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Analysis of perfluorinated chemicals in umbilical cord blood by ultra-high performance liquid chromatography/tandem mass spectrometry

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ABSTRACT

Perfluorinated compounds (PFCs) can cross the placental barrier and enter fetal circulation. This study aimed at developing a fast and sensitive ultra-high performance liquid chromatography/tandem mass spectrometry method for the determination of twelve perfluorinated compounds in cord blood. Samples were processed with protein precipitation using formic acid and methanol, mixed with stable isotope labeled standard, followed by sonication and centrifugation, and were analyzed using a Waters ACQUITY UPLC coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer. The instrument was operated in selected reaction monitoring (SRM) with negative electrospray ionization. Using BEH C_{18} column (2.1 mm × 50 mm, 1.7 μm) with 10-mM *N*-methylmorpholine/methanol gradient elution provided a fast chromatographic separation (5.5 min) and sharp peaks. Intra- and inter-day calibration bias was less than 7% and intra- and inter-day calibration of relative standard deviations were within 0.02–8.22% for all the analytes and concentrations. The recoveries of PFCs spiked into bovine serum ranged from 85 to 104% with relative standard deviations from 0.02 to 6.37%. The limits of quantitation (LOQs), defined as a signal-to-noise ratio of ten, ranged from 0.15 to 3.1 ng/mL for the twelve PFCs. Perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluoroundecanoic acid (PFUA) and perfluorononanoic acid (PFNA) were detected in up to 68% of umbilical cord plasma (n = 444) in Taiwan Birth Panel Study and the health effect of these chemicals on children developmental deserves further investigation.

1. Introduction

The perfluorinated compounds (PFCs) are synthetic chemicals that have been widely used in the manufacturing of consumer products (e.g. polymers, surfactants and plastic additives) since the 1950s. The fluorine moiety of PFCs provides extremely low surface tension and uniquely hydrophobic and hydrophilic properties. However, these chemicals have ubiquitously distributed in the environment, human body and biota [1–5]. The two most commonly measured PFCs, perfluorooctanoic acid (PFOA) and perfluorooctyl sulfonate (PFOS), contain an eight-carbon backbone, and have been observed in retired fluorochemical industry workers with mean elimination half-lives of 3–5 years [6]. After subchronic exposure to PFOS and PFOA, several species manifest significant weight loss, liver impairment, and reduced levels of serum cholesterol and thyroid hormones [7–11]. Although the industry has put efforts to reduce and phase out the production of certain PFCs, including PFOS and PFOA since 2002 [12], these PFCs may still exist in older products. Also, other PFCs are still being widely used.

PFOS and PFOA as well as certain PFCs have been detected in umbilical cord blood, indicating that these chemicals can cross the placenta. Studies by Monroy et al. showed that the median levels of PFOA, PFOS, PFNA (perfluorononanoic acid), and PFHxS (perfluorohexanesulfonate) in cord blood serum (n = 105) in Canada were 1.58, 6.08, 0.72, and 2.07 ng/mL, respectively [13]. Inoue et al. found that the median concentration of PFOS in cord blood serum (n = 15) in Japan was 2.5 ng/mL, and both PFOA and PFOSA were not detected [14]. Midasch et al. reported the median levels of PFOA and PFOS in cord blood plasma in Germany were 3.4 and 7.3 ng/mL, respectively [15]. Apelberg et al. indicated that the mean levels of PFOA and PFOS in cord blood serum in USA were 4.9 and 1.6 ng/mL, respectively [16]. Hanssen et al. observed that the levels of PFOA, PFOS, and PFHxS in cord blood serum in South Africa were 1.3, 0.7, 0.3 ng/mL, respectively [17]. However, reports on the profile of other PFCs in cord blood are very limited, and little is known about their levels and potential developmental effects on children.

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There are several existing methods for analyzing PFCs. Using off-line or on-line solid phase extraction (SPE) is a current trend due to its lower matrix effects and improvement on signal intensity [18,19]. However, SPE methods involve steps of conditioning, loading, washing off of interferences, eluting analytes, evaporating and reconstituting the elute before the analysis, which are tedious for sample preparation and are less appropriate for high-throughput analysis if samples are not processed using automatic SPE extractors. The protein precipitation is commonly used for fast sample clean-up and disrupting protein–analyte binding, and it can also be used as a pretreatment to other methods to reduce sample viscosity and formation of clots or precipitates. To our best knowledge, none has used protein precipitation directly to analyze PFCs in plasma/serum samples. Moreover, more and more studies have used ultra-high performance liquid chromatography (UHPLC) combined with tandem mass spectrometry (MS/MS) for fast chromatography and increase of signal-to-noise (S/N) ratios without sacrificing separation efficiency. Recently, the limits of quantification (LOQs) of the selected PFCs in milk or tissue samples using UHPLC/MS/MS have been reported to be lower than 0.06 ng/mL except for PFOA at 0.5 ng/mL [3].

This study aimed to establish a qualitative and quantitative method for determining twelve PFCs in plasma/serum samples employing protein precipitation approach coupled with UHPLC/MS/MS method. This reliable method was subsequently applied to determine the levels of twelve PFCs within umbilical cord blood in Taiwan Birth Panel Study.

2. Experimental

2.1. Chemicals and reagents

Potassium perfluorohexanesulfonate (PFHxS; purity > 98%), perfluoroheptanoic acid (PFHpA; purity > 99%), perfluorononanoic acid (PFNA; purity > 95%), perfluorooctanoic acid (PFOA; purity > 90%), perfluorooctyl sulfonate (PFOS; purity > 98%), perfluorodecanoic acid (PFDeA; purity > 98%), perfluoroundecanoic acid (PFUA; purity > 95%), perfluorododecanoic acid (PFDoA; purity > 95%), 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (Me–PFOSA–AcOH; purity > 99%) and 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (Et–PFOSA–AcOH; purity > 99%) were bought from Sigma-Aldrich (St. Louis, MO, USA). Perfluorohexanoic acid (PFHxA; purity > 98%) and perfluorooctane sulfonamide (PFOSA; purity > 98%) were purchased from Chiron AS (Trondheim, Norway). 1,2-$^{13}C_8$-perfluorooctanoic acid ($^{13}C_8$-PFOA) was supplied by Cambridge Isotope Laboratories (Andover, MA, USA; purity > 98%). Stock solutions of each target compound were prepared at a concentration of 50 μg/mL in methanol and stored at 4 °C. Milli-Q water was obtained from a Millipore water purification system (Billeria, MA, USA). Formic acid (purity > 98%) and *N*-methylmorpholine (purity > 99.5%) were provided by J.T. Baker (Phillipsburg, NJ, USA). Methanol and acetonitrile were LC/MS grade from J.T. Baker. Bovine plasma and serum were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample collection and sample preparation

The specimens of umbilical cord blood were taken from Taiwan Birth Panel Study (TBPS), which were recruited in one medical hospital in Taipei City, one area hospital, and two clinics in the Taipei County from April 2004 to January 2005. The cord blood samples were collected by the hospital personnel at the time of child delivery using tubes containing ethylenediaminetetraacetic acid (EDTA). The collected samples were transported to a laboratory immediately and were separated into blood cells and plasma by centrifuged for 15 min, and then were frozen at −80 °C until the analysis. The study has been approved by the Independent Ethics Committee of National Taiwan University Hospital and a total of 444 subjects were included in this study.

All samples were primarily prepared using protein precipitation. Firstly, the frozen samples were thawed at room temperature and then were vortexed for 30 s to ensure homogeneity. 100 μL of plasma sample in polypropylene centrifuge tube was vortexed with 100 μL of 1% formic acid (pH 2.8) for 30 s. Then 80 μL of methanol and 20 μL of 0.375 ng/mL internal standard solution ($^{13}C_8$-PFOA) were added to each sample before the second vortex. The mixture was sonicated for 20 min and then centrifuged at 14,000 rpm (14,462 × *g*) for 20 min using a bench top centrifuge (Beckman Microfuge 16, Fullerton, CA, USA). The supernatant was collected (~150 μL) and then was filtered through 0.22-μm PVDF syringe filter into a 2.0 mL auto-sampler vial.

Ten calibration standard solutions were prepared in 100 μL of bovine plasma and went through sample preparation under the same procedure, which the concentrations of all analytes were equivalent to 0.15–300 ng/mL in bovine plasma with a fixed amount of internal standard (75 ng/mL).

2.3. Instrumental analysis and quantification

The separation and detection of analytes were performed on a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) coupled with a Waters Quattro Premier XE triple quadrupole mass spectrometer. The UHPLC/MS/MS system was controlled by MassLynx V.4.1 with QuanLynx Application Manager and the data were acquired and processed using MassLynx V.4.1. An ACQUITY UPLC BEH C_{18} column (2.1 mm × 50 mm, 1.7 μm) was used and the column temperature was maintained at 30 °C. The mobile phase was composed of 10 mM *N*-methylmorpholine (pH 9.5) and methanol. The LC system was held at initial composition of 10% methanol for 1.0 min followed by a linear gradient to 65% methanol in 0.5 min, then 90% methanol in 1.5 min, at which point it was held at 90% methanol for 1.0 min before being returned to the initial condition. The column was re-equilibrated for 1.5 min. The total run time was 5.5 min and the flow rate was maintained constantly at 0.5 mL/min throughout the run. Ten-microliter solution was injected into UHPLC/MS/MS.

The mass spectrometer was operated in electrospray negative ion mode with capillary voltage 1.5 kV. Cone- and desolvation-gas flows were kept at 50 and 750 L/h, respectively. Source and desolvation temperatures were kept at 150 °C and 450 °C, respectively. Both ion energy 1 and 2 were set at 1 and collision gas pressure of 3×10^{-3} mbar with argon gas. The multiplier voltage was set at 650 V. The dwell times were 20 ms for all the analytes including the internal standard, and the data points across the peak were no less than 10 to ensure precise integration of peak area. Ions were monitored by selected reaction monitoring (SRM) as shown in Table 1.

All glassware was rinsed with methanol before being used for experiments. Three duplicates of bovine serum spiked at two different levels (7.5 and 30 ng/mL) and with the stable isotope-labeled chemical (75 ng/mL) were processed with every batch of forty samples to check the method reproducibility. The linear dynamic ranges in plasma with $1/\chi$ weighted were as follows: 0.15–300 ng/mL for PFHxA, PFHxS, PFOS, PFDeA, PFOSA and PFDoA; 0.75–300 ng/mL for PFHpA, PFNA and Et–PFOSA–AcOH; 1.5–300 ng/mL for PFOA and Me–PFOSA–AcOH; 3–300 ng/mL for PFUA. The square of the correlation coefficient (R^2) of each curve was equal to or greater than 0.996 (Table 1).

Table 1
Selected reaction monitoring (SRM) transitions, collision energy, cone voltage, and linear range of the analytes.

Analytes	MW	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Collision energy (V)	Cone voltage (V)	Linear range (ng/mL)	R^2
PFHxA	314	312.9	268.7	8	15	0.15–300	0.997
PFHpA	364	362.6	318.8	10	20	0.75–300	0.998
PFHxS	438	398.7	79.8	32	47	0.15–300	0.999
PFOA	414	412.8	368.9	10	14	1.50–300	0.999
PFNA	464	462.7	418.8	12	18	0.75–300	0.996
PFOS	500	498.9	79.8	42	60	0.15–300	0.999
PFDeA	514	512.9	468.9	9	15	0.15–300	0.998
Me–PFOSA–AcOH	571	570.0	511.7	20	20	1.50–300	0.996
Et–PFOSA–AcOH	585	583.9	525.5	20	24	0.75–300	0.996
PFUA	564	562.6	518.7	11	20	3.00–300	0.996
PFOSA	499	497.6	77.8	30	37	0.15–300	0.999
PFDoA	614	612.6	568.6	11	19	0.15–300	0.998
Internal standard							
$^{13}C_8$-PFOA	422	420.8	375.8	10	14		

2.4. Evaluation of matrix effect and extraction efficiency of sample pretreatment

Three types of blood-based matrices, bovine plasma, bovine serum and pooled human plasma, were used for assessing matrix effects using the post-extraction addition method at two different concentrations (2.5 and 25 ng/mL). The backgrounds of samples for spiking were subtracted if an analyte was detectable. The percent matrix effect (%ME) was calculated by the peak area of post-extraction spiking divided by the peak area of standard × 100%. In order to distinguish the analyte loss during the extraction from matrix suppression, co-precipitation was tested by adding analytes to samples of bovine plasma before and after protein precipitation with two different concentrations (7.5 and 30 ng/mL). The extraction efficiency (%) was determined by peak area of pre-extraction spiking divided by the peak area of post-extraction spiking × 100%.

2.5. Method validation

The protein precipitation with UHPLC/MS/MS method was validated regarding the precision, accuracy, and detection limits. For the intra- and inter-day precision and accuracy, three duplicates of calibration standards in bovine plasma were analyzed on the same day and on three different days. The recovery of the method was determined by spiking known amounts of analytes and an internal standard into bovine plasma, and was calculated by dividing the measured quantities with the theoretical (spiked) quantities. In addition, blank matrix was simultaneously quantified and the levels of endogenous analytes were subtracted. The limits of detection (LODs) and the limits of quantitation (LOQs) were determined with the lowest concentration ($n = 6$) at the linear range of the calibration curve and were defined as signal-to-noise (S/N) ratios equaling to 3 and 10, respectively; the signal-to-noise calculation was carried out by MassLynx software using the peak-to-peak option. Because PFOA, PFNA and PFUA were found in blanks, their LODs and LOQs were calculated using their mean levels of backgrounds in cord blood plasma samples plus three and five times of standard deviations, respectively ($n = 10$ for PFOA and PFUA, and $n = 5$ for PFNA).

3. Results and discussion

3.1. Method performance, matrix effect and recovery

The application of protein precipitation was successful in determining the twelve PFCs in plasma/serum samples. Even after 1000 injections of plasma samples that only with a simple treatment of protein precipitation, the UPLC BEH C_{18} column was still with stable column back pressure (~6300 psi) and provided good peak shapes; the method did not reduce column lifetime and make instrument down. The combination of *N*-methylmorpholine/methanol was chosen as the mobile phases after testing several compositions. Use of the basic buffer (pH 9.5) as the aqueous mobile phase increased the dissociation of these acidic analytes and improved the ionization efficiency of negative ESI, which offered better peak shapes and 5–11 times higher signal intensities than that of 5-mM ammonium acetate. Methanol as the organic mobile phase provided better signal intensities of the analytes comparing with that of acetonitrile. A fast gradient elution of the UPLC much shortened the separation without compromise of the quantification by combining with the specificity of MS/MS detection; the response factors of all analytes were similar within the range of standard calibration curve, which manifested no ion suppression resulting from peak overlapping.

The %ME in three matrices (bovine plasma, serum and human plasma) was compared among different concentrations (Table 2). The %ME for all analytes in bovine plasma was from 21 to 72%, with exception to PFOSA, which showed ion enhancement at high level up to 136%. In bovine serum, the matrix effect ranged from 16 to 88% for all the analytes; however, the %ME was inconsistent among different spiked levels for PFHxA, PFOS and PFDeA. In pooled human plasma, the %ME were 22–99%, but the PFOSA showed ion enhancement at high level up to 168%; the %ME of PFOA, PFDeA, Me–PFOSA–AcOH, PFUA and PFOSA were different in two spiked levels. Overall, we did not observe serious ion suppression among these matrices on most analytes, and the results also showed that the %ME using protein precipitation was highly reproducible with standard deviations lower than 8% for all analytes (Table 2).

The extraction efficiencies of the twelve analytes in bovine plasma were 30–81% (Table 3). Using the quantitative method of matrix-matched calibration with one internal standard, the recoveries of the twelve analytes in bovine serum were 85–104% with relative standard deviations from 0.02 to 6.37% (Table 3), which demonstrated a good accuracy and precision for measuring perfluorinated chemicals in plasma or serum samples.

3.2. Method validation

Controlling background contamination is crucial for reliable measurement of PFCs. The background levels of the analytes in methanol, reagent and bovine plasma blank were examined. The existing backgrounds of PFOA, PFNA and PFUA in these blank samples were found and their levels were less than 1.5, 0.75 and 3.0 ng/mL, respectively. Because we observed similar signal intensities of these three analytes in the three types of blanks, the possible sources of contamination could be polytetrafluoroethylene (PTFE) and perfluoroalkoxy components originated from the LC system [5] instead of labware. On the other hand, the backgrounds of these

Table 2
Matrix effect (%) of analytes in bovine plasma, bovine serum and human plasma samples using post-extraction spike (mean ± SD, $n = 3$).

Analytes	Bovine plasma		Bovine serum		Pooled human plasma	
	2.5 ng/mL	25 ng/mL	2.5 ng/mL	25 ng/mL	2.5 ng/mL	25 ng/mL
PFHxA	72.2 ± 0.14	64.5 ± 0.87	71.8 ± 1.60	49.4 ± 0.07	98.3 ± 1.50	90.3 ± 6.81
PFHpA	28.2 ± 0.19	28.0 ± 0.36	24.4 ± 0.45	17.9 ± 0.05	38.1 ± 0.20	42.0 ± 0.29
PFHxS	66.9 ± 0.16	64.5 ± 0.07	50.6 ± 0.03	38.4 ± 0.57	68.1 ± 1.03	74.9 ± 0.21
PFOA	35.5 ± 2.60	39.8 ± 0.33	24.3 ± 0.22	17.5 ± 0.04	34.5 ± 0.15	50.2 ± 1.43
PFNA	25.3 ± 4.07	26.7 ± 1.06	20.8 ± 1.38	15.9 ± 0.17	32.7 ± 1.33	31.4 ± 4.43
PFOS	57.7 ± 0.85	62.3 ± 0.25	70.5 ± 0.74	39.3 ± 0.05	51.0 ± 1.42	55.3 ± 7.90
PFDeA	30.6 ± 0.11	42.3 ± 5.88	22.4 ± 0.11	55.3 ± 7.90	27.1 ± 0.32	46.8 ± 0.34
Me–PFOSA–AcOH	27.5 ± 0.29	21.2 ± 0.40	17.4 ± 0.46	18.6 ± 0.42	45.6 ± 0.59	26.0 ± 0.13
Et–PFOSA–AcOH	27.4 ± 0.35	25.6 ± 3.89	18.9 ± 0.30	19.0 ± 1.27	23.6 ± 0.62	33.2 ± 0.73
PFUA	46.1 ± 0.19	30.5 ± 0.05	31.5 ± 0.03	24.8 ± 1.22	65.3 ± 0.04	36.2 ± 3.29
PFOSA	109 ± 1.24	136 ± 1.95	84.2 ± 0.49	88.4 ± 0.76	99.1 ± 7.76	168 ± 1.61
PFDoA	23.4 ± 0.24	31.8 ± 0.62	18.5 ± 0.30	25.1 ± 0.02	21.6 ± 0.13	35.9 ± 0.61

Table 3
Extraction efficiency and recovery percentages of the spiked samples with different concentration in bovine plasma and serum (mean ± SD (RSD %)).

Analytes	Extraction efficiency (bovine plasma, $n = 3$)		Recovery (bovine serum, $n = 3$)	
	7.5 ng/mL	30 ng/mL	7.5 ng/mL	30 ng/mL
PFHxA	64.4 ± 9.20 (14.3)	77.8 ± 0.79 (1.01)	101 ± 1.73 (1.71)	104 ± 2.00 (1.93)
PFHpA	50.9 ± 14.6 (28.8)	50.5 ± 9.78 (19.4)	102 ± 2.80 (2.75)	103 ± 1.58 (1.54)
PFHxS	34.0 ± 1.27 (3.72)	30.0 ± 5.85 (19.5)	89.6 ± 3.21 (3.59)	96.6 ± 3.11 (3.22)
PFOA	66.6 ± 9.78 (14.7)	61.0 ± 6.70 (11.0)	103 ± 0.92 (0.90)	101 ± 0.12 (0.02)
PFNA	61.0 ± 8.42 (13.8)	63.8 ± 4.66 (7.31)	99.8 ± 1.54 (1.54)	101 ± 0.68 (0.67)
PFOS	79.7 ± 3.67 (7.61)	62.4 ± 8.54 (13.7)	101 ± 1.29 (1.27)	101 ± 1.87 (1.84)
PFDeA	57.3 ± 16.1 (28.2)	66.7 ± 7.54 (11.3)	102 ± 0.66 (0.64)	100 ± 0.16 (0.16)
Me–PFOSA–AcOH	57.1 ± 6.12 (10.7)	65.2 ± 3.03 (4.65)	100 ± 2.39 (2.38)	101 ± 2.16 (2.15)
Et–PFOSA–AcOH	42.6 ± 2.12 (4.97)	48.6 ± 7.12 (14.6)	84.9 ± 4.57 (5.38)	102 ± 1.78 (1.75)
PFUA	76.6 ± 18.2 (23.7)	81.2 ± 19.1 (23.5)	99.6 ± 0.40 (0.40)	101 ± 0.76 (0.75)
PFOSA	47.0 ± 8.19 (17.4)	50.8 ± 8.89 (17.5)	94.8 ± 6.13 (6.37)	95.6 ± 2.71 (2.83)
PFDoA	60.1 ± 9.76 (16.2)	79.6 ± 23.6 (29.7)	98.9 ± 1.09 (1.10)	97.3 ± 0.54 (0.56)

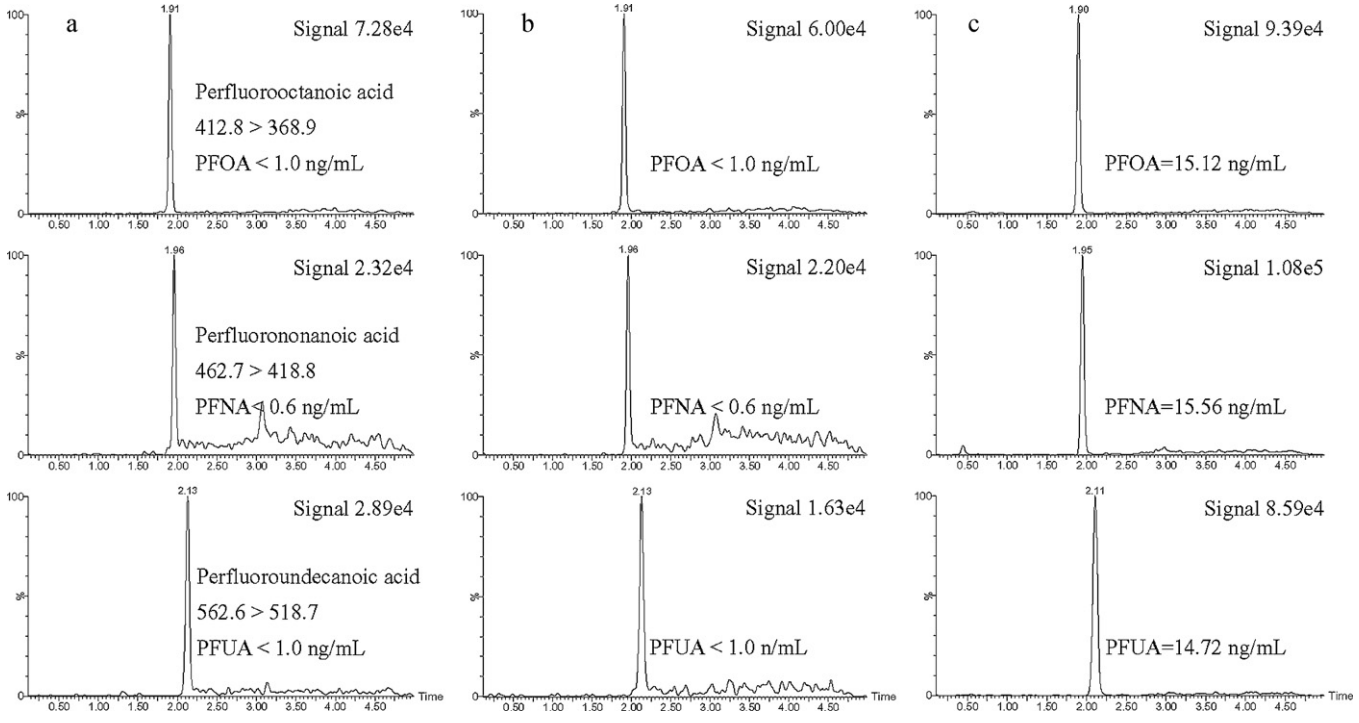

Fig. 1. The concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) in samples (10-μL injection): (a) reagent blank (b) bovine plasma blank and (c) spiked bovine plasma (15 ng/mL).

chemicals did not significantly influence the quantification of these compounds in plasma samples (Fig. 1).

Good accuracy and precision were obtained using matrix-matched calibration with one internal standard. The intra-day and inter-day biases for all the analytes were within ±3% and ±6%, respectively; the intra-day and inter-day variations (RSDs%) ranged from 0.15 to 6.61% and 0.09 to 5.28%, respectively. For example, the intra- and inter-day biases of PFUA, PFOA, PFNA

Table 4
Intra- and inter-day accuracy and precision for PFUA, PFOA, PFNA, and PFOS in bovine plasma ($n = 3$).

Concentration (ng/mL)		PFUA		PFOA		PFNA		PFOS	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.15	Mean							0.15	0.15
	RSD%							2.28	1.14
	Bias%							1.33	1.33
0.75	Mean					0.76	0.76	0.76	0.75
	RSD%					1.65	2.86	3.3	1.8
	Bias%					0.93	1.07	1.33	1.47
1.5	Mean			1.50	1.47	1.49	1.48	1.50	1.48
	RSD%			4.53	2.87	3.01	2.57	1.31	0.2
	Bias%			−0.2	−2.33	−0.8	−1.07	0.2	−1.2
3.0	Mean	3.02	3.07	3.02	2.98	2.96	2.94	2.98	2.96
	RSD%	1.14	0.8	2.37	1.56	2.54	2.15	1.8	2.5
	Bias%	0.8	2.3	0.7	−0.57	−1.47	−1.8	−0.6	−1.4
15	Mean	15.15	14.90	15.20	15.15	15.23	15.27	14.87	15.08
	RSD%	1.79	0.15	1.95	3.16	1.66	1.79	4.57	0.54
	Bias%	1.03	−0.65	1.34	0.99	1.54	1.83	−0.81	0.51
30	Mean	29.80	29.83	29.74	30.49	30.04	29.97	29.94	29.71
	RSD%	1.24	1.07	2.87	0.44	0.83	0.68	0.6	1.27
	Bias%	−0.66	−0.56	−0.85	1.62	0.12	−0.11	−0.2	−0.96
75	Mean	74.45	74.73	74.63	74.46	74.98	75.86	74.63	76.13
	RSD%	0.35	0.8	1.53	0.49	1.12	0.24	1.71	0.92
	Bias%	−0.73	−0.36	−0.5	0.61	−0.02	1.15	−0.5	1.5
150	Mean	148.2	147.3	148.3	150.2	149.3	147.9	149.7	147.8
	RSD%	1.93	1.21	1.64	0.84	1.15	0.57	1.99	1.1
	Bias%	−1.22	−1.78	−1.16	0.15	−0.45	−1.37	−0.2	−1.46
300	Mean	302.4	303.1	302.2	298.7	300.5	301.0	300.9	301.3
	RSD%	0.93	0.55	1.25	0.6	0.43	0.25	0.54	0.61
	Bias%	0.8	1.05	0.72	−0.42	0.16	0.34	0.28	0.43

Table 5
Concentration of the twelve PFCs in cord blood plasma (ng/mL, $n = 444$).

Analytes	Detection rate %	Median	Mean (SD)	90th percentile	Maximum	LOD	LOQ
PFHxA	9.9	0.75	0.78 (0.24)	1.23	1.29	0.093	0.30
PFHpA	0	–	–	–	–	0.24	0.78
PFHxS	20.5	0.36	0.45 (0.27)	0.72	2.67	0.072	0.24
PFOA	81.9	3.75	4.42 (2.51)	7.78	18.9	1.23	1.58
PFNA	67.6	7.70	10.5 (9.54)	21.7	64.5	0.67	0.84
PFOS	98.9	5.67	7.65 (7.35)	13.8	67.8	0.066	0.22
PFDeA	2.7	1.92	1.98 (0.99)	3.57	3.81	0.042	0.14
Me–PFOSA–AcOH	2.9	1.26	2.85 (5.10)	13.1	19.6	0.12	0.39
Et–PFOSA–AcOH	0.2	–	–	–	–	0.075	0.25
PFUA	85.1	19.0	22.4 (15.4)	43.2	105	2.4	3.1
PFOSA	9.9	0.21	0.24 (0.06)	0.30	0.57	0.042	0.14
PFDoA	1.8	6.30	6.24 (3.24)	11.8	11.8	0.063	0.21

Note: Samples with values below the LOQ were excluded in the calculation of median, mean and 90th percentile.

and PFOS were −1.47 to 1.54% and −2.33 to 2.30%, respectively; their intra- and inter-day RSD% were 0.35–4.57% and 0.15–3.16% (Table 4). The LODs and LOQs of PFHxA, PFHpA, PFHxS, PFOS, PFDeA, Me–PFOSA–AcOH, Et–PFOSA–AcOH, PFOSA and PFDoA, which ranged from 0.042 to 0.237 ng/mL and 0.138 to 0.789 ng/mL, respectively (Table 5). The LODs and LOQs of PFOA, PFNA and PFUA were higher than other analytes because of their background levels in cord blood plasma samples; the estimated LODs and LOQs were 1.23 and 1.58 ng/mL for PFOA ($n = 10$), 0.67 and 0.84 ng/mL for PFNA ($n = 5$), and 2.4 and 3.1 ng/mL for PFUA ($n = 10$), respectively.

The study showed that the LODs of PFCs in plasma using UHPLC/MS/MS with protein precipitation were similar or better than those of Kuklenyik et al. [18] who used HPLC/MS/MS with automated solid-phase extraction (0.1–1.0 ng/mL) and those of Inoue et al. [20] with column-switching extraction (0.05–0.25 ng/mL). In addition, Haug et al. [21] showed excellent LODs of 0.002–0.020 ng/mL, which may result from a large-volume injection (400 μL) and less matrix effect using the column-switching procedure. Overall, this study is not only at low cost and solvent-saving but also provides good detection sensitivity for PFC in plasma samples.

3.3. Levels of PFCs in umbilical cord blood plasma in Taiwan Birth Panel Study

PFOA, PFNA, PFOS and PFUA were detected the most frequently in all examined samples (Fig. 2). The level profile of twelve PFCs were listed in Table 5 (the reported value did not deduct background levels), and those values below the LOQs were excluded for calculating median, mean, and the 90th percentile. The detection rates of PFOA, PFNA, PFOS and PFUA were 82, 68, 99 and 85%, respectively. PFHxS was observed in 21% of the samples. PFHxA, PFDeA, Me–PFOSA–AcOH, PFOSA and PFDoA were detected in a few samples (<10%), and no PFHpA was observed in any samples. In previous studies, PFOS and PFOA were detected in over 99% of cord blood samples in US, Canada and Germany [9,13,15], and certain PFCs such as PFNA and PFHxS were detected in less than 30% of cord blood samples in Canada [13]. PFOSA, Et–PFOSA–AcOH, Me–PFOSA–AcOH, PFDeA, PFUA and PFDoA were found in 26, 1, 40, 24, 34 and 5% of cord blood samples of the US, respectively [16]. Overall, the detection profile of these PFCs in cord blood in Taiwan is consistent with other countries except for relatively higher detection rates of PFNA and PFUA.

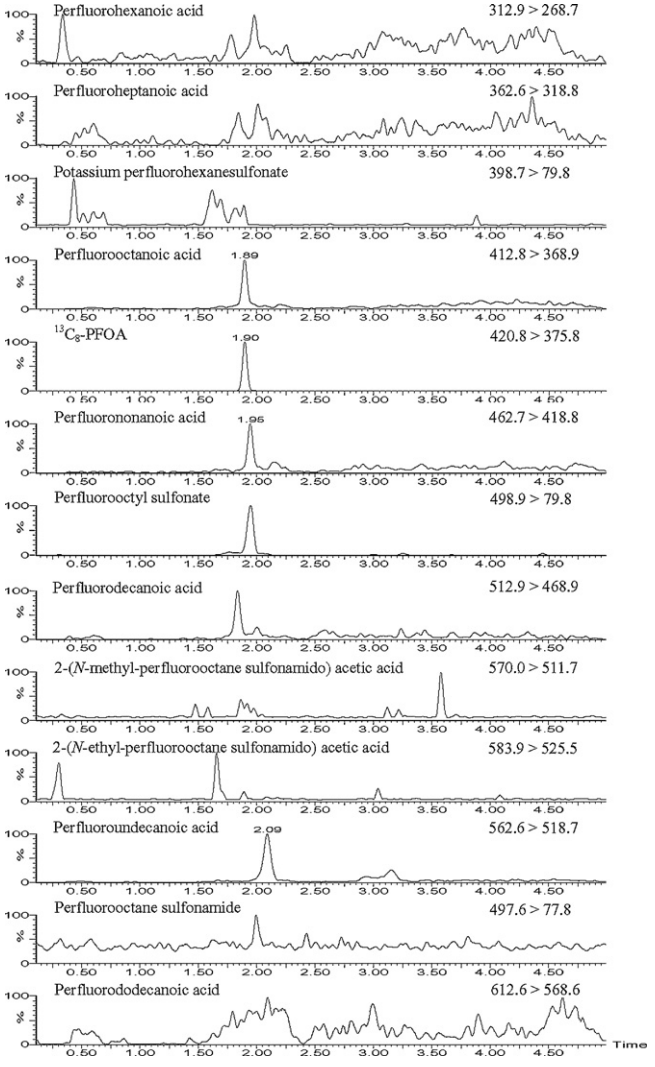

Fig. 2. Chromatogram of the 12 analytes in a cord blood sample from Taiwan Birth Panel Study. The concentrations of perfluorooctanoic acid, perfluorononanoic acid, perfluorooctyl sulfonate, and perfluoroundecanoic acid in the sample were 4.14, 7.83, 9.57, and 21.2 ng/mL, respectively; other chemicals were not detected.

In terms of the maximal levels, the highest was PFUA at 105 ng/mL, and the next highest was PFNA (64.5 ng/mL) and PFOS (67.8 ng/mL), followed by Me–PFOSA–AcOH (19.6 ng/mL), PFOA (18.9 ng/mL), PFDoA (11.8 ng/mL) and PFDeA (3.81 ng/mL). Et–PFOSA–AcOH was detected in only one sample (1.41 ng/mL), and the rest compounds were less than 3.0 ng/mL (Table 5). The median concentrations for PFOA, PFNA, PFOS and PFUA excluding values below LOQ were 3.75, 7.70, 5.67 and 19.0 ng/mL, respectively. The PFOA levels in Taiwan were similar with those reported in Germany (3.4 ng/mL) [15] and US (4.9 ng/mL)[16]; however, the levels were higher than that in Canada (1.58 ng/mL) [13] and South Africa (1.3 ng/mL) [17]. Moreover, PFOS levels in Taiwan were similar with those reported in Canada (6.08 ng/mL) [13] and Germany (7.3 ng/mL) [15]. Nevertheless, the PFNA and PFUA were detected at relatively higher levels in cord bloods in Taiwan than those in US [16]. To explore the possible exposure sources of these PFCs, performing analysis in maternal blood or intake food samples in Taiwan deserved further investigation.

4. Conclusions

We have developed a high-throughput method for measuring trace levels of twelve PFCs in human plasma/serum. Because the sample volume was small at 100 μL and was diluted for three times, this would be why a simple protein precipitation worked. PFOS, PFOA, PFNA and PFUA are the most frequently detected PFCs in umbilical cord bloods among Taiwan Birth Panel Study, and the health impacts of these compounds deserve further exploration.

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