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High Speed Separation of PFCs in Human Serum by C18-Monolithic Column Liquid Chromatography-Tandem Mass Spectrometry

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An analytical method has been developed for the rapid determination of perfluorinated compounds (PFCs) in human serum samples. The extraction and purification of PFCs from human serum were performed by the modified method of previous report. Ten PFCs were rapidly separated within 3.3 min by C18-monolithic column liquid chromatography (LC) and detected by electrospray ionization (ESI) tandem mass spectrometry (MS/MS) in negative ion mode. The runtime of PFCs on monolithic column LC was up to 4-fold faster than that on conventional column LC. The effect of triethylamine (TEA) to the mobile phase has investigated on the overall MS detection sensitivity of PFCs in ESI ionization. Quantification was performed by LC-MS/MS in multiple-ion reaction monitoring (MRM) mode, using ^{13}C-labeled internal standards. Method validation was performed to determine recovery, linearity, precision, and limits of quantification, followed by, the analysis of a standard reference material (SRM 1957 from NIST). The overall recoveries ranged between 81.5 and 106.3% with RSDs of 3.4 to 16.2% for the entire procedure. The calibration range extended from 0.33 to 50 ng mL^{-1}, with a correlation coefficient (R^2) greater than 0.995 and the limits of quantification with 0.08 to 0.46 ng mL^{-1}. This approach can be used for rapid and sensitive quantitative analysis of 10 PFCs in human serum with high performance and accuracy.

Key Words : Perfluorinated compounds, Serum, Monolithic column, LC-MS/MS, Method validation

Introduction

Perfluorinated compounds (PFCs) and related compounds have been produced in significant amounts for decades and are widely used in a variety of chemical industries due to their unique physiochemical properties such as stability, rigidity, and low chemical reaction.[1] PFCs are known to be environmental persistent substances and bioaccumulation and showed adverse health effects as well as environment ecosystem.[2-4] Recently, PFCs have been frequently found at significant exposure level in human blood,[5-7] and serum[8-10] due to their extensive usage as industrial materials. Thus, a rapid analytical method is necessary to perform high-throughput human biomonitoring for determining a general population's PFC exposure level.

Numerous analytical methods have been introduced to measure PFC concentrations with both precision and accuracy in biological and environmental samples. Several analytical tools for measurement of PFCs include liquid chromatography (LC) using spectrophotometeric detection,[11,12] ^{19}F-NMR,[13] gas chromatography-mass spectrometry (GC-MS),[14] and LC-MS[15] or tandem mass spectrometry (MS/MS).[6-10] Among them, a LC-MS/MS based on MRM method has been recommended to minimize interference effects in complex sample matrices, enabling to enhance sensitivity, selectivity, and specificity. LC-tandem mass spectrometry following an appropriate solid-phase extraction (SPE) preparation step has been used to analyze PFCs with promising results. However, when performing repeated analyses, sample throughput was limited by a long LC separation time with a conventional packed column. These complex and time-consuming separation procedures make this technique less than ideal to handle large number of samples for the rapid determination of PFCs in a clinical laboratory. For this reason, noble separation technique using acetonitrile mobile phase and C8 column has been introduced for the determination of PFCs in serum.[10] Recently, ultra-performance liquid chromatography (UPLC) methods providing higher peak capacity, resolution, and sensitivity have been successfully applied for the determination of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and other PFCs in various samples including human blood and environmental samples.[16,17]

Monolithic columns have proven to be a new alternative to conventional packed column for high efficiency separations in LC analysis.[18,19] Due to their small-sized skeletons and wide pore structure, higher separation efficiency can be achieved. Main advantages of monolithic column included the use of a high flow-rate of mobile phase, a low back pressure, and a short time for column equilibration.[20-22] Recently, several reports have described the practical application of monolithic columns towards the rapid determination or separation of several kinds of chemicals.[23,24] However, no previous applications of C18-monolithic column LC-MS/MS method for high speed PFC analysis in serum have been reported up-to-date.

This report describes a high speed separation for the quantification of 10 PFCs in human serum by on a C18-monolithic LC/MS/MS-MRM mode. The effect of triethylamine (TEA) addition in mobile phase has been studied on the

sensitivity and peak resolution of PFCs on a monolithic C18 column. The PFC chromatographic behavior on monolithic column LC was also compared with the conventional packed column LC. The analytical method has been validated by the determination of PFC levels in a standard reference material (SRM 1957) and in quality control samples prepared in our laboratory.

Experimental

Reagents and Chemicals. The PFC standards (PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFHxS, PFHpS, PFOS, and PFNS) were purchased from Aldrich (Steinheim, Germany). PFOS was purchased from Tokyo Chemical Industries (Tokyo, Japan). The isotopically-labeled internal standards, perfluoro-*n*-[1,2,3,4-$^{13}C_4$]octanoic acid ($^{13}C_4$-PFOA) and perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate potassium salt ($^{13}C_4$-PFOS) were purchased from Wellington Laboratories (Guelph, Ontario, Canada) as 50 μg mL^{-1} methanol solutions (1.2 mL). Standard solutions were prepared in acetone at a concentration of 10 μg mL^{-1} and diluted with acetone as necessary.

Organic solvents (methanol, water, and ammonium hydroxide) were of analytical grade and were supplied by J.T. Baker (Phillipsburg, NJ, USA). Formic acid was purchased from Junsei (Chuo-ku, Tokyo, Japan). Methyl-*tert*-butyl ether (MTBE) and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI, USA) and Fisher Scientific (NYSE, TMO), respectively. Ammonium acetate and triethylamine were purchased from Sigma-Aldrich (Steinheim, Germany) and Acros Organics (Geel, Belgium), respectively. All chemicals were tested for the presence of PFCs prior to use by LC/MS/MS. All lab-wares were cleaned with laboratory detergent, sequentially rinsed with distilled water, acetone, and methanol, and baked in an oven at 300 °C prior to use. For sample cleanup, several SPE methods were tested and compared. Oasis HLB (0.5 g), Sep-Pak C18, and Oasis®WAX (weak anion exchanger, 30 mg) cartridges were supplied by Waters (Milford, MA, USA). Before use, all cartridges were checked for PFC contamination due to the presence of polytetrafluoroethylene or perfluoroalkoxy compounds used in labware.

Sample Extraction and Purification. The sample preparation used procedure in this study was slightly modified from the previously reported methods.[25] The human serum sample (0.75 mL) was measured by autopipette, and isotopically labeled internal standards (each 5 μL) were added prior to the precipitation of proteins with acetonitrile (0.75 mL). The resulting mixture was then homogenized with 0.3 mL of 0.1% formic acid in water. The sample was centrifuged at 13,000 rpm for 10 min to separate proteins from the sample matrix. Finally, the sample supernatant was subjected to solid-phase extraction with an Oasis WAX cartridge.

For SPE purification, WAX-SPE cartridges were conditioned with 2 mL of 2% ammonium hydroxide in methanol/MTBE (1:9, v/v) and air-dried for 1 min. Subsequently, 2 mL of 2% formic acid was used to condition the cartridge. After addition of the sample, the WAX-SPE cartridge was washed with 2 mL of 2% formic acid in water, followed by 2 mL of methanol. PFCs were eluted from the anion-exchange sorbent using 2 mL of 2% ammonium hydroxide in methanol/MTBE (1:9, v/v) at a flow rate of 1 mL min^{-1}. The SPE extract was dried under nitrogen gas and reconstituted with 0.5 mL of 10% acetonitrile in water. The eluent was collected in a polypropylene tube to minimize background contamination and stored in a refrigerator prior to analysis. The overall sample preparation scheme is outlined in Figure 1.

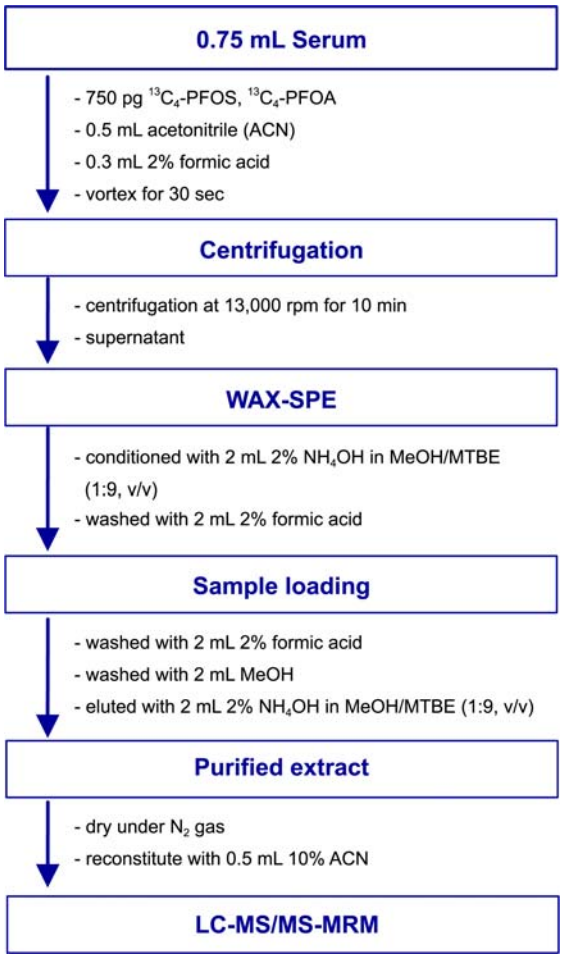

Figure 1. Sample preparation method for the analysis of PFCs in serum sample.

Preparation of Standard Reference Material and Real Samples. Human serum standard reference material (SRM 1957) was obtained from the National Institute of Standards & Technology (Gaithersburg, Maryland, USA). SRM 1957 is intended for the evaluation of analytical methods that determine levels of selected polychlorinated biphenyl congeners, chlorinated pesticides, polybrominated diphenylether congeners, dibenzo-*p*-dioxin and furan congeners and PFCs (PFHpA, PFOA, PFNA, PFDA, PFUnA, PFHxS, and PFOS) in human serum. Human blood samples were collected from volunteers, who lived near urban and industrial areas, and were centrifuged at 13,000 rpm for 10 min to separate cell and coagulation factors from the sample matrix. The obtained serum samples (0.75 mL) were transferred to polypropylene tubes and stored at –20 °C until use.

LC-MS/MS Analysis. Perfluorinated compounds were analyzed by an Agilent 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) coupled directly to a triple quadrupole mass spectrometer (API 3200, MDS Sciex, Concord, ON, Canada). Separations were accomplished on a Chromolith® RP-e18 analytical column (100 mm × 3 mm, 100^{-3} mm) from Merck (Darmstadt, Germany) at room temperature. The mobile phase was composed of 20 mM ammonium acetate and 0.05% triethylamine in water (solvent A) and acetonitrile (solvent B), and it was delivered at a flow rate of 0.6 mL min^{-1}. The linear gradient elution program was as follows: 10% B for 0.5 min, then 10-40% B for 0.1 min, then 40-56% B for 0.9 min, then 56-90% B for 0.3 min followed by an isocratic hold at 90% B for 1.2 min. At 3 min, B was returned to 10% over 0.1 min, and followed by an isocratic hold at 10% B for 1.9 min. The total run time for each injection was 5 min and the injection volume was 10 μL.

The mass spectrometer was operated in negative ion mode with a TurboIonSpray ionization source. Negative electrospray was used as the means of ionization. Instrument control, data acquisition, and data analysis were carried out with Analyst 1.5 software (Applied Biosystems/MDS Sciex). Nitrogen was used as the collision gas. The mass spectrometer was optimized while in multiple reaction-monitoring (MRM) mode by diffusing 10 ng mL^{-1} of PFOA or PFOS standard solutions. Secondary ions corresponding to fragments of *m/z* 80, and $[M\text{-}CO_2]^-$, were used to monitor perfluorinated sulfonic acids and perfluorinated carboxylic acids, respectively. The other ionization parameters were as follows: desolvation gas, 20 psi; nebulizing gas, 60 psi; heating gas, 60 psi; source temperature, 600 °C; electron voltage, −4500 V; entrance potential, −10 V; collision cell exit potential, 15 V and collision-activated dissociation (CAD), 3.0. The dwell time of each MRM transition was 50 ms. The chemical formula of PFCs used in this study, their retention times, and their LC-MS/MS conditions are summarized in Table 1.

A seven-point linear calibration curve was established using two isotopically labeled internal standards over a range of 0.33-50 ng mL^{-1}. ^{13}C-Isotopically labeled internal standards (0.75 ng) were added into each 0.75 mL of serum sample or 100 μL of calibration standard. Two internal standards ($^{13}C_4$-PFOA and $^{13}C_4$-PFOS) were used for the quantitation of perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids (PFSA), respectively. Calibration curves were constructed from the peak-area ratios of each analyte to isotopically labeled internal standards using a least-squares regression method.

Five replicates of quality control (QC) samples at three concentrations (3.0, 10.0, and 50.0 ng mL^{-1}) were included in each run to determine the intra-day and inter-day precision and accuracy of this method. Accurate concentrations of QC working solutions were used in the method validation. Relative standard deviations (RSD) of the concentrations were used as an index of precision. Accuracy was calculated by comparing the mean experimental concentrations of analyzed QC samples with their nominal values. Percentage values were used as the index.

The limit of detection (LOD) and quantification (LOQ) based on a signal/noise ratio of 3 and 10, respectively, was defined as the lowest working solution concentration analyzed with accuracy to within 80-120% of its reference value, and with a precision better than 20% RSD.[26] The LOQ was determined by calculating precision and accuracy for five samples that were independent of the calibration curve.

Results and Discussion

Separation of PFCs by Monolithic C18 LC-MS/MS. It is essential to rapid separation the PFCs to obtain accurate estimates of levels of PFCs in serum for high-throughput analysis. To optimize the separation conditions of PFCs by monolithic column LC analysis, mixed mobile phases (acetonitrile-water and methanol-water), and concentration of ion-pair agent were tested and compared. It is already reported that the composition of mobile phase could be considerably affected the retention of PFCs on conventional packed reversed-phase column LC.[10] In this study, an

Table 1. Chemical formula, retention times, and LC-MS/MS-MRM conditions for PFCs used in this study

	Chemical formula	RT (min)	Precursor ion $[M\text{-}H]^-$	MS/MS Q1Q3	CID (eV)	Other product ions[a] (*m/z*)
PFPA	$C_5HF_9O_2$	2.55	263	263 → 219	-10	-
PFHxA	$C_6HF_{11}O_2$	2.65	313	313 → 269	-10	119
PFHpA	$C_7HF_{13}O_2$	2.77	363	363 → 319	-10	169, 119
PFOA	$C_8HF_{15}O_2$	2.90	413	413 → 369	-10	169, 219
PFNA	$C_9HF_{17}O_2$	3.05	463	463 → 419	-10	169, 219, 269
PFDA	$C_{10}HF_{19}O_2$	3.18	513	513 → 469	-10	219, 269, 169
PFHxS	$C_6HF_{13}O_3S$	3.01	399	399 → 80	-70	99, 119, 169
PFHpS	$C_7HF_{15}O_3S$	3.15	449	449 → 80	-70	99, 119, 169
PFOS	$C_8HF_{17}O_3S$	3.25	499	499 → 80	-70	99, 130, 169
PFNS	$C_9HF_{19}O_3S$	3.32	549	549 → 80	-70	99, 130, 169
$^{13}C_4$PFOA	$^{13}C_4C_4HF_{15}O_2$	2.89	417	417 → 372	-10	169, 219, 135
$^{13}C_4$PFOS	$^{13}C_4C_4HF_{17}O_3S$	3.24	503	503 → 80	-70	99, 131, 119

[a]not included in the quantification assay

acetonitrile-water mixture as mobile phase provided better separation efficiency and shorter LC run-time than methanol-water mixture on conventional particulate C18 column, as shown in Figure 2(a) and (b). The overall retention of PFCs using acetonitrile-water as mobile phase could be significantly reduced, compared to using methanol-water. This is consistent with previous report used by conventional packed-C8 column.[10]

Similarly, compared to methanol-water, the LC run time using acetonitrile-water could be also reduced from 5.5 to 3.3 min on monolithic C18 column. The LC elution order of PFCs on monolithic C18 column was similar to those on conventional particulate C18 column. The retention times of PACAs and PASAs on monolithic C18 column were proportional with the chain length (Figure 2(c)). It can be explained that increasing F atoms of PFCs associated with chain length might lead to reducing the polarity of carboxylic acid and sulfonic acid groups, resulting in increasing hydrophobic. Notably, the overall retention times of PACAs using acetonitrile-water were shorter than those of PASAs due probably to the difference in delocalization capacity of partial charge between carboxylic and sulfonic acid groups. The retention times of all PFCs have shown to be reduced as the portion of acetonitrile increased. In this study, the portion of acetonitrile on mobile phase was varied in the range 10-90% to improve PFCs separation and to reduce LC run time. The flow rate was set at 0.6 mL/min.

As shown in Figure 2(c), chromatographic separation of a standard mixture of PFCs (1.0 ng mL^{-1} of each analyte) with good symmetrical peak shapes was achieved within 3.3 min on C18-monolithic column. While, using conventional C18 column, the separation of 10 PFCs could be performed within 14.5 min (Figure 2(b)). From these observation, the separation of PFC mixture containing 10 unlabeled and two ^{13}C-labeled internal standards on monolithic C18 column could be reduced the run time of the analysis 4-fold. This rapid and efficient separation of PFCs was obtained with a C18 monolithic column that gave a high separation speed at a modest operating pressure. Total run time of monolithic LC-MS/MS method, including equilibrium time, was within 5 min, while that of C18-packed column HPLC method was at least 20 min. Also compared to UPLC method,[16,17] total run time was also almost equivalent. Moreover, monolithic LC-MS/MS method provided almost equivalent or more robust separation compared to both conventional packed-C18 HPLC and UPLC methods, in terms of the consistency of retention times and peak areas. The chromatographic stability and injection accuracy of monolithic HPLC system was tested in detail by 10 consecutive measurements of PFCs, and coefficients of variation for the retention times were found to range between 0.12% and 0.27% RSD.

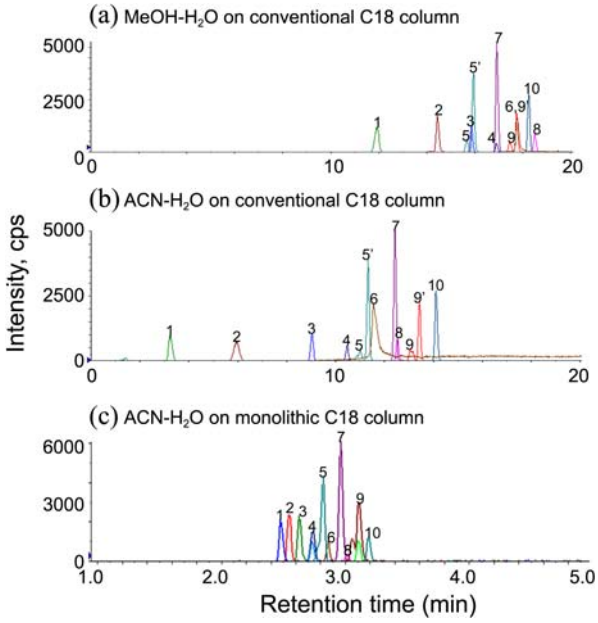

Figure 2. LC-ESI-MS/MS chromatograms of PFCs standard mixture obtained by (a) methanol-water, (b) acetonitrile-water on conventional C18 column, and (c) acetonitrile-water on monolithic C18 column. Peak identities are as follows: 1. PFPA, 2. PFHxA, 3. PFHpA, 4. PFOA, 5. PFHxS, 6. PFNA, 7. PFHpS, 8. PFDA, 9. PFOS, 10. PFNS, 11. $^{13}C_4$-PFOA, and 12. $^{13}C_4$-PFOS.

Moreover, the addition of ammonium acetate to the mobile phase greatly affected the separation efficiency of PFCs by the monolithic C18 column. In general, the increment of ammonium acetate concentration used as additive of mobile phase increased the overall separation efficiency of PFCs and could significantly reduce the peak tailing of PFCs on monolithic C18 column. The separation efficiency of PFCs did not greatly improve at more than 20 mM ammonia acetate. However, the concentration of ammonium acetate could alter the peak responses of some PFCs under ESI-MS detection. As the concentration of ammonium acetate increases from 2 to 20 mM, the overall detection sensitivity of PFCs appeared to gradually increase, as shown in Figure 3(a). Using 30 mM ammonium acetate, the overall detection sensitivity of PFCs appeared to decrease due primarily to the increased ionic strength. Thus, addition of 20 mM ammonium

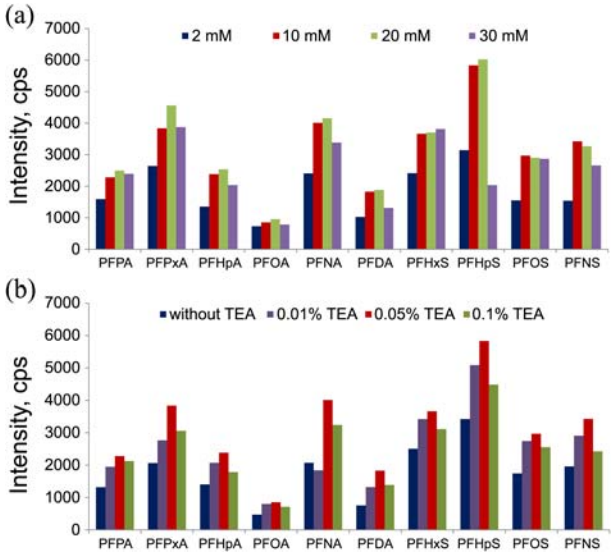

Figure 3. The influence of (a) ammonium acetate concentration (b) addition of TEA on MS detection response of PFCs.

acetate to an acetonitrile-water mixture provided the most efficient mobile phase for PFC separation on C18 monolithic LC.

In this study, to enhance the ionization efficiency of PFCs during the ESI process, TEA was added to an acetonitrile-water mobile phase containing 20 mM ammonium acetate. The overall response of PASAs (bearing sulfonic acid group) was shown to be a better than that of PACAs (bearing carboxylic acid group), owing to lower pK_a of PASAs than PACAs. The addition of TEA greatly improved the MS ionization efficiency of PFCs in comparison to TEA-free mobile phases, as shown in Figure 3(b). This is likely due to the ability of TEA to facilitate deprotonation of PFCs during the ESI process. The use of 0.05% TEA had shown higher detection sensitivity and did not show any reducing the chromatographic separation of PFCs, but TEA at more than 0.1% did not show any significant detection improvement for all PFCs.

Extraction and Purification of PFCs from Serum Sample. The satisfactory determination of PFCs from serum samples was mainly dependent on the methods of extraction and purification. A complex mixture containing several types of co-extracted interferents is inevitably created during the extraction of PFCs from serum samples. These interferents seriously complicate efforts to analyze trace amounts of PFCs and tend to prematurely plug the LC inlet and column. To reduce such interference effect, the weak anion-exchange (WAX)-SPE method was shown to be the most effective cleanup efficiency for the elimination of interferents in serum extracts [Supplementary Fig. 1]. The sample preparation used modified WAX-SPE cleanup from previous reports[25,28] to effectively remove the interferences by adjusting pH 3.0-3.5 of extract with formic acid. The adjustment pH 3.0 could lead to the protection of ionization of PFCs with low pK_a, resulting in reproducible interaction with WAX adsorbent. Though not shown data here, PFCs in an extract could be sufficiently purified by the WAX-SPE cartridge, eluting them with 2% ammonium hydroxide in methanol/MTBE (1:9, v/v). It can be explained that PFCs with an anionic character might be strongly retained at doubly charged dihydropiperazine of the WAX adsorbent surface, but neutral and cationic compounds eluted freely. However, acidic interferences such as fatty acids could not be effectively eliminated due to their strong interaction on the adsorbent of WAX-SPE. Thus, the collection volume of SPE eluent should be carefully selected in WAX-SPE cleanup procedure, and 2 mL of eluent was suitable to minimize the interferences. The percentage recovery of PFCs from a spiked serum sample

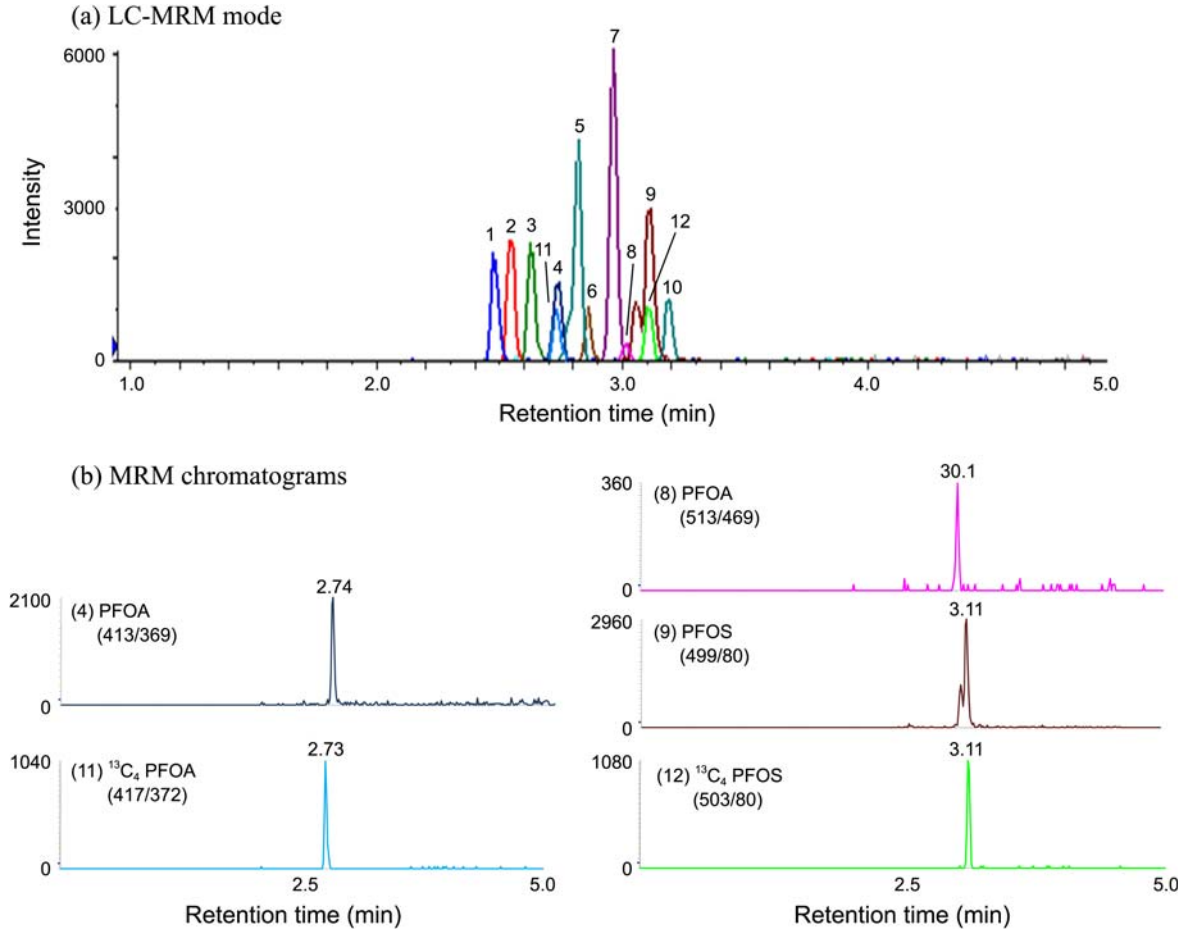

Figure 4. (a) TIC and (b) MRM chromatograms of PFCs at an 1 ng mL^{-1} level using a monolithic C18 column. Peak identities are the same as in Fig. 2.

after centrifugation and subsequent WAX-SPE ranged from 87 to 119% with less than 7.5% RSD. The recovery of PFCs obtained by this method was shown to be the most effective, compared to other methods (Supplementary Table 1).

LC/MS/MS Analysis of PFCs in Serum Sample. Although appropriate further purification methods were applied, several matrix-derived interferents (*e.g.*, lipids and fatty acids in serum) as well as other background contamination were present in the serum extract. To reduce such matrix effect, LC-MS/MS-multiple-reaction monitoring (MRM) mode was applied for the determination of PFCs in samples with complex sample matrices. After the addition of $^{13}C_4$-PFOS and $^{13}C_4$-PFOA as internal standards, the serum sample underwent the same SPE purification step followed by analysis with other LC-MS/MS protocols, as described in the experimental section. The typical LC-MS/MS chromatograms of PFCs obtained using MRM detection and a spiked serum sample, containing 1.0 ng mL^{-1} of each PFC and internal standards, are shown in Figure 4(a). As shown in Figure 4(a), a rapid and effective separation of PFCs was obtained within 3.3 min and no interferences were observed using monolithic column LC-MS/MS-MRM mode. It indicated that monolithic column LC-MS/MS-MRM mode can accurately quantify the PFCs compounds in serum sample. Although some of PFCs such as the peaks PFOA and $^{13}C_4$-PFOA, and the peaks PFDA, PFOS, and $^{13}C_4$-PFOS were co-eluted or overlapped, these peaks could be successfully characterized and qualified by their specific MRM chromatograms (Figure 4(b)). The concentration was calculated from the peak area in MRM chromatograms, and the ratio of each PACA to $^{13}C_4$-PFOA and each PASA to $^{13}C_4$-PFOS, respectively, was used to estimate the concentration of PFCs in serum sample.

This method provided an equivalent or greater detection sensitivity compared to previous reports.[10,29] Also, this is the first approach enabling to the high-throughput analysis of PFCs in serum sample.

Method Validation. To evaluate the present method, spiked human serum samples were repeatedly analyzed to determine the percentage recovery, reproducibility, and detection limits of the method. Blank samples were spiked with analytes at 1 ng mL^{-1} and 10 ng mL^{-1} levels before extraction. These spiked samples were extracted and purified by the method mentioned above. Five sample replicates at each spiking level were analyzed by LC/MS/MS in MRM detection. As indicated in Table 2, the mean percentage recoveries and relative standard deviations (RSDs) were as follows; recoveries ranged from 81.5% to 102.3% at the 1 ng mL^{-1} level and from 90.2% to 106.3% at the 10 ng mL^{-1} level, with RSD values ranging from 3.4% to 16.2%. Precision assays were carried out five times using control serum samples at three different concentrations (3, 10, and 50 ng mL^{-1}) on the same day and over five different days. The percentages of intra-day and inter-day variation were within 15.5% and 15.6%, respectively (Supplementary Table 2).

Calibration standards consisted of 0.75 mL samples of serum spiked with 0.75 ng of $^{13}C_4$-PFOA, $^{13}C_4$-PFOS, and PFC standards in amounts of 0.25, 0.5, 1.5, 2.5, 5, 15, and 37.5 ng. The calibration curves were generated by a linear least-squares regression analysis of the PCA/$^{13}C_4$-PFOA and PSA/$^{13}C_4$-PFOS peak area ratio versus the amount of spiked PFCs. The correlation coefficient (R^2) for each PFC was greater than 0.995, indicating excellent linearity (Table 2).

Limits of detection (LODs) and quantitation (LOQs) were evaluated using LC/MS/MS and MRM detection at a signal-to-noise ratio of three and ten, respectively. A summary of LODs and LOQs obtained using LC/MS-MRM is given in Table 2. These detection limit data suggest that monolithic C18 column LC-MS/MS-MRM combined with WAX-SPE gives an equivalent or better sensitivity than previously reported methods.[8-10,29] The good linearity, sensitivity, recovery, and precision of this method suggest that it is well-suited for the high-throughput analysis of serum samples.

The developed method was also validated using SRM 1957, a standard reference material for organic pollutants. SRM 1957 contains certified concentrations of seven PFCs (PFHpA, PFOA, PFNA, PFDA, PFUnA, PFHxS, and PFOS) in human serum. The results obtained by this method (n = 5) were satisfactory for all six species analyzed (Table 3), within an accuracy of 9.2%. The reproducibility of the complete analytical method was determined by replicate analyses of the SRM standard on different days. The reproducibility of the method was within 1.52 standard deviations for all compounds. Therefore, the developed method, which is based on centrifugation, WAX-SPE purification, and monolithic

Table 2. Calibration curves, detection limits, and quantitation limits of PFCs spiked in blank serum samples (n = 5)

Compound name	Range (ng mL^{-1})	Calibration equations	Correlation coefficient	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
PFPA	0.33-50	y = 0.5810x+0.1480	0.9998	0.10	0.30
PFHxA	0.33-50	y = 0.9732x+0.4232	0.9969	0.03	0.10
PFHpA	0.33-50	y = 1.2023x+0.6228	0.9986	0.03	0.08
PFOA	0.33-50	y = 0.6095x+0.1326	0.9999	0.09	0.30
PFNA	0.33-50	y = 0.7133x+0.1654	0.9992	0.04	0.12
PFDA	0.33-50	y = 0.3400x–0.1771	0.9978	0.10	0.31
PFHxS	0.33-50	y = 2.0727x–0.2071	0.9998	0.05	0.18
PFHpS	0.33-50	y = 1.6592x+0.0755	0.9982	0.05	0.17
PFOS	0.33-50	y = 1.3149x–0.7059	0.9998	0.15	0.46
PFNS	0.33-50	y = 0.5873x–0.7610	0.9957	0.07	0.23

Table 3. Comparison of certified SRM (NIST SRM 1957) values with measured values of PFCs (ng mL^{-1}) obtained by the proposed method (n = 5)

Compound name	NIST standard values (SD)	Measured values (SD)	Rec. (%)
PFPA	-	-	
PFHxA	-	-	
PFHpA	0.305 (0.036)	0.33 (0.04)	108.9
PFOA	5.00 (0.40)	5.46 (0.37)	109.2
PFNA	0.880 (0.068)	0.82 (0.14)	93.5
PFDA	0.39 (0.10)	0.36 (0.11)	91.6
PFHxS	4.00 (0.75)	3.81 (0.36)	95.3
PFHpS	-	-	
PFOS	21.1 (1.2)	22.31 (1.52)	105.7
PFNS	-	-	

C18-LC/MS-MRM, provides reliable confirmation and quantitation of PFCs in biological samples.

Method Application. The developed and validated method was applied to the analysis of PFCs in serum samples collected from volunteers living near a Korean industrial area. Typical total ion chromatogram and MRM ion chromatograms of PFCs from this sample set are shown in Figure 5. To ensure correct identification and to prevent overestimation of PFCs, two or more different ions were selected for each analyte, and background PFCs levels were carefully checked as PFCs can originate from lab-ware and instrument parts composed of fluorinated compounds.

As can be seen in Figure 5, four compounds including PFOS, PFOA, PFDA, and PFHxS were primarily detected by LC-MS/MS in MRM mode. Besides these compounds, some PFCs were also observed at trace levels. Among them, PFOA and PFOS were detected at 13.3 and 32.2 ng mL^{-1}, respectively. These are as relatively significant amounts compared to other PFCs, and they are commonly observed together in biological samples and environmental samples due to their extensive production and usage in industry. Trace amounts of PFCs at sub-ppb level in serum sample could be readily determined by the established method.

Conclusion

An analytical method for the extraction, purification, and detection of PFCs from human serum samples has been developed and evaluated. The use of a WAX-SPE cartridge for preliminary sample purification permitted the efficient removal of potentially interfering species. A monolithic

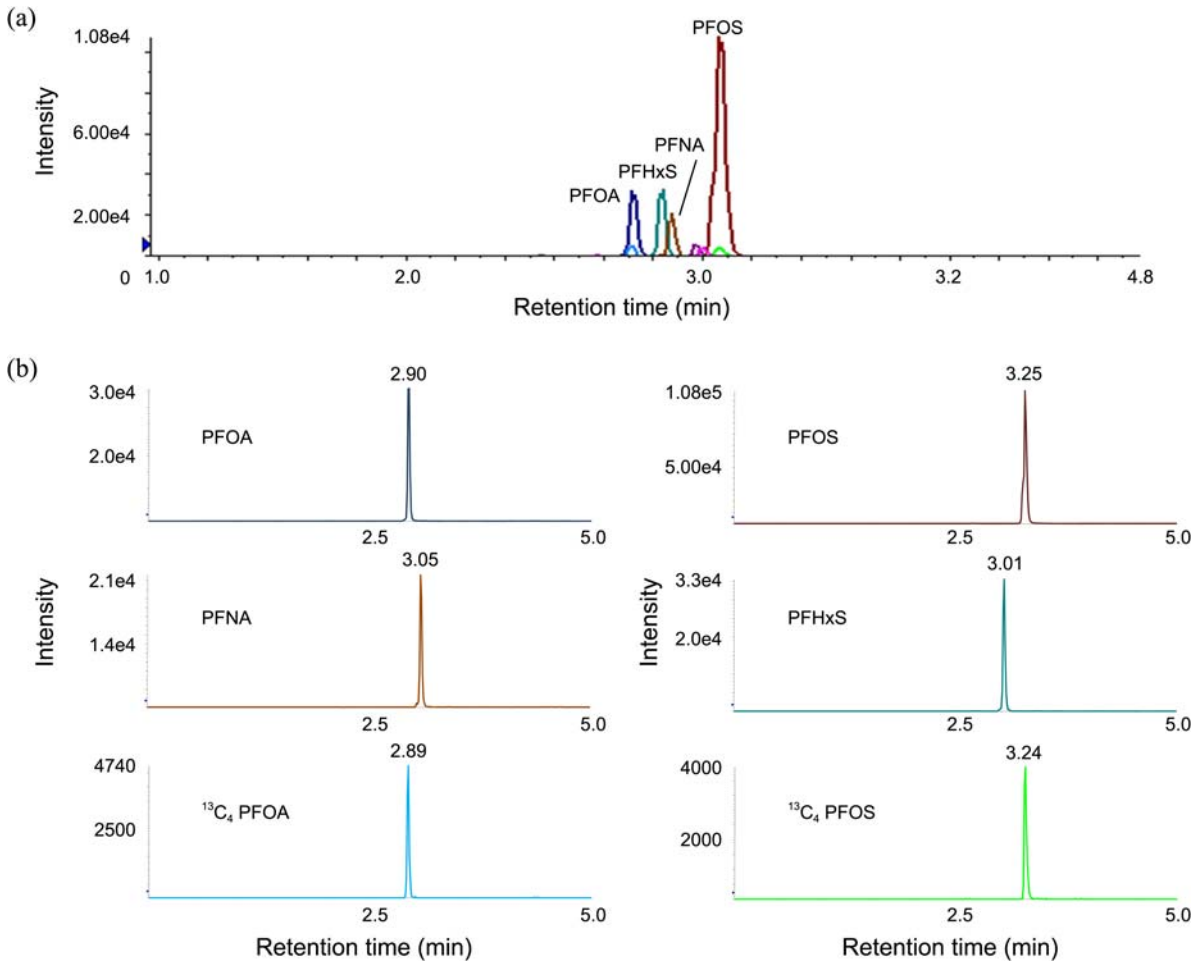

Figure 5. (a) TIC and (b) MRM chromatograms of PFCs detected in human serum sample.

column LC-MS/MS-MRM mode could greatly enhance the specificity, selectivity, and sensitivity in terms of rapid qualitative and quantitative analysis of PFCs in serum sample. Compared to a conventional packed-column, analysis time and solvent was saved for high-throughput analyses of PFCs.

Additionally, the addition of TEA to the mobile phase significantly improved the detection sensitivity and retained chromatographic separation power of PFCs. Validation studies of the analytical method using analyte spiking and recovery from human serum and a SRM standard resulted in greater than 81.5% recovery. Thus, this method is suitable for the high throughput evaluation of accumulation levels in human body fluid.

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