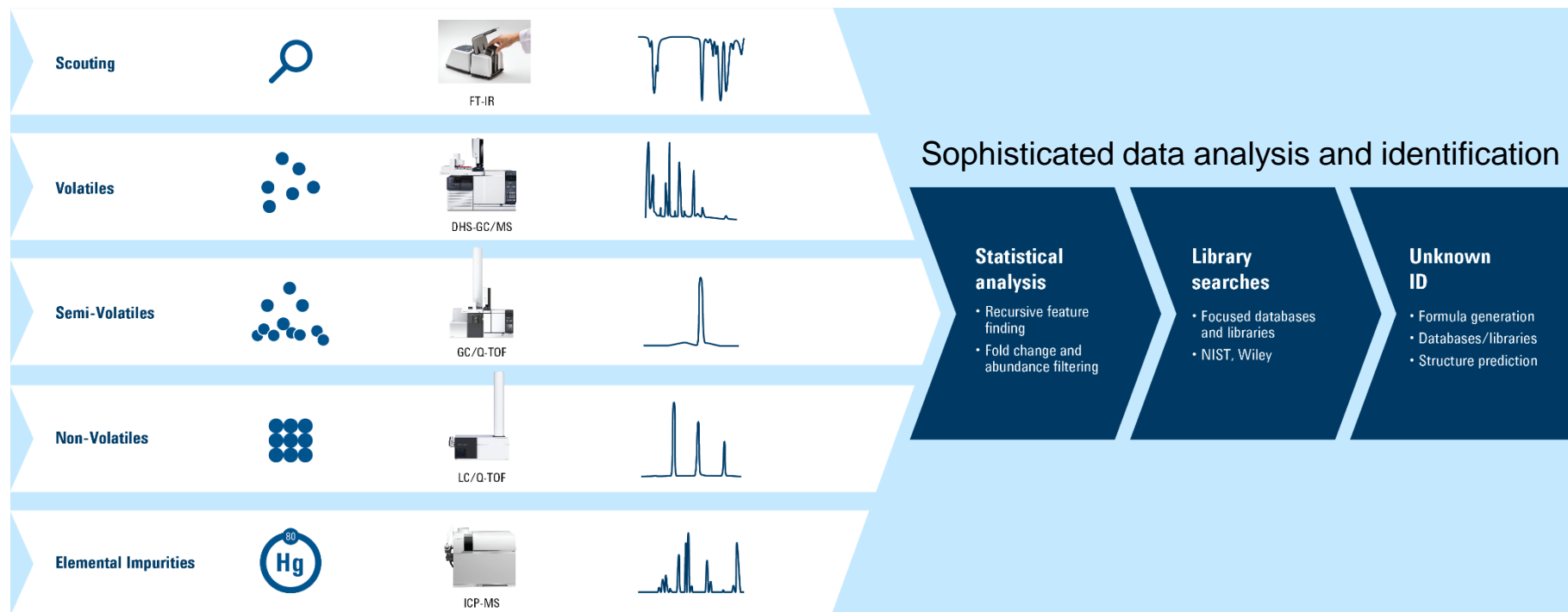


# E&L: Streamlining LC/MS and GC/MS Workflows

ASMS 2018 San Diego

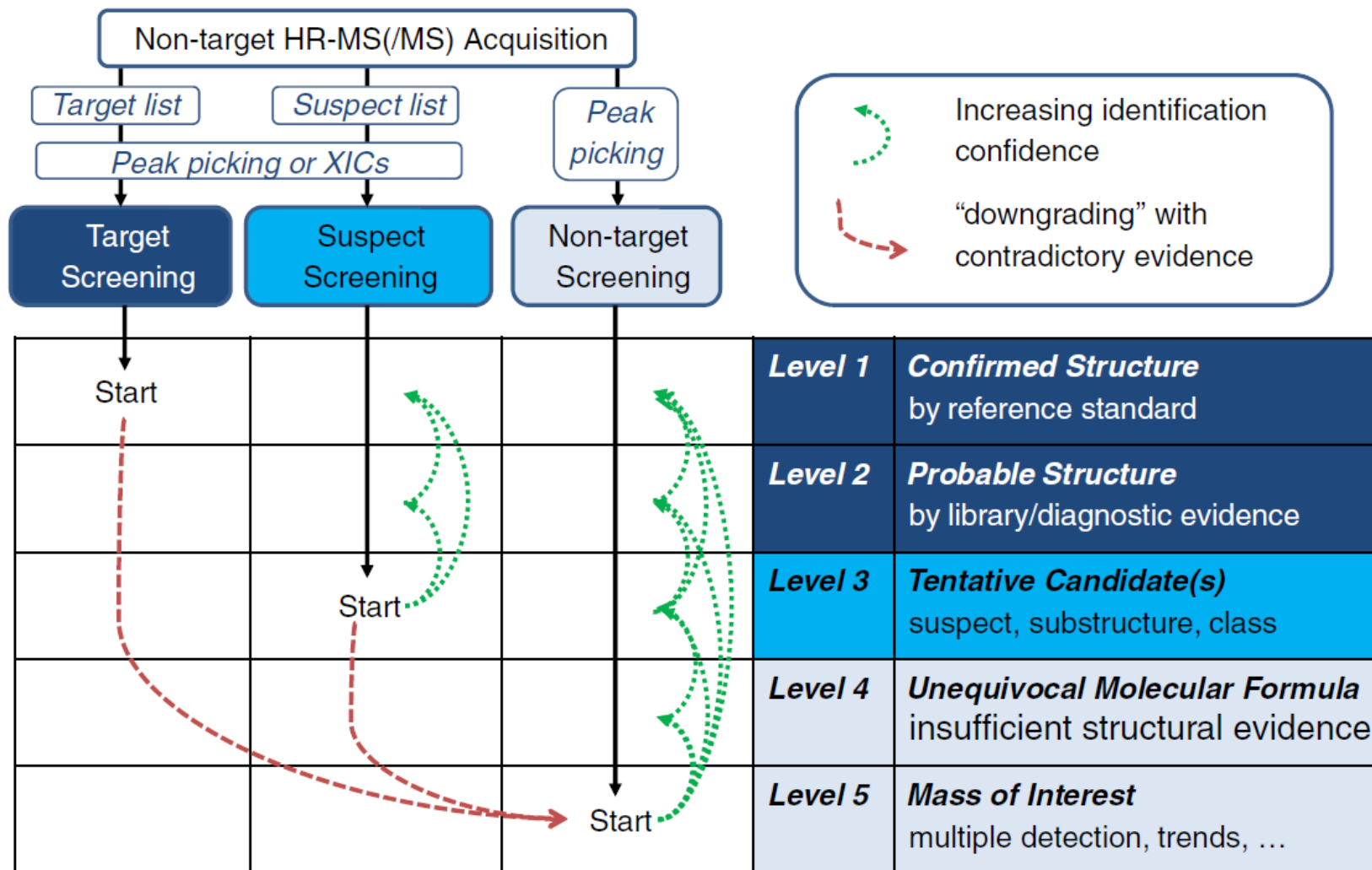
Smriti Khera, Ph.D.  
Pharma Segment Manager  
[Smriti\\_khera@Agilent.com](mailto:Smriti_khera@Agilent.com)

# Agilent's Comprehensive Solutions for Extractable Profiling



# Identification Strategy and Confidence

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



# Generalized MassHunter Workflow for Extractables Profiling

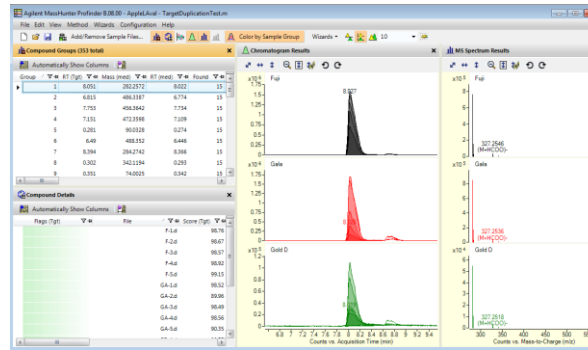
Acquire



Native support for all Agilent LCMS, GCMS and ICPMS instruments

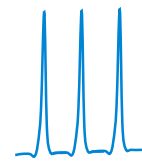
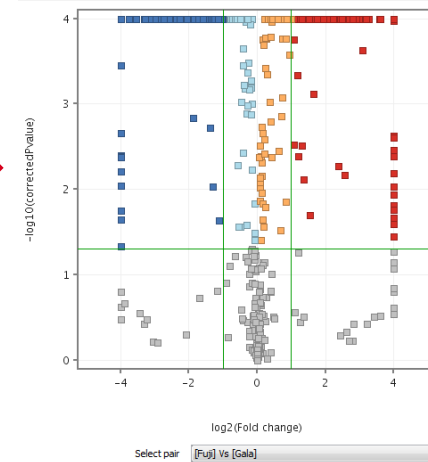
Extract

Feature Detection Profinder

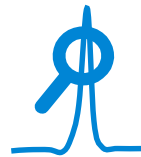


Analyze

Differential Analysis Mass Profiler Professional



MassHunter Acquisition



Qualitative Analysis Profinder  
Unknowns Analysis



PCDLs, Molecular Structure Correlator



Online Resources

Identification  
E&L PCDL  
MH Qual

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

For many labs this is a manual process:

.. lack of standardization..

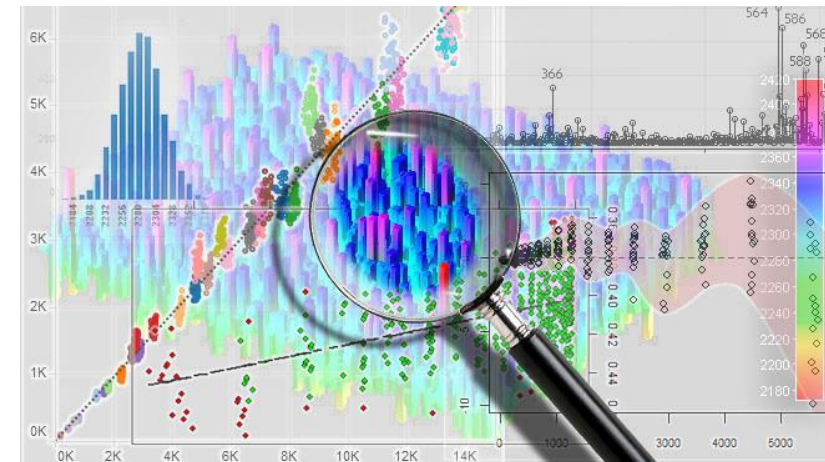
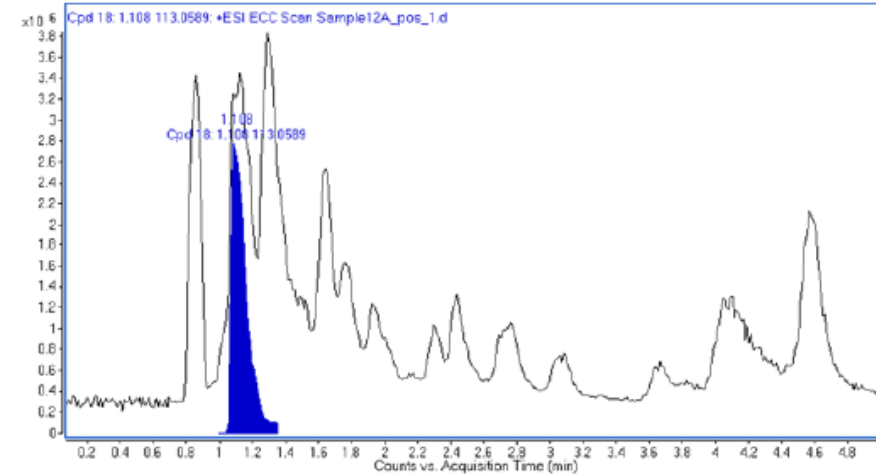
.. process single datafiles, walk across TIC, background subtraction, export m/z values to excel, use custom macros..

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



[This Photo](#) by Unknown Author is licensed under [CC BY-NC](#)

# Generalized MassHunter Workflow for Extractables Profiling

Acquire



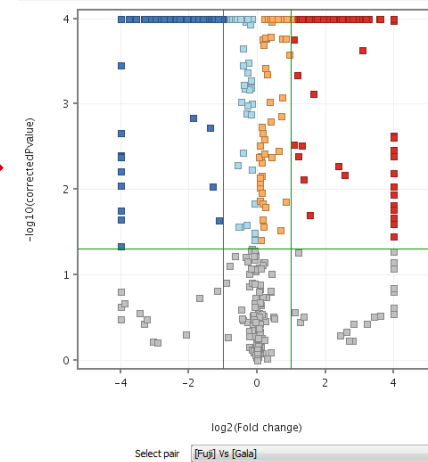
Native support for all Agilent LCMS, GCMS and ICPMS instruments

Extract

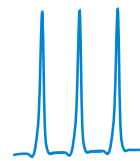
### Feature Detection Profinder

Analyze

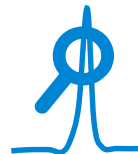
Differential Analysis  
Mass Profiler Professional



Identification  
E&L PCDL



MassHunter Acquisition



Qualitative Analysis Profinder  
Unknowns Analysis



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 ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^2\text{H}$ ,  $^{18}\text{O}$ )
- Include adducts, such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$
- Include dimers, such as  $(2\text{M}+\text{H})^+$
- Create a compound chromatogram (ECC)

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

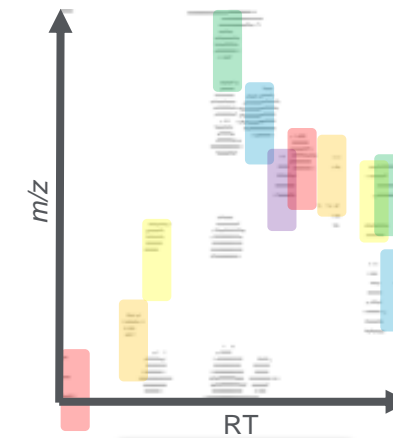
–Create a compound spectra for the group of ions

–Report results as retention time and neutral mass

–Fully automated processing

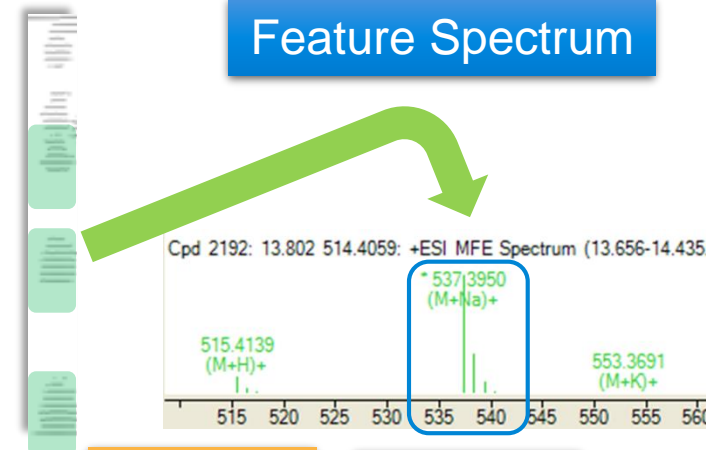
- Filters noise

Performed in MH Qual or Profinder or Mass Profiler



coelution  
grouping

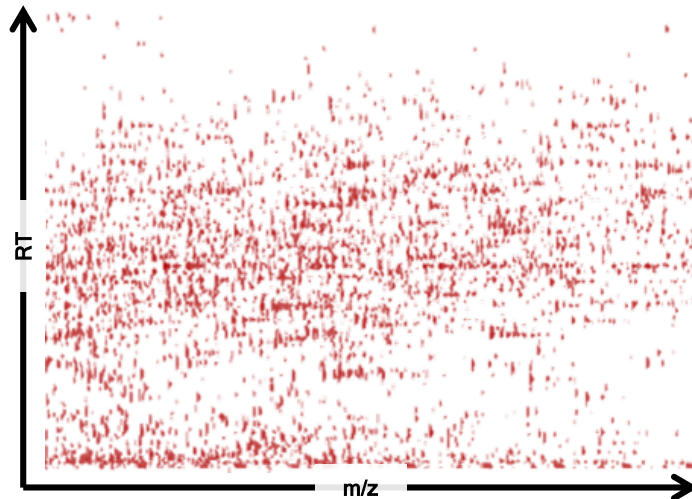
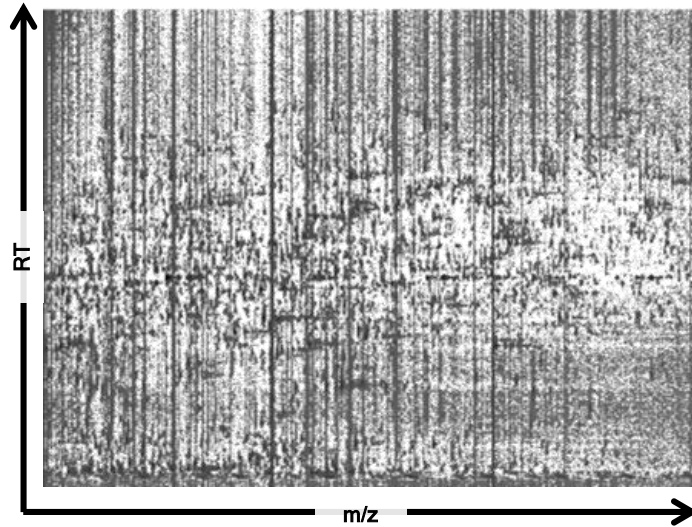
Feature Spectrum



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

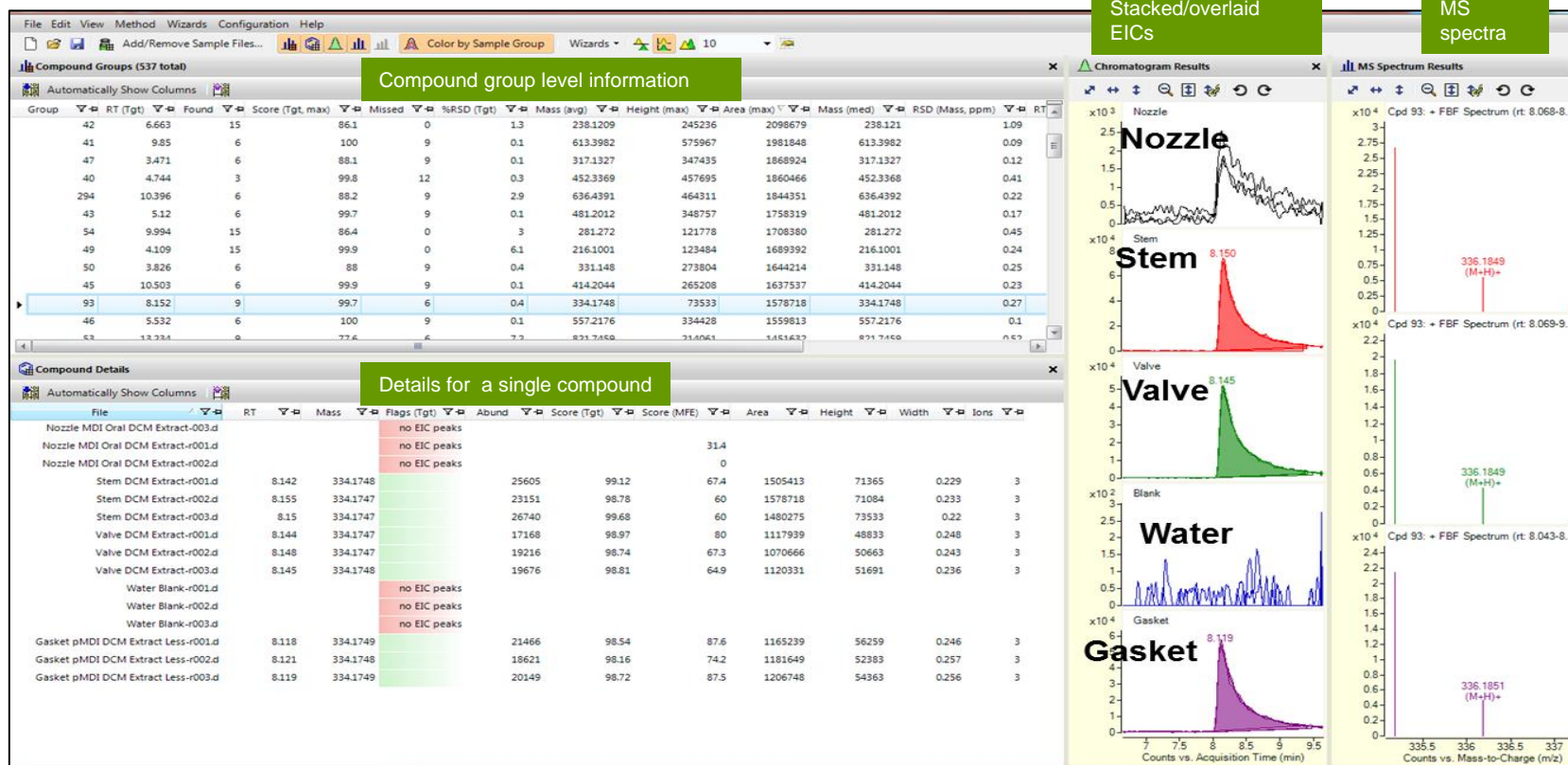
## For High-throughput Compound Finding

MassHunter Profinder is a productivity tool for processing multiple samples

## Profinder Feature Extraction

- Batch Molecular Feature Extraction
- Batch Recursive Feature Extraction (small molecules / peptides)
- Batch Recursive Feature Extraction (large molecules)
- Batch Targeted Feature Extraction
- Batch Isotopologue 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

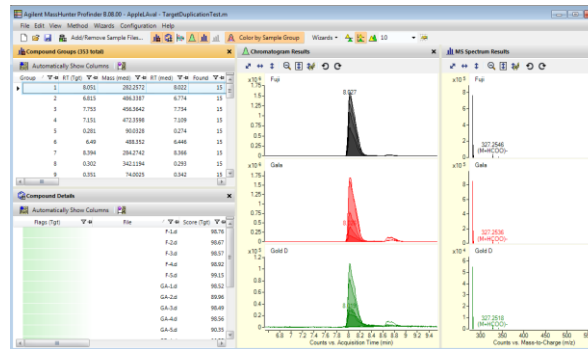
Acquire



Native support for all Agilent LCMS, GCMS and ICPMS instruments

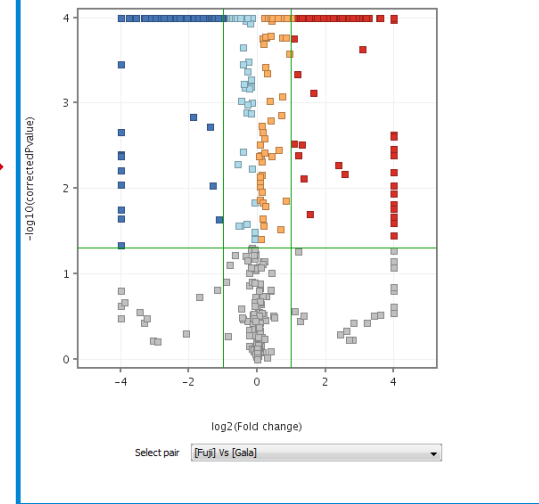
Extract

Feature Detection Profinder

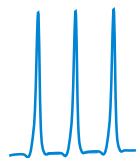


Analyze

Differential Analysis Mass Profiler Professional



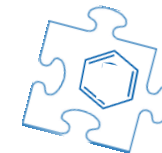
Identification E&L PCDL



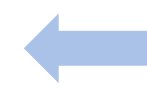
MassHunter Acquisition



Qualitative Analysis Profinder Unknowns Analysis



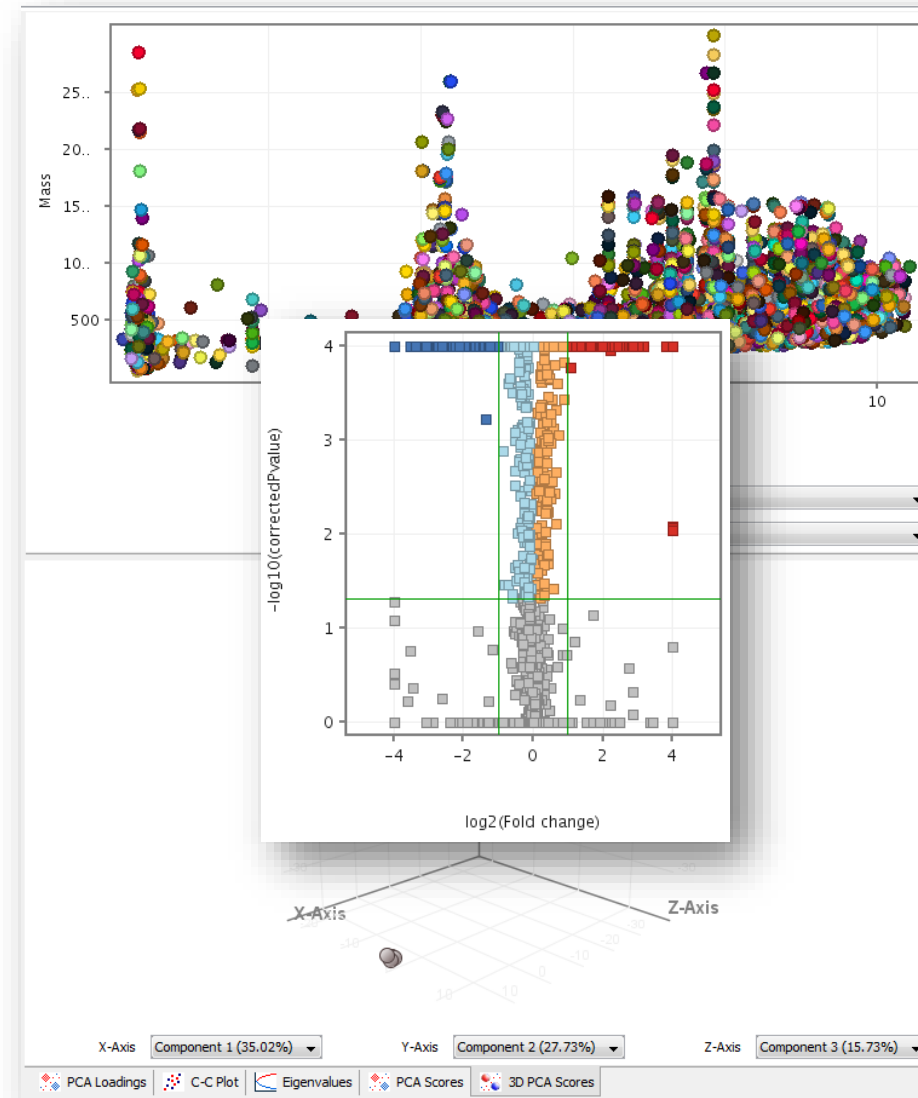
PCDLs, Molecular Structure Correlator



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



# Generalized MassHunter Workflow for Extractables Profiling

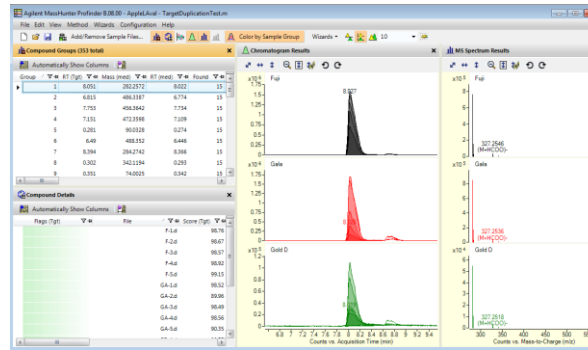
Acquire



Native support for all Agilent LCMS, GCMS and ICPMS instruments

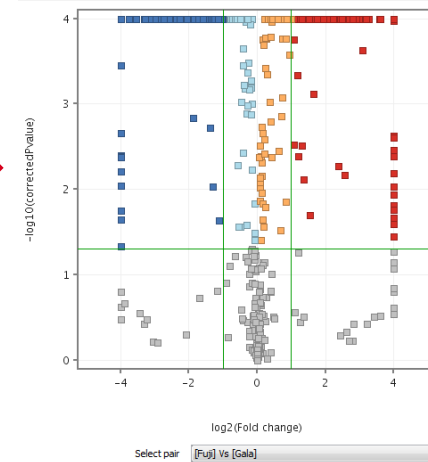
Extract

Feature Detection Profinder

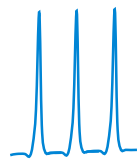


Analyze

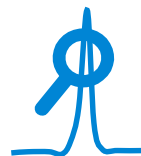
Differential Analysis Mass Profiler Professional



Identification E&L PCDL



MassHunter Acquisition



Qualitative Analysis Profinder Unknowns Analysis



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)

Chemical List

MassHunter PCDL Manager - Pesticides C:\MassHunter\PCDL\Water Screening PCDL 8.07.00\Water\_AMRT\_PCDL.cdb

File View PCDL Configuration Links Help

Find Compounds Spectra Ion Mobility Import

Compounds search criteria: epa

Must also contain: [ ]

Must not contain: [ ]

Ion search mode:  Include neutrals,  Include anions,  Include cations

Tolerances: Mass: 10.0 ppm, RT: 0.1 min, RI: 10.00

Search only visible columns | Search all columns | With spectra | With CCS

Compound Results: 1451 hits

Name	Formula	Mass	Retention Time	Cation	Anion	CAS	ChemSpider	IUPAC	NumSpectra
Indoxacarb	C22H17O3F3N3	527.07071	14.125	<input type="checkbox"/>	<input type="checkbox"/>	173584-44-6	96889	Methyl (4aS)-7-chloro-2-((methoxycarbonyl)(4-(trifluoromethoxy)phenyl)...	3
Bromadiolone	C30H23BrO4	526.07797		<input type="checkbox"/>	<input type="checkbox"/>	28772-56-7	10606098	3-[3-(4'-bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-chr...	0
Virginiamycin M1 (Mikamycin A)	C28H35N3O7	525.2475		<input type="checkbox"/>	<input type="checkbox"/>	21411-53-0	10222381	(10R,11R,12E,17E,19E,21S)-21-Hydroxy-10-isopropyl-11,19-dimethyl-9...	3
Cefotiam	C18H23N9O4S3	525.10351		<input type="checkbox"/>	<input type="checkbox"/>	61622-34-2	39831	(6R,7R)-7-(((2-Amino-1,3-thiazol-4-yl)acetyl)amino)-3-(((1-(2-dimethylam...	3
Cefoselis	C19H22N8O6S2	522.11037		<input type="checkbox"/>	<input type="checkbox"/>	122841-10-5	16736411	5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[2-amino-4-thi...	0
Brodifacoum	C31H23BrO3	522.08306		<input type="checkbox"/>	<input type="checkbox"/>	66052-95-7	10444663	3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]-4-hydroxy...	3
Mometasone furoate	C27H30Cl2O6	520.14194		<input type="checkbox"/>	<input type="checkbox"/>	83819-23-7	390091	(11beta,16alpha)-9,21-dichloro-11-hydroxy-16-methyl-3,20-dioxopregna...	3
Latamoxef	C20H20N6O9S	520.10125		<input type="checkbox"/>	<input type="checkbox"/>	64952-97-2	43215	(6R,7R)-7-[[Carboxy(4-hydroxyphenyl)acetyl]amino]-7-methoxy-3-[[1-m...	0

Compound - Virginiamycin M1 (Mikamycin A)

Mass: 525.2475 Formula: C28H35N3O7 Save

Notes: Forensic and Toxicology drug; Veterinary drug; Environmental contaminant; PPCP; Antibiotic  
 Synonyms: 维吉尼霉素; Factor M1; Ostreoglycin A; Pristinamycin IIA; Staphylomycin M1; Streptogramin A; Vemamycin A; Virginiamycine  
 Also found in mixture of virginiamycins M1 and S1, CAS:11006-76-1  
 EPA 1694; CN-NY-235; CN-NY-265

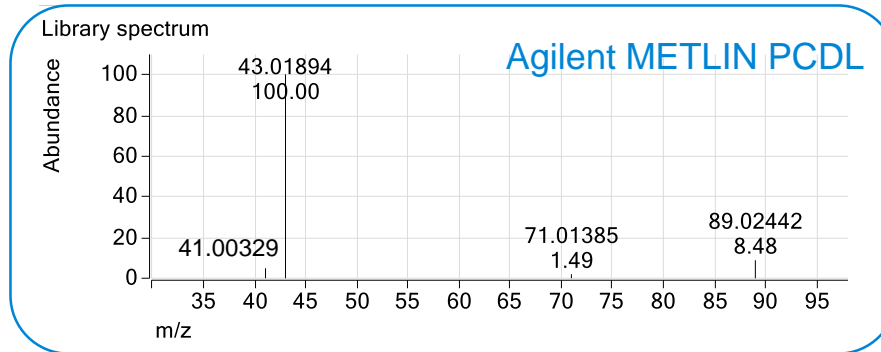
Structure MOL Text

Compound View

# Benefits of the Curation Process

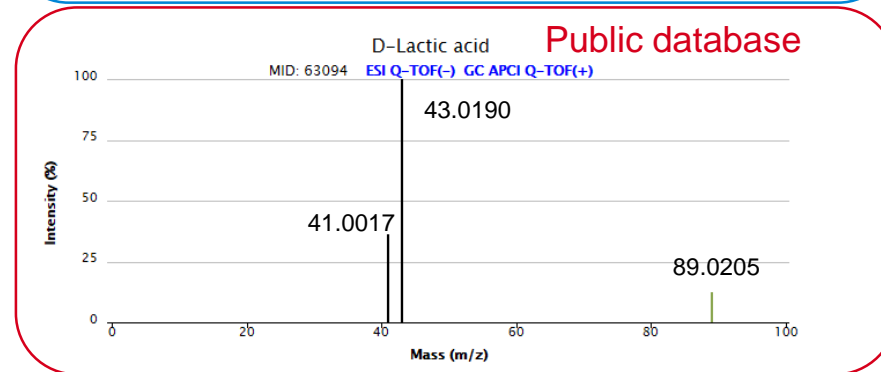
## Correction to theoretical accurate mass eliminates mass error

METLIN Metabolomics PCDL



D-lactic acid

METLIN ID: 63094  
Mass 90.03169  
Formula: C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>  
HMDB01311

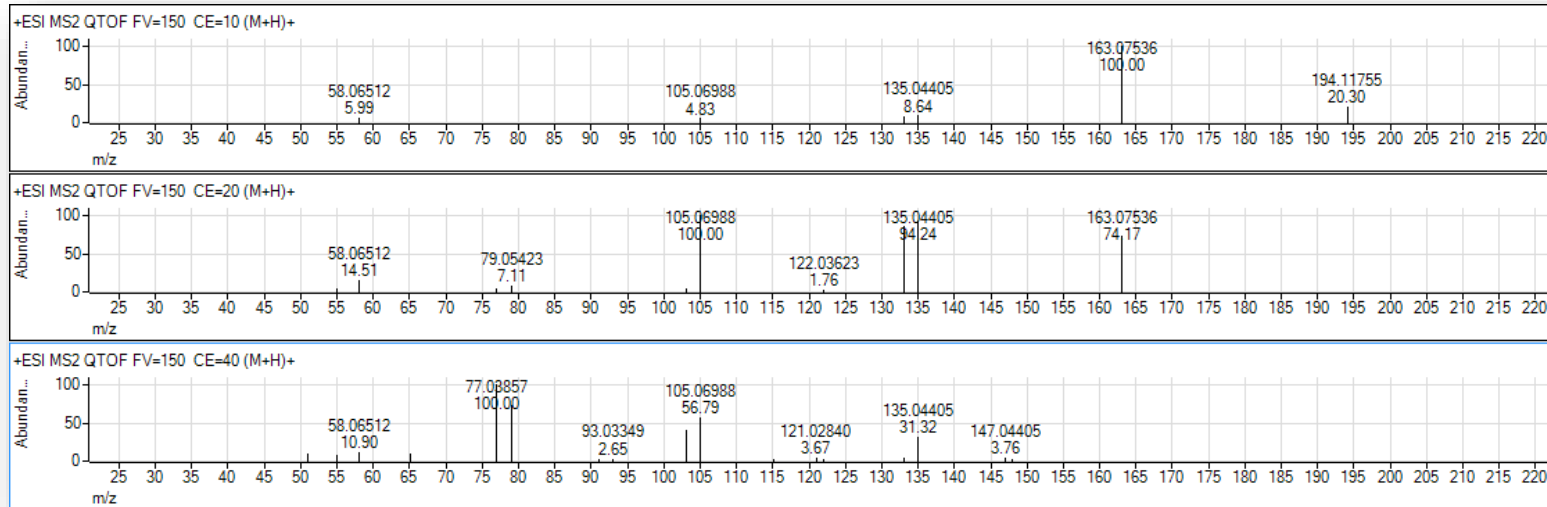


Agilent METLIN*	Uncurated MS/MS Spectrum	Mass Error (ppm)
41.00329	41.0017	38.74
43.01894	43.0190	-1.43
71.01385	Missing	
89.02442	89.0205	44.01

\*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 collision energies, polarities, and ion species

## Curation

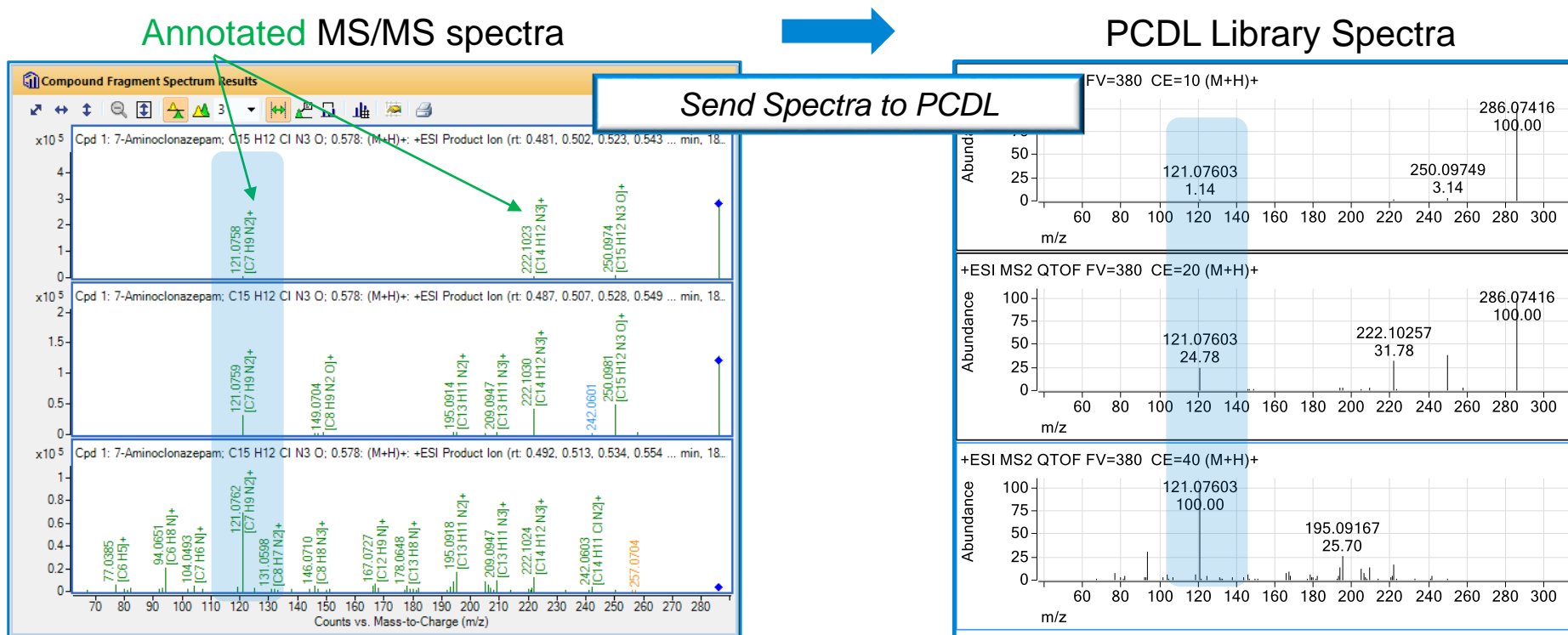
Correction to Theoretical accurate mass

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



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 11 <sup>th</sup> / NIST 2017	>730,000	1,007,924
Maurer, Pfeleger, Weber	10,430 + 7,800 of their metabolites	10,430

# 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