

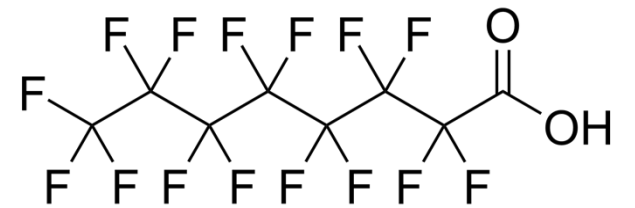


# High Resolution Mass Spectrometry (HRMS) Techniques for Screening of PFAS in Environmental Samples

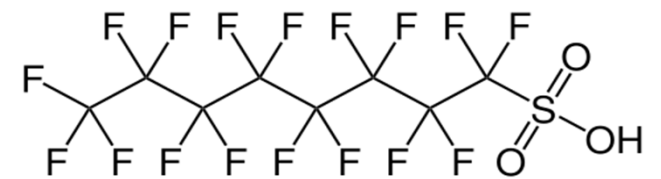
**Kari Organtini, Ph.D**  
**Principal Scientist**

# Per- and Polyfluoroalkyl Substances (PFAS)

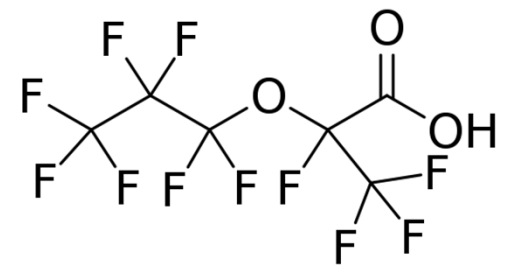
Waters  
THE SCIENCE OF WHAT'S POSSIBLE.™



PFOA

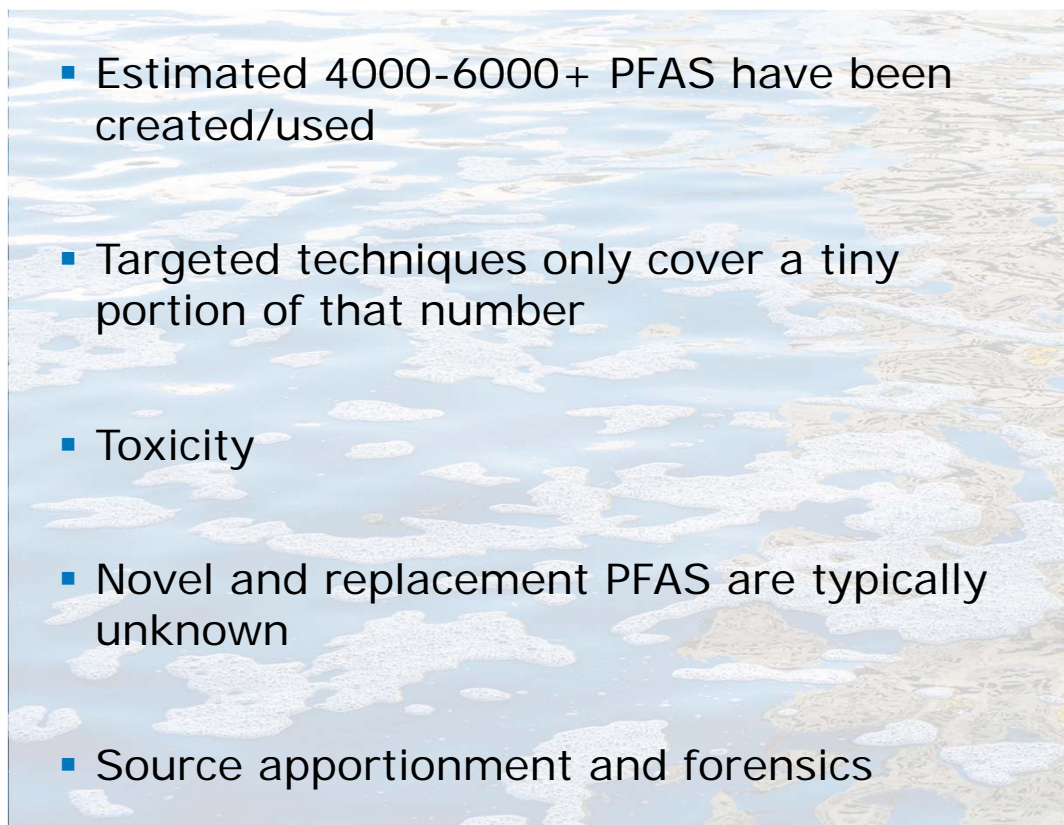


PFOS



GenX (HFPO-DA)

## Why is High Resolution Screening Helpful for PFAS?

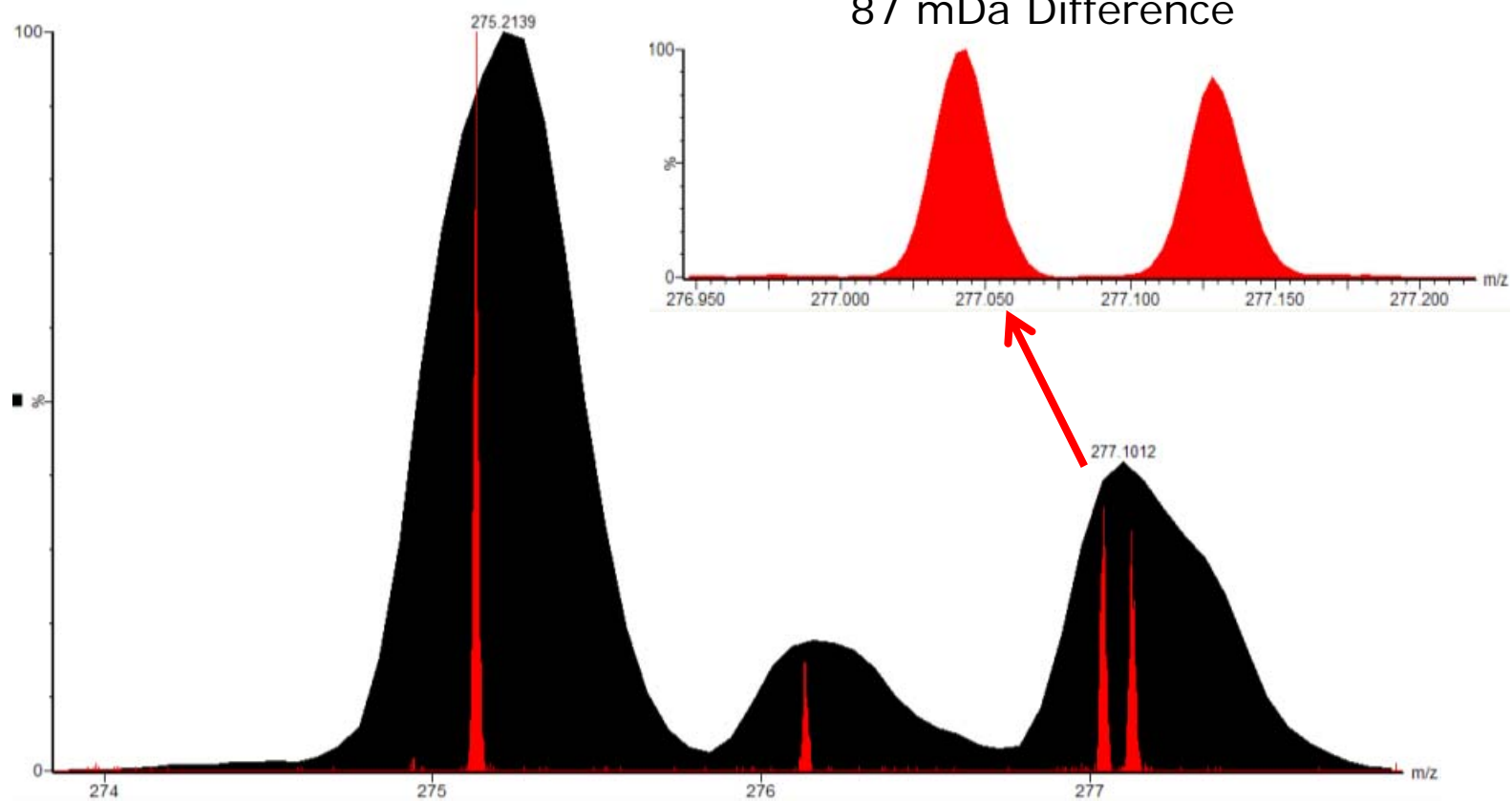


Method	# PFAS
EPA 537.1	18
EPA 533	25
EPA 8327	24
ISO 21675	30
EU DWD	20

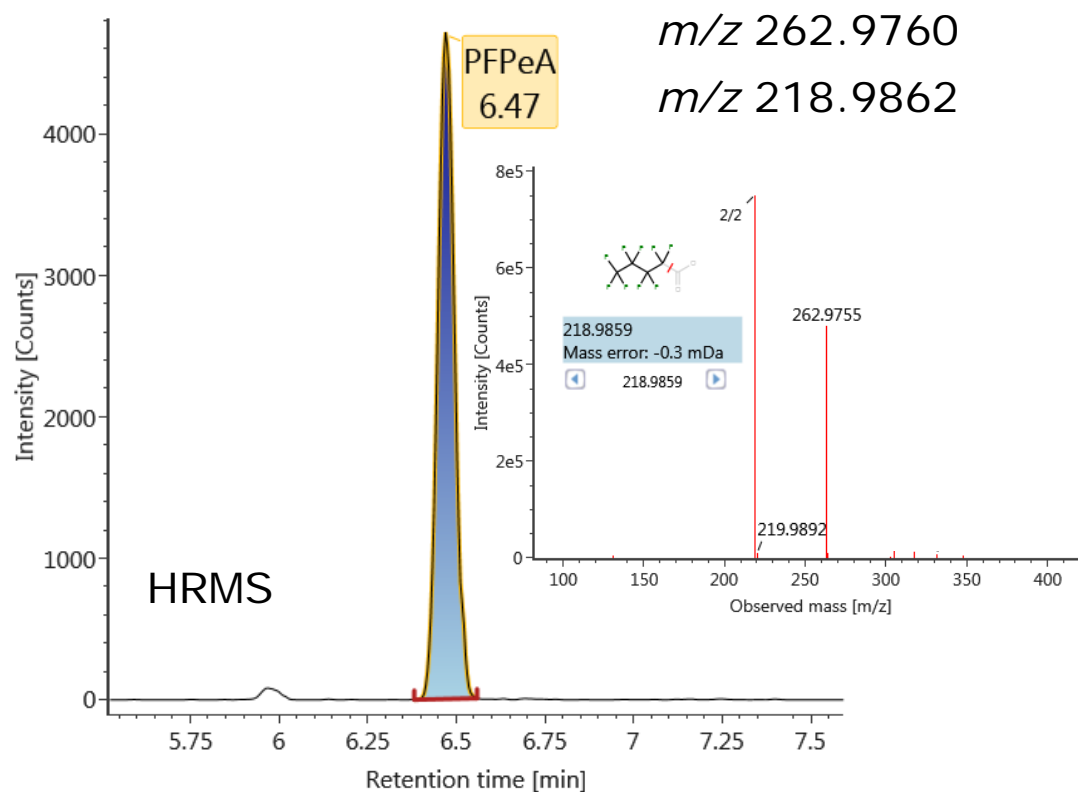
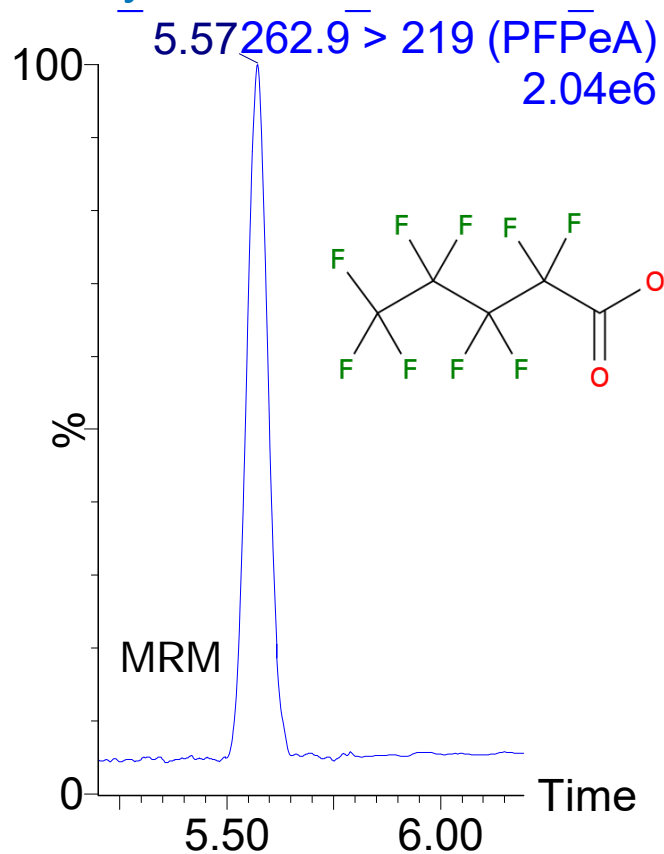
## Advantages of accurate mass screening?

- Over recent years high resolution mass spectrometry has gained in popularity as a screening tool
  - ✓ **Ability to perform non-targeted analysis**
    - The freedom to measure compounds without prior compound specific tuning
  - ✓ **Ability to perform historical data review**
    - The capability of performing structural elucidations of unknowns or suspect compounds
  - ✓ **Ability to perform full spectral analysis**
    - Providing greater insight into the composition of a complex sample
  - ✓ **Ability to screen for larger number of compounds and adducts**
    - Compared to QqQ based screening

# Power of High-Resolution MS



# Power of High-Resolution MS Analysis of PFPeA: MRM Vs. HRMS



## Four Fundamental Questions

Are these compounds in my sample?

Targeted analysis

How much is in my sample?

Quantitation

What else is in my sample?

Non-Targeted Analysis

What are the differences between samples?

Comparison



# Collecting a Comprehensive Profile

UltraPerformance Separation

A vertical stack of three black laboratory modules, likely a chromatography system, with a central handle on the middle unit.

Universal Ion Sources

A complex assembly of scientific instruments, including a central orange and silver unit flanked by two black rectangular modules with screens.

Xevo G2-XS QTof

A large, dark-colored laboratory instrument with a tall vertical component on the right side and a control panel on the left.

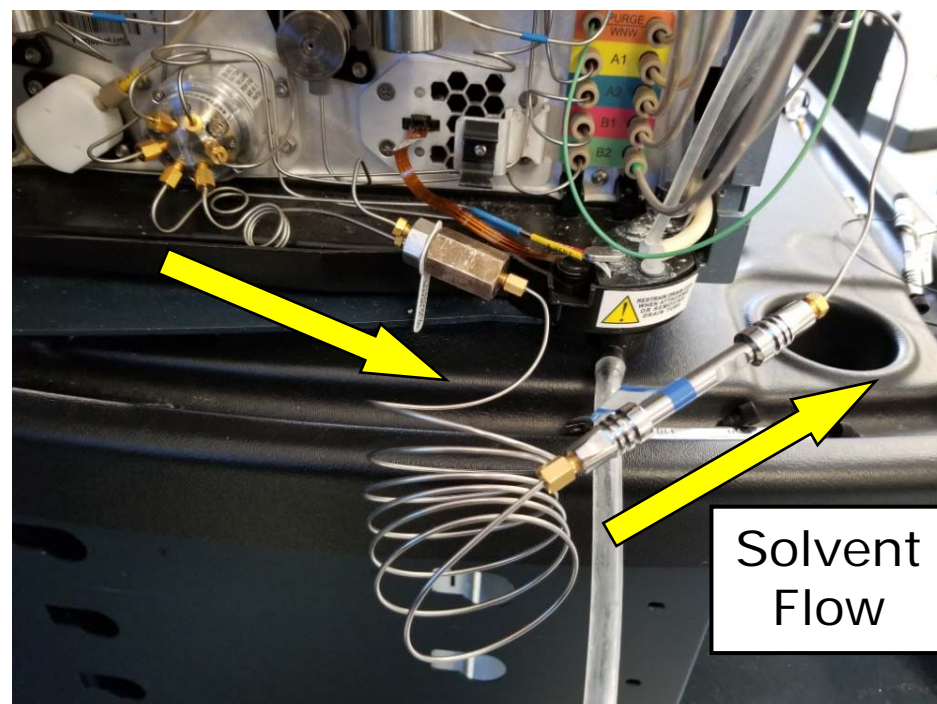
Scientific Information System

A graphic featuring a blue and white wavy line on the left, transitioning into the word "UNIFI" in a blue, sans-serif font on the right.

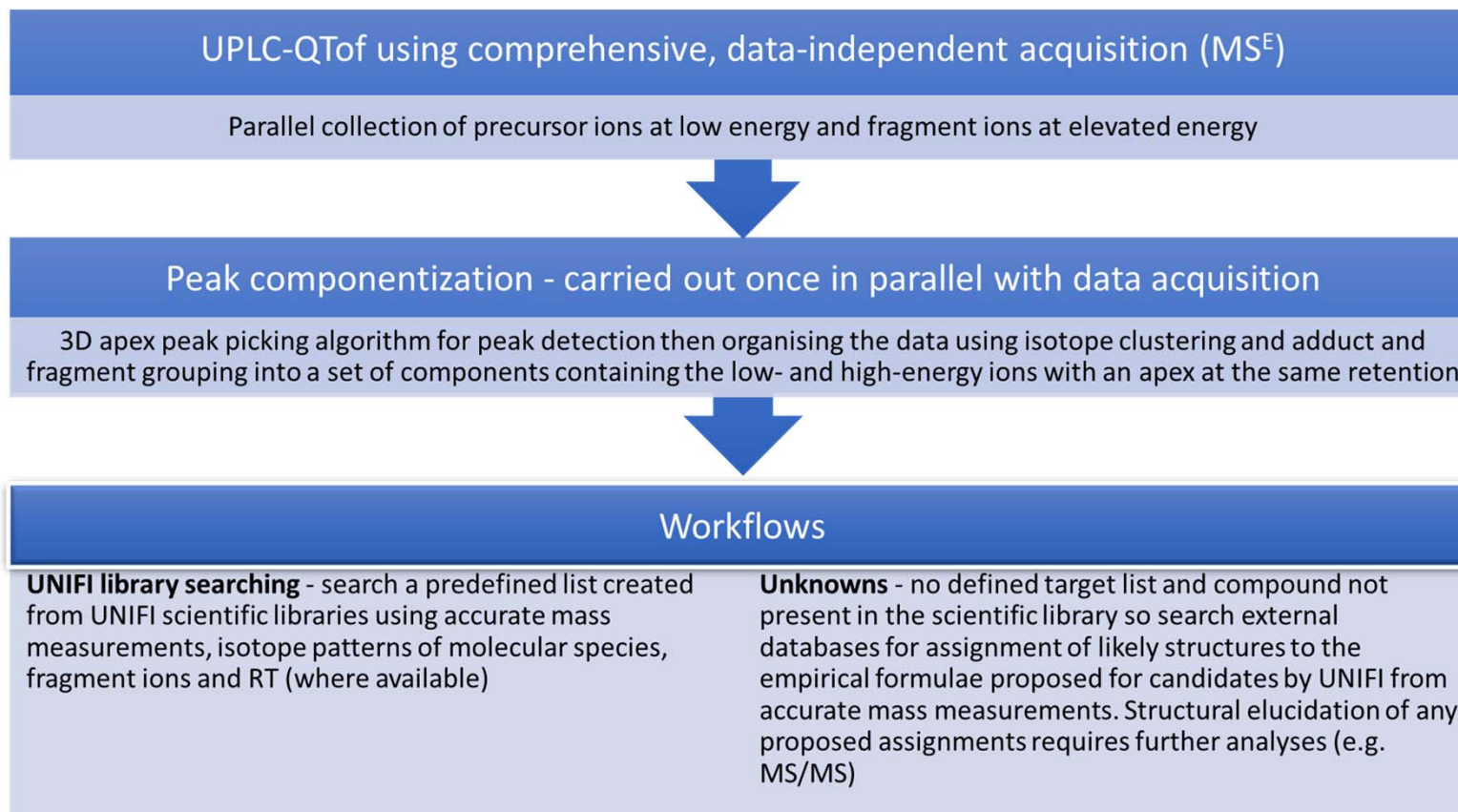


## LC modifications for PFAS analysis

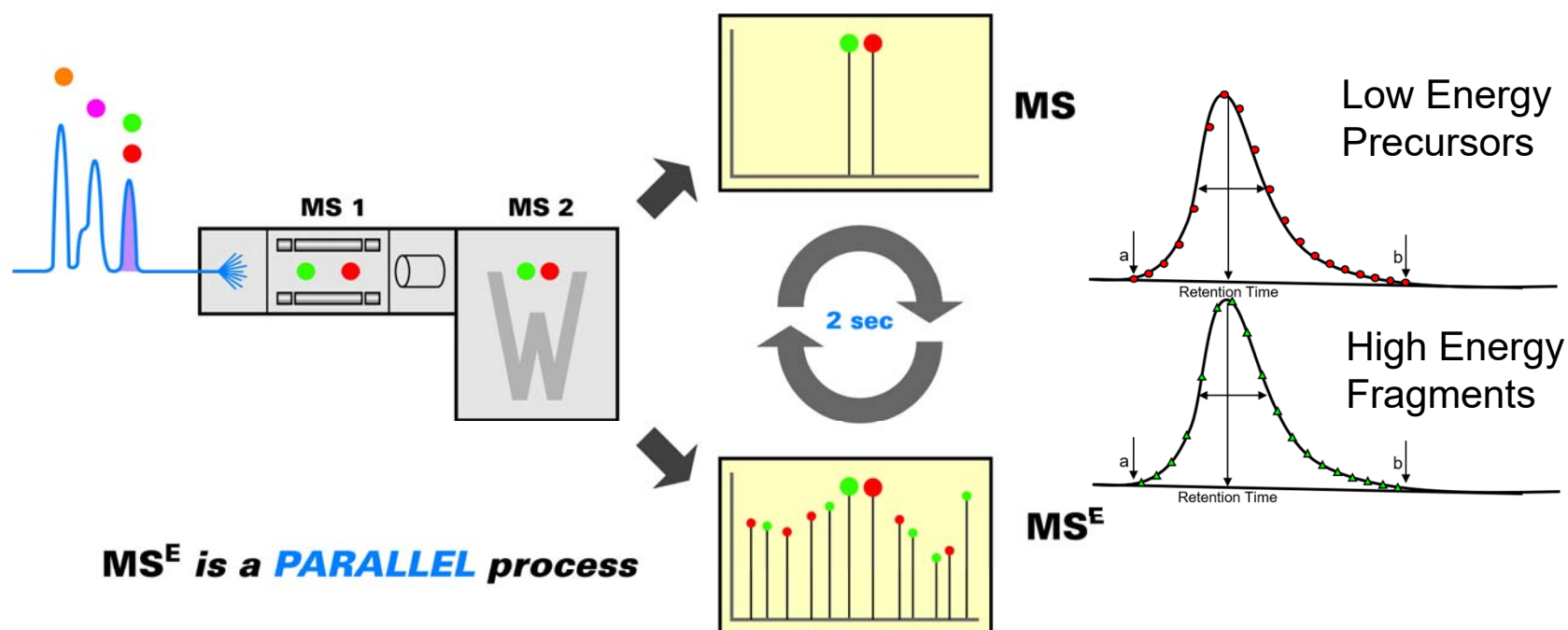
- Care must be taken when analyzing samples for PFAS compounds to avoid procedural and instrument contamination.
- System contamination can be reduced by taking the appropriate steps.
- PFAS Kit must be installed on the UPLC system prior to use for PFAS analysis.
- The kit is comprised of PFAS-free components and an isolator column.



# Separation, Detection and Identification Workflow



# MS<sup>E</sup>: Data Independent Acquisition



# Sample Preparation

**WATER**



5 mL sample,  
Spike surrogate,  
Dilute

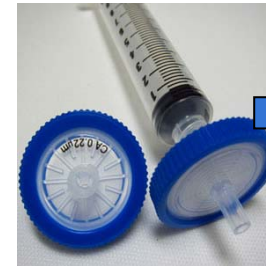
**SOIL**



2 g sample,  
Spike surrogate,  
Add Solvent

Centrifuge

Water  
Surface  
Ground  
Influent  
Effluent



Syringe filter entire  
sample



Acidify and transfer to  
polypropylene vial

Soils  
Sand  
Silt  
Lean Clay  
Fat Clay

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# Targeted Analysis

# UNIFI Library Generation

- Gather structures (mol files) → UNIFI automatically determines exact mass
- Inject standards for RTs and fragments

PFTrDA [110820, PFAS, Library]

Property	Value
Name	Compound
Item description	PFAS; carboxylate; legacy
IUPAC name	
Formula	C14HF27O2
Hill formula	C14HF27O2
Average molar mass	714.1134
Monoisotopic mass	713.9545
Item tag	
InChI	1S/C14HF27O2/c15-2(16,14(2)4)3(17,18)4(19,20)5(21,22)6(23,24)7(25,26)8(27,28)9(29,30)10(31,32)11(33,34)12(35,36)13(37,38)14(39,40)41/h(H,42,43)

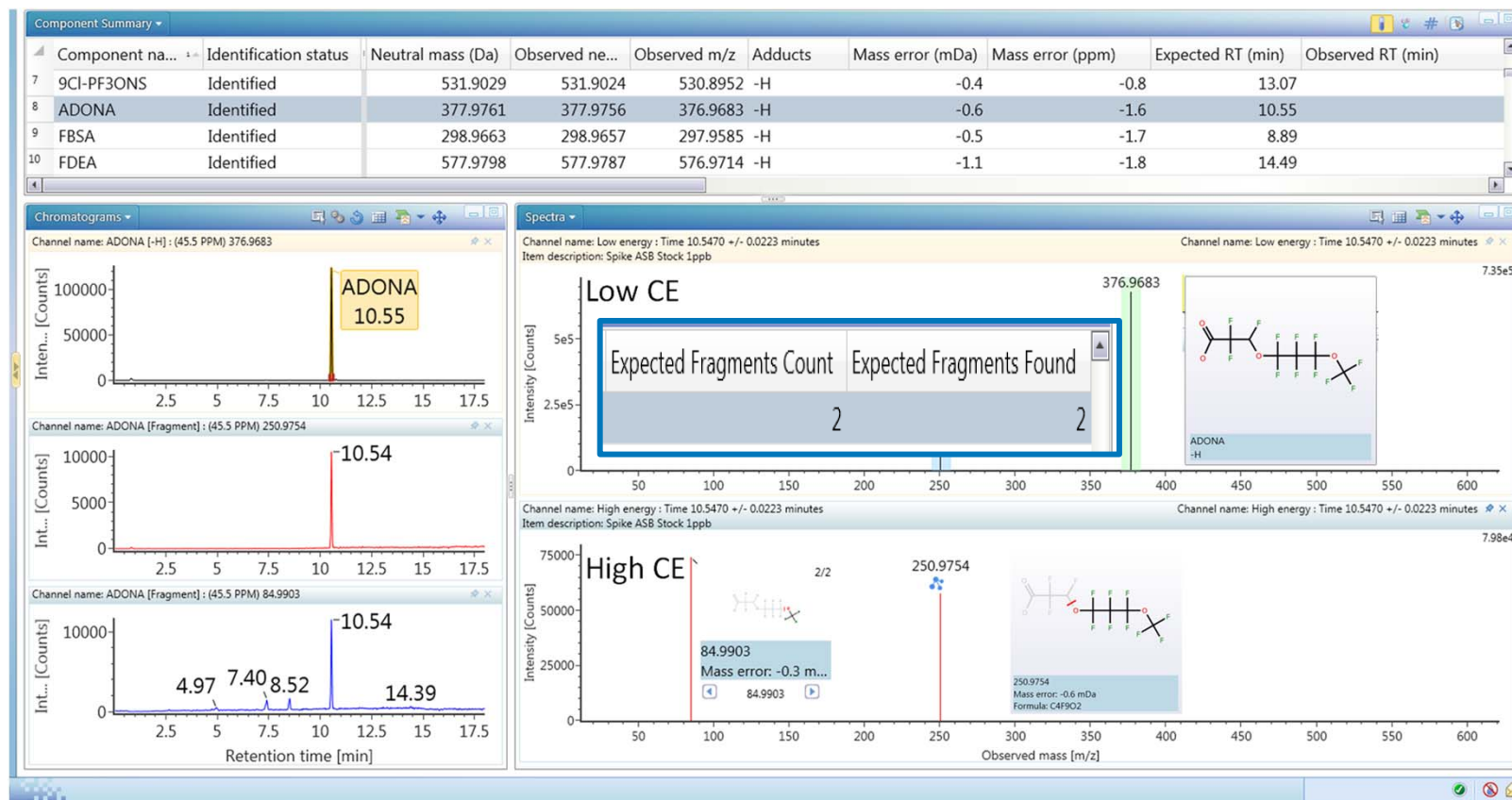
Priority	Neutral Mass (Da)	Adduct	Charge	Fragmentation type	Expected m/z	Observed...	Expected RT (min)	Ionization technique	Detail type
Detection result (Instrument model: Unknown, Instrument serial no.: Manually created, Created by administrator on Aug 11, 2020 (4 items))									
1	713.9545	-H	-1	None	712.9473		15.320	ESI-	MSe
2				CID	668.9574		15.320	ESI-	MSe
4				CID	218.9862		15.320	ESI-	MSe
3				CID	168.9894		15.320	ESI-	MSe

# Library Generation Based on Authentic Standards





# Assessing Identified Components



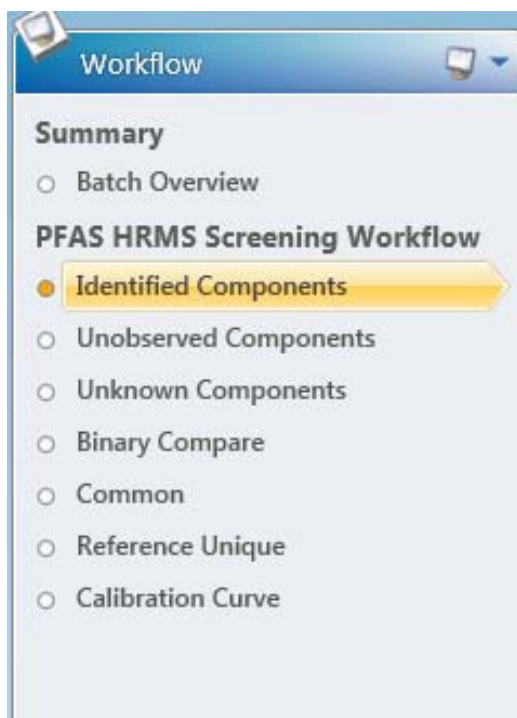
# View Filters for Efficient Data Review

The screenshot displays the Waters software interface for data review. The main window shows a 'Component Summary' table with columns for Component n..., Identification status, Neutral mass (Da), Observed neutral mass (Da), Observed m/z, Mass error (mDa), Mass error (ppm), Expected RT (min), Observed RT (min), Adducts, and Expected Fragments... The table lists 20 identified components, including N-MeFOSAA, PFBA, PFBS, PFDA, PFDoDA, PFDS, PFHpA, PFHpS, PFHxA, PFHxS, PFNA, PFNS, PFOA, PFOA [M+8], PFOS, PFOS [M+8], PFPeA, PFPeS, PFTrDA, PFTriDA, and PFUnDA.

An 'Edit Filter' dialog box is open, allowing users to define filter criteria. The criteria are set to 'Match all groups' and include:
 

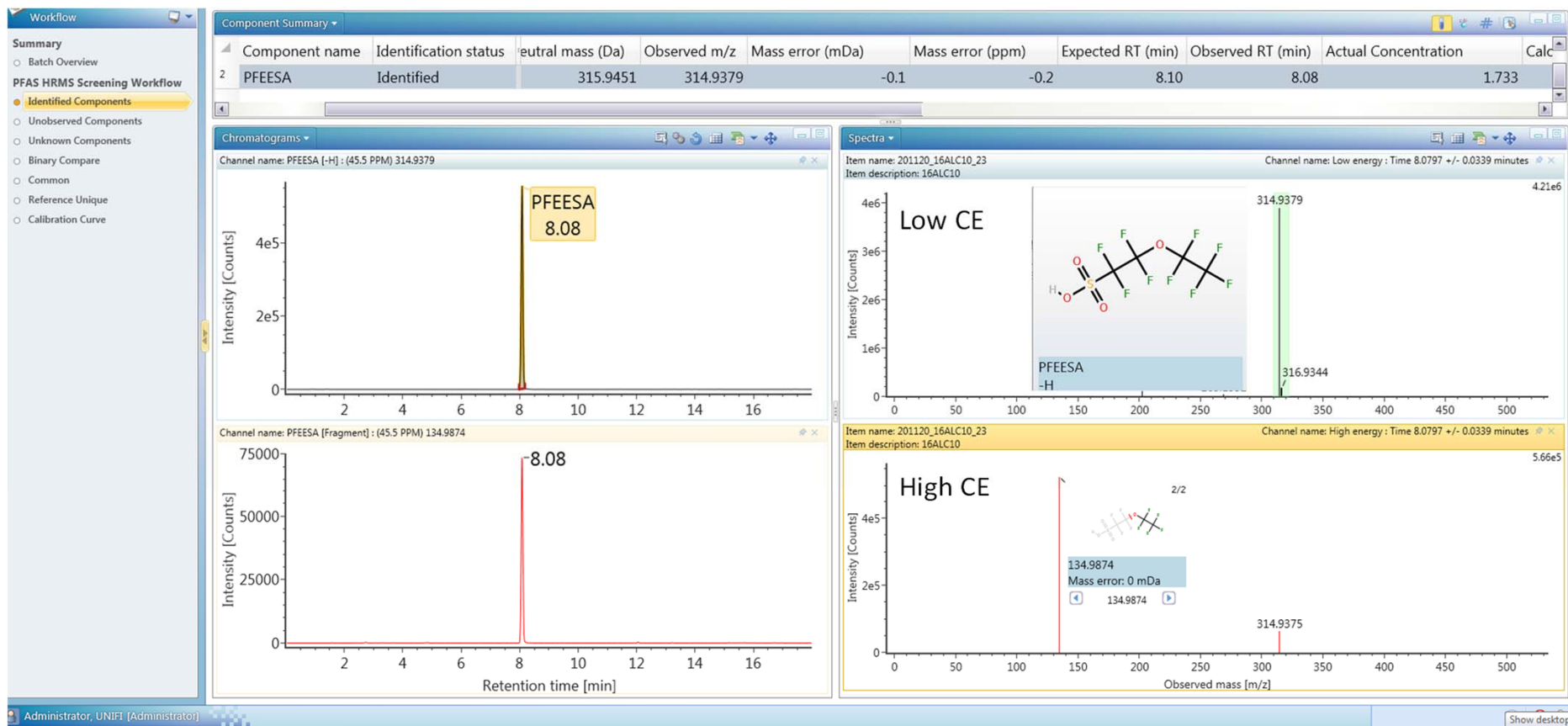
- Mass error (ppm) is less than 10 ppm
- Retention Time Error (min) is less than 0.1 min
- Theoretical Fragments Found is greater than 1
- Has Common Fragment Ions is Yes
- Has Common Neutral Losses is Yes
- Within Mass Defect Region is Yes

At the bottom, two chromatograms are visible. The left chromatogram shows a peak at 14.39 minutes labeled '11Cl-PF3OUdS'. The right chromatogram shows peaks at 576.9719 and 632.8862 m/z, with a label '630.8890'.



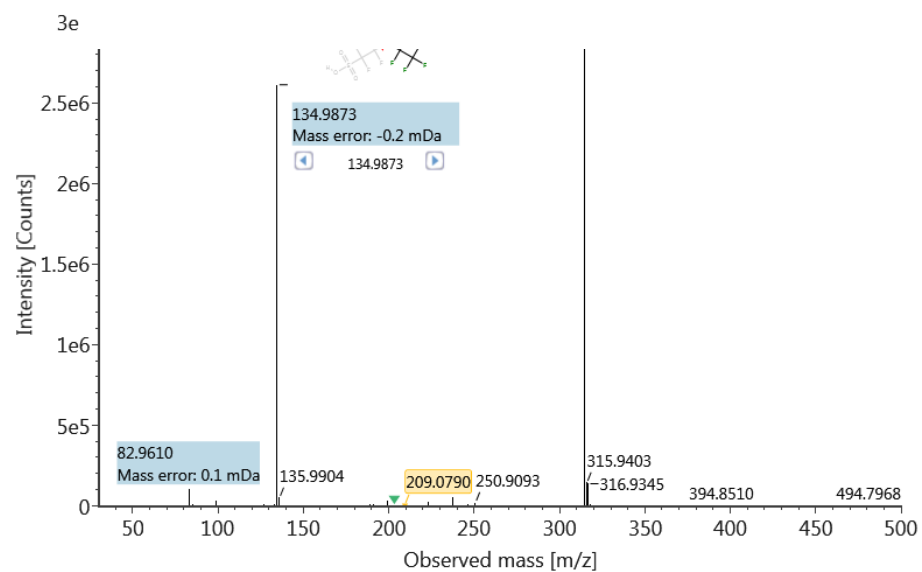
## Identification of PFAS in Soil Samples

# Identification of PFAS in a Soil Sample

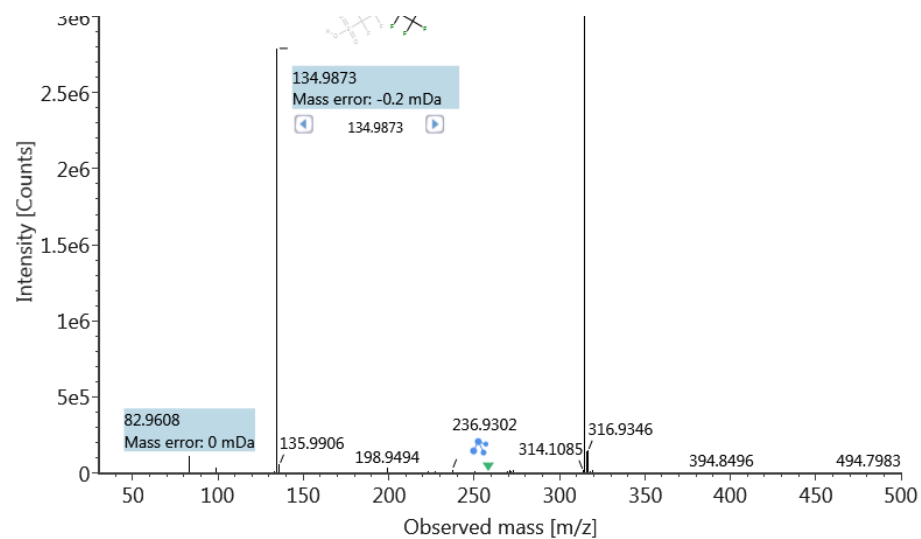


# Confirmatory Targeted MS/MS

Item name Authentic standard  
Item descri MS/MS spectrum for  $m/z$  314.9379 at  $t_R$  8.12

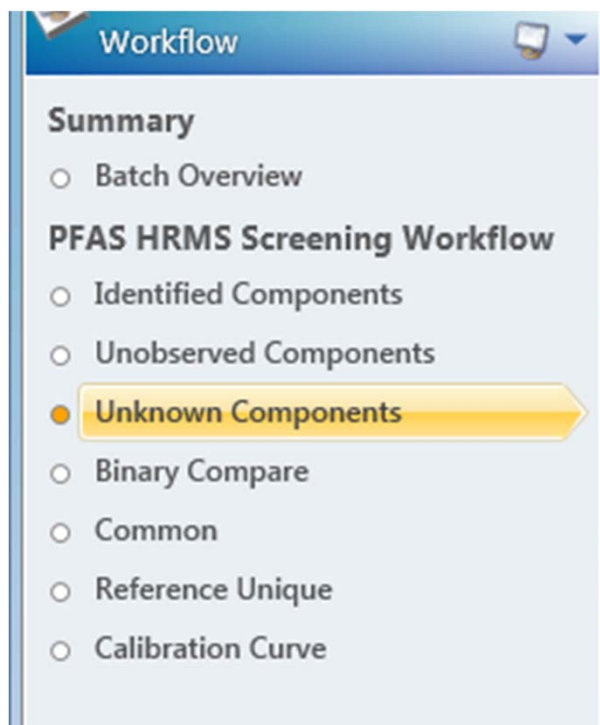


Lean Clay extract  
MS/MS spectrum for  $m/z$  314.9379 at  $t_R$  8.12



# Quantitation of PFEESA in a Soil Sample





## Non-Targeted Analysis



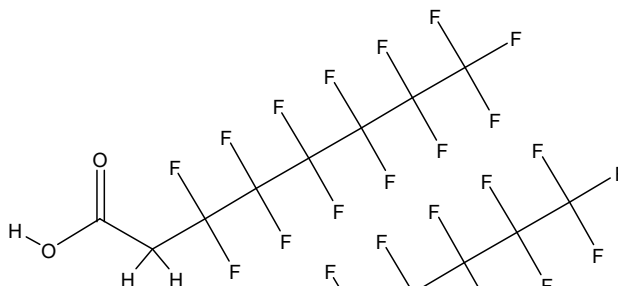
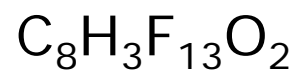


# Structural Elucidation Tools-Common Fragments

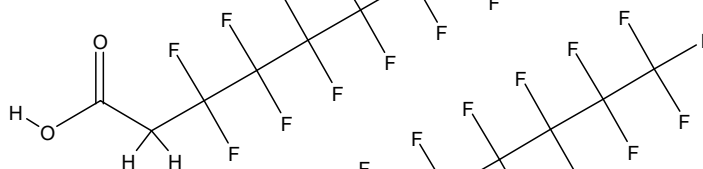
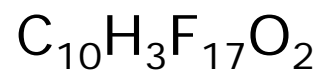


## Structural Elucidation Tools-Neutral Loss

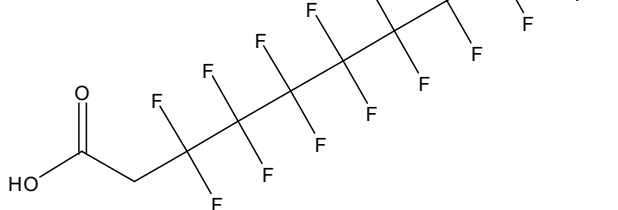
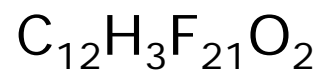
FHEA



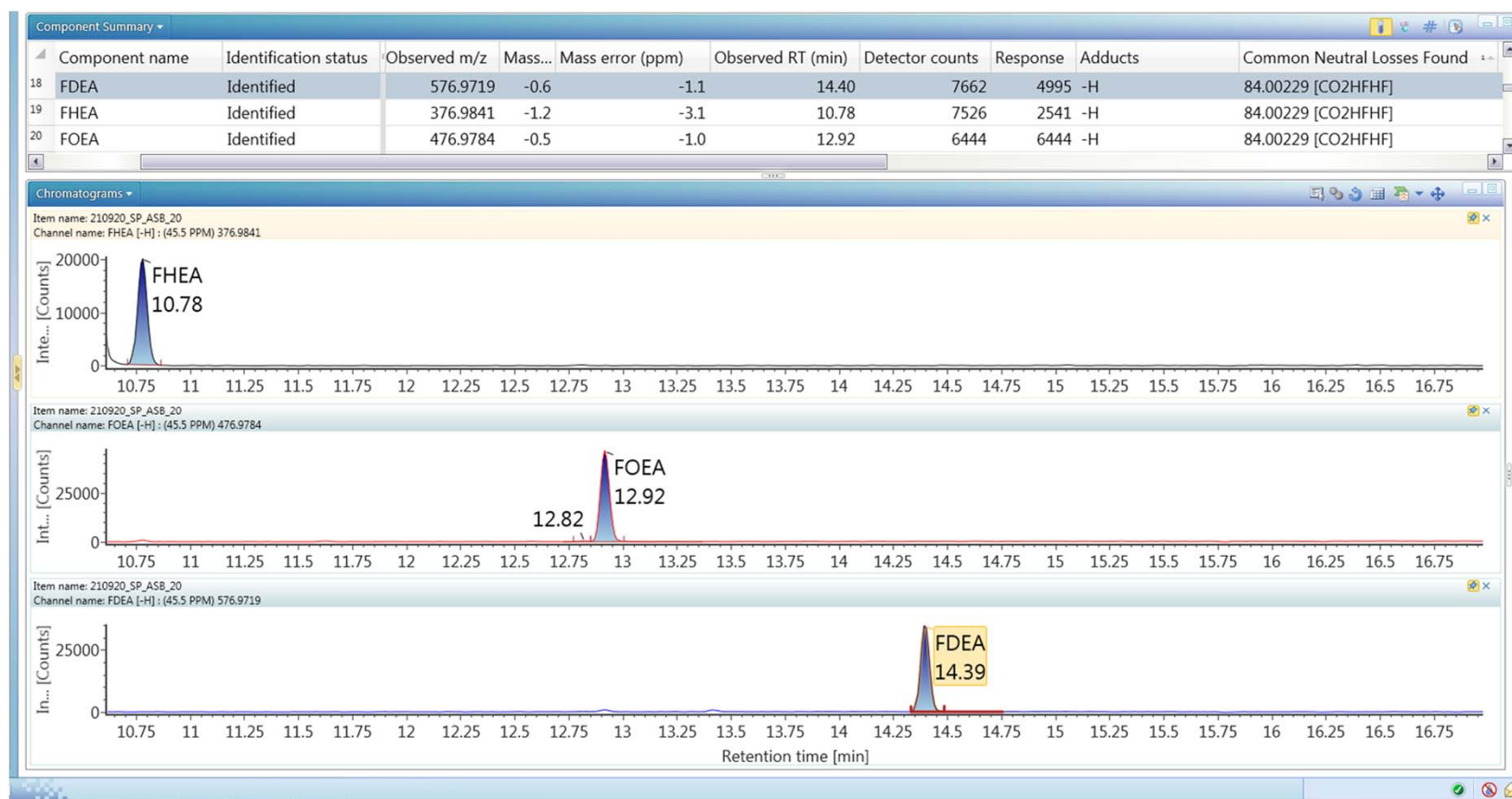
FOEA



FDEA



# Structural Elucidation Tools-Neutral Loss



# Elucidation of a Suspect Component

The screenshot displays the Waters software interface for component elucidation. It is divided into several panels:

- Component Summary:** A table with columns: Component name, Identification status, Observed RT (min), Detector counts, Response, Adducts, Has Common Fragment Ions, Common Fragment Ions Found, and Within Mass Defect Region. The row for Candidate Mass 278.9705 shows: None, 7.41, 30970, 30335, Yes, 84.99122 (CF3O-), and Yes.
- Chromatograms:** A plot of Intensity [Counts] vs Retention time [min]. A single sharp peak is visible at 7.40 minutes. A context menu is open over the peak with options: Elucidate..., Label components..., Assign as in source fragment, View alternate assignments, View component details, Copy, Print, Report selected components, Report all, and Properties...
- Spectra:** Two mass spectra are shown for the peak at 7.4073 minutes.
  - Low CE:** Channel name: Low energy : Time 7.4073 +/- 0.0213 minutes. The x-axis is Observed mass [m/z] (0-1200) and the y-axis is Intensity [Counts] (0-1.5e6). The base peak is at 278.9705. Other labeled peaks are at 84.9905, 340.9708, and 916.1066.
  - High CE:** Channel name: High energy : Time 7.4073 +/- 0.0213 minutes. The x-axis is Observed mass [m/z] (0-1200) and the y-axis is Intensity [Counts] (0-75000). The base peak is at 84.9906. Another labeled peak is at 278.9707.

# Discovery Tool

The image displays four overlapping screenshots of the Discovery Tool software interface, each showing a different tab in the Parameters window. The tabs are: Discovery, Elemental Composition, Chemspider, and Fragment Match.

- Discovery Tab:** Shows the main parameters. The "ChemSpider" radio button is selected. Fields include "Minimum i-FIT Confidence" (10%), "Number of compositions" (25), and "Number of hits" (50). A purple box highlights the "Number of hits" field.
- Elemental Composition Tab:** Shows options for "Automatic elements selection" and "Automatic adducts selection". It includes fields for "m/z Tolerance" (1 mDa), "Electron state" (Even), "Minimum DBE" (-1.5), and "Maximum DBE" (50). Checkboxes for "Use Senior rule", "Use Carbon/Hydrogen ratio filter", "Use Carbon/Hetero-atom ratio filter", and "Use multi-atom filter" are present.
- Chemspider Tab:** Shows a list of "Available libraries" (4C Pharma Scientific, ABU Pharmtech, A1 BioChem Labs, A2Z Chemical, Abacipharm, Abblis Chemicals, Abcam, ABI Chemicals) and a "Selected libraries" field containing "ChEMBL".
- Fragment Match Tab:** Shows advanced matching parameters. It includes checkboxes for "Use smartsScores", "Phenyl" (8), "Aromatic" (6), and "Ring" (2). It also has fields for "Multiple" (4), "Alpha" (5), "Hydrogen difference" (6), "Allow scores below" (8), "Delta (mDa)" (0.5), "DBE minimum" (-1.5), "DBE maximum" (50), "Neutral" (On), and "H Penalty" (0). A "Filter peaks by intensity" checkbox and a "Number of peaks" field (0) are also visible.

# Discovery Tool

**Workflow**

- Summary
  - Batch Overview
  - PFAS HRMS Screening Workflow
    - Identified Components
    - Unobserved Components
    - Unknown Components
    - Elucidation Toolset**
  - Binary Compare
  - Common
  - Reference Unique
  - Calibration Curve

**Spectra**

Channel name: Low energy : Time 7.4073 +/- 0.0213 minutes 1.62e6

Channel name: High energy : Time 7.4073 +/- 0.0213 minutes 9.08e4

Observed mass [m/z]

**Discovery**

Parameters

Results (5 found)

Component Name	m/z	Elemental Composition	Common Name	Fragment Matches	Citations
1 Candidate Mass 278.9704...	278.9705	C5HF9O3	Perfluoro(4-methoxybutanoic) acid	1	20
2 Candidate Mass 278.9704...	278.9705	C5HF9O3	2,2,3,3-Tetrafluoro-3-(pentafluoro...	0	3
3 Candidate Mass 278.9704...	278.9705	C5HF9O3	Difluoro(heptafluoropropoxy)aceti...	0	3

Assign

**Information**

Perfluoro(4-methoxybutanoic) acid

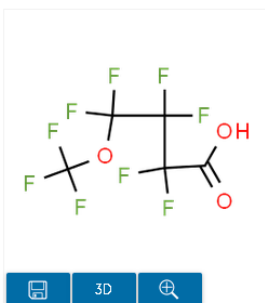
Synonyms

- 1 Butanoic acid, 2,2,3,3,4,4-hexafluoro-4-(trifluoromethoxy)-
- 2 2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid
- 3 863090-89-5
- 4 Perfluoro(4-methoxybutanoic) acid
- 5 2,2,3,3,4,4-Hexafluor-4-(trifluormethoxy)butansäure
- 6 Acide 2,2,3,3,4,4-hexafluoro-4-(trifluorométhoxy)butanoïq..

**C5HF9O3**

S: 0.50 B: 1 H:0

# ChemSpider Review



## Perfluoro(4-methoxybutanoic) acid

Molecular Formula C<sub>5</sub>HF<sub>9</sub>O<sub>3</sub>  
Average mass 280.045 Da  
Monoisotopic mass 279.978210 Da  
ChemSpider ID 23955753



More details:

This record has not been tagged.

+ TAG

Names Properties Searches Spectra Vendors Articles More ▾

Names and Synonyms

Validated by Experts, Validated by Users, Non-Validated, Removed by Users

EDIT

2,2,3,3,4,4-Hexafluor-4-(trifluoromethoxy)butansäure [German] [ACD/IUPAC Name]

2,2,3,3,4,4-Hexafluoro-4-(trifluoromethoxy)butanoic acid [ACD/IUPAC Name]

863090-89-5 [RN]

Acide 2,2,3,3,4,4-hexafluoro-4-(trifluorométhoxy)butanoïque [French] [ACD/IUPAC Name]

Butanoic acid, 2,2,3,3,4,4-hexafluoro-4-(trifluoromethoxy)- [ACD/Index Name]

Perfluoro(4-methoxybutanoic) acid

MFCD07784238 [MDL number]

Perfluoro-4-methoxybutanoic acid

Perfluoro-4-methoxybutanoic acid (PFMOBA)



# Sending Identified Compounds to the Library

The screenshot displays the Waters software interface with the following components:

- Component Summary Table:**

Component name	Identification status	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Expec...	Observed RT (min)	Detector counts	Response
221 N-MeFOSAA	Identified	570.9746	570.9742	569.9670	-0.4	-0.7	13.97	13.78	33209	29€
222 N-MeFOSE	Identified	556.9953	556.9948	616.0087						
223 Perfluoro(4-methoxybutanoic acid)	Identified	279.9782	279.9777	278.9705						
224 PFBA	Identified	213.9865	213.9859	212.9786						
225 PFBS	Identified	299.9503	299.9499	298.9426						

- Chromatograms:** A plot showing Intensity [Counts] vs Retention time [min]. A peak is labeled "Perfluoro(4-methoxybutanoic acid) 7.40".
- Spectra:** A plot showing Intensity [Counts] vs Observed mass [m/z]. A peak is labeled "84.9905".
- Context Menu:** A menu is open over the table with the following options: Send To, Elucidate..., Remove assignment..., Add component comment..., Label components..., Assign as in source fragment, View alternate assignments, View component details, Copy, Print, Report selected components, Report all, Properties... The "Send To" option is highlighted, and a sub-menu is open showing "Scientific Library".

## Summary

- HRMS screening allows for more thorough characterization of environmental samples
- MSe data collection allows for comprehensive data collection in one injection that can be processed and queried in many different ways without needing to re-inject
- A custom library greatly aided in the assignment of the PFAS compounds detected in the wastewater and soil samples.
- The UNIFI software provides a set of tools to aid in the identification of potential unknown PFAS not present in the library, using common fragments, neutral loss, and mass defect searching

## Acknowledgements

- UMass Amherst
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  - Sarah Dowd, Ph.D.
  - Ken Rosnack
  - Simon Hird, Ph.D.



# Waters

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Thank you!