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Tracing per- and polyfluoroalkyl substances (PFASs) in the aquatic environment: Target analysis and beyond

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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) have been manufactured and used for decades. The aquatic environment is a critical transportation and transformation compartment for PFASs. Target analysis is the most used method of tracing PFASs in the aquatic environment but is powerless for the large amounts of unknown PFASs. This review summarizes the advantages of three target analysis supplementary approaches, including extractable organofluorine (EOF) analysis, high-resolution mass spectrometry (HRMS) screening, and PFAS precursor oxidative conversion. For known PFASs, more targets, including emerging PFASs, PFAS isomers, ultrashort-chain PFASs, and cationic/zwitterionic PFASs, should be considered. For unknown PFASs and organofluorines, developing comprehensive and low-contaminated sample treatment strategies is essential yet challenging. We propose including PFASs screened via HRMS at confidence level (CL) 3 and above in EOF mass balance analysis and CL 4 for samples collected from a “known source”. Appropriate approaches should be applied to investigate unknown PFASs beyond target analysis comprehensively.

1. Introduction

Per and polyfluoroalkyl substances (PFASs) are synthetic fluorinated compounds containing at least one fully fluorinated methyl or methylene carbon atom [1]. Their high C–F bonding strength (485 kJ/mol) accounts for their incredible stability, and with hydrophilic and hydrophobic functional groups, they exhibit excellent surfactant properties [2]. Furthermore, their robust stability and high surface activity endow them wide application in various products and processes, such as electroplating, textiles, paints, leathers, and pesticides, for over 70 years [2, 11]. PFASs have attracted significant attention since the early 2000s when the ubiquity and health risk of two PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), were reported [4]. Henceforth, one major group of PFASs, perfluoroalkyl acids (PFAAs), has raised substantial public concern because of their highly persistent, bioaccumulative, and toxic properties, as well as their long-range transport potential [3,5,6]. Consequently, some PFASs (e.g., PFOA, perfluorohexanesulfonic acid (PFHxS)) and their related compounds have been listed under the Stockholm Convention as persistent organic pollutants (POPs) for global elimination or restriction [7–9]. In 2022, it was proposed that long-chain perfluorocarboxylic acids (PFCAs) with perfluorinated carbon chain lengths ranging from 8 to 20 should be listed as POPs [10].

Apart from the direct emission of PFASs, some PFASs can degrade into persistent PFASs through biotic or abiotic pathways (i.e., PFAS precursors) [2,11]. The degradation of PFAS precursors serves as an indirect source of PFASs in the environment, and this topic deserves more research effort due to the limited knowledge on PFAS precursors at present [12]. In addition, many PFASs (e.g., hexafluoropropylene oxide dimer acid (HFPO-DA), also known as GenX) have similar chemical structures with PFAAs [13]. These compounds are often considered as emerging PFASs and are manufactured as substitutes for legacy PFASs that have been phased out in the market. Notably, they might have been used for several decades, but were not reported until recently, attributed...
to the advancement in analytical techniques and increased awareness of the threats of PFAS pollution [14,15].

Over the past two decades, target analysis has been extensively employed to investigate typical PFASs when reference standards are available. Target analysis is the most well-established method for PFAS analysis, featuring high sensitivity and excellent quantitative results. However, PFASs comprise a vast array of compounds with distinct properties, and currently, over 5000 individual PFASs are registered [16]. Thus, developing new methods for detecting and analyzing a broader range of PFASs is necessary, but challenging because of the lack of reference standards. Moreover, only a minute fraction of target PFASs has been subjected to routine monitoring. To address this issue, extractable organofluorine (EOF) analysis was introduced to conduct fluorine mass balance analysis and estimate the amount of unknown organofluorine (UOF) [17]. Adsorbable organofluorine (AOF) is another approach in which sorbent loaded with OF is combusted directly [18]. Considering the limited natural sources of OFs in the aquatic environment, most OFs present in water samples likely originate from anthropogenic sources [19,20]. To better understand UOF profiles, nontarget and suspect screening have been applied using high-resolution mass spectrometry (HRMS) techniques, which can expand the analyte list [21]. Another approach to revealing the UOF profile is to apply the oxidative conversion method to measure the oxidizable precursors that can transform into measurable PFCAs [22]. Combining these analytical methods can provide a comprehensive picture of the PFAS pollution in the aquatic environment (Fig. 1).

PFASs are widely used and continuously released from point and diffuse sources into the aquatic environment, resulting in their ubiquitous distribution in water bodies [23–25]. The aquatic environment is an important transportation and transformation compartment for PFASs, particularly those with relatively high water solubilities (e.g., ionic PFASs) [26]. Due to their relatively high water solubility and bio-accumulation potential, PFASs can pose a potential ecological risk to aquatic organisms, particularly to those at higher trophic levels [23,27]. Studying the occurrence and distribution of PFASs in water bodies can enhance the understanding of the pollution sources and fate of PFASs as well as assess their potential risks to humans and wildlife. The majority of studies have focused on the determination of PFASs in water bodies using target analysis. In recent years, analytical methods beyond target analysis have been increasingly applied, which enable the comprehensive risk evaluation of a wide array of various PFAS species and provide directions for further PFAS research in the aquatic environment.

The present review evaluates the four most commonly used analytical methods for tracing legacy and emerging PFASs in aquatic matrices: target analysis, HRMS screening, oxidative conversion of precursors, and EOF analysis. The pros and cons of these methods are discussed in detail. This review aims to identify the knowledge gaps in the current analytical methods for PFASs in the aquatic environment and to provide directions for future research.

2. Current analytical methods for tracing PFASs in water samples: target analysis and supplementary approaches

2.1. Target analysis

Target analysis is the most used method for the identification and quantification of PFASs in the aquatic environment. In most environmental studies, levels of ionic PFASs in water samples are measured using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). LC–MS/MS is typically operated in the negative electrospray ionization (ESI) mode for analyzing anionic PFASs (e.g., PFAs), commonly combined with multiple reaction monitoring (MRM) to avoid false detection.

Due to the usually trace levels of PFASs in the aquatic environment, an analyte enrichment step is required prior to instrumental analysis. Solid phase extraction (SPE) is the most commonly used sample treatment method, in which a weak anion exchange (WAX) SPE cartridge is conventionally employed since the most concerning PFASs (i.e., PFAAs) are present as strong acids (anionic) in the aquatic environment. The use of mass-labeled standards as surrogate compensates for signal enhancement/suppression or analyte losses during sample treatment, bringing substantial improvement in the result accuracy. Target analysis enables PFAS quantification with high precision and sensitivity [28–30]. Advancement in instrument and better solvent quality together with the available mass-label standard as well as the available standard methods improve the data quality for target analysis substantially. Currently, the method quantification limits (MQLs) of typical PFASs, such as PFOS and PFOA, are at the pg/L level, with procedural recoveries typically ranging from 80 % to 110 %, assuring high-quality data and satisfactory results [25,31]. Numerous inter-laboratory comparisons and standardized operating procedures have been reported [32]. Over the last few decades, the number of PFAS analytes detected using target analysis has considerably increased [5]. Advancement in the PFAS target analysis research is significantly facilitated by the widespread implementation of target analysis techniques across analytical laboratories, offering high sensitivity and accurate linearity across a wide range of PFAS determination experiments [5,31].

The primary limitation of the PFAS target analysis is its relatively low inclusivity, which is highly dependent on the availability of reference standards. With the increasing occurrence of emerging PFASs and PFASs...
precursors (or intermediates) in the aquatic environment, there is an urgent need to broaden the scope of target analysis [33–35]. Another insufficiency of the PFAS target analysis is that the result can be influenced by potential interference compounds (i.e., substances having signals on MRM transitions and retention times (RTs) close to those of the target PFASs) in samples. Such an issue becomes more significant for PFASs of low molecular weight that only have one MS/MS transition, e.g., perfluorobutanoic acid and perfluoropentanoic acid. To obtain reliable results, using HRMS for compound identification becomes requisite.

Another approach is using different columns for chromatographic separation so as to verify the results based on RTs. It is imperative to exercise caution when interpreting target analysis results for complex aquatic matrices, such as wastewater. In view of these considerations, combining supplementary PFAS analytical approaches, including EOF analysis, PFAS precursor oxidative conversion, and HRMS screening, can provide an in-depth understanding of the PFAS pollution status (Table 1).

2.2. Extractable organofluorine (EOF) analysis

The development of analytical standards for newly identified PFASs has fallen behind their production and release, leading to a shortage of reference standards for routine analysis. EOF analysis is generally used as a complementary approach for the target analysis to determine the potential existence of unknown PFASs in water samples.

To enable the comparison between target analysis and EOF analysis, treatment processes for EOF analysis in water samples are generally similar to those for PFAS target analysis (e.g., WAX SPE method). However, SPE with an ion exchanger could capture anions and the free fluoride, and consequently, the separation of from inorganic fluoride (IF) interferences cannot be evaluated using combustion ion chromatography (CIC). The addition of a washing step using 0.01 % ammonia solution, followed by Milli-Q water, has been demonstrated to effectively eliminate the majority of the spiked fluoride (>99.9 %) [17]. Moreover, the addition of a 0.2 mol/L acidic sodium nitrate solution has been demonstrated to eliminate up to 99.97 % of the spiked fluoride in the water (i.e., CIC).

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EOF analysis approach has the advantage of capturing a large number and diversity of PFASs (i.e., high inclusivity). EOFs can be quantified with proven high accuracy using any fluorine-containing standard. In addition, the treatment and analysis processes for determining EOF in water samples (e.g., SPE-CIC) are less complicated as compared with others, and do not rely on complex data-processing algorithms or expensive instruments. These advantages make EOF analysis a very suitable method for measuring the overall PFAS amount in water samples, which could facilitate the selection of “samples of interest.” Nevertheless, the most significant drawback of EOF analysis is that it gives limited information on chemical structure, which calls for the combination with other analytical approaches (e.g., target analysis and suspect screening) to achieve the identification of various PFAS species in samples. The MQLs of EOF analysis using CIC could reach subµg/L levels for water samples [18,19]. However, this MQL is still considerably higher than that of PFAS target analysis (MQLs at pg/L–ng/L levels). The relatively low sensitivity of EOF analysis can also, to some extent, restrict its broad application.

2.3. Oxidative conversion of PFAS precursors

Oxidative conversion of PFAS precursors is a relatively novel method, which aims at additionally revealing PFAS precursor profile beyond traditional target analysis. This method works via specifically oxidizing PFAS precursors (e.g., fluorotelomer sulphonates and perfluoralkyl sulfonamide acetic acids) through hydroxyl radicals to measurable PFAs which are subsequently measured using target analysis (Fig. 2). The oxidative conversion process can be salt-based [22], catalyst-based [42], or photooxidation-based [43]. By comparing the PFCA levels between oxidized and unoxidized extracts, the oxidative conversion method highlights the correlation between PFAS precursors and the utilization of synthetic polystyrene-divinylbenzene-based activated carbon-filled SPE cartridges [18]. Apart from SPE, ion-pair extraction (IPE), a liquid–liquid extraction method, has also been applied to extract OF in water samples. For example, tetrabutylammonium was used to form stable ion pairs with anionic PFASs, which together with other neutral OF are subsequently extracted to the organic phase while IF remains in the aqueous phase [19,36]. Nevertheless, IPE has several disadvantages; for example, the co-extraction of matrix components can occur, and analytical bias can be introduced due to the formation of unstable ion pairs, resulting in a high fluctuation in the recoveries of different PFAs [19,36]. Various analytical methods have been developed to determine the EOF levels in the aquatic environment, e.g., ¹⁹F nuclear magnetic resonance (¹⁹F NMR) spectroscopy used for surface water and wet precipitation samples [37,38]. However, an extensive preconcentration process and prolonged acquisition time (approximately 1 h) are conventionally required because of the low sensitivity of the ¹⁹F NMR technique, which restricts its application to PFAS detection in water samples [37,38]. Continuum source molecular absorption spectrometry (CS-MAS) uses online pyrolysis and the formation of metal monofluorides (e.g., gallium monofluoride) at high temperatures for the fluorine detection. However, the fluoride signal yield by CS-MAS may originate from unspecified EOF substances, which limits its application in PFAS identification in water samples [39]. Particle-induced gamma-ray emission offers rapid analysis and is valuable for quantitative analysis to determine fluoride in water samples; however, the requirement of nuclear excitation (gamma-ray) increases the associated analytical complexity and cost [19]. Given the above, CIC is currently the most recommended approach for the determination of EOF and AOF in water samples, which is relatively sensitive and convenient with a low and consistent background [19,40]. In CIC, the EOF extract can undergo pyrolysis and thermal oxidation in a moisturizer oxygen stream at a high temperature (1000 °C) to convert all OFs into hydrogen fluoride (HF). Subsequently, HF is absorbed in aqueous media (e.g., Milli-Q water, hydroxide or peroxide solution), and the free anions (i.e., fluoride) are determined based on conductivity [41].
and PFAS concentrations in the environment. This method has been applied in many studies on PFAs in various aquatic compartments, including drinking water, wet precipitation, groundwater, and wastewater [44–48]. It can discover the presence of a high amount of PFAS precursors in water matrices that might have been long neglected.

The oxidative conversion method is readily applicable to water samples considering the relatively simple composition of constituents in aquatic matrices [49]. This is a relatively rapid screening technique that measures the total mass of oxidizable precursors converted to PFAs. By oxidizing these PFAS precursors to PFAAs to be analyzed using LC–MS/MS, this method yields precise quantitative data with MQLs as low as PFAS target analysis. The oxidative conversion method can also help to explain UOF in contaminated water samples [50]. In addition, oxidized homologs can offer insights into the structures of oxidizable precursors (e.g., functional groups and carbon chain lengths) and manufacturing methods (electrochemical fluorination versus telomerization), as different classes of PFAS precursors can have different fingerprinting oxidation patterns (Fig. 2) [51, 52].

Nevertheless, the are some drawbacks in the oxidative conversion method. Firstly, certain emerging PFASs cannot be oxidized, such as per- and polyfluoroalkyl ether acids (e.g., 6:2 chlorinated perfluoroalkyl ether sulfonic acid and HPPO-DA), making oxidative conversion approach ineffective in investigating the occurrence of these PFASs. Secondly, some PFAS precursors can be oxidized to non-PFAAs or unmonitored products. For example, ADONA can be oxidized to non-PFCA products, and precursors can be oxidized to ultrashort-chain PFCAAs [53–54], which making the situation complicated. Additionally, there exist a potential for incomplete degradation due to the exhaustion of the oxidant, particularly for complex aquatic matrices (e.g., aqueous film forming foam (AFFF)-contaminated water and raw wastewater). Therefore, sample dilution or an excess in oxidant is required to ensure complete oxidation. In this context, the use of “total” in the term “total oxidizable precursor assay” is incorrect, and the use of mass-labeled PFAS precursors as surrogates is highly recommended to monitor the oxidation completeness.

More specifically, the current problems associated with the oxidative conversion method include the risk of losing volatile precursors during heating, potential post-oxidation blank contamination, matrix effects brought from post-oxidation extracts, potential reproducibility issues, and limited information about zwitterionic and cationic precursors [55]. In future works, a relatively “green” (i.e., chemical-saving reagents) approach, for example, catalyst-based oxidative conversion, is recommended. Other oxidation approaches, e.g., ultraviolet-based photo-oxidative conversion, may have broad application prospects because they are time-saving and could avoid the potential loss of volatile PFASs [42].

2.4. High-resolution mass spectrometry (HRMS) screening

The HRMS technique can provide accurate masses, isotopic distributions, and MS/MS spectra to discover emerging and unknown pollutants without reference standards [56, 57]. The application of PFAS HRMS screening could greatly expand our understanding of the structure and rough concentration of unknown PFASs beyond the limited PFAS targets.

Many classes of emerging/novel/new PFASs have been identified in the aquatic environment in recent years. These studies are mainly focused on water samples collected at (or downstream from) the point source, e.g., AFFF-impacted regions [21, 58, 59], airports [59], fluoroochemical facilities [57, 60–63], and wastewater treatment plants [35, 45, 63]. Considering the relatively low levels of PFASs in water samples, a sample-treatment procedure is needed to concentrate analytes of interest and simultaneously remove unwanted matrix interferences. This is an inherent challenge in unbiased nontarget screening when the analytes are unknown. Thus, any sample treatment process should be kept to a minimum action to prevent unwanted losses or unnecessary contamination. For studies regarding PFAS HRMS analysis, SPE cartridges are the most used treatment tools for concentrating and purifying PFASs from water samples. The sample treatment methods generally followed the PFAS target analysis sample treatment process where the WAX cartridge has been widely applied to extract PFAs and PFAA-related PFAS precursors and alternatives. In addition, depending on the PFAS class of interest, an additional extraction method could be applied to capture PFASs with different properties from the water samples. For example, to simultaneously extract cationic and zwitterionic PFASs, more SPE cartridges, such as weak cation exchange (WCX) and hydrophilic–lipophilic balanced (HLB) cartridges, have been used in tandem [35, 63].

Regarding the instrumental analysis, the ESI+ ion source was commonly used, and there are two major data acquisition modes: data-dependent acquisition (DDA) and data-independent acquisition (DIA). In the DDA mode, MS/MS analysis is performed on selected parent ions based on their intensities (e.g., top 10) in full scan. With the background and dynamic exclusion, DDA is theoretically most applicable to enriched
samples whereby PFAS signals dominate. Nevertheless, although DDA effectively identifies suspects, the potential “target compounds” could sometimes be limited in water samples because the MS/MS information is lost for ions with low MS abundance [64]. In the DIA mode, all parent ions are introduced to the collision cell for fragmentation. This mode potentially better suits PFASs at low levels in water samples, which does not involve preselection by the quadrupole, and is expected to be highly effective for flagging the presence of unknown PFAS ions. DIA delivers many coeluting ions in the full-scan spectra at specific RTs due to complex environmental matrices. Therefore, differentiating the fragment ions of the parent ion based only on RTs is challenging because of the coeluting ions. Fortunately, software tools, such as MS-DIAL, can partially help to solve this problem through spectral deconvolution analysis [65].

PFAS nontarget screening strategies can be generally divided into homolog-based and fragment-based approaches. Homolog patterns can be employed to identify PFAS homologs based on the accurate mass and the elution order of homologs since PFASs always occur as homologs (e.g., the difference in -CF₂-, -C₆H₄FO₂-, -CF₃-, and -CF₂O- units). This method is time-saving and widely used to discover PFASs in the environment [35,66-68]. However, one problem is that some PFASs without homologs may not be detected (e.g., sodium perfluoro-n-exonybenzenesulfonate, OBS). For the fragment-based screening method, featured fluorine fragments, such as C₂F₅ and C₂F₄O⁻, are frequently detected, and these fragments can differentiate PFASs from other non-fluorinated chemicals. Using this method, it is theoretically possible to detect all PFASs, and this method has been widely applied in water samples which have a relatively weak matrix effect [61,69,70]. A complex matrix may limit the discovery of PFAS-related MS² fragments. The CF₂-based mass-defect filter is an effective tool that is commonly used during nontarget screening to save time, and conventionally, only a feature with a mass defect >0.85 or <0.15 is retained. Nevertheless, despite mass-defect detection approach well suits highly fluorinated PFASs, this method does not work for all PFASs according to the latest definition from OECD (i.e., at least one fully fluorinated methyl or methylene carbon atom) [1]. Unlike nontarget screening, suspect screening is easier to perform and can also provide detailed information within a specific range of unknown PFASs, depending on the suspect list selected. The results are compared with available libraries, which mainly include (1) open-source databases (e.g., NORMAN PFAS suspect list), (2) commercialized software (e.g., Sciex OS), and (3) recently published papers (i.e., newly reported PFASs). Considering the extensive nature of HRMS data and the considerable amount of data in the PFAS libraries, suspect screening is always conducted using software (e.g., FluroMatch) and programming codes (e.g., R scripts). Eventually, suspect screening data must be checked manually to yield convincing results.

The major limitations of PFAS HRMS screening are that this approach relies on expensive instruments (e.g., LC--Orbitrap), and the data processing of nontarget and suspect screening is much more complicated and time-consuming than target analysis. In addition, the current sample treatment method for PFAS HRMS screening generally follows PFAS target analysis. However, to achieve the high inclusivity of PFASs with different properties, additional efforts are needed. For example, to include as many as possible unknown PFASs in water samples, using additional SPE cartridges like HLB WCX cartridges for sample treatment and an additional sample injection in the positive ESI (ESI⁺) mode injection is needed. This may also increase the workload of PFAS HRMS screening in a way compared with PFAS target analysis. Thus, researchers should choose an appropriate HRMS screening strategy according to their objectives. Moreover, due to the lack of reference standards, using existing standards as substitutes for the semi-quantification of novel PFASs (e.g., using PFOA as the standard for C1-PFOA quantification) may bring uncertainty to the quantification result.

3. Research gaps and perspectives about investigating PFASs in water samples

3.1. Discovering more targets: emerging PFASs, PFAS isomers, cationic and zwitterionic PFASs, and ultrashort-chain PFASs

PFAS target analysis is highly dependent on the availability of reference standards. With the increasing production and use of various PFAS formulas, more and more emerging PFASs were discovered and detected in the aquatic environment, making target analysis inapplicable to these new PFASs. Evidently, the main direction on method development for PFAS analysis is to include more target analytes. When more reference standards of emerging PFASs, such as unsaturated PFCAs (uPFCAs) and chlorine-substituted PFCAs (Cl-PFCAs), become commercially available, these compounds should be included in the PFAS target list to enable a more comprehensive and precise investigation of PFASs. In addition, current PFAS studies on “non-PFAA” compounds usually do not discriminate isomers (i.e., only one isomer is involved), due to the limitation that isomer-specific standards of many PFASs are unavailable. Considering that different PFAS isomers exhibit different behaviors in the aquatic environment and may induce different toxicological effects on aquatic organisms (Schulz et al., 2020), an in-depth investigation of the PFAS isomeric profile can help the source profiling of PFASs and further our understanding of their ecological risks. Moreover, in the aquatic environment, cationic and zwitterionic PFASs (e.g., 6:2 fluorotelomer sulfonamide alkyl betaine, 6:2 FTAB) are much less investigated compared with the anionic ones (e.g., carboxylates and sulfonates). This can be attributed to (1) the limited commercial availability of reference standards (e.g., only five reference standards of cationic and zwitterionic PFASs are available in a commonly used commercial laboratory 2021–2023 catalogue), (2) an unintentional loss of cationic and zwitterionic PFASs during the treatment process of water samples (e.g., using WAX SPE cartridges), and (3) the need for an additional sample injection in the ESI⁺ mode. Many cationic and zwitterionic PFASs (e.g., 6:2 and 8:2 FTABs) show a considerable bio-accumulation potential, and they can be degraded to legacy PFASs (e.g., PFHxS and PFOS) in the environment [58,71,72]. Thus, more investigations on these compounds should be included in future studies.

Another group of PFASs usually neglected in target analysis are ultrashort-chain PFASs, which are usually referred to as PFCAs and PFOSs with carbon chain length no more than 3. They have been frequently found at higher levels than other PFASs in various aquatic compartments, including seas, oceans, rivers, drinking water, ground-water, wet precipitation, and deep polar seas [73,74]. Nevertheless, knowledge regarding the environmental behavior and toxicity of these ultrashort-chain PFASs is still scanty. One notable challenge for the investigation of ultrashort-chain PFASs in water samples is the development of robust analytical methods. Ultrashort-chain PFASs, which are more polar, are less compatible for the simultaneous quantification along with most PFAS analytes using reverse-phase (e.g., octadecylsilane, C18) analytical methods. Considering that ultrashort-chain PFASs frequently elute very early and exhibit poor resolution in the routine PFAS analysis (e.g., for PFOS, PFOA and PFHxS), optimization of the current LC method is required. Improvement in the detection sensitivity and chromatographic resolution of ultrashort-chain PFASs has been the subject of considerable research, with trials undertaken on additional stationary phases or separation mechanisms, particularly hydrophilic-interaction LC, ion exchange chromatography, and super-critical fluid chromatography [19,48].

3.2. Nontarget and suspect screening: workflow and result communication

The confidence in HRMS-based identification varies between studies and substances, and it is challenging to communicate these varying confidence levels (CLs) concisely and accurately to audience. For most
studies on the nontarget and suspect screening of PFASs in water samples, small molecules identified through HRMS are defined with five CLs [75]. CL 1 (a confirmed structure) represents the ideal situation in which the proposed structure has been confirmed via the appropriate measurement of a reference standard with MS, MS/MS, and RT matching. CL 2 (a probable structure) indicates that it is possible to propose an exact structure using other pieces of evidence, while CL 3 (a tentative candidate) describes a gray zone where evidence exists for possible structures; however, insufficient information is available to identify one exact structure (e.g., positional isomers). CL 4 (an unequivocal molecular formula) indicates that a formula can be unambiguously assigned using spectral information (e.g., adduct, isotope, and fragment information), although insufficient evidence exists to propose possible structures. CL 5 (an exact mass m/z) can be measured in a sample and be of specific interest in the investigation; however, it lacks information to assign a formula. Nontarget screening allows tracing these masses in other investigations; however, CL 5 indicates that no specific information about the structure or formula exists. To achieve a CL as high as possible, one should examine the HRMS result in the order of exact mass, isotope distribution, adduct (if any), and MS\(^2\) fragments to determine if the result fits well with the proposed structure or matches the experimental data/library/reference standard (Fig. 3A). Recently, a PFAS-specific HRMS communicating confidence framework has been proposed, which considers the detection of homologous series, specific ranges of mass defects, and RT consistency according to the features of PFAS homologs [76]. For example, for candidate structures with little fragmentation-based evidence, distinct patterns of homologs can constitute sufficient circumstantial evidence to enable identification with CL 3 d confidence. The presence of homologs (identified with CL 2 confidence or greater) and the candidate’s RT are consistent with the homologous series, and can provide strong circumstantial evidence for a candidate that lacks sufficient MS\(^2\) data. This allows a more efficient analysis of PFASs through nontarget and suspect screening (Fig. 3B).

Considering the large PFAS family, many emerging/novel/new PFASs have quite different properties from those of PFAAs (or their precursors and alternatives), and caution should be exercised when identifying these substances in water samples through suspect screening. Many PFASs have relatively high volatility and poor water solubility (e.g., fluorotelomer olefins), and these substances are unlikely to occur in water samples. In addition, the current nontarget and suspect screening methods for PFASs in water samples mainly use ESI as the ion source, and thus, it is challenging to ionize nonpolar PFASs (e.g., perfluorooalkanes), and these substances are likely to be lost during the sample treatment. Occasionally, these substances can be falsely detected at CLs of 3 and 4, possibly due to the coelution in MS\(^2\), in-source fragmentation, or neutral loss. Thus, caution should be exercised when identifying low-water-soluble (or highly volatile) and nonpolar (or difficulty ionized) PFASs. Here, we recommend that PFASs featuring an exact mass of interest but with potentially low water solubility or high ionization difficulty using ESI should be directly classified as CL 5 during the suspect screening of PFASs in water samples.

To avoid false detection in future works on the nontarget and suspect screening of PFASs in water samples, reference standards should be employed as control. In addition, RT is an effective tool for eliminating some false detection results (e.g., in-source fragmentation and dimers), which is recommended to be considered and reported alongside the LC conditions in future PFAS nontarget and suspect screening works. A shortened “aquatic PFAS list” based on the broad spectra (e.g., the OECD PFAS list [16]) is also recommended to avoid false identification and save time in future suspect screening processes. However, considering thousands of candidates present in the current PFAS inventory, establishing an aquatic PFAS list requires the assistance of developing a prediction model for physicochemical properties. Predicting the possible bioactivities for various PFAS species based on structure—activity relationships is recommended in the “aquatic PFAS list” [77]. A unified, broad-spectrum, and easy-to-operate workflow is urgently needed for PFAS nontarget and suspect screening.

3.3. Organofluorine mass balance: standardisation and multi-method integration

EOF analysis could provide a complete picture of PFAS levels in water. To examine unknown PFASs, the EOF analysis approach was usually combined with PFAS target analysis, which offers a “known” OF amount to get the UOF concentration (ng F/L). For comparison, the concentration of PFAS (CPFAS, ng/L) is converted into the fluorine-equivalent concentration (C_{Feq,PFAS} (ng F/L)) according to the following equation:

\[ C_{Feq,PFAS} = \frac{C_{PFAS} \times 19}{16} \]

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\[ C_{Feq,PFAS} = \frac{C_{PFAS} \times 19}{16} \]
where $C_{PFAS}$ is the concentration of PFAS (ng/L), $n_F$ is the total number of fluorine atoms in the compound, $A_F$ is the atomic weight of fluorine (g/mol), and $MW_{PFAS}$ is the molecular weight of the corresponding PFAS. UOF can be calculated by deducting $C_{Feq,PFAS}$ from EOF. Studies have reported a high proportion of UOF in various aquatic matrices [48, 58]. The high proportion of UOF observed globally in the aquatic environment indicates the widespread occurrence of PFASs, and more efforts shall be made on the standardization of the EOF-determination approach, including sample treatment and fluorine mass balance calculation [40]. The proposed workflow for comprehensive PFAS assessment in water samples is given in Fig. 4.

Similar to that in HRMS screening, the sample treatment process in the EOF analysis should be more compatible, and the use of multi-SPE cartridges is recommended to extract OF substances with different properties (as stated in Section 2.4). However, different from the case in HRMS screening, the removal of IF shall be validated during the IF washing step. This is particularly important for samples collected from natural water bodies and drinking water, where the fluoride concentrations can be considerably higher than the PFAS concentrations. Nevertheless, more procedural blank contamination may be introduced when the sample treatment process becomes more complex, and this aspect warrants particular concern.

Due to the limited information obtained from the results of EOF analysis, more analytical approaches should be incorporated to conduct fluorine mass balance analysis. First, target analysis should certainly be included. Oxidative conversion of precursors and HRMS screening can effectively reveal the UOF concentrations in water samples, which can also assist in closing the fluorine mass balance loop. Considering its high sensitivity and quantification accuracy, the oxidative conversion method could provide an accurate fluorine amount for the PFAS precursors in water samples. However, as stated in Section 2.3, this method does not apply to non-oxidizable PFASs (e.g., chlorinated polyfluorinated ether sulfonic acids), and the presence of non-PFCA oxidation products (e.g., some polyfluoroalkyl ether carboxylic acids) may also influence the mass of EOF obtained through the PFAS precursor oxidative conversion process [54]. Apart from the oxidative conversion method, HRMS screening can also provide the fluorine amount through semi-quantification. Considering the high inclusivity of HRMS screening, most PFASs in the extract can be identified. However, there are two significant problems with the HRMS-based EOF mass balance. First, target analysis should certainly be included. Oxidative conversion of precursors and HRMS screening can provide an accurate fluorine amount for the PFAS precursors. As these two approaches can offer complementary information on unknown PFASs, the combination of HRMS screening (i.e., nontarget and suspect screening) with PFAS precursor oxidative conversion can give a more comprehensive understanding of the PFAS pollution profile.

3.4. Oxidative conversion of PFAS precursors: an extension of HRMS screening

The oxidative conversion method can provide a quantitative estimation of the unknown PFAS precursors and their structural characteristics. Nevertheless, such an approach does not work for non-oxidizable PFASs, and the chemical structure of unknown PFAS precursors cannot be identified using this method. In contrast, HRMS screening can provide more specific structure information on most PFASs, but this approach brings about certain uncertainty in the quantification of PFASs. As these two approaches can offer complementary information on unknown PFASs, the combination of HRMS screening (i.e., nontarget and suspect screening) with PFAS precursor oxidative conversion can give a more comprehensive understanding of the PFAS pollution profile.

Embedding PFAS precursor oxidative conversion in HRMS screening can help evidence the presence of unknown PFASs. Considering the time-consuming nature of HRMS data processing, oxidative conversion can also help select samples of interest (i.e., samples with high amount of PFAS precursors with specific carbon chain lengths) for the in-depth compound identification to improve the efficiency of HRMS screening. In addition, as stated in Section 2.3, the oxidative conversion approach can reveal the structures and manufacturing processes of oxidizable

![Fig. 4. A proposed workflow for comprehensive PFAS assessment in water samples. Modified based on Koch et al. [19].](Image)
PFAS precursors, and such knowledge can serve as supplementary information for identifying the structures of unknown PFASs via HRMS screening. Moreover, conducting HRMS screening on the sample extract before and after the PFAS precursor oxidative conversion can provide detailed information on PFAS precursors, the result of which can distinguish whether certain unknown (or emerging) PFASs are oxidizable. Nontarget screening may future reveal a possible new oxidation pathway and untended potential terminal oxidation product. In light of the above, combining the results of PFAS precursor oxidative conversion and HRMS screening has broad prospects for investigating unknown PFASs, especially in contaminated waters.

4. Conclusions

The aquatic environment is one of the most important compartments for PFAS transportation and transformation. Understanding the state-of-the-art analytical techniques available is important to better reveal the occurrence, fate, and transport of PFASs in the environment. In this review, we discuss the four most used analytical methods for tracing PFASs in the aquatic environment: target analysis, EOF analysis, oxidative conversion, and HRMS screening. Target analysis, a traditional method for PFAS analysis, was the most sensitive, convenient, and accurate method; however, it is limited by its relatively low inclusivity and less comprehensive results. EOF analysis, with high inclusivity, generally supplements target analysis to provide a complete picture of the PFAS family, and frequently detect high amounts of UOF in water samples. To reveal the UOF profiles, the oxidative conversion method, a relatively rapid approach with comparable low MQLs close to that in target analysis, is applied to assess the unknown PFAS precursors in water samples. However, this oxidative conversion method falls short in identifying non-oxidizable emerging PFASs. To examine the detailed UOF profiles, HRMS screening, including nontarget and suspect screening, has been increasingly utilized in recent years; nonetheless, this method is complicated and exhibits relatively poor quantification performance. Considering that the four approaches complement each other to reveal the PFAS contamination status, combining these methods is recommended to reveal the PFAS pollution profiles in the aquatic environment comprehensively.

For future PFAS target analysis works, more targets should be considered, including but not limited to emerging PFASs, PFAS isomers, ultrashort-chain PFASs, and cationic and zwitterionic PFASs. In the oxidative conversion method, adding mass-labeled PFASs as surrogate to compensate the matrix effect is highly recommended, and the development of a relatively green and highly effective oxidation approach should be given a high priority. The high amount of UOF found in the waters indicated the necessity to apply multi-method (e.g., oxidation conservation and HRMS screening) for organofluorine mass balance. In addition, a unified and comprehensive sample preparation treatment method with a low risk of contamination is critical for EOF analysis and HRMS screening, although this task is challenging. We suggest that PFASs featuring an exact mass of interest revealed by HRMS screening but poor water solubility or high ionization difficulty using ESI should be directly classified as CL 5 unless confirmed by a reference standard to avoid false detection. A broader target list combined with oxidative conversion of precursors and HRMS screening should be used in the fluorine mass balance calculation. We advise considering PFASs screened via HRMS at CL 3 and above in the fluorine mass balance calculation. Nevertheless, if the samples were collected from a “known source”, PFASs screened at CL 4 can also be considered in the fluorine mass balance calculation. The combination of HRMS screening and oxidative conversion could complementarily provide an in-depth understanding of unknown PFAS characteristics and their environmental behavior, which is ongoing progress with broad application prospects. This review suggests appropriate tools should be applied to best measure the large unknown PFAS fraction after target analysis and supports the idea that regulatory action should be taken by treating PFASs as a class rather than as each compound.

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

Data will be made available on request.

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