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Applying New GC-Orbitrap™ MS Functionalities to Extractables and Leachables and Metabolomics Analyses

Jason Cole
GC-Orbitrap Product Manager

Presentation Overview

- GC-Orbitrap Overview
- Updated Functionality for Unknowns Identification
  - Automated library search with retention indexing
  - New GC-Orbitrap libraries
  - Variable electron energy (VeV) tuning

Thermo Scientific Orbitrap GC-MS Systems: The Technology Inside

Orbitrap mass analyzer

Incredible HRAM performance

Highly regarded Thermo Scientific™ Q Exactive™ GC system platform

Thermo Scientific™ TRACE™ 1310 GC System

Unique modular injector and detector design

Rapid heat cycling

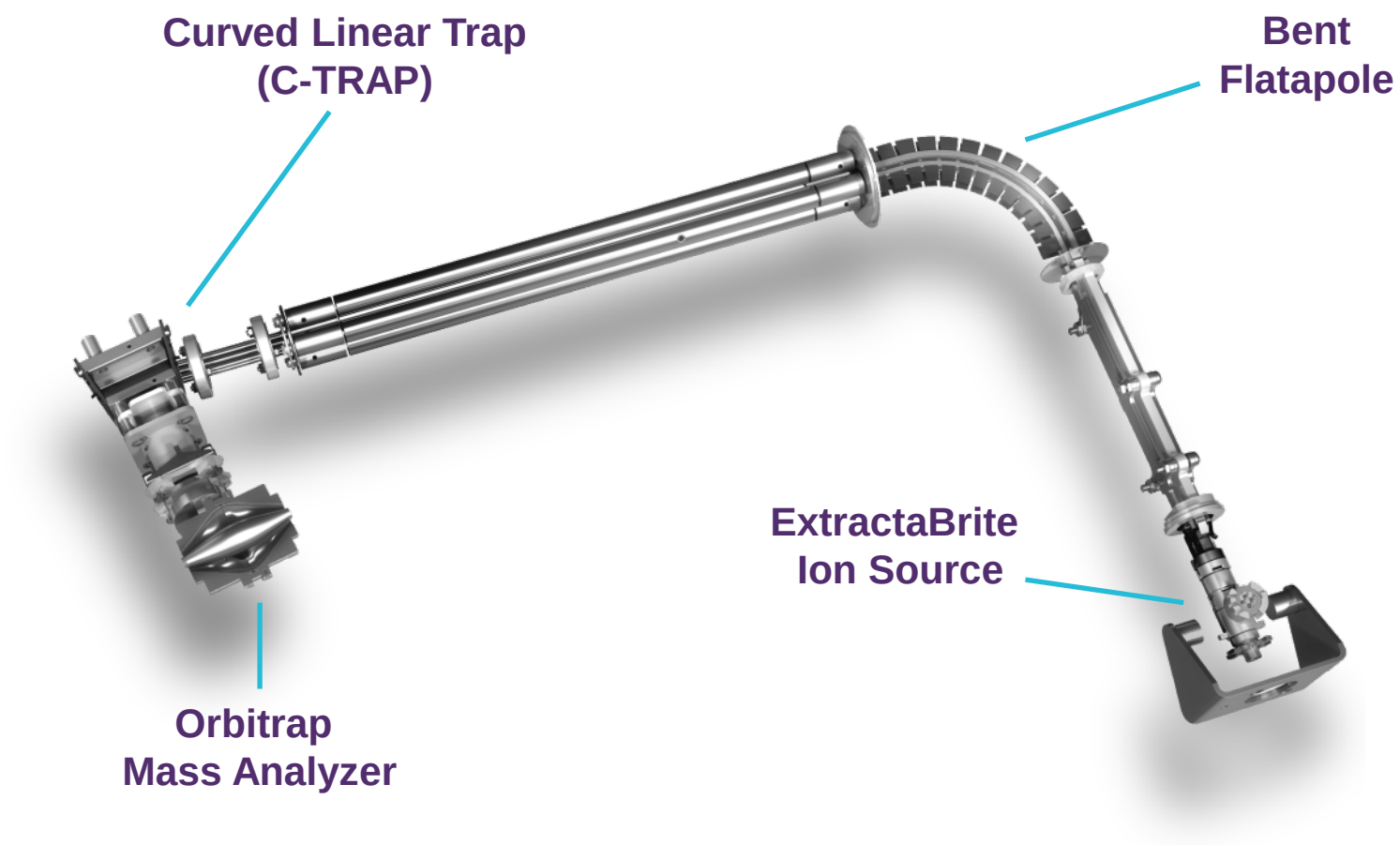
Thermo Scientific™ ExtractaBrite ™ Ion Source technology

Routine grade robustness

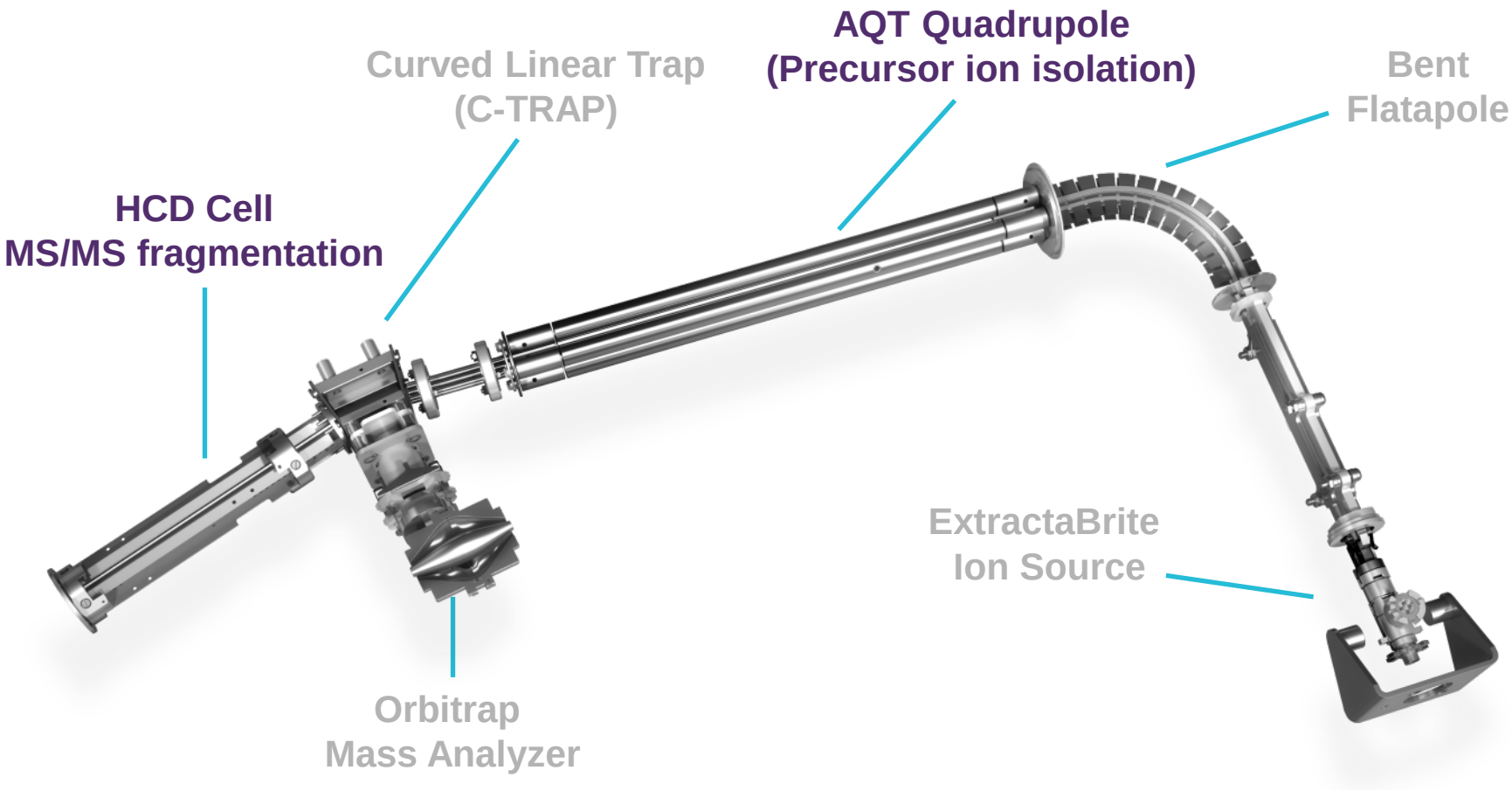
Patented RF lens

Removable without breaking vacuum

Thermo Scientific Exactive GC Orbitrap GC-MS System: The Technology Inside

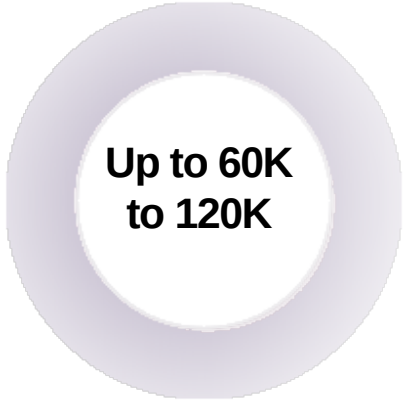


Thermo Scientific Q Exactive Orbitrap GC-MS/MS System: The Technology Inside



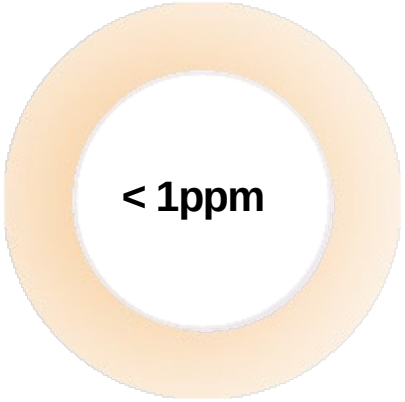
Thermo Scientific Orbitrap GC-MS System Highlights

Resolving Power



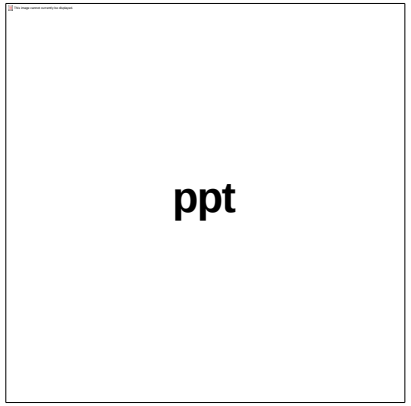
- Maximum selectivity
- Fast enough for GC

Mass Accuracy



- Every scan
- All concentrations
- In complex matrix
- Across the mass range
- Everyday!

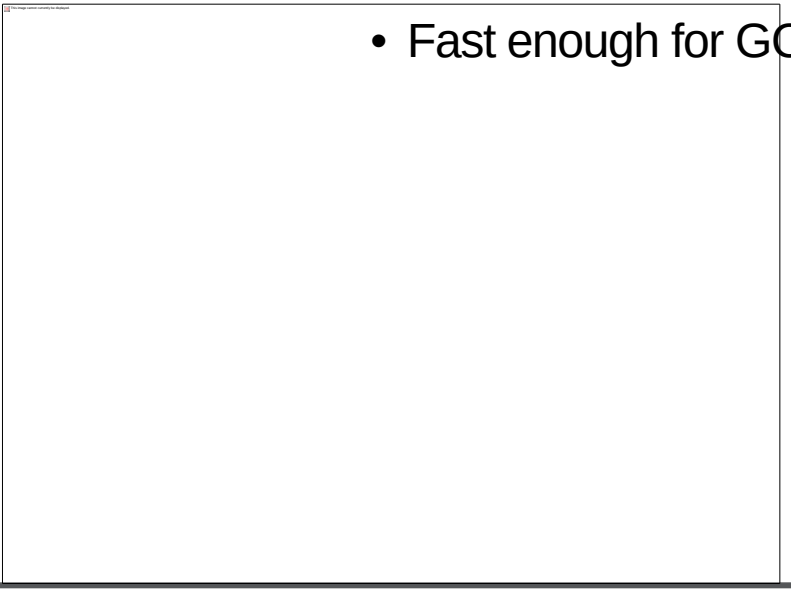
Sensitivity



- In full-scan
- High selectivity
- High spectral fidelity

Dynamic Range

- Excellent coverage in sample profiling
- “Triple quad grade” quantitation in full-scan



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Identification Categories - USP General Chapter <1663>

- Tentative: ID consistent with class of molecule only
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- Confident: Evidence for ID precludes all but related structures
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  - Confirmation of molecular weight and elemental composition
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  - NMR/FTIR confirmation can also increase confidence

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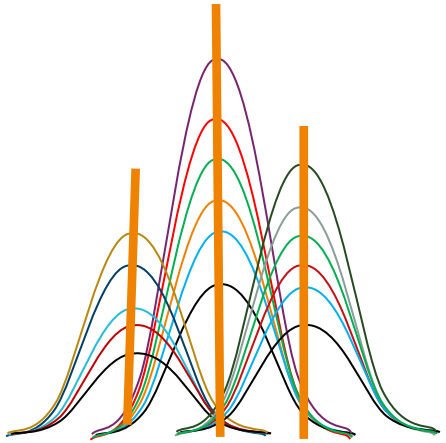
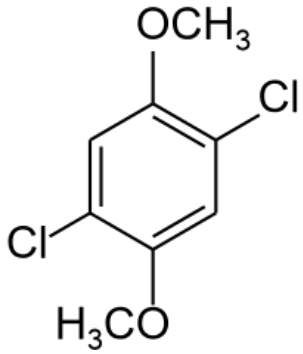
Algorithm for Automated Library Match



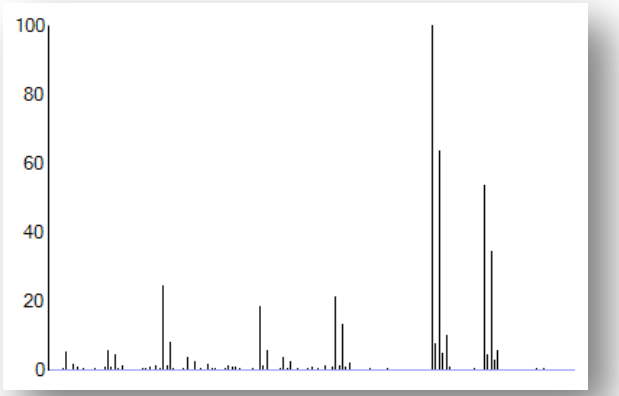
Deconvolve TIC



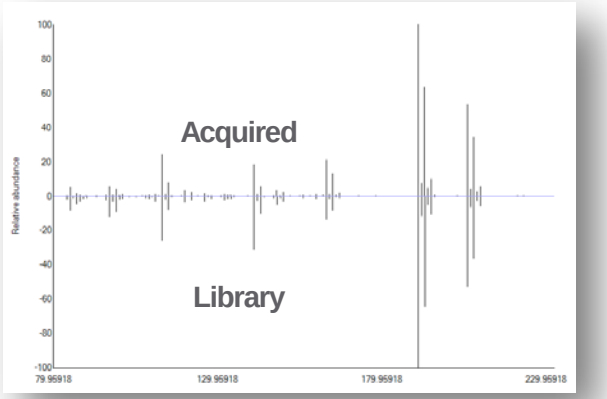
Candidate Compounds

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	$C_5Cl_2H_2O$	147.9477	0.20277
148.9369	$C_5Cl[37]ClHO$	148.9369	0.2679
149.9448	$C_5Cl[37]ClH_2O$	149.9448	0.06602
151.9419	$C_5[37]Cl_2\ H_2O$	151.9418	0.72528
154.9895	$C_7ClH_4O_2$	154.9894	0.38712
155.9974	$C_7ClH_5O_2$	155.9973	0.89745
157.9943	$C_7[37]ClH_5O_2$	157.9943	0.25381
159.9479	$C_6Cl_2H_2O$	159.9477	0.87529
161.9446	$C_6\ Cl[37]ClH_2O$	161.9448	0.80213
162.9711	$C6Cl_2H_5O$	162.9712	0.36816
163.9745	$C_5[13]CCl_2H_5O$	163.9745	0.3342
164.9682	$C_6Cl[37]ClH_5O$	164.9682	0.24186
165.9716	$C_5CCl[37]ClH_5O$	165.9716	0.02832

Formula confirmation with HRF scoring

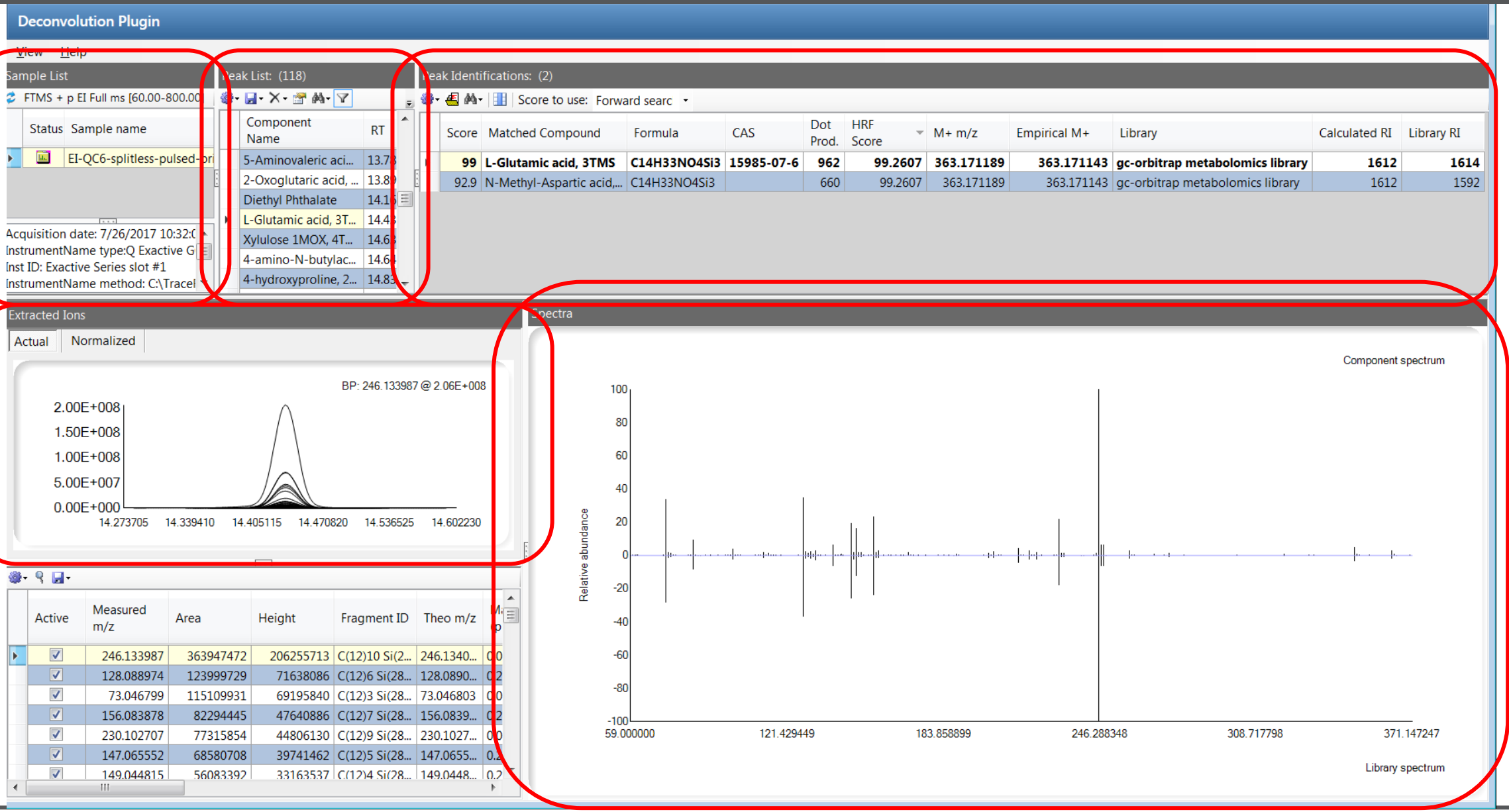


Create “clean” spectrum



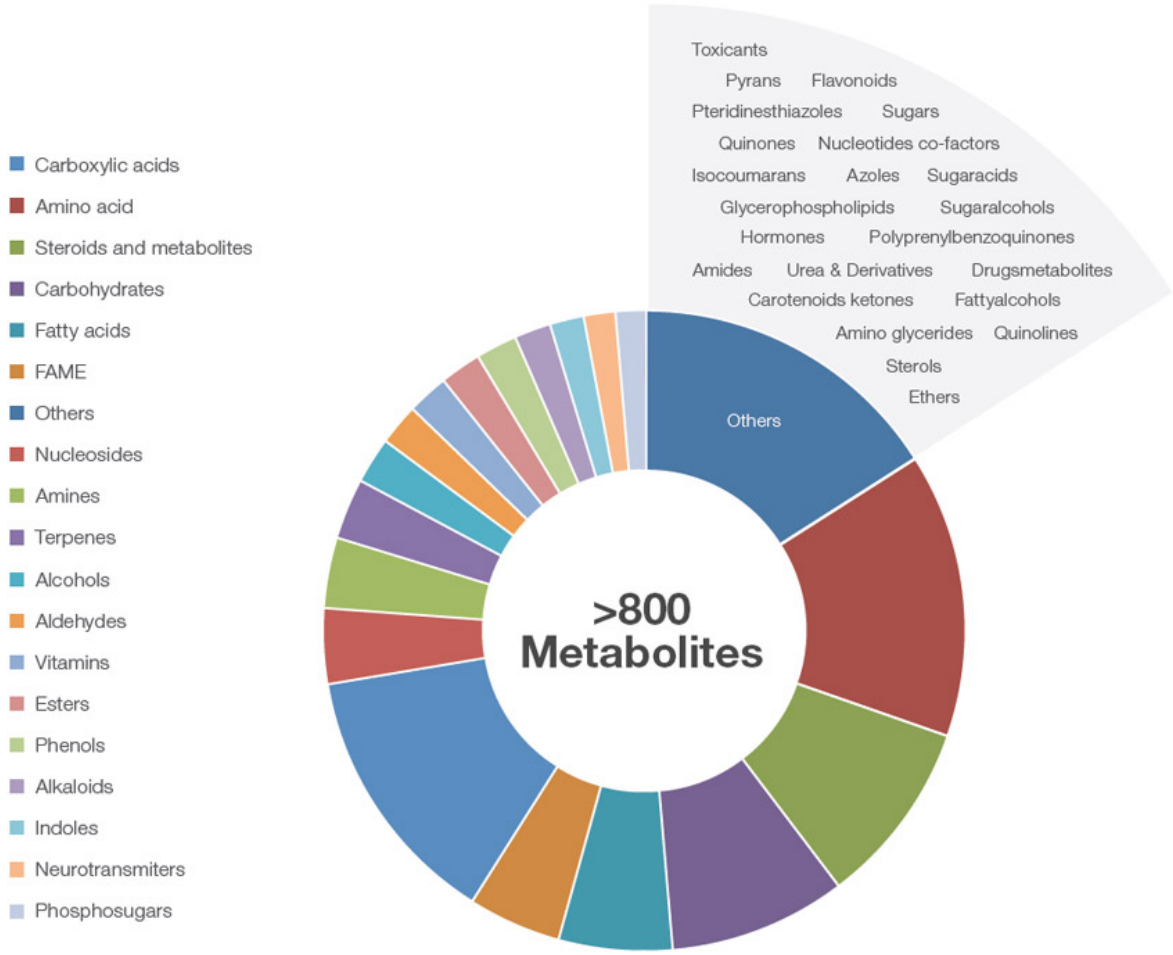
Library search

Thermo Scientific TraceFinder Software Deconvolution Overview



GC-Orbitrap Metabolomics Library

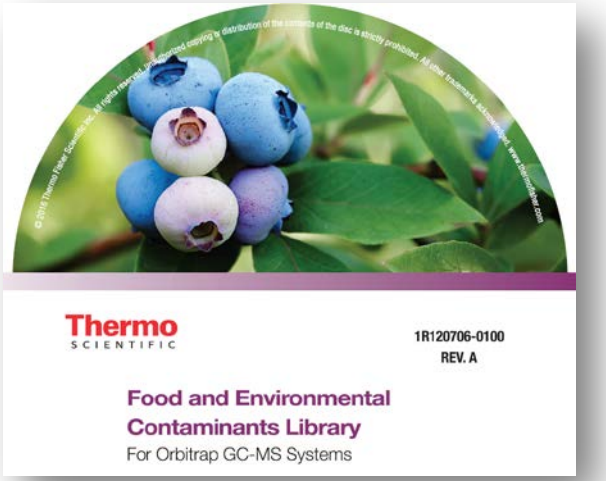
- Over 800 unique metabolites
- Majority with Methoxyamine/MSTFA derivatization
- High resolution, high mass accuracy spectra acquired at 60,000 RP (*m/z* 200)
- Contains Kovats retention indices
- Can be used in combination with existing unit mass libraries



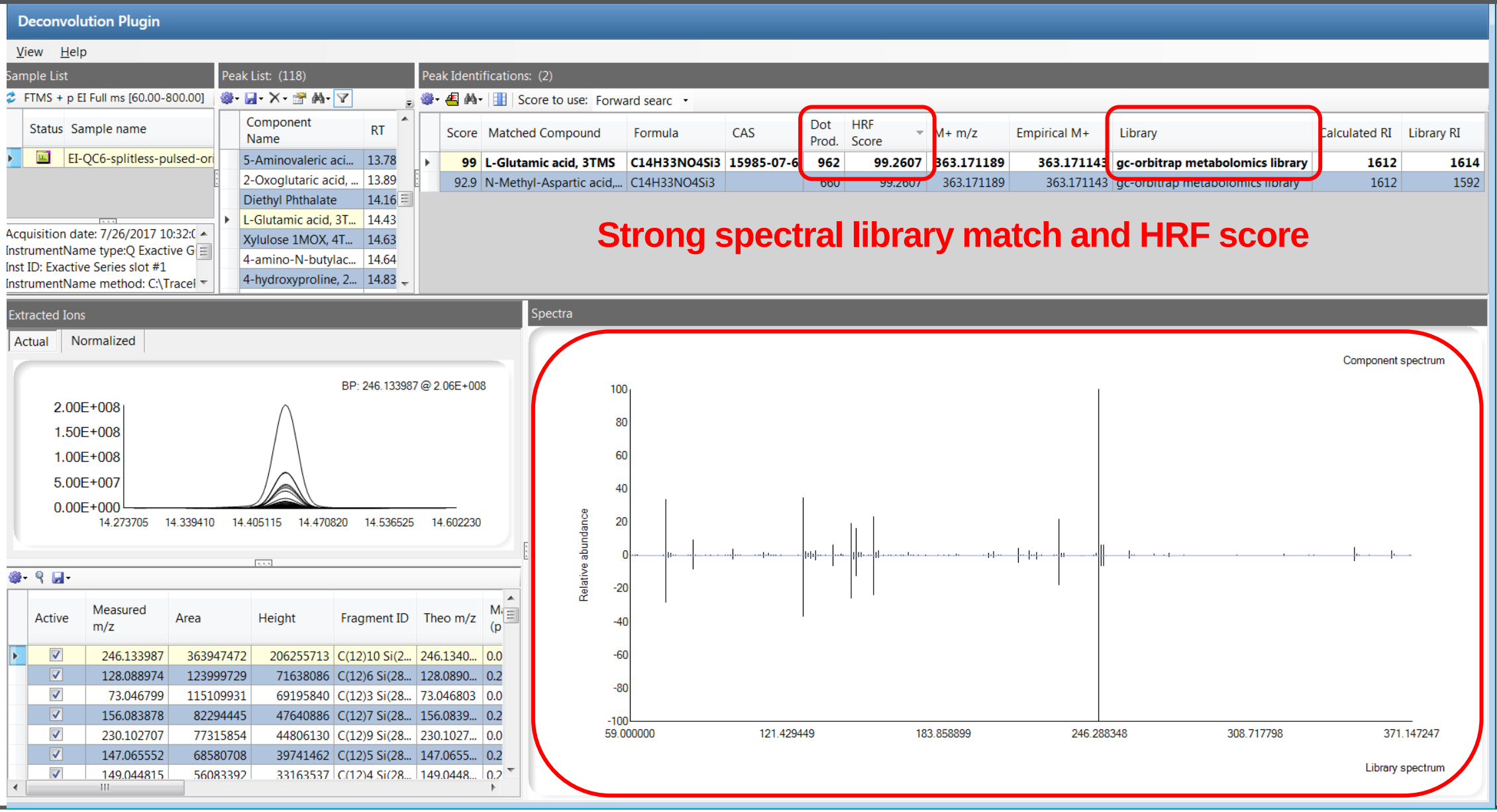
Orbitrap GC-MS Contaminants Library

- Fast start-up of screening and quantitation applications
  - Thermo Scientific™ TraceFinder™ Compound Database >700 food and environmental contaminants
  - HRAM Spectral Library of over 700 food and environmental contaminants
  - User guide detailing how to install and make custom enhancements to library

- Compound classes V1:
  - Pesticides, PAHs, PCBs,Dioxins and Furans. Flame Retardants



Example Unknown Peak Detection and Identification



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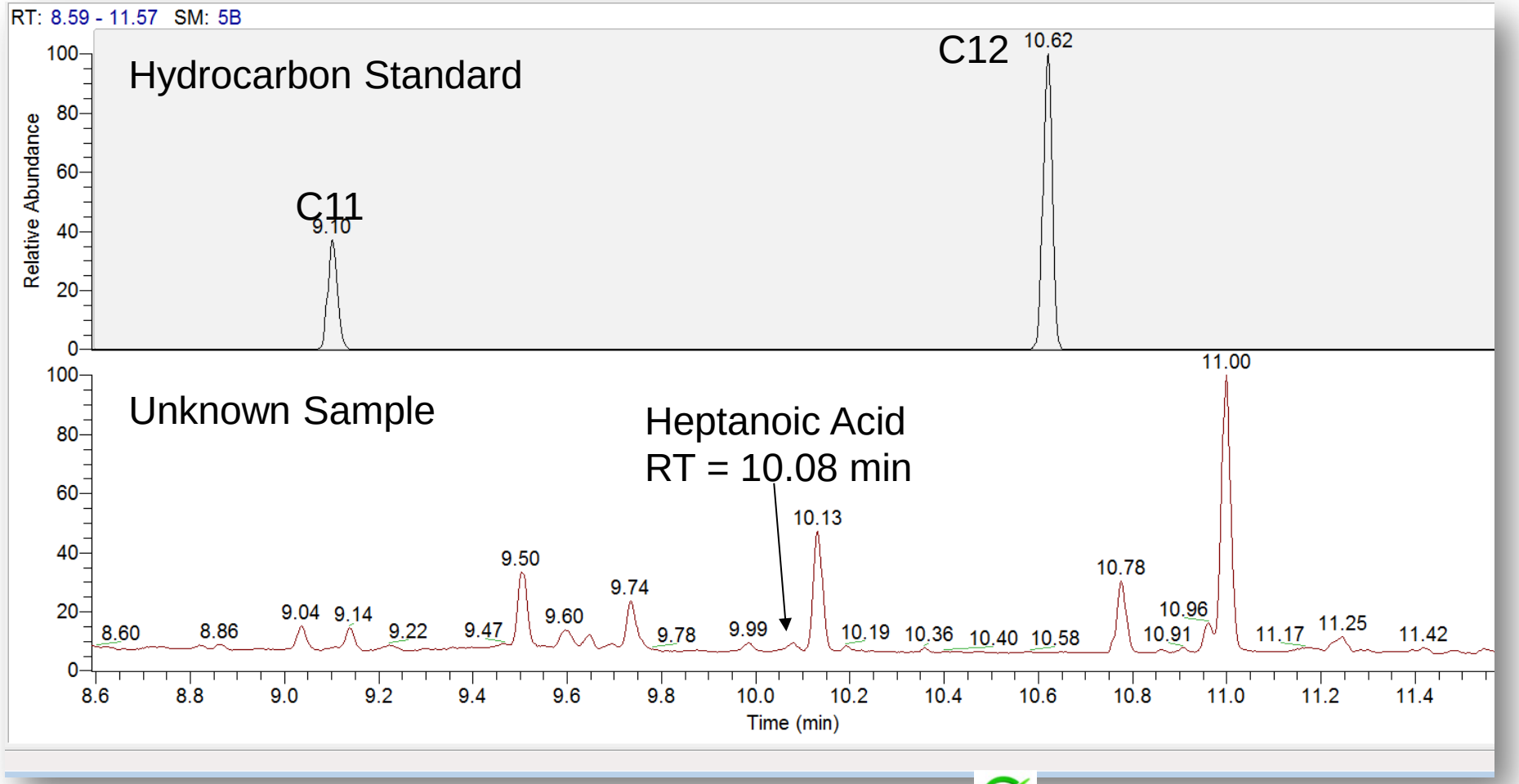
Retention Indexing Background



Retention Index (measured) = 1164

Heptatonic Acid Retention Index (library) = 1169

$$RI = 100x(C1 + \frac{RT(Unk) - RT(C1)}{RT(C2) - RT(C1)})$$

$$RI(Heptanoic\ Acid) = 100x(11 + \frac{10.08\ min\ - 9.10\ min}{10.62\ min - 9.10\ min})$$

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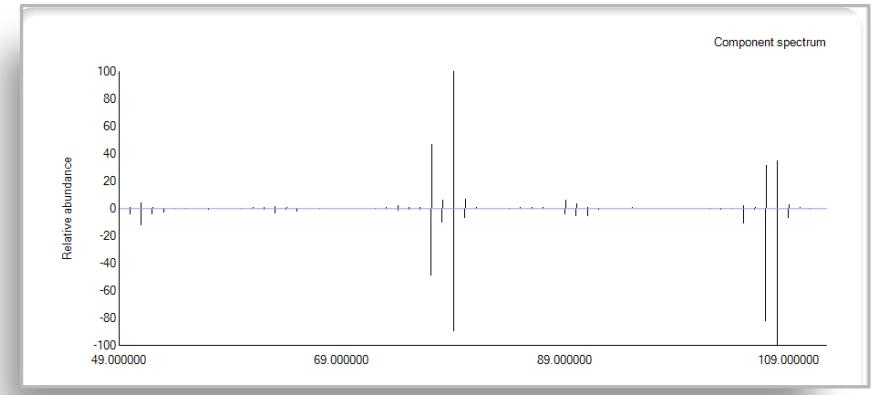
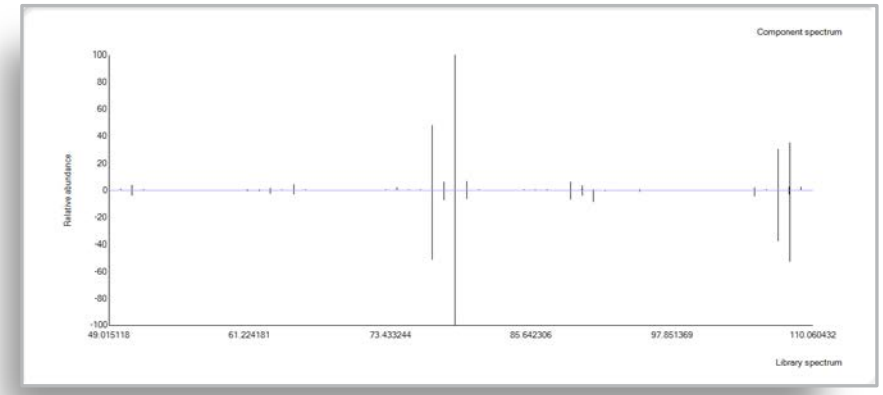
Example of Implementation of RI Scoring



1-phenyl-1,2-propanediol



benzyl alcohol

Spectral scores nearly identical

Retention index clearly indicates benzyl alcohol

Score	Matched Compound	Formula	SI	HRF Score	Calculated RI	Library RI	Δ RI	(%) Δ RI
96	Benzyl alcohol	C7H8O	878	99.4017	1038	1036	2	0.2
96.1	**1,2-Propanediol, 1-phenyl-**	**C9H12O2**	**814**	**99.4017**	**1038**	**1327**	**289**	**27.8**
~~95.9~~	~~dl-Erythro-1-phenyl-1,2-pr...~~	~~C9H12O2~~	~~805~~	~~99.4017~~	~~1038~~	~~N/A~~	~~N/A~~	~~N/A~~
95.8	N-Cbz-glycylglycine p-nitr...	C18H17N3O7	796	99.5983	1038	N/A	N/A	N/A
95.5	L-Arginine, N2-[(phenylmet...	C14H20N4O4	782	99.5983	1038	N/A	N/A	N/A
95.4	1,2-Ethanediol, 1,2-dipheny...	C14H14O2	781	99.4017	1038	N/A	N/A	N/A
95.1	2,6-Pyridinedicarboxaldehy...	C19H19N7O	762	99.5983	1038	N/A	N/A	N/A
94.8	N-α,N-ω-Di-cbz-L-arginine	C22H26N4O6	745	99.5983	1038	N/A	N/A	N/A
94.8	o-Cresol	C7H8O	749	99.4017	1038	N/A	N/A	N/A
94.6	Ethyl (S)-(+)-mandelate	C10H12O3	740	99.4017	1038	N/A	N/A	N/A
94.6	N-carbobenzyloxy-l-tyrosyl...	C22H26N2O6	738	99.5983	1038	N/A	N/A	N/A
94.6	Phenol, 3-methyl-	C7H8O	739	99.4017	1038	1075	37	3.6

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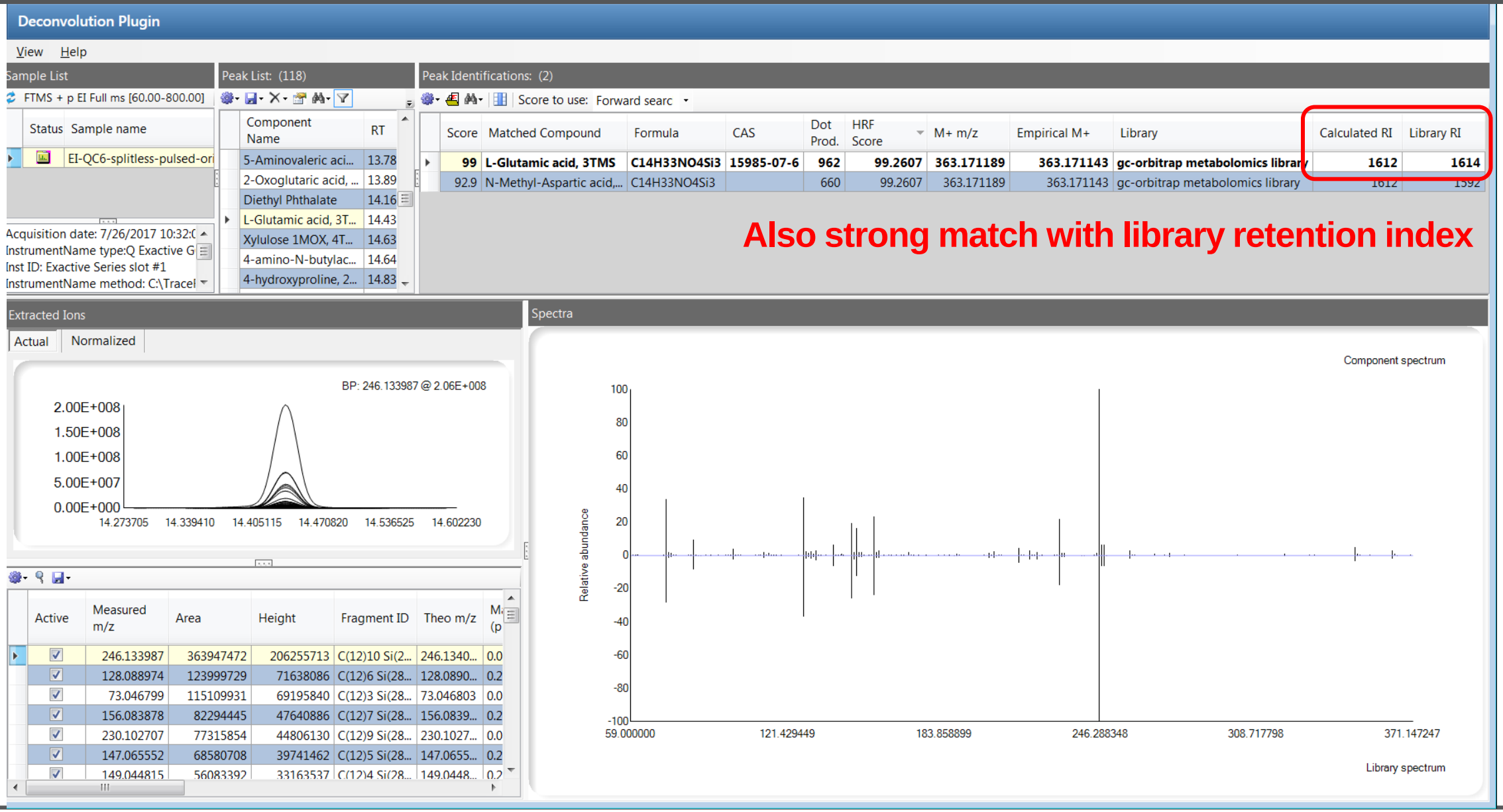

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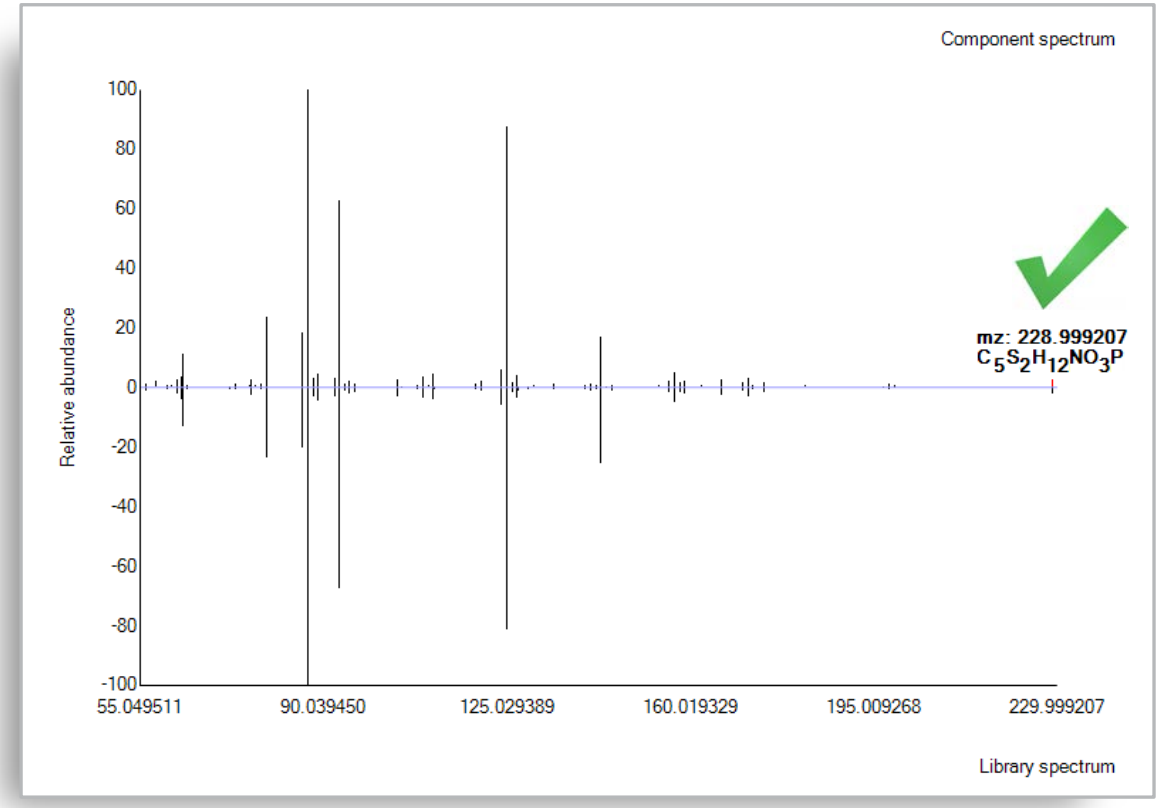
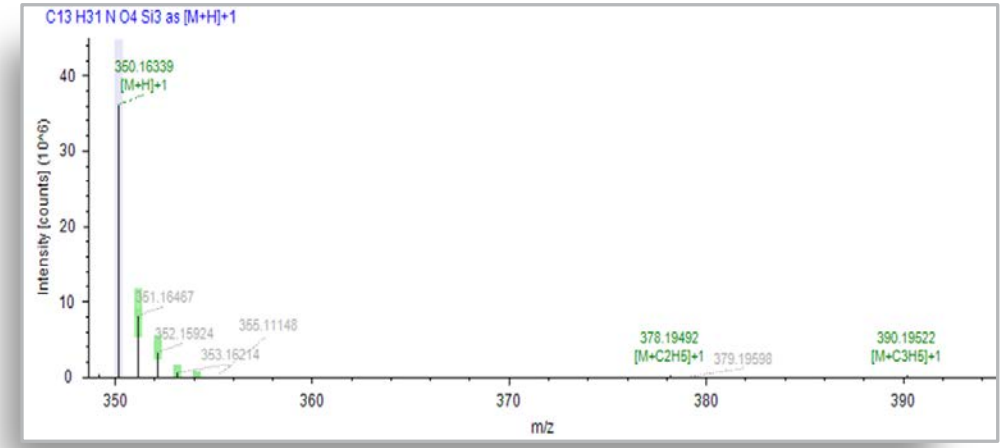
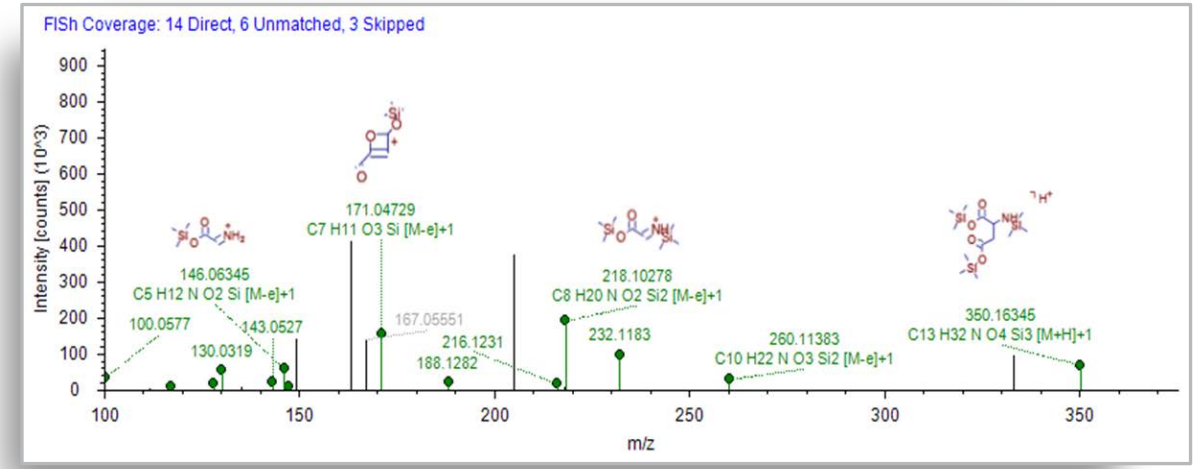
Reasons of Elemental Formula Elucidation

- Increase confidence of library hit



- First step in determining true unknown





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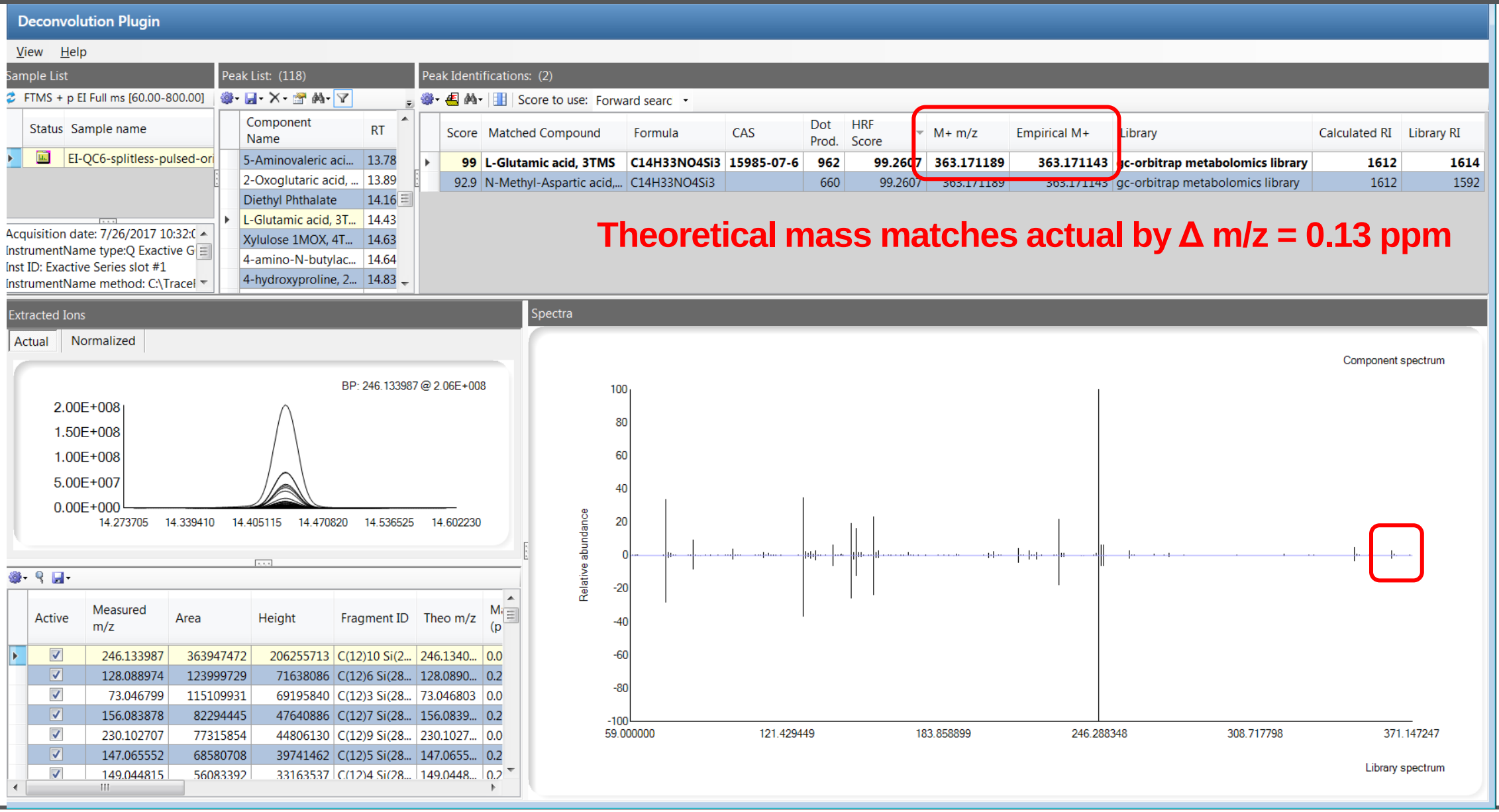
Molecular Ion Confirmation of Library Search



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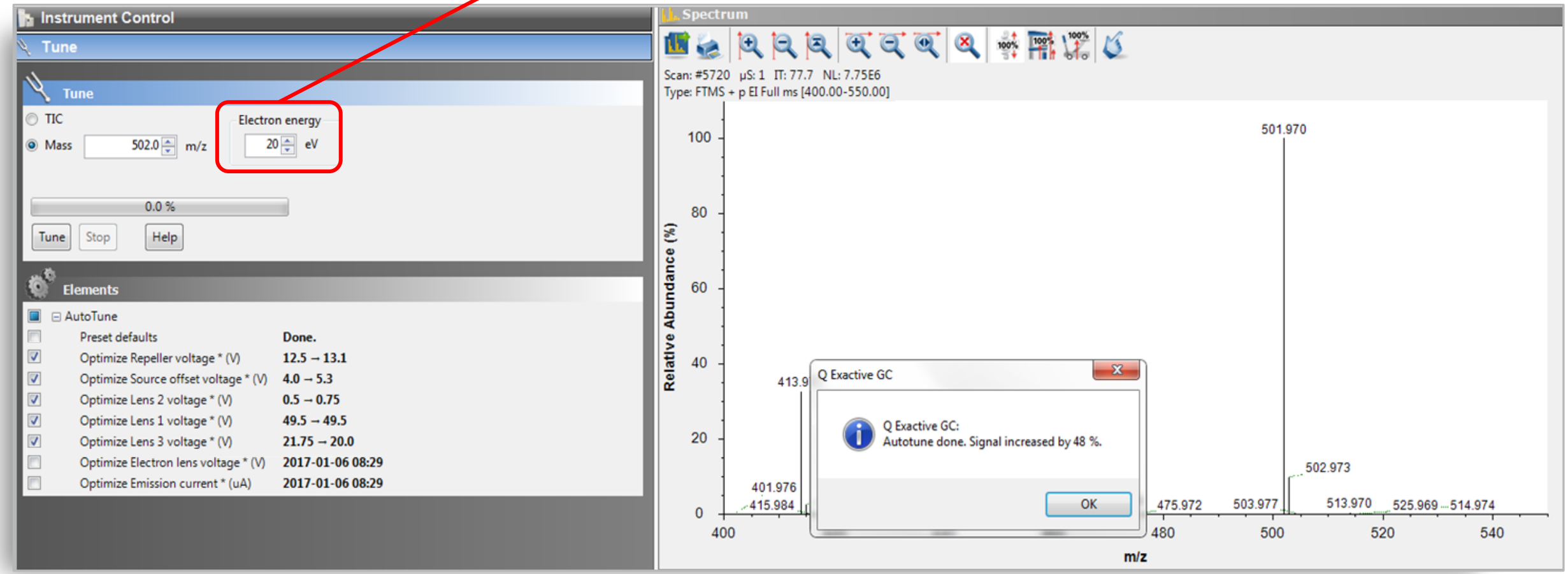
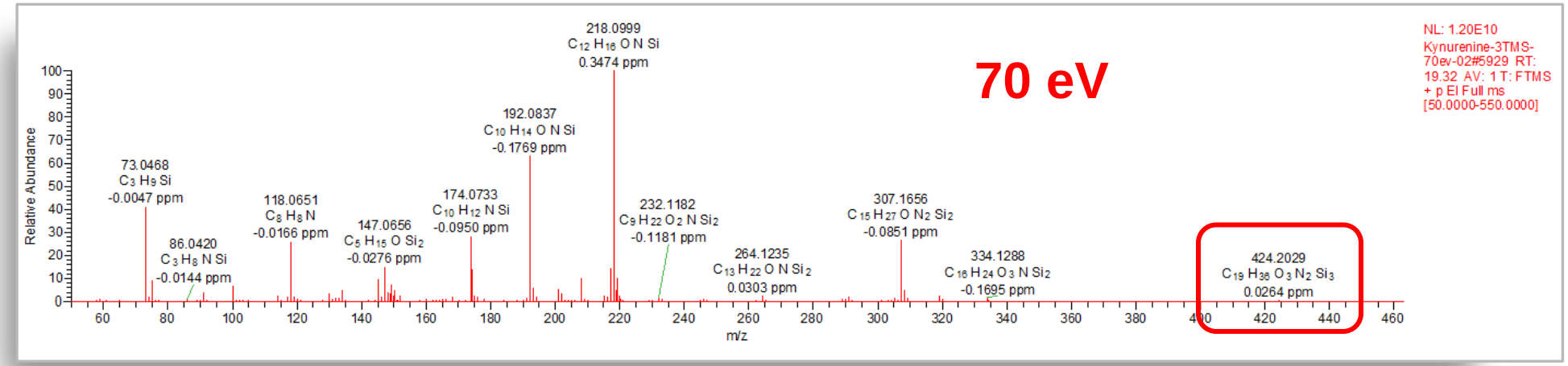
Variable Electron Energy Tuning (VeV)

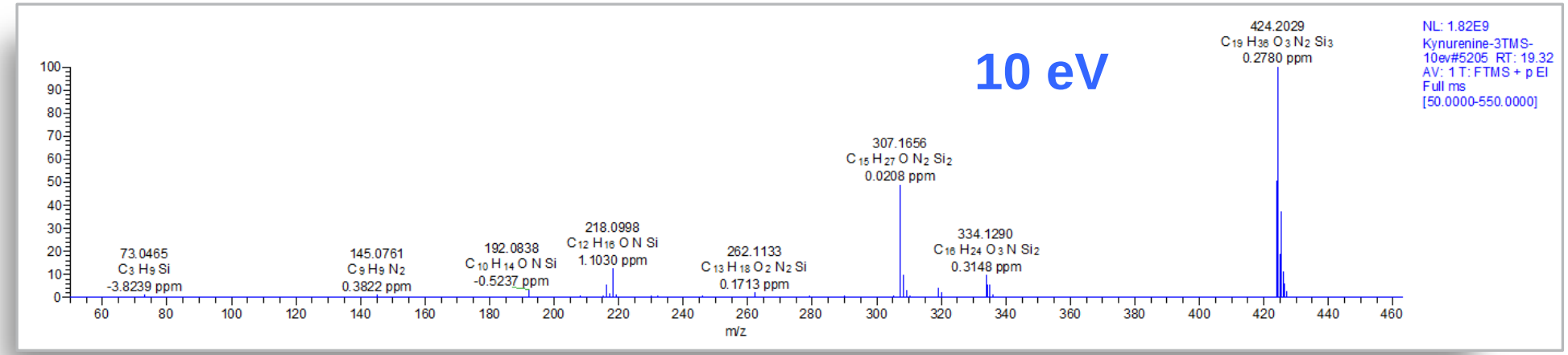
Set electron energy and tune

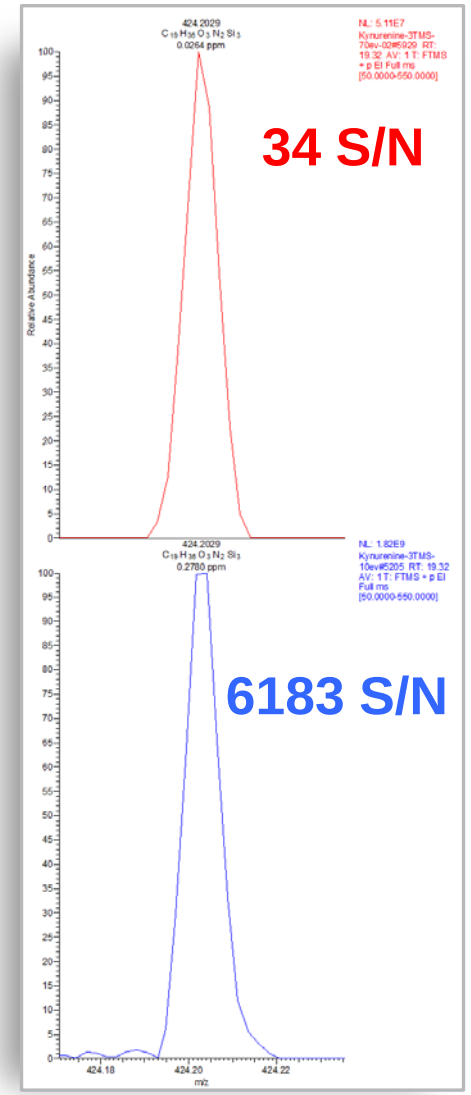


Robust tuning down to near the ionization potential of FC43

Boosting Molecular Ion of Kynurenine with VeV







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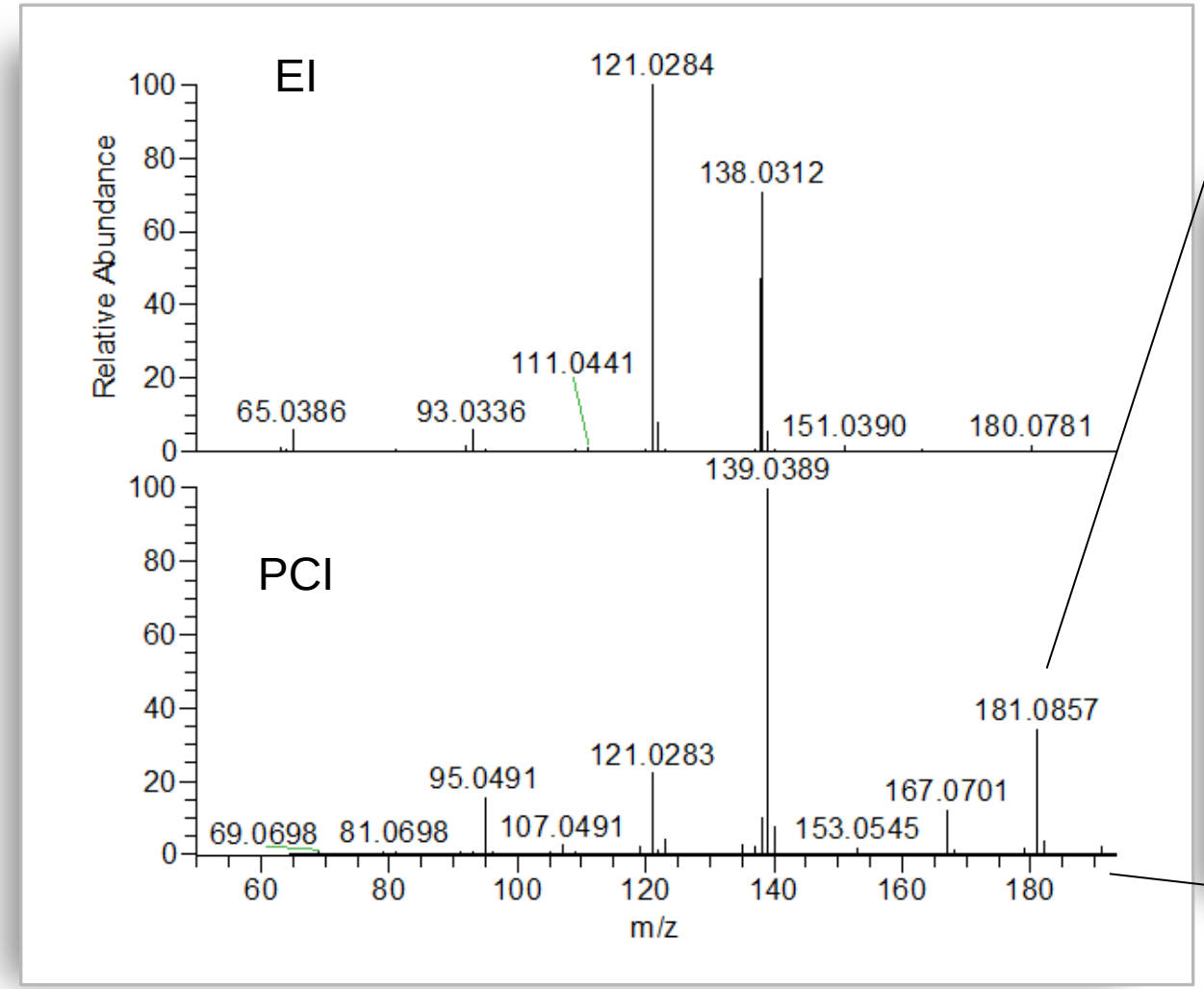
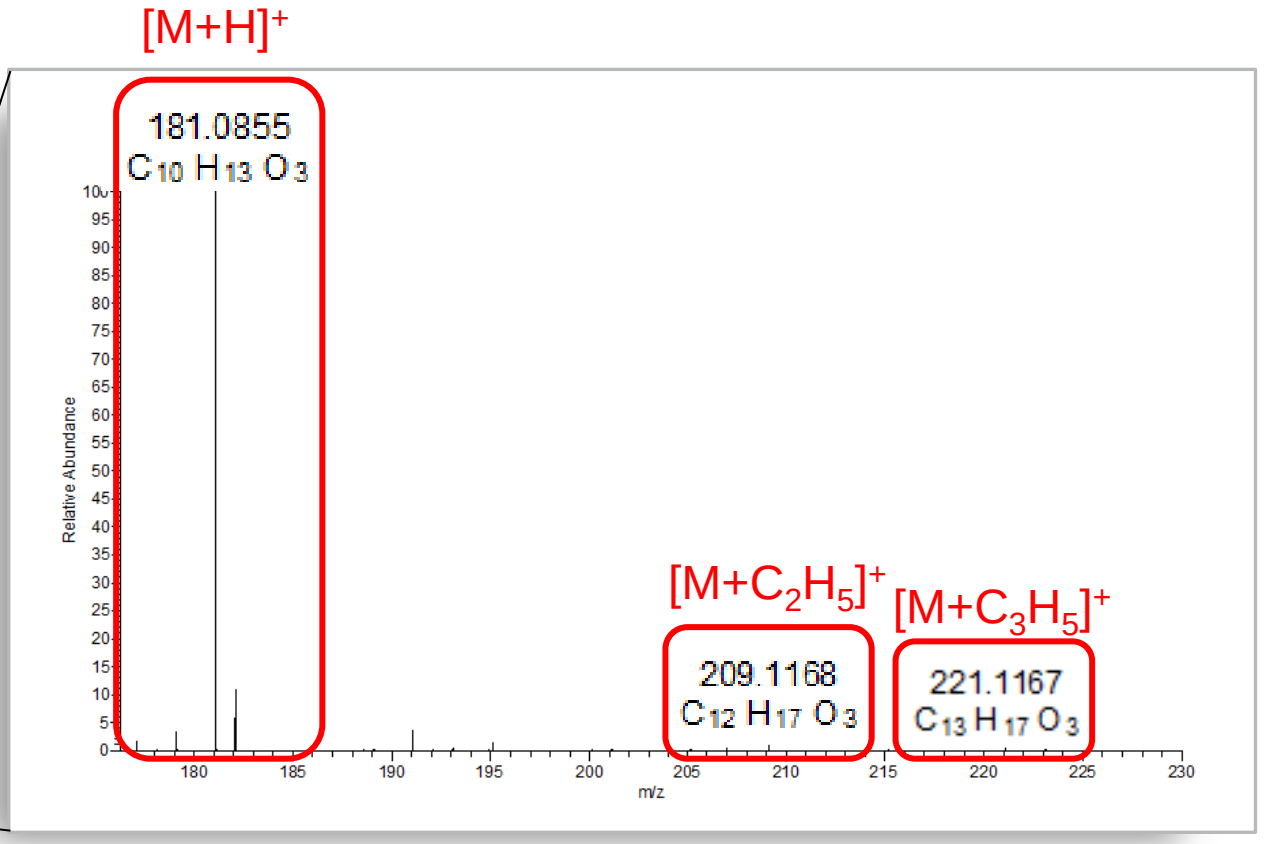
Molecular Formula Confirmation of Propyl Paraben with Chemical Ionization





Courtesy of Dr. Gyorgy Vas, Intertek

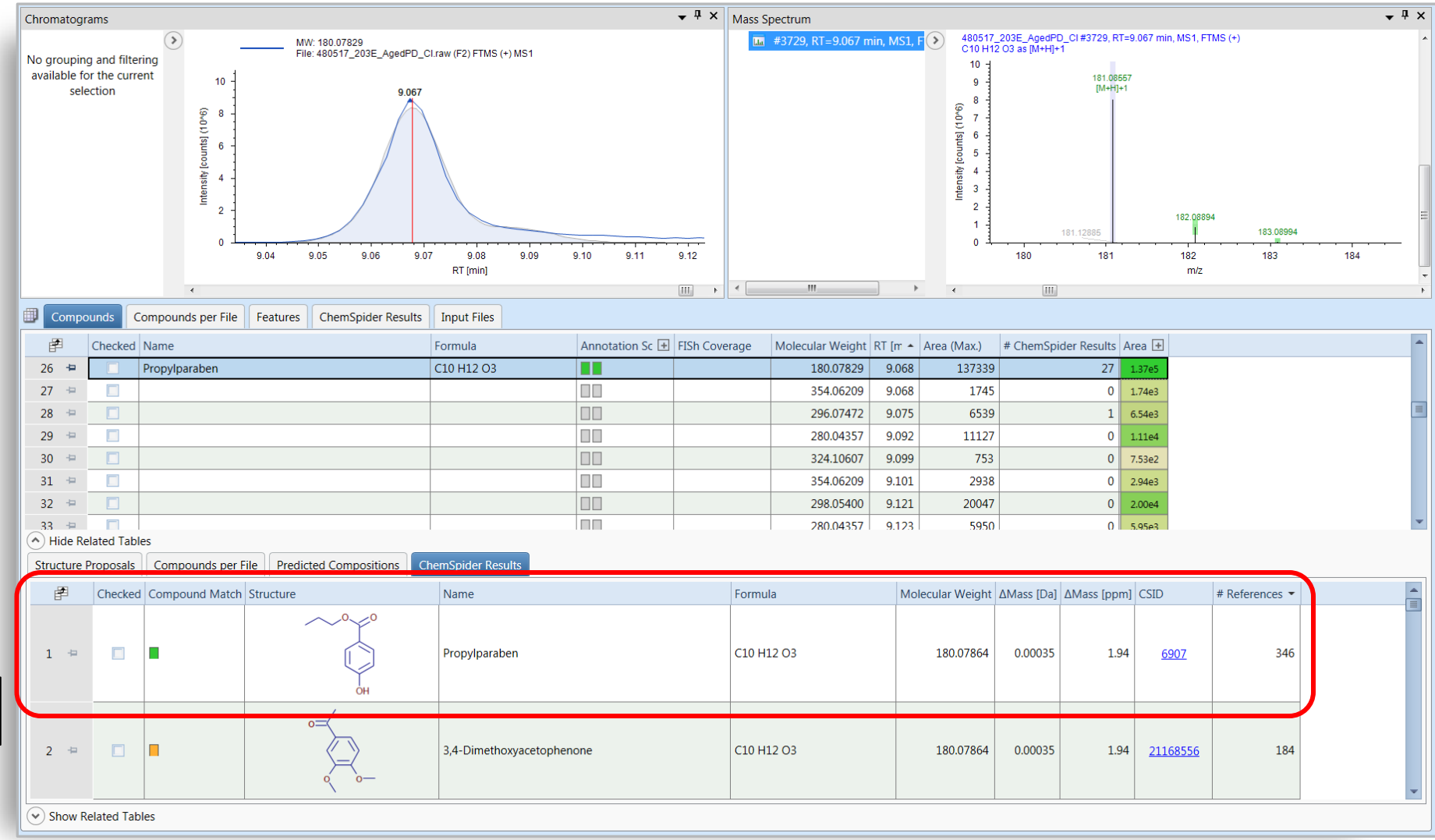
Automated CI Detection in Thermo Scientific Compound Discoverer Software

Thermo Scientific™ Compound Discoverer™ Software

Search ChemSpider

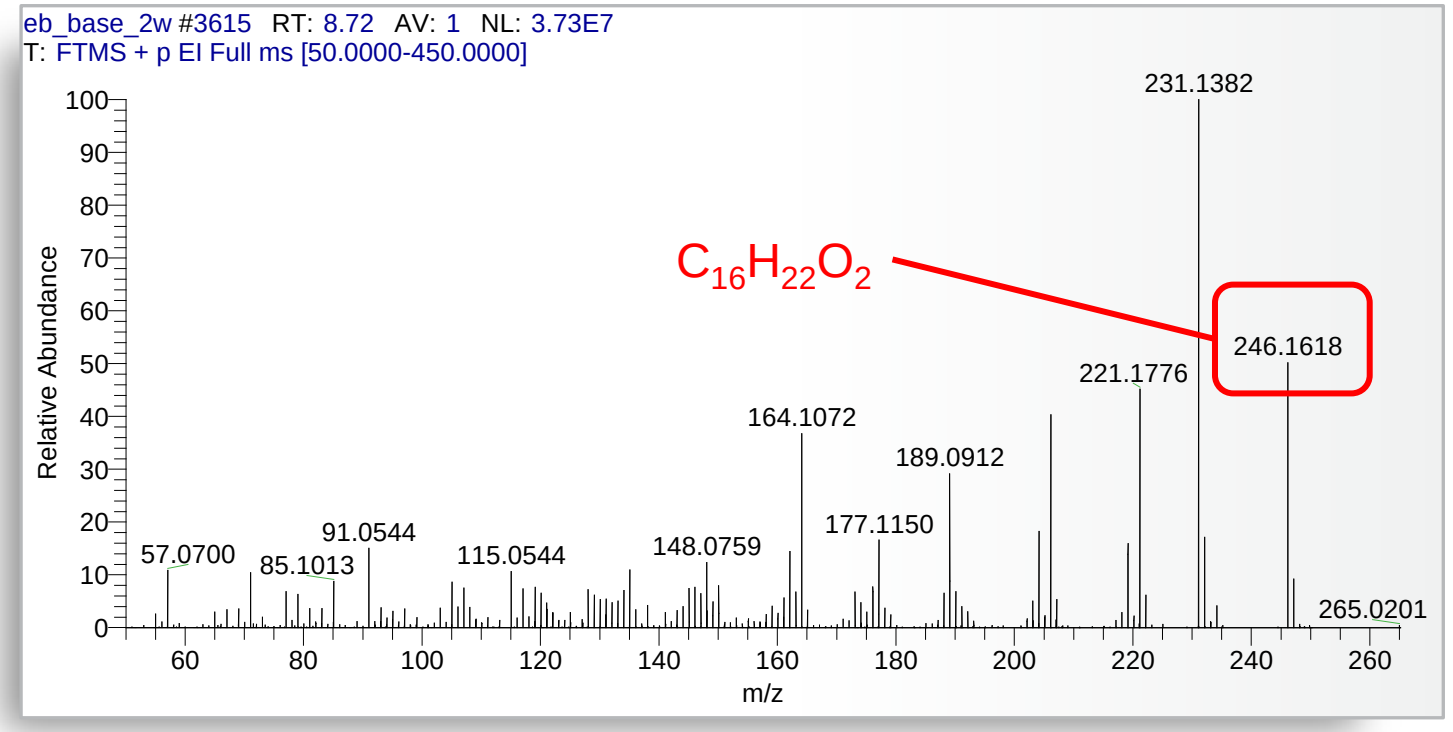
1. Search Settings

Database(s) FDA UNII - NLM



Extractable &Leachable Example of Utility of HRAM

- No reference spectrum found in NIST library
- Identified as 2, 6-di-tert-butyl-r-(prop-1-en-1-yl) phenol in literature – $C_{17}H_{26}O$
- Accurate mass analysis on Thermo Scientific™ Exactive™ GC revealed this was incorrect
  - $C_{17}H_{26}O \longrightarrow \Delta$ 36 mmu
  - $C_{16}H_{22}O_2 \longrightarrow \Delta$ 0.4 mmu



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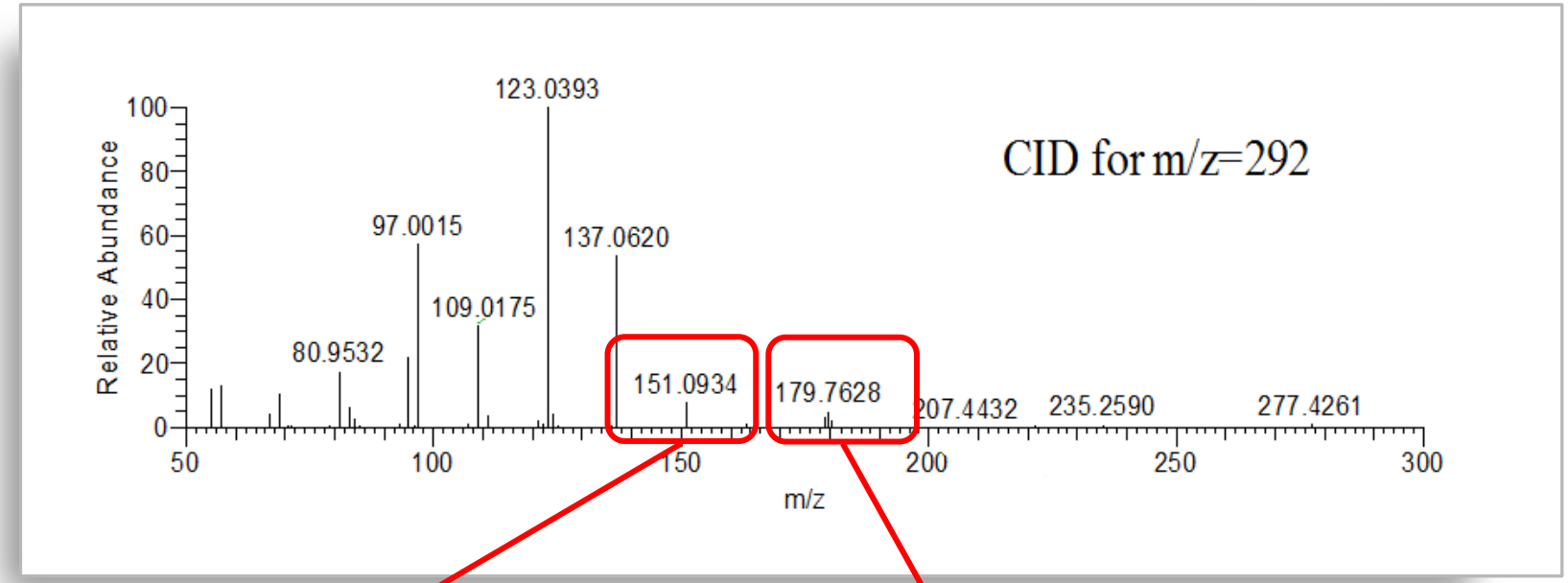
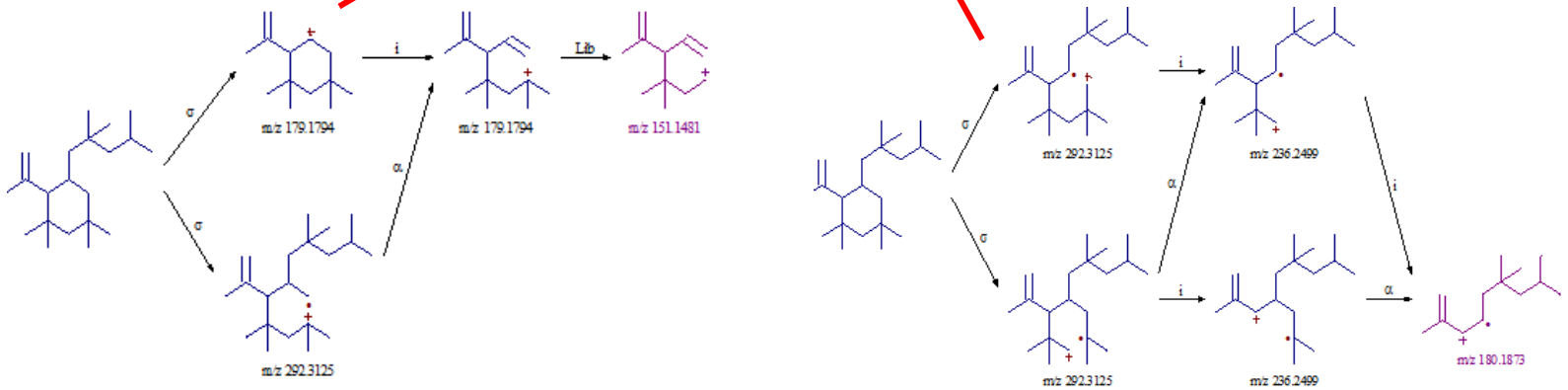
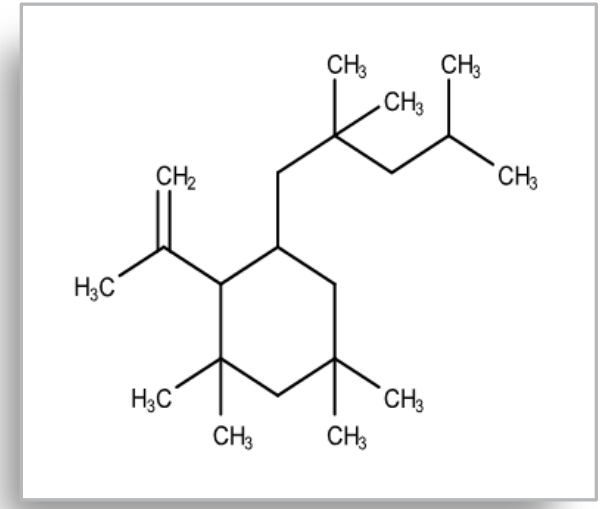
Structural Elucidation of Unknown Rubber Oligomer

Note: Data is from GC-Triple Quad





Thermo Scientific™
Mass Frontier™ Software



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Presentation Summary

- HRAM GC-MS allows for more confident identification
  - Allows formula confirmation of library hits
  - Essential for identification without reference spectrum
- New GC-Orbtrap functionality facilitates unknown ID
  - Retention Indexing added to automated library search
  - New metabolomics library
  - VeV tuning increases molecular ion in EI