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Per- and Polyfluoroalkyl Substances and Fluorinated Alternatives in Urine and Serum by On-line Solid Phase Extraction–Liquid Chromatography–Tandem Mass Spectrometry

Kayoko Kato[*], **Akil A. Kalathil**, **Ayesha M. Patel**, **Xiaoyun Ye**, **Antonia M. Calafat**

Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Abstract

Per- and polyfluoroalkyl substances (PFAS), man-made chemicals with variable length carbon chains containing the perfluoroalkyl moiety (C_nF_{2n+1}–), are used in many commercial applications. Since 1999–2000, several long-chain PFAS, including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), have been detected at trace levels in the blood of most participants of the National Health and Nutrition Examination Survey (NHANES)—representative samples of the U.S. general population—while short-chain PFAS have not. Lower detection frequencies and concentration ranges may reflect lower exposure to short-chain PFAS than to PFOS or PFOA or that, in humans, short-chain PFAS efficiently eliminate in urine. We developed on-line solid phase extraction–HPLC–isotope dilution–MS/MS methods for the quantification in 50 μL of urine or serum of 15 C_3-C_{11} PFAS (C_3 only in urine), and three fluorinated alternatives used as PFOA or PFOS replacements: GenX (ammonium salt of 2,3,3,3,-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoate, also known as HFPO-DA), ADONA (ammonium salt of 4,8-dioxa-3H-perfluorononanoate), and 9Cl-PF3ONS (9-chlorohexadecafluoro-3-oxanonane-1-sulfonate), main component of F53-B. Limit of detection for all analytes was 0.1 ng/mL. To validate the method, we analyzed 50 commercial urine/serum paired samples collected in 2016 from U.S. volunteers with no known exposure to the chemicals. In serum, detection frequency and concentration patterns agreed well with those from NHANES. By contrast, except for perfluorobutanoate, we did not detect long-chain or short-chain PFAS in urine. Also, we did not detect fluorinated alternatives in either urine or serum. Together, these results suggest limited exposure to both short-chain PFAS and select fluorinated alternatives in this convenience population.

[*]Corresponding author: Kayoko Kato, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Mailstop F53, Atlanta, GA 30341, Phone: (770) 488-7295, Fax: (770) 488-4371, kkato1@cdc.gov.

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Keywords

PFAS; urine; short-chain PFAS; PFESAs

1. Introduction

Some per- and polyfluoroalkyl substances (PFAS), including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), persist in humans and the environment and have been detected worldwide in wildlife and the ecosystem [1;2]. Exposure to PFOS and PFOA in the general population is also widespread, although demographic, geographic, and temporal differences exist [1;3–11]. PFOS was used in a wide variety of industrial and consumer products including protective coatings for carpets and apparel, paper coatings, insecticide formulations, and surfactants [1;2]. PFOA has been used primarily to produce its salts, which are used in the production of fluoropolymers and fluoroelastomers. These polymers are used in many industrial and consumer products, including soil, stain, grease, and water resistant coatings on textiles and carpet; uses in the automotive, mechanical, aerospace, chemical, electrical, medical, and building/construction industries; personal care products; and non-stick coatings on cookware [1;2].

In animals, exposure to PFOS and PFOA is associated with adverse health effects, albeit at serum concentrations higher than the concentrations observed in the general population [1;2]. In 2002, 3M, the main worldwide manufacturer of PFOS, voluntarily discontinued the production of PFOS precursors and related compounds in the United States. In 2006, the U.S. EPA invited eight major companies in the PFAS industry to join in a global stewardship program with the goal to eliminate emissions of PFOA and its related products by 2015 [12]. As a result of the above changes in manufacturing practices, PFAS with shorter alkyl chains, and fluorinated alternatives, including perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs) have entered the market [13;14] and the environment [15].

Two PFECAs, GenX (ammonium salt of 2,3,3,3,-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoate [also known as HFPO-DA]) and ADONA (ammonium salt of 4,8-dioxa-3H-perfluorononanoate [DONA]) (Table 1), introduced as replacements for long-chain PFAS [13;14], have shorter elimination half-lives in animals than PFOA (hours versus days) [16;17]. However, previous research suggested toxicity of HFPO-DA and ADONA in experimental animals, with a similar mode of toxicity as that of PFOA [14]. Furthermore, these fluorinated alternatives cannot be metabolized in biota and may have a similar high affinity to proteins, resulting in a potential for bioaccumulation [14]. Last, the detection of GenX in surface waters and drinking water [18–20] has raised concerns about the potential health implications from human exposure to PFECAs through contaminated drinking water.

A chlorinated PFESA (2-[(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexyl)oxy]-1,1,2,2-tetrafluoroethanesulfonic acid potassium salt, F-53B (CAS No. 73606−19−6) [14]) has been used in chrome plating industry in China for decades [21], and recently detected at relatively high concentrations in the environment and biota in China [22–24]. In vitro, in vivo, and in

silico studies suggest that F-53B can disrupt the thyroid endocrine system at environmentally relevant concentrations [25]. Documented uses of F-53B outside China are not known, but the persistence and transport potential of F-53B raise some concerns about a future global contamination problem [24].

PFAS with five or fewer carbon chains could be possible degradation products generated in the environment or during waste water treatment processes [26] and do not seem to bioaccumulate or be toxic [27;28]. For example, perfluorobutane sulfonate (PFBS) appears to be much more efficiently eliminated than perfluorohexane sulfonate (PFHxS) or PFOS [28]. Nonetheless, the potential impact of these short-chain PFAS on human health and the environment is still unclear [28;29]. Short-chain PFAS have been used as replacements of traditional long-chain PFAS. We have measured select short-chain PFAS in U.S. National Health and Nutrition Examination Survey (NHANES) participants' sera since 1999; yet, we infrequently detected these short-chain PFAS, and when we did, concentrations were rather low compared to other PFAS [3]. The low detection frequencies and concentration ranges [8;30] may reflect limited human exposure to short-chain PFAS or the fact that, in humans, these PFAS have relatively short half-lives and are efficiently eliminated in urine. Therefore, urine, not serum, may be the preferred matrix for biomonitoring of short-chain PFAS.

Information on urinary concentrations of PFAS in humans is critical to understand exposure to alternative PFAS. A few methods for the quantification of select PFAS in human urine exist [31–37], but no methods for the concurrent quantification of select PFAS, PFECAs, and PFESAs for population-based biomonitoring programs such as NHANES. Therefore, we developed an on-line solid phase extraction–high performance liquid chromatography–isotope dilution–tandem mass spectrometry (on-line SPE-HPLC-MS/MS) method for the selective analysis of urine for both short- and long- chain PFAS, two PFECAs, and one PFESA. We also updated our current on-line SPE-HPLC-MS/MS serum method so it could include PFAS alternatives. We analyzed 50 urine–serum paired samples to validate the performance of these methods.

2. Experimental

2.1. Reagents

Methanol (MeOH), acetonitrile, and water were HPLC grade purchased from Fisher Scientific (Pittsburgh, PA). Formic acid (99%) was purchased from EM Science (Gibbstown, NJ). Acetic acid (glacial) was purchased from J.T. Baker (Phillipsburg, NJ). The following PFAS (currently accepted acronyms followed, if pertinent, by previously used acronyms) and fluorinated alternatives were purchased form Wellington Laboratories (Guelph, ON, Canada): perfluorooctane sulfonamide (FOSA, PFOSA), N-methylperfluoro-1-octanesulfonamidoacetic acid (MeFOSAA, Me-PFOSA-AcOH), N-ethylperfluoro-1-octanesulfonamidoacetic acid (EtFOSAA, Et-PFOSA-AcOH), sodium perfluoro-1-propanesulfonate (PFPrS), potassium perfluoro 1-butanesulfonate (PFBS, PFBuS), sodium perfluoro-1-hexanesulfonate (PFHxS), sodium perfluoro-1-heptanesulfonate (PFHpS), sodium perfluoro-1-octanesulfonate (n-PFOS), mixture of sodium perfluoro-5-methylheptane sulfonate (Sm-PFOS) and perfluoro-5-methylheptanoic acid (Sb-PFOA) isomers, perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate

(PFHxA), perfluoroheptanoate (PFHpA), ammonium perfluorooctanoate (n-PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA), 2,3,3,3,-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoate (HFPO-DA), dodecafluoro-3H-4,8-dioxanoate (DONA), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS (Table 1), main component of F-53B), N-methyl-d_3-perfluoro-1-octanesulfonamide acetic acid (D_3-MeFOSAA), N-ethyl-d_5-perfluoro-1-octanesulfonamide acetic acid (D_5-EtFOSAA), perfluoro-n-[$^{13}C_4$] butanoic acid ($^{13}C_4$-PFBA), perfluoro-n-[$^{13}C_5$] pentanoic acid ($^{13}C_5$-PFPeA), perfluoro-n-[1,2,3,4,6-$^{13}C_5$] hexanoic acid ($^{13}C_2$-PFHxA), perfluoro-n-[1,2,3,4,5-^{13}C]-heptanoic acid ($^{13}C_5$-PFHpA), perfluoro-n-[1,2,3,4-^{13}C]-octanoic acid ($^{13}C_4$-PFOA), perfluoro-n-[1,2,3,4,5-^{13}C]-nonanoic acid ($^{13}C_5$-PFNA), 2-perfluorooctyl [1,2-^{13}C]-ethanoic acid ($^{13}C_2$-PFDA), 2-perfluorooctyl [1,2-^{13}C]-undecanoic acid ($^{13}C_2$-PFUnDA), sodium perfluoro-1-[2,3,4-^{13}C] butanesulfonate ($^{13}C_3$-PFBS), sodium perfluoro 1-hexane [$^{18}O_2$]-sulfonate ($^{18}O_2$-PFHxS), sodium perfluoro 1-[1,2,3,4-^{13}C]-octanesulfonate ($^{13}C_4$-PFOS), and 2,3,3,3,-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-$^{13}C_3$-propanoate ($^{13}C_3$-HFPO-DA). $^{18}O_2$-perfluorooctane sulfonamide ($^{18}O_2$-FOSA) was purchased from RTI Laboratories (RTP, NC). β-glucuronidase/arylsulfatase (BGALA-RO from Helix Pomatia) was purchased from Sigma Aldrich Laboratories, Inc. (St. Louis, MO). All reagents were used without further purification.

Preparation of Standard Solutions and Quality Control (QC) Materials—Nine working native standard solutions containing the target analytes were prepared in water/methanol (50/50) by serial dilutions from commercial solutions (50 or 2 μg/mL in methanol) of PFPrS, PFBS, PFHpS, PFHxS, n-PFOS, Sm-PFOS, PFBA, PFPeA, PFHxA, PFHpA, n-PFOA, Sb-PFOA, PFNA, PFDA, PFUnDA, HFPO-DA, DONA, 9Cl-PF3ONS, FOSA, MeFOSAA, and EtFOSAA. A 50-μL spike in 50 μL of urine or serum would cover concentration ranges of 0.07–20 ng/mL for all analytes (except for n-PFOS (95 ng/mL) and n-PFOA (50 ng/mL) in serum). All prepared solutions were stored frozen (−70 ºC) in 2.0 mL polypropylene vials until use. Commercial solutions of $^{13}C_3$-PFBS, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_2$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_5$-PFHpA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_3$-HFPO-DA, D_3-MeFOSAA, D_5-EtFOSAA, and $^{18}O_2$-FOSA in methanol (50 μg/mL) were diluted to provide working solutions so that a 50-μL spike provides 2 ng/mL in 50 μL of matrix. Aliquots of this internal standard spiking solution were dispensed into 2.0 mL polypropylene vials and stored frozen (−70 ºC) until use.

To prepare quality control (QC) materials, urine was collected anonymously from adult volunteers at the Centers for Disease Control and Prevention (CDC); no personal or demographic data were available. CDC's Human Subjects Institutional Review Board reviewed and approved the study protocol. A waiver of informed consent was requested under 45 CFR 46.116(d). To make serum QC materials, we used calf serum (Gibco, Grand Island, NY). The urine or serum was pooled, mixed thoroughly, and divided into two subpools for urine and three subpools for serum (which included a serum blank (SB)). The subpools were enriched with the target analytes as needed to afford low concentration (QCL, ~0.7–5.4 ng/mL [serum], ~0.5–2.8 ng/mL [urine]) and high concentration (QCH, ~1.8–11.2 ng/mL [serum], ~1.1–5.8 ng/mL [urine]) materials. QCL and QCH materials were dispensed

into 2-mL polypropylene vials and stored frozen (−70 ℃). The QC materials were characterized to define the mean and the 95% and 99% control limits of target biomarker concentrations.

For method validation, we used 50 paired serum-urine samples, collected in December, 2016 that we purchased from Zenbio INC (http://www.zen-bio.com/). We had no demographic (e.g., age range), or other (e.g., geographical location) information from the persons who provided these commercially-available samples.

2.2. Sample and standards preparation

First, we dispensed 50 μL of urine into a polypropylene snap-cap autosampler vial, added 50 μL of stable isotope-labeled internal (IS) and 25 μL of 1M ammonium acetate buffer (pH 5.5) including 5 μL β-glucuronidase, and incubated for 240 min at 37 ºC; next, we added 400 μL of formic acid. For standards, reagent blanks (RB), and QCs, we aliquoted 0.1M formic acid (425 μL for RBs and standards, 375 μL for QCs) into autosampler vials and added 50 μL of QCs, 50 μL of internal standard, and 50 μL of the appropriate native standard (STDs) (S1–S9) solutions.

For serum, we dispensed 50 μL of serum, calf serum (SBs and STDs), or QCs into the autosampler vials, added 50 μL of IS and 0.1 M formic acid (500 μL for RBs; 450 μL for serum, QCs, SBs; 400 μL for STDs) into appropriate vials; for standards, we added 50 μL of the appropriate STDs (S1-S9).

We vortexed all vials for five seconds before the next analytical steps described below.

2.3. On-line SPE-HPLC-MS/MS

The on-line SPE-HPLC-MS/MS system was built from Symbiosys PICO (Spark Holland, VE Emmen, The Netherlands) coupled with an Sciex Triple Quad 5500 or Qtrap 6500 mass spectrometer (Sciex, Framingham, MA, USA) with a TurboIonSpray ionization (TIS) source. We used HySphere C8-SE (7μM) cartridge (i-Chrome solutions, Plainsboro, NJ) or Oasis WAX 30 μm, 10 mm × 1 mm (Waters) cartridge as solid phase extraction (SPE) cartridges for the serum or urine method, respectively.

We used the same HPLC columns and mobile phases for the chromatographic separation of the target analytes in serum and urine. However, the gradient programs for the SPE and HPLC differed. HPLC and SPE conditions for serum are described in the supporting information (Tables S1–S2). With these conditions, which followed the general approach used for the analysis of the 2013–2014 NHANES serum samples described in https://www.cdc.gov/nchs/data/nhanes/nhanes_13_14/PFAS_H_MET.pdf, we quantified PFBS, C6-C11 PFAS, and fluorinated alternatives in serum. To quantify PFBA and PFPeA in serum, we extracted the serum using the following conditions described for the urine method. For urine, the SPE run starts with the conditioning of an Oasis WAX cartridge with 0.3% NH_4OH-99.7% MeOH (2mL), acetonitrile (2 mL), and 0.1 M formic acid (2 mL). Afterward, 500 μL of matrix (containing 50 μL urine) injected into the 1 mL sample loop is loaded onto the SPE column by use of 2 mL 0.1 M formic acid at 2 mL/min. Next, the SPE

column is washed with 2 mL 50% 0.1 M formic acid / 50% MeOH (flow rate: 2 mL/min). The time of the SPE cleanup (including injection time) is 8 min.

Before the start of the clean-up of the next sample, the cartridge containing the extracted urinary analytes is transferred by a robotic gripper from the left clamp to the right clamp. Therefore, while the right clamp is used for analyte elution and HPLC-MS/MS acquisition, the left clamp is used for the clean-up of the next sample. Once the SPE column is in the right clamp, the HPD syringe pump transfers the analytes from the SPE column by 0.3% NH_4OH-99.7% MeOH 100 μL/min (4 min) and mixing with mobile phase from the HPLC gradient pump to a Chromolith® HighResolution RP-18e guard column (4.6 × 5 mm) followed by a Chromolith® HighResolution RP-18e column (4.6 × 25 mm) (Merck KGaA, Germany) (Figure S1). In addition, the divert valve is turned into 1–2 position (to waste) for the first 2 min. At 1 min, the divert valve is turned into 6–1 position to transfer the analytes from the above HPLC guard precolumns to two Chromolith® HighResolution RP-18e columns (4.6 × 100 mm) in tandem. At 8 min, the RCV is turned back to 1–2 position and the SPE column is returned to the cartridge tray, while the HPLC gradient program continues. The HPLC pump operates at a 1000 μL/min flow rate with 20 mM ammonium acetate (pH 4.0) in 5% acetonitrile as mobile phase A and acetonitrile as mobile phase B, respectively. The HPLC gradient program (18 min) is as follows: start at 1 %B (1 min), increase B content from 1% to 60% (1 to 8 min) and from 60% to 85% (8 to 12 min), hold B content at 85% (4 min), decrease B content to 1% and hold (16 to 18 min); the mobile phase flow rate was 1500 μL/min during the conditioning of the HPLC column (Table S3). The valco valve connecting the HPLC column and the MS/MS is turned into waste position at 6.0 min and after 15 min to minimize contamination of the MS/MS interface.

To delay the elution of PFAS contaminants potentially leaching out from Teflon parts of the HPLC pump, another Chromolith® HighResolution RP-18e Guard column (5 × 4.6 mm) is inserted between the HPLC pump and the RCV. Because a contaminant would have to go through twice the filter column length, the contaminant would elute after the target analyte, and the contaminant corresponding signal would be separated from the signal of the target analyte, and thus would not interfere with the quantification of the main analyte signal [38].

Detection of the target analytes in both urine and serum was conducted in the negative ion mode. The heated turbo ion spray gas temperature was 400 °C. The curtain and collision gas (nitrogen) settings were: collision (CAD=9.0), curtain gas (CUR=25). Ionization parameters and collision cell parameters were optimized individually for each analyte (Table S4). In agreement with past work [39], we identified and monitored one suitable precursor/product ion transition for each analyte and isotope labeled internal standard; for $^{13}C_4$-PFOS, we used two transitions, one as internal standard for n-PFOS and the other as internal standard for Sm-PFOS (Table S4).

Typical chromatograms are shown in Figure 1 (urine) and Figures S2–S3 (serum). The methods included long- and short-chain PFAS as well as fluorinated alternatives; however, because the target analytes encompass a wide range of physicochemical properties which, in turn, strongly influence performance in multi-analyte methods, we could quantify PFPrS only in urine, and FOSA, MeFOSAA and EtFOSAA only in serum.

2.4. Data analysis

Data acquisition were performed by use of the Analyst 1.6.2 software. The data analysis program used was MultiQuan 3.0.2 which automatically selected and integrated the chromatographic signals for each transition of interest. We manually corrected integrations, if necessary. For quantification, we used a response factor (RF), calculated as the peak area of each analyte ion divided by the peak area of its internal standard.

We used nine standard solution concentrations, spiked into 0.1 M formic acid for urine, encompassing the entire linear range of the method (0.07–20 ng/mL) to construct daily calibration curves, weighted by the reciprocal of the standard amount (1/x), of RF versus the standard amount. For serum, we used standard solution concentrations spiked into 50 μL of calf serum and 0.1 M formic acid encompassing the entire linear range of the method (0.07–20 ng/mL, except for n-PFOS (95 ng/mL) and n-PFOA (50 ng/mL)). Calibration curves were linear over up to three orders of magnitude and had correlation coefficients exceeding 0.99. Because standards and unknown samples went through the same extraction procedure, reagent contributions were automatically corrected by the calibration curve intercept.

To monitor the accuracy and precision of the method, two QCL, two QCH and three reagent blanks were analyzed concurrently with 20 samples and calibration standards. The concentrations of the QCs, averaged to obtain one measurement of high- and one of low-concentration QC for each run, were evaluated by use of standard statistical probability rules [40].

3. Results and discussion

3.1. Method Optimization

We optimized chromatographic resolution by examining the separation of PFBA, the first eluting compound, from the urine interferences. Although we tested HySphere C8 and C18 (data not shown), we obtained the best performance when using an Oasis WAX as the SPE column and focusing mode: eluted by 100% MeOH with 0.3% NH_4OH and diluted by the HPLC mobile phase (~94% aqueous) before eluting into the HPLC column. These conditions reduced interferent peaks and high background not only for PFBA but also PFPeA, the first two eluting PFAS (Figure 1). Of interest, the use of isotope labeled standards improved not only the accuracy of the method, but its selectivity by minimizing the chance of misidentifying signals from interferent compounds with those of the target analytes.

To evaluate matrix effects, we analyzed 0.1M formic acid and pooled urine spiked with three different analytical standard concentrations (N = 5). Spearman’s rank correlation coefficients between the urine and the solvent-based calibration curves were above 0.98 and the slopes were ~1 for all analytes, even for PFHpS which doesn’t have an isotope labeled-internal standard (Figure 2). Therefore, we chose to use a calibration curve in formic acid for quantification of target analytes in urine. For serum, details have been discussed before [39].

3.2. Sensitivity, accuracy, and precision of the analytical method

Spiked human urine was analyzed repeatedly to determine the limits of detection (LODs). LODs were calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero. S_0 was determined from 5 repeated measurements of low-level standards spiked onto human urine [41]. The LOD was 0.1 ng/mL for all analytes. Of interest, even under the alkaline conditions required for ionization, these LODs are comparable to or even lower than the LODs we achieved with our previous method in serum [39] for most analytes (Table 2). The method accuracy (93.6 to 106.2%) was also acceptable for all target analytes (Table 2). Interday precision, calculated as the RSD% of 20 repeated measurements in a 2-month period, ranged from 6.2 to 10.4 % for QCL (0.5–2.8 ng/mL) and from 5.6 to 10.4 % for QCH (1.1–5.8 ng/mL) (Table 2). Intraday precision, calculated as the RSD% of 5 repeated measurements within one day, ranged from 3.2 to 5.8 % for QCL and from 2.2 to 6.2 % for QCH. Together, these data suggest that the sensitivity, accuracy, and precision of the method to quantify select PFAS and fluorinated alternatives in urine at sub-parts-per-billion concentration ranges are adequate for biomonitoring purposes.

Method performance parameters for the quantification of PFAS and fluorinated alternatives in serum are shown in Tables 3 and S5. For serum, we used the present method developed in urine for short-chain PFAS (PFBA and PFPeA) (Figure S2); to quantify the rest of PFAS and fluorinated alternatives including HFPO-DA, ADONA, and 9Cl-PF3ONS, we followed the approach used to analyze the 2013–2104 NHANES serum samples (https://www.cdc.gov/nchs/data/nhanes/nhanes_13_14/PFAS_H_MET.pdf).

Analysis of human samples—To validate the method, we analyzed 50 urine-serum paired samples (Table 4). In serum, we rarely detected short-chain PFAS and did not detect fluorinated alternatives, but detected several long-chain PFAS in 42% to 100% of samples, depending on the analyte (Table 4), with concentrations patterns similar to those from 2013–2014 NHANES [3]. In contrast, in urine, at least in this group of samples, collected from persons with no known occupational or accidental exposure to the target analytes, we rarely detected any PFAS (regardless of alkyl chain length) or fluorinated alternatives, with the exception of the short-chain PFBA, detected in 56% of the samples, albeit at concentrations close to the LOD (i.e., median concentration was 0.2 ng/mL).

Of interest, PFBA can be formed by industrial synthesis, metabolism [42], and environmental degradation [43;44] of certain fluorinated chemicals. PFBA has been detected in precipitation, surface waters, and water treatment facility effluents at low ng/L concentrations [45–48]. Furthermore, drinking water has been identified as one of the major routes of human exposure to this compound, and trace concentrations of PFBA have been measured in drinking water sources in several countries [45;49]. PFBA has also been detected at low ng/mL concentrations in the serum or urine of people with potential exposure to PFBA through drinking water or via occupational exposure sources as well as in the general population [32;50]. However, we only detected PFBA at rather low sub-part-per billion concentrations suggesting that exposure to PFBA may be restricted to populations who live or work nearby exposure sources. The higher detection of PFBA in urine compared to serum suggest that for these non-persistent chemicals urine, and not serum, is the most

adequate biomonitoring matrix. By contrast, serum is the most adequate matrix for assessing exposure to long-chain PFAS. Nevertheless, based on the results from this convenience sampling of 50 persons, the limited detection of other short-chain PFAS (e.g., PFBS, PFPeP, PFHxA) or fluorinated alternatives (e.g., HFPO-DA, DONA, 9Cl-PF3ONS) in either serum or urine may suggest limited background exposure to these compounds.

4. Conclusions

We have developed a selective, accurate, and precise analytical method for the separation and trace-level quantification of 15 C_3-C_{11} PFAS, and three fluorinated alternatives in human urine, and updated the currently used method to quantify PFAS in serum to also measure three fluorinated alternatives in human serum. In a convenience collection of samples in 2016 from persons with no known occupational or accidental exposure to the target chemicals, we did not detect fluorinated alternatives in serum or urine, and urinary concentrations of short-chain PFAS were undetectable, except for PFBA. These preliminary results suggest limited exposure to short-chain PFAS and fluorinated alternatives among these persons. Nevertheless, population-representative data would greatly improve our understanding of background exposure to these compounds among the U.S. general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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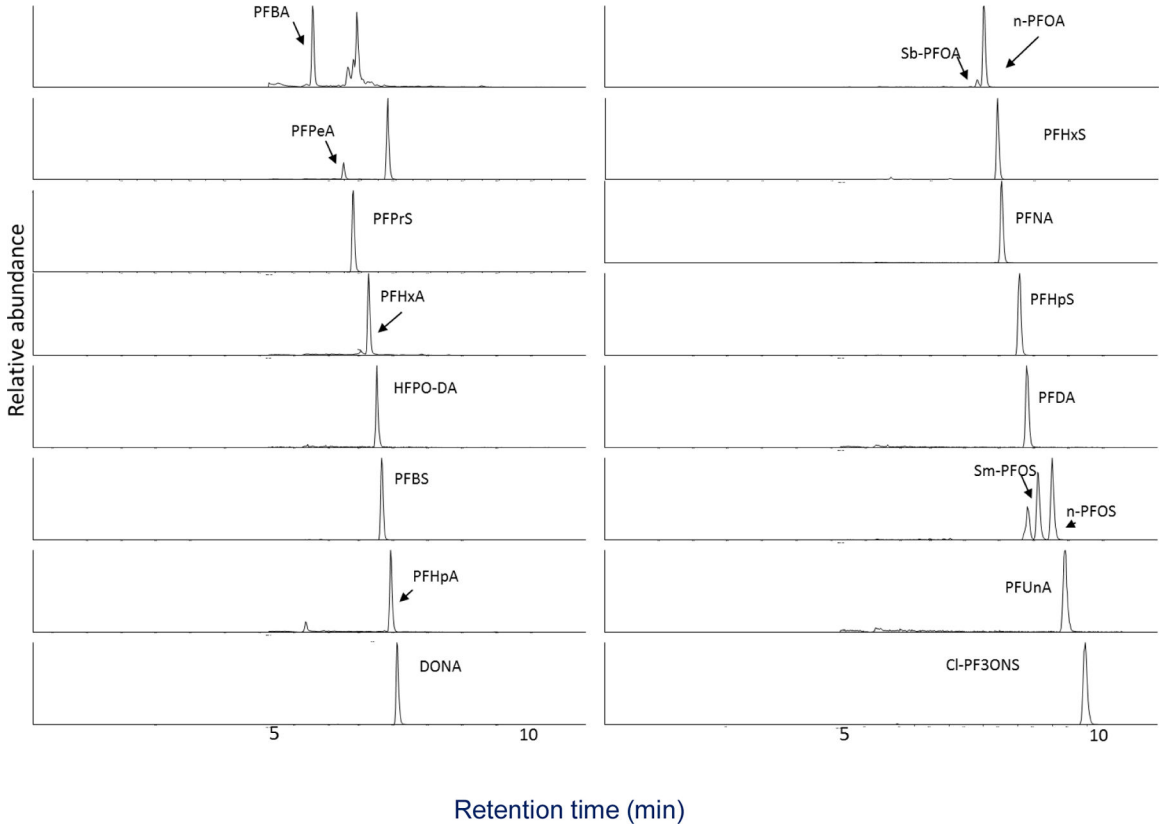


Fig. 1.

Typical HPLC-MS/MS chromatograms of select PFAS in a low concentration (~0.5–2.8 ng/mL, depending of the analyte) quality control urine sample

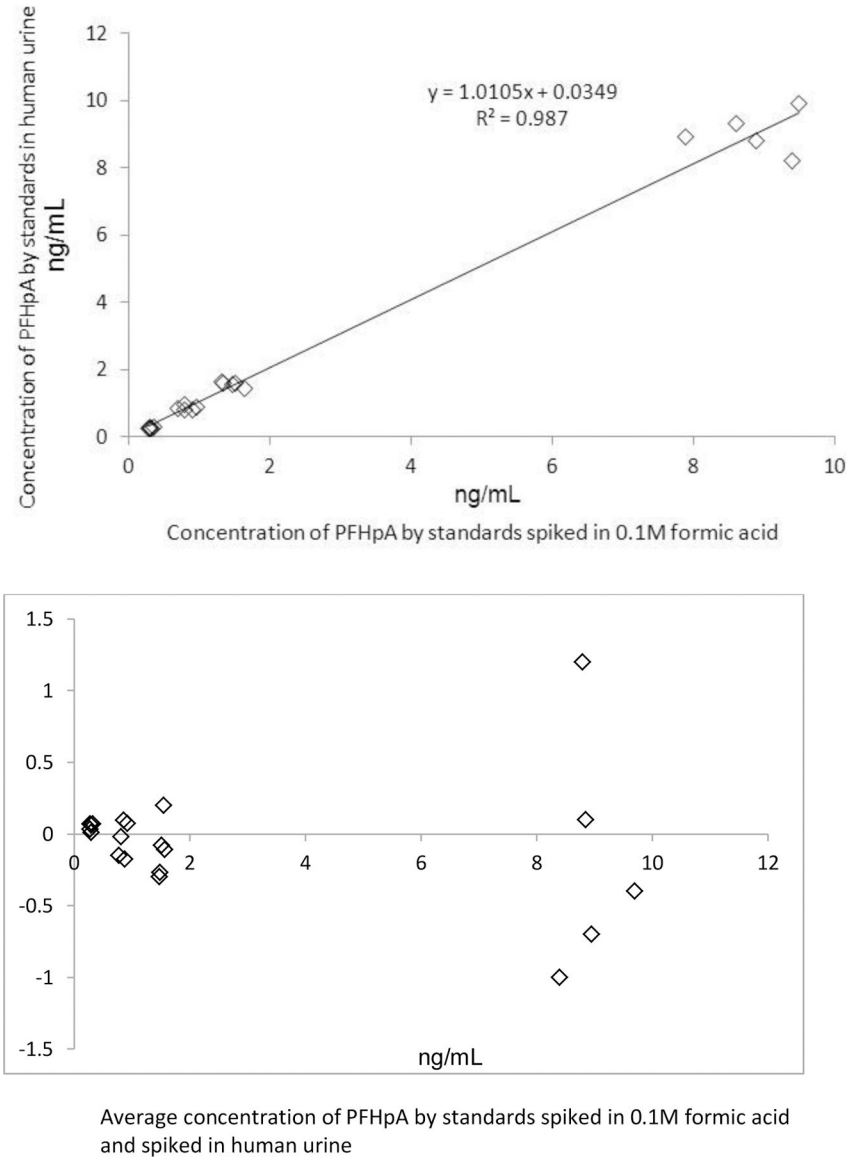


Fig. 2.

Evaluation of matrix effects on the calibration standards. Correlation plot between the concentrations of solvent- and urine-based analytical standards (top). Bland–Altman plot (difference in concentrations vs average of concentrations using both sets of standards) (bottom).

Table 1.

Chemical structures of the biomarkers assessed for the three fluorinated alternatives evaluated in this study.

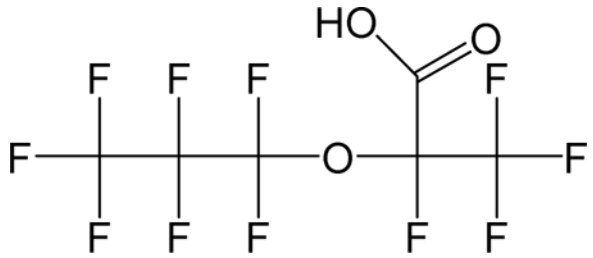
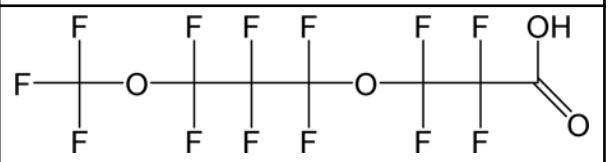
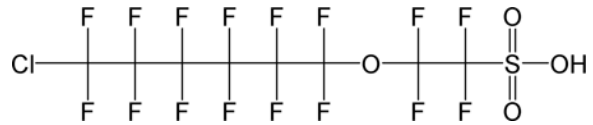
Biomarker	Structure
HFPO-DA	
ADONA	
9Cl-PF3ONS	

Table 2.

Accuracy, sensitivity, and precision of the analytical method for the quantification of PFAS and fluorinated alternatives in urine

	LOD	Precision				Accuracy (%)	
		QCL		QCH			
	ng/mL	ng/mL	RSD (%)	ng/mL	RSD (%)	Low level	High level
PFPrS	0.1	1.5	6.8	2.8	7.1	97.8±4	96.2±3
PFBS	0.1	1.3	7.8	3.2	8.0	98.7±3	98.1±8
PFHxS	0.1	1.6	8.2	3.7	8.5	98.7±6	93.8±7
PFHpS	0.1	1.5	10.2	3.9	9.5	100.3±6	101.5±5
n-PFOS	0.1	1.3	8.9	3.2	8.8	99.1±4	98.8±2
Sm-PFOS	0.1	0.5	9.5	1.8	8.4	96.7±4	98.2±1
PFBA	0.1	1.6	8.2	3.7	8.5	93.6±2	95.0±8
PFPeA	0.1	1.3	8.9	3.2	8.8	101.0±8	106.2±2
PFHxA	0.1	0.5	10.4	1.5	8.2	102.2±6	103.8±5
PFHpA	0.1	0.5	9.2	1.1	9.8	102.7±3	98.5±6
n-PFOA	0.1	2.8	9.5	5.4	8.8	103.1±2	102.3±5
Sb-PFOA	0.1	1.6	7.6	3.4	10.4	98.5±4	100.1±6
PFNA	0.1	1.4	6.2	4.2	6.6	102.1±5	96.5±4
PFDA	0.1	1.4	8.8	3.6	7.2	102.4±7	99.1±5
PFUnDA	0.1	1.5	10.2	3.5	6.5	97.4±2	97.4±4
HFPO-DA	0.1	1.8	7.4	10.8	9.8	102.6±11	97.5±9
DONA	0.1	1.6	8.2	3.7	8.5	96.5±2	98.3±6
9Cl-PF3ONS	0.1	1.3	8.9	3.2	8.8	104.2±10	103.8±7

Accuracy: n=5; Limit of detection (LOD): n=5; Intraday precision: n=20

Table 3.

Accuracy, sensitivity, and precision of the analytical method for the quantification of select PFAS and fluorinated alternatives in serum.[a]

	LOD	Precision				Accuracy (%)	
		QCL		QCH			
	ng/mL	ng/mL	RSD (%)	ng/mL	RSD (%)	Low level	High level
PFBS	0.1	2.2	8.9	4.9	8.7	98±5	101±2
PFHxS	0.1	2.3	7.6	6.3	9.1	98±5	98±6
PFHpS	0.1	2.5	7.2	4.3	9.4	97±8	98±7
n-PFOS	0.1	5.4	13.3	11.2	9.3	99.7±6	97±2
Sm-PFOS	0.1	0.7	8.7	1.8	6.8	92±4	96±6
PFHxA	0.1	2.3	10.4	5.9	10.1	102±6	104±5
PFHpA	0.1	2.4	8.8	6.1	7.3	103±3	99±6
n-PFOA	0.1	2.5	12.7	6.7	8.2	105±5	101±2
Sb-PFOA	0.1	3.0	10.9	8.5	10.8	106±8	102±5
PFNA	0.1	2.3	10.6	6.5	9.0	103±7	102±3
PFDA	0.1	2.5	8.8	6.4	7.2	96±6	98±4
PFUnDA	0.1	2.4	10.2	6.7	6.5	105±3	97±4
HFPO-DA	0.1	2.3	7.4	6.4	9.8	97±4	98±5
DONA	0.1	2.4	8.2	6.4	8.5	96±5	94±5
9Cl-PF3ONS	0.1	2.0	8.9	6.6	8.8	104±7	102±5
FOSA	0.1	2.1	10.8	6.5	8.9	100±5	98±2
MeFOSAA	0.1	1.7	11.7	7.8	9.3	93±5	101±3
EtFOSAA	0.1	1.1	11.8	5.0	8.1	98±8	102±2

[a]We followed the approach used to analyze the 2013–2104 NHANES serum samples (https://www.cdc.gov/nchs/data/nhanes/nhanes_13_14/PFAS_H_MET.pdf)

Table 4.

Frequency of detection (%) and percentile concentrations (ng/mL) of select PFAS in paired human serum and urine (n=50).[a]

	Serum[b]											Urine[c]
	PFHpA	**PFOA**	**Sb-PFOA**	**PFNA**	**PFDA**	**PFUnDA**	**PFHxS**	**PFHpS**	**n-PFOS**	**Sm-PFOS**	**MeFOSAA**	**PFBA**
Frequency (%)	2	98	2	100	40	8	92	96	98	86	42	56
median	<LOD	0. 7	<LOD	0.5	<LOD	<LOD	0.5	0.3	1.7	0.7	<LOD	0.2
75th percentile	<LOD	1.3	<LOD	0.7	0.1	<LOD	0.9	0.4	3.1	1.2	0.2	0.3
90th percentile	<LOD	2.0	<LOD	1.0	0.2	<LOD	1.2	0.7	5.0	2.1	0.3	0.5
95th percentile	<LOD	2.4	<LOD	1.2	0.3	0.1	1.7	0.8	5.1	2.3	0.4	0.6
Maximum	0.1	4.0	0.1	1.3	0.6	0.2	2.0	1.0	10.3	4.4	0.5	0.8

[a] LODs were 0.1 ng/mL in urine and serum for all analytes.

[b] PFBA, PFPeA, PFHxA, PFBS, PFPrS, HFPO-DA, DONA, EtFOSAA, FOSA, and 9Cl-PF3ONS were not detected in any serum samples.

[c] Only PFBA was detected in urine