FluoroMatch Flow and Visualizer are New Tools for Streamlined PFAS Annotation and Visualization

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Introduction

FluoroMatch Flow and Visualizer are open-source tools that simplify suspect and nontarget screening of per- and polyfluoroalkyl substances (PFAS) compounds. The software is optimized to work with liquid chromatography based high-resolution tandem mass spectrometry data. The FluoroMatch tools were developed because many structure elucidation algorithms are focused on the six main chemical elements necessary for life, namely carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), and sulfur (S). This means that they tend to perform poorly when confronted with anthropogenic compounds such as PFAS. FluoroMatch automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, and annotation ranking. The software library contains ~7,000 PFAS fragmentation patterns based on rules derived from standards and literature, and the software automates a process to add more compounds.[1]

FluoroMatch Flow and Visualizer are freely available from innovativeomics.com/software. FluoroMatch Flow directly processes vendor files and includes a systematic scoring framework to communicate confidence for every feature, alongside reporting confidence levels via the Schymanski schema.[2]

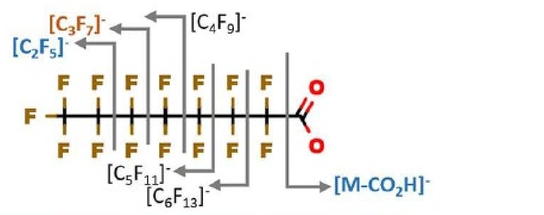


Figure 1: Around 7000 PFAS fragmentation patterns have been evaluated from standards and literature to develop PFAS-specific annotation rules.

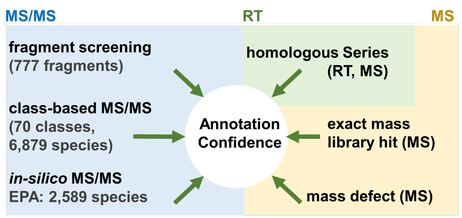


Figure 2: Annotation ranking and confidence assignment, require many lines of evidence.[1]

To aid interpretation by making homologous series more identifiable, we have added a Visualizer tool to the FluoroMatch suite of software utilizing Microsoft PowerBI Desktop to provide users with new graphs, variables, and tables to help with data interpretation. It is also easily customizable for users familiar with the platform. For example, new columns can be added to tables containing information of interest, new plots, for example mass defect versus retention time can be added, and new splicers and filters can be developed.

Materials and Methods

An Aqueous film forming foam (AFFF) mixture was collected from a holding tank containing legacy AFFF products. The sample was diluted 1:100,000 in 70:30 H2O:MeOH. The diluted sample was injected four times for iterative exclusion information-data dependent analysis (iterative MS/MS). Each time, the injection volume was 50 µL. The instrument was an Agilent 1290 Infinity II ultra-high-performance liquid chromatography (UHPLC) system connected to an Agilent 6545 quadrupole time-of-flight mass spectrometer (Q-TOF MS). Blanks were acquired every other injection for blank filtering. PFAS were detected in negative electrospray ionization mode. Data was acquired from m/z 100-1100, with MS/MS collision energy set to 0, 25, and 40 eV. Source parameters and further acquisition parameters for this dataset have been previously described.[3]

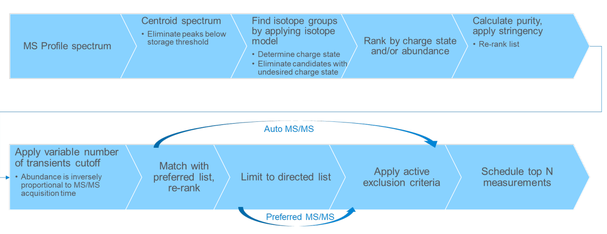
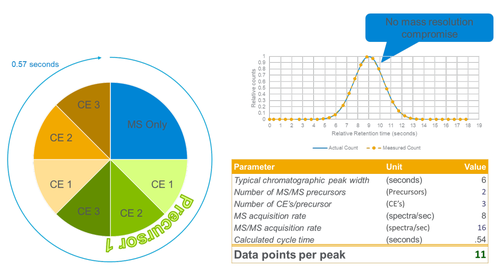


Figure 3: Auto MS/MS Schema.



Parameter	Unit	Value
Typical chromatographic peak width	(seconds)	6
Number of MS/MS precursors	(Precursors)	2
Number of CE's/precursor	(CE's)	3
MS acquisition rate	(spectra/sec)	8
MS/MS acquisition rate	(spectra/sec)	16
Calculated cycle time	(seconds)	.54
Data points per peak		**11**

Figure 4: Example Auto MS/MS Cycle. Data dependent analysis (DDA) modes like Auto MS/MS can be implemented without compromising mass or chromatographic resolution under most conditions.

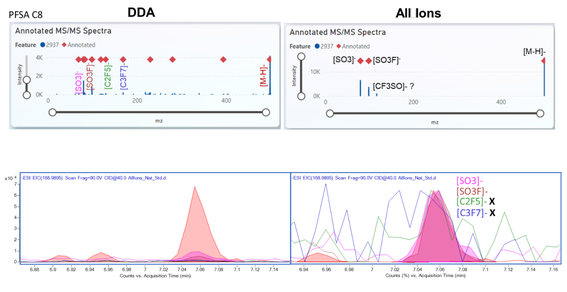


Figure 5: Side-by-side comparison of Auto MS/MS and All Ions (data independent analysis) run under similar conditions.

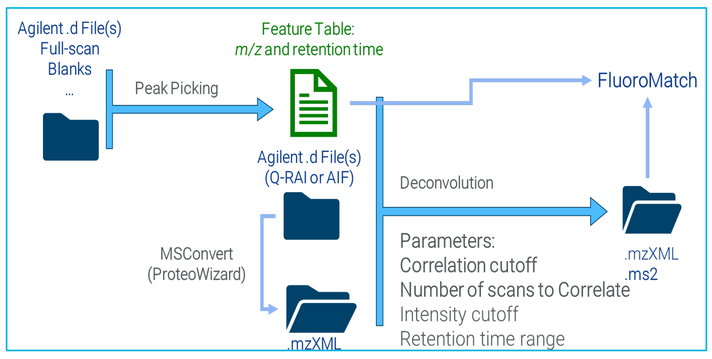


Figure 6: Generic FluoroMatch workflow. FluoroMatch can be run in an automatic manner using Flow, demonstrated here. Flow automatically performs file conversion, peak picking, blank filtering, identification, and combining positive and negative mode data. Alternatively, FluoroMatch Modular allows users to incorporate their own feature-finding-algorithms.

Results and Discussion

Once the MS and MS/MS data has been collected, FluoroMatch Flow directly processes vendor files to generate a systematic scoring framework to communicate confidence for every feature. The integrated steps include file conversion using msConvert, a unique untargeted chromatographic peak picking strategy implementing MZmine 3.0 (users own peak picking workflow can be integrate into FluoroMatch Modular), and blank feature filtering (BFF). It outputs annotations using exact mass and fragment masses, rankings of multiple annotations for features, and compilations of metadata on fragmentation information and peaks used to annotate features.

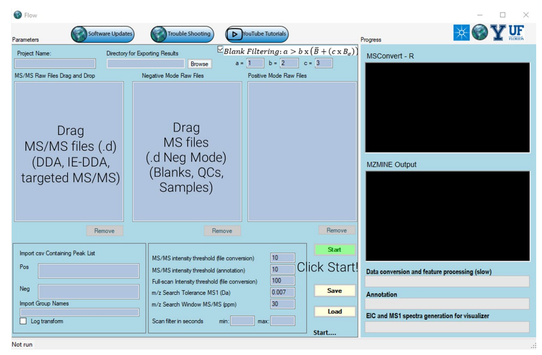


Figure 7: FluoroMatch Flow is designed for ease-of-use with drag-and-drop capability. FluoroMatch Flow directly processes vendor files and includes a systematic scoring framework to communicate confidence for every feature.

The outputs of FluoroMatch Flow are data files. FluoroMatch Visualizer provides interactive mass defect plots, accurate mass vs. retention time plots, MS/MS fragmentation plots, annotation tables, and fragment screening. Selecting a feature in one graph will adjust what is displayed in other views. This interactive cross-filtering allows simplified evaluation of a feature, PFAS series, or other groups of features.

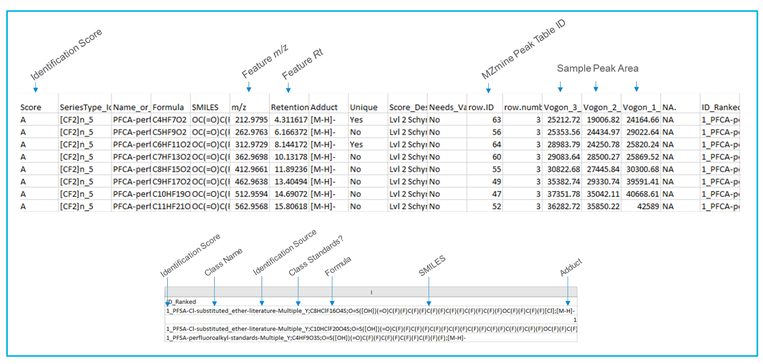


Figure 8: A primary FluoroMatch output file is in the format of a CSV file. It also generates a PBIX file for FluoroMatch Visualizer.

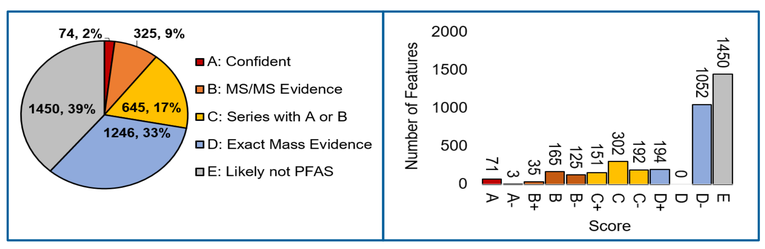


Figure 9: In this AFFF example, iterative MS/MS with a data dependent exclusion list allows for the number of confident identifications to double and the number of tentative identifications with MS/MS evidence to increase by a factor of four. Even so, a third of the components only have exact mass evidence.[4]

The Visualizer PBIX report file was created to provide researchers with a PFAS-specific template. User workflows can be diverse. With the Power BI Desktop, new graphs, variables, and tables can be designed and added. For example, new columns can be added to tables containing information of interest, new plots, for example mass defect versus retention time can be added, and new splicers and filters can be developed.

To aid in interpretation, FluoroMatch Visualizer interface was designed so that all relevant information could be observed simultaneously. Because of the complexity and richness of nontargeted data, users need to prioritize which group of features to investigate. The interface consists of three filters: by MS/MS file, score, and chemical series. Filtering by score and/or fragments allows FluoroMatch Visualizer users to determine which PFAS features to focus on based on features, including annotation quality. It has three graphs: m/z vs retention time, normalized mass defect plot, and MS/MS spectra. It also contains a table of fragments, and table of annotated features, EICs, isotopic pattern, and statistical visualizations.[4]

Figure 10: FluoroMatch Visualizer 1.10 provides interactive mass defect plots, accurate mass vs. retention time plots, MS/MS fragmentation plots, annotation tables, fragment screening, and statistical features. Volcano plot, PCA scores and loading plots are not shown. Selecting and sorting on fragments and features can aid in confirming annotations in homologous series. This AFFF sample is treated with enzymes and is more dilute to avoid contaminating the instrument.

Conclusions

FluoroMatch automated PFAS annotation using in-silico PFAS fragmentation libraries and rule-based annotation. We introduced in-silico fragmentation libraries containing over 7,000 PFAS across 72 PFAS subclasses, built using spectra from literature and authentic standards.

Validating the percent coverage and accuracy of annotations in real-world samples was challenging due to the case of known unknowns and unknown-unknowns. Here, we used all-ion fragmentation to estimate that FluoroMatch covered 71% of CF_2 containing PFAS compounds with fragmentation and CF_2 normalized mass defect plots to estimate 56% coverage of compounds with the remaining being false negatives.

FluoroMatch Visualizer allowed the investigation of trends across PFAS by narrowing down the number of features. One of the most useful approaches was to select individual homologous series, automatically determined using nominal mass and normalized mass defect. When series were selected, all visuals, including MS/MS spectra, were updated to show all members of a series overlaid. Then patterns could easily be observed, and outliers determined. Tens to hundreds of series often exist, and these series can be reduced by those containing high scores or certain characteristic PFAS fragments.

Future Developments

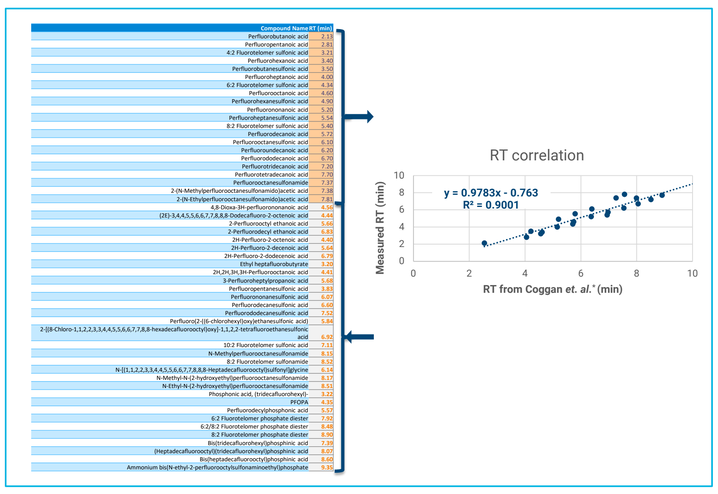


Figure 11: Direct experimental mapping of known PFAS compounds allows for the long-term goal of building in silico libraries. The precision of this approach is approximately ± 30 s.[5]

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