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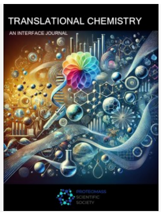

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Determination of four regulated PFASs in drinking water by UHPLC-MS/MS with direct injection: development and validation of a rapid, green analytical procedure

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ABSTRACT

Per- and poly-fluoroalkyl substances (PFAS) are currently being widely used in various industrial applications; however, they represent an environmental and human health concern due to their persistence and bioaccumulation properties. This situation has stimulated the drafting of a European Directive (2020/2184), which regulates PFAS in drinking water. The regulation includes four PFAS of particular concern, whose concentrations must not exceed 20 ng/L as a sum: perfluorooctanoic acid (PFOA), perfluorooctanesulphonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexanesulphonic acid (PFHxS). Aim of this study was to develop and validate an original rapid and sensitive method, without any sample preparation and pre-concentration step, for monitoring the four target analytes in drinking water matrices by UHPLC-MS/MS. Water samples were just diluted 1:1 (*v/v*) with methanol, filtered and directly injected into the analytical system. The method was validated in real drinking water over 0.5-600 ng/L concentration range. Results showed good linearity for all the target analytes ($R^2 > 0.9950$). Accuracy, expressed as recovery and evaluated at three concentration levels, ranged within 96.9-114 % with RSD < 12.9 %. Despite the direct injection, good method detection limits (MDL) were achieved: 0.19 ng/L < MDL < 0.28 ng/L and lower limit of quantification was 0.5 ng/L for all analytes. The validated method was applied to 12 real drinking water samples from North – East Italy. PFOA, PFOS and PFHxS were detected in some samples, while PFNA was never detected. The sum of the four PFAS' concentrations was below the regulatory threshold of 20 ng/L in all samples.

Keywords: direct injection, drinking water, green analytical chemistry, PFAS, UHPLC-MS/MS.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) constitute a large family of synthetic fluorinated compounds that have been widely employed since the 1950s in industrial processes and consumer products due to their exceptional thermal stability, chemical resistance, and surface-active properties. The extremely stable perfluorinated carbon chain confers to these molecules unprecedented persistence in environmental matrices and in biological systems, leading to the common designation 'forever chemicals' [1]. The environmental persistence of PFAS is accompanied by documented toxicological properties. Several studies have associated chronic exposure to PFAS, particularly long-chained ones, with a range of adverse health outcomes, including hepatotoxicity, thyroid hormone disruption, reproductive toxicity, and increased risk of cancer occurrence [2]. The International Agency for Research on Cancer (IARC) classified perfluorooctanoic acid (PFOA) as carcinogenic to humans (Group 1). Perfluorooctanesulphonic acid (PFOS) has similarly been recognised as a persistent organic pollutant (POP) of global concern and a probable carcinogen for humans (IARC's Group 2) [3]. Both PFOA and PFOS exhibit pronounced bioaccumulation along the food chain and have been detected in human serum, breast milk, liver and kidney tissue across all continents [4-7]. International regulations initially focused on the most extensively studied PFAS. The progressive phase-out of these substances, however, has prompted the widespread industrial adoption of substitute or structurally alternative PFAS, a phenomenon commonly referred to as 'regrettable substitution'. Emerging PFAS such as

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perfluorononanoic acid (PFNA) and perfluorohexanesulphonic acid (PFHxS) are now routinely detected in environmental and biological matrices and also display substantial toxicity profiles [8-11]. Among various pathways of human exposure to PFAS, drinking water represents one of the most significant and direct ones, particularly in areas where groundwater or surface water sources have been impacted by industrial discharges or contaminated groundwater. PFAS detection has been reported in water matrices worldwide, including the Arctic [12], ranging from sub-ng/mL to several hundreds of ng/mL in impacted regions [13]. The persistence and mobility of PFAS in aquatic environments, combined with their limited removal by conventional drinking water treatment technologies such as sedimentation and chlorination, render the contamination of finished drinking water a persistent regulatory and public health challenge. Advanced treatment processes, including activated carbon adsorption and nanofiltration or reverse osmosis, can achieve significant PFAS removal [14]. In response to growing evidence of PFAS occurrence in drinking water and their associated health risks, the European Union adopted Directive 2020/2184 [15], which for the first time introduced parametric values for PFAS in drinking water at the European level. The Directive establishes a limit of 100 ng/L for the sum of 20 priority PFAS and a more stringent limit of 20 ng/L for the sum of four substances of particular concern (PFOA, PFOS, PFNA and PFHxS) selected on the basis of their hazard profiles and frequency of detection. The Italian Legislative Decree no. 102/2025, which implements this Directive into national law, reproduces these parametric values. The analytical determination of PFAS in water matrices at the low concentrations required by current and forthcoming regulations poses significant challenges. Reference methods, including EPA Method 537.1 and the European standard UNI EN 17892, typically rely on solid-phase extraction (SPE) using weak anion-exchange (WAX) cartridges followed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode [16-17]. SPE provides high pre-concentration factors and improved limits of quantification (LOQ), but it is often time-consuming, requires large volumes of organic solvents and reagents, and introduces risks of analyte loss, cross-contamination and batch-to-batch variability. In addition, the increasing number of samples required for routine monitoring under the new regulatory schemes calls for high-throughput analytical approaches that minimise labour and solvent consumption while maintaining adequate sensitivity and reliability. Direct injection of filtered water samples into ultra-high performance liquid chromatography coupled to triple-quadrupole tandem mass spectrometry (UHPLC-MS/MS) has been proposed as an alternative to SPE-based workflows for PFAS analysis in water. Direct injection eliminates the SPE step, offering reduced sample preparation time and lower solvent consumption and possibility of contamination, as well as enabling a more sustainable analytical workflow, aligned with the 12 principles of green analytical chemistry (GAC) [18]. However, the number of peer-reviewed studies applying direct injection to PFAS analysis in drinking water remains limited, and the methods reported to date have generally achieved limits of quantification (LOQ) in the range of 10–100 ng/L [19,20] for individual PFAS; these values, while adequate for less stringent regulatory contexts, are insufficient to ensure reliable compliance monitoring against the limit of 20 ng/L imposed by D.Lgs. 102/2025 as the sum of four PFAS, which in turn requires individual LOQs well below 5 ng/L. Furthermore, existing direct injection methods have typically targeted a restricted panel of predominantly long-chain PFAS, without coverage of all the analytes included in the regulatory 'sum of PFAS' parameter, which under D.Lgs. 102/2025 now encompasses a list of approximately 30 compounds subject to a collective limit of 100 ng/L. The development of sensitive, validated direct injection methods capable of simultaneously determining the four priority PFAS, and designed with a view to future extension to the full suite of approximately 30 regulated compounds, therefore represents a pressing analytical need. The present study addresses this gap by developing and validating a rapid, green and reliable UHPLC-MS/MS (QqQ) method for the simultaneous determination of four PFAS explicitly cited in D. Lgs. 102/2025 (PFOA, PFOS, PFNA and PFHxS) in drinking water by direct injection without any pre-treatment other than filtration and dilution. The method is designed to meet the sensitivity requirements for monitoring compliance with the regulatory threshold (20 ng/L as sum). The analytical approach is furthermore conceived as a scalable platform for future extension to the complete panel of approximately 30 PFAS subject to the 100 ng/L collective limit [21], supporting the transition towards comprehensive, high-throughput routine monitoring of drinking water quality in Italy. We report a full validation according to current analytical guidelines [22], including linearity, method detection limits (MDL) and lower limits of quantification, accuracy, precision, recoveries, and we present the application of the method to a set of real drinking water samples collected from drinking treatment plants in North-East Italy.

2. Materials and Methods

2.1. Reagent and standards

Ultrapure water was obtained from a Milli-Q International 10 system (Millipore, Bedford, MA, USA) and used for all solutions. Acetonitrile (ACN) and methanol (MeOH) of MS grade and ammonium formate (NH_4CHOO) and formic acid (FA) of analytical grade were purchased from Merck Sigma-Aldrich (Milan, Italy). Drinking water matrix for calibration was purchased from a commercial brand. Individual standard solutions of PFOA, PFOS, PFNA and PFHxS (≥ 98% purity) and isotopically labelled internal standards of ^{13}C8-PFOA, ^{13}C8-PFOS, ^{13}C9-PFNA and ^{13}C3-PFHxS (≥ 98% atom purity) were purchased from Wellington Laboratories (Guelph, ON, Canada) as 100 µg/mL solutions in methanol. A stock solution containing all analytes at 2 µg/mL was prepared by dilution of the individual standard solutions. Three working standard solutions containing all analytes at 20 µg/L, 1 µg/L and 100 ng/L were prepared in methanol by appropriate dilution of the stock solutions and stored at −20°C. A working internal standard solution containing ^{13}C8-PFOA, ^{13}C8-PFOS, ^{13}C9-PFNA and ^{13}C3-PFHxS at 20 ng/L each was prepared in methanol and used for spiking all samples and calibration solutions.

2.2. Instrumentation and chromatographic conditions

Chromatographic separation was performed using a 1290 Infinity III UHPLC system coupled to a 6495D triple quadrupole mass spectrometer equipped with an electrospray ionisation (ESI) source (both from Agilent, Santa Clara, CA, USA). An Ultra Inert IBD column (2.1 × 100 mm, 3 µm; Restek, Bellefonte, PA, USA) was used for all analyses. The column temperature was maintained at 40 °C. The injection volume was 45 µL for all samples. Mobile phase A consisted of water containing 5 mM ammonium formate (0.1% formic acid), and mobile phase B consisted of acetonitrile. The gradient program was as follows: 0.0 min, 50% B; 0.1-7.0 min, linear increase from 50% to 95% B; 7.0-13.0 min, hold at 95% B; 13.1-13.2 min, decrease from 95% to 50% B; 13.2-18.0 min, hold at 50% B for column re-equilibration. The total run time was 18.0 min and the flow rate was 0.4 mL/min. The instrument setup incorporates a delay column (identical to the main analytical column) between pumps and autosampler to mitigate interference from mobile phase or instrument components. Mass spectrometry was performed in negative ESI mode with the following source parameters: capillary voltage 3.5 kV, source temperature 150 °C, desolvation temperature 180 °C, desolvation gas flow 14 L/min, cone gas flow 11 L/min. Argon was used as collision gas at a pressure of 4.5×10^{-3} mbar in the collision cell. Analytes were quantified using dynamic multiple reaction monitoring (dMRM) of the most intense precursor-product ion transitions. The MRM transitions, collision energy (CE), iFunnel settings and retention times (RT) for all analytes and internal standards are listed in **Table 1**.

Table 1 | Multiple reaction monitoring (MRM) transitions and mass spectrometric parameters for PFHxS, PFOA, PFOS, PFNA and the respective ISs.

Analyte	Molecular formula	Precursor (m/z)	Product [1] (m/z)	CE [1] (eV)	Product [2] (m/z)	CE [2] (eV)	R_t (min)	iFunnel	Polarity
PFHxS	$C_6HF_{13}SO_3$	399	80	48	99	39	4.9	Standard	Negative
PFOA	$C_8HF_{15}O_2$	413	369	6	169	19	5.6	Fragile	Negative
PFOS	$C_8HF_{17}SO_3$	499	99	48	80	52	6.2	Large Molecule	Negative
PFNA	$C_9HF_{17}O_2$	463	419	10	219	16	6.5	Standard	Negative
^{13}C3-PFHxS	$C_6HF_{13}SO_3$	402	80	40			4.9	Standard	Negative
^{13}C8-PFOA	$C_8HF_{15}O_2$	421	376	10			5.6	Fragile	Negative
^{13}C8-PFOS	$C_8HF_{17}SO_3$	507	80	40			6.2	Large Molecule	Negative
^{13}C9-PFNA	$C_9HF_{17}O_2$	472	427	10			6.5	Standard	Negative

[1] Quantifier; [2] Qualifier; CE = collision energy; R_t = retention time.

2.3. Sample collection and preparation

Drinking water samples were collected from 12 different sites of tap water located in North - East Italy, during the November 2025 - January 2026 period. Sampling was performed in pre-cleaned 1 L HDPE bottles according to standard protocols. Samples were transported to the laboratory, stored at 4 °C and analysed within 7 days. Prior to injection, all samples were diluted 1:1 (*v/v*) with methanol and then filtered through 0.45 µm PP membrane filters (Merck Sigma-Aldrich) to remove particulate matter. Filtered samples were then transferred into 2 mL PP vials and spiked with the internal standard solution to give a final concentration of 200 ng/L for each internal standard. Method blank samples (in a 50:50 mixture Milli-Q water:methanol) were processed in the same way to assess background contamination.

2.4. Calibration and quality control

Matrix-matched calibration curves were prepared by spiking drinking water matrix (prepared as described in Section 2.1) with the working standard solution to obtain calibration levels at 0.5, 1, 1.5, 2, 5, 10, 25, 50, 100, 200, 400 and 600 ng/L for each of PFOA, PFOS, PFNA and PFHxS. All calibration solutions were spiked with the internal standard solution at the concentration of 200 ng/L. Calibration curves were set up by plotting the ratio of the analyte peak area to the internal standard peak area against the analyte concentration and fitting by weighted (1/x) linear least-squares regression. Quality control (QC) samples included: (i) method blank (Milli-Q water processed as the samples); (ii) spiked QC samples at low (0.5 ng/L), medium (5 ng/L) and high (400 ng/L) concentration levels for the analytes. QC samples were analysed at the beginning and end of each batch and at a rate of at least one QC *per* ten samples.

2.5. Method validation

Method validation was performed according to Environmental Protection Agency (EPA/537.1) [22] and European (UNI EN 17892) [23] guidelines. The following performance characteristics were evaluated.

- Linearity and calibration range: assessed by the correlation coefficient (R^2), the slope and intercept of the calibration curve, and the residual plot.
- Method detection limit (MDL) and lower limit of quantification (LLOQ): the MDL was estimated from replicate analyses (n = 12) of drinking water samples spiked at low concentration levels, according to the standard deviation approach. The LLOQ was defined as the lowest calibration level meeting predefined acceptance criteria for accuracy and precision [24].
- Accuracy (recovery): evaluated as the percentage of the spiked concentration recovered in spiked drinking water samples at three concentration levels (n = 5 *per* level).

- Precision: intra-day precision (repeatability) and inter-day precision (intermediate precision) expressed as relative standard deviation (RSD %) at the same three concentration levels (n = 5 *per* level, over 3 days).
- Matrix effects: evaluated by post-filtration spiking of authentic drinking water samples at 100 ng/L (n = 12). Since analytes were added directly to the filtered matrix immediately prior to injection, process-related losses were absent; the response of each analyte in the spiked matrix was compared to that obtained from a standard solution in pure solvent at the same nominal concentration, and the matrix effect was expressed as: ME (%) = [(area in matrix − area in solvent) / area in solvent] × 100. Positive and negative values indicate ion enhancement and ion suppression, respectively.

2.6. Data analysis

Chromatographic data were processed with Agilent MassHunter v12.1 software. Quantification was performed using the internal standard method. Statistical analysis was performed using Microsoft Excel 365. Plots were created using GraphPad Prism v 9.5.0.

3. Results and Discussion

3.1. Chromatographic separation and mass spectrometric detection

The selection of the chromatographic column and mobile phase composition was critical to achieve adequate separation of the four analytes within a short run time while maintaining high sensitivity in negative ESI mode. The Restek Ultra Inert column, characterised by a silica-based stationary phase functionalised with a polar (amide) group embedded within an alkyl chain, is specifically designed to minimise secondary interactions with active sites and enhance the formation of hydrogen bonds, optimising the separation of acidic compounds. It provided excellent peak shape and retention for both long-chain (PFOA, PFOS, PFNA) and short-chain (PFHxS) PFAS. All four analytes were well separated within 7 min, with retention times between 4.9 and 6.5 min (**Table 1**). PFHxS (C6 sulfonate), the shortest-chain compound, elutes first at 4.9 min, followed by PFOA (C8 carboxylate) at 5.6 min, PFOS (C8 sulfonate) at 6.2 min, and PFNA (C9 carboxylate) at 6.5 min (**Figure 1**). The MRM transitions were selected based on the most intense precursor-product ion pairs observed in full-scan MS experiments. The sulfonate PFAS (PFHxS, PFOS) showed the characteristic product ion at m/z 80.0 (SO_3^-). Despite the common product ion for the carboxylate series, adequate chromatographic separation prevented cross-talk. The use of isotopically labelled internal standards for all the target analytes compensated for variations in ionisation efficiency.

3.2. Linearity, LLOQ and MDL

Calibration curves for all analytes showed excellent linearity over the investigated concentration ranges. The correlation coefficients (R^2) were ≥ 0.995 for all analytes, and the residuals were randomly distributed around zero, indicating no systematic deviation from linearity (**Table 2**). The calibration model was forced through the origin, as blank samples showed no detectable contamination.

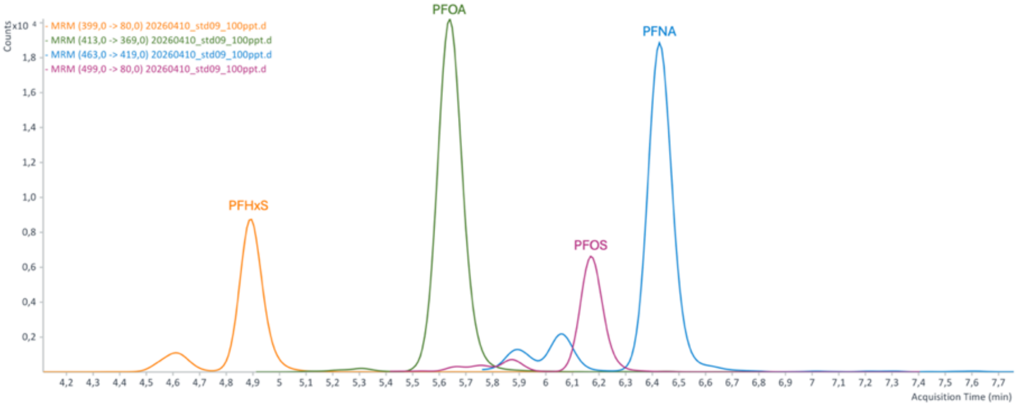

Figure 1 | Chromatographic peaks of four target analytes (PFHxS, PFOA, PFOS and PFNA).

Table 2 | Linearity, lower limits of quantification (LLOQ) and method detection limit (MDL) for PFHxS, PFOA, PFOS and PFNA in drinking water by direct injection UHPLC-MS/MS.

Analyte	Calibration range (ng/L)	R^2	LLOQ (ng/L)	MDL (ng/L)
PFHxS	0.5–600	0.9950	0.5	0.26
PFOA	0.5–600	0.9952	0.5	0.19
PFOS	0.5–600	0.9956	0.5	0.26
PFNA	0.5–600	0.9963	0.5	0.28

The weighted (1/x) regression improved the fit at low concentrations, which is critical for accurate quantification near the LLOQ. The method has shown excellent sensitivity, with MDLs ranging from 0.19 to 0.28 ng/L and LLOQ of 0.5 ng/L for all target compounds (**Table 2**). The LLOQ was established as the lowest calibration level. These values are well below the regulatory limits introduced by D. Lgs. 102/2025 for the sum of PFOA, PFOS, PFNA and PFHxS (20 ng/L) as well as for a presumptive single-compound limit of 5 ng/L, demonstrating that the method is suitable for drinking water compliance monitoring. The LLOQ for PFOA, PFOS, PFNA and PFHxS is also comparable to or better than those reported for SPE-based methods in wastewater, which typically range from 0.5 to 5 ng/L depending on the preconcentration factor [24-26].

3.3. Accuracy, precision and recovery

Accuracy was evaluated as the percentage recovery of spiked analytes in drinking water matrix at three concentration levels (low, medium and high). Recovery values obtained at the three spiking levels (1, 5 and 400 ng/L) ranged from 96.9 % to 114.0 % for all target PFAS, well within the accepted range of 70–130 % according to 2002/657/EC and demonstrating excellent method accuracy across the investigated concentration range. Most recoveries were close to 100 %, indicating negligible systematic bias. Slightly higher recoveries observed at the intermediate concentration level (up to 114.0 % for PFOS) remained within commonly accepted validation criteria, confirming the reliability of the method for quantitative determination of PFAS in water samples. The use of isotopically labelled internal standards improved recovery consistency. Intra-day precision (repeatability) was excellent for all analytes, with RSD values between 2 and 9 % at all concentration levels (**Table 3**). Inter-day precision (intermediate precision) was slightly higher but still within the acceptable limit of 20 %, with RSD values between 4 and 13 %. The highest RSD values were observed for PFNA at the low level (12.8 %).

3.4. Matrix effects

Matrix effects were evaluated by post-filtration spiking of authentic drinking water samples and comparing analyte response in pure solvent (methanol/water 1:1). The results demonstrate negligible to minor matrix effect: ion enhancement for PFOA (+4.80 %), minor ion suppression for PFHxS and PFOS (-6.85 % and -5.09 %, respectively) and negligible effect for PFNA (+1.04 %): see **Figure 2**.

Table 3 | Accuracy (recovery), intra-day precision (repeatability) and inter-day precision (intermediate precision) for the four analytes in drinking water at three concentration levels (n = 5 per level).

Analyte	Level (ng/L)	Recovery (%)	Intra-day RSD (%)*	Inter-day RSD (%)*
PFHxS	1	100.7	5.2	7.7
PFHxS	5	108.6	2.8	8.8
PFHxS	400	96.9	4.0	5.9
PFNA	1	104.1	8.2	12.8
PFNA	5	101.0	3.2	4.6
PFNA	400	104.0	3.8	4.6
PFOA	1	102.1	2.0	6.8
PFOA	5	110.0	3.5	7.2
PFOA	400	102.7	2.2	5.5
PFOS	1	100.3	3.7	8.4
PFOS	5	114.0	5.4	5.6
PFOS	400	100.7	3.3	5.9

*Intra-day precision was assessed on a single day (n = 5); inter-day precision was assessed over 3 days (n = 5 per day).

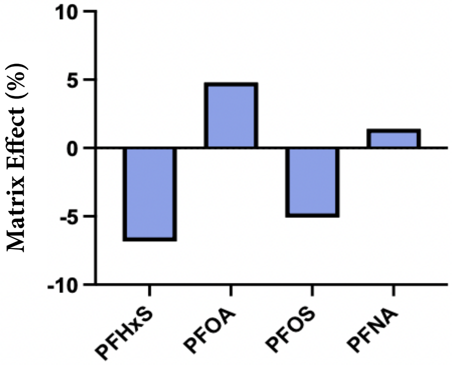

Figure 2 | Matrix effects for the four analytes in drinking water, expressed as percentage ion suppression/enhancement compared to pure solvent.
Note: Matrix effect (%) = [(area in matrix − area in solvent) / area in solvent] × 100. Negative values indicate ion suppression; positive values indicate ion enhancement.

These values are well within the ±20 % acceptance criterion adopted in method validation for environmental water analysis, indicating that the drinking water matrix does not significantly alter the ionisation of the target analytes under the developed UHPLC-MS/MS conditions. The low matrix effects observed are consistent with the use of direct injection of a relatively clean matrix such as drinking water, which contains substantially lower concentrations of co-eluting organic interferents compared to surface water or wastewater matrices. The isotopically labelled internal standards employed (^{13}C8-PFOS, ^{13}C8-PFOA, ^{13}C9-PFNA, ^{13}C3-PFHxS) provided effective compensation for the residual matrix-induced signal variations.

3.5. Application to real drinking water samples

The validated method was applied to the analysis of 12 real drinking water samples collected from tap water sampling sites across North - East Italy. The concentration results are summarised in **Figure 3**. PFOA was the most frequent and most concentrated compound across all sampling sites, with concentrations ranging from 1.36 to 2.0 ng/L and was detected in 6 samples. PFHxS was detected in just 2 out of 12 samples with concentrations of 1.31 and 2.32 ng/L. PFOS was detected in just 1 sampling site, with a concentration of 1.41 ng/L. PFNA was not detected in any sample. In the remaining samples, concentrations were below the LLOQ. The sum of the four regulated PFAS (PFOA + PFOS + PFNA + PFHxS, hereafter PFAS-4 Sum) ranged from 1.36 to 5.73 ng/L across all sites: thus, importantly, all 12 samples displayed PFAS-4 Sum values well below the regulatory limit of 20 ng/L established by D.Lgs. 102/2025 for this parameter, indicating compliance of the monitored drinking water supply with the most stringent PFAS parametric value currently in force in Italy. These concentration levels are consistent with those reported in precedent surveys of PFAS in finished drinking water in northern Italy and other European countries [27]. However, it should be noted that the present method targets exclusively the four PFAS explicitly regulated under the sum-of-four parametric value (PFOA, PFOS, PFNA and PFHxS) and does not provide information on the broader suite of 30 PFAS subject to the collective limit of 100 ng/L under D.Lgs. 102/2025. While the samples analysed here appear compliant with respect to the most critical regulatory threshold, the contribution of the remaining regulated PFAS to the total PFAS burden cannot be assessed from the present dataset. This represents a methodological limitation of the current study, particularly in the context of routine compliance monitoring, where a comprehensive analytical panel covering all regulated compounds is ultimately required. The extension of the developed direct-injection UHPLC-MS/MS platform to the full panel of approximately 30 regulated PFAS represents a feasible next step. Given the sensitivity and throughput advantages demonstrated here, direct injection is a promising candidate approach for this comprehensive monitoring, provided that adequate LOQs (compatible with individual contributions to the 100 ng/L collective limit) can be achieved for all target analytes.

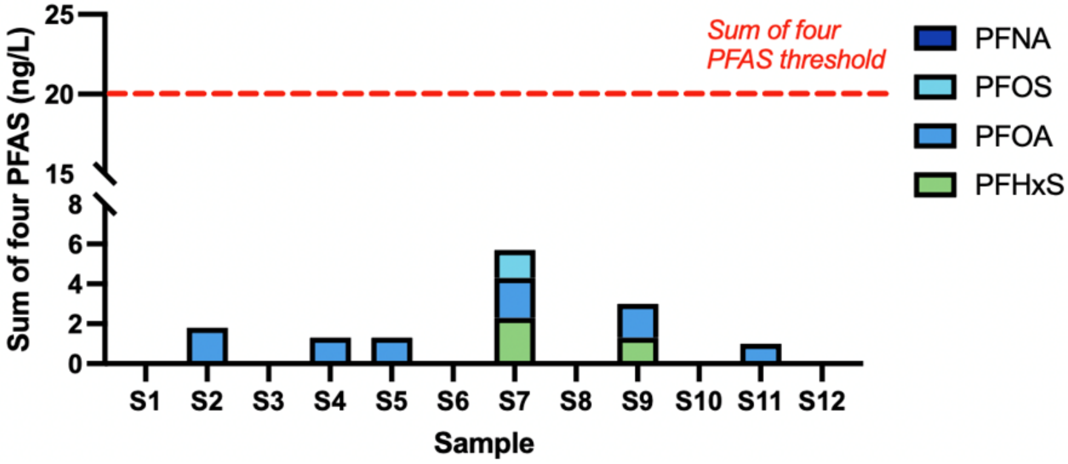

Figure 3 | Concentrations (ng/L) of the four analytes in 12 tap water sampling sites.

3.6. Comparison with SPE-based methods and green analytical chemistry

The method described, based on direct injection, offers different advantages over conventional SPE-based methods for PFAS analysis in drinking water. First, the sample preparation time is reduced from hours to less than 5 min *per* sample, enabling a much higher sample throughput (up to 50 samples *per* day on a single UHPLC-MS/MS system). Second, the method eliminates the use of SPE cartridges, large volumes of organic solvents or other related reagents (typically 10–20 mL *per* sample for SPE elution), reducing both cost and environmental impact of the analytical procedure. Third, the direct injection approach minimises the risk of analyte loss or contamination during sample preparation, which is particularly important for trace analysis of PFAS. In terms of green analytical chemistry, the method aligns with several of the 12 principles: (i) prevention of waste (no SPE waste and less sample waste); (ii) less hazardous chemicals used (only 2 mL of methanol *per* sample); (iii) design for energy efficiency (short run time and no SPE equipment); and (iv) increase in analytical throughput (fewer samples *per* time unit, reducing overall resource consumption). The total solvent consumption *per* sample is reduced from ~15 mL (SPE) to ~0.5 mL (UHPLC mobile phase only), corresponding to a more than 95 % reduction in solvent use. However, the direct injection method has some limitations compared to SPE. The main limitation is the absence of preconcentration factor: SPE methods can achieve LOQ values in the sub-ng/L range (0.1 ng/L) in drinking water. Nevertheless, the LLOQs obtained in this study (0.5 ng/L for PFOA, PFOS, PFNA and PFHxS) are sufficiently low for monitoring compliance with the regulatory limits for drinking water in Italy and the EU, and are comparable to or better than those reported for other direct injection methods in drinking water [20,28,29].

4. Conclusions

A rapid, green and reliable UHPLC-MS/MS (QqQ) method for the simultaneous determination of PFHxS, PFOA, PFOS and PFNA in drinking water by direct injection without pre-concentration has been developed and fully validated. The method provides LLOQs of 0.5 ng/L for each one of the four target compounds. Accuracy (recovery 96-114 %) and precision (intra-day RSD 2.8-8.2 %, inter-day RSD 4.6-12.8 %) meet the requirements of international standard guidelines, and the limited matrix effects are well compensated by isotopically labelled internal standards. The validated method was applied to real samples from twelve drinking water sampling sites in North-East Italy. PFOA was the most frequently detected analyte, PFOS and PFHxS were detected in just a few samples, while PFNA was not detected in any of the analysed samples. The PFAS-4 Sum specifically regulated by the Italian decree did not exceed the limit of 20 ng/L in any of the tested samples. Nevertheless, the sporadic detection of PFOA, PFOS and PFHxS highlights the importance of continued monitoring of these compounds in drinking water, since they may contribute cumulatively to human exposure even at low level contamination. Furthermore, the present method addresses only four of the 30 PFAS compounds subject to regulatory limits under D.Lgs. 102/2025, leaving uncharacterised the potential contribution of the remaining compounds to the collective 100 ng/L threshold. The extension of the developed direct-injection UHPLC-MS/MS platform to the full panel of regulated PFAS therefore represents a priority for future work. The direct injection approach significantly reduces sample preparation time, solvent consumption and risk of contamination compared to SPE-based methods, making it a high-throughput, cost-effective and environmentally sustainable tool for routine monitoring of PFAS in drinking water. The method is suitable for compliance monitoring under D. Lgs. 102/2025 and can be readily adapted for the analysis of drinking water.

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