

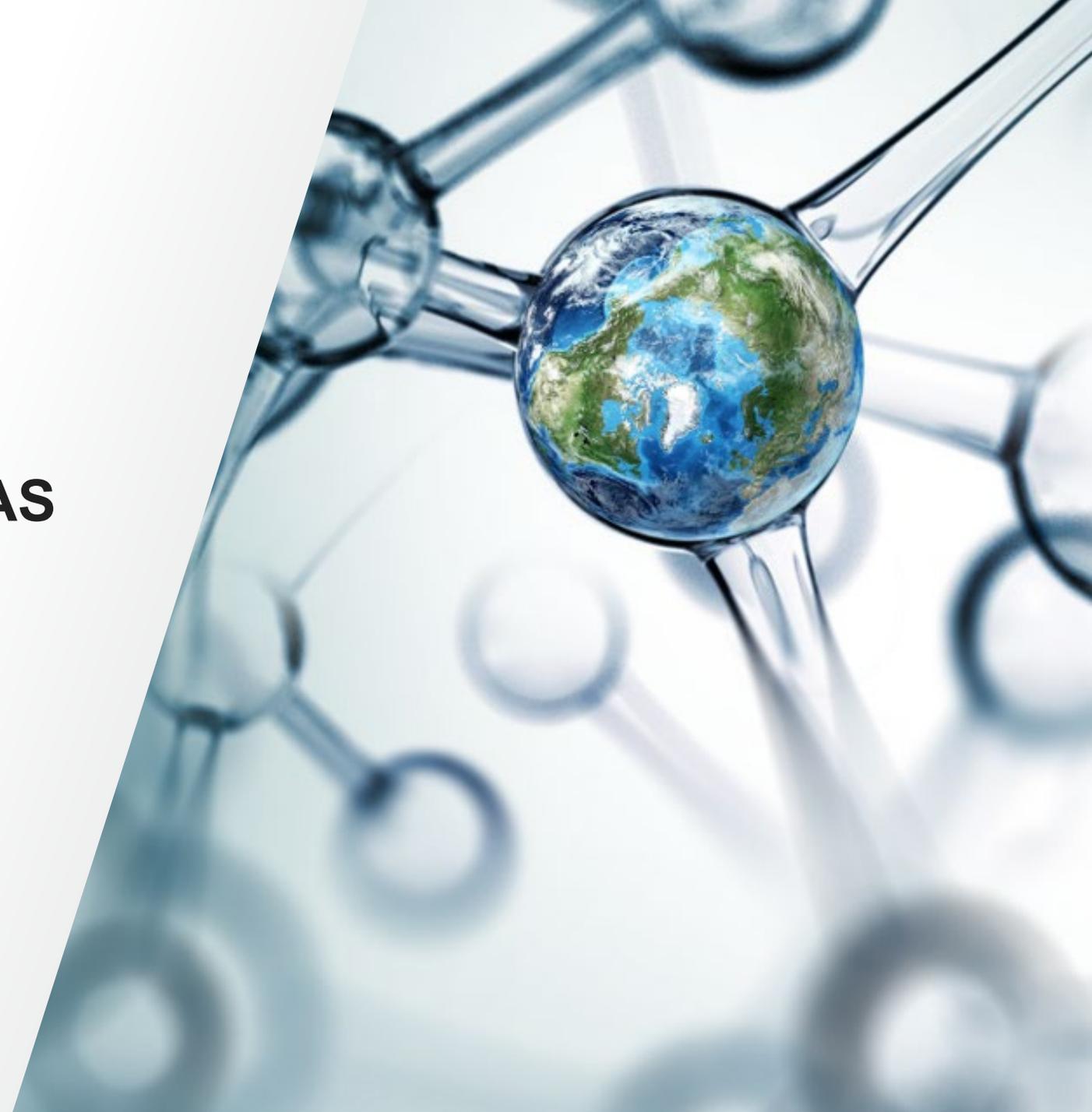
# Implementing new GC-MS and LC-MS technologies to stay ahead with your food safety analysis from pesticides to PFAS and microplastics

**Frans Schoutsen**

Environmental, Food and Beverage Support

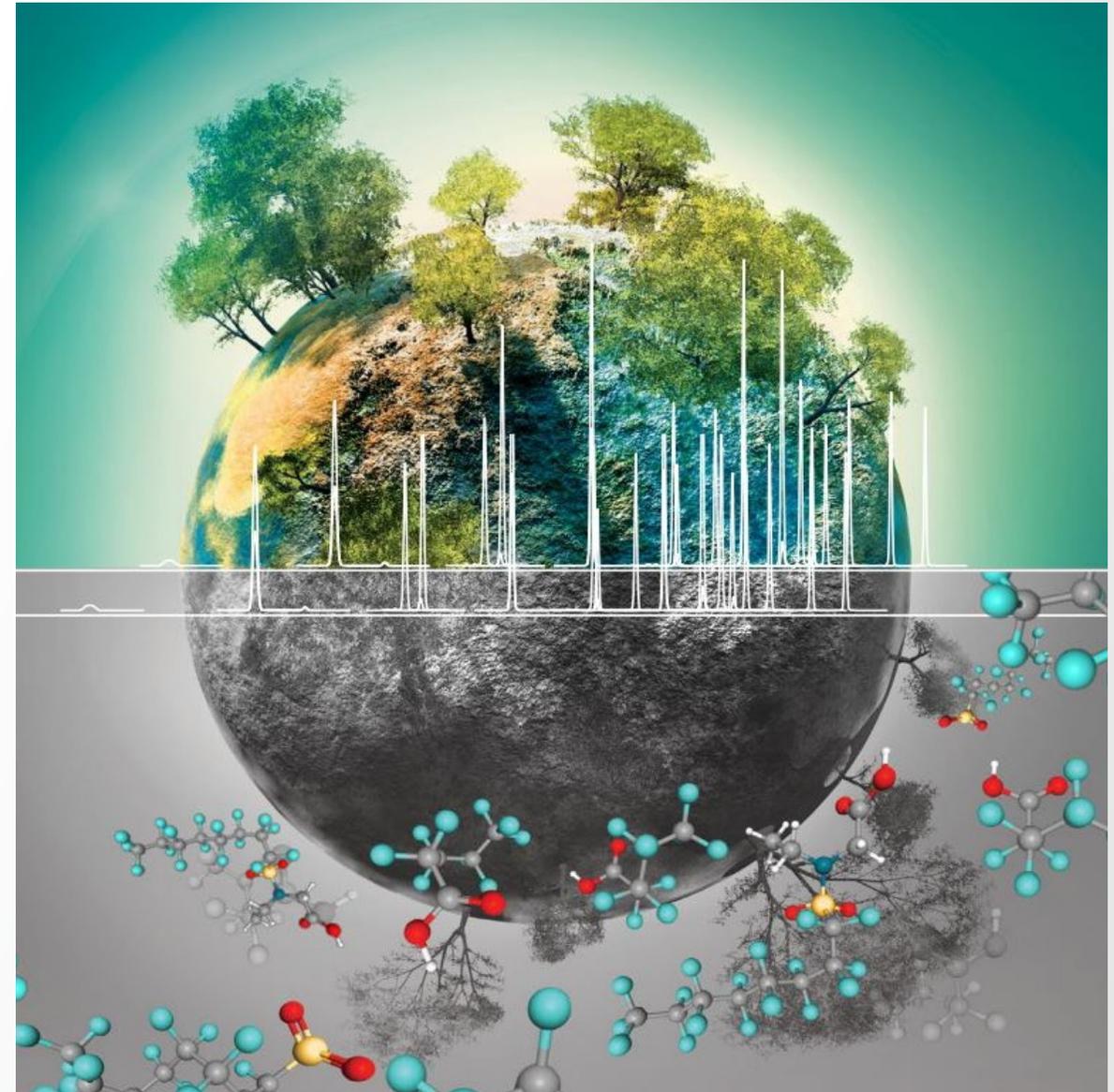
10<sup>th</sup> International Symposium on Recent Advances in Food Analysis, September 6-9, 2022

 The world leader in serving science



# Presentation outline

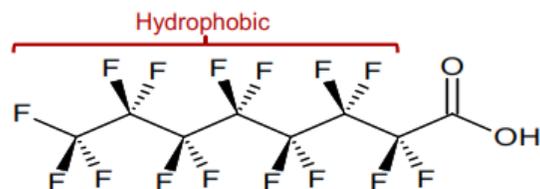
- PFAS background and overview
- Instruments and experiments
- Why HRAM and the use of Thermo Scientific™ myLibrary™ Enterprise to create spectral libraries
- Method description, sample preparation, chromatography, and Thermo Scientific™ Orbitrap Exploris™ MS conditions  
Calibration, recovery, LOQs, and confirmation
- Conclusions



# What are PFAS compounds?

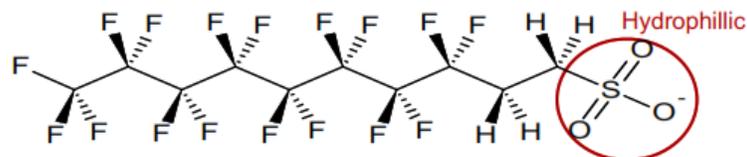
What are PFAS?

- PFASs are **Per-** and **Poly**Fluorinated Alkyl **S**ubstances. Exclusively anthropogenic.
- Structures contain a hydrophobic perfluoroalkyl backbone and a hydrophilic end group
- Include a diverse range of compounds with a variety of chain lengths and end groups



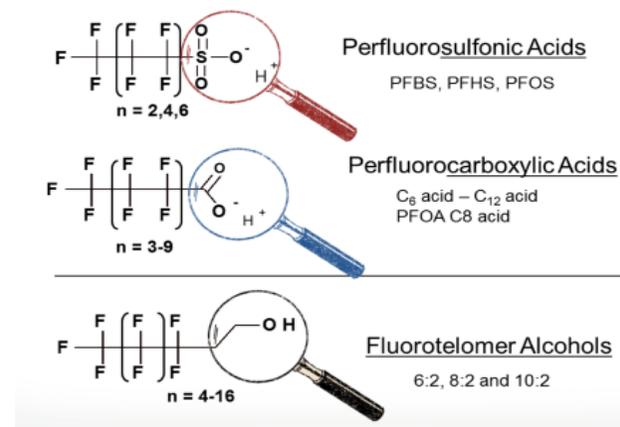
Perfluorooctanoic acid

- PFOA
- Teflon®



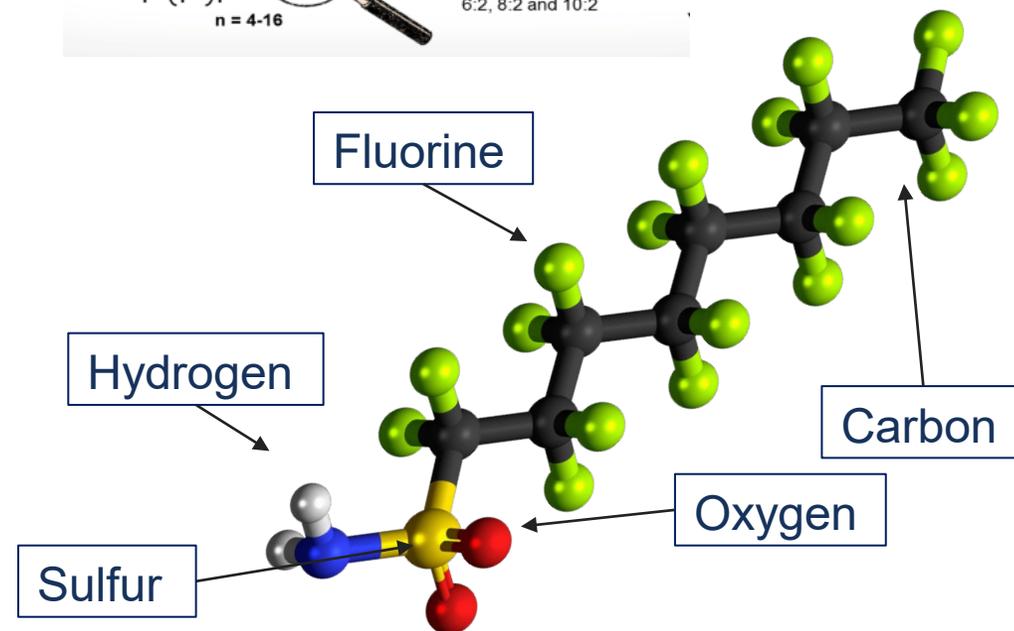
8:2 Fluorotelomer sulfonate

- 8:2 FTS

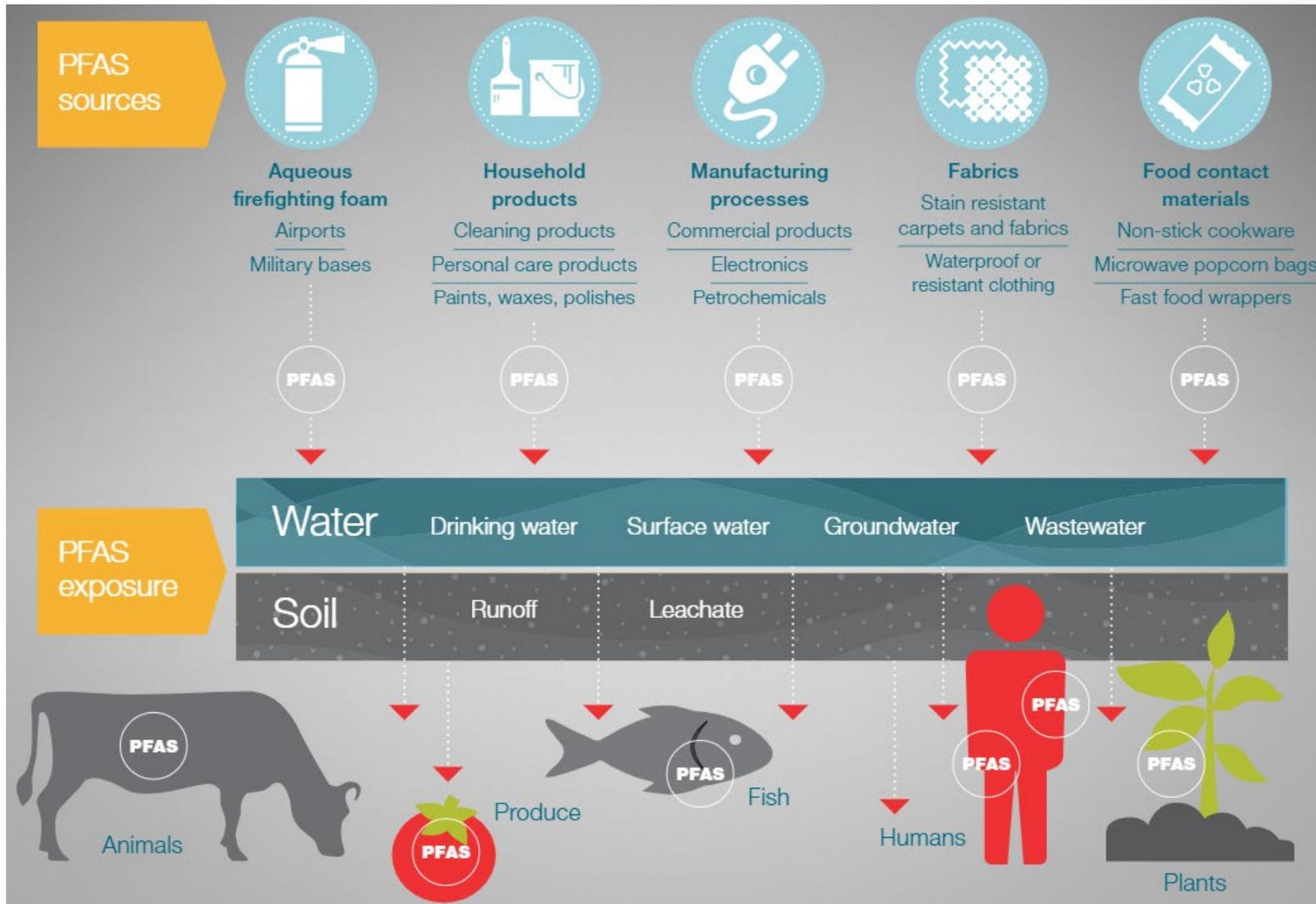


Wide Variety of Industrial Applications

- PFAS are used because of their unique chemical and physical properties. These include:
  - Industrial polymers (Teflon® - PFOA)
  - Stain repellants (Scotchgard® - PFOS)
  - Aqueous film forming foams (AFFF) – fire fighting applications
- Sources can be found anywhere at differing (generally lower) concentrations



# How does PFAS enter the environment?



A high number of sources



Very strong C-F bonds results in bioaccumulation



The workflow for analysing PFAS will depend on the goals of your analysis

# US methods associated with PFAS measurements

## Drinking Water

- US EPA 537.1 – Internal Standard method, 18 analytes
- US EPA 533 – Isotope Dilution method, 25 analytes

## Groundwater/Wastewater/Solids

- US EPA 8327 – External Standard method, 24 analytes
- ASTM D7979-17 – Isotope Dilution method, 21 analytes
- Draft US EPA 1633 - Isotope Dilution method, 40 analytes, includes tissue

## Sediments/Soil Extracts

- US EPA 8327 – External Standard method, 24 analytes
- ASTM 9768-17a – Isotope Dilution method, 21 analytes

## Food

- USDA CLG - PFAS 2.03 - 16 (PFAS) - bovine, porcine, poultry muscle and bovine plasma
- USFDA Method C-010.01 - 16 (PFAS) - milk, bread, cheese, meat, others

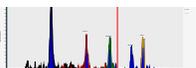
# TSQ separation of perfluorinated alkyl substances, 50 pg/mL



## Thermo Scientific™ Vanquish™ Flex UHPLC System

Delay Column: 3.0 x 50 mm, 5 μm  
BDS Hypersil C8  
Analytical Column: 2.1x100 mm, 2.6  
μm Accucore C18  
Column Temp: 30 C  
Mobile Phase: [A] H<sub>2</sub>O + 10 mM Am.  
Acetate; [B] MeOH  
Injection Volume: 3 uL

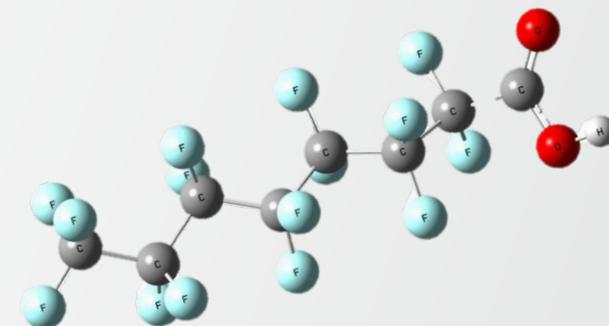
PFASs C4 – C10 at 50 pg/mL (0.15 pg on-column); LODs are ~10 pg/mL (0.03  
pg O.C.)



## Thermo Scientific™ TSQ Quantis™ Mass Spectrometer

Ionization Mode: HESI, Negative ion mode  
MS Acquisition Mode: Selective Reaction  
Monitoring (SRM) **Cycle time: 0.15 s**  
Quad Isolation (Q1,Q3) = Unit (0.7 Da  
FWHM)

C8-PFAS at 50 pg/mL (0.15 pg on-column); LODs are ~10 pg/mL (0.03 pg  
O.C.)





### Thermo Scientific™ TSQ Fortis™ Mass Spectrometer

- Mass Range  $m/z$  2 – 3000
- Max Resolution **0.4 FWHM**
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- **80,000:1 S/N**



### Thermo Scientific™ TSQ Quantis™ Mass Spectrometer

- Mass Range  $m/z$  2 – 3000
- Max Resolution **0.4 FWHM**
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- **200,000:1 S/N**



### Thermo Scientific™ TSQ Altis™ Mass Spectrometer

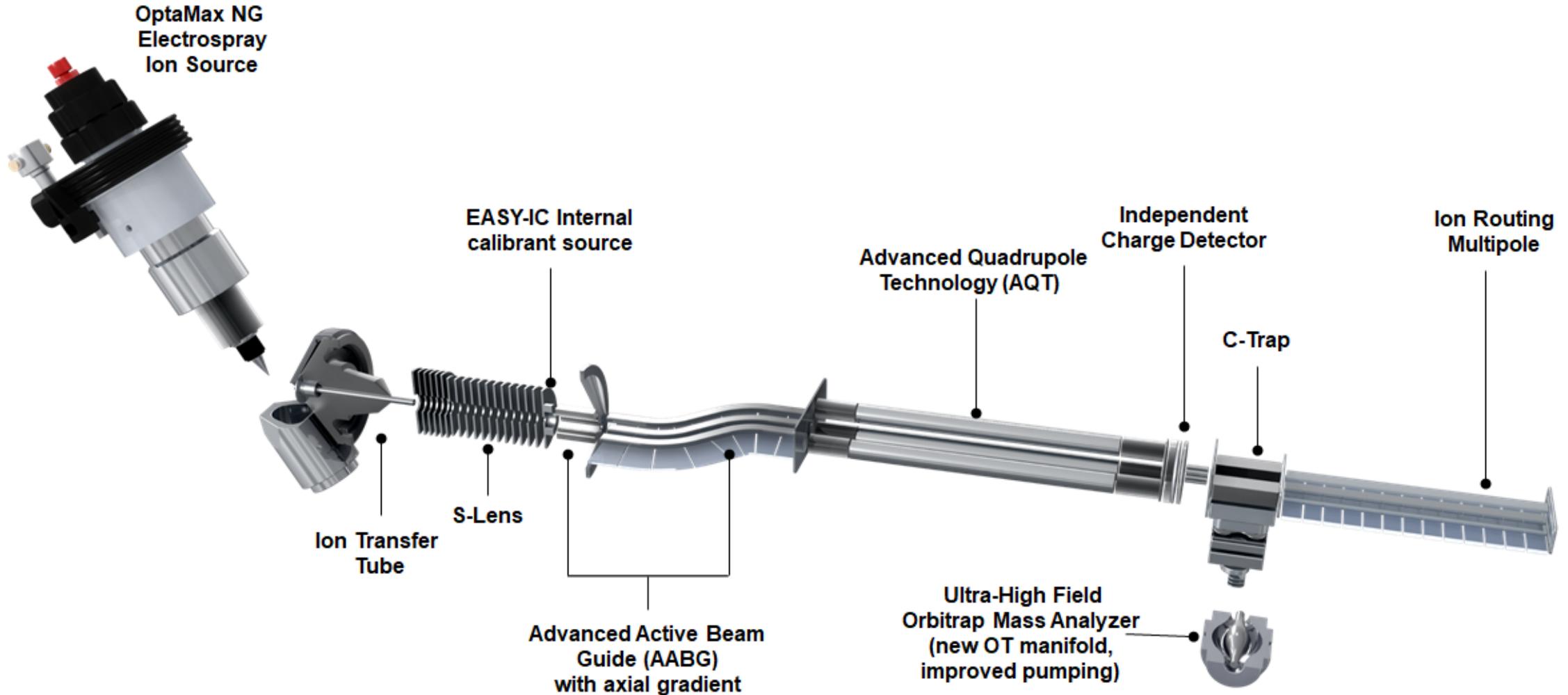
- Mass Range  $m/z$  2 – 2000
- Max Resolution **0.2 FWHM**
- Max 30,000 transitions per run
- Polarity Switching < 20 msec
- Dynamic interscan time
- 600 SRM/sec
- TNG software
- Chromeleon support
- **500,000:1 S/N**

# Why use Orbitrap HRAM for PFAS analysis?

## High Resolution Accurate Mass

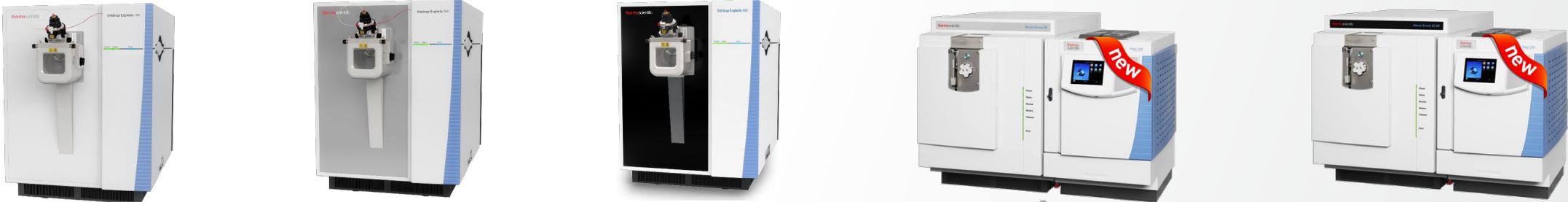
- There are over 9000 known PFAS (with more PFAS being actively discovered) and a very limited number of certified reference standards commercially available for routine targeted analysis.
- HRAM analysis by LC-Orbitrap has an inherent advantage over triple quadrupole MS because it can provide both quantification and identification of target PFAS, along with the option of retrospective analysis on samples that may contain other untargeted PFAS.
- It can also overcome challenges of matrix interferences that have been observed in animal tissue extracts by tandem MS due to the low ppm mass accuracy and high mass resolution capability of orbitrap instrumentation.
- The Orbitrap exhibits excellent sensitivity on par with most triple quadrupole instruments, providing excellent quantitative data at low ppt levels.

# Orbitrap Exploris mass spectrometer schematic

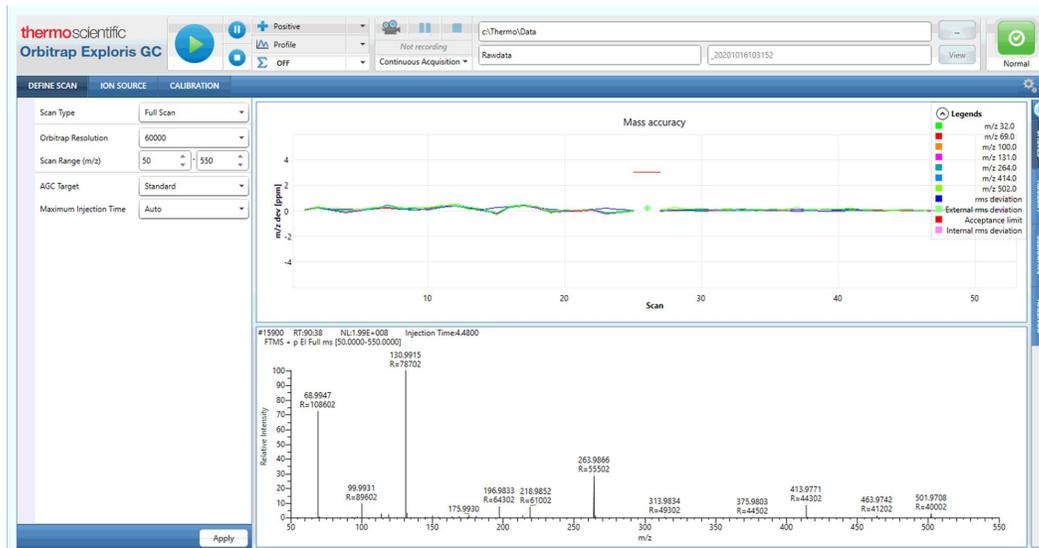


# One instrument series control software (GC- and LC)

A user of one becomes a user of all systems



## Thermo Scientific™ Orbitrap Exploris™ Mass Spectrometers



Instrument Method Editor

Application method templates

# Simplified drag and drop method editor and templates

Reduce method development time and capture the highest data quality every time

The screenshot displays the ThermoFisher method editor interface. At the top, it shows 'Experiment # 1' and 'Time Range (min) 3.5-23'. Below this, there are 'ADD', 'DELETE', 'IMPORT', and 'EXPORT' buttons. The main area features a table with the following data:

	Scan Range (m/z)	Orbitrap Resolution
1	50-550	60000

On the left side, there is a 'System Templates' section with a 'Save as Template' button and a list of application categories: Anti-Doping Control, Flavor and Fragrances, Food Safety and Enviro, Impurity Testing, Metabolomics, PCI Data Dependent MSMS, and POPs. Below these are 'Custom Templates' and 'My Experiments'. A dropdown menu is open over the 'Food Safety and Enviro' category, showing three options: 'Contaminants EI Fullscan', 'Unknown Confirmation PCI Fullscan', and 'Unknown Identification PCI ddMSMS'.

Method templates to enable quick method start up across popular applications including

- Food safety contaminants
- Persistent organic pollutants
- Anti-doping
- Flavor and fragrances
- Impurity testing

# Instrument editor Orbitrap Exploris options

Orbitrap Exploris 240 Method Editor 2.0.182.18 [C:\Thermo\Instruments\test22.meth]

File Orbitrap Exploris 240

### Method Editor

Global Parameters Scan Parameters Summary

#### Method Timeline

Application Mode: Small Molecule

Method Duration (min): 60

Scans: MS, MS<sup>2</sup>

Filters: MIPS, Intensity, Precursor Fit

Experiment # 2

Experiment ACTIONS Settings

30 40 50 60

MS

Settings

Infusion Mode: Liquid Chromatography

Expected LC Peak Width (s): 10

Mild Trapping:

Advanced Peak Determination:

Default Charge State: 2

Enable Xcalibur AcquireX method modifications:

Internal Mass Calibration: EASY-IC™

Time Range (min): 0-60 CLEAR

#### Data-Dependent MS<sup>2</sup> Scan Properties

Show All

Isolation Window (m/z)	1
Collision Energy Mode	Stepped
Collision Energy Type	Normalized
HCD Collision Energies (%)	15,30,45,60,75
Orbitrap Resolution	30000
Scan Range Mode	Auto
Use EASY-IC™	On

Full Scan

Intensity

Targeted Mass

Apex Detection

ddMS<sup>2</sup>

20 scans

# Ampicillin @ 10 ppb in pig muscle

**Analysis** Data Review - FS\_DIA\_70K\_17K\_001\_quan\*

**Batch View**

- Samples
- Auto Samples
- Reference Sample
- Threshold Samples
- Data Review**
- Sample View
- Compound View
- Comparative View
- Qualitative View

**Report View**

- Local Method
- Acquisition
- Quantitation
- Processing
- Compounds
- QAQC
- Groups
- Intel Seq
- Reports

**Compounds**

Compound
1 Abamectin
2 Amoxicillin
3 Ampicillin
4 Cefalexin
5 Cefalonium
6 Cefaperazone
7 Cefapirim
8 Cefquinome
9 Chlorotetracycline
10 Ciprofloxacin
11 Cloxacillin
12 Danofloxacin
13 Dapsone
14 Difloxacin
15 Dimetridazol
16 Doramectin

**Sample Results**

Accr	PK	IR	IP	LS	FI	Confirm	Status	Filename	Height	Area	Actual RT	Formula	m/z (Apex)	m/z (D)
28	28							Avermectins STD 008	N/F	N/F	N/F		N/F	N/F
29	29							Blank005	N/F	N/F	N/F		N/F	N/F
30	30							Blank006	N/F	N/F	N/F		N/F	N/F
31	31							Muscle001	6835815	26949880	5.55	C16H19N3O4S	350.11731	1.1698
32	32							Muscle002	10017380	39573263	5.57	C16H19N3O4S	350.11734	1.2569
33	33							Muscle003	6033173	23927030	5.57	C16H19N3O4S	350.11752	1.7799
34	34							Blank007	N/F	N/F	N/F		N/F	N/F
35	35							Kidney001	1695692	6882638	5.57	C16H19N3O4S	350.11728	1.0826
36	36							Kidney002	3185046	12791714	5.57	C16H19N3O4S	350.11713	0.6468
37	37							Kidney003	6364897	22791845	5.57	C16H19N3O4S	350.11725	0.9954
38	38							Plasma001	55484	153304	5.33	C16H19N3O4S	350.11765	2.1286
39	39							Blank009	N/F	N/F	N/F		N/F	N/F
40	40							Milk001	N/F	N/F	N/F		N/F	N/F
41	41							Milk001	N/F	N/F	N/F		N/F	N/F
42	42							Blank010	N/F	N/F	N/F		N/F	N/F

**Compound Details**

- Quan Peak
- Isotope
- Fragments
- Calibration Curve

**Quan Peak**

Ampicillin RT: 5.55 | Muscle001

RT: 5.55  
AA: 26949880.48  
AH: 6835814.73  
SN: 29984939595637000.00

Relative Intensity vs RT(min)

Apex RT: 5.55  
Area: 26949880

**Isotope**

Scan #: 2917-2977 RT: 5.50 - 5.65  
Muscle001  
F: FTMS +p ESI Full ms [100.00-10 ...

- All Isotopes
- Multi-Isotopes
- #1: 350.11690
- #2: 351.12020
- #3: 352.11276
- #4: 352.12260
- #5: 353.11612

Relative Intensity vs m/z

**Fragments**

Minimum # of fragments needed: 1  
Muscle001 # 2932 RT: 5.55  
F: FTMS + p ESI Full ms2 350.00@ ...

- All Fragments
- #1: 192.04720
- #2: 174.05450
- #3: 106.06480

Intensity vs m/z

**Calibration Curve**

Ampicillin  
Y = 1.721e6X - 2.471e5; R<sup>2</sup>: 0.9993; Origin: Ignore; W: 1/X; Area

Avg vs ppb

# What is myLibrary Enterprise platform?

- **myLibrary Enterprise** is the one of its kind platform that enables a company to **collaboratively create spectral libraries** of proprietary data for **use** within their own organization in a **secure fashion**.
- **myLibrary Enterprise** platform is **SaaS solution** hosted on Amazon Web Services (AWS) cloud within the Thermo Scientific™ Ardia™ platform that will be individually created and dedicated to each customer.
- Built to be fast, scalable, and secure.



# myLibrary Enterprise was inspired by mzCloud

Thermo Scientific™ m/z Cloud™ Library

<https://mzcloud.org>

<https://YourCompanyName.mylibrary.thermofisher.com>

The screenshot shows the mzCloud interface. On the left is a blue sidebar with the 'm/z CLOUD' logo and navigation options: 'Spectral Libraries', 'LC/MS Autoprocessed', 'LC/MS Reference', and 'Library Search'. The main content area is titled 'Spectral Libraries - LC/MS Reference' and features a table with columns for ID, Legacy ID, and Compound Name. A search bar is located above the table.

	ID	Legacy ID	Compound Name
	<input type="text"/>	<input type="text"/>	<input type="text"/>
+	8,159	8,116	6-Deoxy-β-D-galactopyrano...
+	8,218	8,258	6-Deoxyhexopyranosyl-(1->...
+	6,132	6,194	Vancomycin
+	7,296	7,230	Bacitracin A
+	8,160	8,115	6-Deoxy-α-L-mannopyranos...
+	8,127	8,199	Methyl (3Z)-3-ethylidene-4-...
+	8,157	8,118	3-O-[(2S,3R,4R)-3,4-Dihydr...
+	8,163	8,112	β-D-Glucopyranosyl-(1->3)-...
+	5,883	5,843	Amphomycin

The screenshot shows the myLibrary interface. On the left is a green sidebar with the 'myLibrary CLOUD' logo and navigation options: 'Compounds', 'Files', 'Processing', 'Tree Builder', 'Spectral Trees', 'Curation Workflows', 'Libraries', 'Manage Libraries', 'Spectral Libraries', 'Library Search', 'Settings', and 'Metadata Schema'. The main content area is titled 'Spectral Libraries - PFAS' and features a table with columns for ID, Compound Name, and Classes. A search bar is located above the table.

	ID	Compound Name ↓	Classes
	<input type="text"/>	<input type="text"/>	
+	14	11CI-PF3OUdS	PFAS
+	20	4:2 FTS	PFAS
+	17	PF4OPeA	PFAS
+	48	M2-6:2 FTS	PFAS
+	30	PFBA	PFAS
+	47	M2-4:2 FTS	PFAS
+	44	M4PFHpA	PFAS
+	51	M3PFHxS	PFAS
+	53	M6PFDA	PFAS
+	27	PFDA	PFAS
+	33	L-PFDS	PFAS
+	10	PFTrDA	PFAS

# What is mzCloud Library?

- Worlds largest HRAM LC-MS reference spectral library
- Constantly growing with new data
- HRAM MS/MS and MS<sup>n</sup>
- High quality curated data
- Wide chemical diversity
- Searchable web User Interface
- Online at mzCloud.org
- Integrated into Thermo Fisher Scientific software

The screenshot displays the mzCloud web interface. On the left, a navigation menu includes 'Views', 'Reference Library', 'Standard', 'Compare', 'Structures', 'Libraries', 'Search', 'Search Results', and 'Tools'. The 'Reference Library' section shows a list of compounds with their IDs and monoisotopic masses, such as Glycitin (No: 6137, Monois. Mass: 446.12130) and Trilostane (No: 6143, Monois. Mass: 329.19909). The main area features a 'Spectral Tree' and a 'Recalibrated Spectrum' plot. The spectrum shows relative intensity versus m/z, with major peaks at 112.03930, 147.11683, 201.16378, 303.1955, and 330.2064. The precursor structure is identified as [C<sub>20</sub>H<sub>27</sub>NO<sub>3</sub>+H]<sup>+</sup>. The compound name 'Trilostane' is shown in the 'Compound' section, along with its systematic name: (4 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-3,17-Dihydroxy-4,5-epoxvandrost-2-ene-2-carbonitrile.

# myLibrary Enterprise – cloud based spectral library building platform

## Secure access and storage

- Secure storage in cloud instance.
- Control user access and role
  - ✓ Administrator
  - ✓ Manager
  - ✓ Creator
  - ✓ Viewer
- Single tenant – utilizing Customer's own IdP.
- Accessible via major web browsers



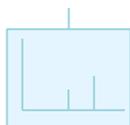
# myLibrary Enterprise – cloud based spectral library building platform



- Define compounds
  - Supports both reference as well as “putative” compounds without structure.



- Upload MS data
  - Thermo .RAW or any other vendor through .mzML format
  - NIST MSP, mzVault or Mass Frontier libraries

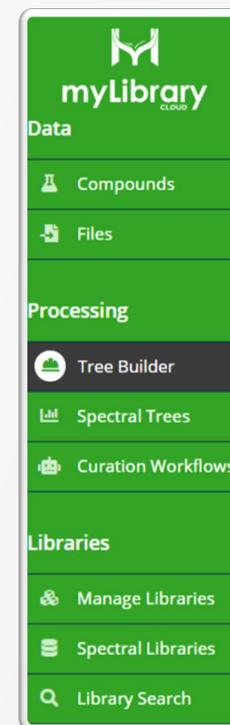


- Create and Curate spectral trees
  - Batch processing and professional curation procedure of spectral trees

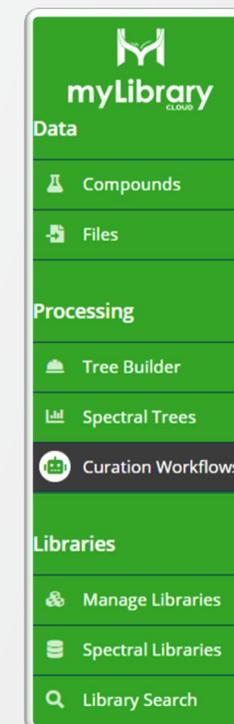


- Build and Manage Libraries
  - Full control over what compounds are in what libraries. Create application or product specific libraries.

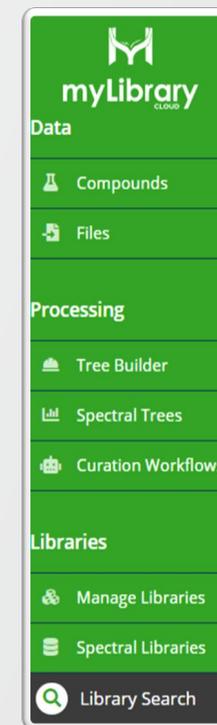
## CREATE



## CURATE



## SEARCH



# myLibrary Enterprise – cloud based spectral library building platform

## Tailored library creation

- Customizable metadata and tags that capture critical information.
  - ✓ Batch/ lot
  - ✓ Matrix/ Tissue
  - ✓ Disease
  - ✓ Operator/ Season



- Flexibly define libraries for specific needs.



**GREATER CONTEXTUAL  
SEARCHING CAPABILITIES**

myLibrary <b>Manage Libraries</b>						
	Name	Owner	Assigned to	Library Type	No. Records	Sync. Status
Data	Pesticides	Bill	All	Reference Manual	466	<span style="background-color: green; width: 20px; height: 10px;"></span>
Compounds	Illicit Drugs	Anna	QC Team	Reference Manual	235	<span style="background-color: green; width: 20px; height: 10px;"></span>
Files	Flavanols	Anna	R&D Team	Reference Manual	333	<span style="background-color: green; width: 20px; height: 10px;"></span>
Processing	Plasma Metabolites	Rob	Clinics Team	Putative Manual	74	<span style="background-color: green; width: 20px; height: 10px;"></span>
Tree Builder	Pathogen Response	Bill	R&D Team	Putative Manual	24	<span style="background-color: green; width: 20px; height: 10px;"></span>
Spectral Trees	Unknown Urine	Rob	Clinics Team	Putative Manual	35	<span style="background-color: green; width: 20px; height: 10px;"></span>
Curation Workflows						
Libraries						
Manage Libraries						
Spectral Libraries						

# myLibrary Enterprise – cloud based spectral library building platform

## Automated Curation Pipeline



The screenshot shows the myLibrary Enterprise interface. On the left is a navigation sidebar with categories: Data (myLibrary logo), Compounds, Files, Processing (Tree Builder, Spectral Trees, Curation Workflows), and Libraries (Manage Libraries, Spectral Libraries, Library Search). The main area displays the 'Curation Workflows' table with columns for Name, Type (Source template), Trees, and Status. The table lists several workflows, all with green progress bars and 'Workflow finished' status.

Name	Type (Source template)	Trees	Status
PFAS_Caroline02_lite	liteCuration	434	Workflow finished. 7:32 PM
PFAS_Caroline04_lite	liteCuration	247	Workflow finished. 11:54 PM
TS01_lite	liteCuration	5	Workflow finished. 9:05 PM
marynka_test_lite	liteCuration	2	Workflow finished. 12:17 PM
Marynka_Full	fullCuration	6	Workflow finished. 4:59 PM
Bhenic acid_full	fullCuration	1	Workflow finished. 12:35 PM
Default_Full_Curation	fullCuration	7	Workflow finished. 7:06 PM
ASMS_Unused	fullCuration	0	Workflow finished. 7:44 PM
Eric_lite	liteCuration	2	Workflow finished. 5:45 PM

## Workflow type

### Lite Curation

- Remove Empty Spectra
- Average Spectra
- Select Significant Spectra

### Advanced Curation

- Lite Curation Steps
- Structure Fragmentation
- Fragment Structure Annotation
- Spectra Recalibration

# myLibrary Enterprise – cloud based spectral library building platform

**myLibrary** Data

- Compounds
- Files
- Processing
  - Tree Builder
  - Spectral Trees
  - Curation Workflows
- Libraries
  - Manage Libraries
  - Spectral Libraries
  - Library Search
- Settings
  - Metadata Schema
  - Ion Species

## Manage Libraries

Name	Owner	Assigned to	Library Type	no. records	
Test123	sayonkumar.ghosh@t...	Testers	Reference Manual	2	
SS5301	suncerae.smith@high...	Testers	Reference Manual	0	
Ref Test	sayonkumar.ghosh@t...	Testers	Reference Manual	1	
Caroline's Putative Library	caroline.ding@thermo...	Product Owners	Putative Manual	11	
Put Test	sayonkumar.ghosh@t...	Testers	Putative Manual	1	
Azaspiracid -New	akhil.p@thermofisher...	Testers	Reference Manual		
Fluoro	suncerae.smith@high...	Testers	Reference Man		
Caroline's Ref Library	caroline.ding@thermo...	Product Owners	Referen		
Aceclofenac	akhil.p@thermofisher...	Testers			
User C					
Load					

Library settings overview

Name: Test123  
Type: Reference  
Allow access for: Testers  
Owner: sayonkumar.ghosh@thermofisher.com  
Number of Records: 2  
Number of Spectral Trees: 2  
Searching:  
By Identifiers: Index Ready 4:27 AM  
By Name: Index Ready 4:27 AM  
By Metadata: Index Ready 4:27 AM  
By Similarity: Index Ready 4:27 AM  
By Identity: Index Ready 4:27 AM

- Add Reference or Putative libraries
- Export and Import libraries:
  - mzVault .db
  - NIST .MSP

# Sample preparation

Based on USFDA Method C-010.01

Step	Action
1	Weigh 5g ground pork sample into a 50 mL polypropylene (PP) centrifuge tube
2	Add isotopically labeled PFAS compounds (500 ppt)
3	Add 5mL UHPLC-MS Ultra Pure Water (P/N W8-1) to the 50 mL PP conical centrifuge tube
4	Add 10 mL acetonitrile (Ultra Pure grade P/N A956-1) to the centrifuge tube
5	Add 150 µL Formic Acid, 99% Ultra-Pure LCMS Grade
6	Vortex for 2 minutes, then add a QuEChERS salt packet (Thermo Fisher Product #60105-210 with 6000 mg MgSO <sub>4</sub> and 1500 mg C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub> )
7	Place on benchtop shaker at 1500 rpm with pulse set to 70 for 5 minutes
8	Centrifuge for 5 minutes at 10000 rcf
9	Add 6 mL supernatant to 15 mL PP conical centrifuge tube with dSPE sorbent (Thermo Scientific #60105-205 900 mg MgSO <sub>4</sub> , 300 mg PSA, 150 mg graphitized carbon black)
10	Vortex/shake for 2 minutes; Centrifuge 5 min at 10000 rfc
11	Transfer 300 µL to a PFAS free polypropylene vial with cap and septa (Thermo Scientific #C4015-100)
12	Add 50 µL Ultra Pure Water, vortex, and place in A/S ready for injection

- Additional SPE clean-up step with a polymeric weak-anion exchange column was not required (recommended in FDA method)
- A contamination study was performed on combinations of reagents, containers, and solvents used in the study.

DSPE rgts+ MeCN+H2O+FA	MeCN+H2O+FA+tube	MeCN+H2O+FA +tube		
DSPE Tube+Reagents	ExtSolventBlank	ExtSolventBlank	MeCN (pure)	Water (pure)

- PFBA and PFOA were detected above 5 ppt. PFBA was present in all solvent blanks and reagents; PFOA was present primarily in the dSPE cleanup tube with reagents.

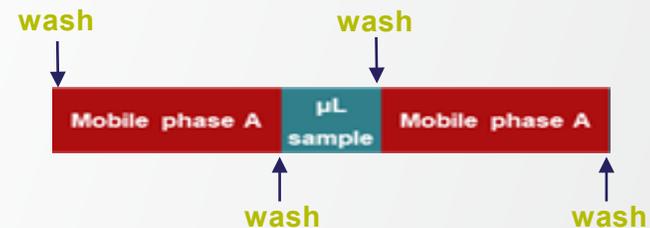


# LC Setup for sandwich injection on Thermo Scientific™ Vanquish™ Flex UHPLC System

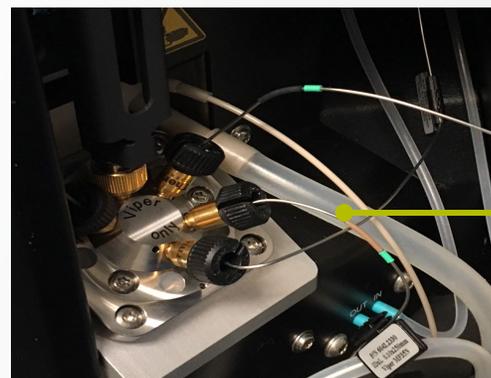


## Custom Injection Program

- Sample loop: **100  $\mu\text{L}$**
- Sandwich injection
- In needle mixing
- Polypropylene A/S vials



Inj. Volume	15 $\mu\text{L}$ sample with 2 plugs 30 $\mu\text{L}$ MF A
Col Temp. and Flowrate	40 C; 400 $\mu\text{L}/\text{min}$
Analytical Column	Thermo Scientific™ Accucore™ C18, 100 x 2.1 mm, 2.6 $\mu\text{m}$
Trap Column	Thermo Scientific™ Hypersil Gold™ C18, 50 x 4.6 mm, 1.9 $\mu\text{m}$
Run Time	19 minutes
Mobile Phase A	5mM Ammonium Acetate in H <sub>2</sub> O
Mobile Phase B	Methanol

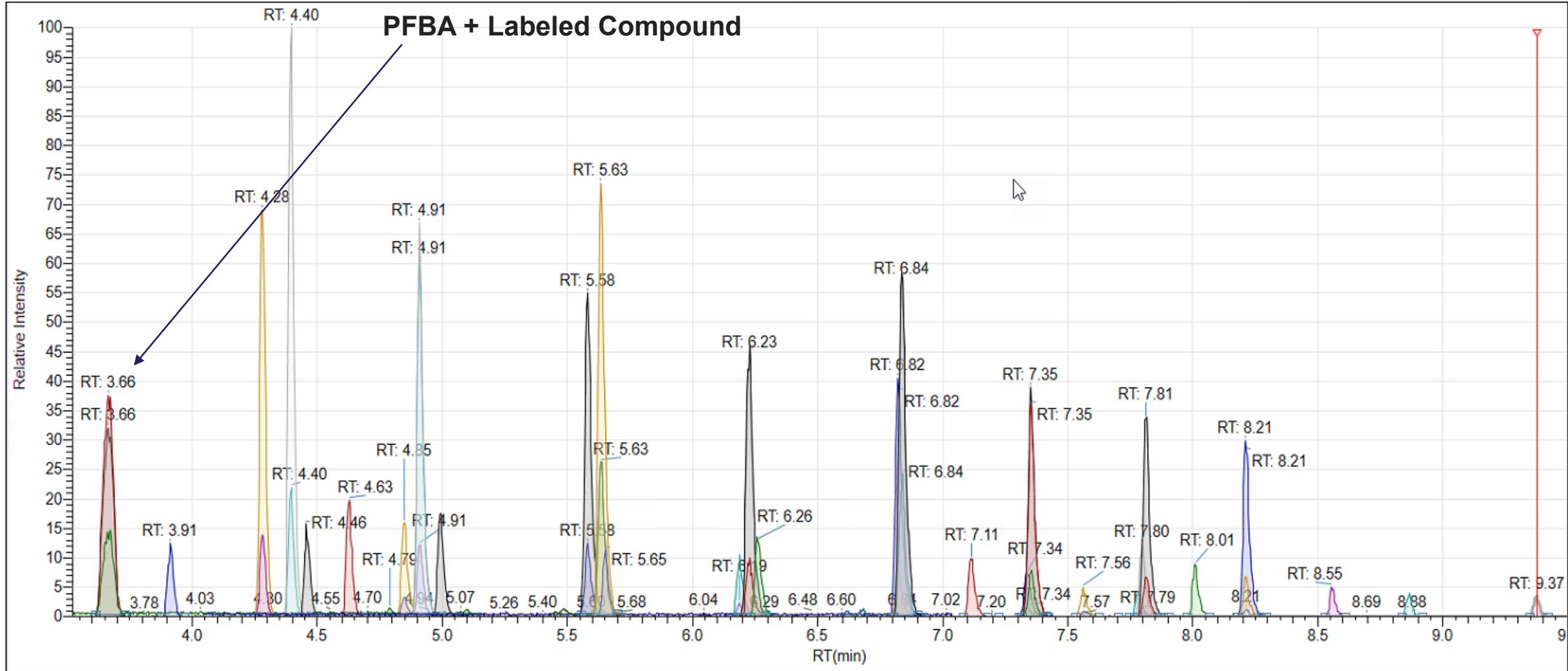


## Capillary

- Sampler valve to column
- Viper capillary 0.18 mm ID x 350 mm

# Optimized peak shapes

Extracted full scan precursor ions of calibration standard 100 ppt



# Orbitrap Exploris 120 MS settings and workflow



Spray voltage	1.0 kV
Sheath gas	35 arb
Aux gas	5 arb
Sweep gas	1 arb
Capillary temp.	220 °C
Vaporizer temp.	450 °C
Ion polarity	Negative

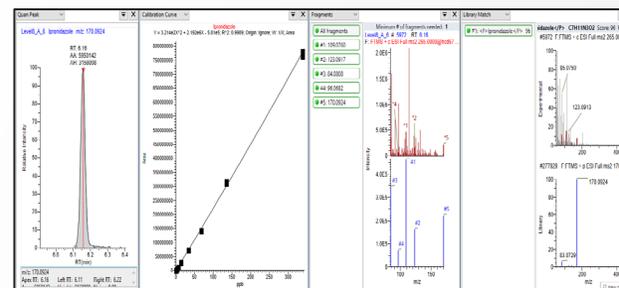
Full scan range	100-1000 m/z
Full scan resolution	60,000
MS2 resolution	15,000
HCD collision energy:	Stepped 10,50
RF Lens	50
DIA m/z windows	5 @ 200 m/z

## Screening and Quantitation

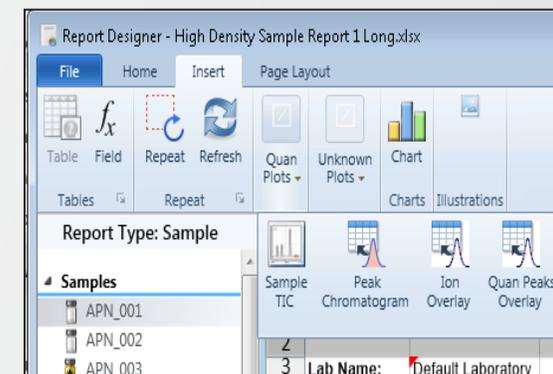


### Data Independent Acquisition (DIA)

- No target list
- Precursor isolation windows w/ stepped NCE
- MS2 triggered across entire peak



## Reporting



## Fragment Match and Library Search

# Calibration

## Prepared in Solvent with Isotopically Labeled Standards

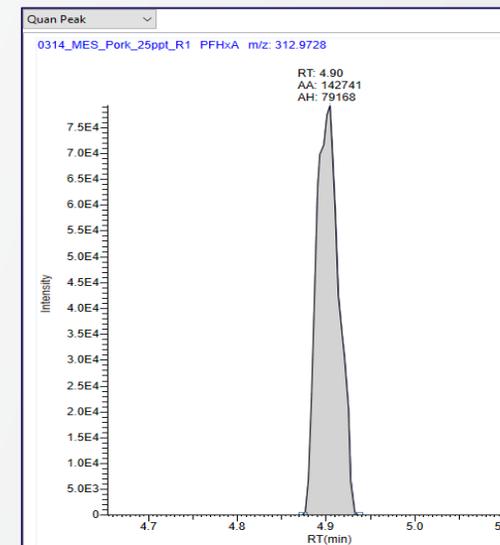
- 34 Target PFAS Compounds + 23 labeled analytes
- Cal Range for most analytes: 5- 5000 ppt (in-vial concentration)
- Standards prepared in neat solvent matching the initial QuEChERS extraction solvent composition (70:30 MeCN:H<sub>2</sub>O + 1% Formic Acid).
- Branched and linear isomers of PFOS and PFHxS were summed together.
- Some labeled compounds were not available for certain targets during the development of the method. In those cases, either an external standard calculation method was used, or another labeled compound was used
- r<sup>2</sup> range: 0.9516 to 0.9993, with Calibration Average % RSDs < 7 % for all compounds

# Recovery experiments

## Pork Muscle Meat

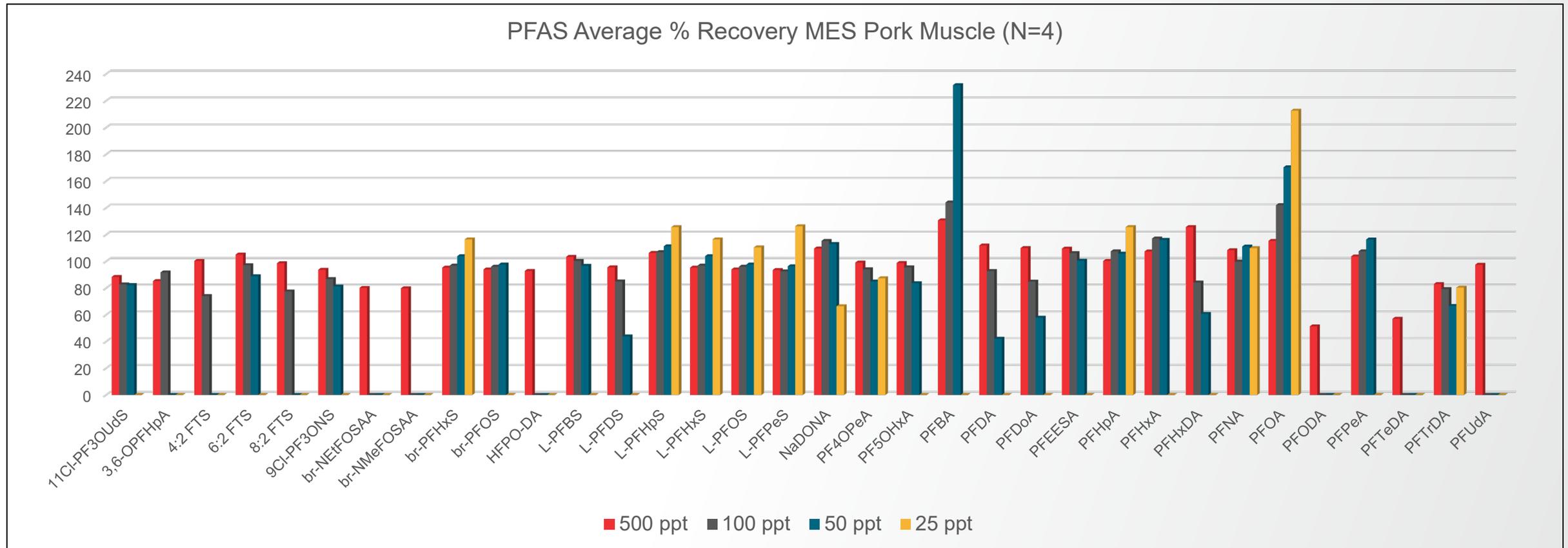
- Biological replicates (N = 4) of ground pork meat samples were spiked with PFAS labeled compounds along with native analytes and taken through the entire extraction and cleanup process (Matrix Extracted Spikes-MES).
- The labeled analytes were spiked at 500 ppt, and the replicates (N=4 at each concentration) had native PFAS levels of 25, 50, 100, and 500 pg/g).

Spike Concentration, pg/g	Final Extract Concentration (ppt)
25	8.3
50	16.7
100	33.3
500	167



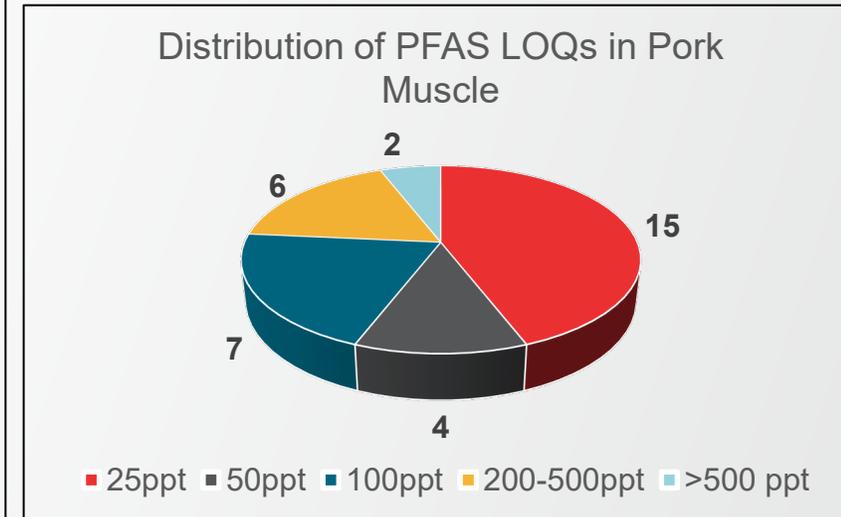
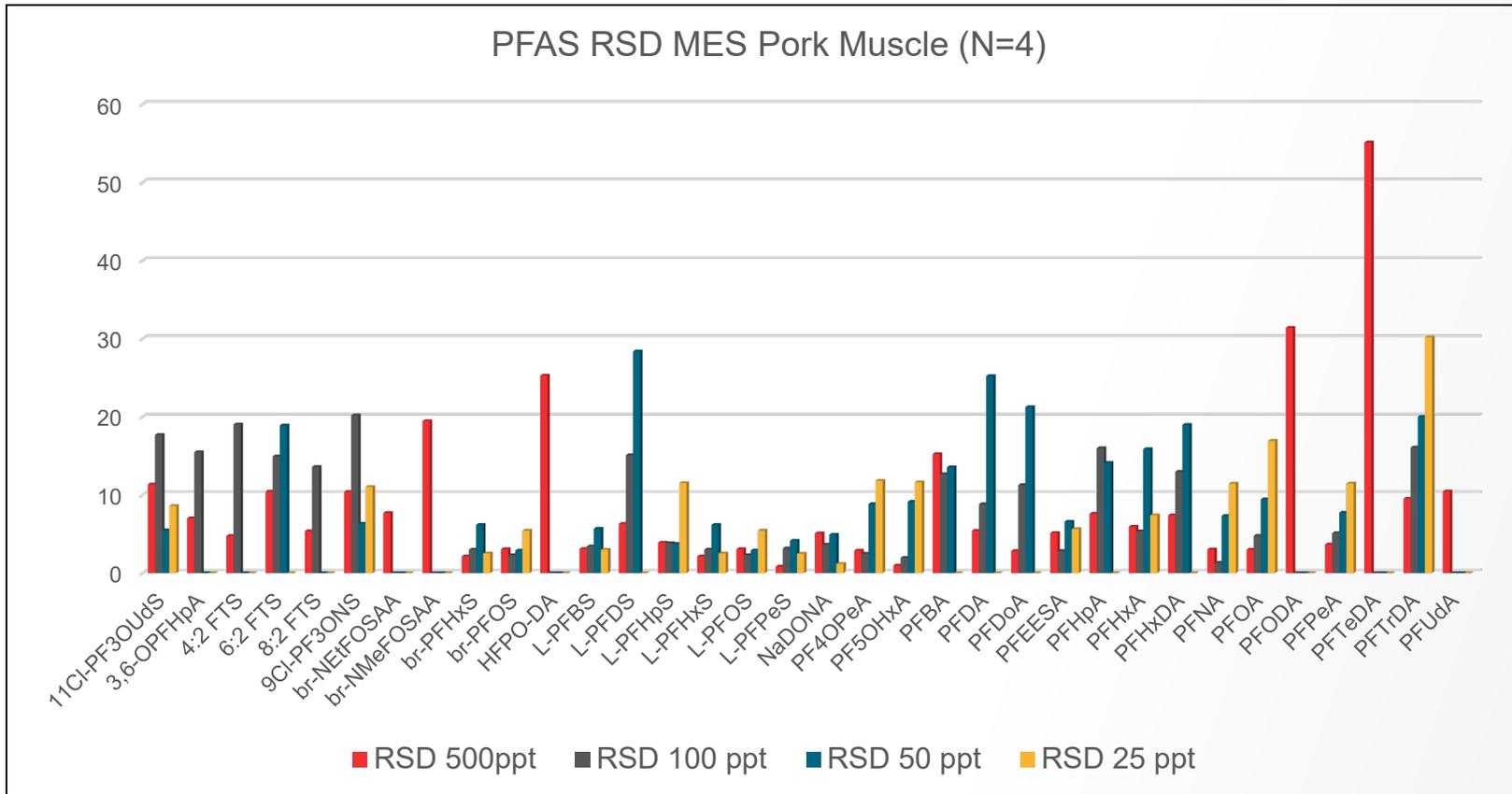
Extracted ion chromatogram for  
PFHxA 25 ppt MES

# Recovery experiments



- Most recoveries between 80-120%
- PFOA and PFDA had high biased recoveries due to reagent contamination
- Some analytes exhibited poor recovery especially at 25 and 50 ppt, could be due to absorption on GCB for the longer chain PFAS

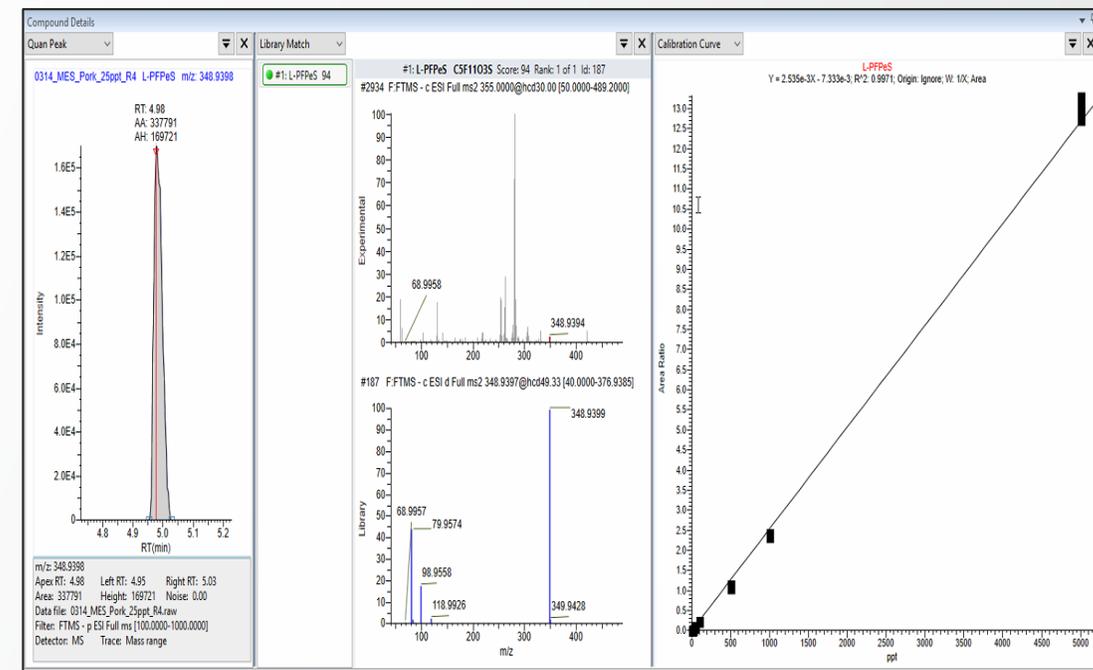
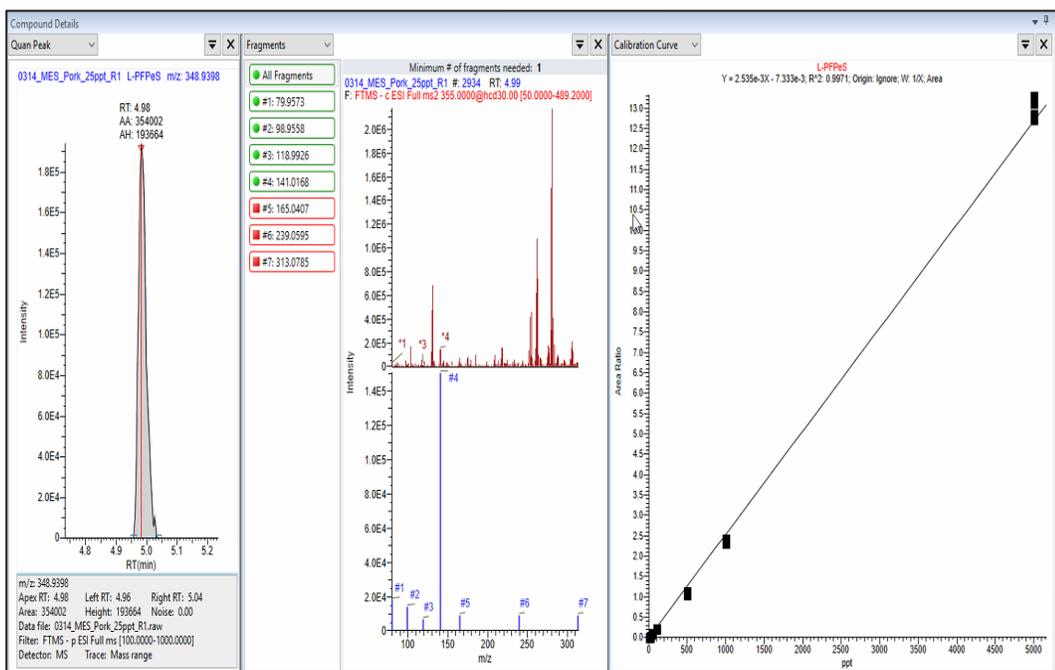
# % RSD and estimated LOQs in pork meat



- Most % RSDs are below 20%
- Higher RSDs (>30%) for PFTeDA and PFODA- also exhibited poor recovery
- Most LOQs observed to be  $\leq$  100ppt

# Identification and library search

- A detected and confirmed analyte is defined as the native PFAS precursor ion detected at <5 ppm mass accuracy with  $S/N \geq 3$ , AND at least one MS2 fragment detected at < 5 ppm.
- A spectral library search result also adds confidence in the confirmation process, using the spectral library created in myLibrary Enterprise and exported to mzVault for use in Thermo Scientific™ TraceFinder™ software.



The left plot shows the fragment ions match for L-PFPeS at 25 ppt in the pork meat MES. The plot on the right is a library search result vs. the user created mzVault spectral library for the same spike level.

- The Vanquish Flex UHPLC system and using solvent sandwich injection technique coupled to the Orbitrap Exploris 120 Mass Spectrometer provided excellent quantitative sensitivity with qualitative confirmation in FS-DIA mode, with most PFAS LOQs in pork meat matrix less than 50 pg/g (16.7 ppt in final extract), without the need for further extract concentration.
- myLibrary Enterprise allows users to easily create highly curated spectral libraries for added confidence in confirmation- and the ability to expand screening and share libraires across organizations.
- The method was shown to be fit-for-purpose and may be explored for future expansion into other food matrices. Further work at USFDA is on-going to improve method performance and expand the scope
- Orbitrap HRAM will always have a clear value proposition in the lab as more complex matrices are being addressed for PFAS analysis (i.e. cosmetics, food contact materials, tissues, plasma, etc.), as well as ability to look for non-targeted PFAS in retrospective analysis

# Tell us how we did

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Together with your pad you will find a survey form.  
Share your comments about today's event, this will help us to improve in the future.

Return your survey at the exit and **you will receive an apron as a thank you gift.**



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What other topics would you like to see presented?							
See reverse side to let us know of any follow-up you would like to receive							

Chromatography & Mass Spectrometry Division - North America and Europe Form - Last Updated May 28, 2022

# Thank you

