Development of an Analytical Method for Perfluorochemicals in Human Plasma and Blood by Liquid Chromatography-Tandem Mass Spectrometry Coupled with Solid-Phase Extraction Using a Column-Switching Technique

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Introduction

Perfluorochemicals (PFCs) are man-made chemicals used as surfactants, polymers, plastic additives, and so on. Their amphiphilic character and their thermal, biological, and chemical stability make them useful for many purposes [1]. On the other hand, evidence of toxic effects and environmental pollution was reported and discussed [2]. Previous reports for the measurement of PFCs in human blood samples were performed by liquid chromatography-mass spectrometry (LC/MS) and LC/MS/MS with liquid-liquid extraction or solid-phase extraction [3-5]. However, these methods sometimes required a complicated process for extraction, clean-up and concentration of these compounds. Therefore, we have developed a simple and rapid method for the determination of PFCs such as perfluorooctanesulfonic acid (PFOS), perfluorooctanesulfonamide (PFOSA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) in human plasma and whole blood by liquid chromatography-tandem mass spectrometry (LC/MS/MS) coupled with solid-phase extraction using column-switching. The PFCs in human plasma and whole blood samples can be measured by LC/MS/MS with an online solid-phase extraction, only after deproteination with acetonitrile. The method enables the precise determination of standards and can be applied to the determination of PFOS, PFOSA, PFOA, PFNA and PFDA in human plasma and whole blood for monitoring human exposure.

Materials and methods

PFOS (M.W. 538.23, 98%), PFOA (M.W. 414.07, >90%) and PFNA (M.W. 464.08, 95%) were obtained from FlukaChemie AG, Buchs, Switzerland. PFOSA (M.W. 499.14, 97%) and PFDA (M.W. 514.08, 97%) were purchased from ABCR GmbH & Co. KG, ImSchlehert and Lancaster Co. Inc., Morecambe, England, respectively. Perfluoroheptanoic acid (PFHpA, 99%) and 1H,1H, 2H, 2H-perfluorooctaneslufonic acid (THPFOS, >90%) that had been used as an internal standard were purchased Sigma Aldrich Laboratories, Inc., St. Louis, MO. and SynQuest lab. Inc., America, respectively.

Instrumentation and analytical conditions of column switching LC/MS/MS coupled with solid-phase extraction. Liquid chromatography-tandem mass spectrometry was performed using a Waters Quattro Micro system. Separation was achieved on an Inertsil ODS-3 column (2.1 x 50 mm, 5 um, GL Sciences Inc., Tokyo, Japan) with a Mightysil RP-18 GP pre-column (2.0 x 5 mm, 5 um, Kanto Chemical Inc., Osaka, Japan). The column oven was maintained at 40^{o}C. The column-switching LC/MS/MS coupled with an on-line extraction system consisted of this LC/MS/MS combined with an LC pump (Shimadzu LC-10ADvp pump: Shimadzu, Kyoto, Japan) and Waters Oasis HLB extraction column (20 x 2.1 mm, 25 um). After a 50 ul sample was injected by an auto-sampler, it was loaded onto the extraction column by flowing 50 mM acetate buffer (pH=4.7)/methanol (90/10, v/v) at a flow rate of 1.0 ml/min. The valve was switched 5 min after sample injection. The sample was eluted by back-flashing extraction column to the analytical column and was introduced to MS/MS. The separation was carried out using a mobile phase of 1.0 mM ammonium acetate in water/acetonitrile (v/v) at a flow rate of 0.2 ml/min. The gradient mode was as follows: 5-12 min using a linear increase from 45 to 85% acetonitrile solution, and holding at 85%. The conditions of MS/MS were as follows: the desolvation and source temperatures were set at 350^{o}C and 100^{o}C, respectively; the capillary was held at a potential of 600 V relative to the counter electrode in the negative-ion mode for all compounds. The cone and desolvation gas flow were 50 and 350 l/hr. The cone and collision voltage were 60 and 65 V for PFOS, and 45 and 35 V for PFOSA, 30 and 18 V for PFOA, 30 and 20 V for PFNA, 30 and 22 V for PFDA, 28 and 18 V for PFHpA, and 35 and 37 V for THPFOS.

Sample preparation. The 0.1 ml of human plasma and whole blood samples were added to 0.3 ml of internal standard solution with acetonitrile. The mixed plasma sample was centrifuged at 1450 x g for 10 min and whole blood

sample was centrifuged 10000 x g for 10 min after sonication for 10 min. The supernatant was removed to the polypropylene tube. The filtered sample solution was determined by the column switching LC/MS/MS.

Results and discussions

Column-switching LC/MS/MS coupled with an on-line extraction system. In the mass spectral analysis using MS/MS system, both molecular and fragment ions were observed as the major peaks. The precursor ions were set *m/z* 499 for PFOS, *m/z* 498 for PFOSA, *m/z* 369 for PFOA, *m/z* 419 for PFNA, *m/z* 469 for PFDA, *m/z* 319 for PFHpA and *m/z* 427 for THPFOS, respectively. The product ions were set *m/z* 499→80 for PFOS, *m/z* 498→78 for PFOSA, *m/z* 369→169 for PFOA, *m/z* 419→169 for PFNA, *m/z* 469→169 for PFDA, *m/z* 319→169 for PFHpA and *m/z* 427→81 for THPFOS, respectively. The capillary voltage and mobile phase were optimized by using set monitoring ion. As for the capillary voltage, the peak area became the maximum in 600 V (Figure 1). The effect of the mobile phase ammonium acetate concentration was shown in Figure 2. When the 1.0 mM ammonium acetate was added to mobile phase, the peak area was the maximum.

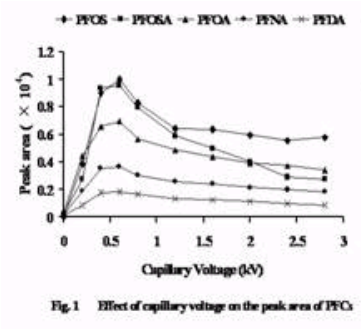

Fig. 1 Effect of capillary voltage on the peak area of PFCs

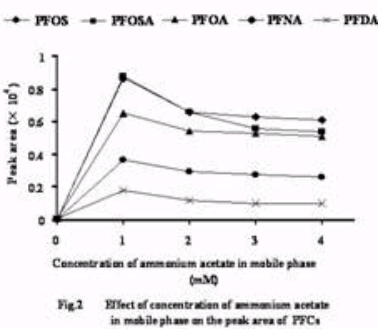

Fig.2 Effect of concentration of ammonium acetate in mobile phase on the peak area of PFCs

Internal standard. Recently, it was recommended that ^{13}C-labeled isotope be used as internal standard for MS. However, obtaining the ^{13}C-labeled isotope of PFCs was very difficult. We evaluated PFHpA and THPFOS as internal standards. PFHpA and THPFOS were evaluated by comparing the PFCs recoveries in human plasma samples spiked with analytes at 10 ng/ml (Figure 3). The recoveries of analytes using THPFOS as internal standard were 43.7 to 53.3%. However, the recoveries of analytes using PFHpA as internal standard were 91.0 to 110%. Therefore, we used PFHpA as internal standard.

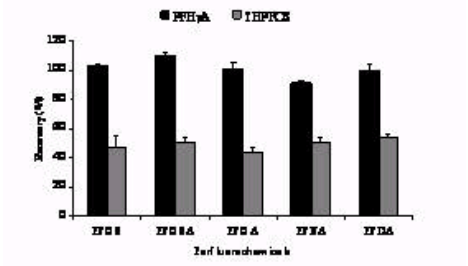

Fig. 3 Recovery levels of PFCs in human plasma spiked the internal standard

Validation and recovery test. The calculated detection limits of PFOS, PFOSA, PFOA, PFNA and PFDA with a signal-to-noise ratio of 3:1 were 0.02, 0.02, 0.025, 0.03 and 0.03 ng/ml, respectively. In addition, the calculated method quantification limits of PFOS, PFOSA, PFOA, PFNA and PFDA were 0.5 ng/ml. The calibration curves (0.5-100 ng/ml) were rectilinear with over 0.999. The average recoveries of PFOS, PFOSA, PFOA, PFNA and PFDA in human plasma were in the range of 93.3 to 105% (RSD: 3.0-8.9%, n=6) (Table 1). Obtaining human whole blood was very difficult. Therefore, horse whole blood was examined instead of human whole blood. The average of recoveries in horse whole blood were in the range of 92.8 to 108% (RSD: 2.5-7.9%, n=6) (Table 2).The method enables the precise determination of standards and can be applied to the determination of PFOS, PFOSA, PFOA, PFNA and PFDA in human plasma and whole blood for monitoring human exposure.

Table 1 Recovery levels of PFOS, PFOSA, PFOA, PFNA and PFDA in human plasma sample spiked with the PFCs and internal standard

Compound	Spiked amount (ng/mL; human plasma sample)	Average recovery(%)	RSD (%)
PFOS	5	99.3	3.0
	50	97.5	6.3
PFOSA	1	98.3	4.2
	10	105	4.2
PFOA	1	100	8.9
	10	97.3	4.8
PFNA	1	98.7	8.4
	10	94.7	3.1
PFDA	1	93.3	8.7
	10	103	4.7
			(n=6)

Table 2 Recovery levels of PFOS, PFOSA, PFOA, PFNA and PFDA in horse whole blood sample spiked with the PFCs and internal standard

Compound	Spiked amount (ng/mL; horse whole blood sample)	Average recovery(%)	RSD (%)
PFOS	5	97.0	2.5
	50	108	4.6
PFOSA	1	92.8	3.3
	10	108	7.1
PFOA	1	101	5.1
	10	96.3	3.7
PFNA	1	101	7.9
	10	106	2.1
PFDA	1	99.5	7.5
	10	108	4.7
			(n=6)

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