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Analysis of perfluorinated alkyl substances in Spanish sewage sludge by liquid chromatography–tandem mass spectrometry

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Abstract The present article describes the development of an analytical method for the determination of 13 perfluorinated alkyl substances (PFAS), as well as its application to real sewage sludge samples to confirm the presence of these compounds. The isolation of the analytes was performed by agitation, sonication and centrifugation techniques, followed by EnviCarb cleanup and weak anion exchange solid-phase extraction. Sensitive and selective determination was carried out by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). Six mass-labelled internal standards were used to ensure the accuracy of the analytical results following isotopic dilution method. Several mobile phases (acetonitrile, methanol, mixtures of both and water with ammonium acetate or acetic acid) have been tested to reach the best resolution and reproducibility results. Other parameters related to MS/MS conditions were optimized. The reliability of the method was confirmed by the evaluation of linearity (R^2=0.995–0.999), accuracy (84–99%) and injection repeatability and reproducibility (relative standard deviation below 19 and 23%, respectively). Limits of detection ranged from 0.007 to 2.217 pg. Recoveries show values higher than 80% for most of the target compounds. The application of this method to twenty real samples demonstrates its efficiency and accuracy, as well as provides for the first time to our knowledge, PFAS levels in sewage sludges from Spain.

Keywords Perfluorinated alkyl substances (PFAS) · PFOS · PFOA · HPLC-MS/MS · Sewage sludge

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Introduction

Perfluorinated alkyl substances (PFAS) have been manufactured for over 50 years. These chemicals have been used as surface protectors in numerous industrial and consumer applications, including coatings for furniture, clothing and carpets and some are active ingredients in cosmetics, household cleaners, firefighting foams and packaged food containers [1]. Due to their chemical structure, these substances are resistant to hydrolysis, photolysis, biodegradation and metabolism, characteristics making them environmentally persistent, bioaccumulative and potentially harmful [2–4]. Several studies in biota [5, 6], air [7], sediments [8] and water [9] demonstrate that these compounds are globally distributed in the environment.

Perfluorinated compounds found in the environment are anthropogenic [3], mainly as a result of human manufacture and use. Release into the environment can occur at each stage of the fluorochemical product's life cycle. They can be released during the synthesis, the incorporation into the product, the distribution of the product to the users, the use of the product by consumers and the disposal [10]. After their utilization, these fluorinated surfactants can reach the aquatic systems either by release into rivers or via wastewater discharge into receiving waters. Although PFAS are considered highly mobile in aqueous phase, significant detection in activated sludge could suggest preferential

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partitioning behaviour of PFAS to solids [11, 12]. In wastewater treatment plants (WWTPs), chemicals being recalcitrant to biodegradation processes are expected to be removed mostly by sorption on suspended solids and subsequent sedimentation [13].

Sewage sludges proceeding from wastewater treatment plants located downstream from industries, which use or have been using PFAS, for instance, chromium plating plants, paper and cardboard facilities or textile plants, are prone to contain elevated PFAS concentrations. Even in case where the industry stops the production or use of PFAS, the persistent nature of these chemicals in combination with their surface properties could cause a significant PFAS concentration years after the use of a PFAS-free alternative. Therefore, sewage sludge could be considered as one of the most important secondary sources of these chemicals because accumulates not only PFAS which are currently used but also those PFAS which have been emitted in the past.

The current waste policy of the European Union (EU) is based on avoiding, reusing, recycling or recovering the waste to the extent possible. The total quantities (i.e. production) of sludge in the EU are currently estimated at 10.13 million tons (dry solids) and nearly 40% is estimated to be spread on land for agricultural use. The recycling of sludge to agriculture varies greatly among Member States. About 1,065,000 tons (dry solid) of sludge were produced in Spain during 2006, and about 687,000 tons (dry solid) were recycled to agriculture, equivalent of 65% of the sludge produced [14]. The application of sludge for land treatment or its disposal on dump sites could lead to a remobilization of these recalcitrant compounds [15]. Therefore, it is necessary to assess the presence of these compounds in sewage sludges to manage them properly and to revise the European Directive 86/278/EEC [16] concerning the application of WWTP sludge to soil for agricultural purposes in order to establish new limits for pollutants considered at first and increase the number of chemicals analysed, mainly in regard to new emerging compounds as perfluorinated compounds. An EU-initiative to improve the present situation for sludge management proposed limit values for concentration of heavy metals and organic compounds (including persistent organic pollutants —POPs—such as polychlorinated biphenyls, dibenzo-*p*-dioxins and furans) in sludge for use on land [17]. Some European countries have already fixed limit concentrations for some organic pollutants but the pollutants regulated and the limits fixed are different from one country to another, supposing a complex and controversial issue [18]. In the case of PFAS, a target value for the sum of perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) of 100 μg/kg dry mass has been established in Germany for agriculturally used sewage sludge [19].

Recently, PFOS has been considered as a persistent organic pollutant under of the Stockholm Convention [20] and listed in Annex B [4]. This fact involves its introduction in POP monitoring studies, including environmental and wildlife compartments, in order to determine and assess its global distribution.

Currently, limited studies regarding perfluorinated alkyl substances have been published. Besides, there are few studies focused on waste matrices, concretely on sewage sludge and there is no adopted any standard analytical method for testing PFAS in this kind of matrices. Due to their relatively low volatility, good solubility in water and lack of chromophores, the analysis of perfluorinated alkyl substances is a challenging task. The analytical problems related to the determination of neutral and anionic PFAS are multiple, including aspects such as physicochemical properties, reliable standards, impurities, ion suppression and possible contamination during all stages of the analytical procedure. Extraction of PFAS from waste matrices and separation from interfering substances extracted together with these compounds is considered to be the main prerequisite for PFAS analysis in waste matrices. In most of the cases, the analytical procedure combines an agitation and centrifugation extraction with a unique clean up step based on solid-phase extraction or activated carbon particles [8, 21–23].

Consequently, due to the gap of knowledge regarding perfluorinated alkyl substances in sewage sludge, in this work, it is proposed an analytical method which allows the determination of 13 PFAS in sewage sludge. Target compounds were perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), PFOS, perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), perfluorooctanesulfonamide (FOSA), *N*-methyl perfluorooctanesulfonamide (*N*-MeFOSA) and *N*-ethyl perfluorooctanesulfonamide (*N*-EtFOSA). The application of the method to real samples demonstrates its efficiency and accuracy, as well as provides for the first time, to our knowledge, PFAS levels in sewage sludges from Spain.

Experimental

Chemicals

Solvents and reagents used in this work were of analytical or HPLC grade and were purchased from Scharlau (Barcelona, Spain).

Standards solutions were provided from Wellington Laboratories Inc. (Guelp, Canada): non-labelled or native

compounds (PFBS, PFHxS, PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, FOSA, *N*-MeFOSA and *N*-EtFOSA), mass-labelled compounds used as surrogates (^{13}C-PFHxS, ^{13}C-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFDA, *N*-d3-MeFOSA and *N*-d5-EtFOSA) and used as internal standard ($^{13}C_5$-PFNA). Non-labelled compounds were used to spike some blank samples (siliceous earth) and the Sludge D to validate the analytical methodology. Surrogate standards were spiked before extraction to quantify and control the analyte losses during all procedure. Internal standard was added to extracts just before analyses in the HPLC-MS/MS. Non-labelled and mass-labelled compounds (surrogates and internal) were also used to prepare the calibration standard solutions.

EnviCarb cartridges (500 mg, 6 ml) used to purify the extracts were provided from Sigma-Aldrich (St. Louis, MO, USA) and Oasis WAX (Weak Anion Exchange mixed mode sorbent) cartridges (500 mg, 6 ml) used to purify and concentrate the analytes were obtained from Waters (Milford, MA, USA).

Sludge D from 12th Round of the International Intercalibration Study was used as reference material to validate the analytical methodology although its content of PFAS has not been yet certified [24].

Sample collection

Sewage sludge samples were collected from 20 wastewater treatment plants of different sizes and geographically distributed all over Spain from April to June 2006. Homogenized samples collected in polypropylene (PP) containers were dried at 40°C until constant weight. The different facilities were classified in three groups depending on the number of inhabitants associated to each plant: L (lowly populated area, <500,000 inhabitants), M (medium populated area, 500,000–1,000,000 inhabitants) and H (highly populated area, >1,000,000 inhabitants).

Sample preparation

One gram of sewage sludge spiked with ^{13}C-PFHxS, ^{13}C-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFDA, *N*-d3-MeFOSA and *N*-d5-EtFOSA solutions was extracted with 10 ml of methanol in a PP tube. Samples were agitated at room temperature for 10 min, ultrasonicated at 40°C for 30 min and centrifuged at 3,000 rpm for 15 min at room temperature. The supernatants were removed and transferred to a second PP tube. The ultrasonication and centrifugation processes were repeated with 9 ml of fresh methanol. The centrifugation process was repeated again with 9 ml of fresh methanol.

Extracts of each sample were then combined and were passed through EnviCarb cartridges (500 mg, 6 ml) to remove the potential matrix interferences. Cartridges were previously activated and conditioned with 5 ml of methanol. Once samples passed through EnviCarb cartridges and were cleaned, the SPE tubes were washed with 1 ml of methanol to elute possible remaining target compounds and avoid possible losses. Then, extracts were diluted with Milli-Q water into 1 L in order to make the methanol content less than 5% and were homogenized. Solutions were loaded in Oasis WAX cartridges (500 mg, 6 ml) to pre-concentrate the analytes. Cartridges were previously activated and conditioned with 12 ml of 0.1% ammonium hydroxide in methanol and 12 ml of Milli-Q water. After loading the samples, SPE tubes were washed with 12 ml of 25 mM sodium acetate buffer (pH 4) and the target analytes were eluted with 8 ml of 0.1% ammonium hydroxide in methanol. An aliquot was taken from the final extract and was spiked with $^{13}C_5$-PFNA solution before the injection in HPLC-MS/MS.

Instrumental analysis and quantification

HPLC conditions

The chromatographic separation was performed using a Varian LC 212 liquid chromatograph (Varian, CA, USA). A 20-μl aliquot of the extract was injected into a Varian Polaris C18 A analytical column (50 mm×2.0 mm and 3 μm particle diameter) kept at 40°C. The oven column temperature was studied in a range of 25–55°C and 40°C was chosen as the suitable temperature for the analyte separation.

Several mobile phases were evaluated: 2 mM ammonium acetate in Milli-Q water (A) and methanol (B), 10 mM ammonium acetate in Milli-Q water (A) and methanol (B), 2 mM ammonium acetate in Milli-Q water (A) and acetonitrile (B), 10 mM ammonium acetate in Milli-Q water (A) and acetonitrile (B), 0.15% acetic acid in Milli-Q water (A) and 50% methanol/acetonitrile with 0.15% acetic acid (B), 10 mM ammonium acetate in Milli-Q water/acetonitrile (90:10; A), and 50% methanol/acetonitrile (B). The mobile phase selected as optimal was 2 mM ammonium acetate in Milli-Q water (A) and methanol (B). The gradient started at 5% B at 200 μl min^{-1} flow rate, then was increased to 80% B in 5 min, changed to 100% B in 5 min and hold at that level for 5 min before reversion to original conditions at the 18 min time point.

Optimization of MS/MS parameters

In the present study, the HPLC was interfaced with a Varian 320 MS triple quadrupole mass spectrometer. Analyses were carried out in negative electrospray ionization (ESI) mode using selected reaction monitoring technique. ESI interface and mass spectrometer parameters were optimized

Table 1 List of target compounds analysed, selected reaction monitoring transitions, capillary voltages and collision energies values

Compound	Formula	MS/MS mass transition monitored (*m*/*z*)	Daughter ion	Capillary voltage (V)	Collision energy (eV)
PFBS	$C_4F_9SO_3^-$	*299> 99*	$[FSO_3]^-$	−45	30
		299>299	$[M\text{-}H]^-$	−45	30
PFHxS	$C_6F_{13}SO_3^-$	*399> 99*	$[FSO_3]^-$	−100	35
		399>399	$[M\text{-}H]^-$	−100	35
PFOS	$C_8F_{17}SO_3^-$	*499> 80*	$[SO_3]^-$	−110	50
		499>499	$[M\text{-}H]^-$	−110	50
PFBA	$C_3F_7CO_2H$	*213> 169*	$[C_3F_7]^-$	−25	5
PFPeA	$C_4F_9CO_2H$	*263> 219*	$[C_4F_9]^-$	−35	10
		263>263	$[M\text{-}H]^-$	−35	5
PFHxA	$C_5F_{11}CO_2H$	*313> 269*	$[C_5F_{11}]^-$	−35	10
		313>313	$[M\text{-}H]^-$	−35	5
PFHpA	$C_6F_{13}CO_2H$	*363> 319*	$[C_6F_{13}]^-$	−35	5
		363>363	$[M\text{-}H]^-$	−35	5
PFOA	$C_7F_{15}CO_2H$	*413> 369*	$[C_7F_{15}]^-$	−40	10
		413>413	$[M\text{-}H]^-$	−40	5
PFNA	$C_8F_{17}CO_2H$	*463> 419*	$[C_8F_{17}]^-$	−40	10
		463>463	$[M\text{-}H]^-$	−40	5
PFDA	$C_9F_{19}CO_2H$	*513> 469*	$[C_9F_{19}]^-$	−40	10
		513>513	$[M\text{-}H]^-$	−40	5
FOSA	$C_8F_{17}SO_2NH_2$	*498> 78*	$[SO_2N]^-$	−95	40
		498>498	$[M\text{-}H]^-$	−95	30
N-MeFOSA	$C_8F_{17}SO_2NHCH_3$	*512> 169*	$[C_3F_7]^-$	−95	25
		512>512	$[M\text{-}H]^-$	−95	30
N-EtFOSA	$C_8F_{17}SO_2NHC_2H_5$	*526> 169*	$[C_3F_7]^-$	−100	30
		526>526	$[M\text{-}H]^-$	−100	30
[$^{18}O_2$]-PFHxS	$C_6F_{13}S^{18}O_2{}^{16}O^-$	*403> 103*	$[FS^{18}O_2{}^{16}O]^-$	−105	30
		403>403	$[M\text{-}H]^-$	−105	35
[$^{13}C_4$]-PFOS	$^{13}C_4{}^{12}C_4F_{17}SO_3^-$	*503> 80*	$[SO_3]^-$	−105	50
		503>503	$[M\text{-}H]^-$	−105	50
[$^{13}C_4$]-PFOA	$^{13}C_4{}^{12}C_4HF_{15}O_2$	*417> 372*	$[M\text{-}^{13}COOH]^-$	−30	10
		417>417	$[M\text{-}H]^-$	−30	5
[$^{13}C_5$]-PFNA	$^{13}C_5{}^{12}C_4HF_{17}O_2$	*468> 423*	$[M\text{-}^{13}COOH]^-$	−45	10
		468>468	$[M\text{-}H]^-$	−45	5
[$^{13}C_2$]-PFDA	$^{13}C_2{}^{12}C_8HF_{19}O_2$	*515> 470*	$[M\text{-}^{13}COOH]^-$	−45	10
		515>515	$[M\text{-}H]^-$	−45	5
N-d3-MeFOSA	$C_9D_3HF_{17}NO_2S$	*515> 169*	$[C_3F_7]^-$	−100	30
		515>515	$[M\text{-}H]^-$	−100	30
N-d5-EtFOSA	$C_{10}D_5HF_{17}NO_2S$	*531> 169*	$[C_3F_7]^-$	−100	30
		531>531	$[M\text{-}H]^-$	−100	30

MS/MS transitions in italics are used for quantification

in order to improve the signal-to-noise (*S*/*N*) relation for each PFC: (N_2) drying gas temperature (range of study: 150–350°C), (N_2) drying gas pressure (range of study: 20–30 psi), (N_2) nebulizing gas pressure (50–65 psi), spray chamber temperature (30–60°C), spray shield voltage (300–600 V), needle voltage (1,000–4,000 V), (Ar) collision gas pressure (1.6–2 mTorr), capillary voltage (20–110 V) and collision energy (5–50 V).

For the mobile phase chosen (2 mM ammonium acetate in Milli-Q water (A) and methanol (B)), the drying gas pressure was set at 25 psi, 270°C drying gas temperature, 55 psi nebulizing gas pressure, 55°C spray chamber

Table 2 List of each target compound related to the proper mass-labelled compound used to quantify and mass-labelled compound used as internal standard

Compound	Mass-labelled compound (surrogate standards)	Mass-labelled compound (internal standard)
PFBS	[$^{13}C_4$]-PFOS	[$^{13}C_5$]-PFNA
PFHxS	[$^{18}O_2$]-PFHxS	[$^{13}C_5$]-PFNA
PFOS	[$^{13}C_4$]-PFOS	[$^{13}C_5$]-PFNA
PFBA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFPeA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFHxA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFHpA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFOA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFNA	[$^{13}C_4$]-PFOA	[$^{13}C_5$]-PFNA
PFDA	[$^{13}C_2$]-PFDA	[$^{13}C_5$]-PFNA
FOSA	[$^{13}C_4$]-PFOS	[$^{13}C_5$]-PFNA
N-MeFOSA	*N*-d3-MeFOSA	[$^{13}C_5$]-PFNA
N-EtFOSA	*N*-d5-EtFOSA	[$^{13}C_5$]-PFNA

temperature, 1.8 mTorr collision gas pressure, −450 V spray shield voltage and −2,000 V needle voltage. The optimal values for capillary voltage and collision energy for each PFC are detailed in Table 1.

Quality control

One of the problems associated with PFAS analysis is background contamination in the analytical blanks [25]. To minimize it, the polytretafluoroethylene tubing of the instrumental equipment was substituted for polyetheretherketone. For samples analysis, procedural blanks (siliceous earth purified and calcined extrapure) were processed identical to samples for monitoring contamination during the extraction and purification steps. Instrumental blanks (100% methanol) were injected every three to four samples to monitor carry-over. Glassware was avoided during all analytical procedure because of the possible PFAS adsorption to the glass, only polypropylene and polyethylene materials were used throughout the treatment and measurement of samples.

Quantification procedure

Quantification of target analytes was carried out by isotopic dilution method if proper standards were available. This method is based on relative response factors between the mass-labelled compounds added to samples prior to extraction and the target compounds, and response factors between the mass-labelled compounds added to samples prior to extraction and the mass-labelled compound added to extracts just before analyses in the HPLC-MS/MS. Calibration curves were prepared in methanol with non-labelled PFAS concentrations of 1, 2, 10, 20, 50, and 100 pg/μl and mass-labelled compounds were maintained at 20 pg/μl. The relative response factor in the calibration standard solutions is calculated using a linear regression. The relative response factor of each non-labelled compound relative to its mass-labelled analogue is determined using the area responses and concentrations for each calibration standard solution. An averaged relative response factor is used for each native compound if the relative response factor is constant (less than 20% coefficient of variation) over the calibration range. The response factor of each

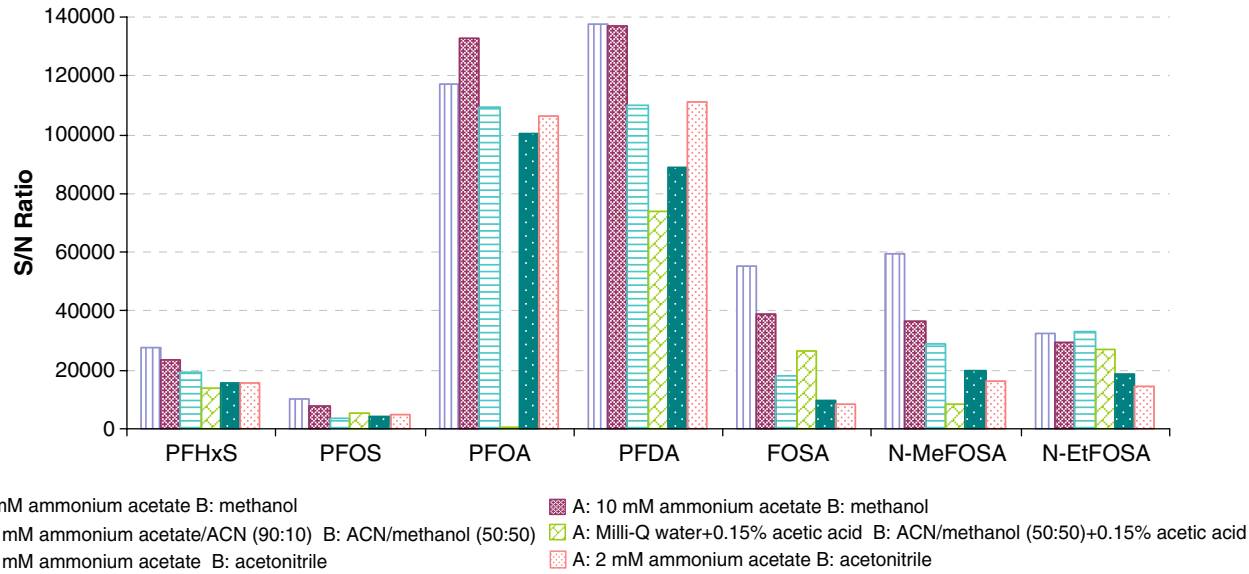

Fig. 1 Evaluation of the several mobile phases for the analysis of PFAS

Table 3 Instrumental parameters of the HPLC-MS/MS method developed for the analysis of PFAS. Standard solutions (n=3)

Compound	R^2	Accuracy (%)	LOD (pg)	LOQ (pg)	Repeatability (RSD%)	Reproducibility (RSD%)
PFBS	0.998	88	0.096	0.321	18	22
PFHxS	0.999	94	0.113	0.377	6	9
PFOS	0.999	84	0.105	0.349	2	20
PFBA	0.999	91	1.945	6.483	2	7
PFPeA	0.998	92	2.217	7.391	2	4
PFHxA	0.999	92	0.166	0.552	17	7
PFHpA	0.999	86	0.417	1.391	4	11
PFOA	0.995	97	0.891	2.971	3	1
PFNA	0.997	99	0.025	0.083	8	3
PFDA	0.998	88	0.011	0.035	11	9
FOSA	0.998	98	0.011	0.036	15	20
N-MeFOSA	0.997	88	0.007	0.024	8	6
N-EtFOSA	0.999	93	0.014	0.048	3	1

mass-labelled compound relative to a selected mass-labelled compound (internal standard) is also determined using the area responses and concentrations for each calibration standard solution. The relative response factors are used to determine the target analyte concentration in the sample and the response factors are used to calculate and control the target analyte losses during all procedure.

Due to absence of proper mass-labelled compound standards to each target compound analysed, some available mass-labelled compound standards had to be selected for quantification and another one was chosen to calculate the recoveries of the mass-labelled compounds during the analytical procedure. Details about standards used are described in Table 2.

Identification was verified by retention time and parent to daughter ion transition. Daughter ion transition was used for quantification and parent ion transition for confirmation of the PFAS selected.

Results and discussion

HPLC-MS/MS conditions

Several mobile phases were considered to reach the best chromatographic separation of target compounds and to achieve the best relation signal/noise, and consequently, the lowest limits of detection (Fig. 1). The mobile phase selected was 2 mM ammonium acetate in Milli-Q water (A) and methanol (B) because they allowed to achieve higher *S*/*N* ratio and an adequate chromatographic separation of the compounds. Good separation and *S*/*N* ratio values were

Table 4 Quality parameters of the methodology developed for the analysis of PFAS. Sludge D (n=4)

Compound	R^2	LOD (ng/g)	LOQ (ng/g)	Repeatability (RSD%)
PFBS	0.992	0.02	0.07	5
PFHxS	0.999	0.02	0.08	9
PFOS	0.997	0.08	0.27	17
PFBA	0.996	0.18	0.59	10
PFPeA	0.991	0.21	0.71	3
PFHxA	0.999	0.08	0.25	4
PFHpA	0.998	0.01	0.01	4
PFOA	0.999	0.08	0.27	4
PFNA	0.994	0.01	0.02	4
PFDA	0.992	0.06	0.21	12
FOSA	0.998	0.01	0.04	16
N-MeFOSA	0.991	0.01	0.02	19
N-EtFOSA	0.998	0.03	0.09	22

obtained also with 10 mM ammonium acetate in Milli-Q water (A) and methanol (B) mobile phase, however, a lower ammonium acetate concentration was preferred to preserve the useful life of the chromatographic column.

Another HPLC parameter as oven column temperature (40°C) and several electrospray ionization chamber parameters as drying gas temperature (270°C), drying gas pressure (25 psi), nebulizing gas pressure (55 psi), spray chamber temperature (55°C), spray shield voltage (−450 V) and needle voltage (−2,000 V) were optimized to obtain the best results. Details are shown in the Electronic supplementary material.

Some MS/MS parameters were optimized to achieve the suitable collision induced dissociation (CID) of the parent ions for formation of the product ions: collision gas pressure (1.8 mTorr; see Electronic supplementary material), capillary voltage and collision energy. The optimal values for capillary voltage and collision energy are detailed in Table 1.

Validation of the analytical method

Firstly, instrumental parameters of the method developed for the analysis of PFAS were evaluated using standards (Table 3). The calibration curves obtained in the concentration range assessed (1–100 pg/μl) were linear for all compounds with determination coefficient (R^2) ranging from 0.995 to 0.999. Method accuracy was tested using a standard solution (non-labelled and mass-labelled analytes at concentrations of 20 pg/μl). This solution was injected directly into HPLC-MS/MS (n=3) and was quantified to determine its concentration. The theoretical concentration value and the values obtained in the analyses were compared to determine the accuracy. Non-labelled values obtained, ranging from 84 to 99%, show good accuracy. The limit of detection (LOD) is defined as the minimum amount of target analyte producing a chromatographic peak with a signal-to-noise ratio of 3 and the limit of quantification (LOQ) as the minimum concentration with a signal-to-noise ratio of 10. Instrumental LOD and LOQ were calculated with the lowest concentration standard solution used in the calibration curve. Repeatability was carried out by three consecutive injections of a standard solution (non-labelled and mass-labelled analytes at concentrations of 20 pg/μl) and reproducibility by three injections made on three different days within a week. The relative standard deviations (RSD) calculated for the target analytes at repeatability were from 2% to 18%. The values obtained at reproducibility were slightly higher, from 1% to 22%. These results indicate a good intra- and inter-assay variation because the values of the RSD are below 20% and 25%, respectively.

Due to the absence of certified reference materials for the analytes and matrices of interest at the time of the experiments, to test the analytical methodology developed, four blank samples constituted for 1 g of siliceous earth extrapure and four replicas of 1 g of Sludge D from the 12th Round of the International Intercalibration Study were spiked with the 13 target analytes (PFBS, PFHxS, PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, FOSA, *N*-MeFOSA and *N*-EtFOSA) and the six surrogate (^{13}C-PFHxS, ^{13}C-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFDA, *N*-d3-MeFOSA and *N*-d5-EtFOSA) standard solutions and were extracted, purified and analysed as detailed previously in the "Sample preparation" section. Sludge D from the 12th Round of the International Intercalibration Study was used as reference material although its content of PFAS has not been yet certified. Therefore, prior to use, this sludge to validate the analytical methodology, four replicas were extracted, purified and analysed as detailed previously to determine its content of PFAS. Only PFOA (0.79±0.11 ng/g d.w.) and PFDA (0.44±0.47 ng/g d.w.) were detected. These values were considered in order to spike adequately the sample and were considered in the calculations.

The accuracy of the methodology was estimated by non-labelled compounds recoveries. These values were obtained considering the theoretical non-labelled compound standard concentration spiked in the blank and Sludge D samples prior to extraction. Recovery values of surrogate standards were calculated using the mass-labelled compound standard

Table 5 Recoveries of non-labelled and surrogate standards spiked in four blank samples (siliceous earth) and four replicas of Sludge D

Compound	Blank samples (siliceous earth; n=4) recovery (%; mean ± SD)	Sludge D samples (n=4) recovery (%; mean ± SD)
PFBS	141±27	129±7
PFHxS	96 ±16	124±11
PFOS	94±6	111±19
PFBA	137±11	122±12
PFPeA	135±22	128±4
PFHxA	80±18	109±4
PFHpA	101±25	122±5
PFOA	104±19	108±4
PFNA	114±20	112±4
PFDA	97±23	113±14
FOSA	69±11	76±12
N-MeFOSA	74±14	64±12
N-EtFOSA	80±24	93±20
[$^{18}O_2$]-PFHxS	59±5	60±7
[$^{13}C_4$]-PFOS	73±11	72±7
[$^{13}C_4$]-PFOA	70±11	73±4
[$^{13}C_2$]-PFDA	79±11	77±2
N-d3-MeFOSA	28±9	46±6
N-d5-EtFOSA	24±13	42±6

selected as internal standard ($^{13}C_5$-PFNA). Results are shown in Table 4.

Considering data obtained for blank samples (siliceous earth), non-labelled compounds recoveries for the majority of the compounds were higher than 80%, ranged from 69% to 141%. The values related to recoveries of surrogate standards were higher than 70% in most of the cases. However, recoveries for *N*-d3-MeFOSA and *N*-d5-EtFOSA were relatively low, 28% and 24%, respectively. As it was shown previously in the "Chemicals" section, $^{13}C_5$-PFNA standard solution was used as internal standard and consequently, used in recovery calculations. $^{13}C_5$-PFNA is a perfluoroalkylcarboxylate while *N*-d3-MeFOSA and *N*-d5-EtFOSA are perfluorooctanesulfonamides, this difference of nature could cause different behaviour during the analysis and explain the low results obtained, indicating that $^{13}C_5$-PFNA may not be optimal for calculating recoveries of certain compounds.

Regarding analyses of Sludge D, results are shown in Tables 4 and 5. LOD ranged from 0.01 to 0.21 ng/g and LOQ ranged from 0.02 to 0.71 ng/g. Repeatability of the sample preparation step was evaluated for four replicas of Sludge D in the same day. The RSD calculated for the target analytes at repeatability were from 4% to 21%. The accuracy of the methodology was estimated by non-labelled compounds recoveries obtained (see Table 5). Non-labelled compounds recoveries were higher than 80% for most of the compounds, ranging from 64% to 129%. The mass-labelled or surrogate standard recoveries were higher than 60% in most of the cases. The recoveries for *N*-d3-MeFOSA and *N*-d5-EtFOSA were also lower than those for the rest of the compounds, 46% and 42%, respectively, but relatively higher than in blank samples (siliceous earth).

Application to sewage sludge samples

Twenty sewage sludge samples were extracted, purified and analysed as has been described. Results are shown in Table 6. PFHxS, PFOS, PFPeA, PFHxA, PFHpA, PFOA, PFNA and PFDA were found in most of the sewage sludge,

Table 6 Levels of perfluorinated compounds in sewage sludge from Spanish WWTPs (ng/g d.w.)

ID sample	PFHxS	PFOS	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA
L1	<0.03	30.04	<0.15	<0.13	<0.07	1.35	<0.08	3.34
L2	<0.14	43.47	1.81	<0.04	<0.03	1.33	<0.26	0.34
L3	<0.02	<0.40	2.66	1.31	<0.02	5.43	<0.02	1.28
L4	18.20	95.77	3.36	<0.10	<0.10	2.85	<0.02	2.44
L5	<0.02	32.73	<0.45	2.60	2.04	7.94	10.23	21.47
L6	<0.02	143.75	2.92	<0.05	<0.13	2.20	<0.01	2.93
L7	<0.05	24.71	<0.14	1.54	<0.01	4.63	<0.18	6.23
L8	<0.02	<0.84	2.45	<0.19	0.87	3.05	<0.01	4.72
L9	<0.01	26.54	<0.15	<0.05	<0.26	1.58	3.01	24.29
L10	<0.01	77.63	<0.26	<0.07	<0.08	2.13	<0.16	4.59
L11	<0.03	<0.03	<0.36	<0.30	<0.18	5.90	1.18	5.55
L12	7.09	<0.03	<0.44	<0.15	<0.30	0.92	<0.01	7.22
M1	<0.01	249.40	<0.18	<0.04	<0.05	4.18	2.13	1.98
M2	<0.01	<0.02	2.58	1.16	<0.05	1.54	<0.02	1.14
M3	<0.04	286.81	<0.35	<0.23	<0.08	2.94	4.06	14.04
M4	<0.02	<0.01	4.69	<0.10	0.96	<0.03	<0.04	<0.04
M5	<0.02	<0.02	<0.22	<0.03	<0.05	1.87	1.10	2.02
M6	<0.01	<0.11	2.07	<0.07	<0.01	1.04	1.01	0.80
H1	3.48	208.81	<0.08	<0.12	<0.28	4.98	<0.15	2.85
H2	<0.01	58.59	<0.05	<0.03	<0.08	1.04	1.00	1.00
Range	<0.01–18.20	<0.01–286.81	<0.05–4.69	<0.03–2.60	<0.01–2.04	<0.03–7.94	<0.01–10.23	<0.04–24.29
Mean	1.46	63.99	1.27	0.42	0.28	2.85	1.23	5.41
SD	4.30	89.05	1.14	0.69	0.49	2.04	2.40	6.78
LOD	0.01–0.29	0.01–1.02	0.05–1.81	0.03–0.67	0.01–0.70	0.03–0.33	0.01–0.26	0.01–0.60

<*x*: below limit of detection

PFBS, PFBA, FOSA, *N*-MeFOSA and *N*-EtFOSA were not detected in any sludge. Limits of detection of these compounds were 0.01–0.72 ng/g d.w., 0.12–1.88 ng/g d.w., 0.01–0.04 ng/g d.w., 0.01–0.46 ng/g d.w. and 0.01–0.02 ng/g d.w. respectively

but PFBS, PFBA, FOSA, *N*-MeFOSA and *N*-EtFOSA were not detected in any samples. PFOA and PFDA were found in 95% of the samples, followed by PFOS (60%), PFPeA and PFNA (40%), PFHxA (20%) and PFHxS and PFHpA (15%). Limits of detection varied depending on the compound and the sample considered. This variability could be explained by the different nature and origin of the sewage sludges evaluated. No PFAS were detected in any instrumental blanks injected every three samples. Nevertheless, PFOA, PFNA and PFDA were found in several procedural blanks at levels below the limit of quantification. Recovery values were calculated by the addition of mass-labelled compounds prior to the extraction (surrogate standards) and mass-labelled compound used as internal standard ($^{13}C_5$-PFNA) just before the injection into HPLC instrument. Values obtained in the analyses of the twenty sludge samples were suitable for most of the compounds: ^{13}C-PFHxS (63%±13%), ^{13}C-PFOS (71%±13%), $^{13}C_4$-PFOA (62%±8%), $^{13}C_2$-PFDA (52%±7%), *N*-d3-MeFOSA (61%±18%) and *N*-d5-EtFOSA (68%±25%).

Variation in relative composition of individual PFAS in sludge was found (see Fig. 2). This variability could depend on the characteristics of the investigated WWTPs and their different treatment processes. WWTPs present a variety of treatment processes and the effects of combination of these processes on PFAS are not clear. PFOS was the compound with higher concentrations, achieving 286.81 ng/g dry weight (d.w.), greater than the perfluorocarboxylates (PFCAs) studied. The contribution of PFOS in the total PFAS concentration is clearly significant, while PFOA was detected in lower levels, ranging from 0.92 to 7.94 ng/g d.w. and PFDA from 0.34 to 24.29 ng/g d.w. Similar behaviour has been reported for sludge coming from different countries, in 2008 in Hong Kong (3.1–7304.9 ng/g PFOS and 1.3–15.7 ng/g PFOA) [23], from The Netherlands in 2009 (35.4–47.7 ng/g PFOS and no detected levels for PFOA) [26], in WWTPs sludge collected in Ontario in 2002 (0.068–460 ng/g PFOS and 0.11–4.5 ng/g PFOA) [27], in sewage sludge samples from Germany collected in 2009 (<1.0–1,910 ng/g PFOS and <1.0–405 ng/g PFOA) [28], in sludge samples from Kentucky WWTP collected during 2005 (8.2–993 ng/g PFOS and 8.3–219 ng/g PFOA) [29], in sludge collected during 2004 in the Pacific Northwest, USA (42–100 ng/g PFOS and <3–7.1 ng/g PFOA) [30] and in WWTPs sludge from USA collected from 1998 to 2004 (14.4–2,610 ng/g PFOS and <6–29.4 ng/g PFOA) [8]. On the contrary, PFOA dominance was detected in some sludge samples collected during 2005 in New York State (<10–65 ng/g PFOS and 18–241 ng/g PFOA) [21] and in sludge samples from Georgia WWTP during 2005 (<2.5–77 ng/g PFOS and 7.0–130 ng/g PFOA) [29].

Total perfluorocarboxylates (∑PFCAs) levels in sludge were consistently lower than the total levels of perfluoroalkylsulfonates (∑PFSAs). Considering the target value established in Germany of 100 μg/kg d.w. for the sum of PFOS and PFOA, the 20% of the Spanish sewage sludges studied exceed it significantly, owing to the contribution of PFOS.

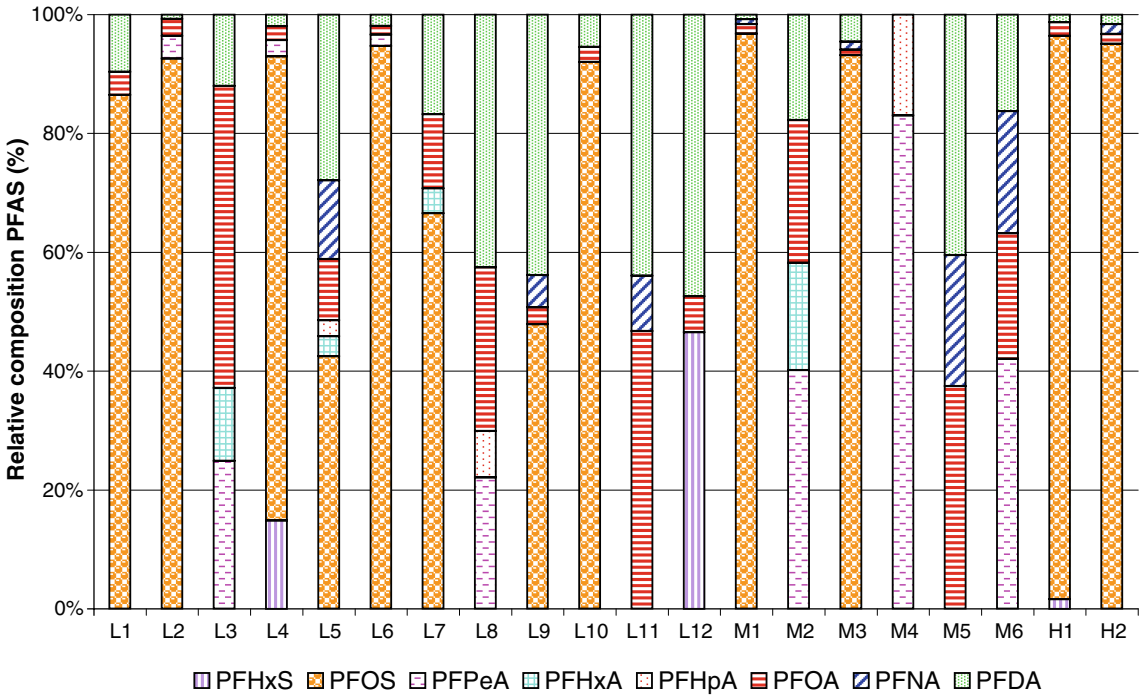

Fig. 2 Relative composition of individual PFAS in sewage sludge

Biodegradation of precursor compounds during activated sludge treatment is a likely source of these PFAS. 2-(*N*-ethyl-perfluorooctanesulfonamido)ethanol (*N*-EtFOSE alcohol) and 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (*N*-EtFOSAA) are biotransformed to PFOS [31, 32]. 8:2 FTOH (fluorotelomer alcohol) may biodegrade to form PFOA and possibly PFNA and 10:2 FTOH may lead to the formation of PFDA and PFUnA (perfluoroundecanoic acid) in wastewater treatment sludge [33]. This fact could explain the predominance of PFOS, PFOA and PFDA in the sewage sludge analysed.

Overall, no correlation between number of inhabitants related to each facility studied and PFAS concentration in the sludges was found.

Consequently, the present study provides an effective analytical method with the capacity to determine, by unique injection, 13 of the most environmentally relevant PFAS in sewage sludge matrices at trace levels. These 13 compounds involve 7 perfluoroalkylcarboxylates, 3 perfluoroalkylsulfonates and 3 perfluorooctanesulfonamides. Due to incorporation of PFOS to Stockholm Convention, the introduction of this compound in POP monitoring studies in all kind of matrices, including environmental compartments and wildlife, is an imminent necessity. Therefore, the methodology developed could be adequate and applied by those laboratories implicated in monitoring studies regarding waste matrices.

Additionally, lines of evidence of the presence of several PFAS in sewage sludge from Spain have been shown. Considering the importance of recycling of sludge to agriculture in Spain and several countries of European Union, further research should be done to evaluate the content of these compounds in sludge in order to avoid the increase of PFAS concentration in soil and their transference to the environmental compartments and food chain.

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