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Solution for Extractables & Leachables Analysis

- Instruments, Software, Database/Spectral Library

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Small molecule and E&L analysis
Pharma/Biopharma Marketing, CMD

Outline

- Introduction
  - Definitions
  - Industries need Extractable & Leachable (E&L) analysis
  - Regulatory and industry groups
- Thermo Fisher Scientific solution for extractable & leachable analysis
  - Multi instrumentations and technologies
  - Data Analysis software for ID, structure elucidation, and quantitation
  - Database and 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

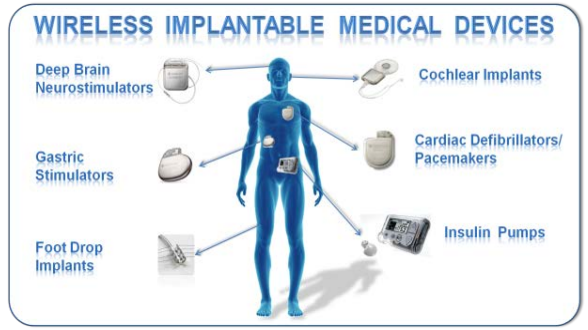
The Broad Market of Extractable & Leachable Analysis




Single-use systems for bioproduction and storage







Medicine container/packaging, implant and diagnostic devices

Food package

Printing ink and adhesives

Wearable Consumer Electronic Products

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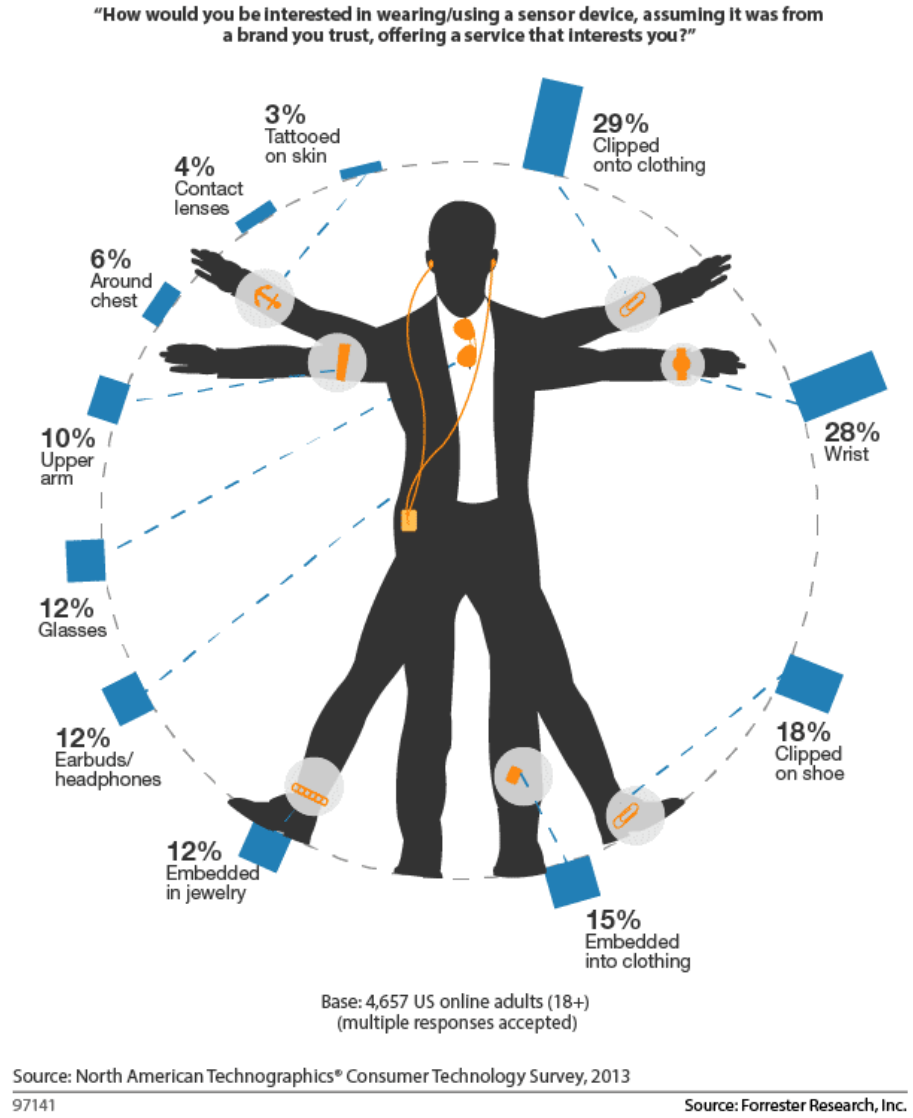
Food Contact Material Migration Test is Mandatory!

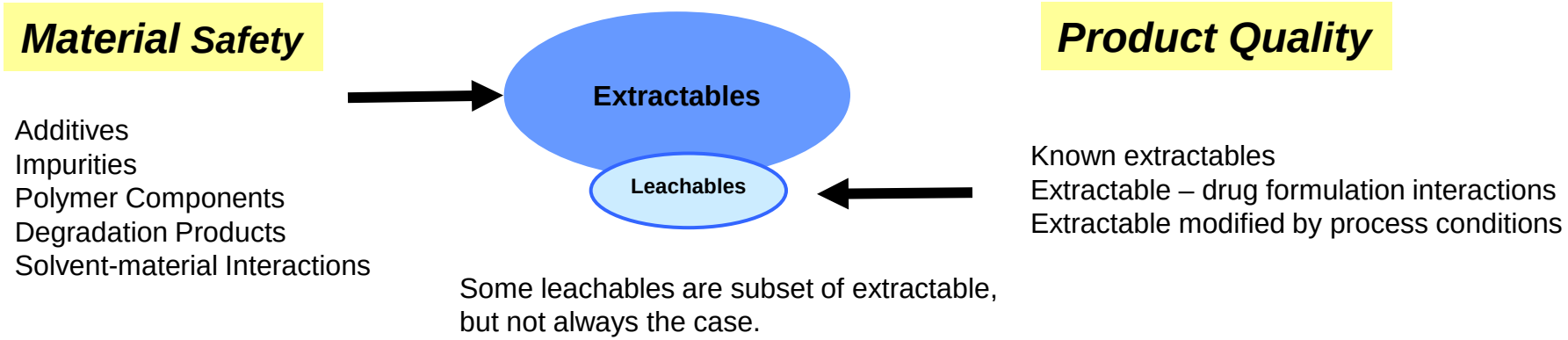
Wearable Consumer Electronic Products



The overall market for wearable technology is expected to reach USD 31.27 Billion by 2020, at a CAGR of 17.8% between 2015 and 2020.

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. *Formulated drug or simulants*



Safety/risk assessment ➡ Submission to regulatory agency ➡ Market

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.

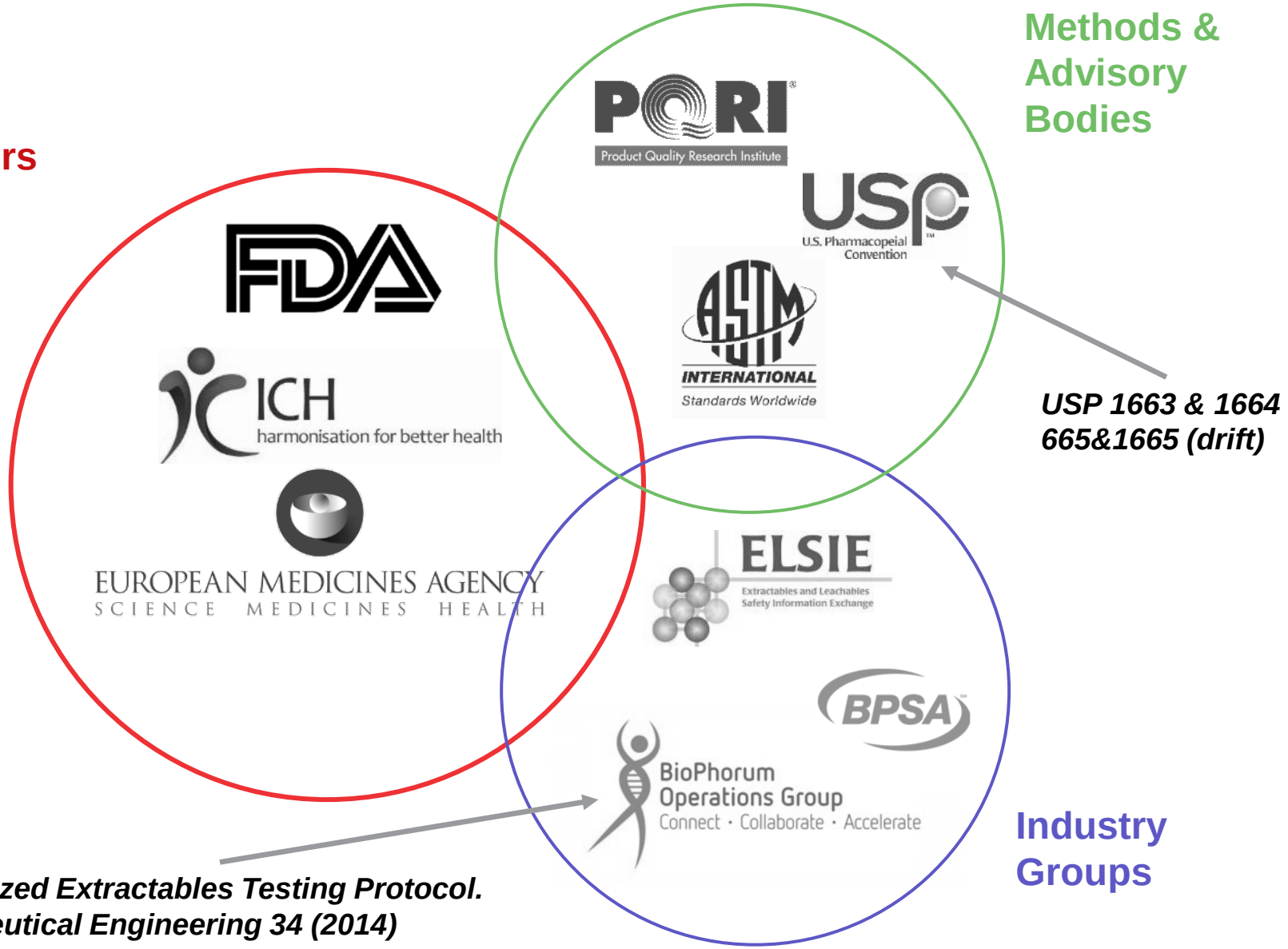
Regulations

- E&L analyses are highly regulated and required for market approval of new products.
- More regulations have been made along the way and will continue into the future.

E&L analyses are 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



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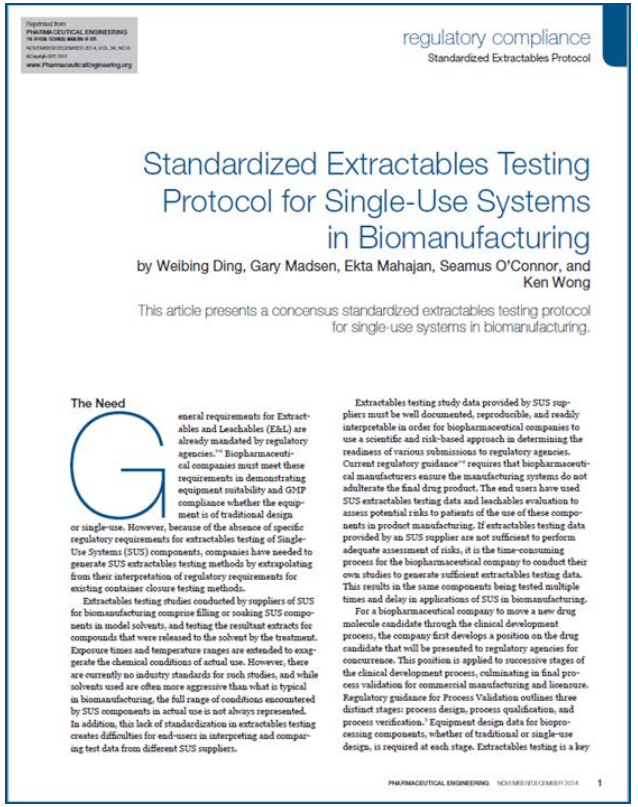
Regulations & Guidelines

- FDA guidance
  - Container Closure Systems for Packaging Human Drugs and Biologics (May 1999)
  - 21 CFR 211.94
- EMA guideline
  - Guideline on plastic media packaging, EMEA/205/04
- ICH guidelines
  - Test procedures and acceptance criteria for new drug substances and drug products (Q6A,B)
  - Pharmaceutical development (ICH Q8)
- PQRI leachables and extractables working group (recommendations to the FDA)
  Safety thresholds and best practices for extractables & leachables in orally inhaled and nasal drugs
- USP Chapters
  - Chapter 87,88 (materials direct or indirect in contact with patients biological reactivity test in vitro, in vivo)
  - Chapter 661 (Containers – Plastics)
  - Chapter 1663 Extractables Associated with Pharmaceutical Packaging/Delivery Systems
  - Chapter 1664 Drug Product Leachables Associated with Pharmaceutical Packaging Delivery Systems
  - Chapter 1665_draft Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products.
- ISO 10993 parts (medical devices)
- Japan, China, and other countries healthy authorities...

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/

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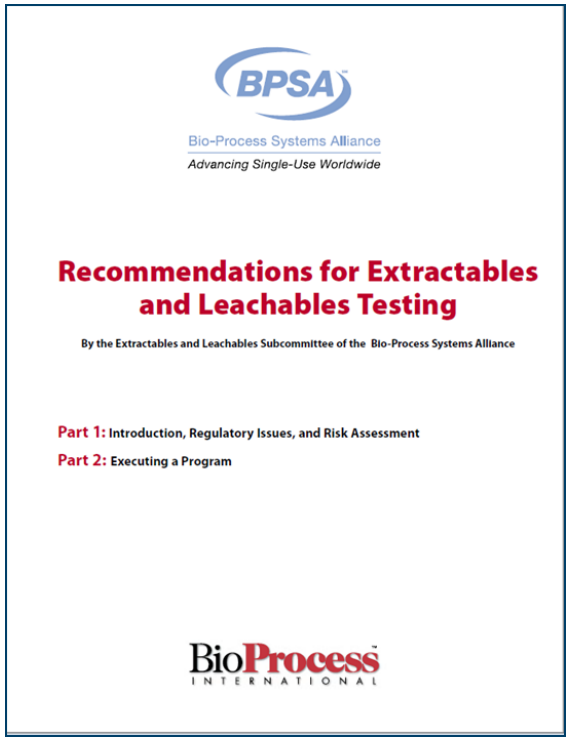
Industry Group: Bio-Process Systems Alliance BPSA Published Technical Guides

http://bpsalliance.org/

BPSA members are industry-led corporate member of single use manufactures. BPSA is encouraging and accelerating the adoption technologies used in the production of biopharmaceuticals and vaccines.

Many Technical Guides on their website
http://bpsalliance.org/



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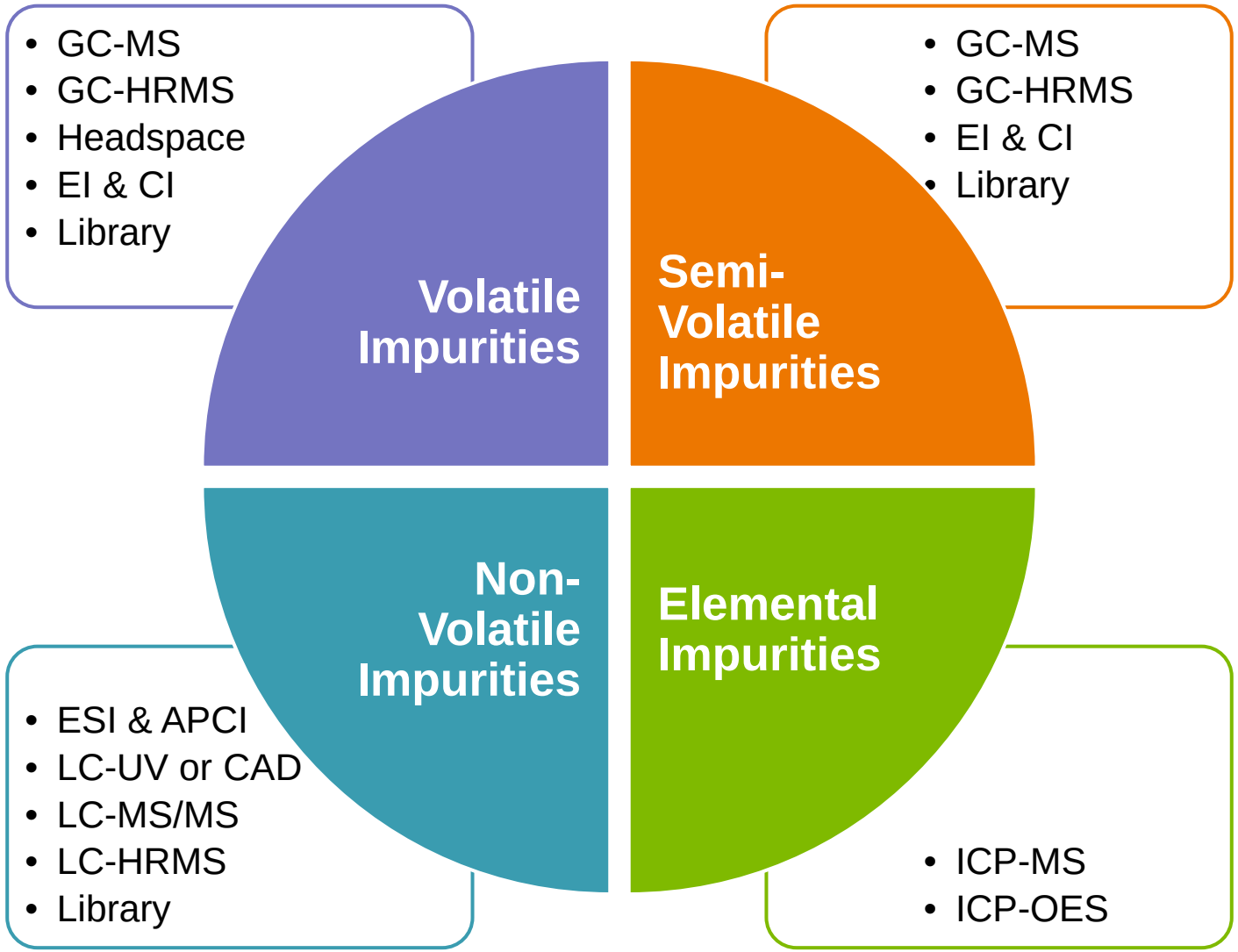
Extractable & Leachable Analysis

Analysis of Extractables & Leachables



Thermo Scientific Instruments and Software for Impurity ID and E&L Analysis

ASE

FTIR

Q Exactive GC-MS/MS

GCMS -Single Quad

GCMS-Triple Quad

IC

ICPMS

Charged Aerosol Detector

Vanquish UHPLC

Triple Quad

Benchtop HRMS
Q Exactive

Hybrid HRMS

Orbitrap Fusion Tribrid MS

Software & Database

U3000 UHPLC

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GC-MS – Volatile and Semi-Volatile

Thermo Scientific™ Q Exactive™ GC Orbitrap GC-MS/MS and Thermo Scientific™ TRACE™ 1310 GC

Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS

Thermo Scientific™ ISQ™ Single Quadrupole GC-MS/MS

Solid Phase Microextraction (SPME)

Thermo Scientific Q Exactive MS Models for E&L Analysis

Thermo Scientific™ Q Exactive Plus MS

Resolving Power: up to 280K @ m/z 200
Scan Range: 50-6,000

Thermo Scientific™ Q Exactive™ MS

Resolving Power: 140K @ m/z 200
Scan Range: 50-6,000

Thermo Scientific™ Q Exactive™ Focus

Resolving Power: 70K @ m/z 200
Scan Range: m/z50-2,000

Elemental Analysis

Thermo Scientific™
iCAP™ 7000 Plus Series ICP-OES

Thermo Scientific™ iCAP Q™ ICP-MS

Thermo Scientific™ iCAP™ RQ ICP-MS Series

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Thermo Scientific iCAP RQ ICP-MS and Thermo Scientific Qtegra Software

Extract | Automated dilution and standard preparation | ICP-MS | Compliant data processing

Thermo Scientific™ iCAP™ RQ ICP-MS Series

Fully compliant Thermo Scientific™ Qtegra™ ISDS software

Full USP 233 & ICH Q3D method capabilities

The current compliance deadline for USP 232 and 233 is January 1, 2018

USP <232>/<233>: Be Prepared for the Changes to Come

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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

HighChem LLC

Thermo Scientific™ mzVault 2.0
Library Search and Manager

E&L Compound Database

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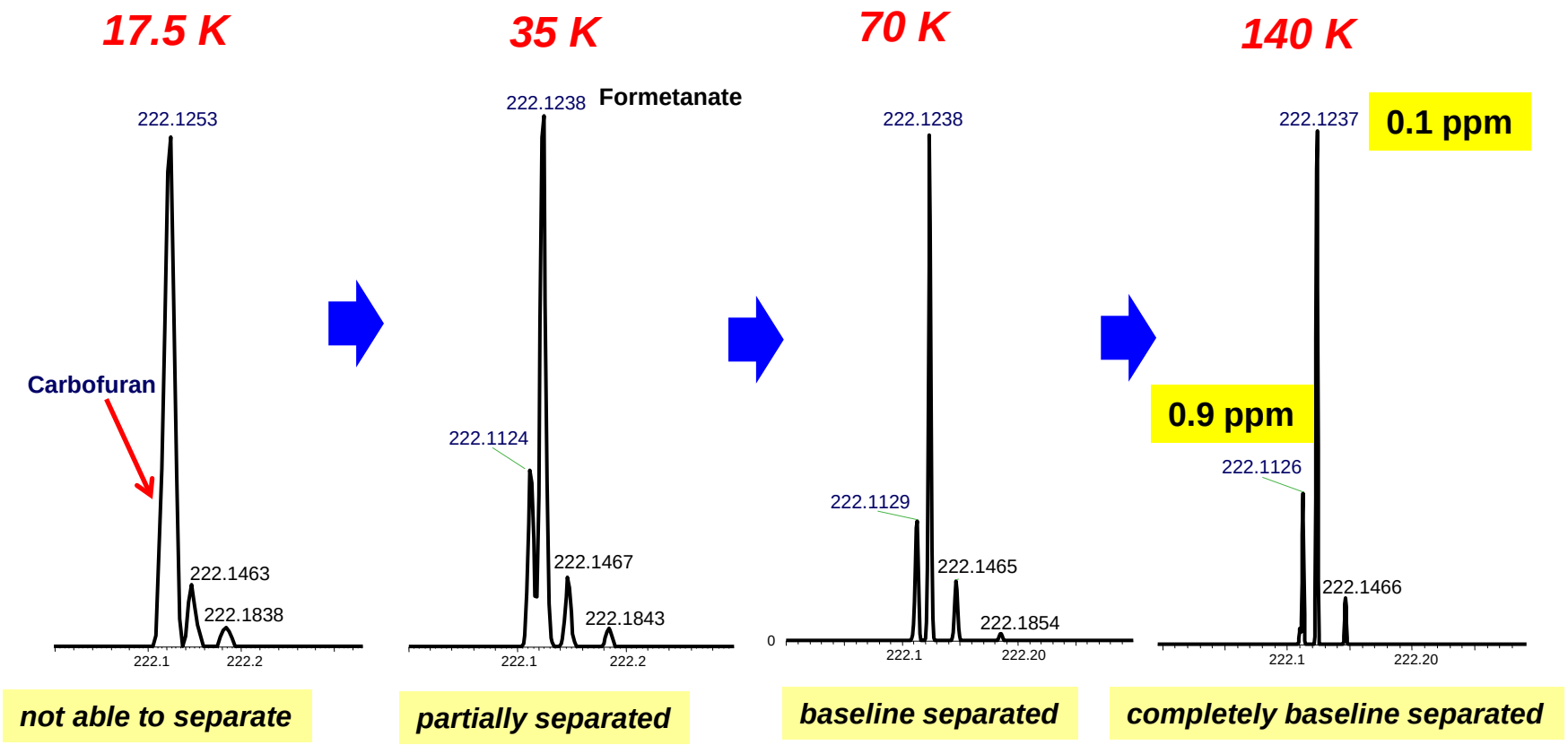
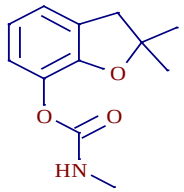
High Resolution Mass Spectrometer for E&L Analysis

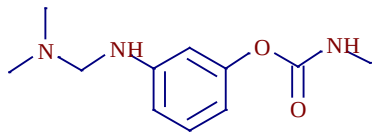
The Power of High Resolution



Carbofuran



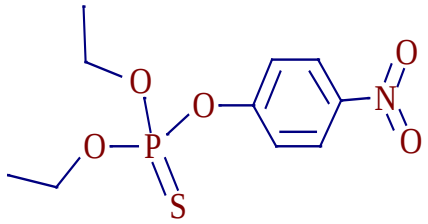
ΔM= 0.0109 Da



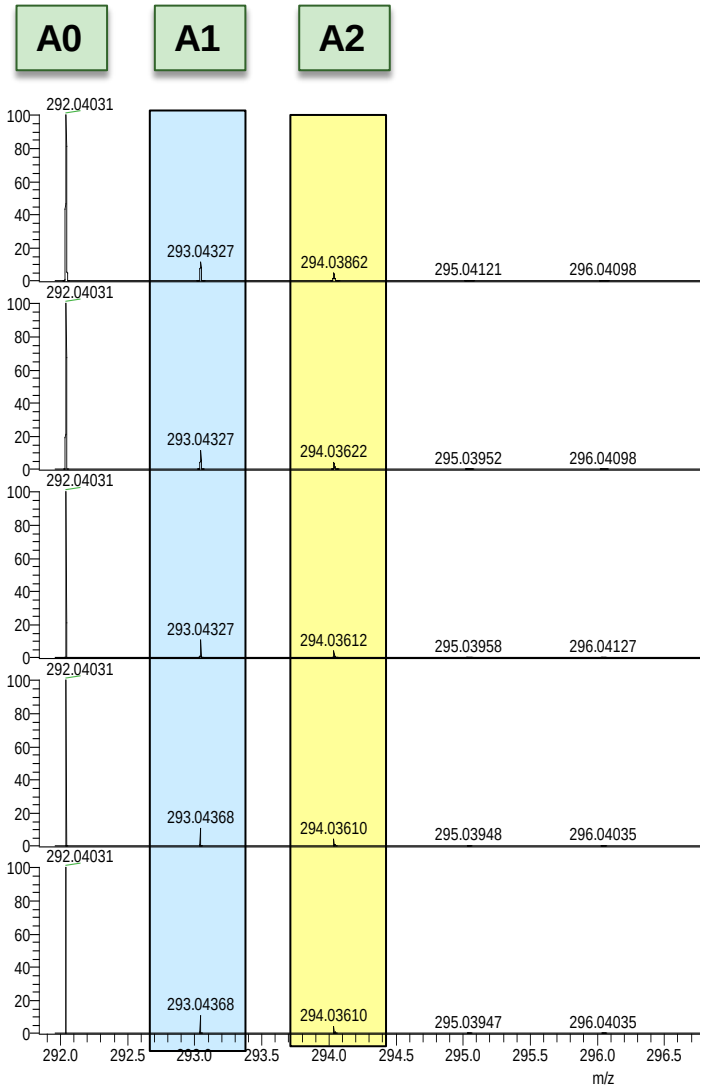
Formetanate

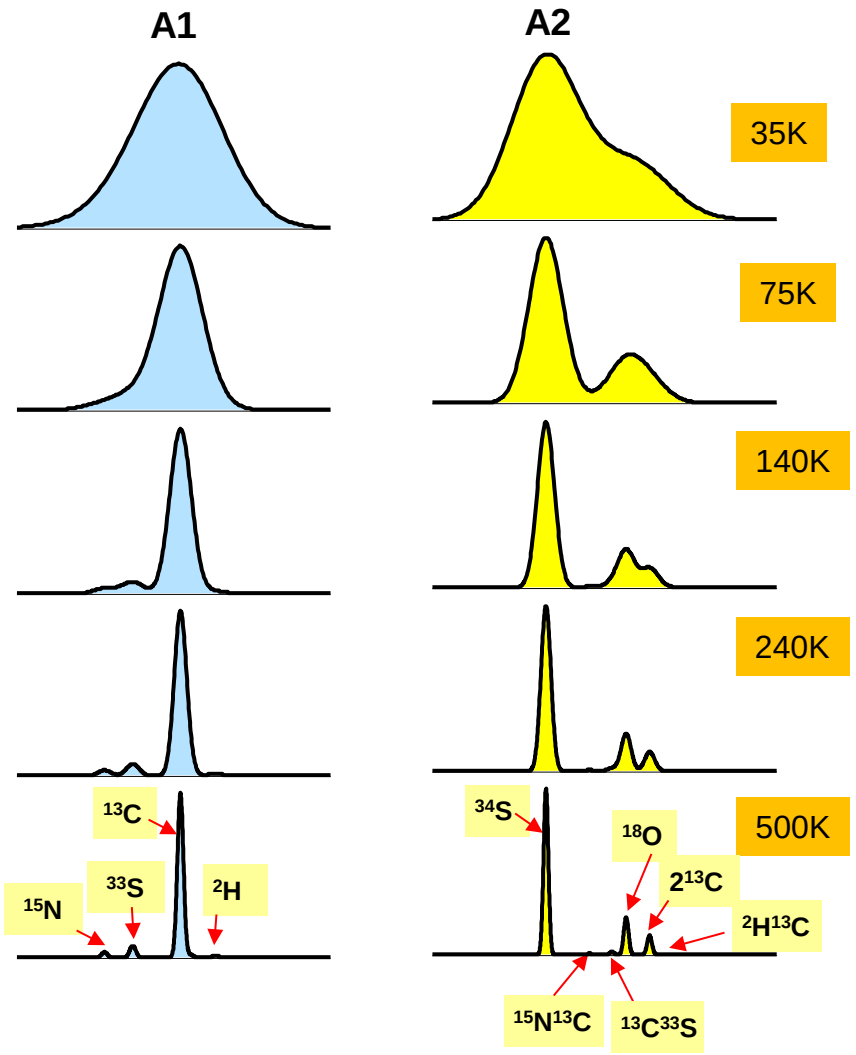
Name	Molecular Formula	$[M+H]^+$
Carbofuran	$C_{12}H_{15}NO_3$	222.1125
Formetanate	$C_{11}H_{15}N_3O_2$	222.1237

Very High Resolution Shows Isotope Fine Structure



Parathion
$C_{10}H_{15}O_5NPS$
$[M+H]^+ = 292.04031$





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MS Requirements for Characterization of Unknowns

- **Mass Accuracy**
- **Isotopic Pattern Fidelity**
- **MS^n Capabilities**

Instrument of Choice: Thermo Scientific™ Orbitrap™ MS

Orbitrap MS High Resolution Accurate Mass (HRAM) data enable confident

- Component identification
- Elemental composition determination
- Structure characterization
- HR Quantitation

Thermo Scientific Q Exactive MS - First Benchtop Orbitrap Mass Spectrometer

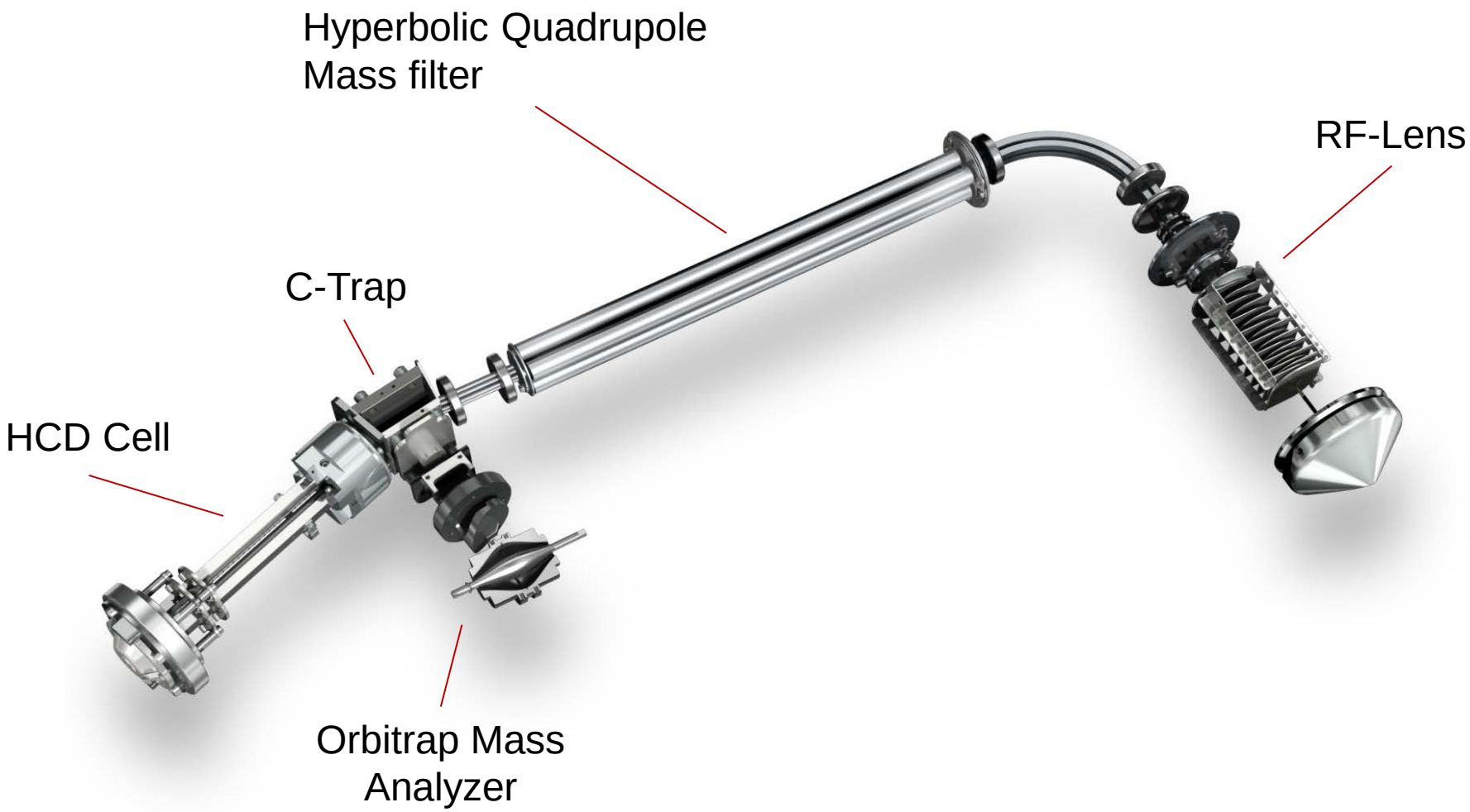
High Performance

- Resolving power
- Sensitivity
- Acquisition speed
- Mass accuracy
- Dynamic range
- Rapid polarity switching
- Multiple ionization modes

Easy to use

- Bench top
- Tune Wizard
- Method templates
- Drag and drop
- Robust
- Plug and play

Thermo Scientific Q Exactive MS Schematics



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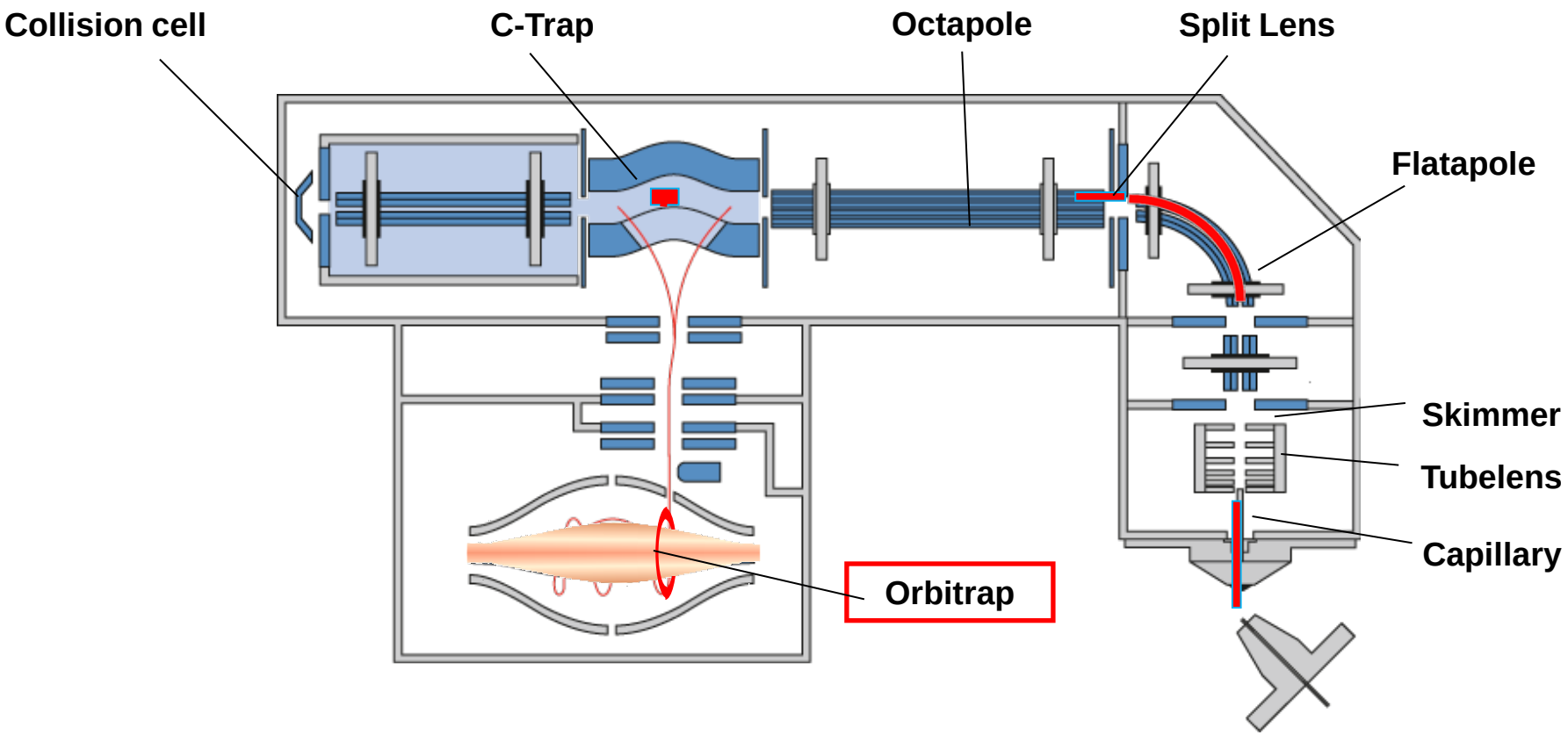
Thermo Scientific Orbitrap MS – Principle of Operation



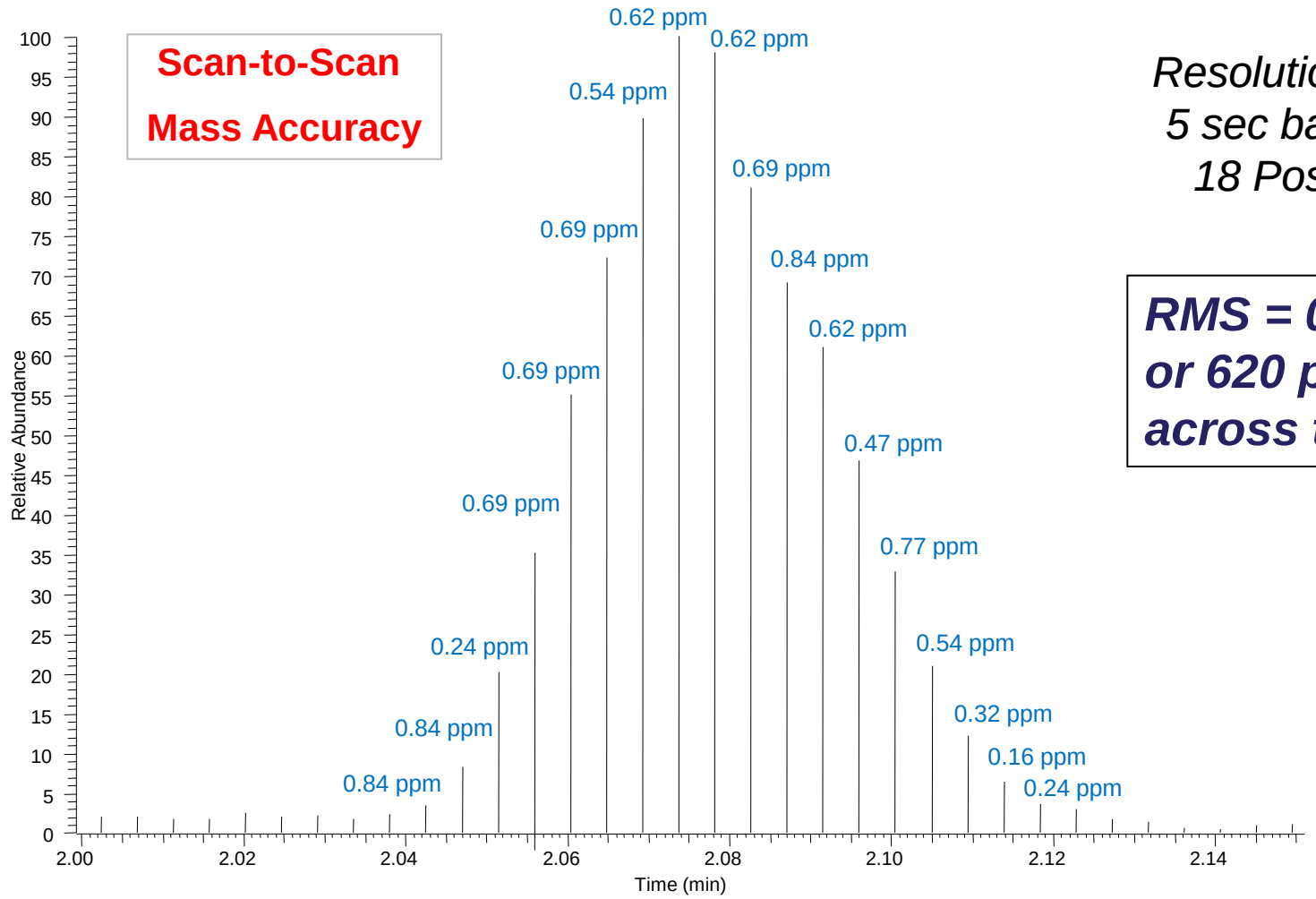
1. Ions are injected through the source
2. …and trapped in the C-trap and squeezed into a smaller cloud
3. …then a voltage pulse across C-trap ejects ions towards the Orbitrap
4. …where they are trapped and detected

Thermo Scientific Q Exactive MS Specifications

- **Max Resolution**
  - 140,000 at *m/z* 200
- **Max Scan Speed**
  - Up to 12 Hz (at 17,500)
- **Mass Accuracy**
  - < 3 ppm external
  - < 1 ppm internal
- **Mass Range**: m/z 50 – 6,000
- **Intra-scan Dynamic Range**: > 5000:1
- **Sensitivity**
  - Full MS: 500 fg Buspirone on column S/N 100:1
  - SIM: 50 fg Buspirone on column S/N 100:1
- **Polarity Switching**
  - One full cycle in < 1 sec (one full scan positive mode and one full scan negative mode at resolution setting of 35,000)

Resolution at *m/z* 200	Max. Scan Speed (Hz)
17,500	12
35,000	7
70,000	3
140,000	1.5

Scan-to-Scan Mass Accuracy | Carbaryl, $C_{12}H_{11}NO_2$



Resolution @ 70k
5 sec base peak
18 Pos scans

RMS = 0.62 ppm or 620 ppb across the peak

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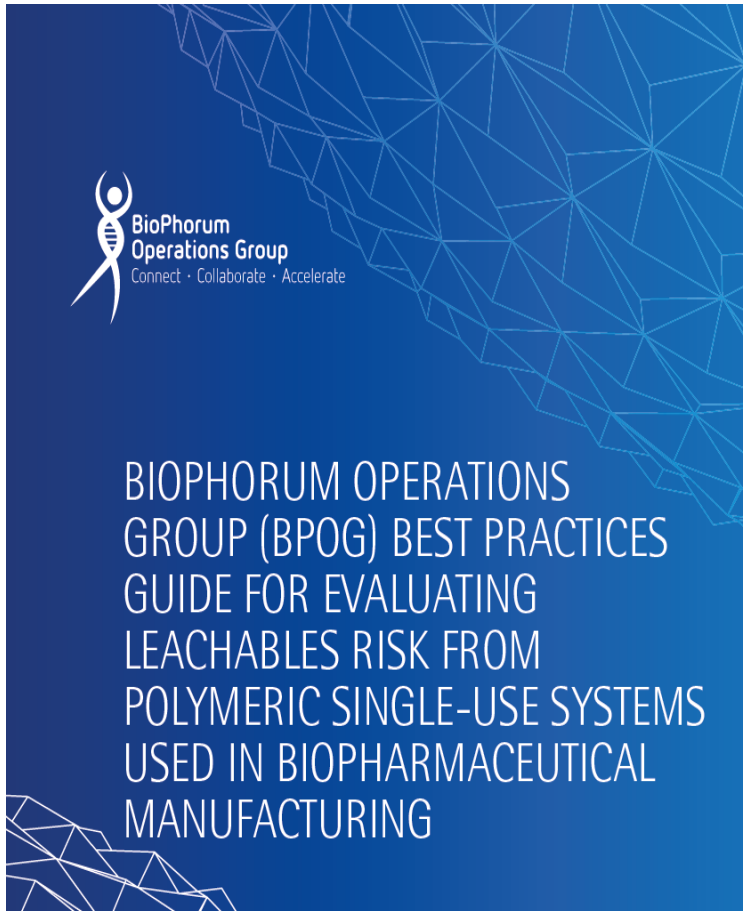
BPOG is Recommending Accurate MS for E&L Studies

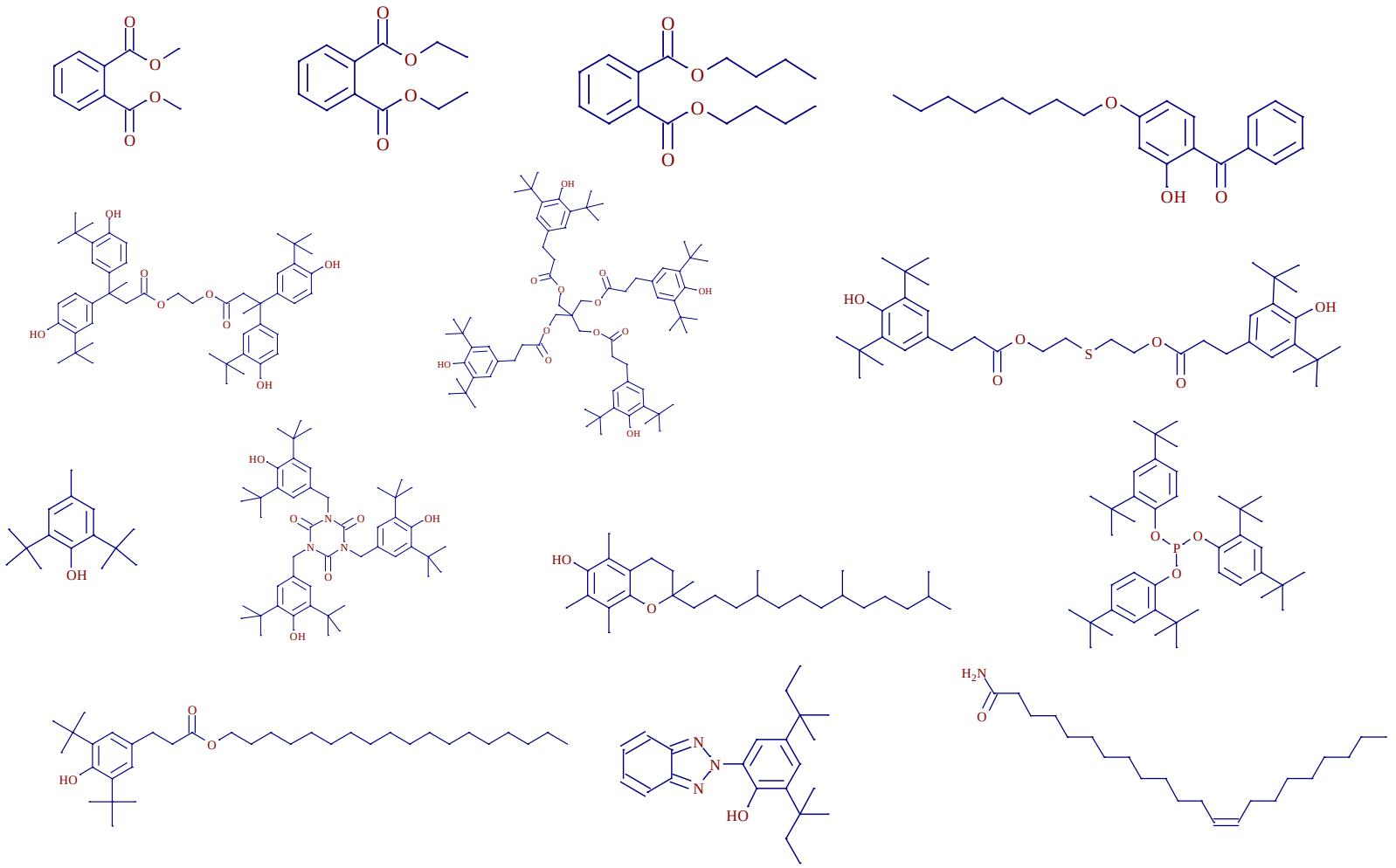


5.4 Analytical methods

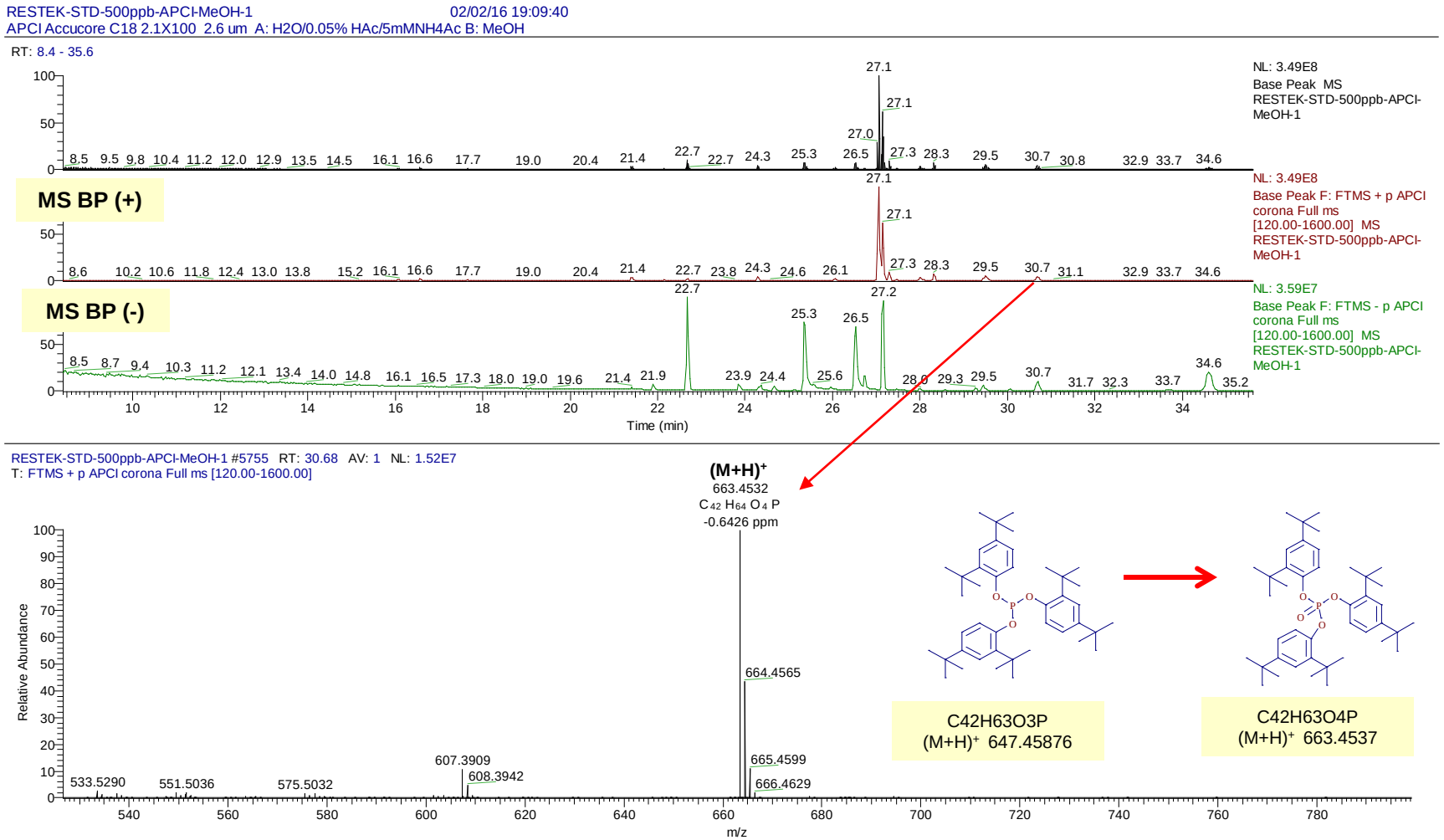
- Know what you need from the data before approaching a CRO to perform leachables studies.
- Define the limits of detection (LOD) and limits of quantitation (LOQ) of your analytical methods. Persist in getting required data from vendors.
- Validate/qualify the analytical methods appropriately in consideration of the test objective.
- Use standard/control samples at the beginning and the end of each run.
- Use accurate mass detectors, even if only conducting a screening study.
- Use appropriate matrix controls to account for degradation of matrix components that could be mistaken for leachables.
- Non-specific analyses are typically not useful for leachables studies.
- Do not make assumptions without data; generate required data.
- Matrix interference from protein/excipients/process components can be significant; ensure that such effects on the methods planned are well understood before performing analysis.
- Determine analytical evaluation thresholds (AET) for your target compounds.
- Where possible, use an MS (or MSn) library to aid in the identification of compounds.

Additives and Their Unknown Degradants are Common Extractables

Antioxidant, plasticizer, slipping agent, light stabilizer… also monomer, oligomer.



Irgafos 168 (Oxidation Product)



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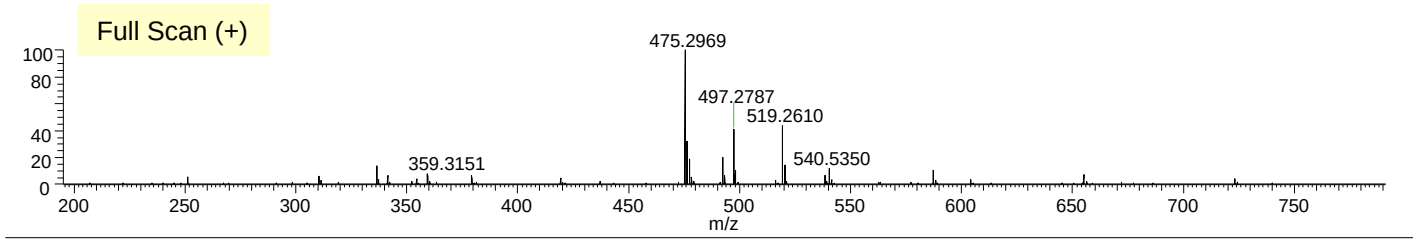
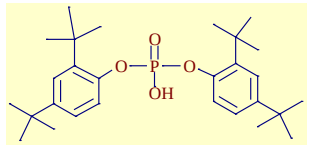
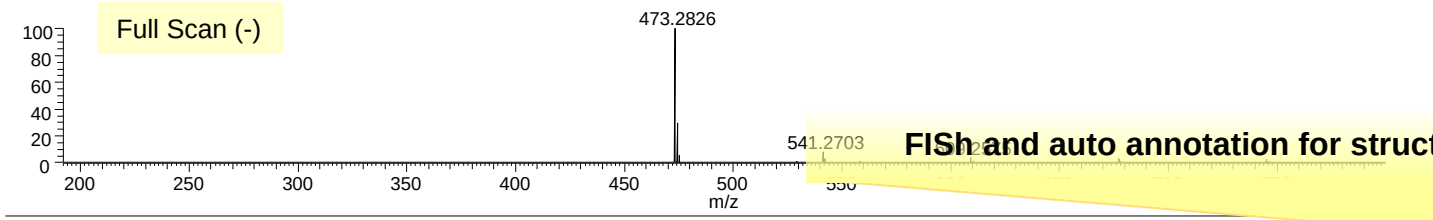
Irgafos 168 Degradant ID by Full Scan (+/-) and MS/MS Spectra

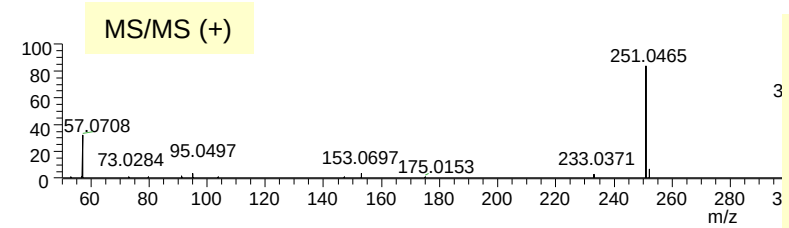


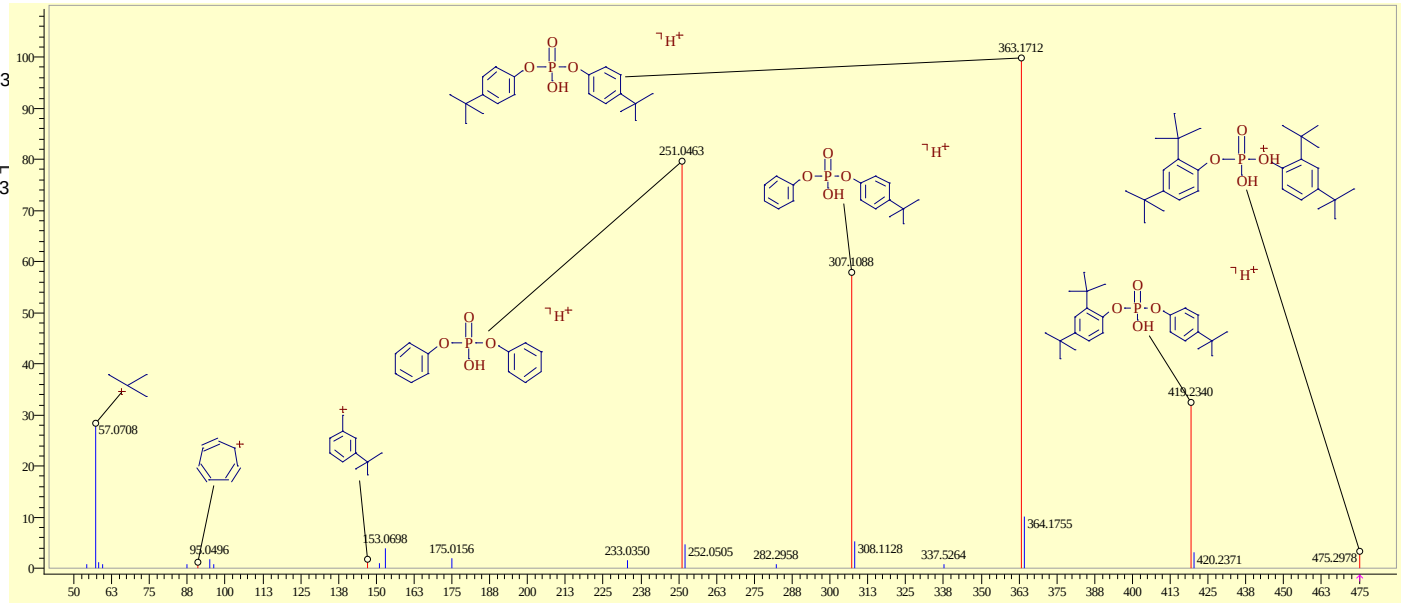


C28H43O4P
$(M+H)^+$ 475.2971
$(M-H)^-$ 473.2826



FISh and auto annotation for structure elucidation of Irgafos 168 Degradant





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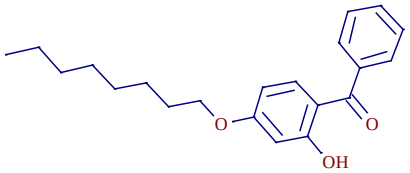
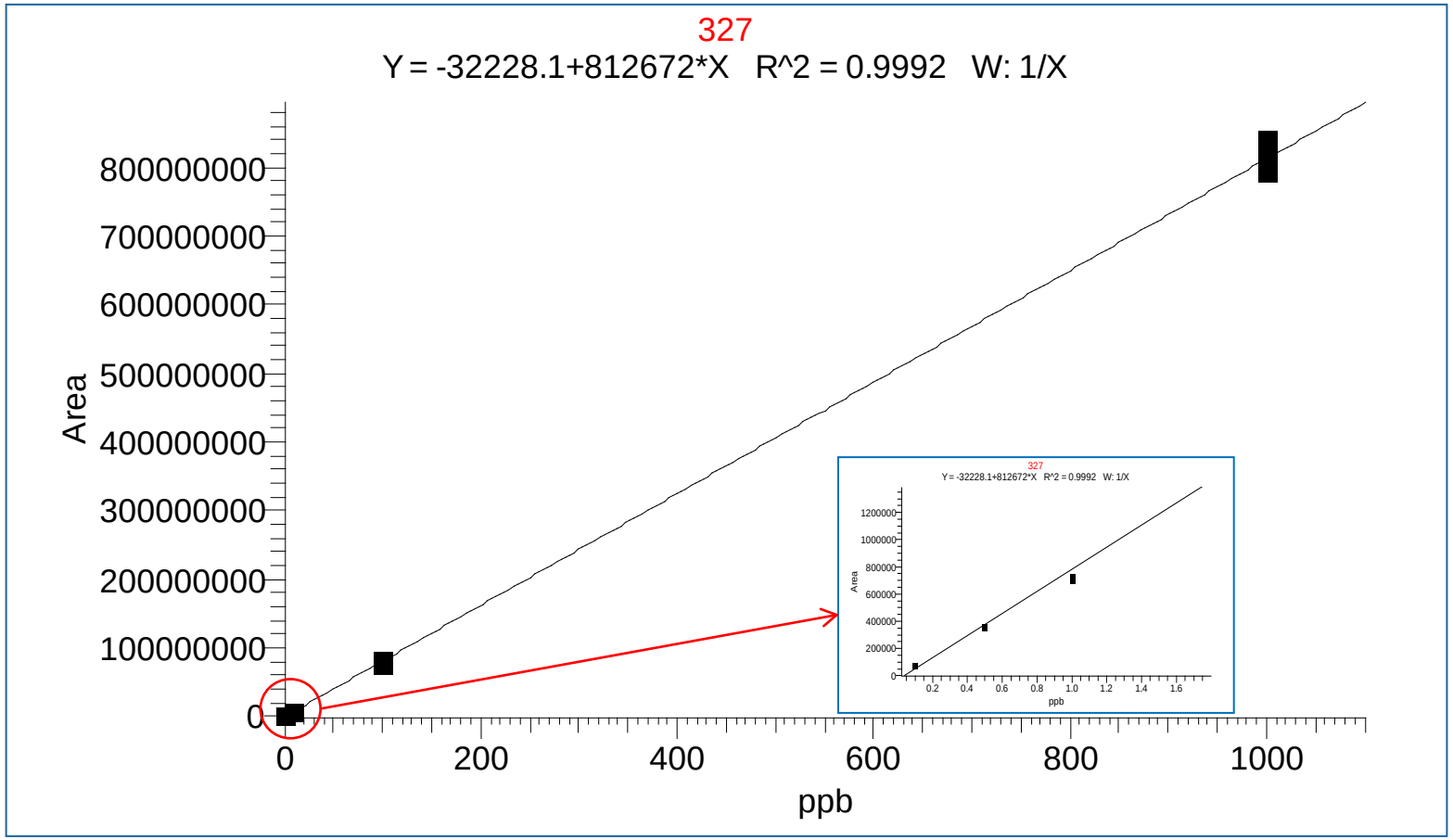
Quantitation of Octabenzone on Thermo Scientific Q Exactive Focus MS

Octabenzone
Cas# 1843-05-6
C21H26O3
(M+H)+ 327.18092





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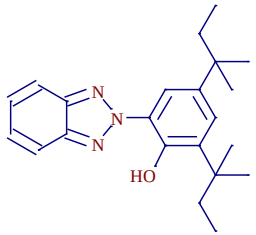
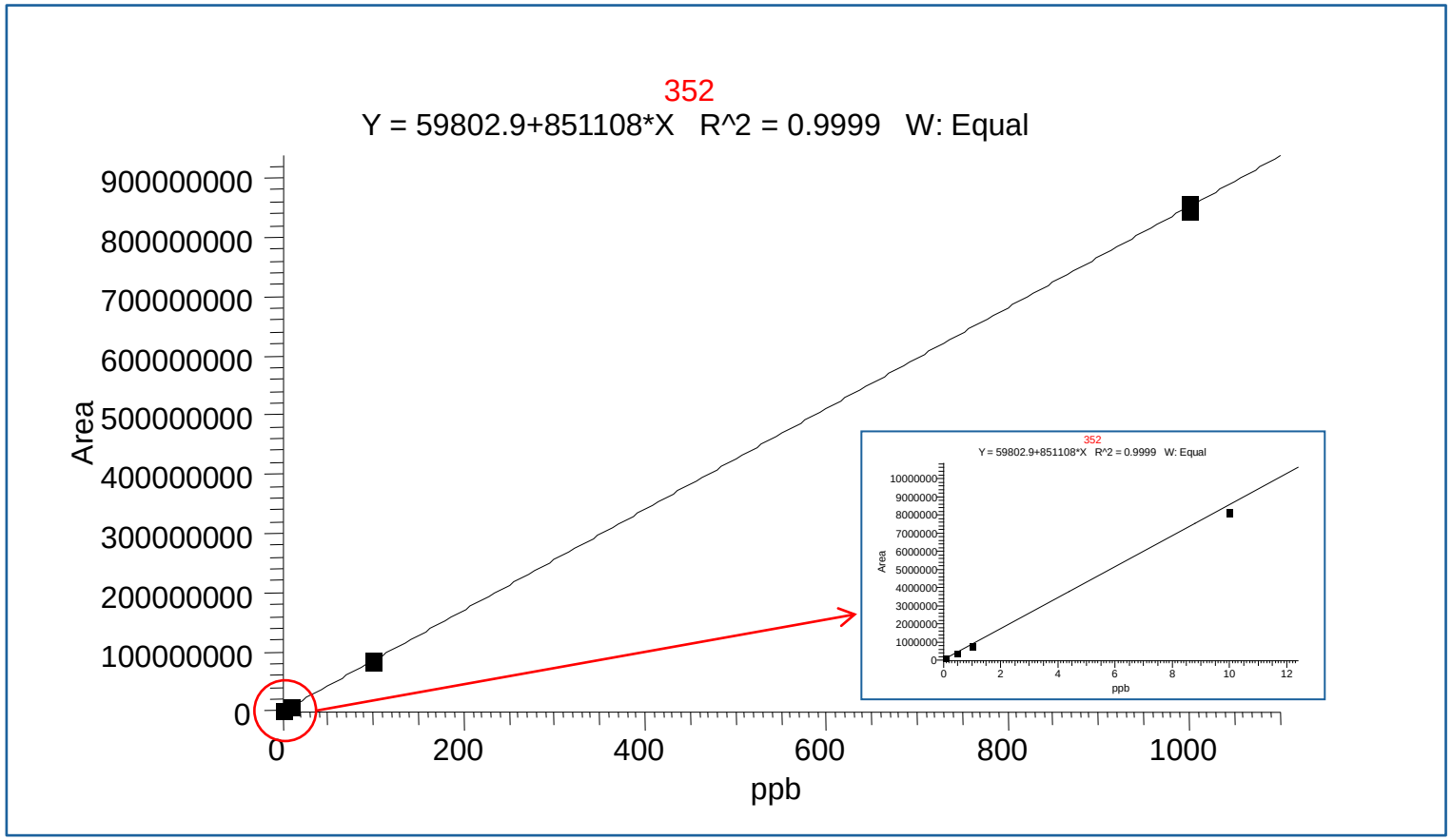
Quantitation of Tinuvin 328 on Thermo Scientific Q Exactive Focus MS

- Tinuvin 328
  Cas# 21615-49-6
  C22H29N3O
  (M+H)+ 352.23834





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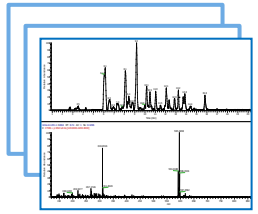
LC-HRMS Analysis for IV Bag Extractables

UHPLC-HRMS

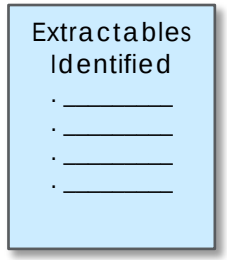
Thermo Scientific™ Q Exactive™ Plus MS and Thermo Scientific™ Vanquish UHPLC

HRAM data



Thermo Scientific™ Compound Discoverer™ 2.1 Software

Result



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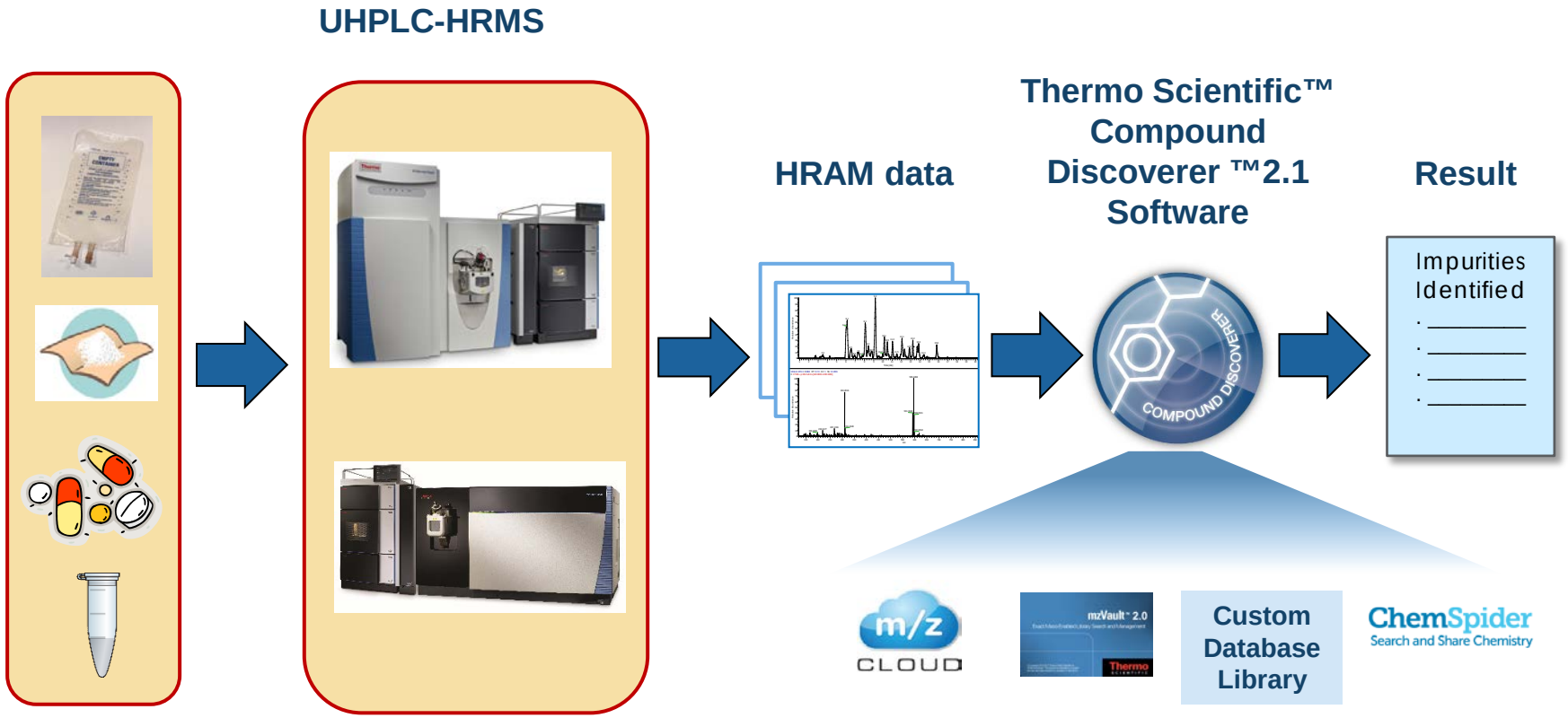
General Workflow for Small Molecule Impurity ID and E&L Analysis



General Workflow for API Impurity and Extractable & Leachable Analysis

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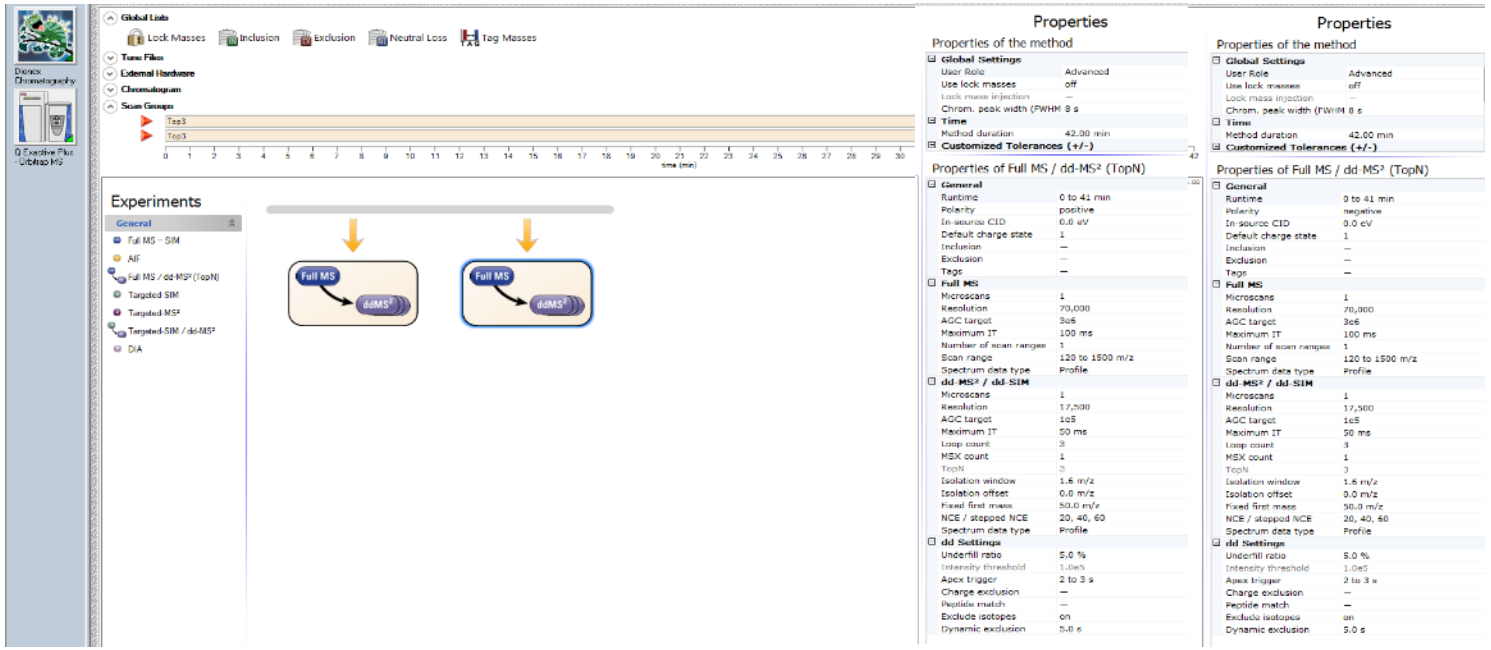
MS Method: High Resolution Accurate Mass Untargeted Screening

- **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



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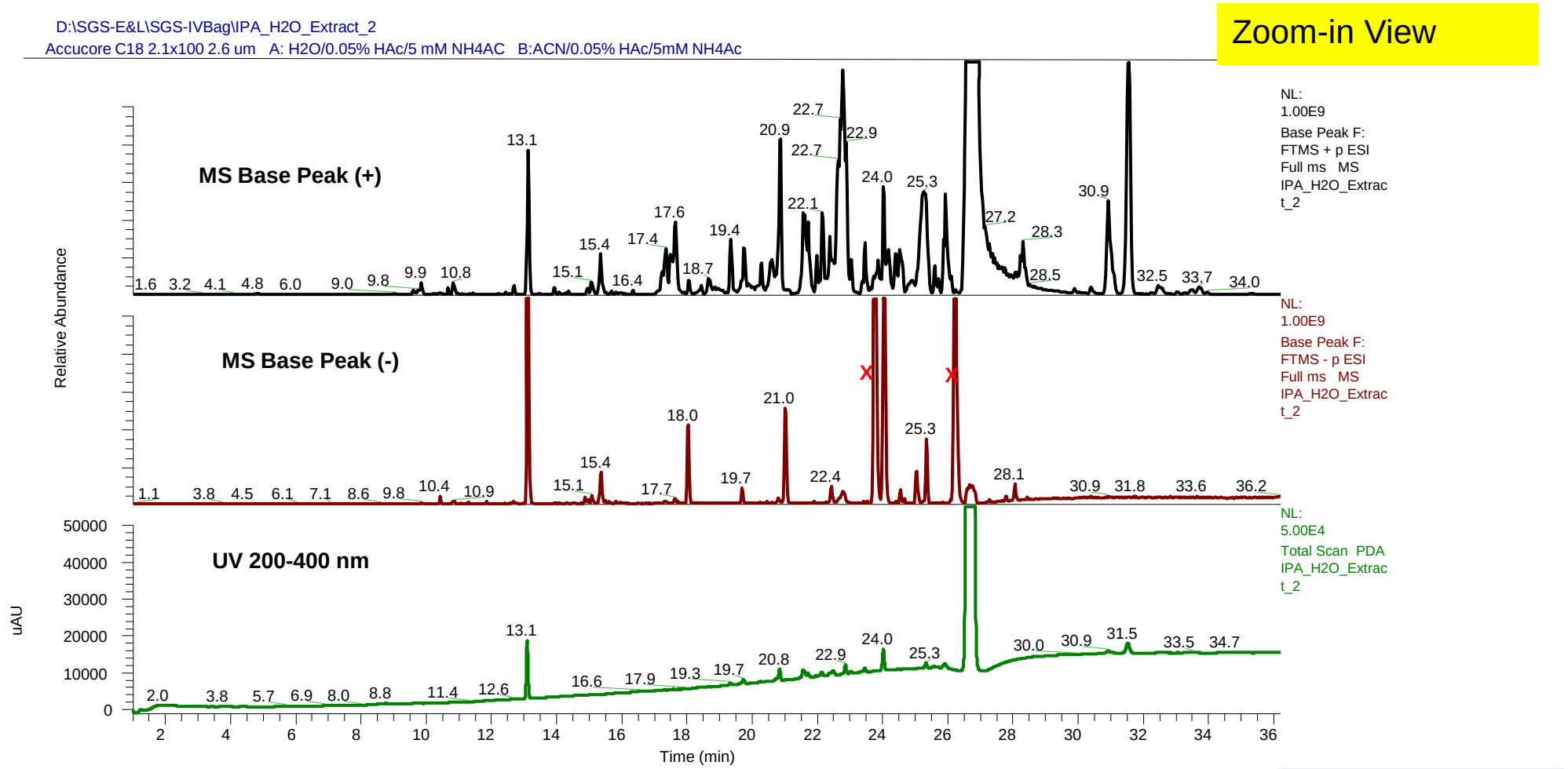
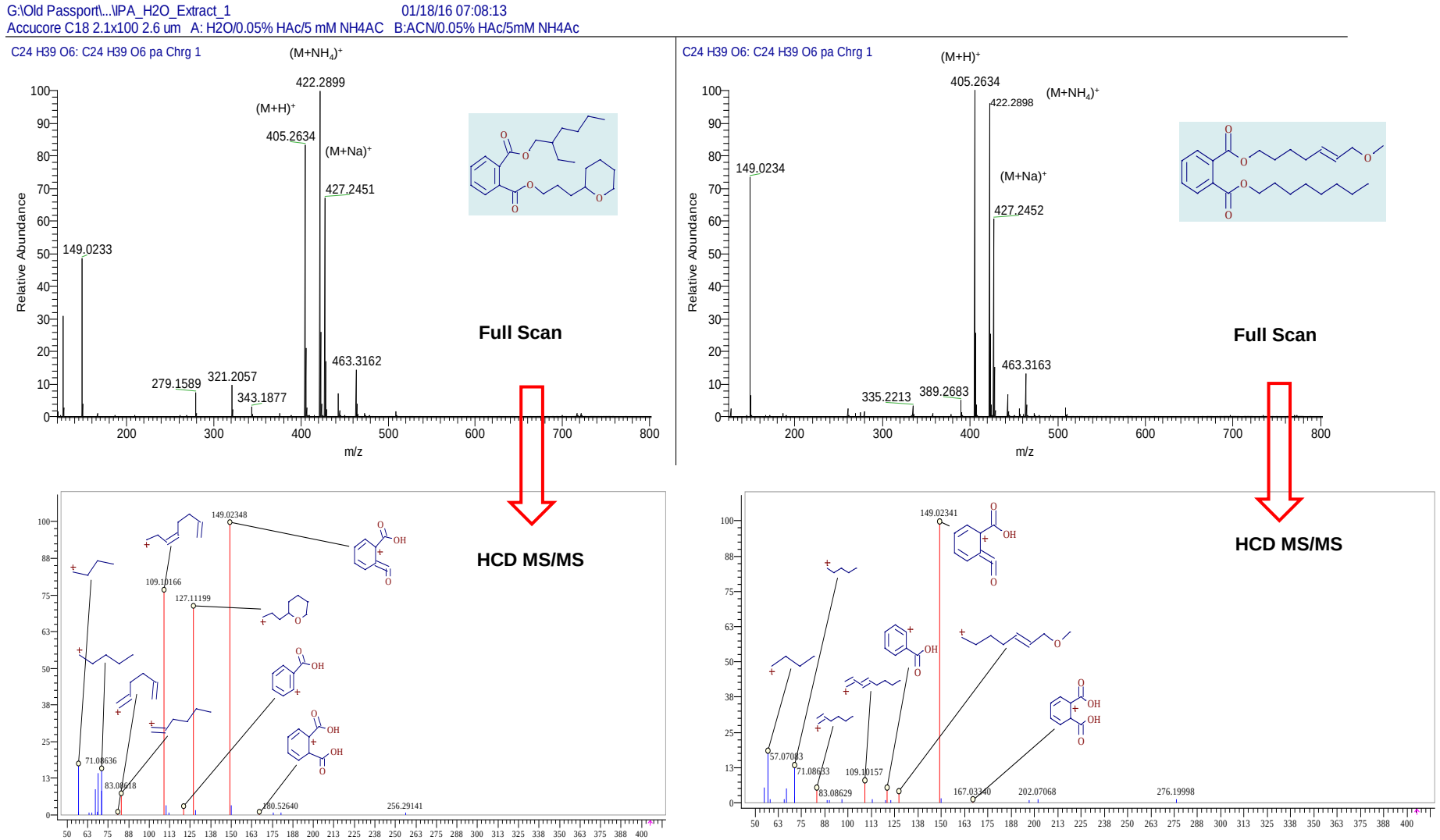
IV Bag IPA-H2O Extract - MS Base Peak Chromatogram and UV



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



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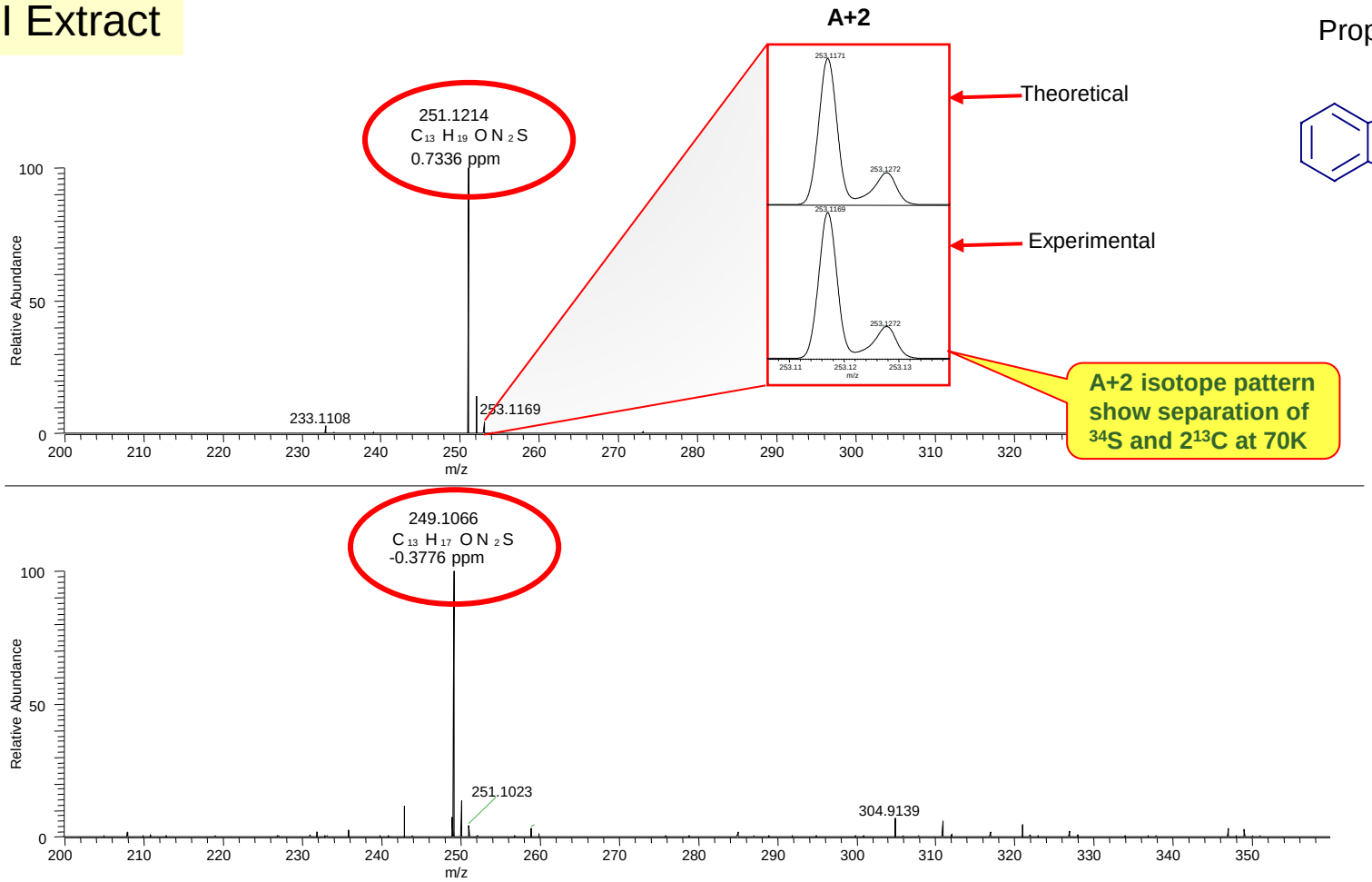
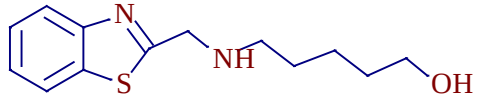
Pos/Neg Switching Maintain High Mass Accurate Mass and Fine Isotope Pattern

From O-Ring WFI Extract



Proposed Structure



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Ultra High Resolution MS Reveals Fine Isotope Structure

HRAM Data

- Accurate Mass
- High Isotope Fidelity
- MS/MS Spectrum
- Fine Isotope Structure

COMPOUND DISCOVERER

- mzCloud
- ChemSpider
- Thermo E&L DB

Structure Elucidation

Thermo Scientific™
Fusion™ Lumos™
Tribrid™ MS
1 Million Resolution

CD 2.1 Result - Predicted Compositions for a unknown component in cosmetic product IPA/H2O extract

	Checked	Name	Formula	Annotation Sc	FISh Coverage	Molecular Wei	RT [min]	Area (Max.)	# ChemSpider Results	# mzCloud Results	mzCloud Best Match
53			C27 H36 N3 O4 P S			529.21404	11.708	2857456	119	0	

Hide Related Tables

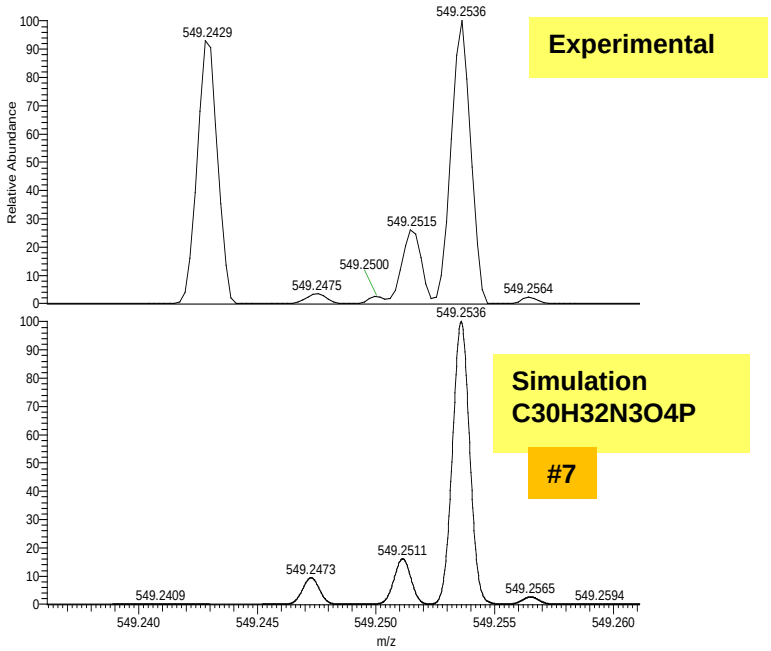
Structure Proposals | Compounds per File | Predicted Compositions | Merged Features | mzCloud Results | ChemSpider Results | Mass List Search Results

	Check	Compound Match	Formula	Molecular Weight	ΔMass [Da]	ΔMass [pp	RDBE	H/C	Rank	# Matched Iso.	# Missed Iso.	# Matched F	SFit [%]	Pattern Cov. [%]	MS Cov. [%]	MSMS Cov. [%]	In ChemSpider
1			C27 H36 N3 O4 P S	529.21641	-0.00238	-4.49	12.0	1.3	1	7	0	23	78	100.00	99.97	99.64	
2			C24 H35 N O12	529.21593	-0.00189	-3.57	8.0	1.5	7	5	0	18	54	100.00	96.51	95.64	X
3			C29 H31 N5 O3 S	529.21476	-0.00072	-1.37	17.0	1.1	2	7	0	22	72	100.00	99.97	98.57	X
4			C28 H37 N O5 P2	529.21470	-0.00066	-1.25	12.0	1.3	5	4	0	20	85	100.00	96.48	97.90	
5			C23 H32 N9 O2 P S	529.21373	0.00031	0.58	13.0	1.4	10	5	2	22	39	97.17	95.98	99.48	
6			C28 H35 N O7 S	529.21342	0.00061	1.16	12.0	1.2	3	8	0	23	63	100.00	100.00	99.64	X
7			C30 H32 N3 O4 P	529.21304	0.00099	1.88	17.0	1.1	6	4	0	19	76	100.00	96.48	96.84	
8			C22 H36 N5 O6 P S	529.21239	0.00164	3.11	8.0	1.6	4	7	0	23	60	100.00	99.97	99.64	
9			C32 H37 N P2 S	529.21219	0.00184	3.48	16.0	1.2	9	7	0	6	65	100.00	99.97	11.05	

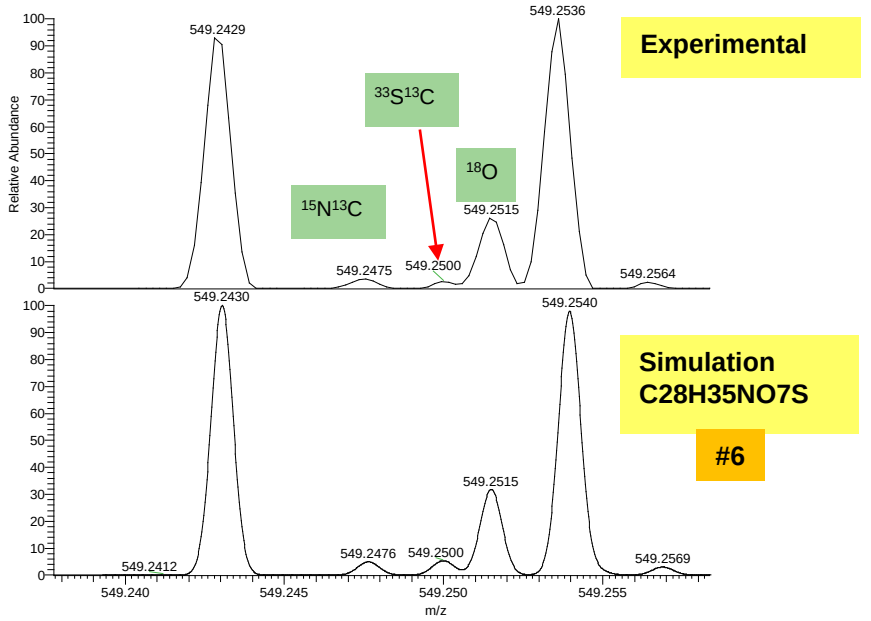
#6 and #7

A2 Isotope Fine Structure Validates Correct Elemental Composition

The incorrect formula doesn't match the result.



The correct formula matches the ana fine isotope structure.



The above results demonstrate that the fine isotope structure made available by ultra high resolution mass spectrometer greatly enhances the ability to confidently determine the correct unknown elemental composition.

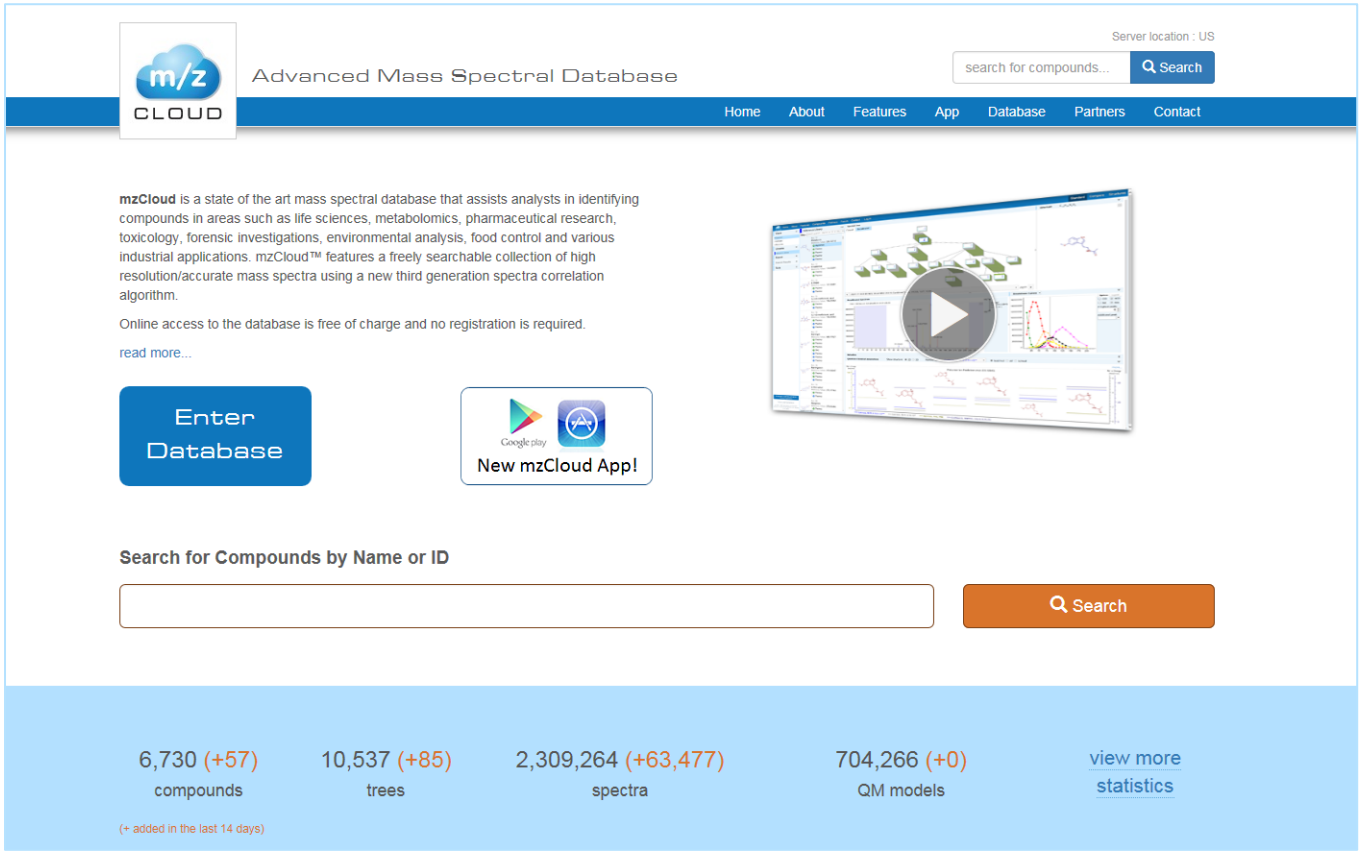
mzCloud - HR Mass Spectral Database

mzCloud™

- Advanced high resolution mass spectral database (Free)
- Very high quality data: standardized acquisition, highly curated data
- Search through spectrum, structure, substructure, m/z, …
- Identifying compounds even when they are not present in the library through substructure search

→ https://www.mzcloud.org

Free and cloud-based



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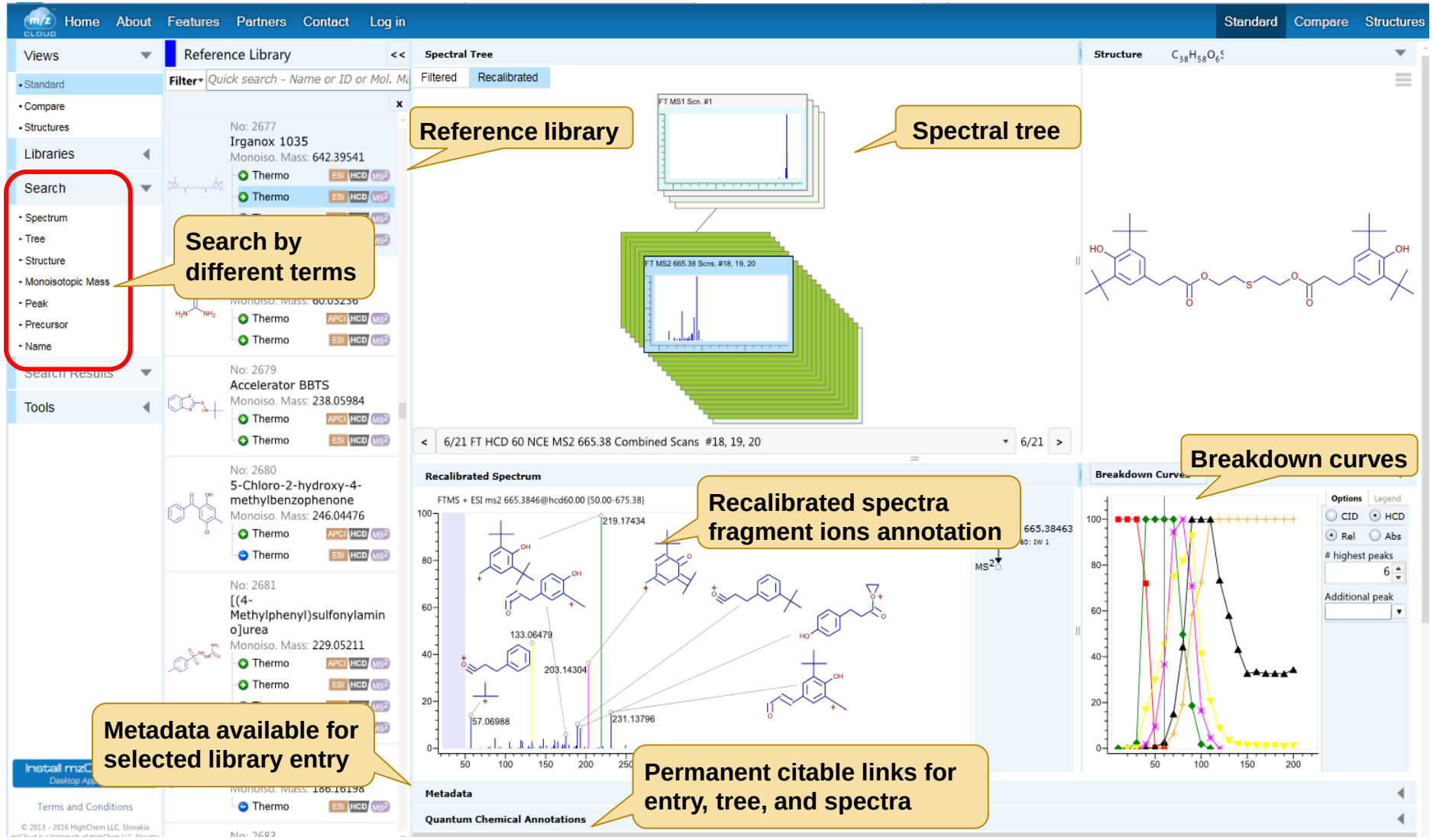
mzCloud Spectral Library



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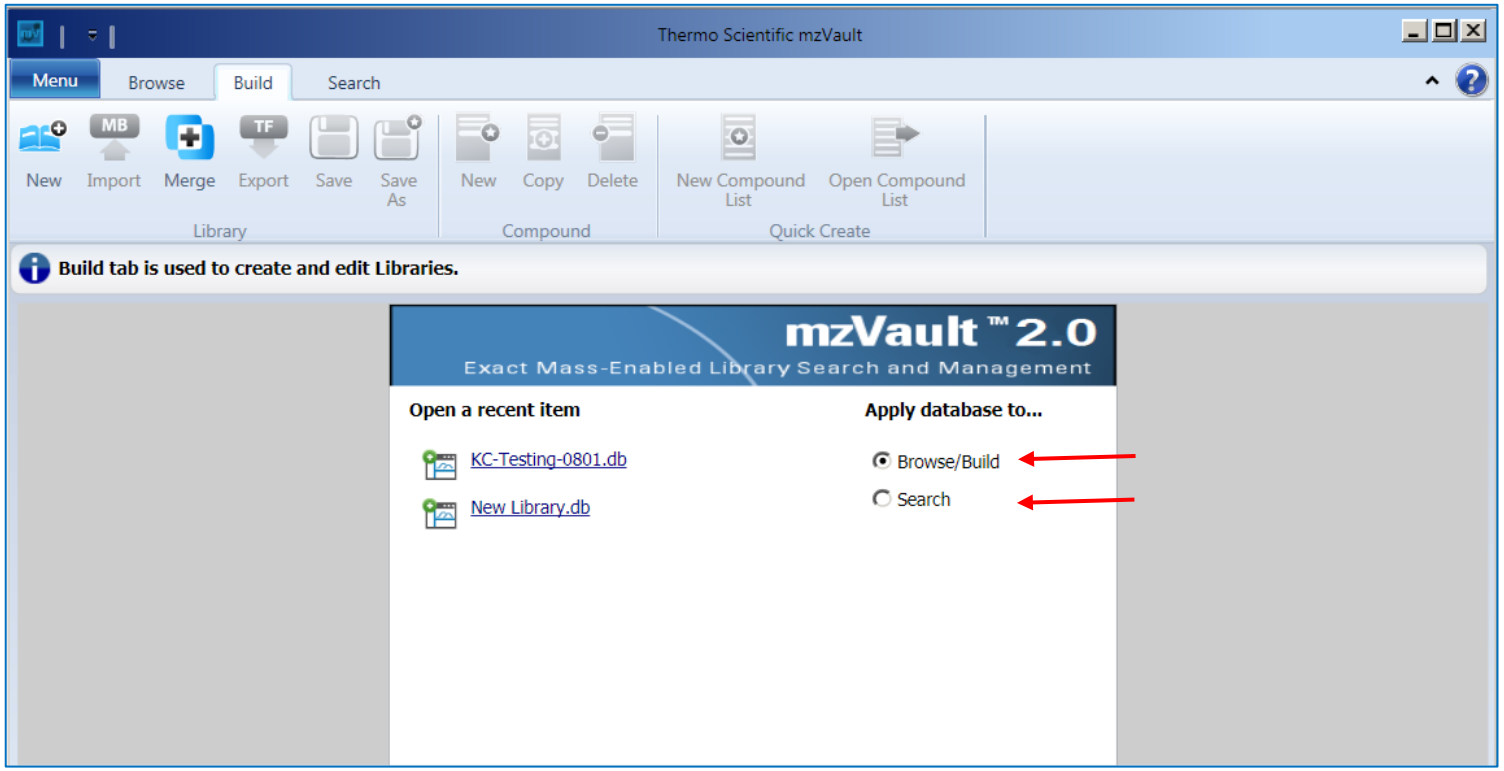
Thermo Scientific mzVault 2.0

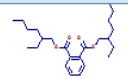
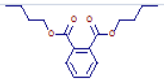
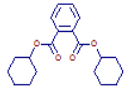
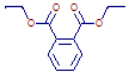
Exact Mass-Enabled Library Search and Management
- local mzCloud
- Creating customer library
- Searching library



ThermoFisher SCIENTIFIC

E&L Compound Database the “E&L Mass List” in CD 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 Thermo Scientific™ Compound Discoverer™ as “E&L Mass List”.

Thermo Scientific Compound Discoverer 2.1 Software for Compound ID and Structure Elucidation

Component extraction and elemental composition prediction

- HRAM data
- Isotope pattern
- *fine isotope structure*
- MS/MS fragments

Unknown identification through multiple databases searching in parallel

Known unknown compound ID

Unknown compound structure elucidation using “Structure Proposals”

- Predicted Compositions
- MS/MS fragment ions
- The similarity search identify the fragment ions or substructures in mzCloud, which facilitated unknown structure elucidation.

De Novo ID Most Challenge Task Need Chemistry knowledge and expertise

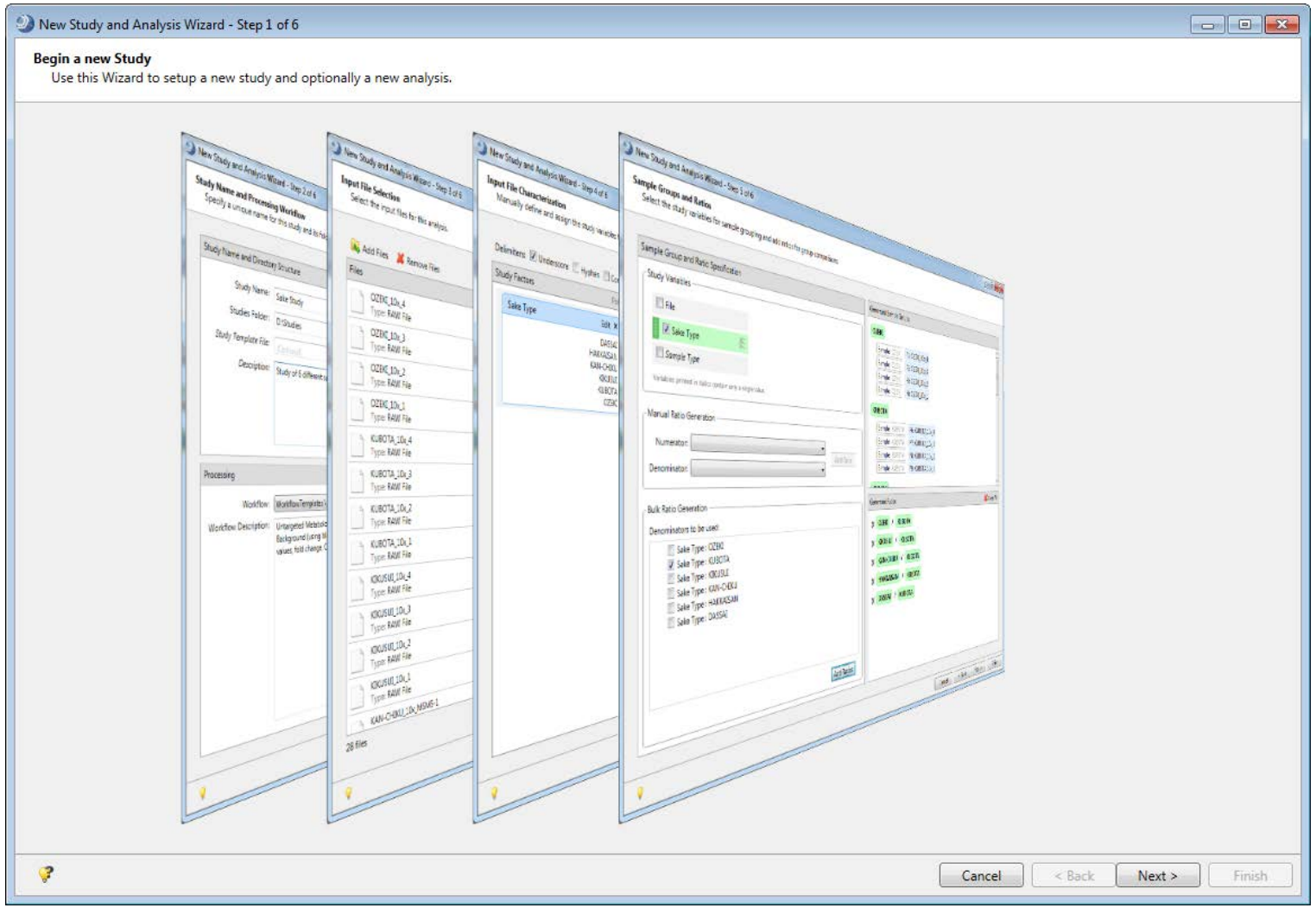
Validity check of the proposed structure

- using “FISh Scoring” feature by searching the embedded “HighChem Fragmentation Library”.

Differential Analysis for different lots, batch, or type samples analyses.

FISh stands for “Fragment Ion Search”

Thermo Scientific Compound Discoverer 2.1 Software Study and Analysis Wizard



Creating a study and analysis use the guided "New Study and Analysis wizard"

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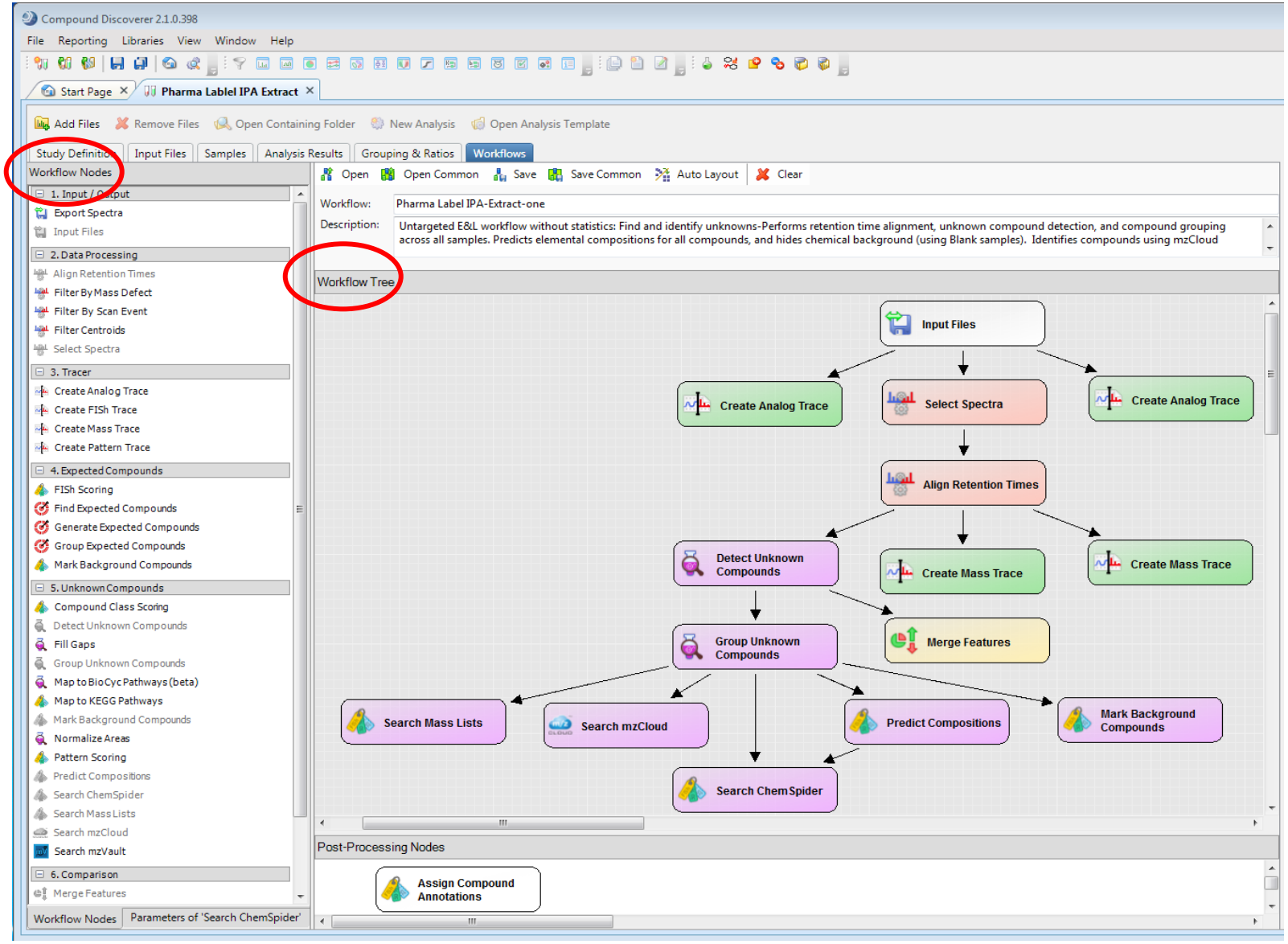
Pharma Label Extractable Study - Nodes-based Processing Workflow



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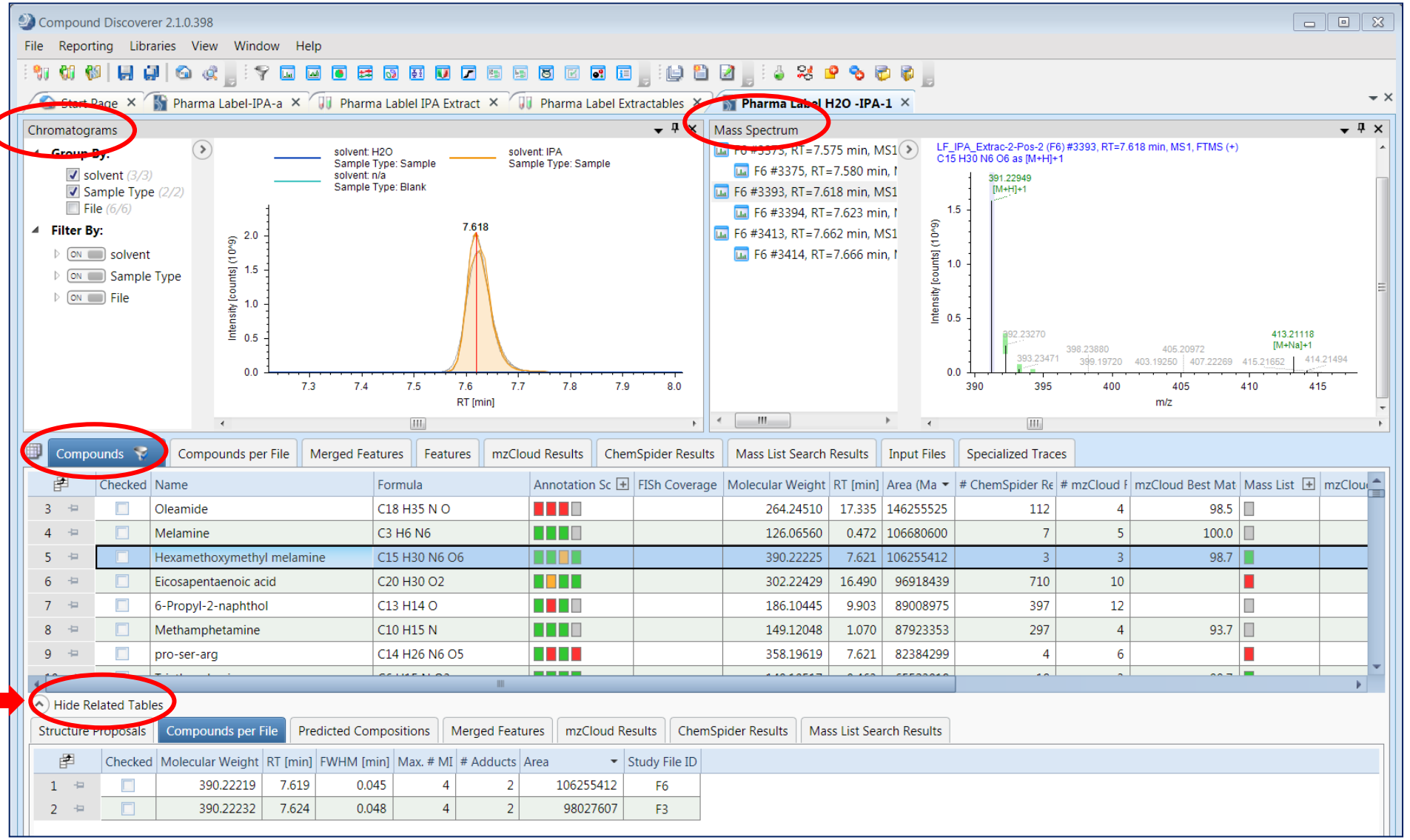
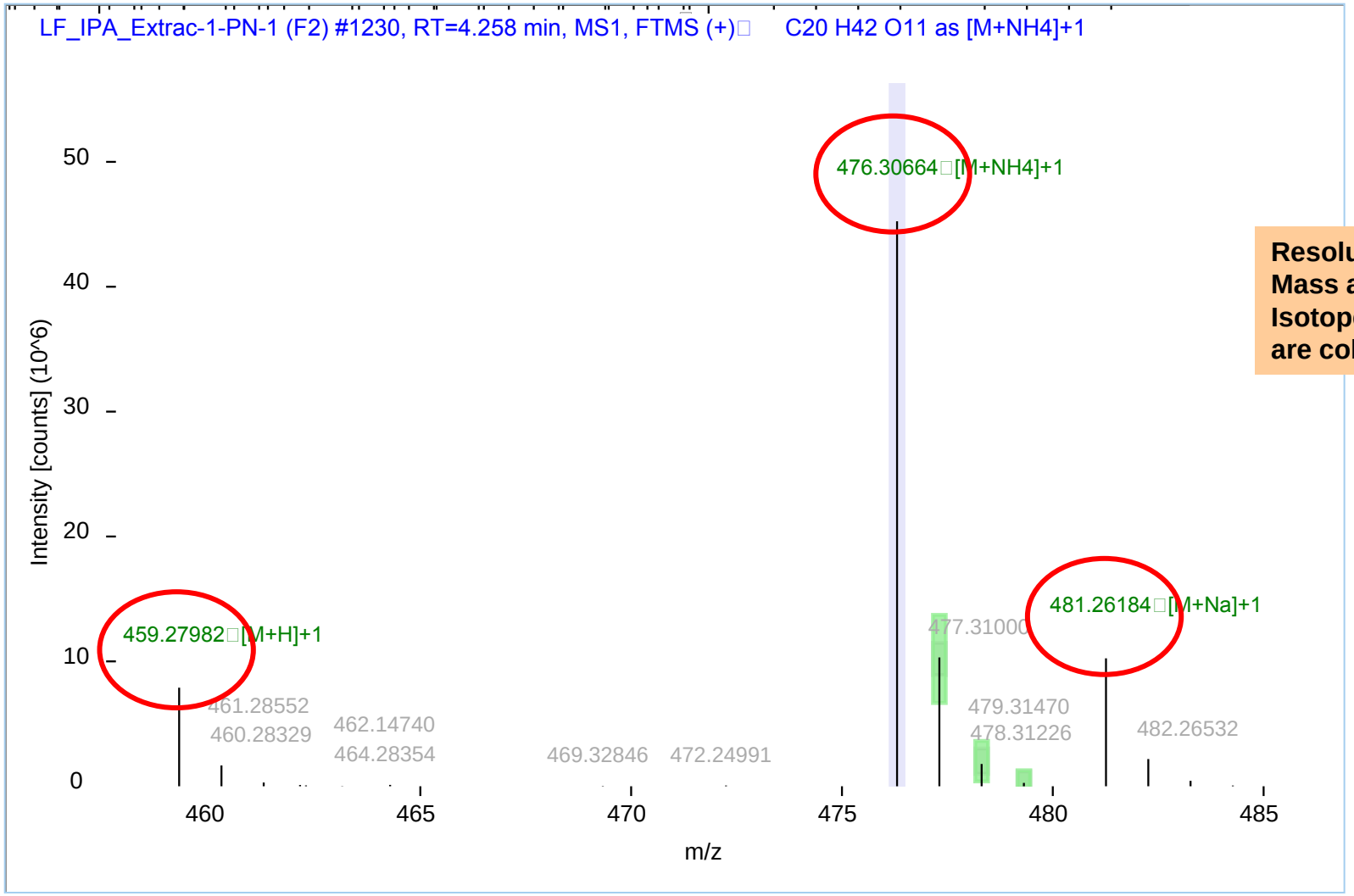
Result View – Data Interpretation



sub-tables

Component Detection with Adducts Grouping



Resolution aware
Mass accuracy
Isotope pattern
are color coded.

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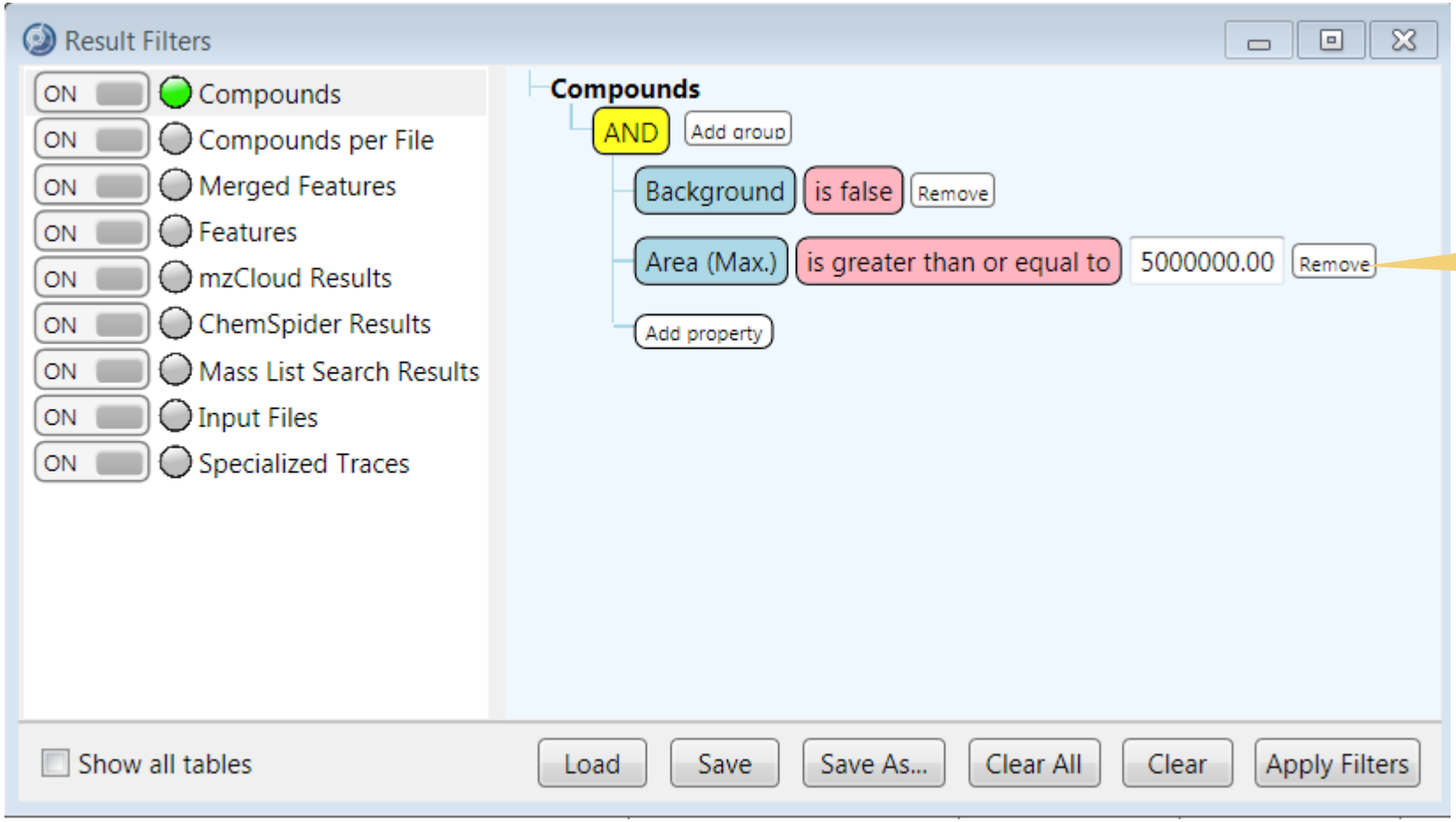
Result Filters - Flexible Result Review



Filtering the result based on your need

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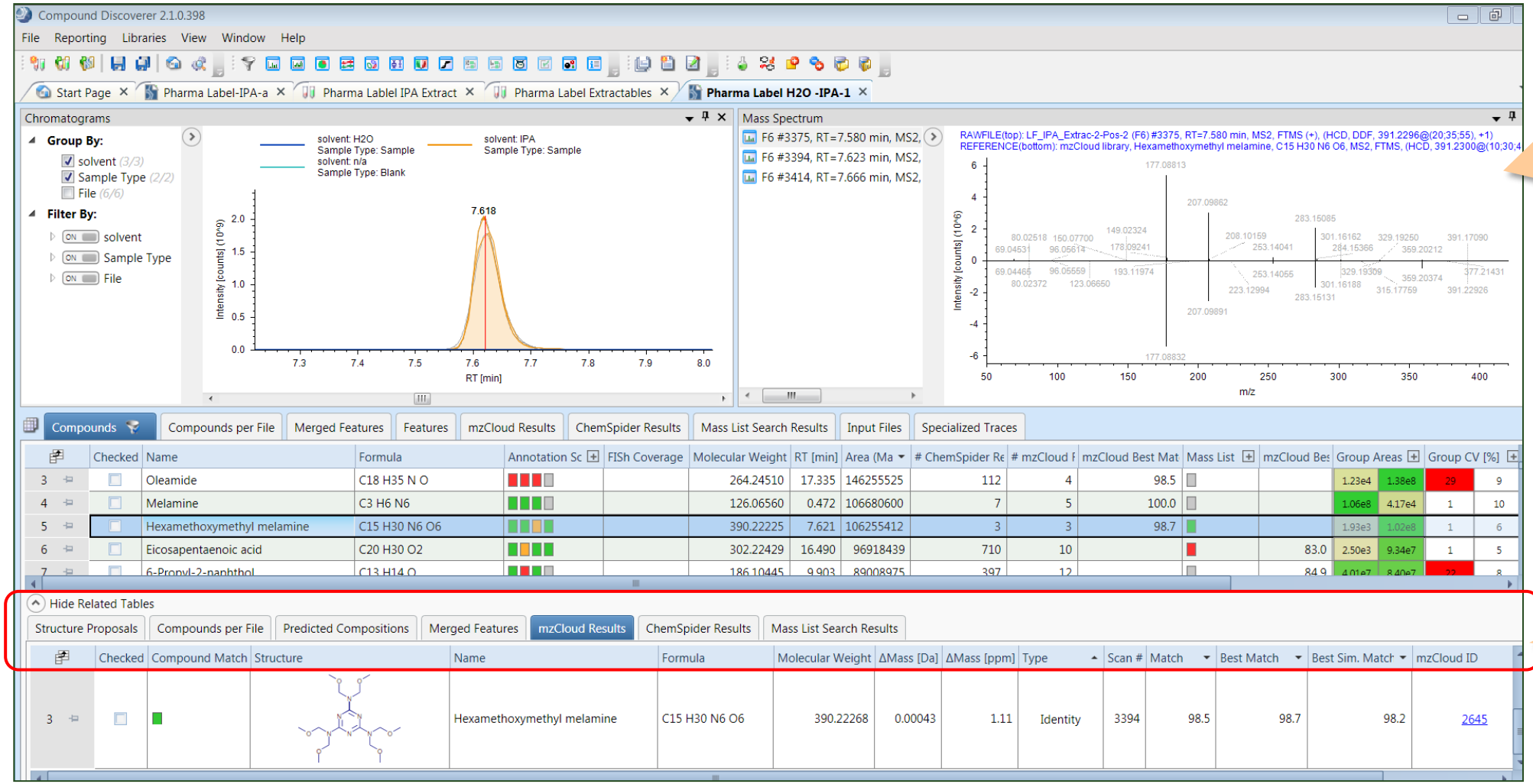
Multiple Database Searching in Parallel to Identify Known Unknowns

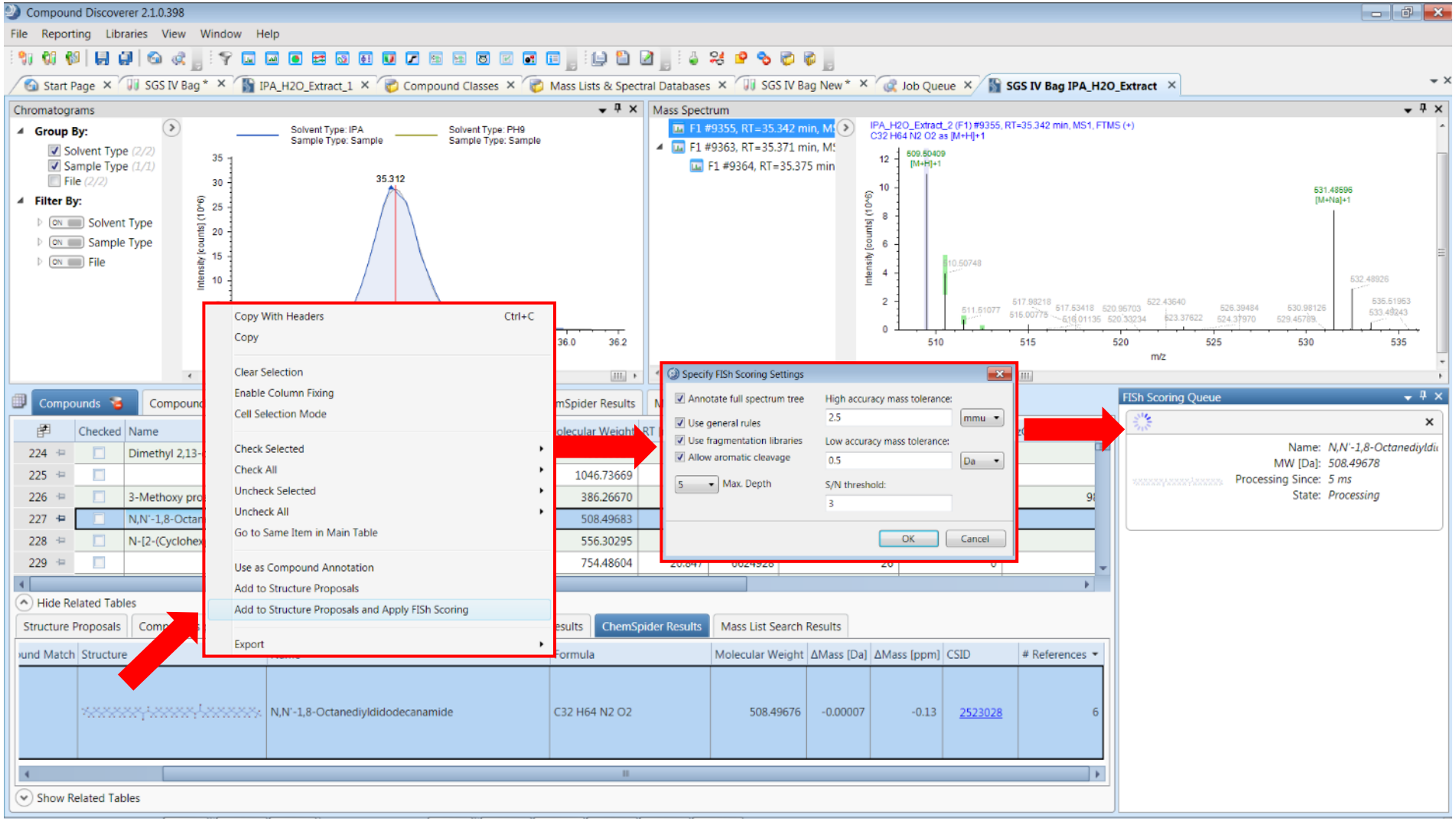


Mirror plot for each mzVault hit: observed MS2 vs. library MS2

- Matches collision energy between raw file MS/MS and reference MS/MS

Sub table show detailed info for compound

Add Chemspider Search Result to “Structure Proposal and Apply FISh” for Unknown ID



FISh stands for “Fragment Ion Search”

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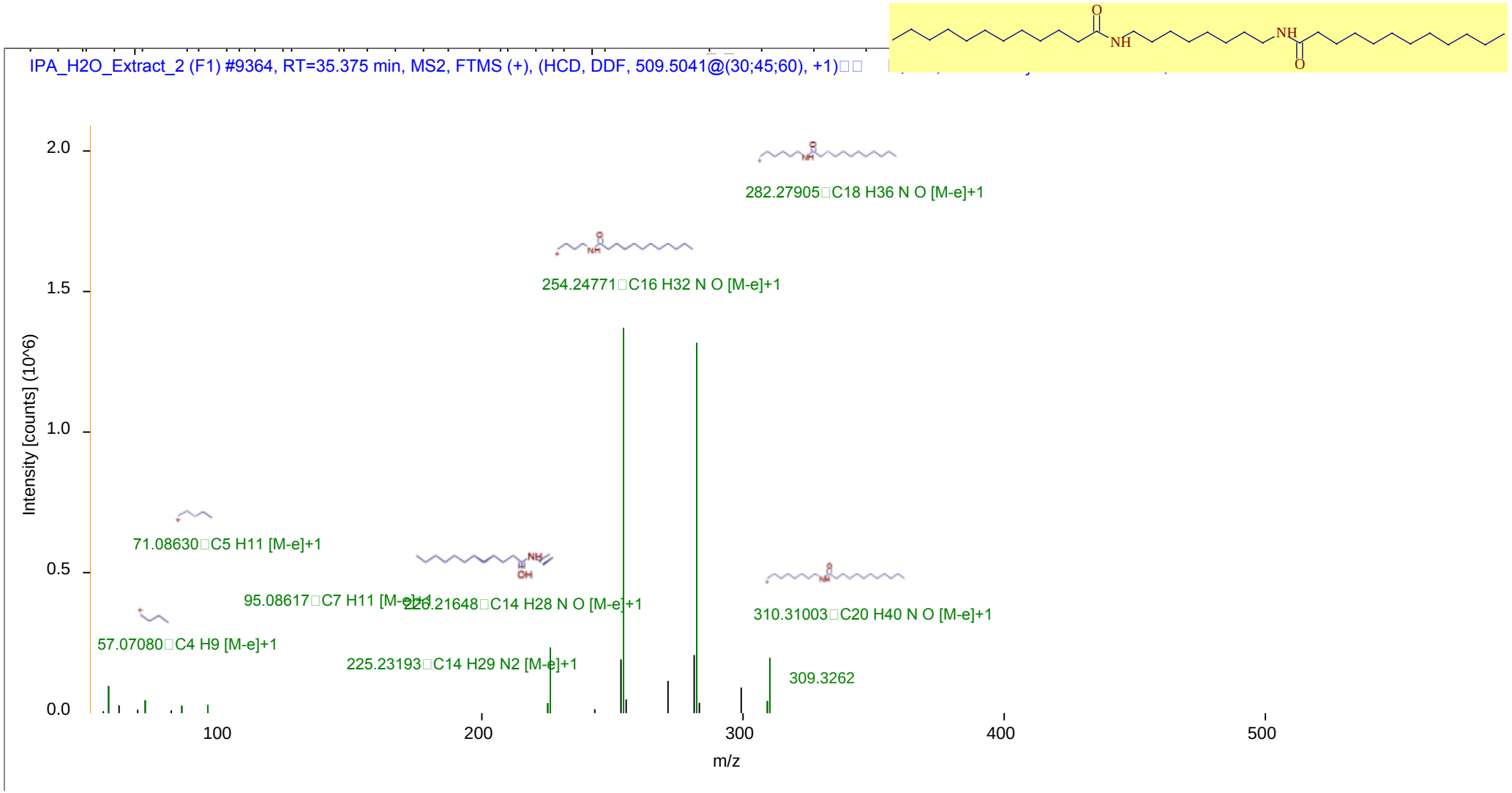
FISh Scoring Result: Fragment Ion Matching and Fragment Structure Annotation



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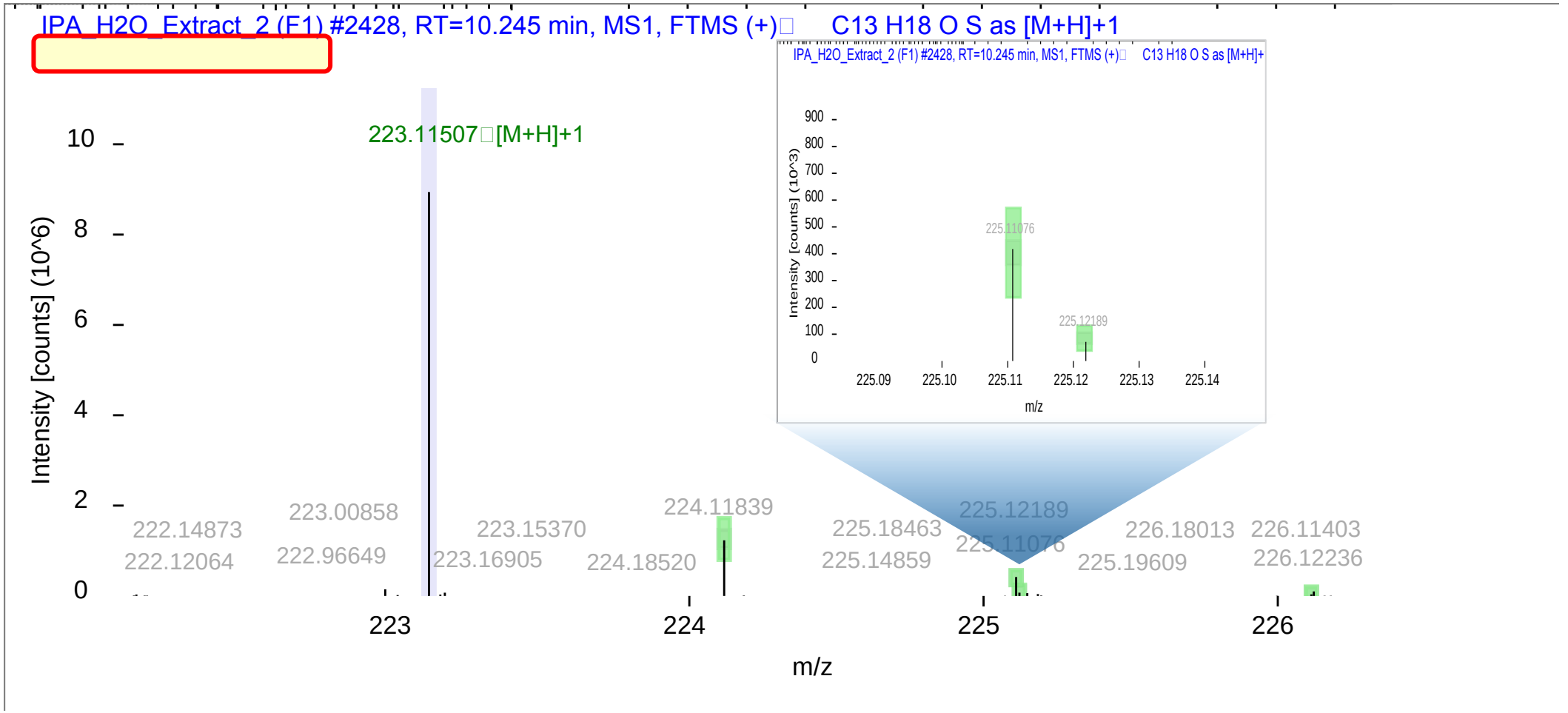
Unknown Structure Elucidation Based on CD 2.1 “Predicted Compositions”



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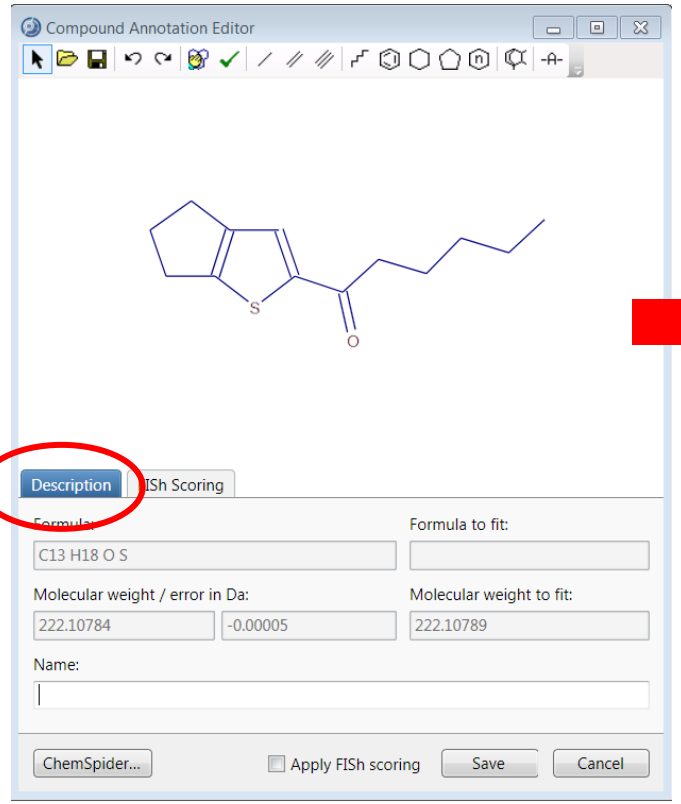
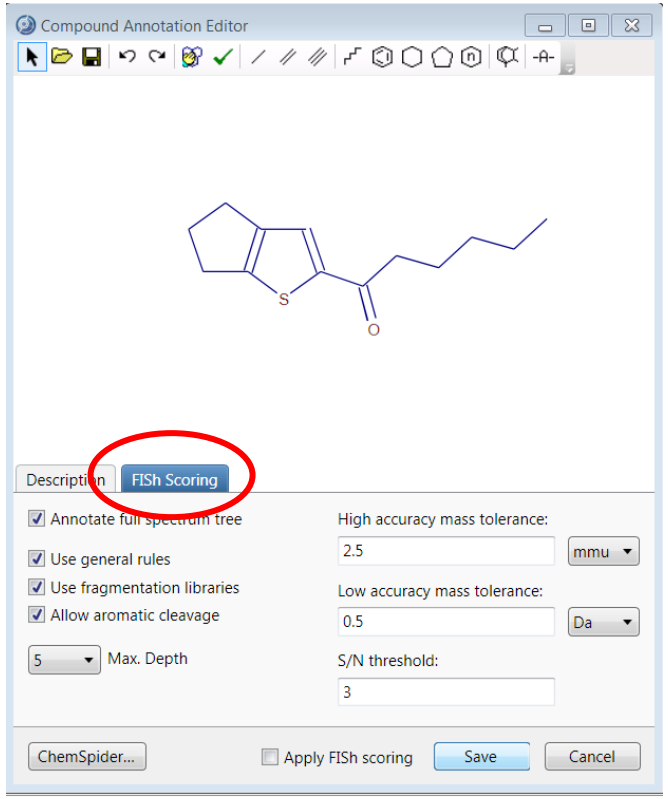
Unknown Structure Elucidation Using Compound Annotation Editor and FISh Scoring

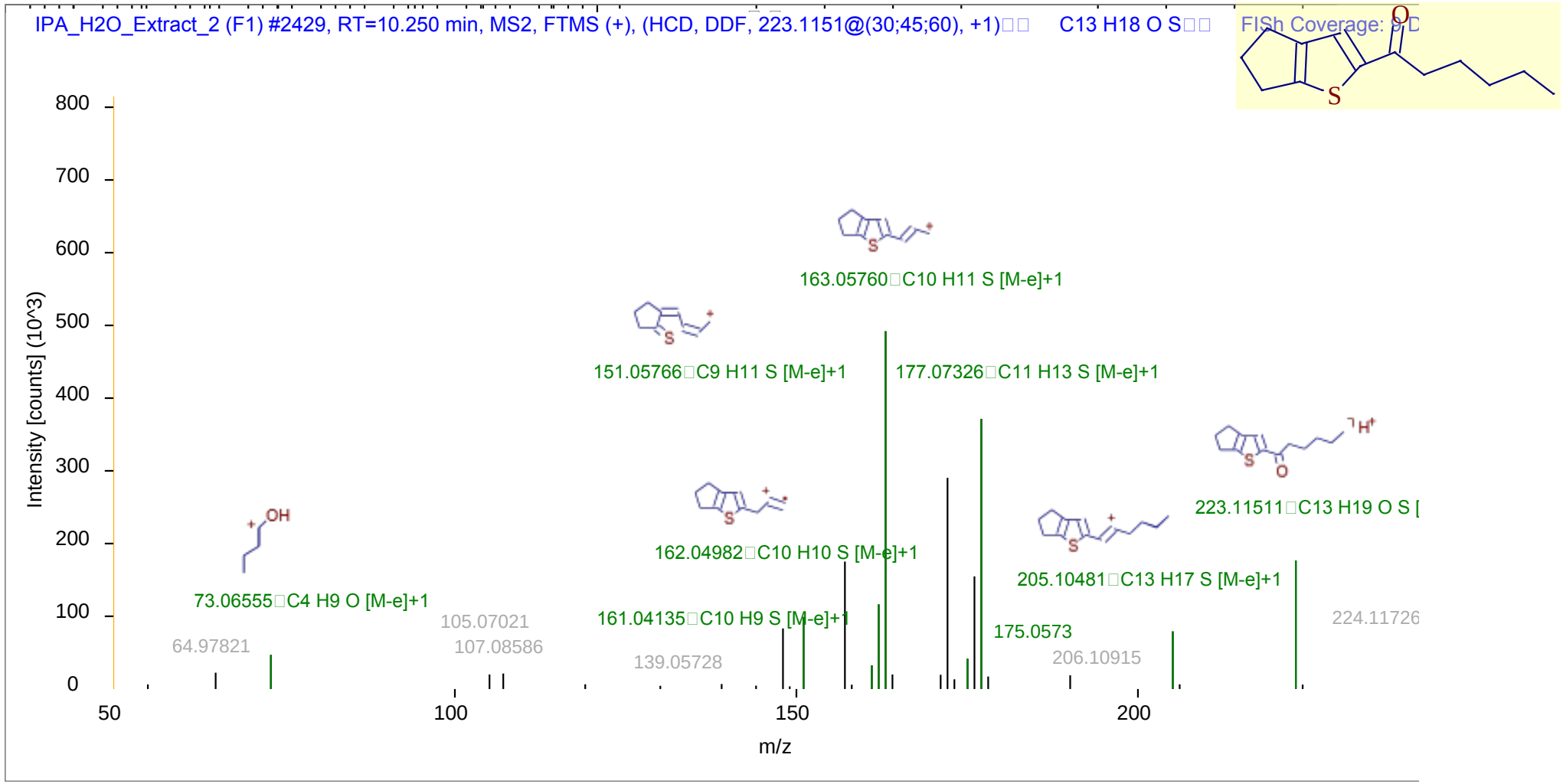


Propose structure based on "Predicted Composition" and MS/MS fragments



Apply "FISh Scoring" to search the embedded "HighChem Fragment Library"

FISh Scoring Result: Fragment Ion Matching and Fragment Structure Annotation



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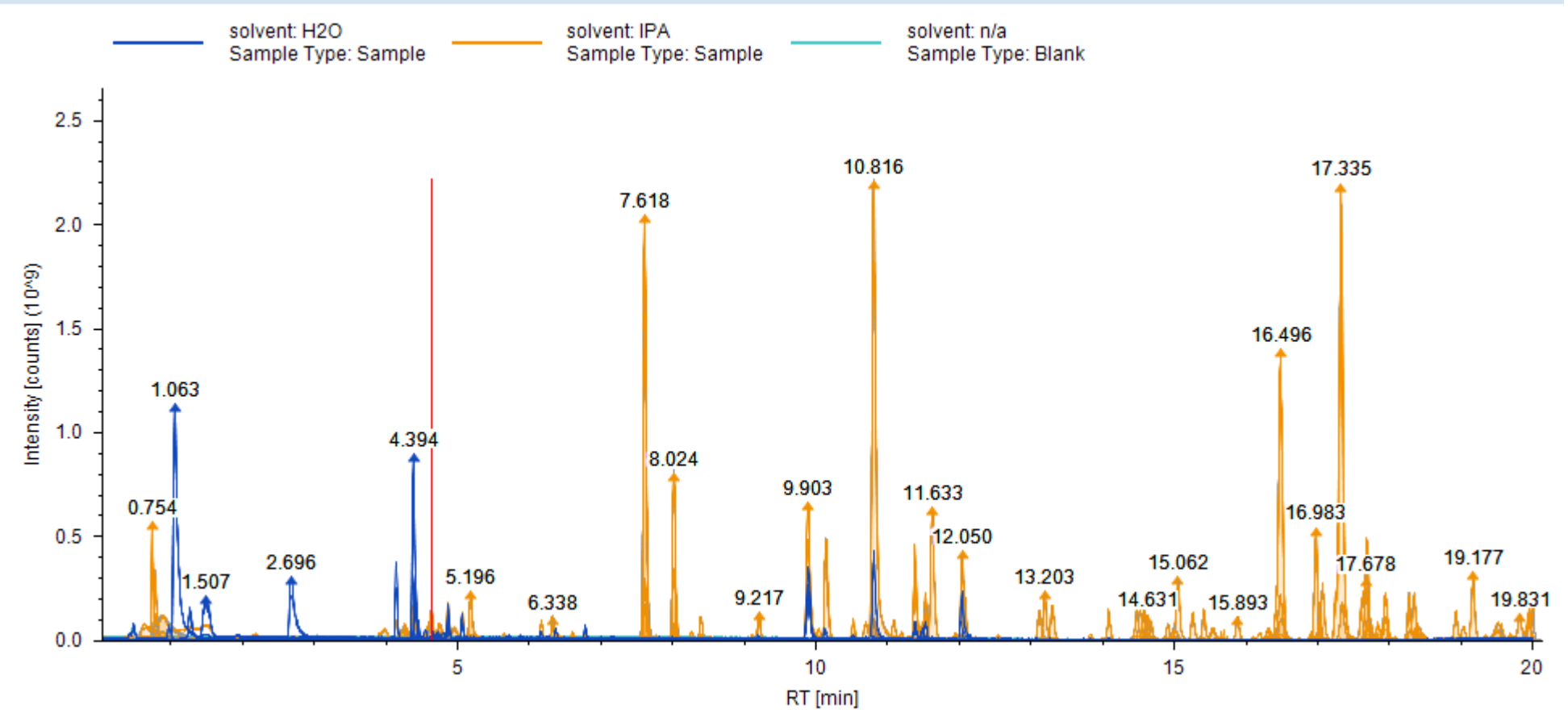
Specialized Traces



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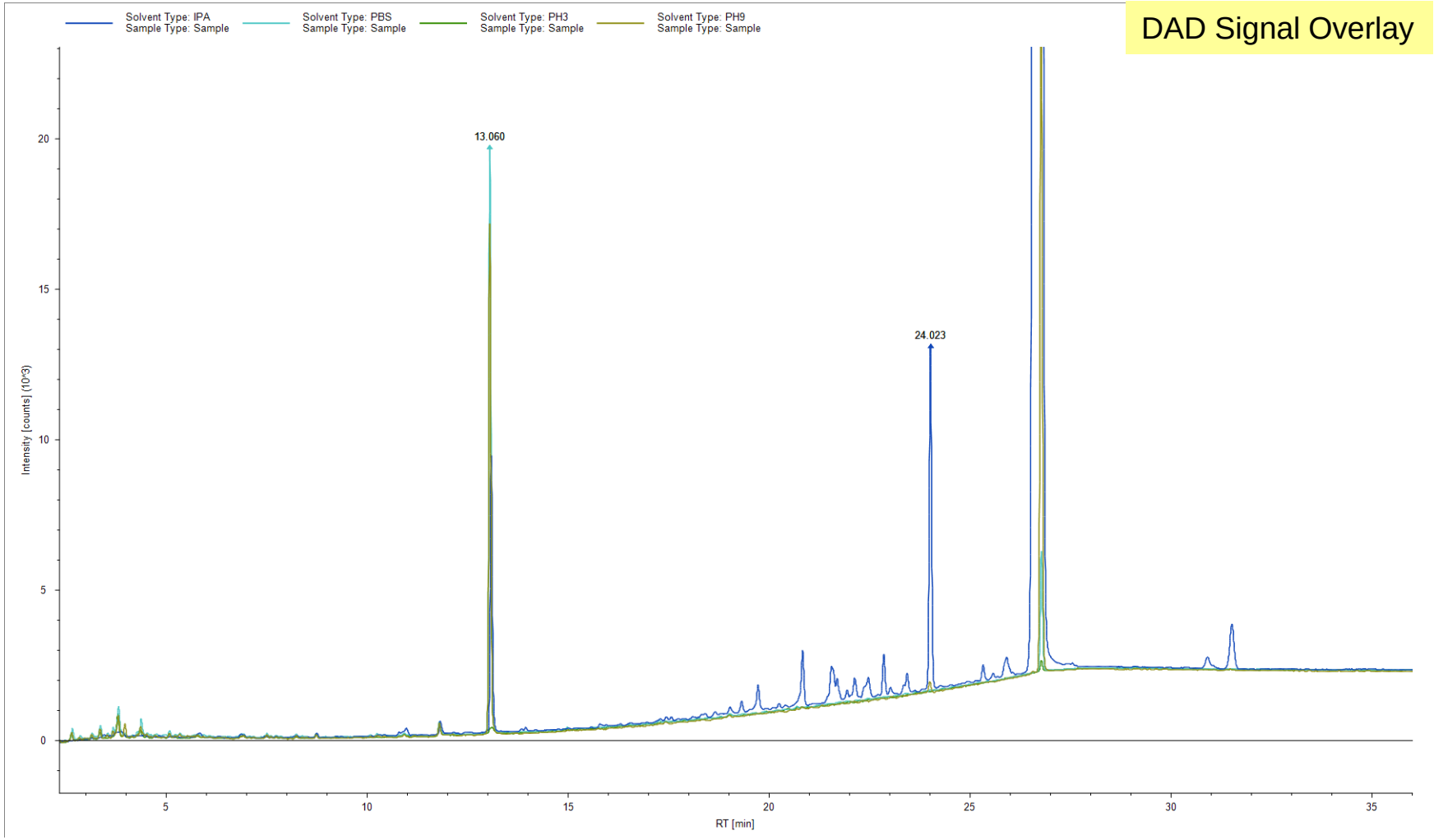
Specialized Traces Provide Additional Information



DAD Signal Overlay

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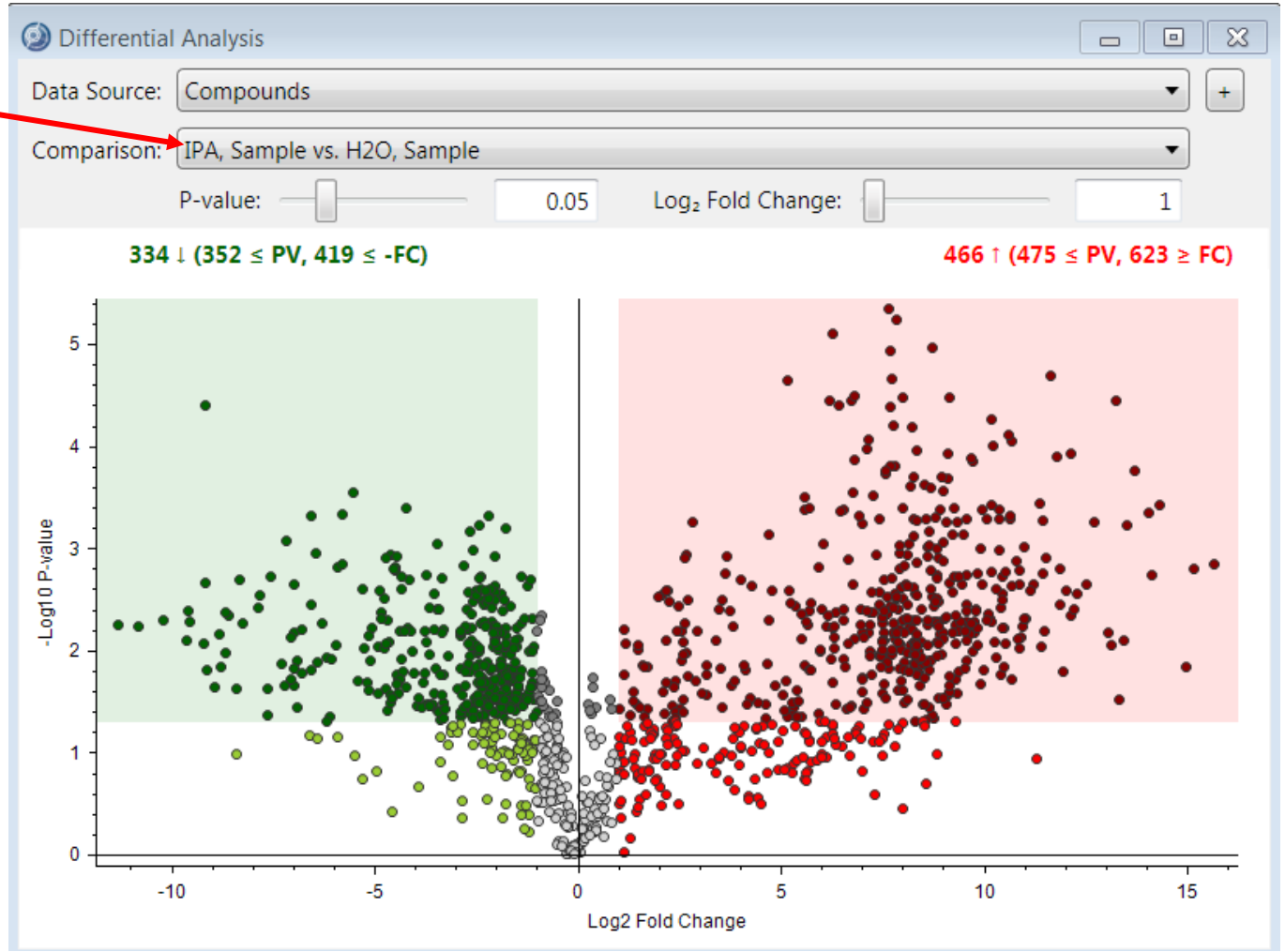
CD 2.1 Differential Analysis Feature: Volcano Plot

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



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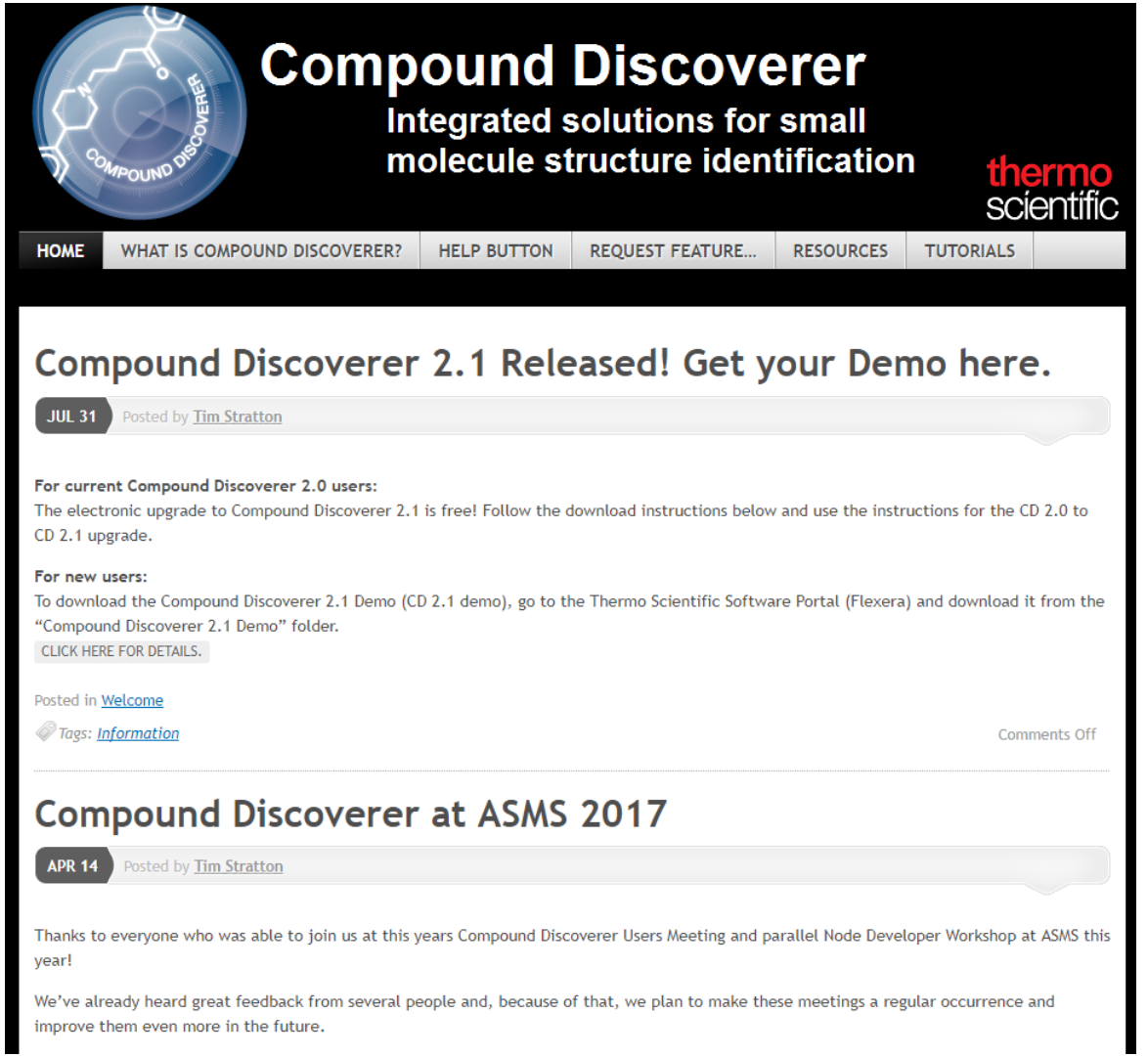
Thermo Scientific Compound Discoverer Software Home Page

http://mycompounddiscoverer.com



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Thank you!

www.thermofisher.com/learnleachables

Kate.comstock@thermofisher.com

Some of the Common Additives

- Plasticizers
- Flame Retardants
- Impact Modifiers
- Antioxidants
- Antimicrobials
- UV Stabilizers
- Colorants
- Lubricants & processing aids