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Development and validation of an extraction method for the analysis of perfluoroalkyl substances in human hair

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HIGHLIGHTS

- Reliable analytical method for PFASs in human hair was developed.
- Solid-phase extraction was found to be the optimal method for PFASs analysis in hair.
- The method accuracy and precision were satisfactory.
- Among 11 PFASs, PFOA and PFHxS were dominant compounds in human hair.

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ABSTRACT

Human hair has many advantages as a non-invasive sample; however, analytical methods for detecting perfluoroalkyl substances (PFASs) in human hair are still in the development stage. Therefore, the aim of this study was to develop and validate a method for monitoring 11 PFASs in human hair. Solid-phase extraction (SPE), ion-pairing extraction (IPE), a combined method (SPE+IPE) and solvent extraction with ENVI-carb clean-up were compared to develop an optimal extraction method using two types of hair sample (powder and piece forms). Analysis of PFASs was performed using liquid chromatography and tandem mass spectrometry. Among the four different extraction procedures, the SPE method using powdered hair showed the best extraction efficiency and recoveries ranged from 85.8 to 102%. The method detection limits for the SPE method were 0.114–0.796 ng/g and good precision (below 10%) and accuracy (66.4–110%) were obtained. In light of these results, SPE is considered the optimal method for PFAS extraction from hair. It was also successfully used to detect PFASs in human hair samples.

1. Introduction

Perfluoroalkyl substances (PFASs) have been used extensively in consumer products and industrial processes, such as food packaging, paper, firefighting foams, water and oil repellents for leather, and waxes (Herzke et al., 2012; Myers et al., 2012). However, due to their potential toxicity to the environment and human body, several countries have begun banning the sale and production of PFASs, and they were registered as persistent organic pollutants (POPs) in 2009.

For now, human biomonitoring (HBM) for PFASs have been conducted by using various human samples. Blood has been widely used for HBM for PFASs, and is considered the ideal matrix because it is in contact with all body tissues and in equilibrium with organs (Smolders et al., 2009). However, the invasiveness and ethical limitations of blood collection are significant challenges. There is therefore a growing need for the use of non-invasive samples (Perez et al., 2012; Li et al., 2013), including urine, fingernails and toenails, and hair. Breastmilk is also frequently used for HBM; however, there is a significant limitation to this in that breastmilk is produced only by nursing mothers. Among other non-invasive samples, hair has been used as bio-indicator for assessing exposure to POPs (Król et al., 2013), such as dioxins (Nakao et al., 2002), organochlorine pollutants (Covaci et al., 2002, 2008) and pesticides (Cirimele et al., 1999). However, there is a potential constraint associated with using hair for HBM, with respect to the difficulty in distinguishing between internal and external contamination. Despite this, hair for HBM has many advantages, such as painless and cost-effective collection, and easy transfer and storage, compared to other invasive human samples.

PFAS monitoring using hair, and development of a hair

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monitoring method for PFAS analysis, is still in the development stage. So far, three studies pertaining to the development of analytical methods for measuring PFASs in human hair have been published (Alves et al., 2015; Li et al., 2013; Perez et al., 2012). These studies employed various extraction methods, such as acid and alkaline digestion and solvent extraction, and affirmed the effectiveness of sample extraction by comparison among various hair types (Alves et al., 2015; Li et al., 2013). However, the PFAS concentration and profiles results were inconsistent among the studies. Perez et al. (2012) and Li et al. (2013) showed similar PFAS concentration ranges (ND [not detected] ~ 6.45 ng/g), except for perfluorodecanoic acid (PFDA), but Alves et al. (2015) reported a very low concentration (in the range of parts per trillion to parts per billion); thus, strikingly different concentrations have been observed even though the samples of all of the studies were drawn from the general population. In addition, these studies showed different dominant compounds and their detection frequency. In Perez et al. (2012), perfluorooctane sulfonate (PFOS) was predominant, but all target PFASs including PFOS were shown low detection frequencies (below 50%). Li et al. (2013) reported perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS) and PFOS were dominant. However, Alves et al. (2015) reported the dominant PFOA and perfluorobutane sulfonate (PFBS). In light of these inconsistent results, more research is needed for analyzing PFASs and confirmation of the PFASs profiles in hair based on accurate analytical method.

Therefore, the aim of this study was to develop and validate a reliable analytical method for measuring PFASs in hair, i.e., a non-invasive human sample, using liquid chromatography and tandem mass spectrometry (LC-MS/MS). To optimize the extraction method, four different extraction procedures were evaluated for hair samples in piece and powder forms: (i) solid-phase extraction (SPE), (ii) ion-pairing extraction (IPE), (iii) a combined method (IPE+SPE) and (iv) solvent extraction with ENVI-carb clean-up. After selecting the optimal method, PFAS exposure in a Korean population was assessed by applying the method to human hair samples.

2. Materials and methods

2.1. Chemicals and reagents

Validated PFASs, namely perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), PFDA, perfluorobutane sulfonate (PFBS), PFHxS, perfluoroheptane sulfonate (PFHpS), PFOS and perfluorodecane sulfonate (PFDS), were purchased from Wellington Laboratories (Guelph, Canada). A mixture of $^{13}C_2$ or $^{18}O_2$-labeled PFASs (Wellington Laboratories) and $^{13}C_8$-labeled PFASs (Wellington Laboratories) were used as internal and recovery standards (Table 1). All solvents (including acetone, acetonitrile, methanol, methyl *tert*-butyl ether [MTBE], and water) were high performance liquid chromatography grade (J.T. Baker Co., Phillipsburg, NY, USA). Aqueous ammonia and formic acid were purchased from Junsei Chemical Co. (Tokyo, Japan) and Merck Co. (Darmstadt, Germany).

2.2. Human hair sampling and preparation

Hair samples were collected from the general adult population (n = 47) of Busan Metropolitan City, South Korea, after approval was granted for the study by the local medical ethics committee. Hair samples were cut as close to the scalp as possible and there was no contamination from hair dye. Collected hair samples were stored in polypropylene (PP) tubes at −20 °C until analysis. To prevent contamination of the hair samples, cleaning to remove external contamination (e.g., dust particles) was required. Therefore, prior to extraction, hair samples were rinsed using HPLC water and acetone and air-dried. To compare the extraction efficiency of the different sample formats, dried hair samples were cut into small pieces (about 5 mm) using scissors and were powdered using a mini-mill grinder (Fritsch GmbH., Rhineland-Palatinate, Germany). To develop the extraction method, some hair samples were selected and a composite hair sample was used. All collected hair samples were analyzed using the optimized extraction method and LC-MS/MS.

2.3. Pretreatment procedure

The piece- and powder-form hair samples (0.2 g) were placed in 15 mL PP tubes. All tested hair samples were spiked with 2.5 ng of PFAS internal standard mixture prior to extraction. The internal standard mixture contained two mass-labeled perfluoroalkylsulfonates ($^{18}O_2$-PFHxS and $^{13}C_4$-PFOS) and four mass-labeled perfluoroalkylcarboxylic acids ($^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA and $^{13}C_2$-PFDA). In this research, the concentrations of 11 PFASs (5 perfluoroalkylsulfonates and 6 perfluoroalkylcarboxylic acid) were analyzed based on the internal standard method. Finally, 2.5 ng of recovery standard ($^{13}C_8$-PFOA and $^{13}C_8$-PFOS) was injected before instrumental analysis to confirm the recoveries of the internal standards.

2.3.1. Extraction method test

In this study, four different extraction methods (Method 1: SPE, Method 2: IP, Method 3: combination of Methods 1 and 2, and Method 4: solvent extraction with ENVI-carb clean-up) were applied to two the types of hair sample (piece- and powder-form). The four extraction methods are described in detail below.

Table 1
MRM transitions, internal and recovery standard for the target perfluoroalkyl substances.

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)		Internal standard	Recovery standard
		Ion for quantification	Ion for identification		
PFPeA	263	263	219	$^{13}C_2$-PFHxA	$^{13}C_8$-PFOA
PFHxA	313	269	119	$^{13}C_2$-PFHxA	
PFHpA	363	319	169	$^{13}C_4$-PFOA	
PFOA	413	369	169	$^{13}C_4$-PFOA	
PFNA	463	419	219	$^{13}C_5$-PFNA	
PFDA	513	469	219	$^{13}C_2$-PFDA	
PFBS	299	99	80	$^{18}O_2$-PFHxS	$^{13}C_8$-PFOS
PFHxS	399	99	80	$^{18}O_2$-PFHxS	
PFHpS	449	99	80	$^{13}C_4$-PFOS	
PFOS	499	99	80	$^{13}C_4$-PFOS	
PFDS	599	99	80	$^{13}C_4$-PFOS	