FluoroMatch 3.0 – Automated PFAS Non-Targeted Analysis and Visualizations Applied to Mammalian Biofluids

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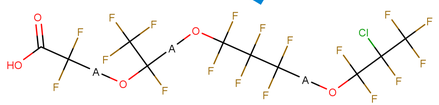
Introduction

Per and poly-fluorinated substances (PFAS) have gained considerable attention from the media, public, and government regulators due to their persistence and toxicity. Most research, media attention, and regulation focus on 2 PFAS (PFOA and PFOS) whereas even broad targeted methods seldom measure over 30 PFAS. Targeted PFAS analysis in serum is incomplete often measuring less than 40% of total PFAS. The portion of uncommonly measured or unknown PFAS is only increasing across time as companies manufacture alternative structures. Therefore, non-targeted PFAS analysis is needed to increase coverage of PFAS measurement to those uncommonly measured or unknown to understand the full implications of PFAS loads on human health. For this purpose, we release FluoroMatch 3.0. Here we present new development in FluoroMatch 3.0 and application to dried blood spots.

Libraries

Class based libraries with fragmentation rules were generated for over 10,000+ species across over 80 different types of PFAS classes. These include biotransformation products, emerging PFAS, and legacy PFAS, as well as predicted structures not currently contained in any database



EPA

In collaboration with the EPA, new PFAS discovered at the 5 major manufacturer sites (surface water) will be added to FluoroMatch libraries continuously

In-Silico Biotransformation Libraries
Predicted Biotransformation Products

CompTox Dashboard
BioTransformer 3.0
mzAnnotation
SMILES2MSMS

7,000+ PFAS
[M-H]⁻ 2,589
Transformation Products: 200,000+

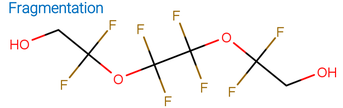
OS(=O)(=O)C1(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C1(F)F

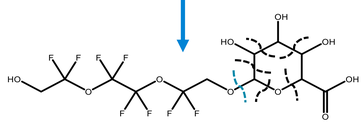
OS(=O)(=O) [SO3H]- [SO3]- [SO3F]- [SO2F]-

C(F)(F)C(F)(F) [C2F5]-

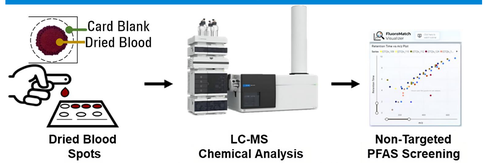
C(F)(F) [CF2]- [CF3]-

Chemical Standards Based biotransformation Libraries
(Biotransformed with Mouse Enzymes)
21 transformers identified across 13 parent PFAS
75 Transformation Products With Class Based Fragmentation



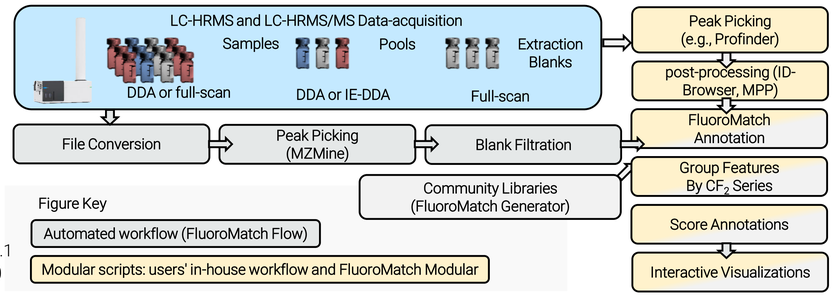


Dried Blood Spot Analysis



Reference whole blood (UTAK; #44600-WB(F)) was dried onto blood spot cards (QIAcard). Cards were spiked with a mixture of 20 native PFAS standards (Accustandard). To account for background contamination a blank portion of the card was analyzed. An Agilent 1290 Infinity II LC with a Poroshell ECC18 (2.1 x 100 mm, 2.7 um) column and PFC Delay Column (4.6 x 30 mm) connected to an Agilent 6546 LC/Q-TOF was used for analysis.

Entire Acquisition and Software Workflow

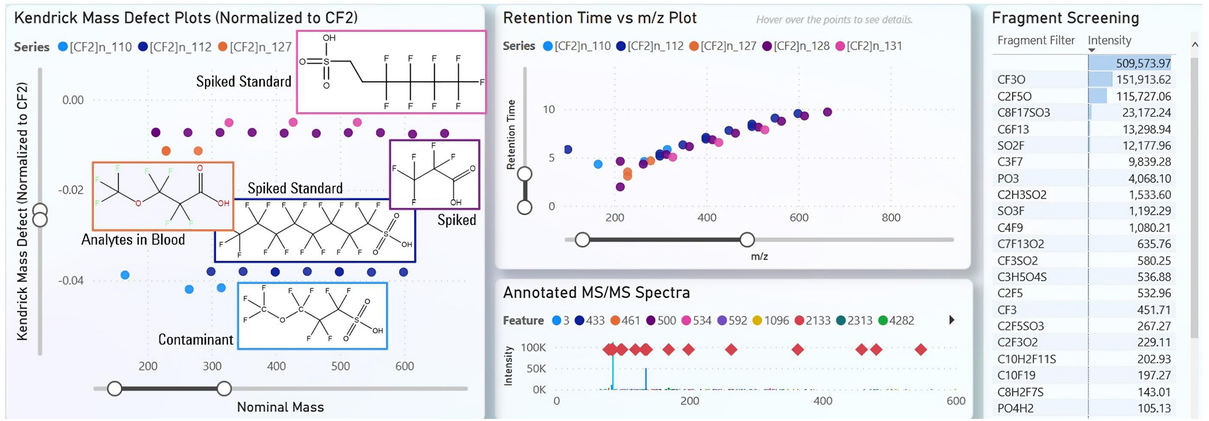


FluoroMatch Flow and FluoroMatch Modular (acquisition and data-processing workflow)

The FluoroMatch software data analysis workflow starts by importing data collected using MS, and MS/MS data dependent (DDA), iterative exclusion MS/MS (IE-DDA), or targeted MS/MS modes from individual, pooled and blank samples. FluoroMatch algorithms cover file conversion, blank filtering, feature annotation, and visualization. FluoroMatch Software also directly imports data processed initially using Agilent's Mass Profiler software or other peak picking software.

In this study, IE-DDA was performed on pooled samples

Visualizer Interface: Dried Blood Spot Results – Homologous Series



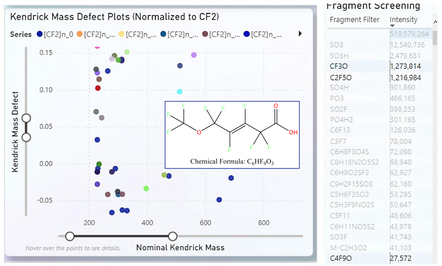
Fragment Screening

Fragment Filter	Intensity
	509,573.97
CF3O	151,913.62
C2F5O	115,727.06
C8F17SO3	23,172.24
C6F13	13,298.94
SO2F	12,177.96
C3F7	9,839.28
PO3	4,068.10
C2H3SO2	1,533.60
SO3F	1,192.29
C4F9	1,080.21
C7F13O2	635.76
CF3SO2	580.25
C3H5O4S	536.88
C2F5	532.96
CF3	451.71
C2F5SO3	267.27
C2F3O2	229.11
C10H2F11S	202.93
C10F19	197.27
C8H2F7S	143.01
PO4H2	105.13

FluoroMatch Visualizer Outputs (Left are All Homologous Series Automatically and Manually Assigned) (Right are All Features After Filtering by Ether Related Fragments)

Left: A total of 28 PFAS across 5 homologous series were annotated in dried blood spot samples. These series were identified as fluorotelomer perfluoroalkyl sulfonic acids (FTS), perfluoroalkyl carboxylic acids (PFCA), perfluoroalkyl ether carboxylic acids (PFECA), perfluoroalkyl sulfonic acids (PFSA), and perfluoroalkyl ether sulfonic acids (PFESA). These annotations captured 95% of the standards spiked onto samples for validation (19 of 20 spiked standards, 5% false negative rate). Three PFECA species (two C4 isomers and C5) were above levels in the card blank and neat standard solution, and hence were likely from the blood and indicative of human exposure.
Right: Fragment screening for $[CF_3O]^-$, $[C_2F_5O]^-$, $[C_3F_7O]^-$, and $[C_4F_9O]^-$, showing many potential ether-linked PFAS in dried blood spots. One additional annotated species is shown.
The interactive dataset can be review for new PFAS at innovativeomics.com/datasets

Visualizer Interface: Fragment Screening



Conclusions

FluoroMatch can be used to classify mixtures, identify compounds, and determine unknowns

- Incorporates MS/MS, MS, EICs, homologous series, and retention time
- Has over 200,000 species with fragmentation in libraries; fragment screening (777) and substructure assignment for unknowns
- Five novel or rarely screened PFAS were found in whole blood using the workflow: PFECA, PFSA branched isomer, and unsaturated PFECA
- < 5% false positive and false negative rate

Innovative Omics

To install the software please visit:
Innovativeomics.com/software
Questions? Trainings? Collaboration?
Contact: jeremykoelmel@gmail.com