Game of Unknowns: Non-Target Analysis Strategies for Identifying Per- and Polyfluoroalkyl Substances (PFAS) in Water

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Introduction

Per/Polyfluoroalkyl substances (PFAS), are compounds that have uniquely desirable properties for use in various industries. However, their wide-ranging use leads to emission into the environment, and as PFAS are persistent and bioaccumulate in the environment and wildlife, they are contaminants of concern. Monitoring PFAS precursors present in an environmental sample may impact decisions in treatment processes at remediation sites and help deduce possible degradation products that could exist in the environment. Consequently, scientists are contributing newly identified PFAS structures and spectra to various publicly available databases: growing the list of precursors and degradation by-products, some listing thousands of PFAS.

There are estimated to be >4,000 PFAS in the environment while analytical standards for only around ~100 are readily available. Thus, we need strategies for screening and identifying a larger array of PFAS using high-resolution accurate mass (HRAM) mass spectrometry like quadrupole time of flight (Q/TOF) and sophisticated yet user friendly software tools. This study summaries some approaches to screen and identify new and emerging PFAS on an LC-Q/TOF instrument without the need for every analytical standard.

Quantification with HRMS

Quantification of a target list of PFAS using a LC-Q/TOF instrument is possible due to its improved sensitivity and robustness. In this study, a 5 mL sample of water was diluted with 5 mL of methanol, acidified with acetic acid, filtered and centrifuged. The a 30 µL injection of the 50/50 methanol/water was injected to the Agilent 1290 Infinity II LC coupled to a 6546 Q-TOF (Santa Clara, USA) to quantify 22 PFAS across multiple classes. Fig 1 denoted the chromatogram of the 22 PFAS.

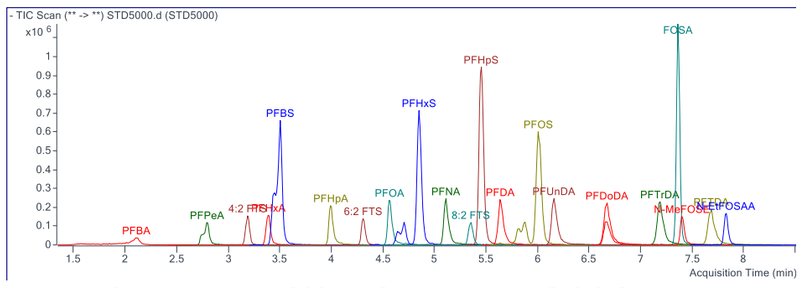


Fig 1. Chromatogram of 22 PFAS analyzed on LC-Q/TOF

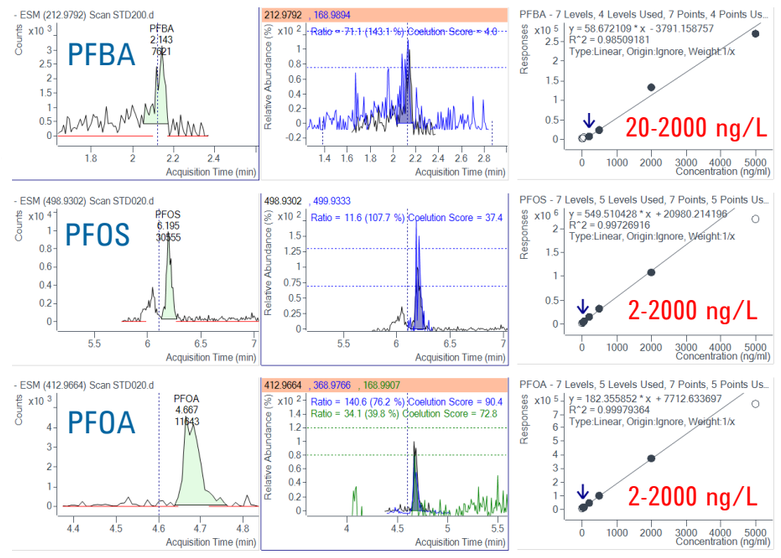


Fig 2. Low level EICs and calibration curves for PFBA, PFOS and PFOA

Screening Strategies for PFAS

Using Libraries & Databases

One of the most common ways to use a LC-Q/TOF and screen for compounds including PFAS is to use personal compound databases (PCD) or libraries (PCDL). There are several different forms of this but typically a PCD is defined as a group of compounds (PFAS in this case) where information including molecular mass, formula and optionally retention time is provided. A PCDL includes the information included in a PCD but in additional also has collected MS/MS spectra to provide fragment information thus adding additional specificity and confidence in identification. These can be commercially curated and available or user-collected.

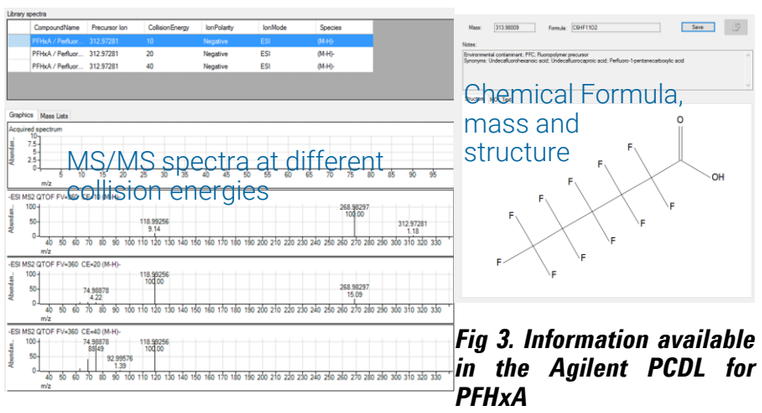
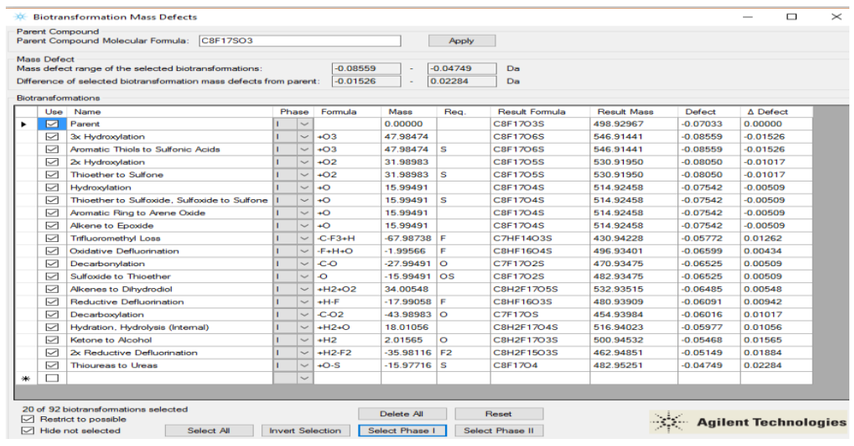


Fig 3. Information available in the Agilent PCDL for PFHxA

Increasing scope with Metabolite and Transformation Products

PFAS are stable in the environment generally but it is possible for them to degrade and transform in the environment especially under harsh conditions or during certain weathering and water treatment processes. To account for this, it can be useful to increase the scope of screening strategies by adding metabolites and transformation products of PFAS. Here, we use a software available in the Agilent MassHunter package that can automatically calculate biotransformation products of a compound given a chemical formula.



Use	Name	Phase	Formula	Mass	Req.	Result Formula	Result Mass	Defect	Δ Defect
✓	Parent	I		0.00000		C8F17O3S	498.92967	-0.07033	0.00000
✓	3x Hydroxylation	I	+O3	47.98474		C8F17O6S	546.91441	-0.08559	-0.01526
✓	Aromatic Thiols to Sulfonic Acids	I	+O3	47.98474	S	C8F17O6S	546.91441	-0.08559	-0.01526
✓	2x Hydroxylation	I	+O2	31.98983		C8F17O5S	530.91950	-0.08050	-0.01017
✓	Thioether to Sulfone	I	+O2	31.98983	S	C8F17O5S	530.91950	-0.08050	-0.01017
✓	Hydroxylation	I	+O	15.99491		C8F17O4S	514.92458	-0.07542	-0.00509
✓	Thioether to Sulfoxide, Sulfoxide to Sulfone	I	+O	15.99491	S	C8F17O4S	514.92458	-0.07542	-0.00509
✓	Aromatic Ring to Arene Oxide	I	+O	15.99491		C8F17O4S	514.92458	-0.07542	-0.00509
✓	Alkene to Epoxide	I	+O	15.99491		C8F17O4S	514.92458	-0.07542	-0.00509
✓	Trifluoromethyl Loss	I	-C-F3+H	-67.98738	F	C7HF14O3S	430.94228	-0.05772	0.01262
✓	Oxidative Defluorination	I	-F+H+O	-1.99566	F	C8HF16O4S	496.93401	-0.06599	0.00434
✓	Decarbonylation	I	-C-O	-27.99491	O	C7F17O2S	470.93475	-0.06525	0.00509
✓	Sulfoxide to Thioether	I	-O	-15.99491	OS	C8F17O2S	482.93475	-0.06525	0.00509
✓	Alkenes to Dihydrodiol	I	+H2+O2	34.00548		C8H2F17O5S	532.93515	-0.06485	0.00548
✓	Reductive Defluorination	I	+H-F	-17.99058	F	C8HF16O3S	480.93909	-0.06091	0.00942
✓	Decarboxylation	I	-C-O2	-43.98983	O	C7F17OS	454.93984	-0.06016	0.01017
✓	Hydration, Hydrolysis (Internal)	I	+H2+O	18.01056		C8H2F17O4S	516.94023	-0.05977	0.01056
✓	Ketone to Alcohol	I	+H2	2.01565	O	C8H2F17O3S	500.94532	-0.05468	0.01565
✓	2x Reductive Defluorination	I	+H2-F2	-35.98116	F2	C8H2F15O3S	462.94851	-0.05149	0.01884
✓	Thioureas to Ureas	I	+O-S	-15.97716	S	C8F17O4	482.95251	-0.04749	0.02284

20 of 92 biotransformations selected
Restrict to possible
Hide not selected
Delete All | Reset | Select All | Invert Selection | Select Phase I | Select Phase II

Fig 4. Agilent Biotransformation software that calculated multiple possible PFOS metabolites for increasing the scope of screening

Identification of 6:2 FTS in a wastewater effluent sample using the database. The mass accuracy was <2 ppm but also, the isotopic fidelity match (shown in inset) was excellent compared to theoretical spectra. Subsequently, an analytical standard was procured and the RT was an exact match too thus adding multiple layers for increased confidence in ID.

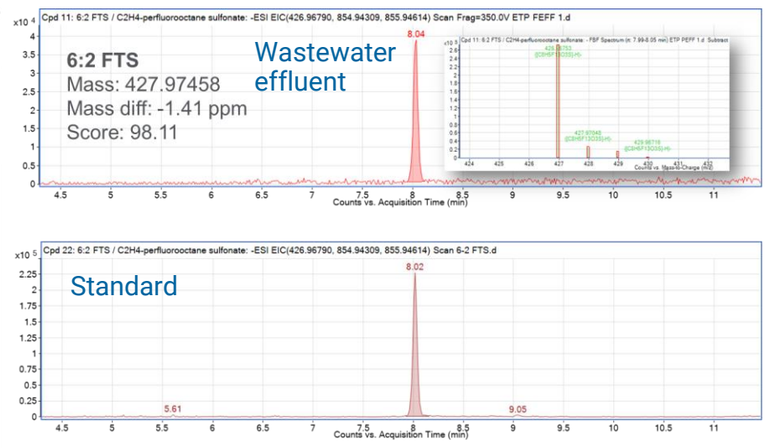


Fig 5. Identification of 6:2 FTS in a wastewater effluent sample with good match to the library for mass and isotope fidelity along with RT confirmation with the standard

Retention Time Prediction for Increased Confidence

Adding retention time information is another parameter that increases confidence in ID. However, PFAS standards are very limited and so make RT collection for many very difficult. A model to predict RTs for a wide range of PFAS was developed by first projecting RT's from a validated method analyzing a chemically diverse set of PFAS (Figure 6A), to increase the model training set size. Measured and projected RT's were then regressed on predicted physiochemical properties, including LogP (Figure 6C) and LogS (Figure 6D) as well as the number of -CF_2- subunits (Figure 6B) in the chemical structure. A weighted average of predicted RT's from individual regressions was calculated.

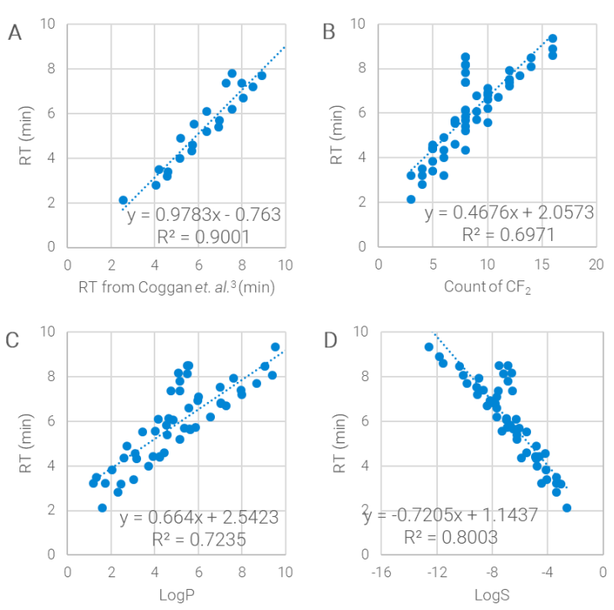


Fig 6. Correlation of other PFAS RT's and chemical properties to RT's measured in this study

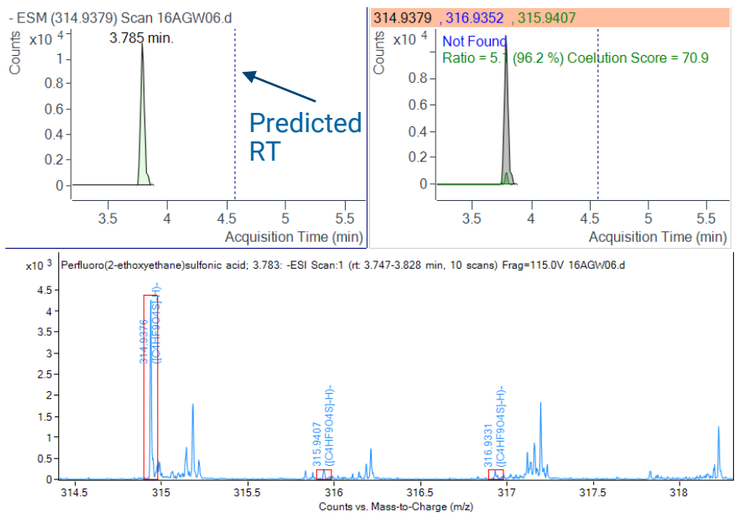


Fig 7. Suspect screening results for Perfluoro(2-ethoxyethane) sulfonic acid using a PCD and RT prediction to increase confidence of ID without need for a standard.

A Personal Compound Database (PCD) containing predicted RT's was curated from US EPA's PFAS Inventory List. These suspect PFAS were appended to the MassHunter Quantitative 10.0 data analysis method, and screened for. The Screening Summary Report is shown in Figure 5 for a selected sample.

Perfluoro (2-ethoxyethane) sulfonic acid had a predicted retention time of 4.56 minutes and was putatively detected at 3.78 minutes in this sample, which is within an expected window given error in the prediction model. This suspect must be subject to further inspection to determine if the putative identifications is real. However, the predicted retention time, accurate mass results, stable isotope pattern provides much data for this inspection.

Identifying Homologous series using Repeating Units & Kendrick Mass Defect

Many PFAS are separated by a $-CF_2$ unit and this is a critical tool in identifying whole series of PFAS in a sample by knowing just one homologue. Since these compounds exhibit a negative mass defect due to the F atom, and making use of the repeating unit of 44 Da; software like the Agilent Mass Profiler Professional can identify repeating series with specified mass units assisting in ID of series of PFAS in a sample.

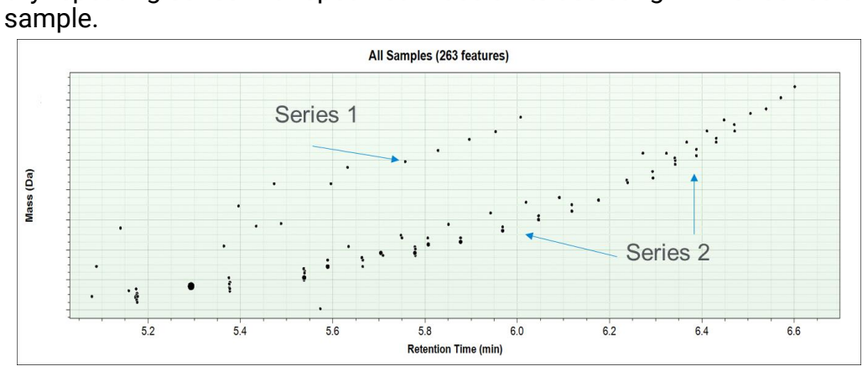


Fig 8. Agilent Mass Profiler Professional identifying series of PFAS using repeating units of -CF_2 in the sample.

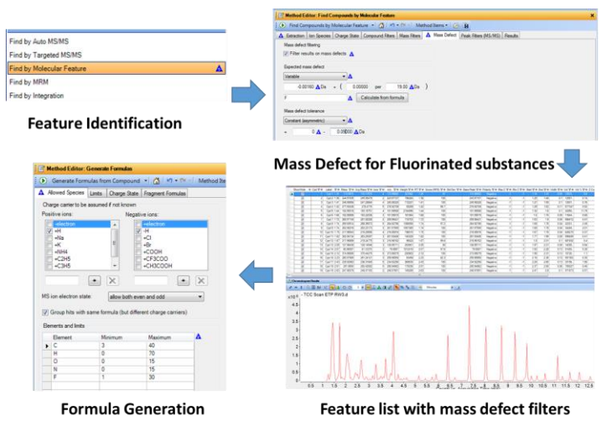


Fig 9. Agilent MassHunter workflow utilizing Kendrick Mass Defect to identify specific features in the sample followed by formula generation

In Silico Fragment Predictors for Structure Identification of Unknown PFAS

Presently, there are >4000 PFAS thought to be created and many thousands of them are still unknown. Further, The availability of PFAS standards of a vast majority of them is non-existent. So, without standards, putative identifications rely on knowledge of fragment annotation and physiochemical properties. Software, such as Agilent's Molecular Structure Correlator can be used to correlate MS/MS spectra with chemical structures.