SHIMADZU

TP 562

Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances in Environmental Extracts by Microflow LC-QToF Coupled with Multi-spray ESI

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1. Introduction

Targeted screening methods are well established for monitoring Per- and polyfluoroalkyl substances (PFAS) in environmental samples. However, PFAS comprise a large and evolving class of compounds, creating a need to screen existing, emerging, and transformation products across diverse matrices. Non-targeted Analysis (NTA) allows for the confirmation of suspected PFAS in the environment, but the sample characterization is often limited by analytical sensitivity. Microflow (0.5 – 40 µL/min) coupled with a multi-nozzle emitter enhances sensitivity by improving desolvation. When this is coupled to a high mass accuracy and resolution Quadrupole Time-of-Flight Mass Spectrometer (QToF), capable of data-dependent and independent MS/MS, PFAS characterization of unknown analytes is possible. In this study, various environmental extracts were processed and screened using NTA for PFAS compounds.

2. Methods

A Shimadzu Nexera Mikros LC was used to introduce the sample to a Newomics DuoESI source with an M3 emitter on a LCMS-9050 QToF MS (**Fig. 1**). A direct injection on an octadecyl column without delay column was performed. Direct injection was chosen over trap-and-elute configuration to minimize selectivity bias caused by the trap column. The mobile phase, gradient, and source conditions were optimized in negative mode using known PFAS standards (**Table 1**).

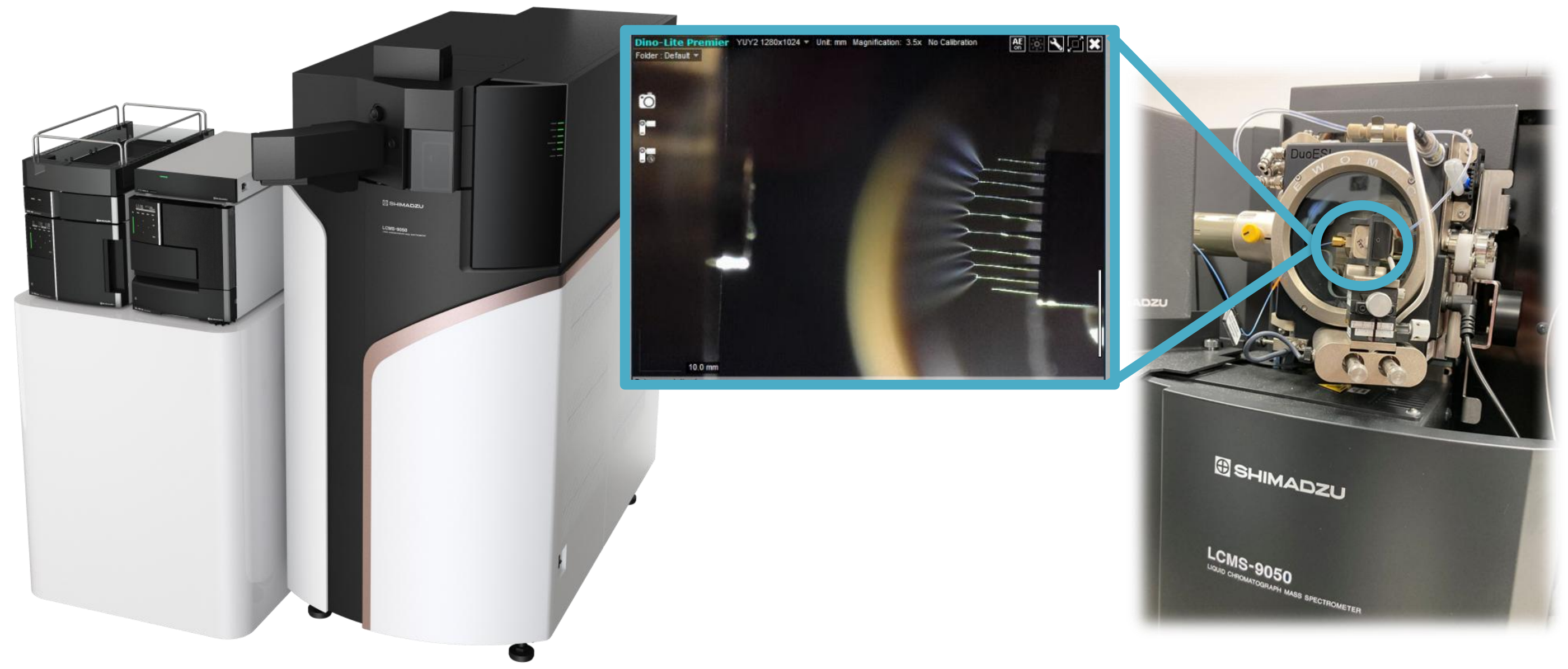
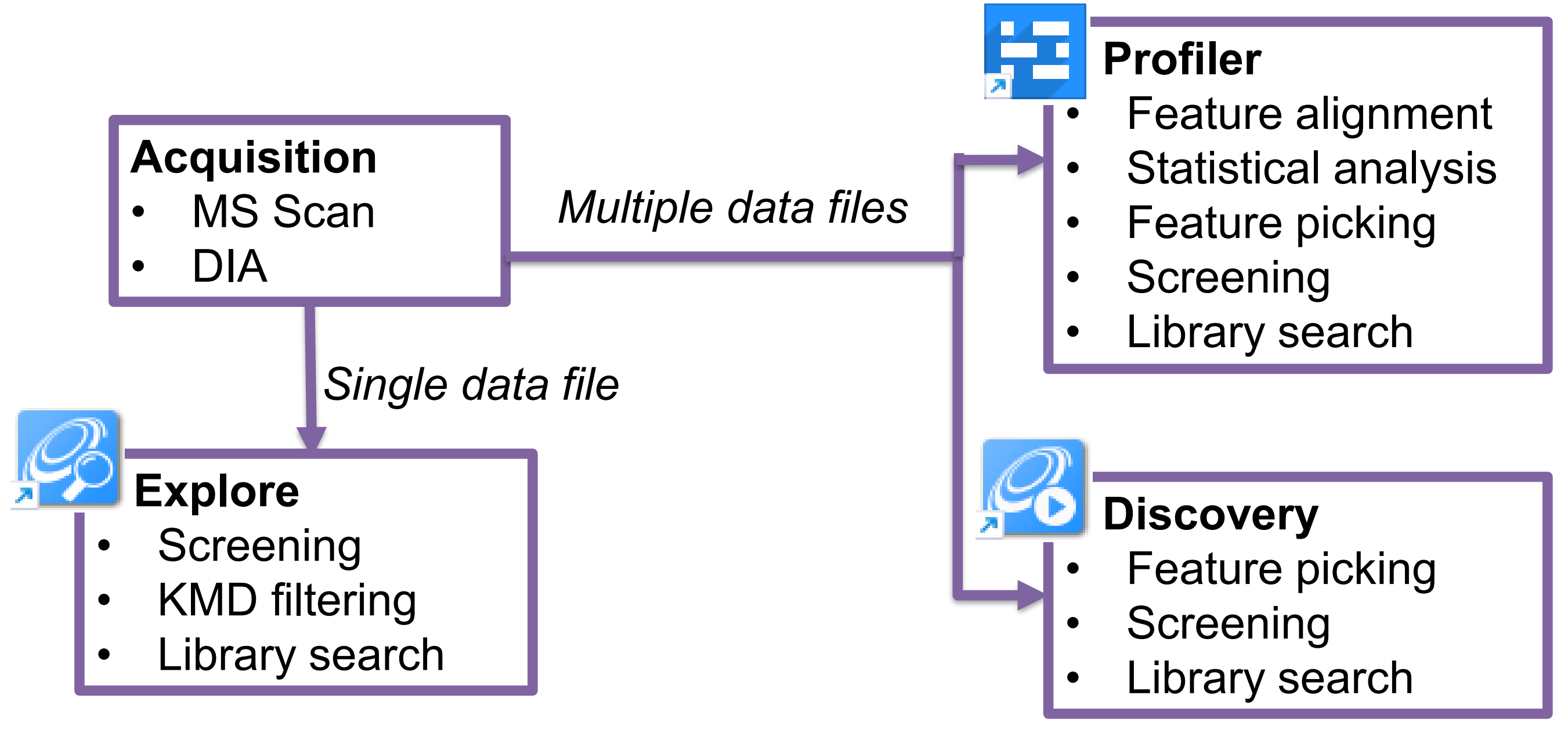


Fig. 1. The Newomics M3 Multi-Nozzle Emitter and the Newomics Source mounted on the Shimadzu LCMS-9050 QToF MS.

Table 1. Acquisition parameters.

Nexera Mikros	
Mobile phase	A: 2mM ammonium acetate in water B: Acetonitrile
Column	Shimadzu Shim-pack MC C18 0.175 mm x 50 mm, 1.9 µm
Gradient (%B)	5% (3.0 min) ⇒ 95% (33.0 -38.0 min) ⇒ 5% (38.1-50 min)
Column oven temp.	50 ℃
Flow rate	5 µL/min
Injection volume	1 µL
Rinsing type	Internal/External
LCMS-9050	
Ionization	DuoESI source, negative mode
Mode	MS *m/z* 100-1000 DIA, isolation window 34.6 Da
Nebulizer gas flow	1 L/min
Interface voltage	-2 kV
DL temp.	150 ℃
Heatblock temp.	150 ℃
Collision energy	25 ±17 V
Loop time	0.93 s

Environmental PFAS samples includes methanolic extraction of AFFF-impacted soil from the NIST NTA interlaboratory study and extracted landfill leachate. Multiple Shimadzu software (e.g. Insight Explore, Insight Discovery, Profiler) were used to process the data acquired under data independent acquisition (DIA) (**Fig 2**).



Acquisition
- MS Scan
- DIA

Multiple data files

Single data file

Profiler
- Feature alignment
- Statistical analysis
- Feature picking
- Screening
- Library search

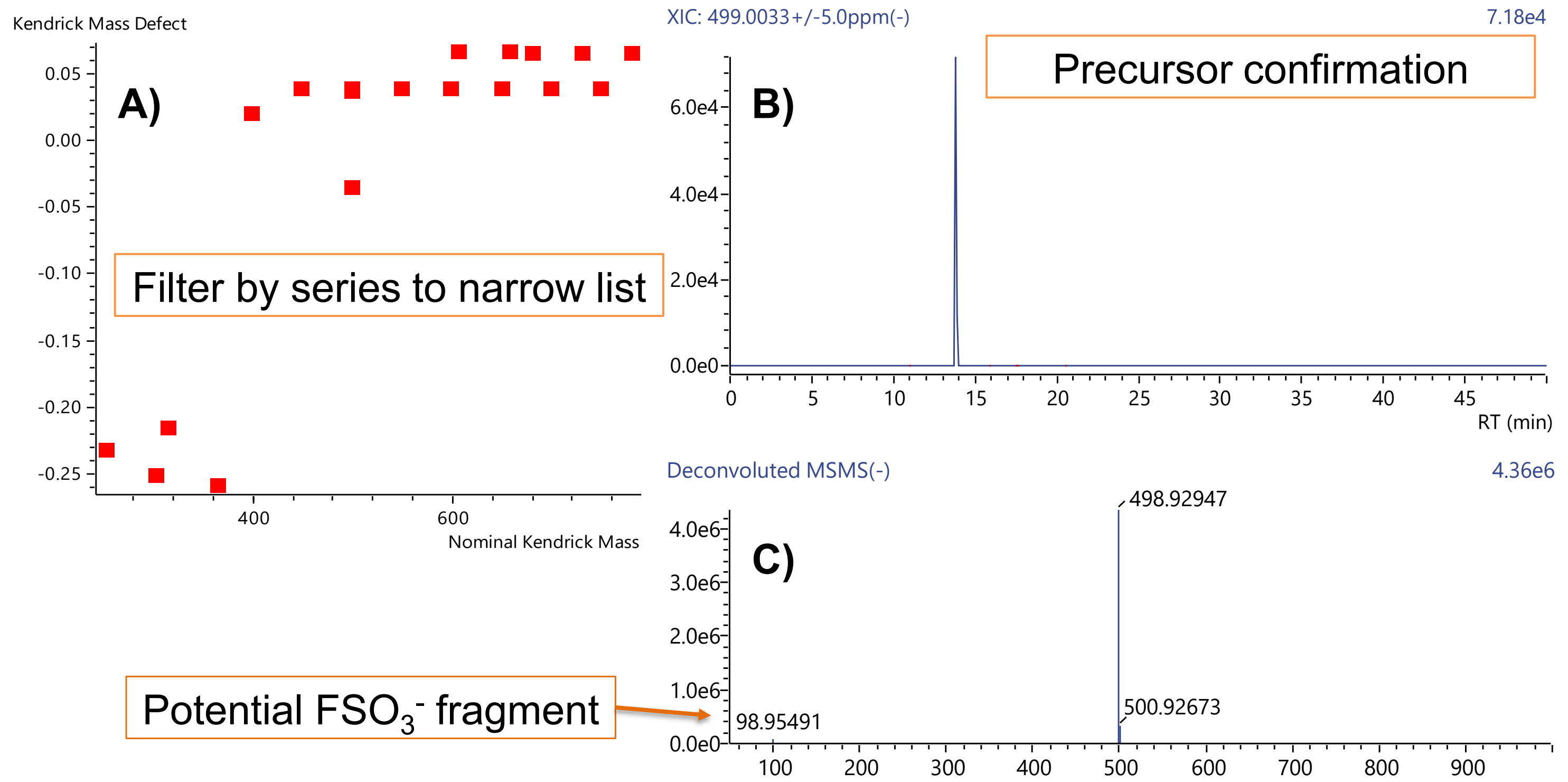
Explore
- Screening
- KMD filtering
- Library search

Discovery
- Feature picking
- Screening
- Library search

Fig. 2. Schematic outlining the workflow and software features.

3. Results and Discussion

Kendrick Mass Defect (KMD) filtering is a feature in Insight Explore to reduce data complexity and find new, putative compounds by identifying the difference between the nominal and exact Kendrick mass. For this work, difluoromethylene was used (CF_2) and 80 mDa KMD tolerance was set to create a list of possible PFAS compounds. Only series are shown for quick filtering (**Fig. 3**).



Potential FSO_3^- fragment

Fig. 3. Workflow demonstrating the utilization of KMD (A) for tentative identification. XIC of *m/z* 499.0033 (B) revealed a clean peak not currently in the library or screening list. Observation of DIA deconvoluted MSMS (C) revealed a diagnostic ion of FSO_3^- suggesting a possible sulfated PFAS.

Further identification can be done by the Assign feature in Insight Explore. Confirmed compounds can be easily added to libraries. Once a screening list and library is established, Insight Discovery can be used to process multiple data files for quick elimination of the known PFAS compounds and to explore other chemicals in the environmental samples (**Fig. 4**). Statistical analyses were used to narrow down potential targets for further identification. PCA was performed on environmental sample extracts and more than 1000 features were aligned with the *m/z* of each feature given on the loading plot (**Fig. 5A**). Further screening and library search yielded that 6:2 FTS (*m/z* 426.966, RT 10.74 min) was among those that contributes to PC2.

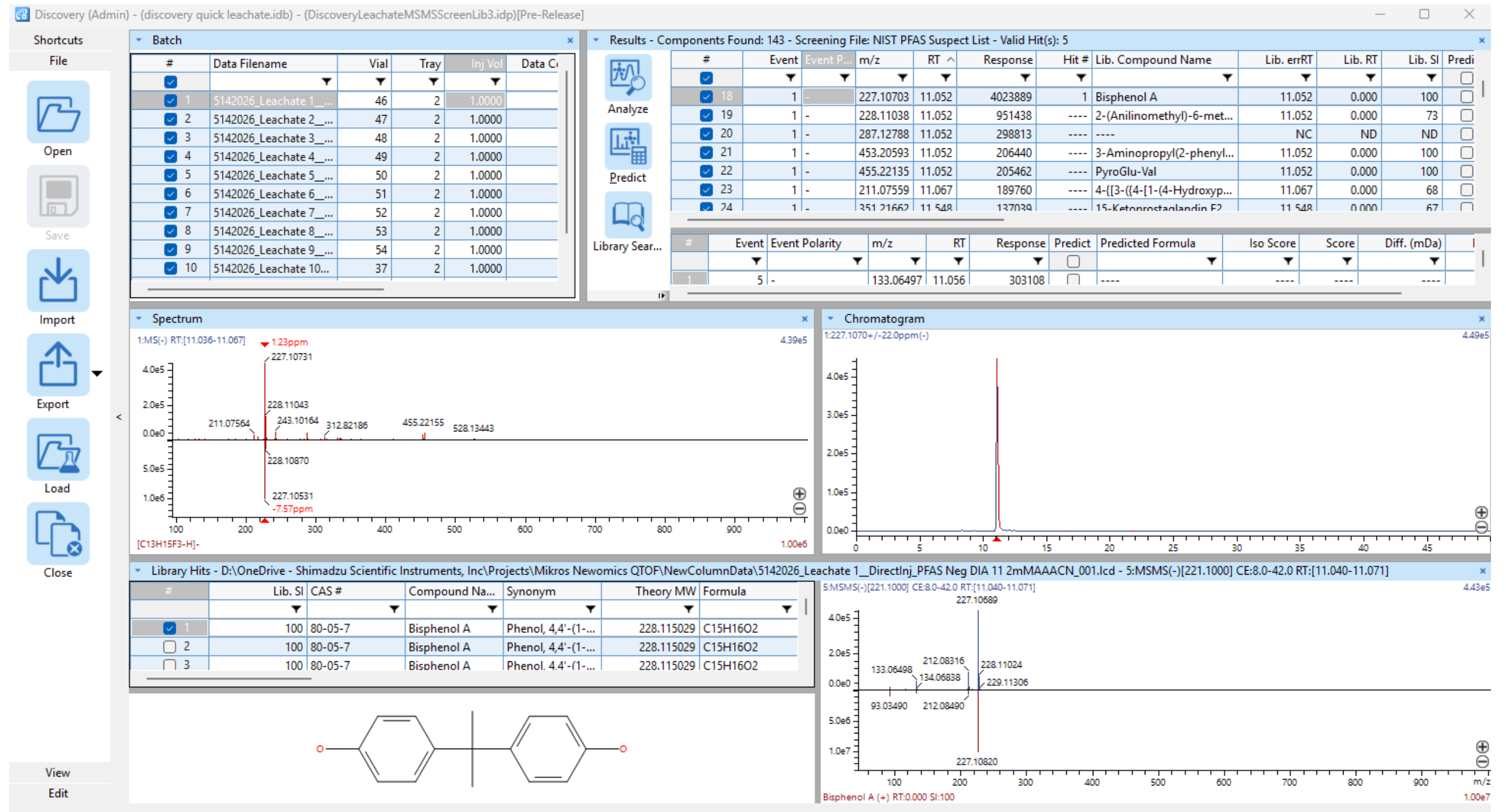


Fig. 4. Multiple samples were processed through Insight Discovery for feature picking, screening, and library search.

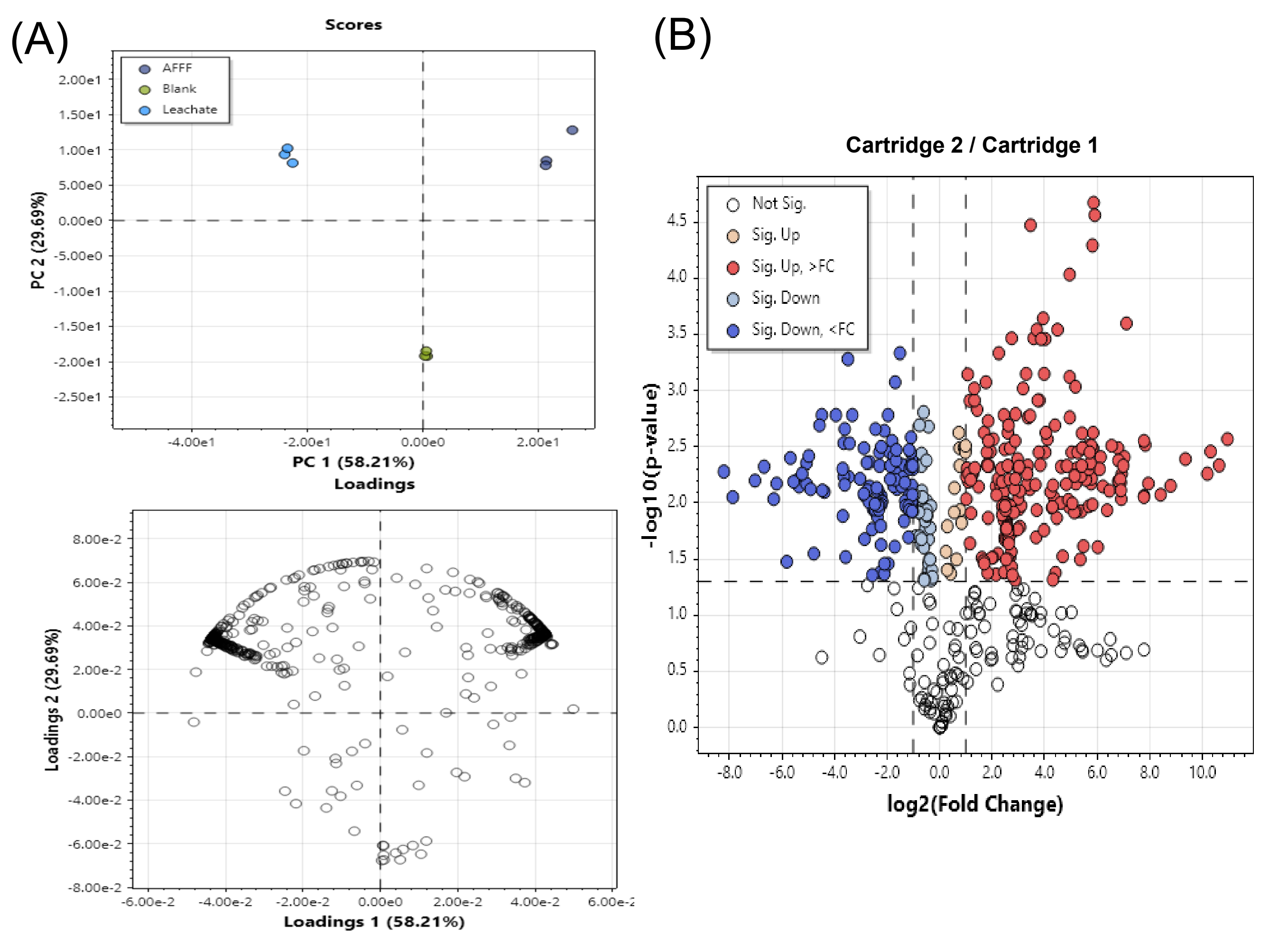


Fig. 5. (A) The scores and loading plot from the PCA of two environmental extracts and blank. (B) Volcano plot comparing two different types of SPE cartridge for leachate extraction.

Two sources of WAX based SPE cartridges were compared for cleaning the leachate sample. Profiler picked 641 aligned features and a volcano plot was generated (**Fig. 5B**). With quick filtering, 302 features were significantly higher in cartridge 2, whereas 129 features were significantly higher in cartridge 1.

4. Conclusion

With the increased sensitivity of microflow LC-QToF coupled with multi-spray ESI with DIA acquisition, Insight Explore aided in NTA PFAS analysis. When paired with Insight Discovery and Profiler, a streamlined batch data processing workflow with statistical analysis was shown to effectively handle complex environmental matrices and their analyses.

References

1. Place, Benjamin J., et al. *Per-and Polyfluoroalkyl Substances-Non-Targeted Analysis Interlaboratory Study Final Report.* US Department of Commerce, National Institute of Standards and Technology, 2024.
2. Benjamin Place (2021), Suspect List of Possible Per- and Polyfluoroalkyl Substances, National Institute of Standards and Technology, https://doi.org/10.18434/mds2-2387 (Version: 1.7)

The authors are affiliated and funded by Shimadzu Corporation.