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High throughput online solid phase extraction-ultra high performance liquid chromatography-tandem mass spectrometry method for polyfluoroalkyl phosphate esters, perfluoroalkyl phosphonates, and other perfluoroalkyl substances in human serum, plasma, and whole blood

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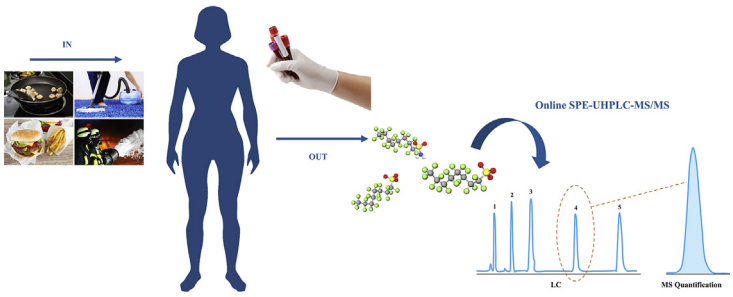
HIGHLIGHTS

- Only 50 μL serum, plasma, or whole blood was used for determination of 25 PFASs.
- The method allowed simultaneous analysis of PFSAs, PFCAs, FOSAs, PAPs, and PFPAs.
- The sample preparation was limited to a protein precipitation by methanol.
- The total method run time was only 14 min using online SPE-UHPLC-MS/MS.
- The method was successfully applied to human serum, plasma, and whole blood.

GRAPHICAL ABSTRACT



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ABSTRACT

A rapid, sensitive and reliable method was developed for the determination of a broad range of poly- and perfluoroalkyl substances (PFASs) in various blood matrices (serum, plasma, and whole blood), and uses only 50 μL of sample material. The method consists of a rapid protein precipitation by methanol followed by high throughput online solid phase extraction (SPE), ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), and negative electrospray ionization detection. The method was developed for simultaneous determination of twenty-five PFASs, including polyfluoroalkyl phosphate esters (PAPs; 6:2, 8:2, 6:2/6:2, and 8:2/8:2), perfluoroalkyl phosphonates (PFPAs; C_6, C_8, and C_{10}), perfluoroalkyl sulfonates (PFSAs; C_4, C_6, C_7, C_8, and C_{10}), perfluoroalkyl carboxylates (PFCAs; C_5–C_{14}), and perfluoroalkyl sulfonamides (FOSAs; C_8, *N*-methyl, and *N*-ethyl). High linearity of matrix-matched calibration standards (correlation coefficients, $R = 0.99$–0.999) were obtained in the range of 0.006–45 ng mL^{-1} blood. Excellent sensitivity was achieved with method detection limits (MDLs) between 0.0018 and 0.09 ng mL^{-1}, depending on the compound and matrix. The method was validated for serum, plasma, and whole blood (n = 5 + 5) at six levels in the range 0.0180–30 ng mL^{-1}.

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The accuracy (n = 5) was on average 102± 12%. The intermediate precision (n = 10) ranged from 2 to 40% with an average between-batch of analyses difference of 10± 10%. Two human serum samples from a former interlaboratory comparison were analyzed and the differences between the applied method and the consensus values were below ≤22% (n = 5). The method was also successfully applied to samples of human plasma and whole blood with coefficients of variation in the range 0.8–15.2% (n = 5).

1. Introduction

Poly- and perfluoroalkyl substances (PFASs) comprise a large group of synthetic organic compounds that have been manufactured and applied in numerous industrial and commercial products due to their unique physicochemical properties. PFASs have been a cause for increasing global concern since they have been reported to persist in the environment and bioaccumulate in both humans and animals, and are of toxicological concern [1–4]. The two most frequently studied PFASs are perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) which belong to the groups of perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs), respectively. Because of the growing concern for these groups of PFASs, the main manufacturer, the 3 M Company, voluntarily phased out the production of PFOS and related compounds during 2000–2002 while providing shorter chain PFASs as replacements [5]. In 2009, PFOS was included as a persistent organic pollutant (POP) in the Stockholm Convention [6]. Moreover, a PFOA stewardship programme was committed to phasing out PFOA and longer chain PFCAs by 2015 [7]. In 2015, a proposal to list PFOA, its salts, and PFOA-related substances in the Stockholm Convention was submitted by the European Union [8], and the United States Environmental Protection Agency is reviewing the substitutes for PFOA, PFOS, and other long-chain PFASs [9].

Because of these actions, decreasing concentrations of PFOS and PFOA have been observed in human blood, while for other PFASs increasing trends have been observed [10–14]. Nevertheless, a study measuring the total organic fluorine in human blood reported that even though known PFSAs and PFCAs continue to make up a large fraction of the organic fluorine found in blood, 15–70% of the total organic fluorine is not accounted for [15]. Other fluorinated chemicals with wide commercial applications may contribute to an unknown percentage of the organofluorine in human blood. Further, the industry tends to replace restricted chemicals with similar non-restricted ones, and thus further research to characterize and assess levels of a broad range of PFASs, in human blood matrices are needed.

In addition, the potential contribution of precursor compounds to the total exposure to certain PFASs has gained considerable attention [16,17]. Polyfluoroalkyl phosphate esters (PAPs) belong to the fluorotelomer-based PFCA precursor classes and biotransformation from PAPs to PFCAs has previously been observed [18,19]. PAPs are used as greaseproof agents in food packaging materials [20,21]. They have been identified in paper food packaging [22,23], and their ability to migrate into food have been demonstrated [23]. Moreover, PAPs have been suggested to contribute to the indirect exposure to PFCAs [24].

Another group of PFASs that has recently emerged as an understudied group of PFASs is perfluoroalkyl phosphonates (PFPAs) [25]. PFPAs have contributed to widespread contamination of surface waters, tap water, wastewaters, and house dust [26]. They are used as a wetting agent in household cleaning products and defoaming agents in pesticide formulations [21].

Most studies on levels of PFASs in humans have been conducted on serum or plasma. However, especially for the emerging PFASs, very little is known about the distribution of these compounds in different blood matrices, and this knowledge is of high importance when evaluating the exposure to PFASs. Even though an extensive number of studies on the determination of PFOS, PFOA and some PFASs in human serum and plasma have been published. To our knowledge no studies have determined a broad range of PFASs, including PFCAs, PFSAs, perfluoroalkyl sulfonamides (FOSAs), PAPs, and PFPAs simultaneously, in addition with no studies have determined this broad range of PFASs using the same method for various blood matrices (serum, plasma, and whole blood). This is because the physicochemical properties of PFASs are different, and they can even vary within in the same class of compounds [21,27]. These differences represent an analytical challenge when a multicomponent method is to be developed. The present method for determination of PFCAs, PFSAs as well as some FOSAs, PAPs and PFPAs in blood matrix is based on an ion-pairing method [24,28,29], modified from a method established by Hansen et al., in 2001 for the determination of four PFASs (PFOS, PFOA, PFHxS, and PFOSA) in serum [30]. Moreover, the recently published temporal trend studies of PFASs utilized two different extraction methods and analytical conditions in order to determine PAPs and other PFASs in serum [17]. For large sample series, it is especially advantageous to use a method which includes a wide range of compounds, as it would save time, costs and sample amount.

The aim of this study was to develop a rapid, sensitive, and reliable method applicable for large-scale biomonitoring of twenty-five different PFASs in human serum, plasma, and whole blood. The included PFASs represent five different groups of compounds; PAPs (6:2, 8:2, 6:2/6:2, and 8:2/8:2), PFPAs (C_6, C_8, and C_{10}), PFSAs (C_4, C_6, C_7, C_8, and C_{10}), PFCAs (C_5–C_{14}), and FOSAs (C_8, *N*-methyl, and *N*-ethyl). An online solid phase extraction (SPE) and ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) method were developed based on prior success in the analysis of PFCAs, PFSAs, and FOSAs in serum using online SPE with column-switching LC technique [31]. The online SPE technique allowed large sample volume injection and rapid analysis. In addition, applying online SPE in the method results in low sample contamination due to limited sample preparation, and good reproducibility. The present method is the first PFASs methodology that can determine a broad range of PFAS target compounds in different blood matrices and without sacrificing throughput. The method was validated for serum, plasma, and whole blood and successfully applied to a selection of human blood samples.

2. Materials and methods

2.1. Chemicals

A list of the twenty-five included PFASs and eleven isotope-labeled internal standards with their abbreviations and formulas are given in Table 1. All native and isotope-labeled PFASs were obtained from Wellington Laboratories (Guelph, Ontario, Canada),

and were delivered in amber glass ampoules in a concentration of 50 μg mL^{-1} in methanol (>98% purity). Formic acid (eluent additive for LC-MS, ~98%) and ammonium hydroxide (≥25% NH_3 in H_2O) were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥99.9% purity), methanol (≥99.99% purity), and water were obtained from J.T. Baker (Deventer, The Netherlands) (Supplementary material, Table S-1).

2.2. Standard solutions

A stock solution of each of the native and isotope-labeled PFASs was prepared in methanol at a concentration of 1 μg mL^{-1}. Further, working solutions including all 25 native PFASs were prepared in methanol at concentrations of 0.01, 0.05, 0.25, 1.0, 5.0, and 25 ng mL^{-1}. The 11 isotope-labeled internal standards were included in a working solution in methanol at 5 ng mL^{-1}. All the standard solutions were stored in amber glass ampoules at −20 °C. All amber glass ampoules were rinsed with methanol and then heated at 450 °C for 4 h before use.

2.3. Matrix-matched calibration standards and samples

The serum, plasma, and whole blood method was applied using matrix-matched calibration standards prepared with newborn calf serum (Invitrogen, Oslo, Norway), calf plasma (Lampire Biological Labs, Pipersville, USA) and calf whole blood (Lampire Biological Labs, Pipperville, USA), respectively. To assess the applicability, the established methods for serum, plasma, and whole blood were applied to samples of human serum (Interlaboratory comparison study organized by Institute National de Sante Publique du Quebec, Canada for the Arctic Monitoring and Assessment Programme, AMAP), human plasma (in-house quality control sample), and human whole blood (in-house quality control samples), respectively. All the blood samples were stored in polypropylene tubes at −20 °C until analysis.

Table 1
Abbreviations, empirical formulas, and MRM data acquisition parameters of PFASs.

Target compound	Abbreviation	Molecular ion	MRM data acquisition			
			Precursor ion (*m/z*)	Product ion (*m/z*)		Collision energy (V)**
				Quantifier	Qualifier	
Native compounds						
Polyfluoroalkyl phosphate esters (PAPs)						
6:2 polyfluoroalkyl phosphate monoester[a]	6:2 PAP	$[C_8H_5F_{13}O_4P]^-$	443	97	79	16 (72)
8:2 polyfluoroalkyl phosphate monoester[a]	8:2 PAP	$[C_{10}H_5F_{17}O_4P]^-$	543	97	79	20 (60)
6:2 polyfluoroalkyl phosphate diester[a]	6:2 diPAP	$[C_{16}H_8F_{26}O_4P]^-$	789	443	97	20 (28)
8:2 polyfluoroalkyl phosphate diester[b]	8:2 diPAP	$[C_{20}H_8F_{34}O_4P]^-$	989	543	97	24 (36)
Perfluoroalkyl phosphonates (PFPAs)						
Perfluorohexylphosphonate[e]	PFHxPA	$[C_6HF_{13}O_3P]^-$	399	79		56
Perfluorooctylphosphonate[e]	PFOPA	$[C_8HF_{17}O_3P]^-$	499	79		72
Perfluorodecylphosphonate[e]	PFDPA	$[C_{10}HF_{21}O_3P]^-$	599	79		44
Perfluoroalkyl sulfonates (PFSAs)						
Perfluorobutanesulfonate[c]	PFBS	$[C_4F_9O_3S]^-$	299	80	99	32 (32)
Perfluorohexanesulfonate[c]	PFHxS	$[C_6F_{13}O_3S]^-$	399	80	99	60 (40)
Perfluoroheptanesulfonate[d]	PFHpS	$[C_7F_{15}O_3S]^-$	449	80	99	56 (44)
Perfluorooctanesulfonate[d]	PFOS	$[C_8F_{17}O_3S]^-$	499	99	80	44 (56)
Perfluorodecanesulfonate[d]	PFDS	$[C_{10}F_{21}O_3S]^-$	599	80	99	64 (52)
Perfluoroalkyl carboxylates (PFCAs)						
Perfluoropentanoate[e]	PFPeA	$[C_5F_9O_2]^-$	263	219		4
Perfluorohexanoate[e]	PFHxA	$[C_6F_{11}O_2]^-$	313	269		4
Perfluoroheptanoate[f]	PFHpA	$[C_7F_{13}O_2]^-$	363	319		4
Perfluorooctanoate[f]	PFOA	$[C_8F_{15}O_2]^-$	413	369		4
Perfluorononanoate[g]	PFNA	$[C_9F_{17}O_2]^-$	463	419		4
Perfluorodecanoate[h]	PFDA	$[C_{10}F_{19}O_2]^-$	513	469		4
Perfluoroundecanoate[i]	PFUnDA	$[C_{11}F_{21}O_2]^-$	563	519		8
Perfluorododecanoate[j]	PFDoDA	$[C_{12}F_{23}O_2]^-$	613	569		8
Perfluorotridecanoate[j]	PFTrDA	$[C_{13}F_{25}O_2]^-$	663	619		8
Perfluorotetradecanoate[j]	PFTeDA	$[C_{14}F_{27}O_2]^-$	713	669		8
Perfluoroalkyl sulfonamides (FOSAs)						
Perfluorooctanesulfonamide[k]	PFOSA	$[C_8HF_{17}NO_2S]^-$	498	78	48	36 (80)
N-methyl perfluorooctanesulfonamide[k]	MeFOSA	$[C_9H_3F_{17}NO_2S]^-$	512	169	219	24 (24)
N-ethyl perfluorooctanesulfonamide[k]	EtFOSA	$[C_{10}H_5F_{17}NO_2S]^-$	526	169	219	28 (24)
Mass-labeled internal standards						
$^{13}C_4$-6:2 polyfluoroalkyl phosphate diester	$^{13}C_4$-6:2 diPAP	$[^{13}C_4^{12}C_{12}H_8F_{26}O_4P]^-$	793	445	97	20 (32)
$^{13}C_4$-8:2 polyfluoroalkyl phosphate diester	$^{13}C_4$-8:2 diPAP	$[^{13}C_4^{12}C_{16}H_8F_{34}O_4P]^-$	993	545	97	20 (36)
$^{18}O_2$-perfluorohexanesulfonate	$^{18}O_2$-PFHxS	$[C_6F_{13}^{18}O_2^{16}OS]^-$	403	84	103	40 (36)
$^{13}C_4$-perfluorooctanesulfonate	$^{13}C_4$-PFOS	$[^{13}C_4^{12}C_4F_{17}O_3S]^-$	503	80	99	60 (48)
$^{13}C_2$-perfluorohexanoate	$^{13}C_2$-PFHxA	$[^{13}C_2^{12}C_4F_{11}O_2]^-$	315	270		4
$^{13}C_4$-perfluorooctanoate	$^{13}C_4$-PFOA	$[^{13}C_4^{12}C_4F_{15}O_2]^-$	417	372		4
$^{13}C_5$-perfluorononanoate	$^{13}C_5$-PFNA	$[^{13}C_5^{12}C_4F_{17}O_2]^-$	468	423		4
$^{13}C_2$-perfluorodecanoate	$^{13}C_2$-PFDA	$[^{13}C_2^{12}C_8F_{19}O_2]^-$	515	470		8
$^{13}C_2$-perfluoroundecanoate	$^{13}C_2$-PFUnDA	$[^{13}C_2^{12}C_9F_{21}O_2]^-$	565	520		4
$^{13}C_2$-perfluorododecanoate	$^{13}C_2$-PFDoDA	$[^{13}C_2^{12}C_{10}F_{23}O_2]^-$	615	570		8
d_3-*N*-methyl perfluorooctanesulfonamide	d_3-N-MeFOSA	$[C_9D_3F_{17}NO_2S]^-$	515	169	219	28 (24)

* Corresponding Internal standard used; a = $^{13}C_4$-6:2 diPAP, b = $^{13}C_4$-8:2 diPAP, c = $^{18}O_2$-PFHxS, d = $^{13}C_4$-PFOS, e = $^{13}C_2$-PFHxA, f = $^{13}C_4$-PFOA, g = $^{13}C_5$-PFNA, h = $^{13}C_2$-PFDA, i = $^{13}C_2$-PFUnDA, j = $^{13}C_2$-PFDoDA, k = d_3-N-MeFOSA.
** Collision energy for quantifier and in the parentheses for the qualifier.

2.4. Sample preparation

Frozen blood samples (serum, plasma or whole blood) were allowed to thaw at room temperature and then homogenized using a whirling mixer. An aliquot of 50 μL thawed blood was transferred into a 2 mL polypropylene centrifuge tube. The matrix-matched calibration standards were made by spiking the blood with 30–90 μL of the PFASs standard solutions. 90 μL of 5 ng mL^{-1} PFASs internal standard were added to matrix-matched calibration standards and samples, along with 0–90 μL methanol to make up a total volume of 180 μL methanol, and then mixed on a whirling mixer. The tubes were centrifuged for 40 min at 14000 RPM at 20 °C to precipitate protein and any suspended particles. The supernatants were subsequently transferred into polypropylene vials (250 μL screw top vial, Agilent Technologies, Palo Alto, CA, USA) for analyses by online SPE-UHPLC-MS/MS.

2.5. Method validation and quality control

For the method validation, matrix-matched calibration standards were prepared at twelve different concentrations corresponding to 0.006, 0.012, 0.03, 0.06, 0.15, 0.3, 0.6, 1.2, 3.0, 6.0, 15, and 45 ng mL^{-1} blood (number of replicates were 5, 5, 3, 5, 3, 5, 3, 5, 3, 5, 3, and 3, respectively). Accordingly, the accuracy and repeatability of the method were examined at six different concentrations ($n = 5$), namely 0.018, 0.90, 0.45, 1.8, 9.0, and 30 ng mL^{-1} blood.

Two to four months after the initial validation, new matrix-matched calibration standards were prepared by the same procedure to investigate the intermediate precision and possible differences in the accuracy between the two-time points. Analytical quality control was performed by including three procedural blanks (90 μL of 5 ng mL^{-1} PFASs internal standard with 90 μL methanol) and three non-spiked calf serum, calf plasma or calf whole blood samples to monitor the PFAS background levels in the blood matrices.

2.6. Online SPE-UHPLC-MS/MS analysis

All analyses were performed using online SPE-UHPLC-MS/MS with an Agilent 1290 UHPLC interfaced to an Agilent 6490 Triple Quadrupole (QqQ) mass spectrometer (MS/MS) equipped with an Agilent Jet-Stream electrospray ionization (ESI) interface (Agilent Technologies, Palo Alto, CA, USA). The column switching system consisted of two columns. A Betasil C8, 10 mm × 3 mm, 5 μm particle size (Thermo scientific, CA, USA) column in a holder (Thermo scientific, CA, USA) was used as online SPE column, and an Agilent ZORBAX Eclipse Plus C18, UHPLC, 50 mm × 2.1 mm, 1.8 μm particle size (Agilent Technologies, California, USA) as analytical column. The columns were maintained at a temperature of 25 °C, and 40 °C for online SPE and analytical column, respectively. The column switching system included a two-position six-port valve (Supplementary material, Fig. S-1).

An aliquot of 80 μL of prepared standard or sample were injected by a CTC PAL autosampler (operated at 4 °C) and loaded onto SPE column with 0.1 M formic acid in water and methanol (95/5,v/v) using the loading pump (1260 Infinity Quaternary pump VL) at a constant flow rate of 1.5 mL min^{-1} (Supplementary material, Fig. S-2). The samples were passed through a stainless steel screen filter (1/8 inches, 2 μm) held in a 316 stainless union (1/16 inches, 0.25 mm bore) both obtained from Valco (Houston, TX,USA). This automatic filtration was installed in front of the column switching system to avoid system clogging, and significantly improved the robustness, allowing for hundreds of blood sample injections before filter replacement was necessary. After the samples passed through the filter, the analytes were trapped on SPE column. Loading and cleanup of the samples required a relatively long time (3 min), after which the position of switching valve was changed to connect the SPE column with the analytical column. The eluting pump (Agilent 1290 Infinity Quaternary Pump) back flushed the analytes from the SPE column, using 0.15% ammonium hydroxide in water (pH 8–9) and acetonitrile (90/10, v/v) at a constant mobile phase flow rate of 0.2 mL min^{-1}. Target analytes were re-focused and eluted using a gradient that increased the acetonitrile to 100% over 4 min. Eight minutes after sample injection the switching valve was automatically switched back to an original position allowing the SPE column to be washed and then reconditioned for 6 min. The mobile phase (100% acetonitrile) continuously passed through the analytical column for 3.5 min and was then ramped down to 10% acetonitrile, and allowed to recondition for 3.5 min prior to the next sample injection. All the analytes were eluted within 9 min. To be able to clean the system and recondition the columns the total run time was set to 14 min. This minimized the carryover and avoided high back pressure when running large samples series.

The mass spectrometer was operated in ESI negative ionization mode. The source-dependent parameters were optimized for PFASs determination; gas temperature was 230 °C with 20 L min^{-1} flow rate; sheath gas heater was set to 400 °C with 10 L min^{-1} flow rate; the capillary voltage was 3500 V and the nebulizer gas was set to 40 psi. The mass analyzer was used in multiple reactions monitoring (MRM) mode. The MRM transitions are given in Table 1. One additional product ion was monitored for all compounds except for PFPAs and PFCAs, for which only one product ion was formed. The precursor and product ions for PFPAs and PFSAs of the same carbon chain length were identical or almost identical, but the compounds were chromatographically separated by the UHPLC column.

3. Results and discussion

3.1. Development of the online SPE-UHPLC-MS/MS method

The chromatographic separation of PFASs was optimized based on an existing method [31]. However, several modifications were necessary in order to extend the method to allow for determination of PAPs and PFPAs. Further, the method development included optimization of the method to make it suitable for analysis of whole blood and plasma in addition to serum. In the following, the term blood is used for all three matrices.

Two ZORBAX Eclipse Plus analytical columns with different stationary phases (C8 and C18) were tested with various mobile phases, flow rates, and gradient programs. The mobile phases examined were combinations of acetonitrile or methanol with ammonium acetate, ammonium formate, formic acid, and ammonium hydroxide. Using a C18 column and mobile phase containing 0.15% ammonium hydroxide in water and acetonitrile as the organic component, considerably improved both the chromatographic resolution and the MS response for PAPs, PFPAs as well as some other PFASs, when compared to using the conditions described in the existing method [31]. The improvements are most probably attributable to different pK_a values of PAPs and PFPAs compared to that of the other PFASs.

The mobile phase program was developed to prevent the blood from clogging and/or accumulating on the column. The method also successfully enriched the analytes on the SPE column. An initial mobile phase combination of 95% of 0.1 M formic acid in water and 5% methanol at 1.5 mL min^{-1} was found to be the most effective in terms of sample cleanup and retention without breakthrough of the analytes. Two different Betasil stationary phases (C8 and C18) were tested for the selection of SPE column. The Betasil C8 was chosen because it provided better peak shapes and increased sensitivity for

Table 2
Detection limits and linearities of the method for the selected PFASs.

	Method limits (ng mL^{-1} blood)						Calibration curves					
	Estimated MDL			Estimated MQL			R*			Range (ng mL^{-1} blood)		
	Serum	Plasma	W. Blood	Serum	Plasma	W. Blood	Serum	Plasma	W. Blood	Serum	Plasma	W. Blood
6:2 PAP	0.09	0.045	0.045	0.3	0.15	0.15	0.996^{f}	0.996^{e}	0.999^{e}	0.3–45	0.15–45	0.15–45
8:2 PAP	0.045	0.018	0.018	0.15	0.06	0.06	0.995^{e}	0.996^{d}	0.996^{d}	0.15–45	0.06–45	0.06–45
6:2 diPAP	0.018	0.018	0.009	0.06	0.06	0.03	0.998^{d}	0.997^{d}	0.997^{c}	0.06–45	0.06–45	0.03–45
8:2 diPAP	0.009	0.018	0.009	0.03	0.06	0.03	0.995^{e}	0.996^{d}	0.997^{c}	0.15–45	0.06–45	0.03–45
PFHxPA	0.045	0.0018	0.009	0.15	0.006	0.03	0.998^{e}	0.997^{a}	0.998^{c}	0.15–45	0.006–45	0.03–45
PFOPA	0.009	0.018	0.045	0.03	0.06	0.15	0.998^{c}	0.996^{d}	0.999^{e}	0.03–45	0.06–45	0.15–45
PFDPA	0.009	0.0036	0.018	0.03	0.012	0.06	0.996^{f}	0.998^{b}	0.998^{d}	0.3–45	0.012–45	0.06–45
PFBS	0.009	0.018	0.009	0.03	0.06	0.03	0.996^{e}	0.990^{e}	0.992^{e}	0.15–45	0.15–45	0.15–45
PFHxS	0.0036	0.0018	0.0018	0.012	0.006	0.006	0.999^{b}	0.996^{c}	0.998^{a}	0.012–45	0.03–45	0.006–45
PFHpS	0.0036	0.009	0.0036	0.012	0.03	0.012	0.998^{b}	0.997^{c}	0.995^{b}	0.012–45	0.03–45	0.012–45
PFOS	0.009	0.009	0.009	0.03	0.03	0.03	0.998^{c}	0.998^{e}	0.998^{c}	0.03–45	0.15–45	0.03–45
PFDS	0.0018	0.009	0.0018	0.006	0.03	0.006	0.998^{a}	0.996^{d}	0.998^{a}	0.006–45	0.06–45	0.006–45
PFPeA	0.09	0.09	0.045	0.3	0.3	0.15	0.998^{f}	0.996^{f}	0.995^{e}	0.3–45	0.3–45	0.15–45
PFHxA	0.045	0.045	0.09	0.15	0.15	0.3	0.997^{e}	0.997^{e}	0.998^{f}	0.15–45	0.15–45	0.3–45
PFHpA	0.045	0.045	0.045	0.15	0.15	0.15	0.996^{e}	0.995^{e}	0.995^{e}	0.15–45	0.15–45	0.15–45
PFOA	0.018	0.009	0.045	0.06	0.03	0.15	0.996^{d}	0.998^{c}	0.997^{e}	0.06–45	0.03–45	0.15–45
PFNA	0.009	0.018	0.009	0.03	0.06	0.03	0.993^{c}	0.993^{d}	0.997^{c}	0.03–45	0.06–45	0.03–45
PFDA	0.045	0.009	0.009	0.15	0.03	0.03	0.995^{e}	0.996^{c}	0.995^{c}	0.15–45	0.03–45	0.03–45
PFUnDA	0.009	0.018	0.009	0.03	0.06	0.03	0.998^{c}	0.997^{d}	0.996^{c}	0.03–45	0.06–45	0.03–45
PFDoDA	0.0036	0.0036	0.0018	0.012	0.012	0.006	0.998^{c}	0.998^{b}	0.996^{a}	0.03–45	0.012–45	0.006–45
PFTrDA	0.018	0.0018	0.018	0.06	0.006	0.06	0.997^{d}	0.999^{a}	0.998^{d}	0.06–45	0.006–45	0.06–45
PFTeDA	0.009	0.09	0.018	0.03	0.3	0.06	0.997^{c}	0.995^{f}	0.993^{d}	0.03–45	0.3–45	0.06–45
PFOSA	0.0018	0.009	0.0018	0.006	0.03	0.006	0.997^{a}	0.995^{c}	0.997^{a}	0.006–45	0.03–45	0.006–45
MeFOSA	0.045	0.009	0.009	0.15	0.03	0.03	0.996^{e}	0.995^{c}	0.995^{c}	0.15–45	0.03–45	0.03–45
EtFOSA	0.045	0.009	0.009	0.15	0.03	0.03	0.996^{e}	0.996^{c}	0.996^{c}	0.15–45	0.03–45	0.03–45

*Number of calibration points used; a = 48, b = 43, c = 38, d = 35, e = 30, f = 27 points.

the PFASs with the short-chain lengths.

The protein precipitated (PPT) sample composition was evaluated to find optimal conditions for the online SPE-UHPLC-MS/MS system. Initially, the PPT samples were diluted with 0.1 M formic acid in water before injection. This acidification gave high peak areas for the less retained PFASs, but the intensity for the PAPs and PFPAs were poor. Because of this, different ratios of non-acidified water and methanol were tested, with the highest peak areas observed when adding no water.

When two or more MRM transitions were identified, the most intense signal was chosen for the quantitative determination (quantifier), while the second product ion was employed to confirm the identification (qualifier). However, due to a known interference with PFOS for the *m/z* transition 499 > 80, the *m/z* transition 499 > 99 was chosen as the quantifier [32]. Further, the *m/z* transition 499 > 99 gave more accurate results in the spiking experiments with lower background compared to using 499 > 80.

3.2. Validation of the developed method

3.2.1. Linearities and method detection limits

Linearity was examined using concentration-weighted (*1/concentration*). To compensate for possible loss of analytes, ion suppression or ion enhancement, appropriate internal standards were selected based on retention time, molecular structure, and accuracy obtained in the spiking experiments (Table 1). For PFPAs no commercial internal standards were available. Thus, $^{13}C_2$PFHxA was selected as an appropriate internal standard for all PFPAs based on retention time, and the accuracy obtained in the spiking experiments. The linearities of calibration curves were in the range 0.006 and 45 ng mL^{-1}, depending on the compound and blood matrix. Non-spiked samples of serum, plasma, and whole blood with added internal standards were used to examine the background levels of PFASs in the blood matrices. Very low levels of a few PFASs (e.g. PFBS, PFHxA, and PFOS) were observed in some of the replicates of the blanks, but this was considered to be negligible because the concentrations were typically less than half of the amount for the lowest calibration level.

Details of the linearity and the concentration ranges are presented in Table 2. The achieved method detection limits (MDLs) and method quantification limits (MQLs) are also summarized in Table 2. The estimated MDLs and MQLs were found by extrapolation using the matrix-matched calibration standards and defined as a signal to noise ratio (S/N) of 3 and 10, respectively. As matrix-matched calibration standards were used, the estimated MDLs and MQLs were directly related to the sensitivity of the overall method. The MDLs obtained ranged between 0.0018 and 0.09 ng mL^{-1} in serum, plasma, and whole blood. The MDLs obtained in this present method were comparable to what has been described in the existing method used in our laboratory [31], despite the lower sample volume (50 μL vs 150 μL) and total injection volume (80 μL vs 400 μL) in the present method. The MDLs in the present method were also lower than in other online SPE column switching methods (Supplementary material, Table S-2). For example, Mocsh et al., in 2010 reported MDLs in the range 0.03–0.1 ng mL^{-1} using online SPE-LC-MS/MS for determination of seven PFASs in serum [33]. Gosetti et al., in 2010 developed an online SPE-UHPLC-MS/MS method for the determination of nine PFASs in serum and plasma and obtained MDLs ranging from 0.009 to 0.75 ng mL^{-1} [34]. Kato et al., in 2011 obtained MDLs in the range 0.1–0.2 ng mL^{-1} in an online SPE-LC-MS/MS method for determination of 13 PFASs in serum and cord serum [35]. Also, a column switching-UHPLC-MS/MS method established for 19 PFASs in human serum by Salihovic et al., in 2013 reported higher MDLs than this present method (0.01–0.17 ng mL^{-1}) [36]. In addition, the MQLs of this present method range from 0.006 to 0.3 ng mL^{-1} in serum, plasma, and whole blood. The MQLs in this present method are also lower when compared with the recently proposed online SPE-LC-MS/MS method for the determination of 6 PFASs in serum by Bartolome et al., in 2016 [37].

Table 3
Accuracy (Acc., %) with repeatability (Rep., % cv) in parenthesis for serum, plasma, and whole blood spiked at six different concentration of PFASs.

a. spiking levels of 0.0180, 0.090, and 0.450 ng mL^{-1} blood

	0.0180 ng mL^{-1} blood, Acc. (Rep.)			0.090 ng mL^{-1} blood, Acc. (Rep.)			0.450 ng mL^{-1} blood, Acc. (Rep.)		
	Serum	Plasma	Whole blood	Serum	Plasma	Whole blood	Serum	Plasma	Whole blood
6:2 PAP							103 (3.9)	111 (7.8)	90 (5.6)
8:2 PAP					149 (16)	140 (13)	96 (17)	97 (2.2)	86 (9.8)
6:2 diPAP				131 (36)	116 (33)	145 (25)	91 (14)	101 (16)	124 (12)
8:2 diPAP					111 (33)	87 (55)	101 (9.4)	106 (11)	100 (13)
PFHxPA		103 (14)			96 (10)	102 (51)	82 (14)	97 (4.2)	114 (4.4)
PFOPA				97 (3.2)	135 (7.0)		89 (9.8)	100 (3.7)	103 (9.7)
PFDPA		128 (13)			101 (8.9)	105 (9.9)	124 (6.2)	103 (2.1)	115 (6.7)
PFBS							121 (7.8)	134 (14)	84 (9.8)
PFHxS	102 (5.5)		96 (37)	95 (7.2)	112 (28)	95 (12)	98 (5.8)	105 (7.2)	99 (5.4)
PFHpS	116 (26)		97 (2.9)	98 (17)	101 (23)	91 (12)	90 (13)	100 (7.7)	104 (10)
PFOS				107 (24)		105 (18)	109 (5.1)	119 (21)	103 (4.9)
PFDS	131 (13)		156 (26)	92 (24)	95 (30)	107 (7.8)	95 (8.4)	105 (14)	114 (11)
PFPeA							95 (6.5)	101 (9.2)	95 (5.8)
PFHxA							93 (16)	99 (5.4)	88 (5.6)
PFHpA							106 (10)	113 (10)	
PFOA				111 (7.3)	105 (7.8)		103 (12)	102 (13)	65 (18)
PFNA				118 (21)	120 (22)	113 (9.9)	97 (7.4)	100 (13)	82 (8.4)
PFDA					96 (14)	89 (23)	107 (10)	102 (11)	105 (4.1)
PFUnDA				119 (15)	112 (13)	105 (12)	98 (7.6)	102 (12)	99 (8.7)
PFDoDA		110 (55)	107 (7.0)	115 (15)	103 (9.8)	92 (21)	101 (4.1)	96 (14)	99 (8.1)
PFTrDA		118 (15)		138 (6.9)	106 (2.7)	104 (15)	105 (5.1)	109 (5.5)	93 (6.8)
PFTeDA				111 (11)		121 (9.7)	107 (6.1)	144 (9.5)	81 (11)
PFOSA	147 (10)		72 (50)	110 (20)	106 (13)	82 (24)	110 (11)	108 (7.6)	93 (14)
MeFOSA					109 (25)	117 (8.8)	112 (6.2)	104 (11)	116 (9.1)
EtFOSA					129 (13)	108 (17)	123 (9.8)	93 (21)	93 (15)

b. Spiking levels of 1.80, 9.0, and 30 ng mL^{-1} blood

	1.80 ng mL^{-1} blood, Acc. (Rep.)			9.0 ng mL^{-1} blood, Acc. (Rep.)			30 ng mL^{-1} blood, Acc. (Rep.)		
	Serum	Plasma	Whole blood	Serum	Plasma	Whole blood	Serum	Plasma	Whole blood
6:2 PAP	98 (3.6)	102 (8.3)	80 (4.6)	96 (4.5)	109 (14)	95 (7.3)	96 (4.2)	112 (7.3)	95 (3.7)
8:2 PAP	103 (6.6)	101 (4.5)	66 (3.1)	104 (4.4)	100 (12)	89 (5.3)	95 (8.6)	103 (5.7)	86 (4.5)
6:2 diPAP	91 (5.5)	95 (6.9)	104 (10)	104 (5.8)	100 (15)	106 (4.0)	100 (2.0)	95 (8.8)	100 (11)
8:2 diPAP	94 (12)	92 (12)	92 (6.3)	95 (0.8)	105 (8.9)	103 (9.8)	92 (7.1)	92 (14)	102 (7.9)
PFHxPA	76 (12)	97 (4.2)	128 (4.1)	96 (6.6)	106 (3.9)	133 (7.6)	103 (13)	102 (2.0)	136 (3.3)
PFOPA	84 (6.4)	92 (5.1)	102 (2.6)	100 (5.5)	96 (4.0)	106 (2.7)	103 (2.4)	96 (4.8)	103 (1.1)
PFDPA	103 (3.5)	105 (5.0)	116 (8.9)	113 (2.4)	100 (5.8)	110 (6.0)	106 (5.2)	99 (6.6)	105 (8.2)
PFBS	98 (5.0)	102 (11)	89 (9.9)	89 (4.8)	101 (15)	78 (11)	95 (5.7)	113 (12)	95 (5.0)
PFHxS	92 (7.8)	91 (8.5)	108 (10)	96 (4.8)	96 (3.8)	98 (6.9)	102 (4.5)	99 (11)	101 (3.3)
PFHpS	88 (1.6)	104 (4.5)	104 (5.3)	92 (14)	106 (4.9)	91 (5.2)	96 (5.4)	106 (9.8)	101 (8.8)
PFOS	87 (3.5)	98 (17)	100 (6.4)	100 (4.4)	100 (11)	99 (3.2)	106 (6.5)	96 (5.2)	103 (7.2)
PFDS	87 (4.0)	103 (8.7)	120 (3.6)	99 (8.2)	108 (9.5)	109 (9.7)	98 (4.5)	95 (6.3)	127 (12)
PFPeA	88 (8.7)	94 (5.7)	96 (3.7)	92 (8.1)	85 (4.4)	93 (7.9)	102 (7.6)	86 (7.8)	106 (3.4)
PFHxA	98 (3.4)	99 (3.0)	112 (1.4)	104 (5.7)	98 (3.4)	102 (6.5)	98 (5.5)	100 (4.3)	104 (5.1)
PFHpA	98 (7.4)	97 (17)	92 (14)	94 (13)	94 (9.1)	97 (15)	98 (7.2)	97 (8.2)	128 (9.4)
PFOA	93 (7.1)	95 (11)	99 (5.1)	100 (6.5)	104 (7.0)	106 (9.8)	97 (5.2)	103 (8.9)	106 (2.6)
PFNA	99 (3.6)	99 (5.0)	97 (7.9)	90 (8.8)	96 (7.0)	104 (8.2)	99 (4.1)	100 (4.9)	111 (12)
PFDA	98 (1.4)	96 (7.0)	107 (13)	100 (3.3)	103 (7.9)	99 (6.1)	101 (2.1)	102 (4.4)	103 (8.2)
PFUnDA	96 (4.0)	97 (7.2)	102 (8.4)	100 (1.3)	102 (3.7)	102 (10)	100 (4.5)	100 (7.0)	99 (4.7)
PFDoDA	102 (4.7)	97 (4.1)	103 (2.9)	102 (3.2)	101 (6.0)	105 (5.1)	99 (4.5)	97 (5.6)	107 (5.0)
PFTrDA	101 (3.6)	102 (7.3)	96 (5.6)	103 (2.0)	102 (11)	95 (9.9)	101 (1.1)	103 (4.1)	99 (6.4)
PFTeDA	100 (4.5)	105 (11)	86 (3.7)	115 (2.4)	107 (6.4)	82 (9.8)	105 (4.1)	102 (6.1)	86 (8.8)
PFOSA	99 (6.3)	98 (5.5)	102 (2.1)	109 (3.3)	106 (11)	93 (8.8)	101 (6.1)	111 (6.8)	101 (12)
MeFOSA	99 (6.2)	104 (5.6)	114 (6.0)	96 (6.8)	104 (8.5)	101 (7.4)	98 (5.4)	111 (8.0)	108 (13)
EtFOSA	103 (3.1)	92 (3.7)	104 (8.6)	105 (5.8)	94 (6.2)	99 (4.3)	93 (2.5)	97 (12)	104 (14)

A number of replicates (n) = 5 for accuracy and repeatability determination.

3.2.2. Accuracy and repeatability

Accuracies were assessed by spiking calf serum, calf plasma, and calf whole blood at six concentrations (n = 5) ranging from 0.0180 to 30 ng mL^{-1} blood. The accuracies (in %) were then calculated by dividing the obtained concentration in the spiked sample by the theoretical spiked concentration and multiplying by 100. An average method accuracy of 102± 12% was obtained, including all spiking levels and blood matrices, confirming that the matrix-matched standards were appropriate for the quantification (Table 3). Most of the compounds were found in the spiked samples at 0.09 ng PFASs mL^{-1} blood, and all compounds, except PFHpA in whole blood, were detected in the spiked samples containing 0.450 ng PFASs mL^{-1} blood (Table 3 a). Accuracies between 65 and 144% were observed in the range of 0.450–30 ng mL^{-1} blood, for the majority of the analytes, the accuracies were close to 100%. A comparison of accuracies in different blood matrices (0.450–30 ng mL^{-1} blood) is illustrated in Fig. 1. As can be seen in the box plot (Fig. 1), less variation in the accuracies were observed for plasma than serum and whole blood. For some PFASs without matching isotope-labeled internal standards, accuracies between

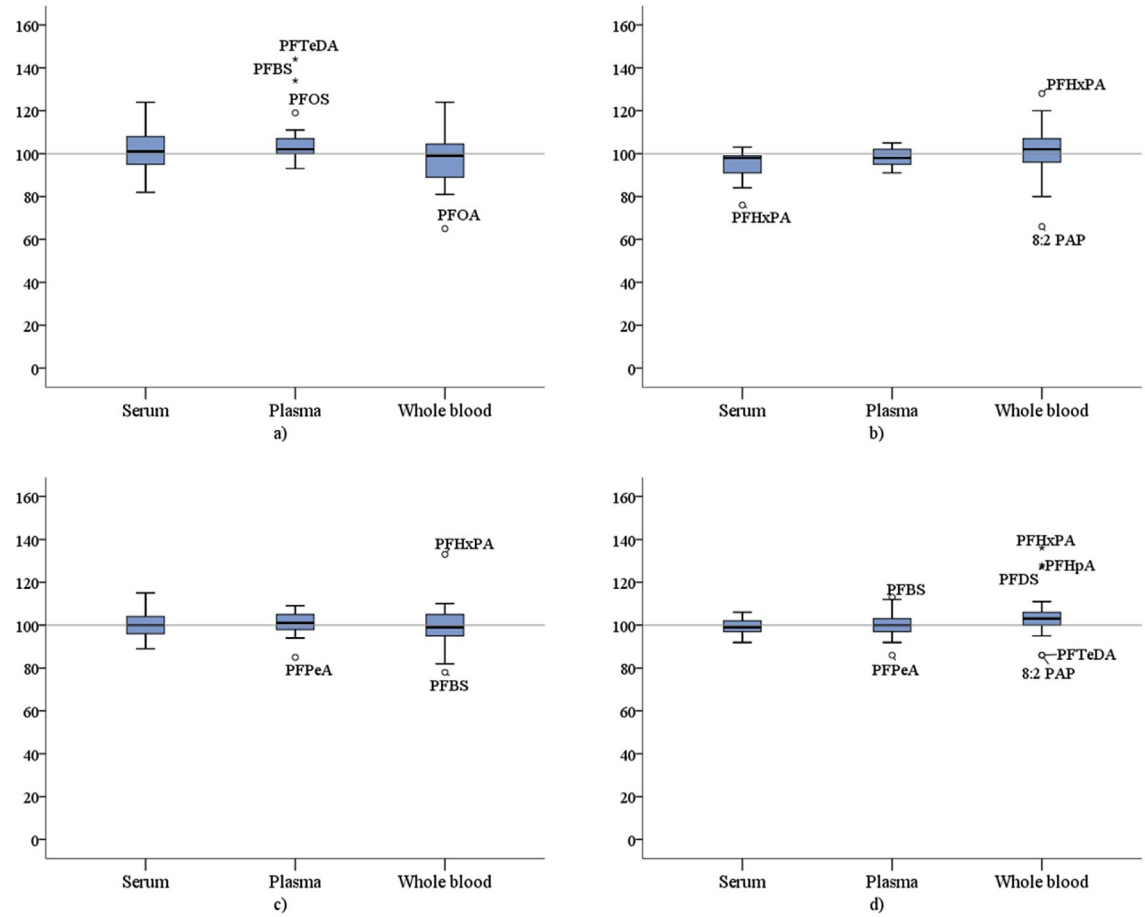

Fig. 1. Box-plot for comparison of PFASs accuracy (%) in different blood matrices a) method accuracy at 0.450 ng mL^{-1} b) method accuracy at 1.80 ng mL^{-1} c) method accuracy at 9.0 ng mL^{-1} d) method accuracy at 30 ng mL^{-1}.

124 and 144% were found, suggesting that the internal standards used over-compensates for matrix suppression. The repeatability of the method (within-run) was evaluated by the coefficients of variation for the obtained analyte concentrations (n = 5). Sufficient coefficients of variation were found, ranging from 0.8 to 21% at the concentration levels between 0.450 and 30 ng mL^{-1} blood.

3.2.3. Intermediate precision and between-batch of analyses differences

Data on the intermediate precisions of the method were obtained by analyzing samples spiked at the same concentrations at two different time points. The second experiment was performed two to four months after the initial validation for each blood matrix. The intermediate precisions were calculated as the coefficients of variation for the determined analyte concentrations from these two spiking experiments (n = 5 + 5). An average intermediate precision of 10± 5.8% was found (Table 4), ranging from 2 to 31% for concentrations from 0.450 to 30 ng mL^{-1}, depending on the spiking level and matrix. The method also showed a satisfactory between-batch of analyses difference (assessed using the normalized difference, $((X_1 - X_2)/((X_1 + X_2)/2)) \times 100$ where X_1 and X_2 are %accuracy of analyte in the first and second experiment, respectively). The average between-batch of analyses difference (%) was 10± 9.5% (Table 4). These results suggest satisfactory robustness of the method for biomonitoring purposes.

3.3. Application to human serum, plasma, and whole blood samples

The developed online SPE-UHPLC-MS/MS method was successfully applied to determine PFASs in samples of whole blood, serum, and plasma (Table 5). Two human serum samples (5 replicates) were analyzed to assess the applicability of the method for this matrix. These samples were from an interlaboratory comparison study organized by the Arctic Monitoring Assessment Program(AMAP). The determined concentrations of the respective compounds were compared to the consensus values of PFHxS, PFOS, PFHxA, PFOA, PFNA, and PFUnDA from the interlaboratory comparison study [38]. The relative difference between the concentrations of the analytes analyzed by this method and the consensus values were between 0 and 13%, except for PFNA which had a relative difference of 22%. For one of the same serum samples (AMSY1303), Huber and Brox in 2015 compared their results obtained by an SPE-UHPLC-MS/MS method with the consensus value given by AMAP and reported differences in the range 6–15% [39]. 18 of the 25 targeted PFASs were observed in the human serum samples obtained from AMAP, indicating sufficient sensitivity of the method. The coefficients of variation for the five replicates ranged from 1.5 to 20% for the detected compounds. No consensus values were available for plasma and the two whole blood samples. The coefficients of variation for all detected compounds ranged from 0.8 to 21.6%, and 2.4–12.8% for human plasma and human whole blood, respectively, demonstrating the applicability of this method.

Table 4
Intermediate precision (Int., %) and between-run differences (Diff., %) in serum, plasma, and whole blood spiked at six different PFAS concentration.

a. Spiking levels of 0.0180, 0.090, and 0.450 ng mL^{-1} blood

	0.0180 ng mL^{-1} blood						0.090 ng mL^{-1} blood						0.450 ng mL^{-1} blood					
	Serum		Plasma		Whole blood		Serum		Plasma		Whole blood		Serum		Plasma		Whole blood	
	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.
6:2 PAP																	8.4	10
8:2 PAP											25	37	16	21	8.1	14	9.3	4.5
6:2 diPAP							30	24	22	8.5	38	54	12	1.9	14	13	21	37
8:2 diPAP											40	27	14	6.3	8.8	5.8	19	24
PFHxPA											34	1.4	14	14	17	30	8.6	14
PFOPA							5.3	7.5	5.5	2.8			11	11	7.0	5.4	13	19
PFDPA											9.1	6.9	18	32	6.2	7.1	15	24
PFBS													9.4	12	19	28	15	24
PFHxS					27	13	6.0	1.9	23	18	8.3	0.2	8.8	12	12	19	7.5	6.8
PFHpS							13	1.8	15	8.0	10	3.2	11	5.8	11	16	11	15
PFOS							22	8.6			17	5.9	12	18	21	31	11	19
PFDS					35	46	17	12	22	5.3	14	22	13	13	14	18	11	12
PFPeA															7.0	4.2	8.6	4.9
PFHxA													11	4.7	4.8	1.8	6.9	9.5
PFHpA													11	8.6	9.6	11		
PFOA							8.9	13	8.6	6.9			9.2	6.6	12	14	24	40
PFNA							14	2.3	16	2.1	11	16	8.7	12	13	10	9.9	12
PFDA											16	14	8.5	8.0	11	3.7	12	21
PFUnDA							21	32	12	0.2	9.1	3.2	7.1	0.6	14	19	8.4	8.5
PFDoDA					13	3.1	14	13	6.8	1.7	20	17	8.5	11	11	8.8	7.4	7.6
PFTrDA							28	52	6.9	7.5	12	6.2	15	26	9.2	12	8.6	0.9
PFTeDA							11	5.6			17	24	7.3	7.8	27	44	13	13
PFOSA							15	1.0	19	16	34	55	10	7.9	11	3.3	13	8.5
MeFOSA													8.1	1.1	9.4	6.9	12	14
EtFOSA									19	18	14	3.3	21	35	16	3.1	13	3.7

b. Spiking levels of 1.80, 9.0, and 30 ng mL^{-1} blood

	1.80 ng mL^{-1} blood						9.0 ng mL^{-1} blood						30 ng mL^{-1} blood					
	Serum		Plasma		Whole blood		Serum		Plasma		Whole blood		Serum		Plasma		Whole blood	
	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.	Int.	Diff.
6:2 PAP	5.7	5.7	9.4	12	5.6	4.8	5.3	3.4	18	28	9.6	15	5.3	7.8	8.1	1.4	4.3	2.1
8:2 PAP	5.9	4.5	7.2	8.6	13	17	5.7	0.8	9.3	3.9	7.1	0.1	7.7	8.7	6.2	3.9	7.2	11
6:2 diPAP	6.1	0.9	8.4	12	10	12	6.4	6.0	10	2.2	6.7	11	3.3	3.4	6.0	2.7	8.9	6.4
8:2 diPAP	9.9	0.7	9.1	6.8	13	19	4.8	6.1	6.8	2.6	10	12	7.4	11	11	13	6.1	2.3
PFHxPA	11	4.1	10	17	18	32	9.6	11	17	28	16	29	14	14	8.6	5.4	17	30
PFOPA	12	16	11	13	8.6	15	14	24	13	20	3.4	0.4	6.7	5.0	14	25	7.2	6.3
PFDPA	4.8	4.5	4.9	0.1	14	21	4.7	3.6	9.9	16	5.3	5.9	6.3	7.9	8.9	14	11	15
PFBS	6.1	4.5	12	3.2	10	16	14	17	11	0.6	15	24	5.0	1.5	11	12	8.2	14
PFHxS	7.1	0.2	10	9.6	12	17	6.6	7.8	4.8	5.5	6.9	4.1	11	19	8.7	2.6	3.7	5.4
PFHpS	5.4	2.1	12	20	12	20	12	11	8.3	13	5.4	5.7	7.7	1.2	8.1	3.0	7.5	2.9
PFOS	10	2.7	18	11	8.6	13	13	14	8.8	0.3	6.8	0.9	13	16	7.8	8.7	7.9	6.4
PFDS	6.8	7.3	11	7.6	13	21	7.1	3.7	9.3	7.4	7.0	1.8	7.6	11	6.7	3.8	14	19
PFPeA	6.7	5.3	5.1	0.5	3.9	4.6	8.2	11	5.9	8.0	7.2	8.1	6.5	5.0	7.0	9.2	3.0	1.6
PFHxA	4.7	0.9	2.4	1.7	7.0	13	5.9	5.8	2.9	0.8	5.2	3.3	4.8	3.2	3.5	2.7	3.8	2.8
PFHpA	7.4	8.2	13	0.3	31	30	18	30	8.7	10	16	19	13	24	12	17	10	1.4
PFOA	8.6	5.2	8.3	1.3	6.1	2.9	5.7	5.3	7.3	8.7	9.3	10	5.3	4.4	6.7	4.2	3.1	4.0
PFNA	6.5	7.4	11	13	7.0	7.0	7.9	9.8	9.5	1.3	7.2	6.6	8.4	0.9	5.1	1.6	11	12
PFDA	7.6	14	5.8	1.4	16	24	5.2	6.4	8.5	1.0	5.0	4.4	4.2	5.5	5.4	8.5	8.2	8.2
PFUnDA	3.7	0.5	6.1	3.8	8.6	9.3	4.2	2.3	7.4	1.6	8.3	0.6	3.7	2.5	7.2	4.1	6.6	0.6
PFDoDA	5.6	0.6	5.7	0.5	9.7	15	4.8	0.1	6.2	6.9	4.2	1.7	3.7	1.3	5.7	7.6	5.6	5.1
PFTrDA	9.3	17	8.4	10	8.9	9.3	12	21	9.3	8.7	7.3	1.1	5.4	9.5	5.2	5.8	6.9	1.9
PFTeDA	6.5	10	28	29	8.8	2.8	4.4	6.5	9.2	10	11	16	5.2	6.3	5.0	3.8	9.8	14
PFOSA	11	19	12	1.8	15	25	7.3	12	11	7.3	6.5	3.7	5.6	5.6	7.2	7.3	9.3	4.2
MeFOSA	10	15	7.8	7.8	17	31	11	19	6.3	3.3	9.8	11	9.0	8.5	8.7	11	10	8.6
EtFOSA	9.3	4.3	4.7	5.2	9.1	7.9	6.3	1.9	8.1	2.0	5.0	0.5	5.4	9.7	12	11	11	6.3

A number of replicates (n) = 10 for intermediate precision and between-run reproducibility determination.

4. Conclusions

An analytical method for determination of twenty-five PFASs with different physicochemical properties in three different blood matrices, requiring only 50 μL of sample, was developed. The method is rapid, sensitive and reliable, and is based on a quick protein precipitation followed by online SPE-UHPLC-MS/MS. A successful validation was conducted, including assessment of important parameters; i.e. linearity, MDLs, MQLs, accuracy, intermediate precision, repeatability, and the relative difference between the batch of analyses. The developed method was successfully applied to samples of human serum, plasma, and

Table 5
PFAS concentrations (ng mL^{-1}) in two human serum samples from an AMAP interlaboratory comparison study, one human plasma sample, and two human whole blood samples (The results from the serum samples applying this method compared to consensus values given by AMAP.).

	Serum								Plasma		Whole blood			
	AMSY1402				AMSY1303				1		1		2	
	Consensus	This method		Diff. (%)	Consensus	This method		Diff. (%)	This method		This method		This method	
	Conc.	Conc.	% cv		Conc.	Conc.	% cv		Conc.	% cv	Conc.	% cv	Conc.	% cv
6:2 PAP		nd				nd			nd		nd		nd	
8:2 PAP		nd				nd			[a]0.21	8.6	nd		nd	
6:2 diPAP		[a]0.07	20			[a]0.04	6.7		0.07	4.6	nd		nd	
8:2 diPAP		[a]0.10	3.2			[a]0.10	4.3		nd		nd		nd	
PFHxPA		0.34	13			0.26	9.8		nd		nd		nd	
PFOPA		nd				nd			nd		nd		nd	
PFDPA		nd				nd			nd		nd		nd	
PFBS		0.18	4.1			0.26	7.5		[a]0.09	14	nd		nd	
PFHxS	1.25	1.21	1.5	3	19.00	19.21	3.4	1	2.59	7.8	0.10	2.5	3.02	3.0
PFHpS		0.07	12			0.06	7.4		0.45	7.6	0.04	7.7	0.08	7.4
PFOS	3.28	3.03	14	8	5.61	5.50	6.6	2	23.76	7.0	1.45	2.4	5.49	6.0
PFDS		0.05	9.0			0.05	9.1		0.37	4.6	0.02	13	0.06	11
PFPeA		nd				nd			nd		nd		nd	
PFHxA	0.46	0.52	5.1	13	2.83	2.79	1.7	2	nd		0.59	13	0.24	5.1
PFHpA		nd				0.24	8.5		0.20	7.4	nd		nd	
PFOA	0.90	0.90	8.7	1	6.30	6.82	6.1	8	4.33	0.8	0.16	5.5	15.28	3.8
PFNA	0.38	0.48	12	22	0.72	0.89	1.9	21	0.67	8.4	0.12	3.0	0.31	5.2
PFDA		0.20	7.3			0.21	12		0.31	8.3	0.10	3.8	0.16	5.9
PFUnDA	0.52	0.52	4.7	0	1.38	1.38	2.0	0	0.36	2.4	0.09	4.7	0.31	5.3
PFDoDA		0.04	17			0.05	9.7		0.07	15	0.02	9.6	0.06	8.3
PFTrDA		[a]0.05	4.5			0.07	5.4		0.13	8.2	0.09	6.9	0.23	4.4
PFTeDA		nd				nd			[a]0.05	11	0.07	3.5	0.10	13
PFOSA		[a]0.03	18			[a]0.03	9.2		[a]0.03	22	0.12	10	0.41	7.8
MeFOSA		[a]0.10	2.6			[a]0.10	8.0		nd		nd		nd	
EtFOSA		nd				nd			0.08	15	nd		nd	

Number of replicates (n) = 5.
nd = non detected.
[a] A higher uncertainty is expected as the value was between MDL and the lowest calibration point.

whole blood. The presented method is suitable for large-scale monitoring of a wide range of PFASs in both human serum, plasma, and whole blood, and is optimal for studying the distribution in blood for the different PFAS. The method's ability to determine PFASs in low volumes of different blood matrices is advantageous in large-scale studies comprising samples from different cohorts, where sample volumes often are limited and the type of blood matrix might differ.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.12.043.

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