MULTICLASS ANALYSIS OF PERFLUOROALKYL SUBSTANCES IN SOIL BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY. COMPARISON OF DIFFERENT EXTRACTION PROCEDURES

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Introduction

Perfluoroalkyl substances (PFASs) have been extensively used in industrial and commercial applications since 1950. During last decades, numerous studies reported their presence in food, water, sediment and biota[1,2]. There is a general concern because of the stability, persistence and bio-accumulative characteristics of these compounds, which can produce adverse effects on humans and wildlife[3]. At present, there are a few well documented cases of how soil can play a central role in the environmental distribution and subsequent human exposure to PFASs.

However, the main limiting factor to enlarge the number of studies is the complexity of soil matrix and the lack of standardized methods with broad applicability. The choice of a suitable sample-preparation technique is fundamental for the accurate and reliable identification of PFASs in trace or ultra-trace concentrations. Because of these compounds' properties, there are a series of factors of paramount importance to be taken into account as: (i) background contamination problems that can be a source of interferences (laboratory materials made of or containing perfluroethylene or perfluoroalkyl compounds, (ii) selection of the analyte-isolation and pre-concentration technique, as well as (iii) careful optimization of the corresponding operational parameters among others[1].

Additional difficulties in the analysis of soil, sediment, sewage sludge and suspended solids are the labour intensive and time-consuming extraction and clean-up required as well as the further matrix effect problems in the determination that could make practically impossible the quantitation of some compounds. Commonly reported, extraction procedures include acetic acid[4], sodium hydroxide[5], ion pair and methanol extraction[6]. In addition, the clean-up step is generally carried out by solid phase extraction (SPE) with different sorbents: C18, Oasis HLB or STRATA-X[1,4-6]. However, there is not information on the features and pitfalls of each method.

The objectives of this work were to develop a robust analytical method for the simultaneous extraction and determination of 23 PFASs in soil, which could easily be enlarged to determine a wide range of similar environmental matrices such as sediments or particulate matter. Different extraction methods (methanol, 1 % acetic acid, sodium hydroxide) were compared and finally the performance of the methods was validated in naturally contaminated samples. To our knowledge, such a broad spectra of methodologies were never assessed for the quantification of PFASs in soil.

Materials and methods

A total of 23 PFASs (C4–C14, C16, C18 carboxylates, C4, C6–C8 and C10 sulfonates, two C8s sulfonamides and C10 native unsaturated telomere acid) were selected. They have a wide variety of uses as well as different physicochemical characteristics and toxicity.

For the combined objectives of complete recovery and minimization of background noise, we have settled on: (1) alkaline pre-treatment; (2) ionic pairing; (3) extraction with 1 % acetic acid; (4) extraction with methanol by sonication.

(1) Alkaline pre-treatment was carried out according to the method described by Yeung et al.[5]. After addition of ISs about 1 g of soil (dry weight, dw) was mixed with 2 mL of 200 mM NaOH in methanol by sonication for 30 min. Then, 20 mL of methanol was added to the mixture and thoroughly shaken. After that, 0.05 mL of 4 M HCl was added. The soil and the methanol were separated by centrifugation. The supernatant was transferred into a new polypropylene tube. The above procedures were repeated with 10 mL of methanol. Then, 10 mL of methanolic extract was reduced to 3 mL and further SPE cleaned up after dilute it with 27 ml of deionized water.

(2) Ion-pairing, similarly as described by Zhang et al.[6] except for some modifications. Briefly, 5 ml of distilled water were added to soil (about 1 g, dw) and spiked with the ISs. After homogenization, 1 ml of TBAS (0.5 M, pH 10) and 2 ml of sodium carbonate solution (0.25 M) were added to the soil sample. The sample solution was

agitated on a vortex and 5 ml MTBE was added. After agitation, the organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4.0 ml) was removed from the solution. The aqueous phase was extracted twice with the MTBE solution (4 ml). The solvent was evaporated at 40º C under a gentle stream of N2 and reconstituted in 0.5 ml of methanol–water both 20 mM ammonium acetate (10/90, v/v).

(3) Acid extraction was carried out according to the method of Higgings et al.[4] on soil sample (1 g dw). The sample was spiked with ISs and homogenized with 10 mL of 1% acetic acid in water. The mixture was agitated intensively, ultra-sounded and centrifuged. The supernatant was passed to a second falcon tube. Then, 2.50 mL of methanol-acetic acid 1% (90:10 v/v) was added to the first falcon tube and the mixture was again agitated, ultra-sounded and centrifuged as previously. The supernatant was poured into the second falcon tube. This procedure was repeated with 10 mL of 1% acetic acid in water. Then, the extract was cleaned-up by SPE.

(4) Methanolic extraction was performed as described by Beškoski et al.[7] and Zhang et al.[6] except for some modifications. About 5g of soil sample spiked with ISs were extracted three times using 10 mL of methanol, vortex agitation and ultrasonic extraction. Then, after reducing the volume to 5 mL under a nitrogen stream, 100 mL of Milli-Q water and 20 µL of formic acid were added and the sample was cleaned-up with SPE.

Very briefly, the SPE clean-up was performed diluting the extracts with water to 250 mL that were vacuum passed through STRATA-X Polymeric Reversed Phase cartridges, previously preconditioned with 4 mL 0.1% of NH_4OH in methanol, 4 mL of methanol and 4 mL of H_2O. The cartridges were air-dried and, then, analytes were eluted with 4 mL of 0.1% of NH_4OH in methanol drop by drop. Extracts were evaporated to dryness, re-constituted with 250 µL of methanol and analysed.

Table 1. MRM conditions for LC-MS/MS determination of PFASs

Target PFAS (IS)	t_R [(a)] (min)	Precursor Ion	SRM_1 [(b)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2[(e)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2/SRM_1 (%)(%RSD)[(f)]
PFBA *(MPFBA)*	8.0	213	169	66	5				
PFPA*(MPFBA)*	8.9	263	219	66	5				
PFBS *(MPFHxA)*	9.2	299	99	142	38	80	142	26	15.3 (2.3)
PFHxA *(MPFHxA)*	13.3	313	269	71	5	119	71	5	10.6 (3.3)
PFHpA *(MPFHxA)*	15.4	363	319	76	5	169	76	5	68.5 (9.2)
PFHxS (*MPHxS)*	15.6	399	99	169	37	80	169	29	65.9 (10.8)
PFOA (*MPFOA)*	17.2	413	369	87	5	169	87	5	46.7 (1.4)
PFHpS (*MPFOA)*	17.3	449	99	179	37	80	179	57	31.9 (8.9)
i,p-PFNA (*MPFNA)*	19.3	463	419	87	5	169	87	5	27.0 (1.2)
PFNA (*MPFNA)*	19.5	463	419	82	5	219	82	5	13.2 (0.9)
PFOS (*MPFOS)*	19.9	499	99	190	41	80	190	65	82.2 (3.2)
PFDA (*MPFDA)*	25.5	513	469	89	5	269	89	13	15.3 (2.2)
i,p-PFNS (*MPFNS*)	25.5	549	99	195	45	80	195	73	21.6 (1.6)
PFUdA (*MPFUdA)*	28.1	563	519	104	5	269	104	13	14.1 (0.6)
PFDS (*MPFNS*)	28.2	599	99	80	80	80	80	80	17.6 (1.3)
PFDoA (*MPFDoA)*	32.7	613	569	94	5	269	94	13	9.0 (0.8)
PFTrA (*MPFDoA)*	33.4	663	619	104	0	169	104	24	8.1 (1.8)
PFTeA (*MPFDoA)*	34.0	713	669	112	5	169	112	25	7.8 (0.2)
PFHxDA (*MPFDoA)*	35.2	813	769	114	8	169	114	28	9.6 (1.1)
PFODA (*MPFDoA)*	35.8	913	869	134	10	169	128	29	

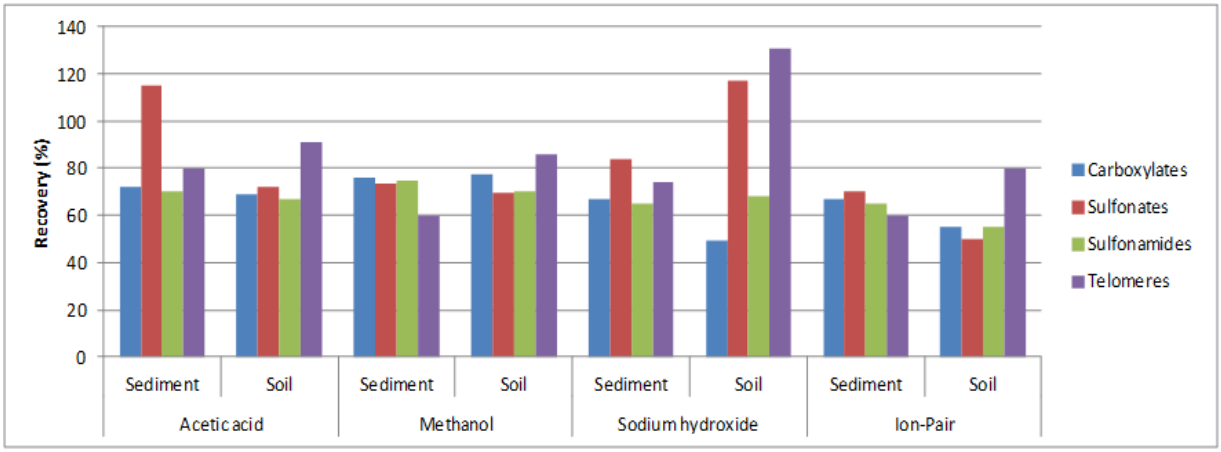
(a) t_R = retention time; (b) SRM_1 = selected product ion for quantification; (c) Frag = fragmentor; (d) CE = collision energy; (e) SRM_2 = selected product ion for qualification; (f) SRM_2/SRM_1 (%RSD) = mean values obtained from the matrix-matched calibration curves and relative standard deviation of the ratio.

The chromatographic instrument was an HP1200 series LC – with an automatic injector, a degasser, a quaternary pump and a column oven – combined with an Agilent 6410 triple quadrupole (QqQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using MassHunter Workstation Software for qualitative and quantitative (internal standard methodology based on peak areas) analysis (A GL Sciences, Tokyo, Japan). PFASs were separated with a Kinetex C18 (5.0 cm × 0.21 cm, 1.7 µm, 100A) from Agilent. The mobile phase consisted of (A) aqueous ammonium formate (0.010 M) and (B) methanol also with a concentration 0.010 M ammonium formate. Following gradient was applied: 0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min). The flow rate was kept at 0.2 ml/min throughout the run, and the sample volume injected was 5 µl. Analysis was performed in negative ion mode. Data acquisition was performed in multiple reaction monitoring (MRM) mode to obtain sufficient quantification points for the confirmation of each analyte. Identification and quantification of the target analytes were carried out using m/z transitions and retention times. Fragmentor and collision energies were optimized for each compound individually. The optimal conditions are reported in Table 1.

Results and discussion

The selection of a previously developed in our laboratory[2] optimized clean-up method was aimed to eliminate or reduce the matrix-induced ion suppression or enhancement. In the present study, our results showed that STRATA cartridge reduced the color of the extracts efficiently, indicating efficient removal of colored substances. Repeated injections of colored extracts (non-treated with STRATA) required frequent cleaning of the interface to maintain sensitivity and to avoid deterioration of the LC analytical column performance. Then, the application of this further step is highly desirable in order to maintain the robustness and reliability of the whole procedure.

Fig. 1. Summary of the results obtained by each method grouped by the different types of PFASs.



Apparently, the best results were obtained using methanol extraction, which minimized ion-suppression effects, improving detection limits down to 0.013-2.667 ng g^{-1} dw and allowed effective quantitation down to 0.04-8 ng g^{-1} dw. Procedure also showed proper relative recoveries ranging between 65% and 102% for all target compounds. The lowest LODs for the analysis of the selected PFASs from soil samples were achieved with the methanol extraction and the highest with the ion pairing method. However, the recoveries for the individual PFASs were better using the method based on acetic acid because we obtained for all the tested PFASs a most homogeneous range of recoveries. The comparison of the four methods showed that all of them are able to recover some the selected PFASs with a good repeatability. Nevertheless, sodium hydroxide an ionic pairing (mean recoveries 70 % and 60%, respectively) followed by the SPE failed to recover low chain PFASs.

The applicability of this extraction method was tested in 22 sediments of the Turia River basin (sampled in 2012). The results showed that of the 23 target compounds, 7 were identified in the analysed samples (PFBA,

PFPeA, PFHxA, PFHpA, PFOA, PFNA and L-PFOS), with concentrations ranging from 0.71 ng g^{-1} dw (PFOA) to 868 ng g^{-1} dw (PFOA) (Table 2). These results suggest that the optimized methodology used in this research is the most feasible and efficient way for systematic PFASs determination in soil samples and similar matrixes (sediment, sewage sludge and suspended solid).

Table 2. Results of the soil monitoring in the Turia River basin (nd: not detected)

Compound	Min (ng g^{-1})	Max (ng g^{-1})	Mean (no zero)	Mean	Frequency (n)	Frequency (%)
PFBA	16,76	287,34	109,46	80,91	17	74
PFPA	16,28	280,60	156,94	20,47	3	13
PFHxA	1,16	1,16	1,16	0,05	1	4
PFHpA	0,00	0,00	nd	0,00	0	0
PFOA	0,32	49,24	12,40	7,01	13	57
PFNA	0,00	0,00	nd	0,00	0	0
i,p-PFNA	0,00	0,00	nd	0,00	0	0
PFDA	0,00	0,00	nd	0,00	0	0
PFUdA	0,00	0,00	nd	0,00	0	0
PFDoA	0,00	0,00	nd	0,00	0	0
PFTrDA	0,00	0,00	nd	0,00	0	0
PFTeDA	0,00	0,00	nd	0,00	0	0
PFHxDA	0,00	0,00	nd	0,00	0	0
PFODA	0,00	0,00	nd	0,00	0	0
L-PFBS	0,00	0,00	nd	0,00	0	0
L-PFHxS	0,00	0,00	nd	0,00	0	0
L-PFHpS	0,00	0,00	nd	0,00	0	0
L-PFOS	13,14	43,86	24,49	3,19	3	13
i,p-PFNS	0,00	0,00	nd	0,00	0	0
L-PFDS	0,00	0,00	nd	0,00	0	0
PFOSA	0,00	0,00	nd	0,00	0	0

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