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Application Note

Direct Injection Analysis of PFAS in Seawater Using the Shimadzu LCMS-8060RX

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals (Fig.1) widely used in industrial processes and consumer products due to their exceptional chemical stability and resistance to heat, water, and oil. However, these same properties make PFAS highly persistent, mobile, and bioaccumulative in the environment. They have been detected in various environmental matrices, including seawater, and have been associated with adverse effects on both human health and aquatic ecosystems. In many coastal regions, seawater is used as the raw water source for desalination plants that produce drinking water, making routine monitoring of PFAS increasingly important in line with the growing regulatory focus, including the EU Drinking Water Directive (EU) 2020/2184[1] and US EPA Method 1633A[2] for environmental water analysis.

The determination of PFAS in seawater is particularly challenging due to its high salt content (approx. 3–4%), which can cause severe matrix effects and reduce LC-MS/MS performance. Conventional analytical methods, including US EPA Method 1633A, typically rely on solid-phase extraction (SPE) to remove salts and concentrate analytes, but these procedures are labor-intensive and increase the risk of background contamination during sample preparation.

Direct injection eliminates extensive sample preparation, reduces the potential for contamination, and significantly improves laboratory throughput. However, introducing high-salinity samples directly into an LC-MS/MS system presents challenges related to salt loading and instrument robustness.

In this study, a direct injection LC-MS/MS method was developed using the Shimadzu LCMS-8060RX coupled with the Nexera™ LC-40 Series UHPLC system (Fig. 2), manufactured by Shimadzu Corporation, providing a rapid, robust, and sensitive solution for the determination of PFAS in seawater while maintaining reliable analytical performance.

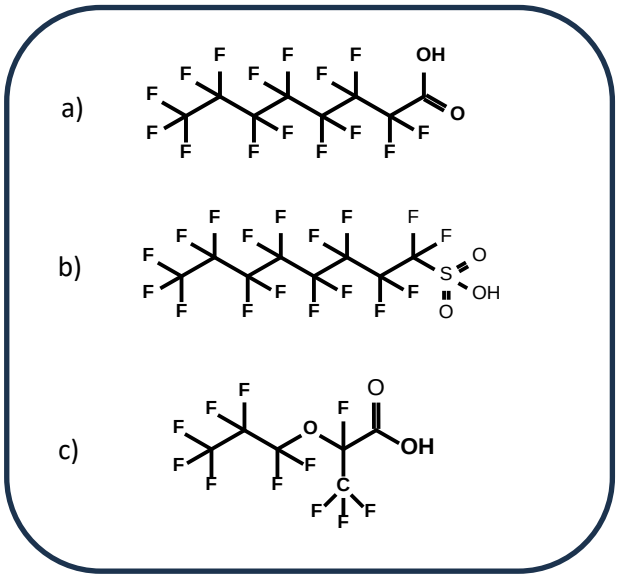

Fig. 1 Representative structures of PFAS group
a) Perfluorooctanoic acid (PFOA), b) Perfluorooctane sulfonate (PFOS), c) Hexafluoropropylene oxide dimer acid (GenX)

2. Materials and methods

The reference standards were procured from Wellington Laboratories with below catalogue numbers:

Isotope Dilution Standard PDS:- EPA-533ES

Native Analyte Primary Dilution Standard:- EPA-533PAR

Seawater samples were collected from a local water supply organization. Calibration standards were prepared and analyzed over a concentration range of 0.5 to 50 ng/L using LC-MS/MS. Calibration curves were generated using the internal standard calibration method with 1/x weighted linear regression, and the intercept was forced through zero.

PFAS contamination can originate from commonly used laboratory materials, including certain pipette tips, sticky notes, vial caps containing LDPE or PTFE (Teflon), and various plastic consumables. To minimize background contamination from these unavoidable sources, the Nexera LC-40 system was equipped with a PFAS-Free Kit, which includes PFAS-free mobile phase tubing, PFAS-free in-line filters, and a delay column. The delay column effectively separates background PFAS originating from the LC system and laboratory materials from the PFAS present in the sample, thereby improving analytical accuracy and confidence in trace-level quantification. The system was used for the identification and quantification of PFAS in seawater.

The analytical method was developed based on Shimadzu's LC/MS/MS Method Package for PFAS in Drinking Water, which provides optimized acquisition parameters for both native and isotopically labeled PFAS compounds. Data acquisition and processing were performed using LabSolutions Insight™, enabling efficient quantification and streamlined evaluation of method performance, including calibration and validation parameters.

2.1. Sample preparation

A 600 µL aliquot of seawater sample was transferred directly into a PFAS-free vial and mixed with 400 µL of an acidified organic reagent containing 25 ng/L isotopically labeled internal standards. The mixture was vortexed for 5 minutes and injected directly into the LC-MS/MS system without further sample preparation.

The ratio of organic solvent to seawater is critical for maintaining sample stability. A higher proportion of organic solvent can cause salt precipitation either immediately after mixing or during storage in the autosampler due to the low operating temperature, potentially affecting analytical performance and system robustness.

Method performance was evaluated by spiking seawater samples at three concentration levels (2, 5, and 10 ng/L) for all 25 PFAS analytes. Calibration standards were prepared over the concentration range of 0.5–50 ng/L using the same water-to-organic solvent ratio as the samples to ensure matrix consistency. The analytical conditions are summarized in Table 1.

Fig. 2 Shimadzu LCMS-8060RX

2.2. Analytical Conditions

Table 1 Analytical conditions for LC-MS/MS

LC	
Stationary Phase	Shim-pack GIST-HP C18 75mm x 3mm, 2µm C18 (Part no. 227-30002-03)
Flow rate	: 0.4 mL/min
Mobile Phase A	: 10 mM Ammonium acetate in water + 0.1% Formic acid
Mobile Phase B	: Methanol
Gradient program	Modified gradient program to remove salt.
Run time	16 min
Injection volume	50 µL (Co-injection with water)
Column Oven temp.	45 ˚C
MS	
Ionization	Heated-ESI
Interface temp.	100 ˚C
Nebulizing gas flow	3 L/min
Heating gas flow	15 L/min
Drying gas flow	5 L/min
DL temp.	150 ˚C
Heating block temp.	250 ˚C

3. Results and Discussion

3.1. Salt Management Strategy for Direct Injection of Seawater

The major challenge associated with the direct injection of seawater is its high salt content, typically ranging from 3-4% (w/v). When seawater is introduced directly into an LC-MS/MS system, the dissolved salts are transported through the LC system into the electrospray ionization (ESI) source. During the ionization process, the mobile phase is evaporated, while the non-volatile salts accumulate on the ESI capillary and other ion source parts. This salt deposition can reduce ionization efficiency, increase instrument maintenance, and adversely affect analytical sensitivity and system robustness. Despite sample dilution, the high salt load from direct injection resulted in significant salt deposition on the ion source components after only two

Fig. 3 Salt buildup in different components of the LC-MS/MS ion source after two direct seawater injections.

50 µL seawater injections (Fig. 3), demonstrating the need for an effective salt management strategy for direct injection analysis.

To overcome this challenge, the chromatographic gradient was optimized to achieve clear temporal separation between the highly polar salt matrix and the first eluting PFAS compound. Under reversed-phase chromatographic conditions, PFAS compounds are retained on the non-polar stationary phase according to their hydrophobicity, whereas the inorganic salts, owing to their high polarity and lack of interaction with the stationary phase, elute near the column void volume. By optimizing the initial gradient conditions, sufficient separation was achieved between the salt fraction and the PFAS analytes.

Following this chromatographic separation, an FCV-20AH$_2$ flow control valve was installed between the LC and the mass spectrometer. During the initial portion of the chromatographic run, corresponding to the salt elution window, the column effluent was diverted to waste. After the salt fraction had completely eluted, the valve was switched to direct the remaining flow containing the PFAS analytes into the mass spectrometer. This valve-switching strategy effectively prevented most of the salt matrix from entering the ion source while maintaining high sensitivity and enabling robust direct injection analysis of seawater samples.

The valve-switching configuration and analytical workflow are illustrated schematically in Fig. 4.

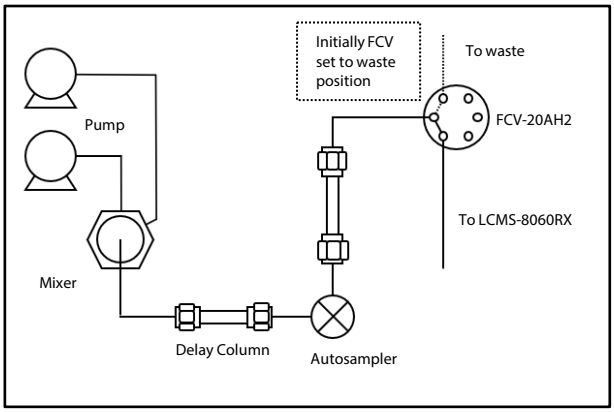

Fig. 4 Schematic of FCV-20AH$_2$ based salt diversion workflow for Direct Injection Analysis of Seawater.

The chromatographic gradient was optimized to achieve complete separation between the salt matrix and the PFAS analytes. Using LabSolutions™ software, the FCV-20AH$_2$ was programmed to switch automatically based on the chromatographic time. During the initial salt elution window, the column effluent was diverted to waste, and after complete elution of the salt fraction, the flow was automatically directed to the mass spectrometer. This automated valve-switching strategy ensured that most of the salt matrix did not enter the mass spectrometer while allowing all target PFAS analytes to be detected without loss.

To verify the salt elution profile, the effluent diverted to waste through the FCV-20AH$_2$ was collected at different time intervals during the chromatographic run and analyzed using the Nexera IC system. As a reference, Type I water and seawater samples were collected over the entire waste diversion period, followed by the collection of individual waste fractions at selected time intervals. The overlaid ion chromatograms of these collected fractions are shown in Fig. 5. Comparison of the conductivity profiles confirmed that the inorganic salts were completely eluted within the designated waste diversion window before the valve switched the flow to the mass spectrometer. These results verified the optimized FCV switching time and ensured that only the PFAS-containing fraction entered the MS, thereby minimizing salt contamination, improving instrument robustness, and maintaining analytical sensitivity.

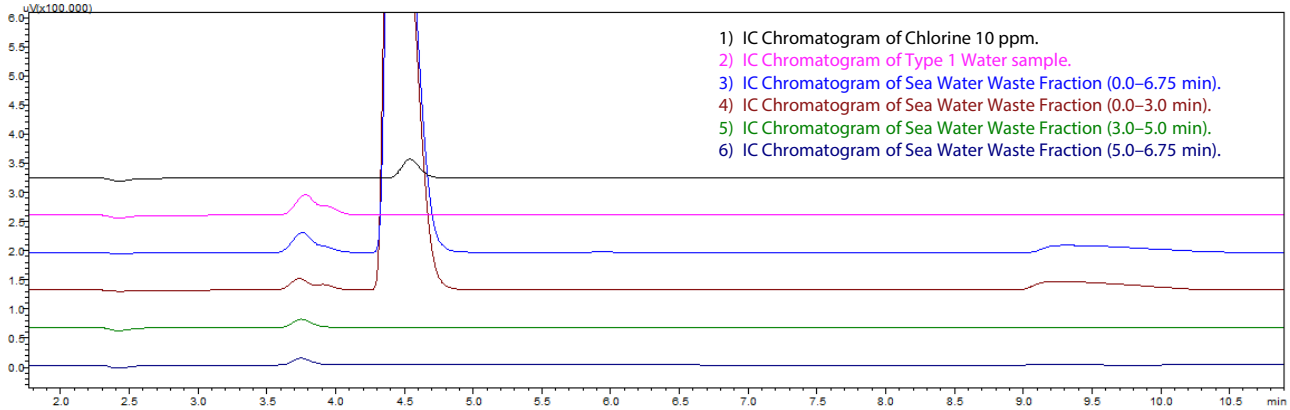

Fig. 5 Overlay of IC Chromatograms of a 10-ppm Chloride Standard, Type I Water, and Time-Resolved Waste Fractions

3.2. Effect of Organic Diluent

Seawater samples were diluted with an organic solvent mixture prior to injection to improve analyte solubility and compatibility with the analytical method. However, the relatively high organic solvent content adversely affected the chromatographic behavior of the early-eluting PFAS compounds. Due to their limited retention under these injection conditions, these analytes exhibited poor interaction with the stationary phase, resulting in distorted peak shapes and reduced chromatographic performance.

To overcome this issue, the "Co-Injection" feature of the Shimadzu SIL-40 autosampler was utilized. This feature allows a secondary solvent (water) to be introduced simultaneously with the sample, thereby reducing the effective organic solvent strength at the column inlet without diluting the analyte concentration. As a result, improved retention and significantly enhanced peak shapes were obtained for the early-eluting PFAS compounds. The effect of water co-injection on chromatographic performance is illustrated in Fig. 6a and 6b.

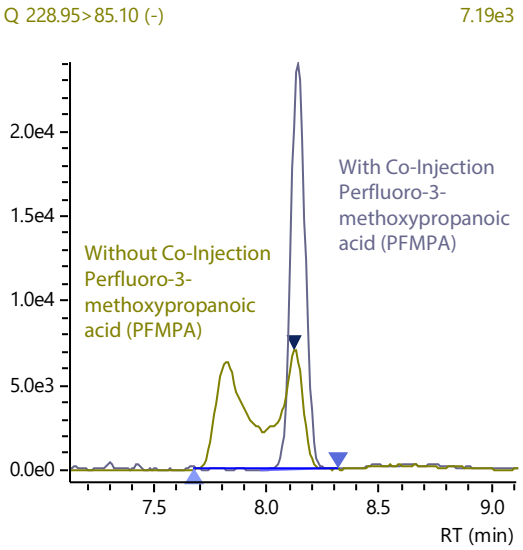

Fig. 6a Effect of Water Co-Injection on Peak Shape of early eluting PFAS.

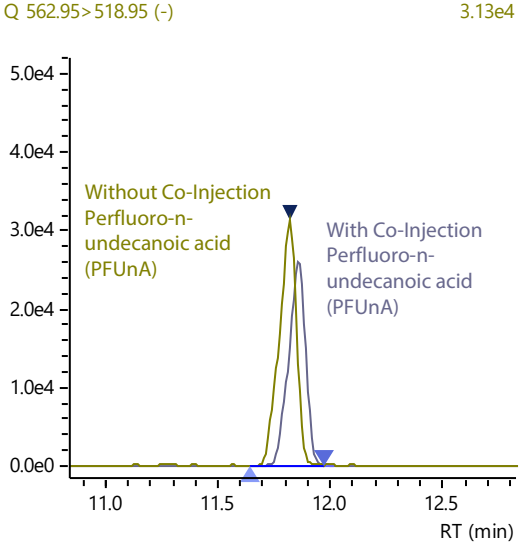

Fig. 6b Effect of Water Co-Injection on Peak Shape of late eluting PFAS.

3.3. Linearity Study

With the optimized instrument configuration and acquisition method, calibration curves were established over the concentration range of 0.5–50 ng/L. Excellent linearity was achieved for all target PFAS compounds, with correlation coefficients (R^2) greater than 0.99 and calibration accuracies within 80–120%. Quantification was performed using the internal standard calibration method with isotopically labeled internal standards. Representative calibration curves for selected PFAS compounds are shown in Fig. 7a and Fig. 7b.

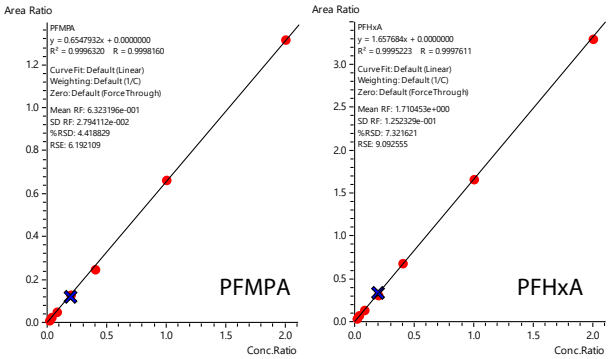

Fig. 7a Representative calibration curves for PFAS ranging from 0.5 ng/L to 50 ng/L.

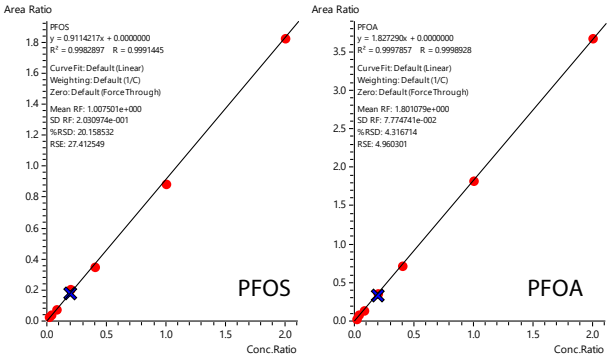

Fig. 7b Representative calibration curves for PFAS ranging from 0.5 ng/L to 50 ng/L.

3.4. Recovery Study

To evaluate the trueness of the developed method, seawater samples were fortified with all 25 target PFAS compounds at 2, 5, and 10 ng/L. Each spiking level was analyzed in triplicate using the optimized direct injection LC-MS/MS method, and recoveries were calculated using isotopically labeled internal standards.

The mean recoveries for the target PFAS compounds ranged from 70–120% across all 3 spiking levels, except for perfluorooctane sulfonate (PFOS; >120% at 2 ng/L) and 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS; <70% at 10 ng/L). The optimized direct injection workflow, chromatographic conditions, and salt management strategy effectively minimized matrix effects while maintaining robust analytical performance. Method precision, evaluated from triplicate analyses, showed relative standard deviations (RSDs) below 10% for 20 of 25 compounds at 2 ng/L and below 15% for the remaining five. At the 5 and 10 ng/L spiking levels, all compounds exhibited RSDs below 10%, demonstrating excellent repeatability. The recoveries for the spiked samples are presented in the statistical plot in Fig. 8.

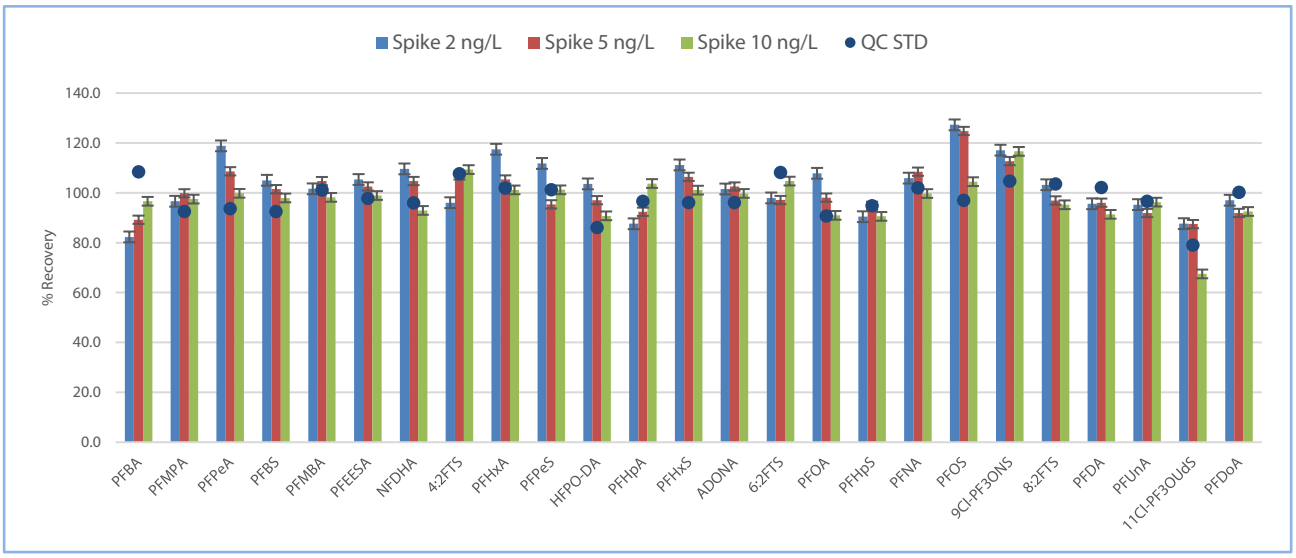

Fig. 8 Mean percentage recoveries of 25 PFAS in seawater at spiking levels of 2, 5, and 10 ng/L (n = 3). Error bars represent the standard deviation of triplicate analyses.

Following the analysis of the complete calibration sequence, seven consecutive seawater sample injections were performed, followed by a QC standard to evaluate the robustness of the optimized method. Despite repeated direct injections of the high-salinity seawater matrix, no visible salt deposition was observed on the ESI ion source components.

This demonstrates that the combination of the optimized chromatographic gradient, automated FCV switching, and water co-injection effectively prevented salt accumulation within the mass spectrometer while maintaining stable analytical performance. Photographs of the ESI ion source components after completion of the analytical sequence are shown in Fig. 9.

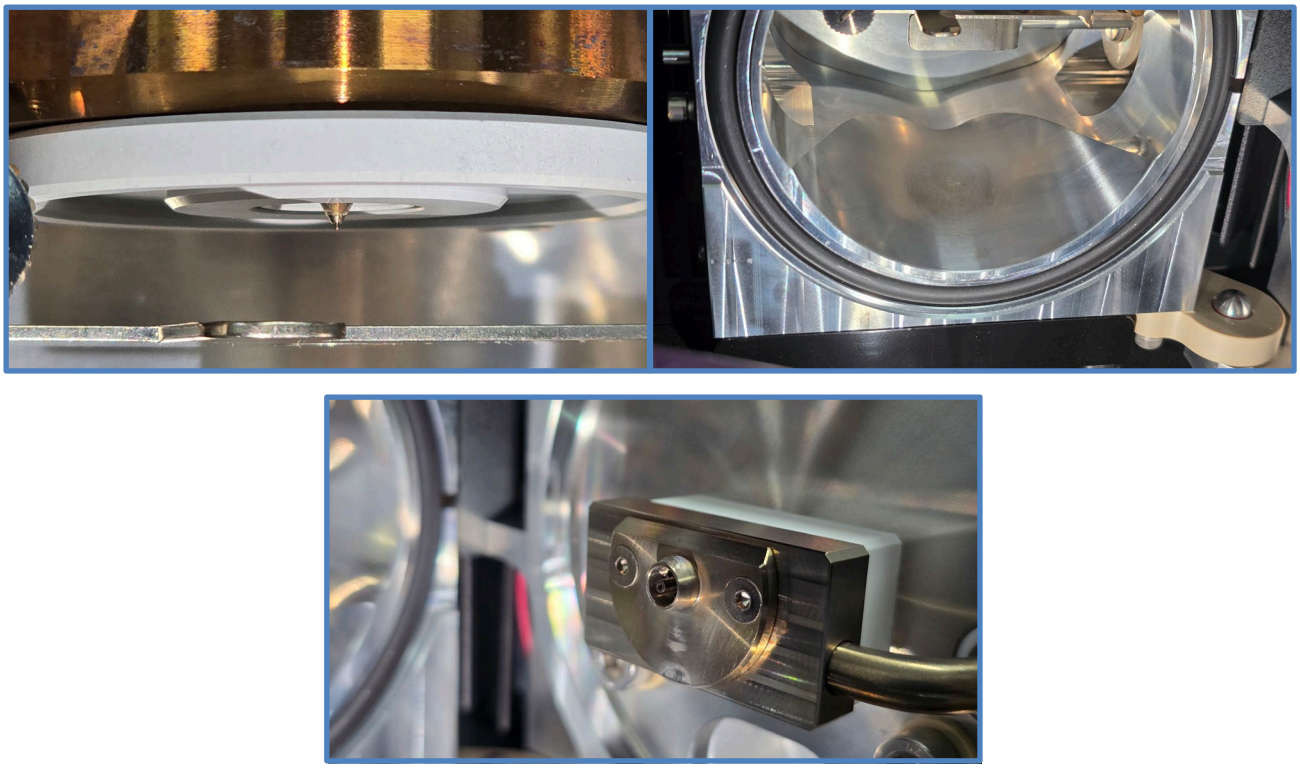

Fig. 9 ESI Ion Source Components After Seven Consecutive Direct Seawater Injections Using the Optimized Workflow.

4. Conclusion

A robust and sensitive direct injection LC-MS/MS method was successfully developed for the determination of 25 PFAS compounds in seawater using the Shimadzu Nexera LC-40 series equipped with the PFAS-Free kit and coupled to the LCMS-8060RX. The high-salt matrix was effectively managed through an optimized chromatographic gradient, automated FCV-20AH$_2$ valve switching, and water co-injection, while the PFAS-Free kit minimized background PFAS contamination, enabling reliable trace-level analysis.

The developed method eliminated the need for labor-intensive sample preparation and significantly reduced the risk of PFAS contamination associated with conventional extraction procedures. Excellent analytical performance was achieved, with linear calibration over the range of 0.5-50 ng/L, recoveries ranged from 70–120% for all target PFAS compounds, except for PFOS (>120% at one spiking level) and 11Cl-PF3OUdS (<70% at one spiking level), while relative standard deviations were below 10% for most compounds, demonstrating good accuracy, precision, and repeatability.

The proposed workflow provides a simple, reliable, and high-throughput solution for routine PFAS monitoring in seawater and can support environmental surveillance, marine pollution assessment, and the monitoring of seawater used as a source for desalinated drinking water.

5. References

1. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption.
2. U.S. EPA. Method 1633A: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. Revision A, December 2024.

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