Leveraging the MS1 Dimension and Formula Prediction in Non-Targeted Analysis of PFAS using New FluoroMatch Algorithms: Assessing Confidence and Coverage

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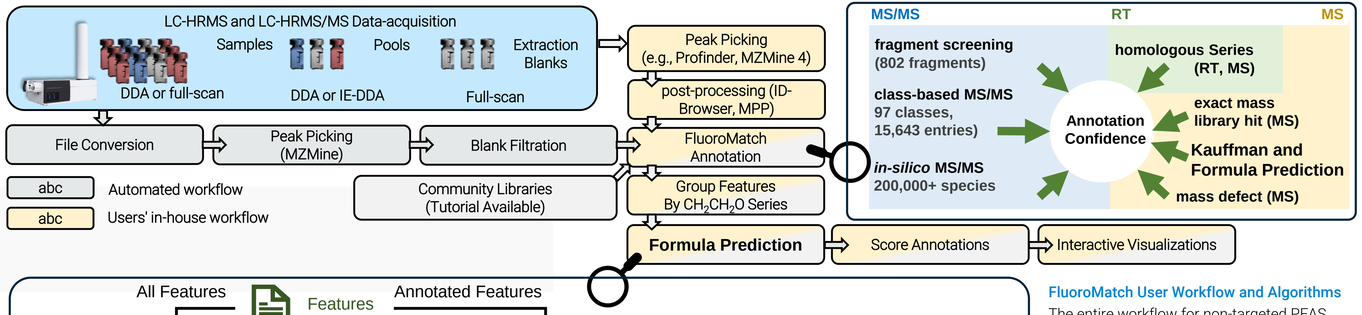
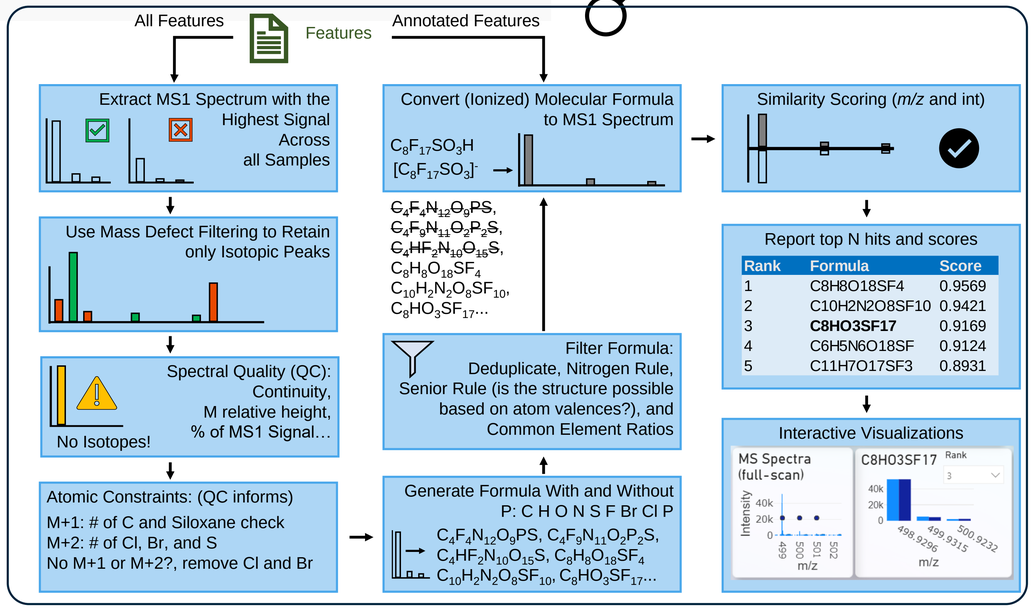
FluoroMatch Covers the Entire Data-Processing Workflow, and Supports Various Acquisition Modes

Data was acquired on an Agilent 6546 LC/Q-TOF for this study to assess PFAS in dried blood spot cards (paper) and blood on dried blood spot cards





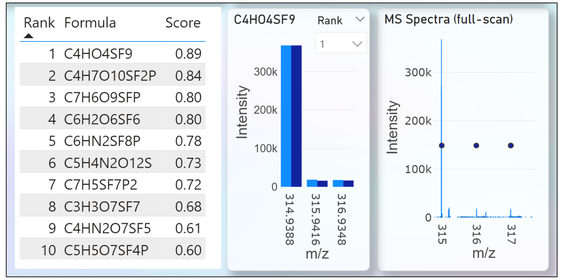
FluoroMatch User Workflow and Algorithms

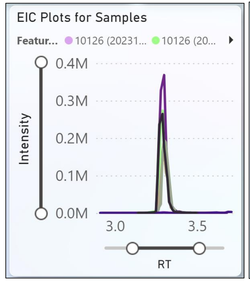
The entire workflow for non-targeted PFAS analysis is covered by FluoroMatch Flow (shown top left). Users simply drag files (including extraction or field blanks) onto the software and click run after choosing their parameters. FluoroMatch [DOI: 10.1007/s00216-021-03392-7] then performs the file conversion, peak picking, annotation, confidence scoring, homologous series detection, formula prediction, and visualizations.

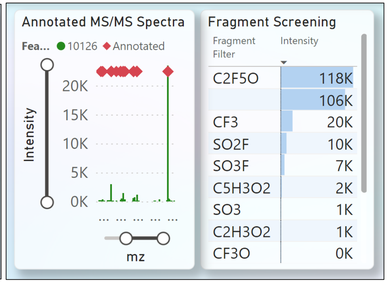
FluoroMatch integrates a wide range of evidence to classify PFAS: mass defect, retention time, and exact mass can be used alongside homologous series to compile groups of chemicals that likely belong to the same class. MS/MS evidence can give structural information pertaining to class or species level assignments. The use of formula prediction is a new feature which we assessed for enhanced coverage in terms of annotation, and enhanced confidence as a further layer of evidence.

The formula prediction algorithm (left) selects the most intense MS1 spectra (based on M ion) and filters the spectra using mass defect to only retain the ions of interest. Atomic constraints are set based on isotopic pattern and the resulting formula are filtered using various criteria (Senior Rule 3 [DOI: 10.1186/1471-2105-8-105], common element ratios [DOI: 10.1186/1471-2105-8-105], nitrogen rule, etc.). The resulting list of formula (after adding MS/MS and database matches from FluoroMatch) are scored based on several criteria including m/z and intensity similarity.

Visualization Interface: All Mass Spectral Evidence, with Cross-Filtering, and Able to be Shared via Web Link



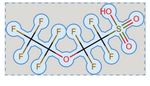


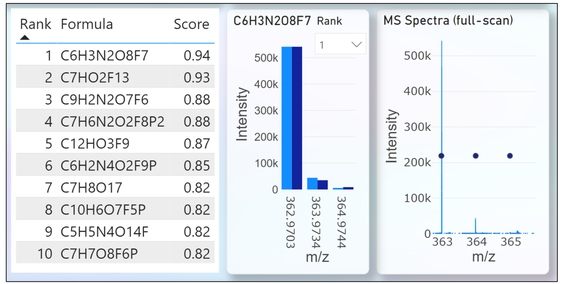


Formula Prediction is a Powerful Tool to Use Alongside All Spectral Evidence

Formula prediction visualizations (above) include tables of ranked candidates, isotopic distributions of experimental (light blue) versus theoretical (dark blue) spectra, and unfiltered MS1 spectra with labeled isotopes and ppm error for isotopic accurate mass match. Furthermore, other visualizations include extracted ion chromatograms (EICs), annotated MS/MS spectra, tables of fragments, Kauffman plots, Kendrick Mass Defect plots, retention time versus *m/z* plots, and statistical visualizations. All plots and charts can be cross filtered and the visual interface with users' data can be shared online in interactive form.

The above plot shows FluoroMatch providing the correct top ranked formula prediction for an ether-linked sulfonic acid (structure shown to the left) (PFESA). PFESAs and PFECAs were discovered both in pooled blood and individuals' blood indicating confirmation via targeted analysis is warranted.





Formula Prediction is highly sensitive to isotopic fidelity

Above is the incorrect top rank for a perfluoroheptanoic acid (PFHpA) $C_7HO_2F_{13}$. The second rank is correct, but both nearly look identical in isotopic pattern, hence making it difficult to discern between formulas when such candidates exist.

Results and Discussion

112 PFAS were discovered without removing PFAS which were close to levels in the blood spot card blank, after blank filtering only 18 remained, signifying the importance of using a matched card blank.
Out of the 18 PFAS discovered in dried blood spots, 13 PFAS were detected using FluoroMatch which were not found using the targeted QqQ screening approach. Of these 13 PFAS, 11 had chemical annotations, and the remaining 2 had evidence of being PFAS, although their exact structures remain unknown. 34% of 30 PFAS in the dried blood spot card with a score of A were assigned the correct formula. 66% of 30 PFAS had the correct formula assigned within the top 10 hits. 4 homologous series were assigned correctly using the most common hits for the series which all had the same formula except the CF2 repeating unit, with 0 incorrectly assigned. Hence, for this study there was a 100% accurate formula prediction using the homologous series approach.

Conclusions

FluoroMatch can be used to rapidly annotate PFAS in an automatic fashion, determine unknowns and expand annotation using an interactive visualizer. Formula prediction provides a significant benefit by providing evidence for annotation for molecules which are considered tentative using other lines of evidence, or for which the signal is too low to get quality MS/MS.
The FluoroMatch formula prediction algorithm is highly sophisticated and has several new or optimized approaches for formula prediction of fluorine containing compounds. With the additional use of homologous series assigning the predicted formula with the most common motifs for all members of a series, the resulting annotations are highly confident (0% false positives in this case for the four series with confident assignments).

Innovative Omics

To install the software please visit:
Innovativeomics.com/software
Questions? Trainings? Collaboration?
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