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Determination of polyfluoroalkyl substances in biological matrices by chromatography techniques: A review focused on the sample preparation techniques - Review

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) present interesting features for several applications and have great commercial relevance. However, PFAS bioaccumulate, persist in the environment, and are toxic, so regulating and controlling their use and evaluating the risks of human exposure to them is vital. Liquid chromatography with tandem mass spectrometry (LC–MS/MS) has been employed to analyze PFAS, especially in biomonitoring studies. Unfortunately, these studies have been met with challenges, such as the low PFAS concentrations in the samples, small sample amount, contamination of labware and reagents, and signal suppression due to the sample matrix. A suitable sample preparation method could preconcentrate PFAS in the sample and remove the matrix before chromatographic analysis, thereby helping to overcome these challenges. Moreover, the right sample preparation method could reduce the analysis time and enhance the selectivity and sensitivity achieved with HPLC–MS/MS and other correlated techniques. Solid Phase Extraction (SPE) is the most common sample preparation technique. SPE is usually employed with protein precipitation (PPT) in multistep sample preparation methods. Nevertheless, depending on the sample type and composition, presence of contaminants, and analytes, other sample preparation techniques may be relevant. In this paper, we provide a critical review of the sample preparation techniques applied for PFAS analysis in biological matrices. We exploit the benefits, challenges, and future perspectives of these techniques.

1. Introduction

In 1938, Plunkett synthesized polytetrafluoroethylene (PTFE, also known as Teflon), a polymer based on a fluorinated carbon chain. The unique properties of this polymer motivated researchers to synthesize and to study new fluorinated substances, creating a class of compounds known as per- and polyfluoroalkyl substances (PFAS), which have been manufactured on a large scale and commercially applied since the 1950s [1]. PFAS have interesting characteristics: they repel oil, water, stain, and soil; are chemically and thermally stable; reduce friction; and display surfactant properties. Currently, PFAS can be found in various industries such as the construction, medical, automotive, electronics, aviation and aerospace, and semiconductor industry, as well as in consumer products, particularly food packaging and repellent clothing and household [2].

Supposedly, PFAS comprise between 5 and 10 thousand chemical substances; over 250 of them are commercially relevant [3]. The large number of molecules belonging to the class of PFAS has called for their names and acronyms to be systematized and subdivisions to be created. In 2011, Buck and coworkers [4], proposed one of the most accepted PFAS classifications, and they recently published a new version of the classification [3]. Roughly, these authors proposed a first division of PFAS into polymers and non-polymers, followed by subdivision of the latter class into perfluoroalkyl substances (where all the hydrogen atoms in the alkyl group are replaced with fluorine atoms) and polyfluoroalkyl substances (where fluorine atoms substitute all the hydrogen atoms in at least one carbon, but not in all of them). Fig. 1 illustrates some examples of PFAS as well as their subdivisions.

Some PFAS, especially polymers like PTFE, are chemically stable and poorly soluble in water. Provided that their production is controlled,

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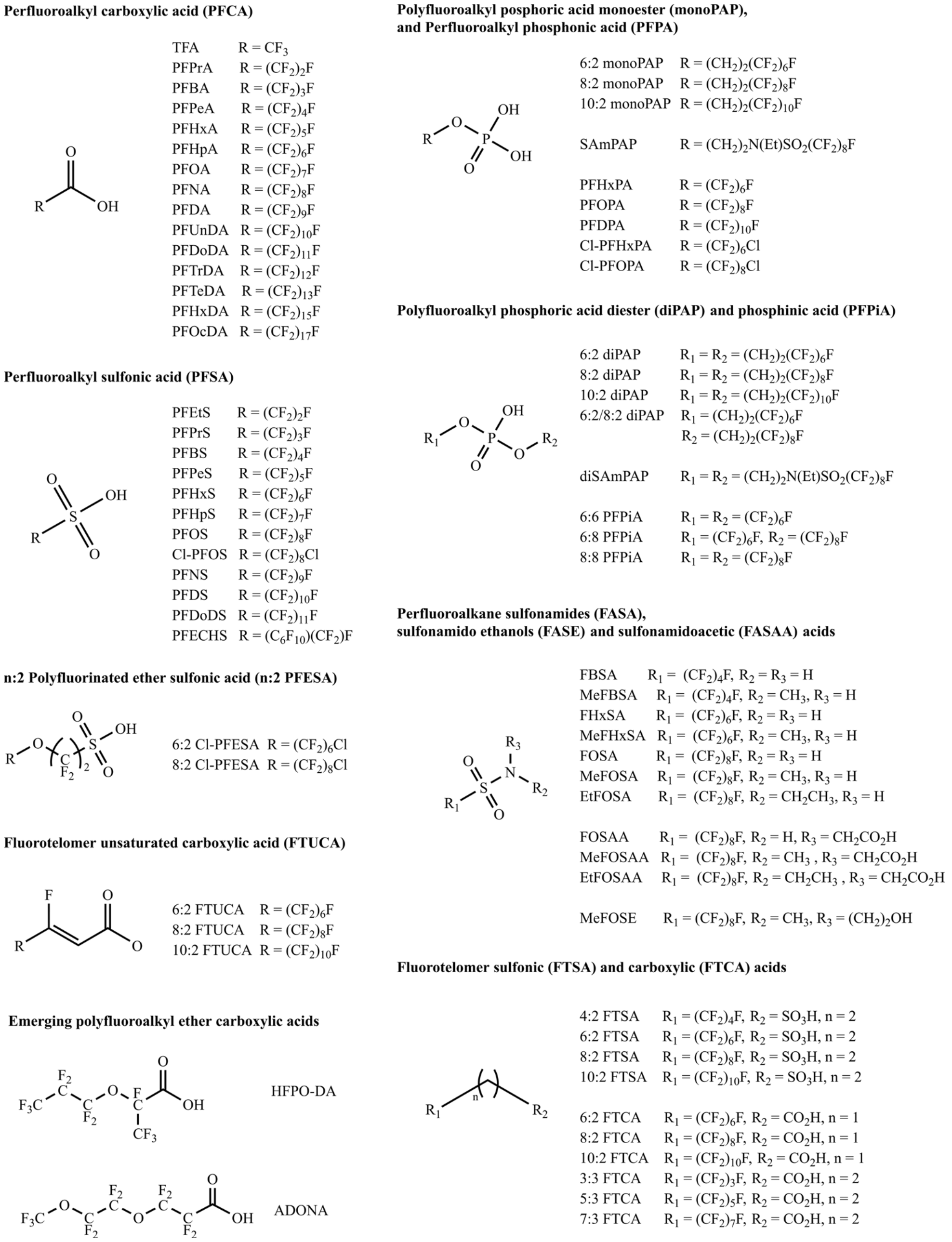

Fig. 1. Some per- and polyfluoroalkyl substances (PFAS) currently investigated in biological samples. PFPrA – Pentafluoropropionic acid, PFBA – Perfluorobutanoic acid, PFPeA – Nonafluoropentanoic acid, PFHxA – Perfluorohexanoic acid, PFHpA - Perfluoroheptanoic acid, PFOA – Perfluorooctanoic acid, PFNA – Perfluorononanoic acid, PFDA – Perfluorodecanoic acid, PFUnDA – Perfluoroundecanoic acid, PFDoDA – Perfluorododecanoic acid, PFTrDA – Pentacosafluorotridecanoic acid, PFTeDA – Perfluorotetradecanoic acid, PFHxDA – Perfluorohexadecanoic acid, PFOcDA – Perfluorooctadecanoic acid, PFEtS – Pentafluoroethanesulfonic acid, PFPrS – Perfluoropropanesulfonic acid, PFBS – Perfluorobunate sulfonate, PFPeS - Perfluoropentanesulfonic acid, PFHxS – Perfluorohexanesulfonic acid, PFHpS – Perfluoroheptanesulfonic acid, PFOS – Perfluorooctanesulfonic acid, Cl-PFOS – 1-Chloro-perfluorooctanesulfonic acid, PFNS – Perfluorononanesulfonic acid, PFDS – Perfluorodecane sulfonic acid, PFDoDS – Perfluorododecane sulfonate, PFECHS – Perfluoroethylcyclohexane sulfonate, 6:2 Cl-PFESA – 6:2 Chlorinated polyfluorinated ether sulfonate, 8:2 Cl-PFESA – 8:2 Chlorinated polyfluorinated ether sulfonate, 6:2 FTUCA - 6:2 Fluorotelomer unsaturated carboxylic acid, 8:2 FTUCA - 8:2 Fluorotelomer unsaturated carboxylic acid, 10:2 FTUCA - 10:2 Fluorotelomer unsaturated carboxylic acid, HFPO-DA Hexafluoropropylene oxide-dimer acid, ADONA – Ammonium 4,8-dioxa-3H-perfluorononanoate, 6:2 monoPAP – 6:2 Fluorotelomer phosphate monoester, 8:2 monoPAP – 8:2 Fluorotelomer phosphate monoester, 10:2 monoPAP – 10:2 Fluorotelomer phosphate monoester, SAmPAP – 2-(N-ethylperfluorooctanesulfonamido)ethyl phosphate, PFHxPA – Perfluorohexylphosphonic acid, PFOPA – Perfluorooctylphosphonic acid, PFDPA – Perfluorodecylphosphonic acid, Cl-PFHxPA – Monochloroperfluorohexylphosphonic acid, Cl-PFOPA – 8-Choloroperfluorooctylphosphonic acid, 6:2 diPAP – 6:2 Fluorotelomer phosphate diester, 8:2 diPAP – 8:2 Fluorotelomer phosphate diester, 10:2 diPAP – 10:2 Fluorotelomer phosphate diester, 6:2/8:2 diPAP - 6:2/8:2 Fluorotelomer phosphate diester, diSAmPAP – bis-[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl] phosphate, 6:6 PFPiA – Bis(perfluorohexyl)phosphinic acid, 6:8 PFPiA – Perfluorohexylperfluorooctylphosphinic acid, 8:8 PFPiA – Bis(perfluorooctyll)phosphinic acid, FBSA – Perfluorobutane sulfonamide, MeFBSA – N-Methylperfluorobutanesulfonamide, FHxSA – Perfluorohexanesulfonamide, MeFHxSA – Tridecafluoro-N-methyl-1-hexasulfonimide, FOSA – Perfluorooctanesulfonimide, MeFOSA – N-methylperfluorooctanesulfonimide, EtFOSA – N-ethylperfluorooctanesulfonimide, FOSAA – Perfluorooctane Sulfonamidoacetic Acid, MeFOSAA – N-methylperfluorooctane Sulfonamido Acetic Acid, MeFOSE – N-Methylperfluorooctanesulfonamidoethanol, 4:2 FTSA – 4:2 Fluorotelomer Sulfonate, 6:2 FTSA – 6:2 Fluorotelomer Sulfonate, 8:2 FTSA – 8:2 Fluorotelomer Sulfonate, 10:2 FTSA – 10:2 Fluorotelomer Sulfonate, 6:2 FTCA – 2-Perfluohexyl Ethanoic Acid, 8:2 FTCA – 2-Perfluooctyl Ethanoic Acid, 10:2 FTCA – 2-Perfluodecyl Ethanoic Acid, 3:3 FTCA – 3-Perfluoropropyl Propanoic Acid, 5:3 FTCA – 2H, 2H, 3H, 3H-Perfluorooctanoic acid, 7:3 FTCA – 3-Perfluoroheptylpropanoic Acid.

Table 1
Main components of biological samples in which PFAS have been investigated.

Biological Sample	Main components	Reference
Whole blood	Water[1] (75–82%), Corpuscles (36–51% (v/v)), Proteins (17.8–24.6%), Fibrinogen (0.1–0.2%), Fat (0.2–2%), Fatty acids (0.29–0.42%), and NaCl (0.45– 0.52%)	[16,17]
Serum / Plasma	Water[1] (90–91.5%), Proteins (5.8–8.6%), Fibrinogen[2] (0.2–0.4%), Fat (0.45–1.2%), Fatty acids (0.19–0.64%), and NaCl (0.56–0.62%)	
Human milk	Water (87%), fat (3.8%), proteins (1.0%), lactose (7%), and minerals (0.2%)	[18]
Human urine	Water (95%), urea (2%), creatinine (0.1%), uric acid (0.03%), and electrolytes (<0.03%)	[19]
Human placenta	Water (87%), protein (77%[3]), fat (3.8%[3]), and minerals (8.6%[3])	[20]

1 – Water content estimated as 100% - Solid content (%). According to Fearon 2014, the solid content is 18–25% in blood and 8.5–10% in plasma. 2 – Serum does not contain fibrinogen. 3 – Relative to dry weight. Dry weight represents 13% of fresh weight.

and that they are correctly discarded, they are not believed to harm the environment or humans [2]. However, other PFAS can persist in the environment and bioaccumulate, causing environmental and health problems [5,6]. Concern about the PFAS threat, mainly in the case of perfluorooctane sulfonate (PFOS) and perfluoroocnanoic acid (PFOA), two of the most produced PFAS, dates from the 1970s (Fig. 1). Not until a few decades later were some PFAS phased out. Nevertheless, they remain a matter of concern because they are present in the environment, and humans are continuously exposed to them [7] . PFAS have been detected in water, food, and biological samples like plasma, blood, semen, milk, and tissues [5,6,8,9]. In addition, some studies have correlated exposure to PFAS with cancer, lack of immune response to vaccines, and impact on human fertility and development [10–15].

Recently, analytical separation techniques including high-performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) have been coupled to mass detection and high-resolution mass spectrometry (HRMS), allowing trace PFAS concentrations ($<$ ng mL^{-1}) to be detected and constituting a great tool for biomonitoring. To enhance the efficiency of analytical techniques, some cautions must be taken. For example, choosing the right sample preparation method for a given biological sample is vital. Moreover, the analyte properties play an important role in detection, quantification limit, and reproducibility.

This review discusses the main sample preparation methods employed for PFAS analysis in biological samples, as well as advances in this field. Papers published from 2018 to 2023 have been evaluated and considered in this review.

2. Initial considerations

The presence of PFAS in biological samples has been extensively reported. These samples are particularly challenging because they have complex composition and vary among individuals. For reference, Table 1 breaks down the main components in some biological samples in which the presence of PFAS has been recently investigated.

By observing Table 1, we can see that the composition of biological samples may vary drastically. Therefore, sample complexity must be considered before deciding which sample preparation method is more suitable. For instance, the dilute-and-shoot (DnS) technique can be used for analyzing urine samples. Because urine is primarily composed of water and other components (Table 1), such as inorganic salts, dilution may reduce the signal of these components [21]. Fig. 2 presents the sample preparation techniques used for analyzing PFAS in biological samples.

Fig. 3 shows that, when it comes to analyzing PFAS in biological matrices, employing a multistep sample preparation method is more common than using a single- step sample preparation method. For example, while plasma samples can be subjected to a single-step sample preparation method, e.g., liquid-liquid extraction (LLE) or protein precipitation (PPT), these methods alone may not remove some matrix components, which can impact the analytical method performance and the feasibility of the validation step. In addition, the large number of samples involved in biomonitoring studies demands more analytical equipment maintenance. In this sense, multistep sample preparation method could provide a “cleaner” extract, reducing the need for maintenance.

Another common concern in the reviewed papers is which analytical technique should be used to detect and to quantify PFAS. Table 2 shows that liquid chromatography with tandem mass spectrometry (LC–MS/MS) is the analytical technique of choice for PFAS analysis in biological samples. Despite the advantages of LC–MS/MS, including its selectivity and sensitivity, analyte ionization is susceptible to the presence of matrix components (inorganic salts, proteins, lipids, etc.) that were not removed during sample preparation, which may lead to matrix effects [22]. For instance, when DnS is applied for preparing urine samples, inorganic salts present in urine may affect the analyte ionization efficiency even though the salts were diluted during sample preparation. If this is the case, different actions can be taken to circumvent matrix effects, as pointed out by Cortese et al. 2020 [22]. For instance, the ionization mode can be changed, the chromatographic conditions can be altered, and the sample preparation procedure can be optimized. However, PFAS have acidic character, so they are primarily analyzed under the negative ionization mode. Additionally, one should remember that the PFAS concentration in biological matrices is usually low, so choosing the right sample preparation method and system detection will dictate whether the analytical work is successful.

3. Sample preparation techniques

3.1. Solid phase extraction (SPE)

In recent years, SPE has been increasingly used for extracting PFAS from biological samples. SPE is a well-established procedure that was introduced in the 1950s for analyzing trace organic molecules in water [23]. This technique works by either trapping the analytes in a solid sorbent or trapping the other sample components and letting the analytes be eluted from the solid phase. In typical SPE procedures, the first step comprises conditioning the extraction phase. Later, this phase is equilibrated with a solvent that has similar properties to the solvent in which the sample has been diluted. At this point, depending on the extraction phase properties, the analytes or interfering components of the matrix could be retained. If the analytes are retained, an elution step is required [24].

Because HPLC and SPE share some similarities, some concepts employed for HPLC separations can also be applied for preparing samples by SPE. As in the case of HPLC, the SPE retention mechanism can be understood as a partition between the eluent and the phase. In this sense, if an octadecylsilyl-based sorbent material is employed and the analytes are in their neutral form, they will be retained by the material. On the other hand, if an ion exchange adsorbent is used and the analytes are in their neutral form, they will not be retained. Different materials like octadecyl (C_{18}), aminopropyl (NH_2), diethylaminopropyl (DEA), and quaternary amine (SAX) have been developed and are commercially available for exploring different separation strategies [24]. Despite their similarities, SPE and HPLC also have critical distinctions. SPE is typically used as a cleanup step, when just an initial separation between the analytes and the matrix interferents is required, whereas the separation between analytes in HPLC is intended for their analysis. For this reason, SPE cartridges can have lower plate count than HPLC columns, which can be achieved mainly by using larger particle sizes in the first case. This helps to decrease the backpressure in the cartridge and allows

Table 2
Summary of the chromatographic methods developed for PFAS analysis in biological matrices (from 2018 to 2023).

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) SPE of this extract using ENVI-Carb cartridge Matrix: Human red blood cells SLE of the red blood cells with acetonitrile	PFBA, PFPeA, PFHxA, PFHpA, n-PFOA, isso-PFOA, 5m-PFOA, 4m-PFOA, 3m-PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFBS, PFHxS, n-PFOS, isoPFOS, 4m-PFOS, 3 + 5m-PFOS, 1 m PFOS, Σm2-PFOS, PFOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA. 6:2 diPAP, 8:2 diPAP	UHPLC	QqQ	Stationary Phase: Ascentis express F5 (100 mm x 2.1 mm, 2.7 µm 90A). Temperature 40 °C, gradient elution with water added with formic acid to pH 6 and methanol	**Plasma** LOD 15–93 pg mL^{-1} **Red Blood Cells** LOD 38–92 pg mL^{-1}	[67]
Matrix: Human urine LLE Ethyl acetate: isopropanol (95:5 v/v) and 5% (v/v) ammonia in water	Untargeted MS range *m/z* 50–1200 MS/MS range *m/z* 40–1000	UHPLC	QTOF	Stationary Phase: Zorbax eclipse plus RRHD C18 (100 mm x 2.1 mm, 2.7 µm). Gradient elution with 2 mmol L^{-1} ammonium acetate buffer and methanol. Mobile phase flow rate 0.25 mL min^{-1}. Total analysis time 35 min	LOD 20 ng mL^{-1} (Trifluoroacetic acid)	[63]
Matrix: Human serum PPT with acetonitrile and filtration through a 0.2 µm RC Membrane	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS PFPeS, PFHxS, PFHxS, PFHps, PFOS, PFNS, PFDoDS, FOSA, N-Me FOSA, N-Et FOSA, FOSAA, N-Et FOSE, N-Me FOSE, PFECHS, 10:2 FTS, 8:2 FTS, 6:2 FTS, 4:2 FTS, 6:2 PAP, 8:2 PAP, SAmPAP, 6:2 DiPAP, 8:2 DiPAP, 6:2 DiPAP	HPLC	QqQ	Stationary Phase: EVO C18 (100 mm x 2 mm, 2.6 µm). Temperature 50 °C, Gradient elution, mobile phase flow rate 0.45 mL min^{-1} Pre-injection column Kinetex C18 (50 mm x 4.6 mm, 5 µm)	MDL 0.04–1.13 ng mL^{-1}	[44]
Matrix: Human serum, plasma, and whole blood *Multistep sample preparation* 1) PPT wth methanol 2) Online SPE using a Betasil C8 (100 mm x 3 mm, 5 µm) at 25 °C and 0.1 mol L^{-1} formic acid in water and methanol mixture 95/5 (v/v) as loading solvent	6:2 PAP, 8:2 PAP, 6:2diPAP, 8:2 diPAP, PFHxPA, PFOPA, PFDPA, PFBS, PFHxS, PFHpS, PFOS, PFDS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA,PFDoDA, PFTrDA, PFTeDA, PFOSA, MeFOSA, EtFOSA	UHPLC	QqQ	Stationary phase: Zorbax Eclipse Plus C18, (50 mm x 2.1 mm, 1.8 µm). Temperature 40 °C. Gradient elution with 0.15% ammonium hydroxide in water (pH 8–9) and acetonitrile. Mobile phase flow rate 0.2 mL min^{-1}. Total analysis time 14 min	LOD 0.002–0.09 ng mL^{-1} LOQ 0.006–0.3 ng mL^{-1}	[74]
Matrix: Human plasma PPT with acetonitrile	PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnDA	UHPLC	QqQ	Stationary Phase: Acquity UPLC BEH C18 (100 mm x 2.1 mm, 1.7 µm). Temperature 60 °C. Gradient elution with 5 mmol L^{-1} ammonium acetate and methanol. Mobile phase flow rate 0.28 mL min^{-1}. Total analysis time 9.5 min	LOD 0.02–0.05 ng mL^{-1}	[43]
Matrix: Human milk *Multistep sample preparation* 1) PPT with formic acid solution and acetonitrile followed by acetonitrile/water 60:40 (v/v) 2) SPE of the supernatant using a SPE-WAX cartridges	PFBS, PFHxA, PFHxS, PFHpA, PFHpS, PFOA, L-PFOS, PFNA, PFDA, PFUdA, PFDS, PFDoA, PFTeDA	UHPLC	QqQ	Stationary Phase: Acquity UPLC BEH C18 (100 mm x 2.1 mm, 1.7 µm). Temperature ambient. Gradient elution with 5 mmol L^{-1} ammonium formate and 2:1 (v/v) acetonitrile/methanol. Mobile phase flow rate 0.15 mL min^{-1}. Total Analysis time 24 min	LOD 5 – 10 ng L^{-1}	[26]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human milk *Multistep sample preparation* 1) Protein digestion overnight using protease and lipase enzyme mixture 2) PPT using methanol and centrifugal filtration Matrix: Serum *Multistep sample preparation* 1) PPT using methanol/water 50:50 (v/v) and acetonitrile 2) Online SPE Supernatants from previous steps were subjected to SPE online to the HPLC using an EC 20/2 Chromabond HR-X (20 × 2 mm, 15 µm) using 2 mmol L^{-1} ammonium acetate solution to load the samples into the cartridge	PFOS, PFHxA, PFOA, PFNA, PFDA, PFDoA, PFBS, PFHxS	HPLC	QqQ	Stationary Phase: ReproSil-Pur 120 ODS-3 (150 mm x 2 mm, 5 µm). Temperature 35 °C. Gradient elution with 2 mmol L^{-1} ammonium acetate and methanol. Mobile phase flow rate 0.4 mL min^{-1}. Total analysis time 13 min	**Human milk** LOQ 25–250 ng L^{-1} **Human serum** LOQ 250 ng L^{-1}	[73]
Matrix: Human milk and plasma SPE using a WAX µ-elution plate of the samples after being pretreated with methanol containing formic acid.	PFOA, L-PFOA, PFHxS, L-PFHxS, PFNA, PFDA	UHPLC	QqQ	Stationary Phase: CSH Phenyl-Hexyl. Gradient Elution with 10 mmol L^{-1} ammonium acetate solution and 10 mmol L^{-1} ammonium acetate solution in methanol.	**Human milk** LLOQ 0.015 ng mL^{-1} **Plasma** LLOQ 0.150 ng mL^{-1}	[30]
Matrix: Human placenta *Multistep sample preparation* 1) SLE using formic acid and acetonitrile 2) SPE using an Oasis WAX cartridge	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, HFPO-DA	HPLC	QqQ	Stationary Phase: Zorbax Eclipse XDB C-18 (50 × 4.6 mm, 1.8 µm). Temperature 45 °C. Gradient elution with 2 mmol L^{-1} ammonium acetate in water and 2 mmol L^{-1} ammonium acetate in methanol. Mobile phase flow rate 0.4 mL min^{-1}. Total analysis time 18 min	LOD 0.01–8.10 ng g^{-1}	[65]
Matrix: Human plasma SPE Captiva EMR lipid cartridge 96-well plate. Samples were spiked with internal standard and loaded into the cartridges	PFBA, PFPeA, PFHxA, PFHpA, PFOA, *n*-PFOA, *br*-PFOA, *5m*-PFOA, isoPFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, *n*-PFOS, *1m*-PFOS, *3m*-PFOS, *5m*-PFOS, Σ*m2*-PFOS, isoPFOS, PFDS, 6:2 PFESA, 8:2 PFESA, 4:2 FTS 6:2 FTS, 6:2 FTS, 8:2 FTS, 6:2 diPAP	UHPLC	QqQ	Stationary Phase: BEH shield RP18 (100 × 2.1 mm, 1.7 µm). Gradient elution with 5 mmol L^{-1} ammonium formate (pH 4) and methanol. Mobile phase flow rate 0.4 mL min^{-1}. Total analysis time 27.5 min	LOQ 0.02–1.1 ng mL^{-1}	[84]
Matrix: Human plasma PPT with methanol	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOSA, N-MeFOSAA, N-EtFOSAA, PFBS, PFPeS, n-PFHxS, Σ-PFHxS, PFHpS, n-PFOS, Σ-PFOS, PFNS, PFDS, PFECHS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, HFPO-DA, ADONA, cC6O4, 6:2 CIPFESA, 8:2 CIPFESA	HPLC	QqQ	Stationary Phase: HSS T3 C18 (100 × 2.1 mm, 1.8 µm). Temperature 45 °C. Gradient elution with 10 mmol L^{-1} ammonium acetate with 0.1% (v/v) acetic acid and acetonitrile. Mobile phase flow rate 0.2 mL min^{-1}. Total analysis time 28.6 min	LLOQ 0.009–0.245 µg L^{-1}	[38]
Matrix: Human serum SPE 96-well OASIS WAX µElution plate. Samples were directly loaded into the cartridge	PFBA, PFPA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS, PFOSA	UHPLC	QqQ	Stationary Phase: UPLC HSS 3T (100 × 2.1 mm, 1.8 µm). Temperature 50 °C. Gradient elution with 2 mmol L^{-1} ammonium acetate aqueous solution and 2 mmol L^{-1} ammonium acetate in methanol. Mobile phase flow varied from 0.3 to 0.7 mL min^{-1} during gradient. Total analysis time 46.6 min	–	[14]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human serum Samples were extracted using IP-LLE the samples pH was adjusted to 10 and TBA was used as ion paring agent, the extraction phase consisted of MTBA. Matrix: Human urine Samples were acidified with 1% formic acid and extracted by ultrasound for 30 min at 60 °C following a SPE step using an Oasis WAX cartridge. The acidified urine samples were directly loaded into the cartridge	PFBA, PFBS, PFOA, PFOS, PFHxA, PFHxS, PFHpA, PFHpS, PFDA, PFDS, PFDoA, PFUdA, PFNA, PFTrDA, PFTeDA, PFPeDA, PFHxPA, PFOPA, PFDPA, 6:2 diPAP, 8:2 diPAP	UHPLC	QqQ	Stationary Phase: BEH C18 (100 × 2.1 mm, 1.7 µm). Gradient elution with water and 2 mmol L^{-1} ammonium acetate in methanol. Mobile phase flow rate 0.2 mL min^{-1}.	**Human serum** LOD 0.0635–2.372 ng mL^{-1} LOQ 0.212– 0.757 ng mL^{-1} **Human Urine** LOD 0.048–1.260 ng mL^{-1} LOQ 0.161–4.216 ng mL^{-1}	[54]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) Online SPE of the supernatant using an Inertsil ODS-3 (33 × 4.5 mm, 5 µm)	PFOS, PFOA, 1m-PFOS, 5m-PFOS, 6m-PFOS, 3,5m2-PFOS, 4,5m2-PFOS, 4,4m2-PFOS, 5,5m2-PFOS, 5m-PFOA, 6m-PFOA, 3,5m2-PFOA, 4,5m2-PFOA, 4,4m2-PFOA, 5,5m2-PFOA	HPLC	QqQ	Stationary Phase: Eclipse Plus C18 (100 × 4.6 mm, 3.5 µm). Gradient elution using 10 mmol L^{-1} ammonium formate solution (pH 3.5) and methanol. Mobile phase flow rate 0.35 mL min^{-1}	LOD 0.002–0.150 ng mL^{-1}	[33]
Matrix: Human whole blood IP-LLE employing TBA as ion paring agent and MTBE extraction phase	PFEtS, PFPrS, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFND, PFDoDS, TFA, PFPrA, PFBA, PFPeA PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, PFHxDA, PFOcDA, 3:3 FTCA, 5:3 FTCA, 7:3 FTCA, 6:2 FTUCA, 8:2 FTSA, 10:2 FTSA, 6:2 monoPAP, 8:2 monoPAP, 10:2 monoPAP, 6:2 diPAP, 8:2 diPAP, 6:2/8:2 diPAP, 10:2 diPAP, PFHxPA, PFOPA, PFDPA, C6/C6 PFiA, C6/C8 PFPiA, C8/C8 PFPiA, FBSA, MeFBSA, PFHxSA, MeFHxSA, FOSA, FOSAA, MeFOSAA, EtFOSAA, PFECHS, ADONA, HFPO-DA, 6:2 Cl-PFESA, 8:2 Cl-PFESA	SFC / UHPLC	QqQ	Short chain compounds **SFC** Stationary Phase: Torus DIOL (100 × 3.0 mm, 1.7 µm). Mobile phase CO_2 and 0.1% ammonium hydroxide in methanol. Other analytes **UHPLC** Stationary Phase: C18 BEH (100 × 2.1 mm, 1.7 µm). Mobile phase consisted of methanol and a 30/70 (v/v) mixture between methanol and water containing 2 mmol L^{-1} ammonium acetate and 5 mmol L^{-1} 1-methylpiperidine.	LOD 5–9834 pg mL^{-1}	[51]
Matrix: Human glioma tissue LLE was performed at pH 10 using TBA as paring agent and MTBA as extraction phase	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUA, PFOoA, PFTrDA, PFBS, PFHxS, PFOS, PFOSA, HFPO-DA, 6:2CL-PFESA, 8:2CL-PFESA,	HPLC	QqQ	Stationary Phase: Ascentis C18 (50 × 2.1, 3 µm). Temperature 40 °C. Mobile phase consisted of acetonitrile and 2 mmol L^{-1} ammonium acetate. Mobile phase flow rate 0.2 mL min^{-1}	LOD 0.5 ng g^{-1}	[52]
Matrix: Manatee whole blood *Multistep sample preparation* 1) Samples were subjected to DBS prior to extraction with extraction with methanol with formic acid Homogenization of the DBS with a 2:1 (v/v) chloroform/methanol and water Homogenization of the DBS with 2:1 (v/v) chloroform/methanol 2) IP-LLE with TBAHS and sodium carbonate/sodium bicarbonate (pH 10) and MTBE	PFBA, PFPrS, PFPeA, PFBS, FBSA, 4:2 FTS, PFHxA, PFHpA, NaDONA, FHEA, N-AP-FHxSA, PFECHS, FHxSA, 6:2 FTS, PFHpS, PFOA, ΣPFOS, PFNA, 8Cl-PFOS, FOEA, FOUEA, 9Cl-FP3ONS, FOSAA, PFNS, 8:2 FTS, FOSA, PFDA, N-MeFOSAA, PFDS, PFUnDA, N-EtFOSAA, FDUEA, FDEA, 11Cl-PF3OUdS, N-MeFOSA-M, PFDoA, 10:2 FTS, 6:6 PFPi, N-EtFOSA-M, PFDoS, PFTrDA, 6:2 diPAP, PFTeDA, 6:8 PFPi, 6:2/8:2 diPAP, PFHxDA, 8:2 diPAP, PFODA, SAmPAP, diSAmPAP	UHPLC	QqQ	Stationary Phase: Gemini C18 (100 × 2 mm, 3 µm). Temperature 40 °C. Gradient elution using 5 mmol L^{-1} ammonium acetate solution and methanol containing 5 mmol L^{-1} ammonium acetate. Mobile phase flow rate 0.5 mL min^{-1}. Total analysis time 30 min	–	[75]

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Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human lung and kidney *Multistep sample preparation* 1) SLE of the tissues with 100 mmol L^{-1} sodium hydroxide in methanol 2) SPE using an Oasis WAX cartridge	PFBA	UHPLC	Orbitrap	Stationary Phase: Hypersil GOLD (150 × 2.1 mm, 1.9 µm). Temperature 40 °C. Gradient elution using 2 mmol L^{-1} ammonium acetate in 95:5 (v/v) water/methanol and 2 mmol L^{-1} ammonium acetate in methanol. Mobile phase flow rate 0.3 mL min^{-1}	LOD 0.03 ng mL^{-1}	[66]
Matrix: Human serum SPE of the serum samples using a Water Oasis HLB cartridge	PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFBuS, PFHxS, PFOS, PFOSA	HPLC	QqQ	Stationary Phase: Kinetex C18 (100 × 4.6 mm, 1.8 µm). Gradient elution	–	[85]
Matrix: Human milk Ultrapure water, acetonitrile and formic acid were added to the human milk samples and afterwards SALLE - DLLME was performed using NaCl, di-sodium hydrogen and tri-sodium hydrogen citrate and TCM/Acetone 4:1 (v/v)	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, PFOS	UHPLC	Q	Stationary Phase: Kinetex PS C18 (100 × 3 mm, 2.6 µm). Temperature 30 °C. Gradient elution using 5 mmol L^{-1} ammonium acetate aqueous solution (pH 4.5) and acetonitrile.	LOD 0.66–0.86 ng L^{-1}	[60]
Matrix: Human serum Samples were subjected to SPE using an SPE-WAX cartridge Matrix: Human placenta *Multistep sample preparation* 1) SLE with acetonitrile, 2) SPE using a Superclean ENVI-Carb cartridge	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, PFHxDA, PFOcDA, PFBS, PFPeS, PFHxS, PFHpS, L-PFOS, PFNS, PFDS, PFDoDS, PFECHS, FBSA, MeFBSA, FHxSA, FOSA, MeFSOA, EtFOSA, MeFOSE, EtFOSE, 3:3 FTCA, 5:3 FTCA, 7:3 FTCA, 6:2 FTUCA, 8:2 FTUCA, 10:2 FTUCA, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 10:2 FTSA, PFHxPA,PFOPA, PFDPA, 6:6 PFPiA, 6:8 PFPiA, 8:8 PFPiA, 8:2 Cl-PFESA, 6:2 Cl-PFESA, ADONA, HFPO-DA, MeFOSAA, EtFOSAA, diSAmPAP, 6:2 PAP, 8:2 PAP	UHPLC	QqQ	Stationary Phase: BEH C18 (100 × 2.1 mm, 1.7 µm). Elution with 70:30 (v/v) water/methanol and methanol, both containing 2 mmol L^{-1} ammonium acetate and 5 mmol L^{-1} n-methylpyridine.	LOD 0.18 – 2,485,000 pg mL^{-1} LOQ 0.36 – 4,970,000 pg mL^{-1}	[86]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with ammonium acetate buffer 2 mmol L^{-1} in acetonitrile 2) Online SPE with the same solvent LiChrosher® RP-8 ADS (24 × 4 mm RAM, 25 µm) for 2 min	PFHxS, PFOA, PFOS, PFNA	HPLC	QqQ	Stationary phase: Luna C8 (150 × 4.6 mm, 3 µm) Gradient elution using ammonium acetate buffer in water pH 4 (2 mmol L^{-1}) and ammonium acetate buffer pH 4 (2 mmol L^{-1}) in acetonitrile (80:20 v/v) Flow rate: 0.3 mL min^{-1}. Total analysis time 20 min	LOQ 0.10–0.20 ng mL^{-1}	[72]
Matrix: Human urine DnS with methanol (1:5)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFHxS, PFOS, PFDS, 6:2 FTS, 8:2 FTS, 10:2 FTS, F-53B, 8:2 FTUCA, 10:2 FTUCA, 8:2 diPAP, PFDPA, CI-PFOPA, CI-PFHxPA, HFPO-DA, FOSA, N-EtFOSA, N-EtFOSAA, N-MeFOSA, N-MeFOSAA	UHPLC	IM-QTOF	Stationary phase: InfinityLab Poroshell 120 EC–C18 (50 × 2.1 mm, 1.8 µm) at 40 °C. Gradient elution with ammonium acetate in water (2 mmol L^{-1}) and methanol. Flow rate: 0.25 mL min^{-1}. Total analysis time: 20 min	–	[62]

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Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human plasma SPE using Captiva EMR-Lipid cartridge followed by filtration with 0.2 µm nylon filter	PFBA, PFHpA, PFNA, PFDA, PFuDA, PFDoA, PFTrDA, PFTeDA, n-PFOA, PFBS, PFHxS, PFHpS, n-PFOS, 1m-PFOS, 4,4m2-PFOS, 4,5m2-PFOS, 3m-PFOS, 4m-PFOS, 5m-PFOS, 6 m (isso)-PFOS, 5m-PFOA, isso-PFOA, 6:2Cl-PFESA, 8:2Cl-PFESA	UHPLC	Q-TRAP	Stationary phase: Acquity UPLC BEH shield RP18 column (100 × 2.1 mm, 1.7 *µ*m). Gradient elution with ammonium formate in water (5 mmol L $^{-1}$) and methanol. Flow rate 0.3 mL min^{-1}. Total analysis time: 35.5 min	LOD 0.006–1.50 ng mL^{-1}	[87]
Matrix: Human plasma Online SPE using Polaris C18 HD (10 mm × 2 mm) cartridge with 2 mL 100 mmol L $^{-1}$ formic acid at 2 mL min $^{-1}$ as loading solvent	PFOA, PFOS, PFDA, MeFOSAA, PFHxS, PFNA	HPLC	QqQ	Stationary phase: Betasil C8 (50 mm × 8 mm, 5 *µ*m) as analysis and pre-injection column. Gradient elution with ammonium acetate in water pH 4 (20 mmol L $^{-1}$) and acetonitrile. Flow rate 0.6 mL min^{-1}. Total analysis time 12 min	LOD 0.1 ng mL^{-1}	[88]
Matrix: Human plasma PPT with acetonitrile (with 1% of formic acid) and methanol (1:1 v/v) and filtration through 0.22 *µ*m Nylon syringe filter	PFBS, PFOSA, PFHpA, PFDoA, PFHxS, PFUA, PFDA, PFNA, PFOS, PFOA	HPLC	QqQ	Stationary phase: ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 *µ*m) at 35 °C Gradient elution with ammonium acetate in water (10 mmol L $^{-1}$) and methanol. Flow rate 0.3 mL min^{-1}. Total analysis time 12 min	LOD 0.009–0.12 ng mL^{-1}	[82]
Matrix: Human Urine PPT with acetonitrile Matrix: Human Hair SLE with MeOH/ACN (1:1)	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFOS, PFDDA	HPLC	QqQ	Stationary phase: C18 (50 × 2.1 mm, 5 *µ*m). Gradient elution with ammonium acetate in water (2 mmol L $^{-1}$) and methanol. Flow rate 0.2 mL min^{-1}. Total analysis time: 10 min	Human Urine LOD 0.01–0.02 ng L $^{-1}$ Human Hair LOD 2–4 pg g $^{-1}$	[46]
Matrix: Human placenta *Multistep sample preparation* 1)Protein digestion with 2 mL of collagenase solution 37 °C for 4 h, 2) SALLE with acetonitrile and NaCl, followed by acidification of supernatant (pH 2) 3) DLLME with trichloromethane	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFHxS, PFOS	HPLC	QTRAP	Stationary phase: Gemini C18 (100 × 2 mm, 3 *µ*m) at 25 °C. Gradient elution with ammonium acetate in water pH 4.5 (5 mmol L $^{-1}$) and acetonitrile. Flow rate 0.35 mL min^{-1}. Total analysis time: 12 min	LOD 0.006 ng g $^{-1}$ LOQ 0.02 ng g $^{-1}$	[59]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) Online SPE with a Xbridge C18 column (20 mm × 2.1 mm, 2.5 µm)	PFHpA, PFNA, PFDA, PFUnDA, PFHxS, L-PFOS, PFOA, PFOSA	UHPLC	QqQ	Stationary phase: Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 *µ*m) and a PFC Isolator as pre-injection trap column. Gradient elution with ammonium acetate in water (2 mmol L $^{-1}$): methanol 9:1 (v/v) and methanol with ammonium acetate (2 mmol L $^{-1}$). Flow rate: 0.3 mL min^{-1} Total analysis time: 26 min	LOQ 0.02–0.22 ng mL^{-1}	[71]
Tested different extraction methods Matrix: Human serum Method 1) SPE with HLB cartridge Method 2) SPE with WAX cartridge SPE with HLB cartridge IP-LLE with tetrabutylammonium bisulfate (TBA) and tert-butyl methyl ether (MTBE, three times) Matrix: Human placenta *Multistep sample preparation* Method 1) 1) SLE with 0.5 mol L $^{-1}$ formic acid/acetonitrile 1:25 (v/v) 2) SPE using EnviCarb Method 2) SLE with 0.5 mol L $^{-1}$ formic acid/acetonitrile 1:40 (v/v) 2) SPE using EnviCarb cartridge	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFOcDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS, FBSA, MeFBSA, FHxSA, MeFHxSA, FOSA, MeFOSA, EtFOSA, MeFOSE, EtFOSE, MeFOSAA, EtFOSAA, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 10:2 FTSA, 3:3 FTCA, 5:3 FTCA, 7:3 FTCA, 6:2 FTUCA, 8:2 FTUCA, 10:2 FTUCA, 6:2 monoPAP, 8:2 monoPAP, 10:2 monoPAP, 6:2 diPAP, 8:2 diPAP, 6:2/8:2 diPAP, 10:2 diPAP, diSAmPAP, ADONA, HFPO-DA, 6:2 CI-PFESA, 8:2 CI-PFESA, PFHxPA, PFOPA, PFDPA, 6:6 PFPiA, 6:8 PFPiA, 8:8 PFPiA, PFECHS	Method 1) HPLC Method 2) UHPLC	**Method 1)** QTRAP **Method 2)** QqQ	**Method 1)** Stationary phase: Luna C18 (100 × 2 mm, 5 *µ*m) at 40 °C. Gradient elution with ammonium acetate in water (10 mmol L $^{-1}$) and methanol. Flow rate: 0.3 mLmin^{-1}. Total analysis time: 23 min **Method 2)** Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 *µ*m) at 50 °C. Gradient elution with methanol: water 30:70 (v/v) and methanol, both with ammonium acetate (2 mmol L $^{-1}$) and n-methylpyridine (5 mmol L $^{-1}$) Flow rate: 0.3 mL min^{-1}. Total analysis time: 17 min	**Method 1)** LOD 0.040–1.5 ng mL^{-1} LOQ 0.080–2.8 ng mL^{-1} **Method 2)** LOD 0.0060–10 ng mL^{-1} LOQ 0.012–20 ng mL^{-1}	[29]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human serum SPE with Oasis WAX cartridge	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFBS, PFHxS, PFOS	UHPLC	QqQ	Stationary phase: Kinetex F5 Core-Shell (100 × 2.1 mm, 1.7 μm) at 40 °C. Gradient elution with ammonium acetate in water (2 mmol L^{-1}) and ammonium acetate (2 mmol L^{-1}) in acetonitrile. Flow rate: 0.4 mL min^{-1}. Total analysis time 16 min.	LOQ 0.1 – 0.5 ng mL^{-1}	[89]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) SPE with ENVI-Carb	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxS, PFOS	UHPLC	QqQ	Stationary phase: BEH C18 (50 × 2.1 mm, 1.7 μm) at 55 °C. Gradient elution using ammonium acetate in water (2 mmol L^{-1}) and ammonium acetate in acetonitrile (2 mmol L^{-1}). Flow rate: 0.3 mL min^{-1}. Total analysis time: 15 min	MDL 0.1 – 0.3 ng mL^{-1}	[34]
Matrix: Human plasma PPT with acetonitrile followed by filtration	PFHxA, PFHpA, PFHxS, PFOA, PFNA, PFOS, PFDA, PFUnDA, PFDoDA, PFTrDA	UHPLC		Stationary phase: Acquity HSS T3 (100 × 2.1 mm, 1.8 μm) at 40 °C. Gradient elution with ammonium acetate in water (2 mmol L^{-1}) and ammonium acetate in methanol (2 mmol L^{-1}). Flow rate: 0.3 mL min^{-1}. Total analysis time: 8.5 min	LOQ 0.1 – 0.5 ng mL^{-1}	[42]
Matrix: Human plasma SPE with Captiva EMR-Lipid cartridge followed by filtration with 0.2 μm Nylon filter	PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFOA, PFBS, PFHxS, PFHpS, PFDS, PFOS, 6:2 Cl-PFESA, 8:2 Cl-PFESA	UHPLC	QTRAP	Stationary phase: Acquity UPLC BEH shield RP18 (100 × 2.1 mm, 1.7 μm) Gradient elution using ammonium formate in water (5 mmol L^{-1}) and methanol Flow rate: 0.2 mL min^{-1}. Total analysis time: 35.5 min	LOQ 0.02 – 0.36 ng mL^{-1}	[90]
Matrix: Human plasma IP-LLE with methyl tert-butyl ether and tetrabutylammonium hydrogen sulfate at alkaline pH	PFOA, PFOS, PFHxS	UHPLC	QqQ	Not reported	LOD 0.1–0.3 μg mL^{-1}	[49]
Matrix: Human serum *Multistep sample preparation* 1) PPT with acetonitrile or methanol 2) SPE with Oasis MCX 3) Online SPE with WAX column (20 × 2.1 mm, 30 μm)	PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFHxS, PFHpS, PFOS, PFDS, MeFOSA-M, EtFOSA-M, MeFOSA-A, EtFOSA-A, 4:2 FTS, 6:2 FTS, 8:2 FTS, 6:2 diPAP, 8:2 diPAP, diSAmPAP	HPLC	QqQ	Stationary phase: Triart C18 column (100 × 2.1 mm, 3 μm) at 40 °C and WAX (20 × 2.1 mm, 30 μm) pre-column after all pumps *Two method system* Gradient elution using ammonium acetate in water (2.5 mmol L^{-1}), methanol, ammonium acetate in 95% methanol (2.5 mmol L^{-1}), ammonium hydroxide in methanol (0.1%, v/v), water, ammonium hydroxide in 90% methanol (0.5%, v/v). Flow rate: 0.3 – 0.5 mL min^{-1}. Total analysis time: Method A) 29 min; Method B) 27 min	MDL 0.0071–0.16 ng mL^{-1}	[70]
Matrix: Human serum and plasma PPT with ice-cold methanol	PFBS, 4,2 FTS, GenX, PFHpA, PFHxS, PFOA, PFOS, PFNA, FTSL, PFOAL, PFOSL, PFNAL	UHPLC	QTRAP	Stationary phase: Atlantis T3 (50 × 2.1 mm, 3 μm) at 35 °C. Gradient elution using ammonium acetate buffer (20 mmol L^{-1}) and methanol Flow rate: 0.2 mL min^{-1}. Total analysis time: 13.51 min	LOD 0.04–0.20 ng mL^{-1}	[39]

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Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human plasma Online SPE with Polaris C18 HD 10 mm × 2 mm with 2 mL 0.1 mol L^{-1} formic acid at 2 mL min^{-1} as loading solvent	PFOS, PFOA, PFHxS, EtFOSAA, MeFOSAA, PFNA	HPLC	QqQ	Stationary phase: Betasil C8 HPLC precolumn (50 × 3 mm, 5 µm) as analysis and pre-injection column. Gradient elution using ammonium acetate in water (20 mmol L^{-1}) and acetonitrile. Flow rate: 0.6 mL min^{-1}. Total analysis time: 12 min	LOD 0.1 ng mL^{-1}	[91]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) Online SPE with Oasis® HLB (20 × 2.1 mm, 25 *µ*m) and the mobile phase as loading solvent	PFOA, PFOS, PFBS, PFHxS, PFHxA, PFNA, PFDA, PFDoDA, ADONA	HPLC	QTRAP	Stationary phase: ReproSil-Pur-ODS-3 (150 × 2 mm, 5 *µ*m) at 35 °C a ReproSil C-18AQ (33 × 3 mm, 5 µm) was used as a pre-injection column. Gradient elution: using ammonium acetate in water (2 mmol L^{-1}) and methanol. Flow rate: 0.4 mL min^{-1}. Total analysis time: 16 min	LOQ 0.25 µg L^{-1}	[69]
Matrix: Human serum Online SPE with HySphere PICO C8-SE cartridge (10 × 2.1 mm, 7 *µ*m) with acetonitrile: formic acid 100 mmol L^{-1} solution (1:10) as loading solvent	PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFBS, PFHxS, PFOS, PFOSA, PFOSAA, MeFOSAA, EtFOSAA	HPLC	QTRAP	Stationary phase: Agilent ZORBAX Eclipse XDB-C8 (100 × 3.0 mm, 3.5 *µ*m). Gradient elution using ammonium acetate in water pH 4.3 (10 mmol L^{-1}) and acetonitrile/methanol 1:1 (v/v). Flow rate: 0.7 mL min^{-1}. Total analysis time: 10.5 min	DL 0.005–0.019 ng mL^{-1} RL 0.018–0.096 ng mL^{-1}	[92]
Matrix: Human serum and semen IP LLE with tetra-*n*-butylammonium hydrogen sulfate, $NaHCO_3/Na_2CO_3$ buffer (pH 10) and methyl tert-butyl ether (three times) (1:2:4)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFBS, PFHxS, PFOS, 6:2 Cl-PFESA, 8:2 Cl-PFESA	UHPLC	QqQ	Stationary phase: Acquity BEH C18 column (100 × 2.1 mm, 1.7 *µ*m) at 50 °C. Gradient elution using ammonium acetate in water (10 mmol L^{-1}) and methanol. Flow rate: 0.4 mL min^{-1}. Total analysis time: 10 min	**Serum** LOQ 0.01–0.20 ng mL^{-1} **Semen** LOQ 0.002–0.10 ng mL^{-1}	[53]
Matrix: Human plasma PPT with acetonitrile after wash/dilute the samples with Sperm Washing Medium (culture medium)	PFOA	HPLC	QqQ	–	LOQ: 0.1 ng mL^{-1}	[45]
Matrix: Human plasma and serum *Multistep sample preparation* PPT multiplexed with SPE with 1% formic acid in acetonitrile by using Ostro Protein Precipitation and Phospholipid Removal plate	PFBA, PFBS, PFDA, PFDoDA, PFDoDS, PFDS, PFECHS, PFHpA, PFHpS, PFHxS, PFNA, PFNS, PFOA, L-PFOS, PFOSA, PFPeA, PFPeS, PFTDA, PFTrDA, and PFUnDA	UHPLC	QqQ	Stationary phase: Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 µm). Gradient elution using 2 mmol L^{-1} ammonium acetate and methanol/ammonium acetate 9:1 (v/v). Flow rate 0.3 mL min^{-1}	MDL 0.01–0.06 ng mL^{-1}	[40]
Matrix: Liver, kidney, and brain *Multistep sample preparation* 1) SLE of the tissues with water 2) Protein digestion of the homogenates overnight with potassium hydroxide in water/methanol 1:1 (v/v) 3) SPE using Strata X-AW cartridges Matrix: Plasma SPE using Strata X-AW cartridges	PFHxA, PFDA, and PFOA	HPLC	QTRAP	Not reported	**Plasma** LOD 1.7–2.8 ng mL^{-1} LOQ 25 ng mL^{-1} **Liver** LOD: 30–211 ng mL^{-1} LOQ: 500 ng mL^{-1} **Kidney** LOD: 31–73 ng mL^{-1} LOQ: 500 ng mL^{-1} **Brain** LOD: 58–368 ng mL^{-1} LOQ: 500 ng mL^{-1}	[80]

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Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human Urine *Multistep sample preparation* 1) Protein digestion using 1 mol L^{-1} acetate buffer (pH 5.5), β-glucuronidase, and formic acid 2) Online SPE using HySphere C8-SE (7 um) cartridge or Oasis WAX (30 mm)	PFHxS, PFHpS, n-PFOS, Sm-PFOS, Sb-PFOA, PFBA, PFPeA, PFHxA, PFHpA, n-PFOA, PFNA, PFDA, PFUnDA, HFPO-DA, DONA, 9Cl-PF3ONS	HPLC	QTRAP	Stationary phase: Chromolith HighResolution RP-18e (100 × 4.6 mm) preceded by Chromolith HighResolution RP-18 (25 × 4.6 mm) and guard column. Gradient elution with 20 mmol L^{-1} ammonium acetate in 5% acetonitrile and acetonitrile. Flow rate 1 mL min^{-1}	LOD: 0.1 ng mL^{-1}	[78]
Matrix: Human umbilical cord plasma PPT using methanol and acetonitrile, 1% formic acid, sonication and filtration of supernatant through a 0.22 μm nylon syringe filter	PFOA, LPFOS, PFNA, PFDA, PFUA, PFHxS, PFOSA, PFDoA, PFBS, PFHpA	HPLC	QqQ	Stationary phase ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm) preceded by guard column (5 × 2.1 mm, 1.8 µm) at 35 °C. Gradient elution using 10 mmol L^{-1} ammonium acetate aqueous solution and methanol	LOD: 0.009–0.12 ng mL^{-1}	[93]
Matrix: Human plasma IP-LLE using TBAS and carbonate buffer at pH 11.8 with 5 mL MTBE. Extraction assisted by sonication	PFHxS, PFOS, FOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, and PFHxDA	HPLC	QqQ	Stationary phase Extend-C18 (100 × 2.1 mm, 1.8 µm) maintained at 45 °C. Gradient elution using of 2 mmol L^{-1} ammonium acetate and methanol. Flow rate 0.3 mL min^{-1}.	LOD: 0.01–0.02 ng mL^{-1}	[50]
Matrix: Human plasma Online SPE with Betasil C8 (10 × 4 mm)	PFBS, nPFHxS, brPFHxS, nPFOS, brPFOS, PFDS, PFPeA, PFHpA, nPFOA, brPFOA, nPFNA, brPFNA, PFDA, PFUnDA, PFDoA	HPLC	QqQ	Stationary phase Betasil C8 (50 × 3 mm, 1.9 µm) with a hypercarb guard column (10 × 4 mm, 5 µm). Gradient elution with acetonitrile and water.	LOD: 0.03 ng mL^{-1}	[50]
Matrix: Human plasma *Multistep sample preparation* 1) PPT in 2 steps, first acetonitrile and then methanol 2) Online SPE using Betasil C8 (50 mm × 2.1 mm, 3 µm).	PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUA, PFBS, PFHxS, PFOS, PFDS, 8:2 FTUCA, FOSA, MeFOSA, EtFOSA, MeFOSE, and EtFOSE	HPLC	QqQ	Not reported	LOD: 0.006–0.1 ng mL^{-1} LOQ: 0.02–0.33 ng mL^{-1}	[68]
Matrix: Human plasma PPT using methanol and L acetonitrile and 1% formic acid	PFOA, PFHpA, PFNA, PFDA, PFUA, PFDoA, PFOSA, PFBS, PFHxS	HPLC	QqQ	Stationary phase: ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm) preceded by guard column (5 × 2.1 mm, 1.8 µm) at 35 °C. Gradient elution using 10 mmol L^{-1} ammonium acetate and methanol	LOD: 0.009–0.12 ng mL^{-1}	[81]
Matrix: Human whole blood in DBS 50 mL for direct online SPE *Multistep sample preparation* 1) DBS blood was spotted on a Whatman 903 protein saver card and were allowed to dry overnight. Methanol was used to clean and extract analytes from DBS 2) Online SPE with betasil C18 (10 × 3 mm, 5 µm) column at 25 °C	6:2PAP, 8:2PAP, 6:2diPAP, 8:2diPAP, PFBS, PFHxS, PFHpS, PFOS, PFDS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxPA, PFOPA, PFDPA, PFOSA, MeFOSA, EtFOSA	UHPLC	QqQ	Stationary phase: ZORBAX Eclipse Plus C18 (50 × 2.1, 1.8 µm) at 40 °C. Gradient elution with acetonitrile and water	LOD: 0.015–0.3 ng mL^{-1} LOQ: 0.025 ng mL^{-1}	[76]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Human serum, placenta, and fetal tissue *Multistep sample preparation* 1) SLE using 3 parts of acetonitrile 70% (v/v) to one part of tissue 2) PPT of the extract was carried out latter with acetonitrile	PFOS, PFOA, PFNA, PFDA, PFUnA, PFHxS	HPLC	QqQ	Stationary phase: Genesis C8 (20 × 2.1 mm, 4 µm). Gradient elution using 0.1% (v/v) ammonia in water and 0.1% (v/v) ammonia in acetonitrile	**Serum** LOQ: 0.03–0.60 ng mL^{-1} **Tissues** LOQ: 0.09–0.60 ng mL^{-1}	[79]
Matrix: Human plasma and cord blood SPE using Oasis HLB cartridge	PFOS, PFOA, PFBS, PFHxS, PFDS, PFOSA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFNA	HPLC	QqQ	Not reported	LOQ: 0.08–0.2 ng mL^{-1}	[94]
Matrix: Human umbilical cord plasma PPT using methanol and acetonitrile, 1% formic acid. The supernatant was collected and then filtered through 0.22 μm Nylon syringe filter	PFOSA, PFHpA, PFOS, PFNA, PFHxS, PFBS, PFOA, PFDA, PFUA, PFDoA	HPLC	QqQ	Stationary phase ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm) preceded by guard column (5 × 2.1 mm, 1.8 µm) at 35 °C. Gradient elution with 10 mmol L^{-1} ammonium acetate and methanol	LOD: 0.02–0.12 ng mL^{-1}	[95]
Matrix: Rat plasma PPT using acetonitrile and methanol Matrix: Liver, kidney, and brain *Multistep sample preparation* 1) SLE of the tissues using water 2) Protein digestion overnight with potassium hydroxide in methanol/water 1:1	PFBS, PFOS, and PFHxS	–	–	Stationary phase Luna C18(2)-HST (100 × 3.0 mm, 2.5 µm). Isocratic elution using 0.1% formic acid in acetonitrile/ 1 mmol L^{-1} ammonium acetate 85:15 (v/v). Flow rate 0.5 mL min^{-1}	**Plasma** LOQ 25 ng mL^{-1} **Tissues** LOQ 5 ng g^{-1}	[41]
Matrix: Human urine and human serum *Multistep sample preparation* 1) Protein digestion 2) Online SPE using HySphere C8-SE (7 um) cartridge or Oasis WAX (30 mm)	PFPrS, PFBS, PFHpS, PFHxS, n-PFOS, Sm-PFOS, PFBA, PFPeA, PFHxA, PFHpA, n-PFOA, Sb-PFOA, PFNA, PFDA, PFUnDA, HFPO-DA, DONA, 9Cl-PF3ONS, FOSA, MeFOSAA, EtFOSAA.	HPLC	QqQ	–	LOD 0.1 ng mL^{-1}	[77]
Matrix: Human plasma PPT using methanol, acetonitrile, and, 1% formic acid. Extraction assisted by sonication	PFOA, PFOS, PFNA, PFHxS, PFDeA, PFUA, PFBS, PFDoA, PFHpA	HPLC	QqQ	–	LOD 0.009–0.12 ng mL^{-1}	[96]
Matrix: Human plasma *Multistep sample preparation* 1) PPT with acetonitrile 2) SPE using 25 mg ENVI-Carb	PFHxS, PFHxA, PFHpA, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA	UHPLC	QqQ	Stationary phase Ethylene-bridged BEH C18 (50 × 2.1 mm, 1.7 µm) and retention gap column (100 × 2.1 mm, 1.7 µm) at 55 °C. Gradient elution with 2 mmol L^{-1} ammonium acetate and 2 mmol L^{-1} ammonium acetate in acetonitrile	LOD 0.1–0.3 ng mL^{-1}	[35]

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analytes	Separation technique	Detection	Separation conditions	LOD/LOQ	Ref
Matrix: Plasma Online SPE with Betasil C8 (10 × 4 mm)	PFOS, PFOA, PFHxS, PFNA, PFDA	HPLC	QqQ	–	LOD 0.015 ng mL^{-1}	[97]
Matrix: Human serum Online SPE using a Turboflow Cyclone-P (50 × 1.0 mm)	4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 10:2 FTSA, 6:2 monoPAP, 8:2 monoPAP, 6:2 diPAP, 6:2/8:2 diPAP, 8:2 diPAP, PFHxPA, PFOPA, 6:6 PFPiA, 6:6/8:8 PFPiA, 8:8 PFPiA, FOSA, N-MeFOSA, N-EtFOSA, FOSAA, N-MeFOSAA, N-EtFOSAA, 6:2Cl-PFAES, 8:2Cl-PFAES, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS	HPLC	QqQ	Stationary phase Acclaim 120 C18 (150 × 4.6 mm, 5 µm). Isocratic elution using 0.1% formic acid in water (pH 4 with ammonia), acetonitrile/methanol 1:1 (v/v) and, water/acetonitrile/methanol/ isopropanol 1:1:1:1 (v/v/v/v)	MLOD 0.013–0.089 ng mL^{-1}	[83]
Matrix: Human breast milk *Multistep sample preparation* 1) SALLE with acetonitrile and sodium chloride 2) DLLME with 1.5 mL trichloromethane and NaCl solution	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFHxS and PFOS	UHPLC	QTRAP	Stationary phase: Gemini C18 column (100 mm × 2 mm, 3 μm). Gradient elution using 5 mmol L^{-1} ammonium acetate (pH 4.5) and acetonitrile	LOD: 0.006 ng mL^{-1} LOQ: 0.02 ng mL^{-1}	[61]
Matrix: Human urine DLLME performed by adding sodium chloride and injecting a mixture of acetonitrile with perfluoro-tert-butanol	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, C8 Cl-PFESA, C10 Cl-PFESA, C12 Cl-PFESA, 4:2FTS, 6:2FTS, 8:2FTS	HPLC	QqQ	Stationary phase: Acclaim 120 C18 column (150 mm × 4.6 mm, 5 μm). Isocratic elution using methanol and 50 mmol L^{-1} of ammonium acetate in water	LOD: 0.28–3.8 ng mL^{-1} LOQ: 0.9–5.0 ng mL^{-1}	[57]

PFAS abbreviations: PFPrA – Pentafluoropropionic acid, PFBA – Perfluorobutanoic acid, PFPeA – Nonafluoropentanoic acid, PFHxA – Perfluorohexanoic acid, PFHpA - Perfluoroheptanoic acid, PFOA – Perfluorooctanoic acid, PFNA – Perfluorononanoic acid, PFDA – Perfluorodecanoic acid, PFUnDA – Perfluoroundecanoic acid, PFDoDA – Perfluorododecanoic acid, PFTrDA – Pentacosafluorotridecanoic acid, PFTeDA – Perfluorotetradecanoic acid, PFHxDA – Perfluorohexadecanoic acid, PFOcDA – Perfluorooctadecanoic acid, PFEtS – Pentafluoroethanesulfonic acid, PFPrS – Perfluoropropanesulfonic acid, PFBS – Perfluorobunate sulfonate, PFPeS - Perfluoropentanesulfonic acid, PFHxS – Perfluorohexanesulfonic acid, PFHpS – Perfluoroheptanesulfonic acid, PFOS – Perfluorooctanesulfonic acid, Cl-PFOS – 1-Chloro-perfluorooctanesulfonic acid, PFNS – Perfluorononanesulfonic acid, PFDS – Perfluorodecane sulfonic acid, PFDoDS – Perfluorododecane sulfonate, PFECHS – Perfluoroethylcyclohexane sulfonate, 6:2 Cl-PFESA and 9Cl-PF3ONS – 6:2 Chlorinated polyfluorinated ether sulfonate, 8:2 Cl-PFESA – 8:2 Chlorinated polyfluorinated ether sulfonate, 6:2 FTUCA - 6:2 Fluorotelomer unsaturated carboxylic acid, 8:2 FTUCA - 8:2 Fluorotelomer unsaturated carboxylic acid, 10:2 FTUCA - 10:2 Fluorotelomer unsaturated carboxylic acid, HFPO-DA Hexafluoropropylene oxide-dimer acid, ADONA – Ammonium 4,8-dioxa-3H-perfluorononanoate, 6:2 monoPAP – 6:2 Fluorotelomer phosphate monoester, 8:2 monoPAP – 8:2 Fluorotelomer phosphate monoester, 10:2 monoPAP – 10:2 Fluorotelomer phosphate monoester, SAmPAP – 2-(N-ethylperfluorooctanesulfonamido)ethyl phosphate, PFHxPA – Perfluorohexylphosphonic acid, PFOPA – Perfluorooctylphosphonic acid, PFDPA – Perfluorodecylphosphonic acid, Cl-PFHxPA – Monochloroperfluorohexylphosphonic acid, Cl-PFOPA – 8-Choloroperfluorooctylphosphonic acid, 6:2 diPAP – 6:2 Fluorotelomer phosphate diester, 8:2 diPAP – 8:2 Fluorotelomer phosphate diester, 10:2 diPAP – 10:2 Fluorotelomer phosphate diester, 6:2/8:2 diPAP - 6:2/8:2 Fluorotelomer phosphate diester, diSAmPAP – bis-[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl] phosphate, 6:6 PFPiA – Bis(perfluorohexyl)phosphinic acid, 6:8 PFPiA – Perfluorohexylperfluorooctylphosphinic acid, 8:8 PFPiA – Bis(perfluorooctyll)phosphinic acid, FBSA – Perfluorobutane sulfonamide, MeFBSA – N-Methylperfluorobutanesulfonamide, FHxSA – Perfluorohexanesulfonamide, MeFHxSA – Tridecafluoro-N-methyl-1-hexasulfonimide, FOSA – Perfluorooctanesulfonimide, MeFOSA – N-methylperfluorooctanesulfonimide, EtFOSA – N-ethylperfluorooctanesulfonimide, FOSAA – Perfluorooctane Sulfonamidoacetic Acid, MeFOSAA – N-methylperfluorooctane Sulfonamido Acetic Acid, MeFOSE – N-Methylperfluorooctanesulfonamidoethanol, 4:2 FTSA – 4:2 Fluorotelomer Sulfonate, 6:2 FTSA – 6:2 Fluorotelomer Sulfonate, 8:2 FTSA – 8:2 Fluorotelomer Sulfonate, 10:2 FTSA – 10:2 Fluorotelomer Sulfonate, 6:2 FTCA – 2-Perfluohexyl Ethanoic Acid, 8:2 FTCA – 2-Perfluooctyl Ethanoic Acid, 10:2 FTCA – 2-Perfluodecyl Ethanoic Acid, 3:3 FTCA – 3-Perfluoropropyl Propanoic Acid, 5:3 FTCA – 2H, 2H, 3H, 3H-Perfluorooctanoic acid, 7:3 FTCA – 3-Perfluoroheptylpropanoic Acid. HPLC – High Performance Liquid Chromatography, UHPLC – Ultra High-Performance Liquid Chromatography, Q – Single Quadrupole, QqQ – Triple Quadrupole Mass Spectrometry, QTOF MS/MS – Quadrupole Time of Flight Mass Spectrometry, QTRAP – Linear ion trap Mass Spectrometry, IM-QTOF – Ion Mobility Quatrupole Time of Flight Mass Spectrometry, SFC – Supercritical Fluid Chromatography, SPE – Solid Phase Extraction, PPT – Protein precipitation, LLE – Liquid-Liquid Extraction, DLLME – Dispersive Liquid-Liquid Extraction, DBS – Dried Blood Spot, DL – Detection Limit, RL – Reporting Limit, LOD – Limit of Detection, LOQ – Limit of Quantification, MDL – Method Detection Limit, MLOD – Method Limit of Detection.

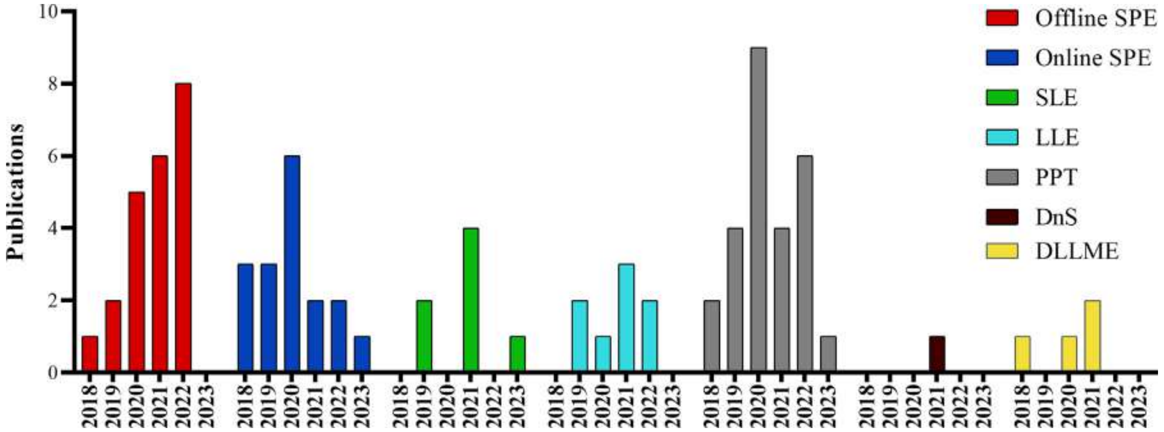

Fig. 2. Different sample preparation techniques used for PFAS analysis in biological samples (papers from 2018 to 2023). SPE – Solid Phase Extraction, SLE – Solid Liquid Extraction, LLE – Liquid-Liquid Extraction, PPT – Protein Precipitation, DnS – Dilute-and-Shoot, DLLME – Dispersive Liquid-Liquid Extraction.

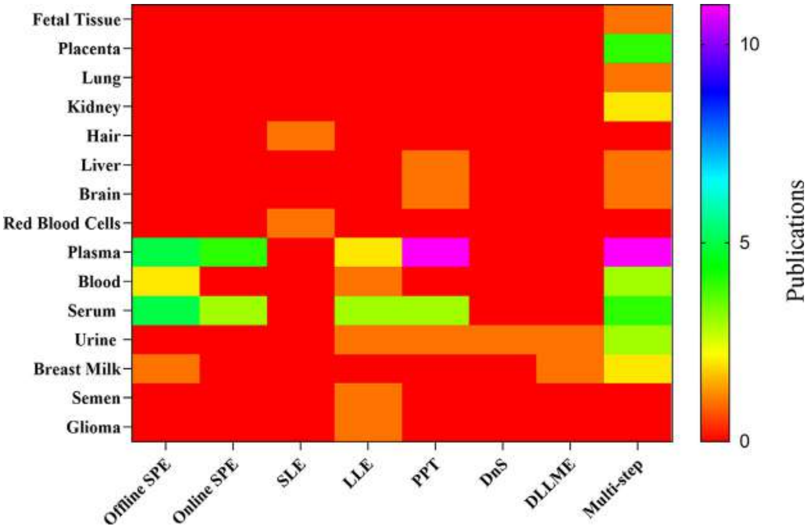

Fig. 3. PFAS analysis by different sample preparation techniques in different matrices (papers from 2018 to 2023). SPE – Solid Phase Extraction, SLE – Solid Liquid Extraction, LLE – Liquid-Liquid Extraction, PPT – Protein Precipitation, DnS – Dilute-and-Shoot, DLLME – Dispersive Liquid-Liquid Extraction.

higher flow rates and other types of adsorbents such as organic synthetic and natural polymers to be employed [24].

Another relevant concept in SPE is the breakthrough volume (V_B). This concept is defined as the volume in which the analyte starts to elute from the SPE cartridge; typically, it is defined as the volume in which 1% of the analyte has eluted from the column. Thus, V_B depends on the analyte and experimental conditions. Understanding this parameter is important when developing an SPE method because it is related to how much sample volume can be loaded into the cartridge. As a best practice, the sample volume should not be large enough to overwhelm the sorbent material, nor should it be larger than V_B. If the sample volume is larger than V_B, the analytes begin to elute from the cartridge before sample loading finishes, which could be translated into poor analyte recovery and reproducibility [24].

As can be seen in Table 2, SPE has been widely applied for PFAS analysis in biological matrices [25]. Inorganic materials such as silica and graphitized carbon or organic materials including polymers are currently used as sorbent materials for PFAS analysis. If the sample preparation strategy relies on trapping the analytes in the material, PFAS could be separated from the matrix components by two mechanisms. PFAS have acidic character, so they could be trapped in the adsorbent through an ion exchange mechanism. This strategy can be adopted by using adsorbents like OASIS WAX® [26] which consists of copolymers of 1,3-divinylbenzene and N-vinyl-pyrrolidone modified with a weak anion exchange moiety containing one primary amine and one secondary amine [24]. The pKa of secondary and tertiary amines is about 10 [27], so the resin needs to be conditioned by employing a solution with pH below 10, so that it is fully charged and able to exchange ions.

Rawn et al. (2022) developed a sample preparation method by using this strategy for analyzing 13 different PFAS in human milk [26]. After a protein precipitation step with formic acid, acetonitrile, and water, the authors conditioned the SPE cartridge by washing the OASIS WAX® cartridge with 0.1% (v/v) ammonium hydroxide solution in methanol followed by another washing step with ultrapure water. This allowed the absorbent material to be charged and hence retain the PFAS. The supernatant of the protein precipitation step was then loaded into the cartridge. After several washes with 20 mmol L^{-1} ammonium acetate in methanol/water 20:80 (v/v), methanol/water 50:50 (v/v), and methanol, the analytes were eluted with 2% (v/v) ammonium hydroxide solution in methanol. Analysis was performed by LC–MS/MS using an Acquity BEH C_{18} column (100 × 2.1 mm, 1.7 µm) and gradient elution was used with 2 mmol L^{-1} ammonium acetate in water and acetonitrile/methanol 2:1 (v/v). The authors achieved LODs ranging from 5 to 10 ng mL^{-1}.

Another possible strategy is to convert PFAS molecules into their neutral form and trap them in the sorbent material through a reversed phase mechanism. This strategy has been applied for PFAS analysis; sorbents like OASIS HLB® and octyl or octadecyl derivatized silica have been employed [24]. OASIS HLB® has been used for PFAS analysis in biological matrices. This sorbent is a copolymer of 1,3-divinyl benzene and N-vinyl-pyrrolidone, and it can interact with molecules of different polarities [24]. Kaiser et al. (2021) adapted a sample preparation method, previously developed by Kukleyik et al. (2004) [28], for PFAS analysis [29]. The authors first added 0.1 mol L^{-1} formic acid solution in water to the samples; this step is important because it protonates the PFAS and converts them back into their neutral form. Then, the OASIS HLB® cartridges were conditioned with methanol and 0.1% (v/v) formic acid solution in water, and the sample was loaded into the cartridge. After several washing steps with 0.1 mol L^{-1} formic acid, formic acid/methanol 60:40 (v/v), and 0.01% (v/v) ammonium hydroxide, the analytes were eluted from the cartridge with 6 mL of 0.0001% (v/v) ammonium hydroxide solution in acetonitrile. Analysis was performed by LC–MS/MS and a C_{18} column (100 × 2 mm, 5 µm) was used. The analytes were eluted by gradient elution with 10 mmol L^{-1} ammonium acetate solution and methanol. By using this method, the authors detected PFAS at concentrations ranging from 0.006 to 10 ng mL^{-1} and quantified them in the range of 0.012–20 ng mL^{-1}.

Some miniaturized SPE systems have also been used for analyzing PFAS in biological samples. These systems are based on a 96-well plate format such as the Oasis WAX 96-well µ-elution plate® by Waters or Captiva EMR-Lipid 96 well-plate® by Agilent. These systems are advantageous because smaller solvent volumes and amounts of sample can be employed in all sample preparation steps. Furthermore, this miniaturized system allows a greater number of samples to be processed simultaneously, accelerating the whole process. Van Beijsterveldt et al. (2022) [30] employed the Oasis WAX 96-well µ-elution plate® for analysis of 7 PFAS in 257 human breast milk samples and 586 plasma samples. For the human breast milk samples, an aliquot of 500 µL was used, while an aliquot of 50 µL was employed for the plasma samples. The authors achieved an impressive lower limit of quantification (LLOQ) of 0.0015 ng mL^{-1} in both human breast milk and plasma samples.

The SPE methods we have described so far were operated offline. SPE is another SPE mode that could be coupled to liquid chromatography systems, allowing the whole process to be automated. As observed in Fig. 3, online SPE has also been employed for preparing biological samples. Online SPE has advantages over offline SPE—it requires little or no sample pretreatment/sample handling, which increases reproducibility and sensitivity, affording low LOD and LOQ [31]. In online SPE, the SPE column is placed before the analytical column; the analytes are injected and trapped in the SPE column, while the other components of the matrix are ideally not retained and directed to the waste [32].

The sample solvent is preferentially water, to avoid breakthrough in the SPE column. Nevertheless, the presence of organic solvents may improve analyte recovery [32]. Octyl and octadecyl are the major sorbents used in online SPE for PFAS quantification or detection. Lin et al. (2021) employed an ODS-3 SPE column and achieved a limit of detection for PFAS ranging from 0.002 to 0.150 ng mL^{-1} [33]. The authors first carried out a protein precipitation step (offline) with 100 µL of acetonitrile, which was followed by online SPE using a gradient between 1% (v/v) methanol with 0.1% (v/v) formic and 90% (v/v) methanol with 0.1% (v/v) formic acid. The samples were analyzed by LC–MS/MS, and a C18 column (50 × 2.1 mm, 5 µm) was used. The analytes were eluted by gradient elution with 2 mmol L^{-1} ammonium acetate and methanol.

3.2. Protein precipitation (PPT)

PPT is one of the simplest sample preparation techniques available to date and has been extensively employed for PFAS analysis in biological samples such as blood, serum, plasma, and even some tissue samples after a digestion process (Fig. 3). The basis of PPT is to overcome the repulsive forces between the proteins present in a sample generally by adding a water-miscible organic solvent like acetonitrile or methanol, to denature, agglomerate, and further remove proteins from the medium with a centrifugation or a filtration step.

Among the papers reviewed herein [34,35], the first developed method was attributed to Okada et al. (2013) [36], who analyzed 11 PFAS (mostly long-chain carboxylic acids) in plasma samples by using ultra-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS). To this end, 2 mL of acetonitrile was vortex-mixed with 500 µL of plasma and centrifuged. The supernatant was transferred to a clean tube, and 25 mg of ENVI-Carb® and 50 µL of acetic acid were added, which was followed by a new centrifugation. The supernatant was collected, dried to 250 µL under nitrogen stream, and diluted with the same volume of methanol before analysis. The method was validated in an inter-laboratory comparison program supported by the university. The recovery and LOD values reported by the authors varied from 75.2 (for PFOS) to 100% (for PFTeDA) and from 0.1 to 0.3 ng mL^{-1}, respectively.

In 2016, Wang et al. (2016) presented a well-established technique [37] and employed it for analyzing 10 PFAS in plasma samples by LC–MS/MS. PPT was carried out in 100 µL of plasma sample with a mixture of acetonitrile/methanol [1:1 (v/v), 300 µL] and 1% (v/v) formic acid followed by ultrasonication, centrifugation, and filtration (0.22-µm nylon filter). The developed method presented the lowest LOD when PPT was used for plasma samples (LOD varied from 0.009 to 0.12 ng mL^{-1}).

In 2022, Frigerio et al. (2022) [38] validated a detailed PPT method for analysis of 30 legacy and emerging PFAS in human plasma by LC–MS/MS. Some advantages of this method were the smaller sample (20 µL) and organic solvent (80 µL of methanol) volumes and the simplicity of the sample preparation technique (the samples were only vortexed and centrifuged). The limit of quantification (0.009–0.245 ng mL^{-1}), was one of the lowest in the reviewed studies. In addition, the authors were careful about PFAS contamination by labware and mobile phase, which may explain their success in obtaining a low LOQ. PFAS contamination should be considered when dealing with biological matrix analysis because these analytes can be present in several devices/instruments present in laboratories. Contamination of the mobile phase or the biological sample (during the sample preparation step) could lead to serious mistakes, mainly false-positive errors, and overestimated concentrations.

Two developed and validated methods that are noteworthy involve an automated system for PPT in serum and plasma samples and use 96-well plates, which are also suitable for SPE. In the first method, Mottaleb et al. (2020) [39] transferred 50 µL of serum or plasma and 100 µL of cold methanol to a 96-well Impact Protein Precipitation Plate followed by 2-min shaking and centrifugation. The supernatant was collected and dried under nitrogen stream for later reconstitution in 2 mmol L^{-1} ammonium buffer before UHPLC–MS/MS analysis. In the second method, Salihovic et al. (2020) [40] used an Ostro Protein Precipitation and Phospholipids Removal 96-well plate to extract 150 µL of plasma or serum. They added the samples in preconditioned plates containing 450 µL of acetonitrile, followed by 450 µL of 1% (v/v) formic acid solution in acetonitrile. The plate was mixed, and extraction was performed under vacuum for 5–7 min. Then, the supernatant was transferred to glass vials and reduced to 190 µL under nitrogen stream, and the performance standards were added along with 300 µL of 2 mmol L^{-1} ammonium acetate solution in water before UHPLC–MS/MS analysis. The authors proposed reducing the sample volume from 150 to 20 µL with slight changes in the method, but sensitivity for some analytes decreased. The authors also warned about signal suppression in the presence of bile acids, which was not significant in the reported analysis, but may play an important role in samples with high contents of this interferent. Both methods are advantageous: they are relatively simple and fast, require small sample volumes (50–150 µL), have low LOD (0.01–0.20 ng mL^{-1}), and allow many samples to be extracted simultaneously, being ideal for biomonitoring analyses.

PFBS, PFHxS, and PFOS have been analyzed in rat plasma and tissue homogenates (liver, kidney, and brain) [41] employing PPT as sample preparation technique after a protein digestion step. The authors prepared a homogenate by mixing 100–300 mg of tissue with water at a 1:10 ratio. After that, 100 µL of homogenate or plasma was mixed with 100–400 µL of internal standard solution in acetonitrile and 0.05 N KOH solution in methanol and water 1:1 (v/v). The samples were rotated for 12 h for protein digestion, which was followed by neutralization with 0.1 N HCl solution. After centrifugation, the supernatant was analyzed by LC–MS/MS. The LOQ was 25 ng mL^{-1} for plasma and 5 ng g $^{-1}$ for tissue samples, and recoveries varied between 61.4 and 129%. Even though PPT helped to clean up the tissue homogenate, the protein content in the tissues was high and, together with other solid components, might elicit matrix effects during chromatographic analysis, leading to higher LOQ and affecting the method selectivity.

Other methods based on PPT as sample preparation technique have been developed for extracting PFAS from different biological matrices (urine, semen, serum, or plasma). However, these methods required larger sample volumes (1 mL of serum or 2 mL of urine), larger solvent volumes (150 µL–30 mL of acetonitrile), and exhaustive sample preparation time (three subsequent extractions or 8–10 h vacuum dry) [42–46]. Anyway, the developed methods were suitable for the desired application and did not affect the quality of the work.

PPT plays an essential part in PFAS analysis in biological matrices because it is inexpensive, easy, and fast to handle, and compatible with different separation techniques (HPLC, UHPLC, and gas chromatography (CG)) and detection systems (quadrupole ion trap (QTRAP), quadrupole couple with time-of-flight (QTOF), and tandem mass spectrometry (MS/MS). Despite these benefits, PPT might provide inefficient protein removal from some biological matrices, and analyte-protein binding can represent a challenge and demand further cleanup and preconcentration steps. In PFAS analysis, especially long-chain carboxylates, interaction with proteins may result in low recovery rates and high LOD and LOQ [6]. PPT can also be employed as a useful step before other sample preparation techniques, like (online)-SPE and DLLME, enhancing analyte recovery and minimizing matrix effects.

3.3. Liquid-liquid extraction (LLE)

LLE, which is based on the distribution of an analyte between two immiscible solvents, has been used for extracting PFAS from biological matrices. Although LLE usually requires large sample quantities (not necessary) and multiple extractions and uses hazardous organic solvents, it is simple and relies on a large number of existing high- purity organic solvent. These factors explain why LLE is still commonly applied for extracting different analytes from serum, plasma, and urine.

Recently, Kim et al. (2022) published a screening procedure based on LLE and UHPLC coupled with quadrupole time-of-flight mass spectrometry (QTOF-MS) for comprehensive PFAS analysis in human urine samples. They optimized LLE by adding formic acid or ammonium hydroxide solutions to extract 12 mass-labeled PFAS from 0.5 mL of urine. They used a mixture of ethyl acetate and isopropanol (95:5 v/v) as solvent and reported that extraction recovery was better for basic extraction, with recovery rates above 70%. Short-chain PFAS such as PFPeA and PFHxA were more easily extracted than longer-chain PFAS, and PFCA was more extractable than the corresponding PFAS. Interestingly, ammonium solution enhanced the partition of acidic PFAS analytes, revealing that solubility is not easily predictable due to the carbon chain and high degree of fluorination, which impart additional hydrophobic tendencies [47]. While this LLE method is simpler than ion-paring liquid-liquid extraction (IP-LLE) methods, it does not assess how matrix components affect the results. Therefore, determining whether the reported responses were affected by the ionization suppression/enhancement effect is difficult.

3.3.1. Ion-pairing liquid-liquid extraction (IP-LLE)

Most PFAS are anionic amphiphilic compounds with low pKa (0.5–3.8)[47], which means they are predominantly present as anions in biological matrices across a wide pH range. This characteristic apparently poses challenges in traditional LLE with aqueous workups given that most reported methods have adopted the IP-LLE technique. Hansen et al. (2001) [48] developed IP-LLE to extract PFOA, PFOS, PFOSA, and PFHxS from serum and liver homogenate samples, which were further analyzed by high performance liquid chromatography with quadrupole time of flight mass spectrometry (LC–QTOF-MS). Since then, this procedure has proven satisfactory for determining various PFAS other than PFCA and PFAS in matrices like plasma [49,50], whole blood [51], glioma tissue [52], and semen [53]. Each of these studies made a few modifications in the sample preparation step, but the modifications only involved sample dilution with ammonium carbonate buffer followed by addition of a salt such as tetrabutylammonium solution for the ion-pairing of target compounds and extraction with methyl-tert-butyl ether (MTBE). This is plausible because addition of an ion-pair reagent causes polar charged compounds to form cation-anion complexes with higher lipophilicity, enhancing their simultaneous extractability with other non-polar analytes. The developed analytical methods generally showed satisfactory recovery for PFAS in the case of serum, liver, and semen samples (51–121% [54], 56–100% [48], and 83–115% [53], respectively).

Kaiser et al. (2021) evaluated 61 PFAS and validated an UHPLC–MS/MS method for analysis of bovine serum samples by using three different sample preparation techniques, IP-LLE and SPE with hydrophilic-lipophilic or weak anion exchange sorbents [29]. Although IP-LLE was a suitable preparation technique for long-chain substances such as PFTeDA, PFDoDS, and diPAP, it gave lower recovery values ranging from 39 to 73% for some PFCA and PFAS. However, the method could be reasonably applicable for PFAS analysis in human serum samples because a highly sensitive and selective system was used. One main limitation of the cited IP-LLE method was the need to carry out multiple extractions for the same aqueous sample. All the IP-LLE methods mentioned in the previous studies involved extraction with MTBE three times, which required a total of 11 –15 mL of solvent and generated a large amount of residue. This is not the best option when large populations need to be biomonitored given the large number of samples that must be analyzed. Among the reviewed papers (2018–2023), no other evaluated ion-pair reagents or extraction solvents in IP-LLE for PFAS analysis in biological matrices. Therefore, this strategy is a gap to be explored if biomonitoring programs and their partner laboratories still wish to use LLE.

Lack of specificity is a challenge of LLE-based methods for trace level and simultaneous detection analysis, especially when it comes to ion-pairing. This results in co-extraction of matrix components that increase the likelihood of ion signal effects, reducing or overestimating the detection capability and affecting precision and accuracy because the sample matrix can vary between samples [55]. Even when multiple extractions were performed (i.e. 6–15 mL in total) and internal standards were employed, Kaiser et al. (2021) [29]reported that the signals of target analytes were suppressed by up to 14.1% (RSD 7–15%) for some PFAS in serum. Similarly, Pan et al. (2019) [53] observed matrix effects of up to 21% for 6:2 Cl-PFESA in semen samples, which could significantly hinder attempts at quantification. Therefore, a further and effective sample cleanup could be necessary after extraction, to reduce matrix effects and to improve method reliability.

3.4. Dispersive liquid-liquid microextraction (DLLME)

Typical DLLME is based on a ternary solvent system, in which the disperser solvent is miscible with both the aqueous matrix and the water-immiscible organic extractant. The main advantage of DLLME is that the contact area, relative to LLE, is higher which increases, improves and accelerates the extraction. This occurs when the extractant and the disperser solvent are rapidly injected into the aqueous solution, generating a cloudy solution with microdroplets of extractant readily dispersed throughout the sample [56].

Wang et al. (2018) [57] developed a method for analyzing 23 PFAS from three different classes: i) PFCAs, ii) PFSAs and iii) FTSs, in human urine samples by LC-MS/MS. In this approach, all the samples were filtered through 0.7-µL filter before extraction of 10-mL sample aliquot by DLLME, which was conducted with 100 µL of perfluoro-tert-butanol and 120 µL of acetonitrile as extractant and disperser solvent, respectively. Phase separation was assisted by addition of 0.3 g of NaCl (3%, w/v), and pH was adjusted to 5 with HCl. In general, the proposed method provided satisfactory recovery (50–90%) for PFAS with over six fluorinated carbons, but extraction efficiency was poor for short-chain PFCA, and FTSA (10–60%). The authors obtained LOD and LOQ of 0.28–1.9 and 0.9–5.0 ng mL^{-1}, respectively. Regarding matrix effects, in this assay, glucose (5 g L^{-1}) and human serum albumin (500 mg L^{-1}) were added to the samples. Additionally, the authors compared the results obtained after extraction by DLLME with the results obtained after extraction by SPE. As a conclusion, they claimed that matrix effects were minimized as judged from the higher peak intensities obtained after DLLME extraction. Although this can be an approach for evaluating the potential influence of organic components in urine samples, further experiments are needed to confirm whether these differences are due to reduced matrix effects, ion signal suppression or enhancement, or recovery loss.

DLLME can be considered a good alternative to LLE or SPE because it requires a smaller volume of organic solvent, extraction is fast, and the operation time is short, which makes sample preparation simpler and less time-consuming [58]. DLLME holds promise for biomonitoring. Additionally, in the case of plasma or another sample matrix with high protein content, combining DLLME with another sample preparation technique may be necessary to improve cleanup. For instance, DLLME has been reported for matrices such as milk or placental tissues, where it is typically combined with salting out liquid-liquid extraction in a multistep approach (SALLE) [59–61].

3.5. Dilute-and-shoot (DnS)

DnS is the simplest sample preparation method—it entails simple sample dilution with water or another solvent before analysis. Some of the advantages of DnS include its low cost, simplicity, rapidity, easy automation, and minimum sample manipulation. Nevertheless, because during DnS no interferents are eliminated from the matrix and the analytes are not concentrated, this technique is restricted to specific applications, especially when it comes to biological sample analysis. Even though DnS can be used for urine samples, matrix effects and lack of sensitivity may pose some challenges. These limitations are in accordance with the scarce literature reports on the use of this technique for PFAS analysis in biological samples.

In the reviewed papers, DnS was reported twice for PFAS analysis in human urine samples. In the first case, to develop an ion mobility mass spectrometry (IM–MS) database, Belova et al. (2021) spiked 1 mL of urine sample with various compounds (including several PFAS) and diluted the samples with methanol (1:5 v/v), followed by centrifugation, overnight frozen cycle, and filtration. According to the authors, because they investigated a low mass range, coelution and false positives were possible, but carryover was not a concern due to the analytical method characteristics [62]. There were no comments about matrix effects, recovery, or LOD/LOQ, which makes it difficult to evaluate the applicability of the sample preparation method.

Kim et al. (2022) evaluated DnS, SPE, and LLE for PFAS analysis in Flemish adolescents' urine by UHPLC–QTOF-MS [63]. These authors diluted 500 µL of sample with the same volume of acetonitrile, which was followed by filtration step. They calculated the extraction efficiency by comparing the internal standard abundances in urine samples with their abundances in a prepared standards mix in methanol, to notice that abundances were > 200% for lower fluorinated compounds and decreased with the carbon chain length. The best sample preparation technique was LLE because it allowed a larger number of analytes to be detected and gave better extraction efficiencies, not to mention that the abundance of the peaks in the sample and the internal standard mix were closer.

The presence of a centrifugation/filtration step during DnS, especially when organic solvents are used, may generate a discussion about the correct name of the employed method (DnS or PPT). Recently, Greer et al. (2021) presented this discussion in their article and considered a possible expansion and redefinition of DnS, to allow previous sample cleanup and/or deproteinization before the dilution step, to make the sample more compatible with chromatographic analysis [21].

3.6. Solid-liquid extraction (SLE)

SLE is typically used when analyzing solid samples. The main goal of this technique is to transfer the analytes from the solid matrix to a liquid phase [64], which is suitable for chromatographic analysis. The extraction solvent plays an important role in SLE: the mechanism ruling SLE is partition of the analytes between the liquid and solid phases. However, other factors can impact analyte recovery in SLE such as penetration of the extractant though the solid matrix, analyte diffusivity, sample homogeneity, and sample humidity [64].

SLE is not a selective sample preparation technique mainly because matrix components other than the analytes can partition to the liquid phase and act as interferents [64]. For this reason, when it comes to PFAS analysis in biological samples, SLE has been mostly employed as a first sample preparation step, or pretreatment step, for different solid samples, including placenta [65], lung, and kidneys [66]. Yet, SLE has also been used as a sole sample preparation step, when the matrix is deemed to be less complex, such as red blood cells [67].

Maceration and percolation are most traditional SLE variations. Despite their simplicity and relatively low cost, these approaches may suffer from reduced extraction yield, being time-consuming and requiring a significant amount of solvent. For this reason, other approaches have been developed to improve the SLE efficiency, e.g., superheated solvent extraction (SHSE), ultrasound-assisted extraction (USAE), and microwave-assisted extraction (MAE) [64]. Even though these SLE variations are available even in automated equipment, only the traditional SLE approaches have been employed to prepare biological samples for PFAS analysis.

As mentioned earlier, Liu et al. (2023) used SLE to extract PFAS from human red blood cells. A total of 30 PFAS were analyzed by LC–MS/MS on Ascentis Express F5 column (100 × 2.1 mm, 2.7 µm). The mobile phase was composed by formic acid aqueous solution (pH 6) and methanol, and the analytes were eluted by the gradient elution mode. The developed method presented good values of extraction recovery and LOD, which ranged from 76 to 100% and from 0.038 – 0.092 ng mL^{-1}, respectively [67]. However, to accomplish that, the authors had to perform the extraction procedure twice in each sample. Some drawbacks of SLE were exemplified in this study. Overall, each sample required 10 mL of acetonitrile, took one hour to be extracted, and took even longer to be concentrated to 100 µL under N_2. When many samples must be processed, these numbers start adding up and may significantly impact to the cost and time needed for method development.

Another interesting work carried out by Li et al. (2021) [46] described PFAS analysis in hair samples after SLE. The authors initially pretreated the samples by washing them with water and acetone. Afterward, the hair samples were cut into small pieces, and 0.1 g was extracted with 10 mL of methanol/acetonitrile 1:1 (v/v) for 10 min. After that, the samples were centrifuged, and the extract was collected, dried under N_2 stream, and reconstituted for chromatographic analysis. The samples were analyzed by LC–MS/MS by using a C_{18} column (50 × 2.1 mm, 5 µm). The mobile phase was composed by 2 mmol L^{-1} ammonium acetate solution and methanol and the analytes were eluted by the gradient elution mode. The extraction recovery and LOD values ranged from 81 to 96% and from 2 to 4 pg g^{-1}, respectively, for the 7 evaluated PFAS.

Other than these two applications, SLE has been primarily used in a multistep sample preparation method as the first step.

3.7. Multistep sample preparation

PFAS analysis becomes even more complex when the limitations of the available extraction techniques are considered. These techniques heavily depend on the sample matrix characteristics and that the analytes be preconcentrated for improved efficiency and detectability. Therefore, improving sample cleanup is necessary to obtain more accurate results. A promising approach to address this issue is to use multiple steps of sample preparation techniques that combine several extractions, to increase efficiency and detectability and to reduce matrix effects. For liquid samples such as plasma, serum, and milk, the initial stage of sample preparation is to eliminate proteins in the sample by using various methods, including PPT with acetonitrile or methanol [26,33,35,67–74], SALLE [60,61], or other techniques such as dried blood spotting [75,76] or protein digestion [53,59,73,77,78]. Human tissues like placenta, lung, or kidney are typically homogenized or directly extracted with SLE [41,65,66,79,80], followed by solid matter removal via centrifugation or filtration. After these steps, the most recent trend is to conduct a cleanup step with online SPE systems, but solvent-based extractions are still commonly used.

Poothong et al. (2019) [76] developed and compared two methods for measuring the concentrations of polyfluoroalkyl phosphate esters (mono- and diPAP) and perfluoroalkane sulfonamides in whole blood and dried blood spot (DBS) samples by using online SPE and ultra-high performance liquid chromatography with tandem mass spectrometry. Additional sample preparation steps, including extraction with methanol and centrifugation, were required before DBS samples were injected into in the online SPE system. The method was applied to 59 volunteers, and a strong correlation was observed between the pairs of dried blood (~30 μL) and whole blood (50 μL) samples, with method quantification limits ranging from 0.025 to 1.0 ng mL^{-1}.

In another study, PFAS were analyzed in 40 μL of manatee DBS samples extracted by four different approaches: (i) IP-LLE (tetrabutylammonium hydroxide, carbonate buffer at pH 10, and 5 mL of MTBE in rotation for 40 min), (ii) methanol crash (300 μL and sonication for 10 min), (iii) Folch (6 mL of 2:1 v/v trichloromethane/methanol for 24 h, then 1 mL water), and (iv) homogenized trichloromethane/methanol (6 mL, 2:1 v/v; 24 h) [75]. While all the methods proved suitable for extracting PFAS from whole blood spotted on DBS cards, the Folch method was chosen because it provided greater recovery of some PFCA, PFSA, FTSA, and diPAP. Using five disks of DBS, the authors achieved LOD of 0.002–0.83 ng mL^{-1} and LOQ ranging from 0.007 to 3.5 ng mL^{-1}. Recovery varied from 0.1 to 14% for short- and long-chain PFCA and PFSA and from 40 to 66% for diPAP, once again showing that the method relies mainly on the sensitivity of the UHPLC–MS/MS system and pretreatments to minimize matrix effects. In both studies, the DBS method facilitated sampling and allowed larger populations to be analyzed.

These two findings suggested that the dried blood spot method can be satisfactorily applied and holds significant potential because self-collected DBS is convenient, and its transport and storage are easy, facilitating extensive biomonitoring investigations. However, the trace levels of some PFAS found by Poothong et al. (2019) [76] Griffin et al. 2021 [75] in DBS substrates, especially PFOA, can significantly affect the measurements, making the method more time-consuming and requiring extra precautions to avoid systematic errors.

Other methods have been developed for multistep sample preparation. Three of them were able to extract PFAS from human breast milk and placenta samples by using DLLME, combined with SALLE and optimized with chemometric tools. For breast milk samples (1-mL aliquot), Vela-Soria et al. (2020) [61] developed a method to determine 10 long-chain PFAS by UHPLC–MS/MS. To precipitate proteins from milk, 5 mL of acetonitrile saturated with hexane and a salt mixture of NaCl and disodium citrate and trisodium citrate was added. Then, the supernatant resulting from manual agitation and centrifugation was transferred to another vial and concentrated to 1 mL. Afterward, DLLME was initiated by adding 1.5 mL of trichloromethane and 10 mL of a 10% (w/v) NaCl aqueous solution at pH 2.0. LOD and LOQ of 0.006 and 0.02 ng mL^{-1}, respectively, and recovery values ranging from 86 to 111% with relative standard deviation (RSD) of 8 to 14% were achieved. In a similar method, Serrano et al. (2021) [60] added formic acid to acetonitrile in the SALLE step and 1.5 mL of a mixture of trichloromethane/acetone (4:1, v/v) to initiate DLLME. By making this minor modification, the LOD and LOQ increased by 100 times, to 0.66–0.86 and to 2.19–2.87 ng mL^{-1}, respectively, and accuracy ranged from 88 to 107% (RSD 2.8–14.4%).

By combining SALLE and DLLME, Vela-Soria et al. (2021) [59] also assessed PFAS in placenta samples by liquid chromatography coupled to a hybrid triple quadrupole linear ion trap. Before 6.0 mL of acetonitrile (used as a disperser solvent) and 600 mg of NaCl were added, 1 g of homogenate was diluted three times with a collagenase solution. Following the same preconcentration and extraction procedure as in the previous SALLE-DLLME methods, the authors used a smaller volume (0.8 mL) of trichloromethane to extract FPCA and PFSA. Recovery values of 88–114% with relative standard deviation (RSD) falling below 14% and LOD and LOQ of 0.006 and 0.02 ng g^{-1}, respectively, were obtained.

All the three mentioned methods were satisfactorily applied to analyze 20 breast milk or placenta samples. The highest concentrations and frequencies were recorded for PFOA, PFOS, and PFHxA. If we compare these methods with the one-step DLLME approach, better recoveries were obtained even though smaller volumes of samples with higher concentrations of potential interferents were used.

To reduce and to simplify the sample preparation step, several strategies such as PPT and SLE have been employed as pretreatment to remove potential interferents before online SPE. Lin et al. (2021) analyzed PFOA/PFOS isomers in plasma samples (50 μL) by coupling PPT to online SPE and an LC–MS/MS system. Coupling both sample preparation techniques proved effective and the LODs varied from 0.002 to 0.15 ng mL^{-1} [33]. Another method developed by Workman et al., (2019) [68] used the same sample preparation strategy for analyzing 17 PFAS by LC–MS/MS. Again, the strategy was attractive because LOD and LOQ lay between 0.006 and 0.1 ng mL^{-1} and between 0.02 and 0.33 ng mL^{-1}, respectively [68]. Compared to the other methods that only used PPT (by using acetonitrile or methanol), LOD varied from 0.02 to 0.5 ng mL^{-1} for the same matrix [39,42,43], but smaller values could be achieved depending on the efficiency of the protein precipitant agent. For instance, by adding a mixture of methanol and acetonitrile (1:1 v/v) containing formic acid, LOD and LOQ of 0.009–0.12 ng mL^{-1} and 0.009–0.245 ng mL^{-1} were achieved [38,81,82]. When only PPT is used, incomplete protein precipitation may occur, to cause variability in the results and loss of reproducibility as well as ion signal issues (suppression/increasing) in mass spectrometry-based techniques. Similar observations have been made for studies that used only SPE as a sample preparation technique for analysis of serum samples [44,70,83].

SLE is necessary for homogenizing wet solid matrices. When combined with other sample preparation techniques such as PPT, IP-LLE, or online SPE, selectivity and sensitivity can be improved due to reduced interference from matrix components. In studies on rat liver, brain, and kidney (0.3 g), researchers used 3 mL of deionized water to extract PFCA such as PFHxA, PFDA, and PFOA. By using a LC–MS/MS system, 100-μL aliquots of the prepared homogenates were subjected to protein digestion with potassium hydroxide in methanol/water (1:1, v/v) for 12 h, and the resulting supernatant was loaded into Strata X-AW cartridges to purify the samples [41]. PFSAs such as PFBS, PFHxS, and PFOS were also quantified in the same matrices by using a simpler extraction protocol where the supernatant from SLE and the digestion step was directly analyzed by HPLC–MS/MS without further cleanup by SPE [80]. In both works, multistep sample preparation yielded reasonable LOQ of 5 ng g^{-1} of organ tissue, being successfully applied in toxicokinetic studies.

Abraham et al. (2021) [66] also developed an interesting method by using SLE as an initial sample treatment method to extract PFBA from 9-g lung homogenate samples. After the SLE step, where 0.5–1.3 g of wet tissue was shaken (30 min) and sonicated (15 min), SPE was carried out for further cleaning the extracts. Although the authors had to perform the extraction three times and used a total of 11 mL of methanol containing sodium hydroxide, they managed to achieve LOD of 0.03 ng mL^{-1} for PFBA.

Therefore, PFAS analysis in biological samples poses challenges that require careful consideration of the sample preparation technique. One-step protocols or multistep approaches seem to be effective to remove interfering matrix components and to improve method sensitivity and selectivity. However, the choice of the sample preparation technique should be tailored to the sample type and target PFAS. A well-designed method must consider both performance and practicality to achieve its intended purpose.

4. Future perspectives and conclusions

When it comes to analyzing PFAS in biological matrices, different sample preparation techniques have been successfully applied for a myriad of biological samples. SPE is the most prominent sample preparation technique employed, especially because it is highly selective compared to other sample preparation techniques. However, it is important to mention that SPE has not been employed alone, but together with other techniques because biological samples are complex, and PFAS concentrations in these samples are low. Given that most of the sample preparation methods reviewed here are multistep, they are frequently time-consuming and, in conjunction with the significant number of samples involved in biomonitoring studies, may overwhelm the resources dedicated for these studies. That is why PPT has been the second most applied sample preparation technique—even though it may lack the SPE selectivity, it is simpler and less expensive. In addition, the automation observed with PPT and SPE can significantly increase sample throughput in biomonitoring studies, which is highly desirable. A noteworthy feature is the use of some miniaturized sample preparation techniques such as DLLME and miniaturized SPE. Special attention has been paid to miniaturized offline SPE systems because they allow a significant number of samples to be processed at once. In conclusion, our perspective for this field is the development, further miniaturization, and implementation of more automated sample preparation solutions that could not only reduce the cost and time involved in the analysis, but also allow automated multiplexing of different sample preparation techniques into one device.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This is a review article. The results described were based on the literature data

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