SHIMADZU

WP 106

# Comprehensive Non-Targeted PFAS Analysis Using Quadrupole Time-of-Flight (QToF) Mass Spectrometry with a Multi-Approach Data Processing Workflow

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## 1. Overview

A comprehensive, multi-approach data processing method was developed for the analysis of non-targeted PFAS in both positive and negative ionization modes.

## 2. Introduction

Per- and polyfluoroalkyl substances (PFAS) are persistent, highly stable chemicals that present possible risks to both environmental systems and human health. Quantitative methods such as triple quadrupole LC-MS/MS are inherently limited by their focus on predefined target analytes, resulting in the exclusion of unknown isomers and broader PFAS chemical classes. To overcome these limitations, non-targeted high-resolution mass spectrometry (HRMS) has emerged as a robust and unbiased alternative for the detection and identification of PFAS and their isomeric forms. In 2024, Shimadzu participated in the PFAS NTA Interlaboratory Study organized by the National Institute of Standards and Technology (NIST) [1], advancing the untargeted LC-MS approach through the utilization of a high-mass-accuracy quadrupole time-of-flight (QToF) instrument. This methodology, combined with an innovative data-processing workflow, enables comprehensive non-targeted screening of PFAS and their associated chemical families.

## 3. Method

Three unknown samples (designated A, B, and C) obtained from the National Institute of Standards and Technology (NIST) were analyzed for PFAS in accordance with the 2024 NTA ILS guidelines [1]. A binary gradient elution method was developed using a C18 reversed-phase column, with a 2 mM aqueous ammonium acetate and acetonitrile for the mobile phase. A 30-minute data-dependent acquisition (DDA) method was developed using a Shimadzu LCMS-9050 quadrupole Time-of-Flight mass spectrometer. Data processing utilized three complementary workflow for PFAS analyte identification (Figure 1).

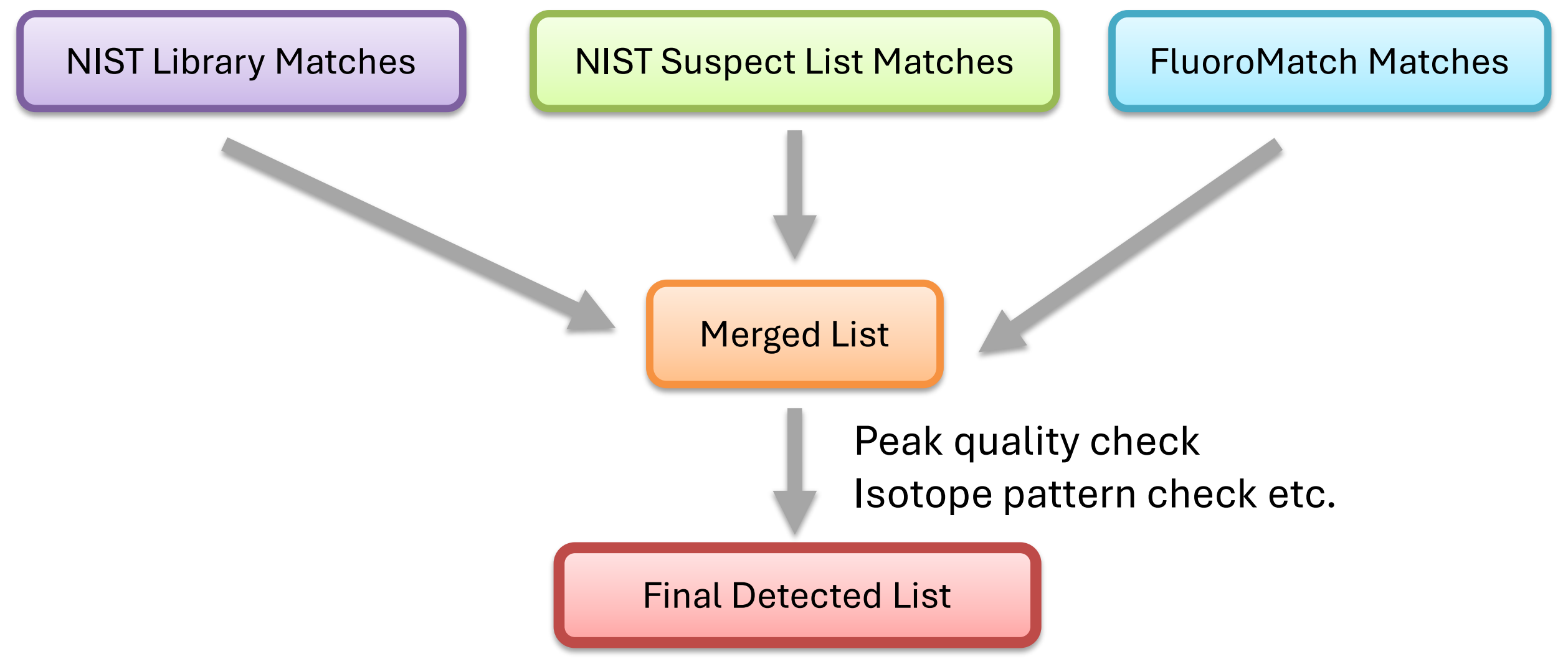

NIST Library Matches
NIST Suspect List Matches
FluoroMatch Matches
Merged List
Peak quality check
Isotope pattern check etc.
Final Detected List

**Figure 1.** Data processing workflow for identification of PFAS analytes

Both positive and negative ionization modes with DDA acquisition were used to analyze all three samples for PFAS screening (Figure 2).

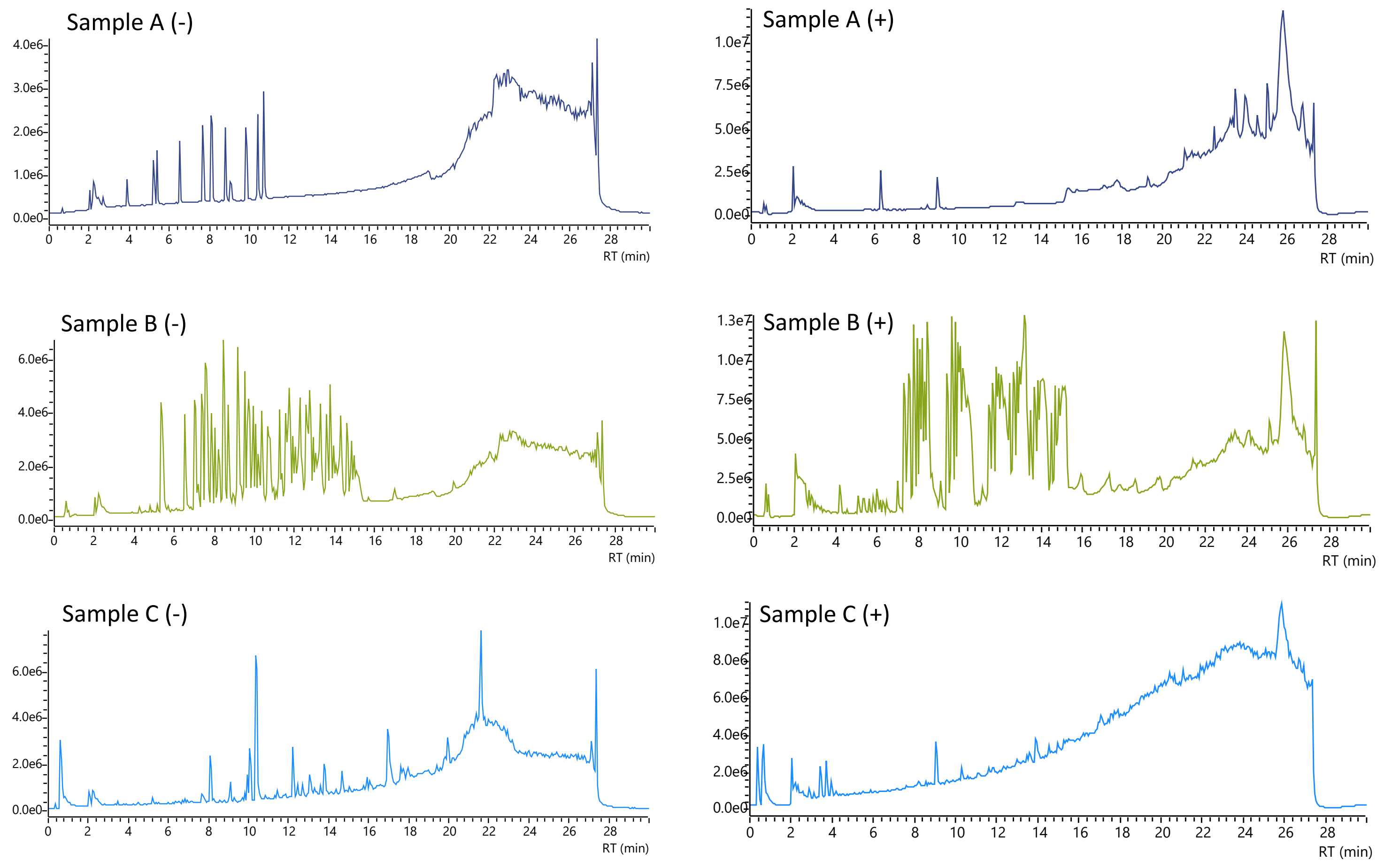
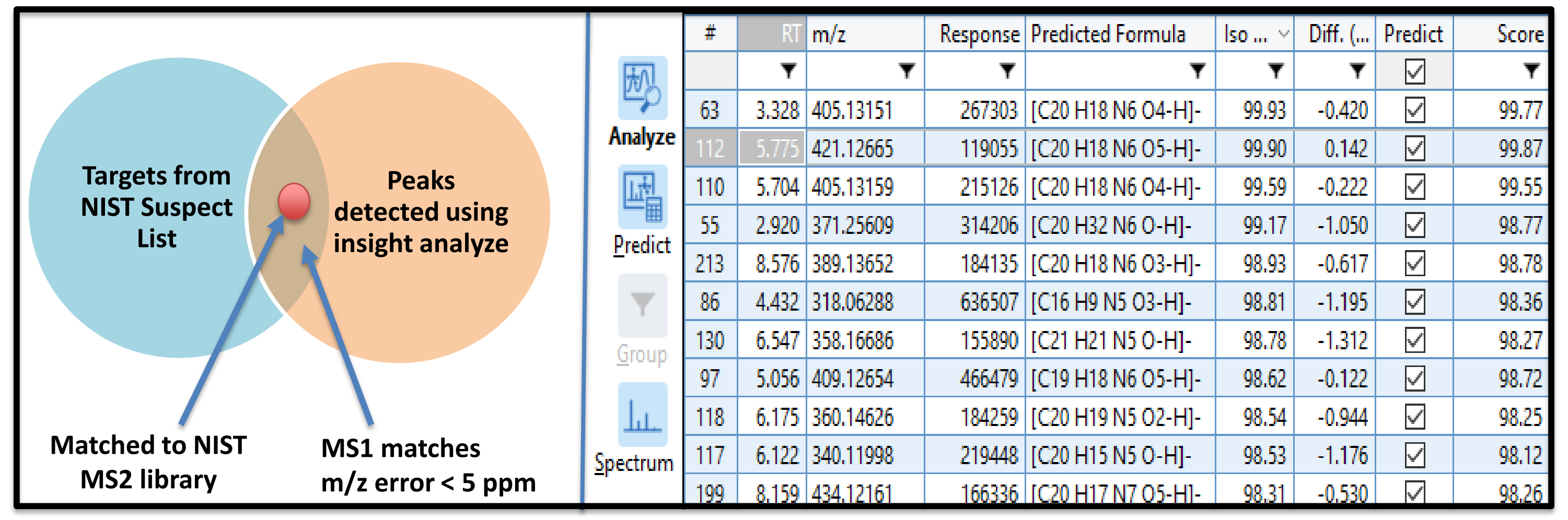


**Figure 2.** TIC chromatographic view of negative and positive mode

All three samples were analyzed using LabSolutions Insight Explore and FluoroMatch software. Identified chromatograms were screened against the NIST suspect list and matched with both the NIST and Shimadzu in-house PFAS library. Results from NIST suspect matches (Figure 3), NIST library matches (Figure 4), and FluoroMatch (Figure 5) were combined to generate a final list of detected compounds (Table 2). The merged list was evaluated for peak quality using Insight Explore chromatograms and isotope patterns. Identified peaks were further refined based on the criteria in Table 1, and NIST IDs were assigned according to NIST NTA guidelines [1,2]. This systematic approach enabled accurate analysis and identification of PFAS analytes in the unknown samples.



| # | RT | m/z | Response | Predicted Formula | Iso ... | Diff. (... | Predict | Score |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | ☑ | |
| 63 | 3.328 | 405.13151 | 267303 | [C20 H18 N6 O4-H]- | 99.93 | -0.420 | ☑ | 99.77 |
| 112 | 5.775 | 421.12665 | 119055 | [C20 H18 N6 O5-H]- | 99.90 | 0.142 | ☑ | 99.87 |
| 110 | 5.704 | 405.13159 | 215126 | [C20 H18 N6 O4-H]- | 99.59 | -0.222 | ☑ | 99.55 |
| 55 | 2.920 | 371.25609 | 314206 | [C20 H32 N6 O-H]- | 99.17 | -1.050 | ☑ | 98.77 |
| 213 | 8.576 | 389.13652 | 184135 | [C20 H18 N6 O3-H]- | 98.93 | -0.617 | ☑ | 98.78 |
| 86 | 4.432 | 318.06288 | 636507 | [C16 H9 N5 O3-H]- | 98.81 | -1.195 | ☑ | 98.36 |
| 130 | 6.547 | 358.16686 | 155890 | [C21 H21 N5 O-H]- | 98.78 | -1.312 | ☑ | 98.27 |
| 97 | 5.056 | 409.12654 | 466479 | [C19 H18 N6 O5-H]- | 98.62 | -0.122 | ☑ | 98.72 |
| 118 | 6.175 | 360.14626 | 184259 | [C20 H19 N5 O2-H]- | 98.54 | -0.944 | ☑ | 98.25 |
| 117 | 6.122 | 340.11998 | 219448 | [C20 H15 N5 O-H]- | 98.53 | -1.176 | ☑ | 98.12 |
| 199 | 8.159 | 434.12161 | 166336 | [C20 H17 N7 O5-H]- | 98.31 | -0.530 | ☑ | 98.26 |

**Figure 3:** LabSolutions Insight Explore matched the suspect list using the Analyze function. MS2 spectra were compared to the NIST database to identify targets from suspect list. In addition, custom C++ program was used to detected common product ions and neutral losses.

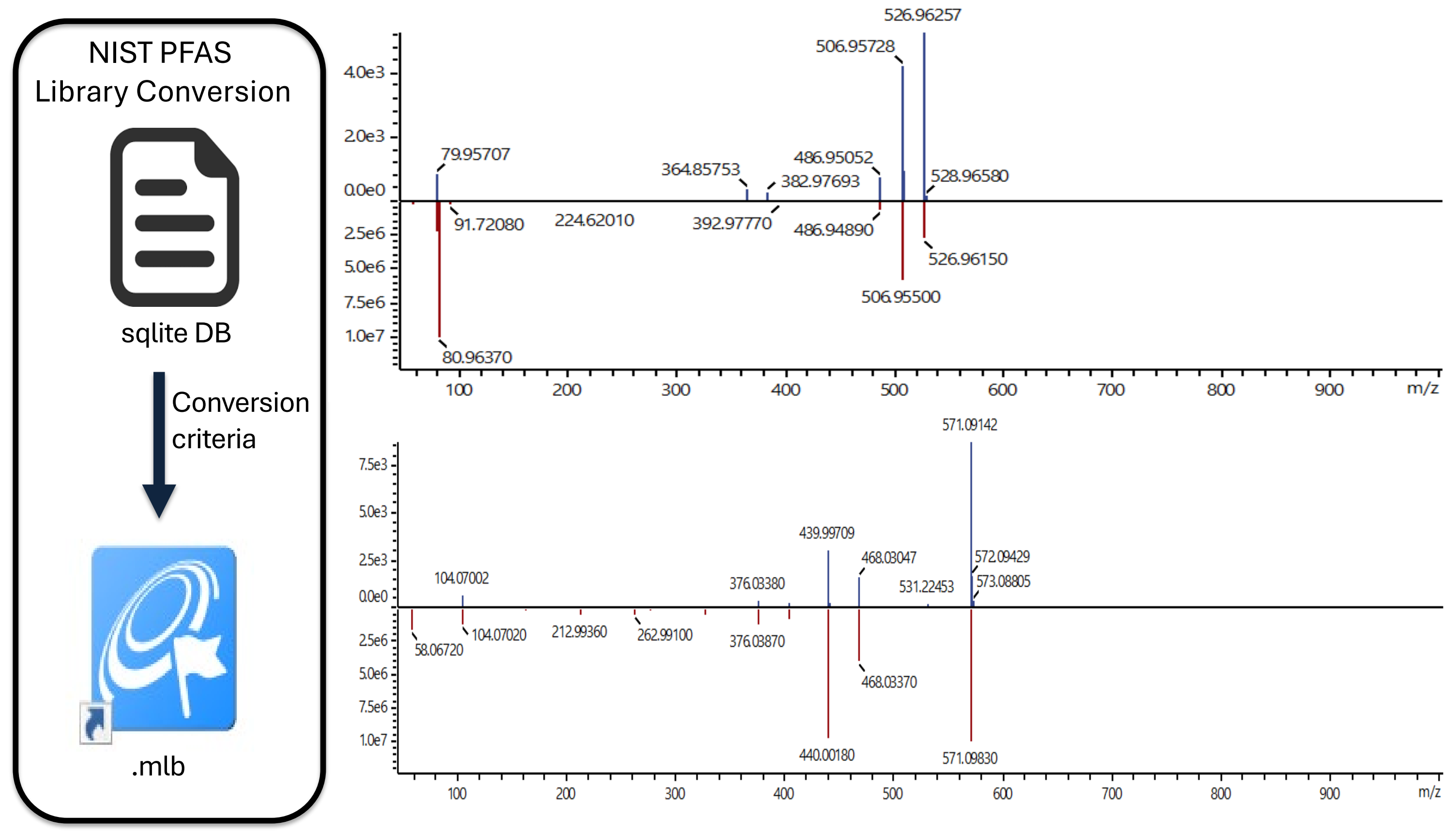


**Figure 4.** The database obtained from NIST in SQLite format was converted to .mlb files compatible with Insight Explore. This library, combined with the Shimadzu in-house PFAS library, was used for matching and identifying untargeted PFAS in samples A, B, and C.

**Figure 5:** Negative mode DDA files from samples A, B, C, and the blank were uploaded to FluoroMatch software, which used its internal library and Kendrick plots to identify PFAS compounds. Results were visualized and further analyzed in Power BI. Each target was evaluated based on recommended scoring criteria. Identified matches were screened for outliers and false positives using Insight Explore.

**Table 1**: Final Assignment Criteria

| NIST Identification criteria | Level 1a-5b |
|---|---|
| Multi platform match | NIST Library Match<br>Suspect List Match<br>FluoroMatch |
| Iso Score | >20 |
| PPM error | <5 |
| Flouromatch Score | >C+ |
| Number of Matching Neutral loss | >1 |

## 4. Results

Data from three unknown samples (A. B and C) were obtained using the DDA method in both positive and negative mode. The EPA 1633 PFAS standard mix was used to validate m/z trigger settings for the DDA method. These data were analyzed using multi approach data processing using NIST Library Match, NIST Suspect Match, and FluoroMatch. Results were dereplicated, and PFAS were identified and scored according to NIST-defined criteria. For samples A, B, and C, there were 40, 57, and 105 negative PFAS matches, respectively, and 9, 44, and 29 positive matches (Table 2). Results from the interlaboratory study were published by NIST and included a results table for each sample. Our laboratory, coded DIM003, was the only one which successfully reported 100% of the analytes in all three tables. Our three-pronged approach gave comprehensive identifications without excess false positives.

**Table 2**: Number of PFAS reported for NIST Interlaboratory study

| Sample | Positive Match | Negative Match | Total |
|---|---|---|---|
| A | 9 | 40 | 49 |
| B | 44 | 57 | 101 |
| C | 29 | 105 | 134 |

## 5. Reference

1. Place, B. , Reiner, J. , Ragland, J. , Rodowa, A. , Burdette, C. , Cuthbertson, A. , Rimmer, C. , Kucklick, J. and Mahynski, N. (2024), Per- and Polyfluoroalkyl Substances &#8211; Non-Targeted Analysis Interlaboratory Study Final Report, NIST Interagency/Internal Report (NISTIR), National Institute of Standards and Technology, Gaithersburg, MD, [online], https://doi.org/10.6028/NIST.IR.8544, https://tsapps.nist.gov/publication/get_pdf.cfm?pub_id=958688 (Accessed May 28, 2025)
2. Joseph A. Charbonnet, Carrie A. McDonough, Feng Xiao, Trever Schwichtenberg, Dunping Cao, Sarit Kaserzon, Kevin V. Thomas, Pradeep Dewapriya, Benjamin J. Place, Emma L. Schymanski, Jennifer A. Field, Damian E. Helbling, and Christopher P. Higgins. "Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry." Environmental Science & Technology Letters 2022 9 (6), 473-481. DOI: 10.1021/acs.estlett.2c00206

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