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Determination of per- and polyfluorinated substances in airborne particulate matter by microwave-assisted extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive and confirmatory analytical method has been developed for the determination of 12 ionic per- and polyfluorinated alkyl substances (PFAS) in fine airborne particulate matter (PM2.5) at trace levels. The proposed method includes extraction of PM2.5-bound PFAS by microwave-assisted extraction (MAE) followed by centrifugation and injection into the liquid chromatograph coupled to a triple quadrupole tandem mass spectrometry system (LC–MS/MS). The main parameters affecting the performance of MAE were optimised using statistical design of experiments (DoE). Recoveries ranged from 83 to 120% and the method quantification limit (MQL) was 1.4 pg m^{-3}, when air volumes of 720 m^3 were sampled. This method was successfully applied to 41 samples collected from five stations of the monitoring network of the Valencian Regional Government (Spain) during April–July 2010. Eight out of 12 PFCs investigated were quantified in at least one sample (PFBA, PFPeA, PFHxS, 6:2 FTS, PFOA, PFNA, PFOS and PFDA). The measured concentrations ranged from 1.4 to 34.3 pg m^{-3}.

1. Introduction

Per- and polyfluorinated alkyl substances (PFAS) comprise a large group of industrial chemicals, consisting of a hydrophobic alkyl chain usually attached to a hydrophilic head. The alkyl chain is partly or fully fluorinated and typically contains between 4 and 18 carbon atoms. Due to their distinctive physical–chemical properties, PFAS have been and are still being used widely in a variety of domestic and industrial applications such as polymerisation aid for production of fluorinated polymers, surface treatment of textiles, paper, carpet and leather or performance chemicals, such as aqueous film forming foam for firefighting and herbicides/insecticides [1].

Concerns about the persistence and bioaccumulative properties of PFAS were raised when the widely used surfactant perfluorooctanate sulfonate (PFOS) was found to be ubiquitous in wildlife and human populations worldwide [2,3]. These compounds have entered the environment from all stages: the production of PFAS, application to products and use and disposal of these products. Jahnke et al. reported that numerous ionic PFAS such as perfluoroalkane sulfonates (PFSAs) and perfluoroalkyl carboxilates (PFCAs), show extreme persistence due to the exceptional stability of the carbon–fluorine bond [4]. Additionally, several of these compounds were found to be accumulative [5] and toxic [6]. Therefore, PFOS, has been added to the persistent organic pollutants list. Findings of non-volatile ionic PFAS in the particle fraction of air samples in remote locations indicate that they undergo significant atmospheric transport on aerosols [7]. Consequently more atmospheric measurements of ionic PFAS are strongly recommended. On the other hand, volatile neutral PFAS are usually not environmentally persistent [4] and they are suggested to be precursors of persistent, ionic ones [8–11].

The distribution of PFAS among gas and particulate phase of air depends on the physical–chemical properties of the compound considered, such as vapour pressure and water solubility. It is also influenced by environmental factors, especially temperature, humidity and the nature and concentration of suspended particulate matter. While neutral PFAS are pre-eminently in the gas phase, ionic PFAS are present mainly in the particulate phase owing to their low volatility [7,12]. However their ambient particle and gas phase concentrations may be biased by the sampling process [13]. Atmospheric particulate matter (PM) is made up of a mixture of solid and aqueous species which enter the atmosphere by anthropogenic and natural pathways and present a range of morphological, physical and chemical properties in different areas [14]. The organic fraction is especially complex and contains hundreds of organic compounds [15]. The selection of PM10 and PM2.5 (fraction of particulate matter with aerodynamic diameter range

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smaller than 10 and 2.5 μm, respectively) rather than total suspended particulate (TSP) matter, as indicators of air pollution are based on health considerations, since the fine fraction of PM is the most dangerous for human health and environment. Likewise, the comparability among studies is improved if PM10 or PM2.5 is used instead of bulk samplers. The regulatory concern is today focused on those particles small enough to enter the thoracic region (respirable fraction) [16,17].

The extraction of ionic PFAS from airborne particulate matter has been carried out by a mechanical shaker using MeOH, by sonication with MeOH as the extraction solvent or by pressurized liquid extraction (PLE) [4]. Also, fluidized bed extraction has been applied [18]. It is well known that microwave assisted extraction (MAE), like PLE, allows reduction of extraction time and organic solvent consumption, and increases sample throughput in the extraction of different pollutants from environmental matrices [19]. Nevertheless, to our knowledge, no articles have been published related to extraction of PFAS from filters (particle phase) or adsorbents (gas phase) using MAE.

PFAS analysis is commonly performed by gas chromatography (GC) (neutral substances) and liquid chromatography (LC) (ionic substances) coupled to mass spectrometry. Environmental monitoring of PFAS has improved by recent developments in LC–MS/MS technology [20]. Jahnke and Berger [4] have recently reviewed the state-of-the-art in trace analysis of PFAS in different environmental matrices, including air.

The purpose of this study was to investigate the feasibility of using microwave energy for the efficient extraction of 12 ionic PFAS from fine airborne particulate matter (PM2.5) and develop a sensitive and confirmatory method that could be used in ambient air monitoring programs and health risk studies. The procedure includes extraction of PM2.5-bound PFAS by MAE followed by a centrifugation step and injection into a liquid chromatograph coupled to triple quadrupole tandem mass spectrometry. The method was applied to several samples of PM2.5 filters collected from the monitoring network of the Valencian Regional Government (Spain).

2. Experimental

2.1. Standards and solvents

Methanol LC–MS grade and diethylene glycol for analysis was supplied by Panreac (Barcelona, Spain) and ultrapure water was obtained from a Milli-Q filter system (Millipore, Bedford, MA, USA). Ammonium acetate, HPLC grade, was from Sharlau (Barcelona, Spain).

Stock standards containing 50 μg mL^{-1} of perfluorobutanoic acid (PFBA, >98%), perfluoropentanoic acid (PFPeA, >98%), perfluorohexanoic acid (PFHxA, >98%), perfluoroheptanoic acid (PFHpA, >98%), perfluorooctanoic acid (PFOA, >98%), perfluorononanoic acid (PFNA, >98%), perfluorodecanoic acid (PFDA, >98%), sodium perfluoro-1-butanesulfonate (PFBS, >98%), sodium perfluoro-1-hexanesulfonate (PFHxS, >98%), 6:2 fluorotelomer sulfonate (6:2 FTS, >98%), sodium perfluoro-1-heptanesulfonate (PFHpS, >98%), and sodium perfluoro-1-octanesulfonate (PFOS, >98%) were obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada). Individual intermediate standard solutions were prepared at a concentration of 5.5 μg mL^{-1} by diluting the stock standards in methanol. A working standard solution (solution 1) containing 275 ng mL^{-1} of each analyte was prepared in methanol from individual intermediate standard solutions. Mass-labelled internal stock standards at a concentration of 50 μg mL^{-1} of perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA, >98%), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA, >98%), and sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (MPFOS, >98%) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). Individual intermediate internal standard solutions of 1 μg mL^{-1} were prepared by diluting the mass-labelled internal stock standards in methanol. A mix labelled standard solution (solution 2) containing 600 ng mL^{-1} of every analyte in methanol was obtained from individual intermediate internal standard solutions.

2.2. Sampling and site characterization

PM2.5 samples were collected using a high-volume sampler from Digitel (Madrid, Spain) and quartz fiber filters (QFF) of 150 mm in diameter were supplied by Munktell filter AB (Falun, Sweden). A sampling flow of 30 m^3 h^{-1} was used over a time period of 24 h providing a total normalized volume of filtered air around 720 m^3.

The filters were individually wrapped with a solvent-rinsed aluminium foil and placed in a precleaned glass jar for shipment to the lab. All samples were stored approximately at −20 °C and analysed within two months after sampling.

Air samples were collected from five stations situated in Southeastern Spain (Alicante province). Two stations were placed in Elche, a town located at the south of Alicante province. One of them was situated in a residential area (P.B., a fire station, 0°43′03″W, 38°15′33″N). The other station (P.A., 0°40′58″W, 38°14′32″N) was placed in an industrial area. The third station was situated in a residential area of Alicante City (El Pla, 0°28′16″W, 38°21′31″N). The fourth station was located in a rural area of Pinoso, a town in the west of the province of Alicante (Pinoso, 1°03′53″W, 38°27′06″N). The last station was installed in a residential area of Alcoy, a town in the Northern Alicante province. A total of 41 samples were collected from April to July 2010.

To perform the PM2.5 determination, a micro-balance MX5 from Mettler-Toledo (Bedford, MA, USA) was used. Filters were previously conditioned according to EN 12341:1998 standard, at temperature (20 ± 1 °C) and at relative humidity (50 ± 5%) conditions for at least 48 h, and then weighed. Prior to weighing, filters were heated during 24 h at 300 °C to eliminate organics.

2.3. MAE conditions

Prior to extraction filters were fortified with 16.7 μL using an electronic pipette with volume range of 2–20 ± 0.02 μL from Mettler Toledo (Barcelona, Spain) of mix labelled standard solution (solution 2). Extractions were carried out using a microwave assisted extraction system (ETHOS EZ, Milestone, Shelton, CT, USA) equipped with 40 mL quartz extraction vessels. Quartz vessels were used instead of Teflon in order to avoid contamination. The optimised MAE conditions were as follows: 120 °C for 2 min, using 1200 W of power and 25 mL of methanol. After cooling, the vessels were taken out of the reactor and the resultant extracts (previously filtered) were evaporated to approximately 1 mL, 100 μL of diethylene glycol were added as keeper and finally the extracts were concentrated on a rotary evaporator at 50 °C (R-205 (BUCHI Labortechnik AG, Postfach, Switzerland). Rotary evaporation was selected after a study of two evaporation methods (see Section 1 and Fig. SC-1 in Supplementary content).

The obtained residues were re-dissolved with 0.5 mL of water:methanol (65:35) and transferred to eppendorf tubes. These were centrifuged for 20 min at 10,000 rpm (5415R-Eppendorf, Hamburg, Germany), and the supernatants were injected as soon as possible in the LC–MS/MS system, because storing extracts in the freezer results in precipitation and potential losses of PFAS.

Table 1
Selected experimental parameters of LC–MS/MS for each PFAS.

Analyte	Retention time (min)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Collision energy (V)	Tube lenses (V)
PFBA	4.38	**212.9**	**169.0**	12	72
		212.9	119.0	25	72
PFPeA	8.10	**262.9**	**219.2**	11	72
PFBS	8.87	**298.9**	**80.0**	38	101
		298.9	98.9	32	101
PFHxA	11.74	312.9	119.0	27	78
		312.9	**269.1**	11	78
PFHpA	14.49	362.9	169.0	18	100
		362.9	**319.0**	12	100
PFHxS	14.73	**398.9**	**79.9**	44	228
		398.9	98.9	39	228
6:2 FTS	16.69	426.8	80.9	44	93
		426.8	**407.1**	26	93
PFOA	16.79	412.9	169.0	20	100
		412.9	**369.1**	13	100
PFHpS	16.83	**448.9**	**79.9**	46	122
		448.9	98.9	44	122
PFNA	18.74	462.9	219.0	20	115
		462.9	**419.2**	13	115
PFOS	18.64	**498.8**	**79.9**	49	129
		498.8	98.9	45	129
PFDA	20.20	512.9	269.1	19	124
		512.9	**469.2**	14	124
MPFHxA	11.64	**314.9**	**270.0**	12	102
MPFNA	18.63	**467.9**	**423.2**	15	113
MPFOS	18.67	**502.9**	**99.0**	48	129

In bold: Quantification transition.

2.4. Liquid chromatography–mass spectrometric determination

The LC–MS system consists of a Finnigan Surveyor Autosampler, a Finnigan Surveyor LC Pump and a Finnigan TSQ Quantum Ultra detector (San José, CA, USA). Separation was accomplished on a Luna C-18 column, 150 mm × 2 mm I.D., 5 μm from Phenomenex (Macclesfield, UK).

Chromatography was performed using water(A)–methanol(B) both containing 5 mM of ammonium acetate at a flow rate of 300 μL min^{-1}. The gradient conditions were as follows: 0–23 min, linear from 35 to 90% B; 23–24 min, isocratic 90% B; 24–27 min, linear from 90% to 35% B; 27–32 min, isocratic 35% B.

The autosampler and column temperatures were set at 15 °C and 25 °C, respectively. The injection volume was 10 μL.

All PFAS were detected using electrospray ionisation (ESI) in negative mode. Multiple reaction monitoring (MRM) was selected as acquisition mode. Nitrogen Alfagaz B50 (99.999%) was used for drying and nebulising. Argon C50 (99.999%) was used as the collision gas. Both gases were from Air Liquide (Madrid, Spain). The optimised operating conditions were as follows: sheath gas 35 psi, capillary temperature 350 °C, spray voltage 3500 V, auxiliary gas value 5 arbitrary units (au) and collision gas pressure was 1.5 mTorr.

Collision energy and tube lens offset voltages were optimised for each compound using the automated optimisation procedure in syringe infusion mode provided by the manufacturer. Table 1 shows these experimental parameters and the monitored transitions for each analyte

For quantification, calibration curves consisted of 6 points that ranged from 2 to 50 ng mL^{-1}. MPFHxA was used as internal standard for PFBA, PFPeA, PFBS, PFHxA, PFHpA and PFHxS; MPFOS was employed for PFOS and PFDA whereas MPFNA was the internal standard for PFHpS and PFNA. Nevertheless, for 6:2 FTS and PFOA appropiate ^{13}C analogues were not available in the laboratory at the time of the study and therefore the quantification of these PFAS was performed by external calibration. All samples concentrations reported were field blank-corrected. As to the samples, diethylen glycol was added to the calibration standars.

2.5. Validation study and quality control protocol

Reference materials for PFAS in fine airborne particulate matter are not available. Thus, method validation was carried out using spiked blank samples (field blank PM2.5 filters). To obtain blank samples, PM2.5 samples were collected in rural stations and then heated at 150 °C for 24 h, before fortification with the working standard solution (solution 1) of PFAS. The absence of detectable quantities of PFAS was checked in each lot of field blank samples prepared.

The following parameters were studied in order to ensure the method quality: linearity, accuracy (measured as mean recovery), precision (expressed as repeatability), selectivity, and method quantification limit (MQL),

Linearity of the method was evaluated with triplicated six point calibration curves (2, 4, 6, 10, 30 and 50 ng mL^{-1}) in water:methanol (65:35). Accuracy and precision were determined by analysing spiked field blank samples at three different concentrations (1.4, 14, 34.7 pg m^{-3}). The analytical schedule was three days and 3 replicates each day.

The MQL was established as the lowest level validated, that is the lowest concentration tested for which recovery and precision were satisfactory (80–120% and <20% RSD, respectively), and the two diagnostic ions fulfil the confirmatory criteria. For a positive identification (confirmation criteria) in accordance with the EU guidelines [21], the following rules have been applied: (i) two transitions per compound must be monitored except for PFPeA in which one transition has been monitored, (ii) the LC retention time of the analyte in the sample must be within 2.5% of retention time in the standard, (iii) the relative abundance of the MRM transitions signals must be within 20% of the ratio obtained for the standards, and (iv) the S/N of the two diagnostic ions must be >3. The method detection limit (MDL), was estimated as the analyte concentration giving a peak of three times the background noise in the chomatograms corresponding to the MQL level.

Each set of filters was analysed under quality assurance protocols, including process blanks, spiked blanks and reagent blanks. In order to determine pollutant backgrounds, a procedural blank was employed as a control and was treated in the same way as the samples. Teflon bottles, Teflon-lined caps, Teflon vessels, and other materials suspected of containing fluoropolymer were avoided throughout the analysis in order to prevent contamination during the process [22].

Statistical data manipulation and numerical analysis of data resulting from experimental design were carried out by means of the statistical package MINITAB for Windows, Release 14 (Minitab Inc., Birmingham, UK).

3. Results and discussion

3.1. Optimisation of LC–MS/MS conditions

3.1.1. LC

Mobile phase composition are known to have a significant effect on LC–MS/MS performance, either in getting suitable chromatographic peaks or in signal sensitivity, consequently adequate modifiers could be added to the mobile phase in order to obtain efficient chromatography and to favour ionisation [23]. For this reason, two different mobile phases and several buffer compositions were tested.

Methanol:water and acetonitrile:water were studied as mobile phases for the PFAS separation, working with the gradient conditions described by Ericson et al. [24] and Powley et al. [25], respectively. Better chomatographic profiles (less background

Table 2
Effect of solvent on absolute recoveries of PFAS from spiked PM2.5 filters ($n = 3$).

	Acetone		MeOH		Ethyl acetate	
	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)
PFBA	91	10	104	8	75	30
PFPeA	130	7	122	6	124	23
PFBS	103	1	106	7	94	24
PFHxA	111	12	97	9	91	35
PFHpA	87	10	82	5	70	25
PFHxS	107	12	95	11	91	20
6:2 FTS	54	23	61	18	23	37
PFOA	81	5	75	11	68	41
PFHpS	45	22	56	8	12	35
PFNA	51	9	57	5	40	32
PFOS	52	17	67	13	19	40
PFDA	49	8	54	7	14	26

RSD: relative standard deviation.

noise and peak widths) were obtained when methanol:water was used, so this mobile phase was selected (see Section 2.4).

Six different buffer compositions were tested: 2 mM ammonium acetate, 5 mM ammonium acetate, 10 mM ammonium acetate, 0.1% acetic acid with 5 mM ammonium acetate, 0.2% acetic acid with 5 mM ammonium acetate and 0.3% acetic acid with 5 mM ammonium acetate. In general, the highest signals were achieved with the 5 mM ammonium acetate buffer, without acetic acid addition. For all analytes, a decreasing in the response was observed when increasing acetic acid concentration, except PFHpA and PFHxS for which significant differences were not found. In accordance with these, 5 mM ammonium acetate was added to both aqueous and organic mobile phases. Some responses of some PFAS are shown, as an example, in Tables SC-1a and 1b.

3.1.2. MS/MS parameters

Precise optimisation of MS/MS parameters is needed in order to maximize the signal of the different PFAS. The first step of the MS/MS optimisation was to select the most abundant ion from the full scan spectra as precursor ion. This was carried out by infusing 5 $\mu g\,mL^{-1}$ standard of each compound prepared in methanol at 10 $\mu L\,min^{-1}$. MS/MS spectra were acquired as well to obtain information about the maximum number of transitions available for each compound. The most sensitive results were always obtained with ESI in the negative ionisation mode, using the $[M-H]^-$ as a precursor ion. The most sensitive transitions were utilized for quantification and the secondary transitions were used for confirmatory purposes. The quantification transitions for PFCAs and PFSAs correspond to the typical fragments $m/z\,[M-COOH]^-$ and $m/z\,[SO_3]^-$, respectively [26], except 6:2 FTS, for which $m/z\,[M-HF]^-$ was chosen. Confirmatory transitions corresponded to $m/z\,[C_2F_5]^-$ for PFBA and PFHxA, $m/z\,[C_3F_7]^-$ for PFHpA and PFOA, $m/z\,[C_4F_9]^-$ for PFNA and $m/z\,[C_5F_{11}]^-$ for PFDA. On the other hand, $m/z\,[FSO_3]^-$ was selected as confirmatory transition for all the PFSAs [26], except in the case of 6:2 FTS for which $m/z\,[HF]^-$ was used.

When acquiring in multiple reaction monitoring (MRM) mode it is important to maximize chromatographic signal to noise ratios and it is also important that the peak to be quantified be defined by at least 12 data points to assure a satisfactory peak shape and a reproducibility of area measurement. Accordingly, three time windows (segments) with dwell time of 500 ms were selected. Likewise, the distributing of the analytes along the time windows try to centre the chromatographic peak in windows, minimizing the risk of peak loss due to unexpected slight changes in retention time. Collision energy and tube lenses were optimised automatically for the different PFAS using the procedure by syringe infusion mode provided by the manufacturer.

3.2. Optimisation of MAE conditions

To obtain acceptable recoveries, an extraction procedure is needed to quantitatively remove PFAS from fine particulate matter. MAE is an attractive alternative to conventional techniques, and it has been successfully applied to the extraction of different families of emerging pollutants (including compounds with very different physicochemical properties), from a wide range of environmental samples [27]. It is a well-known fact that microwave-assisted extraction (MAE) allows reduction of both extraction time and organic solvent consumption and increases sample throughput. However, to our knowledge, MAE has neither been applied to the extraction of PFAS from particulate matter nor to other matrices.

The most commonly used extraction vessels for microwave extraction are made of Teflon, a polyfluorinated polymer, which often results in contamination of the matrix. In this study, Teflon vessels have been replaced by quartz ones in order to avoid contamination of samples. The main parameters influencing MAE performance such as solvent, temperature, time and extraction volume [28] were optimised.

3.2.1. Study of extraction solvents

In order to select an appropriate solvent for quantitative extraction of all PFAS from atmospheric particulate matter, a comparative study was performed using three different solvents commonly employed in extraction of these compounds from environmental matrices: acetone, ethyl acetate and methanol. These three solvents possess a suitable polarity and a proper permittivity (ε) to absorb the microwave energy and to transform it into thermal energy [29]. Furthermore, these solvents have been described to have a high selectivity towards the analytes of interest [4].

Microwave extractions were performed on spiked field blank filters (5 $ng\,filter^{-1}$) with one 15-min cycle, using 30 mL of each solvent and an extraction temperature of 50 °C. Table 2 illustrates the effect of the three solvents on the absolute recoveries of the different PFAS, extracted from PM2.5 filters. Statistical analysis of the obtained data was carried out using a two-sample t-test approach at 95% significance level. Ethyl acetate was discarded as extraction solvent because statistically lower recoveries were obtained for most of the compounds, especially for long chain ones. Significant differences between acetone and methanol were not observed (t-test). Nevertheless, with (5–18% RSD) methanol provided, in general, more precise results and higher signal to noise responses than acetone (1–23% RSD) due to less ion suppression effects. Therefore, it was selected as extraction solvent. This is in accordance with the review carried out by Jahnke and Berger [4], where ionic PFAS were extracted from different matrices using methanol as extraction solvent.

Table 3
Experimental conditions of the central composite design (CCD) used for the optimisation of MAE conditions and obtained responses (as peak area in arbitrary units) for PFHxA, PFOS and PFOA.

Run	Temperature	Time	Volume	Response (10^6)		
Order	(°C)	(min)	(mL)	PFHxA	PFOS	PFOA
1	85	16	20	14.7	0.9	14.2
2	85	16	30	17.9	0.4	16.9
3	85	16	20	7.8	0.3	8.0
4	64	8	14	29.0	0.7	20.0
5	106	8	26	24.2	1.4	22.4
6	85	16	20	20.1	0.2	13.8
7	85	16	20	17.7	0.8	18.2
8	106	24	26	15.3	0.4	9.9
9	85	16	10	5.9	0.4	5.3
10	64	24	26	17.2	1.1	14.2
11	50	16	20	27.0	1.0	25.2
12	120	16	20	29.0	1.5	29.8
13	106	8	14	17.8	0.6	15.3
14	64	8	26	16.5	1.0	11.6
15	106	24	14	25.3	0.9	21.8
16	85	30	20	16.0	0.7	12.6
17	64	24	14	17.0	0.8	17.3
18	85	16	20	16.3	1.1	20.2
19	85	2	20	23.3	1.0	20.3
20	85	16	20	16.0	0.9	16.1

3.2.2. Optimisation of MAE parameters

Once methanol was selected as extraction solvent, the optimisation of the three main factors affecting MAE recoveries (solvent volume, exposure time and temperature) was carried out by a design of experiment approach (DoE) using a central composite design (CCD) [30]. This type of experimental design permits to build the response surface for each compound, and finding the factor settings (operating conditions) that maximize the extraction efficiency. The CCD consisted of a full factorial 2^3 design (8 hypercube points), 6 axial points and 6 central points in the cube. The 20 runs were randomized to provide protection against the effects of hidden variables, and were carried out with spiked field blank PM2.5 filters (5 ng filter^{-1}). The design matrix and the analytical response of three compounds (PFHxA, PFOS and PFOA), as an example, are shown in Table 3. The responses of the remaining nine compounds appear in Table SC-2 of Supplementary content. The microwave power was fixed to 1200 W.

A three-dimensional response surface shows the effect of two independent variables on a given response, at a constant value of the other independent variable. Fig. 1 presents, as an example, some response surfaces developed by the model for PFHxA (m/z 269.1), PFOS (m/z 79.9) and PFOA (m/z 369.1) at a constant value of solvent volume (25 mL).

To select the factors setting that maximize the twelve compound responses, the "response optimiser" from response surface design in MINITAB program was used. This parameter maximizes simultaneously the desirability for each compound. The optimised factor settings were exposure time of 2 min, temperature of 120 °C and solvent volume of 25 mL. These conditions provide a composite desirability of 0.998.

3.3. Matrix effect

Signal suppression or enhancement of the analyte signal as a result of matrix effect (ME) can severely compromise quantitative analysis of the PFAS at trace levels. In LC–MS/MS, both in ESI or APCI ionisation modes, the coextractive matrix components can lead to this effect as a consequence of the interferences in the ionisation mechanism of the analytes [31]. Matrix effects must be evaluated and discussed in the context of method development and appropriate calibration techniques compensating for these effects should be used, if necessary.

The matrix effect was evaluated by comparing the peak areas from standard solutions ($n = 5$) of the 12 PFAS in mobile phase (set A) with the peak areas of spiked blank PM2.5 filter extracts (set B) [32]. The matrix effect was calculated via the formula:

$$\text{ME\%} = \frac{B}{A} \times 100$$

Both A and B sets had concentrations of 10 ng mL^{-1}.

The results obtained are shown in Fig. 2. As can be seen, predominantly ion suppression was found in long chain PFAS. The range of matrix effect (mean values) was from 119% (PFBS) to 20% (PFDA). Strongest suppression was observed for PFNA, PFOS and PFDA. Other PFAS displayed moderate ion suppression ranging (mean values) from 99% (PFPeA) to 75% (6:2 FTS). When the average matrix is lower than 80%, it can generally be considered to have a significant effect on the quantitative analytical results. In general, the ME can be reduced by removing the co-eluting matrix components with an appropriate clean-up step. In this case, with a centrifugation step the ion suppression was removed for the most of PFAS (see

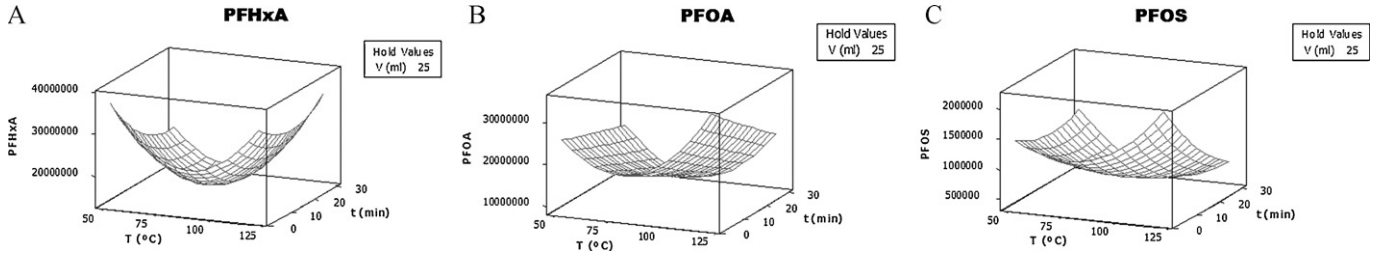

Fig. 1. Response surfaces obtained for (A) PFHxA, (B) PFOA and (C) PFOS. Fixed conditions: volume, 25 mL methanol.

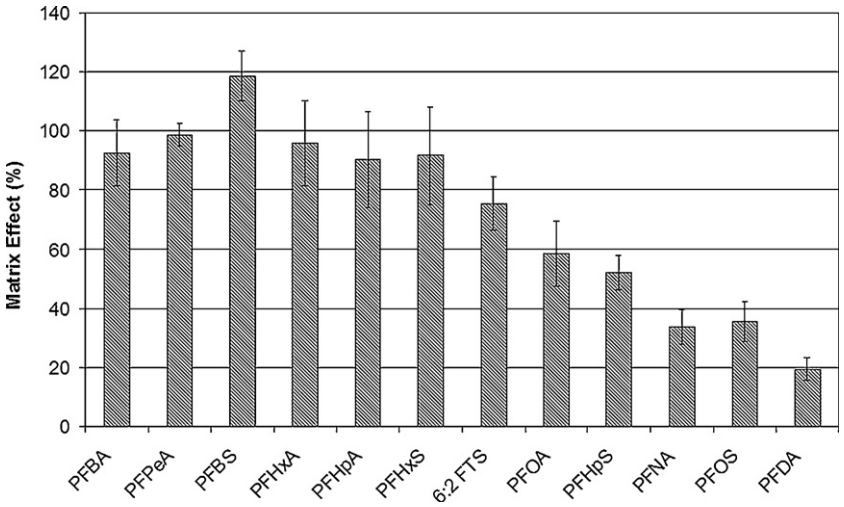

Fig. 2. Experimental matrix effect obtained for PFAS. Mean values are plotted together with the relative standard deviations ($N = 5$).

Table 4
Mean absolute recoveries ($n = 3$) obtained with four tested clean-up.

	HAc-centrifugation		25 mg C18 + HAc-centrifugation		25 mg Cgraphitized + HAc-centrifugation		Centrifugation	
	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)
PFBA	108	21	86	3	113	1	103	3
PFPeA	99	3	83	8	119	1	110	4
PFBS	106	3	83	4	127	2	117	2
PFHxA	101	4	58	10	104	6	112	3
PFHpA	109	7	37	11	107	1	104	5
PFHxS	116	12	42	12	122	2	124	2
6:2 FTS	124	5	23	22	137	6	121	6
PFOA	98	7	15	5	95	3	105	4
PFHpS	111	5	14	20	97	6	114	7
PFNA	91	16	4	20	76	4	88	4
PFOS	105	23	3	85	52	10	83	6
PFDA	107	38	1	141	46	12	60	5

RSD: relative standard deviation; HAc: acetic acid; Cgraph: graphitized carbon.

Section 3.4). A compensation approach, such as the use of internal standards, is also considered a useful method to eliminate also the consequences of matrix effects on the reliability (accuracy and precision) of the data.

3.4. Clean-up procedure

In order to reduce the matrix effect, the introduction of a clean-up step was studied.

Dispersive solid-phase extraction was tested using graphitized carbon and C_{18} as adsorbents, with and without adding 50 μL of glacial acetic acid [33] and the results were compared with those obtained by centrifugation. As an example, mean absolute recoveries obtained for the tested cleanup procedures with acetic acid addition are shown in Table 4. C_{18} was discarded as adsorbent, because significantly lower recoveries ($p < 0.05$) for most of the compounds studied were obtained using it. On the other hand, significant differences between recoveries obtained by graphitized

Table 5a
Accuracy expressed as average relative recovery rate and precision as relative standard deviation (RSD) from spiked PM2.5 filters. Analytical schedule: three days and 3 replicates each day.

Compounds	1 ng filter^{-1} ($n = 9$)		5 ng filter^{-1} ($n = 9$)		25 ng filter^{-1} ($n = 9$)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBA	98	12	104	14	100	12
PFPeA	120	1	105	13	90	5
PFBS	116	10	101	16	105	11
PFHxA	109	8	101	8	88	5
PFHpA	106	8	101	8	85	5
PFHxS	110	13	115	9	92	5
6:2 FTS	118	9	89	7	82	9
PFOA	83	14	98	17	103	12
PFHpS	102	15	92	16	113	13
PFNA	117	8	93	8	96	7
PFOS	115	10	104	14	93	11
PFDA	106	15	83	3	84	12

RSD: relative estándar deviation.

Table 5b
Mean absolute recoveries and relative standard deviation of mass-labelled internal standards from spiked PM2.5 filters. Analytical schedule: three days and 3 replicates each day ($N = 27$).

Compounds	Recovery (%)	RSD (%)
MPFHxA	87	29
MPFOS	43	23
MPFNA	53	35

carbon and those reached by centrifugation, both with acetic acid, were not observed. Similar results were obtained without acetic acid addition. Consequently, only centrifugation was used as the clean up procedure.

3.5. Analytical performance of the method

Calibration curves showed good linearity with correlation coefficients (R^2) > 0.99 between 2 and 50 ng mL^{-1} in water:methanol (65:35), with residues randomly distributed (without trends or patterns) and individual residual deviations less than 20%. Complete regression data are listed in Table SC-3 in the supplementary content. The selectivity of the method was satisfactory and came from the adquisition of two specific SRM transitions for each compound. LC–MS/MS chromatograms did not show the presence of interfering peaks at the analyte retention time for any of the PFAs investigated in this study.

Mean relative recoveries ranged from 83 to 120% (see Table 5a) with coefficients of variation below 20%. Mean absolute recoveries of mass-labelled internal standards are shown in Table 5b.

The MQL of the whole method was 1.4 pg m^{-3}, when air volumes of 720 m^3 were collected. MDLs, estimated as described in Section 2.5, were from 0.03 to 0.27 pg m^{-3} (see Table SC-3 in supplementary content)

3.6. Analysis of real samples

To put the developed method into practice, 41 samples were collected at five different sites (see Section 2.2). It should be pointed out that the sampling was not planned as a monitoring program to find the occurrence and variability of atmospheric PFAS, but to support the applicability of the developed method. An overview about PFAS concentrations measured is presented in Table 6 (see also Tables SC-4–SC-8 in Supplementary content). The results corresponding to blank field samples are reported in Table SC-9 in supplementary content.

Eight out of twelve PFAS investigated were measured in at least one sample: PFBA, PFPeA, PFHxS, 6:2 FTS, PFOA, PFNA, PFOS and PFDA.

The most frequently detected compound was 6:2 fluorotelomer sulfonate (61% of total samples collected), with concentrations ranging from 1.4 to 34.3 pg m^{-3}. PFNA and PFOA were also frequently detected (59 and 54%, respectively) with concentrations ranging from 1.4 to 11.8 pg m^{-3} and from 1.4 to 13.8 pg m^{-3}, respectively. Fig. 3 shows an MRM chromatogram of a sample collected in Elche – P.B during June 2010.

The same compounds were detected in the particle phase of outdoor air samples in a study carried out by Barber et al. [34] in air samples collected from 4 field sites in Europe, except PFBA, PFPeA, which were not included in that study. PFOA was the predominant analyte found in the particulate phase at concentrations ranging from 1 to 818 pg m^{-3}. In other studies carried out in Europe [18,35–37], PFOA was often the predominant analyte measured in the particulate phase. Similar concentrations of PFOA as those found in our study were reported by Dreyer and Ebinghaus in Germany [18], ranging from 1.9 to 6.1 pg m^{-3}

Table 6
Mean concentrations of PM2.5-bound PFAS (pg/m^3) in five stations of the province of Alicante region (Spain).

	Elche		Elche		Alicante		Alicante		Alicante				
	P.B (residencial area)		P.A (industrial area)		El Pla (residencial area)		Pinoso (rural area)		Alcoy (residencial area)		Total		
No. of samples	11		13		11		3		3		41		
	N > MQL[a]	Mean[b]	N > MQL[a]	Mean[b]	N > MQL[a]	Mean[b]	N > MQL[a]	Mean[b]	N > MQL[a]	Mean[b]	N > MQL[a]	Frequency of detection (%)	Range
PFBA	1	2.3	–	<1.4	–	<1.4	–	<1.4	–	<1.4	1	2	2.3
PFPeA	3	1.7	1	1.8	1	1.4	–	<1.4	–	<1.4	5	12	1.4–1.8
PFBS	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	–	–
PFHxA	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	–	–
PFHpA	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	–	–
PFHxS	–	<1.4	1	15.9	–	<1.4	–	<1.4	1	1.5	2	5	1.5–15.9
6:2FTS	8	21.5	4	3.6	9	3.1	1	1.4	3	3.5	25	61	1.4–34.3
PFOA	2	1.6	8	6.1	8	1.9	2	1.5	2	3.3	22	54	1.4–13.8
PFHpS	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	<1.4	–	–	–
PFNA	6	2.7	9	3.8	4	1.7	2	1.65	3	2.2	24	59	1.4–11.8
PFOS	1	4.4	–	<1.4	–	<1.4	–	<1.4	–	<1.4	1	2	4.4
PFDA	2	4.7	5	2.1	7	2.0	2	1.5	3	5.6	19	46	1.4–11.0
ΣPFAS[c]		38.8		33.2		10.1		6.05		16.0			

[a] Number of samples with concentrations above method quantification limit (MQL).
[b] Mean concentrations were calculated from values above MQL
[c] Total concentration of PFAS in each sampling site. <MQL-values are excluded.

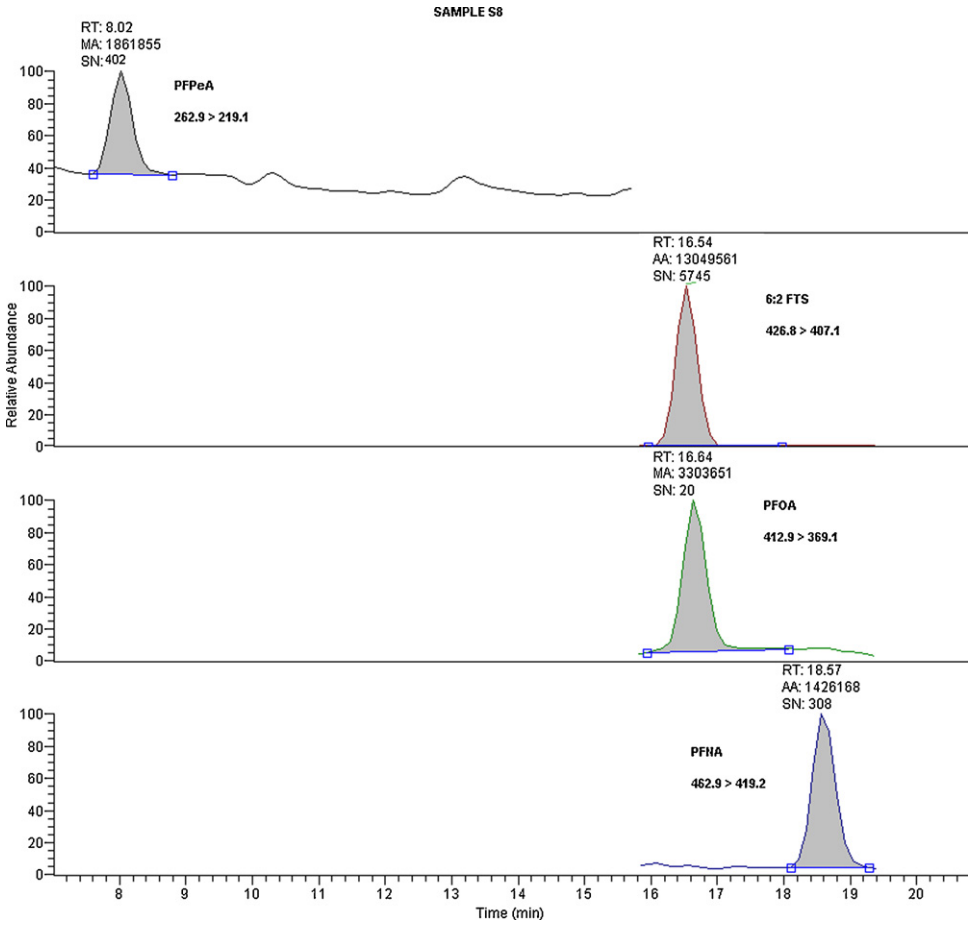

Fig. 3. MRM chromatograms corresponding to a positive sample (S8, Elche-P.B.) for PFPeA (1.6 pg m^{-3}), 6:2 FTS (34.2 pg m^{-3}), PFOA (1.5 pg m^{-3}) and PFNA (1.8 pg m^{-3}).

Levels of PFOS ranged from 1.4 to 4.4 pg m^{-3}, values that are in accordance with those reported in previous studies: PFOS levels of 2–7 pg m^{-3} over the Great Lakes [38]; <1–7 pg m^{-3} in Japan [39]; 0.1–2.3 pg m^{-3} in Germany [18] and <LOQ-7 pg m^{-3} in 4 field sites in Europe [34]. Contrarily, these values are significantly lower than the levels of PFOS founded at Manchester during the period February–March 2005 (46 pg m^{-3}), the highest reported anywhere to date.

The major part of the studies carried out to measure the PFAS concentrations in air are focused on the neutral substances and those centred ionic PFAS usually analyse a reduced number of them (mainly PFOA and PFOS). Only a few authors measured levels of several ionic PFAS in air samples [18,20,34]. Likewise, it has been reported by Dreyer et al. [40] and Kim and Kannan [41] that some ionic PFAS appear in concentrations below 1 pg m^{-3}. In order to quantify these low concentrations using the present analytical method, higher sampling volumes need to be collected (1500–2000 m^3), taking samples for 2–3 days.

One of the main hypotheses for the widespread detection of PFAS in remote locations is the long-range transport of neutral, volatile "precursor" PFAS in the atmosphere, followed by deposition and transformation into less volatile ionic species [8]. Nevertheless, findings of non-volatile ionic PFAS in air samples raise the possibility that they might directly undergo significant atmospheric transport on particles away from source regions [18,34].

4. Conclusions

A sensitive confirmatory method to analyse 12 ionic PFAS in fine airborne particulate matter (PM2.5) has been developed. The method is based on an extraction of PFAS by MAE and determination using LC-ESI(-)–MS/MS.

Three solvents were studied for PFAS extraction. Methanol was selected because it provides more precision and higher signal to noise responses. The majority of compounds presented a matrix effect, so a centrifugation step was added as an effective clean-up method. The main parameters affecting MAE extraction (time, temperature, volume of solvent) were optimised by statistical design of experiment approach. The optimised factor setting was a time of 2 min, a temperature of 120 °C and a volume of 25 ml.

Recoveries ranged from 82 to 120% and the MQL was 1.4 pg m^{-3}, when air volumes of 720 m^3 were collected.

Using the optimised method PFAS concentrations were investigated in 41 samples from five stations in the atmospheric monitoring network of Valencian Regional Government (Spain). Eight out of twelve PFAS investigated were found in at least one sample: the perfluorobutanoic, perfluoropentanoic, perfluorooctanoic, perfluorononanoic and perfluorodecanoic acids and the sulfonates 6:2 fluorotelomer and sodium perfluoro-1-octane. The measured concentrations ranged from 1.4 to 34.3 pg m^{-3}.

This method could be used in the monitoring programs of ionic PFAS in atmospheric air using the network of samplers which already exist in many countries to control fine particulate matter. However, in order to quantify with good accuracy and precision concentrations of PFAs at very low levels (<1 pg m^{-3}), sampling volumes around 1500–2000 m^3 need to be collected.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.04.082.

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