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Analysis of perfluoroalkyl substances (PFAS) in conventional and unconventional matrices: Clinical outcomes

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ABSTRACT

Since 1950, the use of perfluoroalkyl substances (PFAS) increased due to their heat resistance, lipophobic and hydrophobic properties; therefore, these compounds are widely employed to make waterproof and heat resistant coatings, such as food packaging or work wear. However, these chemicals represent a risk to the environment due to their stability to degradation. Moreover, these compounds properties represent a risk also for humans; many studies correlated their concentrations in biological matrices to pathologies, such as hypertension, diabetes, and cancer. To this concern, the analytical detection in different biological matrices plays a crucial role to assess the presence of such analytes in different body districts. We performed a literature search in different scientific databases to review articles reporting the application of PFAS analysis for human exposure monitoring and for possible association with pathologies. The search resulted in 58 studies investigating PFAS presence in conventional matrices, such as blood and urine, and unconventional matrices. Although the solid-phase extraction was preferred for all the considered matrices, liquid-liquid extraction and dilute and shot demonstrated to be suitable extraction approach. The most used instrumental technique was the LC-MS/MS equipped with C18 chromatographic column, electrospray injection source operating in negative mode, and multiple reaction monitoring spectrometric acquisition. The untargeted detection of PFAS was attempted using an LC-HRMS method to elucidate possible new compound structures. Notably, the instruments and laboratory tools may represent an important contamination source due to the PFAS presence in their constituents. The development of an analytical method able to reach low limits of detection (LOD) and suitable for different biological matrices is crucial to study both PFAS health effects and a possible pharmacokinetics. For this purpose, current knowledge about PFAS analytical methods in biological matrices applied to human biomonitoring and pathology studies is reviewed to raise awareness of these chemicals' activities.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are chemical compounds structurally characterized by a fluorinated carbon chain functionalized with carboxylic or sulfonic acid moiety. Based on the alkyl chain length, these substances are classified into short-chain- ($C < 8$) and long-chain-PFAS ($C \geq 8$) [1]. As of the early 2000 s, these compounds were produced by electrochemical fluorination (ECF), allowing the synthesis of both linear and branched isomers. Nowadays, fluorotelomerization is preferred due to the almost exclusive production of linear compounds [2]. Their peculiar physicochemical properties, such as heat resistance and lipophobic and hydrophobic propertie,s are at the basis of the commercial success in different fields [3]. Indeed, several common materials contain perfluorooctanoic acid (PFOA) or perfluorooctane sulfonic acid (PFOS), for example, fluoropolymer coatings [4], food packaging [3], firefighting foams [5], pesticide formulations [6], laboratory tools and consumables. However, their stability to degradation represents a high risk for the environment due to accumulation in the soil, air, and water. In 2002, PFOA was detected in drinking water supplies in the United States of America, as a result of air emissions from a PTFE manufacturing industry [7]. In 2006, the extensive use of PFAS based-soil conditioners provoked considerable soil contamination in Germany, quickly spreading to lakes, streams and drinking water nearby [7]. In 2013, high concentrations of short-chain PFAS were detected both in surface water and drinking water in an industrial area in Veneto Region in Italy [7,8]. Hence, food and drinking water consumption may represent an important direct source of PFAS exposure for humans. Furthermore, the high stability to metabolic degradation

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results in the bioaccumulation in human tissues with a long half-life, estimated of 5–5.4 years for PFOA [3,9].

In the last decades, the alleged toxicity of PFAS raised the attention of the scientific community, which investigated the effects on human health of PFAS bioaccumulation in specific organs. A positive association between the exposure to perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA) and PFOS and increased cholesterol levels was found in pregnant women [10]; moreover, a possible link between these latter compounds and dyslipidemia, hypertension, obesity was showed in a cross-sectional study involving 940 adolescents [11]. Prenatal exposure was investigated in newborns observing a fetal thyroid function disruption with increasing concentrations of PFOS, PFNA, PFDA, perfluoroundecanoic acid (PFUnA) and perfluorododecanoic acid (PFDoA) [12]. Finally, a study demonstrated a correlation between PFOA, PFUnA, PFDoA and gestational and postnatal lower birth weight [13]. In this concern, the PFAS analytical detection in biological matrices plays a crucial role not only to study the PFAS toxidrome, but also to monitor the occupational exposure to those pollutants. In fact, the choice of the most suitable analytical strategy allows to obtain important information on the toxicokinetic to elucidate the pathological role of these molecules. The aim of this review was to provide a summary of currently available analytical methods for PFAS determination in biological samples applied for human exposure biomonitoring or for studying the possible correlations with pathologies, mainly focusing on extraction and analytical techniques.

2. Search strategy

The literature search was performed in PubMed, Scopus and Google Scholar scientific databases selecting articles reporting the application of PFAS analysis for human exposure monitoring and for possible association with pathologies. Search terms, alone or in combination, were “per- and polyfluoroalkyl substances”, “PFAS”, “analysis”, “biological matrices”, “human biomonitoring”, “pathologies”, “bioaccumulation”, “distribution”, “PFOA”, “PFOS”. Only articles sufficiently detailing the PFAS analytical methods applied for clinical purposes were selected. Only articles in English and official reports from national and international organizations were evaluated. Of the eighty-three sources found, seventy-nine were evaluated after eliminating duplicate sources. In addition, the following exclusion criteria were applied: articles reporting only analytical methods without application for the investigation of related pathologies or monitoring of exposure. Finally, only fifty-eight were considered appropriate for our purposes.

3. Results and discussion

Fifty-eight studies on PFAS determination in one or multiple biological matrices, such as perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoA were included in this review (Table 1). Brief descriptions of the analytical methods in plasma (n = 18), serum (n = 25), umbilical cord serum (n = 6), blood (n = 1), urine (n = 5), hair (n = 3), nails (n = 1), semen (n = 2), bone (n = 1), cerebrospinal fluid (n = 1), breast milk (n = 1), and placenta (n = 3) are subsequently reported.

3.1. Serum and plasma and blood

Serum and plasma are blood-based matrices; the first is the liquid portion after blood coagulation, while plasma is obtained through the blood anticoagulant addition. In biomedical sciences, these matrices are usually investigated to assess the recent exposure to exogenous compounds, such as drugs or poisons; hence, they allow to backtrack the exposure source in environmental analysis. In case of PFAS analysis, the accumulation and reabsorption phenomena may lead to a steady state making the PFAS detection in serum or blood not strictly related to recent exposure. For this purpose, plasma PFAS analyses were performed for exposure monitoring [5,25–27,30,31,34,35] or for investigating the possible PFAS correlation with diabetes incidence [28], reproductive function [24,39], child executive function ability and behavioral problems [32], and other health effects [29,33,36–38,40]. The matrices complexity often requires a thorough pretreatment of the biological sample to extract the analytes. Indeed, solid-phase extraction demonstrated to be the most suitable choice for plasma sample pretreatment, both offline [5,24,40] and online [26,34,35,28,32,33,38], allowing the best recoveries in the reported analytical procedures. A dispersive-SPE was performed by Donat-Vargas et al. [29] allowing the separation of PFAS and persistent organic pollutants (POPs). After the protein precipitation, 0.5 mL of activated silica was added to the supernatant and dichloromethane-hexane (1:4) was added as elution solvent for POPs, while activated silica containing PFAS were eluted by a 20 mM ammonium acetate methanolic solution. Interestingly, the largest number (n = 37) of PFAS in plasma was detected by Göckener et al. [31] through an ion-pairing liquid-liquid extraction (LLE), adding 0.5 M tetrabutylammonium hydrogen sulfate in water, 0.25 M Na_2CO_3/$NaHCO_3$ buffer solution and then methyl-tert-butyl ether as extraction solvent. The validated method included GenX, ADONA and other PFAS as sulfonamide-based precursors. However, validation was not successful for perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorooctane sulfonamide (PFOSA), 4:2 fluorotelomer sulfonic acid (4:2 FTS). Though, several authors performed only a protein precipitation before the instrumental analysis, using acetonitrile containing 0.1% formic acid, or methanol [25]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was the gold standard instrumental method, since it represents the most available instrumentation both in environmental and bioanalytical laboratories, requiring also limited sample pretreatment. The reported method reached limits of detection (LOD) and limits of quantification (LOQ) ranges of 0.009–1.2 ng/mL and 0.05–1.0 ng/mL, respectively. Notably, the analytes were ionized in negative polarity mode and the mass spectrometer operated in multiple reaction monitoring (MRM) mode. Nonetheless, Göckener et al. [31] performed a liquid chromatography-high resolution mass spectrometry (LC-HRMS) screening to identify new possible PFAS, exploiting the HRMS resolution for structure elucidation in untargeted analysis with higher LOQ range (0.25–0.5 ng/mL) compared to that obtained with LC-MS/MS methods. Mass spectrometric data were obtained in full scan- all ion fragmentation combined mode (FullMS AIF). Chromatographic separation was mostly achieved through C18 stationary phase columns, eluted with water and methanol at different concentrations of ammonium acetate as organic modifier as mobile phases; notably, different solvents were used only by Donat-Vargas et al. [29], adding 0.001% N-methylpiperidine to a methanol: water (90:10) solution (A) and to methanol (B).

Serum PFAS analysis was performed to monitor human exposure [8,44,46,47,49,18,55,57,59–61,51] or to investigate possible correlations with behavioral attributes [45], serum lipidic profile [48,58], obesity and cardiometabolic health [10,11], or other pathologies [50,41,52–54,56]. SPE was the most common extraction technique, both online [10,11,46,55,52,53,56] and offline [45,51]. Acetonitrile was the most preferred LLE solvent, after the protein precipitation [8,58,50,54], but also methyl terbutyl ether (MTBE) was used, through an ion-pairing LLE. Although LC-MS/MS was the most selected instrumental technique ionizing in negative polarity with an MRM acquisition mode, Itoh et al. [54] applied a gas chromatography-mass spectrometry (GC-MS) method using negative chemical ionization. This allowed to detect 20 PFAS (linear and branched isomers) reaching the lowest method quantification levels (0.007–0.68 ng/mL). Notably, the percentage of not quantified cases were below 30% only for n-PFHpS and iso-PFTrDA, probably due to the low quantification limit. The most used column was C18; the most common mobile phases combination

Table 1
Analytical methods for PFAS determination in biological matrices.

Matrix	N° PFAS	Extraction	Method	LOQ/LOD (ng/mL)	Aim of the study	Ref.
Blood	12	SPE	LC-MS	LOD: 0.1–2	Comparison of PFAS to other persistant organic pollutant	[14]
Bone	11	LLE	LC-MS/MS	LOQ: 0.1–0.3 ng/g	PFAS and bone cell differentiation	[15]
Breast milk	14	LLE + SPE	LC-MS/MS	LOQ: 0.003–0.033	Time-course trend of PFAS in breast milk	[16]
Cerebrospinal fluid	26	LLE	LC-MS/MS	LOD: 0.6–103 pg/mL	PFAS distribution in cerebrospinal fluid	[17]
Hair	8	LLE + SPE	LC-MS/MS	LOD: 0.03–0.07 ng/g LOQ: 0.08–0.22 ng/g	Monitoring PFAS exposure	[18]
	25	LE + SPE	LC-MS/MS	MQL: 0.02–1.72 ng/g	Monitoring PFAS exposure	[19]
	20	SPE	LC-QTOF-MS	LOD: 0.02–0.12 ng/g LOQ: 0.05–0.5 ng/g	Monitoring PFAS exposure	[20]
Nails	8	LLE + SPE	LC-MS/MS	LOD: 0.02–0.09 ng/g LOQ: 0.07–0.30 ng/g	Monitoring PFAS exposure	[18]
Placenta	22	LLE + SPE	LC-MS/MS	RL: 0.001–22.7 ng/g	Identifying sociodemographic risk factors	[21]
	11	SPE	LC-MS/MS	MDL: 0.01–8.10 ng/g	PFAS and birth outcomes	[22]
	11	SPE	LC-QTOF-MS	LOQ: 0.01–0.08 ng/g	PFAS and placental DNA methylation and birth size	[23]
Plasma	2	SPE	LC-MS/MS	LOD: 0.4–1.2	PFAS and human semen quality	[24]
	13	LLE	LC-MS/MS	LOQ: 0.05	Monitoring PFAS exposure	[25]
	6	SPE	LC-MS/MS	LOD: 0.03	Monitoring PFAS exposure	[26]
	13	PP	LC-MS/MS	MDL: 0.01–0.17	Monitoring PFAS exposure	[27]
	9	SPE	LC-MS/MS	LOD: 0.1	PFAS and diabetes incidence	[28]
	13	dSPE	LC-MS/MS	LOQ: 0.15–1.0	PFAS and cardiometabolic risk factors	[29]
	10	PP	LC-MS/MS	LOD: 0.009–0.12	Monitoring PFAS exposure	[30]
	37	LLE	LC-HRMS	LOQ: 0.25–0.5	Monitoring PFAS exposure	[31]
	8	SPE	LC-MS/MS	LOD: 0.1–0.2	PFAS and child executive function and behavioral problems	[32]
	8	SPE	LC-MS/MS	LOD: 0.1	PFAS and body composition	[33]
	9	SPE	LC-MS/MS	LOQ: 0.25	Monitoring PFAS exposure	[34]
	6	SPE	LC-MS/MS	LOD: 0.1	Monitoring PFAS exposure from diet	[35]
	10	PP	LC-MS/MS	LOD: 0.009–0.12	PFAS prenatal exposure and fetal BDNF level	[36]
	8	PP	LC-MS/MS	LOD: 0.01–0.18	PFAS and changes in plasma lipids	[37]
	5	SPE	LC-MS/MS	LOD: 0.03	PFAS and risk of inflammatory bowel diseases	[38]
	15	LLE	LC-MS/MS	LOD: 0.01–0.05	PFAS and male reproductive function	[39]
	5	SPE	LC-MS/MS	LLOQ: 0.05–0.15	Monitoring PFAS exposure	[5]
	9	SPE	LC-MS/MS	LOD: 0.06–0.40	PFAS and dietary profile	[40]
Semen	2	SPE	LC-MS/MS	LOD: 0.4–1.2	PFAS and human semen quality	[24]
	16	LLE	LC-MS/MS	LOQ: 0.002–0.1	PFAS and male reproductive hormones	[41]
Serum	19	LLE	LC-MS/MS	LLOD: 0.05	Monitoring PFAS exposure	[42]
	16	SPE	LC-MS/MS	LOD: 0.0574–0.281	Monitoring PFAS exposure	[43]
	16	LLE	LC-MS/MS	LOD: 0.12	Monitoring PFAS exposure	[44]
	6	SPE	LC-MS/MS	MDL: 0.02–0.18	PFAS and behavioral attributes	[45]
	11	SPE	LC-MS/MS	LOD: 0.081–0.2	Monitoring PFAS exposure	[46]
	12	LLE	LC-MS/MS	LOQ: 0.01–0.5 ng/g	Monitoring PFAS exposure	[47]
	3	LLE	LC-MS/MS	LOD: 0.1–0.5	PFAS and serum lipids	[48]
	21	LLE	LC-MS/MS	LOD: 0.044–2.327 LOQ: 0.148–7.757	Monitoring PFAS exposure and distribution	[49]
	12	PP	LC-MS/MS	LOD: 0.1 LOQ: 0.5	Monitoring PFAS exposure	[8]
	8	LLE + SPE	LC-MS/MS	LOD: 0.01–0.03 LOQ: 0.03–0.11	Monitoring PFAS exposure	[18]
	8	PP	LC-MS/MS	LOD: 0.01–0.06	PFAS and offspring birth weight	[50]
	18	SPE	LC-MS/MS	LOD: 0.01–0.3	PFAS and dyslipidemia, hypertension and obesity	[11]
	14	SPE	LC-MS/MS	LOD: 0.05–0.2	Monitoring PFAS exposure	[51]
	16	LLE	LC-MS/MS	LOQ: 0.01–0.2	PFAS and male reproductive hormones	[41]
	5	SPE	LC-MS/MS	LOQ: 0.03	PFAS and childhood ADHD	[52]
	11	SPE	LC-MS/MS	LOD: 0.1	PFAS and adipokines profile in midlife women	[53]
	6	SPE	LC-MS/MS	LOD: 0.1	PFAS and cardiometabolic health and birth outcomes	[10]
	20	PP	GC-MS	MQL: 0.007–0.68	PFAS and breast cancer risk in Japanese women	[54]
	28	SPE	LC-QTOF-MS	LOQ: 0.01–4	Monitoring PFAS exposure	[55]
	10	SPE	LC-MS/MS	LOD: 0.1	PFAS and renal cell carcinoma	[56]
	15	LLE	LC-MS/MS	LOD: 0.02–0.09	Monitoring PFAS exposure	[57]
	12	PP	LC-MS/MS	LOD: 0.1 LOQ: 0.5	PFAS and lipid profile	[58]
	42	LLE + SPE	LC-MS/MS	LOD: 0.01–0.562 ng/g LOQ: 0.02–3.57 ng/g	Monitoring PFAS exposure	[59]
	12	NA	LC-MS/MS	LOQ: 0.01–0.562	Monitoring PFAS exposure	[60]
	11	NA	NA	LOD: 0.1–1.8	Identifying PFAS sources	[61]
Umbilical cord serum	11	LLE	LC-MS/MS	LOD: 0.01–0.02	PFAS and gestational and postnatal growth	[13]
	10	PP	LC-MS/MS	LOD: 0.009–0.12	PFAS and fetal thyroid hormone levels	[12]
	12	SPE	LC-HRMS	LOQ: 0.01–0.1	PFAS and thyroid function indicators	[62]
	2	SPE	LC-MS/MS	MDL: 0.003	PFAS and body mass index changes	[63]
	2	SPE	LC-MS/MS	MDL: 0.003	PFAS and cognitive development in children	[64]
	12	SPE	LC-HRMS	LOQ: 0.01–0.1	PFAS and adipocytokines	[65]
Urine	19	SPE	LC-MS/MS	LOD: 0.05	Monitoring PFAS exposure	[42]

(*continued on next page*)

Table 1 (*continued*)

Matrix	N° PFAS	Extraction	Method	LOQ/LOD (ng/mL)	Aim of the study	Ref.
Urine	16	LLE	LC-MS/MS	LOD: 0.0875–0.225	Monitoring PFAS exposure	[43]
Urine	5	SPE	LC-MS/MS	LOD: 0.01–0.02	Monitoring PFAS exposure	[46]
Urine	21	SPE	LC-MS/MS	LOD: 0.030–1.26 LOQ: 0.099–4.22	Monitoring PFAS exposure and distribution	[49]
Urine	8	LLE + SPE	LC-MS/MS	LOD: 0.44–3.47 ng/L LOQ: 1.03–10.38 ng/L	Monitoring PFAS exposure	[18]

ADHD, attention-deficit-hyperactivity-disorder; GC-MS, gas chromatography-mass spectrometry; LC-HRMS, liquid chromatography-high resolution mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LC-MS, liquid-chromatography-mass spectrometry; LC-QTOF/MS, liquid chromatography-quadrupole time-of-flight mass spectrometry; LE, liquid extraction; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; MQL, method quantification limit; NA, not available; PFAS, perfluoroalkyl substances; PFHxS, perfluorohexansulfonate; PP, protein precipitation; RL, reporting limit; SPE, solid-phase extraction.

for LC were water, as mobile phase A, and methanol or acetonitrile, as mobile phase B, with different concentration of ammonium acetate as organic modifier [49,59,51,45,48,50,56]. The lowest detection or quantification limits were achieved applying guard security cartridges prior to chromatographic column or a column inserted between the HPLC pump and the right clamp valve to delay the elution of PFAS contaminants from instrument parts [59,45,48,56].

PFAS determination in umbilical cord serum was performed to study the possible correlation of this substances to cognitive development in children [64], fetal thyroid hormone levels [12], and other conditions related to prenatal exposure [13]. As previously described for serum, SPE was the most common technique used for extraction or for a further purification [64,62,63,65], and LC-MS/MS was the most performed instrumental method at the same aforementioned instrumental conditions.

Whole blood analysis was performed by Kärrman et al. [14], which pretreated the samples with a SPE and a filtration step. LC was coupled with single quadrupole operating in selected ion monitoring (SIM) detection mode. In this study, PFAS whole blood concentrations were compared to those in plasma, showing that PFOS, PFOA, PFHxS, and PFNA plasma levels were higher than that in whole blood. In contrast, distribution of PFOSA was different from other congeners (whole blood levels were five-times higher than that in plasma).

3.2. Urine

In biomedical fields, urine analysis provides information of excretion of xenobiotics and may allow the detection of a single drug dose consumption for 1.5–4 days, depending on its pharmacokinetic profile [66]. Anyway, the metabolic stability and physicochemical properties of PFAS enlarge the detecting window in the matrix. PFAS determination in urine was performed to study the exposure to these substances and their excretion in human. Although SPE was the most common extraction approach [46,49,42], LLE was performed using acetonitrile as extraction solvent [43] and dilute and shoot was applied by Wang et al. [18], adding 1 mL of 2% formic acid in methanol. In other cases, further samples purification was obtained with an additional SPE step after LLE [43]; the thorough purification contributed to achieve the best sensitivity, with the lowest reported LOD and LOQ in this study, to our knowledge. Similarly to the other matrices, the C18 functionalized column allowed to satisfactorily separate the analytes. The most common combinations of mobile phases were different concentration of ammonium acetate in water (A) and methanol, or acetonitrile (B) [46,49,18]. The analytes were ionized in negative mode and acquired in MRM by the triple quadrupole mass spectrometer.

3.3. Keratin matrices

As noninvasive matrix, hair is the most preferred for monitoring the exposure to substances in a wide time window, considering the hair slow growth of about 1 cm per month.

The mechanism of incorporation is still unclear, but lipophilic and basic compounds have a major accumulation in hair [67]. Due to the apolar properties of PFAS, hair represents an interesting matrix for the chronic exposure assessment. Three studies [18-20] performed hair analysis to monitor PFAS exposure using similar extraction methods and analytical techniques. A matrix extraction with various solvents followed by SPE clean-up was the most common choice for sample preparation, although the extraction solvents were different; Ruan et al. [19] selected a methanol:water 95:5 (v/v) mixture, while Piva et al. [20] and Wang et al. [18] used only acetonitrile. After a clean-up step by SPE, the latter filtrated the sample using 0.2 µm nylon filter, for a further purification. LC-MS/MS was the most selected instrumental technique, selecting a negative polarity and a MRM acquisition mode; for chromatography, the most common choice for the separation was C18 column [18-20]; mobile phases were 2 mM ammonium acetate in water (A) and methanol (B) [18,19], however Piva et al. [20] chose acetonitrile with 0.1% formic acid as solvent B.

Keratin matrices also included nails, an alternative matrix with the potential to incorporate lipophilic xenobiotics for long periods of time [68,69]. Wang et al. [18] obtained the most performing extraction for human nail sample by methanol with alkaline digestion. LOD and LOQ ranges in keratin matrices were 0.02–0.1 ng/g and 0.02–1.7 ng/g, respectively.

3.4. Semen

Xenobiotics can be transported to the seminal fluid, impacting the male reproductive system functionality; this can lead to physiological, metabolic, or genetic changes [70]. PFAS determination in semen was performed to evaluate the possible PFAS correlation to the male reproductive system. Raymer et al. [24] evaluated the semen quality related to PFOA and PFOS in the matrix using a SPE. However, cases with PFOA concentrations above detection limit were insufficient to statistically study the possible correlations. As expected, higher sensitivity of the analytical method was achieved with the ion-pairing LLE already described for serum and plasma analysis [41]. In this study, LOQ range was 0.002–0.1 ng/mL and PFAS concentrations in real samples ranged between 0.03 and 9.90 ng/mL. Both studies applied LC-MS/MS equipped with C18 chromatographic columns and electrospray ionization (ESI) source for instrumental analyses. While Cui et al. [41] used 2 mM ammonium acetate in water (A) and methanol (B), Raymer et al. [24] added small percentages of methanol in mobile phase A and small percentages of water in mobile phase B, resulting in 95% ammonium acetate in water and 5% methanol (A), and 95% methanol with 5% ammonium acetate in water (B). Mass spectrometer parameters included an ESI- and an MRM acquisition mode.

3.5. Other matrices (bone, breast milk, cerebrospinal fluid, placenta)

Recent studies demonstrated that bone is a sensitive target for some POPs [71]; hence, Koskela et al. [15] determined PFAS concentration in

human femoral bone samples to investigate the possible effects on bone microarchitecture and on differentiation of osteoblasts and osteoclasts. Samples were powdered and extracted with a 20 mM ammonium acetate in methanol solution, followed by LC-MS/MS analysis; PFAS contaminations from inner LC parts were avoided installing a trap column prior to a C18 functionalized column. Negative polarity and an MRM acquisition mode were the most performant parameters for mass spectrometer. The most abundant compounds detected in real samples were PFOA, PFNA and PFOS.

Breast milk and placenta are considered alternative matrices to study children perinatal exposure [72]. In fact, prenatal exposure to xenobiotics may occur through substances placenta crossing. Furthermore, infants may be exposed to xenobiotics from mothers' exposure or consumption through breastfeeding, since xenobiotics can be transported with the nutrients in the breast milk [73,74]. Generally, the analytical investigation of these two matrices is useful for assessment of infant exposure to substances that may be harmful for their cognitive and motor development although the narrow detection window (hours) [72,75,76]. Kim et al. [16] performed a study involving 207 primiparous women. Breast milk samples were ion-paired extracted with methyl tertiary-butyl ether and then cleaned-up by SPE, followed by LC-MS/MS analysis ionizing in negative polarity and acquiring in MRM mode; ammonium acetate (10 mM) and methanol were used as mobile phases A and B, respectively. Alternative PFAS free materials such as nylon and polyetheretherketone were used to prevent the contamination from instrumental parts. However, a PFOA concentration of 0.184 ng/mL was detected in the blank samples, so it was subtracted as baseline concentration from those of the milk samples.

Placenta analysis was performed to investigate prenatal exposure to PFAS related to sociodemographic risk factors [21], birth outcomes [22] and birth size possibly related to PFAS [23]. Bangma et al. [21] detected 22 target analytes using 0.01 M KOH in methanol as extraction solvent followed by a clean-up purification step; the same procedure was applied by Hall et al. [22] but acetonitrile was preferred for extraction. However, SPE allowed Wang et al. [23] to achieve better sensitivity and quantitate lower concentration than other studies. C18 was the most preferred column for LC-MS/MS, while methanol and water both containing 2 mM ammonium acetate were the choice for mobile phases [21,22]. Mass spectrometer operated in MRM acquisition mode and negative polarity ionization. Unfortunately, Wang et al. [23] did not detailed the instrumental parameters. Considering the pre- and perinatal assessment, some difficulties are represented by the differences based on infant sex. Controversial results were obtained in PFOS and PFNA, associated with male lower birth weight while female higher birth weight [22].

Another alternative matrix is cerebrospinal fluid (CSF), the most common matrix used to assess the pollutants presence in human brain. Hu et al. [17] determined 26 PFAS by LLE with ethyl-acetate followed by UPLC-MS/MS analysis, using MRM and negative polarity ionization. C18 was the column of choice; mobile phases were 2 mM ammonium acetate in water (A) and methanol (B), in gradient elution.

4. Conclusions

In the last years, the scientific community focused the attention on biomonitoring of human exposure to investigate the possible etiologic role in some pathologies. As reported in the literature, SPE showed the best suitability for matrix extraction of PFAS in most of the considered biological matrices; on the other hand, LLE extraction allowed to combine highthroughput and good recoveries and sensitivity, saving time and economic resources. LC-MS/MS was the most common instrumental technique, separating the analytes through a C18 column. Moreover, the most used mobile phases were water, as solvent A, and methanol, as solvent B, both with different concentrations of ammonium acetate as organic modifier. One of the main challenges is the high number of existing PFAS (more than 4700); indeed, investigators usually focused on the most popular compounds. As expected, the best signal was obtained with the mass spectrometer operating in negative polarity (since the PFAS acidic properties) and MRM acquisition mode. However, HRMS screening could be useful also for the identification of new perfluoroalkyl compounds, allowing the structural elucidation of analytes thanks to the mass resolution.

Due to the wide use of PFAS also in the laboratory materials manufacture, the operators have to carefully consider any possible source of contamination, during all the analysis steps. To this concern, several PFAS-free chromatographic kits were developed. The most preferred material for laboratory tools such as vials, screw caps, centrifuge tubes were polypropylene and polyethylene. Consequently, the lower background noise results in a higher sensitivity which allows to quantify lower PFAS concentration in the investigated matrices.

For this purpose, mechanism of PFAS toxicity need to be more investigated. Moreover, backtracking the exposure source is not immediate since their widespread use in everyday objects manufacturing. Therefore, a basal concentration represents a difficult parameter to calculate whereas these compounds constitute environmental pollutant. Furthermore, the study of PFAS possible role in the occurrence of certain pathological conditions should be designed as multiparametric studies, to consider also the PFAS fluctuation in the biological matrices during time. To this concern, the analytical method should be properly developed to allow the proper collection of data.

In conclusion, a strong effort is required by the scientific community to expand knowledge about mechanism of toxicity and possible related pathologies, considering the huge spread of this substances and their unclear effects on human health.

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.

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