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# New approach for assessing human perfluoroalkyl exposure via hair

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ABSTRACT

In the recent years hair has been increasingly used as alternative matrix in human biomonitoring (HBM) of environmental pollutants. Sampling advantages and time integration of exposure assessment seems the most attractive features of hair matrix. In the current study, a novel miniaturized method was developed and validated for measuring 15 perfluoroalkyl substances (PFAS), including perfluoro *n*-butanoic acid (PFBA), perfluoro *n*-pentanoic acid (PFPeA), perfluoro *n*-hexanoic acid (PFHxA), perfluoro *n*-heptanoic acid (PFHpA), perfluor *n*-octanoic acid (PFOA), perfluoro *n*-nonanoic acid (PFNA), perfluoro tetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS), perfluoro pentane sulfonic acid (PFPeS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), perfluorooctane sulfonic acid (PFOS), perfluorononane sulfonic acid (PFNS), perfluorodecane sulfonic acid (PFDS) and perfluorododecane sulfonic acid (PFDoS) in human hair by liquid chromatography tandem mass spectrometry (LC–MS/MS). After extraction using ethyl acetate, dispersive ENVI-Carb was used for clean-up. Good intra- and inter-day precision for low (LQ 5 ng/g hair) and high spike (HQ 15 ng/g) levels were achieved (in general RSD $<10\%$). The accuracy was assessed using recoveries (%), which ranged between 68–118% (LQ) and 70–121% (HQ). The instrumental limit of detection ($LOD_i$) and limit of quantification ($LOQ_i$) were between 1–4 pg/g hair and 3–13 pg/g hair, respectively. The method limit of quantification ($LOQ_m$) ranged between 6 and 301 pg/g hair. The PFAS levels were measured in 30 human hair samples indicating that the levels are low (14–1534 pg/g hair). Some PFAS were not present in any hair sample (e.g. PFHpA, PFTeDA, PFNA, PFPeS, PFHpS, PFOS and PFNS), while other PFAS were frequently detected (PFBA, PFPeA, PFHxA, PFOA, PFBS, PFHxS, PFOS, PFDS and PFDoS) in human hair. Although levels in general were low, there is evidence of higher human exposure to some analytes, such as PFBA, PFPeA, PFHxA, PFOA, PFBS, PFHxS, and PFDoS. The current study shows that hair is a suitable alternative non-invasive matrix for exposure assessment of PFAS.



## 1. Introduction

Health authorities around the world try to monitor and control the presence of the most toxic environmental pollutants in environment, wildlife and humans, aiming at reducing their exposure. Among such chemicals are the perfluoroalkyl substances (PFAS), which are included in the list of harmful compounds of the Stockholm Convention since 2009 [1]. These substances are used in diverse everyday consumer and chemical products, such as e.g. cookware, foams, paints, cosmetics, paper, leather and textile coatings, pesticides, electronic devices, adhesives [2–4], consequently leading to a constant and unavoidable human exposure to these chemicals.

Due to their high persistency and bioaccumulation potential [2], human biomonitoring (HBM) tools of PFAS have been widely explored during the last decade, but mainly for invasive samples such as (cord) blood, serum and breast milk [5–10]. Blood perfuses several tissues and organs, so this matrix can be used as an indicator for human exposure to PFAS. However collecting blood holds practical and ethical constraints, especially for children or vulnerable populations [11]. Sampling breast milk and/or cord blood has the same implications as for blood or serum, in addition to the limited exposure assessment (i.e., only mothers who are breastfeeding and who have a newborn child are able to participate).

Meanwhile, the exploitation of alternative non-invasive matrices such as hair brought new insights in HBM of PFAS [12–14] likewise was done in the past for measuring other environmental pollutants [15,16], alcohol, drugs of abuse and their metabolites in hair [17–19]. The main advantages of using hair as matrix in HBM

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are mostly related to the non-invasive sampling, which is easy, fast and not painful. Moreover, the collection is possible for both children/babies and elderly and/or sick people. Also, storage is easy, usually done at room temperature (if the target compounds are not volatile) and for long time period as the matrix stability is higher than for liquid matrices [20]. Further, hair can mirror both the short to long-term exposure (months to years depending on the analyzed hair length), which is considered the major advantage in human exposure assessment.

The main challenge of HBM of PFAS in human hair is the availability of a sufficiently sensitive analytical technique combined with suitable extraction methods. In past studies, the PFAS extraction from hair was performed by accelerated solvent extraction (ASE) or ultrasound extraction using different organic solvents (e.g. acetonitrile, methanol and acid/basic digestion). Then solid-phase extraction (SPE) where weak anion-exchange sorbent combined with a reverse phase (Oasis WAX) is highly suggested as clean-up for strong acidic compounds [12–14]. Instrumental measurement is commonly done using liquid chromatography tandem mass spectrometry (LC–MS/MS) which seems to be a common and more direct analytical approach for both invasive [7,21–23] and non-invasive matrices [12–14]. Although gas chromatography (GC) can be used, there are limitations for PFAS measurement due to their low volatility (especially for the long-chain compounds), leading to a need for derivatization prior to analysis [24]. Capillary electrophoresis (CE) methods could be another choice for detecting polar and ionized PFAS, however the most important drawback is the poor detection sensitivity of this analytical technique, which is mainly due to the low UV/Vis absorption of PFAS [23].

The aim of this study is to overcome pending drawbacks on current extraction methods for HBM of PFAS in human hair [12–14]. The main challenges comprise the development of a sensitive and accurate analytical method for measuring 15 short to long-chain PFAS in hair at pg/g levels by LC–MS/MS. An alternative clean-up method (dispersive ENVI-Carb) is proposed here as easier, faster and more environmental friendly than the conventional SPE methods presented in the literature. In addition, other factors such as hair type and matrix effect influence in the PFAS detection was evaluated for the first time. The assessment of the human exposure to other perfluoroalkyl sulfonic acids than PFOS (until now the most studied), including the perfluoroalkyl pentane sulfonate (PFPeS), perfluoroalkyl heptane sulfonate (PFHpS), perfluoroalkyl nonane sulfonate (PFNS) and perfluoroalkyl dodecane sulfonates (PFDoS) is another innovative aspect of this research. Finally, advantages and drawbacks associated to the hair analysis for HBM of PFAS in general population is discussed.

## 2. Materials and methods

### 2.1. Chemicals and equipment

Fifteen PFAS ( > 98% purity) including perfluoro *n*-butanoic acid (PFBA), perfluoro *n*-pentanoic acid (PFPeA), perfluoro *n*-hexanoic acid (PFHxA), perfluoro *n*-heptanoic acid (PFHpA), perfluoro *n*-octanoic acid (PFOA), perfluoro *n*-nonanoic acid (PFNA), perfluoro tetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS), perfluoro pentane sulfonic acid (PFPeS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), perfluorooctane sulfonic acid (PFOS), perfluorononane sulfonic acid (PFNS), perfluorodecane sulfonic acid (PFDS) and perfluorododecane sulfonic acid (PFDoS) and mass-labelled internal standards (IS) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The mixtures of PFAS and mass-labelled ($^{13}C$ and $^{18}O$) internal standard solutions were prepared in methanol (UPLC grade, Biosolve, Netherlands) from individual stock solutions. Internal standard calibration curves for all analytes considered the concentration and chromatographic area of the respective internal standard (IS); however the PFAS without a corresponding labelled IS were corrected with the antecedent alkyl chain IS (Table 1).

**Table 1**
List of PFAS and IS, MRM transitions and retention time.

| Compound | MRM1[a] | MRM2[b] | Retention time (min) | IS[c] |
|---|---|---|---|---|
| **PFBA** | 213 > 169 | – | 3.10 | $^{13}C_1$–PFBA |
| **PFPeA** | 263 > 219 | – | 6.46 | $^{13}C_5$–PFPeA |
| **PFBS** | 299 > 80 | 299 > 99 | 7.41 | $^{18}O_2$–PFHxS |
| **PFHxA** | 313 > 269 | 313 > 119 | 9.70 | $^{13}C_2$–PFHxA |
| **PFPeS** | 349 > 80 | 349 > 99 | 10.25 | $^{18}O_2$–PFHxS |
| **PFHpA** | 363 > 319 | 363 > 169 | 12.08 | $^{13}C_2$–PFHxA |
| **PFHxS** | 399 > 80 | 399 > 99 | 12.38 | $^{18}O_2$–PFHxS |
| **PFOA** | 413 > 369 | 413 > 169 | 13.86 | $^{13}C_4$–PFOA |
| **PFHpS** | 449 > 80 | 449 > 99 | 14.03 | $^{18}O_2$–PFHxS |
| **PFNA** | 463 > 419 | 463 > 169 | 15.28 | $^{13}C_5$–PFNA |
| **PFOS** | 499 > 80 | 499 > 99 | 15.30 | $^{13}C_4$–PFOS |
| **PFNS** | 549 > 80 | 549 > 99 | 16.47 | $^{13}C_4$–PFOS |
| **PFDS** | 599 > 80 | 599 > 99 | 17.39 | $^{13}C_4$–PFOS |
| **PFDoS** | 699 > 80 | 699 > 99 | 19.09 | $^{13}C_4$–PFOS |
| **PFTeDA** | 713 > 669 | 713 > 319 | 19.84 | $^{13}C_2$–PFTeDA |

[a] Multiple reaction monitoring (MRM) used for quantification.
[b] Multiple reaction monitoring (MRM) used for identification.
[c] Internal Standard.

The ultra-pure water was produced using a Millipore Advantage A10 system (Millipore S.A., Overijse, Belgium). Pure reagents ammonium acetate ($NH_4AC$) and ammonium hydroxide ($NH_4OH$) were purchased from Sigma-Aldrich (Diegem, Belgium and Steinheim, Germany, respectively). UPLC grade organic solvents: acetonitrile, methanol and ethyl acetate (Fisher Scientific, Loughborough, UK), 2-propanol (99.8%), formic acid (98–100%) and tetrahydrofuran (TFH) (99.9%) were supplied by Merck (Darmstadt, Germany). The Envi-Carb 120/400 mesh was obtained from Supelco (Bellefonte, USA) and the Oasis WAX cartridges (150 mg, 3 cc) were supplied by Waters (Massachusetts, USA).

All glass material was washed and baked out in the oven at 450 °C overnight. It was then stored wrapped in aluminum foil to avoid contact with air and dust particles.

### 2.2. Sample collection and decontamination

Hair samples ($n=30$) were collected during 2013 from a general (non-exposed) population. Due to the insufficient amount of hair cut near the scalp (less than 100 mg) per individual, the hair analyses were done using the whole hair shaft. For the method development a pool sample was prepared, unlike the method validation which only one hair sample collected from one individual was used.

The sampling campaign was approved by the Ethical Committee of the University of Antwerp (Reg. B300201316329). The volunteers were duly informed about the purpose of this study giving their consent to participate. In this study, no personal or lifestyle information was collected through questionnaires.

All hair samples were firstly rinsed with ultra-pure water and acetone. Consecutively the samples were dried at room temperature and then cut into small pieces with stainless steel scissors as described in previous studies [12,14]. The samples were stored in aluminum foil at room temperature since the PFAS are stable compounds and non-volatile.

### 2.3. Method description

#### 2.3.1. Extraction solvent test followed by dispersive ENVI-Carb clean-