



ThermoFisher
SCIENTIFIC

**Back to the Future with Thermo Scientific™ Q Exactive™ GC
Orbitrap™ GC-MS/MS**

The world leader in serving science



1 Why GC/MS for Metabolomics?

2 Why High Resolution/Accurate Mass (HRAM) GC/MS for Metabolomics?

3 Tools for HRAM GC/MS-Based Metabolomics





Why GC/MS for Metabolomics?

Example Strategy for Pancreatic Cancer Serum Profiling

GC/MS

Volatiles
Essential oils
Esters
Perfumes
Terpenes
Carotenoids
Flavanoids

Both

Alcohols
Amino acids
Catecholamines
Fatty acids
Phenolics
Prostaglandins
Steroids
Sugar phosphates

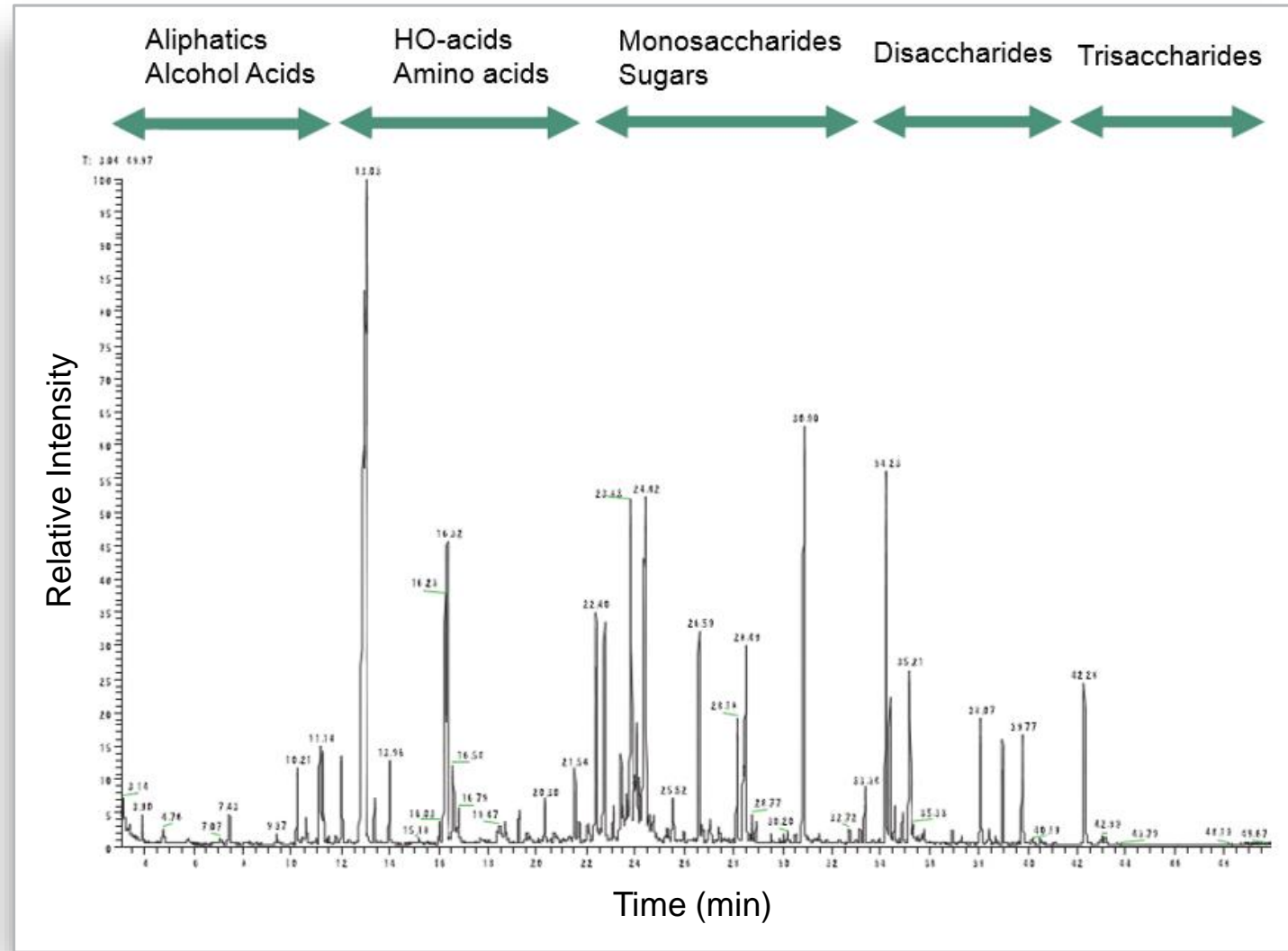
LC/MS

Organic acids
Organic amines
Nucleosides
Nucleotides
Oligosaccharides
Peptides
Co-factors
Polar lipids

Source Dr. Vladimir Tolstikov

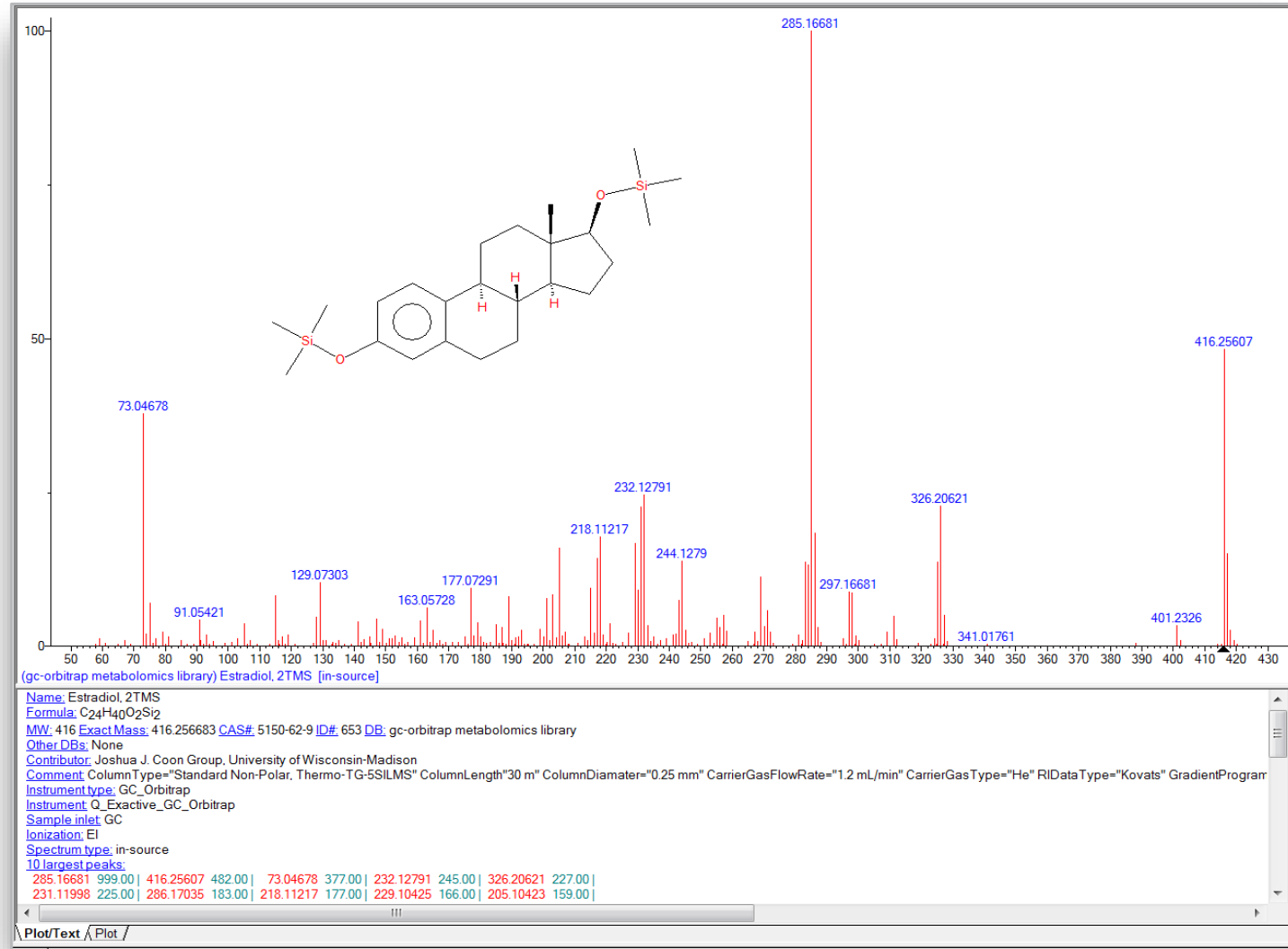
Advantages GC/MS for Metabolomics

- Well characterized for metabolite screening
- EI library - 50 Years of development
- Enhanced chromatographic resolution
- Highly reproducible retention times
- Ideal technique for volatiles
- Matrix independent source response
- Robust/Reliable/Easy to Operate



Considerations Unique to GC/MS

- Requires compounds that evaporate at temperatures $< 350\text{ }^{\circ}\text{C}$
 - Derivatization can increase volatility
 - Online derivatization available
- Molecular ion can be absent in EI
 - Identification by library search
 - Low eV can promote MI in EI
 - CI can be used for soft ionization

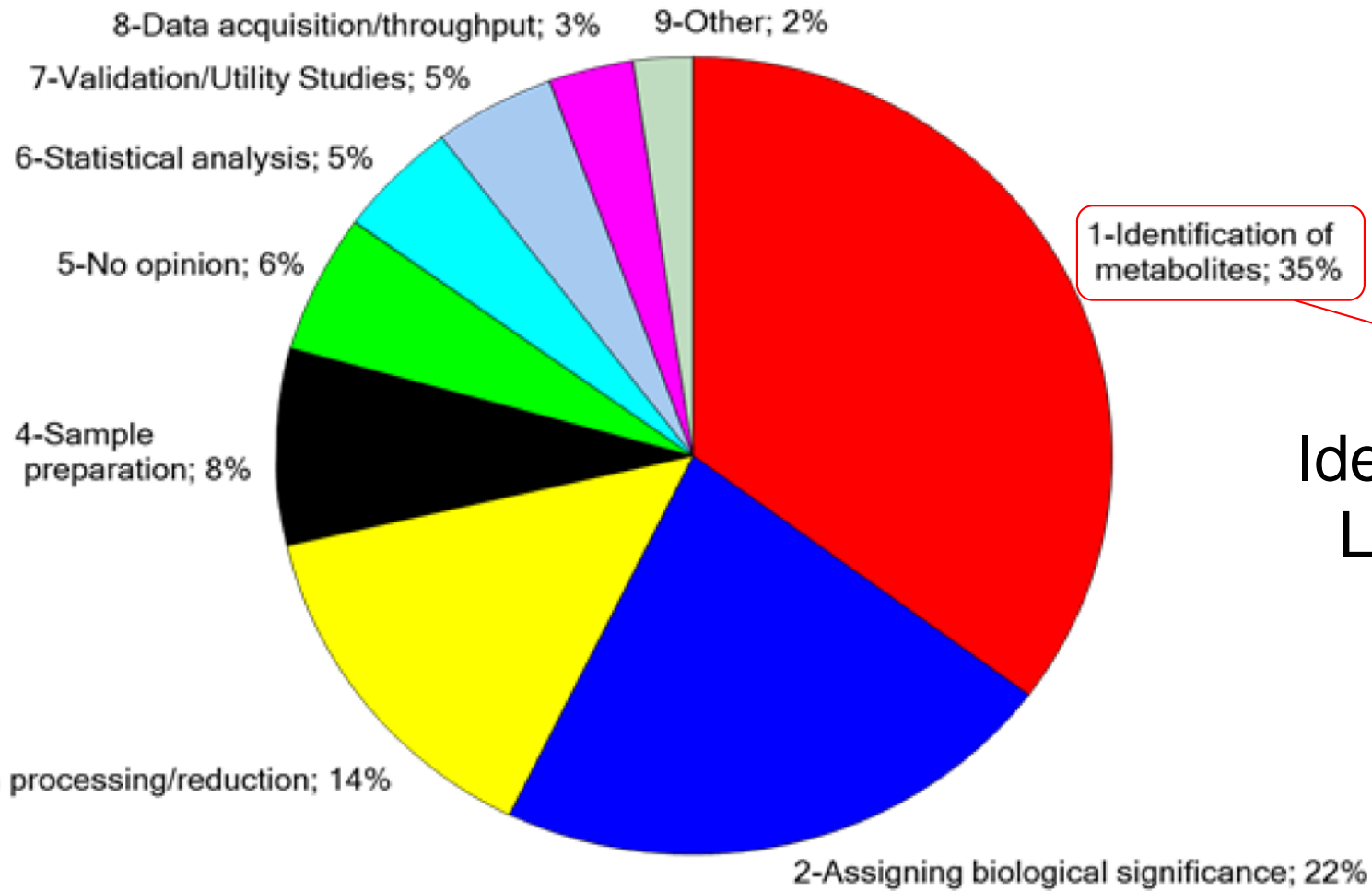




Why HRAM GC/MS for Metabolomics?

Bottlenecks in Metabolomics Studies

*Source: ASMS Metabolomics
Workshop Survey*

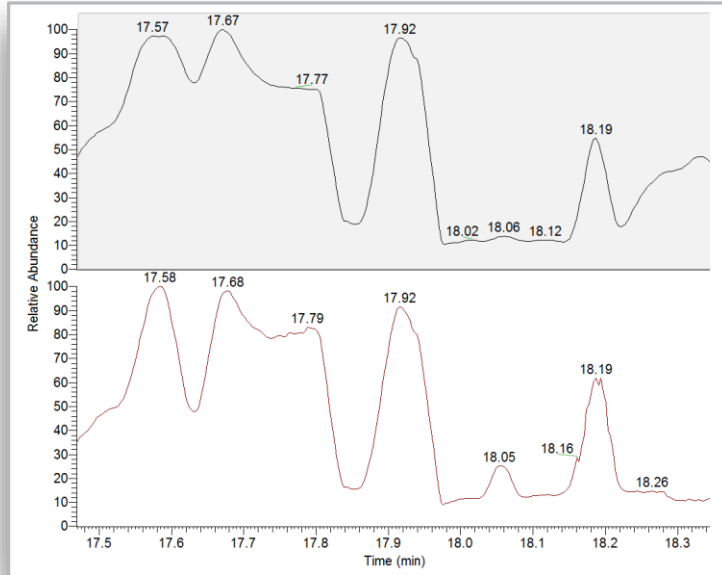


Identification of Unknowns
Listed as #1 Bottleneck

Benefits of HRAM GC/MS to Global Metabolomics

GC-Quad

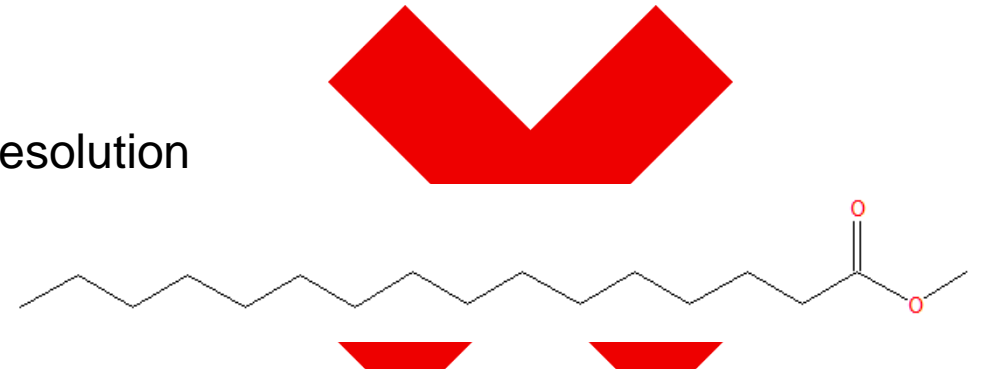
TIC



270 extracted
 ± 0.5 amu

Unit Resolution

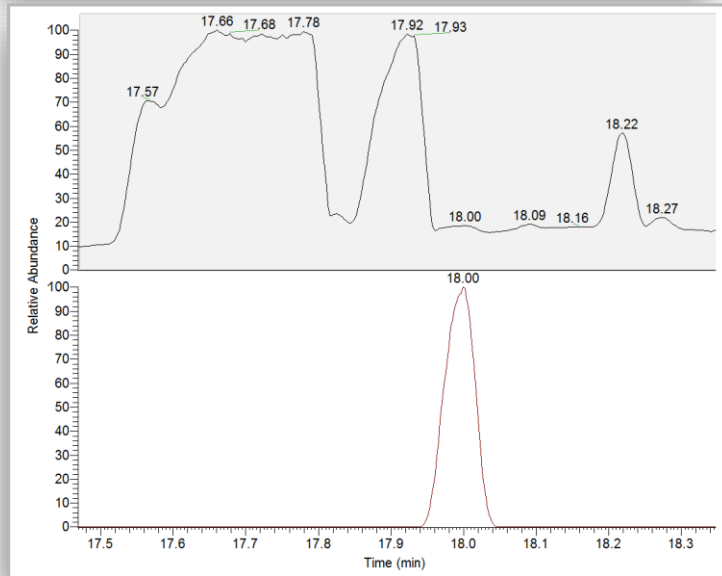
Dec



Hexadecanoic acid methyl ester
molecular ion = 270.2553

GC-Orbitrap

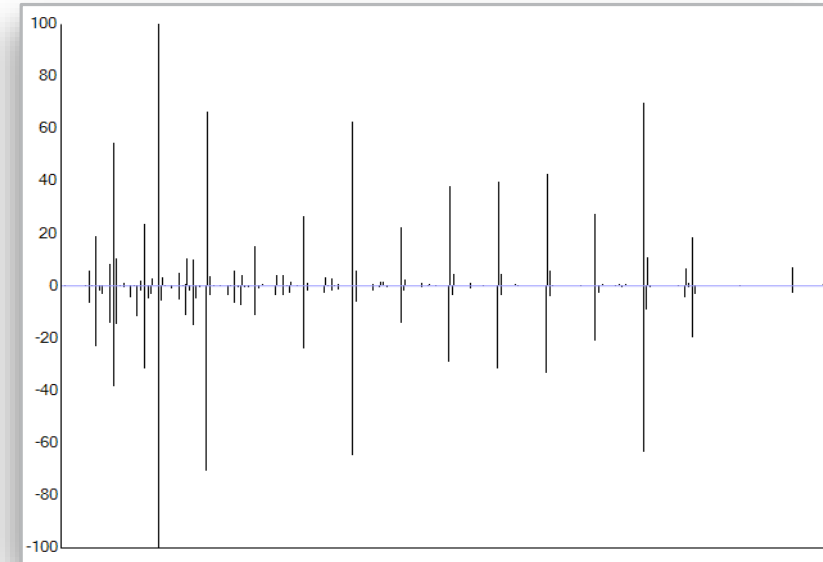
TIC



270.2553 extracted
 ± 5 ppm

High Resolution

Deconvolution



Mass Accuracy for Compound Identification

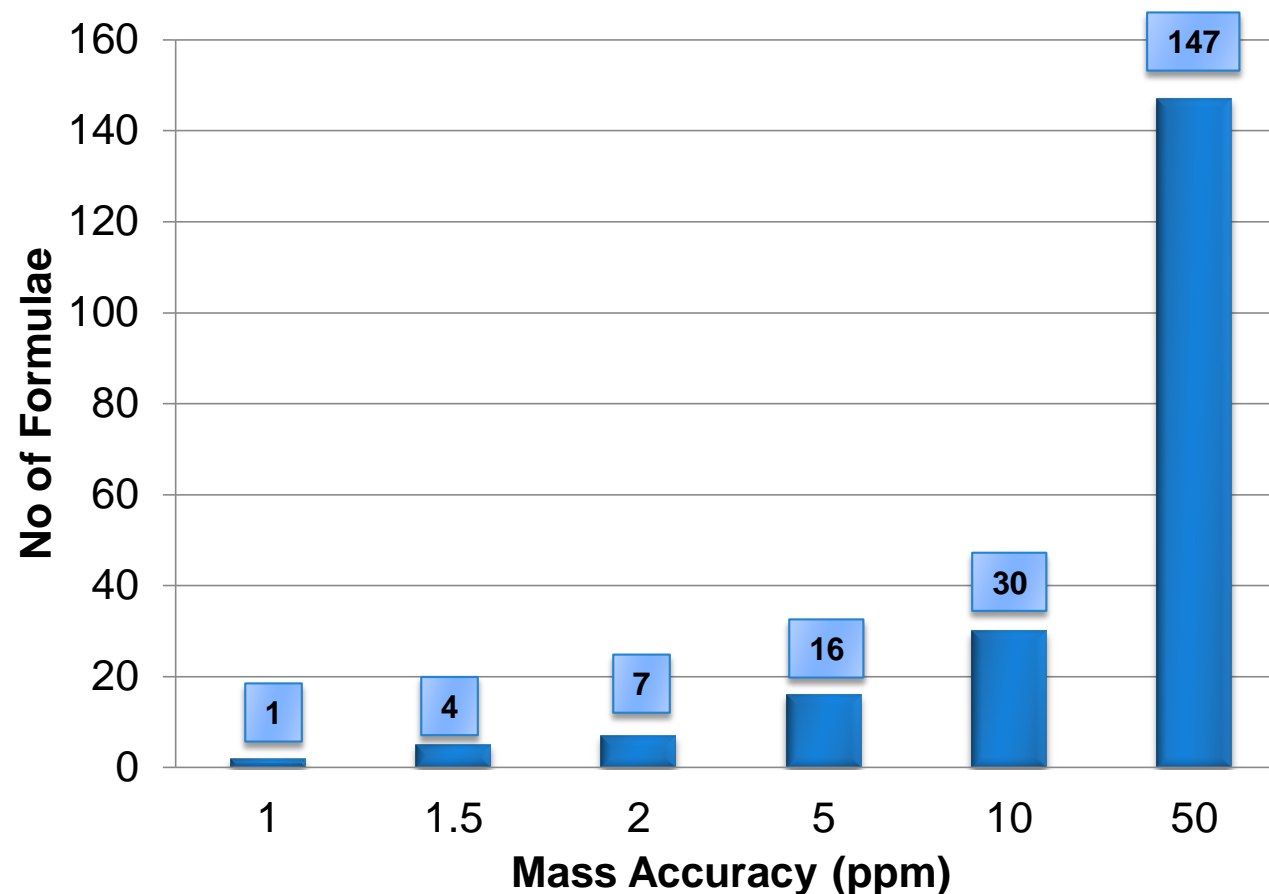
Type	Mass Accuracy
FT-ICR-MS	0.1 - 1 ppm
Orbitrap	0.5 - 1 ppm
Magnetic Sector	1 - 2 ppm
TOF-MS	3 - 5 ppm
Q-TOF	3 - 5 ppm
Linear IonTrap	50-200 ppm (10 ppm in Ultra-Zoom)

Source: Metabolomics Fiehn's lab

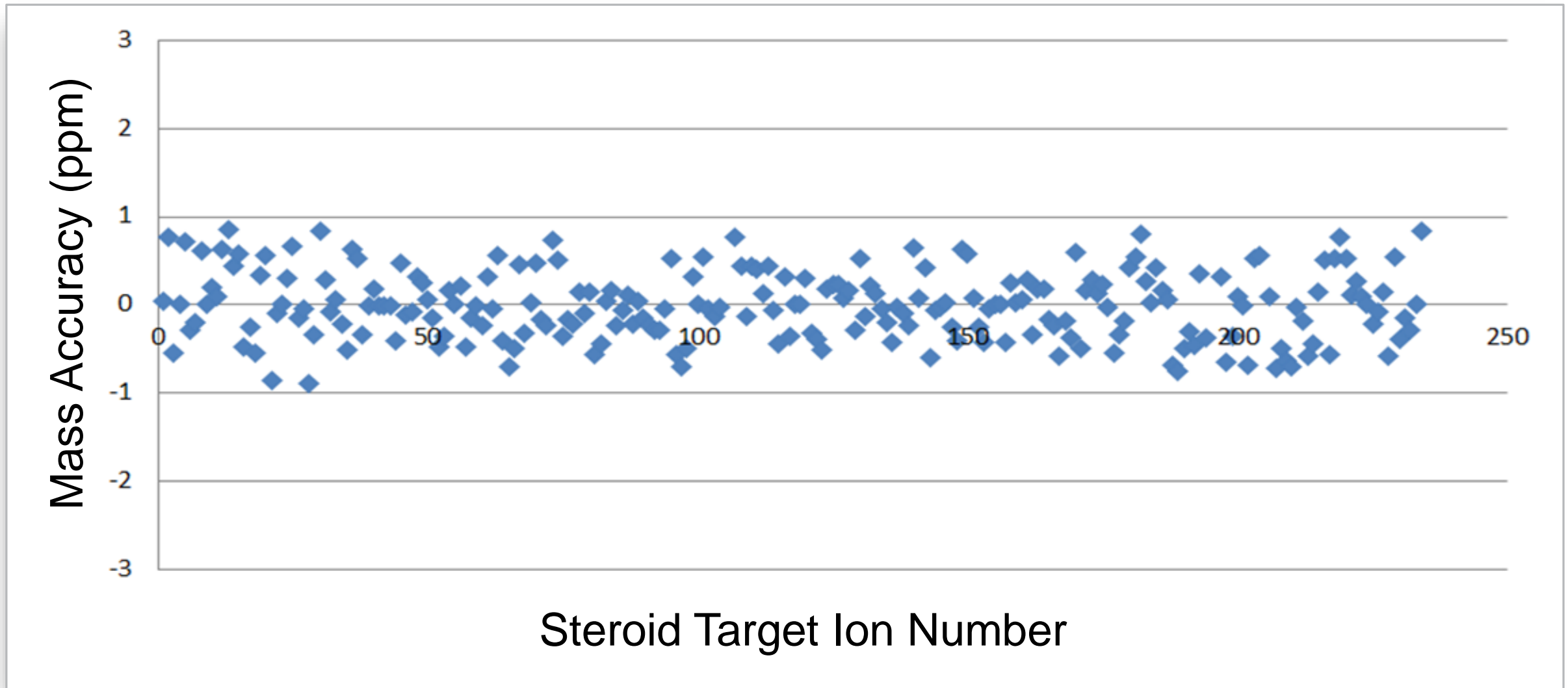
Chemical element	No
C	50
H	100
O	10
N	10
Cl	10

No. of proposed formula for m/z 324.13541

Top hit = $C_{20}H_{20}O_4$



Mass Accuracy of Q Exactive GC for Steroids Analysis



Resolution Can Effect Mass Accuracy

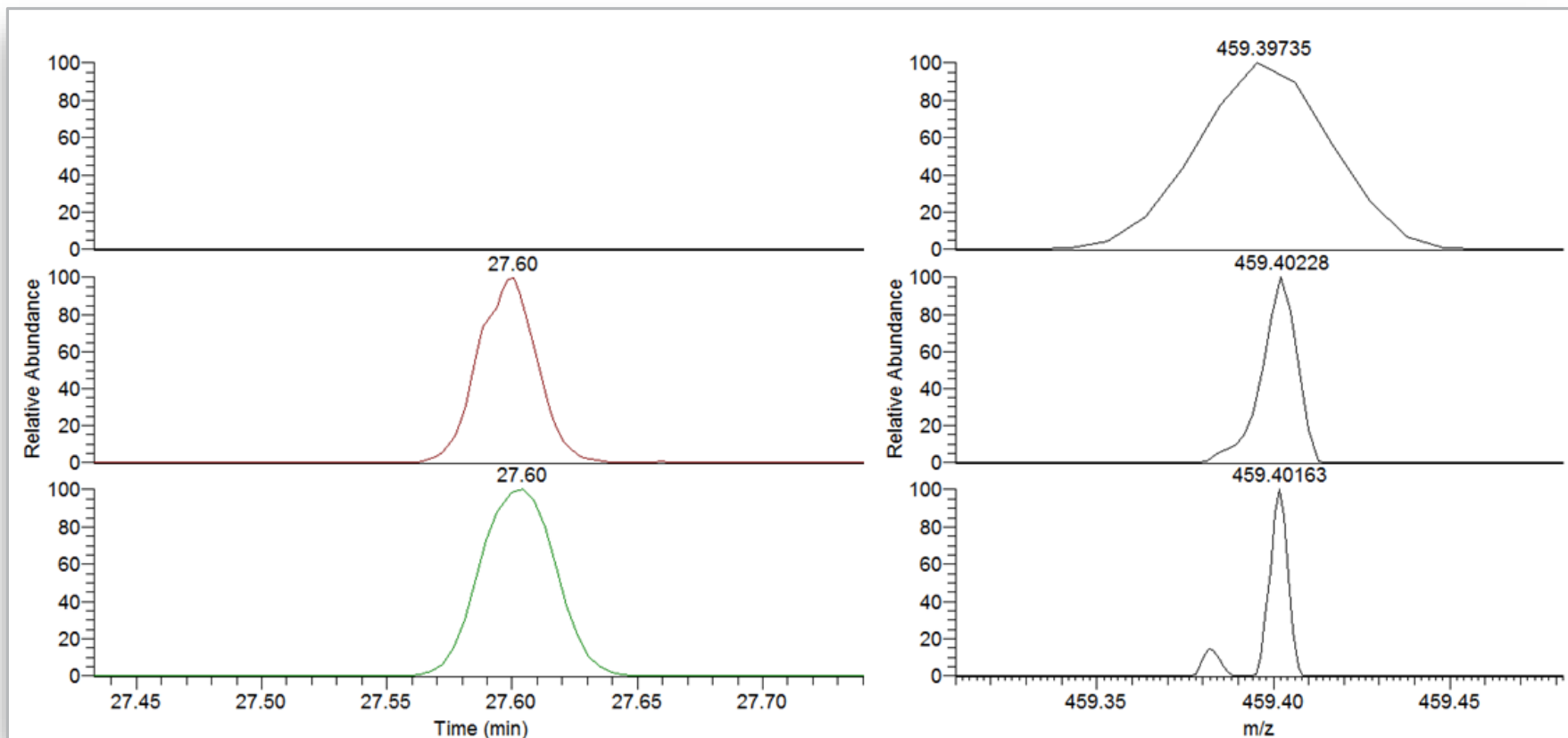
Resolution

5 ppm mass extraction window

M+H Ion

Mass Error

15K



60K

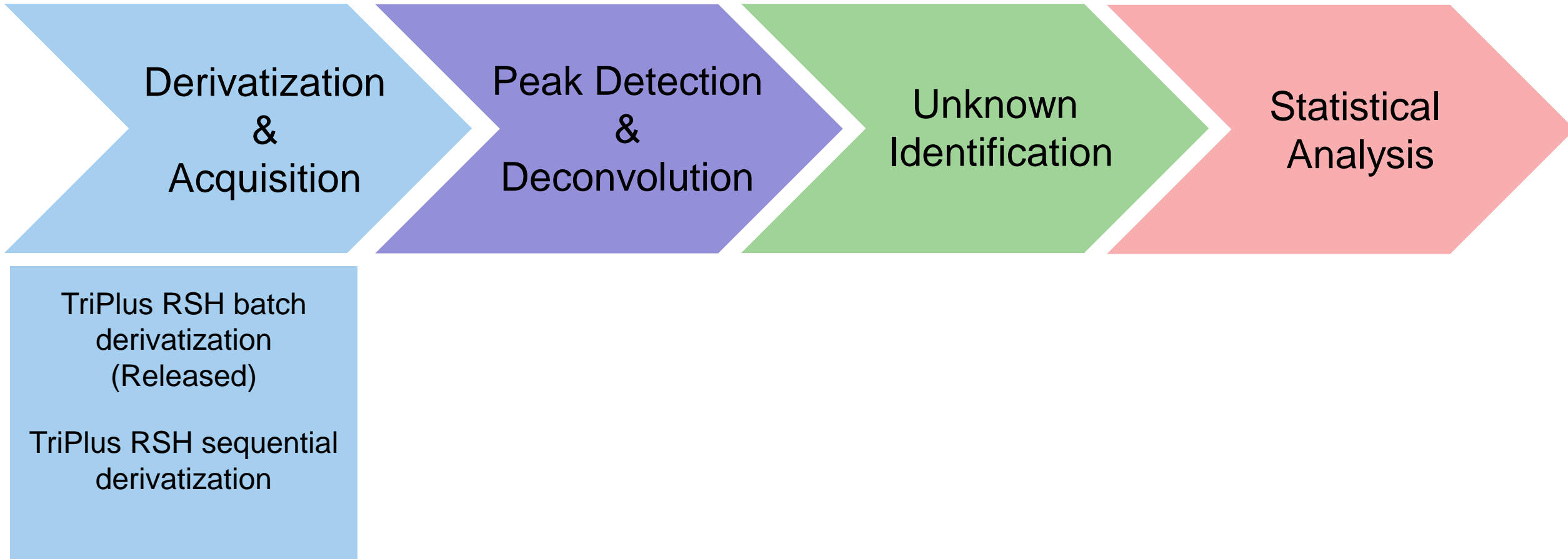
120K

Cholesterol M+H Ion at Different Resolutions

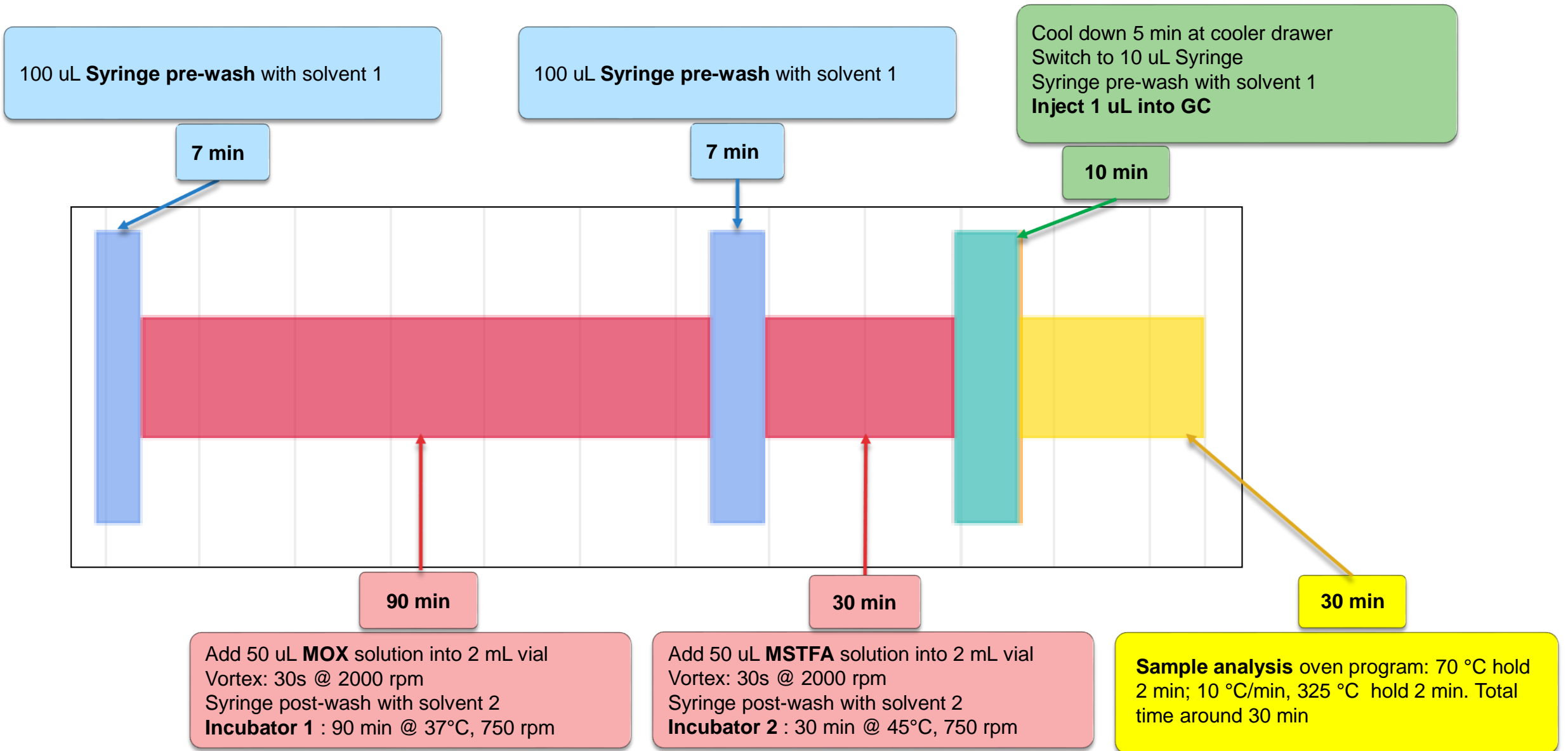


Tools for HRAM GC/MS-Based Metabolomics

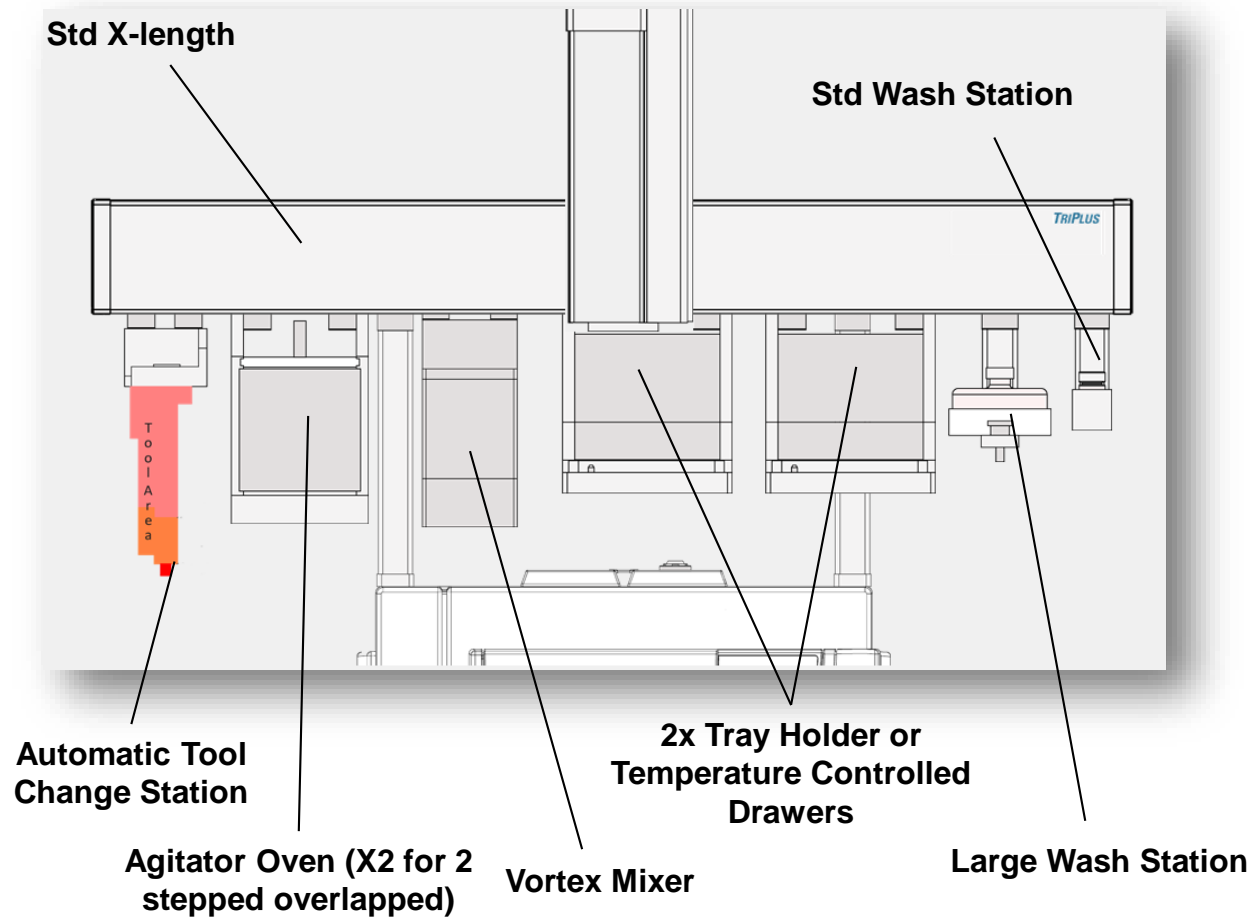
GC/MS Metabolomics Workflow



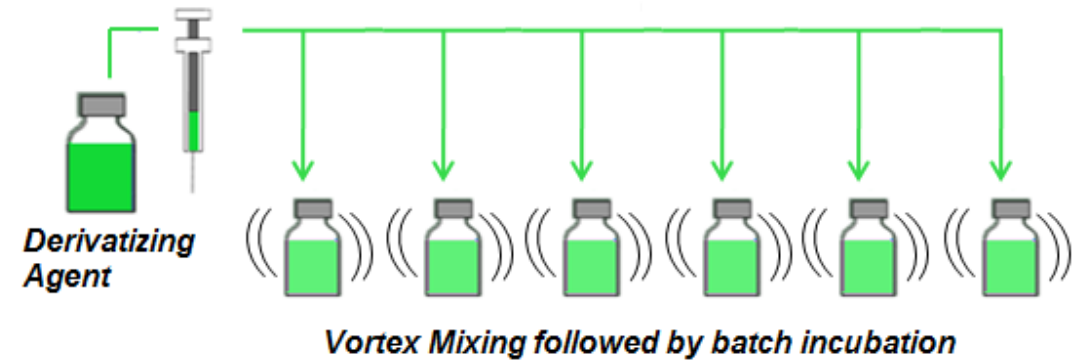
Example GC/MS Metabolomics Derivatization Protocol



Triplus RSH for Automated Derivatization Step



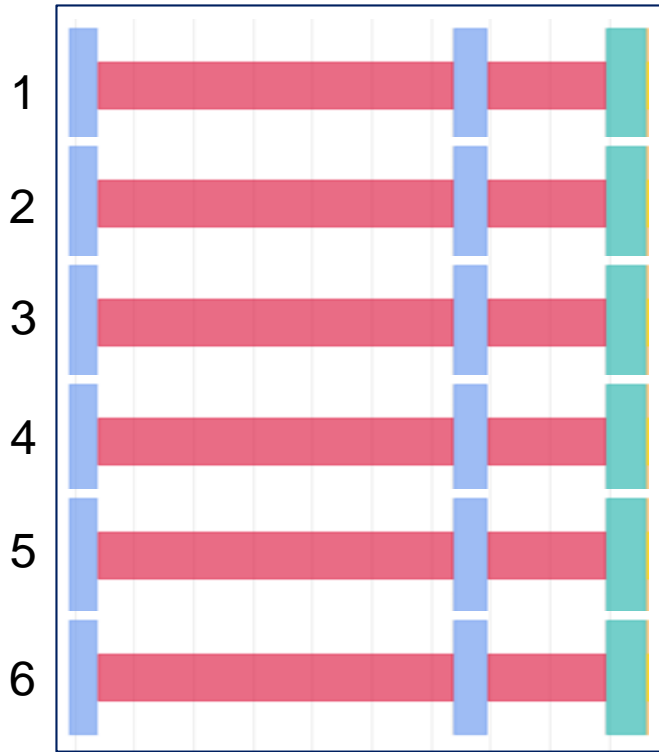
- Prep-Cycle available for Derivatization in batch or sequential



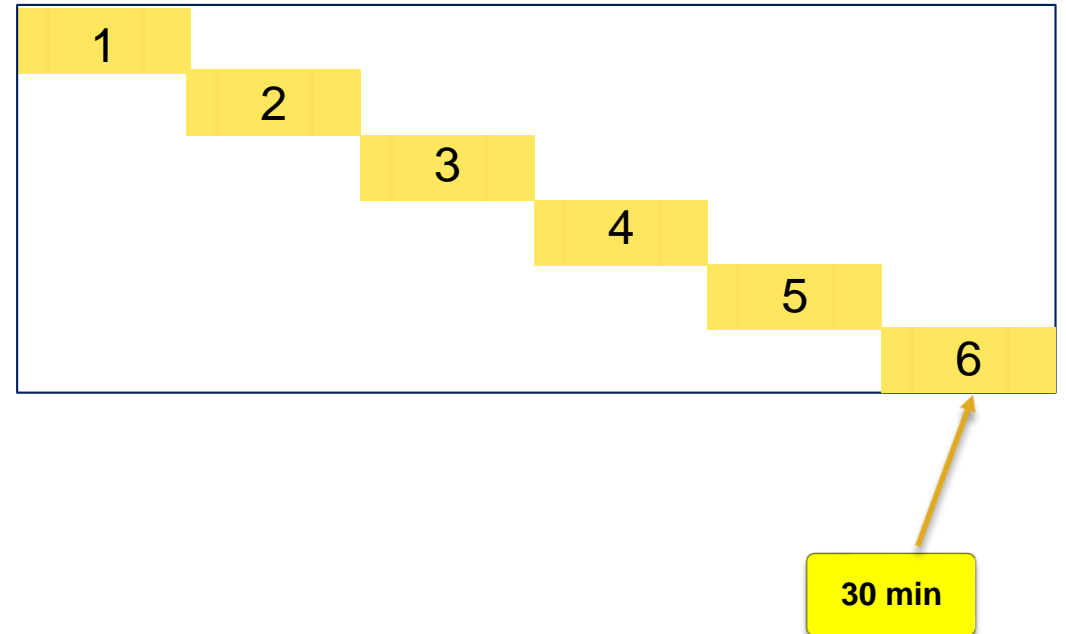
- Both strategies are compatible with methoxyamine/MSTFA derivitization protocol

Batch Online Derivatization

Samples prepared in a batch



Samples analyzed sequentially on GC



7 min

90 min

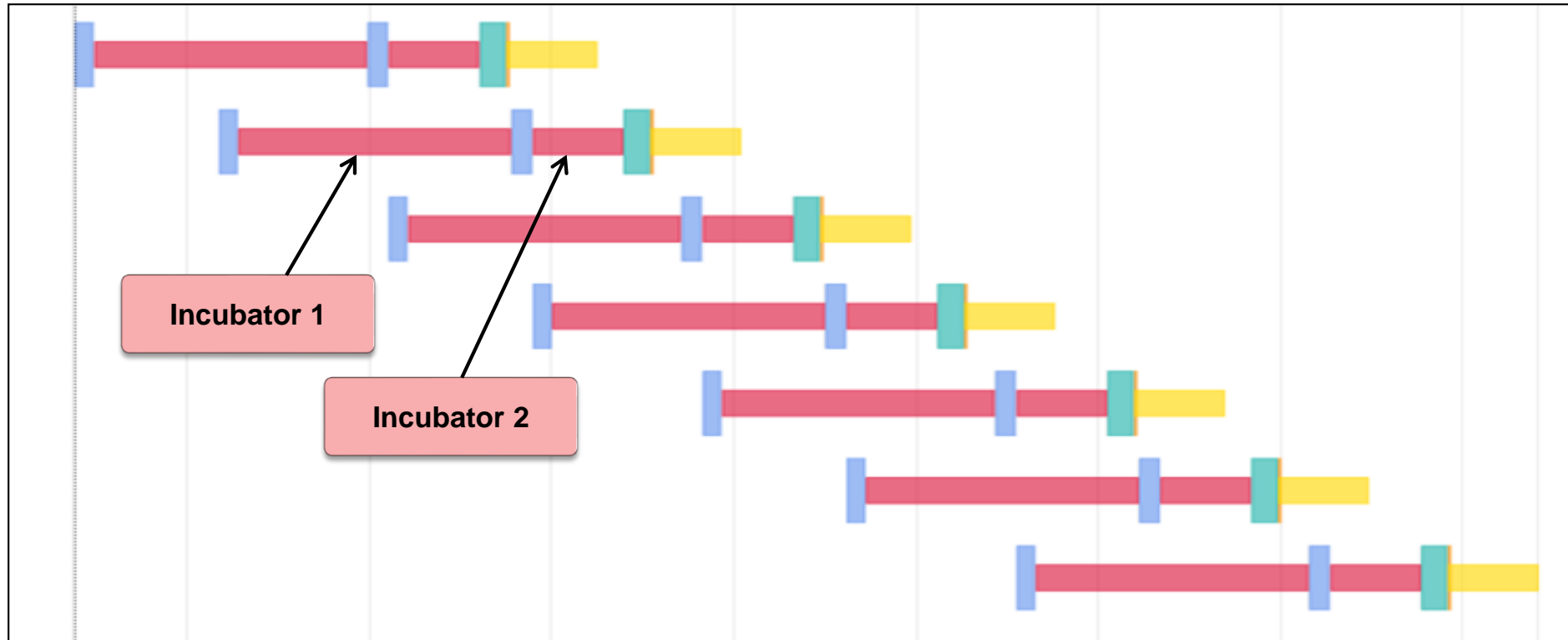
7 min

30 min

10 min

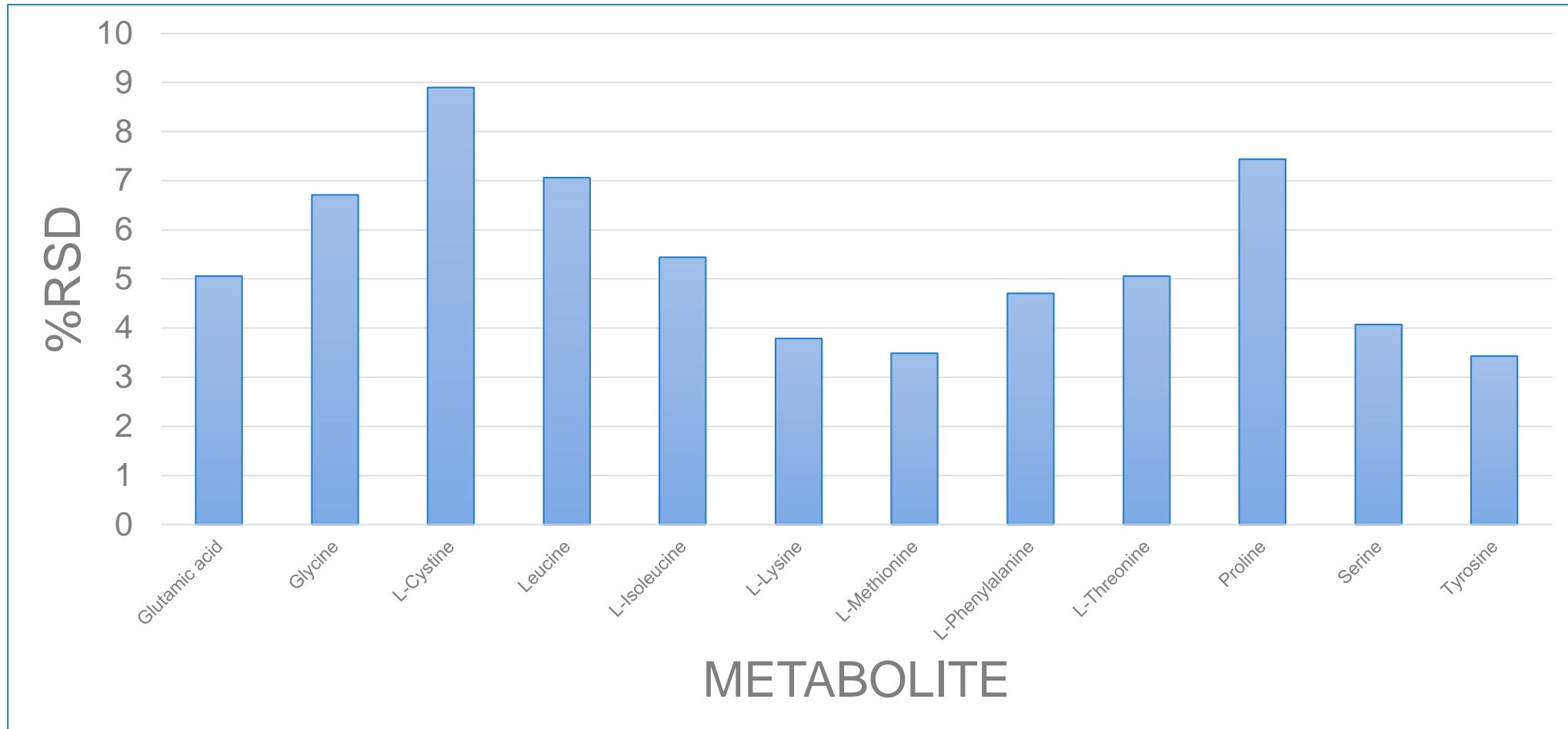
30 min

Two Incubators Sequential Derivatization



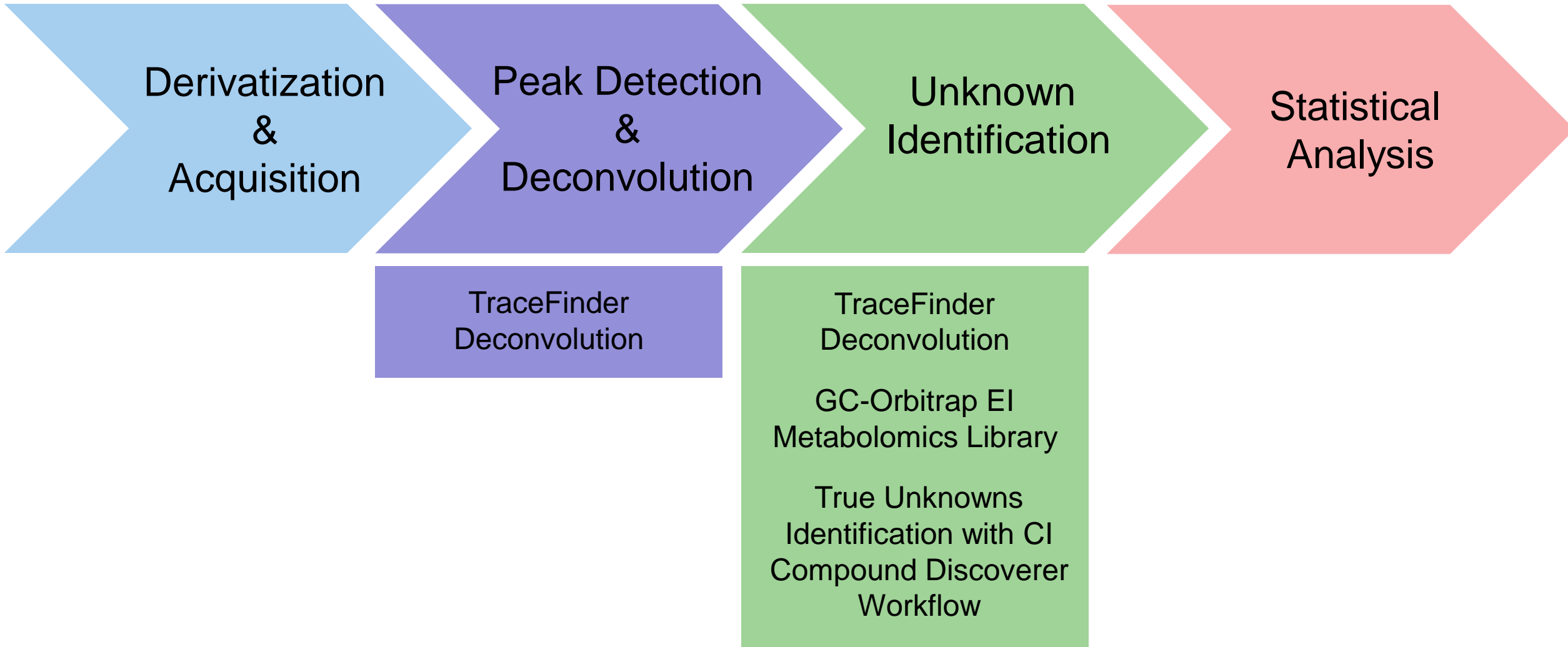
- 24 samples can be prepared and analyzed in 24h
- Two incubators can be used simultaneously
- Minimizes time between derivatization and injection
- Best for labile TMS-metabolites

Preliminary Data from 24 Hour Test with Amino Acids

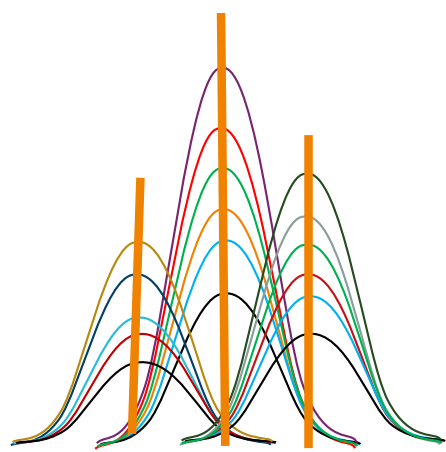


Average 5% RSD over 24 hours without internal standard

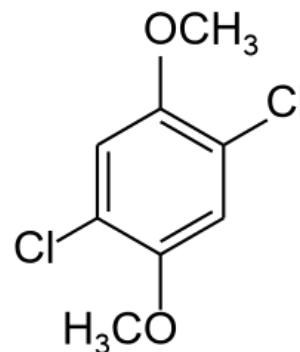
Metabolomics Workflow Tools



Algorithm for Unknowns Identification



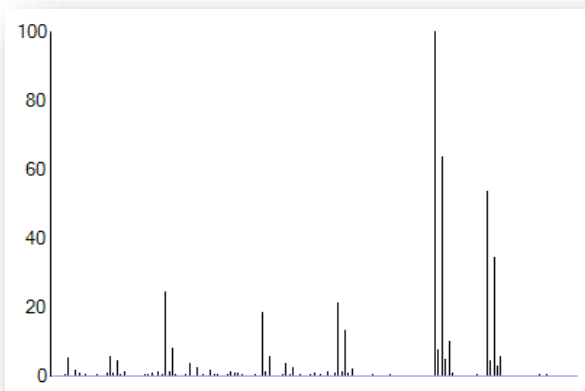
Deconvolve TIC



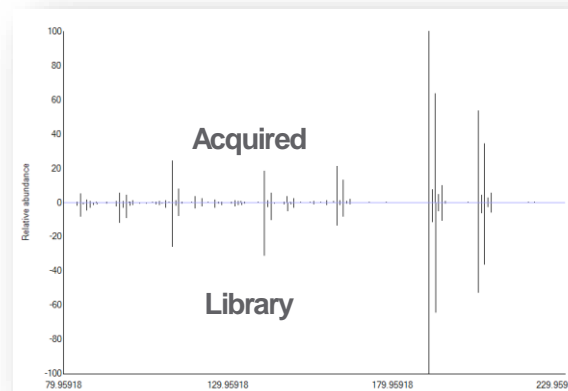
Candidate Compounds

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



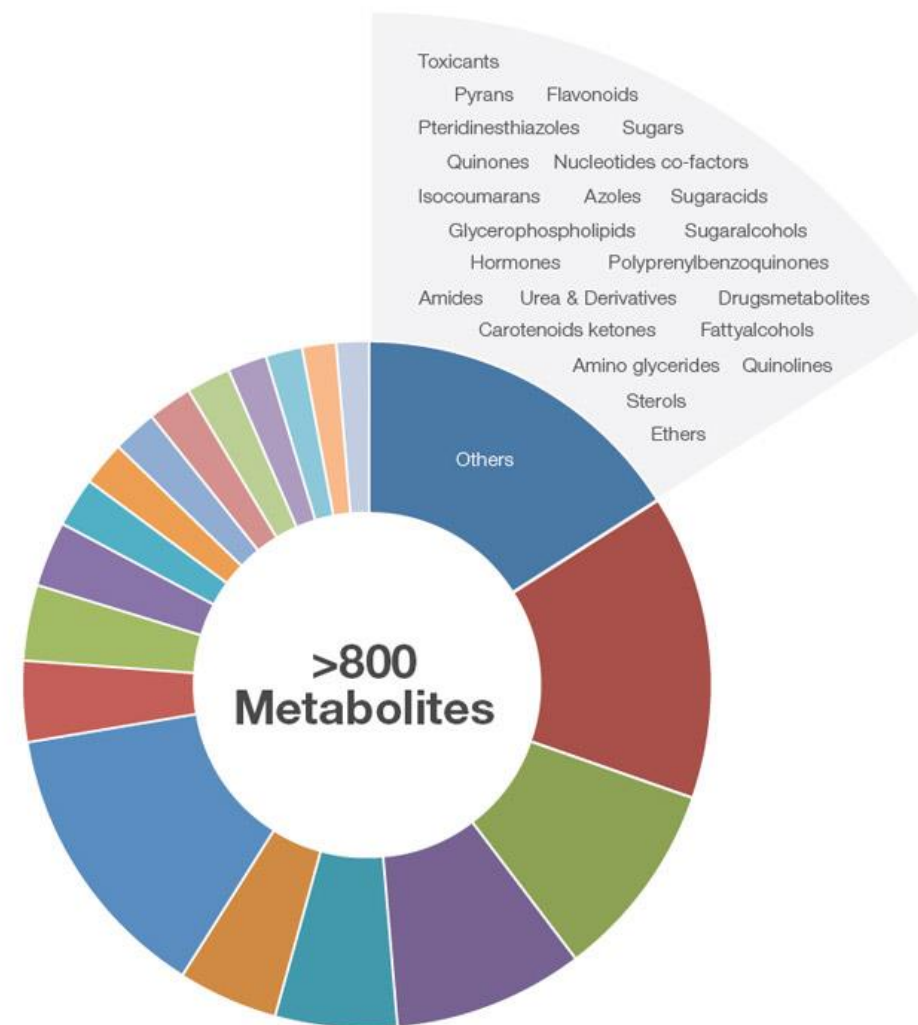
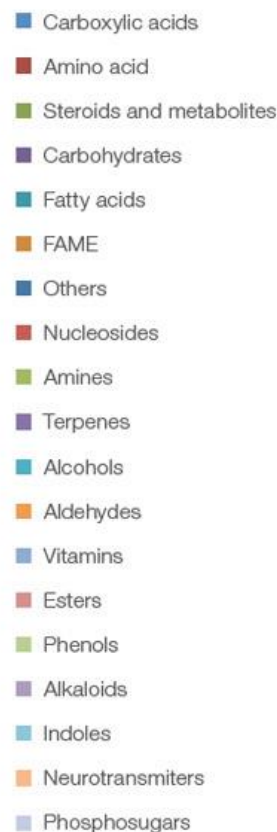
Create "clean" spectrum



Library search

GC-Orbitrap Metabolomics Library

- Nearly 900 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



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		680	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1592

Strong spectral library match and HRF score

Component Name: L-Glutamic acid, 3TMS, RT: 14.43

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

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(28...	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	56083397	33163537	C(12)4 Si(28...	149.0448...	0.2

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		660	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1592

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

Sample List: EI-QC6-splitless-pulsed-or...

Component Name: 5-Aminovaleric aci... RT: 13.78

2-Oxoglutaric acid, ... RT: 13.89

Diethyl Phthalate RT: 14.16

L-Glutamic acid, 3T... RT: 14.43

Xylulose 1MOX, 4T... RT: 14.63

4-amino-N-butylac... RT: 14.64

4-hydroxyproline, 2... RT: 14.83

Acquisition date: 7/26/2017 10:32:00

InstrumentName type: Q Exactive G

Inst ID: Exactive Series slot #1

InstrumentName method: C:\Trace1

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	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	56083397	33163537	C(12)4 Si(28...	149.0448...	0.2

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		660	99.2607	363.171189	363.171143	gc-orbitrap metabolomics library	1612	1592

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
- 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

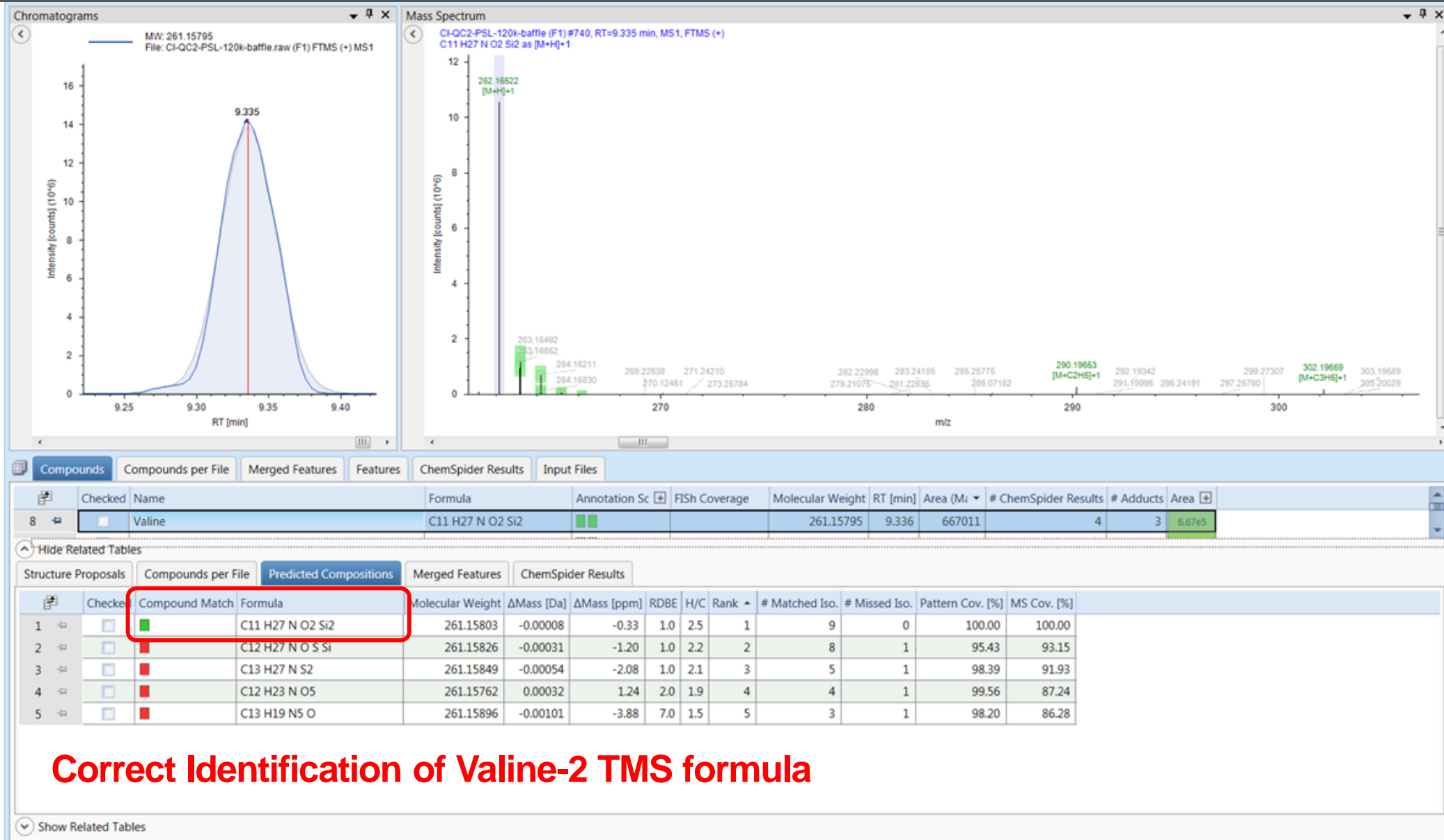
Relative abundance

Component 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	56083397	33163537	C(12)4 Si(28...	149.0448...	0.2

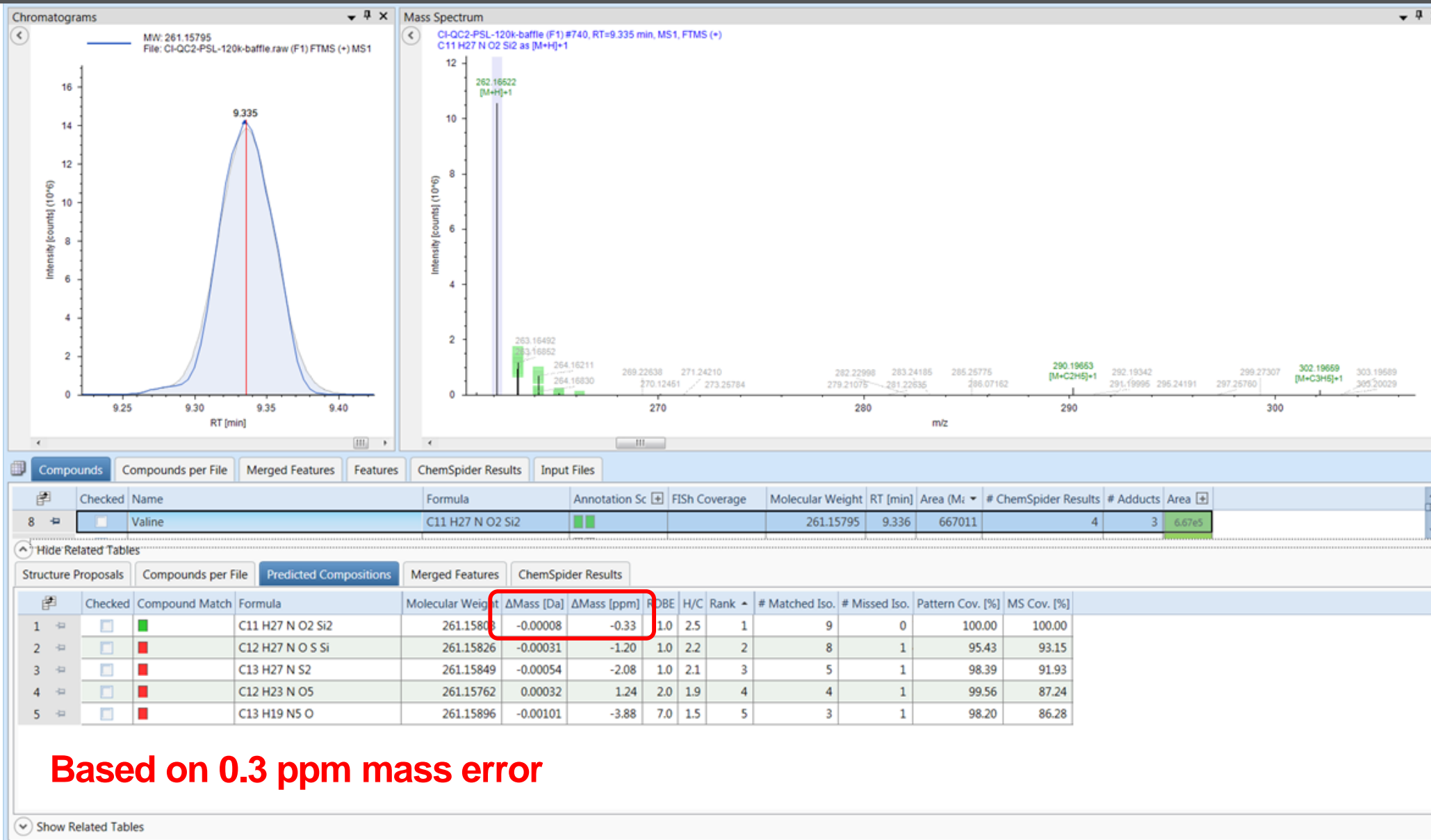
Library spectrum

Automated CI Identification with Thermo Scientific™ Compound Discoverer™ Software



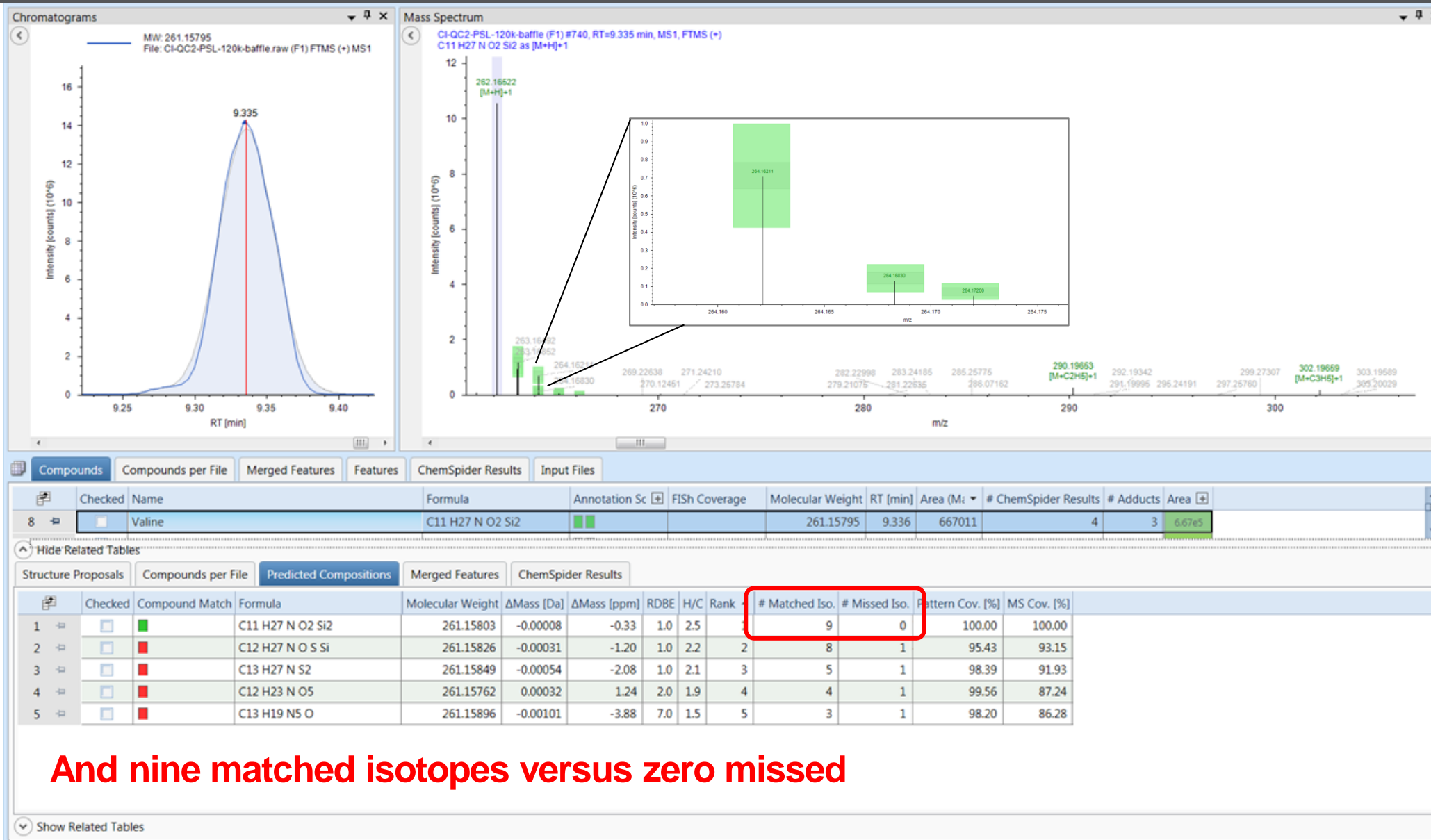
Correct Identification of Valine-2 TMS formula

Automated CI Identification with Compound Discoverer Software



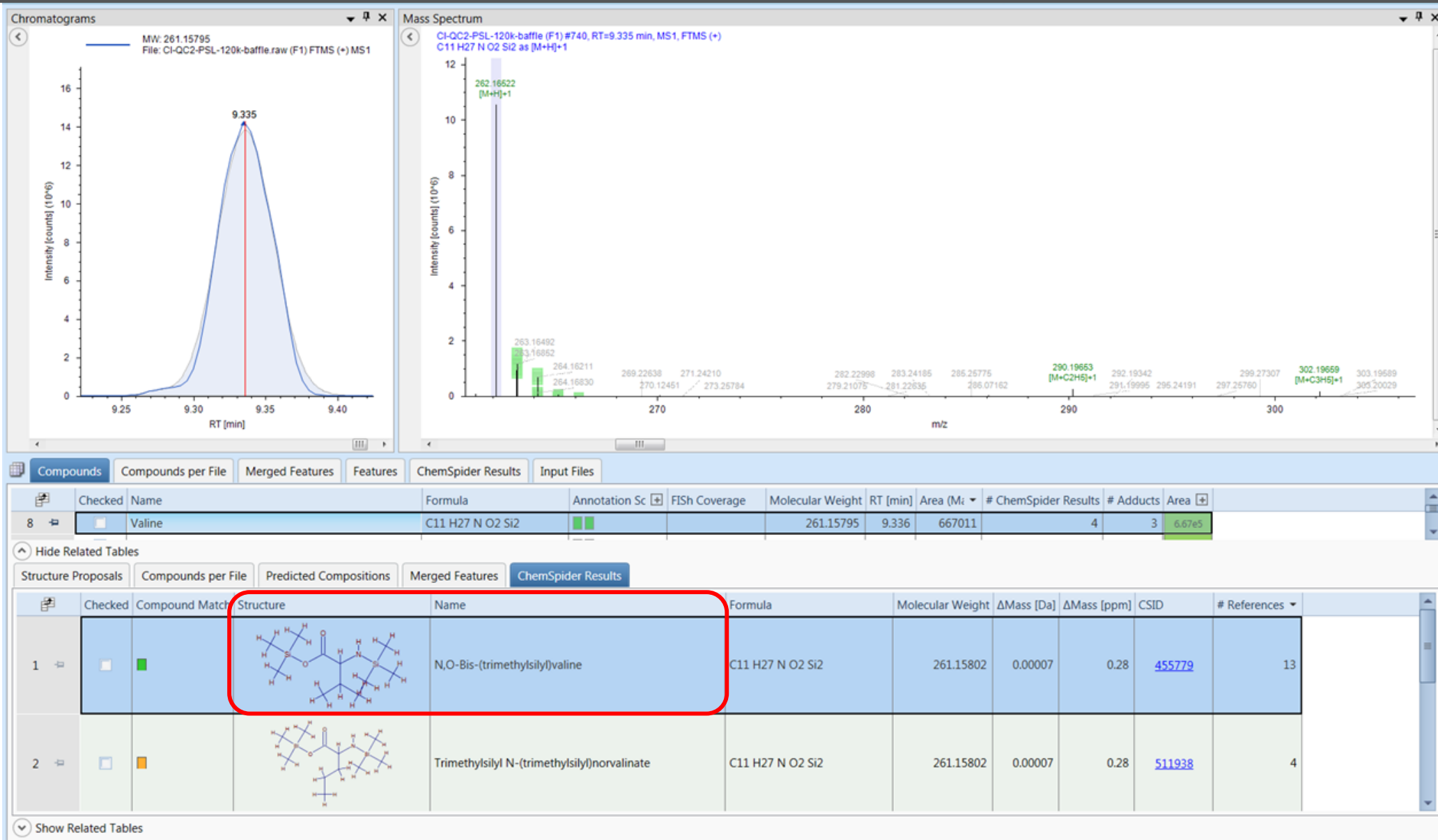
Based on 0.3 ppm mass error

Automated CI Identification with Compound Discoverer Software

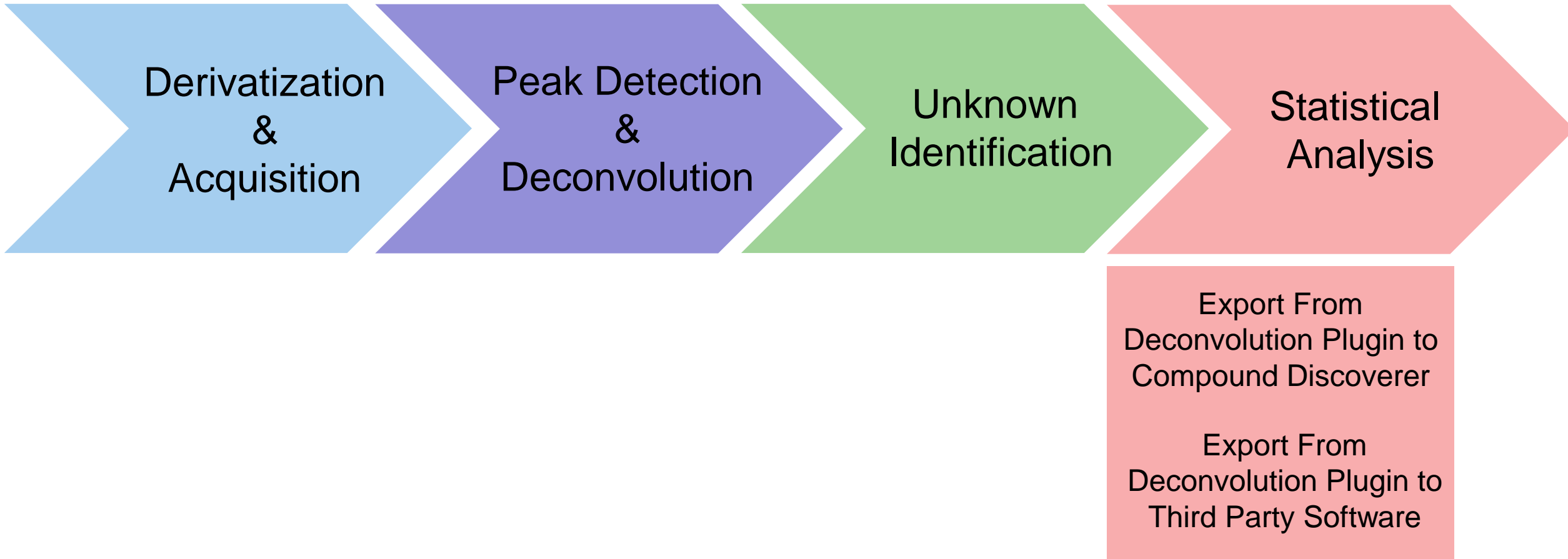


And nine matched isotopes versus zero missed

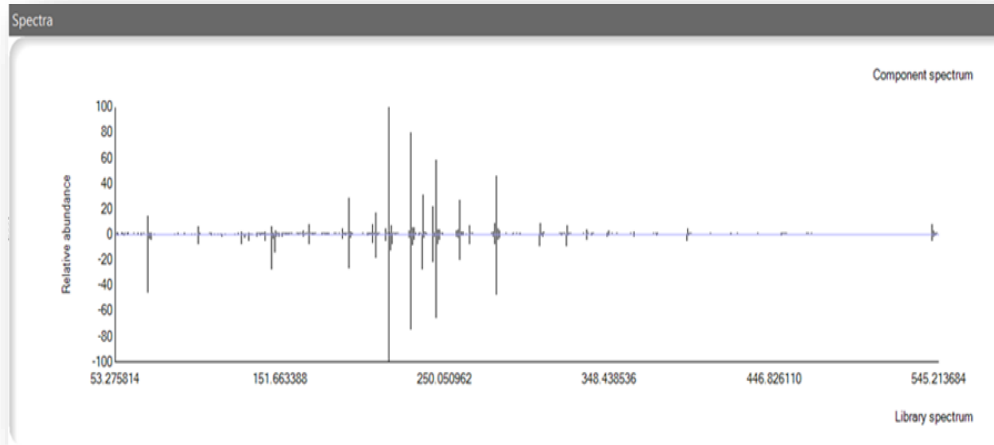
Automated ChemSpider Search Can Suggest Structure



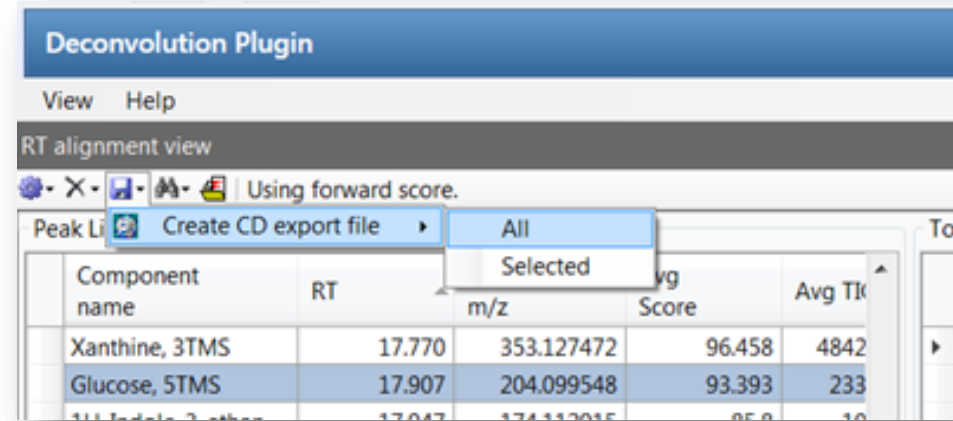
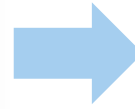
Metabolomics Workflow Tools



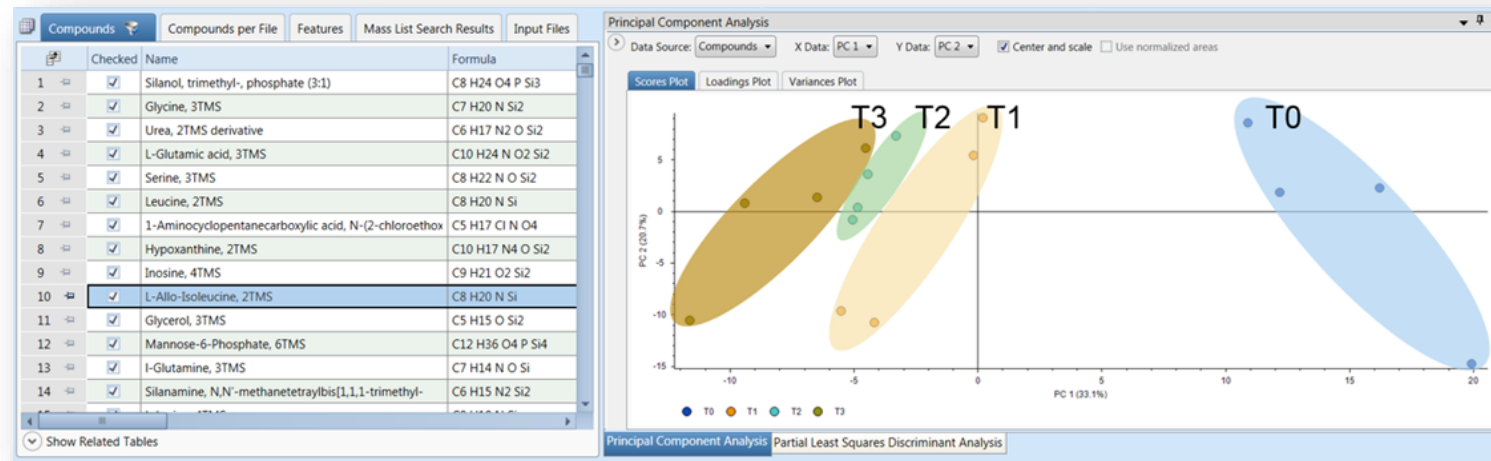
Export from Deconvolution Plugin to Compound Discoverer Software



Identify peaks with Deconvolution Plugin & Metabolomics Library



Create CD peak list with identified peaks



Process in CD assigning metabolite names from peak list

Presentation Summary

- GC/MS increases measurable part of metabolome, increasing biological insight
- Orbitrap GC/MS can provide deepest insight
 - Highest number of detectable metabolites in biological matrix
 - Superior mass accuracy simplifies unknown identification
- Additional tools to facilitate metabolomics workflow
 - Online derivatization with TriPlus RSH
 - Deconvolution with metabolomics library for automated EI identification
 - Compound Discoverer software for automated CI identification

