [8÷10]. However, their recalcitrant properties lead to resistance to degradation [1, 11], resulting in persistence [12] and accumulation [13÷15] in the environment. Over the last decade, reports of PFAS contamination have increased globally, exacerbated by rising industrial and urban development, and significant growth in the manufacturing sector, which drives demand for these chemicals, posing further risks to environmental and human health [16, 17].

PFAS compounds are widely detected in air [18, 19], soil [20], water [21÷23], plants [24], invertebrates [25], and vertebrates [26, 27], due to their extensive use, bioaccumulation [28], and environmental persistence. Atmospheric transport can contaminate both nearby and distant ecosystems, including remote Arctic and Antarctic regions [29, 30]. Their environmental behavior is influenced by chemical properties, structural variations (linear vs. branched, short-chain vs. long-chain), emission levels, local topography, and weather conditions. Ionic PFAS from industrial sources often associate with particles and settle through deposition processes [31], while linear PFAS are more likely to adhere to soil, with branched isomers favoring aqueous retention [32]. Plants bioaccumulate these substances, transferring them through food chains and disrupting ecological functions [24]. PFAS have been found in human blood [33], urine [34, 35], and hair [36], with exposure occurring via drinking water [37], food [38], air [18, 19], and dust [39]. The severity of exposure varies with PFAS type, exposure pathways, intensity, and duration.

Exposure to PFAS has been linked to various negative health consequences in both animals and humans [40]. However, studies on the persistence and accumulation of PFAS throughout the food chain, including new and emerging compounds, are inconsistent and limited. For instance, the branched form of perfluorooctane sulfonic acid (PFOS) shows a high accumulation trend in humans, whereas the linear forms of perfluorooctanoic acid (PFOA) and PFOS accumulate significantly in animals due to their stronger binding to serum albumin [32]. Predicting health impacts on specific organisms is challenging, but evidence suggests harmful implications. Increased exposure has been associated with severe health issues, including cardiovascular [41], hormonal (effects on thyroid hormone levels), reproductive [42], metabolic disorders [43], cancer [44], and birth-related complications, such as low birth weight and BMI [45]. Additionally, exposure to PFAS has been suggested to interfere with neurodevelopment in children, potentially leading to behavioural problems [46].

The presence of PFAS in the environment raises global concerns due to adverse health effects. This has led to the establishment of environmental guidelines for the gradual phase-out of these chemicals at both national and international levels. The Stockholm Convention included PFOS and PFOA on the list of persistent organic pollutants (POPs) in 2009 and 2020, respectively, banning the use of fire-fighting foam containing PFOA [47, 48]. Directive (EU) 2020/2184 sets forth quality standards for water intended for human consumption, with specific regulations concerning PFAS. It mandates the monitoring of PFAS in drinking water, setting maximum allowable concentrations to protect public health. Additionally, the directive requires member states to take necessary measures to reduce the presence of PFAS and ensure the safety of drinking water supplies across the EU [49]. In March 2023, the EPA proposed a national drinking water regulation to set maximum contaminant levels for six PFAS, including PFOA and PFOS [50]. The EU Water Framework Directive imposes limits for PFAS, with a deadline set for January 2024 for new analysis methods [51]. Various countries, including Norway, Germany, Sweden, and the Netherlands, have implemented restrictions on PFAS use and contamination limits in water and soil [52]. In Australia, health guidelines for limiting PFOA and PFOS have been issued [53]. These measures reflect a growing concern for public health and the environment regarding PFAS.

This study aims to develop and validate an LC-MS/MS approach for the detection of non-conventional organic pollutants, particularly PFSA, in wastewater, surface water, and drinking water. To achieve this goal, several activities were undertaken, including optimizing operational parameters for chromatographic separation of the analytes at baseline and ensuring effective mass spectrometric detection. The study focused on refining the parameters of the mass spectrometer and the electrospray ionization (ESI) source to enhance both selectivity and sensitivity. Additionally, the liquid chromatography parameters were optimized for efficient chromatographic separation and elution within a short time frame, maximizing method sensitivity with narrow peaks and high resolution. The solid-phase extraction (SPE) parameters were also optimized to isolate and concentrate target analytes from water samples. Finally, the developed methods were validated to confirm their capability to reliably identify and quantify the compounds of interest in wastewater, surface water, and drinking water matrices at the required sensitivity levels.

This study holds significant importance both nationally and internationally due to the rising awareness

of PFSA as environmental contaminants that pose serious health risks. By developing a reliable method for detecting these pollutants in water sources, the study contributes to enhanced public health safety and environmental protection. It aligns with global efforts to regulate harmful substances and mitigate their impact on ecosystems and human health. Additionally, the validated methods can serve as benchmarks for regulatory agencies and facilitate compliance with evolving environmental standards. This research can also foster international collaboration in monitoring and managing water quality, ultimately paving the way for more comprehensive strategies in addressing water pollution. The findings will support informed decision-making for policymakers and contribute to the ongoing discourse on sustainable water management practices.

MATERIALS AND METHODS

Chemicals and reagents

The standards used were of analytical purity: perfluorobutanoic acid (PFBS), perfluoropentan sulfonic acid (PFPeS), perfluoroheptane sulfonic acid (PFHpS), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorodecane sulfonic acid (PFDS), perfluorononane sulfonic acid (PFNS), perfluorodecan sulfonic acid (PFDS), perfluorooctane sulfonamide (PFOSA), and the internal standard perfluorooctanoic acid 13C8 (PFOA-13C8), all obtained from Sigma-Aldrich (Germany). The solvents used for preparing the mobile phase and for extraction from water samples included methanol (Merck, Darmstadt, Germany), acetic acid, formic acid, and ammonium acetate (Sigma-Aldrich, Germany).

LC-MS/MS equipment

The analysis of PFSA was performed employing an Agilent 1260 series liquid chromatography system linked to an Agilent 6410B triple-quadrupole mass spectrometer (LC-MS/MS). The electrospray ionization source (ESI) was utilized in negative ion mode with a temperature setting of 300°C. This LC-MS/MS configuration incorporated a Zorbax Eclipse C18 chromatographic column measuring 2.1 x 100 mm with a particle size of 3.5 μm [54].

RESULTS AND DISCUSSION

LC Separation Conditions and MS Detection

The optimal parameters for liquid chromatographic (LC) separation of the nine analytes studied were established as follows: a Zorbax Eclipse C18 column (2.1 x 100 mm, 3.5 μm) was operated at a temperature of 30°C. A 10 μl sample volume was injected into the system, utilizing a mobile phase composed of 5 mM ammonium acetate in water (component A) and methanol (component B). The mobile phase was set to flow at a rate of 0.2 ml/min, with water serving as the sample solvent. A gradient elution method was implemented, resulting in a total chromatographic run time of 25 minutes.

These separation conditions emphasize the careful selection of both the chromatographic column and the mobile phase to achieve efficient separation of the target analytes. The use of a gradient elution allows for a tailored approach to enhance the resolution of the compounds, while a controlled temperature and flow rate contribute to the reproducibility and reliability of the results. The combination of these factors is critical for optimizing the detection sensitivity and accuracy in the analysis of complex mixtures.

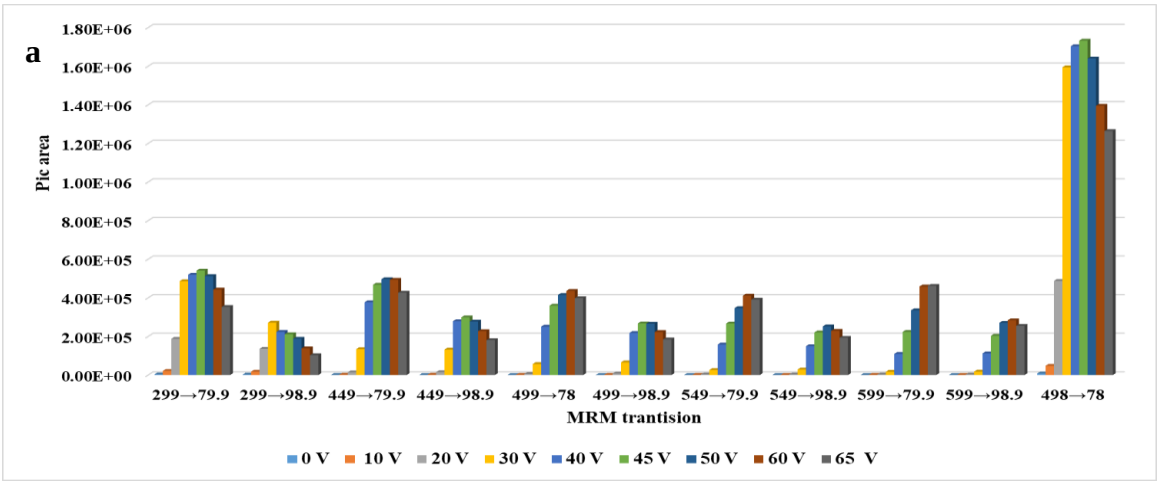
The gradient program outlined in Table 1 was designed to optimize the elution and separation of PFSA compounds. The program consists of several time points, each specifying the composition of two solvents, A and B, along with the flow rate.

Table 1. The gradient program for PFSA elution

Time (min)	A (%)	B (%)	Debit (mL/min)
0	60	40	0.2
1.5	60	40	0.2
2.5	40	60	0.2
12.5	80	20	0.2
17.0	80	20	0.2
17.01	60	40	0.2

At the start (0 minutes), the mobile phase is composed of 60% solvent A and 40% solvent B. Maintaining this composition for the first 1.5 minutes allows the initial analytes to equilibrate within the column, ensuring a stable and reproducible start to the analysis. At 2.5 minutes, the composition shifts to 40% A and 60% B. This modification increases the polarity of the mobile phase, promoting the separation of more polar analytes in the sample. As the gradient progresses, the mobile phase transitions to 80% B and 20% A at 12.5 minutes, which further enhances the elution of less polar components and allows for a more effective separation of the PFSA analytes. After reaching the peak concentration of 80% B, the gradient is held constant until 17.0 minutes. This plateau is crucial as it provides time for any remaining analytes to elute fully from the column, thereby improving peak shapes and resolution. Finally, at 17.01 minutes, the flow returns to the initial gradient of 60% A and 40% B. This rapid adjustment is designed to condition the column for the next injection, ensuring that the system stabilizes before subsequent analyses. This gradient profile is carefully crafted to balance the separation efficiency and the speed of analysis, enhancing the ability to detect and quantify different PFSA compounds effectively. The methodology demonstrates a clear understanding of the interactions between analytes and the stationary phase, which is essential for achieving high-resolution chromatographic separations.

In order to achieve low detection and quantification limits that allow for the determination of extremely low levels (below ng/L) of perfluorinated organic compounds, all detection parameters associated with the triple quadrupole mass spectrometer (QQQ) were optimized. The optimized parameters for the detection of the nine compounds included the fragmentor voltage, collision energy (CE), voltage in the collision cell, resolution on quadrupoles (MS1, MS2 Res), and dwell time per MRM transition. For the optimization of these mass spectrometric detection parameters, a mixed analytical solution with a concentration of 10 mg/L was utilized. Changes in the mass-spectrometric detection parameters were analyzed to assess their impact on peak area and the signal-to-noise ratio (S/N). Following these evaluations, the mass-spectrometric settings that provided the highest sensitivity (as indicated by peak area and S/N) for the compounds under investigation were determined. For every analyte, two signal transitions related to the precursor ion and its two most plentiful product ions were tracked. The ion that showed the greatest abundance was utilized for quantification, while the second most abundant ion served as a confirmatory measure. The fragmentor voltage was varied between 70 and 140 V, with the values chosen that generated the best peak area (Fig. 1a). For the target compounds, a value of 135 V was selected, while the value for the isotopically labeled internal standard was set at 80 V. The voltage applied in the collision cell was tested in the range of 0 to 65 V for the target compounds, and between 0 and 6 V for the internal standard. The results obtained are shown in Fig. 1b. The voltage applied in the acceleration cell was tested in the range of 3 to 6 V. Maximum intensities of the chromatographic peaks were obtained for a voltage of 6 V for the majority of compounds, with the exception of PFOSA and the isotopically labeled standard, which had maximum values at a voltage of 5 V (Fig. 1c).



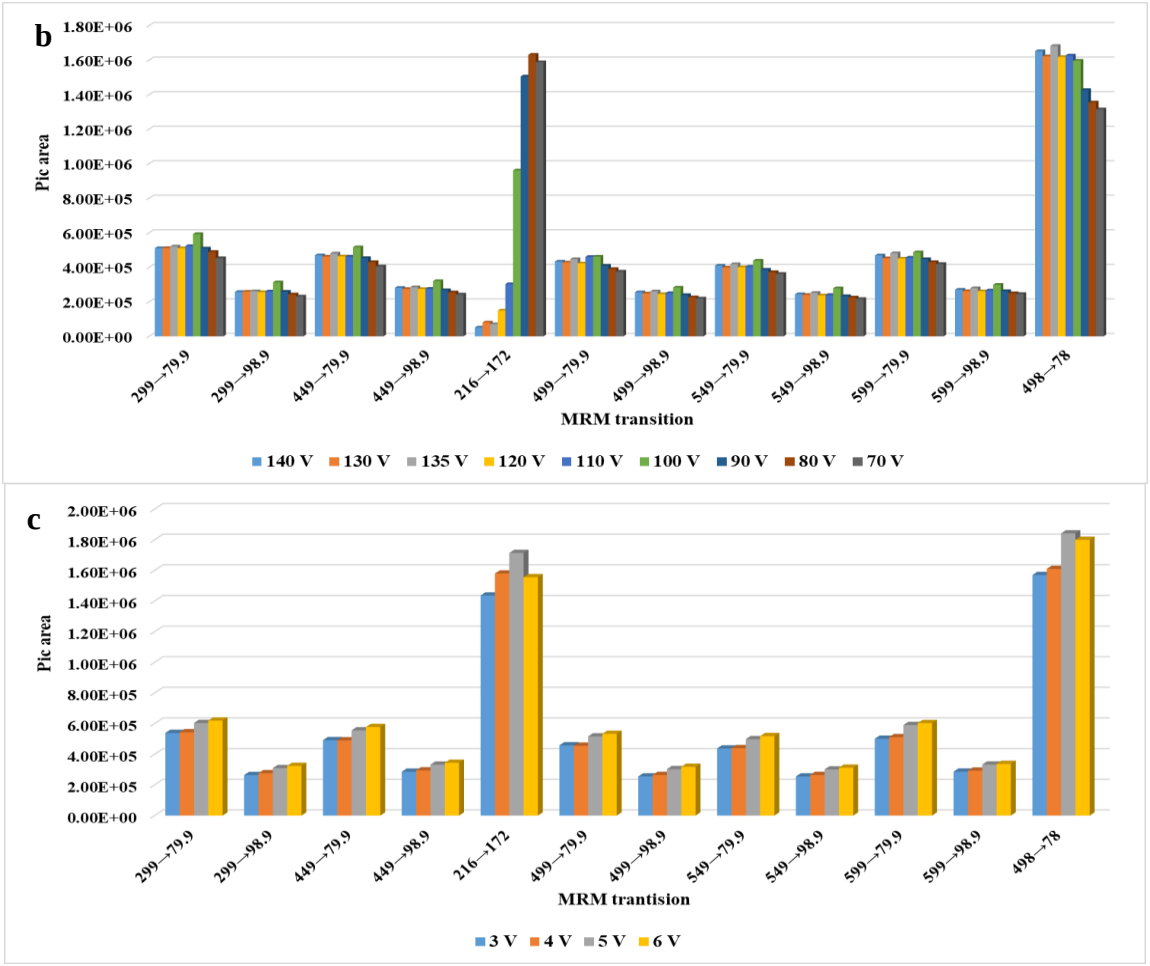


Fig. 1. Optimization of mass spectrometric detection parameters for perfluorinated compounds: (a) Effect of fragmentor voltage on peak area for target compounds; (b) voltage optimization in the collision cell; (c) maximum intensities of chromatographic peaks in relation to the voltages applied in the acceleration cell

This methodical optimization of mass spectrometric parameters is crucial in achieving high sensitivity for quantifying perfluorinated compounds at very low concentrations. The choice of a mixed analytical solution for the optimization process allows for the assessment of how each parameter influences the detection performance comprehensively. Monitoring both peak area and S/N ratio provides a dual approach to evaluating sensitivity, which is particularly important for compounds present at trace levels. By selecting the optimal fragmentor voltage, a balance is achieved between fragmentation of the ions and preservation of the necessary signal for quantification. The significant variation in voltages applied in the collision cell and acceleration cell underscores the necessity of fine-tuning these settings to maximize ionization efficiency and minimize background noise. The strategy of using two product ions for each analyte—one for quantification and another for confirmation—enhances the robustness of the analysis. This confirms that the analytes are being accurately detected while reducing the chances of false positives.

Following the optimization procedure for mass spectrometric detection and the electrospray ionization source, the parameters that generated the maximum sensitivity for both analyzed compounds were selected (table 2).

The optimized parameters for the ionization source that generated the highest signal areas are noteworthy for their impact on mass spectrometric detection. Specifically, the negative electrospray ionization mode (ESI-) was selected as it provides enhanced sensitivity for the compounds of interest. overall.

Tabel 2. Selected optimized parameters for maximum sensitivity in mass spectrometric detection of analysed compounds

Target compounds	RT (min)	MRM	Fragmentor Voltage (V)	Collision energy (V)	Cell Accelerated Voltage (V)	Dwell time (msec)
PFBA-^{13}C3	2.37	421→376 (Q)	110	2	5	35
PFBS	6.00	299→80 (Q)	135	45	4	35
		299→99 (q)	135	30	4	35
PFHpS	11.8	449→80 (Q)	135	50	4	35
		449→99 (q)	135	45	6	35
PFOS	13.2	499→80 (Q)	135	60	6	35
		499→99 (q)	135	45	6	35
PFNS	14.6	549→80 (Q)	135	60	6	35
		549→99 (q)	135	50	6	35
PFDS	146.0	599→80 (Q)	135	65	6	35
		599→99 (q)	135	50	6	35
PFOSA	17.2	498→78 (Q)	135	45	5	35

The drying gas temperature was set at 300°C, which is typically advantageous for ensuring efficient solvent evaporation and ion formation. Additionally, a drying gas flow rate of 8 L/min was chosen, facilitating the removal of solvent molecules from the ionized analytes and improving the signal intensity. The nebulizer pressure was maintained at 50 psi, which is crucial for efficiently generating aerosol droplets from the liquid sample, allowing for optimal ionization. Lastly, a capillary voltage of 2500V was applied, as this high voltage is essential for driving ions into the mass spectrometer, enhancing the ionization efficiency and ultimately contributing to the maximization of the signal detected. Collectively, these parameters illustrate a carefully optimized approach to achieving high sensitivity in mass spectrometric analyses.

Extraction and concentration of PFSA compounds

The extraction process begins with the preparation of necessary equipment and materials. The primary equipment utilized is an automated SPE system, specifically the SPE AutoTrace 280 from Thermo Scientific Dionex. For the extraction, two types of cartridges are employed: Strata-X, which serves general purposes, and Strata-X-WA, which is specially designed for weakly acidic compounds. The solvents required for the procedure include methanol, used in a volume of 10 mL for both conditioning and elution, and ultrapure water, totaling 20 mL (10 mL for conditioning and 10 mL for washing). The sample preparation involves 200 mL of ultra-pure water that has been spiked with 1 mL of a mixed PFSA solution at a concentration of 50 µg/L, along with 100 µg/L of SI. Before commencing the extraction, it is essential to condition the cartridges, which optimizes analyte recovery. The conditioning process involves injecting 10 mL of methanol into each cartridge to activate the sorbent phase, followed by 10 mL of ultrapure water to equilibrate the cartridge. Subsequently, a 200 mL surface water sample, now spiked with the defined concentrations of PFSA and SI, is prepared for loading. The spiked water sample is subsequently processed through the cartridge at a steady flow rate of 5 mL/min. Once the entire sample has exited the cartridge, the next procedure involves rinsing it with 10 mL of ultrapure water at an increased flow rate of 20 mL/min. This washing step is critical for removing interferences, ensuring the target analytes remain retained on the cartridge. Following the washing procedure, the analytes are eluted. First, the adsorptive phase is dried under a nitrogen stream for 20 minutes to eliminate residual water. Analytes are then eluted by passing 2×10 mL of methanol through the cartridge at a reduced flow rate of 5 mL/min, effectively releasing the analytes from the sorbent into a collection vessel. The next stage involves sample reconstitution, where the extracts are evaporated under a gentle nitrogen stream until a concentrated volume is achieved. The dried analytes are then reconstituted in 1.0 mL of ultrapure water, preparing them for subsequent analysis. For the final analysis, the reconstituted sample is transferred into an

autosampler vial and subjected to analysis using ESI(-)-LC-MS/MS for the quantification and identification of the target analytes.
The recovery efficiencies of the process were assessed, showing that Strata-X-WA cartridges achieved greater than 90% recovery for all tested analytes (refer to the detailed data in table 3). These cartridges are particularly effective for weakly acidic organic compounds (pKa ≤ 5), enhancing the analytical outcomes in environmental monitoring applications.

Tabel 3. Recovery efficiency of target analytes using Strata-X-WA cartridges

Compounds	Strata X-WA	Strata-X
PFBS	93	82
PFHpS	96	66
PFOS	98	79
PFNS	103	83
PFDS	95	87
PFOSA	97	88

The data presented in table 3 indicate the recovery efficiencies for various PFSA (per- and polyfluoroalkyl substances) analytes obtained using two different types of cartridges: Strata-X-WA and Strata-X. The results demonstrate that the Strata-X-WA cartridges consistently achieve higher recovery rates across all analytes tested, with results exceeding 90% for every compound listed. Notably, the highest recovery was observed for PFNS, yielding over 100%. In contrast, the Strata-X cartridges exhibited lower recovery percentages, particularly for PFHpS and PFOS, which suggest that these cartridges may not be as well-suited for the extraction of weakly acidic compounds as the Strata-X-WA cartridges. The significant difference in recovery rates can be attributed to the specific selectivity provided by the polymeric sorbent in Strata-X-WA, which is designed to effectively interact with the targeted analytes due to its unique chemical composition. This underscores the importance of selecting appropriate extraction materials for optimizing analytical workflows and achieving accurate quantification of environmental contaminants.

LC-MS/MS method validation
In this section, we detail the validation of the LC-MS/MS method employed for the quantification and identification of PFSA in environmental samples. Method validation is a crucial step in analytical chemistry, ensuring that the developed method is reliable, reproducible, and suitable for its intended purpose. We will outline the specific parameters assessed during the validation process, including selectivity, sensitivity, linearity, accuracy, precision, and limits of detection and quantification. Through rigorous testing and evaluation, we aim to establish the method's robustness for detecting PFSA in complex matrices, ultimately supporting its application in environmental monitoring and regulatory compliance.
The linear regressions obtained for the nine analytes showed that the detector response exhibited linearity over the investigated concentration range (0.01÷100 μg/L). This linear relationship is crucial for quantitative analysis, as it indicates that the response of the detector is directly proportional to the concentration of each analyte within this range. The correlation coefficients obtained for all nine analytes were consistently greater than 0.999, demonstrating an excellent fit to the linear model. Such high correlation coefficients underscore the reliability and accuracy of the method in quantifying the analytes of interest, instilling confidence in its applicability for environmental monitoring and regulatory assessments. This level of linearity is essential for ensuring that the method can produce precise and reproducible results across the desired concentration spectrum.
The precision of the developed method was assessed. The repeatability of the instrument was evaluated by performing six repeated injections of the same sample, which underwent the sample preparation procedure (solid-phase extraction) only once. In contrast, the repeatability of the analysis was determined by preparing and analyzing six sub-samples derived from the same original sample. Intermediate precision was assessed by implementing the sample preparation method on a single

sample, which was then subdivided into six smaller portions. These portions were processed on different days by various analysts, introducing an important degree of variability relevant to practical applications. The results of the precision assessment are summarized in table 4. The instrument's repeatability was characterized by relative standard deviation (RSD%) values ranging from 0.41% to 1.23% for the concentration of 1 µg/L, and from 0.21% to 1.85% for the concentration of 10 µg/L (as presented in table 4). While the RSD% values for both the repeatability (RSD_r) of the analysis and the intermediate precision (RSD_R) were higher, they remained within the acceptable limit of 15% deemed appropriate for LC-MS methods.

Table 4. Summary of precision assessment results (%) for the developed LC-MS method

PFSA	LC-MS/MS		Wastewater				Surface water				Drinking water			
	1µg/L	10µg/L	1 µg/L		10 µg/L		1 µg/L		10 µg/L		1 µg/L		10 µg/L	
	RSD_r	RSD_r	RSD_r	RSD_R	RSD_r	RSD_R	RSD_r	RSD_R	RSD_r	RSD_R	RSD_r	RSD_R	RSD_r	RSD_R
PFBS	1.22	0.85	4.72	9.23	3.84	8.87	4.66	8.95	3.51	8.44	4.23	8.71	3.18	8.02
PFHpS	0.67	0.52	6.55	10.3	5.54	8.92	6.23	9.75	5.22	8.42	5.95	9.51	4.87	8.26
PFOS	1.12	0.83	5.87	9.74	4.93	8.53	5.62	9.38	4.77	8.16	5.45	9.07	4.41	7.76
PFNS	0.41	0.35	4.78	10.5	3.66	9.79	4.53	10.1	3.25	9.41	4.14	9.73	3.95	8.89
PFDS	0.49	0.21	5.74	9.76	4.92	8.55	5.53	9.28	4.49	8.25	5.06	9.02	3.99	8.11
PFOSA	1.23	0.78	5.89	10.3	4.79	9.94	5.61	9.81	4.35	9.53	5.17	9.49	3.86	9.18

To evaluate the selectivity of the method developed for the determination of perfluorinated organic compounds in surface and wastewater, a sample of ultrapure water—devoid of target analytes—was subjected to the sample preparation procedure. The sample underwent solid-phase extraction (SPE) three times to assess potential interferences. For the method to be classified as selective, it is essential that no interferences appear at the retention times of the analytes, or that any potential interferences present a signal-to-noise ratio lower than 3, indicating that they are below the detection limit.

Upon repeating the extraction procedures, no interferences were detected at the retention times corresponding to the analytes of interest, nor were any signal patterns found for the ultrapure water samples above the detection limit. This finding is illustrated in Figure 2.

In conclusion, the developed method meets the criteria for selectivity and is therefore deemed specific for the targeted perfluorinated compounds. Moving forward, we propose a systematic presentation of selectivity data by including a comparative analysis of signal-to-noise ratios across different samples, alongside a graphical representation that highlights retention times, peak areas, and any potential interferences detected during the SPE process. This will provide clearer insights into the method's specificity and enhance the visual communication of the results obtained in relation to selectivity assessments.

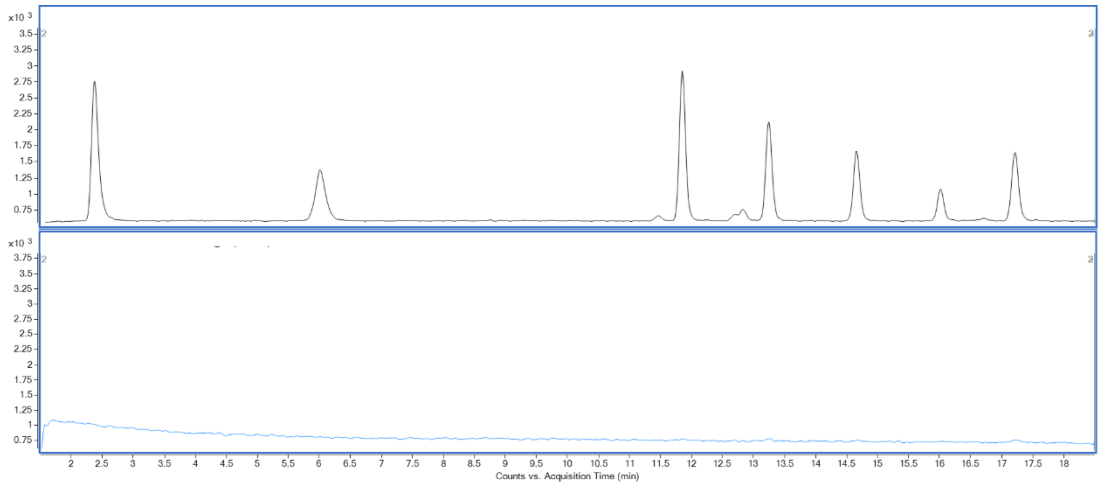


Fig. 3. Evaluation of method selectivity for PFSA compounds: PFSA mix solution 50 µg/L vs. ultra purified water

The recovery efficiency for the nine analytes was determined by applying the extraction procedure to three samples each of wastewater, surface water, and drinking water, all of which were deliberately spiked with a known concentration of the analytes (50 ng/L) and an internal standard. The calculation of recovery efficiency was based on the peak area obtained after extraction, adjusted by subtracting the peak area corresponding to the analytes from the uncontaminated samples. This adjusted peak area was then related to the calibration curve and the peak area of the spiked solution that had not undergone liquid-solid extraction.

Table 5 presents absolute recovery percentages obtained for the analytes in wastewater, surface water, and drinking water samples through the addition of the standard.

Table 5. Absolute recovery rates (%) for analytes in wastewater, surface water, and drinking water samples (spiked at 50 ng/L, n=3)

PFSA compounds	Wastewater	Surface water	Drinking water
PFBS	79 ± 5.37	86 ± 4.82	93 ± 4.46
PFHpS	78 ± 5.30	83 ± 4.65	91 ± 4.37
PFOS	88 ± 5.98	95 ± 5.32	103 ± 4.94
PFNS	84 ± 5.71	94 ± 5.26	101 ± 4.85
PFDS	76 ± 5.17	84 ± 4.70	91 ± 4.37
PFOSA	79 ± 5.37	88 ± 4.93	95 ± 4.56

The recovery values varied across the different matrices, with wastewater samples demonstrating a range of 79% to 88%, surface water samples showing 83% to 95%, and drinking water samples exhibiting a higher range of 91% to 103%. The findings indicate that absolute recovery rates provide a reliable basis for quantifying the target analytes in the respective water samples. Given these recovery ranges, it is possible to apply a correction factor inversely proportional to the observed recovery efficiency, allowing for accurate quantification of the analytes in the contaminated samples. This systematic approach to presenting recovery efficiency data will enhance the transparency and reproducibility of the analytical method, supporting its application across various water matrices.

The instrumental limits of detection (LOD) and quantification (LOQ) for the analytes were determined by injecting solutions with decreasing concentrations until the signal-to-noise ratio reached predetermined thresholds of 3 for LOD and 10 for LOQ. To calculate the overall limits of detection and quantification for the method, which includes all sample preparation procedures, concentration factors of 200 for wastewater and 100 for surface water were applied during the extraction process. Additionally, the absolute recovery rates obtained during method validation were factored into the calculations.

Table 6 provides a comprehensive overview of the LOD and LOQ values corresponding to the nine analyzed compounds across different water matrices, including drinking water, surface water, and wastewater.

Table 6. Instrumental limits of detection (LOD) and quantification (LOQ) for nine analytes in different water matrices

PFSA compounds	LOQ (µg/L)	Wastewater		Surface water		Drinking water	
		LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)
PFBS	0.04	0.18	0.50	0.21	0.59	0.35	0.98
PFHpS	0.03	0.12	0.34	0.14	0.39	0.39	1.09
PFOS	0.04	0.09	0.25	0.15	0.42	0.42	1.18
PFNS	0.05	0.04	0.11	0.06	0.17	0.18	0.50
PFDS	0.03	0.05	0.14	0.08	0.22	0.23	0.64
PFOSA	0.04	0.17	0.48	0.20	0.56	0.51	1.43

The reported LODs and LOQs are expressed in ng/L, reflecting the sensitivity of the method. The results indicate that the method achieved LOD values ranging from 0.04 ng/L to 0.59 ng/L and LOQ values spanning from 0.12 ng/L to 1.43 ng/L, depending on the matrix and specific analyte. Such sensitivity allows for precise monitoring of perfluorinated compounds in various aqueous environments, thereby facilitating the assessment of these contaminants in food webs and human health exposure scenarios. This systematic presentation of sensitivity metrics underscores the analytical method's efficacy and reliability for environmental monitoring purposes.

The LC-MS/MS method developed and optimized in this study was applied to real samples of drinking water, surface water, and wastewater, showcasing its efficacy in detecting PFSA compounds. A total of three samples from each matrix were analyzed in triplicate. The drinking water samples were collected from three residences in Bucharest, while the surface water samples were sourced from the Dambovita River (SW1), Vacaresti Lake (SW2), and Morii Lake (SW3). The wastewater samples were obtained from laboratory services (WW1÷WW3). All samples were collected in polypropylene containers and maintained at 4°C during transport, with refrigeration ensuring stability for a maximum of 72 hours prior to analysis.

The samples underwent solid-phase extraction in accordance with the previously described protocol, followed by LC-MS/MS analysis. The results are summarized in table 7, which presents the concentrations of various PFSA compounds detected in the water samples.

Table 7. Concentrations of PFSA compounds in real water samples from diverse matrices

Sample	PFBS	PFHpS	PFOS	PFNS	PFDS	PFOSA
DW1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
DW2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
DW3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
SW1	0.63±0.022	0.47±0.024	3.53±1.77	0.21±0.011	0.34±0.017	0.72±0.036
SW2	0.71±0.025	0.42±0.021	2.44±0.122	0.51±0.026	0.74±0.037	0.93±0.047
SW3	0.68±0.024	0.68±0.034	3.15±0.158	0.71±0.036	0.96±0.048	0.83±0.042
WW1	2.43±0.122	3.51±0.176	13.3±0.665	0.72±0.036	1.98±0.099	4.22±0.211
WW2	2.18±0.109	4.25±0.213	17.4±0.870	2.12±0.106	3.76±0.188	8.38±0.419
WW3	3.23±0.162	5.28±0.264	15.9±0.795	3.72±0.186	4.26±0.213	5.49±0.275

For drinking water samples, all analytes were found to be below the LOQ, indicating negligible contamination. In contrast, surface water samples showed detectable concentrations of several analytes, with PFOS reaching values up to 3.53±1.77 ng/L, revealing a concerning level of contamination in these environments.

Furthermore, the wastewater samples demonstrated significantly higher concentrations of FFSA compounds, with PFOS concentration peaking at 17.4±0.870 ng/L in WW2, highlighting the potential environmental impact of effluents on water quality. These findings emphasize the importance of ongoing monitoring of PFSA compounds in various water matrices, utilizing the validated LC-MS/MS method as a reliable tool for assessing the presence of these persistent pollutants in the aquatic environment. The data collected not only contribute to the understanding of PFSA contamination levels in these water sources but also underscore the necessity for effective regulatory measures to mitigate such pollution.

CONCLUSIONS

The aim of this study was to develop a sensitive LC-MS/MS method for the determination of nine PFSA compounds in drinking water, surface water, and wastewater. These compounds are widely recognized for their detrimental effects on biological systems, particularly due to their capacity to disrupt endocrine function. Understanding their presence in aquatic environments and potential bioaccumulation in the food chain is crucial, as it can ultimately impact human health. Given the ease with which PFSA compounds infiltrate aquatic ecosystems, the need for reliable identification and quantification from environmental samples cannot be overstated. In response to this challenge, a

robust and precise LC-MS/MS methodology has been developed and validated for the detection of these endocrine-disrupting compounds in water systems. This method facilitates a comprehensive understanding of PFSA contamination levels, thereby helping to inform regulatory efforts across national and international contexts. To achieve this, multiple steps were undertaken, including the establishment of separation and quantification conditions through liquid chromatography coupled with mass spectrometry, optimization of operational parameters to ensure low detection and quantification limits, and the selection of optimal extraction techniques for various water sample types. Additionally, thorough experimental validation and statistical assessment of the method's performance were conducted to establish its reliability. The optimized LC-MS/MS method demonstrates exceptional sensitivity in detecting target organic pollutants at ultra-trace levels, with quantification limits ranging from 0.11 to 0.50 ng/L in drinking water, 0.22 to 0.59 ng/L in surface water, and 0.50 to 1.43 ng/L in wastewater. Extraction recoveries exceeded 75% for all sample types, ensuring high reliability of the analytical results. The successful development of this sensitive method for PFSA compounds not only contributes to environmental monitoring and public health safety at the national level but also aligns with global efforts to assess and mitigate the impacts of chemical pollutants. By enabling timely detection of these hazardous substances, this methodology is poised to support policymakers and regulatory authorities in implementing effective strategies aimed at safeguarding water quality and promoting ecological health on both national and international fronts.

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