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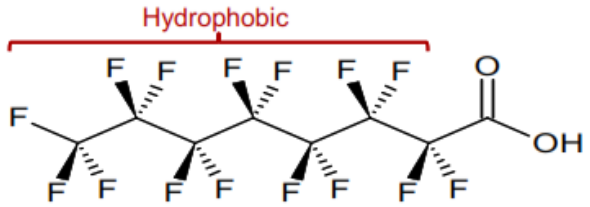
Conquer the Challenges of Small Molecule Analysis

Dr. Richard Jack

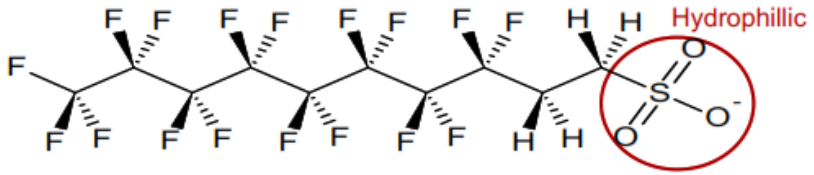
Background

What are PFAS?

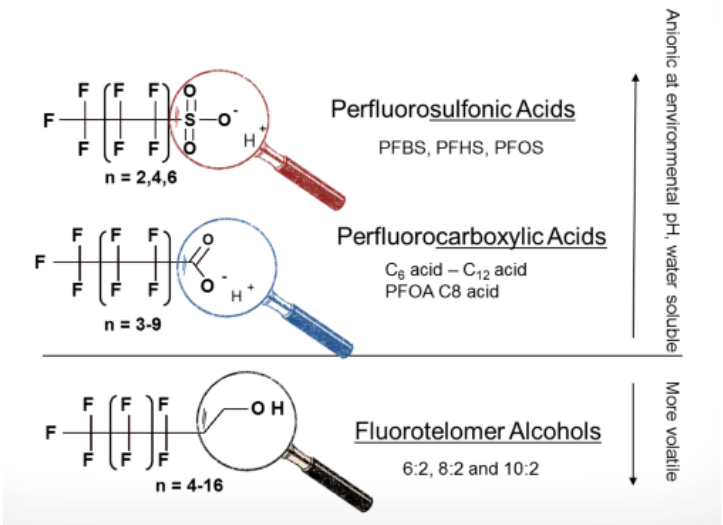
- PFASs are **P**er- and **P**oly**F**luorinated **A**lkyl **S**ubstances. Exclusively anthropogenic.
- Structures contain a hydrophobic perfluoroalkyl backbone and a hydrophillic end group
- Include a diverse range of compounds with a variety of chain lengths and end groups



Perfluorooctanoic acid
- PFOA
- Teflon®



8:2 Fluorotelomer sulfonate
- 8:2 FTS



Industrial Uses

- PFAS are used in a variety of applications because of their chemical and physical properties. These include:
  - Industrial polymers (Teflon® - PFOA)
  - Stain repellants (Scotch Guard® - PFOS)
  - Aqueous film forming foams (AFFF) – fire fighting applications

Sources

- Can be found anywhere at differing (generally lower) concentrations,
- Areas of elevated concentration and concern are:
  - Airports
  - Run-off from incidents of fire
  - Landfill leachate
  - WWTP effluent

PFAA Drinking - , Wastewater and Soil Clean up > Non-targeted and targeted

- **FEDERAL LEVEL ACTIONS**
- Water and Soil Method Validations
- EPA Off. Of Ground Water and Drinking Water
- EPA Office of Water
- Approx. 600 military bases in US have PFC contamination

- **STATE LEVEL ACTIONS**
- PFOS and PFOA in WW regulated in 7 States
- **California** - "*Expressed support for including the **broader panel** of perfluoroalkyl and polyfluoroalkyl substances (PFASs).*"

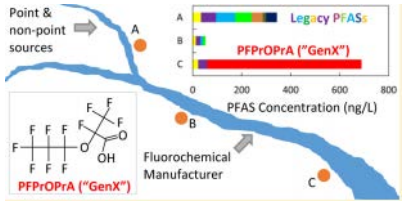
BIOMONITORING CALIFORNIA
MEASURING CHEMICALS IN CALIFORNIANS

- **North Carolina** – New Legislation specifically focused on PFC/PFAA monitoring

SECTION 7.(d) The sum of eight million dollars ($8,000,000) …
(ii) …of essential scientific instruments,
(iii) …sample collection and analysis, training

Dr. P. Lee Ferguson

Assoc. Prof. of Civil
and Environmental Engineering
Duke University



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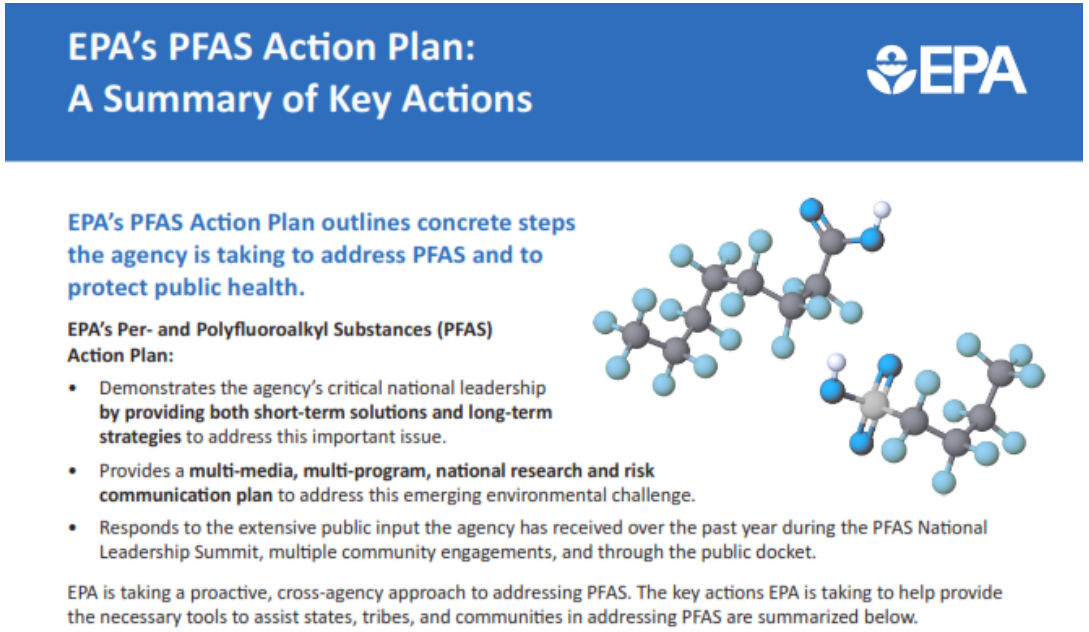
EPA is Proposal to Regulate PFAA in DW

U.S. ENVIRONMENTAL PROTECTION AGENCY
NEWS RELEASE
WWW.EPA.GOV/NEWSROOM

- Announced in Feb. 2019
- First ever comprehensive nationwide Action Plan to help states address concerns.
- Proposing a Federal Maximum Contaminant Level
- Begin the process to **propose** a regulation
- ***This doesn't mean PFAS are regulated!***



EPA's PFAS Action Plan:
A Summary of Key Actions

EPA

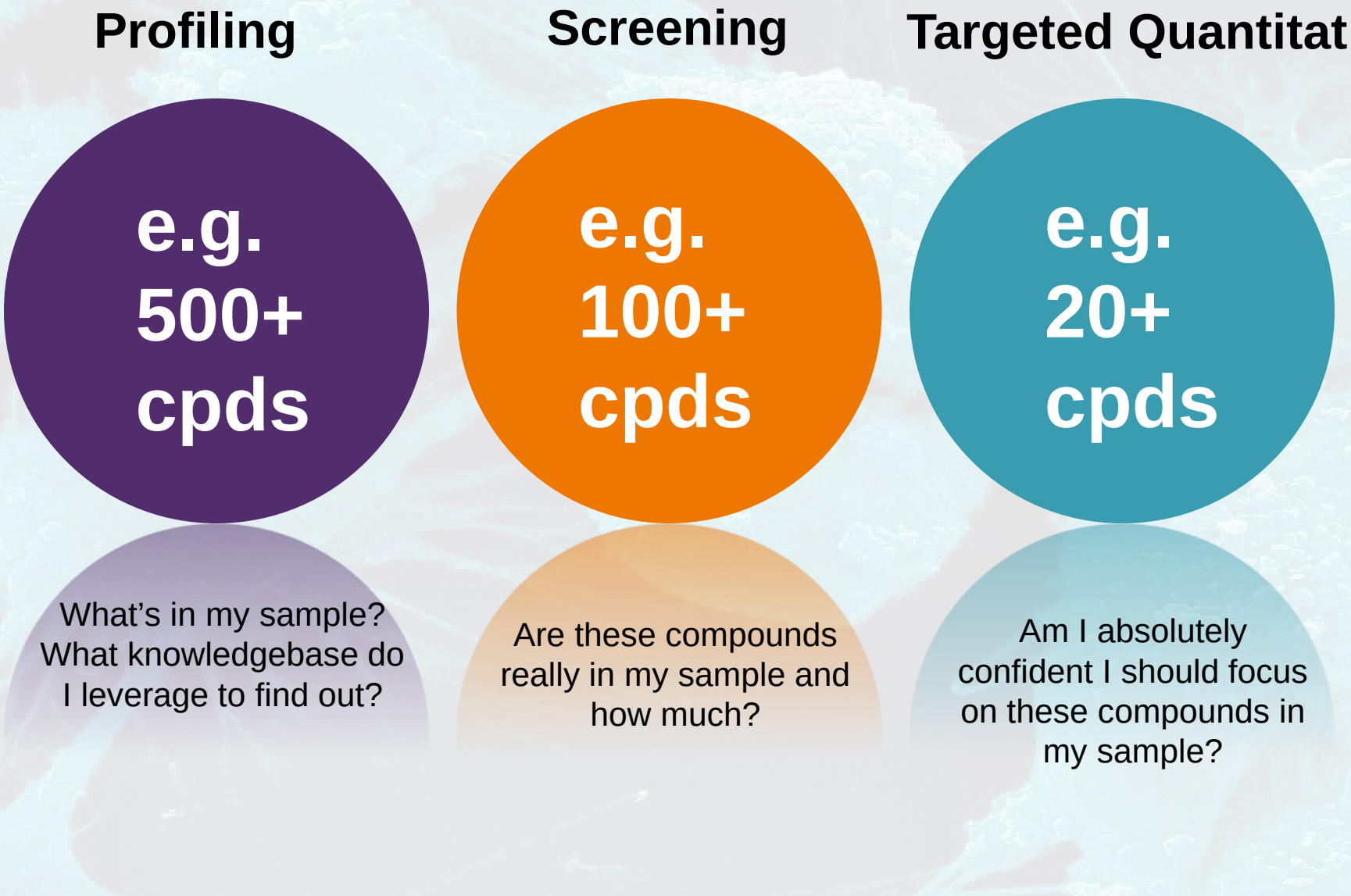
EPA's PFAS Action Plan outlines concrete steps the agency is taking to address PFAS and to protect public health.

EPA's Per- and Polyfluoroalkyl Substances (PFAS) Action Plan:

- Demonstrates the agency's critical national leadership **by providing both short-term solutions and long-term strategies** to address this important issue.
- Provides a **multi-media, multi-program, national research and risk communication plan** to address this emerging environmental challenge.
- Responds to the extensive public input the agency has received over the past year during the PFAS National Leadership Summit, multiple community engagements, and through the public docket.

EPA is taking a proactive, cross-agency approach to addressing PFAS. The key actions EPA is taking to help provide the necessary tools to assist states, tribes, and communities in addressing PFAS are summarized below.

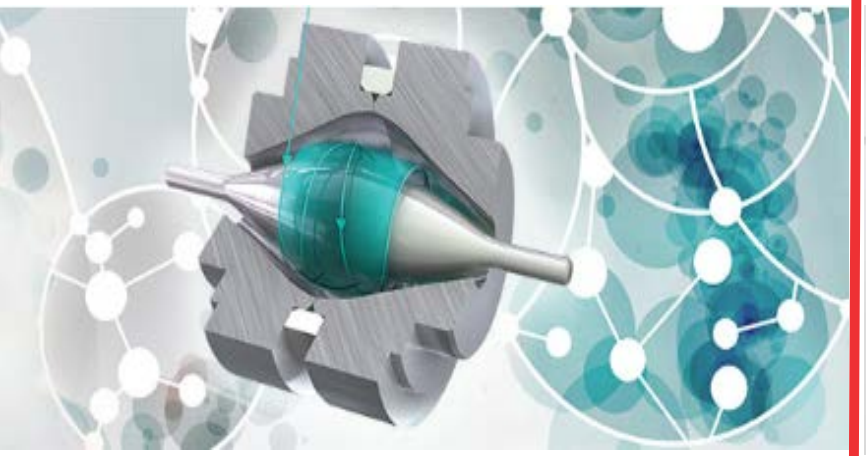
- Drinking Water
- Clean up
- Monitoring
- Research – *Water and Toxicology*
- Enforcement



Tools For Unknown Identification

Thermo Scientific™ Small Molecule Analysis Solutions

Profiling/Screening



Thermo Scientific™ Orbitrap™ Hybrid Mass Spectrometers

Targeted Quantitation

Thermo Scientific™ TSQ Triple Quadrupole Mass Spectrometers

Data Analysis

Thermo Scientific™ Compound Discoverer™ with mzCloud™ mass spectral library

mzCloud is a trademark of HighChem LLC, Slovakia

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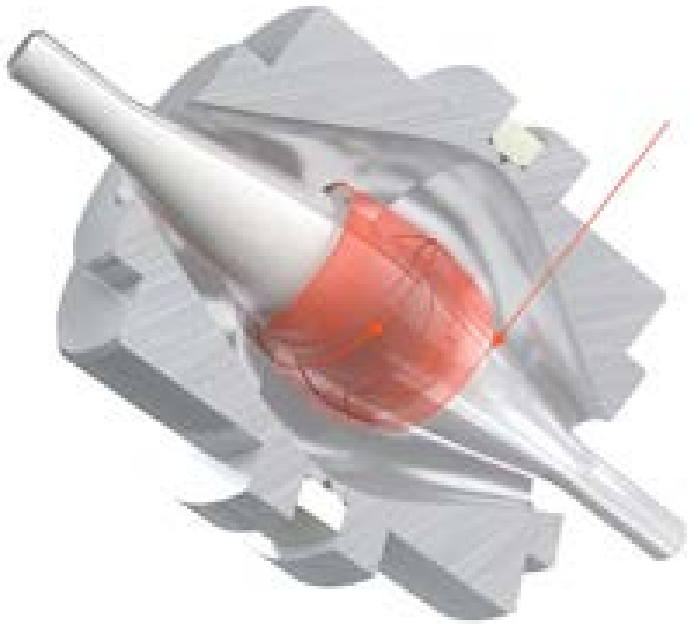
Orbitrap Mass Analyzer Technology for Screening of Unknowns



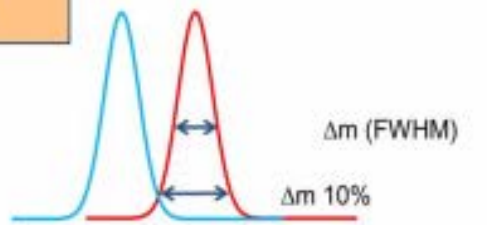
- High and ultra-high resolution enables discrimination between ions of interest and interfering ions in the very low and low *m/z* range
- Mass accuracy – the superior resolution enables accurate mass assignments with sub 1-ppm mass accuracy to eliminate false positives
- Retrospective analysis – enables investigation of new analytes in the same samples because it collects data on all analytes in the sample!

Learn More: **www.thermofisher.com/orbitrap**

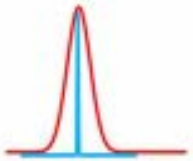
Enhanced Resolution Using Orbitrap Technology

Resolution

$$R = \frac{m}{\Delta m}$$



- Quadrupole MS $R = \frac{m}{\Delta m} = \frac{500}{0.6} = 833$



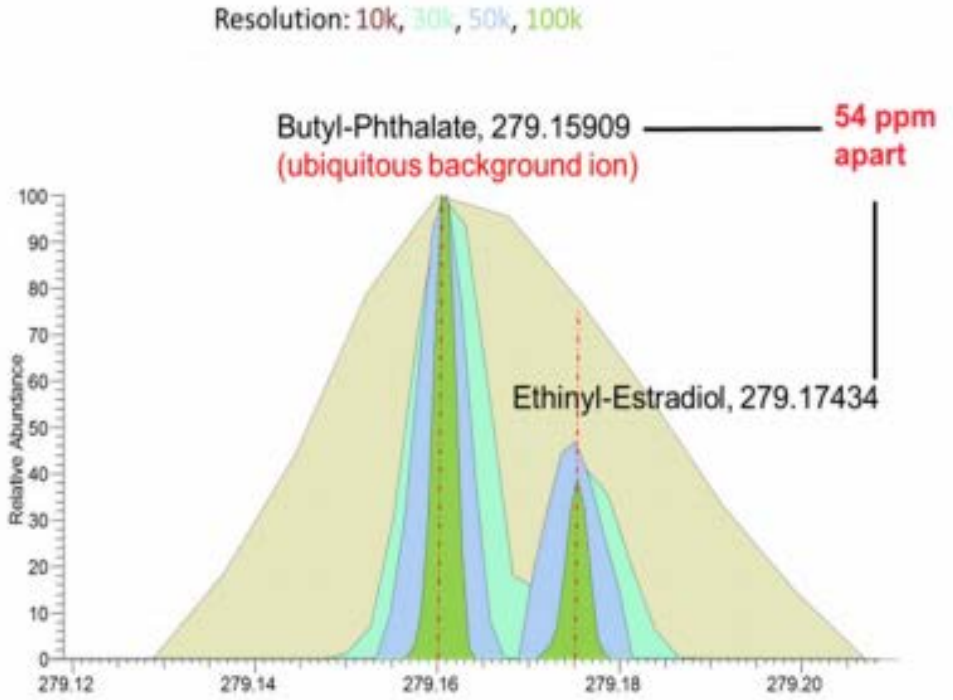
- Orbitrap MS $R = \frac{m}{\Delta m} = \frac{500}{0.0035} = 140000$

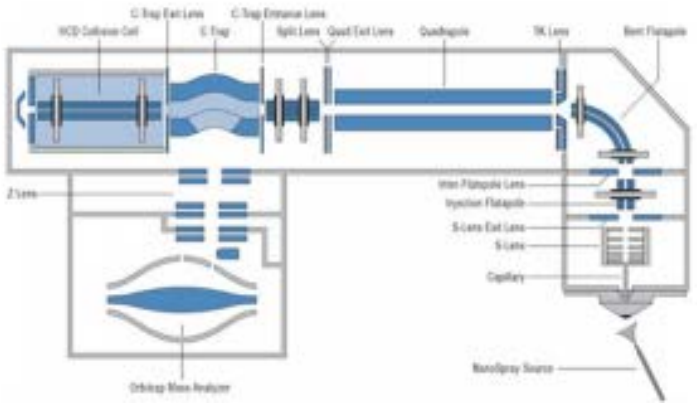
Mass Accuracy

$$\Delta m / z = \frac{m_{meas} - m_{true}}{m_{true}} \cdot 10^6$$

- Quadrupole MS $\Delta m/z = \frac{500.1 - 500.0}{500} \cdot 10^6 = 200 ppm$

- Orbitrap MS / TOF MS $\Delta m/z = \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2 ppm$





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Compounding Insights to Match Analysis Requirements

Compound Discoverer 3.0 Software for Small Molecule Unknown Identification

Efficiently extract high-confidence insights from information-rich small molecule HRAM data

Serves as a hub to seamlessly connect users to the tools they need to analyze productively and confidently

mzCloud Mass Spectral Library

Rank search more **effectively** with industry leading online spectra fragmentation library

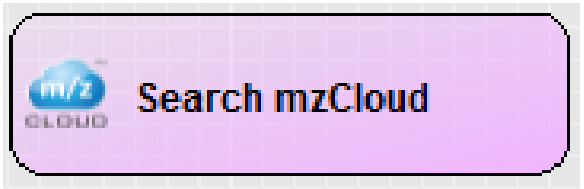
mzCloud is a trademark of HighChem LLC, Slovakia

mzLogic Algorithm

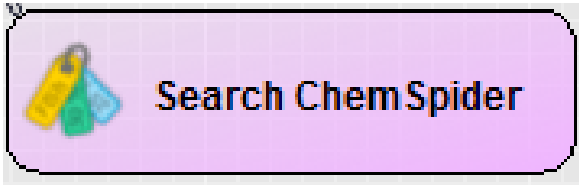
From 1000's of candidates and hours of work to **fast automated logical analysis**

Learn More: **www.thermofisher.com/compounddiscoverer**

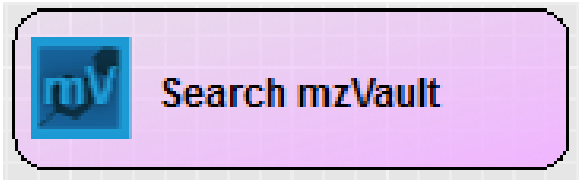
Identifying Unknowns – Spectral Libraries and Compound Databases



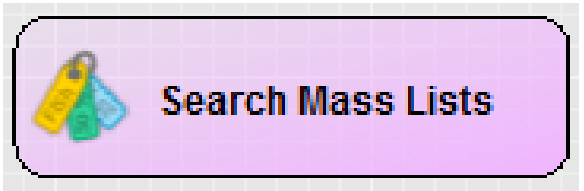
Online spectral library
> 2.8 million spectra



Chemical structure database with >480 data sources, 59 million structures

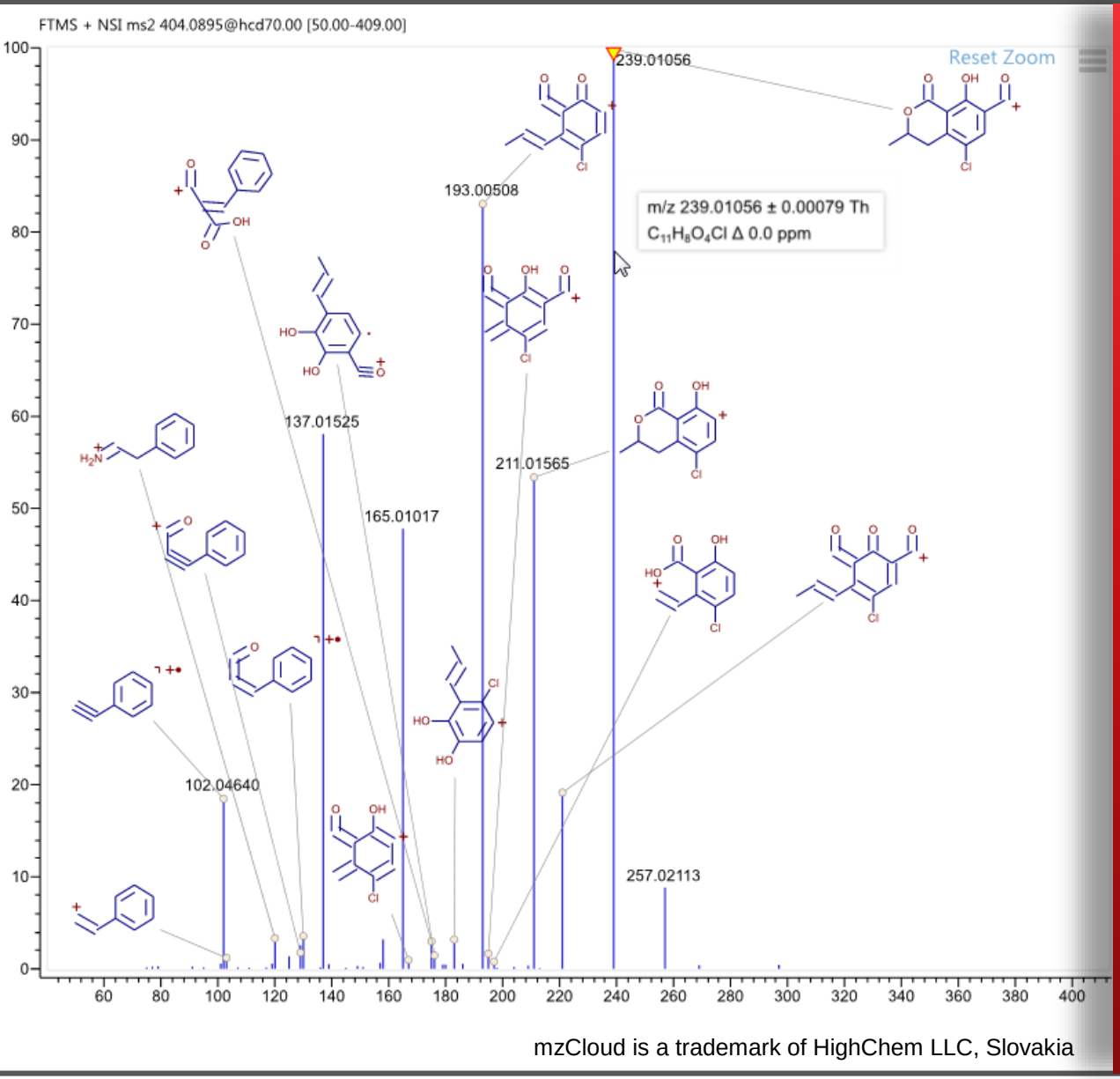


Local spectral libraries



Local databases

mzCloud Library



mzCloud is a trademark of HighChem LLC, Slovakia

HRAM MS/MS and MS^n

HCD and CID fragmentation

Multiple Energy Levels

100% Professionally Curated

>8000 Compounds

>1M Fragment Structures

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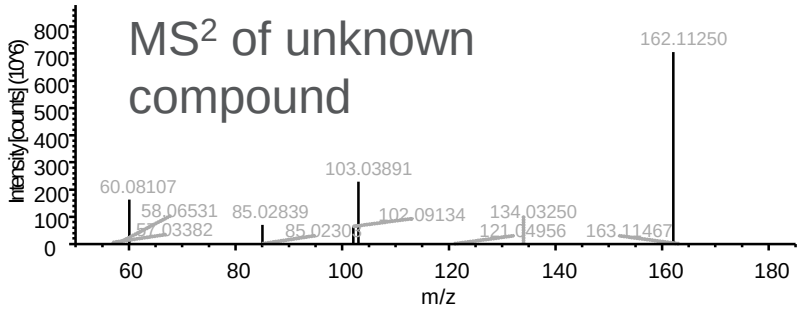
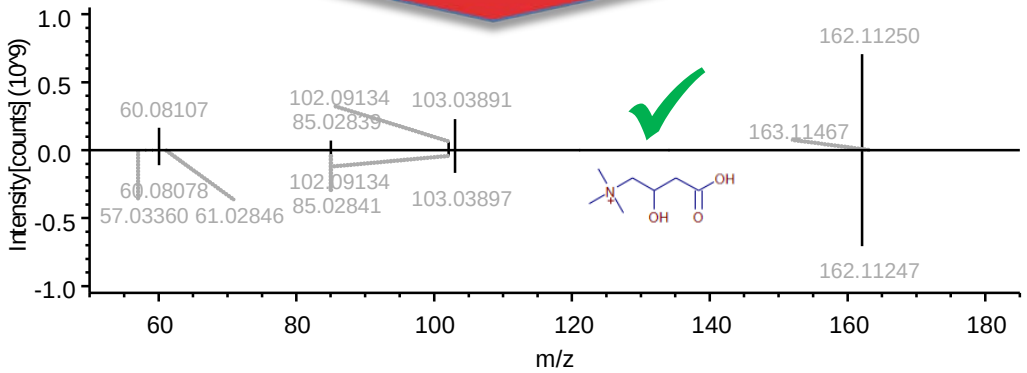
Ideally



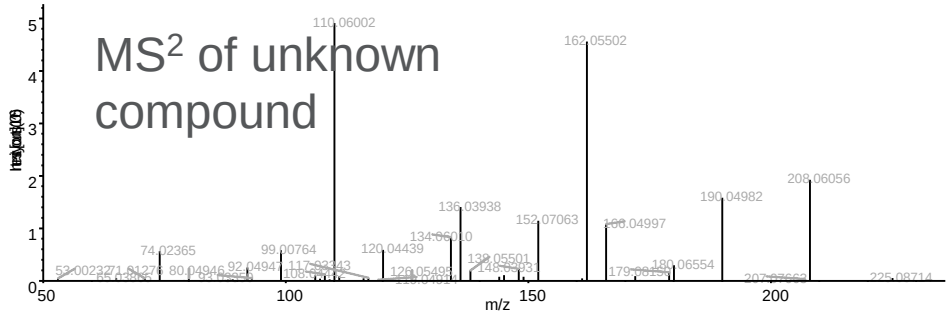
Spectral Library Search



Reference data found in spectral library

→ **Compound identified**

But what if …



Spectral Library Search

NO reference data found in spectral library

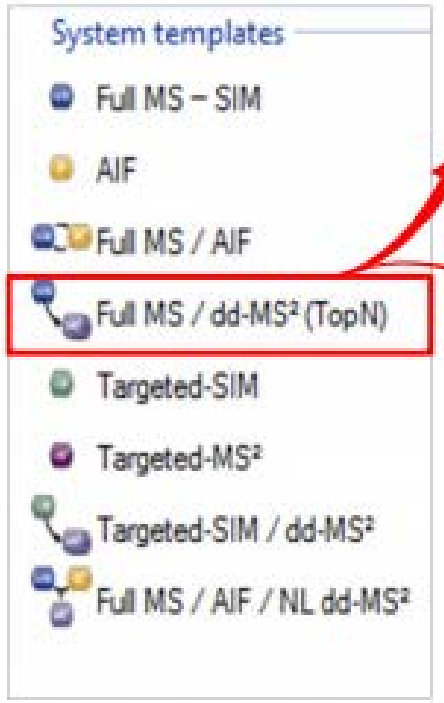
Database Search
(HMDB,KEGG,BioCyc,PubChem)

441 hits
(based on formula)

MS conditions:

Spray voltage 3800V, Capillary temperature 295°C, sheath gas 32 au, Aux gas 7 au, S-lens RF level 55

Full MS/data dependent MS^2



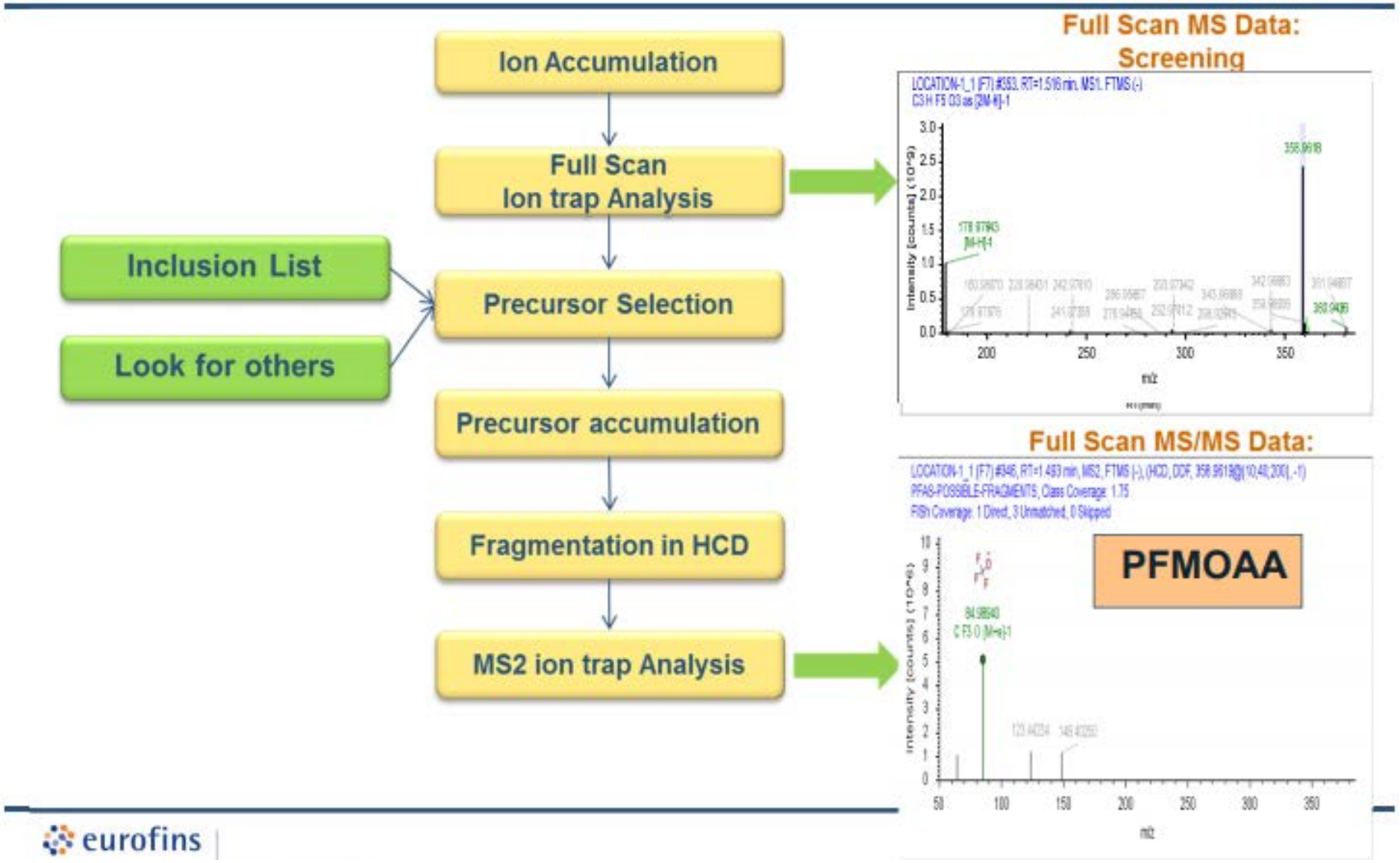
- full MS:resolution 140,000 FWHM
  - scan range 100-1500 m/z
- $ddMS^2$:resolution 35,000 FWHM
  - Inclusion list on
  - isolation 1 m/z
  - exclusion 5 s
  - NCE 10,40,200

List of 146 PFAS target analytes

	Mass [m/z]	Formula [M]	Formula type	Species	CS [z]	Polarity
1	178.97731	C3HF3O3	Chemical formula	M-H	1	Negative
2	112.9907	C2HF3O2	Chemical formula	M-H	1	Negative
3	148.9926	CF3SO3H	Chemical formula	M-H	1	Negative
4	162.9823	C2F5O2H	Chemical formula	M-H	1	Negative
5	178.9773	C3HF3O3	Chemical formula	M-H	1	Negative
6	197.9694	C2HF5SO2NH	Chemical formula	M-H	1	Negative
7	198.9484	C2HF5SO3	Chemical formula	M-H	1	Negative
8	212.9752	C4HF7O2	Chemical formula	M-H	1	Negative
9	228.97411	C4HF7O3	Chemical formula	M-H	1	Negative
10	241.9916	C4H3F5N3O3H	Chemical formula	M-H	1	Negative
11	242.01723	C6H5F7O2	Chemical formula		1	Negative
12	244.96903	C4HF7O4	Chemical formula	M-H	1	Negative
13	247.9616	C3HF7NSO2H	Chemical formula	M-H	1	Negative
14	247.9622	C3HF7SO2NH	Chemical formula	M-H	1	Negative
15	248.9461	C3HF7SO3	Chemical formula	M-H	1	Negative
16	[illegible]	C5HF9O2	Chemical formula	M-H	1	Negative
17	264.02022	C6H5F9O	Chemical formula		1	Negative
18	276.9411	C4F7SO4H	Chemical formula	M-H	1	Negative
19	278.97092	C5HF9O3	Chemical formula	M-H	1	Negative
20	282.9481	C4HF9SO2	Chemical formula	M-H	1	Negative
21	291.9884	C5H3F7N3O3H	Chemical formula	M-H	1	Negative
22	297.9584	C4HF11NSO2H	Chemical formula	M-H	1	Negative
23	298.94299	C4F9SO3	Chemical formula		1	Negative
24	310.9607	C5HF9O5	Chemical formula	M-H	1	Negative
25	310.96079	C5HF9O5	Chemical formula	M-H	1	Negative
26	312.97281	C6HF11O2	Chemical formula	M-H	1	Negative
27	323.96647	C8H4F9O3	Chemical formula	M-H	1	Negative

146

HRAM Strategy for PFAS/PFAA



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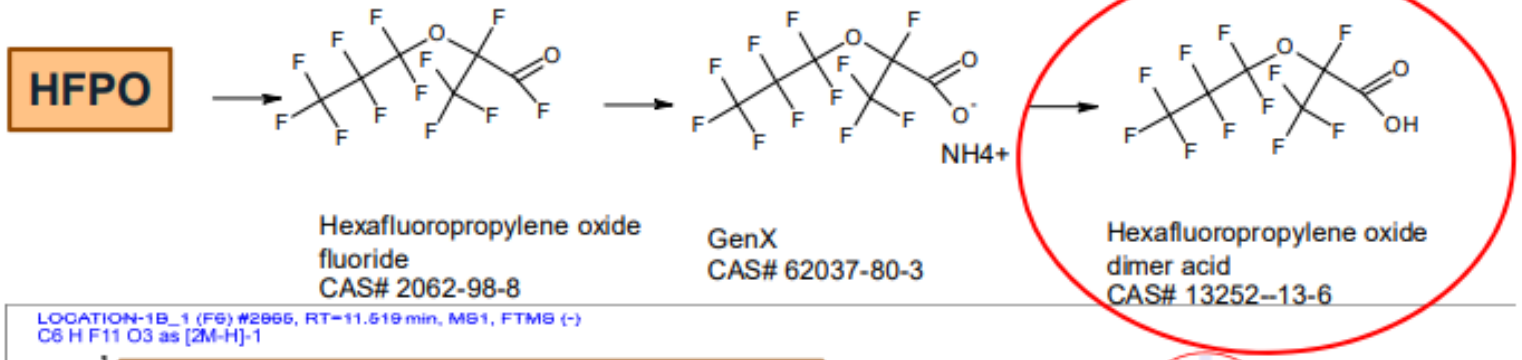
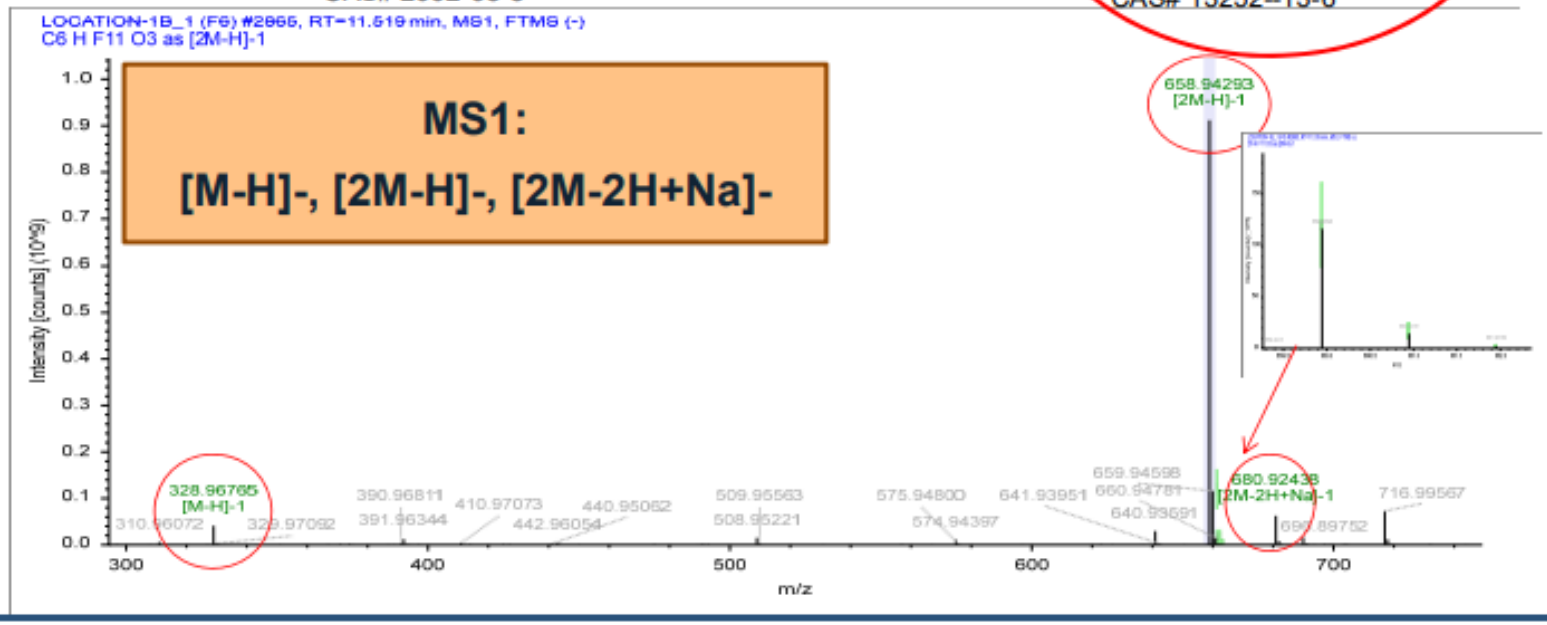
Results for GenX from Inclusion List and dd-MS^2

GenX Gets Reported As HFPO Anion.





eurofins | Eaton Analytical

12

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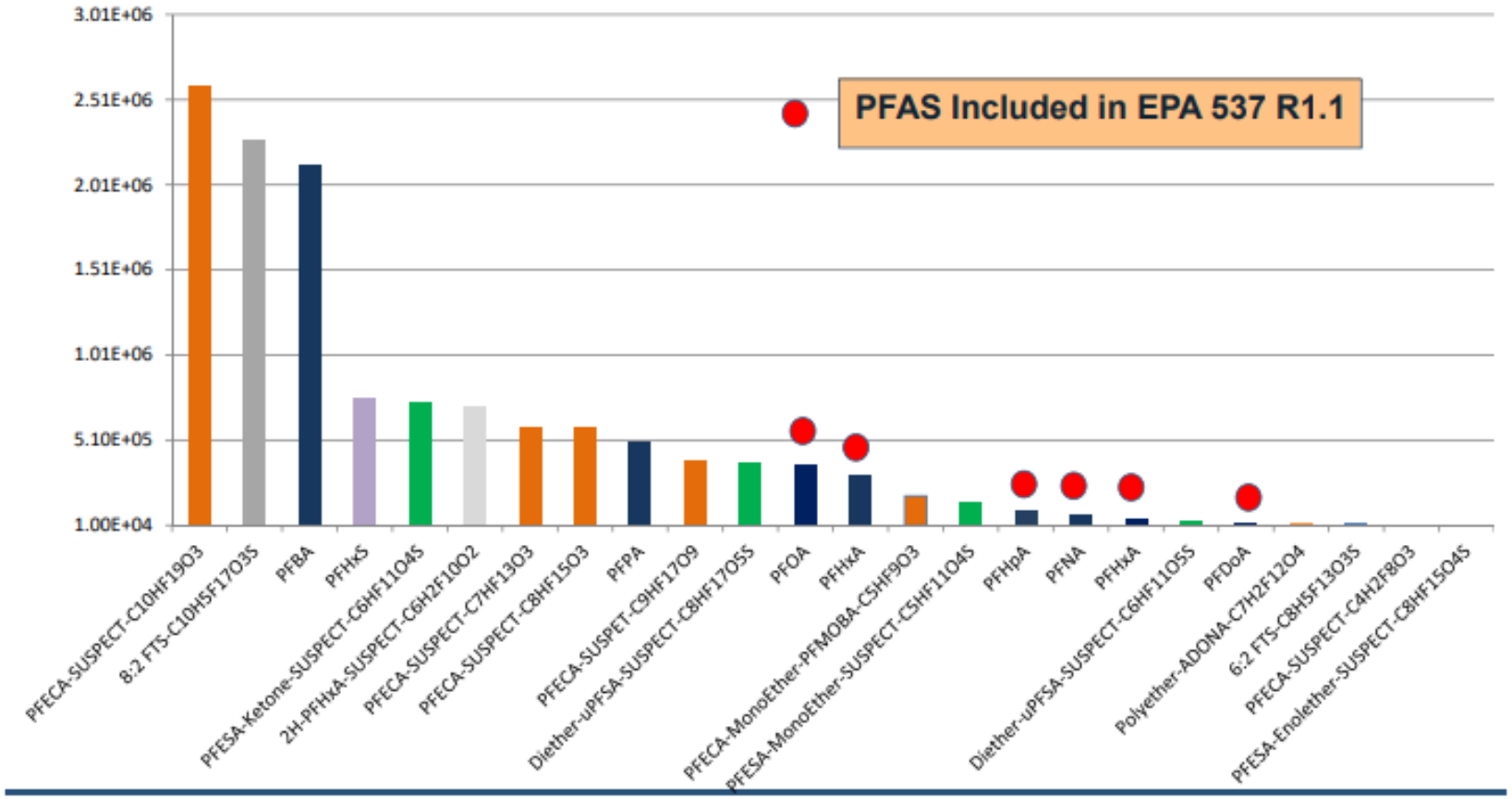
Finding Unknown PFAA's > Conc. than EPA Targeted List

Continued: Showing >1E+04<2.6E+06 area Count.



eurofins | Eaton Analytical

44 Total suspects/hits from a single sample (NC).

23

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Targeted Quantitation Pain Points For Every Analytical Laboratory

Targeted Quantitaton Workflows

Robustness

Consistency in day-to-day performance, sample-to-sample results, and user-to-user productivity

Speed

Enables higher throughput, faster analysis of complex mixtures

Sensitivity

Superior sensitivity for all molecule types regardless of matrix complexity

Resolution

Unusual for QqQs, however, significant benefits for complex mixtures, large molecules in complex matrices

NEW Thermo Scientific TSQ Triple Quadrupole Mass Spectrometers

Confident quantitation for any compound, any matrix, any user

Thermo Scientific™ TSQ Fortis™ Triple Quadrupole MS

New level of confidence

Thermo Scientific™ TSQ Quantis™ Triple Quadrupole MS

Everyday excellence

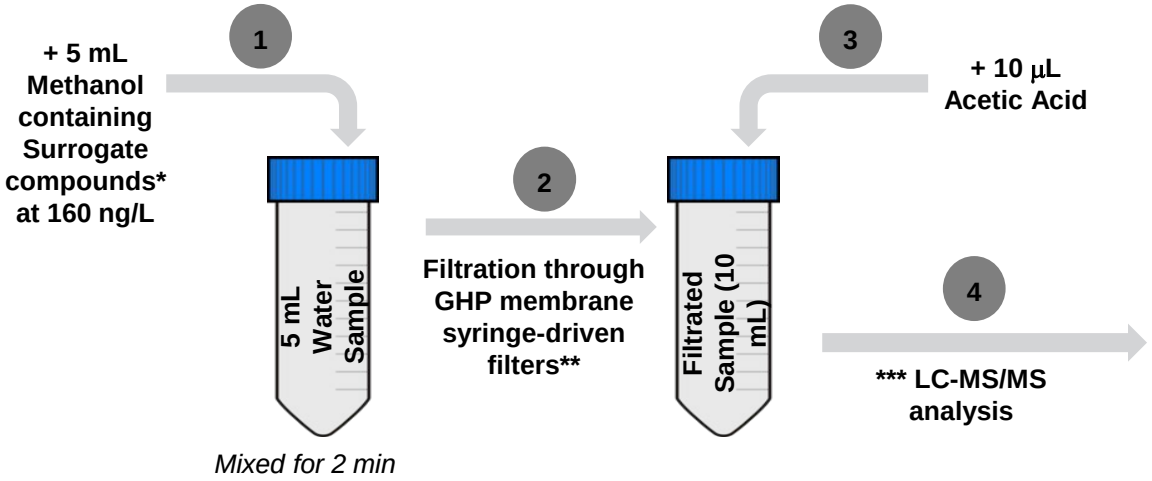
Thermo Scientific™ TSQ Altis™ Triple Quadrupole MS

For what's now and what's next

Learn More: www.thermofisher.com/confidentquan

Sample Preparation

❑ EPA Draft SW-846 Method 8327



Thermo Scientific™ Vanquish™ Flex Binary UHPLC System fitted with PFC-free kit and interfaced with the TSQ Altis Triple Quadrupole Mass Spectrometer

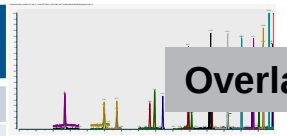
* All standards were obtained form Wellington Laboratories

**Acrodisc GxF/0.2 µm GHP membrane syringe-driven filters were washed twice with LC-MS grade methanol (2x 10 mL) and acetonitrile (2x 10 mL)

*** Silanized-amber glass autosampler vials sealed with polypropylene caps were free of contaminants and interferences

Thermo Scientific TSQ Altis MS – Calibration Curves: Range 5 – 200 ppt

Compounds		LV 1 – 5 ppt % Deviation	LV2 - 10 ppt % Deviation	LV3 – 20 ppt % Deviation	RT (min)	R^2
N-1	PFTreA	15%	7%	-2%	14.83	0.9906
N-2	PFTriA	2%	2%	4%	14.63	0.9966
N-3	PFDoA	-8%	4%	7%	14.3	0.9989
N-4	PFUnA	-3%	4%	-1%	13.72	0.9996
N-5	PFDA	12%	1%	-5%	13.03	0.9979
N-6	PFNA	3%	6%	-2%	12.21	0.9983
N-7	PFOA	13%	-1%	-2%	11.22	0.9972
N-8	PFHpA	1%	6%	-2%	9.91	0.9991
N-9	PFHxA	8%	0%	-1%	7.94	0.9983
N-10	PFPeA	-16%	4%	14%	4.98	0.9984
N-11	PFBA	0%	-1%	3%	2.68	0.9993
N-12	PFDS	4%	-14%	11%	13.7	0.9939
N-13	PFNS	0%	-6%	15%	13.04	0.9976
N-14	PFOS	-7%	-4%	9%	12.24	0.9981
N-15	PFHpS	16%	-7%	-9%	11.3	0.9979
N-16	PFHxS	13%	-5%	0%	10.11	0.9985
N-17	PFPeS	5%	4%	-3%	8.42	0.9991
N-18	PFBS	2%	1%	-2%	5.73	0.9995
N-19	PFOSA	10%	5%	-4%	13.66	0.9931
N-20	FtS 8:2	6%	-3%	-1%	13	0.9997
N-21	FtS 6:2	7%	3%	-7%	11.12	0.9977
N-22	FtS 4:2	23%	-10%	-9%	7.66	0.9976
N-23	NEtFOSAA	4%	-13%	9%	14.04	0.9985
N-24	NMeFOSAA	-10%	8%	6%	13.64	0.9993



Overlay of all PFC compounds analyzed in this method

Calibration curves

- ✓ Linearity over the range 5 – 200 ppt
  (2-fold dilution not taken into consideration)
- ✓ $R^2 > 0.99$ for all compounds
- ✓ Deviation < 20%

EPA Field Samples – External Validation Study

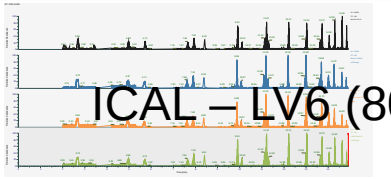
60 EPA water samples

Ground Water	Reagent Water	Surface Water	Waste Water
• 5x 5 mL native analytes • 5x 5 mL low spike • 5x 5 mL high spike	• 5x 5 mL native analytes • 5x 5 mL low spike • 5x 5 mL high spike	• 5x 5 mL native analytes • 5x 5 mL low spike • 5x 5 mL high spike	• 5x 5 mL native analytes • 5x 5 mL low spike • 5x 5 mL high spike

- ✓ Field samples divided into 3 batches of 20 samples
- ✓ 2 Method blanks, 2 LLOQs levels (10 and 20 ppt) and 2 Lab controls (LCS) were prepared for each batch

Perfluorotri- and Tetradecanoic Acids (PFTriDA and PFTeDA): Solubility Issues

Variable recoveries obtained due to their low solubility in water (highest spike concentration was diluted in 50:50 methanol: water)



ICAL – LV6 (80 ppt)

BATCH 1 – LCS (80 ppt)

BATCH 2 – LCS (80 ppt)

BATCH 3 – LCS (80 ppt)

PFDoA
PFTriDA
PFTeDA

EPA Field Samples Results

RSD < 20% for majority of the compounds among different water matrices.
All of the Reagent Water RSD <20%

	GROUND WATER				REAGENT WATER				SURFACE WATER				WASTE WATER			
N = 5	Low level spike		High level spike		Low level spike		High level spike		Low level spike		High level spike		Low level spike		High level spike	
Target compounds	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %	Average (ppt)	RSD %
N1_PFTeDA	26.13	12%	69.5	12%	24.43	10%	71.84	6%	26.78	18%	82.63	13%	23.92	11%	77.91	4%
N2-PFTrDA	22.81	11%	65.76	12%	22.29	9%	74.45	6%	23.8	17%	76.87	15%	22.24	8%	77.47	7%
N3-PFDoA	21.22	8%	64.08	12%	20.01	9%	73.25	9%	22.17	19%	71.55	14%	20.54	13%	75.8	9%
N4_PFUdA	22.05	11%	65.41	7%	21.23	12%	72.08	9%	23.19	21%	69.81	11%	21.74	12%	74.95	9%
N5_PFDA	22.98	9%	64.83	8%	21.63	11%	72.83	8%	23.55	17%	69.98	11%	23.16	3%	76.89	7%
N6-PFNA	22.29	8%	66.83	8%	21.21	9%	73.4	7%	23.15	16%	70.65	10%	22.7	9%	75.64	7%
N7-PFOA	22.89	12%	65.88	10%	21.26	9%	71.72	9%	24.15	15%	69.87	9%	27.08	8%	79.79	7%
N8-PFHpA	23.34	11%	65.96	11%	21.89	10%	72.23	8%	25.51	18%	69.48	11%	26.35	12%	77.48	9%
N9-PFHxA	22.33	12%	64.9	10%	21.05	8%	71.47	8%	23.36	16%	69.43	11%	40.48	7%	93.5	8%
N10_PFPeA	27.41	13%	63.44	11%	23.42	12%	71.5	10%	32.24	23%	67.87	9%	38.32	3%	84.01	10%
N11_PFBA	18.99	24%	64.11	4%	21.04	10%	70.09	6%	19.59	16%	63.82	6%	14.01	45%	66.61	6%
N12-PFDS	20.79	14%	58.96	20%	18.2	14%	60.87	12%	22.02	24%	56.67	13%	19.43	24%	69.12	14%
N13-PFNS	20.32	27%	56.17	16%	19.67	8%	59.42	10%	20.08	17%	57.1	12%	18.19	20%	63.55	9%
N14-PFOS	22.57	20%	60.07	8%	21.26	16%	63.04	9%	24.71	14%	62.52	10%	24.29	10%	75.43	13%
N15-PFHpS	20.81	12%	61.61	9%	19.53	11%	67.95	10%	21.7	17%	63.27	10%	19.43	15%	71.37	10%
N16-PFHxS	19.69	15%	59.56	10%	18.38	10%	63.56	9%	20.89	14%	61.66	7%	19.94	11%	67.58	9%
N17-PFPeS	20.32	11%	62.4	9%	19.35	6%	65.83	9%	21.52	18%	63.48	8%	19.87	10%	68.3	9%
N18-PFBS	22.55	13%	61.07	10%	19.98	11%	63.39	10%	21.34	26%	61.46	9%	24.88	15%	52.03	36%
N19-PFOSA	20.51	15%	60.19	12%	18	10%	60.89	12%	22.6	17%	63.44	10%	19.66	10%	67.62	9%
N20_FtS8_2	22.18	7%	63.6	6%	21.55	10%	69.86	8%	20.12	18%	66.8	8%	21.51	8%	71.42	7%
N21-FtS6_2	20.64	13%	64.29	11%	21.46	17%	69.2	7%	29.54	37%	80.12	13%	33.15	25%	82.55	11%
N22-FtS4_2	21.11	6%	62.04	5%	20.4	11%	69.78	6%	21.02	15%	65.49	11%	19.75	7%	69.17	8%
N23_NEtFOSAA	23.66	12%	63.56	11%	22.96	18%	70.55	7%	23.72	25%	70.56	14%	22.16	16%	76.7	8%
N24_NMeFOSAA	23.88	9%	65.42	9%	21.8	8%	73.34	7%	23.97	17%	71.28	8%	22.9	10%	73.38	7%

Summary

- ✓ **TSQ Altis Triple Quadrupole MS** is able to quantitate at 5 ppt the list of PFCs listed in EPA draft SW-846 method 8327 using direct injection (2-fold dilution not taken into consideration)
- ✓ **TSQ Altis Triple Quadrupole MS** is able to quantitate certain PFCs 5 times lower than the LLOQ reported by EPA
- ✓ **Thermo Scientific™ Accucore™ RP-MS LC Columns** provide similar performance as the Waters Acquity CSH Phenyl-hexyl described in EPA draft SW-846 method 8327
- ✓ Retention time stability was very good inter-batch
- ✓ Large injection volumes overload the analytical columns and chromatographic peak fronting is observed. Reduced injection volumes maintain sensitivity and improve assay robustness.
- ✓ Longest chain perfluorocarboxylic acids showed high variability within batches mainly due their low solubility in water (higher spike concentration)
- ✓ EPA field samples showed RSDs below 20% for most of the compounds among different water matrices

Thank You for Listening

Questions

For more information visit:
http://thermofisher.com/quantitation

Thank You

Please return our survey to receive a drink ticket for our daily networking event where you can continue discussions with our experts!