Agilent MassHunter Qualitative Data Analysis

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Stephen Harnos

MassHunter Qualitative Analysis

Chromatogram Functions

MassHunter Qualitative Analysis Software B.07.00

Topics

- User Interface Configuration
- User Workflows
- Views
  - Navigator
  - Compound Details
- Methods
  - Unified Method Concepts
  - Method Explorer
  - Method Editor
- Working with Chromatograms
  - Anchoring and Scaling
  - Chromatogram Functions
  - Integrators
- Training Resources
- Define Qualitative Analysis

MassHunter Qual - Configurable Software

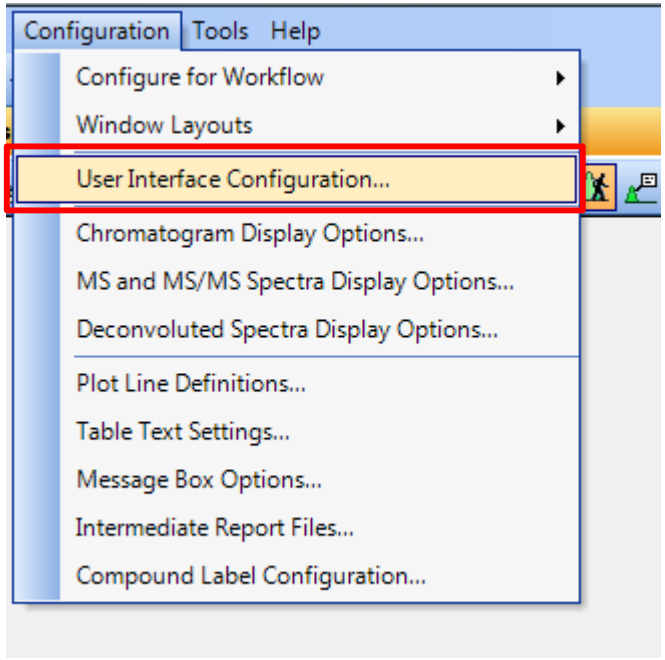
- One program for many instruments and types of data.
  - Single Quad (LC & GC) Unit resolution, Scan, SIM data
  - Triple Quad (LC & GC) Unit Resolution Scan, SIM, MRM (MS/MS) data
  - TOF (LC) High resolution, scan data
  - Q-TOF (LC & GC) High resolution MS/MS data

- Many software features can be used by all data types but many are only useful for a particular instrument type.

- MassHunter Qual MUST be configured to reduce complexity and hide unneeded and potentially misused features.

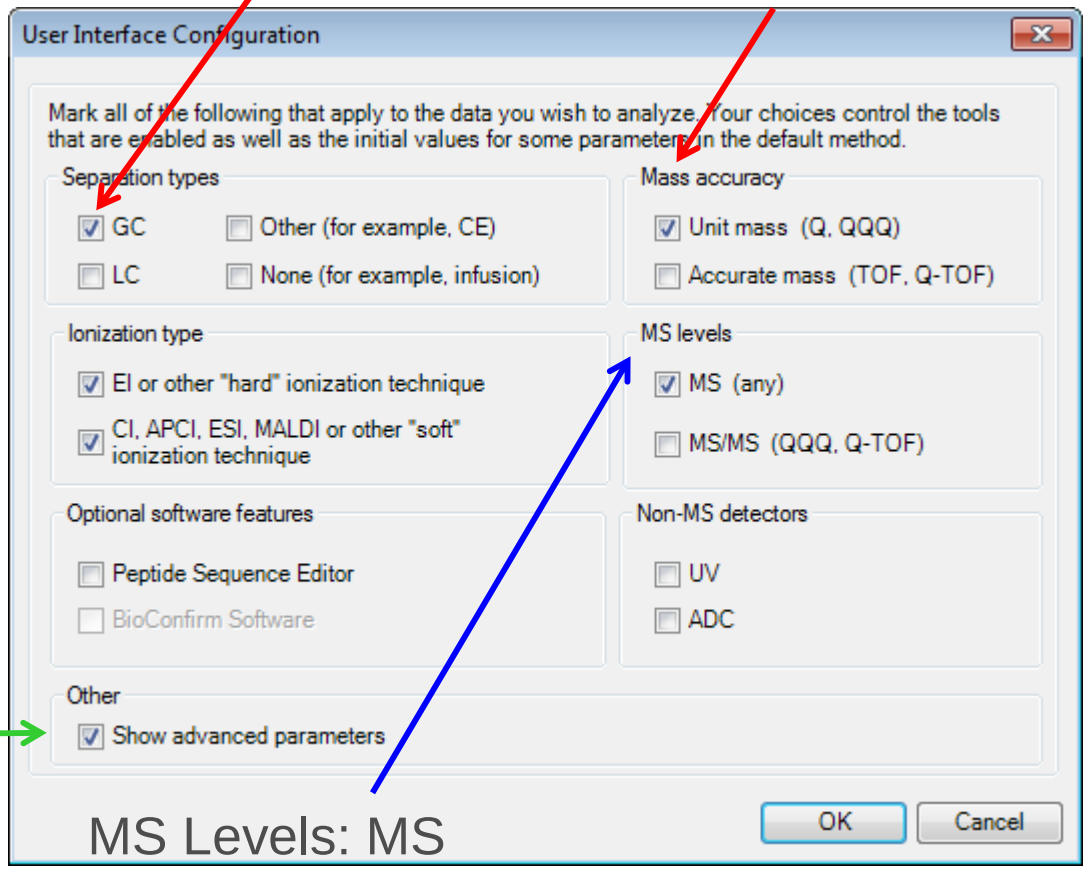
- Even when properly configured some features and parameters for MS/MS and accurate mass are still visible, ignore and avoid them.

User Interface Configuration



Separation types
(Check GC or LC)

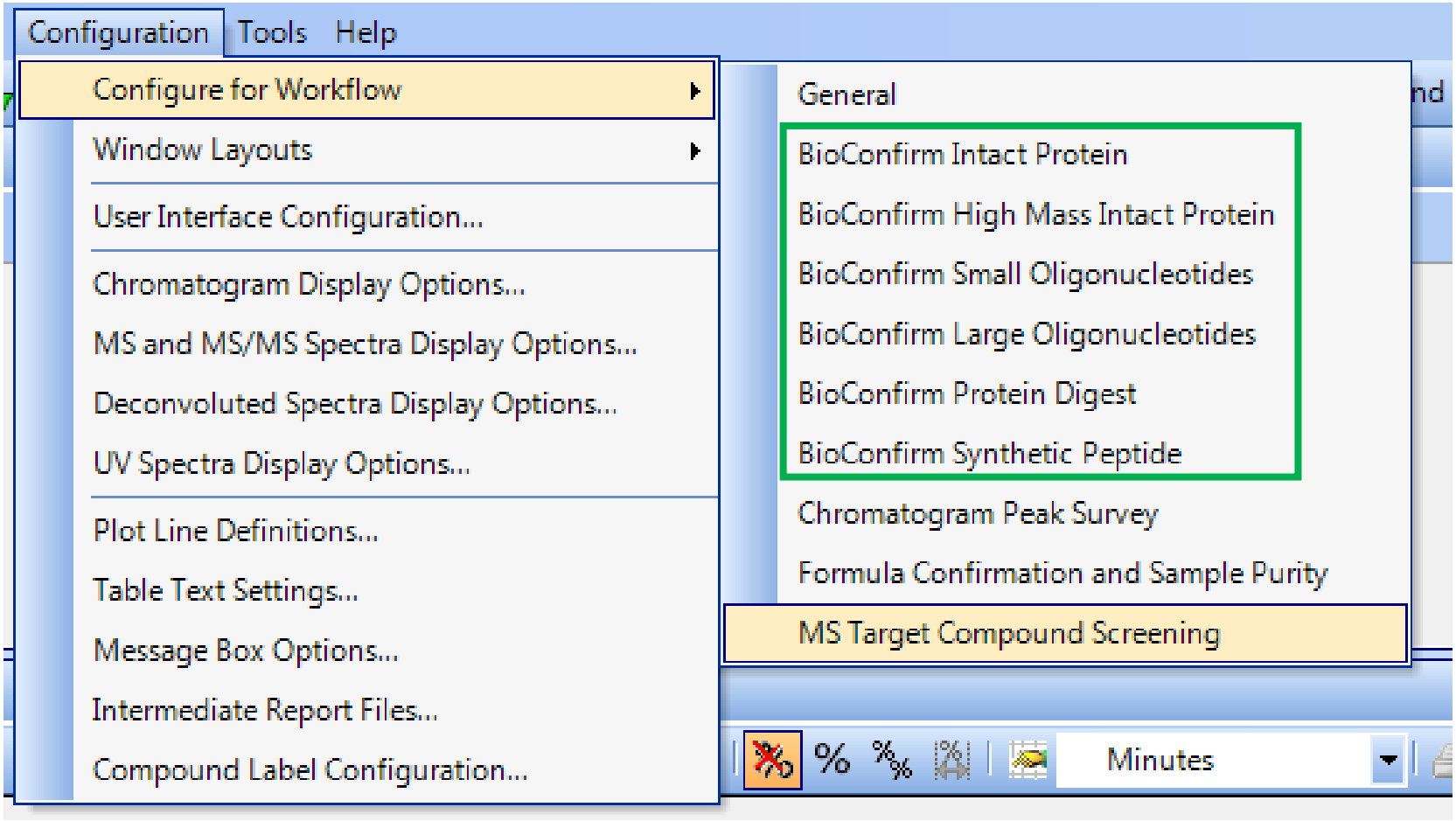
Unit Mass (Q, QQQ)
Accurate Mass
(TOF, QTOF)



Check Show Advanced Parameters

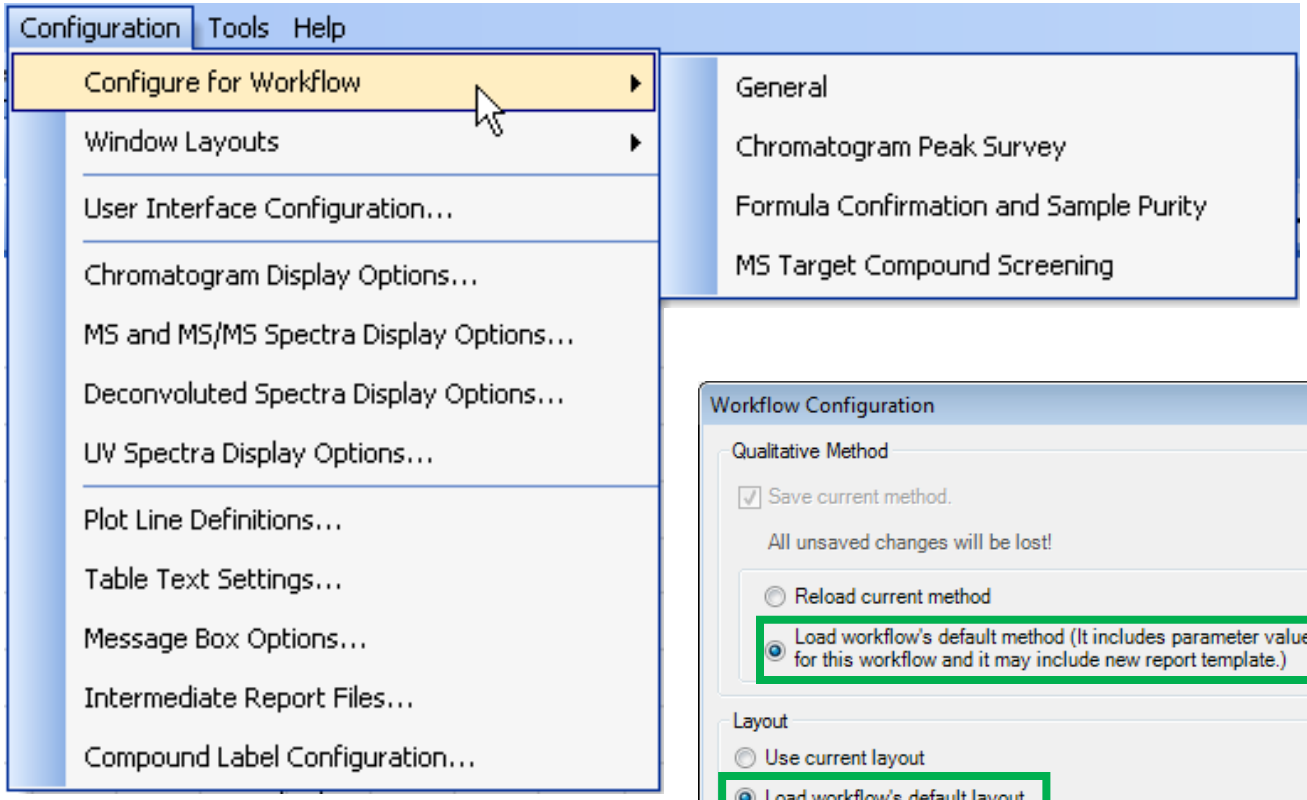
MS Levels: MS

MassHunter Qualitative Analysis Workflows



Depends Upon Software Loaded and Configuration Selected

Configure for Workflow

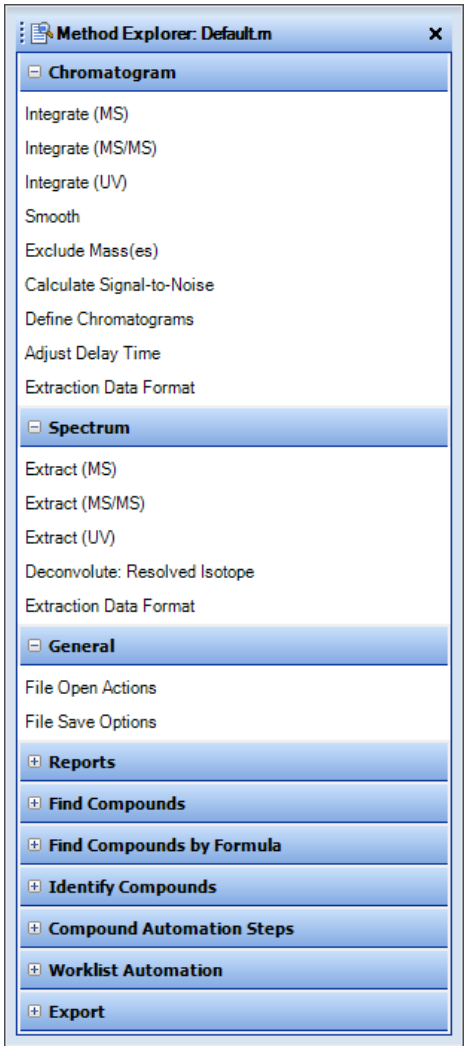


Configuration Changes Graphics, Table, and Method Layouts.

Tip: Load workflow's default method and default layout.

Chromatogram Peak Survey Workflow

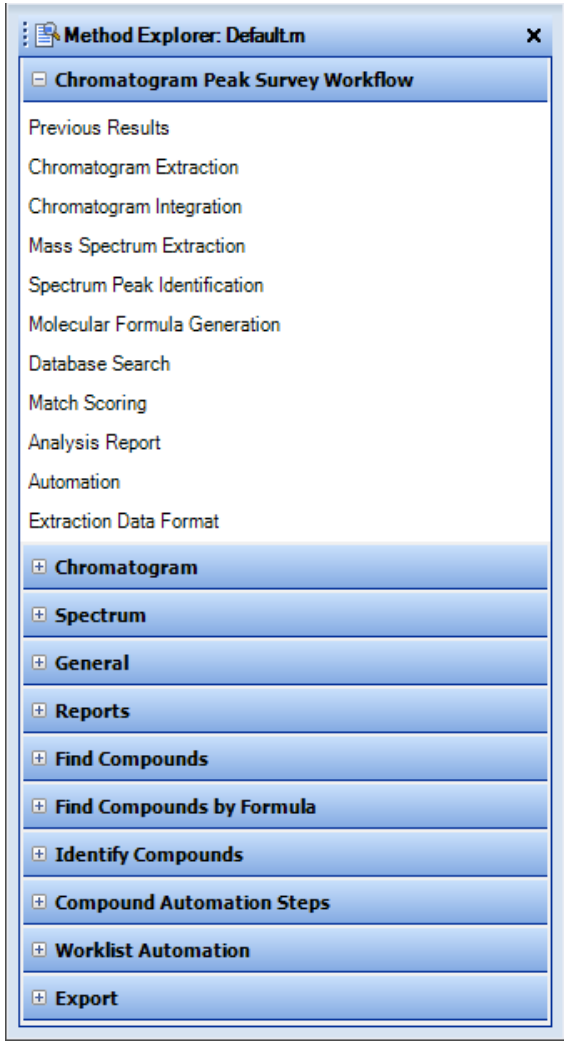
General



Modify settings for chromatogram.

Modify settings for spectral extraction.

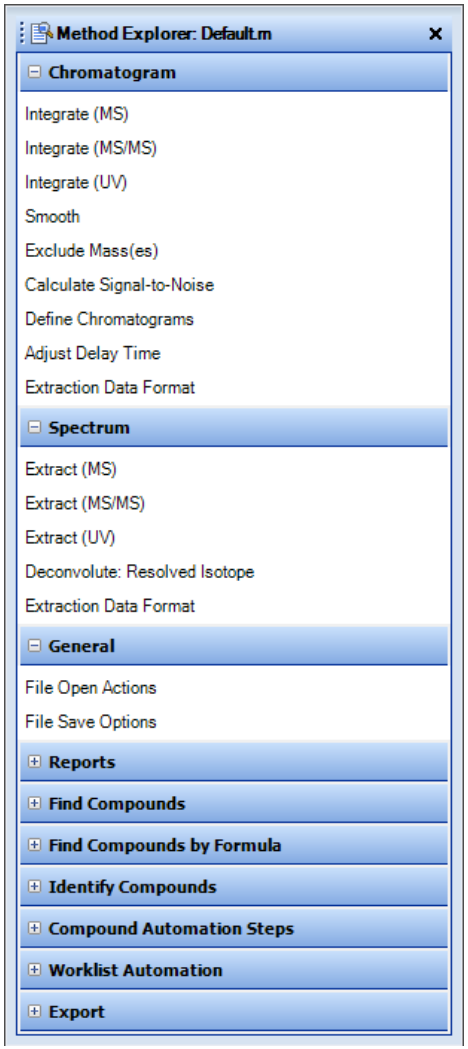
Chromatogram Peak Survey



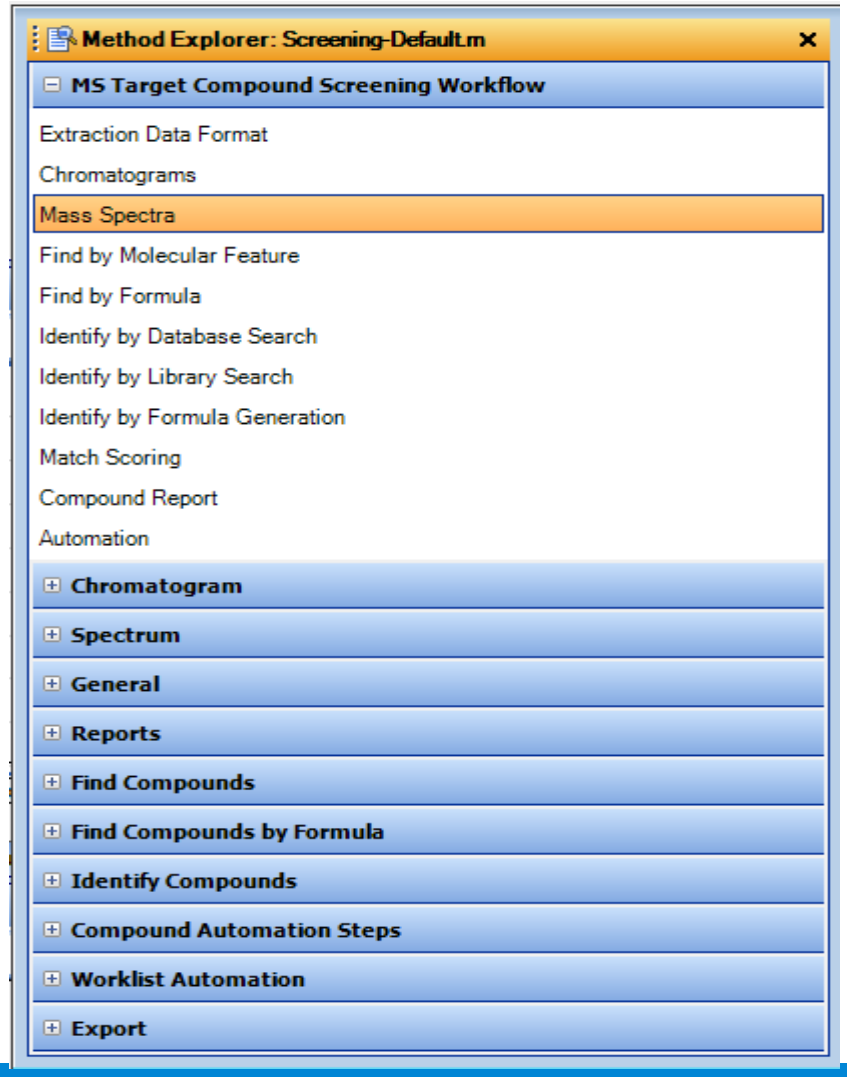
Specified workflow added.

MS Target Compound Screening Workflow

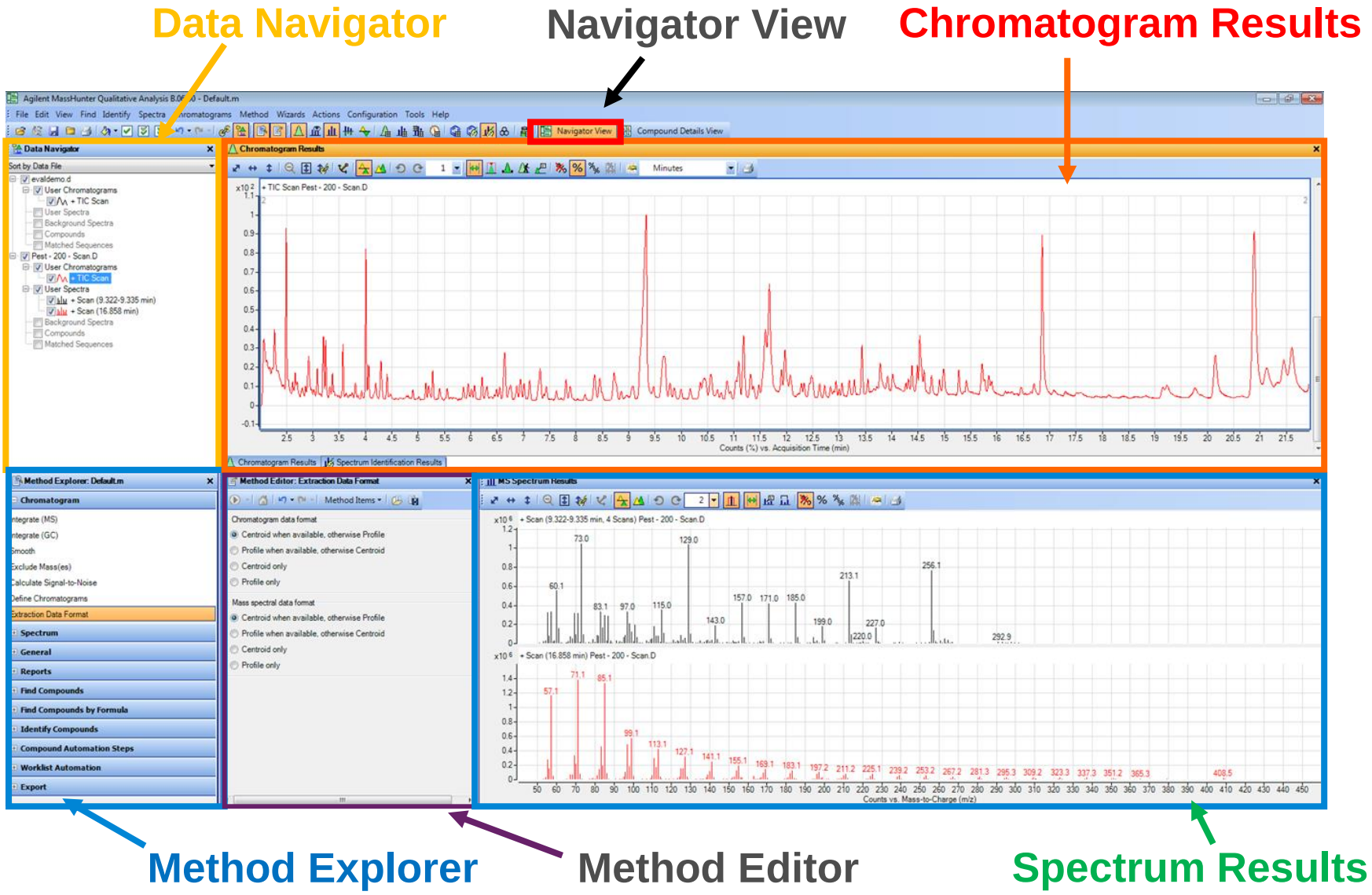
General



MS Target Compound Screening Workflow



Navigator View

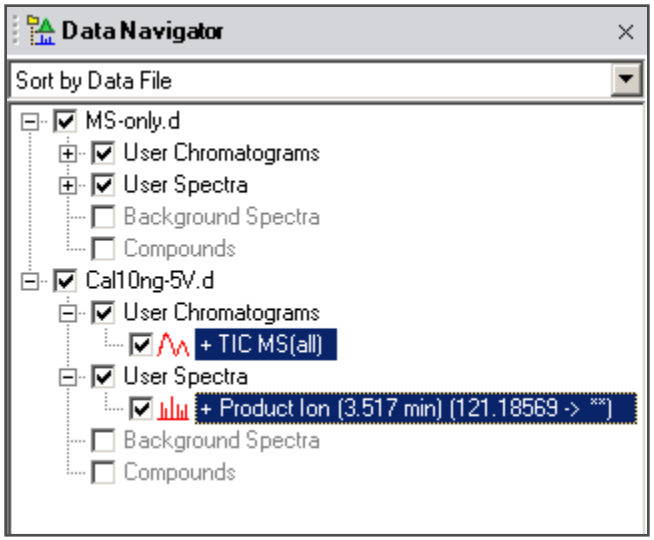


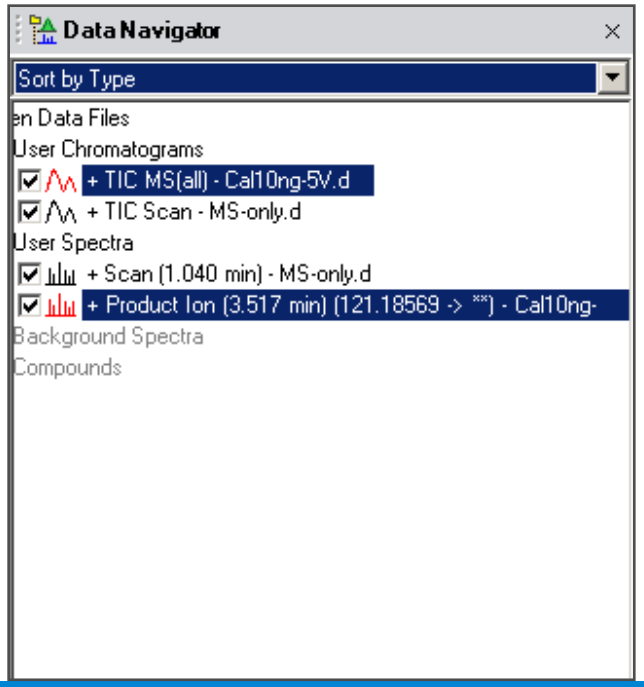
Data Navigator

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

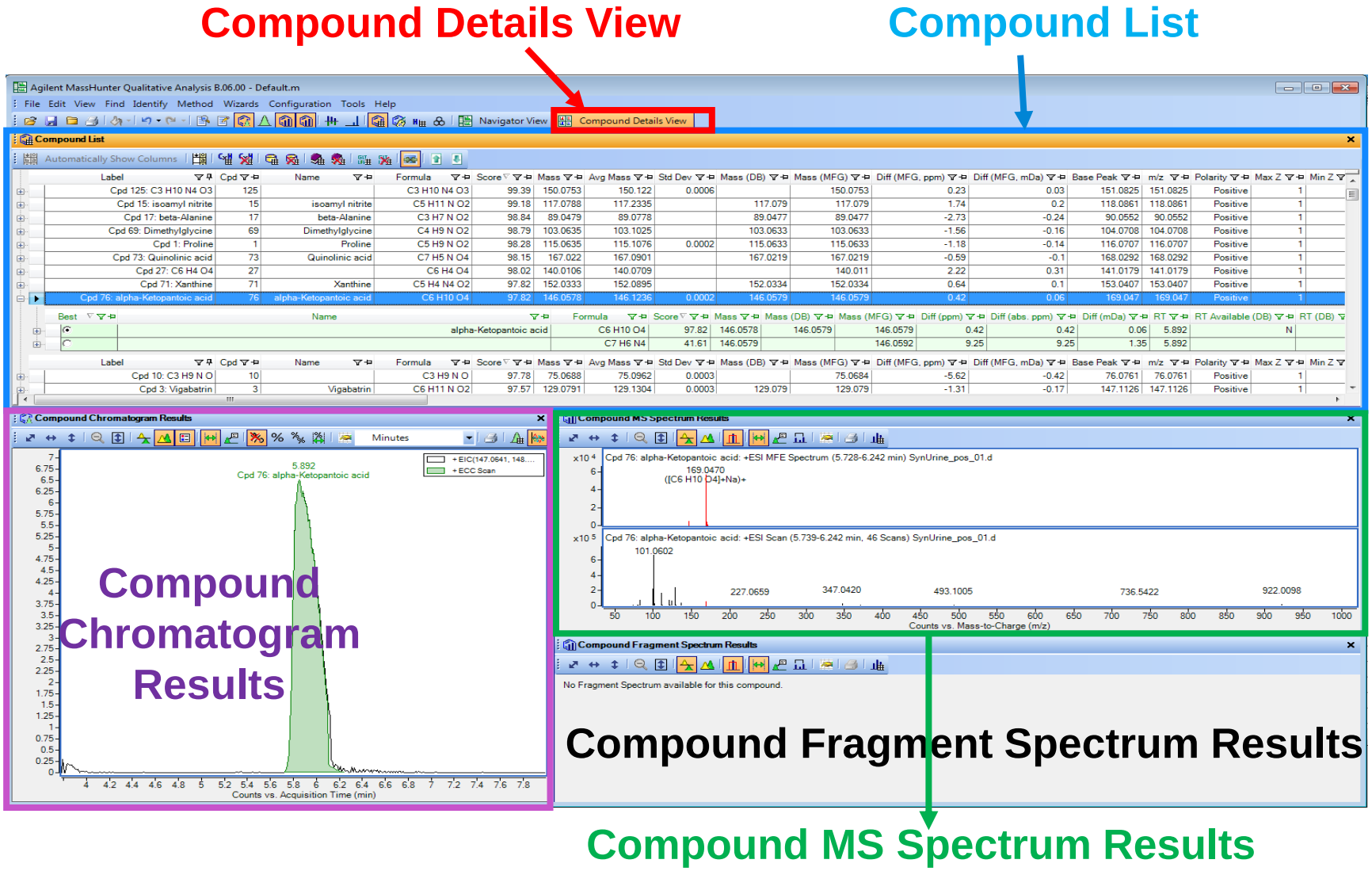
The user can selectively display the information associated with a data file (i.e. chromatograms, spectra, compounds) by selecting/deselecting a checkbox.

In the top drop-down, the user can choose to sort by Data File or Type (i.e. User Chromatogram, etc.)

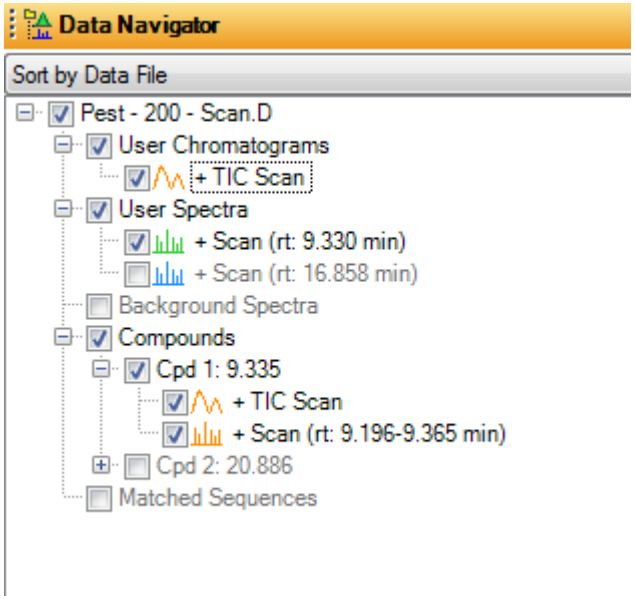




Compound Details View

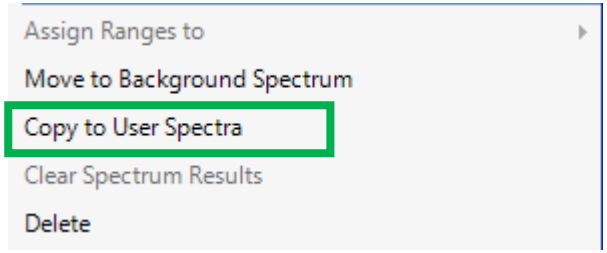


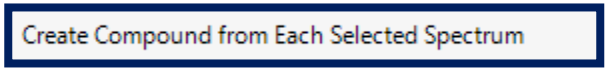
Definitions



User Spectra are mass spectrum that the user creates.

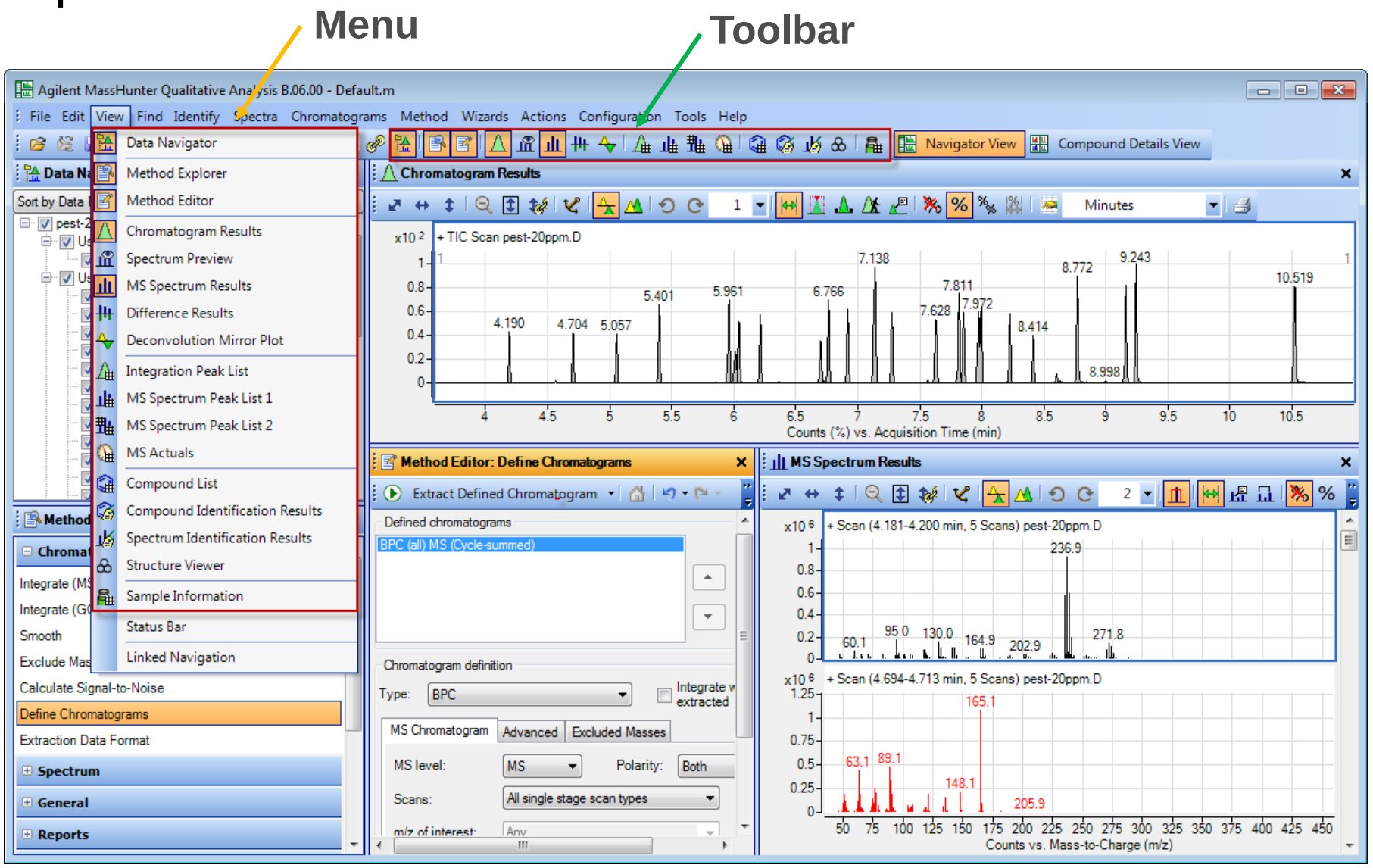
Compounds are generated by one of the 'Find by' algorithms. Compounds are generated by the software.



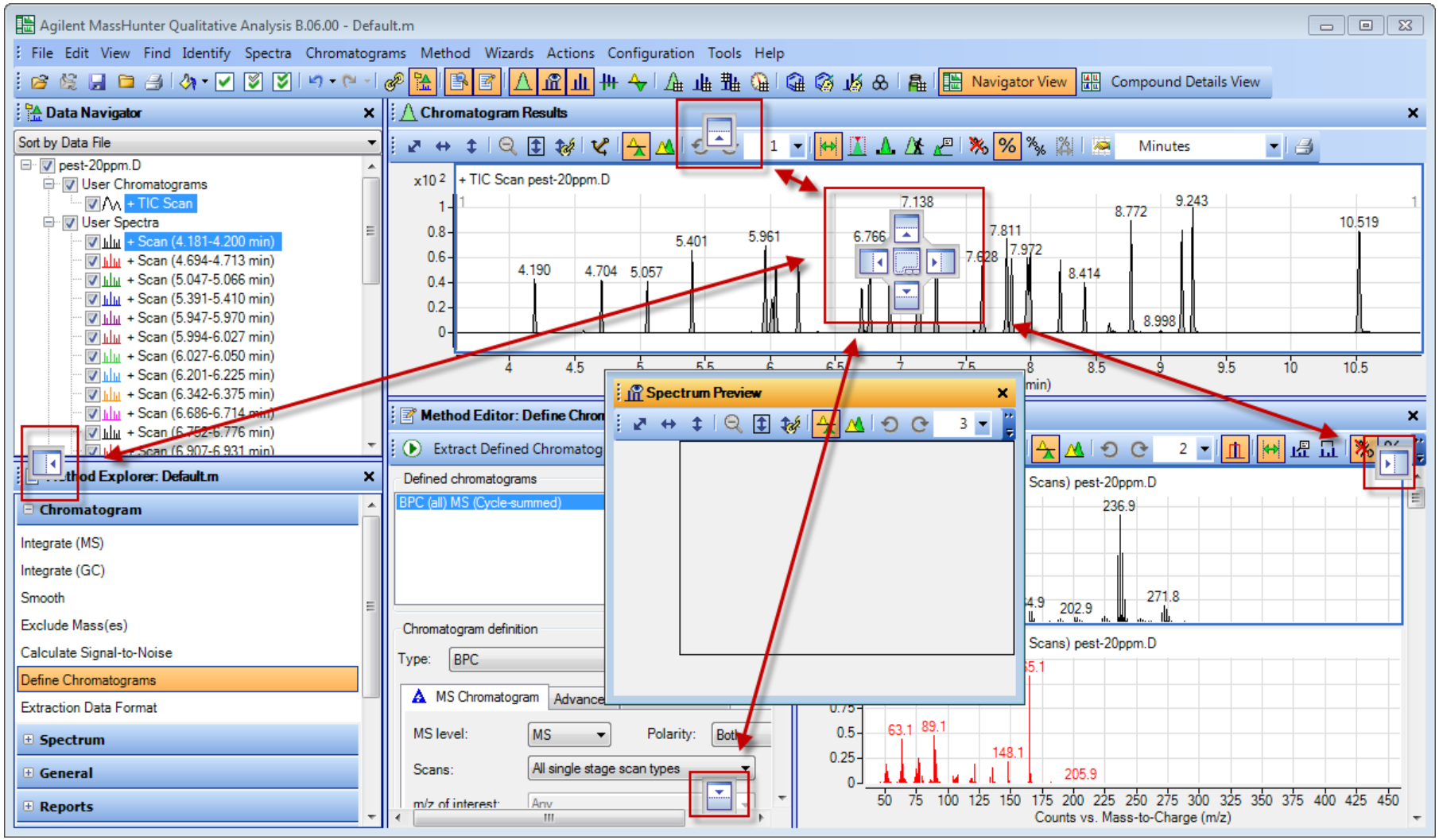


User Spectra and Compounds are readily interchangeable through the context menu (right click on the User or Compound Spectrum in the MS Spectrum Results window).

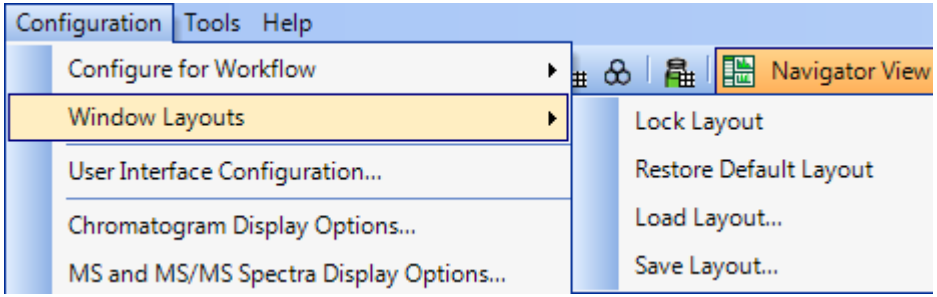
Expose or Hide Windows as Needed



Docking & Undocking Windows



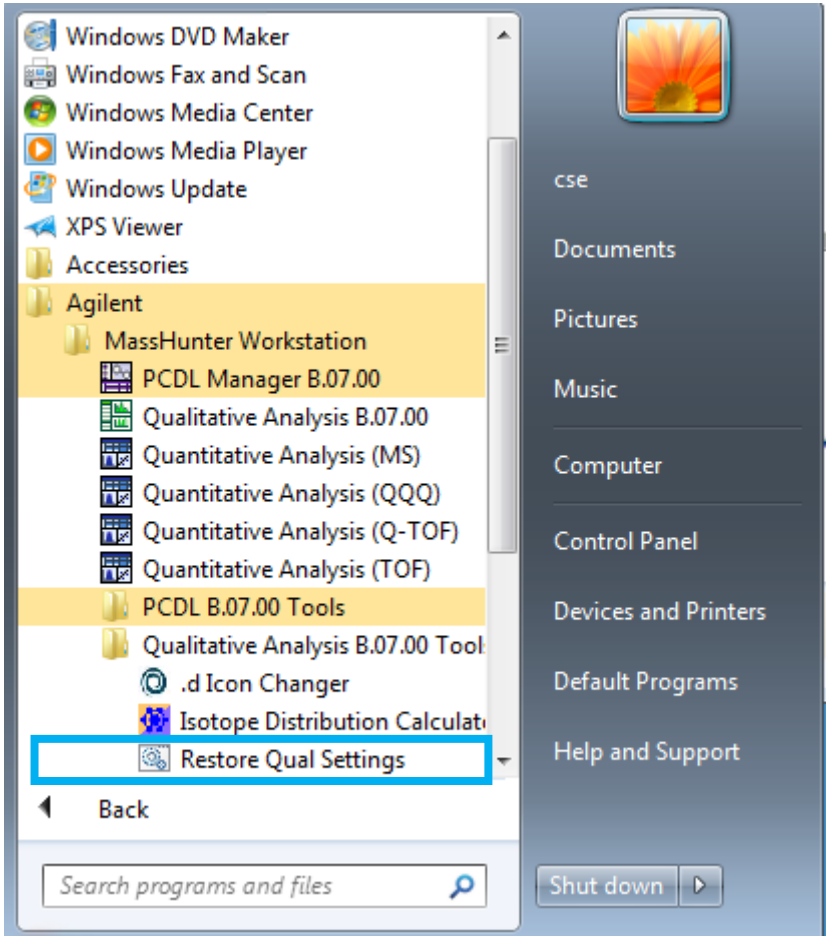
Restore Default Layout

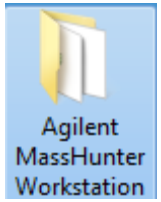


- Complicated windows layouts can be restored to default layout.
- Preferred layouts can be saved and loaded.
- Layouts can be locked.

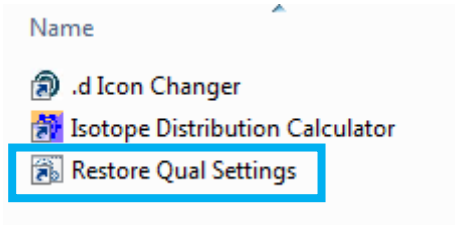
Restore Qual Setting

This may be a useful tool to restore the Qualitative Analysis settings if a configuration problem is suspected.

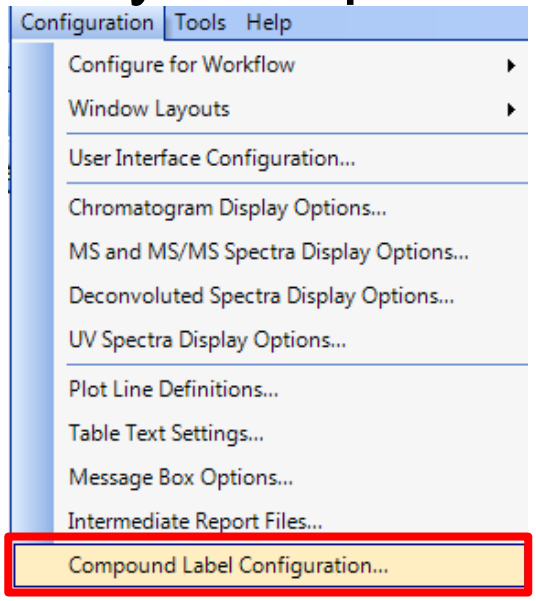




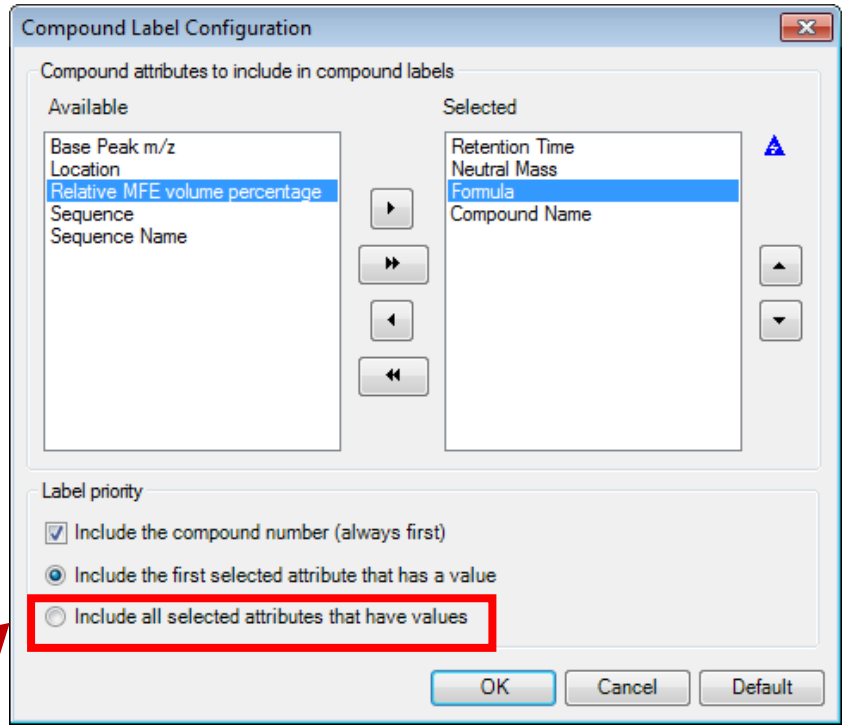
Or Desktop folder



Specify Compound Label Configuration

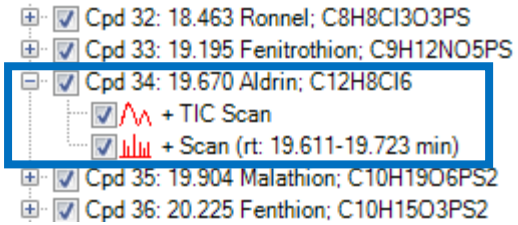


Configuration > Compound Label Configuration

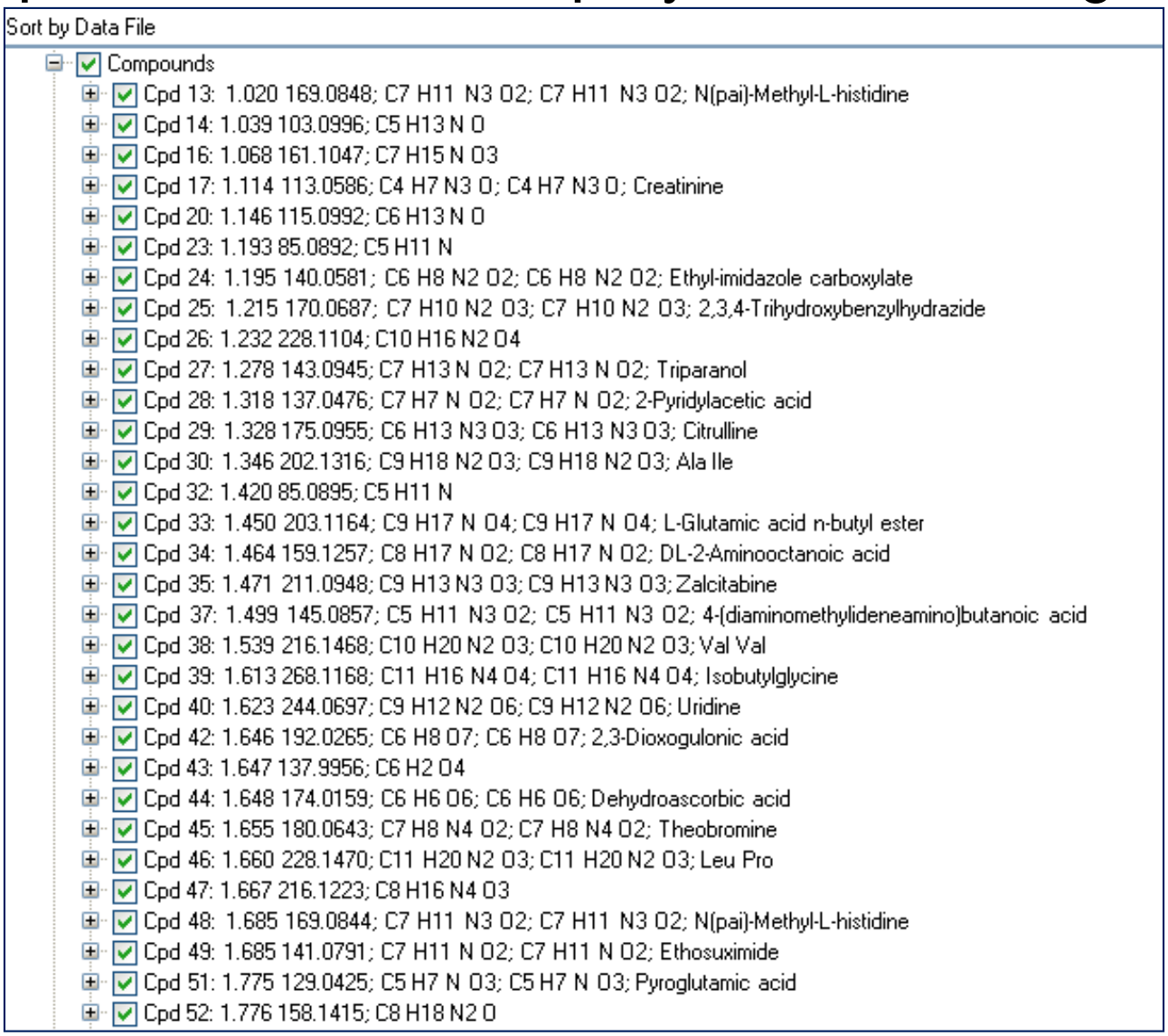


Tip: Select Include all selected attributes that have values.

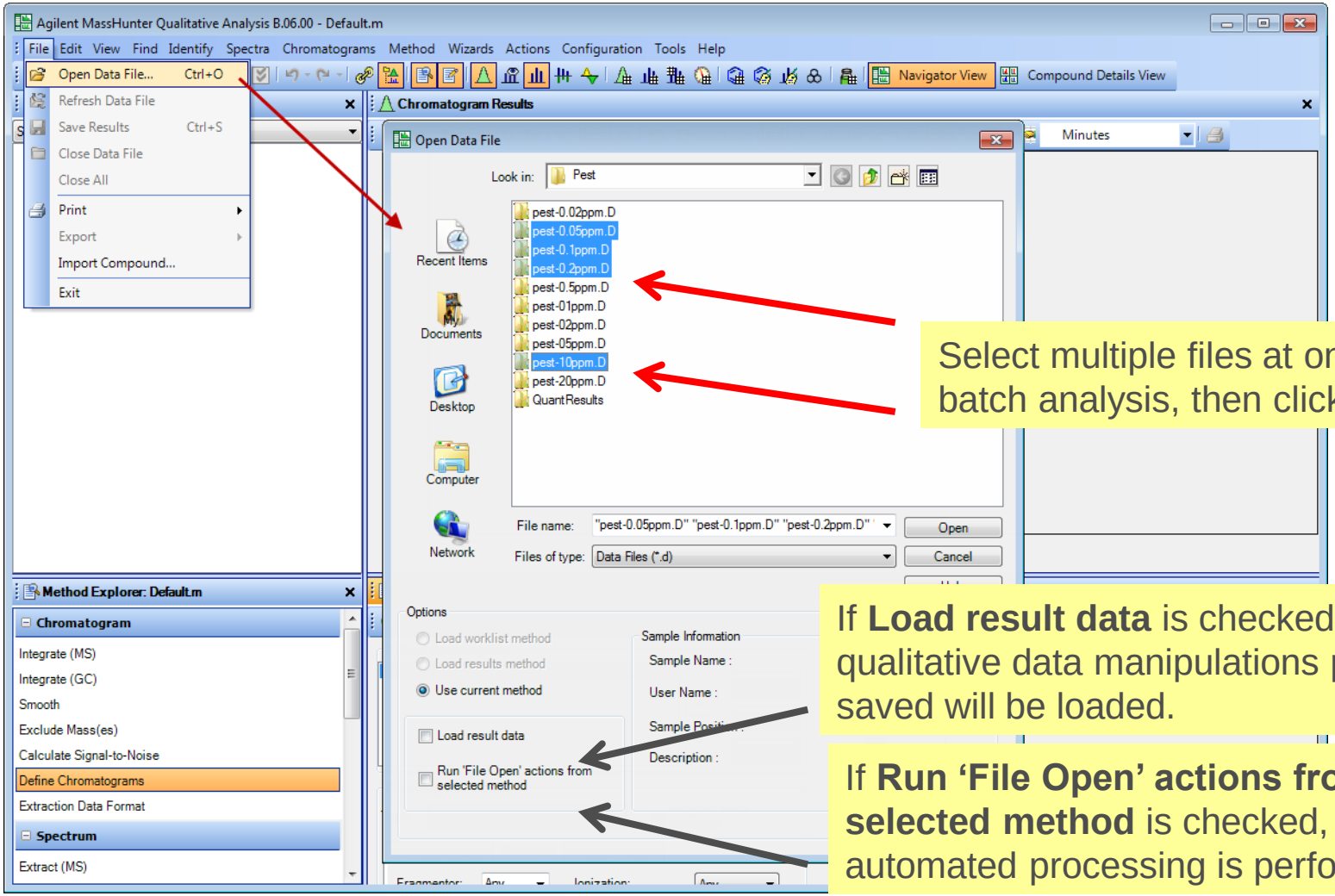
In this example, compounds shows the compound number, RT, compound and formula.



Compounds Labels Display in Data Navigator



Open Data Files



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

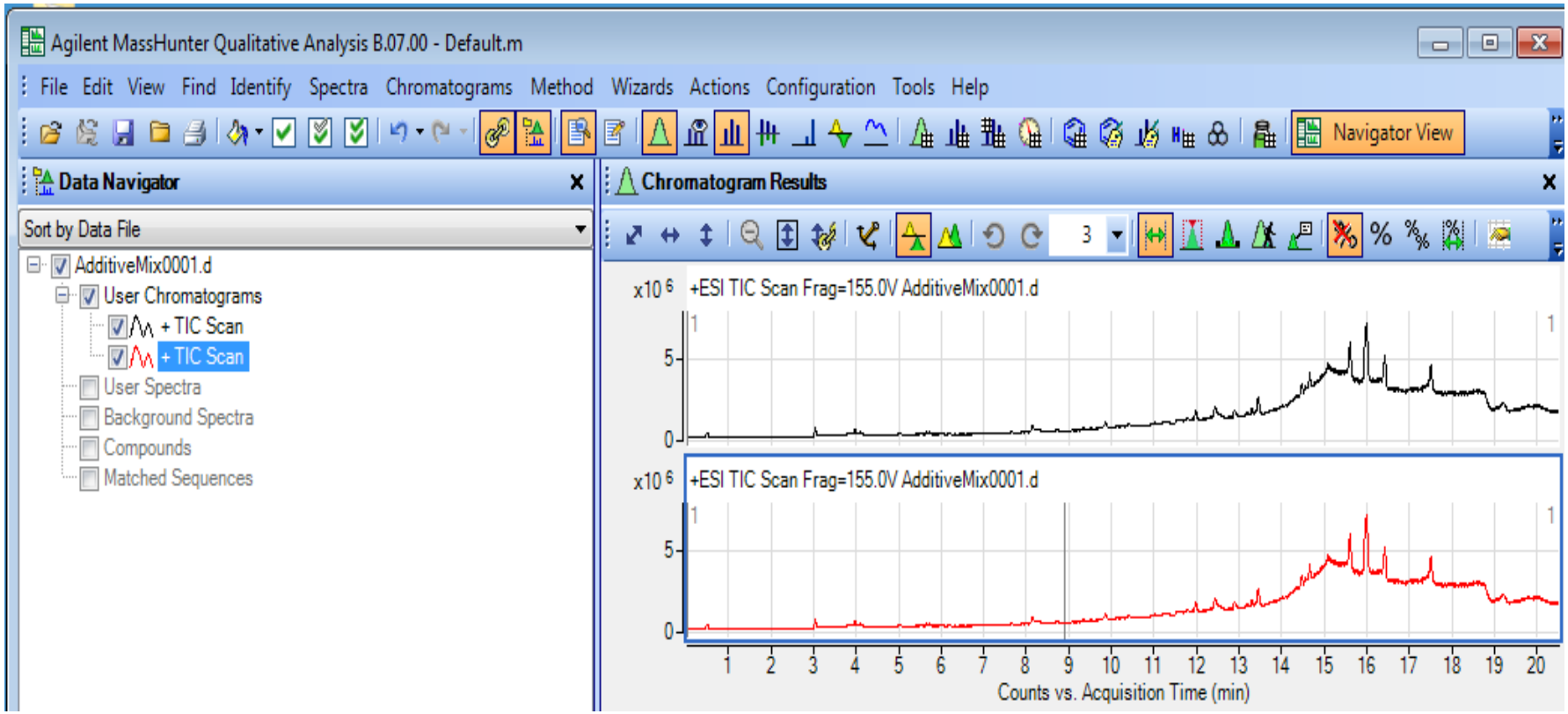
If **Load result data** is checked, the qualitative data manipulations previously saved will be loaded.

If **Run 'File Open' actions from selected method** is checked, automated processing is performed.

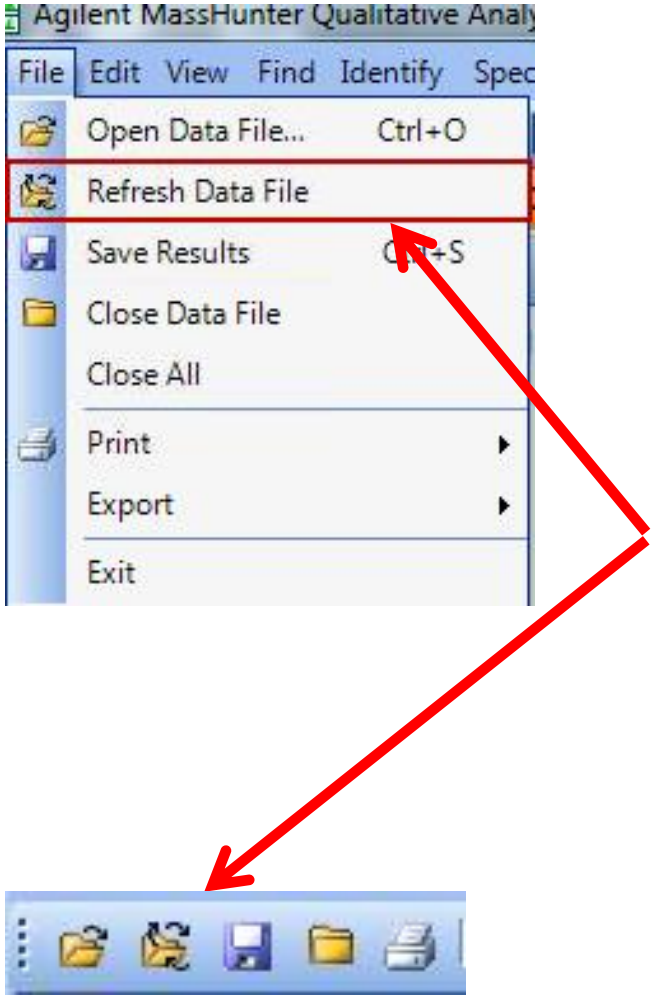
If neither **Load result data** or **Run 'File Open' actions from selected method** is checked, then a TIC is automatically extracted from the data files.

Tip

Every time a data file is loaded see 2 TICs.



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.

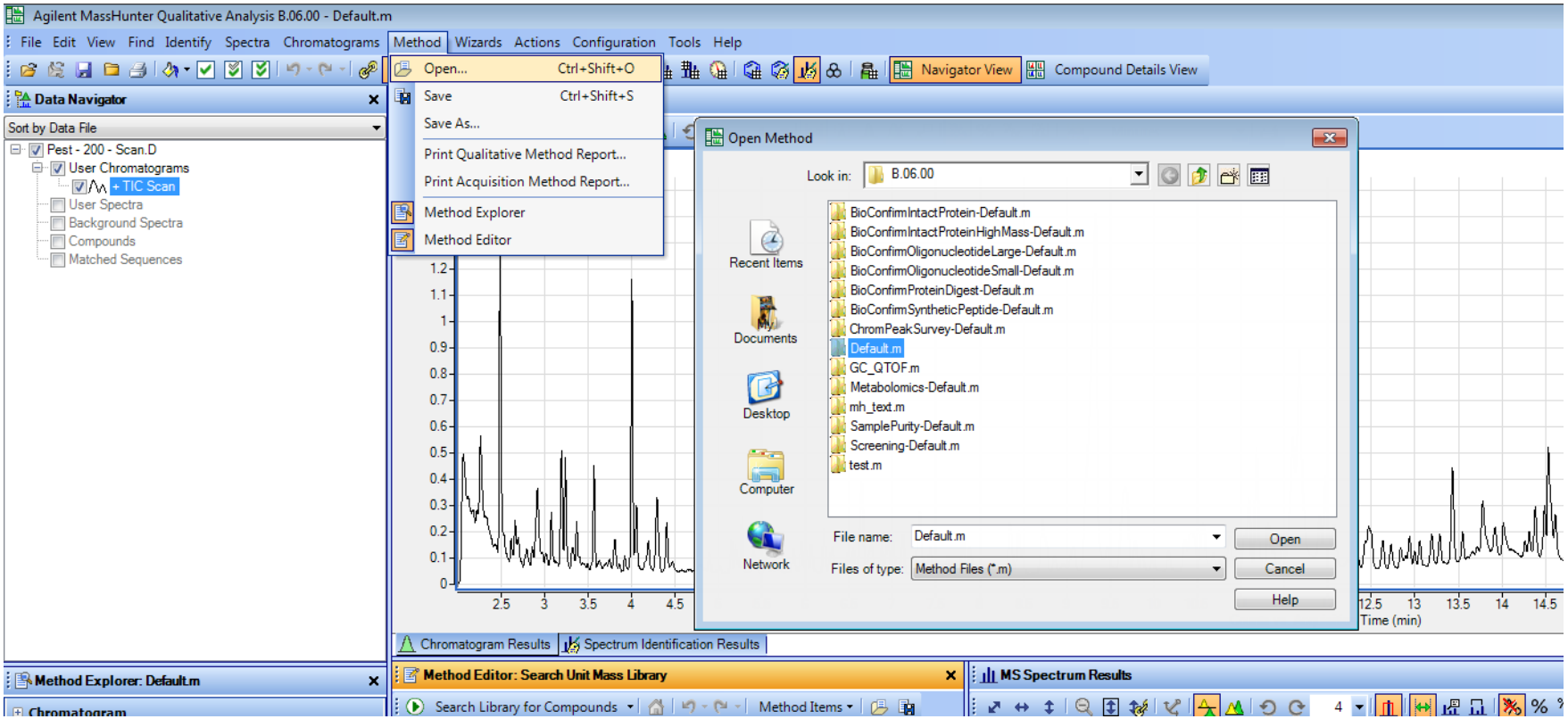
Let’s take a moment for chat questions on Configuration and Layouts.

Up Next:

Qualitative Methods

Demo on Configuration and Layouts.

Qualitative Analysis Methods

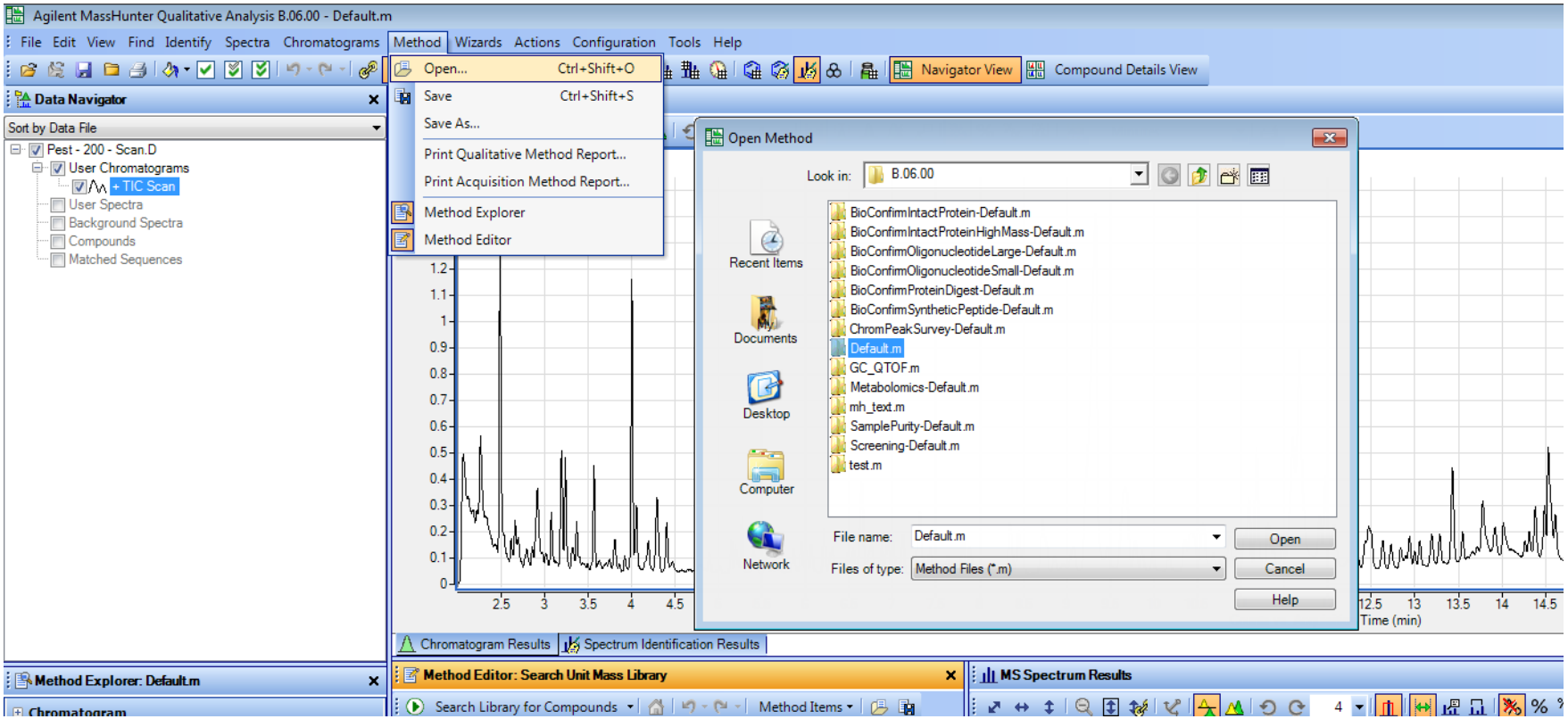


Qualitative Analysis Methods are stored in a .M folder.

Many application & instrument specific methods, generally use Default.m.

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

What is a Method? Unified Method Concept



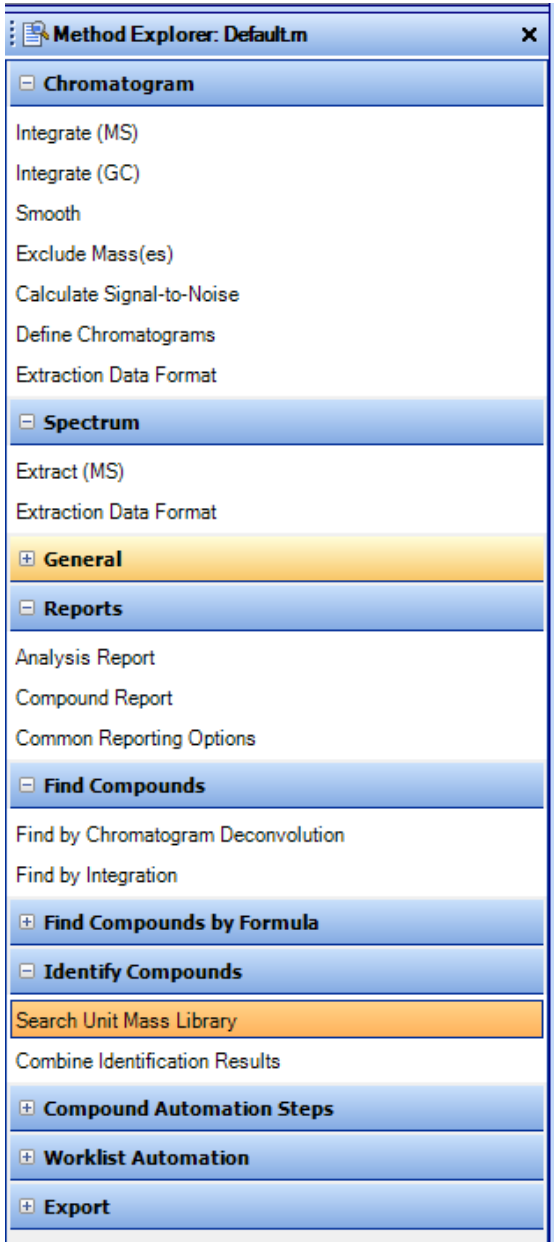
Qualitative Analysis Methods are stored in a .M folder.
Quantitative Analysis Methods are stored in a .M folder.
Quantitative Analysis Reporting Methods are stored in a .M folder.
Unified method can now be automated to run from the sequence/worklist.

Method Explorer

Acts as a table of contents for the method.

Items in Method Explorer automatically display related Method Editor features.

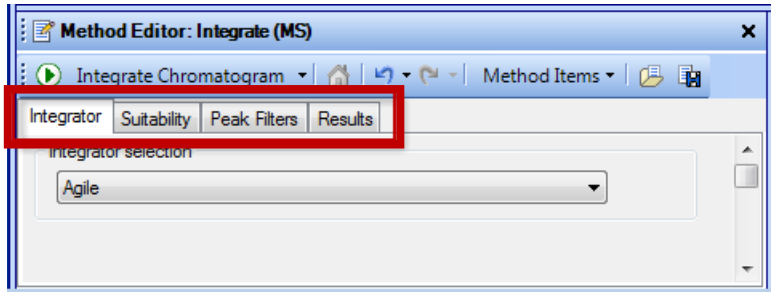
Items are dynamic and controlled by the User Configuration and Workflow setting.



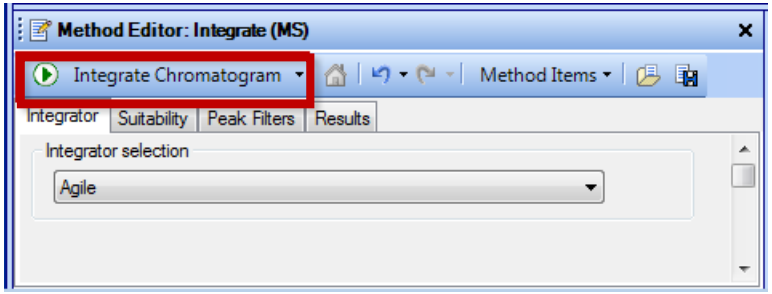
Method Editor

Display and Edit sections of the Method.

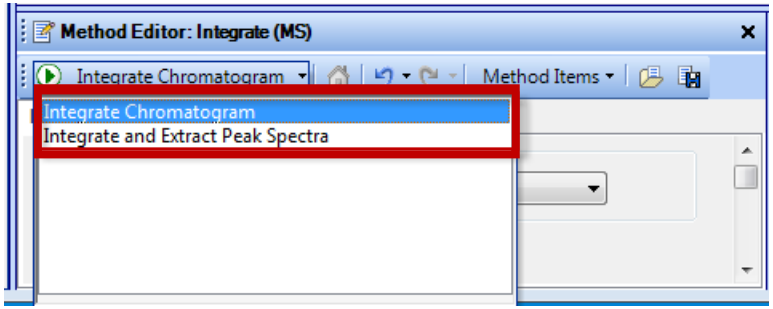
Tabs within the Method Editor further organize method parameters.



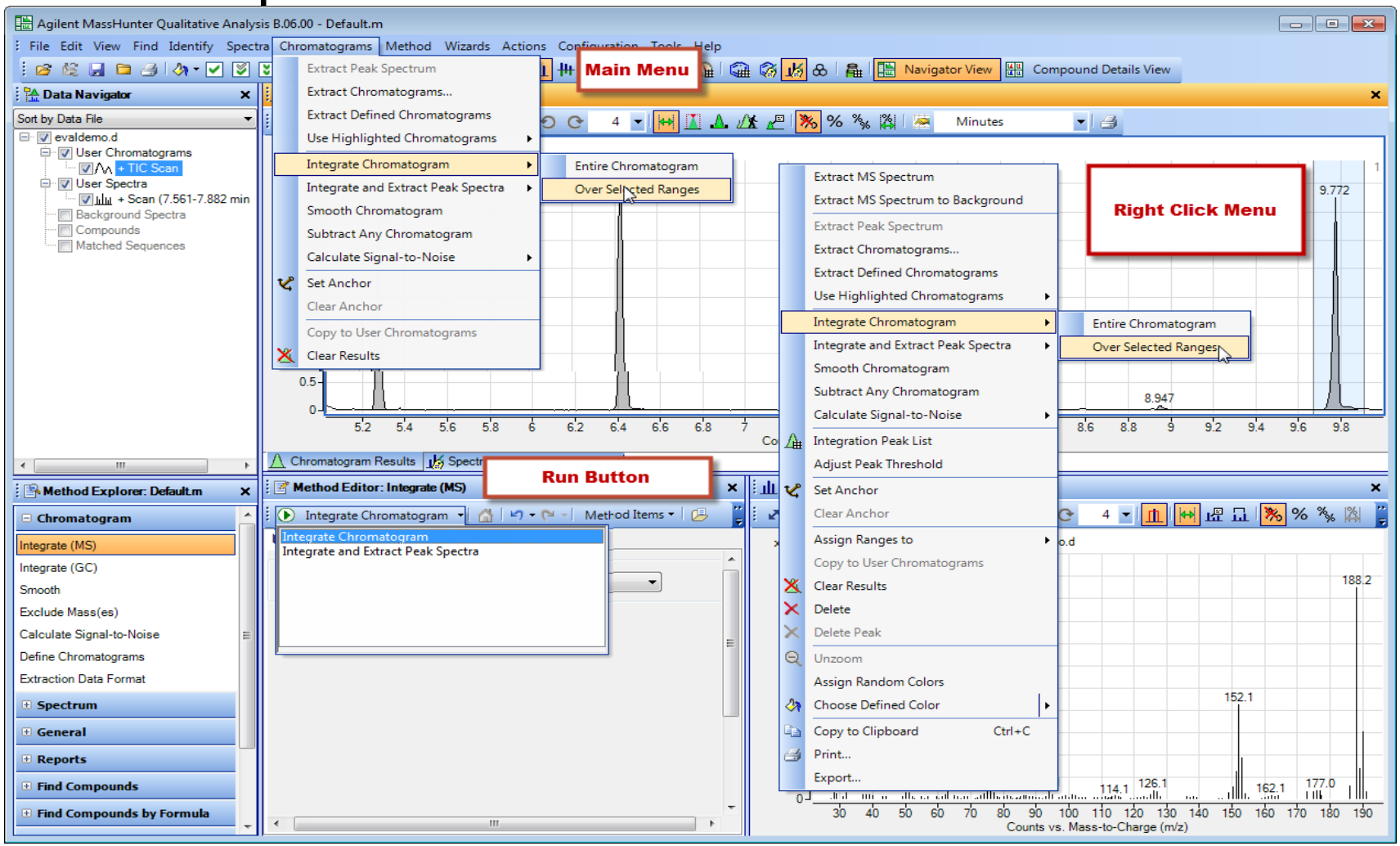
The “Run” icon executes the function associated with this part of the method.



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



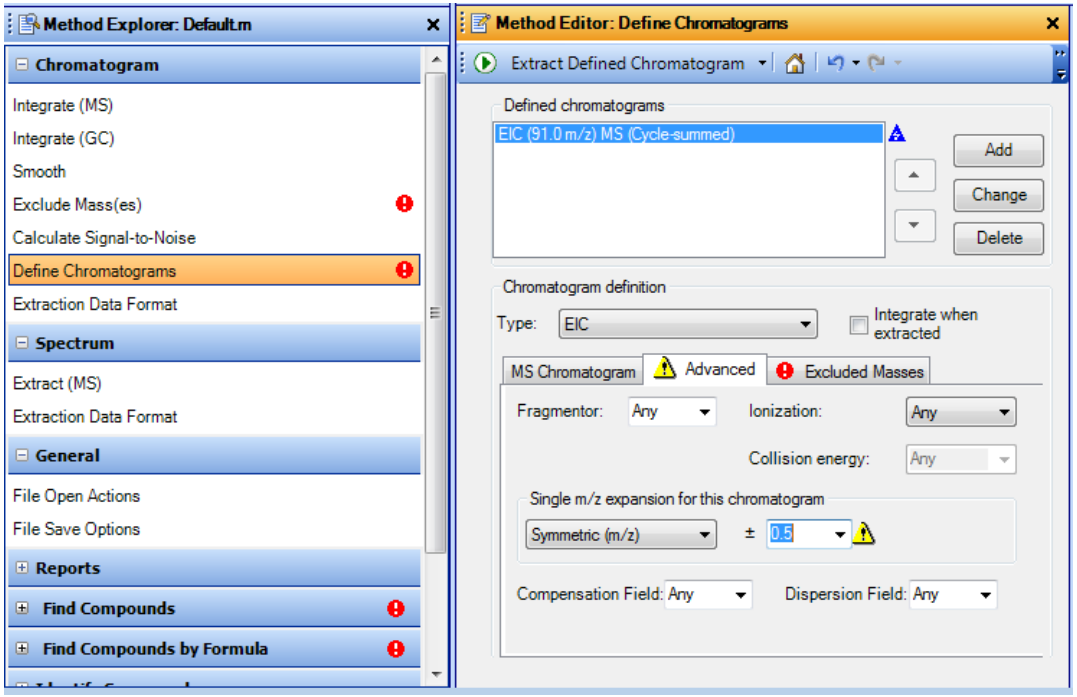
Relationship Between Action and Method Editor



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.**

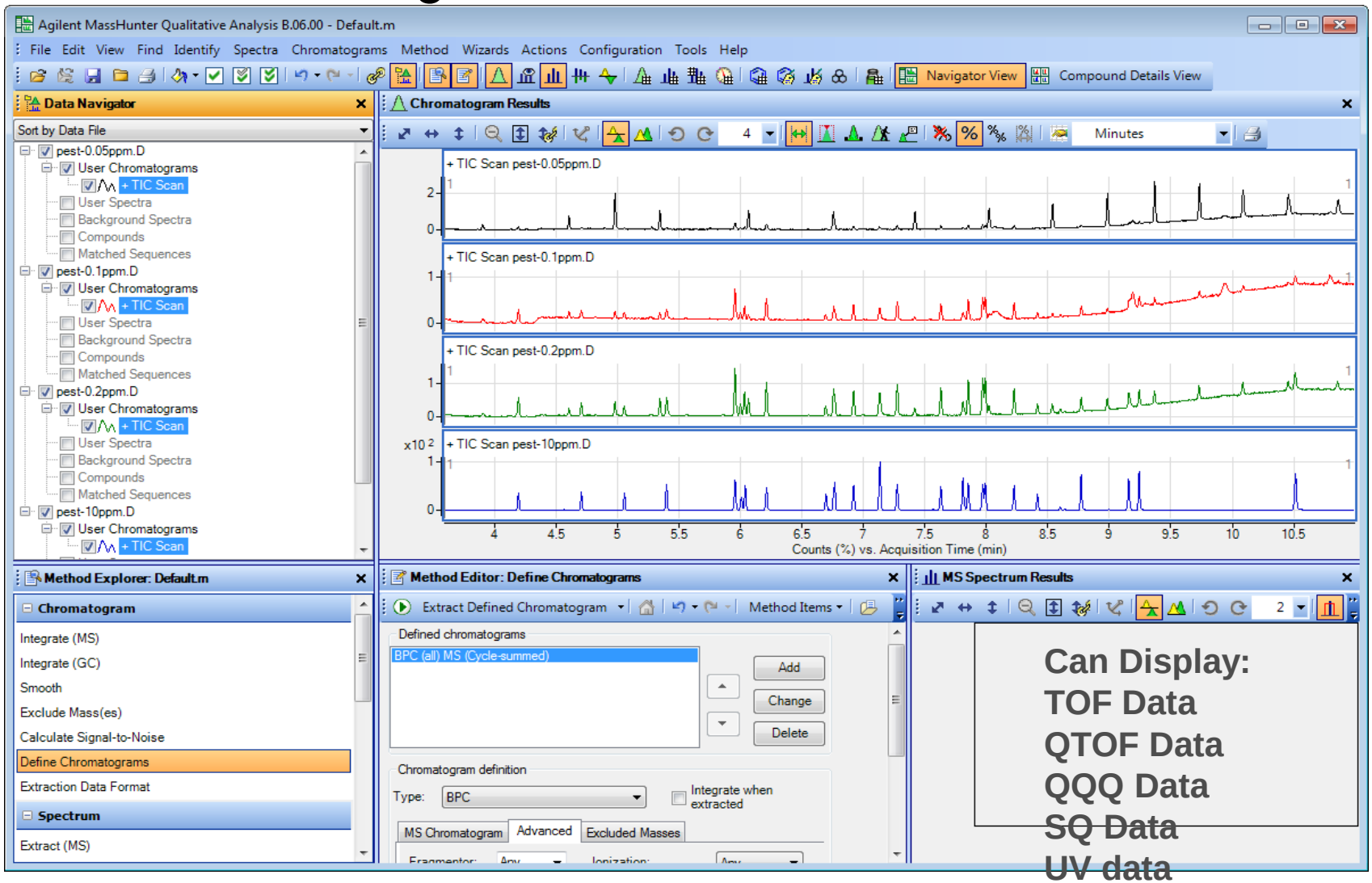
An invalid value has been entered into a field. The field will reset to the last valid value it contained.

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

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

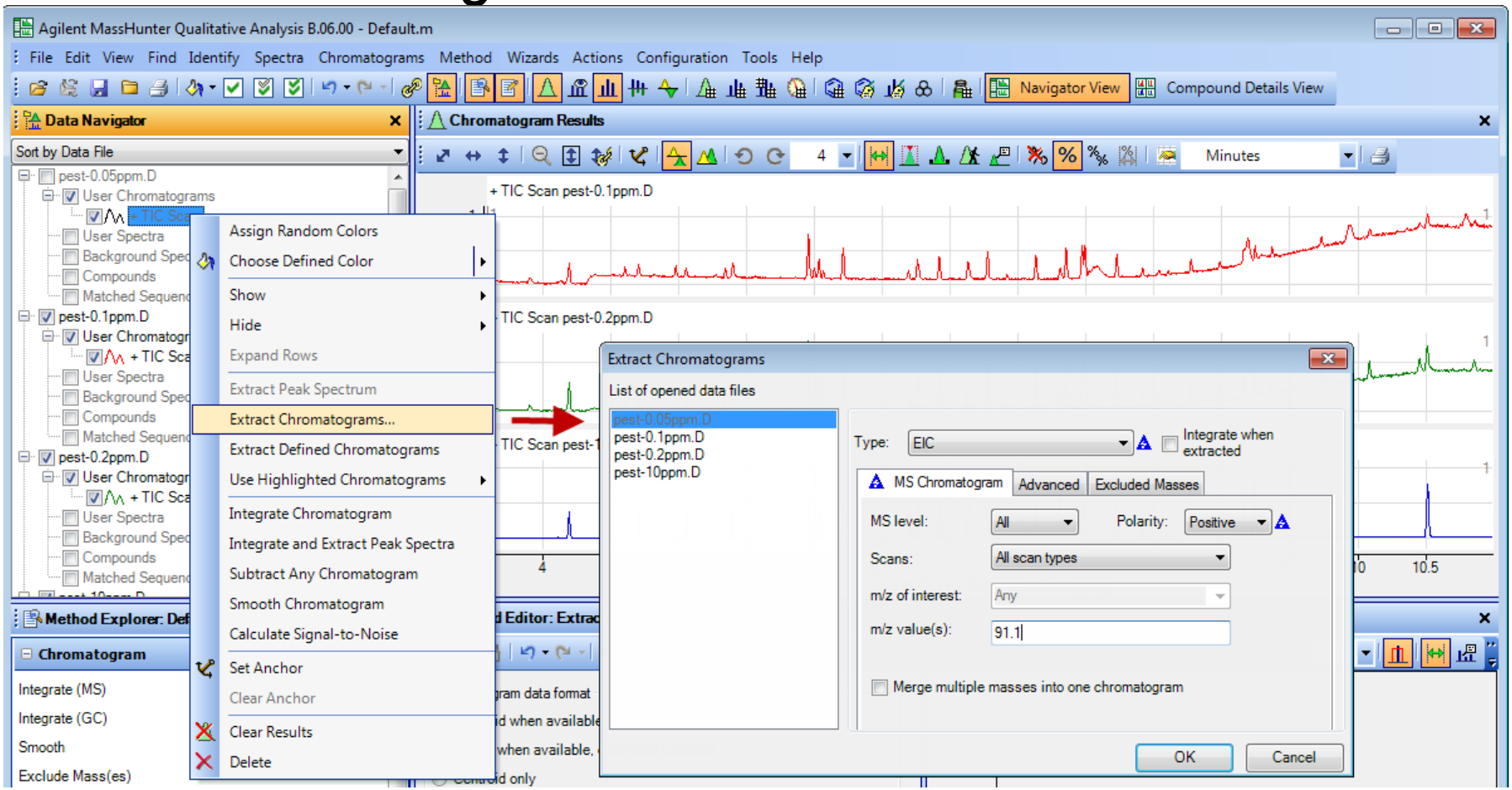
Define Chromatograms



Data Loaded & Displayed

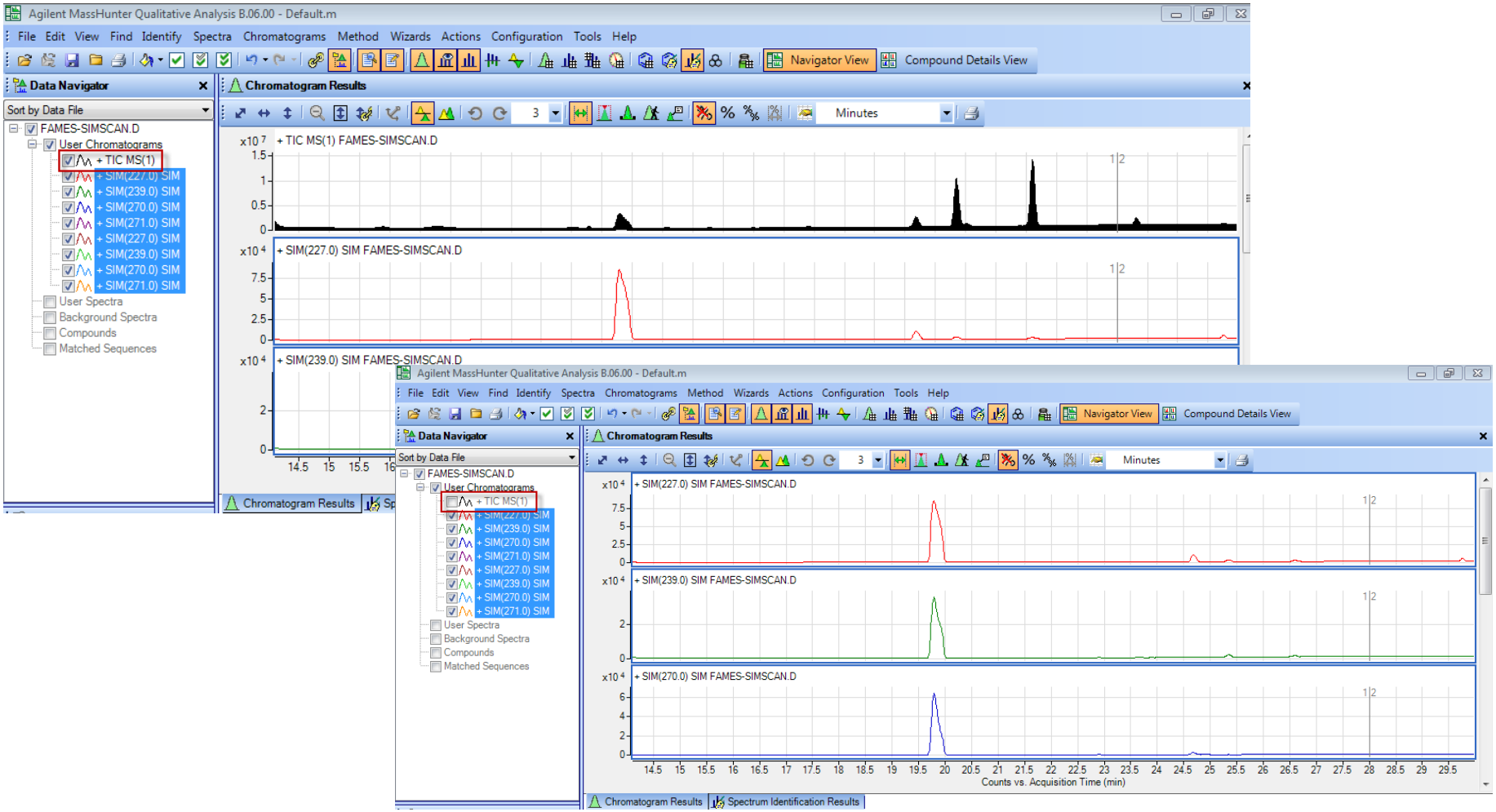
Can Display:
TOF Data
QTOF Data
QQQ Data
SQ Data
UV data
FID Data

Extract Chromatogram



List of Chromatogram types is determined by data in file.

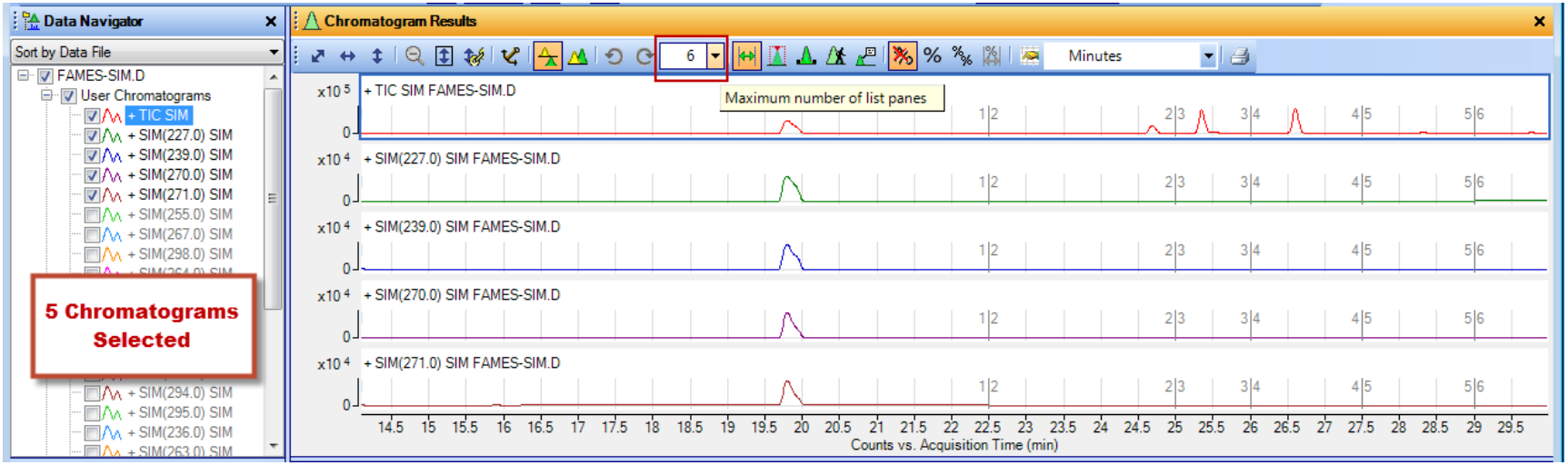
Selecting Chromatograms for Display

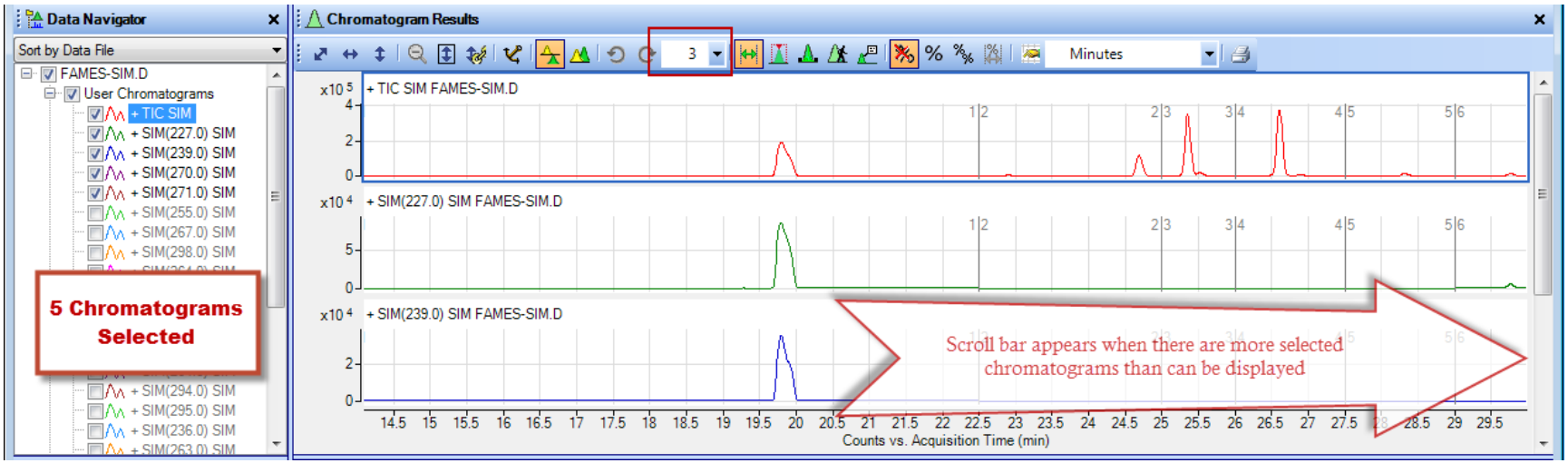


Items in the Data Navigator, like Chromatograms, will be displayed if checked and not displayed if unchecked.

Specify Number of Chromatograms Displayed

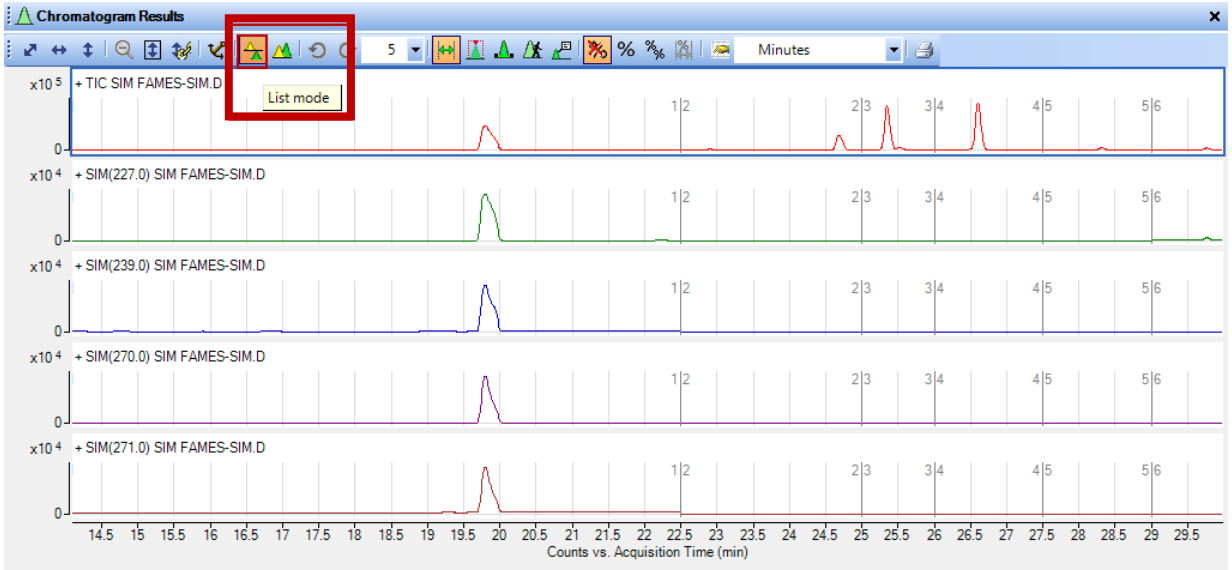
Maximum number of chromatograms to display in window, may be fewer.



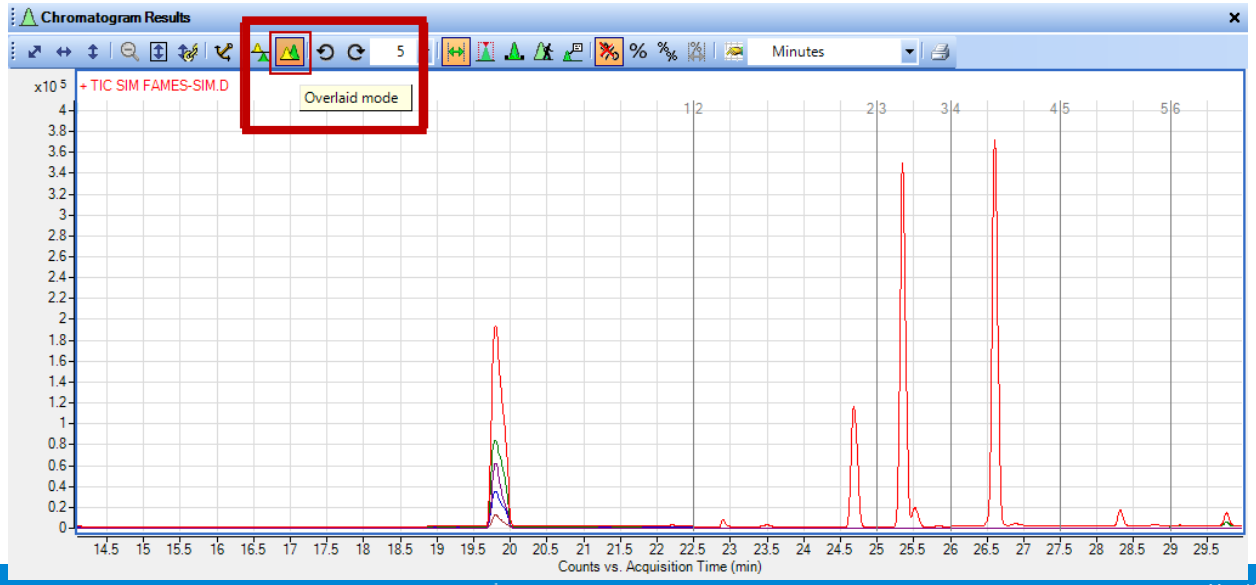


Overlay vs. List Mode Chromatograms

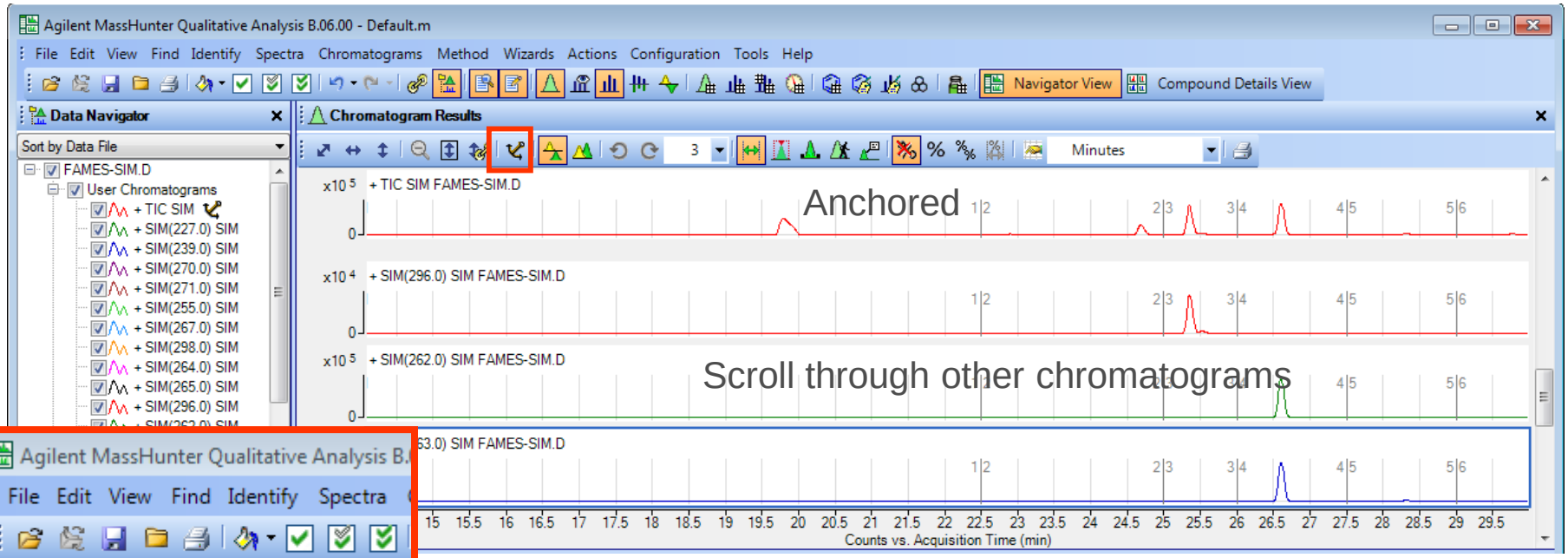
List (Separated)

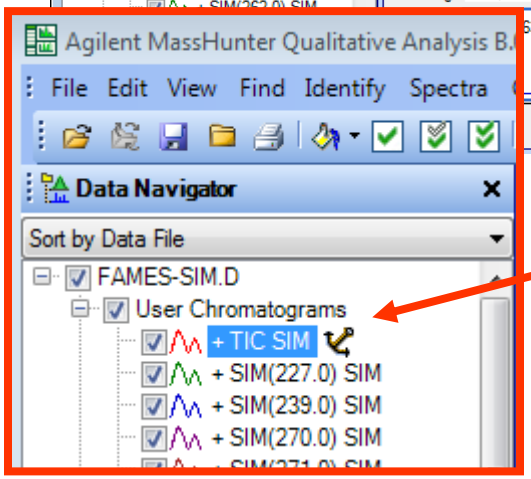


Overlaid



Anchoring

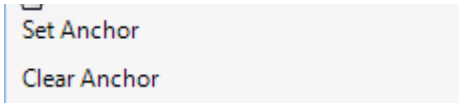




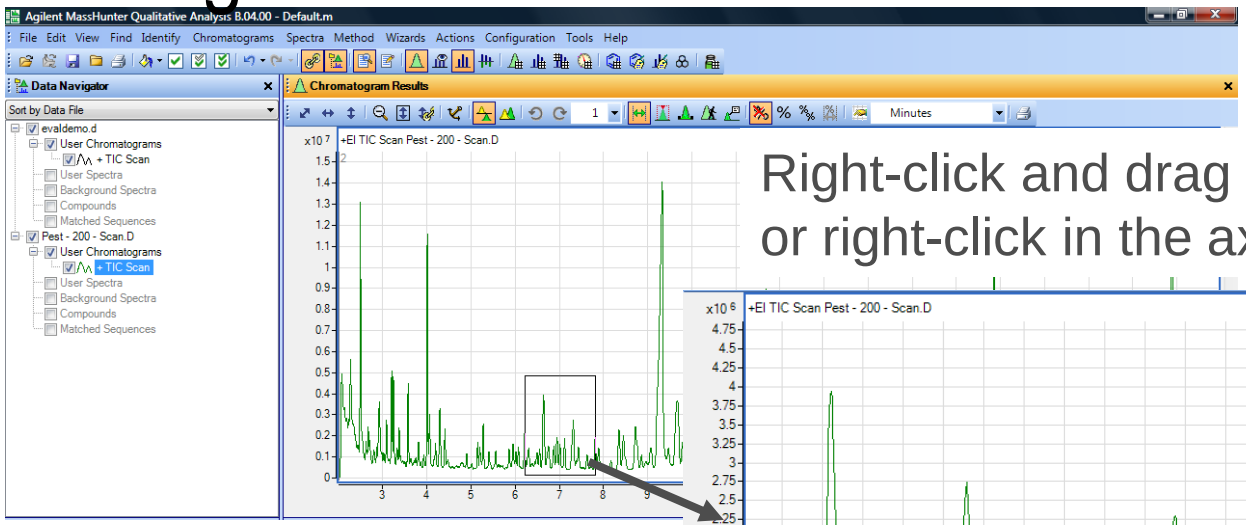
Setting an anchor keeps the anchored chromatogram displayed at all times.

Only one Chromatogram can be anchored at a time.

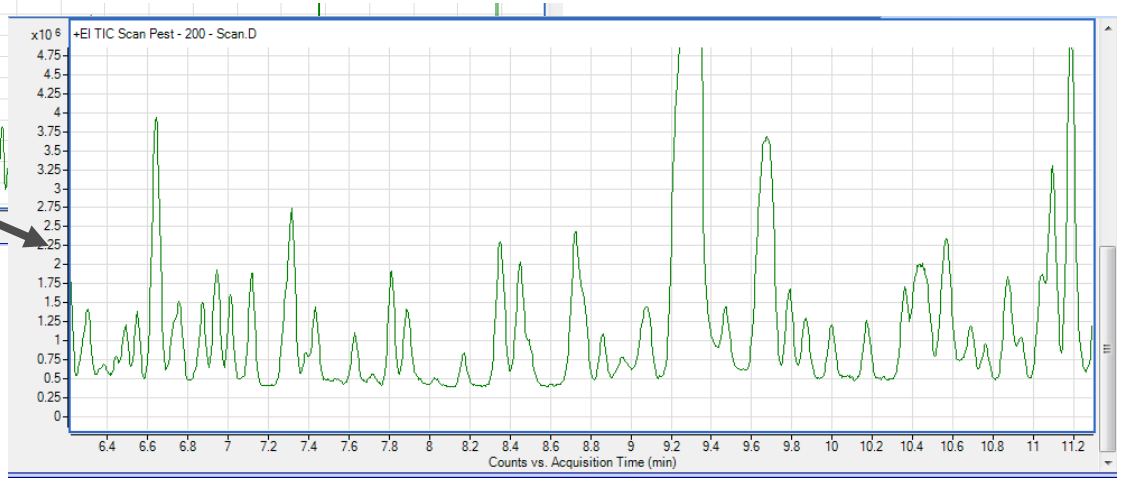
The anchor can be set and cleared from the context menu.



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

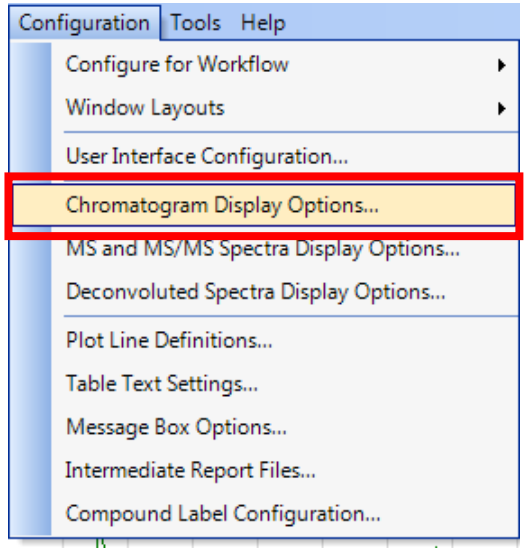
Scale to largest in Each over Selected Range

Scale to largest in Each

Scale to largest in All

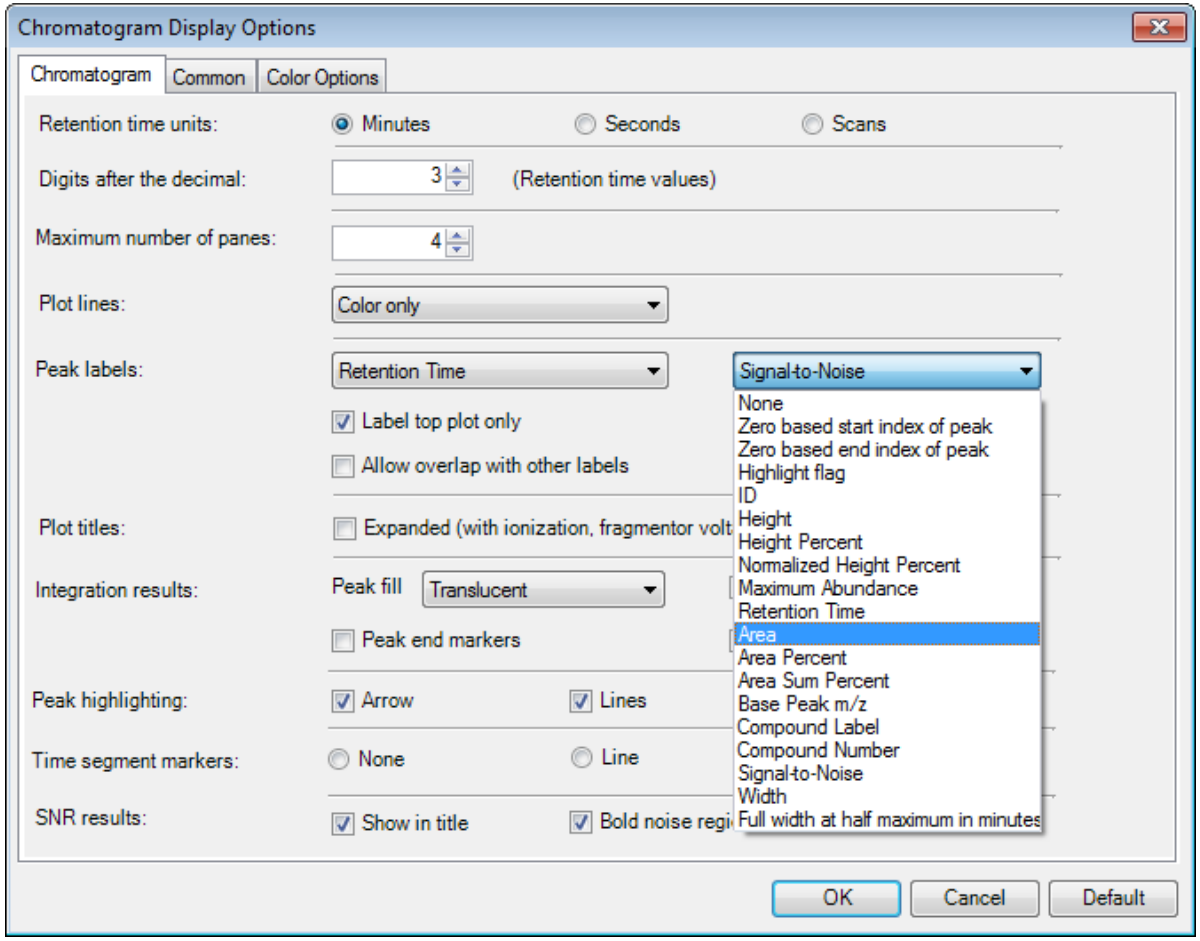
Scale Off

Chromatogram Display Options



Main Menu

Within Display



Customize Appearance of Chromatograms

Let’s take a moment for questions on Chromatogram Display Options.

Up Next:

Chromatogram Functions

Let’s take a moment for a demo on Chromatogram Display Options.

Up Next:

Chromatogram Functions

Chromatogram Functions

Chromatogram

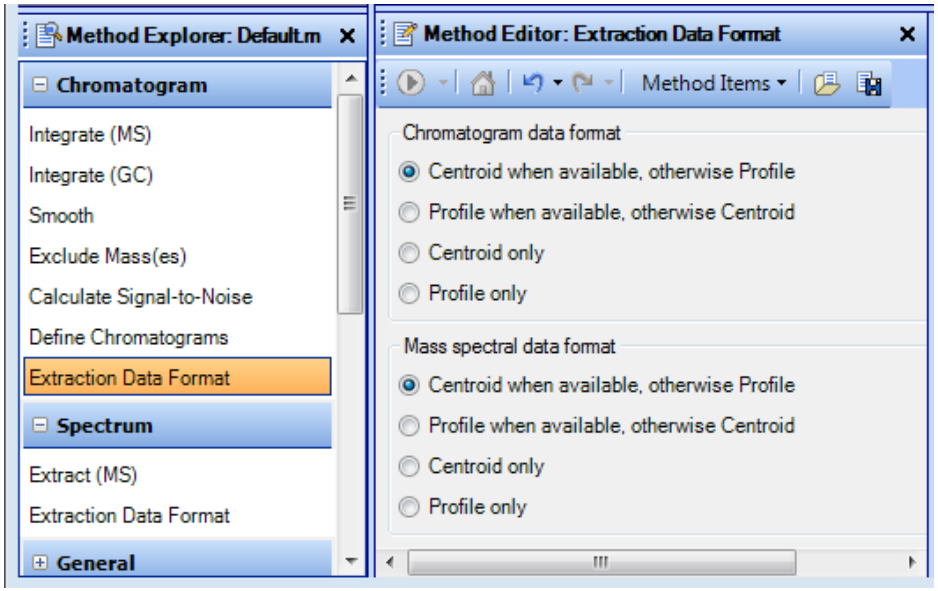
- Integrate (MS)
- Integrate (GC)

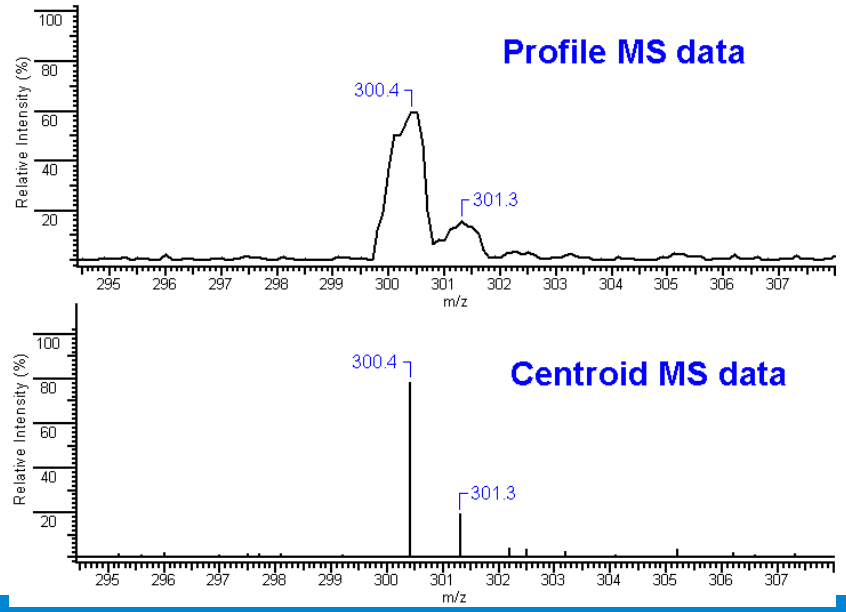
Identify chromatographic peaks for further analysis.

- Smooth
- Exclude Mass(es) — Exclude certain masses when depicting chromatograms.
- Calculate Signal-to-Noise
- Define Chromatograms — Extract chromatograms.
- Extraction Data Format

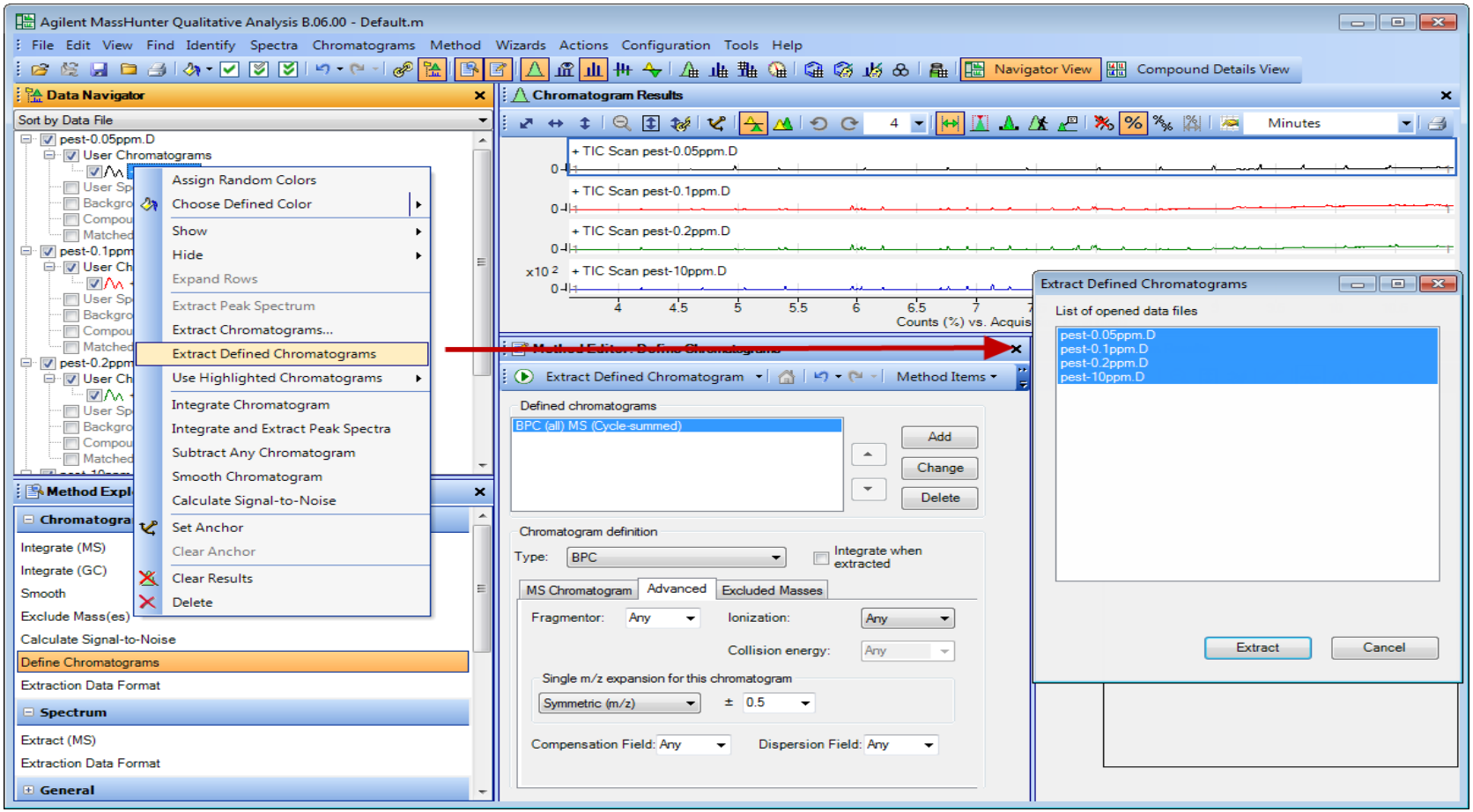
Extraction Data Format - Profile and Centroid

-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?





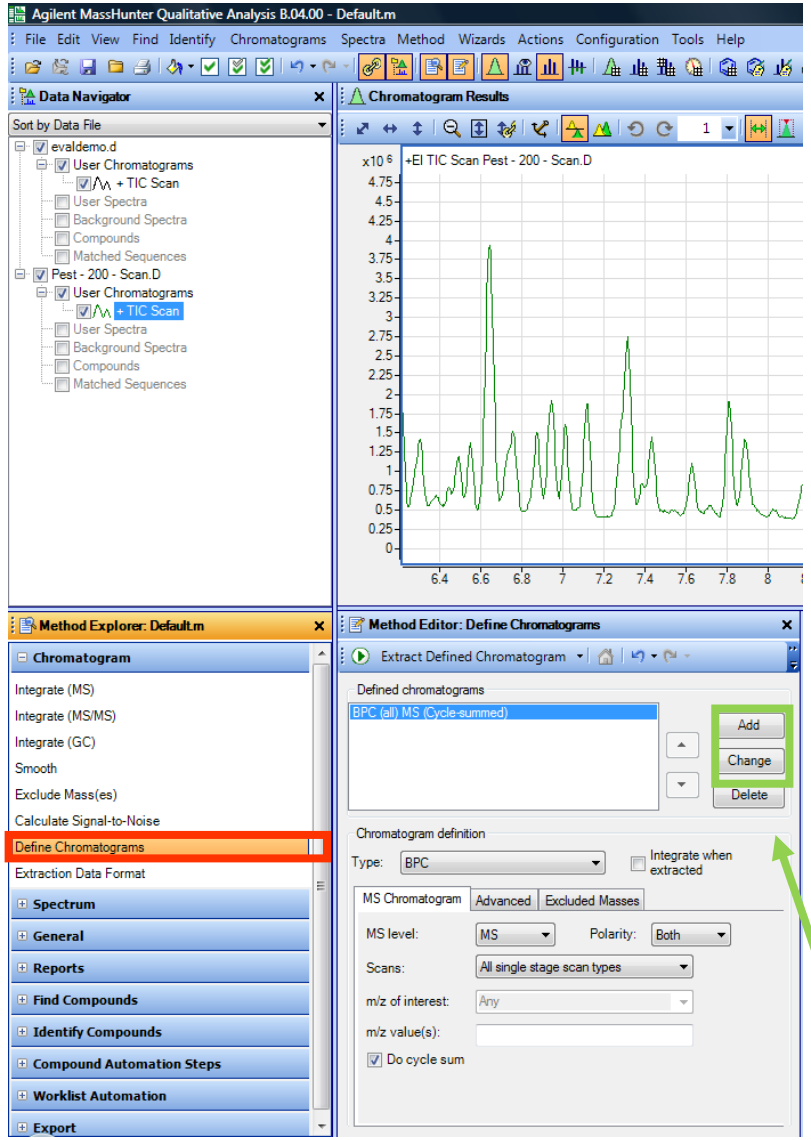
Extract Defined Chromatogram



Software extracts a list of chromatograms which are stored in the Extract Defined Chromatogram section of the method.

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

Extract Define 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

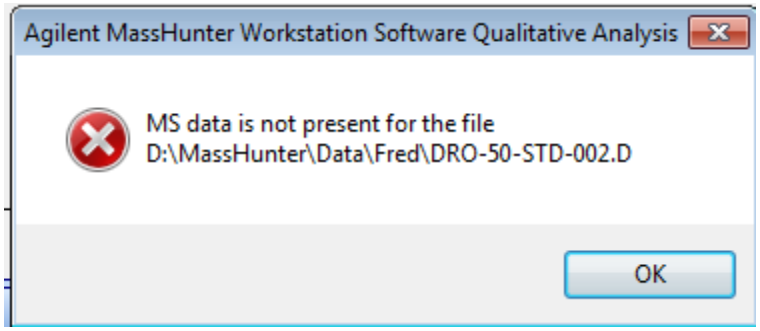
Instrument Curve (LC) - %Comp., Temps, etc.

Triple Quad systems only

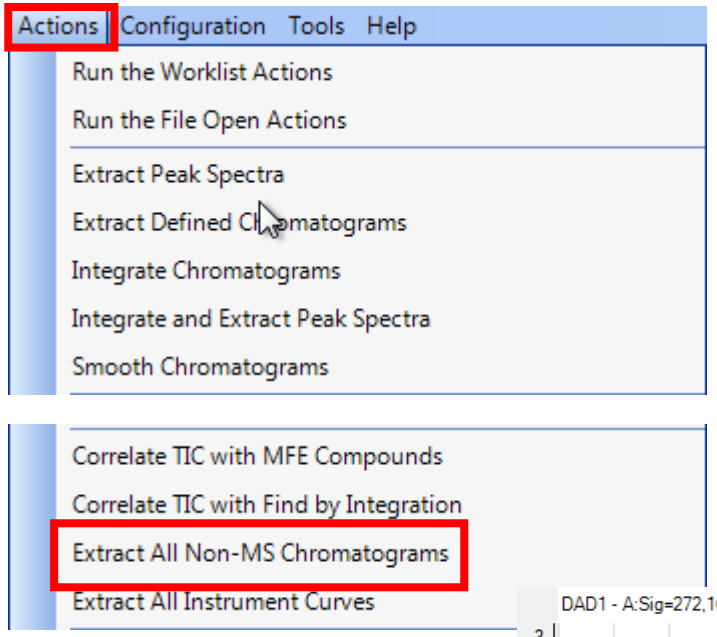
MRM – Multiple Reaction Monitor
pNLC - Precursor Neutral Loss Chromatogram

Tip: Select Change or Add.

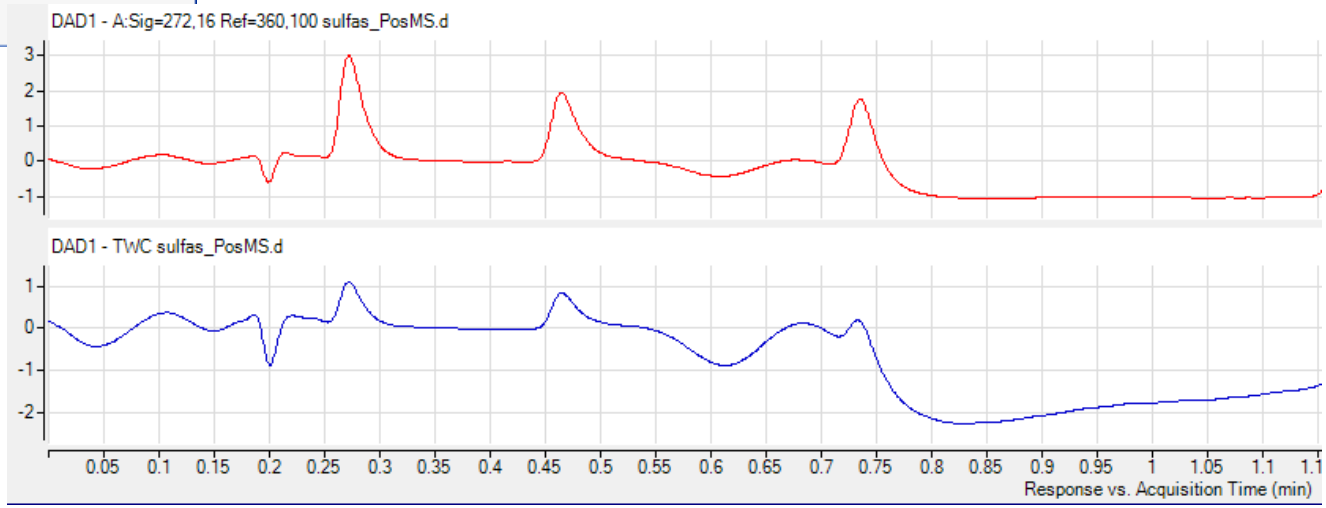
Extracting GC, UV and other Non-MS Signals



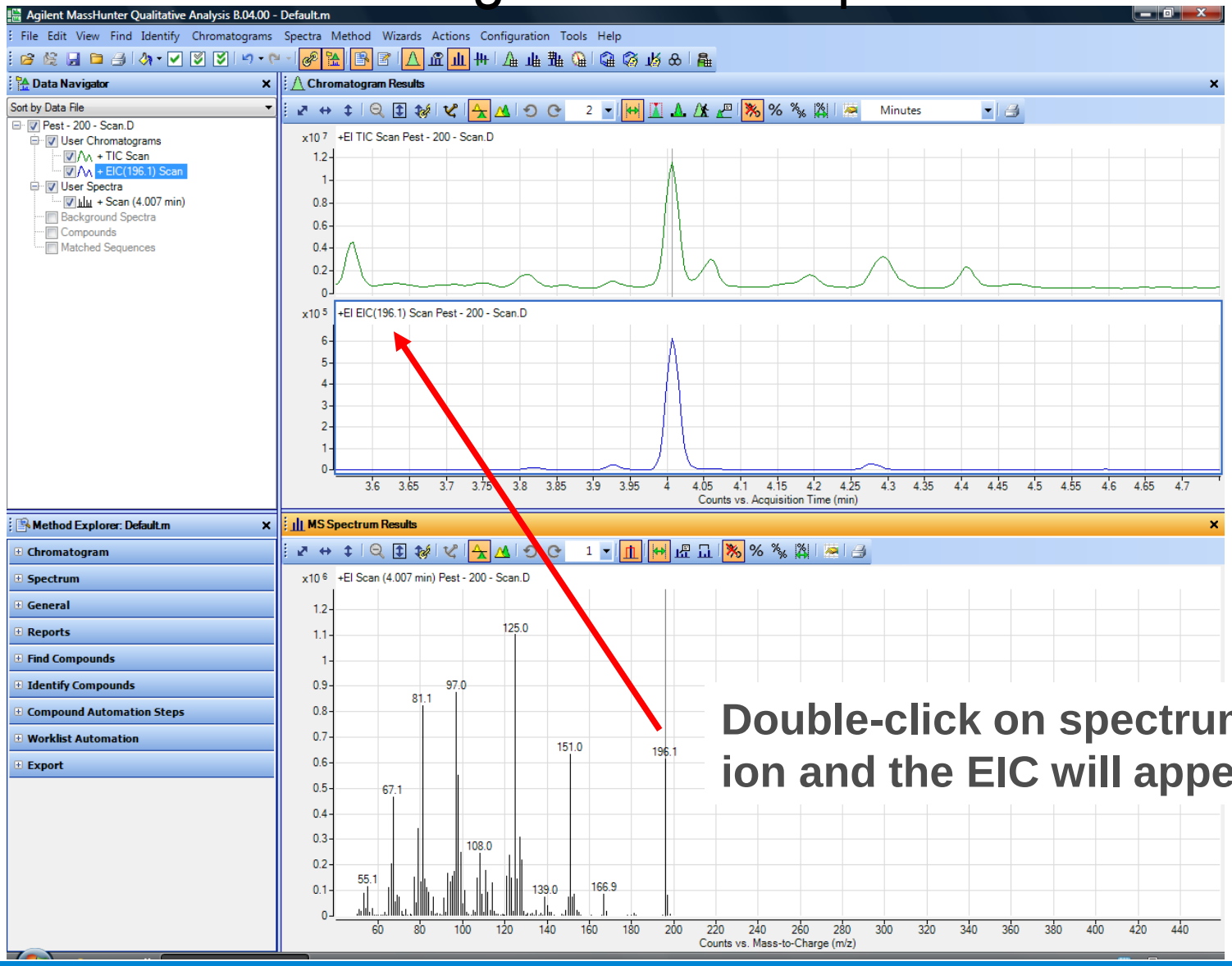
Extract All Non-MS Chromatograms



Select **Actions > Extract All Non-MS Chromatograms.**



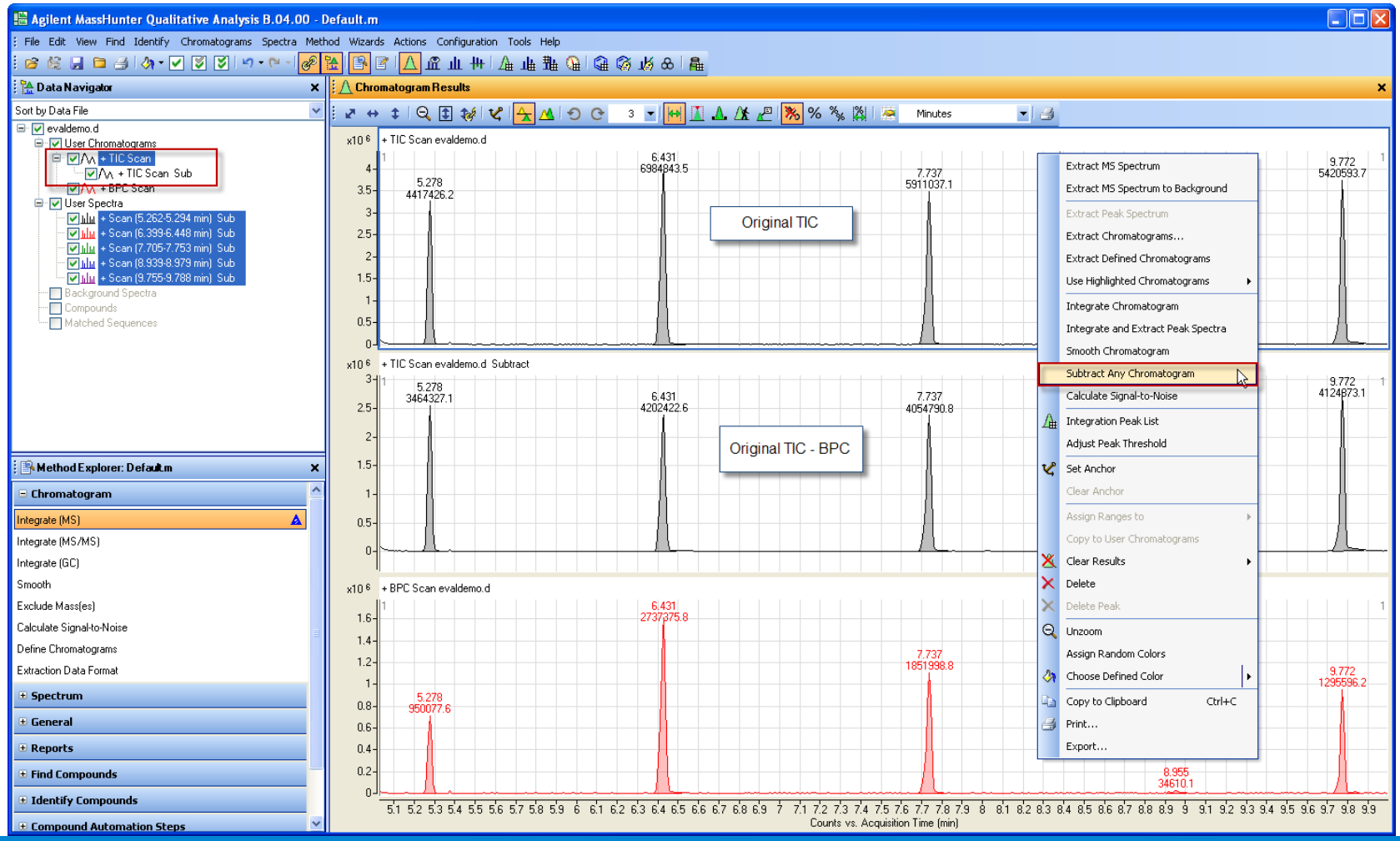
Extract Ion Chromatograms from Spectra



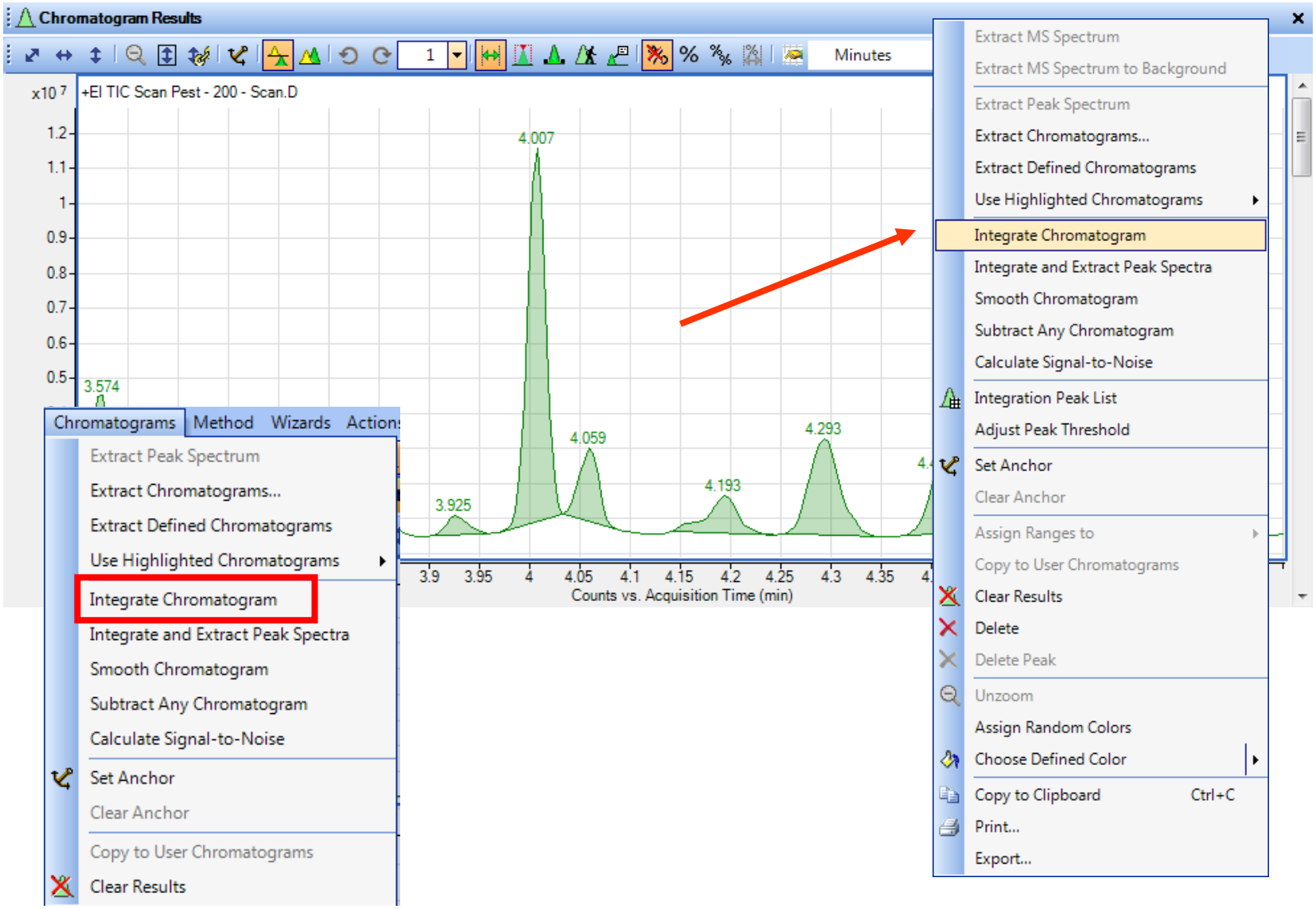
Double-click on spectrum ion and the EIC will appear.

Subtract Any Chromatogram

Right click in Chromatogram, select “Subtract Any Chromatogram”, the next chromatogram you click on will be subtracted from 1st one.

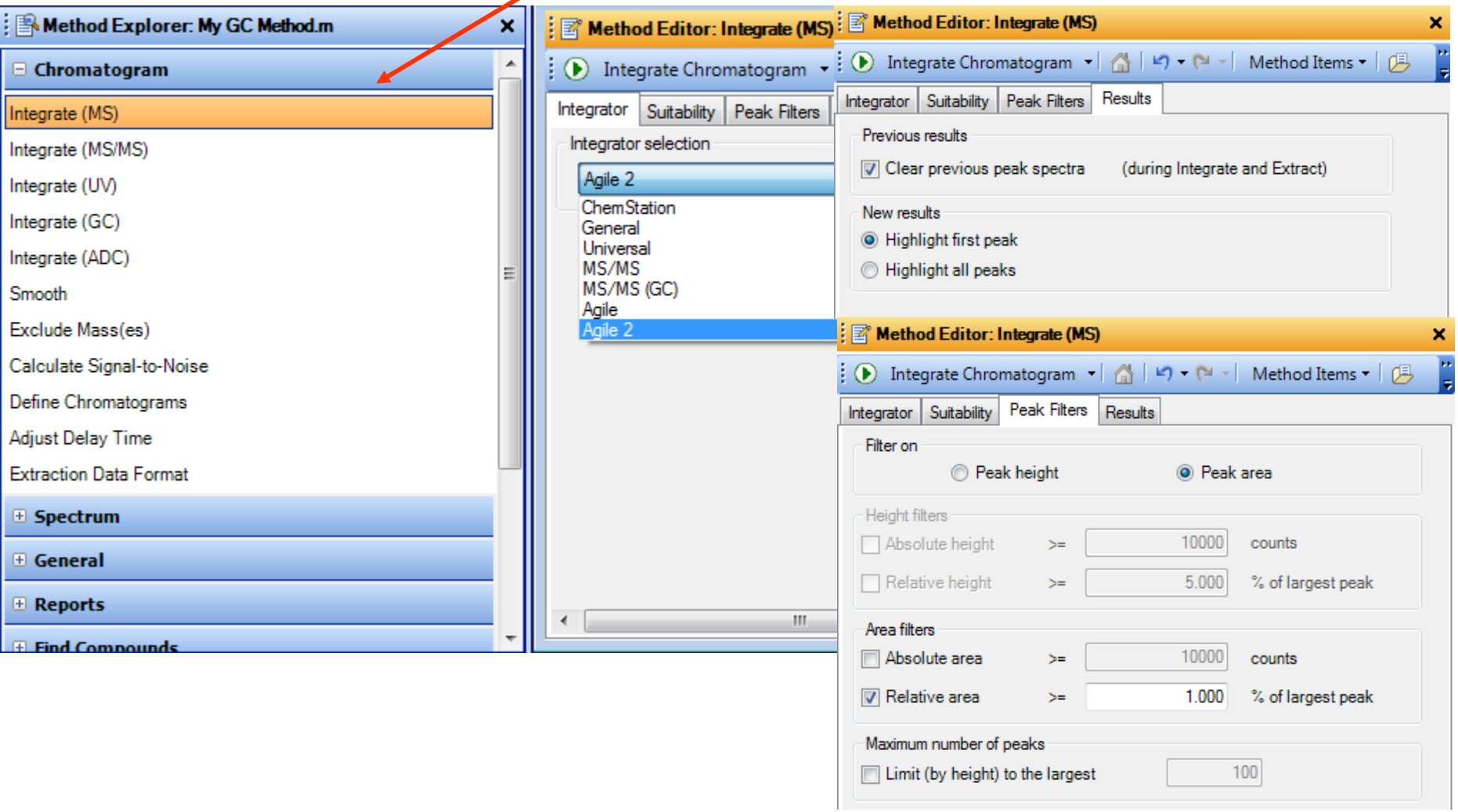


Integrate Chromatogram



Integrate Chromatogram

Independent Integrator for each configuration.



Integrator Types

Agile2

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

Agile

- 2nd generation parameterless integrator

Universal

- 1st generation ChemStation integrator
- Familiar to ChemStation users

General (RTE)

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

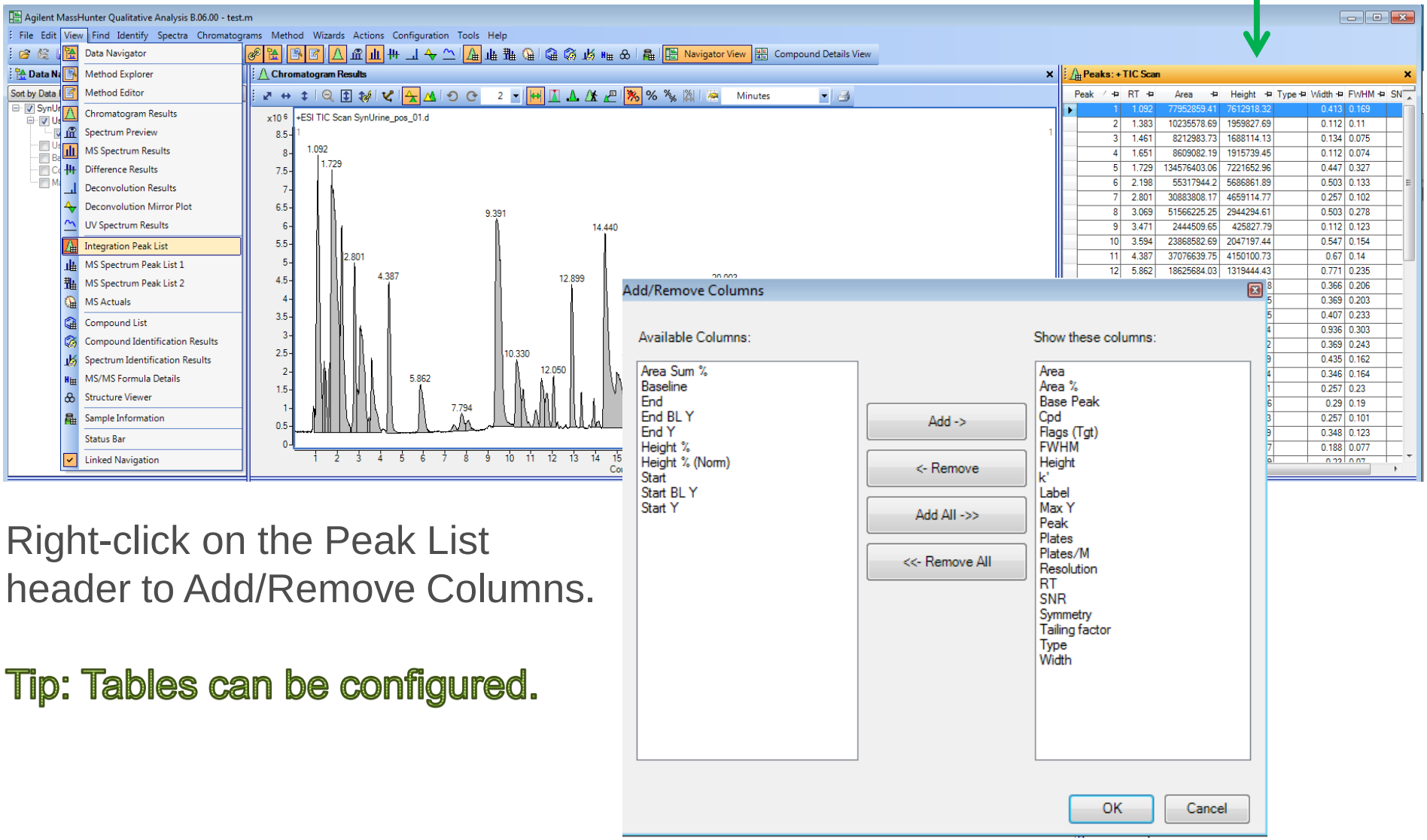
MS/MS and MS/MS (GC)

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

ChemStation

- 2nd generation ChemStation
- Intended for UV

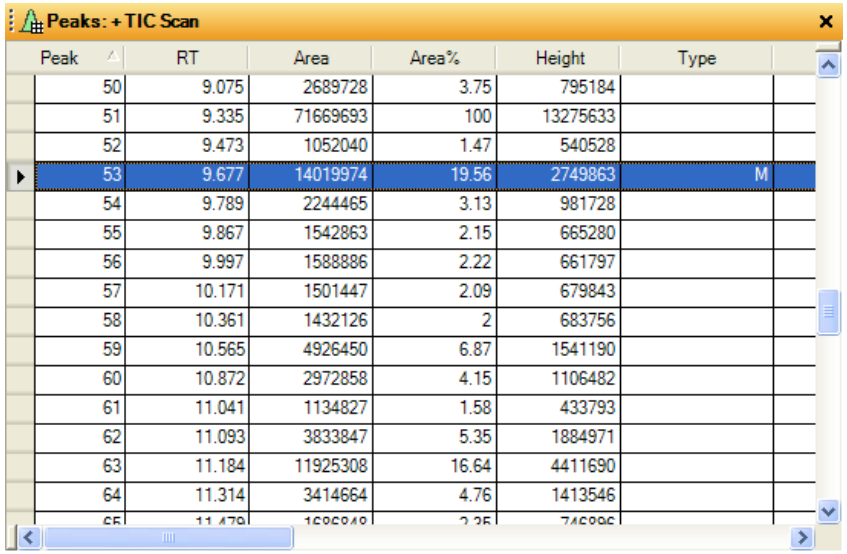
Integration Peak List



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

Tip: Tables can be configured.

Integration Peak Tables (all tables)



Peak	RT	Area	Area%	Height	Type
50	9.075	2689728	3.75	795184	
51	9.335	71669693	100	13275633	
52	9.473	1052040	1.47	540528	
53	9.677	14019974	19.56	2749863	M
54	9.789	2244465	3.13	981728	
55	9.867	1542863	2.15	665280	
56	9.997	1588886	2.22	661797	
57	10.171	1501447	2.09	679843	
58	10.361	1432126	2	683756	
59	10.565	4926450	6.87	1541190	
60	10.872	2972858	4.15	1106482	
61	11.041	1134827	1.58	433793	
62	11.093	3833847	5.35	1884971	
63	11.184	11925308	16.64	4411690	
64	11.314	3414664	4.76	1413546	

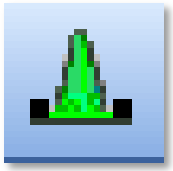
Tables can be moved to different locations.

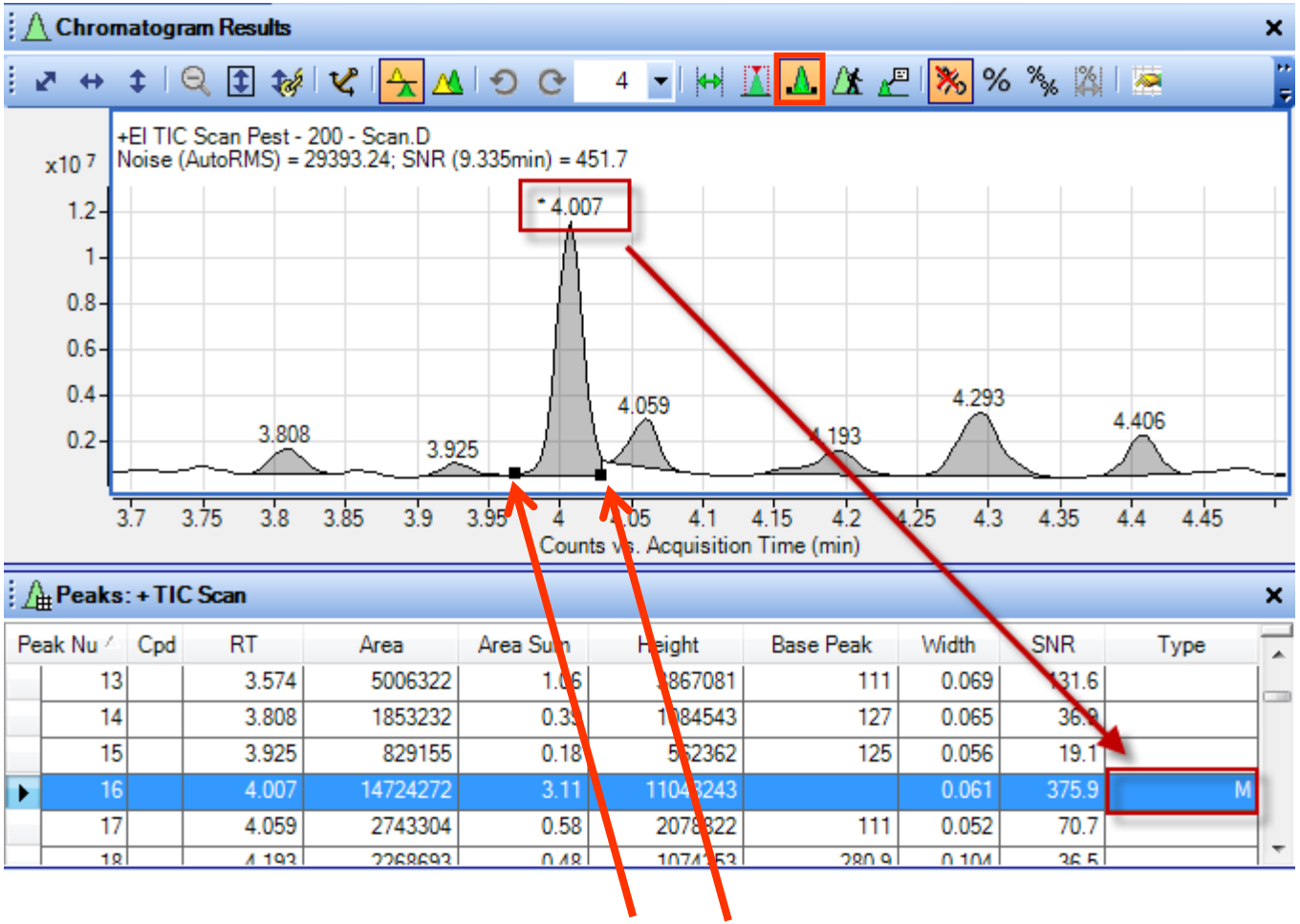
Tables can be split for easy viewing.

Columns can be added, removed, and moved.

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

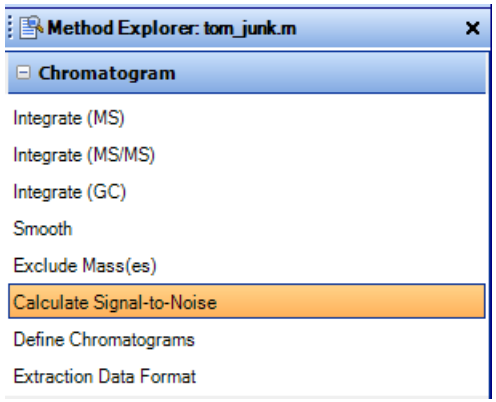
Manual Integration

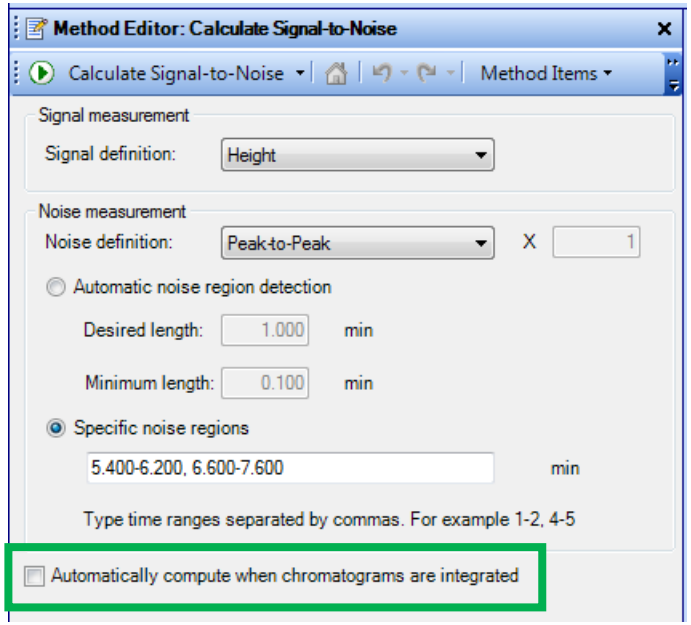




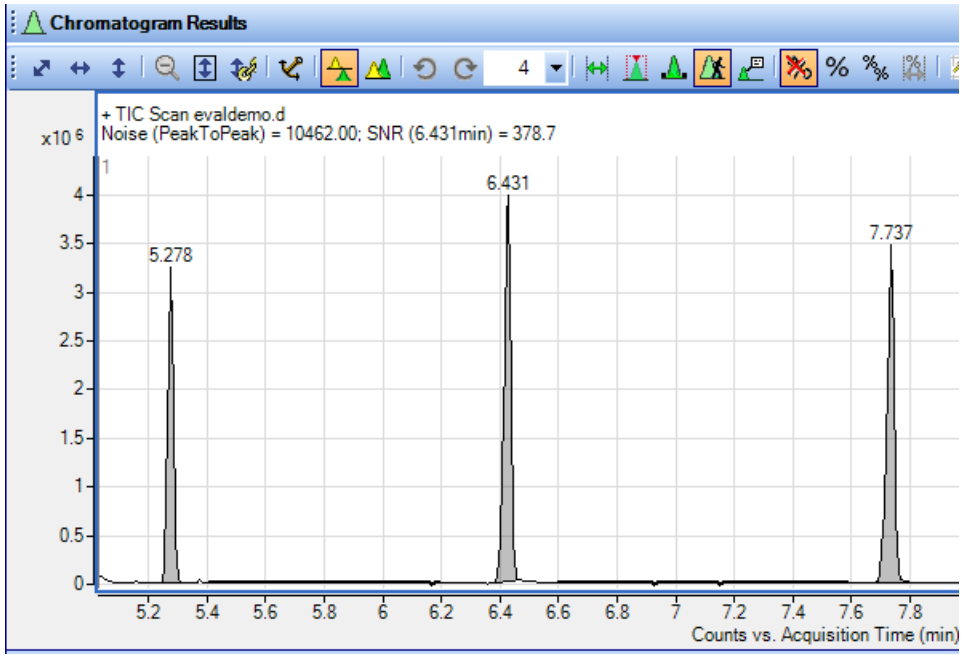
Use mouse cursor to manually integrate peak.

Calculate Signal-to-Noise
Specific Noise Regions





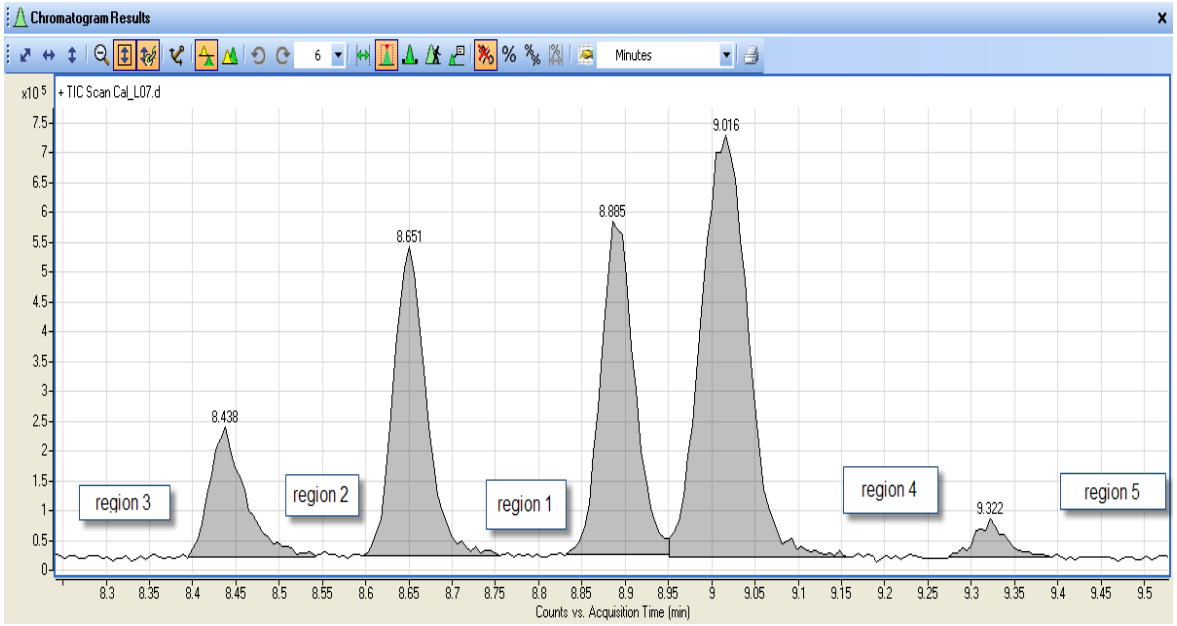
- User defined specific noise regions.
- May be performed automatically when Chromatogram is integrated.

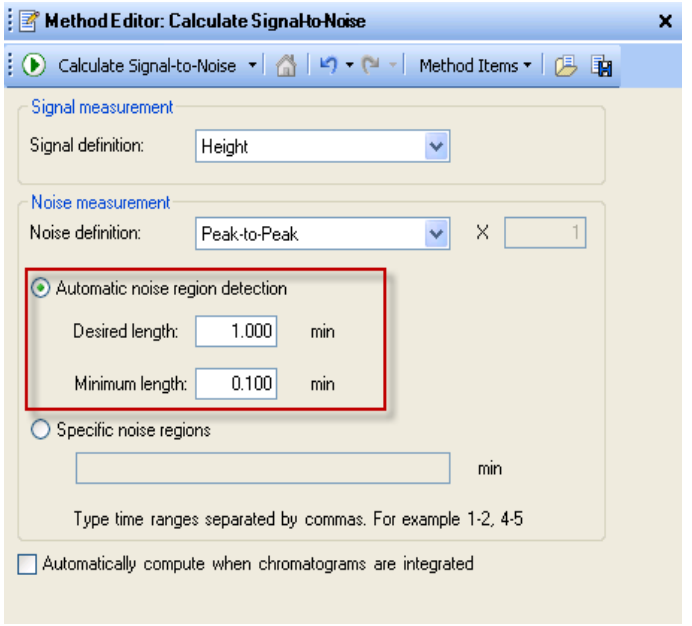


Calculate Signal-to-Noise
Automatic Noise Region Detection

- Alternative to user defined specific noise regions in which the software seeks to locate a “noise region” between the peaks found by the integrator

- User specifies a maximum length (desired) and minimum length of noise region and software locates an acceptable region if one exists





Let’s take a few moments for questions on Chromatogram Functions.

Up Next:

Training Resources.

Time for a demo on Chromatogram Functions.

Up Next:

Training Resources

Training Resources

Training resources that are available.

Convenient Training

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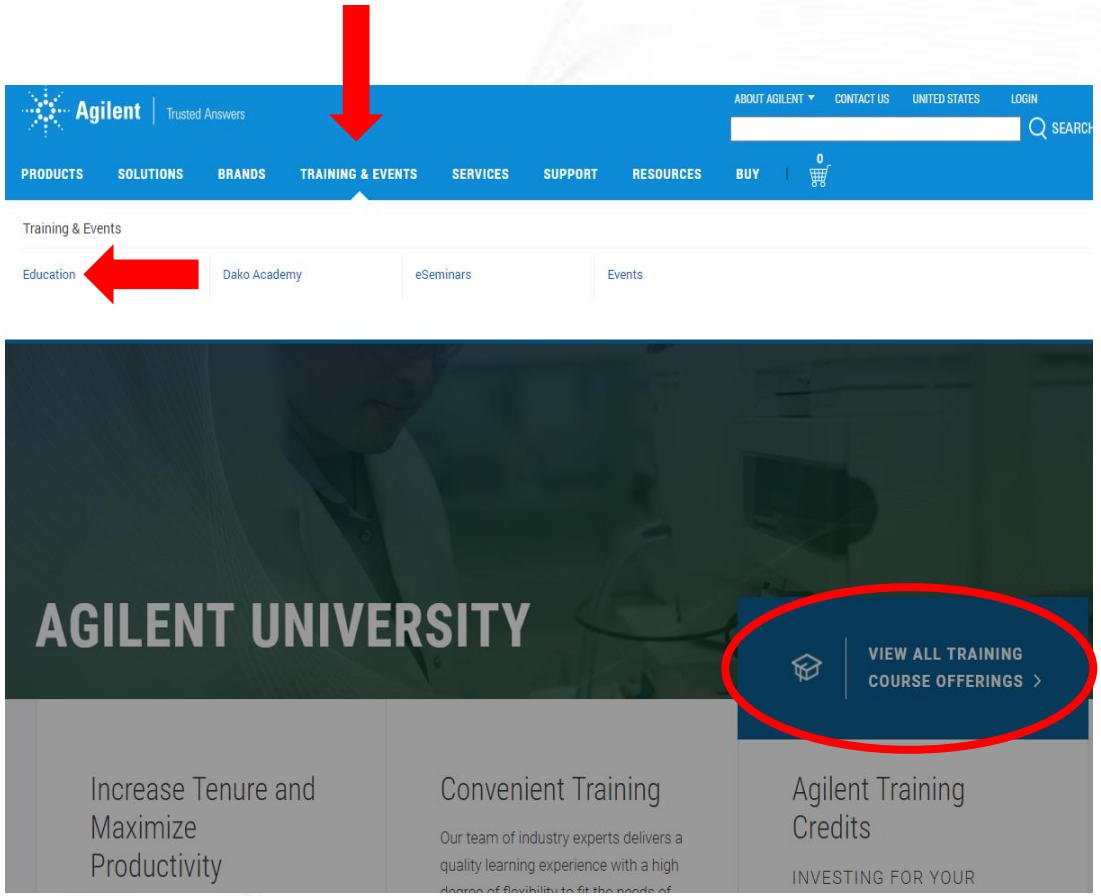

Questions on today's material…

Thank you for your attention.

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