"Simultaneous targeted and non-targeted analysis of per- and polyfluoroalkyl substances in environmental samples by liquid chromatography-ion mobility-quadrupole time of flight-mass spectrometry and mass defect analysis"

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Abstract

Per- and polyfluoroalkyl substances (PFAS) represent a large group of synthetic organic compounds which exhibit unique properties and have been extensively used for consumer and industrial products, resulting in a widespread presence in the environment. Regulation requiring PFAS monitoring has been implemented worldwide due to their potential health and eco-toxicological effects. Targeted methods are commonly used to monitor between twenty to forty PFAS compounds, representing only a small fraction of the number of compounds that may be present. Consequently, there is an increasing interest in complementary non-targeted methods to screen and identify unknown PFAS compounds with the aim to improve knowledge and to generate more accurate models regarding their environmental mobility and persistence. This work details the development of a method that simultaneously provided targeted and non-targeted PFAS analysis. Ultra-high performance liquid chromatography (UHPLC) was coupled to ion mobility-quadrupole time of flight-mass spectrometry (IMS-QTOF-MS) and used to quantify known and screen unknown PFAS in environmental samples collected within the greater Sydney basin (Australia). The method was validated for the quantification of 14 sulfonate-based PFAS, and a non-targeted data analysis workflow was developed using a combination of mass defect analysis with common fragment and neutral loss filtering to identify fluorine-containing species. The optimised method was applied to the environmental samples and enabled the determination of 3-7 compounds from the targeted list and the detection of a further 56-107 untargeted PFAS. This simultaneous analysis reduces the complexity of multiple analyses, and allows for greater interrogation of the full PFAS load in environmental samples.

Keywords

PFAS; non-targeted analysis; mass defect, ion mobility spectrometry; isomer analysis

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a complex family of more than 3000 synthetic fluorinated organic compounds that have been produced since the 1940s [1]. They consist of either a fully (per-) or partially (poly-) fluorinated hydrocarbon chain bonded to a functional group, commonly a sulphonate or a carbonate. Depending on the chemical moieties, different chemical and physical properties can be designed to promote low surface tension, as well as high thermal and chemical stability [2,3]. These unique properties led to the production of a wide range of PFAS compounds for industrial and commercial applications and can be found in cleaners, textiles, leather, paper, paints, fire-fighting foams or wire insulation [4,5].

The widespread use of PFAS has resulted in almost ubiquitous environmental contamination. Major sources involve either the direct release during firefighting training and response sites, industrial sites, landfill sites and wastewater treatment plants, or indirect release by degradation and interconversion of PFAS precursors within the environment [6,7]. Once released, PFAS may absorb on soil or are distributed throughout the environment contaminating even pristine areas and groundwater. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were recently classified as 'possible carcinogens' by the International Agency of Research in Cancer [8] and are known to bioaccumulate [9]. Their abundant presence, along with potential adverse health and environmental impacts have stimulated further scientific enquiries, leading to legislated regulation in many countries. Due to considerable manufacturing and distribution of products containing these chemicals and their persistency within the environment, the 2009 Stockholm Convention listed PFOS and related compounds as persistent organic pollutants candidates [10,11].

Current analytical methods to investigate PFAS contamination or exposure typically target between twenty to forty compounds via gas or liquid chromatography coupled to tandem mass spectrometry (MS/MS) [12–16]. These targeted analyses detect only a subset of a wide range of PFAS, consisting of thousands of variations and isomers of these compounds from variable chain lengths, branching, functional groups and partially fluorinated components and therefore, are generally not able to accurately reflect the actual levels and species of PFAS in a sample [17]. Therefore, non-targeted methods are increasing in use to identify and/or quantify PFAS in the environment [18]. Non-targeted analyses of PFAS often take advantage of high mass resolution mass spectrometers (HRMS) to determine exact masses and predict sum formulas for identification [19]. However, the detection and differentiation of isomers remains challenging. Ion mobility spectrometry (IMS) [20] is an emerging technology in environmental assays, which is compatible with MS and enables a more dedicated structural analysis of PFAS [21–23]. IMS employs an electric field and an inert pressurised gas cell to

separate ions by their charge and collisional cross section (CSS) [23]. Measured drift time values are used to calculate the average CSS (typically measured in $Å^2$) following calibration, which represents the rotationally averaged surface volume of the ion available for interaction with the collision gas [24].

MS/MS data analysis of non-targeted compounds frequently involves the identification of common fragments or neutral losses, also known as fragment ion flagging (FIF) [22,25]. This requires rigorous mass and data filtering, which can be streamlined using purpose-built software packages. Mass defect (MD) analysis of F-containing species is another alternative for non-targeted screening in conjunction with HRMS [26–28]. Mass defect analysis considers exact isotope masses and is defined as the difference between the exact mass and the nominal mass of a molecular compound. For most molecular species, the exact mass is larger than the nominal mass, however, in the case of PFAS, the mass defect is negative, which can be used by data filters to pinpoint all PFAS in a sample [29].

In this work we combined strategies for the targeted and non-targeted analysis of PFAS in environmental samples collected from Sydney, Australia, by employing UHPLC-IMS-QTOF-MS which allowed the characterisation of PFAS in four dimensions: retention time (polarity), CCS, (exact) mass, and fragmentation pattern. A targeted, quantitative analysis was validated for fourteen sulfonated PFAS, and an automated data analysis workflow using MD and FIF was developed to simultaneously identify non-targeted PFAS in the same chromatographic run.

2. Experimental

2.1 Chemicals and Reagents

All reagents used for sample preparation and the mobile phases were of analytical or LC-MS grade. Ultra-pure water (18.2 MΩ cm) was obtained from a Sartorius 611 arium® pro water generation system (Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany). LC-MS grade LiChrosolv® methanol and analytical grade ammonium acetate were obtained from Sigma-Aldrich (St Louis, MO, U.S.).

An analytical standard (2 mg L^{-1}) containing 24 PFAS with carbon chain lengths of C4-C13 perfluroalkylcarboxylic acids, C4-C10 perfluroalkylsulfonates, FOSA, N-MeFOSAA, N-EtFOSAA perflurooctanesulfon- (amide & amidoacetic acids) as well as 4:2, 6:2 and 8:2 fluorinated telomer acids was purchased from Wellington Laboratories (Guelph, Ontario, Canada). Sodium hydroxide, sodium chloride, ammonium hydroxide were purchased from Sigma-Aldrich (Castle Hill, Australia) and glacial acetic acid from Chem-Supply (Gillman,

Australia). PTFE free sample tubes and vials made of polypropylene were used to avoid contamination [30].

2.2 Sample collection and preparation

Water samples were collected from seven locations within the Cooks River catchment area (Sydney) as illustrated in Figure 1. Exact coordinates and sampling dates/times are listed in

Sample preparation was performed via µSPE following the protocol previously described by Lockwood *et al*. [30]. Briefly, 10 mL aliquots of the collected water samples were acidified with 100 µL of glacial acetic acid to approximately pH 3 to increase the interaction of PFAS on the sorbent material. Clean up, extraction and preconcentration of the samples were performed with a digiVOL® Programmable Digital Syringe Driver using ePrep® µSPEed cartridges (Eprep, Mulgrave, VIC, Australia) packed with a mixed mode C18: aminopropyl silica (APS) phase. The mixed mode cartridge was conditioned with 250 µL of 10 mM of NaOH in MeOH, activated with MeOH and equilibrated with 250 µL of 1% acetic acid in water. Subsequently, 2 mL of sample was loaded and washed with 100 µL of ultrapure water before eluting PFAS with 100 µL of 10 mM of NaOH in MeOH. 1 µL of acetic acid was added before injection to neutralise the eluate and improve chromatographic peak symmetry.

2.3 Instrumentation

Chromatographic separation was performed on a Waters ACQUITY UPLC I-Class system coupled to Waters Vion^{TM} IMS-QTOF-MS with high definition MS^{E} data acquisition using the Waters UNIFI software (Waters Corporation, Milford, MA, USA). An Accucore^{TM} Vanquish^{TM} C18+UHPLC (100 x 2.1mm; 1.5µm particle size) column (Thermo Fisher Scientific, Waltham, MA, USA) was used for chromatographic separation. Mobile phase A consisted of ultrapure water and B of methanol, each containing 2 mM ammonium acetate. The initial conditions of 20% B were held for 0.5 min, followed by a linear increase to 60% B at 4.5 min and to 90% B at 11 min. This was maintained for 4 min before returning to the starting conditions and equilibrating for 3 min. The flow rate was set at 0.3 mL min^{-1}, the column temperature at 50 °C, and the injection volume was 5 µL. Internal lock mass was acquired periodically during each injection to compensate for potential drift and to maintain high mass accuracy. The electrospray ionisation (ESI) source was operated in negative ionisation mode and the optimised instrumental parameters are listed in Table 2.

2.4 Data analysis

A standard mix of 24 PFAS (see Table S1 in the Electronic Supplementary Material (ESM)) was used to set up a targeted screening method with compounds identified using a scientific library built in-house. This library contained the exact masses of a set of known PFAS for

identification from a data-independent acquisition (DIA) file. After optimisation of the LC-IMS-QTOF-MS method, the observed exact *m/z*, retention times and CCS values were placed into this library for targeted analysis of the known PFAS standards. The IMS orthogonal separation method allowed the separation of PFAS isomers. Quantification was performed via external calibration.

Non-targeted identification of PFAS compounds was performed through multiple layers of data filtering using the Waters UNIFI data management system. These filtering methods included mass defect analysis, common fragment and neutral losses identification (FIF). Mass defect filtering was the first step to screen and detect PFAS candidates. PFAS comprise of different classes of compounds with one common feature: F atoms (18.9984 Da) replacing some or all H atoms (1.0078 Da) on the C-alkyl chain. As a result, F-rich alkyl PFAS have negative mass defects. A mass padding and a defect padding values were established to identify compounds that potentially contained fluorine atoms. The mass padding was set to 49.997 Da which is equivalent to one $-CF_2^-$ group. This filter was then run against all the detected masses in the DIA chromatogram, reducing the number of possible candidates by ~90%. Common neutral losses are also seen for perfluoro carboxylic acids from the loss of the CO_2 group and its variations plus part of the fluoroalkyl chain. These highly specific fragments can be used as diagnostic ions to search against the DIA spectra for PFAS candidates. Fragmentation of the 24 PFAS contained in the standard mix was used to create an in-house library containing typical common fragments and neutral losses such as $[C_3F_7]^-$, $[O_3S]^-$, or $[CO_2C_2F_4]$ among others. Common fragments and neutral losses used in the in-house library are listed in Tables S2 and S3. Consequently, a combination of multiple layers of data filtering was used for the development of the non-targeted identification workflow. All compounds detected above a threshold of 150 counts were automatically selected and then the developed filter was used to mark candidates that were within the mass defect region and have either common fragments or neutral losses.

3. Results and discussion

3.1 Method development and figures of merit for targeted LC-IMS-QTOF-MS

PFAS comprise of a large group of chemical compounds which consist of several $C\text{-}F_x$ units with different carbon chain lengths and functional groups, including carboxylates and sulfonates, which impact the optimal operating conditions for their separation and detection by LC-MS/MS. In this study, the best instrumental responses and signal-to-noise ratios were achieved for the analysis of the sulfonate-based PFAS, while the experimental conditions where not suited for the trace analysis of the carboxylic acid PFAS. Therefore, in the following, method development was optimised for the targeted quantification of the sulfonated PFAS,

with the carboxylic acids included during method development to identify common fragments and neutral losses for the non-targeted filtering. Sulfonated PFAS exhibited a range of optimum settings in MS/MS which was accommodated by applying ramped potentials to generate high energy fragmentation spectra. Four different ramps (0-20, 20-40, 40-60, 35-75 eV) were tested and a collision energy ramp from 35 to 75 eV provided the optimal figures of merit.

An UHPLC method was developed for the separation of the sulfonated PFAS (see Figure 2). Calibration was performed with a six-point calibration curve with a concentration range of 0.25-10 μg L^{-1}, with R^2 values greater than 0.997 for all compounds. The instrument limits of detection (LOD) and limits of quantification (LOQ) were calculated following the 3σ and 10σ criterion and were between 0.19 to 0.76 μg L^{-1} and 0.56 to 2.30 μg L^{-1} respectively. Instrument response (intensity) repeatability expressed as relative standard deviation (% RSD) was less than 4.8%, and CCS values were repeatable with %RSD < 0.18%. The analytical figures of merit are presented in Table 3.

The current Food Standards Australian New Zealand (FSANZ) guidelines followed the US EPA and European food Safety Authority in respectively limiting the total daily intake of PFOS and PFOA to 20 and 160 ng kg^{-1} d^{-1} (0.070 and 0.560 μg L^{-1} in drinking water) [31]. The instrumental LOQ for PFOS of 0.74 μg L^{-1} reported here, combined with our previously validated μSPE sample preparation protocol which provides a sample pre-concentration factor of 20 [30], is able to reach the required guidelines for analysis.

3.2 Identifying isomers in LC-IMS-QTOF-MS

The acquisition of CCS values and retention times as species-specific parameters enabled the discrimination of isomers which are typically indistinguishable by HRMS. Usually, retention times and fragmentation pattern are used to characterise individual isomers but may complicate the analysis and impact accuracy at low concentrations and when isomers have very similar chemical and physical properties. The additional characterisation of isomers via individual drift times and consequently different CCS values added additional certainty and improved the identification approach. In the following, two representative PFAS (PFHxS and PFOS) were investigated as models to demonstrate the reliable species identification of structural isomers via combined CCS and retention time analysis. Figure 4 shows the chromatogram monitoring m/z 398.937 (PFHxS, black) and m/z 498.930 (PFOS, red). The former detected two species (A and B), which were baseline separated. Analysing the CCS enabled species identification where species A (CCS: 145.97 $Å^2$) corresponded to a branched and B (CCS: 147.15 $Å^2$) to the linear isomer as listed in Table 4. The chromatographic separation of PFOS revealed the presence of three isomers and drift times were calibrated to

identify the respective isomers. Species C had the lowest CCS (161.53 Å^2) and corresponded to the 5-trifluoromethyl isomer and species D (CCS: 163.87 Å^2) corresponded to the 6-trifluoromethyl isomer. The largest CCS (164.75 Å^2) corresponded to the linear isomer (see Table 4). It is evident that the combined information of retention time and drift time (CCS) analysis improved analysis and identification of PFAS isomers and was used in the following non-targeted analysis in environmental samples.

3.3 Evaluation of the untargeted data filtering workflow

PFAS comprise a large group of compounds with similar physical and chemical properties and as such, characterisation commonly requires complementary analytical techniques able to detect and further investigate the species. In this study, PFAS were characterised in four dimensions. A mass defect filter selected PFAS candidates which were then further analysed by comparing exact masses, drift times/CCS values, and fragmentation spectra. Targeting compounds with a negative mass defect, and consequently removing compounds with a positive mass defect, enabled a significant reduction in the number of compounds in the DIA chromatogram, facilitating the analysis of large data sets typically produced in untargeted workflows. However, the detection of compound with a negative mass defect is not a guarantee that it is an F-containing compound, and further multi-dimensional data is required for confirmatory analysis.

In this study, accurate masses were determined and interrogated for mass defect analysis to identify potential PFAS candidates. In a retrospective proof of principle evaluation, 6:2 FTS was removed from the targeted database as an exemplar, and the standard mix of PFAS was analysed to evaluate its performance to detect and characterise unknown PFAS. Applying mass defect analysis selected the 6:2 FTS peak for further analysis. Figure 4 shows its MS analysis with the low energy channel detecting the unfragmented species at m/z 427.9665 together with two isotopic signals. The MS/MS data of these mases were then examined (high energy channel) to identify common mass fragments or neutral losses, confirming it was a PFAS and leading to its structural elucidation. The obtained fragmentation spectrum was then compared against an online data base (ChemSpider) identifying the detected compound as 6:2 FTS. As demonstrated before, drift analysis may further be employed to determine the CCS which may be relevant to distinguish isobars or structural isomers.

Application to environmental samples

In this study, water samples were sourced from freshwater streams as well as from the seawater basin in the Cooks River catchment area. Sampling locations can generally be considered areas which are subject to significant anthropogenic pressures due to the proximity

to industry, canals, stormwater run-off as well as Australia's largest airport and Port Botany, one of Australia's largest deep-water seaports dominated by trade in containerised manufactured goods and bulk liquid imports, including oil and natural gas. To enable the analysis of PFAS in complex matrices, an automated µSPE method was employed to mitigate matrix interferences and to preconcentrate PFAS. Further information on the method validation, recoveries and figures of merit of this method is available elsewhere [30]. For the targeted analysis and quantification of selected PFAS, calibration standards and samples were measured in triplicate, with solvent blanks run periodically to ensure the absence of carry over. The targeted analysis determined between 3 to 7 PFAS in all investigated samples (PFHpS, PFNS, PFOS, PFHxS, PFDS, PFBS and FOSA.) The measured concentrations ranged from 2.9 ± 1.5 to 257.3 ± 11.2 ng L^{-1}, with PFOS the most predominant species detected (see Table 5). Potential sources for this PFAS may be associated with the application of certain firefighting foams around airports [32] and surrounding areas including the Botany Industrial Park [33].

The non-targeted workflow was subsequently applied to the collected DIA data for each sample to detect and identify further PFAS. The application of mass defect filtering returned up to 700 potential PFAS candidates within a sample. The MS/MS data was examined for each candidate to investigate common fragments and neutral losses, reducing the number of potential candidates from 700 to 107. Typical common fragments found in the surface water samples were O_3S^- (m/z 79.9573), $C_5F_5^-$ (m/z 154.9925), $C_5F_8^-$ (m/z 211.9877Da), $C_5F_9^-$ (m/z 230.9861) and $C_6F_{11}^-$ (m/z 280.9829); and common neutral losses such as CO_2 (m/z 43.9898), $CO_2C_2F_4$ (m/z 143.9834), $CO_2C_4F_8$ (m/z 243.9770), $CO_2C_5F_{10}$ (m/z 293.9738), $CO_2C_6F_{12}$ (m/z 343.9706), $CO_2C_7F_{14}$ (m/z 393.9674) or $CO_2C_2F_{16}$ (m/z 443.9642). The detected exact masses, the drift time and corresponding calibrated CCS, the retention time, the signal intensity, matching fragments, and common neutral losses are listed for each sample location in ESM Tables S4 to S10.

Here we developed a combined targeted and non-targeted analysis of PFAS in a single UHPLC-IMS-QTOF-MS run. Targeted analyses remain essential for determining the concentrations of currently regulated compounds, however, given the large number of possible PFAS compounds, targeted analysis may not accurately reflect the actual PFAS abundance in environmental samples. Underestimating the presence of PFAS precludes accurate estimations or conclusions regarding the persistence, and the environmental, eco-toxicological, bioaccumulative, and health impacts of PFAS. This becomes evident when comparing the number of PFAS identified via targeted analysis (3-7) with those via non-targeted analysis (56-107). Among these 56-107, several potential PFAS isomers were observed, which were further discriminated by comparing drift times and the calculated CCS

values (see ESM). LC-HRMS has been used for the identification of PFAS isomers in environmental sample via chromatographic separation [34]. However many PFAS isomers are difficult to separate with reverse-phase chromatography, particularly in non-targeted workflows. IMS provides improved identification and characterisation of isomers via individual drift times and CCS values. Distinguishing PFAS isomers may become more relevant in future studies to correlate compounds across sample locations and to track abundance and occurrence over time.

The major shortcoming of non-targeted PFAS analysis via LC-MS is the inability to quantify total PFAS load in a sample, with only a limited number of species-specific standards available. However, species-unspecific quantification of PFAS is currently an area under investigation. A new paradigm was recently presented in the field of atomic spectroscopy, where any F species may indirectly be quantified by either detecting the emission of F-associated compounds [35], or by analysing polyatomic F-compounds by MS [15,36]. The method presented here identifying the number of PFAS in a sample may therefore be complemented in the future by elemental mass spectrometry to achieve quantitative non-targeted PFAS analysis.

4. Conclusions

This study presented the use of UHPLC-IMS-QTOF-MS for the simultaneous targeted and non-targeted analysis of PFAS in environmental samples, taking advantage of the multi-dimensional features provided by this instrument including drift times/CCS, exact masses, mass defects and mass fragments. Targeted analysis of 14 sulfonated PFAS was validated via MS/MS, with non-targeted analysis using a data analysis workflow that included using the mass defects to identify fluorine-containing compounds, which were further filtered by analysing neutral losses and common fragments. Additionally, the IMS enabled differentiation between isomers of unknown species. The optimised method was applied to surface water samples collected from seven locations across the Cooks River catchment area (Sydney). The targeted component identified 3-7 PFAS, with PFOS the most predominate species. A further 107 PFAS species were identified in one sample via the non-targeted workflow. This work demonstrated that IMS-QTOF-MS is useful for the simultaneous analysis of known PFAS species, along with providing information on the total abundance of emerging PFAS contaminants.

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Conflict of interest

The Vion IMS QTOF is a Waters Australia instrument installed at UTS for collaborative and research use.

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Table 1. Sample information including date, time and coordinates

Sample	Date, Time	Coordinates
A	November 26th 2018, 19:27	-33.903631, 151.097808
B	November 26th 2018, 13:55	-33.929438, 151.138225
C	November 26th 2018, 18:25	-33.923224, 151.153696
D	November 26th 2018, 17:51	-33.930643, 151.162764
E	November 26th 2018, 18:11	-33.930643, 151.162764
F	November 26th 2018, 16:24	-33.948661, 151.167033
G	November 26th 2018, 16:42	-33.958744, 151.198518

501 Table 2. Operating conditions for the Vion IMS-QTOF-MS.

Mass Range	50 to 1000 m/z
Acquisition rate	10 spectra s^{-1}
Collision energy ramp	35 - 75 eV
Capillary voltage	2.3 KV
Cone voltage	20 V
Source temperature	120 °C
Desolvation temperature	450 °C
Cone gas	100 L h^{-1}
Desolvation gas	800 L h^{-1}
Collision gas (for IMS)	N_2

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517 Table 3. Instrument analytical figures of merit for the 14 sulfonate-based PFAS compounds

PFAS compound	Molecular formula	Adduct	Observed m/z	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})	Observed CCS ($Å^2$)	CCS (%RSD)	Instrument response (%RSD)
PFBS	$C_4F_9SO_3H$	[M-H]	298.9441	0.9992	0.19	0.56	129.80	0.06	1.47
PFPeS	$C_5F_{11}SO_3H$	[M-H]	348.9410	0.9972	0.41	1.25	138.10	0.12	1.30
PFHxS[a]	$C_6F_{13}SO_3H$	[M-H]	398.9374	0.9981	0.21	0.65	147.15	0.16	2.14
PFHpS	$C_7F_{15}SO_3H$	[M-H]	448.9338	0.9963	0.36	1.08	155.64	0.09	1.02
PFOS[a]	$C_8F_{17}SO_3H$	[M-H]	498.9377	0.9973	0.24	0.74	164.75	0.09	1.46
PFNS	$C_9F_{19}SO_3H$	[M-H]	548.9278	0.9975	0.42	1.28	173.33	0.02	2.50
PFDS	$C_{10}F_{21}SO_3H$	[M-H]	598.9216	0.9992	0.27	0.81	182.25	0.18	2.56
PFDoA	$C_{11}F_{23}SO_3H$	[M-H]	612.9518	0.9997	0.76	2.30	190.15	0.15	4.82
4:2 FTS	$C_6H_4F_9SO_3H$	[M-H]	326.9750	0.9994	0.32	0.96	148.96	0.16	0.54
6:2 FTS	$C_8H_4F_{13}SO_3H$	[M-H]	426.9683	0.9982	0.57	1.73	165.51	0.04	1.88
8:2 FTS	$C_{10}H_4F_{17}SO_3H$	[M-H]	526.9612	0.9982	0.57	1.74	182.30	0.09	0.99
N-MeFOSAA	$C_{11}H_6F_{17}NSO_4$	[M-H]	569.9677	0.9994	0.34	1.02	189.82	0.08	2.08
N-EtFOSAA	$C_{12}H_8F_{17}NSO_4$	[M-H]	583.9844	0.9990	0.43	1.30	194.22	0.08	3.82
FOSA	$C_8H_2F_{17}NSO_2$	[M-H]	497.9477	0.9984	0.39	1.19	165.91	0.03	2.16

518 [a]All perfluroalkylsulfonates are in the linear form except PFHxS and PFOS which both have linear and various
519 known branched isomers. Here, the most abundant isomer corresponding to the linear form is listed.

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529 Table 4. Summary of observed m/z, retention times, CCS values, drift times and isomer
530 assignment for major PFHxS and PFOS isomers

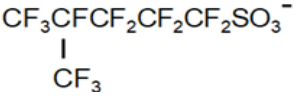
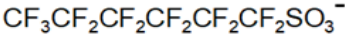
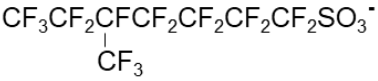
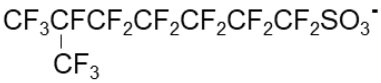
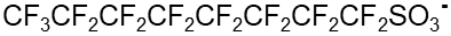
Compound	Peak	Observed m/z	Observed RT (min)	Observed CCS (Å^2)	Observed drift (ms)	Structure
PFHxS	A	398.937	5.65	145.97	3.86	
	B	398.937	5.81	147.15	3.91	
PFOS	C	498.930	7.17	161.53	4.47	
	D	498.930	7.27	163.87	4.56	
	E	498.930	7.56	164.75	4.59	

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Table 5. Targeted PFAS (ng L^{-1}) found in water samples collected from the Cooks River

	PFHpS	PFNS	PFOS	PFHxS	PFDS*	PFBS	FOSA
A	18.6 ± 1.5	21.4 ± 1.2	71.1 ± 3.5	nd	7.7 ± 0.9	nd	26.3 ± 1.6
B	20.8 ± 0.8	23.0 ± 0.4	149.7 ± 2.3	nd	8.5 ± 0.9	45.9 ± 3.1	36.6 ± 0.6
C	nd	21.6 ± 1.6	76.8 ± 8.8	nd	6.9 ± 1.4	19.5 ± 1.9	21.5 ± 0.3
D	nd	20.8 ± 2.7	109.3 ± 9.7	nd	7.5 ± 1.9	nd	20.4 ± 0.6
E	nd	21.7 ± 1.2	257.3 ± 11.2	nd	7.3 ± 1.8	nd	21.8 ± 0.2
F	18.5 ± 1.4	20.4 ± 0.8	80.5 ± 3.3	nd	nd	nd	nd
G	17.6 ± 0.7	21.7 ± 1.5	81.4 ± 3.7	93.6 ± 1.6	7.1 ± 2.3	2.9 ± 1.5*	18.8 ± 0.2

nd= not detected; *=below LOD

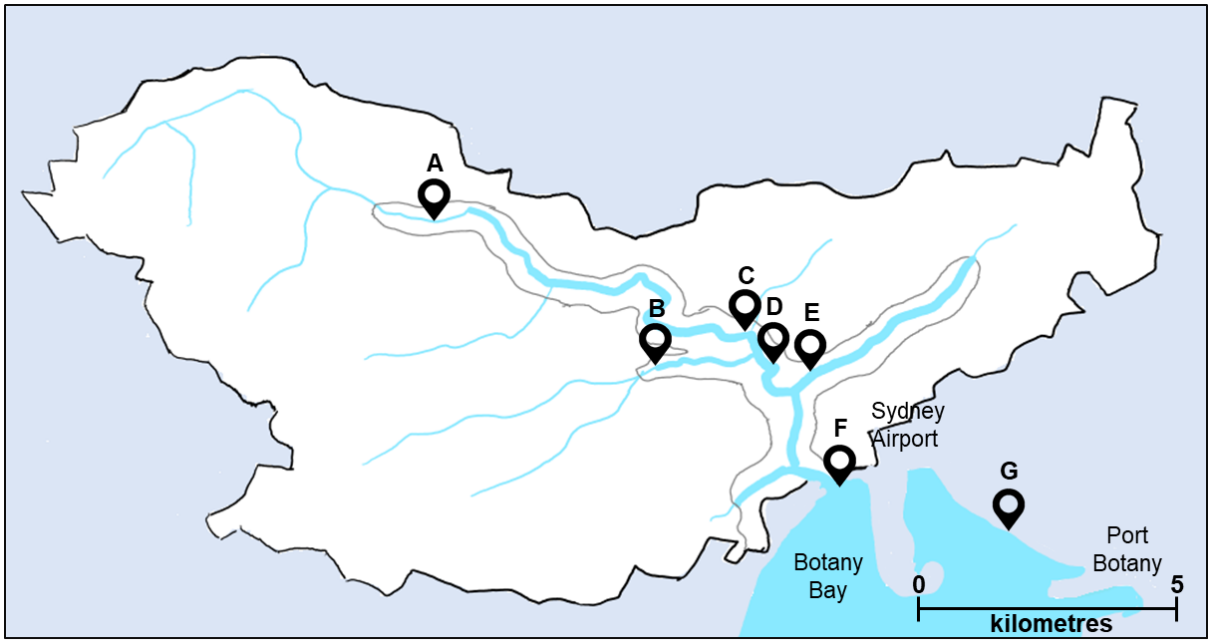


Figure 1. Samples (A-G) collected across the Cooks River catchment area.

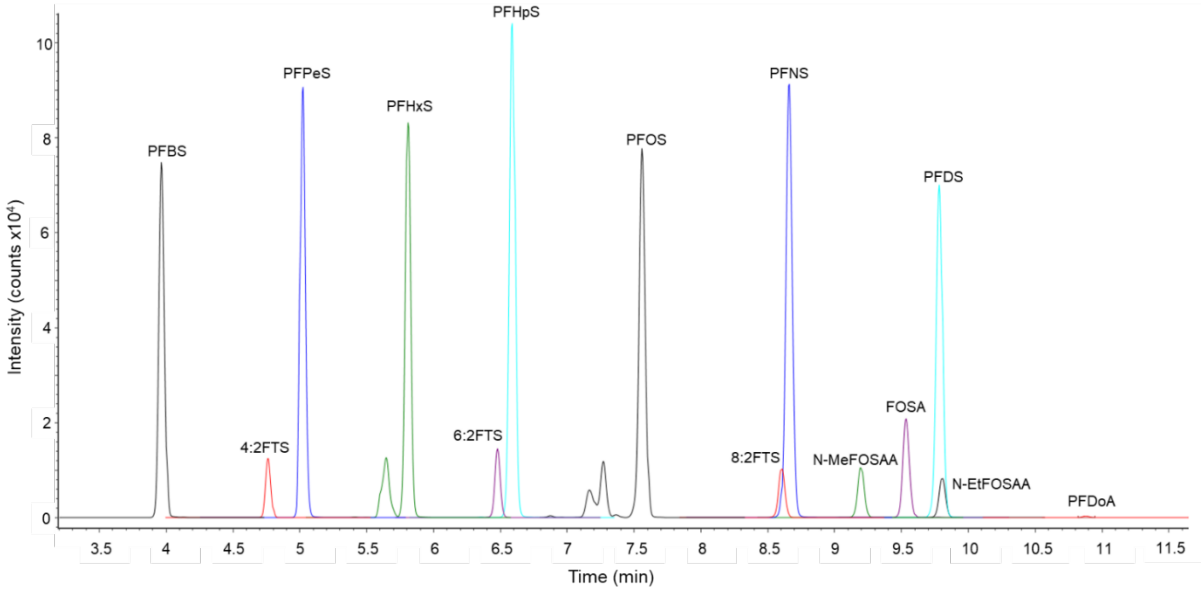
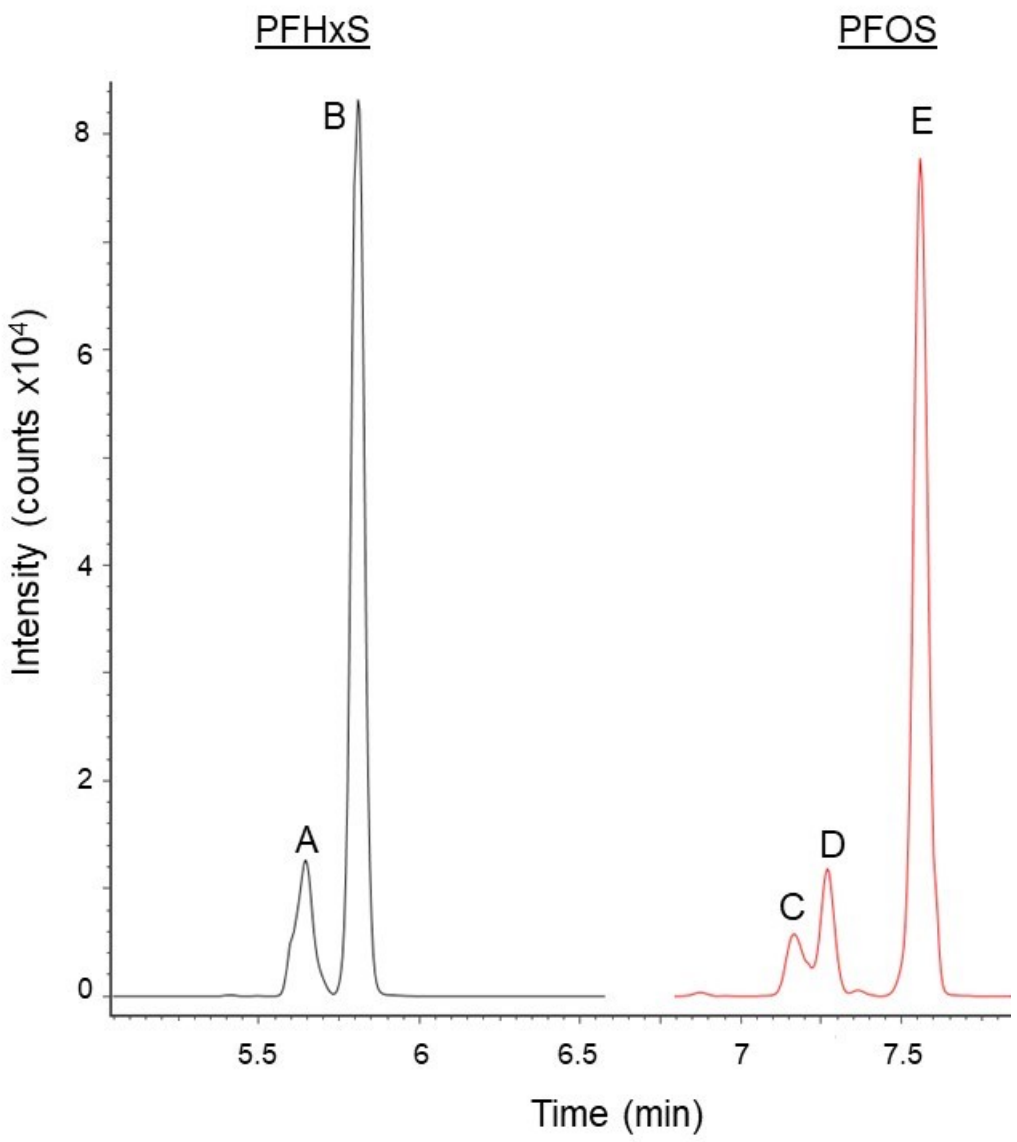


Figure 2. Separated extracted ion chromatograms (XIC) of sulfonate-based PFAS ($C_nF_{2n+1}SO_3H$) following the analysis of a 10 µg L^{-1} standard mix.



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596 Figure 3. Chromatographic separation and detection of linear and branched PFHxS and PFOS
597 isomers via LC-IMS-QTOF.

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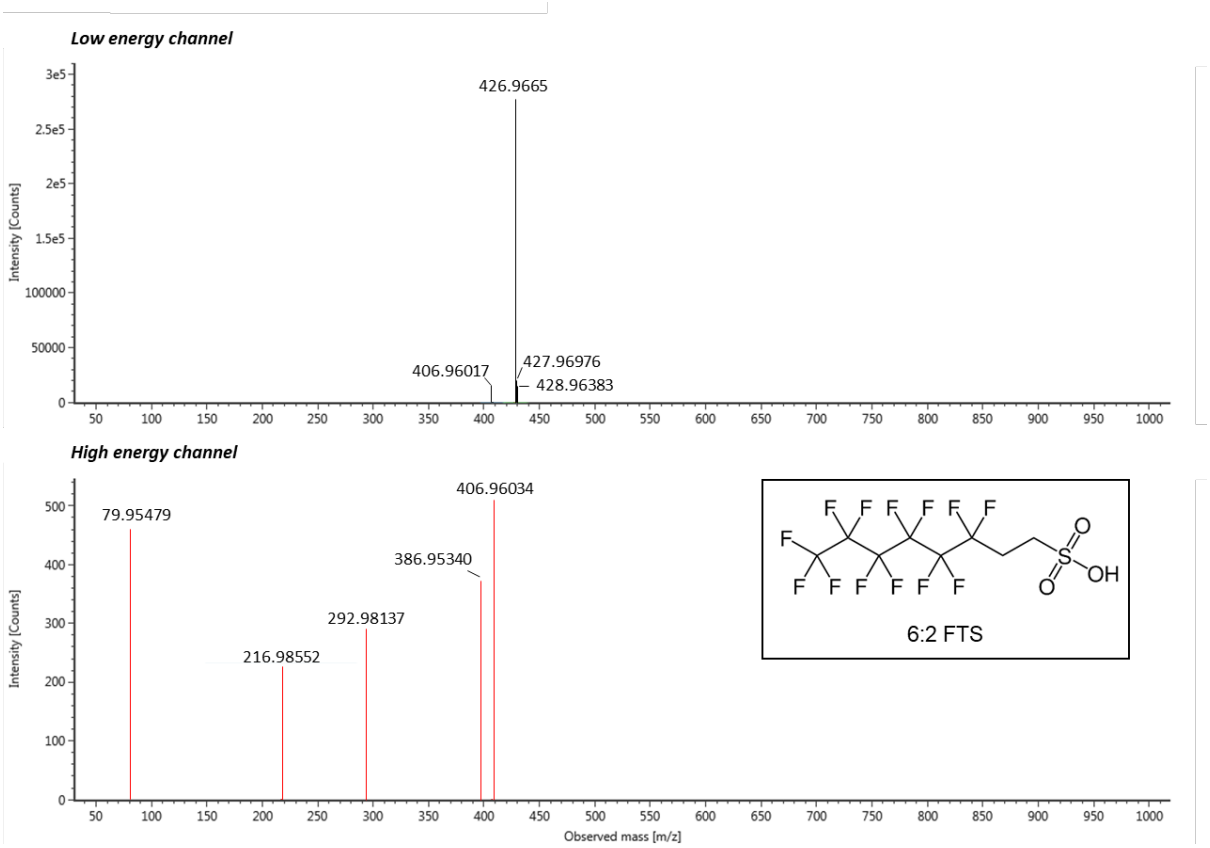

Figure 4. Example of the applicability of the developed workflow. Low (top) and high (bottom) energy m/z CCS curated spectra for 6:2 FTS identified using mass defect and common ion filtering.