



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

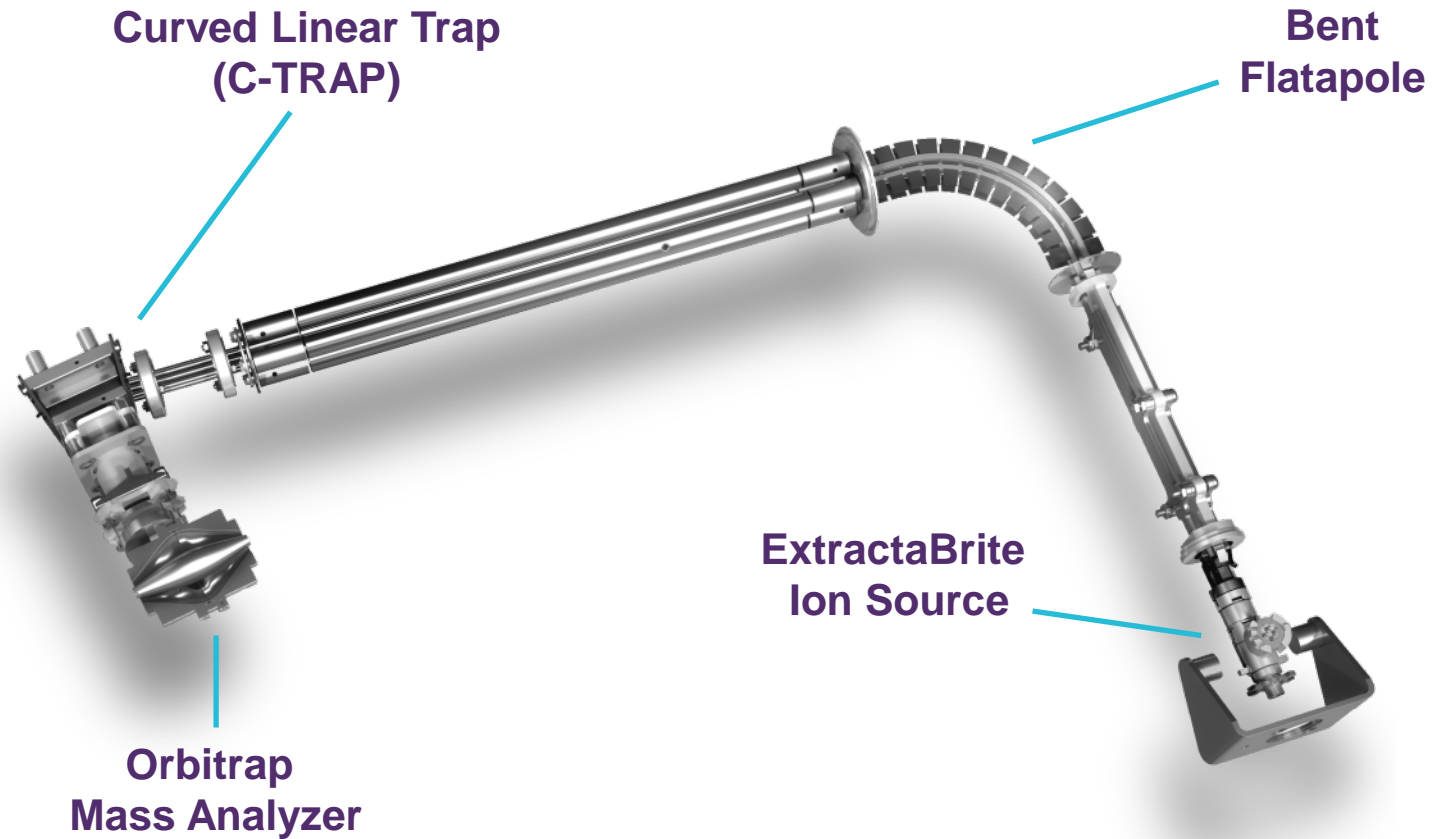


Thermo Scientific™ TRACE™ 1310 GC System
Unique modular injector and detector design
Rapid heat cycling

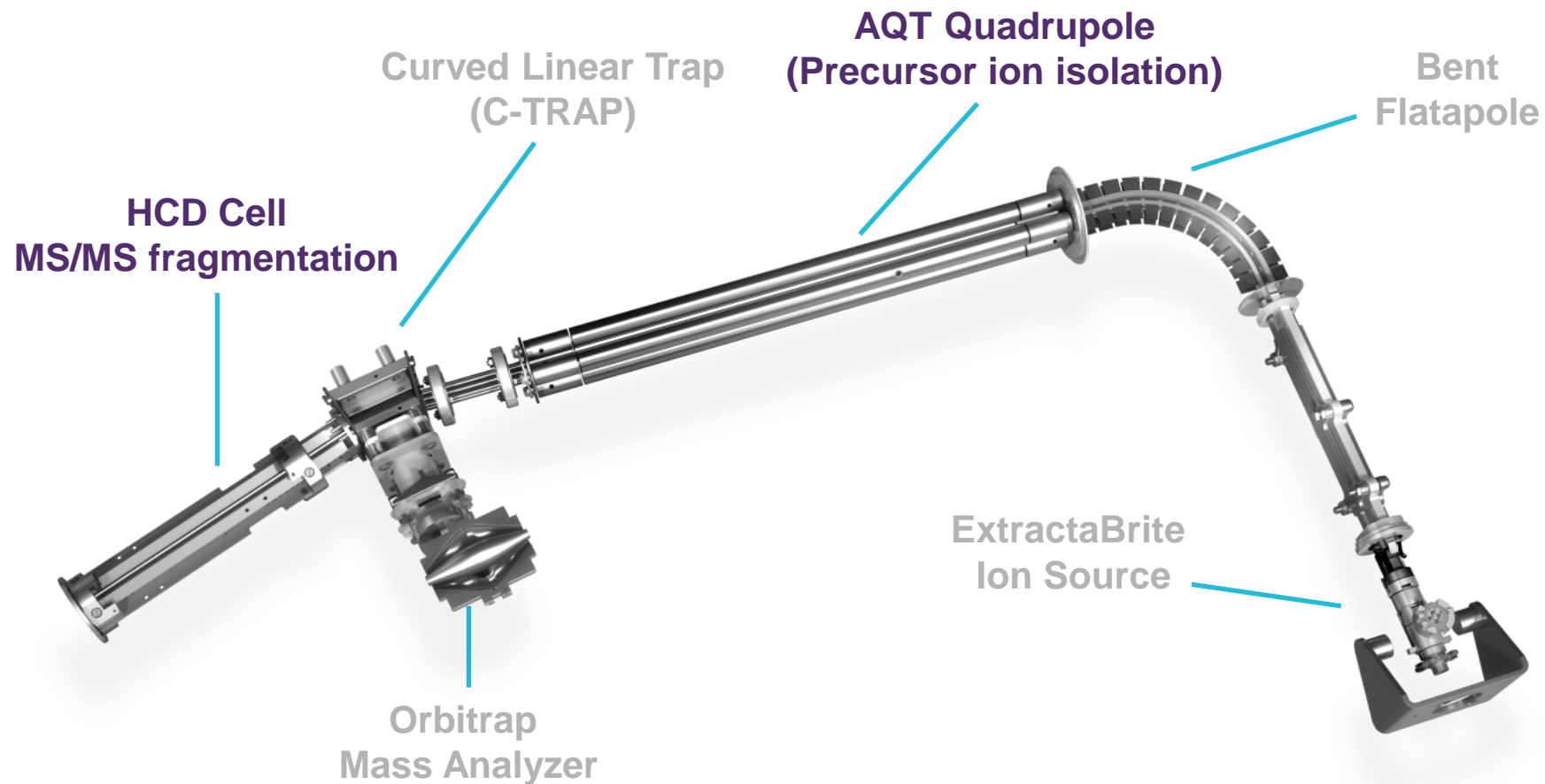
Thermo Scientific™ ExtractaBrite™ Ion Source technology
Routine grade robustness
Patented RF lens
Removable without breaking vacuum



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

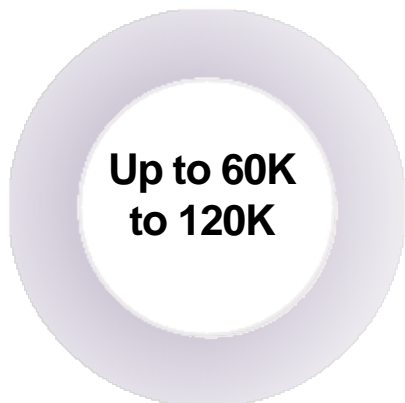


Thermo Scientific Q Exactive Orbitrap GC-MS/MS System: The Technology Inside



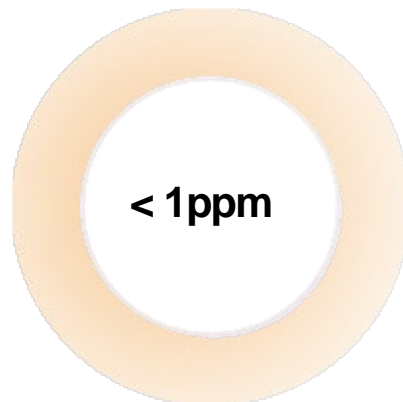
Thermo Scientific Orbitrap GC-MS System Highlights

Resolving Power



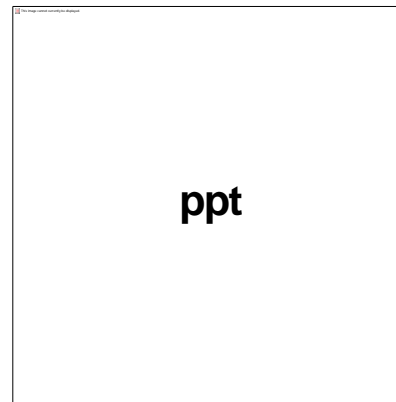
- Maximum selectivity
- Fast enough for GC

Mass Accuracy



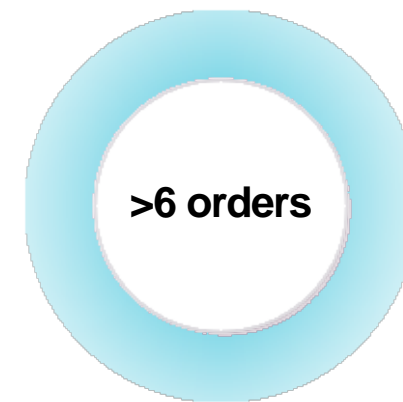
- Every scan
- All concentrations
- In complex matrix
- Across the mass range
- Everyday!

Sensitivity



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

Dynamic Range



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

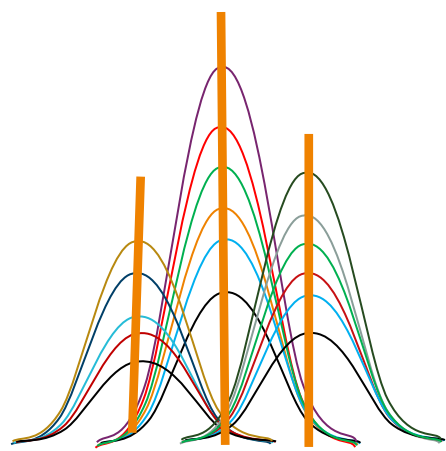
Identification Categories - USP General Chapter <1663>

- 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
 - Fragmentation behavior by expert interpretation
 - NMR/FTIR confirmation can also increase confidence
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 - Retention index match to authentic standard

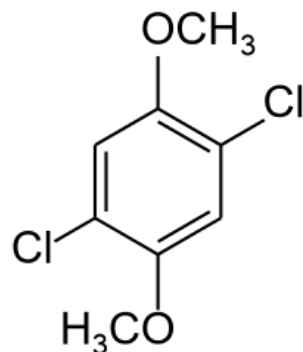
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Algorithm for Automated Library Match



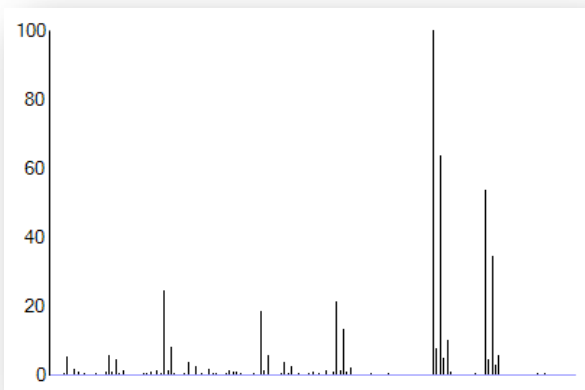
Deconvolve TIC



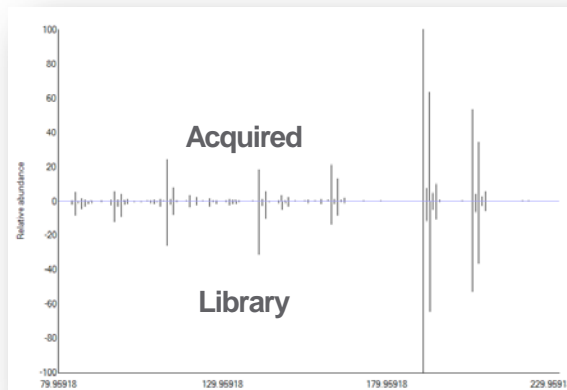
Candidate Compounds

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	C ₅ Cl ₂ H ₂ O	147.9477	0.20277
148.9369	C ₅ Cl[37]ClHO	148.9369	0.2679
149.9448	C ₅ Cl[37]ClH ₂ O	149.9448	0.06602
151.9419	C ₅ [37]Cl ₂ H ₂ O	151.9418	0.72528
154.9895	C ₇ ClH ₄ O ₂	154.9894	0.38712
155.9974	C ₇ ClH ₅ O ₂	155.9973	0.89745
157.9943	C ₇ [37]ClH ₅ O ₂	157.9943	0.25381
159.9479	C ₆ Cl ₂ H ₂ O	159.9477	0.87529
161.9446	C ₆ Cl[37]ClH ₂ O	161.9448	0.80213
162.9711	C ₆ Cl ₂ H ₅ O	162.9712	0.36816
163.9745	C ₅ [13]CCl ₂ H ₅ O	163.9745	0.3342
164.9682	C ₆ Cl[37]ClH ₅ O	164.9682	0.24186
165.9716	C ₅ CCl[37]ClH ₅ O	165.9716	0.02832

Formula confirmation with HRF scoring



Create "clean" spectrum



Library search

Thermo Scientific TraceFinder Software Deconvolution Overview

Deconvolution Plugin

View Help

Sample List: FTMS + p EI Full ms [60.00-800.00]

Peak List: (118)

Peak Identifications: (2)

Score to use: Forward search

Status	Sample name	Component Name	RT	Score	Matched Compound	Formula	CAS	Dot Prod.	HRF Score	M+ m/z	Empirical M+	Library	Calculated RI	Library RI
	EI-QC6-splitless-pulsed-pr...	5-Aminovaleric aci...	13.73	99	L-Glutamic acid, 3TMS	C14H33NO4Si3	15985-07-6	962	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1614
		2-Oxoglutaric acid, ...	13.80	92.9	N-Methyl-Aspartic acid,...	C14H33NO4Si3		660	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1592
		Diethyl Phthalate	14.15											
		L-Glutamic acid, 3T...	14.43											
		Xylulose 1MOX, 4T...	14.63											
		4-amino-N-butylac...	14.63											
		4-hydroxyproline, 2...	14.83											

Acquisition date: 7/26/2017 10:32:00
InstrumentName type:Q Exactive GC
Inst ID: Exactive Series slot #1
InstrumentName method: C:\TraceFinder

Extracted Ions

Actual Normalized

BP: 246.133987 @ 2.06E+008

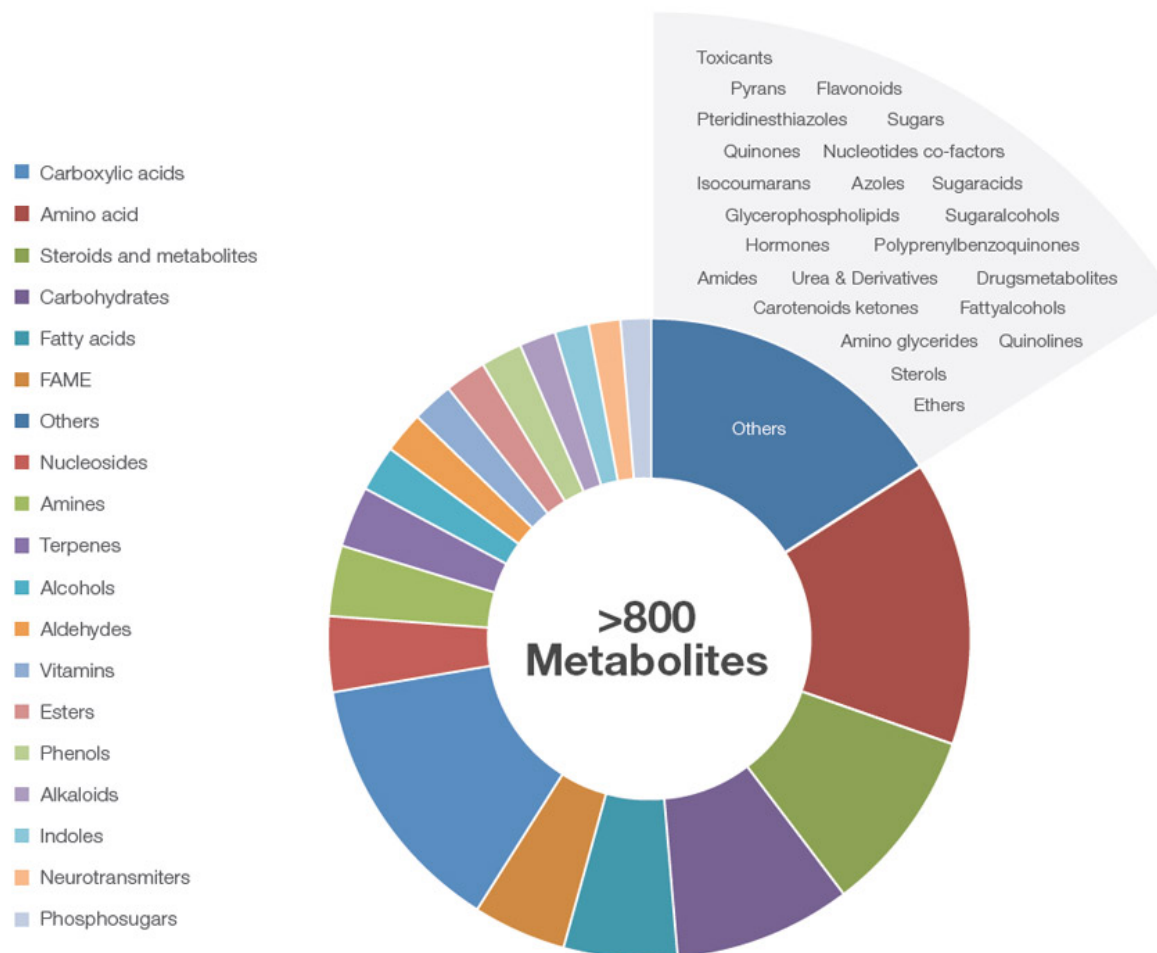
Component spectrum

Library spectrum

Active	Measured m/z	Area	Height	Fragment ID	Theo m/z	Abundance
<input checked="" type="checkbox"/>	246.133987	363947472	206255713	C(12)10 Si(2...	246.1340...	100
<input checked="" type="checkbox"/>	128.088974	123999729	71638086	C(12)6 Si(28...	128.0890...	35
<input checked="" type="checkbox"/>	73.046799	115109931	69195840	C(12)3 Si(28...	73.046803	25
<input checked="" type="checkbox"/>	156.083878	82294445	47640886	C(12)7 Si(28...	156.0839...	25
<input checked="" type="checkbox"/>	230.102707	77315854	44806130	C(12)9 Si(28...	230.1027...	20
<input checked="" type="checkbox"/>	147.065552	68580708	39741462	C(12)5 Si(28...	147.0655...	15
<input checked="" type="checkbox"/>	149.044815	56083397	33163537	C(12)4 Si(28...	149.0448...	10

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

Deconvolution Plugin

View Help

Sample List: FTMS + p EI Full ms [60.00-800.00]

Peak List: (118)

Peak Identifications: (2)

Score	Matched Compound	Formula	CAS	Dot Prod.	HRF Score	M+ m/z	Empirical M+	Library	Calculated RI	Library RI
99	L-Glutamic acid, 3TMS	C14H33NO4Si3	15985-07-6	962	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1614
92.9	N-Methyl-Aspartic acid,...	C14H33NO4Si3		600	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1592

Strong spectral library match and HRF score

Component spectrum

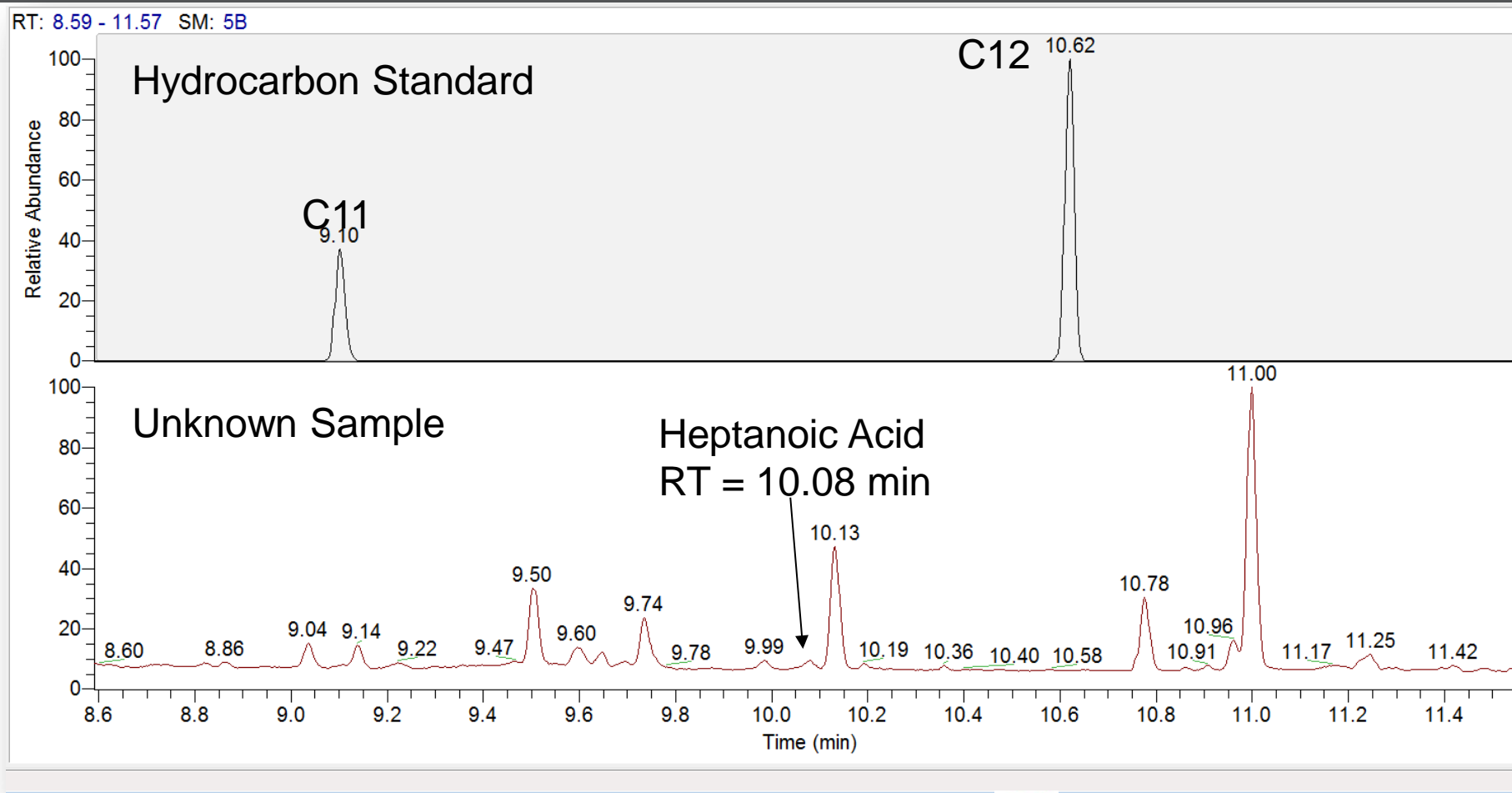
Library spectrum

Active	Measured m/z	Area	Height	Fragment ID	Theo m/z	M _r (p)
<input checked="" type="checkbox"/>	246.133987	363947472	206255713	C(12)10 Si(2...	246.1340...	0.0
<input checked="" type="checkbox"/>	128.088974	123999729	71638086	C(12)6 Si(28...	128.0890...	0.2
<input checked="" type="checkbox"/>	73.046799	115109931	69195840	C(12)3 Si(28...	73.046803	0.0
<input checked="" type="checkbox"/>	156.083878	82294445	47640886	C(12)7 Si(28...	156.0839...	0.2
<input checked="" type="checkbox"/>	230.102707	77315854	44806130	C(12)9 Si(28...	230.1027...	0.0
<input checked="" type="checkbox"/>	147.065552	68580708	39741462	C(12)5 Si(28...	147.0655...	0.2
<input checked="" type="checkbox"/>	149.044815	56083392	33163537	C(12)4 Si(28...	149.0448...	0.2

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 - Retention index match to authentic standard

Retention Indexing Background



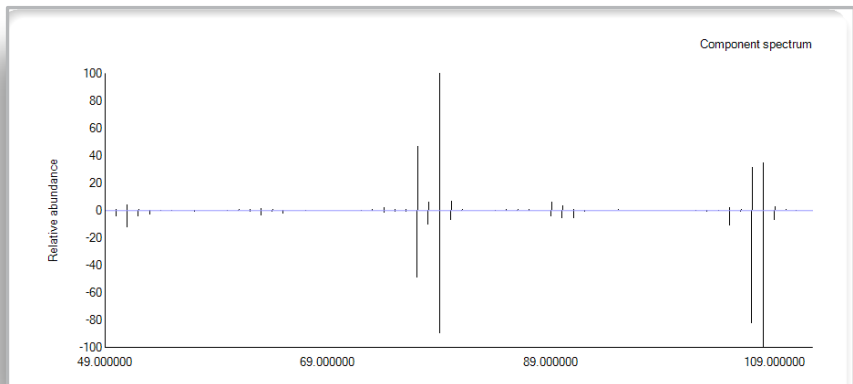
Retention Index (measured) = 1164 

Heptanoic Acid Retention Index (library) = 1169

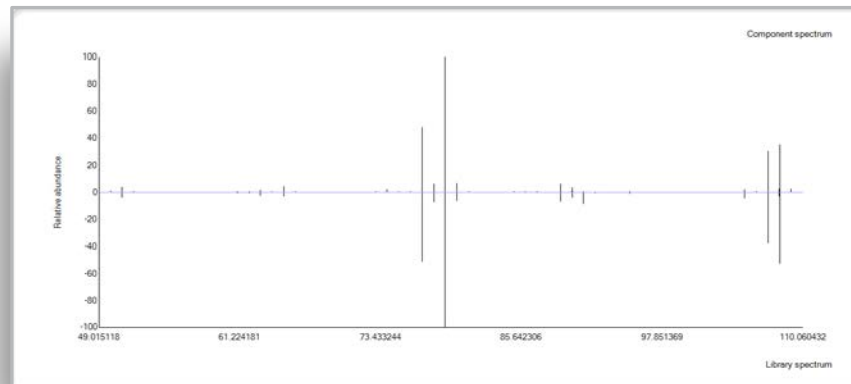
$$RI = 100 \times \left(C1 + \frac{RT(Unk) - RT(C1)}{RT(C2) - RT(C1)} \right)$$

$$RI(Heptanoic Acid) = 100 \times \left(11 + \frac{10.08 \text{ min} - 9.10 \text{ min}}{10.62 \text{ min} - 9.10 \text{ min}} \right)$$

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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Deconvolution Plugin

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		4-amino-N-butylac...	14.64											
		4-hydroxyproline, 2...	14.83											

Acquisition date: 7/26/2017 10:32:00
InstrumentName type: Q Exactive GC
Inst ID: Exactive Series slot #1
InstrumentName method: C:\Trace1

Also strong match with library retention index

Extracted Ions: Actual Normalized

BP: 246.133987 @ 2.06E+008

Spectra: Component spectrum

Library spectrum

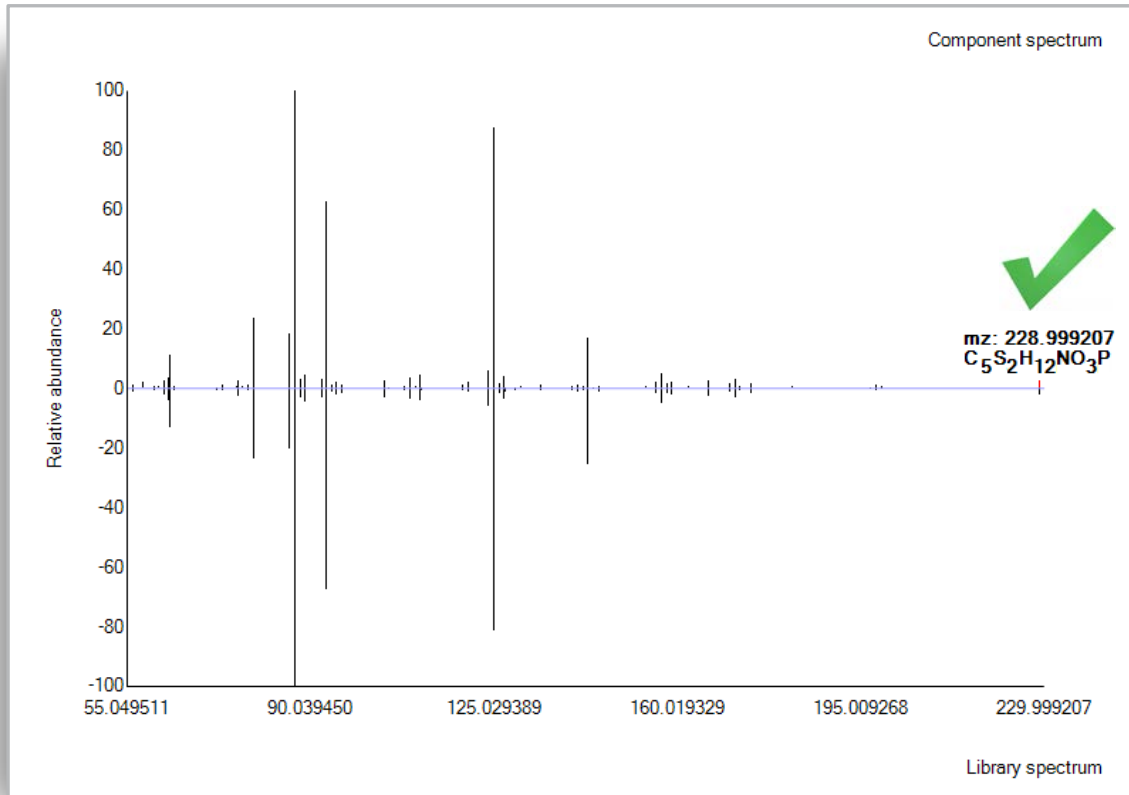
Active	Measured m/z	Area	Height	Fragment ID	Theo m/z	M _r (p)
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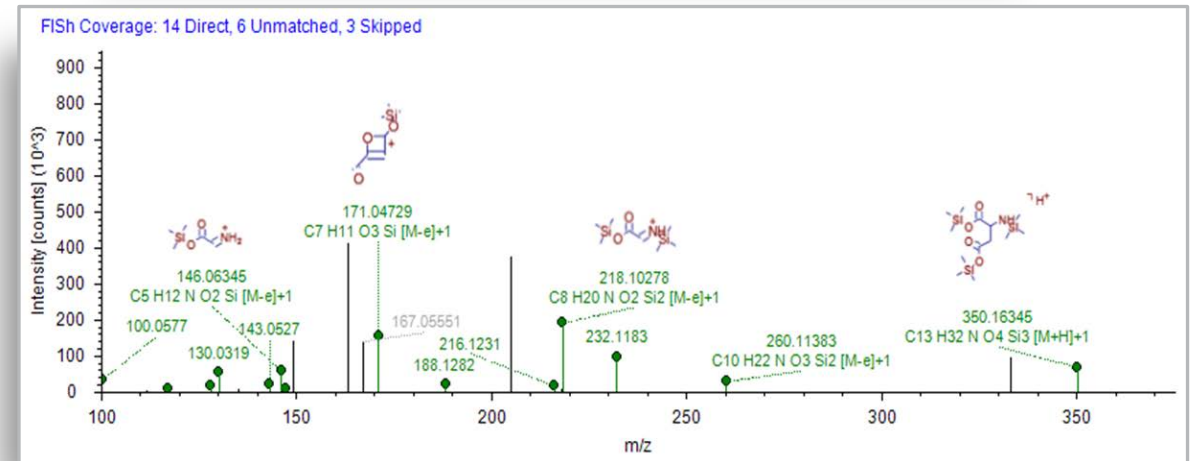
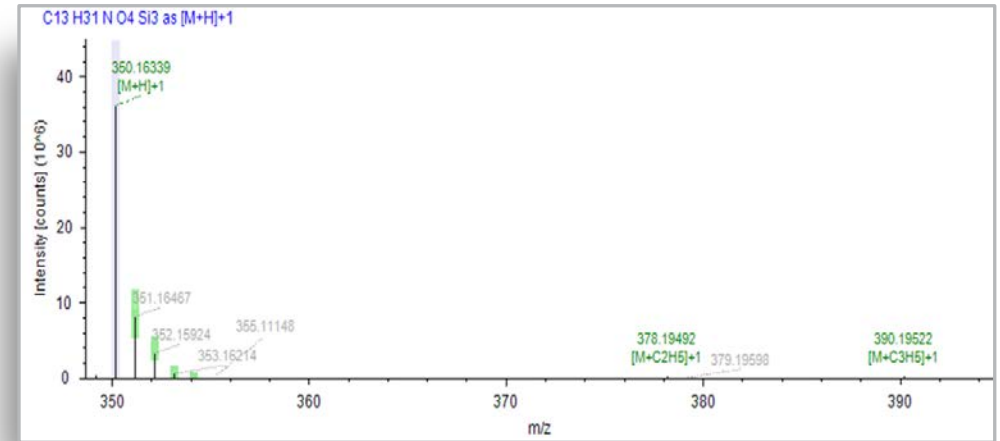
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Reasons of Elemental Formula Elucidation

- Increase confidence of library hit



- First step in determining true unknown



Molecular Ion Confirmation of Library Search

Deconvolution Plugin

View Help

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Component Name | RT

- 5-Aminovaleric aci... | 13.78
- 2-Oxoglutaric acid, ... | 13.89
- Diethyl Phthalate | 14.16
- L-Glutamic acid, 3T... | 14.43
- Xylulose 1MOX, 4T... | 14.63
- 4-amino-N-butylac... | 14.64
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Acquisition date: 7/26/2017 10:32:00
InstrumentName type: Q Exactive GC
Inst ID: Exactive Series slot #1
InstrumentName method: C:\Trace1

Extracted Ions: Actual | Normalized

BP: 246.133987 @ 2.06E+008

Relative abundance vs. m/z

Component spectrum

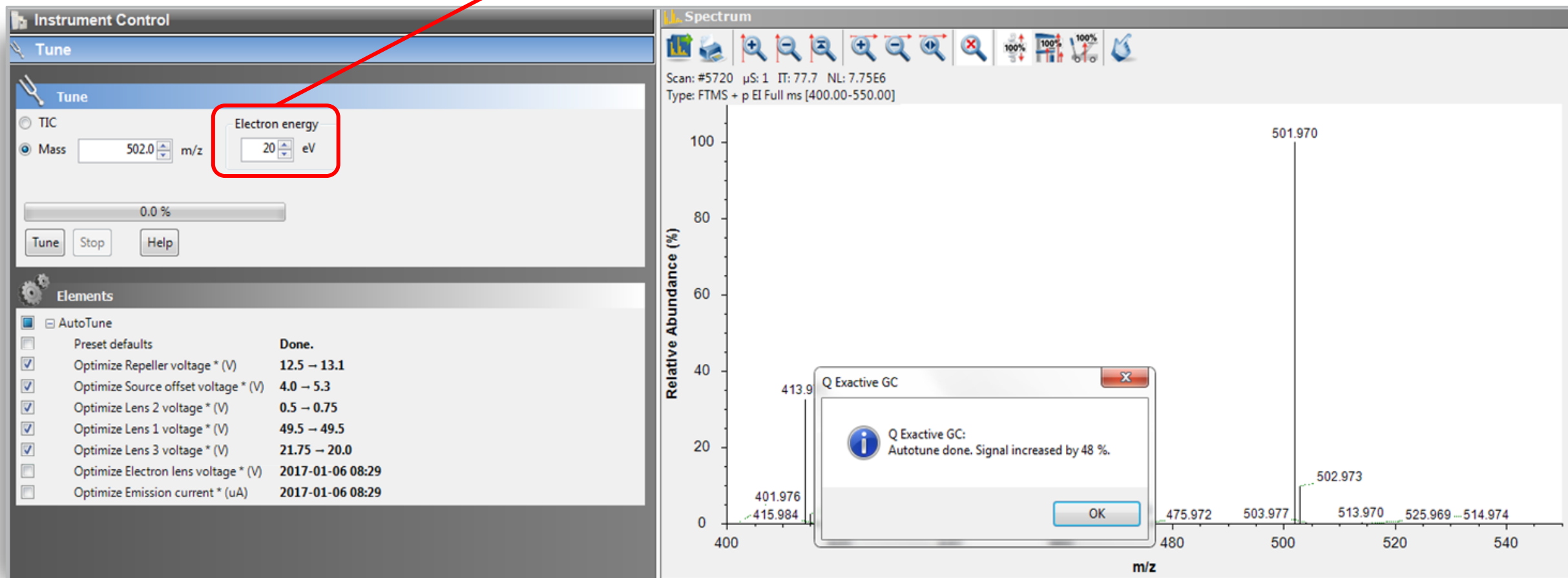
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Theoretical mass matches actual by $\Delta m/z = 0.13$ ppm

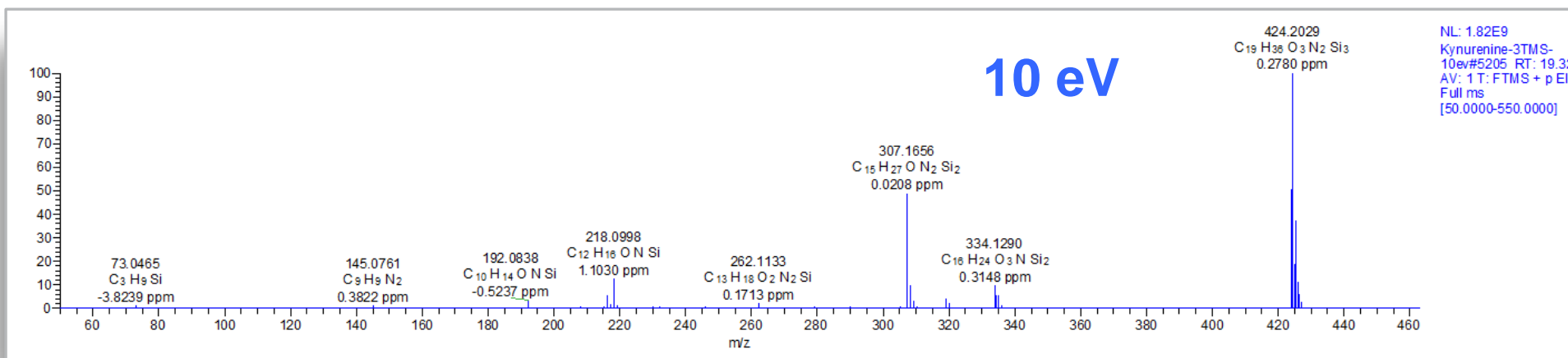
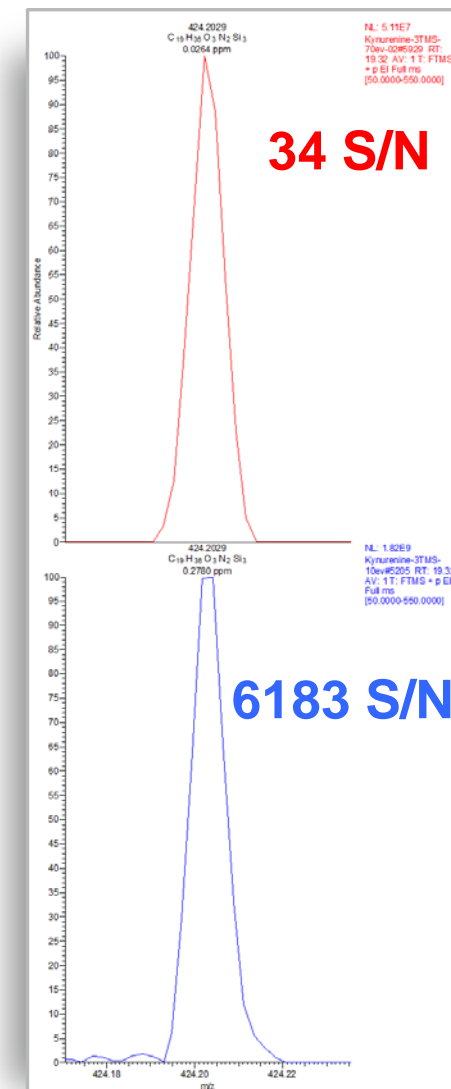
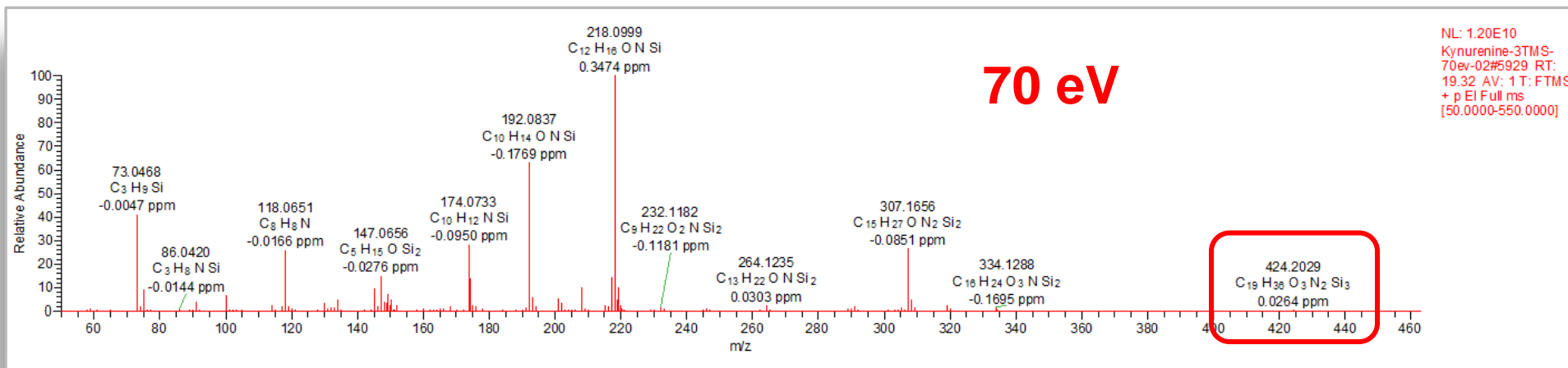
Variable Electron Energy Tuning (VeV)

Set electron energy and tune

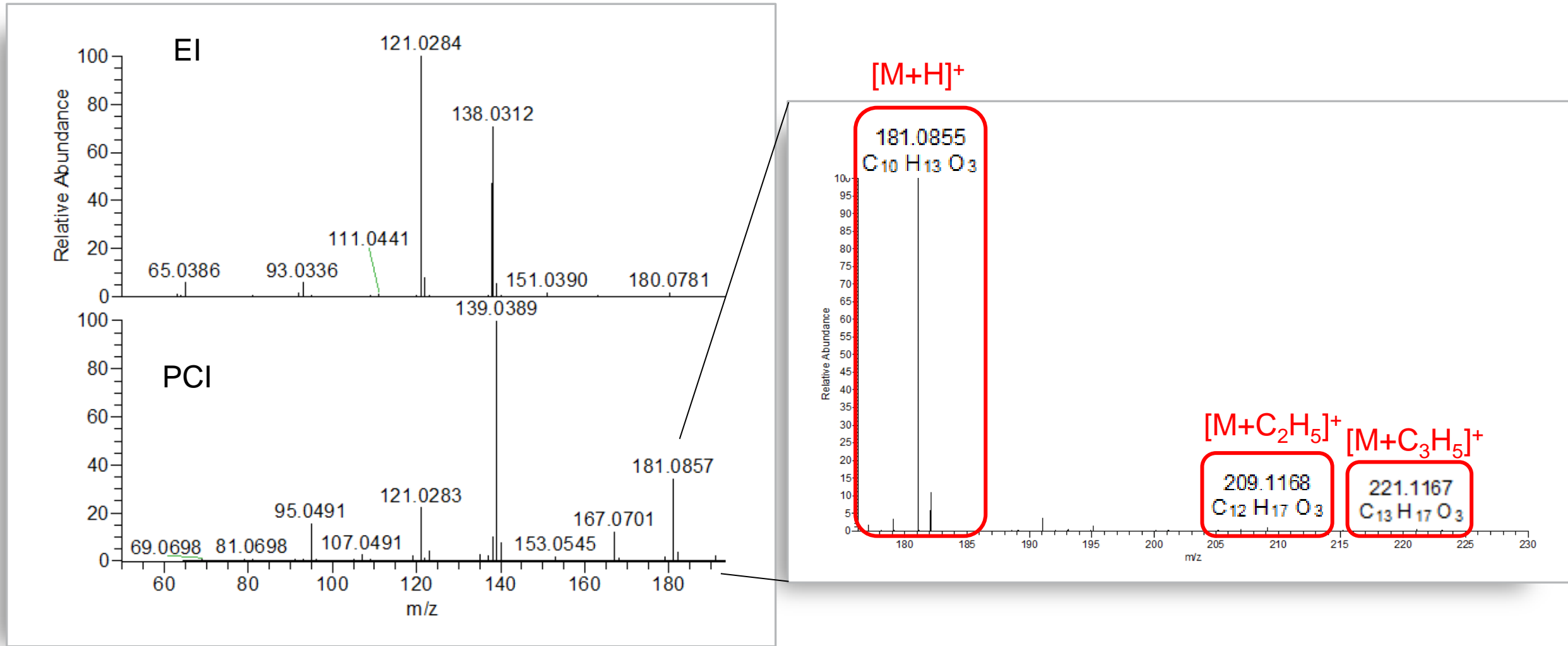


Robust tuning down to near the ionization potential of FC43

Boosting Molecular Ion of Kynurenine with VeV



Molecular Formula Confirmation of Propyl Paraben with Chemical Ionization

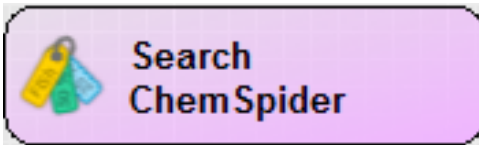


Courtesy of Dr. Gyorgy Vas, Intertek

Automated CI Detection in Thermo Scientific Compound Discoverer Software



Thermo Scientific™
Compound Discoverer™
Software



1. Search Settings

Database(s)

FDA UNII - NLM

Chromatograms

No grouping and filtering available for the current selection

MW: 180.07829
File: 480517_203E_AgedPD_CI.raw (F2) FTMS (+) MS1

Mass Spectrum

#3729, RT=9.067 min, MS1, F

480517_203E_AgedPD_CI#3729, RT=9.067 min, MS1, FTMS (+)
C10 H12 O3 as [M+H]⁺

Compounds

Checked	Name	Formula	Annotation Sc	FISH Coverage	Molecular Weight	RT [r]	Area (Max.)	# ChemSpider Results	Area
<input type="checkbox"/>	Propylparaben	C10 H12 O3	■ ■		180.07829	9.068	137339	27	1.37e5
<input type="checkbox"/>			■ ■		354.06209	9.068	1745	0	1.74e3
<input type="checkbox"/>			■ ■		296.07472	9.075	6539	1	6.54e3
<input type="checkbox"/>			■ ■		280.04357	9.092	11127	0	1.11e4
<input type="checkbox"/>			■ ■		324.10607	9.099	753	0	7.53e2
<input type="checkbox"/>			■ ■		354.06209	9.101	2938	0	2.94e3
<input type="checkbox"/>			■ ■		298.05400	9.121	20047	0	2.00e4
<input type="checkbox"/>			■ ■		280.04357	9.123	5950	0	5.95e3

Hide Related Tables

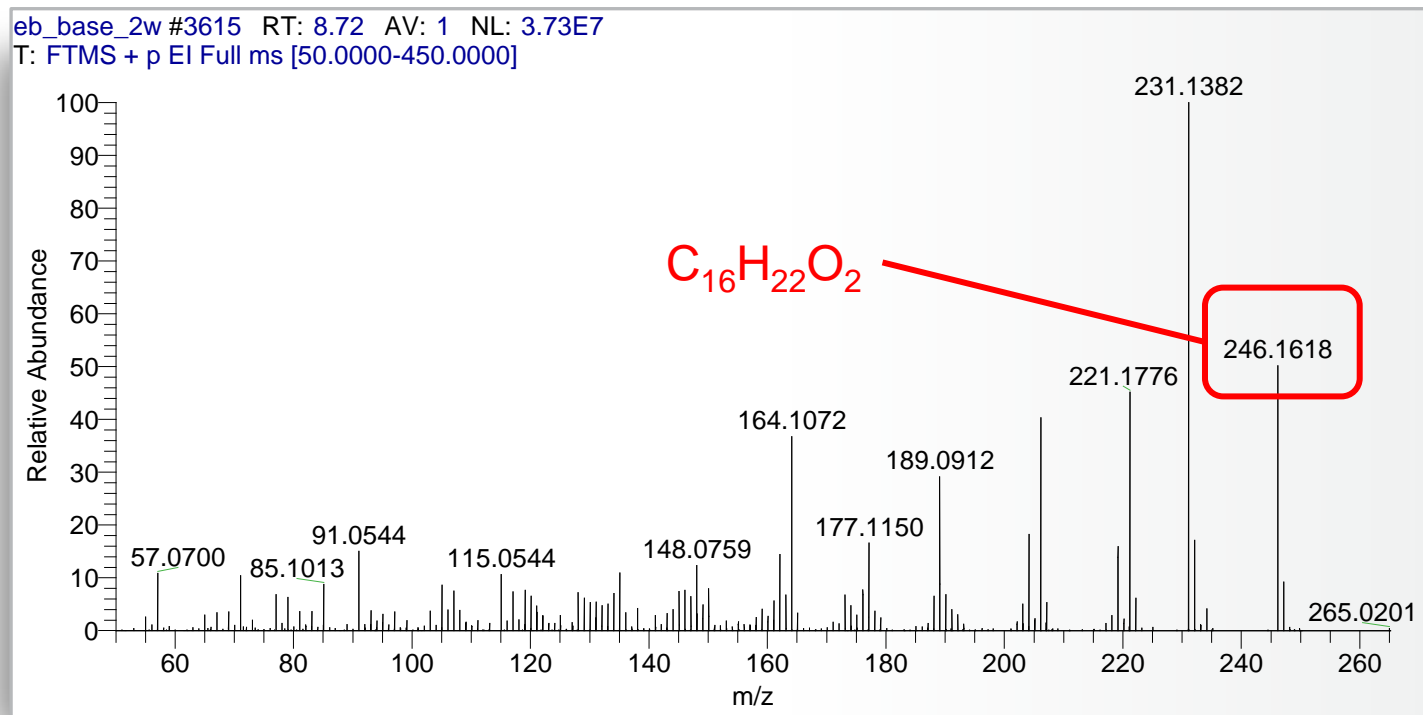
Structure Proposals

Checked	Compound Match	Structure	Name	Formula	Molecular Weight	ΔMass [Da]	ΔMass [ppm]	CSID	# References
<input type="checkbox"/>	■		Propylparaben	C10 H12 O3	180.07864	0.00035	1.94	6907	346
<input type="checkbox"/>	■		3,4-Dimethoxyacetophenone	C10 H12 O3	180.07864	0.00035	1.94	21168556	184

Show Related Tables

Extractable & Leachable Example of Utility of HRAM

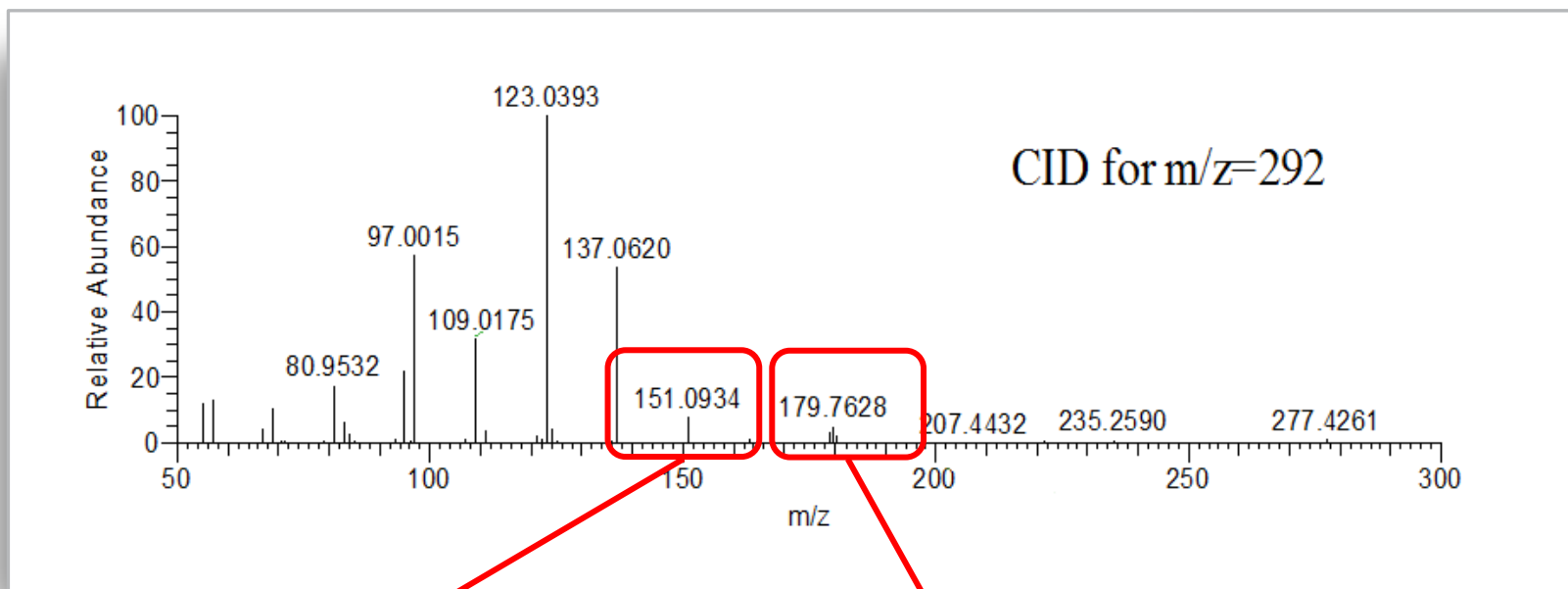
- No reference spectrum found in NIST library
- Identified as 2, 6-di-tert-butyl-r-(prop-1-en-1-yl) phenol in literature – $C_{17}H_{26}O$
- Accurate mass analysis on Thermo Scientific™ Exactive™ GC revealed this was incorrect
 - $C_{17}H_{26}O \rightarrow \Delta 36 \text{ mmu}$
 - $C_{16}H_{22}O_2 \rightarrow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



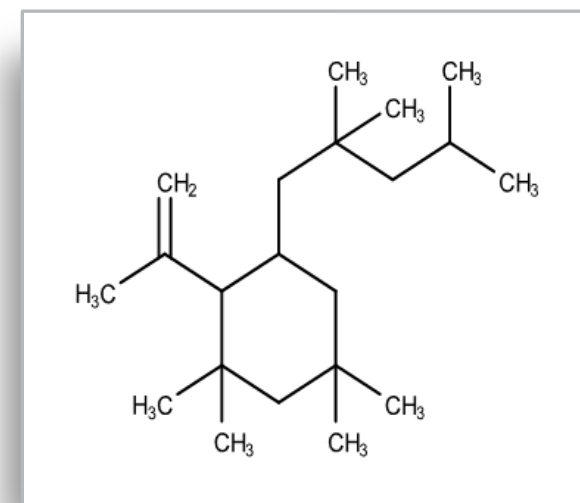
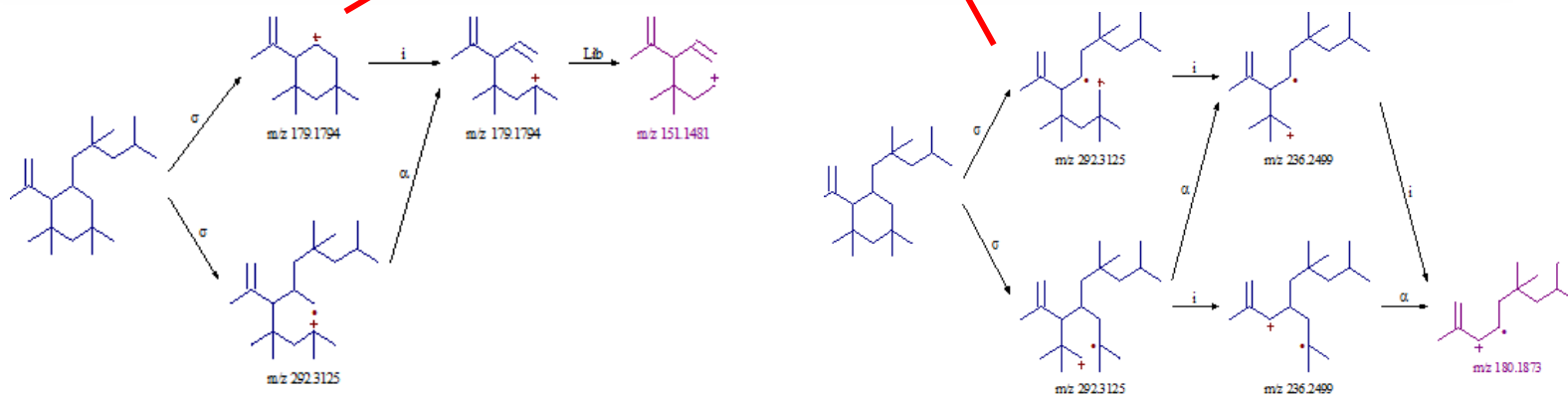
Courtesy of Dr. Gyorgy Vas, Intertek

Structural Elucidation of Unknown Rubber Oligomer

Note: Data is from GC-Triple Quad



Thermo Scientific™
Mass Frontier™ Software



Courtesy of Dr. Gyorgy Vas, Intertek

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



