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Analysis of perfluorinated compounds in sewage sludge by pressurized solvent extraction followed by liquid chromatography–mass spectrometry

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ABSTRACT

Perfluorinated compounds (PFCs) are widely used in everyday life and one of the main recipients of these compounds is waste water treatment plants (WWTPs). Due to the structure and physicochemical properties of PFCs, these compounds could be redistributed from influent water to sludge. This work reports a new validated protocol for the analysis of 13 perfluorinated acids, 4 perfluorosulfonates and the perfluorooctanesulfonamide. The present work has been focused to develop a sensitive and robust method for the analysis of 18 PFCs in sewage sludge, based on pressurized solvent extraction (PSE) followed by solid phase extraction (SPE) clean-up, analytes separation by liquid chromatography and analysis in a hybrid quadrupole-linear ion trap mass spectrometer (LC–QLiT-MS/MS) working in single reaction monitoring (SRM) mode. The final methodology was validated using a blank sewage sludge fortified at different concentration levels. The method limits of detection were ranging in general from 15 to 79 ng/kg. These values were comparable to the decision limit (CCα) and the detection capability (CCβ), which were 17–1134 ng/kg and 18–1347 ng/kg, respectively. The percentage of recovery was from 79 to 111% in the most cases at different spiked levels. Finally, the repeatability of the method was in the range 4% (PFOS and PFOA) to 25% (RSD %). In order to evaluate the applicability of the method, 5 sludge samples were analyzed. The results showed that the 18 PFCs were present in all samples. However, the concentrations for most of them were below the limits of quantification. The compound present at higher concentrations was perfluorooctanesulfonate (PFOS), which was in concentrations from 53.0 to 121.1 μg/kg. The other PFCs were at concentrations between 0.3 and 30.3 μg/kg.

1. Introduction

Perfluorinated compounds (PFCs) comprise a large group of compounds widely used in industrial applications that are characterized by a fully fluorinated hydrophobic linear carbon chain attached to one or more hydrophilic head. They have unique properties to make materials stain, oil, and water resistant, and are widely used in several applications such as stain and water resistant textiles, food packaging, in fire extinguishing formulations, pesticides, paints, personal care products and surfactant agents [1], among others.

PFCs are resistant to breakdown, ubiquitous environmental contaminants, which persist and may be accumulated attached to proteins and biomagnified through the food chain. In recent years, an increasing scientific interest raised due to their widespread distribution. The main direct routes of exposure of PFCs to humans are in their diet and drinking water. PFCs have been found in environment studies of water (at levels of pg/l in lakes [2], ng/l in rivers [3], precipitation water [2]), soils and sediments (at levels of ng/g [4–7]) and biota samples (at levels of μg/kg in fish samples from Germany [8], Spain [9] and North America [2]). Among PFCs, perfluorooctanoic acid (PFOA) and PFOS are regarded as being the terminal degradation end-products, and these are the chemicals that have frequently been detected in environmental samples and often occur at high concentrations. Studies have shown that PFOA and PFOS have potential toxicity to cause liver cancer, affect the lipid metabolism and disturb the immunity system of living organisms [10,11] and human infertility [12] as well. PFCs enter the environment through direct (directly from manufacture wastes or direct application) and indirect sources (due to their decomposition or disposal through products life cycle) [13]. Wastewater treatment plants (WWTPs) have been identified as relevant pathway of PFCs releases into the environment [4,14]. However, few studies have reported the levels of PFCs in sewage sludge. In addition, the routes of introduction of PFCs in sewage sludge remain unclear, but possible ones include the washing residues from treated tex-

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tiles and cooking ware, direct and indirect residues of industrial production and application [5]. Regarding human exposure, these findings are of concern because partially sewage sludge can be used in rural lands. Therefore, these could be an indirect source of PFCs via consumption of crops, air-borne transport, surface waters and ground waters draining from these sites. The concentration levels reported in previous works showed concentrations from 50 to thousands μg/kg for perflurooctanesulfonate (PFOS) [15–20] and ng/kg to hundreds μg/kg for the other PFCs [15–21].

From the analytical point of view the determination of PFCs in sludge and sediments presents a series of limitation in addition to those inherent to their analysis in general such as cross-contamination. The main extra limitations found in the case of sludge and sediment analysis, are the difficulties in their extraction and clean-up steps, because these steps are labor intensive and time consuming, and the high percentage of matrix effects problems (ion-enhancement or ion suppression) which makes practically impossible the quantitative analysis of some compounds. Most previous works were based on extraction procedures using a methanol extraction and alkaline digestion followed by liquid extraction using methanol and acetonitrile [21]. Other procedures that have been applied were ion pair extraction [15,22]. In addition, usually a clean-up step is used in general by solid phase extraction (SPE) with a different retention phase: C_{18}, Oasis HLB or Oasis WAX. However, very few works have reported the use of pressurized liquid extraction for multianalyte analysis of PFCs [7,23].

Under this context, the main objectives of the present study were: (I) to develop an efficient extraction methodology for the analysis of 18 PFCs in sewage sludge based on pressurized solvent, (II) to validate the new developed analytical method extraction followed by analysis by LC–ESI-QqLIT (MS/MS), the most sensitive instrument in our group [24] and (III) to test the good performance of this analytical method by its application in the analysis of real samples.

2. Materials and methods

2.1. Standards and reagents

A mixture of PFCs [MXB; >98%] containing: perfluorobutanoic (PFBA), perfluoropentanoic (PFPA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), perfluoroundecanoic (PFUdA), perfluorododecanoic (PFDoA), perfluorotridecanoic (PFTrA), perfluorotetradecanoic (PFTeA), perfluorohexadecanoic (PFHxDA) and perfluorooctadecanoic (PFODA) acids, perfluorobutanesulfonate (PFBS), perfluorohexasulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluorodecanesulfonate (PFDS) and the perfluorooctanesulfonamide (PFOSA), >99%. A mixture of labeled PFCs [MXA; >98%] containing: [$^{13}C_4$]-perfluorobutanoic acid (MPFBA ($^{13}C_4$)), ion [$^{18}O_2$]-perfluorohexanesulfonate (MPFHxS ($^{18}O_2$)), [$^{13}C_2$]-perfluorohexanoic acid (MPFHxA ($^{13}C_2$)), ion [$^{13}C_4$]-perfluorooctanesulfonate (MPFOS ($^{13}C_4$)), [$^{13}C_4$]-perfluorooctanoic acid (MPFOA ($^{13}C_4$)), [$^{13}C_5$]-perfluorononanoic acid (MPFNA ($^{13}C_5$)), [$^{13}C_2$]-perfluorododecanoic acid (MPFDoA ($^{13}C_2$)), [$^{13}C_2$]-perfluorodecanoic acid (MPFDA ($^{13}C_2$)), [$^{13}C_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}C_2$)), added before the extraction procedure, was used as a surrogate in order to normalize all the analytical process. Labeled PFCs: [1,2-$^{13}C_2$]-perfluorooctanoic acid (M2PFOA ($^{13}C_2$); >98%) and ion [$^{13}C_8$]-perfluorooctanesulfonate (M8PFOS ($^{13}C_8$); >98%), added just before LC injection, were used as internal standards in order to normalize the instrumental analysis response. All analytical and labeled standards were purchased from Wellington Laboratories Inc., Canada. Water and Methanol (MeOH) CHROMASOLV®Plus, for HPLC grade, ammonium acetate salt ($AcNH_4$: MW, 77.08; ≥98%) and Ammonium hydroxide (NH_4OH: MW, 35.05; ≥98%) were obtained from Sigma–Aldrich, Steinheim, Germany. Sodium hydroxide base (NaOH: MW, 39.10; >97%) was purchased from Merck. Ottawa Sand from Applied Separations, Allentown.

2.2. Samples

In order to test the good performance of the developed approach, 5 different sewage sludge samples (sludges 1–5) were collected during April 2010 in a domestic WWTP in Catalonia, Spain. In order to avoid contamination of the samples during sampling and transport those were collected using foil containers. Sludge samples were frozen to −20° C prior to any treatment.

Blank sludge samples were used during optimization process and to assess the non-cross contamination along the analytical process.

2.3. Extraction procedure

The pressurized solvent extraction was carried out in a PSE 240 V (Applied Separations, Allentown).

Sludge samples were frozen at −20° C, lyophilized and homogenized. Approximately 0.5 g of sample was spiked with a surrogate mixture at 3 μg/kg and left to rest for 20 min. The spiked material was homogenized with sand and introduced in a 22 ml extraction cell. The cell was extracted during two consecutive cycles with methanol at 70 °C, 100 bar of pressure. Extracts were dried under a gentle stream of nitrogen and reconstituted in 50 ml of water. Solid phase extraction (SPE, Oasis WAX 3cc) was used as a clean-up step, based on an earlier published method [9]. Very briefly, the conditioning was carried out with 2× 2 ml of MeOH (0.1% NH_4OH), 2× 2 ml of MeOH and 2× 2 ml of water. The reconstituted extract was loaded under gravity conditions and dried under vacuum 20 min. Analytes were eluted in a 2× 2 ml MeOH (0.1% NH_4OH), in PP tubes, and dried under a gentle stream of nitrogen. Extracts were transferred using MeOH in a PP insert LC vial, dried under nitrogen conditions and reconstituted in LC initial conditions (Water/MeOH; 90:10). Internal standards were added at 5 μg/l level, in vial. Samples were analyzed by LC–MS/MS.

2.4. Instrumental analysis

The analysis of selected PFCs was performed by LC–ESI–MS/MS. The chromatographic separation was performed using a Symbiosis™-Pico (Spark Holland, Emmen, The Netherlands) equipped with a LiChroCART® 125-2, Pusopher® STAR, RP-18e (5 μm) analytical column, from Merck, at room temperature. The mobile phase used for the chromatographic separation consisted of aqueous ammonium acetate 20 mM (A) and MeOH (B) and was delivered at flow rate of 0.4 ml/min. The elution gradient condition started at 10% B and rose to 50% B in 2 min, and then it was linearly increased to 70% B in 4 min, and finally increased to 90% B in 8 min. This percentage was maintained for 1 min more. Finally, the mobile phase was returned to initial conditions in 1 min. Initial conditions were maintained for 1 min more. Injection volume was 10 μl.

The LC system was coupled to a quadrupole-linear ion trap mass spectrometer (QqLIT-MS/MS) 4000 QTRAP (Applied Biosystems), equipped with a Turbo Ion Spray source operated in the negative electrospray ionization mode (ESI (−)). The use of this analyzer in the study of PFCs in sludge was decided due to the versatility of the instrument evidences: conventional SRM provides excellent sensitivity and selectivity in the quantitation. Comparing to conventional triple quadrupole (QqQ), the QqLIT system achieved at least 20-fold higher sensitivity than the QqQ system disposed

Table 1
Parent and fragment ions, declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell entrance potential (CEP) optimal conditions for each compound.

Target compounds	Retention times (min)	Precursor ion (m/z)	Product ions (m/z) SRM1 SRM2	DP (V)	CE (V)	EP (V)	CXP (V)	SRM ratio (SRM1/SRM2)
PFBA	4.2	213	**169**	−25	−25	−10	−10	210
			119		−25			
PFPeA	5.3	263	**219**	−25	−15	−10	−10	–
PFHxA	5.8	313	**269**	−25	−25	−10	−10	98.4
			169		−25			
PFHpA	6.2	363	**319**	−25	−25	−10	−10	22.3
			169		−25			
PFOA	6.2	413	**169**	−25	−35	−10	−10	2.6
			369		−25			
PFNA	6.5	463	**219**	−25	−25	−10	−10	5.0
			169		−15			
PFDA	7.5	513	**119**	−25	−35	−10	−10	1.2
			469		−35			
PFUdA	8.0	563	**519**	−25	−35	−10	−10	15.8
			219		−35			
PFDoA	8.7	613	**569**	−25	−35	−10	−10	53.0
			269		−35			
PFTrA	9.4	663	**619**	−25	−35	−10	−10	30.0
			219		−35			
PFTeDA	10.1	713	**669**	−25	−35	−10	−10	36.8
			269		−35			
PFHxDA	11.5	813	**769**	−25	−35	−10	−10	28.8
			269		−35			
PFODA	13.0	913	**869**	−25	−35	−10	−10	26.3
			269		−35			
PFBS	5.4	299	**80**	−25	−80	−10	−10	1.7
			99		−80			
PFHxS	6.2	399	**99**	−25	−80	−10	−10	1.8
			80		−80			
PFOS	6.5	499	**80**	−25	−100	−10	−10	1.2
			99		−100			
PFDS	8.0	599	**80**	−25	−100	−10	−10	0.99
			99		−100			
PFOSA	7.1	498	**78**	−25	−50	−10	−10	64.6
			119		−100			

Bold means "Transitions used for quantification".

in the laboratory as was reported in previous work [24]. Acquisition was performed in single reaction monitoring mode (SRM) to obtain sufficient identification points (IP) for confirming each analyte according to Decision 2002/657/EC [25]. The identification of target analytes was carried out using relation between the highest relative abundances of two m/z transitions and retention times.

The quantification of each compound was carried out using the most intensive m/z^- transition which is indicated in Table 1. Optimized parameters were as follows: curtain gas (CUR), 30 (arbitrary units); ion source gas 1 (GS1), 25 (arbitrary units); ion source gas 2 (GS2), 60 (arbitrary units); source temperature (TEM), 350 °C; ion spray (IS), −4500 V; entrance potential (EP), −10 V, collision cell exit potential (CXP) −10 V and declustering potential (DP) −25 V. The dwell time of each MRM transition was 50 ms.

2.5. Method validation

Validation experiments were performed by spiking blank sludge samples with all selected analytes at three different levels 9, 50, 100 μg/kg (six replicates at each concentration level, $n = 6$). After homogenization the spiked samples were left to balance during 20 min. After this period, the samples were processed as reported in Sections 2.3 and 2.4. For the assessment of all mentioned parameters, the analyte response was always related to the surrogate internal standard responses to compensate for undesirable matrix effects and looses during the extraction step.

The developed method was validated using an "in-house" procedure according to ISO 11843 [26] using spiked materials because no reference material was available. In accordance with the criteria, performance characteristics of a conventional method include recovery, repeatability, with-in-laboratory reproducibility, decision limit (CCα) and detection capability (CCβ), calibration curves and specificity. In addition for comparative purposes, limits of detection (LODs) and limit of quantification (LOQ) were also determined. Positive identification was considered when a ±2.5% retention time agreement was achieved between the analytes in the samples and standards and a 25% relative abundances margin was achieved between the two selected ion transitions for each analyte.

Selectivity was assured by obtaining four identification points for each analyte through the monitoring of two transitions of each precursor ion corresponding to each target analyte and the retention time of each analyte. Linearity was assessed by constructing seven point calibration curves in triplicate at concentration levels ranging from low ng/kg to 150 μg/kg as they are summarized in Table 2. Least-square linear regression analysis was performed by plotting the peak area of the analyte over the analyte concentration and correlation coefficients (R^2) higher than 0.9900 for all compounds.

LOD and LOQ were calculated for each analyte at a signal-to-noise (S/N) ratio of 3 and 10, respectively. LODs were determined using the most intense transition (higher S/N) for each analyte, while for LOQ the second transition was confirmed visible in the chromatogram. The method limits of quantification (MLOQs) were established as the lowest concentration fulfilling all of the following criteria: (1) bias from the calibration curve less than 1.5%, (2) relative standard deviation of four replicates below 19%, (3) peak

Table 2
MLOD, MLOQ, error CCα and error CCβ by calibration curve, according to ISO 11843 when no MPRL is established and matrix effect expressed as % of surrogates used in the extraction method.

	MLOD (ng/kg)	MLOQ (ng/kg)	CCα (ng/kg)	CCβ (ng/kg)	% RSD interday	% of Recoveries			Surrogate (10 μg/l in vial)	Matrix effects (%)
						9 μg/kg	50 μg/kg	100 μg/kg		
PFBA	831	2772	1134	1347	29	102	90	65	MPFBA	−71
PFPeA	69	232	92	108	30	91	80	74		
PFHxA	161	538	184	201	13	110	108	90	MPFHxA	−48
PFHpA	79	264	93	103	15	98	102	80		
PFOA	22	73	31	38	4	114	96	80	MPFOA	−47
PFNA	15	50	17	18	14	106	99	108	MPFNA	−41
PFDA	40	133	61	76	25	102	103	111	MPFDA	−28
PFUdA	57	189	62	66	13	111	99	95	MPFUdA	22
PFDoA	55	183	70	81	16	91	105	86		
PFTrA	65	218	114	149	21	101	107	91		
PFTeDA	69	231	119	154	19	65	109	90	MPFDoA	−46
PFHxDA	67	223	180	260	16	62	110	85		
PFODA	53	176	123	172	21	60	76	70		
PFBS	219	729	262	293	24	105	110	65	MPFHxS	−46
PFHxS	31	102	36	40	24	104	106	93		
PFOS	25	84	34	41	4	99	110	98		
PFDS	45	151	57	65	8	90	97	67	MPFOS	−35
PFOSA	68	228	84	95	8	78	91	89		

Results obtained in spiked sediment at 9 μg/kg level. $n = 6$. Matrix effect (%) = [100 × surrogate peak in extracts ($n = 3$)/surrogate peak in mobile phase ($n = 8$)] − 100. Matrix effects > 0 → ion enhancement. Matrix effects < 0 → ion suppression.

shapes acceptable, and (4) signal-to-noise ratio higher than 10 in sludge spiked material.

CCα and CCβ were calculated according to the ISO 11843 [26] by the calibration curve procedure when no method permitted reference limit (MPRL) is established. CCα was calculated using the sludge blank materials fortified above the minimum required performance level (in this case, 9 μg/kg) in equidistant steps. After analysis of the fortified materials, CCα was calculated as the concentration which after plotting the signal obtained against the added concentration corresponds to the *y*-intercept plus 2.33 times the standard deviation of the within-laboratory reproducibility. On the other hand, CCβ was calculated as minimum detectable value plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured content at the decision limit equals the detection capability ($\beta = 5\%$).

Recovery was assessed for each analyte using fortified blank sludge samples at three levels of concentration 9, 50, 100 μg/kg. Analyte recoveries were calculated from the peak areas obtained for each analyte (average of six replicates for each sample) as percentages of the peak areas obtained from the replicate ($n = 6$) analysis of equivalent standard solutions.

Precision, expressed as repeatability, was calculated by repeated analyses on the same sample sets for calculating interday repeatability.

Table 2 summarizes MLOD, MLOQ, CCα, CCβ, the percentage of recoveries at different levels of fortification and the interday precision.

In order to establish the possible ion enhancement or ion suppression in the matrix, the percentage of matrix effects was calculated in all matrices according to peak areas relation of surrogate added to samples before extraction vs. surrogate in mobile phase, at 10 μg/l level in vial.

3. Results

3.1. Optimization of the PLE procedure

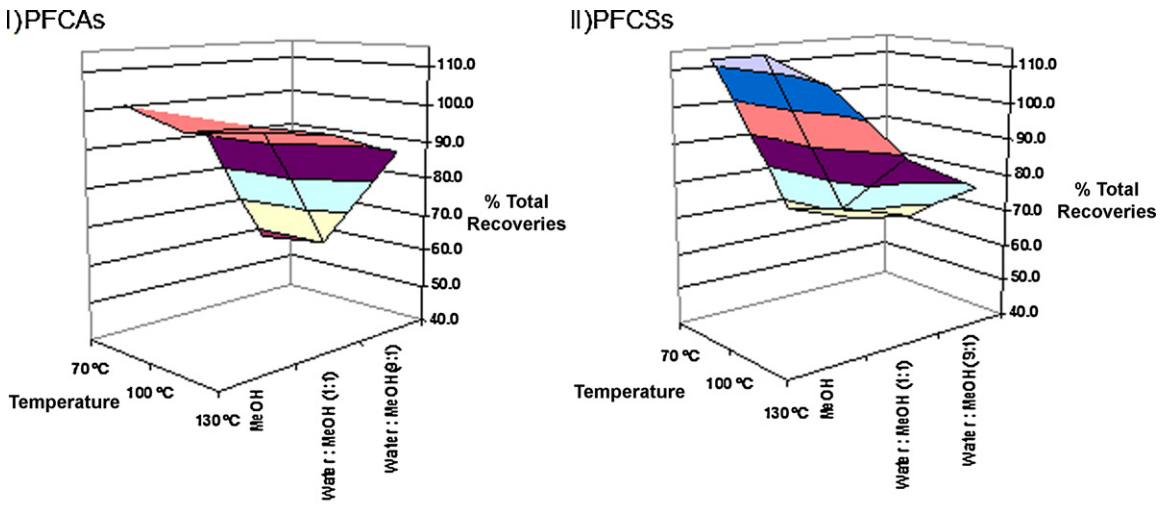
One of the parameters showing the strongest effect on the PSE extraction efficiency is the composition of the extracting solvent, temperature, number of extraction cycles and the cell size. For the selection of the extraction solvent and its composition the following combinations were tested: (1) [water:MeOH (9:1)], (2) [water:MeOH (1:1)] and (3) [MeOH (100%)]. For this series of experiments blank sludge samples were fortified at a 9, 50, 100 μg/kg ($n = 6$). In addition, temperature has played a key role in the extraction procedure development. A series of temperature 70, 100 and 130 °C were evaluated. The minimum temperature was chosen at 5 °C over the MeOH boiling point. In addition, the performance of the extraction was tested using 1–3 extraction cycles. Fig. 1 summarizes these results. As it can be seen, for acids (PFCAs) and sulfate compounds (PFCSs), the percentage of recoveries was increased according to the following order: [water:MeOH (9:1)] < [water:MeOH (1:1)] < [MeOH (100%)]. Therefore, MeOH was selected as optimum extraction solvent. No apparent differences were found at the different temperatures tested, using MeOH as extraction solvent. A percentage of recoveries near 100% was obtained for all PFCs. Due to this reason, the minimum temperature was selected as the optimum one. No significant differences were found using 1, 2 or 3 cycles of extraction. However due to the variety of sludge matrices and in order to assure the better extraction of all compounds in different sewage sludges, 2 cycles of extraction were set for the final procedure.

The cell volume was also evaluated for the best performance. For this experiment, 0.5 g of fortified blank sludge was introduced in 11 ml and 22 ml cells, in both cases filling the void space with sand. It was observed that the smaller cell, although saved time in the process as well as material and solvent consumption, often presented difficulty in processing the sample. From this series of experiments, extraction using the 22 ml cells proved more efficient for most analytes PFAs and PFSs. As a result of this experiment, the method was validated using the 22 ml volume extraction cells.

Summarizing, the following parameters were set in the final method; Cell extraction volume 22 ml, MeOH 100% as a solvent extraction, pressure of 100 bar, temperature of extraction 70 °C and 2 cycles of extraction with 1 min of static time.

3.2. Validation

The validation procedure for the developed method was carried out taking into account the EU requirements. The most significant parameters considered are described in the following points.



	% Recoveries		
PFCAs	70 ºC	100 ºC	130 ºC
Water : MeOH(9:1)	57.5	60.3	88.5
Water : MeOH (1:1)	94.6	94.7	95.2
MeOH	100.3	95.9	98.3

	% Recoveries		
PFC Ss	70 ºC	100 ºC	130 ºC
Water : MeOH(9:1)	59.8	65.7	78.4
Water : MeOH (1:1)	66.9	71.7	89.6
MeOH	111.9	118.6	110.1

Fig. 1. 3D graphic surface combining: temperature, solvent mixture and percentage of total recoveries with the corresponding data table. (I) PFCAs and (II) PFCSs.

After PLE extraction a purification step was carried out using Oasis WAX 3cc cartridges. The final recoveries of the whole extraction and purification procedure were assessed using the blank sludge fortified at a 9, 50, 100 μg/kg (n = 6), Table 2 summarizes the recovery percentage obtained for each PFC at the different concentration levels. For the lowest level of fortification recoveries were ranging from 92 to 111% for PFCAs [excepting PFTeDA (56%), PFHxDA (63%) and PFODA (54%)]. Apart from PFOSA (80%), for PFCSs the percentages of recoveries were 100–105%. At concentrations of 50 μg/kg, the percentages of recovery were between 97 and 111% for PFCAs and PFCSs, the only exception was PFOSA, the recovery of which was 78%. Finally, for the highest fortification level, the percentage of recovery was 80–107% for PFCAs, 76–98% for PFCSs and 91% for PFOSA. Summarizing, in all cases recoveries were between 70 and 110% for the three levels of fortification and the repeatability was lower than 25% for all analytes.

Table 2 reports main values of MLOQ, MLOQ, CCα and CCβ calculated according to ISO 11843 [26] when no MPRL is established, and the percentage of matrix effects on blanks fortified at the 9 μg/kg (n = 6), the lowest spiked level near to MLOQ of PFBA (2.772 μg/kg). The results revealed that MLOQ for acidic compounds were ranging from 50 to 538 ng/kg for most of compounds. PFCSs, in general, presented low MLOQ, with an exception of PFBS (729 ng/kg). In parallel, CCα values were between 17 and 1134 ng/kg and CCβ between 18 and 1347 ng/kg. On the other hand, a strong matrix effect was measured for each compound. Matrix effects produced ion suppression, with exception of MPFUdA for which ion enhancement was observed. In an attempt to compensate for undesirable matrix effects, quantification was carried out using surrogate internal standards added before the extraction.

3.3. Applicability of the method

The method applicability was assessed by analyzing 5 sewage sludge samples, which were collected in an urban WWTP.

Table 3 shows analytical results of the study and Fig. 2 shows an example of chromatogram obtained in a sludge sample. In general, the higher values were found in the sample no. 4.

The concentration levels of PFCAs were ranging from 0.4 to 30.3 μg/kg, in agreement with previous studies by Zhang et al. [15], Guo et al. [17], Li et al. [18] or Ma et al. [19]. PFOA, PFNA, PFDA and PFDoA were present in all the samples at concentrations higher than 1.0 μg/kg. Long chain acidic compounds were not detected in general, as can be expected due to the biodegradation processes, and the high concentration of PFOA can be associated with the biodegradation of other long chain PFCs congeners currently in use [27,28].

PFOSA was detected in three over the five samples analyzed, being one of the more frequently detected compound, with concentrations ranging from 0.3 to 10.7 μg/kg.

It should be pointed out, that the number of PFCs analyzed in sludge or sediments in previous studies is in general 5–6 compounds including PFOS and PFOA, and for other congeners currently in use no previous data was available for comparison purposes.

Table 3
PFCs concentration in sludge samples.

	μg/kg dw (% RSD)				
	Sludge 1	Sludge 2	Sludge 3	Sludge 4	Sludge 5
PFBA	<MLOD	<MLOD	<MLOD	22.6 (16)	14.9 (2)
PFPeA	17.2 (20)	15.6 (14)	2.6 (15)	<MLOQ	<MLOQ
PFHxA	<MLOD	<MLOD	<MLOD	<MLOQ	4.8 (22)
PFHpA	<MLOQ	0.4 (2)	4.5 (7)	<MLOQ	2.0 (25)
PFOA	9.5 (1)	7.0 (12)	9.5 (12)	30.3 (18)	29.7 (6)
PFNA	1.0 (6)	1.2 (19)	1.1 (24)	2.0 (8)	2.4 (21)
PFDA	8.6 (7)	6.1 (16)	7.2 (19)	23.5 (11)	8.2 (25)
PFUdA	3.7 (21)	<MLOQ	<MLOQ	12.2 (25)	7.8 (24)
PFDoA	6.3 (9)	2.7 (13)	3.0 (18)	11.3 (16)	4.0 (25)
PFTrA	<MLOD	<MLOQ	<MLOQ	<MLOD	<MLOD
PFTeDA	5.0 (18)	2.0 (7)	2.0 (19)	<MLOQ	<MLOQ
PFHxDA	<MLOD	<MLOQ	0.4 (18)	4.9 (9)	<MLOQ
PFODA	0.9 (12)	<MLOD	<MLOD	<MLOD	<MLOD
PFBS	<MLOD	0.9 (17)	<MLOD	<MLOQ	7.6 (18)
PFHxS	<MLOQ	<MLOQ	<MLOQ	<MLOQ	<MLOQ
PFOS	101.0 (13)	72.3 (11)	53.0 (19)	121.1 (8)	73.5 (8)
PFDS	<MLOD	<MLOD	<MLOD	7.5 (23)	<MLOQ
PFOSA	0.3 (6)	1.1 (11)	10.7 (16)	<MLOD	<MLOD

n = 3, dw, dry weight; RSD, relative standard deviation.

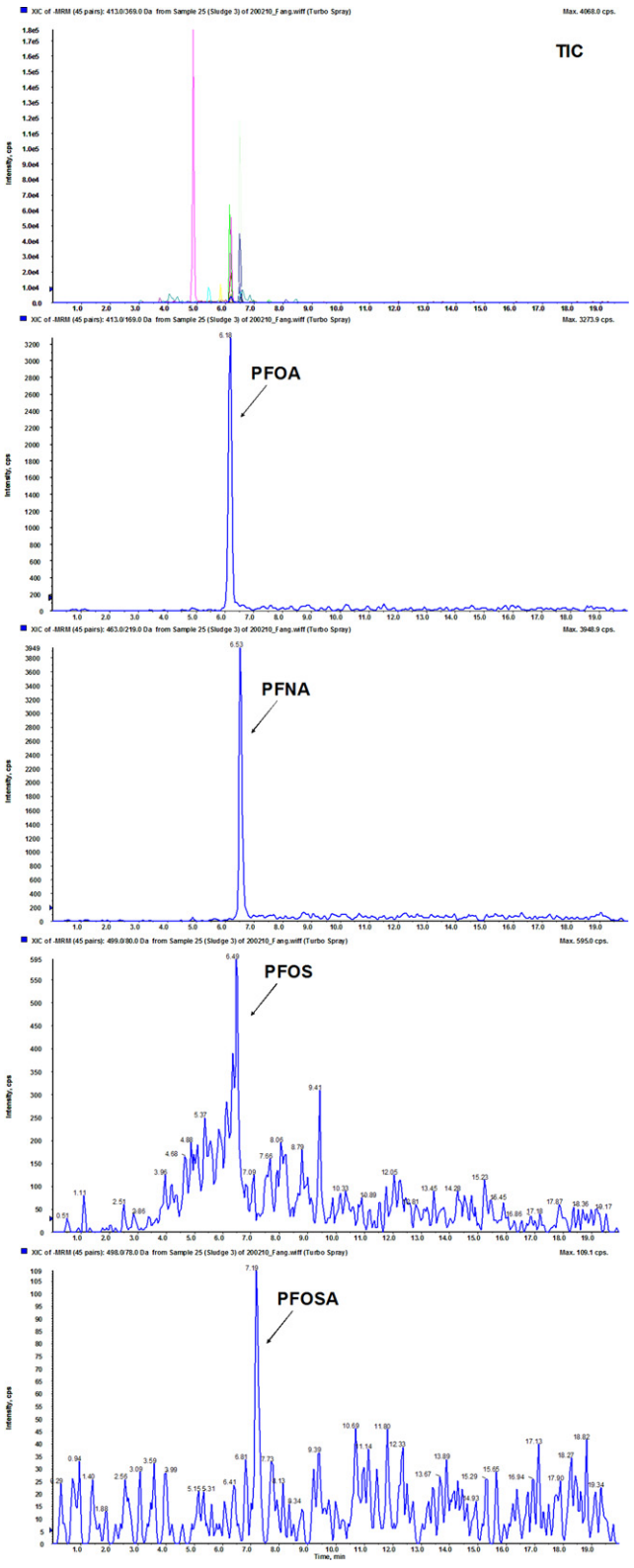


Fig. 2. Total ion chromatogram (TIC) of sludge 3 and extracted ion chromatograms of 4 positive analytes.

In the present study, with exception of PFOS, all compounds belonging to PFCSs were below MLOQ. PFOS was detected in concentrations levels ranging from 53 to 121 μg/kg, being the compound that was found in higher concentrations, in agreement with previous studies [15,17,18,20,22]. Zhou et al. [29] reported the favorable sorption of PFCs on the heterogeneous protein composition of activated sludge, and the different sorption kinetics according to their carbon chain length and different functional groups [30], which could explain the high concentrations of PFOS found in this work, as well as in the previous ones. In addition, in this work the concentration levels of PFOS were between 3 and 10 times higher than those for PFOA. This difference could be associated with the different sorption kinetics in function the different functional groups, in agreement with Zhou et al. [30]. The calculated distribution coefficients indicate that PFOS had a higher sorption tendency to activated sludge than PFOA. On the other hand, Becker et al. [31] showed that in WWTP, the calculated mass flow of PFOA is discharged in a high percentage through the WWTP final effluents while about fifty percent of PFOS is retained in the sewage sludge, fact that also supports the finding of the present work.

4. Conclusions

In the present work a multianalyte method was developed for 18 PFCs in sewage sludge to fulfill requirements for routine analysis. The new robust and sensitive analytical method is based on a PLE step with methanol as solvent followed by SPE (Oasis WAX) clean-up and analysis by LC–MS/MS. The validation showed high recovery rates range between 76 and 111%. The MLOQ were established at ng/kg levels for most compounds. However, for some compounds a high percentage of matrix effect was present, and therefore surrogates internal standards should be used in order to compensate these undesirable effects and to perform a correct quantification.

The applicability of the method was proved by analysis of 5 sewage sludge samples. The results of the analysis of real sewage sludge samples showed and concluded that PFOS was the compound encountered in higher concentrations, and PFOSA and PFCAs were found at levels of μg/kg.

Further studies about the presence and the fate of PFCs into sludge are required, because of the lack of data about some currently in use compounds and in order to elucidate transformation and biodegradation processes, because sewage sludge can be a direct source of PFCs in the environment through their application in soil restoration and agricultural soil, and also an indirect source human exposure through food and groundwater contamination.

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