# Agilent MassHunter Workstation Software

## Qualitative Analysis

## Familiarization Guide for GC/MS

Agilent Technologies

# Notices



## Manual Part Number

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## Software Revision

This guide is valid for B.07.00 and later revisions of the Agilent MassHunter Workstation Software - Qualitative Analysis program, until superseded.

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# In This Guide...

This guide contains information to learn to use your Agilent MassHunter Workstation Software - Qualitative Analysis with GC/MS data.

Before you begin the exercises, please read the instructions in "Before you begin these exercises..." on page 5.

### Exercise 1 Learn basics of qualitative analysis

In this exercise, you explore some of the many powerful capabilities of the Qualitative Analysis program. These tasks are important no matter what data type you are using.

### Exercise 2 Find and identify

In these tasks, you find and identify compounds in GC/MS data files.

### Exercise 3 Use workflows, export and print

In these tasks, you learn to set up and run a qualitative analysis method. Then, you run the actions within the automated method when you open a data file. Each of these tasks is done using a different workflow.

### Reference

In this chapter, you learn some basics about the Qualitative Analysis program.

# What's New

### in B.07.00

- The Agile 2 integrator is supported.
- Spectral library supports multiple ion species per compound. Species information from PCDL is used in the Find by Formula with Fragment Confirmation algorithm, the Find Compounds by MFE, the Find by Auto MS/MS algorithm, and the Find by Targeted MS/MS algorithm.
- MFG fragment annotation on EI data is improved.
- Fragment confirmation supports GC/Q-TOF EI data.
- The Find Compounds by Molecular Feature algorithm now supports All Ions MS/MS data.
- Cleaned HighE spectrum that contains qualified ions gets created with Fragment Confirmation algorithm.
- In Fragment Confirmation in Find by Formula, the options for the Fragment Ion Source are now either the spectral library only or the average fragment spectrum else the spectral library.
- Fragment Confirmation is possible without molecular ion being present.
- The **Score (Frag)** column is available in the Compound Table.
- The **Source** column is available in the Compound Table.
- The Library Search user interface has been greatly simplified and can be customized for LC- or GC- specific workflows.
- Chained library searching is supported for both unit mass and accurate mass libraries.
- You can search accurate mass data against both unit mass and accurate mass libraries.
- The library search algorithm has additional rules for calculating the reverse score (to avoid one hit wonders).
- You can open an IM-MS Browser data file.

- You can import spectra and chromatograms from IM-MS Browser.
- You can send an MS/MS spectrum or a fragment spectrum (GC EI) from Qualitative Analysis to a spectral library easily.
- Chromatograms from the following devices can now be displayed: Compact LC 1220 DAD, High Dynamic Range DAD, Compact LC VWD, and Compact LC 1220 VWD.
- You can automatically launch MassHunter Quantitative Analysis and create a Quant method from Qualitative Analysis.

### in B.06.00 Service Pack 1

- Excel 2013 and Excel 2010 are supported.
- The library PestMix_AIM_PCDL_SP1.cdb is included.
- A new All Ions MS/MS data file (AIM_3CE(0-20-40).d) is included. A new example method is also included.

## Before you begin these exercises...

- Install the software. See the Installation Guide for instructions.
- Copy the folder named **Data** from your installation disk in uncompressed format to any location on your hard disk.

  This folder contains all the data files needed for these exercises. You may need to first extract the data files from their .zip format.

**NOTE** Do not reuse the example data files already on your system unless you know that you copied them from the originals on the disk and you are the only one using them. If the example data files already on the system do not match the original ones on the disk exactly, then the results obtained during these exercises will not match those shown in the guide.

# Contents

# Exercise 1
# Learn basics of qualitative analysis



In this exercise, you explore some of the many powerful capabilities of the Qualitative Analysis program for working with GC/Q-TOF and GC/QQQ data.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

# Task 1. Open the Qualitative Analysis program

In this task you open multiple data files using the current method.

Task 1. Open the Qualitative Analysis program with multiple data files

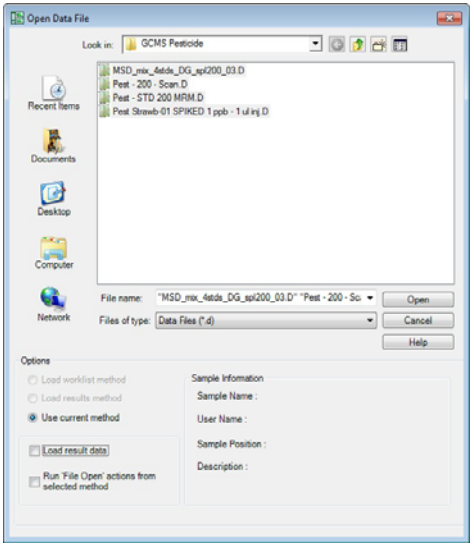
| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the Qualitative Analysis program.<br>• Open the data files, **Pest - 200 - Scan.d**, **Pest - STD 200 MRM.d, Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.d** and **MSD_mix_4stds_DG_spl200_03.d** in the folder **\\MassHunter\Data**, or in the folder where you copied them. | **a** Double-click the **Agilent MassHunter Qualitative Analysis B.07.00** icon . The system displays the Open Data Files dialog box.<br>**b** Go to the folder **\\MassHunter\Data\GCMS Pesticide** or the folder where the example files are located. | • The Pest - 200 - Scan.d file contains MS data, and the Pest - STD 200 MRM.d and Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.d files contain both MS and MS/MS data (all GC/QQQ). MSD_mix_4stds_DG_spl200_03.d contains GC/Q-TOF data.<br>• You can get help for most windows, dialog boxes, and tabs by pressing the F1 key when that window is active.<br>• Click **File > Open Data File** if the files are in different folders. |
| • Make sure that the **Use current method** button is clicked.<br>• Make sure that the **Load result data** check box is clear or grayed out. If the **Load result data** check box is not available, then no results have been saved in the data file. You learn how to save results in "Task 18. Save results" on page 72.<br>• Make sure that the **Run 'File Open' actions from selected method** check box is clear. |  | |

**Figure 1** Open data files when opening software

Task 1. Open the Qualitative Analysis program with multiple data files (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| | **c** Press and hold the **Shift** key while you click **Pest - 200 - Scan.d**, **Pest - STD 200 MRM.d, Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.d** and **MSD_mix_4stds_DB_spl200_03.d**.<br>**d** Click **Open**.<br>All four data files are displayed in the Data Navigator window, and 1 to 3 chromatograms are displayed in the Chromatogram Results window.<br>**e** Click the **List Mode** icon in the Chromatogram Results toolbar. | • If you press the **Ctrl** key, you can pick files which are not directly next to each other in the list.<br>• What you see in the main window at this point depends on the method, layout, display and plot settings used before you opened these files.<br>• When you click the List Mode icon, the background of the icon changes to orange. |

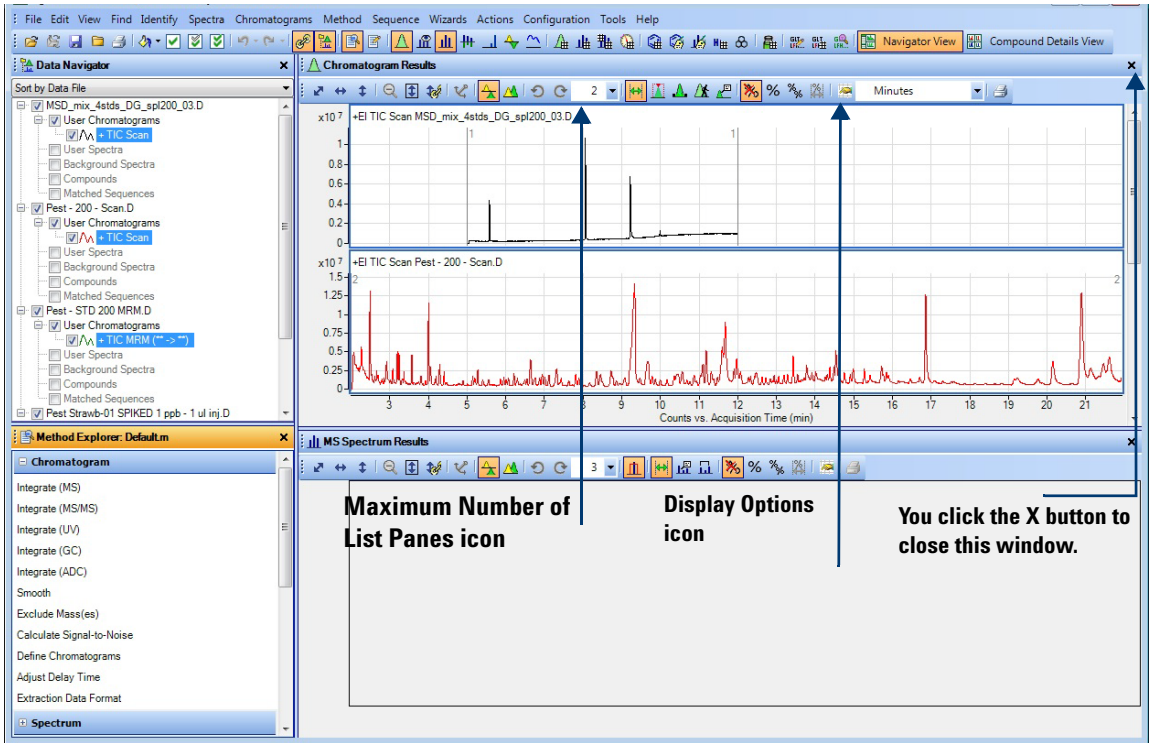



**Figure 2** Qualitative Analysis main window with the General Workflow loaded

# Task 2. Configure User Interface for GC/MS data

In this task, you switch to either the General workflow (for GC/QQQ customers) or the GC/Q-TOF Compound Screening workflow (for GC/Q-TOF customers). These two workflows are the only workflows that support analyzing GC/MS data. Then, you open the **User Interface Configuration** dialog box and mark the appropriate check boxes for a GC/QQQ system or a GC/Q-TOF system.

Task 2. Configure User Interface for GC

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** If necessary, open the Qualitative Analysis program. | **a** Double-click the **Agilent MassHunter Qualitative Analysis** icon . The system displays the Open Data Files dialog box.<br>**b** Click **Cancel** in the Open Data Files dialog box. | • You can get help for any window, dialog box, or tab by pressing the `F1` key when that window is active. |
| **2** Switch to either the General Workflow or the GC/Q-TOF Compound Screening Workflow. | **a** If you have a GC/QQQ instrument, click the **Configuration > Configure for Workflow > General** command. If you have a GC/Q-TOF instrument, click the **Configuration > Configure for Workflow > GC/Q-TOF Compound Screening** command.<br>**b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button.<br>**c** Click **OK**.<br>**d** Click the **List Mode** icon in the Chromatogram Results toolbar. | • If the Data Acquisition program for GC/QQQ or GC/Q-TOF is installed on the same computer, the software configures the User Interface automatically. The GC/Q-TOF Compound Screening section may already be available in the Method Explorer window.<br>• By default, chromatograms are overlaid. For these examples, the chromatograms are shown in **List Mode**. |

Task 2. Configure User Interface for GC

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** If you have a GC/QQQ, configure the user interface to show GC/QQQ features only. | **a** Click **Configuration > User Interface Configuration**.<br>**b** Under Separation types, only mark the **GC** check box.<br>**c** If you have a GC/QQQ instrument, then under Ionization type, mark the **EI or other "hard" ionization technique** check box, and clear the **CI, APCI, ESI, MADLDI or other "soft" ionization technique** check box.<br>**d** Under Mass accuracy, clear the **Accurate mass (TOF, Q-TOF)** check box. Mark the **Unit mass (Q, QQQ)** check box.<br>**e** Under Optional software features, clear the **Peptide Sequence Editor** check box and the **BioConfirm Software** check box.<br>**f** Under Non-MS detectors, clear the **UV** and **ADC** check boxes.<br>**g** Mark the **Show advanced parameters** check box.<br>**h** Click **OK**. | • You change which commands are available in the **User Interface Configuration** dialog box.<br>• If a feature is not visible, it probably was hidden when a check box was cleared in the User Interface Configuration dialog box. |

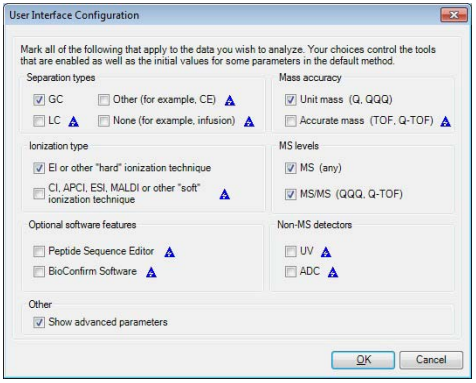



**Figure 3** Configuring the user interface to use with GC/QQQ data

Task 2. Configure User Interface for GC

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **4** If you have a GC/Q-TOF instrument, configure the user interface to show GC/Q-TOF features only. | **a** Click **Configuration > User Interface Configuration**.<br>**b** Under Separation types, only mark the **GC** check box.<br>**c** Under Ionization type, mark both check boxes.<br>**d** Under MS levels, mark both check boxes.<br>**e** Under Mass accuracy, mark the **Accurate mass (TOF, Q-TOF)** check box. Clear the **Unit mass (Q, QQQ)** check box.<br>**f** Under Optional software features, clear the **Peptide Sequence Editor** check box and the **BioConfirm Software** check box.<br>**g** Under Non-MS detectors, clear the **UV** and **ADC** check boxes.<br>**h** Mark the **Show advanced parameters** check box.<br>**i** Click **OK**. | • You change which commands are available in the **User Interface Configuration** dialog box.<br>• If a feature is not visible, it probably was hidden when a check box was cleared in the User Interface Configuration dialog box. |

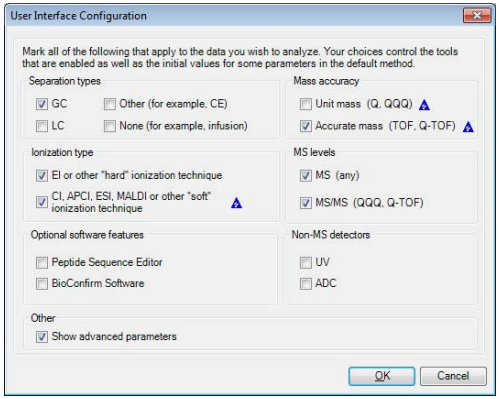



**Figure 4** Configuring the user interface for a GC/Q-TOF

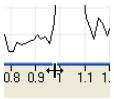
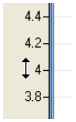
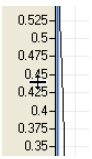
# Task 3. Zoom in and out of the chromatogram

In this task, you become familiar with the zoom in and zoom out features of the Qualitative Analysis program.

Task 3. Zoom in and out of the chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Practice zooming in and out of only one of the three chromatograms (both x and y axes).<br>• Hide the others.<br>• Zoom in twice on last peak.<br>• Zoom in one more time autoscaling the y-axis.<br>• Zoom out once to the previous zoom position.<br>• Completely zoom out to the original chromatogram. | **a** Clear the check boxes in the Data Navigator window for the chromatograms you want to hide.<br>**b** Click the right mouse button and drag over an area on the last peak.<br>Make sure that the **Autoscale Y-axis during Zoom** icon,  , is not selected for this step.<br>**c** Repeat step b.<br>**d** Click the **Autoscale Y-axis during Zoom** icon,  , in the toolbar.<br>**e** Click the right mouse button again and drag over an area of the last peak for the third time.<br>The Quality Analysis program automatically scales the y-axis to the largest point in the range.<br>**f** Click the **Unzoom** icon  to undo the last zoom operation.<br>You can undo the last fifteen zoom operations.<br>**g** Click the **Autoscale X-axis and Y-axis** icon  to zoom out completely. | • If a line is not checked in the Data Navigator window, that information is not displayed in any other window in the Qualitative Analysis program. You simply mark the check box for that information in the Data Navigator window, and the information is displayed in the other windows again.<br>• You can also use these zoom features on spectra in the Spectrum Preview window, the MS Spectrum Results window and the Difference Results window.<br>• A selected icon has an orange background color. |

Task 3. Zoom in and out of the chromatogram (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Practice zooming in and out on each axis separately.<br>• Zoom in only along the x-axis. Hint: Right-click the x-axis values and move cursor from left to right.<br>• Partially zoom out the x-axis. Hint: Move cursor in opposite direction.<br>• Completely zoom out of the x-axis.<br>• Repeat the previous steps for the y-axis. | **a** To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears.<br>**b** Click the right mouse button and drag the new cursor from left to right across the x-axis values.<br>**c** To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values.<br>**d** Click the **Autoscale X-axis** icon to completely zoom out on the x-axis.<br>**a** To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears.<br>**b** Click the right mouse button and drag the new cursor from bottom to top across the y-axis values.<br>**c** To zoom out on the y-axis, click the right mouse button and drag from the top towards the bottom of the y-axis values.<br>**d** Click the **Autoscale Y-axis** icon to completely zoom out on the y-axis. |  <br>**Horizontal Double Arrow**<br>  <br>**New cursor appears when you right-click the x-axis values.**<br>  <br>**Vertical Double Arrow**<br>  <br>**New cursor appears when you right-click the y-axis values.** |

# Task 4. Anchor a chromatogram

In this task, you anchor a chromatogram. When you anchor a chromatogram, the anchored chromatogram remains permanently on display as you scroll through the other chromatograms to display them.

Task 4. Anchor a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|
| • Anchor a chromatogram.<br>  ◦ Show all chromatograms.<br>  ◦ Make sure the chromatogram viewing list is set to 1.<br>  ◦ In the Chromatogram Results window, select the second TIC.<br>  ◦ Anchor this TIC.<br>  ◦ Scroll through the chromatograms.<br>  ◦ Clear the anchor. | **a** In Data Navigator mark the check boxes for the chromatograms you hid in the previous task.<br>**b** Make sure the maximum number of panes is set to 1 in the Chromatogram Results window.<br>**c** In the Chromatogram Results window, select the second TIC.<br>**d** Right-click inside the chromatogram, and click **Set Anchor.**<br>**e** Use the scroll bar in the Chromatogram Results window to scroll through the list of chromatograms. The second TIC stays visible always as the first chromatogram.<br>**f** Click **Chromatograms > Clear Anchor.** | • When you set an anchor for a chromatogram, an anchor icon appears in the Data Navigator window next to the name of the anchored chromatogram.<br>• Two chromatograms appear in the Chromatogram Results window after you anchor one even though the viewing list says 1. This now means you view one chromatogram in addition to the anchored chromatogram.<br>• You can also right-click the chromatogram and click **Clear Anchor** in the shortcut menu. |

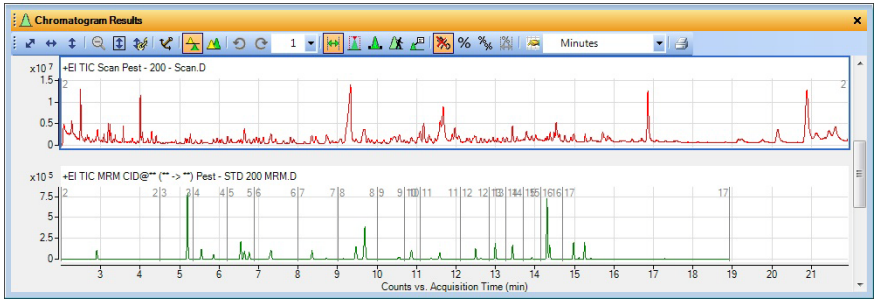



**Figure 5** Anchored TIC in the Chromatogram Results window

# Task 5. Change window layouts

In this task, you move windows within the main view and create various window layouts.

Task 5. Change window layout

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Change the window layout:<br>• Change the window size.<br>• Save a window layout.<br>• Unlock the layout.<br>• Change the Chromatogram Results window to be floating.<br>• Move the Chromatogram Results window.<br>• Display the tools for repositioning the windows. | • To change the size of a window, drag the boundary between the windows.<br>• To save a window layout, click **Configuration > Window Layouts > Save Layout**.<br>• To unlock a layout, click **Configuration > Window Layouts > Lock Layout**.<br>• To make a window float, right-click the title bar of the window, and click **Floating** from the shortcut menu.<br>• To move a window, click the title bar of the window and drag the window to the desired location.<br>• To display the repositioning tools, drag the window over one of the other windows. When one window is overlapped with another, the program displays several layout tools, as shown in Figure 6. | • If the layout is unlocked, the system does not display a check mark next to the Lock Layout menu.<br>• You can only use the repositioning tools when the layout is unlocked.<br>• You can also make a window float by double-clicking the title bar of the window.<br>• The software has many different layouts created. You can also try loading different layouts.<br>• The software has several different workflows. Each workflow loads a different layout. Switching to a different workflow also changes the layout. |

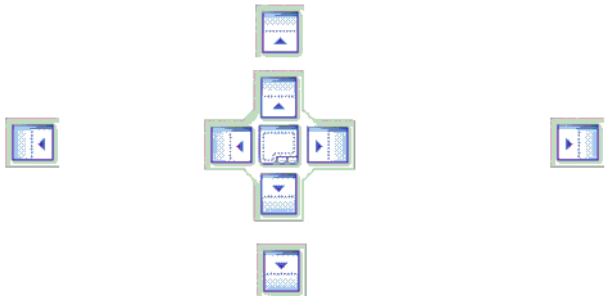


**Figure 6** Window repositioning tools

Task 5. Change window layout (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Reposition the Chromatogram Results window.<br>• Move the window so that it is at the top, to the left, to the right and then at the bottom of the other windows.<br>• Move two windows together so that they are on top of one another and available only through the tabs at the bottom.<br>• Restore the default layout. | • If you drag the cursor over one of the smaller icons, the window you are dragging will be placed above, to the right, below, or to the left of all of the other windows.<br>• Drag the cursor over the larger icon. The window can also be placed above, to the right, below, or to the left of the other window by dragging the cursor over the edges of the larger icon.<br>• To tab two windows together, drag the cursor over the center of the larger icon. You will see a shadow version of the two windows tabbed together. Stop dragging the mouse. The two windows will be tabbed together.<br>• Click **Configuration > Window Layouts > Restore Default Layout**. | • The cursor must be over one of the arrows in a box in order for repositioning to occur.<br>• Clicking the **Restore Default Layout** command restores the layout that is used with the General workflow and the GC/Q-TOF Compound Screening workflow. If you are using a different workflow, you need to load the layout that is used with that workflow. |

# Task 6. Extract chromatograms

In this task, you extract and merge chromatograms from the original TIC.

Task 6. Extract chromatograms

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Extract and merge extracted ion chromatograms (EICs) from two masses in the **Pest - 200 - Scan.d** data file.<br>• The m/z values are 129.0 and 414.2.<br>• Do not merge the peaks from the individual masses into one chromatogram. | **a** In the Data Navigator window, clear the check boxes for the data files except for **Pest - 200 - Scan.d**.<br>**b** Open the Extract Chromatograms dialog box, using the option below or one of the options to the right:<br>• Click **Chromatograms > Extract Chromatograms**.<br>**c** In the **List of opened data files**, click **Pest - 200 - Scan.d.**<br>**d** In the **Type** list box, select **EIC**.<br>**e** In the **m/z value(s)** box, type `129.0, 414.2`<br>**f** If necessary, clear the **Merge multiple masses into one chromatogram** check box to merge the EICs.<br>**g** Click **OK**.<br>**h** Set the **Maximum number of list panes** to 3 in the Chromatogram Results toolbar. | • You can also extract chromatograms in one of the following ways:<br>• Right-click inside the chromatogram, and click **Extract Chromatograms**.<br>• From Data Navigator, highlight the **TIC Scan** for **Pest - 200 - Scan.d**; then, right-click **TIC Scan** and click **Extract Chromatograms**.<br>• You can use an MS level of either **All** or **MS**.<br>• Note that you can also choose to have the extracted chromatogram automatically integrated after extraction.<br>• You can also extract a chromatogram from a mass spectrum. |

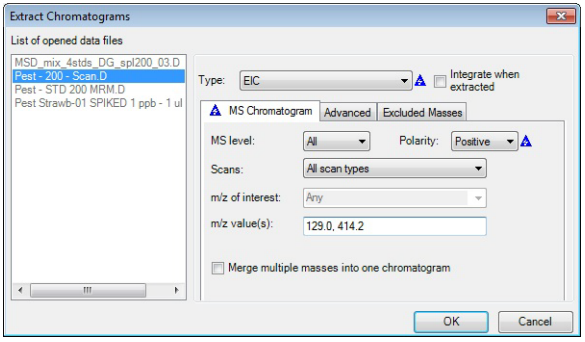



**Figure 7** The Extract Chromatograms dialog box

Task 6. Extract chromatograms (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|

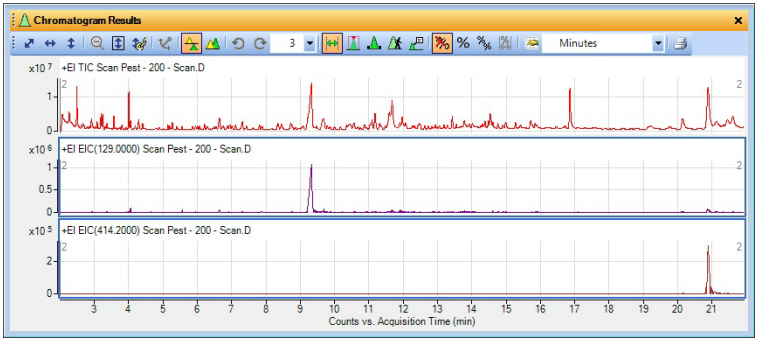



**Figure 8** Merged extracted ion chromatograms (EICs) compared to the original TIC

# Task 7. Interactively integrate a GC/MS chromatogram

In this task, you learn different ways to integrate a chromatogram, change integration parameters to modify the results and calculate the Signal-to-Noise for the integrated peaks for MS/MS data.

Task 7. Interactively integrate a chromatogram (GC/MS)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Integrate the TIC Scan chromatogram for the **Pest - 200 - Scan.d** data file, using any of the options listed at right. | **a** Mark the Pest - 200 - Scan.D data file in the Data Navigator window.<br>**b** Highlight the TIC Scan chromatogram, and use one of the following commands:<br>• From the menu bar click **Chromatograms > Integrate Chromatogram**.<br>• Right-click anywhere in the chromatogram window, and click **Integrate Chromatogram**.<br>• In the Data Navigator window, select **Pest - 200 - Scan.D > User Chromatograms > TIC Scan;** then, right-click the **TIC Scan**, and click **Integrate Chromatogram**. | • Note that the program integrated practically all the peaks in the chromatogram.<br>• You select the integrator to use for MS data, MS/MS data, and GC data in the Method Editor window.<br>• This chromatogram is an MS chromatogram, so the values that are set in the Integrate (MS) section of the Method Editor are used when integrating this chromatogram. |
| **2** Display only two chromatograms at the same time. | • Select **2** in the **Maximum number of list panes** box in the Chromatogram Results Toolbar. | |
|  | | **Many small peaks are integrated.** |

**Figure 9** Integrated TIC Scan Chromatogram with many small peaks

Task 7. Interactively integrate a chromatogram (GC/MS) (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Change the threshold to integrate fewer peaks.<br>• Change the threshold to retain only the three largest peaks. | **a** From the Method Explorer window, click **Chromatogram > Integrate (MS)** to display the Integrate (MS) tab.<br>**b** Select the **Agile 2** integrator.<br>**c** Click the **Peak Filters** tab.<br>**d** Under Maximum number of peaks, mark **Limit (by height) to the largest,** and type 3. | • Note the blue triangle that appears when you change a setting from the value saved in the current method. When you save the method, the triangles disappear. |

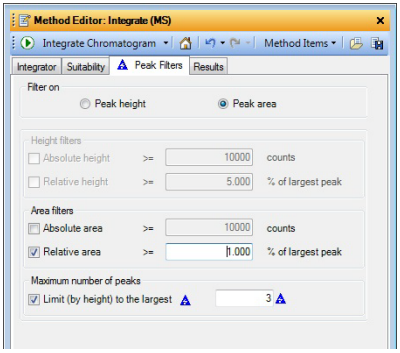



**Figure 10** Peak Filters tab with **Limit (by height) to the largest** marked

| | | |
|---|---|---|
| **4** Reintegrate the chromatogram | **e** Click the button on the Method Editor toolbar to integrate using the new setting. | • Note that only the three peaks with the highest height are now integrated. |

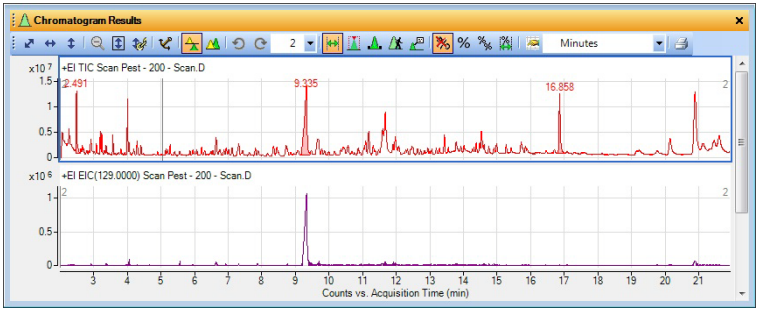



**Figure 11** Integrated TIC Scan chromatogram when limiting the number of peaks

Task 7. Interactively integrate a chromatogram (GC/MS) (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **5** Integrate the TIC MRM chromatogram for the **Pest - STD 200 MRM.D** data file. | **a** In the Data Navigator window, select the **TIC MRM** for the Pest - STD 200 MRM.d data file.<br>**b** Use one of the following commands to integrate the chromatograms.<br>• From the menu bar click **Chromatograms > Integrate Chromatogram**.<br>• Right-click anywhere in the chromatogram window, and click **Integrate Chromatogram**.<br>• In the Data Navigator window, right-click the highlighted chromatogram and click **Integrate Chromatogram**.<br>**c** Zoom in from 5.8 to 8.5 minutes.<br>**d** Set the **Maximum number of list panes** to 2. | • Press the **Ctrl** key to highlight more than one chromatogram in the Data Navigator window.<br>• Note that the program integrated practically all the peaks in the chromatogram.<br>• These chromatograms are MS/MS chromatograms, so the values that are set in the Integrate (MS/MS) section of the Method Editor window are used when integrating this chromatogram. You can select one integrator to use to integrate MS chromatograms and a different integrator to use to integrate MS/MS chromatograms. |

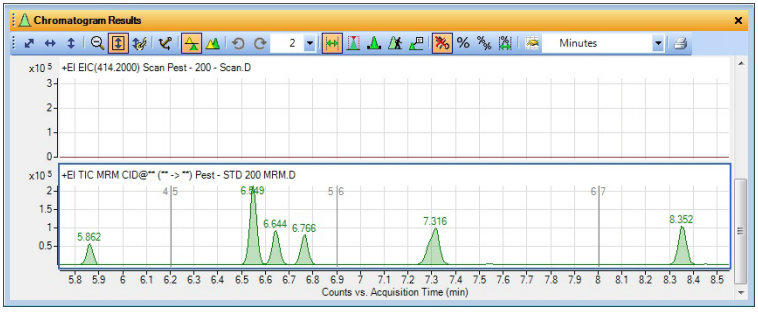
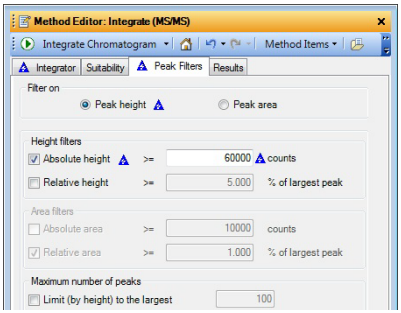
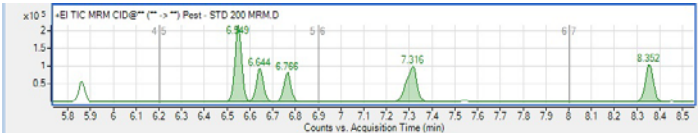



**Figure 12** Integrated MRM chromatograms

| | | |
|---|---|---|
| **6** Select the **MS/MS (GC)** integrator. Change the filter to only accept peaks with an absolute height greater or equal to 20,000. | **a** From the Method Explorer window, select **Chromatogram > Integrate (MS/MS)**.<br>**b** Select **MS/MS (GC)** as the **Integrator**.<br>**c** Click the **Peak Filters** tab.<br>**d** Under **Filter on**, click **Peak height**.<br>**e** Under Height filters, mark the **Absolute height** check box.<br>**f** Type `60000` as the **Absolute height**. | • Note the blue triangle that appears when you change a setting from the value saved in the current method. When you save the method, the triangles disappear. |

Task 7. Interactively integrate a chromatogram (GC/MS) (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| |  | |
| | **Figure 13** Peak Filters tab with **Absolute height** marked | |
| **7** Reintegrate the chromatogram | **g** Click the button on the Method Editor toolbar. | • Note that only the largest peaks are now integrated. |
| |  | The smaller peak at 5.8 minutes is not included in the integration results any longer because the absolute height for this peak is |
| | **Figure 14** Integrated TIC and EIC MS/MS chromatograms with higher threshold setting | |
| **8** Restore the settings that are saved for the current method and close Method Editor. | **a** Select the Chromatogram > Integrate (MS/MS) section in the Method Explorer.<br>**b** Click the icon in the Method Editor.<br>**c** Select the Chromatogram > Integrate (MS) section in the Method Explorer.<br>**d** Click the . icon in the Method Editor.<br>**e** Close the **Method Editor** window. | • To cancel your changes and restore the values from the method that is loaded, click the **Restore to last saved values from file** icon on the Method Editor toolbar. |
| **9** Delete all chromatograms except the original. Delete the integration results from the original chromatogram. | **a** Under User Chromatograms in the Data Navigator window, highlight all the chromatograms except the original.<br>**b** Right-click the highlighted chromatograms, and click **Delete**.<br>**c** Select all of the TIC chromatograms.<br>**d** Click **Chromatograms >Clear Results**. | • When you use the **Clear Results** command, the chromatograms are not deleted; the results that are connected to the chromatograms are removed. In this case, the integration values are cleared. |

# Task 8. Calculate System Suitability values

In this task, you learn different ways to interactively integrate a chromatogram, change integration parameters to modify the results and view the signal-to-noise ratio for each peak. You also learn how to enable System Suitability calculations.

Task 8. Interactively integrate a chromatogram (MS)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Integrate the **MSD_mix_4stds_DB_spl200_03.d** and **Pest - 200 - Scan.d** chromatogram and using any of the options listed at right. | **a** Mark the check box next to the MSD_mix_4stds_DB_spl200_03.d data file in the Data Navigator window.<br>**b** Mark the check box next to the Pest - 200 - Scan.d data file in the Data Navigator window.<br>**c** Highlight both TICs.<br>**d** Integrate the **TIC Scan** for these two files, using any of the following options.<br>• From the main menu, click **Chromatograms > Integrate Chromatogram**.<br>• Highlight the chromatogram. Then, right-click the chromatogram, and click **Integrate Chromatogram**.<br>• In Data Navigator, highlight the **TIC Scan** for both data files. Then, right-click either chromatogram and click **Integrate Chromatogram**. | • For the General workflow and the GC/Q-TOF workflow, the integration uses the **Agile 2** Integrator because that is the integrator selected in the default method for that workflow.<br>• You can change this value in the Chromatogram > Integrate (MS) > Integrator tab.<br>• Note that the integration with default parameters is detecting very small peaks. |

Task 8. Interactively integrate a chromatogram (MS) (continued)

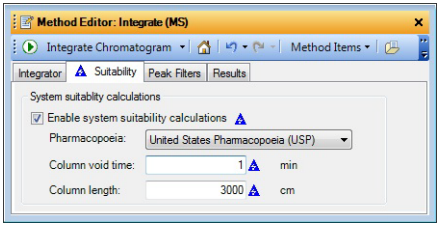
| Steps | Detailed Instructions | Comments |
|---|---|---|


**Figure 15** One of the shortcut menus in the Data Navigator and the integrated chromatograms

**2** Enable system suitability calculations for the MS chromatograms.

**a** From Method Explorer, select **Chromatogram > Integrate (MS)** to display the Integrator tab.
**b** Click the **Suitability** tab.
**c** Mark **Enable system suitability calculations**.
**d** Select the **United States Pharmacopoeia (USP)**.
**e** In the **Column void time** box, type `1`.
**f** In the **Column length** box, type `3000`.

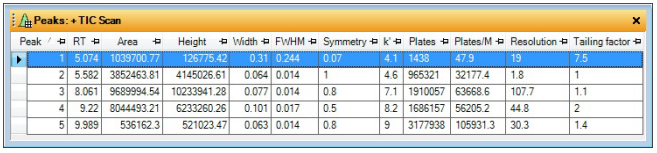
- Note the blue triangle that appears when you change a setting from the value that is saved in the current method. When you save the method, the triangles disappear.
- The algorithms that are used to set several of the columns in the Integration Peak List change, depending on the selected pharmacopoeia. See the online Help for more information.




**The actual column void time and column length for these data files is different than these values. These are only used for this example.**

**Figure 16** Chromatogram > Integrate (MS) Suitability tab

Task 8. Interactively integrate a chromatogram (MS) (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Reintegrate the chromatogram. | • Click the **Integrate Chromatogram** icon on the Method Editor toolbar to integrate using the new setting. | |
| **4** View the system suitability calculations.<br>• Open the Integration Peak List window.<br>• Review the values for the noise region, and calculate the signal-to-noise ratio for the integrated peaks. | **a** Click **View > Integration Peak List**.<br>**b** Right-click the header of the Peaks window and click **Floating**.<br>**c** Right-click the column header of any column that you do not want to see and click **Remove Column**.<br>**d** Right-click any column header and click **Add/Remove Columns** to change the columns that are visible. | • The system suitability calculations are included in the Integration Peak List table.<br>• These values include k', Tailing factor, Plates, Plates/M, and Symmetry.<br>• You can also enable system suitability calculations for an MS, an MS/MS and a GC chromatogram. |
| |  | |
| **5** Restore the settings for the default method, and close the Method Editor window and the Integration Peak List window. | **a** To cancel your changes and restore the values from the default method, click the **Restore to last saved values from file** icon on the Method Editor toolbar.<br>**b** Close the **Method Editor** window.<br>**c** Right-click the title of the Integration Peak List window and click **Floating**.<br>**d** Click **View > Integration Peak List**. | • When you click the Floating command in the shortcut menu the second time, the Integration Peak List window is docked where it was originally. |

Peaks: + TIC Scan

| Peak | RT | Area | Height | Width | FWHM | Symmetry | k' | Plates | Plates/M | Resolution | Tailing factor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.074 | 1039700.77 | 126775.42 | 0.31 | 0.244 | 0.07 | 4.1 | 1438 | 47.9 | 19 | 7.5 |
| 2 | 5.582 | 3852463.81 | 4145026.61 | 0.064 | 0.014 | 1 | 4.6 | 965321 | 32177.4 | 1.8 | 1 |
| 3 | 8.061 | 9689994.54 | 10233941.28 | 0.077 | 0.014 | 0.8 | 7.1 | 1910057 | 63668.6 | 107.7 | 1.1 |
| 4 | 9.22 | 8044493.21 | 6233260.26 | 0.101 | 0.017 | 0.5 | 8.2 | 1686157 | 56205.2 | 44.8 | 2 |
| 5 | 9.989 | 536162.3 | 521023.47 | 0.063 | 0.014 | 0.8 | 9 | 3177938 | 105931.3 | 30.3 | 1.4 |

**Figure 17** Integrated Peaks table with system suitability values

# Task 9. Extract spectra from a chromatogram

In this task, you extract a spectrum from exactly where you specify in the chromatogram. The Qualitative Analysis program extracts a spectrum from a specific data point or extract an average spectrum from an average of multiple data points or ranges.

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Walk a chromatogram to view the precursor ion and product ion for the last few peaks of **Pest - STD 200 MRM.d**.<br>• Zoom in on the region between 13 and 16 minutes.<br>• Use the Walk Chromatogram icon.<br>• Review the spectra starting at about 13 minutes, and move the arrow to the right. | **a** Mark the **Pest - STD 200 - MRM.D** line in the Data Navigator window.<br>**b** Close the Method Editor window.<br>**c** Close the MS Spectrum Results window.<br>**d** Click the **TIC MRM** chromatogram in the Data Navigator window.<br>**e** Click the **Autoscale Y-axis during Zoom** icon  in the Chromatogram Results toolbar.<br>**f** Select **1** for the Maximum number of list panes.<br>**g** To zoom in on a few peaks, right-click the mouse above the peak at 13 minutes and drag it to 16 minutes, and then release.<br>**h** Click the **Walk Chromatogram** icon  in the Chromatogram Results toolbar.<br>**i** Move the Walk Chromatogram cursor to above the X axis at about 13 minutes, and click.<br>**j** To navigate from spectrum to spectrum, use the right and left arrow keys on your keyboard. | • The Walk Chromatogram tool is particularly useful on MS/MS data for identifying precursor and product ions.<br>• The spectrum for each point you click in the Chromatogram Results window is automatically displayed in the Spectrum Preview window, which is opened automatically.<br>• Sometimes, two spectra are displayed in the Spectrum Preview window. For example, two spectra are shown in the Spectrum Preview window for each point you click near the peak at 13.431 minutes. |

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|

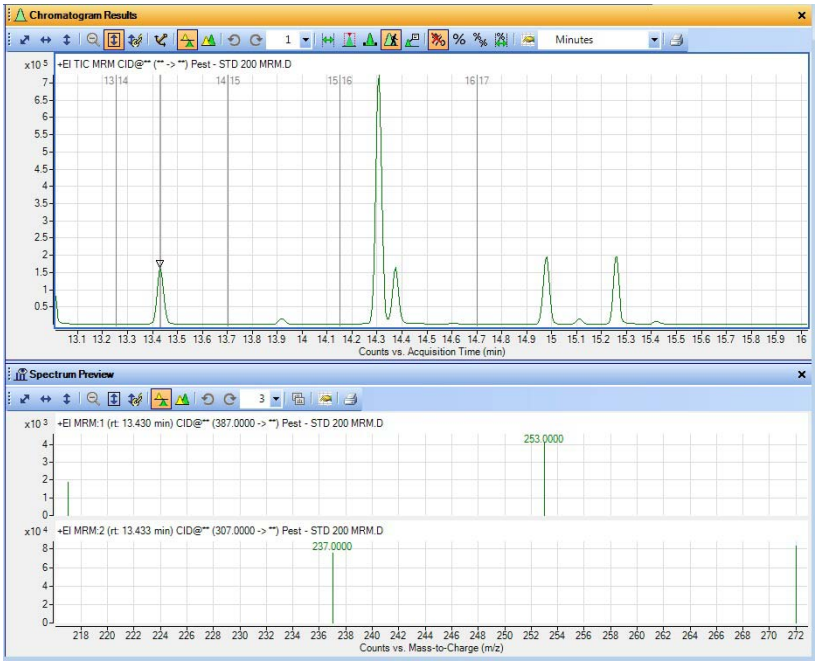



**Figure 18** Walk chromatogram to view the two MRM spectra for the peak at 13. 43 minutes

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Extract spectra on specific data points for the peak at 5.2 minutes and the peak at 14.3 minutes of the **Pest - STD 200 MRM.d** data file.<br>• Extract a spectrum from the peak at or near 5.2 min. and then one of the valleys, using any one of the options described under Comments.<br>• Extract a spectrum from the peak at or near 14.3 minutes. (not the valley yet)<br>• Change the display to show at least three spectra. | **a** Click the **Range Select** icon from the Chromatogram Results toolbar.<br>**b** Close the Spectrum Preview window.<br>**c** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.<br>**d** To zoom in to the peak at 5.2 minutes, right-click the mouse above the peak at 4.0 min. and drag it to 6.0 min., then release.<br>**e** On a peak near 5.2 min. extract a spectrum in any of the ways listed in the Comments column.<br>**f** On a valley near 5.1 min., extract the spectrum.<br>**g** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.<br>**h** Zoom into the region between 14 and 15 min.<br>**i** On a peak near 14.3 minutes, extract a spectrum in any of the ways listed in the Comments column. (Do not extract the valley spectrum yet.)<br>**j** If necessary, select 4 in the **Maximum number of list panes** icon in the MS Spectrum Results toolbar. | • When you zoom, make sure the AutoScale Y-axis during Zoom icon, is "on". The background of the icon is orange when it is on.<br>• You can extract a spectrum in any of the following ways:<br>  • Double-click the data point in the chromatogram.<br>  • Click the data point in the chromatogram, then right-click anywhere in the chromatogram. Click **Extract MS Spectrum**. The Extract Spectrum dialog box is displayed. Make sure the **Pest - STD 200 MRM.d** file is selected, and click **Extract** in the Extract Spectrum dialog box.<br>• Note that when you first extract a spectrum, the MS Spectrum Results window appears containing the spectrum, and the type of spectrum and retention time appear under User Spectra. All subsequent extracted spectra appear in both places as well.<br>• When you extract an MS spectrum from the peak near 14.3 minutes, two spectra are extracted because two transitions occur at that peak. |

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|

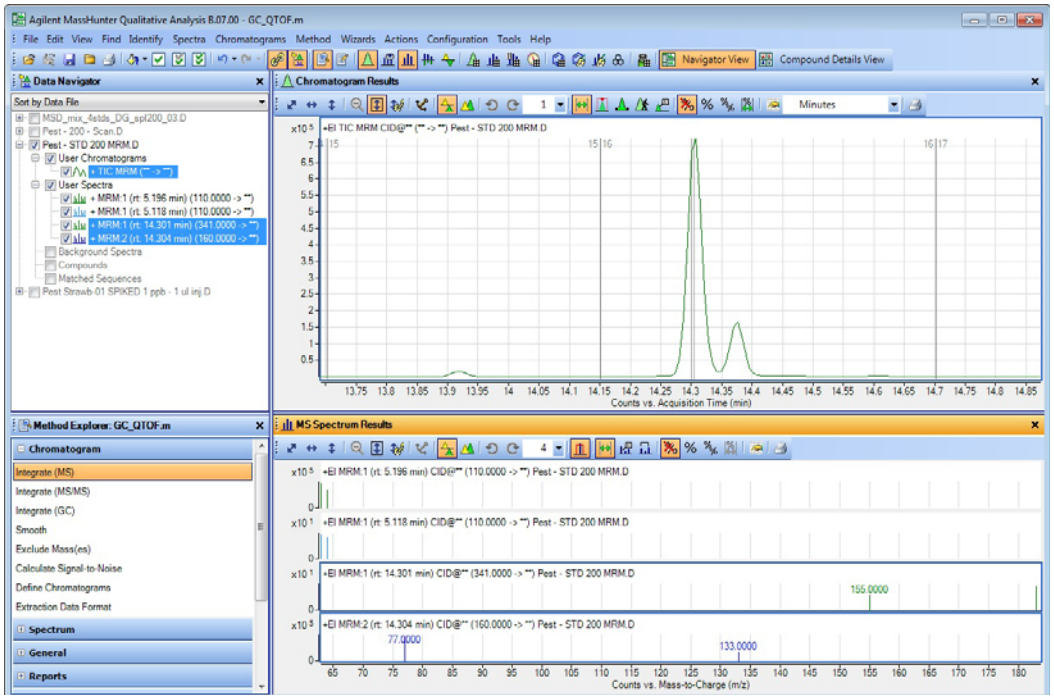



**Figure 19** Main window with two MRM spectra from the peak at 5.2 minutes and two MRM spectra from the peak at 14.3 minutes

**3** Extract an MS Spectrum for the valley at 14.35 minutes of the **Pest - STD 200 MRM.d** data file.

- Bring up Spectrum Preview.
- Extract a spectrum from the valley at RT 14.3 minutes.
- Copy this spectrum to the User Spectra folder.
- Change the display to show 6 spectra.
- Turn off Spectrum Preview.

**a** Click the **Spectrum Preview** icon, .

**b** On a valley near 14.3 minutes extract a spectrum.

**c** Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**. The spectra are copied to the User Spectra section in the Data Navigator and are shown in the MS Spectrum Results window.

**d** Click the down arrow next to the spectrum pane list, and select **6**.

**e** Close the Spectrum Preview window.

- When Spectrum Preview is enabled, the system displays any manually-selected spectrum in the Spectrum Preview window but not in the User Spectra section of Data Navigator.
- With Spectrum Preview on, Qualitative Analysis overwrites the previous spectrum when you extract a new spectrum.
- Spectrum Preview mode is useful when you quickly want to review the spectra in your chromatogram and save only a few of the spectra.

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|


**Figure 20** Chromatogram Results and MS Spectrum Results windows

**4** Extract a spectrum that averages all points within a specified range for the peak at 14.3 minutes for the **Pest - STD 200 MRM.d** data file:

- Zoom out.
- Use the Range Select icon on the Chromatogram toolbar.
- Set the range across the entire peak.
- Extract the spectrum, using any of the options listed.

**a** Click the **Range Select** icon on the Chromatogram toolbar.

**b** Click at the left side of the base of the peak at 14.3 minutes and drag to the base of that peak on the right.

**c** Extract the average spectrum using one of the options on the right.

**d** Select **2** in the Maximum number of list panes in the MS Spectrum Results window.

- You can extract an average spectrum by double-clicking the selected range in the chromatogram.
- Or, right-click anywhere in the chromatogram, and click **Extract MS Spectrum** from the shortcut menu.
- Note that two averaged MRM spectra appear.

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|

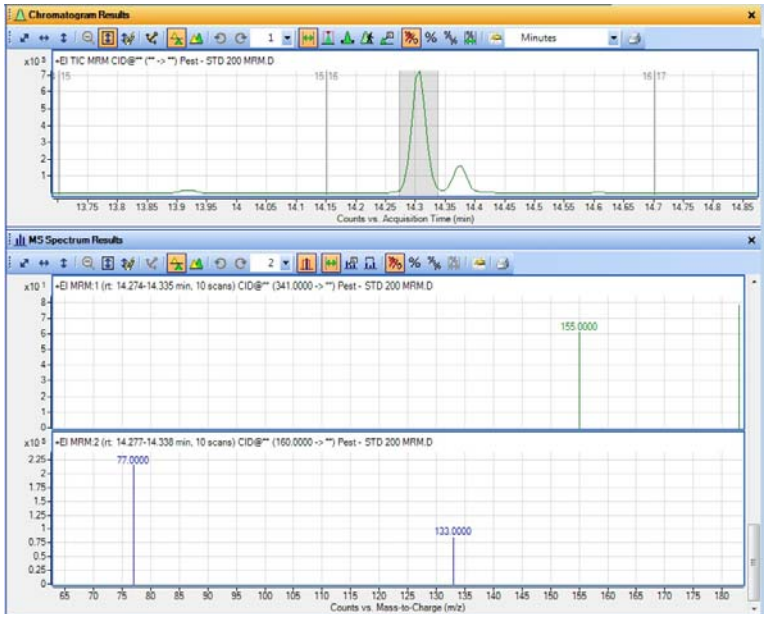



**Figure 21** Chromatogram Results and MS Spectrum Results showing two averaged spectra

**5** Extract spectra that average the ranges of peaks at 5.2 minutes and at 14.3 minutes together for the **Pest - STD 200 MRM.d** data file.

- Hint: Use the Range Select icon and the **Ctrl** key to select the Peak 1 range taken from the halfway point.
- Extract the spectra, using any of the options on the right.

**a** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.
**b** Press the **Ctrl** key.
**c** Click at the left side of the peak at 5.2 minutes and drag to the right of that peak, and release the mouse.
**d** Release the **Ctrl** key.
**e** Extract the averaged spectra using this option or the one on the right:

- Double-click inside the selected range in either peak.
- Remember that the second peak already has a range selected from step 4.
- To extract spectra, you can also right-click anywhere in the chromatogram and clicking **Extract MS Spectrum**. The Extract Spectrum dialog box is shown. Click **Extract**.

Task 9. Extract spectra from a chromatogram

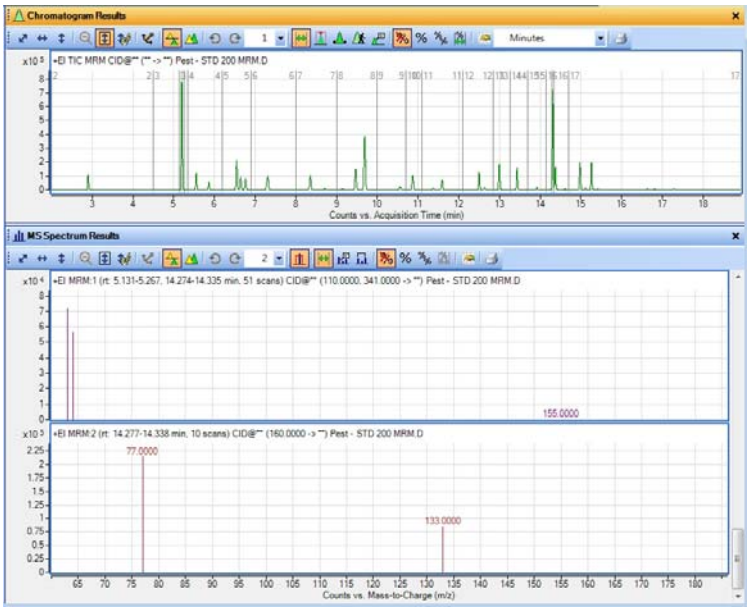
| Steps | Detailed Instructions | Comments |
|---|---|---|
|  | | **The first spectrum has transitions from both time ranges. The second spectrum only has one time range because the 160.00 -> ** transition is not present in the peak at 5.2 minutes.** |



**Figure 22** Two averaged spectra from two different ranges in the chromatogram

**6** Subtract a background spectrum every time you extract a peak spectrum from **Pest - STD 200 MRM.d**.

- Delete any scans under User Spectra in Data Navigator.
- Extract a background spectrum that is the average of a spectrum at the start of the peak and a spectrum at the end of the peak.
- Extract a peak spectrum from the integrated peaks.

**a** Click the User Spectra line in the Data Navigator. Right-click the User Spectra line, and click **Delete**.
**b** Click **Yes**.
**c** In Method Explorer, select **Spectrum > Extract (MS/MS)**.
**d** Click the **Peak Spectrum Extraction (MS/MS)** tab, if not visible.
**e** In the **Peak spectrum background MS/MS** box, select **Average of spectra at peak start and end**.
**f** In the Chromatogram Results toolbar, click the **Peak Select** icon, .
**g** Click the **Chromatograms > Integrate** command.
**h** Select the peak at 5.206 minutes.
**i** Right-click and click **Extract Peak Spectrum** from the shortcut menu.

- Note that at the end of this process, all extracted peak spectra will automatically have the designated background spectrum subtracted.

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|

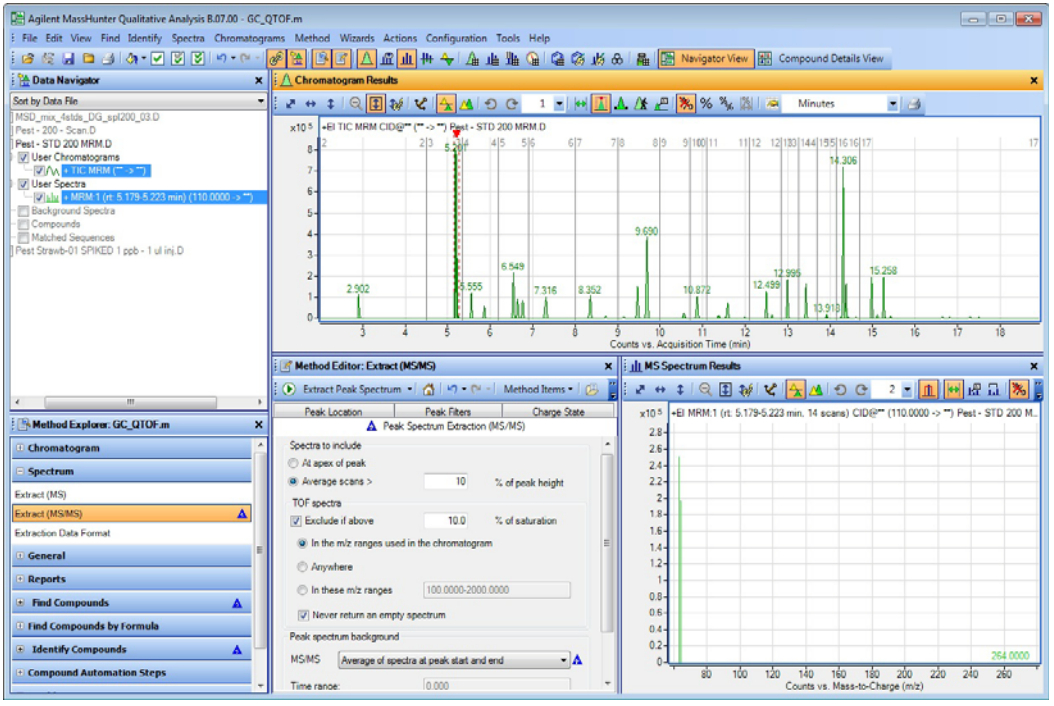



**Figure 23** Peak spectra with a background peak spectrum subtracted

Task 9. Extract spectra from a chromatogram

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **7** Integrate and extract peak spectra from the Pest - STD 200 MRM.d data file. | **a** Click the TIC MRM chromatogram in the Data Navigator window.<br>**b** Click **Chromatograms > Integrate and Extract Peak Spectra**. | • The peak spectra that you extracted manually in the previous step is deleted automatically because by default the **Clear previous peak spectra** check box is marked in the Chromatograms > Integrate (MS/MS) > Results tab. |

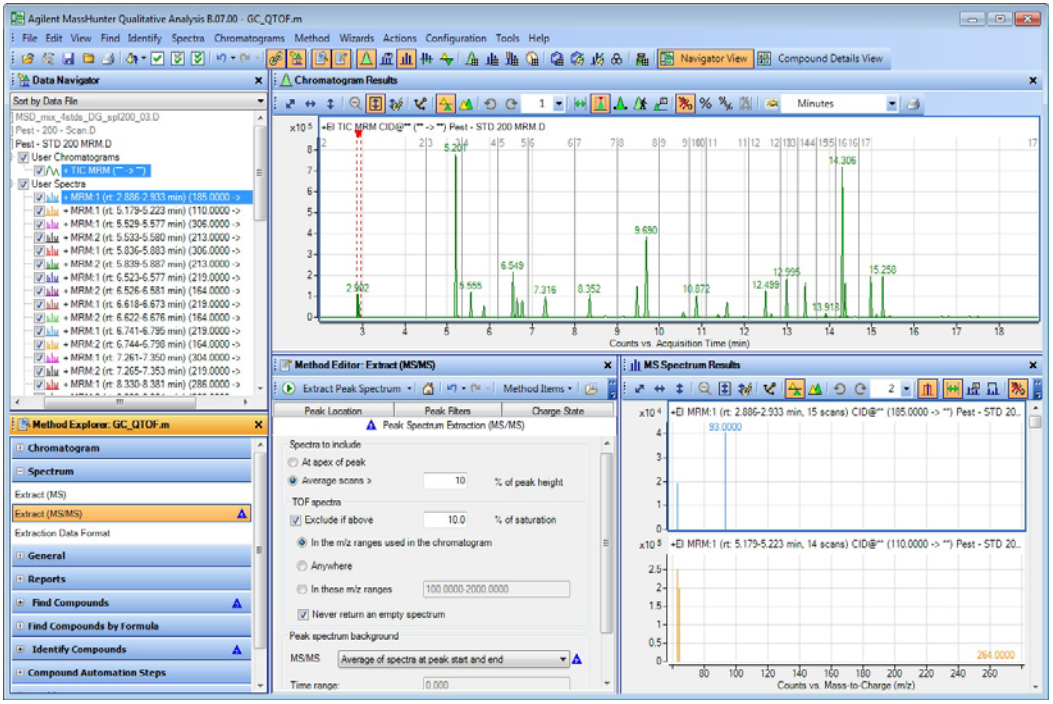



**Figure 24** Integrate and Extract Peak Spectra

| | | |
|---|---|---|
| **8** Remove the integration results and the peak spectra. | **a** Select the Pest - Std 200 MRM.d data file.<br>**b** Click **Chromatograms > Clear Results > Include Peak Spectra**. | • You can instead click **Chromatograms > Clear Results > Only Chromatograms** if you do not want to delete the peak spectra. |

# Task 10. Add annotations

You can add an image annotation or a text annotation to the following graphics windows:

- Chromatogram Results window
- MS Spectrum Results window

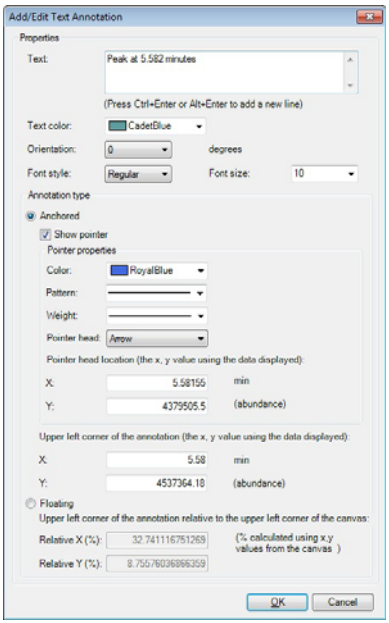
If you save the results for the data file, annotations are also saved.

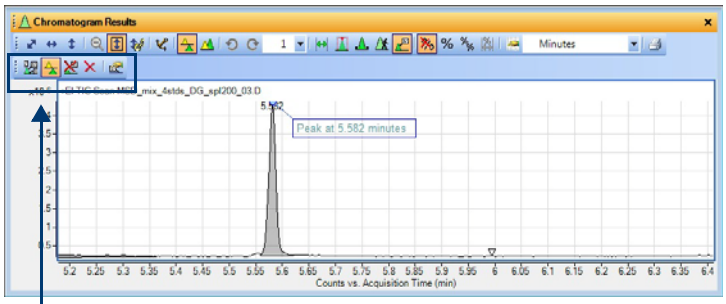
Task 10. Add an annotation

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Select the MSD_mix_4stds_DG_spl200_03.d data file. Hide the other chromatograms. | **a** Mark the check box next to MSD_mix_4stds_DG_spl200_03.D in the Data Navigator window.<br>**b** Click **Edit > Show > Only Highlighted**. | • The chromatograms for the other data files are automatically hidden. |
| **2** Select the location in the chromatogram to add a text annotation. | **a** In the Chromatogram Results window, click the **Annotation** tool (  )in the toolbar.<br>**b** Move the cursor to the location in the chromatogram pane where you want to add the annotation.<br>**c** Right-click and then click **Add Text Annotation**. | • The cursor changes to a cross-hair. You use this cursor to select the exact location to add the annotation.<br>• The Annotate toolbar is available in the Chromatogram Results window.<br>• You can also add annotations to the MS Spectrum Results window. |
| **3** Add the information about the text annotation in the Add/Edit Text Annotation dialog box. | **a** Type the **Text** for the annotation.<br>**b** Select the **Text color**.<br>**c** Select the **Orientation**.<br>**d** Select the **Font style** and **Font size**.<br>**e** Click either **Anchored** or **Floating**. If you click **Anchored**, select the options for the pointer to the text annotation. If you click **Floating**, you can change the relative position. It is easier to change the position interactively in the graphics window.<br>**f** Click **OK**. | • You can add multiple annotations to a chromatogram or spectrum.<br>• You can use the icons in the Annotate toolbar to select all of the annotations, delete annotations and edit annotations. |

Task 10. Add an annotation (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|




**The Annotate Toolbar is only available when the Annotate tool is selected.**

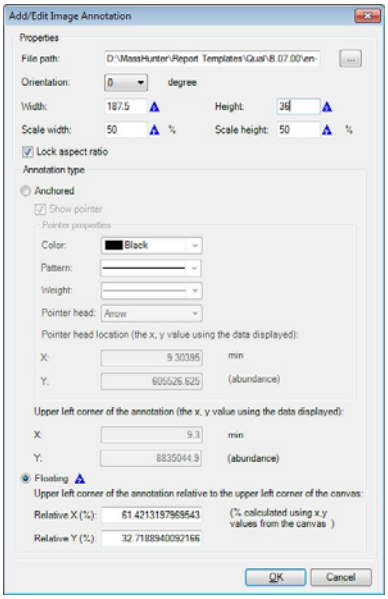
**You can move the annotation to a new location when you click and drag the annotation.**

**Figure 25** Add/Edit Text Annotation dialog box and the Chromatogram Results window

| | | |
|---|---|---|
| **4** Select the location in the chromatogram to add the image annotation. | **a** Move the cursor to the location in the chromatogram pane where you want to add the annotation.<br>**b** Right-click and then click **Add Image Annotation**. | • You can add a JPG or a MOL image file. |
| **5** Add the information about the text annotation in the Add/Edit Text Annotation dialog box. | **a** Select the image annotation.<br>**b** Type 50 for the Scale width.<br>**c** Mark the **Lock aspect ratio** check box.<br>**d** Click **Floating**. You can change the relative position. It is easier to change the position interactively in the graphics window.<br>**e** Click **OK**.<br>**f** Move the image to the upper, right corner of the chromatogram. | • The Agilent_Logo.tif file is included in the \\MassHunter\Report Templates\Qual\B.05.00\en-US\ Letter folder. You need to convert it to a JPG file.<br>• You can add multiple annotations to a chromatogram or spectrum. |

Task 10. Add an annotation (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|



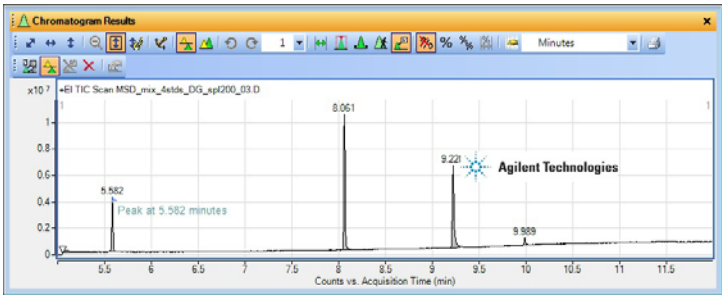
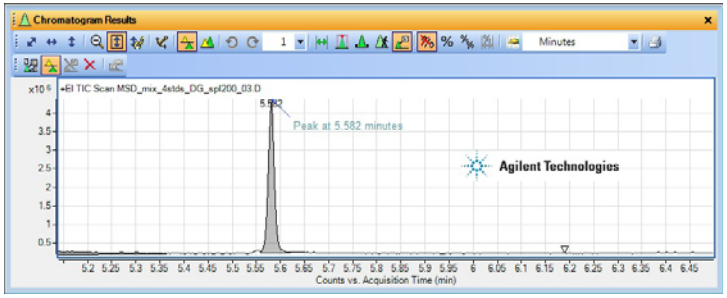




**Figure 26** Add/Edit Image Annotation dialog box and the Chromatogram Results window

**6** Zoom in to the first peak.

- Zoom to an area around the first peak at 5.5 minutes




**If an annotation is anchored, it stays attached at the position where it is anchored. If you zoom into a different peak, an anchored annotation may not be visible. If an annotation is floating, then the annotation is always shown in the same position relative to the upper left corner of the window.**

**Figure 27** Add/Edit Image Annotation dialog box and the Chromatogram Results window

Task 10. Add an annotation (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **7** Switch back to the Range Select tool in the Chromatogram Results window. Delete the annotation first. | **a** Click the  icon to remove all annotations.<br>**b** Click the  (Range Select) icon in the Chromatogram Results toolbar. | • If you want to save the annotations with the data file results, see "Task 18. Save results" on page 72.<br>• You can switch between five different tools in the Chromatogram Results toolbar. Refer to the online Help for more information. The five tools are:<br>◦ Range Select<br>◦ Peak Select<br>◦ Manual Integration<br>◦ Walk Chromatogram<br>◦ Annotation Mouse |

# Task 11. Add a mass caliper

A caliper shows the difference between two points in a spectrum. You can add a caliper to the MS Spectrum Results window.

If you save the results for the data file, calipers are also saved.

Task 11. Add a mass caliper

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Integrate and extract peak spectra from MSD_mix_4stds_DG_spl200_03.d. | **a** Mark the check box next to MSD_mix_4stds_DG_spl200_03.D in the Data Navigator window.<br>**b** Click **Edit > Show > Only Highlighted**.<br>**c** Click **Chromatograms > Integrate and Extract Peak Spectra**.<br>**d** Close the Method Editor window. | |
| **2** Add the caliper to the peak spectrum created in the previous task. | **a** In the MS Spectrum Results window, click the **Delta Mass Caliper** tool ( )in the toolbar.<br>**b** (optional) Select **Profile Point to Point** for the type of caliper in the Caliper toolbar.<br>**c** Zoom in from 79 to 99 *m/z*.<br>**d** Move the cursor to the location in the spectrum pane where you want to add the caliper.<br>**e** Drag the cursor to the end point of caliper in the spectrum. As you drag the cursor, the value of the delta mass changes. When you release the mouse button, the caliper is added. | • The cursor changes to an arrow. You use this cursor to select the start and end point of the caliper.<br>• You cannot select the type of caliper if the spectrum is centroided because **Profile Point to Point** has no effect on centroid data.<br>• The "triangle" cursor is set to the top of the peak that is selected. |
| **3** Modify the caliper to use a different color. | **a** Click the caliper created in the previous step.<br>**b** Click the Caliper Properties button ( ) in the MS Spectrum Results Caliper toolbar.<br>**c** (optional) Type the **Start X** and **Start Y** values.<br>**d** Select the **Text color**.<br>**e** Select the **Font style** and **Font size**.<br>**f** Click **OK**. | • You can add multiple calipers to a spectrum.<br>• You can use the icons in the Caliper toolbar to select all of the calipers, delete calipers and edit calipers. |

Task 11. Add a mass caliper (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|

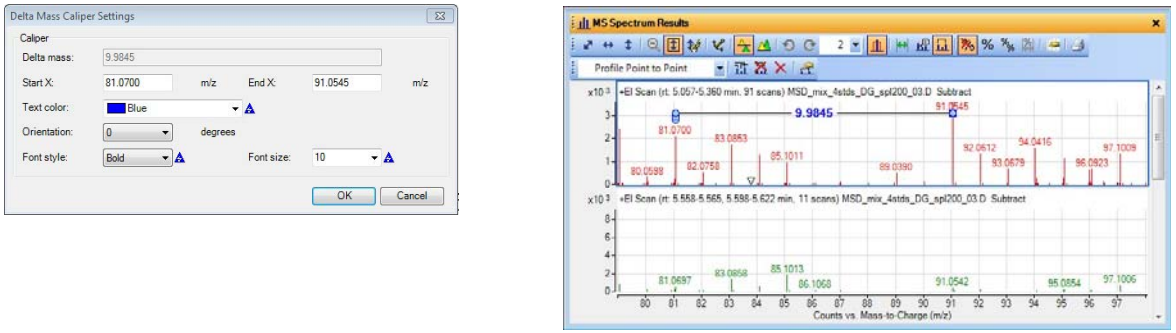



**Figure 28** Delta Mass Caliper Settings dialog box and the MS Spectrum Results window

**4** Delete integration results and spectra.

**a** Click **Chromatograms > Clear Results > Include Peak Spectra**.

**b** Click the **Range Select** tool in the MS Spectrum Results window.

- If you want to save the calipers with the data file results, see "Task 18. Save results" on page 72.

# Exercise 2
# Find and identify



In these tasks, you find and identify compounds in GC/MS data files.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

## Task 12. Find Compounds by Chromatogram Deconvolution

This Find Compounds algorithm identifies compounds in GC/MS data and creates a cleaned MS spectrum for each compound. This functionality is an easy way to “mine” information from complex data. You can only use the Find Compounds by Chromatogram Deconvolution algorithm on GC/MS sample data acquired in Scan, Product Ion scan or Neutral Loss scan mode.

This task shows finding compounds by chromatogram deconvolution with accurate mass data. You can also find compounds by chromatogram deconvolution with unit mass data after you first change the extraction window.

Task 12. Find compounds using Chromatogram Deconvolution (GC/MS)

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **MSD_mix_4stds_DG_spl200_03.d** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • The Find Compounds by Chromatogram Deconvolution algorithm works with both GC/QQQ and GC/Q-TOF data files. |

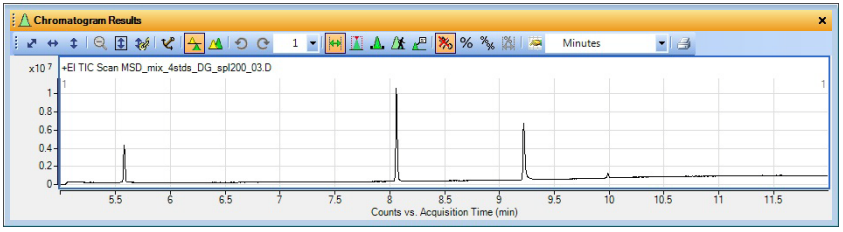
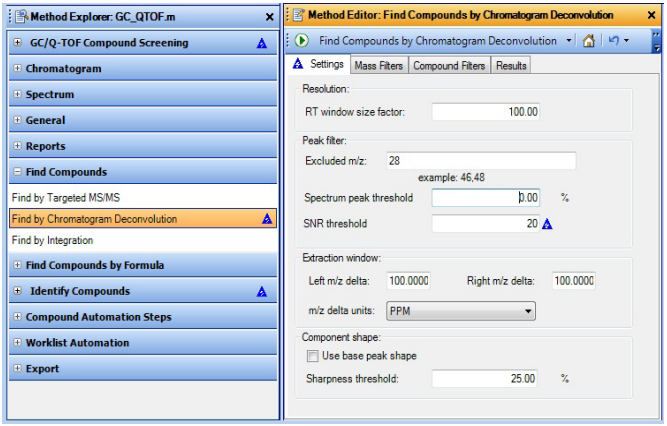



**Figure 29** TIC chromatogram from Pest - 200 - Scan.d

| | | |
|---|---|---|
| **2** Configure the user interface to work with GC data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | • For these examples, load the GC/Q-TOF Compound Screening workflow. |

Task 12. Find compounds using Chromatogram Deconvolution (GC/MS)

| Step | Detailed Instructions | Comments |
|---|---|---|
| **3** Find compounds using the chromatogram deconvolution algorithm.<br>• Select the Agile integrator.<br>• Enter an SNR threshold of 20.<br>• Enter 100 ppm for the **Left m/z delta** and **Right m/z delta** values. | **a** In the Method Explorer window, select **Find Compounds > Find by Chromatogram Deconvolution.**<br>**b** On the Settings tab under Peak filter, type `20` for the **SNR threshold**.<br>**c** Select **PPM** for the **m/z delta units**.<br>**d** Enter `100` for the **Left m/z delta**, and enter `100` for the **Right m/z delta**. | • The Find by Chromatogram Deconvolution section is also available in the GC/Q-TOF Compound Screening section.<br>• If you have unit mass data, you enter `0.3` AMU for the **Left m/z delta** value and `0.7` AMU for the **Right m/z delta** value<br>• You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** menu item when a compound is highlighted. |
| |  | |
| | **Figure 30** Settings tab in the Find by Chromatogram Deconvolution section | |
| • Select to extract EIC, MS spectra and MS/MS spectra. | **e** Click the **Results** tab.<br>**f** Mark the **Extract EIC, Extract ECC, Extract cleaned spectrum** and **Extract raw spectrum** check boxes.<br>**g** Click to run the **Find Compounds by Chromatogram Deconvolution** algorithm on the data file.<br>**h** If necessary, click the **View > Compound List** command. | • The Qualitative Analysis program finds 4 compounds under these conditions.<br>• If the data file is not indexed, it can take a long time when you run this algorithm. |

Task 12. Find compounds using Chromatogram Deconvolution (GC/MS)

| Step | Detailed Instructions | Comments |
|---|---|---|
| **4** Examine the compounds. See Figure 31 on page 48. | **a** Select **2** in the **Maximum number of list panes** box in the MS Spectrum Results toolbar.<br>**b** Click the **Hide Empty Columns** icon in the Compound List window.<br>**c** Click the first compound in the Data Navigator window.<br>**d** When the Data Navigator window is selected, use the arrow keys to switch compounds. | • Showing both spectra is a convenient way to display all the information for a single compound.<br>• Note that both the cleaned spectrum and the raw spectrum are shown. |

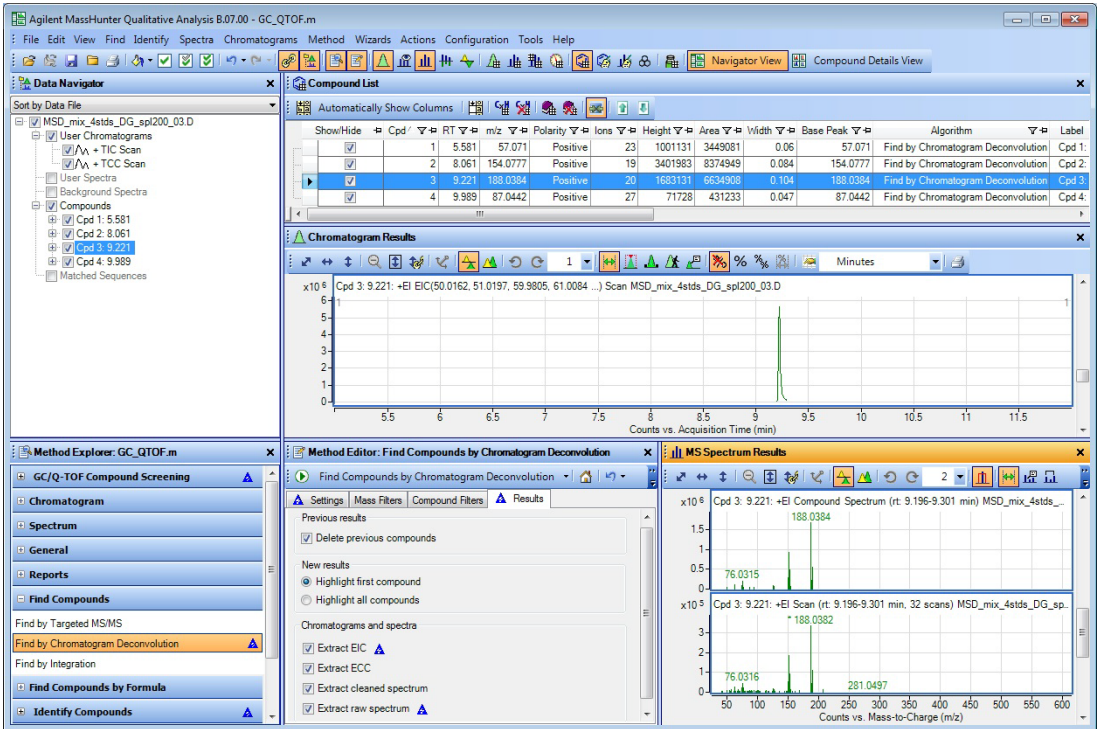



**Figure 31** Find Compounds by Chromatogram Deconvolution results

# Task 13. Identify compounds using the Search Library algorithm

In this task, you identify and generate formulas for the compounds found in "Task 12. Find Compounds by Chromatogram Deconvolution" on page 45. You can do this task if you have purchased the *NIST11.l* library (or a later version) or if you use the *demo.l* library. If you have two libraries, you can even select both libraries.

Task 13. Identify compounds using the Search Library algorithm

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Do a library search of all of the compounds in the MSD_mix_4stds_DG_spl200_03.d data file. | **a** Highlight the compounds in the MSD_mix_4stds_DG_spl200_03.D data file in the Data Navigator window.<br>**b** In the Method Explorer window, click **Identify Compounds > Search Library**.<br>**c** In the Settings tab, click the **Add Library** button. Select the **demo.l** library and click the **OK** button.<br>**d** (optional) In the Settings tab, click the **Add Library** button. Select the **NIST11.l** library and click the **OK** button.<br>**e** (optional) Select **Stop at first library match** for the **Multi-library search type**.<br>**f** Click **Identify > Search Library for Compounds** from the main menu. You can instead click the **Search Library for Compounds** icon to run the algorithm.<br>**g** Click **View > Difference Results**.<br>**h** Click **View > Structure Viewer**.<br>**i** Click **View > Compound Identification Results**, if necessary to display this window.<br>**j** If necessary, click the tab for the **Compound Identification Results** window. This window is tabbed with the Chromatogram Results window. | • You can also click GC/Q-TOF Compound Screening > Identify by Library Search in the Method Explorer. The same section in the Method Editor window is displayed.<br>• Demo.l and Nist11should be installed in the \MassHunter\ Library folder.<br>• Note that many of the compounds are identified after searching the *NIST11.l* library.<br>• If you do not have the *NIST11.l* library, then select a second library if you have one available.<br>• If you have two or more libraries selected and you select **Stop at first library match**, the library search algorithm searches the first library in the list. If the compound is identified, then it stops. If the compound is not identified, then it searches the next library until the compound is identified or the last library is searched.<br>• You use the Library Editor program to modify **.L** libraries that you use with the Search Library algorithm. This program is installed with the Agilent MassHunter Quantitative Analysis program. You click the  icon to start this program. |

Task 13. Identify compounds using the Search Library algorithm

| Step | Detailed Instructions | Comments |
|---|---|---|
| **2** Display the Spectral Library Results columns in the Compound List window and the Compound Identification Results window. | **a** Click the Show Library Search Columns button ( ) in the Compound List toolbar and in the Compound Identification Results toolbar.<br>**b** Click the Hide Empty Columns button ( ) in the Compound List toolbar and in the Compound Identification Results window. | |

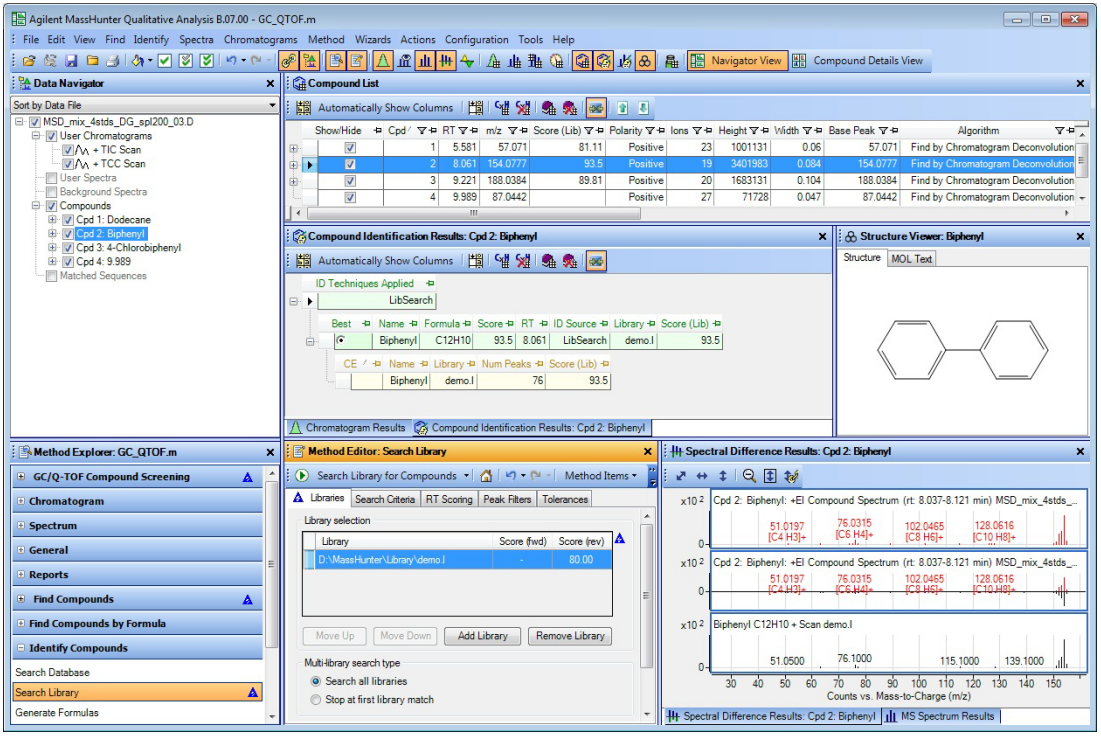



**Figure 32** Compounds in MSD_mix_4stds_DG_spl200_03.D data file and the library search results

| | | |
|---|---|---|
| **3** Switch to the Compound Details View to review the compounds. | **a** Click Compound Details View in the main toolbar.<br>**b** Close the **Compound Fragment Spectrum Results** window. | • The Compound Fragment Spectrum Results window only has results if you used fragment confirmation with the Find by Formula algorithm. |

Task 13. Identify compounds using the Search Library algorithm

| Step | Detailed Instructions | Comments |
|---|---|---|
| **4** Review the results in the Compound Details View. | **a** Click the Overlaid icon in the Compound Chromatogram Results window.<br>**b** Expand the results in the Compound Identification Results window. | • You can find out more about the Compound Details View in the online Help. The Compound Details View is very useful when looking at the results of the Find by Formula algorithm with a data file acquired in All Ions mode. |

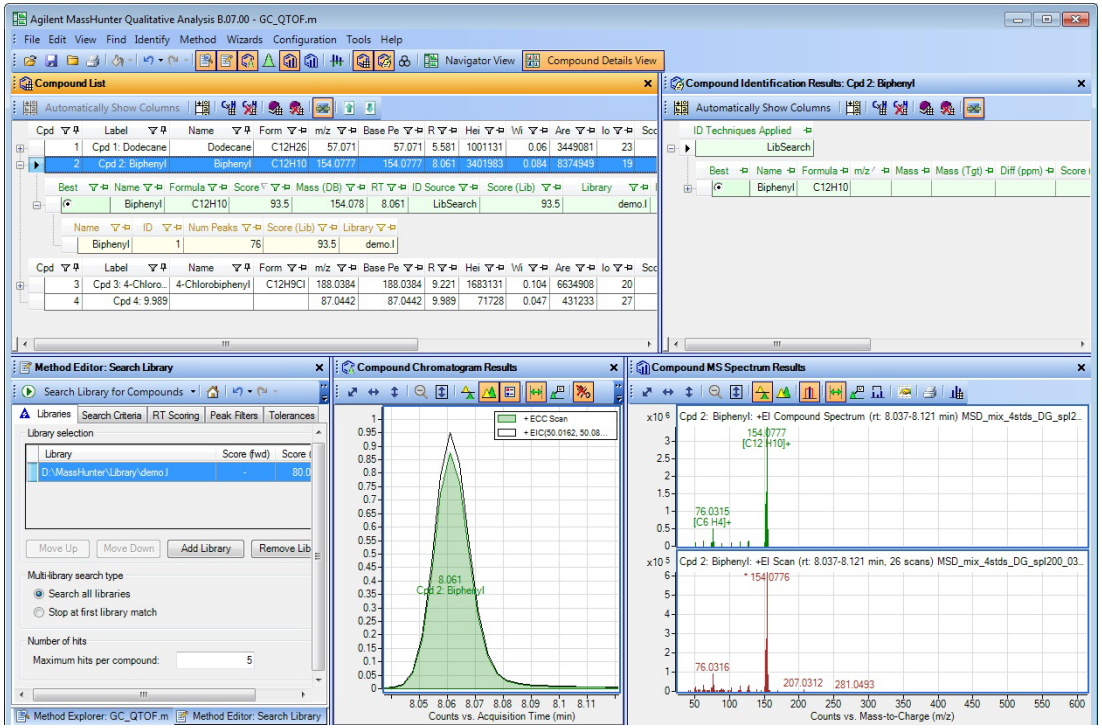



**Figure 33** Compound Details View showing compounds in MSD_mix_4stds_DG_spl200_03.D data file

| | | |
|---|---|---|
| **5** Switch back to the Navigator View. | • Click the Navigator View button in the main toolbar. | |
| **6** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **No** when you are asked if you want to save results. | • If you want to save these results, see "Task 18. Save results" on page 72. |

# Task 14. Find Compounds using MRM (MRM only)

The Find Compounds by MRM algorithm identifies compounds in MRM data from a Triple Quadrupole. The algorithm searches for compounds using the MRM transitions. All of the compounds in the acquisition method are extracted and shown in the Compound List. Compounds are not eliminated based on chromatogram integration results. You can only use the Find Compounds by MRM algorithm on data that was acquired using MRM transitions. The MRM algorithm uses information that is found in the data file if the data file is an MRM data file.

Task 14. Find compounds using MRM (MRM only)

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **Pest - STD 200 MRM.d** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **Pest - STD 200 MRM.d** data file in the **GC Pesticides** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • You use the General Workflow when working with GC/QQQ data. You can use either the General Workflow or the GC/Q-TOF Compound Screening workflow when working with GC/Q-TOF data. |

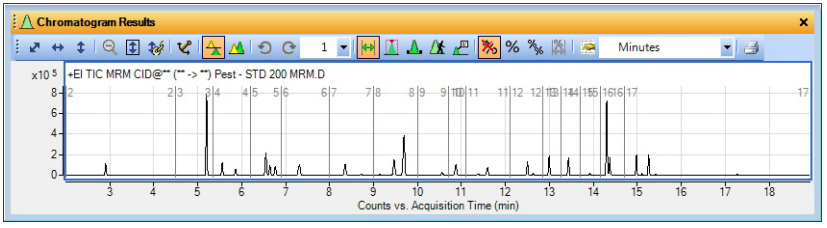



**Figure 34** TIC chromatogram from Pest - STD 200 MRM.d

| | | |
|---|---|---|
| **2** Configure the user interface to work with GC QQQ data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | |

Task 14. Find compounds using MRM (MRM only)

| Step | Detailed Instructions | Comments |
|---|---|---|
| **3** Find compounds using the MRM algorithm. | **a** In the Method Explorer window, select **Find Compounds > Find by MRM.**<br>**b** Click the **Group transitions by compound name** button.<br>**c** Click the **Integrator** tab.<br>**d** Select the **Agile 2** integrator. | • You can choose the region of the chromatogram from which you intend to find compounds.<br>• You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** menu item when a compound is highlighted. |

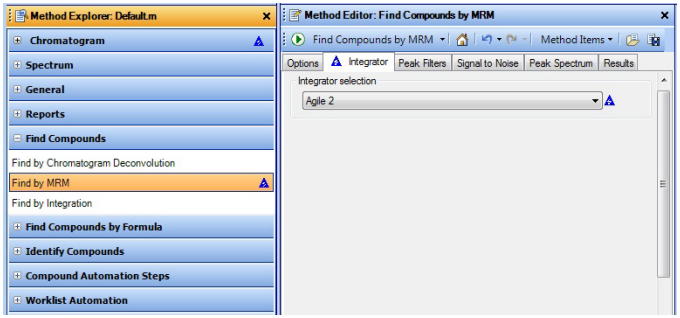



**Figure 35** Integrator tab in the Find by MRM section of the Method Editor

| | | |
|---|---|---|
| | **e** Click ▶ to run the **Find Compounds by MRM** algorithm on the data file.<br>**f** If necessary, click the **View > Compound List** command.<br>**g** If necessary, click the **View > Compound Identification Results**. | • The Qualitative Analysis program finds 28 compounds under these conditions. |
| **4** Examine the compounds. See Figure 36 on page 54. | **a** Select **2** in the **Maximum number of list panes** box in the MS Spectrum Results toolbar.<br>**b** Click the **Automatically Show Columns** icon in the Compound List window and in the Compound Identification Results window.<br>**c** Click the first compound in the Data Navigator window.<br>**d** When the Data Navigator window is selected, use the arrow keys to switch compounds. | • The precursor ion is displayed in the Precursor (Acq Method) column, and the product ion is displayed in the Product (Acq Method) column in the Compound Identification Results window. |

Task 14. Find compounds using MRM (MRM only)

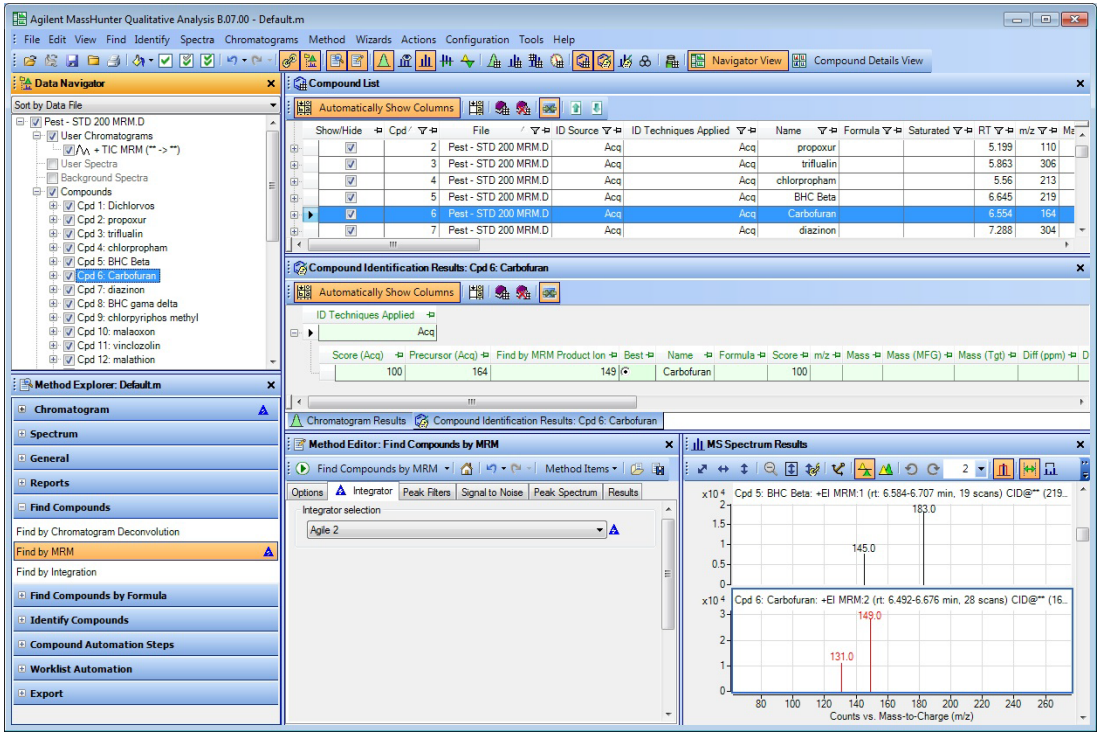
| Step | Detailed Instructions | Comments |
|---|---|---|
| |  | |

**Figure 36** Find by MRM results

| | | |
|---|---|---|
| **5** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **Close**. | • If you want to save these results, see "Task 18. Save results" on page 72. |

# Task 15. Find Compounds by Integration

The Find Compounds by Integration algorithm identifies compounds based on the integration results. A compound is created for each peak that is identified by the integrator.

Task 15. Find compounds using Integration

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **MSD_mix_4stds_DG_spl200_03.D** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • You use the General Workflow when working with GC/QQQ data. You can use either the General Workflow or the GC/Q-TOF Compound Screening workflow when working with GC/Q-TOF data. |
| **2** Configure the user interface to work with GC data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | |
| **3** Find compounds using the Find by Integration algorithm. | **a** In the Method Explorer window, select **Find Compounds > Find by Integration.**<br>**b** Select the **MS/MS (GC)** integrator. | • You can choose the region of the chromatogram from which you intend to find compounds.<br>• You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** command when a compound is highlighted. |

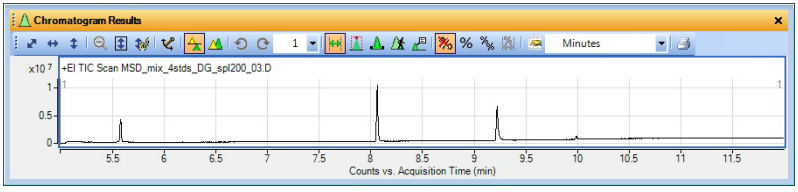



**Figure 37** TIC chromatogram from MSD_mix_4stds_DG_spl200_03.d

Task 15. Find compounds using Integration

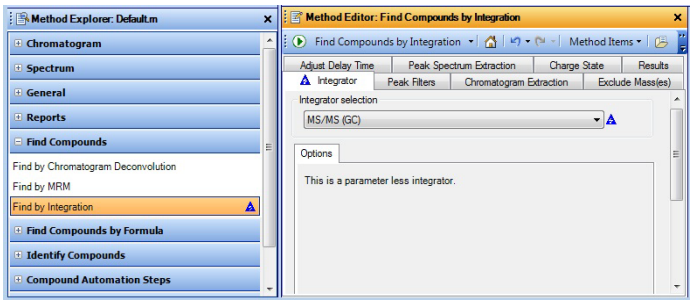
| Step | Detailed Instructions | Comments |
|---|---|---|
| |  | |

**Figure 38** Integrator tab in the Find by Integration section of the Method Editor

| | | |
|---|---|---|
| | **c** Click to run the **Find Compounds by Integration** algorithm on the data file.<br>**d** If necessary, click the **View > Compound List** command. | • The Qualitative Analysis program finds six compounds under these conditions. |
| **4** Examine the compounds. See Figure 36 on page 54. | **a** Select **2** in the **Maximum number of list panes** box in the MS Spectrum Results toolbar.<br>**b** Click the **Automatically Show Columns** icon in the Compound List window.<br>**c** Click the **Hide any currently empty columns** icon in the Compound List window.<br>**d** Click the first compound in the Data Navigator window.<br>**e** When the Data Navigator window is selected, use the arrow keys to switch compounds. | |

Task 15. Find compounds using Integration

| Step | Detailed Instructions | Comments |
|---|---|---|

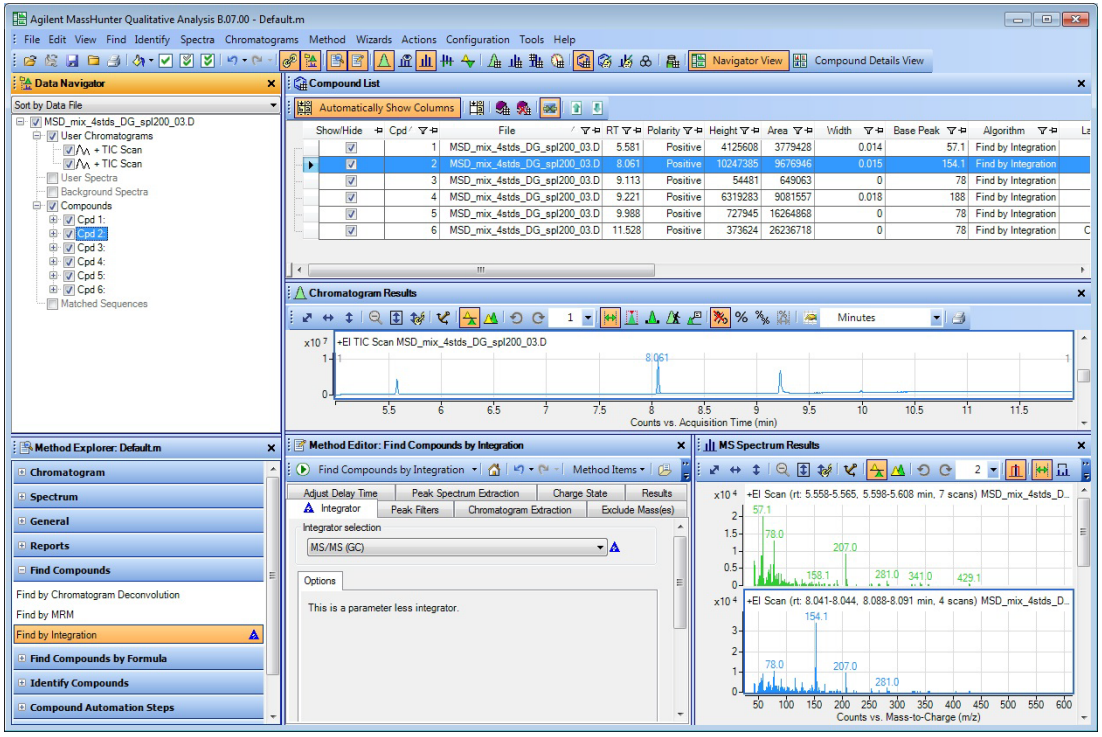



**Figure 39** Find by Integration results

| | | |
|---|---|---|
| **5** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **No** when asked whether or not to save results.<br>**c** Click **Close**. | • If you want to save these results, see "Task 18. Save results" on page 72. |

# Task 16. Find Compounds by Formula with Fragment Confirmation

Fragment confirmation of target compounds can be conducted on LC/MS data files that are acquired in All Ions MS/MS mode. On an LC/Q-TOF instrument this is done by alternating the acquisition between 2 to 4 different collision energies. The recommended collision energies to use are 0 V, 20 V, and 40 V. The 0 V spectrum is considered the "low energy channel" which predominantly shows the precursor ions of the eluting compounds, while the 20 V and 40 V spectra are considered the "high energy channel(s)", which exhibit fragment ions of all compounds eluting at the time. Hence, the name All Ions MS/MS. A similar experiment can be conducted on an LC/TOF instrument by alternating between 2 to 4 fragmentor voltages, (for example, 125 V, 200V and 275 V). For the "low energy channel" the fragmentor voltage is set to avoid in-source fragmentation of most of the target compounds, while the "high energy channel" spectra exhibit fragment ions of the eluting compounds. Using more than one high energy channel provides fragmentation across different compound stabilities.

Fragment confirmation is also possible for GC/Q-TOF EI data, which inherently shows mostly fragment ions in each spectrum. Here, only a high energy channel is present, and most of the time molecular ions are not present in the spectra. Therefore, the **Molecular ion optional** check box needs to be marked. The algorithm first selects “n” fragment ions from the EI-MS spectral library based on abundance and *m/z* value (higher *m/z* fragment ions are given preference because they contain more structural information). The algorithm then extracts ion chromatograms of those ions in a time window around the target retention times in the library and creates a list of target chromatographic peaks. It then attempts to find groups of peaks that cluster by RT and selects a reference ion and confirming fragment ions. The reference ion can be the molecular ion if present, but it does not have to be. The algorithm then calculates how well the selected chromatographic peaks co-elute. The target compound is qualified, if a user settable minimum number of ions is found to have a coelution score above a set threshold.

The **Molecular ion optional** mode can also be used for LC/MS data, if the precursor ion of a compound in the "low energy channel" shows a split peak due to saturation. In that case the molecular ion will not be used as the reference ion; instead, the reference ion and confirming fragment ions are chosen from the high energy channel(s).

In all cases a "Cleaned HighE Scan" is generated which only shows the reference ion and confirming fragment ions, optionally annotated with their sub formulas.

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **Tomato_spiked.D** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **Tomato_spiked.d** data file in the **GCMS Pesticide** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • You use the General Workflow when working with GC/QQQ data.<br>• You can use either the General Workflow or the GC/Q-TOF Compound Screening workflow when working with GC/Q-TOF data. |
| **2** Configure the user interface to work with GC data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | |
| **3** Load the GCQTOF_Pesticide_Example.m method file. | **a** Click **Method > Open.**<br>**b** Select the **GCQTOF_Pesticide_Example.m** method and click **Open**. | • This method is installed in the \\MassHunter\methods\B.07.00 folder.<br>• If you see any blue triangles when you load the method, you can ignore them for now. |
| **4** Save the method to *iii*_GCQTOF_Pesticide_Example.m, where "*iii*" are your initials. | **a** From the top menu, click **Method > Save As**.<br>**b** Type **iii_GCQTOF_Pesticide_Example.m**.<br>**c** Click the **Save** button. | • Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear. |

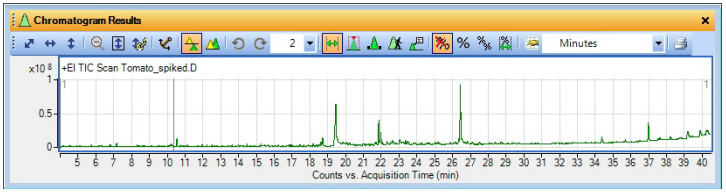



**Figure 40** TIC chromatogram from Tomato_spiked.d

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| **5** Verify the parameters for Find Compounds by Formula. | **a** In the Method Explorer window, select **Find Compounds by Formula > Find by Formula - Options.**<br>**b** Click the **Formula Source** tab.<br>**c** Click **Database/Library**.<br>**d** Select the **Pesticide_Example.cdb** library in the PCDL folder.<br>**e** Click the **Formula Matching** tab.<br>**f** Select **Symmetric (ppm)** for the **Possible m/z** and review the value.<br>**g** Mark the **Limit EIC extraction range** check box, select **Symmetric**, and type `1.0` for the **Expected retention time**. | • You can run the Find by Formula (FbF) algorithm on GC/Q-TOF EI data files. You can also use this algorithm on LC/MS data files acquired in All Ions MS/MS mode.<br>• These values are already set in this example method.<br>• The value selected for **Possible m/z** may depend on whether you are running your acquisition method in high resolution mode or dual gain mode. |

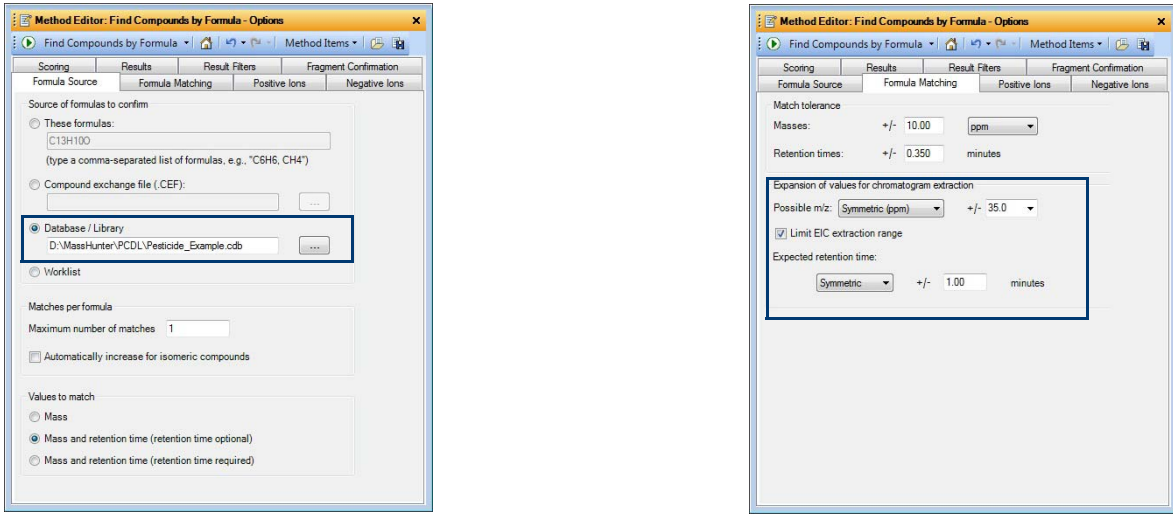
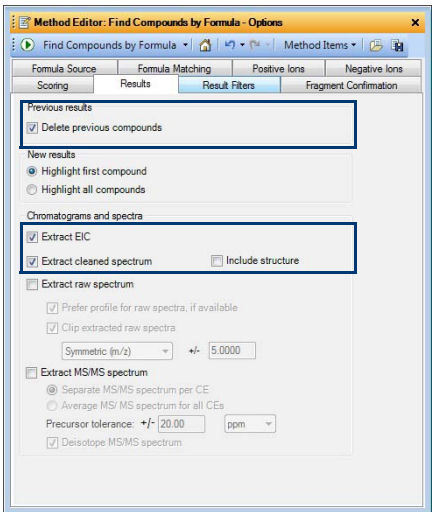



**Figure 41** **Formula Source** tab and **Formula Matching** tab in the Find by Formula - Options section

| | | |
|---|---|---|
| | **h** Click the **Results** tab.<br>**i** Mark **Delete previous compounds**.<br>**j** Mark **Extract EIC** and **Extract cleaned spectrum**.<br>**k** Click the **Result Filters** tab.<br>**l** Mark the **Only generate compounds for matched formulas** check box. | • If you clear **Only generate compounds for matched formulas**, then compounds that are not found are also displayed in the results. |

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|



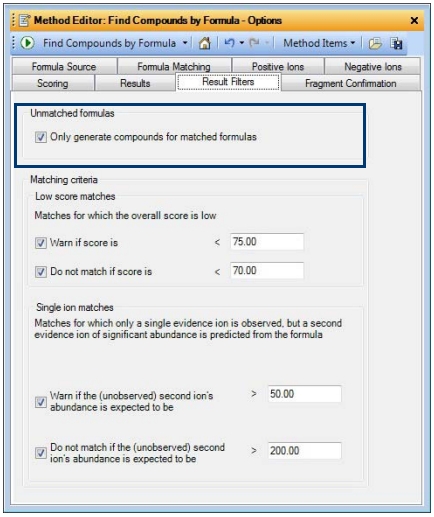




**Figure 42** **Results** tab and **Result Filters** tab in the Find by Formula - Options section

**m** Click the **Fragment Confirmation** tab.

**n** Mark **Confirm with fragment ions**.

**o** Mark **Molecular ion optional**.

**p** Click **Use spectral library only** and type `7` for the **Number of most specific ions from spectral library**.

**q** Type `0.2` for the **RT difference**.

- For GC/Q-TOF data, you mark the **Molecular ion optional** check box.
- A higher number of ions produces a greater specificity and more confidence in the results; however, a higher number of ions results in a longer program run time.
- The recommended range for the **RT difference** is 0.1 to 0.2 This value is the difference that is allowed for the retention time shift of the reference ion. The reference ion is automatically chosen by the Qualitative Analysis program.

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| | **r** Clear the **S/N ratio** check box.<br>**s** Type `70` for the **Coelution score**.<br>**t** Click **Minimum number of qualified fragments** and type `1`. | • If the **S/N ratio** check box is marked, you have a high probability of producing false negatives (if you the ratio too low).<br>• The recommended starting value is 1 to 3. A setting of 1 requires two qualified fragments: a reference ion and a qualified ion. |

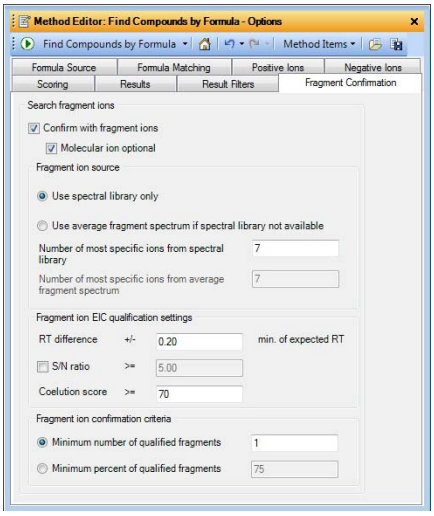



**Figure 43** **Fragment Confirmation** tab in the Find by Formula - Options section

| | | |
|---|---|---|
| **6** Run the Find Compounds by Formula algorithm. | • Click to run the **Find Compounds by Formula** algorithm on the data file.<br>• Click **Find > Find Compounds by Formula**. | • The Qualitative Analysis program finds five compounds under these parameter values.<br>• Leave the values in the other tabs the same. |
| **7** Save the method. | • Save the method in one of three ways:<br>◦ Click the **Save Method** icon in the Method Editor.<br>◦ Right-click the Method Editor, and click **Save Method**.<br>◦ From the top menu click **Method > Save**. | |

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| **8** Examine the compounds. See Figure 36 on page 54. | **a** Click Compound Details View in the main toolbar.<br>**b** If visible, close the **Method Editor** and the **Method Explorer** windows.<br>**c** In the Compound List window, right-click the header of any column that you want to remove, and click Remove Column.<br>**d** Move the **Flags (Tgt)** column in the Compound List window to next to the **Label**. | • Selecting a compound from the Compound List window displays results in the other windows in this view.<br>• See the online Help for more information.<br>• Two of the windows are shown in more detail. |

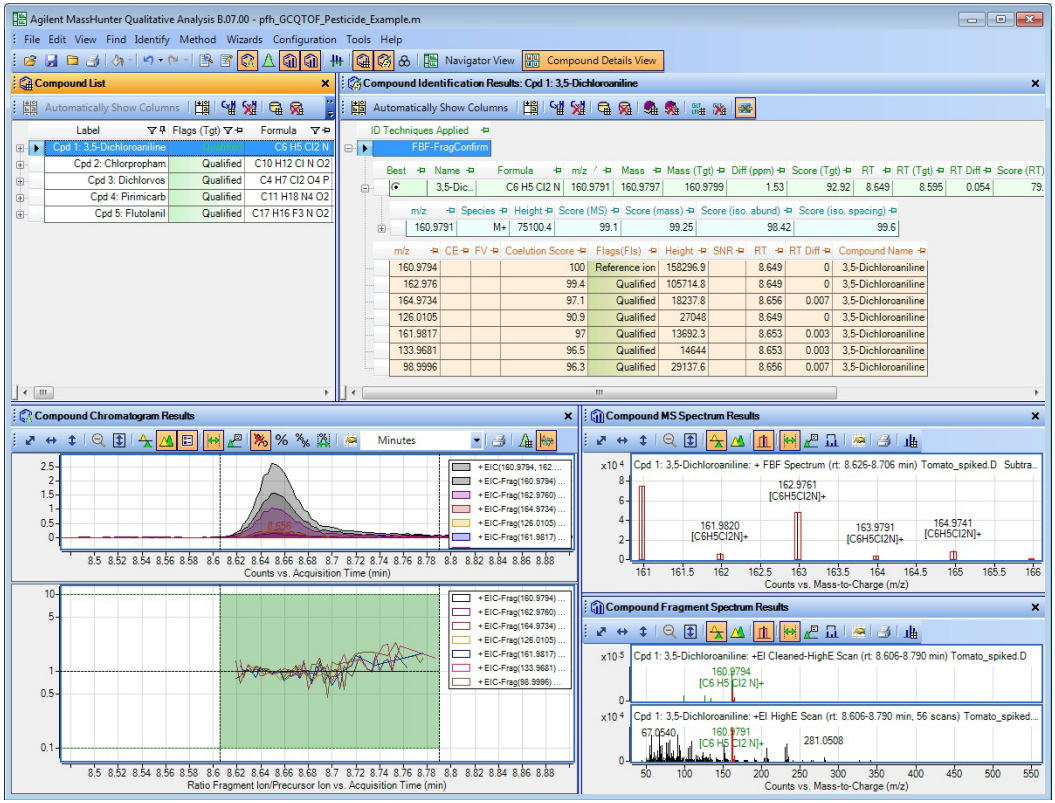



**Figure 44** Find by Formula results including Fragment Confirmation results

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| | **e** Click or use the arrow keys to change compounds in the Compound List to review one compound at a time.<br>**f** Review the information in the Compound Identification Results window.<br>**g** Click the + icon to expand a level of the table. When the level of the table is expanded, the icon changes to a - icon. | • The first level of the table shows the summary information for all of the identification algorithms that you ran.<br>• The second level (blue) shows individual scores that were used to create the overall score. This row is only present when a molecular ion is found and reflects Find by Formula results.<br>• The table at the bottom shows the fragment ions and their coelution scores. It also shows whether or not the fragment ion is qualified. |

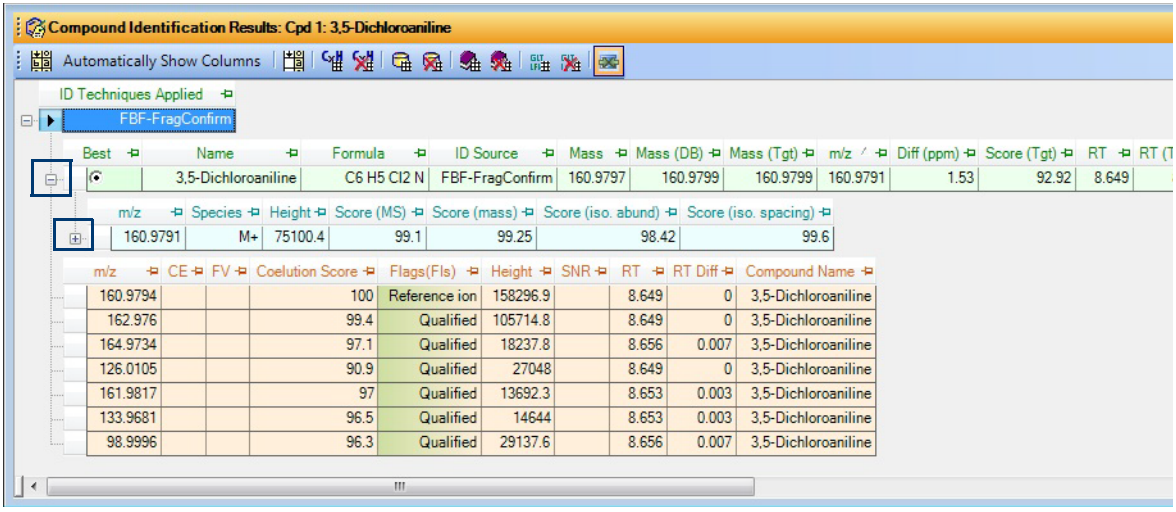



**Figure 45** Compound Identification Results window

Task 16. Find Compounds by Formula with Fragment Confirmation

| Step | Detailed Instructions | Comments |
|---|---|---|
| | **h** Review the results in the Compound Chromatogram Results window.<br>**i** Verify that the Coelution Plot pane is visible.<br>**j** Verify that the chromatograms are overlaid. The icons in the toolbar are set like this:<br>  | • The Compound Chromatogram Results window shows individual ion traces for each fragment ion.<br>• It also shows the Coelution Plot which displays how closely the Fragment ions coelute with the compound. For reference a black line is shown with the y-value of 1. A value of 1 shows the qualifier ions are exactly coeluting with the reference ion chromatogram. As the ratio approaches 1, the qualifier ion is more closely coeluting with the reference ion. |

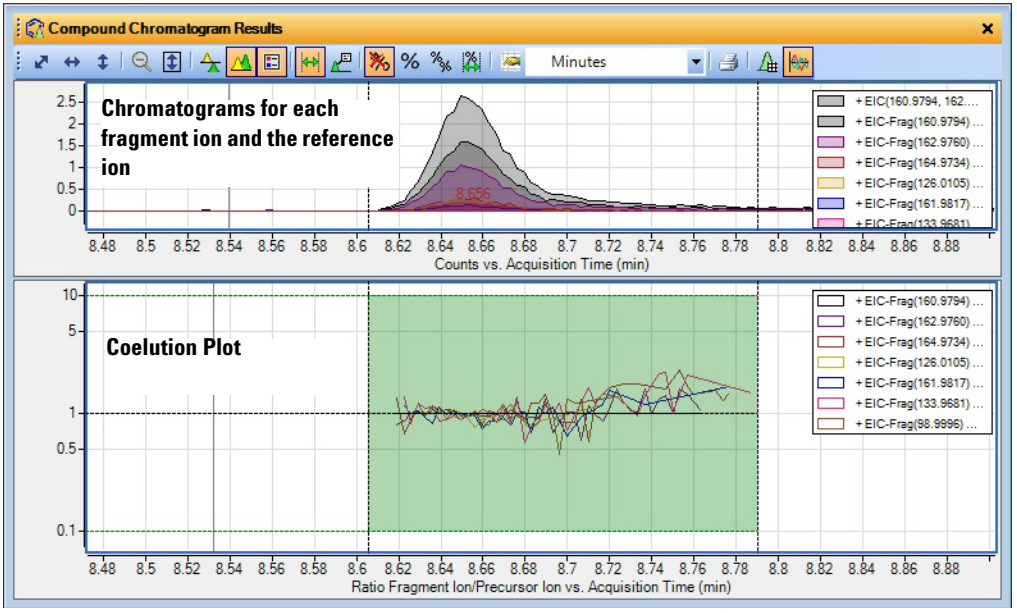



**Figure 46** Compound Identification Results window

| | | |
|---|---|---|
| **9** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **No** when asked whether or not to save results. | • If you want to save these results, see "Task 18. Save results" on page 72. |

# Task 17. Generate formulas and search library for peak spectra

In this task, you first integrate and extract peak spectra from a GC/Q-TOF data file. Then, you generate possible formulas for each of the peak spectra.

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **MSD_mix_4stds_DB_spl200_03.d** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DB_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • If the **Load result data** check box is not available, then no results have been saved in the data file. See "Task 18. Save results" on page 72 for instructions on how to save results.<br>• The General workflow is loaded. |
| **2** Integrate and extract peak spectra. | **a** Click the Chromatograms > Integrate (MS) section in the Method Explorer window.<br>**b** Click the **Peak Filters** tab.<br>**c** Click the **Peak height** button.<br>**d** Mark the **Relative height** check box.<br>**e** Mark the **Limit (by height) to the largest** check box and type 4.<br>**f** Click **Chromatograms > Integrate and Extract Peak Spectra**. | |

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|
| **3** Generate formulas for each peak spectra.<br>• View the Spectrum Identification Results List.<br>• Close the MS Spectrum Results window.<br><br>Hint: To obtain the same results as in Figure 48, make sure you have selected **Common organic molecules** as the Isotope model. | **a** In the Method Explorer window, click **Identify Compounds > Generate Formulas**.<br>**b** In the Method Editor window, click the **Charge State** tab, and select **Common organic molecules** as the **Isotope model**.<br>**c** In the Data Navigator window, highlight all of the spectra in the **User Spectra** section.<br>**d** Click the **Identify > Generate Formulas from Spectrum Peaks** command or the **Generate Formulas from Spectrum Peaks** button to run the algorithm.<br>**e** If necessary, click the Spectrum Identification Results icon, , or click the **View > Spectrum Identification Results** command.<br>**f** In the Spectrum Identification Results window, click the **Automatically Show Columns** button in the toolbar.<br>**g** Click the Hide Empty Columns icon, ,in the Spectrum Identification Results window.<br>**h** In the Data Navigator window, select the spectrum near 5.558 minutes.<br>**i** Select **C6 H7** as the **Best** result.<br>**j** Expand the table for that row.<br>**k** Close the Method Editor window.<br>**l** Review the Formula and Ion Species that are shown above many peaks in the MS Spectrum Results window. All of the Formula and Ion Species are the same color as the spectrum. | • You can see the predicted isotope abundance ratios on the spectrum plot when you zoom in at the appropriate m/z. See the online Help for more information.<br>• The Run icon in the Method Editor toolbar sometimes allows you to choose an action from a set of possible actions. For example, two different actions are possible when you click the Run icon in this section. If you click the arrow, a list of possible actions is shown, and you can choose which action to do. Choosing a different action from the list changes the default action. If you simply click the Run button, the default action is performed.<br>• You can change the width of a column by dragging the line that separates adjacent columns.<br>• You can move a column by dragging the column header.<br>• You can delete a column by clicking **Remove column** in the shortcut menu in the table. |

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|

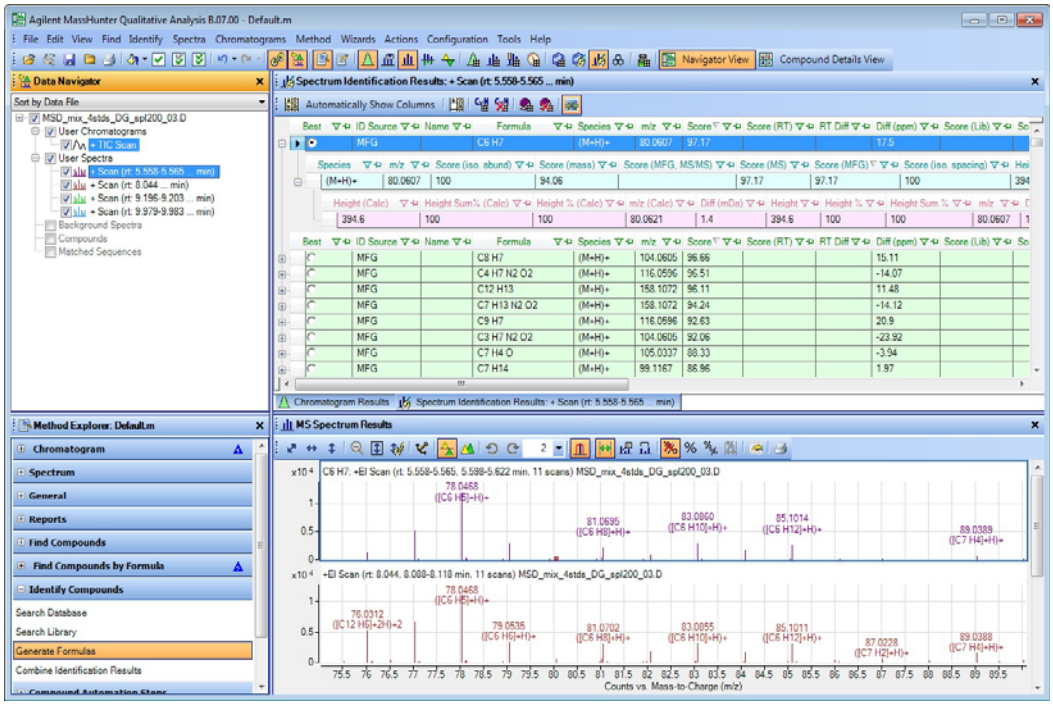



**Figure 47** Generate Formula results for peaks 1 to 4

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|
| **4** Do a library search for peak spectra 1 to 4. | **a** In the Data Navigator window, click **User Spectra**.<br>**b** In the Method Explorer window, click **Identify Compounds > Search Library**.<br>**c** Add a valid library. The GCQTOF_pesticide_matrix_RT.cdb library is selected.<br>**d** Type 50 for the **Score (rev)**.<br>**e** Clear the **Instrument type** and **Collision energy** check boxes on the Search Criteria tab.<br>**f** Clear the **Absolute Height** and **Relative Height** check boxes on the Peak Filters tab.<br>**g** Click **Identify > Search Library for Spectra** in the main menu.<br>**h** Close the Method Editor window. | • The Method Editor is opened automatically when you click a section in the Method Explorer. |
| **5** Modify the columns that are visible. | **a** Right-click the Spectrum Identification Results window and click **Add/Remove Columns**. In the "(Enhanced) Add/Remove Columns" dialog box, mark the columns that you want to display. Click **OK**.<br>**b** Close the Method Editor window<br>**c** Click the Hide Empty Columns icon,  ,in the Spectrum Identification Results window.<br>**d** Review the Formula & Ion Species that is shown above each peak in the MS Spectrum Results window. | • If you use the **Remove Column** command and remove a column that contains data, the software automatically redisplays this column if the Automatically Show Columns feature is on.<br>• The LibSearch algorithm is weighted heavily in the Combine Identification Results section of the method. You can manually choose the best MFG result or change how identification results are combined. |

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|

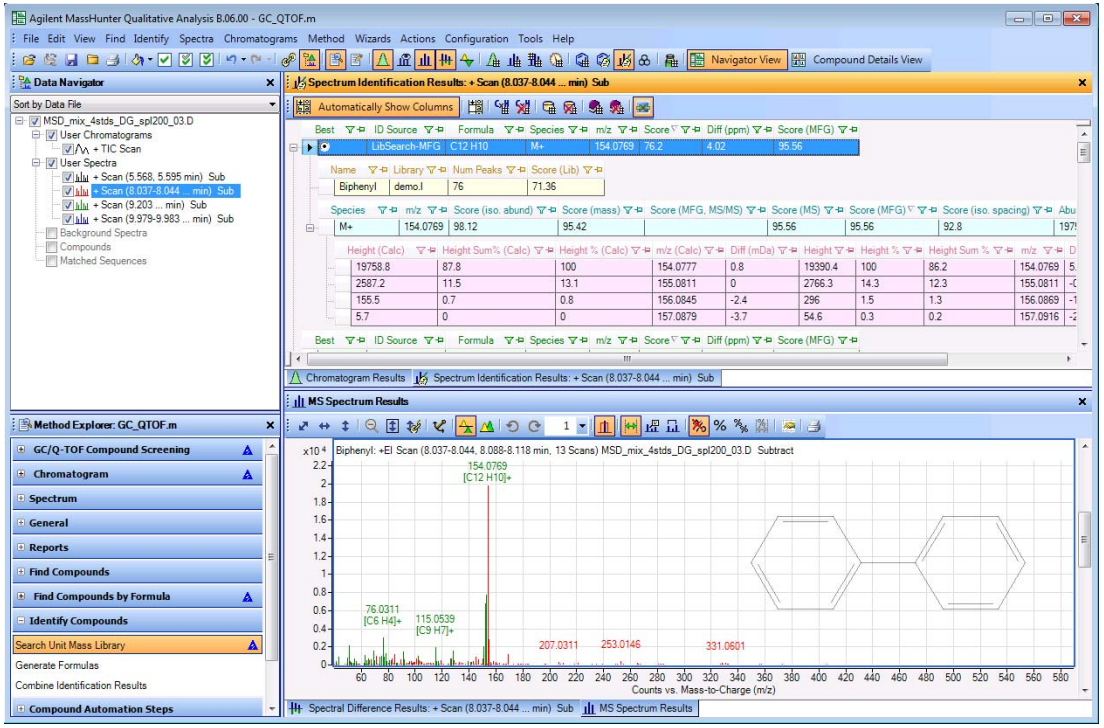



**Figure 48** Results for Library Search and Generate Formulas for first peak spectra

**6** Review results for each spectrum in the MS Peaks One window.

**a** Click **View >MS Spectrum Peak List 1**.

**b** Right-click and click Add/Remove Columns.

**c** Verify that the columns shown in Figure 49 are in the **Show these columns** list.

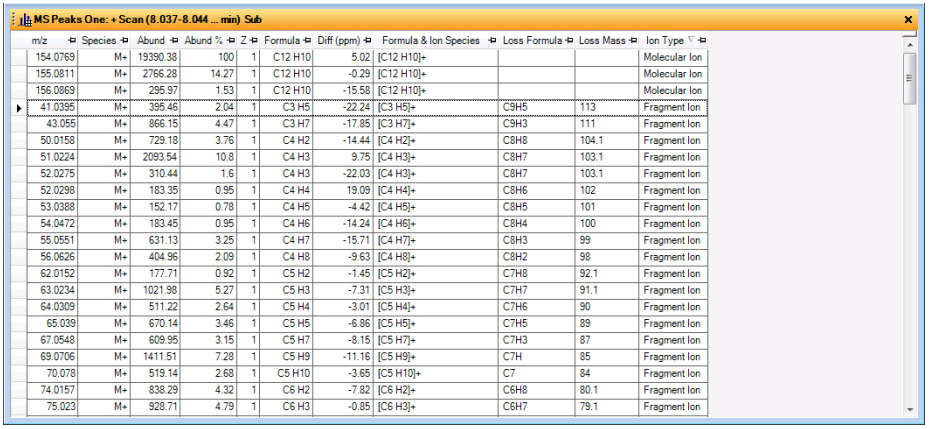
**d** Sort by the **Ion Type** column.

**e** If the Ion Type is Fragment Ion, then the Formula & Ion Species is shown in green on each peak in the MS Spectrum Results window.

- The fragment ions are displayed in green in the MS Spectrum Results window.
- The **Ion Type** can be Molecular Ion, Fragment Ion or blank. If it is a Fragment Ion, then the Loss Formula and Loss Mass column shows the Formula and Mass that accounts for getting to that ion from the Molecular Ion. The Formula & Ion Species shows the formula and ion species for that ion.

Task 17. Generate formulas and search library for peak spectra

| Step | Detailed Instructions | Comments |
|---|---|---|



MS Peaks One: + Scan (8.037-8.044 ... min) Sub

| m/z | Species | Abund | Abund % | Z | Formula | Diff (ppm) | Formula & Ion Species | Loss Formula | Loss Mass | Ion Type |
|---|---|---|---|---|---|---|---|---|---|---|
| 154.0769 | M+ | 19390.38 | 100 | 1 | C12 H10 | 5.02 | [C12 H10]+ | | | Molecular Ion |
| 155.0811 | M+ | 2766.28 | 14.27 | 1 | C12 H10 | -0.29 | [C12 H10]+ | | | Molecular Ion |
| 156.0869 | M+ | 295.97 | 1.53 | 1 | C12 H10 | -15.58 | [C12 H10]+ | | | Molecular Ion |
| 41.0395 | M+ | 395.46 | 2.04 | 1 | C3 H5 | -22.24 | [C3 H5]+ | C9H5 | 113 | Fragment Ion |
| 43.055 | M+ | 866.15 | 4.47 | 1 | C3 H7 | -17.85 | [C3 H7]+ | C9H3 | 111 | Fragment Ion |
| 50.0158 | M+ | 729.18 | 3.76 | 1 | C4 H2 | -14.44 | [C4 H2]+ | C8H8 | 104.1 | Fragment Ion |
| 51.0224 | M+ | 2093.54 | 10.8 | 1 | C4 H3 | 9.75 | [C4 H3]+ | C8H7 | 103.1 | Fragment Ion |
| 52.0275 | M+ | 310.44 | 1.6 | 1 | C4 H3 | -22.03 | [C4 H3]+ | C8H7 | 103.1 | Fragment Ion |
| 52.0298 | M+ | 183.35 | 0.95 | 1 | C4 H4 | 19.09 | [C4 H4]+ | C8H6 | 102 | Fragment Ion |
| 53.0388 | M+ | 152.17 | 0.78 | 1 | C4 H5 | -4.42 | [C4 H5]+ | C8H5 | 101 | Fragment Ion |
| 54.0472 | M+ | 183.45 | 0.95 | 1 | C4 H6 | -14.24 | [C4 H6]+ | C8H4 | 100 | Fragment Ion |
| 55.0551 | M+ | 631.13 | 3.25 | 1 | C4 H7 | -15.71 | [C4 H7]+ | C8H3 | 99 | Fragment Ion |
| 56.0626 | M+ | 404.96 | 2.09 | 1 | C4 H8 | -9.63 | [C4 H8]+ | C8H2 | 98 | Fragment Ion |
| 62.0152 | M+ | 177.71 | 0.92 | 1 | C5 H2 | -1.45 | [C5 H2]+ | C7H8 | 92.1 | Fragment Ion |
| 63.0234 | M+ | 1021.98 | 5.27 | 1 | C5 H3 | -7.31 | [C5 H3]+ | C7H7 | 91.1 | Fragment Ion |
| 64.0309 | M+ | 511.22 | 2.64 | 1 | C5 H4 | -3.01 | [C5 H4]+ | C7H6 | 90 | Fragment Ion |
| 65.039 | M+ | 670.14 | 3.46 | 1 | C5 H5 | -6.86 | [C5 H5]+ | C7H5 | 89 | Fragment Ion |
| 67.0548 | M+ | 609.95 | 3.15 | 1 | C5 H7 | -8.15 | [C5 H7]+ | C7H3 | 87 | Fragment Ion |
| 69.0706 | M+ | 1411.51 | 7.28 | 1 | C5 H9 | -11.16 | [C5 H9]+ | C7H | 85 | Fragment Ion |
| 70.078 | M+ | 519.14 | 2.68 | 1 | C5 H10 | -3.65 | [C5 H10]+ | C7 | 84 | Fragment Ion |
| 74.0157 | M+ | 838.29 | 4.32 | 1 | C6 H2 | -7.82 | [C6 H2]+ | C6H8 | 80.1 | Fragment Ion |
| 75.023 | M+ | 928.71 | 4.79 | 1 | C6 H3 | -0.85 | [C6 H3]+ | C6H7 | 79.1 | Fragment Ion |

**Figure 49** MS Peaks One table with **Ion Type**, **Loss Formula**, **Loss Mass**, and **Formula & Ion Species** columns

**7** (optional) Close the data file.

- You can proceed to the next task to learn how to save results.

**a** Click **File > Close Data File**.

**b** Click **Close**.

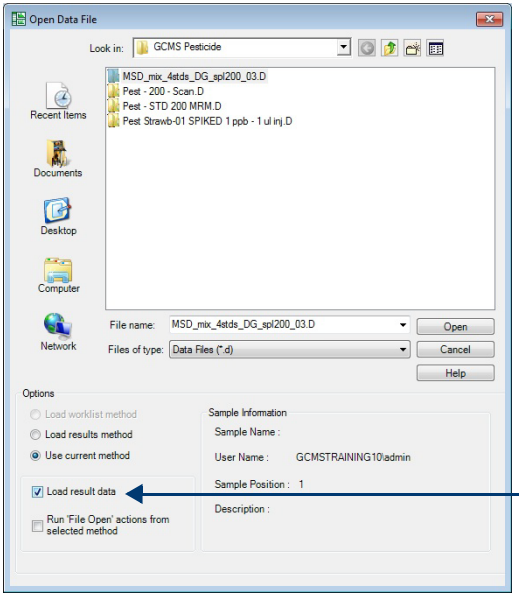
- If you want to save these results, see "Task 18. Save results" on page 72.

# Task 18. Save results

In this task, you save the results for the current data file.

Task 18. Save results

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** Save the results for the current data file and close the data file. | **a** Click **File > Save Results**.<br>**b** Click **File > Close Data File**. | • You can only save one set of results with a data file. If you already have saved results with the current data file, then these results are overwritten when you click **File > Save Results**. |
| **2** Open the data file and load the results. | **a** Click **File > Open Data File**. The "Open Data File" dialog box opens.<br>**b** Select a data file. For this example, select the data file MSD_mix_4stds_DG_spl200_03.d.<br>**c** Mark the **Load result data** check box.<br>**d** Click the **Open** button. | |




**The Load result data check box is marked.**

**Figure 50** Open Data File dialog box

Task 18. Save results

| Step | Detailed Instructions | Comments |
|---|---|---|
| **3** Examine the results. | **a** Click the **Spectrum Identification Results** window.<br>**b** Review the results. | |

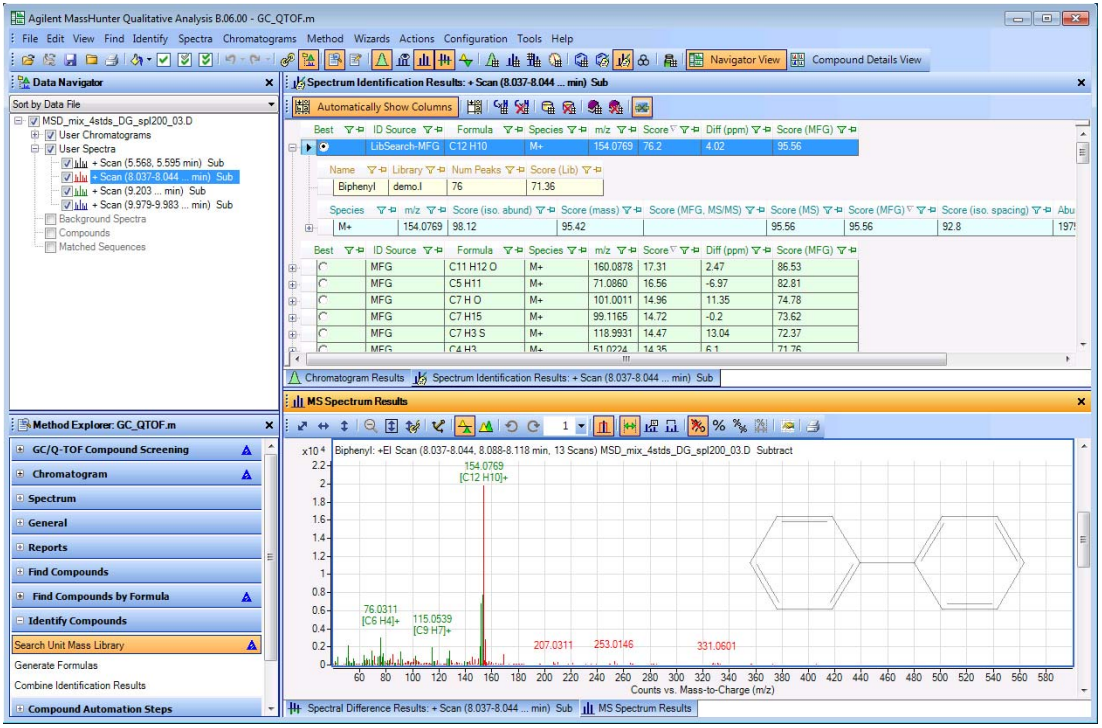



**Figure 51** Results for Library Search and Generate Formulas for first peak spectra

| | | |
|---|---|---|
| **4** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **No** when asked whether or not to save results. | |

Task 18. Save results

| Step | Detailed Instructions | Comments |
|---|---|---|
| **5** Open the data file again and do not load the results. | **a** Click **File > Open**. The **Open Data File** dialog box opens.<br>**b** Select a data file. For this example, select the data file MSD_mix_4stds_DG_spl200_03.d.<br>**c** Clear the **Load result data** check box.<br>**d** Click the **Open** button. | • If you do not load results, then by default a TIC is opened when you open a data file. If you mark the Run 'File Open' actions from selected method check box, then the File Open actions are run, instead. See the online help for more information. |

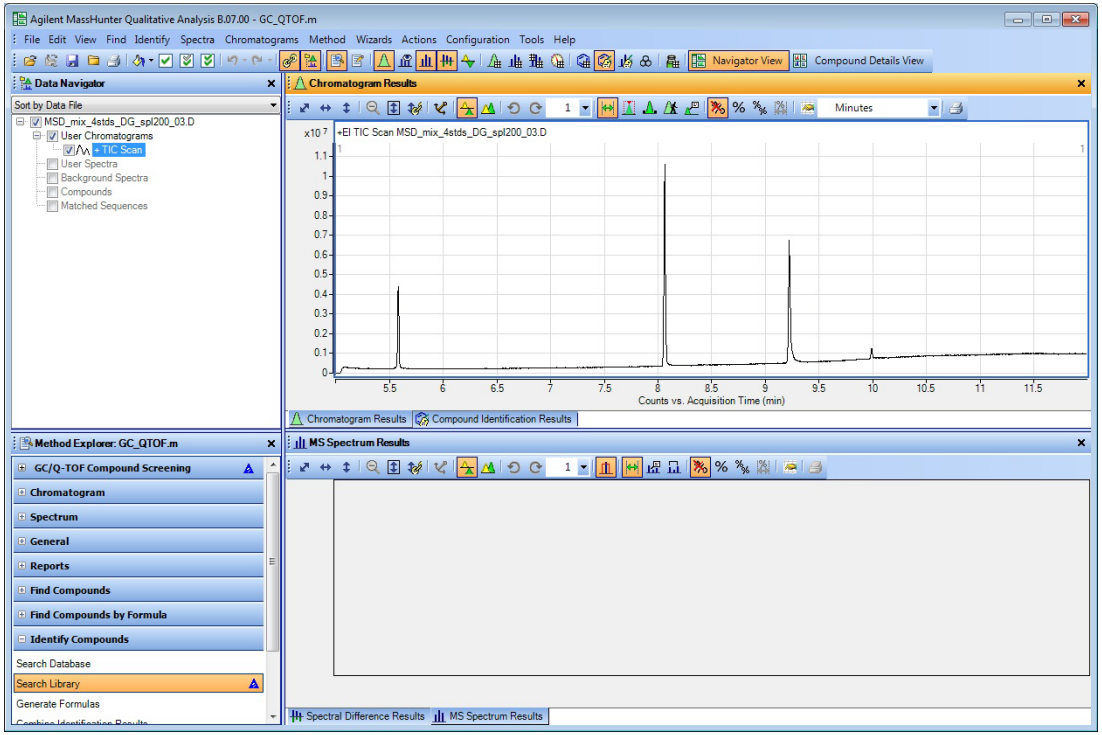



**Figure 52** Results for Library Search and Generate Formulas for first peak spectra

| | | |
|---|---|---|
| **6** Close the data file. | **a** Click **File > Close Data File**.<br>**b** Click **No**. | |

# Exercise 3
# Use workflows, export and print



In these tasks, you learn to set up and run a qualitative analysis method. Then, you run the actions within the automated method when you open a data file.

Two different workflows are used for these examples. See "Workflows" on page 104 for more information.

The General workflow supports GC/QQQ, GC/Q-TOF and LC/MS data. The GC/Q-TOF Compound Screening workflow supports GC/Q-TOF data.

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

# Task 19. Set up and run a qualitative analysis method using the general workflow

When you first start to use the Qualitative Analysis program, the method default.m is loaded. You can make changes to the opened method and save it, or open a new method, make changes and save the method. You cannot overwrite the method default.m.

You can also set up to run specific actions in the method when you open a data file. When you open a data file, you can also load the method that was used to create the results that are stored with the data file. This method is automatically saved whenever you save the results with the data file. The General workflow can be used with either GC/MS or LC/MS data files.

Task 19. Set up and run a qualitative analysis method using the General workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **Pest - STD 200 MRM.d** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **Pest - STD 200 MRM.d** data file in the **GCMS Pesticide** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | • You use either the General Workflow or the GC/Q-TOF Compound Screening workflow when working with GC/MS data. |
| **2** Configure the user interface to work with GC data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | • For this example, select the General workflow. |
| **3** Set up the method to extract a TIC chromatogram.<br>• Define a TIC chromatogram for MS/MS data. | **a** In the Method Explorer window, select **Chromatogram > Define Chromatograms**.<br>**b** Delete the **BPC** chromatogram from the **Defined chromatograms** list.<br>**c** Select **TIC** as the **Type**.<br>**d** Make sure the MS Level is **MS/MS**.<br>**e** Click **Add**. | |

Task 19. Set up and run a qualitative analysis method using the General workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **4** Edit the method to integrate the data.<br>• Limit the integration to the four highest peaks. | **a** In the Method Explorer window, click **Chromatogram > Integrate (MS/MS)**.<br>**b** Click the **Peak Filters** tab.<br>**c** In the Maximum number of peaks section, mark the **Limit (by height) to the largest** check box.<br>**d** Type 4. | • Updating a value in the Peak Filters tab in the Chromatogram > Integrate (MS) section also updates values in other sections of the Method Explorer. Blue triangles appear to show these other sections. |

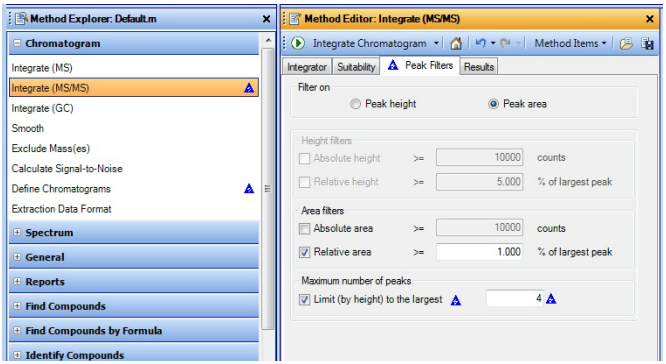



**Figure 53** The Chromatogram > Integrate (MS/MS) > Peak Filters tab

**You can click the Save Method icon to save the current method.**

| | | |
|---|---|---|
| **5** Test the integration to make sure that only 4 integrated peaks appear. | • Click the **Integrate Chromatogram** icon to integrate the data file. | |
| **6** Save the method to *iii*_GCexercise1, where "*iii*" are your initials. | **a** From the top menu, click **Method > Save As**.<br>**b** Type **iii_GCexercise1**.<br>**c** Click the **Save** button. | • Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear. |
| **7** Change the peak spectrum background to use the spectrum at the start of a peak. | **a** In the Method Explorer window, click **Spectrum > Extract (MS/MS)**.<br>**b** Click **Peak Spectrum Extraction (MS/MS)**.<br>**c** For the Peak spectrum background, select **Spectrum at peak start**. | • If you make any additional changes after saving the method, then the blue triangles are added. |

Task 19. Set up and run a qualitative analysis method using the General workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|

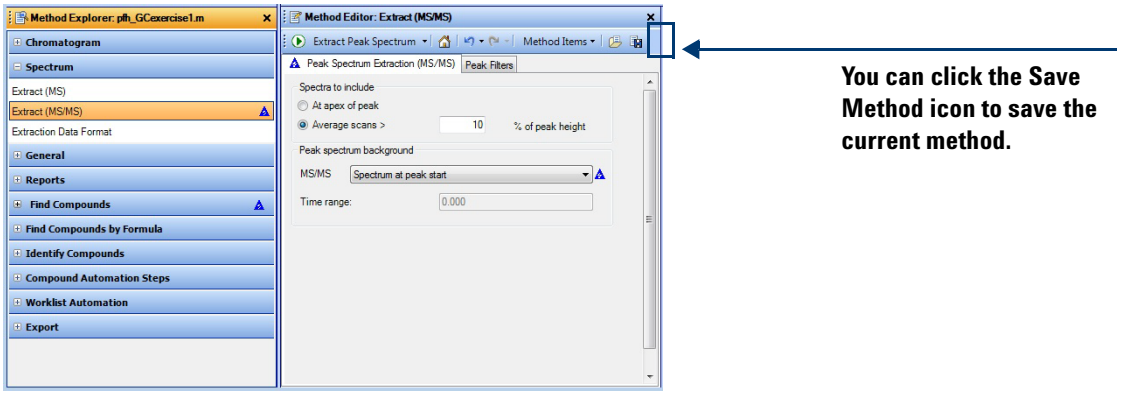
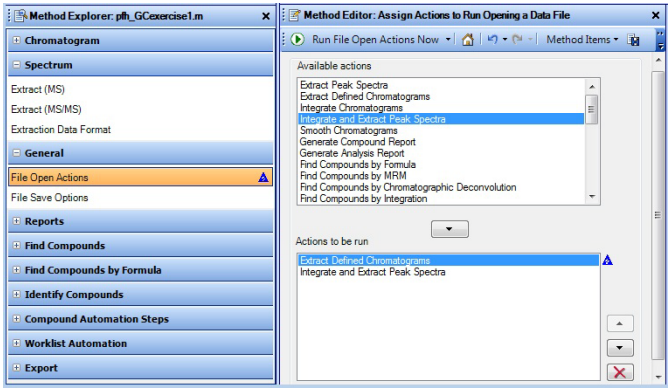



**Figure 54** The Spectrum > Extract (MS/MS) > Peak Spectrum Extraction (MS/MS) tab

| | | |
|---|---|---|
| **8** Test the MS spectrum extraction to make sure a background spectrum is subtracted. | • Click the **Extract Peak Spectrum** icon to run the action on the selected peak in the data file. | |
| **9** Save the method. | • Save the method in one of three ways:<br>  • Click the **Save Method** icon in the Method Editor.<br>  • Right-click the Method Editor, and click **Save Method**.<br>  • From the top menu click **Method > Save**. | • The Save Method icon is shown in Figure 54 on page 78 |
| **10** Set up the method to automate the actions whose parameters you just changed when you open a data file.<br>  • List the actions to be performed when this or another data file is opened.<br><br>Hint: Look under General in Method Explorer. | **a** In the Method Explorer window, select **General > File Open Actions**.<br>**b** Select **Integrate and Extract Peak Spectra** from the **Available actions** list.<br>**c** Click the **Add** button, , to move the selected action to the **Actions to be run** list.<br>You can also double-click on the selected action to move it to the other list. | • The action Extract Defined Chromatograms is in the Actions to be run list by default. The Extract Defined Chromatograms action needs to be first in the list because you first need to extract chromatograms, and then you can integrate and extract peak spectra. |

Task 19. Set up and run a qualitative analysis method using the General workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **11** Test the File Open Actions. | • Click the **Run File Open Actions Now** icon to run the actions on the data file. | • The chromatograms and spectra are not overwritten. New chromatograms and spectra are added. |




**Two different actions are part of the Actions to be run list. The first action is to extract the defined chromatograms. Then, that chromatogram is integrated and peaks are extracted.**

**Figure 55** The General > File Open Actions section in the Method Editor

| | | |
|---|---|---|
| **12** Save the method. | • Click the **Save Method** icon in the Method Editor window, | |
| **13** Set up the method to automate the actions when the method is run during a worklist.<br>• List the actions to be performed when this or another data file is opened.<br><br>Hint: Look under Worklist Automation in the Method Explorer window | **a** In the Method Explorer window, select **Worklist Automation > Worklist Actions**.<br>**b** Remove **Generate Analysis Report** from the **Actions to be run** list. | |
| **14** Test the Worklist Actions. | • Click the **Run Worklist Actions Now** icon to run the actions on the data file. | • The chromatograms and spectra are not overwritten. New chromatograms and spectra are added. |

Task 19. Set up and run a qualitative analysis method using the General workflow

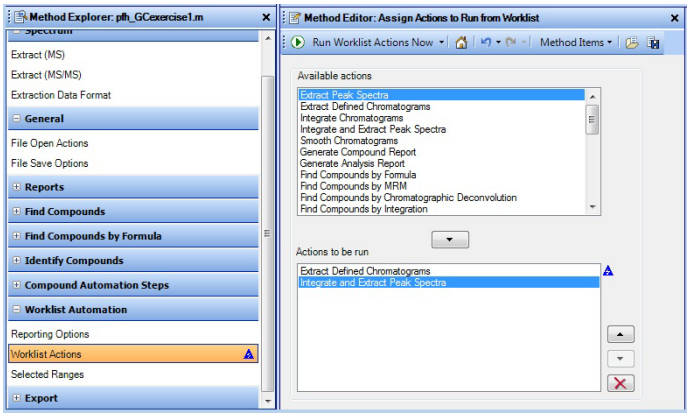
| Steps | Detailed Instructions | Comments |
|---|---|---|
|  | | **Two different lists of actions are included in a method. The first list of actions (File Open Actions) can be run when a data file is opened. The second list of actions (Worklist Actions) is run when the method is run.** |
| **15** Save the method and close the data file without saving results. | **a** Click the **Save Method** icon in Method Editor,<br>**b** Click **File > Close Data File,** and click **No** when asked to save results. | |



**Figure 56** The Worklist Automation > Worklist Actions section in the Method Editor

# Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow

In this task you set up a qualitative analysis method that contains a list of analysis actions to run in a specific order. These include extracting and integrating chromatograms, extracting spectra, searching a library for peak spectra, generating formulas for spectra and printing an analysis report.

Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the TIC for the **MSD_mix_4stds_DG_spl200_03.d** data file. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box and click **Open**. | |
| **2** Configure the user interface to work with GC data. | • Follow the instructions in "Task 2. Configure User Interface for GC/MS data" on page 12. | • For this example, select the GC/Q-TOF Compound Screening workflow. |
| **3** Make sure that a TIC is extracted. | **a** In the Method Explorer window, select **Chromatogram**.<br>**b** Click the **Define Chromatograms** section.<br>**c** In the Method Editor window, verify that the chromatogram in the **Defined chromatograms** section is a TIC. If it is not, select **TIC** as the **Type**. Click the **Change** button. | • |

Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **4** Review parameters for the Find by Chromatogram Deconvolution algorithm. | **a** Click the **GC/Q-TOF Compound Screening > Find by Chromatogram Deconvolution** section in the Method Explorer window.<br>**b** Click the **Mass Filter** tab.<br>**c** Set the **Absolute height** value to `13000`.<br>**d** Click the **Results** tab.<br>**e** Click the **Highlight all compounds** button.<br>**f** Review the results on each tab. | • Look at the sections for the GC/Q-TOF Compound Screening workflow.<br>• Note the six sections in this workflow. All of these sections are duplicates of sections that are already part of the method explorer.<br>• Note that blue triangles appear in other sections of Method Explorer. These indicate that the same parameter values have been changed elsewhere as well. |
| **5** Review parameters for the Identify by Library Search algorithm. | **a** Click the **GC/Q-TOF Compound Screening > Identify by library search** section in the Method Explorer window.<br>**b** Click the **Add Library** button. Select a library and click **Open**.<br>**c** (optional) Click the **Remove Library** button to remove a library if you do not want to use it.<br>**d** Review the parameters on each tab. | • The demo.l library is installed in the \MassHunter\Library folder.<br>• The NIST11.l (or other version of the NIST library) may also be installed in this folder. |
| **6** Save the method to *iii*_GCexercise2, where "*iii*" are your initials. | **a** From the top menu, click **Method > Save As**.<br>**b** Type **iii_GCexercise2**.<br>**c** Click the **Save** button. | |
| **7** Set up the method to automate the actions when a data file is opened.<br>• List the actions to be performed when this or another data file is opened.<br><br>Hint: Look under General in the Method Explorer window | **a** In the Method Explorer window, select **General > File Open Actions**.<br>**b** Remove all actions from the **Actions to be run** list.<br>**c** Add **Extract Defined Chromatograms**.<br>**d** Add **Find Compounds by Chromatographic Deconvolution**.<br>**e** Add **Search Library for Compounds**. | |
| **8** Test the File Open Actions. | • Click the **Run 'File Open' Actions Now** icon  to run the actions on the data file. | • The chromatograms and spectra are not overwritten. New chromatograms and spectra are added. |

Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow

| Steps | Detailed Instructions | Comments |
|---|---|---|

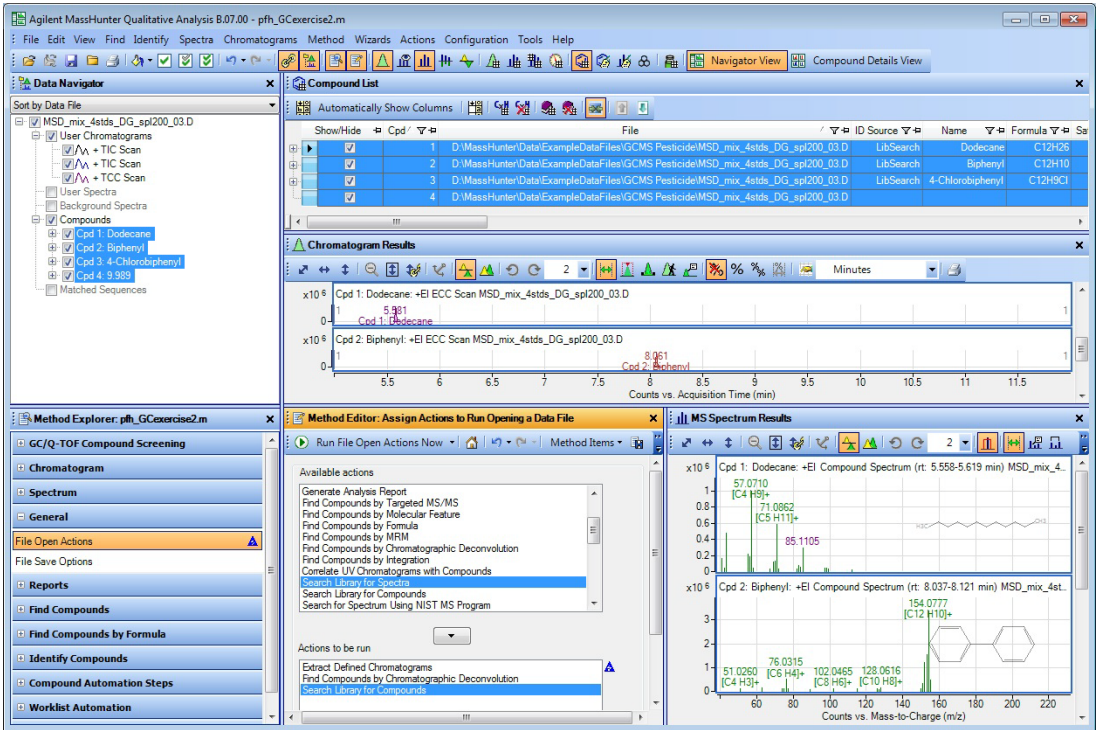



**Figure 57** Results from running worklist actions on the GC/Q-TOF data

| | | |
|---|---|---|
| **9** Save the method to *iii*_GCexercise2, where "*iii*" are your initials. | • Save the method in one of three ways:<br>•Click the **Save Method** icon in the Method Editor.<br>•Right-click the Method Editor, and click **Save Method**.<br>• From the top menu click **Method > Save**. | • If this method is run during a Data Acquisition worklist, then the Worklist Actions on this tab are executed in the given order. |
| **10** Close the data file without saving results. | **a** Click **File > Close Data File**.<br>**b** Click **No** when asked to save results. | |

# Task 21. Export a CEF file

You can export a CEF file containing compound information. This CEF file can be imported into other programs such as MassHunter Quantitative Analysis and Mass Profiler Professional. You can also import compounds that were exported in a CEF file.

Task 21. Export a CEF file

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the **MSD_mix_4stds_DG_spl200_03.d** data file and run the File Open actions for the method iii_GCexercise2.m which was created in "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box.<br>**d** Mark the **Run 'File Open' actions from selected method** check box.<br>**e** Click the **Use current method** button, and click **Open**. | • If you finished "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81, then the current method is iii_GCexercise2.m. This method is set up to run the Find Compounds by Chromatogram Deconvolution algorithm and then run the Search Library algorithm on each compound. |
| **2** Export a CEF file. | **a** To interactively export the file, click **File > Export > as CEF.**<br>**b** Click the **All results** button.<br>**c** Select the location of the export file.<br>**d** Click **OK**. | • A CEF file is used to export compounds. |

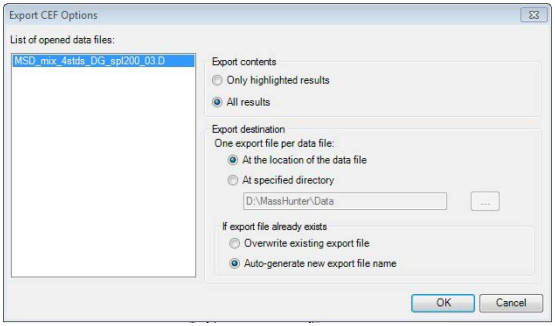



**Figure 58** Export CEF Options dialog box

# Task 22. Print an analysis report

Whenever you want to print an analysis report after performing any of the tasks in this exercise or the next one, use these instructions.

An analysis report can contain the results from extracting and integrating chromatograms, extracting spectra, finding compounds, searching the database for peak spectra or generating formulas from peak spectra.

Task 22. Print an analysis report

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** If the **MSD_mix_4stds_DG_spl200_03.d** data file is not loaded, then open this data file and run the File Open actions for the method iii_GCexercise2.m which was created in "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box.<br>**d** Mark the **Run 'File Open' actions from selected method** check box.<br>**e** Click the **Use current method** button and click **Open**. | • If you finished "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81, then the current method is iii_GCexercise2.m. This method is set up to run the Find Compounds by Chromatogram Deconvolution algorithm and then run the Search Library algorithm on each compound. |
| **2** Change the analysis report selections in the method:<br>• Mark the check boxes for the chromatograms, spectra or tables you want to print.<br>• Clear the check boxes for the chromatograms, spectra or tables which you do not want to print. | **a** In the Method Explorer window, click **Reports > Analysis Report**.<br>**b** Mark the check boxes for any additional selections you want to print.<br>**c** Clear any check boxes for items which you do not want to print. | • The Analysis report only contains the information that you mark in this section.<br>• If some results are not available, then those results are not included, even if those results are marked in this section. For example, if you have not integrated the chromatogram, then the peak table is not included. |

Task 22. Print an analysis report (continued)

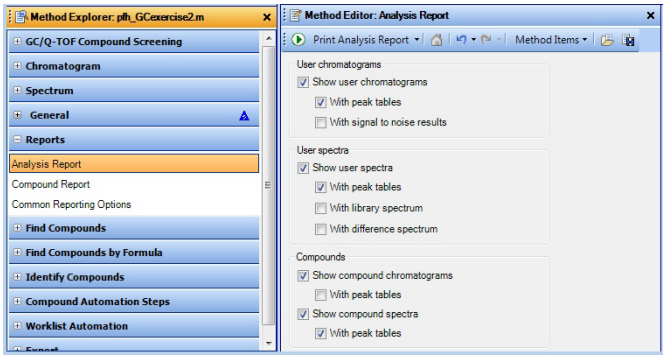
| Steps | Detailed Instructions | Comments |
|---|---|---|
|  | | **By default, the Method Editor window is floating. It is visible as a separate window from the rest of the Qualitative Analysis program. To anchor the window, right-click the title of the window and click Floating. You can also double-click the title bar to anchor the window.** |



**Figure 59** Analysis Report section in the Method Explorer and Method Editor windows

**3** Print the report.

**a** You can interactively print the report in multiple ways:
- From the main menu, click **File > Print > Analysis Report.**
- From the main toolbar, click the Printer icon.
- Click the **Print Analysis Report** icon,  in the Method Editor toolbar when the Analysis Report section is selected.
- Right-click the Analysis Report section in the Method Editor, and click **Print Analysis Report**.
- From the data file shortcut menu in the Data Navigator, click **Analysis Report**.

**b** Click one of the options under **Report contents**.

**c** (optional) Mark the **Separate report per data file** check box.

**d** Mark the **Print report** check box and select a printer.

**e** Mark the **Print preview** check box.

**f** Click the **OK** button.

- The Run icon  in the Method Editor toolbar sometimes allows you to choose an action from a set of possible actions. For example, if you switch to the Reports > Common Reporting Options section of the Method Editor window, four different actions are possible when you click the Run icon. If you click the arrow, a list of possible actions is shown, and you can choose which action to do. Choosing a different action from the list changes the default action. If you simply click the Run button, the current default action is performed.

Task 22. Print an analysis report (continued)

| Steps | Detailed Instructions | Comments |
|---|---|---|

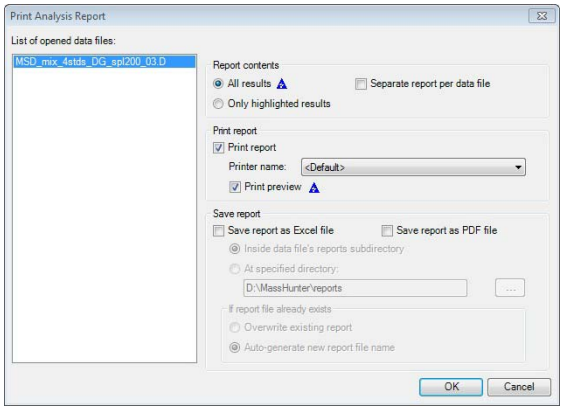



**Figure 60** Print Analysis Report dialog box

**g** Review the report.

**h** Click the **Close Print Preview** icon in the toolbar.

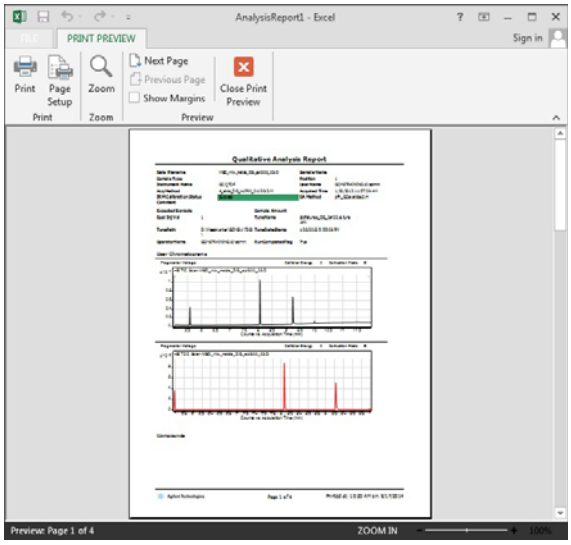



**Figure 61** Print Preview window with Analysis Report

# Task 23. Print a compound report

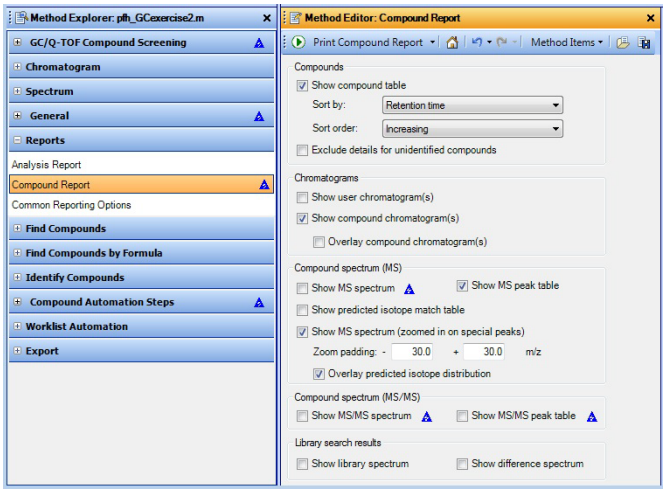
Whenever you want to print a compound report, use these instructions.

Task 23. Print a compound report

| Step | Detailed Instructions | Comments |
|---|---|---|
| **1** If the **MSD_mix_4stds_DG_spl200_03.d** data file is not loaded, then open this data file and run the File Open actions for the method iii_GCexercise2.m which was created in "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81. | **a** If the program is not open, double-click the **MassHunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**.<br>**b** Click the **MSD_mix_4stds_DG_spl200_03.d** data file in the **GC** example data file folder.<br>**c** Clear the **Load result data** check box.<br>**d** Mark the **Run 'File Open' actions from selected method** check box.<br>**e** Click the **Use current method** button and click **Open**. | • If you finished "Task 20. Set up and run a method using the GC/Q-TOF Compound Screening workflow" on page 81, then the current method is iii_GCexercise2.m. This method is set up to run the Find Compounds by Chromatogram Deconvolution algorithm and then run the Search Library algorithm on each compound. |
| **2** Change some of the selections in the method for compound reports:<br>• Turn off viewing the MS spectra zoomed in on special peaks.<br>• Turn off the MS/MS options in the report. | **a** In Method Explorer, click **Reports > Compound Report**.<br>**b** (optional) Clear the **Show MS spectrum** check box.<br>**c** (optional) Clear the **Show MS/MS spectrum** check box.<br>**d** (optional) Clear the **Show MS/MS peak table** check box. | • These check boxes allow you to specify what information to include in a report if it is available. If the information is not available, that section is automatically skipped. For example, MS/MS results are never included when the data file only has MS data. |

Task 23. Print a compound report

| Step | Detailed Instructions | Comments |
|---|---|---|




The Overlay compound chromatograms check box should be cleared for GC/Q-TOF data.

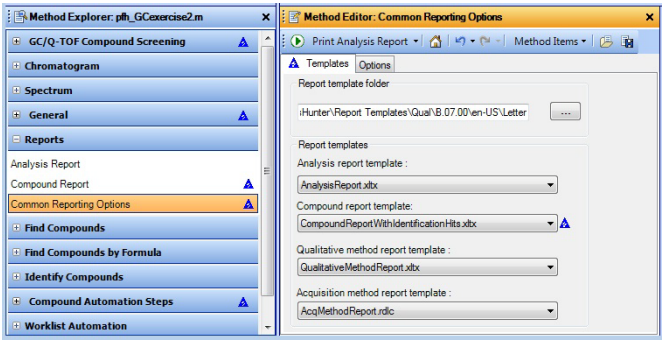
**Figure 62** Compound Report section in the Method Editor

**3** (optional) Choose a different compound report template.

**a** In the Method Explorer window, click **Reports > Common Reporting Options.**

**b** Select **CompoundReport WithIdentificationHits.xltx** as the **Compound report template**.

- Several different report templates are included with the software.
- You can customize a report template using Excel and the Report Designer add-in.




**You can use Excel and the Report Designer add-in to customize any of the templates that have the extension XLTX. You cannot customize the acquisition method report.**

**Figure 63** Common Reporting Options section in the Method Editor

Task 23. Print a compound report

| Step | Detailed Instructions | Comments |
|---|---|---|
| **4** Print the report. | **a** Click **File > Print > Compound Report** or click the arrow in the **Print Analysis Report** icon and click **Print Compound Report** to print the compound report.<br>**b** Mark the **Print preview** check box.<br>**c** Click **OK**. Examine the report.<br>**d** Click the **Close Print Preview** icon. | • In the Print Compound Report dialog box, you can select a different printer, select to save the report to a PDF or Excel file, select whether to print all results or only the highlighted results, and whether or not to combine different data files into one report.<br>• See the online Help or the Report Designer Training DVD for additional information. |

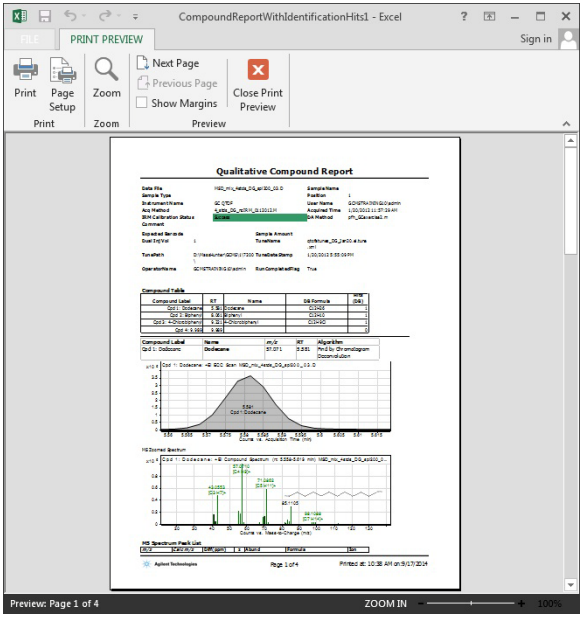



**Figure 64** Print Preview window with the Compound Report

| | |
|---|---|
| **5** Close the data file without saving results. | **a** Click **File > Close Data File**.<br>**b** Click **No** when asked if you want to save the results. |

# Reference

# Navigator View and Compound Details View

The Qualitative Analysis software has two different views. Different windows are available in each of these views. You select which view to use in the main toolbar. The following windows are available in both views:

- Method Explorer
- Method Editor
- Difference Results
- Compound List
- Compound Identification Results
- MS/MS Formula Details
- Structure Viewer

## Navigator View

The Navigator View is the default view. In this view, you can use the Data Navigator window to select different compounds, spectra and chromatograms.

If you are looking at multiple data files or at spectra, then you want to use this view. If you are looking at compounds, you can use this view or the Compound Details View.

## Compound Details View

This view provides a compound centric view of one data file. You can look at information on a single compound in different windows. You change the selected compound in the Compound List window.

If you are reviewing compounds that were found with the Find by Formula algorithm, then you want to use this view, especially if they were found with Fragment Confirmation. If you are reviewing other types of compounds, you can also use this view.

## Work with windows

When you first open the Qualitative Analysis program, you see four windows in the default layout: Data Navigator, Method Explorer, Chromatogram Results and MS Spectrum Results. You can switch between the Navigator View and the Compound Details View.

You can bring up seventeen other windows in the Navigator View using the View menu:

- Method Editor - allows you to edit method parameters separated into different tabs
- Spectrum Preview - allows you to quickly scan the spectra in a data file
- MS Spectrum Results - shows the MS and MS/MS spectra
- Difference Results - shows the difference results after a library search
- Deconvolution Results - shows the deconvoluted spectra
- Deconvolution Mirror Plot - shows two deconvoluted spectra in mirror image
- UV Spectrum Results - shows the UV spectra - only available for LC/MS data
- Integration Peak List - shows the integration results in a table
- MS Spectrum Peak List 1 - shows the peak table for the first spectrum selected
- MS Spectrum Peak List 2 - shows the peak table for the second spectrum selected
- MS Actuals - shows acquisition information for the highlighted spectrum
- Compound List - shows the compounds that are found using one of the Find Compounds algorithms
- Compound Identification Results - shows the identification information for the selected compound
- Spectrum Identification Results - shows the identification information for the selected spectra
- MS/MS Formula Details - shows a table containing possible formulas calculated for fragments seen in an MS/MS spectrum

- Structure Viewer - shows the structure associated with the current compound or spectra
- Sample Information - shows information about the highlighted data file
- Sequence Editor - allows you to edit a method sequence

You can also display three tool windows which are displayed when you start using the associated tool:

- Formula Calculator
- Mass Calculator
- Recalibrate

### Window Icons in the Main Toolbar

You open and close the windows with these icons on the main toolbar. Additional icons are available when the MassHunter BioConfirm software is installed. Commands in the View menu can also be used to open these windows.

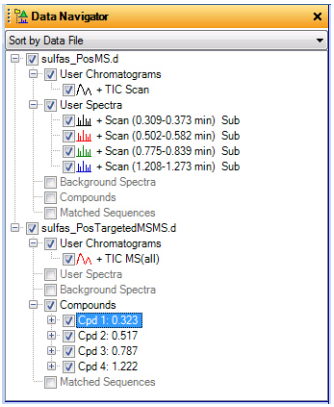
| Toolbar Icon | Window |
|---|---|
|  | Data Navigator window<br>Method Explorer window<br>Method Editor window |
|  | Chromatogram Results window<br>Spectrum Preview window<br>MS Spectrum Results window<br>Different Results window<br>Deconvolution Results window<br>Deconvolution Mirror Plot window<br>UV Spectrum Results window |
|  | Integration Peak List window<br>MS Spectrum Peak List 1 window<br>MS Spectrum Peak List 2 window<br>MS Actuals window |

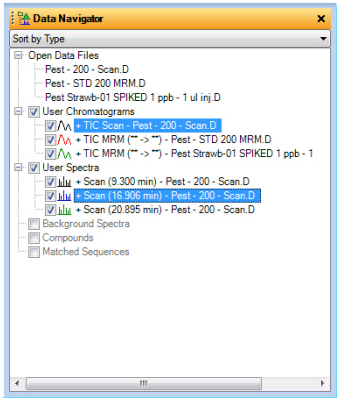
| Toolbar Icon | Window |
| --- | --- |
|  | Compound List window<br>Compound Identification Results window<br>Spectrum Identification Results window<br>MS/MS Formula Details window<br>Structure Viewer window<br>Sample Information window<br>Sequence Editor window |

# Work with result data in Data Navigator

## Data Navigator window and tools

The Data Navigator organizes all the results of extraction and spectrum selection either by data file or by data type. This window is only available in the Navigator View.




**Linked Navigation Icon**

When activated (default), highlighting a chromatogram in Data Navigator also highlights the corresponding spectra. The corresponding chromatogram and spectrum graphic results are also highlighted. Linked Navigation only works if you have used the Integrate and Extract Peak Spectra menu item from the Chromatograms Menu or have run any of the Compounds algorithms.

**Check Mark Tools**

**Single check mark** – Marks check boxes of all highlighted data**.**

**Dual check marks, one gray** – Marks check boxes of highlighted data and clears the other check boxes.

**Dual check marks** – Marks all check boxes.

Chromatograms and spectra are displayed when their check boxes are marked.

# Perform operations on the chromatogram

You can perform the following operations on the whole chromatogram or on a selected region of the chromatogram by using the menu items:

| Action | Menu Item |
|---|---|
| Change peak labels in chromatogram | Configuration > Chromatogram Display Options |
| Extract a chromatogram | Chromatograms > Extract Chromatograms |
| Extract defined chromatograms | Chromatograms > Extract Defined Chromatograms |
| Integrate the chromatogram | Chromatograms > Integrate Chromatogram |
| Integrate and extract peak spectra | Chromatograms > Integrate and Extract Peak Spectra |
| Integrate and Deconvolute Peak Spectra | Chromatograms > Integrate and Deconvolute Peak Spectra |
| Smooth the chromatogram | Chromatograms > Smooth Chromatogram |
| Subtract any chromatogram | Chromatograms > Subtract Any Chromatogram |
| Calculate Signal-to-Noise | Chromatograms > Calculate Signal-to-Noise |
| Find compounds from auto MS/MS data | Find > Find Compounds by Auto MS/MS |
| Find compounds from targeted MS/MS data | Find > Find Compounds by Targeted MS/MS |
| Find compounds for MS(1) data | Find > Find Compounds by Molecular Feature |
| Find compounds for GC/MS data | Find > Find Compounds by Chromatogram Deconvolution |
| Find compounds for MRM data | Find > Find Compounds by MRM |
| Find compounds by integration results | Find > Find Compounds by Integration |
| Find compounds that match specific formulas | Find > Find Compounds by Formula |

## Select range operations from shortcut menu

When you have selected a chromatographic range, you can also extract a spectrum and extract a spectrum to background, in addition to the operations mentioned above and others not mentioned.

1 To access these operations, click the Range Select tool (  ) in the Chromatogram Results toolbar.
2 Click at the point where you want to start the range, drag the cursor over a range, and release the mouse button.
3 Right-click anywhere in the chromatogram, and click the operation from the shortcut menu.

### Save results to the data file(s)

- Click the **Save** icon (  ), or click **File > Save Results**.

  When you exit the program, it also asks if you want to save the results to the data file, unless you have turned off this feature (you turn off this feature in the Message Box Options dialog box).

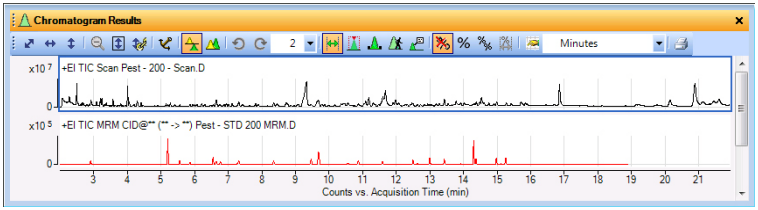
## Perform operations on an MS or MS/MS spectrum

You can perform the following operations on an MS or MS/MS spectrum or on a selected region of an MS or MS/MS spectrum by using the menu items:

| Action | Menu Item |
|---|---|
| View the m/z, abundance, charge state and other information about peaks in a spectrum | View > MS Spectrum Peak List 1 |
| Change the spectral peak labels | Configuration > MS and MS/MS Spectra Display Options |
| Subtract the background spectrum | Spectra > Subtract Background Spectrum |
| Subtract any spectrum | Spectra > Subtract Any Spectrum (and then click another spectrum) |
| Add two spectra together | Spectra > Add Any Spectrum (and then click another spectrum) |
| Search a database for entries that match specific masses in a spectrum | Spectra > Search Database for Spectrum Peaks |
| Generate formulas for the masses in the selected range in a spectrum | Spectra > Generate Formulas from Spectrum Peaks (when a range is selected in the MS spectrum) |
| Search Library | Identify > Search Library for Spectra or Spectra > Search Library for Spectra |

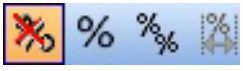
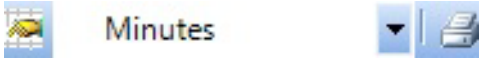
# Work with chromatographic visual data

## Chromatogram Results Window



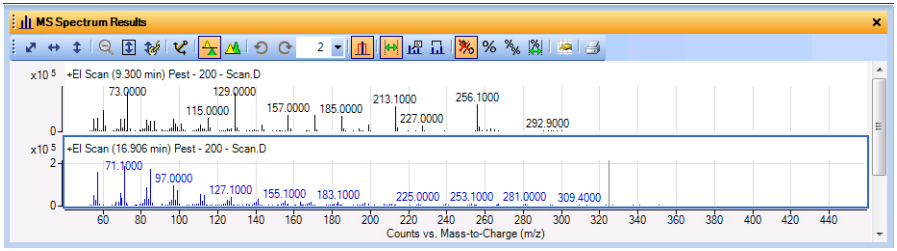

## Chromatogram Results Tools

| Toolbar Icon | Action |
|---|---|
| Zoom tools  | • Autoscale X-axis and Y-axis<br>• Autoscale X-axis<br>• Autoscale Y-axis<br>• Unzoom<br>• Autoscale Y-axis during Zoom<br>• Linked Y-axis mode |
|  | • **Anchor chromatogram** - the current chromatogram is always visible until you click the **Clear Anchor** command.<br>• **List mode** - chromatograms are drawn with each chromatogram having a separate Y-axis.<br>• **Overlay mode** - chromatograms are drawn with the same X-axis and the same Y-axis<br>• **Switches to previous plot**. This button is only available in Overlay mode.<br>• **Switches to next plot**. This button is only available in Overlay mode.<br>• **Number of spectra to show** at the same time before adding a scroll bar. |

| Toolbar Icon | Action |
|---|---|
| Select tools in order<br>  <br>One of these tools always has to be selected. The Range Select tool is selected in this image. The selected tool has an orange background. | • **Range Select** – When On, you can draw a range for chromatogram, for which you can perform actions.<br>• **Peak Select** – When On, you can select spectrum of an integrated peak at apex.<br>• **Manual Integration** – When On, you can integrate interactively.<br>• **Walk Chromatogram** – When On, you can see individual spectra as you click each point or use the left and right arrows on the keyboard.<br>• **Annotation** – When On, you can add image and text annotations to the chromatograms. |
| Normalization tools<br>  | • Stops normalizing chromatograms<br>• Normalizes all chromatograms to the largest peak in any of the chromatograms<br>• Normalizes all chromatograms to the largest peak in itself<br>• Normalizes each chromatogram to the highest peak within the selected range |
| Other tools<br>  | • Opens **Chromatogram Display Options** dialog box<br>• Sets the units used to display the chromatograms<br>• Prints the displayed chromatograms |

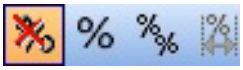
# Work with spectral visual data

## MS Spectrum Results Window




## MS Spectrum Results Tools

| Toolbar Icon | Action |
|---|---|
| Zoom tools  | • Autoscale X-axis and Y-axis<br>• Autoscale X-axis<br>• Autoscale Y-axis<br>• Unzoom<br>• Autoscale Y-axis during Zoom<br>• Linked Y-axis mode |
|  | • **Anchor spectrum** - the current spectrum is always visible until you click the Clear Anchor command<br>• **List mode** - spectra are drawn with each spectrum having a separate Y-axis<br>• **Overlay mode** - spectra are drawn with the same X-axis and the same Y-axis<br>• **Switches to previous plot**. This button is only available in Overlay mode.<br>• **Switches to next plot**. This button is only available in Overlay mode.<br>• **Number of spectra** to show at the same time before adding a scroll bar. |

| Toolbar Icon | Action |
|---|---|
| Select tools in order<br>  <br>One of these tools always has to be selected. The Range Select tool is selected in this image. The selected tool has an orange background. | • **Range Select** – When **On**, you can draw a range for spectra, for which you can perform actions<br>• **Annotation** – When **On**, you can add image and text annotations to the spectra.<br>• **Calipers** – When **On**, you can add a Delta Mass caliper to the selected spectrum. In the Deconvolution Results window, you can also add an Amino Acid caliper or a Modifications caliper. See the online Help for more information. |
| Normalization tools<br>  | • Stops normalizing spectra<br>• Normalizes all spectra to the largest peak in any of the spectra<br>• Normalizes all spectra to the largest peak in itself<br>• Normalizes each spectra to the highest peak within the selected range |
| Other tools<br>  | • Opens **MS and MS/MS Spectra Display Options** dialog box<br>• Prints the displayed spectra |

## Workflows

Workflows help you to customize the user interface for your application. Each workflow loads a different method that has parameters that are appropriate for that workflow. Also, each workflow loads a different layout; these layouts include customizing the columns shown in each table. Lastly, four of the layouts also add a special method editor section which contains copies of the sections in the method editor that are important for that workflow. Grouping the features that are used in a specific workflow together makes it easier for you to customize your method.

Several different workflows are available in the Qualitative Analysis program. They are:

- General

- **BioConfirm** - These workflows are only available if the BioConfirm software is installed, and the BioConfirm check box is marked in the **User Interface Configuration** dialog box. BioConfirm has several possible workflows, depending on the type of analysis that you want to do. BioConfirm is used with LC/MS data files.
- **Chromatogram Peak Survey**
- **Formula Confirmation and Sample Purity**
- **MS Target Compound Screening**
- **GC/Q-TOF Compound Screening**

If you are working with GC/MS data, you can select the **General** workflow or the **GC/Q-TOF Compound Screening** workflow. If you are working with LC/MS data, you can select any of the workflows except for the **GC Q-TOF Compound Screening** workflow.

### Specific Method

Each workflow loads a specific default method with appropriate settings for that workflow. For example, if you switch to one of the BioConfirm workflows, the **Target data type** for the Find Compounds by Molecular Feature algorithm is set to **Large molecules (proteins, oligos)**. This setting is appropriate for the BioConfirm workflows but not, by default, for the other workflows.

### Specific Layout

In addition, each workflow loads a specific layout. A layout consists of the following:

- Each window's position and size
- Which windows are tabbed
- Which windows are floating
- Which tabbed window is on top
- Which windows are visible by default
- Whether the status bar is visible

For each plot window (the Chromatogram Results window, the Spectrum Preview window, the MS Spectrum Results window, the Deconvolution window, the UV Results window, the Compound Chromatogram Results window, the Overall Chromatogram Results window, the Compound MS Spectrum Results window, and the Compound Fragment Spectrum Results window), the following are saved:

- Whether or not the graphics are overlaid
- Whether or not the Autoscale Y-Axis during Zoom mode is on
- Whether or not the Linked Y-Axis mode is on

For each table window, the following are saved

- Which columns are visible
- The order of the columns
- The width of each column
- Any filter that has been added to the table (only available for the Compound List table, the Compound Identification Results table, and the Spectrum Identification Results window).

### Specific section in the Method Explorer and Method Editor

Using the Method Editor with the General workflow, you can change almost all of the parameters in the method.

Each of the other workflows add a section to the Method Explorer. Each new section contains only the Method Editor tabs and sections that are useful in that workflow. Changing a parameter in the workflow section also changes the parameter in the corresponding section in the general Method Editor sections.

Two tabs are not repeated in the general Method Editor sections. The **Chromatogram Peak Survey Workflow > Spectrum Peak Identification** section and the **Chromatogram Peak Survey Workflow > Chromatogram Extraction > Chromatograms** tab are only included in the Chromatogram Peak Survey workflow. These sections only affect the Chromatogram Peak Survey algorithm. This algorithm is only used in this workflow, and in the **Chromatogram Peak Survey without Report** action and in the **Chromatogram Peak Survey with Analysis Report** action.

### Workflow methods and layouts

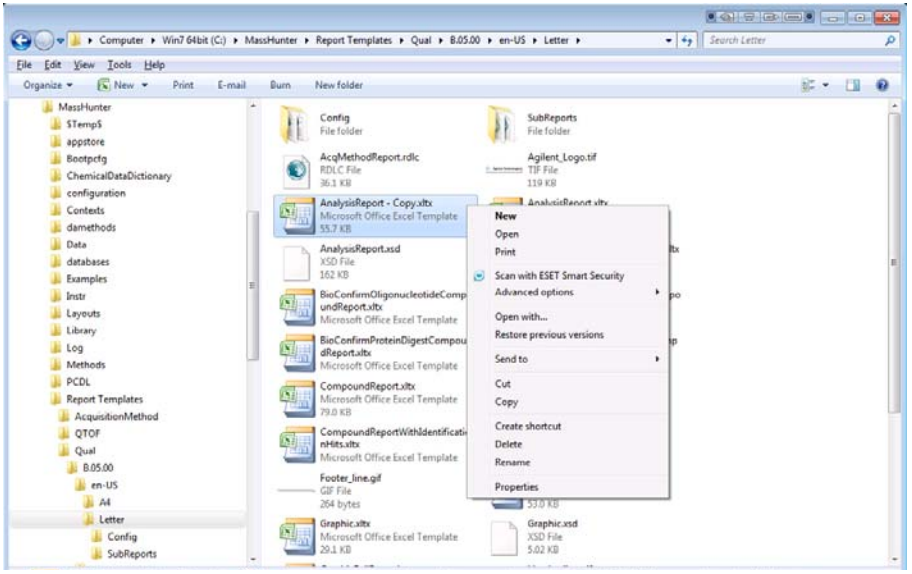
Additional default methods and layouts are provided for each workflow.

| Workflow | Method | Layout | Method Editor Section |
|---|---|---|---|
| General | default.m | Default.xml | None |
| Chromatogram Peak Survey | ChromPeakSurvey-Default.m | Default.xml | Chromatogram Peak Survey Workflow |
| Formula Confirmation and Sample Purity | SamplePurity-Default.m | SamplePurity-Default.xml | Formula Confirmation and Sample Purity Workflow |
| MS Target Compound Screening | Screening-Default.m | Screening-Default.xml | MS Target Compound Screening Workflow |
| GC Q-TOF Compound Screening | GC_Q-TOF.m | QTOFData.xml | GC/Q-TOF Compound Screening |

## Customize a report template

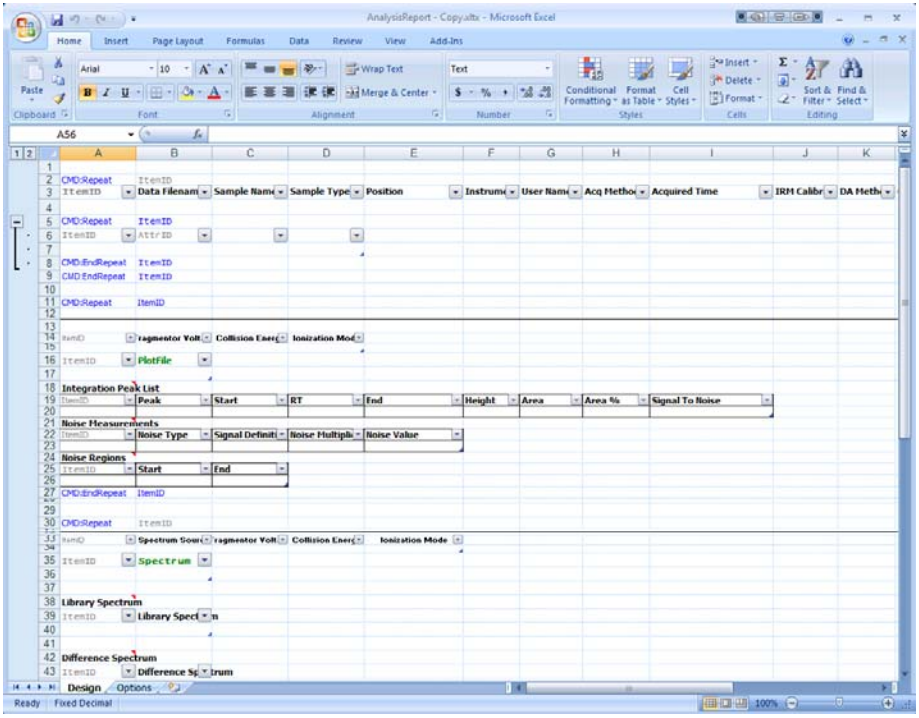
Please refer to either the online Help for the MassHunter Report Designer Add-in, the Report Designer Familiarization Guide or the Reporting Training DVD for detailed information on how to modify a report template. The following steps give you a quick look at what it means to customize a template.

1. Go to the folder that contains the report templates. By default, this folder is **\MassHunter\Report Templates\Qual\B.07.00\en-US\Letter**. You can select a different folder in the Method Explorer in the General > Common Reporting Options > Templates tab.
2. Make a copy of the template which you intend to modify.
3. Right-click the copy and click **Properties**. If necessary, clear the **Read-only** check box. Then, right-click the copy and click **Open** from the shortcut menu.




When the template is open, you can modify headers and footers. You can also add, remove or move parameter columns. You can refer to the online Help for more information.

Many templates are installed with the Qualitative Analysis program.



4 Make the changes you want to make.

   For more information on how to modify a template, see either the online Help for the MassHunter Report Designer add-in, or the *Agilent MassHunter Reporting - Training DVD*.

5 To save the new template, either click **Save** or click **Save As > Other Formats** from the Microsoft Office button.

6 Type an identifying name, and click **Save**.

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## In This Book

This guide contains information to learn to use your Agilent MassHunter Workstation Software - Qualitative Analysis with GC/MS data.



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