METHOD DEVELOPMENT FOR THE DETERMINATION OF PERFLUORINATED COMPOUNDS IN HUMAN URINE

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Abstract
There is increasing research focusing on and public interest in perfluorinated compounds (PFCs), including perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and other structurally related analogues, primarily because they are bioaccumulative, persistent, and toxic. To explore the possibility of a non-invasive way to assess human exposures to these materials, a new method has been developed and evaluated for the quantitation of trace levels of 10 PFCs in human urine. In preliminary studies using a synthetic human urine analogue, the limit of quantitation (LOQ) was determined as 1–2 ng/L with precision and accuracy ranging from 5%–16% and 75%–125%, respectively, for all compounds. The application of this method using human urine samples will be presented at the conference. The ability to detect and quantify these compounds in human samples may be a significant new tool for the non-invasive biological monitoring of PFC exposure. Application of this method in human studies will be of great interest for physiologically-based pharmacokinetic modelling efforts which are intended to describe the disposition of these materials in humans.

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Introduction

Perfluorinated compounds (PFCs) have now been detected worldwide in a range of environmental and biological matrices.[1-3] Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the best known of these compounds as they have been shown to be bioaccumulative, persistent, and toxic.[4, 5] A small number of studies have examined the pharmacokinetic parameters of some of these compounds, with their respective half-lives being of particular concern.[4, 5] In order to develop a better understanding of human exposures to the PFCs and to help determine potential human health effects, it is quite important to measure body burden of these compounds in human populations. While there are many reports detailing PFC measurements in circulating blood in human, no research has been published concerning their presence in human urine. As with other xenobiotoc compounds, urinary measurements are likely to be very useful for biological monitoring as well as a pharmacokinetic modelling. A non-invasive method of evaluating the presence of these materials is preferred to facilitate large scale investigations involving vulnerable subpopulations (e.g., children). However, the performance of such a method must be thoroughly evaluated in terms of accuracy and precision as levels measured in human populations are likely to be very low, and its utility will be based on its ability to differentiate between distinct subpopulations of exposed individuals. The purpose of this study was to develop a sensitive and accurate method for the determination of PFCs in human urine and validate the method with real human urine samples.

Materials and Methods

Standards and reagents: Potassium salts of perfluorobutane sulfonate (PFBS, 98% purity), perfluorohexane sulfonate (PFHS, 93%), and perfluorooctane sulfonate (PFOS, 93%) were provided by 3M Company (St. Paul, MN). Perfluorohexanoic acid (C6, 97%), perfluoroheptanoic acid (C7, 99%), perfluorooctanoic acid (C8 or PFOA, 96%), perfluorononanoic acid (C9, 97%), and perfluorodecanoic acid (C10, 98%) were purchased from Sigma-Aldrich (St. Louis, MO). Perfluoroundecanoic acid (C11, 96%), and perfluorododecanoic acid (C12, 96%) were purchased from Oakwood Products (West Columbia, SC). $^{18}O_2$-Ammonium perfluorooctane sul-

fonate (18O-PFOS) was purchased from Research Triangle Institute (Research Triangle Park, NC). 1,2-$^{13}C_2$-labelled PFOA (13C-PFOA) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). Deionized (DI) water, HPLC grade methanol, and ammonium acetate were determined to be free of PFCs prior to use.

PFC measurement: This method was initially developed using synthetic human urine. Samples were extracted with solid phase extraction (SPE) cartridges. The extract was then introduced to the high-performance liquid chromatography coupled with quadrupole tandem mass spectrometer (LC/MS/MS) for quantitation. Mass transitions of each analyte and HPLC/MS/MS conditions are listed in Table 1 and 2, respectively. Quantitation was carried out using synthetic urine fortified with a series of PFC standards (6 points). Recoveries were calculated using matrix matched solutions. Quality control (QC) samples were prepared by spiking two different amounts of PFCs (10 and 100 ng/L) into bulk samples of synthetic urine. The QC samples were then distributed into small vials, stored at –80°C, and run with each batch of analysis. Intra-day and inter-day accuracies and precisions were determined using these QC measurements.

Creatinine normalization: For normalization, creatinine concentrations were determined using a creatinine assay kit. One hundred µL of urine was sub-sampled from the well homogenized original sample and assayed with the kit.

Table 1. Mass transitions of each analyte

Perfluorinated Compounds	Parent Ion, m/z	Fragment Ion, m/z	Perfluorinated Compounds	Parent Ion, m/z	Fragment Ion, m/z
C6 (perfluorohexanoic acid)	313	269	PFBS (perfluorobutane sulfonate)	299	80
C7 (perfluoroheptanoic acid)	363	319	PFHS (perfluorohexane sulfonate)	399	80
C8 (perfluorooctanoic acid)	413	369	PFOS (perfluorooctane sulfonate)	499	80
C9 (perfluorononanoic acid)	463	419		Parent	Fragment
C10 (perfluorodecanoic acid)	513	469	Isotopically Labelled Compounds	Ion, m/z	Ion, m/z
C11 (perfluoroundecanoic acid)	563	519	$^{13}C_2$-PFOA (^{13}C labelled PFOA)	415	370
C12 (perfluorododecanoic acid)	613	569	$^{18}O_2$-PFOS (^{18}O labelled PFOS)	503	84

Table 2. HPLC and MS/MS conditions

HPLC		MS/MS	
Instrument	Agilent 1100	Instrument	API 3000
Column	Phenomenex Luna C18(2)	Ionization	Negative ESI
	5 µm, 3.0×50 mm	Scan mode	MRM
		Curtain gas	N2 (9 arbitrary units, au)
Mobile phase	75:25 methanol:2 mM ammonium acetate	Nebulizer gas	N2 (8 au)
		Dryer gas	Zero air (8 L/min, 350˚C)
Flow rate	200 µL/min	Ion spay	−1500 V
Injection volume	10 µL	Ionization and collision cell voltages	Optimized for individual analytes

Results and Discussion

This is the first report of a method for the measurement of PFCs in human urine. A detailed description of the method performance characteristics is provided to help evaluate the method's potential utility.

Blank synthetic urine showed no significant peaks for any analyte. The lower limit of quantitation (LOQ) was determined to be 1–2 ng/L for all targeted compounds by computing the standard deviation of a series of injections of the lowest possible standard in synthetic urine and multiplying the standard deviation by ten. The matrix matched recoveries ranged from 55%–105% with less than 10% of the precision for all compounds. Method accuracy, based on the nominal values of the QC samples, ranged between 75%–125% for all compounds. The coefficient of correlation of each calibration curve was greater than or equal to 0.99 for all compounds, with a linear range from 1 or 2 ng/L to 200 ng/L (compound specific).

These method performance characteristics indicate that the method provides sufficient reliability for use in the analysis of perfluorinated alkyl compounds in human urine. The actual utility of the method will be evaluated with the analysis of human urine samples collected from various populations across the USA. Its use in human exposure studies and pharmacokinetic evaluations will be discussed.

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References
1. Yamashita N; Kannan K; Taniyasu S; Horii Y; Petrick G; Gamo T. *Mar Pollut Bull* 2005; 51:658-668.
2. Giesy JP; Kannan K. *Environ Sci Technol* 2001; 35:1339-1342.
3. Kannan K; Koistinen J; Beckmen K; Evans T; Gorzelany JF; Hansen KJ; Jones PD; Helle E; Nyman M; Giesy JP. *Environ Sci Technol* 2001; 35:1593-1598.
4. OECD. *Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts*; ENV/JM/RD(2002)17/FINAL; Organisation for Economic Co-operation and Development: Paris, France, 2002.
5. USEPA. *Revised draft, hazard assessment of perfluorooctanoic acid and its salts*; U.S. Environmental Protection Agency: Washington, DC, November 4, 2002.