MassHunter Software Overview, Tips, & Tricks

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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

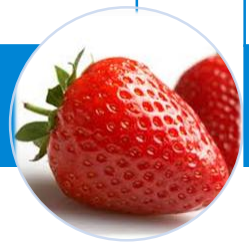
ICP-MS

- **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

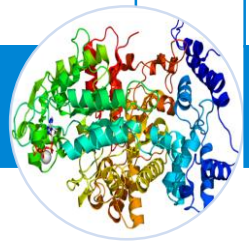
Veterinary Drugs

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

Forensic Toxicology

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

Proteomics

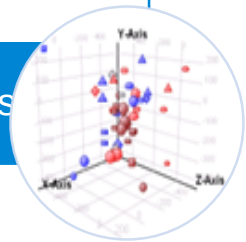


- **BioConfirm** for characterization of intact proteins, peptides, and monoclonal antibodies

Biopharma

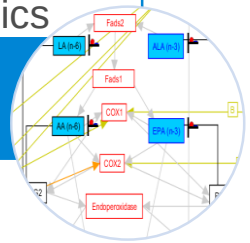
- **Mass Profiler Professional** (MPP) for differential profiling

Metabolomics



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

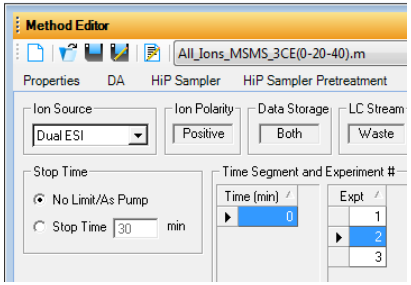
Integrated Biology



Agilent MassHunter Core Programs

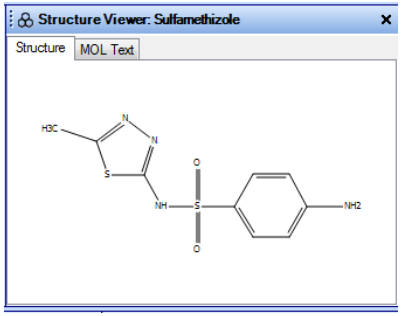
Acquisition

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



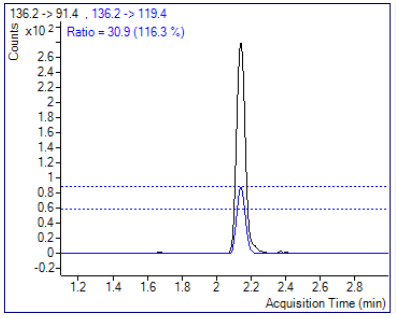
Qualitative Analysis

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

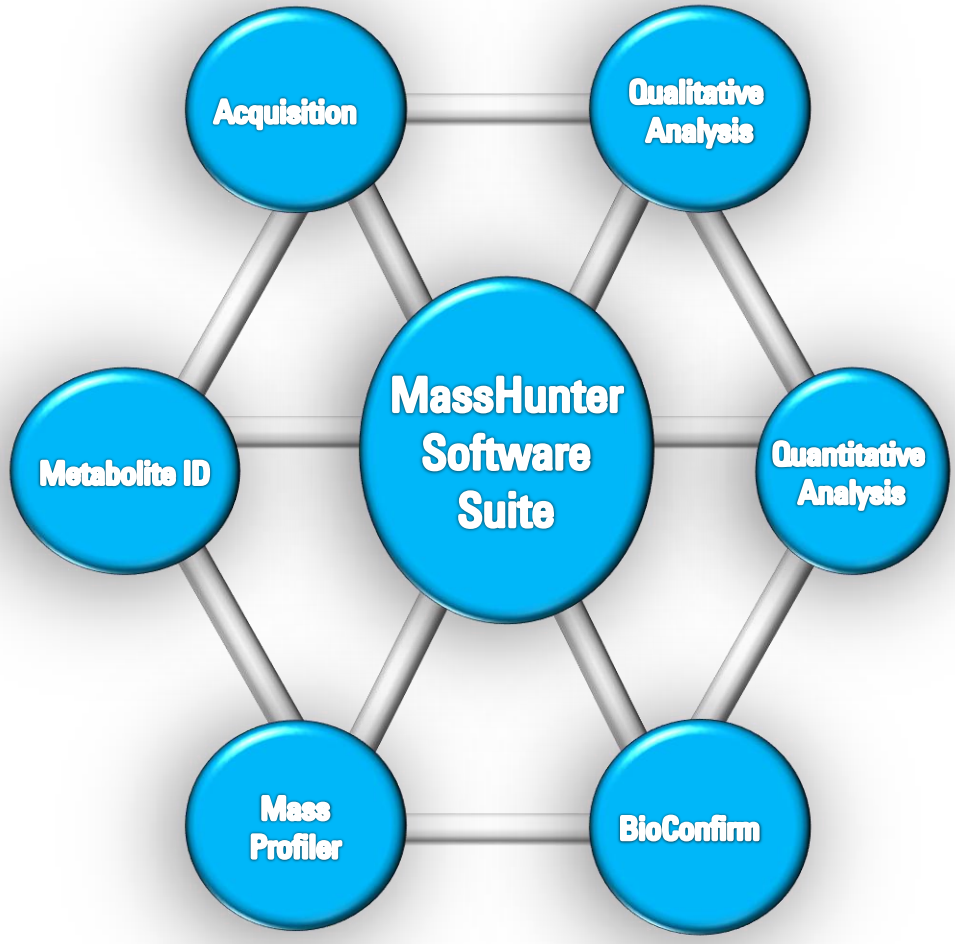


Quantitative Analysis

- 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

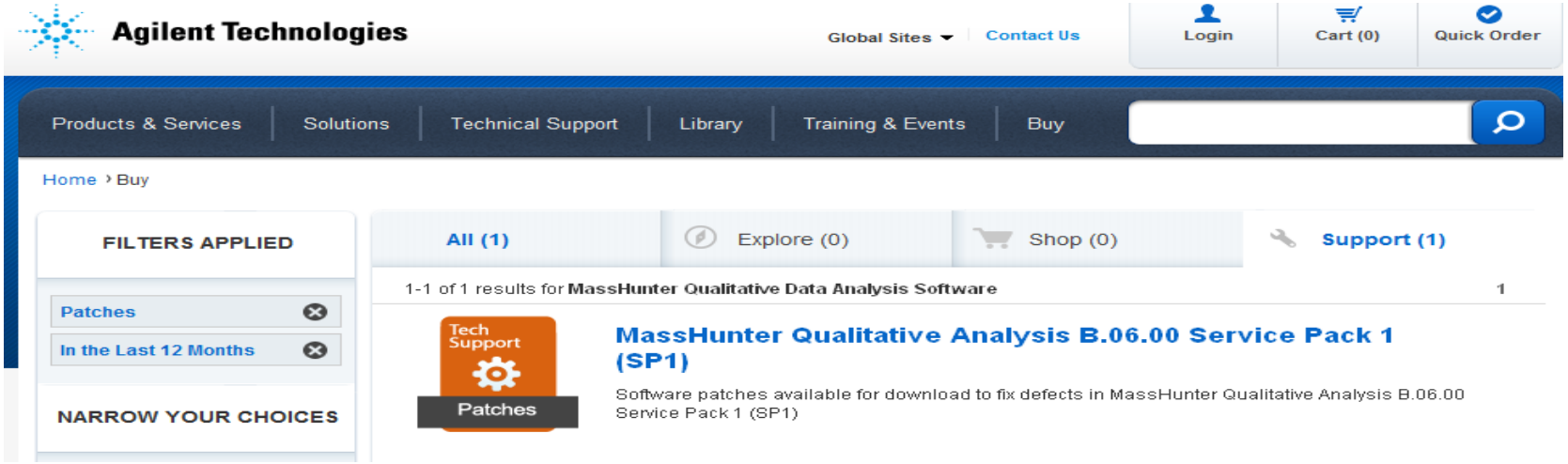
Current Versions (May 2014)

- 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



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

Acq
- High resolution
- Accurate mass
- LC/TOF and Q-TOF

Qual
- Find compounds
- Characterize
- Identify

PCDLs
- Market-focused databases and libraries
- Application Kits for quick startup

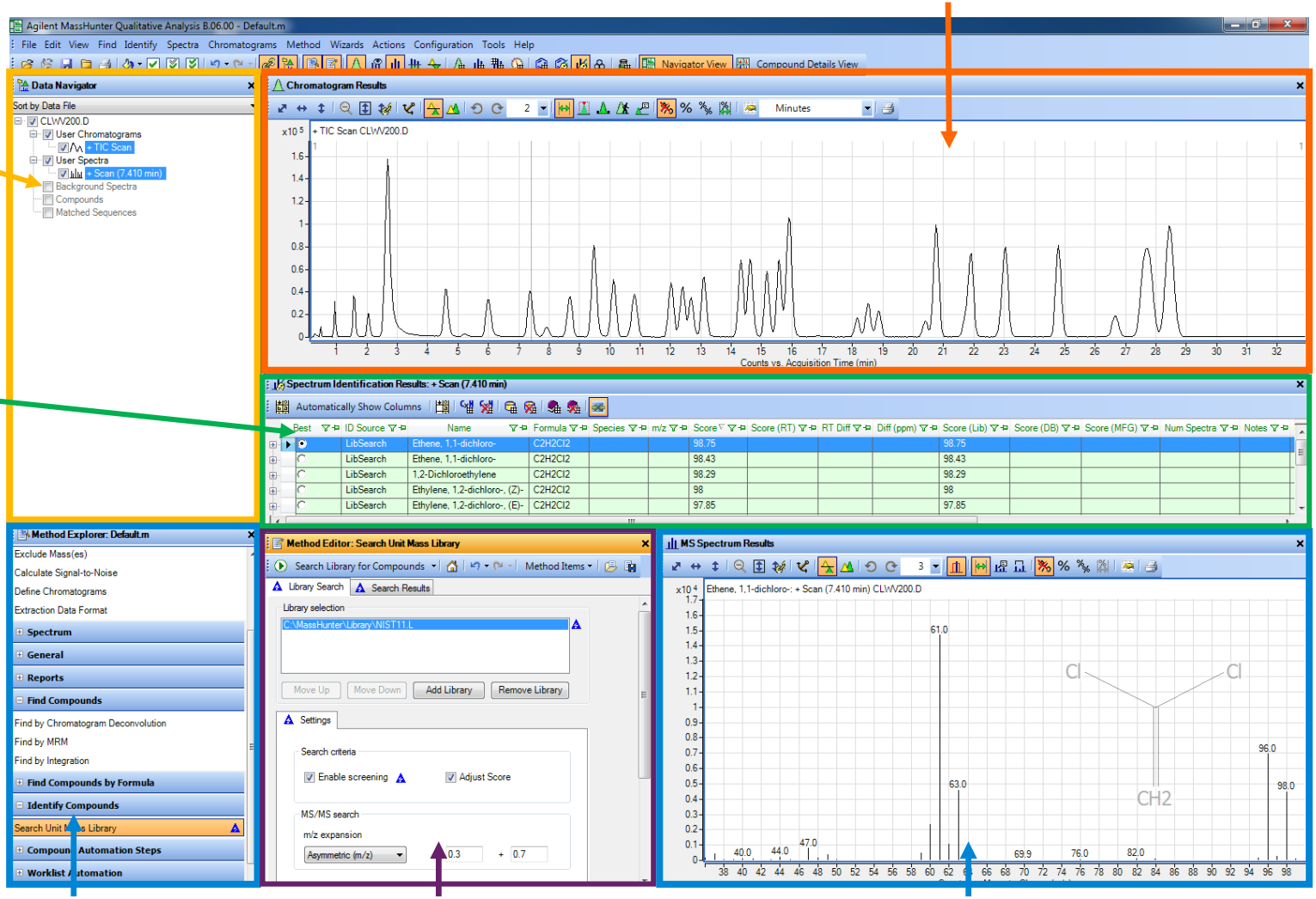
Agilent provides a complete suite of tools, consumables and consulting to set up for the rapid identification of unknowns

Qualitative Analysis

Chromatogram Display

Data Navigator

Results Table



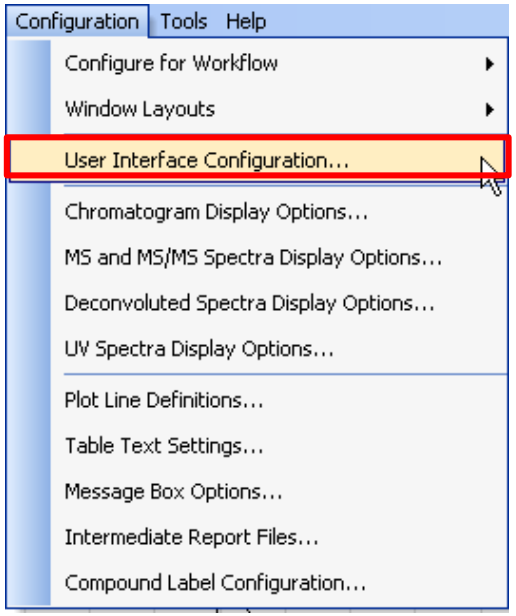
Method Explorer

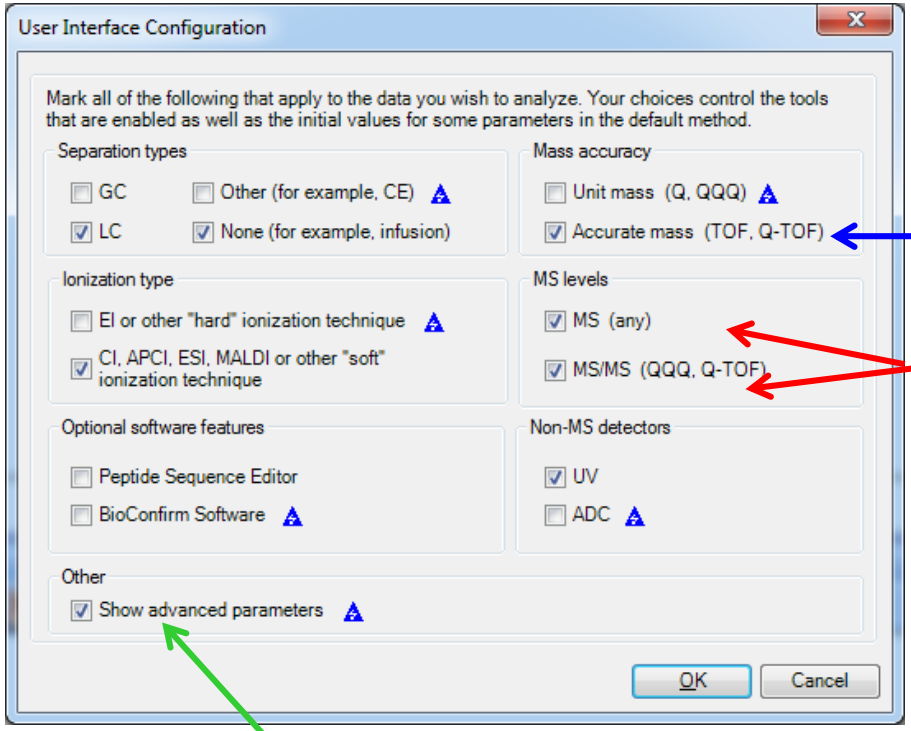
Method Editor

Spectrum Display

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

So Setup User Interface for Data File Type





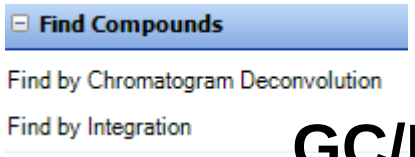
Accurate Mass

Both Levels

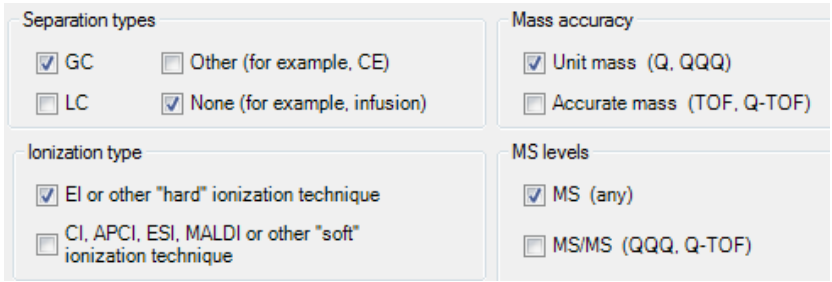
Show Advanced Parameters

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

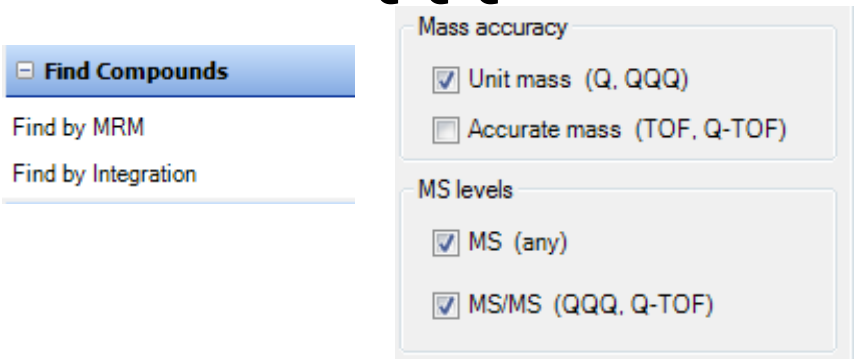
Selecting Data Types Changes Display



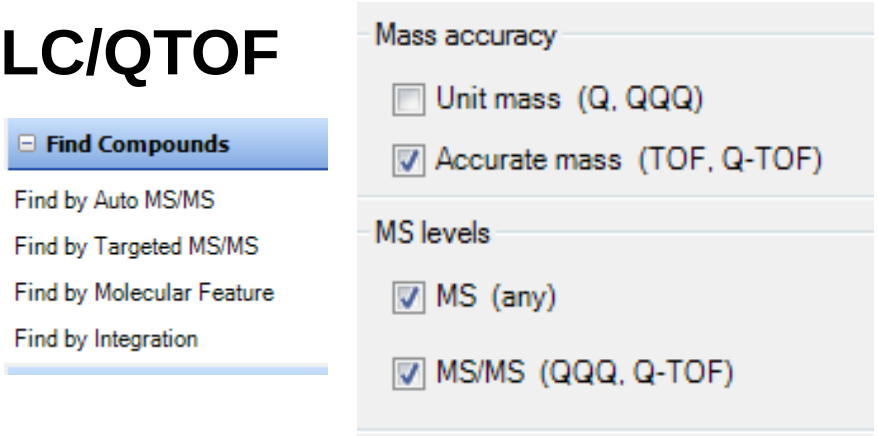
GC/MSD



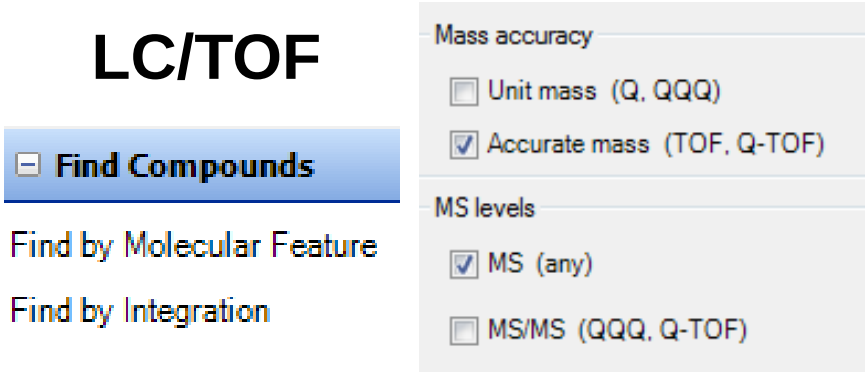
LC/QQQ



LC/QTOF



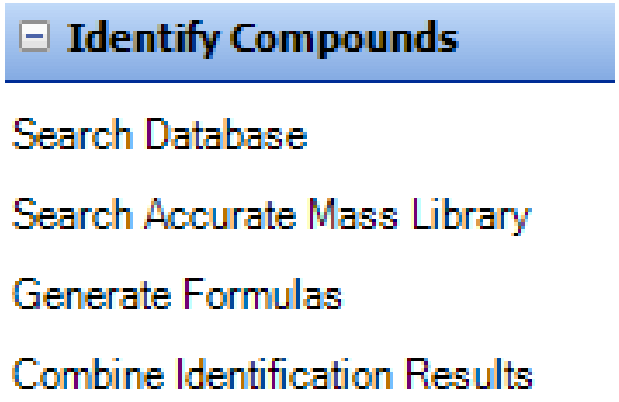
LC/TOF



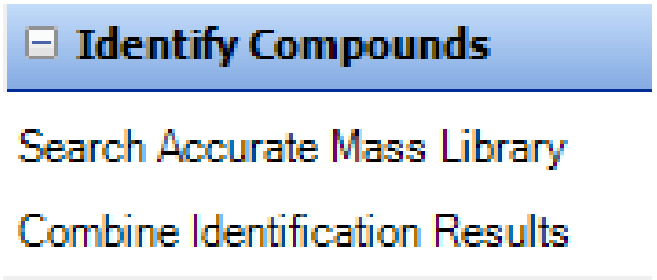
TIP: To use low res libraries you must have the GC checked

And Changes Compound Identification

Accurate Mass Q(TOF)



Unit Mass MSD/QQQ



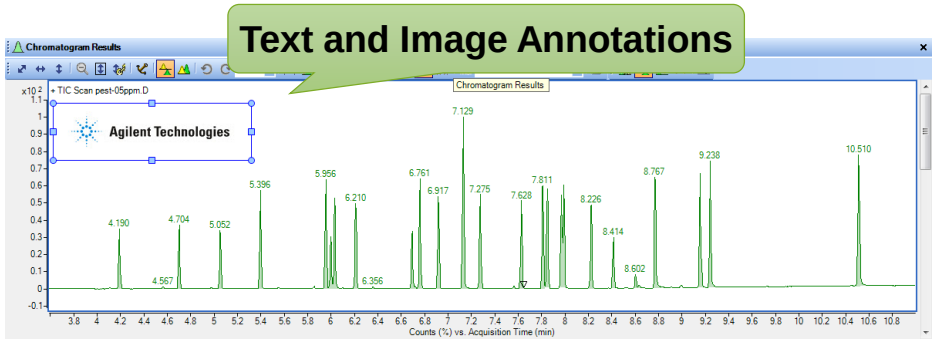
Tip: The low resolution *.L file should be copied to the X:\\MassHunter\Library\ subdirectory and one can use the NIST library if you have a license

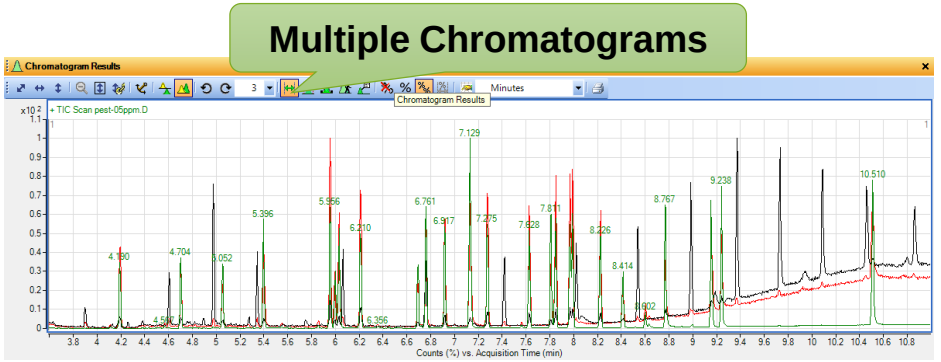
Chromatogram Display

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

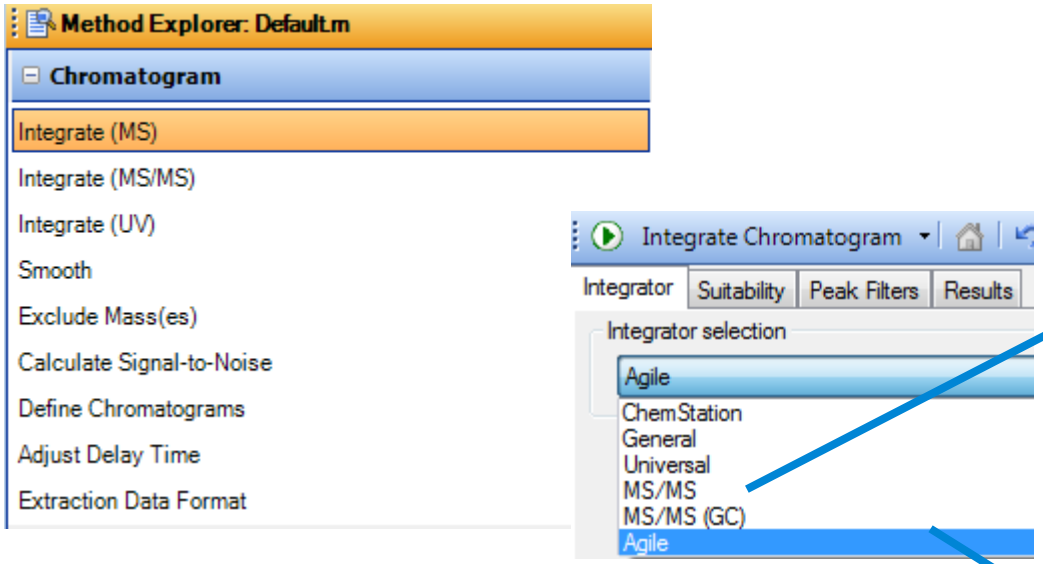
Definition of chromatograms can be stored in the method and used with automation.

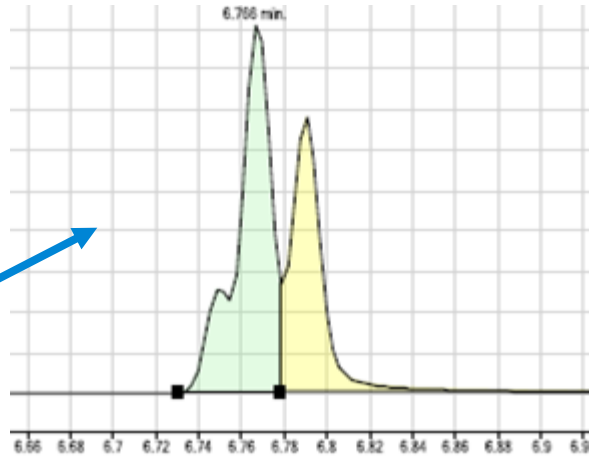




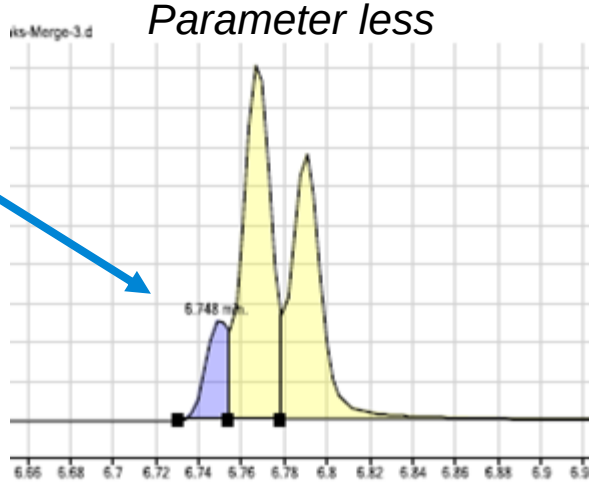
Improved Parameter-less Integrators

MS/MS and Agile what's the difference?





Parameter less

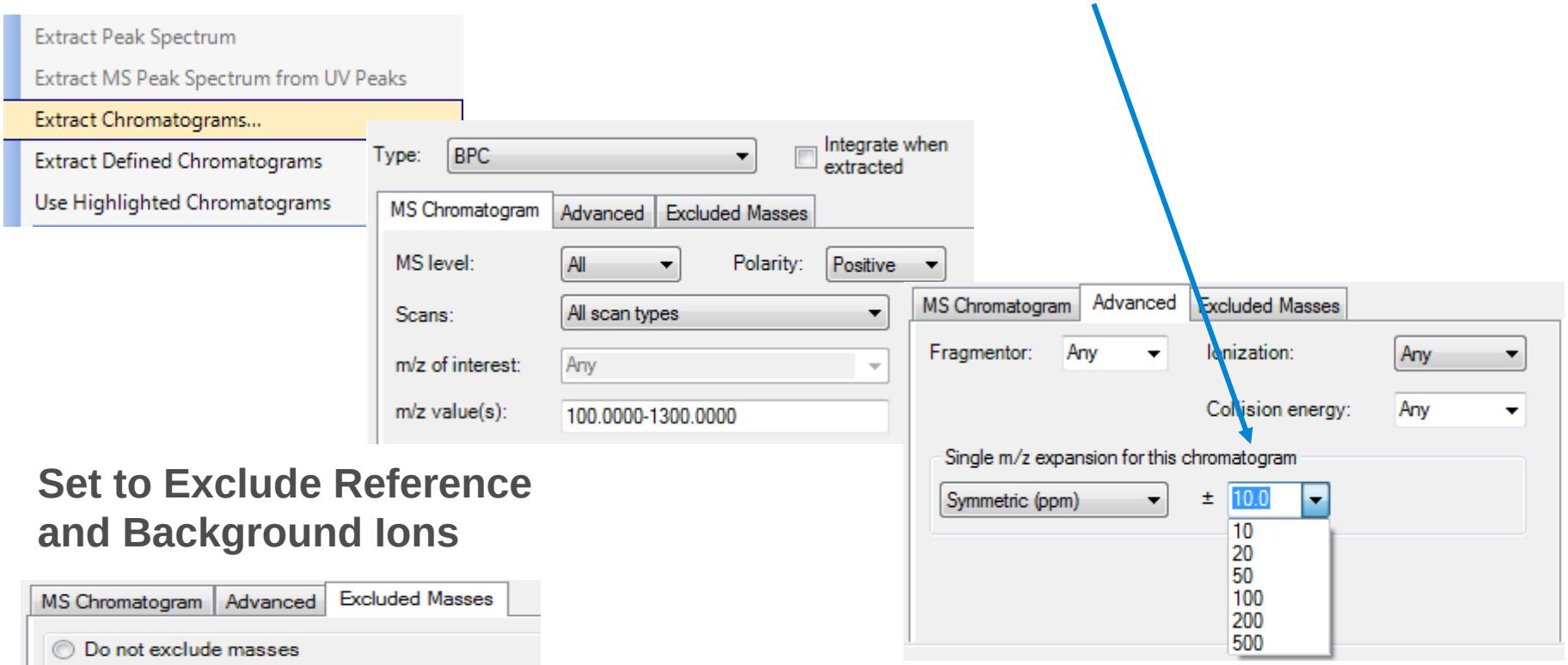


The Agile integrator requires
75% fewer data points for Integration
typically 15 points minimum

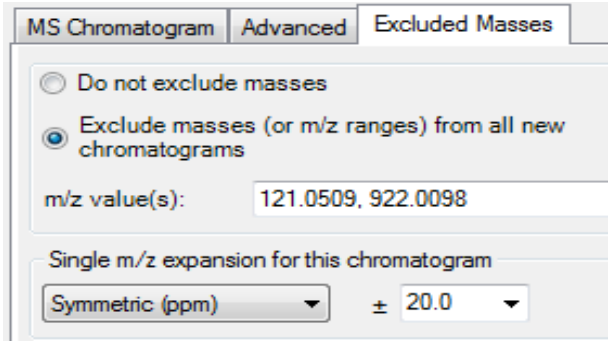
Agile2 Now in QUANT B.07

For Accurate Mass Systems

Use Advanced Tab to Narrow Extraction Limits (10 ppm)



Set to Exclude Reference and Background Ions



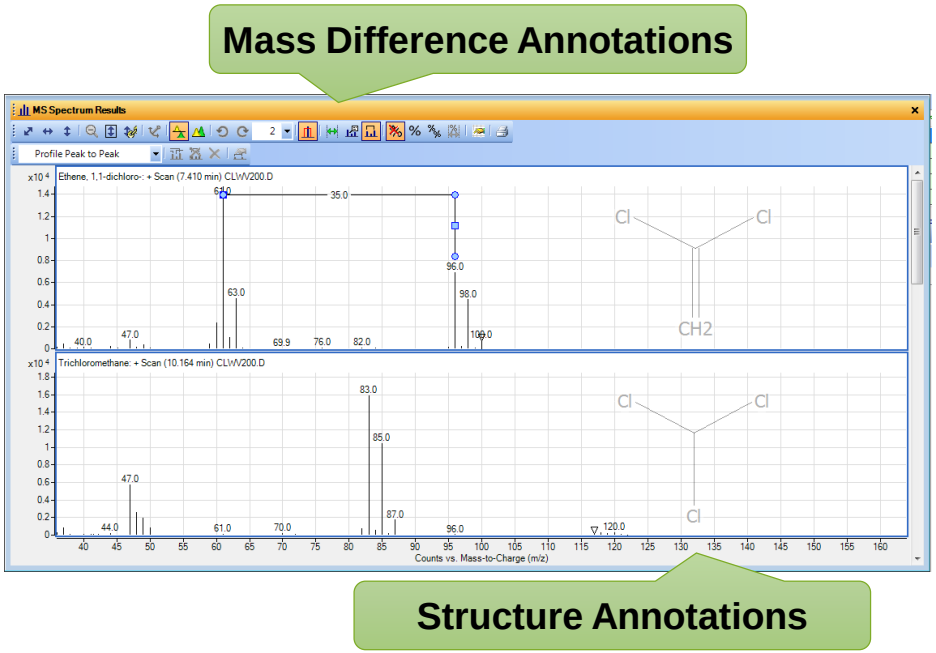
Spectra Display

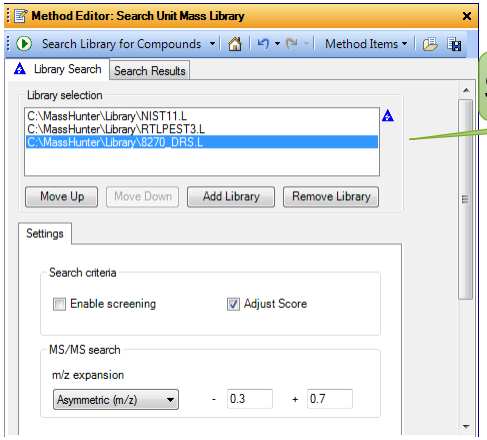
Extract Spectra via:

- 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

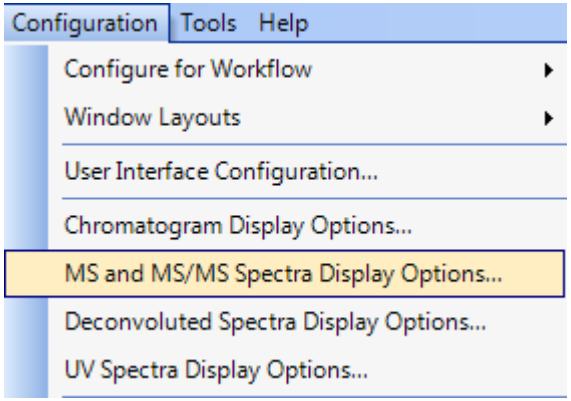
- Multiple Libraries (*.L)
- Link to NIST MS Search

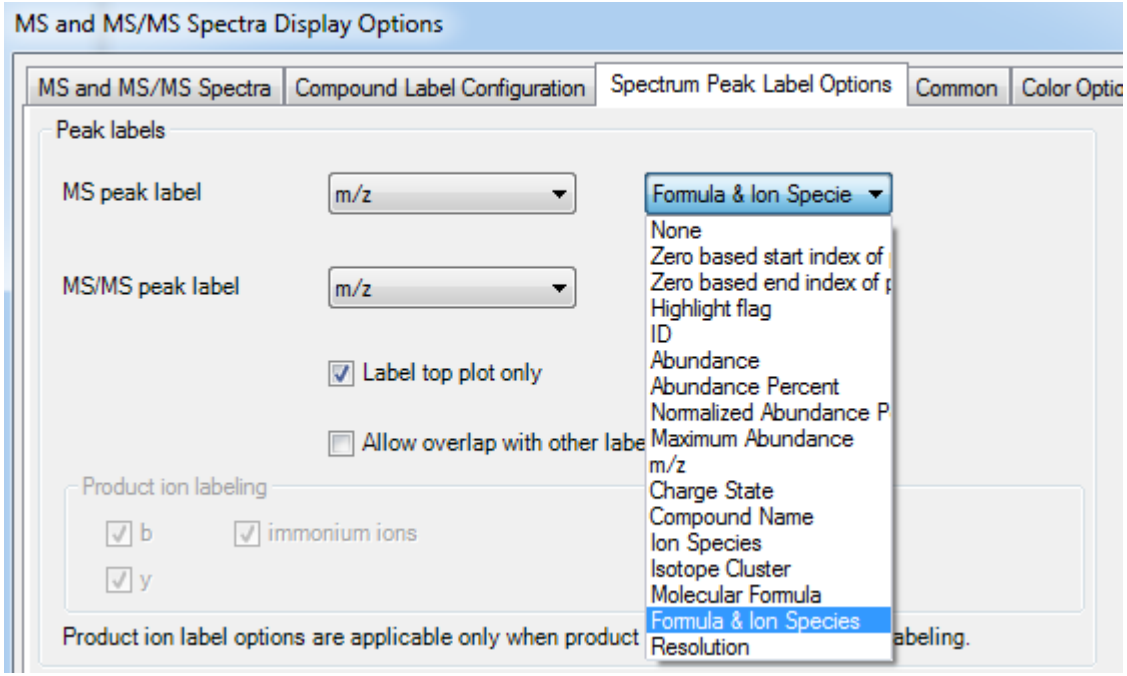




Search Multiple Libraries

How to Set Labels on MS and MS/MS Spectra

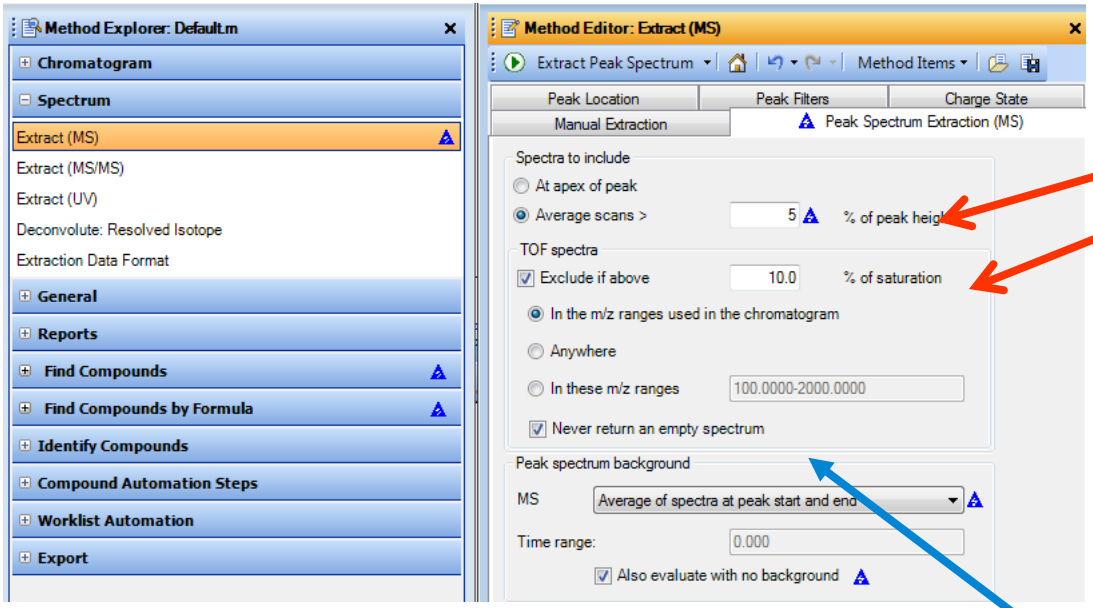




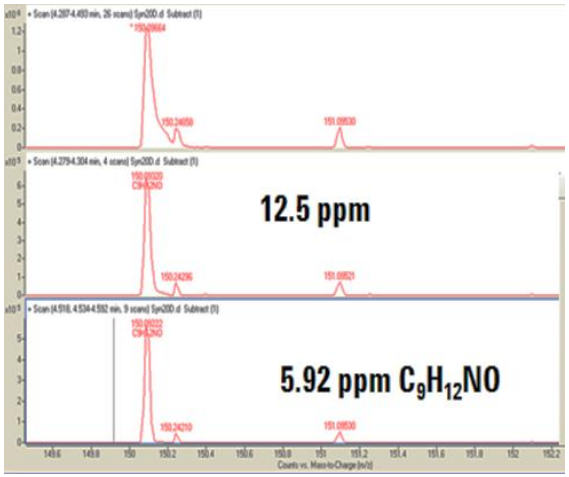
Multiply Charged Species

In Profile Mode Resolution of MS Peak

Using Extract Peak Parameters –Saturation Correction



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

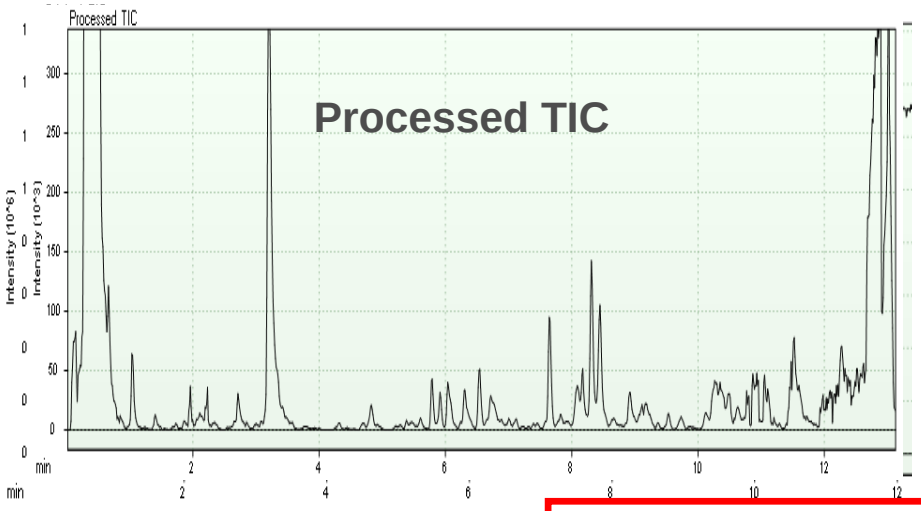


For Narrow Peaks Remember to Check Never Return an Empty Spectrum

Unsupervised Naïve data mining

Molecular Feature Extraction (MFE)

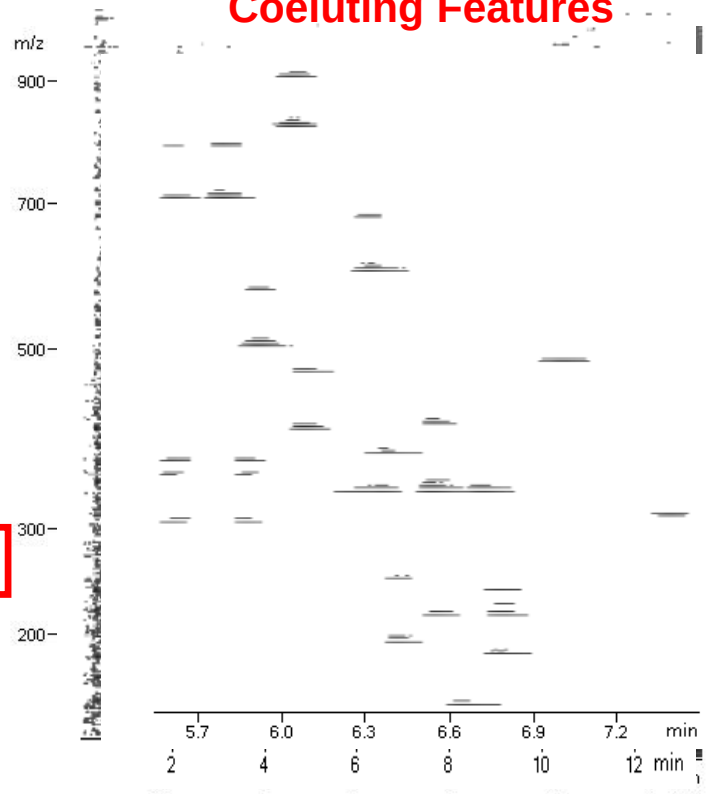
Finds Features in TOF/QTOF Data



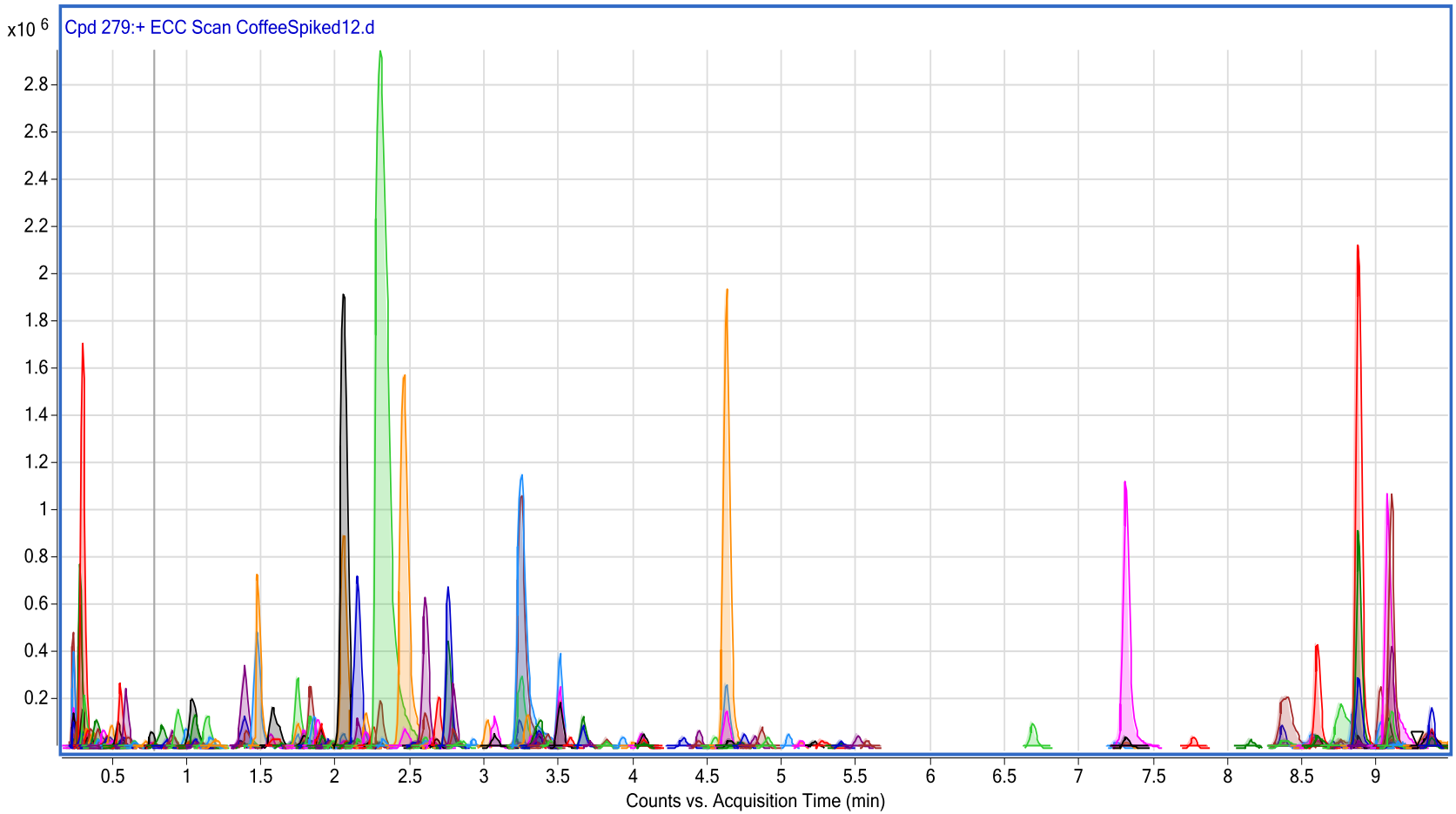
species	RT	m/z	mass	abund.
M	8.162		342.1467	130643
M+H	8.165	343.1547	342.1474	11889
M+H+1	8.162	344.1581		2290
M+H+2	8.157	345.1748		369
M+H4N	8.164	360.1807	342.1469	8420
M+H4N+·	8.156	361.1893		1227
M+Na	8.162	365.1359	342.1466	75678
M+Na+1	8.163	366.1394		15324
M+Na+2	8.162	367.1429		1901
2M+Na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

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

3D Plot Before Coeluting Features

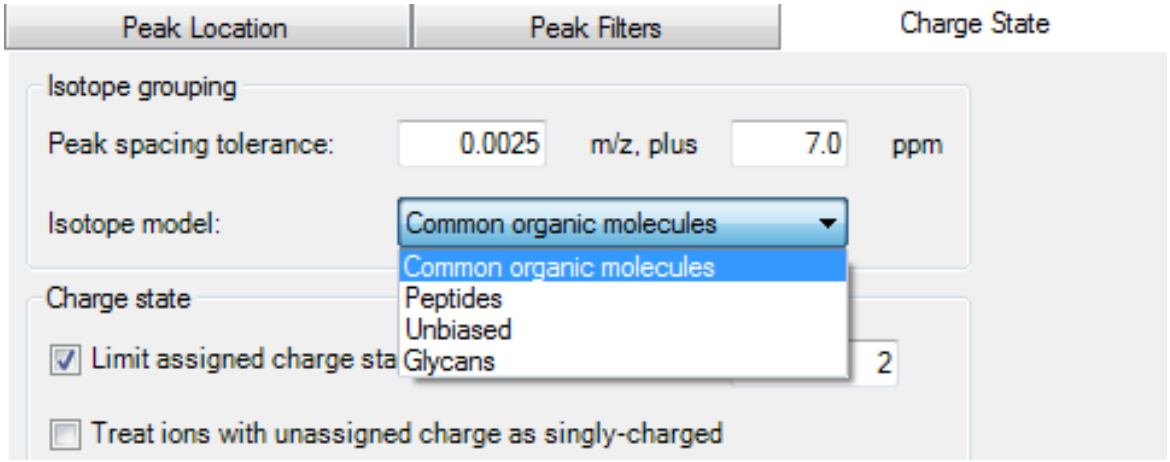


Graphical Results Displayed



Over 280 Compounds Found in Coffee Spiked with Pesticide

Choose Isotope Model to Match Application



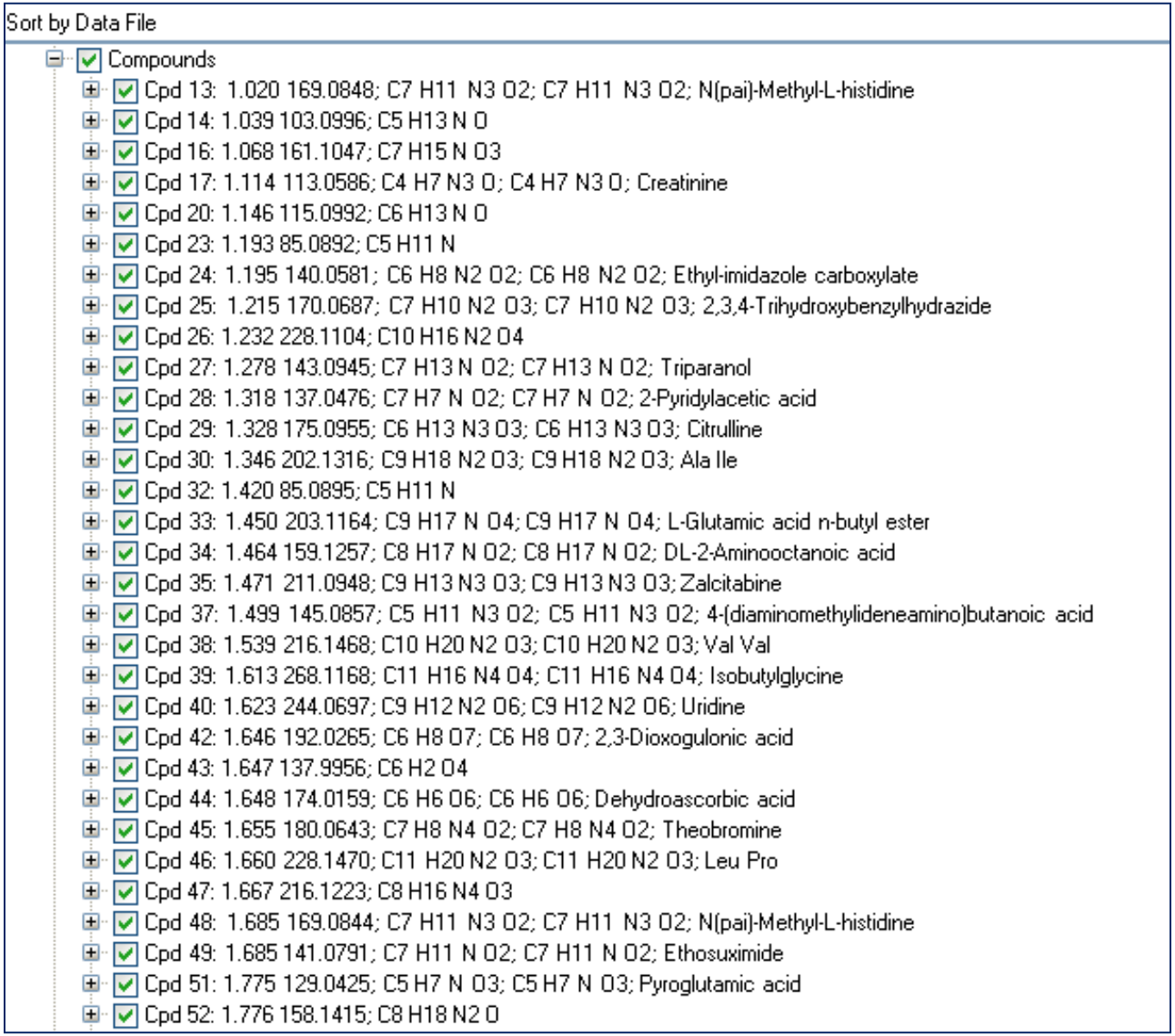
TIP:
For Compounds containing metals or elemental such as B, Li, Si select **Unbiased**

Check Limit Assigned Charge States Maximum Values

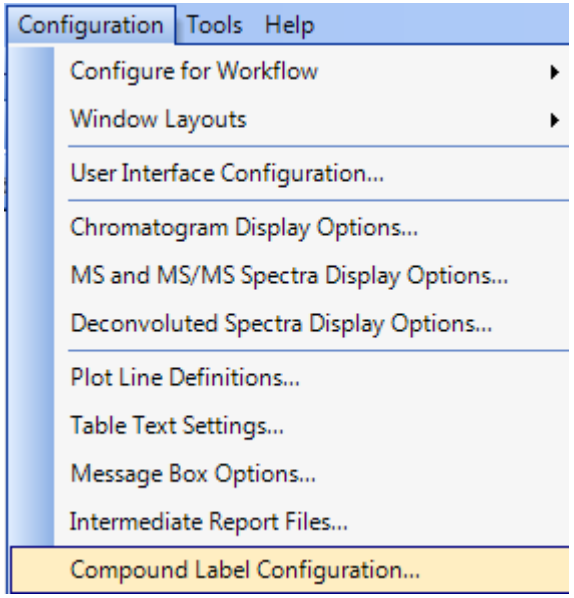
For Small Molecule Applications: Set to 2

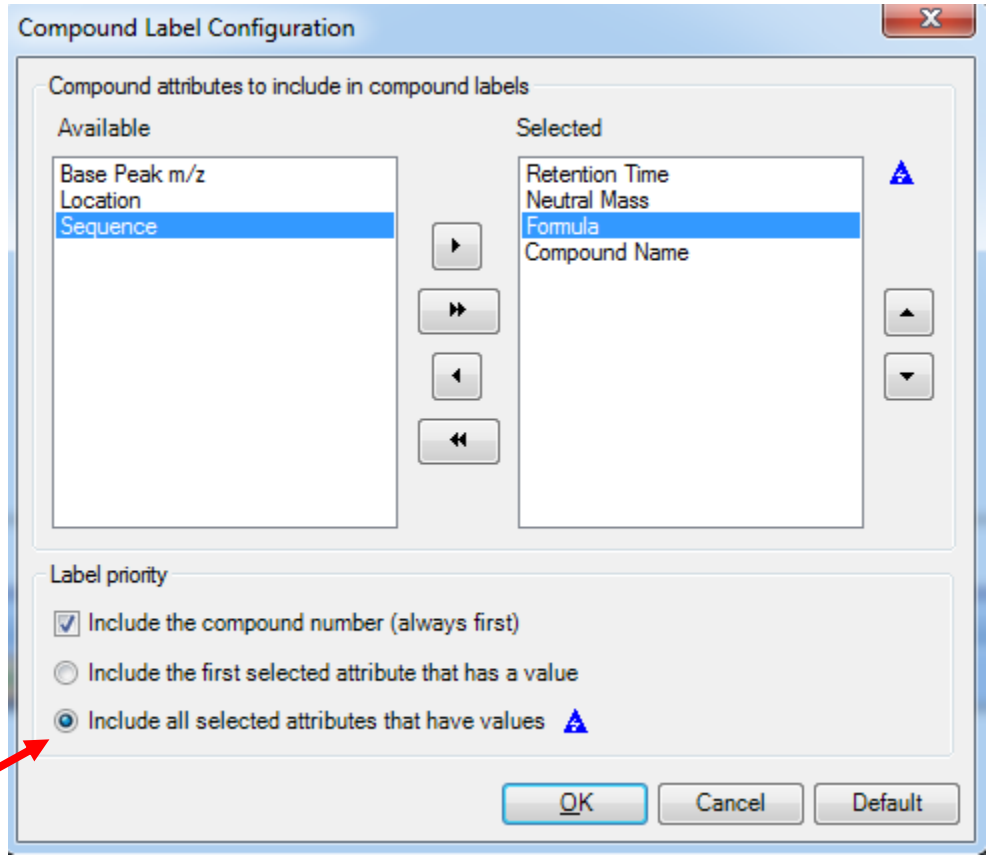
For High Molecular Weight Apps: Uncheck or Max 10

Compounds Labels Display



Specify Compound Label Configuration



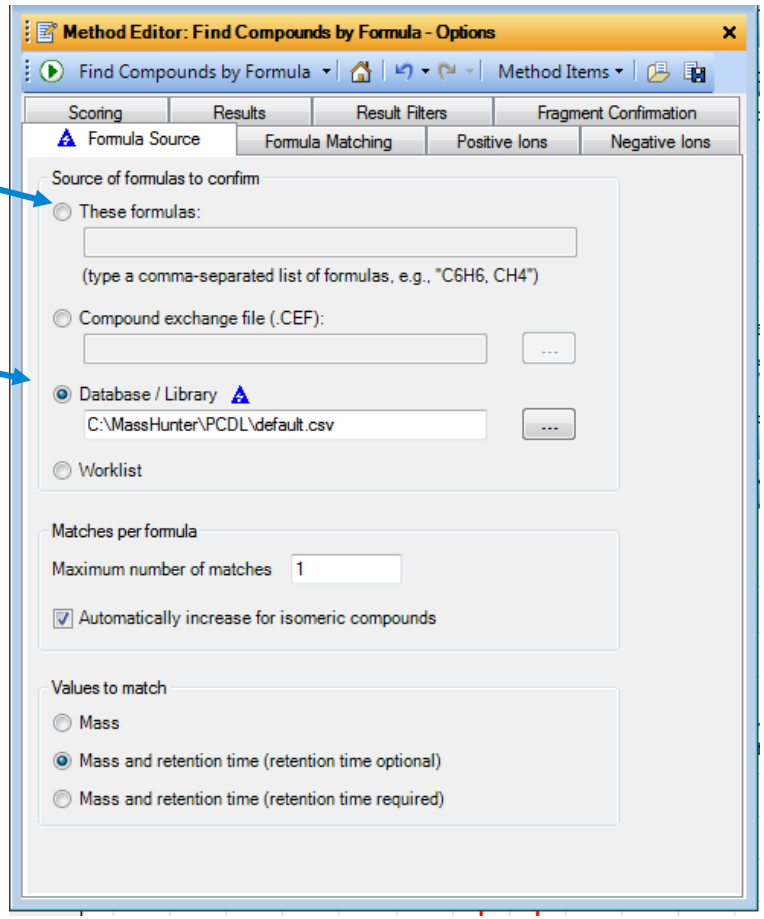


Highlight Parameter in list and use **>** and **<** to move to and from **Selected**.

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

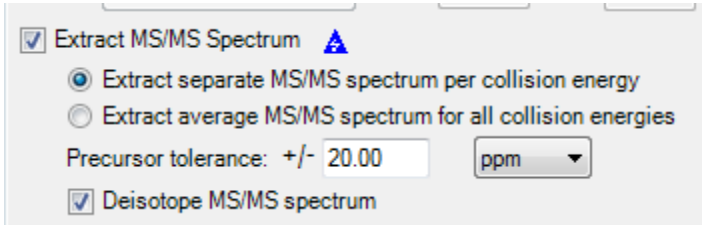
Benefit:

- 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

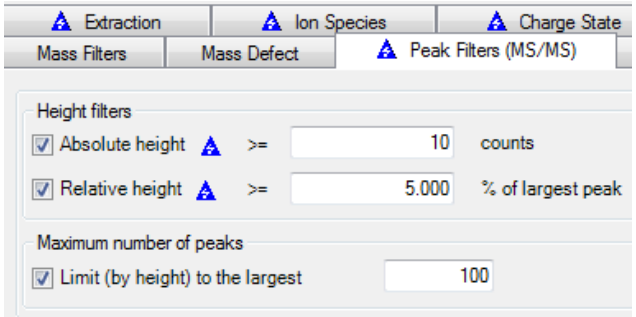
Disadvantage:

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

Results Tab



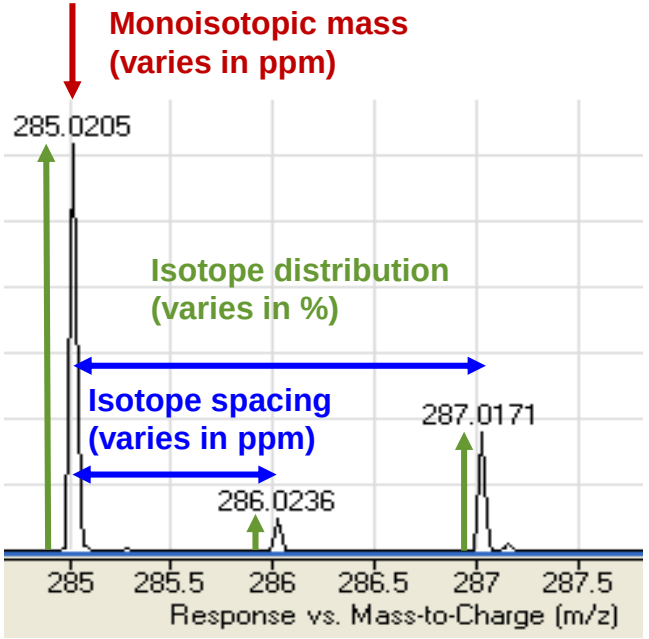
Remember to set MS/MS Peak Filters



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

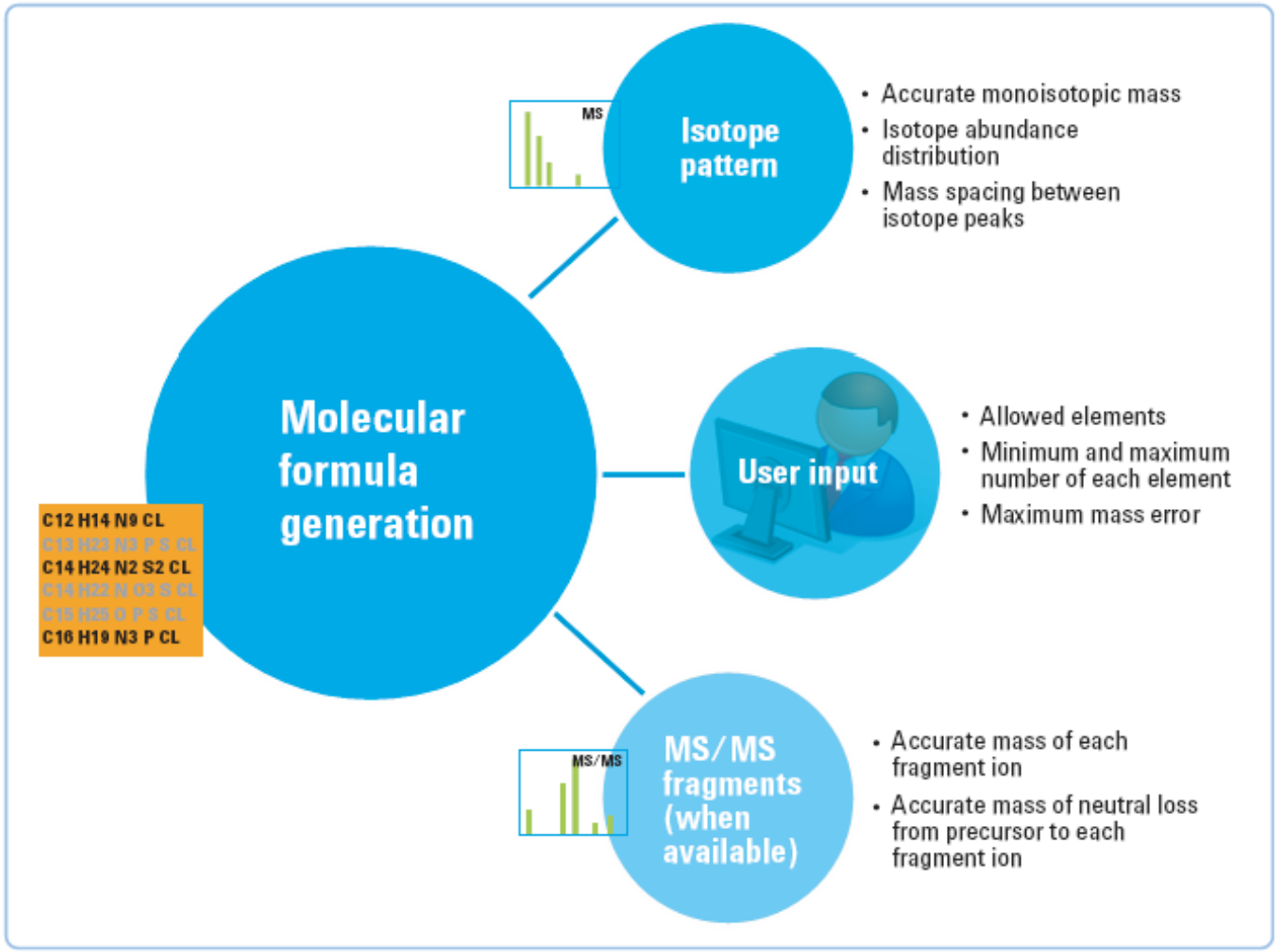
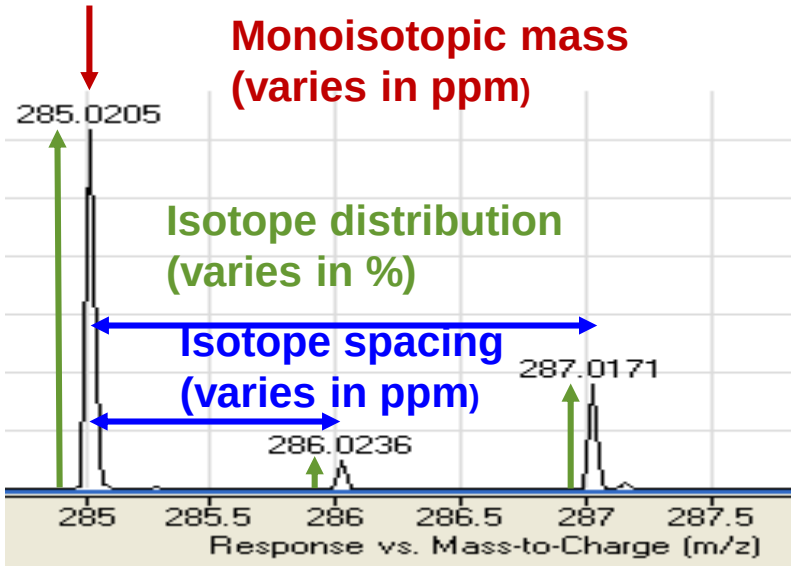


Figure 1. Agilent's molecular formula generation software uses multiple dimensions of information to generate and score lists of possible molecular formulas. It has been optimized for analysis of accurate-mass data from Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS systems.

Agilent's Molecular Formula Generation Software

Scoring based on



Mass Match +

Abund. Match +

Spacing Match =

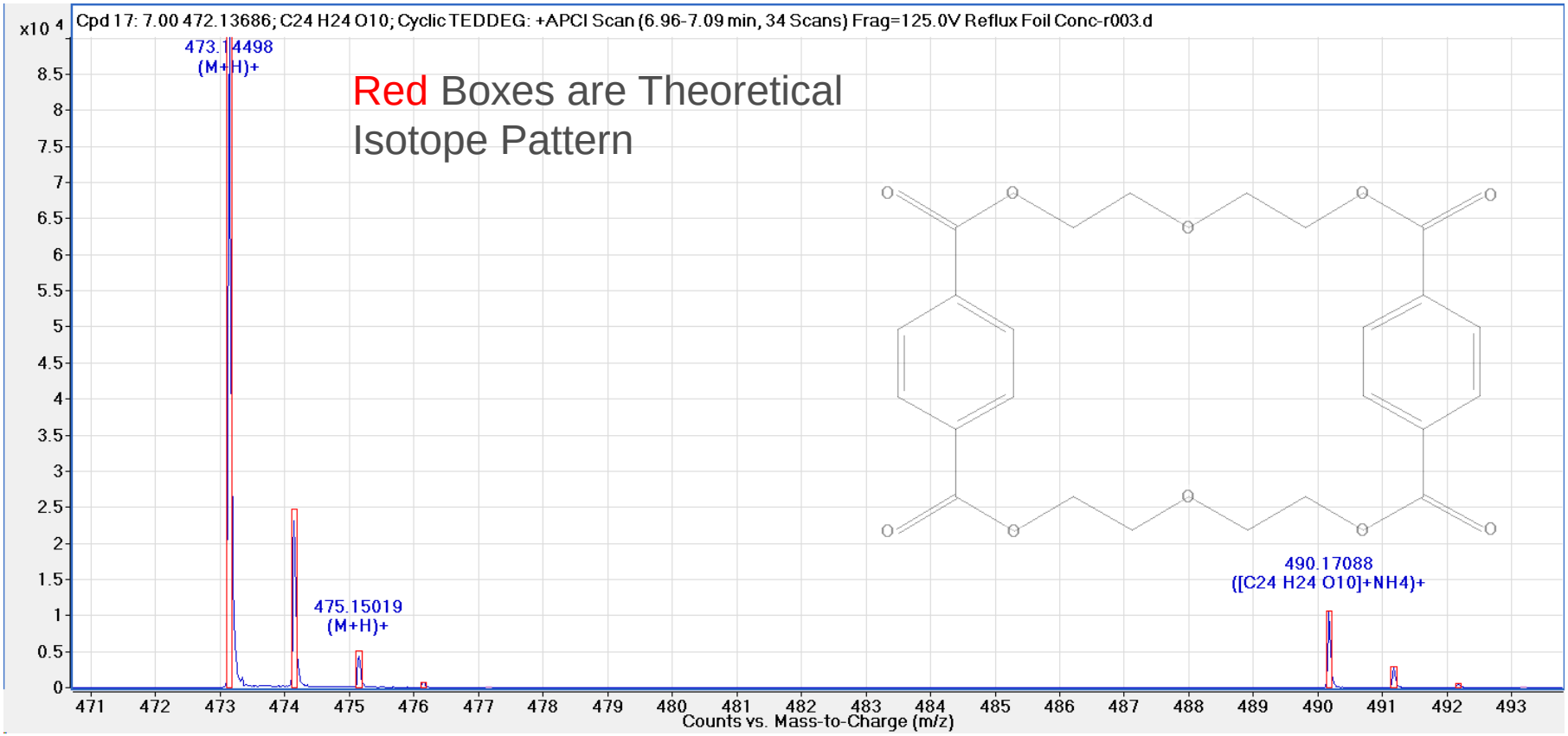
Overall Score

MS Formula Results: Cpd 2: C10H9ClN4O2S

m/z	Ion	Formula	Abundance
285.021	(M+H)+	C10 H10 Cl N4 O2 S	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
✓	C10 H9 Cl N4 O2 S	285.0208	99.55		284.0137	284.0135	-0.71	0.71	99.19	99.26	99.69	285.021	8
	C7 H12 N2 O6 S2	285.021	77.28		284.0137	284.0137	0.01	0.01	99.54	1.93	100	285.021	3
	C7 H13 Cl N4 O2 S2	285.0241	75.57		284.0137	284.0168	11.12	11.12	99.87	83.87	46.22	285.021	3

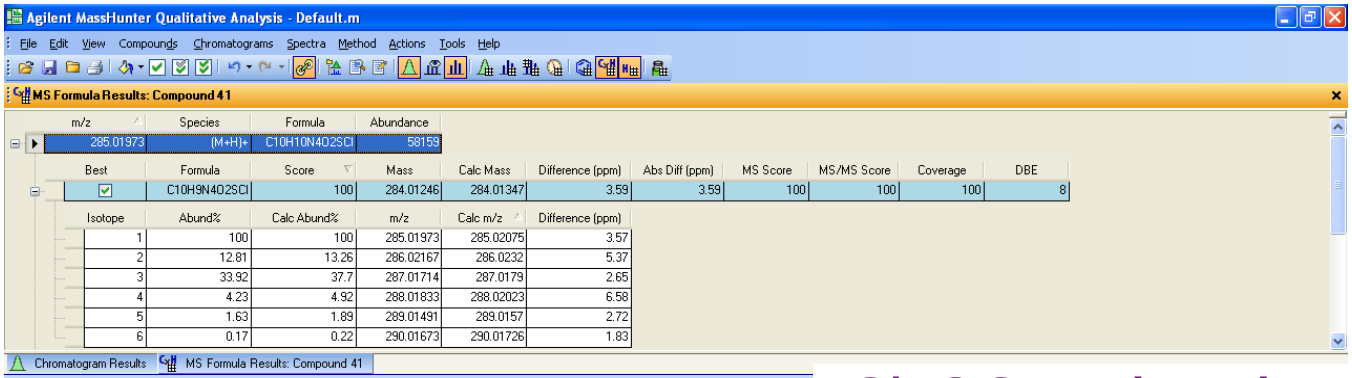
Isotope Pattern Matching



Molecular Formula Generation

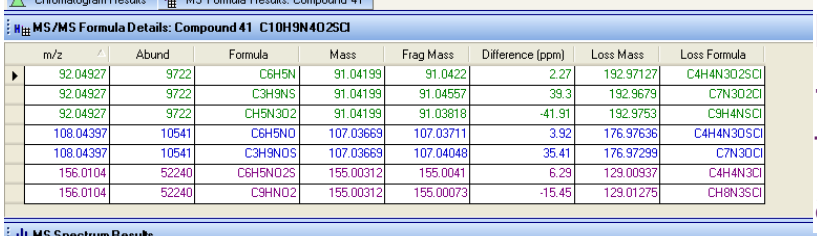
Identify Compounds

Isotope Pattern Matching and MS/MS for Formula confirmation



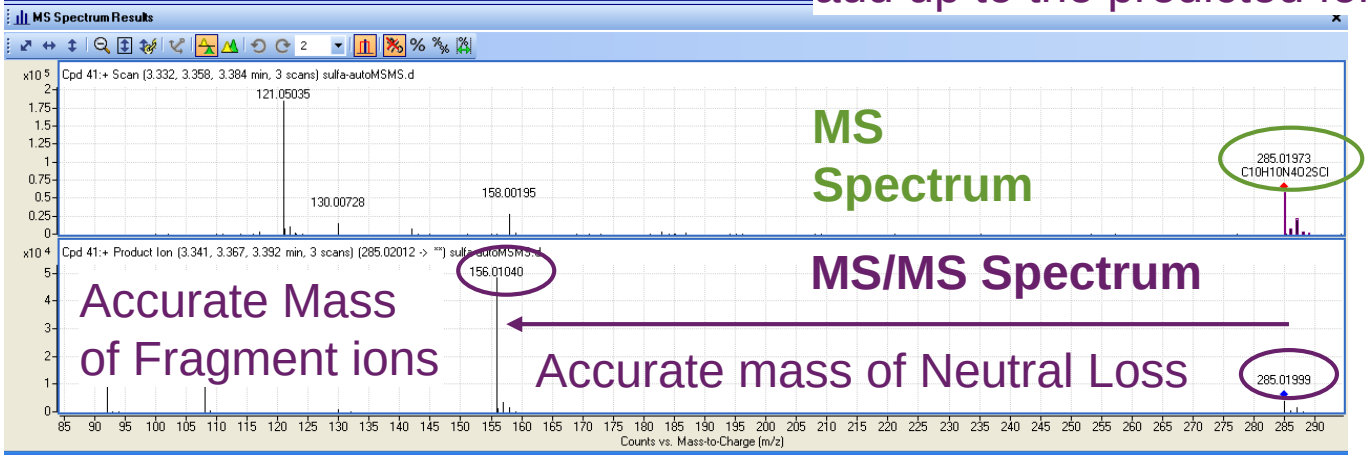
MS Score based on:

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



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:

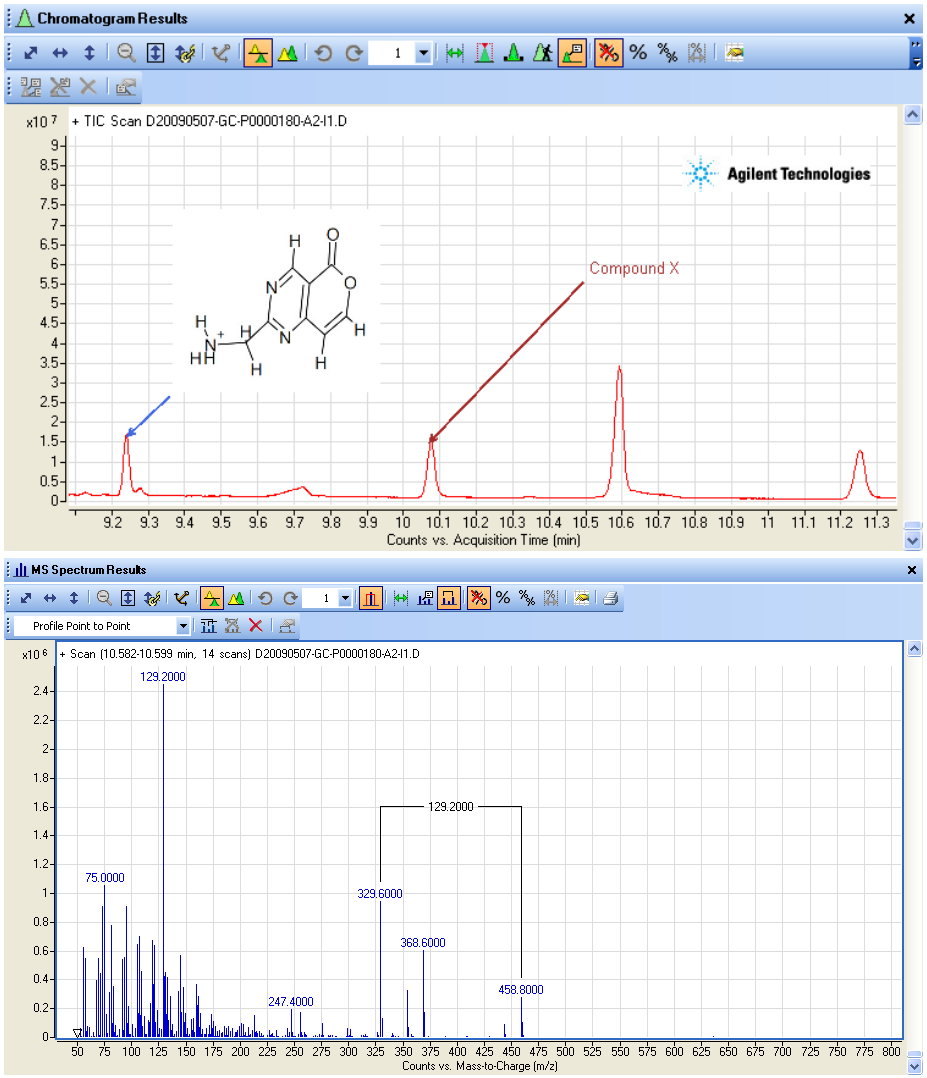
- Precursor ion
- Isotopes M+1, M+2
- Fragment ion
- Neutral loss

Annotate and Document

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

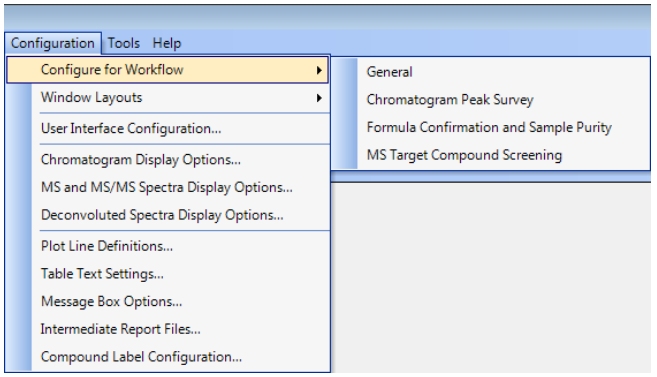
Use *Mass Caliper* to document fragmentation and losses in spectrum

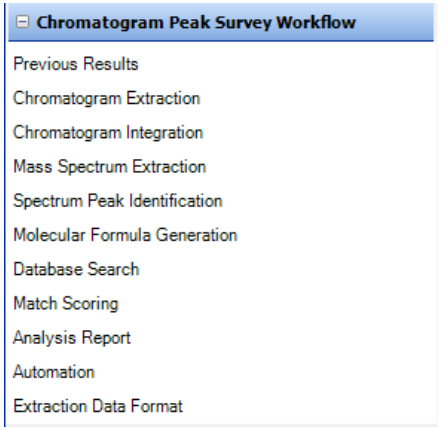
Export or use copy & paste to add graphics or results into presentations and documents



Workflows in Qual

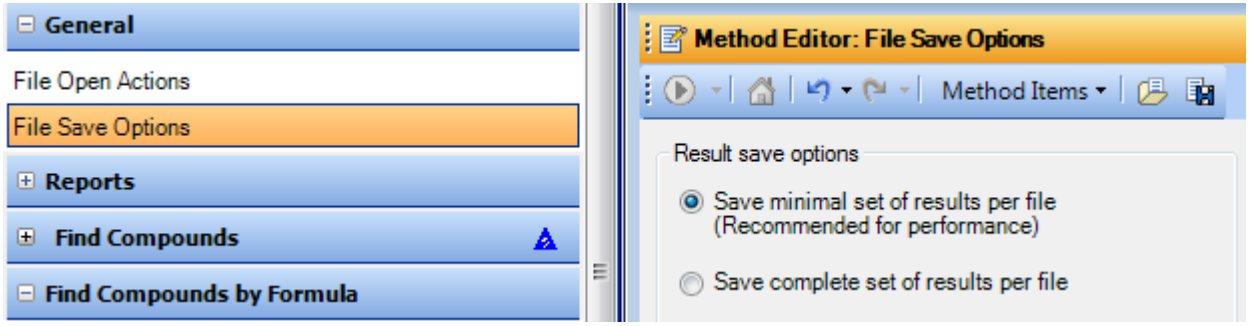
- **Workflows guide users through Qual functions needed for specific tasks**
  - Load via Configuration Menu on Toolbar
  - Default method with default parameters and report templates
  - Specified layout of user interface limited to needed windows
  - Section at the top of the Method Explorer grouping 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

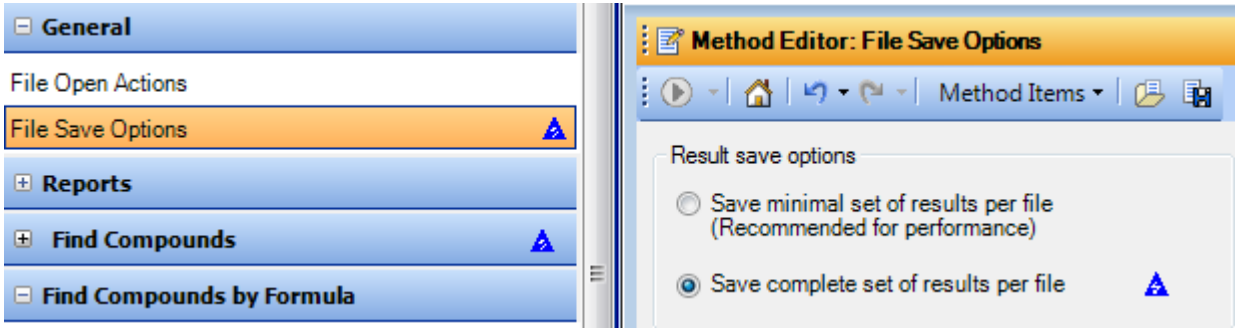
The Method default saves Minimal Amount of Graphics



Default Setting

Graphics not saved only compound list

Change to “Save Complete Results”



All Graphics Saved

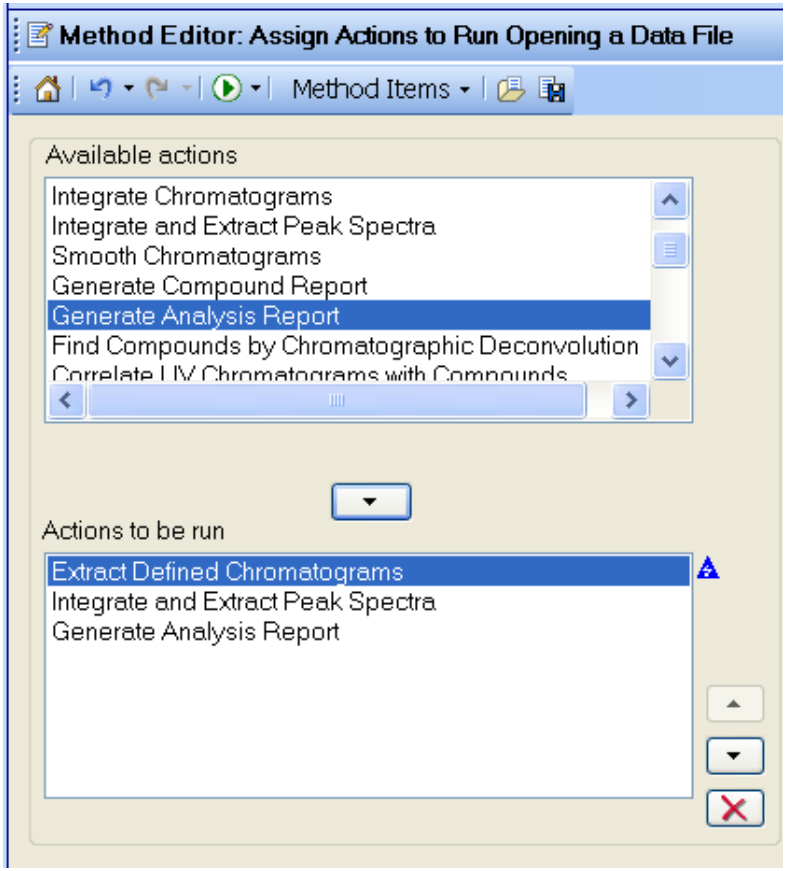
Automation - File Open Actions & Post Acquisition Worklist Processing

Automate common file open actions in manual review

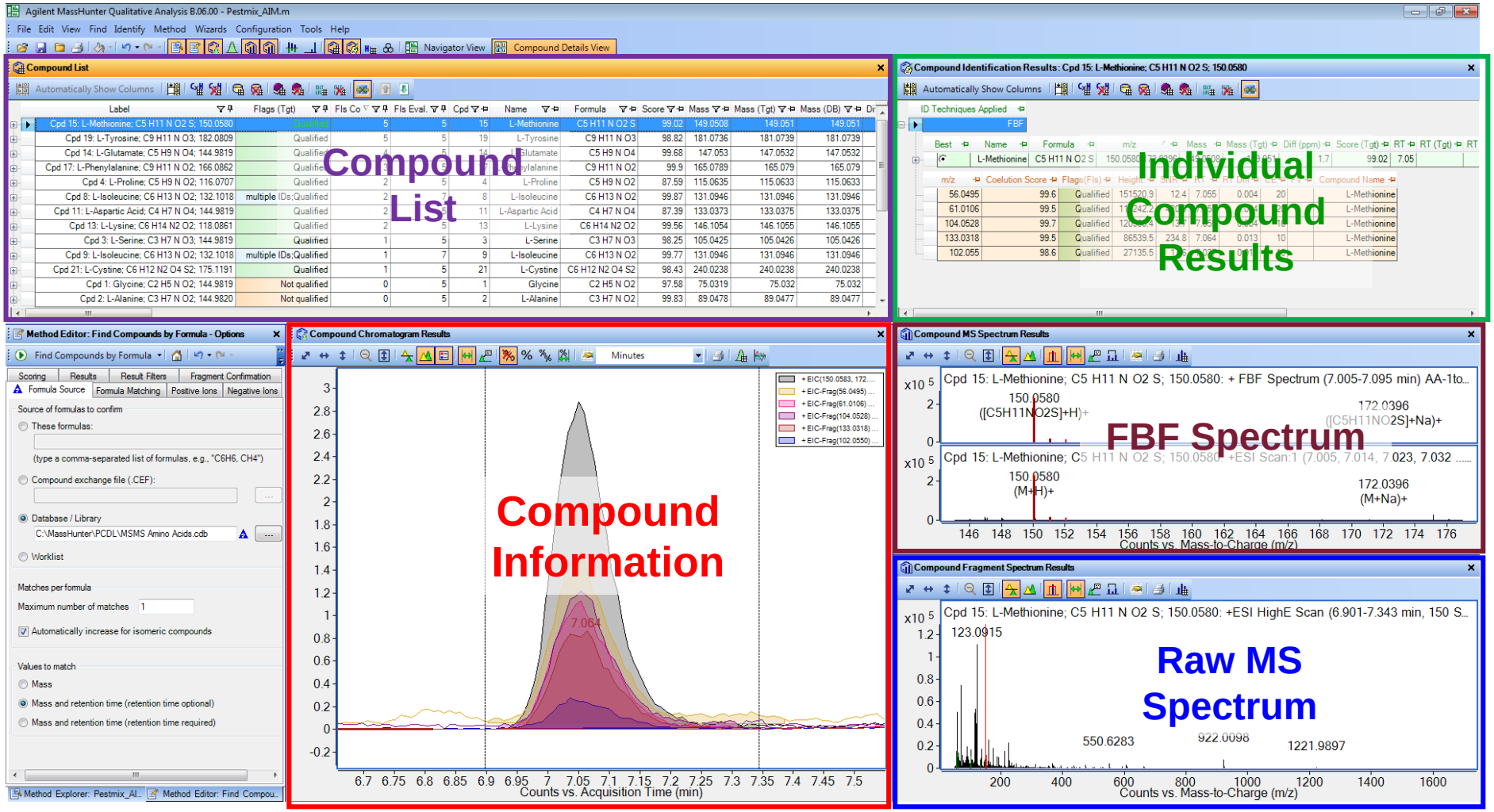
- Standardize review processes
- Automate common actions, i.e. extract EIC.

Define Qualitative methodology for sequence computation.

- No user interaction required.

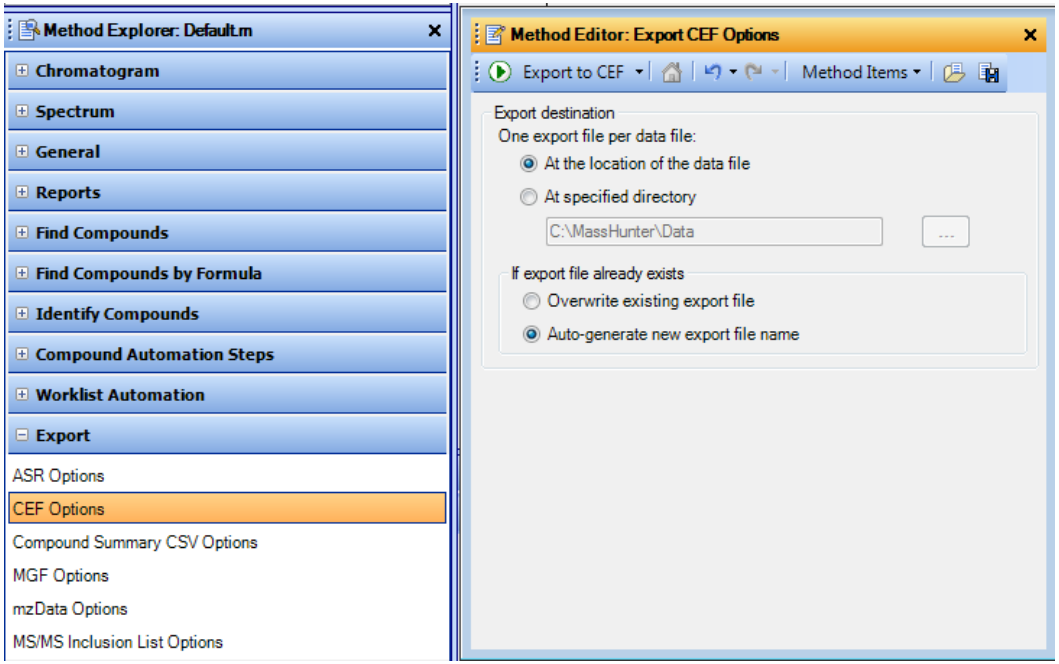


Compound Details View

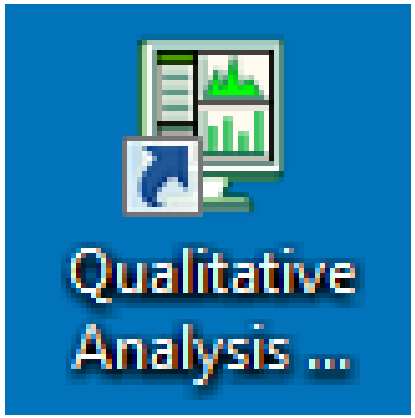


What is a .cef file?

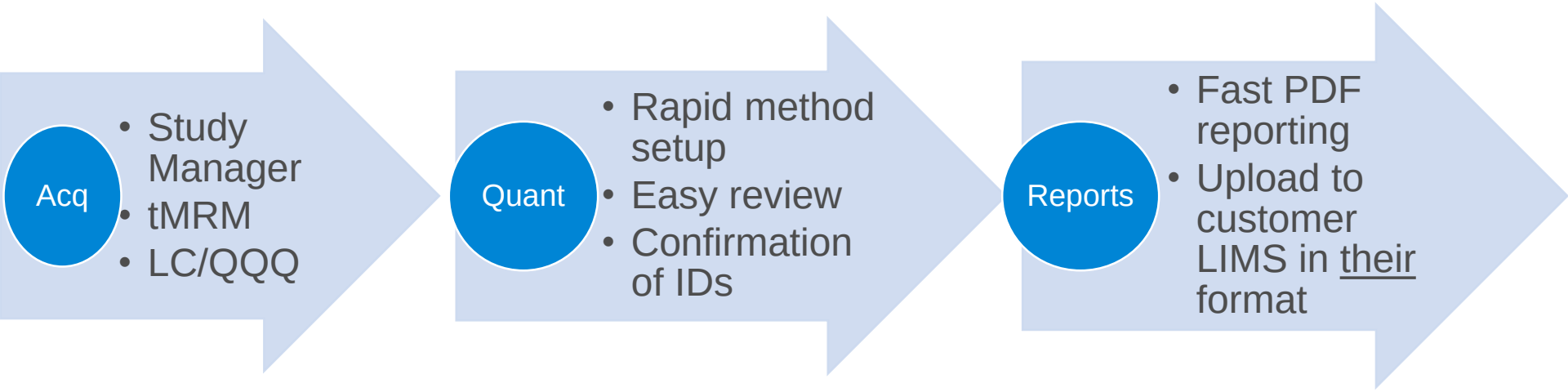
- **C**ompound **E**xchange **F**ormat file
- Agilent-specific file format that moves compound information back and forth between Qual, MPP, and other Agilent software
- XML-based – the amount of information contained depends on what the user has extracted
  - Accurate mass, retention time
  - Formulas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



Questions about MassHunter Qual?



The MassHunter Quantitative Workflow
Quantify with confidence

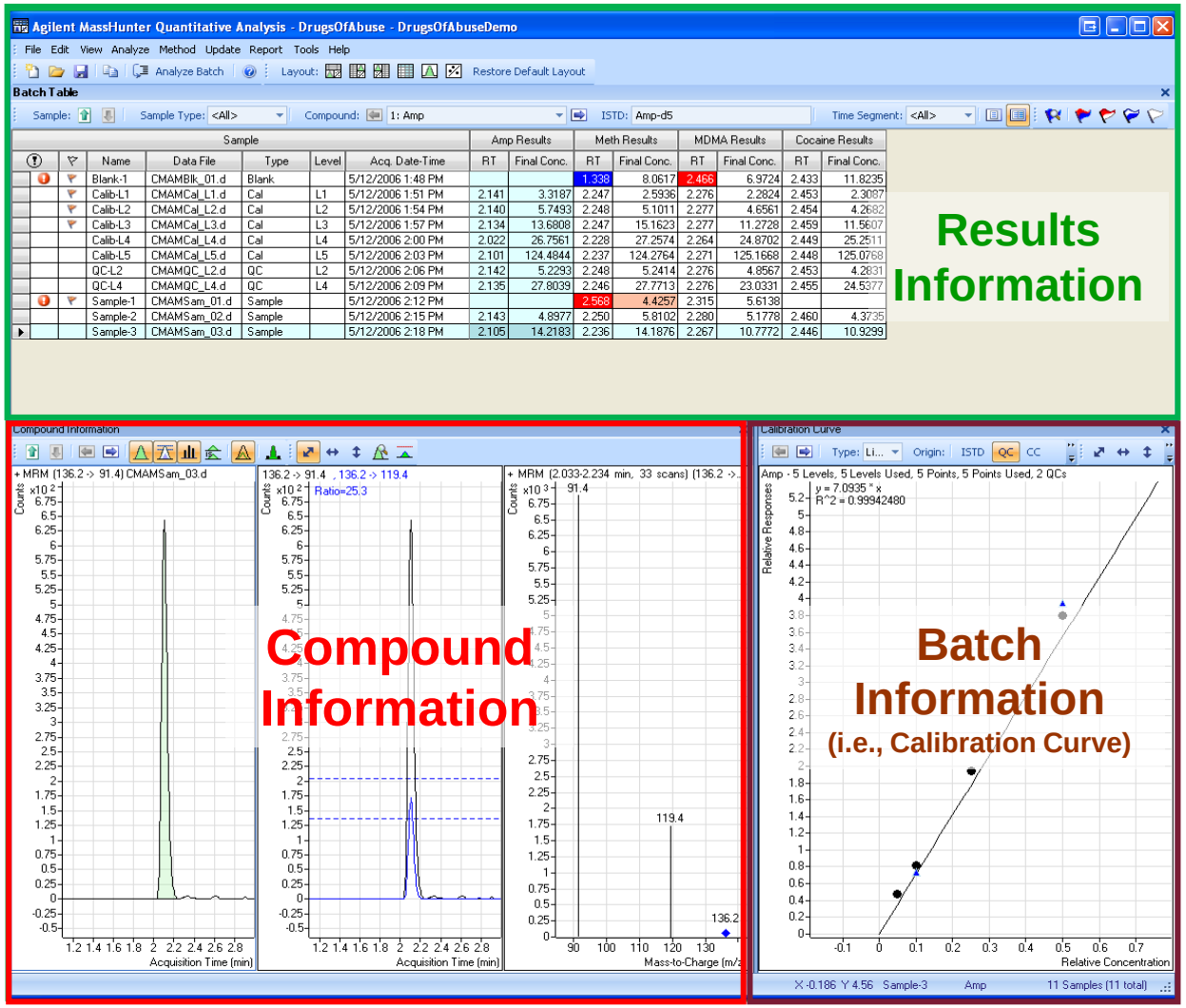


Agilent provides fast setup and running of quantitative methods from acquiring samples to the final report

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	29.7	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	34.17		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.[illegible]	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	70.54		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

These icons filter outliers in the display.

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

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

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

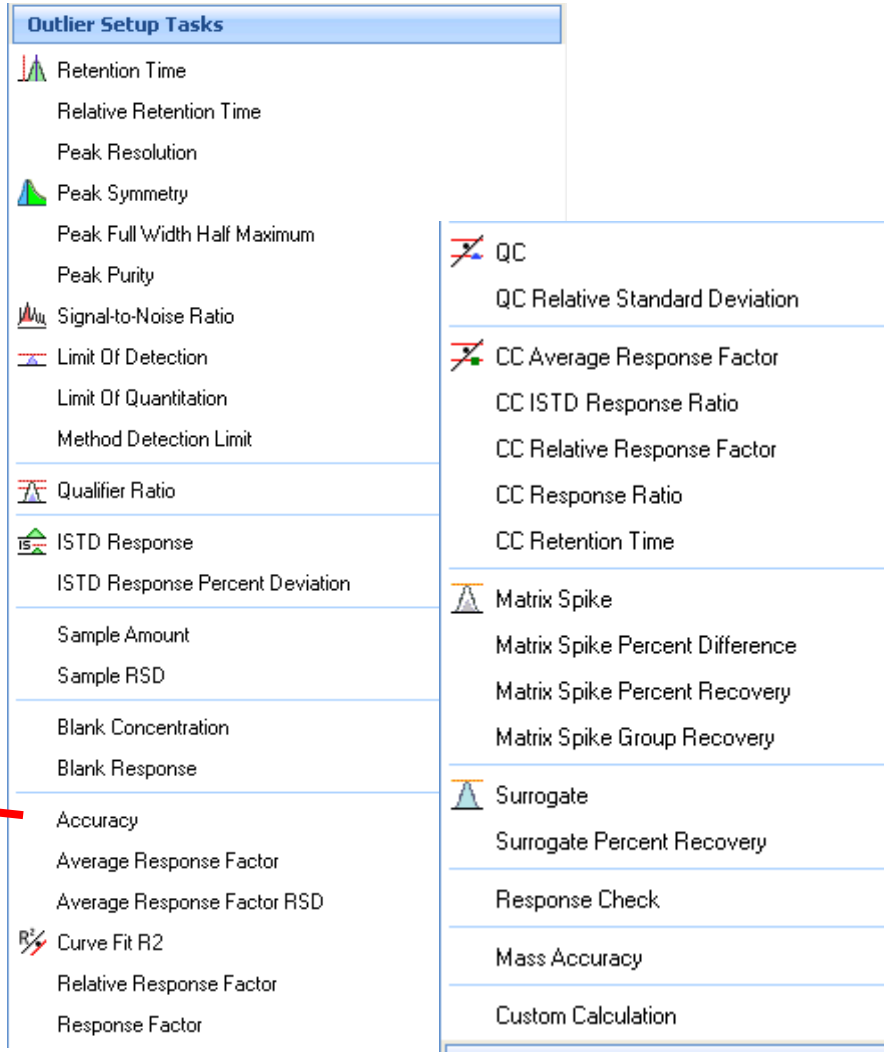
Results Information: Outlier Options

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

- **Set high and low limits.**

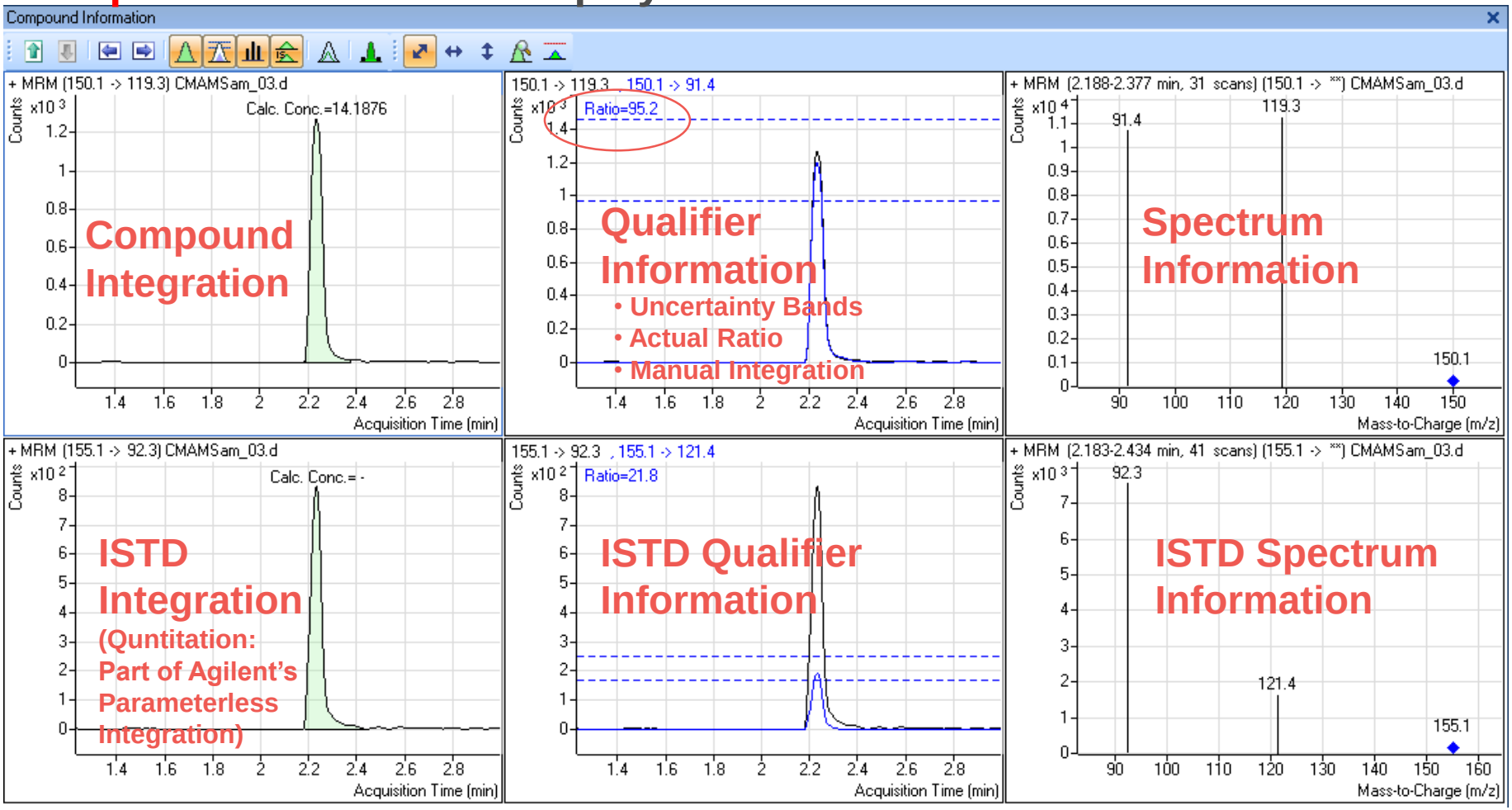
Custom Calculations can be added for User defined quality checks

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	



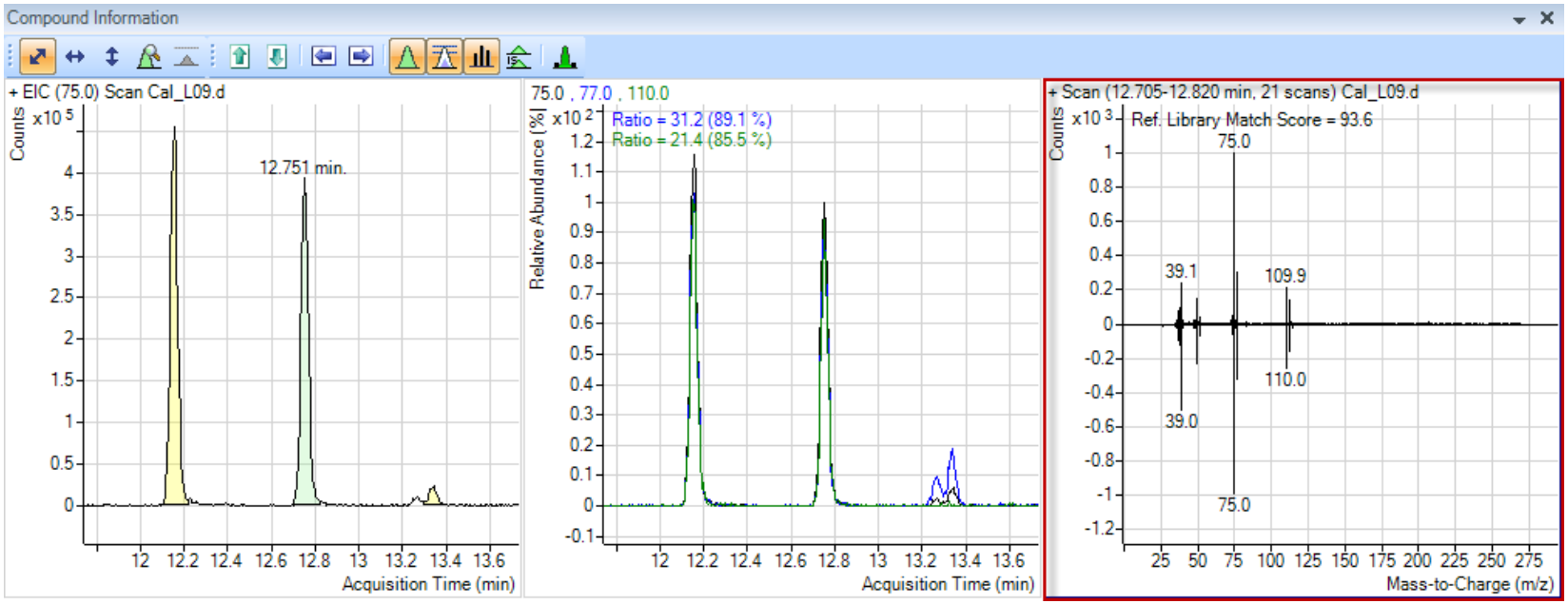
Batch Screen - Compound Information

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)



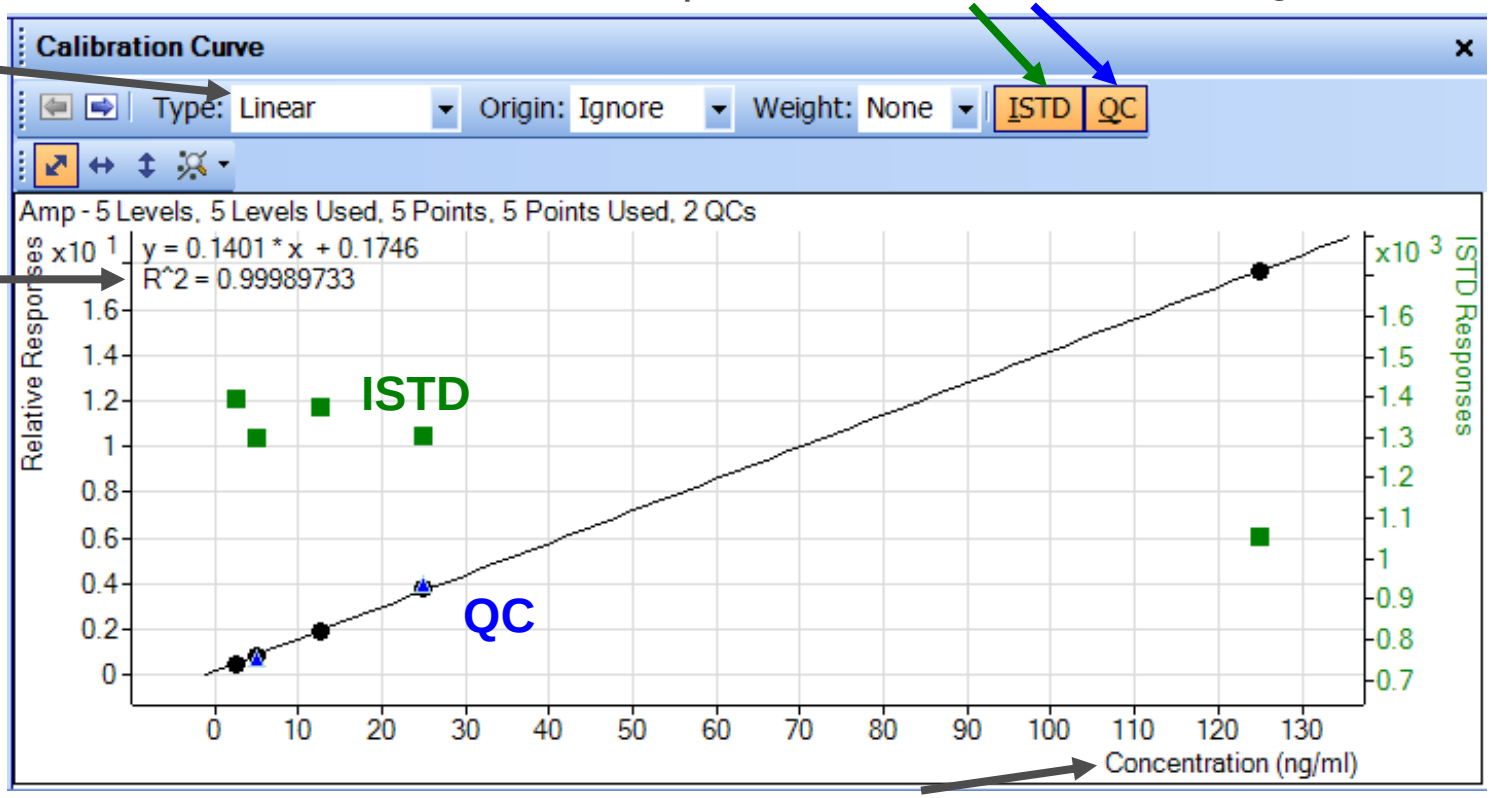
Batch Screen – Calibration Curve

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 ISD or QC button.

Change Curve Fit

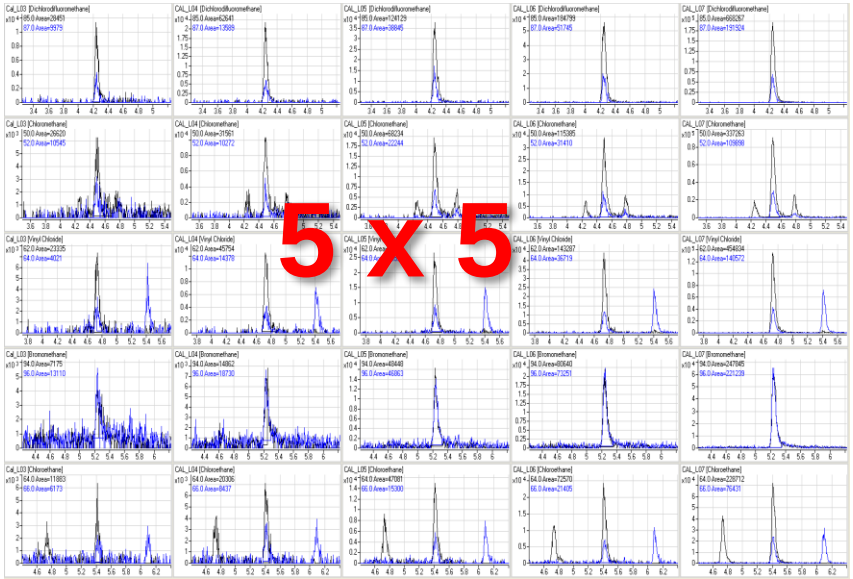
R^2



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

Compounds-at-a-Glance

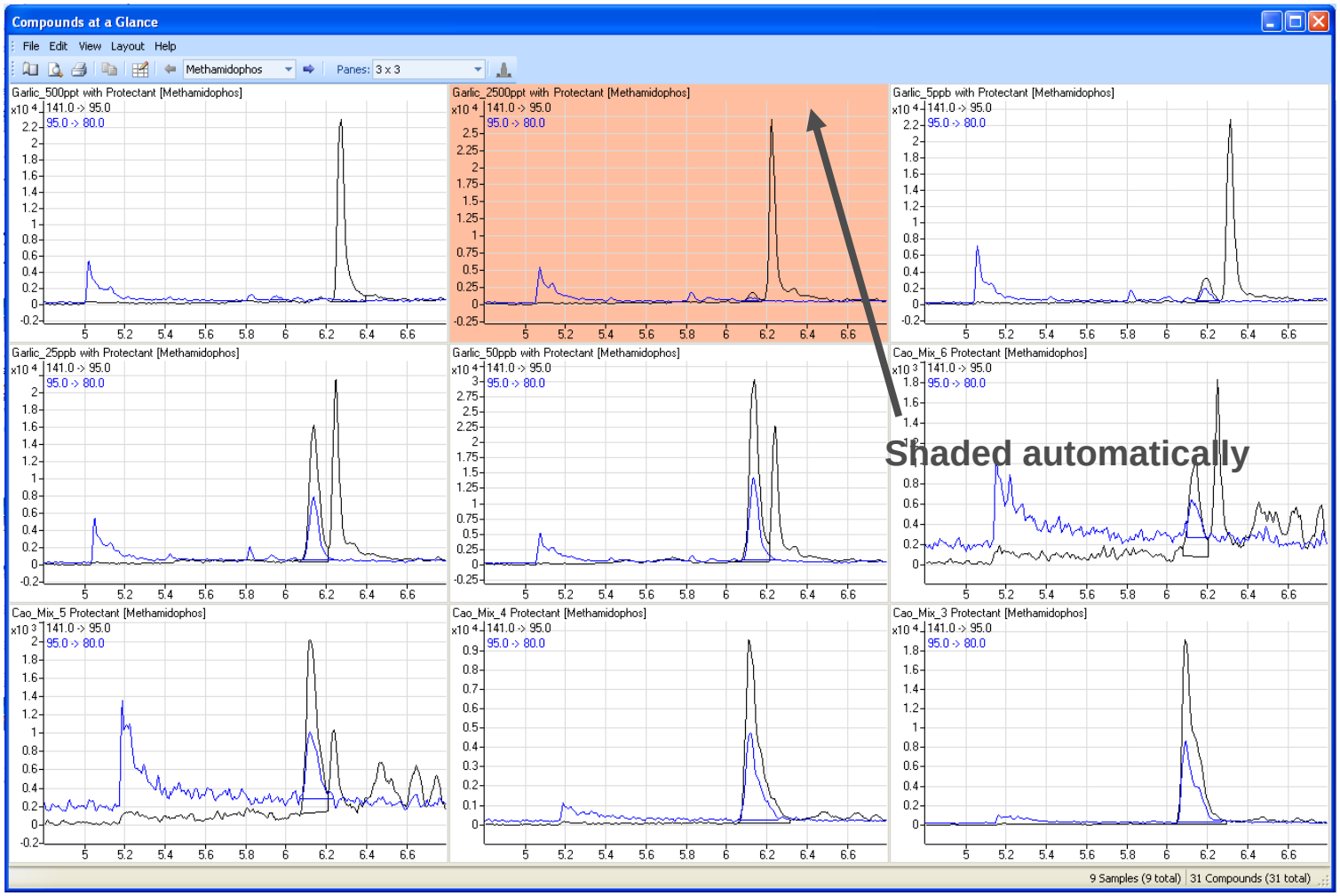
Compounds-at-a-Glance allows you to view multiple traces of compounds at a single glance.



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

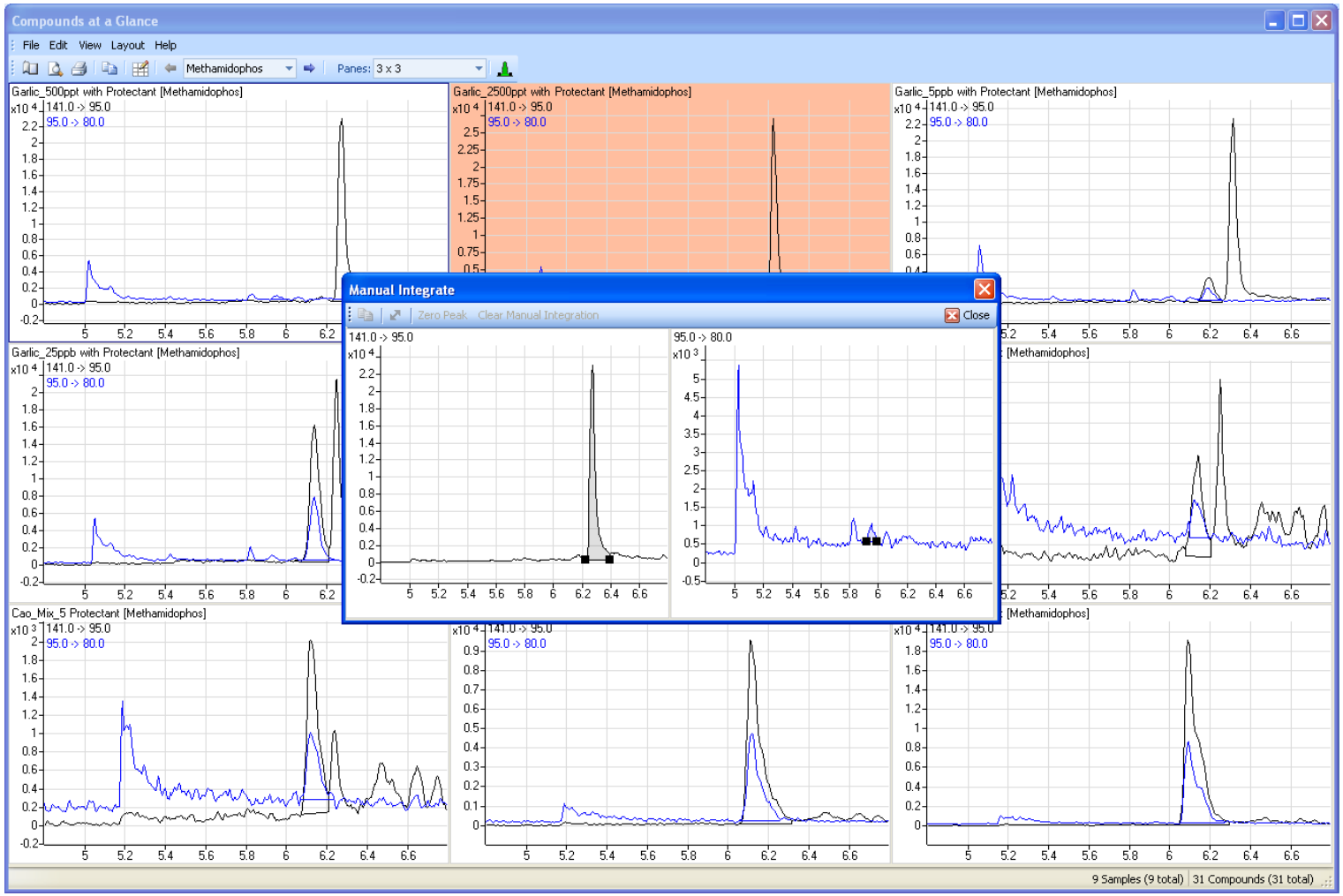
Compounds-at-a-Glance

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



Compounds-at-a-Glance

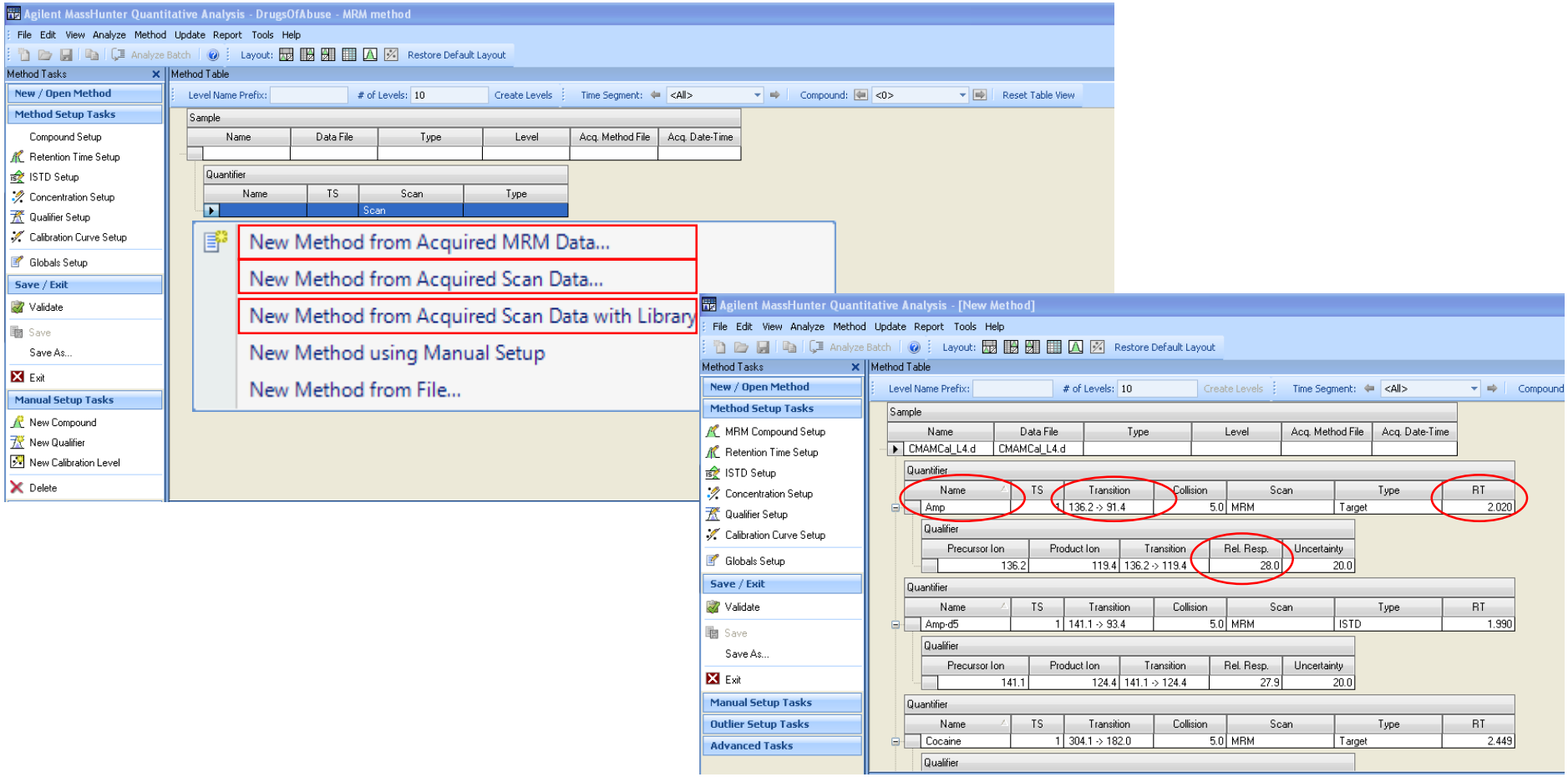
You can perform manual integration of compounds-at-a-glance.



Simplify Quantitation Method Creation

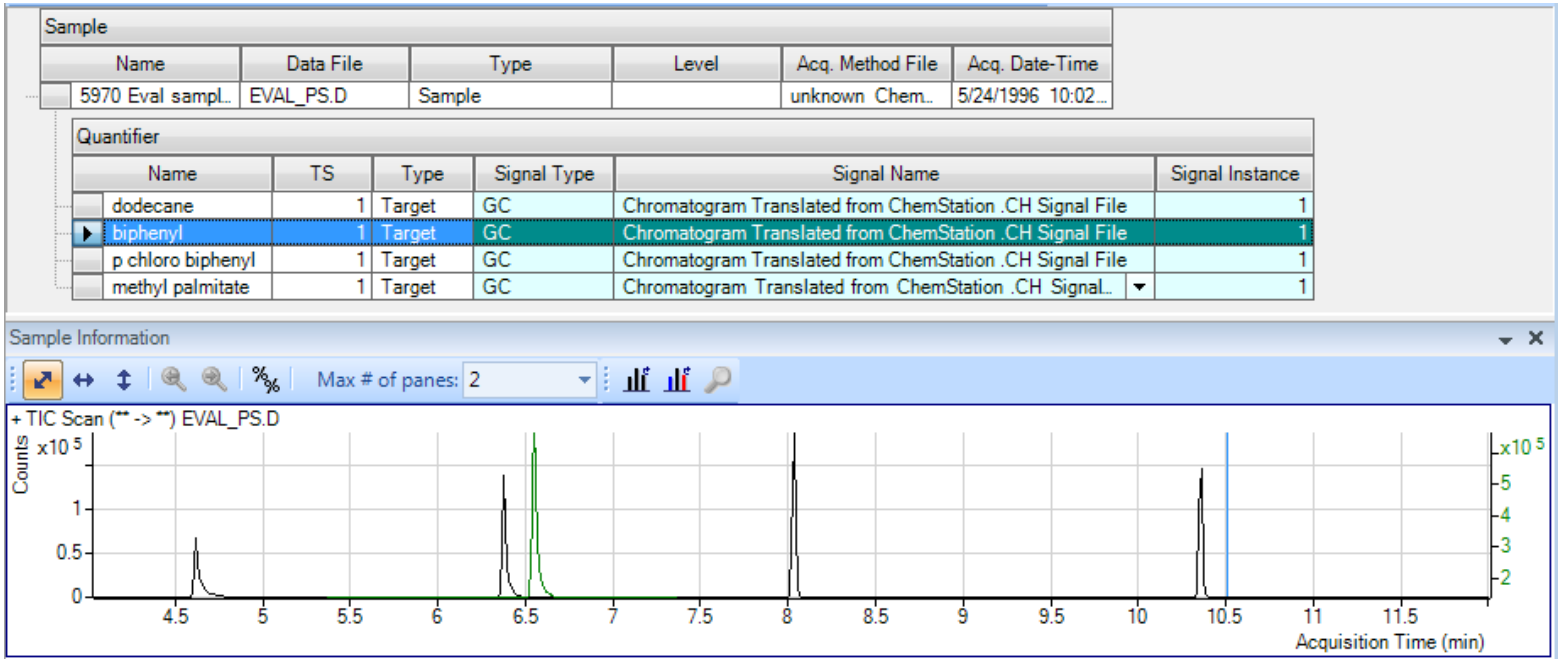
Easily create a target method from acquired data (SIM or Scan).

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



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.



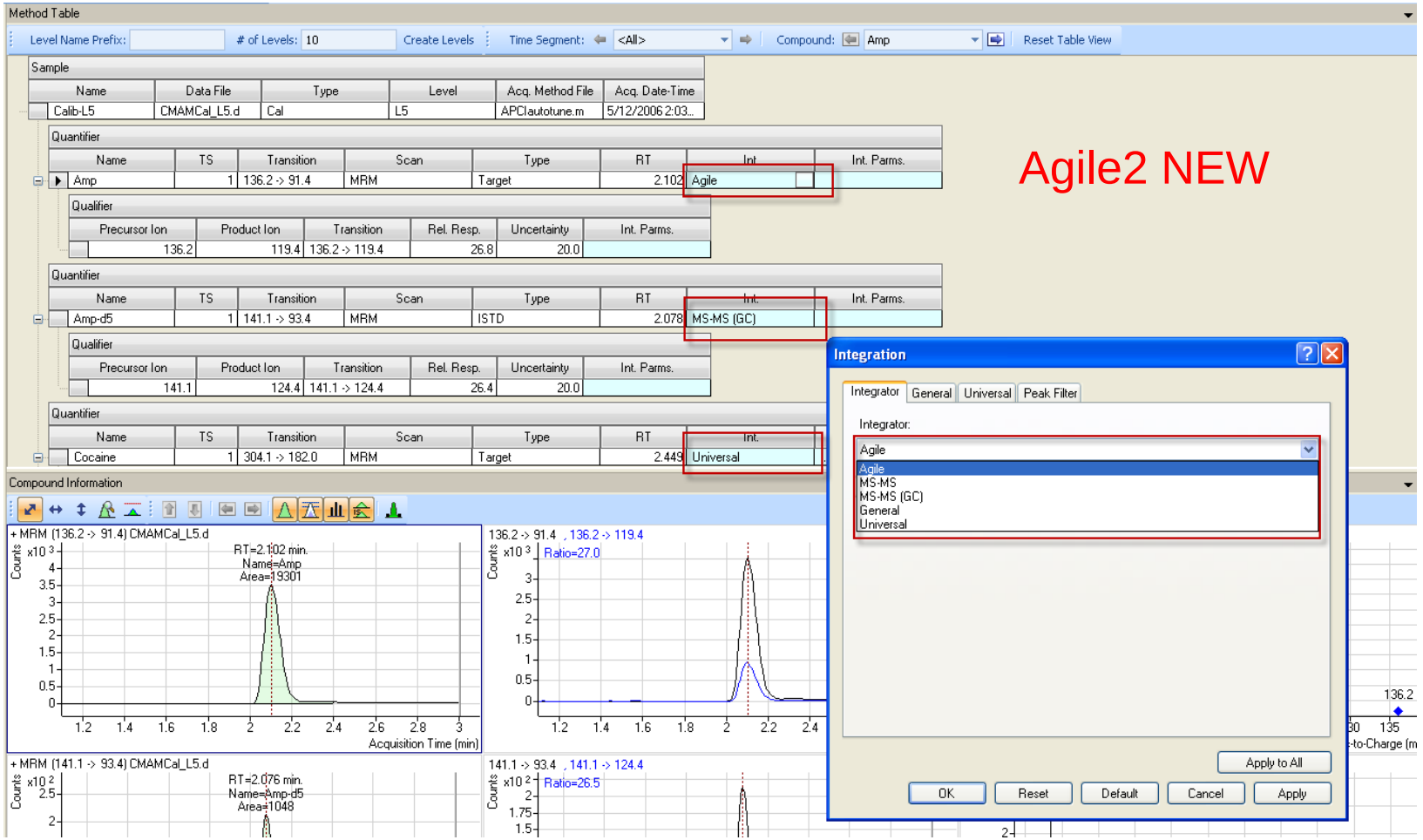
Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
5970 Eval sampl..	EVAL_PS.D	Sample		unknown Chem..	5/24/1996 10:02...

Quantifier

Name	TS	Type	Signal Type	Signal Name	Signal Instance
dodecane	1	Target	GC	Chromatogram Translated from ChemStation .CH Signal File	1
biphenyl	1	Target	GC	Chromatogram Translated from ChemStation .CH Signal File	1
p chloro biphenyl	1	Target	GC	Chromatogram Translated from ChemStation .CH Signal File	1
methyl palmitate	1	Target	GC	Chromatogram Translated from ChemStation .CH Signal..	1

Compound Specific Integration Choice: Including parameterless integration



Questions about Quant?

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

Personal Compound Databases and Libraries

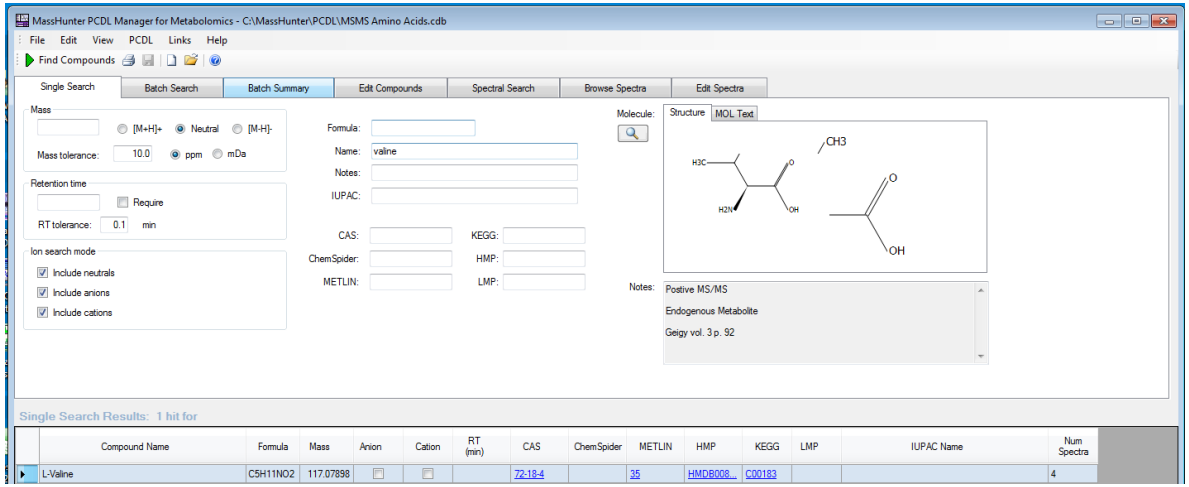
Confident identification using your mass spec

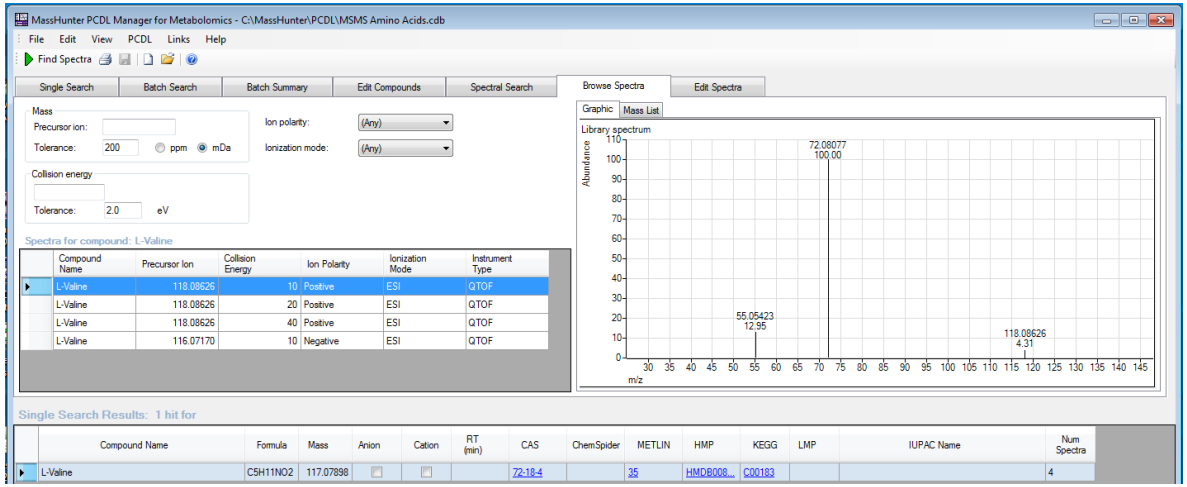
PCDs (accurate mass database) and PCDLs (MS/MS libraries) allow you to search your data to identify compounds using accurate mass against reference spectra

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 Additves, Coffee, Lipids, HMDB, Natural Products

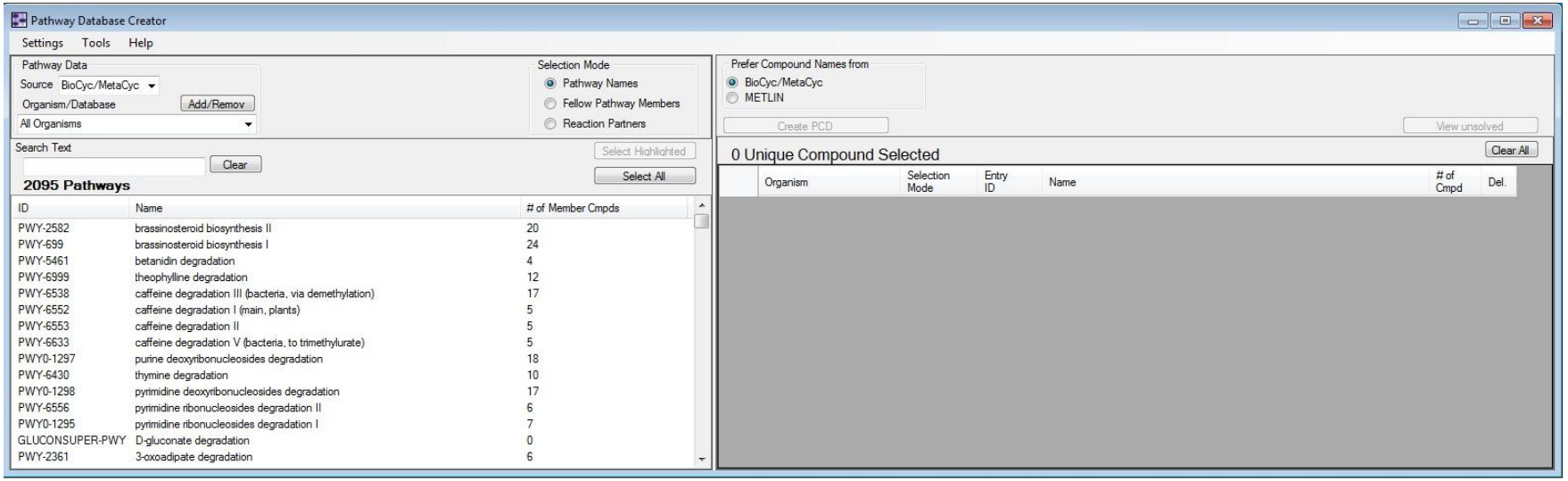




PCD versus PCDL

- 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



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

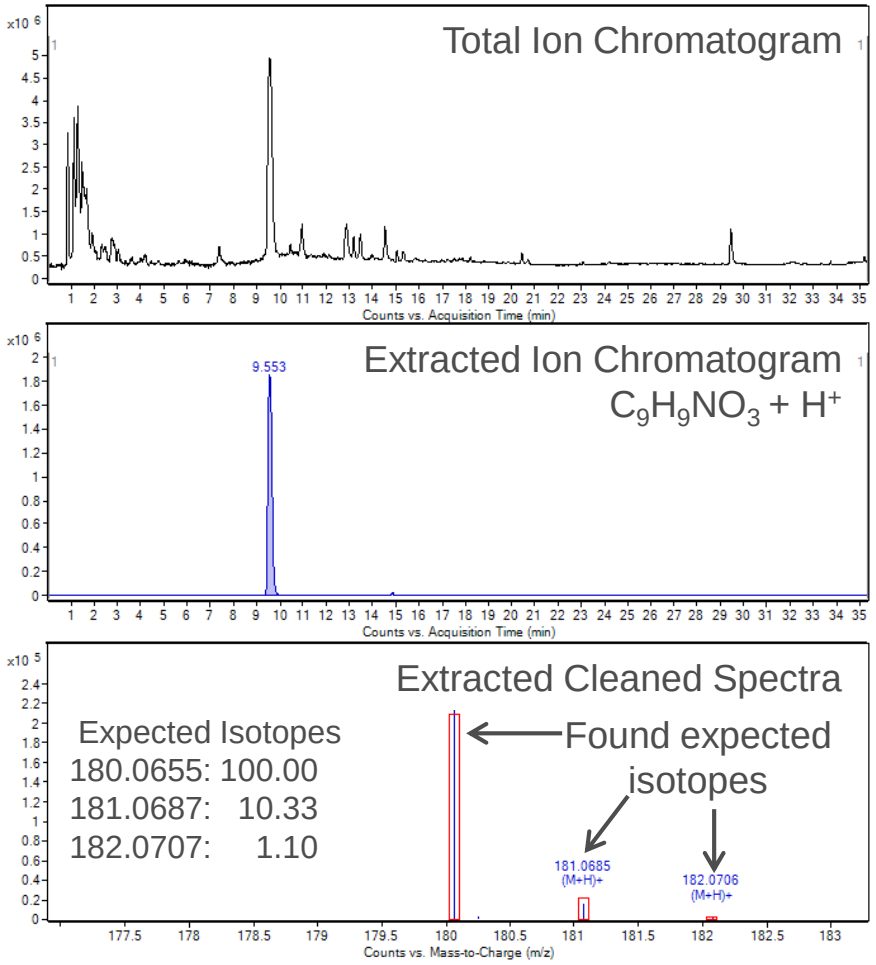
Can link to METLIN PCDL to add compound information

- 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 specifity
- User can review and edit results
- Produce a CEF file for import into MPP



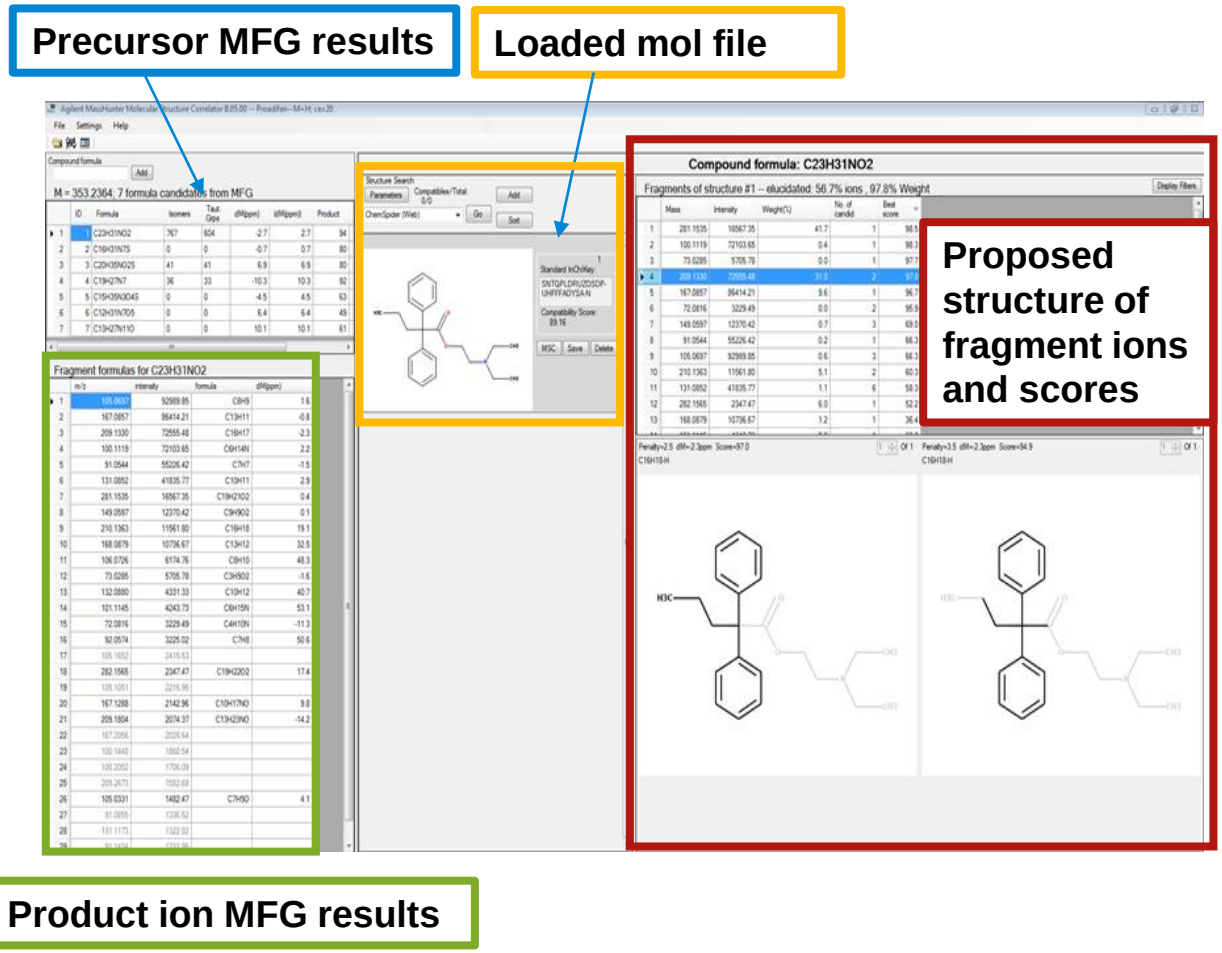
Targeted Data Mining of Qualitative Data for Greater Specificity

Questions about PCDL Manager or Pathways to PCDL?

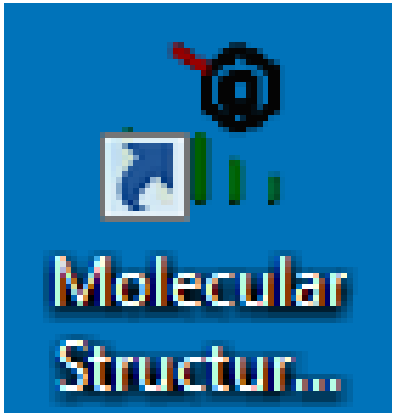
Molecular Structure Correlator (MSC)

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



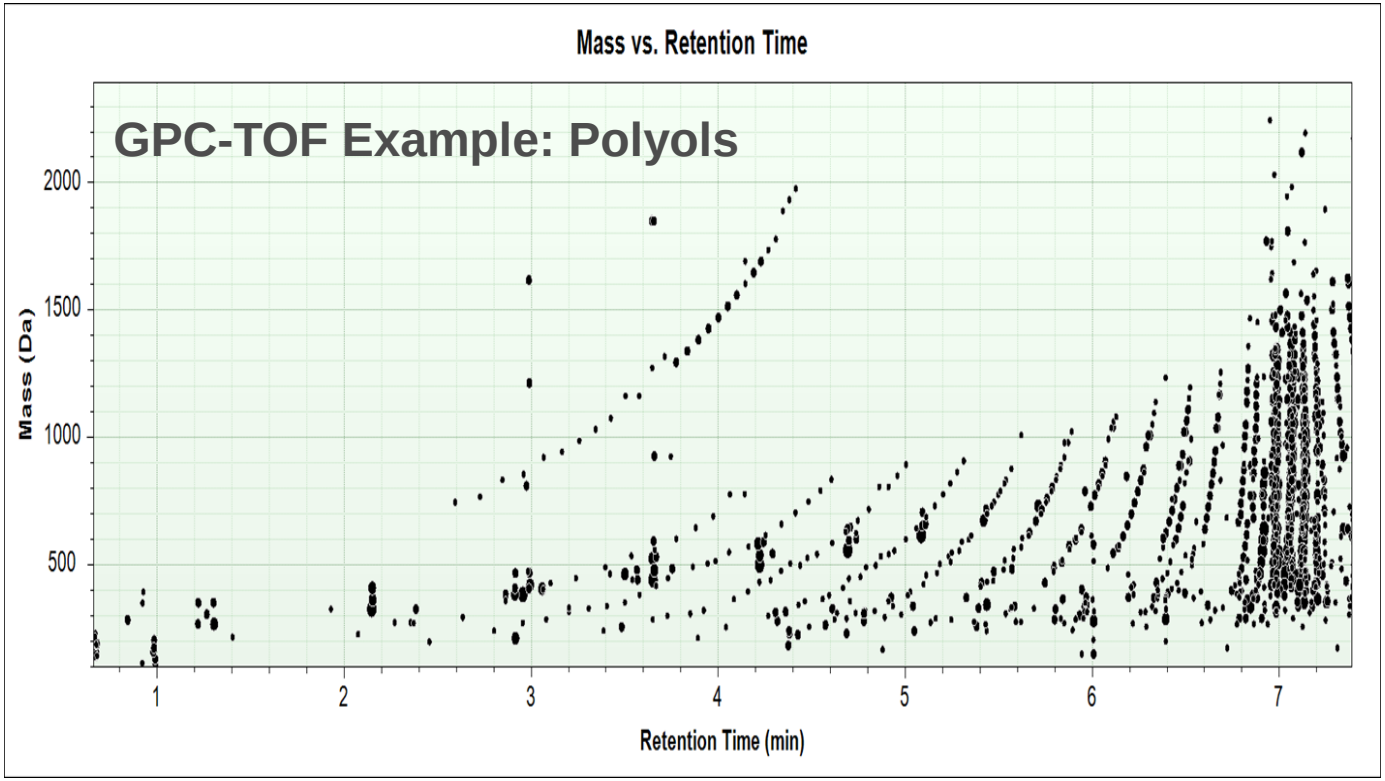
Questions about MSC?



Mass Profiler (MP) Differential Analysis Program

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

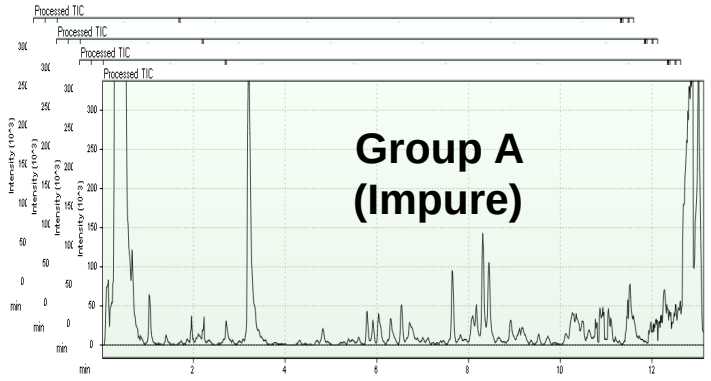
Clearly see repeating units of 44 and 58 m/z

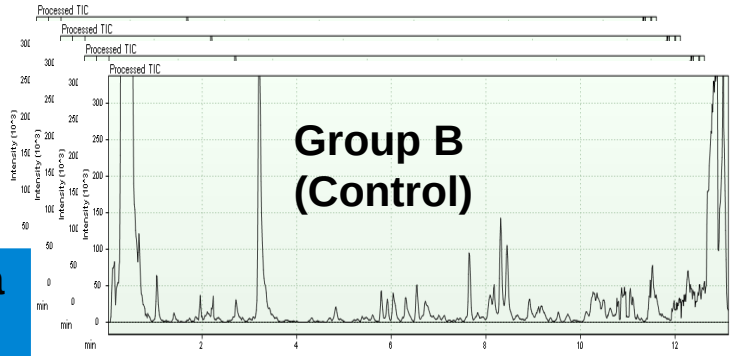


Mass Profiler

- 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

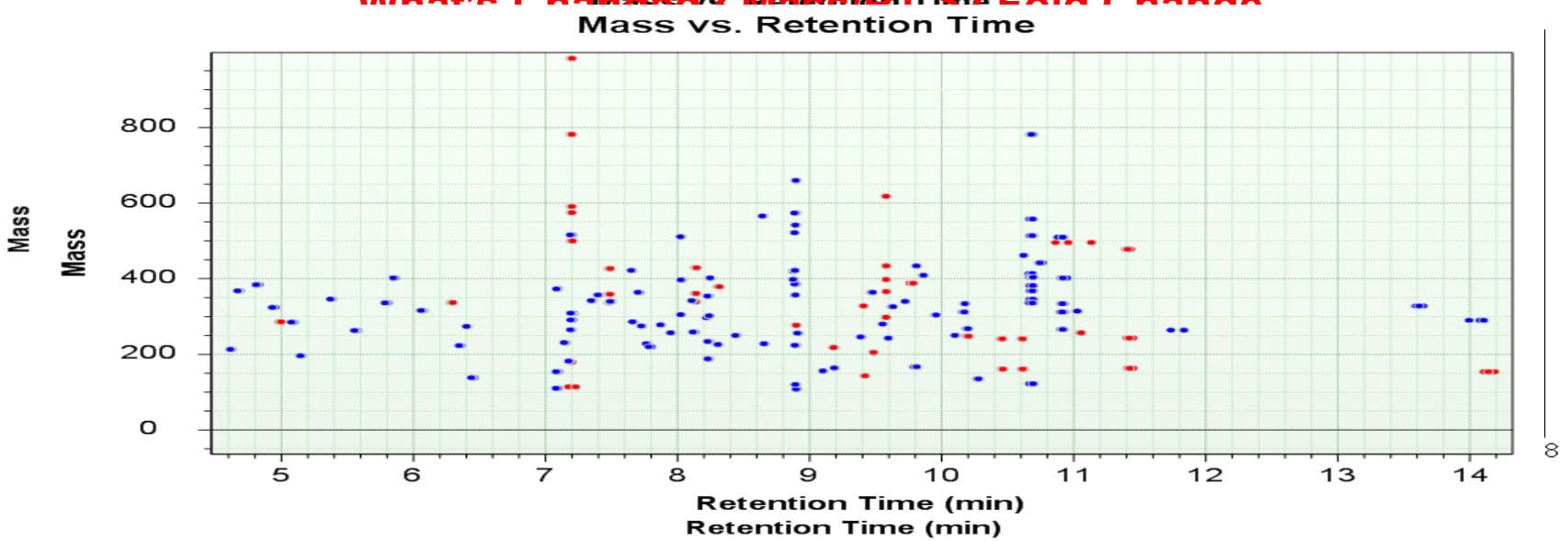
Mass Profiling Software





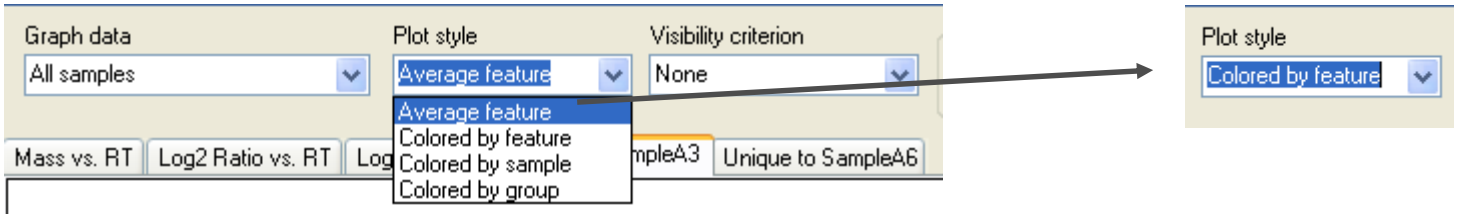
Aligns Data RT, Mass, Abundance

What's Changed? Mass/RT >2 Fold Change

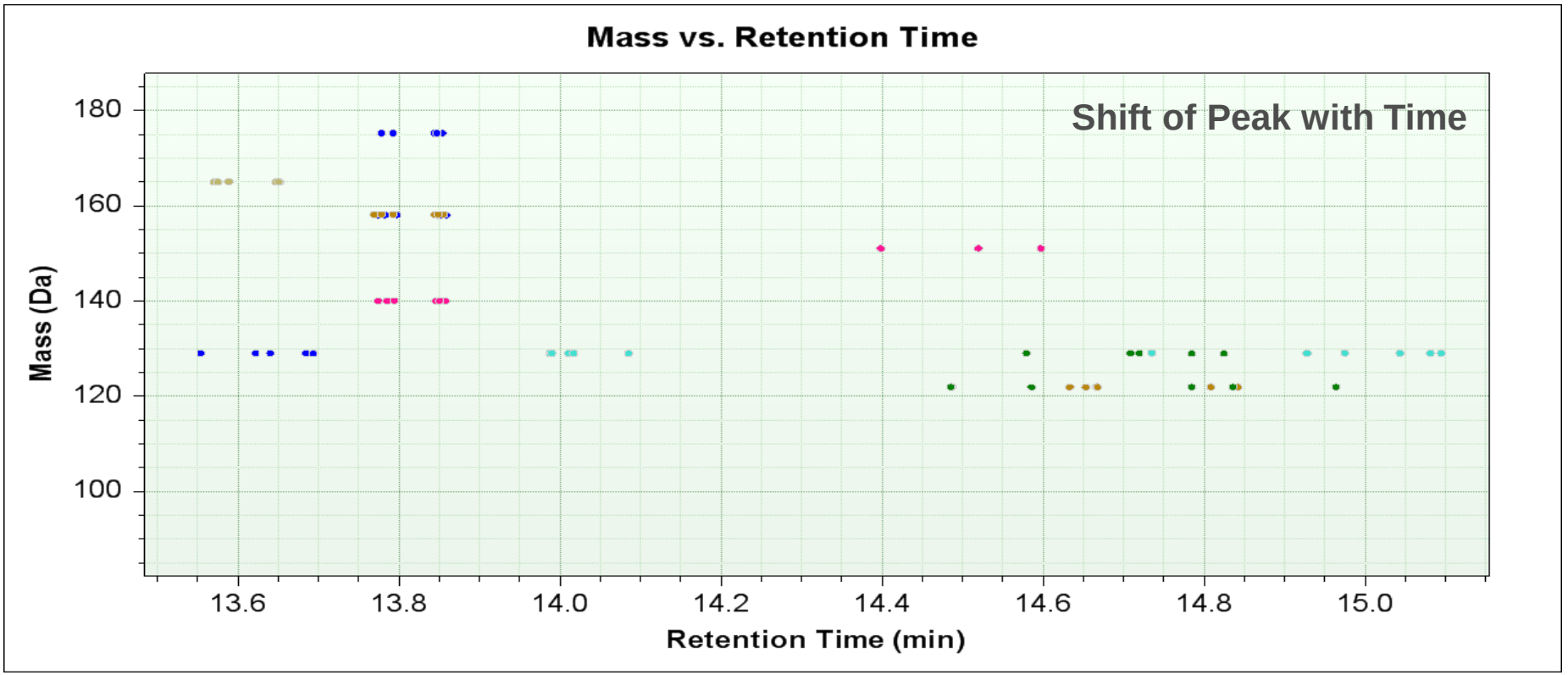


What's Unique? Impurity Control

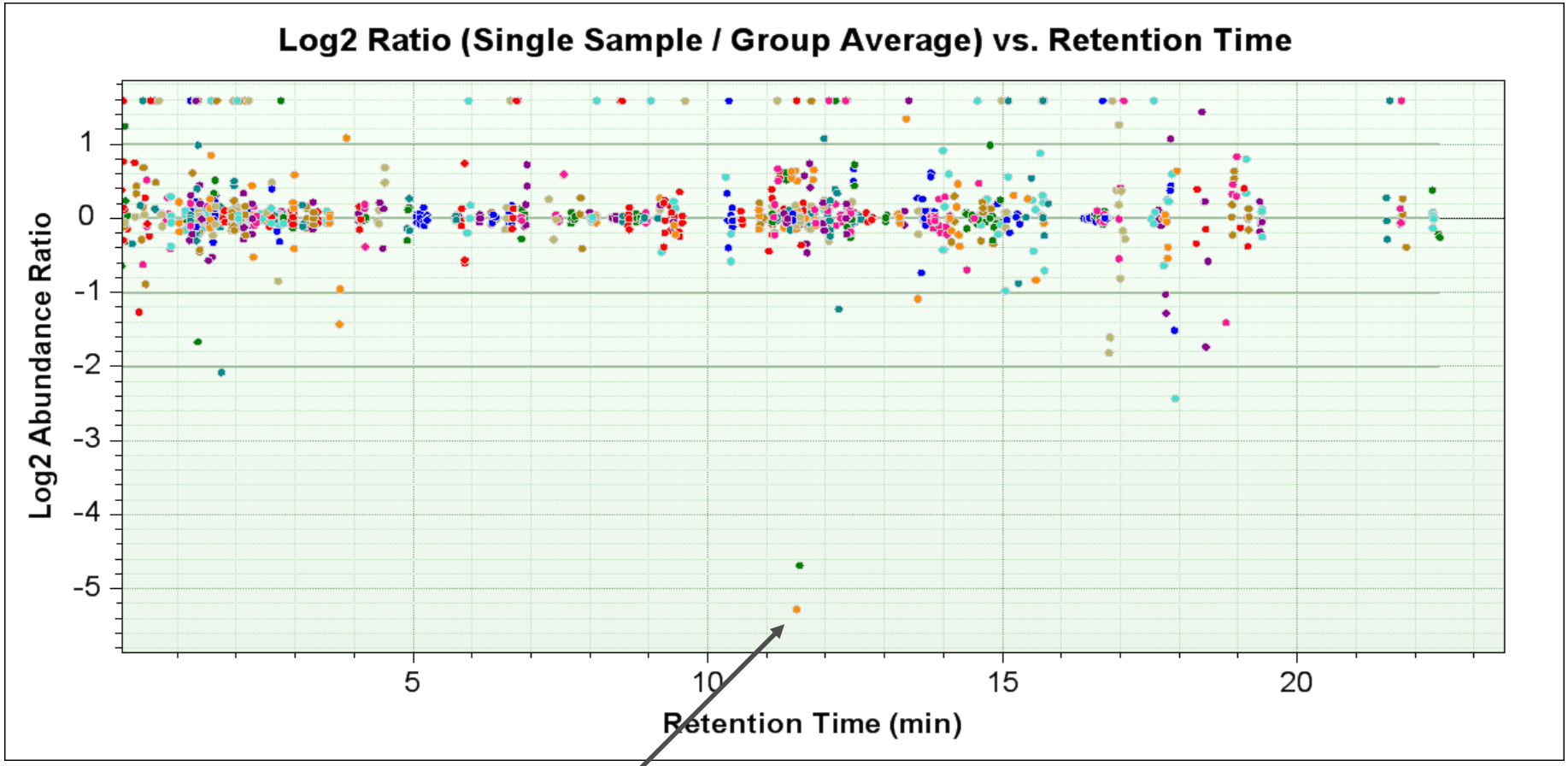
Mass Profiler – Reproducibility of Results



Reproducible Retention Time Stability

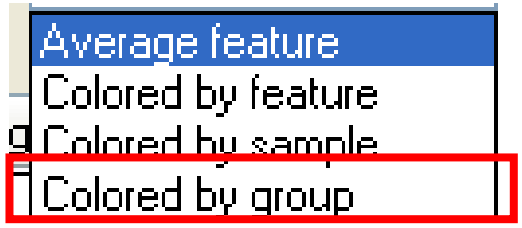


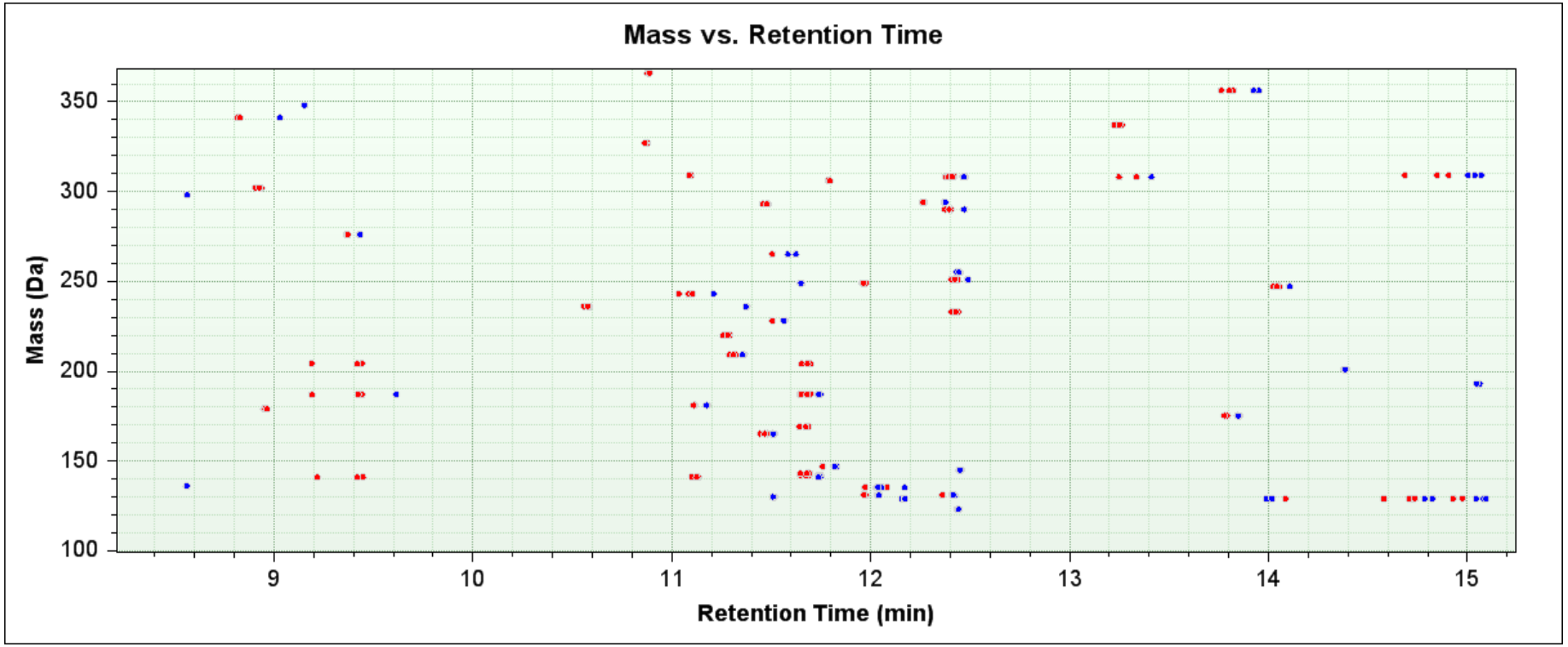
Mass Profiler – Reproducibility of Results



Outlier Sample or Feature

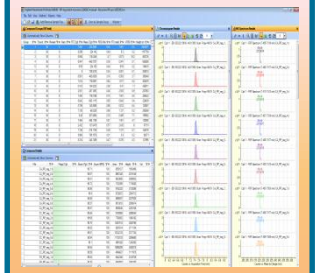
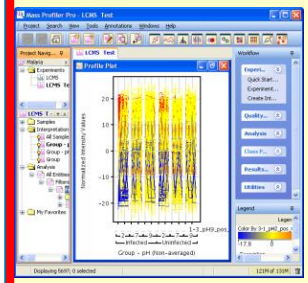
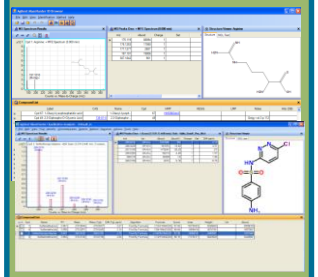
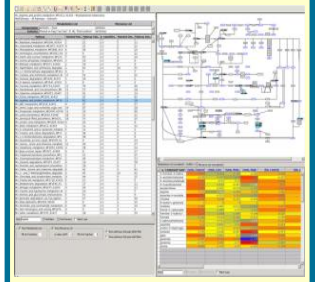
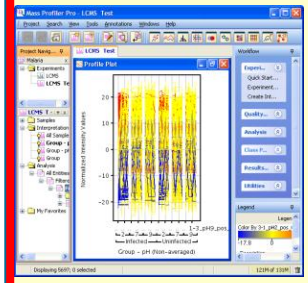
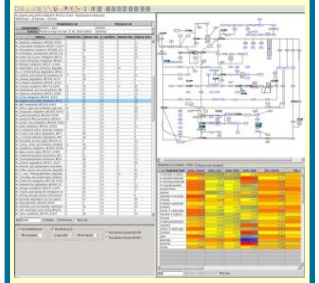
Unique Group 1 and Unique Group 2





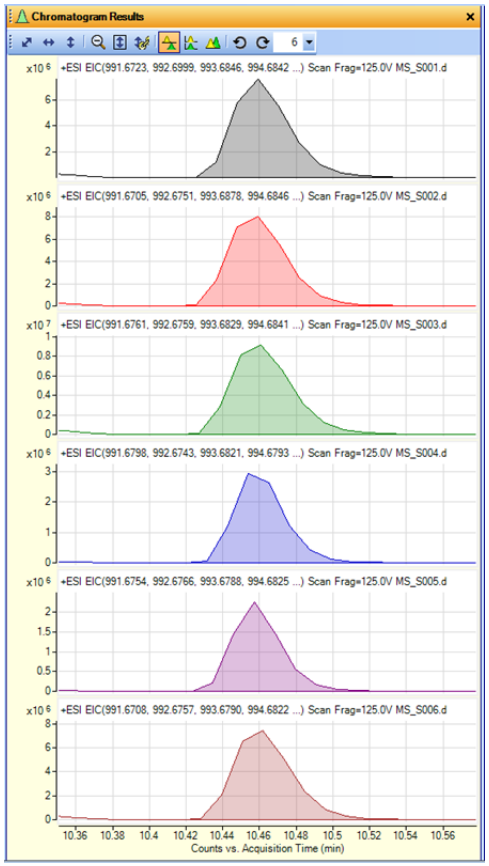
Questions about Mass Profiler?

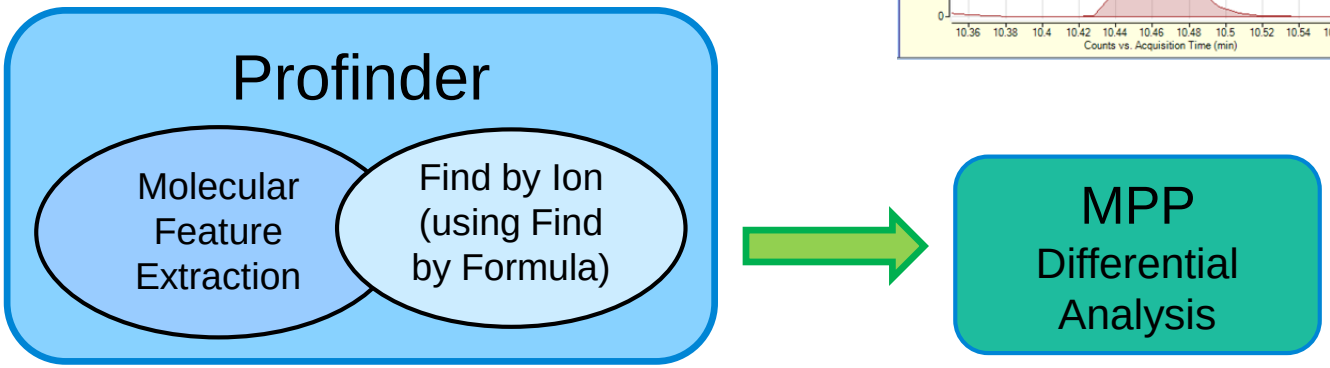
Untargeted Data Acquisition & Analysis

	Separate & Detect	Feature Finding	Alignment & Statistics	Identify	Pathways
Untargeted	 LC-TOF/QTOF	 Profinder	 Mass Profiler Professional	 ID Browser	 Pathway Architect
Pathway Targeted	 LC-TOF/QTOF	 Profinder	 Mass Profiler Professional		 Pathway Architect

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

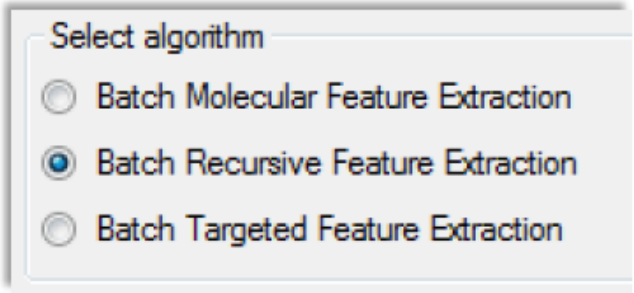




Profinder Workflows

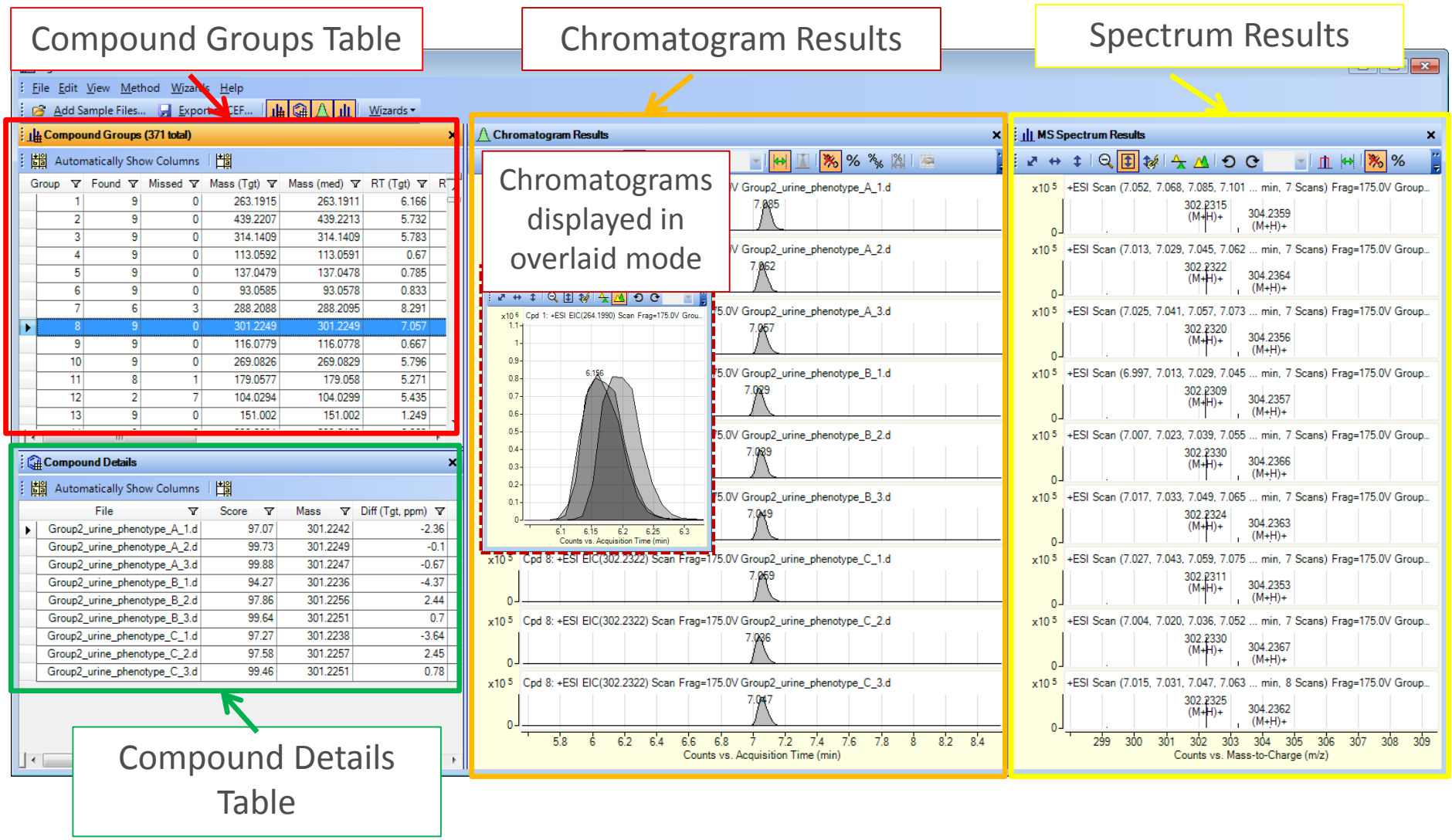
1. Batch Molecular Feature Extraction
   - Reduces False Positives, No Editing

2. Batch Recursive Feature Extraction
   - Reduces False Negatives, Allows Editing



3. Batch Targeted Feature Extraction
   - Uses database targets, Allows Editing

Profinder Results Navigation

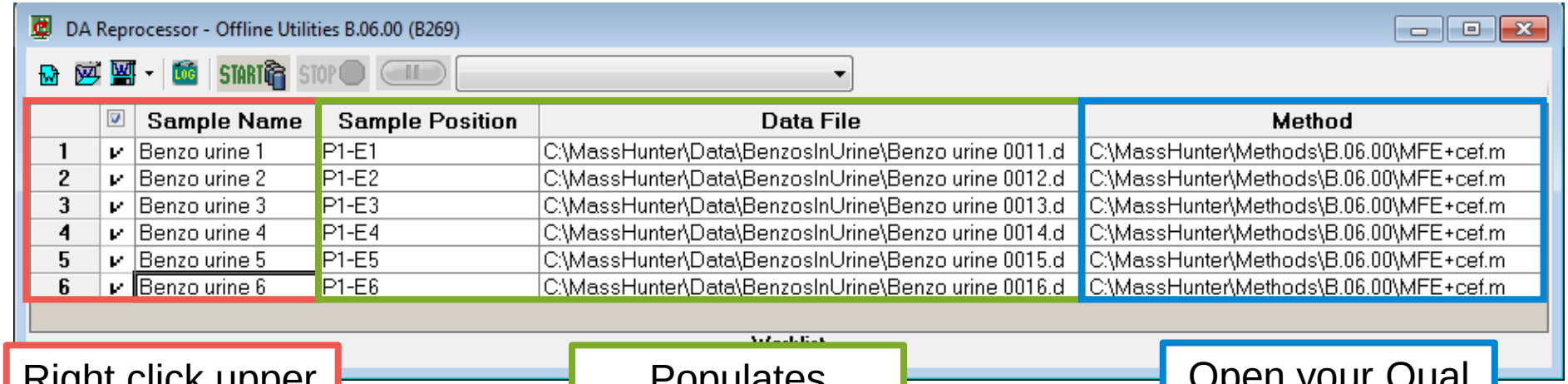


Questions about Profinder?

DA Reprocessor

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

Run Qual method on a batch of samples

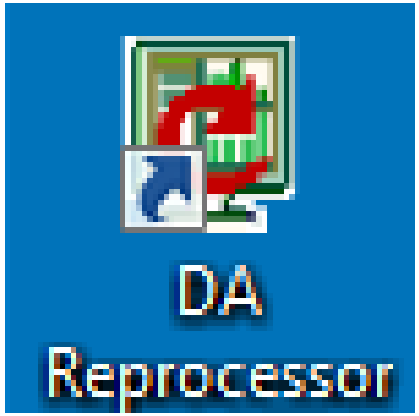


Right click upper left corner to add one or multiple samples

Populates automatically

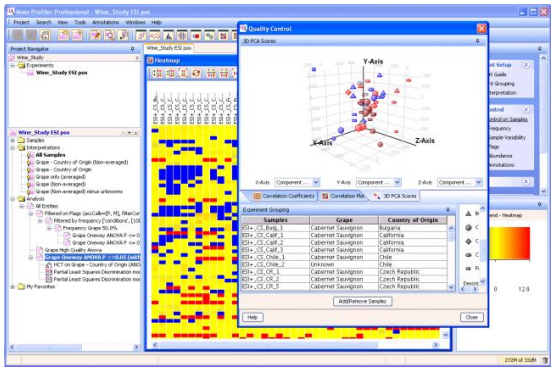
Open your Qual method

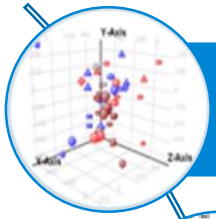
Questions about DA Reprocessor?



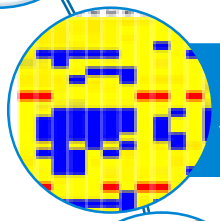
Mass Profiler Professional

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

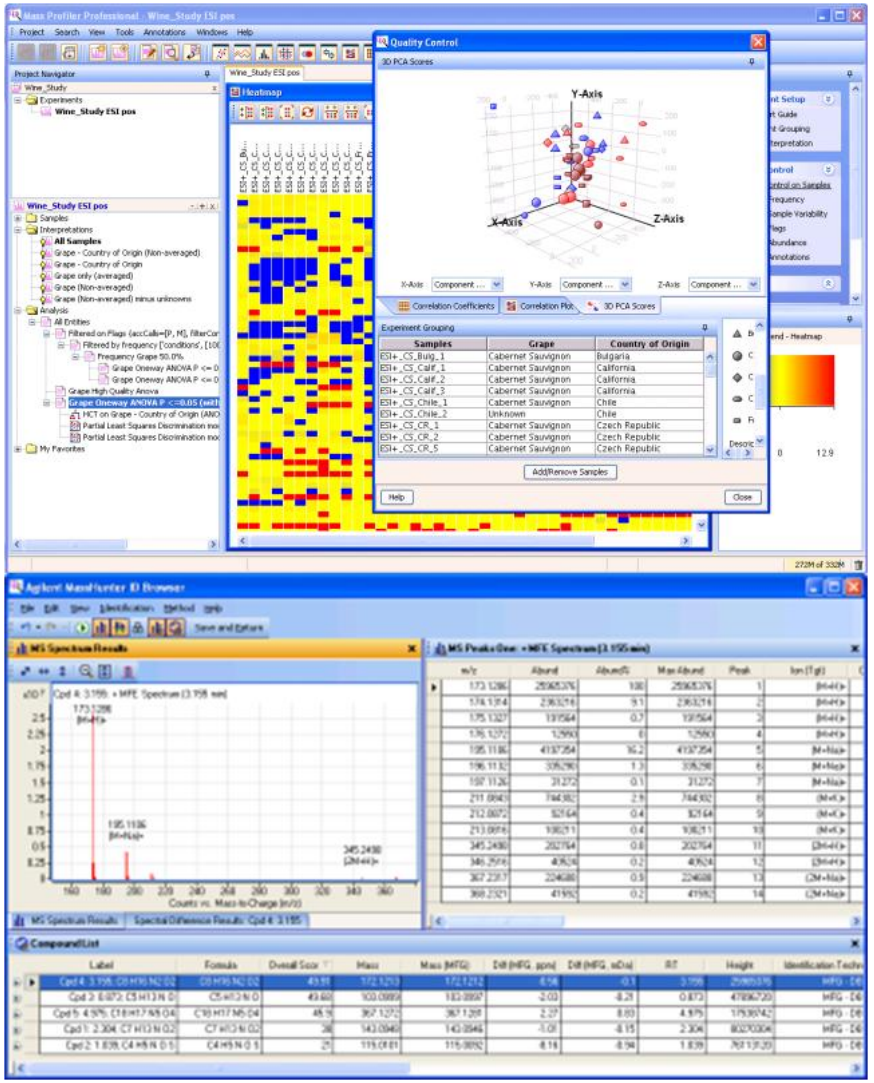
Use data from LC/MS, GC/MS, ICP-MS, and NMR

Run QC assays using the Sample Class Predictor

Bring genomics, proteomics, and metabolomics together

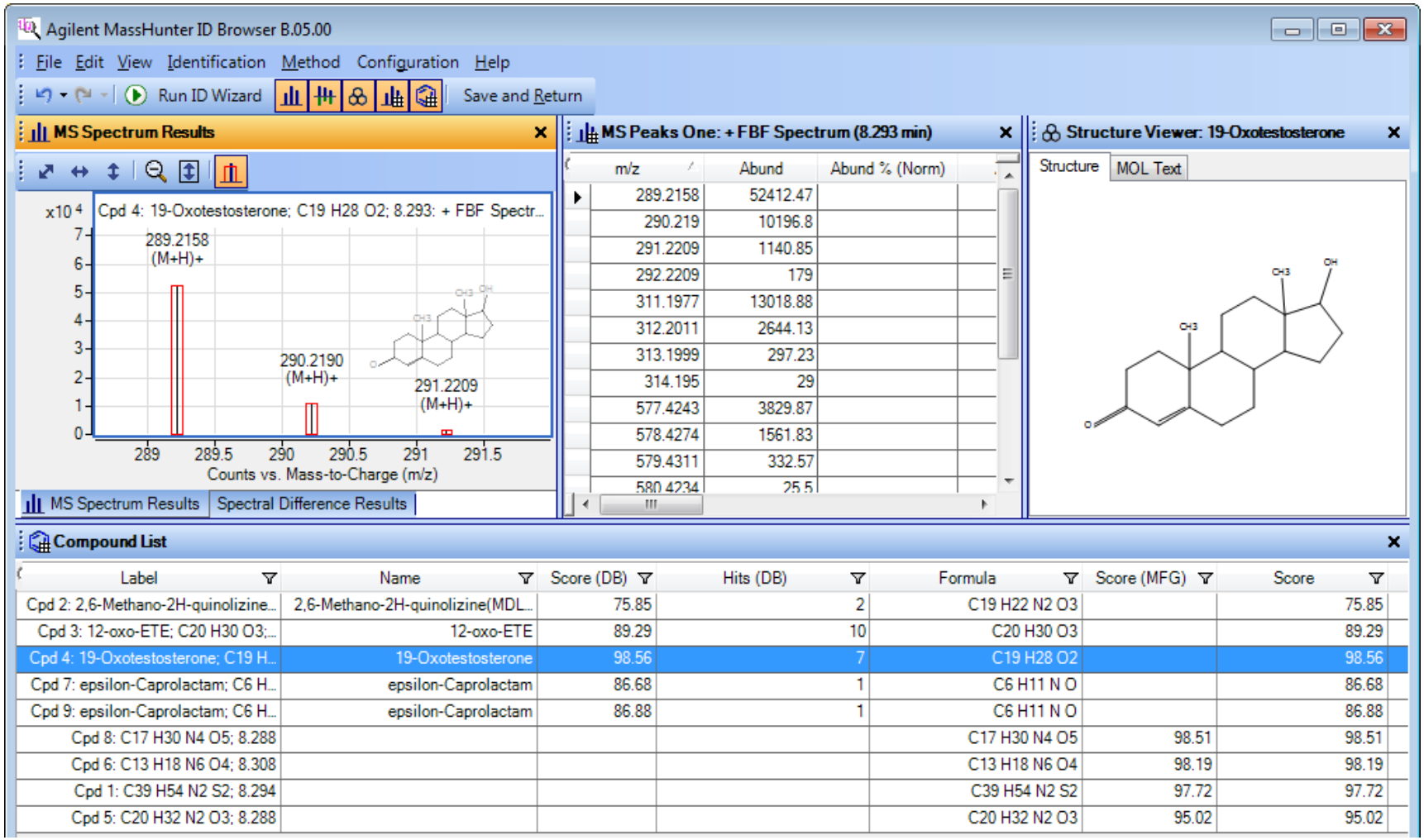
Mass Profiler Professional (MPP)

- 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



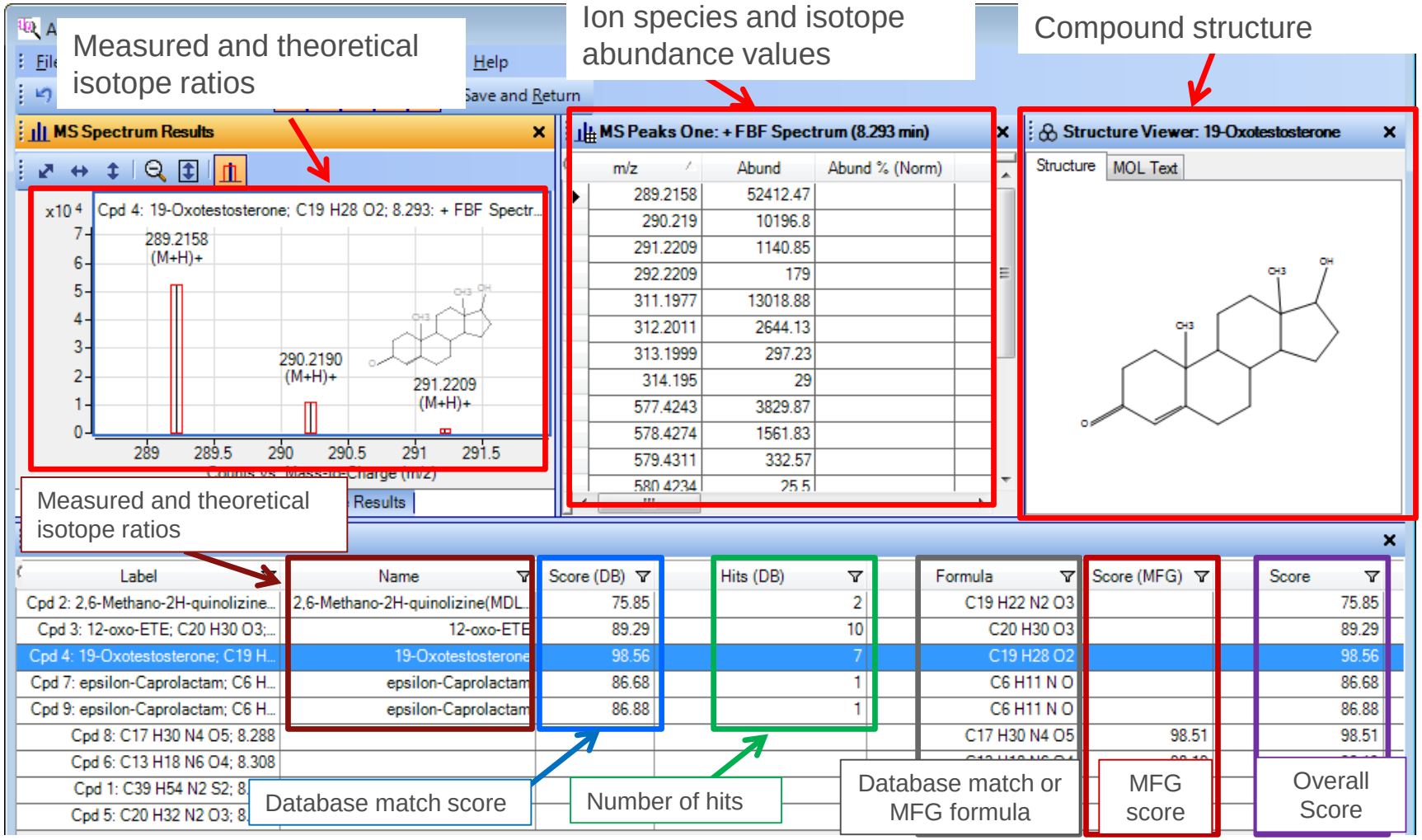
ID Browser

Searches against csv or PCD files to identify compounds



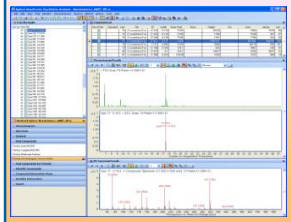
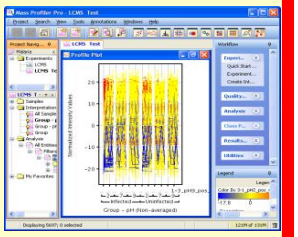
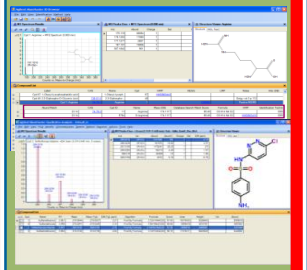
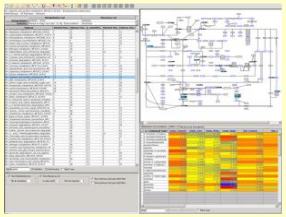
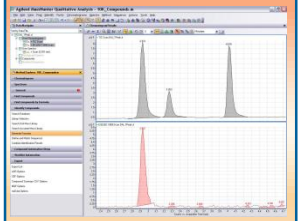
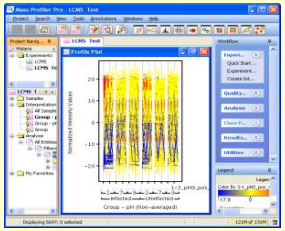
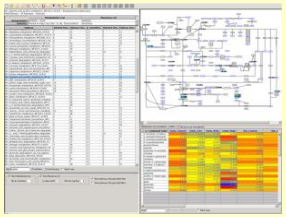
ID Browser

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”

	Separate & Detect	Feature Finding	Alignment & Statistics	Identify	Pathways
Untargeted	 LC-TOF/QTOF	 **MassHunter Qual** *~~MFE~~*	 **Mass Profiler Professional**	 **ID Browser**	 **Pathway Architect**
Pathway Targeted	 LC-TOF/QTOF	 **MassHunter Qual** *Find by Formula*	 **Mass Profiler Professional**		 **Pathway Architect**

SimLipid vs. METLIN PCD

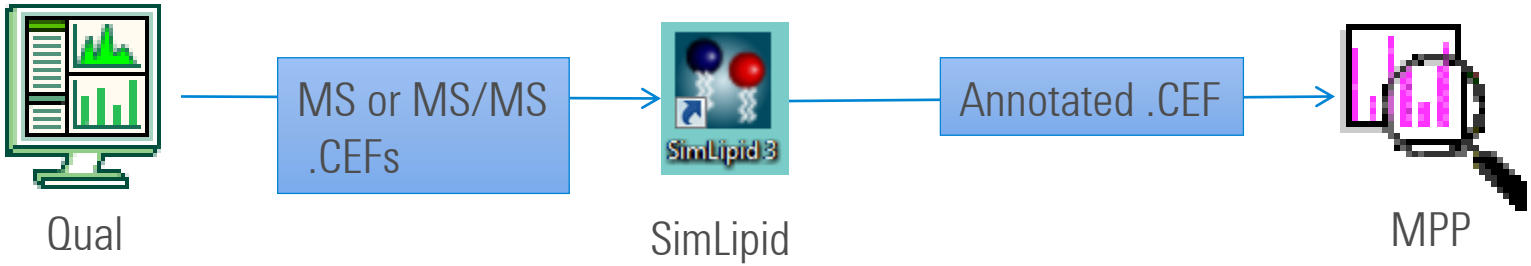
METLIN PCD has 31,011 lipid compounds. It is suited in lipidomics for customers who:

- 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



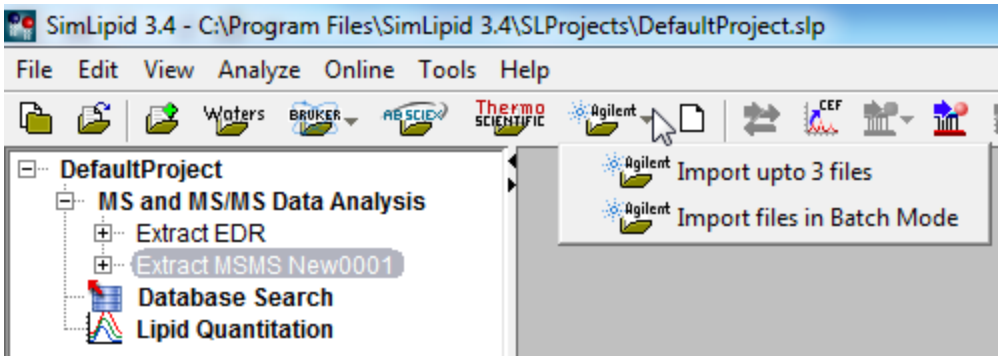
- SimLipid 3.3 and higher supports import and export of Agilent's Compound Exchange Format (.CEF) file. Compounds found in Qual and exported as .CEF can be
  - annotated in SimLipid as lipid compounds using
    - MS database searching
    - MS/MS pattern matching for features that have MS/MS
  - export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

Overview of SimLipid Process

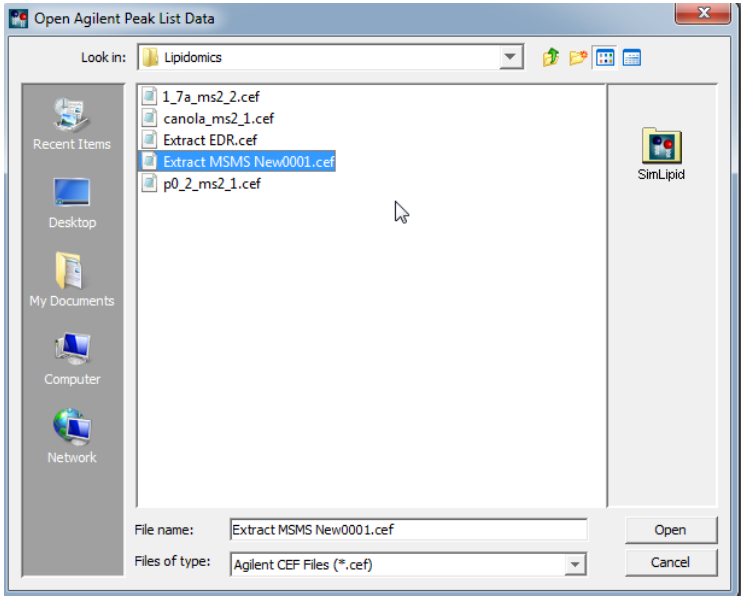
- Import CEF files into SimLipid
- Run High Throughput Search on CEF files
- Load HTP Results from SimLipid server
- Generate HTP Report (optional)
- Export annotated CEF files for analysis in MPP

Import Agilent CEF Files

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

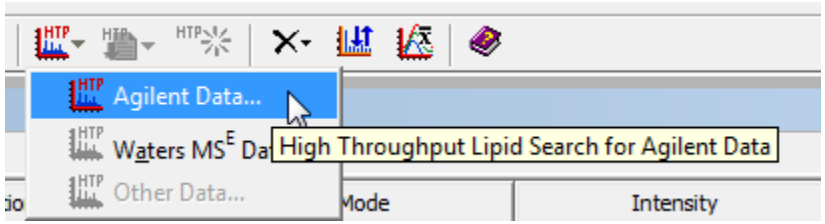


Select your CEF files.

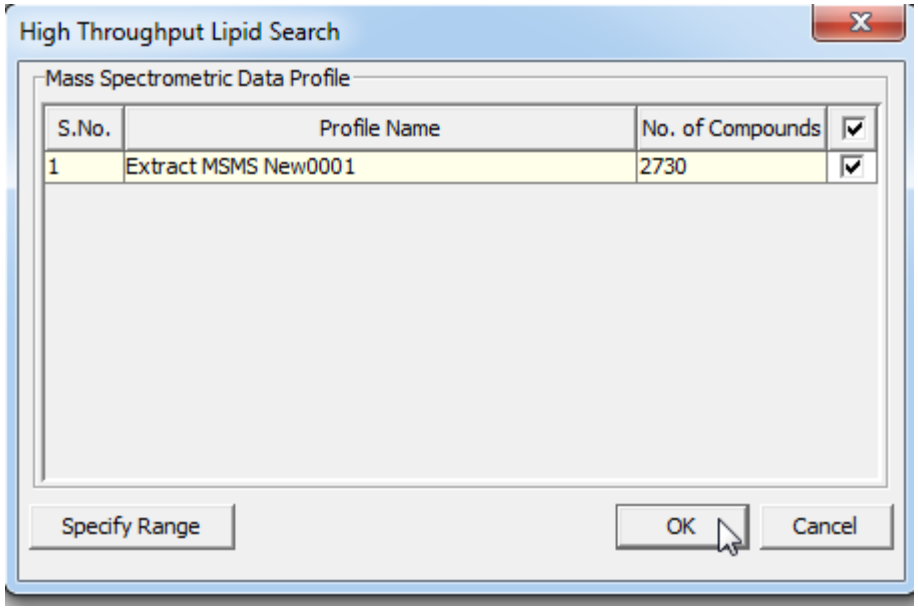


Run High Throughput Search on the CEF Files

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

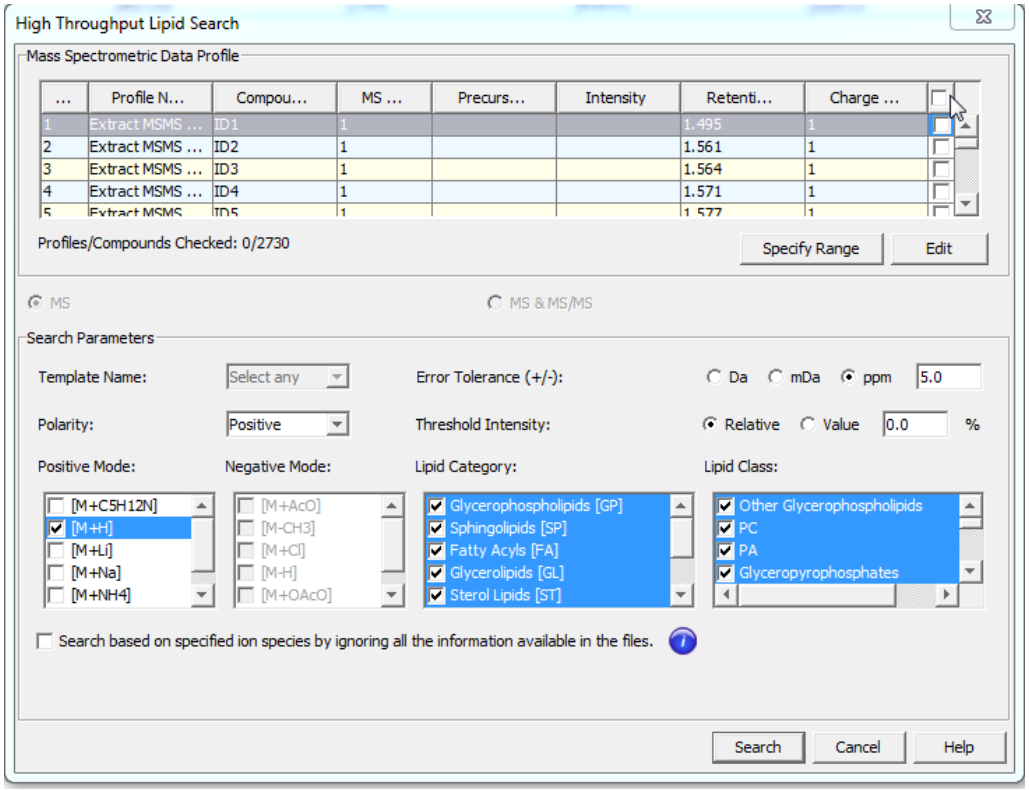


Select CEF files to run High Throughput search on and click OK.



Run High Throughput Search on the CEF Files

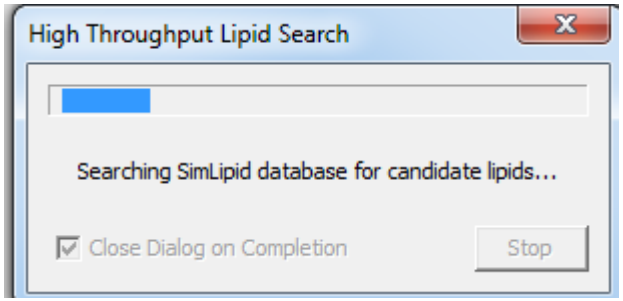
Select the compounds to run HTP search on and the specific search parameters, then press the Search button.

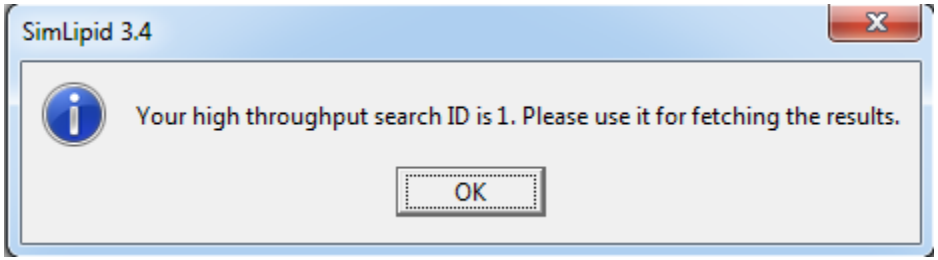


2000 compounds (aka “profiles” in SimLipid) can be searched at one time

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)

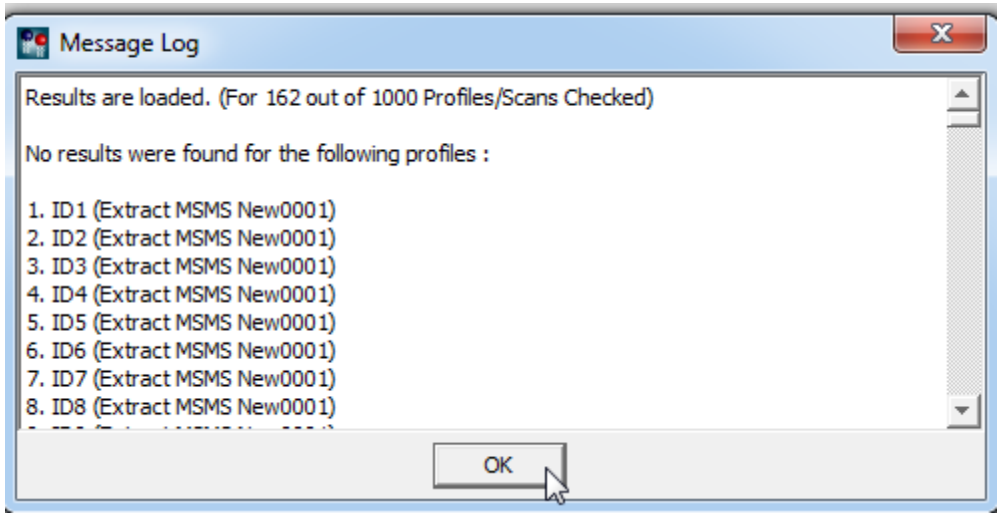




Load HTP Results

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)

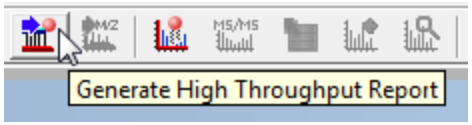


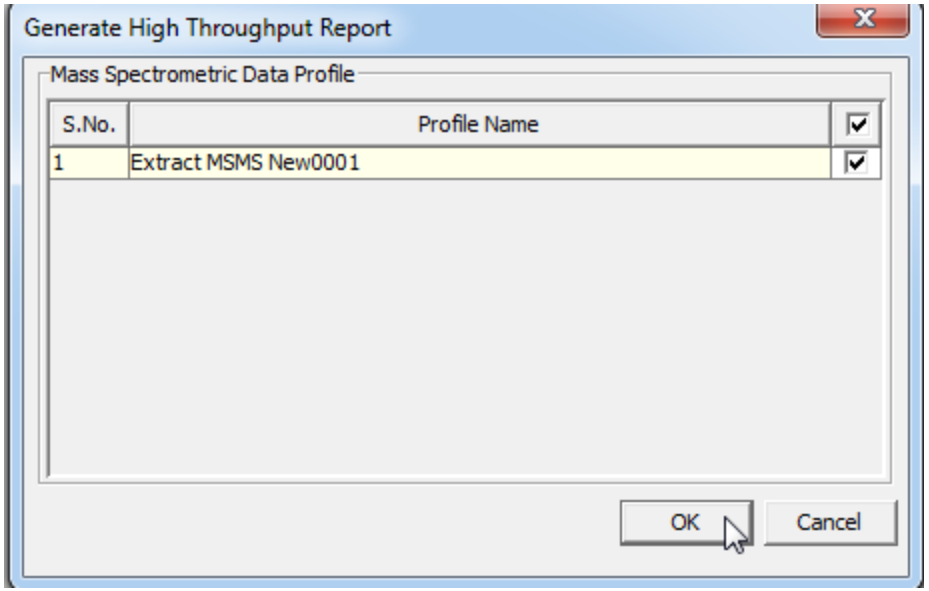
Searches are cumulative—the next time you search it will automatically select the second 1000 compounds.

Generate High Throughput Report (optional)

Select the Generate High Throughput Report icon in the menu bar

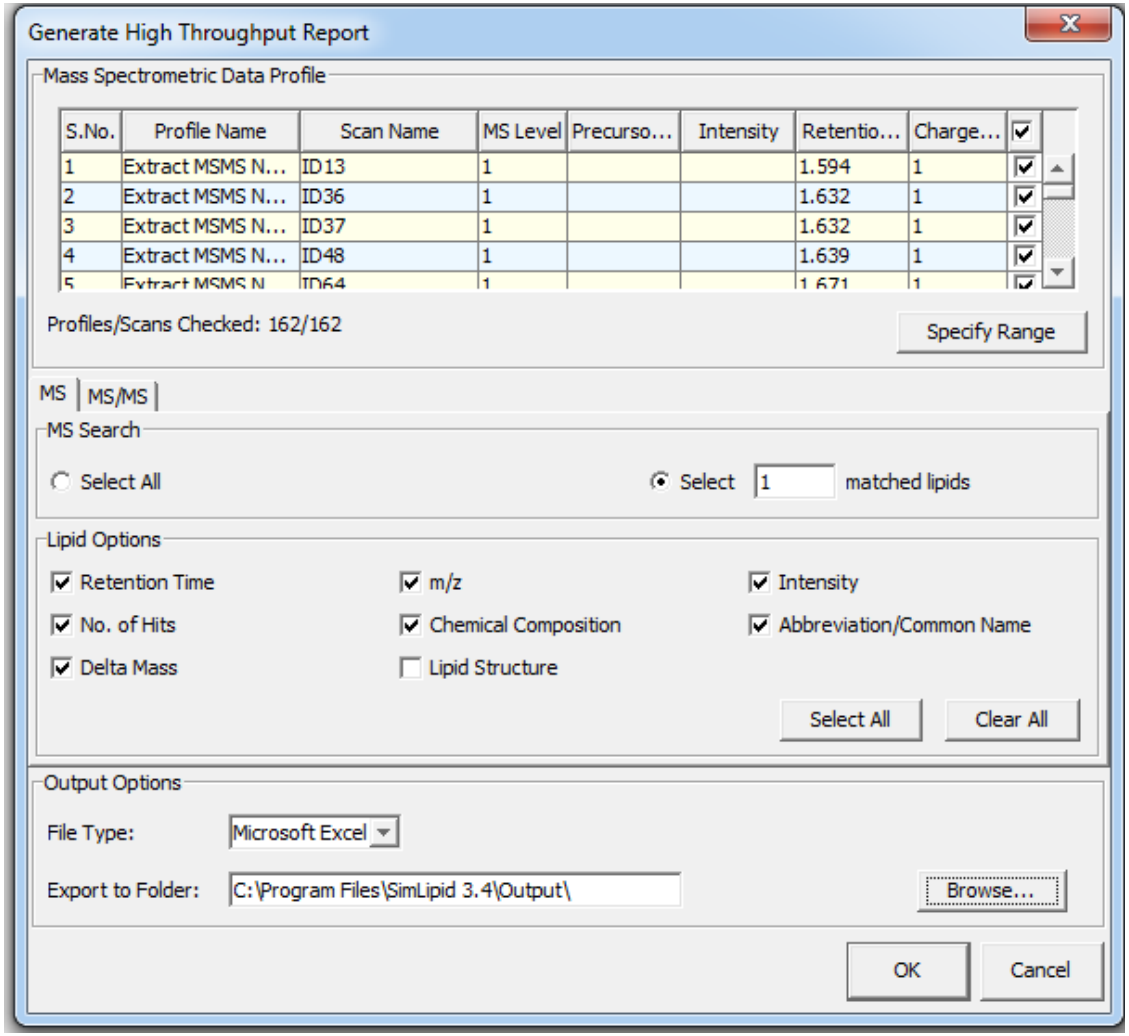
Select CEF files to generate High Throughput report on and click OK.





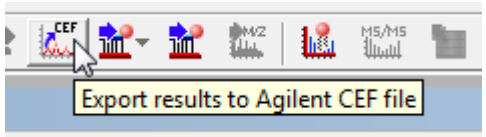
Generate High Throughput Report (optional)

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

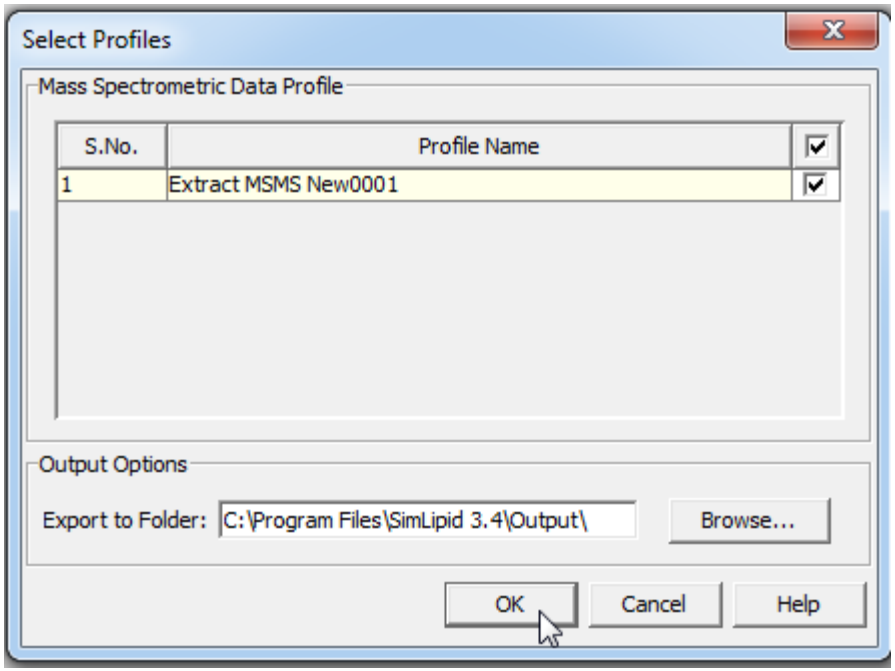


Export Annotated CEF files for Analysis in MPP

Select the CEF icon in the menubar.



Select CEF files to export and click OK.



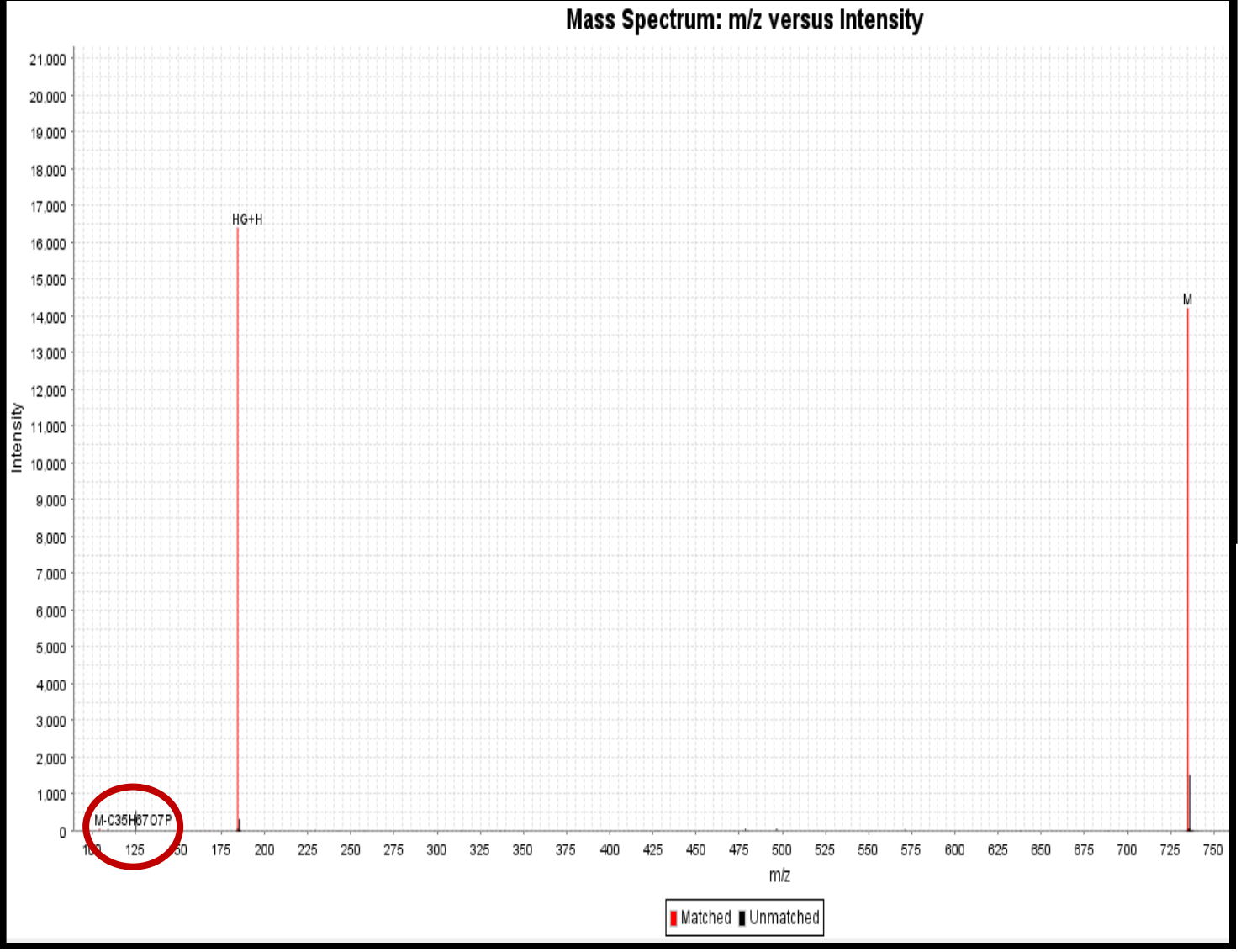
LC/MS/MS Identification of 1,2-dipalmitoyl-sn-glycero-3-PC

Lipid Fragments(LMGP01010564)

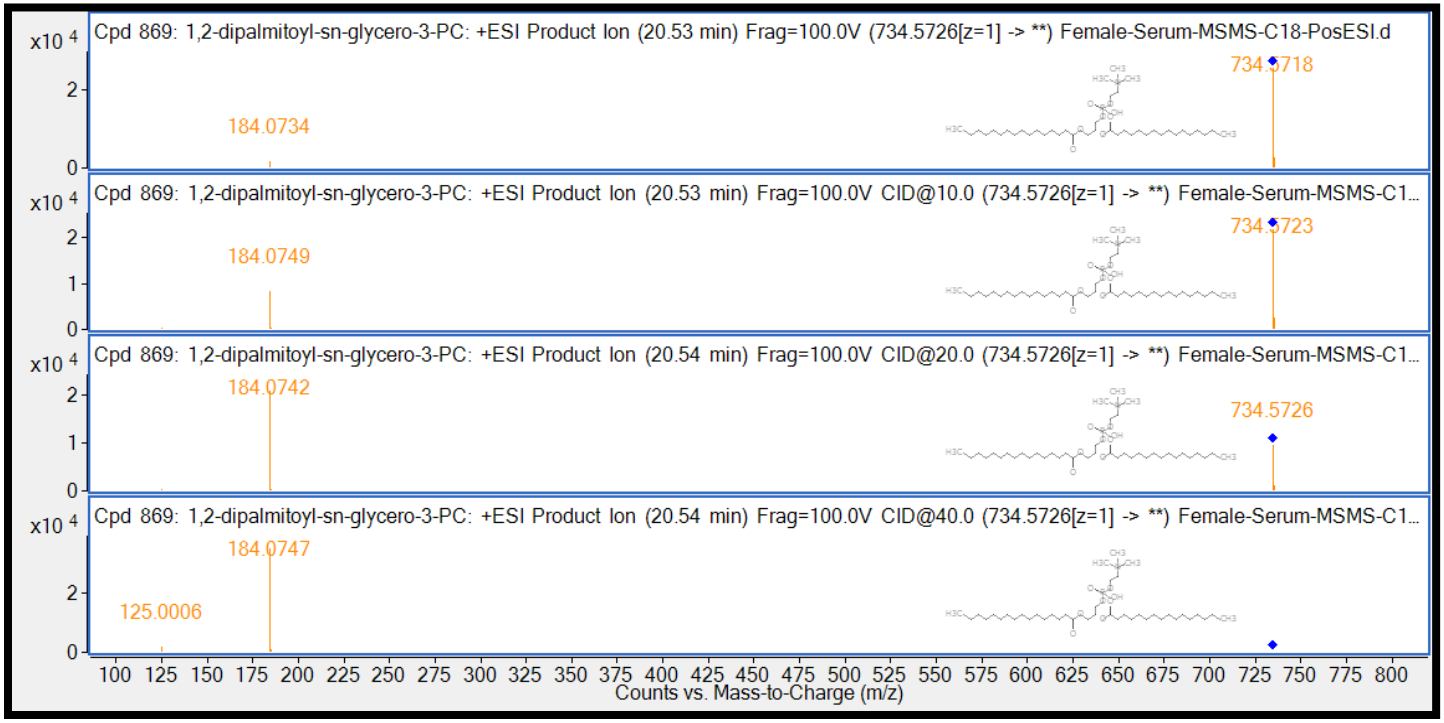
ID869#_PC(16:0/16:0)@734.572

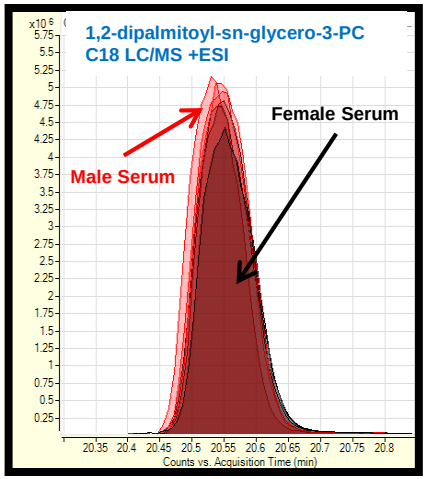
MS/MS Profile | Search Results | MS/M

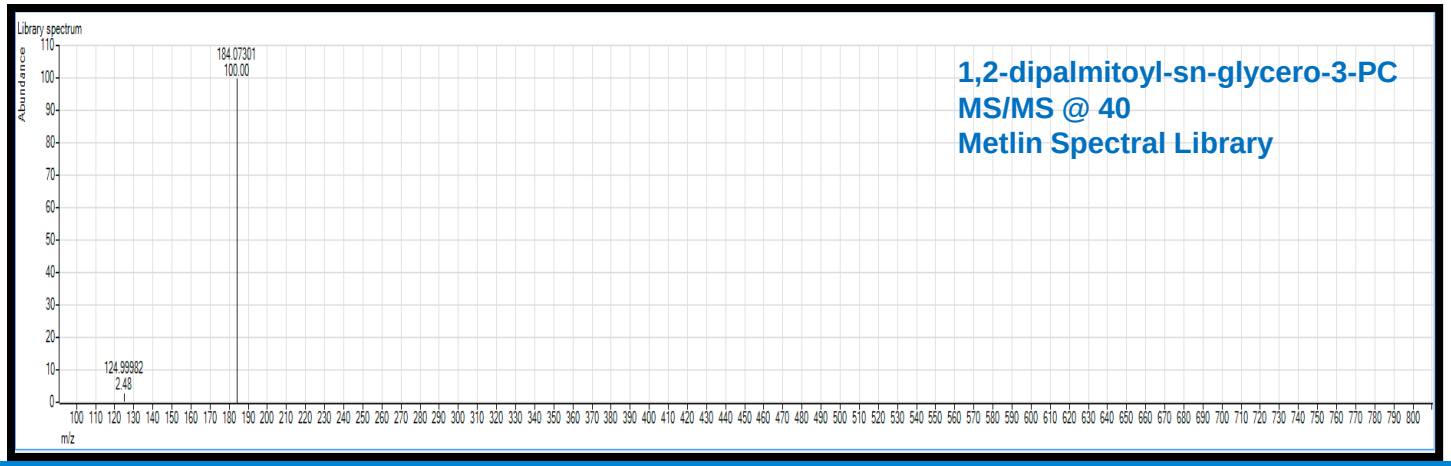
S.No.	m/z
1	104.1074
107	734.5721
11	166.0638
18	184.0745
11.1	166.0638
2	104.7271
3	109.1006
4	113.1999
5	124.7027
6	125.0003
7	125.0562
8	125.1304
9	125.5472
10	142.1619
12	167.8584
13	171.3158
14	183.4557
15	183.4651
16	183.5501



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







1,2-dipalmitoyl-sn-glycero-3-PC
MS/MS @ 40
Metlin Spectral Library

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

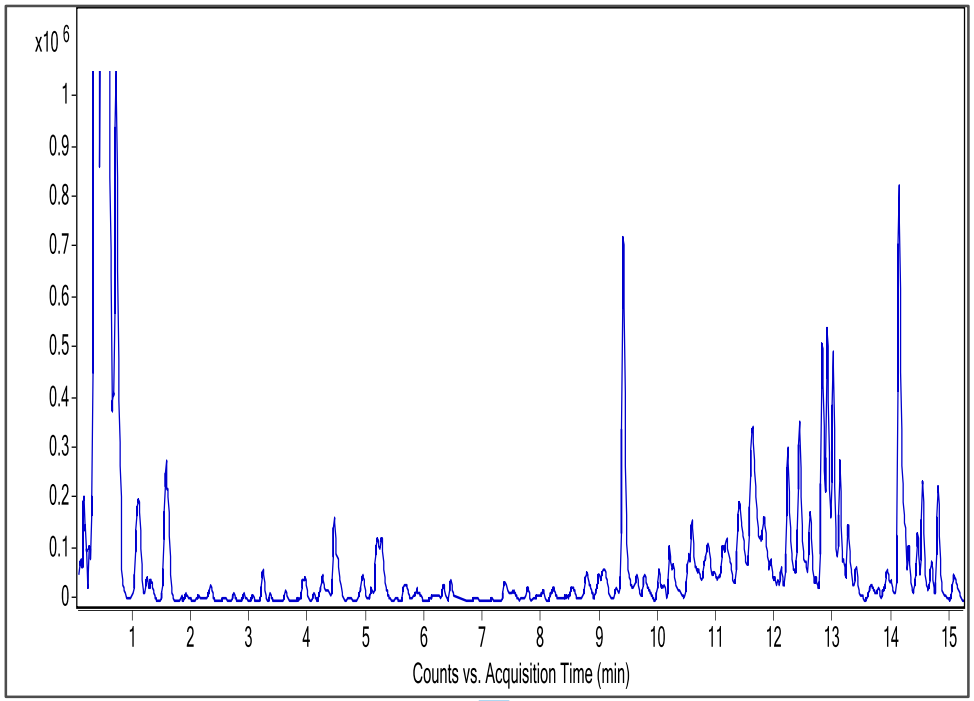
Phone: +1-650-856-2703

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

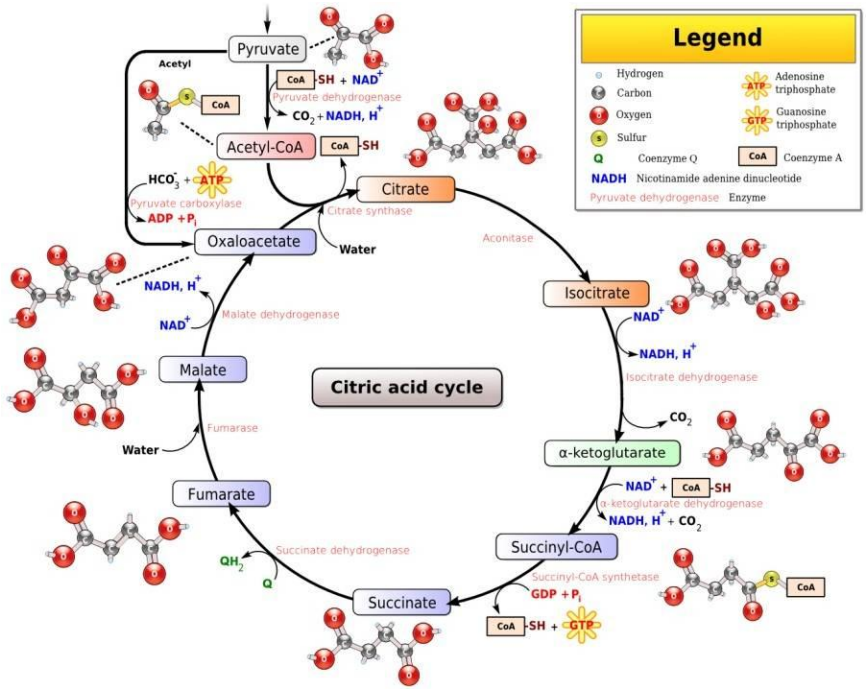
Pathway Architect

Pathway analysis using multi-omic data

Start here



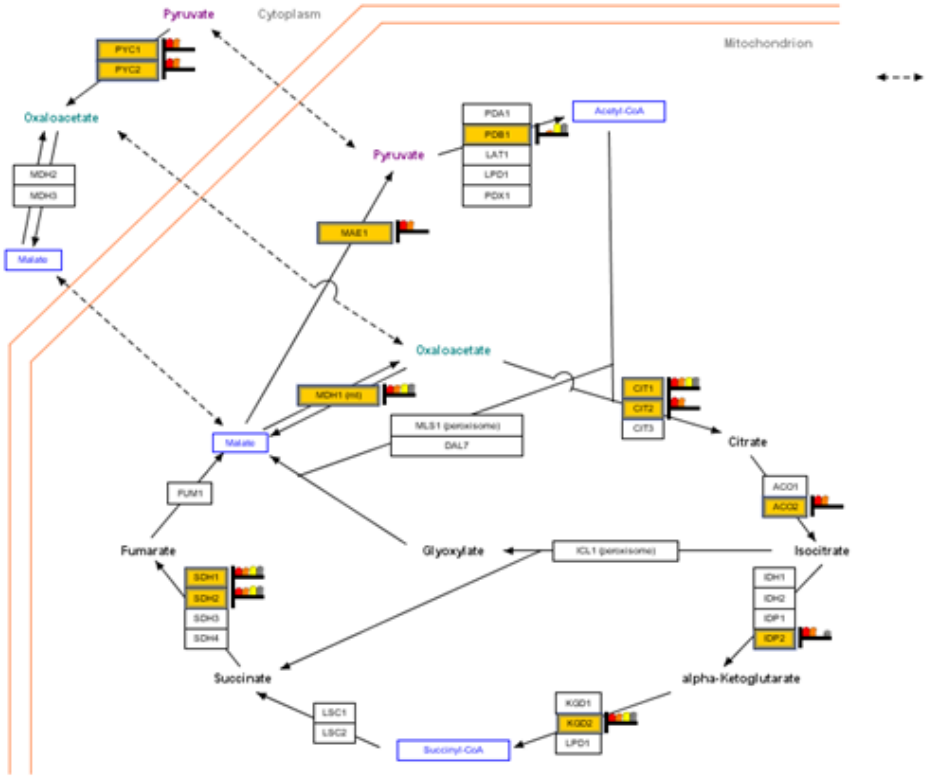
Finish here



Pathway Architect

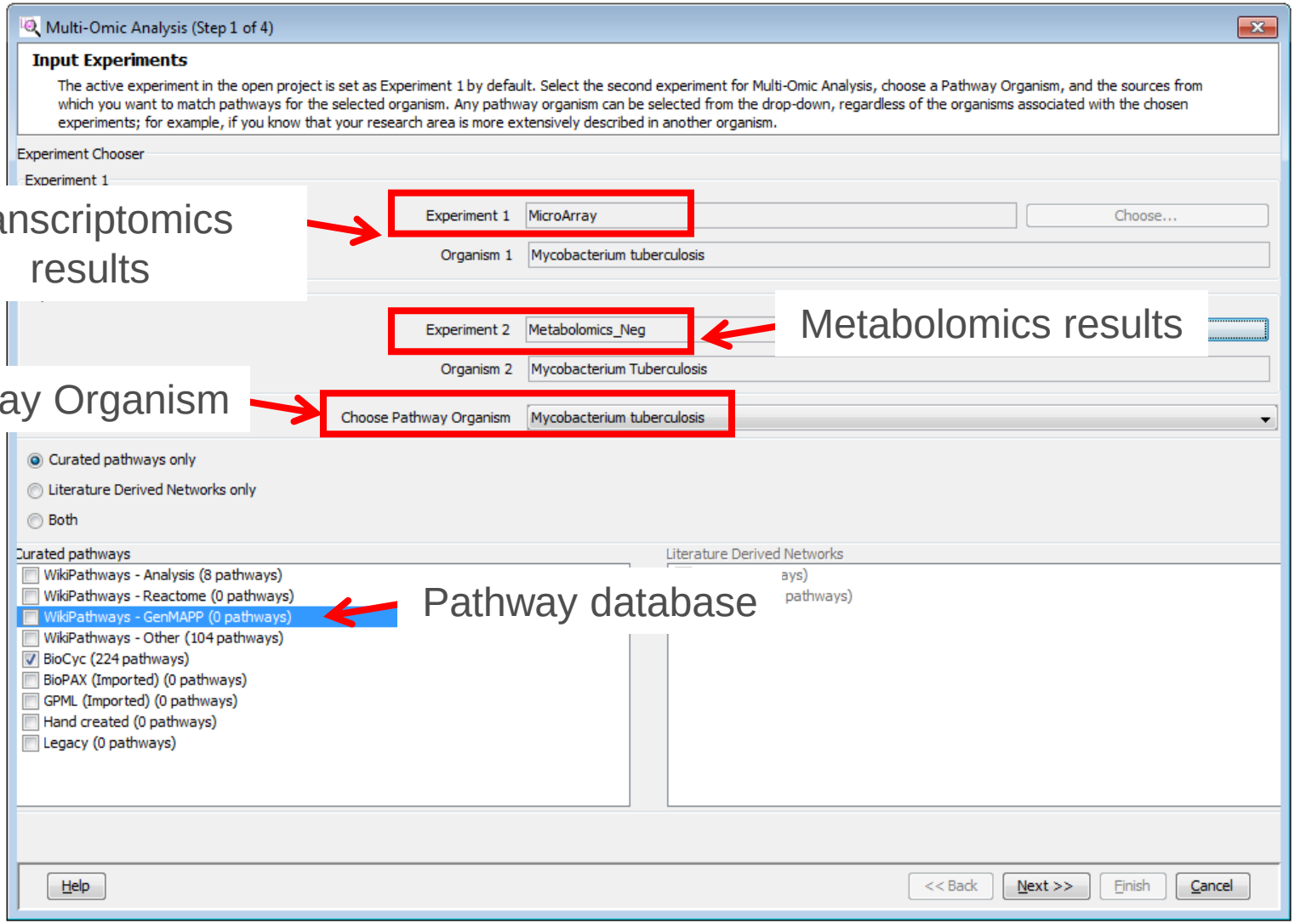
Customers can take the value of their data to another level. Pathway Architect helps them make sense of the data by:

- 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



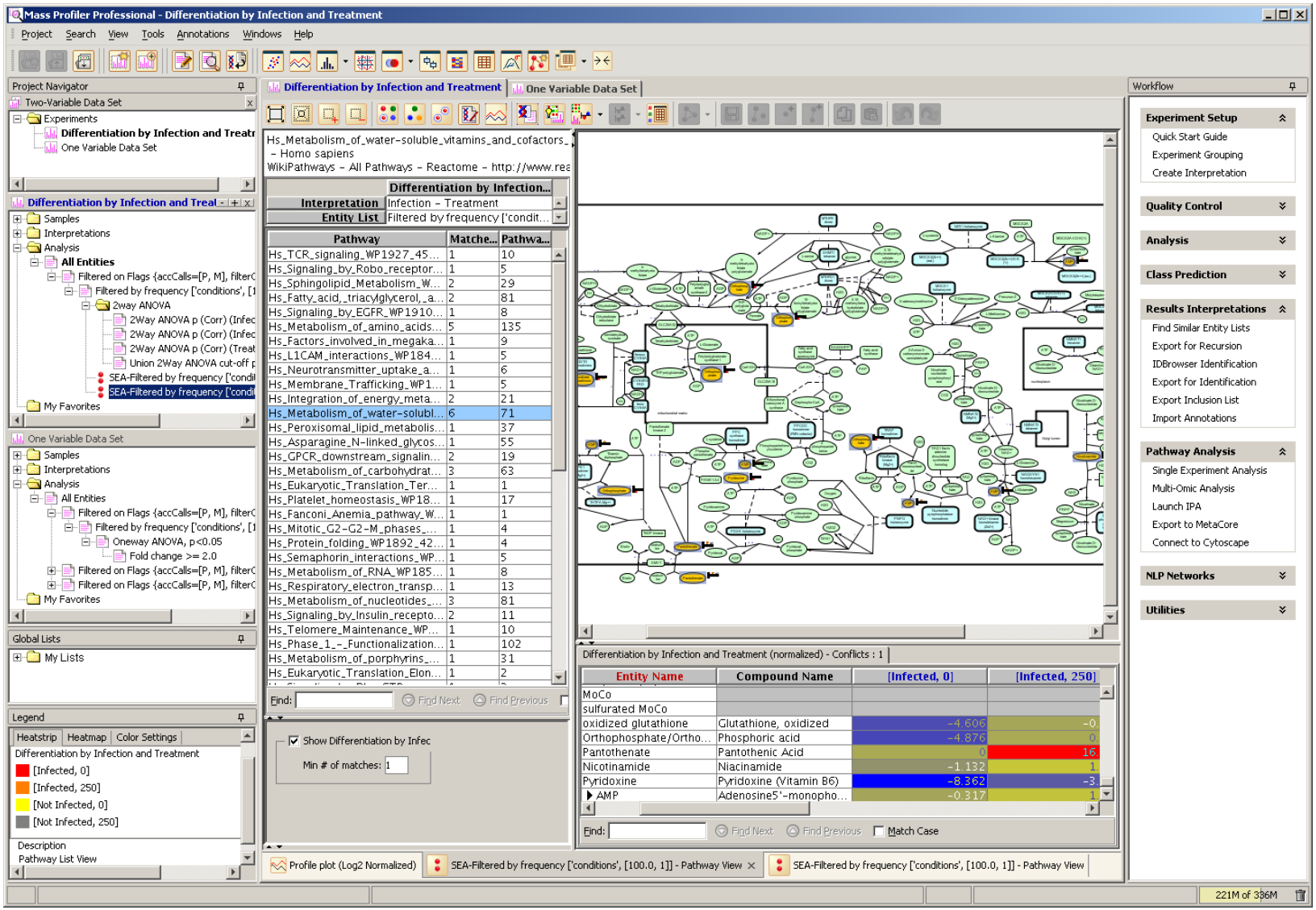
Transcriptomics results

Metabolomics results

Pathway Organism

Pathway database

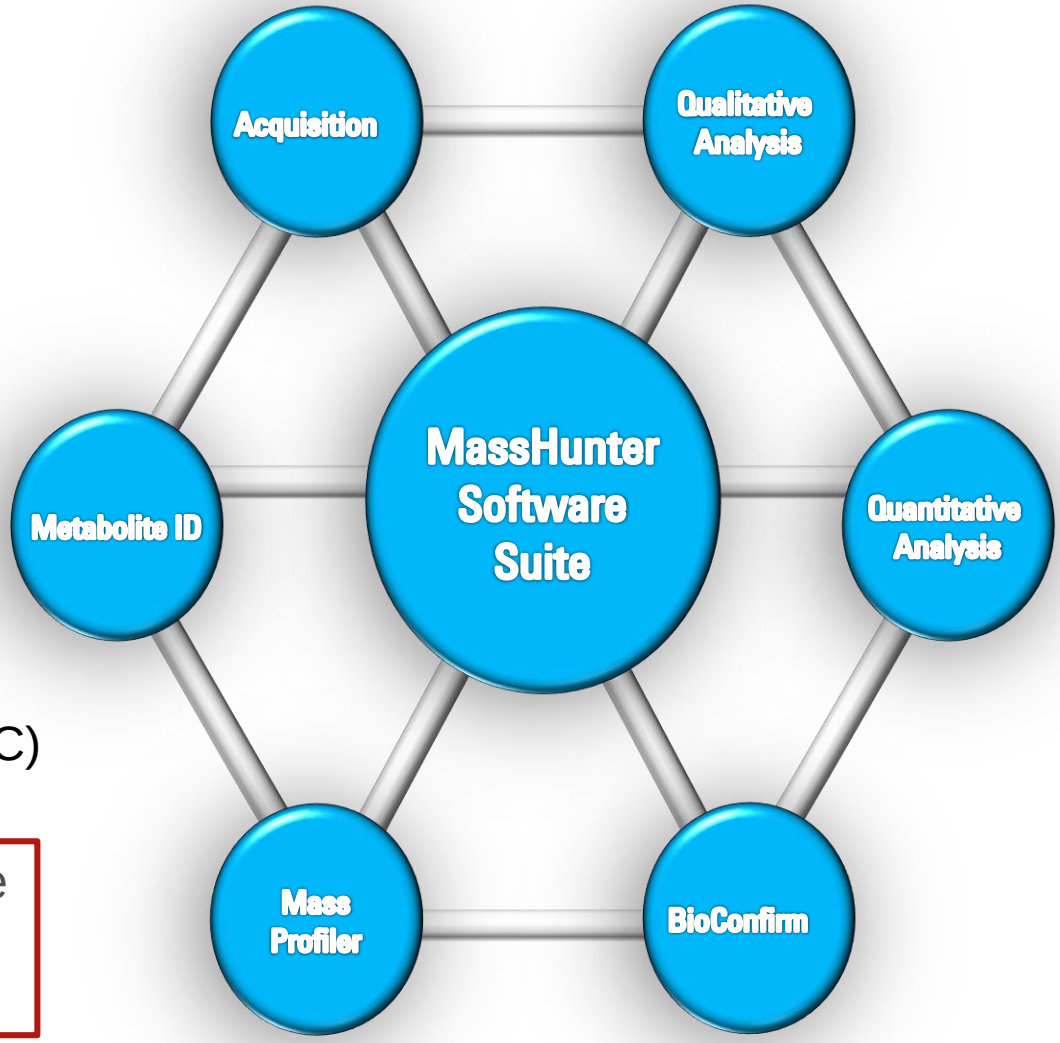
Pathway Architect Navigation



Mass Hunter Qualitative Analysis and Its “Accessories”

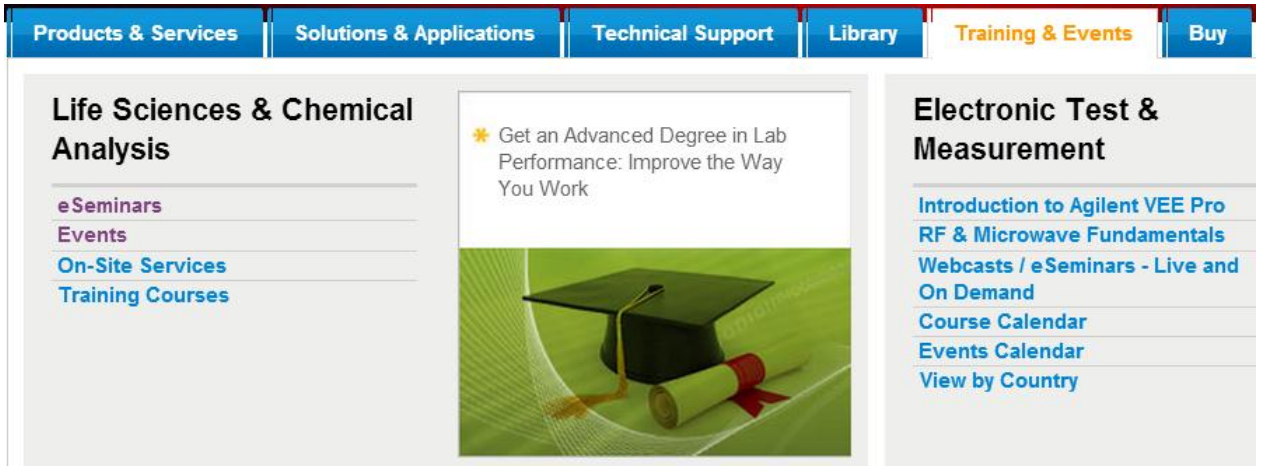
- Qualitative Analysis (Qual)
- PCDL Manager
- Pathways to PCDL
- Profinder
- Mass Profiler (MP)
- Mass Profiler Professional (MPP)
- ID Browser
- SimLipid (Premier Biosoft)
- Molecular Structure Correlator (MSC)

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



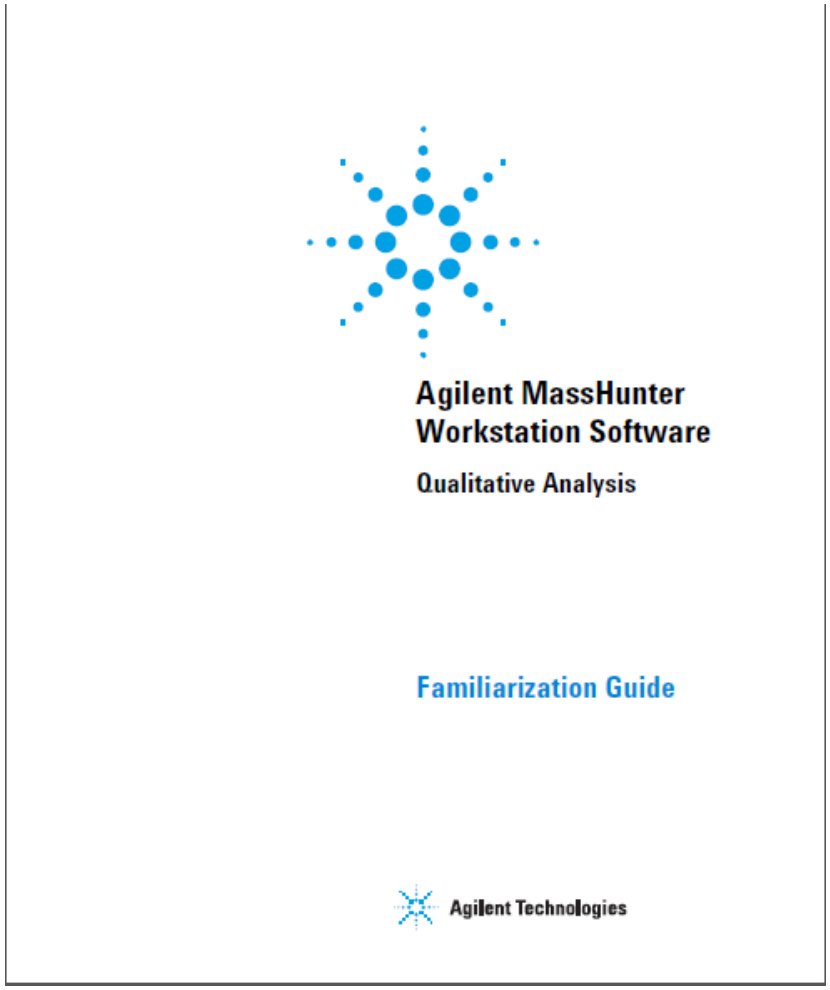
How can you learn more about MassHunter software?

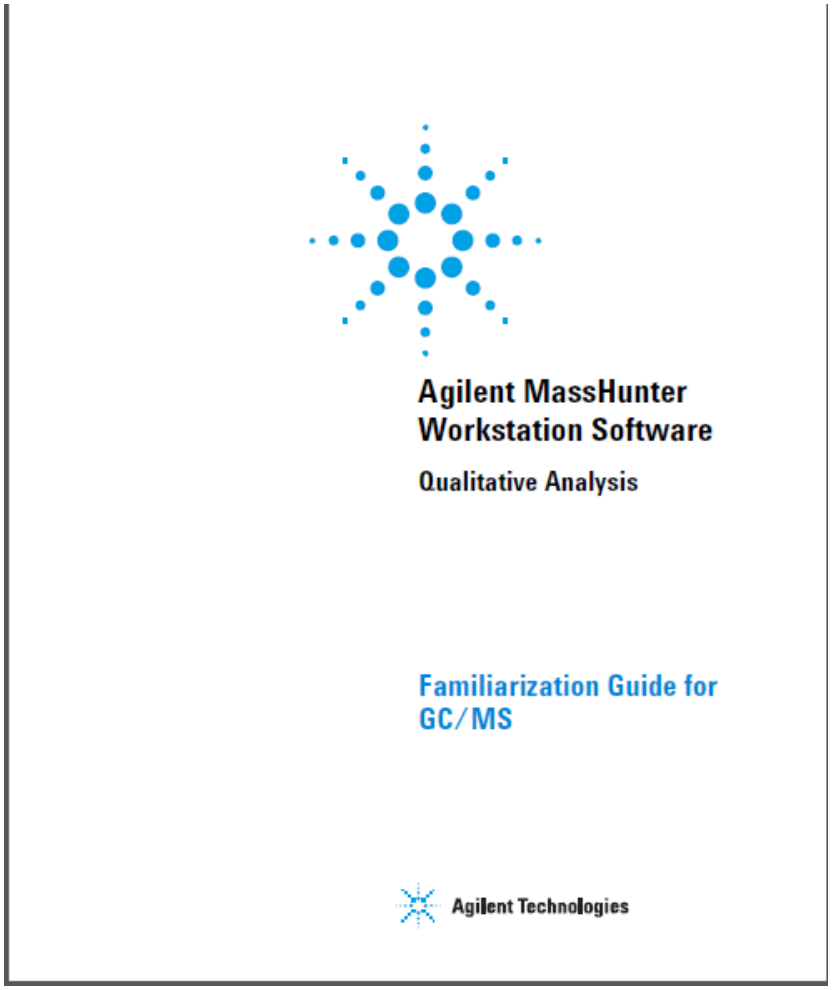
- Read the PDF manuals and guides that shipped with your instrument or software
- Watch the videos that shipped with your instrument or software
- Read the Workflow Overviews and Workflow Guides specific to your application area
- Use the Help feature in the software!
  - Especially good for unfamiliar 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

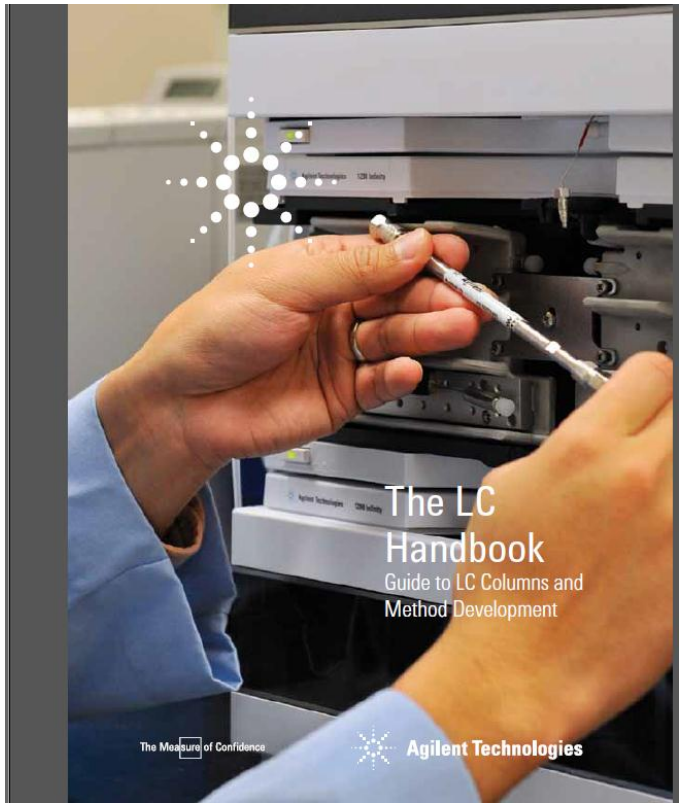
Remember LC/MS and GC/MS Versions!

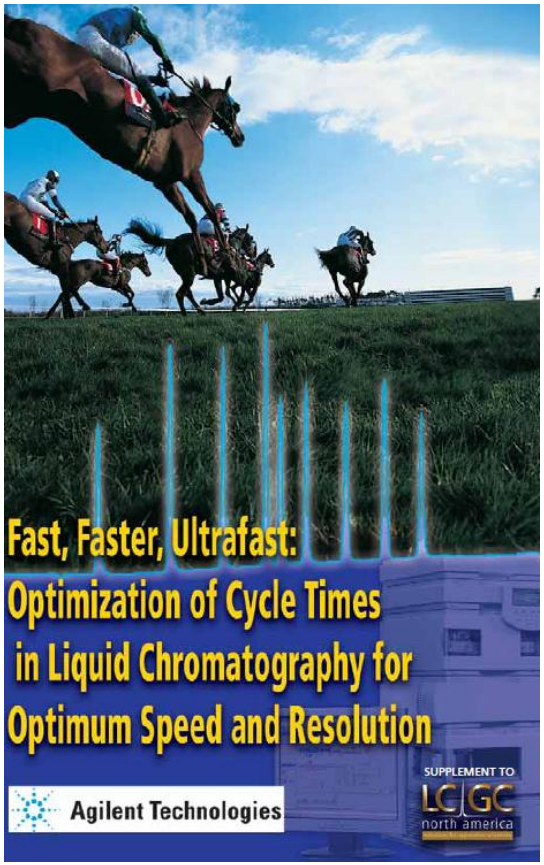




Training: Learn about Liquid Chromatography

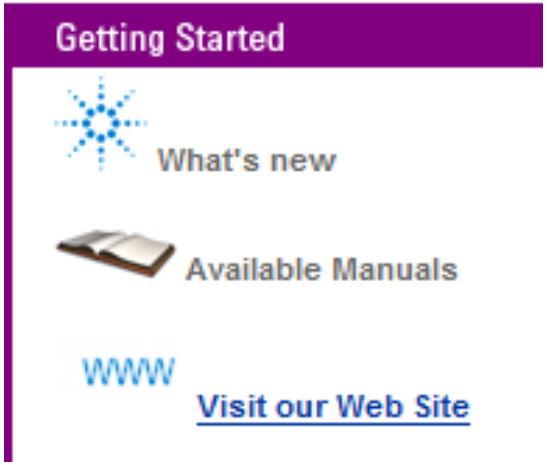
Online Primers and Basic Concept Guides





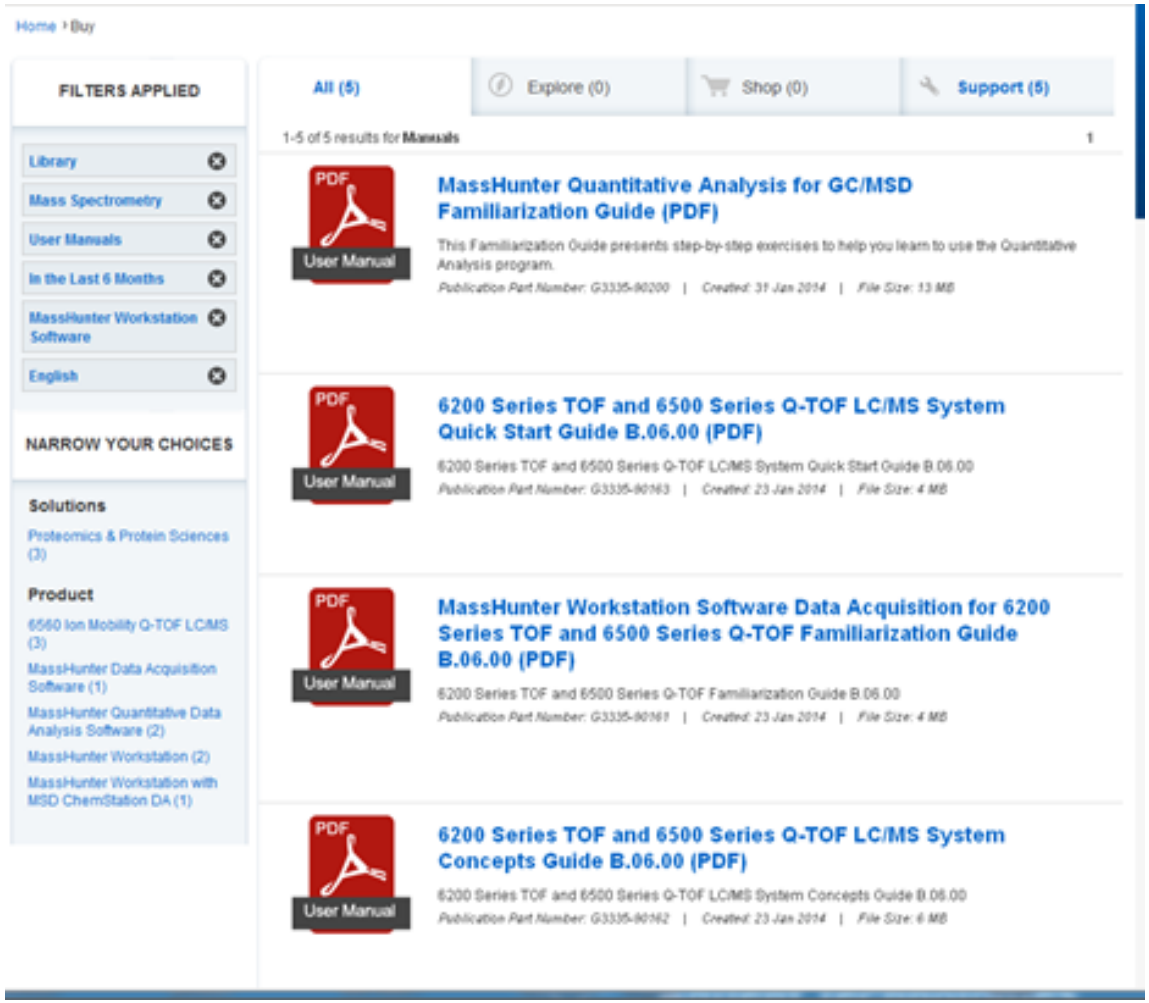
http://www.chem.agilent.com/search/?N=68+4294964917&Nr=OR%28part_language%3Aen%2Cg_rec_type%3ASharePoint%29&Ntt=primers

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



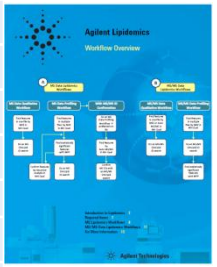
Searching
- Literature
- Mass Spectrometry
- Manuals
- Last 6 Months

PDF's of new manuals



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?