



Enhancement of per- and Polyfluoroalkyl Substances (PFAS) quantification on surface waters from marinas in the douro river, Portugal

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ABSTRACT

PFAS, known as "forever" compounds, are prevalent in various environments, including soils and aquatic systems, due to extensive usage. Surface waters in several European countries, especially marinas and ports with high boat traffic, require further study as potential contamination sources. Reliable methods for the extraction and quantification of these emergent compounds are essential. This study aimed to improve an existent solid phase extraction method to analyse marinas and ports' surface waters with variable salinities (2, 9 and 17 PSU). The objectives were to: 1) optimise the solid phase extraction method, considering matrix salinity effects and cross-contaminations, 2) validate the extraction and quantification method of 18 EPA 537.1 PFAS in estuarine surface waters, using the Ultra-High Performance Liquid Chromatography – Quadrupole Time – Of – Flight – Tandem Mass spectrometry, and 3) apply the optimised method for PFAS quantification in three Portuguese marinas. All ICH criteria were successfully validated considering 9 PSU. Limits of quantification ranged from 117.80 ng/L to 385 ng/L, except for PFHpA (645.85 ng/L). PFAS levels (PFOA, HFPO-DA, PFBS, PFHxS and PFOS) were relatively low, reaching a maximum of 0.32 ng/L only for the PFOA. In Freixo marina, total average concentrations were slightly higher ($\sum$ PFAS = 1.02 ng/L) when compared to the ones found in Cais da Ribeira Port ($\sum$ PFAS = 0.94 ng/L) and Afurada marina ($\sum$ PFAS = 0.81 ng/L). PFOS concentrations are below the limit values set by the Environmental Quality Standards (36000 ng/L of PFOS for inland surface water, respectively), similar to other Portuguese river studies. This study enabled the development of a precise and reliable extraction and quantification method to quantify PFAS in estuarine surface waters, particularly from marinas. This method can be readily applied to analyse PFAS in other estuarine samples.

1. Introduction

Per- and Polyfluoroalkyl Substances (PFAS) are a group of synthetic organic molecules produced over the last 70 years (Prevedouros et al., 2006). Due to environmental concerns and human health effects (Buck et al., 2011), the Stockholm Convention, under the European Union Persistent Organic Pollutants Regulation, has restricted the use of

perfluorooctanesulfonate (PFOS) (European Commission, 2019), perfluorooctanoic acid (PFOA) (European Commission, 2020), perfluorohexanesulfonic acid (PFHxS) (European Commission, 2023) and their salts and related compounds. To address concerns and restrictions, long-chain PFAS have been replaced by shorter-chain alternatives, such as perfluorobutanesulfonic acid (PFBS) and hexafluoropropylene oxide dimer acid (HFPO-DA, commonly referred to as genX), as they are

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presumed to be safer. However, their intense use in recent years has led to contamination in aquatic environments (Pan et al., 2018) and bioaccumulation in organisms (Guillette et al., 2020).

PFAS are characterised by their high thermal and chemical stability (Buck et al., 2011) and, for this reason, used in different industries, such as paints, non-stick kitchen utensils, surfactants, water repellents sprays and clothing, insecticides, coatings and firefighting foams (Ahrens, 2011; Cui et al., 2020). Other industries, like nautical materials, can also contain PFAS, namely in the interior (e.g., boat furniture upholstery, seat and console cover) and the exterior (e.g., awning and boat lacquers) of the boats (Knepper and Janousek, 2019; Janousek et al., 2019). From the existent literature, some studies described the presence of PFAS in surface waters in several European countries, such as in the Rhine River in The Netherlands with a maximum ΣPFAS concentration of 498 ng/L (Möller et al., 2010), various rivers and lakes in France with a median ΣPFAS of 7.9 ng/L (with a maximum of 725 ng/L) (Munoz et al., 2015), and in the Júcar River in Spain, concentrations between 0.04 and 83.1 ng/L were detected (Campo et al., 2016). Other non-European countries, such as the USA (Viticoski et al., 2022), India (Sharma et al., 2016), China (Wang et al., 2022), and South Africa (Groffen et al., 2018), have also reported the presence of these compounds in their aquatic systems (varying between 35.2 ng/L and 390 ng/L), demonstrating their ubiquitous presence.

Specific locations, such as marinas and ports with high boat traffic, still need to be further studied since they can be a potential source of contamination for the aquatic systems due to the direct release from the boats or indirectly through their activities. Boats require regular maintenance and cleaning, and some cleaning products may contain PFAS (Gaines, 2023), contributing to the contamination of aquatic systems when used. Other sources of contamination of PFAS are the use of anti-fouling paints on the exterior of the boats to protect them from external environmental factors, such as marine abrasion, and the use of Aqueous Film-Forming Foam (AFFF) with a high concentration of PFAS (Moody and Field, 2000), which are used on firefighting. To evaluate the source of contamination of PFAS and the risk of contamination, it is essential to quantify PFAS in marinas and ports.

Reliable methods are necessary to extract and quantify these emergent compounds. The most frequently utilised approach for the pre-concentration of PFAS in water samples is Solid Phase Extraction (SPE). This methodology is also employed in several EPA methods, including 537, 537.1, 533 and 1633 (Teymoorian et al., 2023) and successfully used in different water matrices, such as WWTP waters (Winchell et al., 2021), groundwater (Yong et al., 2021), surface water (Bai and Son, 2021), and seawater (Ali et al., 2021) for PFAS extraction. High-resolution instruments are necessary for quantification to obtain accurate results (Aceña et al., 2015). The Quadrupole Time-of-Flight Mass Spectrometry (qTOF-MS/MS) is an analytical technique that provides a fragmentation pattern of compounds with high resolution, facilitating their identification and quantification in environmental samples (Xie et al., 2012). Due to the ubiquitous presence of PFAS in our daily lives, it is important to establish accurate and reliable detection and quantification methods. More recently has been given a special attention to potential sources of contamination, such as laboratory materials, reagents and solvents, sample preparation and analytical equipment, which must be understood and mitigated. One of the strategies is to test all the steps (solvents and devices) of extraction and use a delay column in the LC system to delay contaminants from the mobile phase and tubes (Coggan et al., 2019; Borrell et al., 2020), allowing the separation of target PFAS from those present in the system.

Thus, the main objectives of this study were to 1) optimise the SPE considering matrix salinity effects and cross-contamination, 2) validate the extraction and quantification method of 18 EPA 537.1 PFAS in estuarine surface waters, and 3) apply the optimised method to determine the concentrations of PFAS in the surface waters of three marinas in the Douro River (Portugal), using Ultra-High Performance Liquid Chromatography (UHPLC) coupled to qTOF-MS/MS.

This was achieved by employing the 18 EPA 537.1 PFAS to quantify PFAS in marinas' surface waters located at various points along the Douro River estuary.

2. Materials and methods

2.1. Chemicals and solvents

PFAS and isotope-labelled internal standard mixtures were purchased from LGC standards (Teddington, UK). The EPA Method 537.1 PFAS mixture (100 µg/mL) contained perfluorohexanesulfonic acid (PFHxS, 95%), perfluoroheptanoic acid (PFHpA, 99%), pentadecafluorooctanoic acid hydrate (PFOA, 95%), perfluorononanoic acid (PFNA, 99%), perfluorodecanoic acid (PFDeA, 98%), perfluoroundecanoic acid (PFUA, 101%), perfluorododecanoic acid (PFDoA, 100%), perfluorotridecanoic acid (PFTrDA, 99%), perfluorotetradecanoic acid (PFTeDA, 96%), undecafluoro-2-methyl-3-oxahexanoic acid (HFPO-DA, 97%), 2,2,3-trifluoro-3-[1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy]propanoic acid (ADONA, 93%), perfluorobutanesulfonic acid (PFBS, 102%), perfluorohexanoic acid (PFHxA, 99%), perfluorooctane sulfonic acid (PFOS, 90%), 9-Chloro-perfluoro-3-oxanonanesulfonic acid potassium (9Cl-PF3ONS, 98%), potassium 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS, 98%), 2-(N-Methylperfluorooctanesulfonamido)acetic acid (Me-PFOSA-AcOH, 97%) and 2-(N-Ethylperfluorooctanesulfonamido)acetic acid (Et-PFOSA-AcOH, 98%) (Acronym, CAS number, subgroups and chemical formula of each compound are in Table S1, supplementary material). The EPA Method 537 isotope-labelled internal standard mixture consisted of [¹³C₈] PFOA (1000 ng/mL), [¹³C₈] PFOS (3000 ng/mL) and N-methyl-D₃ (4000 ng/mL).

PFAS stock solution was prepared at 1000 µg/L, while the internal standards mixture was set at 130, 390, and 520 µg/L (for [¹³C₈] PFOA, [¹³C₈] PFOS, N-methyl-D₃, respectively), all dissolved in methanol. These solutions were stored at -20 °C, with a maximum storage period of one month. Chemicals reagents: methanol hypergrade (CAS 67-56-1, Honeywell, ≥99.9%, Germany) for liquid chromatography-mass spectrometry (LC-MS), ultrapure water (CAS 7732-18-5, resistivity 18.2 MΩ cm 25 °C, Milli-Q® Advantage A10 Water Purification System, Millipore, USA), ammonium acetate Optima® LC/MS (CAS 540-69-2, purity, ≥99 %, Fisher Chemical, USA). For solid-phase extraction (SPE), methanol HPLC (CAS 67-56-1, ≥99.9%, Honeywell, Germany), ammonium hydroxide (CAS 1336-21-6, Sigma-Aldrich, USA), and sodium chloride (NaCl) (CAS 7647-14-5, VWR, USA) were used.

For internal calibration, a mixture of isopropanol Optima® LC/MS (CAS 67-63-0, ≥99.9%, Fisher Chemical, USA), sodium hydroxide 1M (CAS 1310-73-2, Fisher Chemical, USA) and formic acid reagent grade (CAS 64-18-6, ≥95%, Sigma-Aldrich, USA) was used.

2.2. Study sites and sampling procedure

Surface water samples were collected in two marinas and one port of the Douro River: Afurada marina (41°08'31.5"N 8°38'58.2"W), Freixo marina (41°08'35.5"N 8°34'40.2"W) and Cais da Ribeira port (41°08'31.5"N 8°38'58.2"W) were the chosen locations (Fig. 1).

Afurada marina (Site 1), located on the South margin of the Douro River, is the largest recreational boating structure between Cascais and Galicia, with 300 yacht spaces (<https://gotosailing.com/pt/douro-marina-marina-da-afurada-vila-nova-de-gaia>). Freixo marina (Site 2) and Cais da Ribeira port (Site 3) are located on the North margin of Douro River. Freixo marina has space for 76 boats up to 16 m and docking for boats up to 100 m (https://visitporto.travel/pt-PT/poi/5cd04b4df979e000019f80a0#), and Cais da Ribeira port, despite not being considered a marina, is a place with high boat traffic, due to tourism.

During low tide, the sampling was performed on two consecutive days (22nd and November 23, 2023). At each sampling location, two

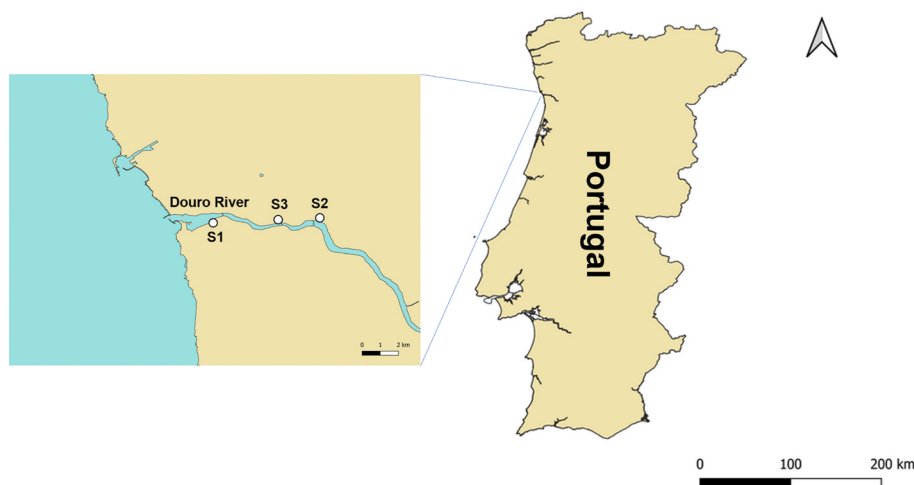


Fig. 1. Schematic representation of the study sites in Douro River, Portugal. S1) Afurada marina (41°08'31.5"N 8°38'58.2"W); S2) Freixo marina (41°08'35.5"N 8°34'40.2"W); S3) Cais da Ribeira port (41°08'31.5"N 8°38'58.2"W). Map source: QGIS software.

replicates (1.5L each) of water were collected in different areas of each site to represent it better. Before collection, high-density polyethylene (HDPE) bottles were washed with local water. Samples were immediately stored in a cool box until the laboratory and physicochemical parameters were measured *in situ* (pH, temperature, salinity and total dissolved solids (TDS)) by a multiparameter probe (HI98494, HANNA instruments, USA) (data in Table S2, supplementary material). In the laboratory, samples were preserved at 4 °C for a maximum period of 24h until extraction.

2.3. Sample extraction

The PFAS extraction procedure was adapted from Zhao et al. (2016), with some modifications to include the effect of salinity on the matrix. PFAS extraction was performed by SPE using Oasis WAX cartridges (ref. 186009568, Waters®, 6 cc, 150 mg, USA).

Briefly, 1) cartridges were pre-conditioned adding 4 mL of methanol with 0.1% ammonium hydroxide, 4 mL of methanol: water (40:60) and 4 mL of ultrapure water; 2) 500 mL of water sample was loaded at approximately one drop per second; 3) cartridge was washed with 4 mL of ultrapure water and 4) dried in vacuum for around 1 h (to confirm that they were completely dry, the reagents in the cartridge had to be completely loose); 4) eluted into a PP falcon tube with 4 mL of methanol with 0.1% ammonium hydroxide, 5) elution solution was completely dried under high-purity nitrogen gas, and 6) reconstituted with 100% of methanol. During the sample extraction, the flow was constant, preventing it from drying out to increase reproducibility and recovery. Then, 200 µL of the final sample was transferred to a polypropylene vial for analysis and injected into the LC instrument. Extracted samples were stored at −20 °C.

2.4. Analytical instrument setup and mass spectrometer conditions

Samples were injected via an UHPLC Focused Dionex ultimate 3000 (Thermo Fisher, USA) coupled to a Quadrupole Time-Of-Flight (qTOF) instrument Impact II (Bruker Daltonics, USA), equipped with Vacuum Insulated Probe Heated Electrospray Ionisation (VIP-HESI) source.

The ion polarity used was in negative mode, generating $[M-H]^-$ precursor and product ions. The UHPLC conditions for the separation were as follows: mobile phase A was ultrapure water with 2 mM ammonium acetate, and mobile phase B was methanol with 2 mM ammonium acetate. The gradient of mobile phases started with a constant 20% B for 1.5 min, increased to 100% B for 11.5 min, held at 100% B for 4 min (to clean the system to the next run) and then decreased at

20% B for 2 min (for equilibration of the system to the next run).

For each run, the sample (5 µL) was injected through a HALO PFAS column (Part number: 92812-613, 2.7 µm, 2.1 mm × 100 mm, Advanced Materials Technology, USA), at a flow rate of 0.2 mL/min and a column oven temperature of 40 °C. Gas temperature and capillary voltage were maintained at 300 °C and 3500 V, respectively.

The flow and temperature of dry gas were 10 L/min and 240 °C, respectively, and the nebulisation gas pressure was 4 bar. The endplate offset potential was 500 V, and the probe dry flow and temperature were 5L/min and 300 °C, respectively.

The internal calibration reagent calibrated the masses in each injection consisting of 12.5 mL ultrapure water, 12.5 mL isopropanol, 50 µL formic acid (95%), and 250 µL sodium hydroxide. Detection using MS/MS was executed with specific parameters detailed in Table 1; each compound was characterised by its retention time and at least two ions (precursor and product ions). Data was processed using the Compass Data Analysis version 5.0 software.

2.5. Method validation

2.5.1. Contamination of PFAS and precautions

The PFAS analysis requires particular care regarding contamination issues, so special attention was paid to the materials used in sampling, extraction, storage of stock solutions, samples, and analysis of the UHPLC-qTOF-MS/MS.

For sampling, beakers of polypropylene (PP) and commercial water bottles of HDPE were used. Physicochemical analyses were performed on an independent water sample to avoid cross-contamination of the sensor.

In the laboratory, all Teflon® (polytetrafluoroethylene, PTFE) materials were avoided, as they are sources of contamination. Therefore, polypropylene materials were chosen, namely tips (1000 µL, catalogue number: 70.3050, PP, Sarstedt, Germany; 200 µL, ref: 1-121-50-1, PP, AHN, Germany) and falcon tubes (catalogue number: 339659, PP, Thermo Fisher, USA).

As for the SPE manifold (Catalogue number: 57030-U, Supelco, USA), the needles were replaced with adapted 200 µL tips and washed with 4 mL of methanol between extraction series. During the SPE procedure, samples without spiked PFAS (matrix-matched blanks) were processed to consider the potential level of contamination of the extraction procedure.

Vials made of polypropylene (C4011-13, Thermo Scientific, USA) were used for analysis in the UHPLC-qTOF-MS/MS.

Finally, a HALO® PFAS Delay column (Part number: 92113-415,

Table 1

Mass spectrometry conditions of EPA 573.1 PFAS mixture: Molecular mass (g/mol), retention time (RT), precursor and product ions [M-H]⁻ of EPA 573.1 PFAS mixture and IS, and their collision energies (eV) used in UHPLC-VIP-HESI-QTOF-MS/MS analyses. PFAS are listed according to chemical structure complexity.

PFAS	Molecular mass (g/mol)	RT (min)	Precursor [M-H] ⁻	→	Product ions	CE (eV)
PFHpA	363.9769	11.7	362.9690	→	318.9618 [*] 168.9805	10
PFOA	413.9737	12.6	412.9661	→	368.9562 [*] 168.9811	10
PFNA	463.9705	13.1	462.9628	→	418.9495 [*]	10
PFDeA	513.9673	13.7	512.9642	→	468.9423 [*]	10
PFUA	563.9641	14.1	562.9563	→	518.9368 [*]	10
PFDoA	613.9609	13.7	612.9537	→	568.9298 [*]	10
PFTTrDA	663.9577	14.9	662.9497	→	618.9246 [*] 354.9411 284.9651 328.9497	10
PFTeDA	713.9545	15.2	712.9467	→	668.9180 [*] 404.9346	13
HFPO-DA	329.975	11.8	318.9795 ⁽¹⁾	→	118.986 168.980	10
ADONA	377.9761	11.9	376.9890	→	250.9700 [*]	10
PFBS	299.9503	9.6	298.9432	→	98.9517 79.954498	40
PFHxS	399.9439	11.8	398.9367	→	98.9511 79.9537 118.9863 168.9807	46
PFOS	499.9375	13.1	498.9304	→	98.9515 79.9537 129.9481 229.9362	58.2
9Cl-PF3ONS	569.8587	14.5	568.9642	→	418.9486 [*] 168.9804	28
11Cl-PF3OUdS	669.8524	15.2	668.9568	→	354.9398 [*] 530.8633	25
Me-PFOSA-AcOH	570.9746	13.9	569.9673	→	168.9813 218.9747 418.9502 268.9689	10
Et-PFOSA-AcOH	583.9461	14.1	583.9830	→	418.9481 525.9439 580.8566	25
[¹³ C ₈] PFOA	422.0005	12.6	420.9681	→	375.9771 [*]	10
[¹³ C ₈] PFOS	529.9460	13.1	506.9570 ⁽²⁾	→	98.9501 79.9527 171.9888 232.9439 [*]	52.2
N-methyl-D ₃	573.9934	13.9	572.9861	→	168.9793 218.9727 418.9466 [*] 514.9479	40

Upperscript symbols.

⁽¹⁾ [M-H-K]⁻.

⁽²⁾ [M-H-Na]⁻.

^(*) used as quantification ion.

particle size 2.7 µm, 3.0 mm × 50 mm, Advanced Materials Technology, USA), placed after the mixer and before the sample injector, was used. When the standard sample is injected, the chromatogram shows two peaks: one originating from the sample and one from the system (Fig. S1).

Despite using the delay column, the PFOA was always present. So, to understand the source of this contamination, different components (i.e., methanol, methanol with 0.1% ammonium hydroxide, water, the manifold and cartridges) were assessed. For this, several steps were tested: 1) methanol not evaporated, 2) 10 mL of methanol evaporated, 3) 10 mL of methanol 0.1% ammonium hydroxide evaporated, 4) 10 mL ultra-pure water evaporated, 5) 10 mL of methanol passed only through the manifold without cartridge and then 6) 10 mL of methanol passed only through the cartridge by gravity. All evaporated samples were reconstituted in methanol.

Carry-over between injections was also checked by performing an

analysis of four consecutive injections of standard solution (2500 ng/L) together with an internal standard mixture (1000, 3000 and 4000 ng/L), followed by methanol blanks (eq. S1, Supplementary material).

2.5.2. Salinity effects on the matrix

A pre-validation step was carried out to test the salinity effect on the SPE recoveries (Fig. 2). Commercial drinking mineral water was used to test the salinity effect on the SPE recoveries. Three different salinities (2, 9 and 17 PSU) were tested, each with four replicates. These values were calculated considering the average of high, medium and low salinity values measured previously in low tide. Before the SPE, samples were spiked with 100 µL of 2 ng/L to obtain 2000 ng/L in the final extract (SPE concentration factor was 1000x).

Recovery assessment (eq. S4, supplementary material) was performed through the quotient between commercial water spiked with PFAS before SPE and matrix-matched blanks spiked after SPE, with the

Salinity matrix optimization and extraction of PFAS

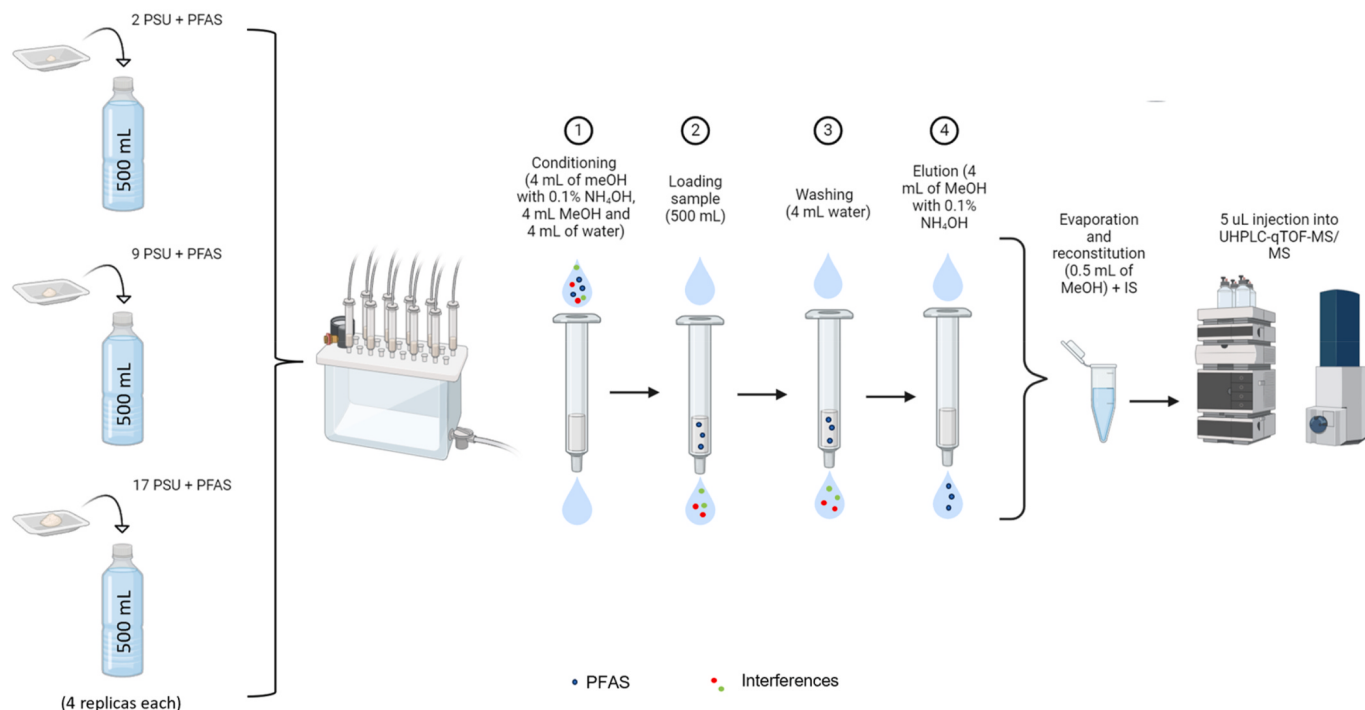


Fig. 2. Schematic representation of the methodology employed to assess the effects of varying salt concentrations on SPE recovery process.

same salt concentration.

2.5.3. Validation criteria

The validation procedure for identifying and quantifying 18 different EPA 573.1 PFAS mixture followed the ICH Guidelines for method validation (EMEA, 2022). For each compound, standardised parameters were evaluated: the limits of detection (LODs, ng/L), limits of quantification (LOQs, ng/L), recovery (%), accuracy (%), intra-day precision (% RSD), inter-day precision (% RSD), matrix effect (%) and stability (%) for over 90 days. Standard formulas for calculating these parameters can be found in the supplementary material (eqs. S1-S9). A pre-determined matrix-matched sample (commercial drinking mineral water) with a salt content of 9 PSU, was used to validate all these criteria.

The limits of detection (LODs) and quantification (LOQs) were evaluated based on the standard deviation of 10 independent matrix-matched blanks, achieved through 10 SPE of commercial water ($n = 10$), injected three times, and determined using eq. S2 and eq. S3, where α is the standard deviation of the blanks, and S is the average slope of the calibration curve, ranging from 20 ng/L to 2500 ng/L. The area of the blanks was determined at the same retention times as the compounds. Afterwards, the LODs and LOQs were reconfirmed with the signal/noise (S/N) calculated by Bruker's Data Analysis software.

For linearity, three ($n = 3$) independent calibration matrix-matched curves with nine points, ranging from 40 ng/L to 5120 ng/L, were used in the final extract. The ISs were set in 1300, 3900 and 5200 ng/L for [¹³C₈] PFOA, [¹³C₈] PFOS, N-methyl-D₃, respectively. Final peak areas were integrated manually, and the mathematical curve was obtained using the ratio between the standard and IS areas.

Recoveries (%), matrix effect (%), intra-precision (%RSD), accuracy (%), and stability of the target PFAS were calculated at three levels: LOQ (385.4 ng/L), 2LOQ (770.8 ng/L) and 5LOQ (1927.2 ng/L) using three independent replicates ($n = 3$). PFHpA only has two levels because its LOQ value (645.85 ng/L) is above the first level (LOQ: 385.4 ng/L).

Recoveries (%) were calculated from the quotient between samples spiked before and after SPE, as previously described (eq. S4). The matrix

effect (ME, %) was determined by comparing the area ratio of matrix-matched spiked after extraction and the area of methanol spiked with PFAS ($n=3$) at 3 levels (eq. S5). Precision on the same day (intra-day precision, $n = 3$) and between three consecutive days (inter-day precision, $n = 3$) were expressed as the relative standard deviation (%RSD). Intra-day was calculated from the quotient between the standard deviation (SD) ($n = 3$) and the average ($n = 3$) at three different levels (eq. S6), while the inter-day precision was calculated through the quotient between the mean of the SD ($n = 3$) and the mean of the average (3) of the different days (day 1, 2 and 3) at a concentration of 2000 ng/L (eq. S7). Accuracy was evaluated as the percentage (%) from the calibration curve in the matrix and the nominal concentration at three levels (eq. S8). Finally, the stability (%) of the selected PFAS in the extracts was evaluated at -4 °C, over time (Day 0, 1, 2, 3, 7, 14, 30, 60, and 90) (eq. S9). To assure the quality of the run, a quality control (QC) as 2LOQ was injected in every 10th injection, and a methanol blank was injected in every 20th injection.

2.6. Quantification of target compounds in the environment

The nine-point calibration curve (0, 40, 80, 160, 320, 640, 1280, 2560, and 5120 ng/L) was generated by spiking mineral water with the pre-selected intermediate salinity (9 PSU), using the mixture standard stock solution (at 1000 µg/L) to spike before extraction procedure. After extraction, the IS mixture (5 µL) was added, resulting in a final concentration in the extract of 1300 ng/L of [¹³C₈] PFOA, 3900 ng/L of [¹³C₈] PFOS and 5300 ng/L of N-methyl-D₃.

All validation procedures and environmental samples corrected the integrated areas by subtracting the matched-matrix blanks. As mentioned above, QC was injected in every 10th injection, and a methanol blank was injected in every 20th injection.

2.7. Data and statistical analyses

Calibration curves and linearity parameters were performed using Microsoft® Excel 2016 (Redmond, WA, USA). Linear regression of

matrix-matched calibration curves was assessed using an F-test (significance level of 5 %).

The cumulative PFAS concentration (Σ PFAS) from each sampling location was calculated by summing the median concentration of the quantified PFAS.

To avoid losing environmental results, samples falling between LOD and LOQ values were recalculated following the method Beal (2001) outlined as LOQ/2; values below their respective LOD were not considered.

One-way ANOVAs were performed to evaluate statistical differences in the potential source of LC contamination, the stability of the extracted samples over time, and the differences between the sampled sites. Prior to that, normality using the Kolmogorov–Smirnov test and homogeneity of variances using Levene's test (Zar, 1999) were checked. Statistical analyses were performed in STATISTICA version 7 software.

3. Results

3.1. Method validation

The LC performance was assessed using different concentrations of ammonium acetate in both mobile phases (2 mM and 10 mM), and the results indicated that a lower concentration was more effective.

The optimisation for PFAS quantification in UHPLC-qTOF-MS/MS showed a double PFOA peak in the methanol blanks (Fig. S1). To identify contamination sources in the SPE methodology (Fig. 2), different reagents and devices were systematically tested (Fig. 3) and compared with methanol blanks. No significant differences ($p > 0.05$) were found between the areas of the tested samples, indicating no external contamination during PFAS extraction, evaporation and reconstitution. Thus, contamination was originated from the UHPLC-qTOF-MS/MS system.

To support this hypothesis, internal background contamination was evaluated by injecting consecutive matrix-matched blanks, and results showed constant areas of PFOA contamination (11.80% RSD).

Regarding carry-over results between samples, a relatively low value (0.70%) was registered, providing assurance that no carry-over occurred within at least the four sample injections. This was assured by injecting methanol blanks between every four injections.

PFHxA was excluded from the subsequent validation steps due to poor ionisation, leading to low or undetectable peaks at 2000 ng/L concentrations. So, 17 PFAS were completely validated.

The impact of salinity on PFAS recovery at different NaCl concentrations was assessed. Results revealed no significant differences (one-

way ANOVA, $p > 0.05$) between almost all the PFAS. PFHpA, PFOA, PFOS, HFPO-DA, and 9Cl-PF3ONS recoveries at 17 PSU were significantly lower than those at 2 and 9 PSU (see Fig. 4).

The LOD values for the 17 PFAS ranged between 14.20 ng/L to 127.70 ng/L and LOQ values between 117.80 ng/L to 385.00 ng/L. The LOD and LOQ values of PFHpA were substantially higher than the other studied PFAS, i.e. 213.13 ng/L and 645.85 ng/L, respectively.

The linearity of the nine-point calibration curves showed a r^2 ranging from 0.989 to 0.998 (Table 2), passing successfully at F-test ($p < 0.001$).

PFAS recoveries ranged between 33.73% (for PFBS) and 113.77% (for HFPO-DA), with most of the compounds (76.47% PFAS) having recoveries above 80%. The results showed higher recovery rates (average of 99.31 %) at the LOQ level and lower recoveries (average of 83.76%) at 5LOQ level. PFBS had higher recoveries (68.06%) at lower concentrations (LOQ) compared to 33.73% (2LOQ) and 34.92% (5LOQ).

Regarding the matrix effect, 82.35% of compounds have a signal suppression at lower concentrations (LOQ and/or 2LOQ) and an increase in signal at higher concentrations (5LOQ). The other compounds (PFTrIDA, PFTeDA and ADONA) enhance the signal for the three studied levels (Fig. 5).

The precision results for intra-day ranged from 2.39% to 14.91%, and inter-day precision ranged between 3.85% and 7.38% (Fig. 6).

Accuracy results ranged between 82.00% and 115.68%, except for PFBS at 5LOQ, which had lower accuracy (63.05%).

The PFAS stability ranged from 86.01% to 126.56%, with no significant differences (one-way ANOVA, $p > 0.05$) among the studied days (Fig. 7).

3.2. Quantification of PFAS in marinas

In the three sampling sites, only 5 PFAS of different subgroups were possible to quantify: PFOA, HFPO-DA, PFBS, PFHxS and PFOS (Fig. 8). In general, the concentrations found were relatively low (average values of 0.19 ng/L) in the different sampling sites, with a maximum Σ PFAS at Freixo marina (Σ PFAS = 1.02 ng/L). However, no statistically significant differences (one-way ANOVA, $p > 0.05$) between sites were detected.

In more detail, concentrations in S1 ranged between 0.13 ng/L (PFOS) and 0.22 ng/L (PFBS), in S2 oscillated between 0.13 ng/L (PFOA) and 0.30 ng/L (PFBS) and in S3 they ranged between 0.07 ng/L (PFOS) and 0.32 ng/L (PFOA). Detailed information on the concentrations of PFAS in the different sampling locations can be assessed in the supplementary material (Table S3).

For more details on physicochemical information at different

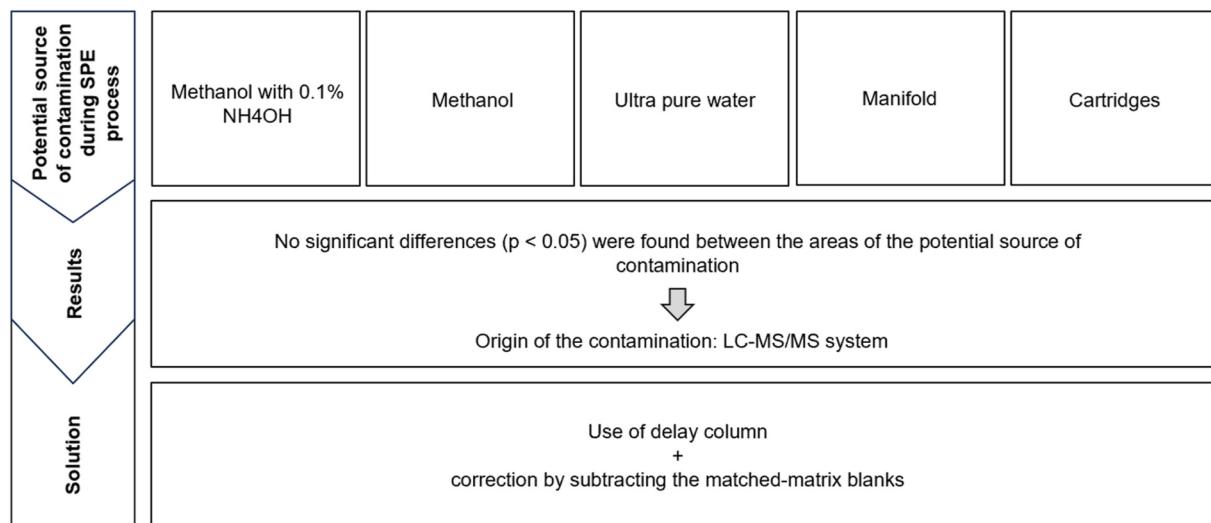


Fig. 3. Schematic figure of potential sources of PFAS contamination and results during the SPE process.

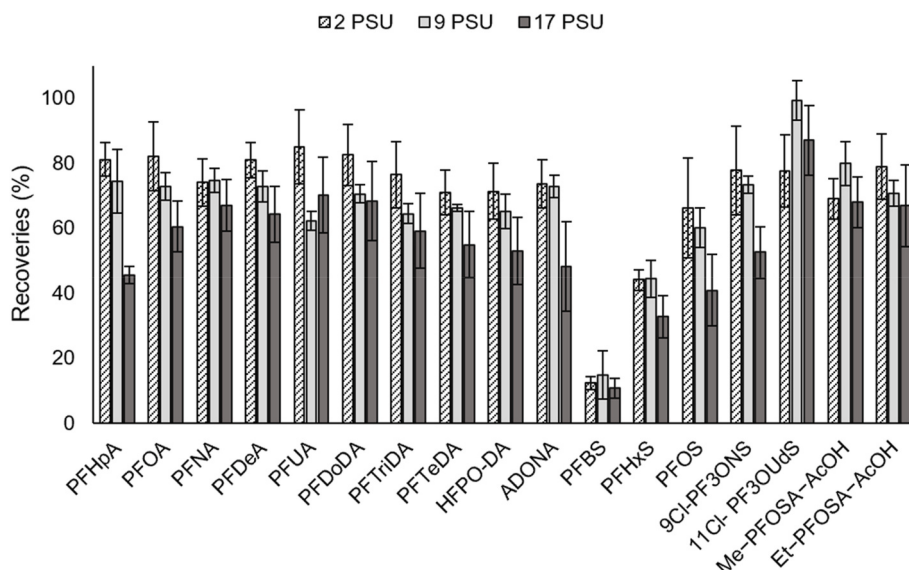


Fig. 4. The effect of salinity on the recovery of 17 EPA 537.1 PFAS mixture ($n = 4$) at 2000 ng/L. Values represent mean $\pm$ SD (%).

sampling sites, see Table S2 in the supplementary material.

4. Discussion

4.1. Method validation

EPA Method 537.1 was developed in 2018 to determine 18 PFAS in drinking water by LC-MS/MS. Since then, several studies have used this method for environmental water samples (Prakash et al., 2019; Mottaleb et al., 2021; Li et al., 2022). This study successfully validated the SPE method for 17 PFAS in surface waters from marina samples.

The addition of ammonium acetate into the mobile phases is important to improve the ionisation in the MS (Ariffin and Anderson, 2006) and guarantee a good peak performance. Therefore, two concentrations of ammonium acetate (2 mM and 10 mM) were tested based on previous studies (de Vega et al., 2021; Nassazzi et al., 2022). While both concentrations had a good peak performance, the lower concentration was preferred because elevated instrument pressures were observed at the highest concentration, posing potential risks to column integrity and autosampler functionality.

Contamination by PFAS of the LC system is well known, so a delay column was used (Coggan et al., 2019; Ali et al., 2021; Olomukoro et al., 2021). Due to the PFOA constant peak, LODs and LOQs were determined from matrix-matched blanks to attain more reliable and accurate results. Comparing LODs and LOQs obtained in this study with those of other studies poses challenges due to variations in equipment sensitivity and calculation methods. In general, most of the environmental studies calculated these parameters through the signal-to-noise (S/N) of the equipment with the target compounds, resulting in lower LOD and LOQ values (Cheng et al., 2021; Mulabagal et al., 2018). However, employing a matched matrix approach, which considers the background of extraction and equipment, may increase the S/N and lead to higher LOD and LOQ values. Nevertheless, it decreases the risk of overestimation of PFAS concentrations in environmental matrices. Additionally, the current SPE method concentrates the sample 1000x, enabling the quantification of environmentally relevant concentrations.

The NaCl content, when quantifying PFAS, can impact the recovery of compounds in SPE and alter the signal in LC-MS/MS, ultimately affecting the precision of the quantitative results (Liu et al., 2020). Brumovský et al. (2018) reported differences in the recoveries between salinities (0%, 50% and 100% of seawater) using WAX cartridges. The PFCA subgroup showed lower recovery rates with the increase of salinity

(approximately 48.00%, 38.00% and 41.00%, respectively, to the salinity indicated above), while the PFSA subgroup did not have significant differences ($p > 0.05$) between salinities (recoveries above 80%). In the present study, some PFAS also showed significant differences ($p < 0.05$) but were not necessarily associated with subgroups like the study above. Therefore, the chosen matrix for validating the remaining criteria had an intermediate salinity (9 PSU) to be coherent with higher salinity samples. Salinities of marina samples varied between 0.1 PSU and 9.6 PSU (Table 2, supplementary material); consequently, no corrections were made in the final concentration of the environmental samples. One of the advantages of this method is the use of a matrix with the presence of salt, which enhances the reliability of environmental sample quantification. However, the method results in higher LODs and LOQs for the target compounds. This issue can be addressed by concentrating the sample using SPE followed by evaporation. Assessing matrix effects in the matched matrix was an important step in understanding the potential alterations in the ionisation efficiency of the target analytes. So, matrix-matched effects were carried out, showing that the matrix (at the selected salinity: 9 PSU) had effects on ionisation at different levels (LOQ, 2LOQ and 5LOQ) and could affect the accuracy of the method. For this reason, validation criteria were always evaluated in matched-matrix to decrease the matrix effects. This approach guarantees higher reliable results and prevents the sub-estimation or overestimation of the target compounds.

This study proposed nine calibration points to cover all compounds since they have different LOQs values. Compared to other environmental studies, where calibration curves ranged from 50 to 10,000 ng/L (Mulabagal et al., 2018) to 250 to 50,000 ng/L (Gardiner et al., 2022), the range used in this study falls within these established ranges, ensuring compliance with relevant standards.

The accuracy obtained in this study is similar to Partington et al. (2023) (97.1–117.2% for 25 PFAS in a LC-qTOF-MS/MS), showing a good method efficiency.

Most of the 17 PFAS had high recovery rates, which were similar to the ones reported by Zhao et al. (2016), which ranged from 81.00% to 124.00% for and Partington et al. (2023) that ranged from 70.00 to 130.00% for 25 PFAS. PFBS showed lower recoveries at 33.73% (2LOQ) and 34.92% (5LOQ), which does not represent a concern because the calibration curve was performed in matrix-matched, covering the losses of the method.

The intra-day precision and inter-day precision (%RSD) results obtained between replicates and injections consistently fell below the

Table 2

Method validation parameters: linearity, LODs (ng/L), LOQs (ng/L), recovery (%), accuracy (%), intra-day precision (%RSD) for the 17 EPA 573.1 PFAS mixture at three levels (LOQ, 2LOQ and 5LOQ).

PFAS	Level (ng/L)	Linear equation	R ²	p-value of F test	LOD (ng/L)	LOQ (ng/L)	Recovery (%)	SD	Accuracy (%)	SD	Intra-day (%)	SD
PFHpA*	1	y = 0.0008x + 0.6691	0.994	<0.0001	213.13	645.85	101.52	4.50	109.78	6.28	10.75	3.52
	2						92.37	8.06	100.03	6.49	9.29	1.90
	3						110.45	16.72	95.06	12.64	7.23	2.31
PFOA	1	y = 0.0051x + 1.58	0.994	<0.0001	38.87	117.80	107.85	6.83	110.01	5.77	9.61	2.32
	2						89.39	8.39	107.08	11.48	5.49	3.05
	3						95.38	9.20	110.94	8.20	14.91	6.81
PFNA	1	y = 0.0013x + 0.2431	0.994	<0.0001	114.26	346.23	80.82	0.25	114.92	8.29	4.12	1.75
	2						94.51	9.83	100.64	7.79	4.23	2.99
	3						107.15	2.61	97.10	18.30	13.59	8.18
PFDeA	1	y = 0.002x + 0.3661	0.990	<0.0001	63.21	191.56	86.10	8.09	93.78	13.90	12.17	0.45
	2						95.45	10.52	105.22	10.94	4.58	2.91
	3						103.40	6.35	105.97	9.66	10.32	3.36
PFUA	1	y = 0.0021x + 0.461	0.992	<0.0001	76.37	231.42	99.97	2.92	96.37	4.35	6.54	3.61
	2						111.25	11.48	107.72	10.33	4.79	1.95
	3						102.59	5.30	96.70	11.03	9.51	4.73
PFDoDA	1	y = 0.0025x + 0.784	0.994	<0.0001	56.98	172.66	90.73	8.55	110.20	4.81	10.64	2.02
	2						93.49	7.11	97.09	9.15	7.46	0.80
	3						103.89	1.19	109.28	9.66	8.01	6.69
PFTriDA	1	y = 0.0027x + 0.7675	0.992	<0.0001	75.96	230.17	92.66	8.32	102.19	12.25	8.10	4.95
	2						88.05	10.16	100.95	9.52	3.33	1.79
	3						101.48	2.92	106.41	8.66	11.85	3.95
PFTeDA	1	y = 0.0022x + 0.7929	0.991	0.0003	41.51	125.80	84.66	4.07	101.89	10.64	11.93	2.06
	2						80.80	8.26	105.25	11.46	4.65	1.70
	3						113.77	13.58	82.00	14.73	2.39	1.63
HFPO-DA	1	y = 0.0054x + 0.3485	0.991	0.0002	113.54	344.05	60.78	4.46	92.35	10.79	10.47	1.16
	2						73.35	10.73	101.42	8.91	7.43	4.36
	3						99.73	6.07	92.23	9.57	7.30	0.60
ADONA	1	y = 0.0052x + 0.6038	0.992	<0.0001	57.11	173.06	82.03	5.09	98.49	6.89	4.80	2.40
	2						89.75	8.97	103.84	8.14	2.69	1.99
	3						68.06	7.06	96.67	15.05	11.45	0.43
PFBS	1	y = 0.0002x - 0.0232	0.991	<0.0001	14.20	43.04	33.73	5.30	82.27	15.05	4.52	0.60
	2						34.92	1.18	63.05	6.28	4.44	2.86
	3						87.51	7.56	106.25	6.49	7.56	1.08
PFHxS	1	y = 0.0002x + 0.0012	0.997	<0.0001	28.83	87.36	55.56	5.94	100.60	19.85	3.83	1.20
	2						64.63	6.47	106.40	4.47	4.03	2.12
	3						95.72	6.48	106.28	7.12	11.84	0.34
PFOS	1	y = 0.0003x + 0.0384	0.998	<0.0001	45.22	137.02	70.16	9.07	105.10	9.95	3.56	1.54
	2						82.37	7.40	112.03	9.45	4.38	2.96
	3						101.48	2.92	107.01	4.86	14.17	1.16
9Chloro 3F	1	y = 1E-04x + 0.025	0.991	<0.0001	46.77	141.74	84.66	4.07	108.88	9.07	7.62	5.52
	2						80.80	8.26	115.68	7.17	5.50	2.04
	3						92.45	6.96	112.26	10.14	6.49	0.32
11 chloro	1	y = 5E-05x + 0.0174	0.989	<0.0001	127.17	385.38	64.03	7.67	107.61	7.34	4.75	1.25
	2						77.12	5.31	115.64	3.44	3.73	3.49
	3						100.00	9.48	103.36	16.61	11.21	2.47
Me PFOSA	1	y = 0.0002x + 0.0464	0.991	0.0002	123.79	375.13	79.08	6.82	95.74	13.90	6.13	2.57
	2						90.92	9.84	91.33	9.55	4.82	1.33
	3						103.76	8.62	105.81	8.25	11.16	3.90
Et PFOSA AcOH	1	y = 0.0002x + 0.0464	0.991	<0.0001	73.20	221.83	82.87	8.75	102.62	8.75	6.84	2.70
	2						84.80	6.21	105.44	11.15	4.65	2.61
	3											

* Due to the higher LOQ, this compound has only two concentration levels (Level 1: 770.8 and level 2: 1927 ng/L).

established threshold (RSD <20%), indicating that the equipment has a high degree of reliability being capable of consistently producing accurate results over time (Pizzutti et al., 2009). Therefore, the precision criteria were successfully met. These results showed the method's reliability without using internal standards at the beginning of the extraction. Therefore, samples were strategically spiked with the IS at the end of the extraction process to address potential variations in instrumental analysis. After extraction, the high stability of the samples allows the user to reinject samples if necessary and store them in vials, reducing storage space and energy costs.

These findings are important to validate a robust method, mainly when analysing matched-matrix and environmental samples.

4.2. Quantification of PFAS in marinas' surface waters of douro river

Five different PFAS were quantified in the three sampling sites, presenting similar concentrations among sites. The ΣPFAS concentration

at each location was relatively low (1.02 ng/L; S3). Comparing these results with other studies was challenging due to the limited available literature, though a few relevant studies were identified. For example, Kaserzon et al. (2012) detected PFAS in the Sydney Harbour, ranging between 0.25 ng/L (PFUA) and 16 ng/L (PFOS). Their presence in the ecosystem was attributed to the use of AFFF in firefighting and other industries. Another common practice in the marine industry is using waxed materials to protect boats from degradation (Glüge et al., 2020). It is already known that some of the waxes also used in the ski boards contain PFAS. For example, Müller et al. (2023) demonstrated that most fluorinated waxes (81.82%) for skis contained PFOA above the European Union regulatory limit of 25 ng/g. They proved that those PFAS could be transferred to snow and soils, with concentrations ranging from <LOQ to 143 ng/L and <LOQ to 5.35 ng/g dry weight, respectively. Following the same analogy, there is a possibility of PFAS transferring to water. Once released, PFAS can be dispersed and transported. Through a hydrodynamic model, Hodgkins et al. (2019) showed that these

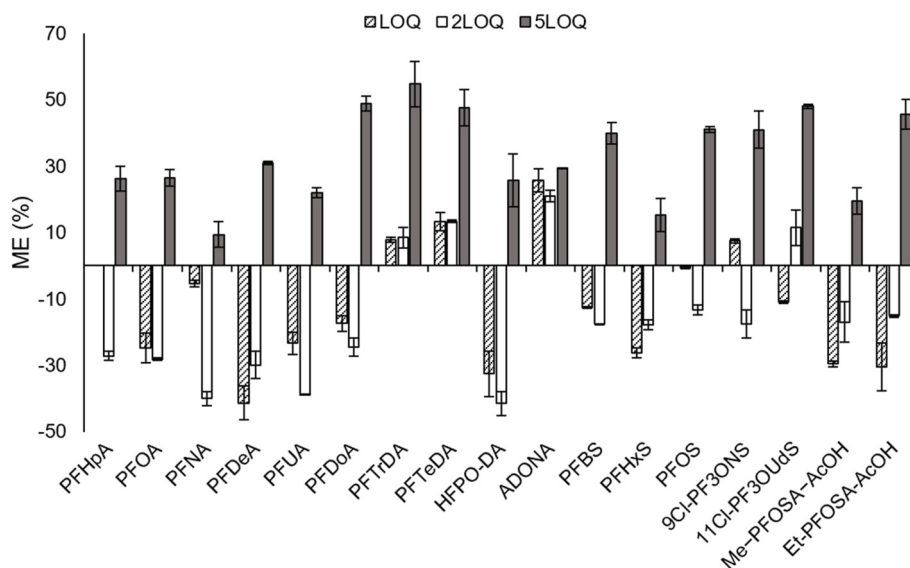


Fig. 5. Matrix effect (%) of PFAS ($n = 3$) at three levels. Values represent mean $\pm$ SD (%).

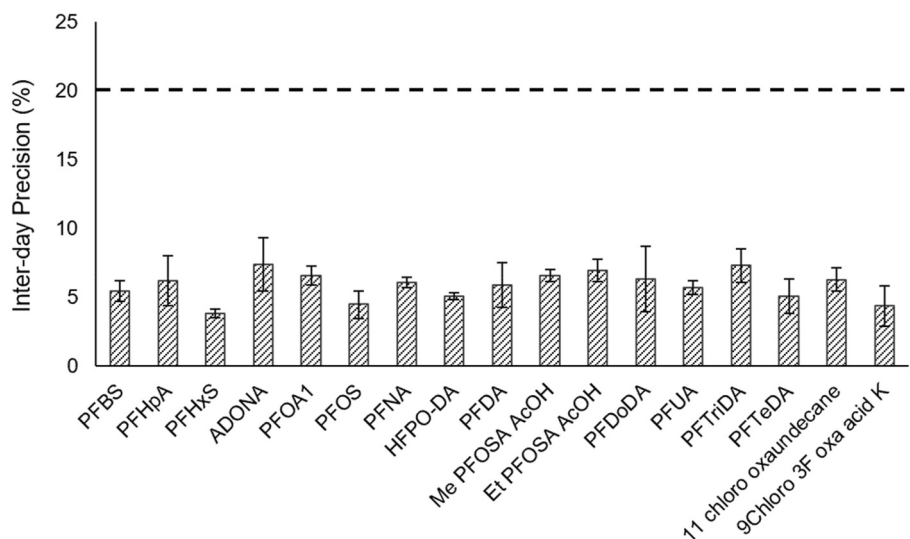


Fig. 6. Inter-day precision of 17 EPA 573.1 PFAS mixture across three consecutive days ($n = 3$) at 2000 ng/L. Values represent mean $\pm$ SD (%). The dashed line represents the maximum % RSD required (<20 % RSD) (Pizzutti et al., 2009).

compounds released from a harbour (Halifax Harbour, Nova Scotia) can easily travel up to 31 km in 2 days during strong winds and waves.

Furthermore, studies of PFAS concentration in Portuguese rivers are very recent. Barbosa et al. (2023) showed similar concentration results for some compounds, such as PFBS (from 0.23 to 0.36 ng/L) and PFHxS (from not detected levels to 0.07 ng/L), across different rivers in Northern Portugal. These values align with the range of PFAS levels found in the Douro system, demonstrating a similar impact on these aquatic systems. For S1 and S2, PFBS was the most dominant PFAS. Its higher solubility in water (0.0017 mol/L) compared to longer-chain PFAS explains its prevalence, as it more readily reaches aquatic environments (Ahrens et al., 2015).

According to Environmental Quality Standards (EQS) (European Union Directive, 2013), the maximum allowable concentration of PFOS is 36000 ng/L, and the annual average is 0.65 ng/L, both for inland surface waters. In this study, PFOS levels were below 0.24 ng/L (S2), which is under the allowed concentration.

Notably, the presence of PFAS or related products used on boats does not necessarily imply that all boats are a significant source of PFAS

contamination in marinas. However, the cumulative impact of multiple sources, including boats, may contribute to global PFAS contamination in aquatic ecosystems.

5. Conclusion

This study developed an accurate extraction and quantification method for 17 PFAS, considering the salinity of the samples. This optimised method enables reliable quantification of these compounds in surface estuarine waters and is applicable to other aquatic environmental samples. The research underscores the significant impact of salinity on method validation, highlighting the need for future studies to consistently consider salinity effects, especially in environments exceeding 9 PSU. Additionally, future research should include seasonal sampling and explore other aquatic systems for comparison, as increased boat activity during summer may influence results.

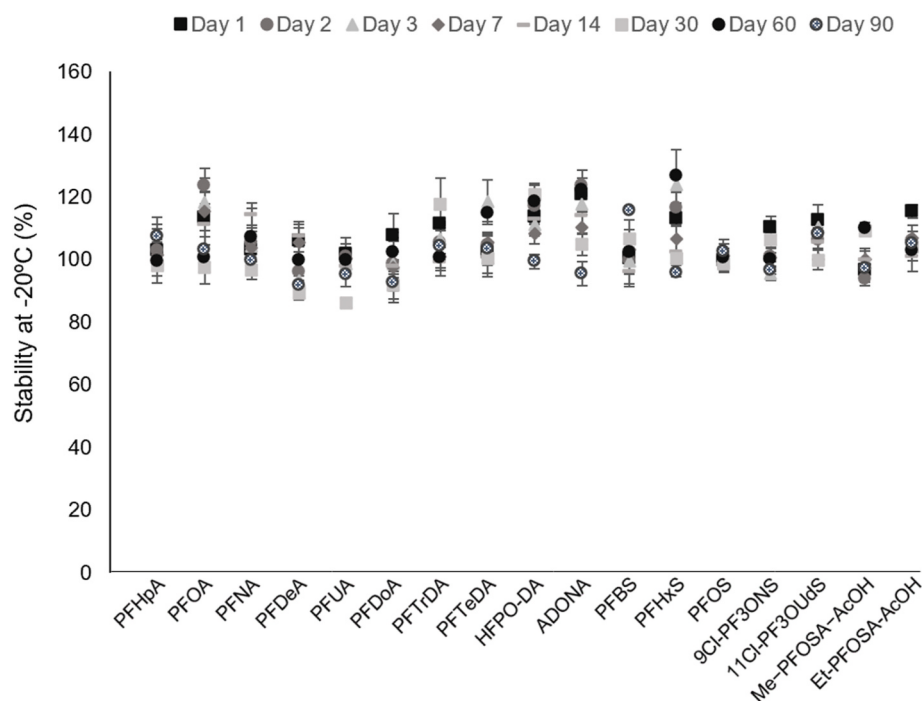


Fig. 7. Stability of EPA 573.1 PFAS mixture (n = 3) at 2000 ng/L over 90 days. Values represent mean $\pm$ SD (%). The dashed line represents the maximum stability (%) required (120%).

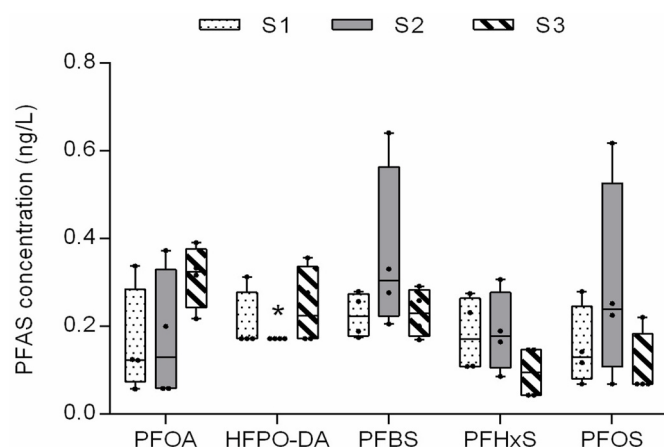


Fig. 8. PFAS real concentration (ng/L) in the different sampling locations divided by the SPE concentration factor (1000x). Values are represented as median $\pm$ SD (n = 4); S1: Afurada marina; S2: Freixo marina; S3: Cais da Ribeira port. (*) represent concentrations LOQ/2.

CRediT authorship contribution statement

V.E. Amorim: Writing – original draft, Methodology, Investigation, Data curation. **A.C. Silva Ferreira:** Writing – review & editing, Validation, Supervision. **C. Cruzeiro:** Writing – review & editing, Validation, Supervision, Methodology. **P.G. Cardoso:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Patricia Cardoso reports financial support was provided by FCT. Patricia Cardoso reports financial support was provided by NORTE2020. If there

are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.119805>.

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