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Development of extraction methods for the analysis of perfluorinated compounds in human hair and nail by high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

Perfluorinated compounds (PFCs) are ubiquitous in the environment and are becoming a public health concern. It is desirable to develop sensitive and accurate methods to measure PFCs in non-invasive matrices such as hair and nail for biomonitoring of body burden. Different extraction methods coupled with solid phase extraction were investigated for extraction efficiency. The extracts were separated, identified and quantified by liquid chromatography-tandem mass spectrometry. Extraction with acetonitrile proved to be the most efficient extraction method for human hair sample, while extraction by methanol with alkaline digestion performed best for human nail sample. The matrix recoveries of the optimized methods ranged from 78% to 116% for hair and from 87% to 126% for nail sample. The ranges of the limit of detection (LOD) were 0.026–0.069 ng/g and 0.023–0.094 ng/g for hair and nail, respectively. These methods were validated by evaluating LOD, accuracy and precision and were proven to be useful for measuring paired human hair and nail samples collected from the general population.

1. Introduction

Perfluorinated compounds (PFCs) have been industrially manufactured since the 1960s and used in a variety of consumer products such as protective coatings for carpets and textiles, surfactants, lubricants and food packaging [1]. The strong carbon–fluorine (C–F) bonds of PFCs make them resistant to hydrolysis, photolysis, metabolism, and biodegradation. Since PFCs have a long biological half-life and are highly bioaccumulative [2,3], they are found in the environment [4], wildlife [5,6], biota [7], and humans [8] throughout the world. Toxicological studies on animals have indicated that two of the most common PFCs, perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), may disturb fatty acid metabolism, affect the reproductive system and induce adverse effects in liver and other tissues [9,10]. Human biomonitoring data is vital for the assessment of human health risk from PFCs exposure.

PFCs do not accumulate preferentially in adipose tissues, but instead bind to proteins [11–13]. For this reason, blood and breast milk have been the most utilized matrices for PFCs biomonitoring in humans. However, sample collection for these two specimens can be challenging and are often unacceptable to participants. Collecting blood is invasive and may have adverse effects on the participants [14], especially for newborns and children. Concentrations of PFCs in breast milk can only provide information concerning the exposure levels of lactating women over a limited age range. Therefore, it is desirable to explore other matrices that are less invasive such as urine, hair and nail. In a previous study, a non-invasive method of measuring PFCs in human urine was developed [15]. However, little data have been reported concerning PFCs in human hair and nail. Hair and nail have been employed as biological matrices to assess the concentration of heavy metals [16,17], drugs [18,19] and organic pollutants [20] in humans. Hair and nail provide numerous advantages for human biomonitoring, such as painless collection, low cost, and easy transport and storage. They also potentially offer information regarding short- and long-term exposure to contaminants as well as temporal exposure patterns through segmental analysis [21]. Therefore, both hair and nail can

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potentially be useful matrices for the biomonitoring of PFCs in the general population.

The purpose of this study was to develop and validate methods for the determination of PFCs in human hair and nail samples. Multiple extraction methods including accelerated solvent extraction (ASE), acid digestion, alkaline digestion, and organic solvents extraction followed by solid phase extraction (SPE) were examined for the analysis of PFCs in these matrices. The hair and nail samples collected from individuals with no occupational exposure to PFCs were chosen for the evaluation of the extraction efficiency. Finally, the developed methods were applied to measure PFCs in 15 paired human hair and nail samples collected from the general population of China.

2. Experimental

2.1. Chemicals

Eight PFCs were analyzed in this study, the standard solutions containing perfluorohexanesulfonate (PFHxS), PFOS, perfluorohexanoic acid (PFHxA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), and perfluorododecanoic acid (PFDoA) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada) with chemical purities of ≥98%. Sodium perfluoro-1-hexane [$^{18}O_2$] sulfonate ($^{18}O_2$-PFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate ($^{13}C_4$-PFOS), perfluoro-*n*-[1,2-$^{13}C_2$] hexanoic acid ($^{13}C_2$-PFHxA), perfluoro-*n*-[1,2,3,4-$^{13}C_4$] octanoic acid ($^{13}C_4$-PFOA), perfluoro-*n*-[1,2,3,4,5-$^{13}C_5$] nonanoic acid ($^{13}C_5$-PFNA), perfluoro-*n*-[1,2-$^{13}C_2$] decanoic acid ($^{13}C_2$-PFDA), perfluoro-*n*-[1,2-$^{13}C_2$] undecanoic acid ($^{13}C_2$-PFUdA), perfluoro-*n*-[1,2-$^{13}C_2$] dodecanoic acid ($^{13}C_2$-PFDoA) were used as internal standards, and also purchased from Wellington Laboratories Inc. (Guelph, Canada) with chemical purities of ≥98% and isotopic purities of ≥99% (^{13}C) and >94% (^{18}O). High performance liquid chromatography (HPLC) grade methanol and acetonitrile (ACN) were purchased from Fisher Scientific (USA), HPLC grade acetone was supplied by J.T. Baker (Phillipsburg, USA) and Milli-Q water was used throughout the study. Tetra-*n*-butylammonium hydrogen sulfate (TBA) was purchased from J&K Chemical company, and methyl-tert-butyl ether (MTBE) was purchased from Sigma–Aldrich (St. Louis, MO). HPLC grade ammonium acetate and formic acid were purchased from Dikma Pure (Richmond Hill, USA), and analytical grade sodium carbonate and guarantee grade sodium hydrogen carbonate (99%) were purchased from the Jinke Institute of Fine Chemicals (Tianjin, China).

2.2. Samples collection and preparation

The hair and nail samples were collected from the general population of the Shanxi province of China. Individual informed consent was obtained and the study was approved by the Research Ethics Board of National Institute of Nutrition and Food Safety. The hair and nail samples were not contaminated by hair dye or nail polish. Hair samples were cut as close to the scalp as possible. Nail samples were obtained by cutting overhang of the nail plate using cosmetic nail clippers. Hair and nail samples were stored in 50 mL polypropylene (PP) centrifuge tubes at room temperature until analysis.

To remove the external contamination, about 20 mL of water was added to each of the individual hair or nail sample and let soaked for 10 min to remove any surface dirt that may have interfered with the analysis. The water was then discarded and the samples were washed twice with acetone, and then air-dried. It has been reported that the analysis of samples on a powdered state increases the efficiency of extraction [22,23]. In order to compare the extraction efficiency of the hair and nail samples in two different forms, the dried samples were divided into two portions. One portion was cut into small pieces (3–5 mm) and the other portion was ground to powder using a Mini-mill Grinder (FRITSCH, "Pulverisette 23", Germany).

2.3. Evaluation of sample extraction methods

The efficiencies of different extraction methods were compared using parallel samples without spiking (triplicate analysis for each method).

2.3.1. ASE extraction

A 0.1 g sample of hair or nail powder was weighed and homogenized with 6 g florisil. It was believed that the dispersion of the sample over a large surface area would result in better extraction efficiency. The sample mixture was transferred to an extraction cell (33 mL). After addition of 5 μL of the internal standard solution (100 pg/μL for each labeled compound), extraction was performed using an ASE 300 system (Dionex Corporation, Sunnyvale, CA, USA). The ASE 300 was operated using the following conditions: preheat – 6 min, heat – 8 min, static – 10 min, flush% – 60% (volume), purge – 1 min, pressure – 1500 psi, temperature – 150 °C, solvent – 100% methanol. The final extract was concentrated to 1 mL under a stream of high-purity nitrogen, and then made up to 10 mL with water prior to SPE cleanup.

2.3.2. Water extraction with acid digestion

An acid digestion method previously used for extracting PFCs from bird feathers [24] was adapted for this study. One mL of HNO_3 (69%, w/w) and 5 μL of internal standard solution were added to 0.1 g sample of hair or nail powder in 15 mL PP tube. After 24 h of digestion at room temperature, 10 mL of water was added. The mixture was sonificated and filtered to a 15 mL PP tube using a 0.45 μm nylon filter (Sartorius, Goettingen, Germany) for SPE cleanup.

2.3.3. Methanol extraction with alkaline digestion

Alkaline digestion has been utilized for PFCs extraction from some biological samples such as fish, human blood and beaver liver [25–27]. In our study, 0.1 g sample of hair or nail powder and 5 μL of internal standard solution were put into a 15 mL PP tube. 10 mL of NaOH in methanol (0.05 mol/L) was added into the tube, and then the mixture was shaken at room temperature for 8 h. The mixture was further digested for 30 min at 55 °C, and then was centrifuged (9384 × *g*) for 15 min. The supernatant was transferred into a clean 50 mL PP tube. Then the residue was further extracted with 10 mL methanol. The two extracts were combined and concentrated to near dryness under a stream of high-purity nitrogen. Milli-Q water was added to the concentrated extract to achieve a final volume of 10 mL before SPE cleanup.

2.3.4. Organic solvents extraction

To evaluate the efficiency of different solvents for extracting PFCs from hair or nail samples, 10 mL of the organic solvent (methanol or 2% formic acid in methanol or acetonitrile) was added to 0.1 g sample of the hair and nail powder spiked with 5 μL of internal standard solution in a 15 mL pre-washed PP tube, respectively. The mixture was sonicated at 55 °C for 2 h. The extract was then centrifuged (9384 × *g*) for 25 min and the supernatant was transferred to a 50 mL PP tube. The extraction process was repeated three times. The combined extract was concentrated to near dryness under a stream of high-purity nitrogen. Milli-Q water was added to the concentrated extract to achieve a final volume of 10 mL before SPE cleanup.

Table 1
The mass condition for compounds.

Compound	Mass transition (m/z)	Cone voltages (V)	Collision energies (eV)
PFHxA	313 → 269	15	12
MPFHxA	315 → 270	15	12
PFOA	413 → 369	15	10
MPFOA	417 → 372	15	10
PFNA	463 → 419	13	12
MPFNA	468 → 423	13	12
PFDA	513 → 469	15	10
MPFDA	515 → 470	15	10
PFUdA	563 → 519	15	10
MPFUdA	565 → 519	13	13
PFDoA	613 → 569	13	13
MPFDoA	615 → 569	15	14
PFHxS	399 → 80	50	35
	399 → 99		30
MPFHxS	403 → 84	50	35
	403 → 103		35
PFOS	499–80	50	48
	499–99		35
MPFOS	503 → 80	50	48
	503 → 99		35

2.4. Cleanup method for extracts

Oasis WAX (150 mg, 6cc) cartridges (Waters Corporation, Milfor, MS, USA) have been used as a clean-up step for PFCs analysis in our previous study [28]. Briefly, the cartridge was preconditioned with 6 mL of 9% ammonium hydroxide in methanol, followed by 6 mL of methanol, and 6 mL of water. The sample (extract) was loaded onto the cartridge and then washed with 2 mL of 2% formic acid in water and 2 mL of 50:50 2% aqueous formic acid solution: methanol. The target compounds were eluted with 3 mL of 9% ammonium hydroxide in methanol and evaporated to dryness. A solution of methanol/water (50:50) was used to reconstitute the sample to a final volume of 200 μL. Particulate matter in the final solution was removed by filtration using 0.2 μm nylon syringe filter (Sartorius, Goettingen, Germany).

2.5. Instrumental analysis

The samples were analyzed using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Separation of analytes was performed by a Waters Acquity UPLC system (Waters, Milford, MA). A 20 μL aliquot of the sample extract was injected using a full loop injection onto a 2.1 × 50 mm BEH C_{18} column (1.7 μm; Waters, USA) heated to 50 °C. A gradient program was employed using 2 mM aqueous ammonium acetate solution and methanol mobile phases and a flow rate of 0.4 mL/min. The gradient started at 20% methanol followed by a 5 min ramp to 90% methanol. At 5.1 min, the ramp was increased to 100% methanol and this was maintained until 6 min. The method then reverted back to initial conditions and a 3 min stabilization time was maintained before the next injection. The triple quadrupole mass spectrometer was operated in the negative electrospray (ESI) mode with multiple-reaction-monitoring (MRM). The mass conditions including the mass transition, cone voltage and collision energy are listed in Table 1.

2.6. Method validation

Linear calibration curves were established using standard solution consisting of a concentration series of 50, 100, 200, 1000, 10 000 and 20 000 pg/mL for each analyte. The matrix matched calibration solution was not needed because the isotope-labeled surrogate was used as internal standard for each target PFC.

The limit of detection (LOD) was defined according to the U.S. Environmental Protection Agency (EPA) Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11) [29] with minor modification. Seven replicates of hair or nail sample spiked at 0.1 ng/g were analyzed using the developed methods to calculate the LOD for each PFC in hair or nail sample. However, PFOA and PFOS were always found in the collected samples, which made it difficult to prepare real blank samples. Therefore the LODs of PFOA and PFOS were estimated from unspiked hair or nail samples with appropriate concentrations. The limit of quantification was mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection [30].

Since certified reference material is currently not available for PFCs in hair and nail, the accuracy and precision of the methods were checked by using the human hair and nail powder samples spiked with the known amounts of PFCs before extraction. The hair or nail powder sample (0.1 g for each) was weighed into a 15 mL PP tubes and spiked with the native standard solutions at two spiking levels (1 ng/g and 10 ng/g). The samples were then left to sit at ambient temperature for 24 h. The spiked samples were subsequently processed using the optimized extraction methods. The extracts were cleaned up and analyzed according to Sections 2.4 and 2.5. Six replicates of sample preparation and analysis were performed for intraday repeatability and four replicates were for interday precision. The amount of detected PFCs (PFOA and PFOS) in the unspiked sample was subtracted for recovery calculation.

2.7. Quality assurance

All accessible polytetrafluoroethylene (PTFE) components were removed from the instruments and related apparatus to minimize the background signal arising from contamination. Furthermore, two tandem C_{18} guard columns were inserted between the pump and injector to separate target PFCs in the sample from contamination originating from the LC system [31]. No PFC contamination was found in the chemical reagents used in this analysis. Procedural blank tests using Milli-Q water were conducted along with each batch of samples. The syringes and filters used during sample preparation were all washed with methanol before use.

ASE or Pressurized liquid extraction (PLE) have already been utilized for extracting PFCs from abiotic and biotic matrices (e.g. dust [32], articles of commerce [33] and fish [34]). However, in the present study, the results obtained from the blank tests indicated that the ASE apparatus was a source of contamination for PFOA (100 pg), PFNA (50 pg), PFHxA (72 pg), PFDA (85 pg) and PFUdA (37 pg). This might be due to the presence of Teflon parts in the ASE instrument. Moreover, in our preliminary examination of this method, the concentration obtained for PFOS (0.31 ng/g) in a hair sample was comparable to a simple methanol extraction (0.39 ng/g). This indicated that normal methanol solvent extraction can achieve similar extraction efficiency to ASE for the matrices. Therefore, ASE extraction was not chosen as the optimal sample preparation method for our matrices of interest.

3. Results and discussion

3.1. Solvents selection for the removal of surface contamination

The removal of surface contamination is related to the selection of the cleaning solvents and washing conditions [35]. The cleaning solvents should remove external contamination as much as

Table 2
The average concentration of PFCs (ng/g) detected in hair and nail pieces and powder samples.

Compound	Hair sample		Nail sample	
	Pieces	Powder	Pieces	Powder
PFHxA	n.d.	n.d.	n.d.	0.45 (17)
PFOA	0.81 (7.2)	0.92 (12)	0.14 (15)	0.18 (8.8)
PFNA	0.24 (6.9)	0.26 (7.1)	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.
PFUdA	0.34 (8.0)	0.32 (9.3)	n.d.	0.26 (6.1)
PFDoA	n.d.	0.26 (2.2)	n.d.	n.d.
PFHxS	n.d.	0.19 (9.9)	n.d.	n.d.
PFOS	0.84 (4.8)	0.98 (13)	0.77 (19)	0.86 (11)

RSD% are given in parentheses ($n = 3$). n.d.: not detected.

possible, but not destroy the matrix of samples or remove analytes of interest [36]. In general, two types of wash solutions have been widely used for removing external contaminations: solutions of surfactants in water (e.g., 0.1–5% solution of sodium dodecyl sulfate; Triton-X 100 [37]), hydrophilic organic solvents such as methanol [19] and acetone [38]. In this study, we selected hydrophilic organic solvents. Methanol was not a suitable choice because it can extract the target compounds from different matrices [28,38–41]. Acetone has been reported to be an inefficient solvent for extracting PFCs [39]. Therefore, the hair and nail samples were first washed with water and subsequently washed twice for 10 min with acetone in an ultrasonic bath. After cleaning, the collected acetone was analyzed for all analytes, and no PFC was detected.

3.2. Comparison of extraction efficiency for samples in pieces (3–5 mm) and powder form

To effectively extract PFCs from human hair and nails, two different forms of samples were evaluated by extraction with methanol that has been used for PFCs extraction [38]. The results are given in Table 2. For the hair samples, six PFCs were detected in powder form, while only four PFCs were detected in hair pieces (3–5 mm). The concentrations determined for the hair pieces were also lower than those in the hair powder samples for all of the detected PFCs except PFUdA. Similarly, four PFCs were found in nail powder, while only two PFCs were detected in nail pieces. The results suggested that the use of the powdered samples provided better extraction efficiency. Therefore, the powdered hair and nail samples were chosen in the present study.

3.3. Comparison of extraction efficiency for different extraction methods

Based on the number and amount of detected PFCs from the unspiked nail and hair samples, the optimal methods were selected for nail and hair, respectively.

3.3.1. Extraction for hair sample

The results of the different extraction methods for the hair powder samples are shown in Table 3. The organic solvent extraction was found to be the most efficient in extracting PFCs from hair samples. Both the acid and alkaline digestions proved to be unsuitable for extracting PFCs from hair samples. This may be due to interferences released when the acid or base destroyed the biomineral structure of the hair samples. When comparing different organic solvents, seven PFCs were detected in the ACN extracts, which was found to be superior over both methanol (4 PFCs detected) and 2% formic acid in methanol (6 PFCs detected).

3.3.2. Extraction for nail sample

Results of the nail sample extractions using different methods are presented in Table 4. The acid digestion was found to be unsuitable for extracting PFCs from nail samples as no PFC was detected using this method. Using alkaline digestion, 2–3 fold higher amounts of detected PFCs were extracted from nail samples than those using organic solvent extractions (2% formic acid in methanol, ACN). The concentrations of detected PFCs obtained by methanol extraction were also lower than those of the alkaline digestion for all of the PFCs except PFUdA. The alkaline digestion followed by methanol extraction was determined to be the optimal extraction method for PFCs in nail samples.

Table 3
The average concentrations of PFCs in hair sample with different extraction methods (ng/g).

Compound	Acid digestion	Alkaline digestion	Organic solvents		
			Methanol	2% formic acid in methanol	ACN
PFHxA	n.d.	n.d.	n.d	n.d	n.d
PFOA	n.d.	n.d.	0.39 (13)	0.58 (8.3)	0.88 (11)
PFNA	n.d.	n.d.	n.d.	0.12 (12)	0.27 (10)
PFDA	n.d.	n.d.	n.d.	n.d.	0.18 (15)
PFUdA	n.d.	n.d.	n.d.	0.23 (11)	0.31 (2.3)
PFDoA	n.d.	n.d.	0.11 (6.8)	0.12 (9.4)	0.20 (3.8)
PFHxS	n.d.	n.d.	0.10 (9.9)	0.19 (16)	0.14 (1.0)
PFOS	n.d.	n.d.	0.34 (26)	0.39 (11)	0.90 (3.8)

RSD % are given in parentheses ($n = 3$). n.d.: not detected.

Table 4
The average concentrations of PFCs in nail sample by different extraction methods (ng/g).

Compound	Acid digestion	Alkaline digestion	Organic solvents		
			Methanol	2% formic acid in methanol	ACN
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	0.19 (8.9)	0.15 (10)	0.12 (9.8)	0.08 (4.0)
PFNA	n.d.	0.25 (3.0)	0.19 (21)	0.24 (20)	0.14 (3.1)
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.
PFUdA	n.d.	0.11 (14)	0.13 (4.6)	0.08 (15)	0.06 (19)
PFDoA	n.d.	0.14 (9.9)	0.14 (14)	0.08 (9.4)	0.05 (4.8)
PFHxS	n.d.	0.30 (5.7)	0.20 (12)	n.d.	n.d.
PFOS	n.d.	0.50 (8.3)	0.36 (12)	0.27 (17)	0.15 (22)

RSD% are given in parentheses ($n = 3$). n.d.: not detected.

Table 5
Linearity, LOD and LOQ of PFCs in hair and nail (ng/g).

Compound	R^2	Hair		Nail	
		LOD	LOQ	LOD	LOQ
PFHxA	0.993	0.069	0.22	0.045	0.143
PFOA	0.998	0.034	0.108	0.043	0.137
PFNA	0.997	0.038	0.121	0.023	0.073
PFDA	0.995	0.057	0.181	0.074	0.235
PFUdA	0.990	0.062	0.197	0.041	0.130
PFDoA	0.990	0.045	0.143	0.05	0.159
PFHxS	0.995	0.058	0.185	0.094	0.299
PFOS	0.999	0.026	0.083	0.048	0.153

3.4. Linearity, LOD and limit of quantification (LOQ) for the optimized extraction methods

The standard calibration curves showed strong linearity with high correlation coefficients as shown in Table 5.

The LODs were calculated from replicates of spiked hair or nail sample (0.1 ng/g) except for PFOA and PFOS. It is difficult to get real blank sample for PFOA and PFOS because of their ubiquitous exposure in human. The samples with appropriate levels of PFOA and PFOS were used. The concentrations of PFOA and PFOS in hair sample were 0.19 ng/g and 0.28 ng/g. For nail sample the concentrations were 0.13 ng/g for PFOA and 0.29 ng/g for PFOS, respectively. The chromatograms of experiments for LOD calculation are presented in the supporting information. The estimated LODs and LOQs are presented in Table 5. The LODs ranged from 0.026 ng/g to 0.069 ng/g for hair sample and from 0.023 ng/g to 0.094 ng/g for nail sample.

3.5. Accuracy and precision

The accuracy and precision of the methods were examined by analyzing hair and nail samples ($n = 6$ for intraday test, $n = 4$ for interday test) spiked at two different concentration levels (1 ng/g and 10 ng/g). The average recoveries for PFCs at different concentrations are shown in Table 6. For hair samples, the results showed that all obtained recoveries ranged from 78% to 116%. The range for recoveries in nail sample is from 87% to 126%. The repeatability given as intraday RSD and interday RSD is shown in Table 6. For lower concentration, the RSDs were higher in both hair and nail samples. The highest RSD was 18.5% for PFHxA in the hair samples at the concentration of 1 ng/g.

3.6. Application of the optimized methods for the analysis of PFCs in nail and hair samples

To investigate the applicability of the extraction methods for the analysis of PFCs, 15 paired nail (N1–N15) and hair (H1–H15) samples collected from the general population were analyzed. The results are summarized in Table 7. For nail samples, eight PFCs (PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFHxS and PFOS) were detected. PFOA, PFHxS and PFOS were detected in all nail samples. The concentration ranges of PFOA, PFHxS and PFOS were <LOQ–0.43 ng/g, 0.36–2.79 ng/g and 0.15–5.09 ng/g, respectively. As shown in Table 7, five PFCs were detected in hair samples. PFOA and PFOS were detected in all hair samples. The concentration ranges of PFOA and PFOS were <LOQ–1.68 ng/g and <LOQ–6.74 ng/g, respectively. Figs. 1 and 2 show the detected PFCs from a hair sample (H3) and a nail sample (N3), respectively. In H3, five PFCs were detected to be above the LODs, and the concentrations of detected PFCs ranged from <LOQ to 2.24 ng/g. In N3, the concentrations range of eight PFCs detected was from 0.17 ng/g to 2.02 ng/g.

In conclusion, an optimized ACN extraction method has been developed for analysis of PFCs in hair. PFCs in nail can be extracted efficiently by the developed method using alkaline digestion. These

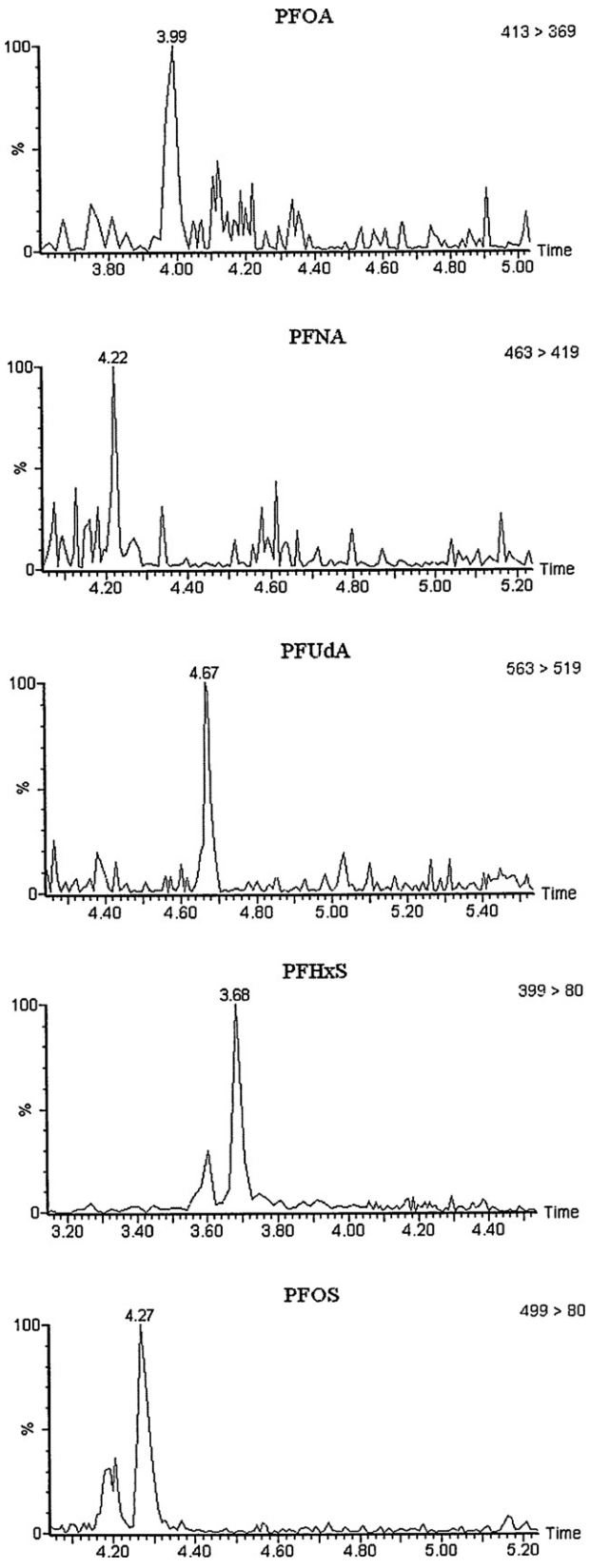

Fig. 1. Chromatograms of detected PFCs in a hair sample.

Table 6
Recovery (%) and RSD (%) for spiked sample ($n = 6$).

Compound	Hair sample								Nail sample							
	1 ng/g				10 ng/g				1 ng/g				10 ng/g			
	Intraday		Interday		Intraday		Interday		Intraday		Interday		Intraday		Interday	
	Rec.	RSD	Rec.	RSD	Rec.	RSD	Rec.	RSD	Rec.	RSD	Rec.	RSD	Rec.	RSD	Rec.	RSD
PFHxA	110	18.1	99	18.5	110	12	114	9.7	111	4.6	113	11.1	91	2.4	97	6.6
PFOA	111	5.8	110	14.3	102	8.1	106	12	112	5.4	103	15.1	94	3.4	98	8.4
PFNA	108	11.0	98	15.3	110	4.3	79	7.6	103	3.1	95	10.2	94	4.2	91	8.6
PFDA	112	13.3	102	17.8	96	6.7	115	5.5	104	6.9	92	12.5	105	5.2	93	4.6
PFUdA	90	8.9	87	18.3	95	6.4	78	12.2	99	2.6	126	9.2	97	5.1	93	10.1
PFDoA	101	6.5	96	14.2	106	8.5	91	13.4	104	3.6	89	8.5	96	5.2	87	6.4
PFHxS	114	12.5	114	14.1	116	8.5	96	9.7	91	11	114	17.7	103	8.9	94	8.3
PFOS	110	4.4	96	8.9	102	6.8	107	10.2	98	4.5	108	10.1	95	8.0	103	9.2

Rec.: recovery, $n = 6$ for intraday, $n = 4$ for interday.

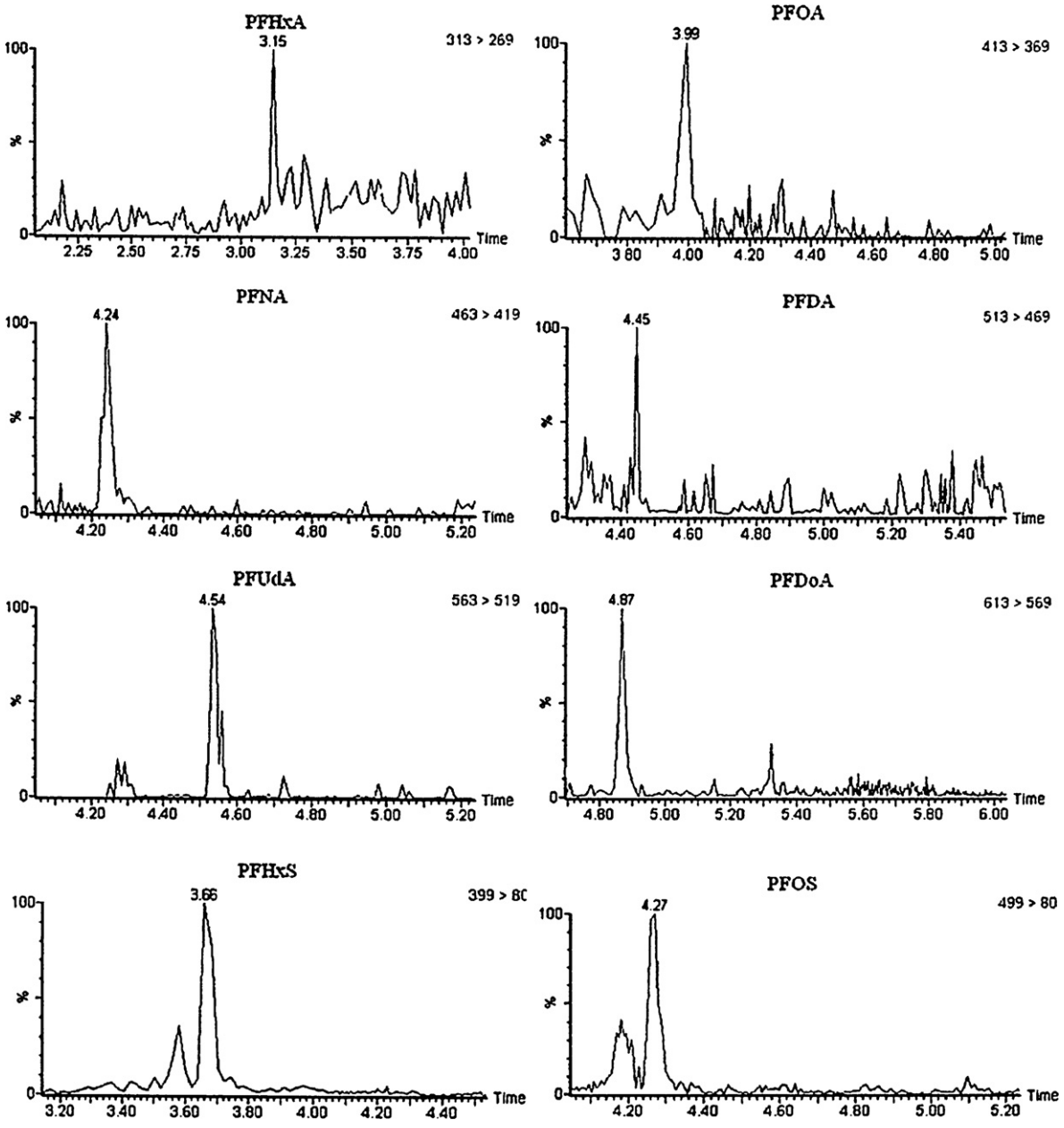

Fig. 2. Chromatograms of detected PFCs in a nail sample.

Table 7
The summary of the PFCs in human hair and nail samples (ng/g).

Compound	Hair sample		Nail sample	
	Detected frequency	Range (ng/g)	Detected frequency	Range (ng/g)
PFHxA	0/15	n.d.	6/15	n.d.–0.55
PFOA	15/15	<LOQ–1.68	15/15	<LOQ–0.43
PFNA	5/15	n.d.–0.58	7/15	n.d.–0.18
PFDA	0/15	n.d.	5/15	n.d.–0.29
PFUdA	6/15	n.d.–1.93	11/15	n.d.–0.43
PFDoA	0/15	n.d.	10/15	n.d.–0.39
PFHxS	9/15	n.d.–2.24	15/15	0.36–2.79
PFOS	15/15	<LOQ–6.74	15/15	0.15–5.09

n.d.: not detected.

methods were validated by evaluating LODs, LOQs, precisions and recoveries. The applicability of the methods was also evaluated with the analysis of paired human hair and nail samples collected from general population. To our knowledge, this is the first comprehensive report of the extraction methods for the analysis of PFCs in human hair and nail. These sensitive methods will be helpful for future biomonitoring studies of PFCs exposure.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.11.015.

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