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Analytical method for biomonitoring of perfluoroalkyl acids in human urine

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ABSTRACT

Perfluoroalkyl acids are an important class of synthetic compounds widely used in commercial and residential settings, which may have potential adverse health effects. The objective of this study was to monitor 6 perfluorocarboxylic acids and perfluorooctane sulphonate in human urine to obtain a way to asses exposure. The target analytes were extracted from urine by using a semi automated solid-phase extraction module and derivatised with isobutyl chloroformate by catalysis with 3% N,N-dicyclohexylcarbodiimide in pyridine. Determination and quantisation were achieved by gas chromatography with a mass spectrometer detector operating in the selected-ion monitoring mode. The developed approach is fast and provided low limits of detection (0.2–1.0 ng L^{-1}) with good precision (relative standard deviation lower than 7.5%, within-day and between day). Recoveries from urine samples, which were spiked with the studied compounds at levels of 10 and 50 ng L^{-1} ranged from 93% to 96%. Perfluorohexanoic ($\lesssim$ 70 ng L^{-1}) and perfluoroheptanoic acids ($<$ 2 ng L^{-1}) were found in the urine samples from exposed researchers taken after handling these compounds. From the calculation of the excretion kinetics it was found that the dosage absorbed was eliminated within 15 h after exposure.

1. Introduction

The ubiquitous occurrence of perfluoroalkyl acids (PFAAs) in the environment as well as in humans has been confirmed in an increasing number of studies conducted by laboratories worldwide. These compounds are used in many commercial products, including lubricants, paints, polishes, food packaging, kitchenware, insecticide formulations, cosmetics, and fire-retarding foams, among others. As a result, workers and the general population are exposed to these compounds through inhalation, ingestion, and dermal absorption [1–3]. Because of their nature, PFAAs bind to serum proteins and thus accumulate in the liver and blood of exposed organisms [4]. Further toxicological studies indicated that they can affect the reproductive system, disturb the fatty acid metabolism, disrupt hormones and induce adverse effects in the liver and in the kidneys. PFAAs are extremely persistent in the human body and have a considerable range of elimination and half-lives, which depend on the carbon-chain length and the functional moiety [5]. For instance, the median human serum half-lives for perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) are 5.4 years and 3.8 years, respectively [3]. The Science Advisory Board of the Environmental Protection Agency has recommended PFOA and its salts to be classified as a "likely human carcinogen" [6]. As a result, increasing research has been conducted to assess human exposure to these compounds.

Because of their nature, PFAAs do not accumulate in lipids but rather bind to proteins. For this reason, blood and breast milk are the most common matrices for the assessment of human exposure to PFAAs [7,8]. Nevertheless, the collection of blood samples is invasive and stressful and breast milk samples can only provide information concerning the exposure levels of a limited segment of the population. Hence, recent trends in risk assessment of PFAAs are exploring the use of other non-invasive samples such as hair, nails and to a lesser extent, urine, for the biomonitoring of these compounds [9–11]. In particular, human urine is readily available in large volumes, allowing the determination of very low concentrations of PFAAs. Animal tests have showed that urinary excretion is the major process of elimination for either perfluoroheptanoic acid (PFHpA) or PFOA [12] and PFOS [13]. The results from these studies have also indicated that PFAAs that have shorter carbon chain lengths are more quickly eliminated in urine. With regard to humans, results from a recent study performed by Zhang et al. [14] show that shorter PFCAs are more easily eliminated than longer PFCAs via human urine.

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The primary challenge in analysing PFAAs in biological matrices is the development of simple extraction and clean-up methods that can span the physical–chemical properties range of these compounds (i.e. from hydrophilic short-chain to hydrophobic long-chain), with control of procedural blank contamination. Most methods involve an isolation step using ion-pair liquid extraction [15–17] or solid-phase extraction (SPE) with polymeric [11,13] or RP-C_{18} sorbents [18] prior to chromatographic determination. With the use of continuous SPE systems, the productivity in the laboratory as well as the quality of analytical results can be greatly improved, but only a few attempts have been made in this direction [18,19]. Liquid chromatography–mass spectrometry is the predominant technique utilised in the determination of PFAAs in biological matrices, but it is problematic due to background contamination arising from fluoropolymers in the equipment [20]. Gas chromatography–mass spectrometry (GC–MS) has a better resolving power and avoids the above mentioned contamination, but PFAAs are not amenable to direct analysis because of their high polarity. Derivatisation of PFAAs has been successfully achieved by esterification [21,22], benzylation [23] or by the formation of difluoroanilide derivatives [24]. In this context, the use of isobutyl chloroformate for derivatisation has several advantages such as mild reaction conditions, speed (2–3 min) and stability of the derivatives [22].

To date, the vast majority of studies concerning the human exposure to PFAAs use invasive sampling protocols [2,7,18,24,25] or only provide information regarding lactating women [8,26]. In addition, there is a lack of information about the possible occurrence of these pollutants in other types of non-invasive human samples such as urine. The two most recent reports addressing the urinary excretion of these compounds do not provide data about occupationally exposed workers [10,11]. To overcome these drawbacks, the aim of this paper is to address the gap of knowledge about this topic by assessing PFAAs levels in human urine samples from two researchers exposed by handling 7 long and short chain PFAAs. For this purpose, we use a tailor-made SPE module (free of PTFE materials), recently developed by our research group [27], which have been proved to be useful to overcome important constraints of most analytical approaches available for PFAAs determination: background contamination and sophisticated equipment requirements. In this way, reliable and useful information about the half-lives of these compounds in humans could be obtained using non-invasive urine samples. Also, this is a unique study about exposure to both short and long chain PFAAs as a whole, which will demonstrate if the urine is a suitable matrix for biomonitoring of these compounds in occupational exposed workers.

2. Materials and methods

2.1. Chemicals and standards

All products were handled with care, using adequate respiratory protection (fume hood, gas mask) and protective gloves. Perfluorohexanoic acid (PFHxA), PFHpA, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA) and PFOS were provided by Sigma-Aldrich (Madrid, Spain), at the highest purity available. LiChrolut EN (particle size 40–120 μm) and chromatographic grade solvents (isobutanol, ethyl acetate, acetonitrile, acetone and methanol) were purchased from Merck (Darmstadt, Germany). Tetradecane (internal standard, IS), the derivatising reagent isobutylchloroformate (IBCF), pyridine and N,N-dicyclohexylcarbodiimide (DCC) were purchased from Fluka (Madrid, Spain). A laboratory-made PTFE filter furnished with a paper disk (4-cm^2 filtration area) was also employed.

Standard stock solutions were prepared by dissolving the appropriate amount of the individual acid in acetonitrile to a concentration of 500 mg L^{-1}. Working solutions were prepared daily by diluting the stock solution to the appropriate concentration with uncontaminated urine or water previously purified by passage through a Milli-Q system (Millipore, Bedford, MA, USA). The urine used for the preparation of these solutions was a pool of urine from volunteers (which was found to be free of the 7 PFAAs). All these solutions were stored at 4 °C in pre-cleaned polypropylene tubes (with hermetical close). Freshly made solutions of ethyl acetate/isobutanol (9:1) containing 7.5% (v/v) of IBCF and IS (60 μg L^{-1}) were used as eluent for continuous SPE system. All PTFE materials were avoided in order to minimise/eliminate the background signal arising from contamination. All labware was washed with methanol before use.

2.2. Apparatus and conditions

The GC–MS system consisted of a Focus gas chromatograph coupled to a DSQ II mass spectrometer detector (Thermo Electron, Madrid, Spain). Chromatographic separation was performed using a DB-5 MS capillary column (30 m × 0.25 mm i.d. with 0.25 μm of film thickness) from Supelco (Madrid, Spain). The oven temperature was first set at 50 °C for 3 min, then raised to 170 °C at 10 °C/min, held for 1 min, and finally at 45 °C/min to 300 °C (total run time, 18.9 min). The carrier gas was helium (purity 6.0), at a constant flow of 1 mL min^{-1}. In all analyses, 1 μL of organic extracts was injected in the split mode (1:20 ratio). The injector port, transfer line and ion source temperatures were maintained at 250, 300 and 200 °C, respectively; the time for solvent delay was set at 4 min. Selected-ion monitoring mode (ionisation energy, 70 eV) was used for quantitative analysis, and the ions of *m*/*z* 69, 131, 169, 181, in addition to the $[M–CH_3–CO]^+$ (for perfluorocarboxylic acids) and $[M–CH_3–SO_2]^+$ ions (for perfluorooctane sulphonate) were used for detection of the corresponding ester derivatives. For tetradecane, the ions of *m*/*z* 43, 57 and 198 were monitored.

A Gilson Minipuls-3 (Villiers-le-Bel, France) and two modified Rheodyne 5041 injection valves (Cotati, CA, USA), in which all PTFE tubing was replaced with polyether ether ketone tubing (1/16 in. × 0.5 mm i.d., VICI AG International, Switzerland), were used throughout. All Teflon based materials were also replaced with polyethylene. A laboratory-made LiChrolut EN sorbent column was prepared by packing a commercial deactivated-glass column (3 mm i.d., Omnifit, UK) with 70 mg of the sorbent material. A laboratory made deactivated glass filter (3 cm × 3 mm i.d.) packed with glass wool was used to pass the aqueous phase into the SPE unit; the filter was replaced after 20–30 analyses.

2.3. Sampling

Urine samples from two researchers who routinely worked with these compounds were taken. Sample collection is described in detail in Fig. 1. First, samples from exposed researchers were collected before, within 15 min after exposure (sample considered at time 0) and 0.5, 1, 2, 3, 5, 6, 8, 12 and 15 h after exposure in sterilised polyethylene bottles of 100 mL (with hermetical close) in an area separated from the site of exposure in order to avoid the risk of contamination. Simultaneously, urine samples of five researchers who worked in other laboratories and did not manipulate PFAAs were also collected following the same procedure. The average time required for sample collection was estimated to be ~5 min. Secondly, each urine samples were analysed directly in triplicate (n=3) after collection or stored at 4 °C for up to 48 h. When the time between sample collection and analysis exceeded 48 h, samples were stored at

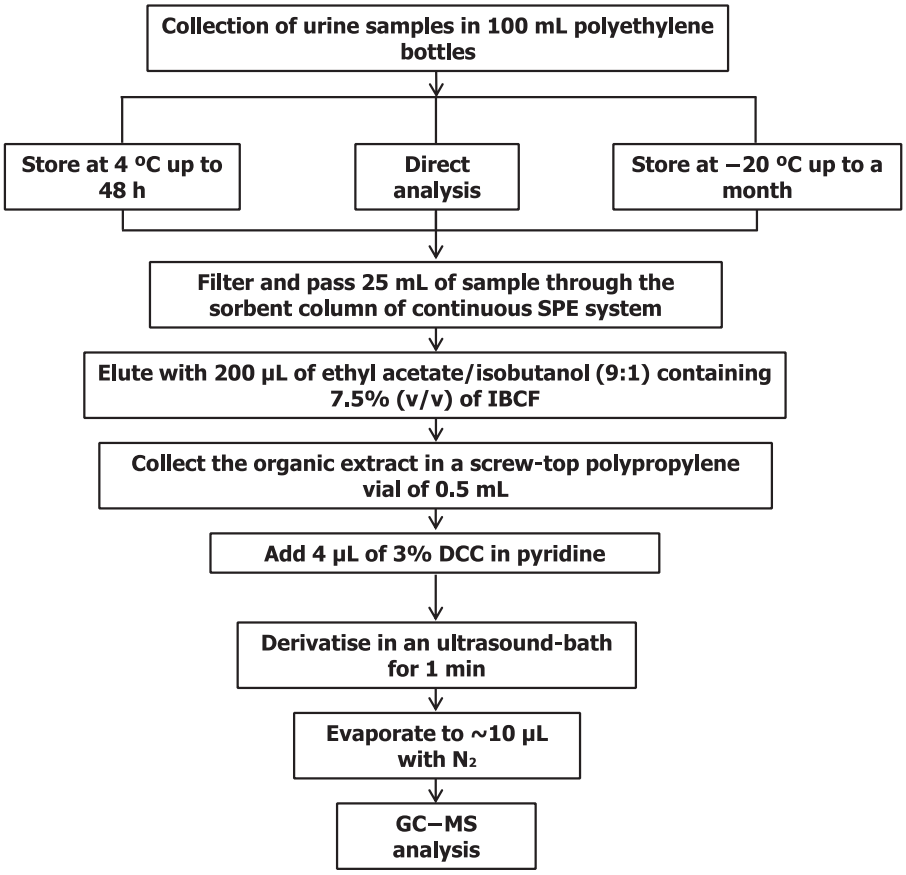

Fig. 1. Flow diagram representing the whole protocol carried out in the determination of perflurocarboxylic acids in human urine. SPE: solid-phase extraction; IBCF: isobutyl chloroformate; DCC: N,N-dicyclohexylcarbodiimide; GC–MS: gas chromatograph with a mass spectrometric detector.

−20 °C for up to a month to avoid storage degradation. The frozen samples were left at room temperature until completely thawed prior to analysis.

2.4. Analytical procedure

The whole procedure followed in this work for the determination of perfluorocarboxylic acids in human urine is schematically depicted in Fig. 1. After collection, 25 mL of urine sample or standard solutions containing 0.7–200 ng L^{-1} of the analytes at pH ~1.0 (adjusted with 0.2 mL of 12 M HCl) were filtered to prevent suspended particles from reaching the continuous unit and passed at 5.0 mL min^{-1} through the sorbent column, located in the loop of first injection valve (IV_1) in the SPE unit. All PFAAs were sorbed and the sample matrix was discarded. Next, IV_1 was switched and the sorbent column was dried for 2 min with an air stream at 3 mL/min; simultaneously the loop of second valve (IV_2) was filled with the eluent [200 µL of ethyl acetate/isobutanol (9:1) containing 7.5% (v/v) of IBCF and 60 µg L^{-1} IS] by means of a syringe. In the elution step, the loop of the IV_2 was switched to pass its content through the column in the opposite direction of the sample aspiration. Then, the whole organic extract was collected in a polypropylene vial and processed as described in Fig. 1. Potential errors in measuring the final extract volume were avoided by using the internal standard. Between each analysis, the sorbent column was cleaned and conditioned with 0.5 mL of methanol, 0.5 mL of acetone and 1 mL of purified water. Under these conditions, no memory effects were observed and the column was serviceable for at least 1 month without any change in its properties.

3. Results and discussions

3.1. Optimisation of the solid-phase extraction and derivatisation

In a previous work, we developed a GC–MS method for the determination of 7 PFAAs at ng L^{-1} level in water samples [27]. To minimise as much as possible the handling of the samples, while at the same time eliminating any major matrix effects, the above mentioned method was initially adopted in this paper, but the chemical and flow variables influencing the extraction process must again be optimised taking into account the different matrices. Uncontaminated urine samples or purified water samples were each spiked with 50 ng L^{-1} of PFAA to optimise chemical and flow variables which are summarised in Table 1. Adsorption of PFAA onto the filter material was found to be negligible by conducting a recovery study, obtaining nearly 100% recoveries of the spiked PFAA. The results for both types of samples (uncontaminated urine or purified spiked with 50 ng L^{-1}) were very similar, so we chose not to pretreat the urine samples and simply filter them in order to prevent any solid particles from reaching the continuous SPE system. Since a compound's ionisation state can drastically change its retention and elution characteristics on the reversed phase sorbents, the first chemical variable evaluated was the sample pH. Therefore, the effect of sample pH was studied over the range 1–5 and was adjusted with diluted HCl. The highest sorption efficiency for all compounds was obtained at pH 1–1.3 (two units below pK_a of the PFAAs). The elution efficiency was also evaluated with several organic sorbents of variable polarity, finding that a mixture of ethyl acetate/isobutanol (9:1) was the most effective. After elution, the analytes

were converted into less polar isobutyl derivatives by using isobutyl chloroformate catalysed by pyridine and N,N-dicyclohexylcarbodiimide [22]. In the first step a mixed anhydride is produced, which decarboxylates in the second step, to produce the corresponding isobutyl ester. DCC act as a coupling agent, eliminating the HCl liberated in the first step. In order to simultaneously elute/derivatise the target analytes, the eluent consisted of a solution of IBCF (7.5% v/v) prepared in ethyl acetate/isobutanol (9:1). Finally, 4 μL of 3% DCC in pyridine were added to the extract for derivatisation. Flow variables (sample and eluent flow-rates and eluent volume) were also optimised and are listed in Table 1. The breakthrough volume, which is directly related to the sensitivity of the method, was examined by using uncontaminated urine samples at pH 1 containing 1 ng of each PFAA at different volumes (from 10 to 100 mL), for insertion into the SPE system. A sorption efficiency of ca. 100% was obtained with urine samples up to 100 mL.

Finally, to increase the sensitivity of the method, the final solution of the isobutyl esters derivatives was evaporated to ~10 μL with a stream of N_2. Potential errors in measuring the final volume after the evaporation of the extract obtained after elution and derivatisation steps can be avoided with the use of the internal standard (tetradecane). Volatilization effects of target analytes at this step were not found.

3.2. Method validation

Procedural blank tests were conducted using purified water in every 3 batches of samples in order to check for any contamination of laboratory materials used in the analyses. Quality parameters such as linearity, limits of detection (LODs) and precision were established for the proposed SPE–GC–MS method. For this purpose, 25 mL blank urine samples were fortified with microlitres of standard solutions containing all the compounds studied in the interval 0.7–200 ng L^{-1} and passed through the sorbent column of continuous SPE system. The analytical figures of merit obtained are summarised in Table 2. Standard curves were constructed by plotting the analyte-to-internal peak area ratio against the analyte concentration. Correlation coefficients were higher than 0.995 in all cases, which testifies the good linearity within the studied range. The LODs were calculated as a concentration providing a chromatographic signal three times higher than background noise, and ranged between 0.2 and 1.0 ng L^{-1}. The precision of the developed method, as relative standard deviation (RSD), was evaluated by analysing 12 individual uncontaminated urine samples containing the PFAAs at a concentration of 10 ng L^{-1} on the same day (within-day precision) as well as on three different days (between-day precision). The RSD obtained was found to be satisfactory with RSD lower than 7.5% in both cases.

In order to validate the developed method for the determination of the perfluorocarboxylic acids in human urine samples, a recovery study was conducted. Since certified reference material was not available, three urine samples from unexposed individuals (blank samples) were spiked with the PFAAs at two different concentrations (10 and 50 ng L^{-1}) and analysed in triplicate. The average recoveries obtained were satisfactory, ranging between 93% and 96%, which testify the applicability of the proposed method in urine samples.

Table 1
Chemical and flow variables of solid-phase extraction and derivatisation processes of perfluoroalkyl acids.

Variable	Optimum range (selected value)
Solid-phase extraction	
Amount of LiChrolut (mg)	55–150 (70)
Eluent volume (μL)	175–250 (200)
Sample pH	1.0–1.3 (1.0)
Sample flow-rate (mL min^{-1})	0.5–5.5 (5.0)
Eluent flow-rate (mL min^{-1})	3.0
Breakthrough volume (mL)	10–100 (25)
Derivatisation	
Isobutyl chloroformate volume (μL)	14–20 (15)
N,N-dicyclohexylcarbodiimide (%) in pyridine	2–5 (3)
Volume of catalyst (μL)	3–5 (4)
Ultrasonic shaking time (min)	1–5 (1)

3.3. Study of human exposure to perfluorocarboxylic acids through the use of urine samples

As an alternative to blood and milk samples, urine is a promising matrix for biomonitoring of human exposure to PFAAs. Thus, Pérez et al. studied the occurrence of 18 PFAAs in 30 urine samples from donors living in regions with point sources of exposure to these compounds. PFHxA (in 3 samples), PFHpA (6 samples), PFOA (17 samples), PFDA (4 samples) and PFUnA (2 samples) were detected at concentrations ranging from 1 to 50, 0.5 to 5, 0.1 to 3, 0.7 to 0.9 and 2 to 5 μg L^{-1}, respectively [10]. Lower concentrations of PFOA (0.005 to 0.06 μg L^{-1}) and PFOS ($<$ 0.007 to 0.16 μg L^{-1}) has been found in 35 and 60 of 63 urine samples, respectively from donors living in industrialised regions [11]. Major routes of exposure have been identified as drinking water, fish consumption, and dust inhalation [10].

In the present study, the exposure to PFAAs in the workplace has been evaluated for the first time by selecting the urinary excretion of these compounds as biomarkers. In order to study the kinetics of excretion of these compounds, urine samples from two exposed researchers, who handled these compounds during the

Table 2
Linearity, limits of detection, precision and recoveries of perfluoroalkyl acids using the developed method.

Compound	Linear range (ng L^{-1})	LODs (ng L^{-1})	RSD (%) ($n=12$)[a]		Recoveries (%)[b]	
			Within-day	Between-day	10 ng L^{-1}	50 ng L^{-1}
Perfluorohexanoic acid (PFHxA)	3.3–200	1	6.3	7.2	95 ± 6	96 ± 7
Perfluoroheptanoic acid (PFHpA)	2.0–200	0.6	6.5	7.1	94 ± 6	94 ± 7
Perfluorooctanoic acid (PFOA)	3.4–200	1	6.7	7.3	95 ± 7	96 ± 7
Perfluorononanoic acid (PFNA)	0.7–200	0.2	6.0	7.0	94 ± 7	95 ± 6
Perfluorodecanoic acid (PFDA)	3.2–200	1	6.4	6.9	93 ± 7	93 ± 6
Perfluoroundecanoic acid (PFUnA)	1.4–200	0.4	6.2	6.8	96 ± 7	95 ± 7
Pefluorooctane sulphonate (PFOS)	0.7–200	0.2	6.1	6.6	95 ± 6	96 ± 6

[a] Relative standard deviation ($n=12$). Values obtained for samples fortified with 10 ng L^{-1} of each compound.
[b] Percent recovery ± standard deviation ($n=3$).

preparations of stocks solutions (0.5 g L^{-1} in acetonitrile), were collected before exposure and 0, 0.5, 1, 2, 3, 4, 5 6, 8, 12 and 15 h after termination of exposure. The sample collected within 15 min after exposure is considered the sample at time 0 and the rest follow this initial sample at determined intervals of time. PFHxA was the only compound found at a concentration above the LOQ in the urine samples collected at the moment of exposure until 12 h of exposure. As can be seen in Fig. 2 for the PFHxA, the elimination curve followed as first-order kinetic, which can be adjusted to an exponential decay functions, $y=y_0+A\times\exp(-kt)$. The rate constant ($k=0.26\ h^{-1}$) indicates the fraction of the determination in the body removal per unit of time. The biological half-life ($t_{1/2}=2.66$ h) of a substance in an organ, tissue or body fluid denotes the time needed to reduce the biological level of the substance in half, which is inversely related to the elimination constant ($k=\ln 2/t_{1/2}$) [28]. In addition, PFHpA was also present in the urine samples from the two researchers at a concentration below the LOQ (2 ng L^{-1}) within 15 min of exposure. In light of these results, the first 15 min after exposure were selected as the sampling time. Accordingly, and as can be seen in Table 3, urine samples from two exposed researchers were collected when preparing stocks solutions of 0.5 g L^{-1} (the six first columns), when handling spiked samples (the others columns) from stocks solutions and from five non-exposed researchers (data not shown). PFHxA was detected at a mean concentration of 62 and 9 ng L^{-1} after preparation of stock solutions or when handling these stocks, respectively. None of the analytes were found in the urine samples of researchers taken before exposure (after a week without any contact with the laboratory), the same as in unexposed people. Therefore, the presence of PFHxA (in 100% of the samples) and PFHpA (in the 50% of the samples; < LOQ) in urine was attributed to exposure during standard solution preparation and analysis of the spiked samples, due to the urine samples of researchers who worked in other laboratories and did not manipulate these compounds were not contaminated with PFAAs. The rest of the PFAAs studied were not detected probably due to their low volatility, which prevent their inhalation or by the effectiveness of the protection devices used. To verify these conclusions, we analysed several urine samples (30) from unexposed people before preparing the pool of urine used for the preparation of working solutions and we did not detected any of the target analytes in this samples. Finally, Fig. 3 shows the chromatograms obtained from the analysis of the urine sample of exposed research 1 (A) and an uncontaminated urine sample spiked with 20 ng L^{-1} of each PFAAs (B).

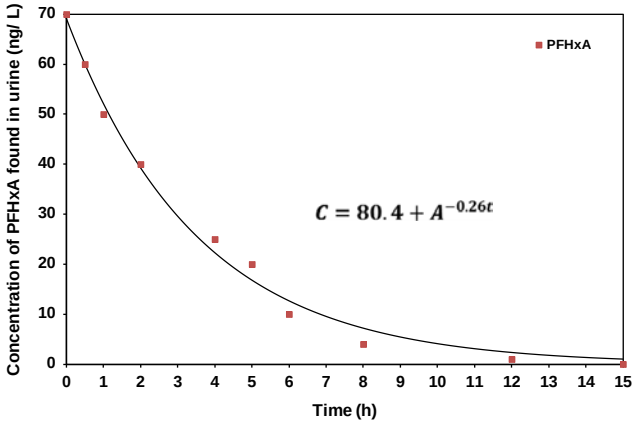

Fig. 2. Kinetic curve and exponential decay function corresponding to the excretion of perfluorohexanoic acid (PFHxA) in the urine of a researcher after exposure.

Table 3
Perfluoroalkyl acids concentration (± SD, ng L^{-1}, $n=3$) found in exposed researchers' urine after exposure.

Compound	Researcher 1[a]	Researcher 1[a]	Researcher 2[a]	Researcher 2[a]	Researcher 2[a]	Researcher 1[b]	Researcher 1[b]	Researcher 2[b]	Researcher 2[b]	Researcher 2[b]
PFHxA	70 ± 5	54 ± 4	62 ± 5	65 ± 5	68 ± 5	55 ± 4	23 ± 1	13 ± 1	2.3 ± 0.2	6.0 ± 0.5	9.0 ± 0.7	1.2 ± 0.1
PFHpA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Urine from researchers exposed to PFCs during the preparation of stocks solutions (0.5 g L^{-1}).
[b] Urine from researchers exposed to PFCs when preparing diluted solutions from stocks solutions (0.5 g L^{-1}).
[c] Not detected.

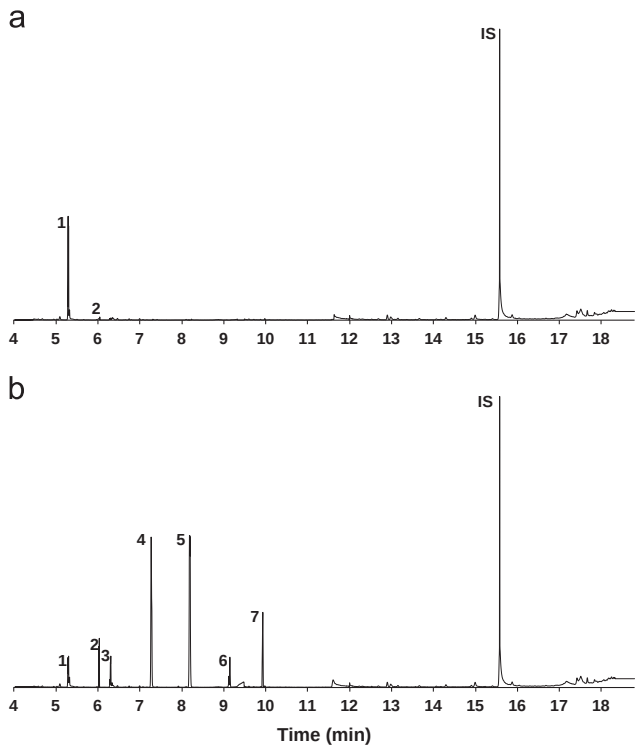

Fig. 3. GC–MS chromatograms (SIM mode) for 25 mL of an urine sample of exposed researcher 1 (a) and an uncontaminated urine sample spiked with 20 ng L^{-1} of each PFAAs (b). 1: PFHxA; 2: PFHpA; 3: PFOA; 4: PFOS; 5: PFNA; 6: PFDA; 7: PFUnA; IS: internal standard (tetradecane). For compounds abbreviation, see Table 2.

4. Conclusions

In the absence of comprehensive methods to assess human exposure to PFAAs using non-invasive samples, the developed method allows the simultaneous determination of 7 perfluorocarboxylic acids in urine samples at nanogram-per-litre levels. The advantages of this method are reduced sample preparation time, high sample throughput (~10 min for the preconcentration and derivatisation steps) and elimination of background PFAAs contamination. Also, the LODs (between 0.2 and 1 ng L^{-1}) are better or similar than those provided by other methods employing on-line turbulent flow liquid-chromatography and tandem mass-spectrometry (10–2700 ng L^{-1}) [10] and solid-phase extraction following liquid-chromatography–mass-spectrometry (1–2 ng L^{-1}) [11].

Currently, the biological indicators associated with exposure to chemical compounds have become increasingly important to complement the environmental control of occupational exposure of workers. Biological control is a much better environmental assessment, because it allows for the determination of the efficacy of personal protective equipment as well as the detection of potential dermal absorption in addition to inhalation. In this work, we have studied for the first time in urine the exposure to PFAAs of two researchers that manipulate these compounds during the preparation of stock solutions in a laboratory. The analysis of urine samples from the exposed researchers shows that PFHxA and PFHpA are absorbed (by inhalation or by dermal absorption during contact) rapidly and eliminated in urine in ~15 h. Also, from the results obtained in this study it can be concluded that urine is a suitable matrix for biomonitoring of PFHxA and PFHpA in occupational exposed workers, since these compounds are readily excreted through urine after 15 min of exposure. The ingestion of PFHxA and PFHpA, unlike the other PFAAs studied, was mainly by inhalation of airborne material [4] despite the researchers use of respiratory protective devices and work in extraction chambers, probably due to the low effectiveness of the masks used for protection.

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