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HIGHLIGHTS

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- 89–1175 per- and polyfluoroalkyl substances identified at Confidence Level 4.
- 33 per- and polyfluoroalkyl substances at Confidence Level 3, 10 at Level 2.
- Perfluorooctanoic acid (12,100 ng L⁻¹) exceeds Environmental Quality Standard.
- Only 8 per- and polyfluoroalkyl substances (17% total) regularly monitored.
- Discovery of new sub-class of unsaturated perfluoroalkyl ether carboxylic acids.



ABSTRACT

There are now over 7 million recognised per- and polyfluoroalkyl substances (PFAS), however the majority of routine monitoring programmes and policy decisions are based on just a handful of these. There is need for a shift towards gaining a better understanding of the total PFAS present in a sample rather than relying on targeted analysis alone. Total PFAS methods help us to understand if targeted methods are missing a mass of PFAS, but they do not identify which PFAS are missing. Non-targeted methods fill this knowledge gap by using high resolution mass spectrometry to identify the PFAS present in a sample. In this manuscript we use complimentary targeted analysis (NTA) to detect hundreds of PFAS in five freshwater samples obtained from the Northwest of the UK. Targeted analysis revealed PFOA at a maximum concentration of 12,100 ng L^{-1} , over

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three orders of magnitude greater than the proposed environmental quality standard (EQS) of 100 ng L⁻¹. A conservative assessment calculated an average total PFAS concentration of approximately 40 μ g L⁻¹ across all samples. A suspect screening approach identified between 1175 (least conservative) to 89 (most conservative) PFAS at confidence level 4. Exploratory data analysis was used to identify 33 PFAS at confidence level 3 and 10 PFAS at a confidence level of 2. Only 8 of these 43 PFAS (representing 17% of the total PFAS peak area) are regularly monitored in the UK as part of the UK DWI 47 PFAS. Our results suggested the presence of a novel group of unsaturated perfluoroalkyl ether carboxylic acids (U-PFECAs) related to EEA-NH₄, a perfluoroalkyl ether carboxylic acid (PFECA), providing an example of the benefits of non-targeted screening. This study highlights the merits of non-targeted methods and demonstrates that future monitoring programmes and regulations would benefit from incorporating a non-targeted element.

1. Introduction

There are now over 7 million recognised per- and polyfluoroalkyl substances (PFAS) using the Organisation for Economic Co-operation and Development (OECD) revised definition (OECD, 2021; Schymanski et al., 2023). This includes any chemical containing at least one saturated CF_2 or CF_3 moiety. Some of these long-chain PFAS (e.g. PFOA and PFOS) have largely been phased out in the developed world, but are still regularly detected in environmental samples (Hensema et al., 2021; Houde et al., 2011). Despite the large number of potential PFAS in the environment most environmental investigations that target PFAS only determine a relatively short list of compounds and are blind to the hundreds of other PFAS that have been detected by non-targeted methods (Liu et al., 2019).

A targeted PFAS approach has generally been favoured by policy makers and regulators, but this is a rapidly emerging field and different regulators are continually having to update their regulations to screen for more PFAS. For instance, the United Kingdom's Environment Agency (UK EA) regularly monitored 16 PFAS in UK watercourses (EA, 2021), until they updated their list following the UK Drinking Water Inspectorate (DWI) recommending a list of 47 PFAS for drinking water (DWI, 2021a; EA, 2023a). The EU Water Directive and the U.S. Environmental Protection Agency (EPA) regularly monitored 20 PFAS (EPA, 2023; EU, 2020; Sims et al., 2022), the U.S. EPA then updated this to 29 PF AS, before increasing it to include 40 PFAS to coincide with release of USEPA method 1633 (EPA, 2024a). Overall, it is expected that the list of PFAS components in (inter)national legislation will continue to be extended with improving understanding of the environmental behaviour and human health risks (EA, 2021).

Some studies are starting to perform a fluorine mass balance calculation in combination with targeted assessments (Spaan et al., 2020). Different techniques for total fluorine measurement, such as Combustion Ion Chromatography (CIC), Fluorine nuclear magnetic resonance spectroscopy (F-19) NMR, total oxidizable precursor (TOP) Assay or Particle-Induced Gamma Ray Emission (PIGE) spectroscopy (Al Amin et al., 2020), each have their own advantages (Al Amin et al., 2020; Al Amin et al., 2020; McDonough et al., 2019). The use of a CIC approach to more comprehensively assess the total PFAS mass, using the Adsorbable Organic Fluorine (AOF) method has been included in a recently released USEPA method 1621 (EPA, 2024b). Information on perfluoroalkyl chain length of polyfluoroalkyl PFAS (usually C4-C12) can be gathered using the total oxidizable precursor (TOP) assay (Houtz and Sedlak, 2012), by measuring detectable perfluoroalkyl acids (PFAAs) (C4-C12) after an oxidative digest (Houtz et al., 2018; Houtz and Sedlak, 2012). A large number publications have now highlighted the presence of PFAS, beyond those assessed using targeted (Androulakakis et al., 2022; Koch et al., 2020; Shen et al., 2023) analysis. These investigations that go beyond using targeted methods have identified that there is the potential to miss a significant number of individual PFAS that contribute to the total concentration present. These studies regularly report that a significant mass of the total fluorine content continues to go unreported by targeted methods, with a 276% increase in PFAS mass reported in some samples of surface water, using TOP assay (Ross et al., 2019).

Targeted analytical methods clearly have their place as it would be

cost- and time-prohibitive to continuously perform non-targeted screening by high resolution mass spectrometry on every environmental sample. Additionally, targeted analyses provide rapid, accurate data which can directly be compared against environmental standards to aid with environmental and human health risk assessments. However, it is important to perform complementary non-targeted screening to understand what else might be in environmental matrices and ensure that the PFAS included in regulations and on targeted monitoring programmes are appropriate and future-proofed. Total PFAS methods are useful to identify if PFAS are being missed by targeted methods, but nontargeted analysis has the advantage of being able to identify what some of those unknown PFAS are. Non-targeted screening methods employing high resolution mass spectrometry have been established in an attempt to identify the large range of PFAS that are present in our environment (Bugsel et al., 2023; Manz et al., 2023). There is no standardised method for non-targeted analysis which makes comparison of different studies challenging. Attempts at harmonising the language used in PFAS non-targeted assessments have helped but there is still ambiguity in reporting (Charbonnet et al., 2022). Some studies have reported the presence of hundreds of PFAS, determined at low confidence levels (Liu et al., 2019), whereas other studies focus on determining PFAS at higher degrees of confidence.

Environmental legislation (e.g. discharge consents) and routine environmental monitoring programmes are generally limited to a handful of specific PFAS. The aim of this current study was to understand how appropriate this is by gaining a better understanding on the number of PFAS that may be present in the environment. To achieve this, we analysed surface water samples collected in the vicinity of potential point source of PFAS (a fluorochemical plant) located in the Northwest of the United Kingdom. Samples were analysed by ultra highperformance liquid chromatography with high resolution mass spectrometry (uHPLC-ESI-HRqToFMS). They were processed using a suspect screening approach using the NIST PFAS database and MassHunter, as well as exploratory analysis using FluoroMatch.

2. Methodology

2.1. Sample collection

Five surface water samples were collected from lagoons and small streams impacted by industrial discharge pipes from a fluorochemical plant located in Thornton-Cleveleys in the Northwest of the United Kingdom. Samples were obtained in May 2021, all samples were collected in 500 mL plastic containers (HDPE), placed in a dark cool box at \leq 6 °C and delivered to a commercial laboratory within 24 h in line with guidance from EPA (2021).

2.2. Sample extraction

Samples were extracted by ALS laboratories (Hawarden, UK) using accredited method TM337 (ALS, 2022a). In brief, samples were spiked with isotopically-labelled standards then extracted by a solid-phase extraction (SPE) containing polystyrenedivinylbenzene (SDVB). This was performed by conditioning of the 6 mL SPE cartridge with 4 mL

ammonia/methanol solution (0.1%), 4 mL methanol (HPLC grade) and 4 mL de-ionised/ultrapure water. The sample was then loaded, washed with 4 mL acetate buffer (0.025 mol L⁻¹; pH 4) and centrifuged at 1500 rpm for 2 min to remove residual wash solution. It was then eluted with 4 mL methanol followed by 4 mL 0.1% ammonia/methanol solution. Eluates were evaporated under a stream of nitrogen gas (purity >99.996%) to 500 µL for analysis (ISO 25101: 2009).

2.3. Sample analysis

TM377 is certified for the extraction of 9 perfluoroalkyl carboxylic acids (PFCAs), 7 perfluoroalky sulphonic acids (PFSAs), perfluorooctanesulfonamide (PFOSA) and 6:2 fluorotelomer sulfonate (6:2 FTS). However, targeted analysis was only performed and reported for PFOA, linear PFOS and branched PFOS. This was undertaken at ALS laboratories (Hawarden, UK) in accordance with accredited methods TM 337 (ALS, 2022a) and TM 434 (ALS, 2022b). Labelled calibration standards of PFOS and PFHxS were used for quantitation of PFAS components.

Non-targeted analysis was performed using two different mass spectrometry methods. Firstly, a full scan method using uHPLC-ESI-HRqToFMS was employed to produce the data that was used for suspect screening via MassHunter and the NIST database. Secondly a MS/ MS method was used to generate data for exploratory screening using FluoroMatch. Data files were generated using the auto-MS/MS acquisition mode, a data-dependent mode acquisition mode that generates data files containing full scan MS data as well as MS/MS spectra for the highest abundance ions during each acquisition cycle.

For the full scan MS measurement, a 25 μ L sample was injected onto a ZORBAX Eclipse Plus C18 (100 \times 2.1 mm, 1.8 μ m; Agilent Technologies, UK) at 40 °C. Mobile phase A was 5 mM ammonium acetate in water and mobile phase B was methanol. The analytical gradient was held at 10 % B for 0.5 min, then increased to 50 % B at 2 min, then increased to 85 % B at 16 min, then finally increased to 100 % B at 18 min and held at 100 % for 3 min. The MS parameters were: negative ion mode, drying gas temperature 230 °C, drying gas flow 6 L min^{-1}, nebulizer pressure 20 psi, sheath gas temperature 375 °C, sheath gas flow 12 L min^{-1}, capillary voltage 2500 V, nozzle voltage 0 V, fragmentor voltage 130 V.

For the MS/MS method, a 10 μ L sample was injected onto a ZORBAX Eclipse Plus C18 (150 \times 2.1 mm, 1.8 μ m; Agilent Technologies, UK) at 40 °C. Mobile phase A was 5 mM ammonium acetate and 0.1 % acetic acid in water, and mobile phase B was 5 mM ammonium acetate and 0.1 % acetic acid in methanol. The analytical gradient was held at 5 % B for 0.5 min, then increased to 40 % B at 3 min, then increased to 100 % B at 17 min and held at 100 % B until 21 min. MS parameters (for auto-MS/MS) were: negative ion mode, drying gas temperature 200 °C, drying gas flow 9 L min⁻¹, nebulizer pressure 35 psi, sheath gas temperature 400 °C, sheath gas flow 12 L min⁻¹, capillary voltage 3000 V, nozzle voltage 0 V, fragmentor voltage 100 V. For MS/MS acquisition, collision energies of 15 V and 50 V were used.

2.4. Non-targeted data processing

Non-targeted data analysis was performed using two complementary approaches with different software programs. Suspect screening was performed using MassHunter (Agilent) to identify compounds present on the NIST PFAS database (NIST, 2021). Exploratory screening was performed using FluoroMatch (version 2.6) to identify any potential PFAS (open-source vendor neutral software), which could then be further investigated in the MassHunter Qualitative Analysis software.

Suspect screening was performed on the full scan MS datafile. Samples were blank-subtracted and hits considered based on comparison of an accurate mass (<5 PPM), with additional circumstantial evidence based on isotopic patterns.

A data-dependent acquisition mode (DDA) containing full scan MS

and MS/MS spectra (auto-MS/MS acquisition mode) was used to obtain data files for the FluoroMatch workflow, as suggested by Koelmel et al. (2020). Data files, including blank MS data for blank filtering were loaded into the software generating a list of hits for features that potentially represent PFAS. Subsequently, results were opened in the FluoroMatch Visualiser tool and hits were filtered with a score of A to B-, corresponding to Schymanski confidence levels 2–3 (Schymanski et al., 2014), and exported to an Excel spreadsheet. These features were curated through individual examination in the MassHunter Qualitative Analysis software and reported as confidence levels reported by Charbonnet et al. (2022).

2.5. Data quality

Sample extraction and targeted analysis was performed at ALS laboratories (Hawarden, UK) in accordance with their accredited methods, detailed QA/QC protocols for sample preparation and analysis are provided within TM 337 (ALS, 2022a) and TM 434 (ALS, 2022b). This includes the use of laboratory blanks and isotope labelled standards. Levels of background contamination and extraction and recovery rates were all within the accepted accredited guidelines. For non-targeted analysis details of data processing optimisation and QA/QC are discussed within the results.

3. Results and discussion

3.1. Targeted analysis

The five samples were analysed for PFOA, linear PFOS and branched PFOS. Elevated concentrations of PFOA (838–12,100 ng L⁻¹) were detected in all samples with maximum concentrations over three orders of magnitude above proposed environmental quality standards (100 ng L⁻¹; DWI, 2021b; EA, 2021). Much lower concentrations of PFOS were recorded with concentrations of branched PFOS ranging from <1 to 6.03 ng L⁻¹ and concentrations of linear PFOS ranging from 0.93 to 7.12 ng L⁻¹. All results still exceeded the PFOS-EQS of 0.65 ng L⁻¹ suggesting potential environmental and human health risks. However, all concentrations were below the 'maximum allowable concentration' (MAC) for PFOS of 36,000 ng L⁻¹ (inland surface water; EA, 2021), which is based on short-term toxic risks to the aquatic environment. The levels of PFOA and PFOS alone would be a cause for concern; however, these only represents two of the thousands of PFAS that may be present.

3.2. Suspect screening using MassHunter and NIST PFAS library

There are over 14,000 PFAS but unfortunately reference standards are only available for less than 2% of these (Nason et al., 2021). Specific mass spectral libraries for PFAS are in their infancy and although accurate mass data is available there are not often MS/MS spectra or MS/MS spectra at different ionisation energies. This makes exact compound identification a challenge as assignments are based on limited lines of evidence, therefore assignments for many compounds cannot be stated at confidence levels of greater than 2 or 3 from those described by Schymanski et al. (2014) and Charbonnet et al. (2022).

Initial data processing was performed using MassHunter Qualitative Analysis (V10.0, Agilent, UK). Suspect screening was performed by searching against the NIST PFAS library (NIST, 2021). This initial identification was performed at confidence level 4 based on mass accuracy and isotope pattern. In total, 1175 hits were recorded across all five samples with a match score >80. This large number of hits highlights a limitation of non-targeted screening based solely on accurate mass and isotope patterns; many of these hits are likely to be false positives, which can be generated by incorrect assignments and the assignment of unique compounds to PFAS fragments created during ionisation. Increasing the tolerance on the match factor to >90 reduced the number of hits down to 697. This made the dataset more manageable, however it is important to note that the reason for the <90 score for many of those hits was a low abundance of secondary isotopes. Therefore, it is possible that valid hits for PFAS present at low concentrations were removed during this process.

Two methanol blank samples were analysed as instrument blanks, a total of 524 hits were recorded within the blank samples, as a highly conservative measure all these hits were removed. Further data processing was performed by investigating fragments created during ionisation by considering the retention time for each hit on a sample-by-sample basis. Any hits occurring at the same retention time were investigated by visual inspection of chromatograms and mass spectra. Any hits that could be attributed to a specific fragment of another PFAS detected at the same retention time were removed. This resulted in a total of 89 PFAS across the five samples at confidence level 4. Whilst this step provides increased confidence in the final PFAS reported it does mean that some valid hits are likely to have been removed.

3.3. Exploratory data analysis using FluoroMatch

FluoroMatch (version 2.6: Koelmel et al., 2021, 2020) was used to generate a list of hits for features that represent PFAS. Auto-MS/MS data files from the pooled sample plus MS¹ data files from a blank sample (for blank filtering) were loaded into FluoroMatch Flow. The results were opened in the FluoroMatch Visualizer tool. The list of hits was filtered to only show hits with a FluoroMatch score of A to B-, roughly corresponding to confidence levels 2-3 (Charbonnet et al., 2022; Koelmel et al., 2021; Schymanski et al., 2014). In total 207 unique hits were recorded across all five samples. Each hit was manually inspected both in FluoroMatch Visualizer (Fig. 1) and in MassHunter Qualitative Analysis (version 10.0). Ninety-one (91) hits were removed after inspection of the mass spectra as the feature was not believed to represent a PFAS. A further 75 hits were removed as they represented features that were associated with PFAS but were not thought to correspond to native molecular ions of a PFAS (for example, suspected in-source fragments of PFAS or fragments of isotope labelled standards). When manually interrogating the data, MassHunter Qualitative Analysis was used to propose formulae for precursor and fragment ions. Hits were only accepted if they met the following criteria: there was at least one proposed molecular formula containing at least two F atoms with mass error <5 ppm; there was agreement between the theoretical isotope pattern of the proposed formula and the observed isotope pattern; the MS/MS spectra contained at least one proposed fragment formula that included CF_2 with a mass accuracy of <5 ppm; and the feature retention time, mass and mass defect were logical and in alignment. This resulted in the identification of 33 unique PFAS detected at confidence level 3 and 10 PFAS at confidence level 2. These identified compounds were then screened for in MS¹ data files of the five individual samples to determine number of detections and abundance in each sample.

3.4. Final reporting and implications

Any non-targeted screening assessment contains an element of subjectivity and therefore it can be a challenge to communicate the findings accurately and confidently. In this assessment we followed the confidence levels stipulated by Charbonnet et al. (2022) and Schymanski et al. (2014). When applying a suspect screening approach, we identified between 1175 (least conservative estimate) to 89 (most conservative estimate) PFAS at confidence level 4. When applying an exploratory data analysis, we identified 33 PFAS at confidence level 3 and 10 PFAS at confidence level 2. No standards were used for confirmatory analysis and so no PFAS were determined at confidence level 1. The PFAS identified at confidence levels 2 and 3 from the exploratory data analysis are summarised in Table 1 and detailed in the supplementary information (SI Table 1).

In total five different PFAS classes were identified, these included PFCAs (perfluoroalkyl carboxylic acids), H–PFCAs (hydrogen substituted perfluoroalkyl carboxylic acids), Cl–PFCAs (chlorine substituted perfluoroalkyl carboxylic acids), PFECAs (perfluoroether carboxylic acids), and an unknown group believed to represent unsaturated perfluoroether carboxylic acids (UPFECAs). Nine (9) PFCAs were identified from C4–C11, with the addition of one branched C8 PFOA. Thirteen (13) H–PFCAs were identified from C5, C6, C8–C15, three C5 isomers were identified along with two C10 isomers. Fourteen Cl–PFCAs were identified for C4–C16 with two isomers identified for C4. One PFECA was identified and assigned as perfluoro(2-ethoxy-2-fluoroethoxy)-acetic acid, ammonium salt (EEA-NH₄).

Six PFAS could not be confidently assigned to a known class. They were all tentatively assigned the formula C₄HF₇O₂ based on the presence of m/z 212.9792. The abundances of all six C₄HF₇O₂ peaks were observed to vary proportionally with the abundance of the assigned EEA-NH₄ peak in the five individual samples. Two C₄HF₇O₂ peaks at 9.06 and 9.15 min were abundant enough to trigger MS/MS acquisition. The resultant MS/MS spectra contained three different fragments with proposed formulae that support the C₄HF₇O₂ precursor formula, with mass errors of <5 ppm. The most abundant fragment ion in the MS/MS spectra acquired for precursor m/z 212.9792 is proposed to correspond to $[C_2F_5O]^-$, which is also present in MS/MS from the suspected EEA- NH_4 peak, suggesting a structural similarity between EEA- NH_4 (C₆HF₁₁O₄) and the unknown C₄HF₇O₂ PFAS (Fig. 2). A possible structure for C₄HF₇O₂ based on the MS/MS evidence at 9.06 and 9.15 min would be an unsaturated PFECA (U-PFECA) fragment with a carbon double bond. Although m/z 212.9792 was selected as the precursor ion in the auto-MS/MS acquisition, this ion may not correspond to a deprotonated molecular ion but may instead be an in-source fragment ion of a larger PFAS. For example, m/z 306.9658 was observed to coelute with m/z 212.9792 at 9.06 and 9.15 min, and m/z 306.9658 has a proposed formula of C₆HF₉O₄ based on MS¹ evidence. The MS/MS spectra (Fig. 2) contained a proposed C₃F₅O₂ fragment, is unclear through what mechanism $C_4F_7O_2^-$ would fragment to $C_3F_5O_2^-$, as this requires a loss of CF₂, but the proposed ion formulae are strongly supported by the low mass errors of both ions. PFECAs have been found to rapidly fragment during ionisation due to the ether linkages (Pan et al., 2020; Yao et al., 2021, 2022). Overall, it was not possible to ascertain the precise molecular formula of the compounds that were observed as C4HF7O2 detections, but the common MS/MS fragments shared with EEA-NH₄ plus the relationship between the abundances of EEA-NH₄ and the unknown C₄HF₇O₂ peaks suggests that these unknown compounds are in some way related to EEA-NH₄ and represent U-PFECAs. U-PFECAs have been reported elsewhere in non-targeted screening studies (Jiang et al., 2023; Shojaei et al., 2022; Song et al., 2023; Yao et al., 2022).

PFECAs are PFAS of emerging concern and have been widely used as replacements for PFCAs (Wang et al., 2013). High fragmentation rates and lack of analytical standards are leading to concerns that these PFAS may be under-reported in the current literature (Yao et al., 2022), which is a large concern considering that some PFECAs may be more toxic than the PFAS they were meant to replace (Chen et al., 2021; Conley et al., 2021; Gomis et al., 2018; Li et al., 2022; Sheng et al., 2018).

This study provides further evidence that highlights the need for more comprehensive PFAS monitoring programmes. The levels of PFOA alone are a cause for concern (838–12,100 ng L⁻¹), however the total PFAS concentration is likely to be a much larger concern. We used the data available to estimate total PFAS concentrations by assuming all of the 43 PFAS detected in Table 1 had the same response factor as PFOA (We accept there is a large degree of uncertainty in this method due to different ionisation efficiencies and fragments produced by other PFAS, however it can serve as a useful ballpark estimate). Using this method, we calculated an average total PFAS concentration of approximately 38 μ g L⁻¹. Concentrations of PFAS in some samples were so high that they saturated the chromatographic column. This was observed for EEA-NH4 (difluoro[1,1,2,2-tetrafluoro-2-(pentafluoroethoxy)ethoxy]-acetate), which had the highest average peak area of all PFAS reported. In one

which had the highest average peak area of all PFAS reported. In one sample the extracted ion chromatogram of [M - H]- m/z 344.9626 was



Fig. 1. Kendrick mass defect plots of features identified as potential PFAS by FluoroMatch (features that represent PFAS that belong to a series that vary only in fluorocarbon chain length align horizontally). A) shows all features that were ranked as A + - B- by FluoroMatch, and B) shows a suspected PFAS series, where MS/MS data supports the association of these features as a series.

Class	Proposed Abbreviation	Proposed Formula	RT	m/z	Mean area in positive samples	Number of Detections	Confidence Level
PFCA	PFBA	C4HF7O2	5.01	212.9791	7140110	5	2
	PFPeA	C5HF9O2	7.03	262.9758	6023634	5	2
	PFHxA	C6HF11O2	8.90	312.9723	1195086	5	2
	PFHpA	C7HF13O2	10.45	362.9696	879659	5	2
	PFOA	C8HF15O2	11.40	412.9660	2922394	5	2
	PFOA	C8HF15O2	11.69	412.9660	14854131	5	2
	PFNA	C9HF17O2	12.74	462.9632	138584	5	2
	PFDA	C10HF19O2	13.62	512.9600	39731	5	2
	PFUnDA	C11HF21O2	14.37	562.9568	16755	5	2
H-PFCA	H-PFBA	C5H2F8O2	5.25	244.9852	13897676	5	2
	H-PFPeA	C5H2F8O2	5.73	244.9852	10133800	2	3
	H-PFPeA	C5H2F8O2	5.91	244.9850	10327801	2	3
	H-PFHxA	C6H2F10O2	6.65	294.9820	12114576	5	3
	H-PFOA	C8H2F14O2	9.52	530.9491	142250	1	3
	H-PFNA	C9H2F16O2	10.78	444.9718	13290252	3	3
	H-PFDA	C10H2F18O2	11.70	494.9687	315330	5	3
	H-PFDA	C10H2F18O2	11.84	494.9686	11068219	3	3
	H-PFUnDA	C11H2F20O2	12.75	480.9696	1708573	2	3
	H-PFDoDA	C12H2F22O2	13.54	594.9631	1633344	3	3
	H-PFTrDA	C13H2F24O2	14.21	644.9597	488361	2	3
	H-PFTeDA	C14H2F26O2	14.80	694.9567	373491	3	3
	H-PFPeDA	C15H2F28O2	15.32	744.9535	133075	4	3
CI-PFCA	CI-PFBA	C4HCIF6O2	5.37	364.9242	5483428	3	3
	CI-PFBA	C4HCIF6O2	5.49	364.9246	9252776	5	3
	CI-PFPeA	C5HCIF8O2	7.49	414.9213	7555999	5	3
	CI-PFHxA	C6HCIF10O2	9.23	328.9433	7885601	4	3
	Cl-PFHpA	C7HClF12O2	10.70	378.9401	4069038	5	3
	CI-PFOA	C8HCIF14O2	11.92	564.9107	56671	3	3
	CI-PFNA	C9HCIF16O2	12.91	614.9083	86498	2	3
	CI-PFDA	C10HCIF18O2	13.76	664.9041	47160	3	3
	Cl-PFUnDA	C11HClF20O2	14.50	578.9273	356279	5	3
	CI-PFDoDA	C12HClF22O2	15.12	628.9241	432410	4	3
	CI-PFTrDA	C13HClF24O2	15.67	678.9209	643264	1	3
	CI-PFTrDA	C14HCIF26O2	16.15	728.9177	535300	1	3
	CI-PFPeDA	C15HClF28O2	16.54	778.9145	363909	1	3
	CI-PFHxDA	C16HClF30O2	16.90	828.9113	402694	1	3
PFECA	EEA-NH4	C6HF11O4	10.24	344.9624	24943982	5	3
Unknown (U-PFECA)	N/A	C4HF7O2	8.14	212.9793	836990	2	3
	N/A	C4HF7O2	8.38	212.9793	6119994	2	3
	N/A	C4HF7O2	8.63	212.9793	1128173	2	3
	N/A	C4HF7O2	8.87	212.9793	2422784	2	3
	N/A	C4HF7O2	9.06	212.9793	6728799	3	3
	N/A	C4HF7O2	9.15	212,9793	6807180	3	3

Table 1

Summary of non-targeted PFAS hits (N = 43) with a confidence level of 3 or better.

so abundant it resulted in a broad, flattened peak that would not provide accurate quantification (Fig. 3). EEA-NH4 is a processing aid in the manufacturing process of fluoropolymers (e.g. PTFE) that is currently being legally discharged directly in proximity to the location that samples were obtained from. EEA-NH₄ is expected to be very environmentally persistent with high mobility in water and soil, thus potentially contaminating drinking water sources (BAuA, 2022). It is chemically similar to ADONA, which has high mobility in the environment and a widespread occurrence far from point sources (BAuA, 2022; Schreiber, 2014). EEA-NH4 has been previously detected in the vicinity at an average concentration of 2.24 mg L⁻¹ between 2016 and 2020 (EA, 2023b), which again raises serious cause for concern.

This research shows that there are more than a handful of PFAS present in our environment, this has been well understood globally but this is the first NTS manuscript of its kind for PFAS in the UK. Monitoring programs continue to expand targeted analyte lists in an attempt to manage the ever-increasing numbers of PFAS detected in the environment. A recent example of this is the UK DWI 47 which targets 47 specific PFAS (DWI, 2021b). This is one of the largest targeted PFAS analyte lists and so is considered by some to be a comprehensive PFAS monitoring procedure. However, when comparing our list of 43 PFAS at confidence level 2 and 3 against this list, only 8 of the UK DWI 47 PFAS were identified. This corresponded to only 17% of the total PFAS peak area and indicates that targeted methods alone are not an appropriate

MS/MS of EEA-NH4 assignment

 $C_6 HF_{11}O_4 [M-H]^{-1}$



 $C_4HF_7O_2[M-H]^2$



Fig. 2. Extracted ion chromatogram of m/z 212.9792 showing 6 unknown PFAS, proposed to be U-PFECAs (C₄HF₇O₂) with associated MS/MS high (50v) and low (15v) collision spectra acquired at 9.05. MS/MS spectra from the suspected EEA-NH₄ peak (precursor 344.9626) is provided for to demonstrate the presence of common fragment ions.

tool for measuring PFAS in the environment. This is just data from one specific point source of pollution, there are likely to be many more locations where novel PFAS continue to go unmonitored. This study provides strong evidence to suggest that non-targeted and total PFAS methods should be integrated within routine targeted monitoring programmes. This would help identify specific PFAS that could be beneficial in source identification investigations, as well as better understanding the total PFAS load and what environmental or health risks it may pose. The high abundance of H- and Cl-substituted PFAS in our dataset, along with the presence of PFECAs such as EEA-NH₄ demonstrates how little we know about the PFAS in our environment from performing targeted analysis in isolation.

There was one notable absence from our dataset, which highlights the potential for false negatives with non-targeted analysis. PFOS was detected in the samples during the targeted analysis but was not detected in the final list of 43 PFAS. PFOS was not detected during the non-targeted analysis because FluoroMatch identifies features that triggered MS/MS, but in auto-MS/MS mode, only a limited number of candidates at each retention time can trigger MS/MS. Therefore, if an analyte co-elutes with multiple other, more abundant ions, it is possible that this analyte will not trigger MS/MS and therefore will not be found by FluoroMatch. As PFOS was present at low concentrations and eluted at a similar time to other more abundant features, MS/MS was not triggered for this feature and so was not detected. However, when the samples were evaluated using full scan MS data, PFOS could be identified at confidence level 4. We were only able to identify this false negative due to information from the targeted analysis. It is considered likely that many more false negatives may have gone unnoticed and so the total reported number of PFAS at confidence level 2 and 3 are likely to be an underestimate of the true number of PFAS in these samples.

3.5. Accepting and embracing the limitations of non-targeted analysis

Despite an analyst's best efforts, all "non-targeted" methods have



Fig. 3. Extracted ion chromatograms of m/z 344.9629, corresponding to the $[M - H]^-$ ion of EEA-NH₄, from the five individual samples. Note the high peak area and chromatographic saturation of the final chromatogram.

some degree of targeting. Here we have documented how each of the steps in our preparation and analytical procedure may have impacted the results obtained. Sample preparation was performed in a commercial laboratory using a certified extraction method for 9 perfluoroalkyl carboxylic acids (PFCAs), 7 perfluoroalky sulphonic acids (PFSAs), perfluorooctanesulfonamide (PFOSA) and 6:2 fluorotelomer sulfonate (6:2 FTS) (ALS, 2022b, 2022a). Whilst the method is accredited and recovery assessed for the target analytes, acceptable recovery cannot be assured for other PFAS classes.

The extraction method used in this study involved solid-phase extractions (SPE) to isolate, purify and concentrate PFAS using different SPE sorbents (polar, non-polar and ion exchange), e.g. polystyrenedivinylbenzene (SDVB), mixed-mode SDVB or weak anion exchange (WAX) (EPA, 2019, 2018; Winchell et al., 2021). These sorbents may potentially introduce interferences and contamination into the sample preparation process (Winchell et al., 2021). Other standardised methodologies are available that report the use of dilute and filter approaches for liquid sample clean-up and purification (e.g. EPA 8327, ASTM 7968 or ASTM7979). However, these can be susceptible to losses of PFOA and PFOS to syringe and filter materials (glass and plastic, particularly for increasing chain lengths >12 carbon atoms; Sörengård et al., 2020; Winchell et al., 2021). Large-volume injection (LVI) and/or direct injection (DI) for PFAS analyses in liquid matrices with little to no pre-treatment has also been reported. Whilst this restricts potential contamination, these methods may have limitations with detection limits and need for more regular chromatographic maintenance (Backe and Field, 2012; Barzen-Hanson et al., 2017; Benskin et al., 2010; Binetti et al., 2019; Busetti et al., 2012; Gottschall et al., 2017; Huset and M. Barzen-Hanson et al., 2017; Nakayama et al., 2019; Schultz et al., 2006; Winchell et al., 2021).

The ionisation method applied was solely negative ESI-mode (ESI⁻), thus suggesting the potential to "exclude" neutral PFAS and compounds detectable in positive ESI-mode (ESI⁺). For instance, Barzen-Hanson

et al. (2017) reported five fragments that can only be observed in in ESI⁺-mode for PFAS in aqueous film-forming foams (AFFFs) and commercial products (CPs), including perfluoroalkyl sulfonamido-based compounds (Barzen-Hanson et al., 2017; Kuehl and Rozynov, 2003) and electrochemical fluorination (ECF)-derived PFAS (Barzen-Hanson et al., 2017; D'Agostino and Mabury, 2014). To address this limitation, further analysis would be required using positive ionisation mode as well as complementary analysis using gas chromatography (GC) to capture volatile, neutral and semi volatile PFAS, such as TFA, FTOH, FOSEs and FOSAs (Al Amin et al., 2020; EA, 2023a; Scott et al., 2006).

PFAS may be present as mixtures of linear and branched isomers, i.e. chemicals with same formula but different molecular structures (Londhe et al., 2022) that may hinder the "correct" identification of compounds. There are tools that can help separate and identify structural isomers such as ion mobility spectrometry (IMS), however these methods require additional data processing and may not be able to achieve the same limits of detection Díaz-Galiano et al. (2023). Our data suggested the presence of several branched PFAS but there are likely more that we were unable to separate.

Within this study, the complementary use of instrument (distributordependent) and open-source (FluoroMatch) software aided the PFAS workflow up to confidence level 2. Nonetheless, the criteria used were set on (subjective) confidence criteria, i.e. for suspect screening our list of PFAS identified at confidence level 4 decreased from 1175 to 89 compounds depending when we applied more stringent controls on selecting a match factor, performing blank subtraction and assigning insource fragments. Whilst this process likely removed many false positive, the aggressive filtering method may have also removed 'valid' PFAS, especially those at lower concentrations or those that ionise relatively poorly by ESI, since low abundance diagnostic ions may not have been recorded.

This manuscript only focused on using mass spectrometry based techniques for the determination of PFAS. This is an excellent tool to help identify what specific PFAS exist in samples. However, there are several total PFAS methods that could have been used to compliment this study such as CIC, F-19 NMR, TOP Assay or PIGE. Performing a total analysis using these methods would have helped to establish if there are yet more PFAS in those samples that are going undiscovered.

4. Conclusions

Five surface water samples collected in the vicinity of a fluorochemical plant in the Northwest of England were analysed for PFAS by complimentary targeted and non-targeted methods. Targeted analysis revealed PFOA at a maximum concentration of 12,100 ng L⁻¹, which is over three orders of magnitude greater than the proposed EQS of 100 ng L⁻¹. A conservative assessment (assuming the same response factor of PFOA for all PFAS) would result in a total average PFAS concentration across the 5 samples of approximately 38 μ g L⁻¹ (0.038 mg L⁻¹). A suspect screening approach identified between 1175 (least conservative) to 89 (most conservative) PFAS at confidence level 4. Exploratory data analysis was then used to identify 33 PFAS at confidence level 3 and 10 PFAS at a confidence level of 2. Detailed toxicological data is not available for many of these PFAS which makes establishing the magnitude of environmental and health risks challenging.

Our results suggested the presence of a new class of U-PFECAs, linked with EEA-NH4, which we have reported in the environment for the first time. Evidence for this designation was supported at confidence level 3 and included; a common ion at 212.9792 which was believed to represent $[C_2F_5O]^-$, three different fragments with proposed formulae that support the $[C_2F_5O]^-$ precursor formula, with mass errors of <5 ppm. The most likely candidate formula was deemed to be $C_4HF_7O_2$ [M – H]⁻, although larger PFAS such as $C_6HF_9O_4$ [M – H]⁻ are also possible as PFECAs have been found to rapidly fragment during ionisation due to the ether linkages.

Regular (inter)national PFAS monitoring requirements include a variety of priority substances, which already have been expanded, and likely will be extended further in the future. These targeted programmes are required to evaluate environmental and human health risks. Our results highlight the limitations of relying solely on targeted analysis for routine monitoring. Only 8 of these 43 PFAS (representing 17% of the total PFAS peak area) are regularly monitored in the UK as part of the UK DWI 47 PFAS. To our knowledge this is the first published study focused on determining PFAS in the UK using non-targeted analysis. The results of our suspect screen revealed that 100s of PFAS may be present within these samples which highlights the vast potential for different PFAS to be present in the environment. Future monitoring programmes that rely solely on targeted monitoring would surely benefit from incorporating a non-targeted approach.

CRediT authorship contribution statement

David Megson: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. Daniel Niepsch: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. Jonathan Spencer: Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. Claudio dos Santos: Writing – review & editing, Formal analysis, Data curation. Hannah Florance: Writing – review & editing, Formal analysis, Data curation. Cecilia L. MacLeod: Writing – review & editing. Ian Ross: Writing – review & editing, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143645.

Data availability

Data will be made available on request.

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