

# Agilent MassHunter Qualitative Data Analysis

Qualitative Navigator B.08.00

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# MassHunter Qualitative Navigator

## Topics

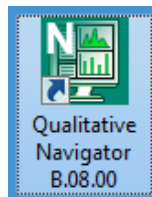
- Navigator and Workflows
- Navigator View
  - Adaptive User interface
- Methods
  - Definition
  - Unified Method Concepts
- Working with Chromatograms
  - Anchoring
  - Integration peak List
- Working with Spectra
  - MS Display Options
  - Background Subtraction
- Annotations of Chromatograms and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

# MassHunter Qualitative Analysis Software B.08.00 SP1

## Navigator and Workflows

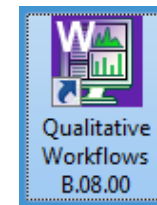
### Navigator

- 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?



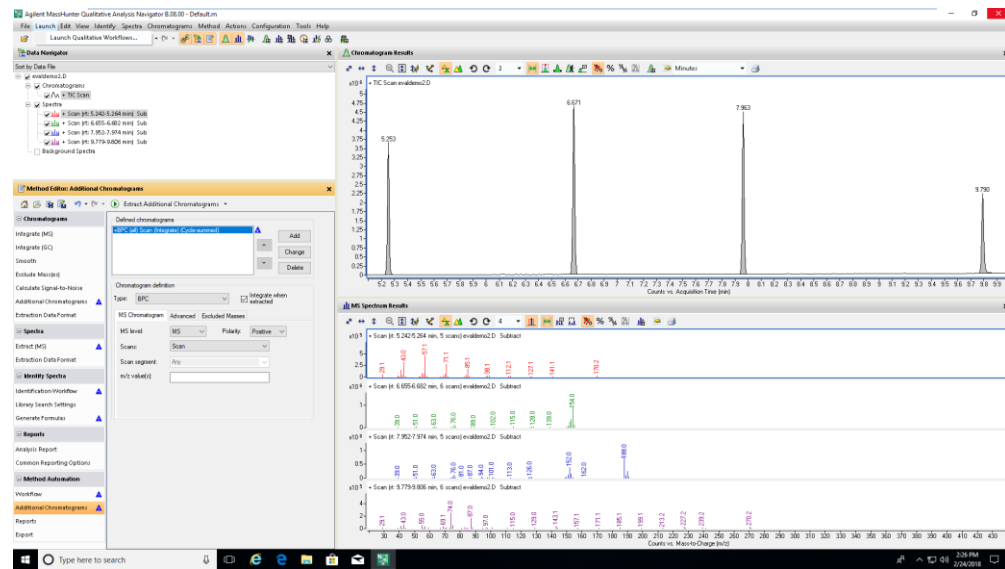
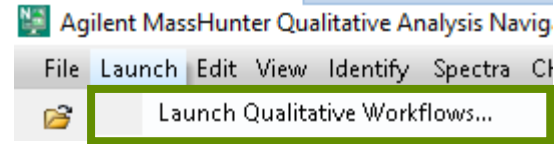
### Workflows

- 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?



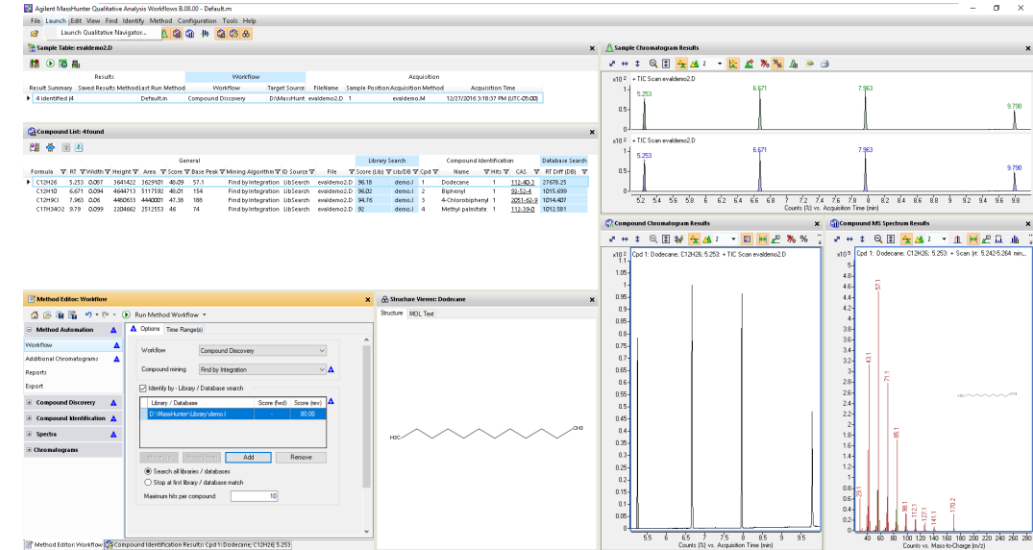
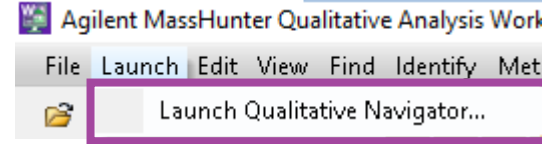
# MassHunter Qualitative Analysis Software B.08.00 SP1

## Navigator and Workflows



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

## Workflows

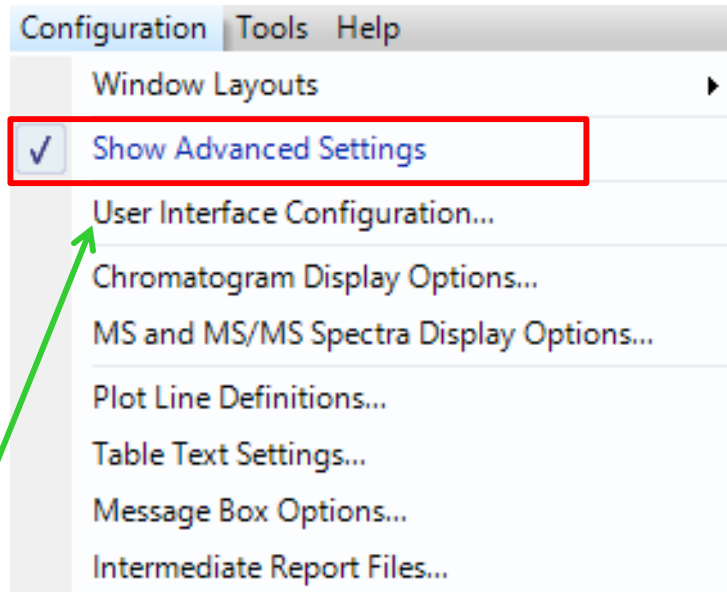


File opens in Navigator.  
The sample chromatogram(s) are extracted.  
No compound results.  
Method is NOT passed to other context.

# 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.

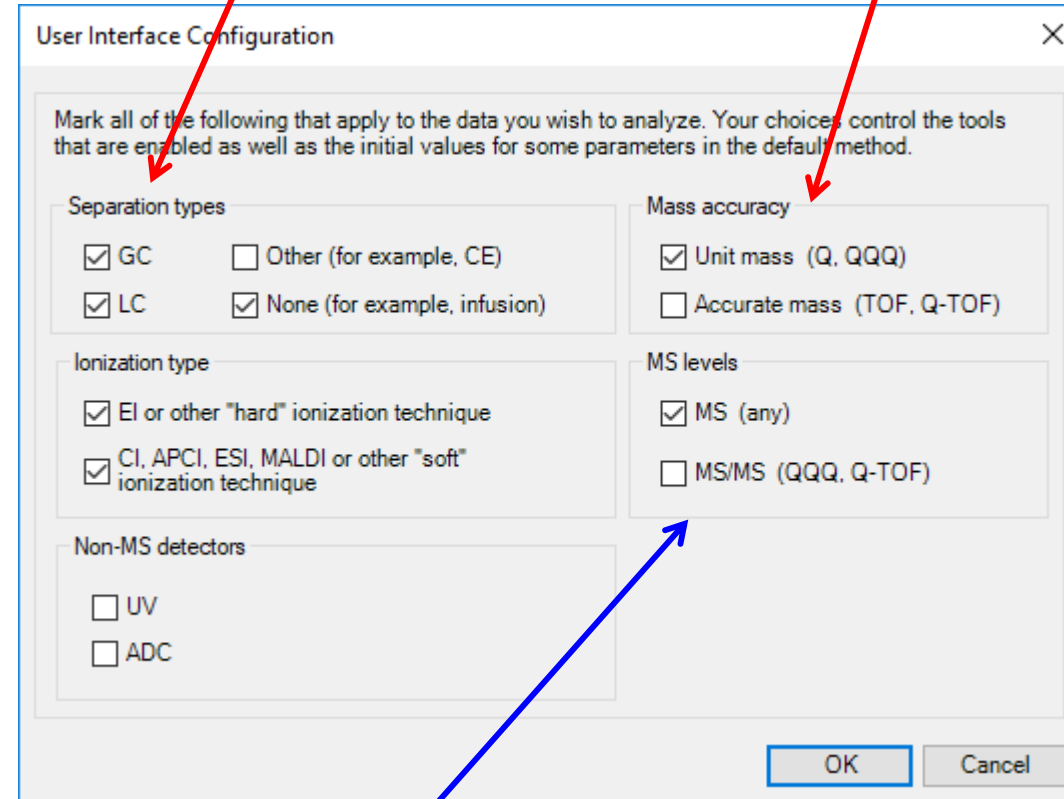
# User Interface Configuration



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)

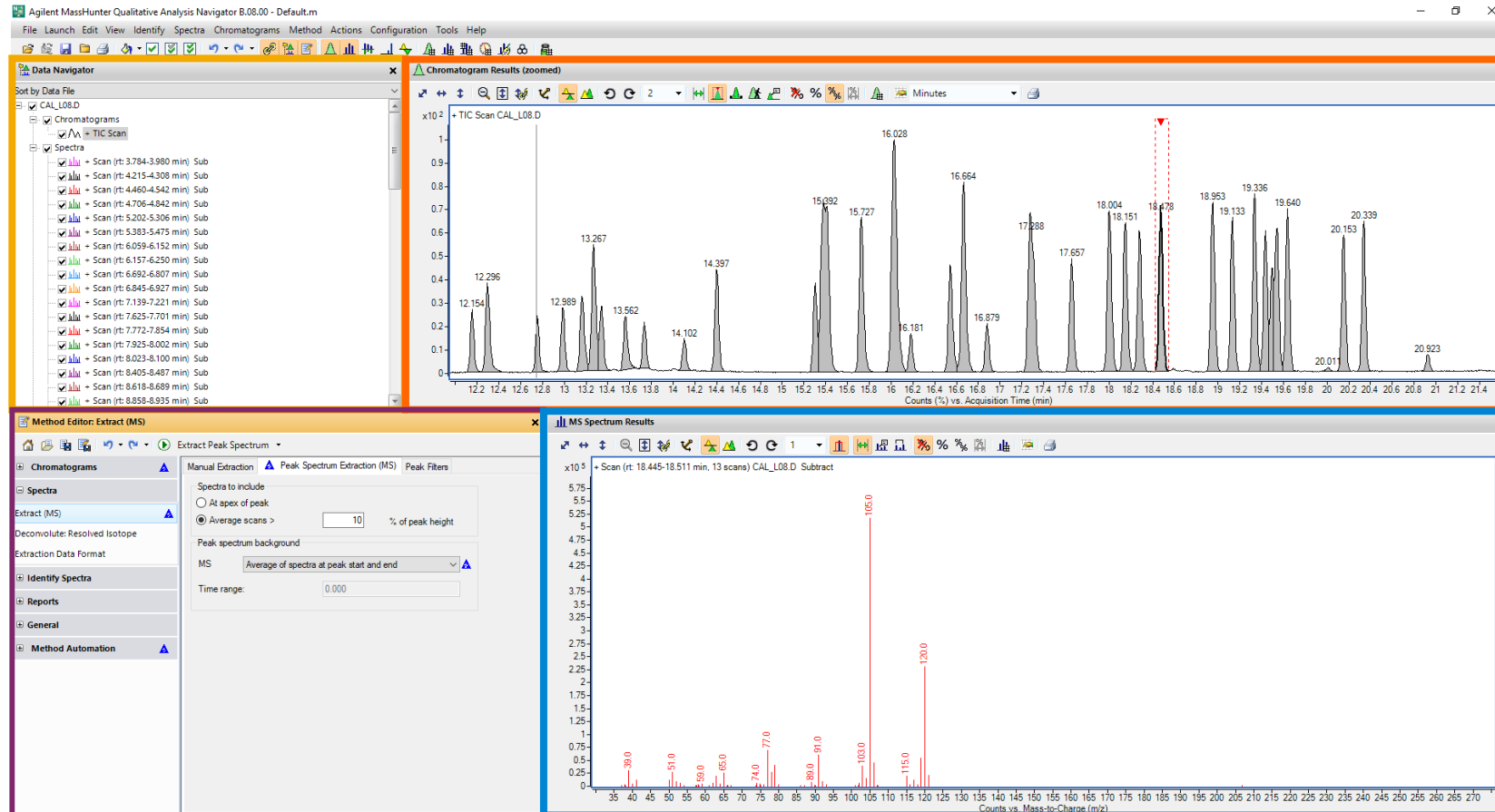


MS Levels: MS or MS/MS

# Navigator View

## Data Navigator

## Chromatogram Results



## Method Editor

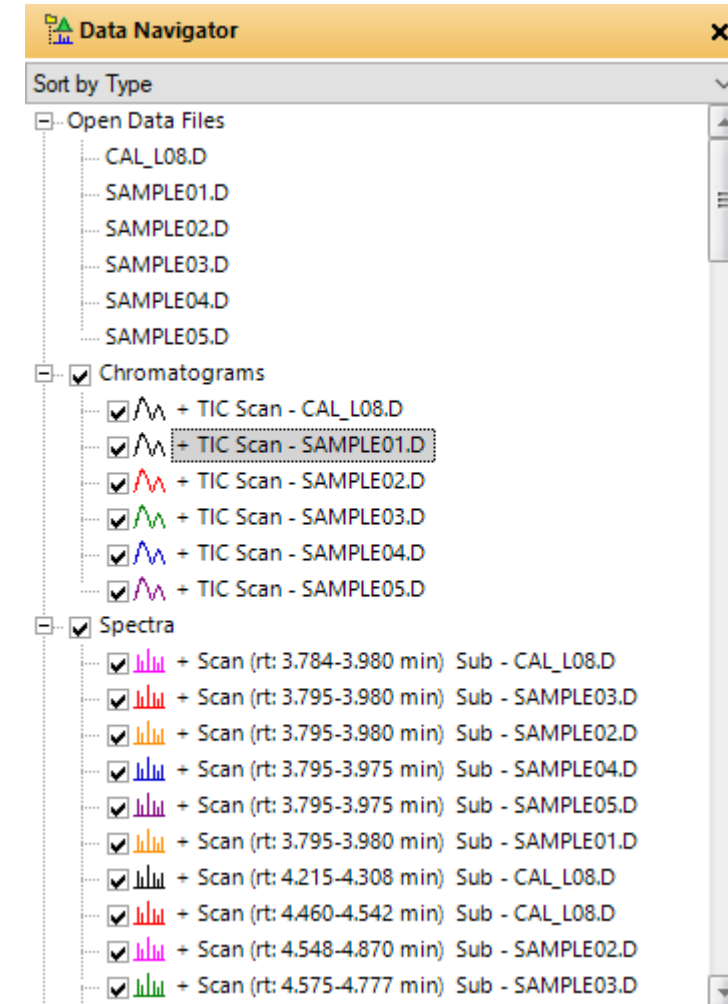
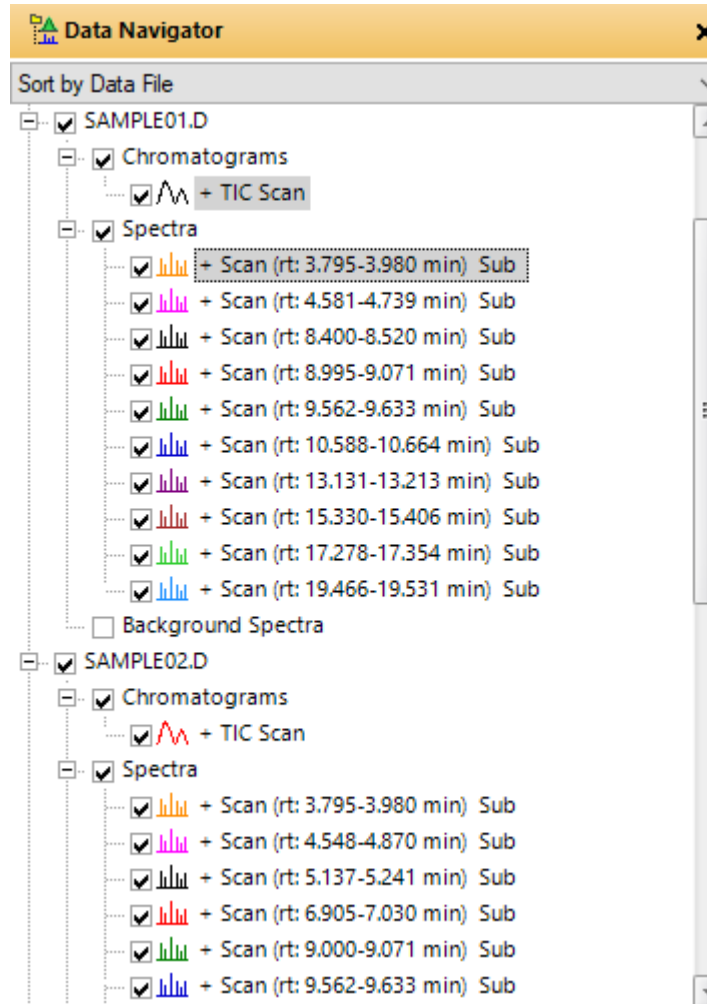
## Spectrum Results

# 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.

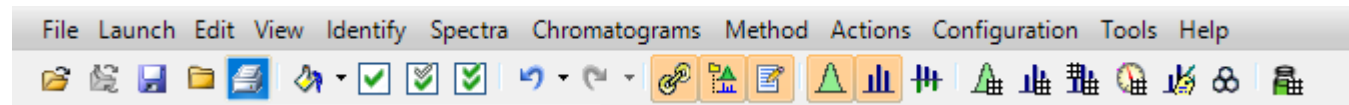
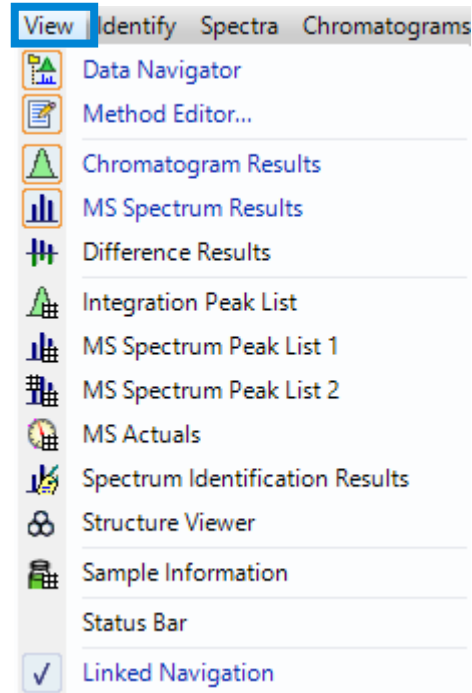
In the top drop-down, the user can choose to sort by Data File or Type.





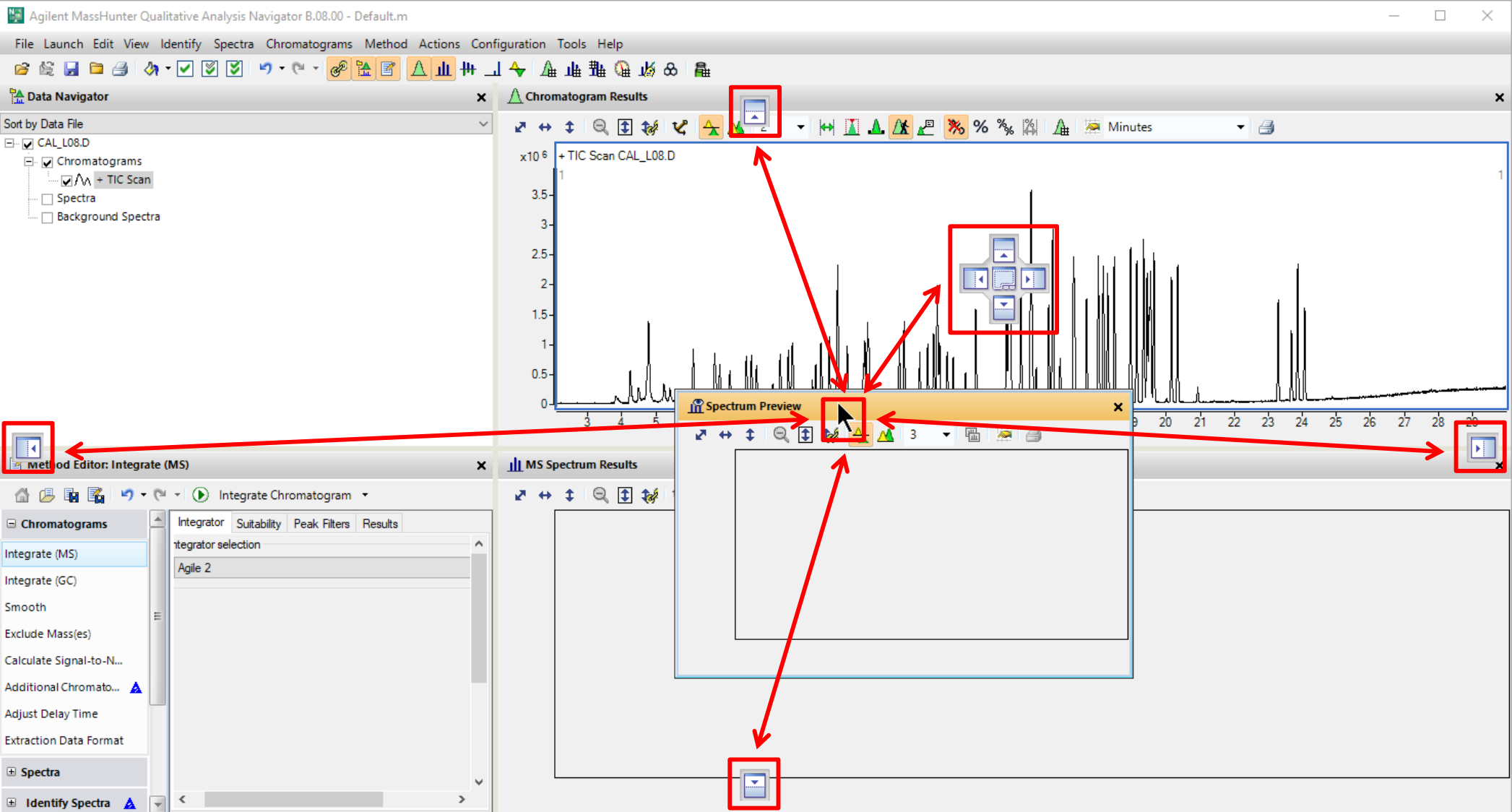
# Expose or Hide Windows as Needed

From the Menu...

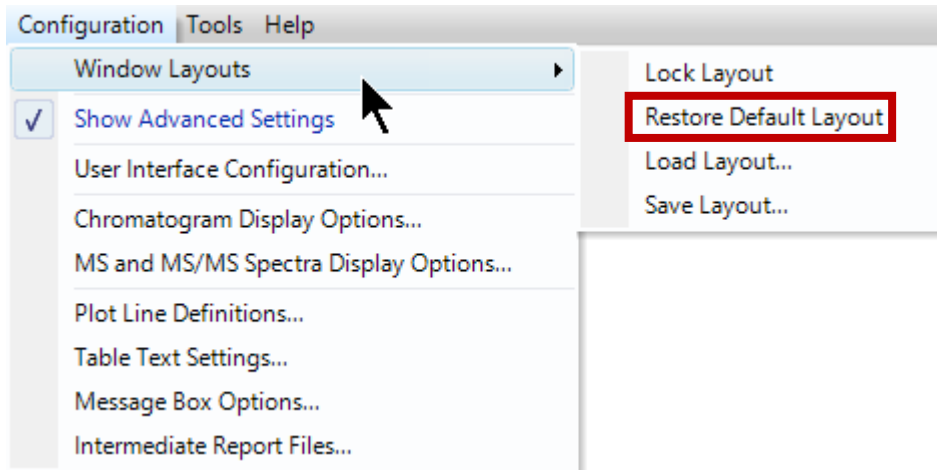


...or from the Toolbar

# Docking & Undocking Windows

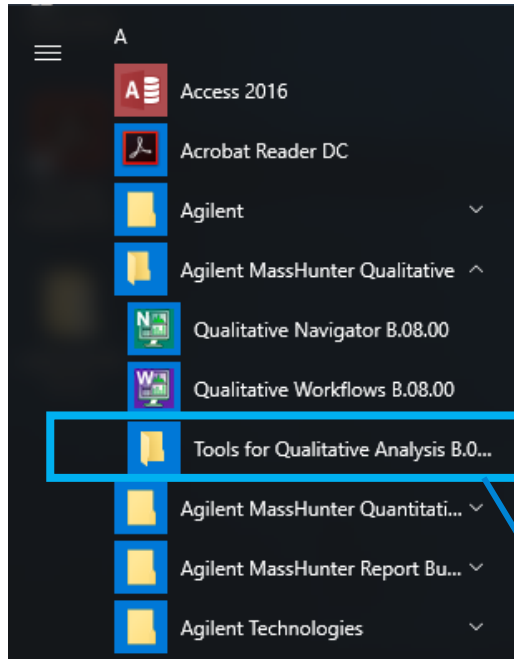


# Restore Default Layout

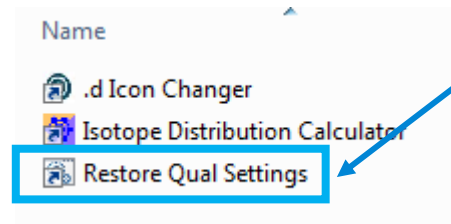
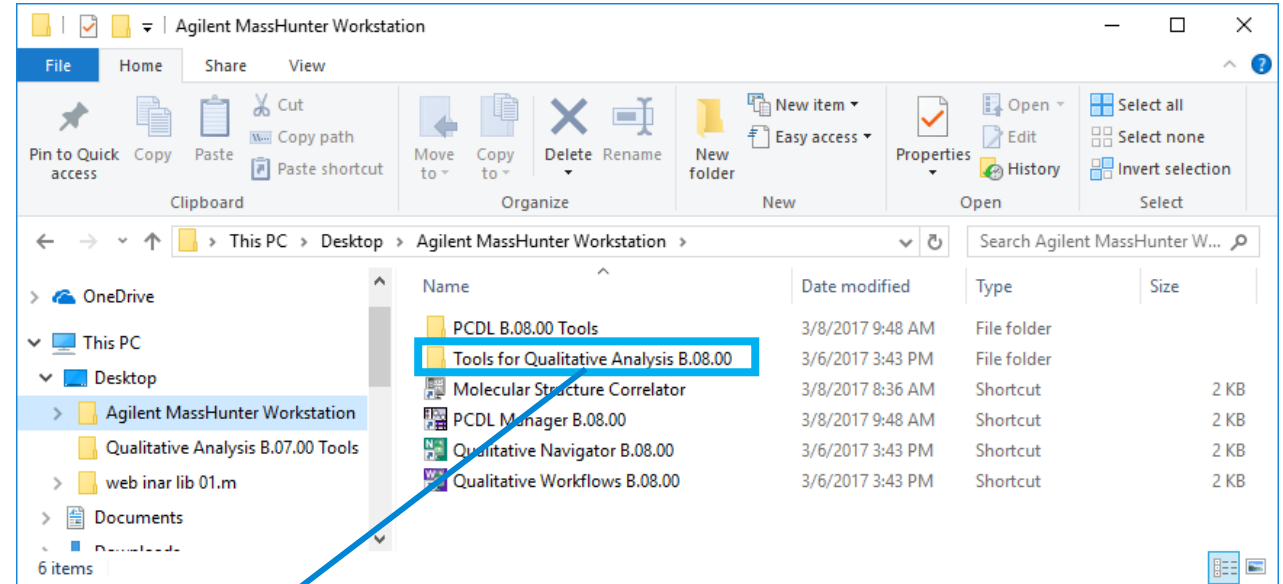


- Complicated windows layouts can be restored to default layout.
- Preferred layouts can be saved and loaded.
- Layouts can be locked.

# 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

File Launch Edit View Identify Spectra Chromatograms Method Actions Configuration Tools Help

Open Data File... Ctrl+O  
Refresh Data File  
Save Results Ctrl+S  
Close Data File  
Close All  
Print  
Exit

Look in: VOA

Recent Items  
Documents  
Desktop  
This PC  
Network

BLANK01.D  
BLANK02.D  
CAL\_L03.D  
CAL\_L04.D  
CAL\_L05.D  
CAL\_L06.D  
CAL\_L07.D  
CAL\_L08.D  
CAL\_L09.D  
CAL\_L10.D  
CAL\_L11.D  
CAL\_L12.D  
CC\_L07.D  
QC\_L06.D

QuantResults  
SAMPLE01.D  
SAMPLE02.D  
SAMPLE03.D  
SAMPLE04.D  
SAMPLE05.D

File name: "CAL\_L08.D" "SAMPLE01.D" "SAMPLE02.D" "SAMPLE03.D" "SAMPLE04.D"  
Files of type: Data Files (\*.d)

Options  
 Load worklist method  
 Load results method  
 Use current method  
 Load result data  
 Run method workflow

Sample Information  
Sample Name :  
User Name :  
Sample Position :  
Description :

Method Editor: Integrate (MS)  
Integrator Suitability Peak Filters Results  
Integrator selection  
Agile 2

Chromatograms  
Integrate (MS)  
Integrate (MS/MS)  
Integrate (UV)  
Integrate (GC)  
Integrate (ADC)  
Smooth  
Exclude Mass(es)  
Calculate Signal...  
Additional Chrom...  
Adjust Delay Time

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

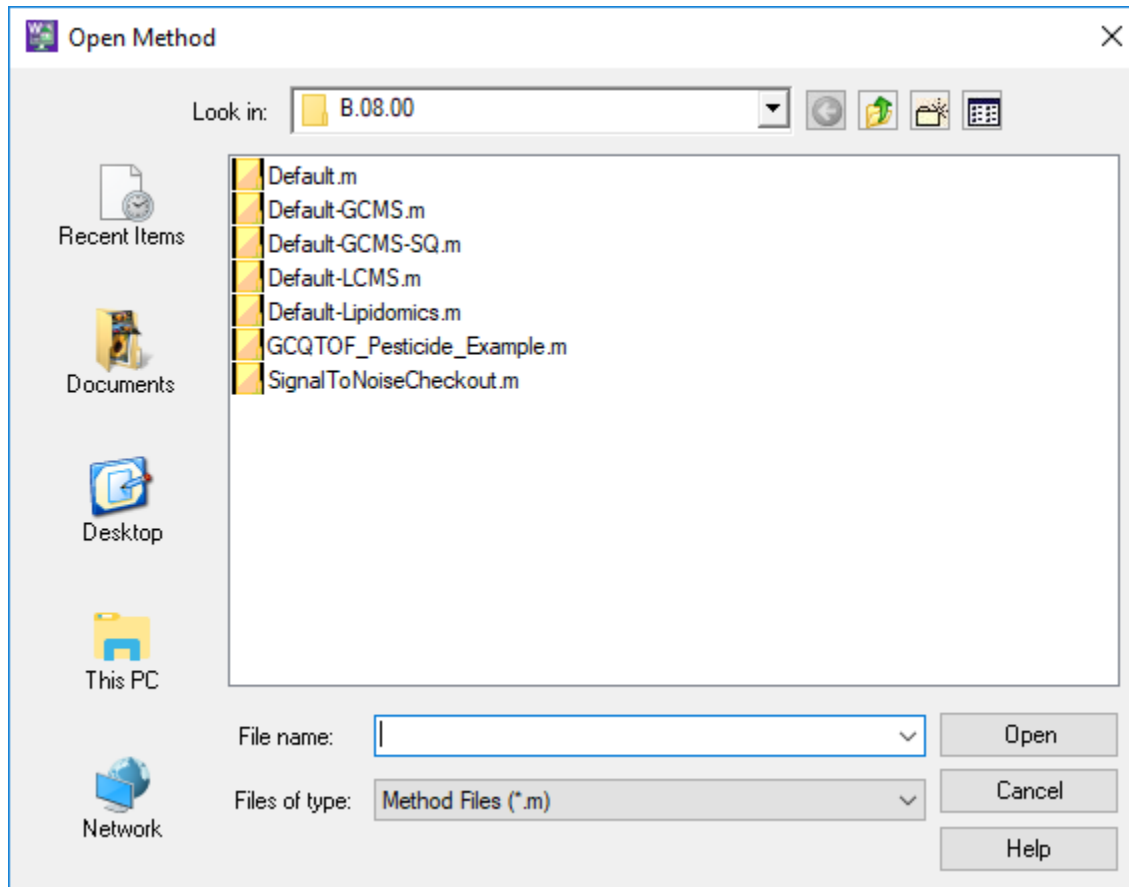
If **Load result data** is checked, the qualitative data manipulations previously saved will be loaded.

**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



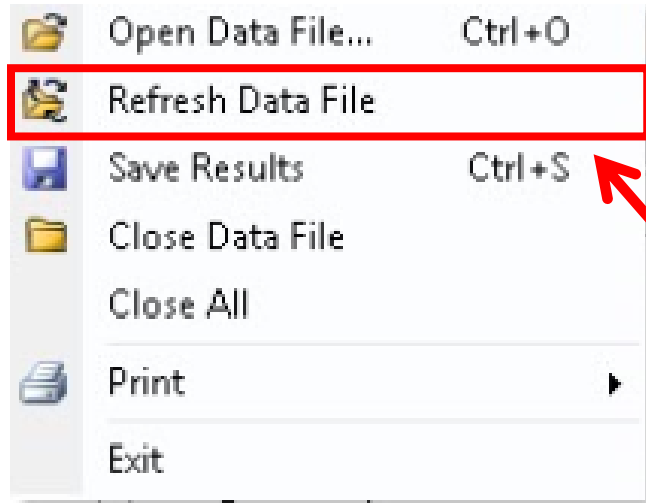
## Open Method

- 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

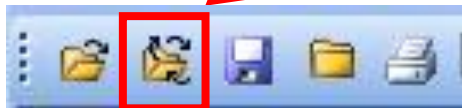
Feature is useful when it is desired to view data as the data file is being acquired.

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

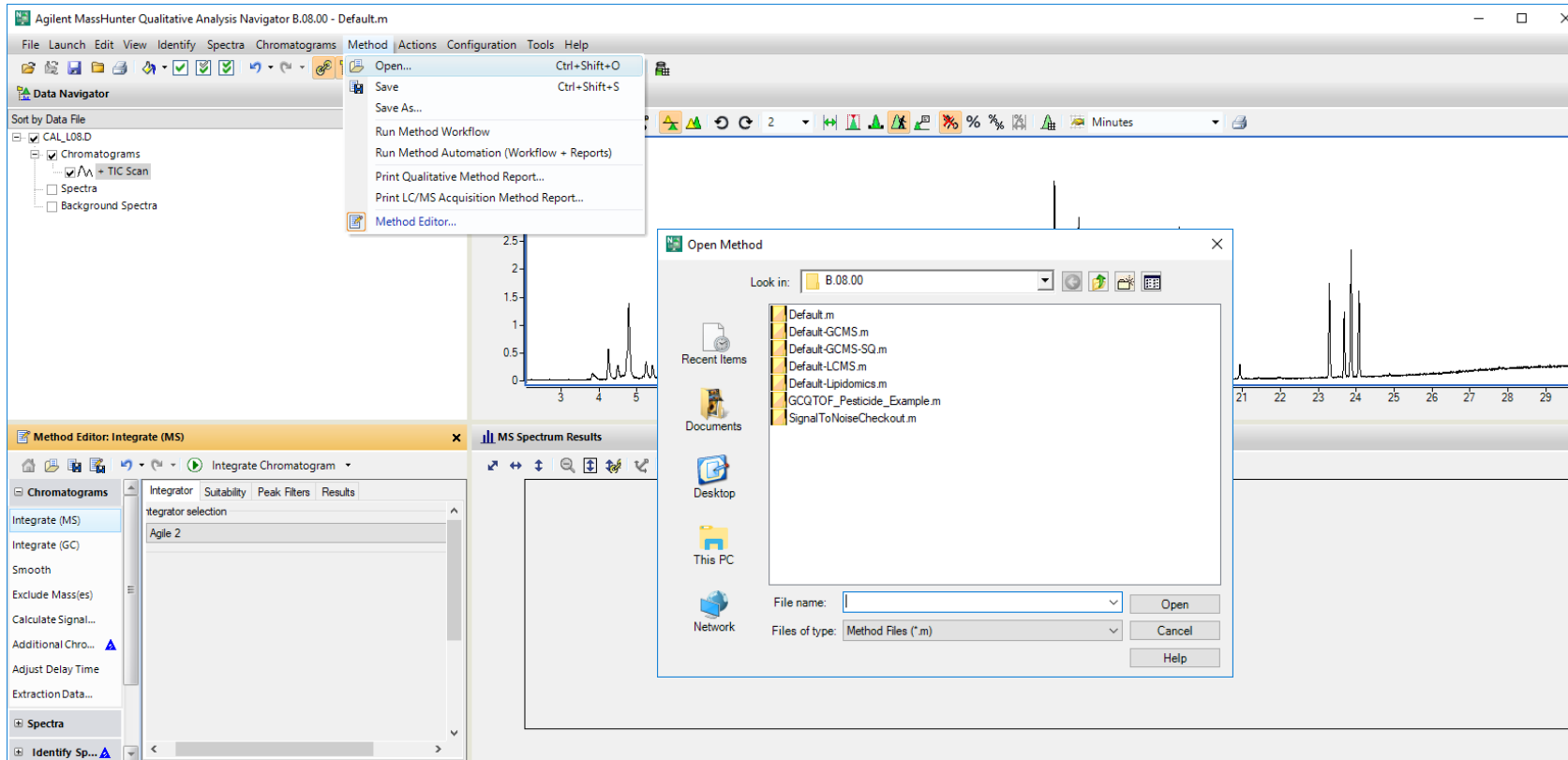
Then use **Refresh Data File** to update the view and add the most recently acquired data.

**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



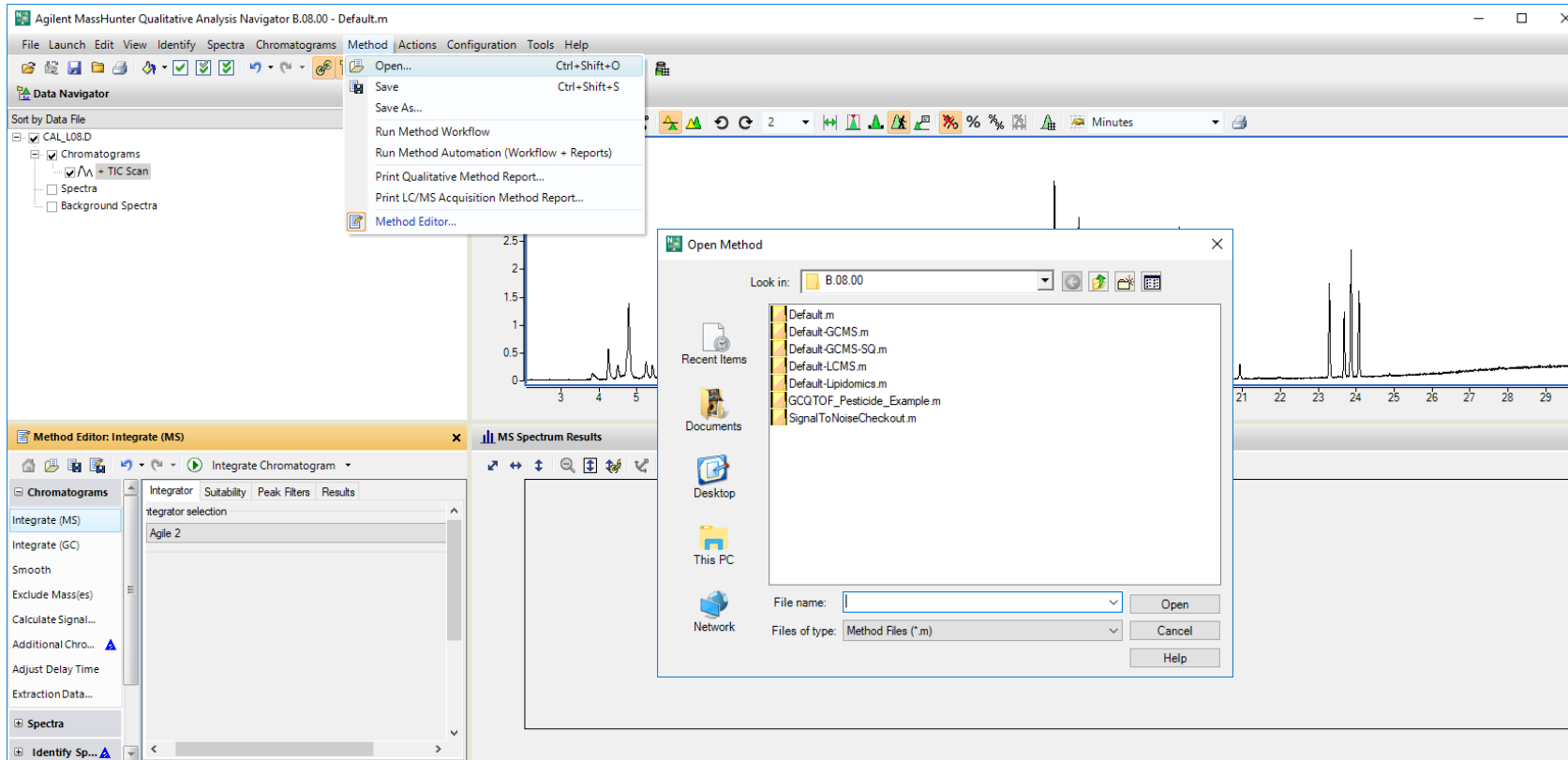
Qualitative Analysis Methods are stored in a .M folder.

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

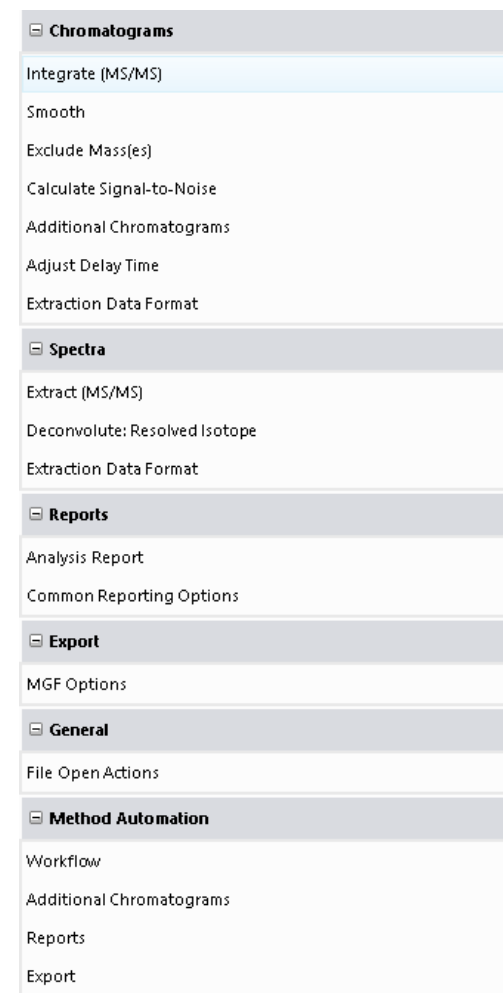
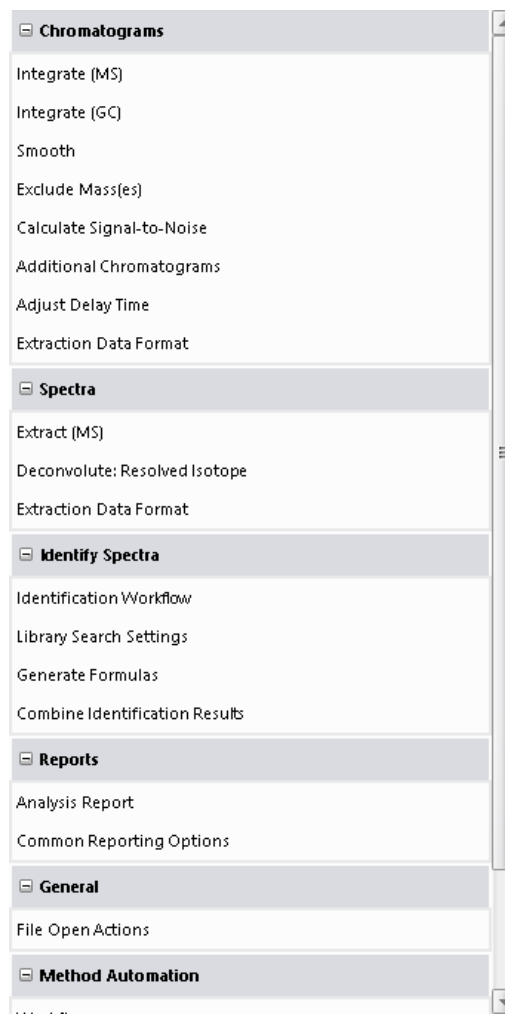
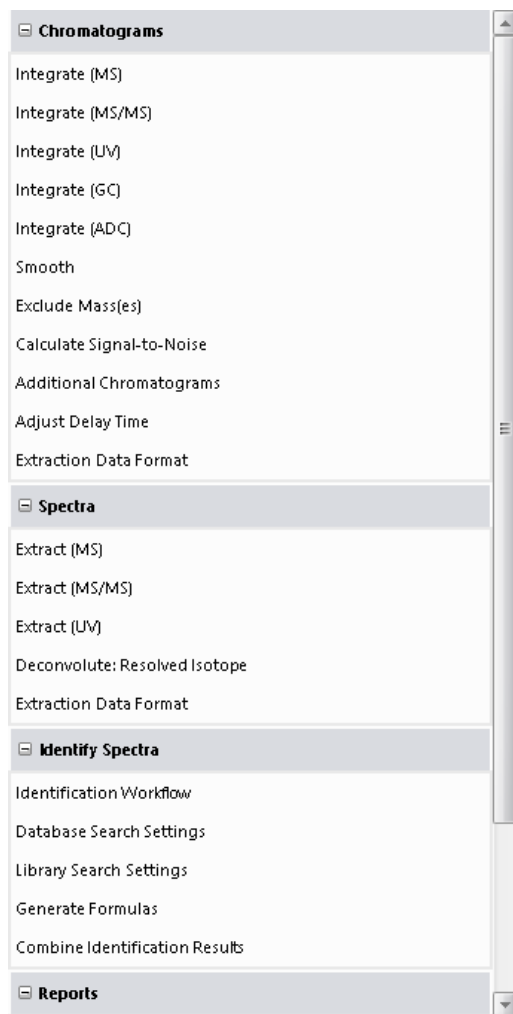


Qualitative Analysis Navigator and Workflows Methods are stored in a .M folder.  
Quantitative Analysis Methods are stored in a .M folder.  
Quantitative Analysis Reporting Methods are stored in a .M folder.

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

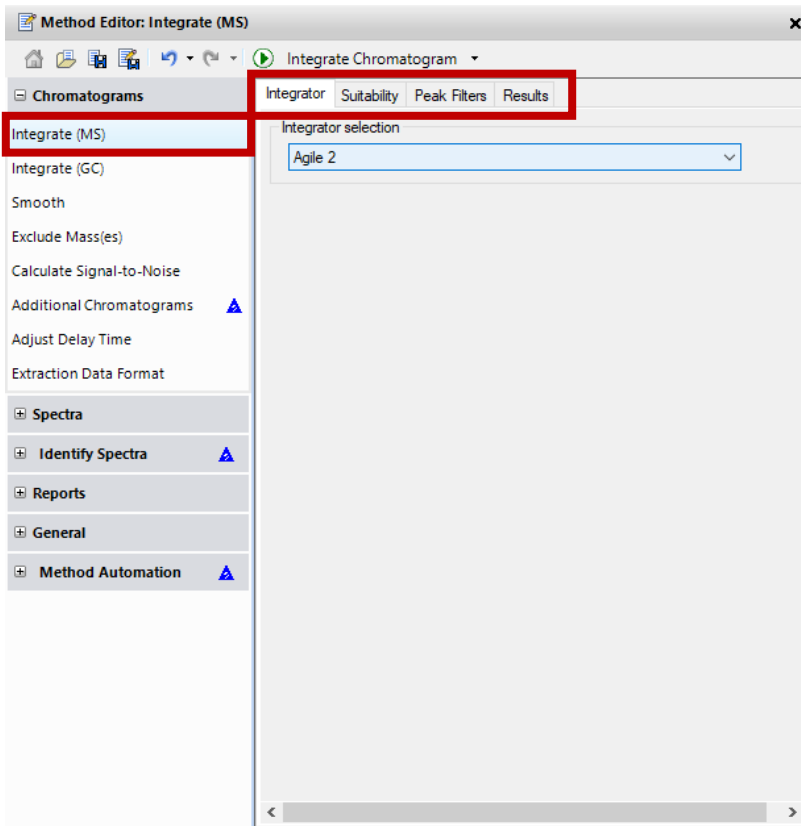
LC QQQ MRM Data Loaded

# Method Editor

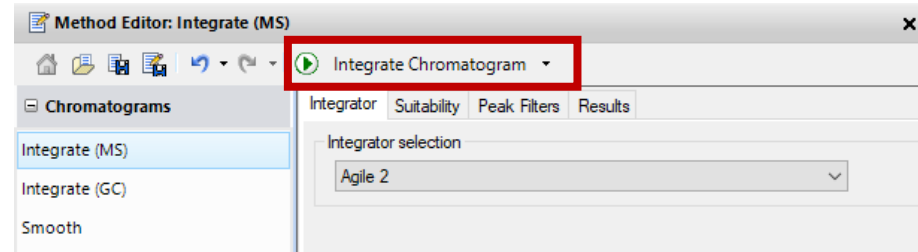
## View > Method Editor

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

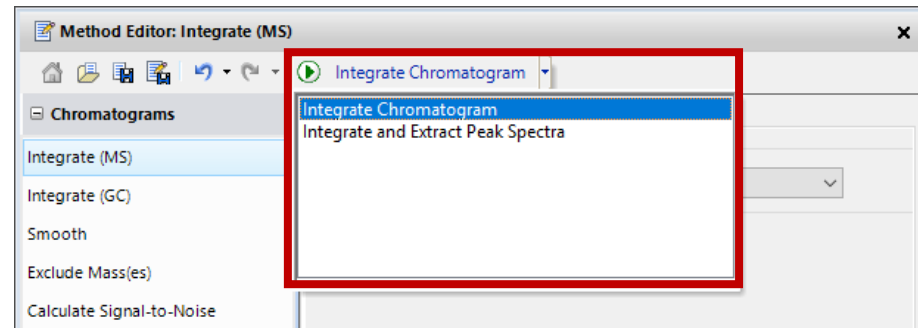
Tabs within a section further organize method parameters.



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 then display them for selection.

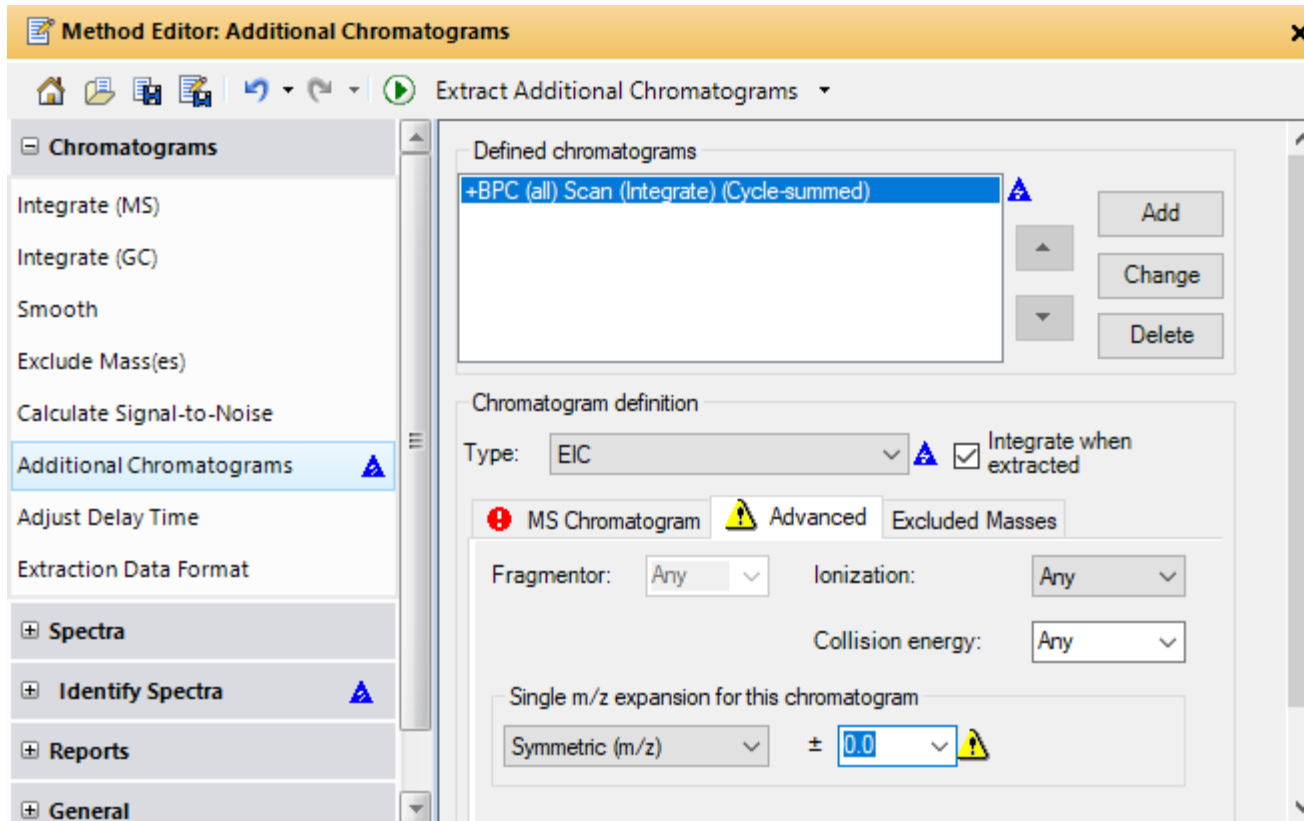


# Relationship Between Action and Method Editor

The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator B.08.00 interface. The 'Main Menu' is open, showing options like 'Integrate Chromatogram' and 'Integrate and Extract Peak Spectra'. The 'Right Click Menu' is also open, showing options like 'Integrate Chromatogram' and 'Integrate and Extract Peak Spectra'. The 'Run Button' is highlighted in the 'Method Editor: Integrate (MS)' window. The background shows a chromatogram plot of TIC Scan CAL\_L08.D and an MS Spectrum Results plot.

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:

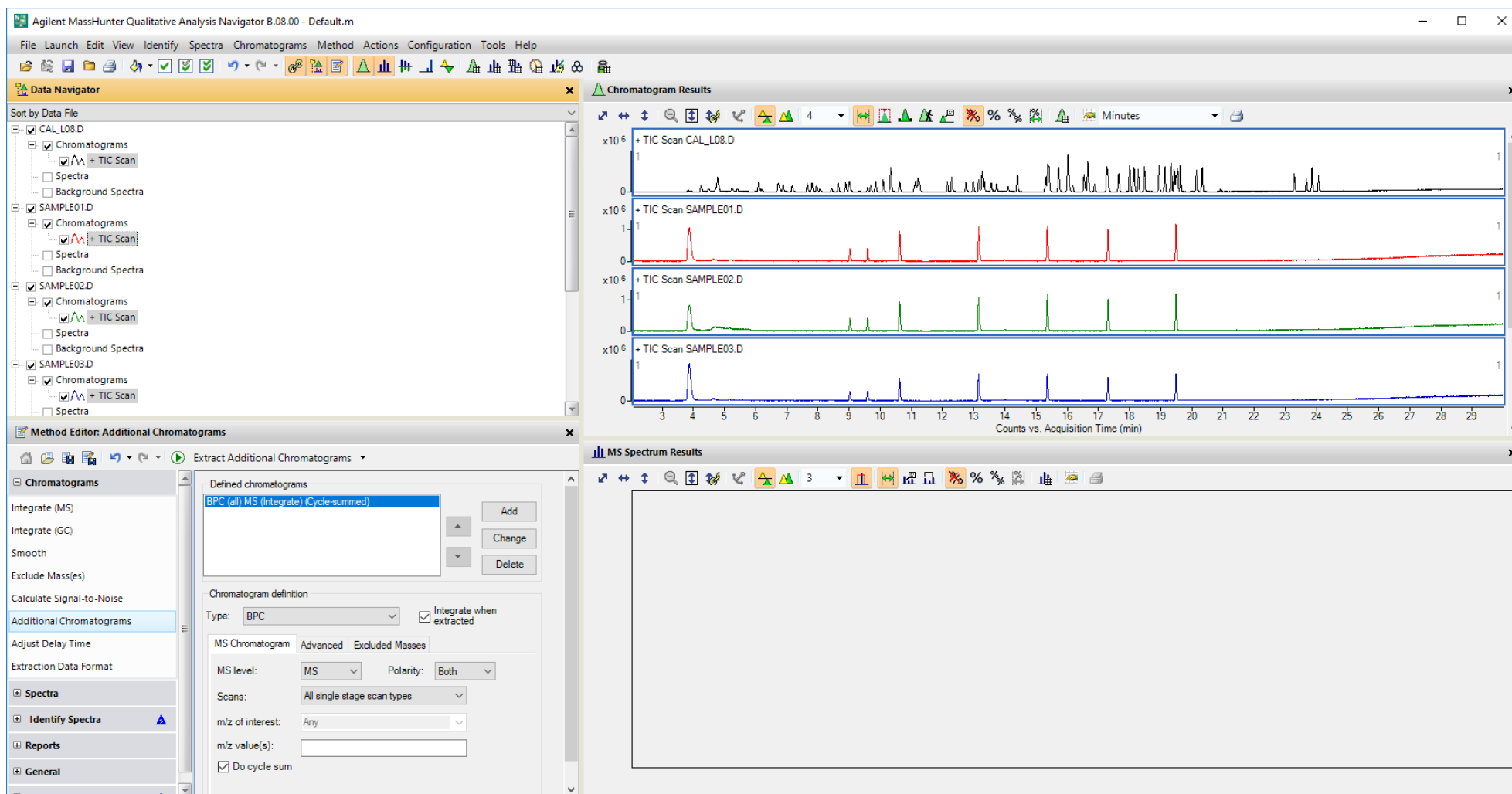
Working with Chromatograms



# 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

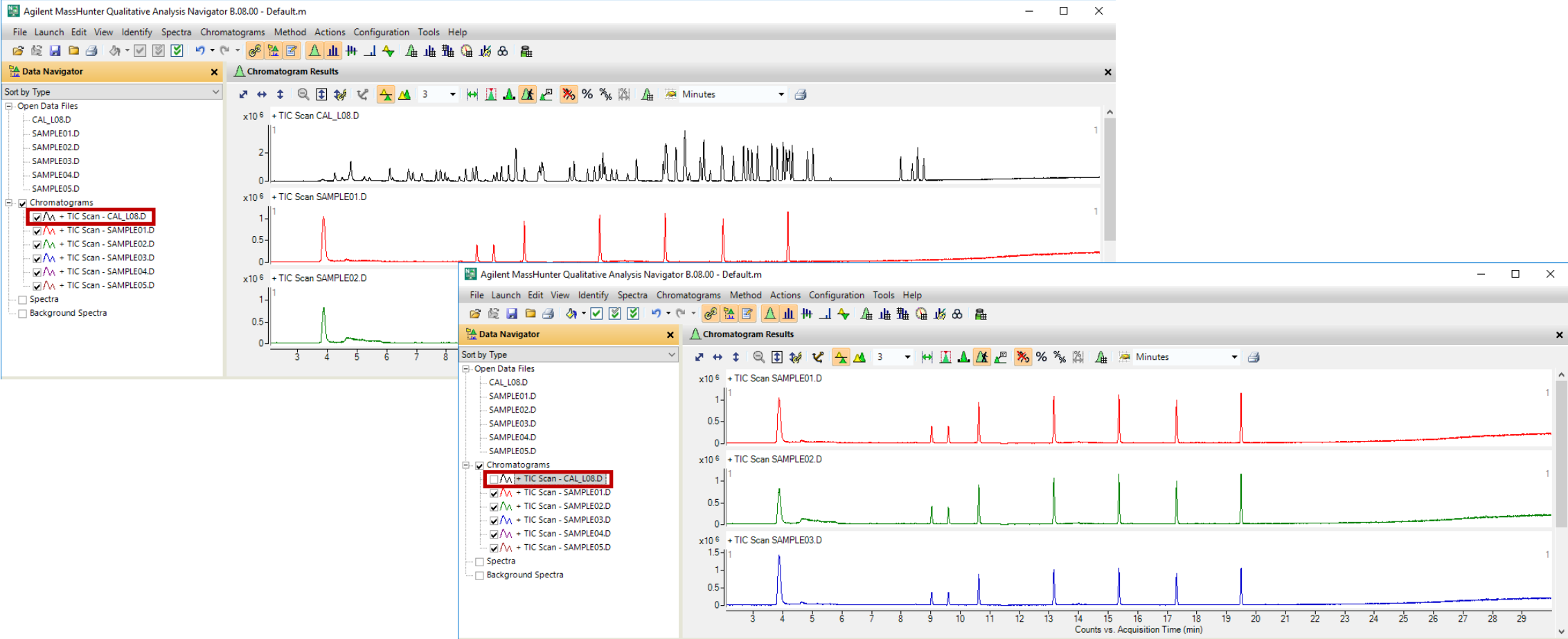


# Extract Chromatogram

The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator interface. On the left, the 'Data Navigator' tree shows a project structure with files like CAL\_L08.D and SAMPLE01.D through SAMPLE05.D. A context menu is open over the 'Chromatograms' folder, with 'Extract Chromatograms...' selected. A red arrow points from this menu item to the 'Extract Chromatograms' dialog box. The dialog box is titled 'Extract Chromatograms' and contains a 'List of opened data files' section listing the project files. Below this, there are configuration options: 'Type' is set to 'EIC', 'Integrate when extracted' is checked, 'MS Chromatogram' is selected, 'Advanced' and 'Excluded Masses' tabs are visible, 'MS level' is 'All', 'Polarity' is 'Positive', 'Scans' is 'All scan types', 'm/z of interest' is 'Any', and 'm/z value(s)' is '78'. There are 'OK' and 'Cancel' buttons at the bottom of the dialog. In the background, a 'Chromatogram Results' window shows several Total Ion Chromatogram (TIC) traces for the different data files, with a time axis from 17 to 29 minutes.

Available Chromatogram types are determined by data in file.

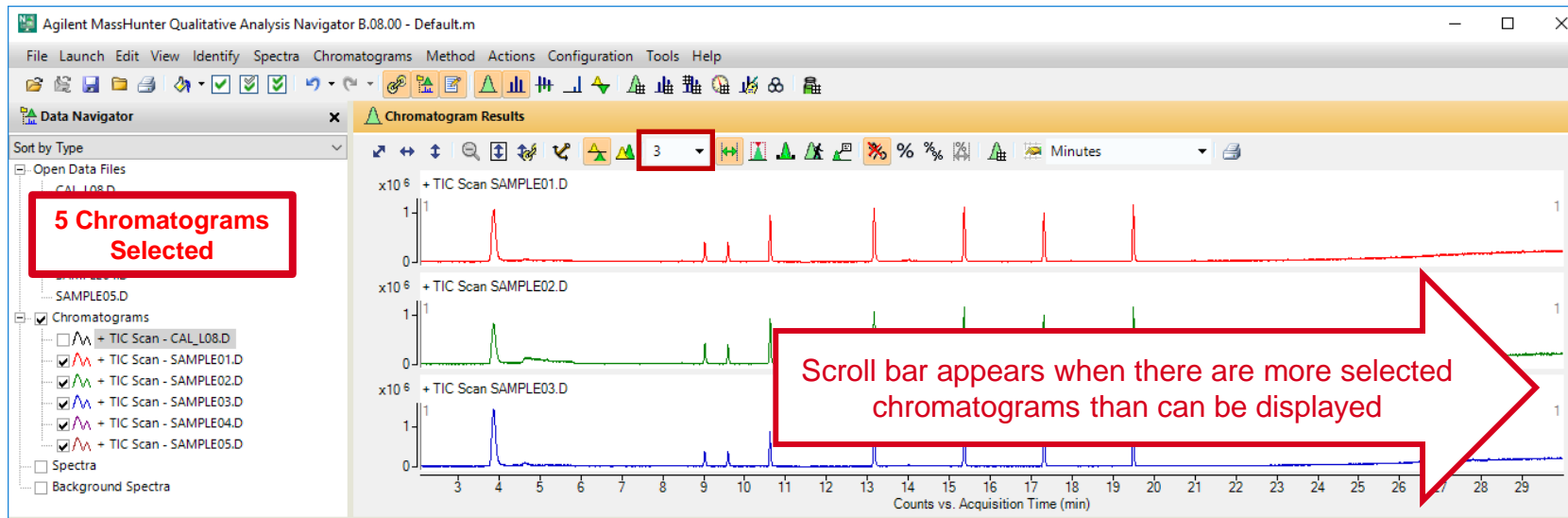
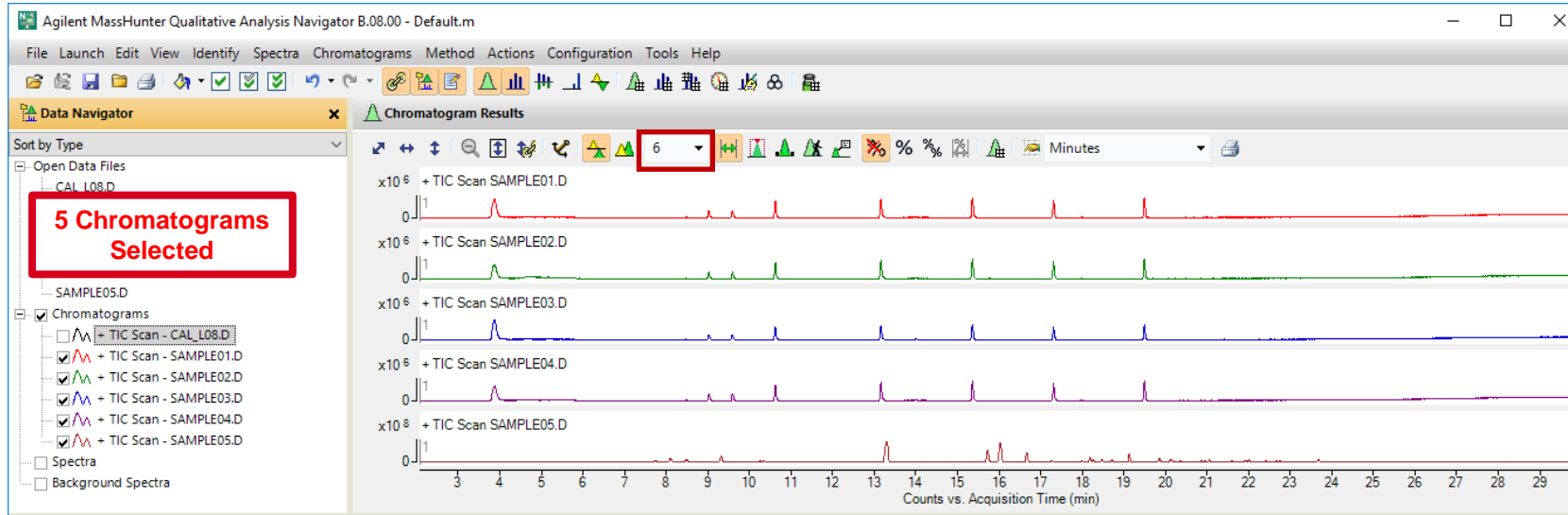
# Selecting Chromatograms for Display



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

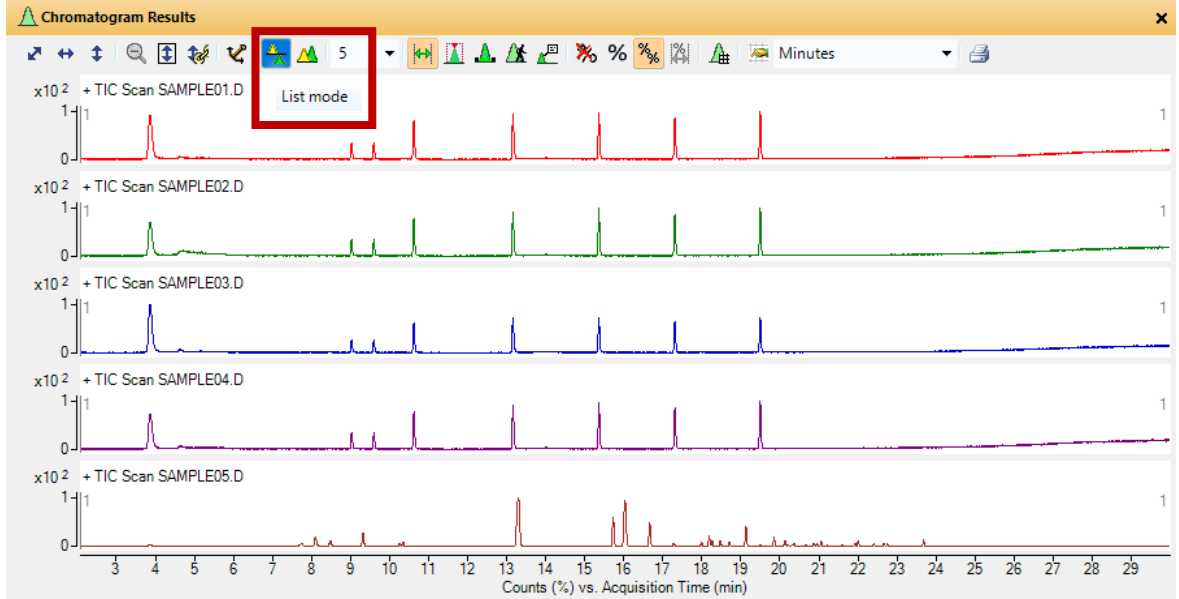
# Specify Number of Chromatograms Displayed

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

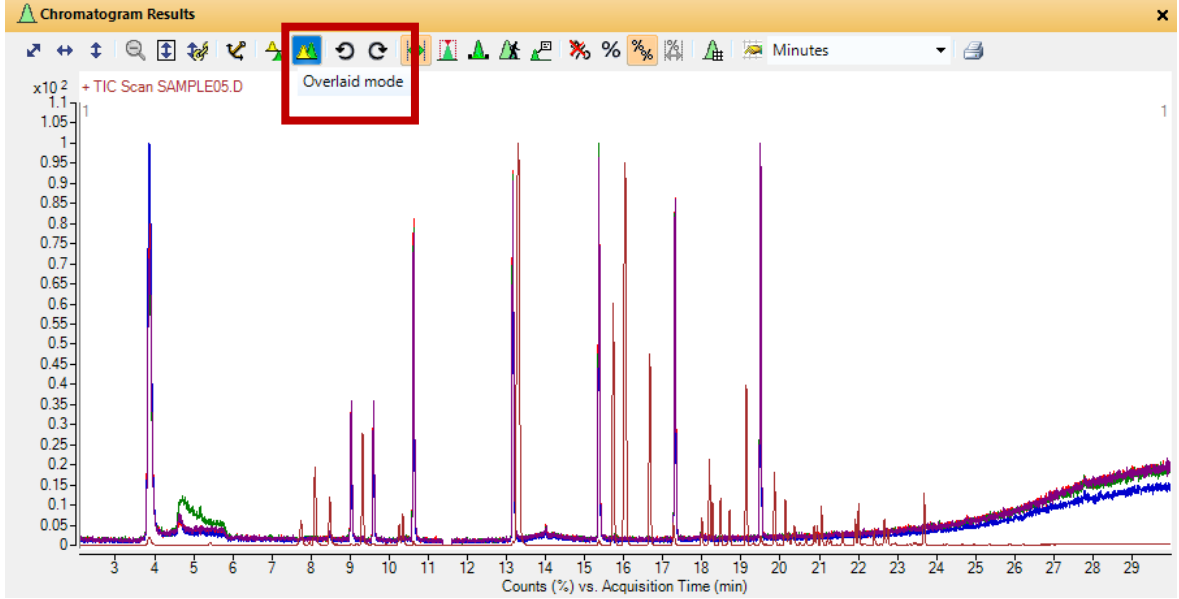
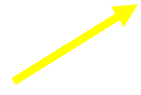


# Overlay vs. List Mode Chromatograms

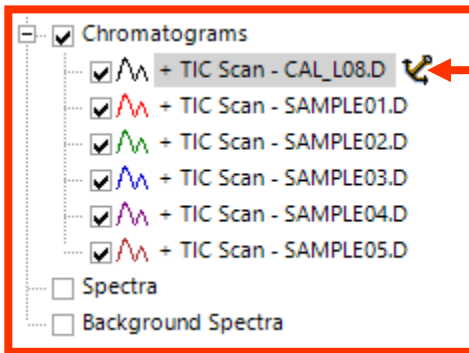
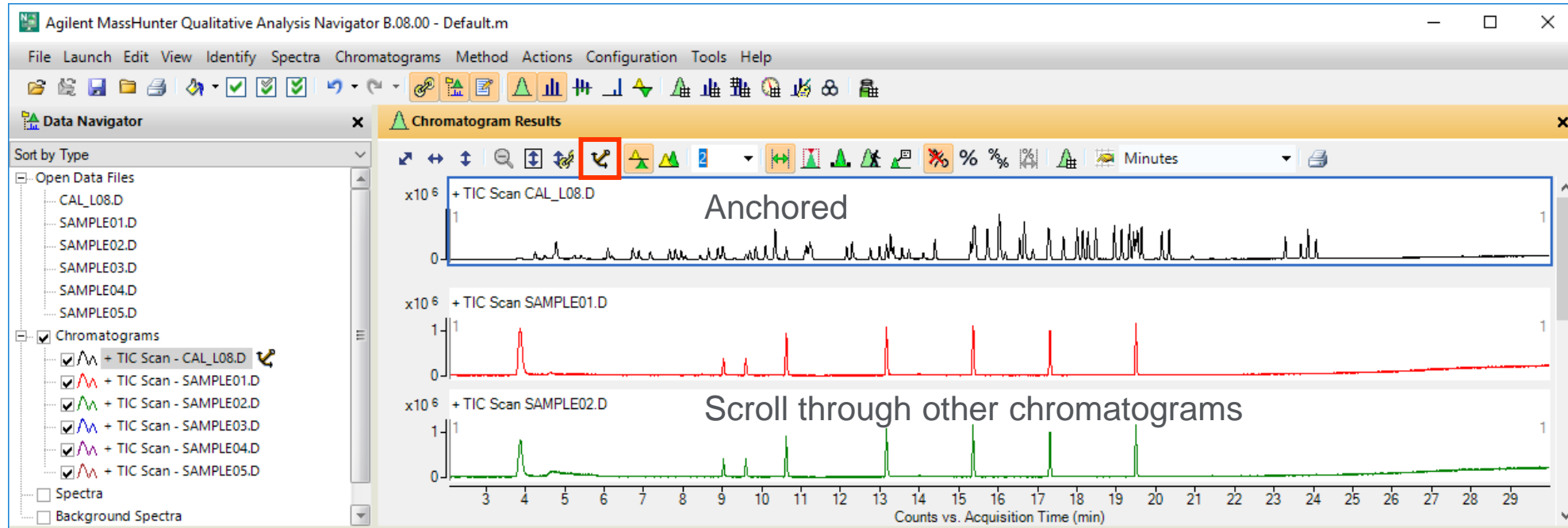
List  
(Separated)



Overlaid



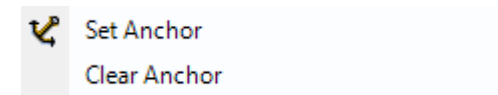
# Anchoring



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

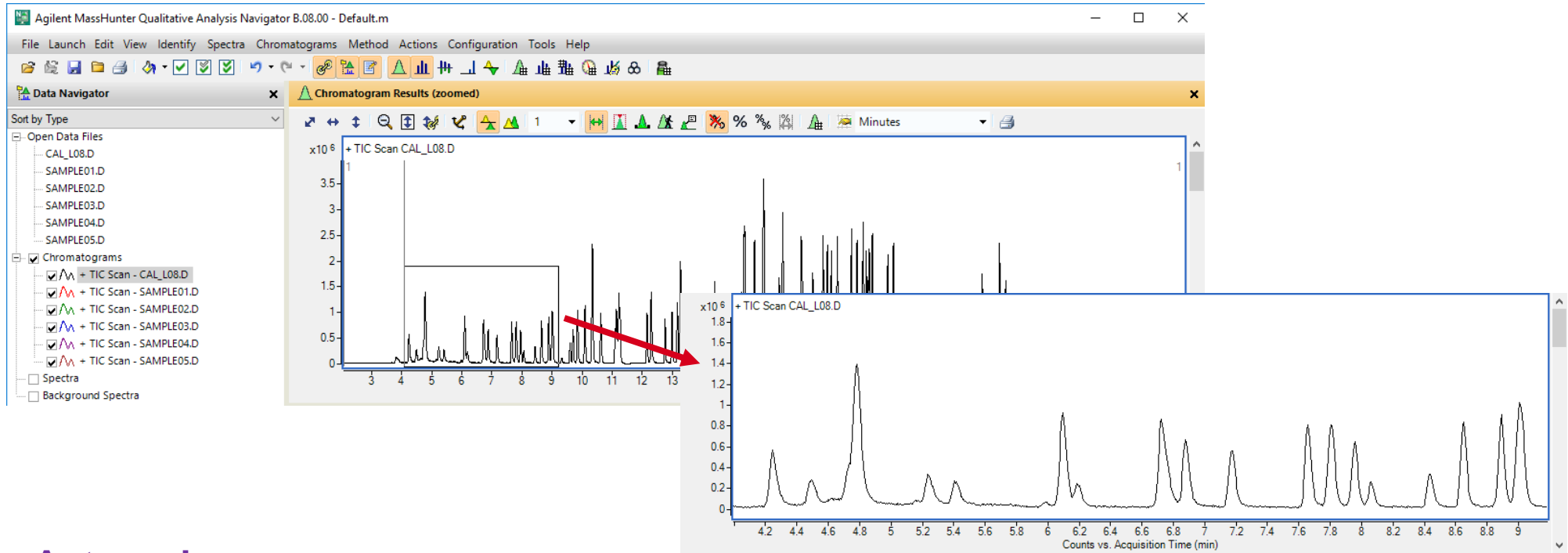
Only one Chromatogram can be anchored at a time.

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

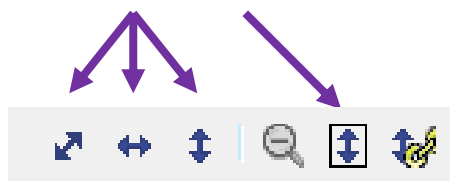


# Zooming

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



**Autoscale**



**Unzoom  
(multiple levels)**

**Linked Y-axis**

**Normalization**



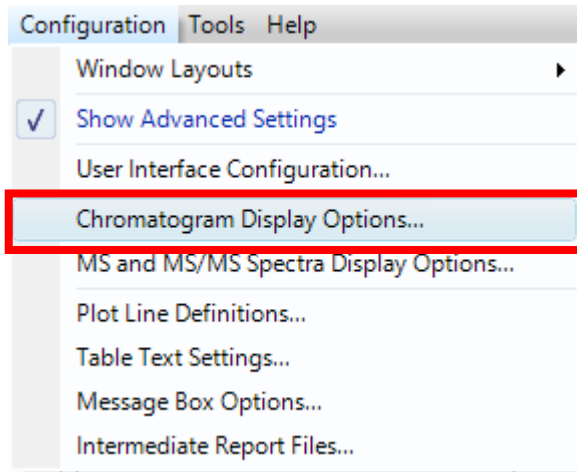
Scale to largest in Each  
over Selected Range

Scale to largest in Each

Scale to largest in All

Scale Off

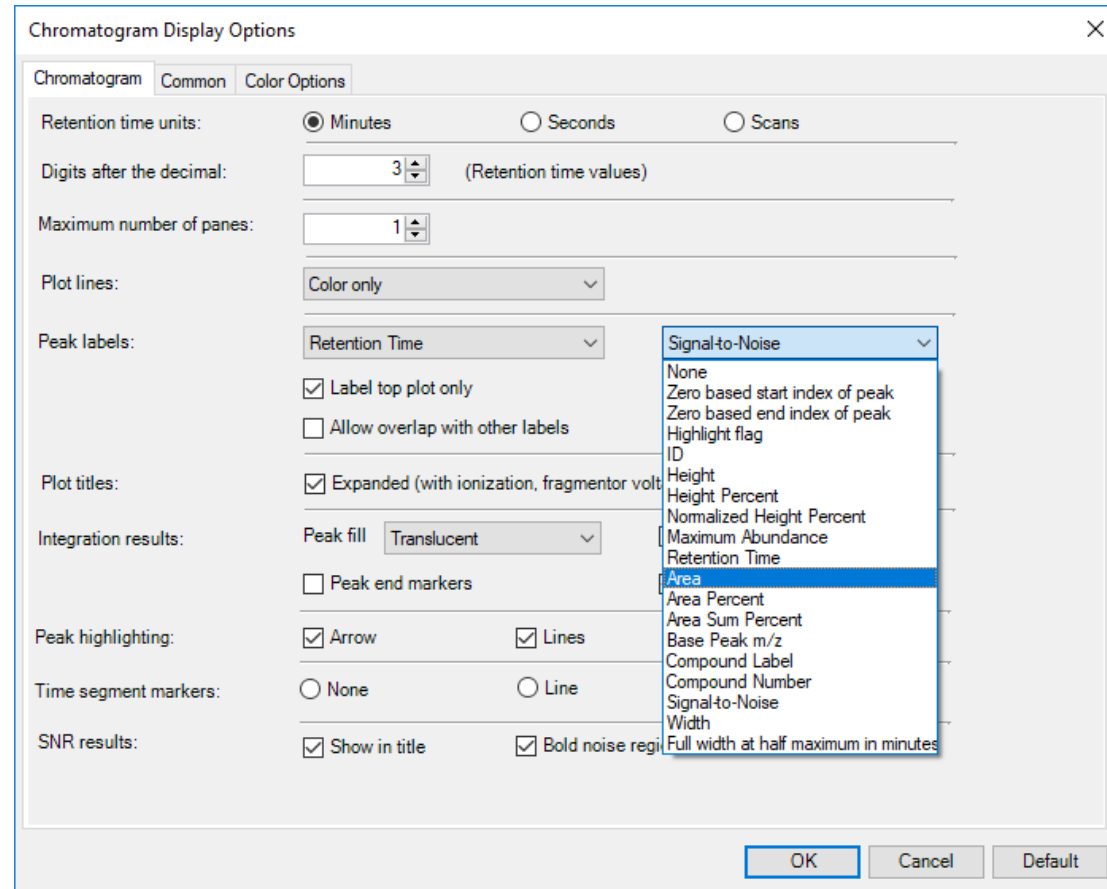
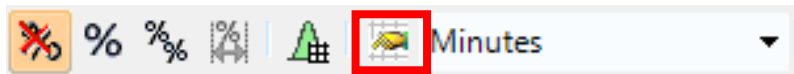
# Chromatogram Display Options



## Main Menu

Show Advanced Settings is checked.

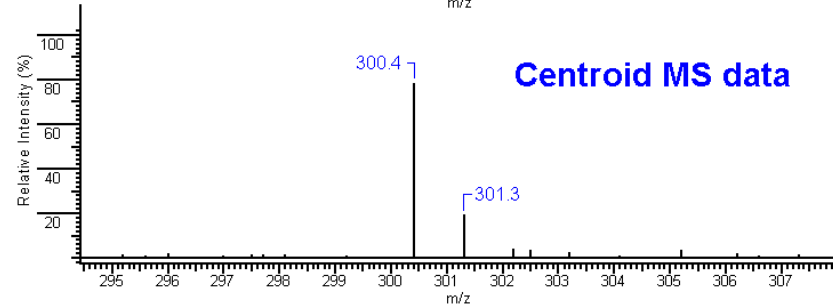
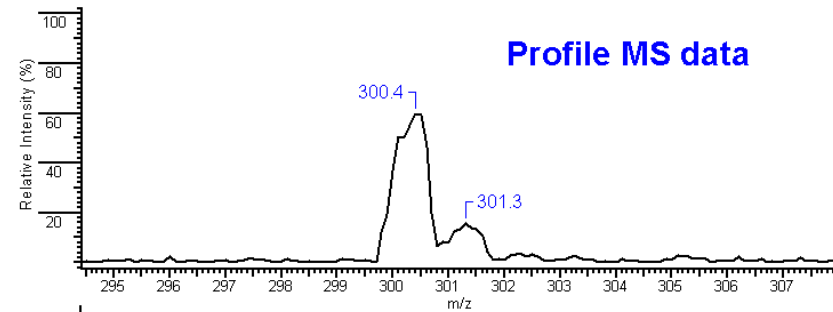
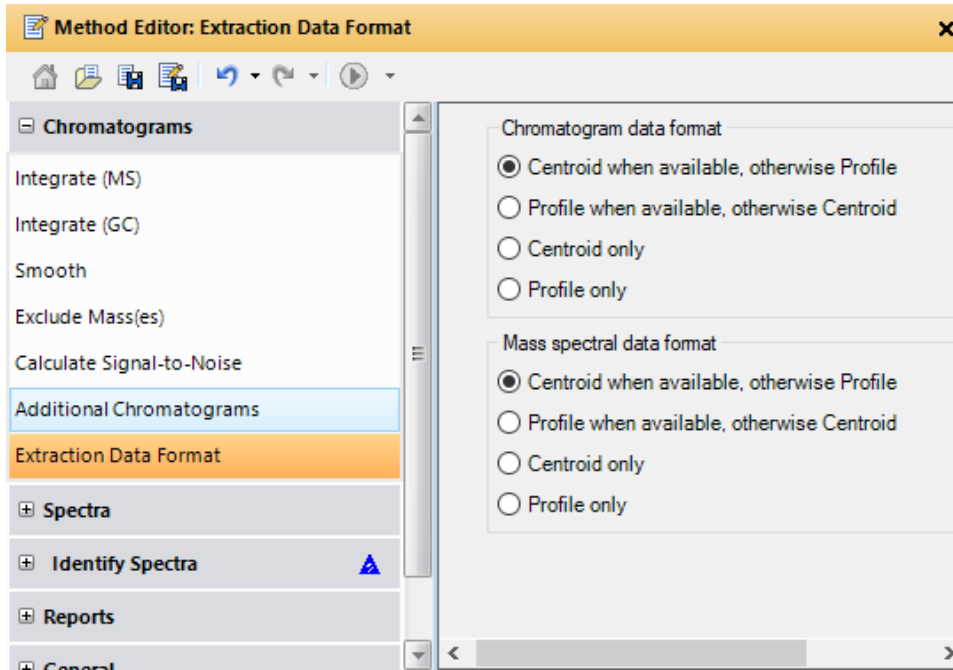
## Within Toolbar



Customize Appearance of Chromatograms

# Extraction Data Format - Profile and Centroid

- Data files may contain Centroid, 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

The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator B.08.00 interface. The main window shows three Total Ion Chromatograms (TIC) for samples CAL\_L08.D, SAMPLE01.D, and SAMPLE02.D. A context menu is open over the chromatograms, with the 'Extract Additional Chromatograms' option selected. A red arrow points from this menu option to the 'Extract Defined Chromatograms' dialog box. The dialog box lists the following data files: CAL\_L08.D, SAMPLE01.D, SAMPLE02.D, SAMPLE03.D, SAMPLE04.D, and SAMPLE05.D. The 'Extract' button is highlighted.

**Method Editor: Additional Chromatograms**

**Chromatograms**

- Integrate (MS)
- Integrate (GC)
- Smooth
- Exclude Mass(es)
- Calculate Signal-to-Noise
- Additional Chromatograms**
- Adjust Delay Time
- Extraction Data Format

**Defined chromatograms**

- EIC (78.0 m/z) MS (Integrate) (Cycle-summed)

**Chromatogram definition**

Type: EIC  Integrate when extracted

MS Chromatogram: Advanced Excluded Masses

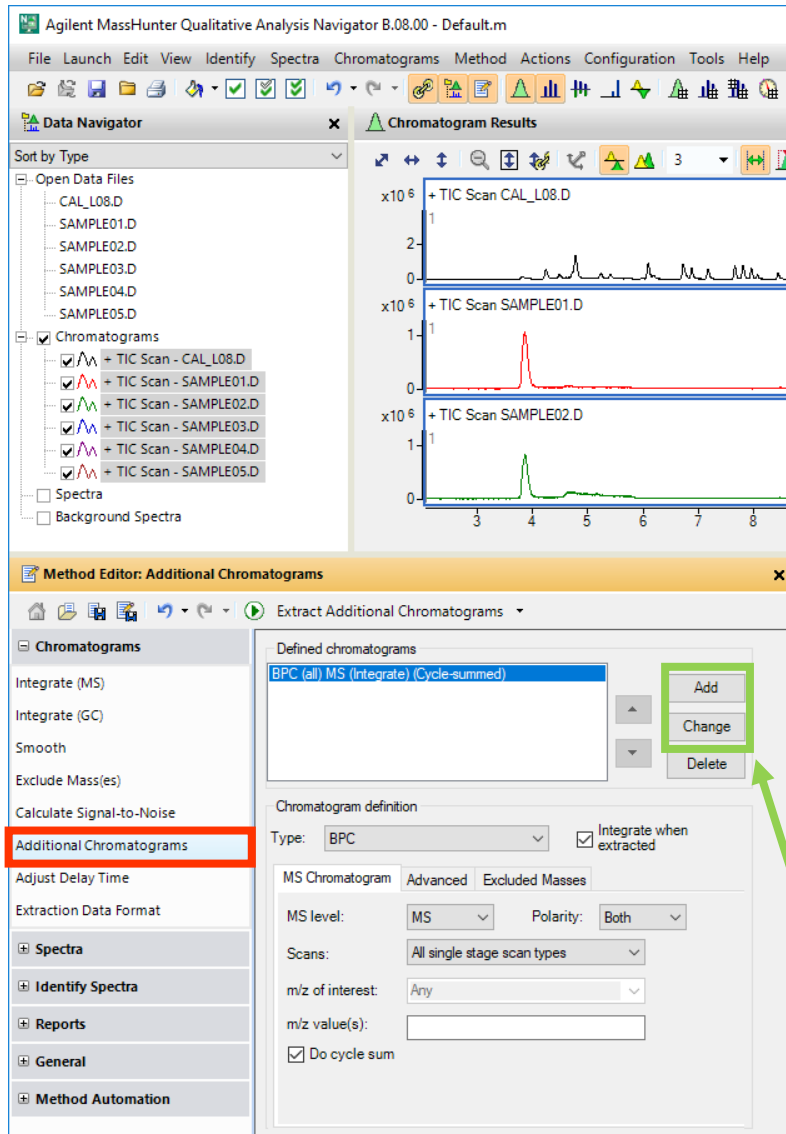
MS level: MS Polarity: Both

Scans: All single stage scan types

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

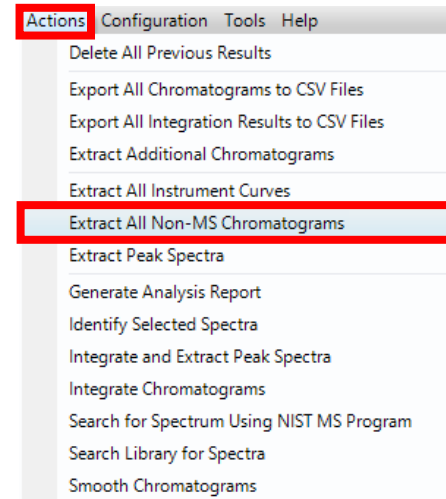
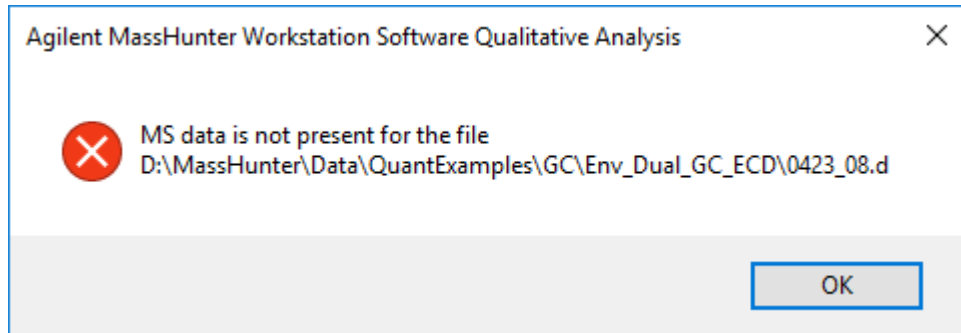
**MRM** – Multiple Reaction Monitor

**pNLC** - Precursor Neutral Loss Chromatogram

**Tip: Select Change or Add.**

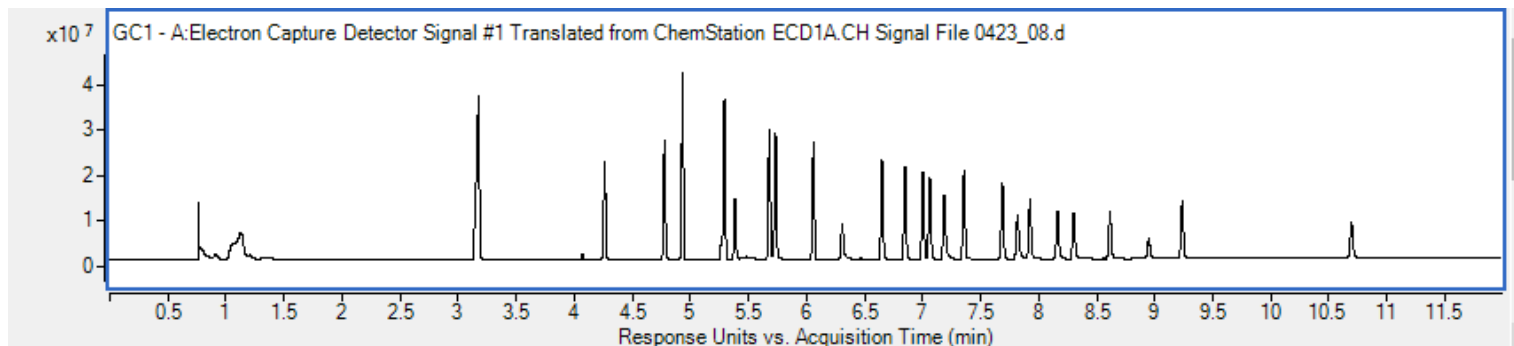
# Extracting GC, UV and other Non-MS Signals

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.

Method Editor: Integrate (MS)

Integrate Chromatogram

Chromatograms

- Integrate (MS)
- Integrate (MS/MS)
- Integrate (UV)
- Integrate (GC)
- Integrate (ADC)
- Smooth
- Exclude Mass(es)
- Calculate Signal-to-Noise
- Additional Chromatograms
- Adjust Delay Time
- Extraction Data Format

Integrator selection

- Agile 2
- Agile 2
- Agile
- MS/MS
- MS/MS (GC)
- ChemStation
- General
- Universal

Integrator Suitability Peak Filters Results

Previous results

Clear previous results

New results

Highlight first peak

Highlight all peaks

Integrator Suitability Peak Filters Results

System suitability calculations

Enable system suitability calculations

Pharmacopoeia: United States Pharmacopoeia (USP)

Column void time: 0.0 min

Column length: 0.0 cm

Integrator Suitability Peak Filters Results

Filter on

Peak height  Peak area

Height filters

Absolute height  $\geq$  10000 counts

Relative height  $\geq$  5.000 % of largest peak

Area filters

Absolute area  $\geq$  10000 counts

Relative area  $\geq$  1.000 % of largest peak

Maximum number of peaks

Limit (by height) to the largest 100

# Integrators

## Agile2

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

## Agile

- 2<sup>nd</sup> generation parameter-less integrator

## Universal

- 1<sup>st</sup> 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)

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

## ChemStation

- 2<sup>nd</sup> generation ChemStation
- Intended for UV

# Integration Peak List



The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator interface. The main window shows a chromatogram titled '+ TIC Scan SAMPLE04.D' with several peaks labeled with their retention times: 3.877, 4.624, 10.626, 13.164, 15.866, 17.316, and 19.493. A table titled 'Peaks: + TIC Scan - SAMPLE04.D' is visible on the right, listing peak data. A green arrow points to the 'Add/Remove Columns' dialog box, which is used to configure the columns displayed in the table.

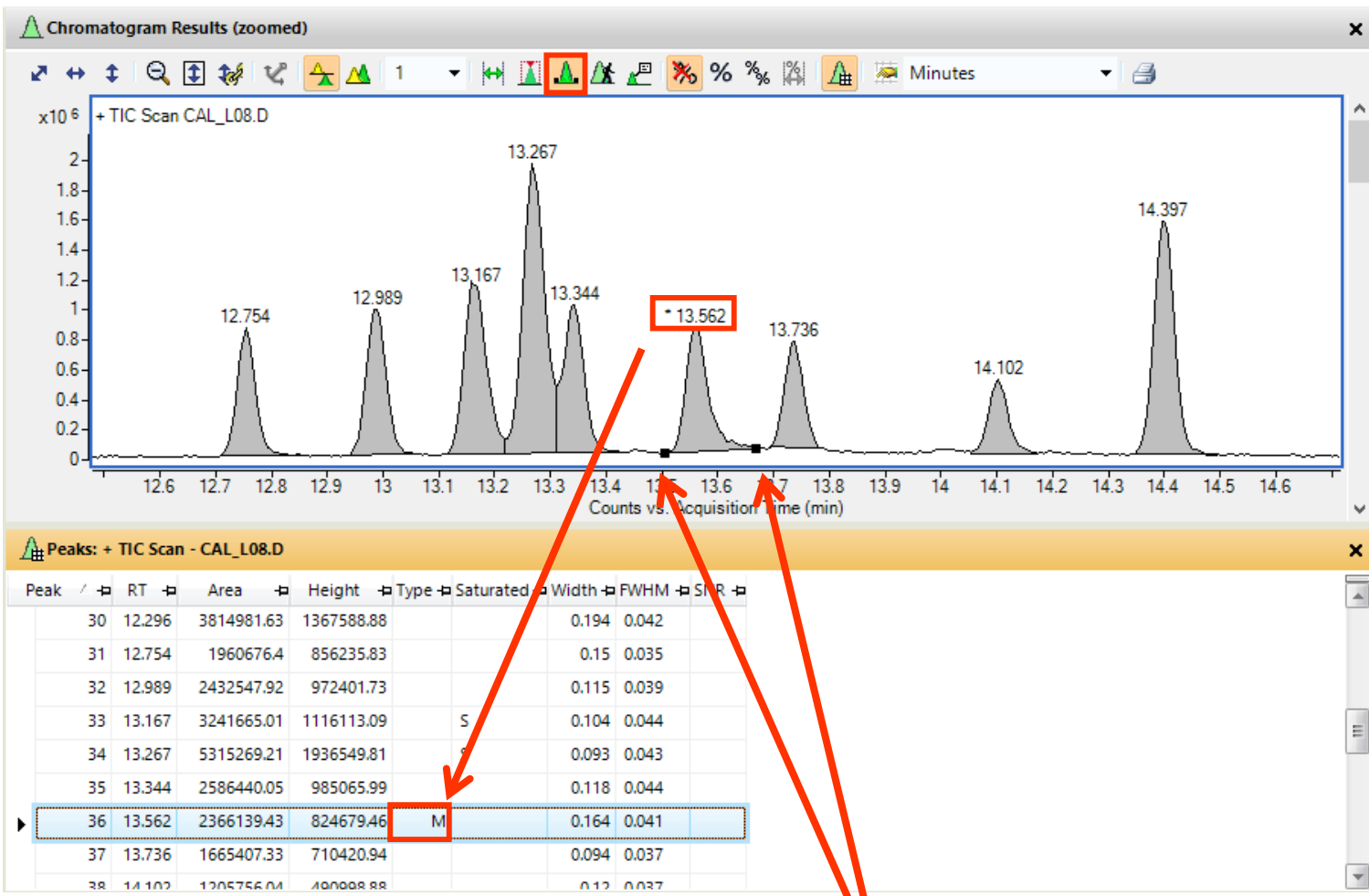
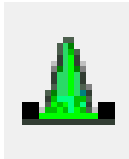
Peak	RT	Area	Height	Type	Saturated	Width	FWHM
1	3.877	4988605.48	741821.51			0.282	0.10
2	4.624	230422.65	43592.25			0.178	0.10
3	9.033	969979.9	361994.16			0.133	0.04
4	9.6	927392.36	360655.54			0.119	0.04
5	10.626	2146507.9	802990.62			0.159	0.04
6	11.728	75656.3	14486.71			0.138	0.12
7	13.164	2744267.99	935588.98			0.173	0.04
8	14.02	102004.41	31527.6			0.125	0.06
9	15.366	2605944.15	955546.39	S		0.135	0.04
10	17.316	2344258.3	890563.62			0.135	0.04
11	19.493	2589160.99	1032025.48			0.134	0.03

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

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

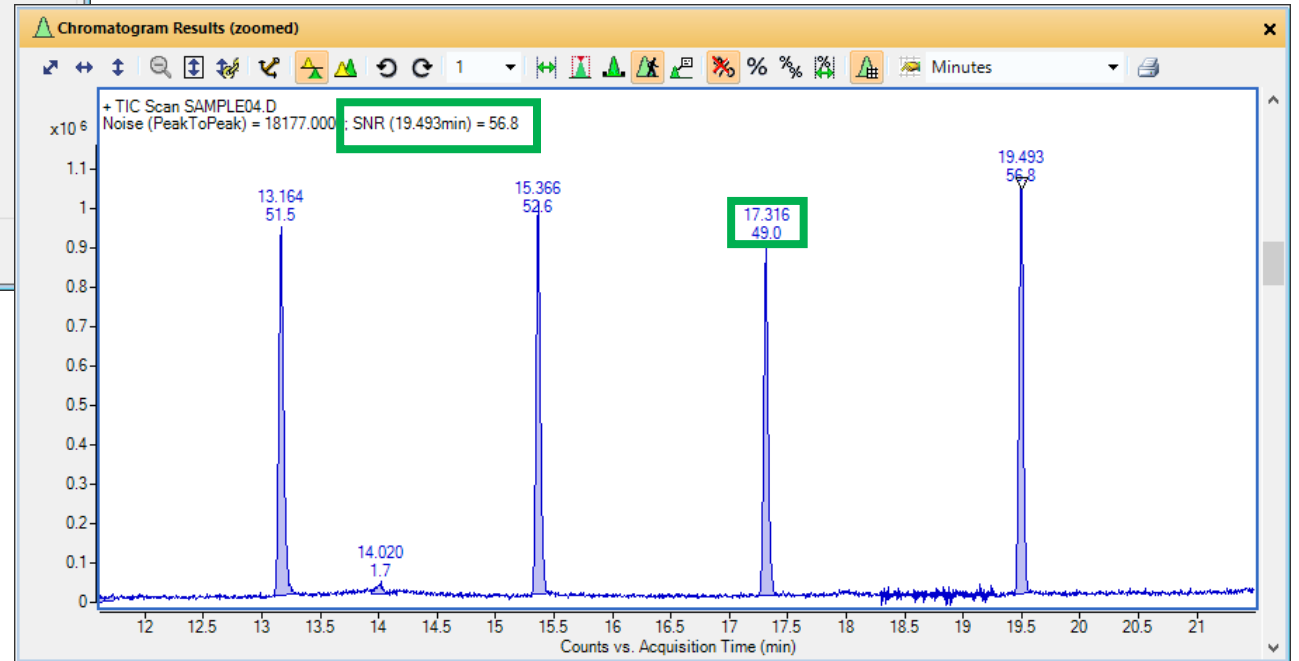
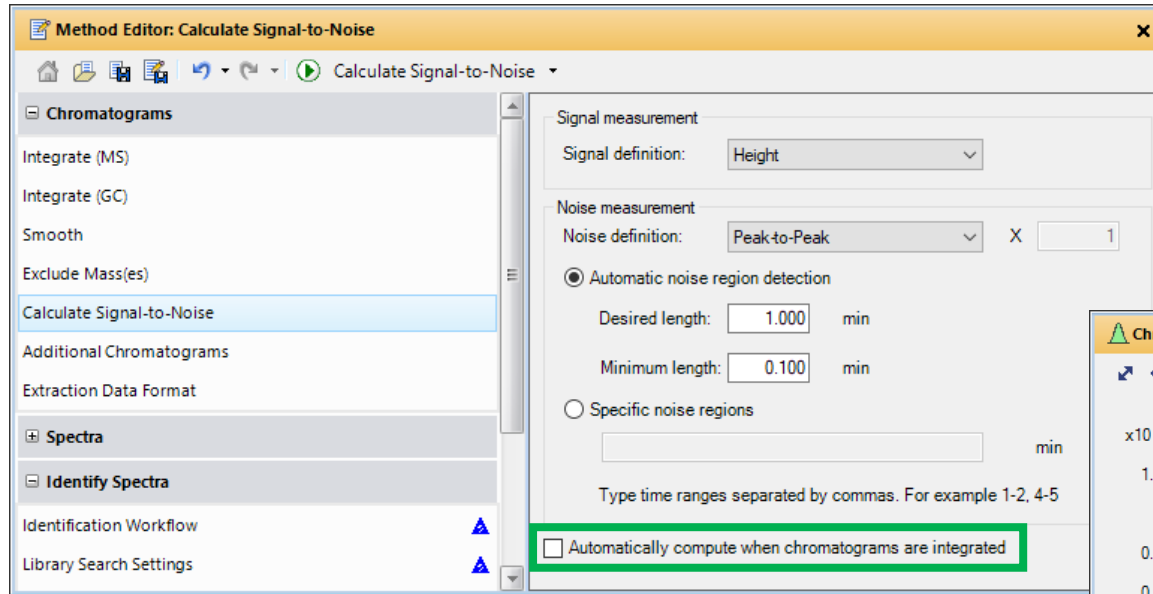
**Tip: Tables can be configured.**

# 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.



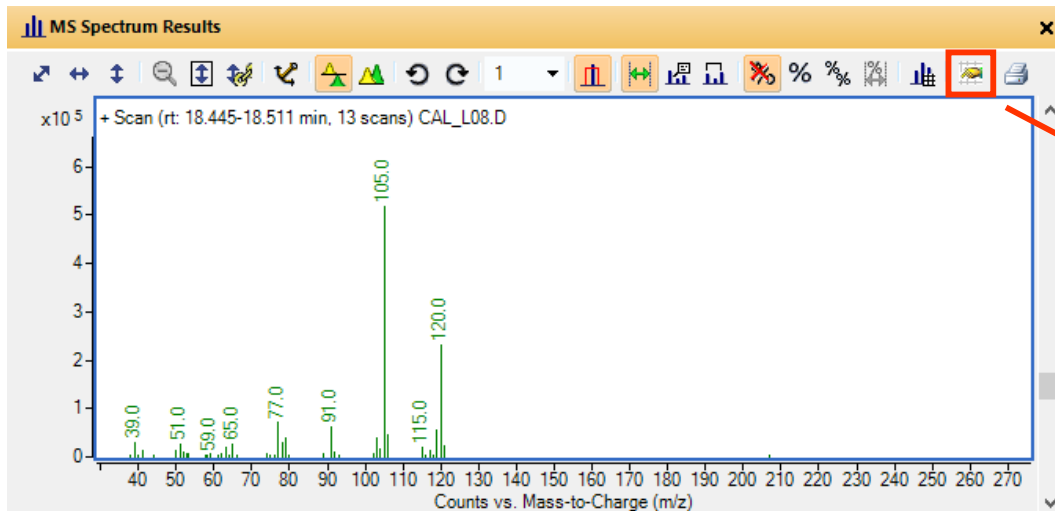
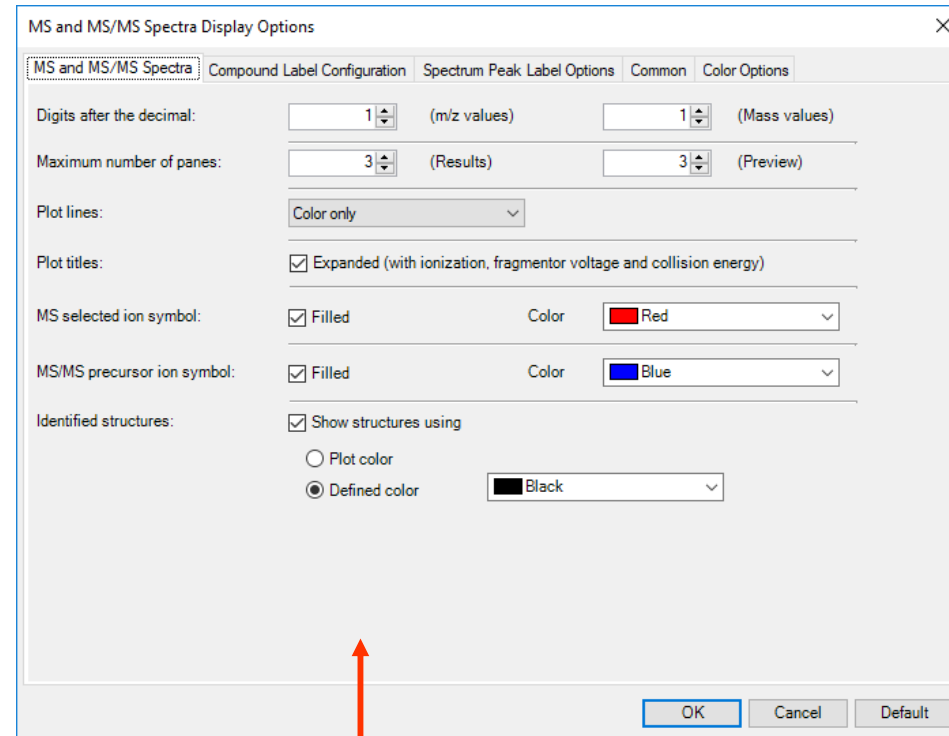
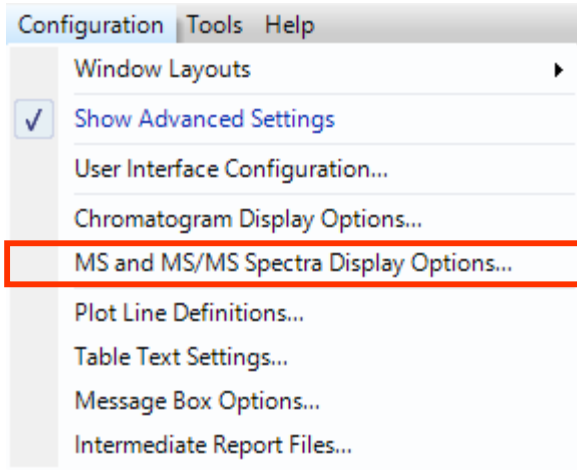
Let's take a few moments for questions on Working with Chromatograms.

Up Next:

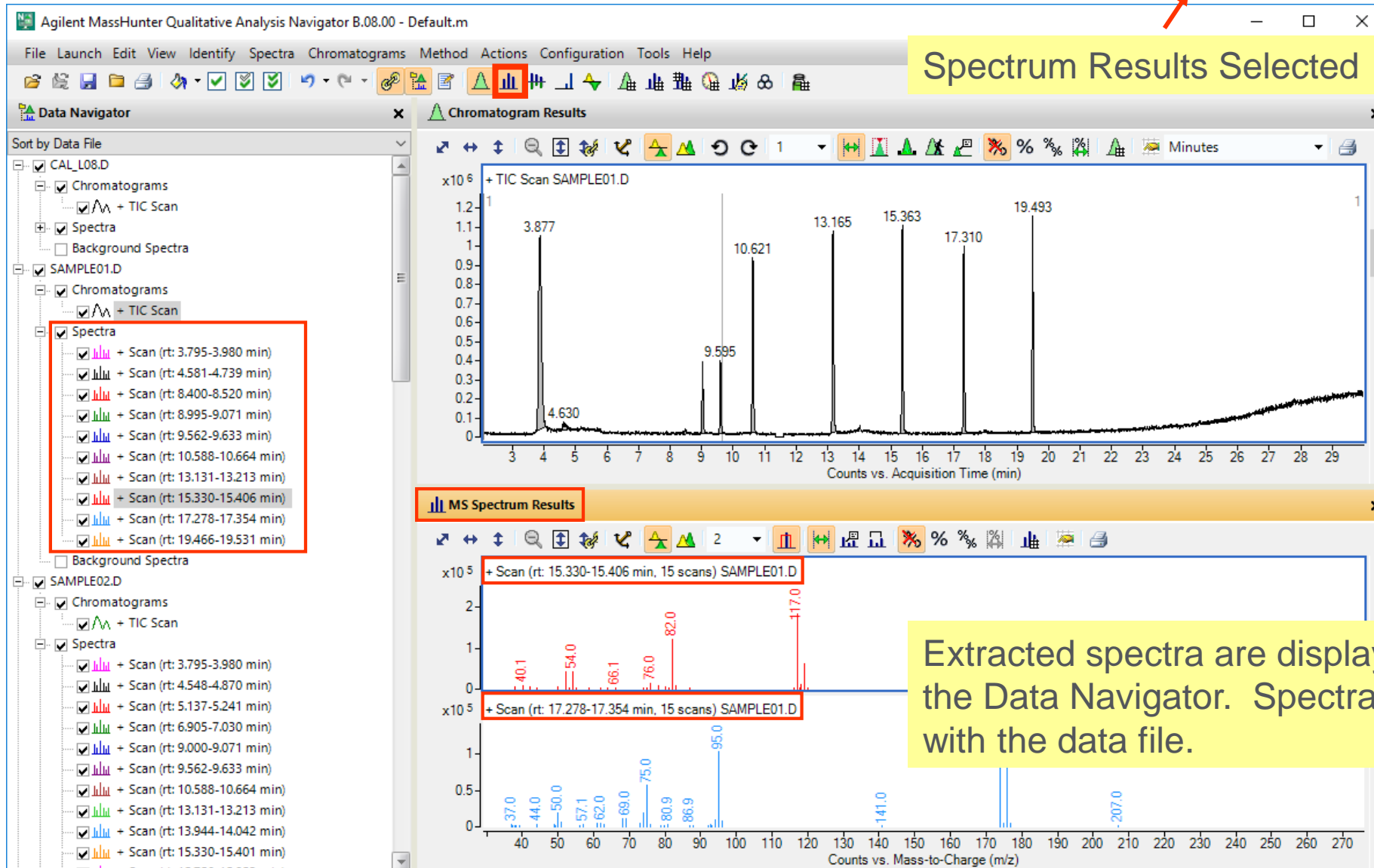
Working with Mass Spectra



# Mass Spectral Display Options



# Spectra Display – MS Spectrum Results



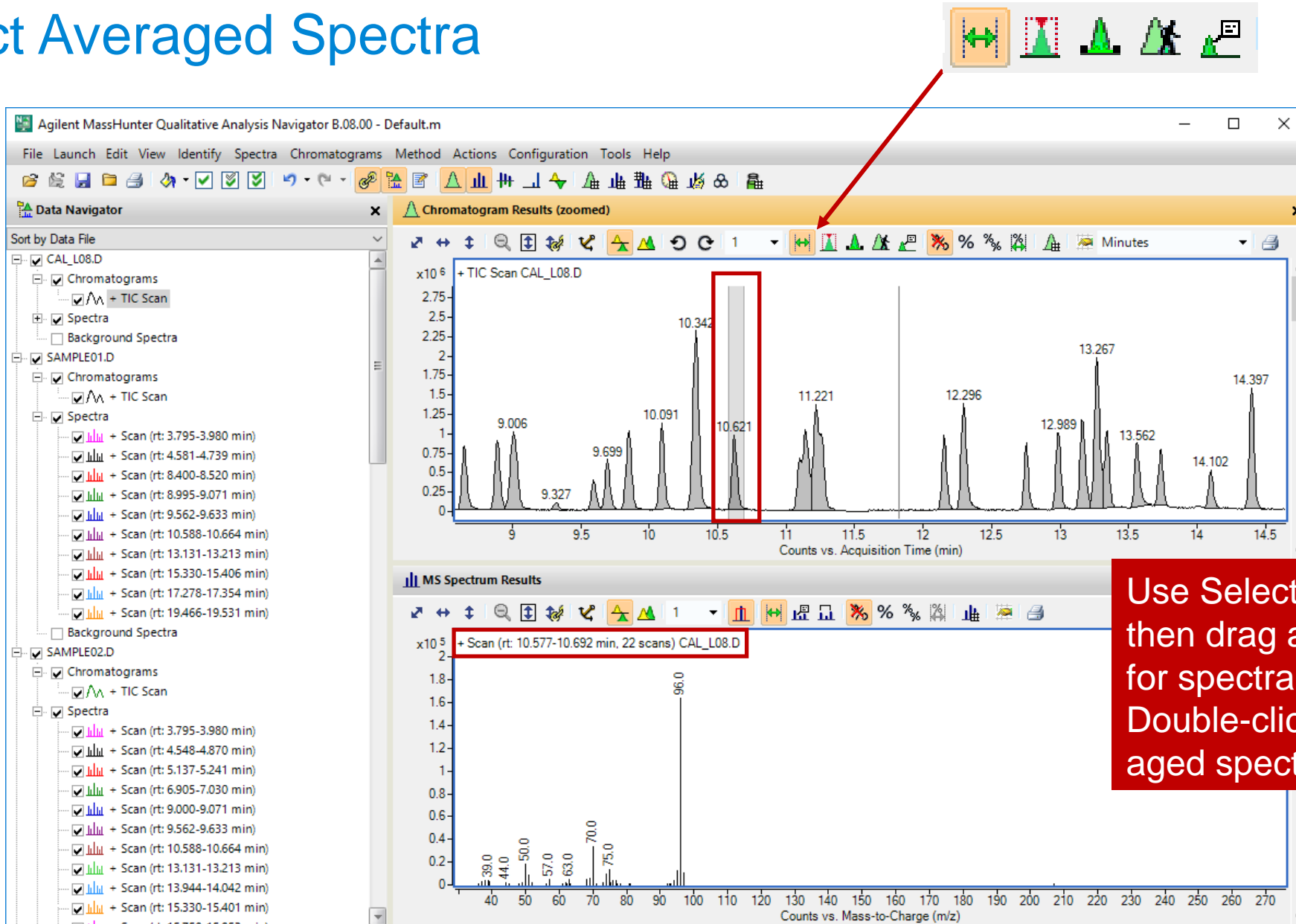
# Extract Single Spectrum

The screenshot shows the Agilent MassHunter Qualitative Analysis Navigator interface. On the left, the Data Navigator tree shows a project with three data files (CAL\_L08.D, SAMPLE01.D, SAMPLE02.D) and their respective chromatograms and spectra. The main window displays a zoomed-in Total Ion Chromatogram (TIC) for CAL\_L08.D. A context menu is open over a peak at 10.091 minutes, with 'Extract MS Spectrum' highlighted. Below the chromatogram, the MS Spectrum Results panel shows the mass spectrum for the selected scan (rt: 3.784-3.980 min, 37 scans), with a base peak at m/z 44.0 and other significant peaks at 37.0, 50.0, 66.0, 84.9, and 100.0.

## Extract Spectra

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

# Extract Averaged Spectra



# Background Subtraction for Manual Spectra

Use the Ctrl Key to average multiple range selections then Extract Spectrum.

①

②

③

Change manual spectrum background to **Current background spectrum**.

Method Editor: Extract (MS)

Manual spectrum background: Current background spectrum

MS Spectrum Results

- Extract EIC
- Extract Chromatograms...
- Subtract Background Spectrum
- Subtract Any Spectrum
- Add Any Spectrum
- Convert Profile to Centroid
- Convert Profile to Centroid and Replace
- Find Spectrum Peaks
- Edit Peak Annotations...
- Adjust Peak Threshold
- Identify Spectra
- Search Library/DB for Spectra
- Add/Edit Manual Identification...
- Clear Spectrum Identification Results
- Search Using NIST MS Program...
- Send Spectra to PCDL
- Deconvolute (Resolved Isotope)
- Set Anchor
- Clear Anchor
- Assign Ranges to
- Move to Background Spectrum**
- Delete
- Unzoom
- Assign Random Colors
- Choose Defined Color
- Copy to Clipboard
- Paste
- Print...
- Export...

# Background Subtraction for Manual Spectra

The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator B.08.00 interface. The **Data Navigator** on the left lists various scan ranges, with the scan at **rt: 10.593-10.653 min** highlighted and a **Sub** button next to it. The **Chromatogram Results (zoomed)** window shows a Total Ion Chromatogram (TIC) for **Scan CAL\_L08.D** with a peak at **10.621** minutes. A green box labeled **Extract Spectrum** points to this peak. The **Method Editor: Extract (MS)** window shows the **Manual Extraction** tab with **Manual spectrum background** set to **Current background spectrum**. The **MS Spectrum Results** window shows the mass spectrum for **Scan (rt: 10.593-10.653 min, 12 scans) CAL\_L08.D**, with a **Subtract** checkbox checked. A green box labeled **Description indicates subtraction occurred.** points to this checkbox. The mass spectrum shows a base peak at **m/z 96.0** and other peaks at **m/z 39.0, 50.0, 63.0, and 70.0**.

# Extract Peak Spectrum

Extracts an averaged spectrum from a chromatographic peak automatically. Must integrate to define chromatographic peaks.

① Integrate the chromatogram to locate chromatographic peaks. Four step process.

Integrate Chromatogram

Integrate Chromatogram

Integrator selection  
Agile 2

Method Editor: Integrate (MS)

Chromatograms

Integrate (MS)

Integrate (GC)

Smooth

Exclude Mass(es)

Calculate Signal-to-No...

Additional Chromatogr...

Adjust Delay Time

Extraction Data Format

Spectra

Agilent MassHunter Qualitative Analysis Navigator B.08.00 - Default.m

File Launch Edit View Identify Spectra Chromatograms Method Actions Configuration Tools Help

Data Navigator

Sort by Data File

CAL\_L08.D

Chromatograms

+ TIC Scan

Spectra

Background Spectra

Chromatogram Results

+ TIC Scan CAL\_L08.D

Counts vs. Acquisition Time

805 9.006 10.342 12.296 13.267 14.397 16.028 18.0

Extract MS Spectrum

Extract MS Spectrum to Background

Extract Peak Spectrum

Extract Chromatograms...

Extract Additional Chromatograms

Use Highlighted Chromatograms

Integrate Chromatogram

Integrate and Extract Peak Spectra

Smooth Chromatogram

Subtract Any Chromatogram

Calculate Signal-to-Noise

Adjust Peak Threshold

Set Anchor

Clear Anchor

Assign Ranges to

Clear Results

Delete

Delete Peak

Unzoom

Assign Random Colors

Choose Defined Color

Copy to Clipboard Ctrl+C

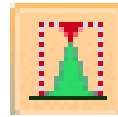
Paste Ctrl+V

Print...

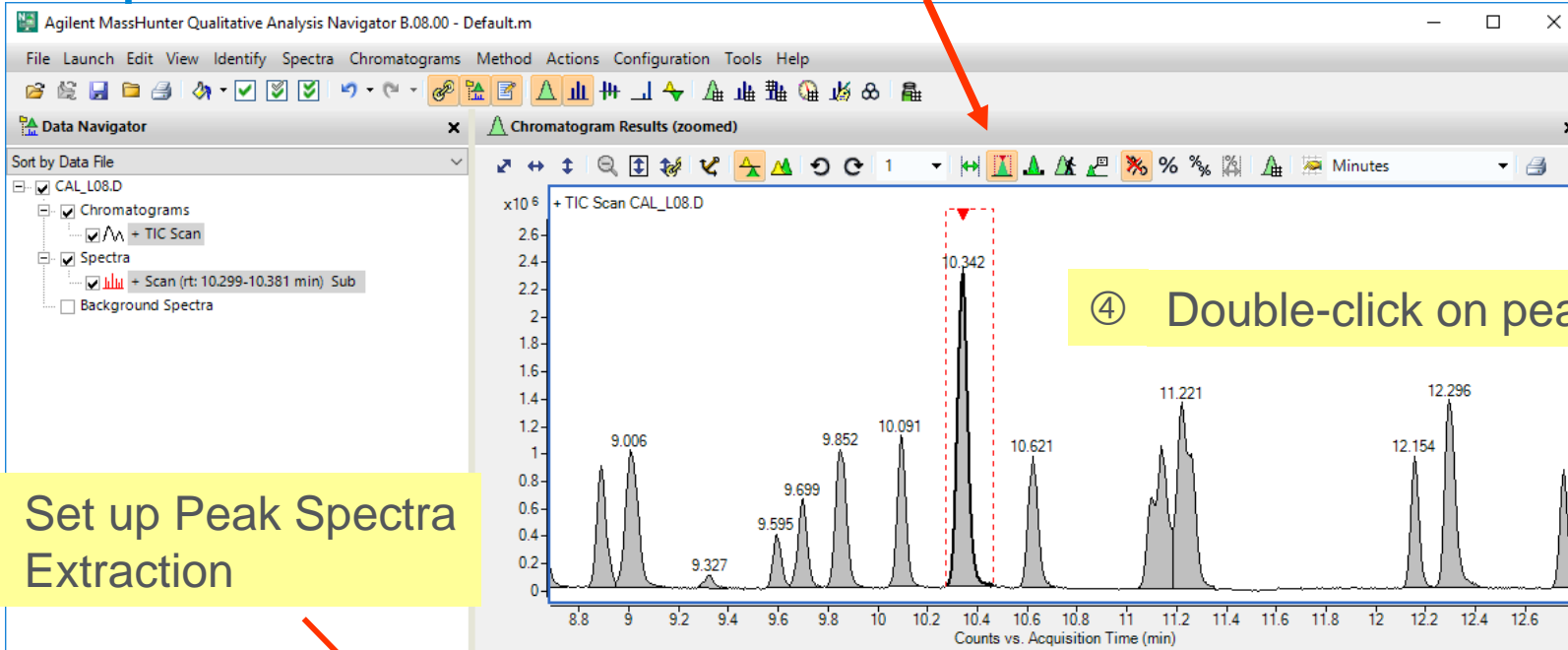
Export...



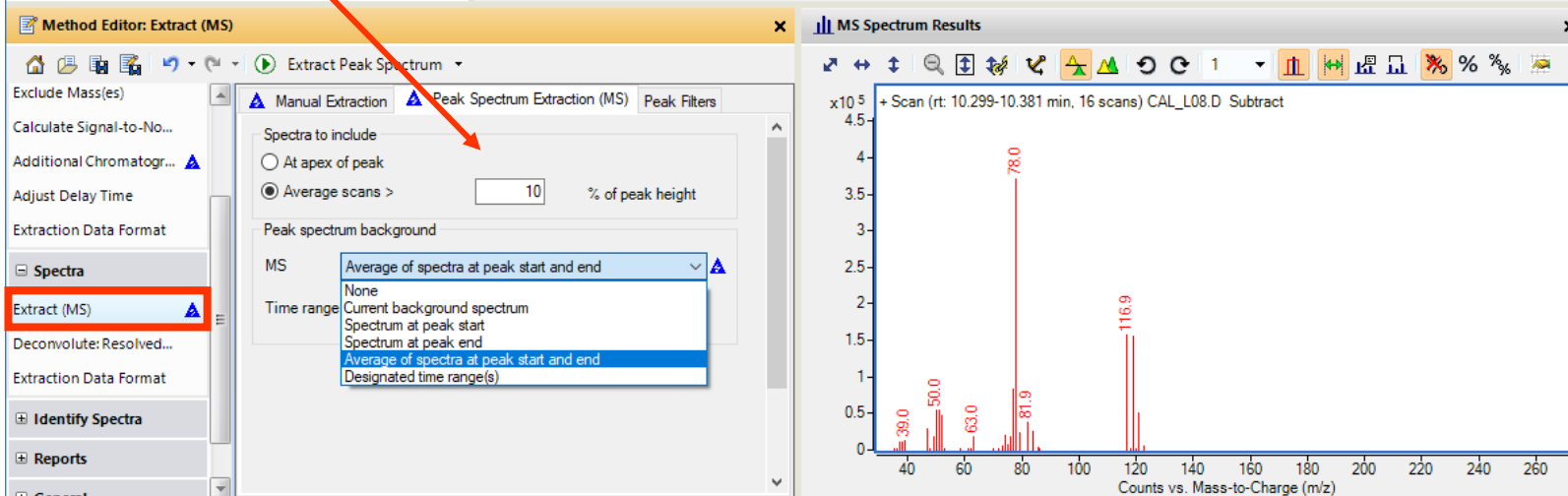
# Extract Peak Spectrum



③ Enable Peak Select tool.



② Set up Peak Spectra Extraction



# Extract Peak Spectra Automatically

**Spectra Results from each Integrated Peak**

Tip: Set integration parameters and Extraction parameters in the Method Editor first. Select Integrate and Extract Peak Spectra from the menu.

Method Editor: Extract (MS)

- Exclude Mass(es)
- Calculate Signal-to-Noise
- Additional Chromatogram
- Adjust Delay Time
- Extraction Data Format
- Spectra
  - Extract (MS)
  - Deconvolute: Resolved Is
  - Extraction Data Format
- Identify Spectra
- Reports

MS Spectrum Results

- + Scan (rt: 7.772-7.854 min, 16 scans) CAL\_L08.D Subtract
- + Scan (rt: 7.925-8.002 min, 15 scans) CAL\_L08.D Subtract
- + Scan (rt: 8.023-8.100 min, 15 scans) CAL\_L08.D Subtract

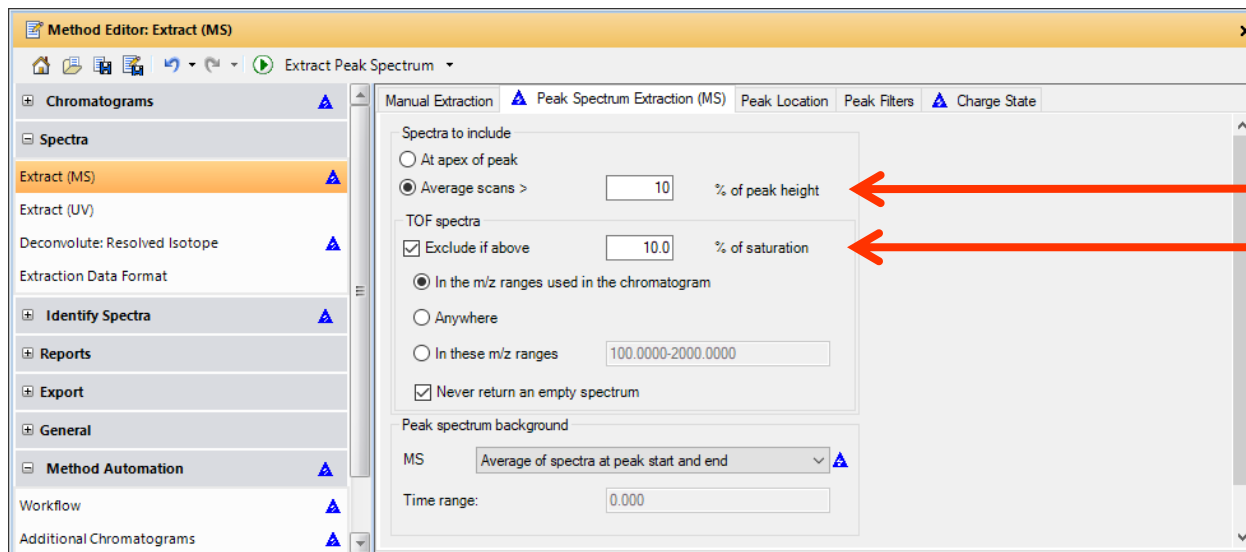
Chromatogram: TIC Scan CAL\_L08.D

Peaks labeled: 6.785, 6.878, 7.178, 7.805, 7.958, 8.062, 8.433, 8.651, 9.006, 9.327, 9.595, 9.699, 10.342

Context Menu:

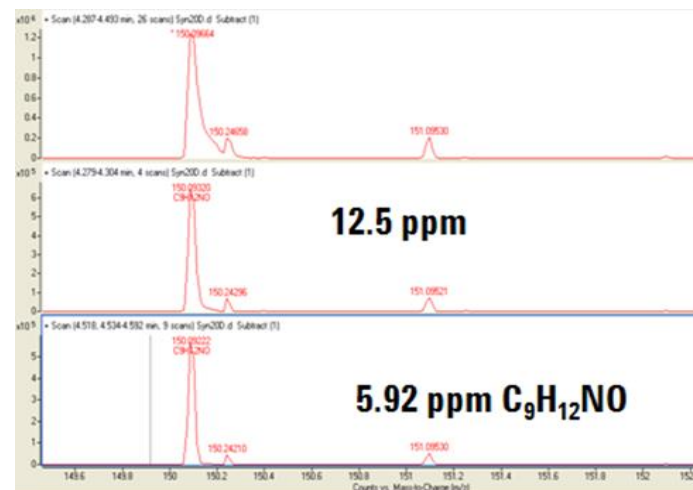
- Extract MS Spectrum
- Extract MS Spectrum to Background
- Extract Peak Spectrum
- Extract Chromatograms...
- Extract Additional Chromatograms
- Use Highlighted Chromatograms
- Integrate Chromatogram
- Integrate and Extract Peak Spectra**
- Smooth Chromatogram
- Subtract Any Chromatogram
- Calculate Signal-to-Noise
- Adjust Peak Threshold
- Set Anchor
- Clear Anchor
- Assign Ranges to
- Clear Results
- Delete
- Delete Peak
- Unzoom
- Assign Random Colors
- Choose Defined Color
- Copy to Clipboard (Ctrl+C)
- Paste (Ctrl+V)
- Print...
- Export...

# Considerations for Accurate Mass Data Using Extract Peak Parameters – Saturation



Change to 5%  
Change to 20%

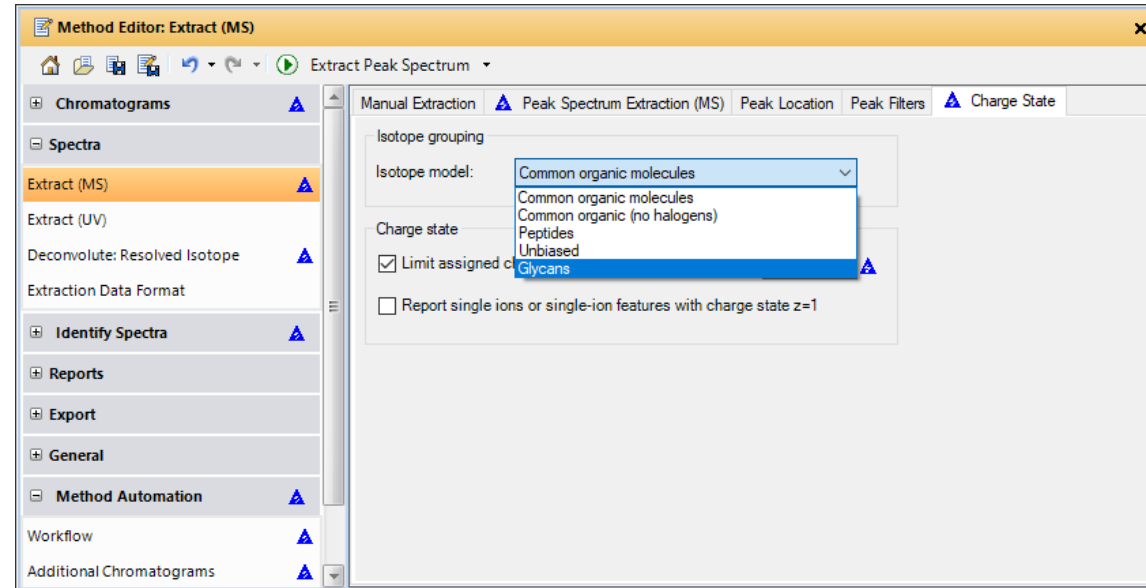
Never return an empty spectrum finds the spectrum with the least saturation.



# Using Extract Peak Parameters – Isotope Model

Chose Isotope model that  
Corresponds to workflow.

Common organic molecules  
Peptides  
Unbiased  
Glycans

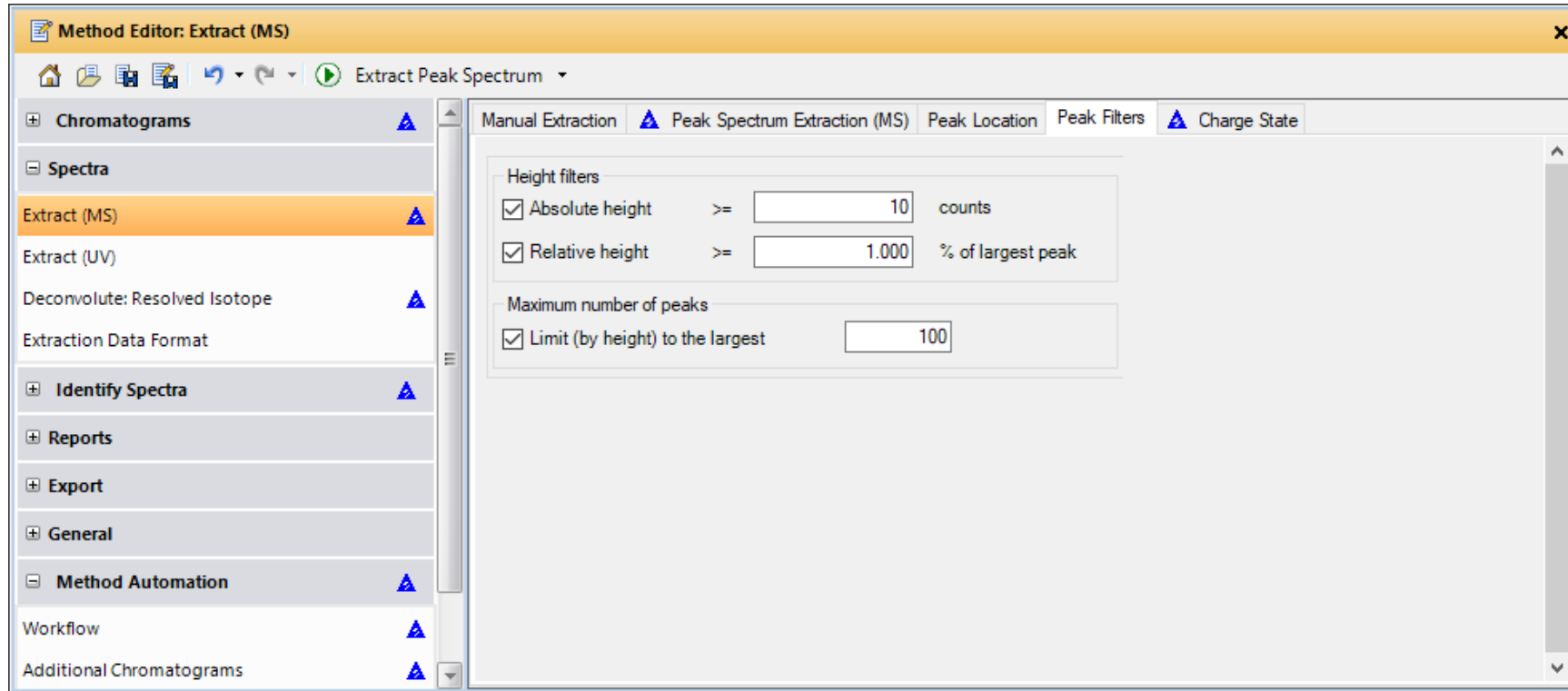


**Tip: Check Limit Assigned Charge States Maximum Values.**

**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



Agilent MassHunter Qualitative Analysis Navigator B.08.00 - Default.m

File Launch Edit View Identify Spectra Chromatograms Method Actions Configuration Tools Help

Data Navigator

Sort by Data File

CAL\_L08.D

Chromatograms

Chromatograms + TIC Scan

Spectra

Background Spectra

Chromatogram Results

TIC Scan CAL\_L08.D

Counts vs. Acquisition Time (min)

Method Editor: Extract (MS)

Extract Peak Spectrum

Chromatograms

Spectra

Extract (MS)

Deconvolute: Resolved...

Extraction Data Format

Identify Spectra

Reports

General

Method Automa...

Manual Extraction Peak Spectrum Extraction (MS) Peak Filters

Manual spectrum background

MS None

Walk Chromatogram Use left and right arrows on keyboard to step through single spectrum.

Spectrum Preview

Scan (rt: 11.221 min) CAL\_L08.D

Counts vs. Mass-to-Charge (m/z)

Walk Chromatogram Can be useful for co-eluting peaks.

# Add Preview Spectrum to Results

The screenshot displays the Agilent MassHunter Qualitative Analysis Navigator interface. The main window shows a chromatogram titled '+ TIC Scan CAL\_L08.D' with various peaks labeled with retention times. A green arrow points from the 'Scan (rt: 11.221 min)' entry in the 'Data Navigator' panel to the 'Spectrum Preview' window. In the 'Spectrum Preview' window, the spectrum for the selected scan is shown, and a right-click context menu is open. The 'Copy to Spectra' option is highlighted in green. A green callout box contains the following instructions:

- Right-click
- Select Copy to Spectra

The 'Spectrum Preview' window also shows a list of peaks with their retention times: 37.0, 47.0, 64.9, 82.9, 94.9, and 129.8. The x-axis is labeled 'Counts vs. Mass-to-Charge'.

# MS Spectrum Peak List One



Agilent MassHunter Qualitative Analysis Navigator B.08.00 - Default.m

File Launch Edit View Identify Spectra Chromatograms Method Actions Configuration Tools Help

Data Navigator

Sort by Data File

- + Scan (rt: 19.466-19.509 min)
- + Scan (rt: 19.509-19.575 min)
- + Scan (rt: 19.608-19.673 min)
- + Scan (rt: 19.973-20.044 min)
- + Scan (rt: 20.120-20.186 min)
- + Scan (rt: 20.306-20.371 min)
- + Scan (rt: 20.895-20.961 min)
- + Scan (rt: 21.888-22.036 min)
- + Scan (rt: 23.258-23.318 min)
- + Scan (rt: 23.651-23.716 min)
- + Scan (rt: 23.831-23.896 min)
- + Scan (rt: 24.038-24.104 min)
- + Scan (rt: 11.221 min)

Background Spectra

Chromatogram Results

+ TIC Scan CAL\_L08.D

Counts vs. Acquisition Time (min)

MS Spectrum Results

+ Scan (rt: 23.831-23.896 min, 13 scans) CAL\_L08.D

Counts vs. Mass-to-Charge (m/z)

Method Editor: Extract (MS)

Peak Spectrum Extraction (MS) Peak Filters

Manual Extraction

Manual spectrum background

MS None

MS Peaks One: + Scan (rt: 23.831-23.896 min)

m/z	Abund	Abund % (Norm)	Max Abund	Z	Sat	Spec
36	5754.69		5754.69			
37	2609.62		2609.62			
44	3866.85		3866.85			
47	20473.92		20473.92			
48	5423.62		5423.62			
49	6869.77		6869.77			
71	15970.85		15970.85			
73	6116		6116			
81.9	7805.92		7805.92			
83	29287.85		29287.85			
83.9	6734.31		6734.31			
85	9683.77		9683.77			
93.9	15391		15391			
94.9	3362.46		3362.46			
95.9	10776.77		10776.77			
105.9	15770.46		15770.46			
107.9	9613.46		9613.46			

•Right-click spectrum > MS Spectrum Peak List One.

•Tool is also available.



# MS Spectrum Peak List Two New



Agilent MassHunter Qualitative Analysis Navigator B.08.00 - Default.m

File Launch Edit View Identify Spectra Chromatograms Method Actions Configuration Tools Help

Data Navigator Chromatogram Results (zoomed)

Sort by Data File

- + Scan (rt: 11.068-11.183 min) Sub
- + Scan (rt: 11.183-11.297 min) Sub
- + Scan (rt: 12.127-12.187 min) Sub
- + Scan (rt: 12.263-12.340 min) Sub
- + Scan (rt: 12.727-12.787 min) Sub
- + Scan (rt: 12.956-13.022 min) Sub
- + Scan (rt: 13.131-13.213 min) Sub
- + Scan (rt: 13.234-13.311 min) Sub
- + Scan (rt: 13.311-13.376 min) Sub
- + Scan (rt: 13.529-13.611 min) Sub
- + Scan (rt: 13.704-13.769 min) Sub
- + Scan (rt: 14.069-14.135 min) Sub
- + Scan (rt: 14.369-14.435 min) Sub**
- + Scan (rt: 15.270-15.355 min) Sub
- + Scan (rt: 15.335-15.455 min) Sub
- + Scan (rt: 15.695-15.766 min) Sub
- + Scan (rt: 15.984-16.088 min) Sub
- + Scan (rt: 16.148-16.219 min) Sub
- + Scan (rt: 16.508-16.590 min) Sub

+ TIC Scan CAL\_L08.D

Counts vs. Acquisition Time (min)

MS Spectrum Results

MS Peaks One: + Scan (rt: 13.704-13.769 min) Sub

m/z	Species	Abund	Abund %	Z	Sat	m/z (prod.)	Diff (ppm)	Formula & Ion Species
46.9		1619.33	1.28					
47		9695.72	7.69					
48		12663.46	10.05					
48.9		1863.31	1.48					

MS Peaks Two: + Scan (rt: 14.369-14.435 min) Sub

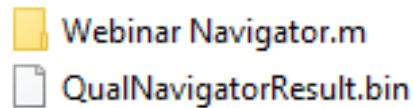
m/z	Species	Abund	Abund %	Z	Sat	m/z (prod.)	Diff (ppm)	Formula & Ion Species
37		5272.54	3.33					
47		35142	22.19					
49		11150.46	7.04					
59		27583.77	17.42					
60.9		8665.42	5.47					
81.9		16679.15	10.53	1				
82.9		1688.08	1.07	1				
83.9		10426.46	6.58	1				
93.9		78286.16	49.43					
95.9		50869	32.12					
97.9		8441.23	5.33					
128.8		105760.62	66.78					
130.9		100131.7	63.22					
132.8		34088.54	21.52					
134.8		3181.23	2.01					
163.8		123693.04	78.1	1				
164.8		2942.46	1.86	1				
165.8		158377.97	100	1				
166.8		2839.31	1.79	1				
167.8		81205.23	51.27	1				
168.8		1678	1.06	1				
169.8		15698.54	9.91	1				

CTRL click on 2<sup>nd</sup> spectrum  
> MS Spectrum Peak List Two  
Tool is also available.

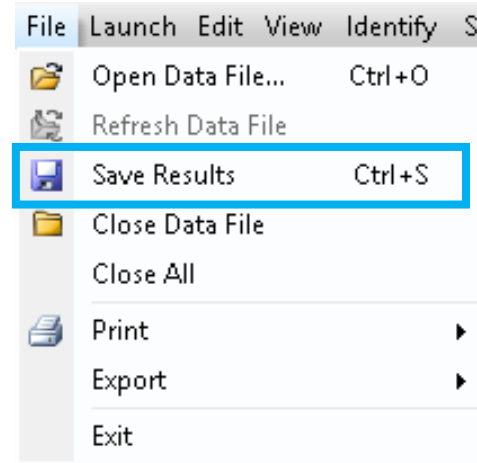
# Saving the Results

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

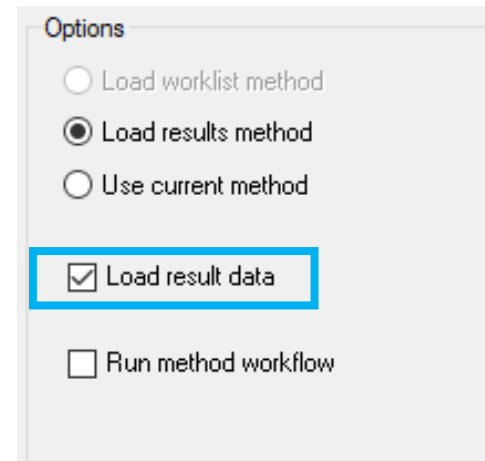
Only one results file can be saved per data file.



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



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



# 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.

**Method Editor: Identification Workflow**

Identify Selected Spectra

Identify by - Library / Database search

Library / Database	Score (fwd)	Score (rev)
D:\MassHunter\Library\demo.I	-	80.00

Move Up Move Down Add Remove

Search all libraries / databases  
 Stop at first library / database match

Maximum hits per compound:

**Method Editor: Generate Formulas**

Generate Formulas from Spectrum Peaks

Allowed Species Limits Charge State Fragment Formulas Scoring

Charge carrier to be assumed if not known

Positive ions:  -electron  +H  +Na  +K  +NH4  +C2H5  +C3H5

Negative ions:  +electron  -H  +Cl  +Br  +HCOO  +CH3COO  +CF3COO

MS ion electron state:

Group hits with same formula (but different charge carriers)

Elements and limits

Element	Minimum	Maximum
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

# 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

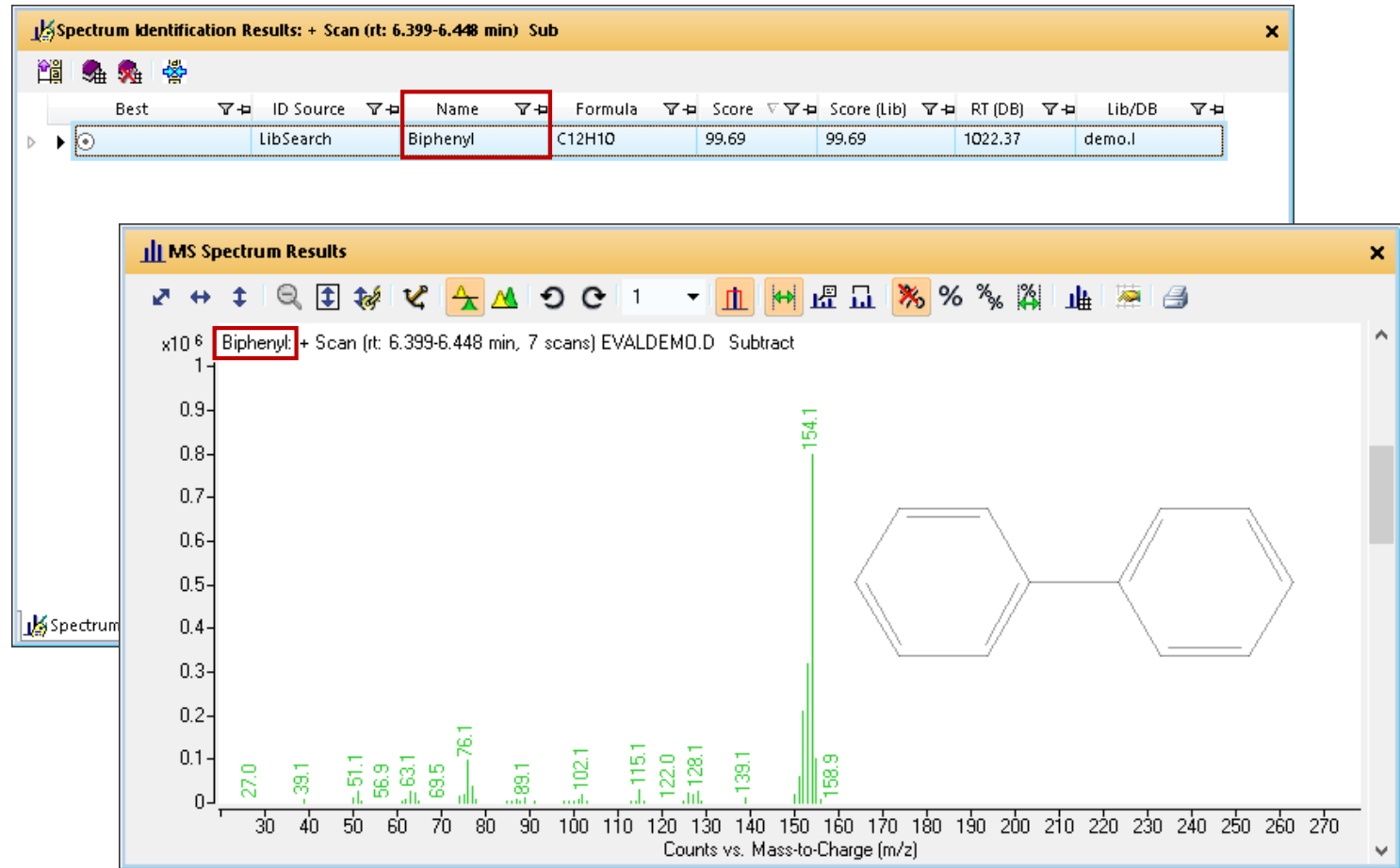
Right click in spectrum window and select Identify Spectra.

The image shows two overlapping software windows. The top window is the 'Method Editor: Identification Workflow' window. It has a sidebar on the left with a tree view containing 'Chromatograms', 'Spectra', 'Identify Spectra', 'Reports', and 'General'. The 'Identify Spectra' section is expanded, showing options like 'Identification Workflow', 'Library Search Settings', 'Generate Formulas', and 'Combine Identification Results'. A red box highlights the 'Identify Selected Spectra' button in the top toolbar. The main area of this window shows a table with columns 'Library / Database', 'Score (fwd)', and 'Score (rev)'. A single entry is visible: 'D:\MassHunter\Library\demo.I' with a score of 80.00. Below the table are buttons for 'Move Up', 'Move Down', 'Add', and 'Remove'. The bottom window is the 'MS Spectrum Results' window. It displays two mass spectra. The top spectrum is for a scan at 5.262-5.294 min, and the bottom is for a scan at 5.399-5.448 min. Both spectra show relative intensity on the y-axis and mass-to-charge ratio on the x-axis. A context menu is open over the bottom spectrum, with 'Identify Spectra' highlighted by a red box. Other menu items include 'Extract EIC', 'Extract Chromatograms...', 'Subtract Background Spectrum', 'Subtract Any Spectrum', 'Add Any Spectrum', 'Convert Profile to Centroid', 'Convert Profile to Centroid and Replace', 'Find Spectrum Peaks', 'Edit Peak Annotations...', 'Adjust Peak Threshold', 'Search Library/DB for Spectra', 'Add/Edit Manual Identification...', 'Clear Spectrum Identification Results', 'Search Using NIST MS Program...', 'Send Spectra to PCDL', 'Deconvolute (Resolved Isotope)', 'Set Anchor', and 'Clear Anchor'.

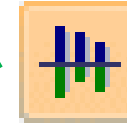
**Note :** Details on these settings will be covered in the Qualitative Analysis Workflows Webinar.

# EI Library Search – GCMS

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

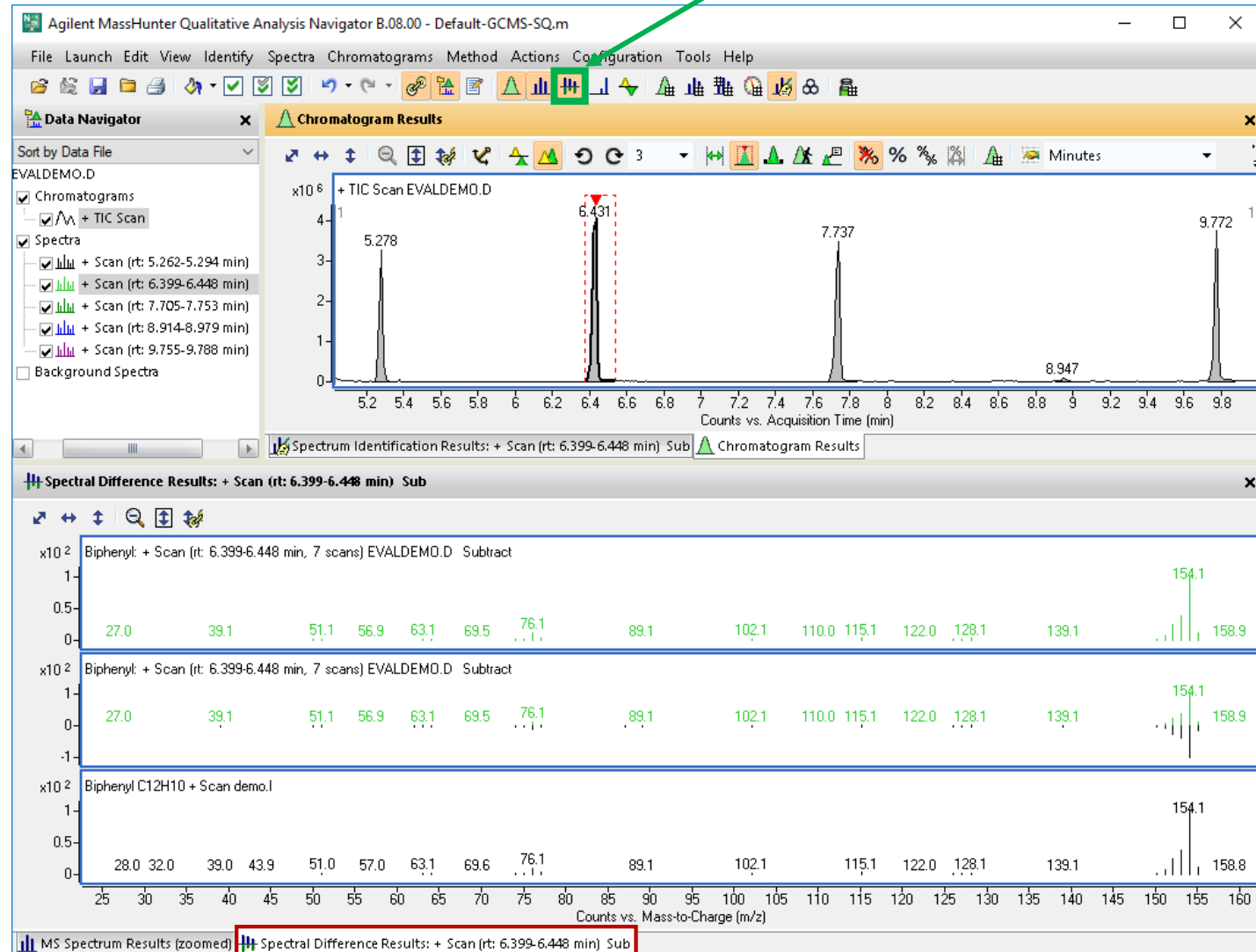


# Difference Results– GCMS



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.

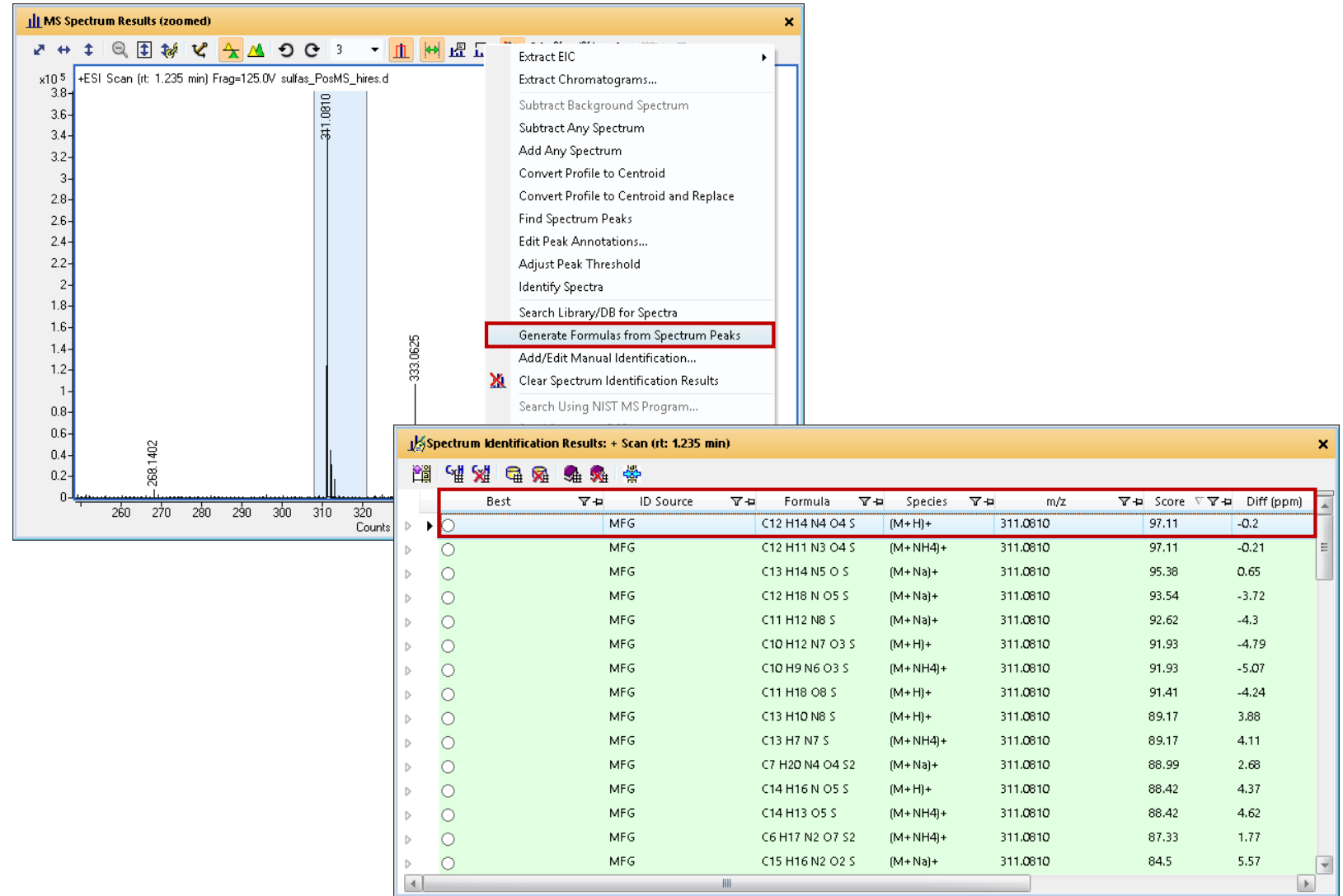


# Formula Generation

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.



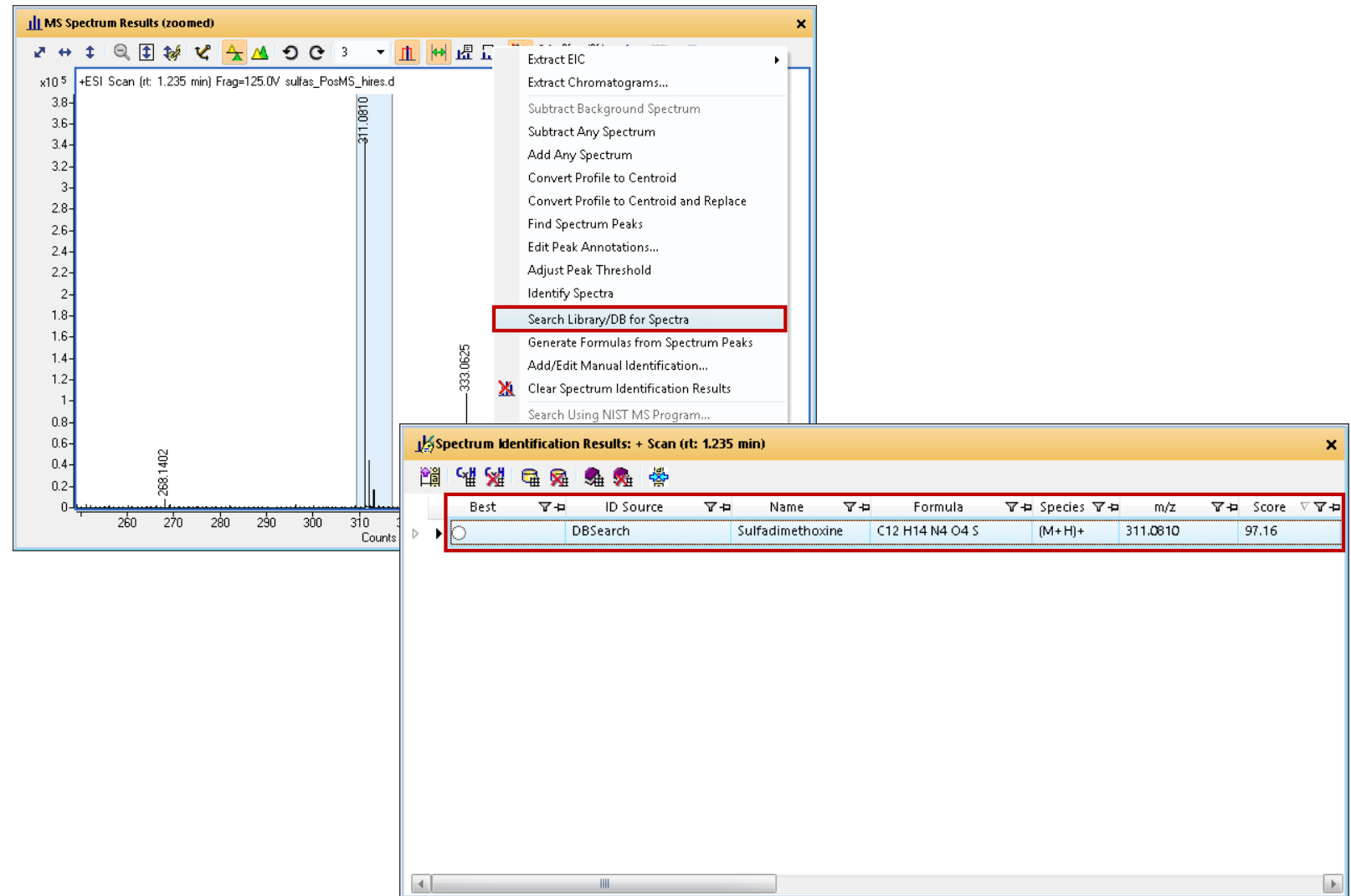


# Library/DB Searching

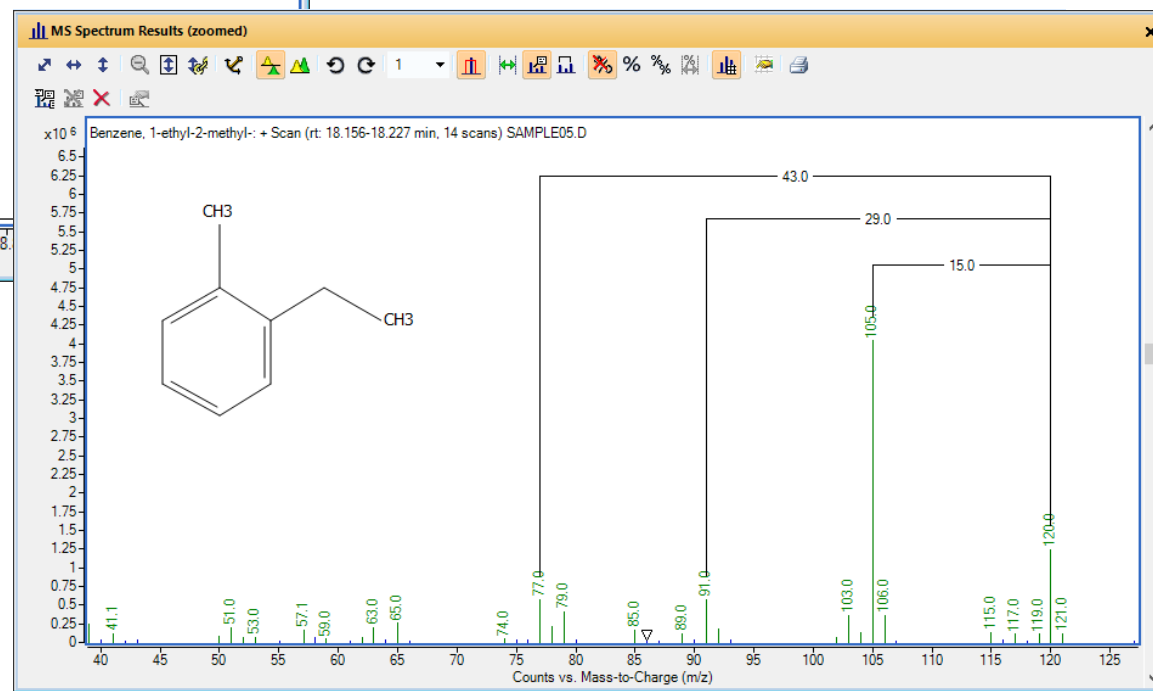
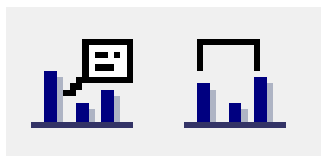
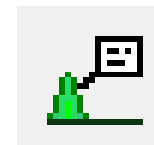
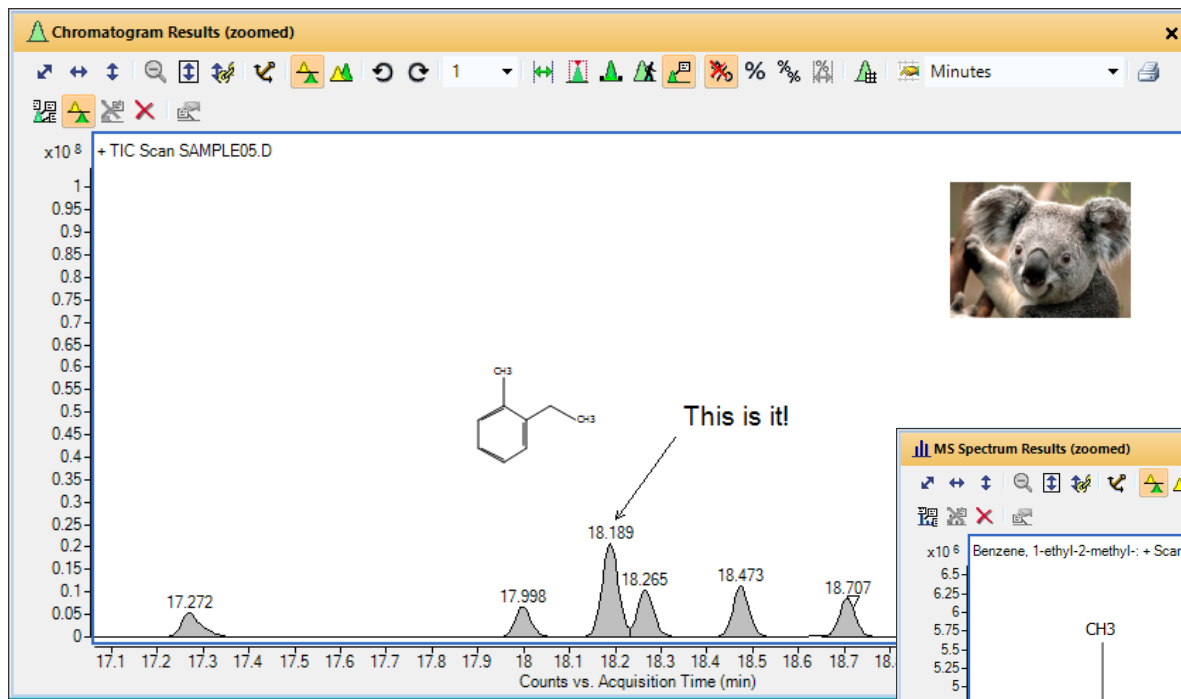
Or, for a library or database search, again use the range select tool to highlight the ion of interest, along with its isotopes.

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.

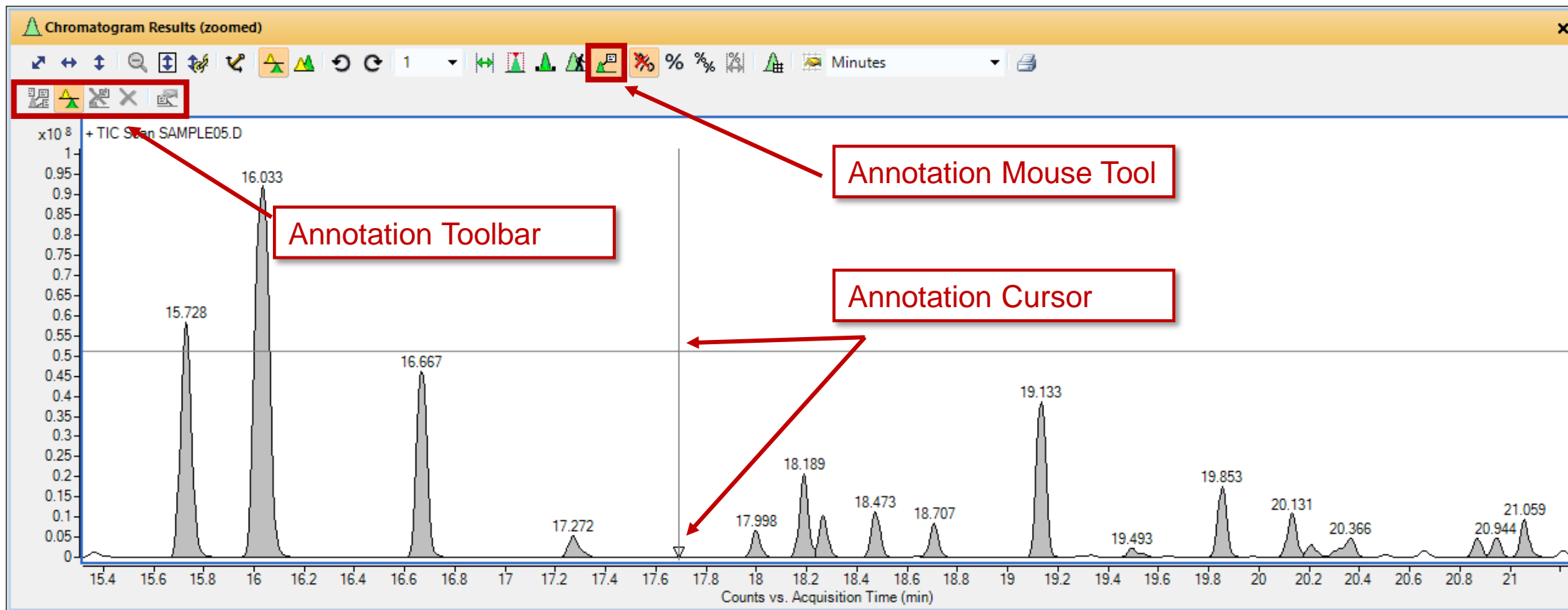


# Annotation of Chromatograms and Spectra



# Place Graphic into Annotation Mode

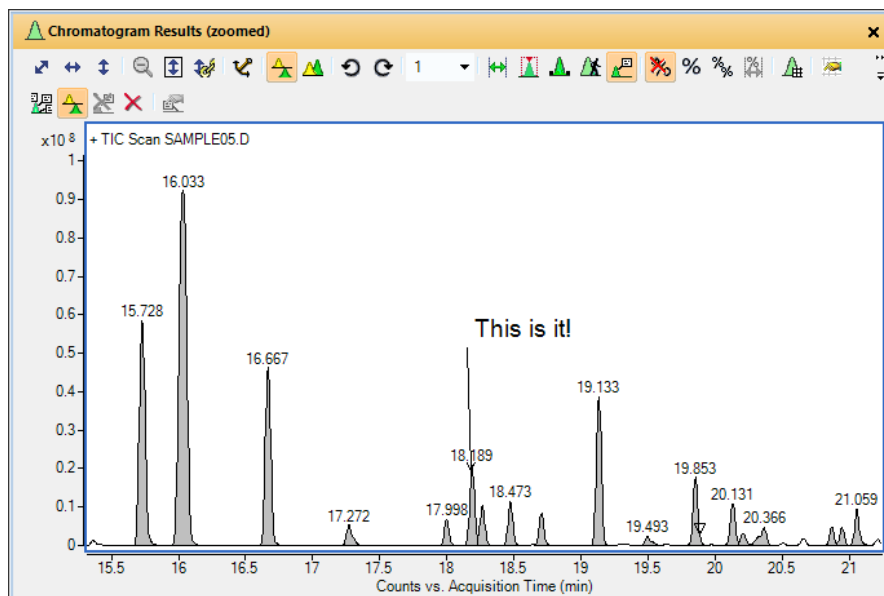
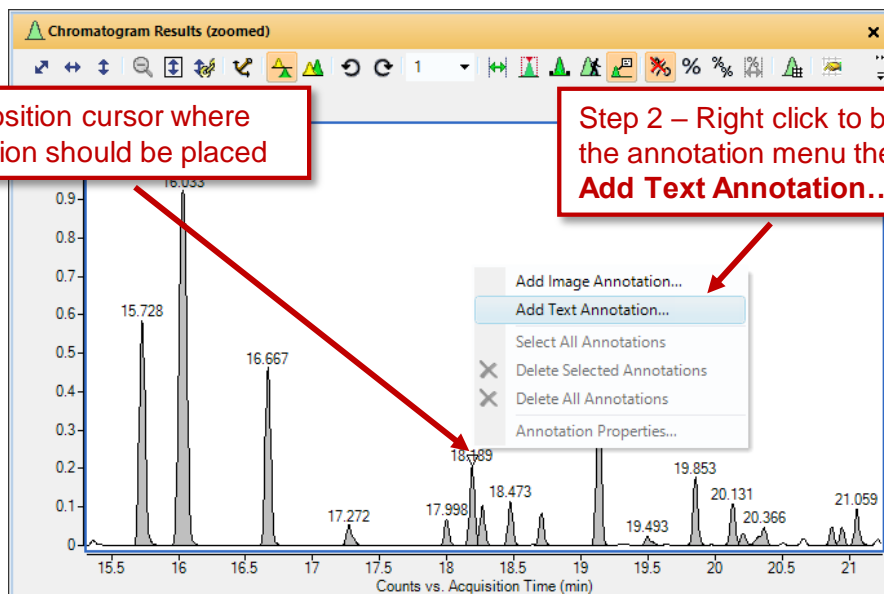
- Click Annotation Mouse Tool
- Annotation Toolbar and Annotation cursor appear



# 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

Add/Edit Text Annotation

Properties

Text:

(Press Ctrl+Enter or Alt+Enter to add a new line)

Text color:

Orientation:  degrees

Font style:  Font size:

Annotation type

Anchored

Show pointer

Pointer properties

Color:

Pattern:

Weight:

Pointer head:

Pointer head location (the x, y value using the data display)

X:  min

Y:  (abundance)

Upper left corner of the annotation (the x, y value using the data display)

X:  min

Y:  (abundance)

Floating

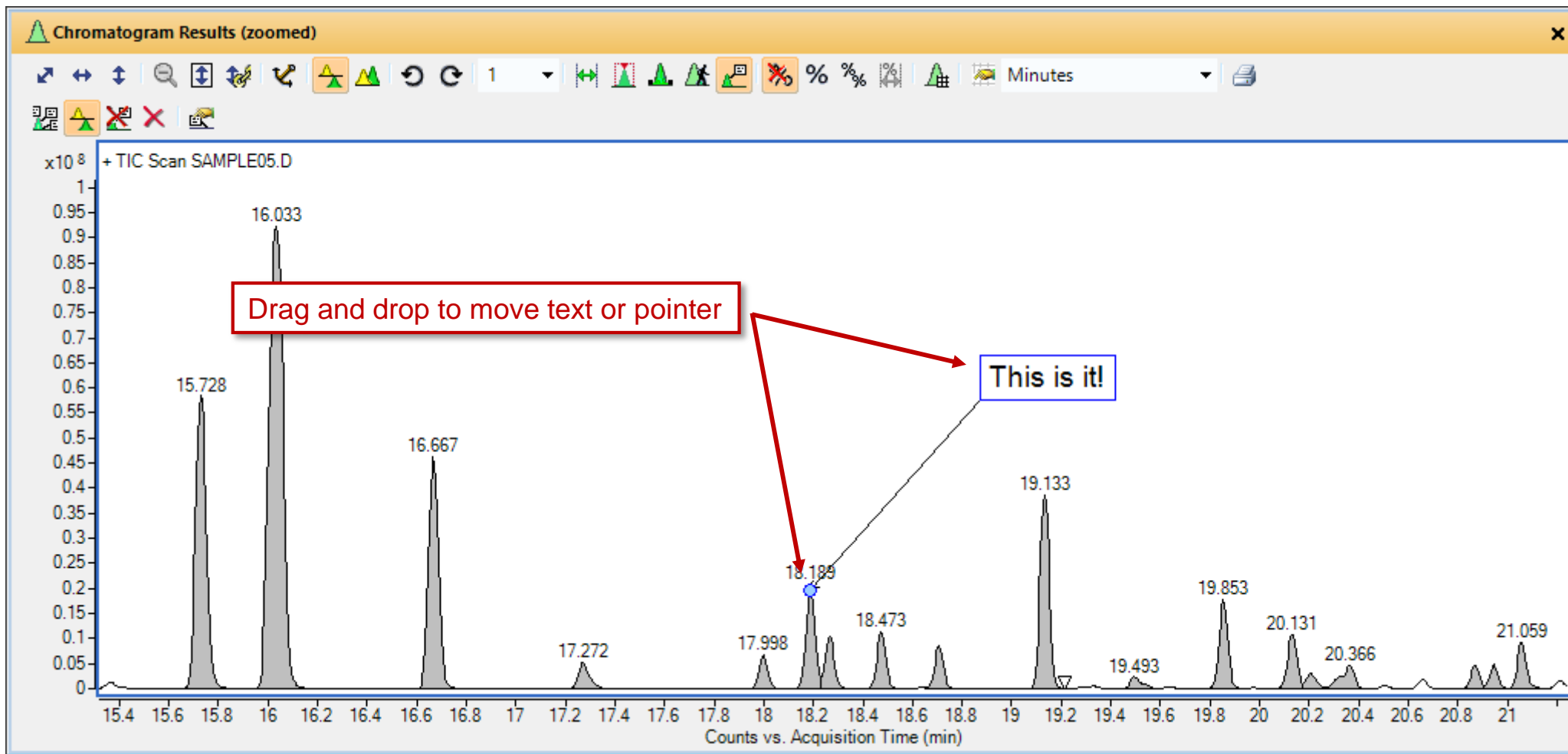
Upper left corner of the annotation relative to the upper left corner of the canvas:

Relative X (%):  (% calculated using x,y values from the canvas)

Relative Y (%):

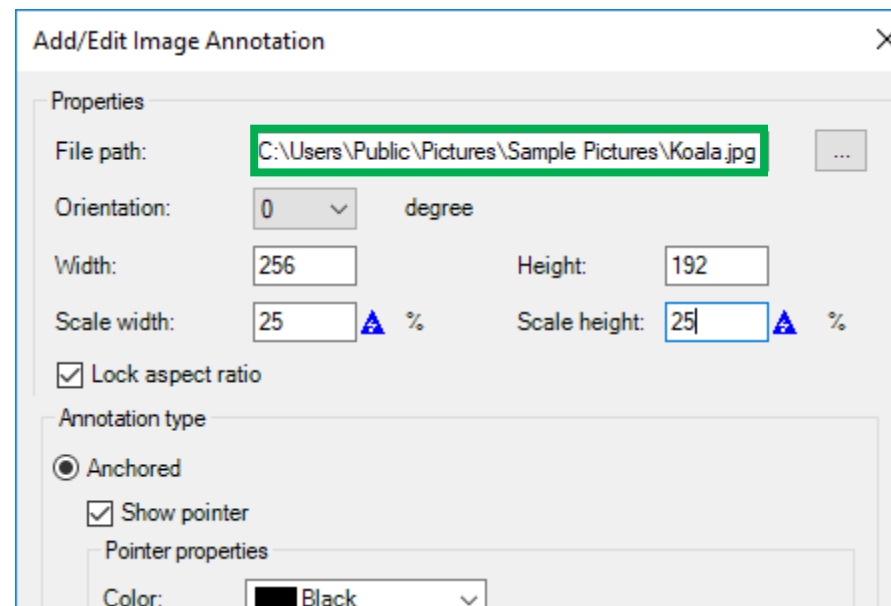
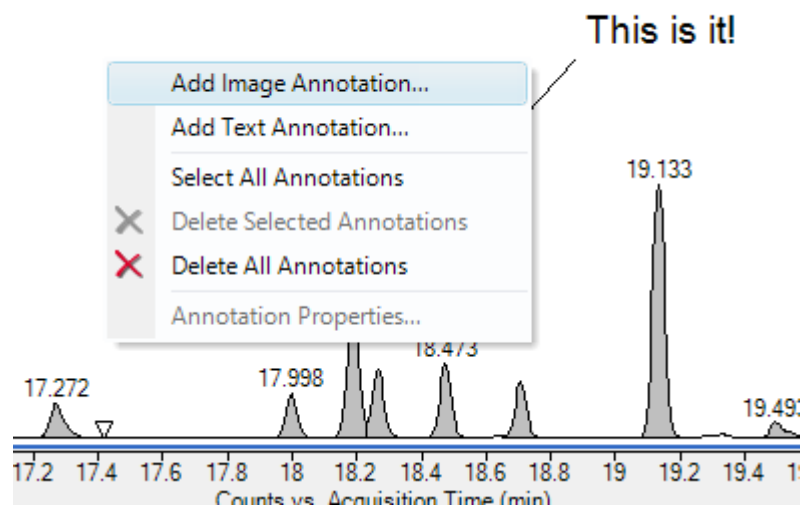
Step 4 Click OK

# Text and Pointer Can Be Repositioned

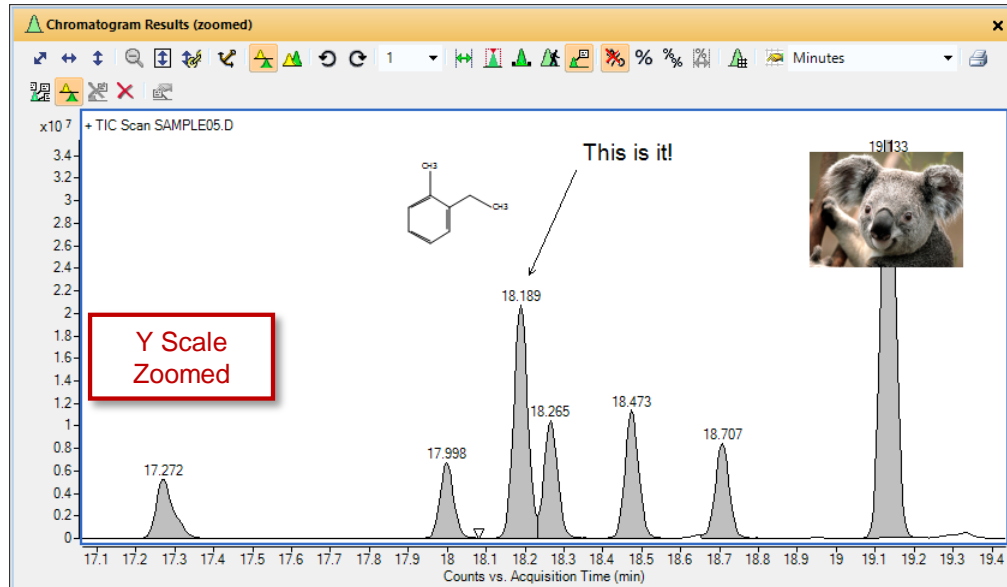
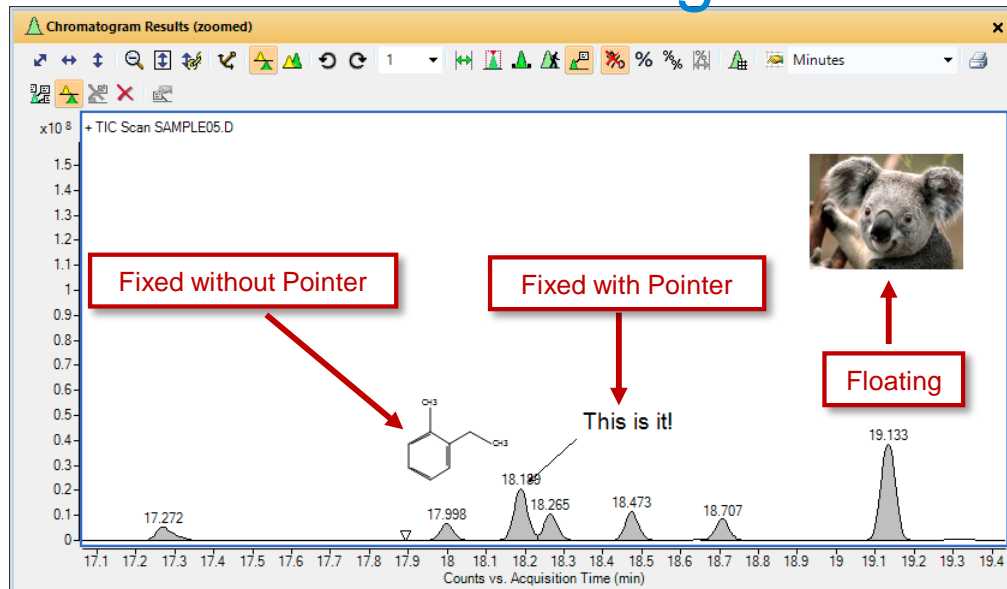


# Image Annotation

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



# Anchored vs. Floating Annotation



Add/Edit Text Annotation

Properties

Text:

(Press Ctrl+Enter or Alt+Enter to add a new line)

Text color:

Orientation:  degrees

Font style:  Font size:

Annotation type

Anchored

Show pointer

Pointer properties

Color:

Pattern:

Weight:

Pointer head:

Pointer head location (the x, y value using the data displayed):

X:  min

Y:  (abundance)

min

Y:  (abundance)

Floating

Upper left corner of the annotation relative to the upper left corner of the canvas:

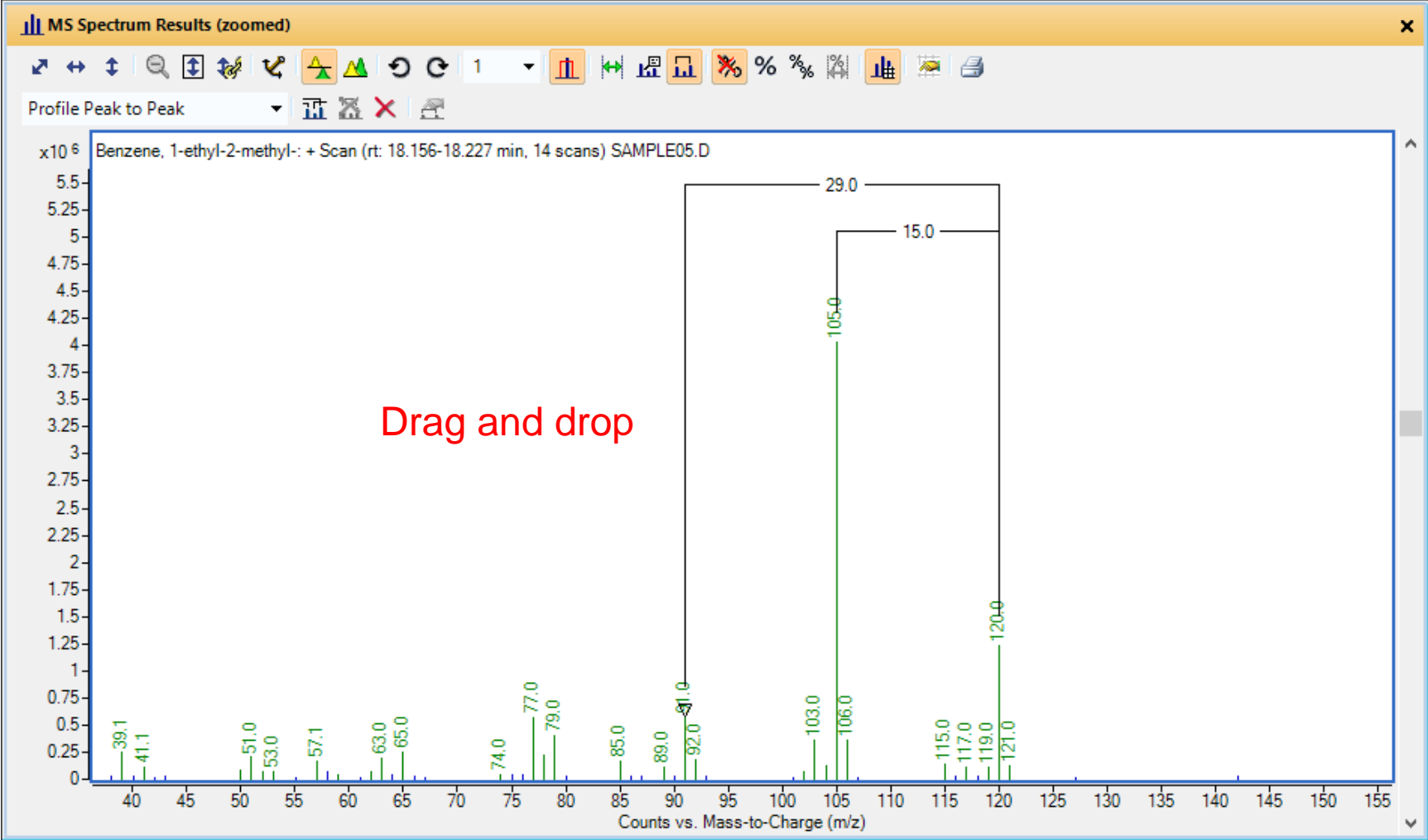
Relative X (%):  (% calculated using x,y values from the canvas )

Relative Y (%):

OK Cancel

# Delta Mass Caliper

Used to calculate and display mass differences between two ions.





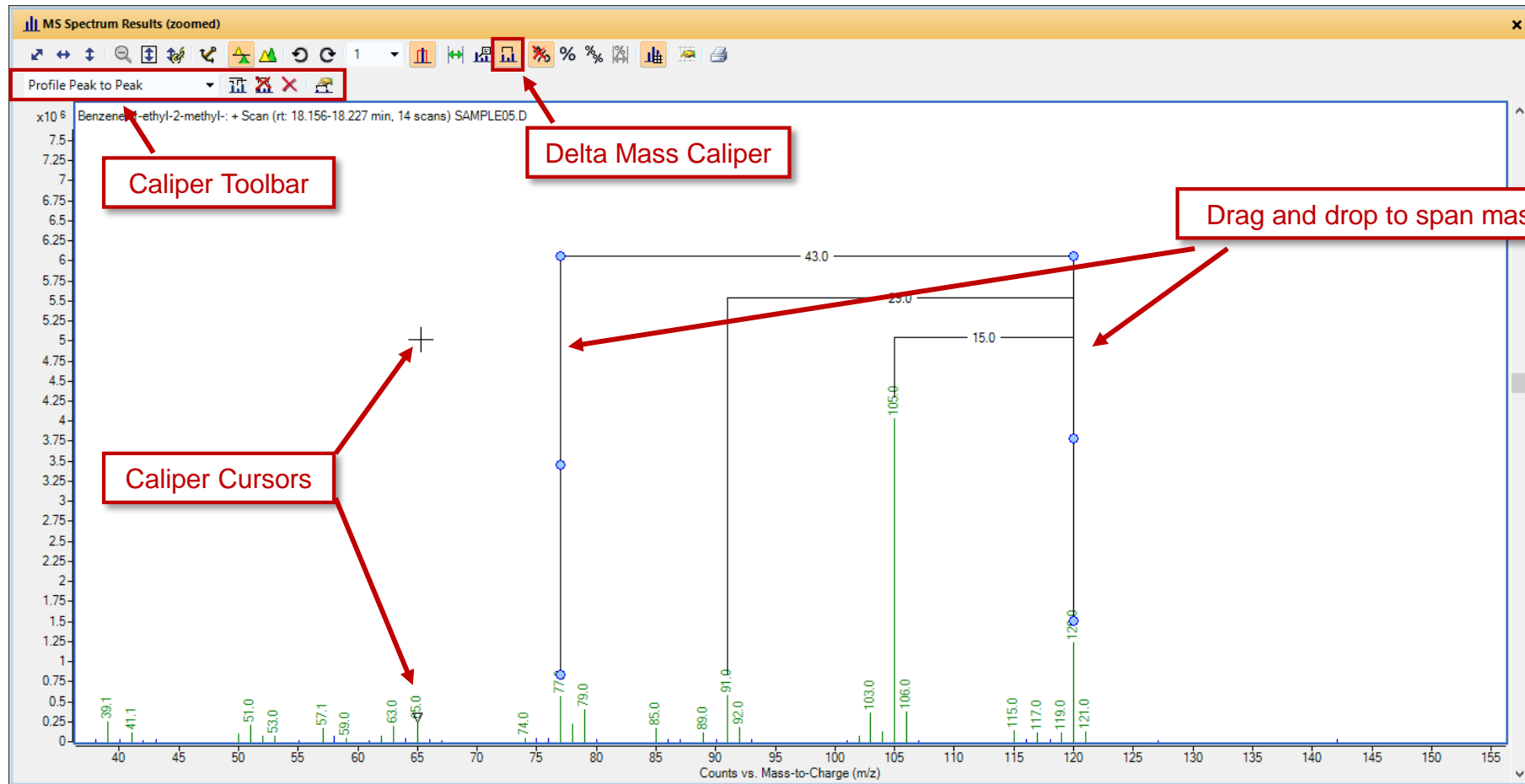
# Delta Mass Caliper - Mass Caliper Mode

Click **Delta Mass Caliper Mouse** icon

Caliber Toolbar and Caliper cursor appear

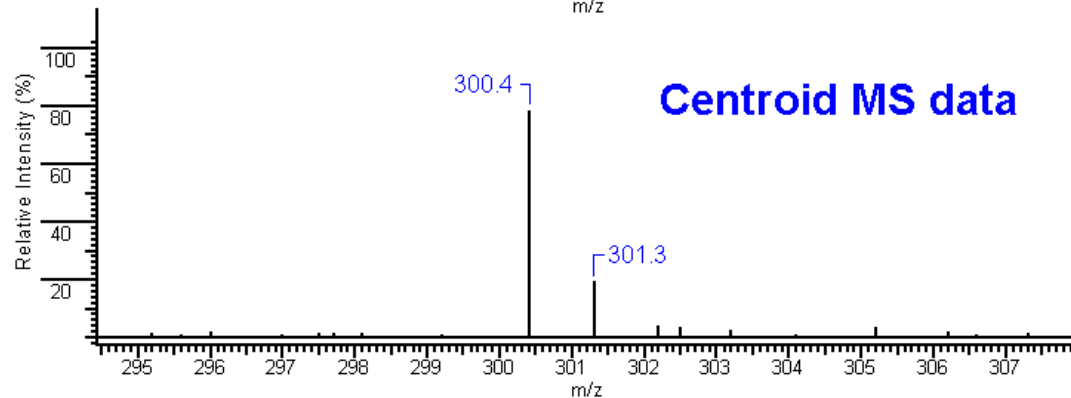
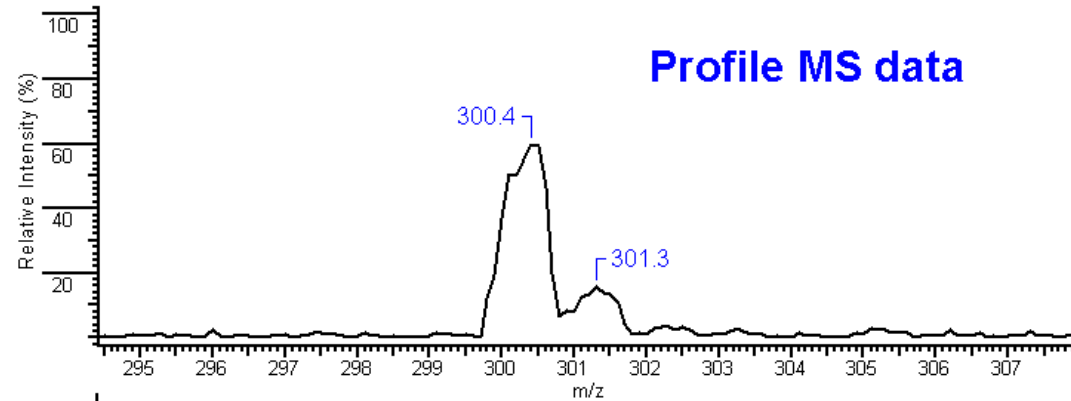
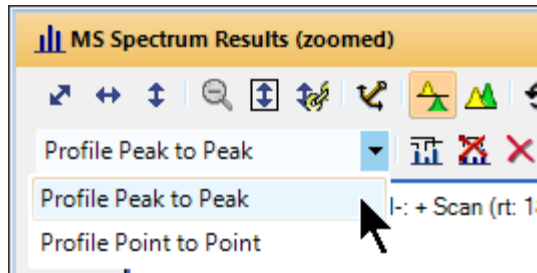
Add or Edit Calipers

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:  
Training Resources



# Training Resources

## Available Training Resources

### Convenient Training

In our classrooms, at your site or online.

From a team of industry experts that deliver a high quality learning experience.

### Classroom Training

Introductory level to in-depth, hands-on for laboratory instrumentation and software.

### Customized On-Site Training

Effective learning environment designed to achieve operational excellence and employ development without the need to travel.

### Online

Offerings from foundation level to expert delivered at your own pace.

# Agilent University

## Access From Home Page

### Upgraded customer experience

Search and find courses that meet your interests and needs in the format they require.

### Introduce new eLearning capabilities

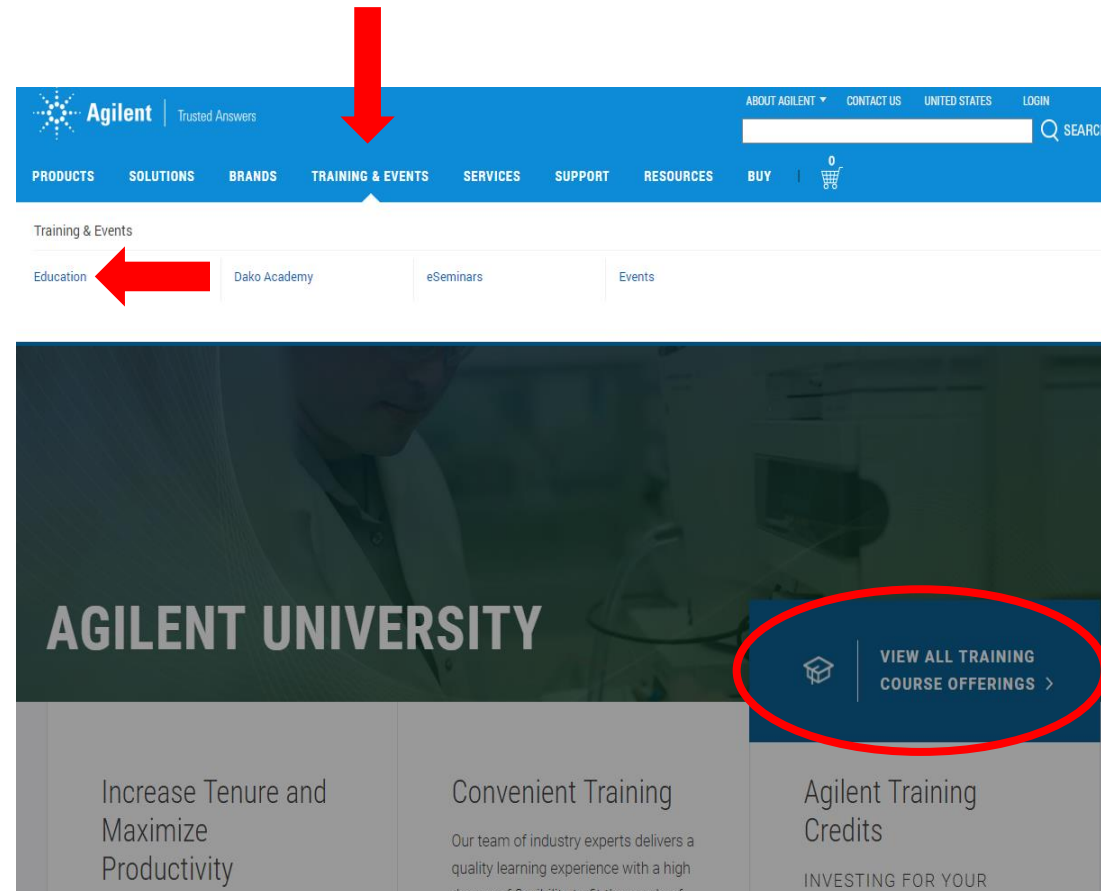
Recorded and video-based learning  
Virtual online classes

### Expanded portfolio

Foundational subjects  
Intermediate subjects  
Advanced subjects  
Workflow and applications

### Helping customers

Educate your employees on Agilent instruments and software.  
From new hires to the most seasoned scientists.



# Agilent Community



**Collaborate** - Ask and answer questions.

**Connect** - Interact with other Agilent users.

**Discover** - Find relevant discussions, documents, and videos.

**Share** - Contribute your insights.

