



MassHunter Software Overview, Tips, & Tricks

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ASTS – Vancouver, BC May 8th

La Jolla, CA May 20th

MassHunter Workstation

One software for all your Agilent mass specs

Minimize the learning and optimize the use of software in your lab across different mass spec instrument platforms

- Control and data processing for Agilent GC/MS, LC/MS, and ICP-MS instruments



LC/MS



GC/MS



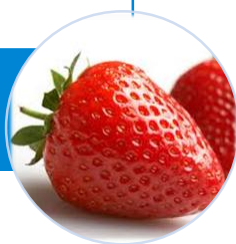
ICP-MS

- **From GC and LC Single Quad to Accurate Mass QTOF's**

MassHunter Software for your Key Applications

- **Qualitative Analysis** for confident identifications and to set up methods

Food



- **Quantitative Analysis** for fast and accurate results

Pharma



- **Acquisition** for innovative techniques such as tMRM to confirm compounds

Veterinary
Drugs



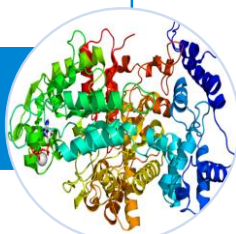
- **Personal Compound Database and Libraries (PCDLs)** for rapid identification

Forensic
Toxicology



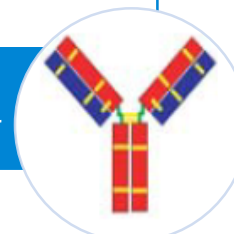
- **Spectrum Mill** for accurate identification of proteins

Proteomics



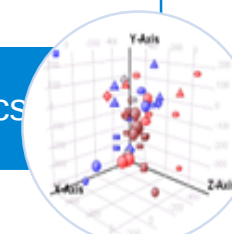
- **BioConfirm** for characterization of intact proteins, peptides, and monoclonal antibodies

Biopharma



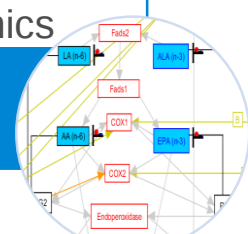
- **Mass Profiler Professional (MPP)** for differential profiling

Metabolomics



- **Pathway Architect** for bringing together genomics, proteomics, and metabolomics

Integrated
Biology



Agilent Technologies

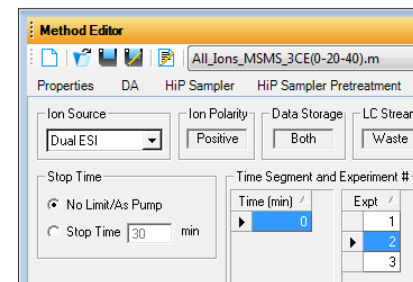
ASTS - La Jolla

May 20, 2014

Agilent MassHunter Core Programs

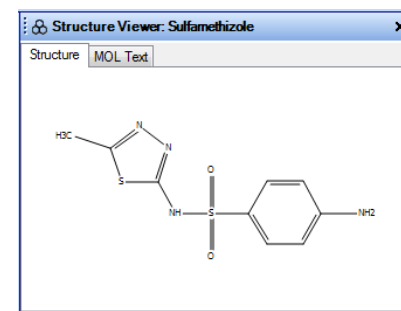
Acquisition

- Support for all Agilent LCs, GCs, and mass spectrometers
- Superior compound detection (LC/MS) tMRM, All Ions MS/MS, and Ion Mobility



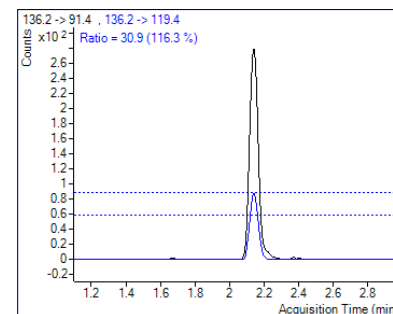
Qualitative Analysis

- Application Focused Solutions Kits: Software, Libraries, Methods
- Patented Data mining & identification software algorithms formula/structure

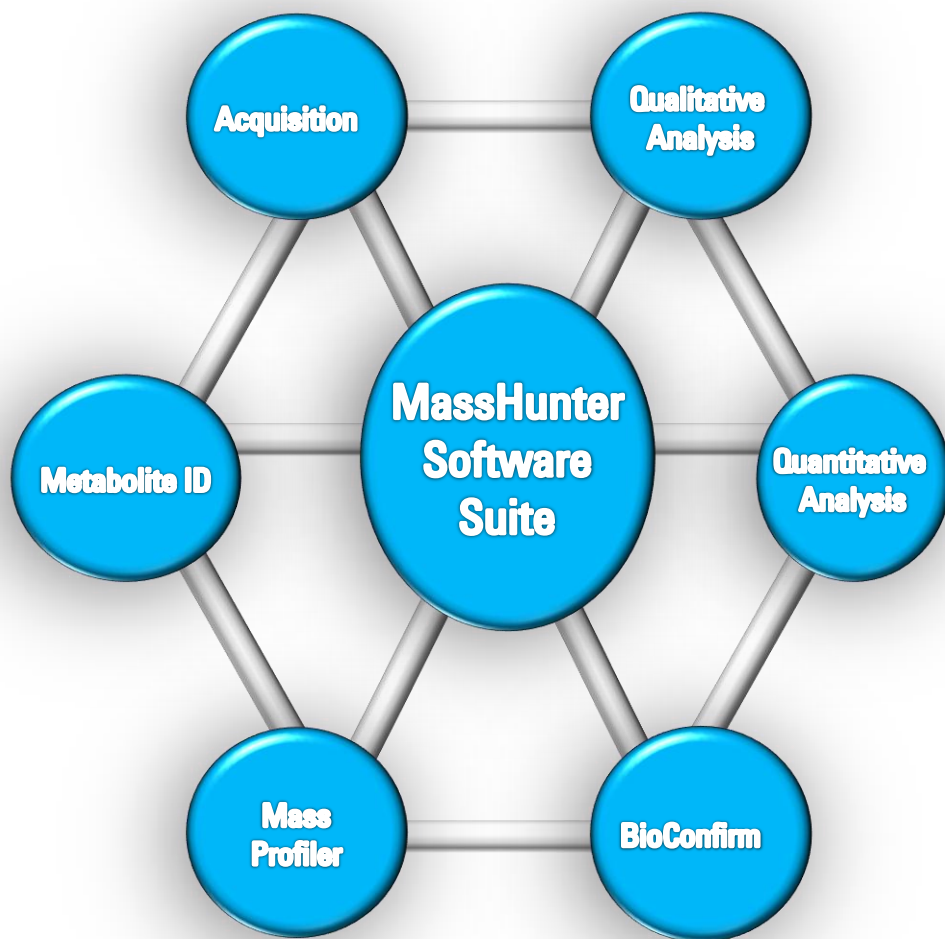


Quantitative Analysis

- High-throughput quantitation of target compounds
- Easily visualize results using the Batch Table or Compounds-at-a-Glance from ALL Agilent LCs, GCs, and Mass Specs



MassHunter Suite of Software:



- Qualitative Analysis (Qual)
- BioConfirm
- Spectrum Mill B.04.01
- Molecular Structure Correlator (MSC)
- PCDL Manager
- Profinder
- Pathways to PCDL
- Mass Profiler (MP)
- Mass Profiler Professional (MPP)
- SimLipid (Premier Biosoft)

Fully integrated workflows to enable you to identify, plan, and execute your next experiment



MassHunter Software Window 7

Current Versions (May 2014)

- MassHunter Acquisition for QQQ B.07.00 **NEW**
- MassHunter Qualitative Analysis B.06.00 SP1*
- MassHunter Quantitative Analysis B.07.00 **NEW**
- MassHunter BioConfirm B.06.00
- MassHunter PCDL Manager B.04.00 SP1
- MassHunter METLIN Metabolite PCDL B.05.00
- MassHunter Mass Profiler Professional 12.65 **NEW**
- MassHunter Profinder B.06 **NEW**
- Accurate Mass Libraries Pesticide/Vet Drugs/Forensics
- Pathway to Database Creator Software
- ChemStation SQ (LC/MS and GC/MS) to MassHunter file translator

All run on Windows 7 Pro 64 bit with Excel 2013



Keep Your MassHunter Current!

Online service patches and updates found at www.agilent.com

The screenshot shows the Agilent Technologies website interface. The top navigation bar includes links for Products & Services, Solutions, Technical Support, Library, Training & Events, and Buy. The top right corner has links for Login, Cart (0), and Quick Order. The main content area displays a search result for 'MassHunter Qualitative Data Analysis Software'. The left sidebar shows filters applied: 'Patches' and 'In the Last 12 Months'. The search results show '1-1 of 1 results for MassHunter Qualitative Data Analysis Software'. The result is 'MassHunter Qualitative Analysis B.06.00 Service Pack 1 (SP1)', which is a software patch available for download to fix defects in MassHunter Qualitative Analysis B.06.00 Service Pack 1 (SP1).

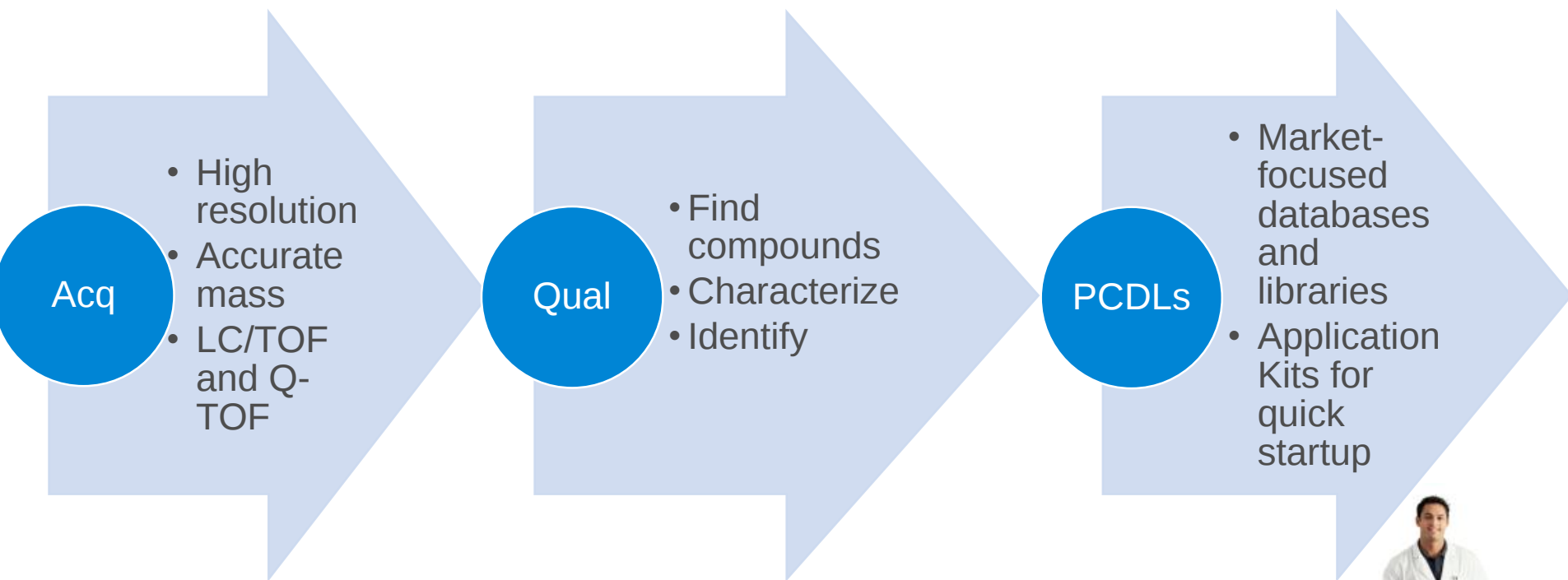
<http://www.chem.agilent.com/en-US/Technical-Support/Software-Informatics/MassHunter-Qualitative-Data-Analysis-Software/Pages/default.aspx>

General Software Technical Support with Patches and Updates

<http://www.chem.agilent.com/en-US/Technical-Support/Software-Informatics/Pages/default.aspx>

The MassHunter Qualitative Workflow

Identify with confidence



Agilent provides a complete suite of tools, consumables and consulting to set up for the rapid identification of unknowns

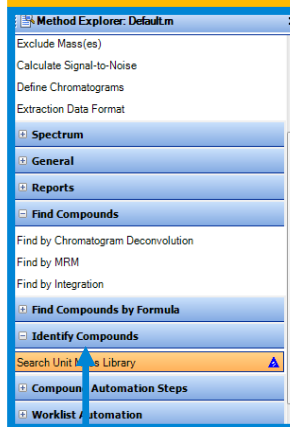
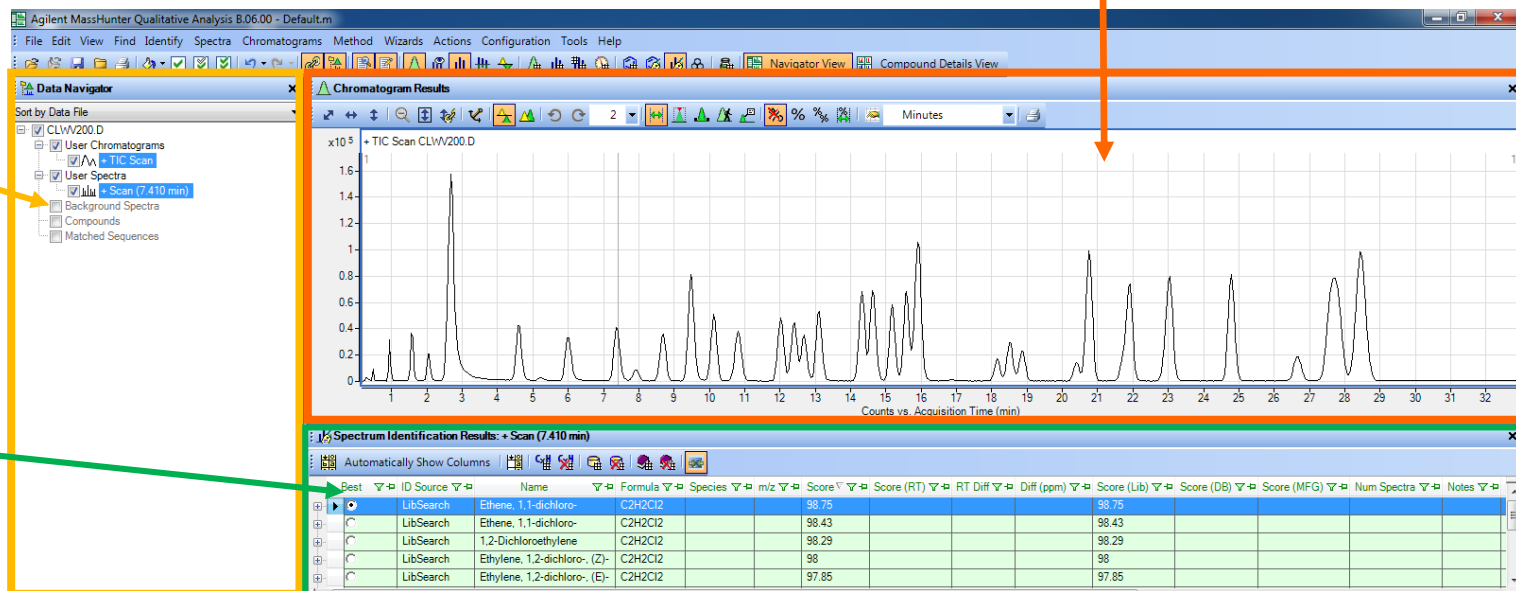


Qualitative Analysis

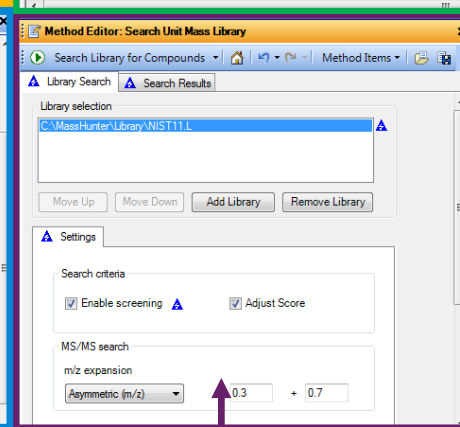
Chromatogram Display

Data Navigator

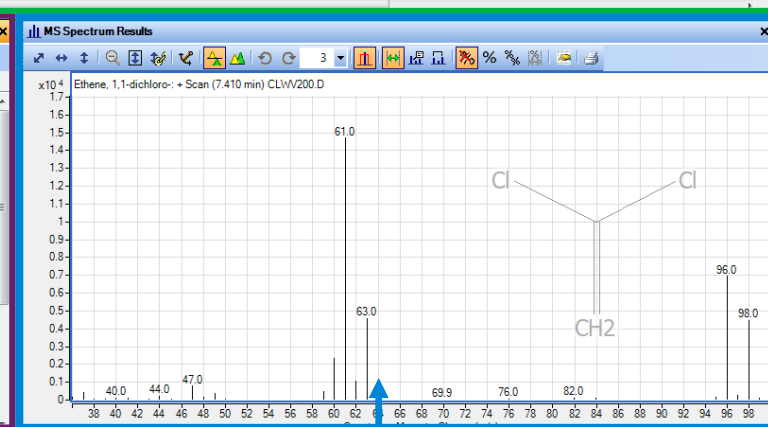
Results Table



Method Explorer



Method Editor



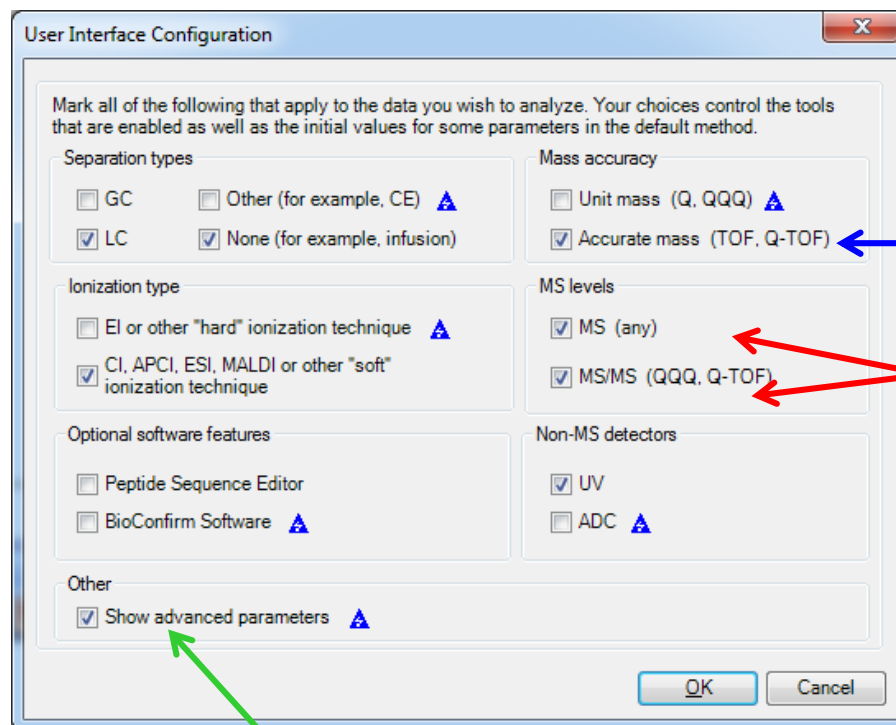
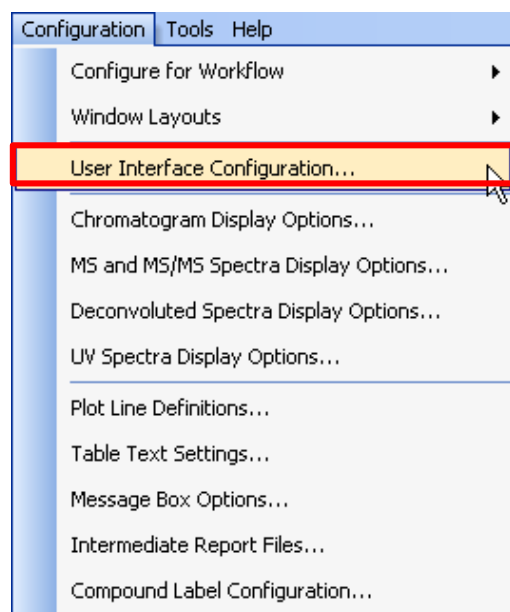
Spectrum Display



Agilent Technologies

Qual is used for All Data Type: MSD, QQQ, TOF

So Setup User Interface for Data File Type



**Accurate
Mass**

Both Levels

Show Advanced Parameters

Setting data file type automatically changes the method options, display and search capabilities

Selecting Data Types Changes Display

Find Compounds

Find by Chromatogram Deconvolution
Find by Integration

GC/MSD

Separation types	Mass accuracy
☒ GC ☐ Other (for example, CE)	☒ Unit mass (Q, QQQ)
☐ LC ☒ None (for example, infusion)	☐ Accurate mass (TOF, Q-TOF)
Ionization type	MS levels
☒ EI or other "hard" ionization technique	☒ MS (any)
☐ CI, APCI, ESI, MALDI or other "soft" ionization technique	☐ MS/MS (QQQ, Q-TOF)

LC/QQQ

Find Compounds

Find by MRM
Find by Integration

Mass accuracy
☒ Unit mass (Q, QQQ)
☐ Accurate mass (TOF, Q-TOF)
MS levels
☒ MS (any)
☒ MS/MS (QQQ, Q-TOF)

LC/QTOF

Find Compounds

Find by Auto MS/MS
Find by Targeted MS/MS
Find by Molecular Feature
Find by Integration

Mass accuracy
☐ Unit mass (Q, QQQ)
☒ Accurate mass (TOF, Q-TOF)
MS levels
☒ MS (any)
☒ MS/MS (QQQ, Q-TOF)

LC/TOF

Find Compounds

Find by Molecular Feature
Find by Integration

Mass accuracy
☐ Unit mass (Q, QQQ)
☒ Accurate mass (TOF, Q-TOF)
MS levels
☒ MS (any)
☐ MS/MS (QQQ, Q-TOF)

TIP: To use low res libraries you must have the GC checked

And Changes Compound Identification

Accurate Mass Q(TOF)

Identify Compounds

Search Database

Search Accurate Mass Library

Generate Formulas

Combine Identification Results

Unit Mass MSD/QQQ

Identify Compounds

Search Accurate Mass Library

Combine Identification Results

Tip: The low resolution *.L file should be copied to the X:\\MassHunter\\Library\\ subdirectory and one can use the NIST library if you have a license

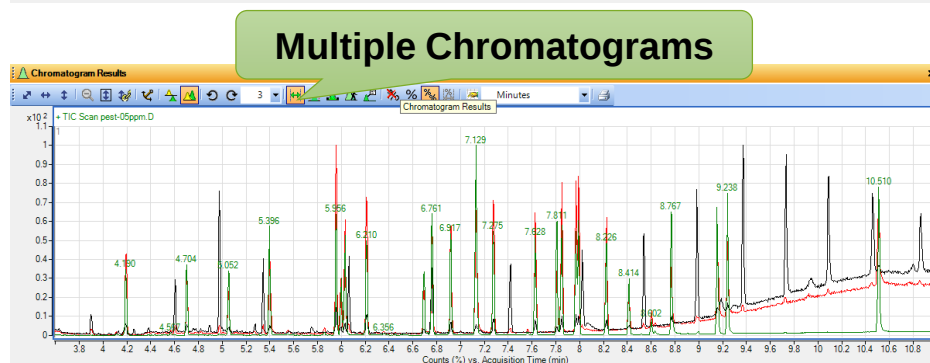
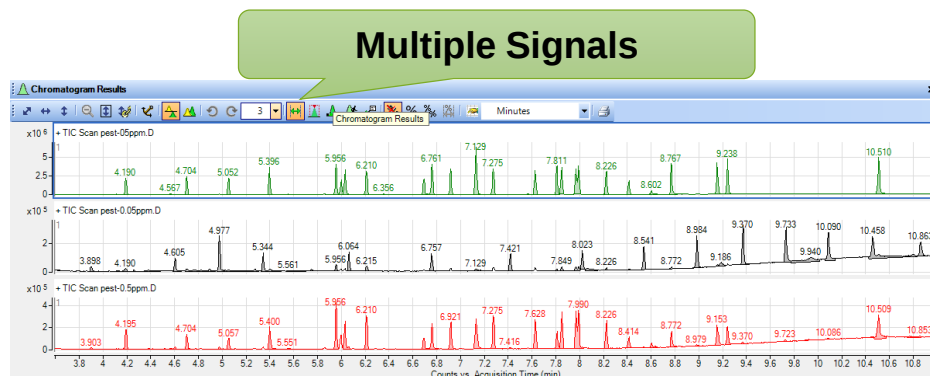
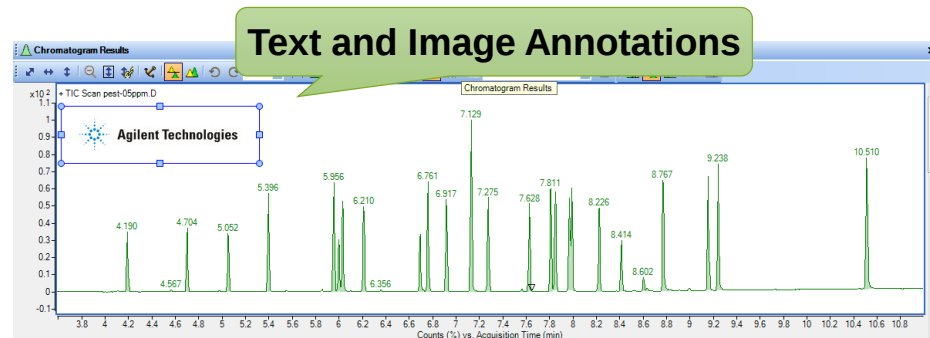


Chromatogram Display

Comprehensive Display Options

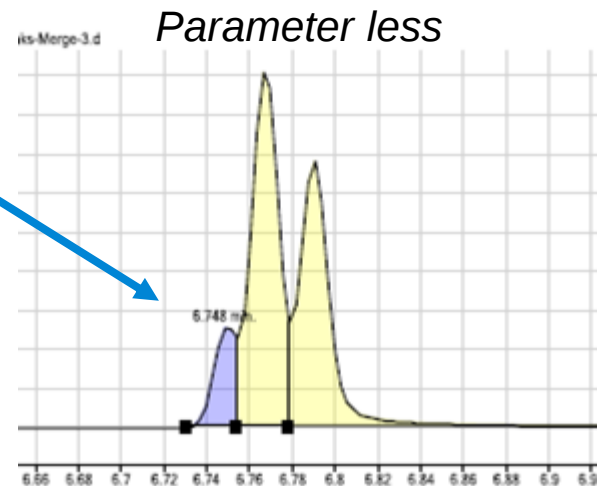
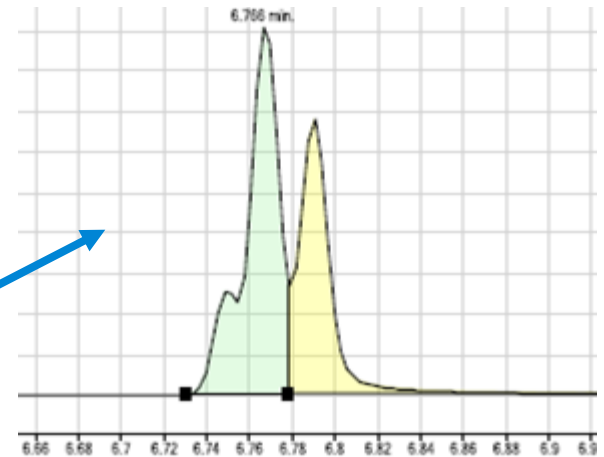
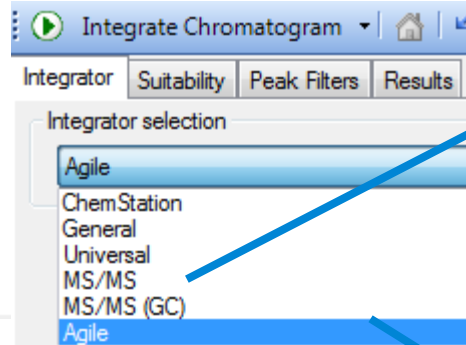
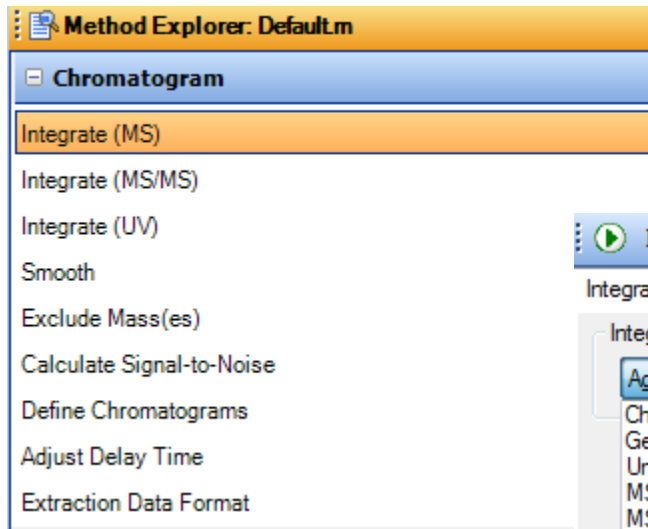
- Display **multiple** Chromatograms, from one or many data files.
- Extract **multiple signal types**, i.e. TIC, EIC, UV, FID, Instrument curves, etc.
- Annotate peaks or chromatograms with text or images

Definition of chromatograms can be stored in the method and used with automation.



Improved Parameter-less Integrators

MS/MS and Agile what's the difference?



The Agile integrator requires 75% fewer data points for Integration typically 15 points minimum

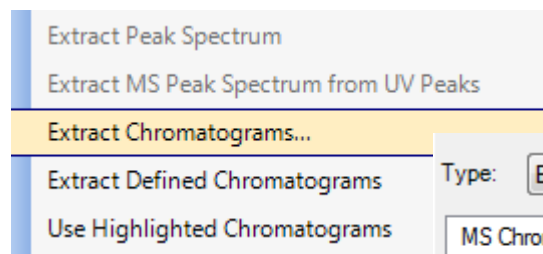
Agile2 Now in QUANT B.07



Agilent Technologies

For Accurate Mass Systems

Use Advanced Tab to Narrow Extraction Limits (10 ppm)



Type: **BPC** ☐ Integrate when extracted

MS Chromatogram **Advanced** Excluded Masses

MS level: **All** Polarity: **Positive**

Scans: **All scan types**

m/z of interest: **Any**

m/z value(s): **100.0000-1300.0000**

Set to Exclude Reference and Background Ions

MS Chromatogram **Advanced** Excluded Masses

☐ Do not exclude masses
☒ Exclude masses (or m/z ranges) from all new chromatograms

m/z value(s): **121.0509, 922.0098**

Single m/z expansion for this chromatogram
Symmetric (ppm) ± **20.0**

MS Chromatogram **Advanced** Excluded Masses

Fragmentor: **Any** Ionization: **Any**

Collision energy: **Any**

Single m/z expansion for this chromatogram
Symmetric (ppm) ± **10.0**

- 10
- 20
- 50
- 100
- 200
- 500



Spectra Display

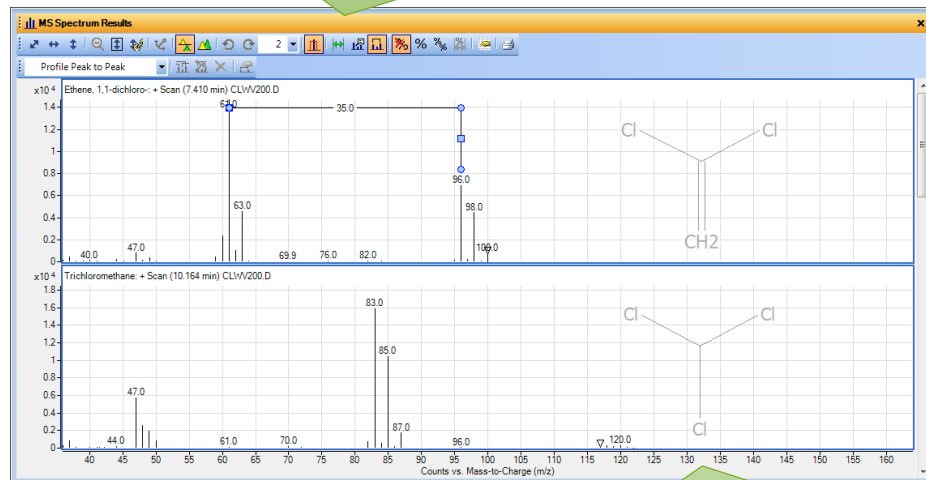
Extract Spectra via:

- Manual spectra selection
- Integrated peaks
- **Multiple Find-by-Methods**
 - By Deconvolution
 - By Integration
 - By Molecular Feature
 - Find by Formula (Ion)

Identify from Library Searching GCMS

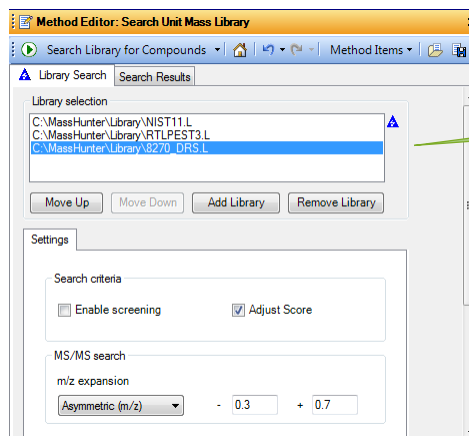
- Multiple Libraries (*.L)
- Link to NIST MS Search

Mass Difference Annotations

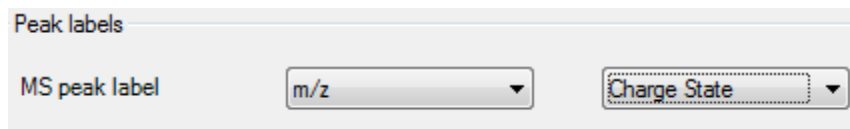
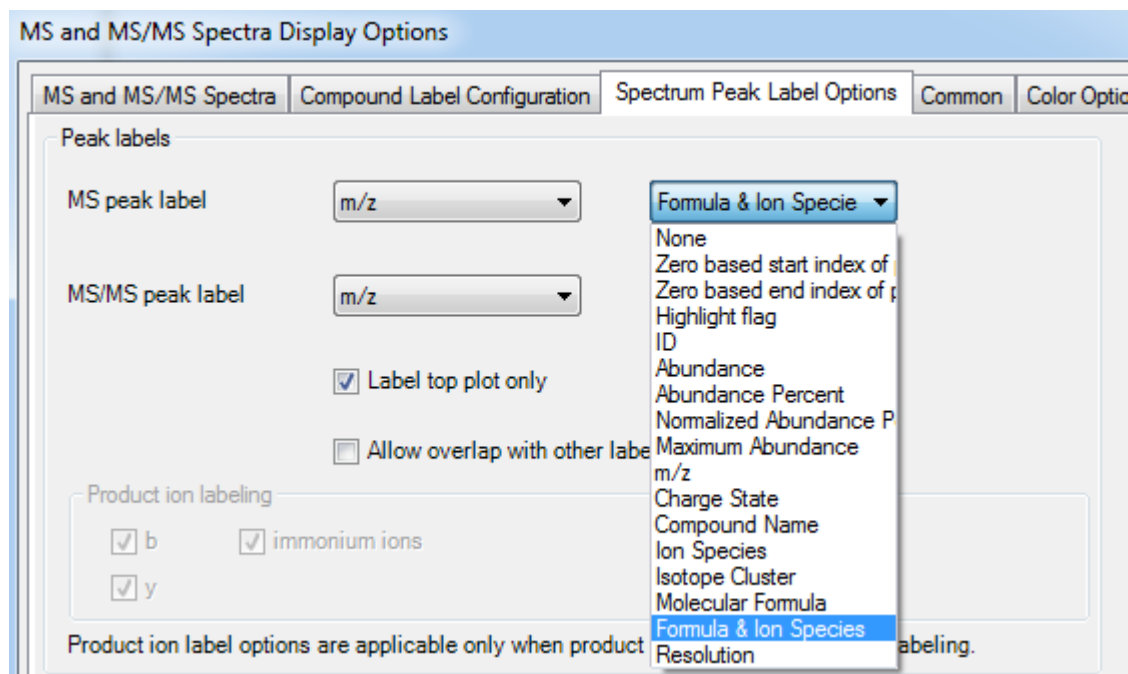
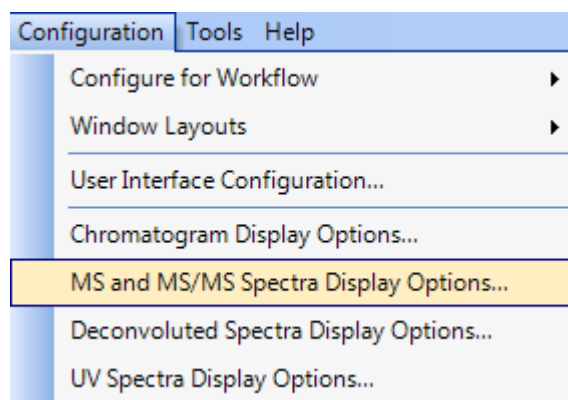


Structure Annotations

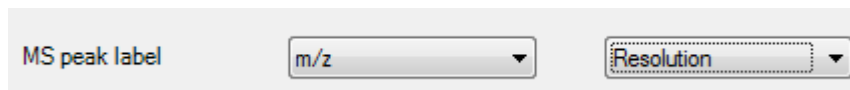
Search Multiple Libraries



How to Set Labels on MS and MS/MS Spectra



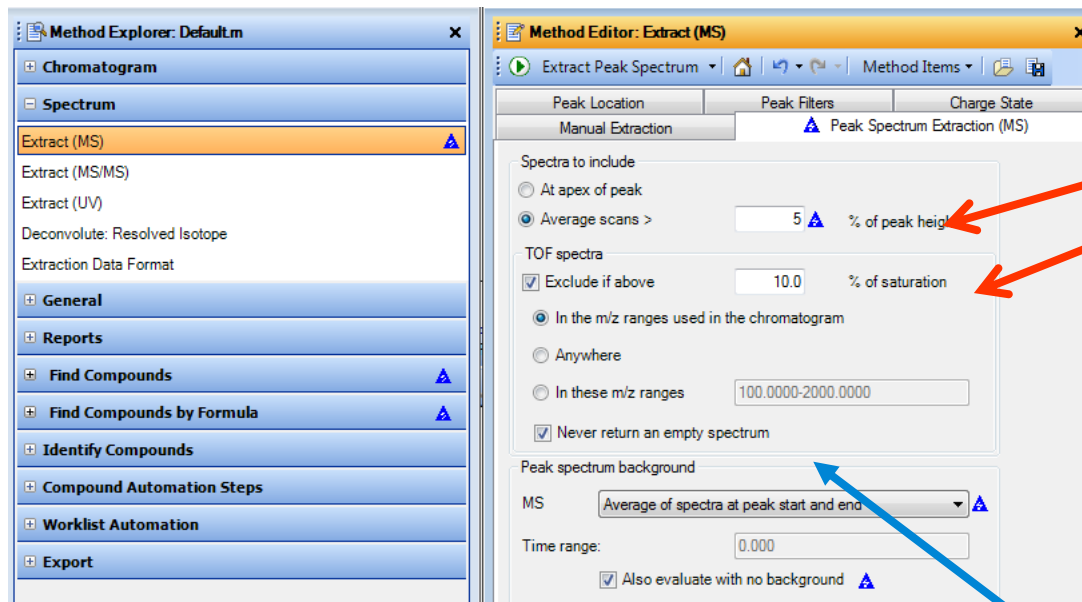
Multiply Charged Species



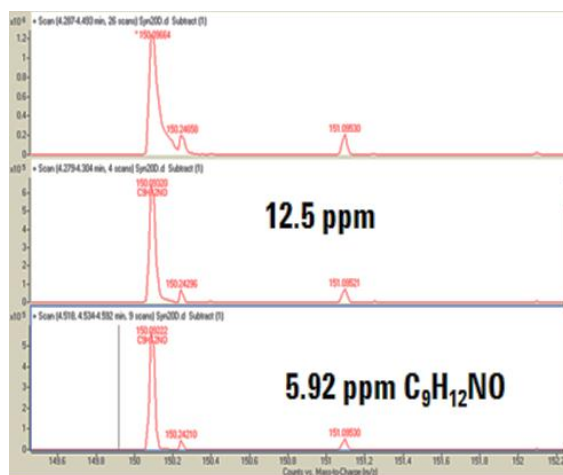
In Profile Mode Resolution of MS Peak



Using Extract Peak Parameters – Saturation Correction



Change 10% to 5%
Change 40% to 10%



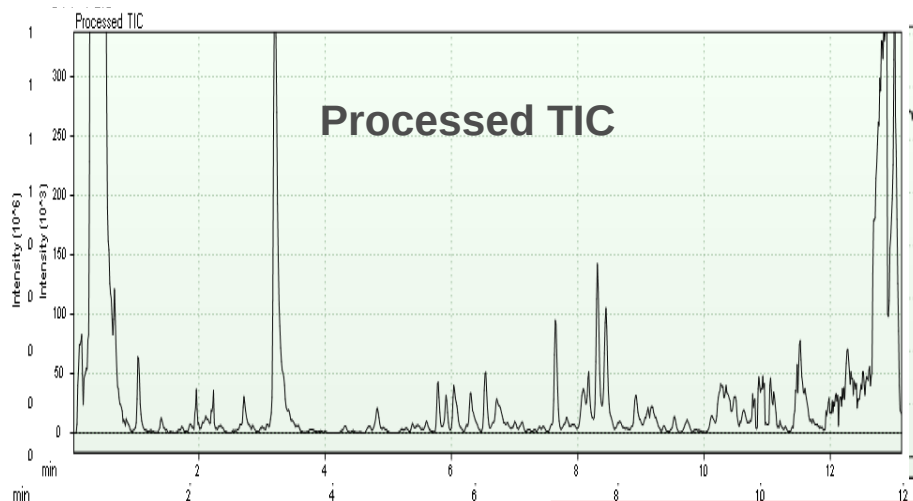
For Narrow Peaks
Remember to Check Never
Return an Empty Spectrum



Unsupervised Naïve data mining

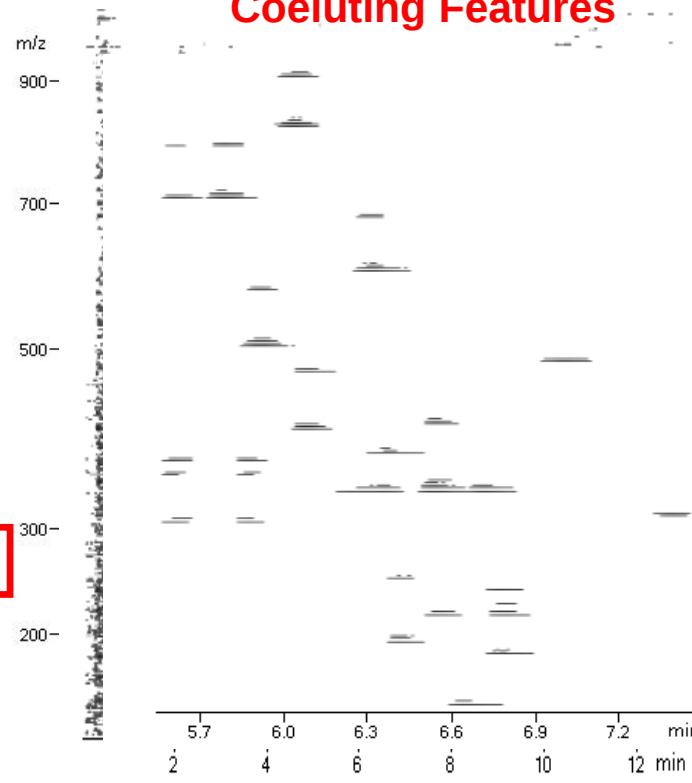
Molecular Feature Extraction (MFE)

Finds Features in TOF/QTOF Data



species	RT	m/z	mass	abund.
M	8.162		342.1467	130643
M+H	8.165	343.1547	342.1474	11889
M+H+1	8.162	344.1581		2290
M+H+2	8.157	345.1748		369
M+H4N	8.164	360.1807	342.1469	8420
M+H4N+	8.156	361.1893		1227
M+Na	8.162	365.1359	342.1466	75678
M+Na+1	8.163	366.1394		15324
M+Na+2	8.162	367.1429		1901
2M+Na	8.164	707.2810	342.1459	4629
2M+Na+1	8.162	708.2860		1808
2M+Na+2	8.173	709.2895		336

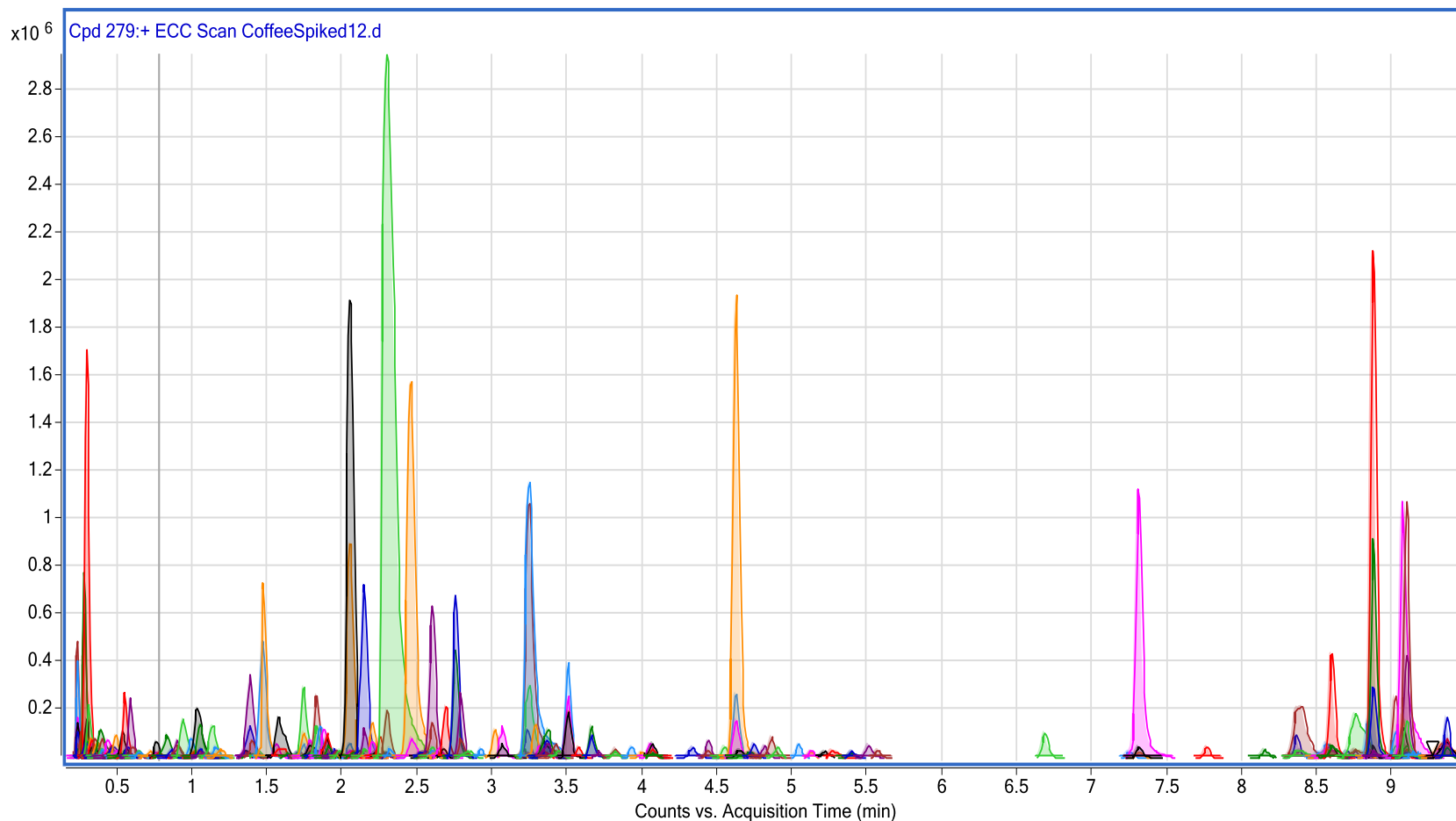
3D Plot Before Coeluting Features



Data Reduced sum intensities of isotopes, adducts, clusters and multiply charges ions together.



Graphical Results Displayed



Over 280 Compounds Found in Coffee Spiked with Pesticide



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May 20, 2014

Choose Isotope Model to Match Application

Peak Location | Peak Filters | Charge State

Isotope grouping

Peak spacing tolerance: 0.0025 m/z, plus 7.0 ppm

Isotope model: Common organic molecules
Common organic molecules
Peptides
Unbiased
Glycans

Charge state

☒ Limit assigned charge state 2

☐ Treat ions with unassigned charge as singly-charged

TIP:

For Compounds containing metals or elemental such as B, Li, Si select **Unbiased**

Check Limit Assigned Charge States Maximum Values

For Small Molecule Applications: Set to 2

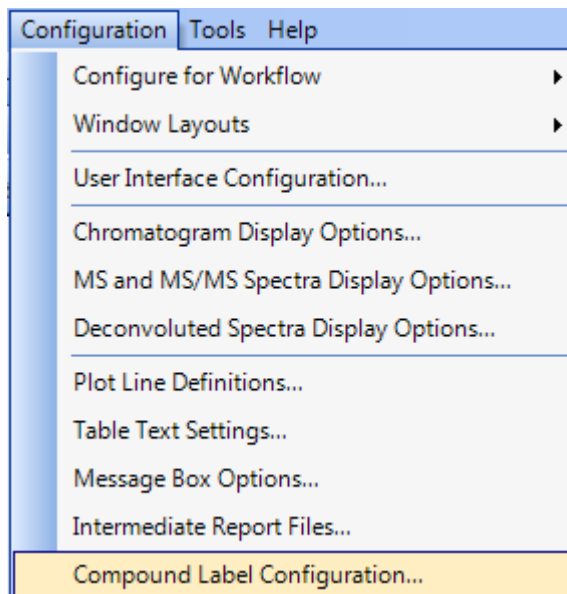
For High Molecular Weight Apps: Uncheck or Max 10



Compounds Labels Display

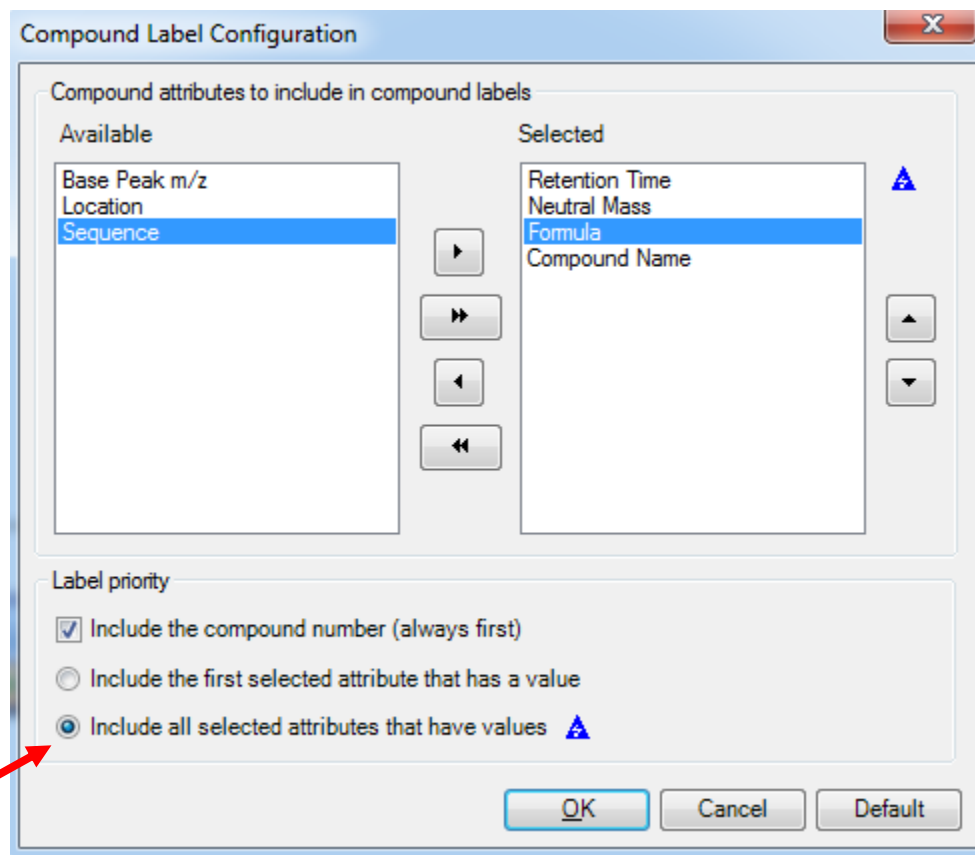
Sort by Data File	
☒	Compounds
☒	Cpd 13: 1.020 169.0848; C7 H11 N3 O2; C7 H11 N3 O2; N(pai)-Methyl-L-histidine
☒	Cpd 14: 1.039 103.0996; C5 H13 N O
☒	Cpd 16: 1.068 161.1047; C7 H15 N O3
☒	Cpd 17: 1.114 113.0586; C4 H7 N3 O; C4 H7 N3 O; Creatinine
☒	Cpd 20: 1.146 115.0992; C6 H13 N O
☒	Cpd 23: 1.193 85.0892; C5 H11 N
☒	Cpd 24: 1.195 140.0581; C6 H8 N2 O2; C6 H8 N2 O2; Ethyl-imidazole carboxylate
☒	Cpd 25: 1.215 170.0687; C7 H10 N2 O3; C7 H10 N2 O3; 2,3,4-Trihydroxybenzylhydrazide
☒	Cpd 26: 1.232 228.1104; C10 H16 N2 O4
☒	Cpd 27: 1.278 143.0945; C7 H13 N O2; C7 H13 N O2; Triparanol
☒	Cpd 28: 1.318 137.0476; C7 H7 N O2; C7 H7 N O2; 2-Pyridylacetic acid
☒	Cpd 29: 1.328 175.0955; C6 H13 N3 O3; C6 H13 N3 O3; Citrulline
☒	Cpd 30: 1.346 202.1316; C9 H18 N2 O3; C9 H18 N2 O3; Ala Ile
☒	Cpd 32: 1.420 85.0895; C5 H11 N
☒	Cpd 33: 1.450 203.1164; C9 H17 N O4; C9 H17 N O4; L-Glutamic acid n-butyl ester
☒	Cpd 34: 1.464 159.1257; C8 H17 N O2; C8 H17 N O2; DL-2-Aminooctanoic acid
☒	Cpd 35: 1.471 211.0948; C9 H13 N3 O3; C9 H13 N3 O3; Zalcitabine
☒	Cpd 37: 1.499 145.0857; C5 H11 N3 O2; C5 H11 N3 O2; 4-(diaminomethylideneamino)butanoic acid
☒	Cpd 38: 1.539 216.1468; C10 H20 N2 O3; C10 H20 N2 O3; Val Val
☒	Cpd 39: 1.613 268.1168; C11 H16 N4 O4; C11 H16 N4 O4; Isobutylglycine
☒	Cpd 40: 1.623 244.0697; C9 H12 N2 O6; C9 H12 N2 O6; Uridine
☒	Cpd 42: 1.646 192.0265; C6 H8 O7; C6 H8 O7; 2,3-Dioxogulonic acid
☒	Cpd 43: 1.647 137.9956; C6 H2 O4
☒	Cpd 44: 1.648 174.0159; C6 H6 O6; C6 H6 O6; Dehydroascorbic acid
☒	Cpd 45: 1.655 180.0643; C7 H8 N4 O2; C7 H8 N4 O2; Theobromine
☒	Cpd 46: 1.660 228.1470; C11 H20 N2 O3; C11 H20 N2 O3; Leu Pro
☒	Cpd 47: 1.667 216.1223; C8 H16 N4 O3
☒	Cpd 48: 1.685 169.0844; C7 H11 N3 O2; C7 H11 N3 O2; N(pai)-Methyl-L-histidine
☒	Cpd 49: 1.685 141.0791; C7 H11 N O2; C7 H11 N O2; Ethosuximide
☒	Cpd 51: 1.775 129.0425; C5 H7 N O3; C5 H7 N O3; Pyroglutamic acid
☒	Cpd 52: 1.776 158.1415; C8 H18 N2 O

Specify Compound Label Configuration



Highlight Parameter in list and use > and < to move to and from **Selected**.

Check **Include all selected attributes that have values** to display all attributes in table.



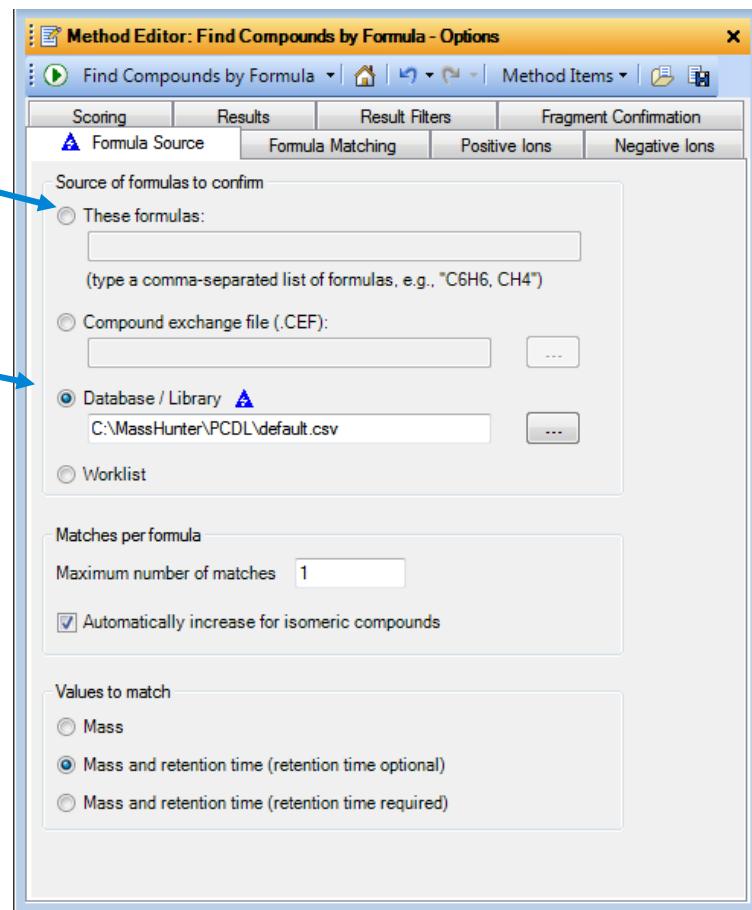
Targeted Data Mining: Find by Formula (FBF)

- **Sources from:**

- Chemical formulae
- CEF file
- PCD/PCDL

- Takes input formula, calculates mono-isotopic mass and isotope pattern filtering
- Extracts and integrates EICs from the data, extracts peak spectra
- Calculates score based on accurate mass, isotope abundance pattern, and isotope spacing

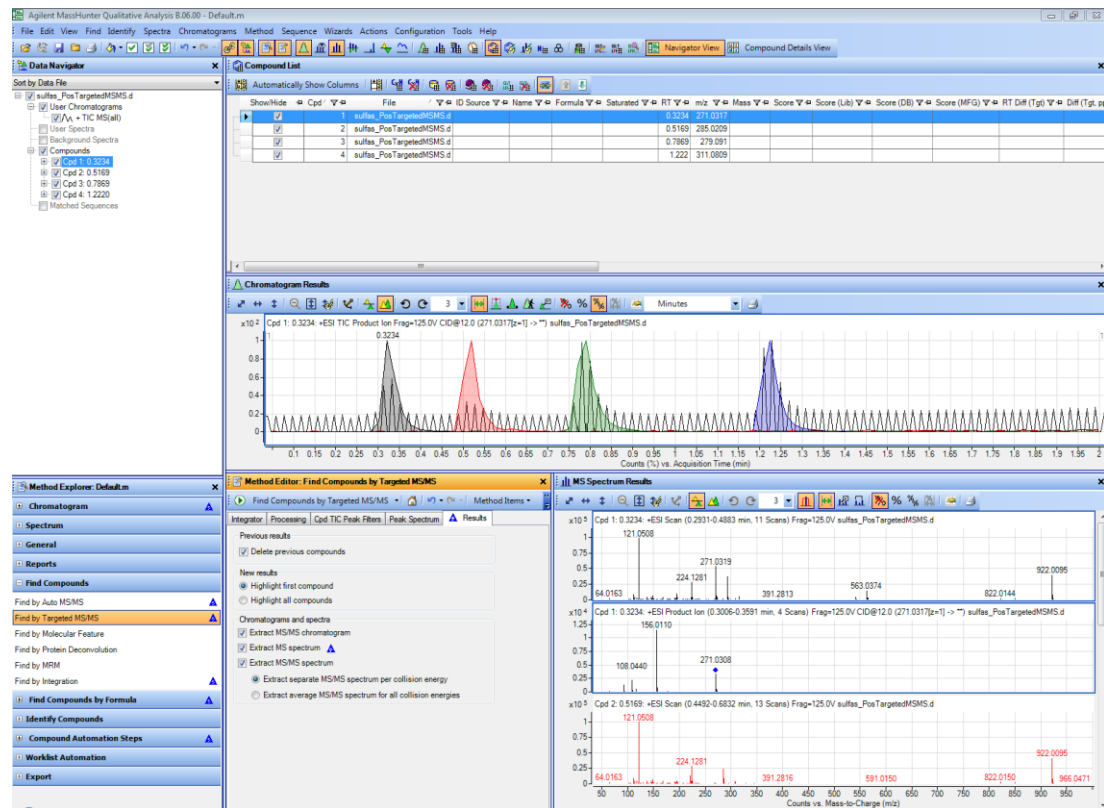
- **Retention Time Matching Optional**



Analysis of MS/MS Data: Multiple Options

Depends on how the data was acquired:

- **MRM for QQQ Data**
- **MFE Extracting MS/MS Data**
- **Auto MS/MS for QTOF**
- **Targeted MS/MS for QTOF**
 - Extracts chromatogram for each targeted mass listed in the acquisition method
- Each compound can have an associated EIC, MS spectrum and MS/MS spectra either an **average** of collision energy or **separated** by collision energy



MFE Extracting MS/MS Data

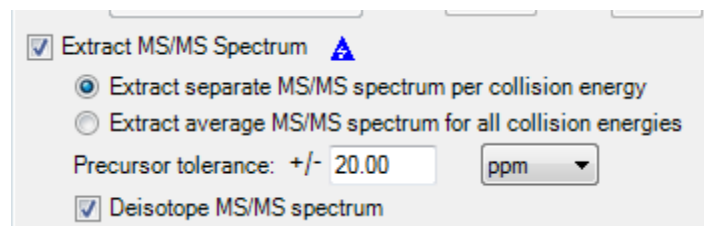
Benefit:

- Identifies Adducts and Groups Them Together
- MS/MS Spectra separated by CE or Combined
- Deisotope MS/MS Spectrum
- Identifies Compounds in which MS/MS Missed
- Easy to Setup MS/MS Inclusion List

Disadvantage:

- No MS/MS Fragment ion Filtering
- Compound List Larger with MS and MS/MS

Results Tab



☒ Extract MS/MS Spectrum ▲

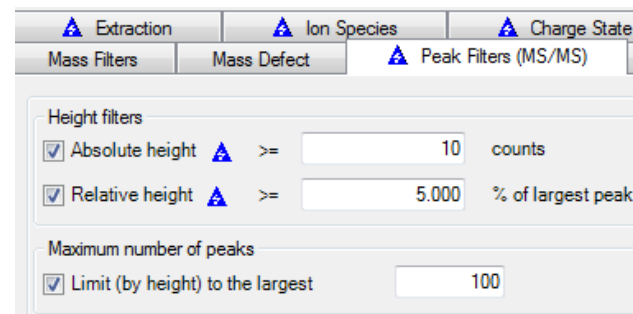
☒ Extract separate MS/MS spectrum per collision energy

☐ Extract average MS/MS spectrum for all collision energies

Precursor tolerance: +/- 20.00 ppm

☒ Deisotope MS/MS spectrum

**Remember to set
MS/MS Peak Filters**



▲ Extraction ▲ Ion Species ▲ Charge State

Mass Filters Mass Defect ▲ Peak Filters (MS/MS)

Height filters

☒ Absolute height ▲ ≥ 10 counts

☒ Relative height ▲ ≥ 5.000 % of largest peak

Maximum number of peaks

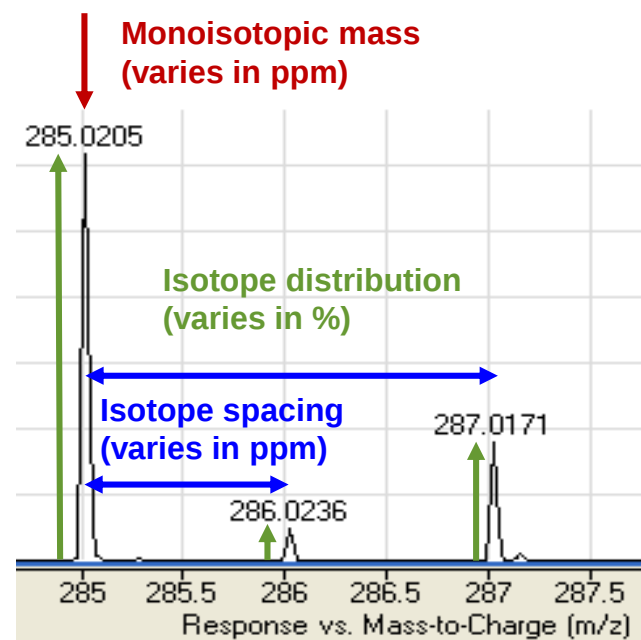
☒ Limit (by height) to the largest 100



Database Searching

- After feature extraction, search for identifications
- Search Database: searches .csv file or PCD
- Search Accurate Mass Library: searches PCDL
- Scoring based on
 - Accurate mass match
 - Isotope abundance
 - Isotope spacing
 - Retention time (if selected)
 - Dot product scoring of MS/MS spectral match
- Forward and/or reverse scoring

Scoring based on



Molecular Formula Generation

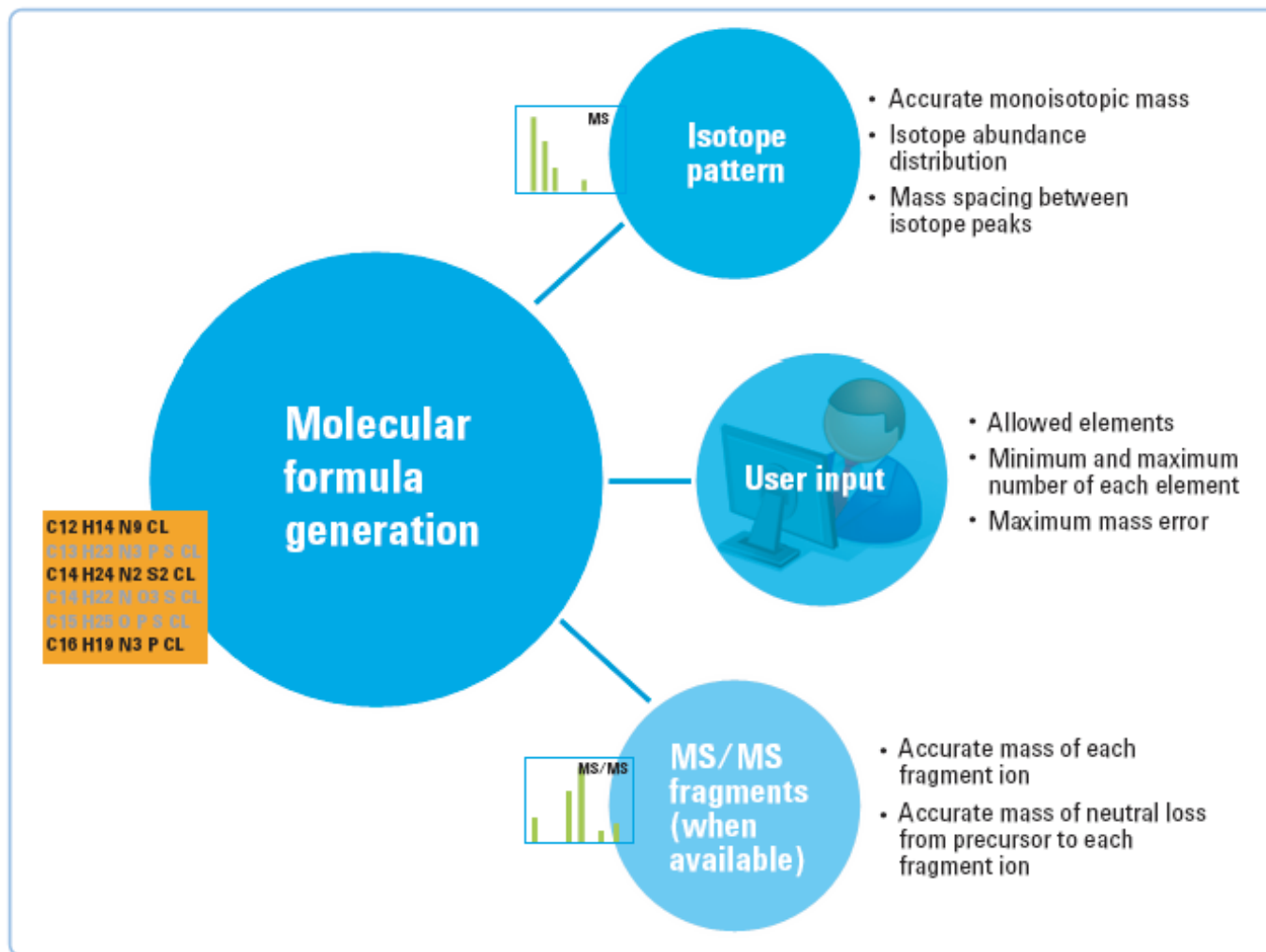
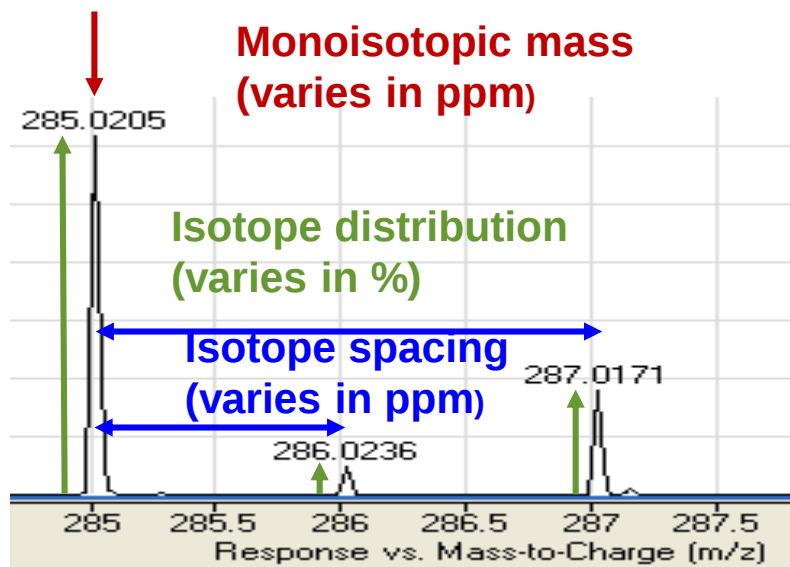


Figure 1. Agilent's molecular formula generation software uses multiple dimensions of information to generate and score lists of possible molecular formulas. It has been optimized for analysis of accurate-mass data from Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS systems.



Agilent's Molecular Formula Generation Software

Scoring based on



Mass Match +

Abund. Match +

Spacing Match =

Overall Score

MS Formula Results: Cpd2: C10H9ClN4O2S

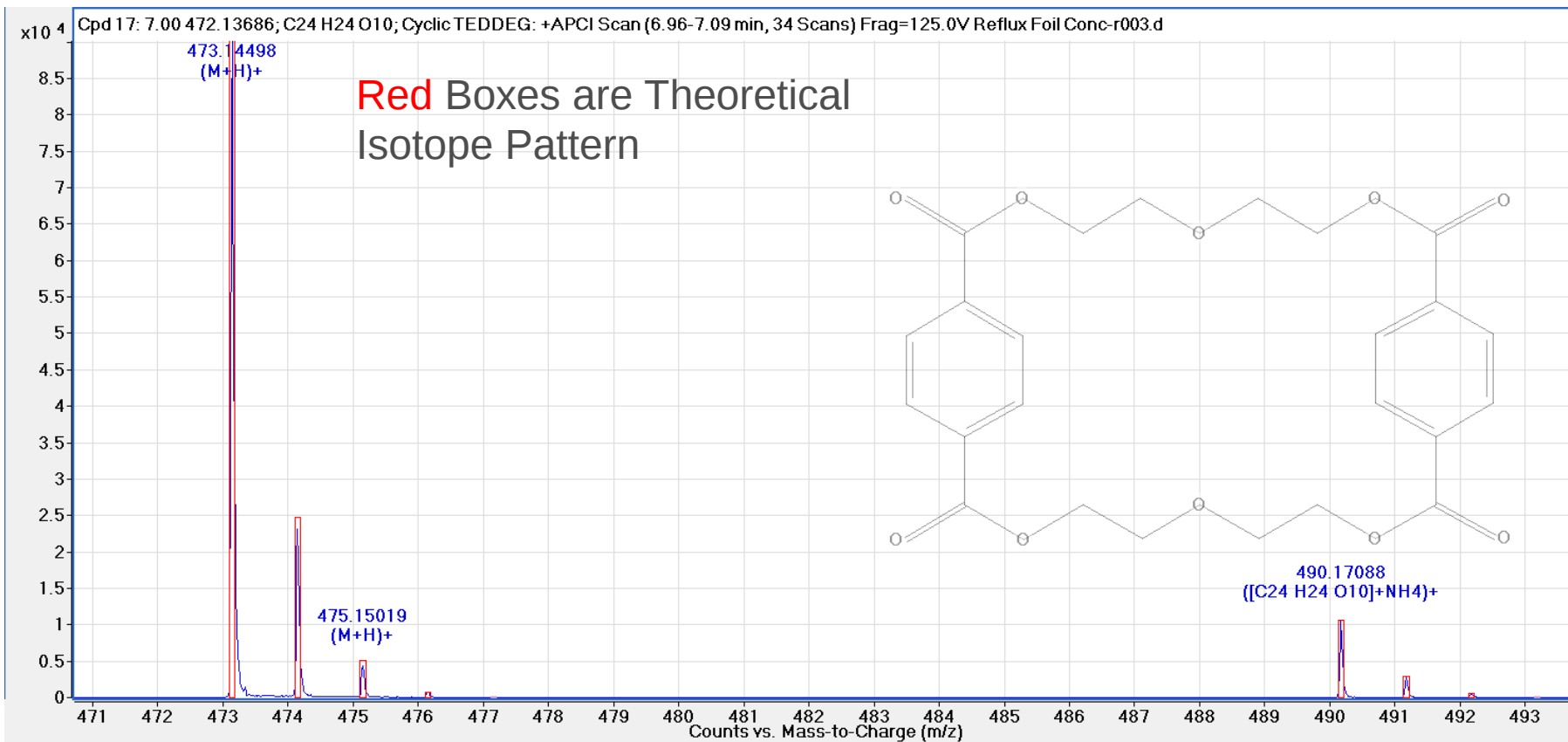
	m/z	Ion	Formula	Abundance
	285.021	(M+H)+	C10H10ClN4O2S	24506.1

Best	Formula (M)	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (p)	Spacing Matc	Abund Matc	Mass Match	m/z	DBE
☒	C10H9ClN4O2S	285.0208	99.55		284.0137	284.0135	-0.71	0.71	99.19	99.26	99.69	285.021	8
☐	C7H12N2O6S2	285.021	77.28		284.0137	284.0137	0.01	0.01	99.54	1.93	100	285.021	3
☐	C7H13ClN4O2S2	285.0241	75.57		284.0137	284.0168	11.12	11.12	99.87	83.87	46.22	285.021	3



Agilent Technologies

Isotope Pattern Matching



Molecular Formula Generation

Isotope Pattern Matching and MS/MS for Formula confirmation

Identify
Compounds

Agilent MassHunter Qualitative Analysis - Default.m

MS Formula Results: Compound 41

m/z	Species	Formula	Abundance
285.01973	(M+H) ⁺	C ₁₀ H ₁₀ N ₄ O ₂ SCl	58159

Best	Formula	Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm)	MS Score	MS/MS Score	Coverage	DBE
☒	C ₁₀ H ₉ N ₄ O ₂ SCl	100	284.01246	284.01347	3.59	3.59	100	100	100	8

Isotope	Abund%	Calc Abund%	m/z	Calc m/z	Difference (ppm)
1	100	100	285.01973	285.02075	3.57
2	12.81	13.26	286.02167	286.0232	5.37
3	33.92	37.7	287.01714	287.0179	2.65
4	4.23	4.92	288.01833	288.02023	6.58
5	1.63	1.89	289.01491	289.0157	2.72
6	0.17	0.22	290.01673	290.01726	1.83

MS Score based on:

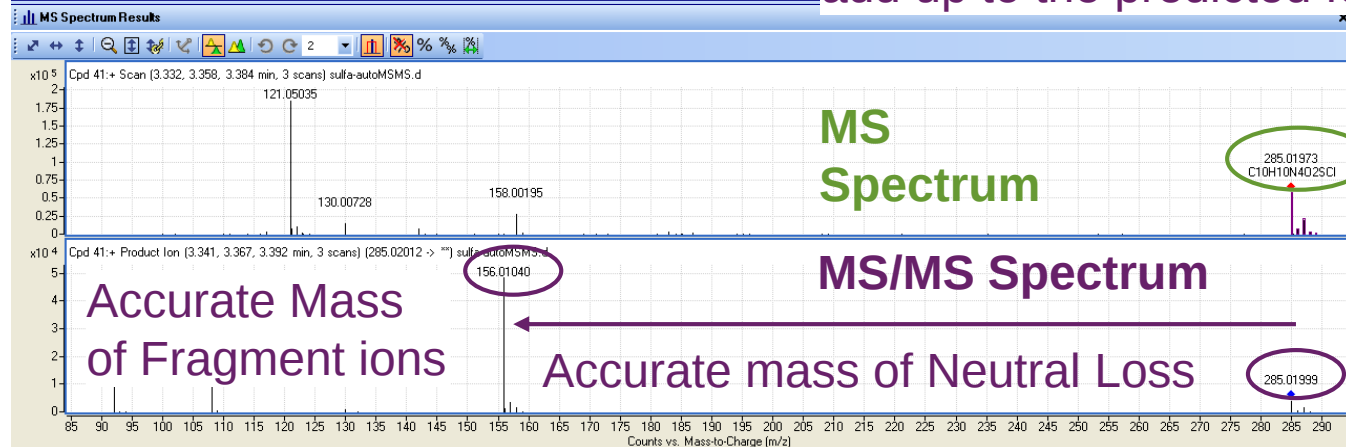
- Mass main Isotope
- Isotope Pattern
- Mass M+1 and M+2

MS/MS Formula Details: Compound 41 C₁₀H₉N₄O₂SCl

m/z	Abund	Formula	Mass	Frag Mass	Difference (ppm)	Loss Mass	Loss Formula
92.04927	9722	C ₆ H ₅ N	91.04199	91.0422	2.27	192.97127	C ₄ H ₄ N ₃ O ₂ SCl
92.04927	9722	C ₅ H ₅ N ₂	91.04199	91.04567	39.3	192.9679	C ₇ N ₃ O ₂ Cl
92.04927	9722	CH ₅ N ₃ O ₂	91.04199	91.03818	-41.91	192.9753	C ₉ H ₄ N ₅ Cl
108.04397	10541	C ₆ H ₅ NO	107.03669	107.03711	3.92	176.97636	C ₄ H ₄ N ₃ O ₂ SCl
108.04397	10541	C ₃ H ₉ NO ₅	107.03669	107.04048	35.41	176.97299	C ₇ N ₃ O ₂ Cl
156.0104	52240	C ₆ H ₅ NO ₂ S	155.00312	155.0041	6.29	129.00937	C ₄ H ₄ N ₃ O ₂ SCl
156.0104	52240	C ₉ H ₉ NO ₂	155.00312	155.00073	-15.45	129.01275	CH ₈ N ₃ SCl

MS/MS Score based on:

- Ability to calculate molecular formulas for each fragment & the corresponding neutral loss which add up to the predicted formula for the precursor



Accurate mass
information used:

- Precursor ion
- Isotopes M+1, M+2
- Fragment ion
- Neutral loss



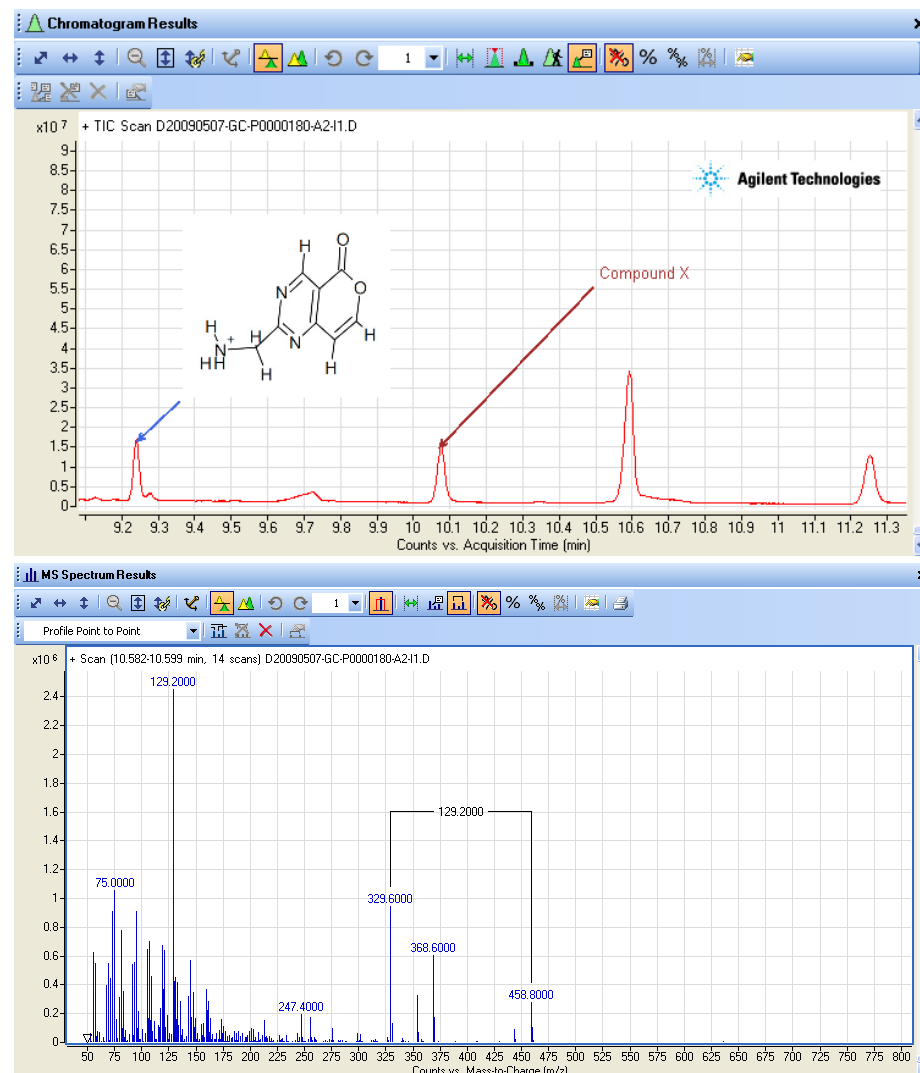
Agilent Technologies

Annotate and Document

Annotate chromatograms and spectra using graphics (*.jpg), text, and chemical structures (.mol)

Use *Mass Caliper* to document fragmentation and losses in spectrum

Export or use copy & paste to add graphics or results into presentations and documents

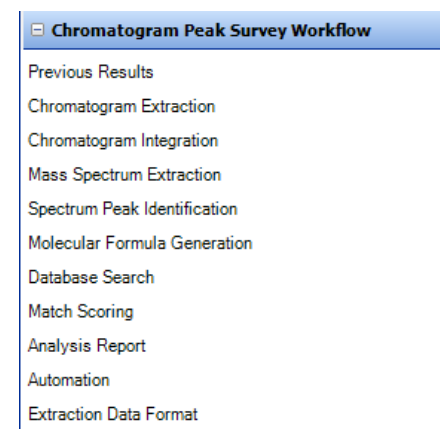
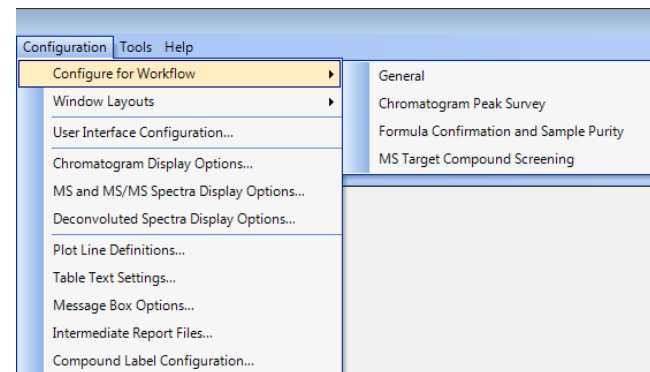


Workflows in Qual

- **Workflows guide users through Qual functions needed for specific tasks**

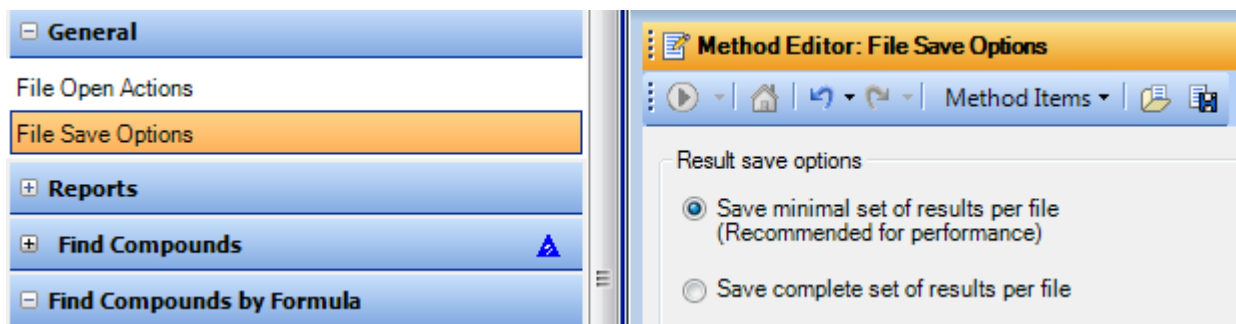
- Load via Configuration Menu on Toolbar
- Default method with default parameters and report templates
- Specified layout of user interface limited to needed windows
- Section at the top of the Method Explorer grouping together the relevant functions

- **Helpful as a starting point for new users and as a preliminary analysis before “deep dive” type data analysis**



Saving Results

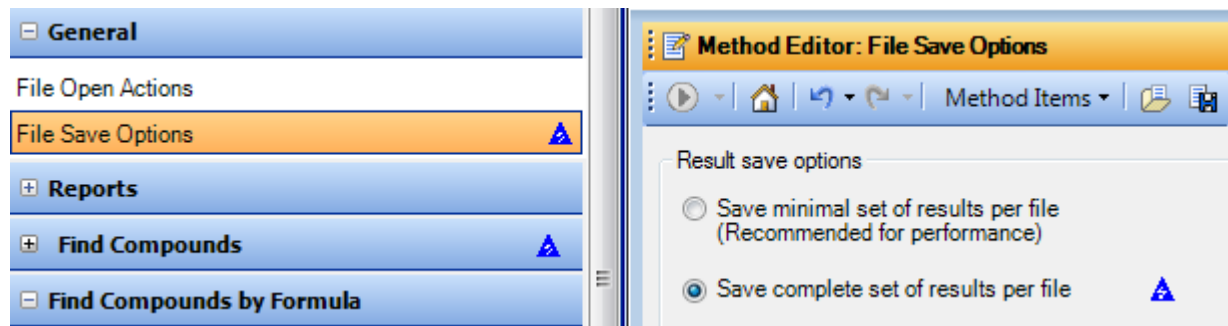
The Method default saves Minimal Amount of Graphics



Default Setting

**Graphics not saved
only compound list**

Change to “Save Complete Results”



All Graphics Saved

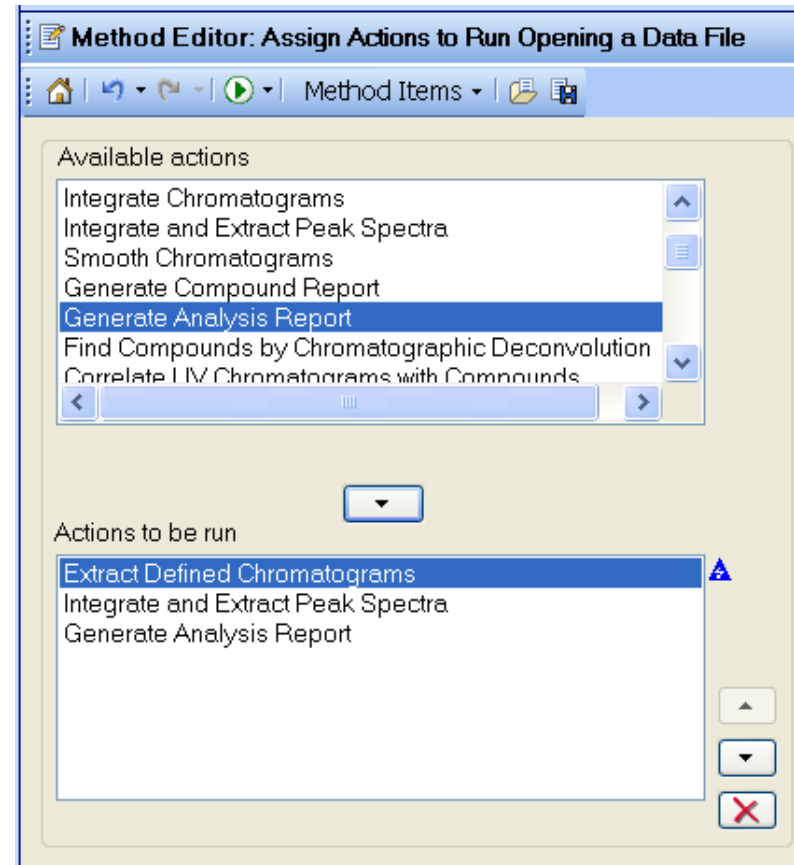
Automation - File Open Actions & Post Acquisition Worklist Processing

Automate common file open actions in manual review

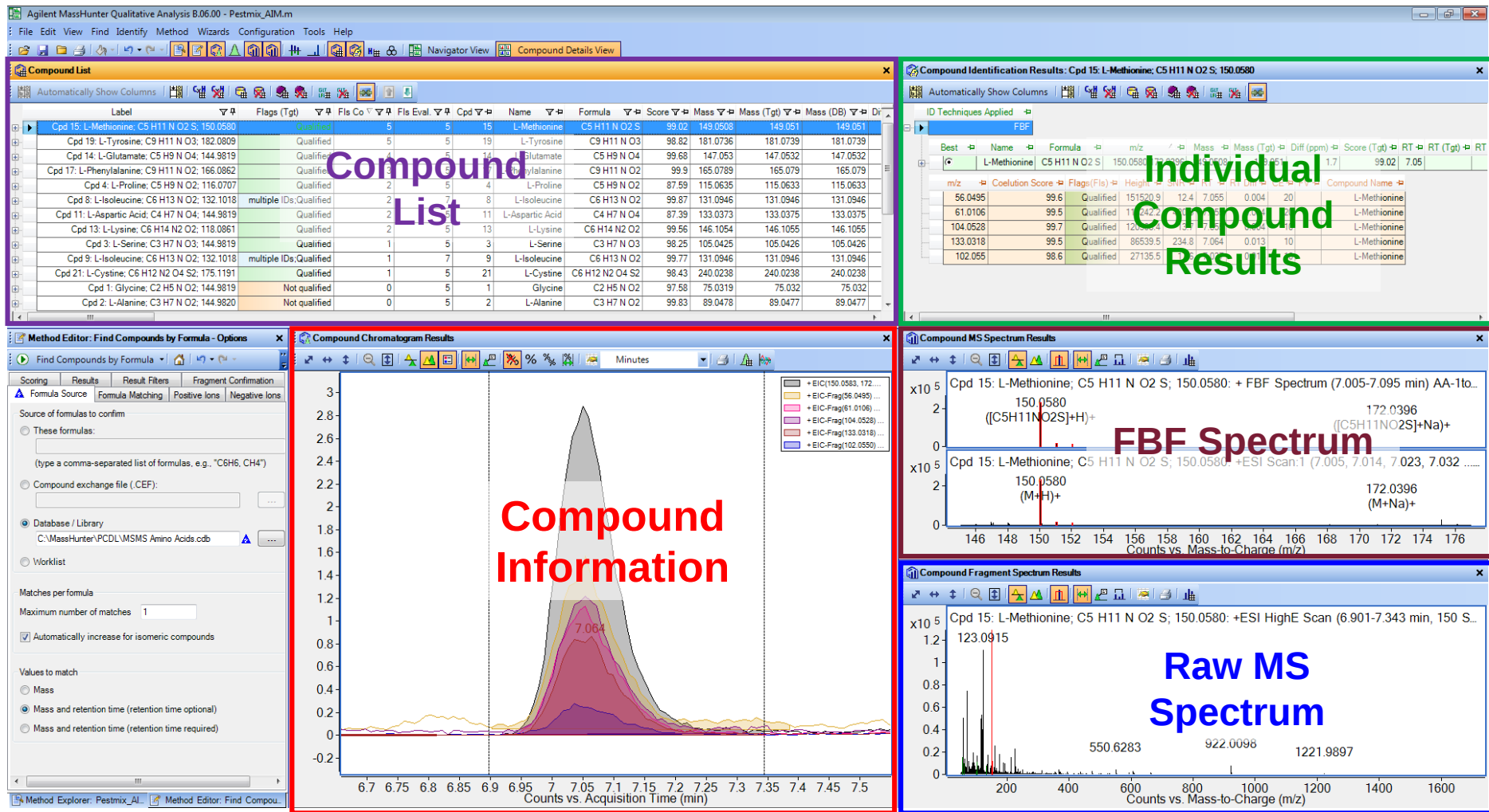
- Standardize review processes
- Automate common actions, i.e. extract EIC.

Define Qualitative methodology for sequence computation.

- No user interaction required.

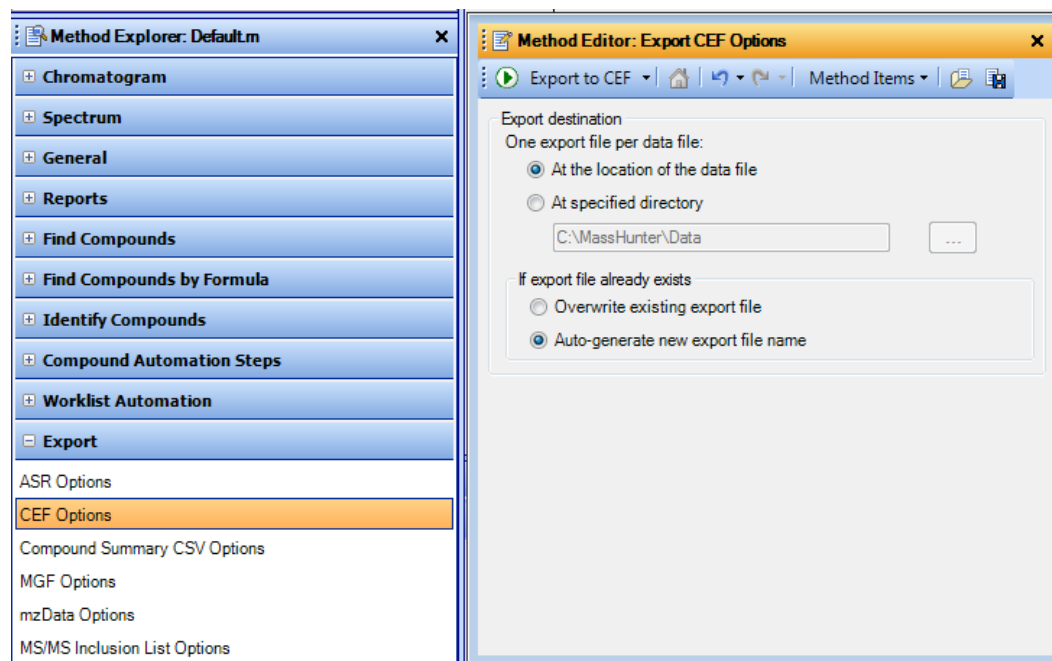


Compound Details View

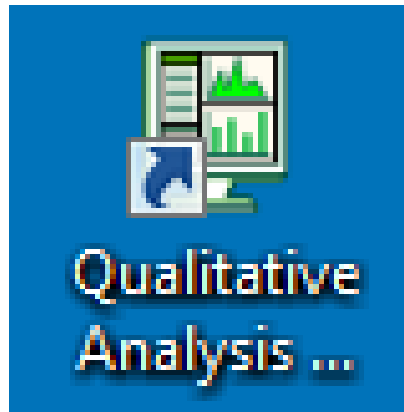


What is a .cef file?

- **C**ompound **E**xchange **F**ormat file
- Agilent-specific file format that moves compound information back and forth between Qual, MPP, and other Agilent software
- XML-based – the amount of information contained depends on what the user has extracted
 - Accurate mass, retention time
 - Formulas
 - Spectra
- Can be edited manually

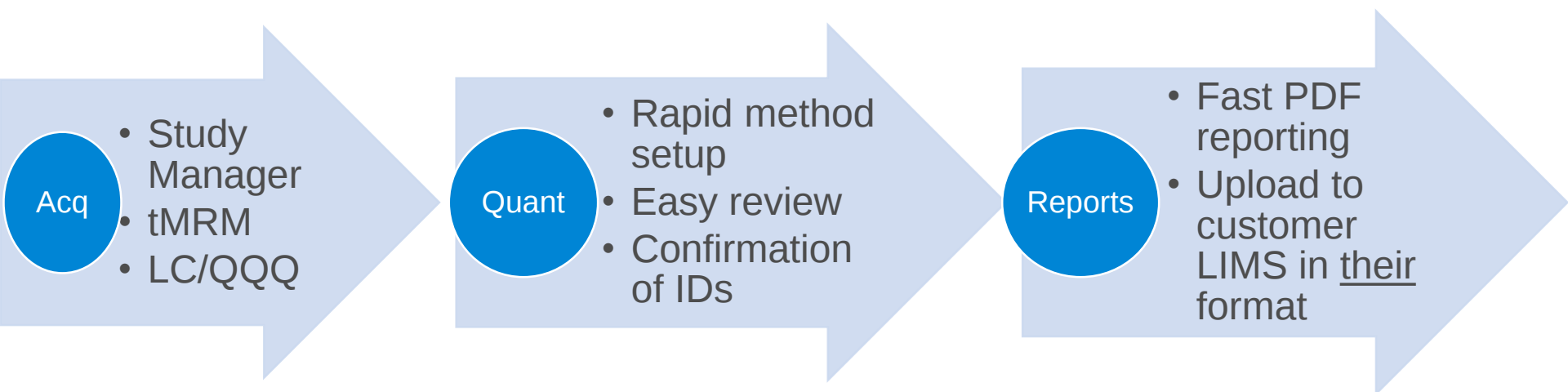


Questions about MassHunter Qual?



The MassHunter Quantitative Workflow

Quantify with confidence



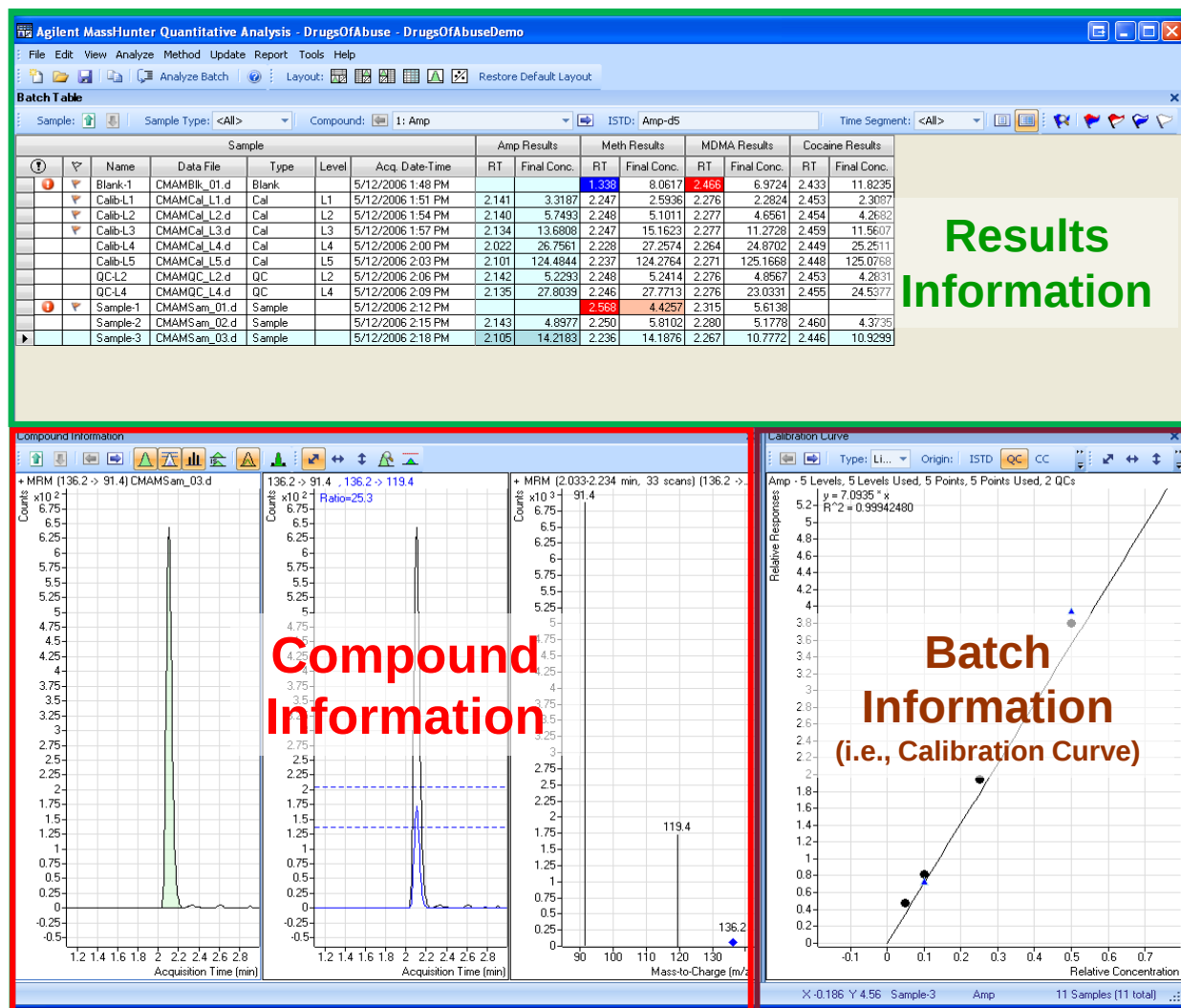
Agilent provides fast setup and running of quantitative methods from acquiring samples to the final report



Quantitative Analysis

The Quant Batch screen:

- Results Information** for the batch can be displayed. Visual guides highlight specific data that fall outside specific, predefined conditions.
- Compound Information** displays graphical representation of the peak, qualifier information, spectral information, and the ISTD. Visual guides help identify associated data problems.
- Batch Information** provides easy visualization and customization of relevant desired data.



Batch Screen – Sample & Results Information

In **Results Information**, the Quantitation Message contains a list of error messages that result from a compound's quantitation.

Sample:		Sample Type: <All>		Compound: 1: Amp		ISTD: Amp-d5		Time Segment: <All>														
Compound Group: <All>																						
Sample						Amp Met...	Amp Results							Qualifier (136.2 - ...)			Amp-d5 (IS...		Qualifier (141.1 - ...)			
		Name	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
		Blank-1	Blank		11/11/2006 11:05 AM	1.0																
		Calib-L1	Cal	L1	11/11/2006 11:10 AM	1.0	2.5000	2.141	658	49.10		2.1151	2.1151	84.6	24.3	45.47		2.129	1397	25.9	Infinity	
		Calib-L2	Cal	L2	11/11/2006 11:15 AM	1.0	5.0000	2.140	1059	42.25		4.5770	4.5770	91.5	33.5	Infinity		2.128	1298	25.9	48.46	
		Calib-L3	Cal	L3	11/11/2006 11:20 AM	1.0	12.5000	2.134	2673	107.28		12.6107	12.6107	100.9	27.7	146.48		2.121	1377	26.3	46.09	
		Calib-L4	Cal	L4	11/11/2006 11:25 AM	1.0	25.0000	2.022	4952	20.26		25.8545	25.8545	103.4	29.1	49.40		1.990	1304	28.8	21.54	
		Calib-L5	Cal	L5	11/11/2006 11:30 AM	1.0	125.0000	2.101	18605	47.90		124.8426	124.8426	99.9	27.0	39.22		2.076	1053	26.4	Infinity	
		QC-L2	QC	L2	11/11/2006 11:35 AM	1.0	5.0000	2.142	1006	81.00		4.0502	4.0502	81.0	27.7	34.47		2.131	1356	31.1	42.99	
		QC-L4	QC	L4	11/11/2006 11:40 AM	1.0	25.0000	2.135	4716	91.48		26.9159	26.9159	107.7	25.3	60.79		2.121	1196	31.1	91.84	
		Sample-1	Sample		11/11/2006 11:50 AM	1.0																
		Sample-2	Sample		11/11/2006 11:55 AM	1.0		2.143	1004	80.65		3.7144	3.7144		30.9	70.64		2.130	1445	25.7	29.89	
		Sample-3	Sample		11/11/2006 11:59 AM	1.0		2.105	2590	74.97		13.1551	13.1551		25.3	65.40		2.089	1284	29.8	129.91	

Quantitation Message(s)

Amp-d5: Integrator did not find any peaks
 Amp-d5: Qualifier M/Z = 124.4: Qualifier peak not found or does not match quantitation criteria
 Cocaine-d3: Integrator did not find any peaks
 Cocaine-d3: Qualifier M/Z = 85.0: Qualifier peak not found or does not match quantitation criteria
 Meth-d5: Qualifier M/Z = 121.4: Integrator did not find any peaks

Outlier(s) **Blue** = low / **Red** = high

Amp: Qualifier ratio = 33.5 is outside the allowed range [21.2, 31.8]

These icons filter outliers in the display.

"Outliers" define and specify results of known problem samples/substances that fall outside predefined conditions.



Results Information: Outlier Options

More than 40 quality checks can be specified to highlight outliers in **results**.

- Set high and low limits.

Custom Calculations can be added for User defined quality checks

Amp Results		
RT	Final Conc.	Accuracy
2.141	3.3187	132.7
2.140	5.7493	115.0
2.134	13.6808	109.4
2.022	26.7561	107.0
2.101	124.4844	99.6
2.142	5.2293	104.6
2.135	27.8039	111.2
2.143	4.8977	
2.105	14.2183	

Outlier Setup Tasks

Retention Time

Relative Retention Time

Peak Resolution

Peak Symmetry

Peak Full Width Half Maximum

Peak Purity

Signal-to-Noise Ratio

Limit Of Detection

Limit Of Quantitation

Method Detection Limit

Qualifier Ratio

ISTD Response

ISTD Response Percent Deviation

Sample Amount

Sample RSD

Blank Concentration

Blank Response

Accuracy

Average Response Factor

Average Response Factor RSD

Curve Fit R2

Relative Response Factor

Response Factor

QC

QC Relative Standard Deviation

CC Average Response Factor

CC ISTD Response Ratio

CC Relative Response Factor

CC Response Ratio

CC Retention Time

Matrix Spike

Matrix Spike Percent Difference

Matrix Spike Percent Recovery

Matrix Spike Group Recovery

Surrogate

Surrogate Percent Recovery

Response Check

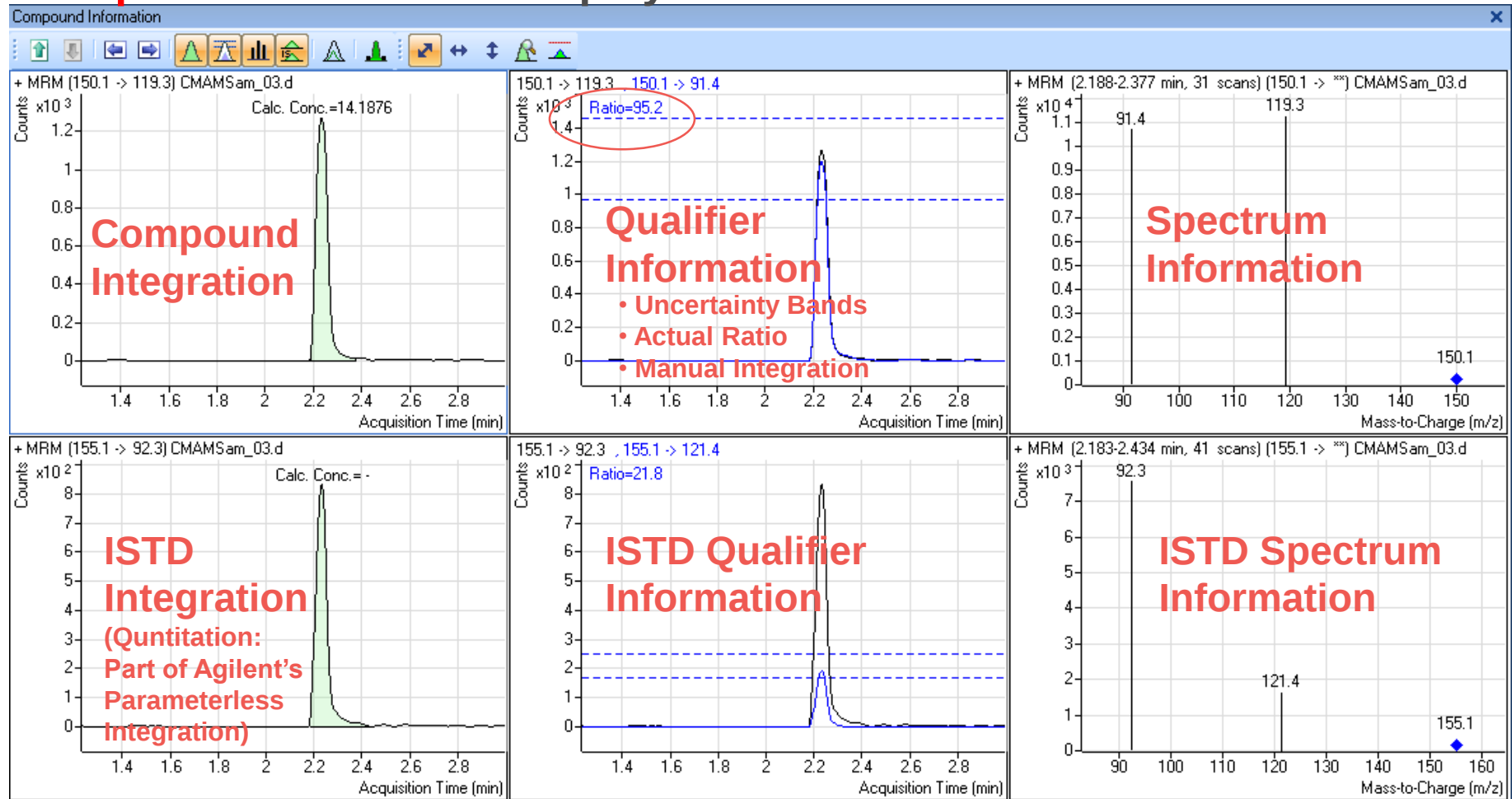
Mass Accuracy

Custom Calculation



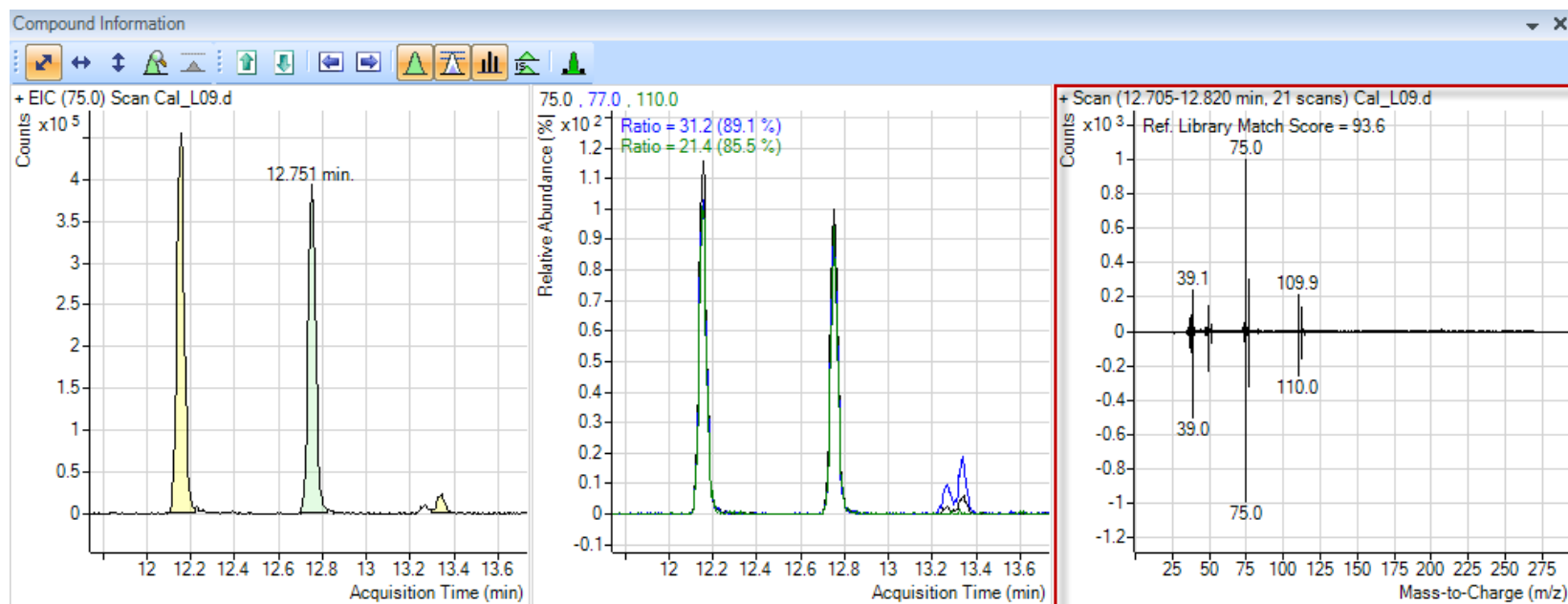
Batch Screen - Compound Information

Compound information displays all relevant information in one view.



Batch Screen - Library Reference Spectra

- Confirmation of Compound Identification
- Visual comparison of Sample and Library Spectra
- Seen in Batch-at-a-Glance and on Reports
- Extracts Spectra from Library by matching on CAS number based in Quant method and creates a small reference library (reflibrary.xml)

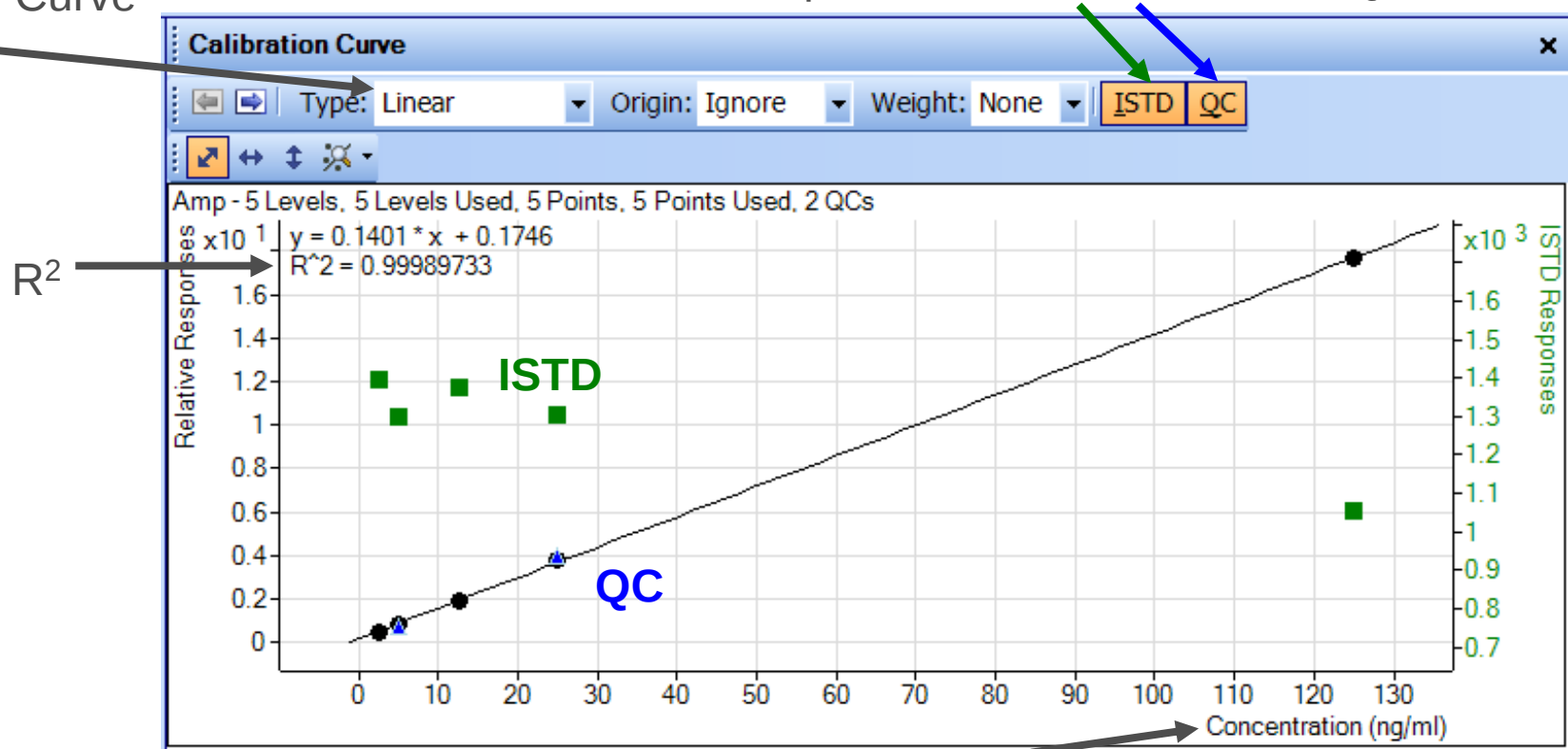


Batch Screen – Calibration Curve

An example of **Batch Information** is this view of the Calibration Curve. The Curve fit can be changed and data can be updated instantaneously.

To view ISTD responses or display QC samples click either the ISTD or QC button.

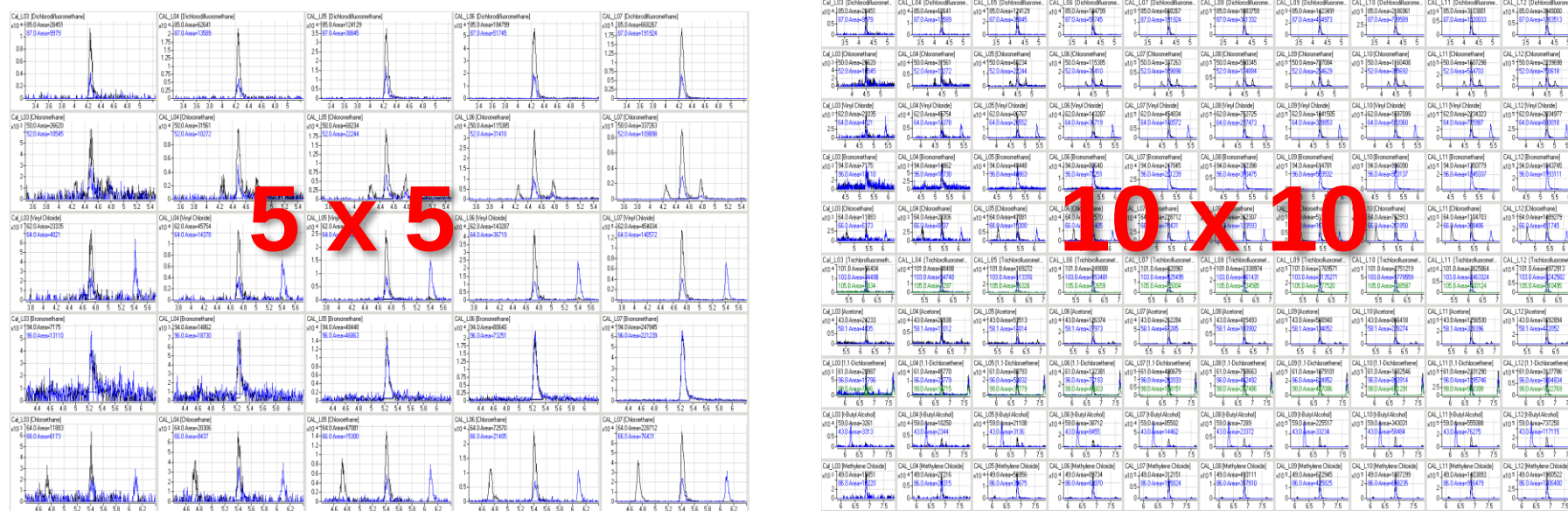
Change Curve
Fit



Concentration can be set as relative (to ISTD) or actual.

Compounds-at-a-Glance

Compounds-at-a-Glance allows you to view multiple traces of compounds at a single glance.

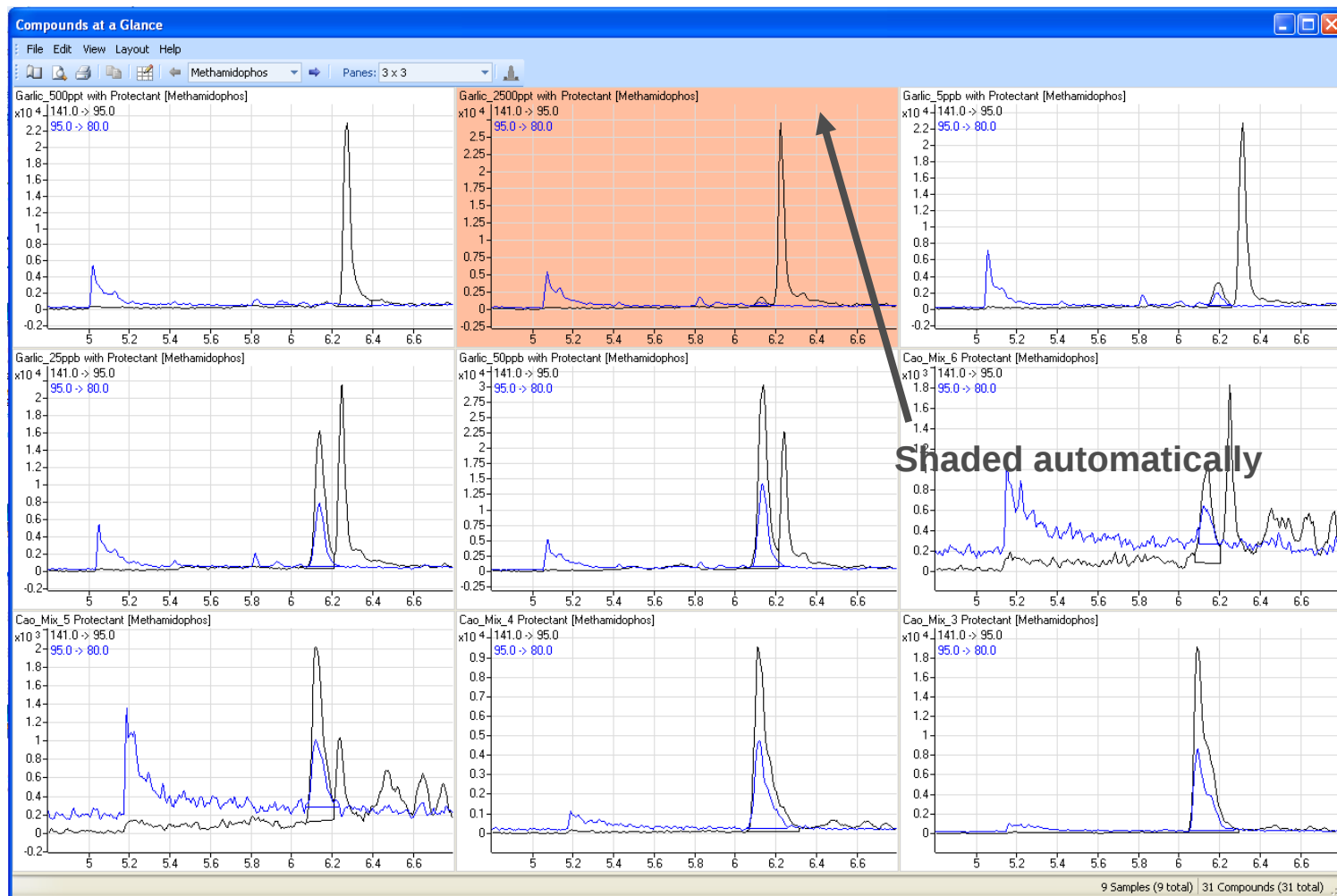


- View up to 10 x 10 chromatograms
- Overlay Target with ISTD
- Overlay Quantifier with Qualifiers
- View chromatograms across 100 samples



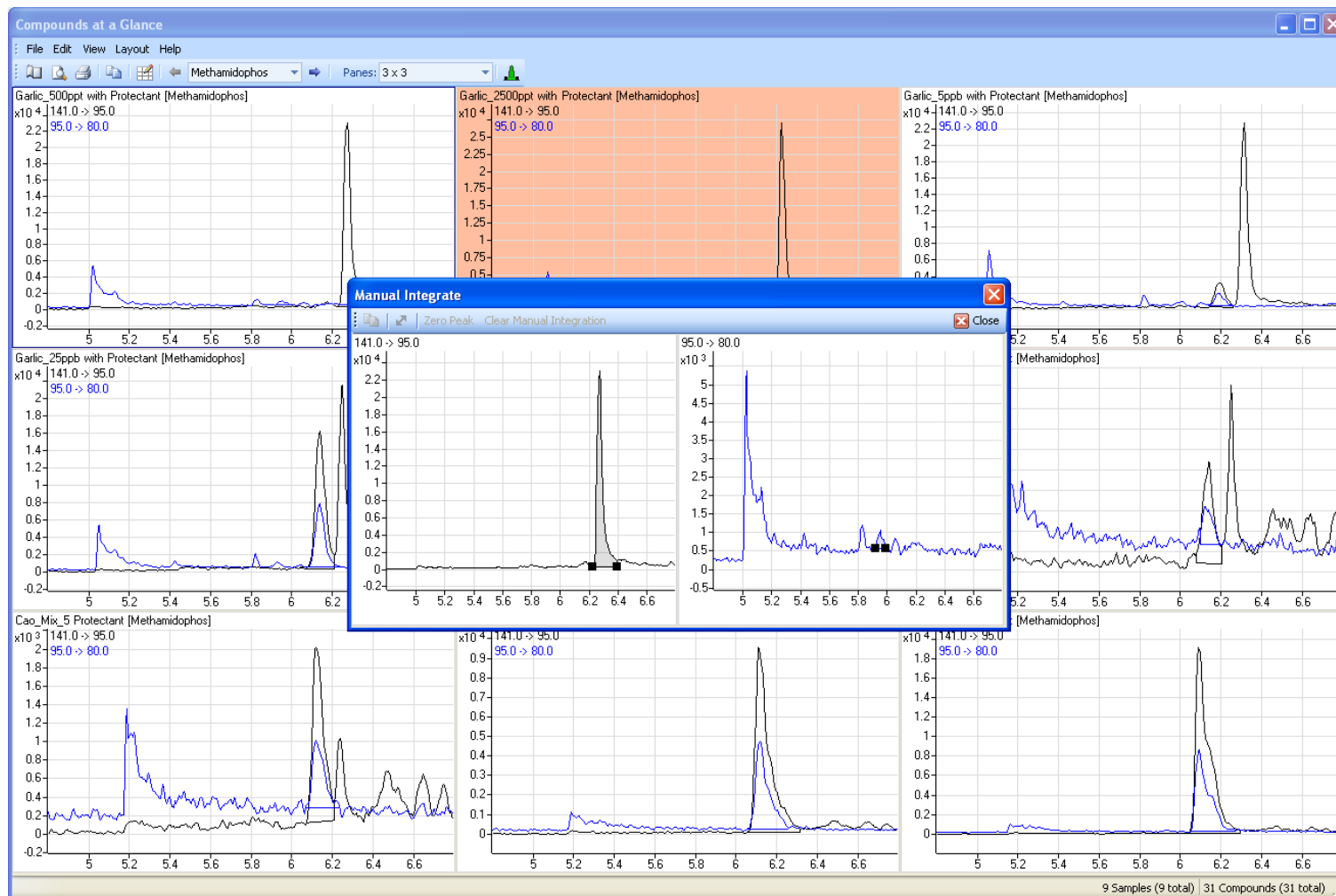
Compounds-at-a-Glance

You can also view compound outliers at-a-glance.



Compounds-at-a-Glance

You can perform manual integration of compounds-at-a-glance.



Simplify Quantitation Method Creation

Easily create a target method from acquired data (SIM or Scan).

- Compound Information from a data file - populates Compound Name, Retention Time, Quant and Qual ions, and ion ratios automatically.

The screenshot displays the Agilent MassHunter Quantitative Analysis software interface. The 'New Method' dialog is open, showing options to create a new method from acquired MRM or Scan data, or from a file. The 'Method Table' is also visible, showing a list of compounds and their associated data.

Method Table Data:

Sample	Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
CMAMCal_L4.d	Amp	CMAMCal_L4.d	MRM	5.0	Target	2.020
	Cocaine	CMAMCal_L4.d	MRM	5.0	Target	2.449

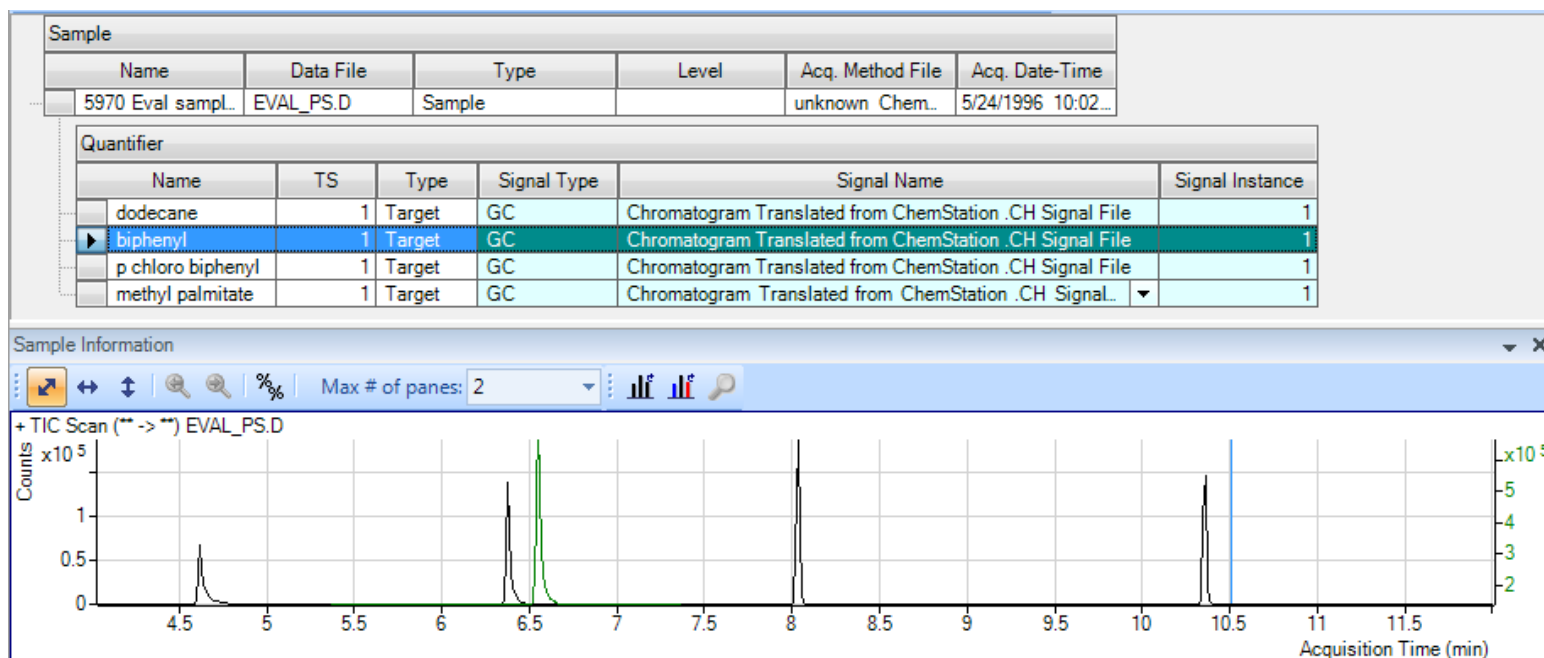
Quantifier Data:

Quantifier	Name	TS	Transition	Collision	Scan	Type	RT
Amp	136.2	91.4	5.0	MRM	Target	2.020	
	136.2	119.4	28.0	20.0			
Amp-d5	141.1	93.4	5.0	MRM	ISTD	1.990	
	141.1	124.4	27.9	20.0			
Cocaine	304.1	182.0	5.0	MRM	Target	2.449	
	304.1	182.0	5.0	MRM	Target	2.449	



Quantitate Using 2-D Signals (GC and LC)

In addition to Scan and SIM data, MassHunter can also quantitate with 2-D signals, such as FID and UV. Set-up and Quantitation of GC and LC signals is the same as for GC/MS or LC/MS data.



Compound Specific Integration Choice: Including parameterless integration

Method Table

Level Name Prefix: # of Levels: 10 Create Levels Time Segment: <All> Compound: Amp Reset Table View

Sample	Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Calib-L5	CMAMCal_L5.d	Cal	L5	APCautotune.m	5/12/2006 2:03...	

Quantifier

Name	TS	Transition	Scan	Type	RT	Int.	Int. Params.
Amp	1	136.2 → 91.4	MRM	Target	2.102	Agile	

Qualifier

Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Int. Params.
136.2	119.4	136.2 → 119.4	26.8	20.0	

Quantifier

Name	TS	Transition	Scan	Type	RT	Int.	Int. Params.
Amp-d5	1	141.1 → 93.4	MRM	ISTD	2.078	MS-MS (GC)	

Qualifier

Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Int. Params.
141.1	124.4	141.1 → 124.4	26.4	20.0	

Quantifier

Name	TS	Transition	Scan	Type	RT	Int.	Int. Params.
Cocaine	1	304.1 → 182.0	MRM	Target	2.449	Universal	

Compound Information

+ MRM (136.2 → 91.4) CMAMCal_L5.d

RT=2.102 min.
Name=Amp
Area=19301

+ MRM (141.1 → 93.4) CMAMCal_L5.d

RT=2.078 min.
Name=Amp-d5
Area=1048

Integration

Integrator: Agile, MS-MS, MS-MS (GC), General, Universal

OK Reset Default Cancel Apply

Agile2 NEW

Questions about Quant?



Look for “Quant Schema” on your Quant install disc
for very detailed help and descriptions!

Personal Compound Databases and Libraries

Confident identification using your mass spec

Identify
Compounds

PCDs (accurate mass database) and PCDLs (MS/MS libraries) allow you to search your data to identify compounds using accurate mass against reference spectra

PCD/PCDL	Market	Compounds	Compounds with MS/MS Spectra
Pesticides	Food Safety	1669	733
Forensics/Tox	Forensics/Tox	9008	3019
Veterinary Drugs	Food / Forensics	1049	630
METLIN	Metabolomics	64,092	8040

PCDL Manager

- Allows user to view, edit, and rearrange PCDs and PCDLs
- Can create custom PCDs and PCDLs, including addition of MS/MS spectra from acquired data
- **Custom Databases:** Polymer Additives, Coffee, Lipids, HMDB, Natural Products

MassHunter PCDL Manager for Metabolomics - C:\MassHunter\PCDL\MSMS Amino Acids.cdb

File Edit View PCDL Links Help

Find Compounds

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Mass: [] (M+H)⁺ Neutral (M-H)⁻

Mass tolerance: 10.0 ppm mDa

Retention time: [] Require

RT tolerance: 0.1 min

Ion search mode:

- ☒ Include neutrals
- ☒ Include anions
- ☒ Include cations

Formula: [] Name: valine Notes: [] IUPAC: []

CAS: [] KEGG: [] ChemSpider: [] HMP: [] METLIN: [] LMP: []

Molecule: Structure MOL Text

Notes: Positive MS/MS Endogenous Metabolite Geigy vol. 3 p. 92

Single Search Results: 1 hit for

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	METLIN	HMP	KEGG	LMP	IUPAC Name	Num Spectra
L-Valine	C5H11NO2	117.07898			72.18-4		35		HMDB008...	C00183			4

MassHunter PCDL Manager for Metabolomics - C:\MassHunter\PCDL\MSMS Amino Acids.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Mass: [] Precursor ion: [] Ion polarity: (Any)

Tolerance: 200 ppm mDa

Collision energy: []

Tolerance: 2.0 eV

Ionization mode: (Any)

Spectra for compound: L-Valine

Compound Name	Precursor Ion	Collision Energy	Ion Polarity	Ionization Mode	Instrument Type
L-Valine	118.08626	10	Positive	ESI	QTOF
L-Valine	118.08626	20	Positive	ESI	QTOF
L-Valine	118.08626	40	Positive	ESI	QTOF
L-Valine	116.07170	10	Negative	ESI	QTOF

Graphic: Mass List

Library spectrum

Abundance

m/z

Single Search Results: 1 hit for

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	METLIN	HMP	KEGG	LMP	IUPAC Name	Num Spectra
L-Valine	C5H11NO2	117.07898			72.18-4		35		HMDB008...	C00183			4



PCD versus PCDL

- Personal Compound Database (PCD)
 - Required: name, accurate mass
 - Optional: structure, retention time, formula, structure, CAS or other ID numbers
- Personal Compound Database Library (PCDL)
 - All of the above, *plus* MS/MS spectra
- Scoring based on
 - Accurate mass match
 - Isotope abundance
 - Isotope spacing
 - Retention time (if selected)
 - Dot product scoring of MS/MS spectra match
 - Forward and/or reverse scoring



Pathways to PCDL for Metabolomics

Pathway Database Creator

Settings Tools Help

Pathway Data

Source: BioCyc/MetaCyc

Organism/Database: Add/Remove

All Organisms

Search Text: Clear

2095 Pathways

ID	Name	# of Member Cmps
PWY-2582	brassinosteroid biosynthesis II	20
PWY-699	brassinosteroid biosynthesis I	24
PWY-5461	betanidin degradation	4
PWY-6999	theophylline degradation	12
PWY-6538	caffeine degradation III (bacteria, via demethylation)	17
PWY-6552	caffeine degradation I (main, plants)	5
PWY-6553	caffeine degradation II	5
PWY-6633	caffeine degradation V (bacteria, to trimethylurate)	5
PWY0-1297	purine deoxyribonucleosides degradation	18
PWY-6430	thymine degradation	10
PWY0-1298	pyrimidine deoxyribonucleosides degradation	17
PWY-6556	pyrimidine ribonucleosides degradation II	6
PWY0-1295	pyrimidine ribonucleosides degradation I	7
GLUCONSUPER-PWY	D-gluconate degradation	0
PWY-2361	3-oxoadipate degradation	6

Selection Mode

☒ Pathway Names

☐ Fellow Pathway Members

☐ Reaction Partners

Prefer Compound Names from

☒ BioCyc/MetaCyc

☐ METLIN

Create PCD

View unsolved

0 Unique Compound Selected

Clear All

Organism	Selection Mode	Entry ID	Name	# of Cmpd	Del.
----------	----------------	----------	------	-----------	------

Convert pathway metabolite information into Agilent personal compound databases

- Pathway database source - WikiPathways, BioCyc and KEGG
- Select one to many pathways
- Removes redundant metabolites
- Adds compound information – **Formula**, Compound ID(s), Name, Structure

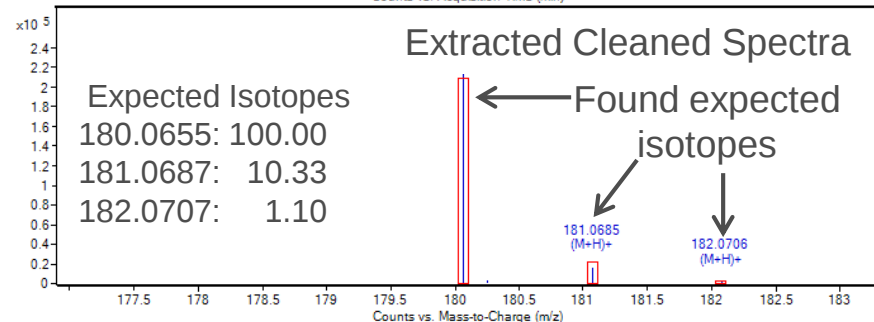
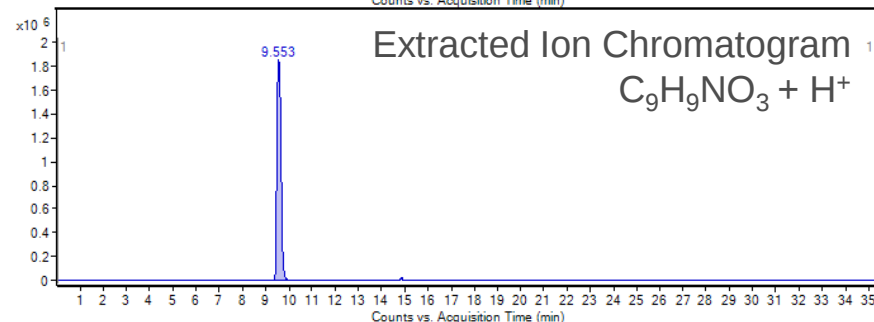
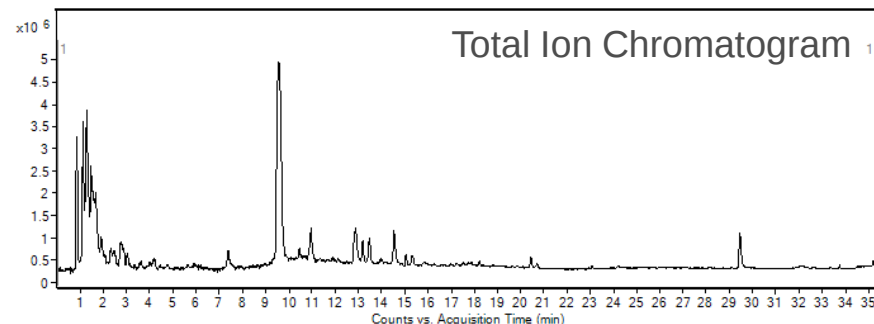
Can link to METLIN PCDL to add compound information

- Retention time or MS/MS spectra

Power of Pathway Directed Data Mining

Mine Data Using Find by Formula and Database

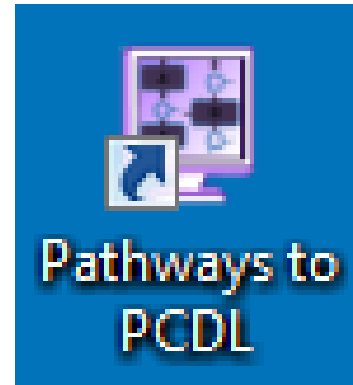
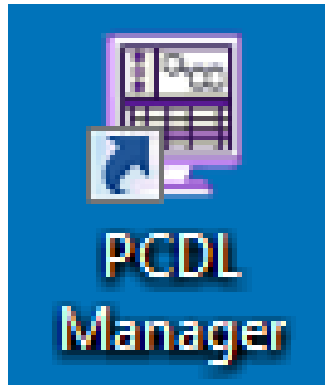
- Extract chromatogram and spectra using empirical formula and user-set rules
 - Use a metabolite database created by Pathways to PCDL
- Create Find by Formula method
- Chromatograms extracted and integrated
- Spectra scored from empirical formula
- User specifies match criteria threshold
 - Spectra score
 - Retention time (optional) increases specificity
- User can review and edit results
- Produce a CEF file for import into MPP



Targeted Data Mining of Qualitative Data for Greater Specificity



Questions about PCDL Manager or Pathways to PCDL?



Molecular Structure Correlator (MSC)

Identify
Compounds

- Utilizes centroid MS/MS data to help elucidate structures for unknown compounds that are *not* found in PCDs or PCDLs by:

- Calculating formulas for a precursor, fragment ions, and neutral losses
- Can Search Online Databases: Chemspider for possible structures
- Matches experimental fragments those from proposed structure by a systematic bond breaking, and displaying the best matches

Precursor MFG results

Loaded mol file

Compound formula: C23H31NO2

Fragment formulas for C23H31NO2

m/z	Intensity	Formula	Δ(Mass)
105.069	52989.85	C8H9	1.6
167.087	86414.21	C13H11	-0.8
209.1330	72555.43	C18H17	-2.3
130.1119	72103.65	C8H14N	2.2
91.0544	55225.42	C7H7	-1.5
131.0862	41825.77	C13H11	2.9
281.1535	16967.35	C19H21O2	0.4
149.0587	12370.42	C9H10O2	0.1
210.1363	11961.80	C18H18	19.1
168.0879	10736.67	C13H12	32.5
156.0726	8174.76	C8H10	48.3
73.0285	5705.78	C3H5O2	-1.6
132.0880	4331.33	C13H12	40.7
121.1145	4243.73	C8H15N	53.1
72.0816	3229.49	C4H10N	-11.3
92.0574	3225.02	C7H8	50.6
133.1402	2475.53		
282.1565	2347.47	C19H22O2	17.4
138.1071	2216.91		
167.1288	2142.96	C18H17N	9.8
208.1604	2074.37	C13H23N	-14.2
117.2056	2035.64		
135.1440	1885.64		
132.2252	1756.18		
219.2672	1552.03		
105.0331	1482.47	C7H9O	4.3
91.0885	1236.52		
131.1172	1202.02		
95.0478	1053.88		

Proposed structure of fragment ions and scores

Compound formula: C23H31NO2

Fragments of structure #1 - elucidated: 56.7% ions, 97.8% Weight

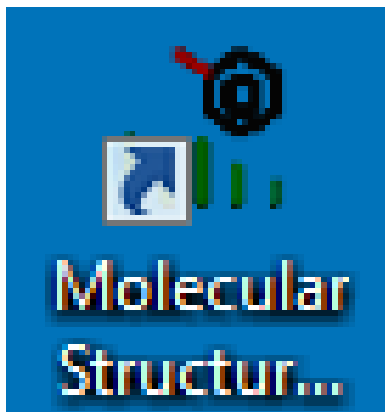
Mass	Intensity	Weight	No. of ions	Score
1	281.1535	16967.35	43.7	98.5
2	130.1119	72103.65	0.4	98.3
3	73.0285	5705.78	0.0	97.7
4	209.1330	72555.43	31.6	97.0
5	167.087	86414.21	9.6	96.7
6	72.0816	3229.49	0.0	95.9
7	149.0587	12370.42	0.7	93.0
8	91.0544	55225.42	0.2	91.3
9	131.0862	41825.77	0.4	90.3
10	210.1363	11961.80	5.1	89.3
11	131.0862	41825.77	1.1	88.3
12	282.1565	2347.47	6.0	87.2
13	168.0879	10736.67	1.2	86.4

Product ion MFG results



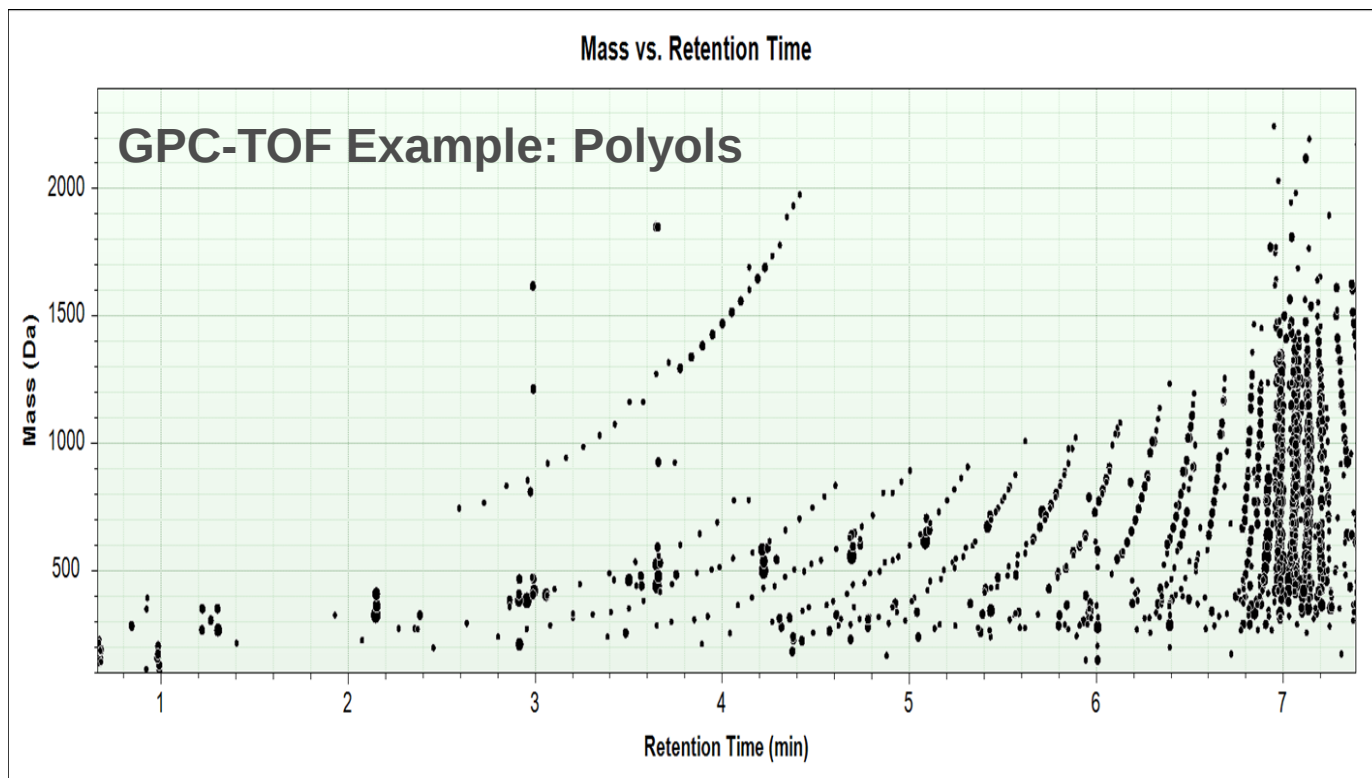
Agilent Technologies

Questions about MSC?



Mass Profiler (MP) Differential Analysis Program

- Feature alignment
- Single-variate statistics for binary experiments
- IDBrowser for compound identification



Clearly see
repeating
units of 44
and 58 m/z

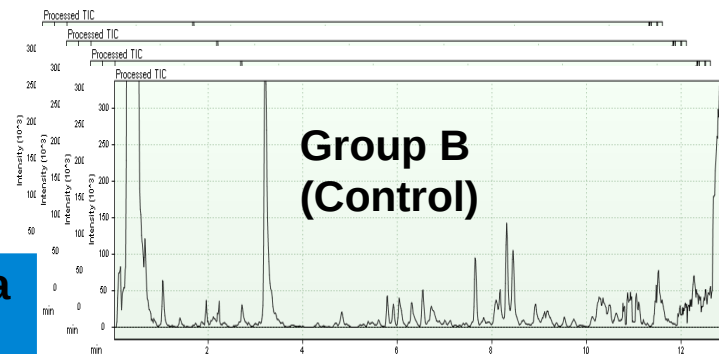
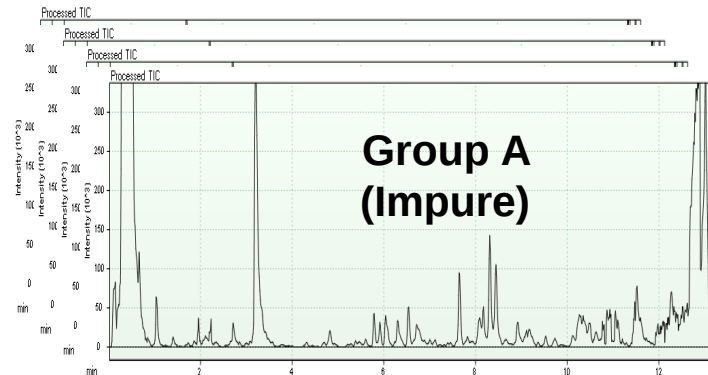


Mass Profiler

- Impurity Profiling/Differential Analysis Software
- Graphical Displays
 - Mass vs Retention Time
 - Log2 Ratio (Group1/Group2) vs Retention Time
 - Log2 Abundance Group 2 vs Log2 Abundance Group 1
 - Unique to Group 1 vs RT and Unique to Group 2 vs RT (New)
- Feature Identification
 - Molecular Formula Generation and AMRT database
 - Single Feature or Batch Processing (summary report)
 - Web Internet Database Searching

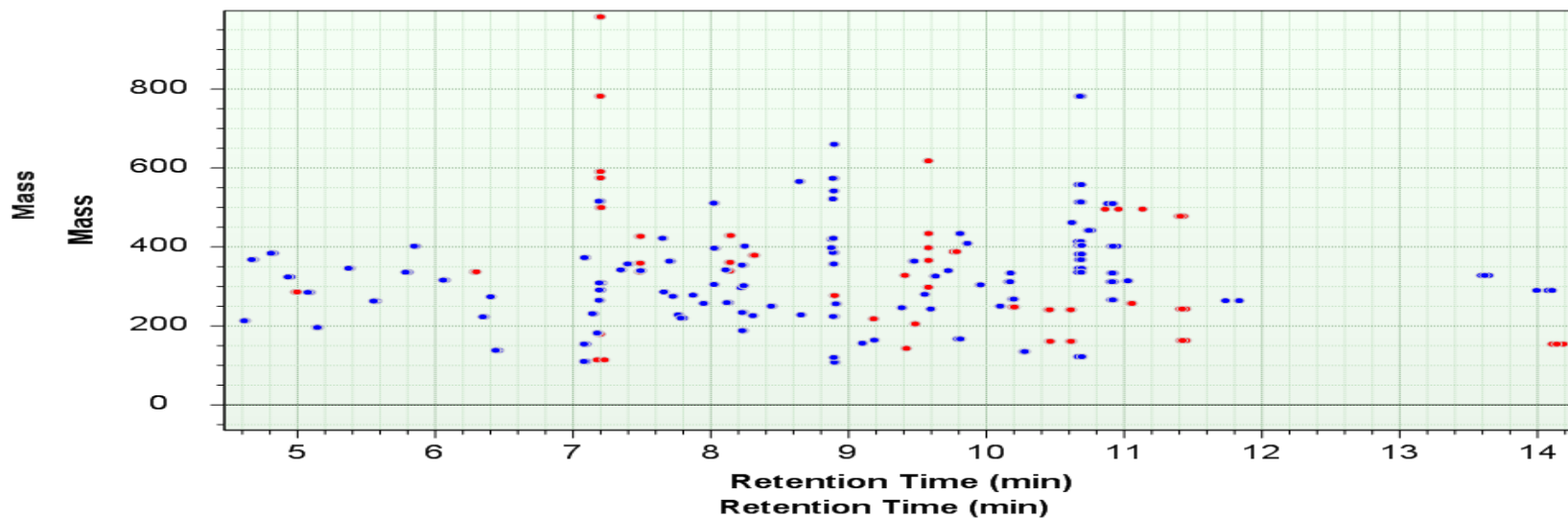


Mass Profiling Software



Aligns Data
RT, Mass,
Abundance

What's Changed? Mass/RT no Fold Change
Mass vs. Retention Time

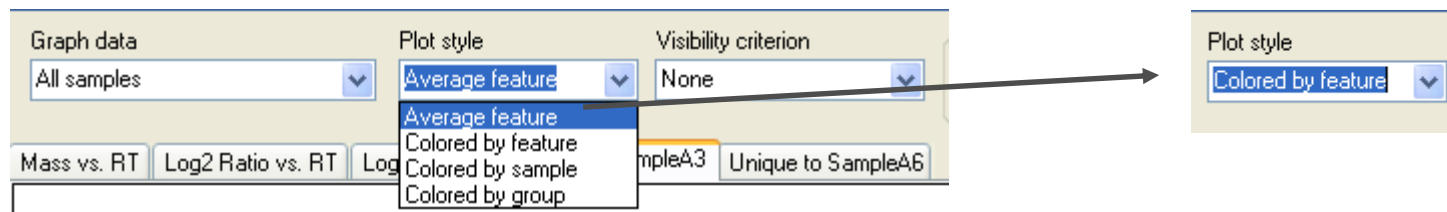


What's Unique? Impurity Control

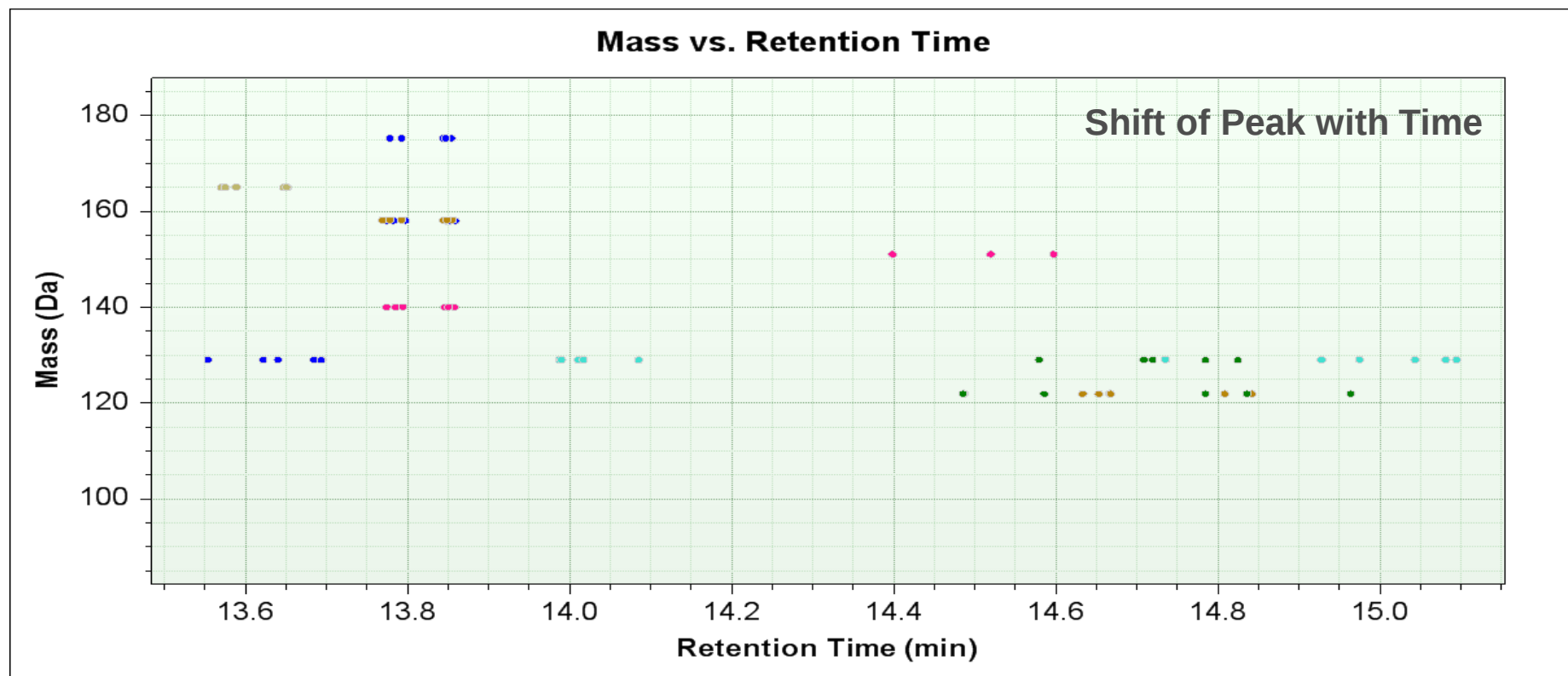


Agilent Technologies

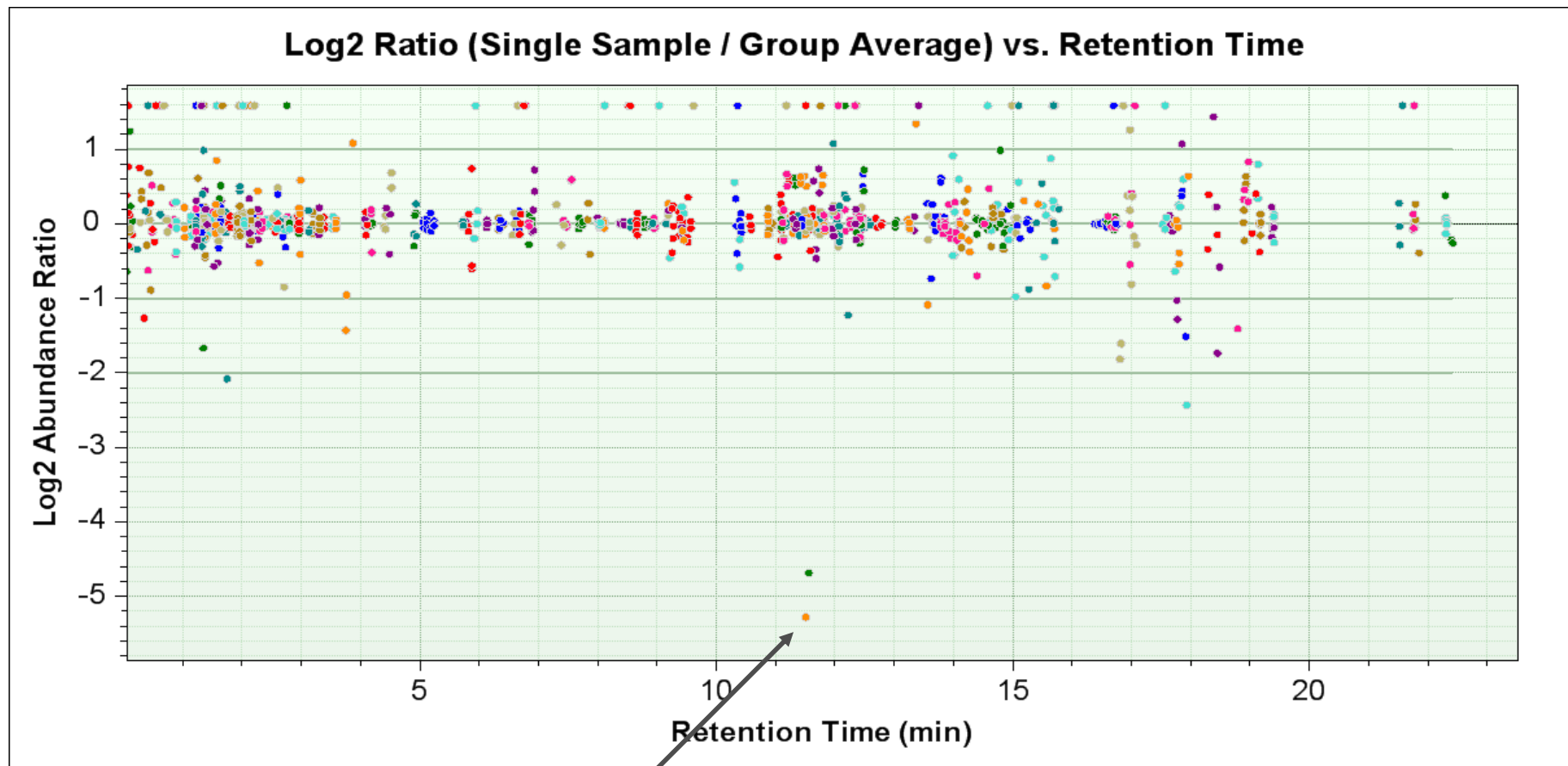
Mass Profiler – Reproducibility of Results



Reproducible Retention Time Stability

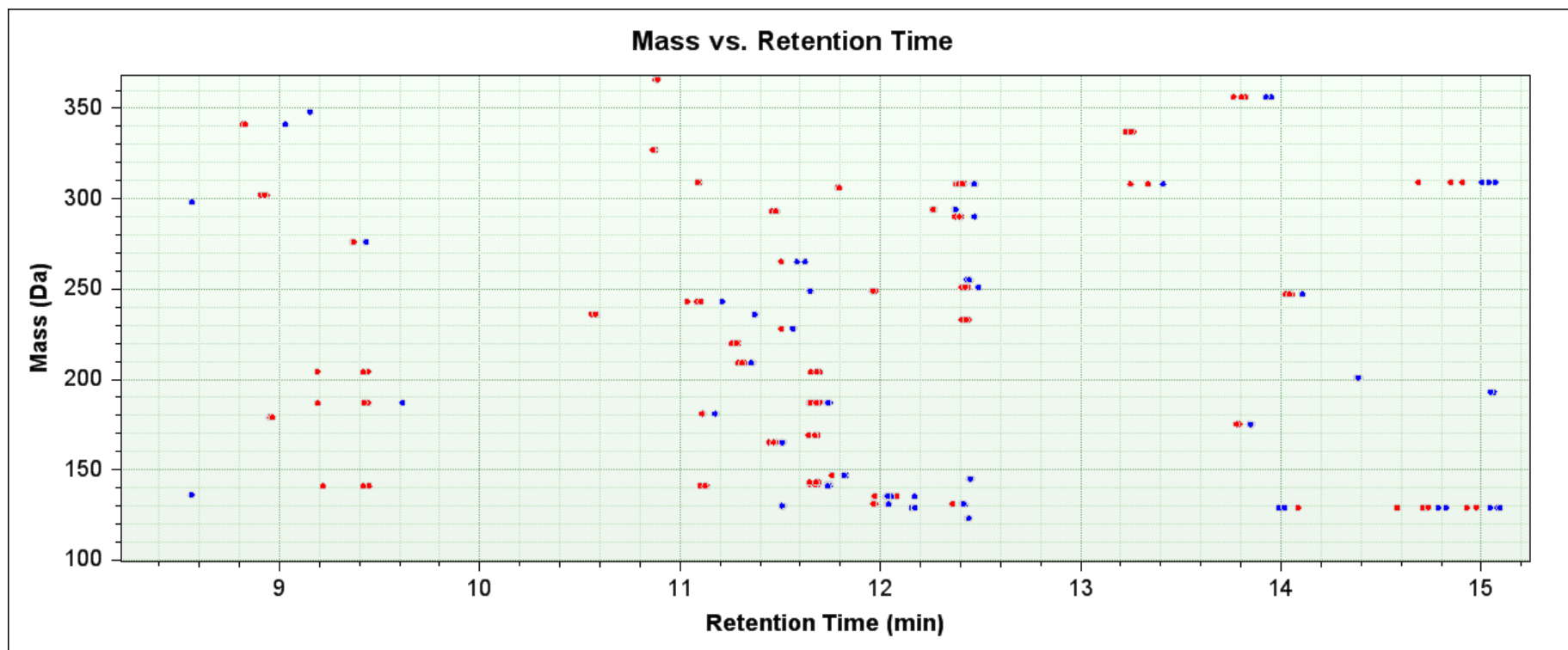
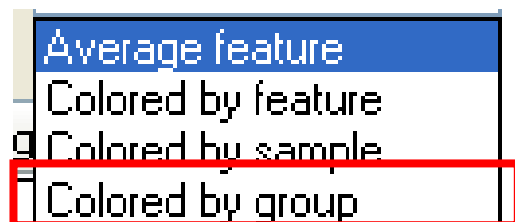


Mass Profiler – Reproducibility of Results



Outlier Sample or Feature

Unique Group 1 and Unique Group 2



Questions about Mass Profiler?



Untargeted Data Acquisition & Analysis

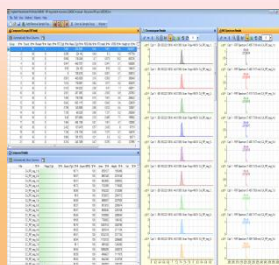
Untargeted

Separate & Detect



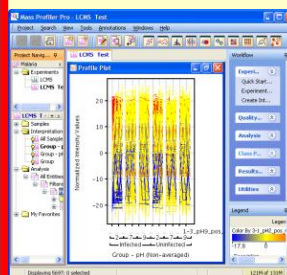
LC-TOF/QTOF

Feature Finding



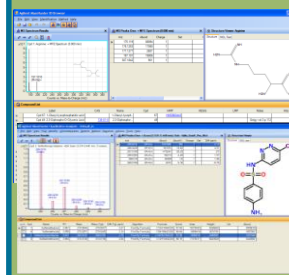
Profinder

Alignment & Statistics



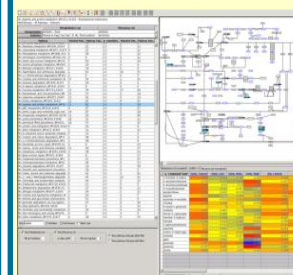
Mass Profiler Professional

Identify



ID Browser

Pathways



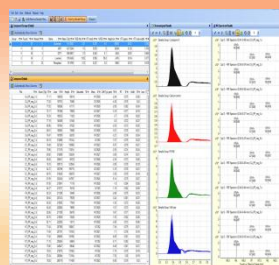
Pathway Architect

Pathway Targeted

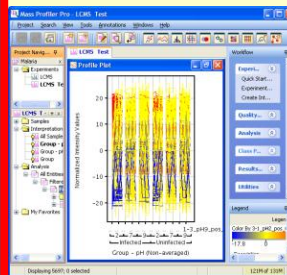
LC-TOF/QTOF



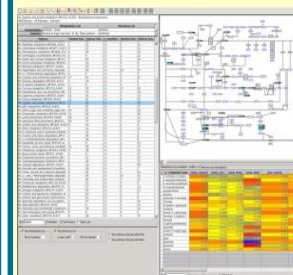
Profinder



Mass Profiler Professional



Pathway Architect



Agilent Technologies

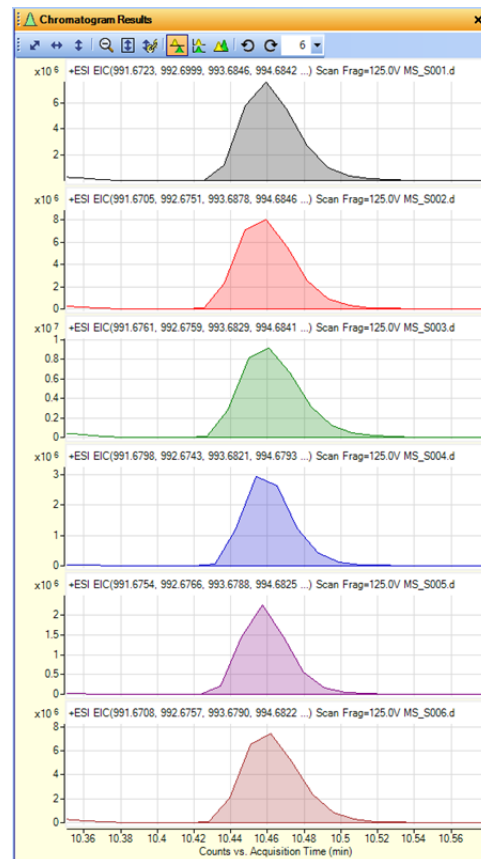
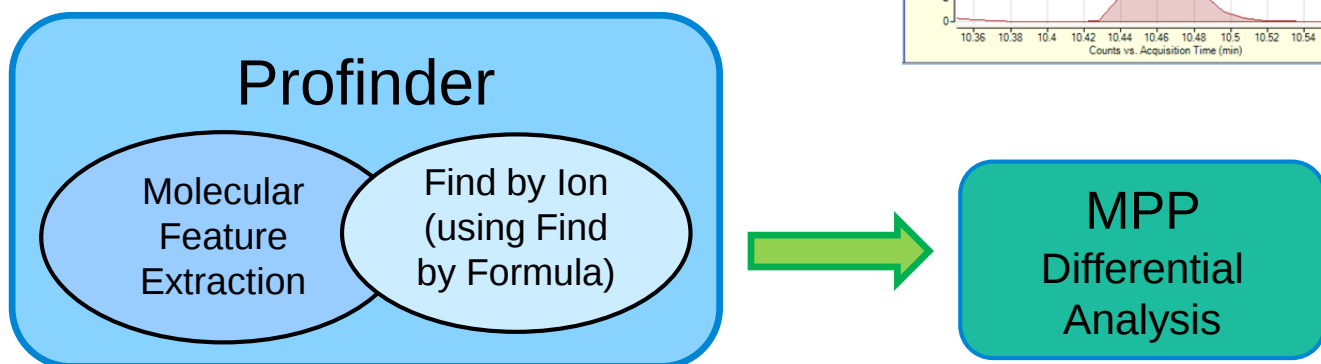
ASTS La Jolla MassHunter Session

May 28, 2014

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Mass Hunter Profinder - **NEW**

- Batch based or “project” based logic
- Extracts and aligns features prior to statistical analysis in MPP
- Can replace previous recursive workflow in Qual
- More efficient, fewer manual steps
- Visualize, review, and edit results across the batch for higher quality results



Profiler Workflows

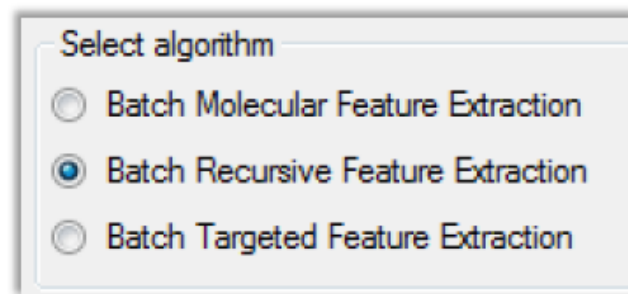
1. Batch **Molecular** Feature Extraction

- Reduces False Positives, No Editing



2. Batch **Recursive** Feature Extraction

- Reduces False Negatives, Allows Editing



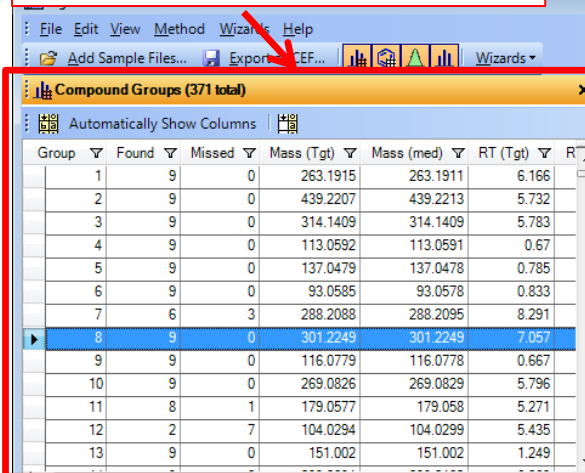
3. Batch **Targeted** Feature Extraction

- Uses database targets, Allows Editing



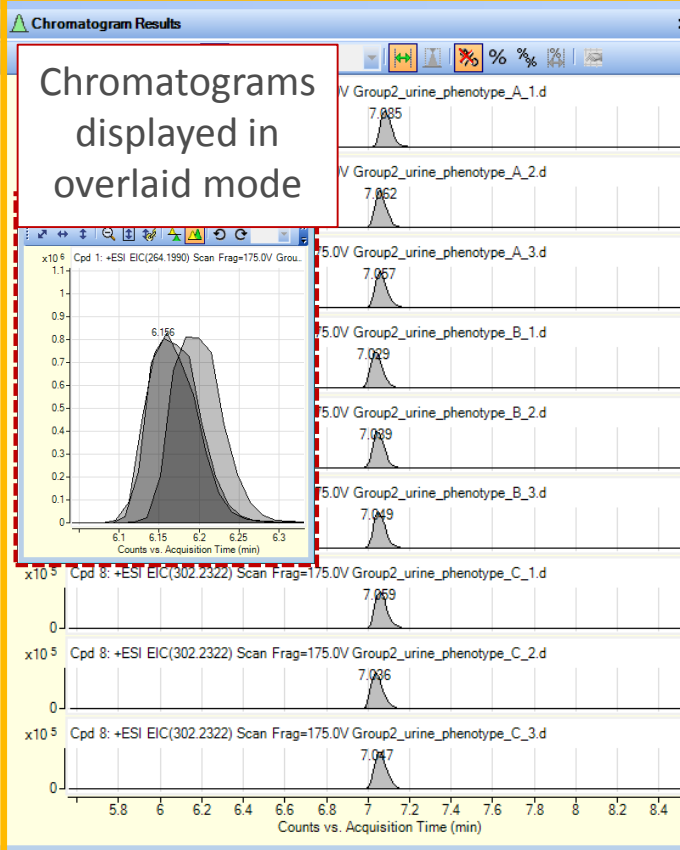
Profinder Results Navigation

Compound Groups Table

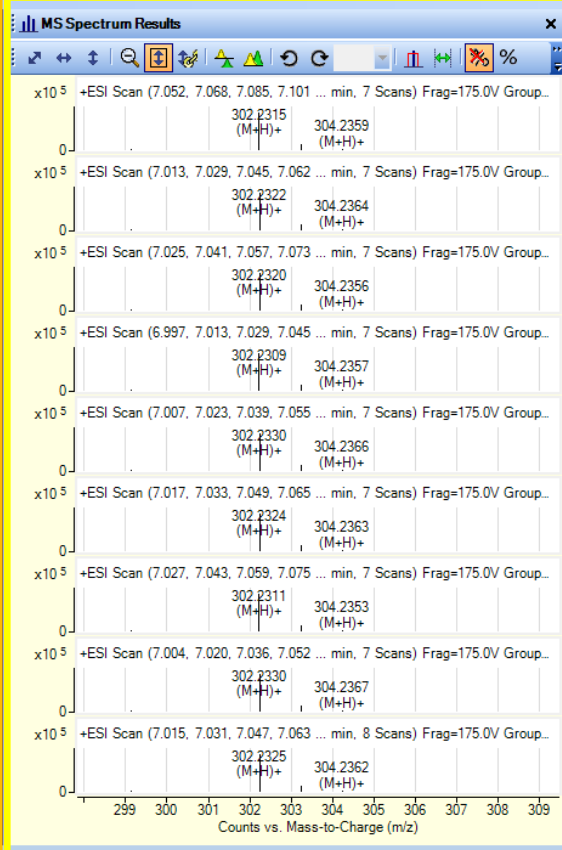


Group	Found	Missed	Mass (Tgt)	Mass (med)	RT (Tgt)	RT (med)
1	9	0	263.1915	263.1911	6.166	
2	9	0	439.2207	439.2213	5.732	
3	9	0	314.1409	314.1409	5.783	
4	9	0	113.0592	113.0591	0.67	
5	9	0	137.0479	137.0478	0.785	
6	9	0	93.0585	93.0578	0.833	
7	6	3	288.2088	288.2095	8.291	
8	9	0	301.2249	301.2249	7.057	
9	9	0	116.0779	116.0778	0.667	
10	9	0	269.0826	269.0829	5.796	
11	8	1	179.0577	179.058	5.271	
12	2	7	104.0294	104.0299	5.435	
13	9	0	151.002	151.002	1.249	

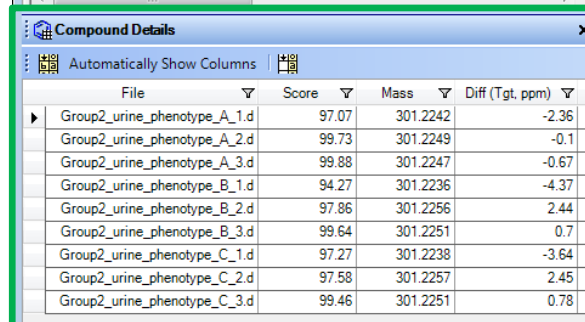
Chromatogram Results



Spectrum Results



Compound Details Table



File	Score	Mass	Diff (Tgt, ppm)
Group2_urine_phenotype_A_1.d	97.07	301.2242	-2.36
Group2_urine_phenotype_A_2.d	99.73	301.2249	-0.1
Group2_urine_phenotype_A_3.d	99.88	301.2247	-0.67
Group2_urine_phenotype_B_1.d	94.27	301.2236	-4.37
Group2_urine_phenotype_B_2.d	97.86	301.2256	2.44
Group2_urine_phenotype_B_3.d	99.64	301.2251	0.7
Group2_urine_phenotype_C_1.d	97.27	301.2238	-3.64
Group2_urine_phenotype_C_2.d	97.58	301.2257	2.45
Group2_urine_phenotype_C_3.d	99.46	301.2251	0.78



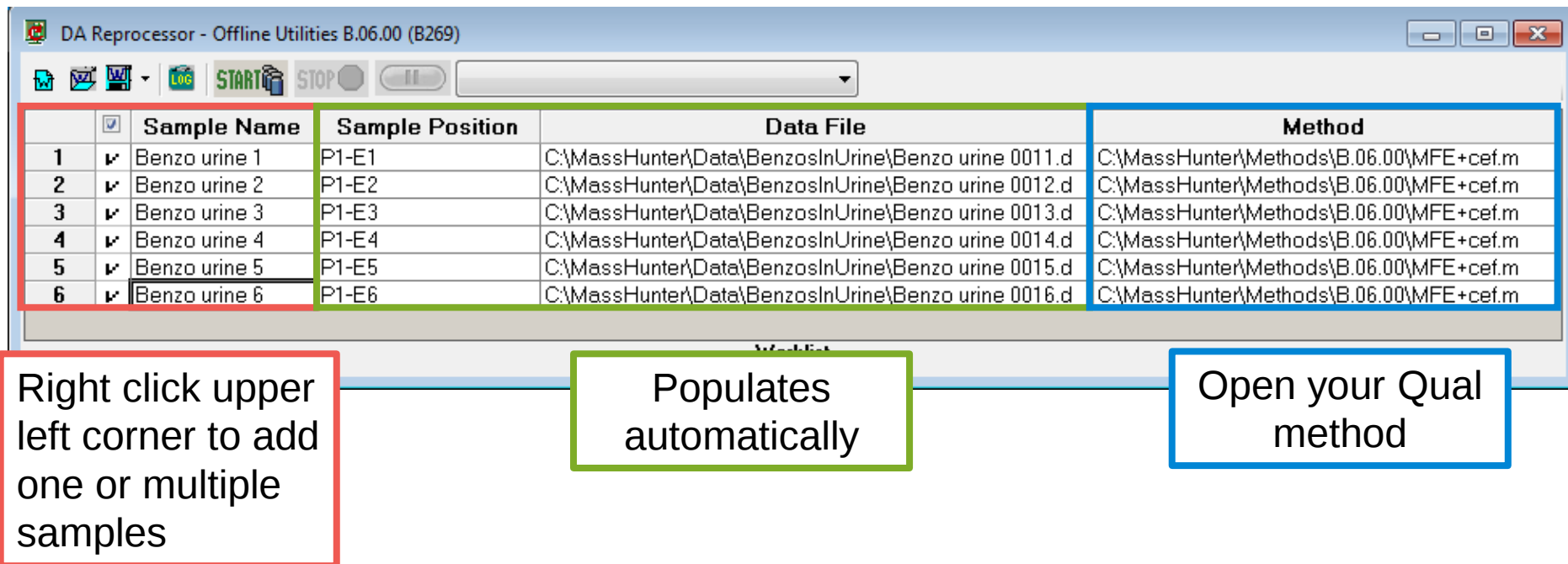
Questions about Profinder?



DA Reprocessor

Use this, it saves memory and works in the background!

Run Qual method on a batch of samples



DA Reprocessor - Offline Utilities B.06.00 (B269)

	☒	Sample Name	Sample Position	Data File	Method
1	☒	Benzo urine 1	P1-E1	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0011.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m
2	☒	Benzo urine 2	P1-E2	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0012.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m
3	☒	Benzo urine 3	P1-E3	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0013.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m
4	☒	Benzo urine 4	P1-E4	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0014.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m
5	☒	Benzo urine 5	P1-E5	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0015.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m
6	☒	Benzo urine 6	P1-E6	C:\MassHunter\Data\BenzosInUrine\Benzo urine 0016.d	C:\MassHunter\Methods\B.06.00\MFE+cef.m

Right click upper left corner to add one or multiple samples

Populates automatically

Open your Qual method

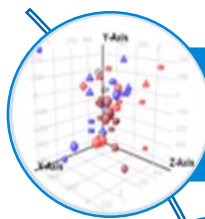
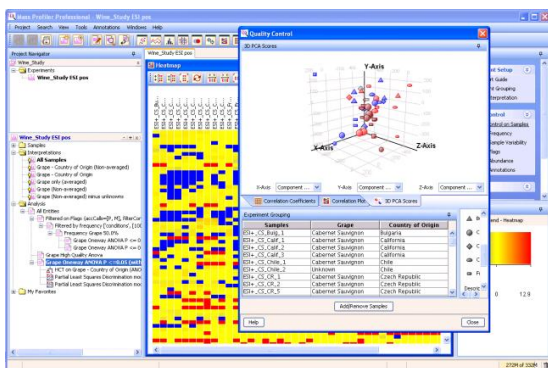


Questions about DA Reprocessor?

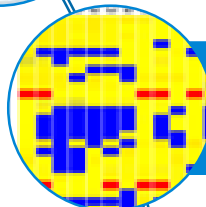


Mass Profiler Professional

Find differences in mass spec data sets and reach statistically valid conclusions



Get from data to answers using **statistical tests** such as PCA



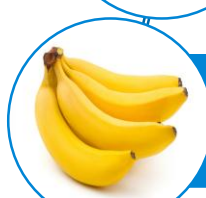
Dive deeper into results using **visualizations** e.g. heat maps



Accurate identifications using ID Browser



Use data from LC/MS, GC/MS, ICP-MS, and NMR



Run QC assays using the **Sample Class Predictor**



Bring genomics, proteomics, and metabolomics together



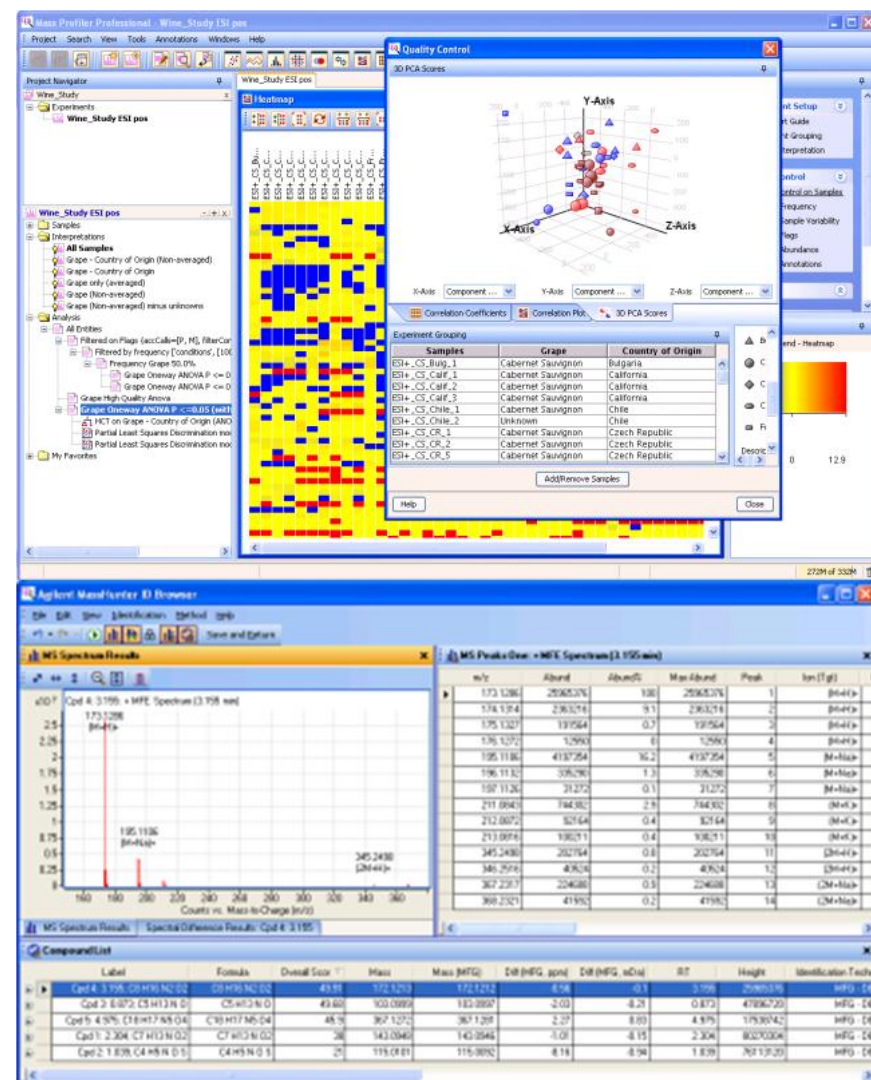
Agilent Technologies

ASTS - La Jolla

May 20, 2014

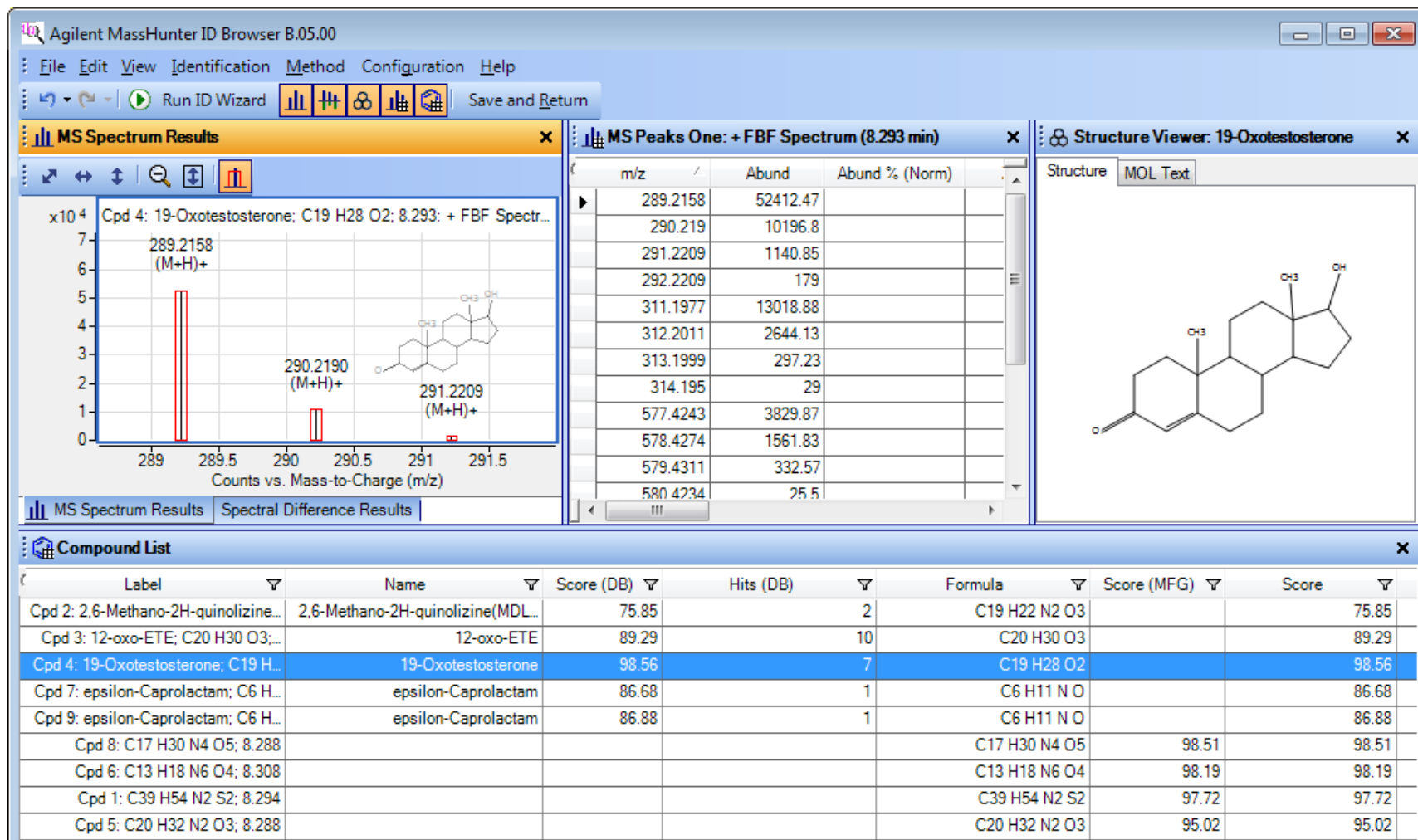
Mass Profiler Professional (MPP)

- Compound alignment
- Filtering
- Single- and multi-variate statistics
 - t-test, ANOVA, clustering, fold change, PCA
- IDBrowser for compound identification
- Sample Class Prediction
- Pathway Architect
- Multiple data types and sources
 - Metabolomics, lipidomics, proteomics, other small molecule profiling
 - LC-MS, GC-MS, ICP-MS, generic import
 - Targeted, untargeted



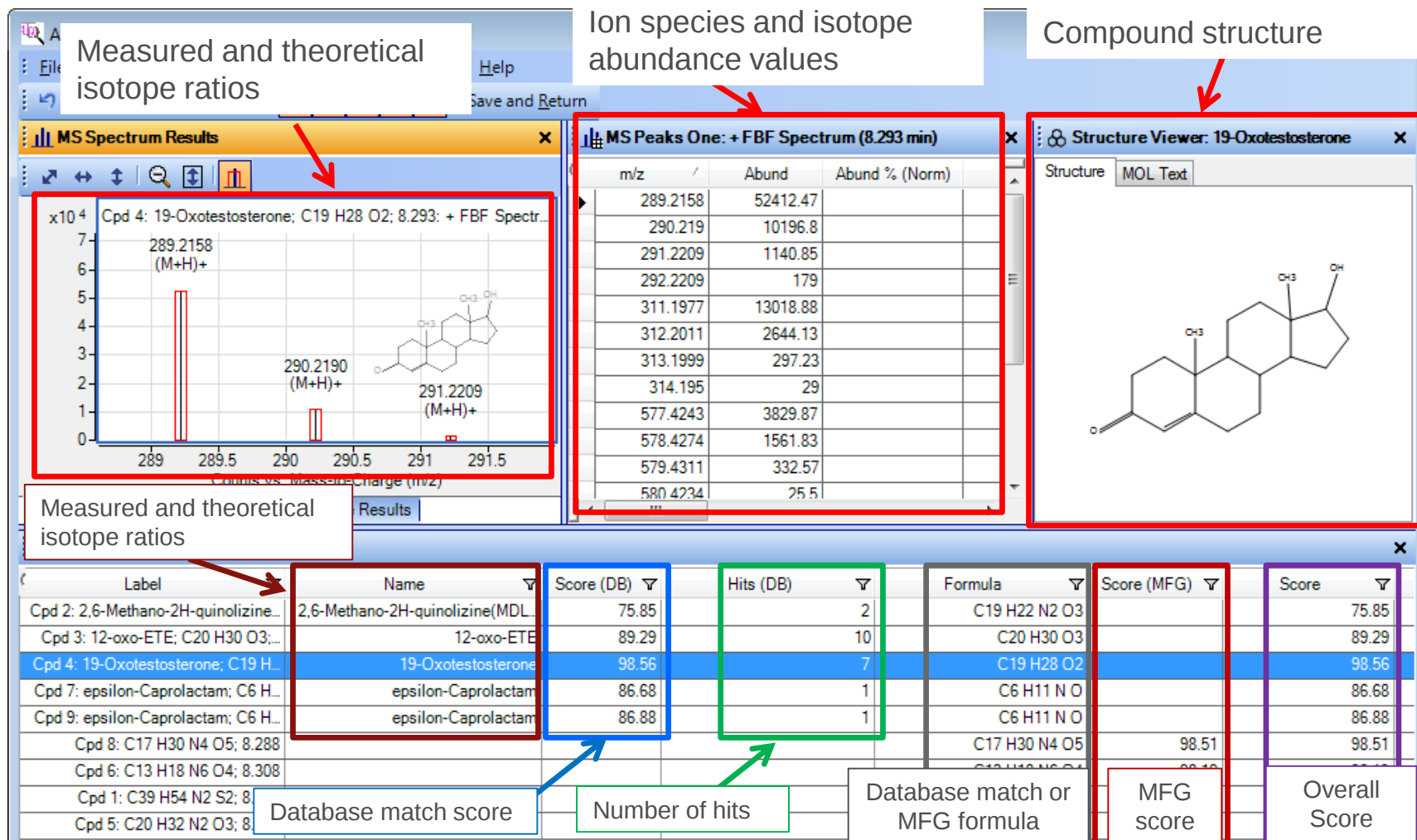
ID Browser

Searches against csv or PCD files to identify compounds



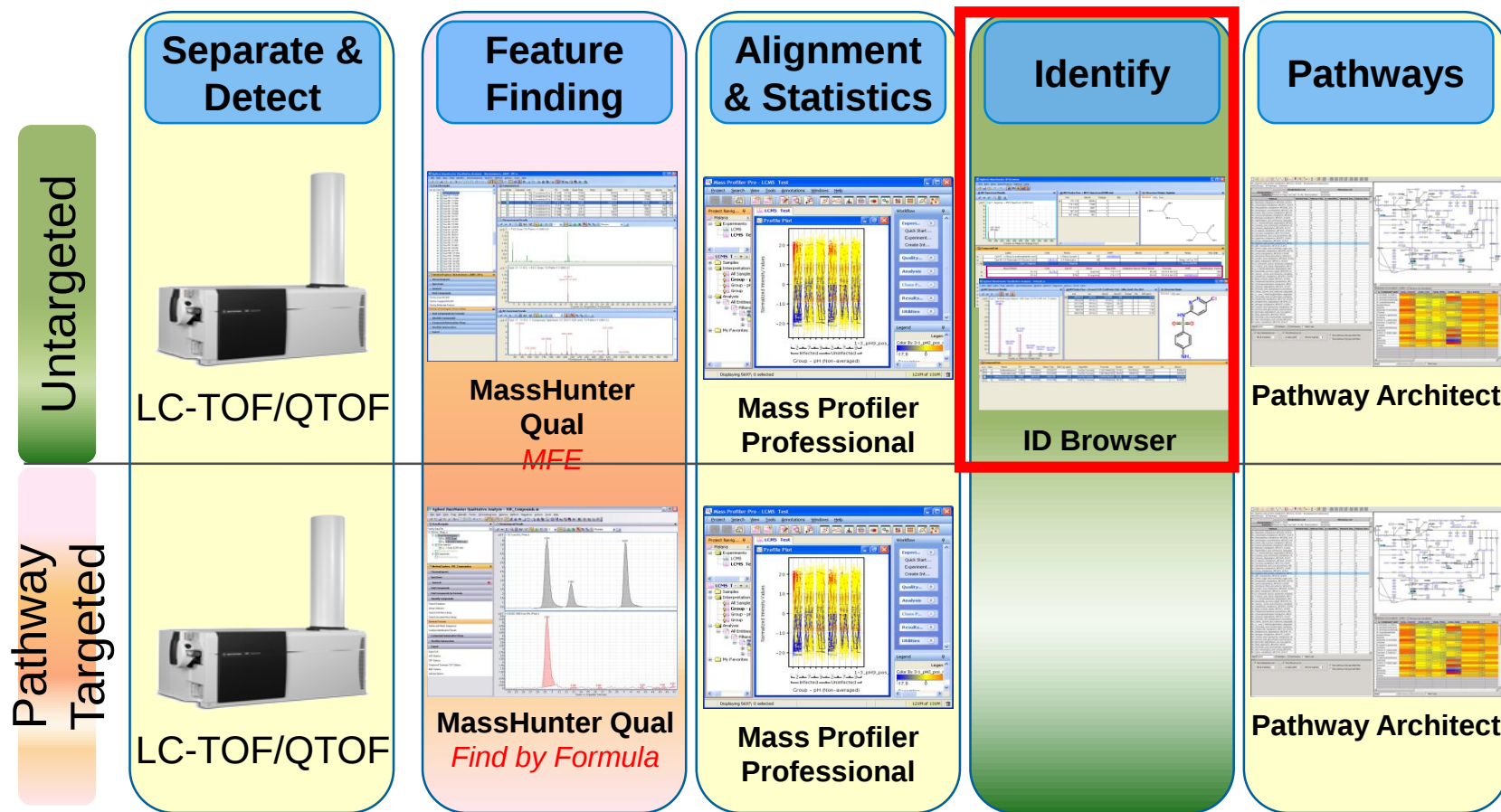
ID Browser

Searches against csv or PCD files to identify compounds



SimLipid from PREMIER Biosoft International

One way to think of SimLipid is like “ID Browser for Lipids”



SimLipid vs. METLIN PCD

METLIN PCD has 31,011 lipid compounds. It is suited in lipidomics for customers who:

- Are agreeable to an **MS-only workflow** (i.e. database search)
- Want to use Agilent's ID Browser for identifications (part of MPP) instead of SimLipid
- Want to have the other ~33,000 metabolite compounds in METLIN
- Lower cost solution – perpetual license

SimLipid has 36,224 lipids in its database. It is suited for customers who:

- Want to identify lipids using **MS/MS pattern matching** (in addition to database search)
- Have instruments other than Agilent's
- Are familiar with or already own SimLipid for lipid identifications
- Requires an annual subscription

The MPP Lipidomics Workflow



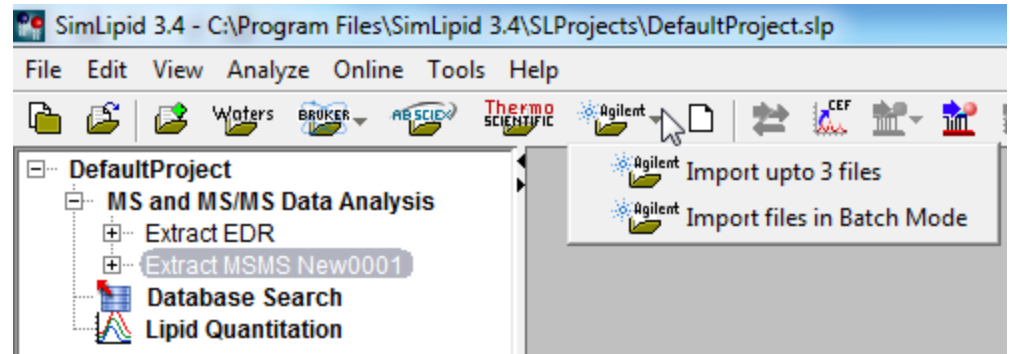
- SimLipid 3.3 and higher supports import and export of Agilent's Compound Exchange Format (.CEF) file. Compounds found in Qual and exported as .CEF can be
 - annotated in SimLipid as lipid compounds using
 - MS database searching
 - MS/MS pattern matching for features that have MS/MS
 - export annotated results in. CEF file format
 - import annotated .CEF file into MPP,
 - use Pathway Architect to give biological contextualization.

Overview of SimLipid Process

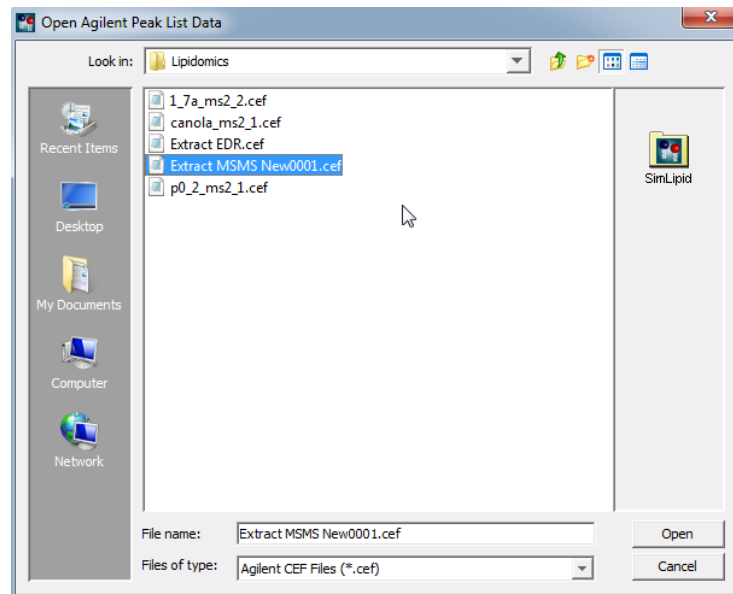
- Import CEF files into SimLipid
- Run High Throughput Search on CEF files
- Load HTP Results from SimLipid server
- Generate HTP Report (optional)
- Export annotated CEF files for analysis in MPP

Import Agilent CEF Files

Choose the Agilent icon on the SimLipid menu bar and select either Import up to 3 files or Batch Mode.

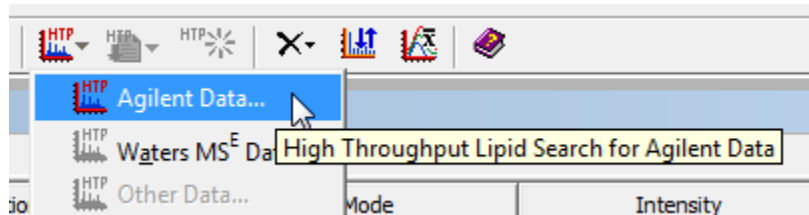


Select your CEF files.

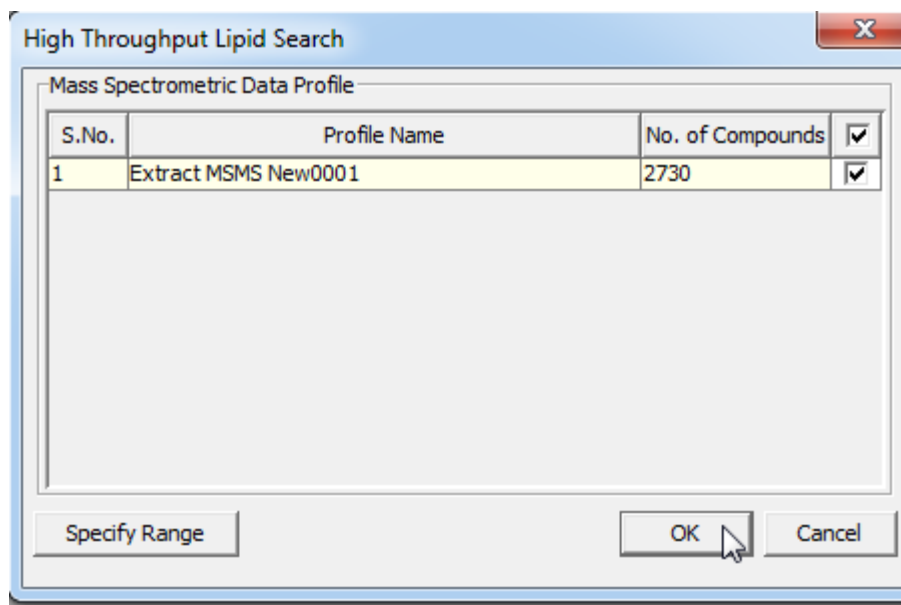


Run High Throughput Search on the CEF Files

Select High Throughput Lipid Search (“HTP”) in the menu bar

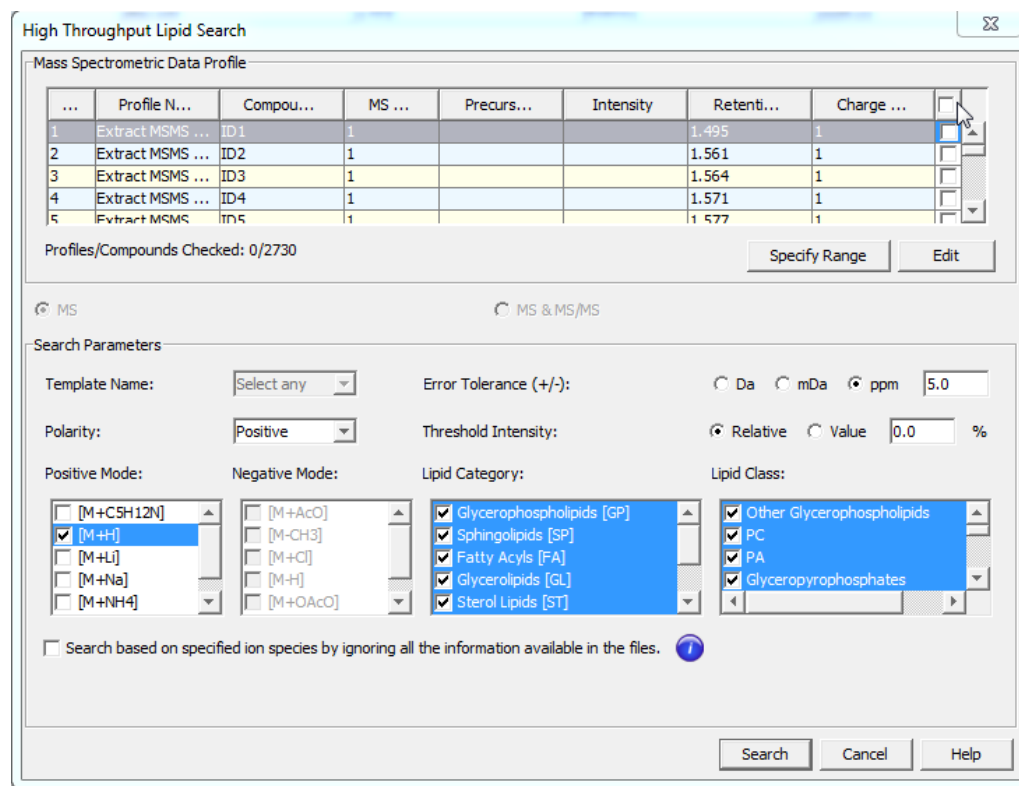


Select CEF files to run High Throughput search on and click OK.



Run High Throughput Search on the CEF Files

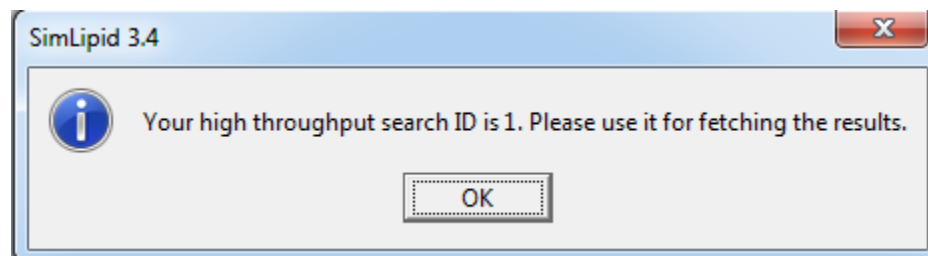
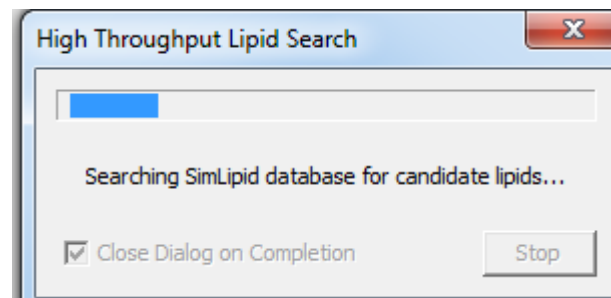
Select the compounds to run HTP search on and the specific search parameters, then press the Search button.



2000 compounds (aka “profiles” in SimLipid) can be searched at one time

Run High Throughput Search on the CEF Files

The High Throughput search runs and then you are given a search ID (even on individually installed instance, i.e. non-server)

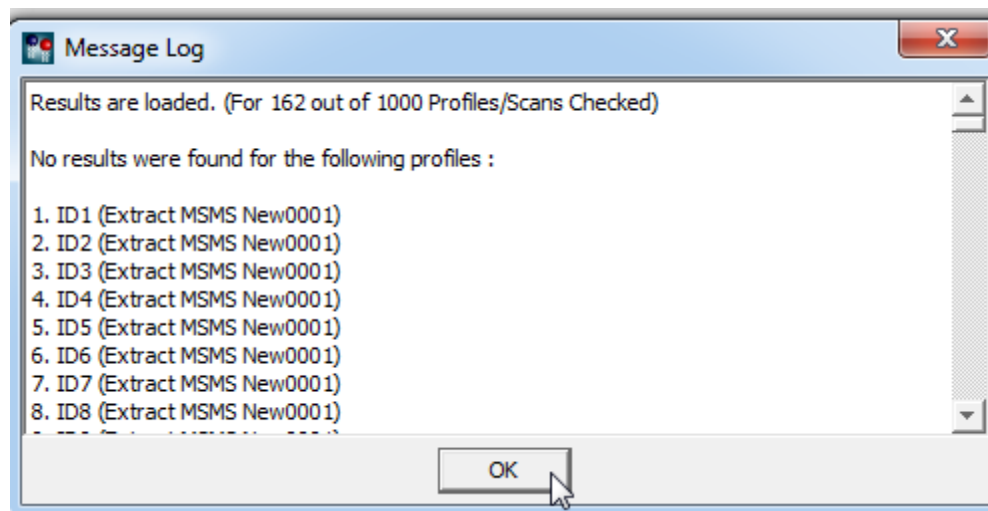


Load HTP Results

Go to the HTP icon on the menu bar and select to load your HTP search request.

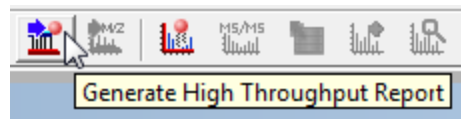
A message log will return how many of the compounds were identified (e.g. 162 out of 1000)

Searches are cumulative—the next time you search it will automatically select the second 1000 compounds.

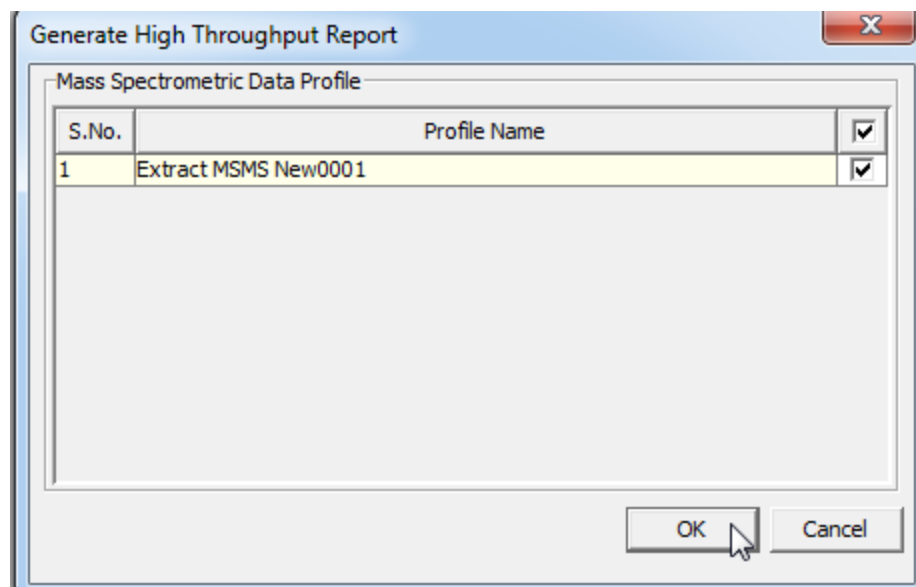


Generate High Throughput Report (optional)

Select the Generate High Throughput Report icon in the menu bar



Select CEF files to generate High Throughput report on and click OK.



Generate High Throughput Report (optional)

Select the compounds (aka profiles) to include in the report and the parameters to be displayed.

Generate High Throughput Report

Mass Spectrometric Data Profile

S.No.	Profile Name	Scan Name	MS Level	Precurso...	Intensity	Retentio...	Charge...	☒
1	Extract MSMS N...	ID13	1			1.594	1	☒
2	Extract MSMS N...	ID36	1			1.632	1	☒
3	Extract MSMS N...	ID37	1			1.632	1	☒
4	Extract MSMS N...	ID48	1			1.639	1	☒
5	Extract MSMS N...	ID64	1			1.671	1	☒

Profiles/Scans Checked: 162/162 Specify Range

MS | MS/MS |

MS Search

☐ Select All ☒ Select matched lipids

Lipid Options

☒ Retention Time ☒ m/z ☒ Intensity
☒ No. of Hits ☒ Chemical Composition ☒ Abbreviation/Common Name
☒ Delta Mass ☐ Lipid Structure

Select All Clear All

Output Options

File Type:

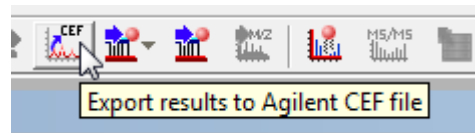
Export to Folder: Browse...

OK Cancel

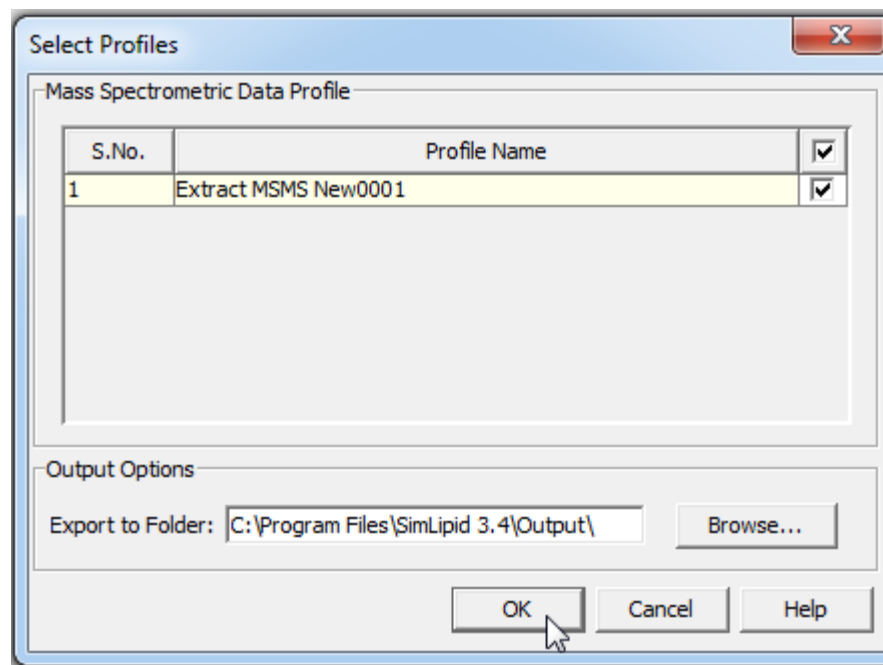


Export Annotated CEF files for Analysis in MPP

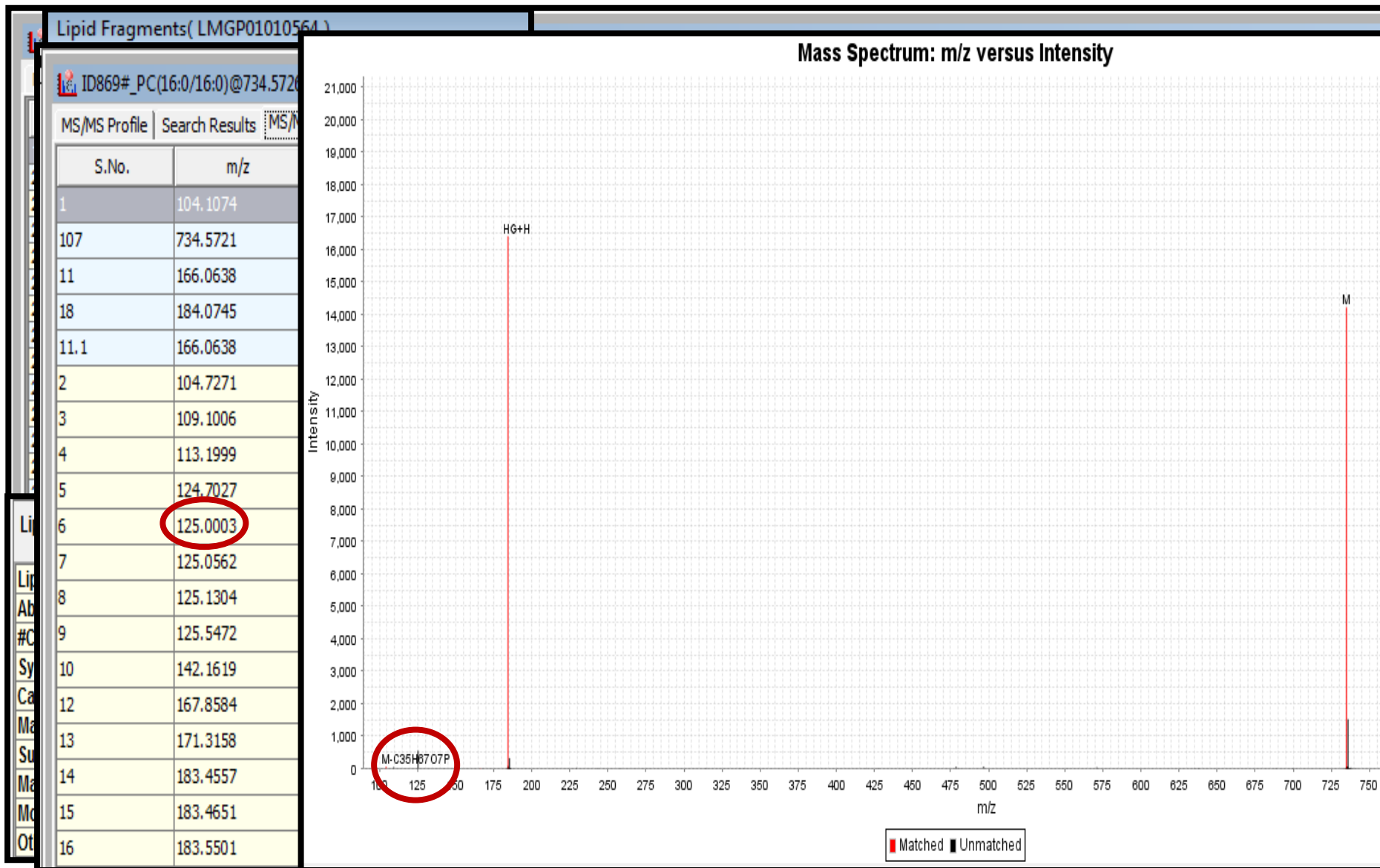
Select the CEF icon in the menubar.



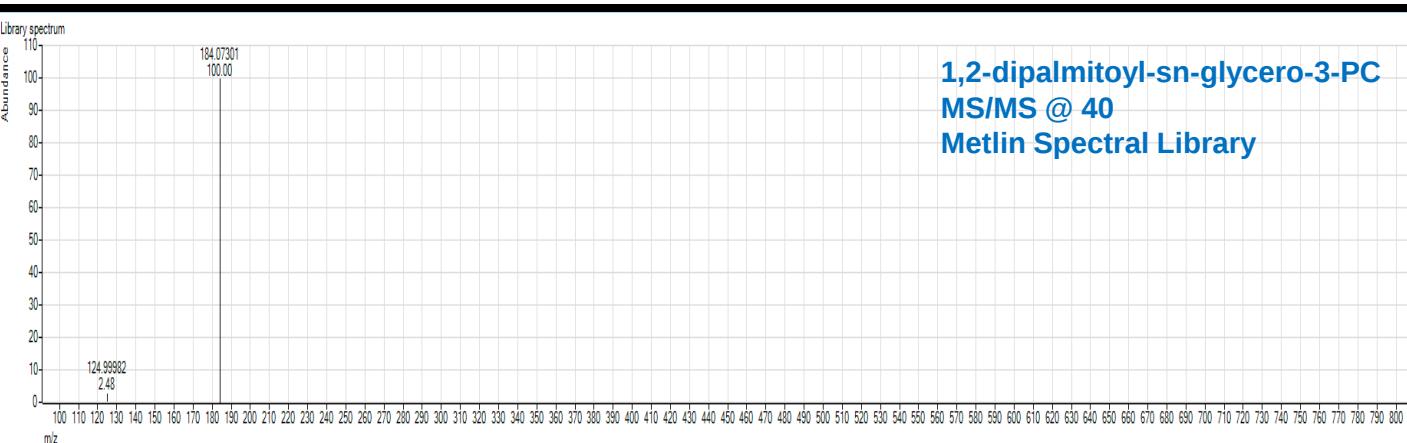
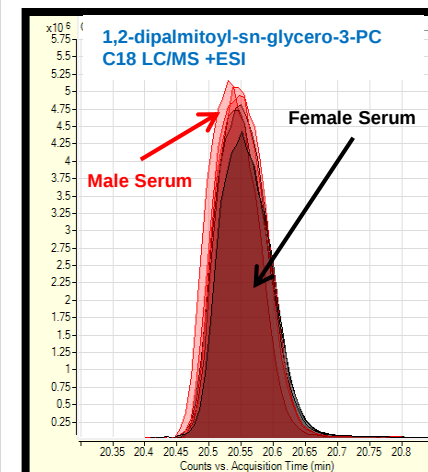
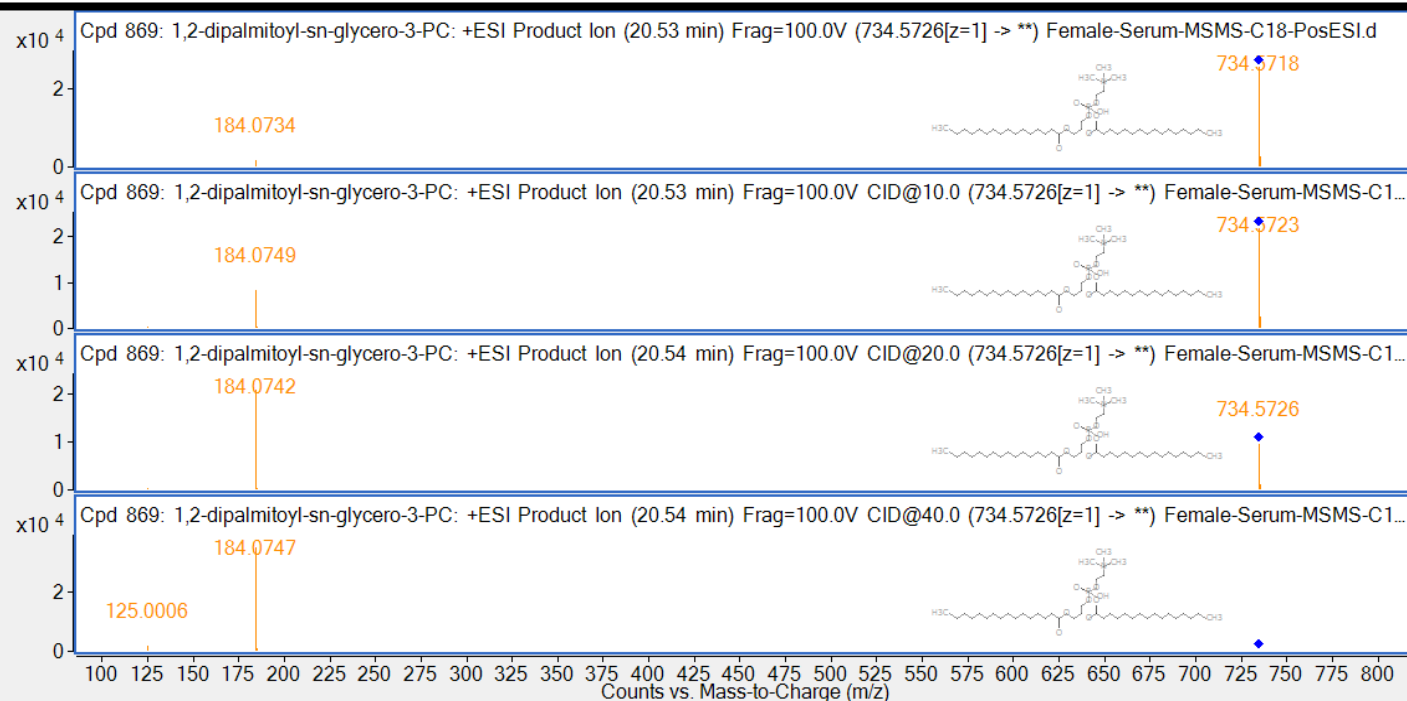
Select CEF files to export and click OK.



LC/MS/MS Identification of 1,2-dipalmitoyl-sn-glycero-3-PC



LC/MS/MS Identification of 1,2-dipalmitoyl-sn-glycero-3-PC



Ordering SimLipid

SimLipid is available from PREMIER Biosoft International through their website:

<http://www.premierbiosoft.com/lipid/index.html>

They can be contacted through sales@premierbiosoft.com

Phone: +1-650-856-2703

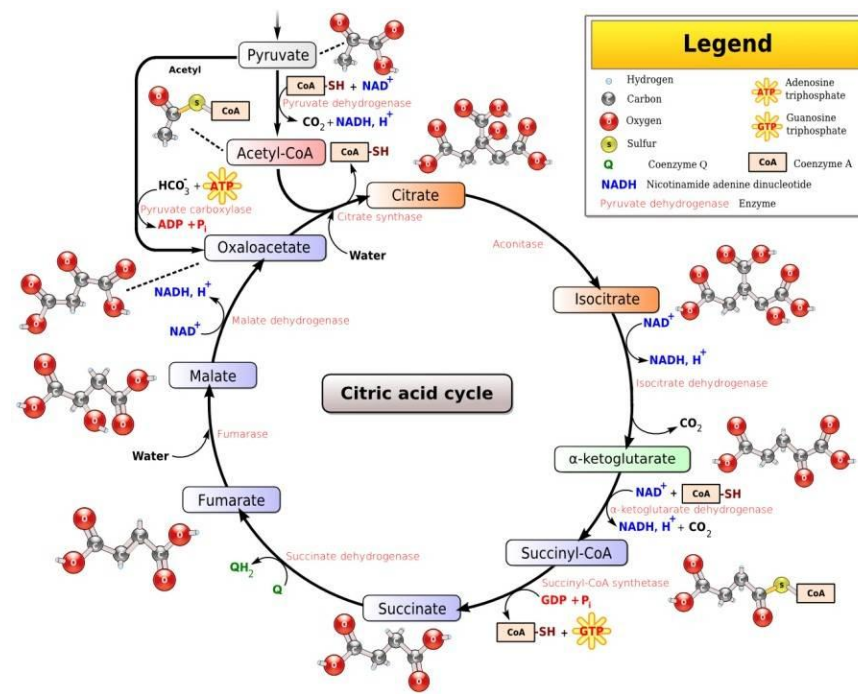
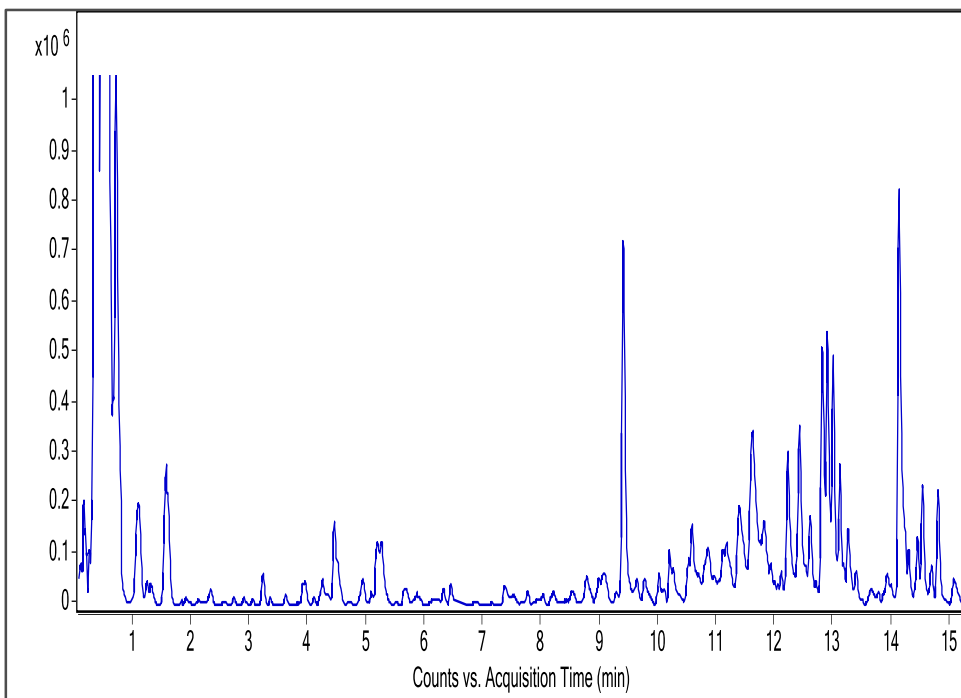
On their website, they offer temporary licenses for customers to try out the product before buying.

Pathway Architect

Pathway analysis using multi-omic data

Start here

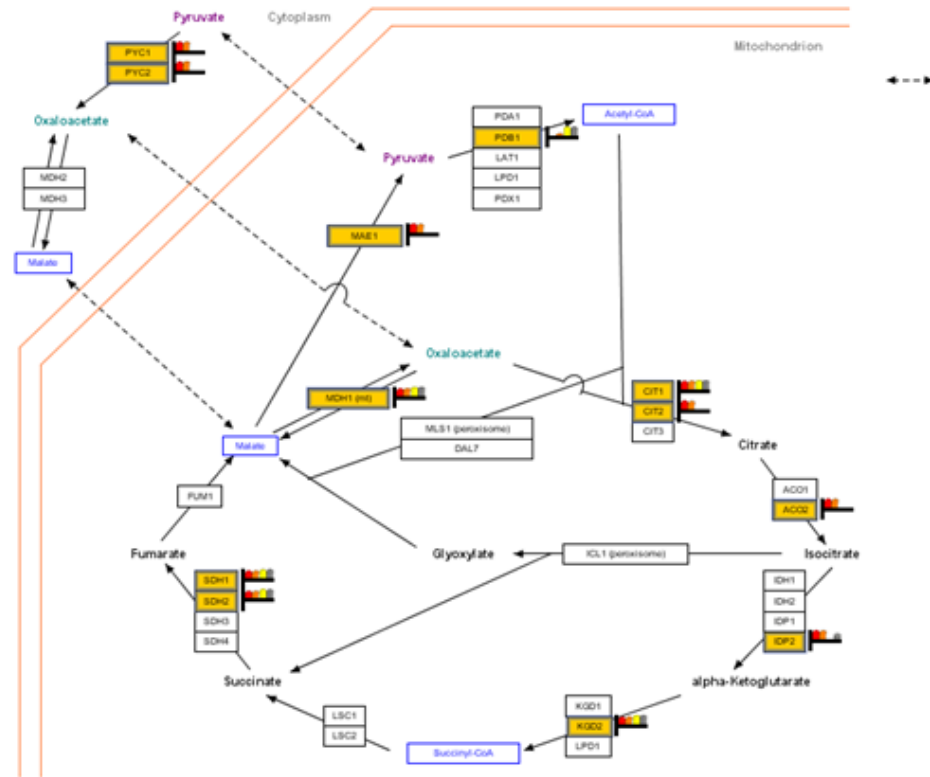
Finish here



Pathway Architect

Customers can take the value of their data to another level. Pathway Architect helps them make sense of the data by:

- Visualizing it on
 - Wikipathways
 - BioCyc/MetaCyc
- Overlaying genomics, proteomics and metabolomics data
- Designing their next experiment for MS/MS analysis or custom microarrays



TCA Cycle



Starting Pathway Architect

Multi-Omic Analysis (Step 1 of 4)

Input Experiments

The active experiment in the open project is set as Experiment 1 by default. Select the second experiment for Multi-Omic Analysis, choose a Pathway Organism, and the sources from which you want to match pathways for the selected organism. Any pathway organism can be selected from the drop-down, regardless of the organisms associated with the chosen experiments; for example, if you know that your research area is more extensively described in another organism.

Experiment Chooser

Experiment 1

Experiment 1 MicroArray Choose...

Organism 1 Mycobacterium tuberculosis

Experiment 2 Metabolomics_Neg

Organism 2 Mycobacterium Tuberculosis

Choose Pathway Organism Mycobacterium tuberculosis

☒ Curated pathways only
☐ Literature Derived Networks only
☐ Both

Curated pathways

- ☐ WikiPathways - Analysis (8 pathways)
- ☐ WikiPathways - Reactome (0 pathways)
- ☐ WikiPathways - GenMAPP (0 pathways)
- ☐ WikiPathways - Other (104 pathways)
- ☒ BioCyc (224 pathways)
- ☐ BioPAX (Imported) (0 pathways)
- ☐ GPML (Imported) (0 pathways)
- ☐ Hand created (0 pathways)
- ☐ Legacy (0 pathways)

Literature Derived Networks

ays)
pathways)

Help << Back Next >> Finish Cancel

Transcriptomics results

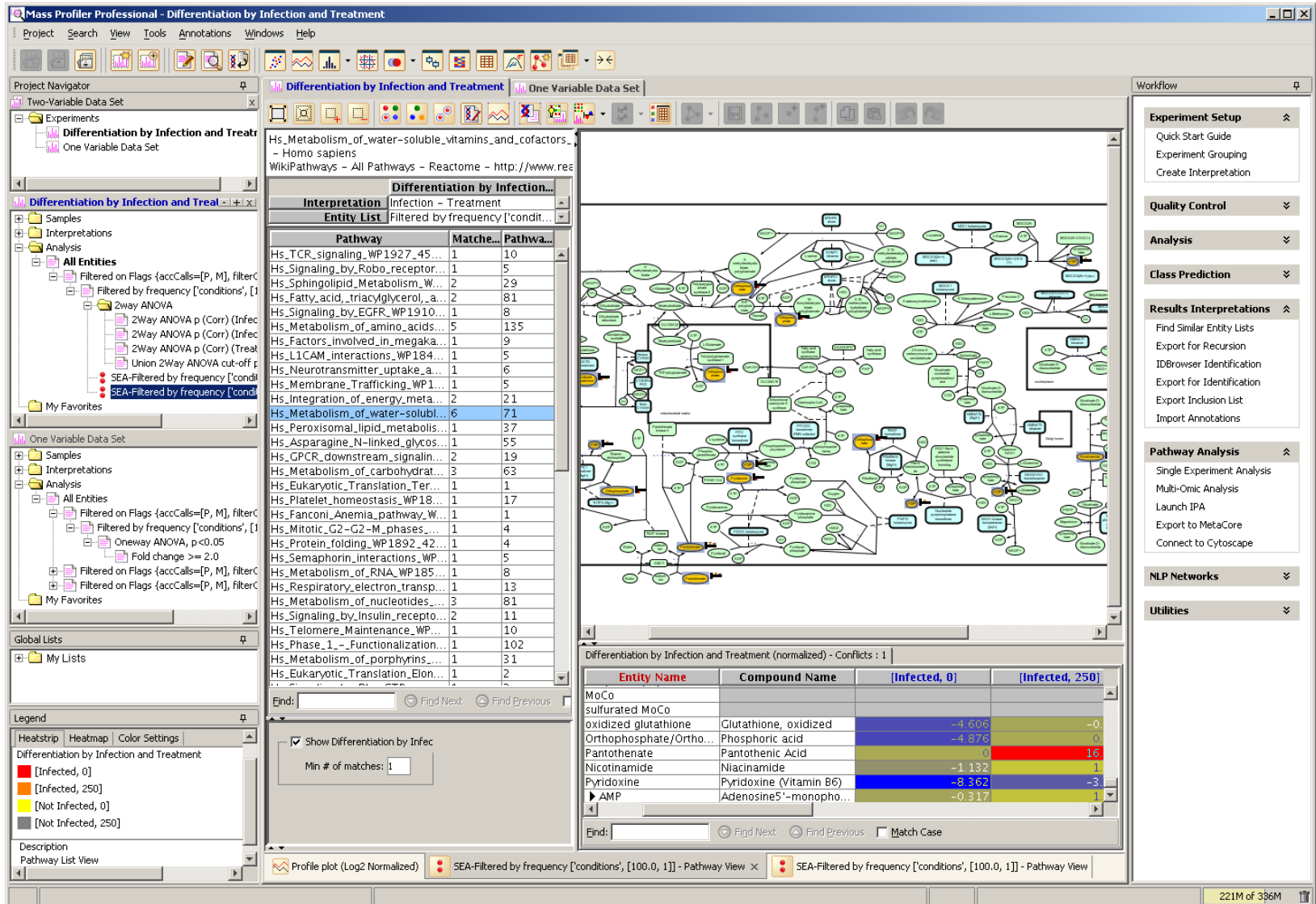
Metabolomics results

Pathway Organism

Pathway database



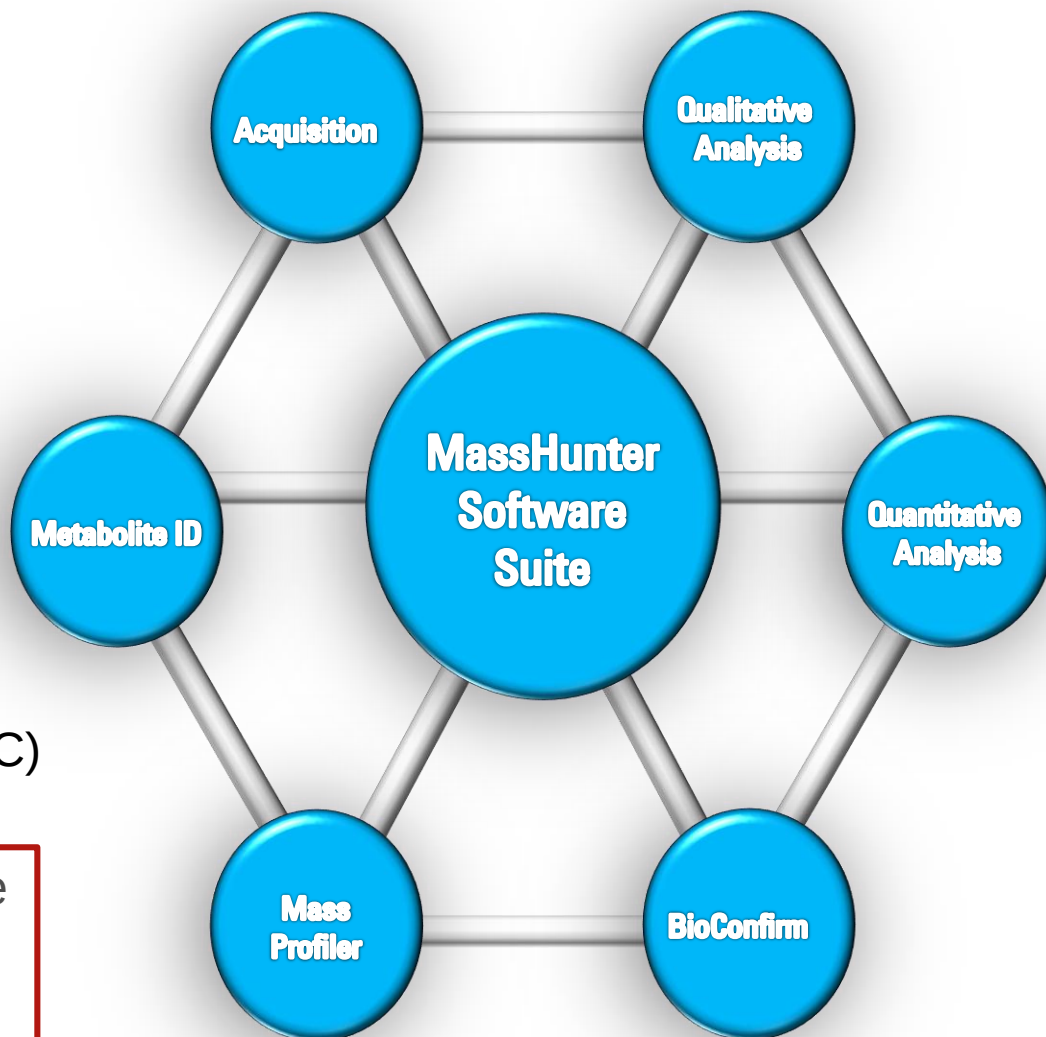
Pathway Architect Navigation



Mass Hunter Qualitative Analysis and Its “Accessories”

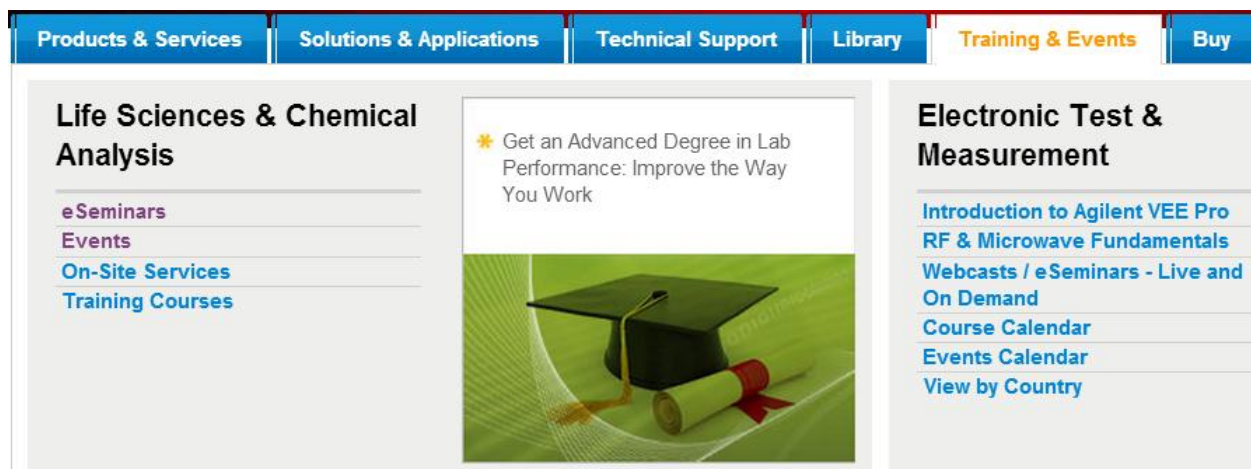
- Qualitative Analysis (Qual)
- PCDL Manager
- Pathways to PCDL
- Profinder
- Mass Profiler (MP)
- Mass Profiler Professional (MPP)
- ID Browser
- SimLipid (Premier Biosoft)
- Molecular Structure Correlator (MSC)

Fully integrated workflows to enable you to identify, plan, and execute your next experiment



How can you learn more about MassHunter software?

- Read the PDF manuals and guides that shipped with your instrument or software
- Watch the videos that shipped with your instrument or software
- Read the Workflow Overviews and Workflow Guides specific to your application area
- Use the Help feature in the software!
 - Especially good for unfamiliar terms or software features
- Attend a training class – contact your Account Manager if interested
 - QTOF operation
 - QQQ operation
 - MPP
- View eSeminars – search Agilent website



Familiarization Guides

Remember LC/MS and GC/MS Versions!



**Agilent MassHunter
Workstation Software**
Qualitative Analysis

Familiarization Guide



Agilent Technologies



**Agilent MassHunter
Workstation Software**
Qualitative Analysis

**Familiarization Guide for
GC/MS**



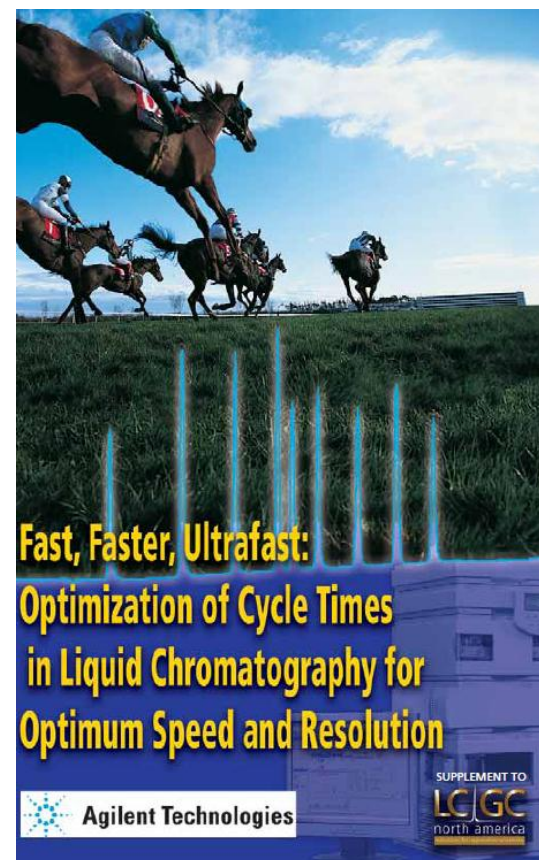
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Training: Learn about Liquid Chromatography

Online Primers and Basic Concept Guides



http://www.chem.agilent.com/search/?N=68+4294964917&Nr=OR%28part_language%3Aen%2Cg_rec_type%3ASharePoint%29&Ntt=primers

Updated Manuals/Training Materials Available on Website directly from the Help Page

Getting Started

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- English

NARROW YOUR CHOICES

Solutions

- Proteomics & Protein Sciences (3)

Product

- 6560 Ion Mobility Q-TOF LC/MS (3)
- MassHunter Data Acquisition Software (1)
- MassHunter Quantitative Data Analysis Software (2)
- MassHunter Workstation (2)
- MassHunter Workstation with MSD ChemStation DA (1)

1-5 of 5 results for Manuals

MassHunter Quantitative Analysis for GC/MSD Familiarization Guide (PDF)

This Familiarization Guide presents step-by-step exercises to help you learn to use the Quantitative Analysis program.

Publication Part Number: G3335-90200 | Created: 31 Jan 2014 | File Size: 13 MB

6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide B.06.00 (PDF)

6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide B.06.00

Publication Part Number: G3335-90163 | Created: 23 Jan 2014 | File Size: 4 MB

MassHunter Workstation Software Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide B.06.00 (PDF)

6200 Series TOF and 6500 Series Q-TOF Familiarization Guide B.06.00

Publication Part Number: G3335-90161 | Created: 23 Jan 2014 | File Size: 4 MB

6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide B.06.00 (PDF)

6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide B.06.00

Publication Part Number: G3335-90162 | Created: 23 Jan 2014 | File Size: 6 MB



Application Specific LC/MS Workflow Guides:

Publ #	Title	Pages
5990-7061EN	QQQ LC/MS Bioanalysis Workflow Overview	9
5990-7060EN	QQQ LC/MS Bioanalysis Workflow Guide	40
5990-7063EN	Drug Discovery Screening Workflow Overview	12
5990-7062EN	Drug Discovery Screening Workflow Guide	50
5990-7065EN	TOF, QTOF LC/MS BioPharma Workflow Overview	12
5990-7064EN	TOF, QTOF LC/MS BioPharma Workflow Guide	50
5990-7068EN	TOF, QTOF LC/MS Metabolomics Discovery Workflow Overview	16
5990-7067EN	TOF, QTOF LC/MS Metabolomics Discovery Workflow Guide	168
5990-7069EN	TOF, QTOF LC/MS Screening of Pesticides Workflow Overview	12
5990-7072EN	TOF, QTOF LC/MS Screening of Pesticides Workflow Guide	84
5990-7074EN	QQQ LC/MS Quantitation of Pesticides Workflow Overview	8
5990-7073EN	QQQ LC/MS Quantitation of Pesticides Workflow Guide	54
5990-9886EN	QQQ LC/MS Peptide Quantitation with Skyline Workflow Overview	12
5990-9887EN	QQQ LC/MS Peptide Quantitation with Skyline Workflow Guide	54
5991-1644EN	Agilent Lipidomics Workflow Overview	14
5991-1643EN	Agilent Lipidomics Workflow Guide	66
5991-1910EN	Integrated Biology with Mass Profiler Professional Workflow Overview	18
5991-1909EN	Integrated Biology with Mass Profiler Professional Workflow Guide	124
5991-1995EN	Agilent All Ions MS/MS - Workflow Overview	8
5991-1994EN	Agilent All Ions MS/MS Workflow Guide	36



<http://www.chem.agilent.com/en-us/search/library/Pages/default.aspx>

Any Other Questions?



Qualitative
Analysis ...



PCDL
Manager



Pathways to
PCDL



Profinder
B.06.00



Mass Profiler
Professional



Molecular
Structur...



DA
Reprocessor



SimLipid 4



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