Application Note
Environmental

Expanding PFAS Coverage in Nontargeted Analysis Using Data-Independent Analysis

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Abstract

This study evaluates the performance of Agilent FluoroMatch software in enhancing data-independent analysis (DIA) for the detection and characterization of per- and polyfluoroalkyl substances (PFAS) in complex samples. Using samples from NIST PFAS Interlaboratory Studies, the software capabilities in processing fragmentation data from an Agilent Revident LC/Q-TOF system were assessed. FluoroMatch, with its extensive PFAS fragmentation library, automates several data analysis steps including peak picking, blank filtering, and annotation. Results showed that All Ions MS/MS mode significantly improved compound coverage in less complex samples, while iterative exclusion provided additional coverage in more complex matrices. The integration of IonDecon and FluoroMatch Visualizer enhances PFAS analysis, offering a comprehensive approach for identifying and characterizing environmental contaminants.[1]

Introduction

PFAS are a group of synthetic chemicals widely used in various industrial and consumer products due to their resistance to heat, water, and oil. However, their persistence in the environment and potential health risks have raised significant concerns, necessitating robust analytical methods for their detection and characterization.

This study used samples from NIST PFAS Interlaboratory Studies to assess the capabilities of the FluoroMatch IonDecon software in processing Agilent DIA files. The objective was to evaluate the performance of fragmentation from the Agilent All Ions MS/MS mode using an Agilent Revident quadrupole time-of-flight LC/MS (LC/Q-TOF) system.

The FluoroMatch suite is an open-source set of tools designed to streamline the suspect and nontarget screening of PFAS compounds. It automates several processes including file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, and annotation ranking. The software library contains 15,643 PFAS fragmentation patterns, with the capability to add more.[2]

IonDecon, the latest enhancement to FluoroMatch, filters All Ions MS/MS data to retain only fragments correlating with precursor ions. This software can deconvolute any All Ions files and generate open-source data-dependent acquisition (DDA) formatted files for downstream nontargeted analysis workflows. In complex samples, incorporating All Ions fragmentation (AIF) and IonDecon can enhance MS/MS coverage of PFAS.

This methodology aims to improve the accuracy and efficiency of nontargeted PFAS analysis, providing a comprehensive approach to identifying and characterizing these persistent environmental contaminants.

Experimental

Three test methanolic solutions of NIST Interlaboratory Studies[3] samples were analyzed using an Agilent 1290 Infinity II LC interfaced with a high-resolution Agilent Revident LC/Q-TOF with an Agilent Dual Jet Stream ESI source.

NIST A was comprised of a methanolic dilution of several commercially available calibration solutions. NIST B was a solution consisting of a methanolic dilution of two aqueous film-forming foam (AFFF) commercial solutions. NIST C was created by extracting, fortifying, and filtering a candidate soil material impacted by AFFF into methanol. Only one additional PFAS, commercially available, was used to fortify NIST C.

Data were acquired in negative mode ionization using All Ions MS/MS and iterative exclusion information data-dependent analysis (iterative MS/MS) with four consecutive injections. Blanks were acquired every other injection for blank filtering. Data were acquired from *m/z* 50 to 1,500, with collision energy for All Ions set at 0, 20, and 40 V, and, for Auto MS/MS, set at 40 eV. Table 1 details the LC and MS method parameters used for this analysis.

Table 1. Agilent 1290 Infinity II LC and Agilent Revident LC/Q-TOF method parameters.

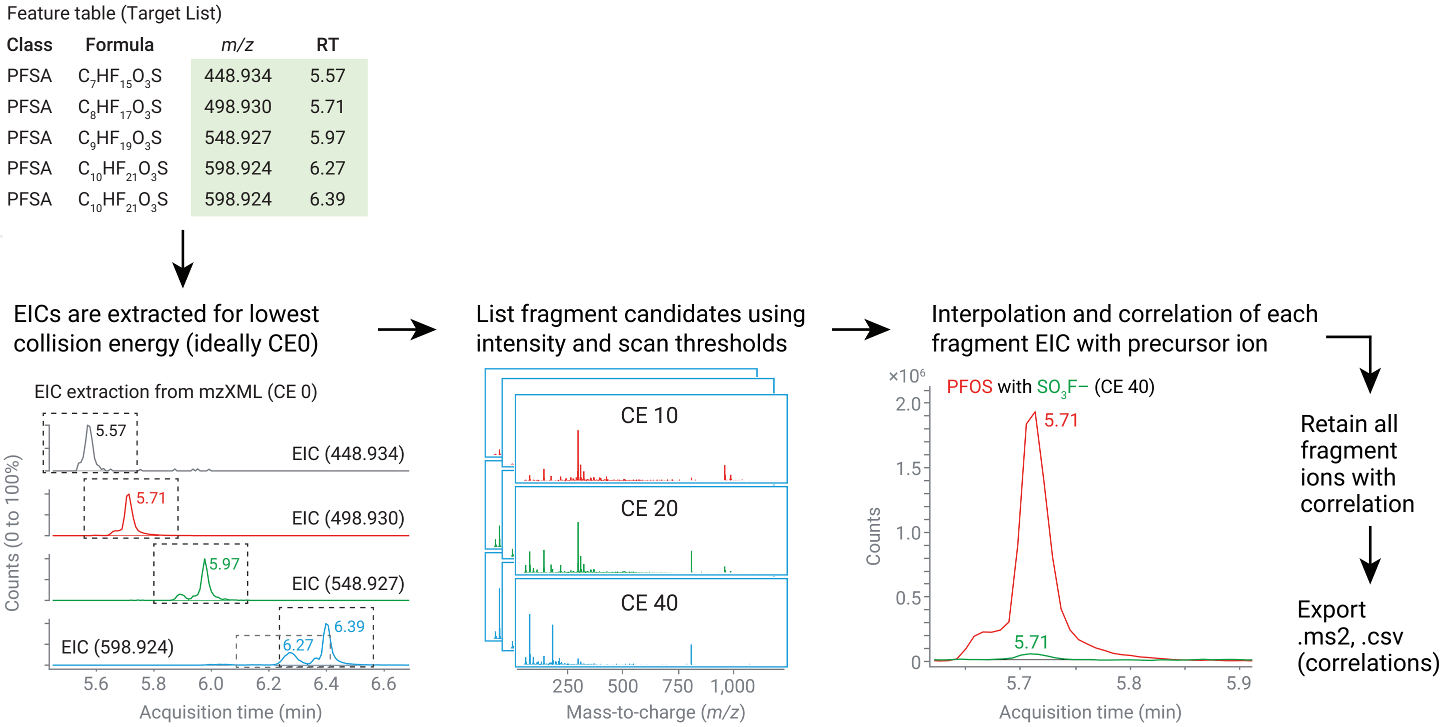
LC Conditions	
Column	– Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) – Agilent ZORBAX RRHD Eclipse Plus C18 guard column, 2.1 × 5 mm, 1.8 µm (p/n 821725-901) – Agilent InfinityLab PFC delay column, 4.6 × 30 mm (p/n 5062-8100)
Column Temperature	45 °C
Autosampler Temperature	4 °C
Injection Volume	7.5 µL
Mobile Phase	A) 2 mM Ammonium acetate in water B) Acetonitrile
Flow Rate	0.4 mL/min
Gradient	Time (min) %B 0.0 10 0.5 10 1.8 40 9.5 95 11.5 95 11.6 10
Post Time	0.5 min
Total Run Time	12.5 min
LC/Q-TOF Conditions	
Gas Temperature, Flow	120 °C, 10 L/min
Nebulizer Pressure	25 psi
Sheath Gas Temperature, Flow	290 °C, 12 L/min
Nozzle Voltage	0 V
Capillary Voltage	2,500 V

Results and discussion

Many structure elucidation algorithms focus on the six main chemical elements essential for life: carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), and sulfur (S). Consequently, these algorithms often perform poorly when analyzing anthropogenic compounds such as PFAS.

In this study, FluoroMatch software enabled the use of the Agilent peak picking method. FluoroMatch then performed blanks filtering, isotope grouping, alignment, and gap filling using mzXML format data. IonDecon was employed to remove fragment ions unlikely to be related to the precursor ion, resulting in an open-source data-dependent acquisition (DDA) file; the basic algorithm is depicted in Figure 1.

Once the MS and MS/MS data were collected, FluoroMatch generated a systematic scoring framework to communicate confidence for every feature. FluoroMatch Visualizer, developed using Microsoft Power BI Desktop software, provided users with customizable graphs, variables, and tables to aid in data interpretation. For example, new columns could be added to tables containing information of interest, new plots could be added, and new splicers and filters could be developed.



Feature table (Target List)

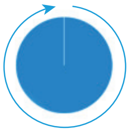
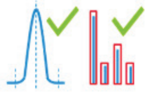
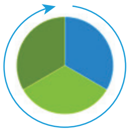
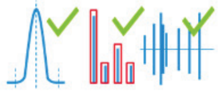
Class	Formula	*m/z*	RT
PFSA	$C_7HF_{15}O_3S$	448.934	5.57
PFSA	$C_8HF_{17}O_3S$	498.930	5.71
PFSA	$C_9HF_{19}O_3S$	548.927	5.97
PFSA	$C_{10}HF_{21}O_3S$	598.924	6.27
PFSA	$C_{10}HF_{21}O_3S$	598.924	6.39

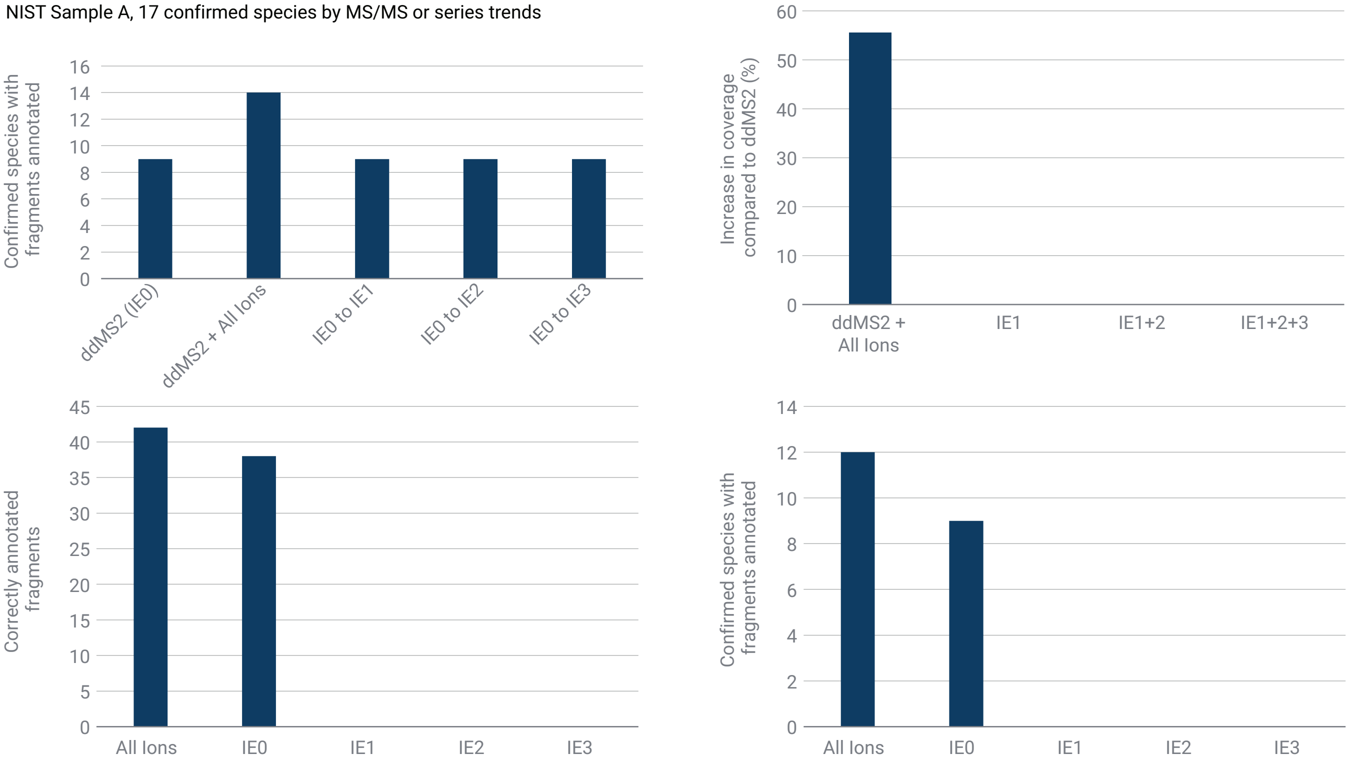
Figure 1. IonDecon basic algorithm from full-scan EIC extraction to deconvolution of related fragmentation and export.

FluoroMatch data processing was standardized across all acquisition modes with a Pearson's correlation threshold of 0.7, a retention time window of 0.11 minutes centered around the peak apex for correlating fragments to precursor ions, and a minimum extracted ion chromatogram (EIC) length of six scans. An overview of the LC/MS acquisition modes is shown in Table 2.

Confirmed species data for the three NIST samples are shown in Figures 2 to 7. Figures 2 and 3 refer to NIST Sample A. Figures 4 and 5 refer to NIST Sample B. Figures 6 and 7 refer to NIST Sample C.

Table 2. Overview of All Ions and Auto MS/MS acquisition modes.

Acquisition Mode		Cycle Distribution	Acquisition Rate	Cycle Time (s)	Data Points Per 6 s Peak	DA Workflow
MS Only			2 Hz	0.50	12	
All Ions			6 Hz for MS 6 Hz for All Ions	0.50	12	
Auto MS/MS			8 Hz for MS 16 Hz for MS/MS	0.54	11	



NIST Sample A, 17 confirmed species by MS/MS or series trends

Figure 2. Comparing confirmed NIST Sample A species with All Ions and Auto MS/MS (ddMDS2) with iterative exclusion.

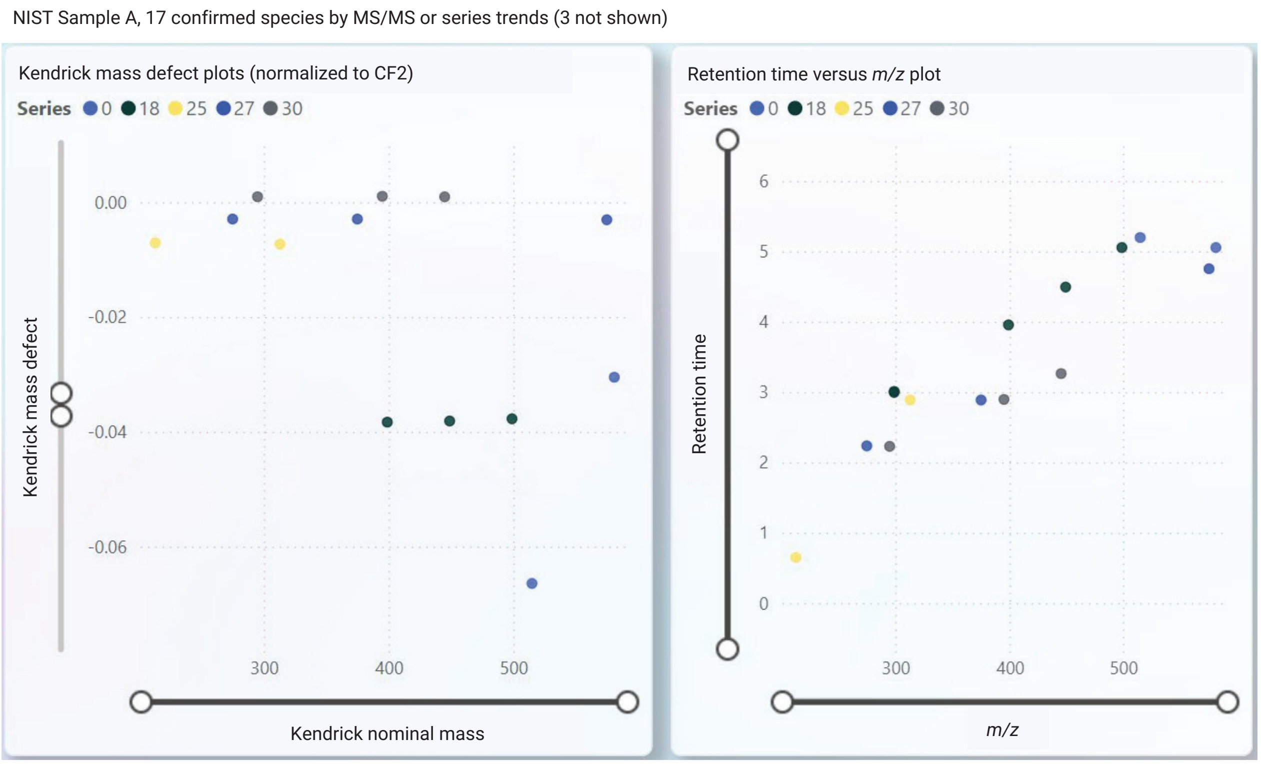


Figure 3. FluoroMatch Visualizer results for NIST Sample A, with 16 confirmed species by MS/MS or series trends.

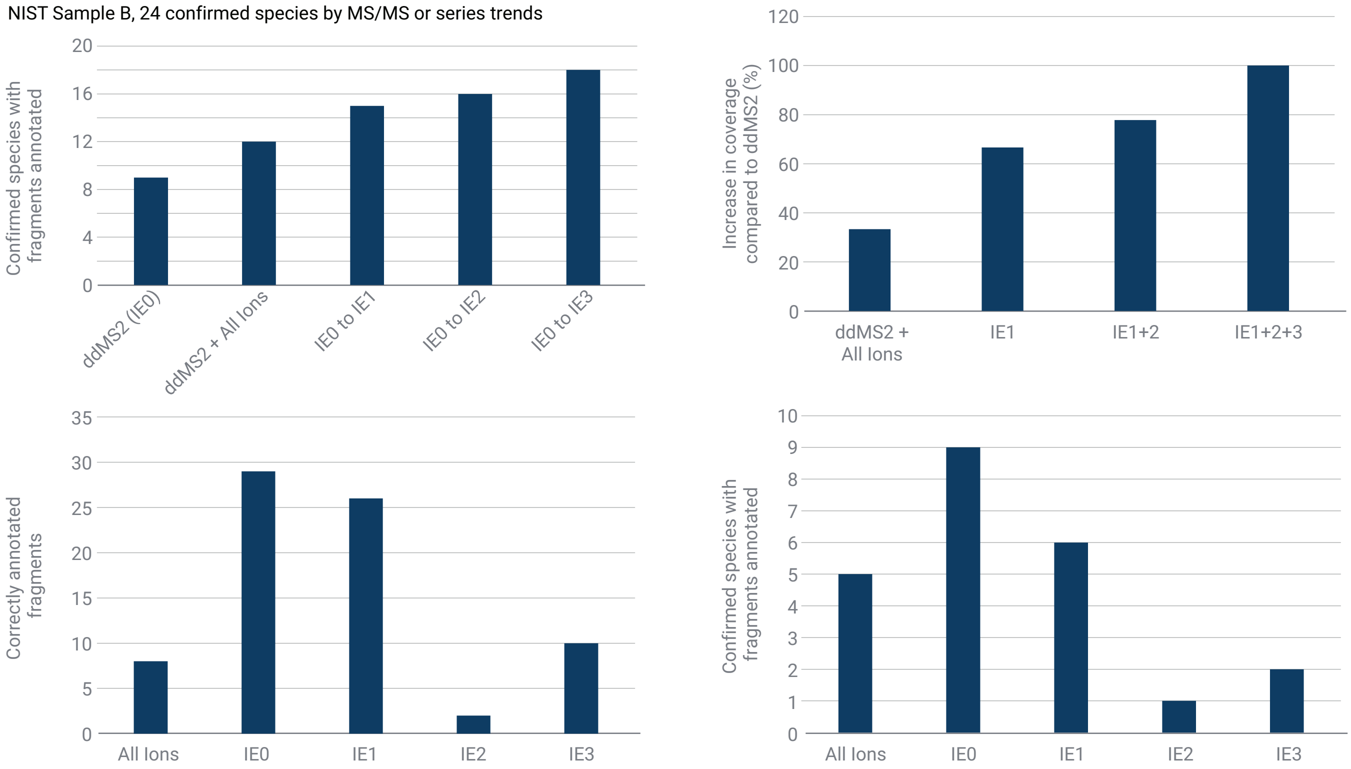


Figure 4. Comparing confirmed NIST Sample B species with All Ions and Auto MS/MS (ddMDS2) with iterative exclusion.

NIST Sample B, 24 confirmed species by MS/MS or series trends

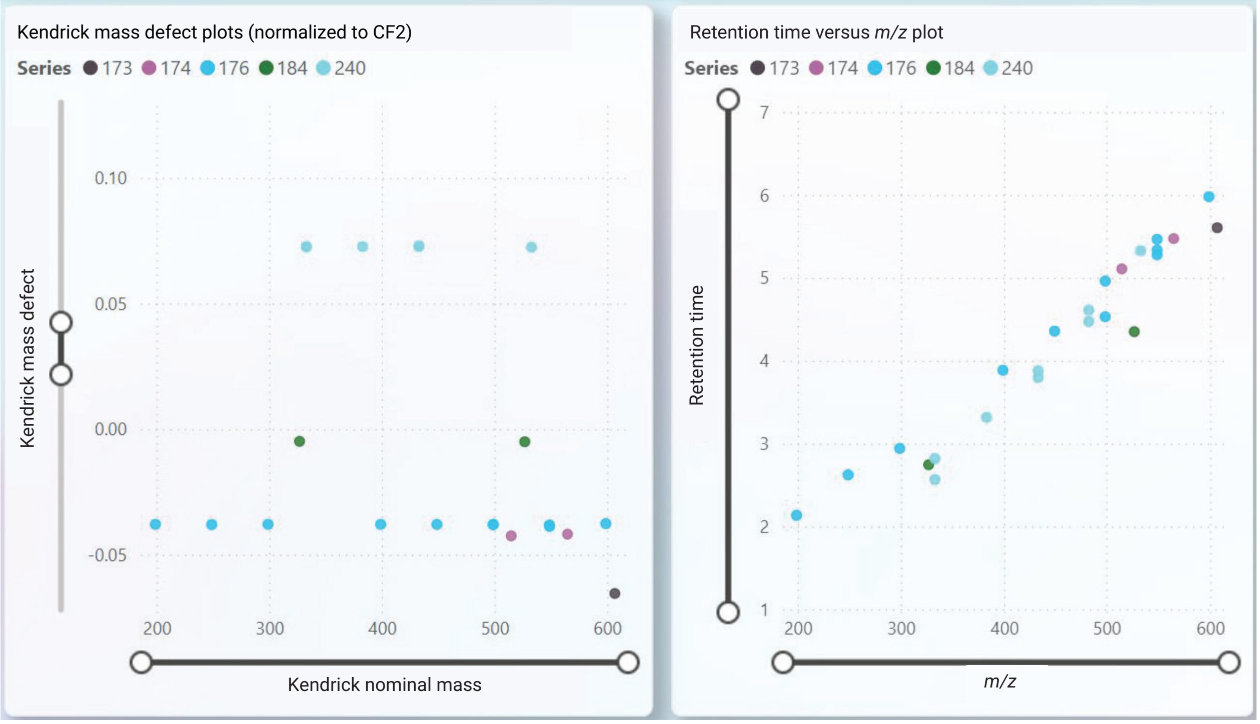


Figure 5. FluoroMatch Visualizer results for NIST Sample B, with 24 confirmed species by MS/MS or series trends.

NIST Sample C, 192 confirmed species by MS/MS or series trends

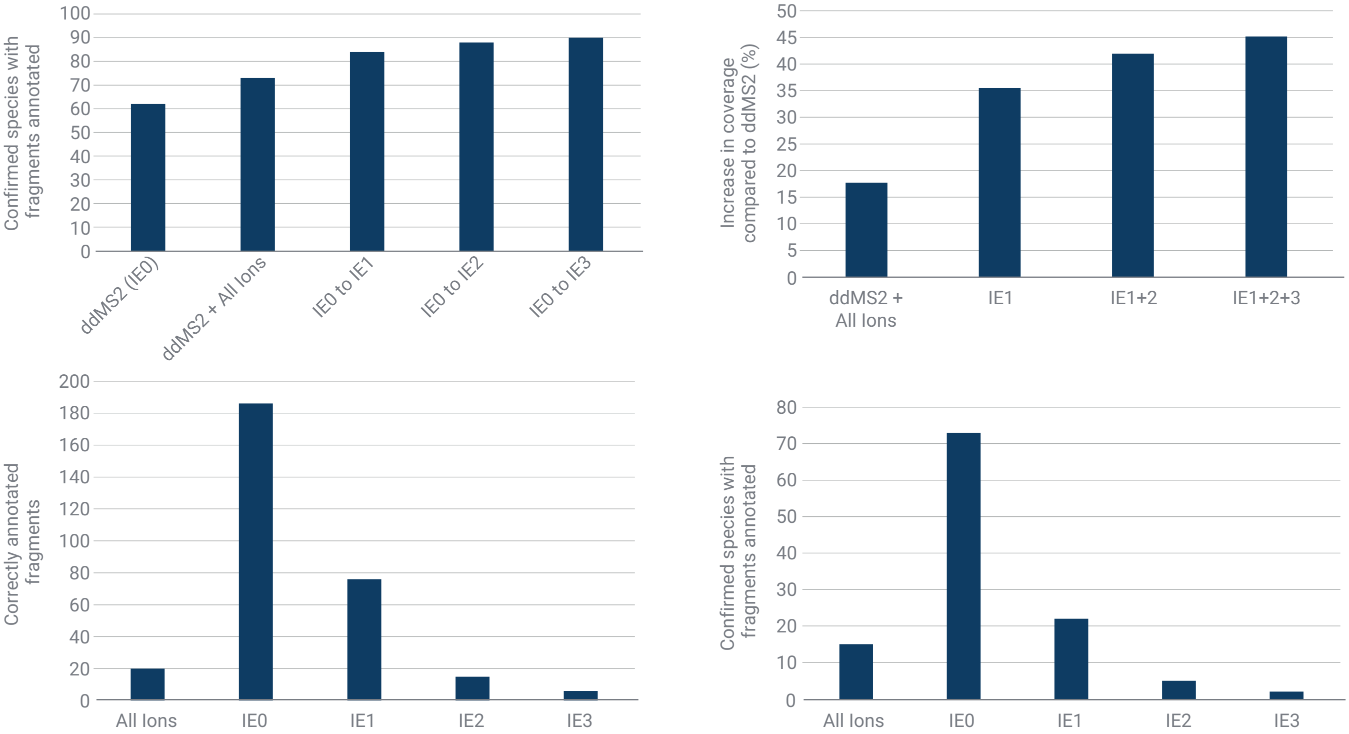


Figure 6. Comparing confirmed NIST Sample C species with All Ions and Auto MS/MS (ddMDS2) with iterative exclusion.

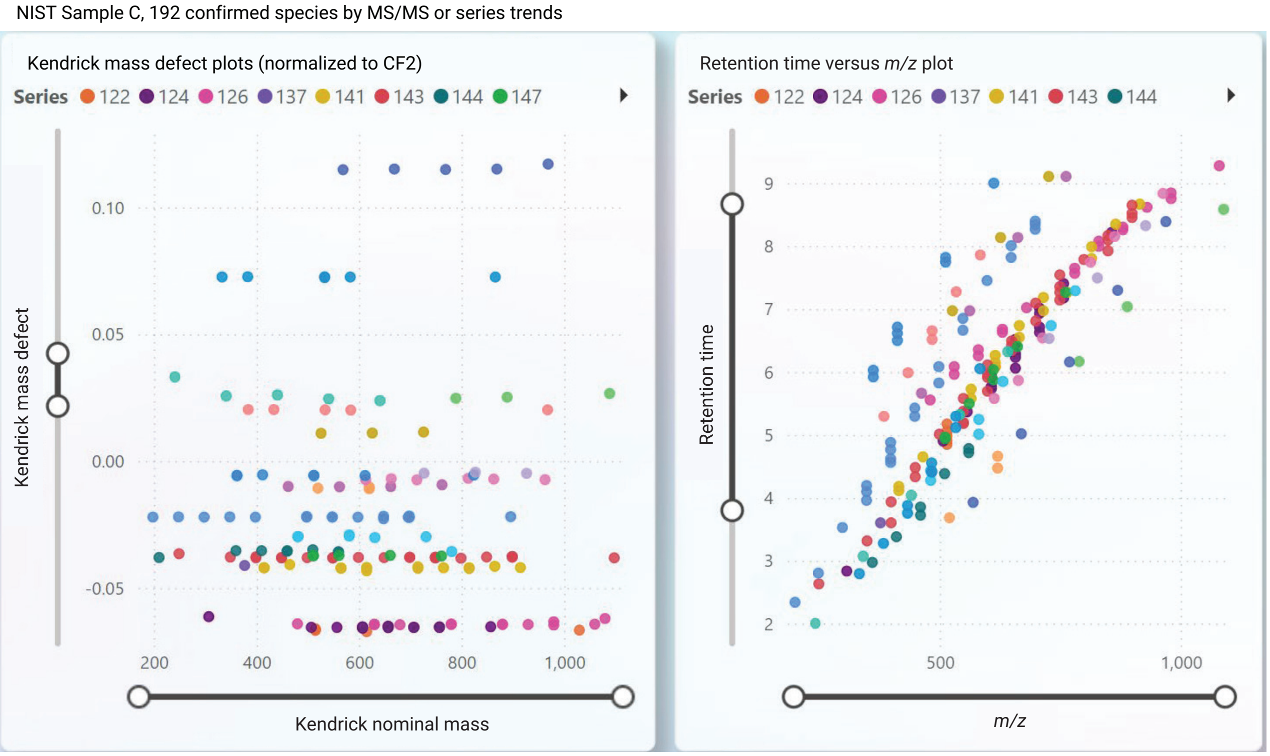


Figure 7. FluoroMatch Visualizer results for NIST Sample C, with 192 confirmed species by MS/MS or series trends.

In NIST Sample A, the overall coverage of the 16 annotated compounds was higher using All Ions compared to other acquisition modes. Given the lower complexity of this sample, iterative exclusion (IE) provided no additional hits, whereas All Ions increased coverage by 56%, making it nearly essential for this sample.

Among the three samples provided, NIST Sample C was the most intriguing due to significant matrix interference and the presence of many PFAS (192 confirmed species). In NIST Sample C, a 45% increase in coverage was observed using IE, with a 35% increase using just IE1. Additionally, there were five unique hits with All Ions.

The FluoroMatch Visualizer interface was designed to display all relevant information simultaneously, accommodating the complexity and richness of nontargeted data. Users need to prioritize which groups of features to investigate. For instance, filtering by score allows users to focus on PFAS features based on annotation quality.

One of the most useful approaches with FluoroMatch Visualizer is selecting individual homologous series, automatically determined using nominal mass and normalized mass defect. When a series is selected, all visuals, including MS/MS spectra, are updated to show all members of the series overlaid. This enables easy observation of patterns and identification of outliers. In Figure 7, more than a dozen series are displayed. These series can be filtered to highlight those with high scores or specific characteristic PFAS fragments. Furthermore, even for PFAS that do not form homologous series or confident assignments, filtering using fragments screening, Kaufmann region, isotopic pattern, mass defect, and other filters available in the Visualizer can be performed to identify novel PFAS.

Conclusion

FluoroMatch software leverages in silico PFAS fragmentation libraries and rule-based annotation to automate the identification of PFAS compounds. With rules derived from over 15,643 PFAS across 97 subclasses, using spectra from literature and authentic standards, FluoroMatch significantly enhances PFAS analysis. In NIST Sample A, Agilent All Ions MS/MS mode increased the coverage of the 16 annotated compounds by 56%. It proved essential for this less complex sample. In contrast, iterative exclusion offered no additional hits in this sample. In the more complex NIST Sample C, containing nearly 200 PFAS, IE improved coverage by 45%, with All Ions providing five unique and correct hits not obtained by other MS/MS modes.

All Ions with IonDecon complements data-dependent acquisition methods such as Auto MS/MS with iterative exclusion. It improves coverage in complex matrices, provides fragmentation for every feature, and aids in the characterization of unknowns through isotopic ratios in fragmentation spectra. FluoroMatch Visualizer further facilitates the investigation of PFAS trends by narrowing down the number of features, making it a powerful tool for comprehensive PFAS analysis.

FluoroMatch was used to identify PFAS classes and highlight homologous series. The tentative identifications were then evaluated through SIRIUS+ CSI:FingerID fragmentation trees. SIRIUS is not optimized for perfluoro compounds, but the CSI:FingerID fragmentation trees were useful in ranking the possible annotations. The power of integrating orthogonal approaches is evident in the DIM004 sample results with a good balance between false negative and positive rates across all three samples. https://www.nist.gov/publications/and-polyfluoroalkyl-substances-non-targeted-analysis-interlaboratory-study-final-report

References

1. Koelmel, J.; *et al.* Expanding Per- and Polyfluoroalkyl Substances Coverage in Nontargeted Analysis Using Data-Independent Analysis and IonDecon. *J. Am. Soc. Mass Spectrom*. **2023**, *34(11)*, 2525–2537.
2. Koelmel, J.; *et al.* Toward Comprehensive Per- and Polyfluoroalkyl Substances Annotation Using FluoroMatch Software and Intelligent High-Resolution Tandem Mass Spectrometry Acquisition. *Analytical Chemistry* **2020**, *92(16)*, 11186–11194.
3. NIST Interlaboratory Studies Home Page. https://www.nist.gov/programs-projects/and-polyfluoroalkyl-substances-pfas/get-involved/interlaboratory-studies (accessed 2025-01-06)

Acknowledgment

The authors would like to thank the unattributed NIST, Yale, and Agilent team members who made this work possible. FluoroMatch is developed by Innovative Omics. The software can be downloaded at innovativeomics.com/software. References, detailed instructions, video tutorials, and links to other Innovative Omics software tools are also available on the website.

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DE-003956

Printed in the USA, February 21, 2025
5994-8148EN

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