# Determination of regulated perfluoroalkyl substances (PFAS) in drinking water according to Directive 2020/2184/EU

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## Abstract

Perfluoroalkyl substances (PFAS) are chemical compounds that have been widely used in industry and manufacture. Occurrence, together with persistence and recent toxicological effects data, have promoted the regulation of 20 PFAS (carboxylic and sulfonic) acids in drinking water through the recent Directive 2020/2184/EU. This Regulation included PFAS with different carbon chain lengths (from $C_4$-$C_{13}$) and limited the total PFAS concentration (as sum) to a maximum of 0.1 µg/L, for which law-enforcement analytical methods are required. In this work, three different methodologies have been developed and evaluated as regards their performance to determine those 20 PFAS in tap and bottled water, based on on-line and off-line solid-phase extraction (SPE) and direct injection. In all cases ultra-high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was used as determination technique. Off-line SPE with Oasis Weak Anion Exchange (WAX) cartridges provided the best results in terms of limits of quantification (LOQ ≤ 0.3 ng/L) and accuracy (R ≥ 70%) in drinking water samples. On-line SPE and direct injection presented some drawbacks such as background contamination problems and lower accuracies for the least polar compounds. These off-line SPE methodology was then applied to the analysis of 46 drinking water samples (11 commercial bottled samples, 23 Spanish and 12 international tap water samples). Ten PFAS were quantified in such samples at concentrations and detection frequencies ranging from 0.1 to 20.1 ng/L and 2 to 91%, respectively. However, the sum concentration did not surpass the established limit in any sample.

**Keywords:** Perfluoroalkyl carboxylic acids (PFCAs), Perfluoroalkyl sulfonic acids (PFSAs), Solid-phase extraction (SPE), Water quality

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# 1. INTRODUCTION

Perfluoroalkyl substances (PFAS) constitute a large family of synthetic chemicals which has been manufactured for more than 80 years (OECD 2018), originally for flame retardant and electronic purposes and later expanded to many other applications (Glüge et al. 2020). Among the different families of PFAS, perfluoroalkyl carboxylic acids (PFCAs) or perfluoroalkyl sulphonic acids (PFSAs) have attracted more interest due to their exceptional chemical stability (Gagliano et al. 2020). Thus, their presence in surface water (Bai et al. 2021, Tang et al. 2022), sewage water (Yan et al. 2012), groundwater (Hepburn et al. 2019, Xu et al. 2021), sea water (Gutiérrez-Martín et al. 2023) and even drinking water (Mak et al. 2009, Gellrich et al. 2013) has been extensively reported.

According to the European Food Safety Agency (EFSA), diet is the main source of human exposure, which could be affected by the PFAS levels in drinking water, non-stick cookware and food packaging materials (EFSA et al. 2020) and several adverse effects on human health derived from this exposure have been reported (EEA 2019). Although in 2013 the European Commission decided to include perfluooctane sulfonic acid (PFOS) in the list of priority substances in the EU Directive 2013/39/EU (EC 2013), i.e. under the Water Framework Directive (WFD), many other PFAS with an important environmental presence still remained unlegislated. However, several recent initiatives from the Organisation for Economic Co-operation and Development (OECD) and the European Chemicals Agency (ECHA) promoted the complete PFAS ban (OECD , ECHA 2023). Recently, the EU Directive 2020/2184/EU (EC 2020) has been implemented to regulate the presence of 20 PFAS in drinking water, stablishing the maximum allowed concentration into 0.1 µg/L (as sum of the 20 regulated PFAS). Therefore, to fulfil this regulation requirements, the analytical methodologies should be able to reach limits of quantification (LOQs) lower than 1.5 ng/L for each of the 20 regulated congener.

The determination of PFAS in aqueous matrices has been extensively addressed, especially for those most well-known congeners, such as PFOS or perfluorooctanoic acid (PFOA) (Al Amin et al. 2020, Zarębska et al. 2023). However, recent concerns on congeners with shorter carbon chains, short-chain ($C_4$-$C_8$) or ultrashort-chain ($C_1$-$C_3$) PFAS, such as perfluorobutanoic acid (PFBA) or perfluoromethane sulfonic acid (PFMS) has challenged method development due to their higher polarity, that hinders both extraction and determination (Gremmel et al. 2017, Montes et al. 2020). Although some methods have addressed the determination of PFAS by gas chromatography coupled to mass spectrometry (GC-MS) with a derivatization step, poor sensitivity was achieved for the most polar ones, such as perfluoropentanoic acid (PFPeA) (Dufková et al. 2012), being the obtained LOQ up to 200 times higher, when compared with less polar congeners. Thus, liquid chromatography coupled to mass spectrometry (LC-MS) is the preferred technique to cover a wide range of PFAS (Mulabagal et al. 2018, Organtini et al. 2018, Montes et al. 2020). Some authors have proposed direct injection of water samples without pre-treatment into the LC-MS system. In this case, although recoveries and limits are acceptable for $C_5$-$C_{14}$ PFAS, such methods failed for the shortest-chain (below $C_5$) ones (Wolf et al. 2013, Organtini et al. 2018). Thus, to improve method sensitivity a sample preconcentration step becomes mandatory. In fact, most standardized and published methods targeting specific PFAS familyuse solid-phase extraction (SPE) as a preconcentration step before LC-MS determination. Styrene-divinylbenzene (SDVB) (USEPA 2009, USEPA 2020), Oasis Hydrophilic-Lipophilic Balance (HLB) (Mulabagal et al. 2018) or Oasis Weak Anion Exchange (WAX) cartridges (USEPA (2019), ISO ISO21675/2019 (2019)) are the most popular sorbents. Among the, the WAX sorbent is the best choice when dealing with short- and ultrashort-chain PFAS (Montes et al. 2020), except for marine/coastal water samples (Villaverde-de-Sáa et al. 2015). Another possibility is the use of on-line SPE, being less time-consuming and labour-intensive (Barreca et al. 2018, Gray et al. 2019, Mottaleb et al. 2021, Brandsch 2022), as it would be desired in order to increase method throughput.

Given the above-mentioned considerations, the objective of the present study was to evaluate different analytical approaches that can be used for law-enforcement according to Directive 2020/2184/EU (EC 2020). That means achieving LOQ ≤ 1.5 ng/L for each PFAS congener, without blank associated issues and validated in the whole drinking-water relevant concentration range (Hernández et al. 2023), keeping method simplicity and potential automation in perspective. Thus, the final proposed methodology could be easily implemented by laboratories to fulfil with the challenging Directive 2020/2184/EU analytical requirements. To reach this objective three different methods based on direct injection and mixed-mode off-line and on-line SPE prior to LC-MS analysis have been tested and the best performing validated.

Figure 1 illustrates the analytical problem, tested methodologies, found drawbacks and main conclusions. Besides, a second objective was obtaining information on the current situation of drinking water pollution as regards the 20 regulated PFAS. Thus, 46 bottled and tap water samples from different geographical origin have also been analysed.

## 2. MATERIALS AND METHODS

### 2.1. Reagents and materials

Analytical solution mixture containing 2 mg/L of 10 PFCAs and 10 PFSAs (reference EU-5813-NSS, see the list of compounds in the Supplementary Material, Table S1) in methanol was purchased from Wellington Laboratories (Guelph, ON, Canada). An internal standard (IS) mixture of 2 mg/L of 10 and 3 isotopically labelled PFCAs and PFSAs, respectively in methanol was also from Wellington Laboratories (ref. MPFAC-C-ES, Table S2). All analytical solutions were stored at 4ºC.

Ultrapure deionized water (18.2 MΩ/cm) was supplied by a Rephile water purification system (Shanghai, China). Methanol (MeOH) LC-MS grade was purchased from Fisher scientific (Portsmouth, NH, USA). Acetonitrile (ACN) LC-MS grade was purchased from VWR (Radnor, PA, USA). Ammonia 7N in methanol was purchased from Acros Organics (Antwerp, Belgium) and 25% ammonium hydroxide (aqueous solution) LC-MS quality was purchased from Scharlab (Barcelona, Spain). Glacial acetic acid (HAc) was obtained from Panreac (Barcelona, Spain) and ammonium fluoride with a purity ≥ 99.99% from Merck (Darmstadt, Germany).

### 2.2. Samples treatment

Bottled drinking water sold in Spanish supermarkets of variable hardness and container material (see Table S3) and tap water samples from different municipalities of Spain and other European and North American countries were analysed. Tap water was collected after letting the tap flow for 5 min with precleaned bottles by the authors or different colleagues (see Acknowledgments). In the last case they were shipped to the University of Santiago for its analysis frozen together with empty bottles of the same materials, as to assess for background contamination. Upon receival, they were kept at -20ºC until their analysis.

## 2.3. Instrumentation

The LC-MS/MS was composed by an Agilent 1290 Infinity II LC system coupled to an Agilent 6495 mass spectrometer (triple quadrupole), through a jet stream electrospray ionization source (ESI). The system includes an autosampler, furnished with a 1.5 mL extended loop in a multidraw valve, and a temperature-controlled column compartment. In addition to the binary analytical pump, it incorporates a quaternary pump to deliver samples, conditioning or clean-up solvents through the on-line connected SPE cartridges. Both SPE cartridge and analytical column, were connected using a 10-port, 2-possition valve located in an Agilent flexible cube module. Nitrogen was employed as nebulizing (30 psi), drying (120ºC, 11 L/min) and sheath gas (350ºC, 10 L/min) in the ESI source. The ESI source was operated in negative ionization (4000 V) and low-pressure and high-pressure radiofrequency voltages were 40 and 70 V, respectively. The fragmentor energy was set at 16 V and collision energy (CE) value was adjusted individually for every compound. Acquisition was performed in dynamic Multiple Reaction Monitoring (dMRM) mode recording one (IS) or two (analytes) precursor/product ion transitions per compound, whenever possible. Selected transitions, together with the CE values, retention times (RT) and IS are listed in the Table S4. Chromatographic conditions will be explained in the following sections together with sample preparation.

## 2.4. Analytical methods

In order to minimize the potential background of PFAS all glassware was baked at 300ºC for 12 hours before use (González-Mariño et al. 2019).

### 2.4.1. Off-line SPE

#### 2.4.1.1. SPE protocol

Off-line SPE was carried out using Oasis WAX-150 mg cartridges (copolymer of divinylbenzene and N-vinyl pyrrolidone, 30 µm particle size, 80 Å pore size) from Waters (Milford, MA, USA) as validated on previous works (Montes et al. 2020). For that purpose, 200 mL of water samples were spiked with 10 ng/L of IS and prior to sample loading, the cartridges were conditioned with 5 mL of MeOH and 5 mL of ultrapure water. Thereupon, samples were passed through the cartridges using a vacuum pump, and after sample loading, cartridges were washed consecutively with 10 mL of ultrapure water thereupon 10 mL of methanol and dried under a nitrogen stream (99.999%) for 30 min. Analytes were recovered with 10 mL of 5% $NH_3$ in MeOH. Eluates were evaporated to dryness under a nitrogen stream, resuspended in 200 µL of MeOH and filtered by 0.22 µm polytetrafluoroethylene (PTFE) hydrophobic filters (Phenomenex, Torrance, CA, USA) prior to analysis by LC-MS/MS.

#### 2.4.1.2. Chromatographic conditions

The separation of PFAS was carried out on a Zorbax Extend $C_{18}$ RRHT column (50 x 2.1 mm x 1.8 µm) from Agilent (Santa Clara, CA, USA) thermostated at 40ºC with a dual eluent system consisting of (A) 1 mM $NH_4F$ in ultrapure water and (B) 1 mM $NH_4F$ in MeOH at a flow rate of 0.3 mL/min. The gradient elution started with 10% B, held for 0.5 min and increasing to 100% B in 9.5 min, held for 2 min. Return to initial conditions (10% B) was performed in 0.1 min and held for 5 min for back-conditioning. The injection volume was set at 2 µL. In order to reduce the PFAS background from the system, a InfinityLab PFC delay column (4.6 x 30 mm) from Agilent was placed before the multisampler valve (Kamuf et al. 2020).

### 2.4.2. On-line SPE

For on-line SPE concentration, water samples were also spiked with IS (50 ng/L) and 0.005% HAc. The injection volume was 1.5 mL by the LC autosampler and loaded onto Strata-X-AW (20 x 2.0 mm, 85 Å pore size, maximum operational pressure 275 bar) 33 µm polymeric weak cartridge purchased from Phenomenex with similar functionalized polymeric sorbent than WAX cartridges. Compounds elution was carried out using 0.05% $NH_3$ with (A) ultrapure water and (B) methanol as mobile phases employed in the UHPLC separation process (see section 2.3).

The compounds were separated using the same analytical column than off-line SPE (Zorbax Extend $C_{18}$ RRHT) thermostated at 40ºC. The mobile phases consisted in ultrapure water (A) and methanol (B), both containing 0.05% $NH_3$, at a flow rate of 0.2 mL/min. The gradient elution started with 10% B, held for 6.5 min and increasing to 100% B in 15.5 min, held for 2 min. Return to initial conditions (10% B) was performed in 0.1 min and held for 5 min for back-conditioning. The injection volume was 1.5 mL, which was loaded onto Strata-X-AW cartridge, delivered by the quaternary pump, the SPE mobile phases were ultrapure water (C), 0.1% HAc in ultrapure water (D), 2.5% $NH_3$ in methanol (E) and methanol (F). The sample was loaded at 1 mL/min using a mixture C:D:F (85:5:10) for 2 min, then the cartridge was washed (the cartridge eluate sent to waste through the flexible cube 10-port valve) consecutively with C:F (90:10) held for 1 min, with C:F (25:75) held for 1 min and with C:F (90:10) held for 2.5 min. At this point (6.5 min), the flexible cube 10-port valve was switched for the elution of PFAS with the binary pump from the cartridge and the subsequent separation in the analytical column using the abovementioned gradient. Meanwhile the quaternary pump remained at 0.05 mL/min for 9 min with C:F (90:10), at 2 mL/min for 2.5 min with 100% E and finally at 1 mL/min for 2 min with C:D:F (85:5:10) for SPE cartridge back-conditioning purposes. As in the case of off-line SPE a delay column to avoid mobile phases contaminations is mandatory, however as the InfinityLab PFC delay column does not support the overly basic conditions of the SPE elution solvent (pH=12), as a replacement, another Strata-X-AW cartridge was used.

### 2.4.3. Direct injection

The direct injection of water samples was also performed spiking the sample vials with IS (50 ng/L) and 5 mM $NH_4F$. The injection volume was set to 1 mL and in this case, to avoid the column overload, and analytical column of higher dimensions was selected. Thus, a Zorbax Eclipse XDB $C_{18}$ column (100 x 2.1 mm x 3.5 µm) from Agilent, thermostated at 40ºC was selected. The mobile phases composition and flow rate were the same than used for off-line SPE method (ultrapure water or MeOH with 1 mM $NH_4F$ and 0.3 mL/min). The gradient elution started with 2% B, held for 0.5 min and increasing to 100% B in 12 min, held for 2 min. Return to initial conditions (2% B) was performed in 0.1 min and held for 5.5 min for conditioning.

## 2.5. Evaluation of analytical methods performance

The methods were evaluated in terms instrumental limits of quantification (iLOQ), repeatability and linearity. Analytes were quantified by internal standard calibration using the isotopic labelled IS. In those (eight) cases where no labelled analogues were available, the labelled compounds providing the best results in terms of trueness were selected (see Table S4).

The calibration curves for linearity were prepared in MeOH between iLOQ and 200 µg/L for off-line SPE and in ultrapure water in the iLOQ-500 ng/L and iLOQ-100 ng/L ranges, for on-line SPE and direct injection, respectively. Due to the detection of PFBA in ultrapure water, for this compound, bottled water A (see Table S3) was used in validation experiments. The IS level was 10 µg/L (off-line SPE) and 50 ng/L (on-line SPE and direct injection). iLOQs were calculated as the highest concentration from two different approaches: a) standard providing a signal-to-noise ratio (S/N) of 10 for the first MRM transition and ≥3 for the confirmation transition (when available) and b) considering the PFAS background as the mean of the blanks signal plus 10 times their standard deviation (Hernández et al. 2023). Instrumental repeatability was assessed following the recommendations of the Association of Official Analytical Chemists (AOAC, 2016) guidelines as the relative standard deviation (%RSD) calculated by dividing the standard deviation (SD) by the mean and expressing it as a percentage of six consecutive injections at two different levels of concentration (0.5 and 50 µg/L) in the case of off-line SPE method and at 10 and 50 ng/L level for on-line SPE and direct injection, respectively.

Trueness and precision of the whole methods were estimated by quintuplicated analysis from recovery experiments performed in tap and bottled water samples spiked at 100 ng/L (high-level), 10 ng/L (medium-level) and 1 ng/L (low-level) of each analyte and 10 ng/L of IS for off-line SPE and at 50 ng/L for on-line SPE and direct injection. Samples were also analysed without analyte addition in order to correct for their native content. Method limits of quantification (mLOQs) were assessed from spiked tap water samples, in analogous way to iLOQs.

Finally, quality control samples (QCs) were prepared and analysed in each sample batch to support the quality of analysis at 10 ng/L addition level. Satisfactory recoveries of QCs were considered between 70 and 120% and reliable identification of positives needed to comply established deviations in ion intensity ratios (≤30%) and retention time (≤0.1 min) in comparison with the reference standard (Hernández et al. 2023).

## 3. RESULTS AND DISCUSSION

### 3.1. Assessment of blank contamination

A key issue in PFAS determination is the background contamination already reported in the literature (González-Barreiro et al. 2006, Janda et al. 2019). To minimize this limitation, an InfinityLab PFC delay column (or Strata-X-AW cartridge for on-line SPE) from Agilent was placed before the multisampler valve in order to delay the retention time of PFAS introduced from the solvent, tubing, and the degasser, enabling the chromatographic separation from those PFAS present in the samples. An example of this separation for PFOS is shown in Figure S1. Subsequently, instrumental blanks were evaluated by running the chromatographic gradient without sample injection (false injection) and also injecting the corresponding solvent MeOH (solvent blank for off-line SPE method) or ultrapure water (solvent blank for on-line SPE and direct injection methods). The results revealed a PFBA background in the false injections from on-line SPE (42.5 ng/L) and in solvent injection (ultrapure water) from on-line SPE and direct injection (57.6 and 12.0 ng/L, respectively). This points to both a potential contamination of the ultrapure water used and the fact that the delay column was not enough to eliminate system blanks in the on-line SPE method since not all the solvents flow through the pre-injector delay column in this configuration. Although other water commercially available water samples were tested, similar results were obtained.

In addition, before performing the recovery studies at different levels, several blanks were assessed at the different stages of the sample preparation off-line SPE protocol to check for PFAS contamination. For this purpose, a cartridge blank (entire off-line SPE protocol without sample loading) and an elution solvent blank (10 mL MeOH 5% $NH_3$ evaporated to dryness and reconstituted) were studied separately (Table S5). PFBA and PFOA were found in both elution solvent and cartridge blanks at concentrations below 0.5 ng/mL in the final extract (which would correspond to 0.5 ng/L referred to 200 mL of sample). Thus, for both compounds off-line SPE protocol blanks were performed in each sample batch and their concentrations subtracted from samples.

## 3.2. Comparison of analytical methods

The validation of the LC-MS/MS methods was performed in terms of linearity, precision and iLOQs (Table 1). It must be noted that they differ on the column and mobile phases used. Thus, although the off-line SPE and on-line SPE method use the same ultrahigh-performance LC (UHPLC) column, the modifier in the case of off-line SPE is ammonium formate (to promote ionization in the negative mode), however this modifier could not be used in the on-line SPE method, since a higher pH is required for elution of the SPE mixed-mode pre-columns, thus 0.05% $NH_3$ was used instead. Also, a regular $C_{18}$ 3.5-µm LC column was used in the direct injection method, to accommodate a larger injection volume.

As presented in Table 1, linearity was satisfactory with determination coefficients ($R^2$) greater than 0.9951. The precision, in terms of intra-day RSD, was evaluated for the chromatographic method used for injecting off-line SPE extracts, through the injection of standards at two concentration levels, 0.5 and 50 µg/L, providing values below 4 and 3%, respectively. For on-line SPE and direct injection where the sample is directly submitted to the instrumental protocol, the repeatability for the injection of standards was satisfactory (below 20%) although a clear worsening in this parameter was observed for longest (C>9) carbon chain congeners. The iLOQs were estimated between 5-50, 0.1-5 and 0.1 to 50 ng/L for off-line SPE, on-line SPE and direct injection instrumental methodologies, respectively. The only exception was PFBA in on-line SPE achieving an iLOQ of 60 ng/L due to the abovementioned blank contamination issues (see section 3.1). It must be noted that iLOQs are very close to mLOQs in on-line SPE and direct injection approaches. All methods presented lower mLOQ for short-chain PFAS in comparison with the high-molecular weight congeners.

In summary, the on-line SPE instrumental method presented some drawbacks due to PFBA contamination and a low sensitivity and repeatability for long-chain PFAS. In direct injection method, the PFBA blank issues were avoided (through the use of the delay column), however sensitivity was not appropriate for C≥11 PFAS, which produced iLOQ >1.5 ng/L set as initial objective of this work (i.e. 30% of the result of dividing the total nominal 100 ng/L limit set by Directive 2020/2184/EU by the 20 regulated congeners). The accuracy and mLOQs of both direct injection and on-line SPE methods are provided in supplementary information (Table S6 and S7). The evaluated accuracy was not adequate for some congeners using both methods, (< 70%) being the lowest values found for the most hydrophobic PFAS. Most of PFASs were corrected with their isotopologues, but the absence of IS for the most hydrophobic PFAS (PFTrDA and PFSAs ($C_9$-$C_{13}$) together with the low sensitivity of these compounds, may could justify the low accuracies. Nevertheless, direct injection could be particularly useful as a fast-screening method due to its simplicity. Although the iLOQs were the highest for off-line SPE (Table 1), after a complete sample processing, a concentration factor of 1000 was achieved allowing the lowest mLOQs. Thus, off-line SPE became the only method that could match the regulatory objectives and was, therefore, fully validated (see section 3.3).

### 3.3. Validation of the off-line SPE-LC-MS/MS methodology

Accuracy, precision and mLOQs were evaluated at 3 concentration levels (1, 10 and 100 ng/L) with real matrices. The experiments at high and medium-levels (10 and 100 ng/L) were performed with tap water, however due to native levels of PFAS in that matrix, experiments at low level (1 ng/L) were performed with bottled water (Table 2). The chromatogram obtained for bottled water at 1 ng/L is shown in Figure 2. In all cases, recoveries between 70 and 120% and an RSD < 20% were obtained. mLOQs, estimated from the lowest spiking level and considering blanks (see section 2.5), were lower than 0.3 ng/L for all congeners, which implies that the off-line SPE proposed method is suitable to fulfil the sensitivity requirements of the Directive 2020/2184/EU and goes far beyond (lower than 30% of Directive regulation).

The influence of the water composition, in terms of salts content (hardness), was evaluated. Thus, matrix effect was calculated for a soft bottled water (A) and hard water (K) (Diggs et al. 2009). Figure S2 shows the matrix effects evaluated depending on sample hardness. In general, PFCAs were more affected by sample hardness than PFSAs and the matrix effects were higher (signal suppression >30%) for long-chain PFAS (C>11). However, when method accuracy was evaluated for both soft and hard waters the obtained values were satisfactory (>80%) which revealed that the selected IS corrected efficiently these eventual matrix effects.

### 3.4. Application to tap water and commercial bottled water

The cumulative concentrations of PFAS concentrations found in the samples (data about the samples is presented Table S3) are shown in Figure 3 and Figure 4, individual concentration of PFAS detected in each sample are reported in Table S8. PFAS were detected in most analysed samples, except in four commercial brands of mineral water where the concentration of PFAS was below mLOQs. In the remaining samples, the total concentrations ranged from 0.4 to 2.2 ng/L for mineral bottled water, and 1.0 to 48 ng/L for tap water samples. Therefore, none of the analysed samples exceed the limits stablished in the Directive 2020/2184/EU (EC 2020). As expected, due to their high mobility/polarity, short chain PFAS (particularly PFCAs) were the most abundant ones. In fact, PFBA and PFHxA contribute, as average, the 70% of total PFAS concentration. PFBA and PFHxA concentrations ranged between 0.2 and 12 ng/L and 0.3 and 20 ng/L, and their detection frequencies were 83% and 91%, respectively. Regarding long-chain PFCAs ($C_8$-$C_{11}$), they presented lower detection frequencies (9-78%) and concentrations between 0.1 and 5.1 ng/L, being PFOA the most abundant one. On the other hand, short-chain PFSAs ($C_4$-$C_7$) presented lower detection frequencies (2 to 39%) and were quantified in concentrations ranging between 0.1 and 8.1 ng/L, being the highest values found for PFBS, analogously to what happened with PFBA. Finally, the only long-chain PFSAs detected was PFOS (detection frequency 20%) with concentrations ranging from 0.2 to 9.3 ng/L. These results, in terms of most abundant PFAS and concentration levels are comparable to those found in drinking water from different countries, including Spain (Schwanz et al. 2016, Wang et al. 2016, Gao et al. 2019, Kurwadkar et al. 2022).

A geospatial distribution of the analysed samples and the sum of PFAS found (grouped by carbon chain length) can be found in supplementary material (Figure S3). From this figure it could be inferred that higher amounts of PFSA congeners are found in north Europe and America samples, in comparison with Spanish samples where short-chain PFCAs are more abundant. However, since the number of samples in each region is different (most samples were taken in Spain) the conclusions derived from this analysis would not be statistically relevant. In general, the PFAS levels in tap water samples (either from Spain or elsewhere) from public facilities were similar with values under 20 ng/L (Figure 4, Table S8). However, Knoxville (TN), Berlin, Milan and Monterrey (at international level) and Santander and Cartelle (at Spanish level) presented higher values. Berlin, Milan and Monterrey are important metropolitan and industrial cities in their countries, conversely to Knoxville and Santander which are mid-industrialised cities with high tourism influx. Surprisingly, Cartelle which is a scarcely industrialised and populated village in the northwest of Spain, hardly affected by tourism, presented also high values of PFAS concentrations, specially PFHxA and PFHpA. In the city of Milan, where previous studies have reported the presence of PFAS in drinking water (Castiglioni et al. 2015), the concentration values are comparatively high, although below Directive 2020/2184/EU limits, pointing to the ubiquitous contamination by PFAS in the city. Knoxville also sought to improve drinking water treatment due to PFDoA contamination (up to 820 ng/L) in drinking water (UCMR3 2021), however in our work moderate levels of PFAS, specially PFBA (11 ng/L) were still detected. A Spearman's correlations study was conducted for those PFAS found with a detection frequency in tap water higher than 50% (i.e. PFBA, PFHxA, PFHpA and PFOA). A substitution method was used for those PFAS below mLOQ by mLOQ/2. A statistically significant correlation was found in the concentration of the four congeners, confirming that such compounds are likely industrially employed as mixtures (Table S9).

The levels found in commercial mineral water are significantly lower than in tap water (PFAS levels for bottled water were below 2.2 ng/L),  in agreement with those reported in literature (Ünlü Endirlik et al. 2019, Chow et al. 2021), pointing to the fact that aquifers used in such cases are much less impacted by PFAS contamination.

## 4. CONCLUSIONS

Direct LC-MS/MS injection, besides determination after on-line and off-line SPE, methodologies were evaluated as potential law-enforcement methods for a total of 20 PFAS ($C_4$-$C_{13}$) included in the EU Directive 2020/2184/EU. Among them, on-line SPE was the worst performing method ought to issues related to blank contamination, while direct injection was close to the required mLOQs, excepting for the longer-chain PFAS congeners, and could thus be useful as screening method, because of its lower cost and being less labour intensive. Therefore, only off-line SPE achieved the required mLOQs to comply the Directive requirements. This method achieved mLOQs between 5 and 10 times lower than required by actual legislation, therefore being capable to deal with potentially more exigent future regulations and provided satisfactory performance in the 1-100 ng/L level (referring to individual PFAS).

The application of the method to 46 samples permitted the detection of 13 PFAS (10 short-chain and 3 long-chain PFAS). Thus, the most polar PFAS, PFBA, PFHxA and PFBS were the most frequently detected congeners of each group (PFCAs and PFSAs), also presenting the highest concentrations. PFOS and PFOA, the most well-known and studied PFAS, although detected in the samples, were found in lower concentrations than short-chain congeners, reinforcing the concern on mobile and persistent chemicals as drinking water pollutants and the possible substitution of longer-chain by shorter-chain PFAS. Bottled mineral water contamination was lower than tap water obtained from public facilities. Yet, none of the samples exceeded the total limit stipulated in Directive 2020/2184/EU.

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## Statements and Declarations

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### Ethical approval

The authors declare that there are no personal data in this work that may require ethics committee approval

### Consent to participate

The authors declare that they have consent from the responsible authorities at the institute/organization where the work has been carried out to publish these results

### Consent for publication

The authors declare that there is no individual person's data in any form in the present work

### Conflict of interest

The authors declared no competing interests

### Availability of data and materials

The authors declare that the results from samples analyzed are compiled in the online repository ZENODO: https://doi.org/ 10.5281/zenodo.10990801

### CRediT authorship contribution statement

Conceptualization: Rosa Montes, Rosario Rodil, José Benito Quintana

Methodology: Rosa Montes, Javier López-Vázquez, Rosario Rodil, José Benito Quintana

Validation: Javier López-Vázquez, Rosa Montes, Rosario Rodil, José Benito Quintana

Formal analysis: Javier López-Vázquez, Rosa Montes, Rosario Rodil, José Benito Quintana

Investigation: Javier López-Vázquez

Resources: Rosario Rodil, José Benito Quintana, Rafael Cela, María Teresa Pena, José Ángel Martínez-Pontevedra

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Writing - Review & Editing: all authors

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Supervision: Rosa Montes, Rosario Rodil, José Benito Quintana, María Teresa Pena, José Ángel Martínez-Pontevedra, Rafael Cela

Project administration: Rosario Rodil, José Benito Quintana

Funding acquisition: Rosario Rodil, José Benito Quintana, Rafael Cela

**Captions to figures:**

**Figure 1:** Graphical abstract - Outline of the analytical problem, approaches and main conclusions

**Figure 2:** Chromatograms obtained after submitting bottled water (A) at low addition level (1 ng/L) to the final off-line SPE-LC-MS/MS methodology. A) PFCAs and B) PFSAs.

**Figure 3:** Stacked barplot sum of PFAS concentrations (ng/L) in bottled mineral water samples.

**Figure 4:** Stacked barplot sum of PFAS concentrations (ng/L) on A) international and B) Spanish tap water samples.

**Captions to tables:**

**Table 1.** Instrumental UHPLC-MS/MS validation parameters for the three evaluated methods.

**Table 2.** Off-line SPE mLOQs and accuracy and precision (n=5) obtained at low (1 ng/L), medium (10 ng/L) and high (100 ng/L) addition level.

**Table 1. Instrumental UHPLC-MS/MS validation parameters for the three evaluated methods.**

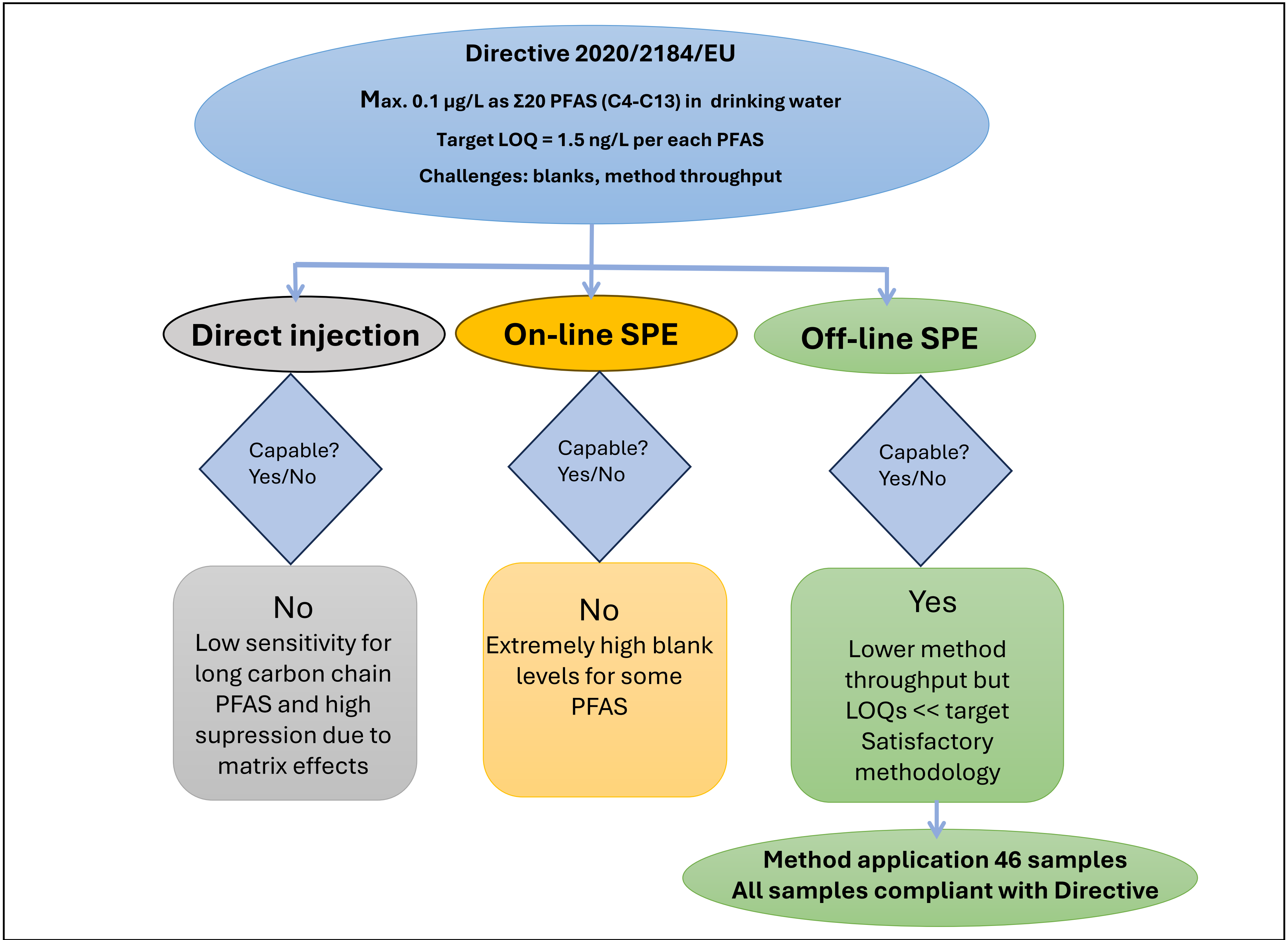
| Compounds | Repeatability n=6 RSD (%) | | | | Determination coefficient ($R^2$) | iLOQ (ng/L) | | |
|---|---|---|---|---|---|---|---|---|
| | Off-line SPE 0.5/50 µg/L | | On-line SPE 10 ng/L | Direct injection 50 ng/L | | Off-line SPE | On-line SPE | Direct injection |
| **PFBA** | 1 | 1 | 7 | 11 | > 0.9981 | 10 | 60 | 1.0 |
| **PFPeA** | 1 | 1 | 3 | 4 | > 0.9978 | 10 | 2.5 | 0.7 |
| **PFHxA** | 2 | 1 | 1 | 3 | > 0.9988 | 10 | 1.5 | 0.7 |
| **PFHpA** | 1 | 1 | 2 | 3 | > 0.9980 | 10 | 1.5 | 0.1 |
| **PFOA** | 1 | 1 | 2 | 6 | > 0.9986 | 10 | 1.0 | 0.9 |
| **PFNA** | 2 | 1 | 7 | 12 | > 0.9989 | 5 | 1.0 | 0.1 |
| **PFDA** | 1 | 1 | 10 | 18 | > 0.9972 | 20 | 1.0 | 0.1 |
| **PFUdA** | 1 | 1 | 7 | 17 | > 0.9984 | 35 | 1.0 | 1.0 |
| **PFDoA** | 2 | 2 | 20 | 20 | > 0.9975 | 35 | 2.0 | 10 |
| **PFTrDA** | 2 | 1 | 3 | 6 | > 0.9953 | 50 | 5.0 | 40 |
| **PFBS** | 1 | 1 | 2 | 1 | > 0.9986 | 10 | 0.5 | 0.5 |
| **PFPeS** | 1 | 1 | 1 | 6 | > 0.9977 | 50 | 0.5 | 0.1 |
| **PFHxS** | 1 | 1 | 1 | 6 | > 0.9988 | 25 | 0.5 | 0.1 |
| **PFHpS** | 1 | 1 | 9 | 7 | > 0.9989 | 10 | 0.1 | 0.1 |
| **PFOS** | 2 | 1 | 8 | 9 | > 0.9977 | 30 | 0.1 | 0.2 |
| **PFNS** | 2 | 1 | 11 | 5 | > 0.9969 | 15 | 0.2 | 0.2 |
| **PFDS** | 2 | 1 | 6 | 10 | > 0.9971 | 20 | 0.1 | 1.3 |
| **PFUdS** | 4 | 3 | 13 | 16 | > 0.9970 | 20 | 5.0 | 10 |
| **PFDoS** | 2 | 1 | 18 | 20 | > 0.9954 | 10 | 5.0 | 15 |
| **PFTrDS** | 3 | 1 | 20 | 20 | > 0.9951 | 25 | 5.0 | 50 |

**Table 2. Off-line SPE mLOQs and accuracy and precision (n=5) obtained at low (1 ng/L), medium (10 ng/L) and high (100 ng/L) addition level.**

| Compounds | mLOQ (ng/L) | Accuracy (%RSD) | | |
|---|---|---|---|---|
| | | Bottled water[1] | Tap water[1] | |
| | | 1 ng/L | 10 ng/L | 100 ng/L |
| **PFBA** | 0.15 | 91 (19) | 107 (4) | 114 (3) |
| **PFPeA** | 0.30 | 113 (8) | 107 (1) | 106 (3) |
| **PFHxA** | 0.10 | 91 (17) | 106 (2) | 109 (3) |
| **PFHpA** | 0.20 | 115 (4) | 107 (2) | 111 (3) |
| **PFOA** | 0.05 | 113 (16) | 113 (13) | 111 (3) |
| **PFNA** | 0.15 | 118 (8) | 104 (5) | 111 (3) |
| **PFDA** | 0.15 | 118 (4) | 111 (2) | 117 (4) |
| **PFUdA** | 0.15 | 115 (8) | 109 (2) | 118 (6) |
| **PFDoA** | 0.20 | 117 (3) | 112 (3) | 119 (10) |
| **PFTrDA** | 0.10 | 112 (13) | 71 (13) | 90 (14) |
| **PFBS** | 0.10 | 109 (7) | 116 (2) | 119 (5) |
| **PFPeS** | 0.10 | 113 (2) | 119 (3) | 119 (8) |
| **PFHxS** | 0.20 | 120 (8) | 107 (3) | 111 (3) |
| **PFHpS** | 0.20 | 101 (4) | 110 (2) | 111 (3) |
| **PFOS** | 0.15 | 112 (10) | 90 (4) | 119 (9) |
| **PFNS** | 0.15 | 100 (15) | 113 (9) | 119 (11) |
| **PFDS** | 0.25 | 104 (14) | 105 (6) | 108 (11) |
| **PFUdS** | 0.10 | 118 (19) | 86 (7) | 83 (17) |
| **PFDoS** | 0.10 | 115 (14) | 73 (17) | 70 (16) |
| **PFTrDS** | 0.30 | 120 (9) | 80 (10) | 91 (8) |

[1] Specific samples used in the validation are shown in Table S2

**Fig. 1. Graphical abstract - Outline of the analytical problem, approaches and main conclusions**



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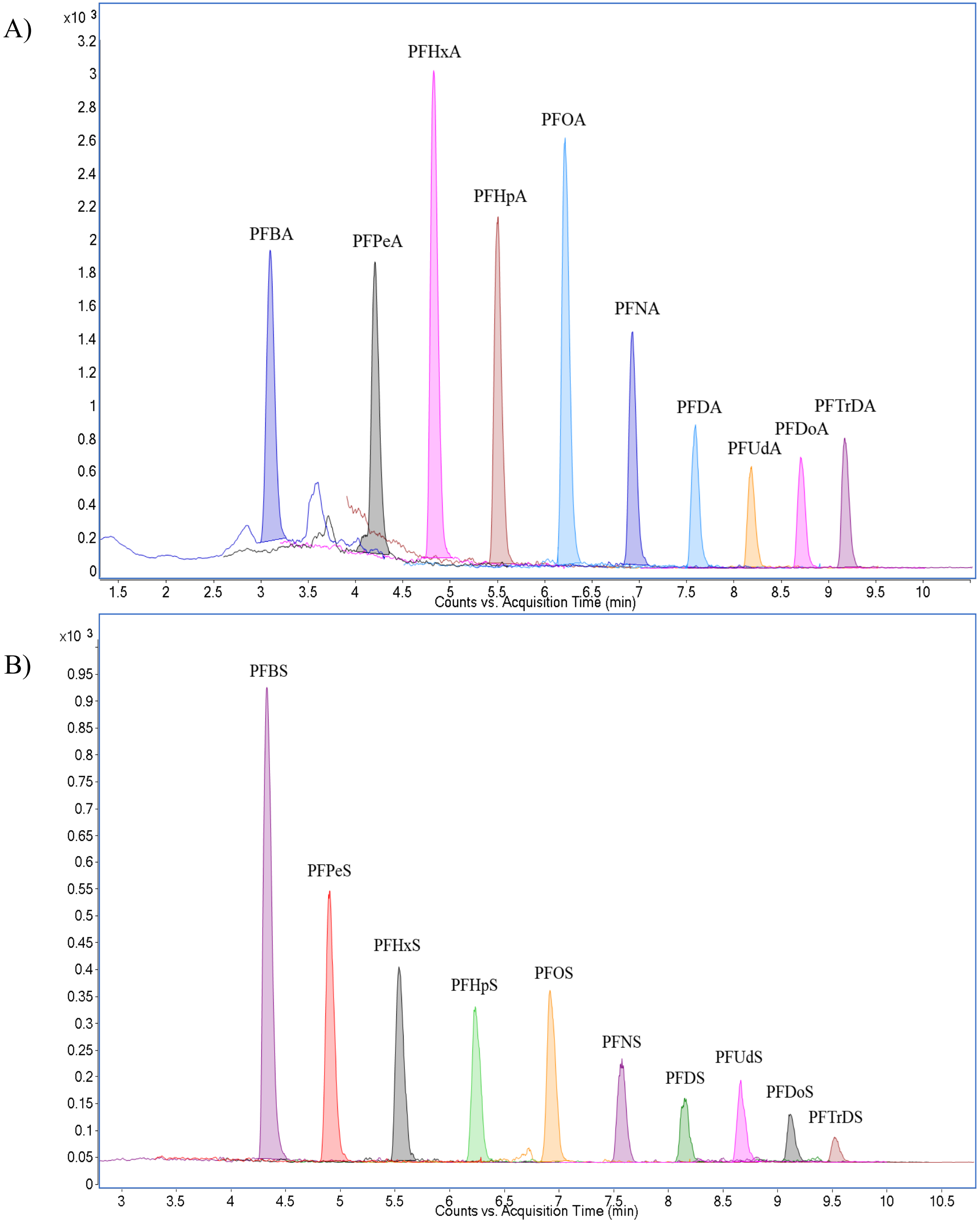

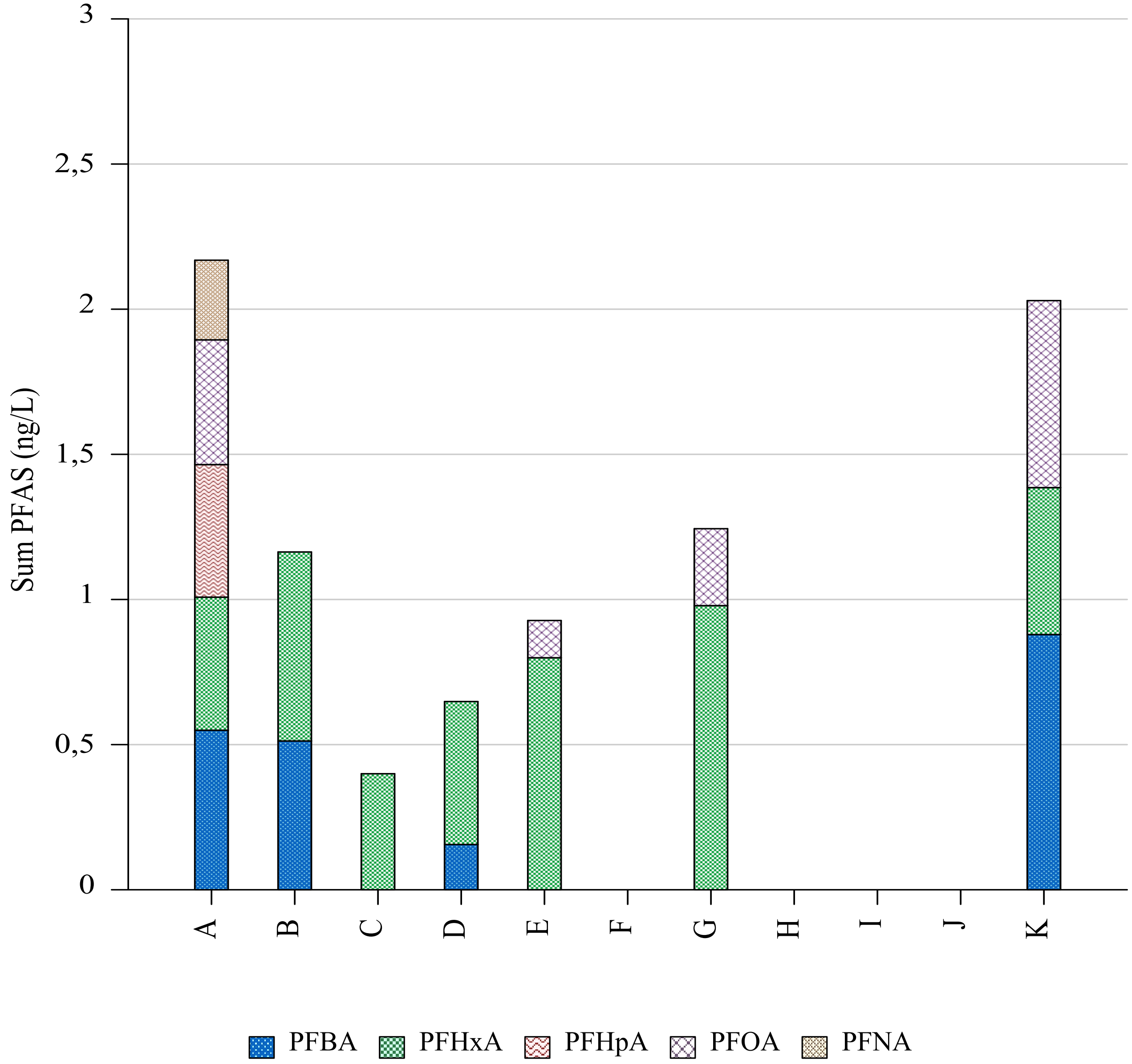

**Fig. 3.** Stacked barplot sum of PFAS concentrations (ng/L) in bottled mineral waters

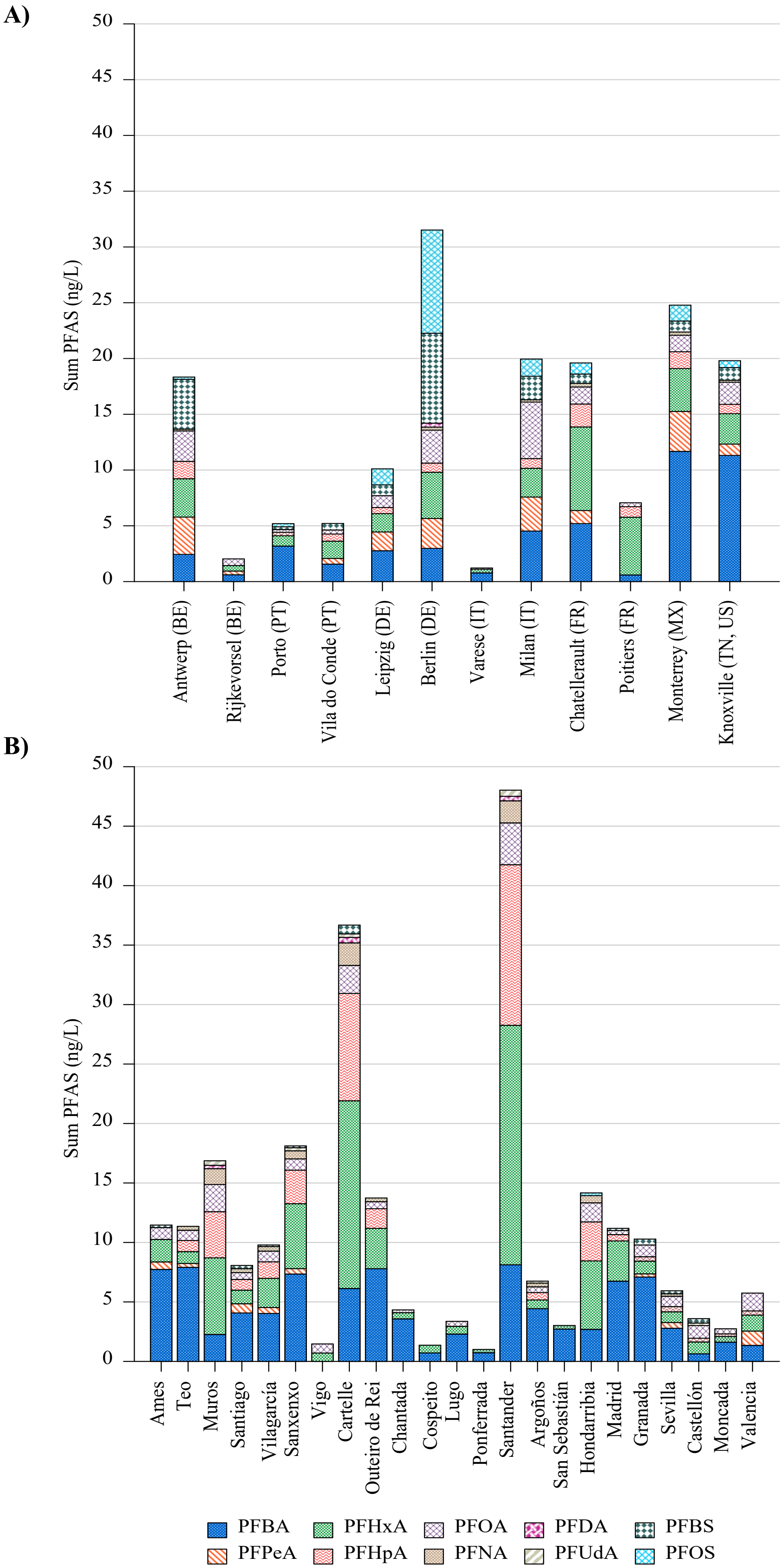

**Fig. 4.** Stacked barplot sum of PFAS concentrations (ng/L) on A) international and B) Spanish tap water samples.

**Supplementary Information**

# Determination of regulated perfluoroalkyl substances (PFAS) in drinking water according to Directive 2020/2184/EU

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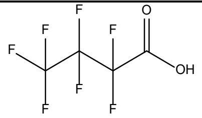
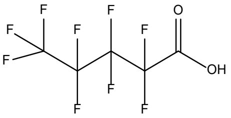
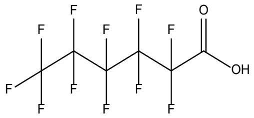
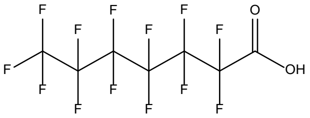
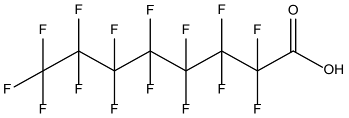
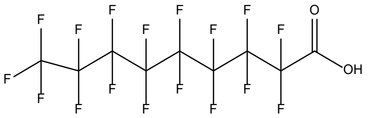
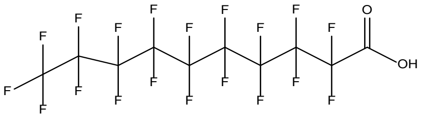
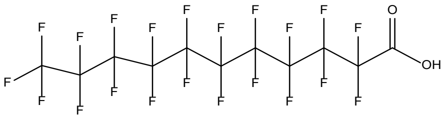
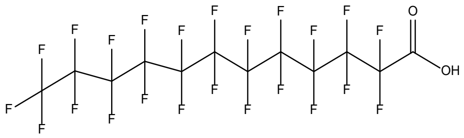
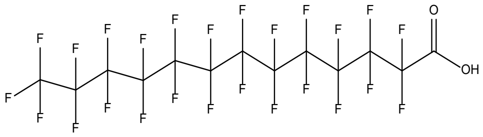
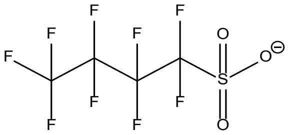
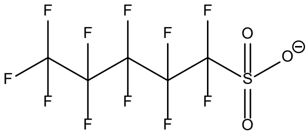
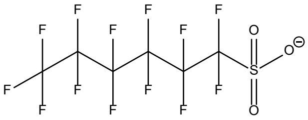
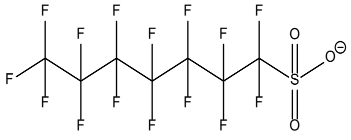
**Number of Pages: 18**

**Number of Tables: 9**

**Number of Figures: 3**

# Contents

**Table S1. Chemical information of PFCAs and PFSAs considered in the study.**

| Name | Acronym | Structure | CAS RN |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA |  | 375-22-4 |
| Perfluoropentanoic acid | PFPeA |  | 2706-90-3 |
| Perfluorohexanoic acid | PFHxA |  | 307-24-4 |
| Perfluoroheptanoic acid | PFHpA |  | 375-85-9 |
| Perfluorooctanoic acid | PFOA |  | 335-67-1 |
| Perfluorononanoic acid | PFNA |  | 375-95-1 |
| Perfluorodecanoic acid | PFDA |  | 335-76-2 |
| Perfluoroundecanoic acid | PFUdA |  | 2058-94-8 |
| Perfluorododecanoic acid | PFDoA |  | 307-55-1 |
| Perfluorotridecanoic acid | PFTrDA |  | 72629-94-8 |
| Perfluorobutanesulfonate | PFBS |  | 29420-49-3 |
| Perfluoropentanesulfonate | PFPeS |  | 630402-22-1 |
| Perfluorohexanesulfonate | PFHxS |  | 3871-99-6 |
| Perfluoroheptanesulfonate | PFHpS |  | 22767-50-6 |

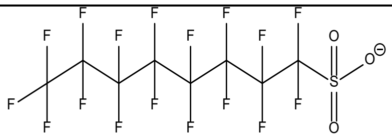
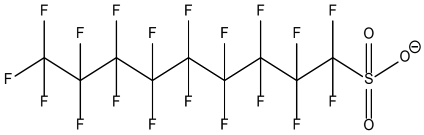
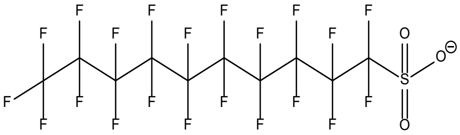
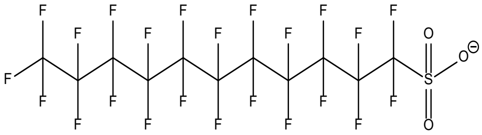
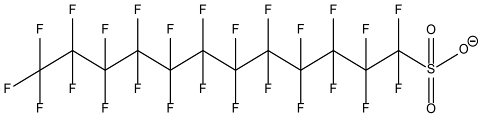
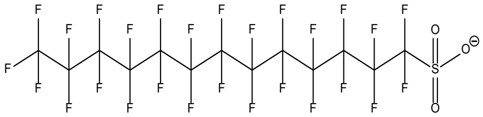
| Name | Acronym | Structure | CAS RN |
|---|---|---|---|
| Perfluorooctanesulfonate | PFOS |  | 4021-47-0 |
| Perfluorononanesulfonate | PFNS |  | 98789-57-2 |
| Perfluorodecanesulfonate | PFDS |  | 2806-15-7 |
| Perfluoroundecanesulfonate | PFUdS |  | 5838-34-6 |
| Perfluorododecanesulfonate | PFDoS |  | 2386-53-0 |
| Perfluorotridecanesulfonate | PFTrDS |  | 5802-89-1 |

**Table S2. Information of the IS used in the study.**

| Name | Acronym |
|---|---|
| Perfluoro-n-[$^{13}C_4$]butanoic acid | $^{13}C_4$PFBA |
| Perfluoro-n-[$^{13}C_5$]pentanoic acid | $^{13}C_5$PFPeA |
| Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid | $^{13}C_5$PFHxA |
| Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid | $^{13}C_4$PFHpA |
| Perfluoro-n-[$^{13}C_8$]octanoic acid | $^{13}C_8$PFOA |
| Perfluoro-n-[$^{13}C_9$]nonanoic acid | $^{13}C_9$PFNA |
| Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid | $^{13}C_6$PFDA |
| Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid | $^{13}C_7$PFUdA |
| Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid | $^{13}C_2$PFDoA |
| Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid | $^{13}C_2$PFTeDA |
| Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate | $^{13}C_3$PFBS |
| Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate | $^{13}C_3$PFHxS |
| Perfluoro-1-[$^{13}C_8$]octanesulfonate | $^{13}C_8$PFOS |

**Table S3. Location of commercial bottled water (A), Spanish tap water (B) and international tap water (C) samples. N.B. [1]Evaluated according to Diggs & Parker, 2009. [2] This water was used in the validation experiments. [3]This water was used in the hardness effect evaluation. SP: Spain, IT: Italy**

| A) | Hardness[1] | Bottle material | Commercial Brand (code) | Manufacturing Location |
|---|---|---|---|---|
| | Soft | Glass | Mondariz[2] (A) | Mondariz (SP) |
| | Soft | Glass | Cabreiroá (B) | Verín (SP) |
| | Soft | Plastic | Sousas (C) | Verín (SP) |
| | Soft | Plastic | Fontecelta (D) | Sarria (SP) |
| | Soft | Plastic | Aquabona (E) | Cospeito (SP) |
| | Soft | Plastic | Bezoya (F) | Segovia (SP) |
| | Soft | Plastic | Aguadoy (G) | Toledo (SP) |
| | Hard | Glass | San Benedetto (H) | San Benedetto del Tronto (IT) |
| | Hard | Plastic | Nestlé (I) | Badajoz (SP) |
| | Hard | Plastic | Solán de Cabras (J) | Cuenca (SP) |
| | Hard | Plastic | Font Vella[3] (K) | Guadalajara (SP) |

| B) | Hardness | Bottle material | Location | Province (SP) |
|---|---|---|---|---|
| | Soft | Glass | Ames | A Coruña |
| | Soft | Glass | Teo | A Coruña |
| | Soft | Glass | Muros | A Coruña |
| | Soft | Glass | Santiago de Compostela[1] | A Coruña |
| | Soft | Glass | Vilagarcía de Arousa | Pontevedra |
| | Soft | Glass | Sanxenxo | Pontevedra |
| | Soft | Glass | Vigo | Pontevedra |
| | Soft | Glass | Cartelle | Ourense |
| | Soft | Glass | Outeiro de Rei | Lugo |
| | Soft | Glass | Chantada | Lugo |
| | Soft | Glass | Cospeito | Lugo |
| | Soft | Glass | Lugo | Lugo |
| | Soft | Glass | Ponferrada | León |
| | Soft | Glass | Santander | Cantabria |
| | Soft | Glass | Argoños | Cantabria |
| | Soft | Glass | San Sebastián | Guipúzcoa |
| | Soft | Glass | Hondarribia | Guipúzcoa |
| | Soft | Glass | Madrid | Madrid |
| | Hard | Glass | Granada | Granada |
| | Hard | Glass | Sevilla | Sevilla |
| | Hard | Glass | Castellón | Castellón |
| | Hard | Plastic | Moncada | Valencia |
| | Hard | Plastic | Valencia | Valencia |

| C) | Hardness | Bottle | Location | Country |
|---|---|---|---|---|
| | Soft | Glass | Antwerp | Belgium |
| | Soft | Glass | Rijkevorsel | Belgium |
| | Soft | Glass | Porto | Portugal |
| | Soft | Glass | Vila do Conde | Portugal |
| | Soft | Glass | Leipzig | Germany |
| | Hard | Glass | Berlin | Germany |
| | Hard | Plastic | Varese | Italy |
| | Hard | Plastic | Milan | Italy |

| | | | |
|---|---|---|---|
| Hard | Plastic | Chatellerault | France |
| Hard | Plastic | Poitiers | France |
| Hard | Plastic | Monterrey | Mexico |
| Soft | Glass | Knoxville (Tennessee) | USA |

### Table S4. dMRM UHPLC-MS/MS analytical method for determination of PFAS.

| Compounds | RT (min) | | | 1st MRM transition (CE) | 2nd MRM transition (CE) | IS |
|---|---|---|---|---|---|---|
| | Off-line SPE | On-line SPE | Direct injection | | | |
| **PFBA** | 3.18 | 8.68 | 8.89 | 213 → 169 (5) | | $^{13}C_4$PFBA |
| **$^{13}C_4$PFBA** | 3.18 | 8.68 | 8.89 | 217 → 172 (5) | | |
| **PFPeA** | 4.24 | 9.35 | 8.90 | 263 → 219 (4) | | $^{13}C_5$PFPeA |
| **$^{13}C_5$PFPeA** | 4.24 | 9.35 | 8.90 | 268 → 223 (4) | | |
| **PFHxA** | 4.97 | 9.79 | 8.91 | 313 → 269 (5) | 313 → 119 (21) | $^{13}C_5$PFHxA |
| **$^{13}C_5$PFHxA** | 4.97 | 9.79 | 8.91 | 318 → 273 (5) | | |
| **PFHpA** | 5.50 | 10.15 | 8.93 | 363 → 319 (8) | 363 → 119 (20) | $^{13}C_4$PFHpA |
| **$^{13}C_4$PFHpA** | 5.50 | 10.15 | 8.93 | 367 → 322 (8) | | |
| **PFOA** | 6.25 | 10.55 | 8.98 | 413 → 369 (5) | 413 → 169 (17) | $^{13}C_8$PFOA |
| **$^{13}C_8$PFOA** | 6.25 | 10.55 | 8.98 | 421 → 376 (5) | | |
| **PFNA** | 6.98 | 11.09 | 9.07 | 463 → 419 (9) | 463 → 219 (17) | $^{13}C_9$PFNA |
| **$^{13}C_9$PFNA** | 6.98 | 11.09 | 9.07 | 472 → 427 (9) | | |
| **PFDA** | 7.67 | 11.69 | 9.21 | 513 → 469 (8) | 513 → 269 (20) | $^{13}C_6$PFDA |
| **$^{13}C_6$PFDA** | 7.67 | 11.69 | 9.21 | 519 → 474 (8) | | |
| **PFUdA** | 8.25 | 12.28 | 9.38 | 563 → 519 (8) | 563 → 319 (20) | $^{13}C_7$PFUdA |
| **$^{13}C_7$PFUdA** | 8.25 | 12.28 | 9.38 | 570 → 525 (8) | | |

| Compounds | RT (min) | | | 1st MRM transition (CE) | 2nd MRM transition (CE) | IS |
|---|---|---|---|---|---|---|
| | Off-line SPE | On-line SPE | Direct injection | | | |
| **PFDoA** | 8.77 | 12.85 | 9.63 | 613 → 569 (12) | 613 → 269 (20) | $^{13}C_2$PFDoA |
| **$^{13}C_2$PFDoA** | 8.77 | 12.85 | 9.63 | 615 → 570 (12) | | |
| **PFTrDA** | 9.22 | 13.36 | 9.96 | 663 → 619 (12) | 663 → 169 (36) | $^{13}C_2$PFDoA |
| **$^{13}C_2$PFTeDA** | 9.71 | 13.84 | 10.40 | 715 → 670 (12) | | |
| **PFBS** | 4.45 | 9.48 | 8.90 | 299 → 80 (41) | 299 → 99 (33) | $^{13}C_3$PFBS |
| **$^{13}C_3$PFBS** | 4.45 | 9.48 | 8.90 | 302 → 80 (41) | | |
| **PFPeS** | 4.96 | 9.86 | 8.91 | 349 → 80 (48) | 349 → 99 (36) | $^{13}C_3$PFBS |
| **PFHxS** | 5.59 | 10.18 | 8.94 | 399 → 80 (44) | 399 → 99 (40) | $^{13}C_3$PFHxS |
| **$^{13}C_3$PFHxS** | 5.59 | 10.18 | 8.94 | 402 → 80 (44) | | |
| **PFHpS** | 6.27 | 10.61 | 8.98 | 449 → 80 (52) | 449 → 99 (44) | $^{13}C_3$PFHxS |
| **PFOS** | 6.98 | 11.13 | 9.06 | 499 → 80 (60) | 499 → 99 (49) | $^{13}C_8$PFOS |
| **$^{13}C_8$PFOS** | 6.98 | 11.13 | 9.06 | 507 → 80 (60) | | |
| **PFNS** | 7.61 | 11.71 | 9.19 | 549 → 80 (60) | 549 → 99 (48) | $^{13}C_9$PFNA |
| **PFDS** | 8.22 | 12.29 | 9.37 | 599 → 80 (56) | 599 → 99 (52) | $^{13}C_6$PFDA |
| **PFUdS** | 8.64 | 12.85 | 9.63 | 649 → 80 (60) | 649 → 99 (56) | $^{13}C_7$PFUdA |
| **PFDoS** | 9.21 | 13.36 | 9.89 | 699 → 80 (60) | 699 → 99 (56) | $^{13}C_2$PFDoA |
| **PFTrDS** | 9.52 | 13.84 | 10.30 | 749 → 80 (60) | 749 → 99 (60) | $^{13}C_2$PFTeDA |

**Table S5. Contamination of PFAS in elution solvent and cartridge blanks for off-line SPE protocol (n=3). Concentration in final extracts (200 µL).**

| Compounds | Blank cartridge, ng/mL (%RSD) | Elution solvent, ng/mL (% RSD) |
|---|---|---|
| **PFBA** | 0.40 (16) | 0.45 (16) |
| **PFPeA** | <iLOQ | <iLOQ |
| **PFHxA** | <iLOQ | <iLOQ |
| **PFHpA** | <iLOQ | <iLOQ |
| **PFOA** | 0.13 (22) | 0.19 (17) |
| **PFNA** | <iLOQ | <iLOQ |
| **PFDA** | <iLOQ | <iLOQ |
| **PFUdA** | <iLOQ | <iLOQ |
| **PFDoA** | <iLOQ | <iLOQ |
| **PFTrDA** | <iLOQ | <iLOQ |
| **PFBS** | <iLOQ | <iLOQ |
| **PFPeS** | <iLOQ | <iLOQ |
| **PFHxS** | <iLOQ | <iLOQ |
| **PFHpS** | <iLOQ | <iLOQ |
| **PFOS** | <iLOQ | <iLOQ |
| **PFNS** | <iLOQ | <iLOQ |
| **PFDS** | <iLOQ | <iLOQ |
| **PFUdS** | <iLOQ | <iLOQ |
| **PFDoS** | <iLOQ | <iLOQ |
| **PFTrDS** | <iLOQ | <iLOQ |

**Table S6. Trueness, precision and mLOQ for direct injection method in different drinking water samples (n=5).**

| Compounds | Accuracy (%RSD)[1] | | mLOQ (ng/L) | |
|---|---|---|---|---|
| | Tap water | Bottled water | Tap water | Bottled water |
| **PFBA** | 119% (17) | 156% (17) | 9.5 | 9.0 |
| **PFPeA** | 82% (5) | 109% (18) | 4.6 | 1.5 |
| **PFHxA** | 86% (5) | 115% (8) | 1.2 | 3.1 |
| **PFHpA** | 88% (10) | 110% (16) | 1.5 | 0.7 |
| **PFOA** | 95% (6) | 125% (11) | 1.1 | 2.3 |
| **PFNA** | 105% (10) | 101% (8) | 2.6 | 1.0 |
| **PFDA** | 78% (2) | 83% (10) | 1.0 | 1.9 |
| **PFUdA** | 46% (9) | 67% (12) | 5.0 | 1.5 |
| **PFDoA** | 31% (9) | 49% (24) | 6.0 | 5.5 |
| **PFTrDA** | 29% (15) | - | 10 | 32 |
| **PFBS** | 94% (6) | 93% (4) | 1.2 | 1.2 |
| **PFPeS** | 83% (5) | 83% (5) | 1.7 | 1.2 |
| **PFHxS** | 90% (5) | 97% (7) | 1.3 | 2.6 |
| **PFHpS** | 106% (6) | 96% (7) | 2.5 | 5.5 |
| **PFOS** | 85% (1) | 96% (8) | 4.5 | 6.4 |
| **PFNS** | 36% (9) | 76% (19) | 4.3 | 5.5 |
| **PFDS** | 41% (11) | 52% (11) | 6.3 | 6.5 |
| **PFUdS** | 36% (19) | 31% (27) | 20 | 24 |
| **PFDoS** | 51% (28) | - | 25 | 45 |
| **PFTrDS** | 52% (20) | - | 70 | 75 |

[1] Evaluated at 50 ng/L addition level

**Table S7. Trueness, precision and mLOQ for on-line SPE method in different drinking water samples (n=5).**

| Compounds | Accuracy (%RSD)[1] | | mLOQ (ng/L) | |
|---|---|---|---|---|
| | Tap water | Bottled water | Tap water | Bottled water |
| **PFBA** | - | - | - | - |
| **PFPeA** | 105% (12) | 98% (11) | 2.3 | 2.2 |
| **PFHxA** | 112% (11) | 101% (15) | 2.5 | 2.0 |
| **PFHpA** | 106% (9) | 92% (8) | 3.2 | 1.5 |
| **PFOA** | 94% (6) | 90% (7) | 1.6 | 1.1 |
| **PFNA** | 97% (12) | 101% (7) | 2.2 | 2.3 |
| **PFDA** | 104% (16) | 88% (5) | 3.8 | 2.3 |
| **PFUdA** | 72% (14) | 80% (9) | 3.1 | 2.9 |
| **PFDoA** | 58% (18) | 71% (12) | 2.0 | 3.8 |
| **PFTrDA** | 41% (20) | 62% (13) | 8.6 | 9.9 |
| **PFBS** | 89% (7) | 90% (11) | 1.6 | 1.3 |
| **PFPeS** | 97% (12) | 102% (8) | 3.9 | 3.1 |
| **PFHxS** | 112% (8) | 101% (13) | 4.3 | 3.0 |
| **PFHpS** | 107% (14) | 98% (11) | 4.1 | 4.3 |
| **PFOS** | 92% (12) | 87% (16) | 5.7 | 6.9 |
| **PFNS** | 80% (14) | 82% (8) | 7.3 | 11 |
| **PFDS** | 74% (16) | 90% (9) | 7.9 | 12 |
| **PFUdS** | 71% (20) | 75% (17) | 8.9 | 17 |
| **PFDoS** | 48% (18) | 53% (12) | 9.1 | 21 |
| **PFTrDS** | 42% (15) | 50% (14) | 10 | 26 |

[1] Evaluated at 50 ng/L addition level

**Table S8. Concentrations of PFAS (ng/L) in commercial bottled water (A-K) and tap water samples. Note: PFDoA, PFTrDA, PFNS, PFDS, PFUdS, PFDoS and PFTrDS concentrations were below mLOQ in all samples.**

| Sample | PFBA | PFPeA | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUdA | PFBS | PFPeS | PFHxS | PFHpS | PFOS | ∑PFAS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.6 | <mLOQ | 0.5 | 0.5 | 0.4 | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **2.2** |
| B | 0.5 | <mLOQ | 0.7 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.2** |
| C | <mLOQ | <mLOQ | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **0.4** |
| D | 0.2 | <mLOQ | 0.5 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **0.7** |
| E | <mLOQ | <mLOQ | 0.8 | <mLOQ | 0.1 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **0.9** |
| F | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **<LOQ** |
| G | <mLOQ | <mLOQ | 1.0 | <mLOQ | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.2** |
| H | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **<LOQ** |
| I | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **<LOQ** |
| J | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **<LOQ** |
| K | 0.9 | <mLOQ | 0.5 | <mLOQ | 0.6 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **2.0** |
| Ames (ES) | 7.7 | 0.6 | 1.9 | <mLOQ | 1.0 | <mLOQ | <mLOQ | <mLOQ | 0.2 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **11.5** |
| Teo (ES) | 7.9 | 0.3 | 1.0 | 0.9 | 0.9 | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **11.4** |
| Muros (ES) | 2.3 | <mLOQ | 6.4 | 3.9 | 2.3 | 1.3 | 0.3 | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **16.9** |
| Santiago (ES) | 4.1 | 0.8 | 1.1 | 0.9 | 0.6 | 0.3 | <mLOQ | <mLOQ | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **8.1** |
| Vilagarcía (ES) | 4.0 | 0.5 | 2.5 | 1.4 | 0.9 | 0.4 | <mLOQ | <mLOQ | 0.1 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **9.8** |
| Sanxenxo (ES) | 7.3 | 0.5 | 5.5 | 2.8 | 1.0 | 0.7 | <LOQ | 0.3 | 0.2 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **18.1** |
| Vigo (ES) | <mLOQ | <mLOQ | 0.70 | <mLOQ | 0.8 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.5** |
| Cartelle (ES) | 6.1 | <mLOQ | 15.8 | 9.0 | 2.4 | 1.9 | 0.5 | 0.3 | 0.7 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **36.7** |
| Outeiro de Rei (ES) | 7.8 | <mLOQ | 3.4 | 1.7 | 0.6 | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **13.7** |
| Chantada (ES) | 3.6 | <mLOQ | 0.5 | <mLOQ | 0.2 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **4.3** |
| Cospeito (ES) | 0.7 | <mLOQ | 0.7 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.4** |
| Lugo (ES) | 2.3 | <mLOQ | 0.7 | <mLOQ | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **3.4** |
| Ponferrada (ES) | 0.7 | <mLOQ | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.0** |
| Santander (ES) | 8.1 | <mLOQ | 20.1 | 13.5 | 3.5 | 1.9 | 0.4 | 0.5 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **48.0** |
| Argoños (ES) | 4.4 | <mLOQ | 0.7 | 0.6 | 0.5 | 0.3 | <mLOQ | 0.2 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **6.8** |

| **Sample** | **PFBA** | **PFPeA** | **PFHxA** | **PFHpA** | **PFOA** | **PFNA** | **PFDA** | **PFUdA** | **PFBS** | **PFPeS** | **PFHxS** | **PFHpS** | **PFOS** | **∑PFAS** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| San Sebastián (ES) | 2.7 | <mLOQ | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **3.0** |
| Hondarribia (ES) | 2.7 | <mLOQ | 5.8 | 3.3 | 1.6 | 0.6 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | 0.2 | **14.2** |
| Madrid (ES) | 6.8 | <mLOQ | 3.4 | 0.5 | 0.3 | <mLOQ | <mLOQ | <mLOQ | 0.2 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **11.2** |
| Granada (ES) | 7.1 | 0.3 | 1.1 | 0.4 | 1.0 | <mLOQ | <mLOQ | <mLOQ | 0.5 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **10.3** |
| Sevilla (ES) | 2.8 | 0.5 | 0.9 | 0.4 | 0.9 | 0.2 | <mLOQ | <mLOQ | 0.3 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **5.9** |
| Castellón (ES) | 0.6 | <mLOQ | 1.0 | 0.3 | 1.1 | <mLOQ | <mLOQ | 0.2 | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **3.6** |
| Moncada (ES) | 1.6 | <mLOQ | 0.5 | 0.2 | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **2.7** |
| Valencia (ES) | 1.4 | 1.2 | 1.3 | 0.4 | 1.5 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **5.7** |
| Antwerp (BE) | 2.4 | 3.3 | 3.4 | 1.6 | 2.7 | 0.2 | <mLOQ | <mLOQ | 4.5 | <mLOQ | 0.4 | <mLOQ | 0.2 | **18.7** |
| Rijkevorsel (BE) | 0.6 | 0.3 | 0.5 | <mLOQ | 0.6 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **2.0** |
| Porto (PT) | 3.2 | <mLOQ | 0.9 | 0.3 | 0.3 | <mLOQ | <mLOQ | <mLOQ | 0.3 | <mLOQ | 0.2 | <mLOQ | 0.3 | **5.4** |
| Vila do Conde (PT) | 1.6 | 0.5 | 1.6 | 0.6 | 0.4 | <mLOQ | <mLOQ | <mLOQ | 0.6 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **5.2** |
| Leipzig (DE) | 2.8 | 1.7 | 1.6 | 0.6 | 1.1 | <mLOQ | <mLOQ | <mLOQ | 1.0 | <mLOQ | 0.3 | <mLOQ | 1.4 | **10.4** |
| Berlin (DE) | 3.0 | 2.7 | 4.1 | 0.8 | 3.0 | 0.3 | 0.4 | <mLOQ | 8.1 | 1.8 | 6.8 | 0.3 | 9.3 | **40.3** |
| Varese (IT) | 0.8 | <mLOQ | 0.3 | <mLOQ | 0.1 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **1.2** |
| Milan (IT) | 4.5 | 3.1 | 2.6 | 0.9 | 5.1 | 0.2 | <mLOQ | <mLOQ | 2.1 | 0.2 | 1.1 | <mLOQ | 1.5 | **21.2** |
| Chatellerault (FR) | 5.2 | 1.2 | 7.5 | 2.1 | 1.5 | 0.3 | <mLOQ | <mLOQ | 0.8 | 0.1 | 1.6 | <mLOQ | 1.0 | **21.4** |
| Poitiers (FR) | 0.6 | <mLOQ | 5.2 | 0.9 | 0.4 | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | <mLOQ | **7.1** |
| Monterrey (MX) | 11.7 | 3.6 | 3.8 | 1.5 | 1.5 | 0.3 | <mLOQ | <mLOQ | 1.0 | 0.4 | 2.1 | <mLOQ | 1.4 | **27.3** |
| Knoxville (USA) | 11.3 | 1.0 | 2.7 | 0.8 | 2.0 | 0.2 | <mLOQ | <mLOQ | 1.1 | <mLOQ | 0.4 | <mLOQ | 0.6 | **20.2** |

**Table S9: Spearman correlations for PFAS found in at least 50% of tap water samples (n=35).**

| | | PFBA | PFHxA | PFHpA | PFOA |
|---|---|---|---|---|---|
| **PFBA** | **Corr.** | | 0.4805 | 0.5101 | 0.3797 |
| | **p-value** | | 0.0051 | 0.0029 | 0.0268 |
| **PFHxA** | **Corr.** | 0.4805 | | 0.8827 | 0.7313 |
| | **p-value** | 0.0051 | | <0.0001 | <0.0001 |
| **PFHpA** | **Corr.** | 0.5101 | 0.8827 | | 0.6814 |
| | **p-value** | 0.0029 | <0.0001 | | 0.0001 |
| **PFOA** | **Corr.** | 0.3797 | 0.7313 | 0.6814 | |
| | **p-value** | 0.0268 | <0.0001 | 0.0001 | |

Concentration values lower than mLOQ were substituted by mLOQ/2 for each congener.

**Figure S1: Comparative chromatograms for native PFOS in methanol with delay column (red line) and without delay column (green line) and PFOS standard in methanol (1 µg/L) with delay column (blue line)**

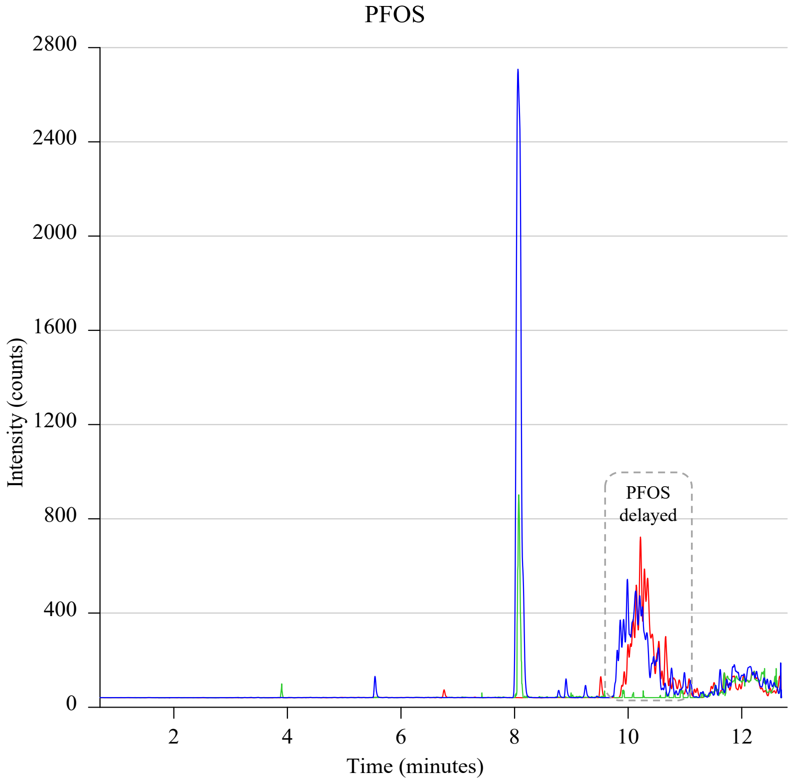


**Figure S2: Influence of sample hardness on method matrix effect. Samples spiked at 100 ng/L (n=3). Error bars represent ± 1 standard deviation.**

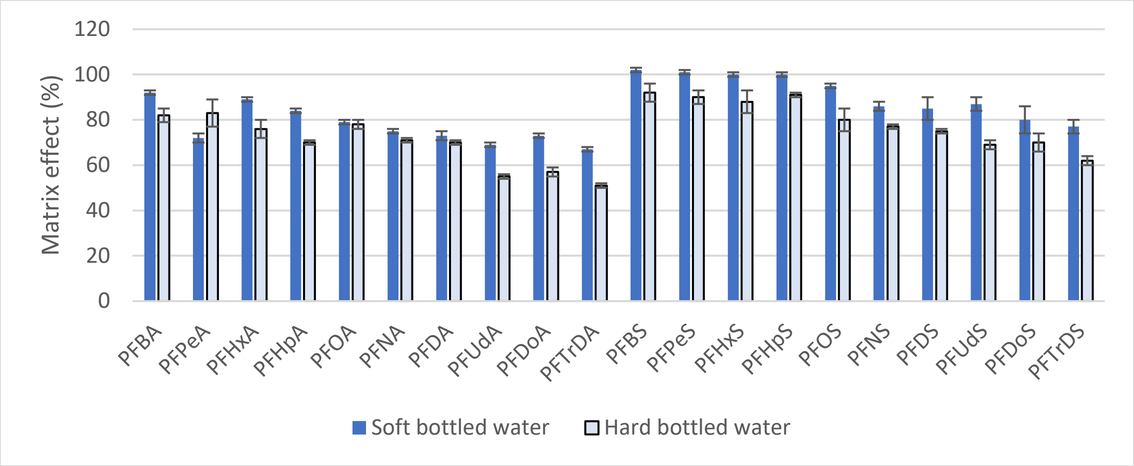


**Figure S3: Geospatial distribution of the tap water samples analyzed and PFAS occurrence (ng/L) grouped by carbon chain length.**