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DEVELOPMENT OF EXTRACTION METHOD FOR THE ANALYSIS OF PERFLUROALKYL SUBSTANCES IN HUMAN HAIR

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1. Introduction

Perfluoroalkyl substances (PFASs), are group of compounds which were registered as persistent organic pollutants (POPs) in 2009. Unlike other POPs, PFASs are both hydrophobic and oleophobic1 and can combine with proteins[2], so the understanding of their fate and mechanism of interaction in the environment and humans have been an increasing concern[3].

For now, many researches on human exposure to PFASs were performed by monitoring of invasive samples like blood. However, blood sampling from human body is very difficult, especially for young generation due to high cost, negative perception and etc. Thus, there is a growing need to use non-invasive samples for human bio-monitoring[4,5]. Among several non-invasive human samples, the hair has been used for assessing human exposure to various compounds such as heavy metals and pesticides. Above all, hair is composed of protein (keratin), so it is similar with blood protein (albumin). However, development of a non-invasive bio-monitoring method for PFASs analysis is still in its infancy.

Therefore, the aim of this study was to develop and validate the extraction method and analysis of PFASs in hair as non-invasive human samples with high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). To develop an optimized extraction method, four different extraction procedures were evaluated with two types of hair samples as hair piece and powdered hair samples. Also based on an optimized method, we investigated the accuracy, precision, instrument detection limits (IDLs) and method detection limits (MDLs).

2. Materials and methods

2.1 Sampling

Individual 47 hair samples from the general adult population of Busan City, South Korea were collected. Hair samples were not contaminated by hair dye and cut as close to the scalp as possible. The study was approved by local medical ethics committee. The samples were stored in polypropylene tube at -20°C until analysis.

2.2 Analysis

Prior to extraction, hair samples were rinsed with HPLC water and acetone and air-dried. In order to compare the extraction efficiency of the various sample forms; dried hair samples were made into small pieces using scissors and powder forms by a mini-mill grinder.

To develop an optimized extraction methods, PFASs were extracted according to the four extraction procedure (Solvent extraction with (i) Solid-phase Extraction (SPE), (ii) Ion-pairing Extraction (IPE), (iii) Combined method (IPE+SPE) and (iv) ENVI-carb clean-up) and analyzed with an Agilent 1200 high-performance liquid chromatography (HPLC) system coupled with an Agilent 6460 electrospray triple quadrupole mass spectrometer (ESI-MS/MS).

2.3 Validation of the method

To obtain and evaluate the extraction efficiency between different samples form and the four extraction procedures, the internal standard method was used. Also accuracy, precision, instrument detection limits (IDLs) and method detection limits (MDLs) of the optimized method were calculated. For accuracy, precision and MDLs calculations, samples spiked with known concentration of the PFASs standards were made and analyzed.

3. Results and discussion

3.1 Optimization of pretreatment method for PFASs in hair

In this study, four different extraction methods (Method 1: Solvent extraction with SPE; Method 2: Solvent extraction with IPE; Method 3: combined method 1 and 2; Method 4: Solvent extraction with ENVI-carb clean-up) with two types of hair samples (piece and powder) were confirmed. All tested hair samples were spiked with 5 ng/mL of 8 PFASs internal standards mixture (MPFAC-MXA, Wellington

laboratories) prior to extraction step. The mixture of internal standard contained two mass-labelled perfluoroalkylsulfonates ($^{18}O_2$-PFHxS and $^{13}C_4$-PFOS) and four mass-labelled perfluoroalkylcarboxylic acids ($^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA and $^{13}C_2$-PFDA). In this research, eleven PFASs (five perfluoroalkylsulfonates and six perfluoroalkylcarboxylic acid) were analyzed, so concentrations of six compounds were measured using each mass-labelled PFASs and other five compounds were selected mass-labelled compound among other six mass-labelled PFASs as appropriate. And 5 ng/mL of recovery standard (2 PFASs recovery standards mixture; M8PFOA [$^{13}C_8$-PFOA] and M8PFOS [$^{13}C_8$-PFOS], Wellington laboratories) were injected before the instrumental analysis to confirm recoveries of internal standard ($^{13}C_8$-PFOA for internal standard of perfluoroalkylcarboxylic acid and $^{13}C_8$-PFOS for internal standard of perfluoroalklysulfonates).
As shown in Figure 1, extraction method 1 and 2 showed good recoveries of internal standards, especially in powdered samples (over 80%). Also, satisfactory range was obtained in method 1. In case of method 2, there was higher recovery of $^{13}C_2$-PFHxA and $^{13}C_2$-PFDA (over 120%). However, method 3 and 4 showed very low recoveries in both powdered and piece samples. In conclusion, extraction method 1 with powdered hair sample is considered the optimal method for PFASs extraction in hair and other tried methods of present research proved to be unsuitable to analysis PFASs in hair.

3.2 Method validation
To evaluate the method accuracy and precision, four same hair samples were analyzed using the optimized extraction method (method 1). Three hair samples were spiked (described in section 2.3) and one was raw sample for confirming background concentration of target PFASs.
The accuracy ranged from 66.4% to 110%. Although the accuracy of only one compound was below 70%, an acceptable result is obtained. All precision values of the optimized method were below 10%, which is satisfactory. Also in this study, IDLs and MDLs of PFASs were confirmed. IDLs were determined by ten calibration standard (0.05 ~ 50 ng/g) and calculated based on a signal to noise ratio of 3:1, and the limits were 0.0143 to 0.0827 ng/g. To determine the MDLs, repeated experiments using seven same hair sample spiked 1 ng of PFASs native standard was conducted and the limit values were 0.114 ~ 0.796 ng/g.

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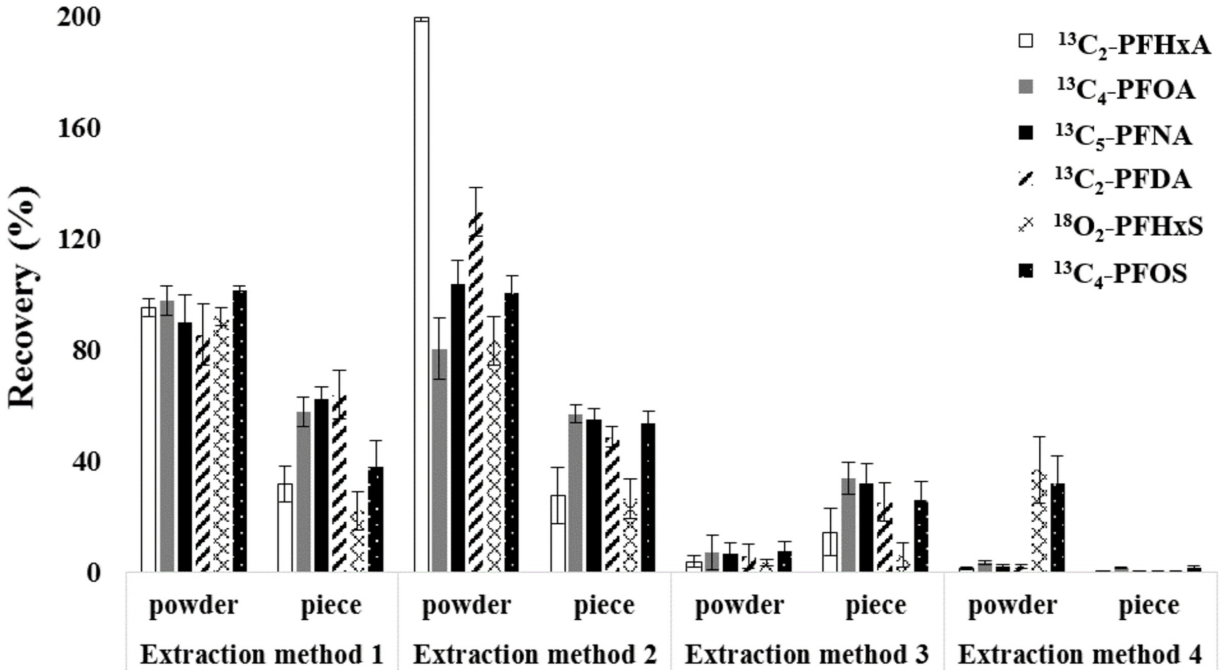
Recovery (%)
200
160
120
80
40
0
$^{13}C_2$-PFHxA
$^{13}C_4$-PFOA
$^{13}C_5$-PFNA
$^{13}C_2$-PFDA
$^{18}O_2$-PFHxS
$^{13}C_4$-PFOS
powder
piece
powder
piece
powder
piece
powder
piece
Extraction method 1
Extraction method 2
Extraction method 3
Extraction method 4