Progress on the Use of Accurate Mass qTOF for PFAS Investigations

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PFAS Forensic Tools

The tools for PFAS forensics are a developing area of applications. We currently have several tools already in use that can be applied towards forensic investigations;

- Chemical Fingerprinting
- Isomer comparison
- Applications of TOP Assay

PFAS Forensic Tools

Additional techniques that are gaining in use and application

- Total Organic Fluorine Analysis
- Non-Target Analysis

The General Classes of Per- and Polyfluoroalkyl Substances (PFAS)

PFAS

Non-polymer

Perfluorinated

- PFAAs
  - PFCAs
  - PFSAs
- FASAs

Perfluoroalkyl acids
- Carboxylates
- Sulfonates

Polyfluorinated

- FTSAs
- FTCAs
- FTOHs
- FASEs
- FASAAs

Fluorotelomers:
- Sulfonates
- Carboxylates
- Alcohols

Polymer

- Fluoropolymers
- Perfluoropolyethers (PFPE)
- Side-chain fluorinated polymers

Source: ITRC Naming Conventions and Physical Chemical Properties fact sheet

Chemical Fingerprinting – PFAS by Isotope Dilution

Matrices

- Potable water
- Nonpotable water
- Soil/sediment
- Tissue/biota
- Dust wipes
- Landfill leachate
- AFFF Formulations

70 Compounds

Solid Phase Extraction/Cleanup using weak anion exchange

Isotope Dilution quantitation

- 25+ isotopically labeled internal standards

Injection Standards for monitoring instrument vs extraction performance

Advantages

- Isotope Dilution offers the highest degree of quantitative accuracy and precision
- Broadest list of compounds and widest range of matrices
- Lowest reporting limits across matrices

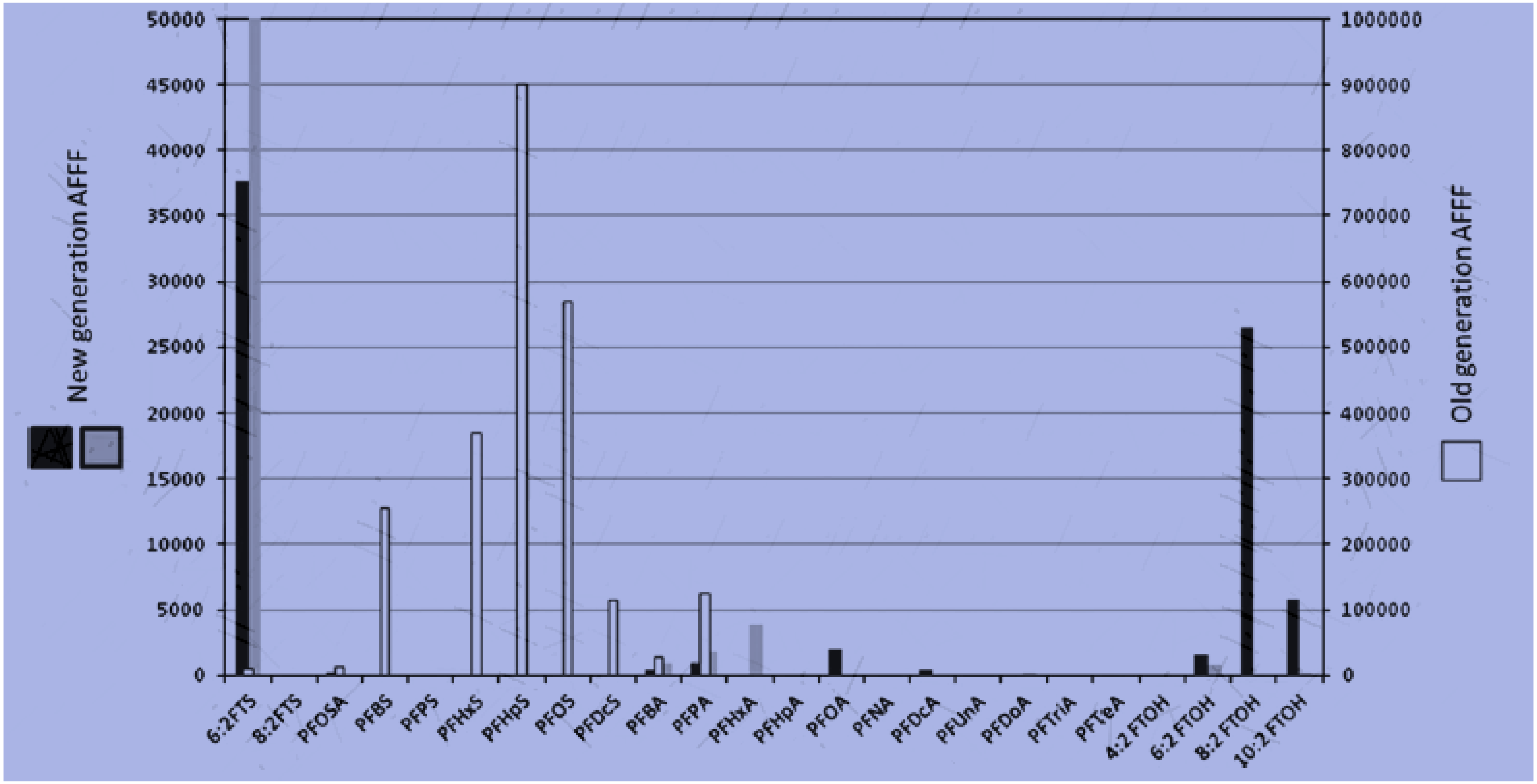
TARGETED PFAS

Perfluorobutanoic acid (PFBA)	Perfluoro-1-nonanesulfonate (PFNS)	NFDHA	PFO4DA
Perfluoropentanoic acid (PFPeA)	Perfluorododecanesulfonic acid (PFDoS)	PFEESA	PFO3OA
Perfluorohexanoic acid (PFHxA)	Perfluoro-n-hexadecanoic acid (PFHxDA)	PFMPA	PFO2HxA
Perfluoroheptanoic acid (PFHpA)	Perfluoro-n-octadecanoic acid (PFODA)	PFMBA	PFO5DA
Perfluorooctanoic acid (PFOA)	NMeFOSAA	3:3 FTCA	R-EVE
Perfluorononanoic acid (PFNA)	NEtFOSAA	5:3 FTCA	NVHOS
Perfluorodecanoic acid (PFDA)	NEtFOSA	7:3 FTCA	Hydro-EVE Acid
Perfluoroundecanoic acid (PFUnA)	NMeFOSA	6:2 FTCA	EVE Acid
Perfluorododecanoic acid (PFDoA)	NMeFOSE	8:2 FTCA	R-PSDA
Perfluorotridecanoic Acid (PFTriA)	NEtFOSE	10:2 FTCA	Hydrolyzed PSDA
Perfluorotetradecanoic acid (PFTeA)	4:2FTS	6:2 FTUCA	R-PSDCA
Perfluorobutanesulfonic acid (PFBS)	6:2FTS	8:2 FTUCA	PS Acid
Perfluorohexanesulfonic acid (PFHxS)	8:2FTS	10:2 FTUCA	Hydro-PS Acid
Perfluoroheptanesulfonic Acid (PFHpS)	10:2FTS	PFECHS	4:2 FTOH
Perfluorooctanesulfonic acid (PFOS)	DONA	PFPrS	6:2 FTOH
Perfluorodecanesulfonic acid (PFDS)	HFPO-DA (GenX)	PFMOAA	7:2S FTOH
Perfluorooctane Sulfonamide (FOSA)	11Cl-PF3OUdS	PFECA G	8:2 FTOH
Perfluoro-1-pentanesulfonate (PFPeS)	9Cl-PF3ONS	MTP	10:2 FTOH
PFPrA	PMPA	PEPA	

Additional PFAS methods

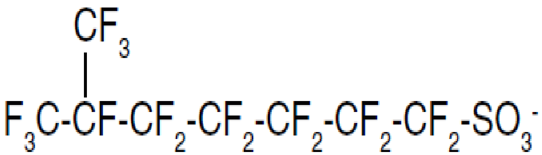
- <u>Fluorotelomer Alcohols</u>
  - GCMSMS method
  - Water and solids
  - Instrumental set-up like 8270E and extractions like 3510 and 3540/50
  - Current compound list
    - 4:2 Fluorotelomer alcohol
    - 6:2 Fluorotelomer alcohol
    - 7:2S Fluorotelomer alcohol
    - 8:2 Fluorotelomer alcohol
    - 10:2 Fluorotelomer alcohol

Chemical Fingerprinting



Herzke, et al., 2012, Chemosphere, 88, 980-987

Isomer Comparison



$F_3C\text{-}CF_2\text{-}CF_2\text{-}CF_2\text{-}CF_2\text{-}CF_2\text{-}CF_2\text{-}CF_2\text{-}SO_3^-$

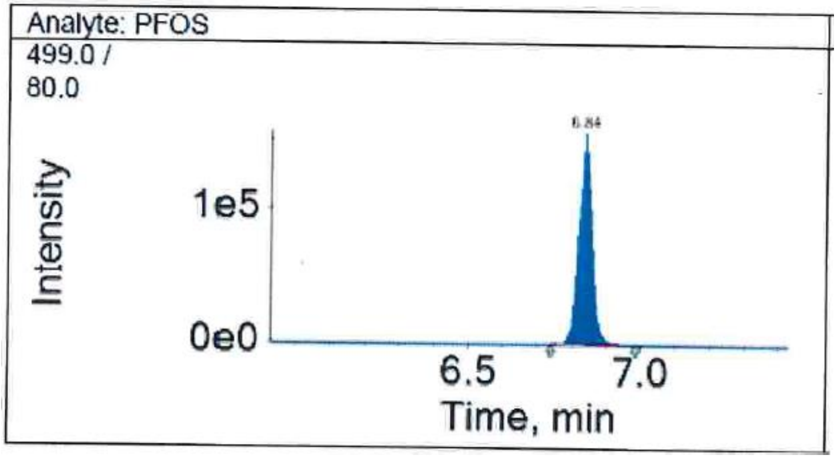
Linear Perfluorooctane sulfonate (PFOS)

Branched Perfluorooctane sulfonate (PFOS)

Figure 4-1. Linear and one branched isomer of PFOS

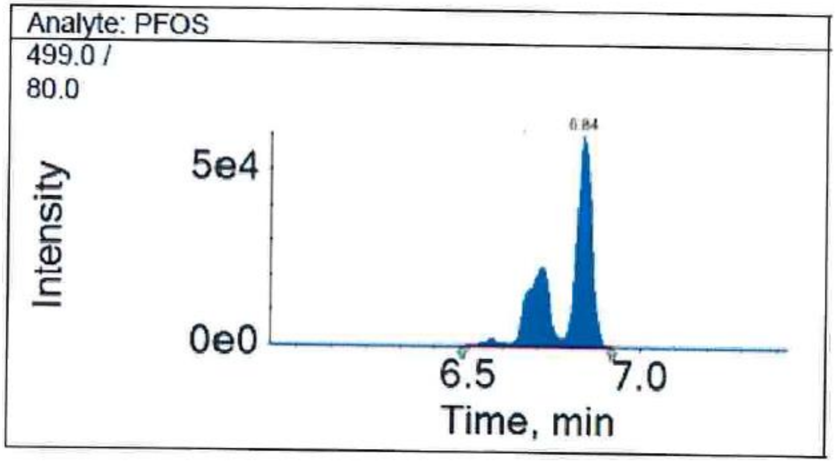
ITRC PFAS Fact Sheet Naming Conventions April 2020

Isomer Comparison

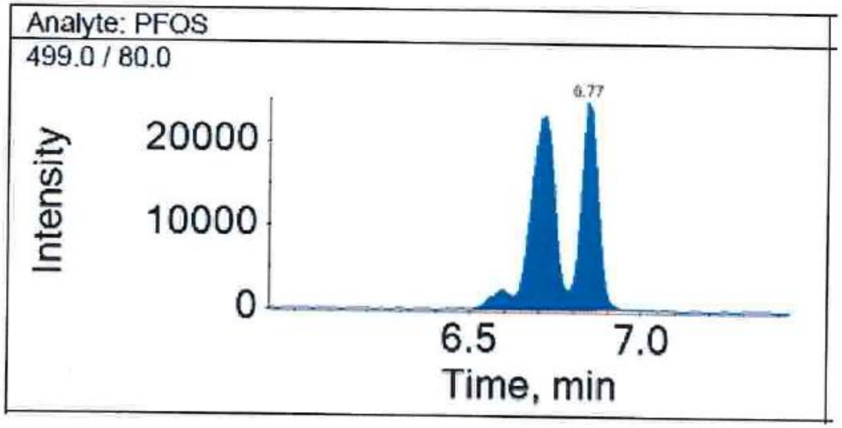


Chromatogram of PFOS Standard of Linear Isomer

Chromatogram of PFOS Standard of Branched/Linear Mix Typical Ratio

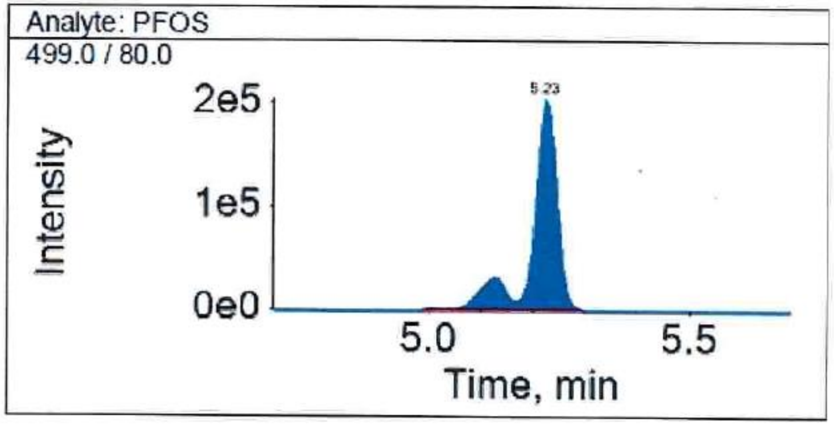


Isomer Comparison



Chromatogram of PFOS Sample with Branched/Linear Mix High Bias Ratio

Chromatogram of PFOS Sample with Branched/Linear Mix Low Bias Ratio

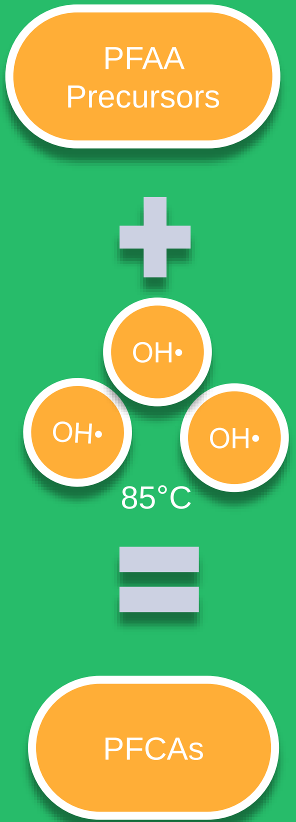


TOP Assay

What is the Total Oxidizable Precursor (TOP) Assay?

- A PFAS sample preparation technique
- Indicates presence of unidentified precursors
- Used in conjunction with standard analysis
- Contrasts pre and post oxidation results

https://pubs.acs.org/doi/10.1021/es302274g



What the TOP Assay is NOT

- A risk assessment tool
- **Total PFAS methodology**
- Identify Unknown PFAS
- **Mass balance PFAS**
- Non-target identification

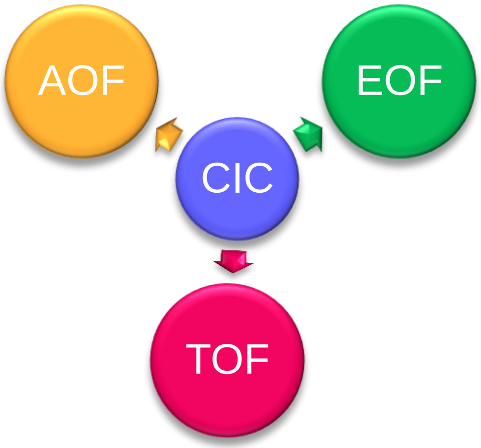
TOP Assay Results

Compound	Pre-Ox	Post-Ox	Difference
PFBA	ND	98 ng/l	98 ng/l
PFPeA	ND	87 ng/l	87 ng/l
PFHxA	5 ng/l	61 ng/l	56 ng/l
6:2 FTS	100 ng/l	ND	- 100 ng/l
PFHpA	11 ng/l	32 ng/l	21 ng/l
PFOA	7 ng/l	26 ng/l	19 ng/l
PFOS	56 ng/l	52 ng/l	- 4 ng/l
8:2 FTS	26 ng/l	ND	- 26 ng/l
PFNA	ND	5 ng/l	5 ng/l

Total Organofluorine (TOF)

CASE STUDY

Total Organic Fluorine (TOF) 410 mg F/kg
Extractable Organic Fluorine (EOF) 390 mg F/kg
LC-MS/MS ΣPFAS (n=28) 120 mg/kg



- Sample (or treated sample) is combusted in a furnace at 900°C – 1100°C
- Effluent collected in buffer and injected into ion chromatograph (IC)
- Quantify fluorine (as fluoride) content
- Compare ratio of total (or extractable) fluorine to total PFAS

CIC: Combustion Ion Chromatography

Non-Target Analysis

Targeted Analysis

Suspect Screening Analysis

Non-Targeted Analysis

Quadrupole
Time of Flight (qToF)

Newer Techniques – Non Targeted Analysis



Technique utilizes LC/MS-qTOF (quadrupole time of flight mass spectrometry)

- Technique allows for determination of accurate mass (0.0001 amu)
- Initial differentiation based on extraction of sample
- Then analysis of targeted compounds (knowns) to remove those from "background"
- Compare remaining peaks to limited mass spectral libraries to identify the known/unknowns
- Remaining peaks are unknowns and would rely on regression of accurate mass determinations for possible identification

Non-Target Analysis



Problems?

Accurate mass solves a variety of PFAS problems

No More Limitations

Precursors without TOP Assay

No LIMS constraints

Want to know all byproducts?

Byproducts?

SWATH uses a moving small mass window for non-target MS/MS spectra; can capture all byproducts

QTOF exact mass analysis for > 40 PFAS analytes

Exact mass confirmation of 'suspect' positive results

Mitigation of matrix effects for short chain analytes

Application for PFAS lacking standards and unknowns (NTAs)

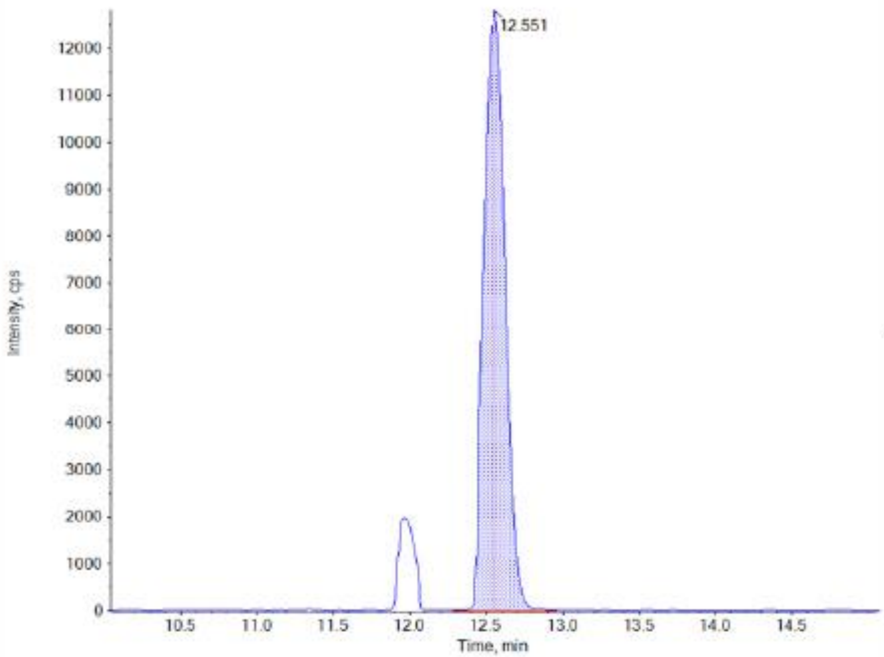
Non-Target Results

#	Analyte Peak Name	Precursor Mass	Found At Mass	Library Hit	Library Score	Formula Finder Results	Formula Finder Score	Combined Score
47	207.1384 / 9.59	207.140	207.1386	Ser-Cys (NIST)	86.3	C13H20O2	77.080	81.702
75	205.1582 / 10.62	205.159	205.1591	Met-Gly (NIST)	82.3	C8H23N4P	83.194	82.724
93	271.2263 / 11.21	271.227	271.2271	DL-.beta.-Hydroxypalmitic acid (NIST)	81.8	C16H32O3	68.518	75.154
119	265.1468 / 12.04	265.148	265.1472	Dodecyl sulfate (NIST)	99.3	C8H24N6P2	78.457	88.862
127	199.1699 / 12.08	199.171	199.1699	Dodecanoic acid (NIST)	93.5	C12H24O2	81.919	87.725
128	297.1516 / 12.16	297.153	297.1520	Ricinoleic acid (NIST)	97.5	C8H21F2N8P	89.209	93.349
129	205.1591 / 12.22	205.160	205.1592	2,6-Di-tert-butylphenol (NIST)	100.0	C8H23N4P	82.310	91.155
130	297.2424 / 12.22	297.243	297.2428	Ricinoleic acid (NIST)	97.5	C18H34O3	71.444	84.466
146	514.9789 / 12.55	514.980	514.9792	Cl-PFOS (chloro-perfluorooctane sulfonate) (neg)	89.8	C8H13FN6O15S2	98.473	94.123
152	309.1728 / 12.64	309.174	309.1733	Ethylene glycol dodecyl ether sulfate (NIST)	100.0	C14H30O5S	73.122	86.561
168	531.0069 / 12.94 M-H-	531.008	531.0081	Cl-PFENS neg	81.5	Too many formula	0.000	40.743
171	353.1999 / 12.94	353.201	353.1996	Diethylene glycol dodecyl ether sulfate (NIST)	99.6	C15H29F3N4S	91.220	95.397
176	241.2162 / 13.06	241.217	241.2165	N2-Trifluoroacetyl-L-glutamine (NIST)	89.3	No formula found	0.000	44.666
192	293.1788 / 13.45	293.180	293.1784	Myristyl sulfate (NIST)	97.8	C14H30O4S	73.162	85.459
216	253.2158 / 14.02	253.217	253.2168	cis-7-Hexadecenoic acid (NIST)	97.8	C16H30O2	77.687	87.726
220	339.1986 / 14.08	339.200	339.1991	Tridecylbenzenesulfonic acid (NIST)	80.2	C13H33N4O2PS	89.239	84.697
260	281.2480 / 14.90	281.249	281.2479	1,4-D-Xylobiose (NIST)	100.0	C18H34O2	73.760	86.880
300	407.2938 / 15.97	407.295	407.2942	.gamma.-Muricholic acid (NIST)	96.5	C21H37FN6O	95.929	96.220
327	311.2943 / 17.36	311.295	311.2943	Benzenesulfonic acid, 4-undecyl- (NIST)	76.3	C16H36N6	53.227	64.777
434	265.1465 / 26.87	265.148	265.1470	Dodecyl sulfate (NIST)	84.6	C13H27FS2	84.840	84.720

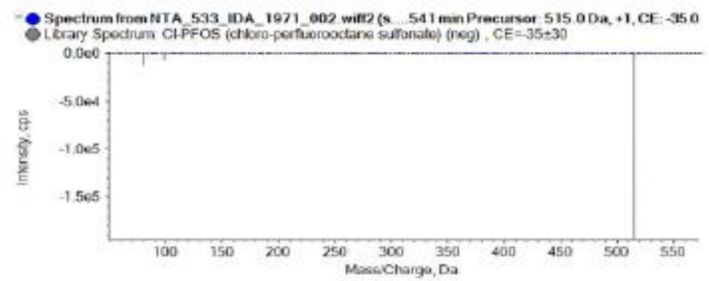
Non-Target Results

514.9789 / 12.55 (Library/Formula) ✓ ✓

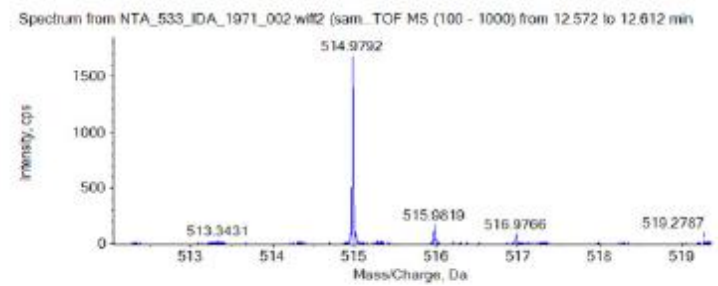
Retention Time: 12.55 minutes **Precursor m/z :** 514.9800 **Fit (%)** 98.6% **RFit (%)** 93.0%	**Analyte Name:** 514.9789 / 12.55



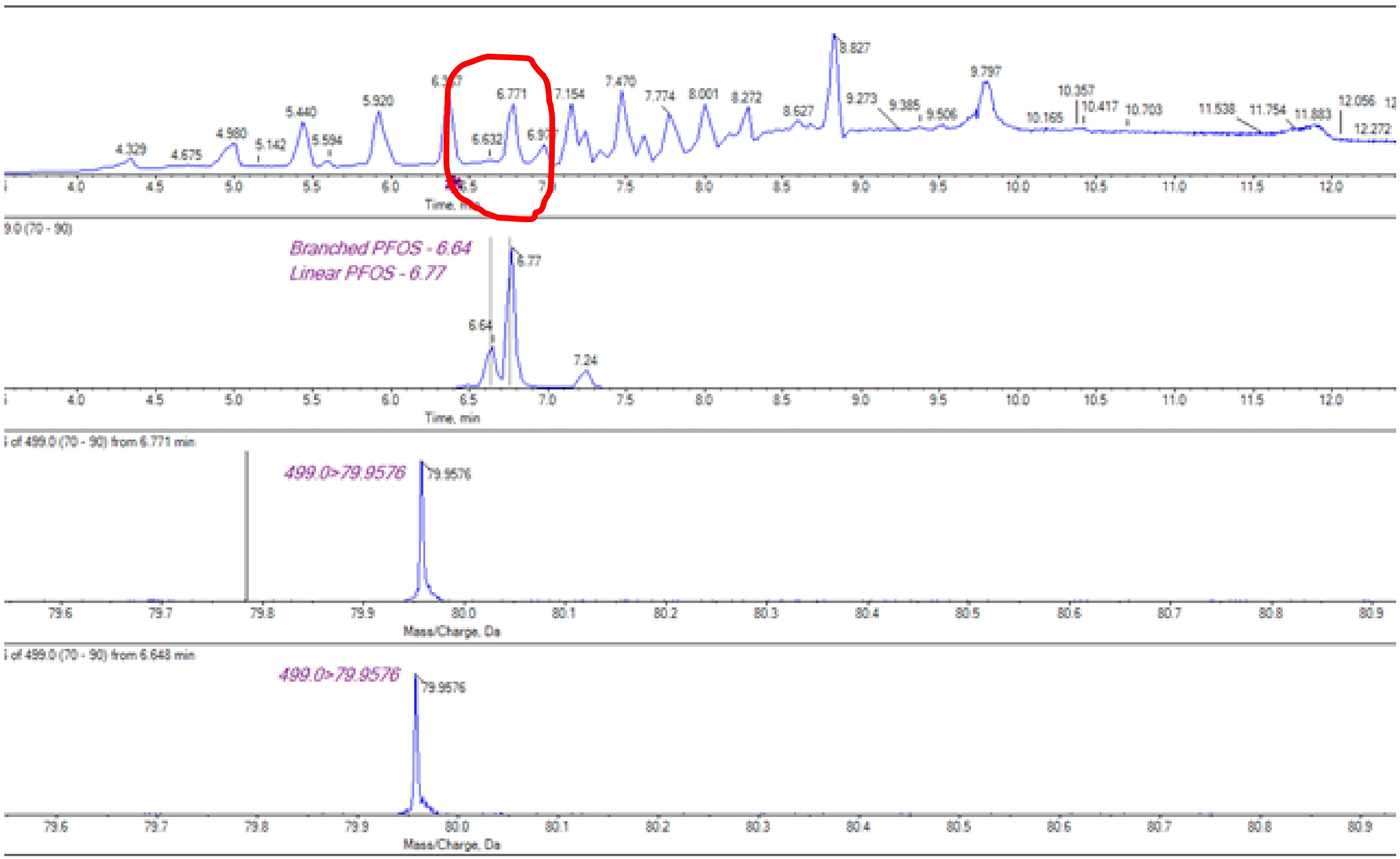
Acquired / Library MSMS



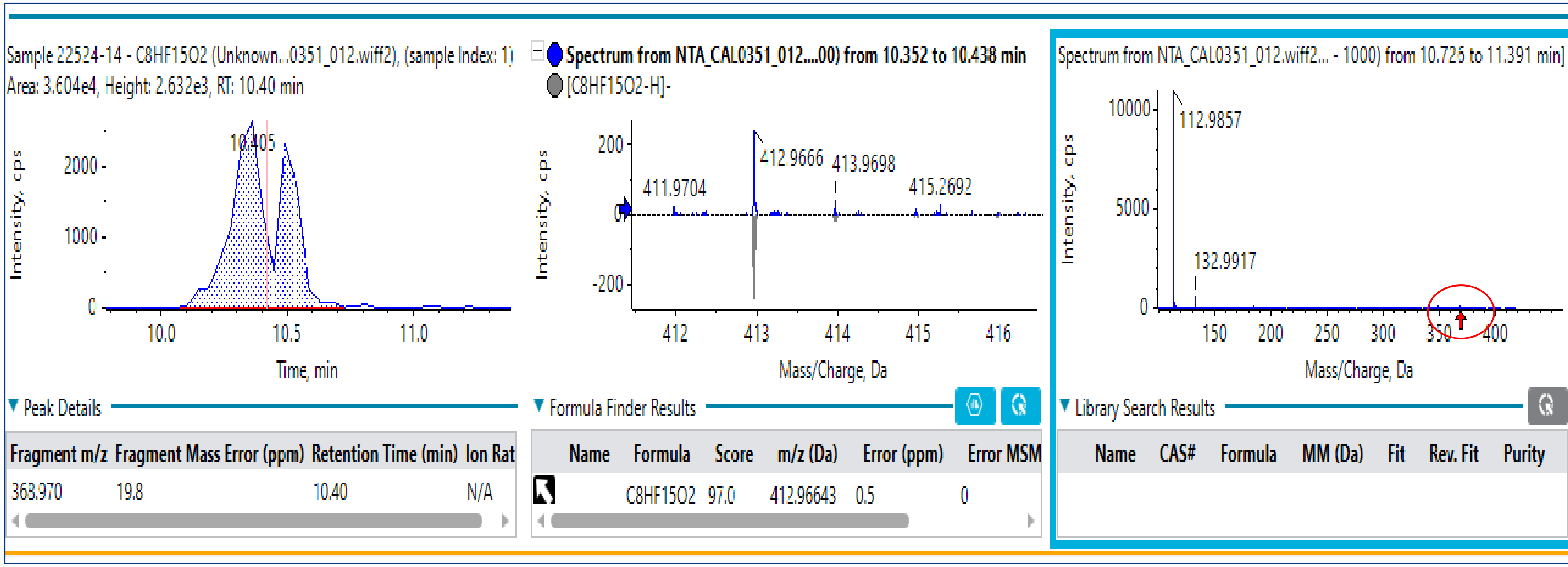
Acquired / Theoretical MS



Non-Targeted Analysis

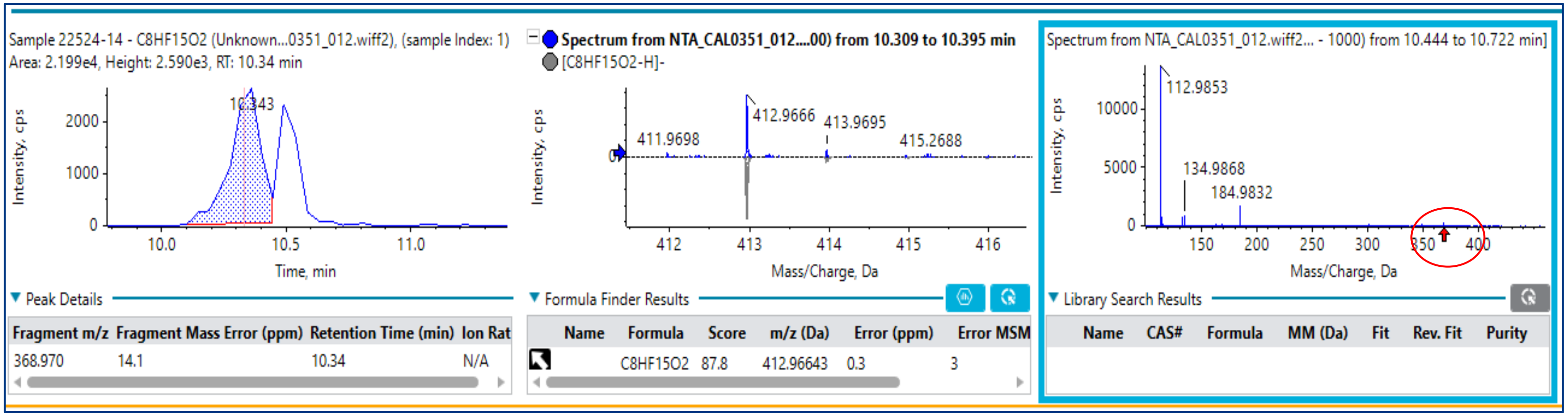


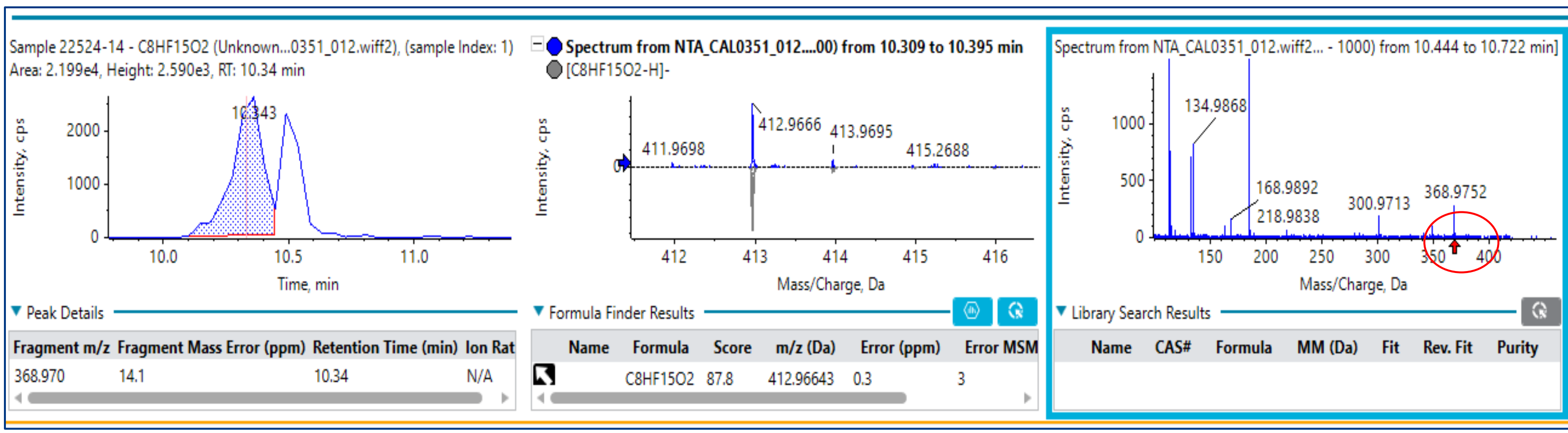
Case Study



Note: the fragmentation pattern of the entire peak, although the 368.9 is present (Red Arrow) the primary fragmentation is 112.98.

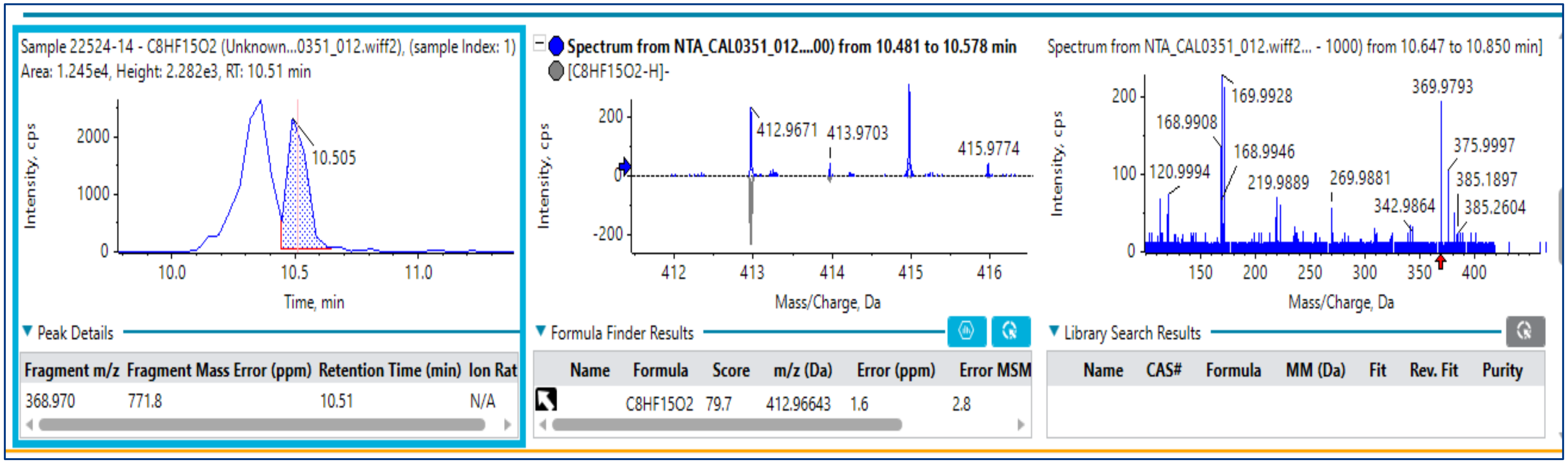
Case Study





Note: The fragmentation of m/z 412.96 for the peak at 10.343 includes the pattern for PFOA, it also includes a predominant fragment at m/z 112.98; 134.98 and 184.95.

Case Study



Note: The fragmentation pattern at 10.50 (RT of peak in CAL standard) – Fragmentation of 412.96 shows the presence of 368.97 however not as the predominant peak. Also in the MS fragmentation, m/z 414.97 is predominant. The fragmentation mass error for m/z 368.98 is extremely high at 771.8 ppm.

Summary

Targeted PFAS

All Matrices – Up to 70 Compounds

Strengths: Selectivity, Sensitivity at ~1-5ppt

Can be used for risk assessment

Weaknesses: Limited list of compounds

TOP Assay

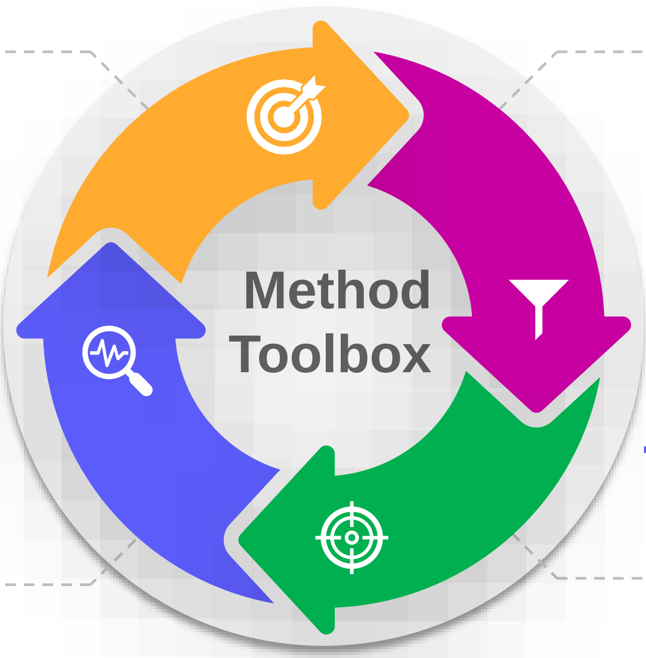
All Matrices – Oxidizable Precursors

Strengths: Sensitivity at ~1-5ppt

Specific to ‘unknowns’ with potential to convert to risk drivers

Weaknesses: Not specific

Does not complete a mass balance



Non-Target Analysis

All Matrices – Unknowns

Strengths: Ability to identify ‘unknowns’ with specificity

Ability to conduct novel compound identification

Weaknesses: Limited to current libraries

Limited quantitation

Total Organic Fluorine

All Matrices – Organic Fluorine

Strengths: Closest to a mass balance

Weaknesses: Sensitivity at ~1-5ppb

No selectivity

Thank You

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