Application Note

Approaches to Non-targeted Analyses of Per- and Polyfluoroalkyl Substances (PFAS) in Environmental Samples

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic compounds that are used in a variety of industrial applications and consumer products and are known environmental pollutants. Targeted methods using tandem quadrupole mass spectrometers provide sensitive detection for these compounds, but focus on a limited number of potential PFAS that could be detected. A non-targeted technique provides a more comprehensive characterization of PFAS contamination in a sample. In this study, workflows for non-targeted analyses for PFAS are demonstrated using a Xevo G2-XS QTof coupled with an ACQUITY UPLC I-Class PLUS modified with PFAS kit components. In-house PFAS reference libraries were used to assign putative identities to the compounds detected in wastewater and soil samples. The libraries consisted of accurate masses of molecular ions, fragment ions, isotope patterns and, in cases where reference standards are available, additional chromatographic properties such as retention times, which were used to confidently assign putative identifications to the components. PFAS components detected in the wastewater and soil samples were subsequently quantified using a similar workflow. Discovery of PFAS not present in the UNIFI libraries can be achieved by automatic searching of external databases, aided using additional software tools including common fragments, neutral loss, and mass defect searching. The presented non-targeted methodology can be useful in various scenarios including, but not limited to, discovery of novel PFAS compounds, a better understanding of PFAS contamination in the environment, and source fingerprinting for remediation purposes.

Benefits

- The direct injection approach provides a simplified sample preparation technique to avoid loss of PFAS and biased results
- Local PFAS accurate mass libraries with over 4,000 compounds that are easily customizable
- An analytical solution that uses multiple attributes for component putative identification increasing confidence in the results for legacy and emerging PFAS in environmental samples

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic compounds that are ubiquitous environmental pollutants. Structurally, they consist of aliphatic chains of carbon with fluorine atoms, fully or partially in place of hydrogens. In addition, these substances contain a terminal functional group, for example a carboxylate, phosphonate, alcohol, sulfonate, or sulphonamide.[1-3] The strength of the carbon-fluorine bond confers excellent stability on highly fluorinated carbon compounds making them resistant to degradation and providing a potential for bioaccumulation. Consequently, these compounds have been widely detected in environmental and biological samples. The environmental persistence of these compounds has led to widespread global interest from scientists trying to understand the potential health and environmental effects for humans and wildlife.[4] Due to known adverse health effects, the two most notorious PFAS compounds, PFOA and PFOS, have been banned from use and production in most of the world. In recent years, alternative product chemistries with shorter chain lengths have been designed by manufacturers, with the aim to preserve the utility of the functional properties while attempting to mitigate the resistance to degradation characteristic of the legacy products.[5] Chemical and toxicological information pertaining to the newer formulated products is often not widely available, therefore, the use of analytical methods that can provide data on non-targeted analytes as well as targeted analytes is beneficial and can help to fully assess the environmental distribution of this class of compounds.[4-10] Furthermore, it is estimated that there have been 4,000–6,000 PFAS-like compounds manufactured since the introduction of these chemicals.[11] Current targeted methods utilizing tandem quadrupole mass spectrometers provide sensitive detection for these compounds, but only focus on a small portion of potential PFAS that could be in a sample. A non-targeted technique provides a more comprehensive characterization of PFAS contamination in a sample.

In this study, two workflows for determination of PFAS in water and soil extracts will be demonstrated using data from non-targeted analysis by UltraPerformance Liquid Chromatography (UPLC) and High-Resolution Time-of-flight Mass Spectrometry (TOF-MS). The first case, in-house PFAS reference libraries including, but not limited to, the subclasses perfluoroalkyl carboxylic acids (PFCA), perfluoroalkyl sulfonic acids (PFSA), perfluoroalkyl ether carboxylic acids (PFECA), and fluorotelomer sulfonic acids (FTSA) were used to assign identities to the compounds detected. These libraries were generated in-silico and from experimental data. Mass spectral information is predicted from chemical structures of known components supported by experimental data generated through the analysis of available authentic reference standards. The libraries consisted of accurate masses of pseudo molecular ions, fragment ions, isotope patterns, and in cases where reference standards are available, additional chromatographic properties such as retention times, which were used to identify components. Then, data from remaining components was used to automatically search external databases via ChemSpider.

Experimental

Authentic PFAS standards were purchased from Wellington Laboratories, Ontario, Canada.

Due to the widespread use of PFAS in manufactured products, care must be taken when analyzing samples for PFAS compounds to avoid procedural and instrument contamination. Challenges in sample collection, preparation, and analysis must all be addressed. Caution should be taken to avoid any Teflon or PTFE containing materials. High-density polypropylene (HDPE) containers and vials should be used from collection through analysis. System contamination can occur in PFAS analysis however it can be reduced by taking the appropriate steps. The Waters' PFAS Solution Installation Kit must be installed on the UPLC system prior to use for PFAS analysis. The kit is comprised of PFAS-free components and an isolator column installed between the mobile phase mixer and the injector which helps to delay residual background interferences from potential co-elution with the analytical peaks.[12]

Wastewater and Soil Sample Preparation

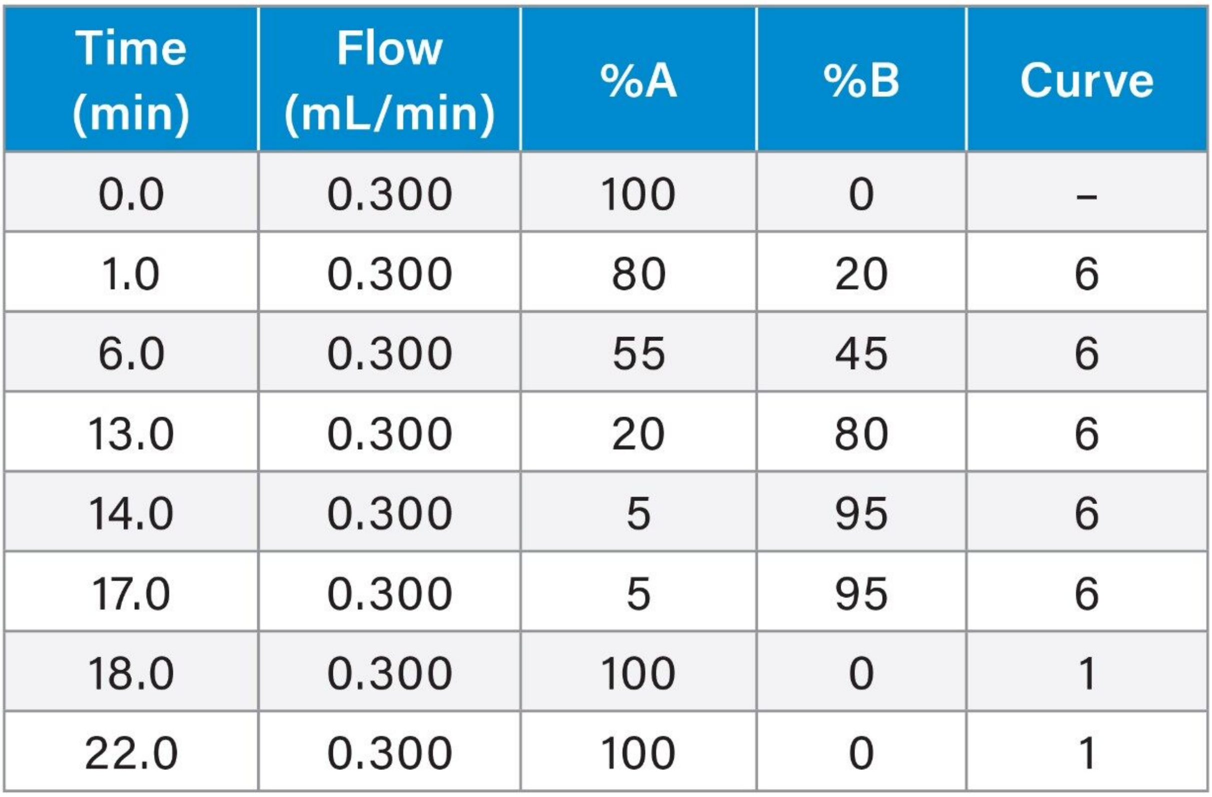
Wastewater and soil samples were kindly provided by collaborators and prepared in accordance with ASTM 7979 and ASTM 7968, respectively. These ASTM methods consist of sample dilution followed by syringe filtration prior to sample analysis. Full details of each method can be found in Waters' application notes 720006329 <https://www.waters.com/content/dam/waters/en/app-notes/2018/720006329/720006329-en.pdf> and 720006764 <https://www.waters.com/content/dam/waters/en/app-notes/2020/720006764/720006764-en.pdf> . Samples were spiked with two ^{13}C isotope labelled internal standards ($^{13}C_8$-PFOA and $^{13}C_8$-PFOS) prior to sample preparation. Water samples studied included surface water, ground water, drinking water, and wastewater. Soil samples studied included sand, silt, lean clay, and fat clay.[13-15]

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS modified with PFAS Kit (p/n: 176004548)
Detection:	Xevo G2-XS QToF Mass Spectrometer ESI Negative Ion
Vials:	Polypropylene autosampler vial and sealed with a polyethylene cap (p/n: 186005230)
Column(s):	ACQUITY UPLC C_{18} 2.1 x 100 mm, 1.7 µm (p/n:

	186002352)
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	30 µL
Flow rate:	0.300 mL/min
Mobile phase A:	95:5 Water:Methanol with 2 mM ammonium acetate
Mobile phase B:	Methanol with 2 mM ammonium acetate

Gradient Table



Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.300	100	0	–
1.0	0.300	80	20	6
6.0	0.300	55	45	6
13.0	0.300	20	80	6
14.0	0.300	5	95	6
17.0	0.300	5	95	6
18.0	0.300	100	0	1
22.0	0.300	100	0	1

MS Conditions

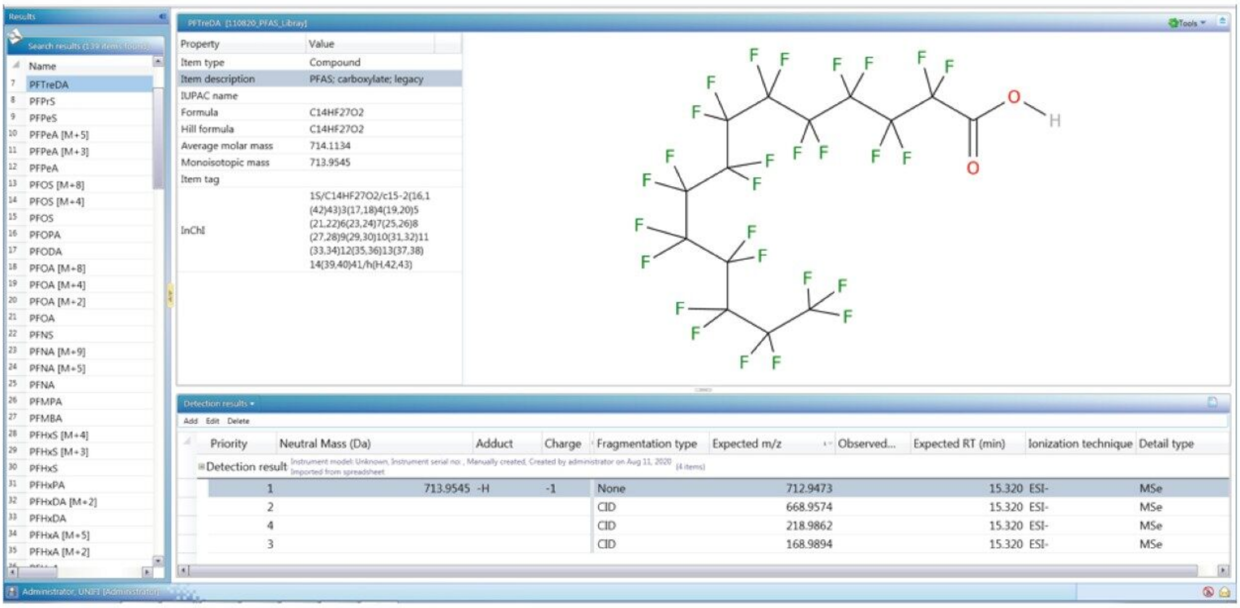
MS system:	Xevo G2-XS QToF Mass Spectrometer
Ionization mode:	ESI- Ion
Acquisition range:	50–1200 Da
Capillary voltage:	0.5 kV
Cone voltage:	10 V
Collision energy:	Low: 4 eV High: 20–70 eV
Desolvation temp.:	350 °C
Source temp.:	100 °C
Desolvation gas flow:	1000 (L/Hr)
Cone gas:	100 (L/Hr)
Lock mass reference:	Leucine Enkephalin, 200 ng/mL

Data Management

Chromatography software:	UNIFI v1.9.4
MS software:	UNIFI v1.9.4
Informatics:	UNIFI v1.9.4

Library Generation

A data independent acquisition mode, known as MS^E, was used to collect accurate mass measurements from precursor and product ions in a single injection.[16] The incidence of false positives is significantly reduced when using multiple attributes (*i.e.* retention time, accurate mass, expected fragments) to search entries in a compound library, greatly increasing confidence in the identifications. The processed data files from the analysis of available authentic standards, in combination with the relevant structural .mol files, were used to create a custom library of compounds of interest. The information can also be imported from a Microsoft Excel file which will then create the library within UNIFI. Additional structures of compounds from the EPA CompTox library, for which no reference standards were available, were used to generate an additional library with the total number of compounds totaling over 4200.[17] These PFAS libraries will be made available for download from Waters' Marketplace (https://marketplace.waters.com/home). An example of a library entry for PFTreDA is shown in Figure 1.



	Name
7	PFTreDA
8	PFPrS
9	PFPeS
10	PFPeA [M+5]
11	PFPeA [M+3]
12	PFPeA
13	PFOS [M+8]
14	PFOS [M+4]
15	PFOS
16	PFOPA
17	PFODA
18	PFOA [M+8]
19	PFOA [M+4]
20	PFOA [M+2]
21	PFOA
22	PFNS
23	PFNA [M+9]
24	PFNA [M+5]
25	PFNA
26	PFMPA
27	PFMBA
28	PFHxS [M+4]
29	PFHxS [M+3]
30	PFHxS
31	PFHxPA
32	PFHxDA [M+2]
33	PFHxDA
34	PFHxA [M+5]
35	PFHxA [M+2]

Property	Value
Item type	Compound
Item description	PFAS; carboxylate; legacy
IUPAC name	
Formula	C14HF27O2
Hill formula	C14HF27O2
Average molar mass	714.1134
Monoisotopic mass	713.9545
Item tag	
InChI	1S/C14HF27O2/c15-2(16,1(42)43)3(17,18)4(19,20)5(21,22)6(23,24)7(25,26)8(27,28)9(29,30)10(31,32)11(33,34)12(35,36)13(37,38)14(39,40)41/h(H,42,43)

Detection result Instrument model: Unknown, Instrument serial no: , Manually created, Created by administrator on Aug 11, 2020 Imported from spreadsheet (4 items)

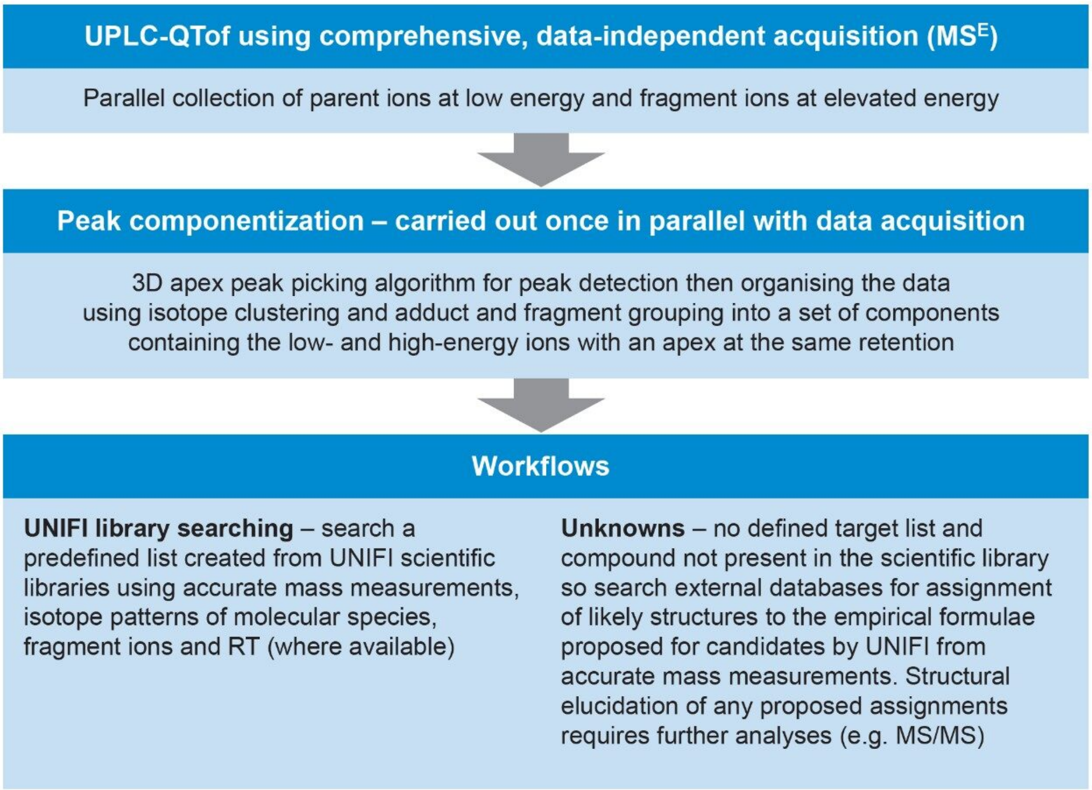
Priority	Neutral Mass (Da)	Adduct	Charge	Fragmentation type	Expected m/z	Observed...	Expected RT (min)	Ionization technique	Detail type
1	713.9545	-H	-1	None	712.9473		15.320	ESI-	MSe
2				CID	668.9574		15.320	ESI-	MSe
4				CID	218.9862		15.320	ESI-	MSe
3				CID	168.9894		15.320	ESI-	MSe

Figure 1. An example of the library entry for PFTreDA (perfluorotetradecanoic acid) showing the expected m/z of the precursor and fragments, adducts, and retention time.

Results and Discussion

The schematic shown in Figure 2 describes the methodology. ACQUITY UPLC coupled with the Xevo G2-XS was used to generate comprehensive datasets for componentization and subsequent evaluation using UNIFI.

Initially, the components are checked against entries from the UNIFI libraries and any positive hits quantified using reference standards if available. The accurate mass measurements of all the ions from each remaining component can then be used to provide elemental compositions to search external databases (e.g. via ChemSpider). Further experiments, such as MS/MS and comparison with data from a reference standard, will ultimately be needed for any confirmation of structure.



UPLC-QTof using comprehensive, data-independent acquisition (MS^E)

Parallel collection of parent ions at low energy and fragment ions at elevated energy

Peak componentization – carried out once in parallel with data acquisition

3D apex peak picking algorithm for peak detection then organising the data using isotope clustering and adduct and fragment grouping into a set of components containing the low- and high-energy ions with an apex at the same retention

Workflows

UNIFI library searching – search a predefined list created from UNIFI scientific libraries using accurate mass measurements, isotope patterns of molecular species, fragment ions and RT (where available)

Unknowns – no defined target list and compound not present in the scientific library so search external databases for assignment of likely structures to the empirical formulae proposed for candidates by UNIFI from accurate mass measurements. Structural elucidation of any proposed assignments requires further analyses (e.g. MS/MS)

Figure 2. An example of the methodology used for non-target analysis.

A range of experiments were carried out using wastewater as well as soil samples. Initially, qualitative experiments were performed to determine which PFAS compounds were detected in the samples. Quantitative experiments were subsequently performed to determine how much of the detected analyte was present.

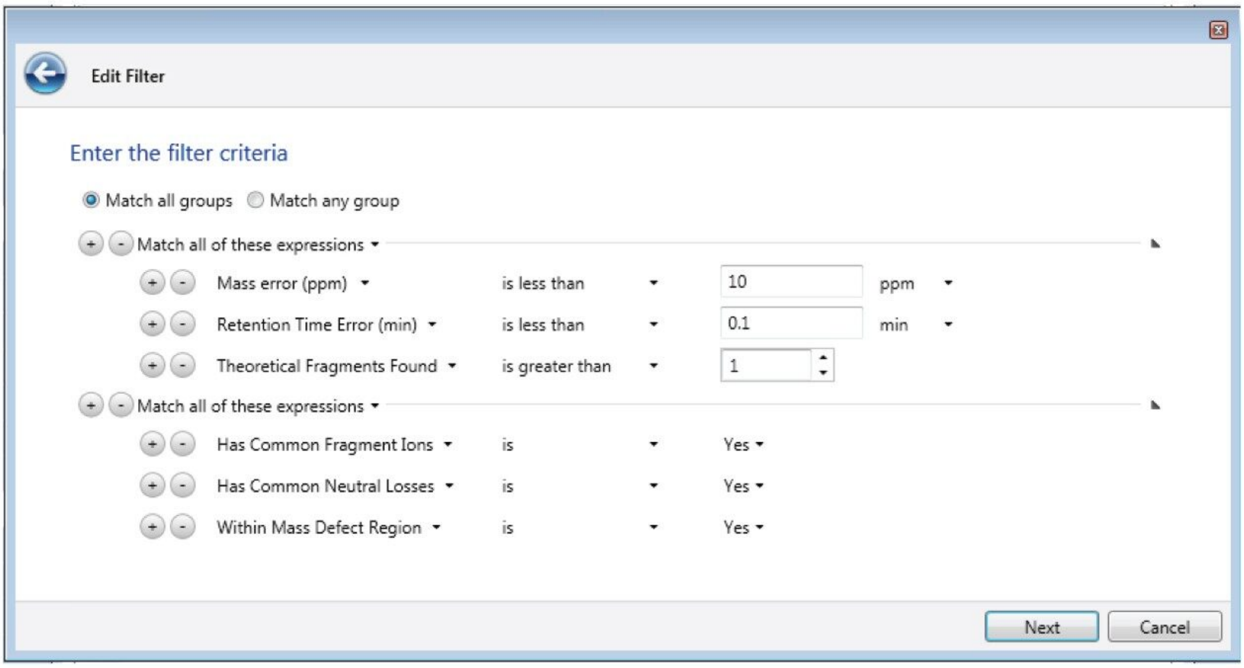
Detection of PFAS in Wastewater Using UNIFI Libraries

The performance of the targeted screening workflow was evaluated by the analysis of wastewater spiked with 30 PFAS compounds. A summary of the criteria used to verify the accuracy of identification of the PFAS detected is shown in Table 1 including the observed *m/z*, mass accuracy, expected and observed retention times, and detected adducts. Each compound spiked into the wastewater blank was detected and correctly assigned with mass accuracy ranging from 0 to -1.1 mDa.

Component name	Neutral mass (Da)	Observed neutral mass (Da)	Observed *m/z*	Mass error (mDa)	Adducts	Expected RT (min)	Observed RT (min)
11Cl-PF3OUdS	631.8965	631.8960	630.8887	-0.5	-H	14.4	14.39
4:2 FTS	327.9816	327.9805	326.9732	-1.1	-H	8.69	8.7
6:2 FTS	427.9752	427.9742	426.967	-0.9	-H	11.57	11.58
8:2 FTS	527.9688	527.9681	526.9609	-0.7	-H	13.43	13.43
9Cl-PF3ONS	531.9029	531.9024	530.8952	-0.4	-H	13.07	13.06
ADONA	377.9762	377.9756	376.9683	-0.6	-H	10.55	10.55
FBSA	298.9663	298.9657	297.9585	-0.5	-H	8.89	8.85
FHxSA	398.9599	398.9596	397.9523	-0.2	-H	12.04	12
FOSA	498.9535	498.9530	497.9458	-0.5	-H	13.92	13.92
GenX	329.9750	329.9749	284.9778	-0.1	-HCO2, -H	9.31	9.31
N-EtFOSAA	584.9903	584.9896	583.9824	-0.6	-H	14.13	14.11
N-MeFOSAA	570.9746	570.9735	569.9662	-1.1	-H	13.78	13.78
PFBA	213.9865	213.9856	168.9885	-0.9	-HCO2, -H	3.96	3.96
PFBS	299.9503	299.9497	298.9424	-0.6	-H	7.32	7.31
PFDA	513.9673	513.9670	512.9597	-0.3	-H	13.43	13.42
PFDoDA	613.9609	613.9606	612.9533	-0.4	-H	14.7	14.71
PFDS	599.9311	599.9308	598.9235	-0.3	-H	14.14	14.11
PFHpA	363.9769	363.9762	362.9689	-0.7	-H	10.43	10.42
PFHpS	449.9407	449.9405	448.9333	-0.2	-H	11.71	11.7
PFHxA	313.9801	313.9790	312.9717	-1.1	-H	8.86	8.85
PFHxS	399.9439	399.9435	398.9362	-0.4	-H	10.59	10.57
PFNA	463.9705	463.9702	462.9629	-0.3	-H	12.6	12.6
PFNS	549.9343	549.9339	548.9266	-0.4	-H	13.43	13.43
PFOA	413.9737	413.9731	412.9658	-0.6	-H	11.64	11.62
PFOA [M+8]	422.0005	422.0003	420.993	-0.3	-H	11.64	11.62
PFOS	499.9375	499.9374	498.9302	-0.1	-H	12.63	12.64
PFOS [M+8]	507.9643	507.9645	506.9572	0.1	-H	12.63	12.63
PFPeA	263.9833	263.9822	262.9749	-1.1	-H	6.7	6.69
PFPeS	349.9471	349.9462	348.939	-0.8	-H	9.15	9.17
PFTreDA	713.9545	713.9545	712.9472	0	-H	15.32	15.32
PFTriDA	663.9577	663.9573	662.95	-0.5	-H	15.09	15.09
PFUnDA	563.9641	563.9634	562.9562	-0.7	-H	14.12	14.12

Table 1. Component summary table showing information for 30 PFAS compounds and two internal standards spiked into wastewater matrix at 1000 ng/L (0.03 ng injected).

To facilitate efficient data review, customizable view filters can be applied to the dataset to limit assignments that only meet certain criteria such as, within specified retention time window, has detected fragments, is within the mass defect region. Using such filters will display only the compounds/components that meet these relevant features (Figure 3).



Edit Filter

Enter the filter criteria

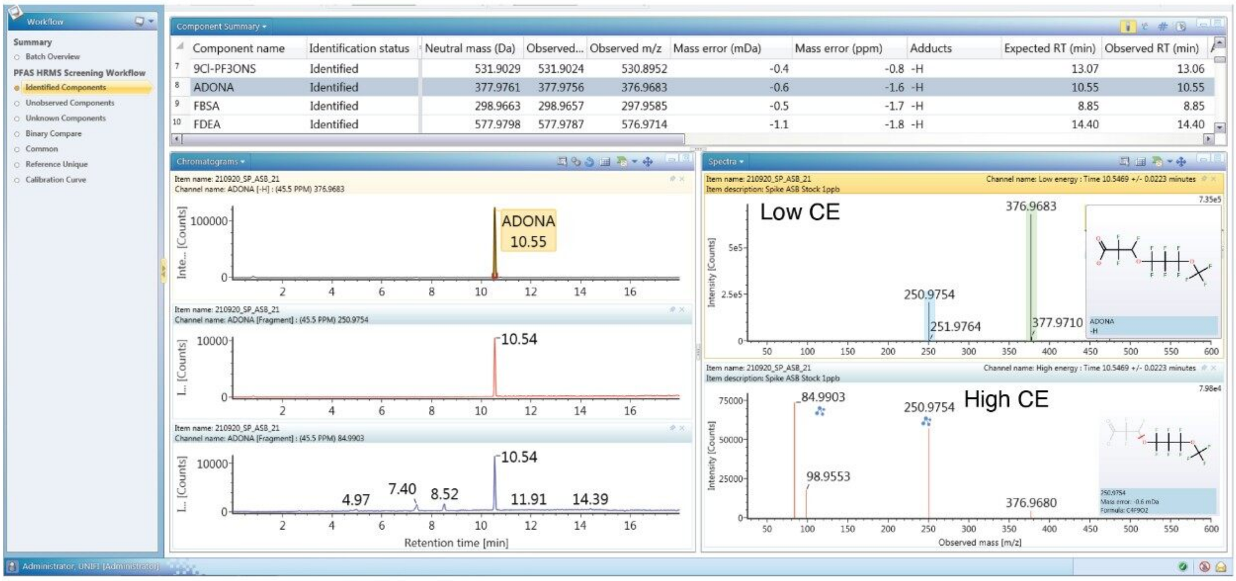
● Match all groups ○ Match any group

Match all of these expressions
- Mass error (ppm) is less than 10 ppm
- Retention Time Error (min) is less than 0.1 min
- Theoretical Fragments Found is greater than 1

Match all of these expressions
- Has Common Fragment Ions is Yes
- Has Common Neutral Losses is Yes
- Within Mass Defect Region is Yes

Figure 3. The View Filter window can be customized to control the way data is presented in the component summary.

The component summary for one of the compounds detected from analysis of wastewater extract spiked with a series of PFAS at 1000 ng/L is shown in Figure 4. The entry for ADONA is highlighted, displaying the precursor and fragment EIC, associated spectral data and structural assignments. A customizable workflow is shown on the left side of the figure. Each entry in the list can be changed to represent the natural progression of data review for any analysis. The Identified Components step is selected automatically displaying all library components that have been identified by the software. The component summary table shows the information pertaining to the identified analyte including, *m/z*, mass error, retention time, and detected adduct. The structure of the analyte is displayed as well as the predicted fragment structure and associated mass error. Availability of this information in one view allows for easy interpretation of the results and increases confidence in the identifications.

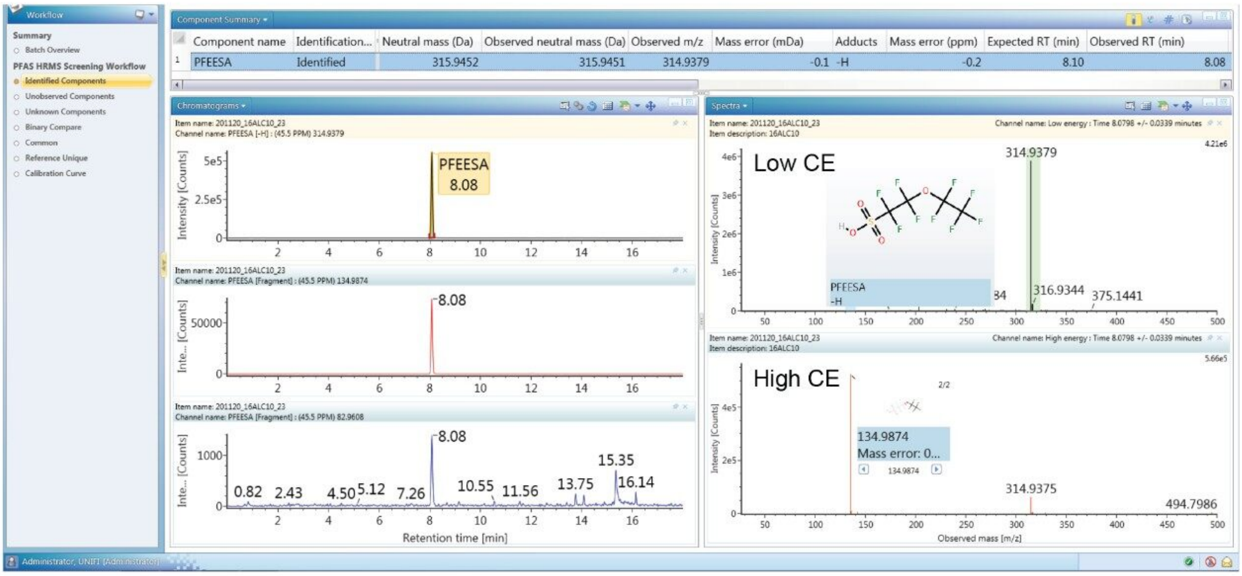


	Component name	Identification status	Neutral mass (Da)	Observed...	Observed m/z	Mass error (mDa)	Mass error (ppm)	Adducts	Expected RT (min)	Observed RT (min)
7	9Cl-PF3ONS	Identified	531.9029	531.9024	530.8952	-0.4	-0.8	-H	13.07	13.06
8	ADONA	Identified	377.9761	377.9756	376.9683	-0.6	-1.6	-H	10.55	10.55
9	FBSA	Identified	298.9663	298.9657	297.9585	-0.5	-1.7	-H	8.85	8.85
10	FDEA	Identified	577.9798	577.9787	576.9714	-1.1	-1.8	-H	14.40	14.40

Figure 4. Identification of ADONA in a wastewater sample extract at 1000 ng/L, 30 µL injection (0.03 ng injected). EIC for the precursor (m/z 376.9683, mass error -0.6 mDa), and the fragments (m/z 250.9754 and 84.9903, -0.6 mDa, and -0.3 mDa respectively). Low collision energy (top) and high collision energy spectra are shown.

Detection of PFEESA in Soil Using UNIFI Libraries

Data from the analysis of a soil sample was processed in the same way. The first step of data review in UNIFI displays all the detected compounds that were assigned from searching the PFAS libraries. These compounds are listed in the component summary table. Figure 5 shows an example of the component summary table for the detection and assignment of PFEESA. In the chromatogram window, extracted ion chromatograms (EIC) are generated for the precursor and any detected fragments recorded in the library. The spectrum for the precursor is displayed in the low collision energy data (top) with the high energy fragmentation spectrum shown beneath. The structure assigned to the fragment is generated using an *in silico* fragmentation algorithm.



	Component name	Identification...	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Adducts	Mass error (ppm)	Expected RT (min)	Observed RT (min)
1	PFEESA	Identified	315.9452	315.9451	314.9379	-0.1	-H	-0.2	8.10	8.08

Item name: 201120_16ALC10_23
Channel name: PFEESA [-H] : (45.5 PPM) 314.9379

Item name: 201120_16ALC10_23
Channel name: PFEESA [Fragment] : (45.5 PPM) 134.9874

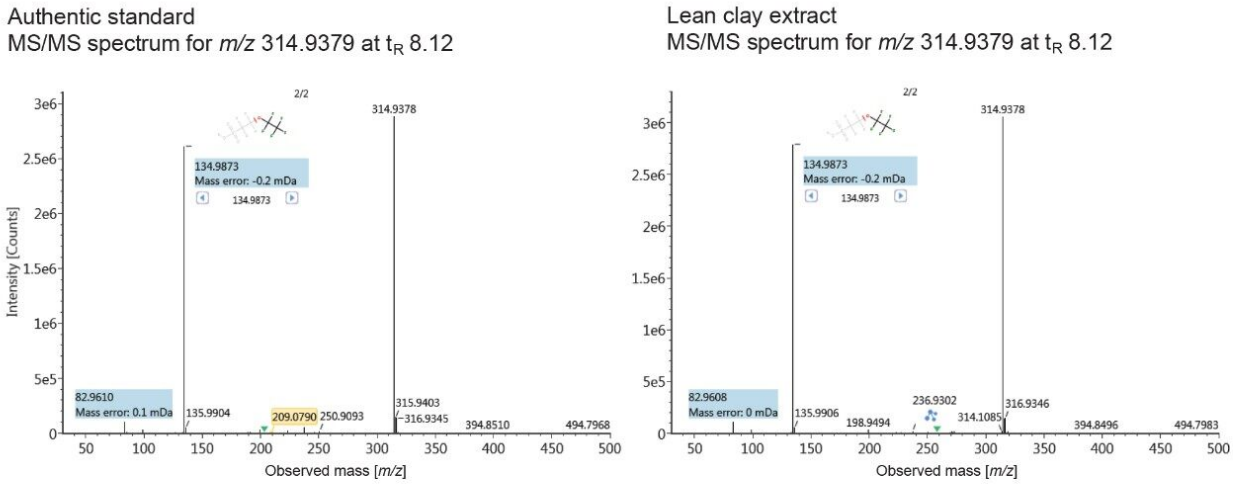
Item name: 201120_16ALC10_23
Channel name: PFEESA [Fragment] : (45.5 PPM) 82.9608

Item name: 201120_16ALC10_23
Item description: 16ALC10
Channel name: Low energy : Time 8.0798 +/- 0.0339 minutes

Item name: 201120_16ALC10_23
Item description: 16ALC10
Channel name: High energy : Time 8.0798 +/- 0.0339 minutes

Figure 5. Identification of PFEESA in extracted lean clay soil sample. EIC for the precursor (m/z 314.9379, mass error -0.1 mDa), and fragments (m/z 134.9874, mass error 0.0 mDa; m/z 82.9608, mass error 0.0 mDa) as well as low collision energy (top) and high collision energy (bottom) spectra and associated structures.

Confirmatory targeted MS/MS experiments were subsequently carried out on the suspect positives from the initial screening data, using a fixed collision energy of 17ev. When the spectrum for the chromatographic peak, eluting at a retention time of 8.10 minutes for an authentic standard of PFEESA, was compared with the peak at the same retention time in the extracted soil sample, they were found to match as is shown in Figure 6.



Authentic standard

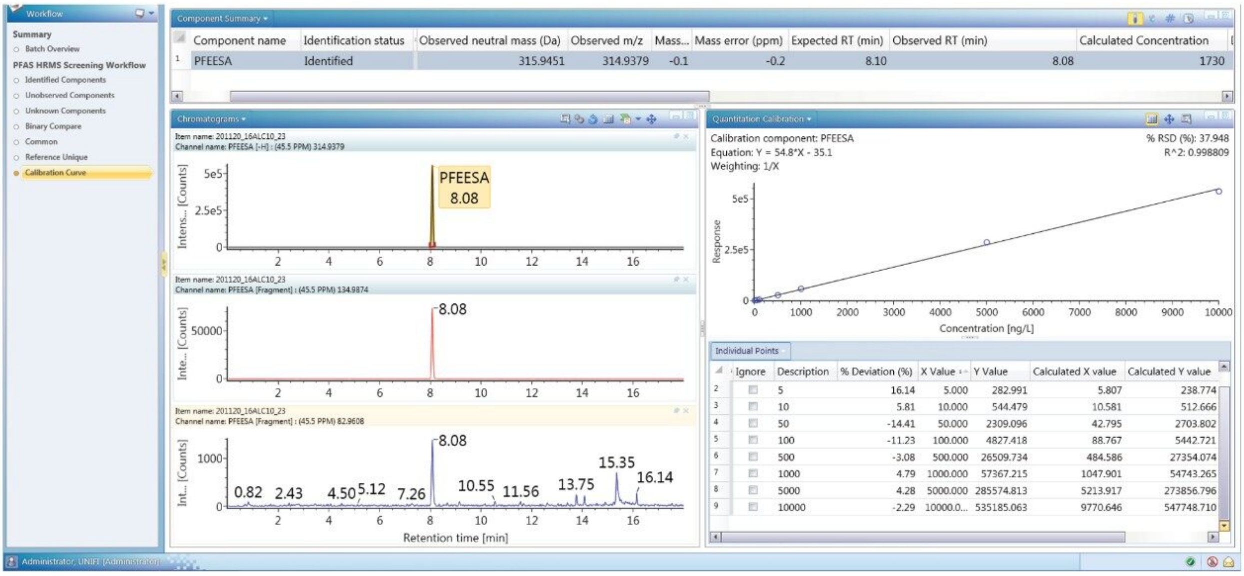
MS/MS spectrum for *m/z* 314.9379 at t_R 8.12

Lean clay extract

MS/MS spectrum for *m/z* 314.9379 at t_R 8.12

Figure 6. Confirmatory targeted MS/MS experiments for the target m/z 314.9379, in an authentic standard and in extracted lean clay soil sample. The predicted fragment structures and mass errors are also shown.

With the same MS and LC methods, quantitative experiments were performed to determine the concentration of PFEESA in the soil sample, based on a calibration curve prepared in solvent (50/50 methanol water with 0.1% acetic acid), using the EIC of the precursor *m/z* (Figure 7). The concentration of PFEESA in the sample extract was determined to be 1730 ng/L or 8.65 ng/g in the original soil sample.



	Component name	Identification status	Observed neutral mass (Da)	Observed m/z	Mass...	Mass error (ppm)	Expected RT (min)	Observed RT (min)	Calculated Concentration
1	PFEESA	Identified	315.9451	314.9379	-0.1	-0.2	8.10	8.08	1730

Item name: 201120_16ALC10_23
Channel name: PFEESA [-H] : (45.5 PPM) 314.9379

Item name: 201120_16ALC10_23
Channel name: PFEESA [Fragment] : (45.5 PPM) 134.9874

Item name: 201120_16ALC10_23
Channel name: PFEESA [Fragment] : (45.5 PPM) 82.9608

Calibration component: PFEESA
Equation: Y = 54.8*X - 35.1
Weighting: 1/X
% RSD (%): 37.948
R^2: 0.998809

	Ignore	Description	% Deviation (%)	X Value	Y Value	Calculated X value	Calculated Y value
2	☐	5	16.14	5.000	282.991	5.807	238.774
3	☐	10	5.81	10.000	544.479	10.581	512.666
4	☐	50	-14.41	50.000	2309.096	42.795	2703.802
5	☐	100	-11.23	100.000	4827.418	88.767	5442.721
6	☐	500	-3.08	500.000	26509.734	484.586	27354.074
7	☐	1000	4.79	1000.000	57367.215	1047.901	54743.265
8	☐	5000	4.28	5000.000	285574.813	5213.917	273856.796
9	☐	10000	-2.29	10000.0...	535185.063	9770.646	547748.710

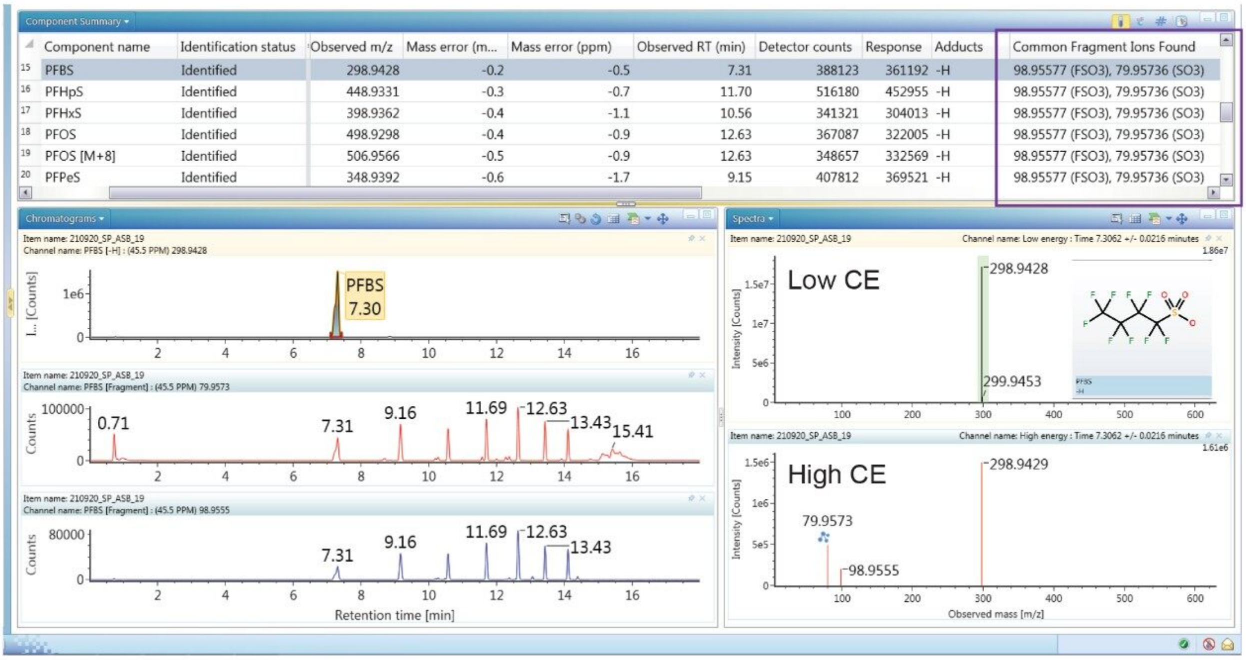
Figure 7. EIC for PFEESA precursor (m/z 314.9379) and fragments (m/z 134.9874, m/z 82.9608) PFEESA measured in a lean clay soil extract at 1730 ng/L (8.65 ng/g in the soil). A linear calibration curve over the range (5–10,000 ng/L) is also shown.

Structural Elucidation Tools

Common Fragment Searching

UNIFI Software has a suite of tools that can aid in identifying and elucidating PFAS present in samples, but which are absent from the UNIFI libraries. The tools most beneficial to helping assign any remaining PFAS in the sample include common fragments, neutral loss, and mass defect searching. Utilization of all the elucidation tools is employed after componentization, so a single set of data can be continuously queried and interpreted in new ways without having to re-process samples.

The chemical structures for many PFAS subclasses often have similar substructures which can lead to shared diagnostic fragment ions when they are subjected to collision induced dissociation (CID). The common structural information can be used in suspect analyses. Once common fragments have been identified they can be entered in the search criteria of the processing method for automated searching of these shared structural features. The Component Summary table, in Figure 8 displays the identifications for PFAS with the common fragments, *m/z* 79.9573 (SO_3) and *m/z* 98.9557 (FSO_3). The fragment EIC also reflects the multiple components in the sample that possess these common features. Filtering for common fragments is one strategy in the identification of potential unknown PFAS that may not be present in the library. Once a potential identification has been made using common fragment filtering, the component can be interrogated using the Discovery tool to identify a potential structure for the unknown component.



	Component name	Identification status	Observed m/z	Mass error (m...	Mass error (ppm)	Observed RT (min)	Detector counts	Response	Adducts	Common Fragment Ions Found
15	PFBS	Identified	298.9428	-0.2	-0.5	7.31	388123	361192	-H	98.95577 (FSO3), 79.95736 (SO3)
16	PFHpS	Identified	448.9331	-0.3	-0.7	11.70	516180	452955	-H	98.95577 (FSO3), 79.95736 (SO3)
17	PFHxS	Identified	398.9362	-0.4	-1.1	10.56	341321	304013	-H	98.95577 (FSO3), 79.95736 (SO3)
18	PFOS	Identified	498.9298	-0.4	-0.9	12.63	367087	322005	-H	98.95577 (FSO3), 79.95736 (SO3)
19	PFOS [M+8]	Identified	506.9566	-0.5	-0.9	12.63	348657	332569	-H	98.95577 (FSO3), 79.95736 (SO3)
20	PFPeS	Identified	348.9392	-0.6	-1.7	9.15	407812	369521	-H	98.95577 (FSO3), 79.95736 (SO3)

Figure 8. Component Summary showing the list of analytes that share the common fragments m/z 79.9573 and 98.9557 in the spiked wastewater sample. Fragment EIC show additional components possessing one or both fragments.

Neutral Loss and Mass Defect Searching

Neutral loss searching can extract data pertaining to subclasses of PFAS that share structural features and undergo loss of common neutral moieties during CID. The software algorithm searches within a defined mass tolerance range, for the precursor accurate mass – neutral loss accurate masses that has been specified in the target list. This approach can be useful in discovery situations to determine novel components with similar substructures to known compounds.

The loss of CO2HFHF is common to the telomer acids, FHEA, FOEA, and FDEA. All three components are identified and grouped together based on target neutral loss list in the processing method and a view filter to display components where neutral losses were detected (Figure 9). Information from the reference library can be used to populate the processing method to aid in identifying related compounds and homologous series.

Mass defect screening can also be used to search for potential unknown PFAS compounds. Many of the hydrogens are substituted for fluorine atoms leading to low or negative mass defects which, when combined with other data filtering methods, can aid in discovery and identification.[7]

These structural elucidation tools can be used to help interrogate the data further, to look for the presence of novel PFAS that are not in the scientific library.

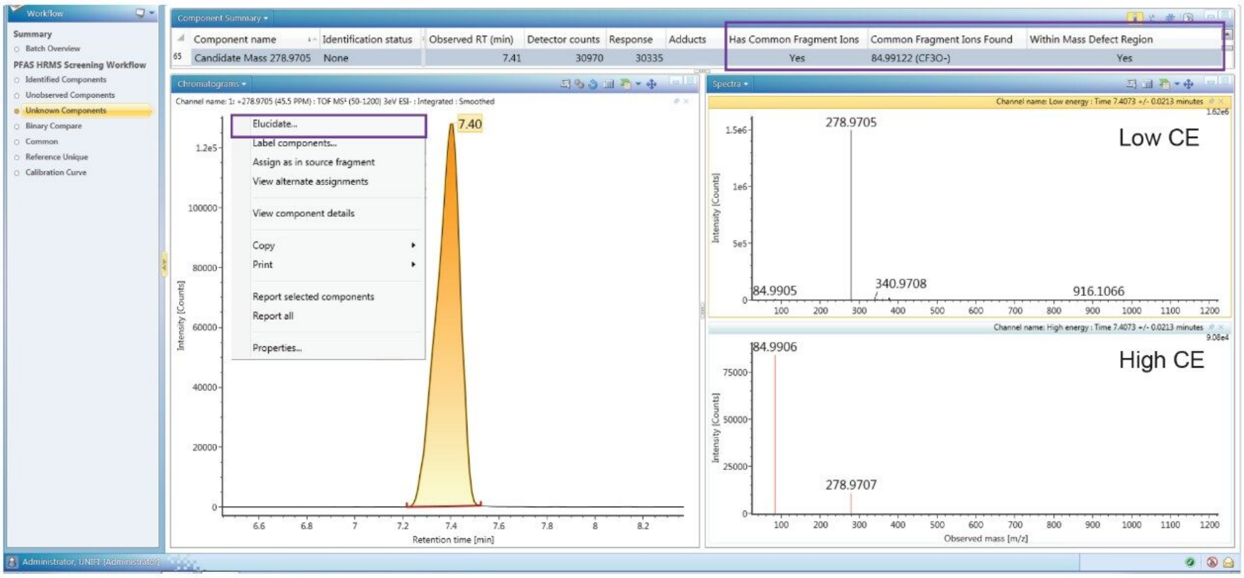
Component Summary

	Component name	Identification status	Observed m/z	Mass...	Mass error (ppm)	Observed RT (min)	Detector counts	Response	Adducts	Common Neutral Losses Found
18	FDEA	Identified	576.9719	-0.6	-1.1	14.40	7662	4995	-H	84.00229 [CO2HFHF]
19	FHEA	Identified	376.9841	-1.2	-3.1	10.78	7526	2541	-H	84.00229 [CO2HFHF]
20	FOEA	Identified	476.9784	-0.5	-1.0	12.92	6444	6444	-H	84.00229 [CO2HFHF]

Figure 9. Component summary displays the telomer acids FDEA, FHEA, and FOEA which have been identified. The common loss of CO2HFHF has been noted by the software.

Detection of other PFAS in Wastewater that are not in the UNIFI Libraries

The UNIFI Discovery tool can be used to identify components that are not present in an existing UNIFI library. The Discovery tool investigates the unknown candidate masses of interest by using all the information provided in the raw data including the accurate mass of the precursor and that of the fragments to generate a list of possible molecular formulae to use to automatically search an external database, such as ChemSpider. Data review of the unidentified components detected in a wastewater sample revealed a suspect entity that was within the mass defect region and had been flagged by the software as having a common fragment (Figure 10).



	Component name	Identification status	Observed RT (min)	Detector counts	Response	Adducts	Has Common Fragment Ions	Common Fragment Ions Found	Within Mass Defect Region
65	Candidate Mass 278.9705	None	7.41	30970	30335		Yes	84.99122 (CF3O-)	Yes

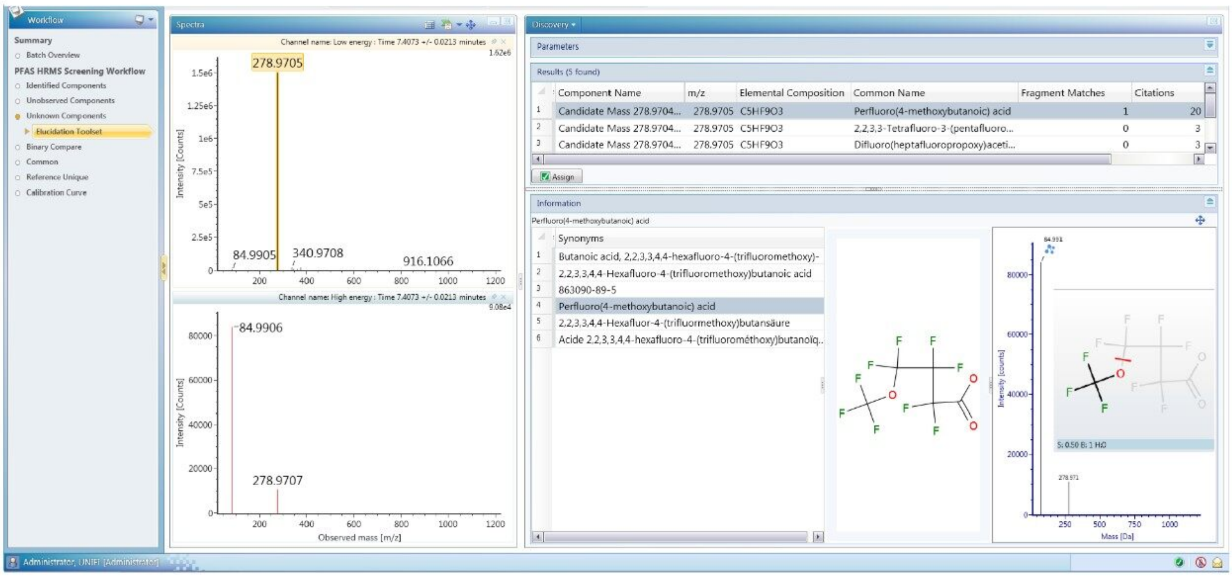
Channel name: 1: +278.9705 (45.5 PPM) : TOF MS² (50-1200) 3eV ESI- : Integrated : Smoothed

Channel name: Low energy : Time 7.4073 +/- 0.0213 minutes

Channel name: High energy : Time 7.4073 +/- 0.0213 minutes

Figure 10. Component summary displays an unknown component detected in wastewater with m/z 278.9705 at a retention time of 7.41 min. Common fragment and mass defect searching have flagged the candidate as a component of interest.

Elucidation of the suspect component revealed several possible proposals, however, only one proposed structure from the list had a matching fragment. The top match proposed, with its elemental composition of $C_5HF_9O_3$, was perfluoro(4-methoxybutanoic) acid. The number of citations for this component was also the highest, indicating how often the proposal has been referenced in the database. In addition to the elemental composition and common name of the proposal, the table provides information on the number of fragment matches and citations. The proposals can be ranked to reveal which structure had the most fragment matches and/or citations which help to prioritize the most likely candidate in the list (Figure 11). The synonyms for each of the proposals are also listed which can be useful for identification if the common name is not the most frequently used identifier.



Results (5 found)

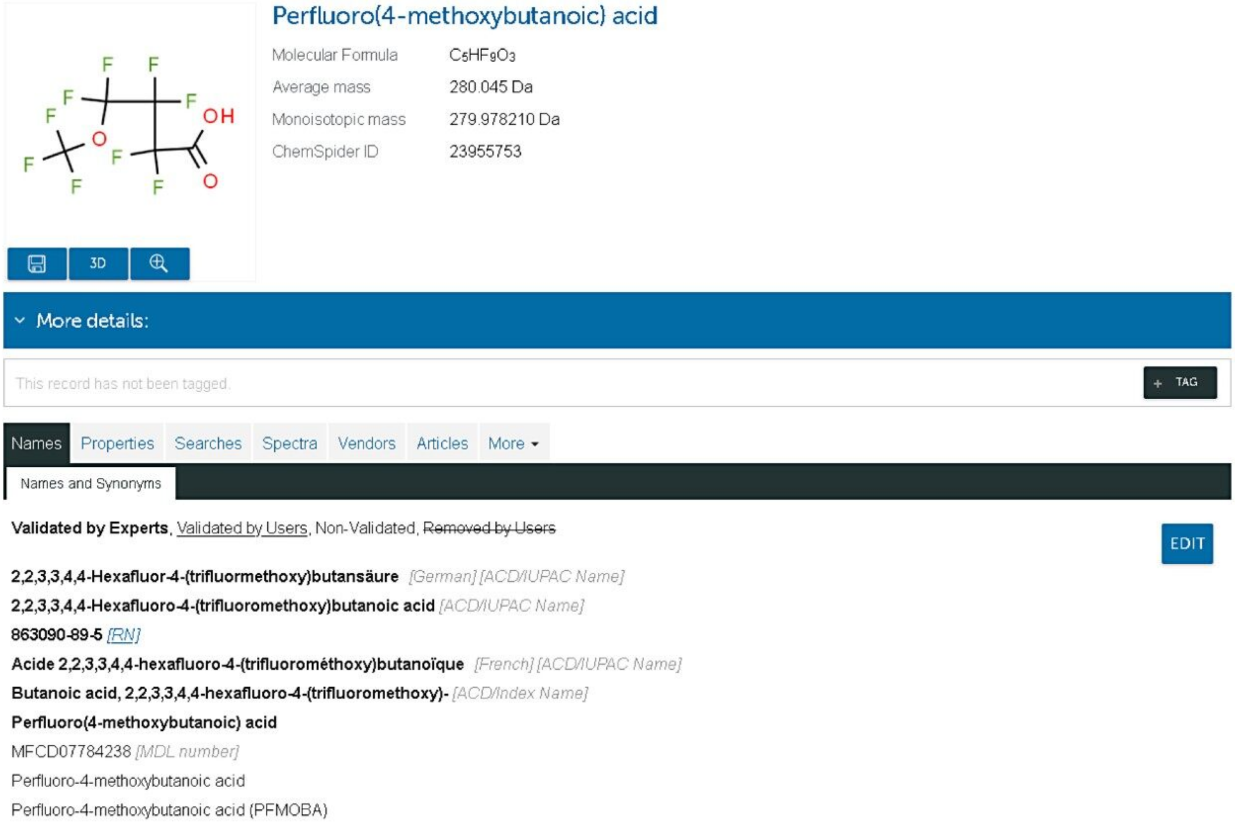
	Component Name	m/z	Elemental Composition	Common Name	Fragment Matches	Citations
1	Candidate Mass 278.9704...	278.9705	C5HF9O3	Perfluoro(4-methoxybutanoic) acid	1	20
2	Candidate Mass 278.9704...	278.9705	C5HF9O3	2,2,3,3-Tetrafluoro-3-(pentafluoro...	0	3
3	Candidate Mass 278.9704...	278.9705	C5HF9O3	Difluoro(heptafluoropropoxy)aceti...	0	3

Perfluoro(4-methoxybutanoic) acid

	Synonyms
1	Butanoic acid, 2,2,3,3,4,4-hexafluoro-4-(trifluoromethoxy)-
2	2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid
3	863090-89-5
4	Perfluoro(4-methoxybutanoic) acid
5	2,2,3,3,4,4-Hexafluor-4-(trifluormethoxy)butansäure
6	Acide 2,2,3,3,4,4-hexafluoro-4-(trifluorométhoxy)butanoïq...

Figure 11. The UNIFI Discovery Tool can be used to view suspect candidates. The common name, number of fragments matched, and citations are listed in the table. The proposed precursor and product fragment structures are also shown.

More information about the proposed candidates can be found by accessing the ChemSpider entry from within the UNIFI Software (Figure 12).



Perfluoro(4-methoxybutanoic) acid

Molecular Formula $C_5HF_9O_3$
Average mass 280.045 Da
Monoisotopic mass 279.978210 Da
ChemSpider ID 23955753

Validated by Experts, Validated by Users, Non-Validated, ~~Removed by Users~~

2,2,3,3,4,4-Hexafluor-4-(trifluormethoxy)butansäure *[German] [ACD/IUPAC Name]*
2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid *[ACD/IUPAC Name]*
863090-89-5 *[RN]*
Acide 2,2,3,3,4,4-hexafluoro-4-(trifluorométhoxy)butanoïque *[French] [ACD/IUPAC Name]*
Butanoic acid, 2,2,3,3,4,4-hexafluoro-4-(trifluoromethoxy)- *[ACD/Index Name]*
Perfluoro(4-methoxybutanoic) acid
MFCD07784238 *[MDL number]*
Perfluoro-4-methoxybutanoic acid
Perfluoro-4-methoxybutanoic acid (PFMOBA)

Figure 12. ChemSpider database entry for perfluoro(4-methoxybutanoic) acid.

Confirmation of the compound identity was made by matching the retention time and fragmentation using confirmatory MS/MS experiments and an authentic standard of perfluoro(4-methoxybutanoic) acid (data not shown). Once unknown components have been identified, the detection results can be saved to the UNIFI library and targeted in subsequent searches.

Conclusion

A custom library greatly aided in the assignment of the PFAS compounds detected in the wastewater and soil samples. The library consisted of compound structures, accurate mass as well as additional properties such as retention time and fragment ions which were created based on available authentic standards. Component identification was based on multiple attributes including retention time, accurate mass precursors, and fragmentation patterns as well as isotopic distributions, increasing confidence in the assignments made. Additional PFAS structural files can easily be added to the library to facilitate screening of larger numbers of analytes. The generation of libraries containing component specific spectral and chromatographic fingerprints combined with information pertaining to known structural features can be a useful tool in the discovery of legacy and novel PFAS compounds providing increased knowledge of their presence and distribution in the environment.

Additionally, the UNIFI Software provides a set of tools to aid in the identification of potential unknown PFAS not present in the library, using common fragments, neutral loss, and mass defect searching. Utilization of these tools allows for easy filtering of potential unknown PFAS to decrease the number of possible identifications to a manageable list of the most likely candidates. All processing is independent of data collection which means a single method and injection can be used to collect data, but data can continuously be queried and filtered in different ways to mine the data for unknown PFAS. The combination of library searching, and elucidation tools allow for in depth screening of environmental samples for a broad range of PFAS that is not achievable using targeted MS/MS techniques. A non-targeted technique utilizing the Xevo G2-XS QTof in combination with UNIFI data processing can be employed in various scenarios including, but not limited to, discovery of new PFAS compounds, a better understanding of PFAS contamination in the environment, and source fingerprinting for remediation purposes.

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