Tutor/s

Dr. Francisco Javier Santos Vicente
Departament d'Enginyeria Química i Química Analítica

Treball Final de Grau

Determination of semi-volatile fluorinated compounds in environmental samples by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

Determinación de compuestos fluorados semi-volátiles en muestras ambientales mediante microextracción en fase sólida (SPME) y cromatografía de gases-espectrometría de masas (GC-MS)

Albert Contreras Llin

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"La verdadera ciencia enseña, sobre todo, a dudar y a ser ignorante"

Ernest Rutherford

M'agradaria dedicar unes paraules d'agraïment a totes aquelles persones que m'han ajudat i recolzat durant aquest tram de la meva vida i que sense elles tot això no hauria estat possible. Primerament agrair al Dr. Francisco Javier Santos per la seva dedicació i paciència, guiant-me en tot moment durant la realització d'aquest projecte per tal de fer-lo possible. També agrair especialment al Juan Francisco Ayala Cabrera per la infinita paciència i la seva disposició a ajudar-me en tot moment quan ho necessitava i del qual he après moltíssimes coses, sense oblidar-me de tot el grup de recerca CECEM que m'han fet sentir-me un més durant la meva estada. Finalment m'agradaria fer una petita menció a la meva família pel seu recolzament i donar-me la possibilitat de fer el que realment m'agrada .

Report

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1. SUMMARY

Fluorinated organic compounds (FOCs) constitute a wide group of organic compounds, which are partially or totally saturated by fluorine atoms. Over the last years, perfluoroalkyl and polyfluoroalkyl substances (PFASs), which constitute a huge group of hazardous organic contaminants, have drawn great attention due to their persistence, potential toxicity and ubiquitous presence in the environment. Thus, PFASs have been substituted by semi-volatile PFASs in many different applications such as fire-fighting foams, paintings, coating and packaging materials. Although these compounds do not present a strong toxicity, their degradation into persistent perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) makes necessary to control their presence in the environment as well as in wildlife and humans.

In the present work, a fast and simple analytical method for the simultaneous determination of several families of semi-volatile PFASs, including fluorotelomer alcohols (FTOHs), perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamide ethanol (FOSE) and fluorotelomer olefins (FTOs), in water samples has been optimised and validated. The proposed method consists on headspace solid-phase microextraction (HS-SPME) technique for the target compounds extraction and determination by gas chromatography-mass spectrometry (GC-MS), which provided good trueness, sensitivity and precision achieving very low method limits of detection ranging from 0.10 ng L^{-1} to 1.93 µg L^{-1}. The validity of the method has been demonstrated for the analysis of water samples. The established method was applied to the analysis of semi-volatile PFASs precursors in water samples collected from different sources such as the Llobregat River, Barcelona tap water and seawater for alimentary purposes.

Keywords: per-polyfluoroalkyl substances, persistent organic pollutants, headspace solid-phase microextraction, electron ionisation, gas chromatography-mass spectrometry, water.

2. RESUM

Els compostos orgànics fluorats (FOCs) constitueixen un ampli grup de compostos químics orgànics que es troben parcialment o totalment saturats per àtoms de fluor. Durant els últims anys, les substàncies perfluoroalquil i polifluoroalquil (PFASs), que constitueixen un gran grup de contaminants orgànics perillosos, han estat el focus d'atenció degut a la seva persistència, toxicitat potencial i presència en el medi ambient. Per aquest motiu, les PFASs han estat substituïdes per precursors semi-volàtils de les mateixes en diverses aplicacions com ara escumes anti foc, pintures, recobriments i materials d'empaquetatge. Tot i que aquests compostos no presenten una toxicitat destacable, la seva degradació en els persistents àcids perfluorooctanosulfonic (PFOS) i perfluorooctanoic (PFOA), fa necessari controlar la seva presència en el medi ambient, així com també en la vida salvatge i humans.

En aquest treball, un mètode simple i ràpid per a la determinació simultània de diverses famílies de PFASs semi-volàtils incloent fluorotelòmer alcohols (FTOHs), perfluorooctansulfonamida (FOSAs), perfluorooctansulfonamida etanol (FOSEs) i fluorotelòmer olefines (FTOs), en mostres d'aigua ha sigut optimitzat i validat. El mètode proposat consisteix en la tècnica de microextracció en fase sòlida en espai de cap (HS-SPME) per a l'extracció dels compostos i la seva quantificació mitjançant cromatografia de gasos-espectrometria de masses (GC-MS), el qual va proporcionar una bona veracitat, sensibilitat i precisió amb límits de detecció molt baixos compresos entre 0,10 ng L^{-1} to 1,93 µg L^{-1}. La validació del mètode ha sigut demostrada per a l'anàlisi de mostres d'aigua. El mètode establert va ser aplicat a l'anàlisi dels precursors semi-volàtils de PFASs en mostres d'aigua obtingudes de diferents fonts com ara del Riu Llobregat, aigua de la xarxa de distribució de Barcelona i aigua de mar d'ús alimentari.

Paraules clau: per-polifluoroalquil, contaminants orgànics persistents, microextracció en fase sòlida en espai de cap, ionització electrònica, cromatografia de gasos-espectrometria de masses, aigua.

3. INTRODUCTION

3.1. ENVIRONMENTAL POLLUTION

One of the greatest problems that the world is facing today is environmental pollution, which is causing irreparable damage to the earth through multiple ways such as air, soil, water, noise and light. Environmental pollution occurs when the environment cannot destroy or neutralize harmful anthropogenic or even natural products without creating structural or functional damage to itself. As human beings increase their power and control over nature, new necessities arise from a widespread and rapidly evolving society. While living beings, such as animals or plants, find their way to adapt to a continuously changing environment, human beings adapt the land resources to their own profit, in order to satisfy global necessities. Both technological progress and demographic growth, lead to the disruption of the environment and even in some cases the alteration of the biological equilibrium might unfortunately follow. Finding the balance between technological development, the advance of the civilisation and the maintenance of the ecological equilibrium should be the main priority in order to preserve life in our planet.

Environmental pollution severely affects human health, being air and water pollution the most common ones. Air pollution leads to respiratory problems including asthma and lung cancer, while contamination by water sources may trigger skin irritation or the so called *chemical diseases* such as fluorosis, among multiple other afflictions.

Particular attention must be paid to a specific group of pollutants called persistent organic pollutants (POPs). This denomination covers a wide range of toxic organic compounds characterized by their resistance to multiple forms of degradation such as chemical, biological or photolytic degradation and their capability to be transported for long distances through different media. These compounds remain intact in the environment for long time periods and can also be accumulated in the fatty tissue of living organisms due to their high solubility in adipose tissue. Because of their persistence and mobility, they are therefore present anywhere on the planet [1].

Due to the necessity of tackling this global problem at source, the Stockholm Convention on Persistent Organic Pollutants was adopted by the Conference of Plenipotentiaries on 22nd May

2001 in Stockholm (Sweden) [2] and signed by 172 countries around world with the aim to protect human health and the environment from POPs. The POP list encompassed several classes of organic pollutants, such as pesticides, industrial chemicals and by-products, which included compounds such as chlordane, dichlorodiphenyl trichloroethane (DDT), hexachlorobenzene, polychlorinated biphenyls (PCBs), and some flame retardants and fluorinated compounds.

3.2. Fluorinated organic compounds

Fluorinated organic compounds (FOCs) are a wide group of organic chemicals which contain the carbon-fluorine bond. FOCs have multiple physical, biological and chemical properties generally due to [3]:

- high electronegativity of fluorine atoms;
- high energy of C-F bond;
- small diameter of fluorine atoms.

Over the last years, a huge group of hazardous organic contaminants has been evaluated as possible contaminants: perfluoroalkyl and polyfluoroalkyl substances (PFASs). As defined by Buck *et al*. (2011), "*PFASs are aliphatic substances containing one or more C atoms on which all the H substituents present in the nonfluorinated analogues from which they are notionally derived have been replaced by F atoms, in such a manner that PFASs contain the perfluoroalkyl moiety* C_nF_{2n+1}-" [4].

Thus, PFASs have drawn great attention due to their persistence, toxicity and bioaccumulative potential, all of which may lead to various harmful effects on humans, wildlife or even the environment. PFASs remain intact for long periods of time due to their high resistance to degradation because of their very strong carbon-fluorine bonds. As a consequence of their high persistence and water solubility, this class of anthropogenic pollutants can be transported for long distances through water currents or the atmosphere thus increasing the possibilities of creating certain risks for animals and human health in a wide range of geographical locations. For decades and based on their functionality and production capability, they have been used in numerous industrial applications and consumer products, such as carpets, fire-fighting foams, lubricants, household products, packaging materials, textiles, insecticides, among others. Many PFASs are also used as surfactants, lowering the surface tension of a liquid, or the interfacial tension between

two liquids. This property makes these chemicals very popular in terms of wetting and levelling agents, emulsifiers or dispersants [4].

Regarding PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) (Figure 1) are well-known world-wide pollutants, which are distributed throughout the environment as well as accumulated in humans. The combination of their high aqueous solubility and low vapour pressure makes it unlikely for these compounds to be transported through air over large distances [5]. In addition, PFOS and PFOA are persistent in the environment as degradation compounds of other PFASs, therefore rising global concern on its regulation.

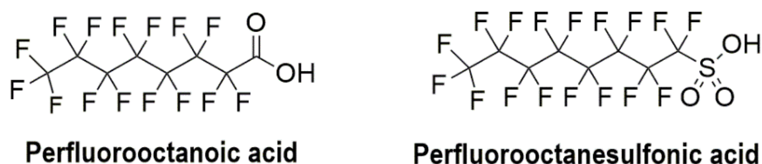


Figure 1. Structure of both perfluorooctanoic and perfluorooctanesulfonic acids.

PFOS in particular, has been included into Annex B of the Stockholm Convention [2] and therefore identified as POP, while PFOA has already been proposed as candidate for listing.

Moreover, there are other groups of fluorinated compounds which are considered potential precursors of some PFASs, including PFOA and PFOS, reported by Backe *et al*. (2013) [6]:

- fluorotelomers;
- perfluoroalkyl sulfonamido amines;
- perfluoroalkyl sulfonamide amino carboxylates;
- perfluoroalkyl sulfonates;
- perfluoroalkyl carboxylates;
- perfluoro betaines;
- perfluoro sulfonamide ketones, aldehydes and ethers.

The PFASs precursors determined in this study are focused to fluorotelomer alcohols (FTOHs), perfluorooctanesulfonamides (FOSAs), perfluorooctanesulfonamide ethanols (FOSEs) and fluorotelomer olefins (FTOs) (Figure 2). These compounds are released into the environment through volatilization and could undergo long-range atmospheric transport according to Fromme

et al. (2009) or even through water currents. Thus, they allow a widespread distribution in the environment as the persistent PFOA and PFOS [7].

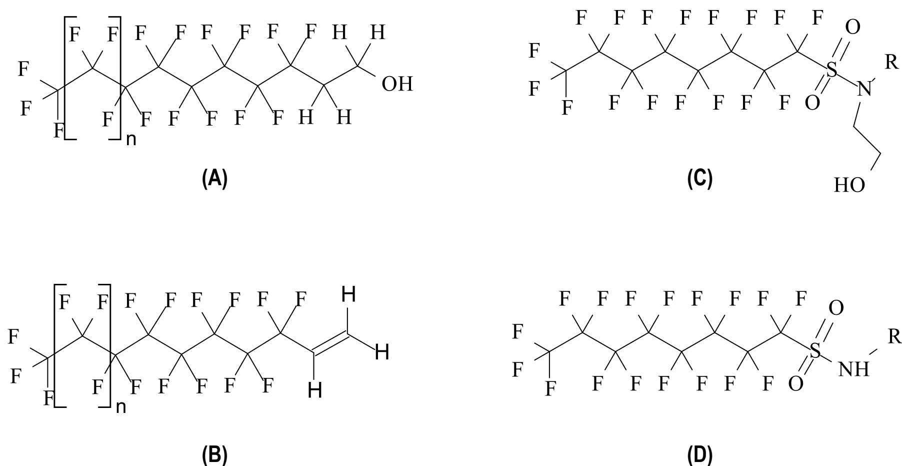


Figure 2. PFASs precursor chemical structures (A: FTOHs; B: FTOs; C: FOSEs; D: FOSAs).

Physicochemical properties for the target compounds present in this project are shown in Table 1.

As a general trend for the same family of compounds, the boiling points increase with the chain carbon length and, consequently, their vapour pressure decreases [8]. This behaviour is fully in accordance with the values given in Table 1. In addition, an increase of the polarity of the molecules causes a decrease on the vapour pressure due to dipole–dipole and dipole-induced dipole interactions. Thus, compounds of high polarity, such as FOSAs and FOSEs, have a relatively lower vapour pressure than the fluorotelomer alcohols [9].

Compound	**Acronym**	**CAS Registry Number**	**Molecular Weight [g/mol]**	**Boiling Point [ºC]** [8]	**Vapour Pressure at 25º ºC [Pa]** [9]
1H,1H,2H-Perfluorohexene	4:2 FTO	19430–93–4	246.08	59	-
1H,1H,2H-Perfluoro-1-octene	6:2 FTO	25291–17–2	346.09	102	-
1H,1H,2H-Perfluoro-1-decene	8:2 FTO	21652–58–4	446.11	146-147	-
1H,1H,2H,2H-Perfluorohexan-1-ol	4:2 FTOH	2043-47-2	264.09	140-143	214
1H,1H,2H,2H-Perfluorooctan-1-ol	6:2 FTOH	647-42-7	364.11	172	18.2
1H,1H,2H,2H-Perfluoro-7-methyloctan-1-ol	7-Me-6:2 FTOH	20015-46-7	414.11	180-184	-
1H,1H,2H,2H-Perfluorodecan-1-ol	8:2 FTOH	865-86-1	464.12	188	3.98
1H,1H,2H,2H-Perfluorododecan-1-ol	10:2 FTOH	678-39-8	564.14	222.4	0.20
N-Methyl-perfluorooctane-1-sufhonamide	*N*-MeFOSA	31506-32-8	513.17	227	0.30
N-Ethyl-perfluorooctane-1-sulfonamide	*N*-EtFOSA	4151-50-2	527.20	247	0.12
2–(*N*–methylperfluoro–1–octanesulfonamido)–ethanol	*N*-MeFOSE	24448-09-7	557.22	300	0.0004
2–(*N*–ethylperfluoro–1–octanesulfonamido)–ethanol	*N*-EtFOSE	1691-99-2	571.25	317	0.002

Table 1. Physicochemical properties of some of the PFASs studied in the present project.

As a general trend for the same family of compounds, the boiling points increase with the chain carbon length and, consequently, their vapour pressure decreases [8]. This behaviour is fully in accordance with the values given in Table 1. In addition, an increase of the polarity of the molecules causes a decrease on the vapour pressure due to dipole–dipole and dipole-induced dipole interactions. Thus, compounds of high polarity, such as FOSAs and FOSEs, have a relatively lower vapour pressure than the fluorotelomer alcohols [9].

3.2.1. Toxicity

Although PFASs precursors do not present a strong toxicity, their degradation to form the persistent PFOS and PFOA requires the monitoring of their levels in the environment. Regarding PFOS and PFOA, they have been the focus of the vast majority of toxicity studies. The most common exposure to PFASs is mainly via ingestion. In 2008, the European Food Safety Authority established a Tolerable Daily Intake value for PFOS and PFOA of 150 and 1.500 ng kg^{-1} of body weight (bw) day^{-1}, respectively. Regarding recent toxicity data, these reference doses have been lowered and set by the United States Environmental Protection Agency (U.S. EPA) to 30 ng kg^{-1} bw day^{-1} for PFOS and 20 ng kg^{-1} bw day^{-1} for PFOA (2014) [10,11].

Both PFOS and PFOA are well absorbed orally and are slowly eliminated from the human body with a half-life of approximately 8.7 and 3.8-4.4 years, respectively [12]. These chemicals are distributed to liver, plasma, and kidney. Neither of them have mutagenic properties, unless exposure at relatively high doses, as seen in studies with animals, which happened to induce tumoral activity.

Some data on acute toxicity of PFOS and PFOA have been carried out mainly in rats, which shown a wide range of symptoms, after inhalation and ingestion exposure, such as nasal discharge, breathing disturbances, hypoactivity, stomach distension and lung congestion due to PFOS (cited in the Organisation for Economic Co-operation and Development, OECD, 2002), and enlarged livers, gastrointestinal irritation and weigh loss after PFOA ingestion [12].

Carcinogenic effects of PFOS and PFOA has also been an issue of concern lately. Nevertheless, due to existing uncertainties, the U.S. EPA classifies them as “suggestive carcinogens” rather than “proven carcinogens”.

3.2.1.1. Bioaccumulation

As defined by Conder *et al*. (2008): “*bioaccumulation of perfluorinated acids is directly related to the length of each compound’s fluorinated carbon chain*” [13]. In addition, it is defined under REACH that a substance is bioaccumulative if its bioconcentration factor (BCF) in aquatic species is higher than 2000 L Kg^{-1} (Commission Regulation (EU) Nº 253/2011) [14].

Examples of selected bioaccumulation factors, which implies the increase of a chemical concentration due to absorption from food/environment, are shown in Table 2 for both PFOS and PFOA.

Substance	Food/Environment	Bioaccumualtion Factor (BAF) (L/kg)	Reference
PFOS	Zooplankton/water	240	[15]
	Mysis/water	1200	
	Sculpin/water	95000	
	Lake trout/water	16000	
PFOA	Water breathing animals	0.9-266	[16]

Table 2. Selection of bioaccumulation factors in certain organisms for both PFOS and PFOA.

PFOA presents lower values of bioaccumulation when compared with PFOS. This might be explained because of PFOA's notable solubility in water (9.5 g L^{-1} at 25ºC) [17]. Both PFOS and PFOA have been found to be distributed in living organisms including humans, birds and specially fish, where the bioaccumulation factor is considerably high in organisms such as sculpin or the lake trout. In addition, the presence of both compounds in animals, surface and tap water demonstrate their bioaccumulative phenomena in the ecosystems.

3.2.2. Legislation and regulation

Nowadays, there is no legislation about the concentration levels of PFASs precursors such as FTOHs, FTOs, FOSAs and FOSEs. In fact, there is few legislation about persistent fluorinated compounds because, as previously explained, PFOS was recently added to Annex B of the Stockholm Convention on POPs (2009), while PFOA (including PFOA-related compounds and its salts) was proposed for listing from June 2015.

3.2.2.1. Spanish legislation and regulation

Regarding the country legislation, the European Directive 2006/122/EC [18], stipulates that, in order to preserve health and the environment, regulation and restriction on PFOS's use and commercialization is strictly required (excepting situational minor usage of such substance). As a result, it may not be placed in the market or used as a component of any product, any kind of good if the concentration of PFOS exceeds or equals 0.005 % of its total weight (wt). In addition, EU Member States must establish inventories of such uses with the aim of obtaining reliable data on the exact quantities of PFOS that have been used. It also mentions the restriction of semi-finished goods which have PFOS in its structure, being 0.1 % in weight (wt%) the maximum

concentration allowed. However, there are some products such as photosensitive resins, certain anti-reflective coatings for photolithographic processes and hydraulic fluids for aviation that are not affected by the previous considerations. Spain adopted the present European Directive with the consequent modification of the Directive 76/769/CEE, stablishing PRE/374/2008 on PFOS use restriction [19].

3.2.2.2. European Union legislation

European Union's legislation is focused mainly on the use of PFOS and its derivatives. As previously described, PFOS was banned on its use in finished and semi-finished products excepting the above-mentioned. The restriction of PFOS was reinforced through Regulation 850/2004/EC, where the current threshold was lowered to or below 10 mg kg^{-1} (0.001 wt%). The existing Regulation (EC) nº 1342/2014 also sets a maximum value for PFOS of 50 mg kg^{-1}.

3.2.2.3. Legislation outside European Union

Advisory levels were set by the U.S. EPA in 2009: 0.4 µg L^{-1} and 0.2 µg L^{-1} for PFOA and PFOS respectively [11]. Therefore, household water consumption should be avoided above these limits. PFOS and PFOA are also included by this institution on the Draft Contaminant Chemical List 4 [20].

3.3. METHODS FOR THE DETERMINATION OF FLUORINATED ORGANIC COMPOUNDS

The breaking point which initiated a wide study and the development of improved methods and techniques was the finding of relatively high levels of PFOS in blood of several employees involved in fluorochemical production [21], and most important, also regarding mainly PFOS, its presence in wildlife [22]. Because of the occurrence all throughout the environment of these compounds, several analytical methods have been developed. As mentioned above, restrictions and legislation control over PFOS and PFOA caused their substitution in many applications by the PFASs precursors. Nevertheless, their capability to be degraded into persistent PFAS requires to dispose of sensitive analytical methods for their determination. Although some works have been published during last years, greater and improved instrumental simplicity of these methods is yet being the principal focus of researchers in order to facilitate their application on a regular basis.

The techniques most commonly used for the determination of FOCs are liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS).

Although other techniques such as capillary electrophoresis or the method used for the determination of the Total Organic Fluorine (TOF) are also employed, their use is quite limited.

3.3.1. Liquid chromatography methods

Liquid chromatography is generally the separation technique of choice when simultaneously determining persistent PFASs and their precursors (Table 3). It is generally employed with mass spectrometry using electrospray ionisation (ESI) source as the main ionisation technique. These methods require acid conditions to allow the chromatographic separation of the persistent PFASs. Nevertheless, the use of acidic species hindered the ionisation efficiency of the precursor compounds giving place to unselective formation of adduct ions, thus preventing their determination. Other ionisation techniques, like atmospheric pressure photoionisation (APPI), are alternatively employed [23], but their application is still very limited. It is important to note the extensive use of the liquid chromatography coupled to tandem mass spectrometry system (LC-MS/MS), which provides great selectivity and a significant improvement on the method limit of detection (mLOD). For instance, Yamashita *et al.* (2004) reported LOD values of 0.4-5.2 pg L^{-1} for PFASs in seawater [24], demonstrating the high capacity offered by LC-MS/MS for the determination of these compounds at low concentration levels.

Matrix	Analytes	Extraction/ Enrichment	Determination	LOD/LOQ	Ref.
Water	PFASs	Aria TLX-1 (online enrichment) SPE (Oasis® WAX)	HPLC-ESI(-)-MS/MS	0.83-10 ng L^{-1} (LOD)	[25]
Water	FTOHs	ACN addition LLE (MTBE)	HPLC-MS/MS	60-90 ng L^{-1} (LOD)	[26]
Lake water	Perfluorooctane surfactants	SPE (C_{18})	HPLC-ESI(-)MS	0.2-13 ng L^{-1} (LOQ)	[27]
River water	PFOS	On-line extraction (turbulent flow chromatography)	HPLC-APPI(-)-MS	5.35 ng L^{-1} (LOD)	[23]
Seawater	Perfluorinted acids (PFAs)	SPE (C_{18})	HPLC-MS/MS	0.4-5.2 pg L^{-1} (LOD)	[24]
Human serum	PFASs	SPE	HPLC-ESI(-)-MS/MS	1-6 ng L^{-1} (LOD)	[28]

Human serum	PFOS, perfluorohexane sulfonate (PFHxS), carboxylates, *N*-MeFOSA	SPE (TurboFlow)	HPLC-ESI(-)-MS/MS	0.16-0.34 μg L^{-1} (LOQ)	[29]
Indoor dust	PFASs	SPE (Oasis® HLB DCHP)	UHPLC-ESI(-)-MS	0.008-0.846 ng g^{-1} (mLOD)	[30]
Sediments and sludge	PFASs	SLE (MeOH/acetic acid in water)	HPLC-ESI(-)-MS/MS	0.011-2.2 ng g^{-1} (LOD)	[31]

Table 3. Selected methods employing liquid chromatography developed for the determination of PFAS in environmental matrices.

3.3.2. Gas chromatography methods

Gas chromatography, although less commonly used than LC, is a reliable option when determining PFASs precursors in different matrices. GC shows larger efficiency compared to other methods of separation but presents an important limitation which is the volatility of the analytes that are going to be determined. Thus, persistent PFASs cannot be determined by GC-MS without a previous derivatisation. However, the analytes determined in the present project are highly volatile and therefore GC methods are suitable for their identification and determination. As well as in LC, GC methods are often used coupled to MS, usually using electron ionisation (EI) or chemical ionisation (CI) techniques, including positive chemical ionisation and negative ion chemical ionisation (NICI) [32,33].

Table 4 shows a description of a selected GC methods reported in the last few years for the determination of PFASs. Recently, it has to be highlighted the use of the atmospheric pressure chemical ionisation (APCI) technique for the determination of non-ionic PFASs in water samples by GC-high resolution MS [34]. APCI is an alternative source for the ionisation of neutral compounds in LC-MS coupling and its application in that particular GC-MS systems provided a soft ionisation method which allowed a selective determination and identification of the target compounds.

Matrix	Analytes	Extraction/ Enrichment	Determination	LOD/LOQ	Ref.
Groundwater	Perfluorocarboxylates	SPE (SAX)	GC-EI-MS	18 μg L^{-1} (LOD)	[35]
Wastewater and seawater	Perfluorocarboxylic acids (PFCAs)	Ion-pair SPME	GC-NICI-MS	50-2,500 ng L^{-1} (LOQ)	[36]
Surface water and rainwater	Perfluorocarboxylic acids/anions	LLE (EtOAc)	GC-MS	0,5 ng L^{-1} (mLOD)	[37]
River water, influent and effluent wastewater	FTOHs, FOSAs,FOSEs	SPE (Oasis HLB™)	GC-APCI (+)-MS/MS	1-5 fg (LOD)	[34]
Water and sediments	PFASs, FTOHs, FOSAs	HS-SPME (50/30 μm DVB/CAR/PDMS)	GC-EI-MS	20-100 ng L^{-1} (LOQ)	[38]
Wastewater	PFASs	SPE (Oasis HLB™)	GC-EI-MS	0.5-2 pg (LOD)	[39]
Air	PFASs	SPE (Oasis HLB™)	GC-EI-MS	0.5-2 pg (LOD) 1 ng m^{-3}	[39]
Air	FTOHs, FOSAs, FOSEs	GFF PUF/XAD/PUF	GC-NICI-MS	0.2-2.5 pg m^{-3} (mLOQ)	[40]
Air	FTOHs, FOSAs, FOSEs	SPE (Isolute ENV +)	GC-(CI/NICI)-MS	10-300 pg m^{-3} (mLOD-indoor method) 0.3-2.1 pg m^{-3} (mLOD-outdoor method)	[41]
Air	FTOHs, FOSAs, FOSEs	Tenax/Carbograph 1TD tube	GC-MS	0.4-2.9 pg (LOD) 45-166 pg m^{-3} (mLOD)	[42]
Popcorn bags	PFCAs, FTOHs	SLE (MeOH or EtOAc)	GC-MS	2.5-25 ng/extracted item (LOQ)	[43]

Table 4. Selected methods employing gas chromatography for the determination of PFAS in environmental and food packaging samples.

3.3.3. Extraction techniques

The analysis of environmental contaminants requires determination of these compounds at trace concentration levels. Thus, a previous extraction and pre-concentration technique is necessary to achieve this goal. The matrix in which the analytes are confined is typically one of the most important factors which determines the election of the appropriate extraction technique. In aqueous matrices, solid-phase extraction (SPE) is undoubtedly the prevailing enrichment or clean-up method, which can also be automated for increasing sample throughput. An alternative to SPE is the liquid-liquid extraction (LLE) but its automatisation is quite more limited. For air samples, a pre-concentration of the sample in a solid sorbent is almost in all cases required. Biota samples are analysed with methods based on the formation of ion pairing ionic PFASs with tetra-*n*-butylammonium hydrogensulfate, followed by solid-liquid extraction (SLE), as reported by Hansen *et al.* (2001) [44]. However, ion pair extraction (IPE) is a time consuming method and shows certain limitations on its automatisation. Regarding extraction of analytes from sludge, suspended matter or sediments, SLE using different organic solvents is the method of choice in many applications. For the extraction of the most polar and/or ionic PFASs, the use of moderately polar solvents such as methanol or acetonitrile is required, while for compounds with a polar group but long carbon chains non-polar solvents, like n-hexane, are often used.

Since 1990, new extraction techniques were introduced to simplify the extraction procedure and also to minimise or eliminate the use of organic solvents. These techniques have acquired in the last years a considerable relevance compared to the classical solvent extraction or SPE and had been rapidly growing because of their wide applicability and sensitivity. Among them, solid-phase microextraction (SPME) has been used in numerous fields, including environment and food analysis, forensic analysis, characterisation of natural products, and pharmaceuticals. Moreover, SPME matches with the main principles of green chemistry.

3.3.3.1. Solid-phase extraction

As previously mentioned, SPE is generally the method of choice when analysing aqueous matrices. It usually may require a pre-enrichment of the sample, which can be provided by both SPE and LLE methods, therefore several studies employed Oasis-HLB [30,34,39], C_{18} [27] and Oasis-WAX [25] as sorbents. When analysing blood or human serum, SPE is also considered, although the application of LLE provided better results for the analysis of these specific matrices. For instance, Szostek and Pricket successfully extracted the telomere alcohol 8:2 FTOH from a

rat's plasma using LLE [45] with excellent results. In most cases a sample pre-treatment and clean-up should be accurately included in the sample treatment, mainly to avoid clogging of the SPE phases. SPE extraction provides numerous advantages when compared to other extraction techniques such as LLE, such as good sensitivity, relatively high selectivity avoiding many of matrix effects on the identification and quantification of the target compounds, and it can also be automatised. Nevertheless, SPE also has some common problems when compared to other extraction techniques, such as reproducibility issues, extensive handling time of the sample, and the potential clogging of the sorbent.

3.3.3.2. Solid phase microextraction

As it was mentioned above, microextraction techniques were introduced as an alternative to the classical extraction techniques such as LLE or SPE. SPME was first proposed by Arthur and Pawilszyn (1990) [46] and has been rapidly gaining interest and popularity since then. SPME is an example of sorbent extraction, solvent-free sample preparation technique, which reduces the drag of sample preparation and consequently reducing analysis time. In this technique the SPME fibre can be directly immersed into the sample solution or hangs into the headspace of the recipient for sampling of the target compounds. Consecutively analytes are enriched in the exposed coating of the fibre until their equilibrium states are reached between both phases. Then, they are usually transferred to an injector of a gas chromatograph, as the fibre can be directly used as a syringe, and the analytes are thermally desorbed into the GC system for their separation and detection. The principal concern of the SPME is to minimise as maximum as possible the time it takes to the system to reach the equilibrium of the analytes between the sample and the extraction fibre [47]. The type of fibre employed depends upon the nature of the analyte to be extracted. The most commonly used fibres are: polydimethylsiloxane (PDMS), divinylbenzene (DVB)/PDMS, Carboxene (CAR)/PDMS, polyacrylate (PA) and the triple fibre DVB/CAR/PDMS.

As previously stated, extraction can be performed through multiple ways:

- **Direct extraction:** the coated fibre is immersed directly into the sample solution [48]. Stirring may be desirable in order to facilitate the extraction.
- **Headspace extraction:** an equilibrium between the headspace and the extracting fibre coexists with the one reached between the sample and the headspace itself. This extraction method prevents and protects the fibre from deterioration due to the

sample matrix. This procedure might be suitable for the analytes determined in this study considering they all are highly volatile.

- **Membrane protection extraction:** the extraction is performed using a selective membrane which allows certain compounds to pass while others remain blocked [49]. This method, unlike headspace extraction, facilitates the extraction of the non-volatile analytes.

To summarise, SPME presents major advantages such as simplified sample preparation and handling since it is a solvent-free method, reduced cost and time of analysis and increased reliability, selectivity and sensitivity. Nevertheless, certain considerations regarding this technique should not be overlooked. High selectivity of SPME fibres towards analytes does exist, for that reason a proper knowledge of the matrix is essential when choosing the fibre. In addition, the ageing of the extraction fibre may result in low reproducibility thus affecting the posterior quantitative measurements.

Until now, only one method using SPME has been reported for the analysis of some PFASs in water and sediments samples [38], but the real applicability of this technique for the determination of a wide range of PFASs precursors, such as FTOs, FTOHs, FOSAs, and FOSEs, has not been evaluated yet.

4. OBJECTIVES

The main objective of the present research project is to develop and validate an analytical method for the determination of semi-volatile PFASs in water samples based on headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). To accomplish the main goal of this project, the research work was conducted through the following sub-objectives:

- To develop a reliable analytical method for the simultaneous determination of different families of semi-volatile perfluoroalkyl substances, such as fluorotelomer alcohols, perfluorooctanesulfonamide, perfluorooctanesulfonamide ethanol and fluorotelomer olefins, in water of different origin at low ng L^{-1} levels.
- To ensure, through a method optimisation procedure, the establishment of the optimal conditions for both sample treatment through the HS-SPME technique and adequate determination by GC-MS to accomplish maximum selectivity and sensitivity on the quantification of the target compounds.
- To determine the quality parameters of the method, in order to prove the method’s suitability and validity for its application to real water samples.
- To assess the applicability of the present method for the determination of semi-volatile PFASs in water samples of different origin.

All these objectives and the research work performed during the realisation of the present project will be further detailed in the following sections in conjunction with the results obtained and their respective discussion.

5. EXPERIMENTAL SECTION

This section includes the development of the experimental procedure as well as the instrumentation required for the optimisation and validation of the whole method.

5.1. MATERIALS AND METHODS

5.1.1. Reagents and standards

The fluorotelomer olefins: 4:2 FTO (1H, 1H, 2H–perfluoro–1–hexene), 6:2 FTO (1H, 1H, 2H–perfluoro–1–octene), 8:2 FTO (1H, 1H, 2H–perfluoro–1–decene), and the fluorotelomer alcohols: 4:2 FTOH (1H, 1H, 2H, 2H–perfluorohexan–1–ol), 6:2 FTOH (1H, 1H, 2H, 2H–perfluorooctan–1–ol) and 7-Me-6:2 FTOH (1H, 1H, 2H, 2H–perfluoro–7–trifluoromethyl–octan–1–ol) were obtained at a purity over 97% from Fluorochem Ltd. (Derbyshire, UK), while 8:2 FTOH (1H, 1H, 2H, 2H–perfluoro–1–decanol) and 10:2 FTOH (1H, 1H, 2H, 2H–perfluoro–1–dodecanol) were obtained from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). Moreover, individual *N*-MeFOSA (*N*–methylperfluoro–1–octanesulfonamide), *N*-MeFOSE (2–(*N*–methylperfluoro–1–octane sulfonamido)–ethanol) and *N*-EtFOSE (2–(N–ethylperfluoro–1–octanesulfonamido)–ethanol) certified standard solutions of 50 mg L^{-1} in methanol were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada), while *N*-EtFOSA (*N*–ethylperfluoro–1–octanesulfonamide) pure standard was acquired from Dr. Ehrenstorfer Gmbh (Augsburg, Germany). Individual solid standards of 7:1 FA (1H, 1H–pentadecafluoro–1–octanol), 8:1 FA (1H, 1H–perfluoro–1–nonanol), 9:1 FA (1H, 1H–perfluoro–1–decanol) and 11:1 FA (1H, 1H–perfluoro–1–dodecanol), used as internal standards, were also provided by Fluorochem Ltd. at a purity higher than 96%.

Methanol was purchased from Sigma Aldrich (St Louis, MO, USA), sodium chloride (99.99%) was supplied from Panreac (Montcada i Reixac, Spain), hydrochloric acid (37%, w/v) and sulfuric acid (purity ≥ 95-97%) for analysis grade were both provided by Merck KGaA (Darmstadt, Germany). In addition, Milli-Q water was obtained through an Elix ® Millipore system. Helium 5.3

(purity ≥ 99.9993 %) and methane (purity ≥ 99.995 %) were obtained from Linde España (Barcelona, Spain) and Air Liquide España (Madrid, Spain), respectively.

Stock solutions of individual standards were each prepared a concentration of 5000 mg L^{-1} in methanol. Intermediate individual solutions of 500 and 100 mg L^{-1} were also prepared in methanol from individual stock solutions. Work standard solutions were prepared by mixing and dilution of the intermediate individual solutions. For quantification, aqueous calibration solutions at concentrations between 0.005 and 5 µg L^{-1} containing the internal standards at a concentration of 1 µg L^{-1} were prepared by adding adequate amounts of the work standard solutions. All solutions have been stored in a refrigerator at 4 ºC while not being used. Before use, all glassware was treated and cleaned with a 1M hydrochloric acid solution and rinsed with Milli-Q water and acetone.

5.1.2. Samples

The developed method was applied to the identification and quantification of PFASs precursors in water samples. In this project, water samples from three different sources, river, tap, and seawater, were analysed. The river sample was collected from the Llobregat River, which runs through several industrialised areas, receiving significant amounts of wastewater from the population inhabiting its surroundings, before flowing into the Mediterranean Sea. This sample was brought in a 1000 mL glass bottle without leaving headspace before being stored in a refrigerator at 4 ºC for its posterior analysis. Tap water samples were obtained from Barcelona's water supply networks at the Faculty of Chemistry (Barcelona, Spain), and a seawater sample for alimentary purposes purchased from Marevendis Agua de Mar S.L (Alicante, Spain), were also analysed.

5.1.3. Sample treatment

The target compounds were extracted from water samples using headspace solid-phase microextraction technique. Prior to the first use, SPME fibre was conditioned for 60 minutes at 250 ºC. After optimisation, the HS-SPME method applied for the determination of the analytes was as follows: 10 mL water sample previously filtered (if needed) or aqueous calibration solution were placed into a 20 mL headspace vial, which is fitted with polytetrafluoroethylene (PTFE)/silicone septa containing a 10 mm x 5 mm PTFE-coated stir bar and an appropriate amount of the internal standards (1 µg L^{-1} of 7:1 FA, 8:1 FA, 9:1 FA and 11:1 FA). Before the

HS-SPME analysis, the sample vial was vortex mixed for 3 min and conditioned for 15 min in a thermostatic water bath at the extraction temperature (60ºC). This time was considered enough to reach equilibrium of the analytes between headspace and aqueous phase. Afterwards, the fibre (50/30 µm divinyilbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS) was exposed into the vial for 25 minutes to perform headspace extraction. Extraction was in all cases performed under constant stirring (750 rpm). Thermal desorption of the target compounds was carried out by exposing the fibre in the GC injector port at 250 ºC for a splitless time of 3 minutes. Finally, the fibre was kept in the injector port for an additional time of 15 minutes, with split mode, for cleaning and preventing possible carryover between samples and then it was saved in a free-analyte vial until the next extraction. Further details about the optimisation of the HS-SPME procedure are given in section 6.2.

5.1.4. Instrumentation

5.1.4.1. Basic instrumentation

Simple instrumentation used during the realisation of the present project is listed hereunder:

- Vortex mixer (Stuart, Staffordshire, UK)
- Tectron Bio immersion thermostat (JP Selecta S.A., Barcelona, Spain)
- Branson 5510 ultrasonic cleaner (Branson Ultrasonics Corporation, Danbury, Connecticut, USA)
- PB1502-L grain weighing scale (Mettler Toledo, Hospitalet de Llobregat, Spain)
- AB204 analytical balance (Mettler Toledo, Hospitalet de Llobregat, Spain)
- pH meter Basic 20 (Hach Lange Spain, S.L.U., Hospitalet de Llobregat, Spain)
- 10, 25, 50 and 100 µL micro-syringes (SGE Analytical Science, Melbourne, Australia)
- 10 mL syringe (SGE Analytical Science, Melbourne, Australia)
- 20 mL headspace vial (CromLab S.L., Barcelona, Spain)
- 20 mm PTFE/Silicone Septum (CromLab S.L., Barcelona, Spain)
- 20 mm crimp caps (CromLab S.L., Barcelona, Spain)

In addition, the following SPME fibres were tested for the extraction's optimisation: 50/30 µm DVB/CAR/PDMS, 85 µm PDMS/CAR, 65 µm PDMS/DVB, 100 µm PDMS and 85 µm PA, all of them supplied from SUPELCO® (Bellefonte, Pennsylvania, USA). HS-SPME experiments were performed with a manual fibre holder supplied from Supelco. Before use, each fibre was conditioned in the GC injection port under helium flow according to the manufacturer's recommendation. After conditioning, fibre blanks were periodically run to ensure there were no contaminants or carryover.

5.1.4.2. Gas chromatography-mass spectrometry

Separation and quantification of PFASs in both samples and standards was performed using a Varian CP-3800 GC system coupled to a Varian Saturn 2200 mass spectrometer system (Varian, Inc., Palo Alto, California, USA) with an ion-trap (IT) analyser operating in electron ionisation (EI) mode. Standard injections were carried out using a PAL system autosampler (CTC Analytics AG, Zwingen, Switzerland) while SPME sample injections were performed directly from the fibre into the system. The GC system was equipped with a DB-624 column (6% cyanopropyl phenyl 94% polydimethylsiloxane, Agilent Technologies, Santa Clara, California, USA) fused silica capillary column, 60m x 0.25 mm I.D., 1.40 µm film thickness. The injector temperature was kept at 250 ºC working at splitless injection mode keeping the split valve closed for 3 minutes after injection. The oven temperature was programmed as follows: from 50 ºC (3 min) to 120 ºC at a rate of 10 ºC min^{-1}, to finally reach 250 ºC (10 min) at a rate of 25 ºC min^{-1}. Helium was used as the carrier gas at a constant flow rate of 1.3 mL min^{-1} held by electronic flow control. A glass inlet liner (I.D., 0.75 mm, SGE Europe) was used for the SPME-GC analysis. The ion-trap MS was operated in electron ionisation mode with an electron energy of 70 eV and an emission current of 20 µA, and in chemical ionisation mode using methane as reagent gas at flow rate of 2.5 mL min^{-1}. Ion trap, transfer line and manifold temperatures were held at 180 ºC, 250 ºC and 80 ºC, respectively. For data acquisition, the selected ion storage (SIS) mode monitoring the most abundant ion for each compound was used at a scan time of 0.7 s/scan (5-10 µscans). Quantification of the target compounds was carried out by internal standard method, using 7:1 FA for FTOs, 4:2 FTOH and 6:2 FTOH; 8:1 FA for 7–Me–6:2 FTOH; 9:1 FA for 8:2 FTOH and 11:1 FA for 10:2 FTOH, FOSAs and FOSEs. All data and the whole operating system was acquired and processed using Saturn® GC/MS Workstation (version 6.41) computer software.

6. RESULTS AND DISCUSSION

6.1. GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION

This section includes the cromatographic conditions and the mass-spectrometry optimisation procedure employed for the determination and quantification of the target compounds.

6.1.1. Gas chromatography separation

As it was mentioned before, gas chromatography had been the technique most widely used for the separation of the target compounds. Thus, a GC-MS method was developed for the determination of these analytes. In previous works from the research group, different chromatography columns were tested in order to proof their efficacy for the separation of the semi-volatile PFASs precursors. Three types of capillary columns with stationary phases of different polarity and characteristics were studied. A non-polar (DB-5MS, 5% phenyl 95% dimethyl polysiloxane), a semi-polar (DB-624, 6% cyanopropyl phenyl 94% dimethyl polysiloxane) and a polar column (TG-WAX, 100% polyethylene glycol) were assessed. The more polar column had trouble retaining FTOs while the apolar one had the same issue with FOSAs and FOSEs. In addition, the fact that these two columns had an internal diameter smaller than the moderately polar column (0.25 µm film thickness) created difficulties for the analytes determination. For that reason, and given the fact that semi-volatile PFASs precursors cover a wide family of compounds with different polarity profiles, the DB-624 fused silica capillary column of 60m of length, 0.250 mm I.D. and 1.40 µm of film thickness was selected for the respective analysis.

Another important factor to consider is the initial temperature for the separation. The oven temperature employed had already been optimised in previous works of the research group, however initial temperature required to be established in order to improve mainly the peak shape of FTOs, which showed a low retention with this stationary phase. For this reason, the effect of the initial oven temperature on the peak shape was evaluated at two different values: 40 ºC and 50 ºC. Although lower initial temperatures may achieve better peak shapes, no significant

differences were obtained using both temperatures. Therefore, 50 ºC was selected to reduce the analysis time as the optimal value.

6.1.2. Mass spectrometry optimisation

6.1.2.1. Selection of the MS ionisation mode

Two different ionisation techniques were tested in the present project: electron ionisation and positive chemical ionisation. When the electron ionisation technique is used, an energy of 70 eV is applied for the ionisation of the target compounds producing generally a high fragmentation. Meanwhile, when positive chemical ionisation is employed, a soft energy is applied in the ion source causing the ionisation of a reagent gas (generally methane) which reacts with the target compounds by proton or charge transferences. Taking into account the data obtained from each full scan spectrum, EI showed a higher in source fragmentation pattern compared to CI as expected. For instance, as can be observed in Figure 3, the base peak ion produced for FTOHs under EI conditions was $[CF_3]^+$ corresponding to a *m/z* of 69. In addition, the ion given at *m/z* 95 was also one of the most intense peak present in all spectra of FTOHs, belonging to the $[CF_2CH_2CH_2OH]^+$ ion. Nevertheless, when CI was employed, the protonated molecule belonged to the base peak of the mass spectrum, although a characteristic ion corresponding to the loss of H_2O+HF from the protonated molecule was also observed. Regarding FTOs, the prevailing ion produced under EI conditions was clearly the one corresponding to $[CF_2CHCH_2]^+$ with a *m/z* of 77 while concerning *N*-MeFOSA and *N*-EtFOSA the most intense ions were $[CH_3NHSO_2]^+$ (94 *m/z*) and $[CH_3CH_2NHSO_2]$ (108 *m/z*) which implied the loss of the perfluorinated alkyl chain, as can be seen in Figure 4 for *N*-MeFOSA. Finally *N*-MeFOSE and *N*-EtFOSE showed a common fragmentation ion for all the compounds of this family at 526 and 540 *m/z* respectively, which corresponded to the $[M-CH_2OH]^+$ ion in both cases.

When under CI conditions, less fragmentation occurred as previously mentioned in the FTOHs example. FTOs exhibited the loss of a fluoride ion, $[M-F]^+$, as its most intense peak, which can be seen on Figure 4 for 8:2 FTO. In contrast, FOSAs and FOSEs underwent the protonation of the precursor ion $[M+H]^+$, as happened with FTOHs.

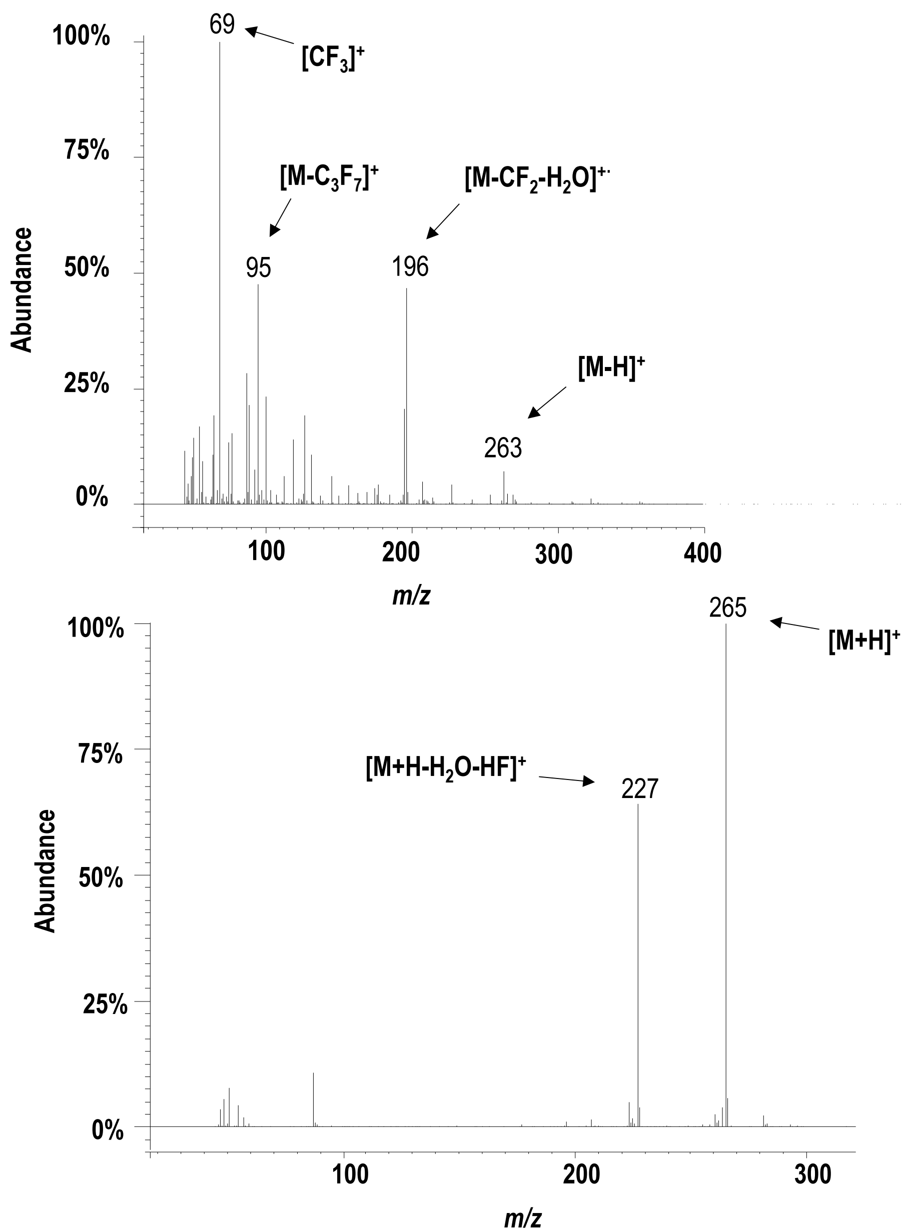


Figure 3. Spectra of 4:2 FTOH under EI (up) and CI (bottom) MS conditions.

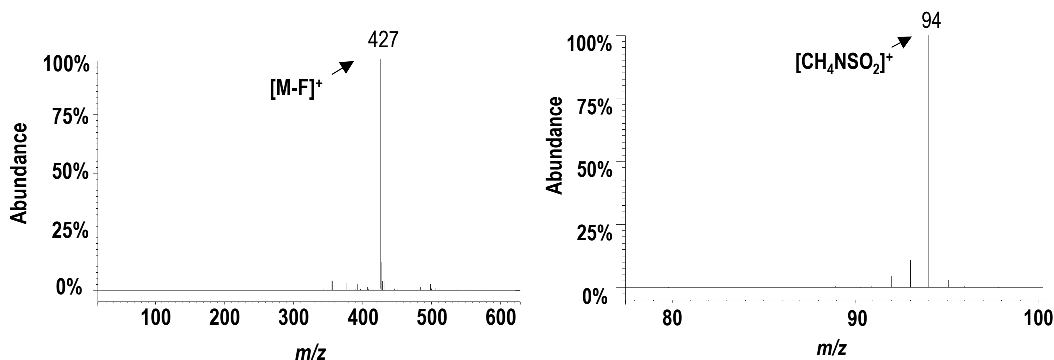


Figure 4. Spectra of 8:2 FTO (left) and *N*-MeFOSA (right) under EI (right) and CI (left) MS conditions.

After the identification of the most abundant ions for each ionisation technique, the chromatogram was divided in 5 different segments taking into account the retention time of the target compounds. The establishment of segments allows the mass spectrometer detector to look for a specific analyte only in a fixed time range where the elution of this compound is expected to occur. For instance, we fixed a first segment from 1 to 10 minutes for the determination of FTOs that eluted during this time while from 10 to 16 minutes we only monitored the ions for the determination of FTOHs. Thus, we could improve the capability of the mass spectrometer detector to determine the target compounds. Moreover, all spectra were acquired in the selected ion storage mode. This acquisition mode consists on the acquisition of small *m/*z ranges around the selected ions for each analyte rather than the entire spectrum, offering a considerable improvement in sensitivity when compared to the full scan mode. Besides the selection of the quantification ions, which are shown in Table 5, the selection of mass ranges for each family of compounds was accurately stablished for the SIS acquisition mode as follows: 60-90 *m/z* (FTOs), 50-80 *m/z* (FTOHs), 80-120 *m/z* (FOSAs), 510-540 *m/z* (*N*-MeFOSE) and 530-550 *m/z* (*N*-EtFOSE).

		Electron ionisation (EI)		Positive chemical ionisation (CI)	
Analyte	**t_R (min)**	**Ion (*m/z*)**	**Ion assignment**	**Ion (*m/z*)**	**Ion assignment**
4:2 FTO	5.07	77	$[C_3H_3F_2]^+$	227	$[C_6H_3F_8]^+$
6:2 FTO	6.86	77	$[C_3H_3F_2]^+$	327	$[C_8H_3F_{12}]^+$
8:2 FTO	9.16	77	$[C_3H_3F_2]^+$	427	$[C_{10}H_3F_{16}]^+$
4:2 FTOH	11.79	69	$[CF_3]^+$	265	$[C_6H_6F_9O]^+$
6:2 FTOH	13.31	69	$[CF_3]^+$	365	$[C_8H_6F_{13}O]^+$
7-Me-6:2 FTOH	14.05	69	$[CF_3]^+$	415	$[C_9H_6F_{15}O]^+$
8:2 FTOH	14.41	69	$[CF_3]^+$	465	$[C_{10}H_6F_{17}O]^+$
10:2 FTOH	15.25	69	$[CF_3]^+$	565	$[C_{12}H_6F_{21}O]^+$
***N*-MeFOSA**	17.41	94	$[SO_2NCH_4]^+$	514	$[SO_2NC_9H_5F_{17}]^+$
***N*-EtFOSA**	17.57	108	$[SO_2NC_2H_6]^+$	528	$[SO_2NC_{10}H_7F_{17}]^+$
***N*-MeFOSE**	19.46	526	$[SO_2NC_{10}H_5F_{17}]^+$	558	$[SO_3NC_{11}H_9F_{17}]^+$
***N*-EtFOSE**	20.03	540	$[SO_2NC_{11}H_7F_{17}]^+$	572	$[SO_3NC_{12}H_{11}F_{17}]^+$

Table 5. Retention times and the most intense ions obtained for all the compounds under both EI and CI MS conditions.

6.1.2.2. Mass spectrometry optimisation

In order to find the most optimal conditions to achieve the best ionisation efficiency of the selected ions for the determination of the target compounds, different MS parameters were optimised. For EI mode, the optimal conditions were previously established by the research group and they did not require further optimisation. For that reason, all the optimisation process was carried out under CI conditions. Thus, the following parameters were optimised: reagent gas flow, emission current, total ion current (TIC), maximum reaction time (MRT), maximum ionisation time (MIT) and the ion source temperature. All these parameters were sequentially studied and the optimal values obtained are shown in Table 6.

Parameter	Optimal value	Parameter	Optimal value
Reagent gas flow	2.5 mL min^{-1}	**Maximum reaction time**	100 msec
Emission current	50 µA	**Maximum ionisation time**	2500 µsec
Total ion current	2,000 counts	**Ion trap temperature**	165 ºC

Table 6. Optimal values for the different mass spectrometry CI-MS parameters.

Emission current can be understood as the current measured between the filament and the electron entry slit. It is essential to maintain this parameter at a constant level, therefore the number of ions produced will be directly related to the number of available molecules in the source region. It is important to mention that under EI conditions emission current is set as default value at 20 µA in our particular case with a fixed electron energy of 70 eV. For its optimisation under CI conditions, replicate experiments were performed at the following emission current values: 30, 50 and 70 µA. Comparing the results obtained at the different conditions, 50 µA was finally selected as the optimal intensity regarding the values of peak area obtained. Afterwards, the total ion current was considered. This parameter determines the amount of ions that are stored into the ion trap and it was evaluated at 2,000, 3,000, 5,000 and 7,000 counts. As shown in Figure 5, the best results were obtained at 2,000 counts, thus we selected that value as the optimal TIC.

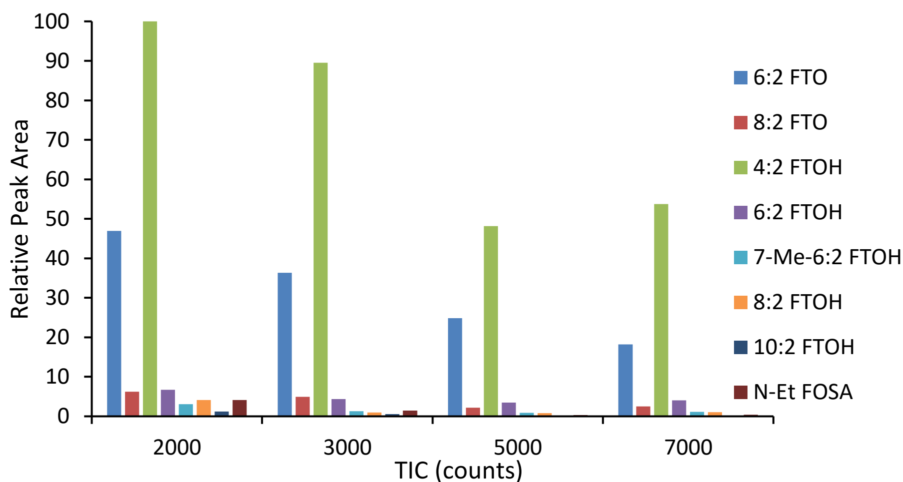


Figure 5. Effect of the total ion current in terms of relative peak area for FTOs, FTOHs and *N*-EtFOSA using CI mode.

Another important parameter that affects the sensitivity of the MS system is the ion trap temperature, since it affects the fragmentation degree of the analytes. This parameter was evaluated at three different temperatures: 165 ºC, 170 ºC and 180 ºC. As can be seen in Figure 6, an ion-trap temperature of 165 ºC seemed to slightly provide better results.

Therefore, this temperature was selected as optimal value for further experiments.

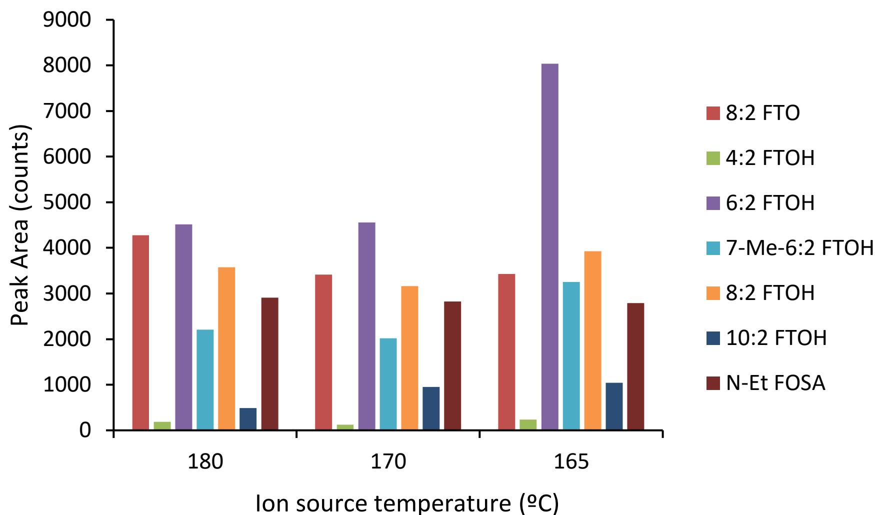


Figure 6. Effect of the ion source temperature in terms of peak area for 8:2 FTO, FTOHs and *N*-EtFOSA using CI mode.

Finally, maximum reaction time and maximum ionisation time, which respectively refer to the time necessary for the interaction of the analyte molecules with the reagent gas and the time required for the ionisation of the reagent gas (methane), were assessed. Triplicate experiments at 60, 80 and 100 msec as MRT were performed, while MIT was evaluated at 1,500, 2,000 and 2,500 msec. Once all spectra were acquired, best response was observed at both highest values studied, those being 100 msec as MRT and 2,500 msec for MIT. This constituted a logical conclusion as the more time methane can be ionised and the more time ions can interact with the reagent gas, the higher ionisation efficiency they experimented.

Comparing the response obtained using both ionisation techniques, CI provided considerably less peak intensity than that obtained with EI as can be seen in Figure 7, in which the GC-MS chromatograms obtained under EI and CI optimal conditions are given. Therefore, EI was the ionisation of choice for the determination and quantification of the target compounds because it provided a better ionisation efficiency and thus it was consequently selected as the most suitable ionisation mode.

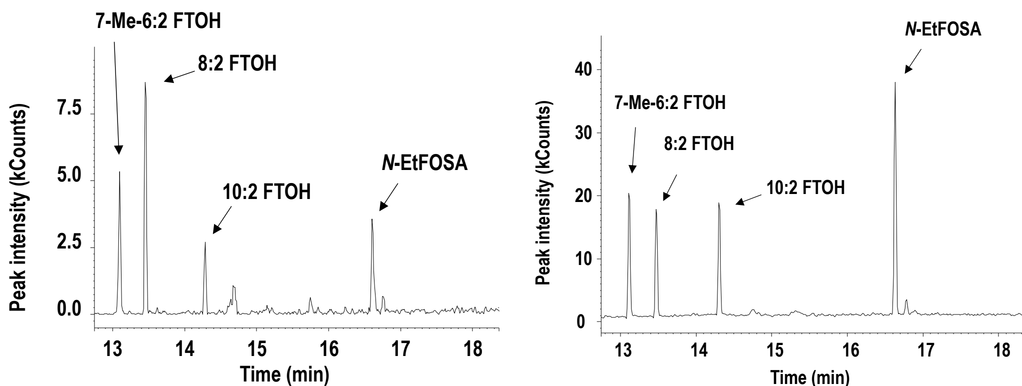
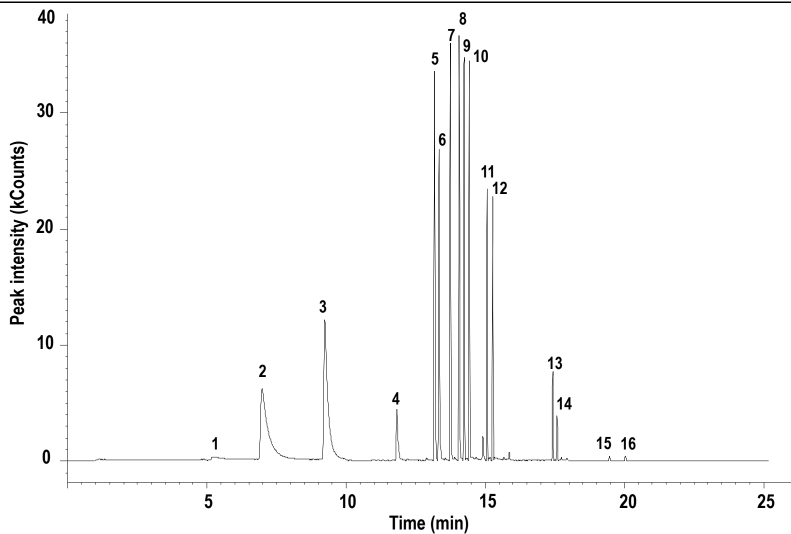


Figure 7. Selected GC-MS chromatograms of 7-Me-6:2 FTOH, 8:2 FTOH, 10:2 FTOH and *N*-EtFOSA obtained under CI (left) and EI (right) optimal conditions.

6.1.2.3. Influence of the glass liner in the chromatographic separation.

The choice of glass liner is also an important factor to consider when trying to improve the method's performance. The liner is directly related with peak broadening, for that reason, two types of glass liner were evaluated: a conventional splitless glass liner (3 mm I.D.) and a specific kind of liner for SPME-use (0.75 mm I.D.). The small internal diameter of the SPME liner results in a higher linear rate of the carrier gas during the injection process that allows a rapid transference of the analytes from the injector inlet to the column head. The injector was configured in the splitless mode to ensure full transference of the analytes from the fibre to the column. Figure 8 shows the effect of both liners in the peak shapes of the target compounds. As can be seen, there was a significant improvement in terms of peak broadening and tailing when the SPME glass liner is employed, being FTOs the ones benefiting the most out of this optimisation. This fact could be explained due to the best transference of these first eluted compounds into the column. Thus, we performed all the following optimisations using the specific SPME glass liner.



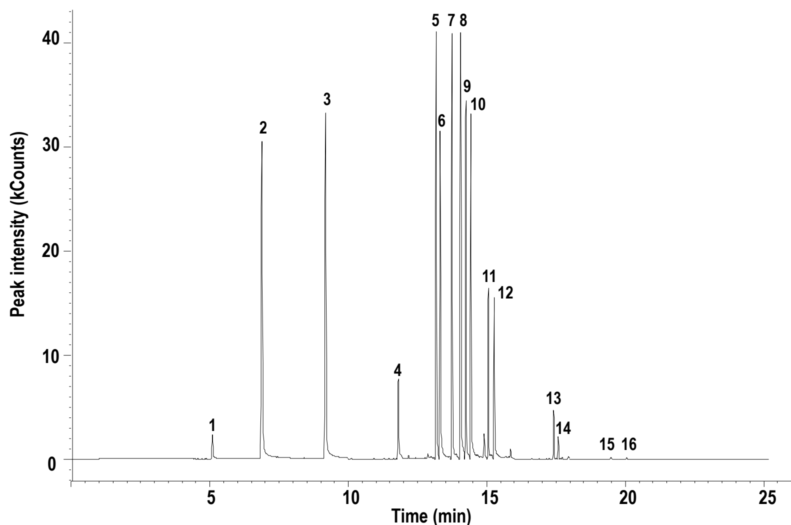


Figure 8. Influence of the glass liner on peak broadening when using a conventional splitless (upper) and a special SPME glass liner (bottom) in the GC-MS system (Compounds: 1: 4:2 FTO; 2: 6:2 FTO; 3: 8:2 FTO; 4: 4:2 FTOH; 5: 7:1 FA; 6: 6:2 FTOH; 7: 8:1 FA; 8: 7-Me-6:2 FTOH; 9: 9:1 FA; 10: 8:2 FTOH; 11: 11:1 FA; 12: 10:2 FTOH; 13: *N*-MeFOSA; 14: *N*-EtFOSA; 15:*N*-MeFOSE; 16: *N*-EtFOSE)

6.2. SPME OPTIMISATION

As previously mentioned, the main objective of this project is to develop and optimise a reliable method for the determination of PFASs semi-volatile precursors in water samples. SPME has been the extraction technique selected in the present work, which represents a key step in the process. For that reason, several operational parameters such as selection of the fibre stationary phase, time and temperature of extraction, pH media, salt addition and desorption time and temperature, were assessed and optimised under GC-EI-MS conditions which were previously optimised. It is important to highlight that we did not optimise the volume of sample taken for the analytes extraction because of the wish of a posterior automatisation of the technique, which requires a specific 20 mL headspace vial. In addition, headspace extraction requires the exposure of the fibre without being directly immersed into the sample, thus the amount of sample taken was fixed at 10 mL. Finally, desorption temperature was held according to the fibre manufacturer's instructions at 250 ºC and all extractions were performed under constant stirring at 750 rpm. It is important to note that the present optimisation also allowed the adequate extraction of the internal standards spiked in each sample.

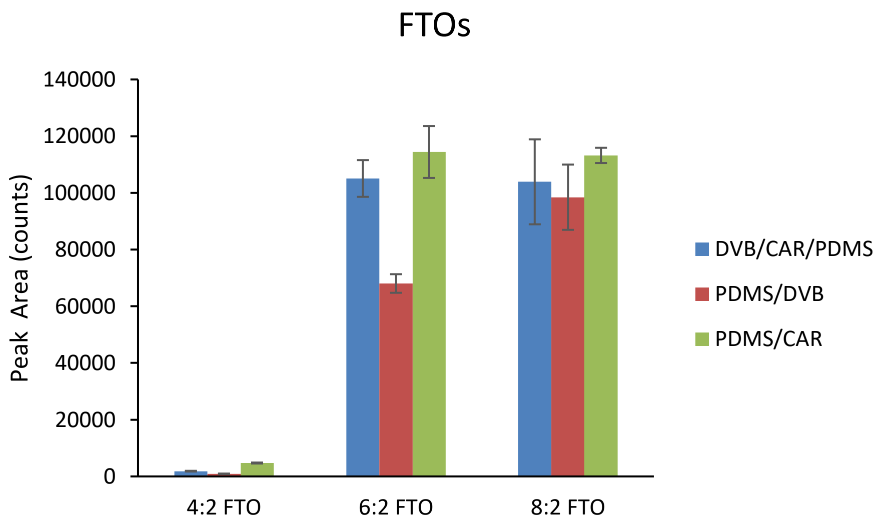
6.2.1. Selection of SPME fibre

Fibre selection is one of the most important factors to consider when determining the target compounds and in most cases, the chemical nature of the analytes is the prime factor involved. Volatility and polarity are generally the defining properties when selecting the appropriate kind of fibre, hence non-polar analytes may require less polar fibres while ionic compounds for instance, might rather require more polar ones. The fibres assessed in this study were: 50/30 µm DVB/CAR/PDMS, 85 µm PDMS/CAR, 65 µm PDMS/DVB, 100 µm PDMS and 85 µm PA, all of them supplied from SUPELCO® (Bellefonte, Pennsylvania, USA). These fibres were selected taking into account their affinity for retaining the target compounds with the aim of obtaining maximum extraction efficiency.

To select the most appropriate fibre, each one of them was tested under the same experimental conditions of extraction: 30 minutes of extraction time with a minimum time of 15 minutes for the system to reach equilibrium (incubation time) and a temperature of extraction of 30 ºC with neither salt addition nor varying solution's pH. In all experiments, 10 mL of Milli-Q

water sample previously spiked with 20 µL of a 1 mg L^{-1} standard solution containing all analytes was used for optimisation.

Since the analytes determined in this study range from non-polar (FTOs), moderately polar (FTOHs) to more polar compounds (FOSAs and FOSEs), a fibre containing sorbents of mixed polarity was expected to be the most suitable in this case. Figure 9 shows the responses, expressed as mean peak area of two replicate experiments, obtained for all the compounds and with the five fibres studied. As can be seen, PDMS and PA fibres, which are respectively the most apolar and polar phases, presented a major disadvantage when simultaneously determining compounds with a wide range of polarity. While PDMS fibre favoured the extraction of FTOs, PA fibre improved the extraction of the more polar compounds (FOSAS and FOSEs). Nevertheless, these two fibres showed low affinity for the rest of compounds and therefore they were discarded for the extraction of the target compounds. For FTOHs, which have a medium-polarity, similar behaviour and non-significant differences in the peak areas were observed using the mixed fibres, DVB/CAR/PDMS, PDMS/CAR and PDMS/DVB. PDMS/DVB fibre showed less extraction efficiency for both FTOs and FOSAs/FOSEs. Since DVB/CAR/PDMS and PDMS/CAR had similar responses for FTOs, the fact that DVB/CAR/PDMS demonstrated higher affinity for FOSAs and FOSEs, made this fibre to be the most appropriate for the determination of all analytes and it was selected for the posterior optimization of the extraction procedure.



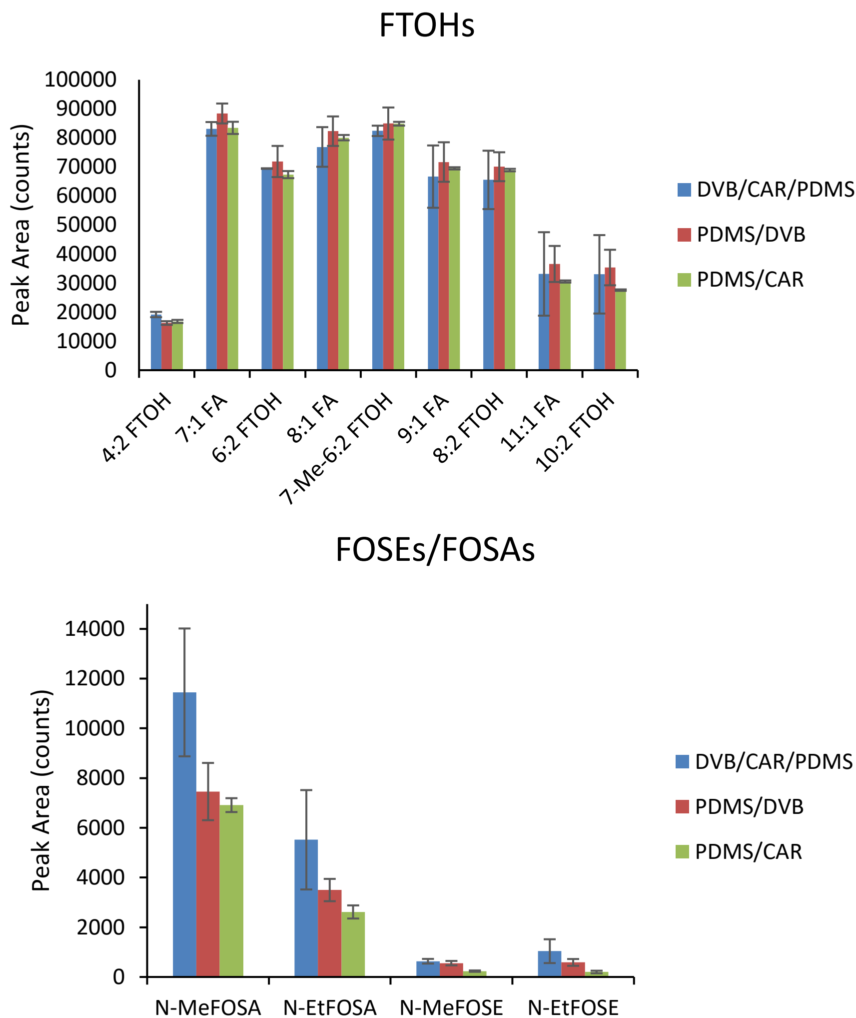


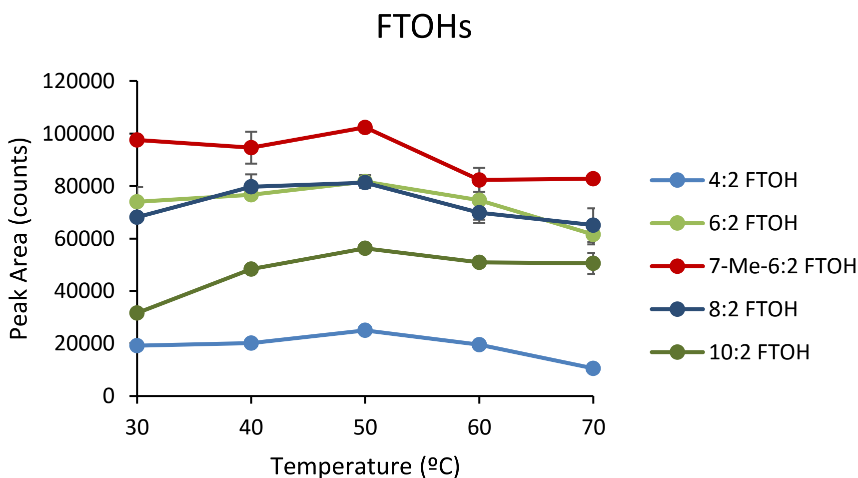
Figure 9. Effect of the SPME fibre on the extraction efficiency, expressed as peak area, for FTOs, FTOHs, FOSAs and FOSEs.

6.2.2. Optimisation of extraction temperature

Extraction temperature is an important parameter when optimising the extraction process. Temperature heavily affects the volatility of the analytes; the higher the temperature, the higher diffusion they experience. When increasing the temperature we are simultaneously decreasing equilibrium time, as the analytes concentration in the headspace significantly increases, but at

the same time compounds are also more rapidly desorbed from the fibre because the absorption of the analytes into the fibre is an exothermal process. Therefore, a workable compromise with the extraction temperature must be made in order to allow the maximum extraction efficiency of the target compounds. To determine the optimal extraction temperature, duplicate experiments were performed at temperatures of: 30 ºC, 40 ºC, 50 ºC, 60 ºC and 70 ºC; maintaining an extraction time of 30 minutes and spiking 10 mL of Milli-Q water with 20 μL of a 1 mg L^{-1} standard solution containing all analytes in each vial. In order to maintain the temperature at the desired value in each case, the vial was immersed in a water bath which is thermostatically controlled with a Tectron Bio immersion thermostat.

Figure 10 shows the effect of the extraction temperature for FTOs, FTOHs, FOSAs and FOSEs. The profile for FTOs clearly decreased as temperature increased, which may be a logical conclusion given the high volatility of such compounds. In contrast, FOSAs and FOSEs experienced a reverse effect probably as consequence of their low volatility. On the other hand, FTOHs experienced no substantial differences when varying the extraction temperature, partly due to their relatively moderate volatility.



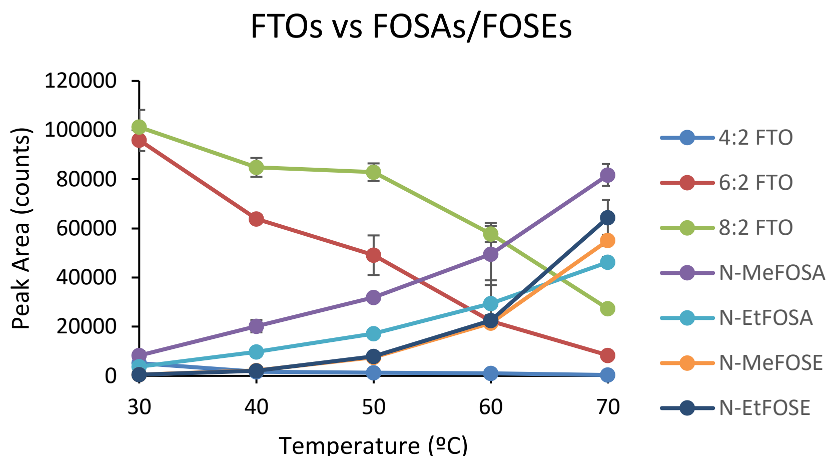


Figure 10. Effect of extraction temperature for FTOHs (upper), FTOs, FOSAs and FOSEs (bottom). (Fibre: DVB/CAR/PDMS; extraction time: 30 min; no salt addition, desorption time: 3 min; desorption temperature: 250 ºC).

Due to the different influence of this factor on the response of the different families of compounds, a compromise situation had to be reached. Although peak area for FTOs at the selected temperature presents lower values, it was of major importance to maximise the extraction of the least volatile compounds (FOSAs and FOSEs). In addition, those last ones are far more likely to be found in water samples than FTOs, mainly due to their volatility. Thus, 60 ºC was finally selected as the optimal extraction temperature.

6.2.3. Extraction time

Extraction time is another important parameter which is related with the diffusion process that occurs between both liquid and gas phases and also between the gas phase and the fibre to finally reach equilibrium. To study the effect of this parameter on the extraction efficiency of the target compounds different exposure times were evaluated: 1, 5, 10, 20, 30 and 40 minutes. In this case, the extraction temperature was set at 60 ºC according to the previous optimisation. As previously mentioned, extraction was performed under constant stirring (450 rpm), which allows the equilibrium state between the liquid and the gas phase to be reached faster. In Figure 11, the behavior of FTOHs towards the extraction time is shown. From these results, it can be concluded that 20 minutes was enough to reach the equilibrium for all of the compounds. For FTOs, due to

their high volatility and low molecular weight, they already achieved the equilibrium after 10 minutes of extraction time. In contrast, FOSAs and FOSEs required more time in order to reach equilibrium, which was the expected behavior as they are heavier and less volatile than the other analytes involved.

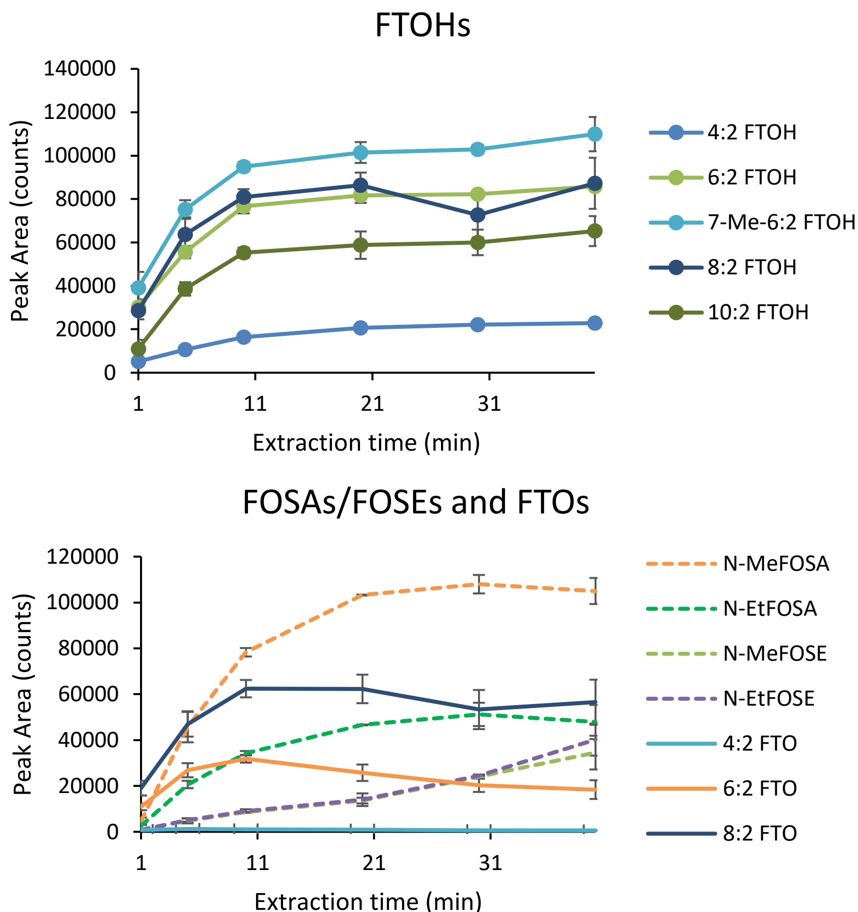


Figure 11. Effect of extraction time in terms of peak area for FTOs, FTOHs, FOSAs and FOSEs.

As compromise, we therefore chose an extraction time of 25 minutes, which allowed all analytes to be absorbed onto the fibre and ensured the complete achievement of the equilibrium

for all compounds except for FOSEs, which, due to their low vapour pressure, required further time for their complete extraction.

6.2.4. Salt addition

An additional parameter we did consider was the salt addition to increase the ionic strength of the sample. Generally salt addition causes a salting-out effect in which neutral molecules pass more readily from the aqueous phase to the vapour phase. NaCl (99.99%) was the salt of choice for the optimisation of this parameter. Several experiments conducted to assess the effect of salts in the extraction yield were performed at 0%, 10%, 20% and 30% of NaCl (m/v). For the preparation of the respective solutions, different quantities (1 g, 2 g and 3 g) of NaCl were each added into the vial along with 10 mL Milli-Q water and extraction was performed in accordance to prior optimisations, setting an extraction time of 25 minutes at 60 ºC. Contrary to expectations, as can be shown in Figure 12, salt addition did not appear to have significant influence in terms of peak area for neither FTOs, FTOHs nor FOSAs/FOSEs. As conclusion, no salt addition was considered for the development of the present method.

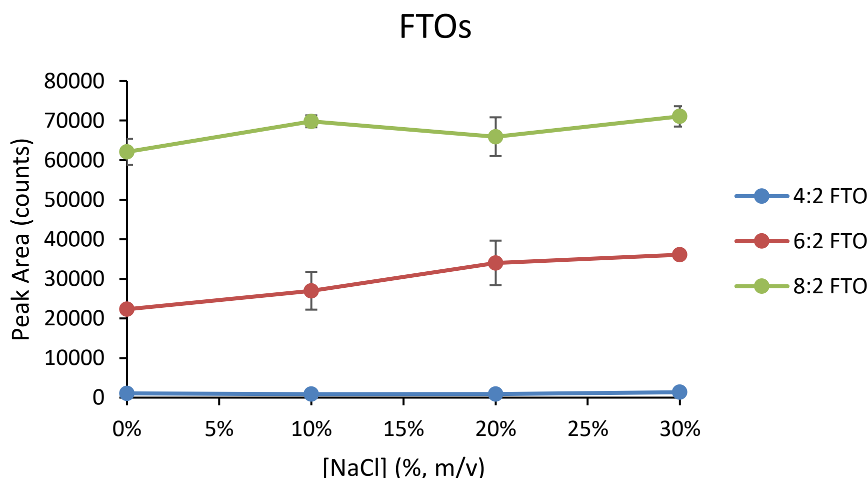


Figure 12. Effect of salt addition in terms of peak area for FTOs.

6.2.5. Optimisation of the pH value

The pH must not be overlooked since only compounds in their neutral form can be transferred to the vapour phase. For that reason, pH should be ideally kept two units below the pKa of the acidic compounds and two units above the basic ones to ensure all analytes are present in their neutral structures. The kind of fibre employed is also to be considered when varying the pH since it might have a negative effect on the fibre and consequently induce its deterioration by the presence of acid/basic molecules in the vapour phase. In the present project, FOSAs were the most acidic species with a pKa value around 8.4. The fact that the optimisation of the extraction procedure was performed employing 10 mL of Milli-Q water, which had a measured pH value of 6.06, suggested that no pH adjustment was required since all species should be found in its neutral form. Nevertheless, we performed an extraction at a pH of 5 to support that thought. To do so, a $2 \cdot 10^{-5}$ mol L^{-1} solution of sulfuric acid 95-97% was prepared in Milli-Q water with a measured pH of 5. After the respective injections, no meaningful differences were obtained at the selected pH value, for that reason we did not consider varying the pH value.

6.2.6. Desorption time

Desorption time can be understood as the minimum time required for the complete desorption of all analytes in the injector port of the GC. This parameter was evaluated by varying the time the valve of the injector remains closed, so in splitless mode, injections were performed at : 1, 2, 3 and 4 minutes. This parameter was assessed maintaining the optimal conditions that had been previously adjusted. Figure 13 shows, for a compound of each family in this case, that no substantial differences did exist when considering desorption time, thus we kept 3 minutes as the selected value.

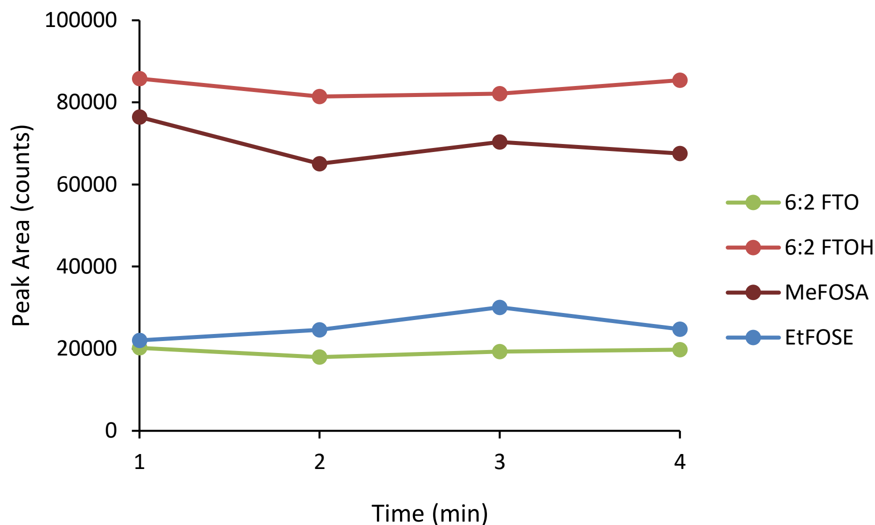


Figure 13. Effect of desorption time in terms of peak area for selected compounds of each family.

6.3. Quality parameters of the HS-SPME GC-EI-MS method

Quality parameters of the present method were studied and its validation was performed throughout seven working days. Several tests were carried out in order to determine the method's limits of detection and quantification (mLOD and mLOQ). In addition, linearity was also assessed by injecting six calibration standards at different concentrations while a river water blank sample was spiked at two concentration levels for the subsequent study of both precision and trueness.

6.3.1. Limits of detection and quantification

In order to evaluate the capability of the developed method to quantify at trace levels of concentration, the method limits of detection and quantification ion were determined in river water. To do so, 10 µL of a 1 µg L^{-1} work standard solution containing all analytes were spiked in 10 mL of river water. Afterwards, dilutions from the respective working solutions were performed until the signal to noise ratio (S/N) of the selected ion for each analyte was equal to 3 and 10 for the mLOD and mLOQ estimation, respectively. The obtained results are shown in the Table 7.

	River water	
Analyte	**mLOD (ng L^{-1})**	**mLOQ (ng L^{-1})**
4:2 FTO	1927	6423
6:2 FTO	11	37
8:2 FTO	10	33
4:2 FTOH	10	34
6:2 FTOH	0.10	0.34
7-Me-6:2 FTOH	0.10	0.35
8:2 FTOH	0.10	0.34
10:2 FTOH	0.10	0.35
***N*-MeFOSA**	0.10	0.33
***N*-EtFOSA**	0.10	0.32
***N*-MeFOSE**	0.10	0.33
***N*-EtFOSE**	0.10	0.33

Table 7. Method limits of detection (mLODs) and quantification (mLOQs) in river water.

As may be observed in Table 7, FTOs exhibit higher LOD and LOQ values when compared with the rest of analytes. This might be explained because of their high volatility, moreover, the extraction temperature was previously fixed at 60 ºC which fosters the facility of such compounds to be either simultaneously absorbed or desorbed from the fibre, thus hindering their posterior determination and quantification. In conclusion, the mLOD and mLOQ obtained ranging from 0.10 ng L^{-1} to 1.93 µg L^{-1} and from 0.33 ng L^{-1} to 6.42 µg L^{-1} respectively, were low enough for the appropriate detection and quantification of semi-volatile PFASs precursors in water samples. These values are lower than those reported in the literature. For instance, as reported by Szostek *et al.* mLODs ranged from 60-90 ng/L for FTOHs while Bach *et al.* obtained mLODs ranging from 20-100 ng L^{-1} for some FTOHs and FOSAs.

6.3.2. Linearity

Linearity was tested by repeatedly HS-SPME analysis of six aqueous calibration solutions containing all analytes at different concentrations ranging from 0.005 µg L^{-1} to 100 µg L^{-1}. However, linearity loss was observed at higher concentrations (higher than 5 µg L^{-1} for all the analytes except to 4:2 FTO). For that reason, the upper level of the linearity range was narrowed from 0.005 µg L^{-1} to 5 µg L^{-1} while the range for 4:2 FTO was stablished at 0.005 µg L^{-1} to

50 µg L^{-1}. As an example, Figure 14 shows one of the calibration curves belonging to 10:2 FTOH relative to 11:1 FA used as internal standard. Taking into account that the SPME fibre was injected manually into the gas chromatograph and the equilibrium sample treatment developed, internal standard method was selected as the most suitable for the quantification of the target compounds. As it was mentioned in the experimental section, the internal standards employed were: 7:1 FA for FTOs, 4:2 FTOH and 6:2 FTOH; 8:1 FA for 7-Me-6:2 FTOH; 9:1 FA for 8:2 FTOH; and 11:1 FA for 10:2 FTOH, FOSAs and FOSEs. All the internal standards were spiked at a concentration level of 1 µg L^{-1}.

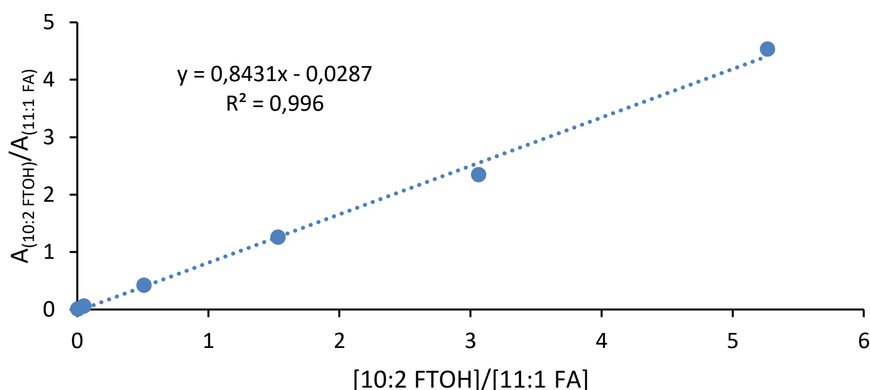


Figure 14. Linear regression of the 10:2 FTOH relative to 11:1 FA used as internal standard.

In conclusion, good correlation of the calibration curves were obtained, with an R factor always higher than 0.99 for all analytes. Although linearity loss occurred as the concentration increases until relatively high values, the linear regression was good enough to provide reliable results, given the fact that the concentration of semi-volatile PFASs precursors are expected to be around ng L^{-1} levels in water samples.

6.3.3. Intra-day precision

The intra-day precision of the method, also known as run-to-run precision or repetitively, is a way of expressing the repeatability of the method. This parameter was studied analysing (6 independent analysis) a blank river water sample which was spiked at two different concentration levels, low level (4:2 FTO at 10 µg L^{-1} ; 6:2 FTO, 8:2 FTO, FTOHs, and FOSAs/FOSEs at 0.05 µg L^{-1}) and high level (4:2 FTO at 35 µg L^{-1}); 6:2 FTO, 8:2 FTO, FTOHs

and FOSAs/FOSEs at 2 µg L^{-1}). Table 8 shows the precision achieved, expressed as relative standard deviation (%), for both the retention time (t_R) and the concentration found (Conc.) value between respective replicates.

Analyte	Intra-day precision of the method			
	Low level [a] (RSD%)		High level [b] (RSD%)	
	RSD t_R (%)	RSD Conc. (%)	RSD t_R (%)	RSD Conc. (%)
4:2 FTO	0.14	8	0.14	9
6:2 FTO	0.17	7	0.10	5
8:2 FTO	0.06	4	0.07	4
4:2 FTOH	0.05	5	0.04	6
6:2 FTOH	0.01	6	0.03	5
7-Me-6:2 FTOH	0.01	10	0.02	2
8:2 FTOH	0.01	10	0.02	6
10:2 FTOH	0.02	5	0.01	4
***N*-MeFOSA**	0.04	10	0.01	8
***N*-EtFOSA**	0.003	9	0.01	8
***N*-MeFOSE**	0.01	12	0.04	8
***N*-EtFOSE**	0.01	10	0.003	8

(a) 4:2 FTO: 10 µg L^{-1} ; 6:2 FTO, 8:2 FTO, FTOHs, FOSAs/FOSEs: 0.05 µg L^{-1}

(b) 4:2 FTO: 35 µg L^{-1} ; 6:2 FTO, 8:2 FTO, FTOHs, FOSAs/FOSEs: 2 µg L^{-1}

Table 8. Run to run precision of the method at two different concentration levels.

As shown in Table 8, a good intra-day precision was achieved with RSD (%) values lower than 0.17% for the retention time and lower than 12% for all the target compounds. As it could be expected, lower values of concentration caused an overall increase of variability due to the higher dispersion showed for the lowest part of the calibration curve. Variability regarding retention time was expected to be minimal, manifesting the high precision achieved for the chromatographic separation.

To sum up, after the whole validation of the method and considering the results obtained all throughout the process, we concluded that the present method is suitable for the determination of semi-volatile PFASs in water samples.

6.4. ANALYSIS OF SEMI-VOLATILE PFAS PRECURSORS IN WATER SAMPLES

The present method was developed and applied to three different and independent water samples collected from different locations. The first sample was collected from the Llobregat river and a tap water sample, which was obtained from the Faculty of Chemistry (Barcelona, Spain), and a seawater sample for alimentary purposes purchased from Marevendis Agua de Mar S.L (Alicante, Spain), were also analysed. To evaluate the presence of the target compounds in the three water matrices, 10 mL of each sample were spiked with a mixture of internal standards (10 µL of a 1 mg L^{-1} work standard solution) and the developed method was applied. After analysis of the samples none of the semi-volatile PFASs precursors were detected. For that reason, river water, which might be considered the most complex matrix among the studied ones, was spiked with the aim of evaluating the trueness of the method.

Trueness was determined considering the relative error (RE %) between the theoretical analyte concentration expected according to the amount previously spiked and the concentration after applying the analytical methodology. Trueness was studied at two concentrations: low level (4:2 FTO at 10 µg L^{-1} and 6:2 FTO, 8:2 FTO, FTOHs and FOSAs/FOSEs at 0.05 µg L^{-1}) and the high level (4:2 FTO at 35 µg L^{-1} and 6:2 FTO, 8:2 FTO, FTOHs and FOSAs/FOSEs at a concentration of 2 µg L^{-1}). The results obtained are summarised in Table 9.

In conclusion, the highest relative error (%) values were obtained for 4:2 FTO, mainly due to its relatively high volatility, which hindered the establishment of a high extraction yield due to its desorption of the fibre at 60ºC. Aside of this compound, the rest of analytes provided good enough RE% demonstrating the validity and good performance of the method for the determination of semi-volatile PFASs precursos on water samples.

	RE %	
Analyte	**Low level**	**High level**
4:2 FTO	-16.3	-15.5
6:2 FTO	6.1	7.2
8:2 FTO	2.2	6.6
4:2 FTOH	5.3	5.2
6:2 FTOH	4.6	8.5
7-Me-6:2 FTOH	9.1	9.2
8:2 FTOH	-9.5	-6.7
10:2 FTOH	-6.9	6.5
***N*-MeFOSA**	-4.3	-9.3
***N*-EtFOSA**	6.4	-9.3
***N*-MeFOSE**	11.0	9.5
***N*-EtFOSE**	11.4	9.5

(a) 4:2 FTO: 10 μg L^{-1} ; 6:2 FTO, 8:2 FTO, FTOHs, FOSAs/FOSEs: 0.05 μg L^{-1}

(b) 4:2 FTO: 35 μg L^{-1} ; 6:2 FTO, 8:2 FTO, FTOHs, FOSAs/FOSEs: 2 μg L^{-1}

Table 9. Relative error (RE%) of the method at two concentration levels.

7. CONCLUSIONS

The following lines summarize the work performed in order to develop and validate an analytical method for the determination of several families of semi-volatile PFASs in water samples. The main conclusions of the research work are the following:

- A simple and fast analytical method based on HS-SPME technique coupled to GC-MS has been developed for the simultaneous determination of semi-volatile PFASs in water samples as an alternative to solid-phase extraction methods.
- For the determination of the target compounds by GC-MS, electron ionisation was found to be the most suitable technique since it provided higher peak intensities compared to those achieved with chemical ionisation technique. In addition, the use of selected ion storage mode for acquisition allowed the improvement of the method's sensitivity.
- The DVB/CAR/PDMS fibre was found to be the most effective coating for the extraction of the whole families of compounds. The use as a compromise of an extraction temperature of 60 ^{o}C for 25 minutes allowed an adequate extraction efficiency for all the compounds. Moreover, a time of 3 minutes at 250 ^{o}C in the GC injector port was found to be enough to guarantee the quantitative thermal desorption of the target compounds from the fibre. No meaningful response differences are obtained with neither pH nor salt addition respective optimisations for the analysis of these compounds in water samples. The absence of matrix effect using the HS-SPME has been demonstrated allowing to propose the internal standard method with water standard as calibration solutions for the quantification of the target compounds.
- The proposed method provided very low limits of detection (0.10 ng L^{-1} - 2.0 µg L^{-1}) and quantification (0.33 ng L^{-1} - 6.67 µg L^{-1}) and precise results (RSD (%) < 12%) for the analysis of river water. In addition, the HS-SPME GC-MS method showed a good linearity with correlation coefficients higher than 0.99 (between 0.005 and 5 µg L^{-1} for all the

compounds and from 5 to 50 µg L^{-1} for 4:2 FTO). Concerning the trueness, relative errors (%) lower than 12% were obtained for all the analytes, except for 4:2 FTO, which was slightly higher (RE%: 16%), probably due to its high volatility causing a partial desorption from the fibre during the extraction. These findings demonstrated the validity and good performance of the method.

- Although the presence of the semi-volatile fluorinated compounds were not detected in any of the tap, river and seawater sample analysed, the applicability of the HS-SPME-GC-MS method has been demonstrated, allowing the accurate quantification of the target compounds in spiked river water samples at low ng L^{-1}. Further studies should be performed to evaluate the real impact of these pollutants in surface waters.

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9. ACRONYMS

ACN	Acetonitrile
APCI	Atmospheric Pressure Chemical Ionisation
APPI	Atmospheric Pressure Photoionisation
BAF	Bioaccumulative Factor
BCF	Bioconcentration Factor
CAR	Carboxene
CI	Chemical Ionisation
DDT	Dichlorodiphenyl trichloroethane
DVB	Divinylbenzene
ECNI	Electron-Capture Negative Ionisation
EI	Electron Ionisation
ESI	Electrospray Ionisation
EtOAc	Ethyl acetate
FA	Fluorinated Alcohol
FOC	Fluorinated Organic Compound
FTO	Fluorotelomer olefin
FTOH	Fluorotelomer alcohol
GC	Gas Chromatography
GC-MS	Gas chromatography-Mass spectrometry
GFF	Glass Fibre Filter
HPLC	High Performance Liquid Chromatography
HS	Headspace
HS-SPME	Headspace Solid-Phase Microextraction
IPE	Ion-Pair Extraction
IT	Ion-Trap
LC	Liquid Chromatography
LC-MS/MS	Liquid chromatography-Tandem mass spectrometry
LLE	Liquid Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MIT	Maximum Ionisation Time
MLOD	Method Limit of Detection
MRT	Maximum Reaction Time
MS	Mass-Spectrometry
MTBE	Methyl tert-butil ether

NICI	Negative Ion Chemical Ionisation
OECD	Organisation for Economic Co-operation and Development
PA	Polyacrylate
PCB	Polychlorinated biphenyls
PDMS	Polydimethylsiloxane
PFA	Perfluorinated Acid
PFAS	Per/Poly Fluoroalkyl Substance
PFHxS	Perfluorohexane sulfonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFOSE	Perfluorooctanesulfonamide ethanol
POP	Persistent Organic Pollutant
PTFE	Polytetrafluoroethylene
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SIS	Selected Ion Storage
SLE	Solid-Liquid Extraction
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
TIC	Total Ion Current
TOF	Total Organic Fluorine
U.S. EPA	United States Environmental Protection Agency