



# Agilent MassHunter Qualitative Data Analysis

Presenters: Howard Sanford

Stephen Harnos

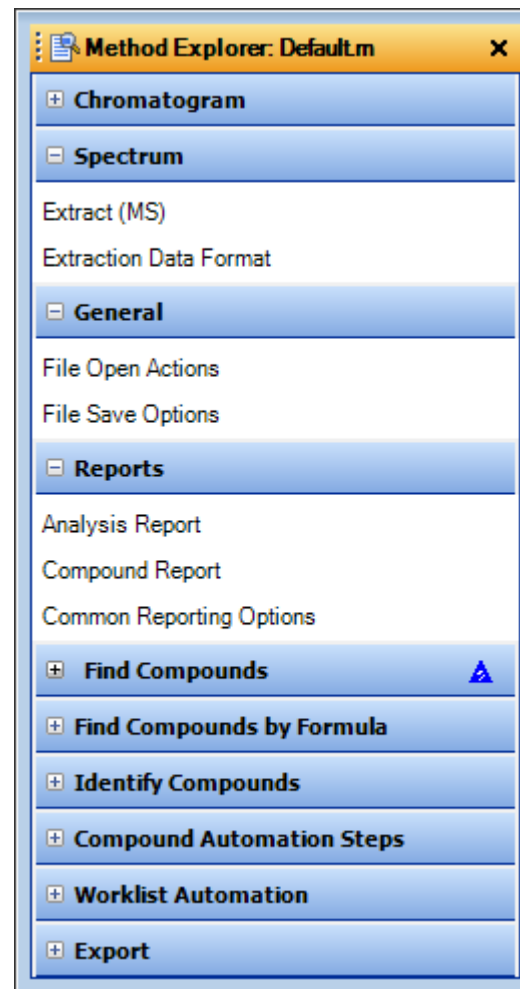
MassHunter Qualitative Analysis

Spectrum Functions

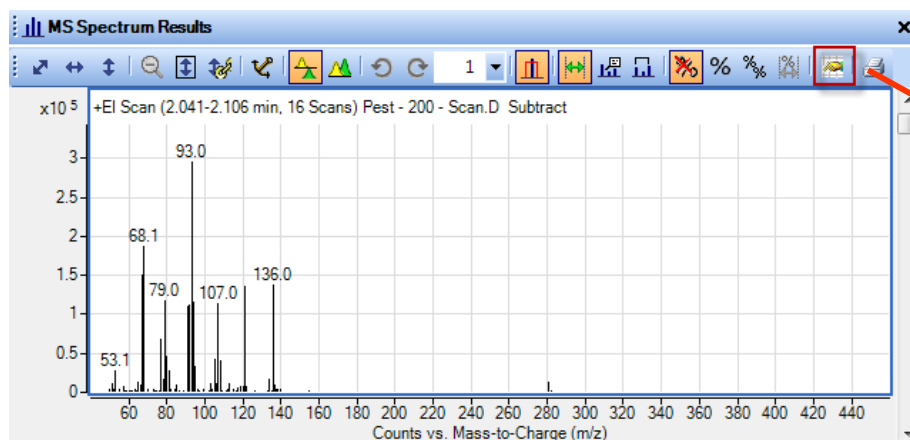
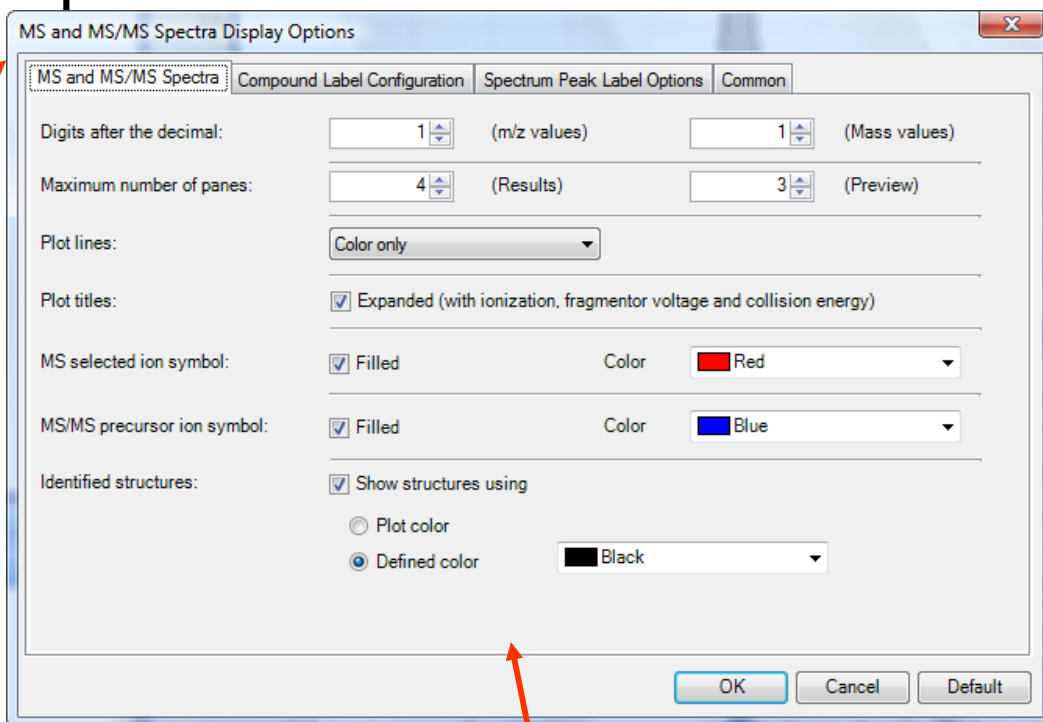
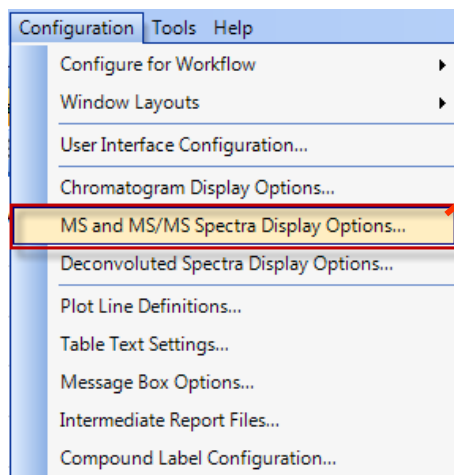
# MassHunter Qualitative Analysis Software B.07.00

## Topics

- Review Chromatogram Functions
- Spectrum Functions
  - Spectral Display
  - Extract a Spectrum and Averaging
  - Background Subtraction (MS Spectrum)
- Accurate Mass Considerations
  - Isotopic Model
- Spectrum Peak List
- Annotation
  - Text and Graphics
  - Anchoring and Floating
- Report Generation
  - Analysis and Compound Reports
- File Save Options
- Tools
- Training Resources



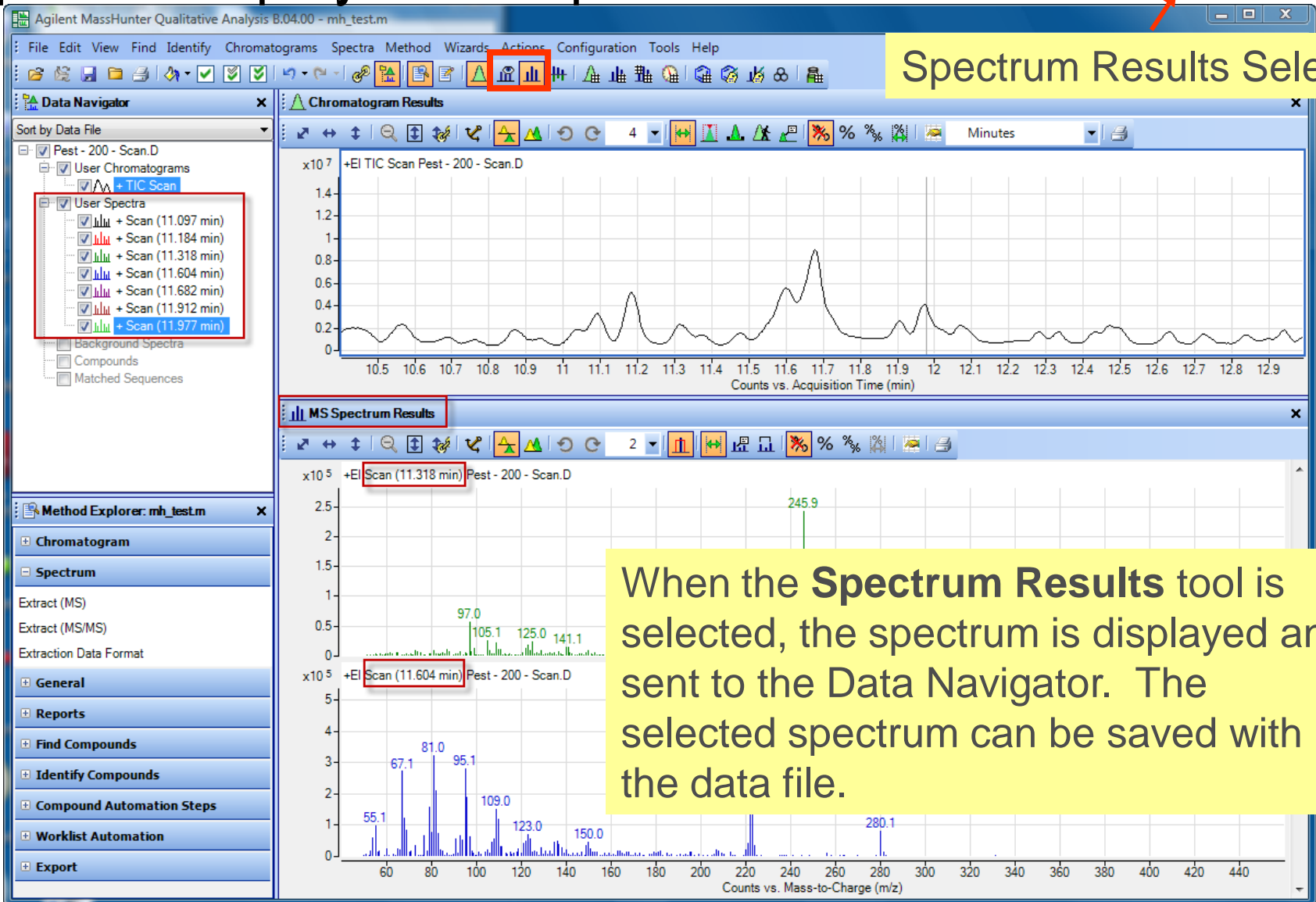
# Mass Spectral Display Options



# Spectra Display – MS Spectrum Results



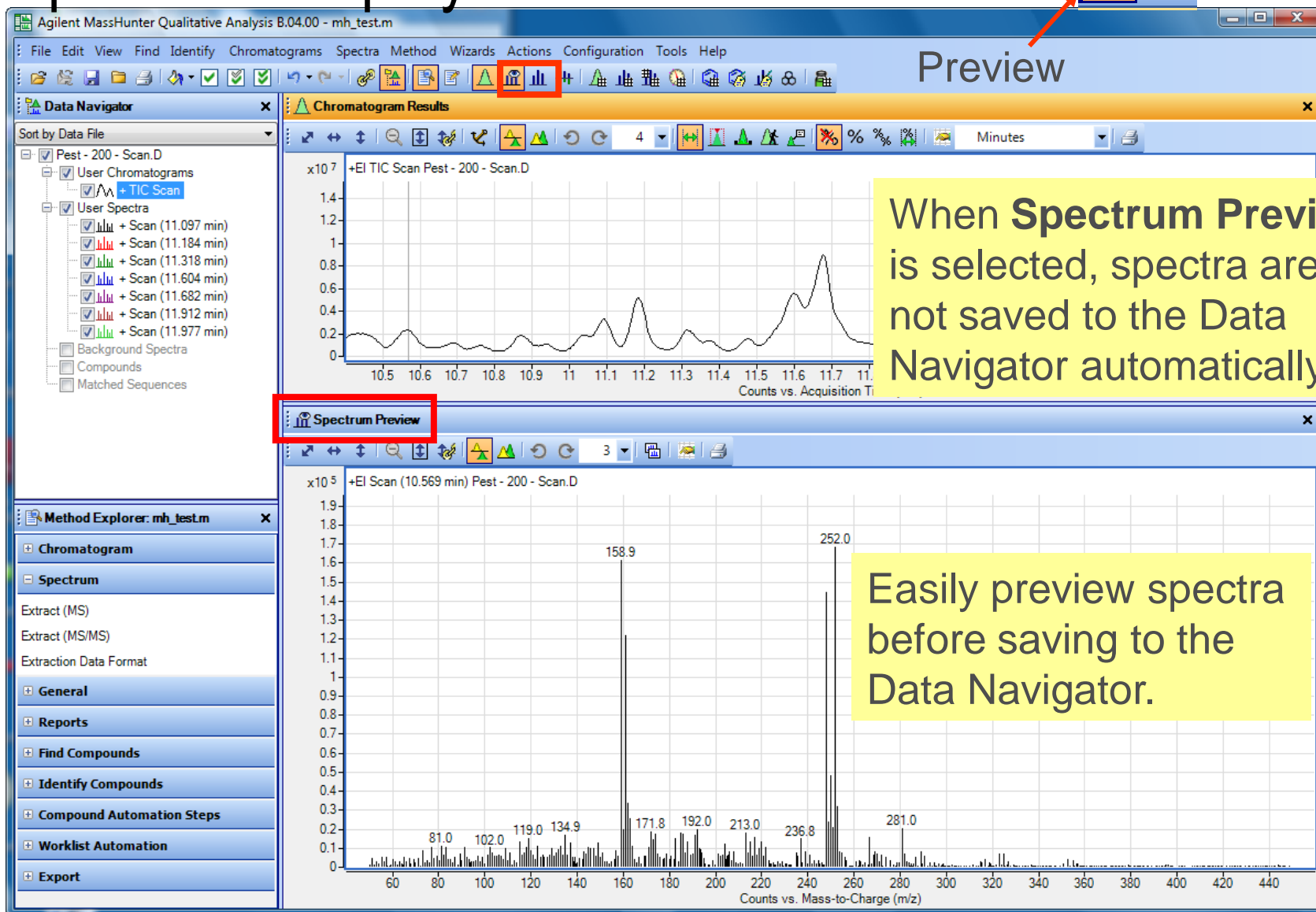
Spectrum Results Selected



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# Spectrum Display - Preview



# Add Preview Spectrum to Results

The screenshot displays the Agilent MassHunter Qualitative Analysis 8.04.00 interface. The 'Data Navigator' on the left shows a tree structure with 'Pest - 200 - Scan.D' expanded, containing 'User Chromatograms' and 'User Spectra'. A red box highlights the entry '+ Scan (10.569 min)' under 'User Spectra', with a green arrow pointing to the 'Spectrum Preview' window. The 'Spectrum Preview' window shows a mass spectrum for 'Scan (10.569 min)' with major peaks at 158.9 and 252.0 m/z. A yellow callout box with the text '•Right-click' and '•Select Copy to User Spectra' is overlaid on the spectrum. A right-click context menu is open, showing options like 'Extract EIC', 'Extract Chromatograms...', 'MS Spectrum Peak List 1', 'MS Spectrum Peak List 2', 'MS Actuals', 'Assign Ranges to', 'Move to Background Spectrum', 'Copy to User Spectra' (highlighted with a green box), 'Unzoom', 'Assign Random Colors', 'Choose Defined Color', 'Copy to Clipboard', 'Print...', and 'Export...'.

Agilent MassHunter Qualitative Analysis 8.04.00 - mh\_test.m

File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help

Data Navigator

Sort by Data File

- ✓ Pest - 200 - Scan.D
  - ✓ User Chromatograms
    - ✓ + TIC Scan
  - ✓ User Spectra
    - ✓ + Scan (11.097 min)
    - ✓ + Scan (11.184 min)
    - ✓ + Scan (11.318 min)
    - ✓ + Scan (11.604 min)
    - ✓ + Scan (11.682 min)
    - ✓ + Scan (11.912 min)
    - ✓ + Scan (11.977 min)
    - ✓ + Scan (10.569 min)
    - Background Spectra
    - Compounds
    - Matched Sequences

Method Explorer: mh\_test.m

- Chromatogram
- Spectrum
  - Extract (MS)
  - Extract (MS/MS)
  - Extraction Data Format
- General
- Reports
- Find Compounds
- Identify Compounds
- Compound Automation Steps
- Worklist Automation
- Export

Chromatogram Results

4 Minutes

+EI TIC Scan Pest - 200 - Scan.D

Counts vs. Acquisition Time (min)

Spectrum Preview

3

+EI Scan (10.569 min) Pest - 200 - Scan.D

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

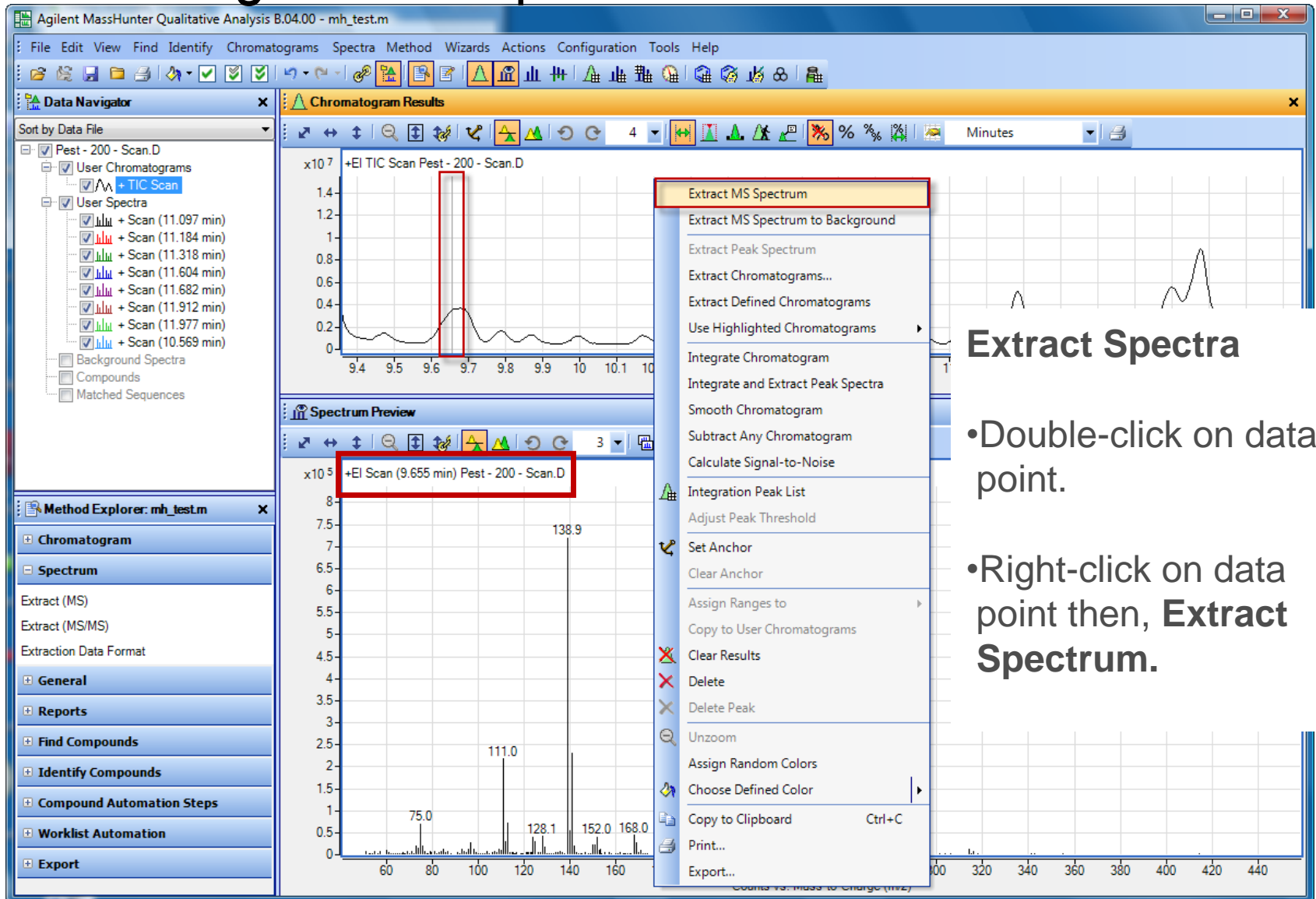
158.9 252.0

81.0 102.0 119.0 134.9 171.8 192.0 213.0 236.8 281.0

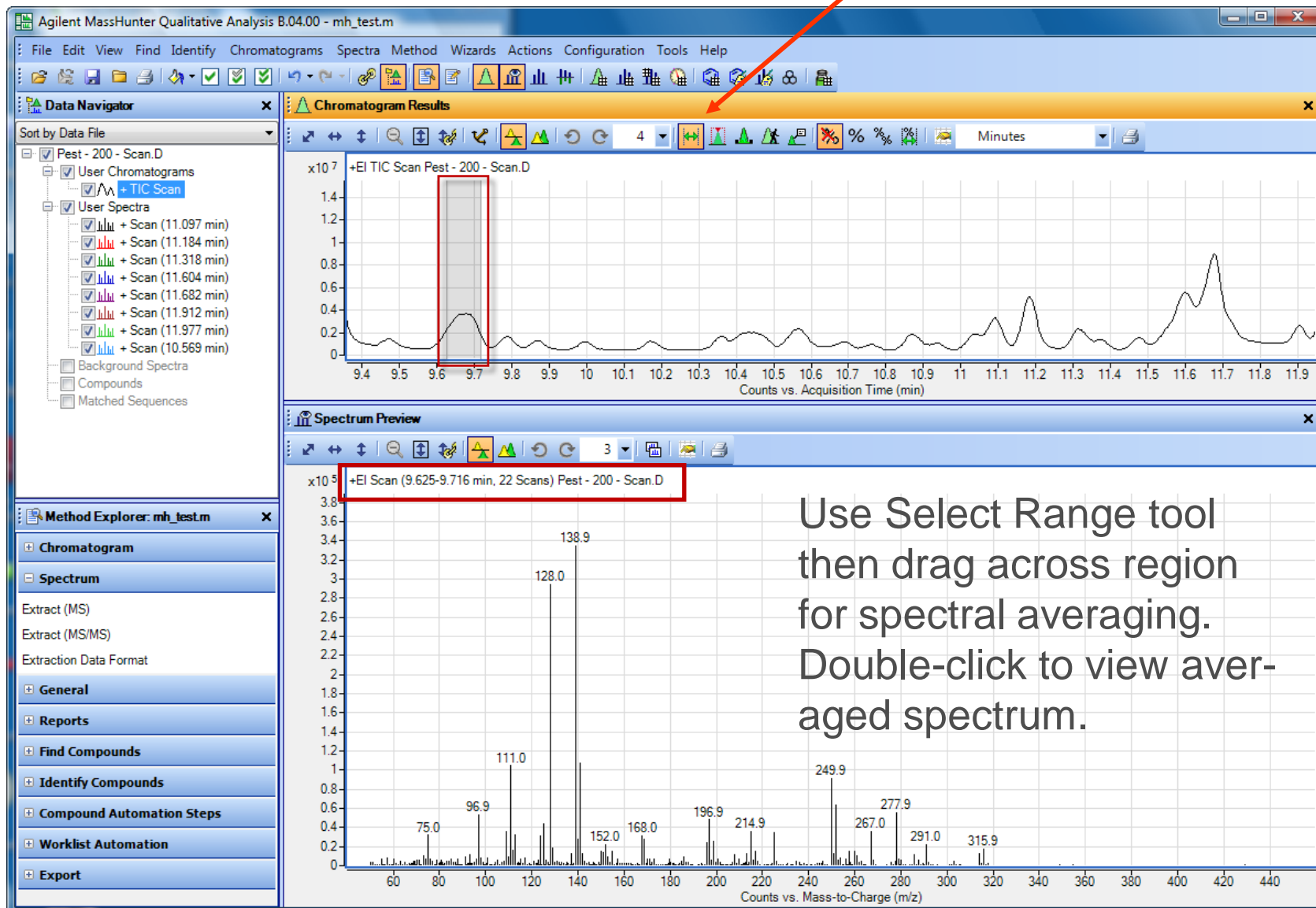
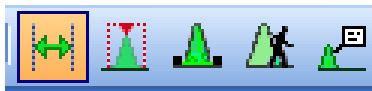
•Right-click  
•Select Copy to User Spectra

Extract EIC  
Extract Chromatograms...  
MS Spectrum Peak List 1  
MS Spectrum Peak List 2  
MS Actuals  
Assign Ranges to  
Move to Background Spectrum  
Copy to User Spectra  
Unzoom  
Assign Random Colors  
Choose Defined Color  
Copy to Clipboard Ctrl+C  
Print...  
Export...

# Extract Single Scan Spectrum



# Extract Averaged Spectra





# Background Subtraction for Manual Spectra

The screenshot displays the Agilent MassHunter Qualitative Analysis B.04.00 interface. The 'Data Navigator' on the left shows a tree structure with 'Pest - 200 - Scan.D' expanded, containing 'User Chromatograms' and 'User Spectra'. The 'Chromatogram Results' window shows a Total Ion Chromatogram (TIC) with a peak at 16.9 minutes. The 'Method Editor: Extract (MS)' window is open, showing the 'Manual Extraction' tab. The 'Manual spectrum background' dropdown is set to 'Current background spectrum'. The 'Spectrum Preview' window shows a mass spectrum with peaks at 57.1, 81.0, 95.1, 125.0, 181.0, and 224.0 m/z. A context menu is open over the spectrum, with 'Move to Background Spectrum' highlighted. A red arrow points from the 'Move to Background Spectrum' option to the 'Current background spectrum' dropdown in the Method Editor. A green box with the number 1 is in the top right corner, and a green box with the number 2 is in the middle right corner. A green box with the number 3 is in the bottom left corner.

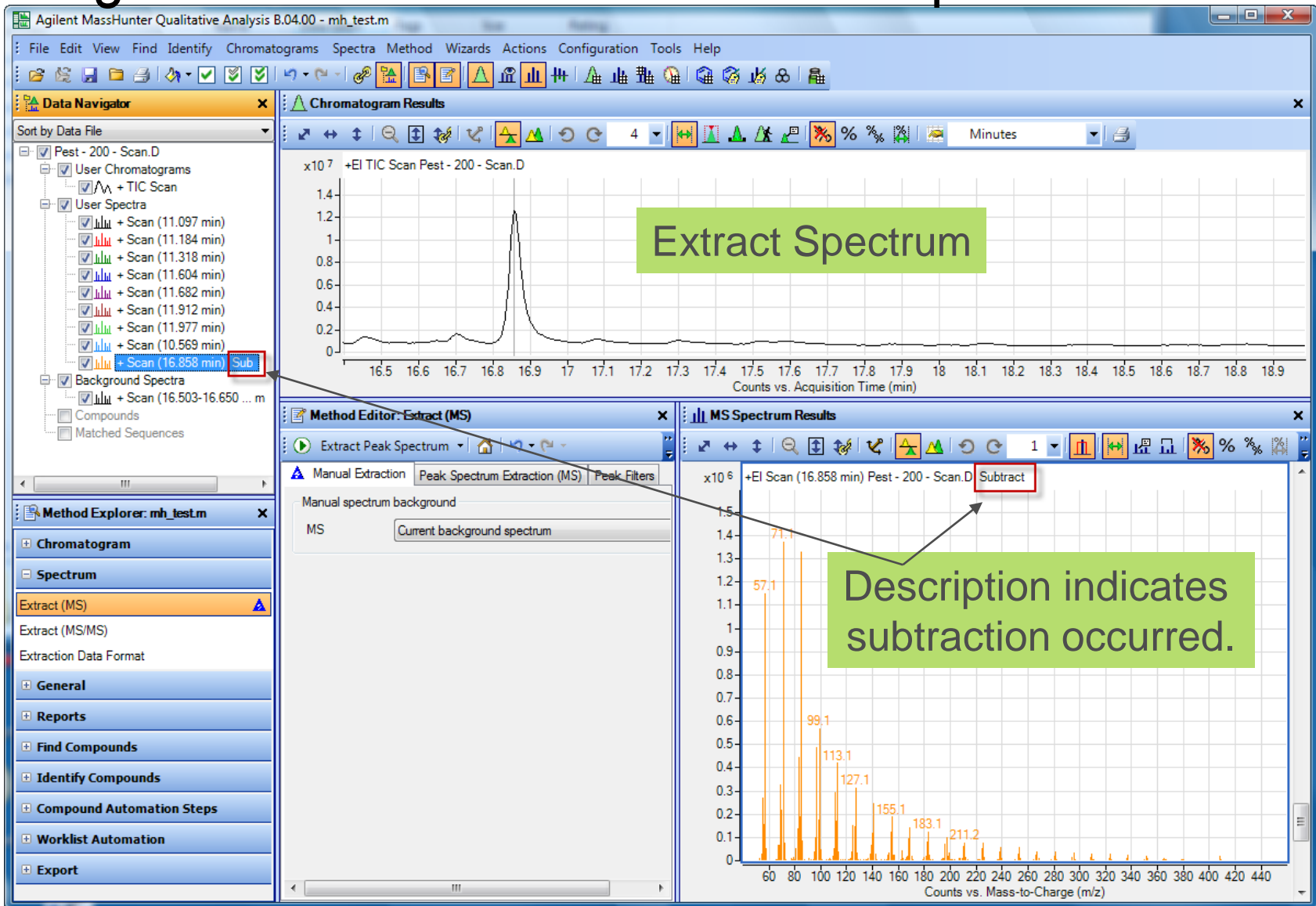
① Use the Ctrl Key to average multiple range selections then Extract Spectrum.

②

③ Change manual spectrum background to **Current background spectrum**.



# Background Subtraction for Manual Spectra



# Extract Peak Spectrum

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

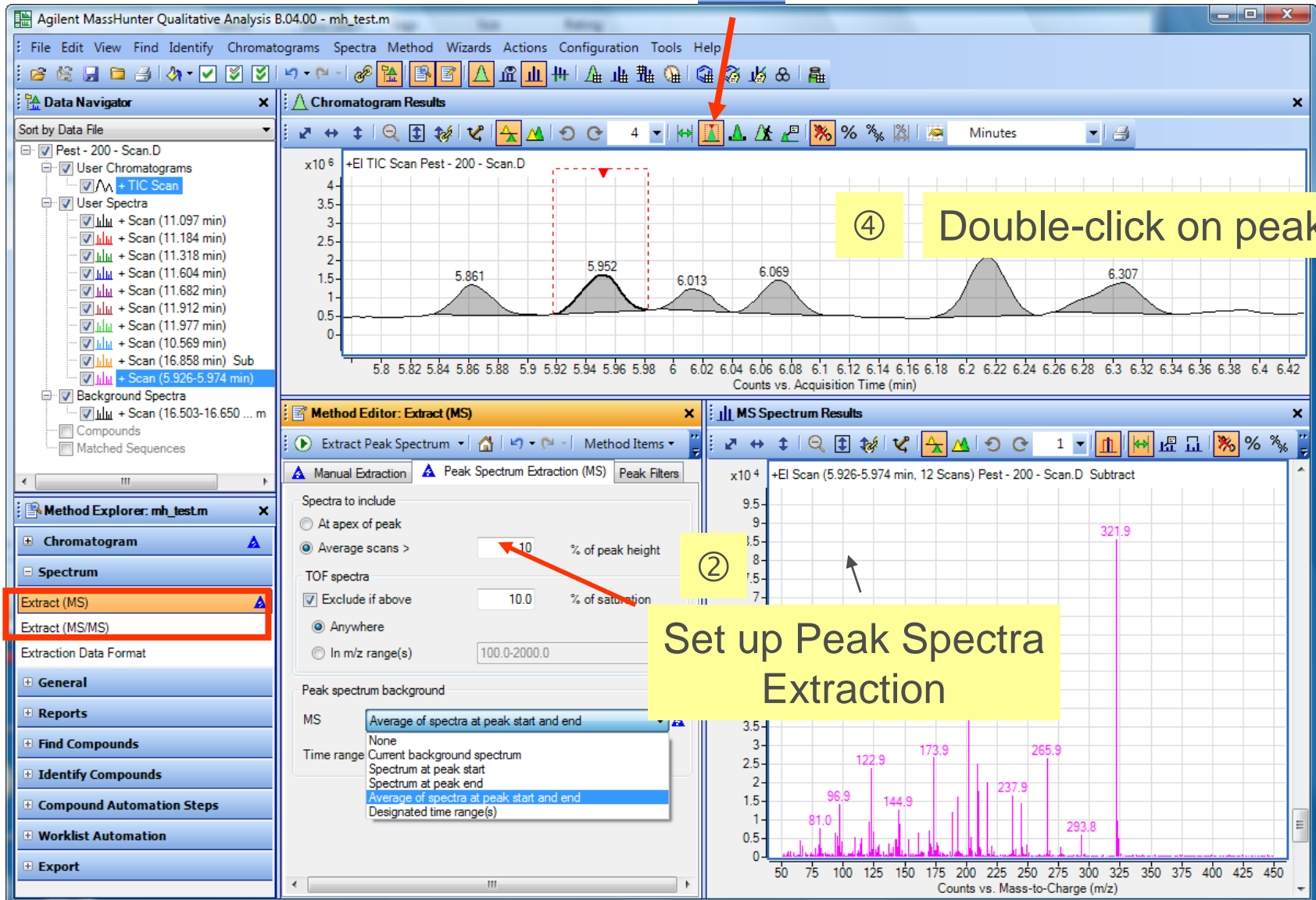
The screenshot displays the Agilent MassHunter Qualitative Analysis 8.04.00 interface. The 'Data Navigator' on the left shows a project named 'Pest - 200 - Scan.D' with a list of scans. The 'Chromatogram Results' window shows a Total Ion Chromatogram (TIC) with several peaks labeled with retention times: 5.861, 5.952, 6.013, 6.069, and 6.216 minutes. The 'Method Editor: Integrate (MS)' window is open, showing the 'Integrate Chromatogram' button highlighted with a red box. The 'MS Spectrum Results' window shows a mass spectrum for the selected peak at 16.858 minutes, with peaks labeled at m/z 57.1, 71.1, 83.1, and 211.2. A context menu is open over the 'Integrate Chromatogram' button, listing various actions such as 'Extract MS Spectrum', 'Extract Peak Spectrum', and 'Integrate Chromatogram', with the latter highlighted by a red box.

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

# Extract Peak Spectrum



③ Enable Peak Select tool.



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# Extract Peak Spectra Automatically

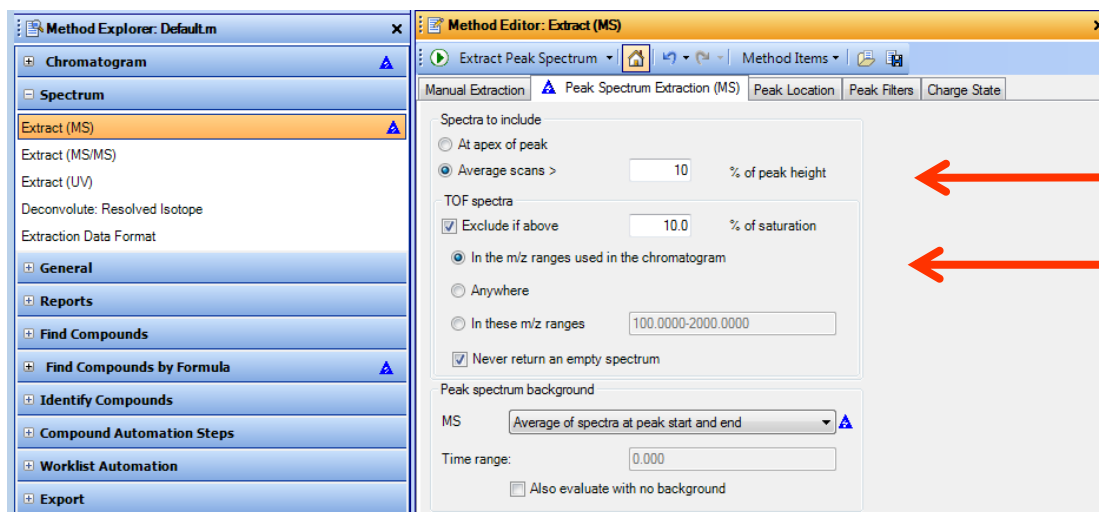
The screenshot displays the Agilent MassHunter Qualitative Analysis 8.04.00 interface. The 'Data Navigator' on the left lists various scans. The 'Chromatogram Results' window shows a Total Ion Chromatogram (TIC) with peaks labeled at retention times 5.861, 5.952, 6.013, and 6.069 minutes. A red arrow points from the 'Integrate and Extract Peak Spectra' menu option to the 'Method Editor: Extract (MS)' window. This window contains settings for 'Spectra to include' (Average scans > 10, % of peak height) and 'TOF spectra' (Exclude if above 10.0, % of saturation). The 'MS Spectrum Results' window shows a mass spectrum for a selected peak with major peaks at m/z 93.0, 121.0, and 136.1. A yellow callout box contains the following text:

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

**Spectra Results from each Integrated Peak**

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

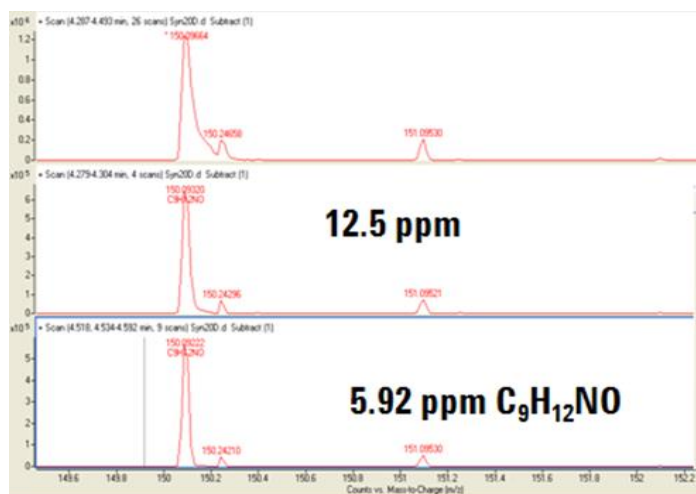
# Considerations for Accurate Mass Data Using Extract Peak Parameters – Saturation



Change 5%

Change 20%

For very narrow chromatographic peaks that are completely saturated you may have to turn this function off or error occurs: No Data Found.





# Using Extract Peak Parameters – Isotope Model

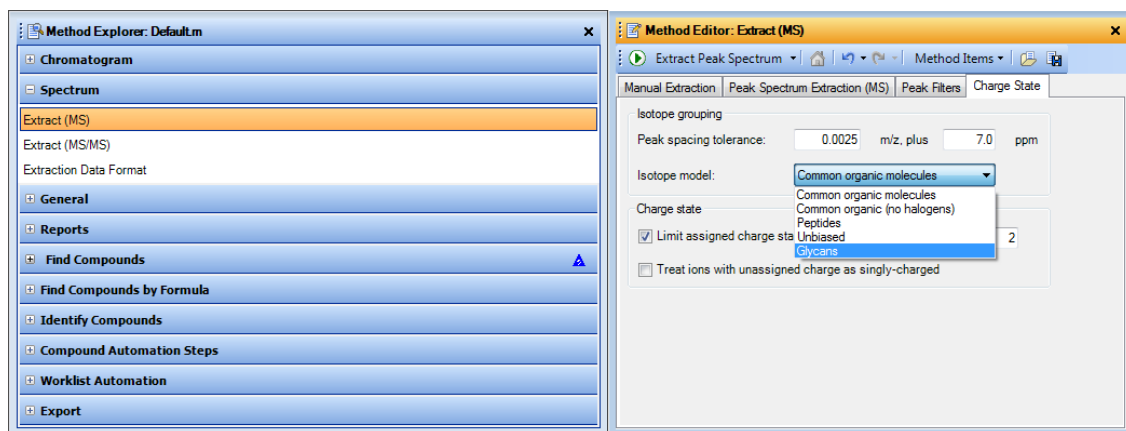
**Chose Isotope model that  
Corresponds to workflow.**

**Common organic molecules**

**Peptides**

**Unbiased**

**Glycans**

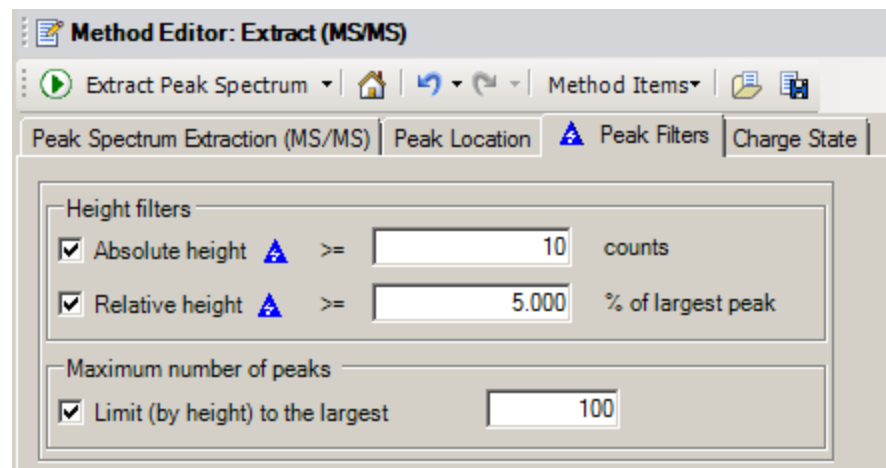
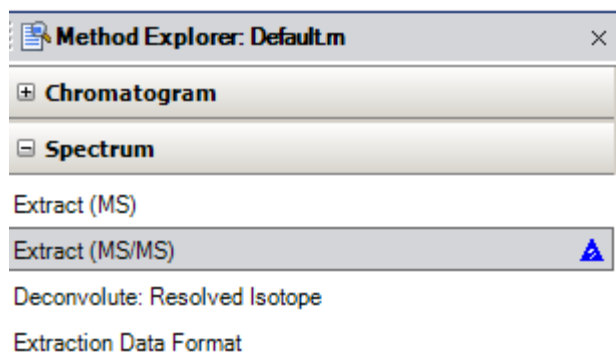


**Tip: Check Limit Assigned Charge States Maximum Values.**

**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.



# Context Sensitive Help

?

Height filters

☒ Absolute height  >=  counts

☐ Relative height >=  % of largest peak

Maximum number of peaks

☒ Limit (by height) to the largest

Peak spectrum background

MS

Time range

Current background spectrum  
Spectrum at peak start  
Spectrum at peak end  
Average of spectra at peak start and end  
Designated time range(s)

Spectra to include

☐ At apex of peak

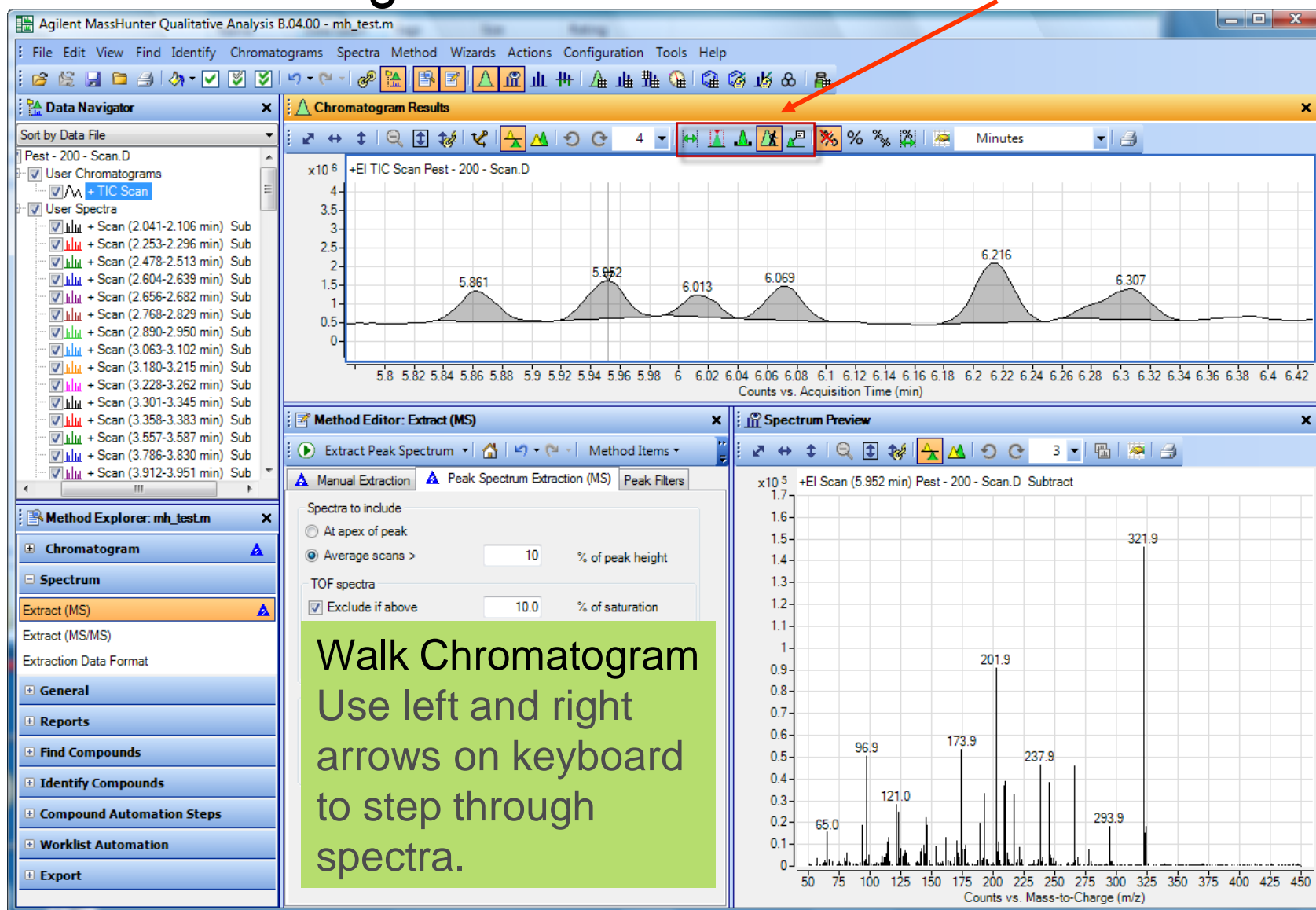
☒ Average scans >  % of peak height

Common organic molecules  
Common organic (no halogens)  
Peptides  
Unbiased  
Glycans

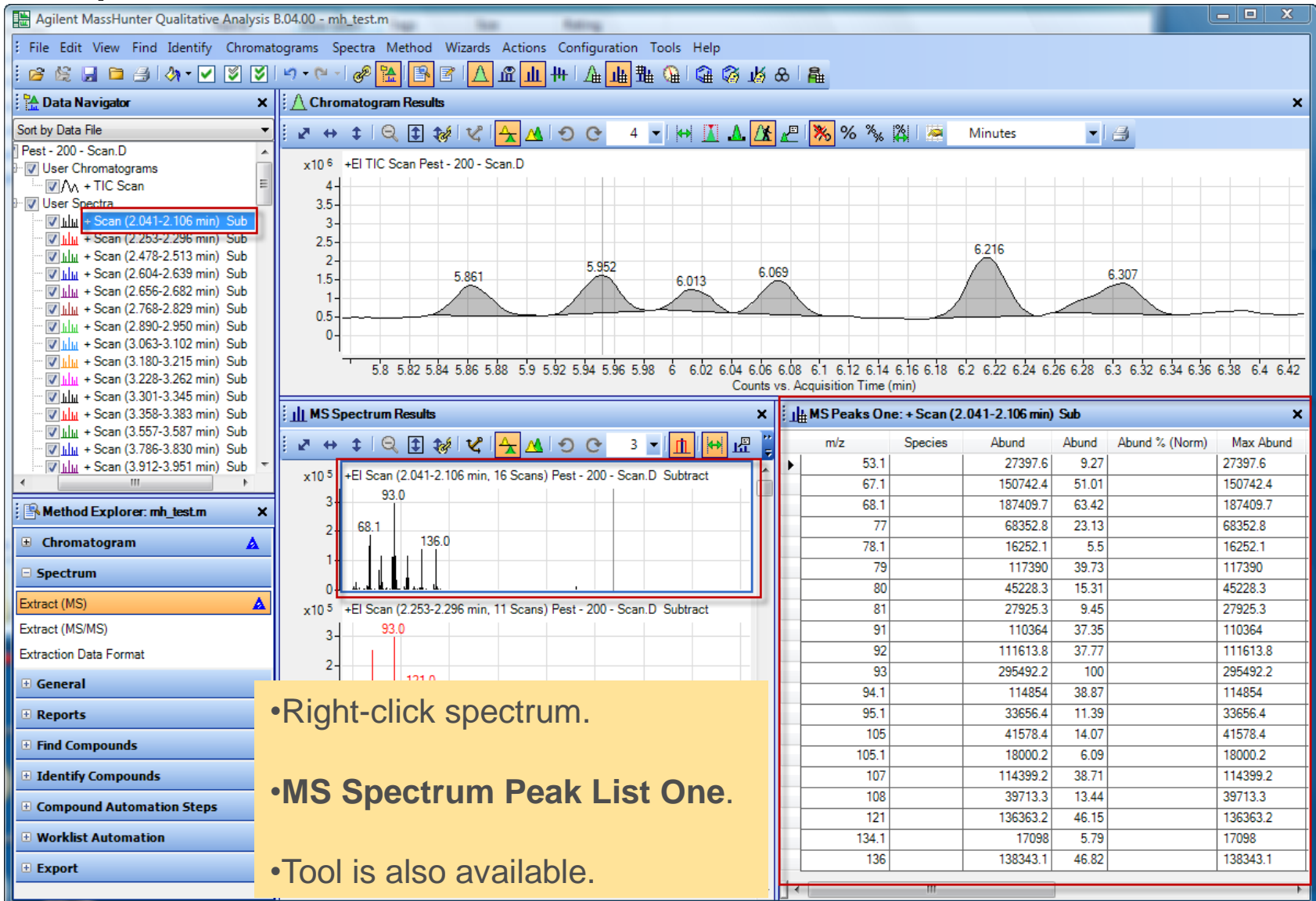
**Tip: Context Sensitive Help is available through the F1 Key.**



# Walk Chromatogram



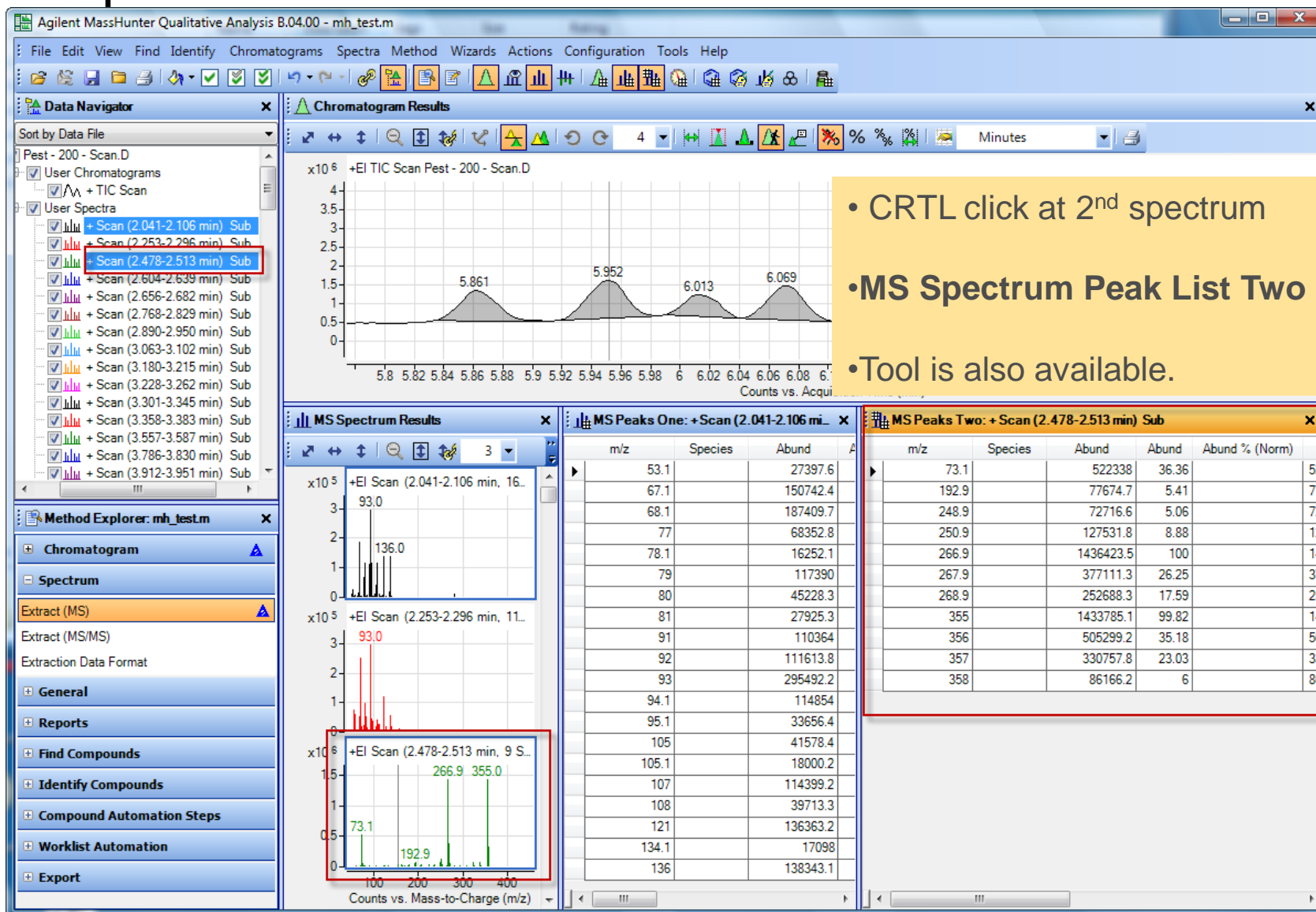
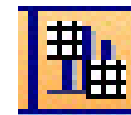
# MS Spectrum Peak List One



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



# MS Spectrum Peak List Two



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



# MS Spectrum Peak List

MS Peaks One: + Scan (2.041-2.106 min) Sub

m/z	Species	Abund	Abund	Abund % (Norm)	Max Abund
53.1		27397.6	9.27		27397.6
67.1		150742.4	51.01		150742.4
68.1		187409.7	63.42		187409.7
77		68352.8	23.13		68352.8
78.1					16252.1
79					117390
80					45228.3
81					27925.3
91					110364
92					111613.8
93					295492.2
94.1					114854
95.1		33656.4	11.39		33656.4
105		41578.4	14.07		41578.4
105.1		18000.2	6.09		18000.2
107		114399.2	38.71		114399.2
108		39713.3	13.44		39713.3
121		136363.2	46.15		136363.2
134.1		17098	5.79		17098
136		138343.1	46.82		138343.1

For relative abundance, right-click table header. and **Add/Remove Columns**. Select desired columns.

Add/Remove Columns

Available Columns:

- Cluster
- Diff (mDa)
- Formula
- Label
- m/z (Calc)
- Peak Number

Show these columns:

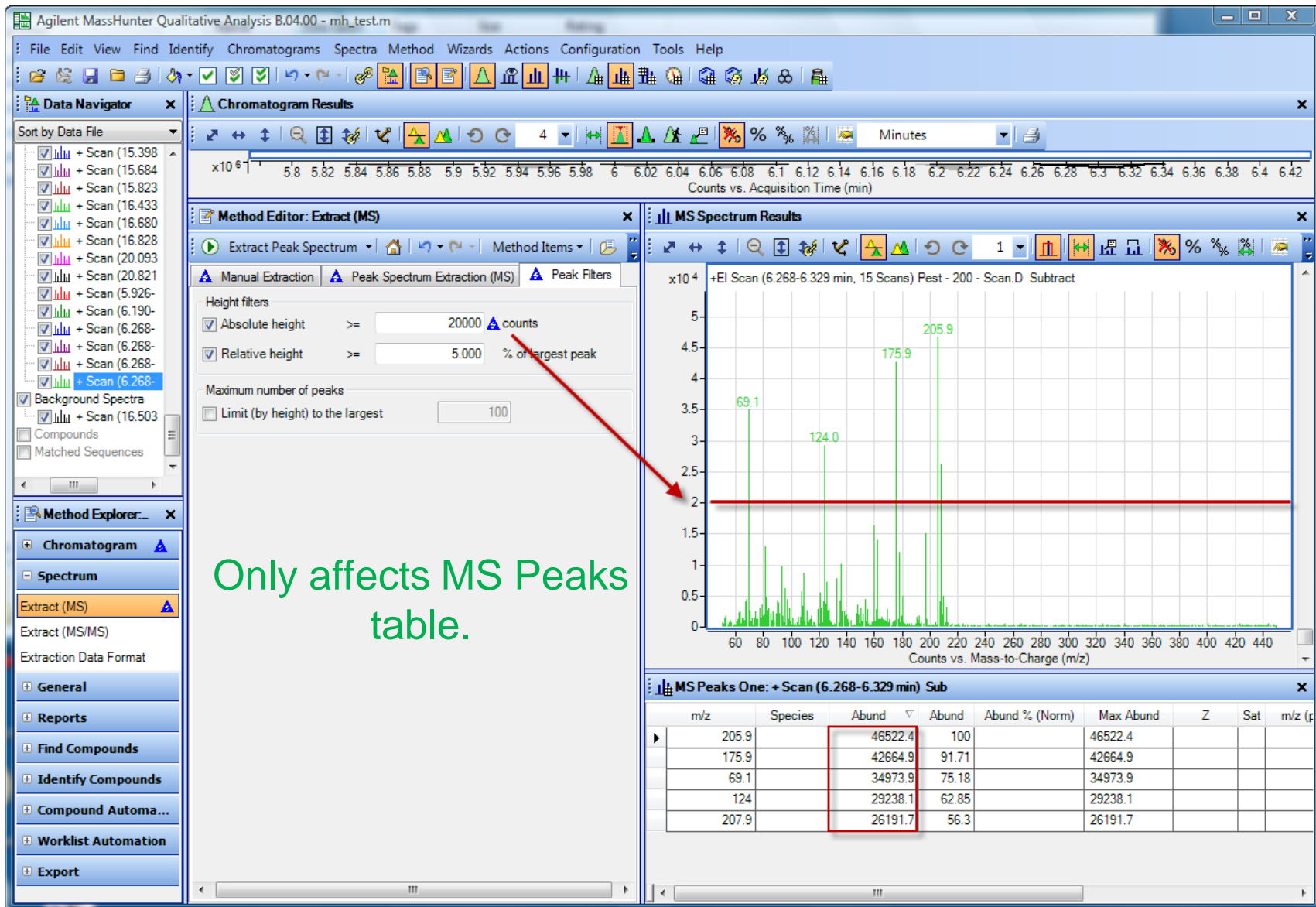
- Abund
- Abund %
- Abund % (Norm)
- Diff (ppm)
- Ion
- m/z
- m/z (prod.)
- Max Abund
- Sat
- Species
- Z
- Z (prod.)

Buttons: Add ->, <- Remove, Add All ->>, <<- Remove All

OK Cancel

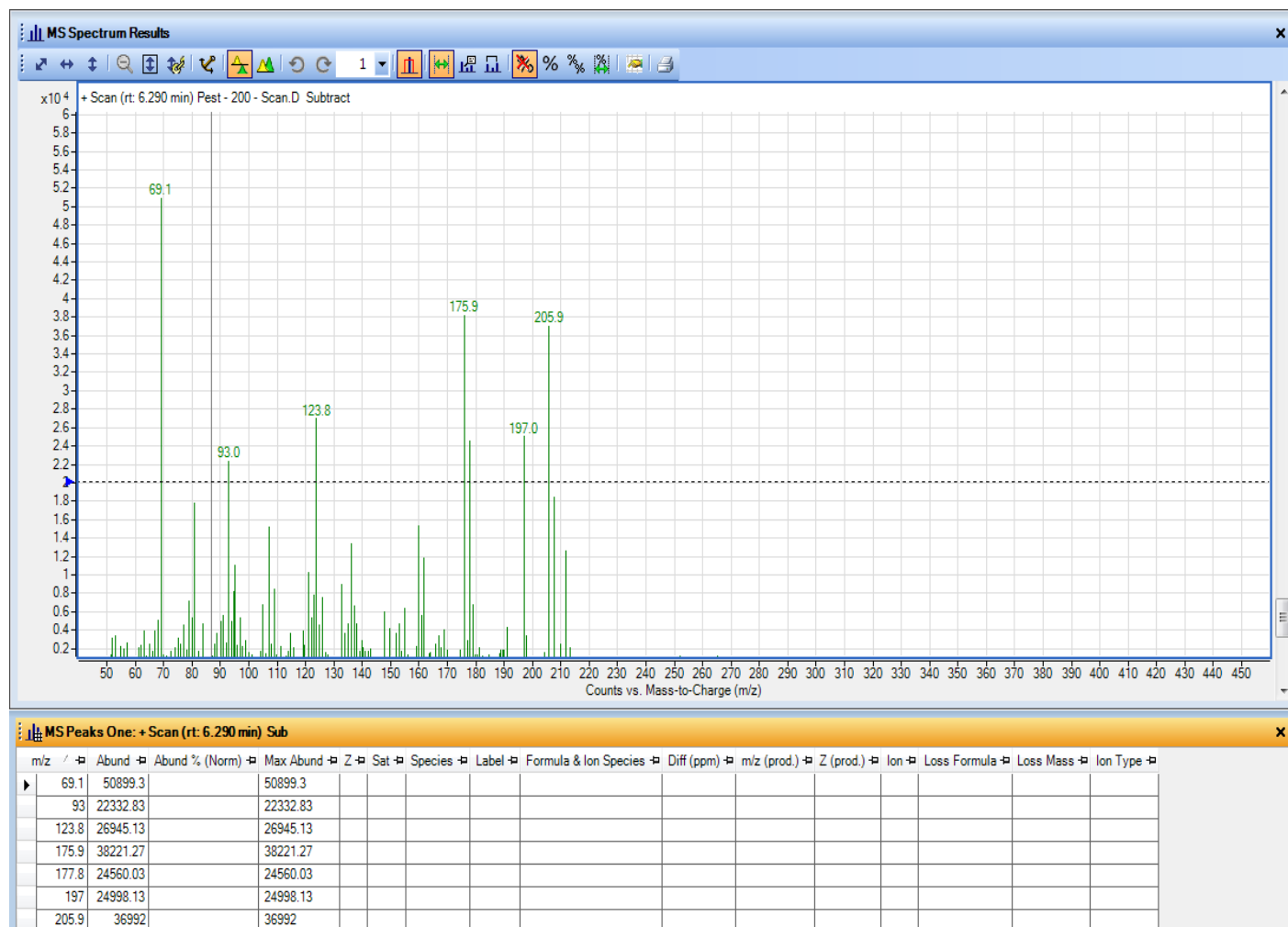


# MS Spectrum Peak Filter



# MS Spectrum Peak Filter Interactive Setup

- Position the cursor at the location for the threshold.
- Right click and select **Adjust Peak Threshold**.
- Tip: Scale Spectra must be off.



MS Spectrum Peak List 1



MS Spectrum Peak List 2



MS Actuals

Adjust Peak Threshold



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# Accurate Mass Considerations

## How to Recalibrate a Mass Spectrum

Use on **User Spectra** only.

Do not background subtract.

Right-click in MS Spectrum Results window and select **Recalibrate**.

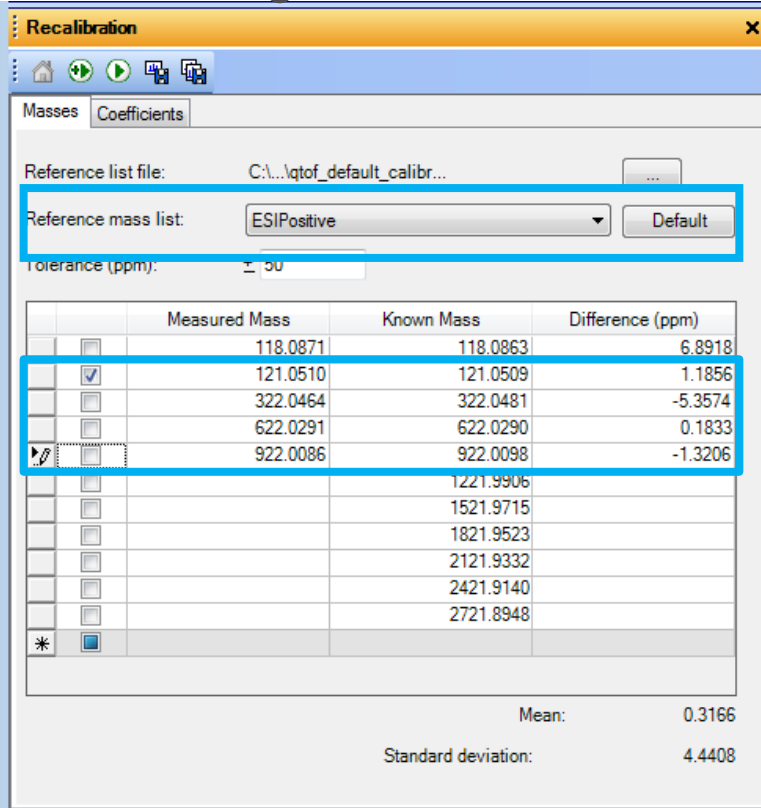
Calculates and applies new calibration to highlighted spectra. 

Select  to apply current recalibration to highlighted spectra.

Saves new calibration to highlighted spectra. 

Save new calibration to whole datafile. 

Restores original calibration. 



The Recalibration dialog box displays the following information:

- Reference list file: C:\...\qtof\_default\_calibr...
- Reference mass list: ESIPositive (dropdown menu)
- Tolerance (ppm): 50
- Table of Measured Mass, Known Mass, and Difference (ppm):

	Measured Mass	Known Mass	Difference (ppm)
<input type="checkbox"/>	118.0871	118.0863	6.8918
<input checked="" type="checkbox"/>	121.0510	121.0509	1.1856
<input type="checkbox"/>	322.0464	322.0481	-5.3574
<input type="checkbox"/>	622.0291	622.0290	0.1833
<input checked="" type="checkbox"/>	922.0086	922.0098	-1.3206
<input type="checkbox"/>		1221.9906	
<input type="checkbox"/>		1521.9715	
<input type="checkbox"/>		1821.9523	
<input type="checkbox"/>		2121.9332	
<input type="checkbox"/>		2421.9140	
<input type="checkbox"/>		2721.8948	
*			

Mean: 0.3166  
Standard deviation: 4.4408





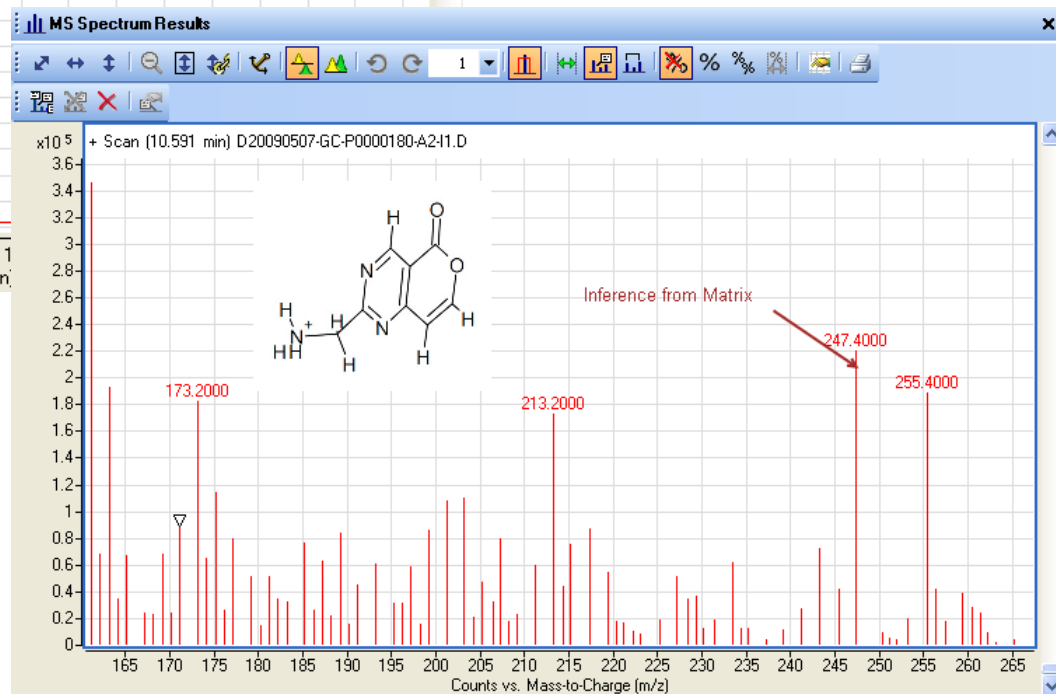
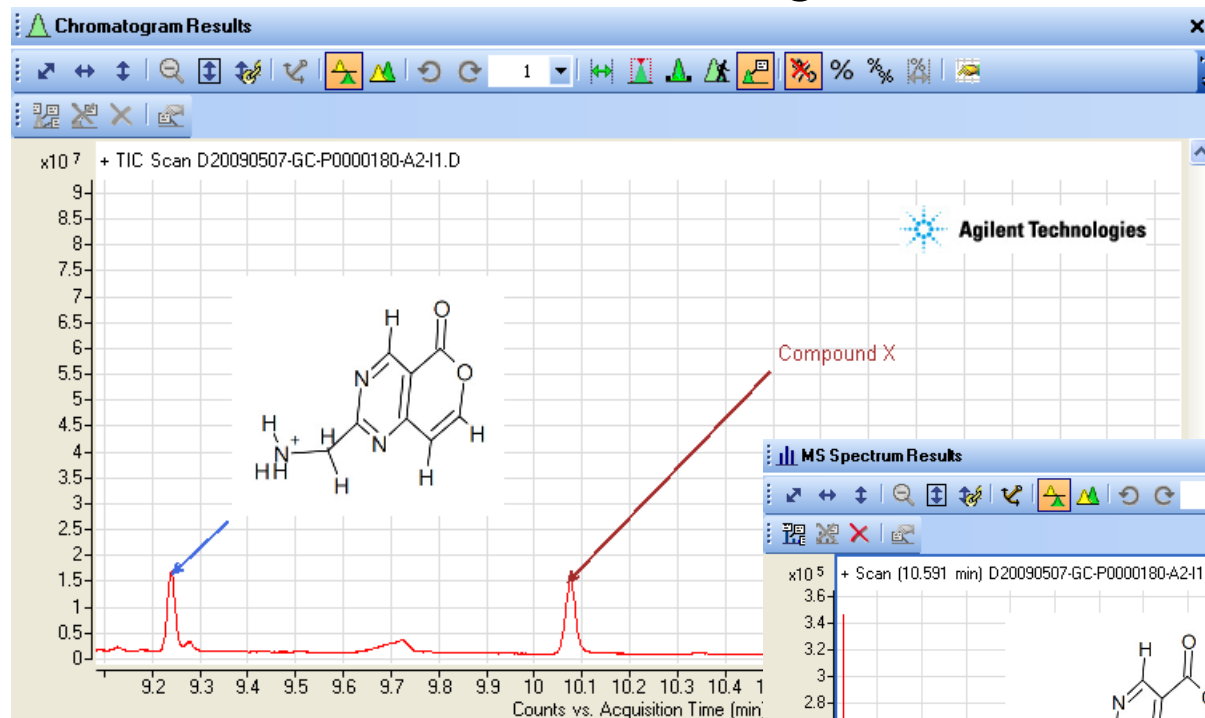


Let's take a moment  
for questions on  
Working with Mass  
Spectra

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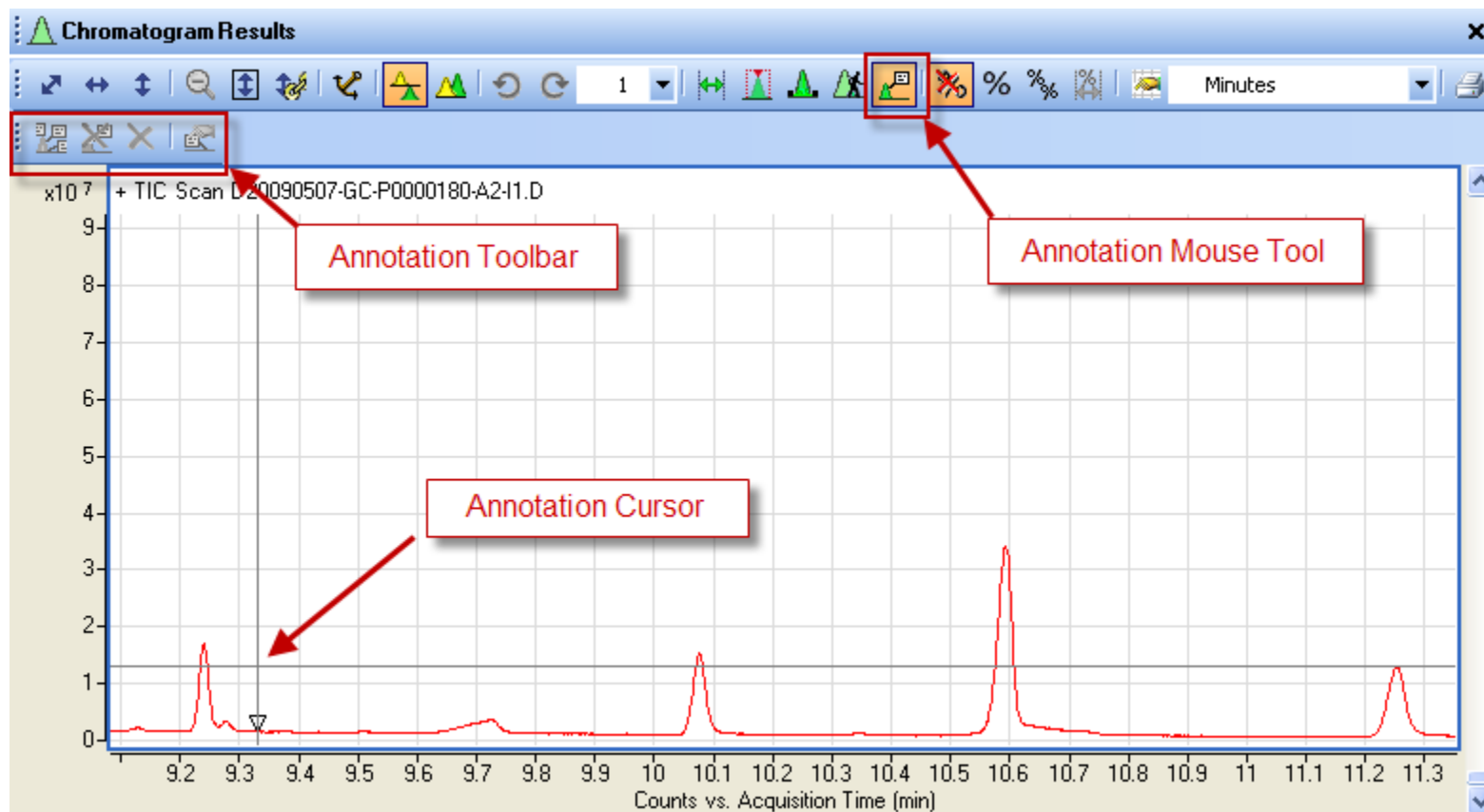
Up Next:  
Annotations

# Annotation of Chromatograms and Spectra

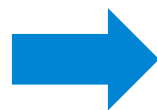
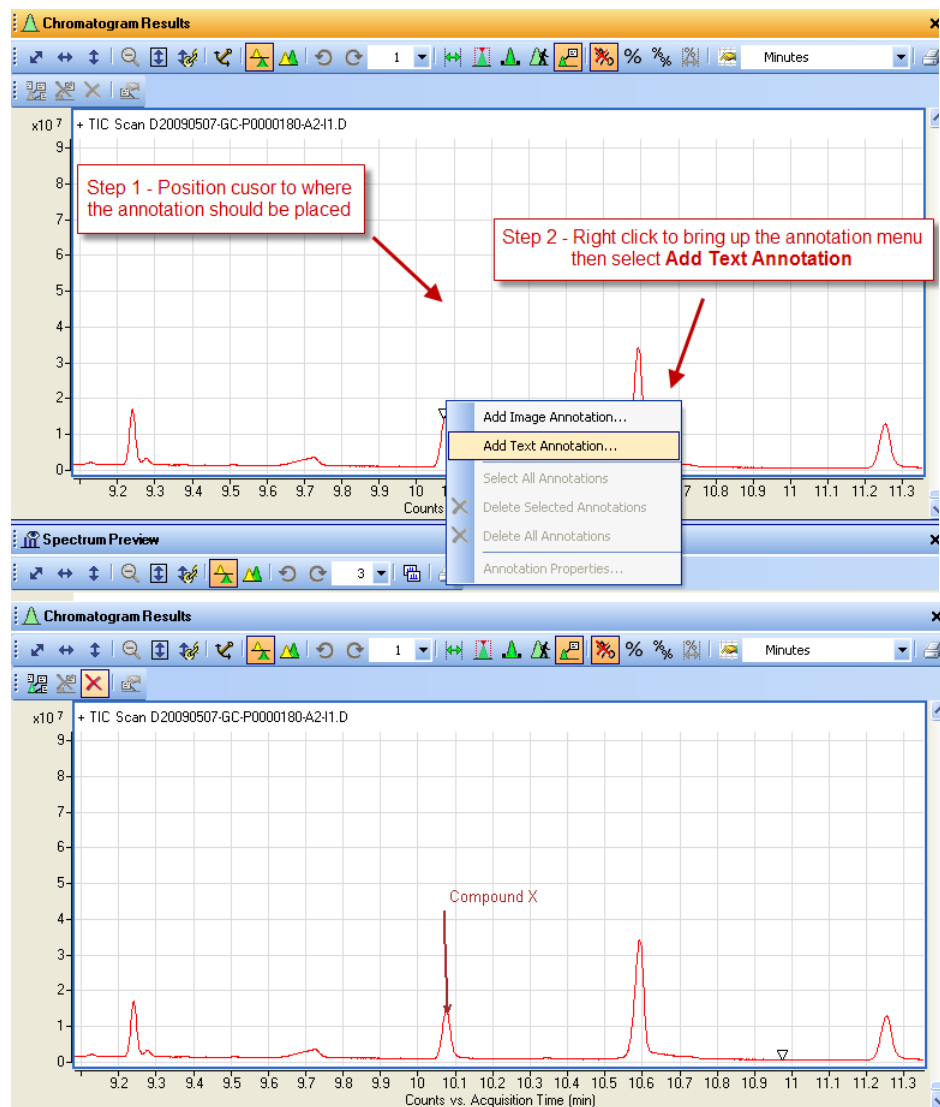


# Place Graphic into Annotation Mode

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



# Add a Text Annotation



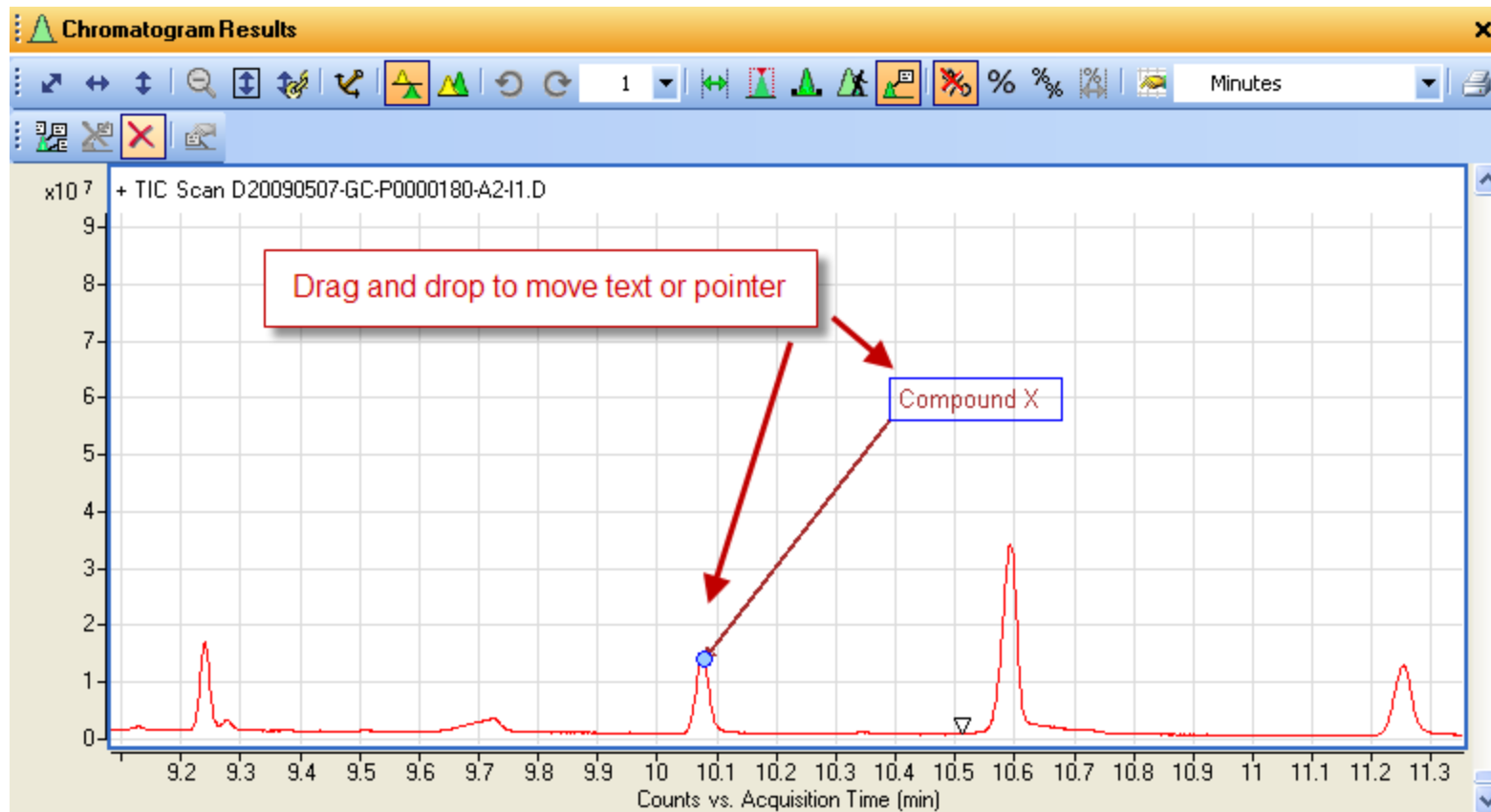
The 'Add/Edit Text Annotation' dialog box is shown with the following settings:

- Text:** Compound X
- Text color:** Brown
- Orientation:** 0 degrees
- Font style:** Regular
- Font size:** 10
- Annotation type:** Anchored
- ☒ Show pointer
- Pointer properties:**
  - Color:** Brown
  - Pattern:** Solid line
  - Weight:** Medium
  - Pointer head:** Arrow
  - Pointer head location (the x, y value using the data displayed):**
    - X: 10.079183333333 min
    - Y: 14256440 (abundance)
  - Upper left corner of the annotation (the x, y value using the data displayed):**
    - X: 10.08 min
    - Y: 49063600.57 (abundance)
- ☐ Floating
- Upper left corner of the annotation relative to the upper left corner of the canvas:**
  - Relative X (%):** 43.8202247191011 (% calculated using x,y values from the canvas)
  - Relative Y (%):** 50.4885993485342

At the bottom, there are three buttons: 'Step 4 - Click OK', 'OK', and 'Cancel'. A red arrow points from the 'Step 4 - Click OK' button to the 'OK' button.

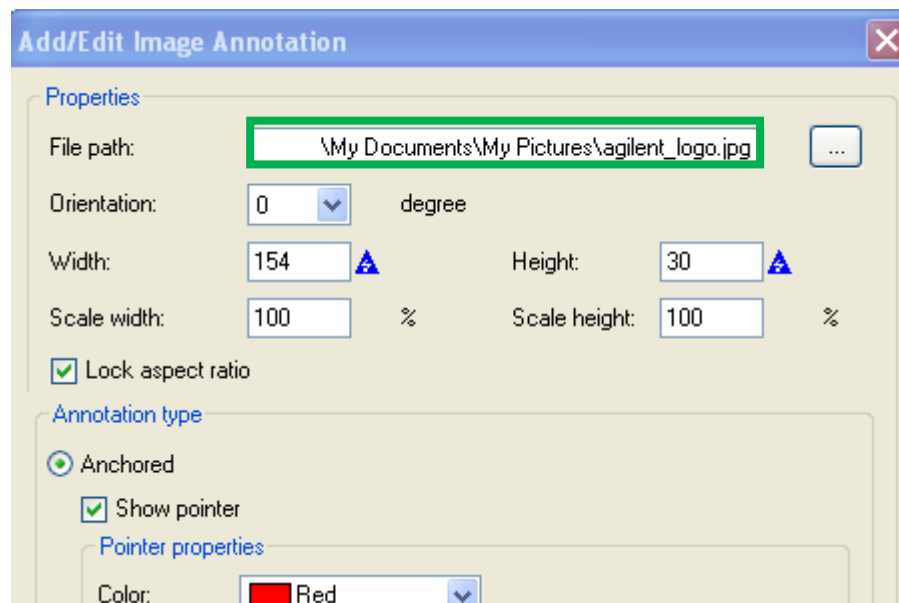
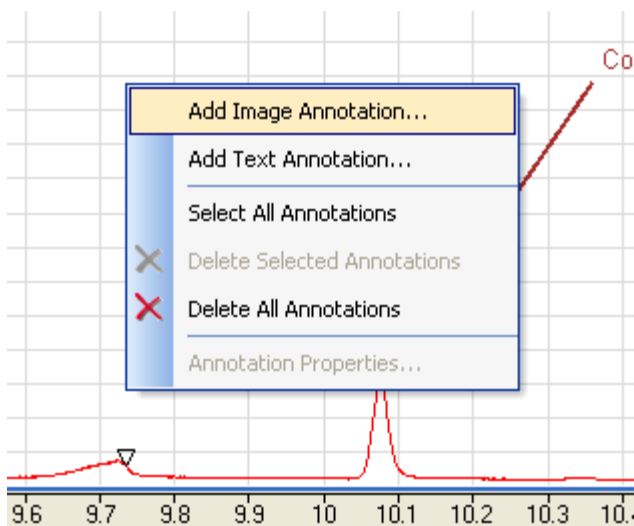


# Text and Pointer Can Be Repositioned



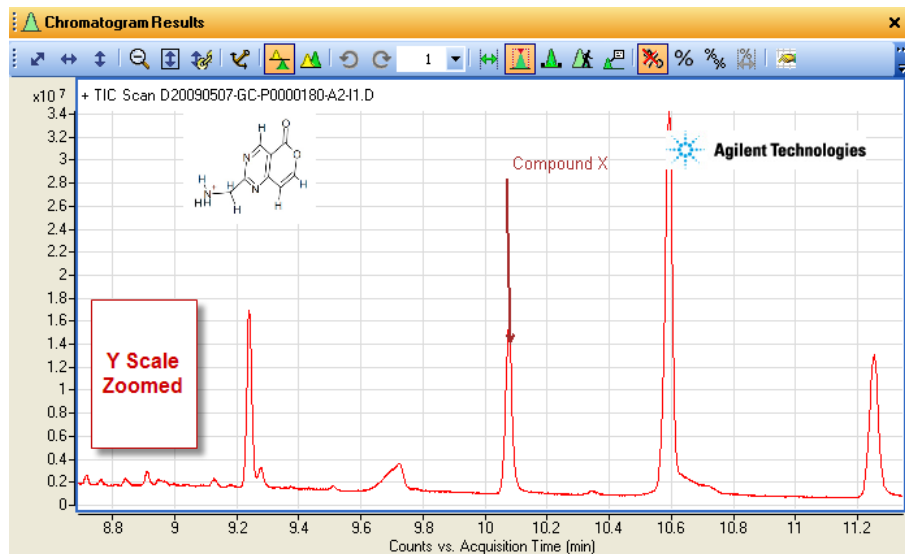
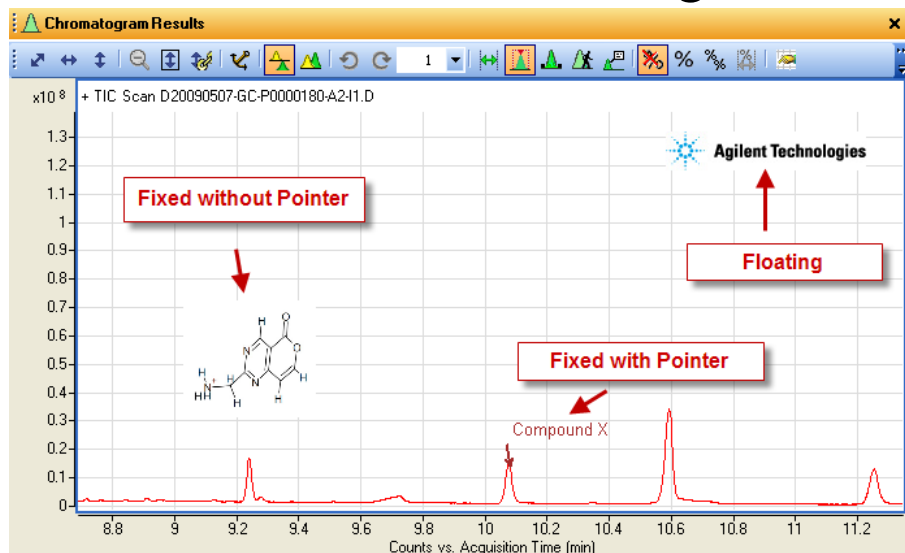
# Image Annotation

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



The 'Add/Edit Image Annotation' dialog box is shown. It has a blue title bar with a close button. The 'Properties' section includes: 'File path' (text box with '\My Documents\My Pictures\agilent\_logo.jpg' and a browse button), 'Orientation' (0 degree), 'Width' (154), 'Height' (30), 'Scale width' (100 %), and 'Scale height' (100 %). There is a checked 'Lock aspect ratio' checkbox. The 'Annotation type' section has a radio button for 'Anchored' and a checked 'Show pointer' checkbox. The 'Pointer properties' section has a 'Color' dropdown set to 'Red'.

# 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)

**Upper left corner of the annotation (the x, y value using the data displayed):**

X:  min

Y:  (abundance)

☐ Floating

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

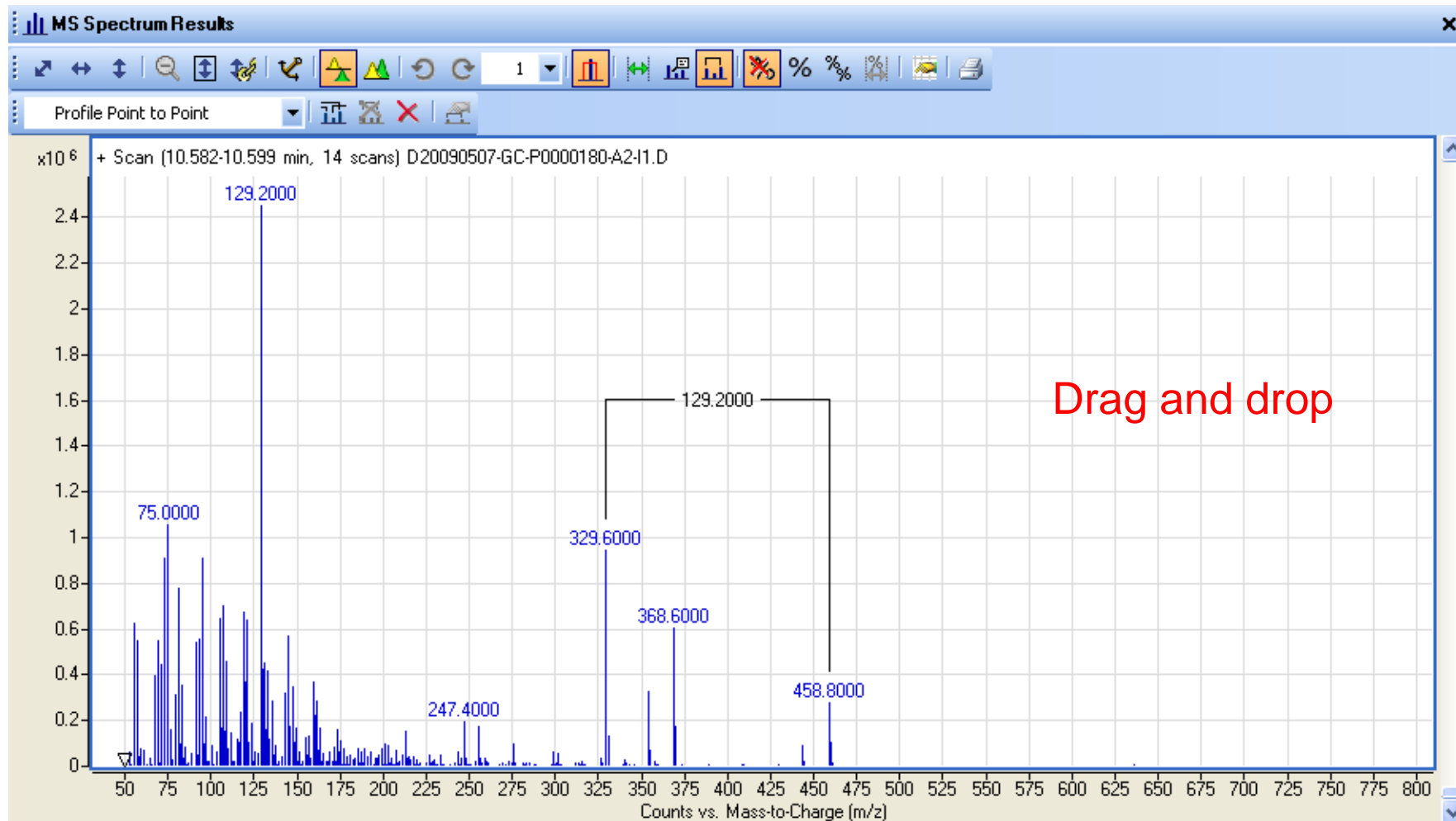
Relative X (%):  (% calculated using x,y values from the canvas )

Relative Y (%):



# Delta Mass Caliper

- New tool to calculate and display mass differences between two ions.





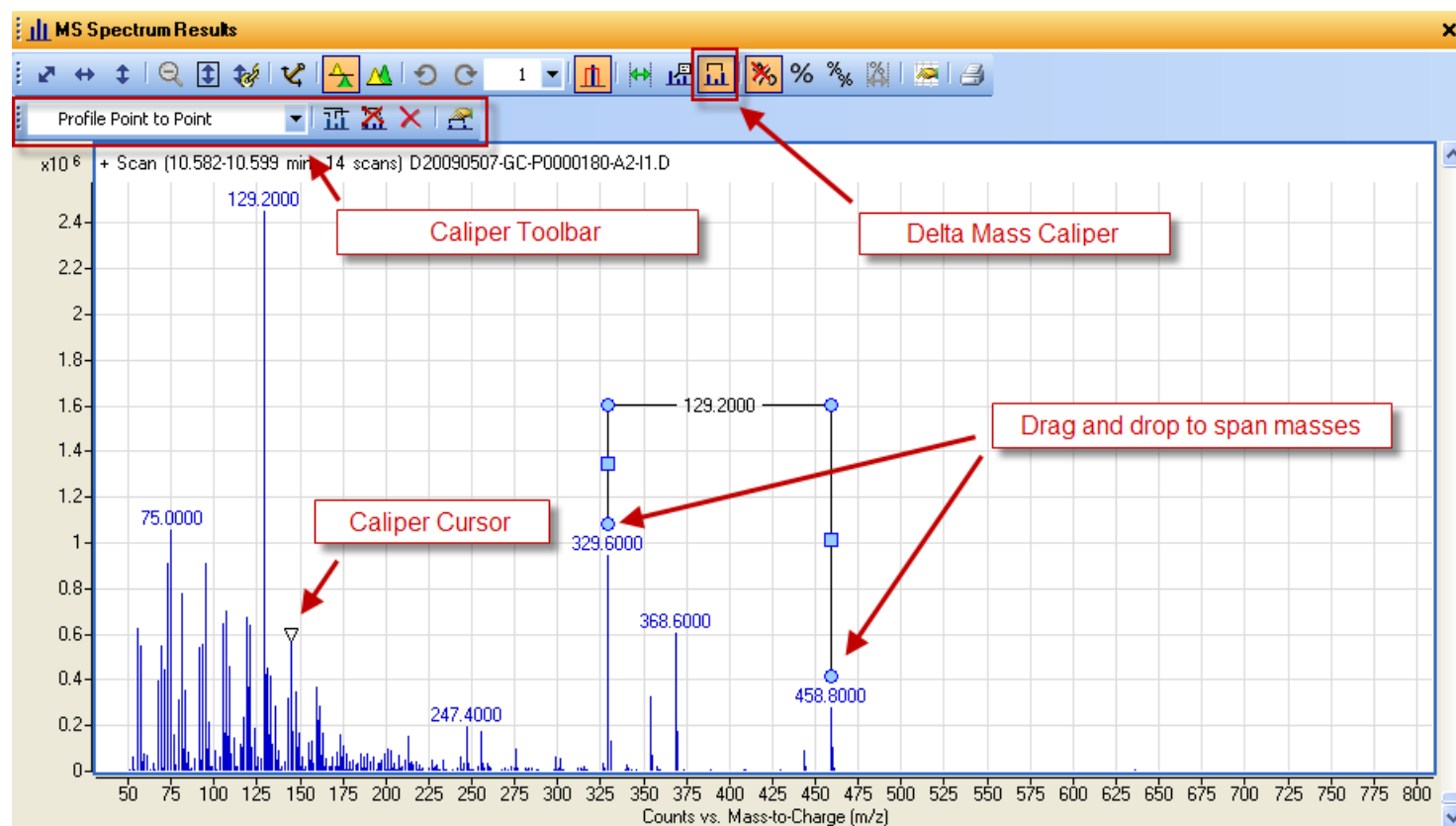
# Delta Mass Caliper - Mass Caliper Mode

Click **Delta Mass Caliper Mouse** icon

Caliber Toolbar and Caliper cursor appear

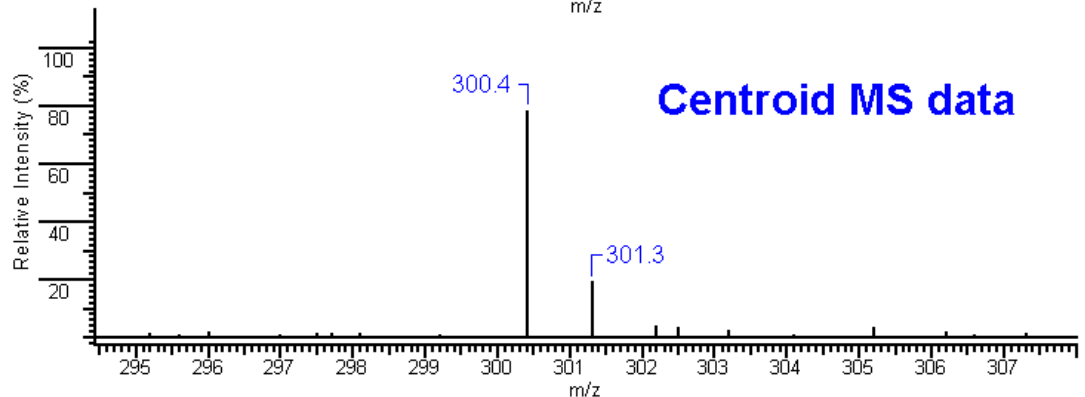
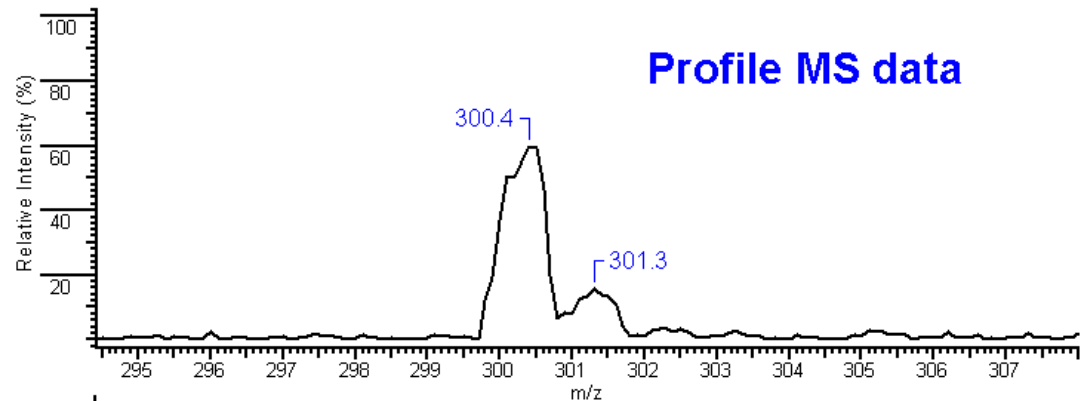
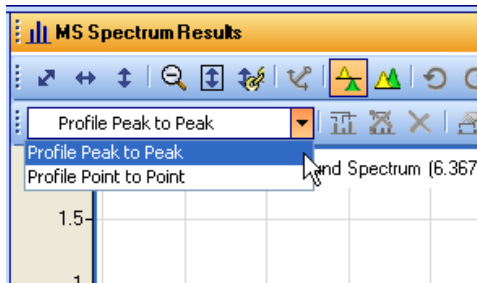
Add or edit Calipers

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



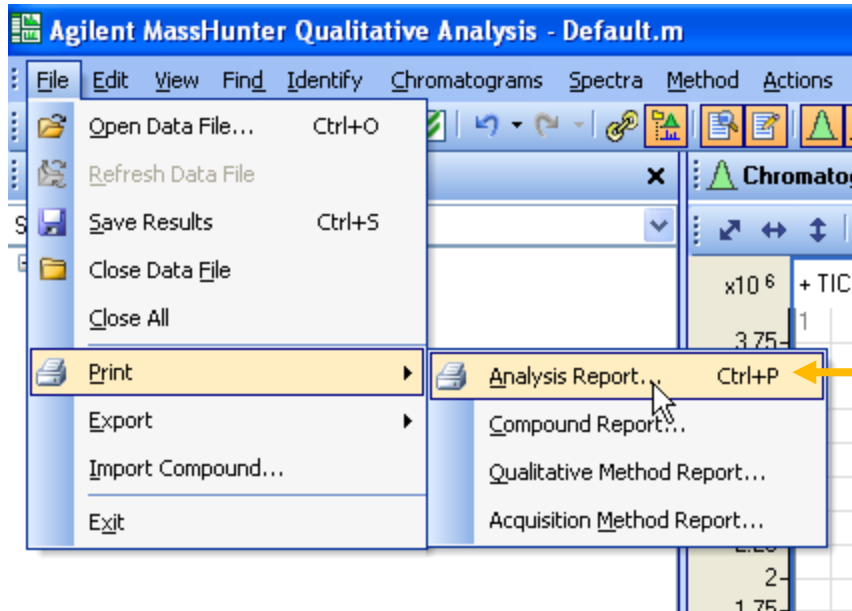


Let's take another  
moment for  
questions on  
Annotations

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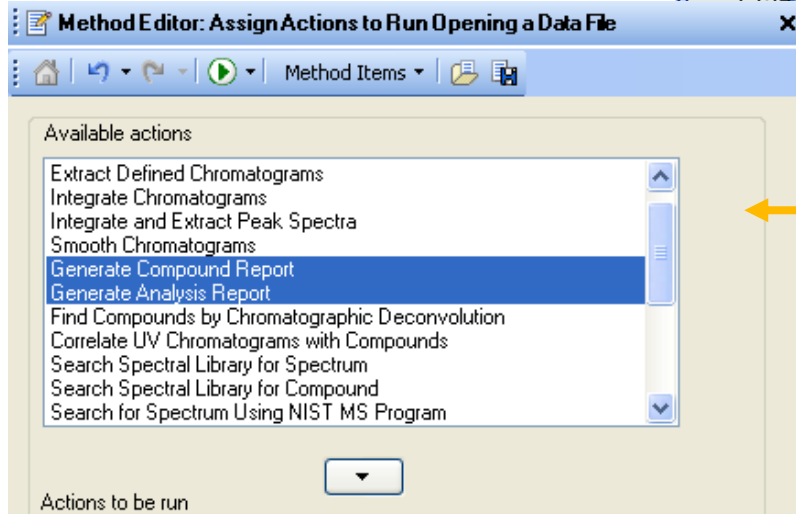
Up Next:  
Reporting

# Print a Report from Qualitative Analysis

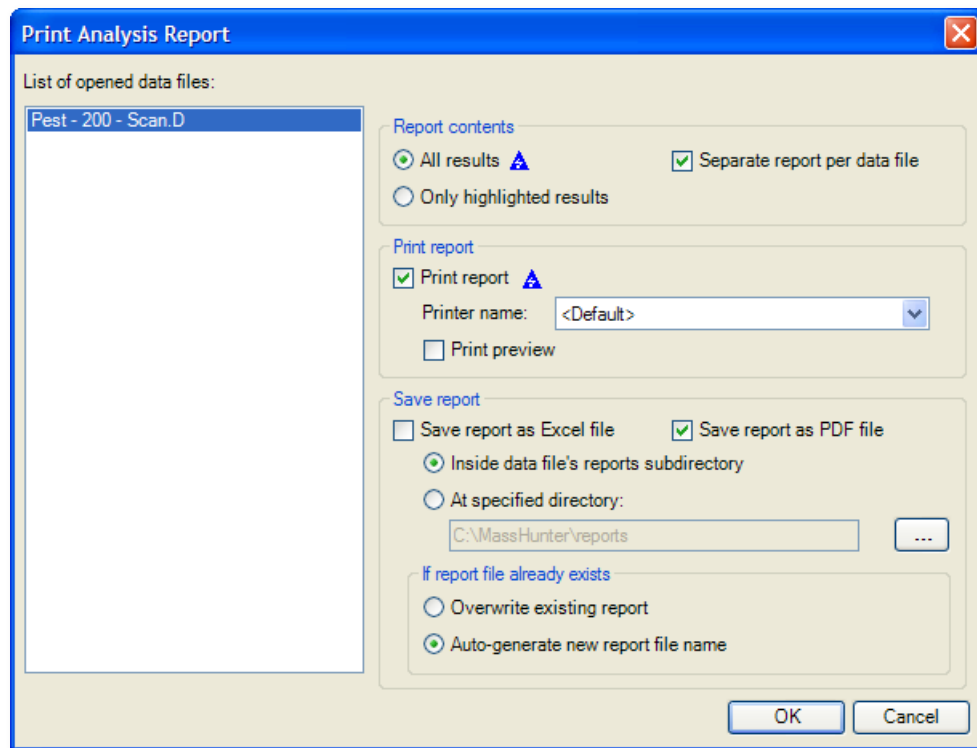
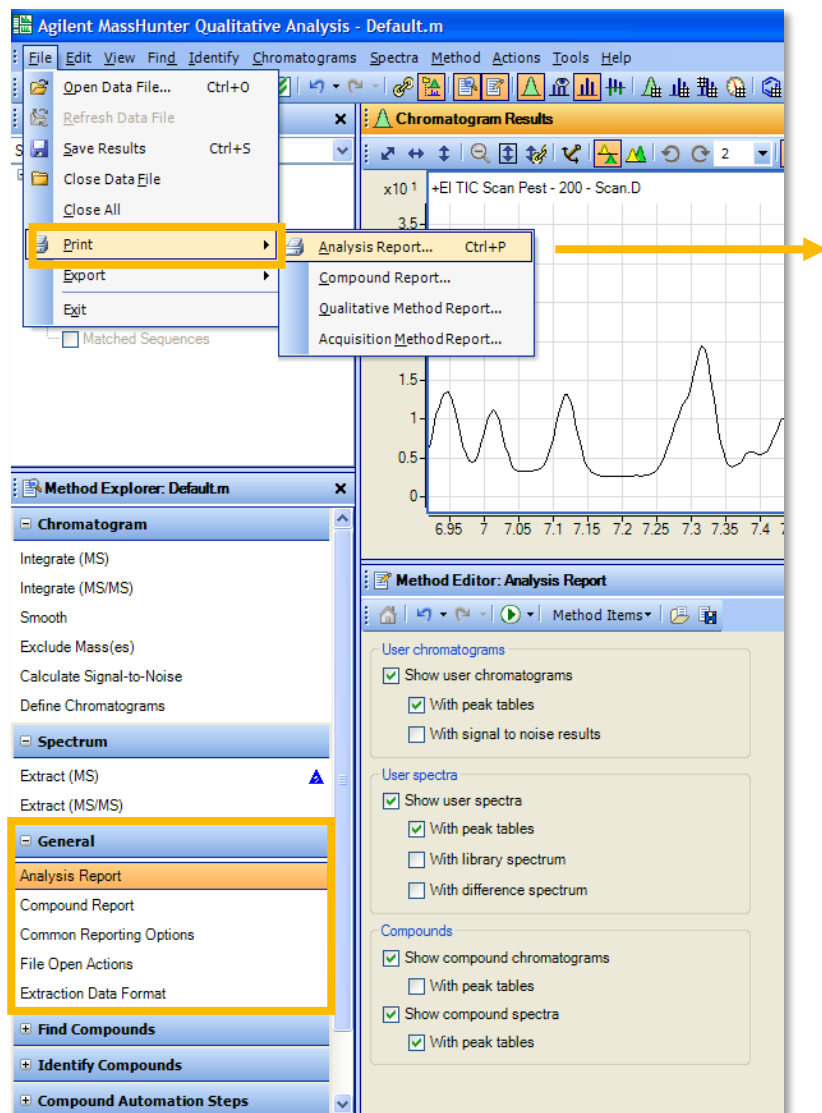


## Print Qualitative Reports:

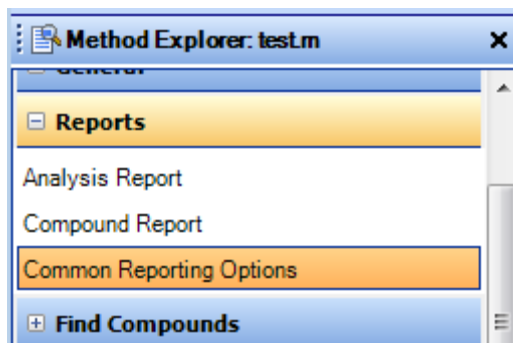
- Directly from menu.
- Indirectly from Automation
  - File Open Actions
  - Worklist Automation
  - Covered in the Familiarization Guides.



# Report Generation Options



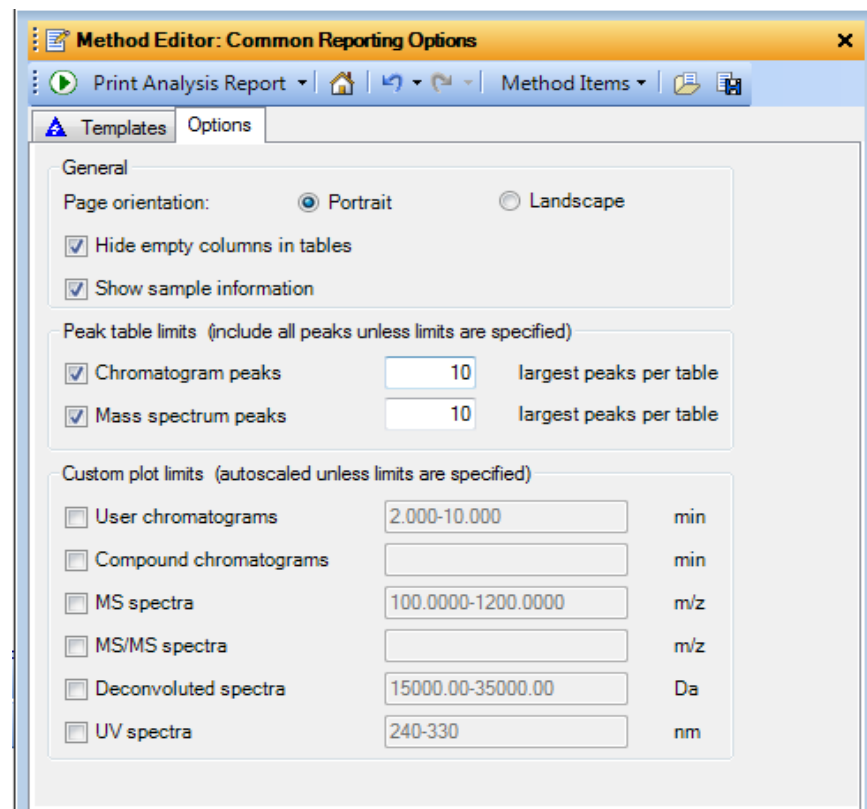
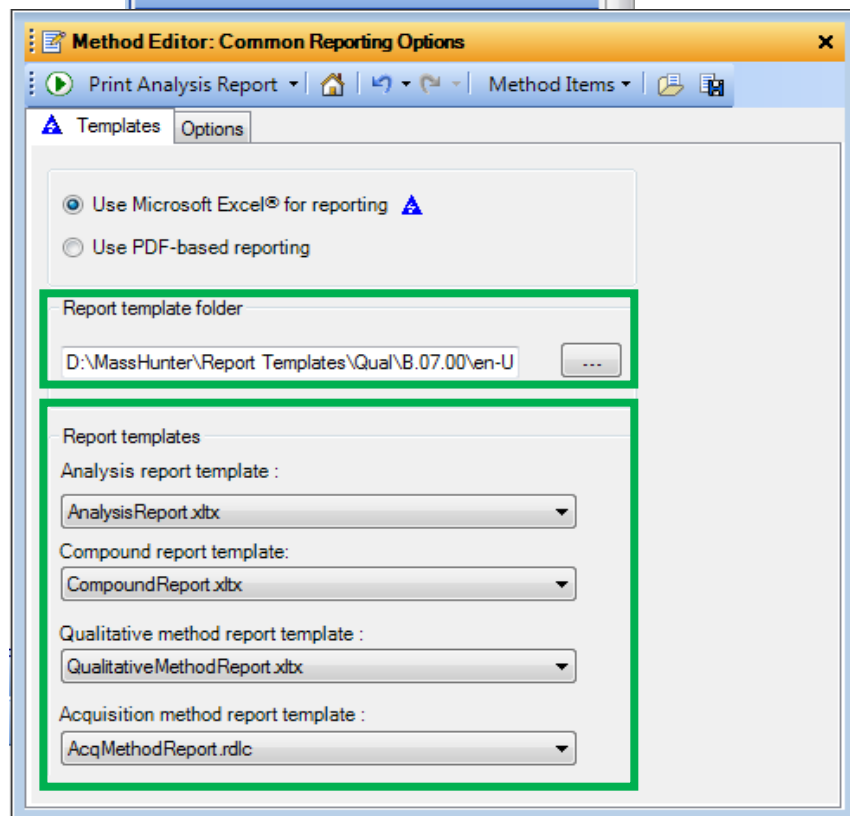
# Common Reporting Options



Excel Based Reporting

Uses xlsx files

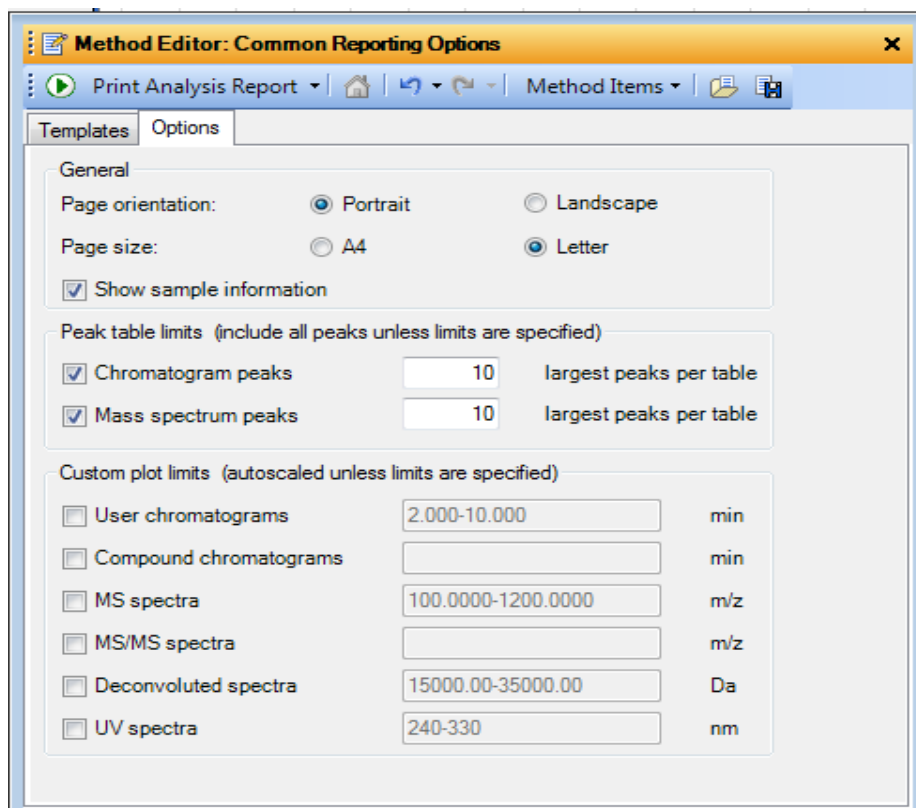
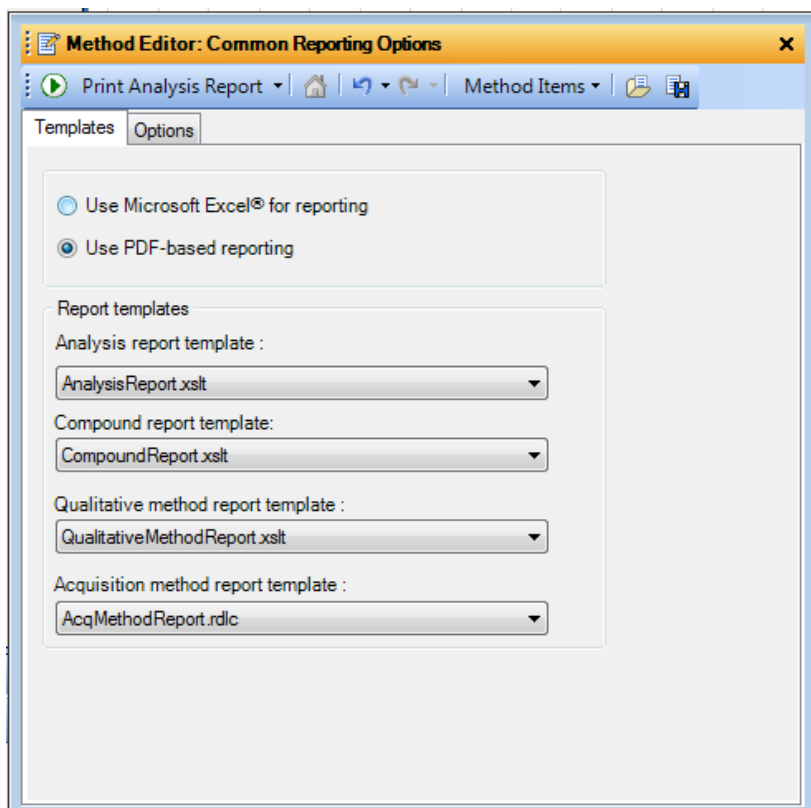
**Tip: Review Common Reporting Options first.**



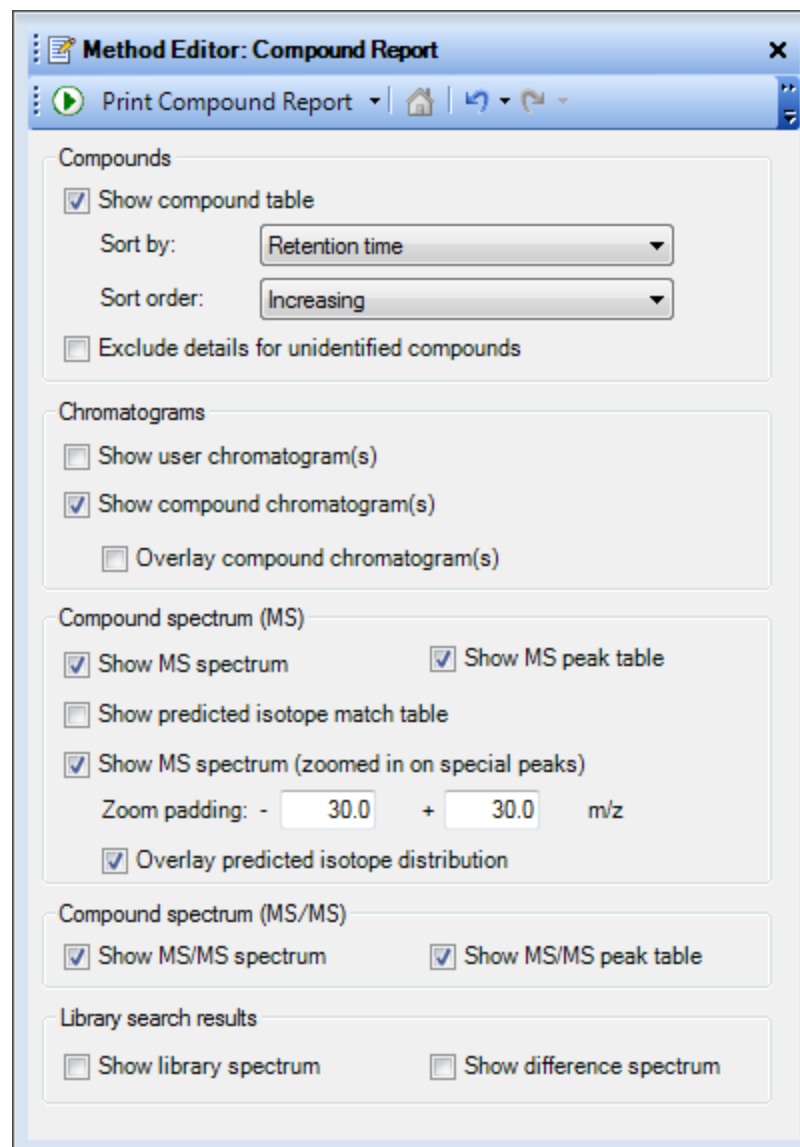
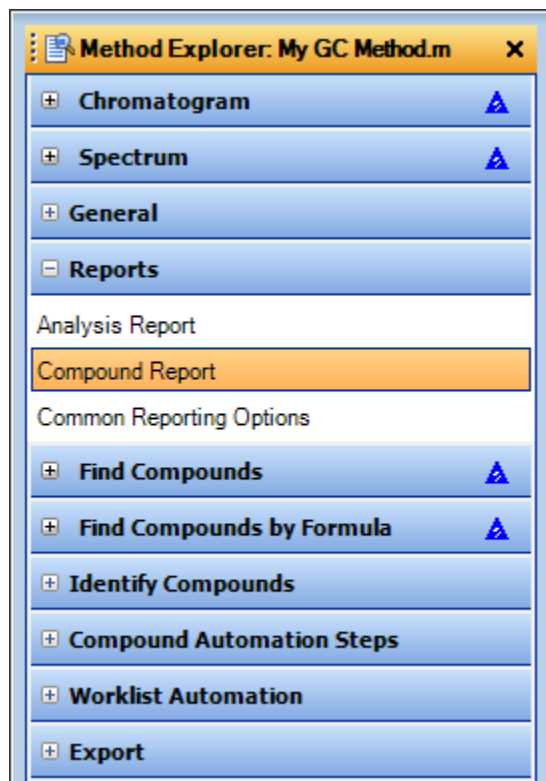
# Common Reporting Options

New to Qualitative Analysis  
B.07.00 SP1

PDF Based Reporting  
Uses xslt files



# Compound Report Options





# Tools

The screenshot displays the Agilent MassHunter Qualitative Analysis 8.04.00 interface. The top menu bar includes File, Edit, View, Find, Identify, Chromatograms, Spectra, Method, Wizards, Actions, Configuration, Tools, and Help. The toolbar below the menu contains various icons for file operations, analysis, and navigation. The main window is divided into several panes: Data Navigator on the left, Chromatogram Results in the center, and Method Explorer on the bottom left. The Data Navigator pane shows a list of data files with checkboxes for selection. The Chromatogram Results pane displays a chromatogram with peaks labeled with retention times (e.g., 5.8, 5.9, 6.0, 6.2, 6.4). The Method Explorer pane shows a tree view of the analysis method, with 'Extract (MS)' selected. The bottom right pane shows a mass spectrum plot with peaks labeled with m/z values (e.g., 69.1, 105.9). Various annotations are present: a red box highlights the 'Open' icon in the toolbar; a red arrow points to the 'Open data files' icon; a blue arrow points to the 'Save results to data files' icon; a green arrow points to the 'Choose defined color' icon; a green arrow points to the 'Show all items (relates to Data Navigator)' icon; a red arrow points to the 'Close data file' icon; a blue arrow points to the 'Print Qualitative Analysis Report' icon; a red arrow points to the 'Linked Navigation' icon; and two white diagonal banners point to the 'Show all highlighted items' and 'Show only highlighted items' icons.

Agilent MassHunter Qualitative Analysis 8.04.00 - mh\_test.m

File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help

Data Navigator x Chromatogram Results x

Sort by Data File

Open data files

Save results to data files

Choose defined color

Show all items (relates to Data Navigator)

Close data file

Print Qualitative Analysis Report

Show all highlighted items

Show only highlighted items

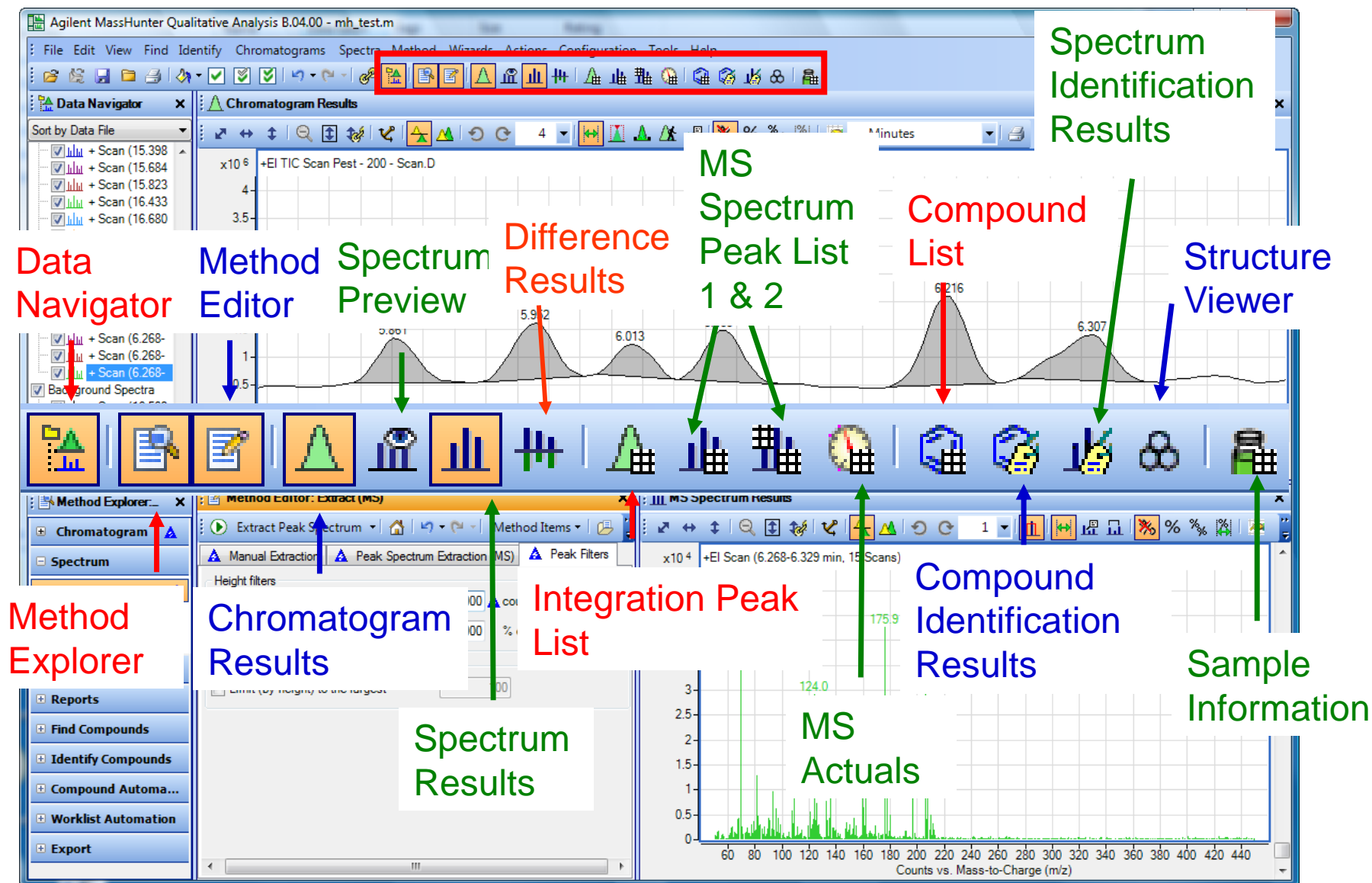
Linked Navigation

MassHunter Webinar Series

Agilent Technologies

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# Tools



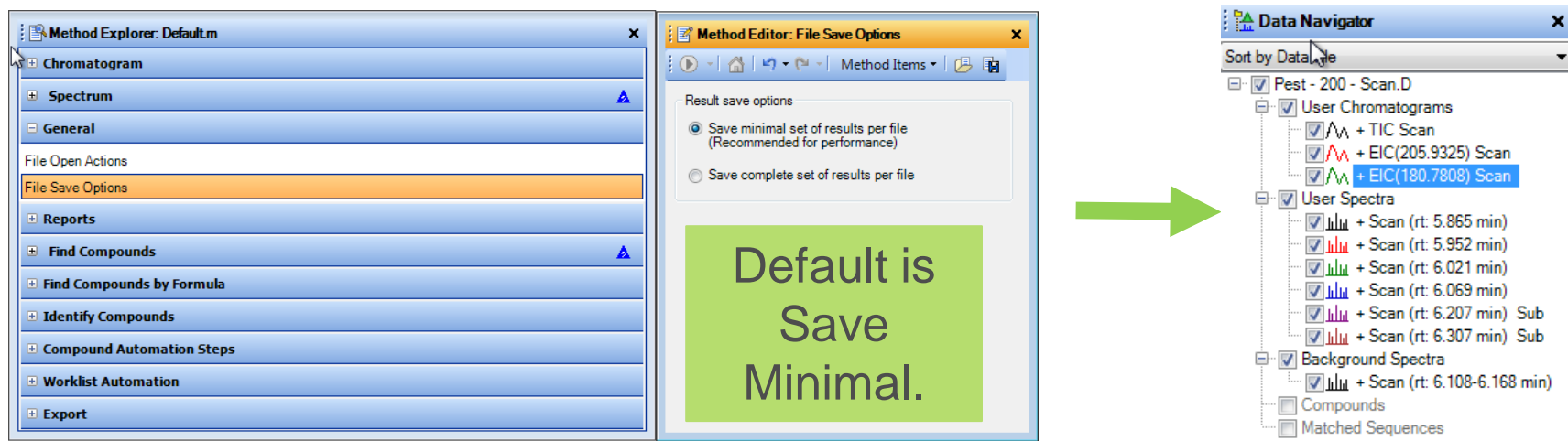
# Saving Data Files and Opening Data Files

The screenshot displays the Agilent MassHunter Qualitative Analysis B.04.00 - mh\_test.m interface. The top menu bar includes File, Edit, View, Find, Identify, Chromatograms, Spectra, Method, Wizards, Actions, Configuration, Tools, and Help. A red box highlights the File menu, which contains icons for opening, saving, and printing files. The Data Navigator on the left shows a list of data files, with a red arrow pointing to the 'Open data files' icon. The Chromatogram Results window shows a chromatogram with peaks labeled at retention times 5.952, 6.069, and 6.216 minutes. A blue arrow points to the 'Save results to data files' icon. The Method Editor: Extract (MS) window shows settings for peak extraction, including height filters (Absolute height >= 20000 counts, Relative height >= 5.000 % of largest peak) and maximum number of peaks (100). The MS Spectrum Results window shows a mass spectrum with peaks labeled at m/z values 69.1, 124.0, 175.9, and 205.9.

Open data files

Save results to data files

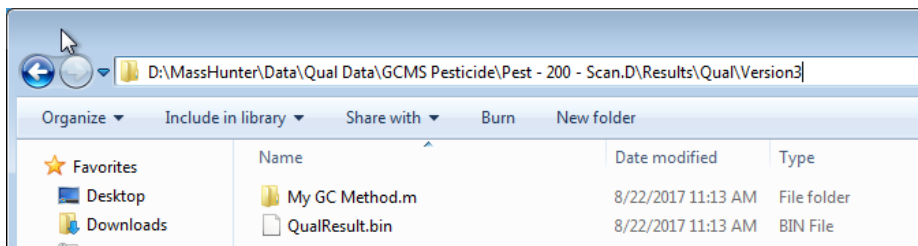
# File Save Options -The Results File



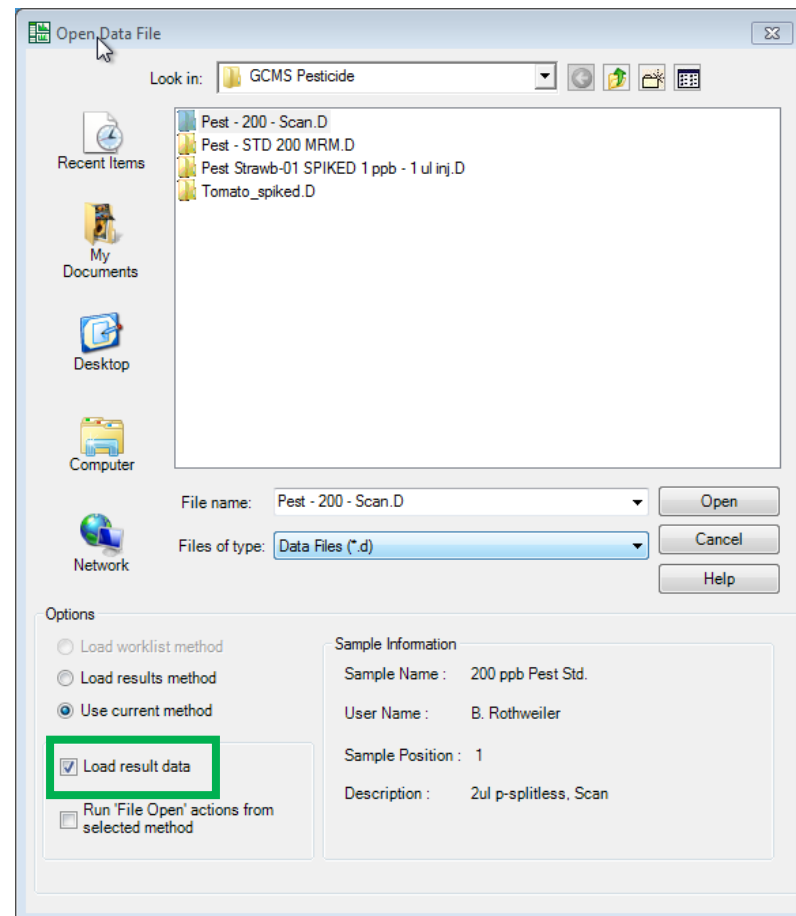
- Save minimal – only the compound level nodes are saved. No spectra or compound chromatograms are saved. If you open again, you will need to re-extract the complete result set.
- Save complete – all compound results are saved including spectra and chromatograms.
- In this example, all the User Chromatograms, User Spectra and Background Spectra are saved.



# The Results File



- The results file is saved within the data file as QualResult.bin.
- A copy of the qualitative analysis method is also saved.
- Only one results file and method can be saved.
- The results file is easily loaded with the data file.
- Complete data archival.





Let's take a few  
moments for questions  
on Reporting.

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Up Next:  
Training Resources.

# Training Resources

Training resources that are available.

## Convenient Training

Our team of industry experts delivers a quality learning experience with a high degree of flexibility to fit the needs of your lab – in our classrooms, at your site or online:

- **Classroom Training** – Introductory level to in-depth, hands-on training for lab hardware or software.
- **Customized On-Site Training** – Effective learning environment designed to achieve operational excellence and employee development without the need to travel.
- **Online** – From foundation to expert offerings when and where you need it at your own pace



# Introducing Agilent University

## Upgraded customer experience:

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

## Introduce new eLearning capabilities:

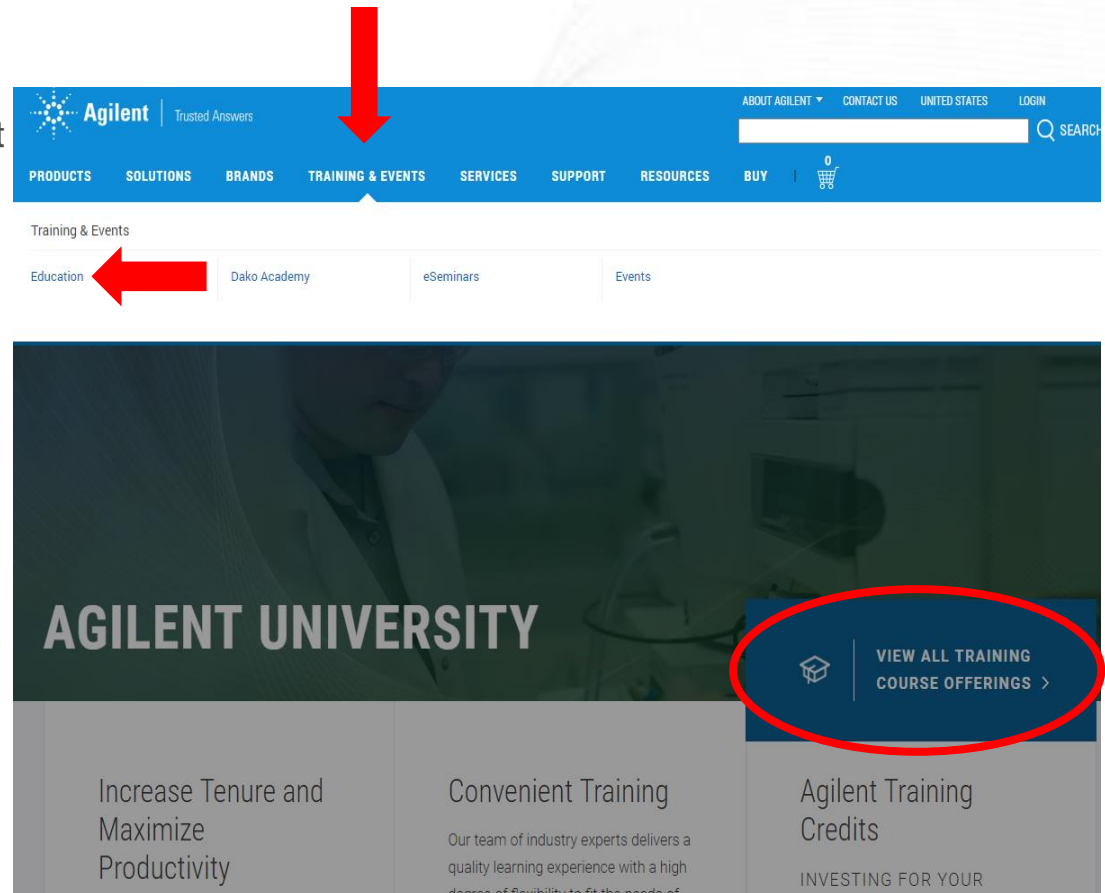
- Recorded and video-based learning
- Virtual online classes

## 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





# Questions on today's material...

## Thank you for your attention.



### MassHunter Qualitative Analysis

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