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Analysis of hexafluoropropylene oxide-dimer acid (HFPO-DA) by Liquid Chromatography-Mass Spectrometry (LC-MS): Review of Current Approaches and Environmental Levels

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Abstract

Emerging per- and polyfluorinated compounds (PFAS) compounds are of increasing interest for environmental monitoring, one being hexafluoropropylene oxide-dimer acid (HFPO-DA), commonly referred to as GenX. The following review describes existing liquid chromatography-mass spectrometry (LC-MS) methods used to analyze HFPO-DA, including sample preparation and method sensitivity relative to other PFAS. Analytical challenges are also described, in particular the significant formation of in-source fragmentation, dimer and dimer adducts which detract from $[M-H]^-$ signal. Lastly, detected levels of HFPO-DA in environmental and biological samples are compared across the limited number of available field exposure studies, which found several $\mu\text{g/L}$ concentrations in water samples taken near fluorochemical plant discharges.

Keywords

HFPO-DA; GenX; negative polarity electrospray ionization; liquid chromatography; mass spectrometry

1. Introduction

Concerns regarding environmental occurrence and biological toxicity for legacy PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS) and their associated salts have led to the production of PFAS alternatives by the fluorochemical industry [1]. One class considered favorable for adoption are perfluoroether carboxylic and

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sulfonic acids (PFECAs and PFESAs, respectively) due to their potential increase in degradation rates from ether insertion in the carbon backbone of per- and polyfluorinated compounds [2, 3, 4, 5]. One such compound is the PFECA commonly referred to by its trade name GenX [6, 7]. GenX refers to the ammonia salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (HFPO-DA) [6, 8], however the name GenX has been used to describe both forms. Developed as a replacement for PFOA, GenX is used as a processing aid in fluoropolymer manufacturing [6, 9]. It is characterized by a low pK_a and high solubility in water relative to PFOA (Figure 1). As of 2018, production levels ranged between 10–100 tons/year in Europe [14, 15]. Within the US, where production has increased following phase-out of PFOA, GenX has been discharged following fluorochemical monomer production into the Cape Fear River (CFR) in North Carolina, USA [6]. As a result, detection of elevated levels of HFPO-DA has been found in surface waters and drinking water of nearby residents [2, 3]. The Department of Health and Human Services in North Carolina following these observations has set a drinking water equivalent level of 140 ng/L as a drinking water health goal for HFPO-DA [6].

Numerous industry reports exist on toxicokinetic and toxicological studies of HFPO-DA performed largely on rodents though also include bobwhite quail, fish, *Daphnia magna*, bacteria, and rabbits as model organisms [1, 9, 10, 16–49]. Toxicological results reported in independent peer-reviewed journals show hepatotoxicity after 28 days at a dose of 1 mg/kg body weight dosing [50] and a no-observable-adverse-effect-level (NOAEL) between 50–500 and 1–50 mg/kg for females and males, respectively [19]. A study by Gomis et al. synthesized available toxicological data for long-chain and alternative PFAS, deducing that HFPO-DA has the higher toxic potency than PFOA [1]. Moreover, the physicochemical properties of HFPO-DA are akin to legacy PFAS, which suggests persistence and potential for long-range transport of HFPO-DA and thus requires further evaluation [1, 4]. As a result, environmental monitoring of HFPO-DA along with legacy PFAS such as PFOA continues to further understand the potential risks of human and environmental exposure.

In general, these compounds ionise well under ESI^- conditions and exhibit negative charge stabilization due to the highly electronegative nature of the fluorine atoms [51, 52]. However, the first study to report environmental detection of GenX/HFPO-DA, using typical LC methodology employed for legacy PFAS coupled with non-targeted MS acquisition, observed ionisation behaviour which yielded very little of the desired $[M-H]^-$ species [2, 53]. Although the ionization conditions used presently require improvement, increasing interest in HFPO-DA has resulted in the incorporation of the compound in existing targeted PFAS analyses [3, 8, 14, 54–60], along with another PFECA, dodecafluoro-3H-4,8-dioxanonoate (ADONA) [55–57, 60]. In late 2018, HFPO-DA was added to the United States Environmental Protection Agency (US EPA) Method 537.1 list of 18 PFAS to be determined in drinking water [61]. The aim of this review is to assess current methods and results of recent studies on the occurrence of HFPO-DA in the environment and biota, despite a limited number of quantitative and qualitative studies published at this time. One contributing factor for the lack of published studies may be the unique analytical challenges HFPO-DA presents when analyzed by current LC-MS methodologies, specifically significant analyte fragmentation in the ionisation source of the mass spectrometer and generation of dimer species upon ionisation, which this review will also explore.

2. Existing LC-MS Electrospray Ionisation (ESI) Methods for HFPO-DA Analysis in the Environment and Biota

To date, few published studies reporting quantitative and qualitative measurement of HFPO-DA in field collected environmental and biological matrices exist. A summary of LC-MS methods employed for the analysis of HFPO-DA are provided in Table 1 and discussed in the following sections. Though current studies utilize slight differences from lab to lab in their methods, the key approach for quantification is tandem quadrupole MS (QqQ), with one or two optimized multi-reaction monitoring (MRM) transitions. Overall, existing methods for HFPO-DA provide suitable chromatographic resolution and use MRM transitions based on loss of $-\text{CO}_2$ and ether linkage break (described further in section 3.1.2). HFPO-DA is relatively less sensitive compared to other PFAS ran concurrently in most of the described methods, as described in Table 2 and discussed in section 2.2.

2.1 Structural characterization and untargeted screening

Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) is the primary tool for detection and structural elucidation of emerging PFAS which in many cases are previously unidentified [53, 62]. The first report of HFPO-DA present in surface water from the CFR watershed resulted from the use of quadrupole time-of-flight MS (QToF-MS) (Table 1) [2, 6]. MS data filtering was performed using negative mass defect, an approach widely used for PFAS and other highly fluorinated compound identification due to the lower exact mass of fluorine (18.9984 m/z) than the nominal mass (19 m/z) [2, 53, 63]. Related species of suspected PFAS were further isolated by searching for the known mass differences representative of $-\text{CF}_2$ (49.9968 m/z) and $-\text{CF}_2\text{O}$ (65.9917 m/z), which are commonly found in PFAS mixtures because of their non-specific manufacturing process [2] such as electrochemical fluorination [63]. Following data filtering, one compound of interest was found to exhibit large dimer ($[2\text{M}-\text{H}]^-$), sodiated dimer adduct ($[2\text{M}-2\text{H}+\text{Na}]^-$) and in-source fragmentation ions, while the desired $[\text{M}-\text{H}]^-$ was barely present [2]. Structural confirmation was supported by a literature search of the proposed formula, $\text{C}_6\text{HF}_{11}\text{O}_3$, which yielded a CAS number and authentic reference standard, confirming the identification of HFPO-DA [2].

Following the inclusion of HFPO-DA in targeted analyses, additional non-targeted analysis of the same samples use repeating mass units and negative mass defect has identified PFAS homologues in other regions as well. For example, 11 PFASs were found in Dutch river samples collected downstream of a fluorochemical production facility [54]. Additional MS/MS experiments acquired with a LC-Orbitrap (Table 1) (Thermo-Fisher Corp. USA) provided fragment information used in structural characterization, supported by a literature search for possible compound candidates. The compounds also followed a similar spatial pattern to HFPO-DA, decreasing in concentration with distance from the manufacturing facility [54]. A similar approach was used by Song et al. in which 42 emerging PFAS were tentatively identified in water near a fluorochemical plant in China [60]. Pan et al. also used HRMS in a study targeting HFPO-DA and other PFAS, where the accurate mass measurement confirmed the presence of HFPO-TA, the trimer acid of HFPO, in water and biological samples [55].

2.2 Tandem quadrupole MS quantification

Tandem quadrupole mass spectrometry (QqQ) is the most widely used MS method for the quantification of PFASs in various matrices (Table 1). Since the first study to quantify HFPO-DA in surface waters [14], all quantitative reports of HFPO-DA using MS utilise QqQ instrumentation to perform multiple reaction monitoring (MRM) experiments. Development of MRM precursor and product ion transitions or optimization of MS conditions for HFPO-DA is not generally described in the literature. To our knowledge, only a recent Master's thesis by Liu et al. [59] describes optimization of source, collision energies and dwell times through full scan, single ion monitoring, and product ion scans. These data were then used to generate appropriate MRM transitions for HFPO-DA analysis. Most studies utilise some nominal mass variation up to one decimal place of the $[M-H]^-$ 329 m/z precursor for the first quadrupole (Q1) isolation followed by collision induced dissociation (CID) with optimized collision energy in the collision cell. Product ions monitored represent the loss of $-CO_2$ (285 m/z), breakage of the ether linkage resulting in C_3F_7 (169 m/z), and further fragmentation of the C-chain leading to C_2F_5 (119 m/z). Brandsma et al. used 285 m/z as the precursor, which is a prominent in-source fragment, for three MRM transitions during acquisition [8]. As a result, the HFPO-DA LOD/Q is lower or equal to LOD/Qs compared to other PFAS monitored in the study [8] (Table 2). Studies surveyed utilise either only one MRM transition for HFPO-DA in its native form [3, 6, 55–57, 60] and two [14, 54, 58, 59] or three described MRMs [8] for quantification/qualification. In these cases, the additional MRM is used for analyte confirmation, as explicitly stated by Gebbink et al. [54]. Generally, the recently introduced stable isotope labeled (SIL) standard for HFPO-DA ($^{13}C_3$ -HFPO-DA) was also included in the MRM schedule and used in isotope dilution quantification [6, 8, 54, 57, 60], or for recovery assessment in sample matrices [section 2.3]. Specific use of $^{13}C_3$ -HFPO-DA in two studies from the same group was not stated, but the SIL was added prior to extraction and was likely used to correct for losses during extraction. McCord et al. [58] highlight the importance of using a SIL standard, where one wastewater sample was found to have 5x suppressed signal of HFPO-DA and $^{13}C_3$ -HFPO-DA due to unknown contamination during sample preparation. Though the sample was removed from the reported results, the quantification results of HFPO-DA were able to be corrected using $^{13}C_3$ -HFPO-DA [58]. Other PFAS SIL standards have been used for HFPO-DA analyte response correction, in part due to $^{13}C_3$ -HFPO-DA being used to measure extraction recovery, such as in the study of the CFR watershed where Sun et al. [3] used $^{13}C_2$ PFHxA. Heydebreck et al. [14] and Gebbink et al. [54] also utilised $^{13}C_2$ - and $^{13}C_8$ -PFOA SIL standards during the analysis of HFPO-DA in water samples.

The establishment of limits-of-detection and quantification (LOD and LOQ) in several studies rely on signal-to-noise (S/N) values of 3 and 10 respectively, or an extrapolation thereof [8, 14, 54–58, 60]. Comparison of the instrument and method sensitivity values reported for other PFAS can be used to deduce HFPO-DA response in existing methods, described in Table 2. Two studies [3, 56] indicated the use of regression value requirements, one determining a single sensitivity delineation for the study method, described as quantitation limit (QL) which was defined as the lowest concentration in the standard curve with a $\pm 30\%$ deviation from the expected concentration [3]. Method detection and/or quantitation limits (MDL or MQL) were determined in some studies based on

environmental/biological sample detection capabilities, specifically using S/N of the lowest concentration detected in matrix [14, 54]. Where blank contamination from SPE cartridges was observed for HFPO-DA, the MDL was calculated from three times the standard deviation of levels in matrix blanks [55, 56]. It is of note that in studies with SPE cartridge contamination, the analysis of both HFPO-DA and HFPO-TA were performed on different QqQ instruments (different manufacturers), due to exceptionally poor sensitivity on one instrument [55, 56]. The reported limits for HFPO-DA and HFPO-TA are from the system showing the best performance for those compounds. However, three studies [2, 8, 57] report a similar detection limit for HFPO-DA as for other PFCA. Like Pan et al., one of these studies [8] also used a different QqQ architecture specifically for HFPO-DA. Overall, improvements in method sensitivity can be achieved, by using pre-concentration steps present in many sample preparation approaches or LC/MS method modifications [58]. However, the often-higher detection limits for HFPO-DA when using the $[M-H]^-$ as the precursor relative to other PFAS as shown in Table 2 indicates additional MS method considerations specific to HFPO-DA could be improved through further method development.

2.3 Sample preparation and chromatographic techniques

2.3.1 Solid phase extraction—Solid phase extraction (SPE) is used in all studies in both water and biological samples for legacy PFAS analysis. Use of weak anionic exchange chemistries (WAX from Waters Corporation or X-AX from Phenomenex) has been increasingly adopted for PFAS analysis which showed a significant improvement on short-chain carboxylate (PFBA, PFPeA and PFHxA) recovery and reproducibility over Hydrophilic-Lipophilic-Balanced (HLB) [64] and other styrene-divinylbenzyl [6] chemistries. These SPE methods have been expanded to include HFPO-DA in most analyses using SPE-WAX/X-AW [2, 6, 8, 14, 54–58, 60], with two cases using HLB-SPE [3, 59]. The few biological matrices in which HFPO-DA levels have been assessed, carp blood, liver and muscle tissue [55], human serum [55, 57] and human urine [57], and grass/leaves [8] incorporate pretreatment steps prior to extraction [55, 57] such as alkaline digestion of fish and plant tissue [8, 55]. Solid matrices such as grass/leaves and sediment were subjected to liquid extraction with methanol also prior to SPE [8, 60]. Challenges existed in two studies with extraction blank contamination; in both cases the source of the contamination was determined to be the SPE cartridge [55, 56]. This contamination was addressed by performing a blank level subtraction from samples [56]. Automation of SPE is described by Kato et al. for HFPO-DA, and demonstrated acceptable performance for the approach when implemented in urine and serum matrices [57]. Similar on-line SPE performance has been demonstrated in previous studies of PFAS in dust [65], human serum [66, 67], and drinking and surface waters [68], and the study is the first to implement it for HFPO-DA as well as other alternative PFAS ADONA and 9Cl-PF3ONS [57].

2.3.2 Recoveries and matrix effects—Extraction recoveries of HFPO-DA using legacy PFAS methods are summarized, where reported, in Table 1 and were evaluated across the cited studies using $^{13}C_3$ -HFPO-DA internal standard spiked into the tested sample [6, 8, 14, 54–56, 58] and/or the unlabeled HFPO-DA certified reference material (CRM) spiked at different concentrations into a representative sample [55, 56, 59, 60]. Matrix effects were

only explicitly reported in two studies by Pan et al. [55, 56], determined by calculating the ratio between post-extraction spike blank matrix to the same spike level in solvent standard. For water and serum, matrix enhancement was found with mean values of 109.3 and 154.5% respectively [55]. Muscle had a mean matrix effect of 95.3%, which is higher than the spike recovery and indicates slight matrix enhancement, while liver showed slight matrix suppression with 97.3% mean matrix effect [55]. It is notable that some enhancement or suppression can also occur for any analyte during electrospray ionisation [69] and may result from matrix constituents' behaviour during ionisation. McCord et al. [58] described the results of extraction recovery in surface (river) water, drinking water and wastewater and found no significant difference in terms of measurement accuracy across the water types for HFPO-DA [58]. However, a significant difference between recoveries measured from a deionised (DI) lab water supply using non-labeled HFPO-DA CRM compared to in surface water samples measured using a $^{13}\text{C}_3$ -HFPO-DA SIL standard in Gebbink et al. [54], indicating matrix interference. Similarly, Heydebreck et al. [14] also observed a low recovery rate (previously noted, 49.8%) when measuring $^{13}\text{C}_3$ -HFPO-DA in river water [14]. Lastly, Brandsma et al. report higher standard deviations for $^{13}\text{C}_3$ -HFPO-DA recoveries in water and grass/leaf extractions than the other PFAS studied (42% and 33% respectively) [8]. In considering extraction recoveries, use of the SIL standard $^{13}\text{C}_3$ -HFPO-DA in environmental samples is recommended to provide further understanding of the matrix constituents' interference with analysis.

2.3.3 Liquid chromatography conditions—Reversed-phase liquid chromatography has been used for legacy PFAS and HFPO-DA. Methods listed in Table 1 primarily use silica based- C_{18} column chemistries, with the exception of one study which used C_8 chemistry [2] and two HRMS analyses performed alongside targeted QqQ analyses which used a more polar retentive phase (Atlantis T3, Waters Corporation) [54] and fluoro-phenyl phase (Acquity HSS PFP, Waters Corporation) [60]. Adoption of ultra-performance (UPLC or UHPLC) versus high performance liquid chromatography (HPLC) is mixed (Table 1). Where described, under reversed-phase conditions, HFPO-DA elutes at a higher proportion of organic (methanol or acetonitrile) than aqueous mobile phase and between PFHxA and PFHpA [54, 57], the latter of which is less polar than HFPO-DA and thus would be expected to be retained on column longer. No LC system contamination has been reported for HFPO-DA yet.

Adjustment of ESI solutions with an MS compatible ionic additive (i.e. an electrolyte such as ammonium acetate) is employed for stabilization of spray dynamics [70] as well as improvement on ionisation of analytes of interest [69, 71–73]. In the case of anionic PFAS, ammonium acetate and occasionally ammonium formate are used as mobile phase additives in most methods [74], particularly for those extended to include HFPO-DA (Table 1). Concentrations range in these methods from 2–20mM ammonium acetate, with ammonium formate also used in three reported methods at concentrations of 0.4–5mM. Few exceptions using different mobile phase additives exist in the wider analyses of PFAS. Experiments with perfluoroalkyl phosphates (PAPs) found that using 1-methyl piperidine as a mobile phase additive increases both chromatographic resolution and analyte response through acting as an ion pairing agent [75]. Trier et al. [52] found raising the pH of the mobile phase

to 9.7 using ammonium and ammonium hydroxide (NH_4OH) resulted in ionisation of non-ionic fluorotelomer alcohols (FTOHs) and fluoroalkoxylates, in conjunction with anionic PFAS ionisation [52]. In this case, raising pH was thought to aid in ionisation of the non-ionic compounds as their pK_a 's are closer to pH 9.7 [52], indicative of why mobile phase assessments and adjustments are useful for the growing array of PFAS and could be considered for HFPO-DA.

3. Analytical challenges

The reported higher detection limits relative to legacy PFAS and widespread commentary regarding the poor performance on some MS instrumental systems [55, 56] raises questions about how the ionisation behaviour of HFPO-DA contributes to instrument functionality and what might mitigate those challenges.

3.1: ESI^- of HFPO-DA using legacy PFAS methods

3.1.1 Dimer formation—Commonly published methods include specifications for the ESI^- full scan spectrum of HFPO-DA [2]; minimal $[\text{M}-\text{H}]^-$ was observed, while homodimer formation via a stable proton- and sodium-bond resulted in the high relative abundance of $[2\text{M}-\text{H}]^-$ and $[2\text{M}-2\text{H}+\text{Na}]^-$ ions [2] which detracts from the desired formation of $[\text{M}-\text{H}]^-$. Figure 2 shows a typical spectrum observed for full scan acquisition for HFPO-DA. Similar homodimer formation in legacy PFAS was only reported by Trier et al. for PFOA and not discussed elsewhere in other ESI^- studies [52]. However, dimer and dimer adduct formation via solvent adducts introduced with mobile phase additives have been well described [52, 53]. Nine additional PFECAs identified in the CFR water sample (and for which analytical standards do not exist currently), also showed significant homodimer formation for both H^+ and Na^+ bound species [2, 53], as well as for mono- and polyether PFECAs in Song et al. [60]. Interestingly, dimer formation was not observed for two proposed PFESAs found in the CFR water sample [2]. Dimer formation is not without precedent in ESI^- , as previous assessment of acidic anti-inflammatory pharmaceuticals which contain a carboxylic acid moiety also showed various degrees of $[2\text{M}-\text{H}]^-$ and $[2\text{M}-2\text{H}+\text{Na}]^-$ formation, albeit on other moieties such as the amine functionality of carprofen [76].

3.1.2 Fragmentation—As illustrated in Figure 3A and B and discussed elsewhere [2], fragmentation of HFPO-DA is initiated by the loss of the $-\text{CO}_2$ functional group, likely preceded by ionisation via deprotonation to generate the $[\text{M}-\text{H}]^-$ ion. Cleavage at the ether linkage results in loss of both $-\text{C}_2\text{F}_4/\text{C}_3\text{F}_4\text{O}_2$ and $-\text{C}_3\text{F}_7\text{O}/\text{C}_3\text{F}_4\text{O}_3$ as well as fragments of 184.9847 m/z and 168.9894 m/z, respectively. Further C-chain breakage results in the loss of $-\text{CF}_2$ and the formation of a fragment of 118.9920 m/z. In-source CID is an observed characteristic of HFPO-DA [2, 8], in which the described fragments are produced upon ionisation and transfer to the detector of the MS without intentional molecule fragmentation induced through elevated collision energies. This characteristic fragmentation of HFPO-DA has been documented with a certified reference material in the low collision energy state of HRMS acquisition [62], as well as in previously described sample analyses. The confirmation of fragment masses via elevated collision energy HRMS accurate mass

measurement is shown in Figure 3B. Common fragment ions with other characterized PFCAs include those previously mentioned, as well as C_2F_5 (118.9920 m/z), which can be seen in Figure 3B [8, 52, 53, 60]. Similarly, PFCAs such as PFOA and PFDoA have also demonstrated in-source CID [51, 52]. In one HRMS study the $-CO_2$ fragment for all 10 surveyed PFCAs was reported to be the base peak in low collision energy full scan spectra [77]. In that study, it was found that the lower molecular weight PFCAs demonstrated the greatest loss of $-CO_2$ [77].

4. Global distribution

Quantitative studies have focused on surface water analyses from rivers [3, 6, 14, 54–56, 60], coastal and estuarine waters [14], wastewater [3], drinking water treatment plants [3, 6, 58] and drinking water [8, 54]. One study also measured HFPO-DA in sediment samples taken from the Xiaoqing, finding significantly lower concentrations at a maximum of 70 ng/g wet wt. [60]. Of the ten published water studies, only one did not detect HFPO-DA in any samples [64]. The highest levels of observed HFPO-DA in the other nine studies were linked to the proximity of fluorochemical manufacturing facilities or point sources directly [3, 8, 6, 14, 54–56, 58, 60]. These levels ranged across the studies from 631 – 9,350 ng/L HFPO-DA from the Xiaoqing River in China [14, 55, 60], lower Rhine River in the Netherlands [14, 54] and the CFR watershed in the USA [3, 6, 58]. Numerous samples in all studies outside of the HFPO-DA discharge ranges were either below detection limits or low ng/L concentrations. Data from global water studies are summarized in Figure 4 and discussed in the following sections, as well as biological sample observations which are currently limited to two reported studies where levels of HFPO-DA could be detected [8, 55]. Sediment samples have also been analyzed in one study of the Xiaoqing River, finding significantly lower concentrations than water samples at a maximum of 70 and 22.3 ng/g wet wt. in 2014 and 2016 [60].

4.1 Reported concentrations and trends in water

The country with the highest reported levels of HFPO-DA detection was China. Mean levels for the most elevated sites in the Xiaoqing River ranged from 118 – 9,350 ng/L during sampling campaigns in 2014 [14, 60], 2015 [55] and 2016 [60], and indicated a large increase in levels of HFPO-DA at the same particular sampling area [14, 55]. This finding was attributed to the presence of a fluorochemical production plant located upstream from the sampling sites, supported by a third study conducted in 2014 and 2016 [55, 60]. HFPO-DA levels in other surface waters in China were found to be significantly lower than the measurements in the Xiaoqing River samples near a fluorochemical plant, and ranged from 0.73–14 ng/L [14, 55]. In each of these studies, the correlation between HFPO-TA levels with HFPO-DA was found to be greater than with other PFAS, suggesting the two PFECAs originated from the same source [55]. The levels of HFPO-TA were found to be higher than HFPO-DA in Xiaoqing River sites downstream of the production plant as well as in the surface water samples measured in 2016 [55, 60]. PFOA concentrations relative to HFPO-DA remain higher across all samples from China, and the highest levels of PFOA globally were reported in China [14, 55, 56, 60]. This would indicate that despite HFPO-DA being used a replacement in other geographies, PFOA production continues in China and has been

attributed to possible manufacturing outsourcing from countries which have phased out PFOA [14].

In the CFR watershed, a large increase in HFPO-DA concentrations was observed in drinking water samples from 2013 ranging from below the QL upstream from a known fluorochemical manufacturing site to 4,560 ng/L downstream of the manufacturing facility, with an average concentration of 631 ng/L at the downstream site [3, 6]. In the downstream sample, only PFBA and PFPeA were also found, while PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS were not observed [3]. HFPO-DA levels at this site were approximately eight times greater than total other PFAS levels in the same study [3, 54]. Two later studies by McCord et al. and Hopkins et al. also are from the CFR watershed, conducted in 2017. Samples taken initially in the study found high HFPO-DA levels still present at approximately 500–700 ng/L range from two drinking water treatment plant locations [58]. These results were above the 140 ng/L North Carolina targeted health goal for HFPO-DA, and prompted a process change by the manufacturer to eliminate the discharge [6, 58]. Further sampling showed HFPO-DA levels after approximately 1 month to fall below the targeted health goal [58]. The same decrease in HFPO-DA, as well as other PFEAs, was observed by Hopkins et al. [6].

Dutch sample sites also contained high levels of HFPO-DA, however proximity to possible point sources was a strong determinant of observed concentrations. Two sampling sites along the lower Rhine and Scheur branch stream in 2013 had elevated concentrations (86 and 73 ng/L, respectively) relative to upstream sites and were linked to possible episodic rather than continuous discharge from known industrial sites [6, 14]. The authors note this is approximately 42 times lower than the aforementioned Xioaqing River samples taken in 2014 [14]. Samples taken in 2016 along the Rhine, albeit from different latitude/longitudinal coordinates than those from 2014 showed significantly higher levels, with a maximum mean concentration of 814 ng/L [54]. The higher level findings from 2016 were linked directly with proximity downstream from a fluorochemical manufacturing plant [54]. In both studies, HFPO-DA was significantly higher proportionally in the elevated samples than legacy PFAS [14, 54].

At sampling sites for which no known large scale fluorochemical manufacturing outfall was present, HFPO-DA levels were significantly lower or not detected. In China, samples taken from the Xioaqing River upstream the fluorochemical plant discharge effluent [14, 55], as well as Laizhou Bay [14], Tai Lake and the Chao, Huai, Pearl, Liao, Yangtze, Yellow and Pearl Rivers [56] were either not detected or contained 0.73 – 44 ng/L HFPO-DA.

Approximately half of the Dutch and German river surface samples spanning from 2013–2016 did not have detectable HFPO-DA [14, 54], and in the case of drinking water samples, three out of twelve were included in this category [54]. The remaining nine drinking water samples had levels ranging from 0.25–11 ng/L [54, 8]. In the USA, surface water samples from the Delaware River had significantly lower concentrations of HFPO-DA than the CFR, and all detected PFAS were below 10 ng/L each [56]. South Korea, Sweden and the UK all showed higher levels of PFOA than HFPO-DA, the later ranging from 1.14 to 1.47 ng/L mean levels, and lower levels of HFPO-TA compared to HFPO-DA [56].

4.2 Biological monitoring

The types of biological matrices tested for HFPO-DA are currently limited in number but include human urine [57] and serum [55, 57] as well as carp muscle, liver tissue and blood from samples taken from/near the Xiaqing river [55], and grass/leaf sampling from near a Dutch fluorochemical plant [8]. Kato et al. [57] focused on paired serum-urine samples purchased from a supplier of biological fluids and found no HFPO-DA in any of the samples, while PFOA was detected in 98% of the serum samples, and only PFBA was detected in any of the urine samples at a frequency of 56% [57]. Carp samples (n=15) were taken from one of the sampling sites along the Xiaqing River and the highest levels of total PFAS concentration were found in the blood, followed by liver and muscle. HFPO-DA was present in all blood samples and 94% of liver and muscle tissues with median levels of 2.09 ng/mL, 1.34 and 1.53 ng/g wet weight respectively [55]. PFOA was found in all samples [55]. Up to 37% of total PFAS concentration in blood, 47% in liver and 51% in muscle consisted of HFPO-DA [55]. Interestingly, this was in inverse proportion to PFOA levels observed across the sample types. Human sera from people who resided near the Xiaqing River were also analyzed for PFAS. In these samples, lower rates of detection were found for HFPO-DA (38%) compared to HFPO-TA (98%) and PFOA (100%) [56]. Grass and leaf samples from the Netherlands all contained detectable HFPO-DA, ranging from 86 ng/g wet weight to <LOQ for grass and 27 ng/g wet weight to <LOQ for leaves. The levels were higher than those of PFOA detected, and both HFPO-DA and PFOA were found to decrease with distance from the fluorochemical plant [8].

5. Conclusions and future perspectives

Increased use of HFPO-DA following the phase-out of PFOA in many countries has resulted in its discharge and detection in various water systems worldwide. Though limited, existing studies clearly describe the incidence of HFPO-DA detection and levels as directly linked with point sources such as fluorochemical manufacturing sites. Efforts to reduce these levels in North Carolina to meet the state drinking water health goal of 140 ng/L in drinking water show relatively rapid success following cessation of discharge [6, 58]. However, monitoring in both environmental and biological compartments is almost certain to continue, in the ongoing studies of total PFAS occurrence and toxicity. Analytical methods used are generally extensions of existing methods for anionic PFAS with the use of HRMS for non-targeted experiments leading to the characterization of HFPO-DA in water, and quantification using QqQ. Highlighted challenges exist with the generation of $[M-H]^-$ ion for HFPO-DA, and yet all discussed QqQ methods in this review rely on MRMs based on initial $[M-H]^-$ formation. Method development targeting more stable $[M-H]^-$ production would potentially aid future studies of HFPO-DA.

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Abbreviations:

ADONA	dodecafluoro-3H-4,8-dioxanonanoate
CFR	Cape Fear River
CRM	certified reference material
ESI⁻	electrospray ionization, negative polarity
FTOH	fluorotelomer alcohols
HFPO-DA	hexafluoropropylene oxide-dimer acid
HFPO-TA	hexafluoropropylene oxide-trimer acid
HLB	hydrophilic-lipophilic-balanced
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MDL	method detection limit
MP	mobile phase
ML	method quantification limit
MRM	multiple reaction monitoring
NOAEL	no-observable-adverse-effect-level
PAPs	polyfluorinated phosphate esters
PFAS	per- and polyfluorinated alkyl substances
PFBA	perfluorobutanoic acid
PFCAs	per- and polyfluorinated carboxylic acids
PFECAs	per- and polyfluorinated ether carboxylic acids
PFESAs	per- and polyfluorinated ether sulfonic acids
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid

PFOA	perfluorooctanoic acid
PFPeA	perfluoropentanoic acid
QL	quantitation limit
QqQ	triple quadrupole mass spectrometer
QTof-MS	quadrupole time-of-flight mass spectrometry
RPLC	reversed-phase liquid chromatography
SIL	stable isotope labeled
SPE	solid-phase extraction
UHPLC (UPLC)	ultra high performance liquid chromatography
WAX	weak anionic exchange

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Highlights:

- HFPO-DA is an alternative PFAS of increasing interest for monitoring studies
- Global distribution varies from low ng/L to µg/L concentrations and are linked to known sources
- LC-MS methods used in 10 currently published studies are discussed

Compound (CAS number)	Abbreviation	CAS number	Formula	pKa	Solubility mg/L
2,2,3,3-Tetrafluoro-3-(heptafluoropropoxy)propanoic acid	HFPO-DA	13252-13-6	C ₆ HF ₁₁ O ₃	2.84*	5.143**
Perfluorooctanoic acid	PFOA	335-67-1	C ₈ HF ₁₅ O ₂	3.8***	0.02595**

*[10]; **[11],[12]; ***[13]

Figure 1:
Structure and physico-chemical characteristics of HFPO-DA (GenX) vs. PFOA

Figure 2:

Spectrum of HFPO-DA derived from full scan acquisition on a Xevo TQ-D (Waters Corporation) following injection using 2mM ammonium acetate (pH 5.0) in approx. 50/50 water/methanol and separation on an Acquity BEH C_{18} UPLC column. Under these typical method conditions, minimal $[MH]^-$ formation is apparent.

Figure 3:
Fragmentation pathway of HFPO-DA (A), with confirmation of masses in elevated energy QToF spectrum derived from solvent standard injection (B).

Figure 4: Mean levels (ng/L) of HFPO-DA, HFPO-TA and PFOA where reported from 2013–2018 global campaigns. Data is provided from references' [3, 8, 14, 54–56, 60]], and log-scale applied to y-axis due to large range in reported concentrations across studies.

Table 1:

Summary of SPE-LC-MS method conditions for the analysis of HFPO-DA.

Matrix	Country	Sample Preparation Protocol	Recovery	RPLC Column	Mobile phase	MS used	Mass(es) Monitored	HFPO-DA Sensitivity	Ref.
Surface Water	USA	500 mL water passed thru Oasis WAX SPE cartridge, eluted with 0.3% NH ₄ OH methanol; concentrated to 1 mL under N ₂	NA	Eclipse Plus C8 (Agilent Technologies) 2.1 × 50 mm 3.5µm	MP A: 0.4mM ammonium formate in 95:5 water:methanol MP B: 0.4mM ammonium formate in 95:5 methanol:water	QTof	Full Scan Acquisition	NA	[2]
Surface Water	USA	500 mL water spiked with SIL passed thru WAX SPE cartridge, eluted with 4mL methanol: NH ₄ OH; concentrated to 1 mL and 100 µL mixed with 300 µL of 2mM ammonium acetate	NA	NA	MP A: 2.5mM ammonium acetate in 95:5 water:methanol MP B: 2.5mM ammonium acetate in 95:5 methanol:water	QqQ	329>284.9	NA	[6]
Surface Water	Netherlands, Germany and China	1 L of water spiked with SIL standard loaded onto Oasis WAX SPE cartridge and eluted with 10 mL of 0.25% NH ₄ OH in methanol; concentrated to 150 µL and 10 µL ¹³ C ₈ -PFOA and ¹³ C ₂ -PFOA added as injection standard	49.8%+/- 8.4 (Mean +/- SD [%])	Synergi Fusion-RP-C18 (Phenomenex) 150 × 2mm 4µm	MP A: 10mM ammonium acetate in water MP B: 10mM ammonium acetate in methanol	QqQ	329>285; 329>169	LOD: 0.4 pg; LOQ: 1.2 pg; MDL: 0.1425 ng/L (average of sampling campaign s); MQL: 0.47ng/L (average of sampling campaigns)	[14]
Drinking Water Source	USA	500 mL of water spiked with SIL standard loaded onto Oasis HLB Plus cartridges, eluted with 2mL methanol; concentrated to 500µL and 200µL of concentrated eluate had 50µL 2mM ammonium acetate added	NA	NA	MP A: 2mM ammonium acetate MP B: methanol	QqQ	NA	QL: 0.2 ng/L	[3]
DWTP Water Samples and Adsorption Experiments	USA	Samples/standards spiked with SIL standards and filtered through 0.45µm glass microfiber syringe filters	NA	Kinetex C18 (Phenomenex) 50 × 4.6 mm 5µm	MP A: 2mM ammonium acetate in 95:5 water:methanol MP B: 2mM ammonium acetate in 95:5 acetonitrile:water	QqQ	329.0>284.7	QL: 10 ng/L	[3]
River and Drinking Water	Netherlands	250 mL water spiked with SIL standards loaded onto a Oasis WAX SPE cartridge, eluted with 3mL 2% NH ₄ OH in acetonitrile; concentrated to dryness, followed by reconstitution in 300 µL acetonitrile and 675 µL 2mM ammonium acetate in water. Lastly, 25µL of ¹³ C ₈ -PFOS and ¹³ C ₈ -PFOA added to 475 µL	MilliQ Water: 5 ng/L=84%; 10 ng/L=81%; 25 ng/L=95% River and Drinking Water: 66+/- 15% (Mean;+/-SD [%])	Acquity UPLC BEH C18 (Waters Corp.) 50 × 2.1 mm 1.7µm	MP A: 2mM ammonium acetate in water MP B: acetonitrile	QqQ	329>169; 329>285	MDL: 0.2 ng/L	[54]

Matrix	Country	Sample Preparation Protocol	Recovery	RPLC Column	Mobile phase	MS used	Mass(es) Monitored	HFPO-DA Sensitivity	Ref.
River and Drinking Water	Netherlands	Same as above	NA	Atlantis T3 (Waters Corp.) 3 × 100 mm 3µm	MP A: 2mM ammonium formate + 0.002% formic acid in water MP B: 2mM ammonium formate + 0.002% formic acid in 95:5 methanol:water	Orbitrap	Full Scan Acquisition: 100–1250m/z	NA	[54]
River Water	China	40 mL water spiked with labeled internal standards loaded on X-AW SPE cartridge, eluted with 4 mL 0.5% ammonium hydroxide in methanol; concentrated to dryness, followed by reconstitution in 200 µL methanol	Spike recovery (2 ng): 102.4+/-6.4 Matrix effect 109.3+/-0.9	Acquity BEH C18 (Waters Corp.) 100 × 2.1 mm 1.7µm	MP A: 2mM ammonium acetate in water MP B: methanol	QqQ	329>169	LOQ: 0.05 ng/L MDL: 0.23 ng/L	[55]
Common Carp and Human Serum	China	0.2 g of sample spike with labeled internal standard and 1 mL of 0.5M tetra-n-butylammonium hydrogen sulfate solution, 2mL of NaHCO ₃ /Na ₂ CO ₃ at pH 10, and 4 mL of MTBE (In the case of fish muscle, 0.2 g were spiked with labeled internal standard and sonicated for 1 hr in 10 mL of methanol/10 mM KOH and shaken overnight); Mixture mixed and centrifuged, followed by removal of supernatant. Remaining pellet extracted 2x more, supernatant combined concentrated to dryness, followed by reconstitution in 200 µL methanol; fish sample extracts were then diluted to 10mL in water to be clean-up further with above SPE procedure	Spike recovery (2 ng): Serum 102.7+/-8.3; Muscle 92.5+/-7.3; Liver 101.6+/-8.1 Matrix effect: Serum 154.4+/-0.9; 95.3+/-5.8; Liver 97.3+/-1.8	Acquity BEH C18 (Waters Corp.) 100 × 2.1 mm 1.7µm	MP A: 2mM ammonium acetate in water MP B: methanol	QqQ	329>169	LOQ: Serum=0.05 (ng/mL); Muscle=0.05 (ng/g); Liver 0.05 (ng/g) MDL: Serum=0.14 (ng/mL); Muscle=0.26 (ng/g); Liver 0.43 (ng/g)	[55]
Surface Water	China, USA, UK, South Korea, Germany, Netherlands and Sweden	(Previously described in [22]); 200 mL of water spiked with labeled internal standard loaded on a X-AW, and eluted with 4mL 0.5% NH ₄ OH in methanol; concentrated to dryness, followed by reconstitution in 200µL methanol	Spike recovery (+/- SD [%]): 0.1 ppb=89.6+/-5.0%; 1 ppb=102.4+/-6.4%; 10 ppb=102.9+/-3.4 Matrix effect: 109.3+/-0.9	Acquity BEH C18 (Waters Corp.) 100 × 2.1 mm 1.7µm	MP A: 2mM ammonium acetate in water MP B: methanol	QqQ	329>169	LOQ: 0.05 ng/L MDL: 0.38 ng/L	[56]
Surface, Drinking and Wastewater	USA *	500 mL samples spiked with labeled internal standard loaded onto Oasis WAX SPE cartridge, and eluted with 4 mL of 0.1%	NA	Acquity BEH C18 (Waters Corp.) 50 × 2.1 mm 1.7µm	MP A: 95:5 2mM ammonium acetate in water: methanol MP B: 95:5 methanol:	QqQ	329.2>168.9; 329.2>286.9	LOD: 16 ng/L	[58]

Matrix	Country	Sample Preparation Protocol	Recovery	RPLC Column	Mobile phase	MS used	Mass(es) Monitored	HFPO-DA Sensitivity	Ref.
		NH ₄ OH in methanol; concentrated to 1 mL and extracts were then diluted 4x in 2.5 mM ammonium acetate			2mM ammonium acetate in water				
Human Urine and Serum	USA	On-line SPE-HPLC-MS/MS system using HySphere C8-SE and Oasis WAX SPE devices for serum and urine, respectively. For serum, 500 µL 1:10 diluted sample loaded onto SPE cartridge with 2mL 0.1 M formic acid and washed with 2mL 90% 0.1 M formic acid/10% acetonitrile. For urine, 500 µL 1:10 diluted sample loaded onto SPE cartridge, and eluted onto HPLC-MS/MS system using 0.3% NH ₄ OH/99.7% MeOH at 100µL/min.	NA	HighResolution RP18e (5 × 4.6 mm) guard column and HighResolution RP-18e (25 × 4.6mm) followed by two HighResolution RP18e (100 × 4.6 mm) in-tandem	MP A: 95:5 20mM ammonium acetate (pH 4) in water: acetonitrile MPB: acetonitrile	QqQ	329>285	LOD: 0.1 ng/mL	[57]
Surface Water	USA	4L of samples filtered using Whatman 1.6 µm glass microfiber filters and loaded onto an Oasis PRiME HLB SPE cartridge, and eluted with 10mL methanol. Samples were then spiked with labeled internal standard	4 replicates at 2ng/mL resulted in mean % recovery of 89.78 and 4 replicates at 10ng/mL resulted in mean % recovery of 107.19	RRHD Eclipse Plus C18 (Agilent) 100 × 2.1 mm 1.8µm	MP A: 5mM ammonium formate in water MP B: methanol	QqQ	328.9>284.9; 328.9>169	LOQ: 0.1pg	[59]
Surface Water	China	Filtered x mL of surface water loaded onto an Oasis WAX SPE cartridge, and eluted with 4mL methanol and 4mL 0.1% NH ₄ OH in methanol; combined eluates were evaporated to 1mL prior to injection	89 +/- 15% (Mean +/- SD [%])	Acclaim 120 C18 (Thermo Fisher Scientific) 150 × 4.6 mm 5µm	NA	QqQ	329.0>285.0	LOD: 0.24 ng/L	[60]
Sediment	China	Sediment was lyophilized and analytes extracted using methanol, and then subjected to SPE protocol above.	83 +/- 9.4% (Mean +/- SD [%])	Acclaim 120 C18 (Thermo Fisher Scientific) 150 × 4.6 mm 5µm	NA	QqQ	329.0>285.0	LOD: 0.13 ng/g dry wt.	[60]
Surface Water	China	Same as above	NA	Acquity HSS PFP (Waters) 50 × 2.1 1.7µm and Acclaim 120 C18 (Thermo Fisher Scientific) 150 × 4.6 mm 5µm	NA	Orbitrap	Full Scan Acquisition: 60–1000m/z	NA	[60]
Sediment	China	Same as above	NA	Acquity HSS PFP (Waters) 50 × 2.1 1.7µm and Acclaim 120 C18 (Thermo Fisher	NA	Orbitrap	Full Scan Acquisition: 60–1000m/z	NA	[60]

Matrix	Country	Sample Preparation Protocol	Recovery	RPLC Column	Mobile phase	MS used	Mass(es) Monitored	HFPO-DA Sensitivity	Ref.
				Scientific) 150 × 4.6 mm 5µm					
Grass/ Leaves	Netherlands	1g grass/leaves spiked with 50mL ¹³ C ₄ -PFOA and ¹³ C ₃ -HFPO-DA and digested with 2mL 200mM NH ₄ OH; 10mL methanol added for extraction and vortexed. Extraction with additional 8mL methanol repeated on supernatant; final volume diluted with 20mL water and 150mL hydrochloric acid. Clean-up of extract using Oasis WAX SPE cartridge, washed with 25mM sodium acetate buffer and tetrahydrofuran:methanol (75:25 v/v), and eluted with 4 mL 0.1% NH ₄ OH in methanol; evaporated to dryness and reconstituted in 100 µL water and 100µL ¹³ C ₈ -PFOA (100ng/mL) added.	121 +/- - 33% (Mean +/- SD [%])	NA	MP A: 5mM ammonium formate in water MP B: methanol	QqQ	285>169; 285>185; 285>119	LOD: <0.1 ng/g wet wt. and LOQ: <0.2 ng/g wet wt.	[8]
Drinking Water	Netherlands	100 mL water extracted following same SPE procedure as above.	135 +/- - 42% (Mean +/- SD [%])	NA	MP A: 5mM ammonium formate in water MP B: methanol	QqQ	285>169; 285>185; 285>119	LOD: <0.1 ng/L and LOQ: <0.3 ng/L	[8]

Table 2:

Detection limits of HFPO-DA and other PFAS across selected studies [8, 14, 54–56, 60], where HFPO-DA values fall in the upper-mean range of both legacy and emerging PFAS.

Compound	Detection Limit Reported [reference]										
	LOD (pg) [14]	MQL (ng/L) [54]	LOQ (ng/L) Water [55]	LOQ (ng/mL) Serum [55]	LOQ (ng/g w.w.) Muscle [55]	LOQ (ng/g w.w.) Liver [55]	LOQ (ng/L) Water [56]	LOD (ng/g w.w.) Grass/Leaves [8]	LOD (ng/L) Water [8]	LOQ (ng/L) Water [60]	LOQ (ng/g dry wt.) Sediment [60]
HFPO-TA	NA	NA	0.05	0.10	0.10	0.10	0.10	NA	NA	0.87	1.41
PFBA	0.70	2.00	0.05 *	0.10	0.10 *	0.10 *	0.05	<0.3	<4	1.25	1.00
PFPeA	0.70	4.00	0.05	0.05	0.05	0.05	0.05	<0.1	<4	2.08	0.70
HFPO-DA	0.40	0.20	0.05 *	0.05 *	0.05 *	0.05 *	0.05 *	<0.1	<0.1	0.24	0.13
PFOA	0.50	0.30	0.02	0.02	0.02	0.02	0.02	<0.2	<0.5	0.72	0.46
PFHxA	0.30	0.10	0.02	0.05	0.10	0.05	0.02	<0.1	<1	1.4	0.54
PFHpA	0.30	0.05	0.02	0.02	0.02	0.02	0.02	<0.1	<1	0.5	0.42
PFOS	0.10	0.03	0.02	0.01	0.01	0.01	0.02	<0.1	<1	0.16	0.10
6:2 Cl-PFESA	NA	0.02	0.01	0.01	0.01	0.01	0.01	NA	NA	0.18	0.10
ADONA	NA	0.01	NA	NA	NA	NA	0.01	NA	NA	0.1	0.08

* present in blank