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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