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

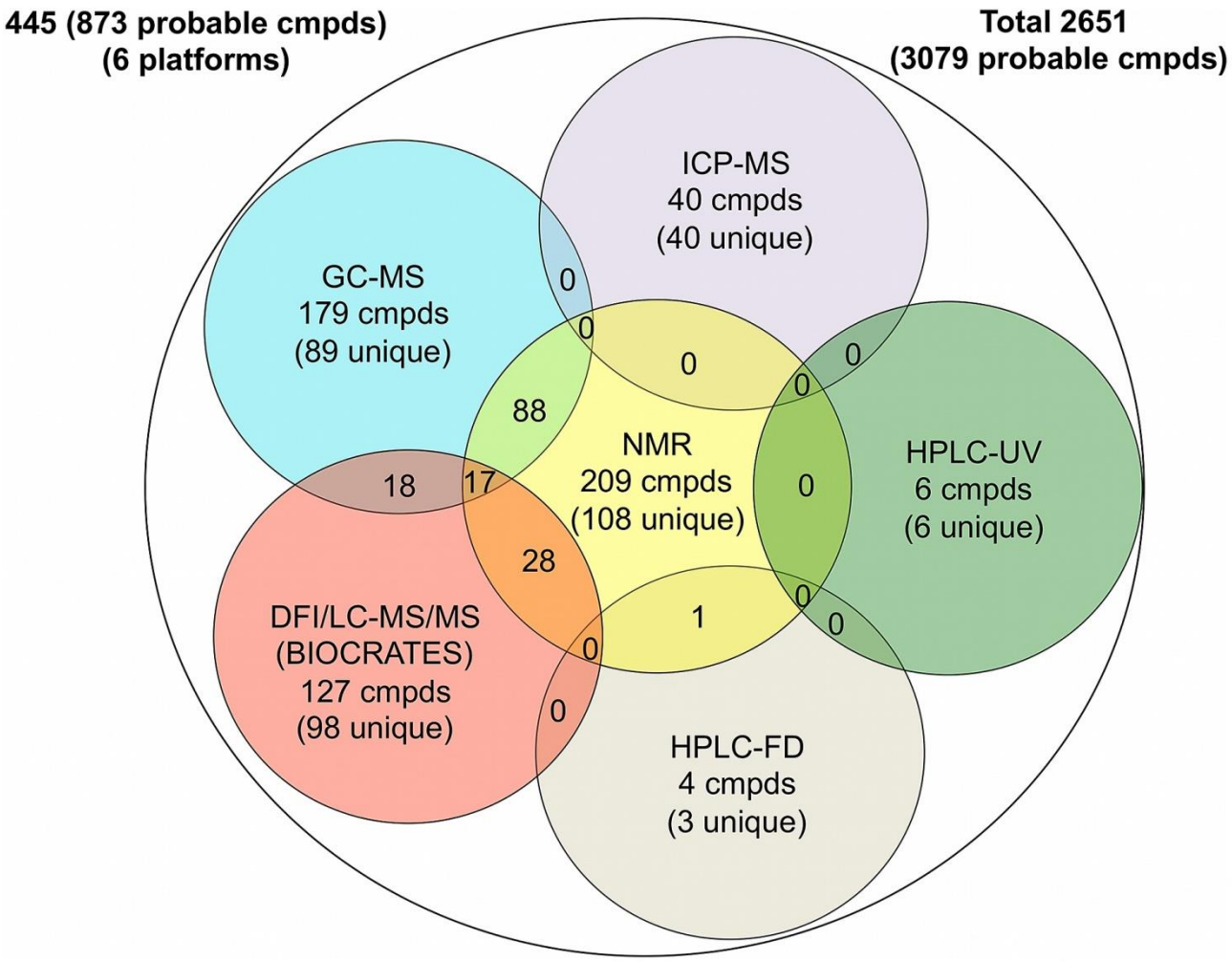
1 **Why GC/MS for Metabolomics?**

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

3 **Tools for HRAM GC/MS-Based Metabolomics**

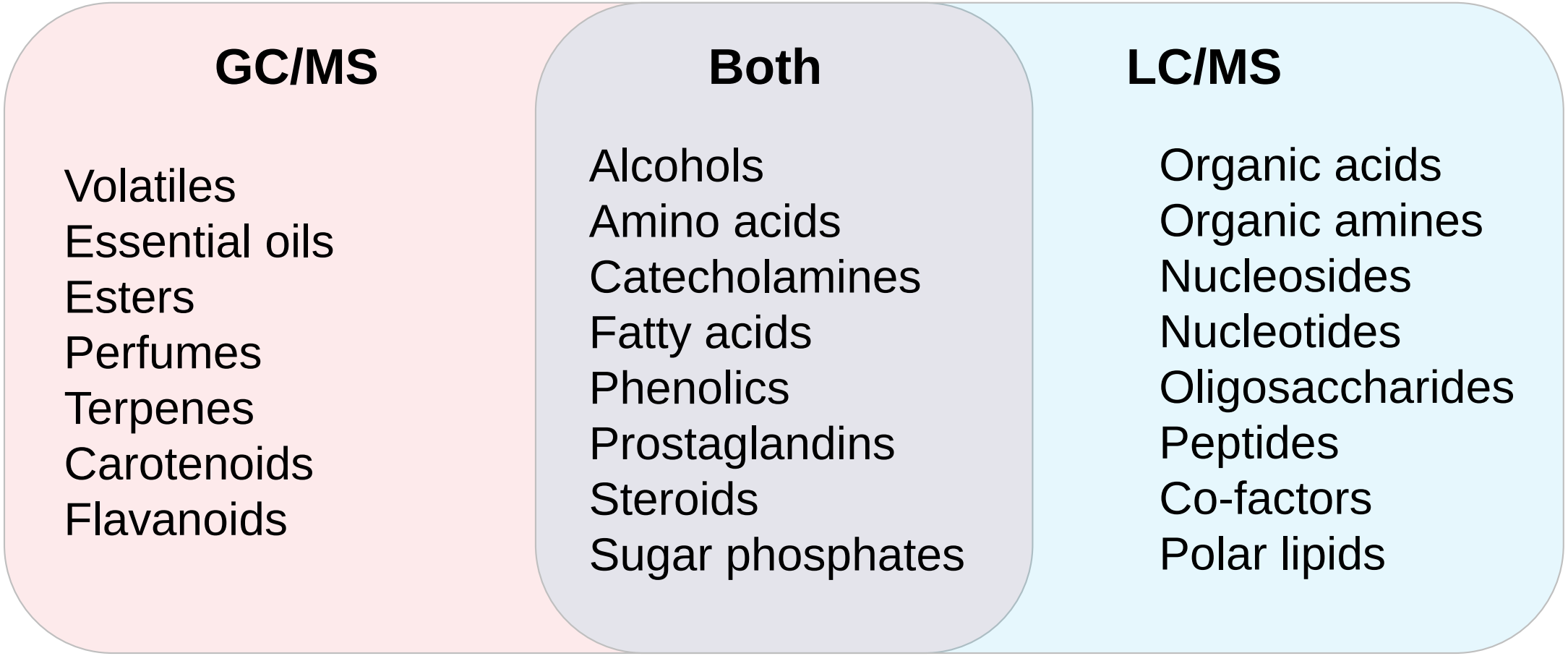
Why GC/MS for Metabolomics?

Urine Metabolome - Compound Identification by Technique



Bouatra, S. et. al., **2013**, The Human Urine Metabolome, *PLOS ONE,* https://doi.org/10.1371/journal.pone.0073076

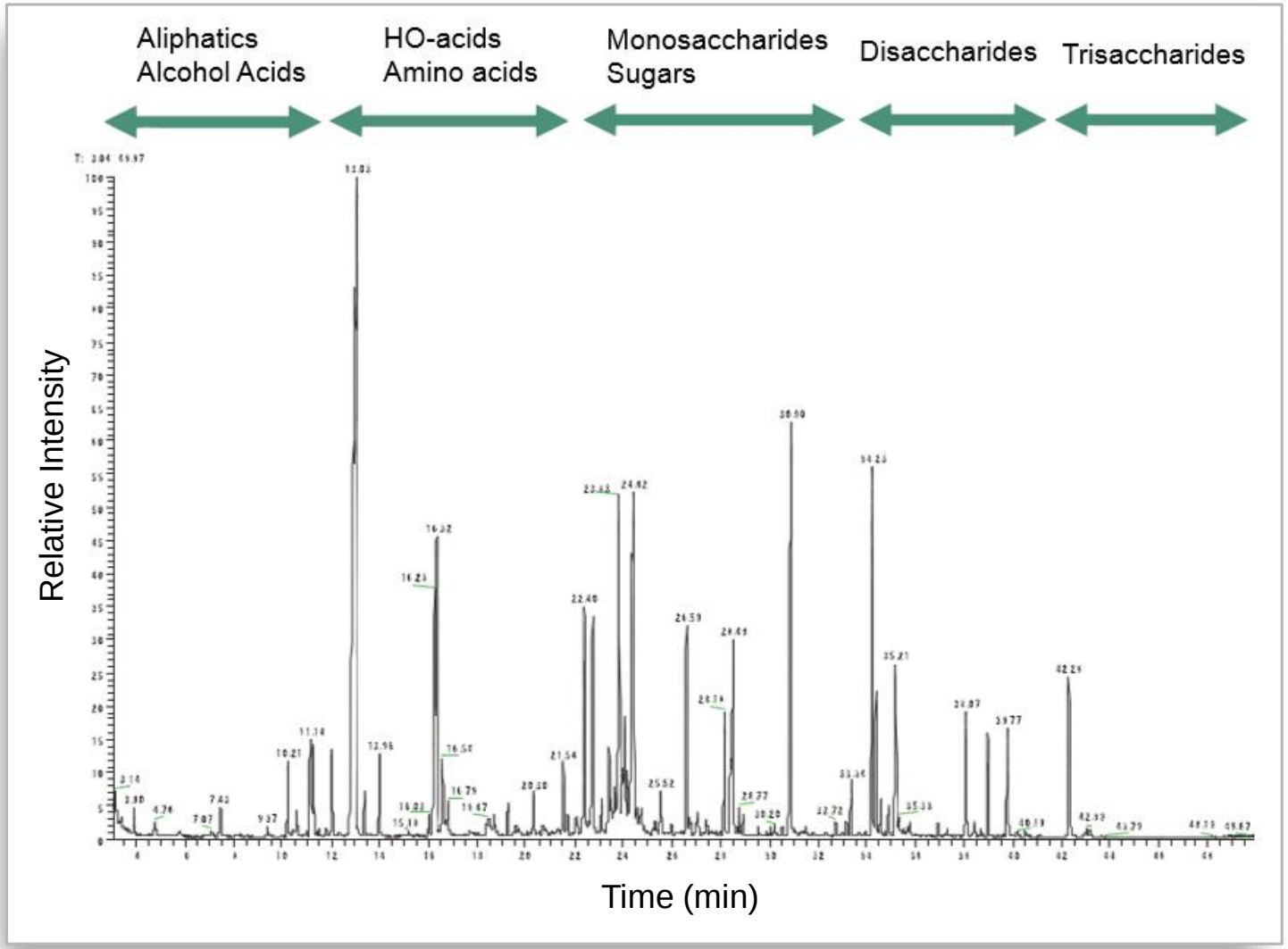
Example Strategy for Pancreatic Cancer Serum Profiling



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



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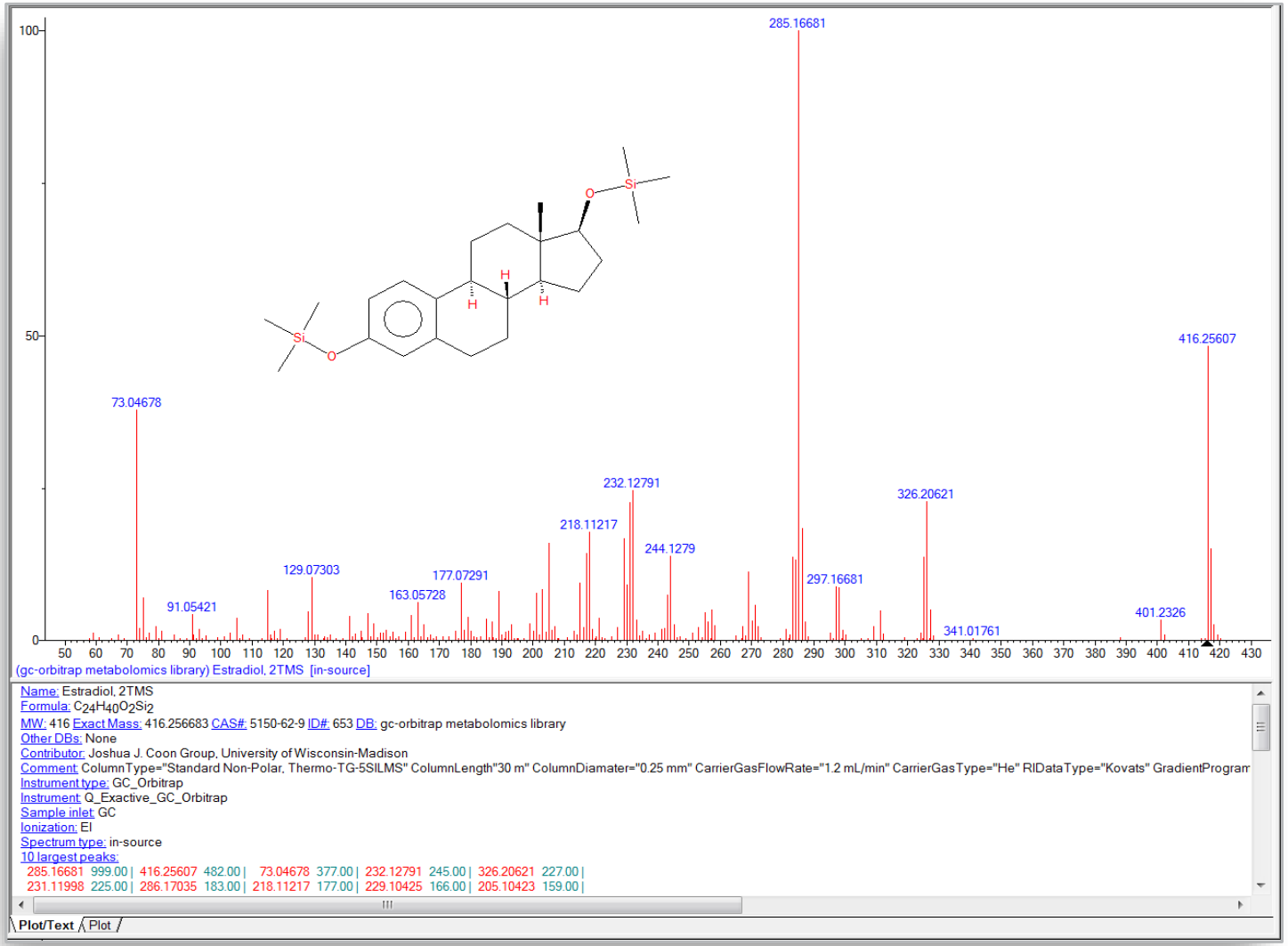
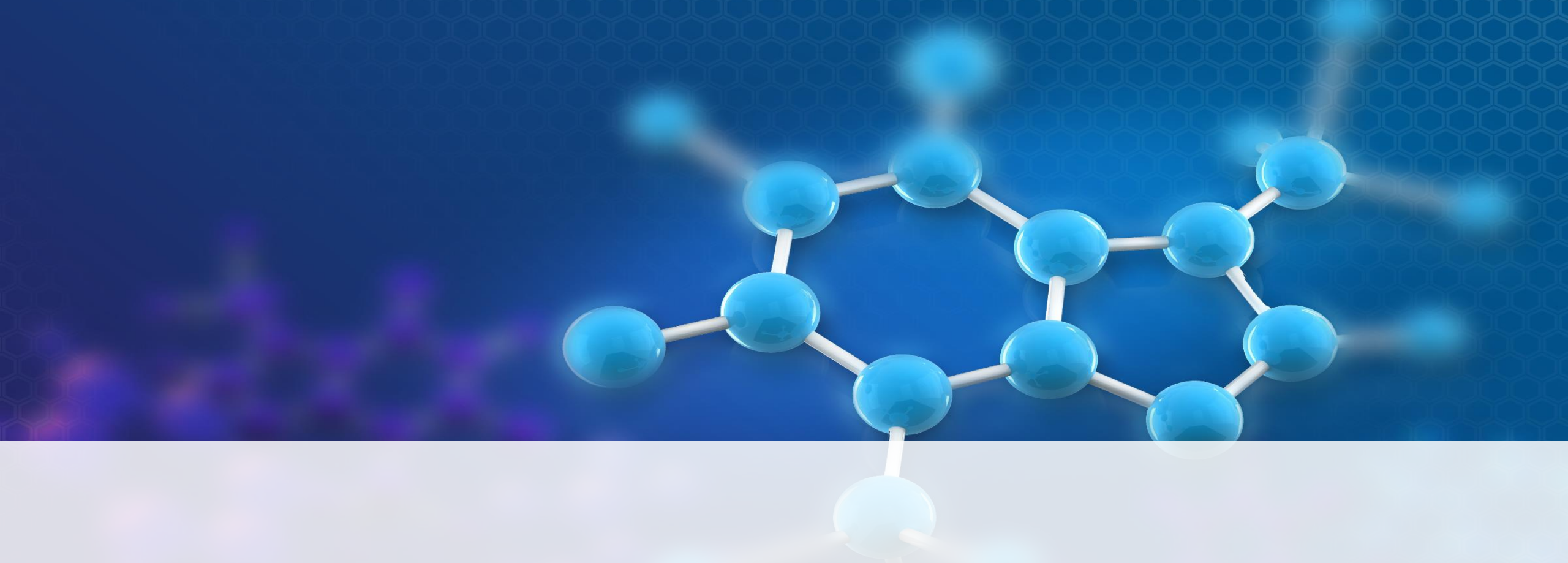
Considerations Unique to GC/MS

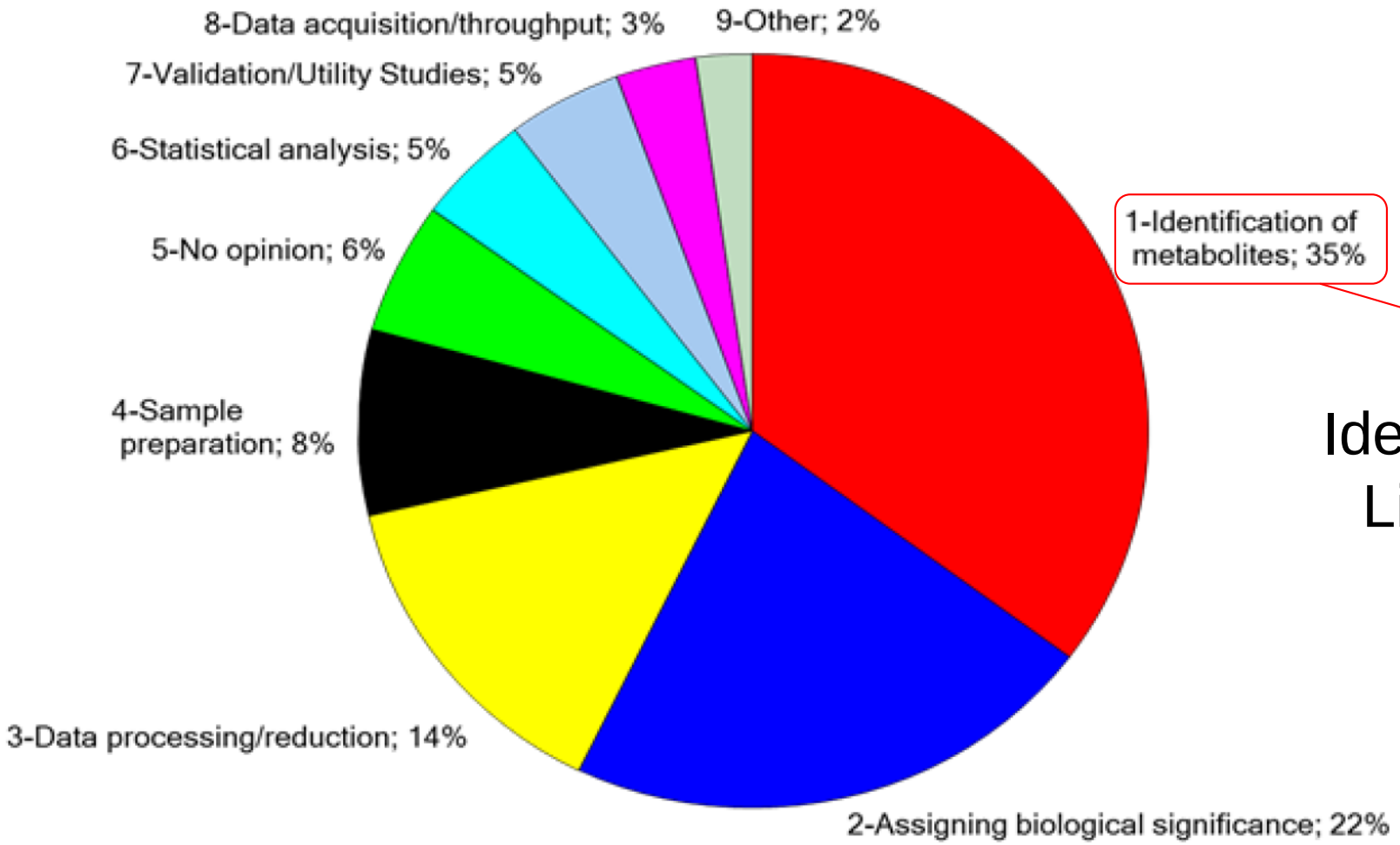
- Requires compounds that evaporate at temperatures < 350 °C
  - Derivatization can increase volatility
  - Online derivatization available
- Molecular ion can be absent in EI
  - Identification by library search
  - Low eV can promote MI in EI
  - CI can be used for soft ionization





Why HRAM GC/MS for Metabolomics?

Bottlenecks in Metabolomics Studies



Source: ASMS Metabolomics Workshop Survey

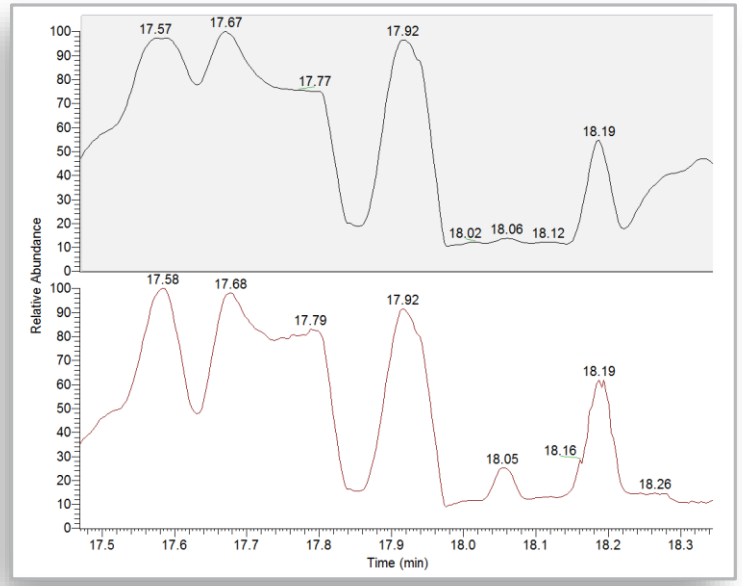
Identification of Unknowns Listed as #1 Bottleneck

Benefits of HRAM GC/MS to Global Metabolomics

GC-Quad

TIC

270 extracted
± 0.5 amu



Unit Resolution

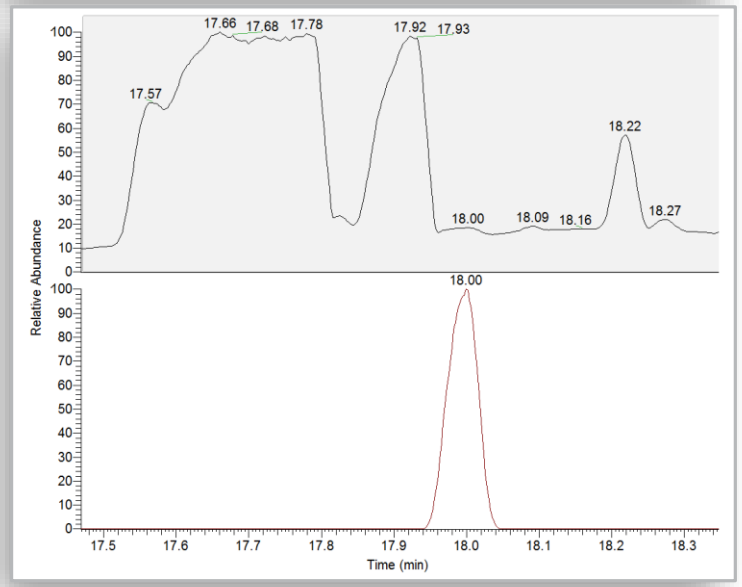
Decc

Hexadecanoic acid methyl ester
molecular Ion = 270.2553

GC-Orbitrap

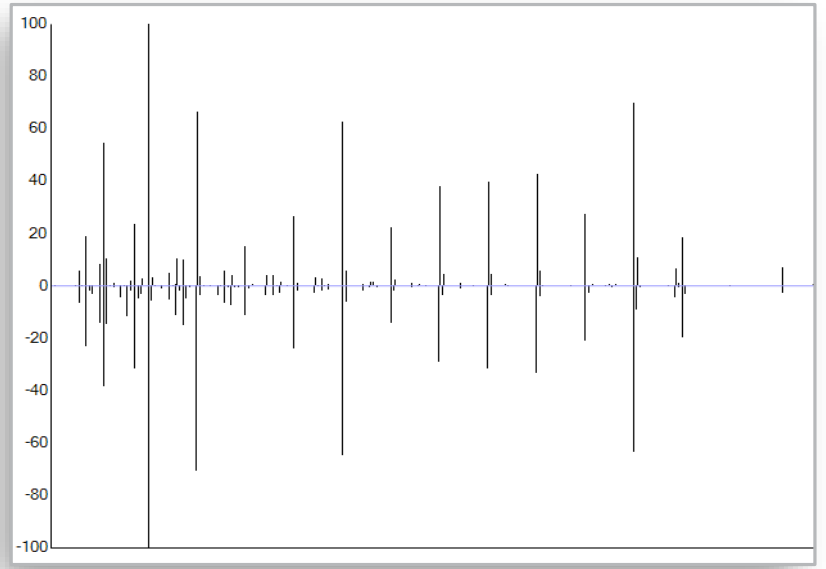
TIC

270.2553 extracted
± 5 ppm



High Resolution

Deconvolution



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Mass Accuracy for Compound Identification

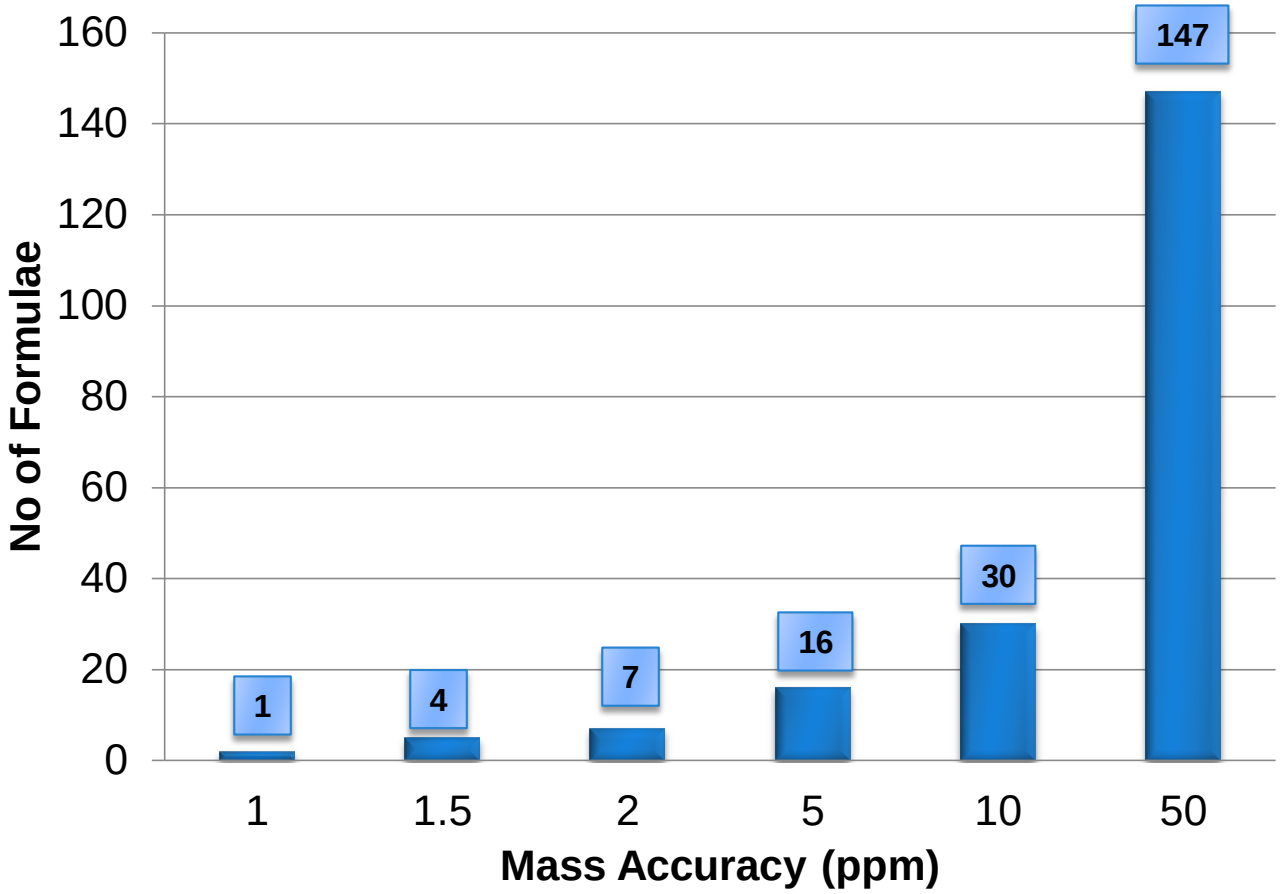
Type	Mass Accuracy
FT-ICR-MS	0.1 - 1 ppm
Orbitrap	0.5 - 1 ppm
Magnetic Sector	1 - 2 ppm
TOF-MS	3 - 5 ppm
Q-TOF	3 - 5 ppm
Linear IonTrap	50-200 ppm (10 ppm in Ultra-Zoom)

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



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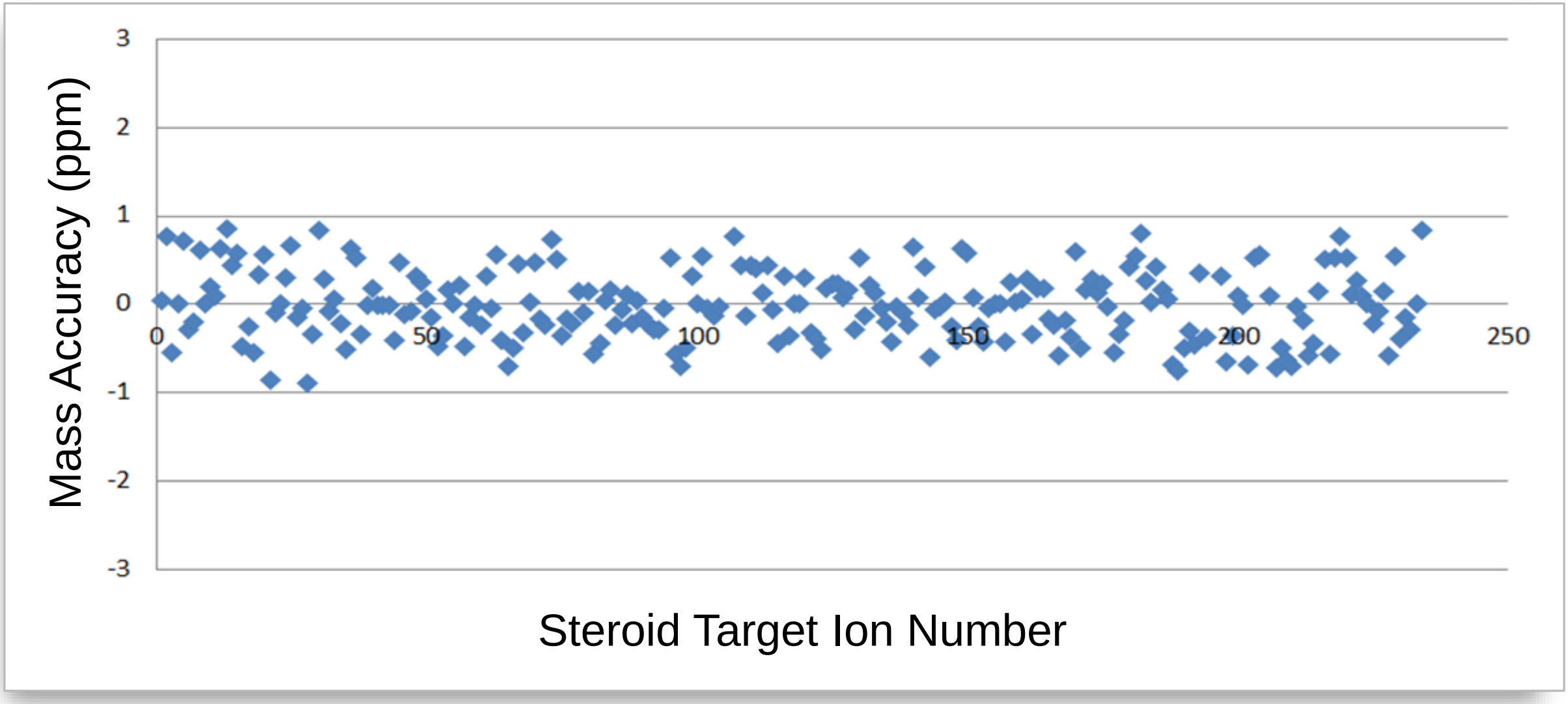
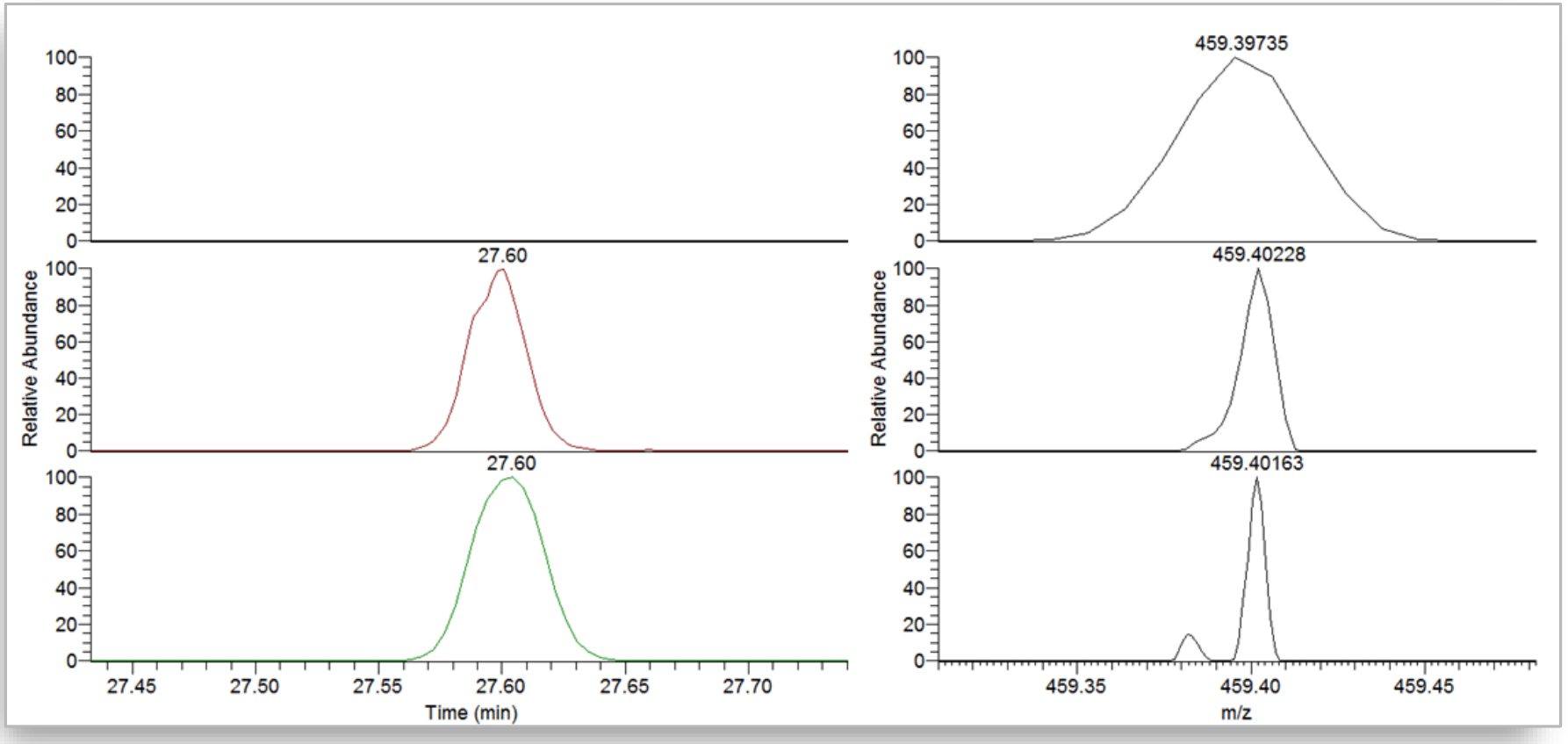
Mass Accuracy of Q Exactive GC for Steroids Analysis



Resolution Can Effect Mass Accuracy

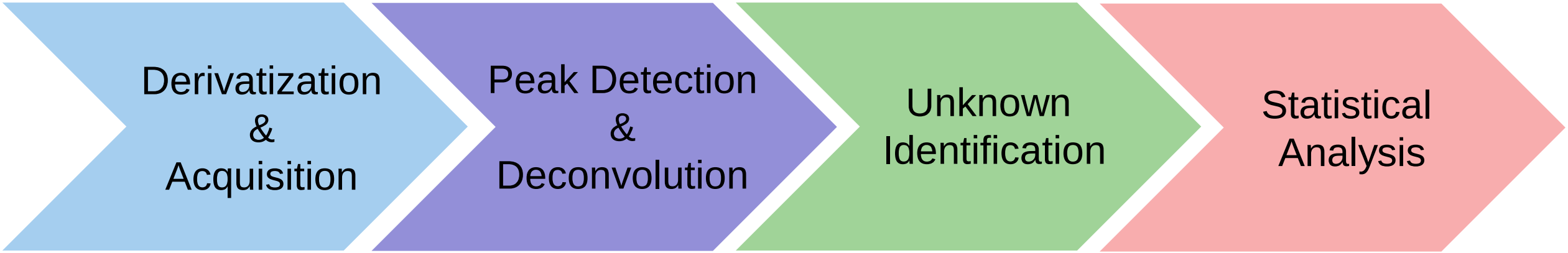
Resolution	5 ppm mass extraction window	M+H Ion	Mass Error
15K			Δ 9.4 ppm ✗
60K			Δ 1.3 ppm ✓
120K			Δ 0.1 ppm ✓ ✓



Cholesterol M+H Ion at Different Resolutions

Tools for HRAM GC/MS-Based Metabolomics

GC/MS Metabolomics Workflow



TriPlus RSH batch derivatization (Released)

TriPlus RSH sequential derivatization

Example GC/MS Metabolomics Derivatization Protocol

100 uL **Syringe pre-wash** with solvent 1

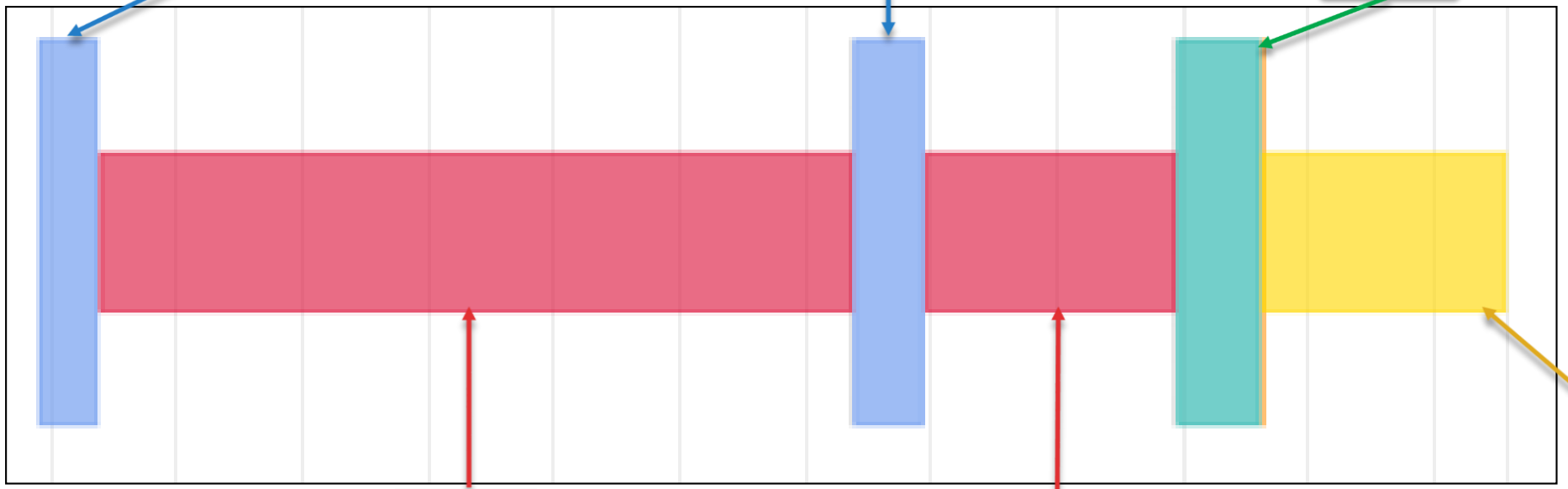
7 min

100 uL **Syringe pre-wash** with solvent 1

7 min

Cool down 5 min at cooler drawer
Switch to 10 uL Syringe
Syringe pre-wash with solvent 1
Inject 1 uL into GC

10 min



90 min

Add 50 uL **MOX** solution into 2 mL vial
Vortex: 30s @ 2000 rpm
Syringe post-wash with solvent 2
Incubator 1 : 90 min @ 37°C, 750 rpm

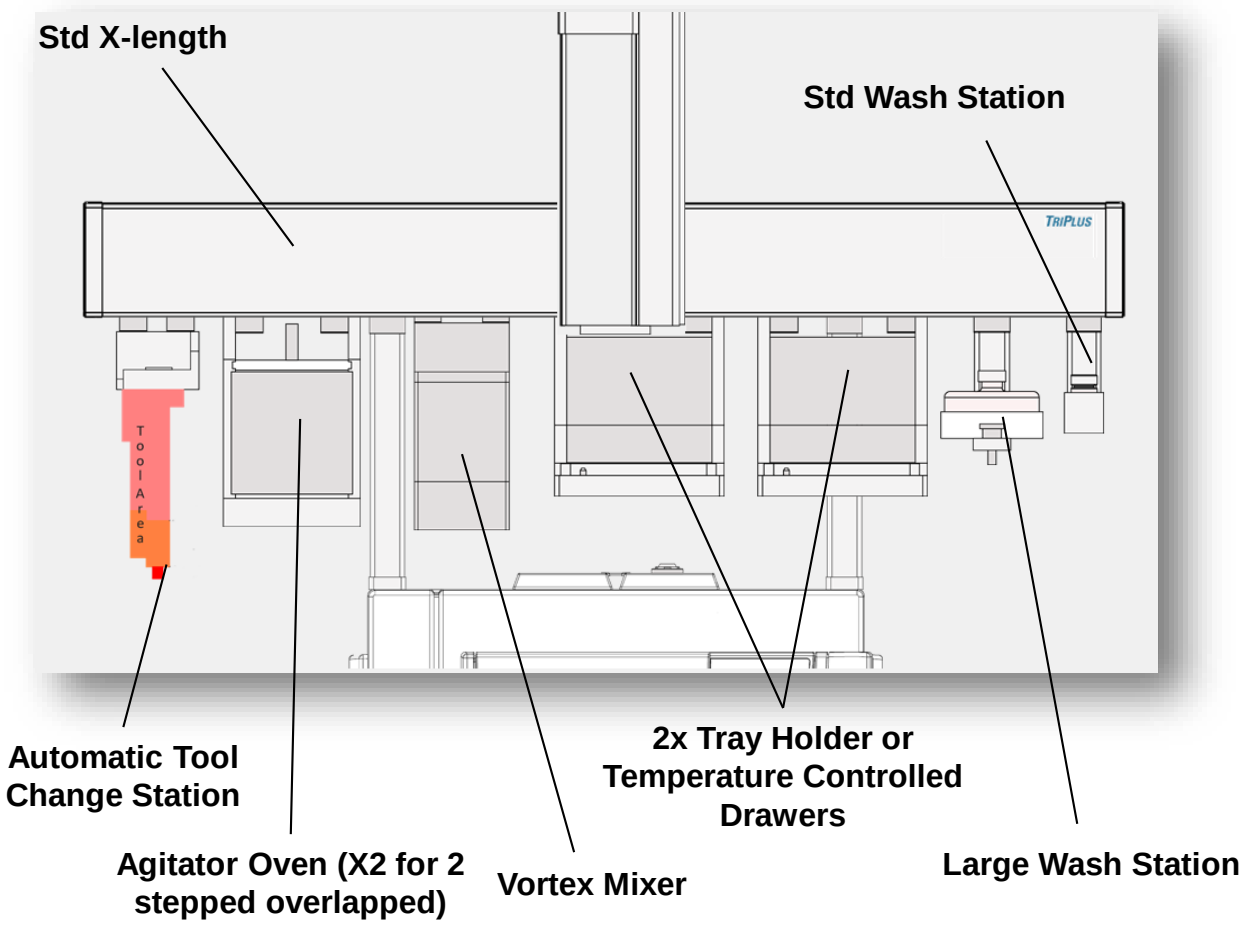
30 min

Add 50 uL **MSTFA** solution into 2 mL vial
Vortex: 30s @ 2000 rpm
Syringe post-wash with solvent 2
Incubator 2 : 30 min @ 45°C, 750 rpm

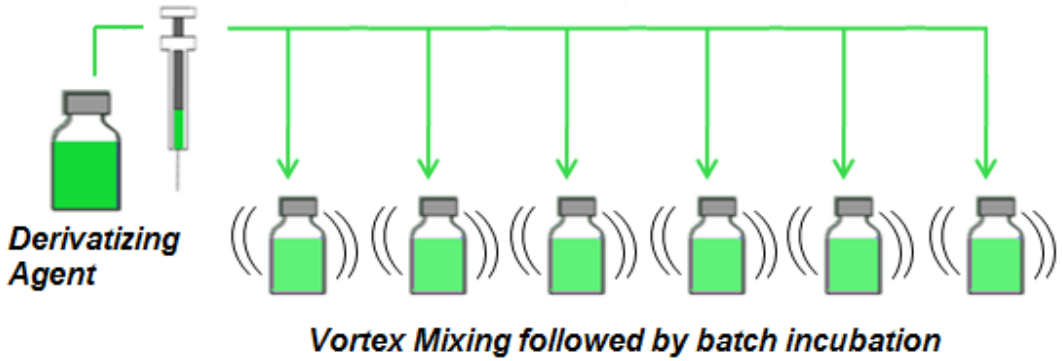
30 min

Sample analysis oven program: 70 °C hold 2 min; 10 °C/min, 325 °C hold 2 min. Total time around 30 min

Triplus RSH for Automated Derivatization Step



- Prep-Cycle available for Derivatization in batch or sequential

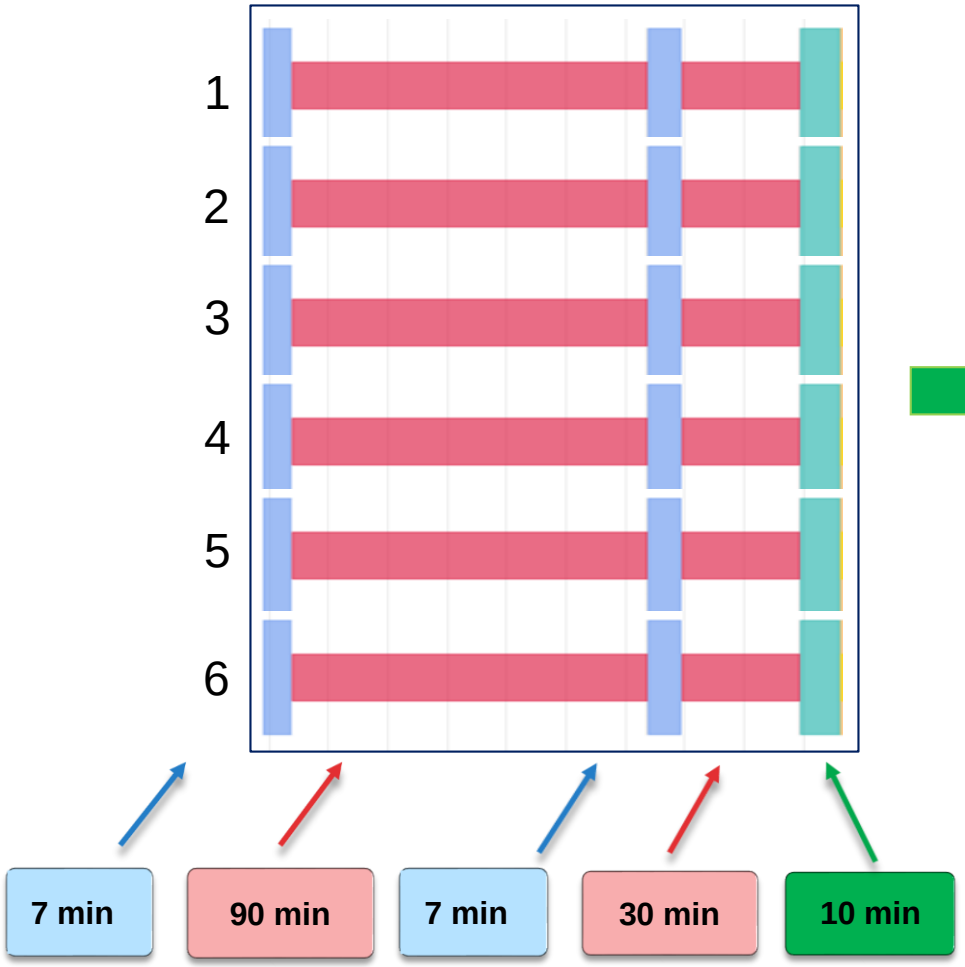


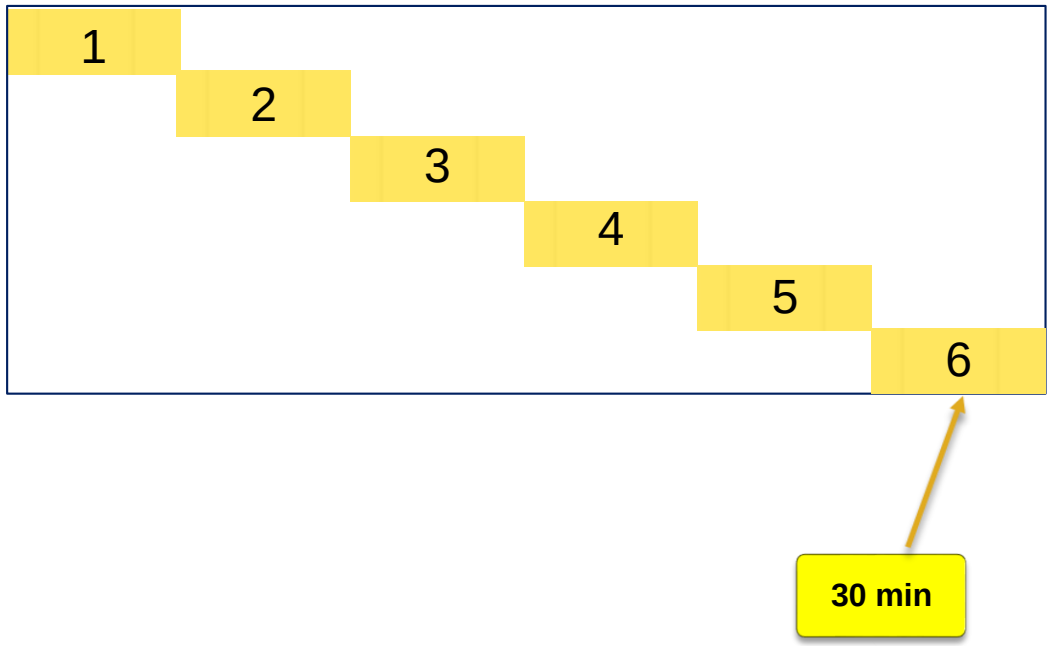
- Both strategies are compatible with methoxyamine/MSTFA derivitization protocol

Batch Online Derivatization

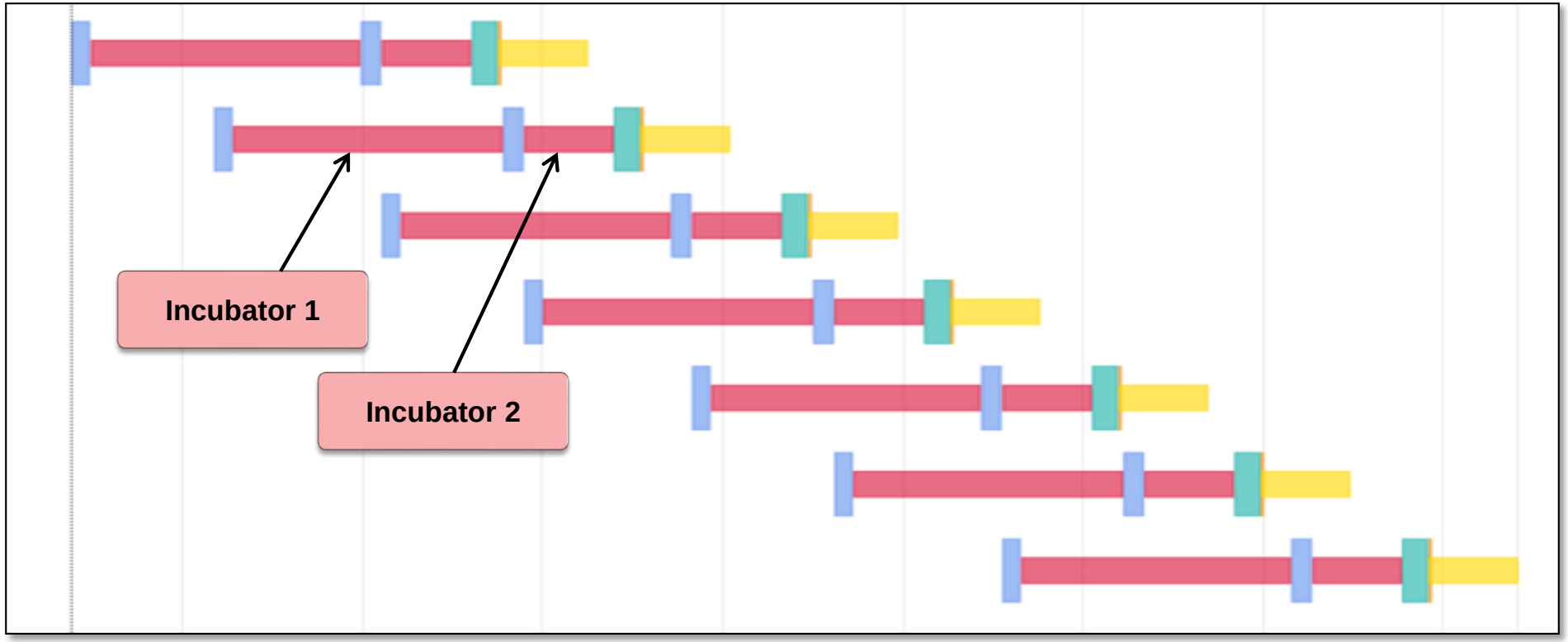
Samples prepared in a batch

Samples analyzed sequentially on GC



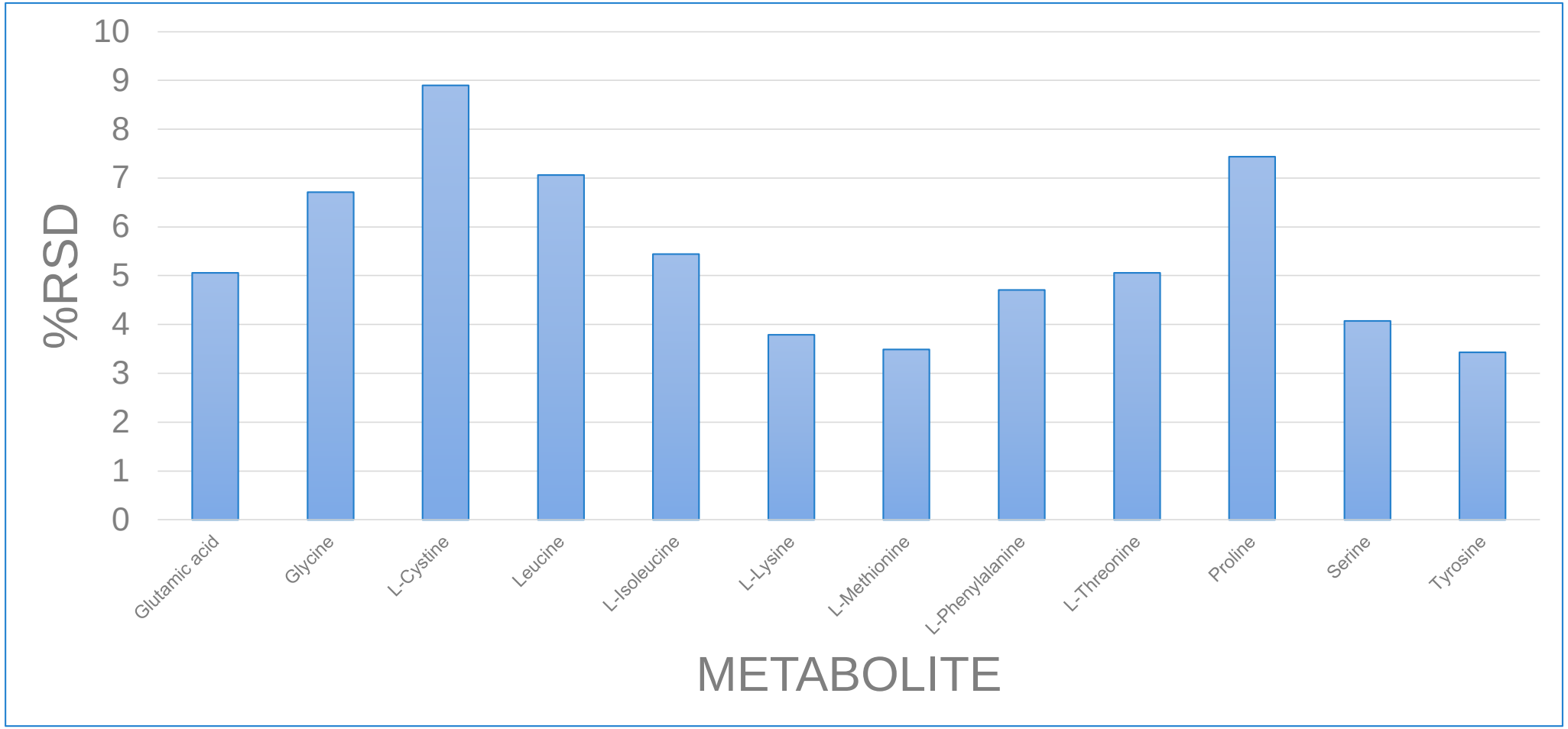


Two Incubators Sequential Derivatization



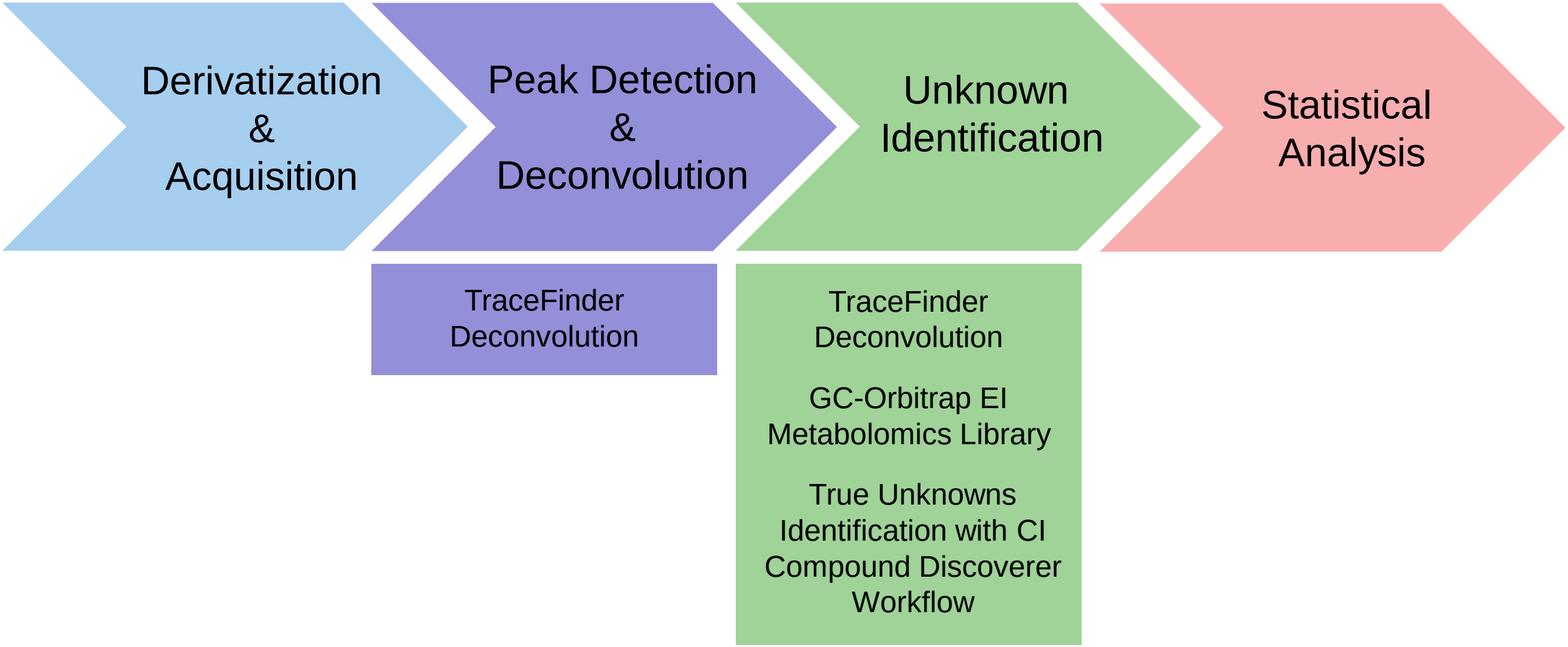
- 24 samples can be prepared and analyzed in 24h
- Two incubators can be used simultaneously
- Minimizes time between derivatization and injection
- Best for labile TMS-metabolites

Preliminary Data from 24 Hour Test with Amino Acids

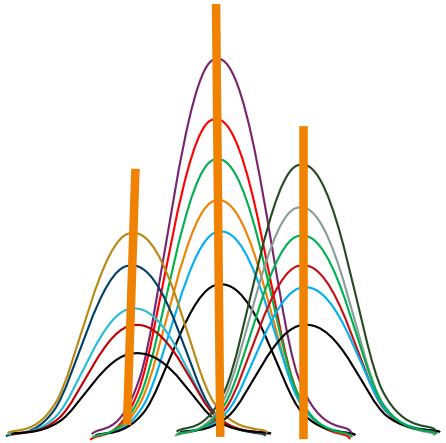


Average 5% RSD over 24 hours without internal standard

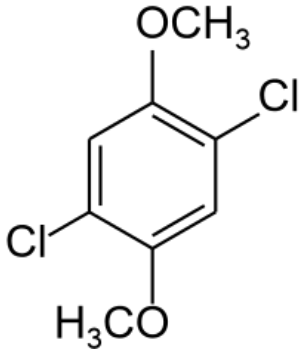
Metabolomics Workflow Tools



Algorithm for Unknowns Identification



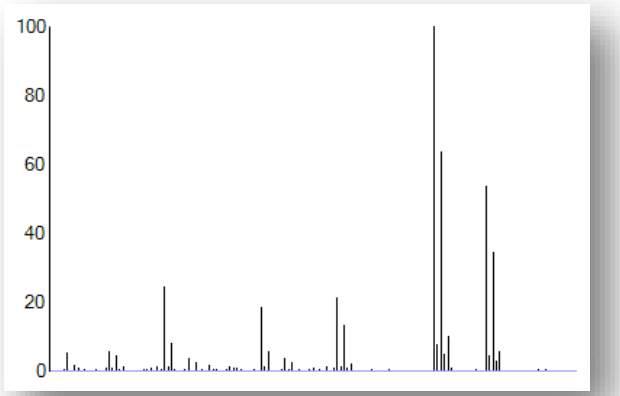
Deconvolve TIC



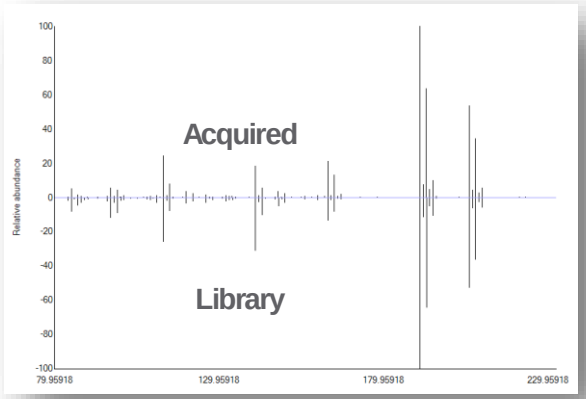
Candidate Compounds

Acq m/z	Fragment ID	Theo m/z	Mass Error (ppm)
147.9477	$C_5Cl_2H_2O$	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



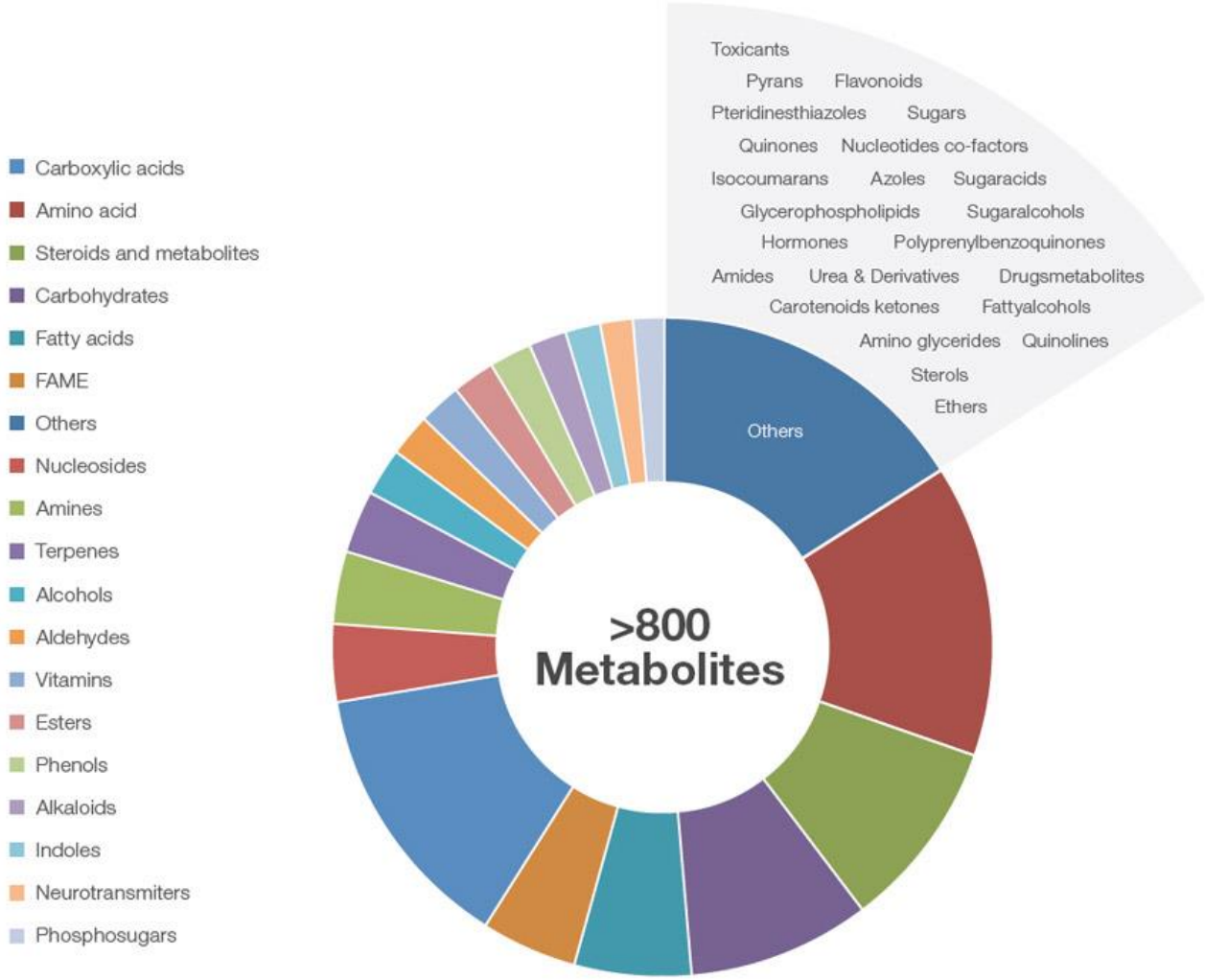
Create “clean” spectrum



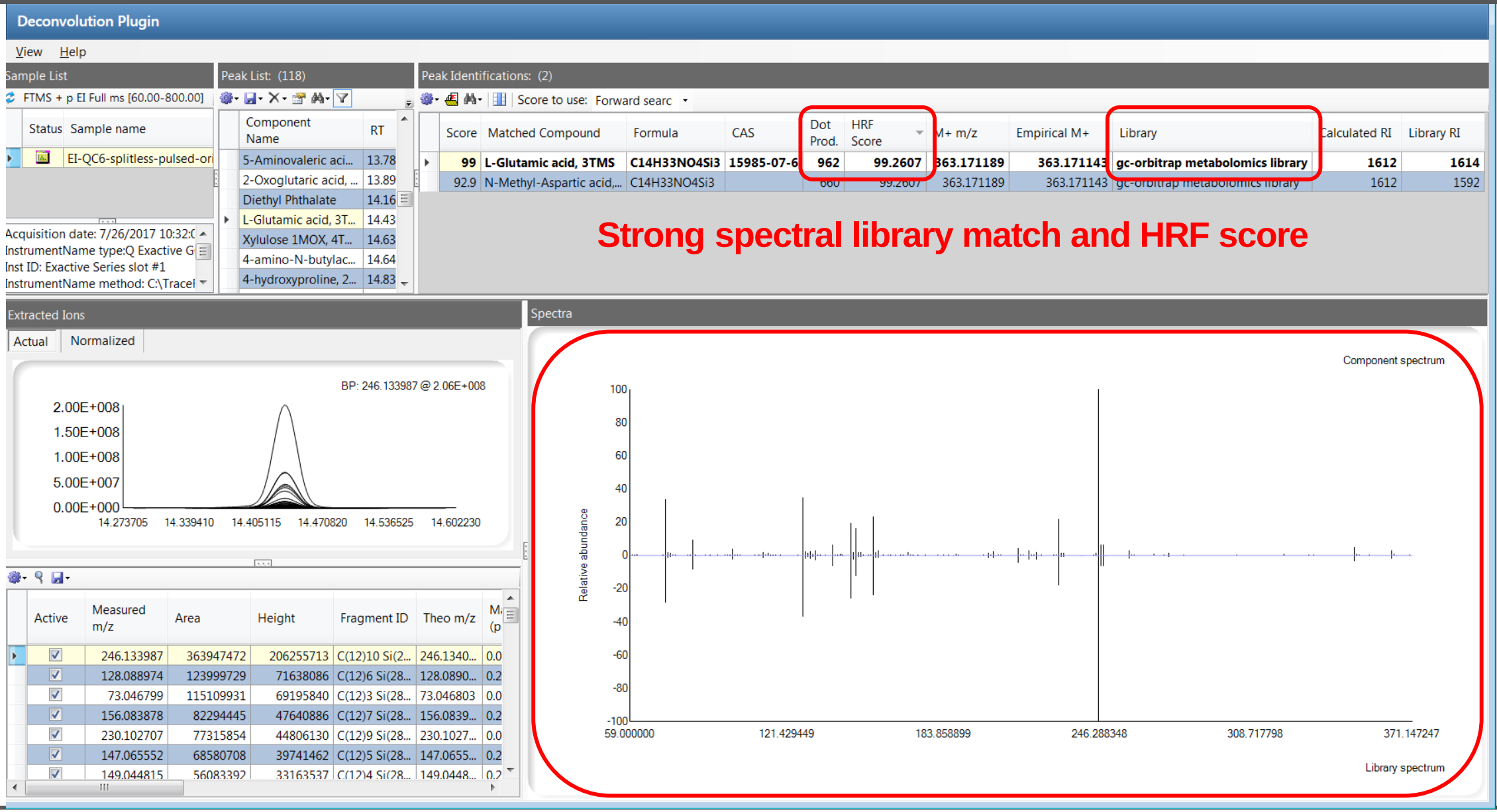
Library search

GC-Orbitrap Metabolomics Library

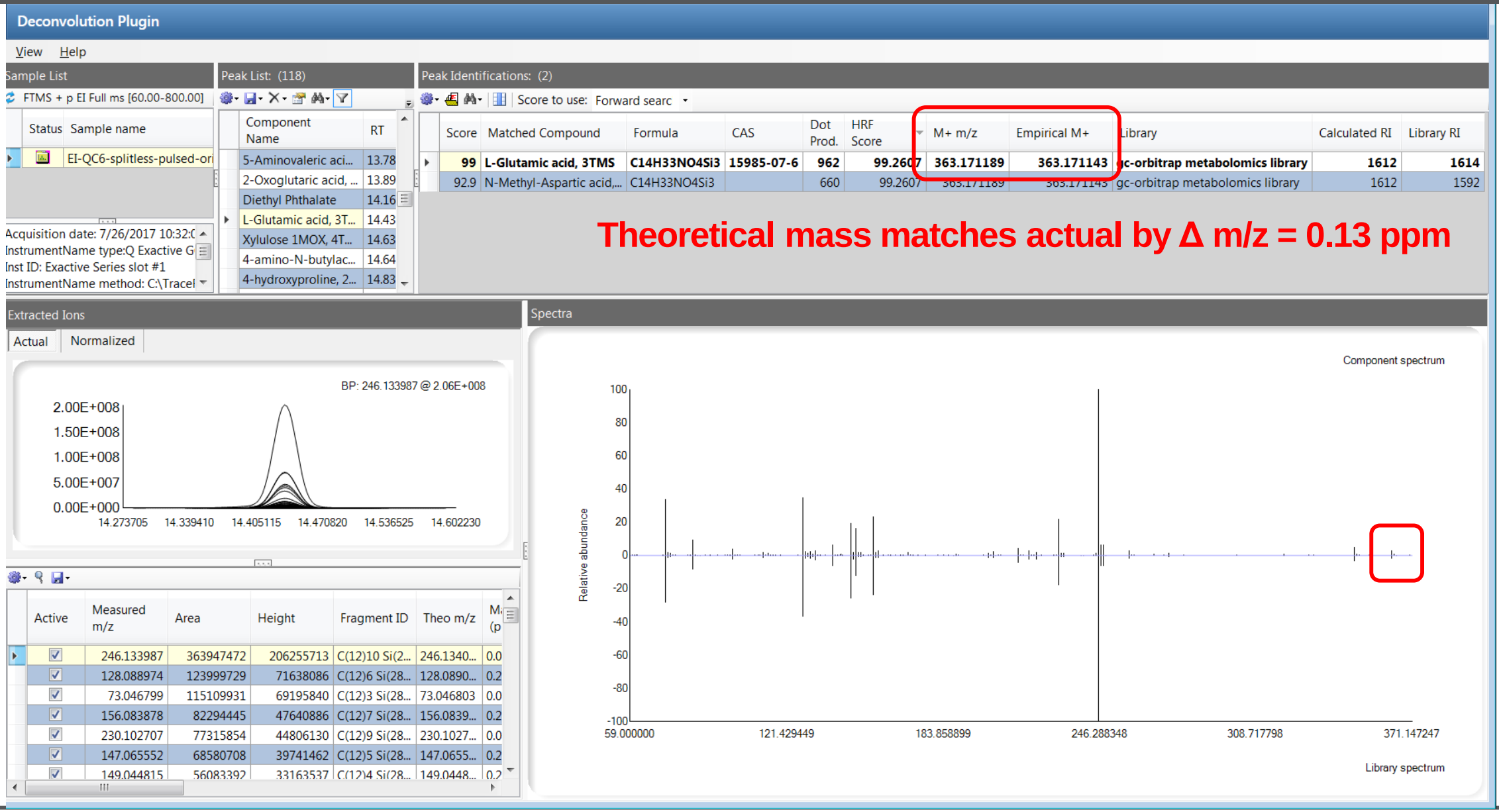
- Nearly 900 unique metabolites
- Majority with Methoxyamine/MSTFA derivatization
- High resolution, high mass accuracy spectra acquired at 60,000 RP (*m/z* 200)
- Contains Kovats retention indices
- Can be used in combination with existing unit mass libraries



Example Unknown Peak Detection and Identification



Example Unknown Peak Detection and Identification



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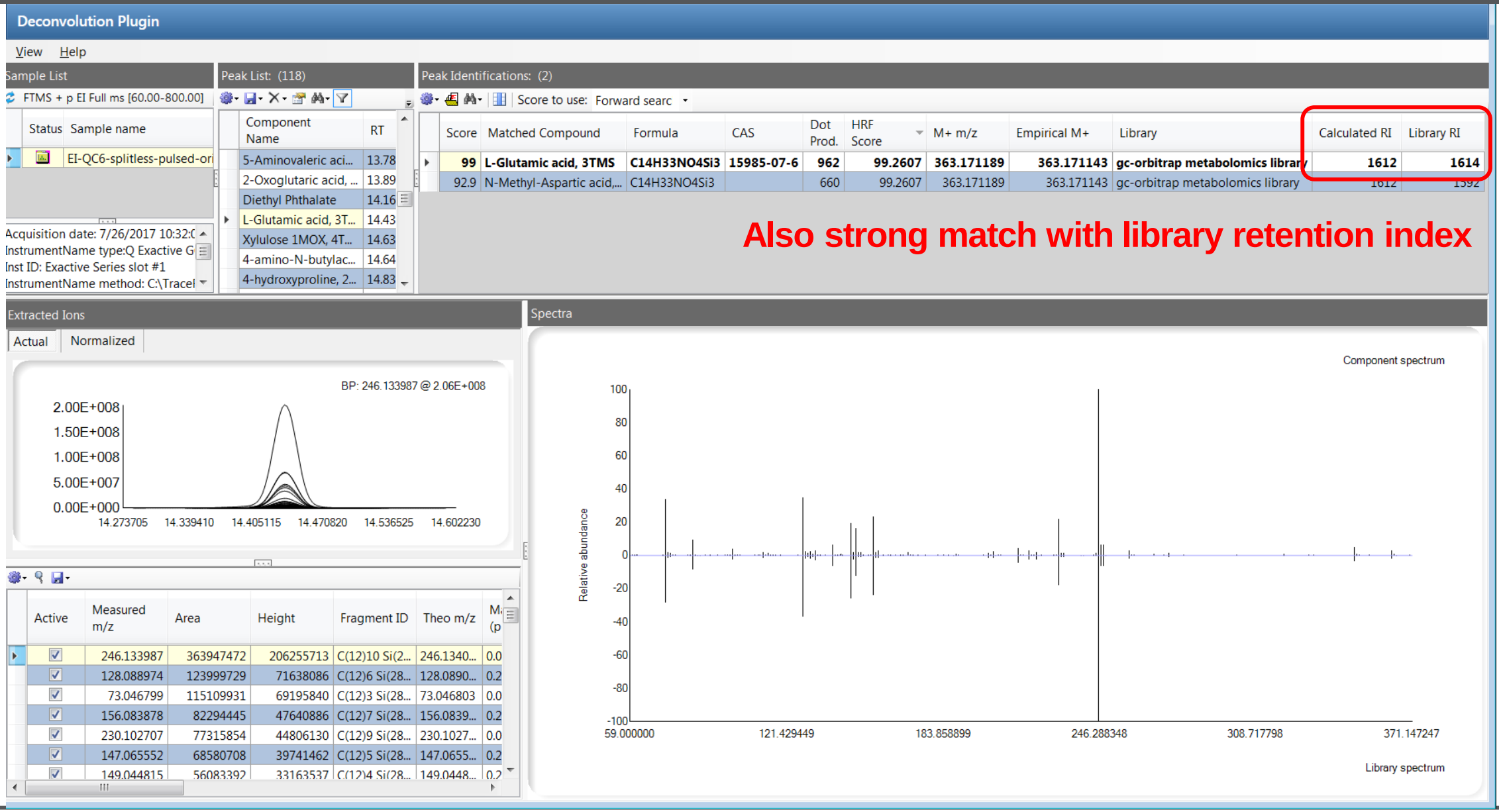
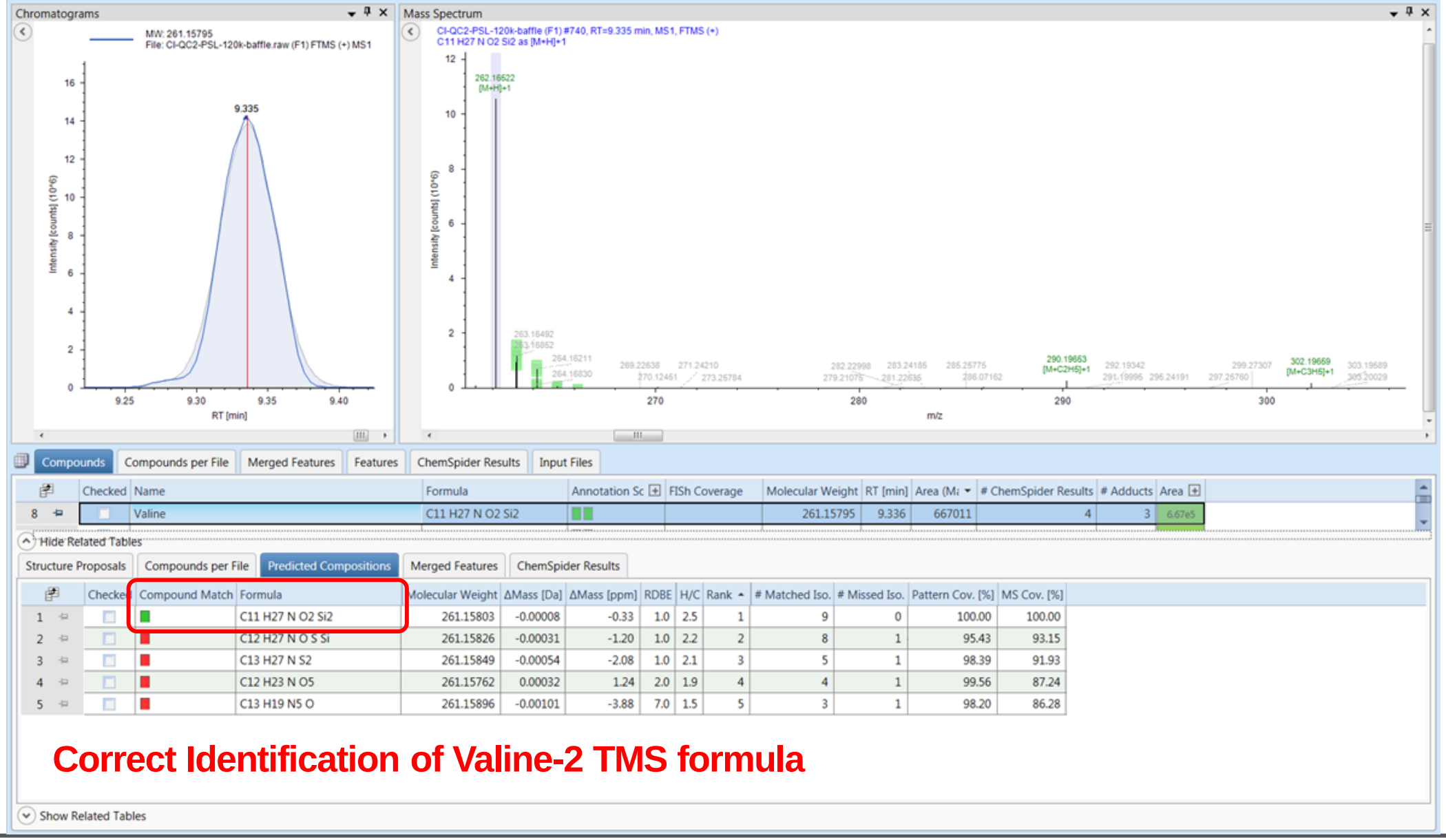
Example Unknown Peak Detection and Identification



Automated CI Identification with Thermo Scientific™ Compound Discoverer™ Software



Correct Identification of Valine-2 TMS formula

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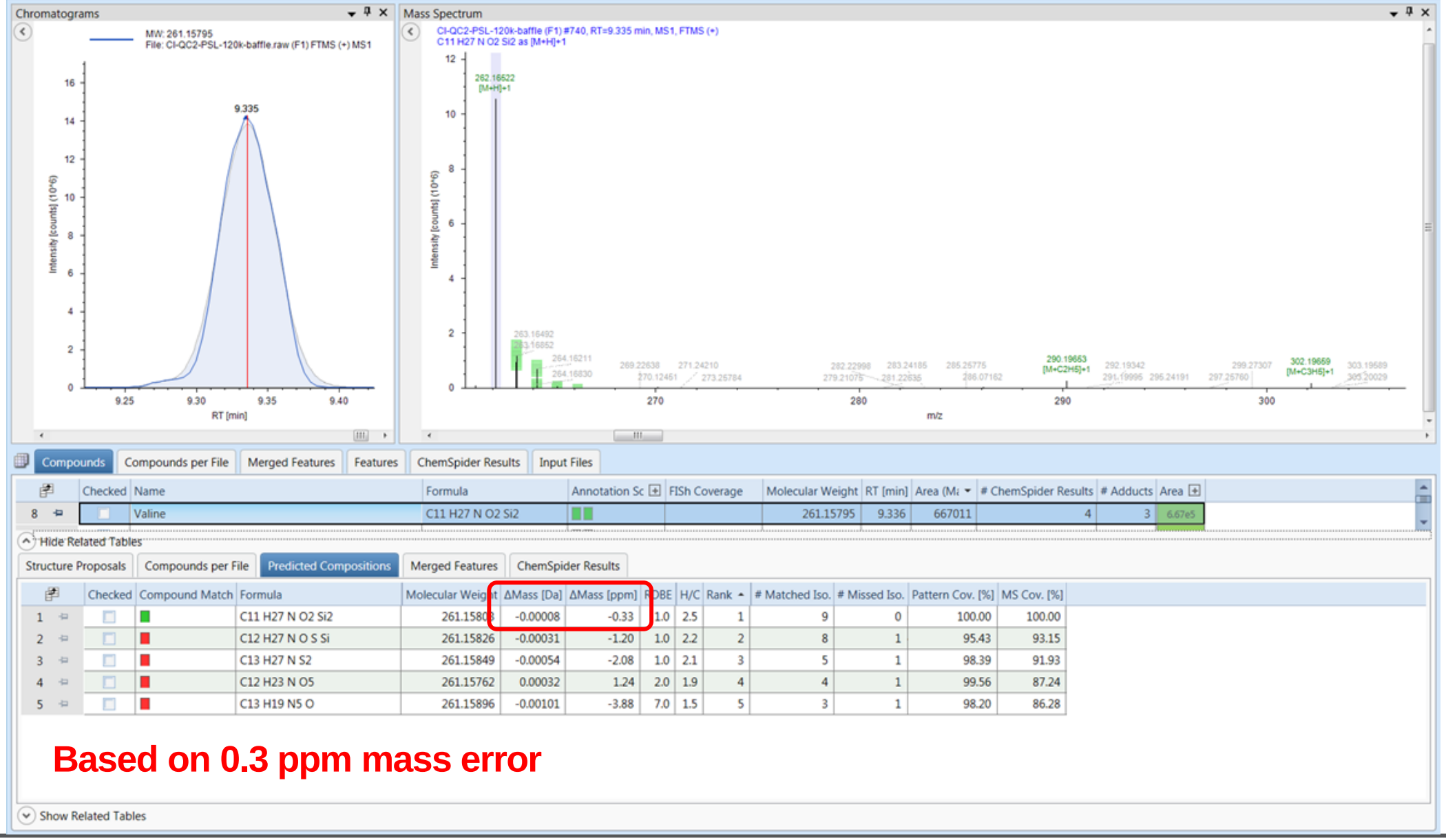
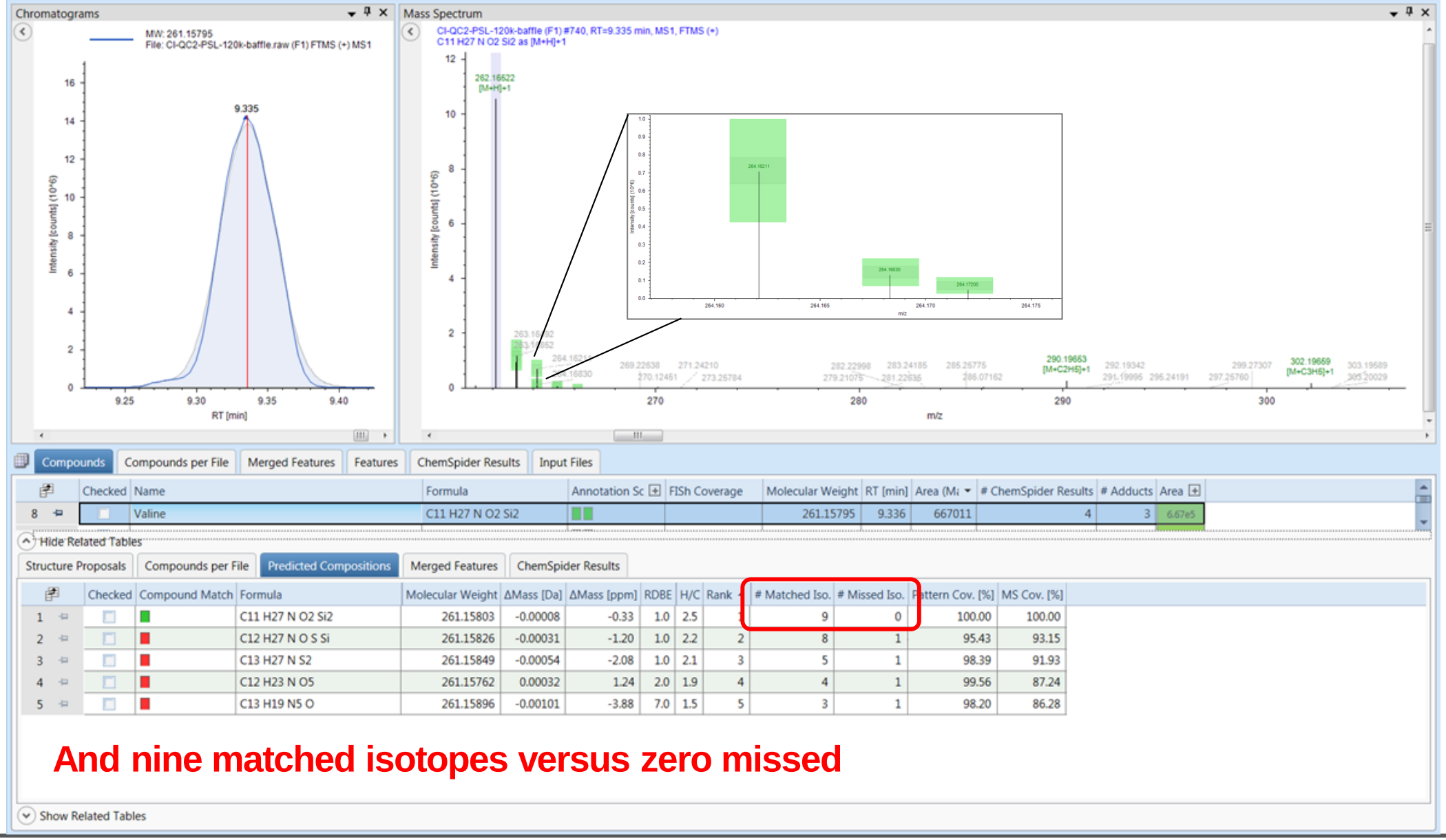
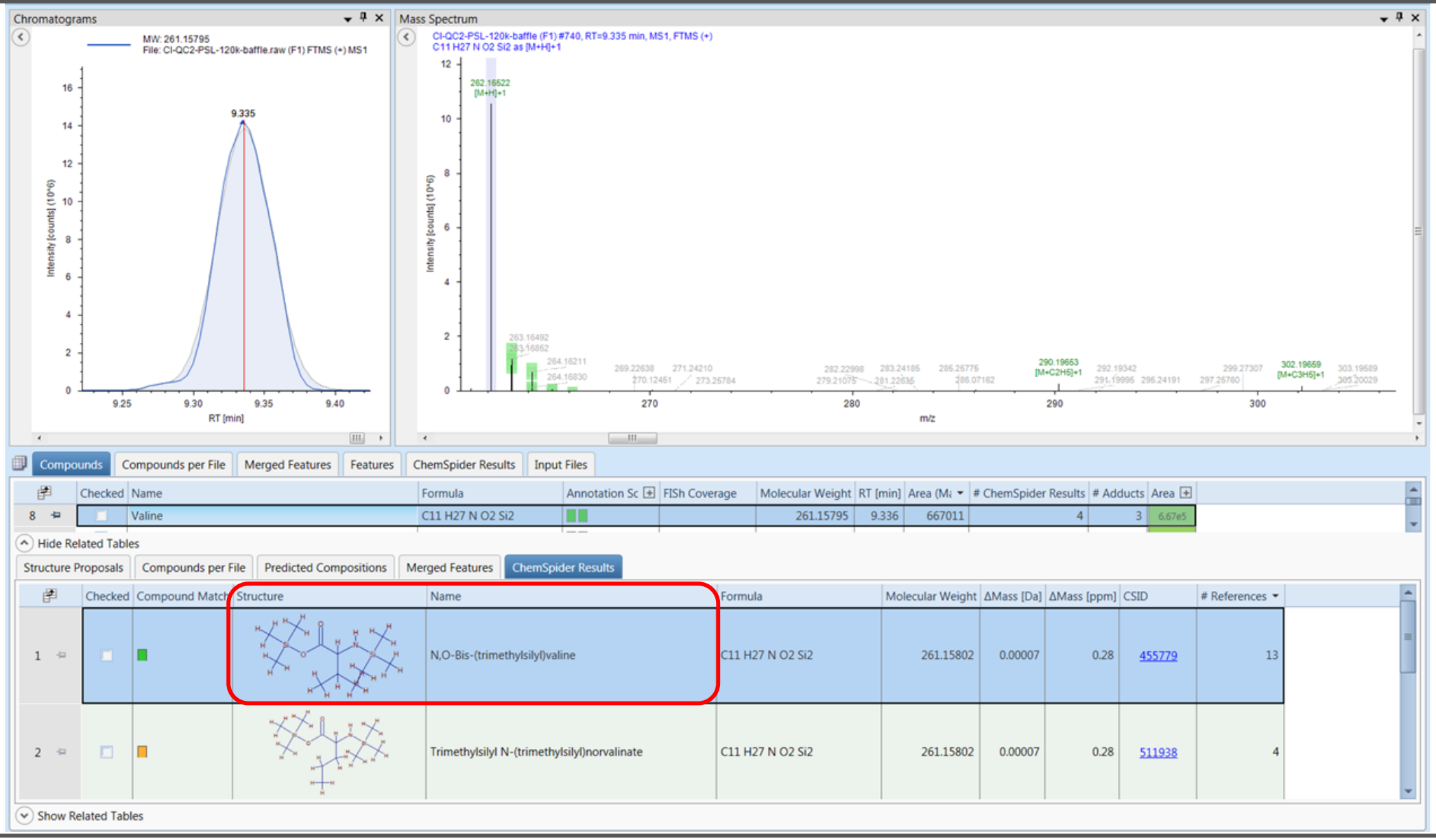
Automated CI Identification with Compound Discoverer Software



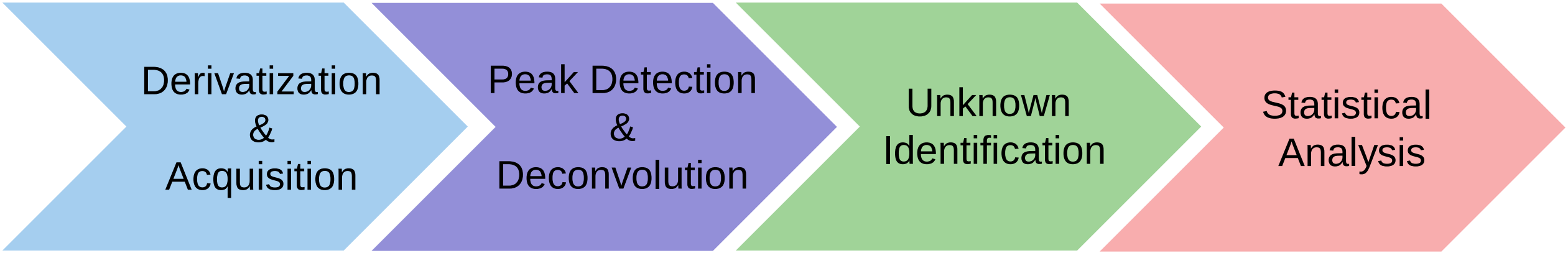
Automated CI Identification with Compound Discoverer Software



Automated ChemSpider Search Can Suggest Structure



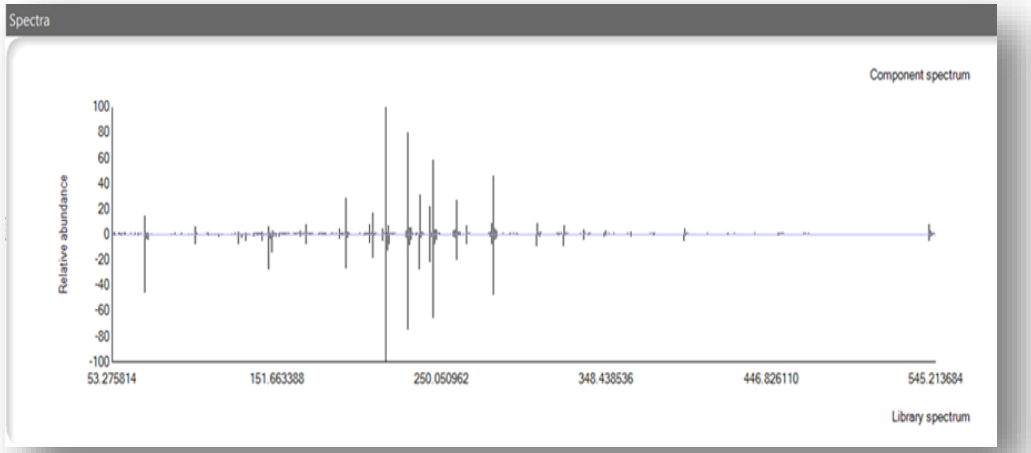
Metabolomics Workflow Tools



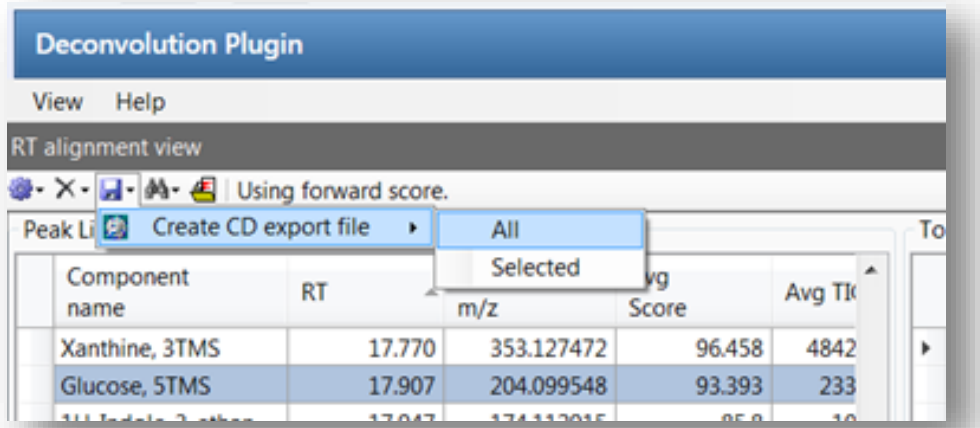
Export From Deconvolution Plugin to Compound Discoverer

Export From Deconvolution Plugin to Third Party Software

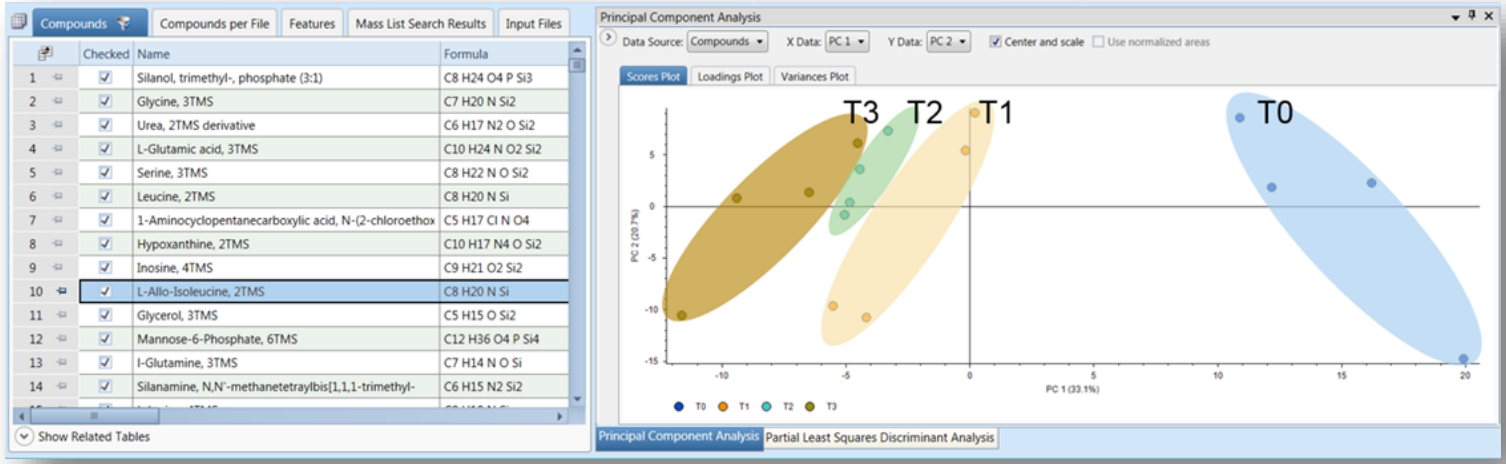
Export from Deconvolution Plugin to Compound Discoverer Software



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 measureable 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