Agilent MassHunter Qualitative Data Analysis

Qualitative Navigator B.08.00

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MassHunter Qualitative Navigator

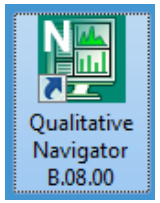
Topics

- Navigator and Workflows
- Navigator View
  - Adaptive User interface
- Methods
  - Definition
  - Unified Method Concepts
- Working with Chromatograms
  - Anchoring
  - Integration peak List
- Working with Spectra
  - MS Display Options
  - Background Subtraction
- Annotations of Chromatograms and Spectra
  - Library Searching and Annotations

MassHunter Qualitative Analysis Software B.08.00 SP1

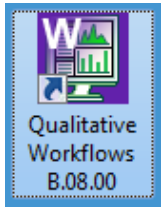
Navigator and Workflows

Navigator



- User centric
- Interactive Browsing
  - Walk the Chromatogram
  - Spectrum Preview
- Spectrum ID
  - Library/Database Search
  - Molecular Formula Generator
- No concept of compounds
- No Feature Finding – ‘Find by…’
- What does the chromatography look like? System Suitability
- What is the mass spectrum for each peak?
- Can the spectrum be identified by searching a library?

Workflows

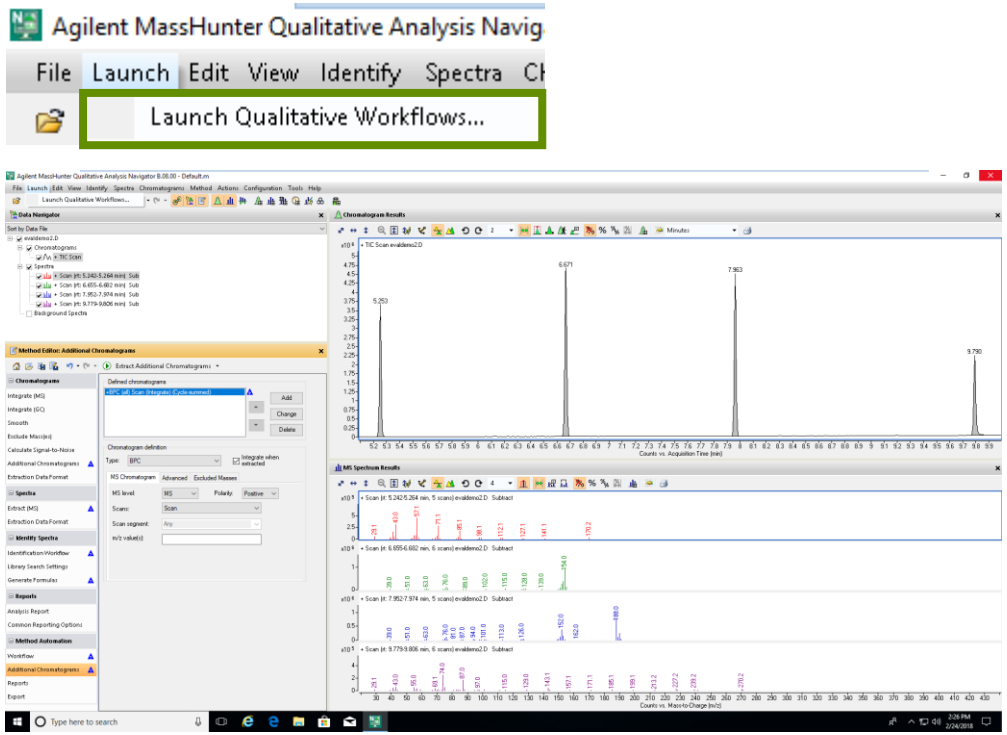


- Compound centric
- Automated Routine Workflows
  - Sample Purity
  - Compound Discovery
  - Compound Identification
- Feature Finding – ‘Find by…’
- No Spectrum Preview
- No ‘User Spectra’
- All spectra are compound based.
- What compounds can be found and identified?

MassHunter Qualitative Analysis Software B.08.00 SP1

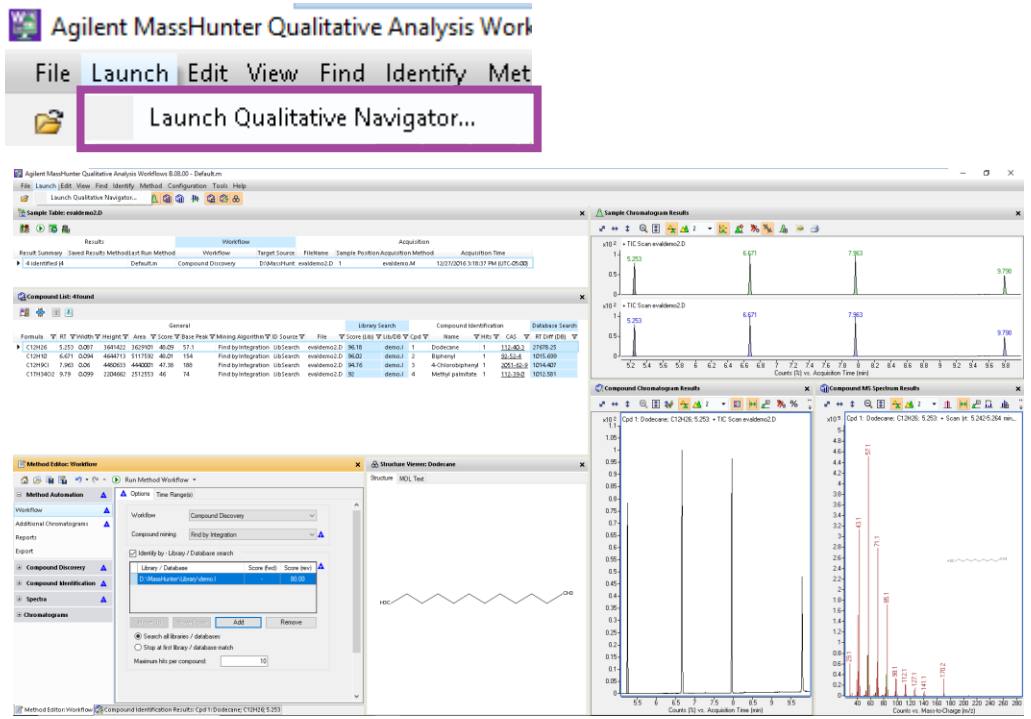
Navigator and Workflows

Navigator



File opens in Workflows.
The same chromatogram(s) extracted.
Spectra not extracted.
Method is NOT passed to other context.

Workflows



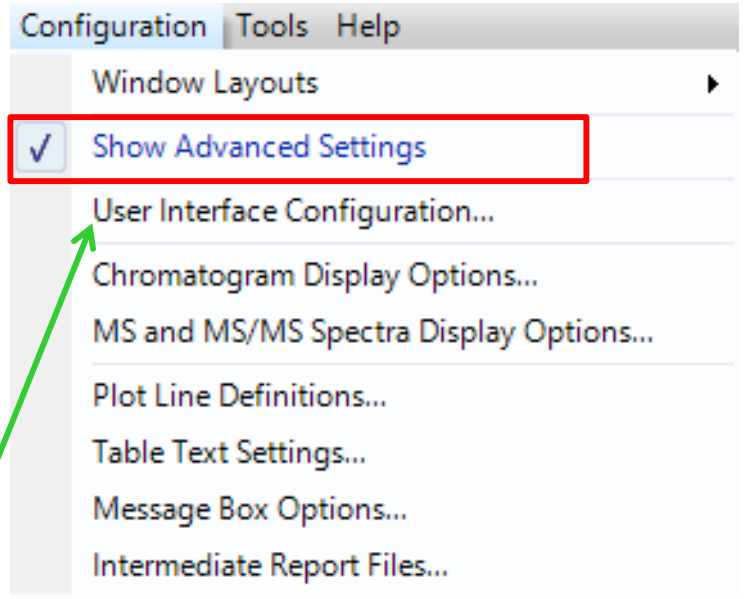
File opens in Navigator.
The sample chromatogram(s) are extracted.
No compound results.
Method is NOT passed to other context.

MassHunter Qual - Configurable Software

- One program for many instruments and types of data.
  - Single Quad (LC & GC) Scan, SIM data
  - Triple Quad (LC & GC) Scan, SIM, MRM (MS/MS)data
  - TOF (LC) High resolution, scan
  - Q-TOF (LC & GC) High resolution MS/MS data

- Qualitative Analysis B.08.00 features the Adaptive User Interface – based on the type of data file opened, MassHunter will attempt to configure the interface with the appropriate options.
- The interface can still be manually configured if necessary.
- Even when properly configured some features and parameters for MS/MS and accurate mass are still visible, ignore and avoid them.

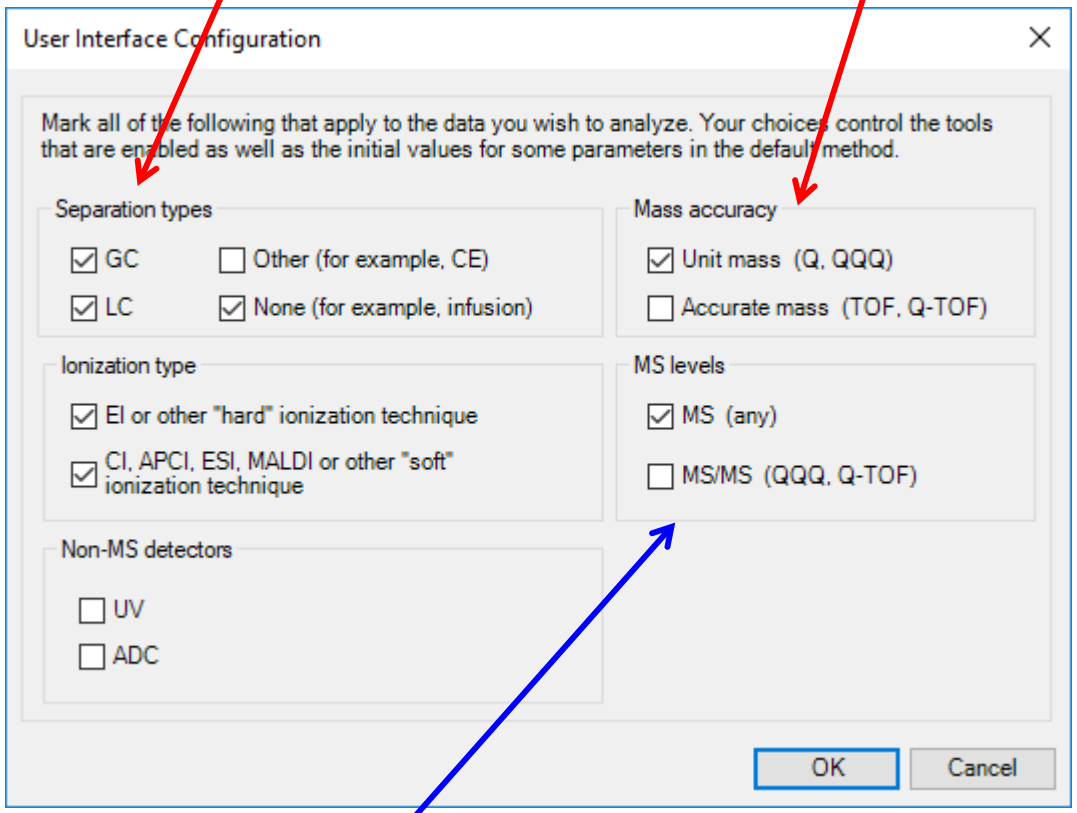
User Interface Configuration



Check Show Advanced Settings from the Configuration menu to access the User Interface Configuration

Separation types (Check GC or LC)

Unit Mass (SQ, TQ)
Accurate Mass (TOF, QTOF)

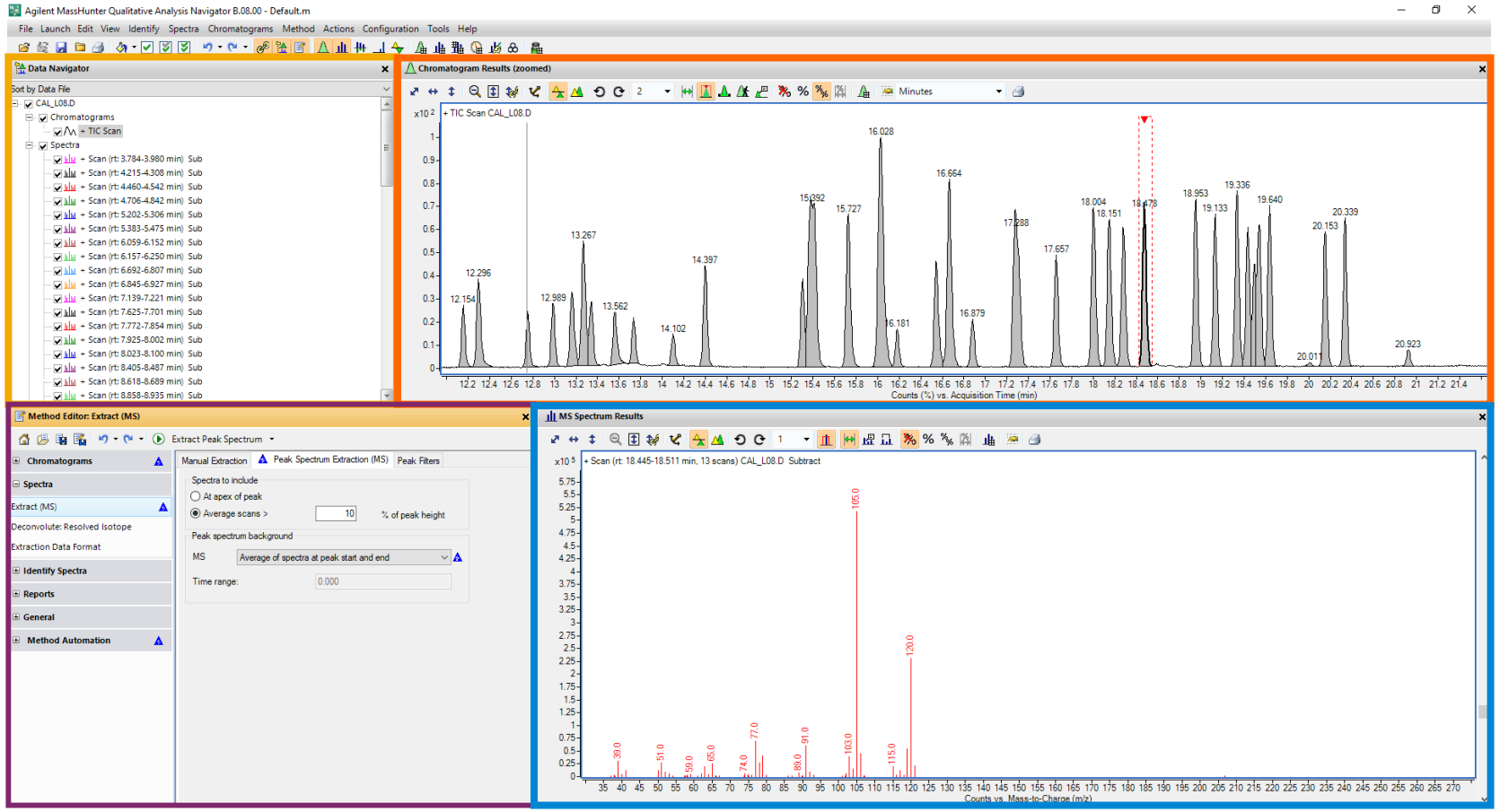


MS Levels: MS or MS/MS

Navigator View

Data Navigator

Chromatogram Results



Method Editor

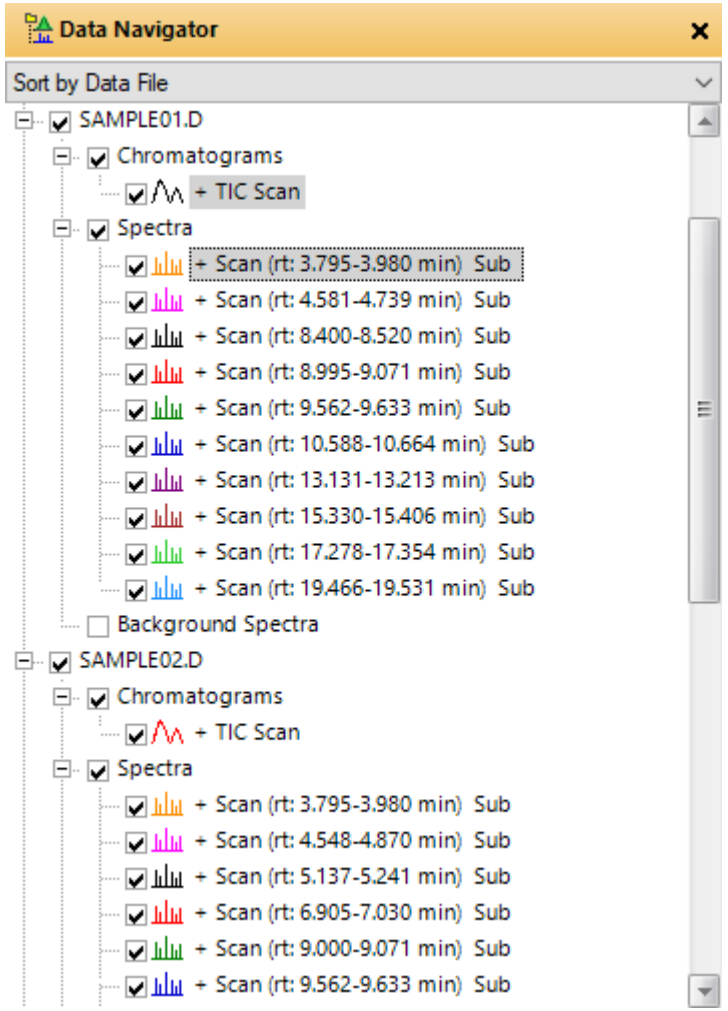
Spectrum Results

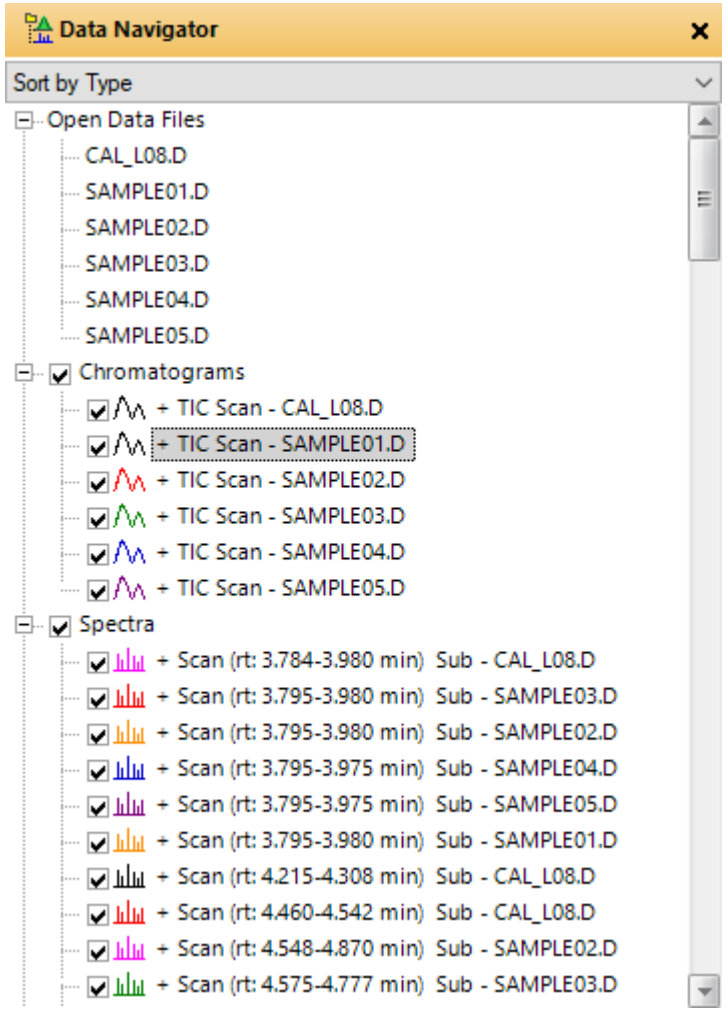
Data Navigator

The Data Navigator pane shows the data files which are loaded into Qualitative Navigator.

The user can selectively display the chromatograms or spectra associated with a data file by selecting/deselecting a checkbox.

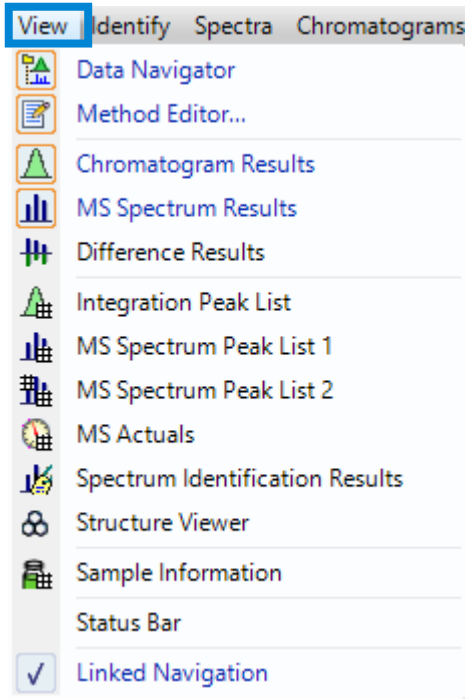
In the top drop-down, the user can choose to sort by Data File or Type.

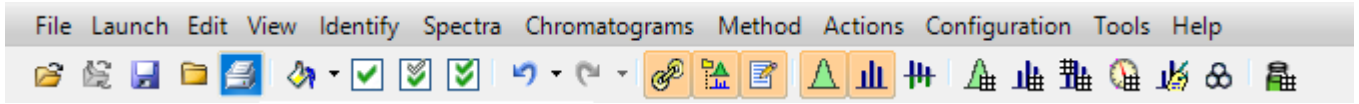




Expose or Hide Windows as Needed

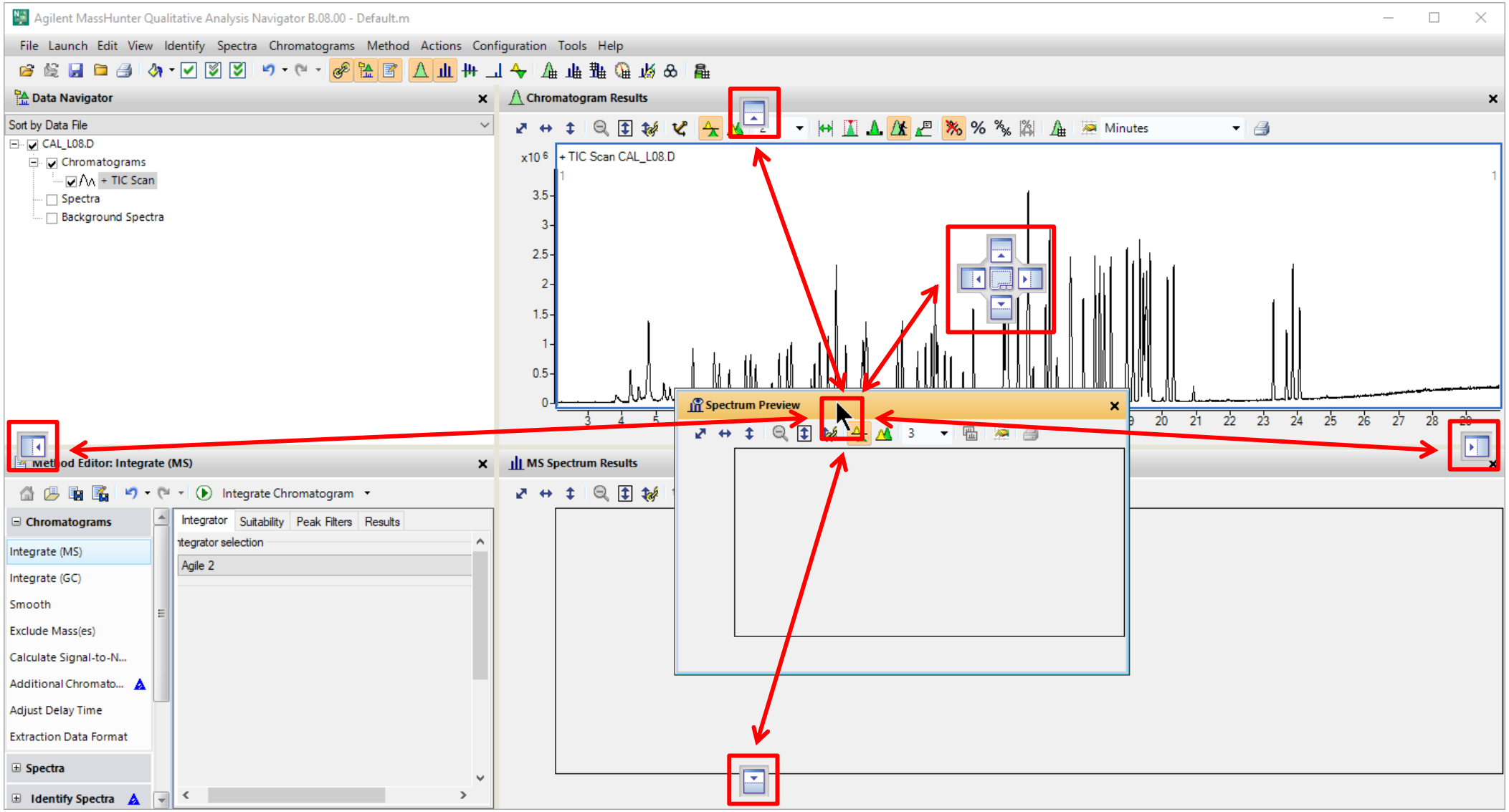
From the Menu…



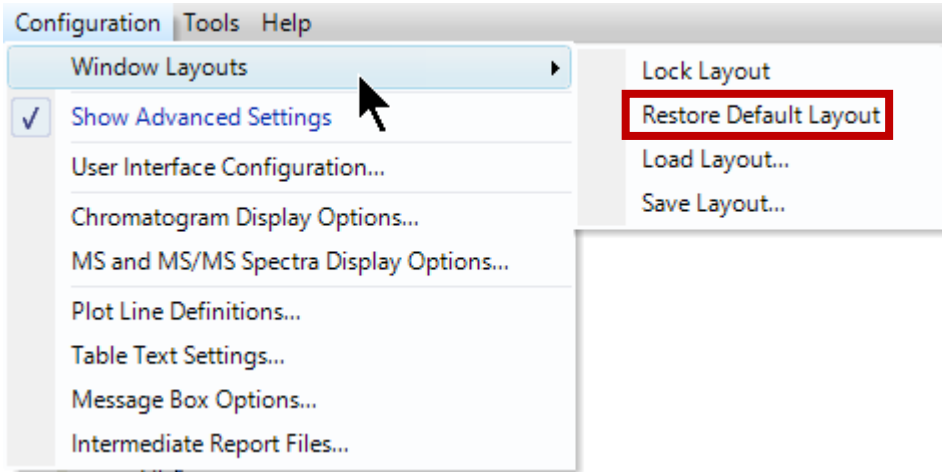


…or from the Toolbar

Docking & Undocking Windows

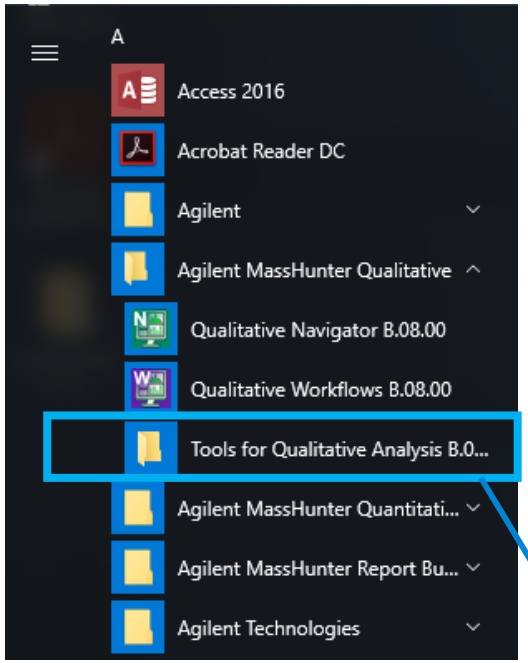


Restore Default Layout

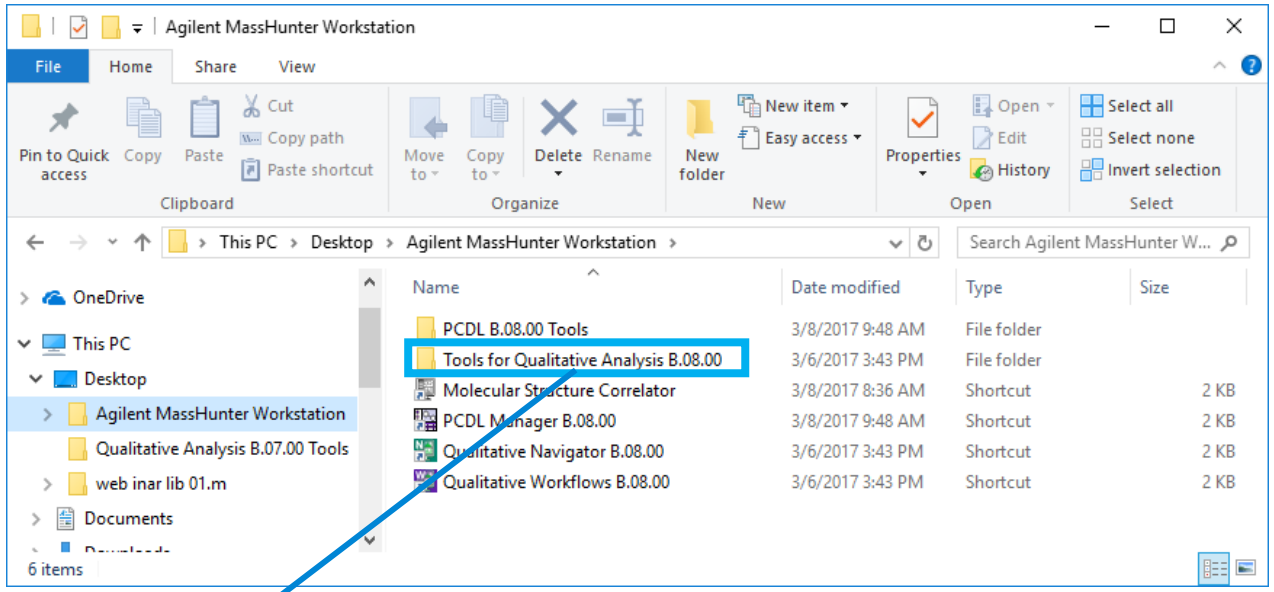


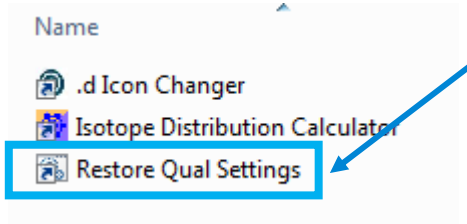
- Complicated windows layouts can be restored to default layout.
- Preferred layouts can be saved and loaded.
- Layouts can be locked.

Restore Qual Setting



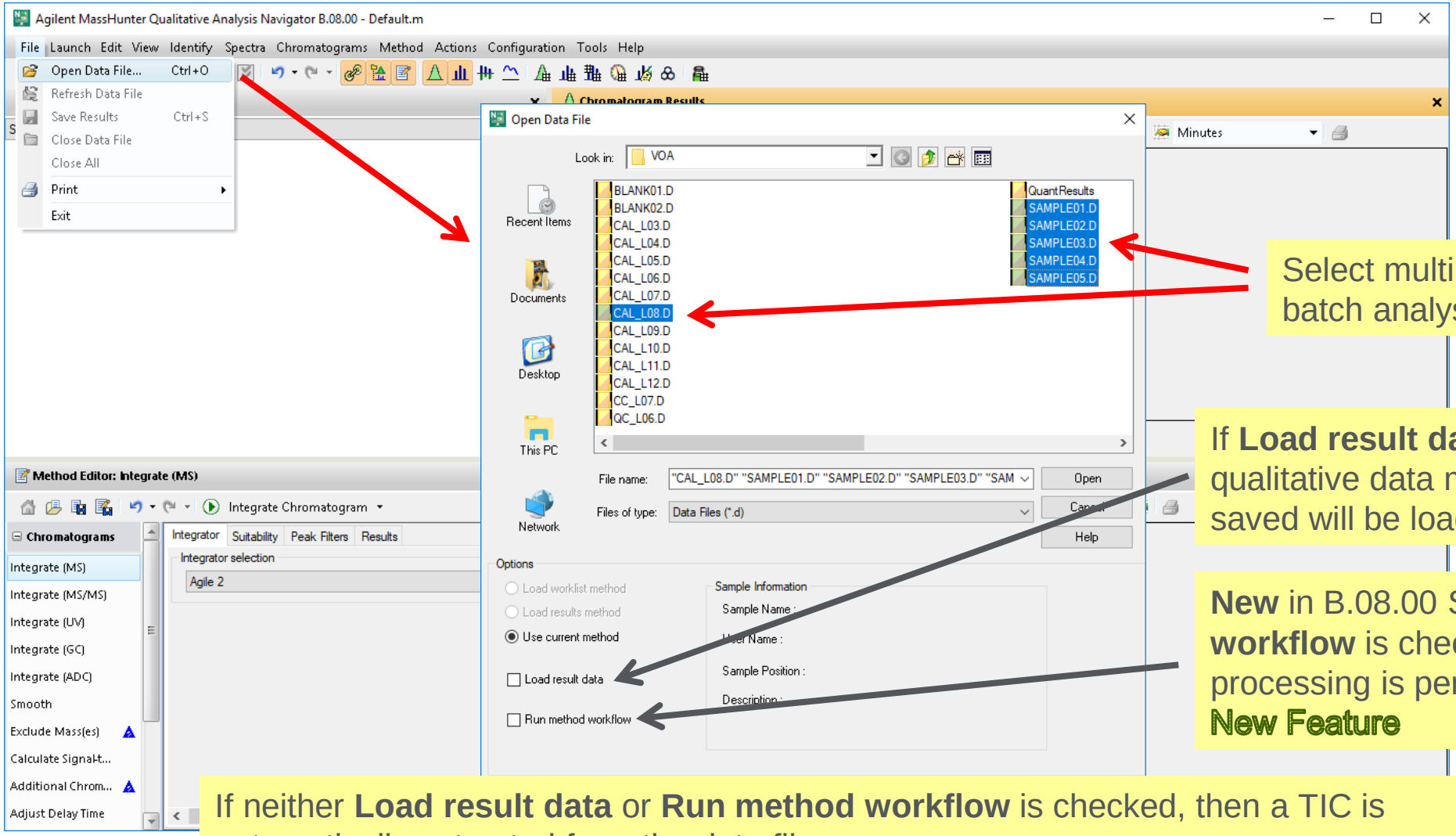
or





- Located in the Tools for Qualitative Analysis B.08.00 folder.
- Accessed from Start Menu or the Agilent MassHunter Workstation desktop folder.
- This can be a useful tool to restore the Qualitative Analysis settings if a configuration problem is suspected.

Open Data Files



Select multiple files at once for batch analysis, then click **Open.**

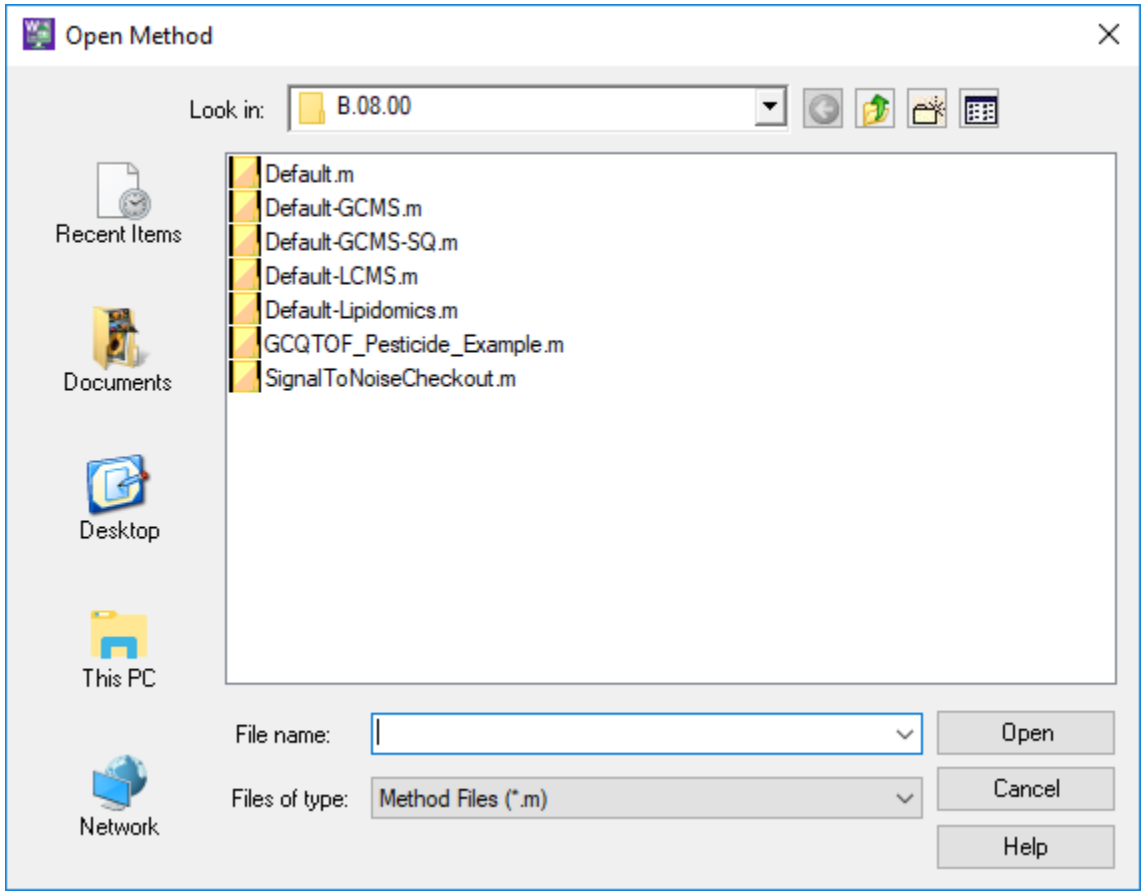
If **Load result data** is checked, the qualitative data manipulations previously saved will be loaded.

New in B.08.00 SP1 - If **Run method workflow** is checked, automated processing is performed.
New Feature

If neither **Load result data** or **Run method workflow** is checked, then a TIC is automatically extracted from the data files.

Qualitative Analysis Methods

Open Method



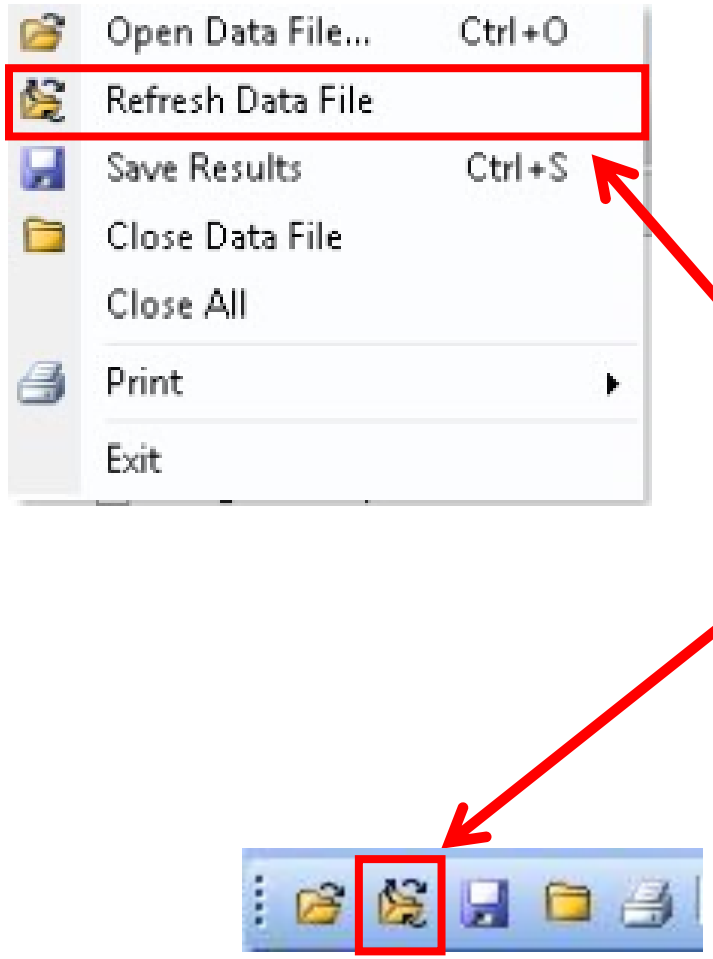
Open Method

- Load the method relevant to the data set.
- Method contains recommended/default parameters.
- Method Editor values can be modified.
- Saves time developing specific values.
- Good starting point.

- **Tip: Load the applicable method.**

- Default-GCMS-SQ.M → GCMS and GC QQQ
- Default-GCMS.M →GC QTOF
- Default-LCMS.M → LCMS

Refresh Data File



File > Refresh Data File

Feature is useful when it is desired to view data as the data file is being acquired.

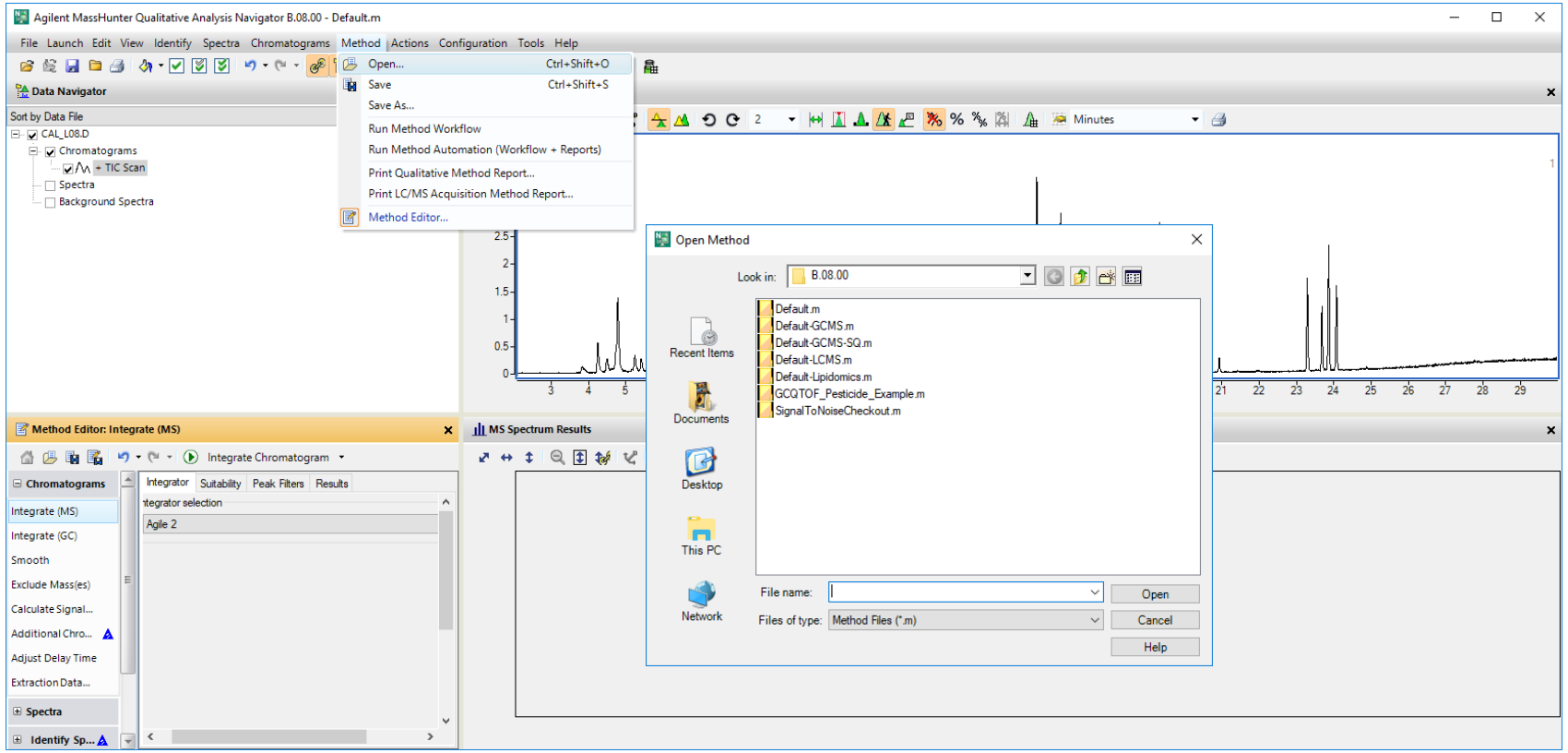
Initially use **Open Data File** as normal to view data file being acquired.

Then use **Refresh Data File** to update the view and add the most recently acquired data.

Refresh Data File is only active if the file is being acquired.

Similar application use for the GC/MSD ChemStation, where it is called SnapShot.

Qualitative Analysis Methods

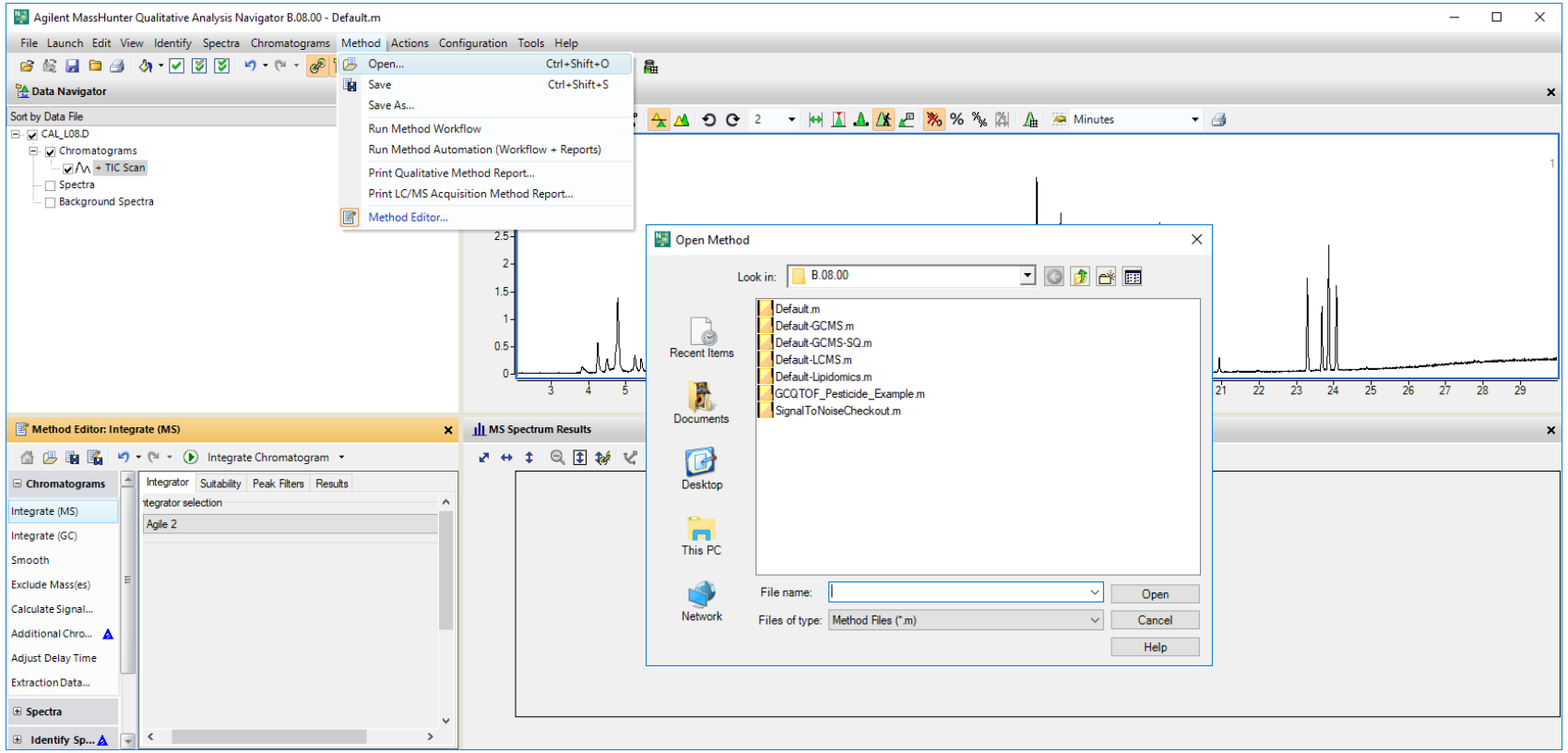


Qualitative Analysis Methods are stored in a .M folder.

Many application & instrument specific methods, generally use the method specific to the dataset..

Default.M is read-only, after editing “Save As” to a customized method.

What is a Method? Unified Method Concept

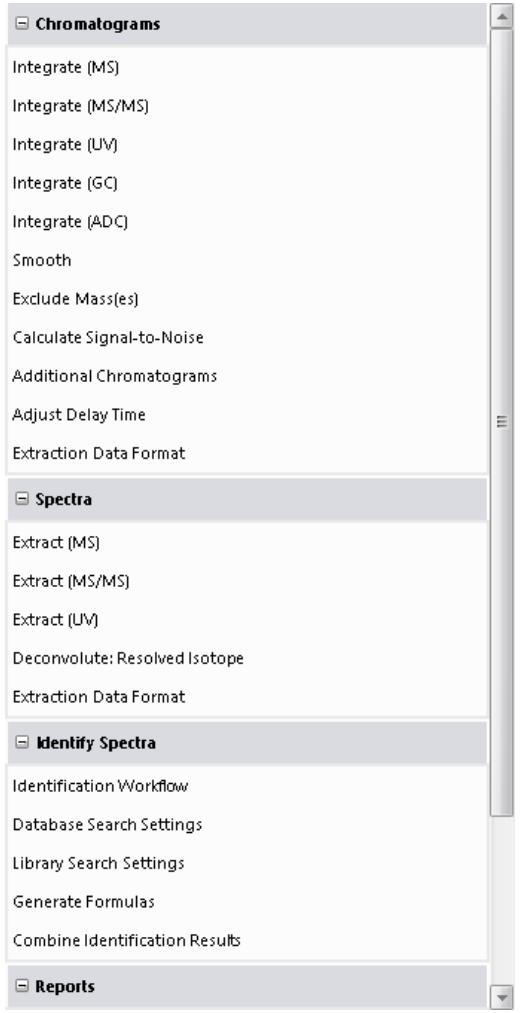


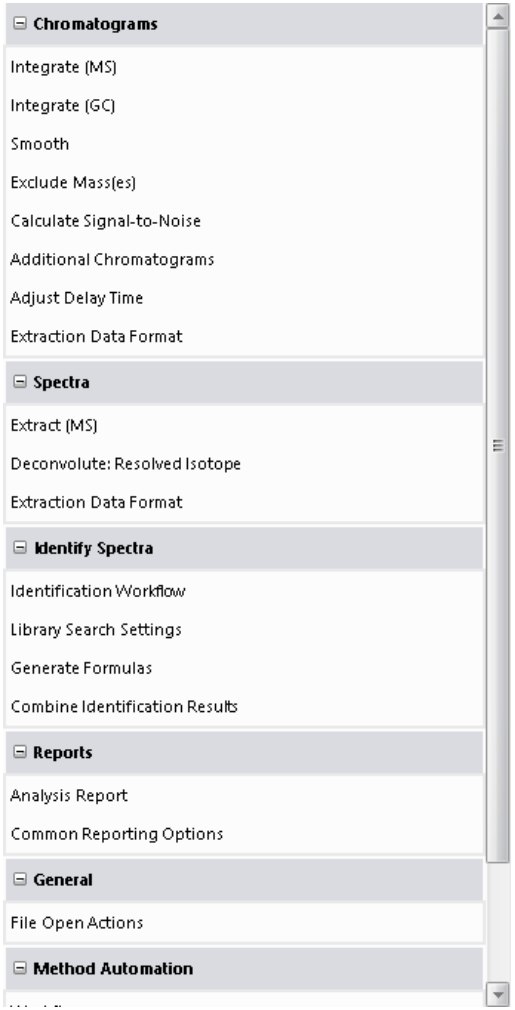
Qualitative Analysis Navigator and Workflows Methods are stored in a .M folder.
Quantitative Analysis Methods are stored in a .M folder.
Quantitative Analysis Reporting Methods are stored in a .M folder.

Unified method can now be automated to run from the sequence/worklist.

Method Editor

Adaptive User Interface– simplifies Method Editor





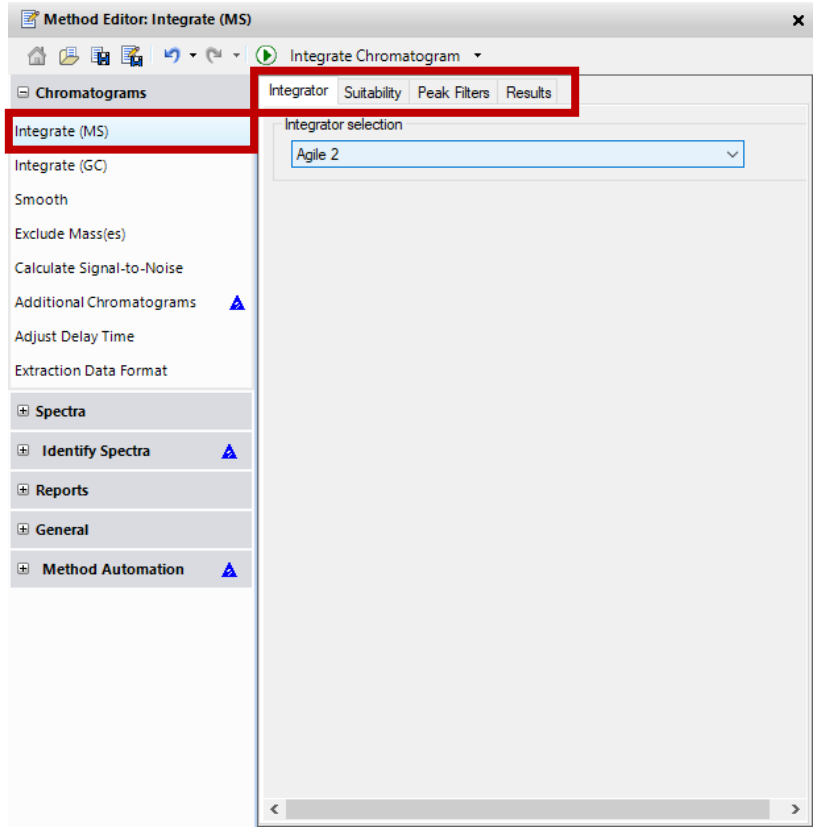
No Data File Loaded | GCMS Data Loaded | LC QQQ MRM Data Loaded

Method Editor

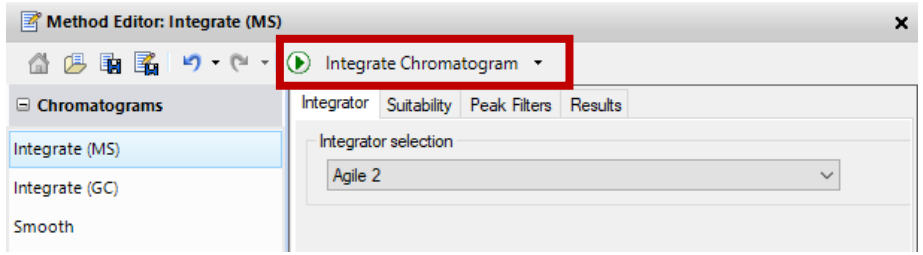
View > Method Editor

Expand nodes on the left side and select a section to edit.

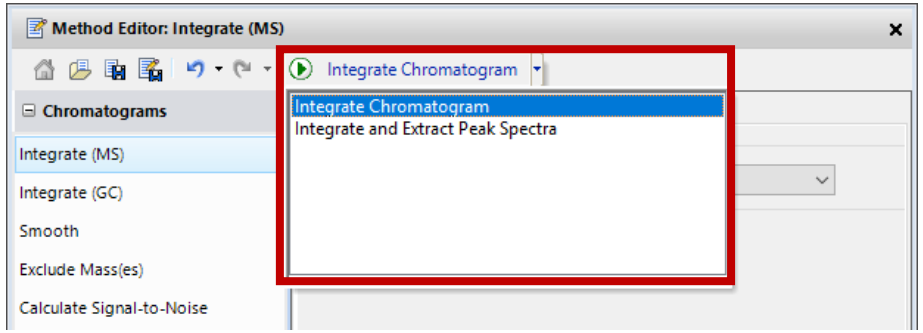
Tabs within a section further organize method parameters.



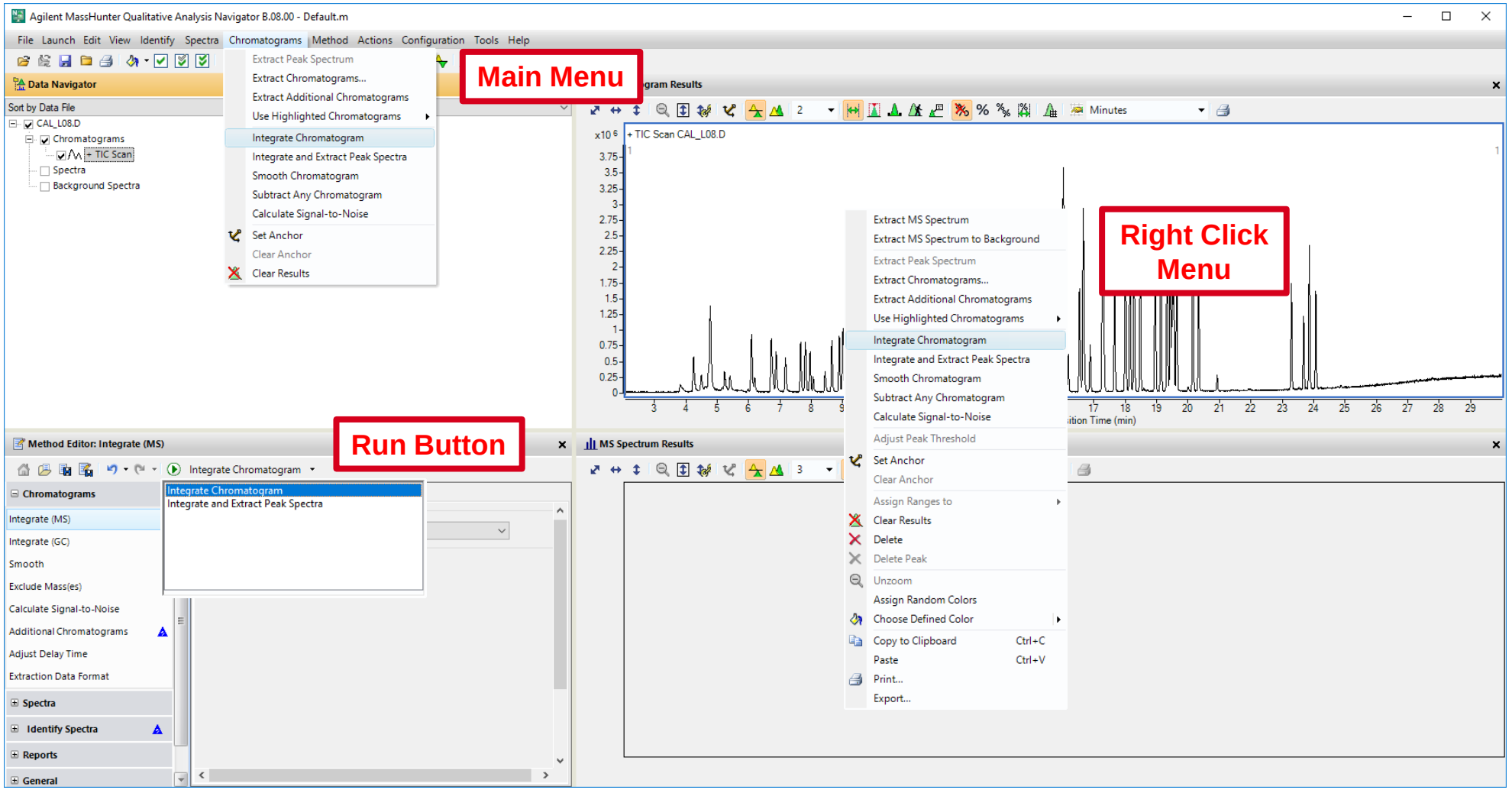
The “Run” icon executes the function associated with the selected part of the method.



In some cases the “Run” icon can have different actions, a drop down list will them display them for selection.

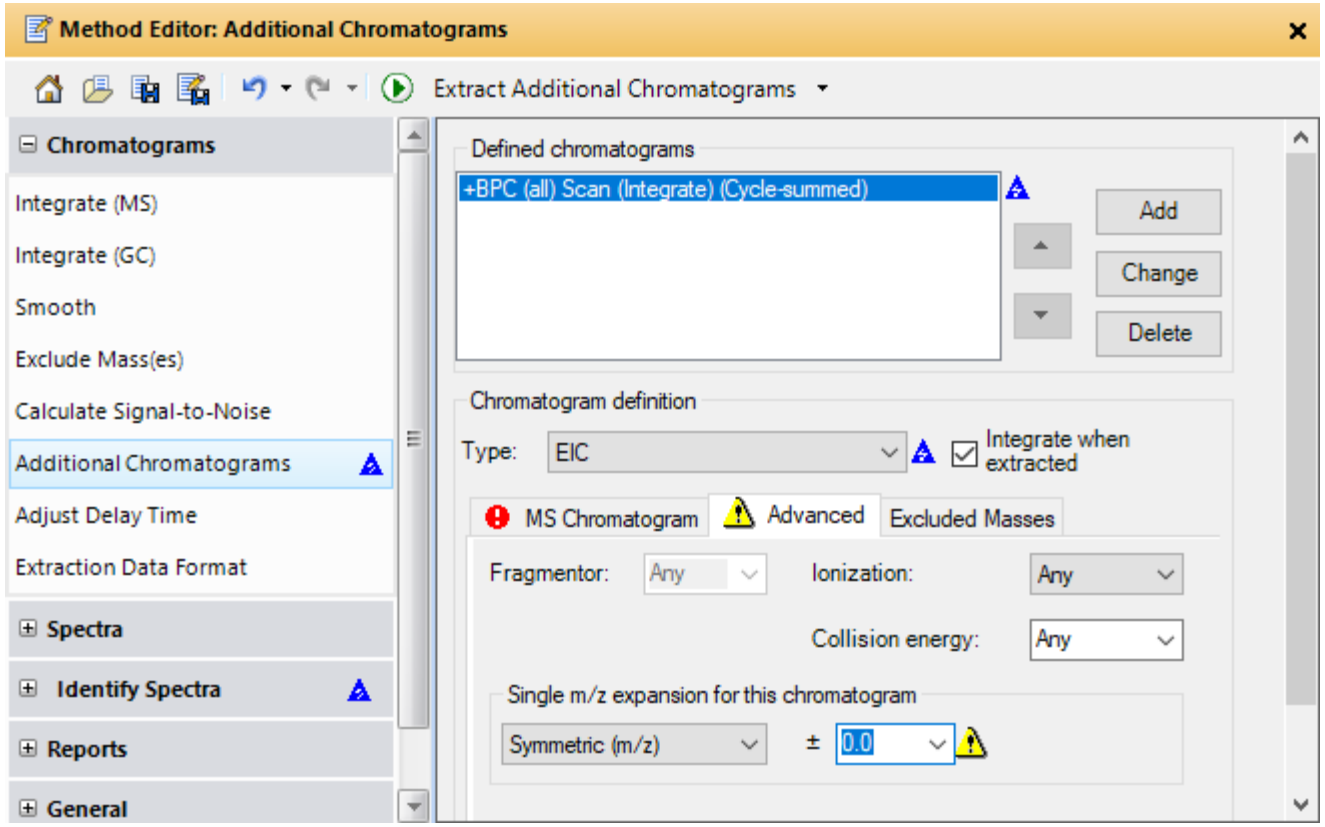


Relationship Between Action and Method Editor



Set parameters for action in Method Editor. Then, perform action.
Note : The action will be performed on ALL selected (highlighted) items!

Change and Error Icons



When you make a change to the current method the change is marked. In addition, all other functions that are affected by this change will be marked. Save the **method to remove the icon.**

An invalid value has been entered into a field. The field will reset to the last valid value it contained.

Additional information is required. The error must be fixed before the algorithm will execute.

Let’s take a moment for questions on Configuration and Methods.

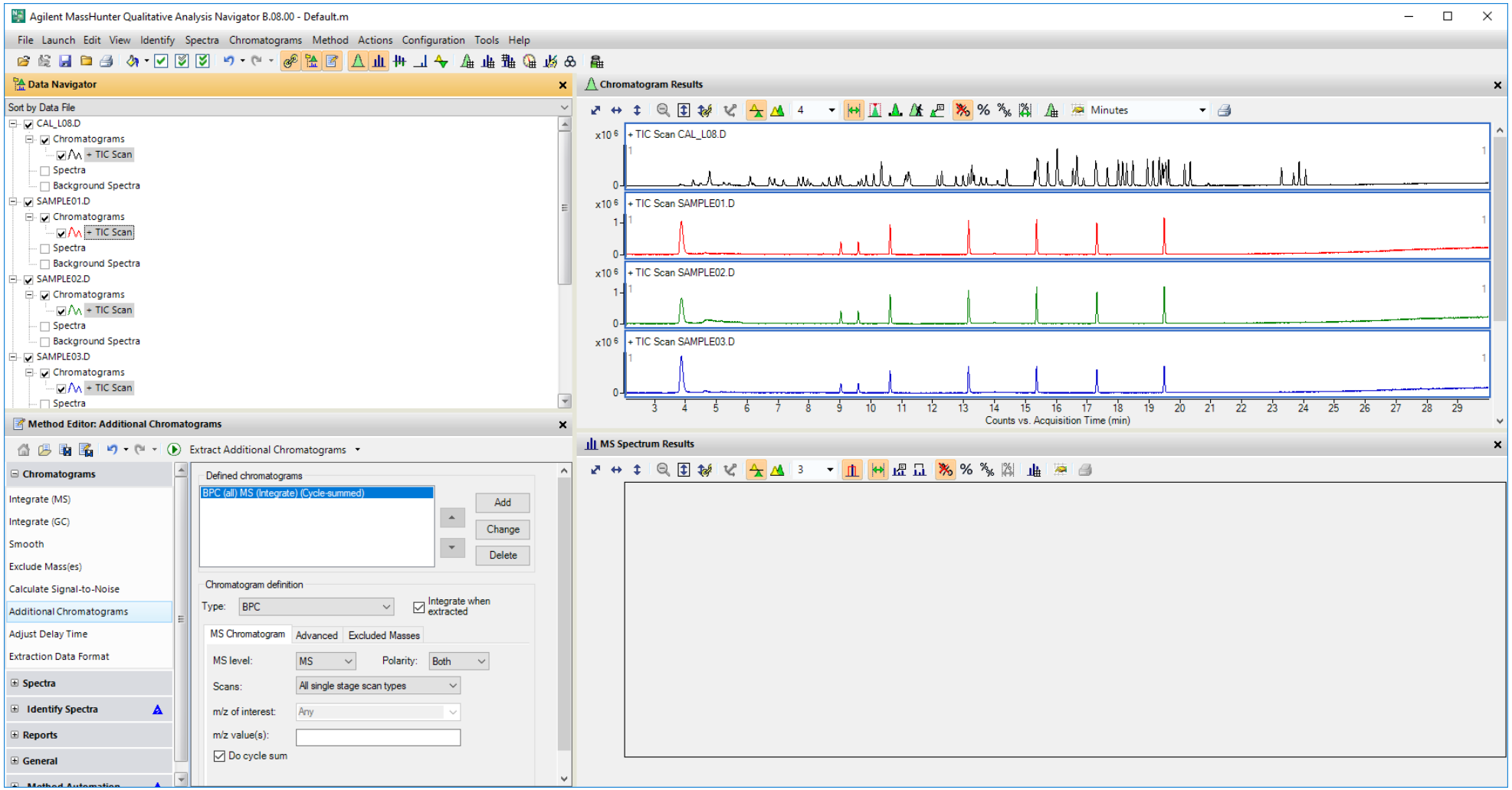
Up Next:

Working with Chromatograms

Working with Chromatograms

- The power of Qualitative analysis is that you can have more than 1 data file open at a time.
- Extract Chromatograms from Data Files.
- Displaying Chromatograms
  - Selecting for display
  - Zooming
  - Scaling
  - Overlay / List mode
  - Anchoring

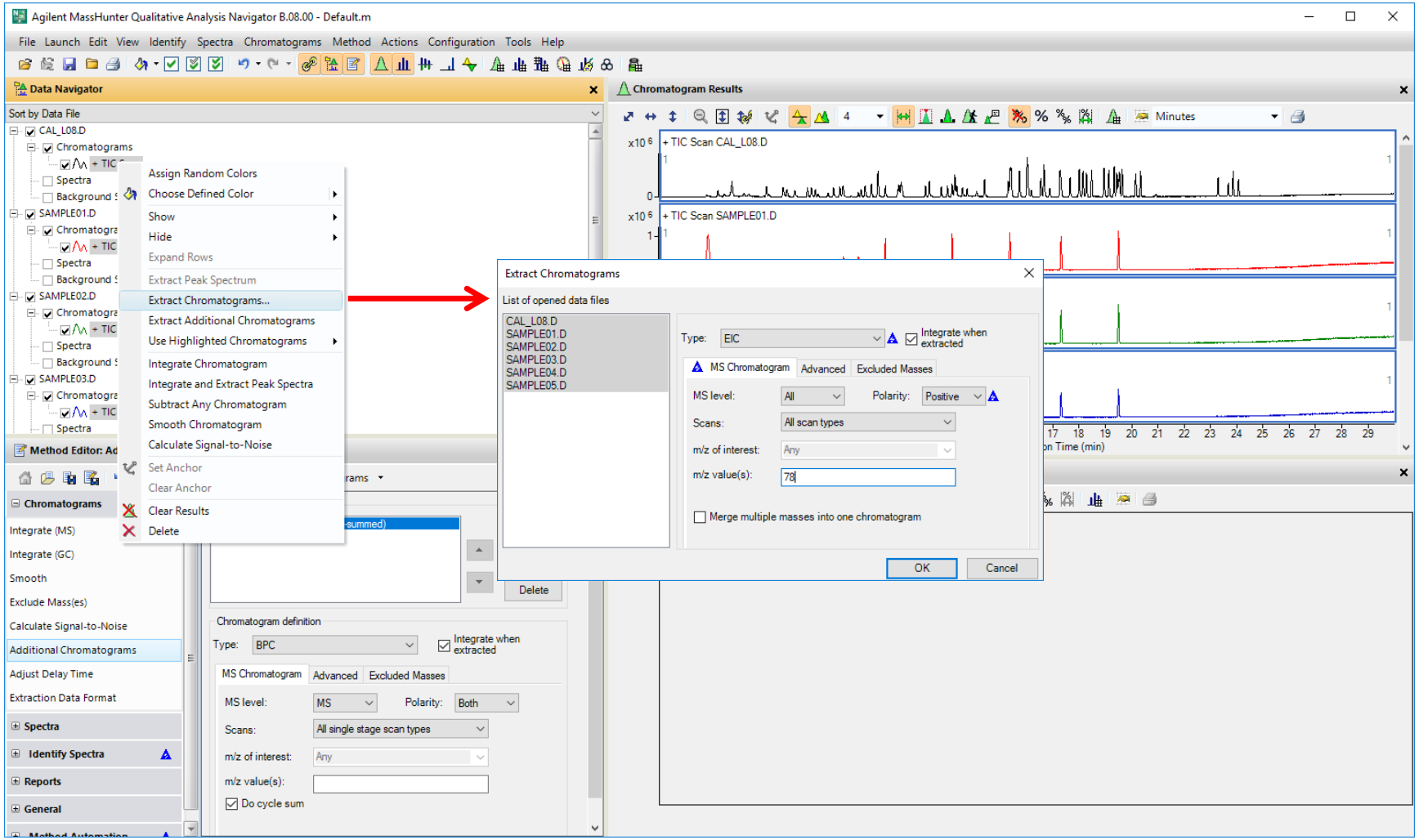
Chromatograms



Can Display:
TOF Data
QTOF Data
QQQ Data
SQ Data
UV data
FID Data

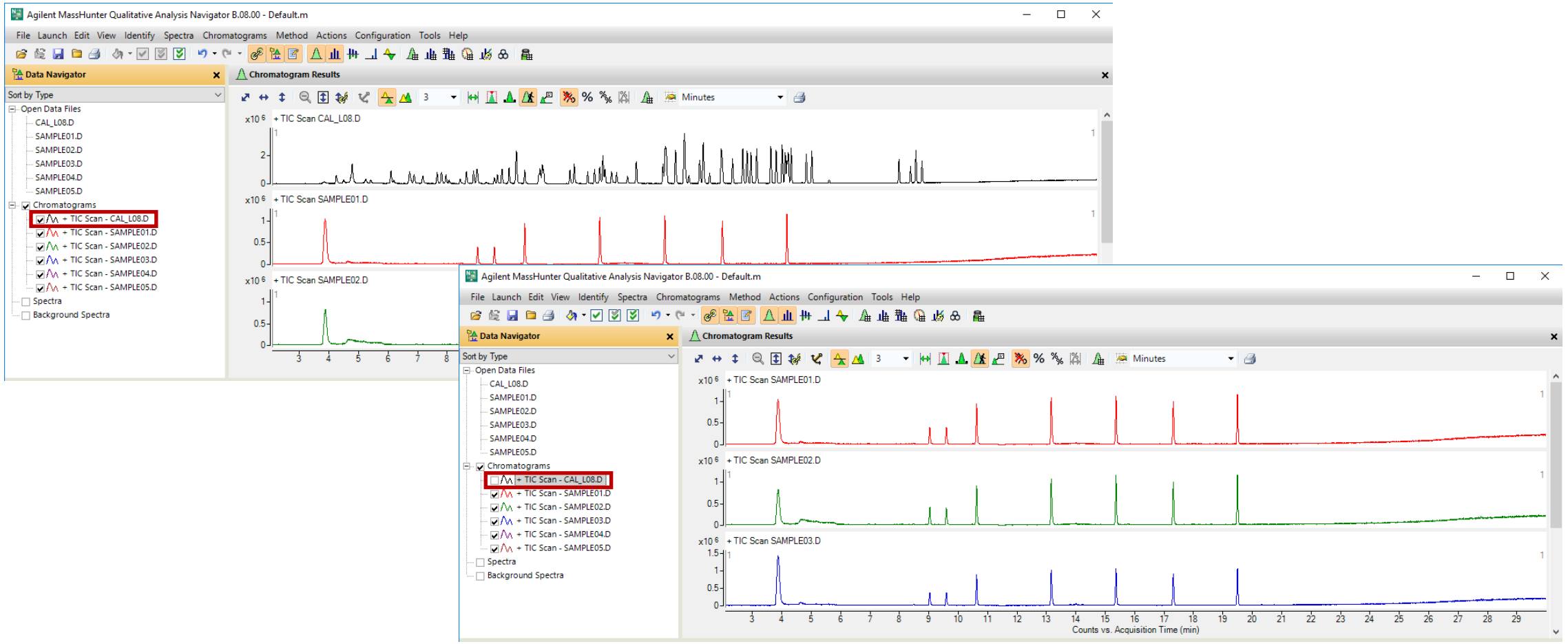
Data Loaded & Displayed

Extract Chromatogram



Available Chromatogram types are determined by data in file.

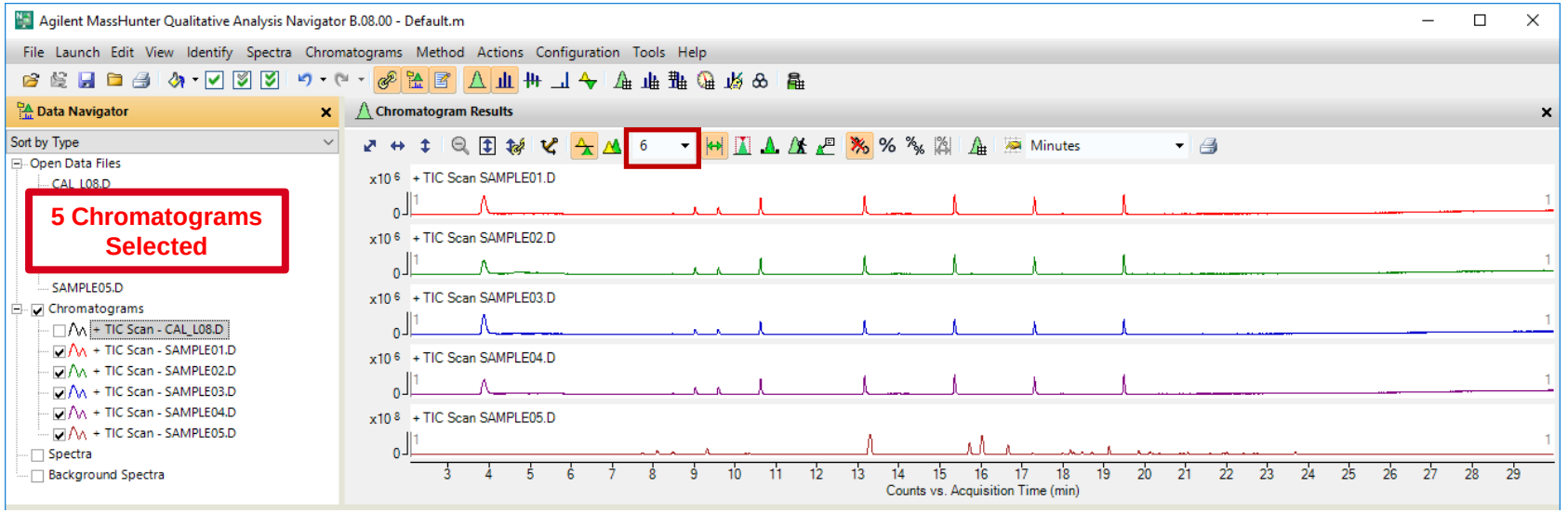
Selecting Chromatograms for Display

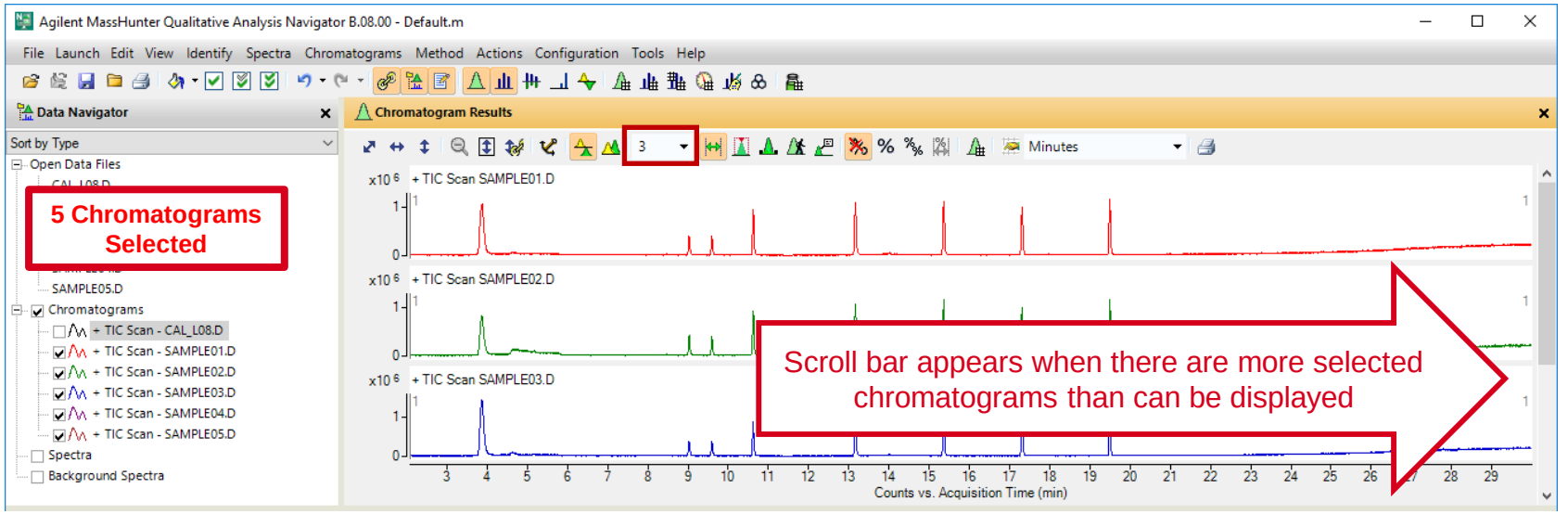


Items in the Data Navigator, like Chromatograms, will be displayed if checked and not displayed if unchecked.

Specify Number of Chromatograms Displayed

Maximum number of chromatograms to display in window, may be fewer.

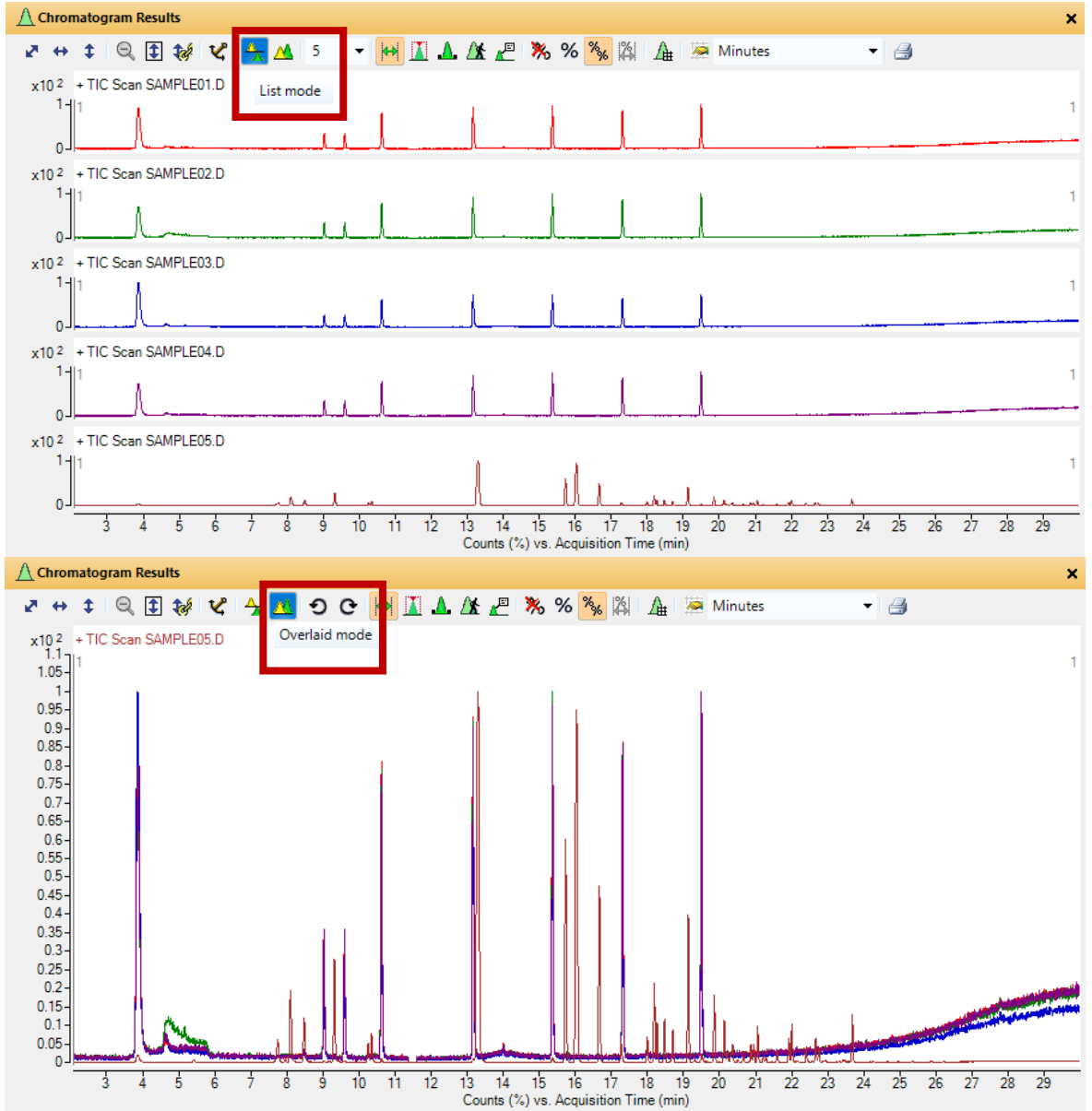




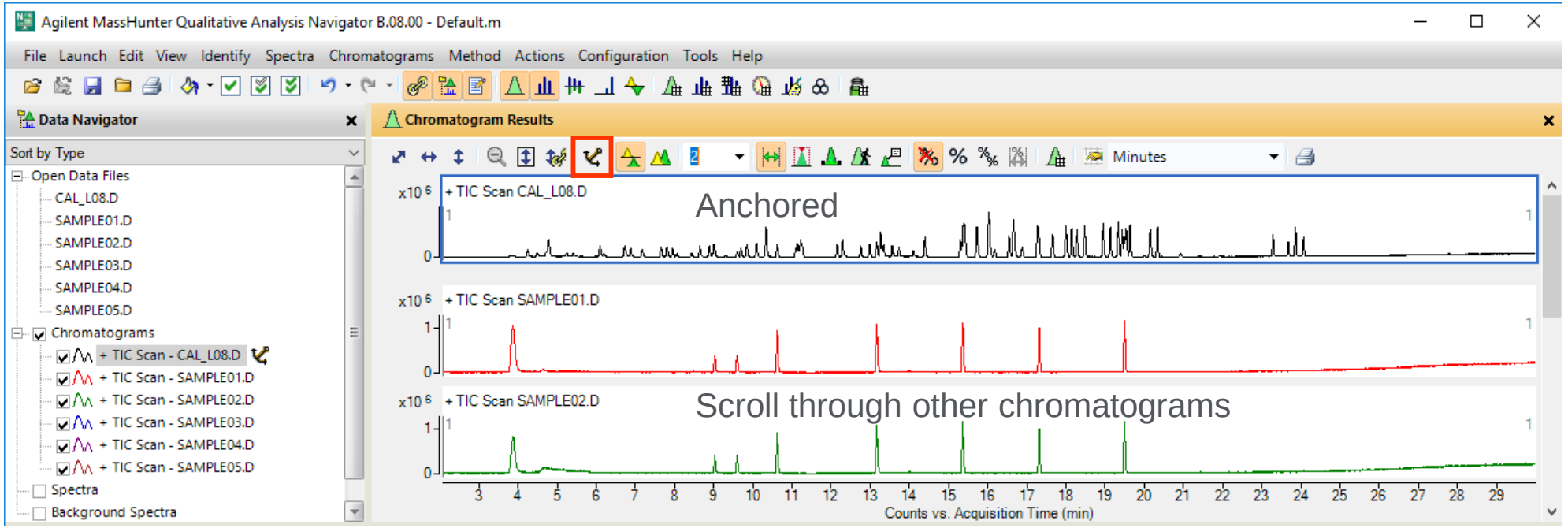
Overlay vs. List Mode Chromatograms

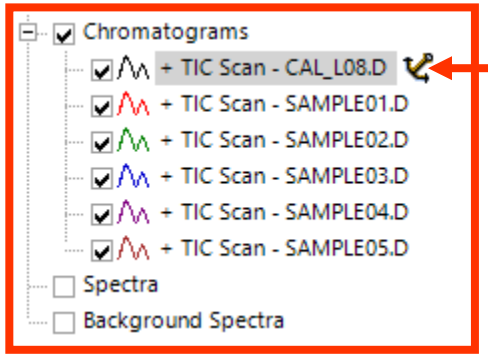
List (Separated)

Overlaid



Anchoring





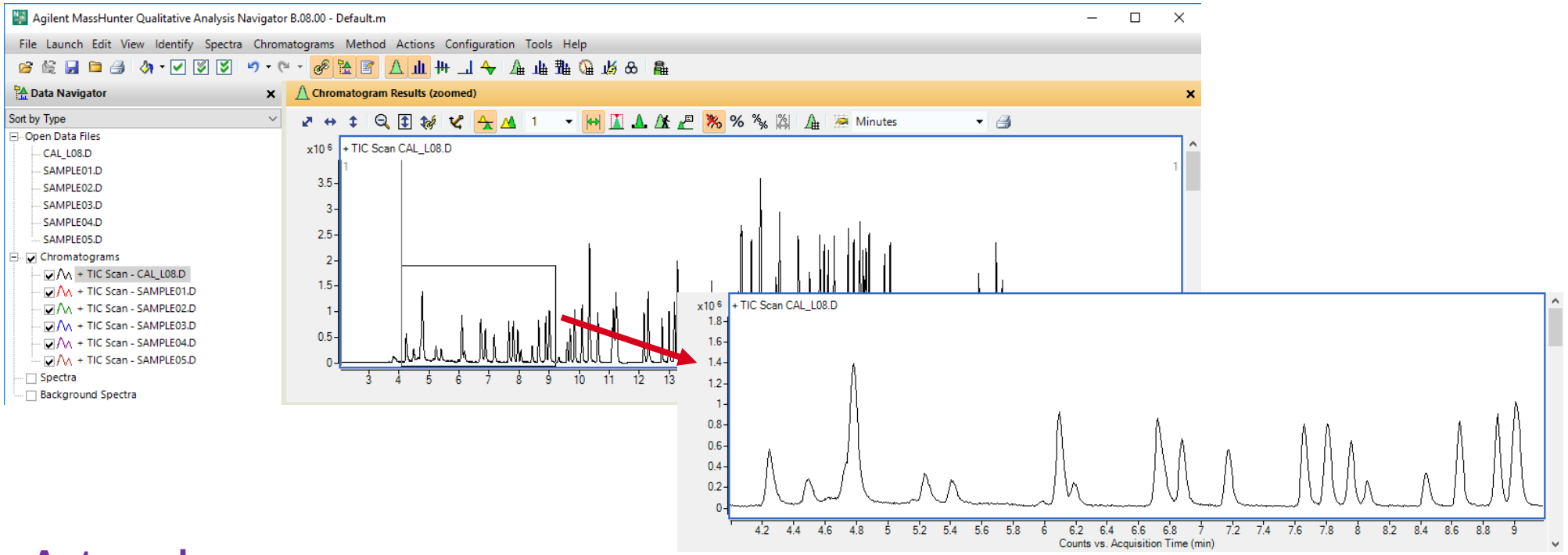
Setting an anchor keeps the anchored chromatogram displayed at all times.

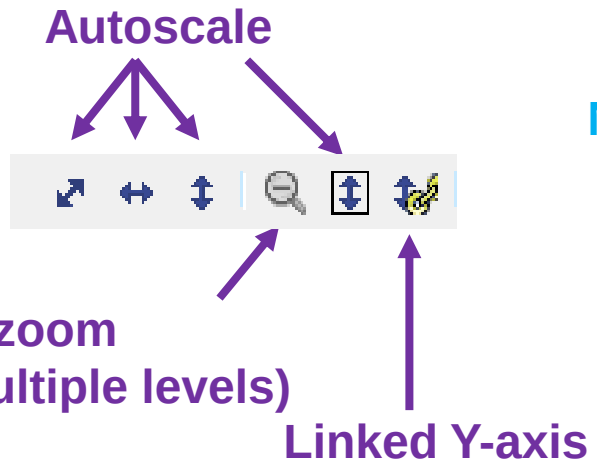
Only one Chromatogram can be anchored at a time.

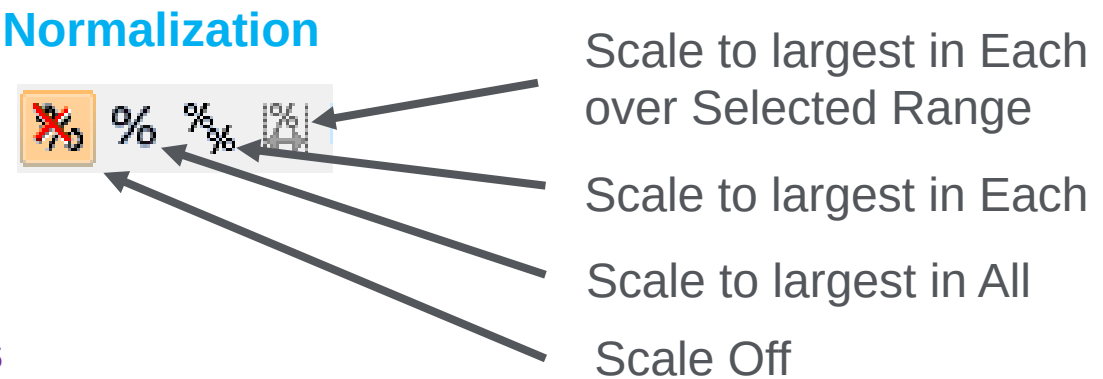
The anchor can be set and cleared from the context menu.

Zooming

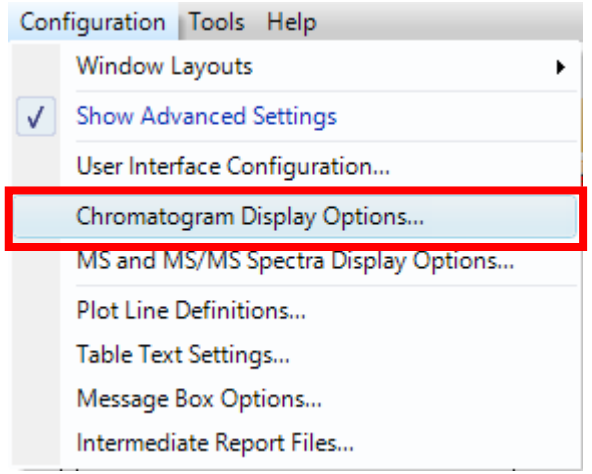
Right-click and drag over the desired area or right-click in the axis and drag to zoom.







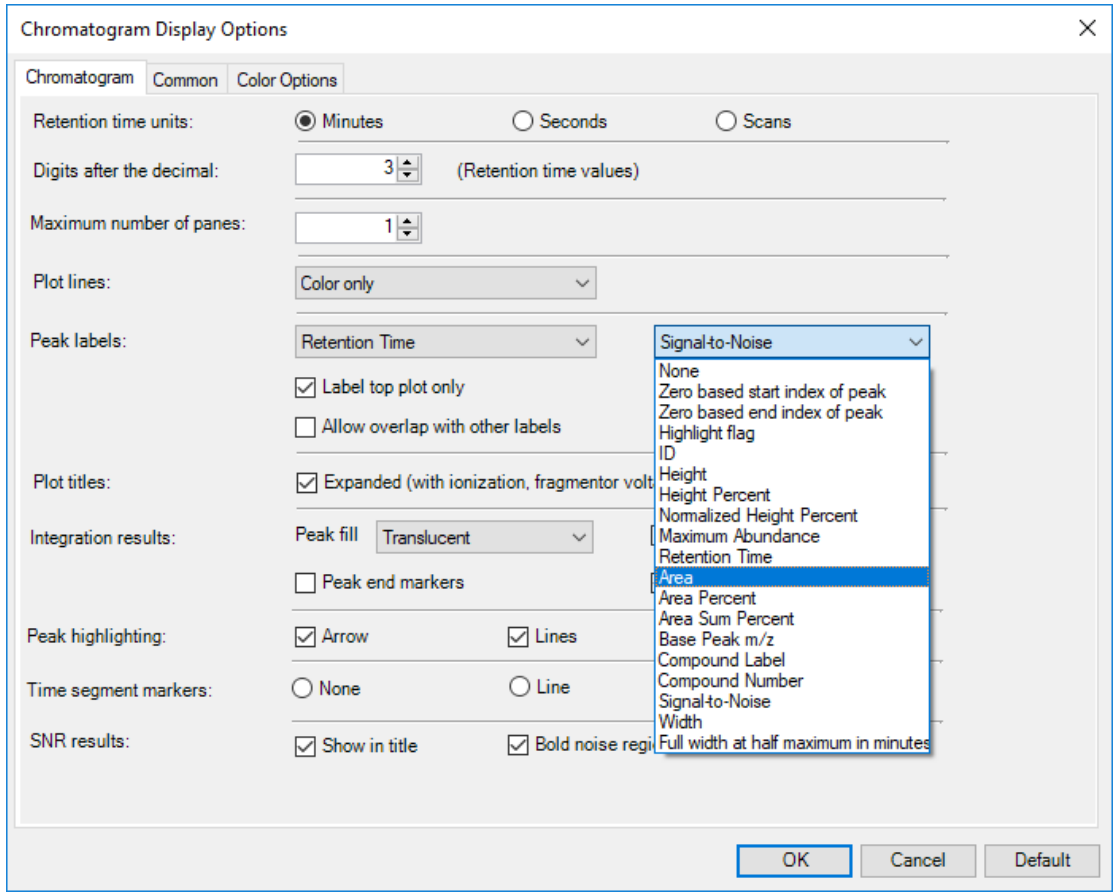
Chromatogram Display Options



Main Menu

Show Advanced Settings is checked.

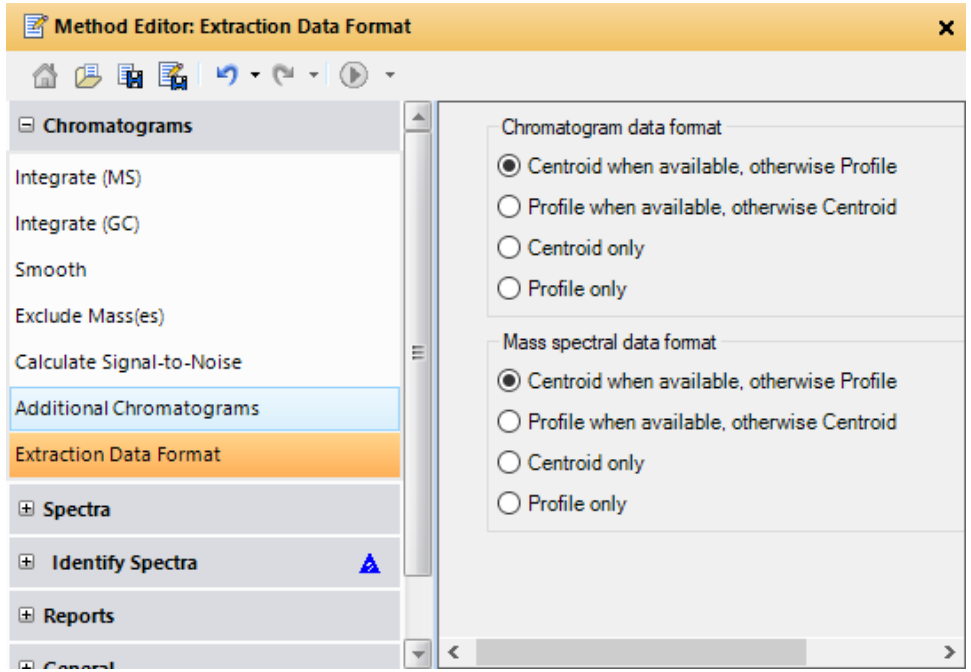
Within Toolbar

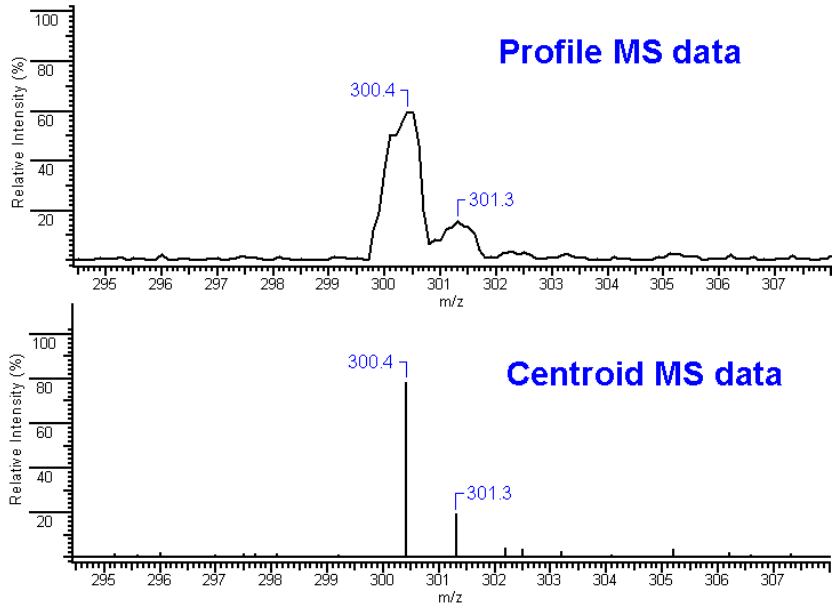


Customize Appearance of Chromatograms

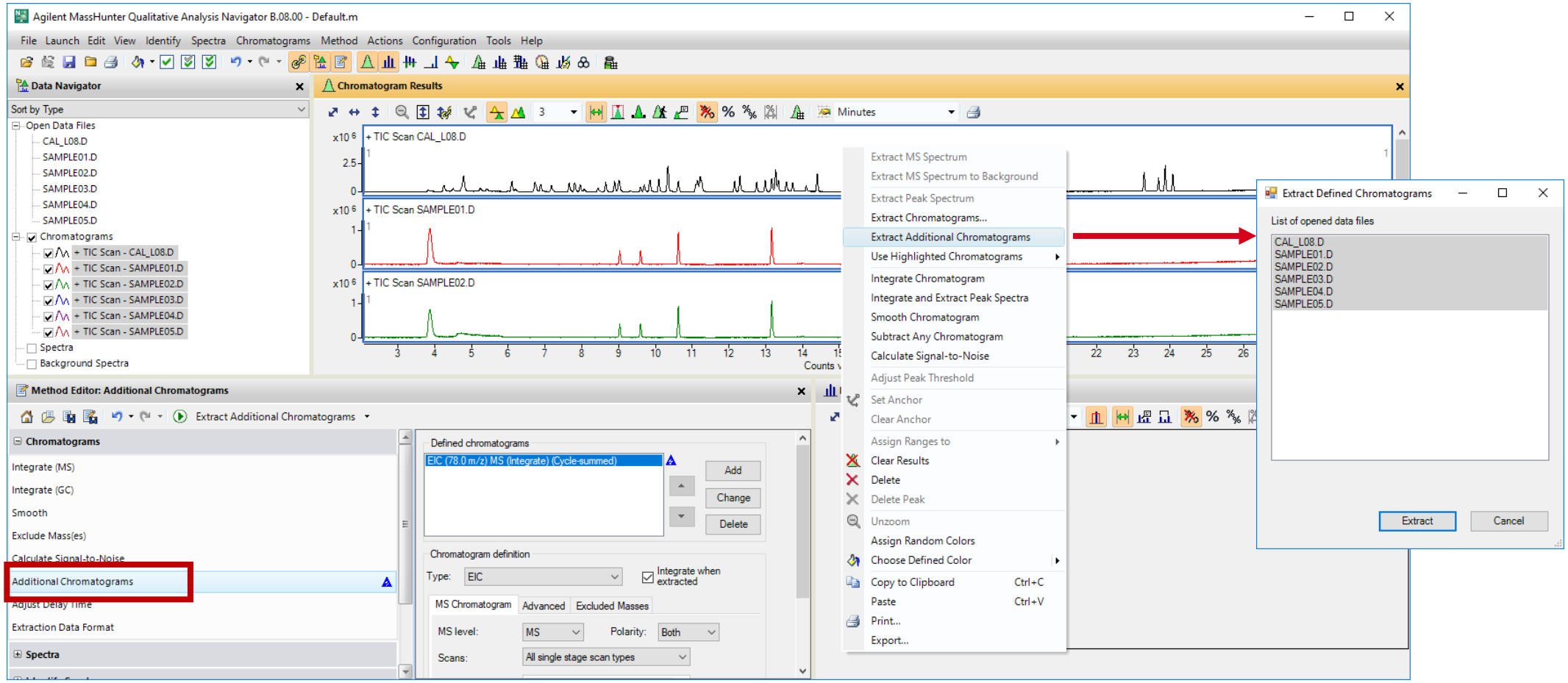
Extraction Data Format - Profile and Centroid

- Data files may contain Centriod, Profile (Raw) or both data types.
- Settings determine which type is used to create chromatograms / spectra.
- Centroid data is the most commonly used, ~10 times smaller than Profile
- Profile is useful for mass peak area comparisons such as when optimizing acquisition parameters, i.e. finding the mass defect or center of mass centroid
- How is Profile Data activated?





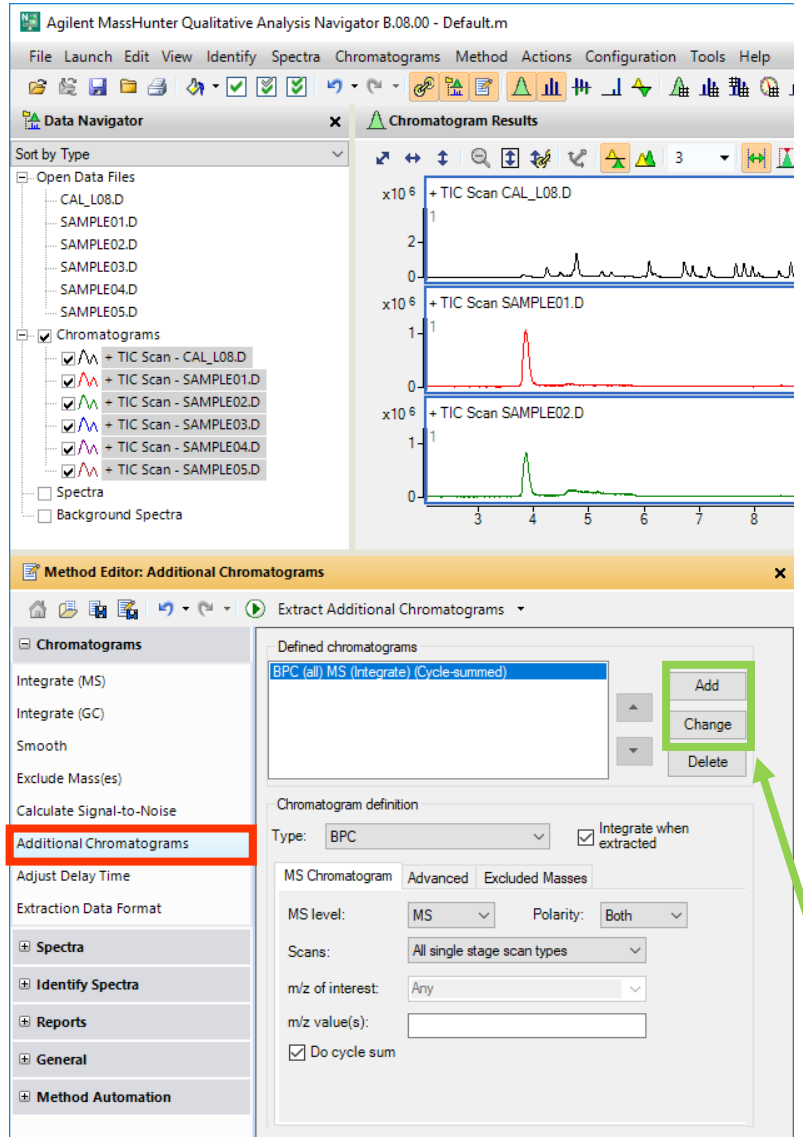
Extract Additional Chromatograms



Software extracts a list of chromatograms which are stored in the Additional Chromatograms section of the method.

List of Chromatogram types is fixed list of all instrument types.

Extract Additional Chromatograms



- Select MS Level based on acquisition scan type.

Types of Chromatograms

TIC – Total Ion Chromatogram

BPC – Base Peak Chromatogram

EIC – Extracted Ion Chromatogram

SIM – Selected Ion Monitor

Other Chromatograms – GC, DAD, ADC

Instrument Curve (LC) - %Comp., Temps, etc.

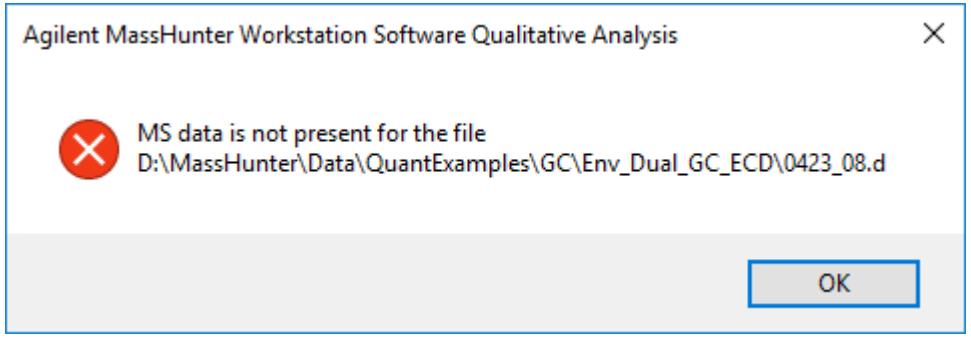
Triple Quad systems only

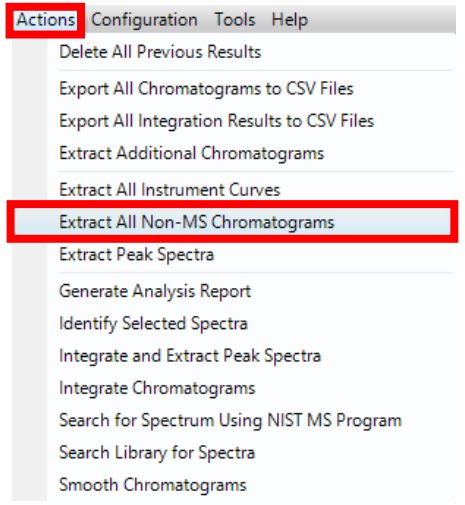
MRM – Multiple Reaction Monitor
pNLC - Precursor Neutral Loss Chromatogram

Tip: Select Change or Add.

Extracting GC, UV and other Non-MS Signals

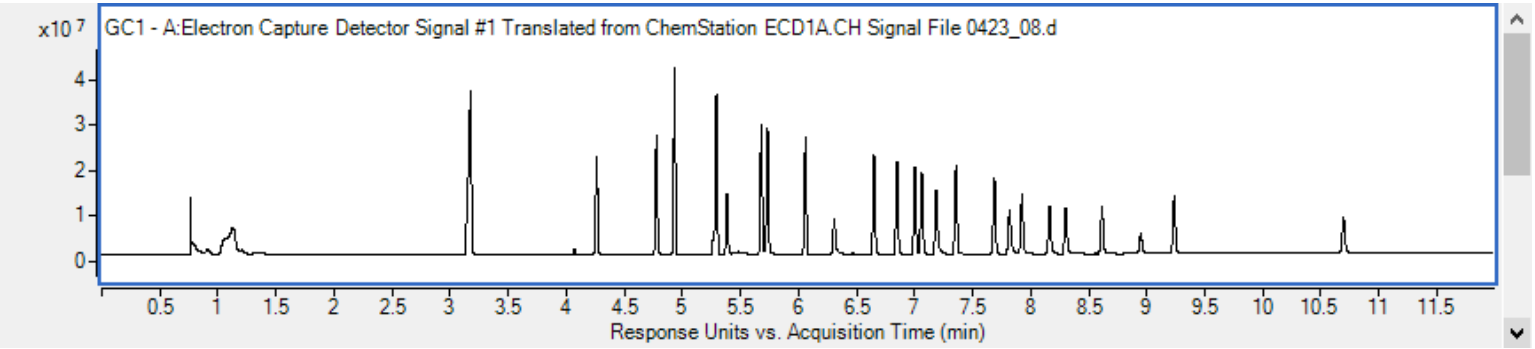
Before SP1, files with only non-MS signals needed to have the signals extracted manually.





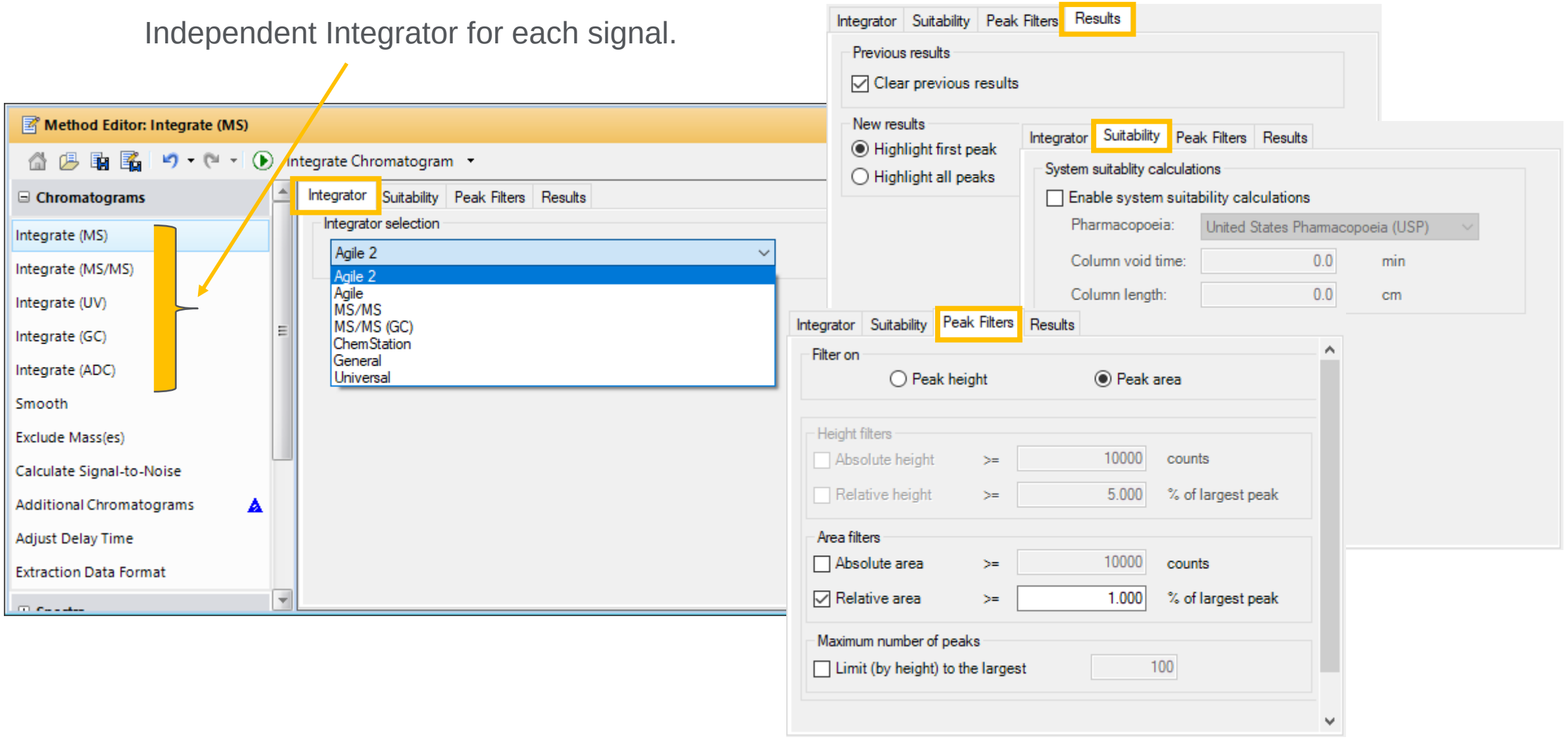
New Feature

With SP1, files with no MS signal automatically extract chromatograms when opened.



Integrate Chromatogram

Independent Integrator for each signal.



Integrators

Agile2

- 3rd generation parameter-less integrator
- Default Integrator
- Better baselines, higher sensitivity to smaller peaks

Agile

- 2nd generation parameter-less integrator

Universal

- 1st generation ChemStation integrator
- Familiar to GC LC ChemStation users

General (RTE)

- Familiar to MSD ChemStation users
- Areas in Universal are 10 time smaller than seen in ChemStation

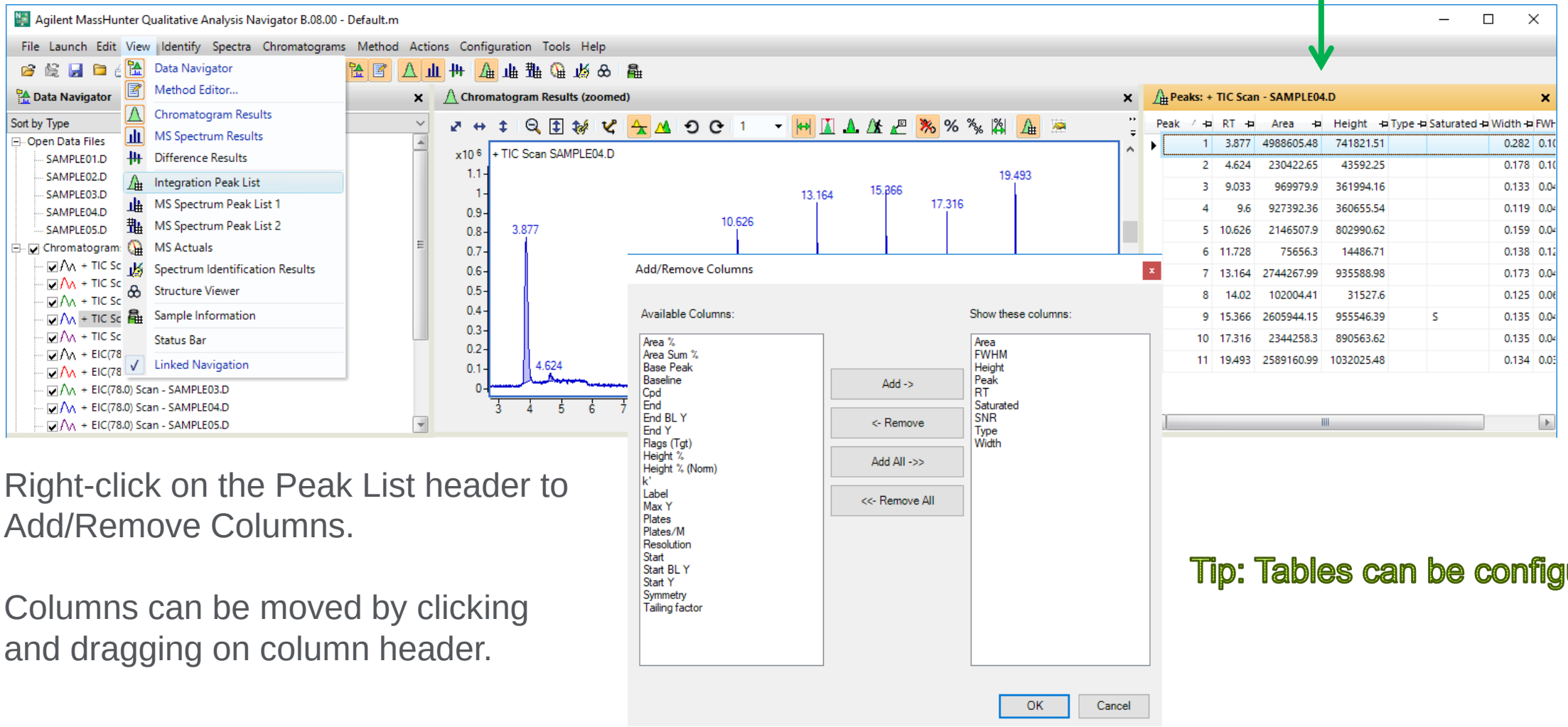
MS/MS and MS/MS (GC)

- 1st generation parameter-less integrator intended for MS/MS systems, not recommended for SQ. Originally required 64 data points.

ChemStation

- 2nd generation ChemStation
- Intended for UV

Integration Peak List

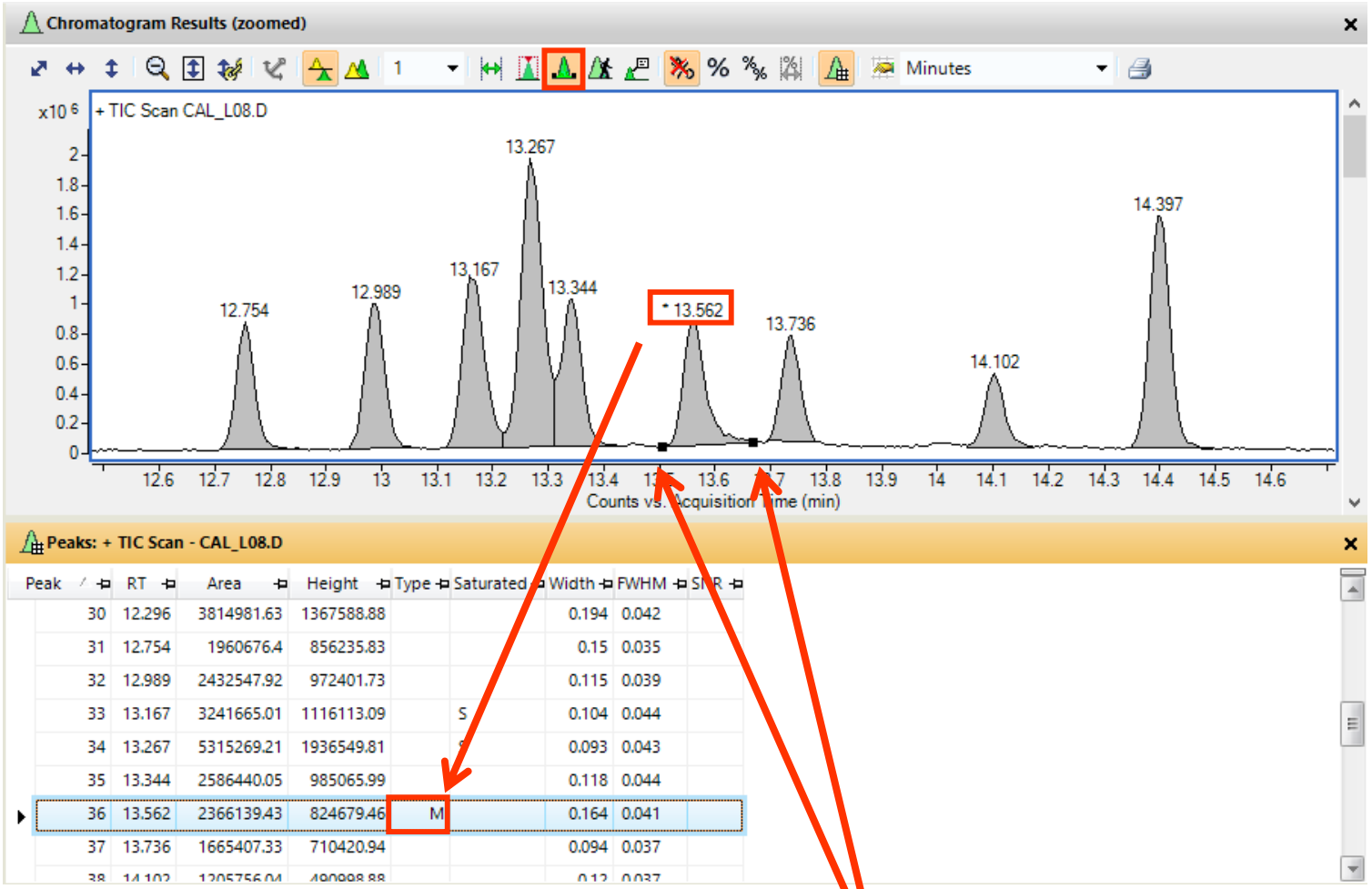


Right-click on the Peak List header to Add/Remove Columns.

Columns can be moved by clicking and dragging on column header.

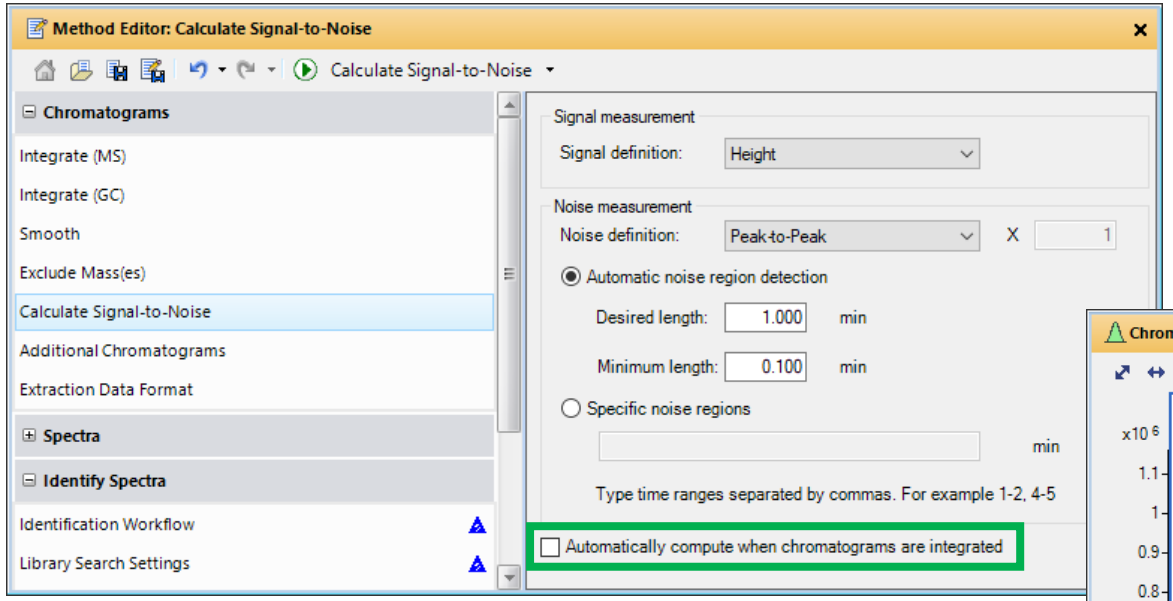
Tip: Tables can be configured.

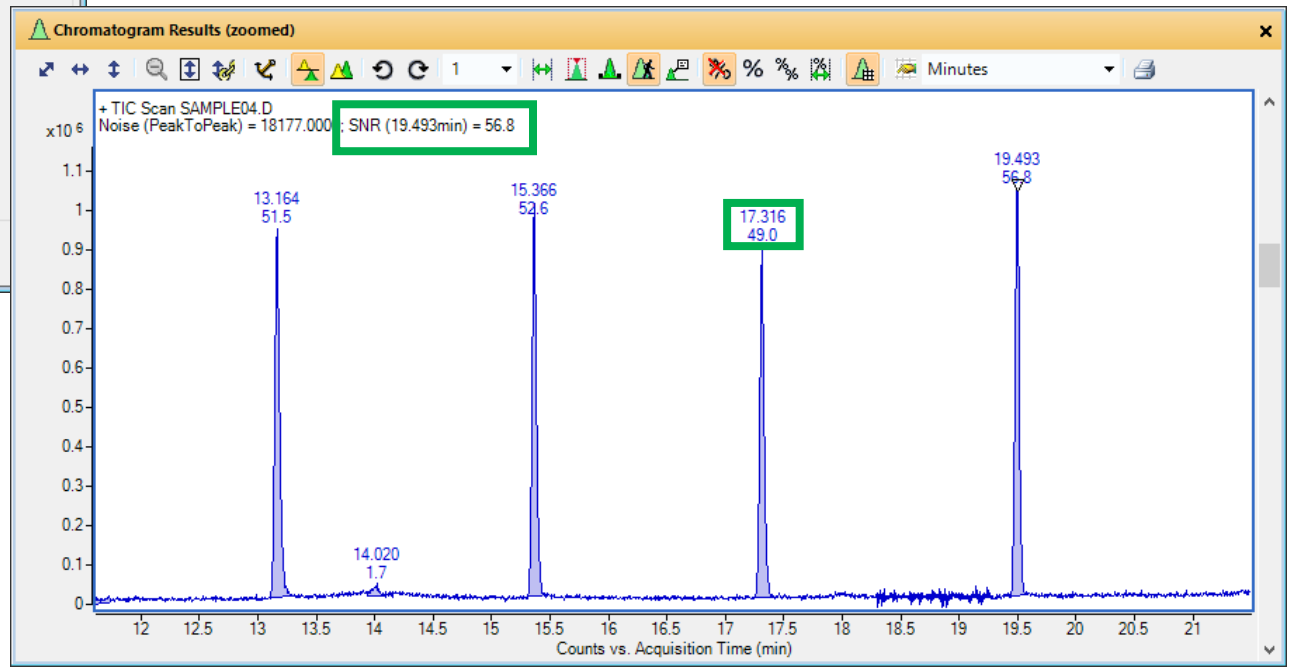
Manual Integration



Use mouse cursor to manually integrate peak.

Calculate Signal-to-Noise
Specific Noise Regions





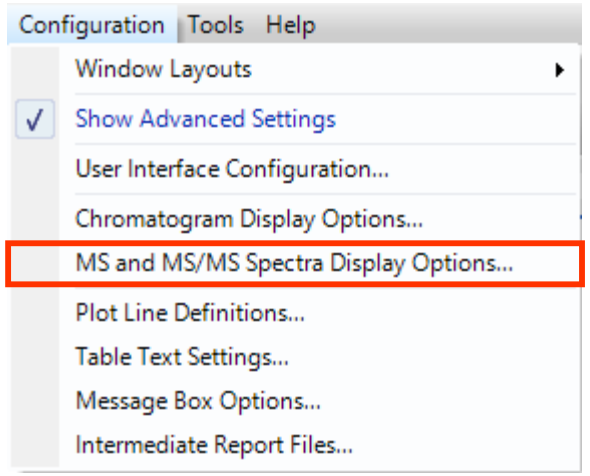
- User defined specific noise regions.
- May be performed automatically when Chromatogram is integrated.

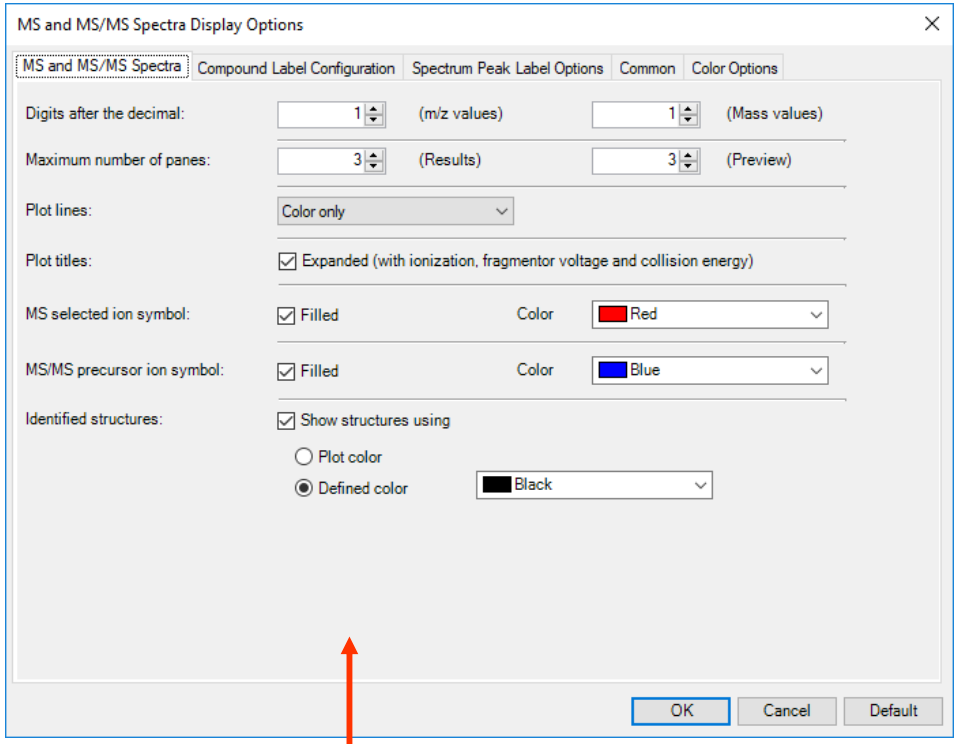
Let’s take a few moments for questions on Working with Chromatograms.

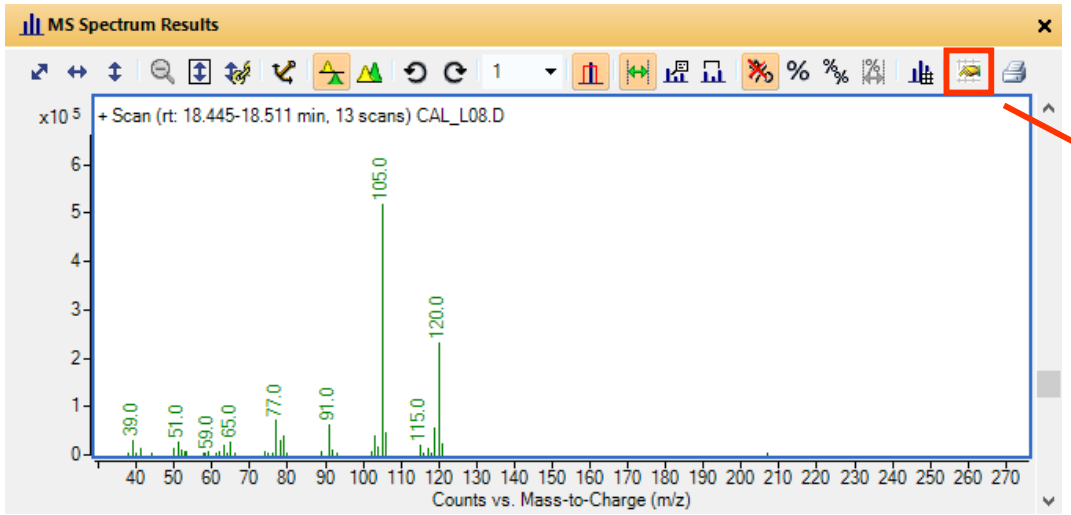
Up Next:

Working with Mass Spectra

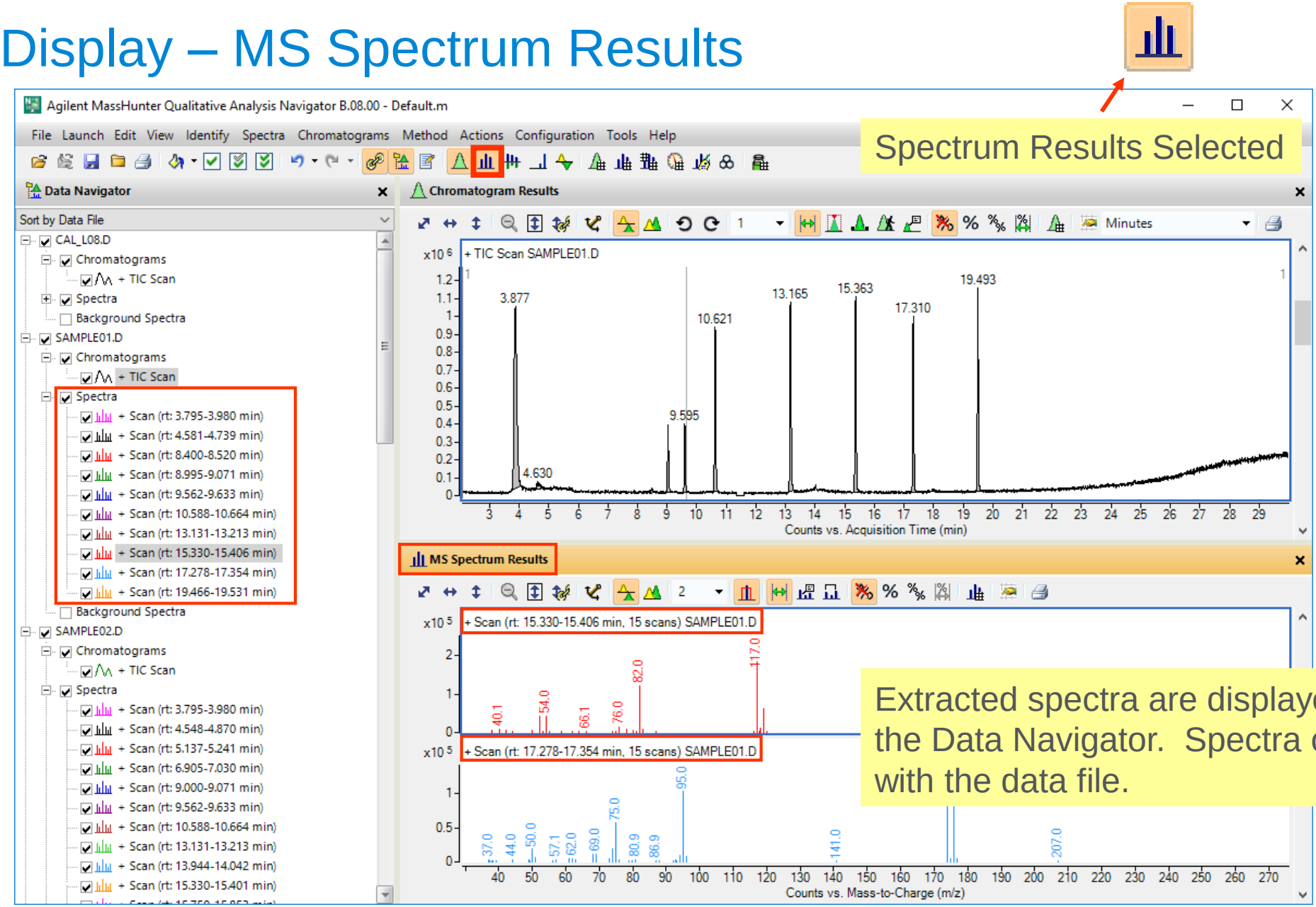
Mass Spectral Display Options



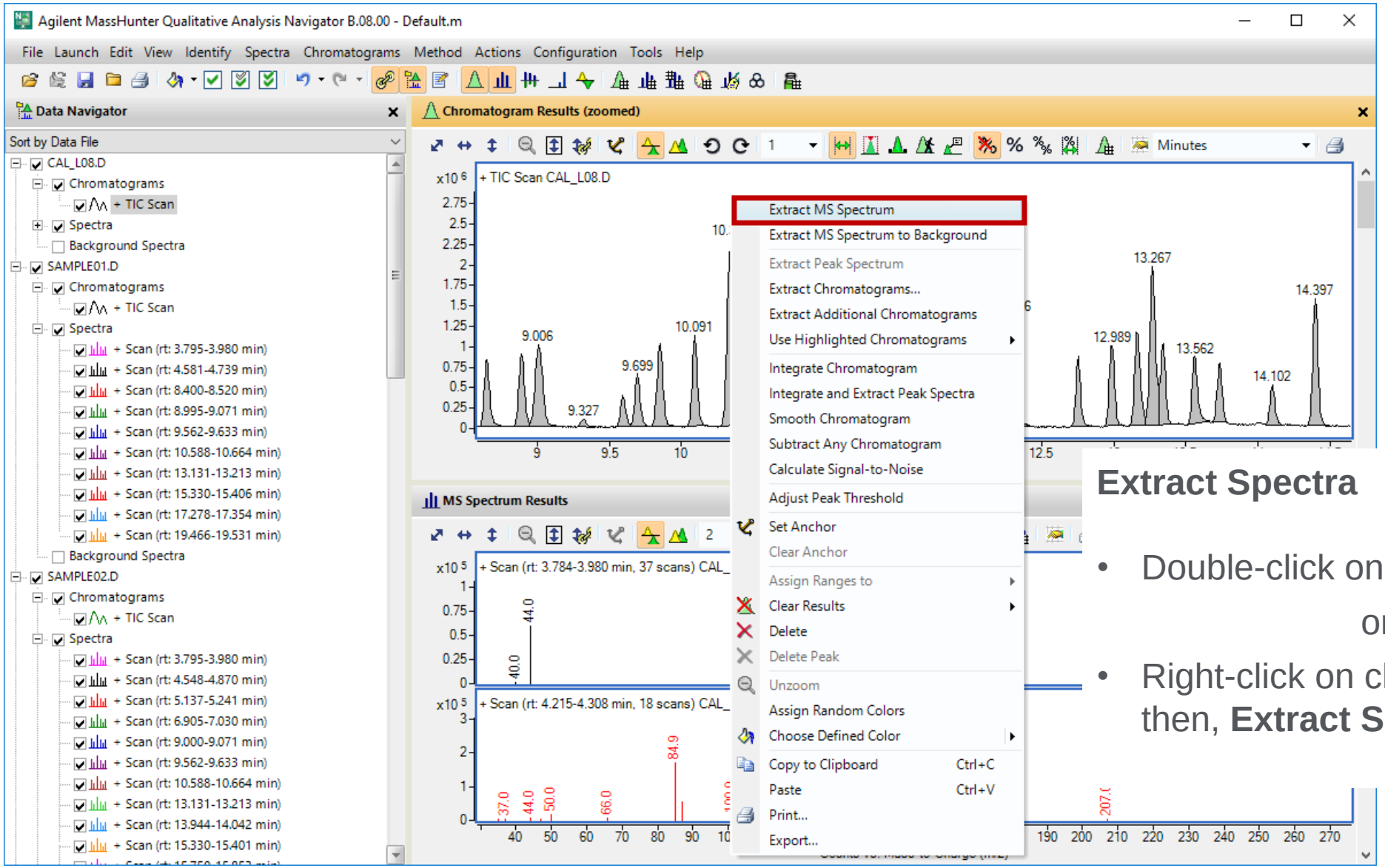




Spectra Display – MS Spectrum Results



Extract Single Spectrum



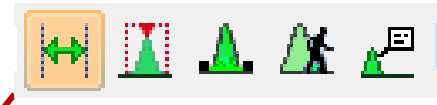
Extract Spectra

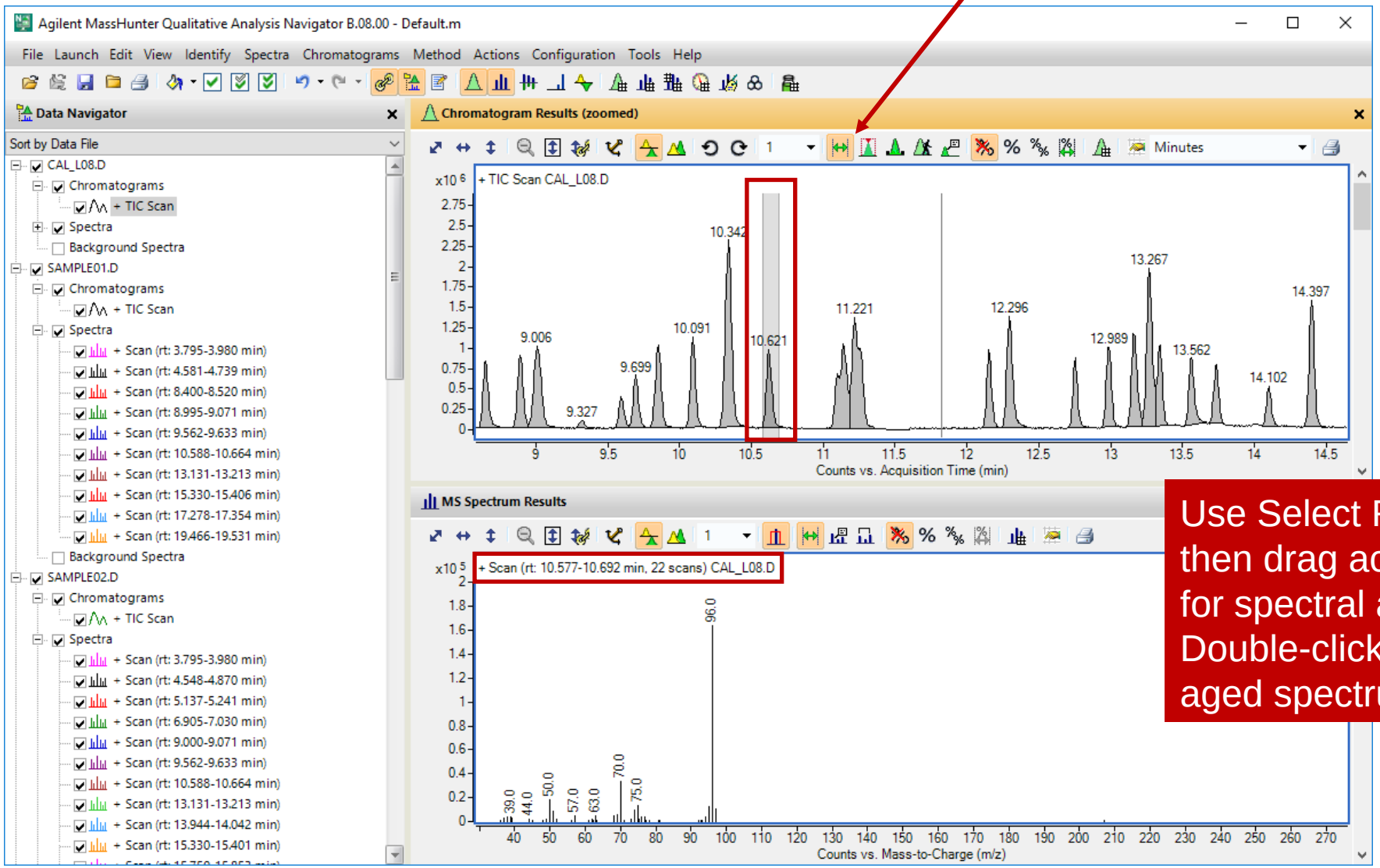
- Double-click on chromatogram

or

- Right-click on chromatogram then, **Extract Spectrum.**

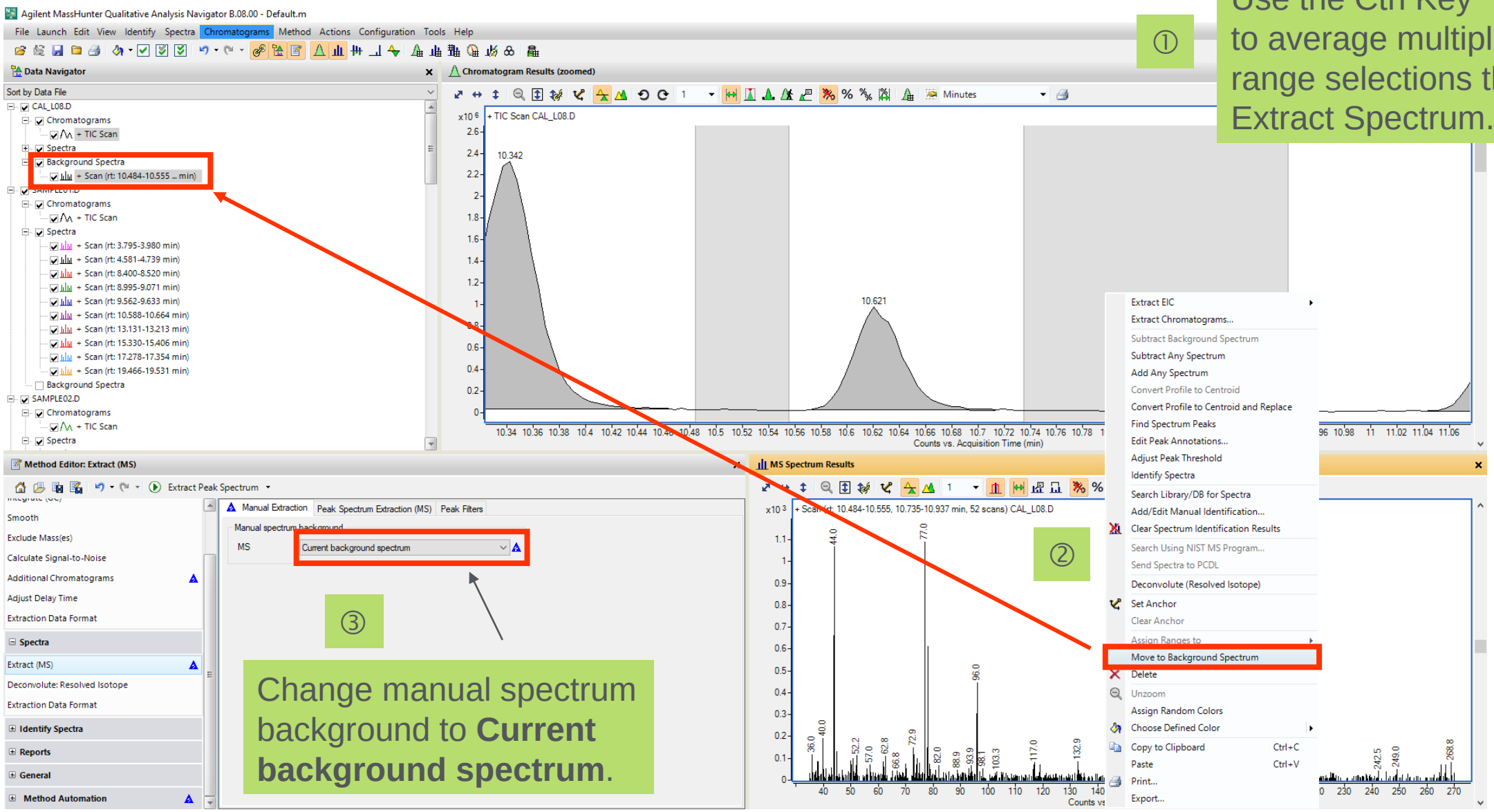
Extract Averaged Spectra



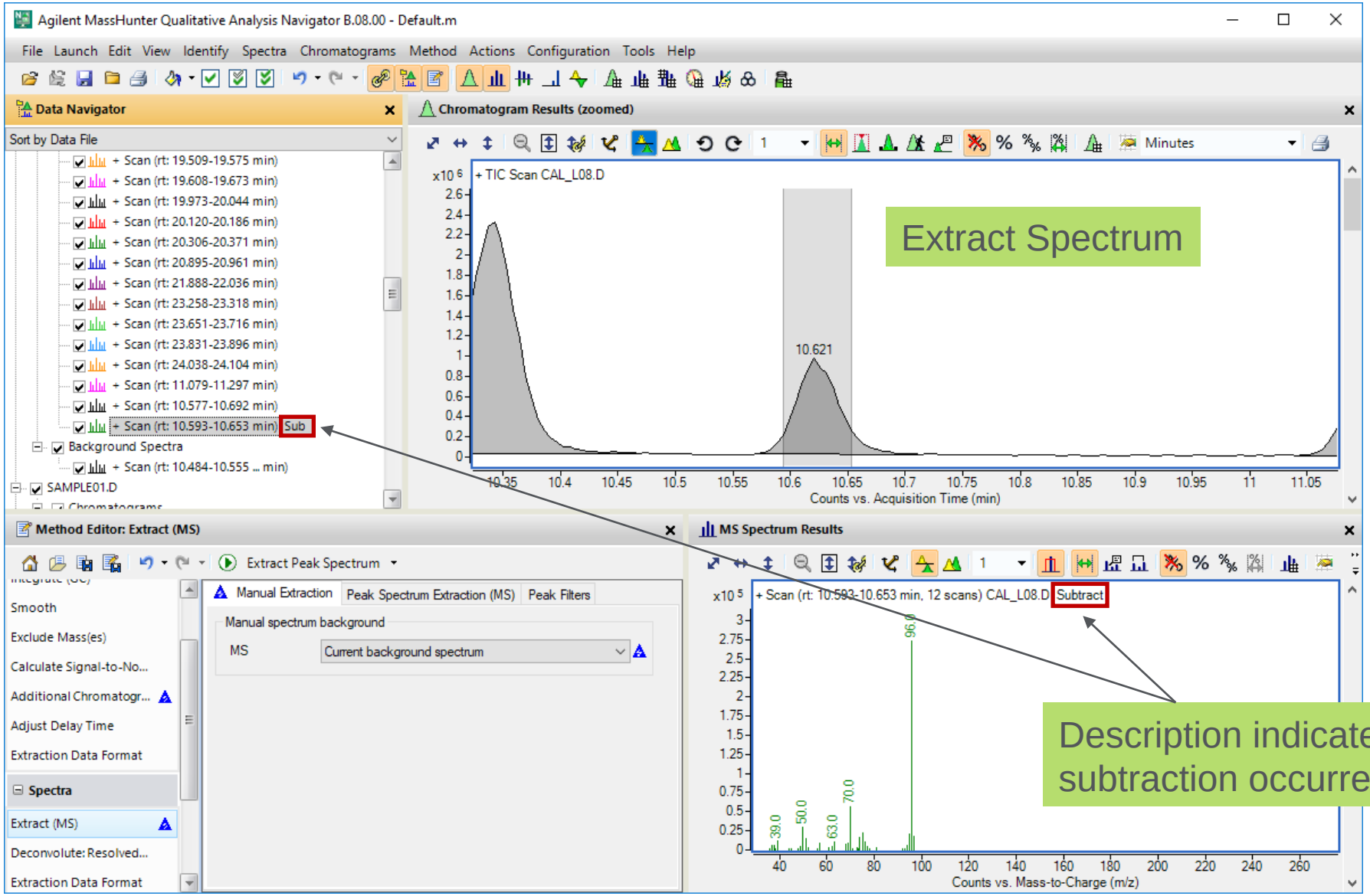


Use Select Range tool then drag across region for spectral averaging. Double-click to view averaged spectrum.

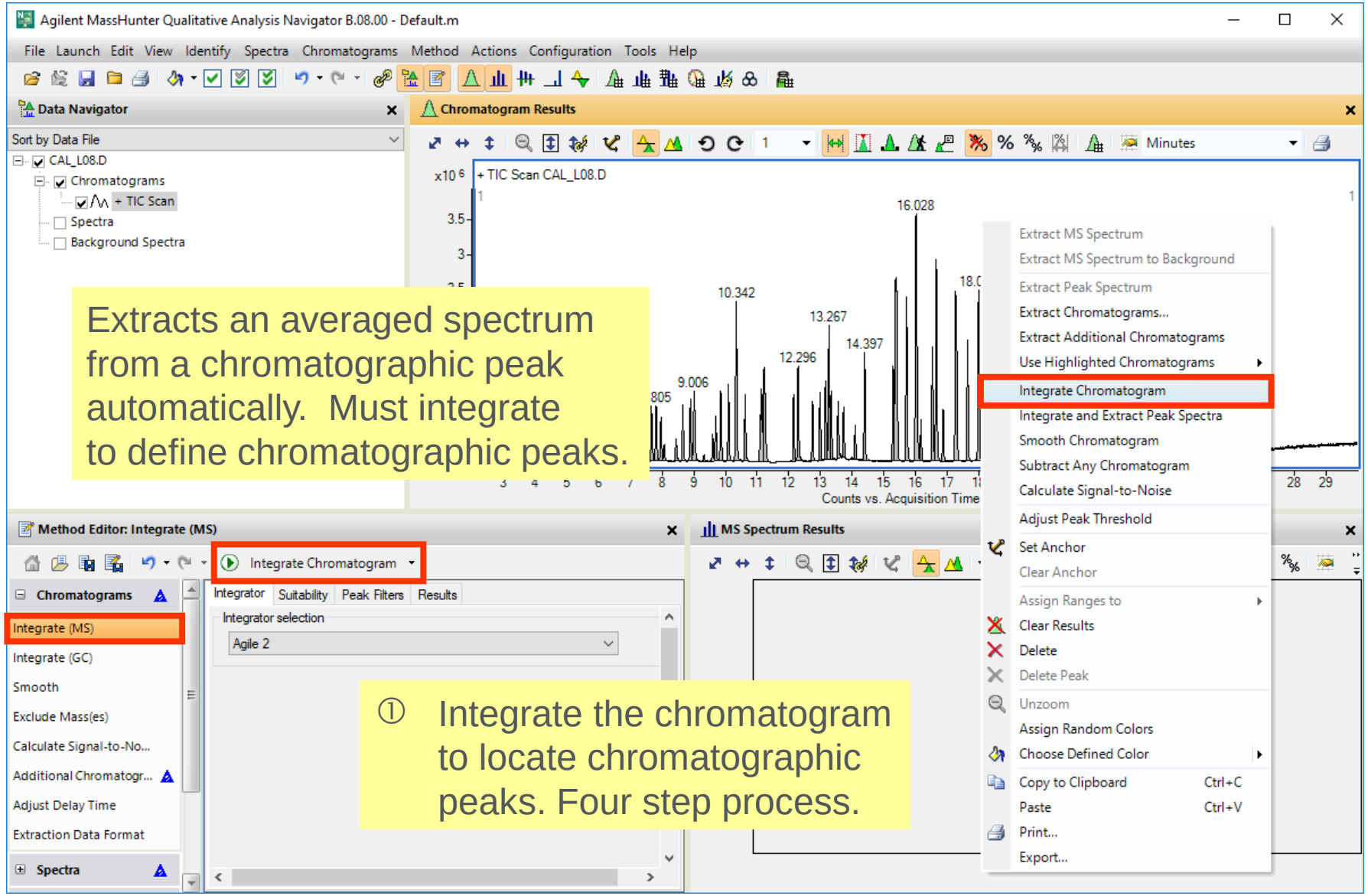
Background Subtraction for Manual Spectra



Background Subtraction for Manual Spectra

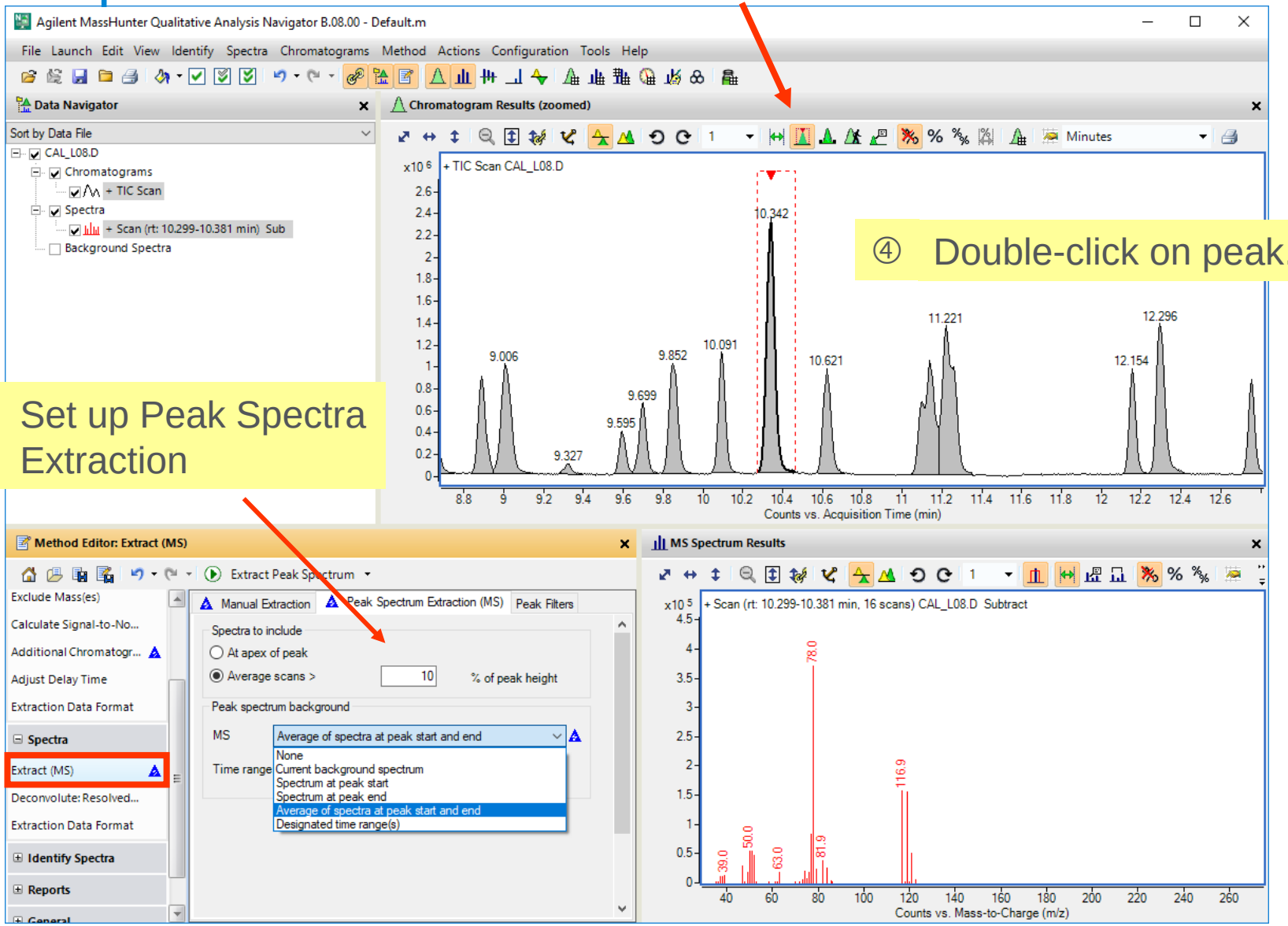


Extract Peak Spectrum

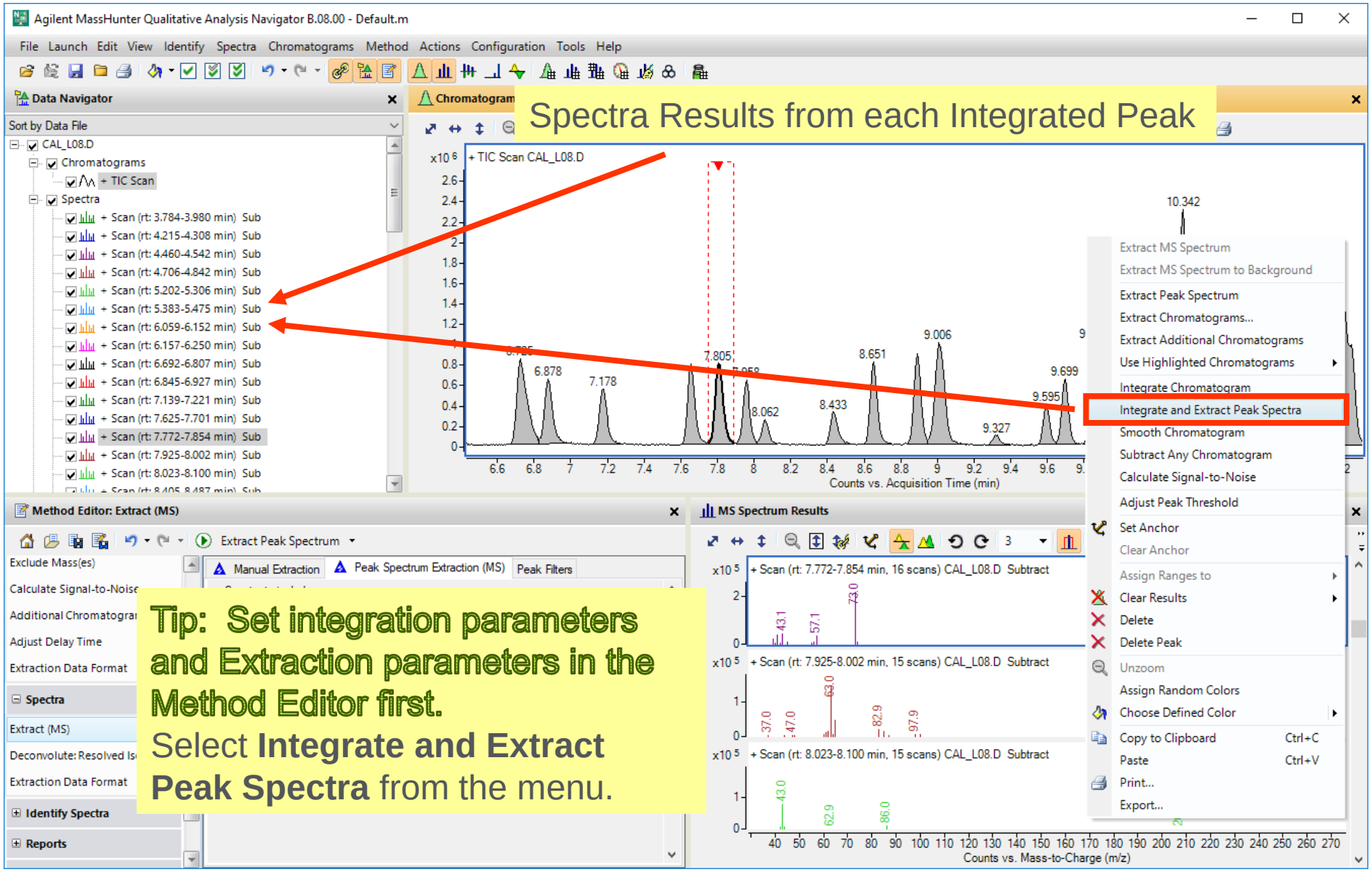


Extract Peak Spectrum

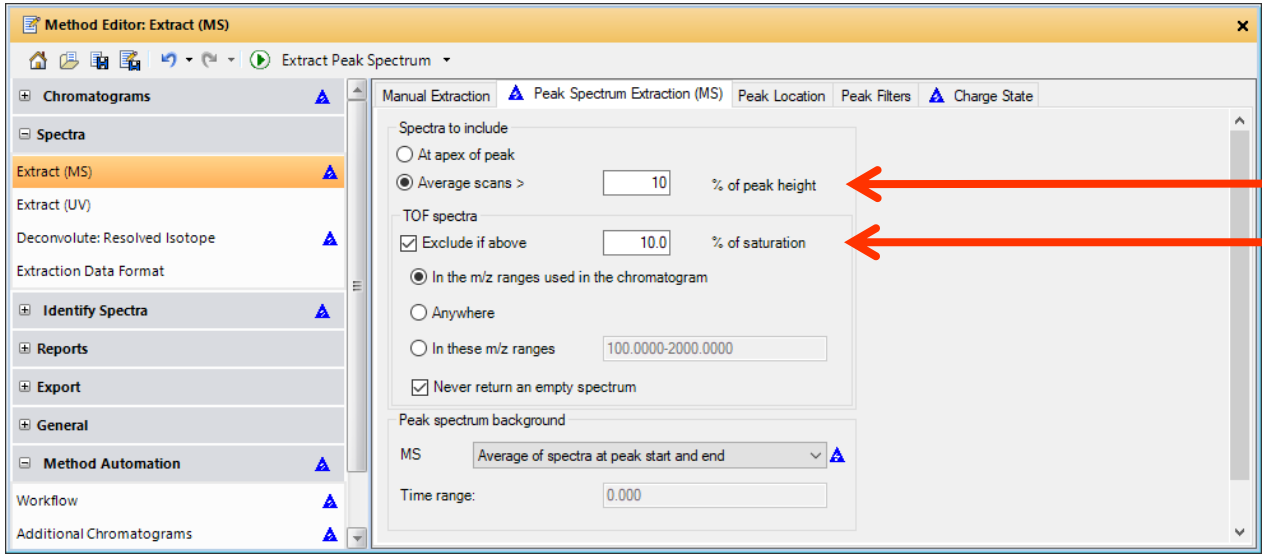
③ Enable Peak Select tool.



Extract Peak Spectra Automatically

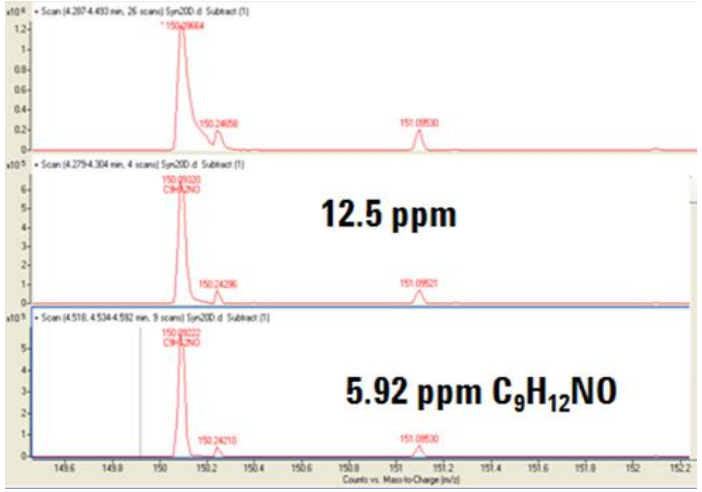


Considerations for Accurate Mass Data Using Extract Peak Parameters – Saturation



Change to 5%

Change to 20%

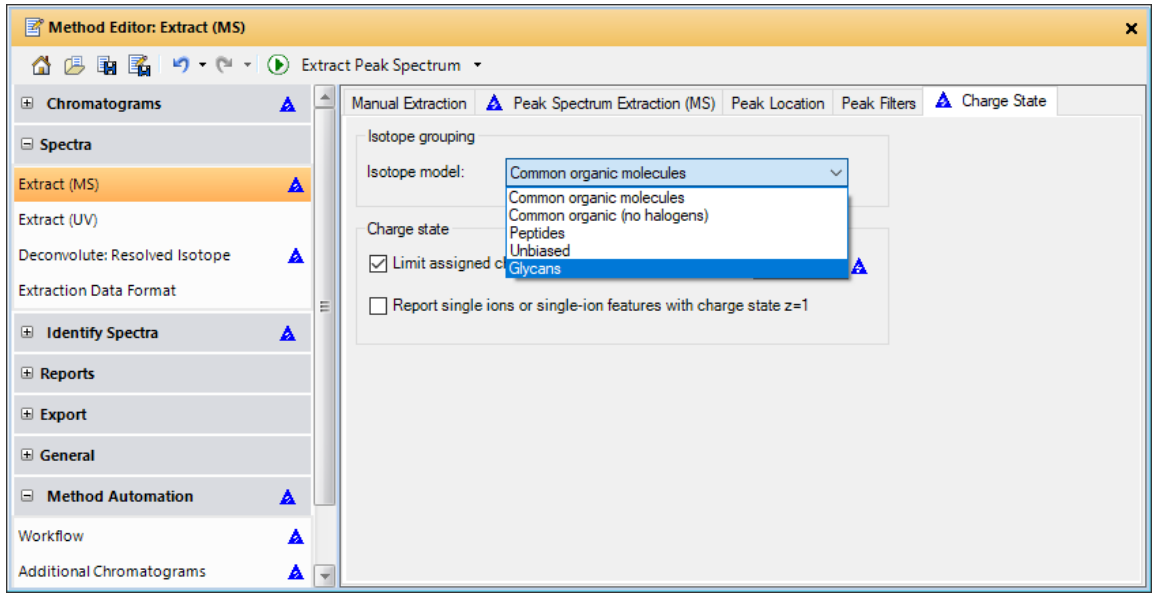


Never return an empty spectrum finds the spectrum with the least saturation.

Using Extract Peak Parameters – Isotope Model

Chose Isotope model that Corresponds to workflow.

Common organic molecules
Peptides
Unbiased
Glycans

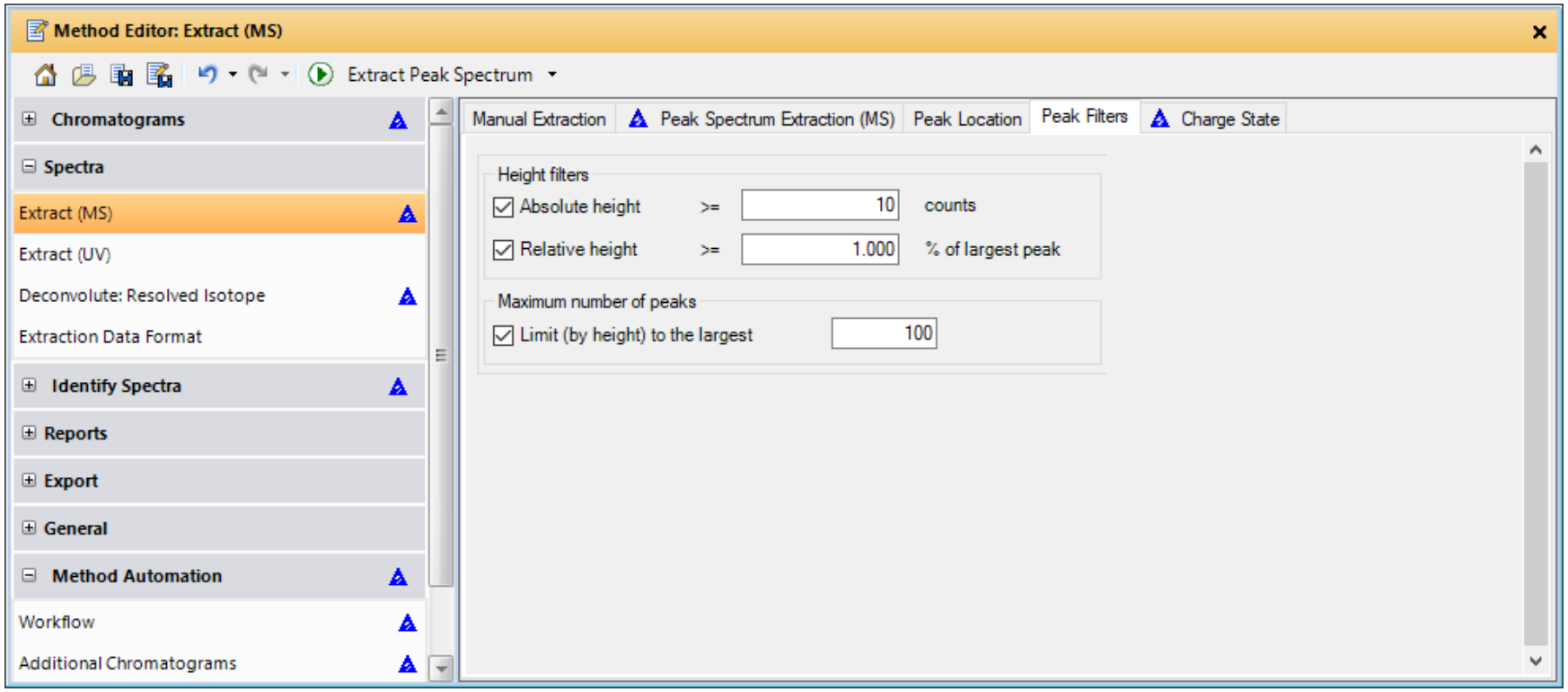


Tip: Check Limit Assigned Charge States Maximum Values.

For Small Molecule Applications: Set to 2

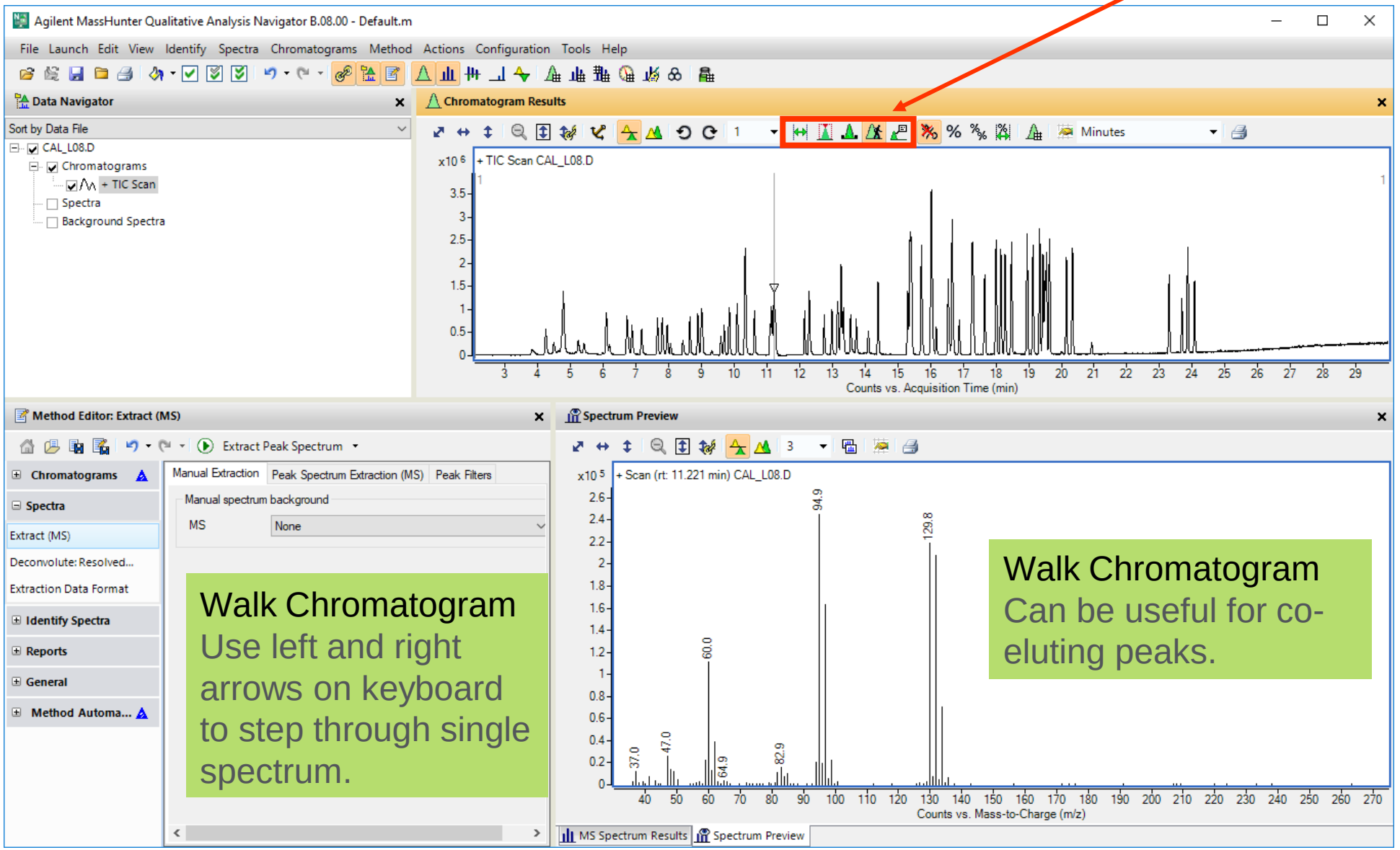
For High Molecular Weight Apps: Uncheck or Max 10

Extract Spectra – Peak Filters

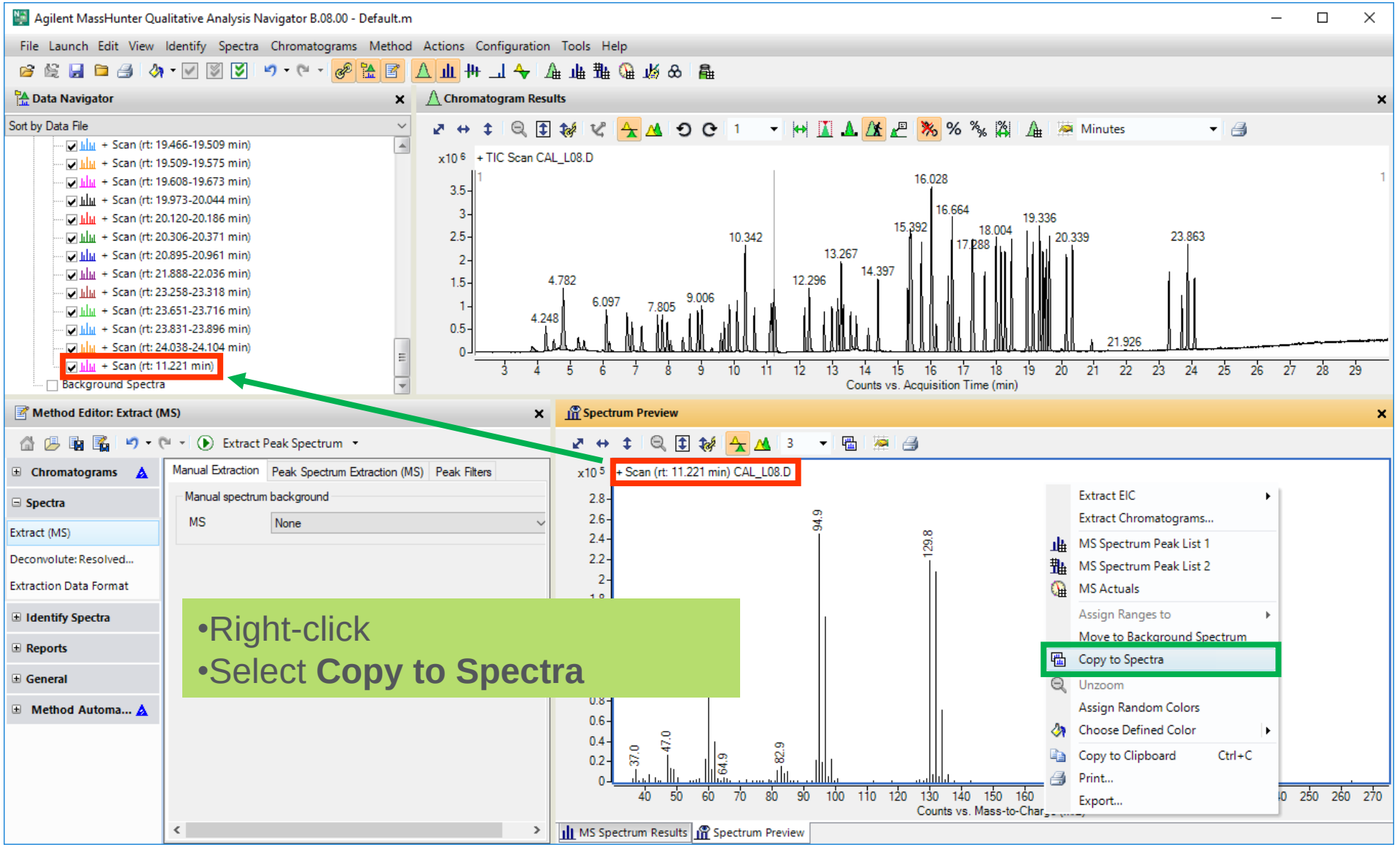


Tip: Always remember to set Peak Filter Limits to reduce noise. Important when library searching.

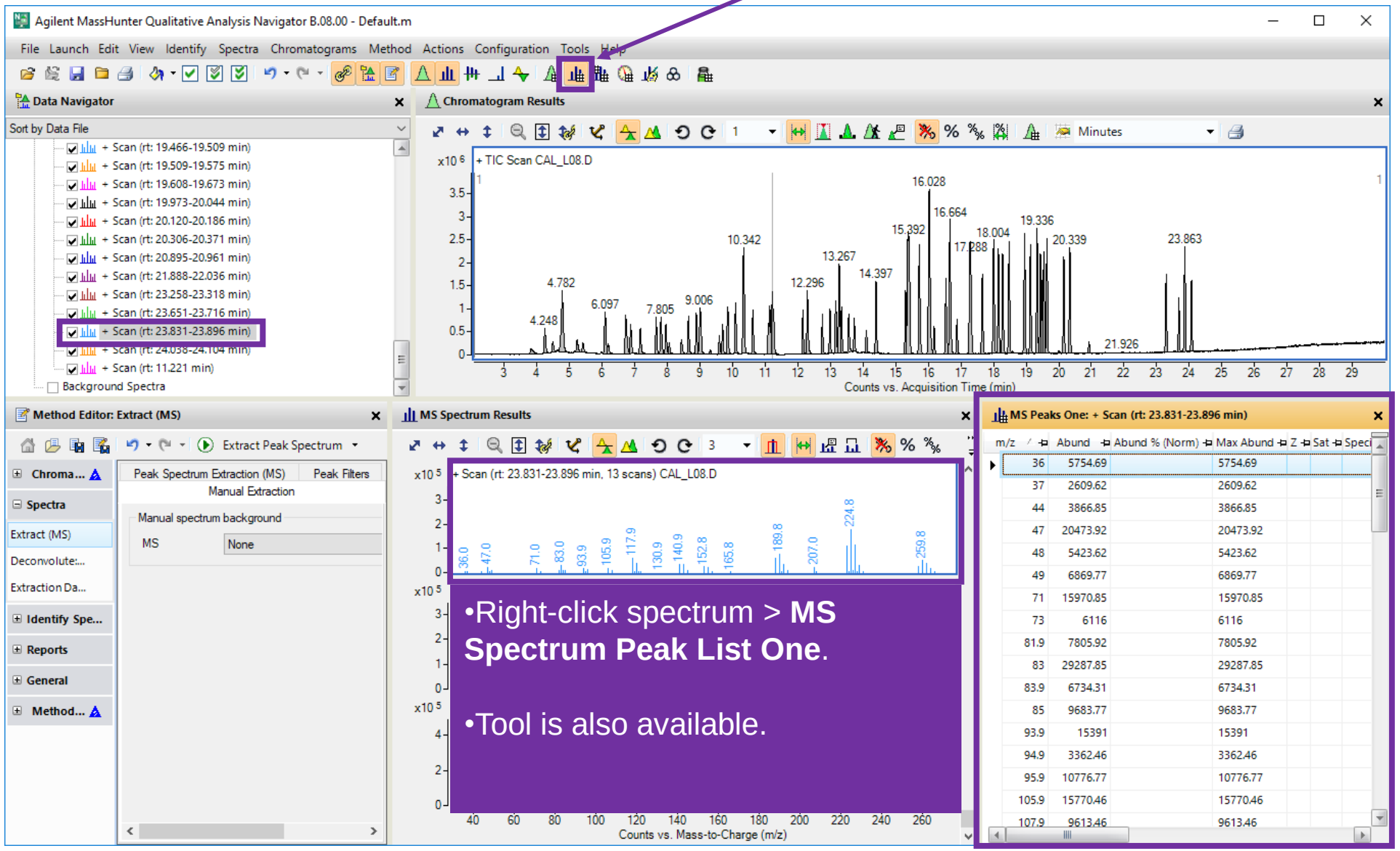
Walk Chromatogram



Add Preview Spectrum to Results

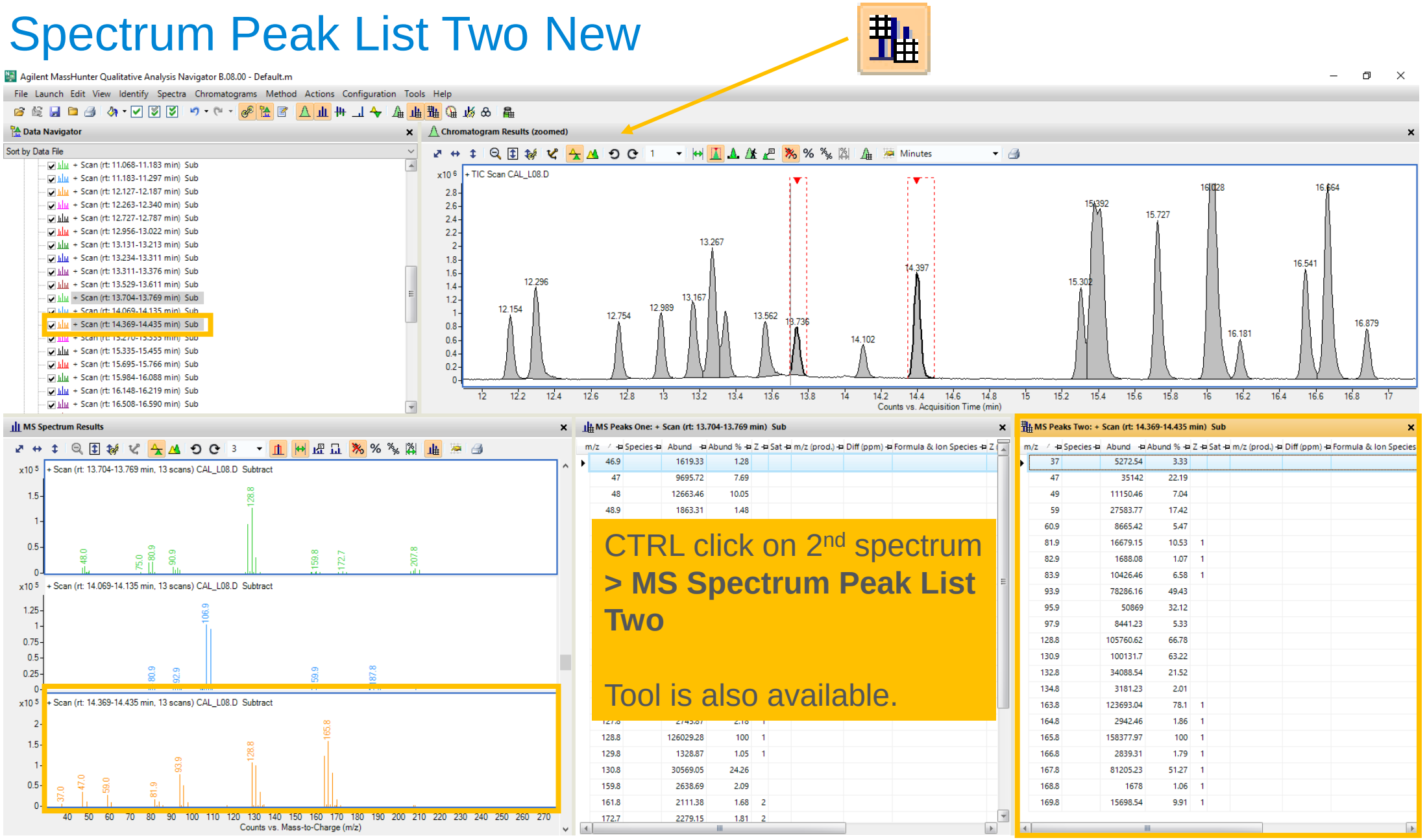


MS Spectrum Peak List One



- Right-click spectrum > **MS Spectrum Peak List One**.
- Tool is also available.

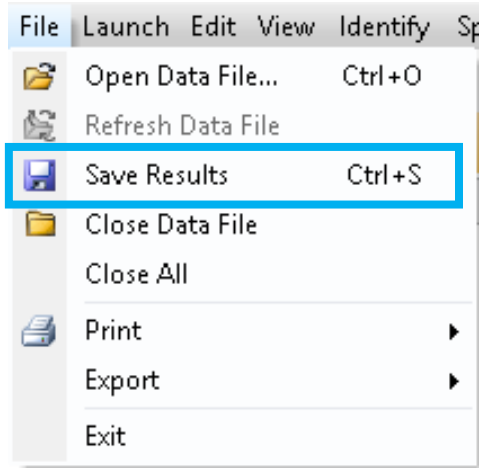
MS Spectrum Peak List Two New

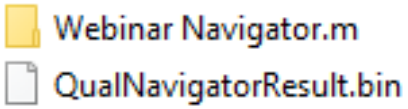


Saving the Results

Results are saved from **File > Save Results** or from icon**.**

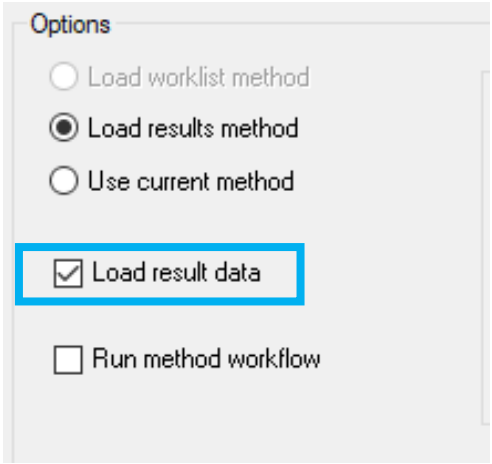
Only one results file can be saved per data file.





The current method and results are saved in the current data folder **> Results > Qual > Version 3** folder

Results can be loaded in the Open Data File dialog under Options.



Let’s take a moment for a demo and questions on Working with Mass Spectra

Up Next:

Searching and Annotations

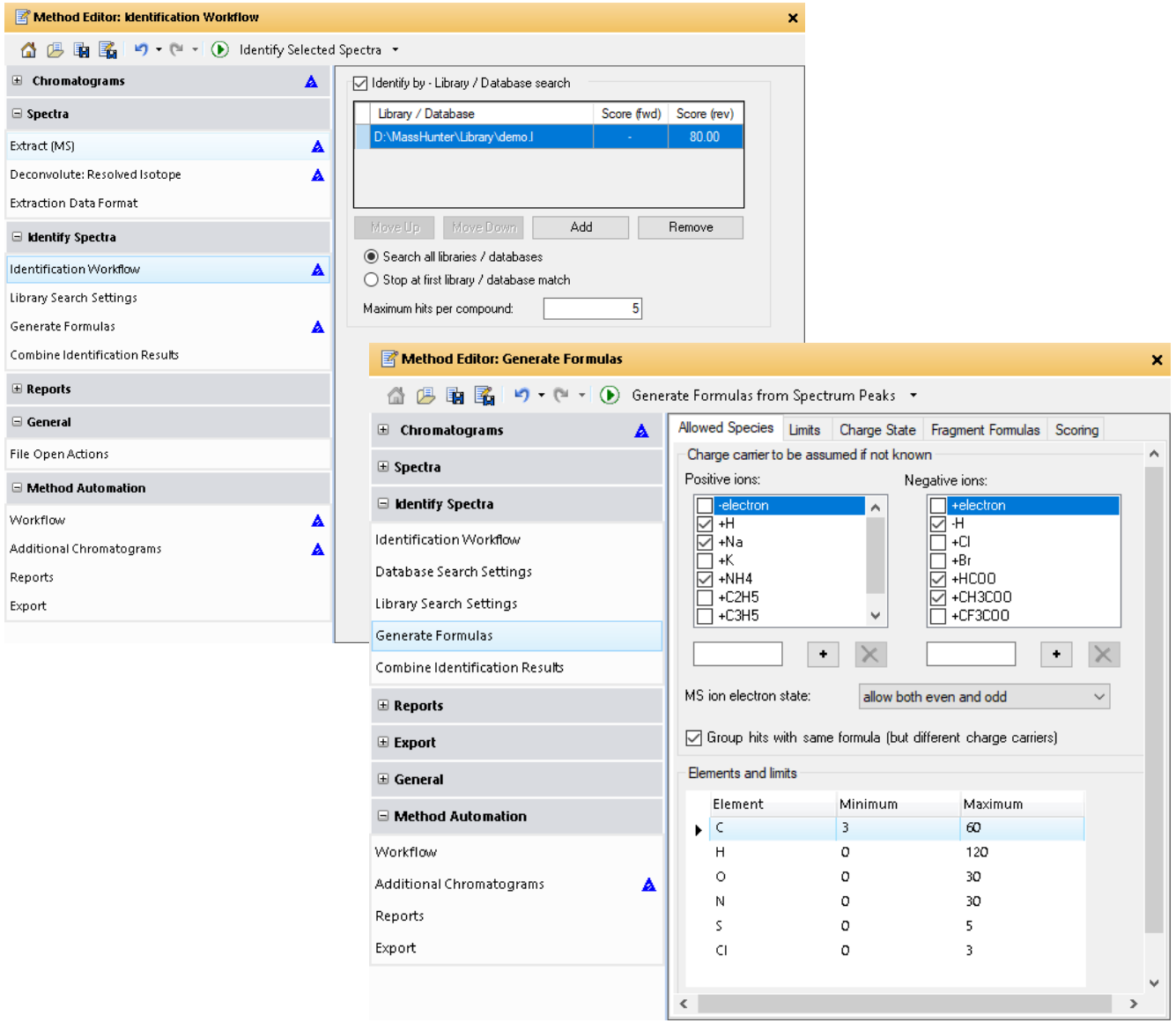
Library/DB Searching and Formula Generation

Qualitative Navigator offers the ability to perform spectral library or database searching and formula generation.

Intended to be used for quick, manual scouting of data files.

For automated compound searching, compound discovery, and compound reports, use Qualitative Analysis Workflows.

Note: No ‘Compounds’ are generated and no Compound Reports are possible in Navigator.



EI Library Search – GCMS

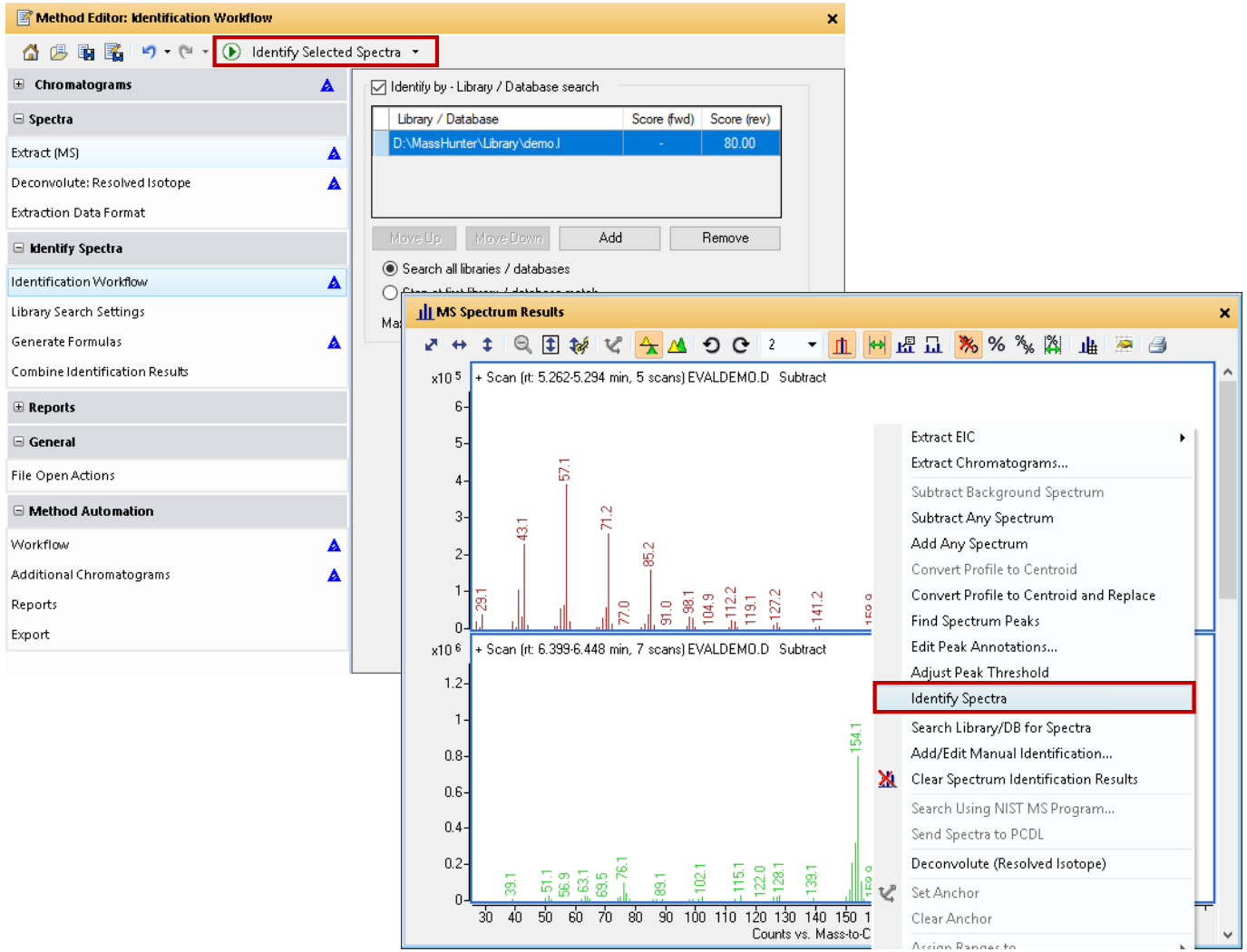
Default-GCMS-SQ.M method has good starting points for EI library searches.

Parameters can be adjusted in Identify Spectra section of Method Editor.

Select the spectra you want to identify and click Identify Selected Spectra.

or

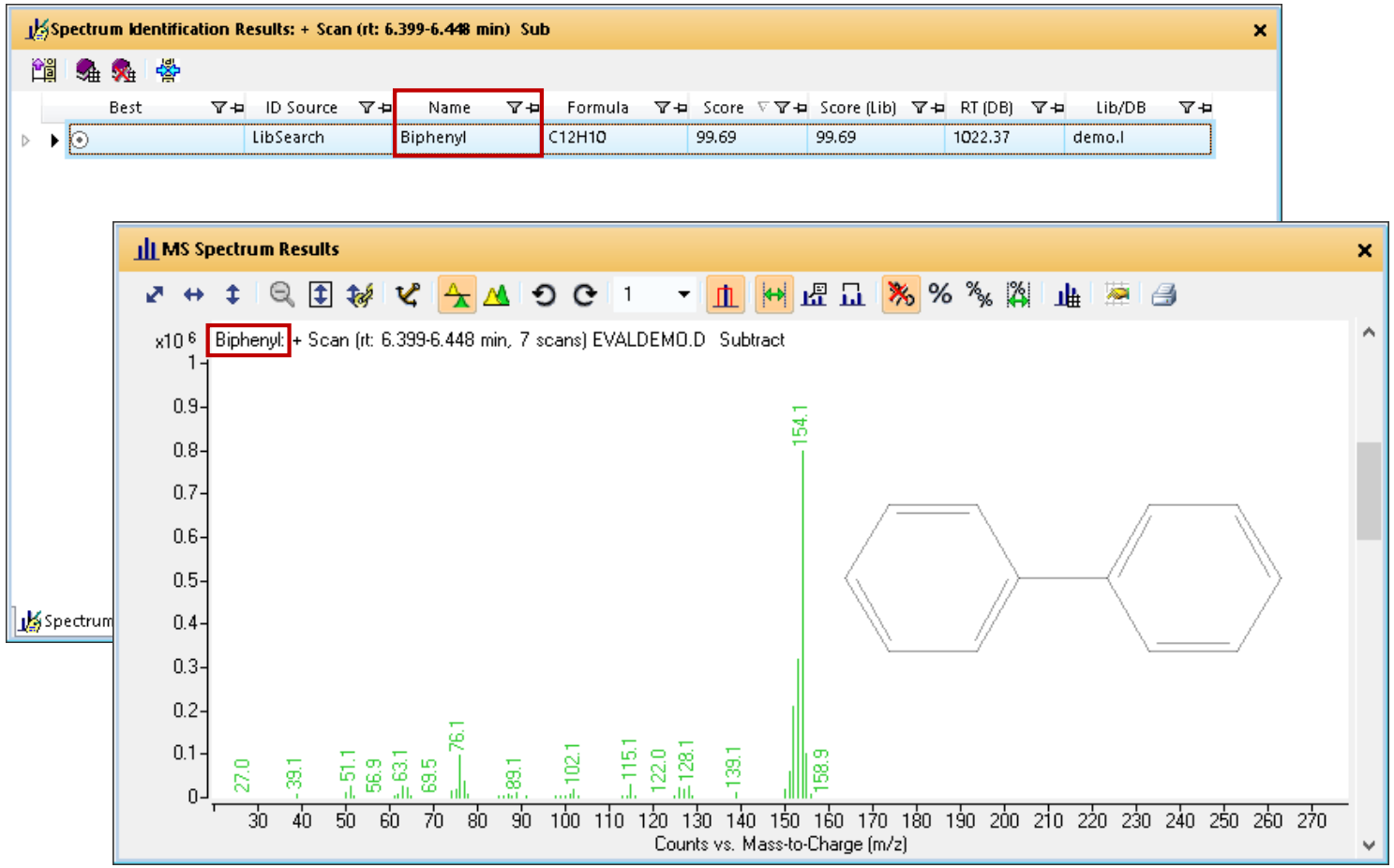
Right click in spectrum window and select Identify Spectra.



Note : Details on these settings will be covered in the Qualitative Analysis Workflows Webinar.

EI Library Search – GCMS

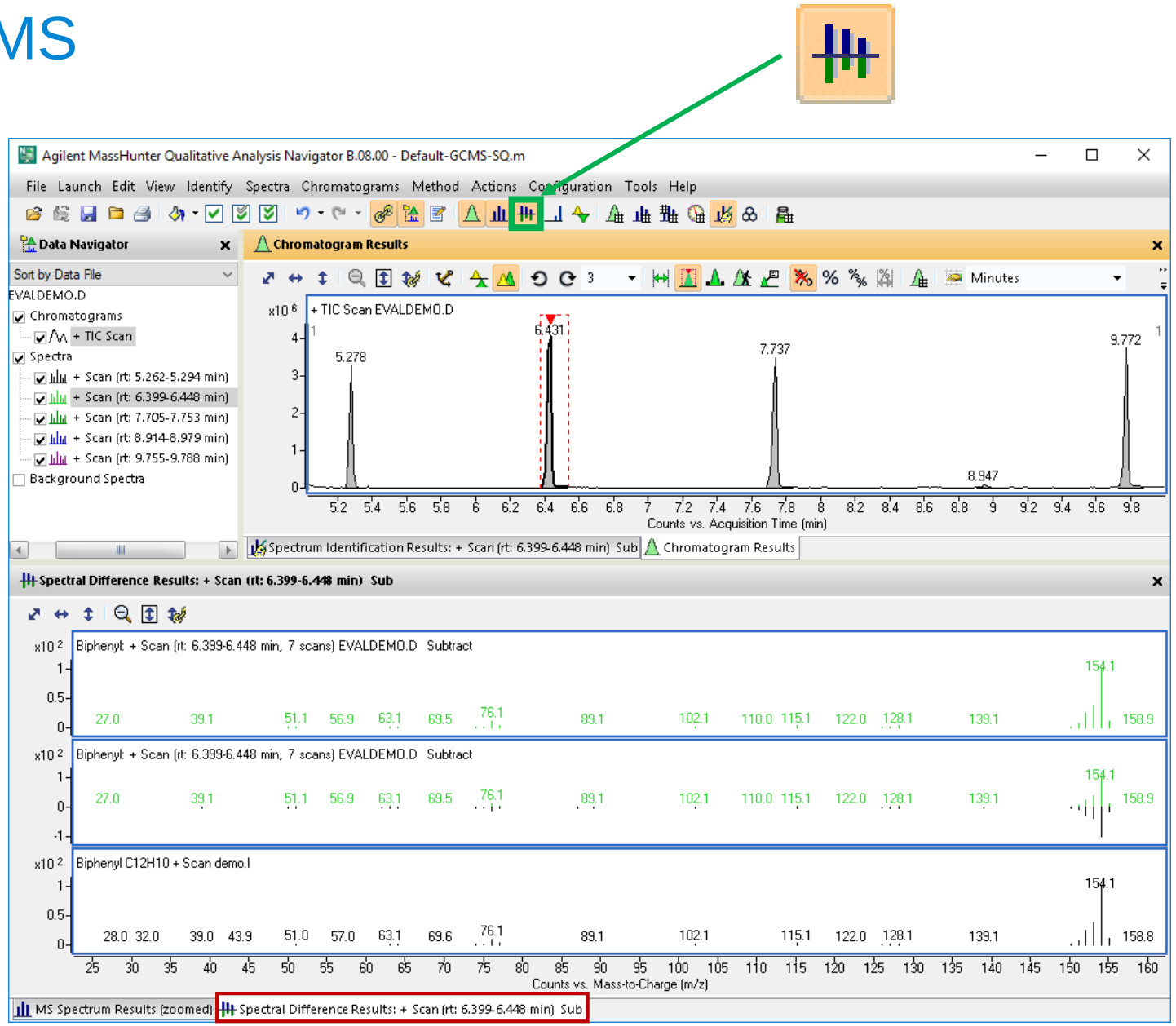
Spectrum Identification Results are shown and the spectrum is labeled with the structure if a library hit is found.



Difference Results– GCMS

Navigator also offers Spectral Difference Results. By default the window is tabbed with the MS Spectrum Results window.

Useful for comparing mass spectrum with library or database spectrum.

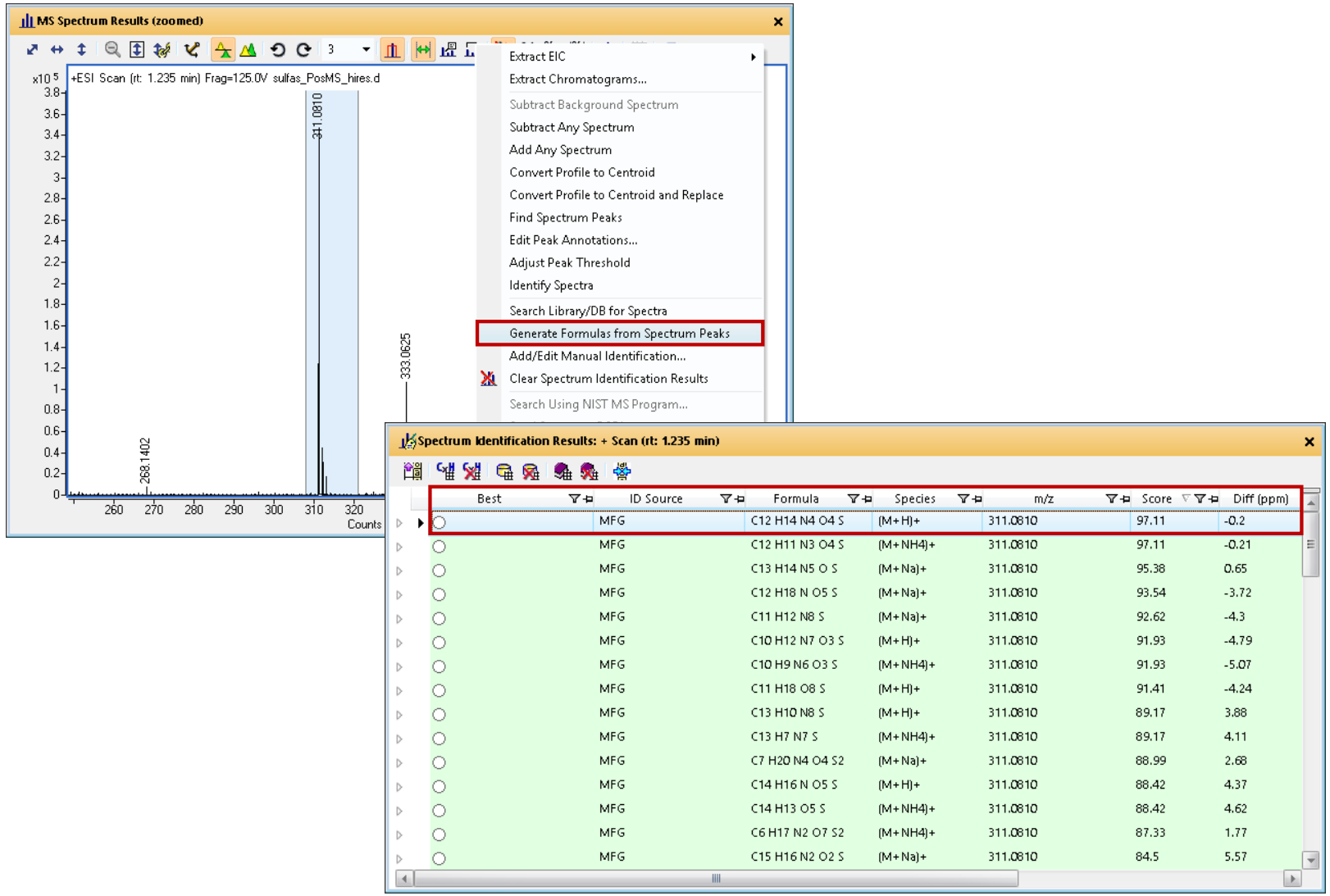


Formula Generation

When working with high resolution data, formulas can be generated from spectrum peaks, or they can be searched against a library or database.

For simply formula generation, use the range select tool to highlight the ion of interest, along with its isotopes.

Right click and choose Generate Formulas from Spectrum Peaks.



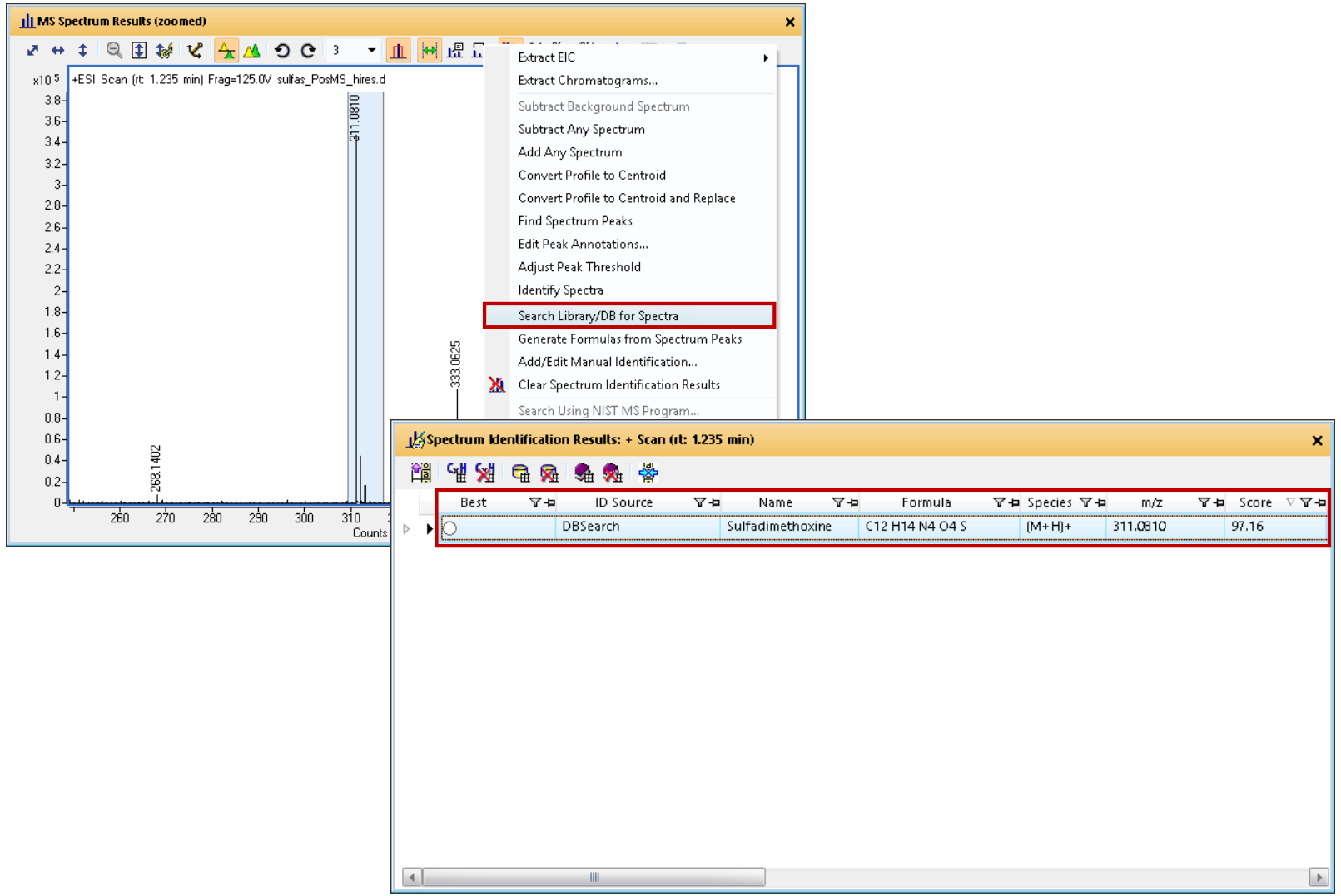
Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)
	MFG	C12 H14 N4 O4 S	(M+H)+	311.0810	97.11	-0.2
	MFG	C12 H11 N3 O4 S	(M+NH4)+	311.0810	97.11	-0.21
	MFG	C13 H14 N5 O S	(M+Na)+	311.0810	95.38	0.65
	MFG	C12 H18 N O5 S	(M+Na)+	311.0810	93.54	-3.72
	MFG	C11 H12 N8 S	(M+Na)+	311.0810	92.62	-4.3
	MFG	C10 H12 N7 O3 S	(M+H)+	311.0810	91.93	-4.79
	MFG	C10 H9 N6 O3 S	(M+NH4)+	311.0810	91.93	-5.07
	MFG	C11 H18 O8 S	(M+H)+	311.0810	91.41	-4.24
	MFG	C13 H10 N8 S	(M+H)+	311.0810	89.17	3.88
	MFG	C13 H7 N7 S	(M+NH4)+	311.0810	89.17	4.11
	MFG	C7 H20 N4 O4 S2	(M+Na)+	311.0810	88.99	2.68
	MFG	C14 H16 N O5 S	(M+H)+	311.0810	88.42	4.37
	MFG	C14 H13 O5 S	(M+NH4)+	311.0810	88.42	4.62
	MFG	C6 H17 N2 O7 S2	(M+NH4)+	311.0810	87.33	1.77
	MFG	C15 H16 N2 O2 S	(M+Na)+	311.0810	84.5	5.57

Library/DB Searching

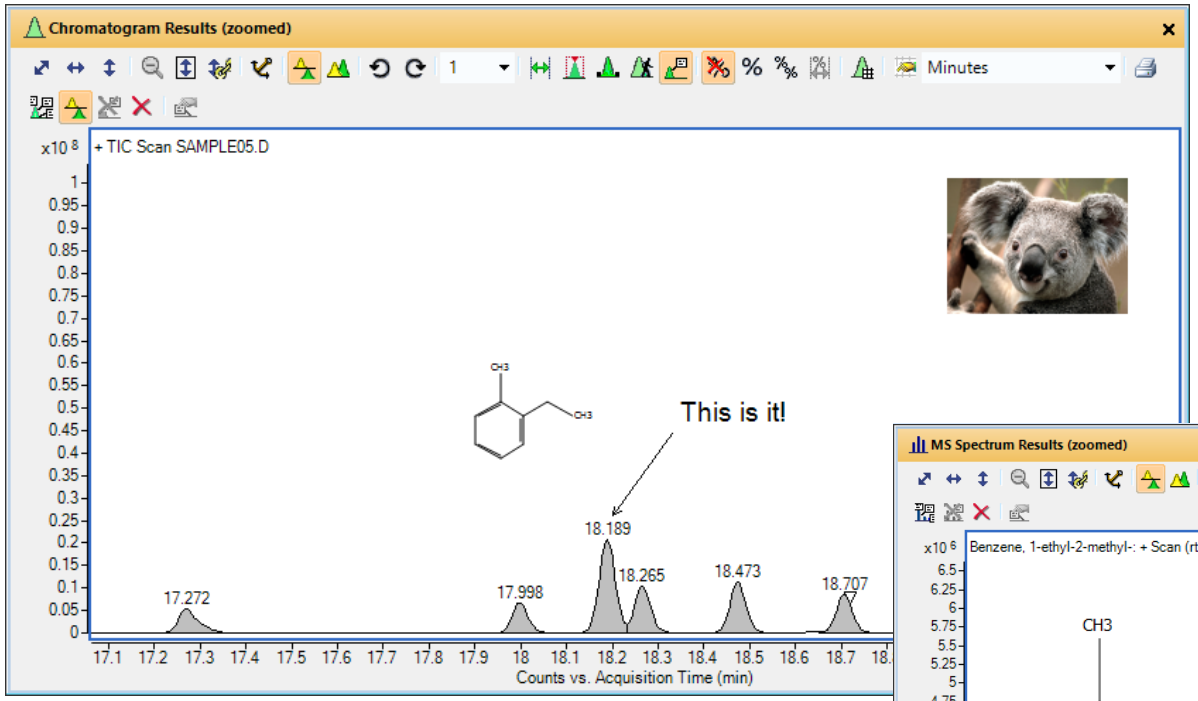
Or, for a library or database search, again use the range select tool to highlight the ion of interest, along with its isotopes.

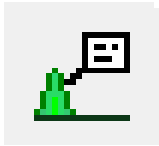
Right click and choose Search Library/DB for Spectra

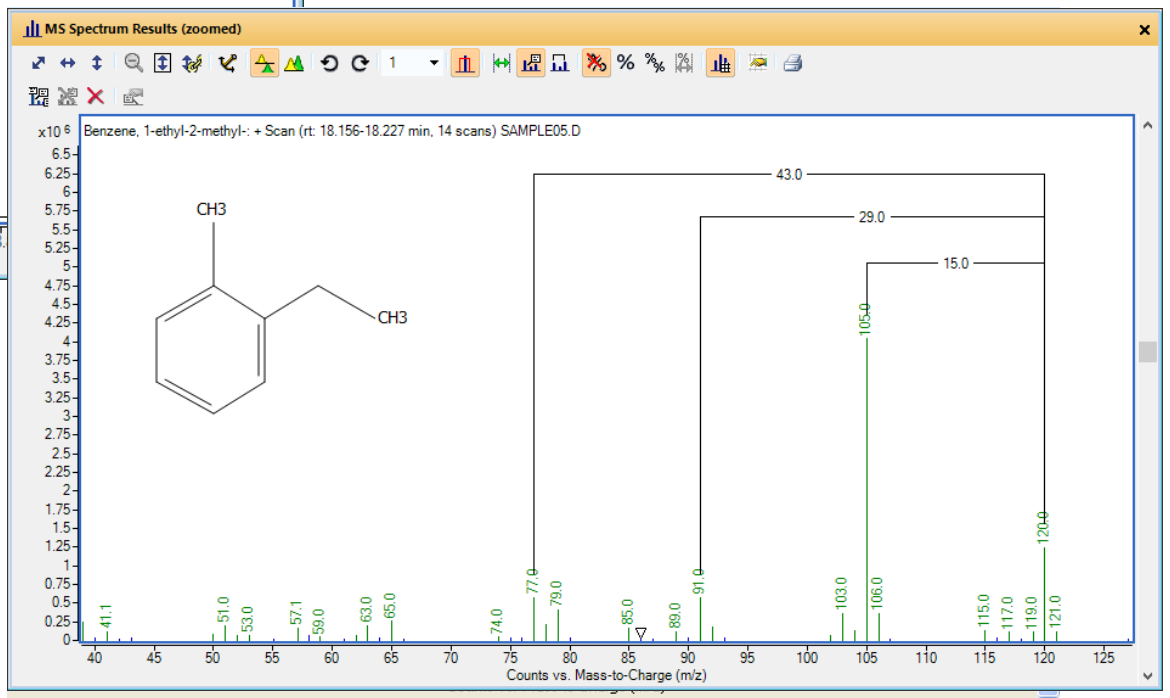
Default-LCMS.M method has good starting points for high resolution data formula generation or Library/DB searching.

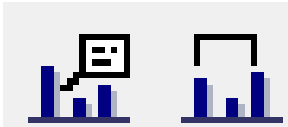


Annotation of Chromatograms and Spectra



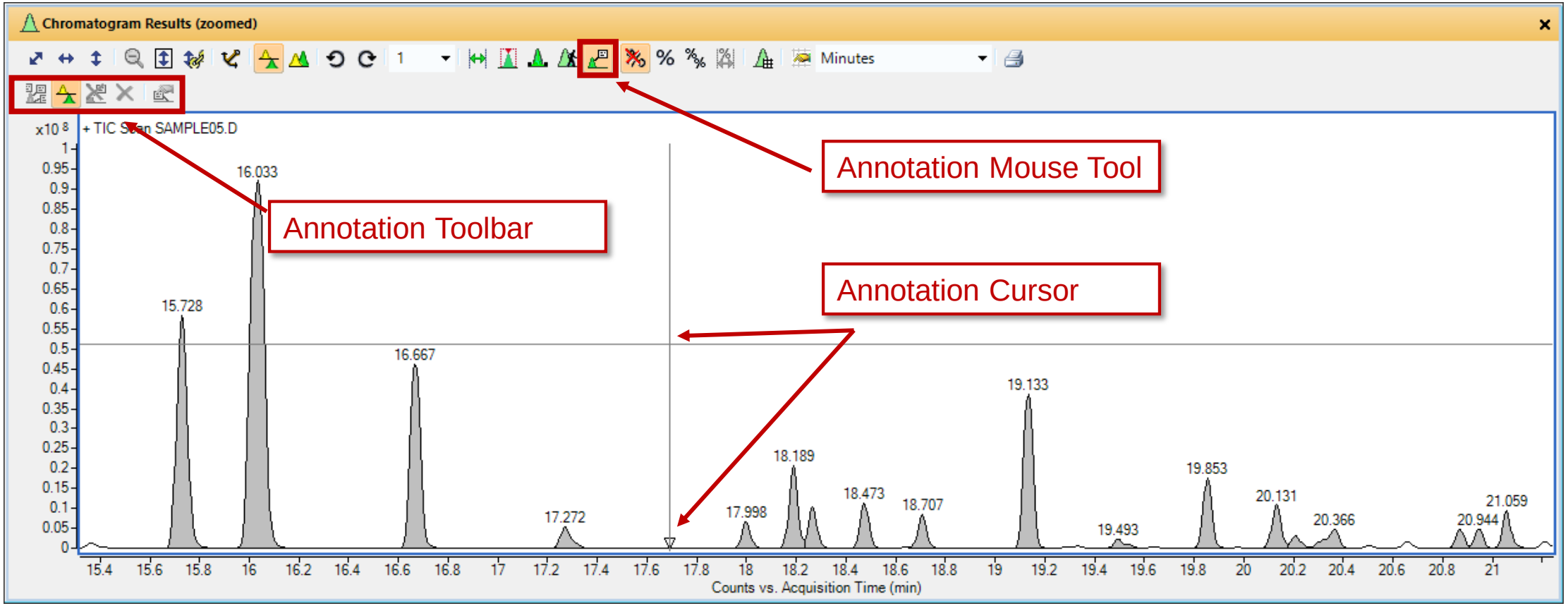






Place Graphic into Annotation Mode

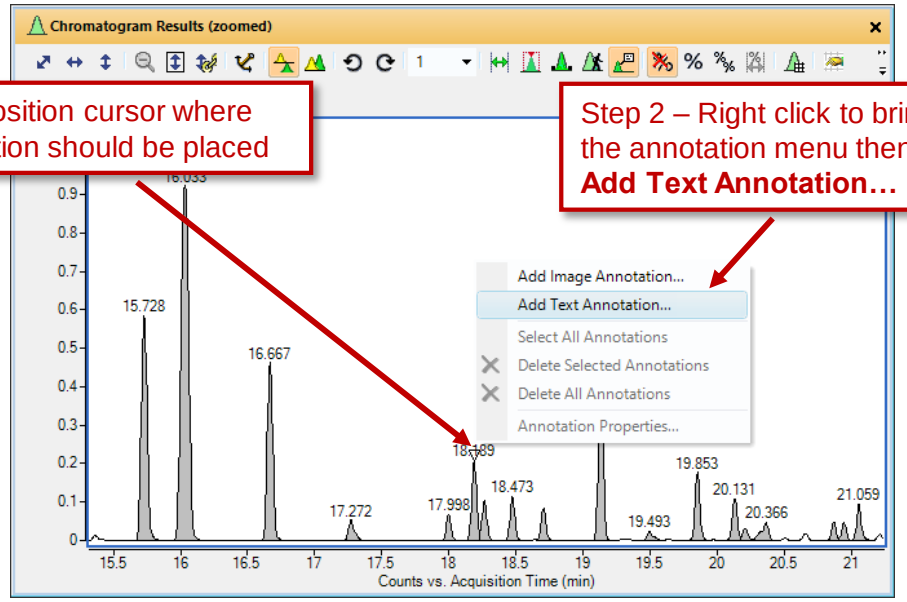
- Click Annotation Mouse Tool
- Annotation Toolbar and Annotation cursor appear

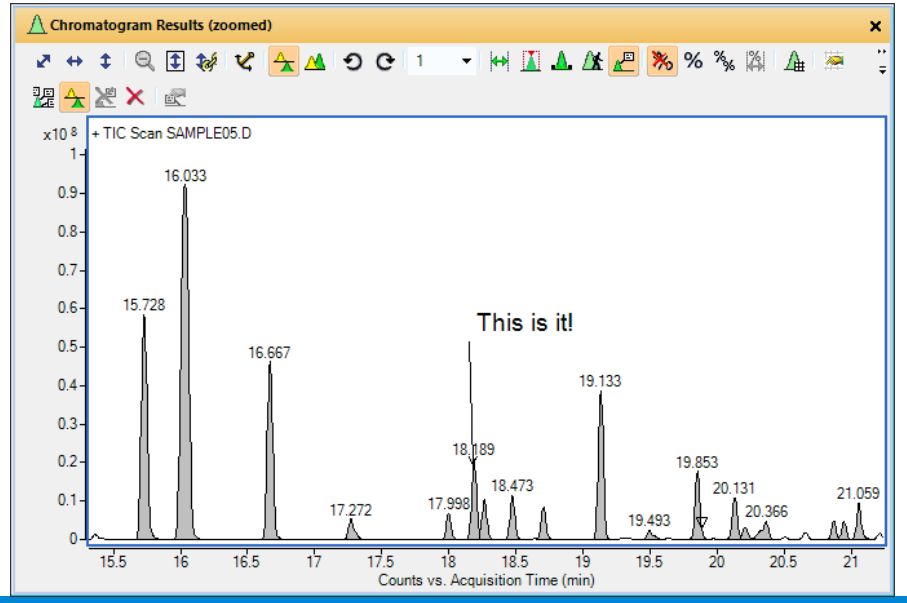


Add a Text Annotation

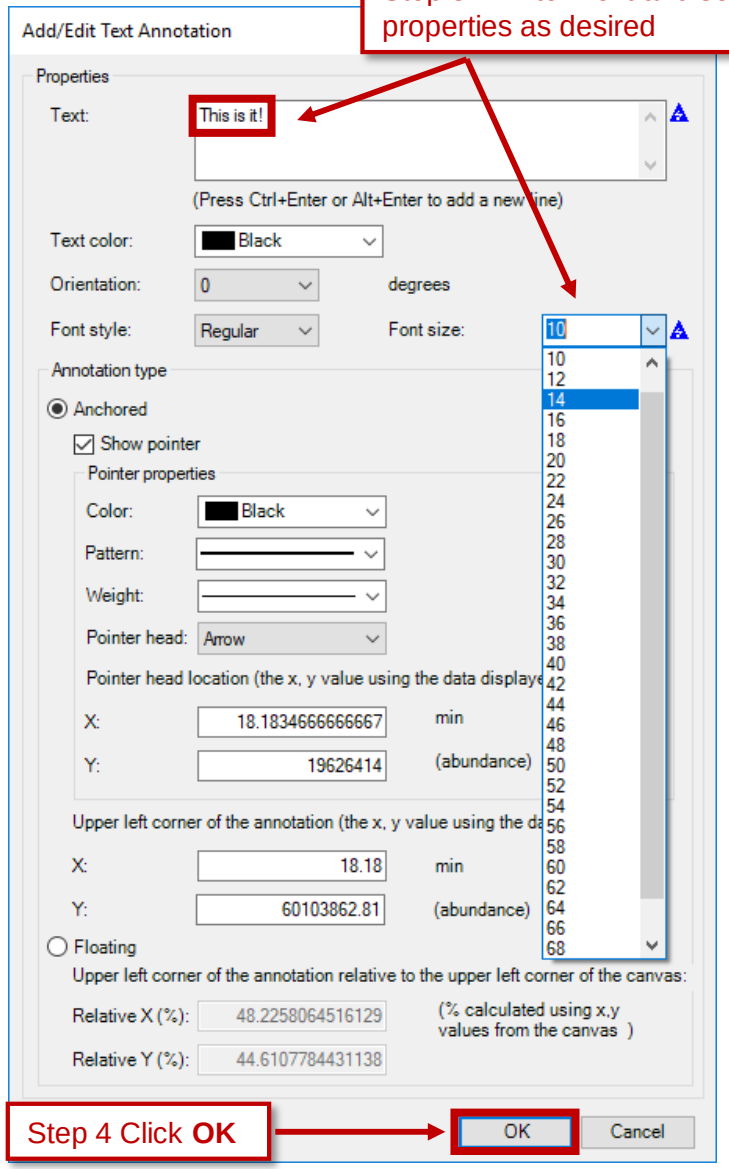
Step 1 – Position cursor where the annotation should be placed

Step 2 – Right click to bring up the annotation menu then select **Add Text Annotation…**





Step 3 – Enter Text and set other properties as desired



Step 4 Click **OK**

Text and Pointer Can Be Repositioned

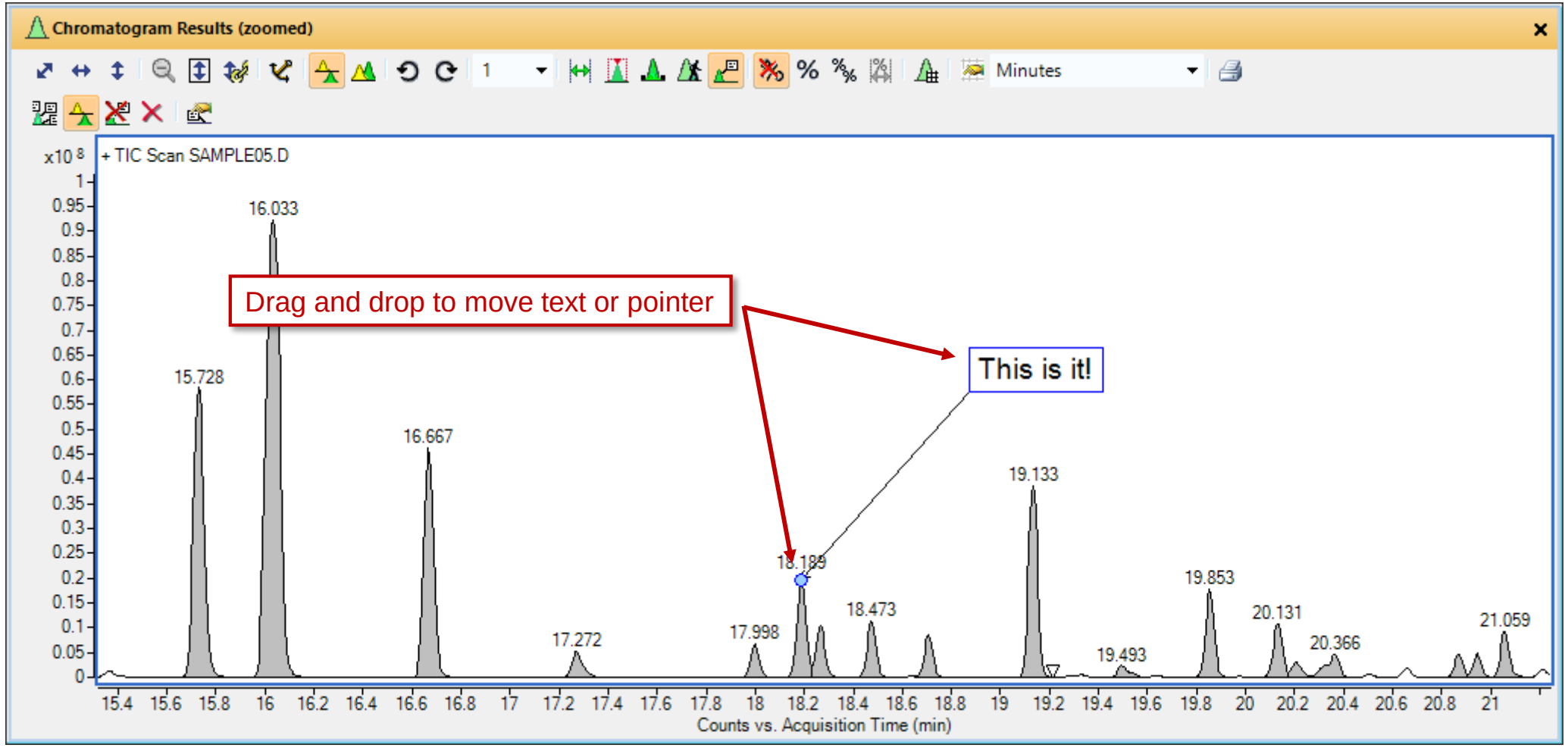
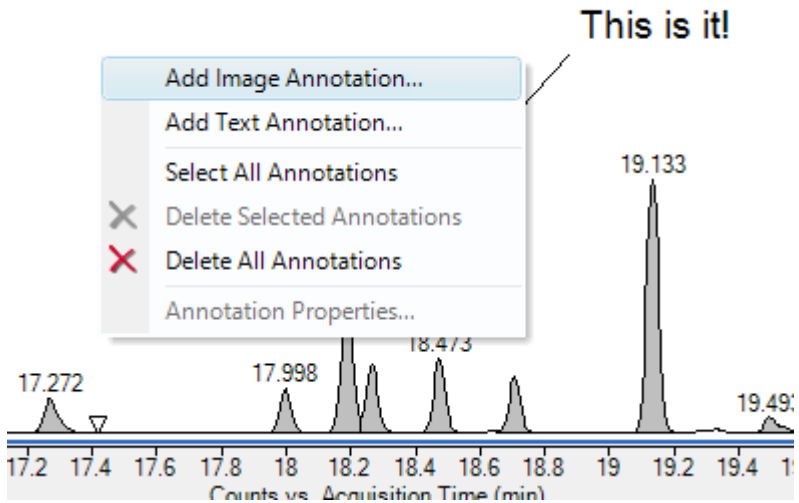
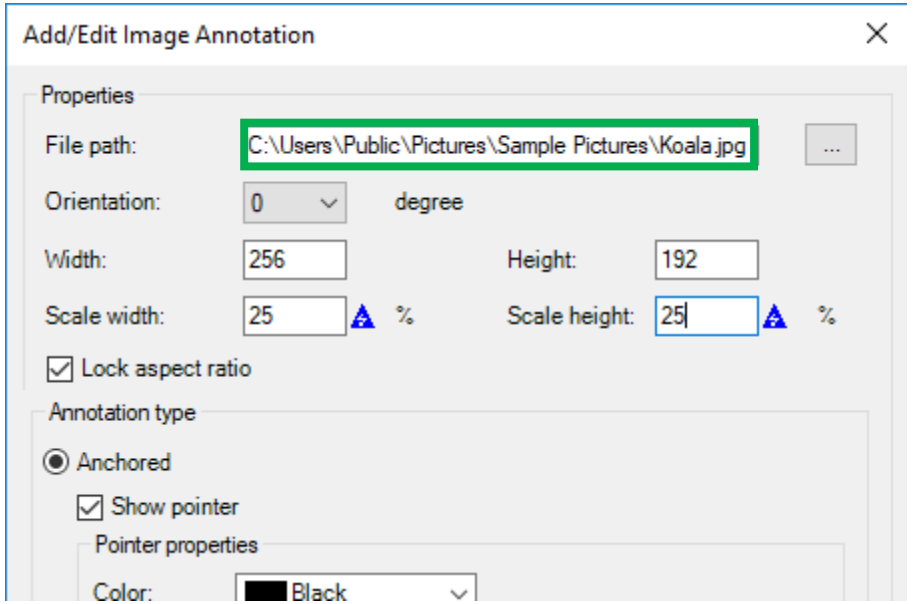


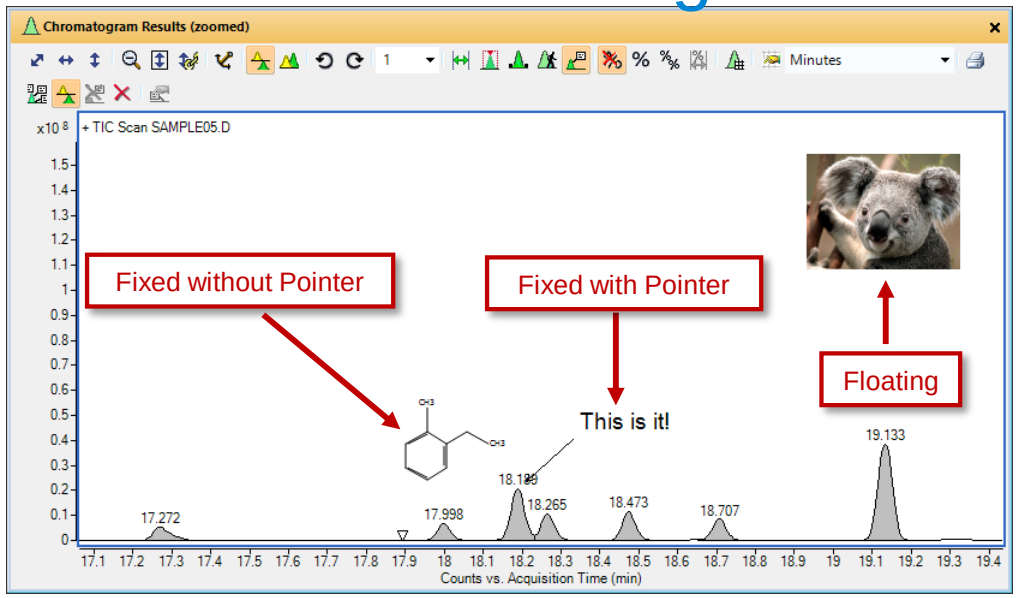
Image Annotation

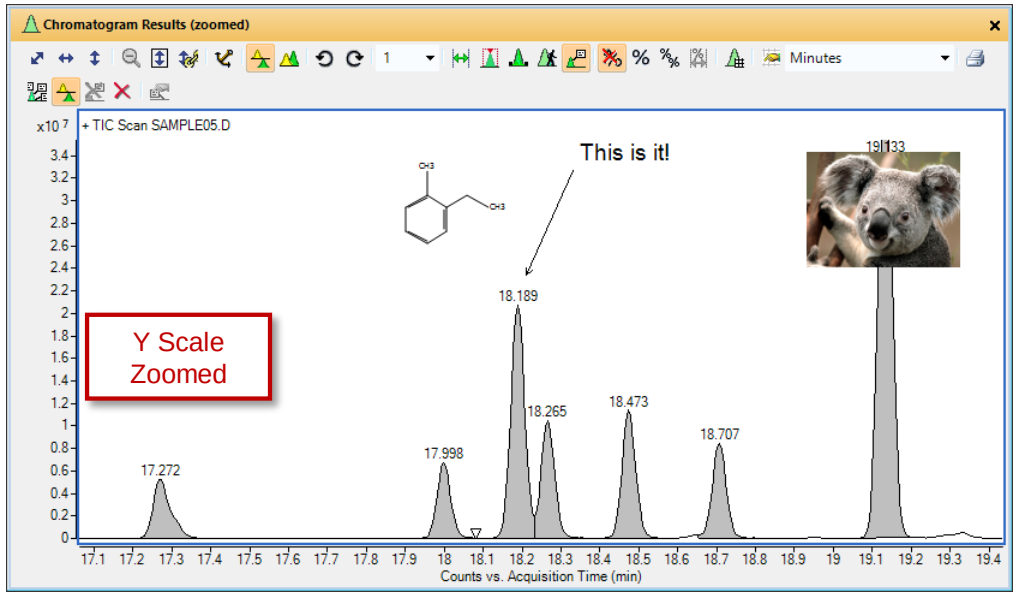
- Same steps as adding Text
- JPEG and MOL (molecular structure) files are supported
- Image may be scaled and pivoted

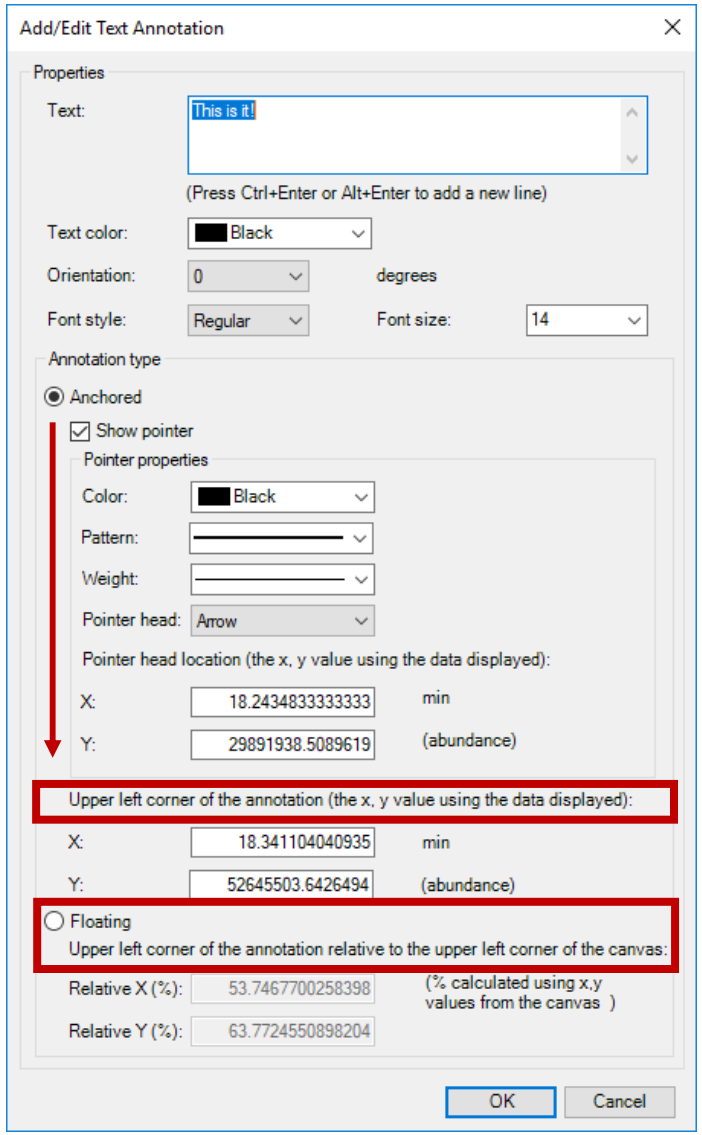




Anchored vs. Floating Annotation

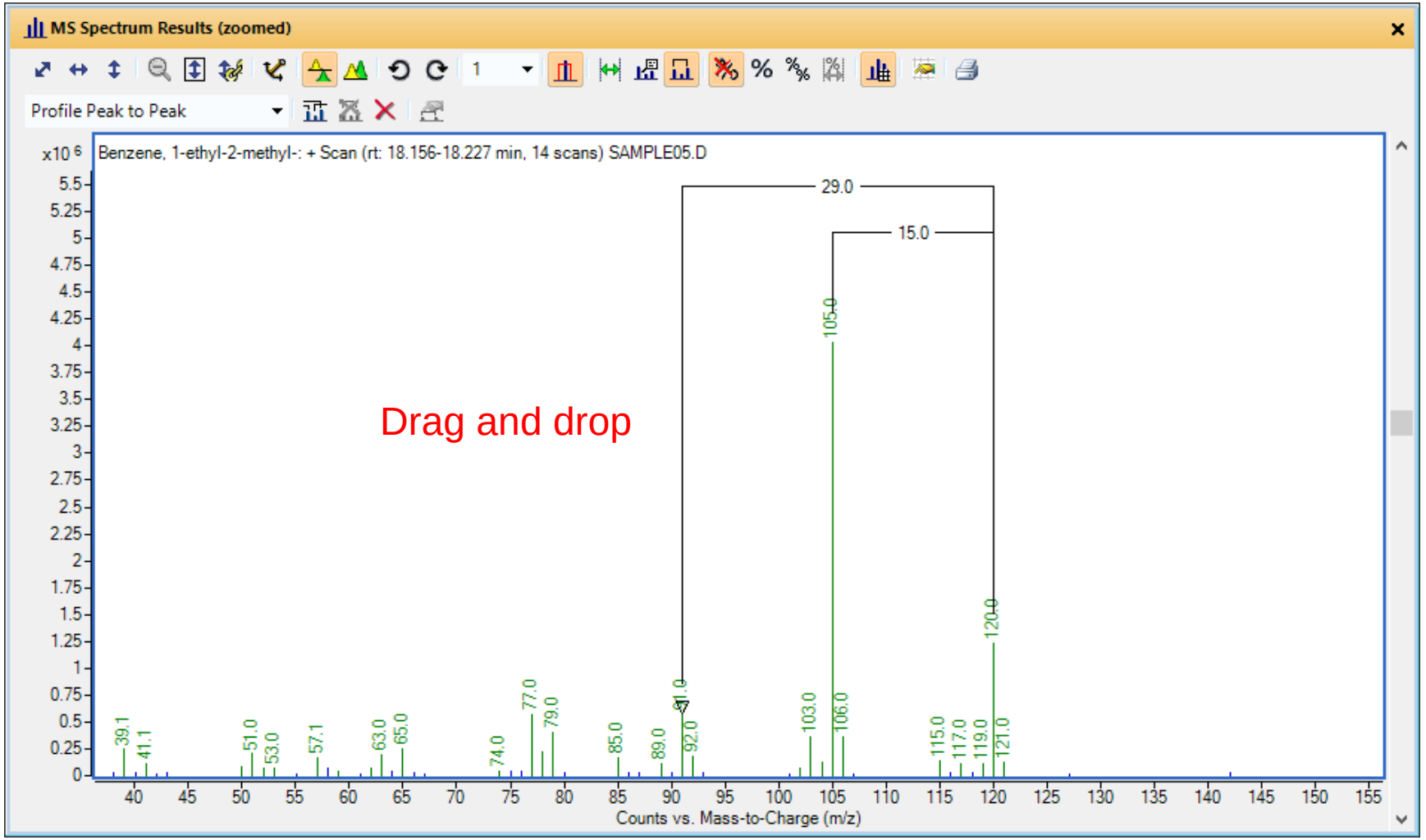






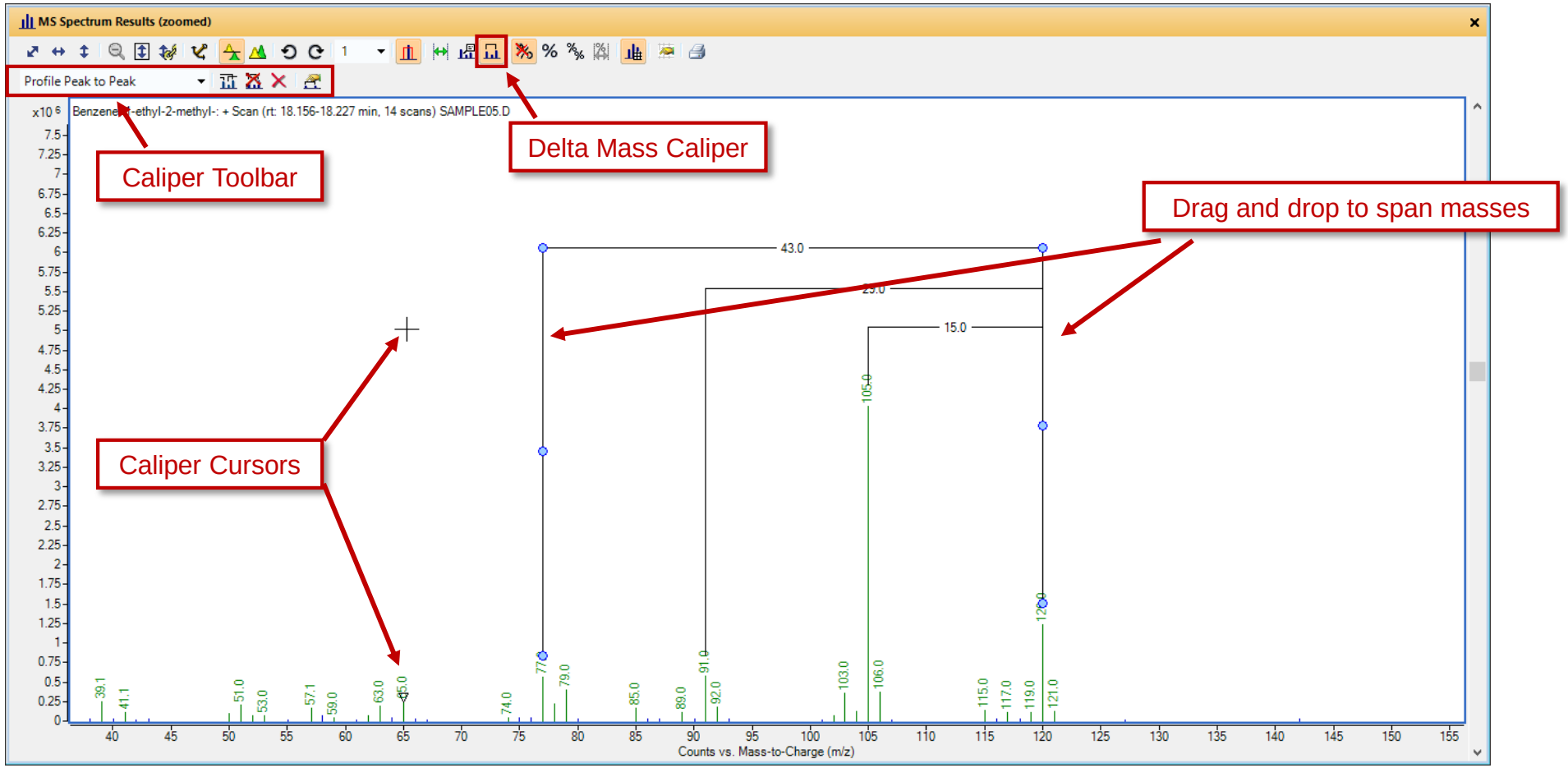
Delta Mass Caliper

Used to calculate and display mass differences between two ions.



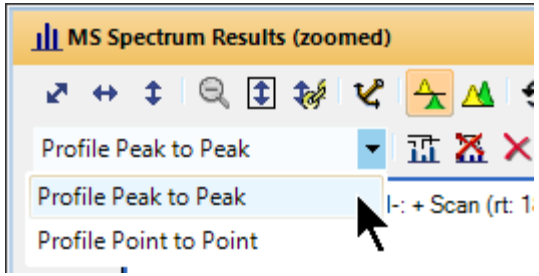
Delta Mass Caliper - Mass Caliper Mode

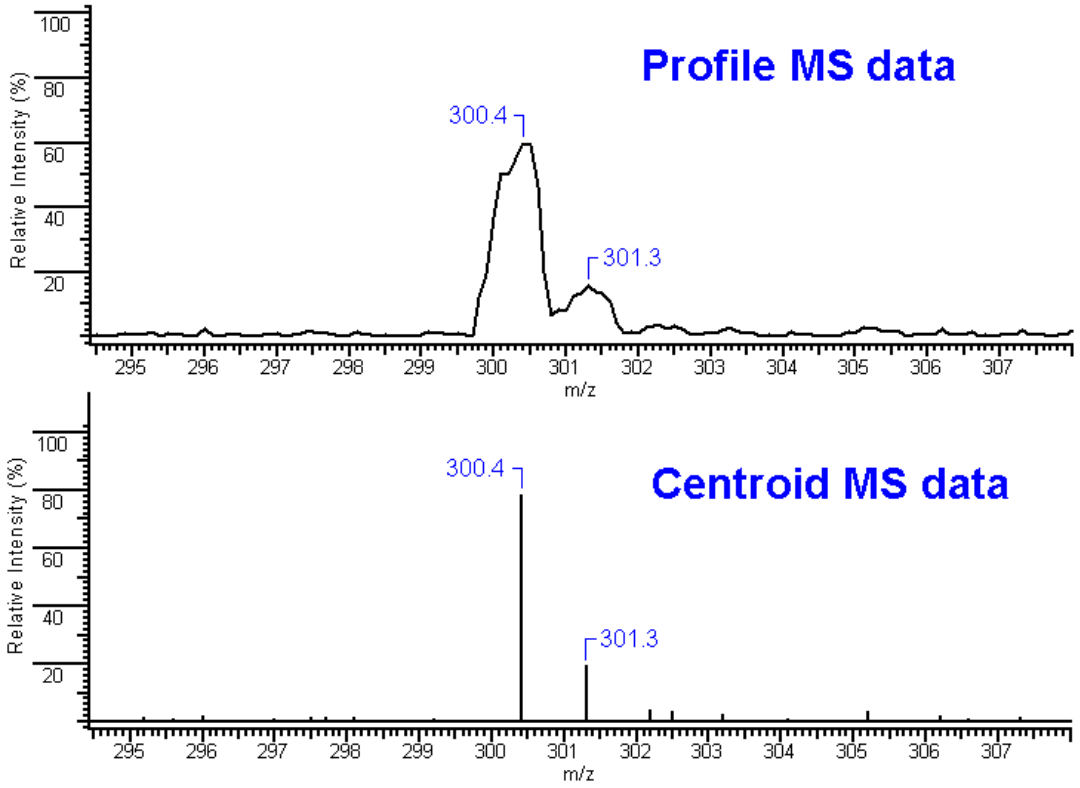
Click **Delta Mass Caliper Mouse** icon
Caliber Toolbar and Caliper cursor appear
Add or Edit Calipers
Calipers can only be placed where there is a signal and “snap” to closest ion



Delta Mass Caliper – Profile Options

- Only used on profile data
- Profile Peak to Peak will “snap” Caliper to profile peak apex
- Profile Point to Point will allow the user to position the Caliper to any point





Let’s take another moment for questions on Searching and Annotations

Up Next:

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Training Resources

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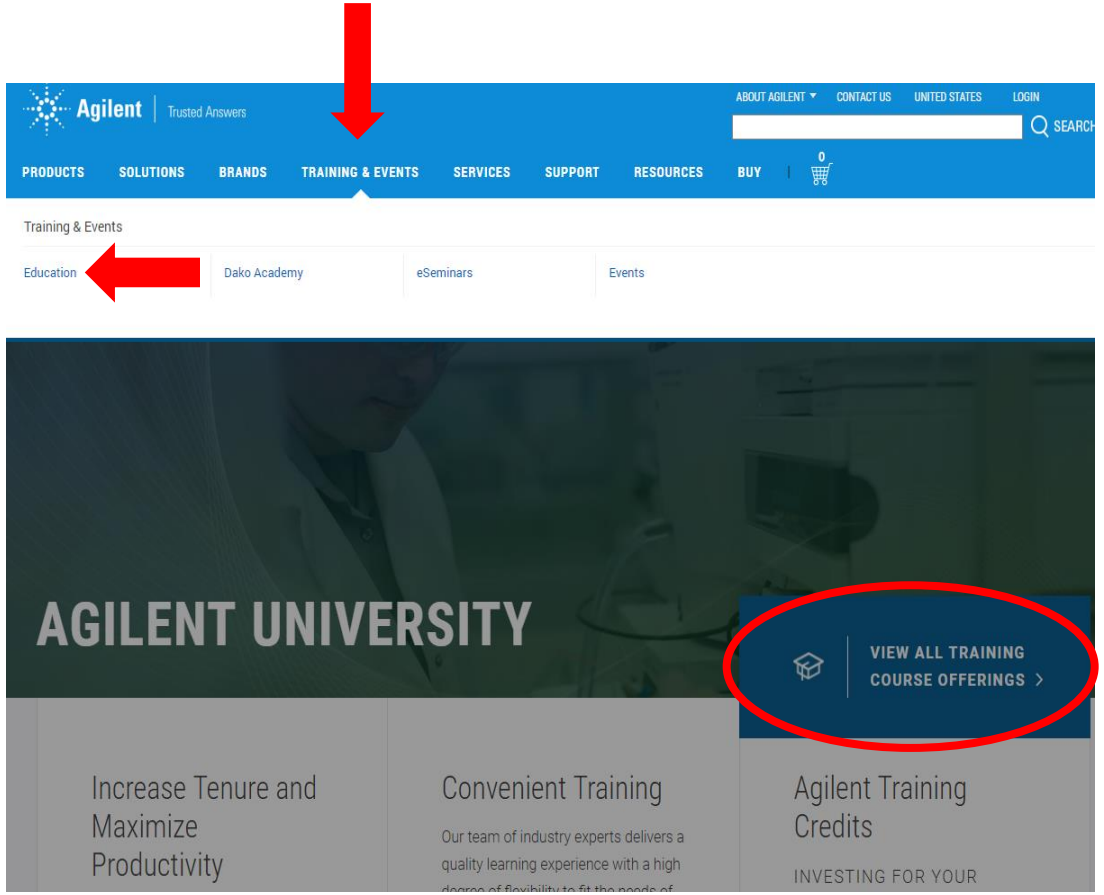

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