ThermoFisher SCIENTIFIC

The Doctor Did Not Prescribe Irgafos: A Solution for Extractables and Leachables Analysis

Outline

- Introduction
  - Why conduct Extractables & Leachable (E&L) analysis?
  - Industries need Extractable & Leachable analysis
  - Regulations and guidelines from agencies and industry consortiums

- Thermo Scientific™ solutions for extractable & leachable analysis
  - Multiple instrumentations and technologies
    - ASE, CAD
    - GCMS
    - LCMS
    - ICP-MS
  - Data analysis software and database/spectral library

Plastics – A Complex Process

Polymer Supply Chain for Pharmaceutically Relevant Materials

Converters

Natural Raw Material	Monomer	Polymer	Masterbatcher	End products & Suppliers
N-4	N-3	N-2	N-1	N
	• Bulk process chemicals • Storage stabilizers	• Initiators/Catalysts • Storage stabilizers • Antioxidants • Processing aids	• Antioxidants • Processing aids • Light stabilizers • Antistatic agents	• Colorants • Processing aids • Lubricants

Components for
- Packaging
- Devices
- Container/closure
- Processing

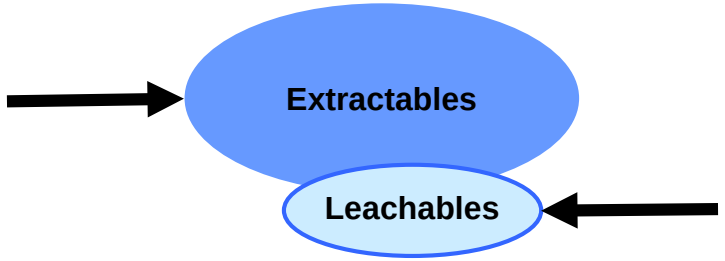
Courtesy of Doug Kiehl, Eli Lilly

Definitions

- Extractable (E)
  - Compounds that migrate from the surface under more aggressive conditions of exposure (solvent, time, and temperature). *Controlled extraction study*
- Leachable (L)
  - Compounds that migrate from the contact surface to drug formulation under normal conditions of exposure. *Bioproduction, formulated drug, or device simulants*

Material Safety

Additives
Impurities
Polymer Components
Degradation Products
Solvent-material Interactions



Product Quality

Known extractables
Extractable – drug formulation interactions
Extractable modified by process conditions

Some leachables are subset of extractables, but this is not always the case.

Safety/risk assessment ➡ Regulatory submission ➡ Market

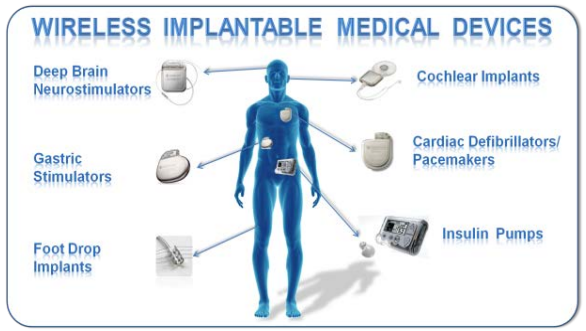
The Broad Market of Extractable & Leachable Analysis




Single-use systems for bioproduction and storage







Medicine container/packaging, implant and diagnostic devices

Food packaging

Printing ink and adhesives

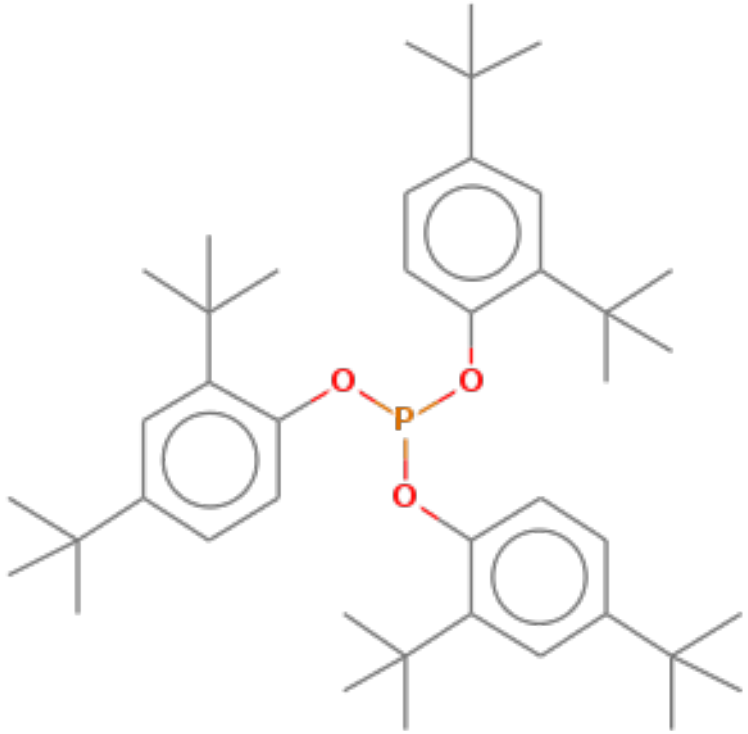
Wearable consumer electronic products

ThermoFisher SCIENTIFIC

What is Irgafos?

Trade name - **Irgafos® 168 (BASF)**

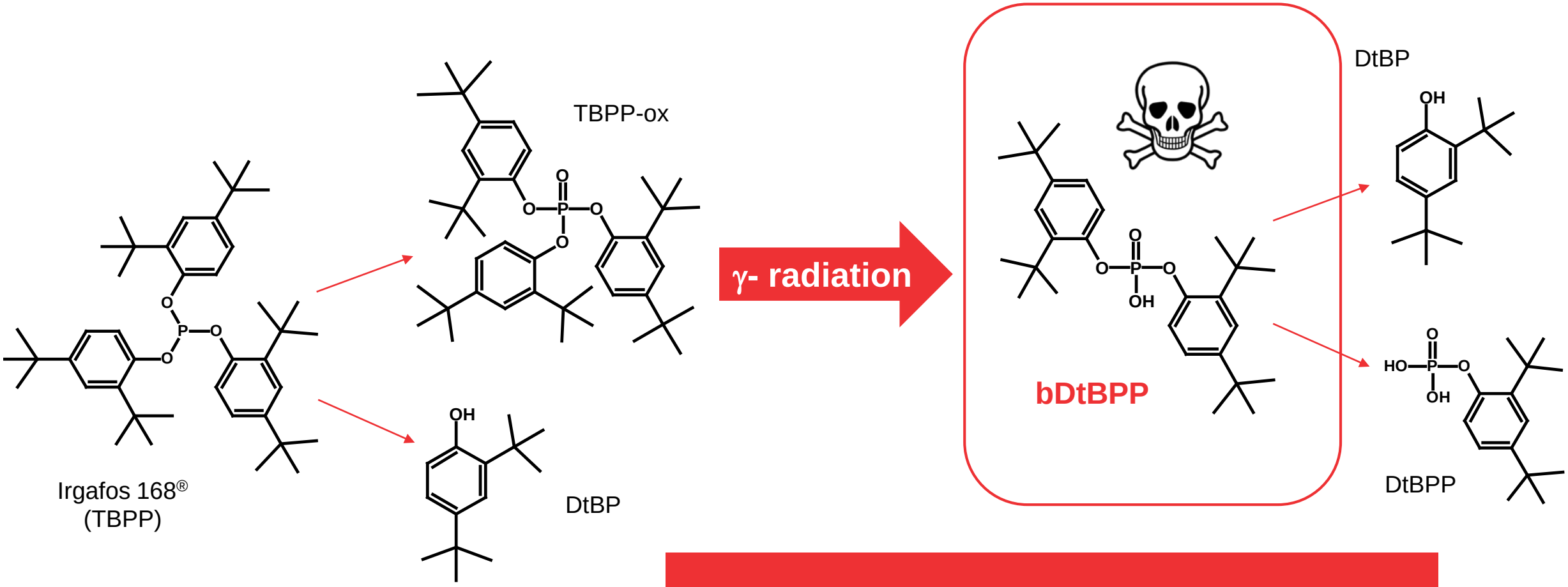
tris(2,4-di-tert.-butylphenyl)phosphite.



- Polymer additive stabilizer
- Protects plastic from thermooxidative degradation
- Improves the strength and durability
- Used in a range of plastic films

including ***Single Use BioProcess films***

Irgafos 168 is safe – until you irradiate it



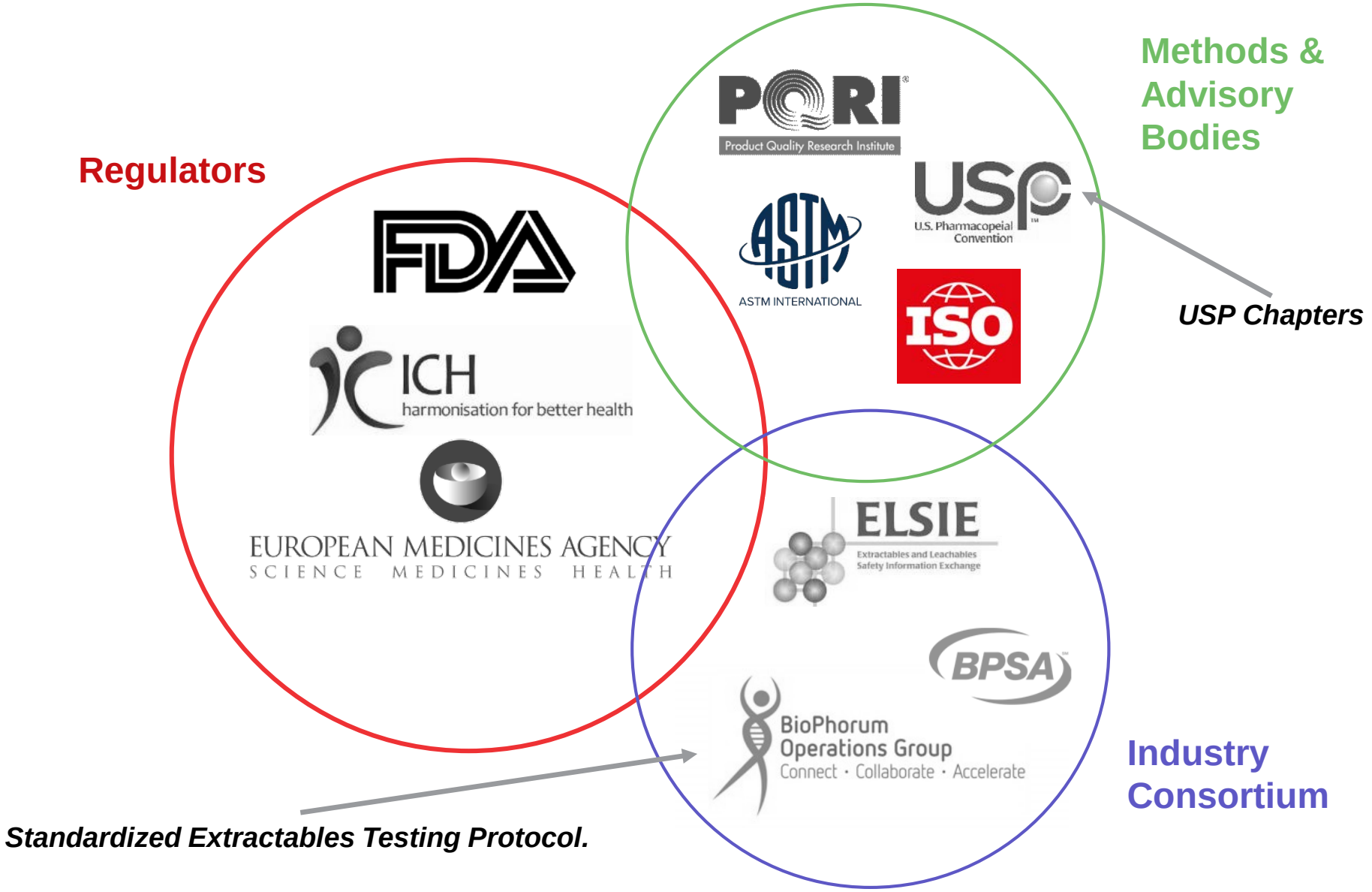
BioProcess bags are sterilized with γ-radiation

[1] Fouyer K et al (2012) *Anal. Chem.* 84 (20): 8642-8649.

The Importance and Challenges of E&L Analyses

- Safety
  - Extractables and Leachables are chemical impurities- they could contaminate medicine, food, and drink, affect product quality, and cause adverse effects for consumers.
- Regulations
  - E&L analyses are highly regulated and required for marketing approval of new products.
  - More regulations have been promulgated along the way and will continue into the future.
- E&L analysis is challenging and complex
  - It requires multiple instruments, expertise, data processing software, and database.
  - It needs both identification and quantitation. Unknown structure elucidation is challenging.
  - It must start at early stage to be ready for product launch.

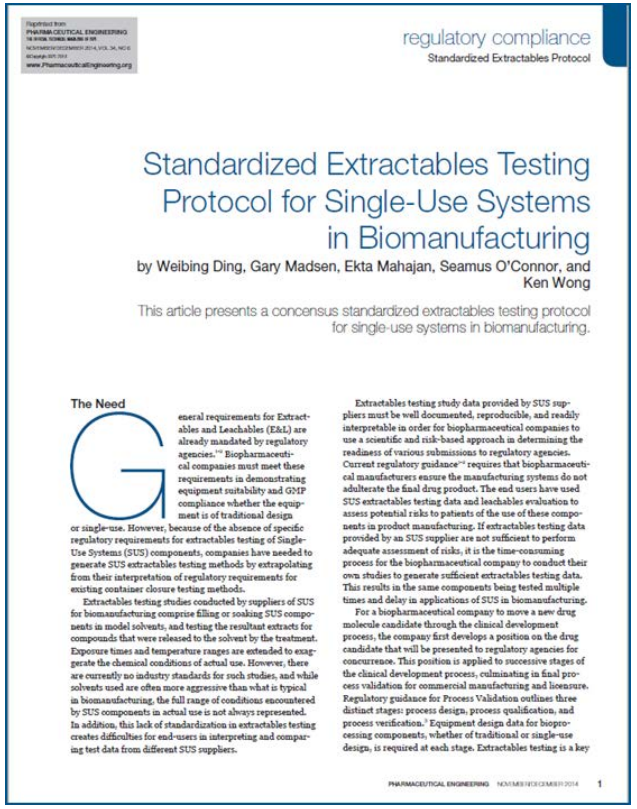
E&L Regulatory and Method landscape



ThermoFisher SCIENTIFIC

Industry Group: BioPhorum Operations Group (BPOG) The Global Community

http://www.biophorum.com/



BPOG members are leading biopharmaceutical companies around the world to create an environment where the global biopharmaceutical industry can collaborate and accelerate their rate of progress, for the benefit of all.

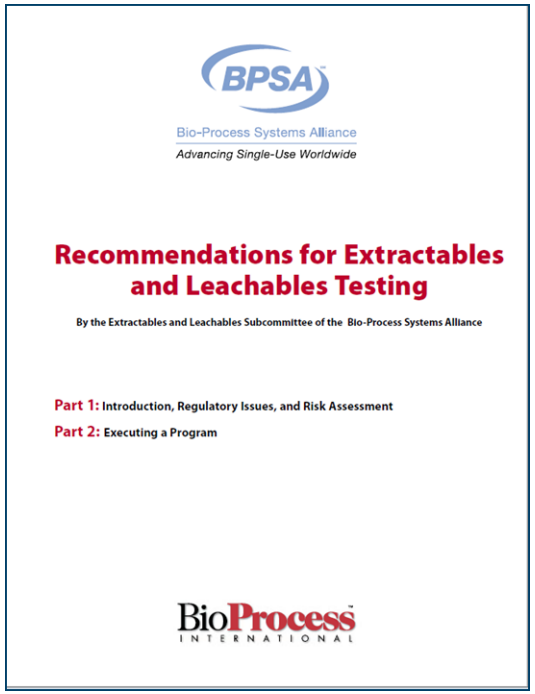
Many Technical Resources on their website http://www.biophorum.com/

ThermoFisher SCIENTIFIC

Industry Group: Bio-Process Systems Alliance (BPSA) - Published Technical Guides

BPSA members include industry-leasing manufacturers of single-use biological processing products. BPSA is encouraging and accelerating the adoption of technologies used in the production of biopharmaceuticals and vaccines.

Many Technical Guides on their website bpsalliance.org/



ThermoFisher SCIENTIFIC

ISO & ASTM International

International Organization for Standardization

ISO 10993

Biological Evaluation of Medical Devices

Sample preparation and Extraction

- Part 12: Sample preparation and reference materials

Material Characterization

- Part 18: Chemical characterization of materials
  - GCMS - SVOC
  - LCMS – NVOC
  - ICP- MS Elemental impurities
  - Other analytical methods – FTIR…

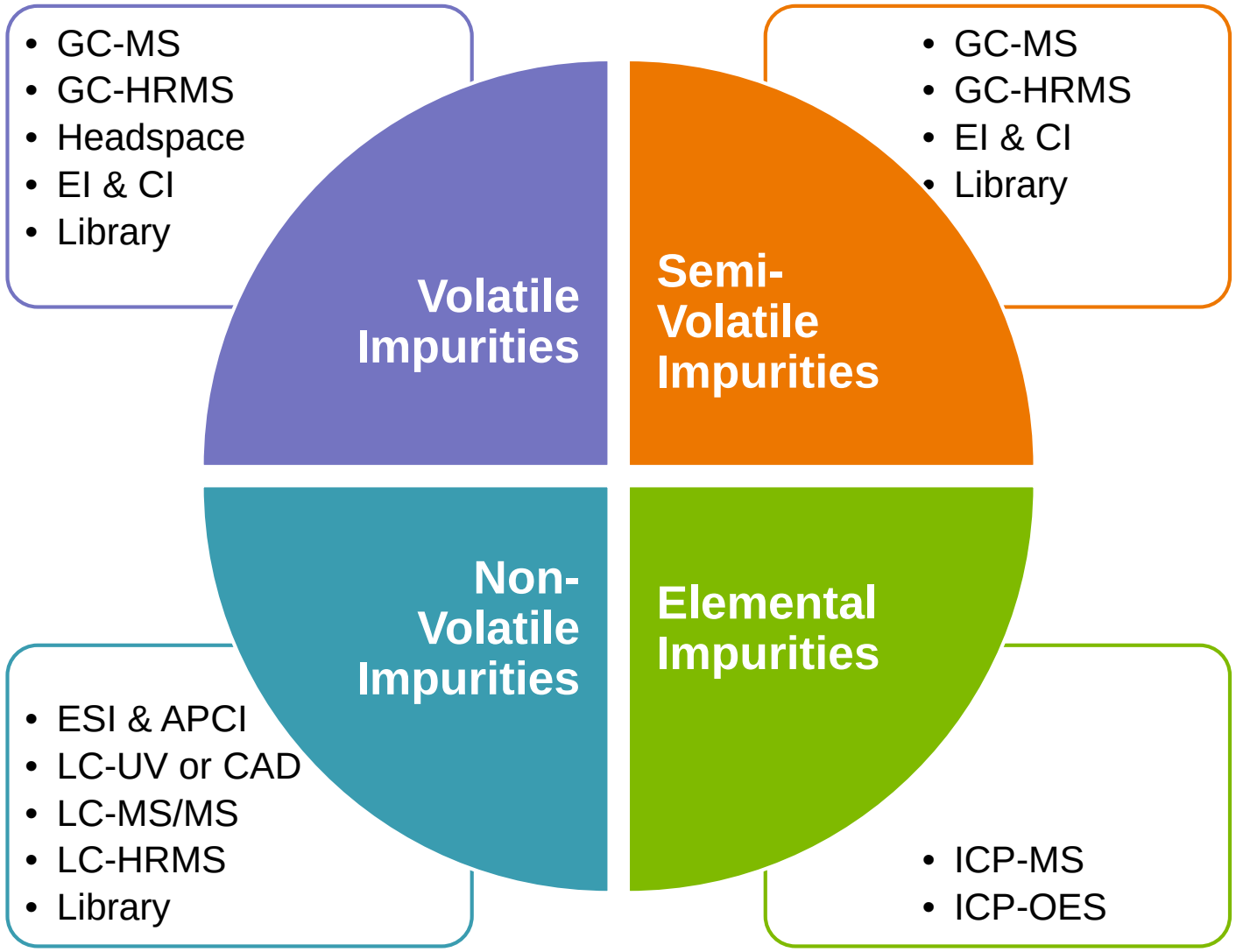
ASTM INTERNATIONAL

ASTM D7210

Standard Practice for Extraction of Additive in Polyolefin Plastics

- USP <1663> lists ASE as a possible option to perform extractable studies
- ASTM D7210 also lists ASE as one of the options to extract antioxidants from polymeric matrices.

Analysis of Extractables & Leachables



Thermo Scientific Instruments, Software, Consumables for Impurity ID & E&L Analysis

Solvent Extraction

FTIR

GCMS- Volatiles and Semi-volatiles

GCMS-Single Quad

GCMS-Triple Quad

Orbitrap GC-MS/MS

ICP-MS

Consumables

UHPLC

Charged Aerosol Detection

IC

LCMS- Non Volatiles

LCMS-Triple Quad

Hybrid Orbitrap LC-MS/MS

Software & Database

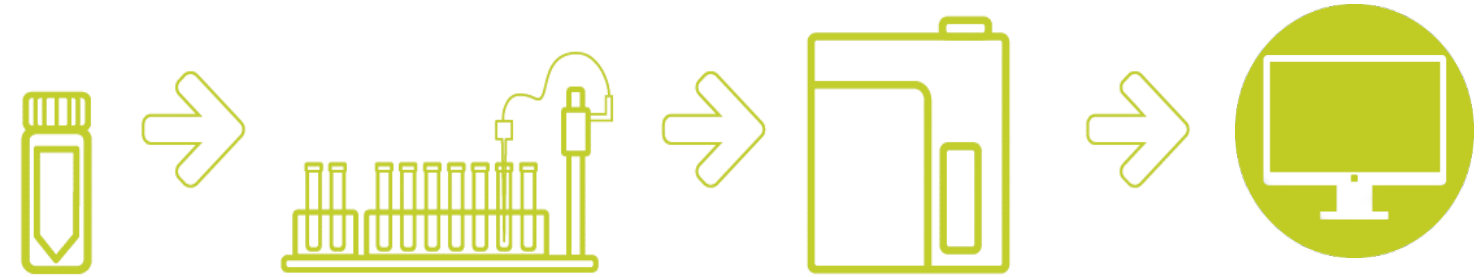
ThermoFisher SCIENTIFIC

Elemental Impurity – Thermo Scientific iCAP RQ ICP-MS and Qtegra Software

Thermo Scientific™
iCAP™ RQ ICP-MS

Thermo Scientific™ Qtegra™ ISDS software.
Integrated CFR compliance tools kit
Full USP 233 & ICH Q3D method capabilities
Compliance with USP <232>/<233>

Thermo Scientific™
iCAP™ 7000 Plus ICP-OES



Using an in-line autodilution and autocalibration system significantly lowers the risk of human error and contamination of the samples, standards, or blanks.

Principle and Best Practices Recommended

Guidelines list ASE as an option for extractable studies:

- USP Chapter <1663> & <1664>
- Product Quality Research Institute (PQRI)
- BioPhorum Operations Group (BPOG)
- ASTM D7210

Accelerated solvent extraction technique (30 minutes)

Soxhlet (24 Hours)

Heated agitation (2-30 days)

Extraction efficiency

Time

Advantage

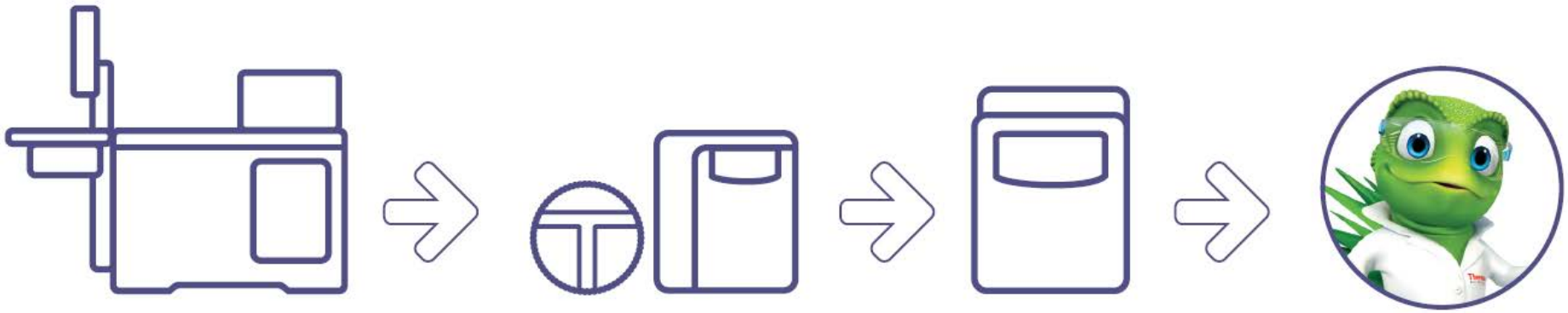
- Automated with intelligent solvent management system
- Reduce extraction times
- Reduce solvent consumption
- Increase extraction efficiency
- Nitrogen flush gas prevents the oxidation of the extractables
- Working very well for wide range of polymers, especially for ultra high molecular weight cross linked polyethylene -Dr. Vas

Comparison of Soxhlet vs. Thermo Scientific Dionex ASE 350 System

Parameter	Soxhlet	Thermo Scientific™ Dionex™ ASE™ 350 System
Extract solvent used per sample (mL)	160	<30
Total extraction time per sample (min)	1440	<30
Extracted compounds	Same	Same
Extracts peak Intensity Ratio Accelerated Solvent Extraction/Soxhlet		**1.4x to 90x**

Dionex ASE 350 system delivers faster extractions using less solvent

Volatile Impurities Workflow



Sample with valve & loop headspace

Robustly separate

Identify and quantify using MS

Compliant data processing

- Analogous to **USP 467** Residual solvents workflow
- Molecules are generally known, or simple to identify
- **Routine compliant quantification**

ThermoFisher SCIENTIFIC

Complete Technologies for Volatile Impurities

Thermo Scientific™ TriPlus™ 300 Headspace Sampler

Thermo Scientific™ Trace™ 1310 GC

Thermo Scientific™ TraceGOLD™ TG-624SilMS GC Columns

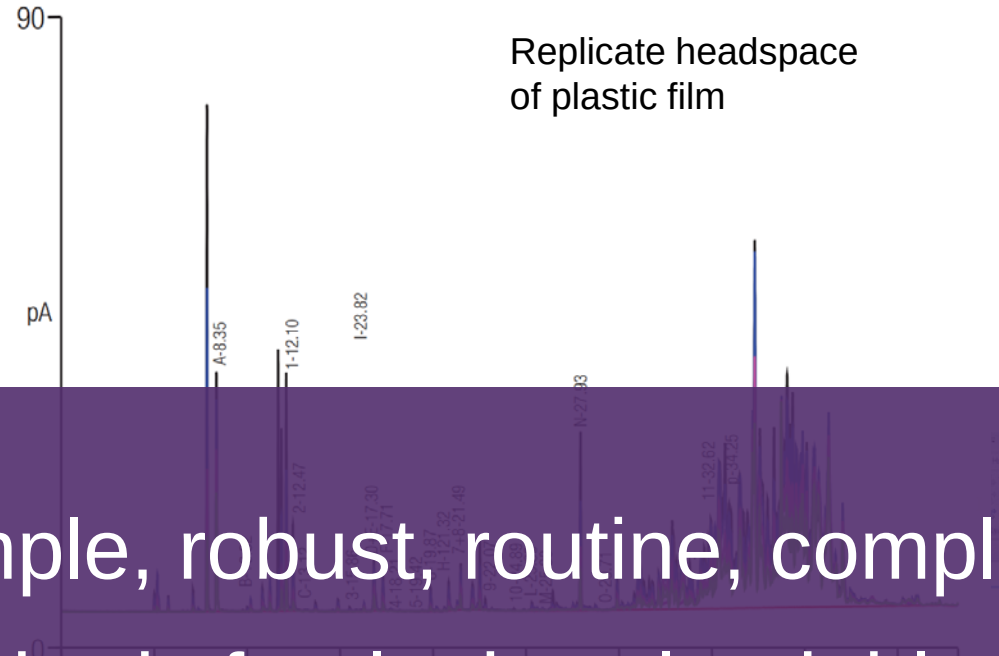
Thermo Scientific™ ISQ™ Series GC-MS

Thermo Scientific™ Chromeleon™ CDS Software

Thermo Scientific™ AppsLab Library

ThermoFisher SCIENTIFIC

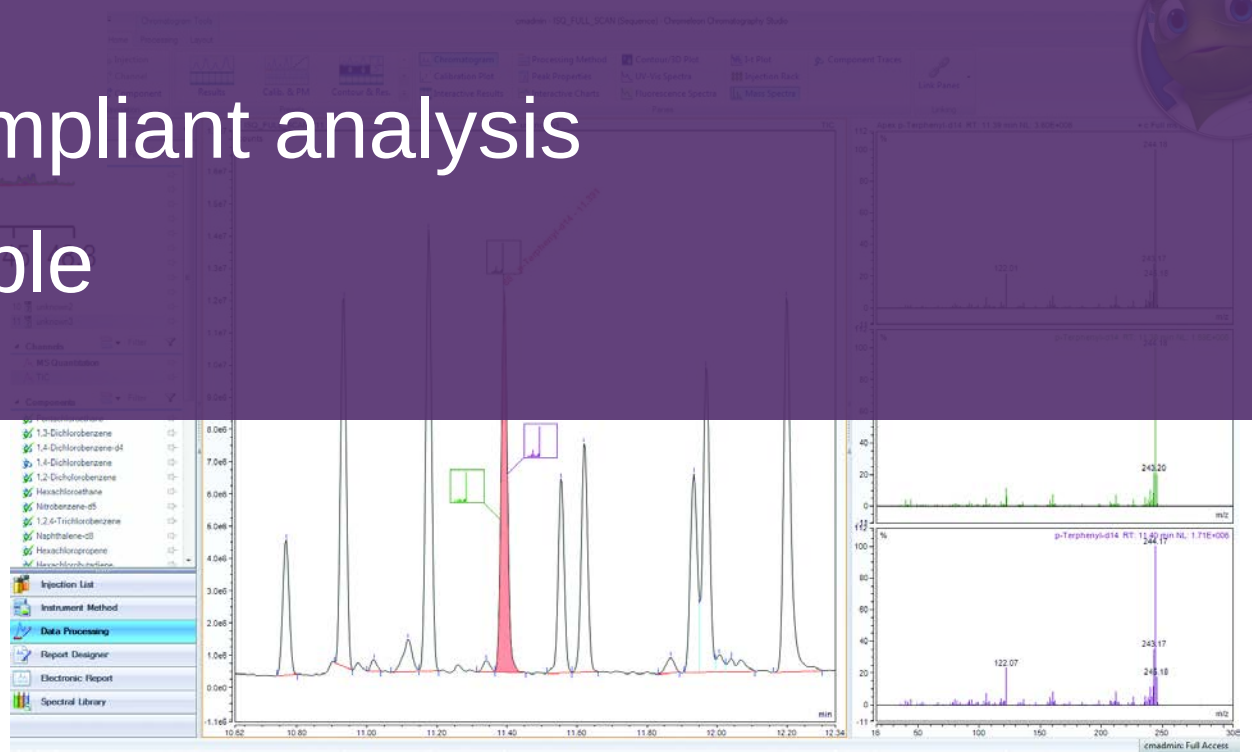
Volatiles Headspace Analysis



Replicate headspace of plastic film

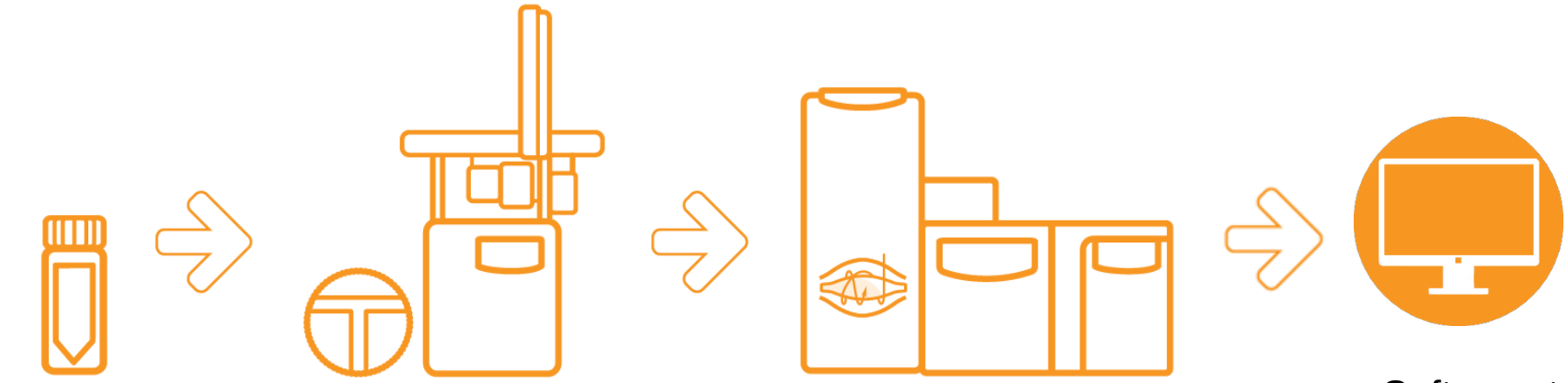
- Methods available on AppsLab
- One click Chromeleon eWorkflow
- Full **quantitative** and **qualitative** mass spectral analysis

- Simple, robust, routine, compliant analysis
- Methods freely downloadable



ThermoFisher SCIENTIFIC

High Resolution GCMS for Impurity Identification and Quantification Workflow



Extract

Separate

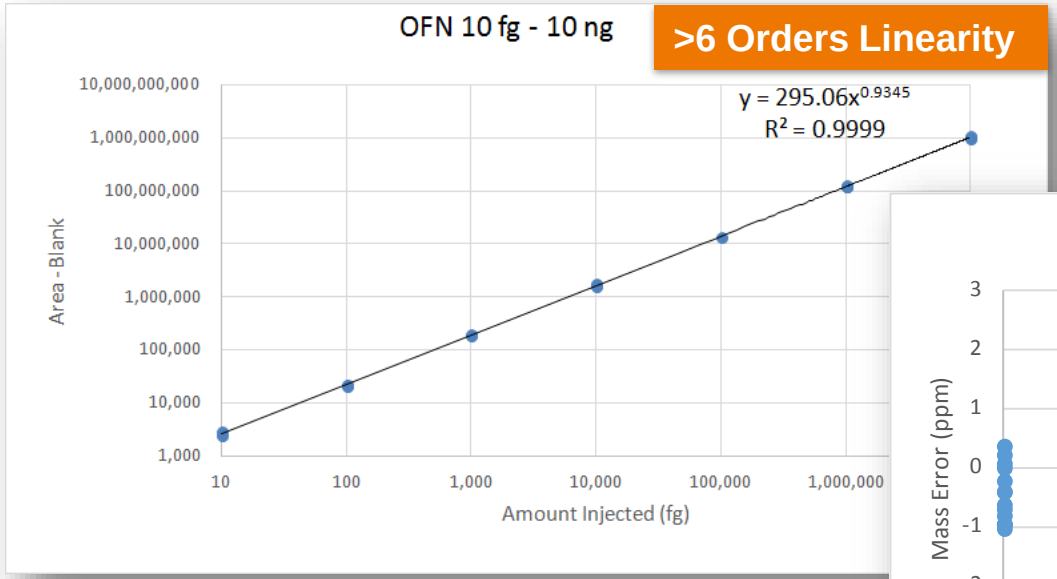
Identify with confidence of HRAM MS/MS

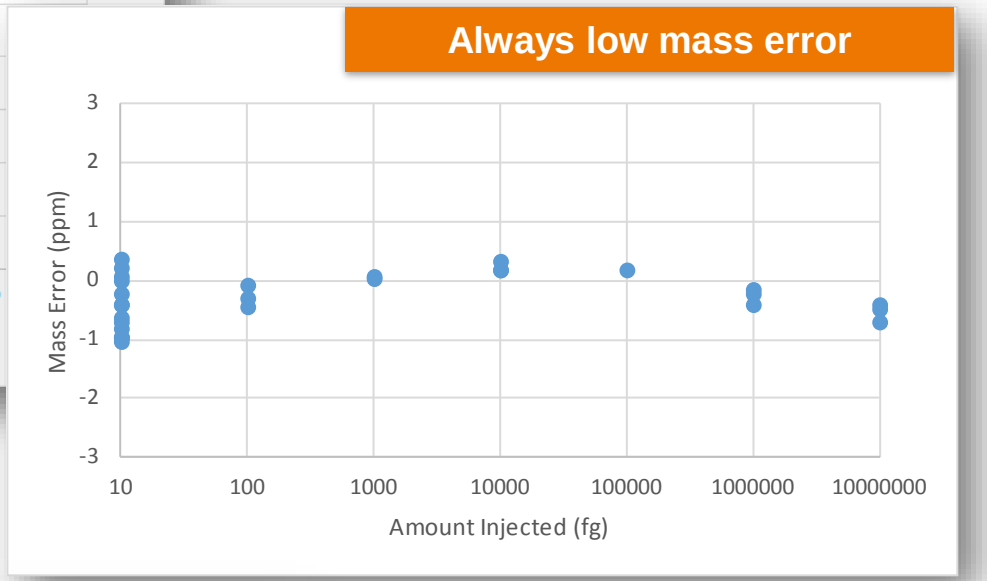
Software to search, elucidate & confirm

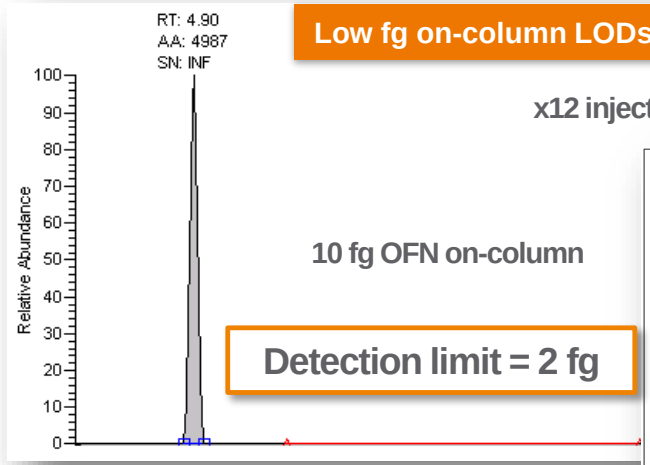
- Deconvolve, identify and quantify even the narrowest GC peaks
- Unambiguously calculate empirical formulae
- No need to average scans
- Simplify data review and report

- Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system
- Thermo Scientific™ TraceFinder™ software

Detect, Quantify and Identify at Any Concentration





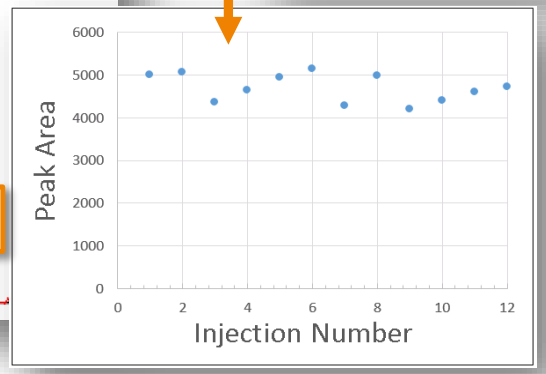


Low fg on-column LODs

x12 injections

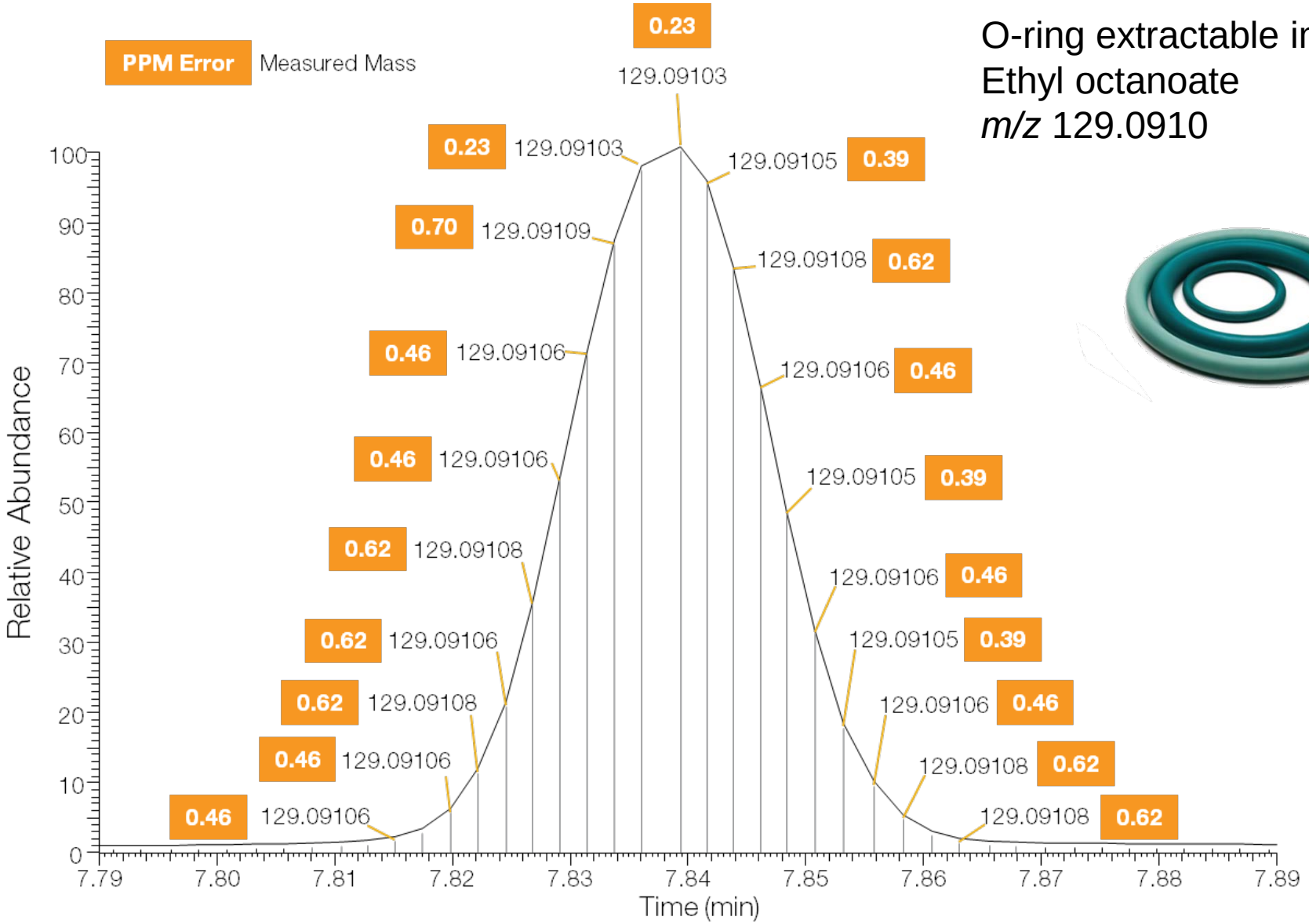
10 fg OFN on-column

Detection limit = 2 fg



ThermoFisher SCIENTIFIC

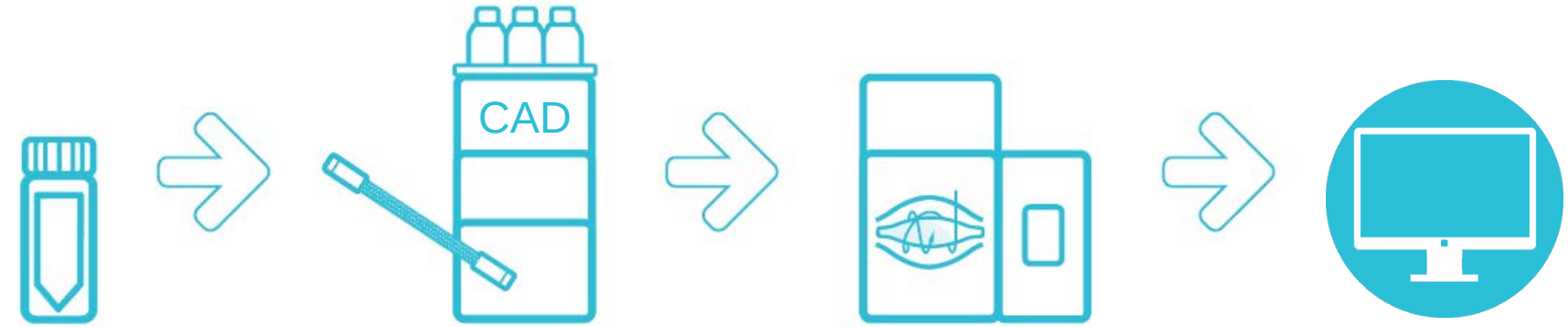
Scan Speed and Accurate Mass Error Across a Peak



O-ring extractable impurity
Ethyl octanoate
m/z 129.0910

ThermoFisher SCIENTIFIC

Non-Volatiles Unknown Identification Workflow



- Catch everything with orthogonal detection
- Detect in both positive and negative ion mode
- Have absolute confidence in elemental composition
- Get full sub-structural information
- Search wide variety of data sources in parallel

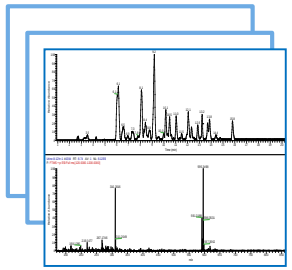
ThermoFisher SCIENTIFIC

LC-HRMS Analysis for IV Bag Extractables

UHPLC-HRMS

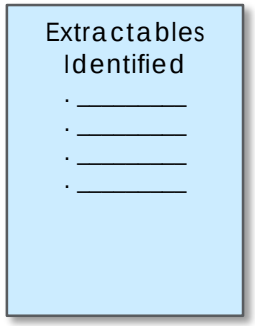
Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
Thermo Scientific™ Vanquish™ UHPLC system

HRAM data



Compound Discoverer 2.1

Result

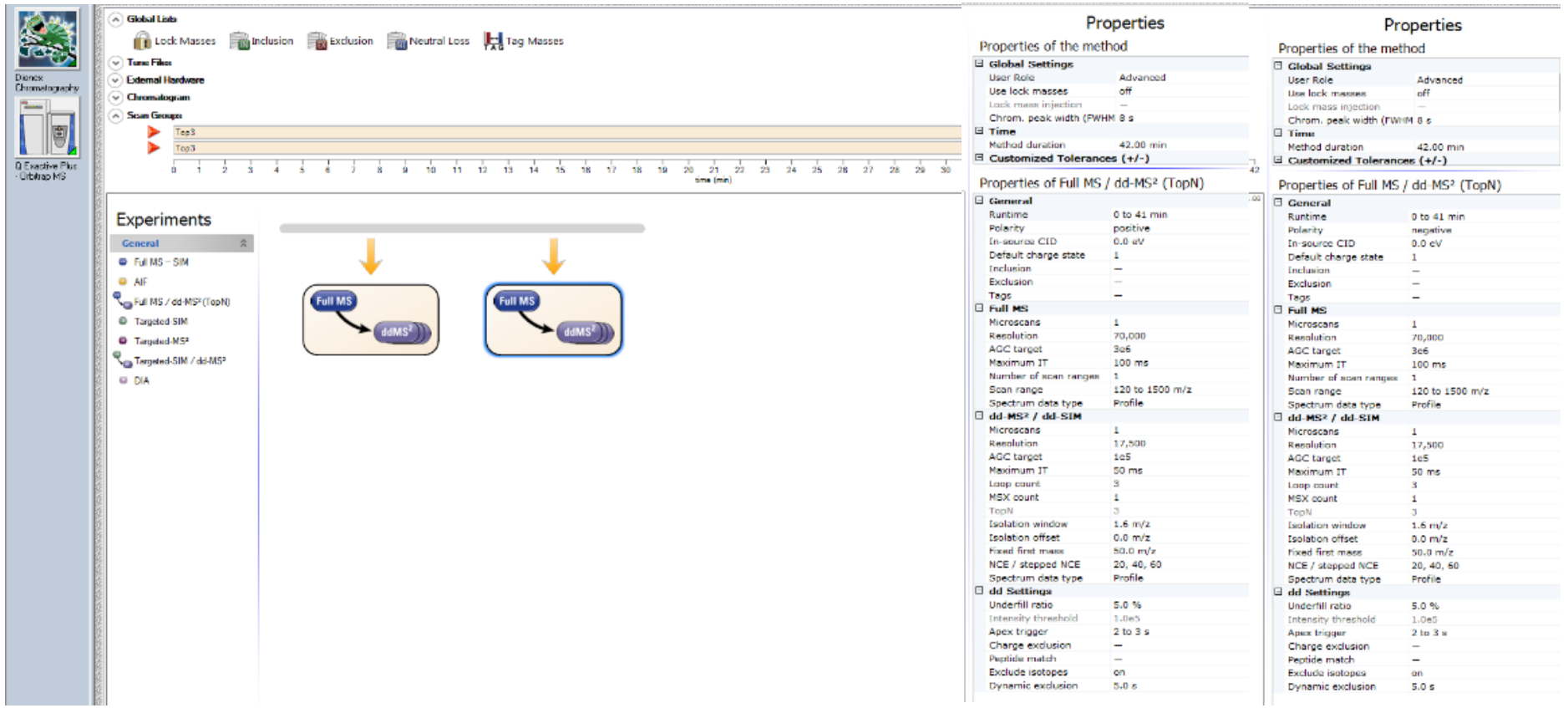


ThermoFisher SCIENTIFIC

MS Method: High Resolution Accurate Mass Untargeted Screening

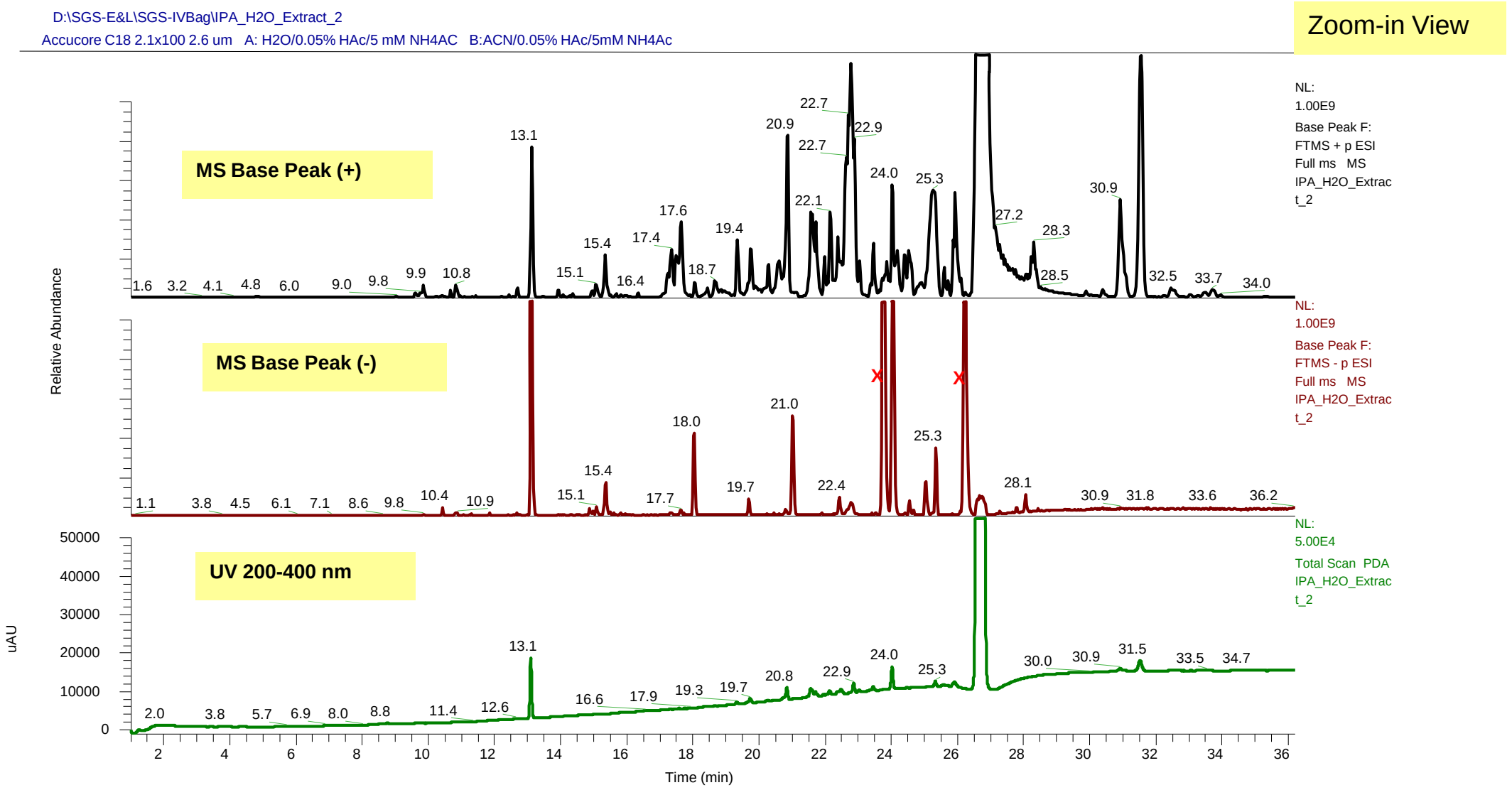
- **HRAM untargeted screening with polarity switching**
- ESI/APCI full scan MS and data-dependent top 3 MS/MS data with polarity switching using 70K &17.5K resolution for FMS and HCD MS^2 respectively. Stepped NCE: 30, 45, 60.

Positive Negative

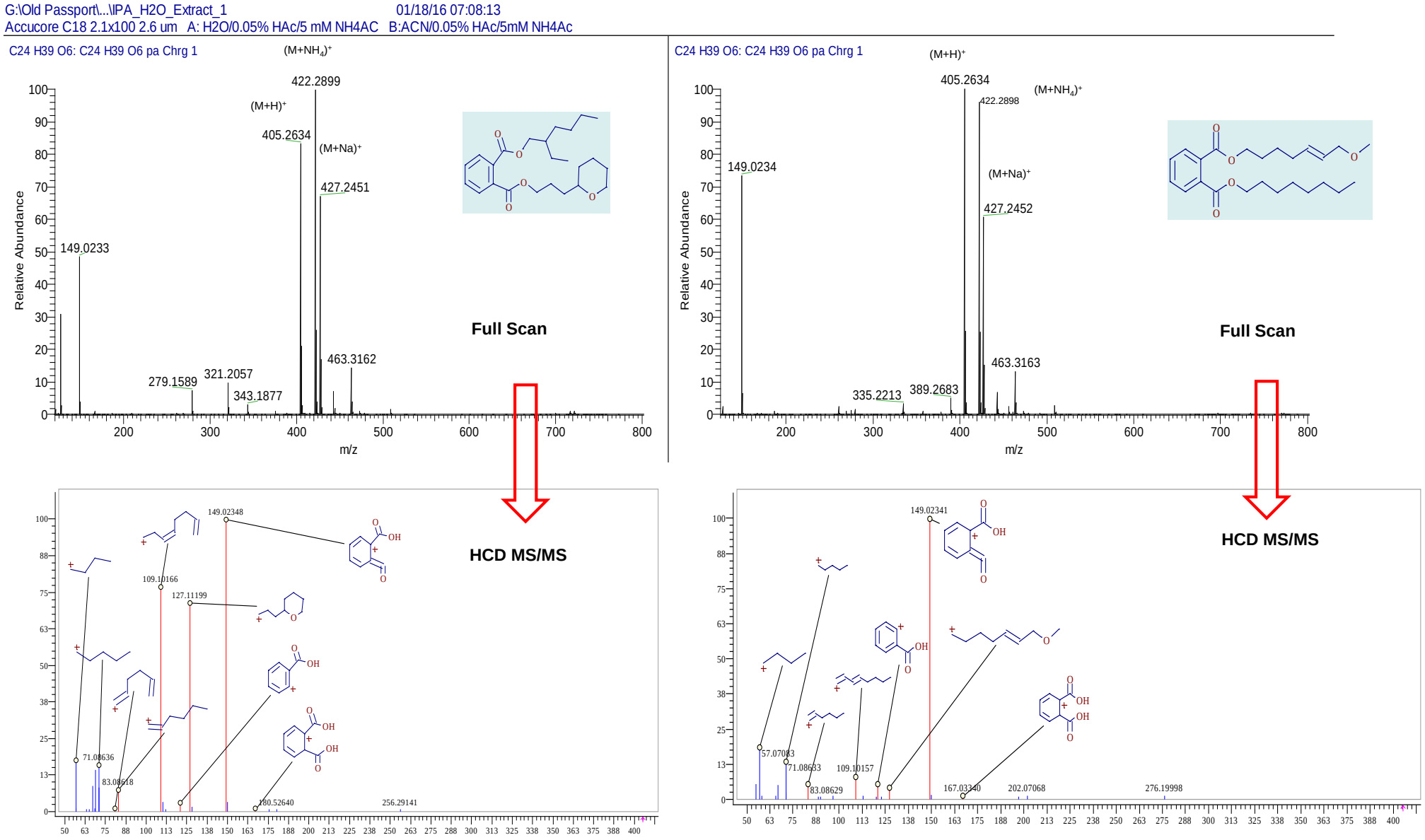


ThermoFisher SCIENTIFIC

IV Bag IPA-H2O Extract - Positive Negative Full Scan, MSMS and UV in One Run

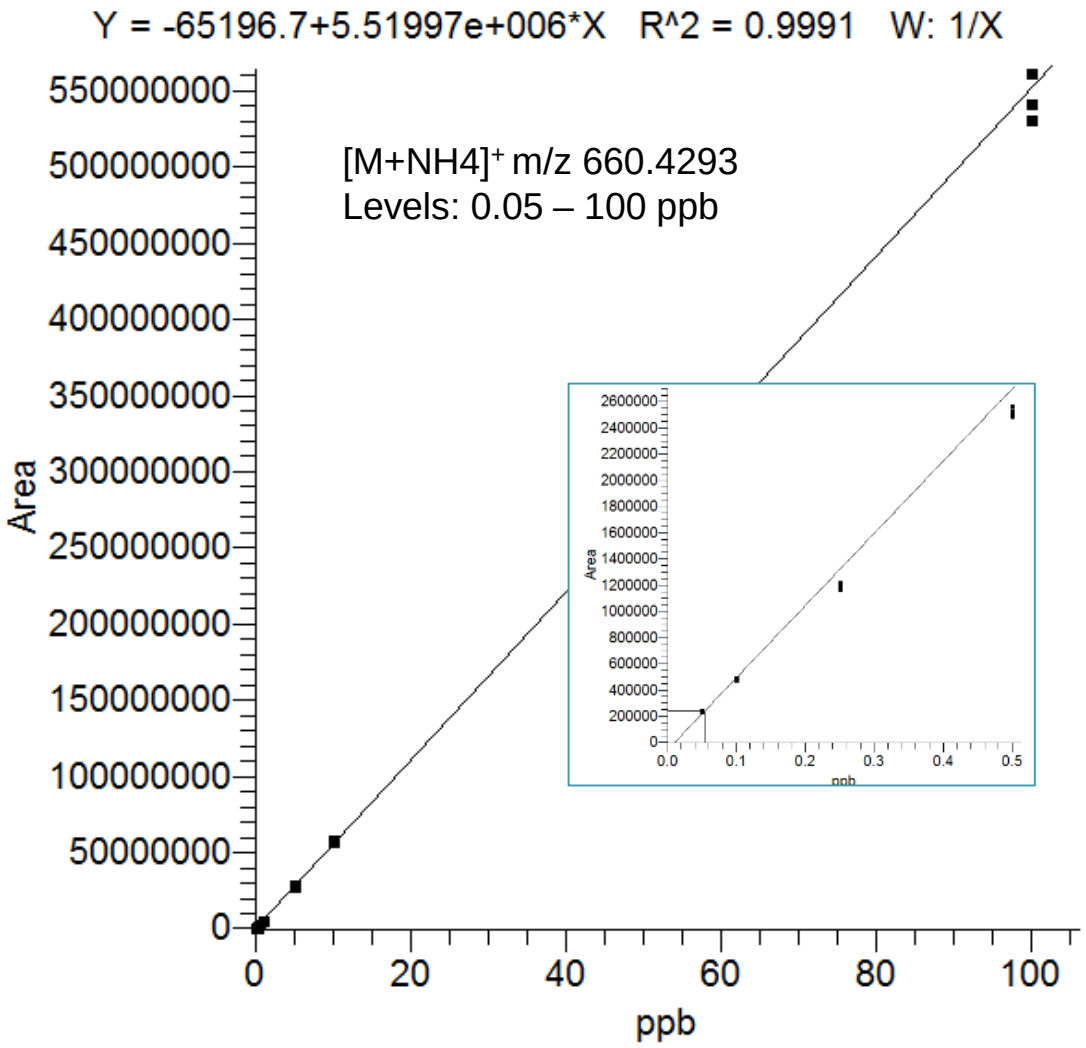


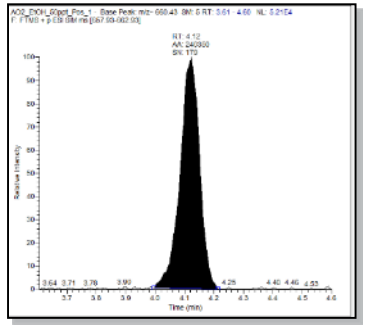
Full Scan and HCD MS/MS Data for Component ID and Structure Elucidation



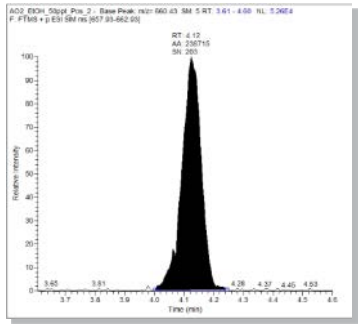
ThermoFisher SCIENTIFIC

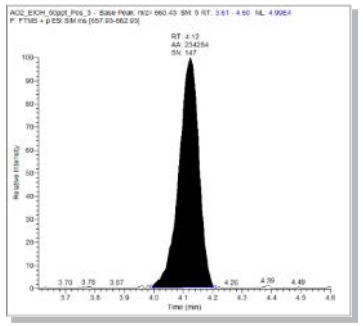
Quantify Non-Volatile Extractables – Irganox1035





+VE



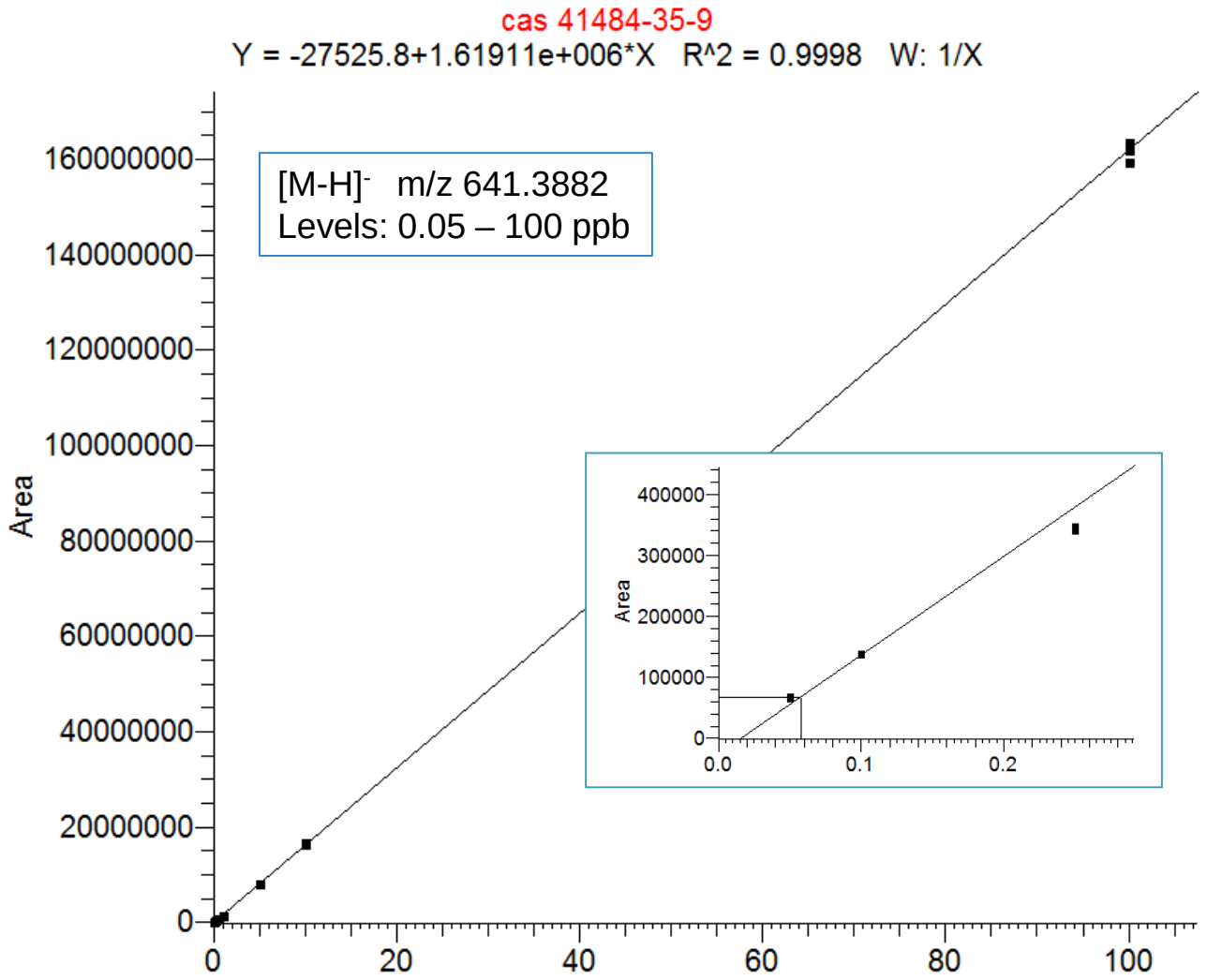


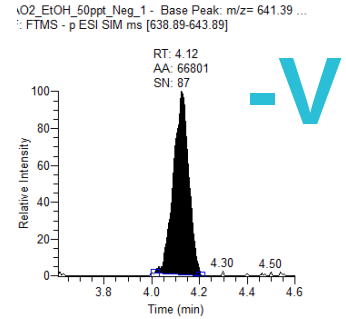
50 ppt
(Triple Injections)

Detect in positive and negative ion mode in the same run

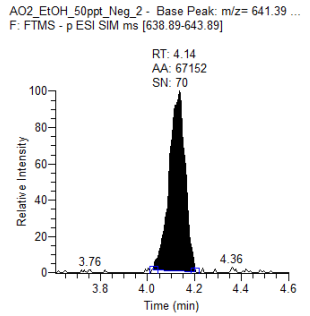
ThermoFisher SCIENTIFIC

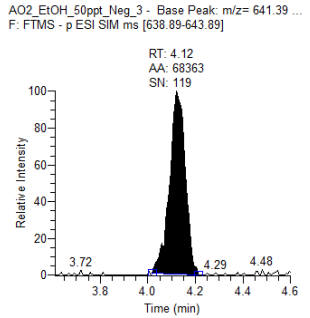
Quantify Non-Volatile Extractables – Example Irganox1035





-VE





50 ppt
(Triple Injections)

Full sensitivity in negative ion mode

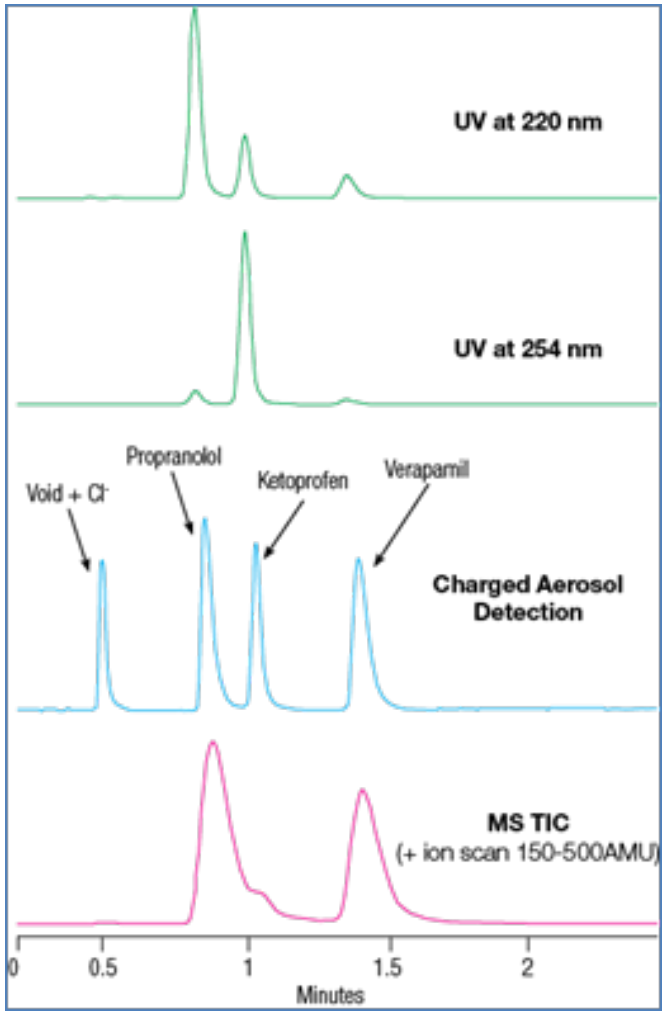
ThermoFisher SCIENTIFIC

See What You're Missing with Charged Aerosol Detection

Thermo Scientific™ Vanquish™ Charged Aerosol Detector

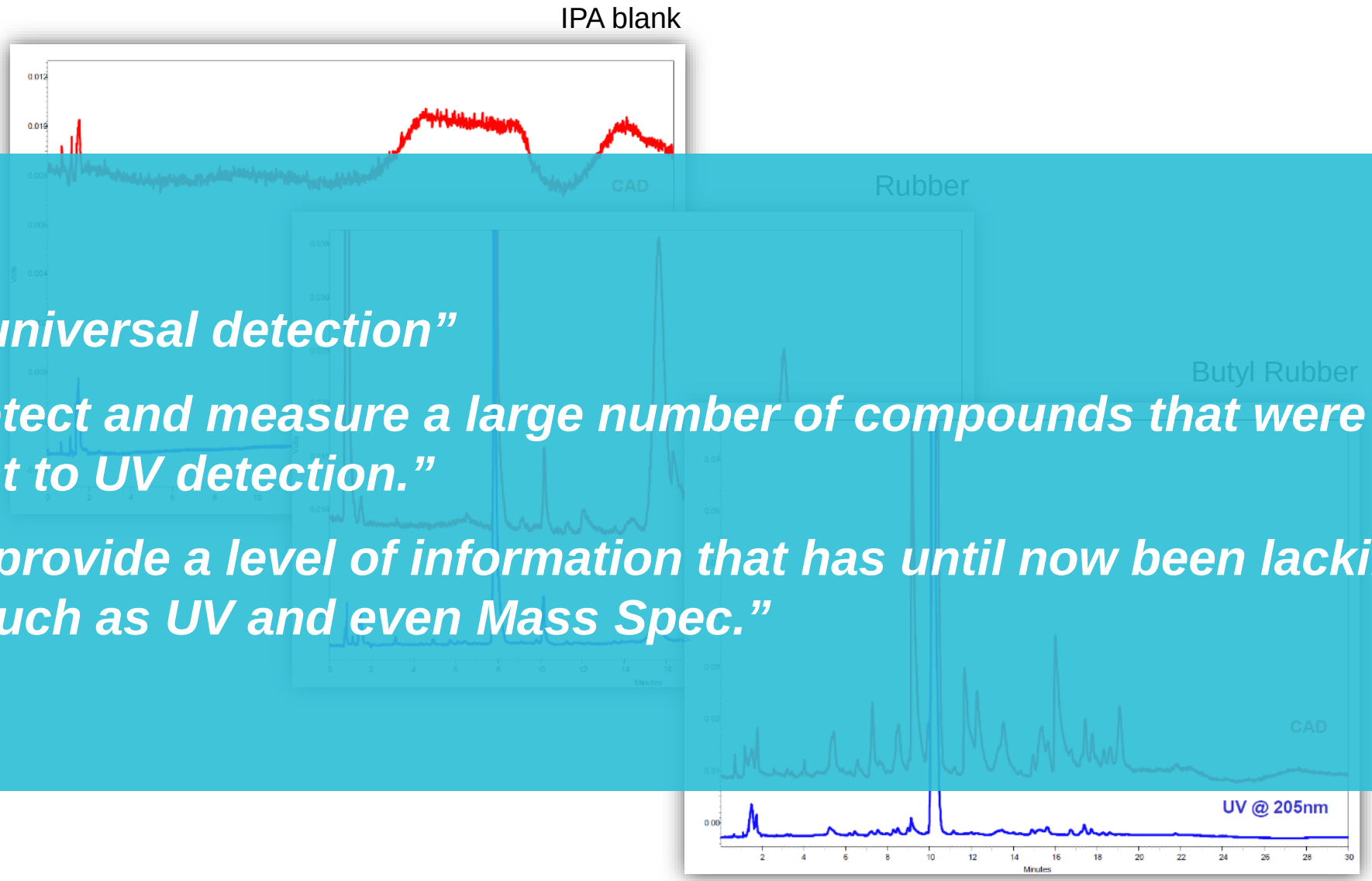
- Detect components without chromophore
- Quantify without exact standards
  - Relative quantification due to consistent response
  - Use virtually any standard for simplified AET calculations
- Consistent analyte response
- Four orders dynamic range
- USP <1663> listed

Comparison of Charged Aerosol Detection Result to UV and MS



ThermoFisher SCIENTIFIC

CAD for Extractables and Leachables



- ***"Virtually universal detection"***
- ***"able to detect and measure a large number of compounds that were completely transparent to UV detection."***
- ***"CAD can provide a level of information that has until now been lacking with methods such as UV and even Mass Spec."***

Data from ESA Biosciences, Inc., Chelmsford, MA

Data Analysis Software, Database and Spectral Library for E&L Analysis

Thermo Scientific™ Compound Discoverer™ 2.1

Thermo Scientific™ TraceFinder™ Targeted Screening and Quan

Thermo Scientific™ Mass Frontier™ Spectral Interpretation software

mzCloud is a trademark of HighChem LLC, Slovakia

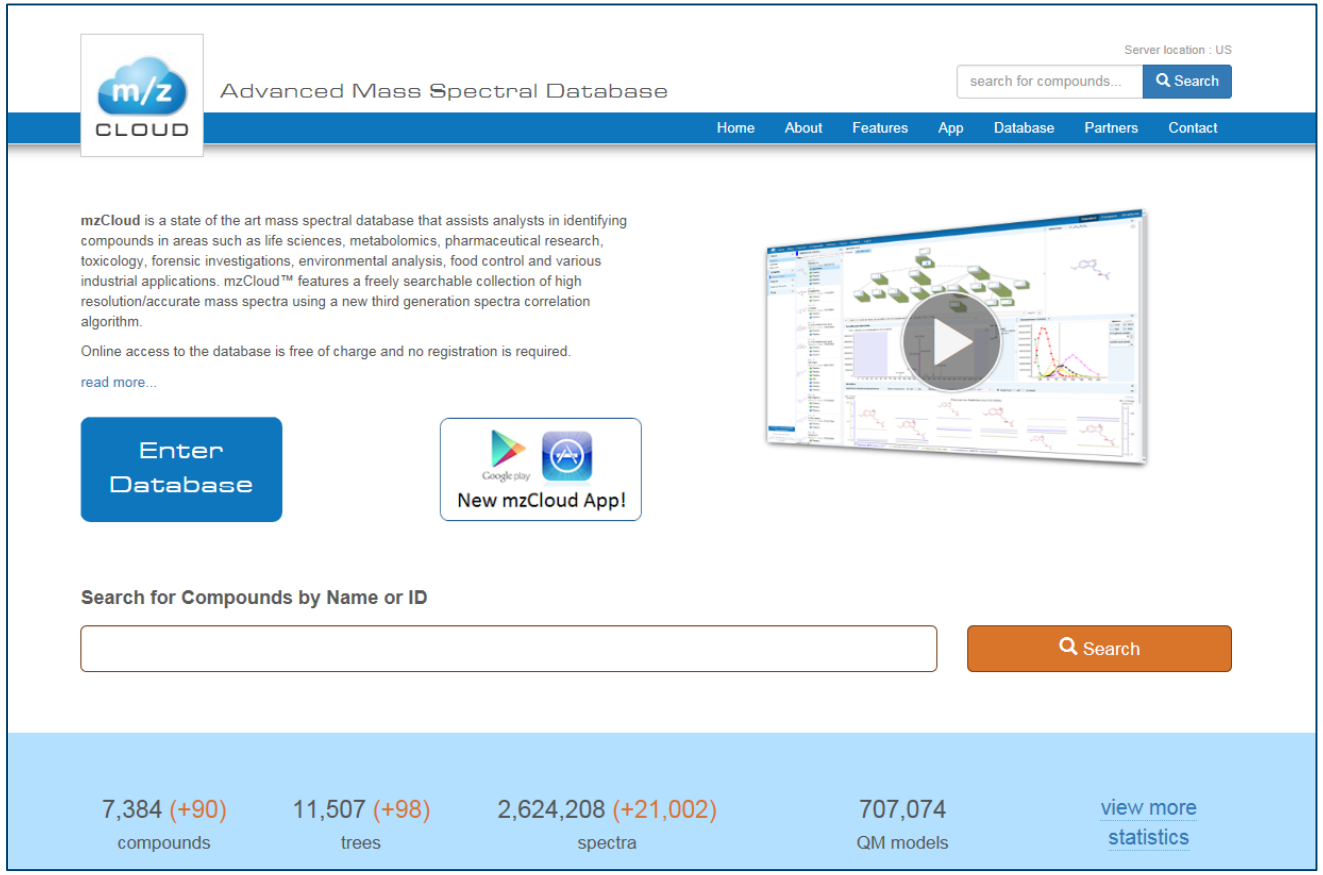
mzVault™ 2.0 Library Search and Manager

E&L Compound Database

mzCloud™ – HR Mass Spectral Database

mzCloud™ Database: Free and cloud-based (www.mzcloud.org)

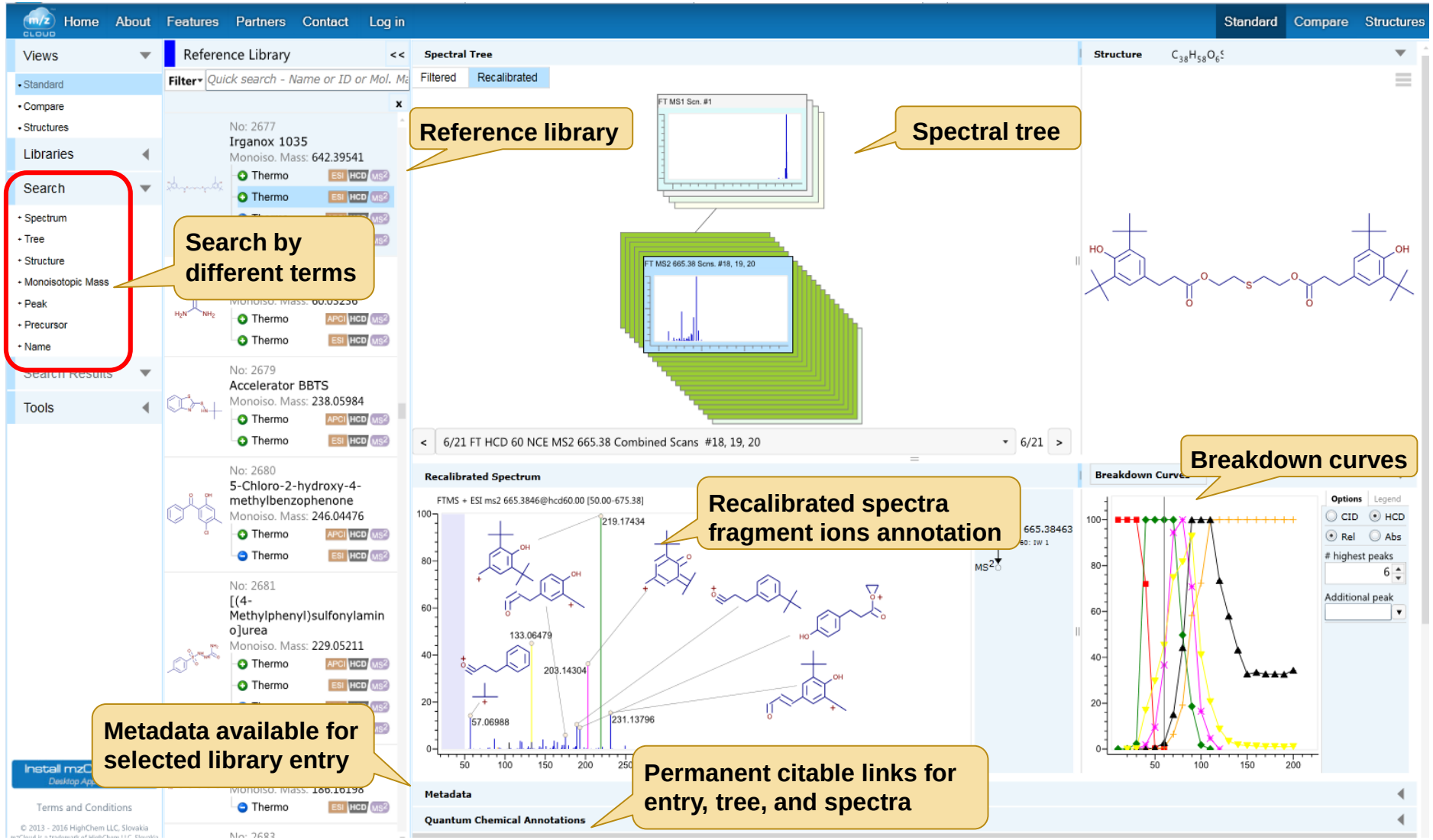
-Advanced high resolution mass spectral database
-Very high quality data: using standardized acquisition, highly curated data
-Identifying compounds, even when they are not present in the library, through substructure search



mzCloud is a trademark of HighChem LLC, Slovakia

ThermoFisher SCIENTIFIC

mzCloud™ Spectral Library

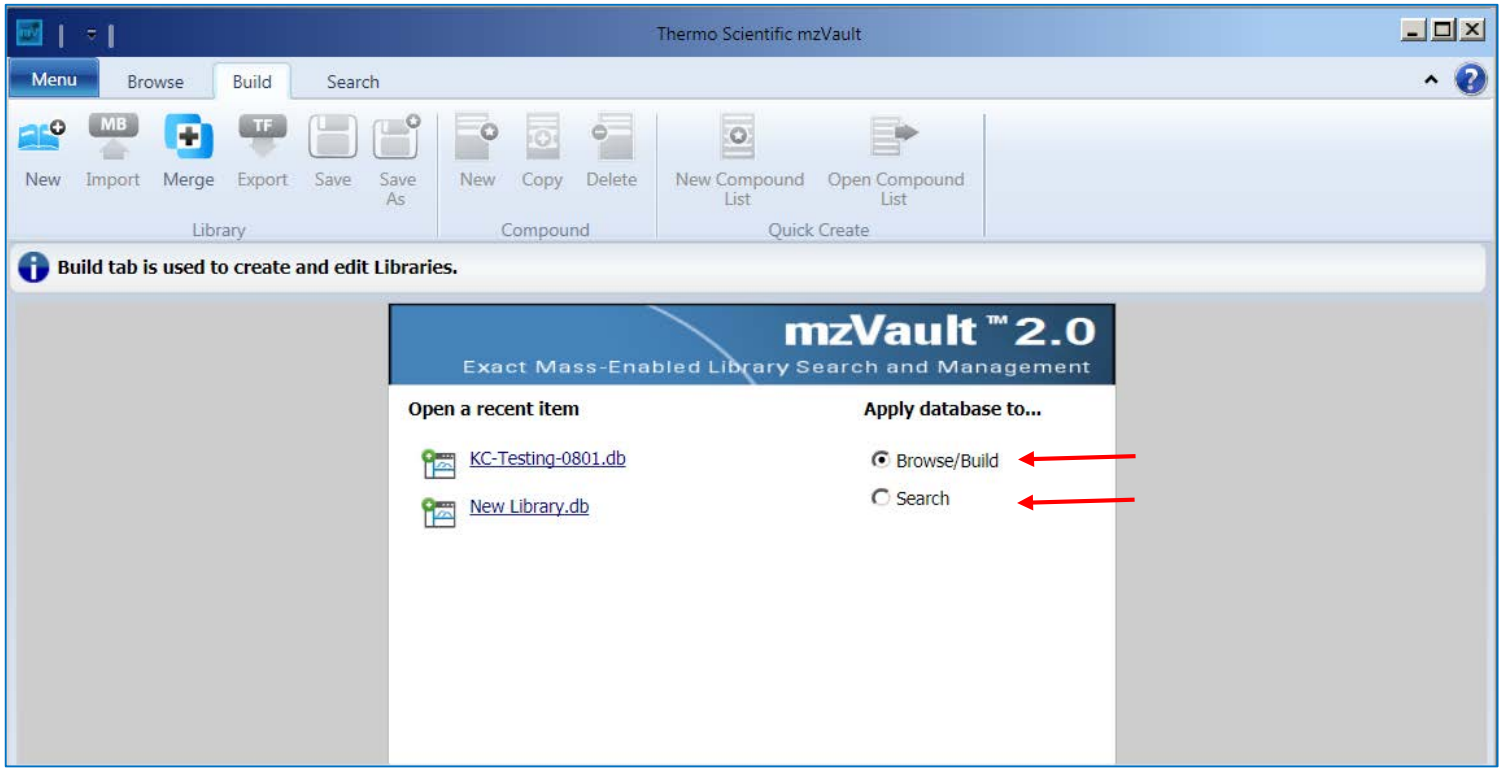


mzCloud is a trademark of HighChem LLC, Slovakia

ThermoFisher SCIENTIFIC

mzVault 2.0

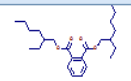
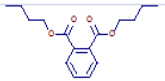
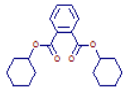
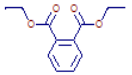
Exact Mass-Enabled Library Search and Management
- Local mzCloud
- Creation of custom library
- Searching library



ThermoFisher SCIENTIFIC

E&L Compound Database in Thermo Scientific Compound Discoverer 2.1

This Excel sheet database contains ~2000 common E&L related compounds.
This is a “living document” and new E&L related compounds are added periodically.

Commercial Name	Chemical Name	Class	CAS No.	Chemical Formula	Formula weight	$(M+H)^+$	$(M-H)^-$	$M+NH_4^+$	$M+Na^+$	$M+K^+$	Structure	mzCLoud link
dioctyl phthalate	ethylhexylphthalate	PL	117-81-7	C24H38O4	390.27701	391.28429	389.26973	408.31083	413.26623	429.24017		https://mzcloud.org/DataViewer.aspx#CReference2
Dibutyl phthalate	Dibutyl phthalate	PL	84-74-2	C16H22O4	278.1518	279.15908	277.14452	296.18562	301.14102	317.11496		https://mzcloud.org/DataViewer.aspx#CReference2
	Dicyclohexyl phthalate		84-61-7	C20H26O4	330.18311	331.19039	329.17583	348.21693	353.17233	369.14627		https://mzcloud.org/DataViewer.aspx#CReference2
	Diethyl phthalate		84-66-2	C12H14O4	222.08921	223.09649	221.08193	240.12303	245.07843	261.05237		https://mzcloud.org/DataViewer.aspx#CReference2
	Dimethyl phthalate		131-11-3	C10H10O4	194.05791	195.06519	193.05063	212.09173	217.04713	233.02107		https://mzcloud.org/DataViewer.aspx#CReference2

This database has been added to Compound Discoverer as “E&L Mass List”.

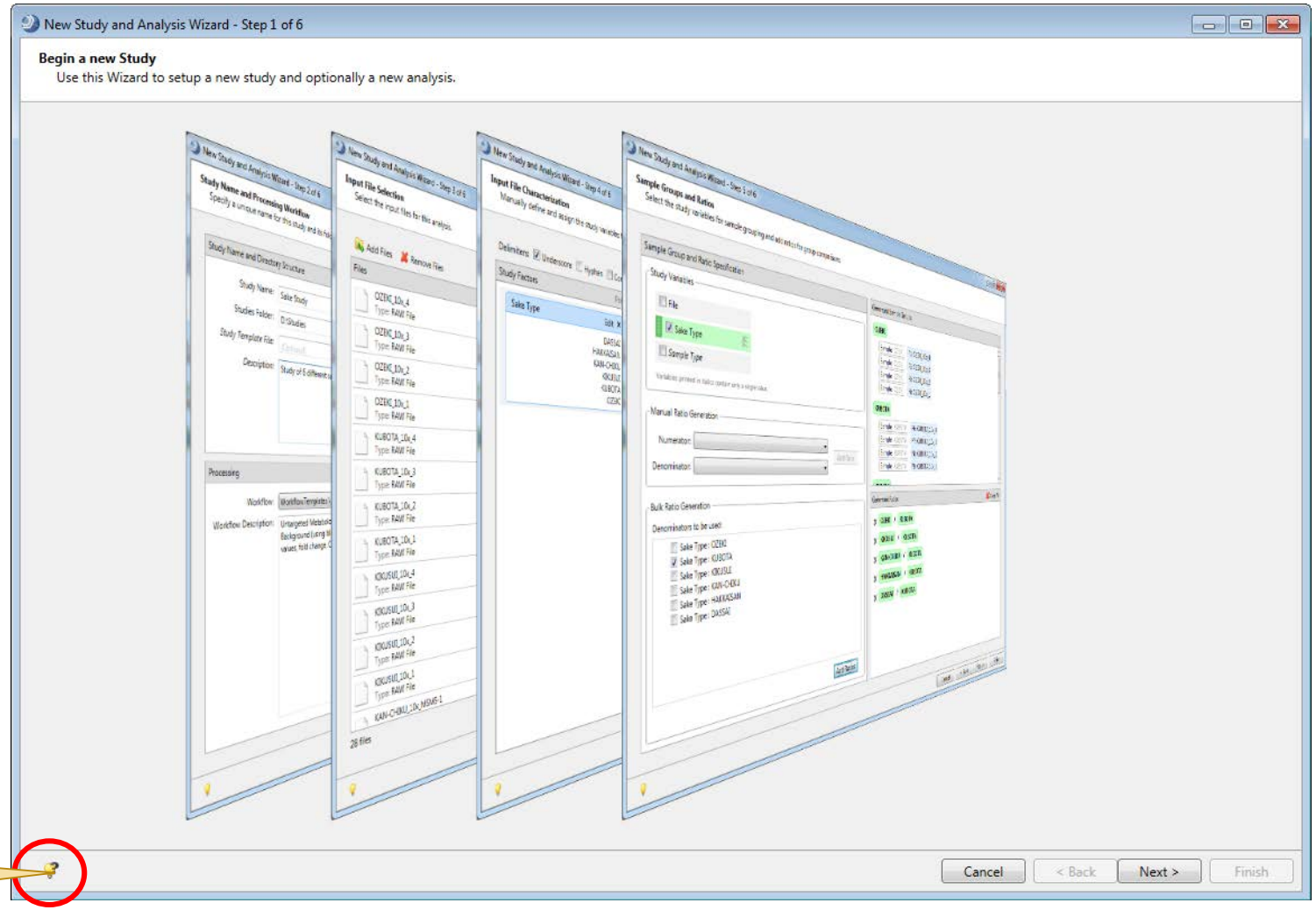
Thermo Scientific Compound Discoverer 2.1: Compound ID and Structure Elucidation

- Compound Discoverer is a small molecule structure analysis software package.
  - It processes high HRAM data generated by Thermo Scientific™ Orbitrap™ MS (LCMS & GCMS)

- It uses flexible node-based processing workflow and HRAM data, isotope pattern matching, conduct component extraction and elemental composition prediction. The component structures are identified using the following techniques:
  - **Known compound ID** through multiple database searching and fragment spectral matching.
  - **Known unknown compound ID** - ChemSpider molecular formula and molecule weight search: using “Structure Proposals” feature to interrogate the fragmentation allows identification of the correct structure.
  - **Total unknown structure elucidation** - For components without database search results, the predicted compositions, MS/MS fragment ions, and similarity search result (mzCloud fragment ions matches) are used to propose putative structure. De Novo structure ID
  - **Validity check of the proposed structure** - Using “FISh Scoring” feature, searching the embedded “HighChem Fragmentation Library”. FISh stands for “Fragment Ion Search”

- Differential analysis for different lots, batch, or type of sample analyses

Thermo Scientific Compound Discoverer 2.1: Wizard for Processing Workflow Build

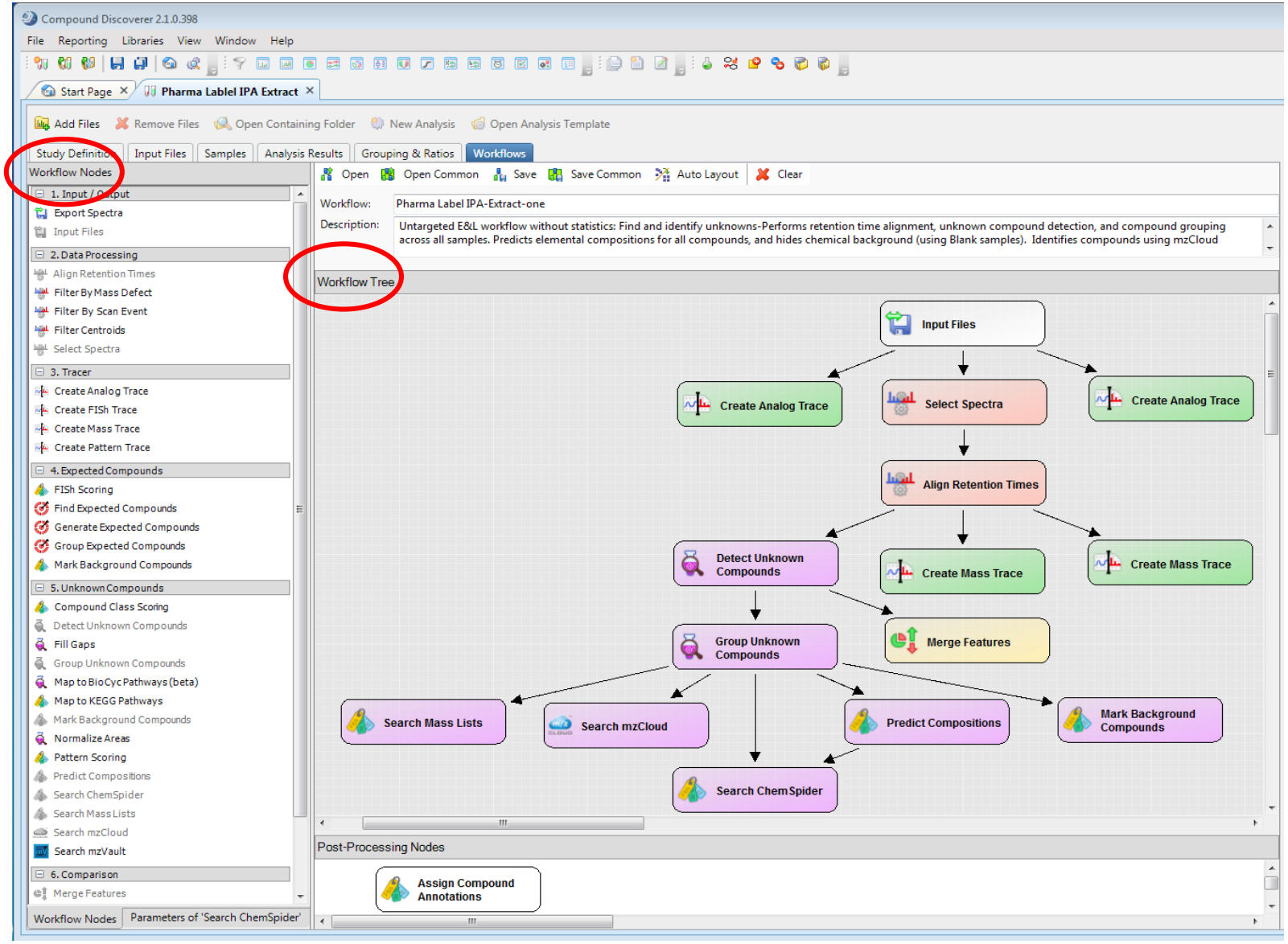


Description for each step

Creating a study and analysis using the guided “New Study and Analysis Wizard” and built-in workflow templates.

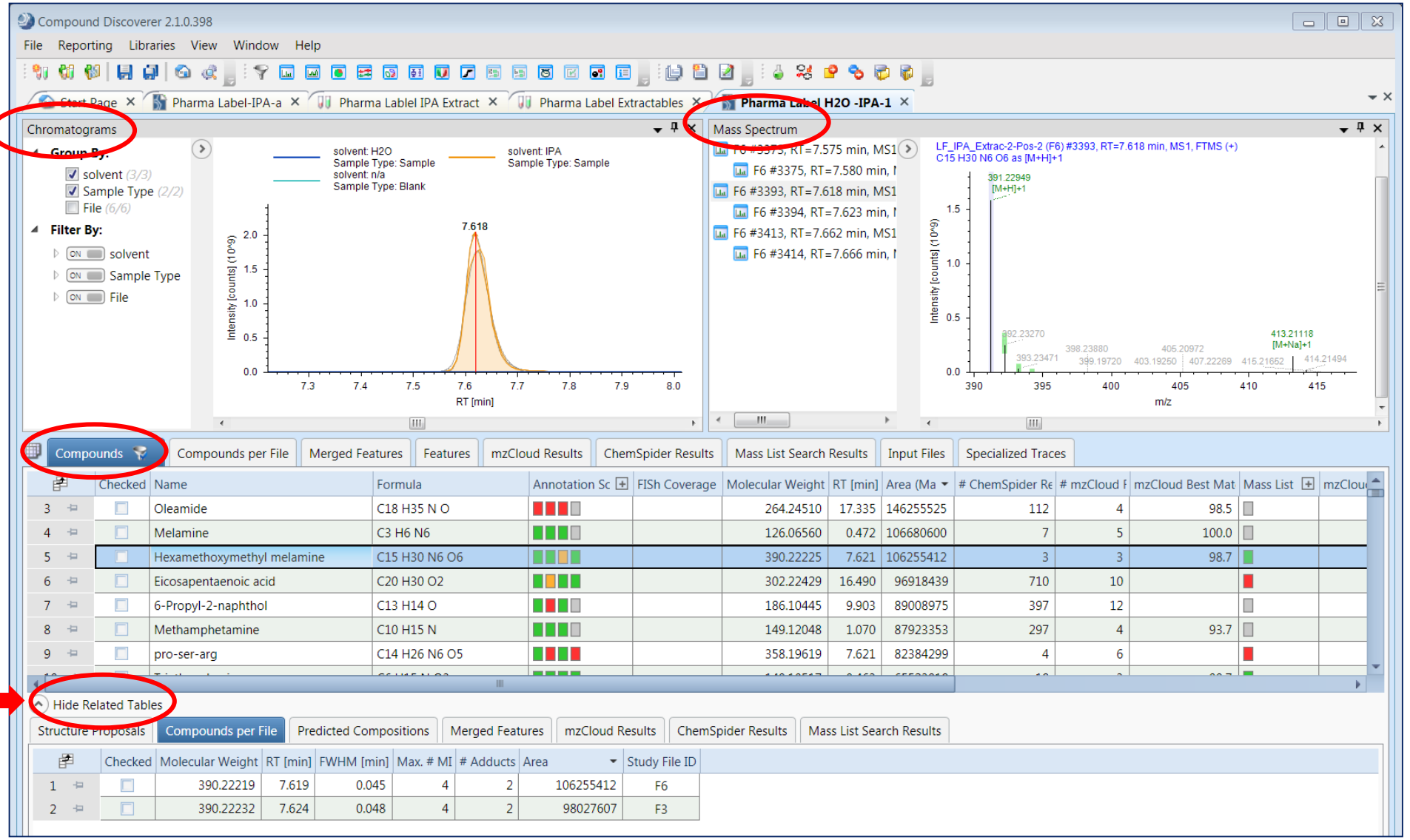
ThermoFisher SCIENTIFIC

Node-Based Processing Workflow



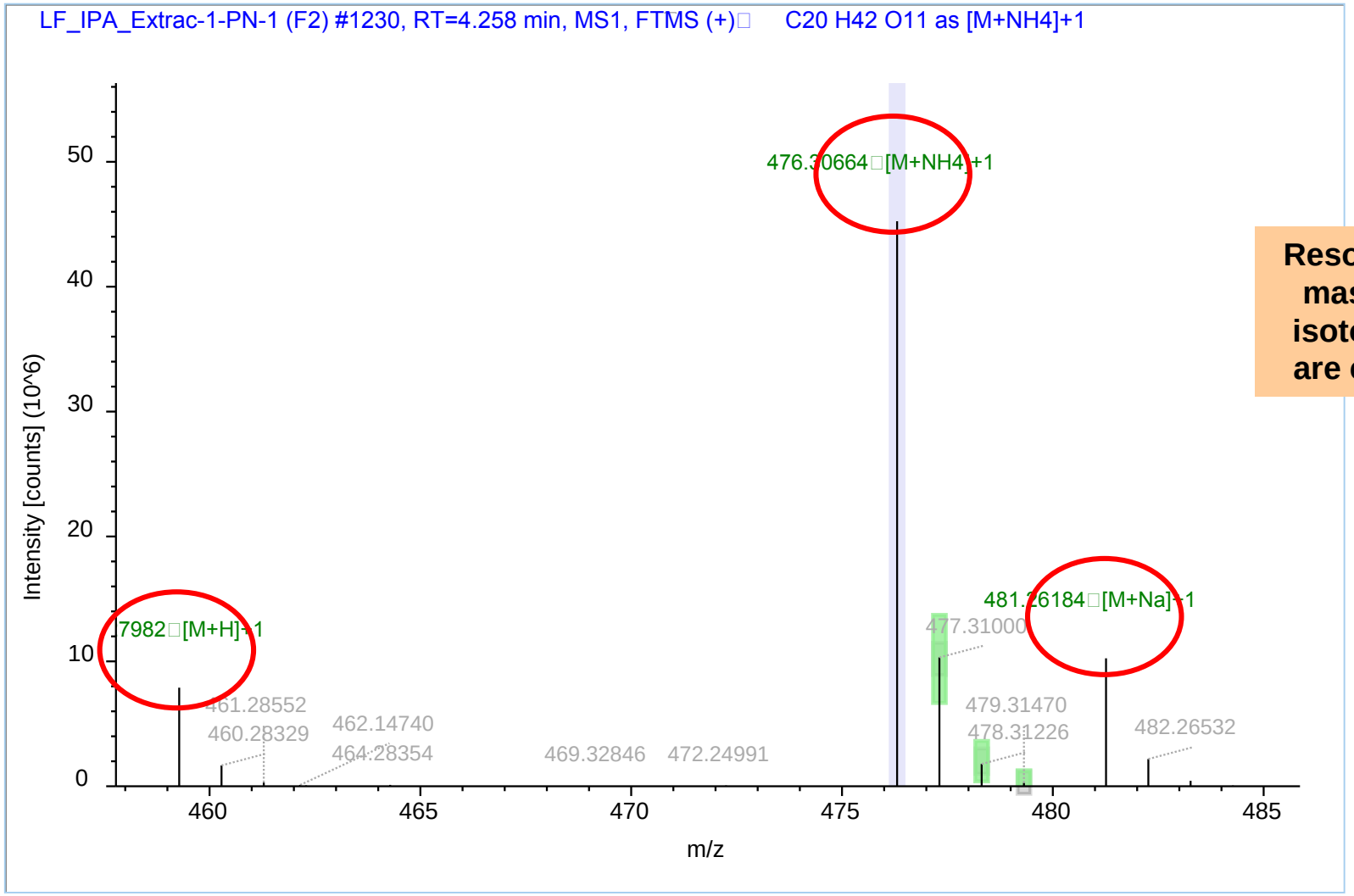
ThermoFisher SCIENTIFIC

Result View – Data Interpretation



Sub-tables

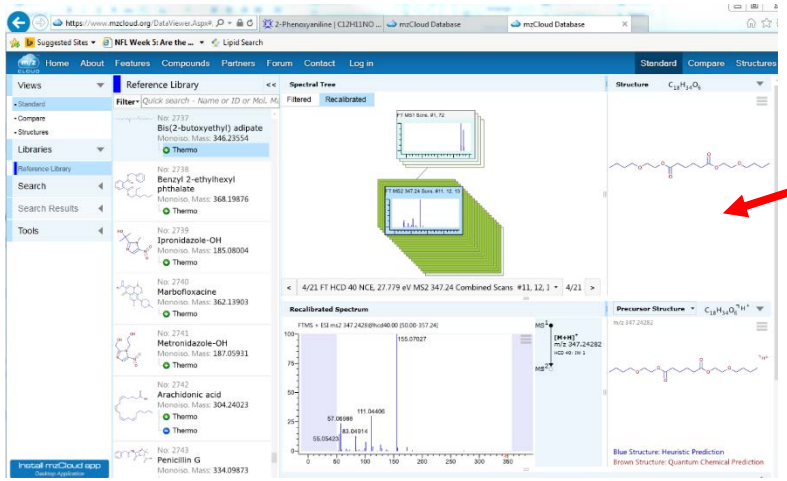
Component Detection with Adducts Grouping

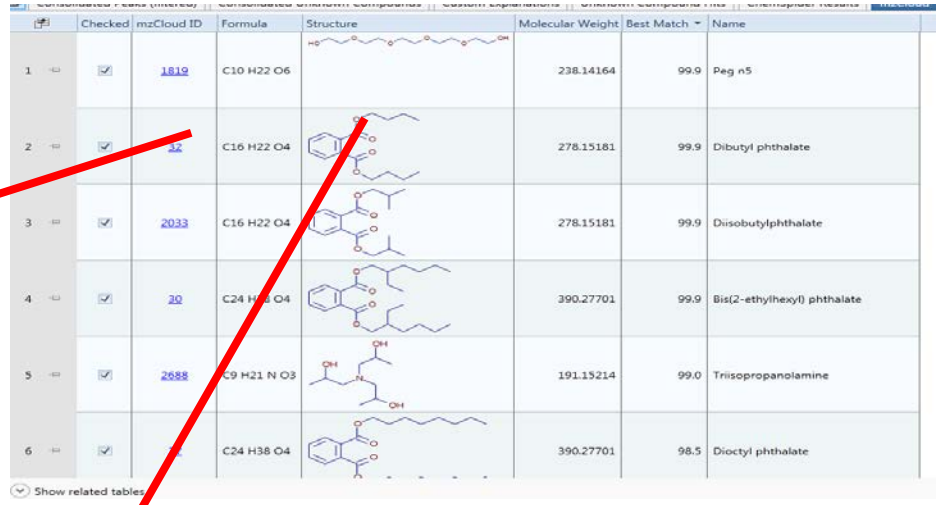


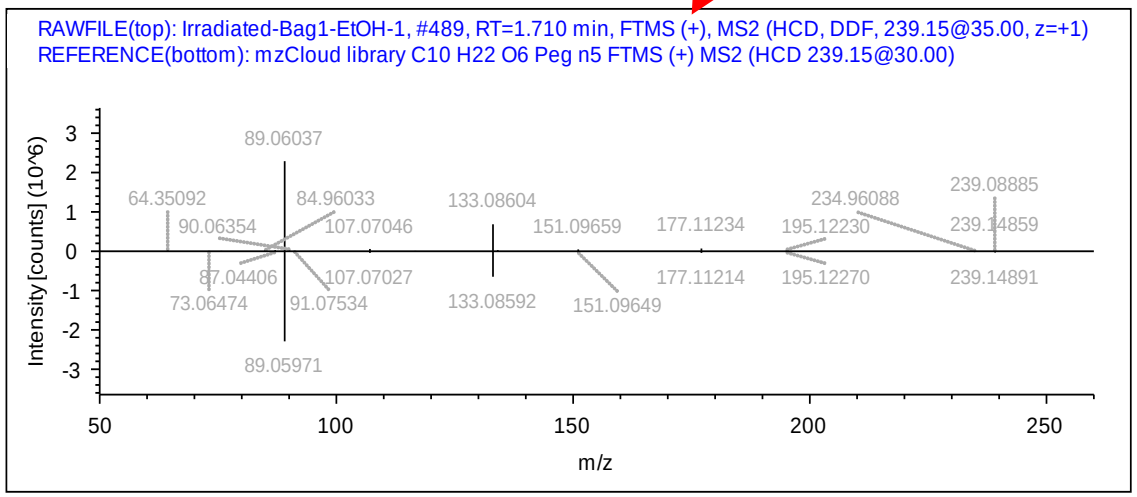
Resolution-aware mass accuracy isotope patterns are color coded.

ThermoFisher SCIENTIFIC

Known Compound ID Through mzCloud™ Database Search







mzCloud™ Mirror plot

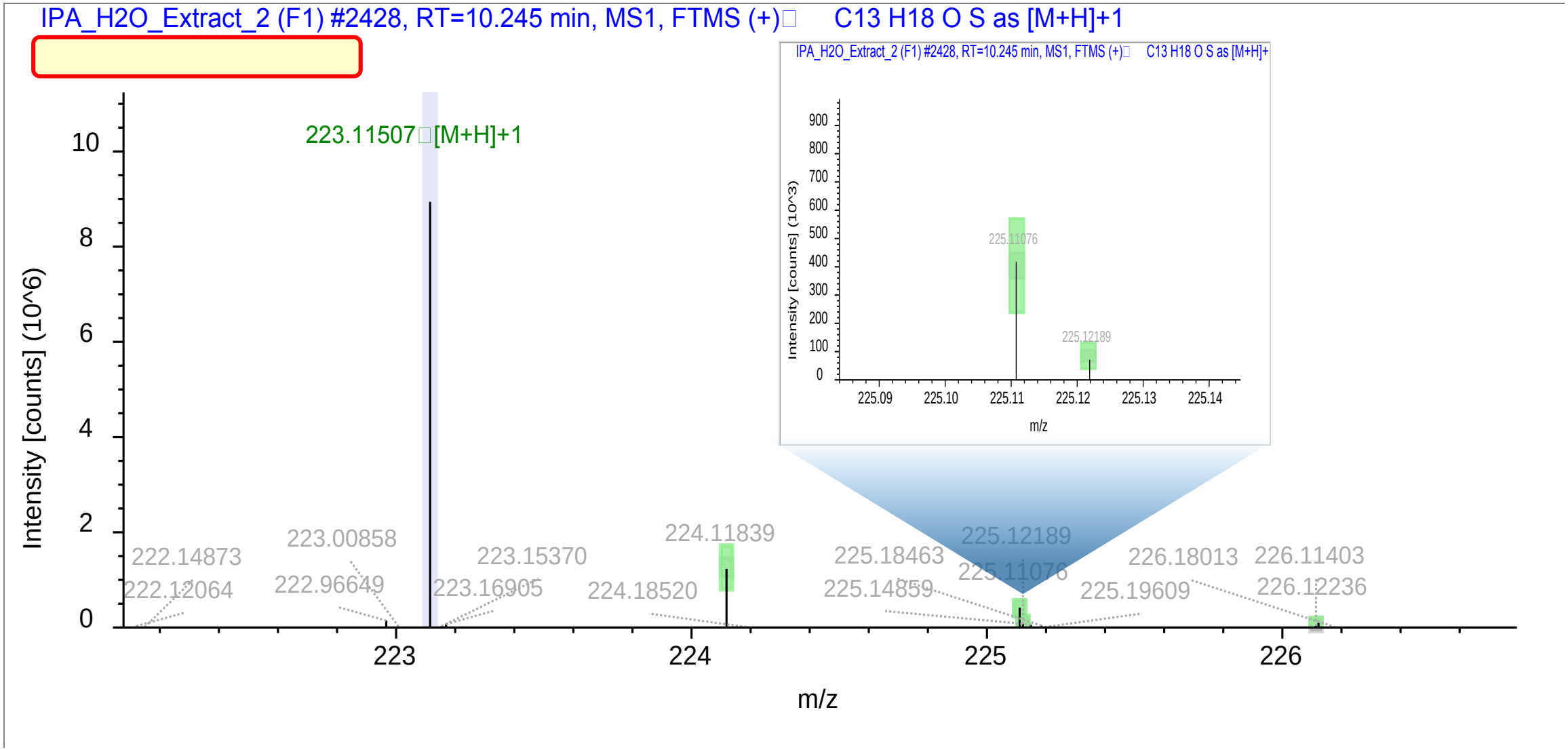
mzCloud is a trademark of HighChem LLC, Slovakia

ThermoFisher SCIENTIFIC

Unknown Structure Elucidation Using Compound Annotation Editor and FISh Scoring

ThermoFisher SCIENTIFIC

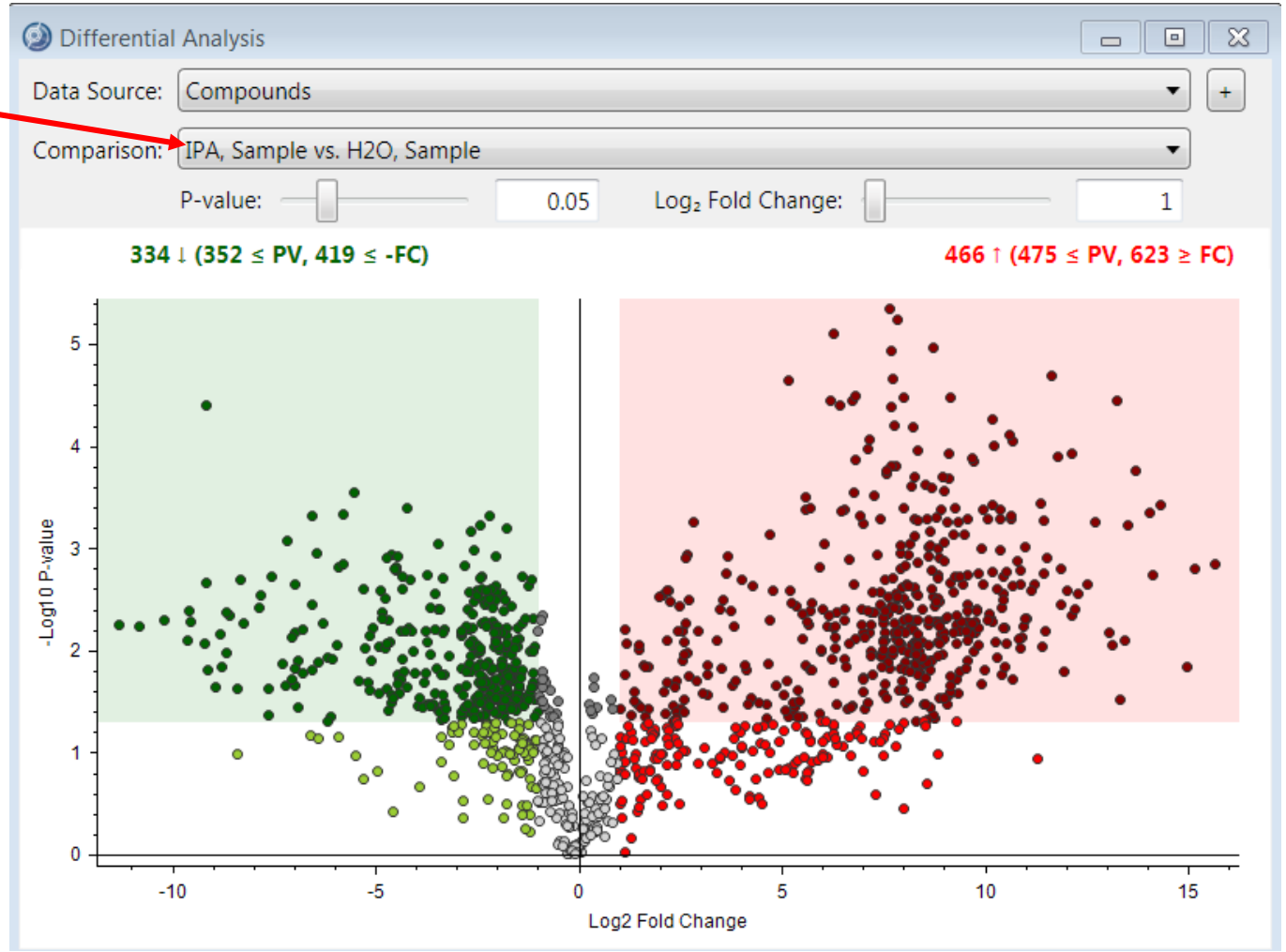
Unknown Structure Elucidation Starting from CD 2.1 “Predicted Compositions”



ThermoFisher SCIENTIFIC

Thermo Fisher Compound Discoverer 2.1 Differential Analysis Feature: Volcano Plot

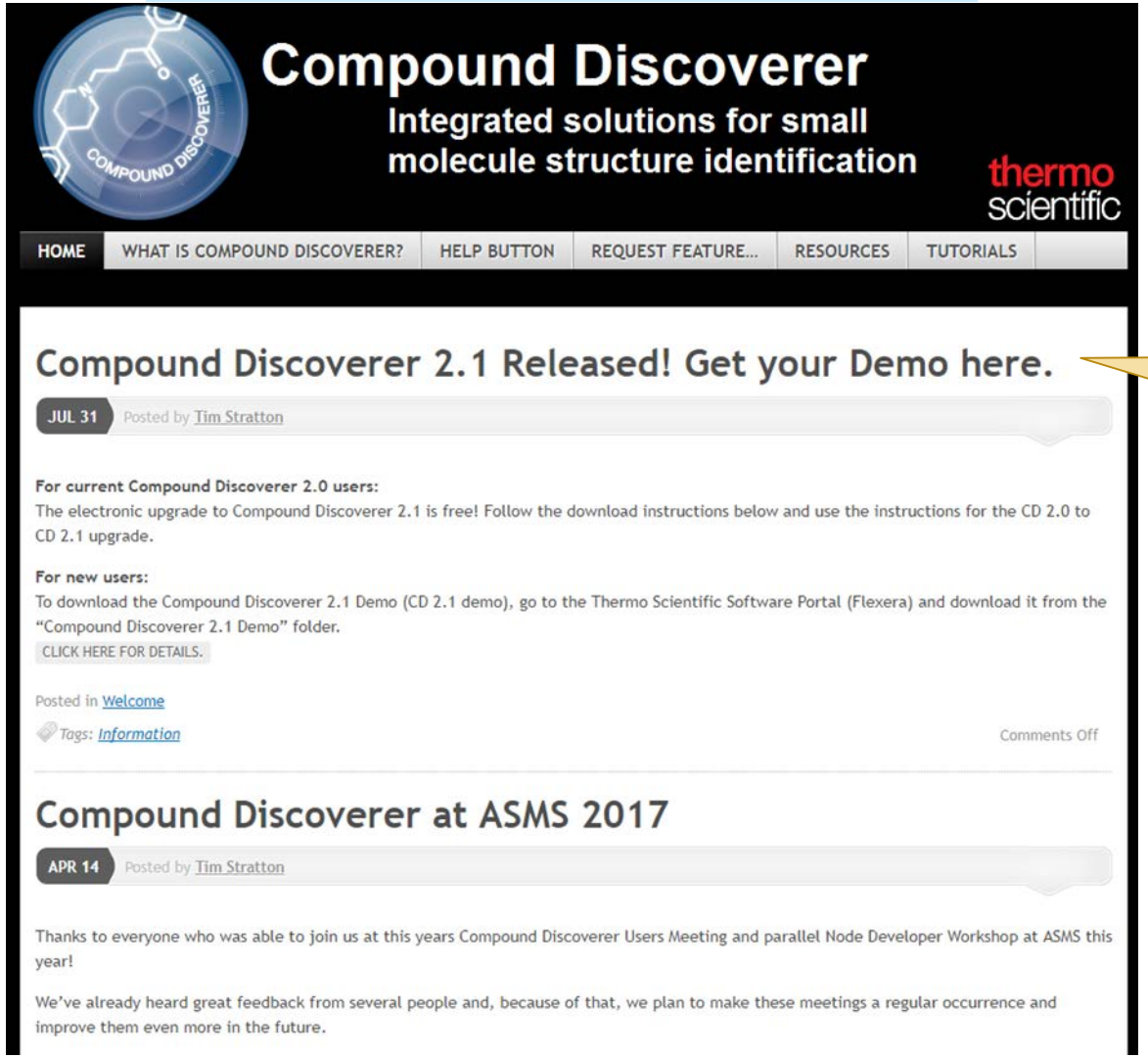
Using the interactive volcano plot to find compounds that are significantly different between two sample groups



ThermoFisher SCIENTIFIC

Thermo Scientific Compound Discoverer (CD) Home Page

http://mycompounddiscoverer.com



Download a DEMO copy, and watch the tutorials videos here.

ThermoFisher SCIENTIFIC

Join the Fun! *Cache a Chromeleon* Game

- Use your mobile device to complete challenges and earn a Charlie Chromeleon plush toy!
- If you are playing, you have earned points for attending this seminar. Be sure to scan the barcode on the desk outside the door.
- Ask booth staff for more details on how to play.

Thank You

Please join me in the
Mass Spectrometry
section of our booth where I'll
address additional comments and questions.