E&L: Streamlining LC/MS and GC/MS Workflows

ASMS 2018 San Diego

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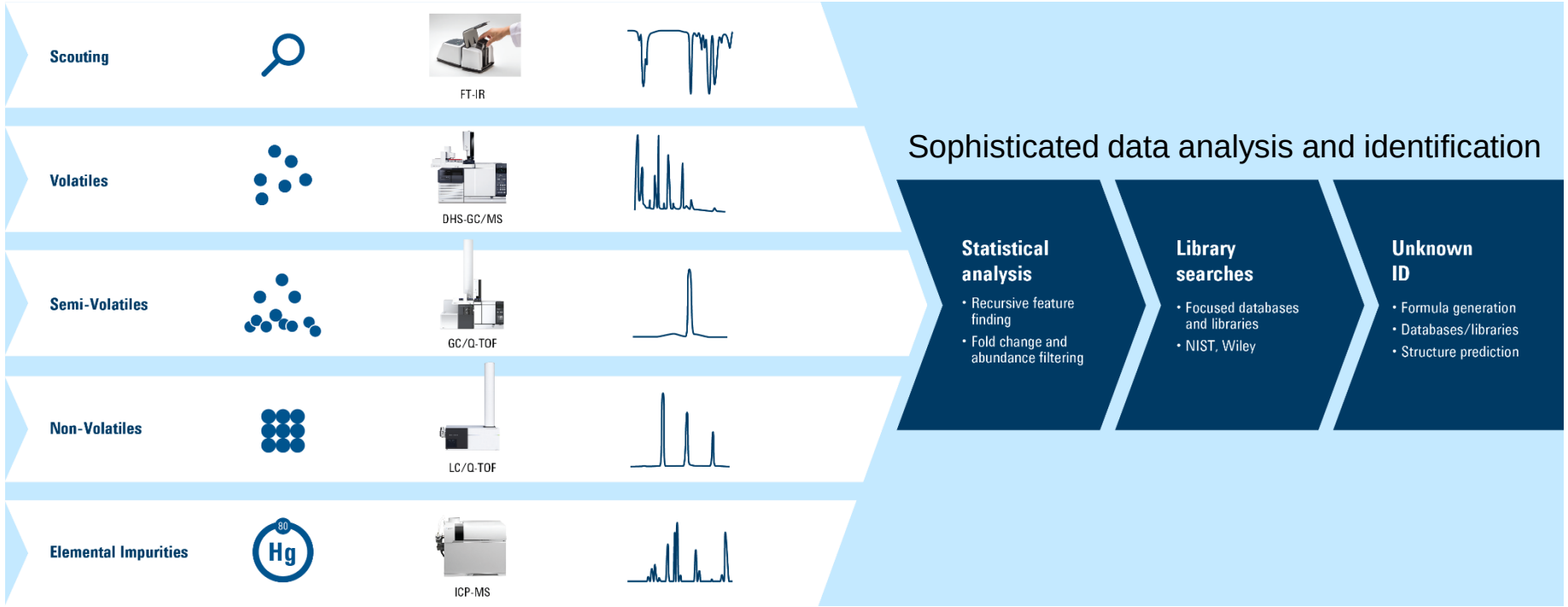
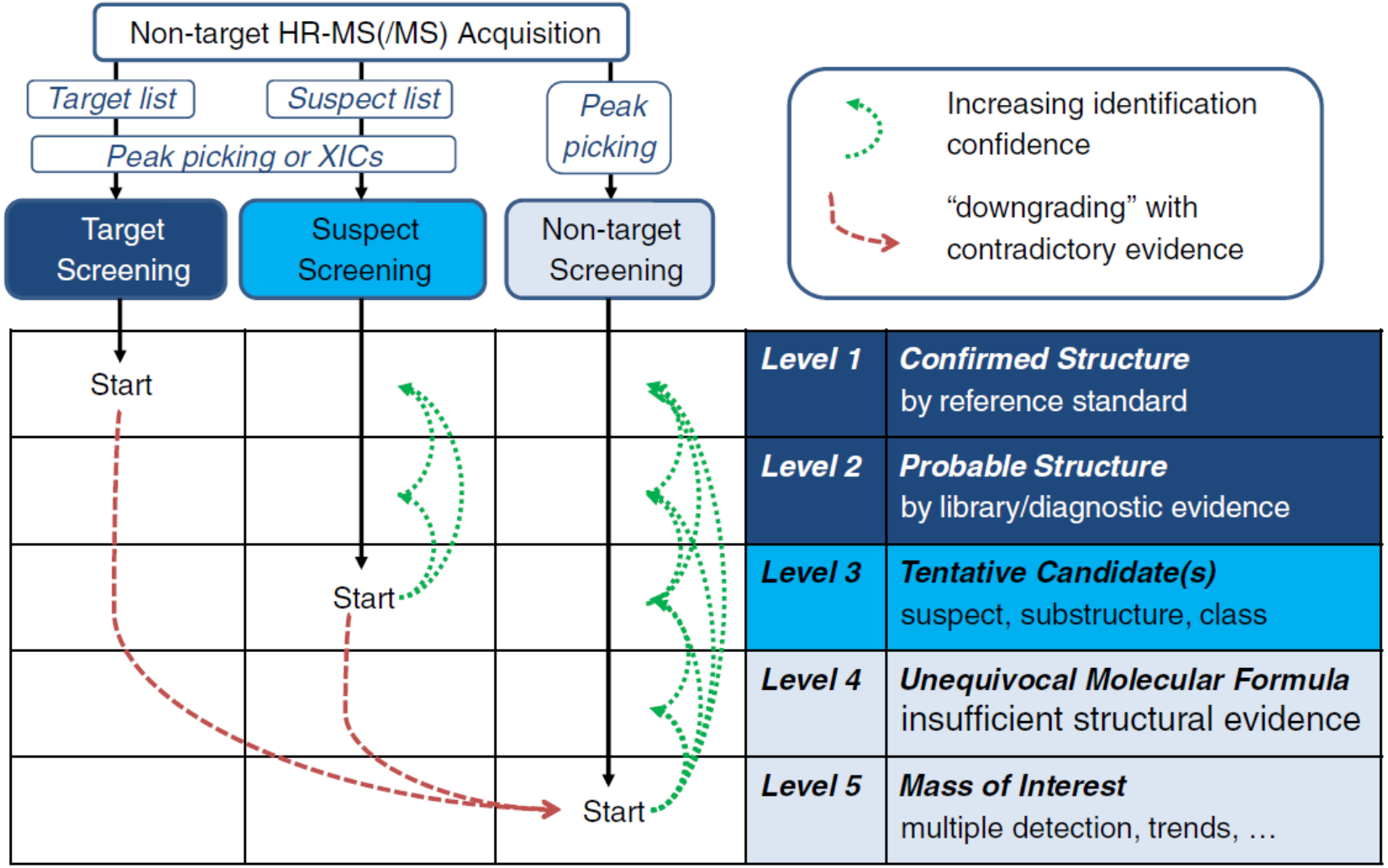
Agilent
Trusted Answers

Agilent’s Comprehensive Solutions for Extractable Profiling



Identification Strategy and Confidence

Schymanski *et* al., Anal. Bioanal. Chem. 2015

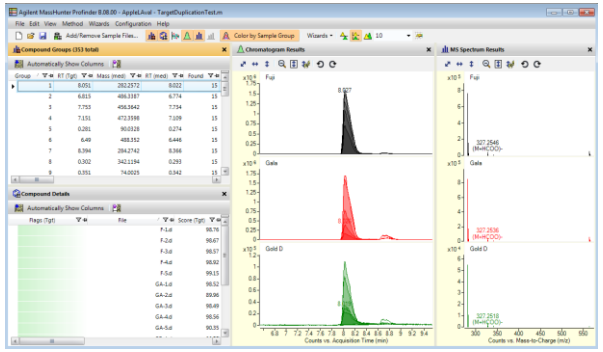


Generalized MassHunter Workflow for Extractables Profiling

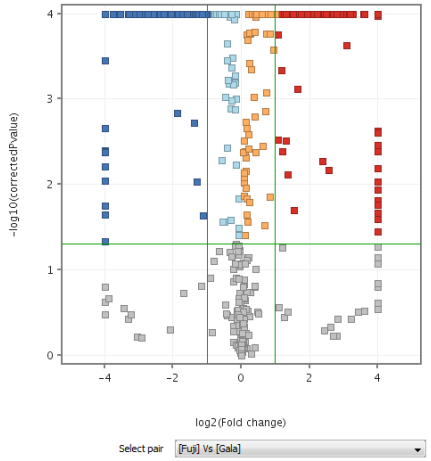
Acquire | Extract | Analyze

Native support for all Agilent LCMS, GCMS and ICPMS instruments

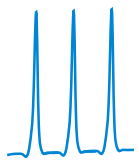
Feature Detection
Profinder



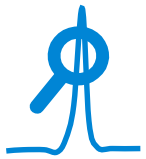
Differential Analysis
Mass Profiler Professional



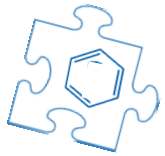
Identification
E&L PCDL
MH Qual



MassHunter Acquisition



Qualitative Analysis
Profinder
Unknowns Analysis



PCDLs, Molecular Structure Correlator

Online Resources

Your Goal: Find all Unique Extractable Peaks Across Your Sample Runs

For many labs this is a manual process:

lack of standardization..

.. process single datafiles, walk across TIC, background subtraction, export m/z values to excel, use custom macros..

Challenges you face:

- Complex chromatograms
- Incomplete peak separation
- Matrix effects
- High background ions
- Other?

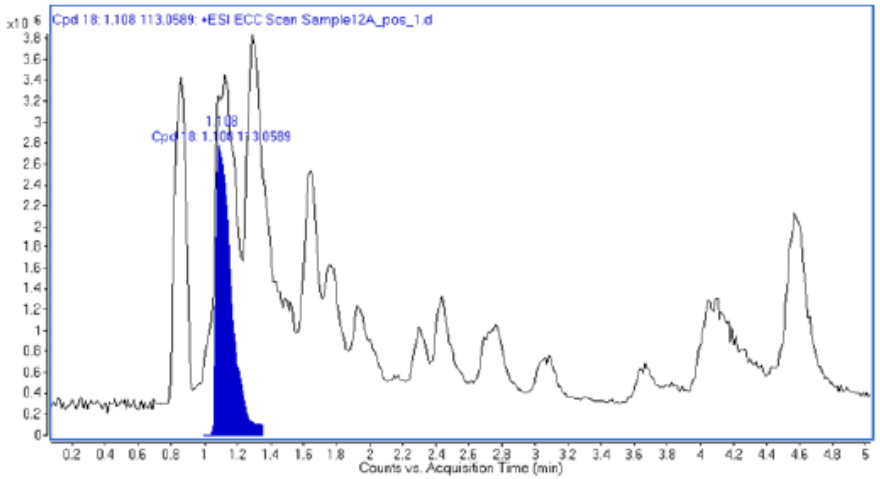
Challenges may contribute to an increase in:

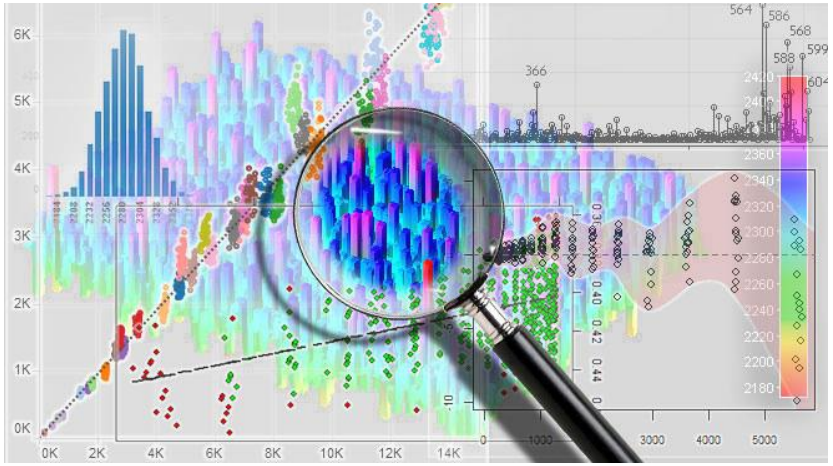
– False positives, False negatives

– Incorrect identifications

– Misdirected and wasted efforts

– Decreased productivity





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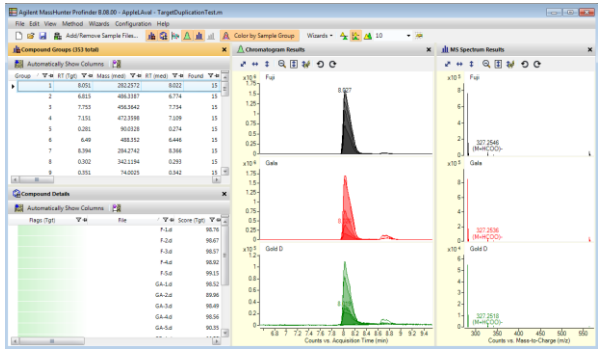

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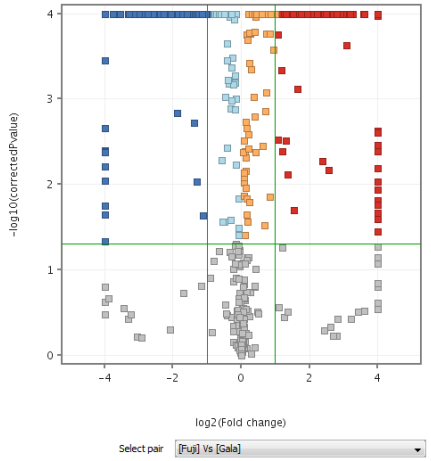
Mass Profiler Professional



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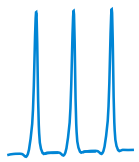
E&L PCDL



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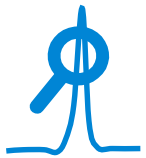

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Profinder
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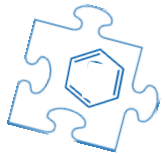


PCDLs, Molecular Structure Correlator

Online Resources

Molecular Feature Extraction

Molecular Feature Extraction (MFE) performs chromatographic deconvolution of RT x *m/z* x abundance and finds molecular features in selected datafiles/runs

–Find co-eluting ions that are related

- •Include isotopes (^{13}C, ^{15}N, ^{2}H, ^{18}O)
- •Include adducts, such as Na^+, K^+, $NH4^+$
- •Include dimers, such as $(2M+H)^+$
- •Create a compound chromatogram (ECC)

–Sum all ion signals into one value (a **Mass Feature**)

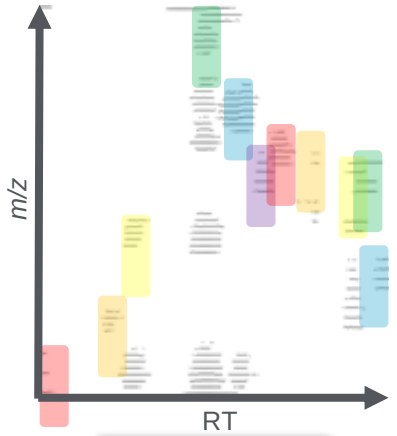
–Create a compound spectra for the group of ions

–Report results as retention time and neutral mass

–Fully automated processing

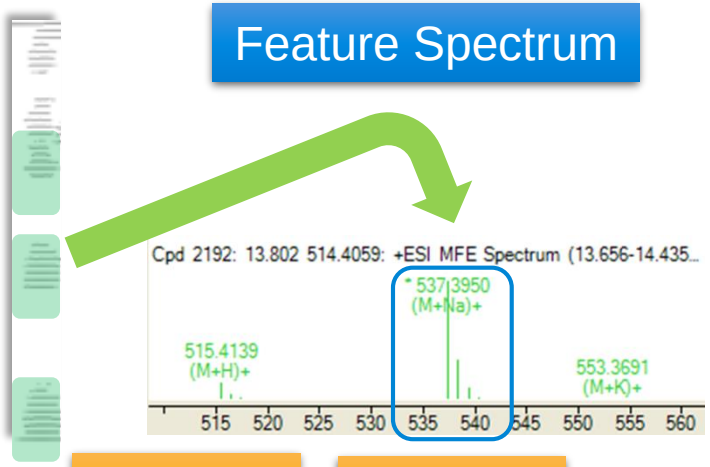
- Filters noise

Performed in MH Qual or Profinder or Mass Profiler



coelution grouping

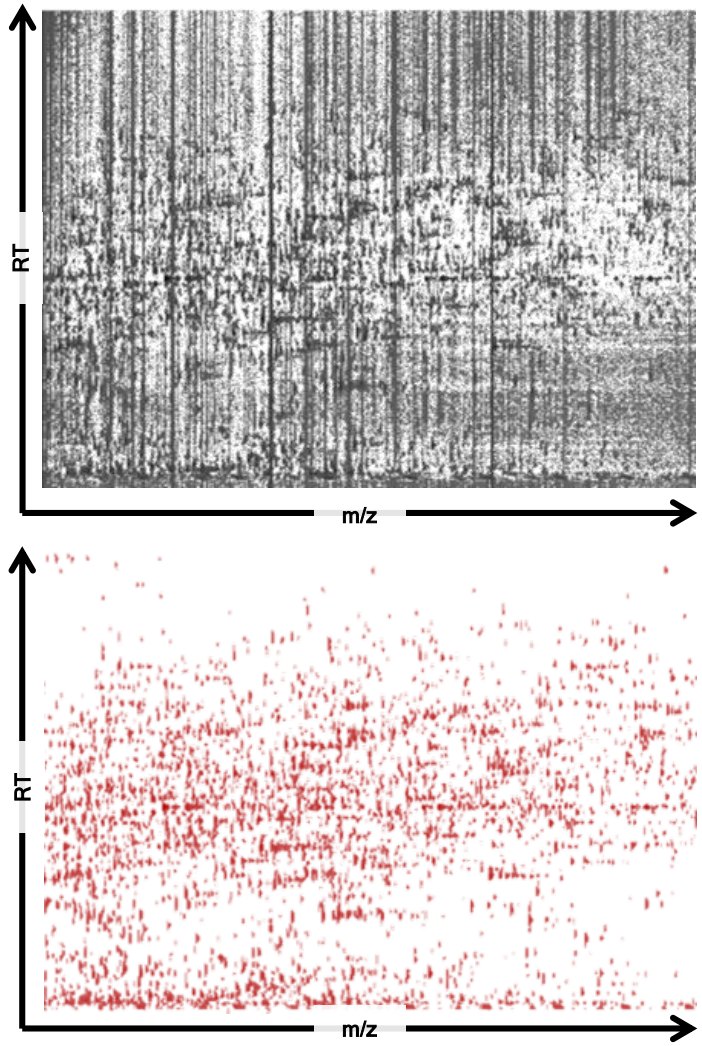
Feature Spectrum



adduct grouping

isotope grouping

Extracting Peaks Across Data File, But Not the Noise



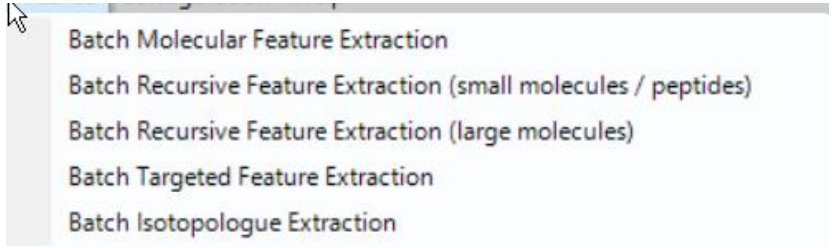
- Effectively removes persistent background ions
- The process is iteratively applied to the entire file, in descending abundance order, until no peak remains above the background.
  => Background is effectively removed
- MH Profinder

MH Profinder: Data Mining SW

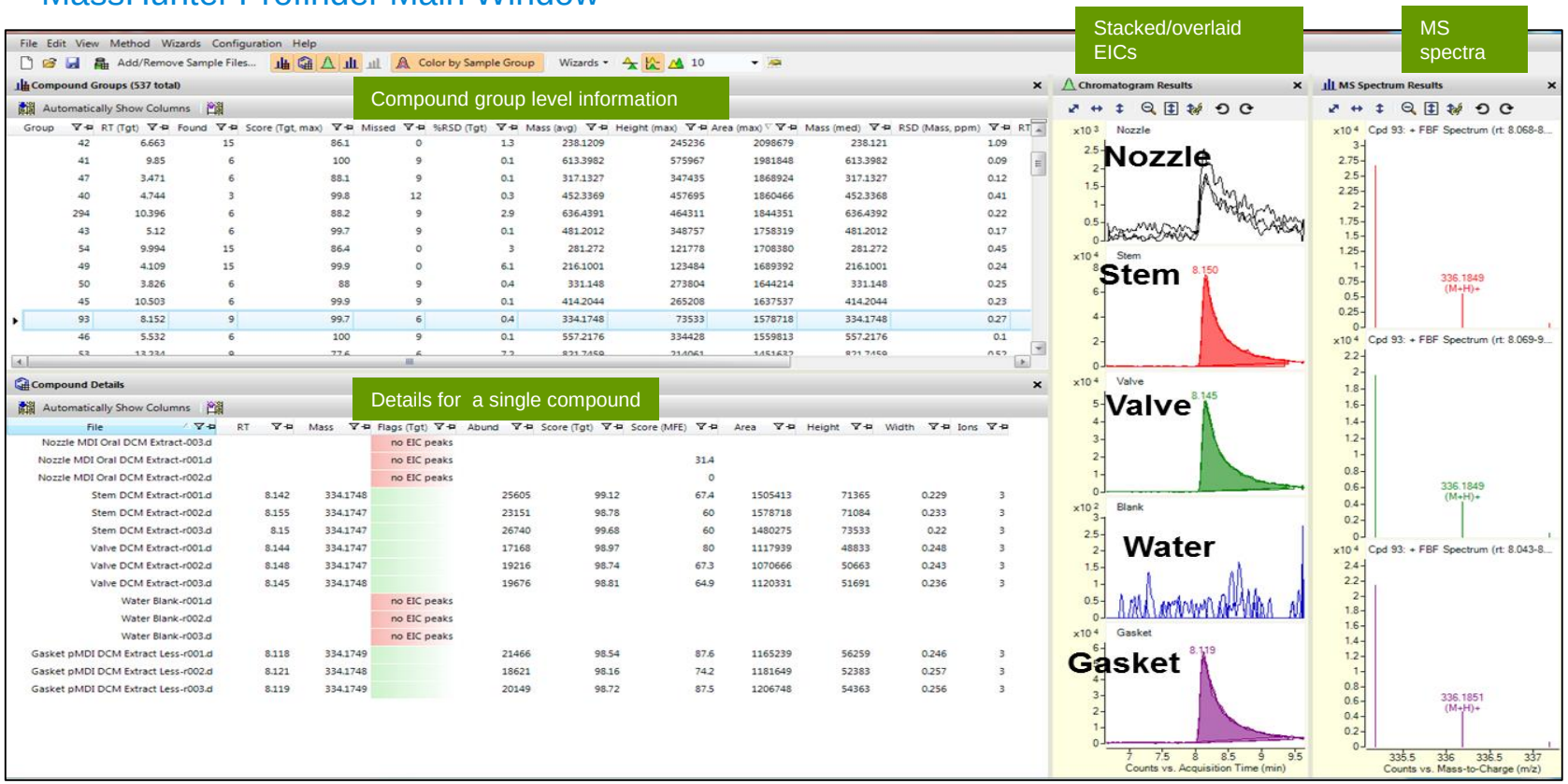
For High-throughput Compound Finding

MassHunter Profinder is a productivity tool for processing multiple samples

Profinder Feature Extraction



MassHunter Profinder Main Window



The feature files generated by Profinder (CEF files, .PFA) can be imported into Mass Profiler Professional (MPP)

Generalized MassHunter Workflow for Extractables Profiling

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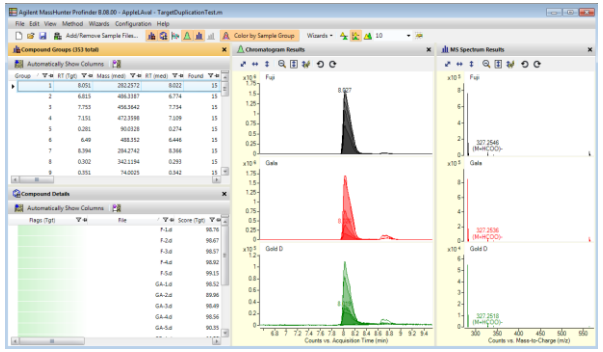
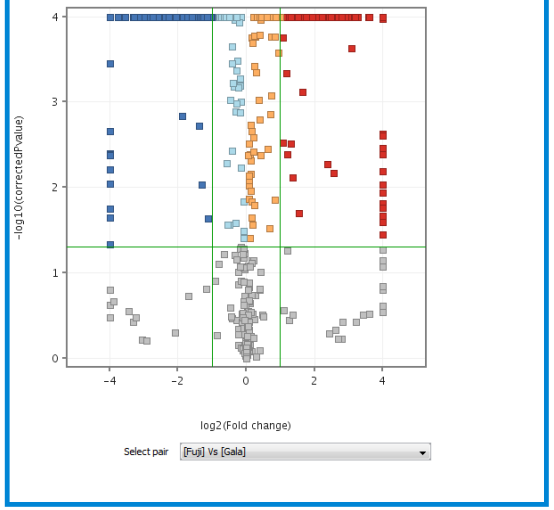
Extract

Feature Detection
Profinder



Analyze

Differential Analysis
Mass Profiler Professional



Identification
E&L PCDL

MassHunter Acquisition

Qualitative Analysis
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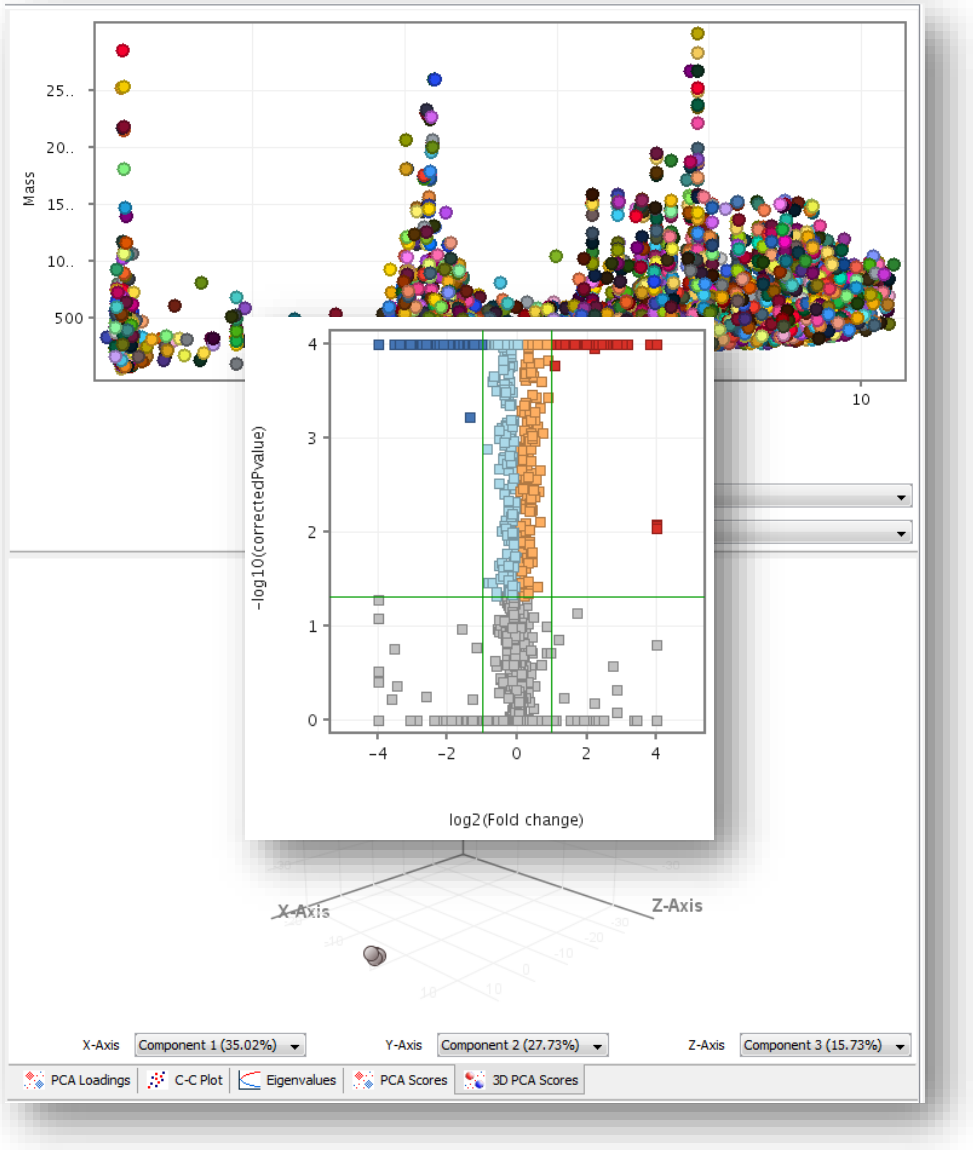
PCDLs, Molecular Structure Correlator

Online Resources

Agilent Profiling Software: Mass Profiler Professional

- Designed for Mass Spectrometry data
- Big users in Metabolomics area but many other applications: Food, E&L..
- Import, store, and visualize
  - Agilent LC/MS TOF, Q-TOF, and QQQ
  - Agilent GC/MS SQ, and Q-TOF
  - Agilent ICP-MS
  - Generic file import (CSV, NMR data)
- Performs many types of simple to complex statistical analysis
  - ANOVA, clustering, PCA, class prediction tools
- ID Browser for compound identification
  - PCDLs
- Pathway Architect for biological contextualization



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Acquire → **Extract** → **Analyze**

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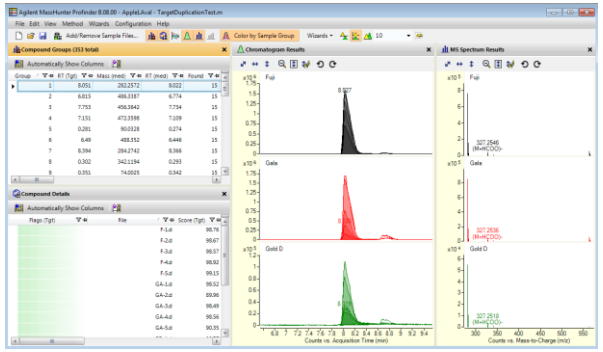
Profinder



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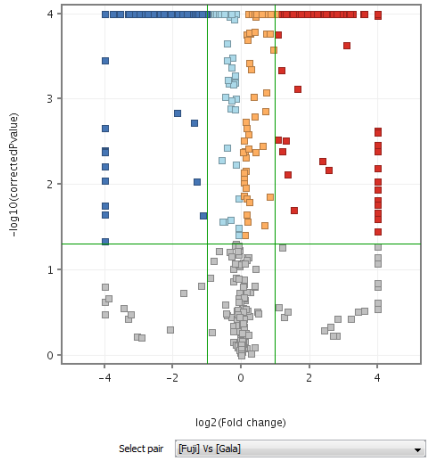

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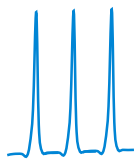

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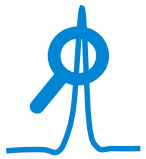

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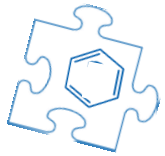


PCDLs, Molecular Structure Correlator

Online Resources

Agilent PCDL Portfolio

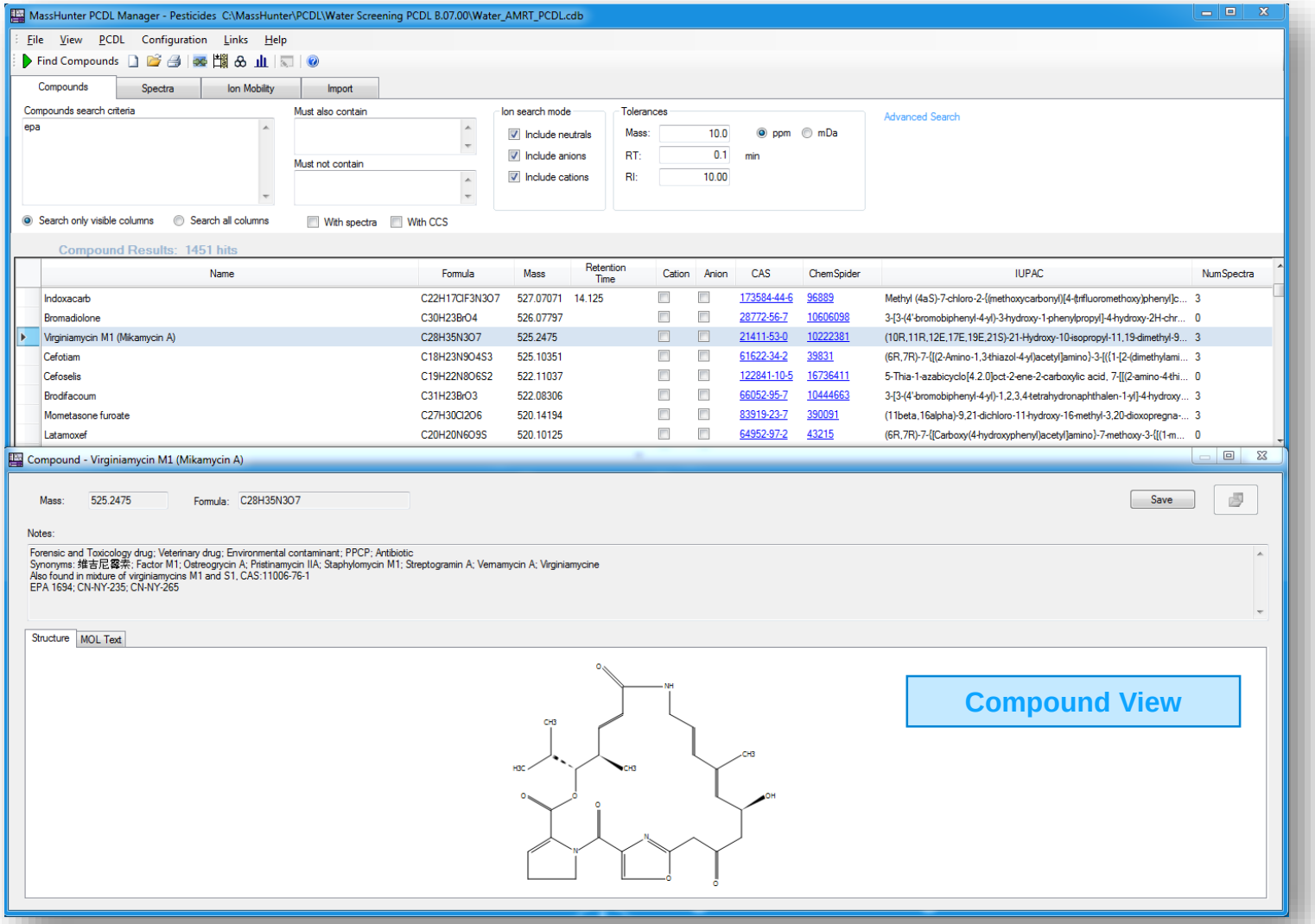
Accurate Mass PCDL for LC/TOF and QTOF	Market Segment	Total Compounds	Compounds with Accurate Mass MS/MS spectra	Total number of spectra	Compounds with RTs
Water contaminants	Environmental	>1,400	>1,000	~3,900	>260
Pesticides	Food Safety, Environmental	1,750	>825	>2,700	0
Veterinary drugs	Food Safety	>2,100	>1,150	>5,200	>120
Mycotoxins and Related Metabolites	Food Safety	>450	>300	>1,350	0
Broecker, Herre, and Pragst Forensic Toxicology*	Forensic Toxicology	>9,200	>3,900	>13,500	0
METLIN Metabolomics**	Metabolomics	>249,450	>11,000	>37,260	>680
Extractables and Leachables	Pharma	>1,000	>360	>1,300	129
NIST LC/MS/MS	General	>13,800	>13,800	>574,825	0
Accurate Mass PCDL for GC/Q-TOF	**Market Segment**	**Total Compounds**	**Compounds with Accurate Mass MS/MS spectra**	**Total number of spectra**	**Compounds with RTs**
Pesticides	Food Safety, Environmental	>1000	>1000	>1000	>1000 (15m x 15m, 20 and 40-minute methods) >1000 (5m x 15m, 20-minute method

* For Forensic Use
** For Research Use Only. Not for use in diagnostic procedures.

PCDL Manager

Water Screening PCDL

Chemical List

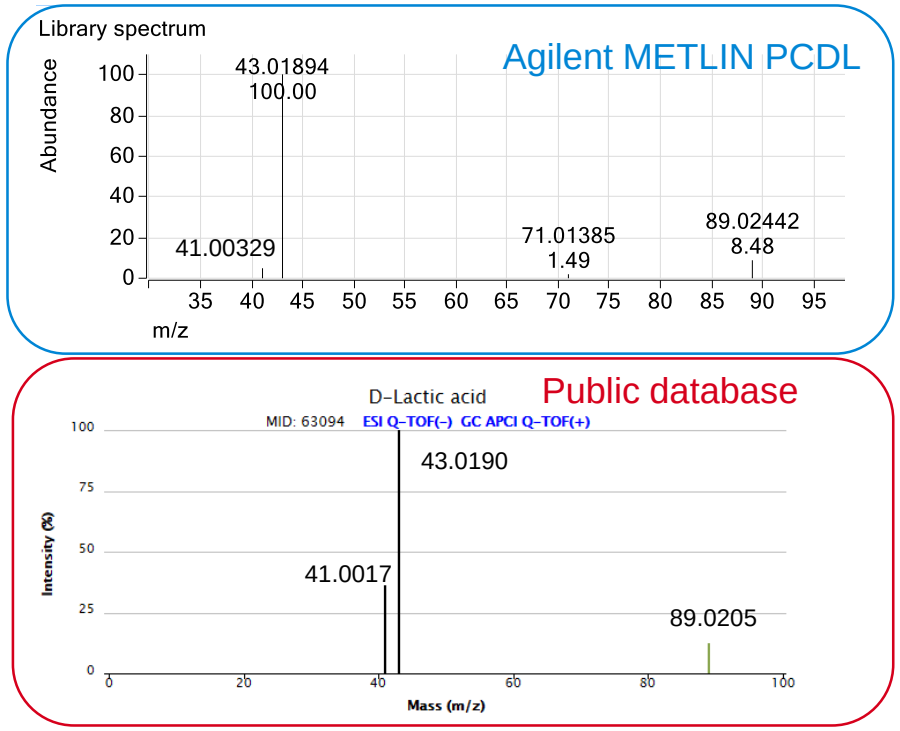


Compound View

Benefits of the Curation Process

Correction to theoretical accurate mass eliminates mass error

METLIN Metabolomics PCDL



D-lactic acid

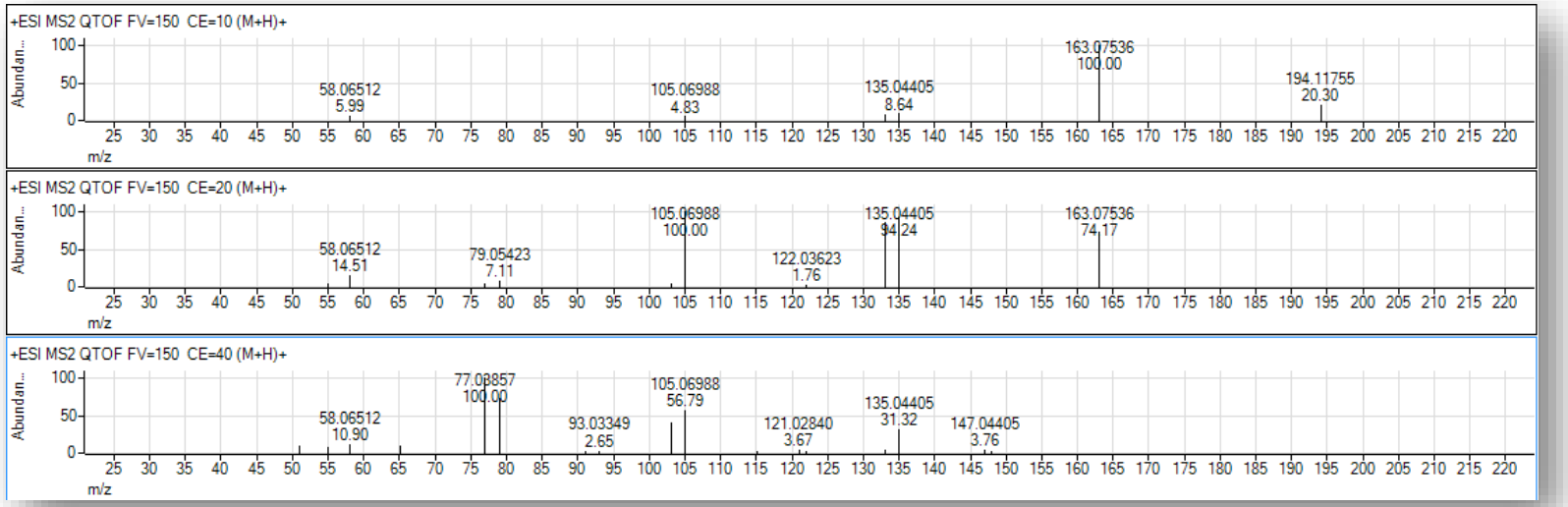
METLIN ID: 63094
Mass 90.03169
Formula: $C_3H_6O_3$
HMDB01311

Agilent METLIN*	Uncurated MS/MS Spectrum	Mass Error (ppm)
41.00329	41.0017	**38.74**
43.01894	43.0190	-1.43
71.01385	Missing	
89.02442	89.0205	**44.01**

*Agilent METLIN has 0 mass error

MS/MS Spectra Collection

Carefully designed experiments and curation protocols



Data Collection

- Flow Injection Analysis (FIA) of **pure standards or purified isolates**
- Inclusion of commonly analyzed adduct species
- Collected at multiple collisions energies, polarities, and ion species

Curation

Correction to Theoretical accurate mass

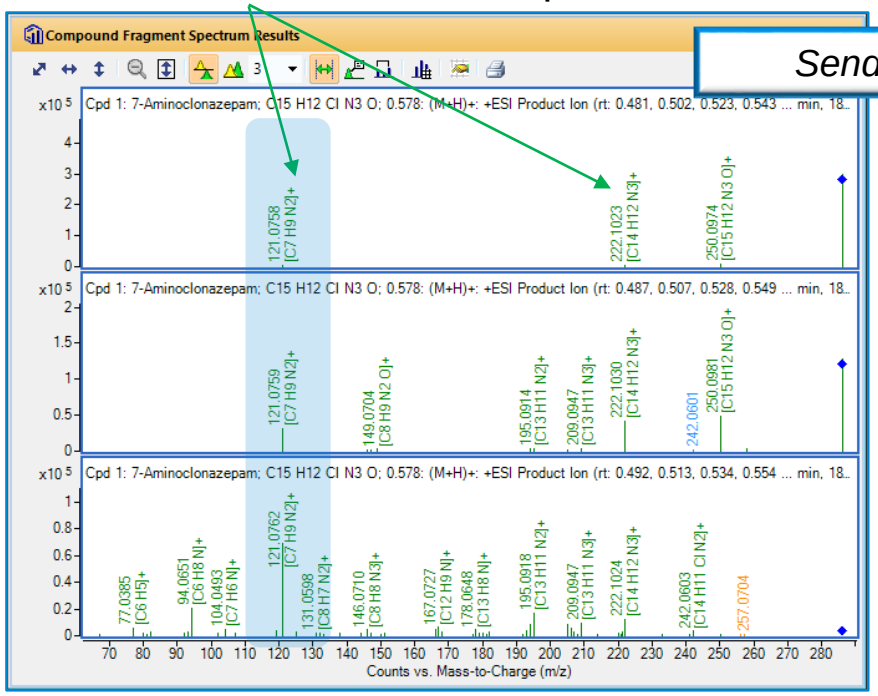
Filtered for signal intensity and curated for spectrum noise and chemical impurities

How to make an Agilent PCDL your own?

User Customizable PCDL – Auto-curate New Spectra

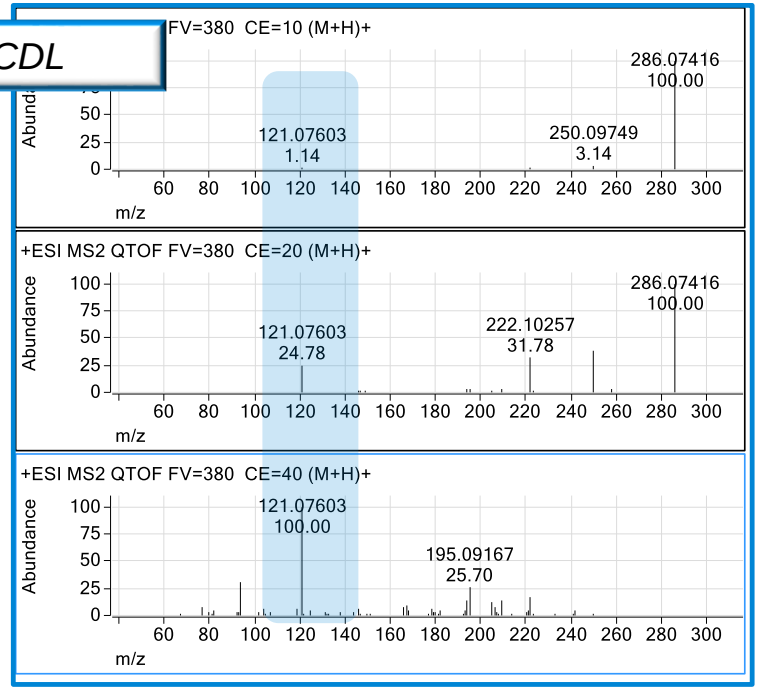
Open data in MH Qual → *Open method (Default-LCMS.m)* → *FBF wt extract MS/MS spectra* → *SEND to PCDL Export curated spectra to customized PCDL*

Annotated MS/MS spectra



Send Spectra to PCDL

PCDL Library Spectra



Untargeted Q-TOF data can be retrospectively reviewed with addition of new compounds

Summary

The right tools for your E&L Needs

- **Detect Unique Extractables**

  Use Molecular Feature Extraction for automated SW assisted feature finding

- **Perform Data Reduction and Differential Analysis**

  Compare sample and control or different extractions

- **LC/MS PCDL for E&L**

  Curated following documented process

- **…No more Unknowns**

  Use PCDL Manager to add compounds and spectra to your PCDL once identified

Acknowledgements

David Weil

Ruben Garnica

Chris Miller

Agilent GC/MSD Library Portfolio

RTL Library for GC/MSD	Unique Compounds	Spectra
Hazardous Chemicals Compounds RTL DB	700	700
Pesticide & Endocrine Disruptor RTL DB	1,000	1,000
Indoor Air Toxics RTL DB	200	200
Forensic Toxicology RTL DB*	700	700
Japanese Positive List RTL DB	450	450
Environmental SVOAs RTL DB	150	150
Solvents Plus RTL DB	400	400
Controlled Substances RTL DB*	450	450
Fiehn RTL Library**	1,200	1,200
NIST 2017	262,157	306,622
Wiley 11th / NIST 2017	>730,000	1,007,924
Maurer, Pfleger, Weber	10,430 + 7,800 of their metabolites	10,430

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Use of Open Source Databases and Libraries and Agilent PCDLs

Agilent PCDL Products

- Comprehensive and relevant
- High quality spectra with defined quality control criteria
- One entry per compound, compatible between Agilent Q-TOF instruments
- *Curated database and library entries*
- Protect proprietary information

Open Source Libraries

- User contributed spectra
- Multiple entries for the same compound (different instruments, different users)
- *Uncontrolled data quality*
- Share results with scientific community
- Tentatively identified compounds