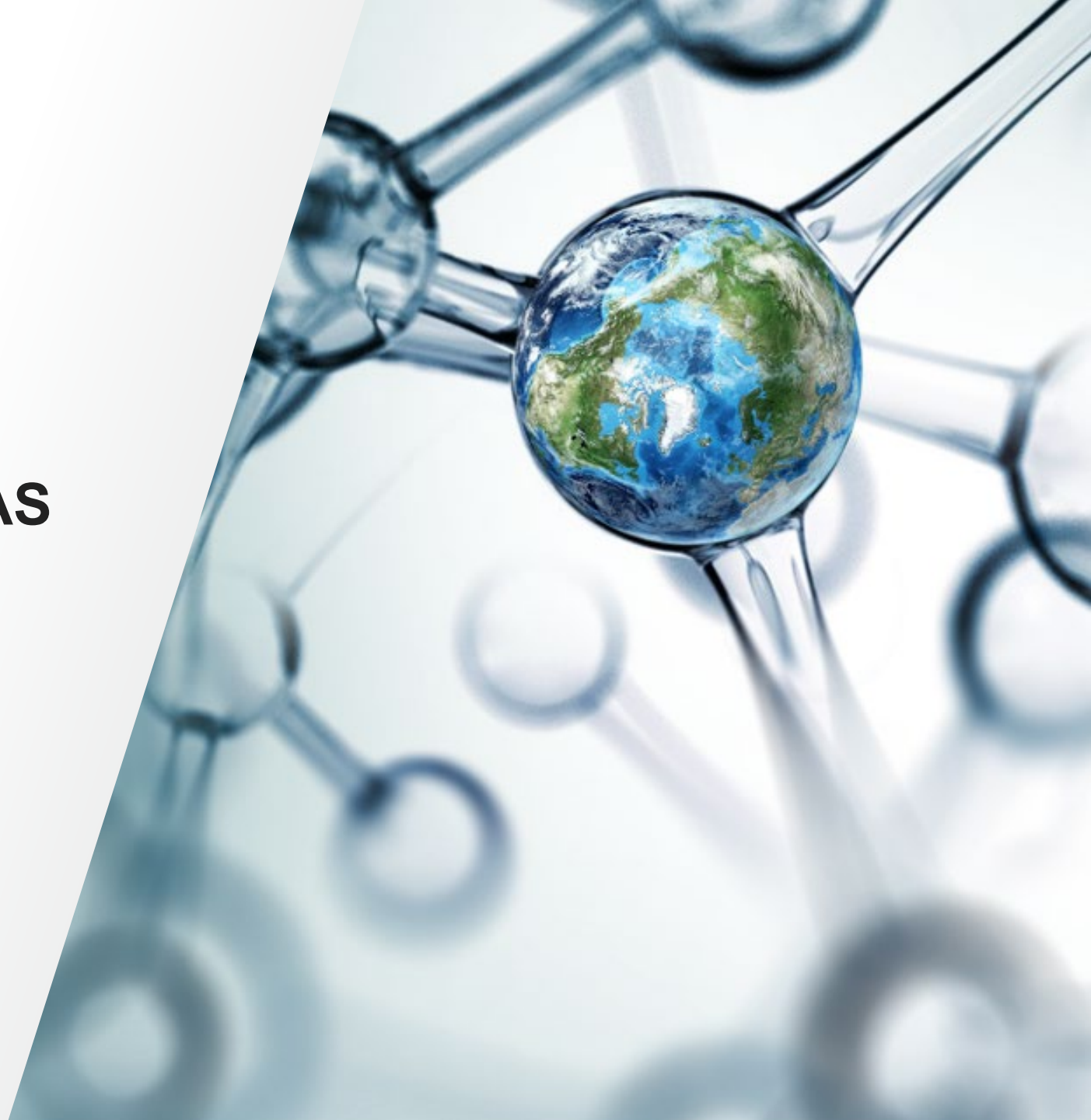
Implementing new GC-MS and LC-MS technologies to stay ahead with your food safety analysis from pesticides to PFAS and microplastics

Frans Schoutsen

Environmental, Food and Beverage Support

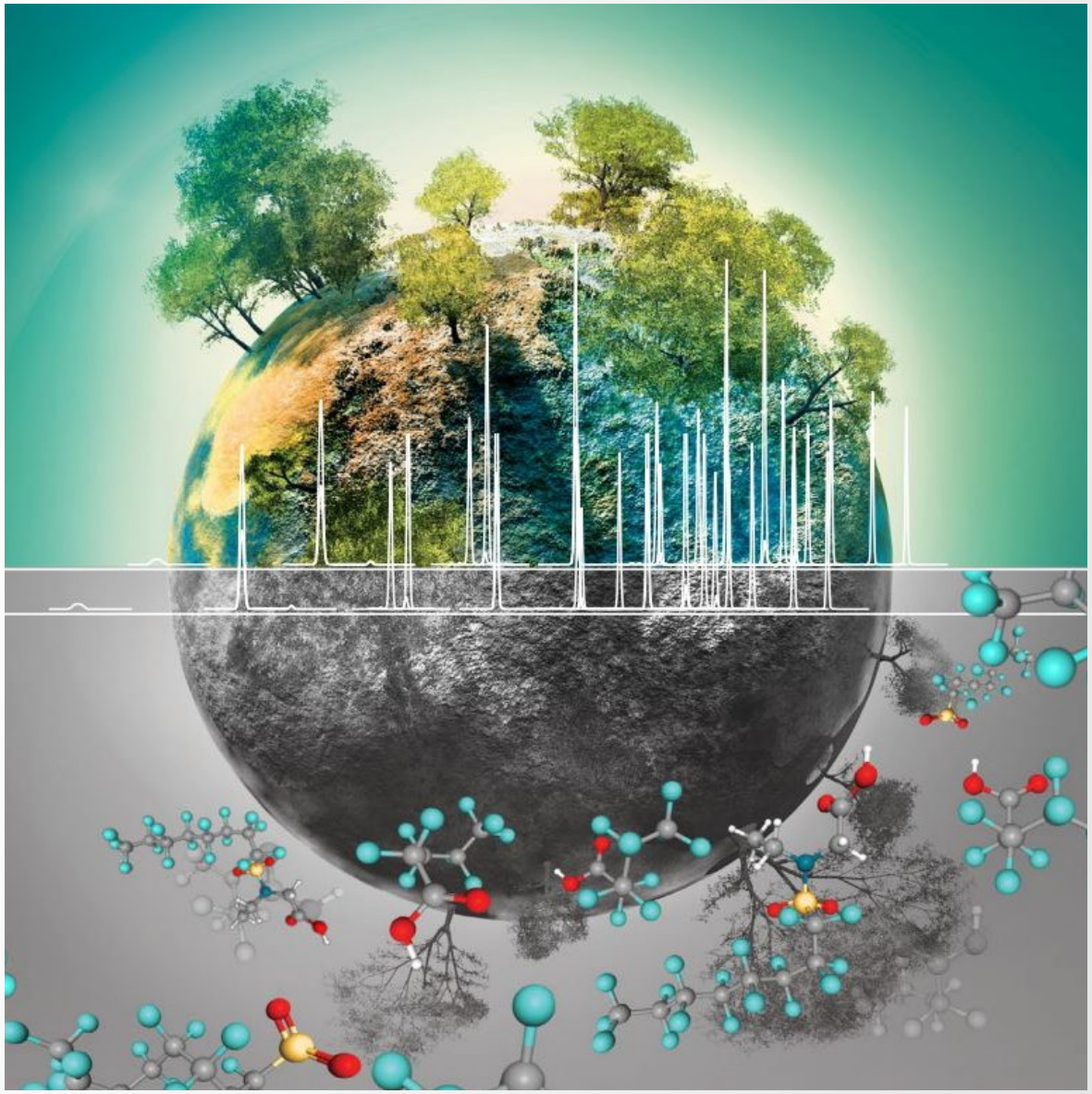
10th International Symposium on Recent Advances in Food Analysis, September 6-9, 2022



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



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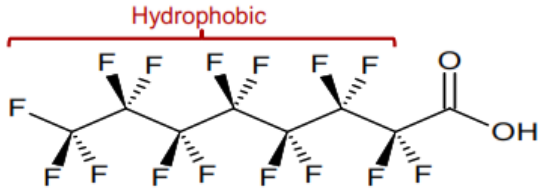
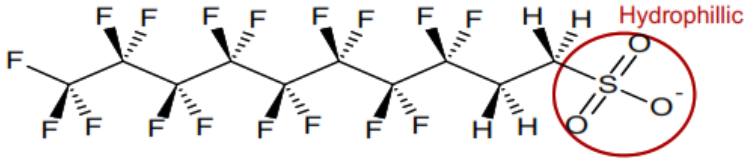
What are PFAS compounds?

What are PFAS?

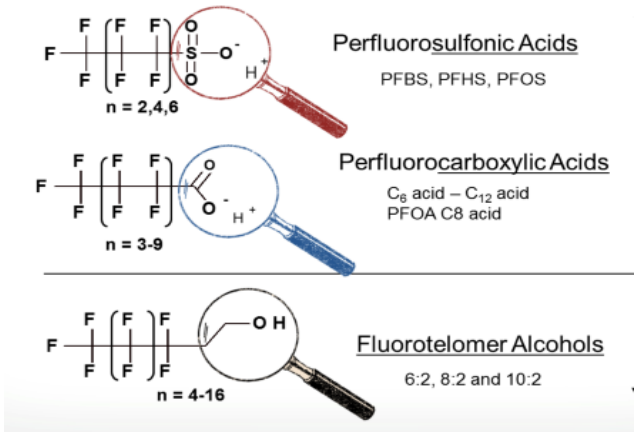
- PFASs are **P**er- and **P**oly**F**luorinated Alkyl **S**ubstances. Exclusively anthropogenic.
- Structures contain a hydrophobic perfluoroalkyl backbone and a hydrophilic end group
- Include a diverse range of compounds with a variety of chain lengths and end groups



Perfluorooctanoic acid
- PFOA
- Teflon®

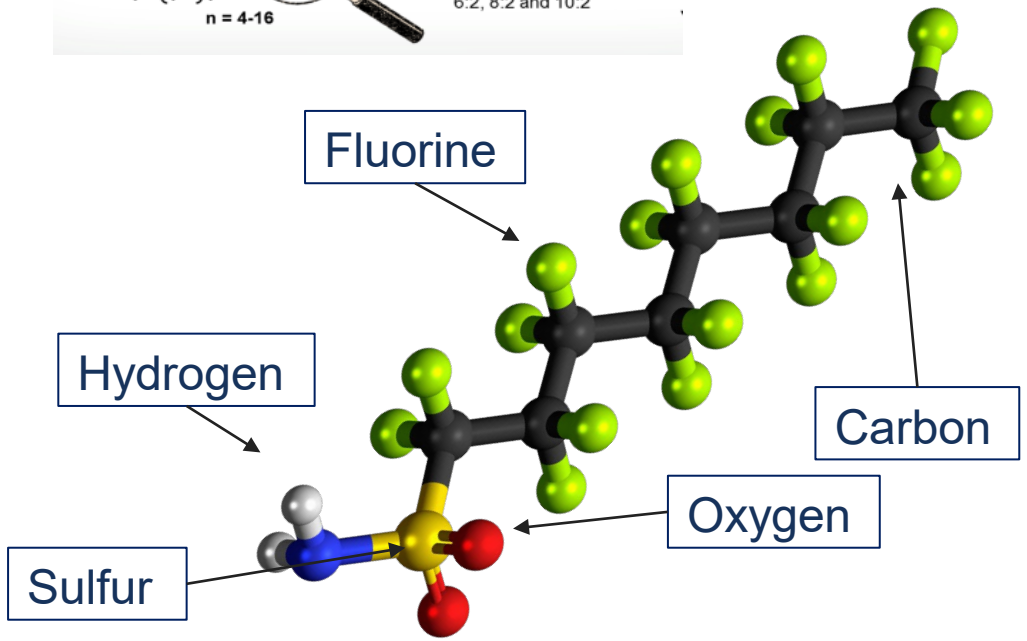


8:2 Fluorotelomer sulfonate
- 8:2 FTS



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



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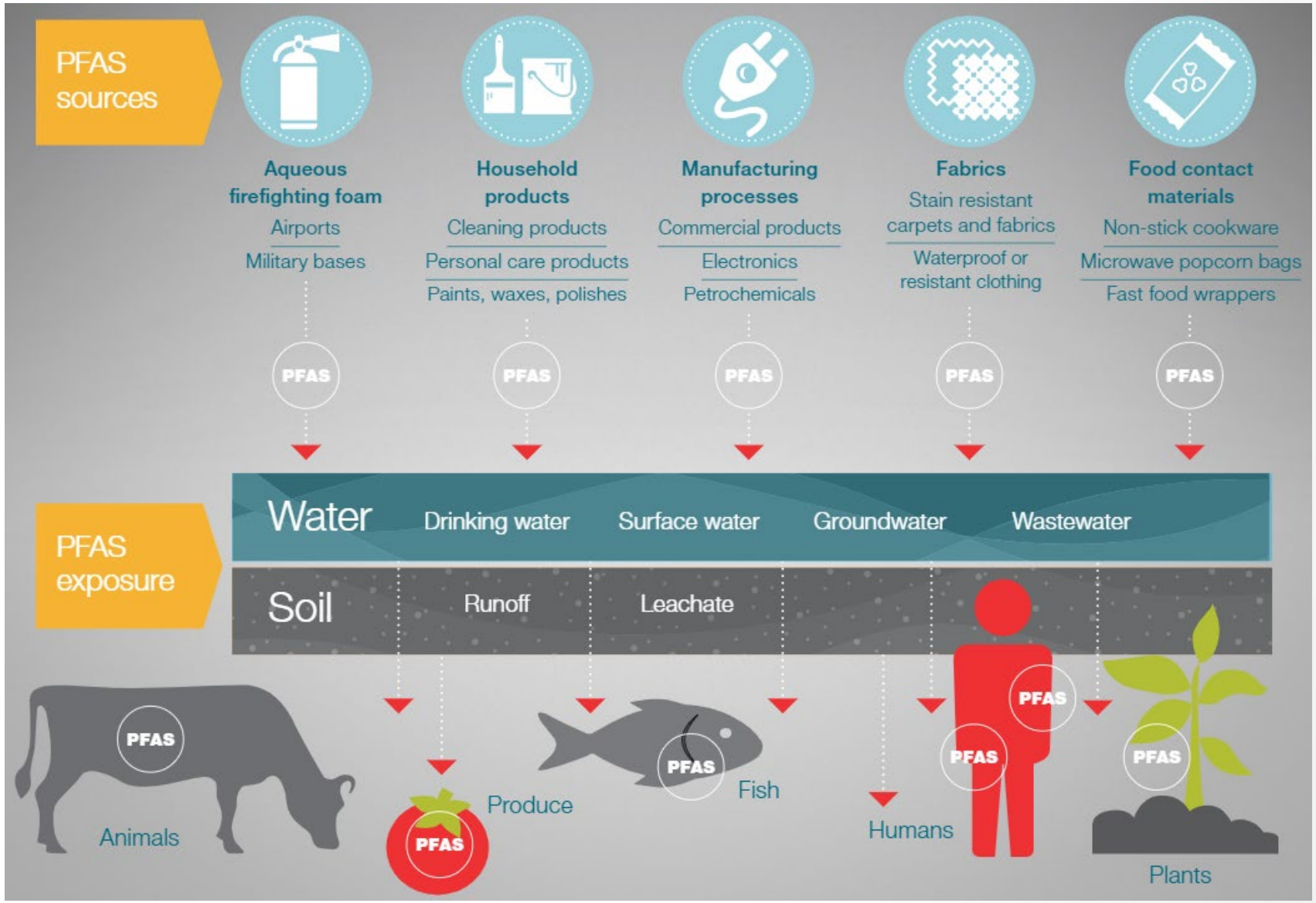
How does PFAS enter the environment?



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

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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

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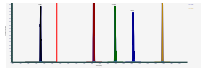
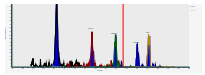
TSQ separation of perfluoronated alkyl substances, 50 pg/mL



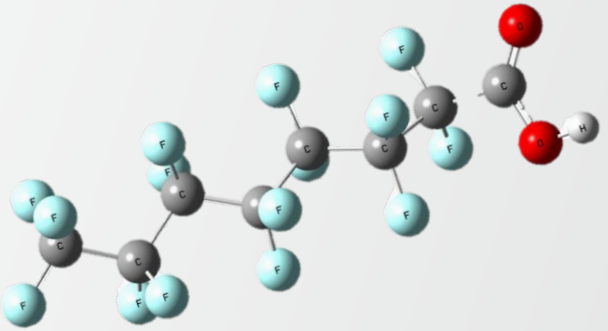
PFASs C4 – C10 at 50 pg/mL (0.15 pg on-column); LODs are ~10 pg/mL (0.03 pg O.C.)



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

Thermo Scientific™ Vanquish™ Flex UHPLC System

Delay Column: 3.0 x 50 mm, 5 um BDS Hypersil C8
Analytical Column: 2.1x100 mm, 2.6 um Accucore C18
Column Temp: 30 C
Mobile Phase: [A] H_2O + 10 mM Am. Acetate; [B] MeOH
Injection Volume: 3 uL



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)

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For Research Use Only

TSQ portfolio

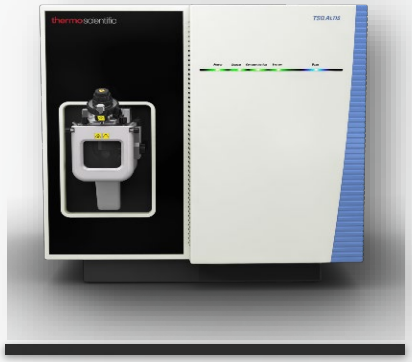
PERFORMANCE

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***

VALUE

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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.

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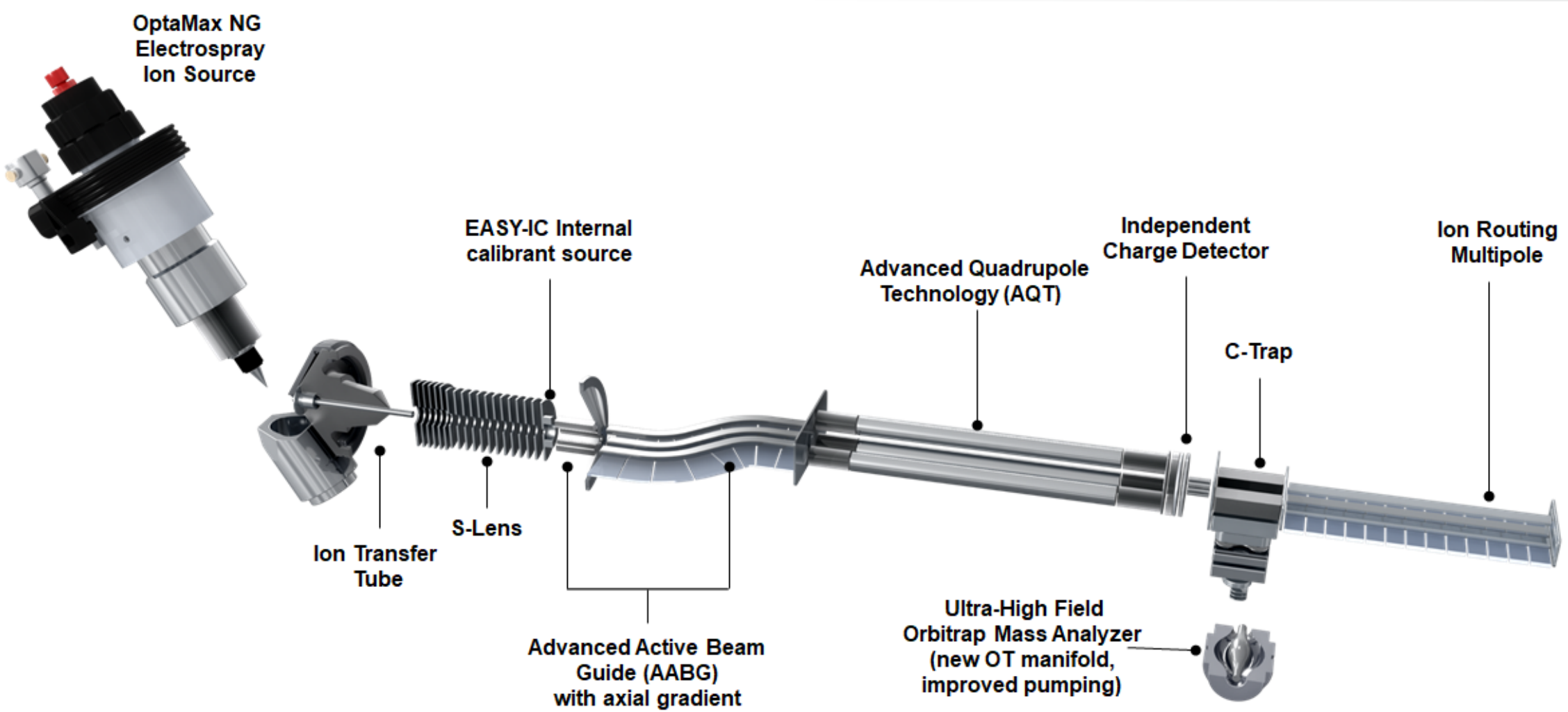
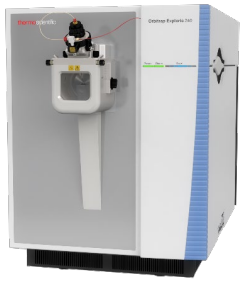
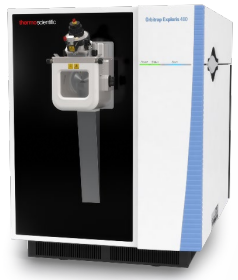
Orbitrap Exploris mass spectrometer schematic

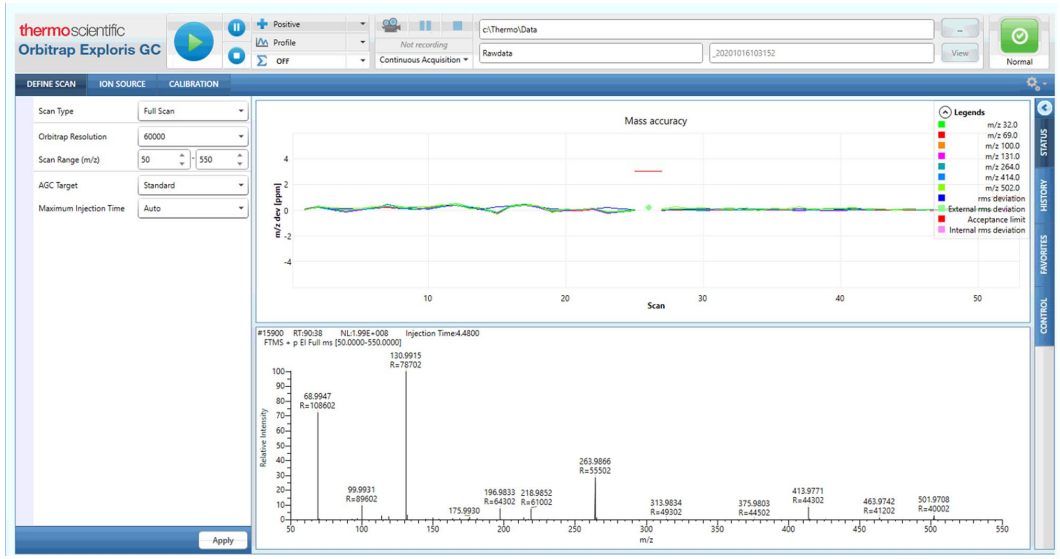


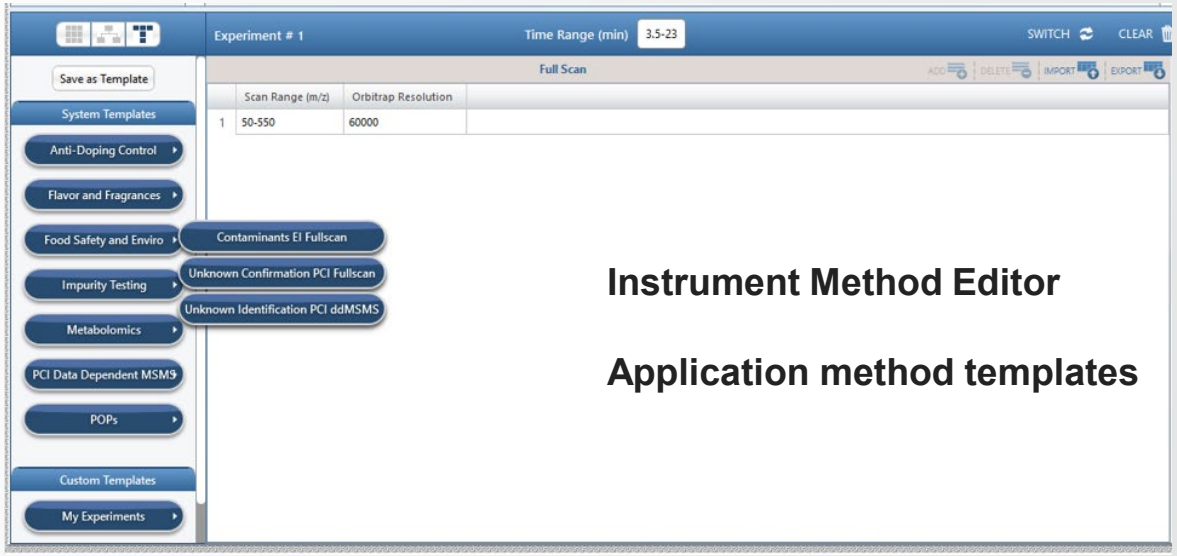
One instrument series control software (GC- and LC)

A user of one becomes a user of all systems


Thermo Scientific™ Orbitrap Exploris™ Mass Spectrometers





Instrument Method Editor

Application method templates

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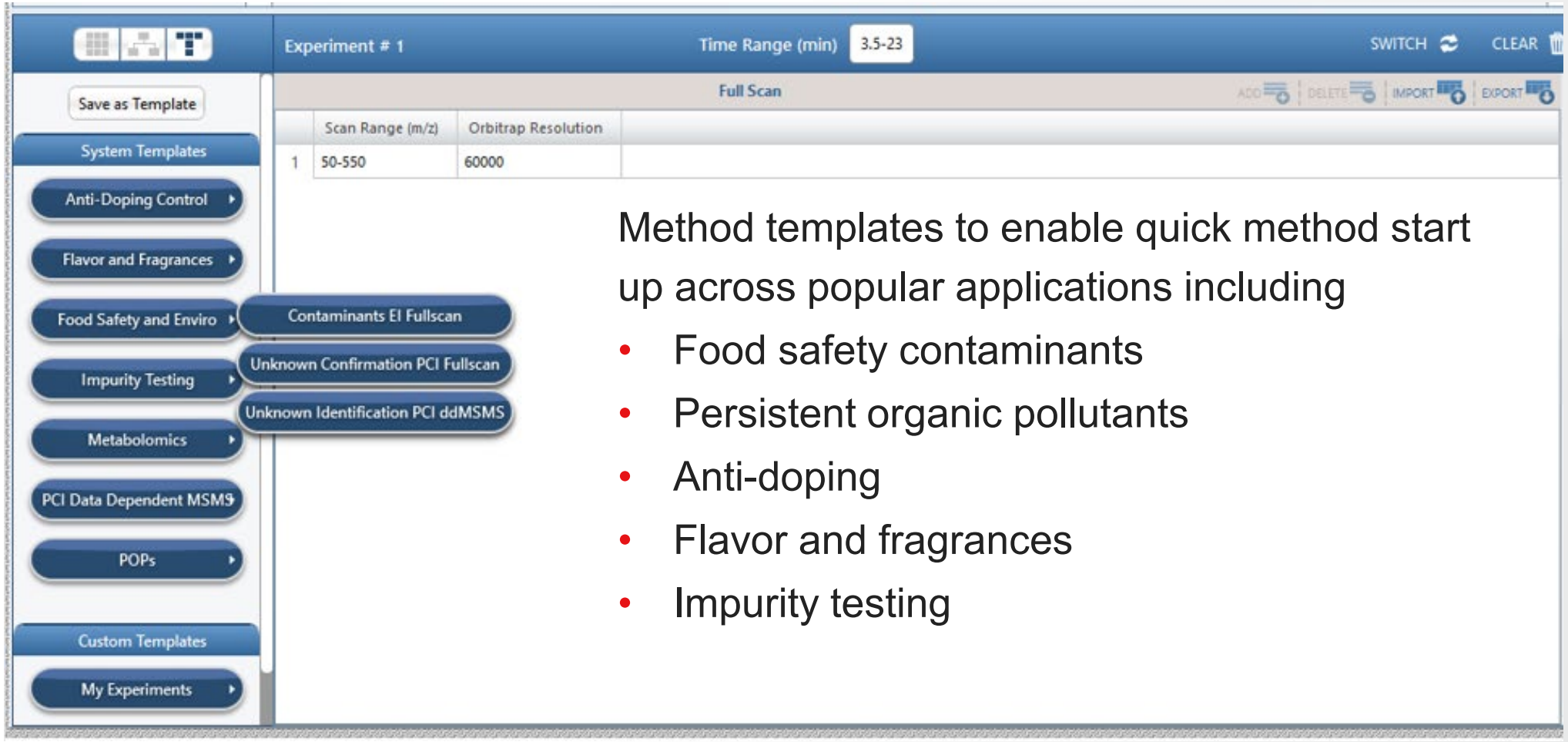
Simplified drag and drop method editor and templates

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



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

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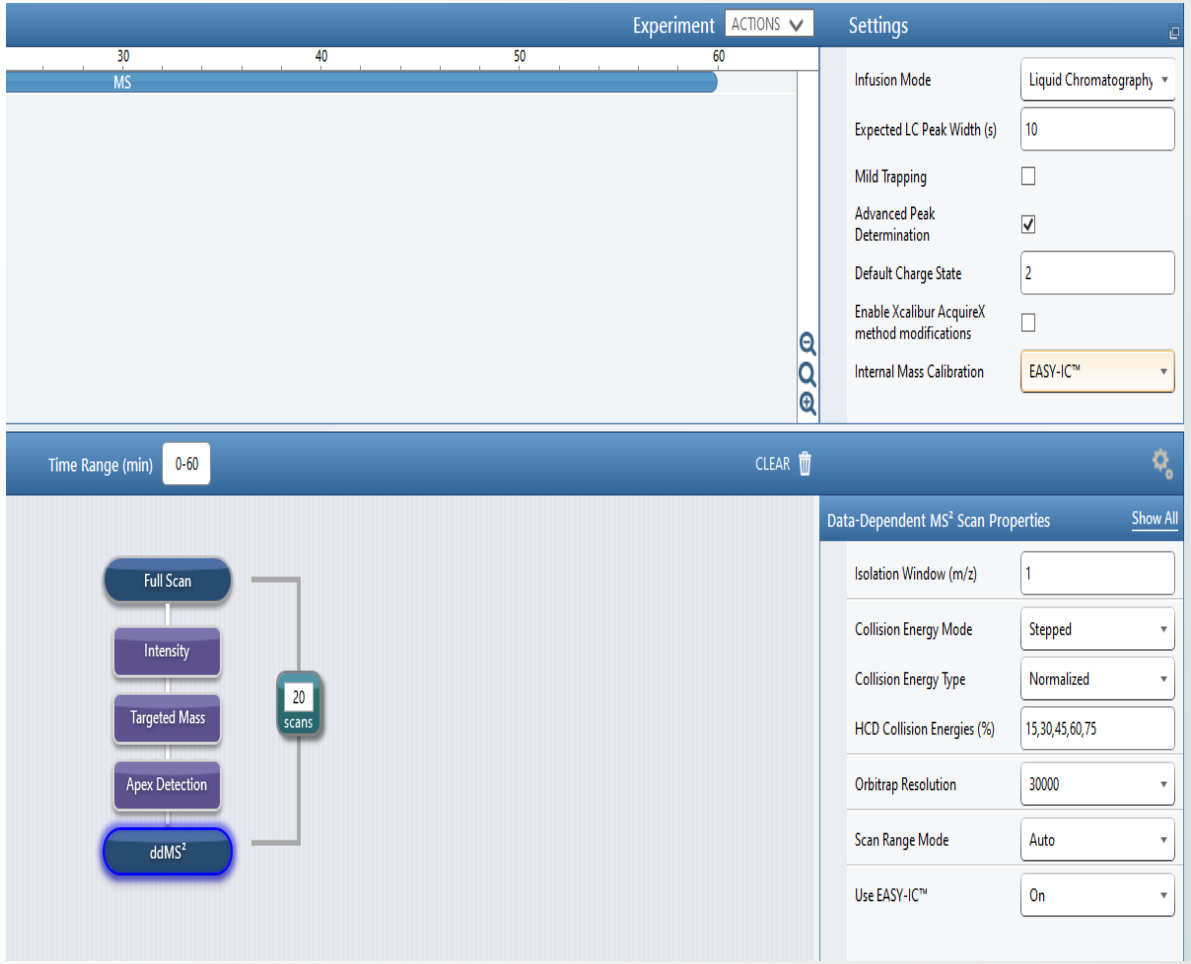
Instrument editor Orbitrap Exploris options



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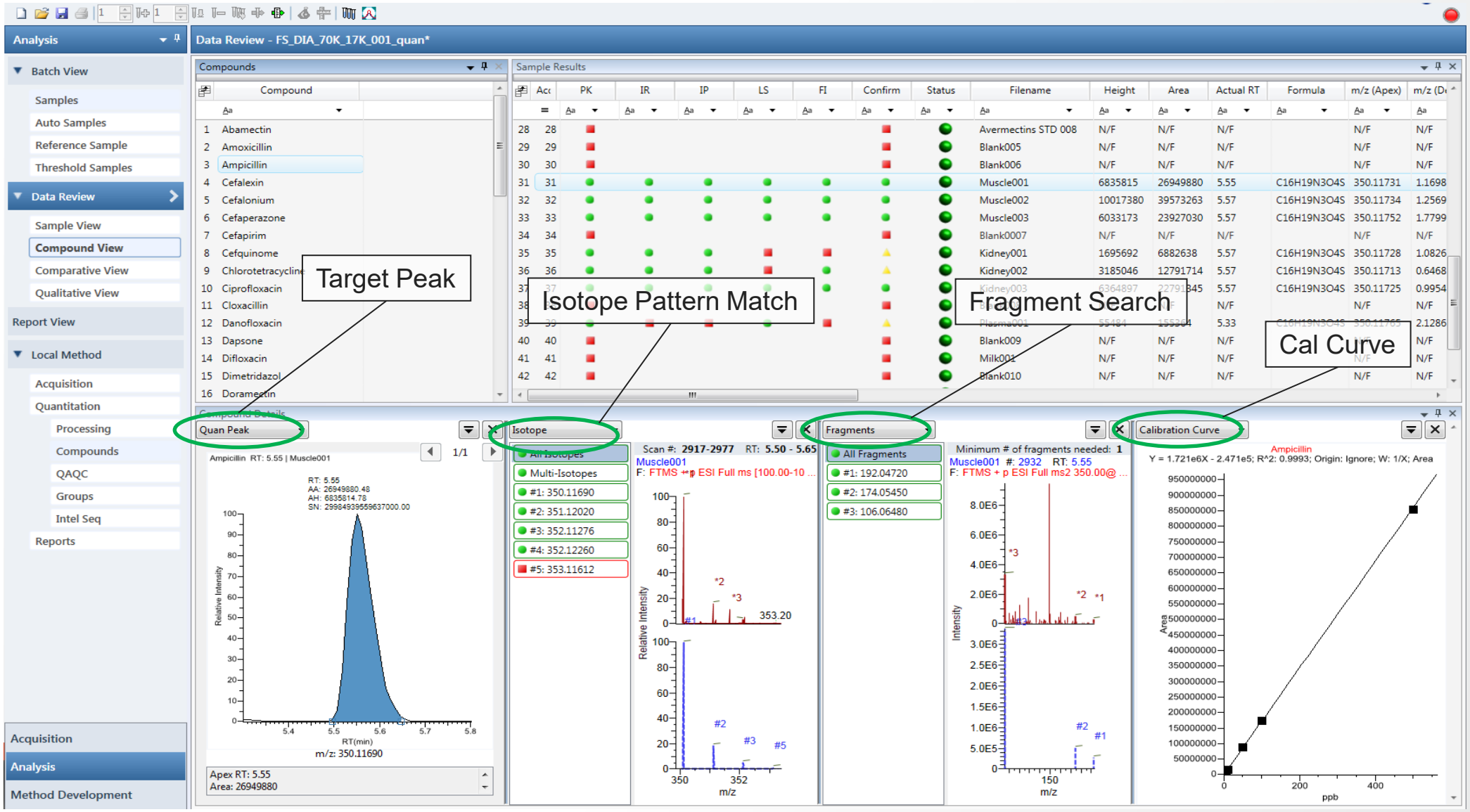
Ampicillin @ 10 ppb in pig muscle



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.

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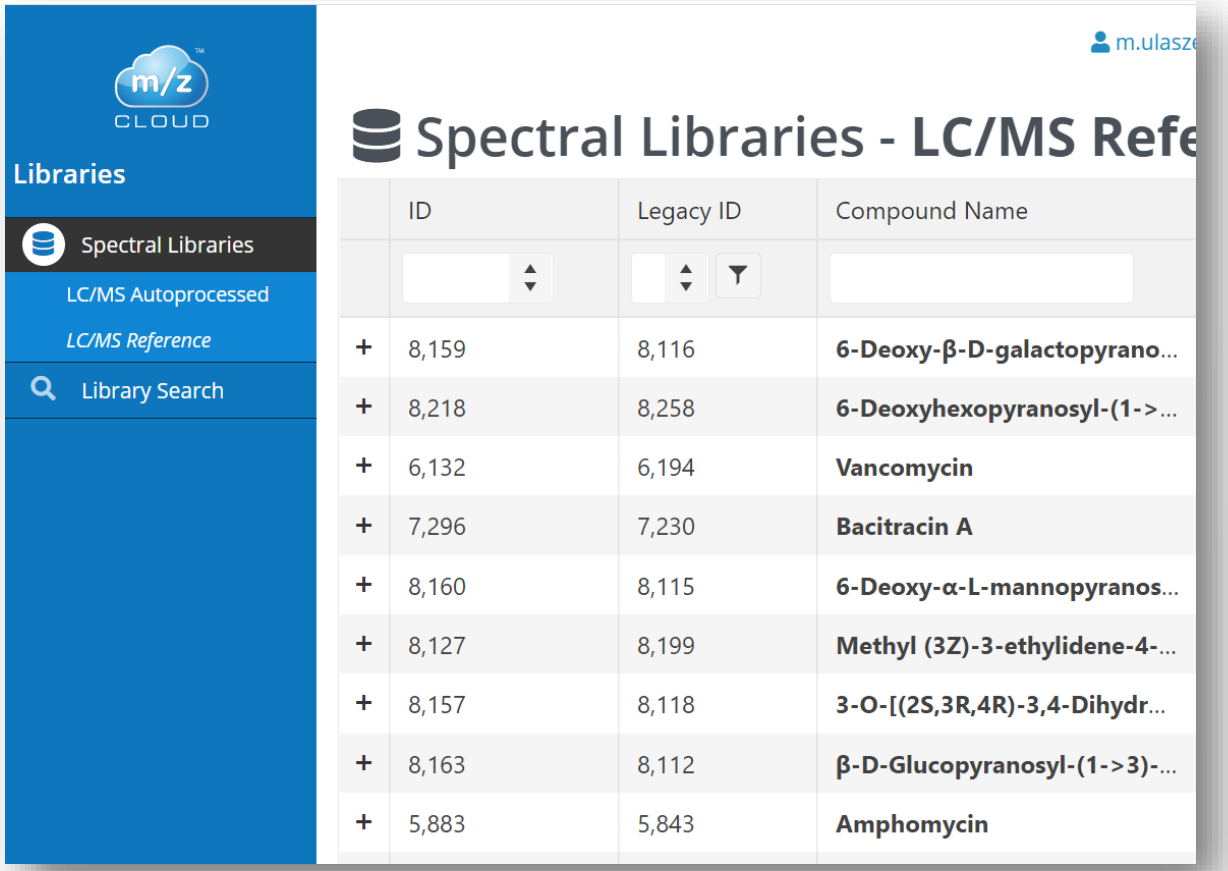
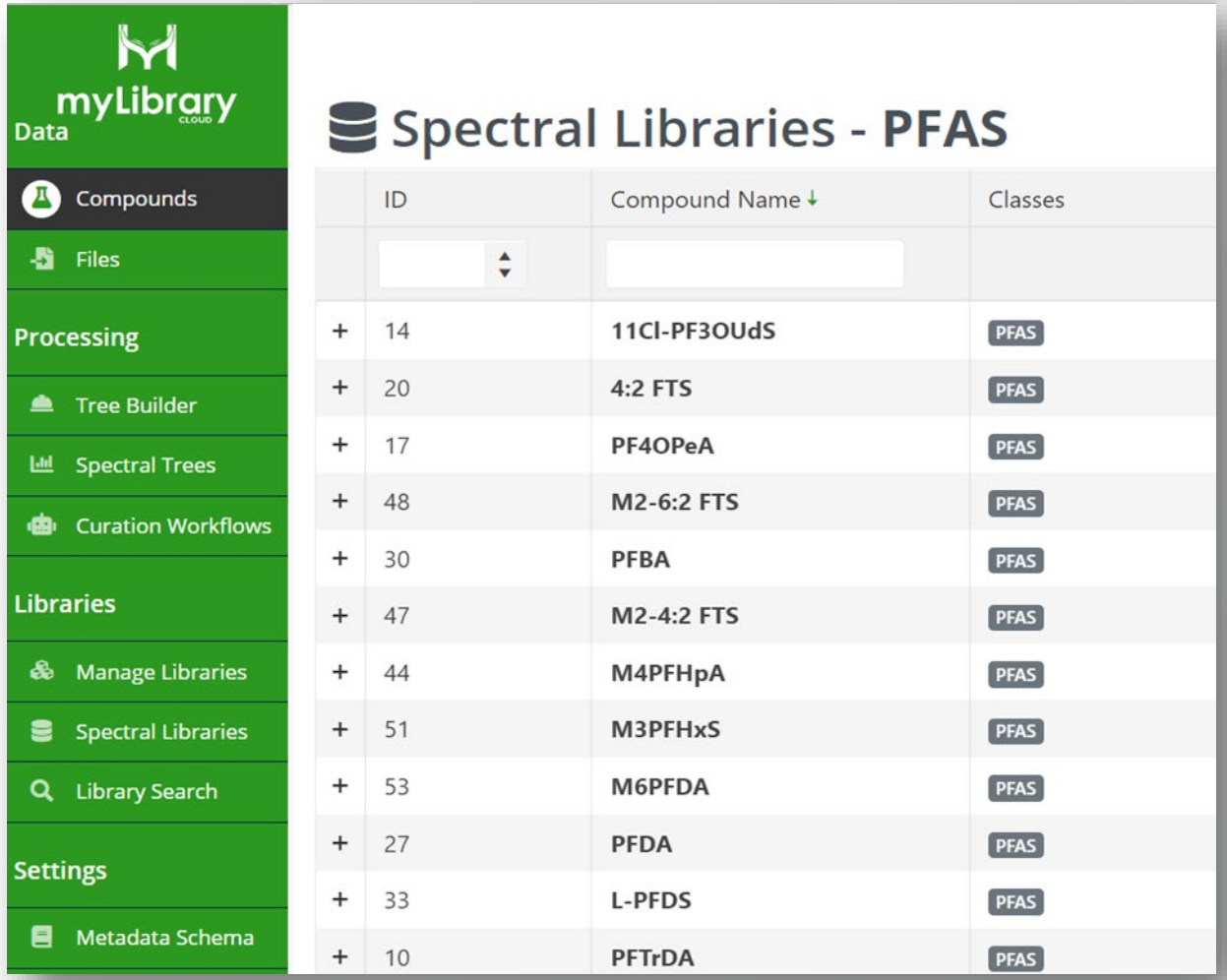
myLibrary Enterprise was inspired by mzCloud

Thermo Scientific™ m/z Cloud™ Library
https://mzcloud.org

https://YourCompanyName.mylibrary.thermofisher.com





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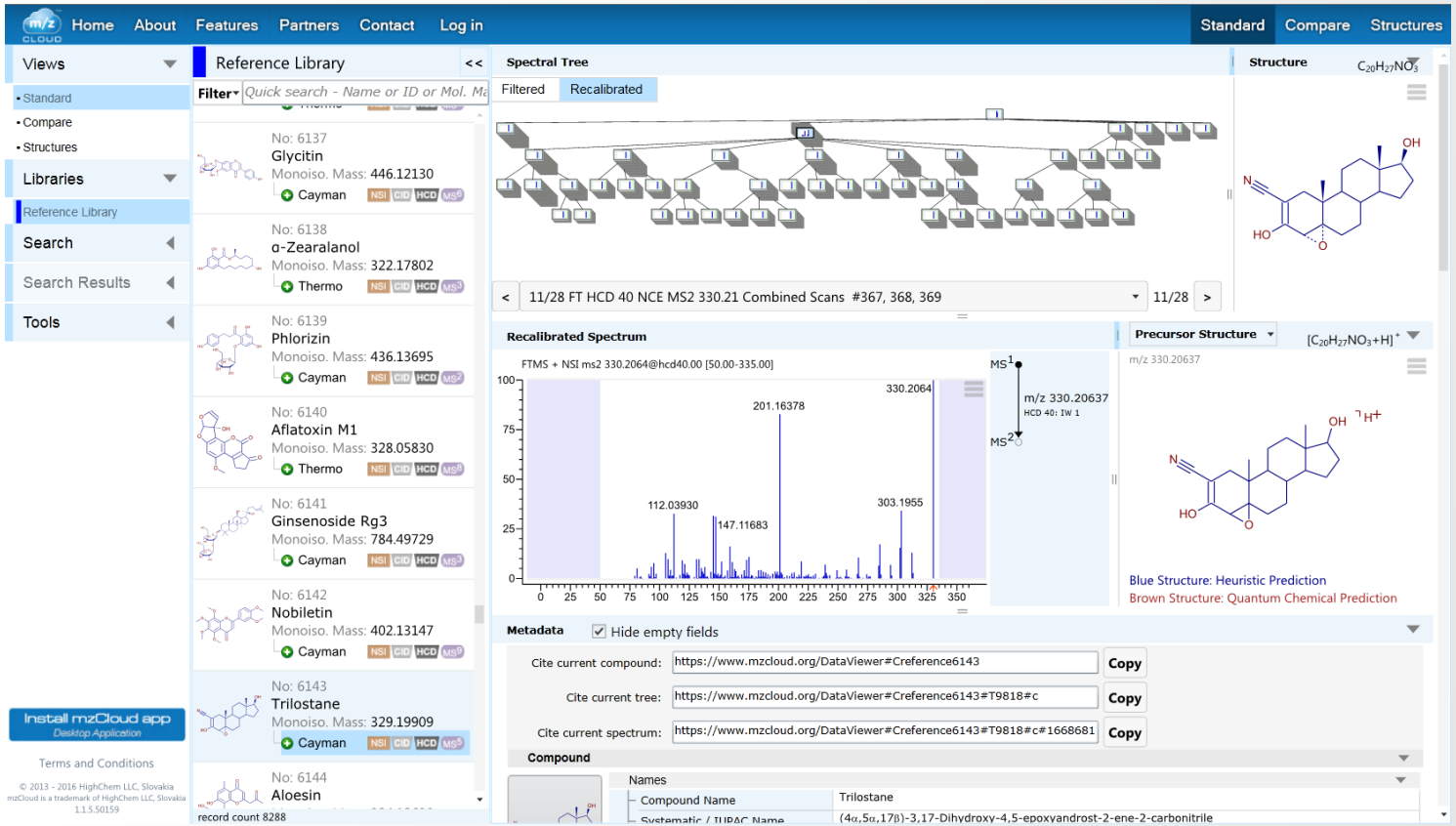
What is mzCloud Library?

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



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

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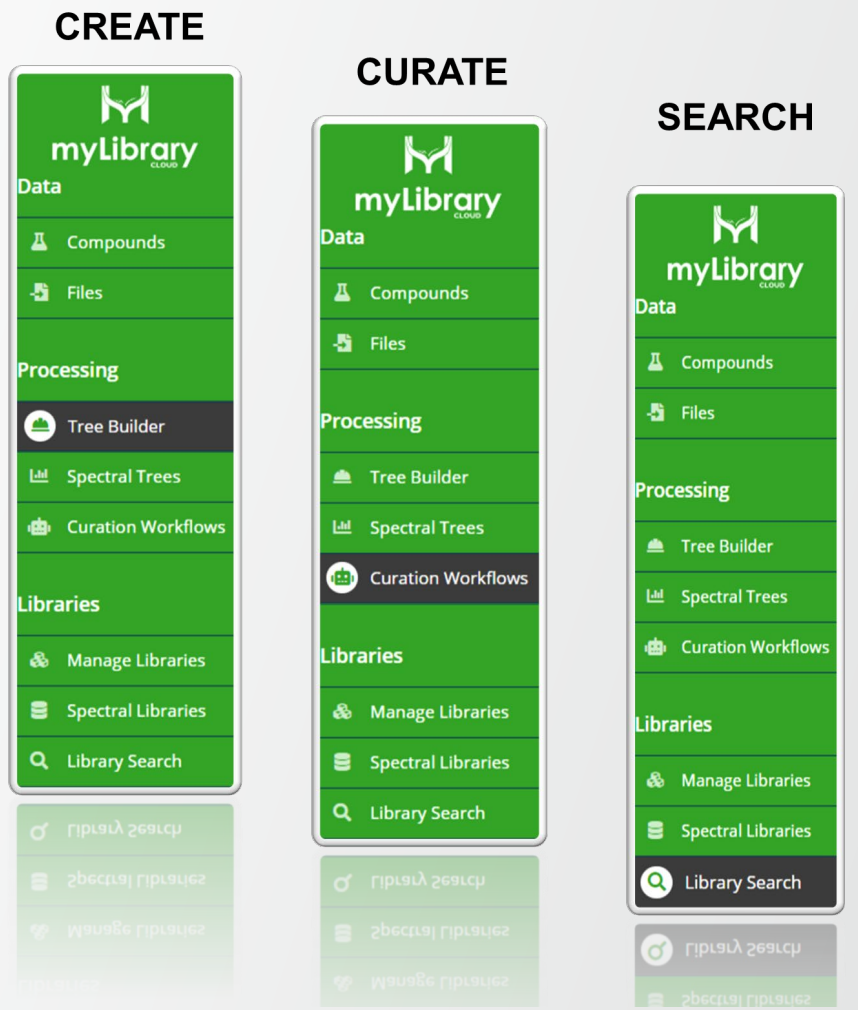
myLibrary Enterprise –
cloud based spectral library building platform

- 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.



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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

Data
- Compounds
- Files

Processing
- Tree Builder
- Spectral Trees
- Curation Workflows

Libraries
- Manage Libraries
- Spectral Libraries

Manage Libraries

Name	Owner	Assigned to	Library Type	No. Records	Sync. Status
Pesticides	Bill	All	Reference Manual	466	
Illicit Drugs	Anna	QC Team	Reference Manual	235	
Flavanols	Anna	R&D Team	Reference Manual	333	
Plasma Metabolites	Rob	Clinics Team	Putative Manual	74	
Pathogen Response	Bill	R&D Team	Putative Manual	24	
Unknown Urine	Rob	Clinics Team	Putative Manual	35	

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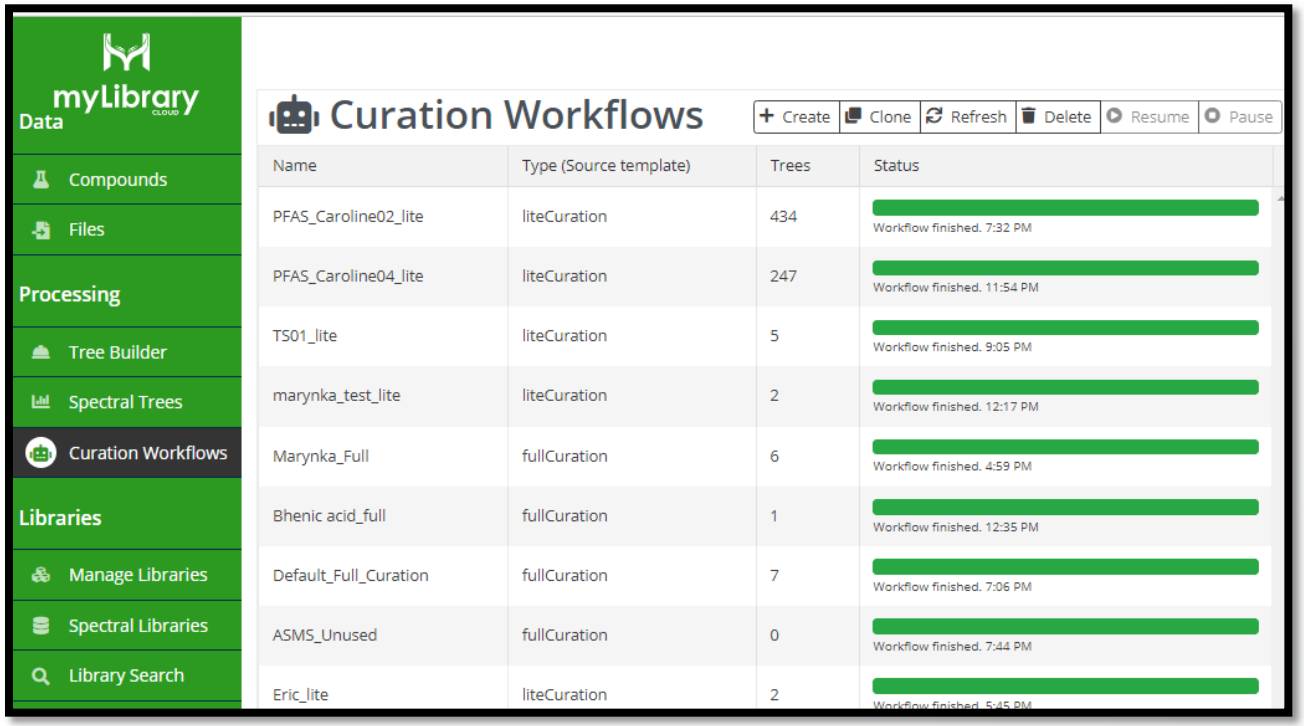
myLibrary Enterprise –
cloud based spectral library building platform

Automated Curation Pipeline



Workflow type

- Lite Curation
  - Remove Empty Spectra
  - Average Spectra
  - Select Significant Spectra
- Advanced Curation
  - Lite Curation Steps
  - Structure Fragmentation
  - Fragment Structure Annotation
  - Spectra Recalibration

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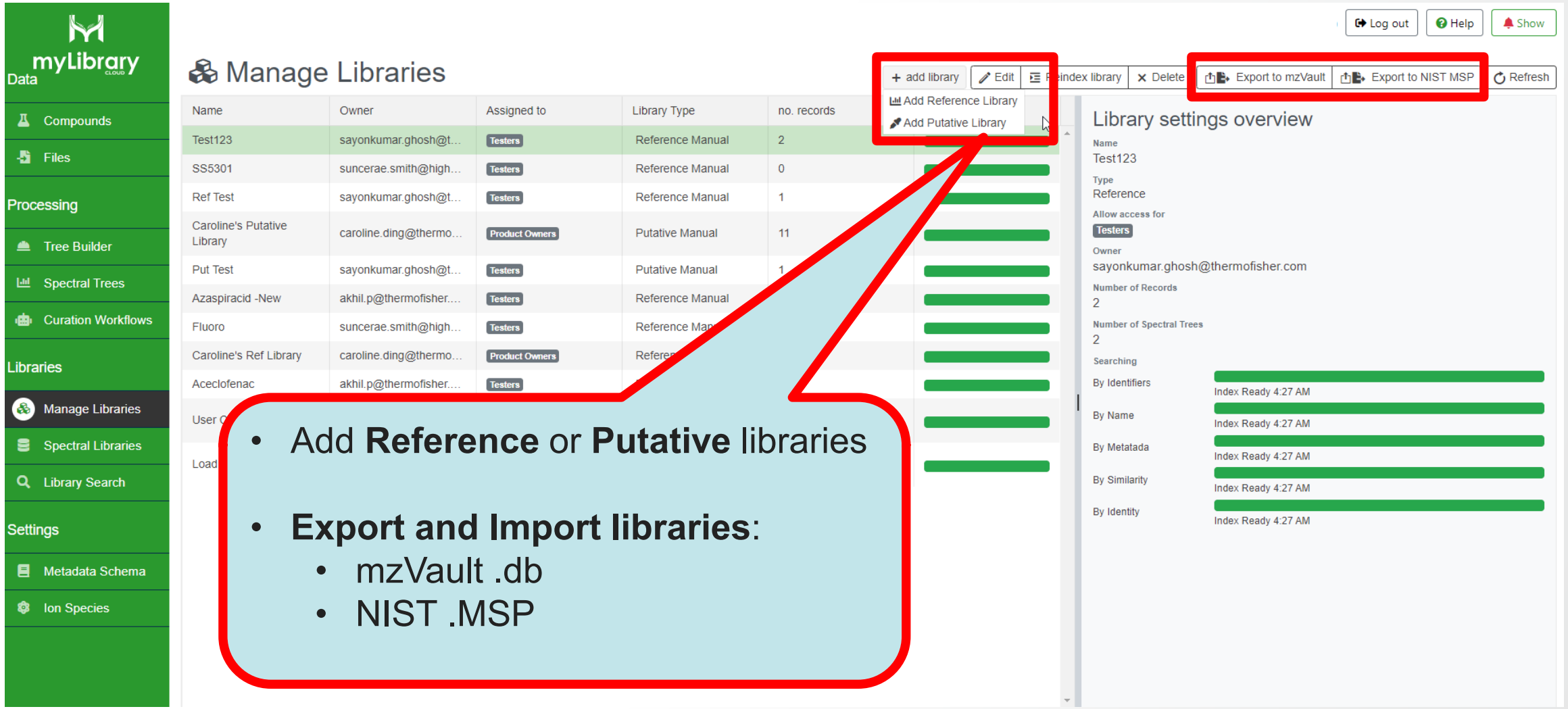

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Sample preparation

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 MgSO4 and 1500 mg C2H3NaO2)
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 MgSO4, 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.

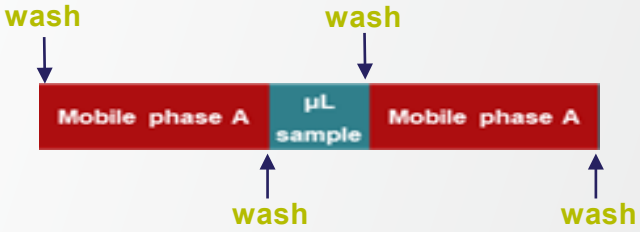
DSPE rgts+ MeCN+H2O+FA	MeCN+H2O+FA+tube	MeCN+H2O+FA +tube		
DSPE Tube+Reagents	ExtSolventBlank	ExtSolventBlank	MeCN (pure)	Water (pure)

- 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.

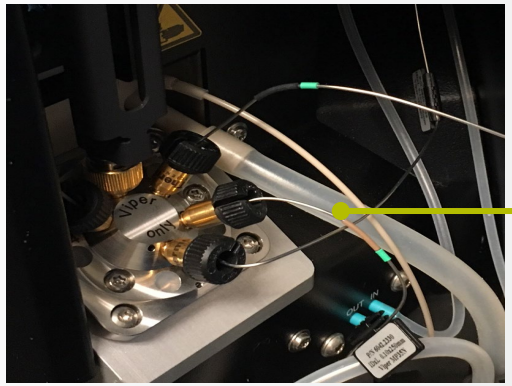
LC Setup for sandwich injection on Thermo Scientific™ Vanquish™ Flex UHPLC System

Custom Injection Program

- Sample loop: **100 µL**
- Sandwich injection
- In needle mixing
- Polypropylene A/S vials



Inj. Volume	15 µL sample with 2 plugs 30 µL MF A
Col Temp. and Flowrate	40 C; 400 uL/min
Analytical Column	Thermo Scientific™ Accucore™ C18, 100 x 2.1 mm, 2.6 µm
Trap Column	Thermo Scientific™ Hypersil Gold™ C18, 50 x 4.6 mm, 1.9 µm
Run Time	19 minutes
Mobile Phase A	5mM Ammonium Acetate in H2O
Mobile Phase B	Methanol



Capillary

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

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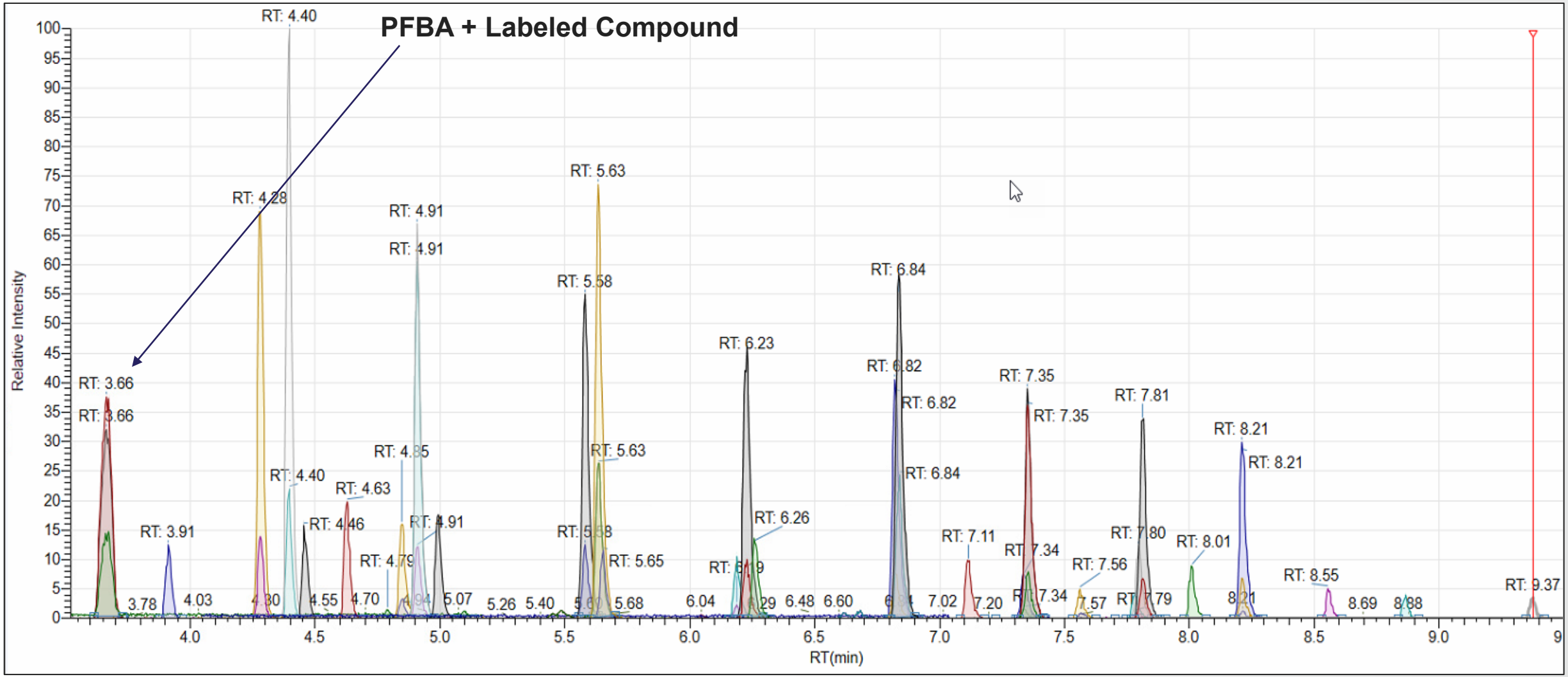
Optimized peak shapes

Extracted full scan precursor ions of calibration standard 100 ppt



Orbitrap Exploris 120 MS settings and workflow

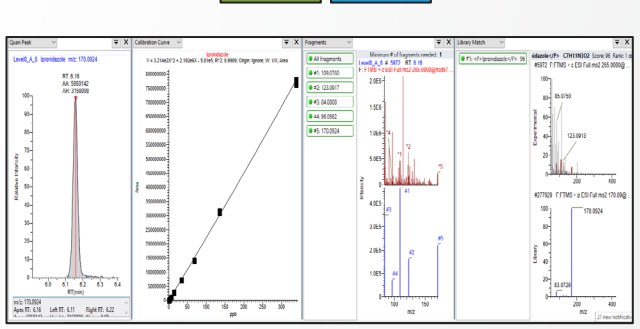
Spray voltage	1.0 kV
Sheath gas	35 arb
Aux gas	5 arb
Sweep gas	1 arb
Capillary temp.	220 °C
Vaporizer temp.	450 °C
Ion polarity	Negative

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

Data Independent Acquisition (DIA)

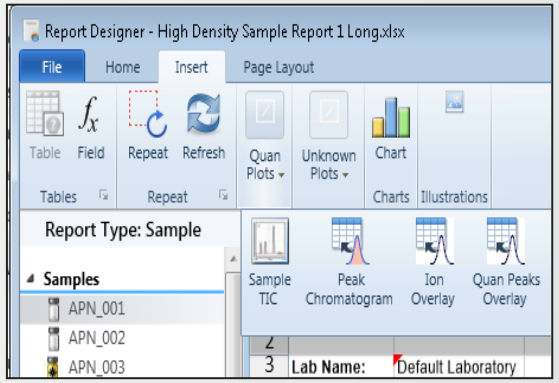
- No target list
- Precursor isolation windows w/ stepped NCE
- MS2 triggered across entire peak

Screening and Quantitation



Fragment Match and Library Search

Reporting



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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:H2O + 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
- r2 range: 0.9516 to 0.9993, with Calibration Average % RSDs < 7 % for all compounds

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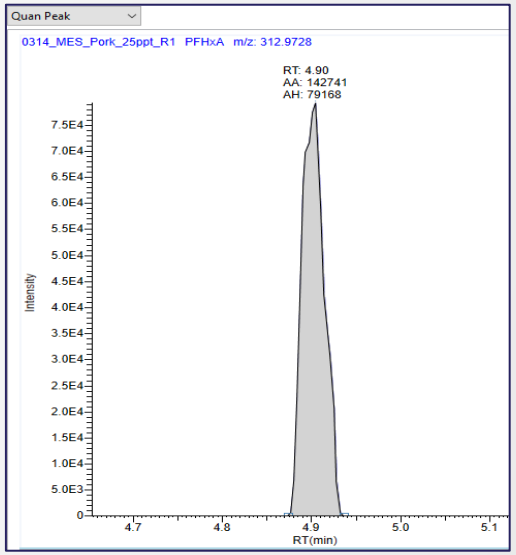
Recovery experiments

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

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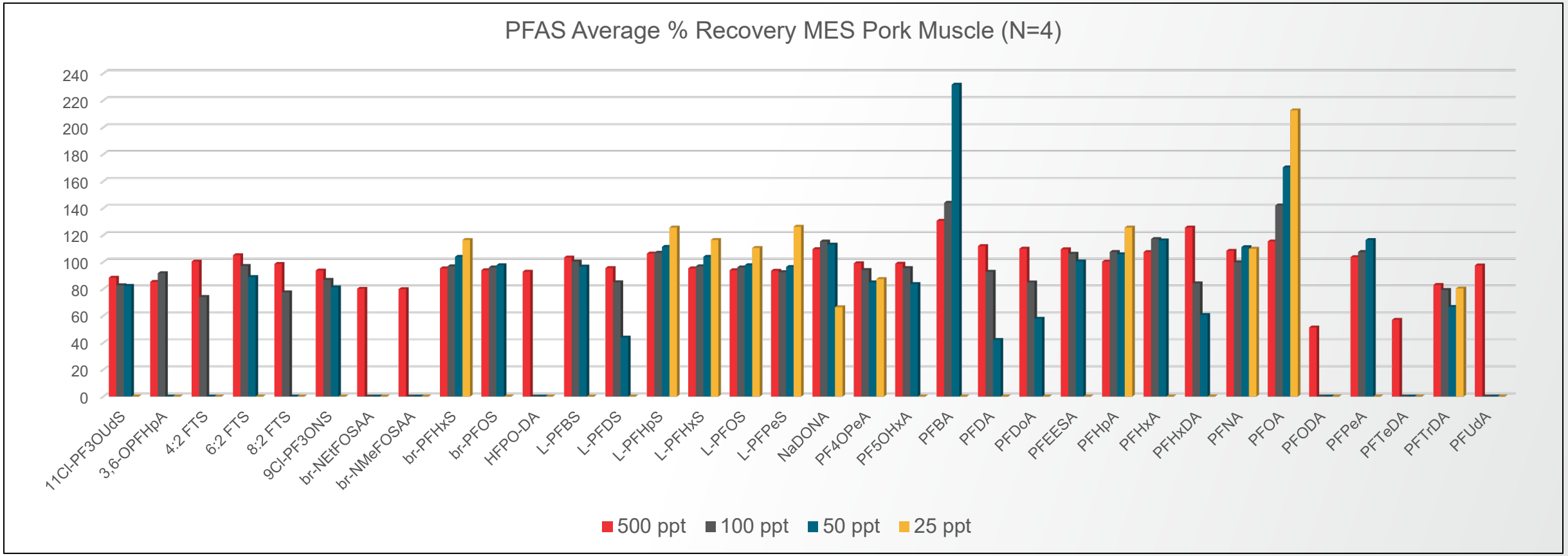
Recovery experiments



- 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

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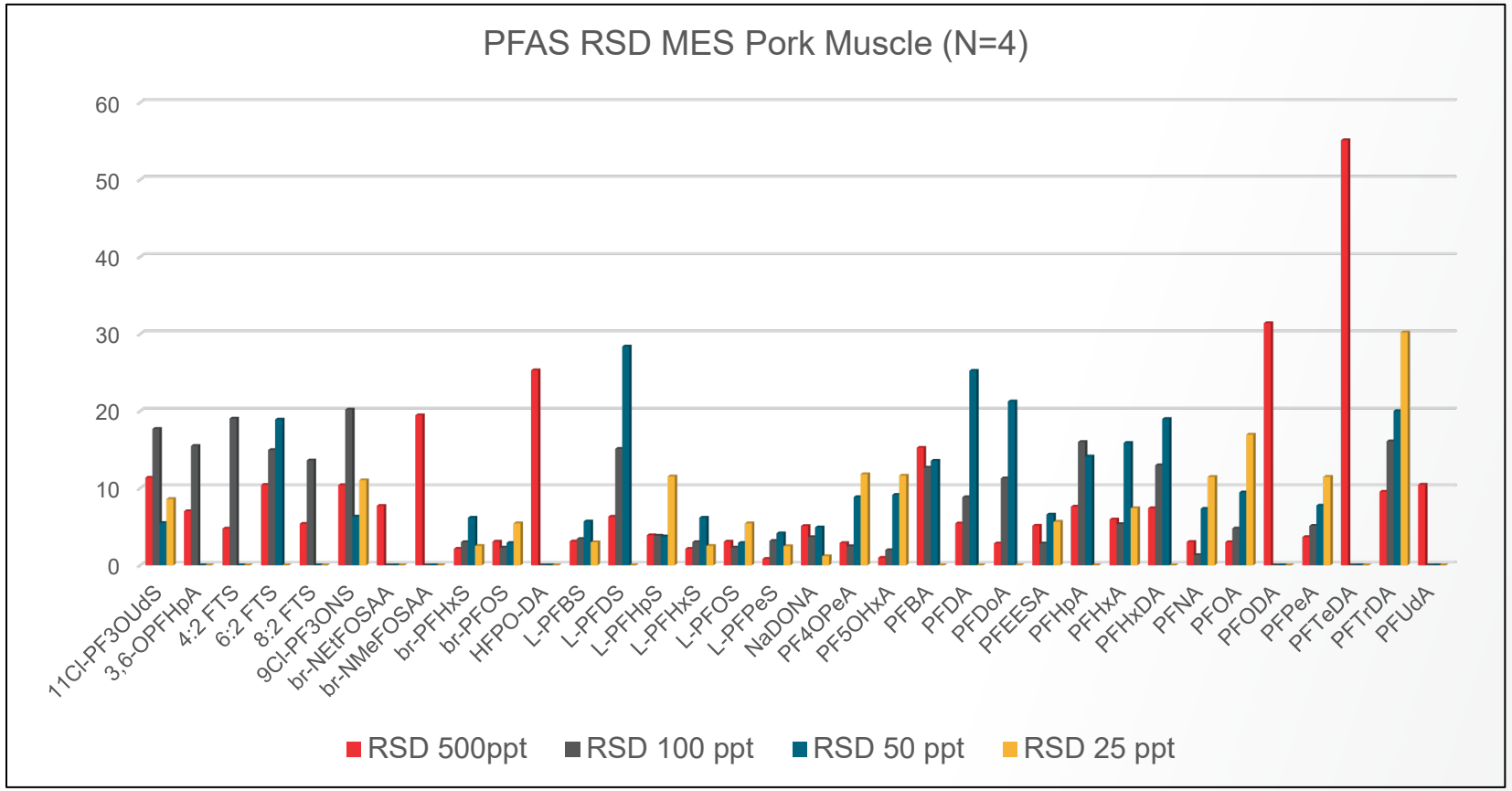
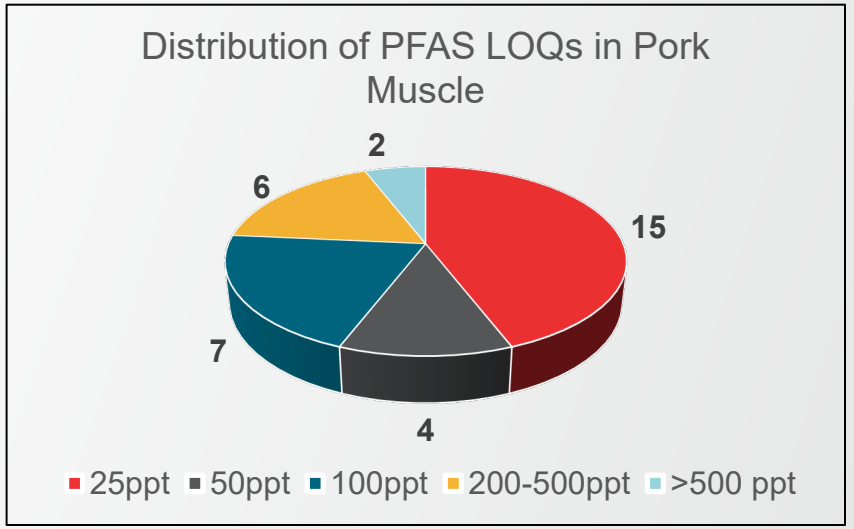
% RSD and estimated LOQs in pork meat





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

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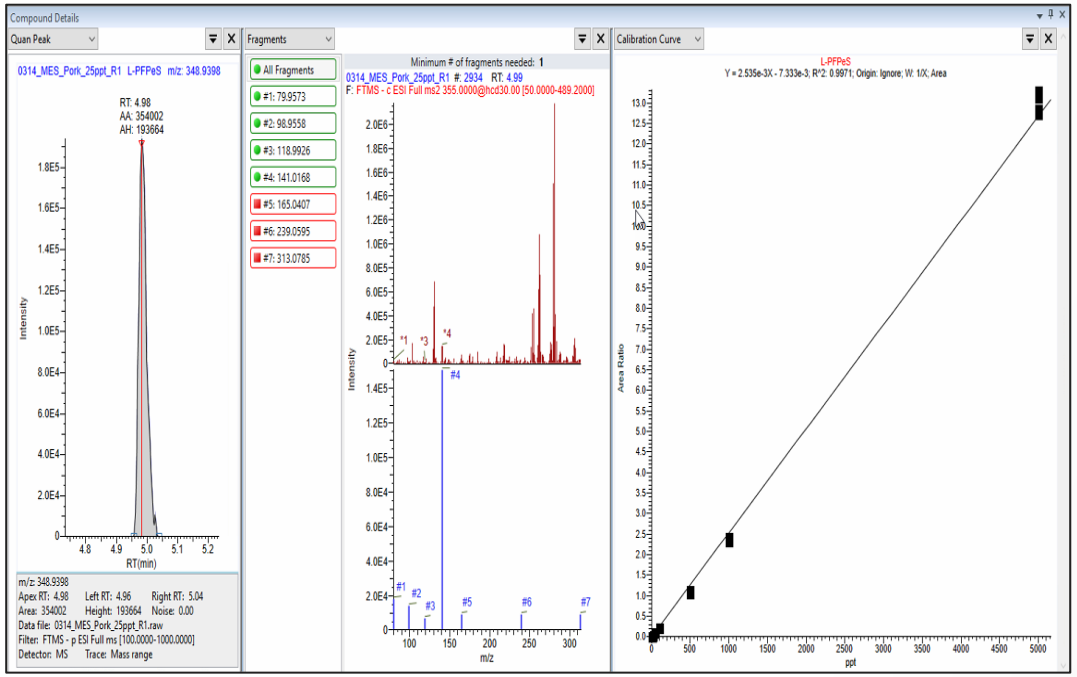
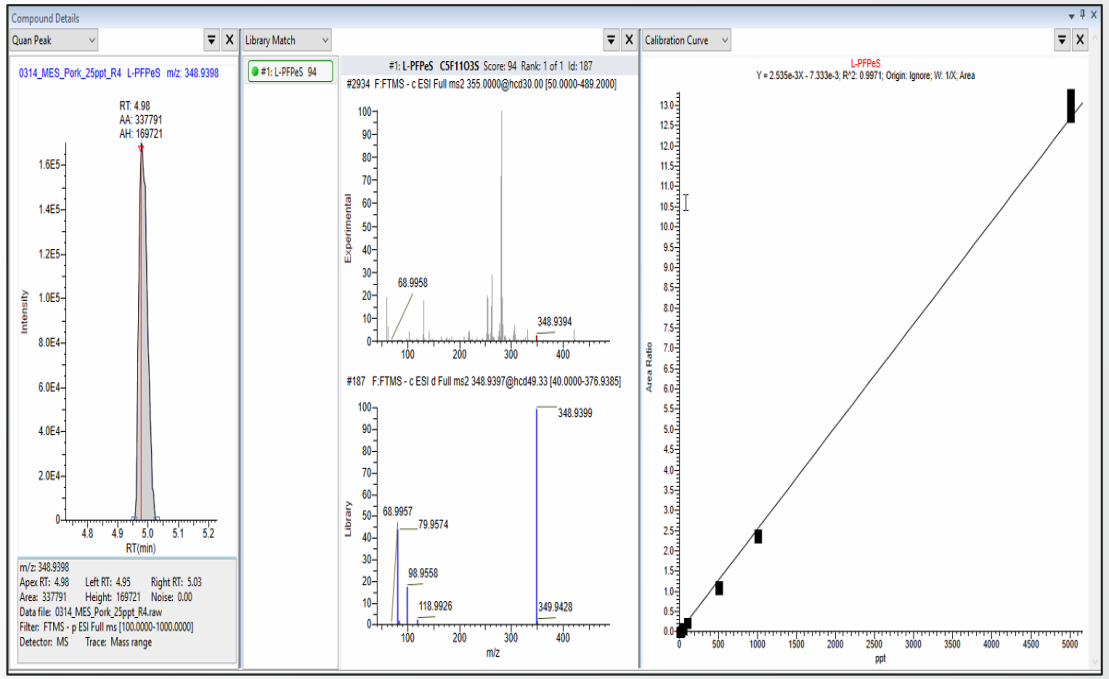
Identification and library search

- A detected and confirmed analyte is defined as the native PFAS precursor ion detected at <5 ppm mass accuracy with S/N ≥ 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 ion the right is a library search result vs. the user created mzVault spectral library for the same spike level.

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Conclusions

- 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

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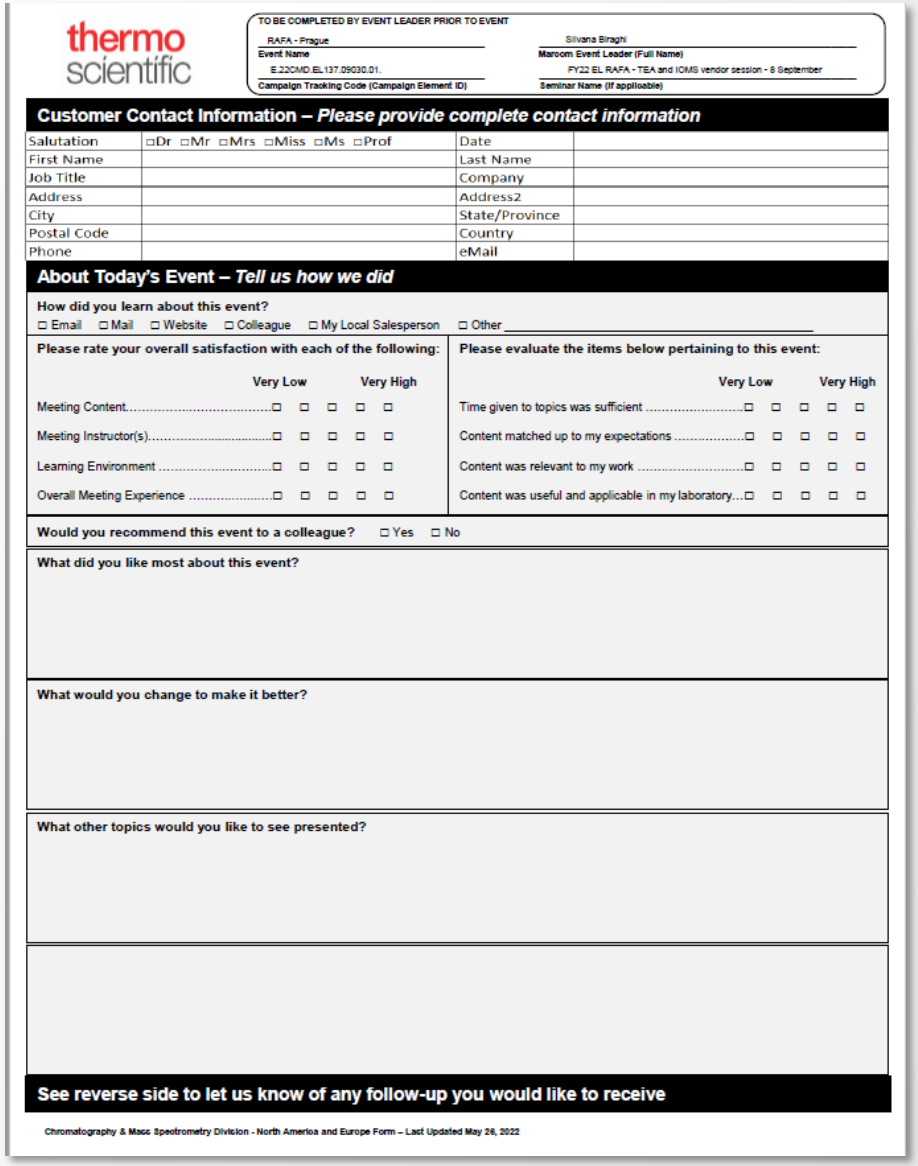
Share your comments about today's event, this will help us to improve in the future.

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



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Please rate your overall satisfaction with each of the following:

	Very Low				Very High
Meeting Content	☐	☐	☐	☐	☐
Meeting Instructor(s)	☐	☐	☐	☐	☐
Learning Environment	☐	☐	☐	☐	☐
Overall Meeting Experience	☐	☐	☐	☐	☐

Please evaluate the items below pertaining to this event:

	Very Low				Very High
Time given to topics was sufficient	☐	☐	☐	☐	☐
Content matched up to my expectations	☐	☐	☐	☐	☐
Content was relevant to my work	☐	☐	☐	☐	☐
Content was useful and applicable in my laboratory…	☐	☐	☐	☐	☐

Would you recommend this event to a colleague? ☐ Yes ☐ No

What did you like most about this event?

What would you change to make it better?

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 26, 2022

Thank you