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Determination of perfluorooctane sulfonate, perfluorooctanoate and perfluorooctane sulfonylamide in human plasma by column-switching liquid chromatography–electrospray mass spectrometry coupled with solid-phase extraction

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Abstract

We report a method for determining fluorinated organic compounds such as perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorooctanesulfonylamide (PFOSA) in human blood samples by column-switching liquid chromatography–electrospray mass spectrometry. The sample preparation prior to solid phase extraction (Waters Oasis HLB extraction column) involved simply mixing plasma sample with internal standard followed by centrifugation and extraction. The compounds were separated by reversed-phase chromatography with a C_8 column, and detected by mass spectrometry using selected ion monitoring in the negative mode. The average recoveries of PFOS, PFOA and PFOSA ranged from 82.2 to 98.7% (R.S.D.: from 2.0 to 5.2%, $n = 6$). The limits of quantitation of PFOS, PFOA and PFOSA at signal to noise (S/N = 10) were 0.5, 0.5 and 1.0 ng ml^{-1}. The method enables the precise determination of standards and can be applied to the detection of PFOS, PFOA and PFOSA in human plasma samples for monitoring human exposure.

Keywords: Perfluorooctane sulfonate; Perfluorooctanoate; Perfluorooctanesulfonylamide

1. Introduction

Perfluorooctane sulfonate (PFOS) is used in industrial applications as a surfactant. In addition, perfluorooctanoate (PFOA) is used to make Teflon. PFOS and related fluorinated organic compounds have been used since the 1950s in stain resistant coatings for fabrics, carpet, leather and paper products. These structures are shown in Fig. 1. Fluorinated organic compounds such as PFOS, PFOA and perfluorooctanesulfonylamide (PFOSA) have been shown to exist in the environment in river water, marine mammals, fishes and birds [1–5]. In addition, evidence of toxic effects and environmental pollution was reported and discussed [6].

The 3M Company, a major manufacturer of sulfonyl-based perfluoro-compounds, announced the phase-out of production of PFOS-based compounds from December 2000, because of concerns about the persistence of PFOS in the environment and the potential for long-term environmental effects [7]. Therefore, various studies on the distribution of fluorinated organic compounds in the environment and our daily lives are needed for risk assessment. The importance of knowing the sources of human exposure is poorly understood. Recently, environmental pollution by PFOS, PFOA and PFOSA has been reported. Therefore, it is possible that healthy humans are exposed to these compounds via a variety of daily activities. There are, however, few studies in the development and study of analytical method for determination of fluorinated organic compounds in human biological sample. Several methods using gas chromatography with mass

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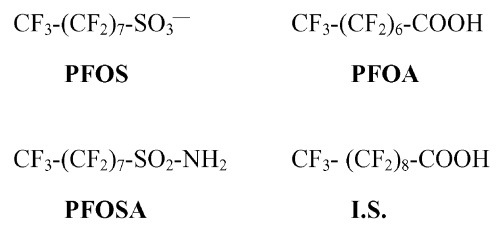

Fig. 1. Structures of analytes and the internal standard. PFOS: perfluorooctane sulfonate, PFOA: perfluorooctanoate, PFOSA: perfluorooctane sulfonylamide, internal standard (IS): perfluorodecanoic acid.

spectrometry, liquid chromatography with mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS/MS) have already been described for the simultaneous determination of PFOS and related compounds [1–5,8–10]. The sample preparation for human blood samples was liquid–liquid extraction [5,11]. However, this may be a complicated process for extraction, clean-up and concentration of these compounds.

Therefore, the development of LC–MS analytical method for the determination of PFOS, PFOA and PFOSA in human biological samples is desired. The novel method described in this paper was successfully used to determinate PFOS, PFOA and PFOSA in human plasma samples from healthy humans.

2. Experimental

2.1. Materials and reagents

PFOS (MW 538.23, 98%), PFOA (MW 414.07, >90%) and PFOSA (MW 199.14, 97%) were purchased from Wako Pure Chemical Inc., Osaka, Japan, Fluka Chemie AG, Buchs, Switzerland, and ABCR GmbH & Co.KG, Im Schlehert. The internal standard (perfluorodecanoic acid) is shown in Fig. 1 and purchased from Lancaster Co. Inc., Morecambe, England. Other reagents and solvents were of HPLC grade and purchased from Wako Pure Chemical Inc., Osaka, Japan. The distilled water purification system was Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solution

Concentrated solutions of each standard were prepared in acetonitrile, and diluted as required by the addition of distilled water with constant amounts of internal standard. The internal standard solution was prepared in acetonitrile.

2.3. Instrumentation and analytical conditions of column-switching LC–MS coupled with solid phase extraction

Liquid chromatography with electrospray mass spectrometry (LC–ESI-MS) was performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). The injection volume was 30 μl. The column used was Inertsil C8-3 (2.1 mm × 100 mm: 5 μm, GL Sciences Inc., Tokyo, Japan) with a Mightysil RP-18 GP pre-column (2.0 mm × 5 mm: 5 μm, Kanto Chemical Inc., Osaka, Japan). The column oven was maintained at 40 °C.

Table 1
Time program of column-switching LC–MS coupled with an on-line extraction condition

Time (min)	Event	Column position (configrations are shown in Fig. 2)	Mobile phase (A–B, v/v)[a]
0.0	Sample injection	Configration A	65:35
5.0	Valve changed gradient	Configration B	65:35
15.0		Configration B	85:15
20.0		Configration A	65:35
30.0	Next analysis		

[a] Pump B solvent: (A) acetonitrile; (B) water.

The working conditions for ESI-MS were as follows: the drying nitrogen gas temperature was set at 350 °C and was introduced into the capillary region at a flow rate of 12 l min^{-1}; the capillary was held at a potential of 3500 V relative to the counter electrode in the negative-ion mode for all compounds. The fragmentor voltages were 220 V for PFOS, 130 V for PFOA, and 170 V for PFOSA during the chromatographic run. When working in the SIM mode, the ions determined were the $[M–K]^-$, $[M–COOH]^-$ and $[M–H]^-$ of PFOS, PFOA and PFOSA. In addition, *m/z* ion of internal standard was designated as the $[M–COOH]^-$ in negative mode.

The column-switching LC–MS coupled with an on-line extraction system consisted of this LC–MS combined with an LC pump (Shimadzu LC-10ADvp pump: Shimadzu, Kyoto, Japan) and Waters Oasis HLB extraction column (20 mm × 2.1 mm, 25 μm). The column-switching LC–MS system, as depicted in Fig. 2, was used for the injection of sample solution. The system program is shown in Table 1. After a sample was injected by an auto-sampler, it was loaded onto the extraction column by flowing water/methanol (90/10, v/v) at a rate of 1.0 ml min^{-1} using pump A for 5 min. After on-line extraction for 5 min, the position of the switching valve was changed. This configuration connected the back-flashing extraction column to the analytical column and the MS detector in the flow path of pump B. After 20 min, the switching valve was returned to its original position. The run time for the assay of the sample mixture was 30 min.

Separation was carried out using a gradient mobile phase of 1.0 mM ammonium acetate in water/acetonitrile (v/v) at a flow rate of 0.2 ml min^{-1}. The gradient mode was as follows: 5–15 min using a linear increase from 65 to 85% acetonitrile solution, and holding at 85%.

In the quantitative procedure, standard solutions of PFOS, PFOA and PFOSA were prepared for the calibration range. Quantitative analysis was performed in the SIM mode in order to maximize sensitivity. PFOS, PFOA and PFOSA concentrations in each sample were calculated relative to the internal standard added to the sample prior to analysis. Calibration

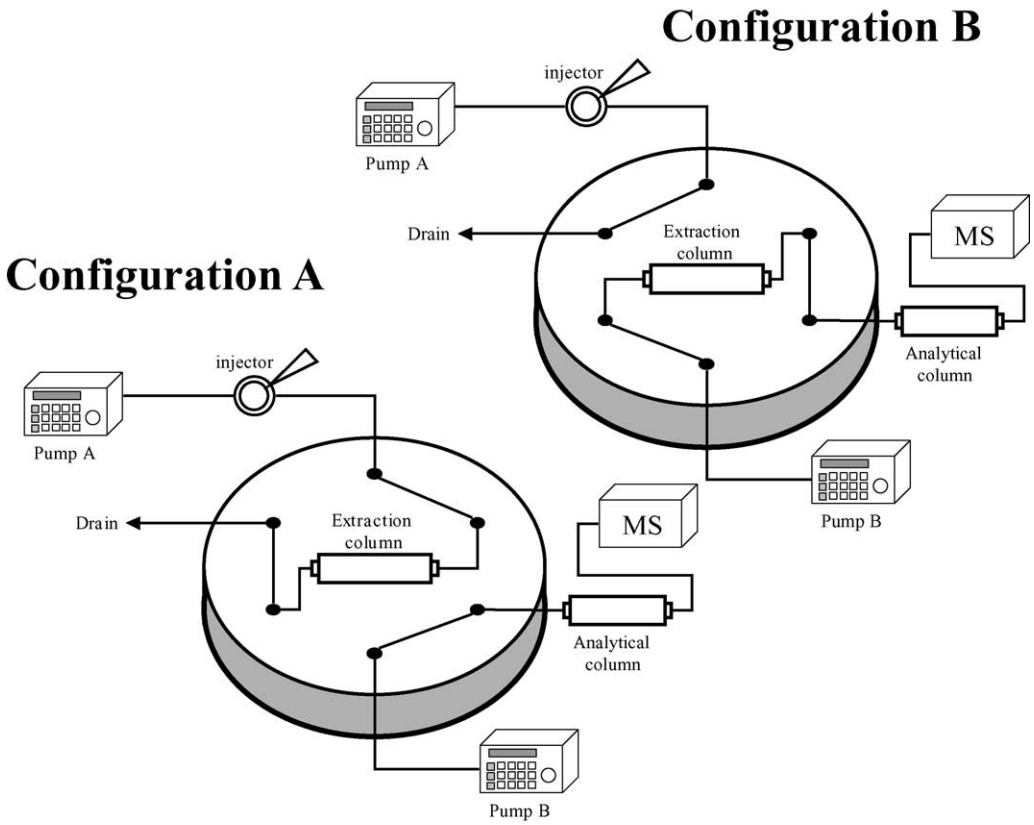

Fig. 2. Schematic representation of the column-switching LC–MS system. LC–ESI-MS was performed using an Agilent 1100 MSD-SL system (Pump B + ESI-MS). Sample preparation system was performed using a Shimadzu LC-10ADvp pump (Pump A). Configuration A: after the sample solution was injected by the auto-sampler, the sample was loaded onto the extraction column for the extraction and cleanup on the on-line column. Configuration B: this configuration connected the back flushing extraction column to the analytical column and MS detector in the flow path of the pump B.

curves of PFOS, PFOA and PFOSA were performed daily for all samples with internal standard.

2.4. Sample preparation

The 0.3 ml of human plasma sample was added to 0.3 ml of internal standard solution. The mixed sample was centrifuged at 3000 rpm (RCF: 1450 × *g*) for 10 min. The top clear layer was removed to the glass tube. This sample solution was filtered (0.45 μm). This solution was analyzed by column-switching LC–MS.

2.5. Qualitative analysis of PFOS and PFOSA standards by LC–MS/MS

Liquid chromatography with electrospray tandem mass spectrometry (LC–ESI-MS/MS) was performed using Waters 2795 and Micromass Quattro micro system (Waters, Tokyo, Japan). The injection volume was 10 μl. The column used was Inertsil ODS-3 (2.1 mm × 50 mm: 3 μm, GL Sciences Inc., Tokyo, Japan). The column oven was maintained at 40 °C. Separation was carried out using a mobile phase of 1.0 mM ammonium acetate in water/acetonitrile (40/60, v/v) at a flow rate of 0.2 ml min^{-1}.

The working conditions for ESI-MS/MS were as follows: the desolvation and source temperatures were set at 350 and 100 °C; the capillary was held at a potential of 2800 V relative to the counter electrode in the negative-ion mode for all compounds. The cone and desolvation gas flows were 50 and 350 l/h. The cone and collision voltages were 55 and 50 V for PFOS, and 40 and 33 V for PFOSA during the scan chromatographic run. When working in the SCAN mode, the ions determined were in the *m*/*z* range from 50 to 600.

2.6. Human plasma samples

Human plasma samples were obtained from 21 healthy volunteers aged 21–56. All samples were stored at −80 °C prior to use.

3. Results and discussion

3.1. Optimization of analytical method for determination of PFOS, PFOA and PFOSA

In the mass spectral analysis using MS system, both molecular and fragment ions were observed as the major

Table 2
Ions monitored (*m/z*) for the determination of analytes and internal standard

Compound	Quantitation ion (*m/z*)	
Perfluorooctane sulfonate (PFOS)	$[M–K]^-$	499
Perfluorooctanoate (PFOA)	$[M–COOH]^-$	369
Perfluorooctane sulfonamide (PFOSA)	$[M–H]^-$	498
Perfluorodecanoic acid (IS)	$[M–COOH]^-$	469

peaks. We examined the optimal ionization for detecting these compounds. The ions monitored for PFOS, PFOA, PFOSA and internal standard are shown in Table 2. The important parameters are the fragmentor voltage and the mobile phase composition. In order to determine the optimum fragmentor voltage for the detection of PFOS, PFOA and PFOSA, the *m/z* signals were plotted against the fragmentor voltage (Fig. 3). The effect of the mobile phase ammonium acetate concentration was investigated (Fig. 4). The main *m/z* signals showed a maximum in 1.0 mM ammonium acetate as

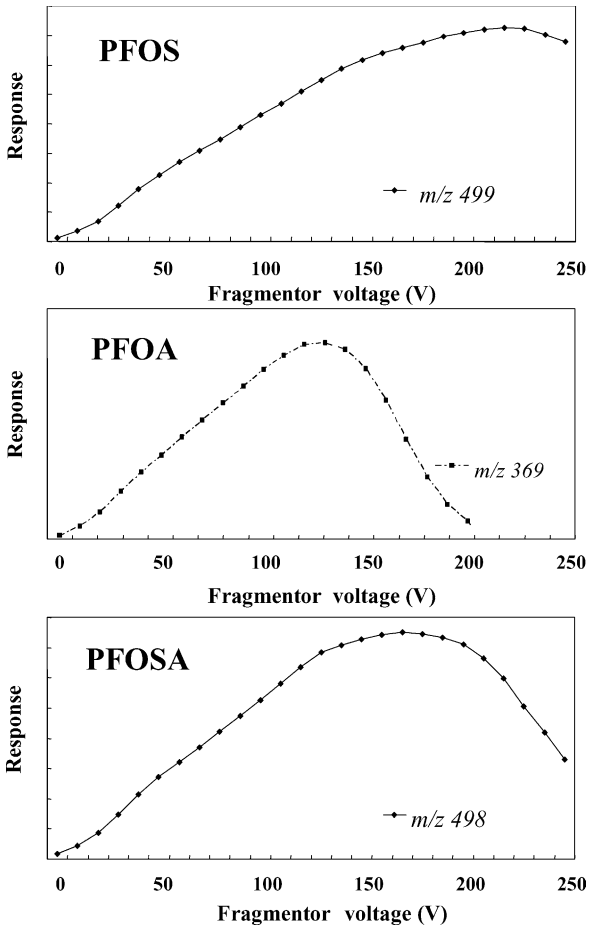

Fig. 3. Effect of fragmentor voltage on the peak responses of PFOS (*m/z* 499), PFOA (*m/z* 369) and PFOSA (*m/z* 498). Analytical conditions: electrospray-MS; drying nitrogen gas temperature set at 350 °C; capillary region flow rate, 12 l min^{-1}; capillary potential 3500 V relative to the counter electrode; negative-ion mode; fragmentor voltage range 0–200 V or 0–250 V; and SIM mode. Carrier solution, 0.1 mM ammonium acetate in water/acetonitrile (40/60, v/v) at a flow rate of 0.2 ml min^{-1}.

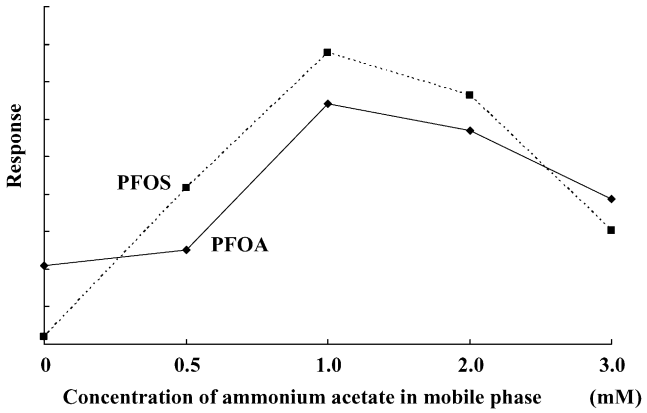

Fig. 4. Effect of mobile phase on the peak responses of PFOS (*m/z* 499) and PFOA (*m/z* 369). Analytical conditions: electrospray-MS (drying nitrogen gas temperature set at 350 °C; capillary region flow rate, 12 l min^{-1}; capillary potential 3500 V relative to the counter electrode; negative-ion mode, fragmentor voltage 220 V (PFOS), 130 V (PFOA); and SIM mode). Carrier solution, 0–3.0 mM ammonium acetate in water/acetonitrile (40/60, v/v) at a flow rate of 0.2 ml min^{-1}.

the mobile phase at 220 V for PFOS ($[M–K]^-$), 130 V for PFOA ($[M–COOH]^-$) and 170 V for PFOSA ($[M–H]^-$).

The calculated detection limits of PFOS, PFOA and PFOSA were 0.05, 0.05 and 0.25 ng ml^{-1}, respectively, for column-switching LC–MS detection with the ratio of the compound's signal of analyte in standard solution to the background noise in standard solution (S/N) = 3 (Table 3). In addition, the limits of quantification calculated when S (signal of analyte in standard solution)/N (background noises of plasma sample used range from 10 to 12 min for PFOS, 12 to 14 min for PFOA and 14 to 16 min for PFOSA) = 10 were 0.5 for PFOS, 0.5 for PFOA and 1.0 ng ml^{-1} for PFOSA in human plasma sample (Table 3).

A peak shoulder appears on the LC–MS chromatograms of PFOS and PFOSA standards analyzed. In a similar method for determination of PFOS in water samples, a shoulder on the peak for PFOS in the LC–MS chromatogram was detected [15]. In this paper [15], the authors explained that the PFOS standard contained impurity isomers of the same alkyl chain lengths. The peak shoulder of PFOS in our method was investigated by LC–MS/MS. Ions of *m/z* 499 ($C_8F_{17}SO_3^-$: primary ion: MS scan mode Fig. 5A) to *m/z* 99 (FSO_3^-: product ion: MS/MS daughter scan mode Fig. 5B) and *m/z* 80 (SO_3^-: product ion: MS/MS daughter scan mode Fig. 5B) were observed in shoulder and main peaks. Therefore, this peak is a PFOS isomer, and thus area counts of the shoulder in the chromatogram of PFOS are integrated for calibration.

Table 3
Validation data of column-swiitching LC–MS system

Compound	Detection limit (S/N = 3) (ng ml^{-1})	Limit of quantitation (ng ml^{-1} plasma sample)	Calibration curve (range: ng ml^{-1}: plasma sample)
PFOS	0.05	0.5	0.999 (0.5–100)
PFOA	0.05	0.5	0.999 (0.5–100)
PFOSA	0.25	1.0	0.999 (1.0–100)

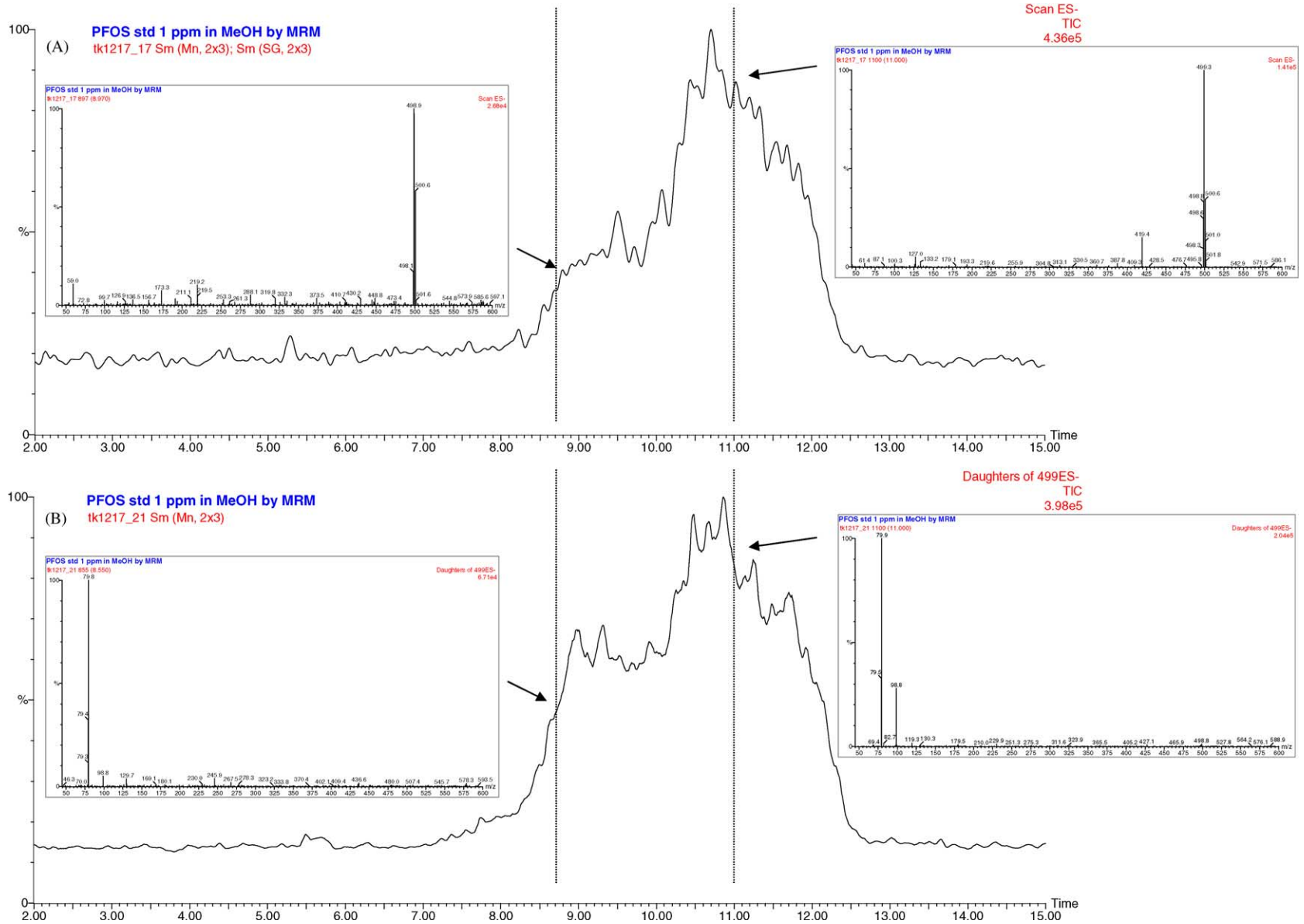

Fig. 5. LC–MS/MS chromatograms and mass spectra of PFOS standard solution, (A) MS scan mode: *m/z* 499 ($C_8F_{17}SO_3^-$: primary ion), (B) MS/MS daughter scan mode: *m/z* 99 (FSO_3^-: product ion) and *m/z* 80 (SO_3^-: product ion).

In the same LC–MS/MS conditions, the shoulder peak of the PFOSA isomer was investigated. The MS/MS spectra of PFOSA shoulder and main peaks show *m*/*z* 498 of primary ion and *m*/*z* 78 of product ion (data not shown). Therefore, the shoulder peak is a PFOSA isomer, and thus the area counts of the shoulder in the chromatogram of PFOSA are integrated for calibration.

In general, it is very important that LC–MS determination of a target compound be accomplished by using stable isotopically labeled internal standards. However, we cannot obtain these internal standard compounds at present. Therefore, a different internal standard is used in this study. For an internal standard, the compound should need the conditions of similar structure, close retention time and easy ionization in the same ionization mode. After investigation of many similar compounds, perfluorodecanoic acid was the most suitable choice. Furthermore, we investigated and evaluate that this internal standard could be used by the following recovery and quality control test, peak area ratios with respect to each internal standard were plotted, and the response was found to be linear over the validated range with correlation coefficients (*r*) higher of 0.999 (Table 3).

3.2. Recovery and quality control from the coupled on-line solid-phase extraction for determination of PFOS, PFOA and PFOSA in plasma samples

We investigated the recoveries of PFOS, PFOA and PFOSA (10 and 100 ng ml^{-1}) in human plasma samples using this method.

To perform recovery tests and quality control, we searched for a blank human plasma sample which was not contaminated by these compounds by using our analytical techniques. PFOS concentrations in human pooled plasma samples available on the market ranged from 45.0 to 52.6 ng ml^{-1} (n = 6). The commonly available human pooled blood samples are thus nor useful for the blank test for recovery and quality control. For that reason, human plasma in a few healthy volunteers was used for blank test with low concentration of PFOS levels (below 10 ng ml^{-1}). We subtracted the background of PFOS concentration from the amount of PFOS in the plasma. Concentrations of these compounds in plasma is adjusted and used for in quality control.

The average recoveries of PFOS, PFOA and PFOSA ranged from 82.2 to 98.7% with R.S.D. < 5.2% (Table 4). In addition, Fig. 6 shows the chromatograms obtained from the recovery test in human plasma samples. Further, a typical daily sample batch of reagent blanks, unknown plasma sample spiked internal standard (n = 8), and plasma sample spiked PFOS solutions (25 ng ml^{-1} concentration in plasma sample with internal standard) were measured for a comparison of with-in run and between run variability (Fig. 7). Quality control materials (spiked samples) were analyzed with each batch of samples on separate days. This material did not deviated from the 99% confidence interval (in this case <UCL = 25.53 ng ml^{-1}, >LCL = 24.17 ng ml^{-1}, Fig. 6B). Therefore, we think that our method can enable precise determination of PFOS, PFOA and PFOSA using the added internal standard, and it was applied to the detection of these compounds in human blood samples.

Table 4
Recovery levels of PFOS, PFOA and PFOS A in human plasma sample spiked with the analytes and the IS

	Spike amount (ng ml^{-1}: plasma samples)	Recovery average (%)	R.S.D. (n = 6) (%)
PFOS[a]	100, 10	95.3, 97.7	3.3, 4.6
PFOA[b]	100, 10	87.2, 82.2	4.0, 2.3
PFOSA[b]	100, 10	95.8, 98.7	5.2, 2.0

[a] Background PFOS levels in the unspiked human plasma were subtracted out of the spiked sample levels to allow easier comparison.

[b] Background PFOA and PFOS A levels in the unspiked human plasma can be neglected.

3.3. Background PFOS, PFOA and PFOSA levels

The analysis of trace levels of PFOS, PFOA and PFOSA in biological samples is complicated by contamination, particularly by leaching from Teflon plastics. Thus, care must be taken to control contamination during experiments and where possible, to eliminate the contamination. Investigations of PFOS, PFOA and PFOSA contamination of the Milli-Q water, plastic tube and LC system gave negative results (<LOQ).

3.4. Measurement of PFOS, PFOA and PFOSA in healthy human plasma samples

We measured the concentrations of PFOS, PFOA and PFOSA in human plasma samples obtained from 21 healthy volunteers. PFOSA could not be detected by this method in any samples (Table 5). On the other hand, PFOS and PFOA were detected (Table 5).

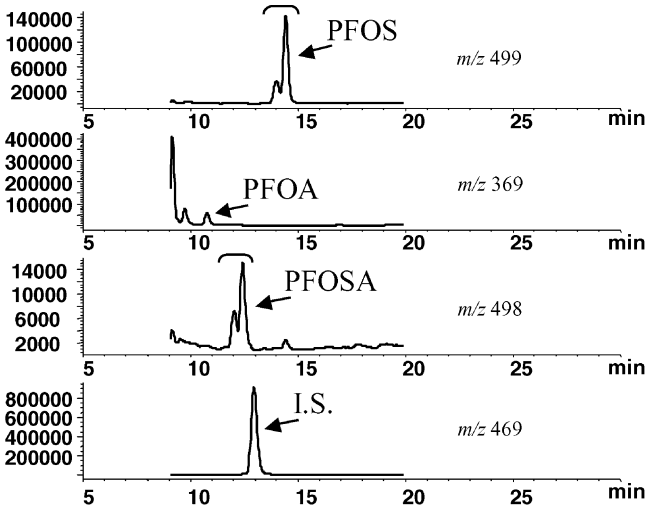

Fig. 6. SIM chromatograms of PFOS, related compounds and internal standard in human plasma sample (recovery test: 10.0 ng ml^{-1}).

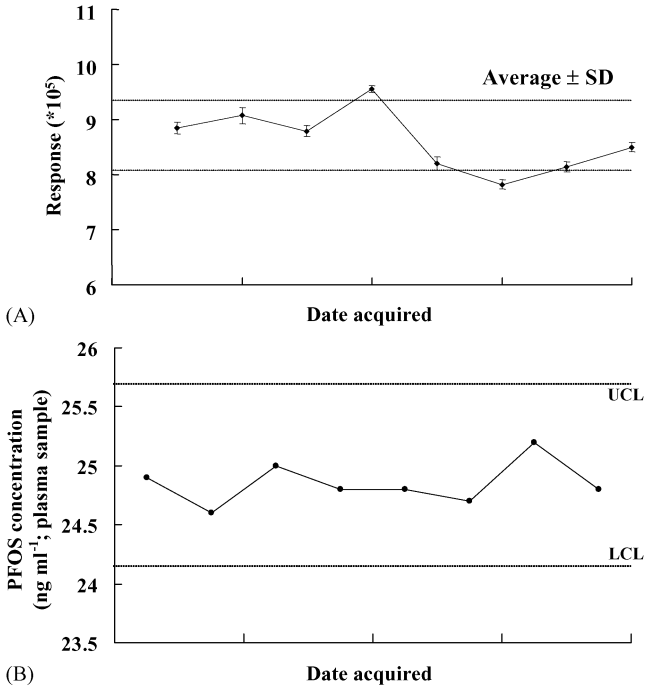

Fig. 7. Quality control plot for PFOS and internals standard levels in plasma sample. (A) Analysis of internal standard (n = 64) on eight separate days. (B) Analysis of PFOS (n = 8) when quality control values dose not deviate outside the 99th confidence interval (in this case <UCL = 25.53 ng ml^{-1}, >LCL = 24.17 ng ml^{-1}).

Table 5
Concentration levels of PFOS, PFOA and PFOSA in plasma samples from healthy volunteers

Volunteer	Sex	Age	Concentration levels (ng ml^{-1})		
			PFOS	PFOA[a]	PFOSA[b]
A	Male	56	31.9	4.1	n.d.
B	Male	49	15.9	n.d.	n.d.
C	Male	22	15.5	1.9	n.d.
D	Female	24	10.8	n.d.	n.d.
E	Female	24	11.6	n.d.	n.d.
F	Female	37	18.3	1.6	n.d.
G	Male	21	17.5	0.7	n.d.
H	Female	21	12.4	n.d.	n.d.
I	Female	21	27.7	3.0	n.d.
J	Male	22	24.6	1.8	n.d.
K	Male	23	15.2	n.d.	n.d.
L	Male	23	18.4	3.9	n.d.
M	Female	21	12.2	0.6	n.d.
N	Female	23	10.4	1.0	n.d.
O	Female	23	19.2	2.7	n.d.
P	Male	24	15.5	1.3	n.d.
Q	Male	32	12.3	3.0	n.d.
R	Male	29	17.9	2.8	n.d.
S	Male	29	19.0	1.4	n.d.
T	Female	25	12.1	n.d.	n.d.
U	Female	22	11.8	1.8	n.d.

[a] n.d. indicates PFOA concentrations lower than 0.5 ng ml^{-1} in samples.
[b] n.d. indicates PFOSA concentrations lower than 1.0 ng ml^{-1} in samples.

We used this method to assess PFOS, PFOA and PFOSA in human plasma samples to provide a reference range. In addition, we evaluated the suitability of the method for detecting PFOS, PFOA and PFOSA levels in human plasma and other biological specimens by analyzing many human samples from a cooperative hospital. Further description of this population and the fluorinated organic compound levels found are discussed in a companion paper [13].

4. Conclusions

In the present study, we developed a method for the simultaneous measurement of PFOS, PFOA and PFOSA in blood samples by column-switching LC–MS coupled with solid phase extraction. Sample preparation prior to the column-switching system included addition of internal standard, pretreatment and centrifugation. The precision was improved by incorporating internal standards. This method will have potential applications in the analysis of human biological samples [13].

In a previous study where the pharmacokinetic behavior and subchronic toxicity of PFOS in monkey was investigated, the elimination half-life from blood was found to be long [12]. In addition, PFOS concentration in a total of 645 adult donor serum samples ranged from the lower limit of quantitation of 4.1–1656 ng ml^{-1} with geometric mean of 34.9 ng ml^{-1} [14]. The results of our experiment clearly show that Olsen et al.'s research regarding the large scale of investigation in American population of PFOS, PFOA and PFOSA can be supported.

Acknowledgments

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