Agilent MassHunter Workstation Software

Qualitative Analysis

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

G3336-90023

Edition

Revision A, September 2014

Printed in USA

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Santa Clara, CA 95051 USA

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for B.07.00 and later revisions of the Agilent MassHunter Workstation Software - Qualitative Analysis program, until superseded.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 LC/MS data.

Before you begin the exercises, please read the instructions in "Before you begin these exercises..." on page 6.

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 compounds

In the first two sets of tasks, you find and identify low-concentration sulfa drugs within a complex matrix and generate their formulas for both TOF and Q-TOF data. You also do a molecular feature extraction on a protein digest with both TOF and Q-TOF data. These tasks can also be performed on Triple Quad data.

Exercise 3 Set up and run qualitative analysis methods using different workflows

In these tasks, you learn to set up and run any qualitative analysis method. You also learn to edit a method to automate the analysis and/or compound identification. Then you run the actions within the automated method when you open a data file. You also learn to create a method to perform automated actions with a worklist. Each of these tasks is done using a different workflow.

Exercise 4 Qualitative Analysis Wizards

Several wizards are included in the Qualitative Analysis program. These wizards lead you through the steps necessary to do certain tasks.

Identify Chromatogram Peaks wizard - This wizard shows you the different method editor sections and tabs that you

modify before running the Chromatogram Peak Survey without Analysis Report action.

Find Targets by: MFE + Database Search wizard - This wizard shows you the different method editor sections and tabs that you modify before running the Find by Molecular Feature algorithm and the Database Search algorithm.

Exercise 5 Analyzing Data Files acquired in All Ions MS/MS Mode

The program can qualify fragment ions when running the Find Compounds by Formula algorithm if the data file is acquired in All Ions MS/MS mode.

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 these exercises, you explore some of the many powerful capabilities of the Qualitative Analysis program for working with TOF, Q-TOF and Triple Quad 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.

Basic Tasks for All Data

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

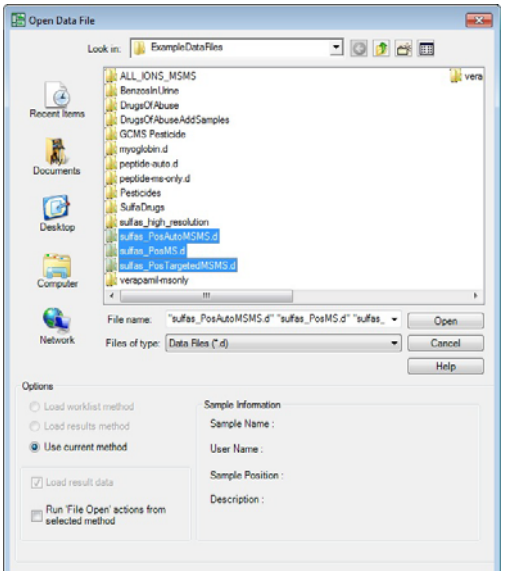
Steps	Detailed Instructions	Comments
1 Open the Qualitative Analysis program. • Open the data files, **sulfas_PosAutoMSMS**.d, **sulfas_PosMS.d** and **sulfas_PosTargetedMSMS.d** in the folder **\\MassHunter\Data**, or in the folder where you copied them.	**a** Double-click the **Agilent MassHunter Qualitative Analysis B.05.00** icon . The system displays the Open Data Files dialog box. **b** Go to the folder **\\MassHunter\Data** or the folder where the example files are located.	• The sulfas_PosMS.d file contains MS (TOF or Q-TOF) data, and the sulfas_PosAutoMSMS.d and sulfas_PosTargetedMSMS.d files contain both MS and MS/MS (Q-TOF) data. • You can get help for any window, dialog box, or tab by pressing the `F1` key when that window is active.
• Make sure that the **Use current method** button is clicked. • Make sure that the **Load result data** check box is clear. • 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 **sulfas_PosAutoMSMS.d**, **sulfas_PosMS.d** and **sulfas_PosTargetedMSMS.d**. **d** Click **Open**. All three data files are displayed in the Data Navigator window, and 1 to 3 chromatograms are displayed in the Chromatogram Results window. **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. • 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. • When you click the List Mode icon, the background of the icon changes to orange.

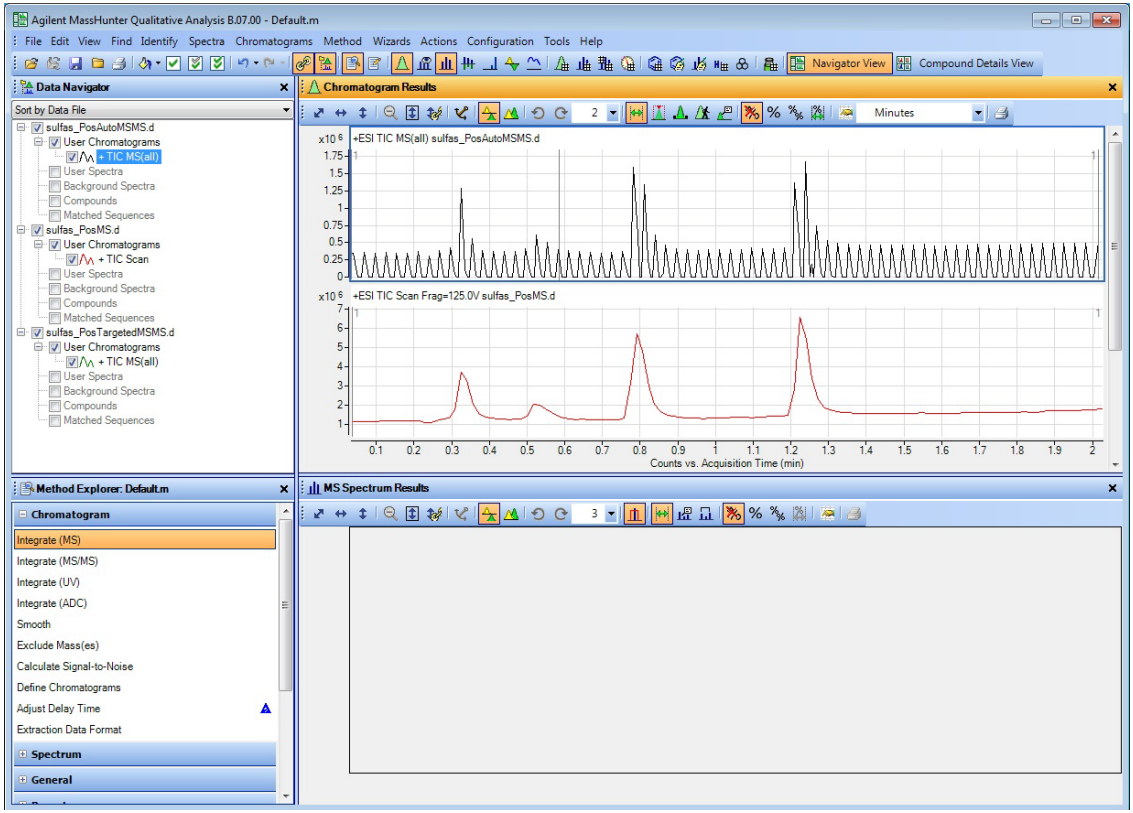


Figure 2 Qualitative Analysis main window

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

Steps	Detailed Instructions	Comments
2 Return the main window to the default workflow, General. The default method and layout are loaded. • Make sure you can see all three chromatograms.	**a** If necessary, click **Configuration > Configure for Workflow > General**. **b** In the Workflow Configuration dialog box, click the **Load workflow's default method** button and the **Load workflow's default layout** button. Clear the **Save current method** check box. Then, click the **OK** button. **c** Click the down arrow next to the Maximum Number of List Panes icon in the Chromatogram Results Toolbar, and select **3**.	• The display and plot settings remain the same even after you switch to the General workflow. These settings are set in Display Options dialog box for each type of data. You click the  button in the graphics window to change the display options. • You can change the layout if you click **Configuration > Window Layouts > Load Layout**.

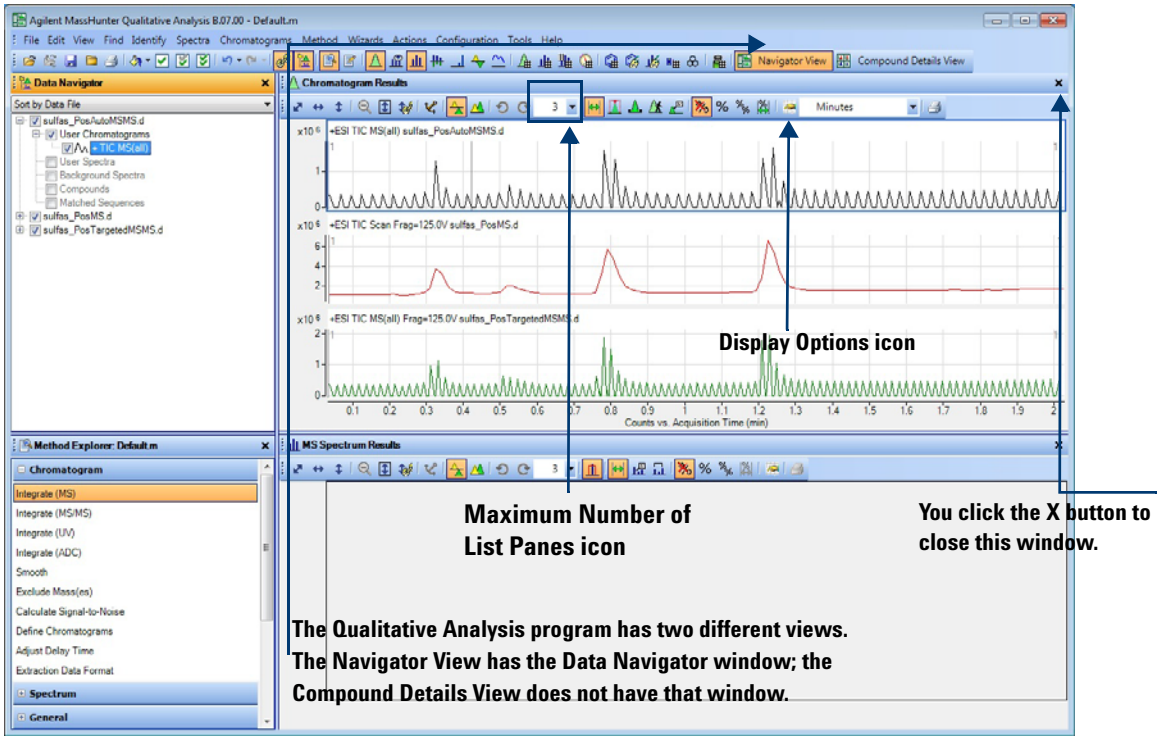


Figure 3 Qualitative Analysis main window in the Data Navigator view with the General Workflow selected.

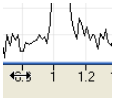
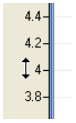
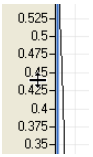
Task 2. Zoom in and out of the chromatogram

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

Task 2. 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). • Hide the others. • Zoom in twice on last peak. • Zoom in one more time autoscaling the y-axis. • Zoom out once to the previous zoom position. • Completely zoom out to the original chromatogram.	**a** Clear the check boxes in the Data Navigator window for the chromatograms you want to hide. **b** Click the right mouse button and drag over an area on the last peak. Make sure that the **Autoscale Y-axis during Zoom** icon,  , is not selected for this step. **c** Repeat step b. **d** Click the **Autoscale Y-axis during Zoom** icon,  , in the toolbar. **e** Click the right mouse button again and drag over an area of the last peak for the third time. The Quality Analysis program automatically scales the y-axis to the largest point in the range. **f** Click the **Unzoom** icon  to undo the last zoom operation. You can undo the last fifteen zoom operations. **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. • You can also use these zoom features on spectra in the Spectrum Preview window, the MS Spectrum Results window, the Deconvolution Results window, the UV Results window and the Difference Results window. • A selected icon has an orange background color.

Task 2. Zoom in and out of the chromatogram (continued)

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

Task 3. 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 3. Anchor a chromatogram

Steps	Detailed Instructions	Comments

• Anchor a chromatogram.
  ◦ Show all three chromatograms.
  ◦ Make sure the chromatogram viewing list is set to 1.
  ◦ In the Chromatogram Results window, select the second TIC.
  ◦ Anchor this TIC.
  ◦ Scroll through the chromatograms.
  ◦ Clear the anchor.
	a In Data Navigator mark the check boxes for the chromatograms which you hid in the previous task.
	b Make sure the maximum number of panes is set to 1 in the Chromatogram Results window.
	c In the Chromatogram Results window, select the second TIC.
	d Right-click inside the chromatogram, and click **Set Anchor.**
	e Use the scroll bar in the Chromatogram Results window to scroll through the list of chromatograms. The second TIC stays visible always.
	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.
		• 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.
		• You can also right-click the chromatogram and click **Clear Anchor** in the shortcut menu.

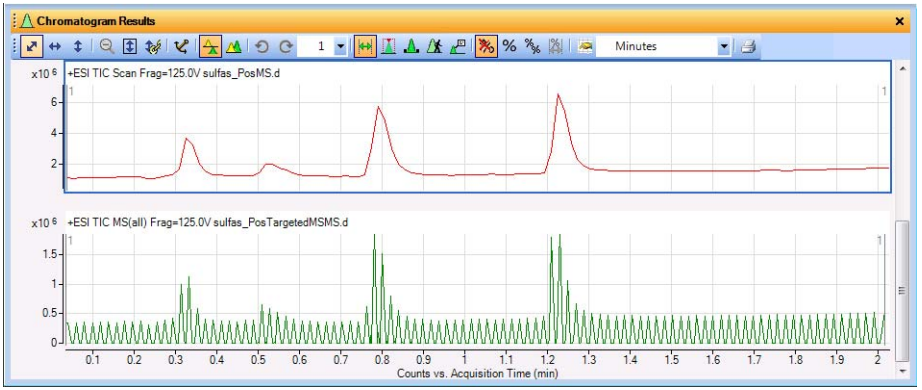


Figure 4 Anchored TIC in the Chromatogram Results window

Task 4. Change window layouts

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

Task 4. Change window layout

Steps	Detailed Instructions	Comments
1 Change the window layout: • Change the window size. • Save a window layout. • Unlock the layout. • Change the Chromatogram Results window to be floating. • Move the Chromatogram Results window. • Display the tools for repositioning the windows.	• To change the size of a window, drag the boundary between the windows. • To save a window layout, click **Configuration > Window Layouts > Save Layout**. • To unlock a layout, click **Configuration > Window Layouts > Lock Layout**. • To make a window float, right-click the title bar of the window, and click **Floating** from the shortcut menu. • To move a window, click the title bar of the window and drag the window to the desired location. • 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 5.	• If the layout is unlocked, the system does not display a check mark next to the Lock Layout menu. • You can only use the repositioning tools when the layout is unlocked. • You can also make a window float by double-clicking the title bar of the window. • The software has many different layouts created. You can also try loading different layouts. • The software has several different workflows. Each workflow loads a different layout. Switching to a different workflow also changes the layout. • If the BioConfirm program is installed, it has several different workflows and layouts.

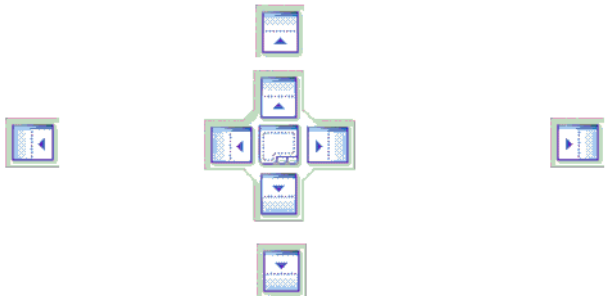


Figure 5 Window repositioning tools

Task 4. Change window layout (continued)

Steps	Detailed Instructions	Comments
2 Reposition the Chromatogram Results window. • 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. • Move two windows together so that they are on top of one another and available only through the tabs at the bottom. • 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. • 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. • 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. • 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. • Clicking the **Restore Default Layout** command restores the layout that is used with the General workflow. If you are using a different workflow, you need to load the layout that is used with that workflow.

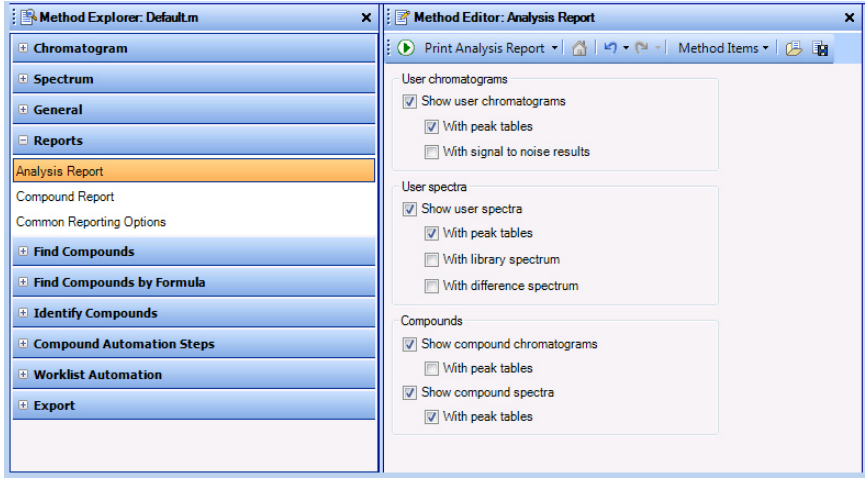
Task 5. Print an analysis report

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 5. Print an analysis report

Steps	Detailed Instructions	Comments
1 Change the analysis report selections: • Mark the check boxes for the chromatograms, spectra or tables you want to print. • Clear the check boxes for the chromatograms, spectra or tables you do not want to print.	**a** In the Method Explorer window, click **Reports > Analysis Report**. **b** Mark the check boxes for any additional selections you want to print. **c** Clear any chromatogram and spectra choices you do not want to print.	• The Analysis report only contains the information that you mark in this section. • 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.



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 or float the window.

Figure 6 Analysis Report section in the Method Explorer and Method Editor windows

Task 5. Print an analysis report (continued)

Steps	Detailed Instructions	Comments
2 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 the **Report contents**. **c** Mark the **Print report** check box and select a printer. **d** Mark the **Print preview** check box. **e** Click the **OK** button. **f** Review the report. **g** Click the **Close Print Preview** icon in the toolbar.	• 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.

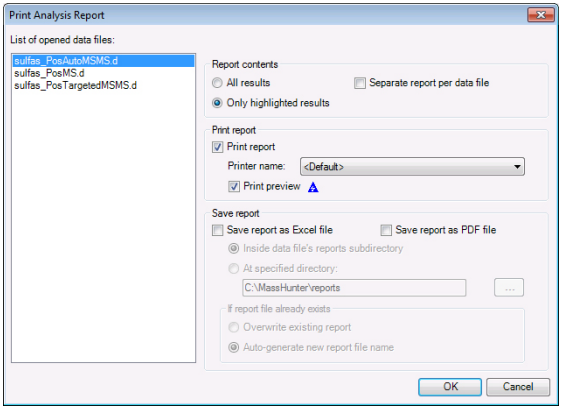


Figure 7 Print Analysis Report dialog box

Task 6. Add an annotation

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

- Chromatogram Results window
- MS Spectrum Results window
- Difference Results window
- Deconvolution Results window
- UV Spectrum Results window

Only in the Compound Details View

- Compound Chromatogram Results window
- Overall Chromatogram Results window
- Compound MS Spectrum Results window
- Compound Fragment Spectrum Results

Task 6. Add an annotation

Steps	Detailed Instructions	Comments
1 Select the location in the chromatogram.	**a** In the Chromatogram Results window, click the **Annotation** tool ()in the toolbar. **b** Move the cursor to the location in the chromatogram pane where you want to add the annotation. **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.

Task 6. Add an annotation (continued)

Steps	Detailed Instructions	Comments
2 Add the information about the text annotation in the Add/Edit Text Annotation dialog box.	**a** Type the **Text** for the annotation. **b** Select the **Text color**. **c** Select the **Orientation**. **d** Select the **Font style** and **Font size**. **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. **f** Click **OK**.	• You can add multiple annotations to a chromatogram or spectrum. • You can use the icons in the Annotate toolbar to select all of the annotations, delete annotations and edit annotations.

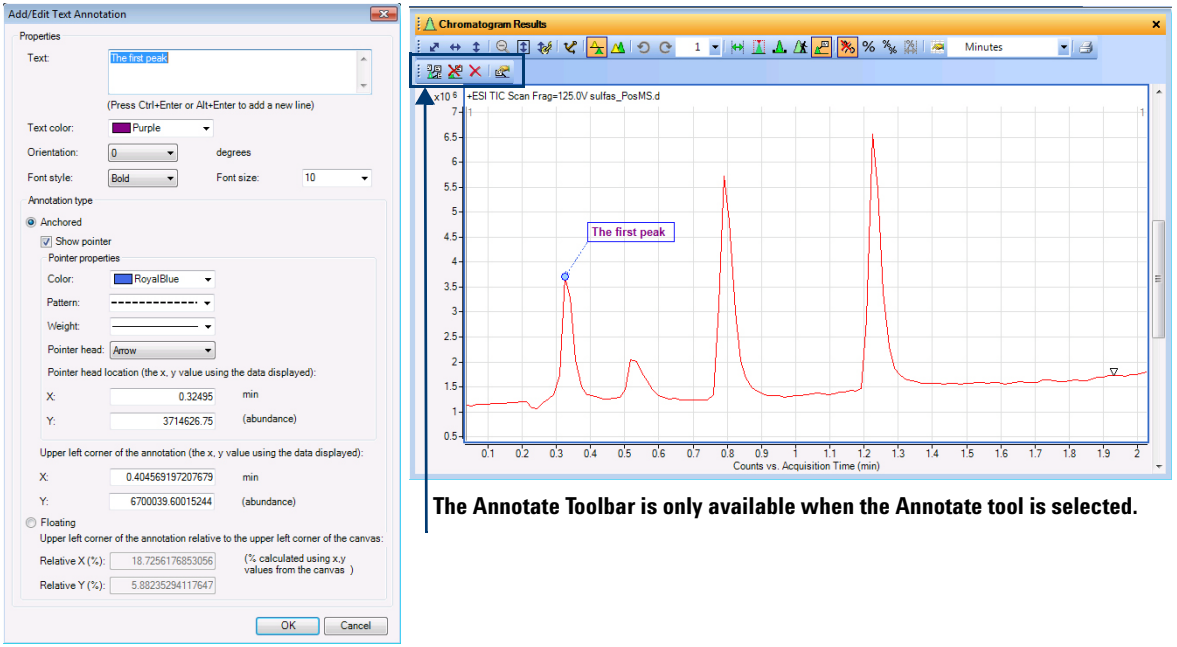


Figure 8 Add/Edit Text Annotation dialog box and the Chromatogram Results window

Task 6. Add an annotation (continued)

Steps	Detailed Instructions	Comments
3 Switch back to the Range Select tool in the Chromatogram Results window. Delete the annotation first.	**a** Click the  icon to remove all annotations. **b** Click the  (Range Select) icon in the Chromatogram Results toolbar.	• You can switch between five different tools in the Chromatogram Results toolbar. Refer to the online Help for more information. The five tools are: ◦ Range Select ◦ Peak Select ◦ Manual Integration ◦ Walk Chromatogram ◦ Annotation

Tasks for MS-Only Data (TOF, Q-TOF or Triple Quad)

Perform these tasks with MS data from a TOF instrument and MS-only data from a Q-TOF or Triple Quad instrument.

Task 7. Extract chromatograms (MS only)

In this task, you extract and merge chromatograms from the original TIC. You extract an EIC (Extracted Ion Chromatogram) and merge multiple *m/z* values into one chromatogram.

Task 7. Extract chromatograms (MS only)

Steps	Detailed Instructions	Comments
1 Extract and merge extracted ion chromatograms (EICs) from two masses in the **sulfas_PosMS.d** data file. • The m/z values are 279.09102 and 311.08085. • 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 **sulfas_PosMS.d**. **b** Open the Extract Chromatograms dialog box, using the option below or one of the options to the right: • Click **Chromatograms > Extract Chromatograms**. **c** In the **List of opened data files**, click **sulfas_PosMS.d.** **d** In the **Type** list box, select **EIC**. **e** In the **m/z value(s)** box, type `279.09102, 311.08085` **f** Mark the **Merge multiple masses into one chromatogram** check box to merge the EICs. **g** Click **OK**. **h** Make sure the **Maximum number of list panes** is set to `3` in the Chromatogram Results toolbar.	• You can also extract chromatograms in one of the following ways: • Right-click inside the chromatogram, and click **Extract Chromatograms**. • From Data Navigator, highlight the **TIC Scan** for **sulfas_PosMS.d**, and then right-click **TIC Scan,** and click **Extract Chromatograms**. • You can use an MS level of either **All** or **MS**. • Note that you can also choose to have the extracted chromatogram automatically integrated after extraction. • You can also extract a chromatogram from a mass spectrum.

Task 7. Extract chromatograms (MS only) (continued)

Steps	Detailed Instructions	Comments

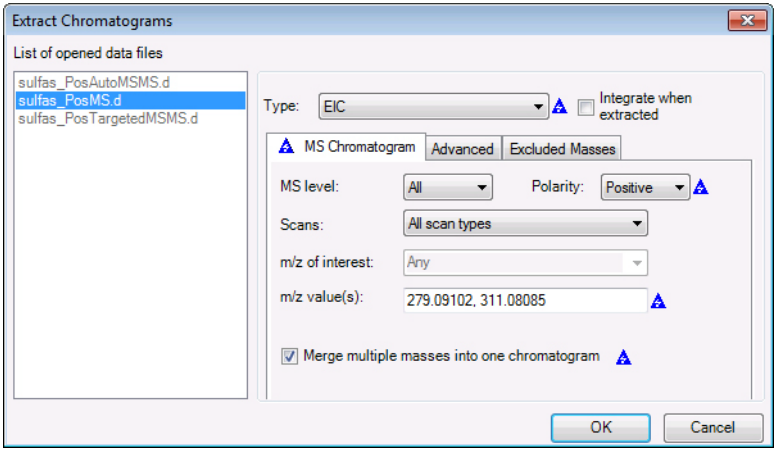


Figure 9 The Extract Chromatograms dialog box

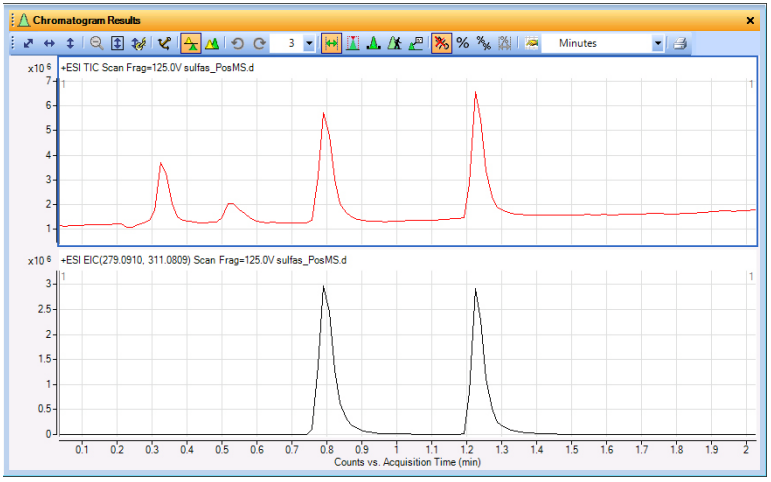


Figure 10 Merged extracted ion chromatograms (EICs) compared to the original TIC

Task 8. Interactively integrate a chromatogram (MS only)

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.

Task 8. Interactively integrate a chromatogram (MS only)

Steps	Detailed Instructions	Comments
1 Integrate the **sulfas_PosMS.d** TIC chromatogram.	• Integrate the **sulfas_PosMS.d** chromatogram, using any of the following options. ◦ From the main menu, click **Chromatograms > Integrate Chromatogram**. ◦ Highlight the chromatogram. Then, right-click the chromatogram, and click **Integrate Chromatogram**. ◦ In Data Navigator, highlight **TIC Scan** in the **sulfas_PosMS.d > User Chromatograms** section. Then, right-click the TIC Scan and click **Integrate Chromatogram**.	• The integration uses the **Agile 2** integrator, because that is the integrator selected in the method default.m. You can change this value in the Chromatogram > Integrate (MS) > Integrator tab in the Method Editor window. • Different integrators will detect different peaks. The **Agile 2** integrator only identifies the four largest peaks. • You can click the **Integrate Chromatogram** button in the Method Editor window to integrate.

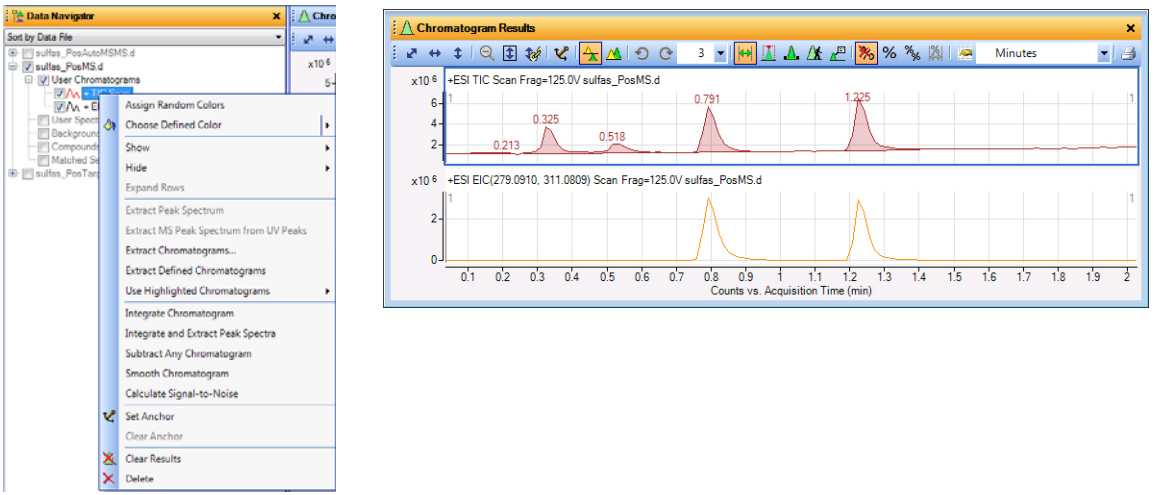


Figure 11 Shortcut menu in the Data Navigator and the integrated sulfas_PosMS.d TIC chromatogram

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

Steps	Detailed Instructions	Comments
2 Integrate the extracted ion chromatogram (EIC) from Task 1.	• Right-click anywhere in the EIC window, and click **Integrate Chromatogram**.	• You can mark the check box, **Integrate when extracted**, in the Extract Chromatogram dialog box when you set up for extraction.
3 Change the filter parameters for the integrated TIC. • Display the Integration Method Editor window from Method Explorer for MS data. • Change the threshold to retain only the two largest peaks.	**a** From Method Explorer, click **Chromatogram > Integrate (MS)** to display the Integrator tab. **b** Click the **Peak Filters** tab. **c** Under Maximum number of peaks, mark the **Limit (by height) to the largest** check box, and type in 2.	• 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.

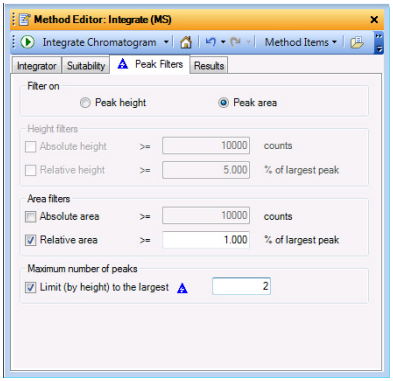


Figure 12 Peak Filters tab with **Limit (by height) to the largest** marked

4 Reintegrate the chromatogram.	**a** Click the **TIC Scan** in the Data Navigator window. **b** Click the **Integrate Chromatogram** icon .	• Note that only the two largest peaks are now integrated.

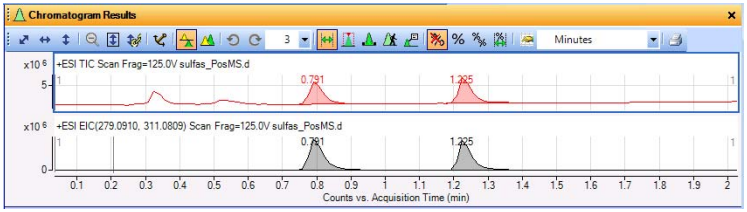
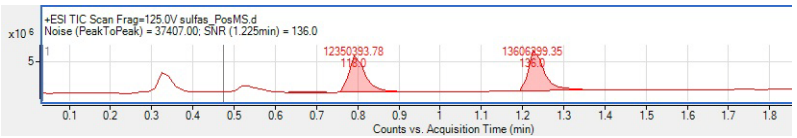


Figure 13 Integration results with limited number of peaks

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

Steps	Detailed Instructions	Comments
5 Calculate the signal-to-noise ratio. • Select the **sulfas_PosMS.d** TIC. • Set the first Peak Label to **Area** and the second Peak Label for the chromatographic peaks to **Signal-to-noise.** • Open the Method Editor. • Use `0.63 – 0.73` for the noise region, and calculate the signal-to-noise ratio for the integrated peaks.	**a** Click **Configuration > Chromatogram Display Options**. **b** Click the **Chromatogram** tab. **c** Set the first **Peak labels** to **Area** and the second **Peak labels** to **Signal-to-Noise**. **d** Click **OK**. **e** In the Method Explorer, click **Chromatogram > Calculate Signal-to-Noise**. **f** Click the **Specific noise regions** button. **g** Type `0.63 – 0.73` for the **Noise regions**, and click the **Calculate Signal to Noise** icon.	• You can also click the icon in the Chromatogram Results window to display the **Chromatogram Display Options** dialog box. • Press **F1** to get help on most windows and dialog boxes. • Make sure the TIC is highlighted before you calculate the signal-to-noise. • The area that you specified to be the noise region is drawn in bold in the Chromatogram Results window.
		
	Figure 14 Integrated TIC with Area and Signal-to-Noise labels	
6 Restore the settings for the default method, and close the Method Editor window.	**a** To cancel your changes and restore the values from the default method, click the **Restore to last saved values from file** button on the Method Editor toolbar. **b** Close the **Method Editor** window.	• The online Help describes each of the Signal-to-Noise algorithms.
7 Return the peak labels to Retention Time.	**a** Click **Configuration > Chromatogram Display Options**. **b** Click the **Chromatogram** tab. **c** Set the first Peak label to **Retention Time** and the second Peak label to **Compound Label**. **d** Click **OK**.	• The **Default** button restores the default values in this dialog box. Those values may not be the same as the values that were selected when the first opened this dialog box.

Task 9. Extract spectra from a chromatogram (MS only)

In this task, you extract a spectrum from exactly where you specify in the chromatogram. You can extract a spectrum from a specific data point or extract an average spectrum from an average of multiple data points or ranges. This task also shows you how to change spectral display options and subtract the background spectrum.

Task 9. Extract spectra from a chromatogram (MS only)

Steps	Detailed Instructions	Comments
1 Extract spectra on specific data points for the peak at 0.79 min. and the last peak of the **sulfas_PosMS.d** data file. • After zooming in on the region between 0.7 and 1.0 minutes, extract a spectrum from the peak at or near 0.79 minutes using any one of the options described under Comments. • Open Spectrum Preview. • After zooming in on the region between 1.1 and 1.4 minutes, extract a spectrum from the peak at or near 1.22 minutes. • Copy this spectrum to the User Spectra section. • Change the display to show at least two spectra.	**a** To zoom in to the peak at 0.79 minutes, right-click the mouse above the peak at 0.70 min. and drag it to below the curve at 1.0 min., then release. **b** On the peak near 0.79 minutes, extract a spectrum in any of the ways listed in the Comments column. **c** Click the **Zoom Out** icon,  , in the Chromatogram Results toolbar. **d** Click the **Range Select** icon,  , in the MS Spectrum Results toolbar. **e** To open Spectrum Preview, click the **Spectrum Preview** button,  . **f** Zoom into the region between 1.1 and 1.4 min. **g** On the peak near 1.22 minutes, extract a spectrum in any of the ways listed in the Comments column. The spectrum is shown in the Spectrum Preview window. **h** Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**. The Spectrum Preview window is not closed. **i** If necessary, click the arrow next to the **Maximum number of list panes** icon in the MS Spectrum Results toolbar, and select **2**. **j** Close the **Method Editor** window.	• 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". • You can extract a spectrum in any of the following ways: ◦ Double-click the data point in the chromatogram. ◦ Click the data point in the chromatogram. Then, right-click anywhere in the chromatogram. Click **Extract MS Spectrum**. The **Extract Chromatogram Analysis** dialog box is displayed. Make sure the **sulfas_PosMS.d** file is selected, and click **Extract**. • When you first extract a spectrum, the MS Spectrum Results window appears containing the spectrum, and in the Data Navigator window, the type of spectrum and retention time appear under **User Spectra**. • When the Spectrum Preview window is open, the system displays any manually-selected spectrum in the Spectrum Preview window but the spectrum is not kept in the User Spectra section. • With Spectrum Preview on, Qualitative Analysis overwrites the previous spectrum when you extract a new spectrum.

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments

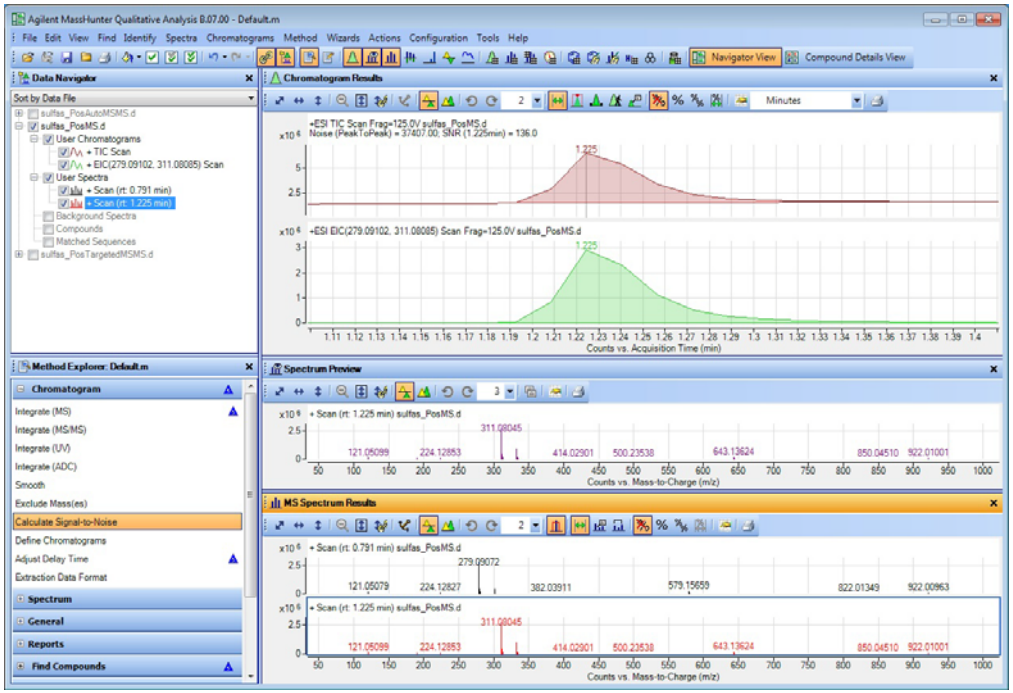


Figure 15 Main window with extracted spectra from both integrated peaks in the sulfas_PosMS.d file

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments
2 Extract a spectrum that averages all points within a specified range for the last integrated peak for the **sulfas_PosMS.d** data file: • Delete any existing User Spectra. • Zoom out of the chromatogram. • Turn off Spectrum Preview. • Use the Range Select icon on the Chromatogram toolbar. • Set the range from the halfway point on the left to the same point on the right of the peak. • Extract the spectrum, using any of the options listed.	**a** Highlight the User Spectra to be deleted (Press **Ctrl** to highlight more than one spectra). **b** Right-click the selected User Spectra, and click **Delete**. **c** Click **Yes** in the Delete dialog box, if it is displayed. **d** Click  in the Chromatogram Results window to zoom out completely. **e** Close the **Spectrum Preview** window. **f** Click the **Range Select** icon  on the Chromatogram toolbar. **g** Click at the halfway point on the left side of the last integrated peak and drag over to the halfway point on the right. **h** Extract the average spectrum using an option below or on the right. • Right-click anywhere in the range of the peak, and click **Extract MS Spectrum** from the shortcut menu. • Click **Extract** in the Extract Spectrum dialog box.	• You can also delete all user spectra if you right-click the User Spectra line in the Data Navigator window and click **Delete**. • You can also extract an average spectrum by double-clicking the selected range in the chromatogram. • You can change whether or not you are asked to confirm every time you delete a chromatogram or spectrum by using the Message Box Options dialog box. This dialog box is displayed when you click the **Configuration > Message Box Options** command. • The Extract Spectrum dialog box is only shown if more than one data file is loaded.

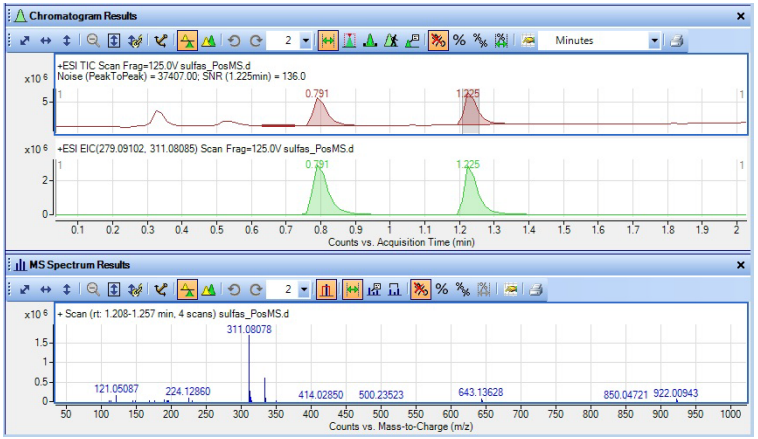


Figure 16 Average spectrum extracted from selected range for last peak

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments
3 Extract a spectrum that averages the ranges of integrated peaks 1 and 2 together for the **sulfas_PosMS.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 spectrum, using any of the options on the right.	**a** Click the **Chromatogram Results** window title bar. The Chromatogram Results window becomes the active window, and the selected area is not lost. **b** Press and hold the **Ctrl** key. **c** Click at the halfway point on the left side of the first integrated peak, and drag over to the halfway point on the right. **d** Release the mouse. **e** Release the **Ctrl** key. **f** Extract the average spectrum 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 2. • You can also extract a spectrum by right-clicking anywhere in the chromatogram, and then click **Extract MS Spectrum**. The Extract Spectrum dialog box is shown. Click **Extract**.

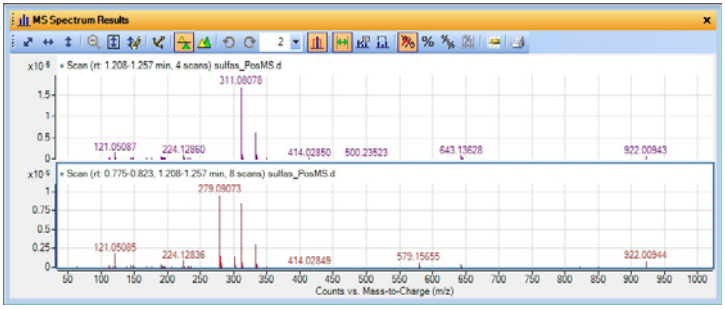
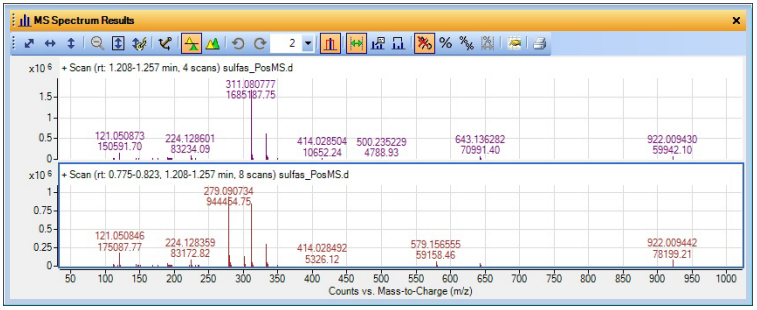


Figure 17 An averaged spectrum created from multiple ranges.

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments
4 Change the spectral display option for **sulfas_PosMS.d**. • Change the digits after the decimal to one more than the current setting. • Change back to the original number of digits.	**a** Click **Configuration > MS and MS/MS Spectra Display Options.** **b** Click the **MS and MS/MS Spectra** tab. **c** Set **Digits after the decimal** to one more than the current setting for the m/z values. **d** Click the **Spectrum Peak Label Options** tab. **e** Select **Abundance** as the second **MS peak label.** **f** Click **OK**.  **g** Repeat steps a and b, and then set **Digits after the decimal** to one less than the current setting. **h** Click the **Spectrum Peak Label Options** tab. **i** Select **Formula & Ion Species** as the second **MS peak label**. **j** Click **OK**.	• You can also click the Display Options icon, , in the MS Spectrum Results window. • Note that the label now shows *m/z* with one more digit.

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments
5 Subtract a background spectrum every time you extract an MS peak spectrum. • Delete any scans under User Spectra in Data Navigator. • Extract a background spectrum in the region of 0.0 to 0.25 minutes and have it appear in the Background Spectrum folder in Data Navigator. • Use the current background MS spectrum for subtraction. • Integrate the chromatogram, limiting the integrated peaks to 4. • Extract a peak spectrum from the third integrated peak.	**a** Under **User Spectra** in Data Navigator, highlight the User Spectra to be deleted (Press the **Ctrl** key). **b** Right-click the spectra, and click **Delete**. Click **Yes**. **c** Drag the cursor between 0.0 and 0.25 min. **d** Right-click within the range, and click **Extract MS Spectrum to Background**. **e** If a dialog box is shown, select the Sulfas_PosMS.d data file and click **Extract**. **f** In Method Explorer click **Spectrum > Extract MS**. **g** Click the **Peak Spectrum Extraction (MS)** tab. **h** Under **Peak spectrum background**, select **Current background spectrum** for the MS spectrum. **i** From Method Explorer click **Chromatogram > Integrate (MS).** **j** Click the **Peak Filters** tab. **k** Mark the **Limit (by height) to the largest** check box, and type 4. **l** From the main menu click **Chromatograms > Integrate Chromatogram > Entire Chromatogram**. **m** Click the **Peak Select** icon,  , in the Chromatogram Results toolbar. **n** Select the third integrated peak, and extract a peak spectrum using one of the following options • Double-click the peak. • Right-click the peak and click **Extract peak spectrum**. • Click **Chromatograms > Extract Peak Spectrum**. • Right-click the chromatogram in the Data Navigator window and click **Extract Peak Spectrum**.	• To set the spectrum to be subtracted when you manually extract a spectrum, select the **Manual spectrum background** in the Manual Extraction tab. This tab does not affect the Peak Spectrum that is extracted. • Note that at the end of this process, all extracted peak spectra will automatically have the designated background spectrum subtracted. • As an alternative way to move a background spectrum to the Background Spectrum folder, follow these steps: • Double-click the selected range to extract an averaged spectrum. • Right-click anywhere in the spectrum window and click **Move to Background Spectrum**.

Task 9. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments

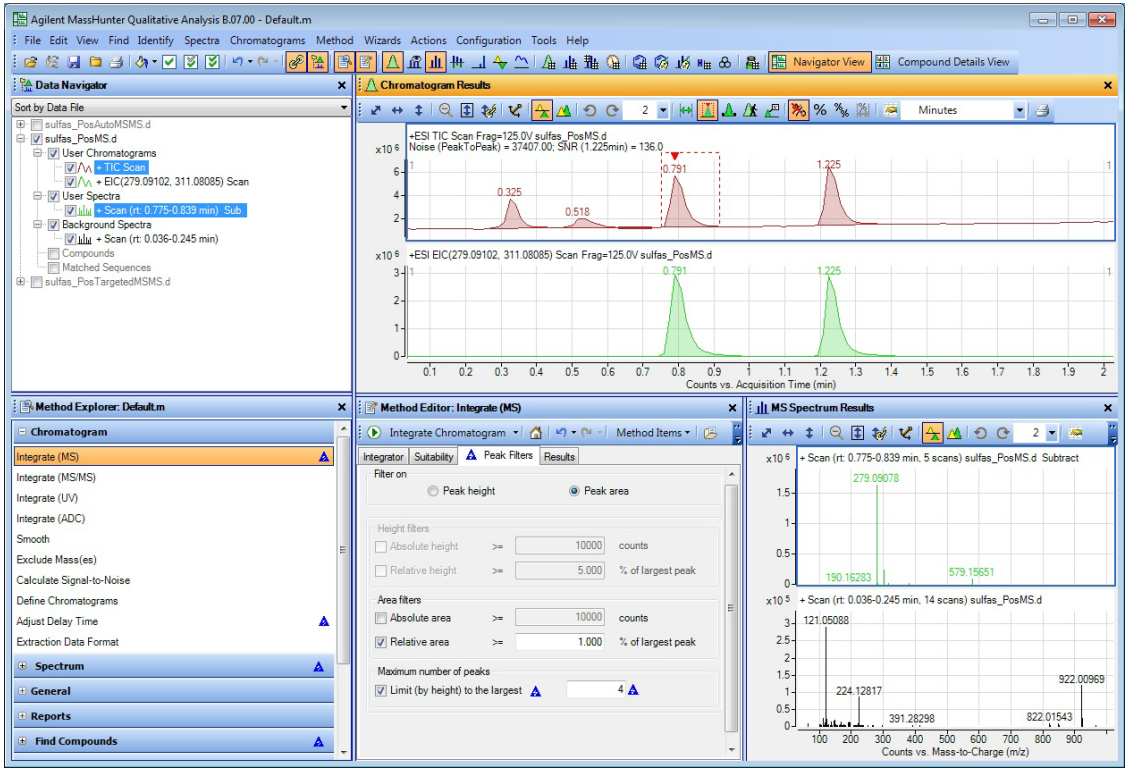


Figure 18 Spectrum with background subtracted

Task 10. Add a caliper

A caliper shows the difference between two points in a spectrum. You can add a caliper to the following graphics windows:

- MS Spectrum Results window
- Deconvolution Results window

You can also add calipers in two windows in the Compound Details View. See "Task 3. Review results in Compound Details View" on page 149 for more information about this view.

You can also add a **Modifications** caliper or an **Amino Acid** caliper to deconvoluted spectra which are displayed in the Deconvolution Results window. If the mass could have changed due to a Modification or an Amino Acid, then the label for the caliper is the Modification or the Amino Acid. Otherwise, the change in the mass (Delta Mass) is reported.

Task 10. Add a caliper

Steps	Detailed Instructions	Comments
1 Add the caliper to the peak spectrum created in the previous task.	**a** In the MS Spectrum Results window, click the **Caliper** tool ()in the toolbar. **b** Select **Profile Point to Point** for the type of caliper in the Caliper toolbar. **c** Move the cursor to the location in the spectrum pane where you want to add the caliper. **d** 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.	• See "Task 9. Extract spectra from a chromatogram (MS only)" on page 31 to extract an MS spectrum. • The cursor changes to an arrow. You use this cursor to select the start and end point of the caliper.

Task 10. Add a caliper (continued)

Steps	Detailed Instructions	Comments
2 Modify the caliper to use a different color.	**a** Click the caliper created in the previous step. **b** Click the Caliper Properties button ( ) in the MS Spectrum Results Caliper toolbar. The **Delta Mass Caliper Settings** dialog box is displayed. **c** (optional) Type the **Start X** and **Start Y** values. **d** Select the **Text color**. **e** Select the **Font style** and **Font size**. **f** Click **OK**. **g** (optional) Delete the caliper. Click the button in the MS Spectrum Results Caliper toolbar. **h** Switch to the range select tool. Click the tool in the MS Spectrum Results toolbar.	• You can add multiple calipers to a spectrum. • You can use the icons in the Caliper toolbar to select all of the calipers, delete calipers and edit calipers.

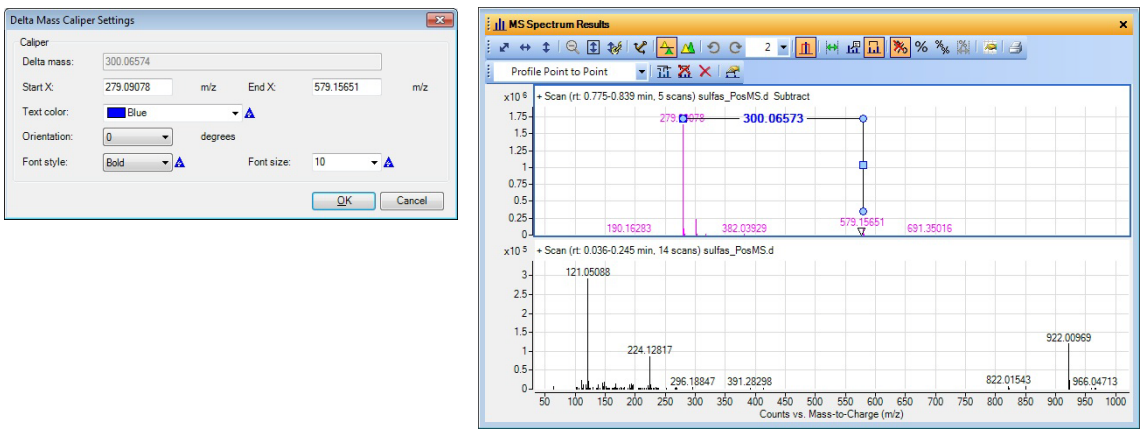


Figure 19 Delta Mass Caliper Settings dialog box and the MS Spectrum Results window

Tasks for LC/MS/MS Data (Q-TOF and Triple Quad)

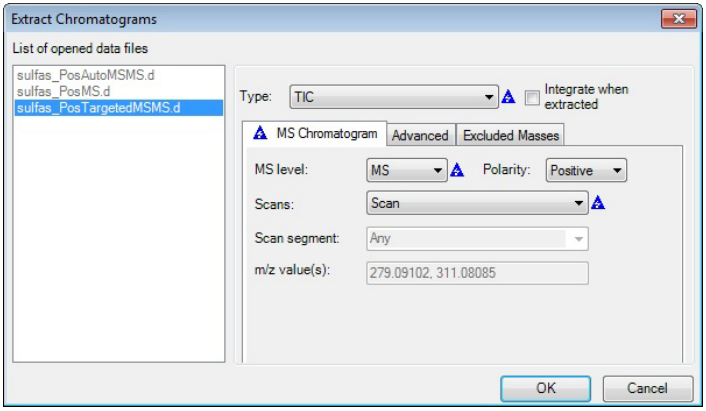
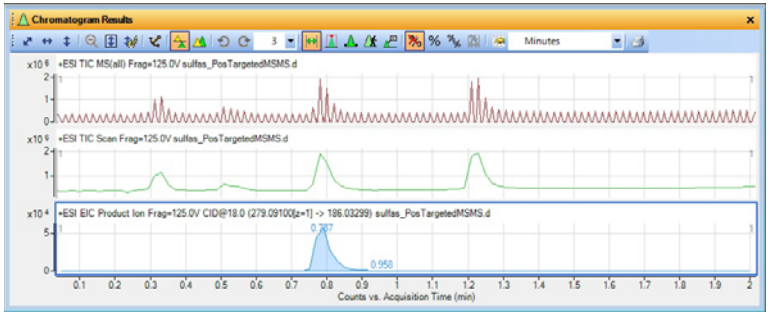
Task 11. Extract chromatograms (LC/MS and LC/MS/MS)

In this task, you extract one chromatogram for MS data and one for MS/MS data in order to integrate the peaks. You cannot integrate the TIC of the original chromatogram because it contains both MS and MS/MS data.

Task 11. Extract chromatograms (MS and MS/MS)

Steps	Detailed Instructions	Comments
1 Extract TICs for the MS data in the **sulfas_PosTargetedMSMS.d** data file.	**a** In the Data Navigator window, mark the check box for **sulfas_PosTargetedMSMS.d** and clear the check boxes for the other data files. **b** Display the Extract Chromatograms dialog box, using the option below or one of the options to the right: • Click **Chromatograms > Extract Chromatograms**. **c** In the **List of opened data files**, click **sulfas_PosTargetedMSMS.d**, if necessary. **d** Make sure the **Type** is **TIC**. **e** From the MS Level list, click **MS**. **f** Click **OK**.	• You can also extract chromatograms in one of the following ways: ◦ Right-click the chromatogram, and click **Extract Chromatograms**. ◦ From Data Navigator, click **User Chromatograms > TIC MS (All)**, then right-click **TIC MS (All)** and click **Extract Chromatograms**. • You can also extract chromatograms starting from a mass spectrum.

Task 11. Extract chromatograms (MS and MS/MS) (continued)

Steps	Detailed Instructions	Comments
		
	Figure 20 Extracting an MS chromatogram	
2 Extract another chromatogram but based on a product ion for the MS/MS data. • This time choose to integrate the extracted chromatogram. • Extract a Product Ion EIC for 279.091 -> 186.03299.	**a** Repeat step b and step c of Step 1. **b** Click **EIC** as the Type. **c** From the **MS Level** list, click **MS/MS**. **d** From the **Scans** list, click **Product ion**. **e** From the **Precursor ion m/z**, select 279.09100. **f** In the m/z value(s) text box, type `186.03299`. **g** Mark the **Integrate when extracted** check box. **h** Click **OK**.	• When you first select EIC, an error icon is displayed next to the m/z value(s) box. You specify the **m/z value(s)** in step e and step f. • In the m/z value(s) text box, you can also type a range (for example, `100 - 300`).
		
	Figure 21 TIC for MS and EIC for MS/MS data compared to the original TIC	

Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS)

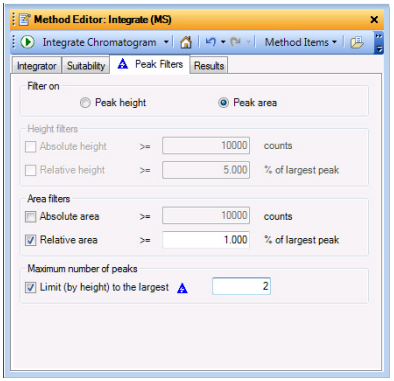
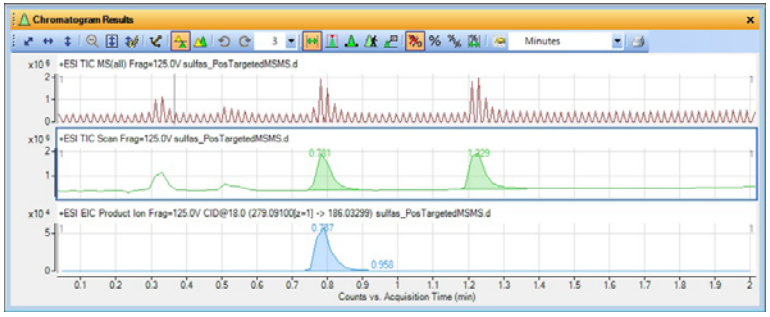
In this task, you learn different ways to integrate a chromatogram, change integration parameters to modify the results and calculate the S/N for the integrated peaks for MS/MS data.

You cannot integrate the original Q-TOF TIC chromatogram because it contains both MS and MS/MS data, possibly in no particular order.

Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS)

Steps	Detailed Instructions	Comments
1 Integrate the TIC Scan chromatogram for the **sulfas_PosTargetedMSMS.d** data file, using any of the options listed at right.	**a** Highlight the TIC Scan chromatogram, and choose from any one of the following commands to integrate the chromatogram. • From the menu bar click **Chromatograms > Integrate Chromatogram**. • Right-click anywhere in the chromatogram window, and click **Integrate Chromatogram**. • In the Data Navigator window, select **sulfas_PosTargetedMSMS.d > User Chromatograms > TIC Scan,** right-click the **TIC Scan**, and click **Integrate Chromatogram**.	• Note that the program integrated 4 peaks in the chromatogram. • You select the integrator to use for MS data, MS/MS data, UV data and ADC data in the Method Editor window. • You cannot integrate the **TIC MS(all)** chromatogram. You cannot integrate a chromatogram that has both MS and MS/MS data.
2 Change the threshold to integrate fewer peaks. • Change the threshold to retain only the two largest peaks.	**a** From the Method Explorer window, click **Chromatogram > Integrate (MS)** to display the Integrator tab. **b** Review the parameters on the Integrator tab. **c** Click the **Peak Filters** tab. **d** Under Maximum number of peaks, mark **Limit (by height) to the largest,** if necessary, and type in 2.	• Press **F1** to see the online Help for more information on the integrators. • 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 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
		
	Figure 22 Peak Filters tab with the **Limit (by height) to the largest** check box marked	
3 Reintegrate the chromatogram.	**e** Click the button on the Method Editor toolbar to integrate using the new setting.	• Note that only the two largest peaks are now integrated.
		
	Figure 23 Integrated TIC MS and MS/MS chromatograms with limited peaks integrated	

Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
4 Integrate the EIC Product Ion chromatogram for the **sulfas_PosTargetedMSMS.d** data file, using any of the options listed at right.	**a** Highlight the EIC Product Ion chromatogram, and choose from any one of the following commands to integrate the chromatogram. • From the menu bar click **Chromatograms > Integrate Chromatogram**. • Right-click anywhere in the chromatogram window, and click **Integrate Chromatogram**. • In the Data Navigator window, select **sulfas_PosTargetedMSMS.d > User Chromatograms > EIC Product Ion** then right-click the **EIC Product Ion** and click **Integrate Chromatogram**.	• Note that the program integrated practically all the peaks in the chromatogram. • You select the integrator to use for MS data, MS/MS data, UV data, GC Data and ADC data in the Method Editor window in the Integrator tabs. You can select a different integrator for MS data, MS/MS data, UV data, GC data, and ADC data.
5 Change the filter to filter on height and set an absolute height limit.	**a** From Method Explorer, click **Chromatogram > Integrate (MS/MS)** to display the Integrator tab. **b** Click the **Peak Filters** tab. **c** Under **Filter on**, click **Peak height**. **d** Under Height filters, mark the **Absolute height** check box.	• The MS/MS integrator is selected by default for MS/MS data. • 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.
6 Reintegrate the chromatogram	**e** Click the  icon on the Method Editor toolbar to integrate using the new setting.	• Note that only the largest peak is now integrated.

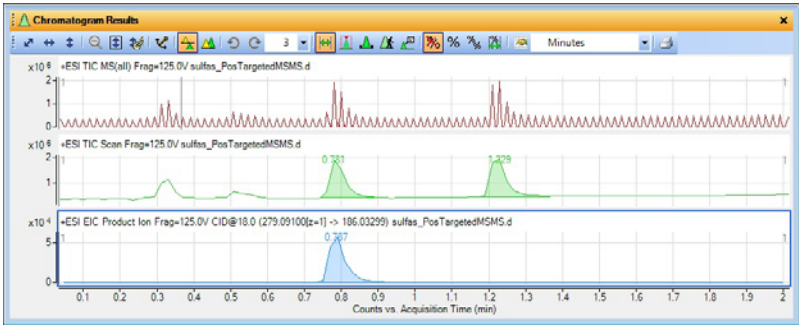


Figure 24 Integrated MS and MS/MS chromatograms

Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
7 Calculate the signal-to-noise ratio for the EIC of the product ion. • Set the first Peak Label to **Area** and the second Peak Label for the chromatographic peaks to **Signal-to-noise.** • Open the Method Editor. • Use `0.0 – 0.76` for the noise region, and calculate the signal-to-noise ratio for the integrated peaks.	**a** Click **Configuration > Chromatogram Display Options**, and set the first Peak label to **Area** and the second Peak label to **Signal-to-Noise**. Click **OK**. **b** In Method Explorer in the Chromatogram section, select **Calculate Signal-to-Noise**. **c** Click the **Specific noise regions** button. Type `0.0 – 0.76` for the **Noise regions**, and click the **Calculate Signal to Noise** icon .	• Make sure the EIC is highlighted before you calculate the signal-to-noise. • The default **Noise definition** algorithm is Peak-to-Peak. See the online Help for information about each **Noise definition**. • The area that you specified to be the noise region is drawn in bold in the Chromatogram Results window.

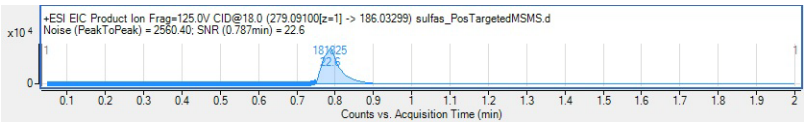


Figure 25 Signal-to-Noise results for MS/MS EIC Product Ion

8 Restore the settings that are saved for the current method and close Method Editor.	**a** Click the **Chromatogram > Calculate Signal-to-Noise** section in the Method Explorer. **b** Click the **Restore to last saved values from file** icon in the Method Editor toolbar. **c** Click the **Chromatogram > Integrate (MS/MS)** section in the Method Explorer. **d** Click the icon. **e** Click the **Chromatogram > Integrate (MS)** section in the Method Explorer. **f** Click the icon. **g** Close **Method Editor**.	• To cancel your changes and restore the values from the method that is loaded, click the **Restore to last saved values from file** icon in the Method Editor toolbar.
9 Return the peak labels for Chromatograms to Retention Time.	**a** Click **Configuration > Chromatogram Display Options**. **b** Select **Retention Time** for the first Peak label and **None** for the second Peak label. **c** Click **OK**.	• You can also click the Display Options icon, , in the Chromatogram Results window to open the Chromatogram Display Options dialog box.

Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
10 Delete all chromatograms except the original.	**a** If you selected "Sort by Type" in the Data Navigator window, then under User Chromatograms, highlight all the chromatograms except the original. Right-click the highlighted chromatograms, and click **Delete**. **b** If you selected "Sort by Data" in the Data Navigator window, then under the Sulfas_PosTargetedMSMS.d data file section in User Chromatograms, highlight all the chromatograms except the original. Right-click the highlighted chromatograms, and click **Delete** **c** Click **Yes** if the **Delete** message box is displayed.	

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS)

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

This task also shows you how to walk a chromatogram, change spectral display options and subtract the background spectrum.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS)

Steps	Detailed Instructions	Comments
1 Walk a chromatogram to view the precursor ion and product ion for the last peak of **sulfas_PosTargetedMSMS.d**. • Zoom in on the region between 1.15 and 1.35 minutes. • Use the Walk Chromatogram icon. • Review the spectra starting at about 1.15 minutes, and move the arrow to the right.	**a** Click the **TIC MS(all)** chromatogram for the sulfas_PosTargetedMSMS.d data file in the Data Navigator window. **b** To zoom in to the last peak, right-click the mouse above the peak at 1.15 minutes and drag it to 1.35 minutes, then release. **c** Close the Method Editor window. **d** Click the **Walk Chromatogram** icon on the Chromatogram Results toolbar. **e** Move the Walk Chromatogram cursor to above the X axis at about 1.15 minutes, and click. The spectrum for that time is automatically displayed in the Spectrum Preview window. **f** To navigate from spectrum to spectrum, press 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. • The Spectrum Preview window is opened automatically when you click the Walk Chromatogram icon. • The spectrum for each point you click in the Chromatogram Results window is automatically displayed in the Spectrum Preview window. • If you click the button (Autoscale Y-axis during zoom), the software automatically sets the Y-axis when you are zooming to include all of the points in the X-axis range that you select.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

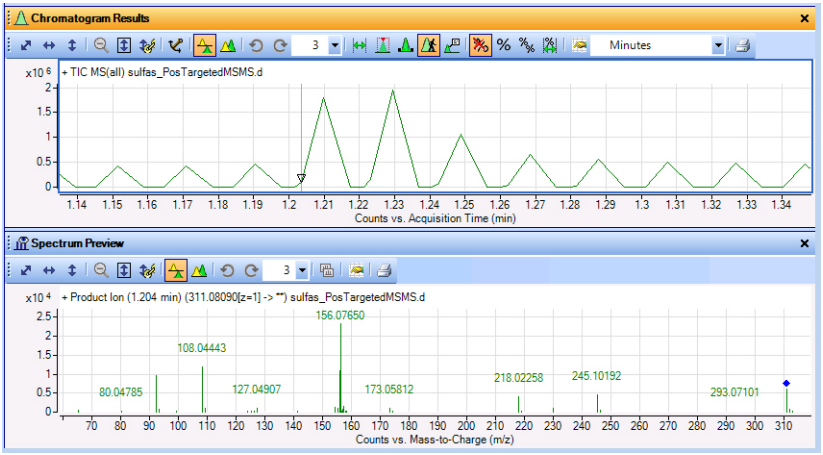


Figure 26 Walk chromatogram to view the MS/MS product ion at 1.204 minutes

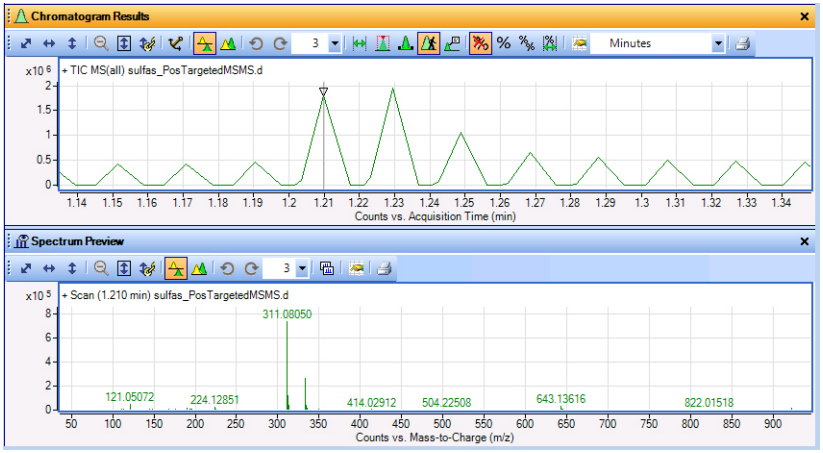


Figure 27 Walk chromatogram to view the MS scan for the peak at 1.210 minutes

If you want the Fragmentor voltage included in the chromatogram title and the spectrum title, you mark the Expanded check box in the Chromatogram Display Options dialog box and in the MS and MS/MS Spectra Display Options dialog box.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

2 Extract spectra on specific data points for the peak at 0.33 minutes and the last peak of the **sulfas_PosTargetedMSMS.d** data file.
• After zooming in on the region between 0.3 and 0.4 min., extract a spectrum from one of the peaks (MS) at or near 0.33 min. and then one of the valleys (MS/MS), using any one of the options described under Comments.
• After zooming in on the region between 1.15 and 1.25 min., extract a spectrum from one of the peaks at or near 1.23 min. (not the valley yet)
• Change the display to show at least three spectra.
	a Click the **Range Select** icon from the Chromatogram Results toolbar.
	b Close the Spectrum Preview window.
	c Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.
	d To zoom in to the first peak, right-click the mouse above the peak at 0.3 min. and drag it to 0.4 min., then release.
	e On a peak near 0.33 min. extract a spectrum in any of the ways listed in the Comments column.
	f On a valley near 0.34 minutes, extract a spectrum.
	g Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.
	h Zoom into the region between 1.15 and 1.25 min.
	i On a peak near 1.23 minutes, extract a spectrum in any of the ways listed in the Comments column. (Do not extract the valley spectrum yet.)
	j If necessary, click the arrow next to the **Maximum number of list panes** icon in the MS Spectrum Results toolbar, and select **3**.
		• The AutoScale Y-axis during Zoom icon, , is "on" if the background of the icon is orange.
		• You can extract a spectrum in any of the following ways:
		  • Double-click the data point in the chromatogram.
		  • 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 **sulfas_PosTargetedMSMS.d** file is selected, and click **Extract** in the Extract Spectrum dialog box.
		• 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** in the Data Navigator window. All subsequent extracted spectra appear in both places as well.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

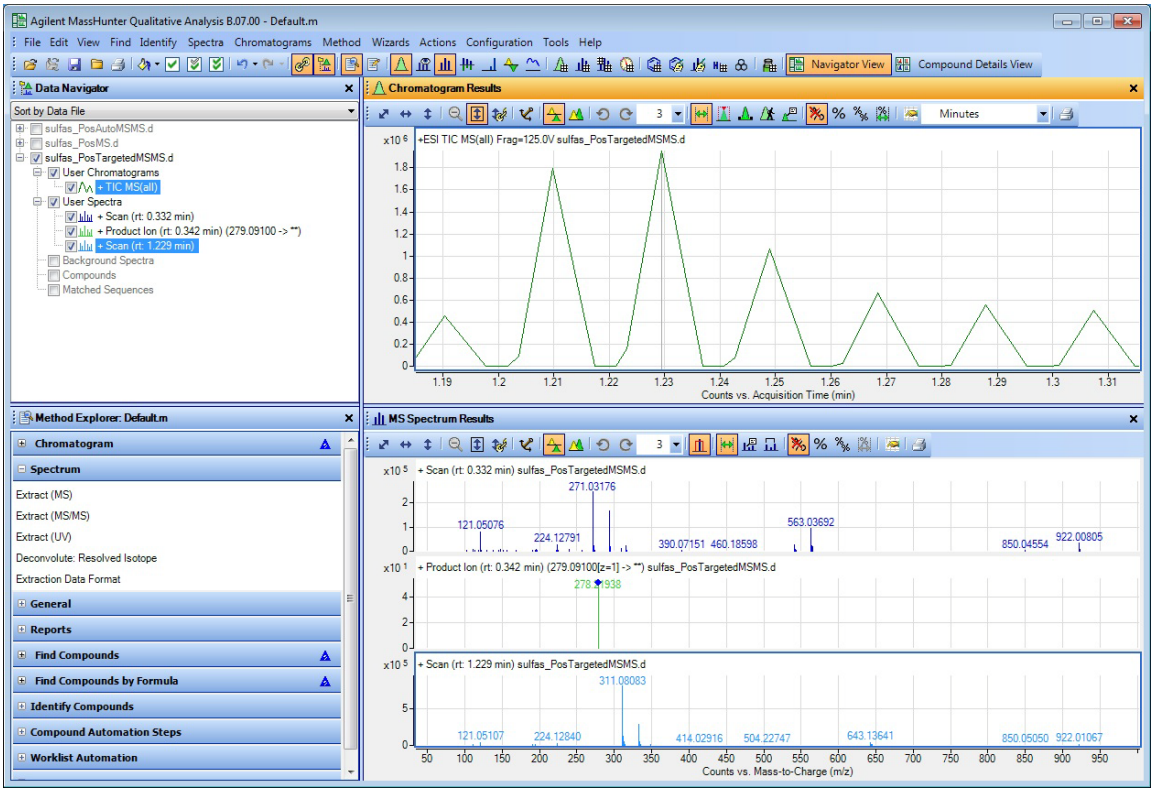


Figure 28 The Qualitative Analysis program with MS Scan and Product Ion spectra from the first peak and MS Scan spectrum from the last peak

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
3 Extract a product ion spectrum for the last peak of the **sulfas_PosTargetedMSMS.d** data file. • View the Spectrum Preview window. • Extract a spectrum from the valley at RT 1.237 min. • Copy this spectrum to the User Spectra folder. • Change the display to show 4 spectra. • Turn off Spectrum Preview.	**a** Click the **Spectrum Preview** icon, in the main toolbar. **b** On a valley near 1.23 minutes extract a spectrum. **c** Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**. **d** Select 4 for the **Maximum number of list panes** in the MS Spectrum Results window. **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 the Spectrum Preview window open, 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.

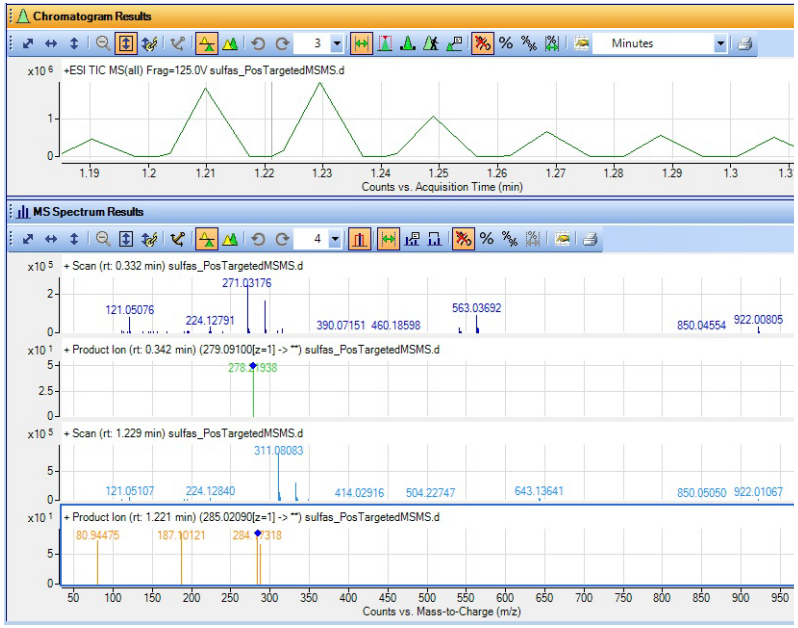


Figure 29 Chromatogram Results and MS Spectrum Results windows with product ion spectrum from the last peak in the chromatogram

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
4 Extract a spectrum that averages all points within a specified range for the last peak for the **sulfas_PosTargeted.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 **Autoscale X-axis and Y-axis** icon in the Chromatogram Results toolbar to zoom out completely. **b** Click the **Range Select** icon on the Chromatogram toolbar. **c** Click at about 1.21 minutes of the last peak and drag over to about 1.229 minutes on the right. **d** Extract the average spectrum using one of the options on the right. **e** Click the down arrow next to the Maximum number of list panes icon in the toolbar, and select **2**.	• 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. Then, click **Extract**. • Note that both the averaged MS spectrum and averaged MS/MS spectrum appear.

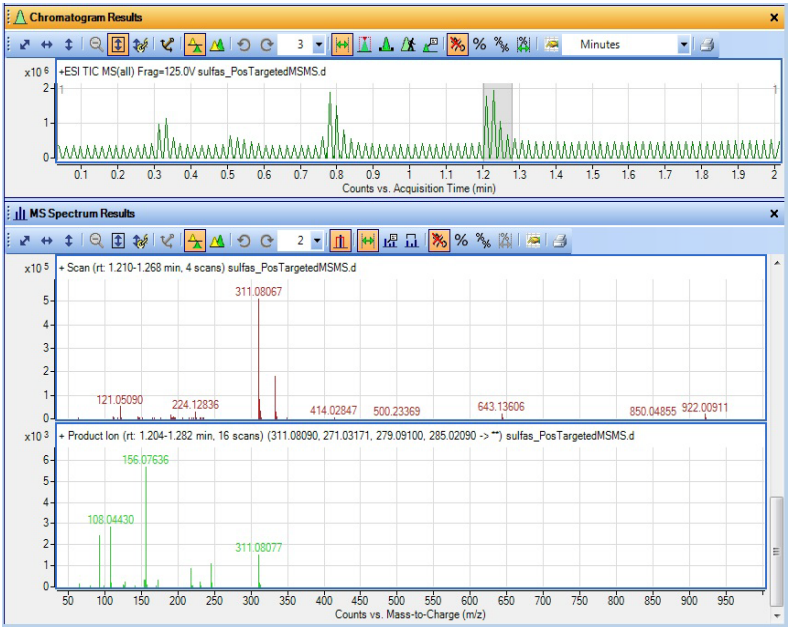


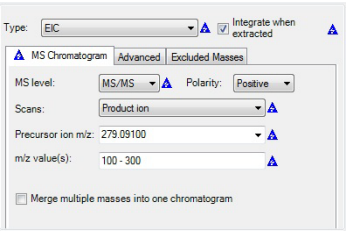
Figure 30 Averaged spectra extracted from selected range for last peak

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
5 Extract spectra that average the ranges of peaks 1 and 4 together for the **sulfas_PosTargetedMSMS.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** Press and hold the **Ctrl** key. **b** Click at about 0.3 min. on the left side of the first peak and drag over to about 0.33 min. on the right, and release the mouse. **c** Release the **Ctrl** key. **d** 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**. • The range that you select is shown in blue. When you use this range, the range that is actually used is shown in gray and the blue range is removed.

Figure 31 Averaged MS and MS/MS spectra created from multiple ranges.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
6 Subtract a background spectrum every time you extract a peak spectrum for an MS/MS EIC extracted from **sulfas_PosTargetedMSMS.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** Under **User Spectra** in Data Navigator, right-click the spectra, and click **Delete**. **b** Click **Yes** in the Delete message box. **c** Extract an integrated MS/MS EIC of ions 279.09100 with an m/z range of 100-300 (see "Task 11. Extract chromatograms (LC/MS and LC/MS/MS)" on page 40) **d** In Method Explorer, select **Spectrum > Extract (MS/MS)**. **e** Click the **Peak Spectrum Extraction (MS/MS)** tab, if not visible. **f** Under **Peak spectrum background**, click **Average of spectra at peak start and end**. **g** In the Chromatogram Results toolbar, click the **Peak Select** icon. **h** Select the peak near 0.8 min. **i** Right-click and click **Extract Peak Spectrum**.	• Note that at the end of this process, all extracted peak spectra will automatically have the designated background spectrum subtracted. • You make the following changes to the **Extract Chromatograms** dialog box.  • You are extracting any transition with a precursor ion of 279.09100 and a product ion between 100 and 300.

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

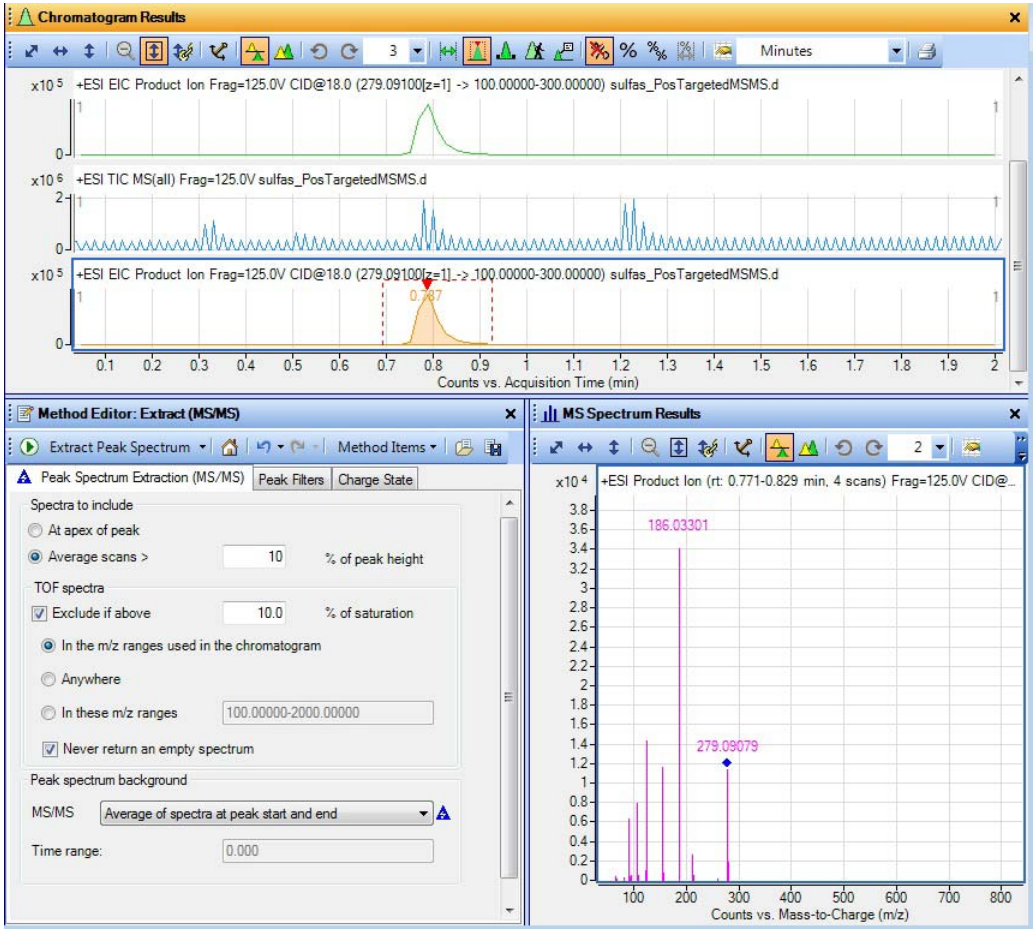


Figure 32 Product ion (MS/MS) spectra with background subtracted

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
7 Extract an MS/MS EIC Product Ion chromatogram specifying the product ions 186.03396 and 156.07760. • Do not integrate when the chromatogram is extracted.	**a** Right-click the Product Ion spectrum. **b** Click **Extract Chromatograms**. **c** From the **Type** list, select **EIC**. **d** Clear the **Integrate when extracted** check box. **e** From the **MS level** list, select **MS/MS**. **f** Select **Any** for the **Precursor ion m/z**. **g** Type `186.03396,156.07760` into the m/z values box. **h** Mark the **Merge multiple masses into one chromatogram** check box. **i** Click **OK**.	• You separate multiple m/z values with a comma. • If you type a single m/z value, then it is changed to a range automatically by using the **Single m/z expansion range for this chromatogram** parameters that are entered on the Advanced tab. • You are extracting a chromatogram that has any precursor ion but only has the product ion of 186.03396 or 156.07760.

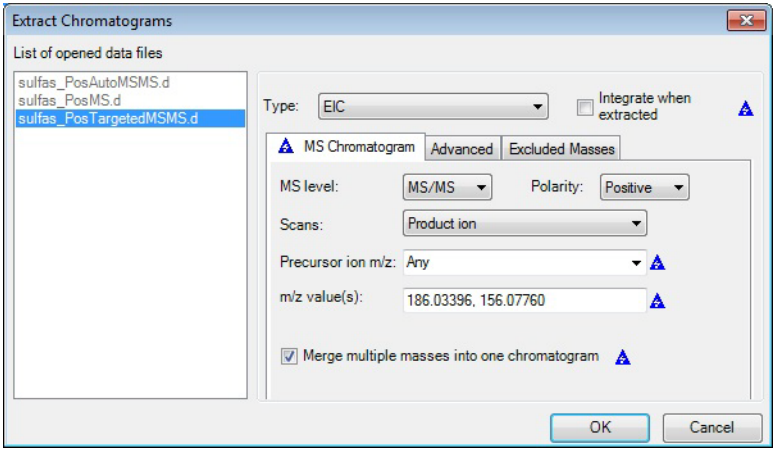


Figure 33 **Extract Chromatograms** dialog box for EIC based on product ions

Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

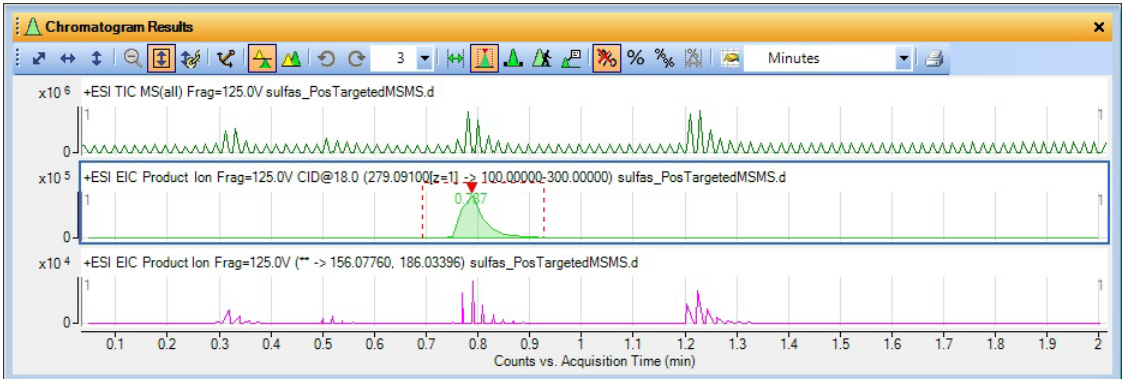
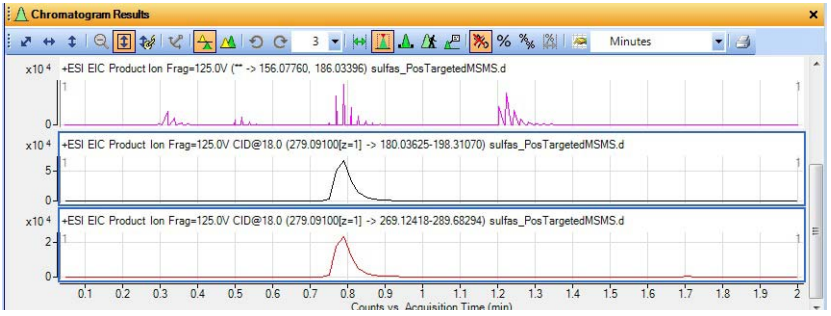


Figure 34 Product Ion EIC

8 Extract an MS/MS EIC using the product ion spectra, 279.091-> ** from Step 6.

a In the MS Spectrum Results window, select a range around the 279.09079 peak.
b Press and hold the **Ctrl** key.
c Select a range around the 186.03301 peak.
d Right-click the spectrum and click **Extract EIC > Over Selected Ranges.**

- A separate chromatogram is extracted for each range in the spectrum.
- The range for the precursor ion is set to the range (or ranges) selected in the MS Spectrum Results window.



Expanded titles are enabled in the Chromatogram Display Options dialog box. The expanded title includes Ionization, Fragmentor voltage and Collision Energy voltage.

Figure 35 Product Ion EIC created directly from the Product Ion spectrum

Tasks for MS and UV Data

Task 14. Extract chromatograms (MS and UV)

In this task, you extract MS and UV chromatograms from a data file.

Task 14. Extract chromatograms (MS and UV)

Steps	Detailed Instructions	Comments
1 Extract UV chromatograms (DAD1 and ADC1) from the **sulfas_PosMS.d** data file. • Hide all data files except sulfas_PosMS.d • Delete all chromatograms except the TIC Scan. • Extract the DAD1 chromatogram. • Extract the ADC1 chromatogram. • Change the number of panes visible to 3.	**a** In the Data Navigator window, clear the check boxes for the data files except for **sulfas_PosMS.d**. **b** Mark the check box for the sulfas_PosMS.d data file. **c** Delete all chromatograms except the **TIC Scan**. **d** Open the Extract Chromatograms dialog box, using the option below or one of the options to the right: • Click **Chromatograms > Extract Chromatograms**. **e** In the **List of opened data files**, click **sulfas_PosMS.d.** **f** In the **Type** list, click **Other chromatograms**. **g** In the **Detector** combo box, select `DAD1`. **h** Click **OK**. **i** Open the Extract Chromatograms dialog box. **j** In the **List of opened data files**, click **sulfas_PosMS.d.** **k** In the **Type** list, select **Other chromatograms**. **l** In the **Detector** combo box, select `ADC1`. **m** Click **OK**. **n** Make sure the **Maximum number of list panes** is set to `3` in the Chromatogram Results toolbar.	• You can also extract chromatograms in one of the following ways: • Right-click the chromatogram, and click **Extract Chromatograms**. • From the Data Navigator window, highlight the **TIC Scan** for **sulfas_PosMS.d**. Then, right-click the **TIC Scan** and click **Extract Chromatograms**. • Note that you can also choose to have the extracted chromatogram automatically integrated after extraction.

Task 14. Extract chromatograms (MS and UV) (continued)

Steps	Detailed Instructions	Comments

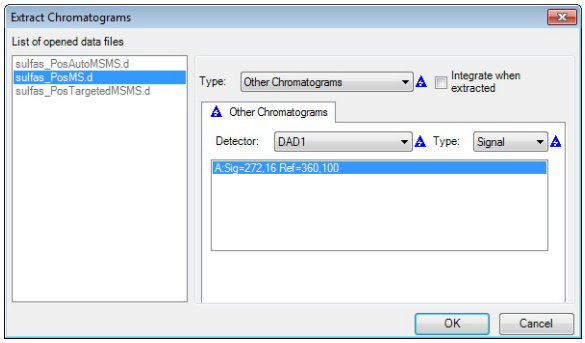


Figure 36 The Extract Chromatograms dialog box with **Type** Other Chromatograms.

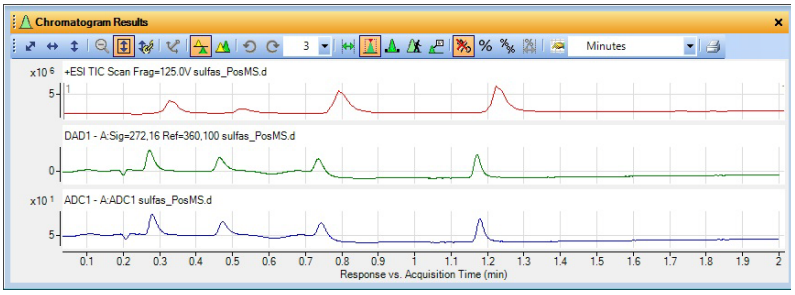


Figure 37 Chromatogram Results window with the original TIC, the DAD1, and the ADC1

Task 15. Interactively integrate a chromatogram (UV) and calculate System Suitability values (MS and UV)

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 15. Interactively integrate a chromatogram (MS and UV)

Steps	Detailed Instructions	Comments
1 Integrate the **sulfas_PosMS.d** UV chromatograms, using any of the options listed at right. • Highlight the DAD1 and ADC1 chromatogram. • Integrate the chromatograms.	**a** Highlight the DAD1 and ADC1 chromatograms. **b** In Method Explorer, select **Chromatogram > Integrate (UV)**. **c** Select the **General** integrator. **d** Integrate the **sulfas_PosMS.d** UV chromatograms, using any of the following options. • From the main menu, click **Chromatograms > Integrate Chromatogram**. • Highlight the chromatogram. Then, right-click the chromatogram, and click **Integrate Chromatogram**. • In Data Navigator, highlight **DAD1** and **ADC1** in the **sulfas_PosMS.d > User Chromatograms** section. Then, right-click either chromatogram and click **Integrate Chromatogram**. **e** If needed, highlight the MS chromatogram and integrate.	• The integration uses the General Integrator, instead of the Agile 2 integrator selected in the method default.m. • If the Chromatogram > Integrate (UV) section is not available, then you need to mark the UV check box in the "User Interface Configuration" dialog box. • Note that using the General integrator with default parameters is detecting very small peaks.
2 Adjust the delay time so that the chromatogram peaks line up.	**a** In Method Explorer, select **Chromatogram > Adjust Delay Time**. **b** Mark the **MS1** check box. **c** Enter 0.325 for the **Retention Time.** **d** Mark the DAD1 check box. **e** Enter 0.272 for the Retention Time. **f** Click **Calculate delay from RT**. **g** Click **Adjust Delay Time in Data** in the Method Editor toolbar.	• In this exercise, the retention times for the MS data are adjusted to match those in the UV trace and so will not match the unadjusted times elsewhere in the guide.

Task 15. Interactively integrate a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments

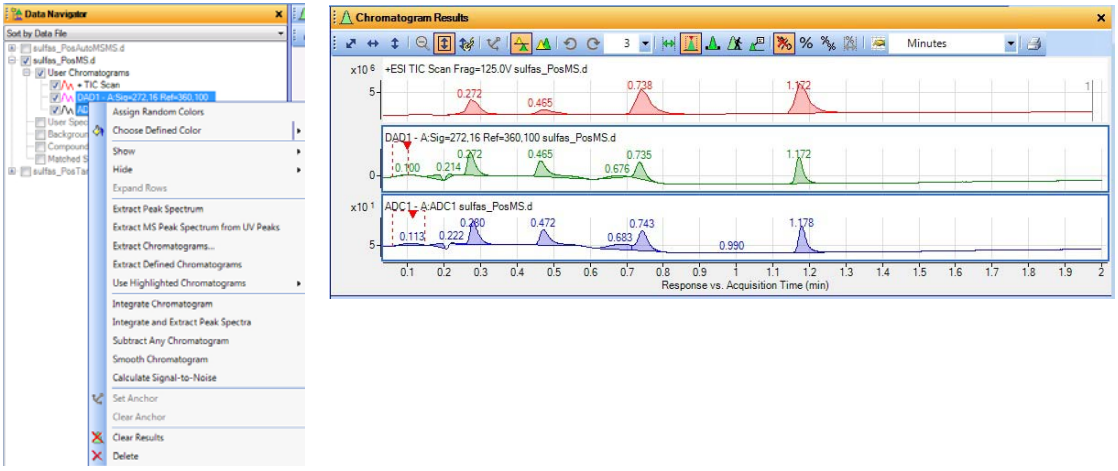


Figure 38 One of the shortcut menus in the Data Navigator and integrated sulfas_PosMS.d chromatograms

3 Enable system suitability calculations for UV chromatograms.

a From Method Explorer, select **Chromatogram > Integrate (UV)** 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 `0.15`.
f In the Column length box, type `5`.

- 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.

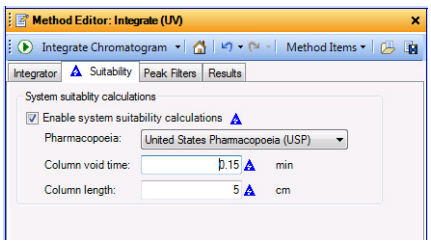


Figure 39 Chromatogram > Integrate (UV) Suitability tab

Task 15. Interactively integrate a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments
4 Reintegrate the chromatogram.	• Click the **Integrate Chromatogram** icon on the Method Editor toolbar to integrate using the new setting.	
5 View the system suitability calculations. • Open the Integration Peak List window. • Review the values for the noise region, and calculate the signal-to-noise ratio for the integrated peaks.	**a** Click **View > Integration peak list**. **b** Right-click the header of the Integration peak list window and click **Floating**. **c** Right-click the column header of any column that you do not want to see and click **Remove Column**. **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. • These values include **k'**, **Tailing factor**, **Plates**, **Plates/M**, and **Symmetry**. • You can also enable system suitability calculations for MS, MS/MS, ADC and GC chromatograms.

Peaks: DAD1 - A:Sig=272,16 Ref=360,100

Peak	RT	Area	Height	Type	Width	FWHM	SNR	Symmetry	k'	Plates	Plates/M	Resolution	Tailing factor
1	0.1	0.1	0.01		0.042			23.5	-0.3	1602	32040	-20	0.5
2	0.185	1.03	0.61		0.057			3.14	0.2	266	5320	4.8	0.7
3	0.214	0.45	0.46		0.028			1	0.4	1325	26500	0.8	1
4	0.272	4.39	2.91		0.089			0.45	0.8	815	16300	1.9	1.7
5	0.465	4.88	2.02		0.169			0.18	2.1	1692	33840	4.6	3.4
6	0.676	0.81	0.25		0.095			1.77	3.5	334	6680	2.2	0.8
7	0.735	3.5	2.11		0.08			0.62	3.9	4688	93760	0.6	1.3
8	1.172	4.72	3.37		0.085			0.58	6.8	19207	384140	11.4	1.5

Figure 40 Integrated Peaks table with system suitability values

6 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. **b** Close the **Method Editor** window. **c** Right-click the title of the Integration Peak List window and click **Floating**. **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. • When you click **View > Integration Peak List** a second time, the window is no longer visible.

Task 16. Extract spectra from a chromatogram (UV)

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

Task 16. Extract spectra from a chromatogram (MS and UV)

Steps	Detailed Instructions	Comments

1 Extract spectra on specific data points for the peak at 0.27 minutes and the last peak (1.22) of the **sulfas_PosMS.d** data file.
• Delete the ADC1 chromatogram.
• After zooming in on the region between 0.17 and 0.31 minutes, extract a spectrum from the peak at or near 0.27 minutes using any one of the options described under Comments.
• Open Spectrum Preview.
• After zooming in on the region between 1.1 and 1.3 minutes, extract a spectrum from the peak at or near 1.17 minutes.
• Copy this spectrum to the User Spectra section.
• Change the display to show at least two spectra.
	a Delete the ADC1 chromatogram.
	b Click the **Autoscale X-axis and Y-axis** icon in the Chromatogram Results toolbar to zoom out completely.
	c Click the **Range Select** icon on the Chromatogram Results toolbar.
	d Highlight the DAD1 chromatogram.
	e To zoom in to the peak at 0.272 min, right-click the mouse above the peak at 0.2 minutes and drag it to below the curve at 0.31 minutes, and release.
	f On the peak near 0.27 minutes, extract a UV spectrum using one of the methods in the Comments column.
	g Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.
	h To open Spectrum Preview, click the **Spectrum Preview** icon,
	i Zoom into the region between 1.1 and 1.3 min.
	j On the peak near 1.17 min. extract a UV spectrum. The spectrum is shown in the Spectrum Preview window.
	k Right-click the spectrum, and click **Copy to User Spectra**. The Spectrum Preview window is tabbed with the UV Spectrum Results window.
	l If necessary, click the arrow next to the **Maximum number of list panes** icon in the UV Spectrum Results toolbar, and select **2**.
	m Close the MS Spectrum Results window.
		• You cannot extract spectra from an ADC chromatogram.
		• 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".
		• You can extract a spectrum in any of the following ways:
		  • Double-click the data point in the chromatogram.
		  • Click the data point in the chromatogram, then right-click anywhere in the chromatogram. Click **Extract UV Spectrum**. The Extract Spectrum dialog box is displayed. Make sure the **sulfas_PosMS.d** file is selected, and click **Extract**.
		• Note that when you first extract a spectrum, the UV Spectrum Results window appears containing the spectrum, and the type of spectrum and retention time appear under User Spectra in the Data Navigator.
		• When Spectrum Preview is enabled, the system displays any manually-selected spectrum but it is not kept in the User Spectra section.
		• With Spectrum Preview open, Qualitative Analysis overwrites the previous spectrum in the Spectrum Preview window when you extract a new spectrum.

Task 16. Extract spectra from a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments

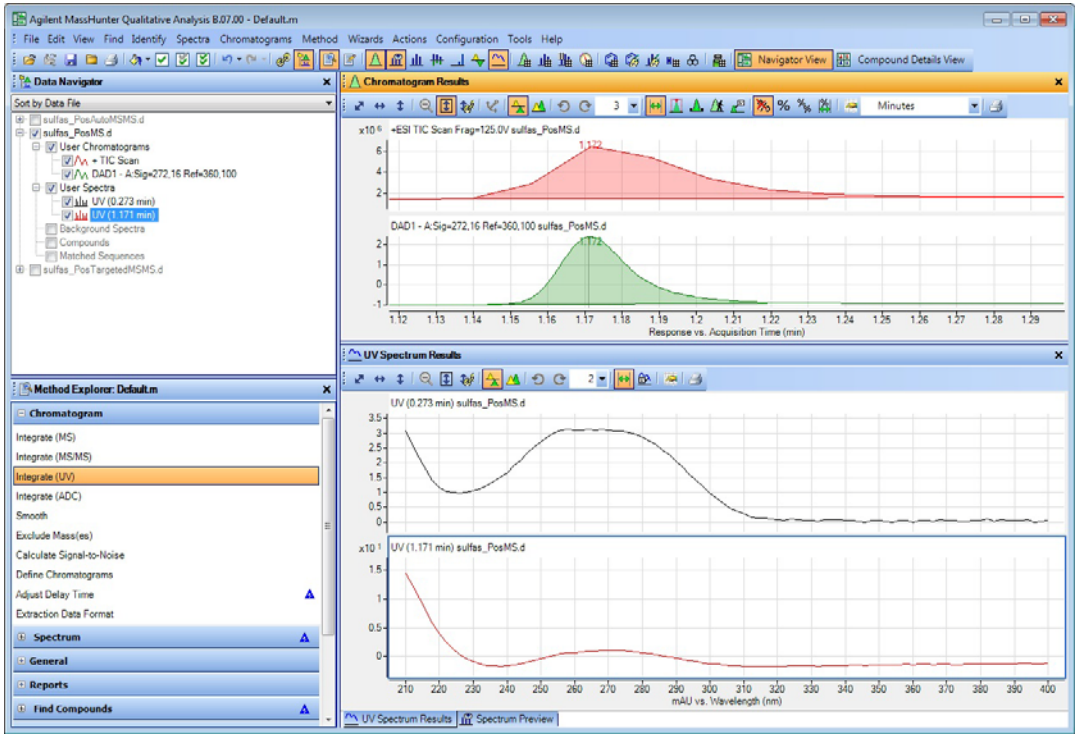


Figure 41 Main window with extracted UV spectra from two integrated peaks in the sulfas_PosMS.d file

Task 16. Extract spectra from a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments
2 Extract a spectrum that averages all UV points within a specified range for the last integrated UV peak for the **sulfas_PosMS.d** data file: • Delete any existing User Spectra. • Zoom out of the chromatogram. • Turn off Spectrum Preview. • Use the Range Select icon on the Chromatogram toolbar. • Set the range from the halfway point on the left to the same point on the right of the peak. • Extract the spectrum, using any of the options listed.	**a** Highlight the User Spectra to be deleted (Use **Ctrl**). **b** Right-click the selected User Spectra, and click **Delete**. **c** Click **Yes** in the Delete dialog box, if it is displayed. **d** Click the **Autoscale X-axis and Y-axis** icon to zoom out completely. **e** Click the **Spectrum Preview** window, then close the window. **f** Click the **Range Select** icon on the Chromatogram toolbar. **g** Click at the halfway point on the left side of the last integrated peak in the DAD1 chromatogram and drag over to the halfway point on the right. **h** Extract the averaged spectrum using the option below or on the right. • Right-click anywhere in the range of the peak, and click **Extract UV Spectrum** from the shortcut menu. • Click **Extract** in the Extract Spectrum dialog box.	• You can also extract an average spectrum by double-clicking the selected range in the chromatogram. • You can change whether or not you are asked to confirm every time you delete a chromatogram or spectrum by using the Message Box Options dialog box. This dialog box is displayed from the **Tools > Message Box Options** command. • The Extract Spectrum dialog box is only shown if more than one data file is loaded.

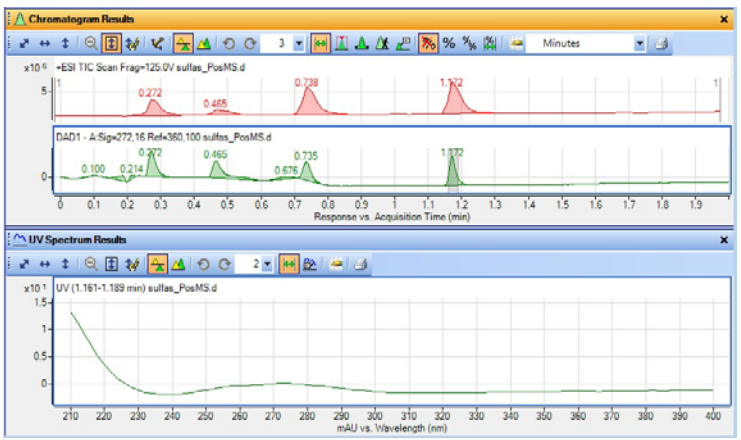


Figure 42 Average spectrum extracted from selected range for last peak

Task 16. Extract spectra from a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments
3 Extract a UV peak spectrum in **sulfas_PosMS.d**. • Delete any scans under User Spectra in Data Navigator. • Integrate the DAD1 chromatogram. • Extract a peak spectrum from the third integrated peak.	**a** Under **User Spectra** in Data Navigator, highlight the User Spectra to be deleted. **b** Right-click the spectra, and click **Delete**. **c** Click **Yes**. **d** Highlight the DAD1 Chromatogram. **e** Click **Chromatograms > Integrate Chromatogram.** **f** Click the **Peak Select** icon in the Chromatogram Results toolbar. **g** Click the integrated peak at 0.272 minutes in the DAD1 chromatogram. **h** Right-click the peak and click **Extract Peak Spectrum**.	• Extracted peak spectra are always put into either the UV Spectrum Results window or the MS Spectrum Results window, even if the Spectrum Preview window is open. • If you extract a peak spectrum from a UV chromatogram, the peak is placed in the UV Spectrum Results window. • If you have an area selected in the chromatogram, you can integrate either over the **Entire Chromatogram** or **Over Selected Ranges**.

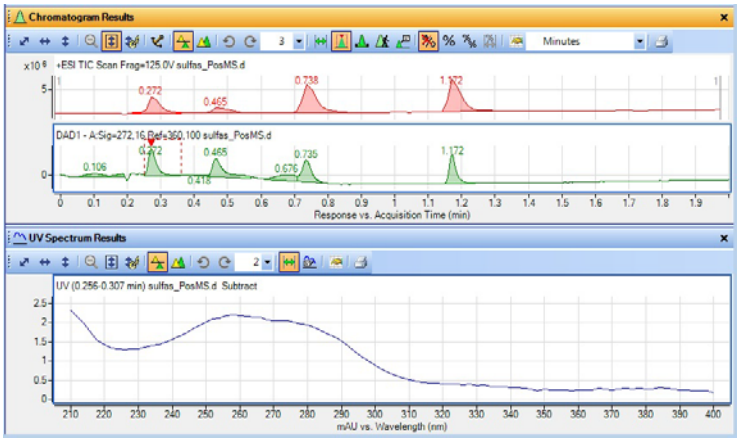


Figure 43 Integrated DAD1 chromatogram and UV Peak Spectrum

4 Close all three data files.	**a** Click **File > Close All**. **b** Click **No** when asked to save the results.	

Exercise 2
Find and identify compounds

In the first two sets of tasks, you find and identify low-concentration sulfa drugs within a complex matrix and generate their formulas for both TOF and Q-TOF data. You also do a molecular feature extraction on a protein digest with both TOF and Q-TOF data. These tasks can also be performed on Triple Quad 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.

Tasks for MS-Only Data (LC/MS - TOF, Q-TOF or Triple Quad)

Task 1. Find compounds by molecular feature (LC/MS - MS only)

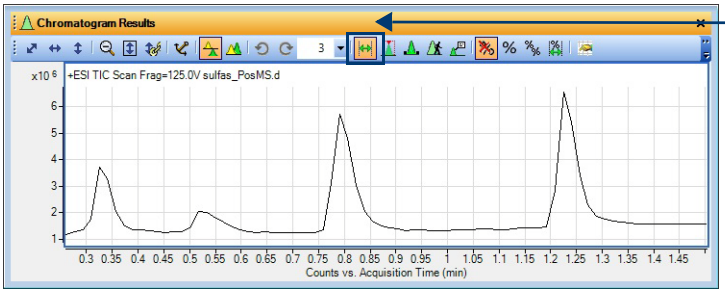
The FindCompounds algorithms find compounds in data and create averaged MS spectra for each compound. This functionality is an easy way to "mine" information from complex data. This algorithm only works with data that contains MS scan data. It does not work on data with unit mass resolution (for example, Triple Quad data).

Task 1. Find compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments
1 Open the **sulfas_PosMS.d** chromatogram. • Use the General workflow • Select a range between 0.24 and 1.5 minutes.	**a** Double-click the **Mass Hunter Qualitative Analysis icon**. **b** Select the **sulfa_PosMS.d** data file in the example data file directory. Clear the **Load result data** check box and click **Open**. **c** Click **Configuration > Configure for Workflow > General**. The Workflow Configuration dialog box is opened. **d** Clear the **Save current method** check box if you don't want to save the changes to the method. **e** Click the **Load workflow's default method** button. **f** Click the **Load workflow's default layout** button. **g** Click **OK**. **h** Click the **Range Select** tool, and select the region from 0.24 to 1.5 minutes.	• The method Default.m is loaded automatically. To load this method interactively, click **Method > Open**. Select Default.m and click **Open**. • You can get help for any window, dialog box, or tab by using the `F1` key when that window is active. • When you switch between workflows, the Workflow Configuration dialog box is opened. • If you mark the **Save current method** check box, the method is automatically saved to the current method name. If the method is default.m, then the Save Method dialog box is opened. (you cannot overwrite the default method).

Task 1. Find compounds (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments



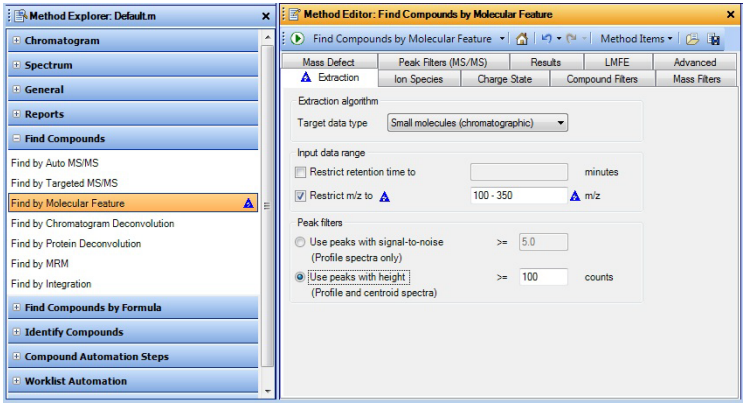
Range Select tool

Figure 44 Selecting a time range in the TIC chromatogram

2 Find compounds in the chromatogram.
- Restrict m/z to 100-350.
- Make sure you can see chromatograms and spectra for all the compounds.

a In the Method Explorer window, click **Find Compounds > Find Compounds by Molecular Feature.**
b Select **Small molecules (chromatographic)** as the **Target data type**.
c Mark the **Restrict m/z to** check box.
d Type `100 - 350`.

- Learn more about **Target data type** in the online Help.
- You choose the region of the chromatogram from which to find compounds. See Figure 44.
- The red circle appears next to the **Restrict m/z** to box until you enter a value. Then, it changes to a blue triangle. The blue triangle is removed when you save the method.



The LMFE and the Advanced tabs are only available if the Advanced check box is marked in the User Interface Configuration dialog box.

The LMFE tab is only available if the MassHunter BioConfirm software is installed.

Figure 45 Restricting mass range for finding compounds by molecular feature

Task 1. Find compounds (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments
	e Click the **Results** tab. **f** Mark the **Extract MFE spectrum** and the **Extract ECC** check boxes. **g** Mark the **Display only the largest** check box and type 4 for the number of compounds.	• You can extract the complete result set for a compound after it is found by using the **Find > Extract Complete Result Set** command when one or more compounds are highlighted. You can also select one or more compounds in the Data Navigator window and click the **Extract Complete Result Set** command in the shortcut menu.

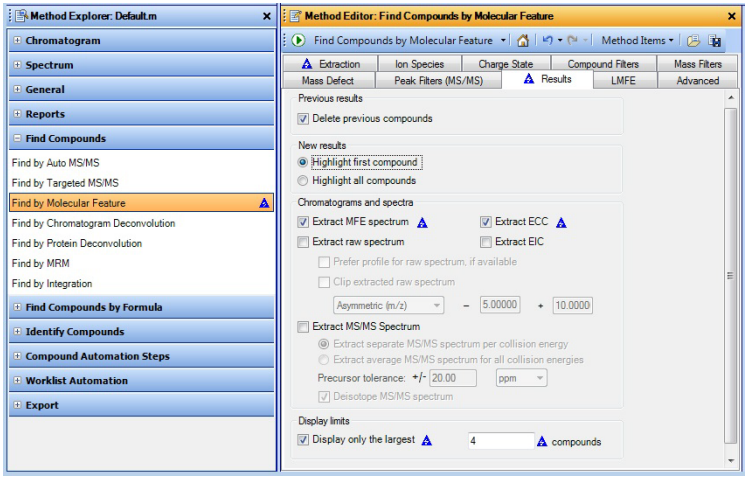


Figure 46 Changing the values in the Find by Molecular Feature > Results tab

Task 1. Find compounds (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments
	h Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file. **i** Select **4** in the **Maximum number of list panes** in the MS Spectrum Results windows. **j** Click the **Autoscale Y-axis during Zoom** icon,  , in the MS Spectrum Results toolbar. **k** Zoom in on the m/z range from 270 to 350. **l** Click the  button in the Chromatogram Results toolbar.	• Four major compounds in the selected range are found. • The selected range is used when you click  in the Method Editor toolbar. In the **Find > Find by Molecular Feature** command, you click either **Entire Chromatogram** or **Over Selected Ranges**. • Click the **Configuration > Chromatogram Display Options** command to change **Label top plot only**. • The Compound List is changed to show different columns.

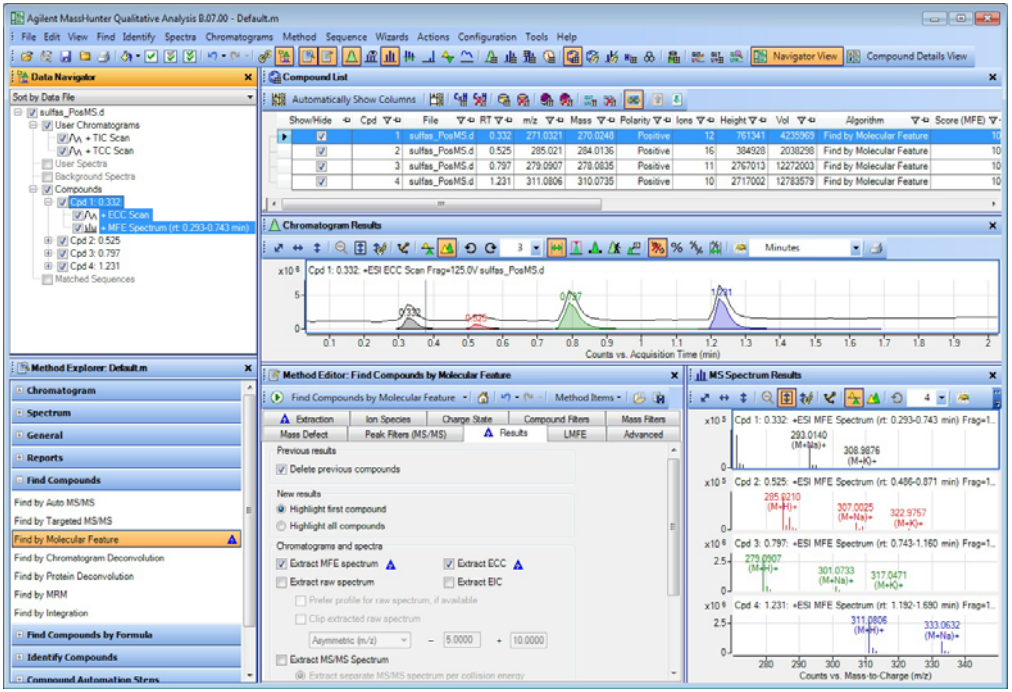


Figure 47 Finding all four compounds in the sulfa drug mix

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

In this task, you generate possible formulas and search for each of those compounds found in Task 1.

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments
1 Generate formulas for Compounds 1-4. • View the MS Formula Results for each compound. • View the Compound List. • Close the MS Spectrum Results window. Hint: To obtain the same results as in Figure 49, make sure you have selected **Common organic molecules** as the Isotope model.	**a** In the Method Explorer window, click **Identify Compounds > Generate Formulas**. **b** In the Method Editor window, click the **Charge State** tab, and select **Common organic molecules** as the **Isotope model**. **c** In the Data Navigator window, click **Compounds** to highlight all of the compounds. **d** Click the **Identify > Generate Formulas from Compound** command or the **Generate Formulas from Compound** icon. **e** If necessary, click the Compound Identification Results icon, , or click the **View > Compound Identification Results** command. **f** If necessary, click **View > Compound List**. **g** In the Compound List window, click the **Automatically Show Columns button** in the toolbar. **h** In the Compound Identification Results window, click the **Automatically Show Columns** button in the toolbar. **i** Click the Hide Empty Columns icon, ,in the Compound List and the Compound Identification Results window. **j** Close the Method Editor window and the MS Spectrum Results window.	• By default, the MS Formula Results window is tabbed with the Chromatogram Results window. Click on the tab at the bottom of the window to switch between windows. • 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. • 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. • You can change the width of a column by dragging the line that separates adjacent columns. • You can move a column by dragging the column header. • You can delete a column by clicking **Remove column** in the shortcut menu.

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments

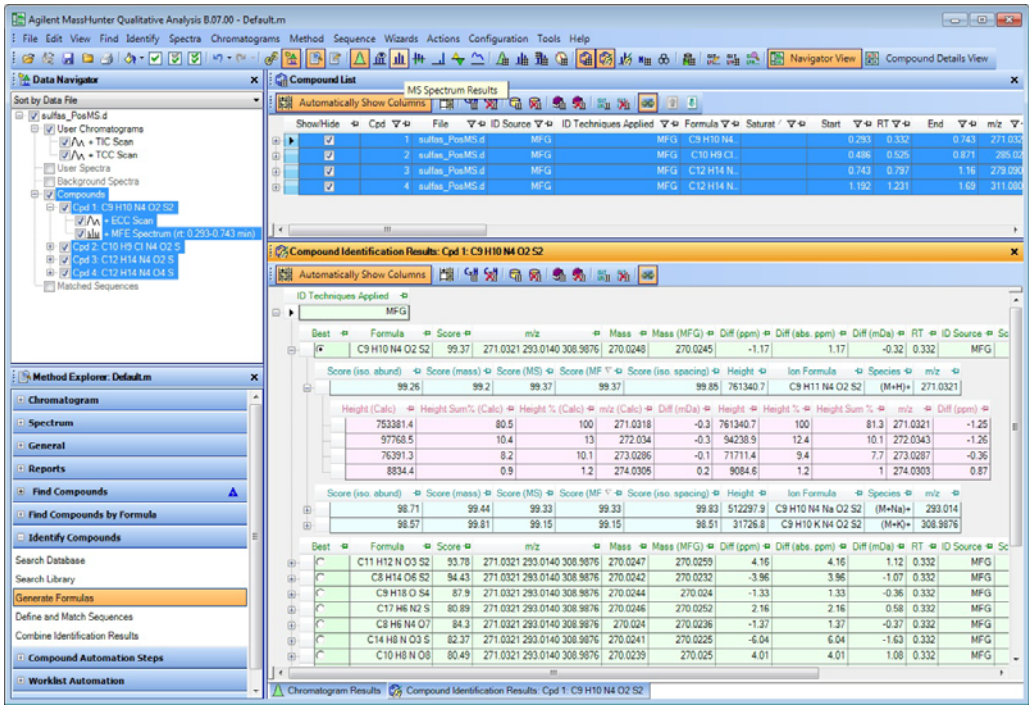


Figure 48 Generate Formula results for Compounds 1 to 4 in sulfas_PosMS.d

2 Do a database search based on formulas for compounds 1 to 4.
- Base search on formula.

a In the Data Navigator window, click **Compounds**.

b In the Method Explorer window, click **Identify Compounds > Search Database**.

c Under **Values to match** click **Molecular formula**.

d Click **Identify > Search Database for Compounds** in the main menu.

e Close the Method Editor and the MS Spectrum Results windows.

- The Method Editor is opened automatically when you click a section in the Method Explorer.
- Note in the Compound List that all four sulfa drugs have been identified (See Figure 49).
- All of the identification results for compounds are shown in the Compound Identification Results window.
- Some identification results are also shown in the Compound List window.

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments
3 Modify the columns that are visible.	**a** Right-click the Compound List window and click **Add/Remove Columns**. **b** Mark the check box next to the CAS value and click **OK**. The CAS column is empty; the software automatically displays any column that contains information. **c** Click the Hide Empty Columns icon,  ,in the Compound List.	• If you use the Remove Column command and remove a column that contains data, the software automatically redisplays this column if you turn off the Automatically Show Columns feature and then turn it back on.

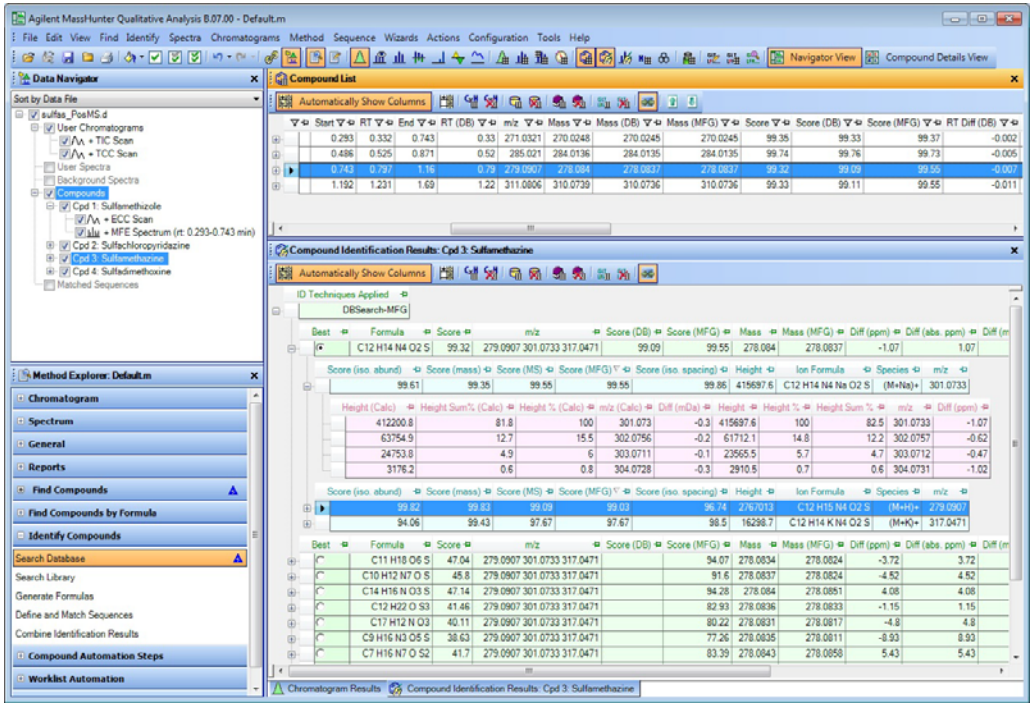


Figure 49 Results for Database Search and Generate Formulas for Compounds 1 to 4 in sulfas_PosMS.d

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments
4 Review results in the Compound Details View.	**a** Click the Compound Details View button in the main toolbar. **b** Close the Compound Fragment Spectrum Results window. Click **View > Compound Fragment Spectrum Results**. **c** Close any other window that does not contain data. **d** In the Compound List window, right-click the header of any column that you want to remove, and click **Remove Column**. **e** In the Compound List window, switch between different compounds.	• The Compound Fragment Spectrum Results window is empty unless you are analyzing All Ions MS/MS data. See "Analyzing Data Files acquired in All Ions MS/MS Mode" on page 137. • As you switch between compounds in the Compound List window, the contents of the other windows are updated automatically to show information on the selected compound. • See the online Help for more information.

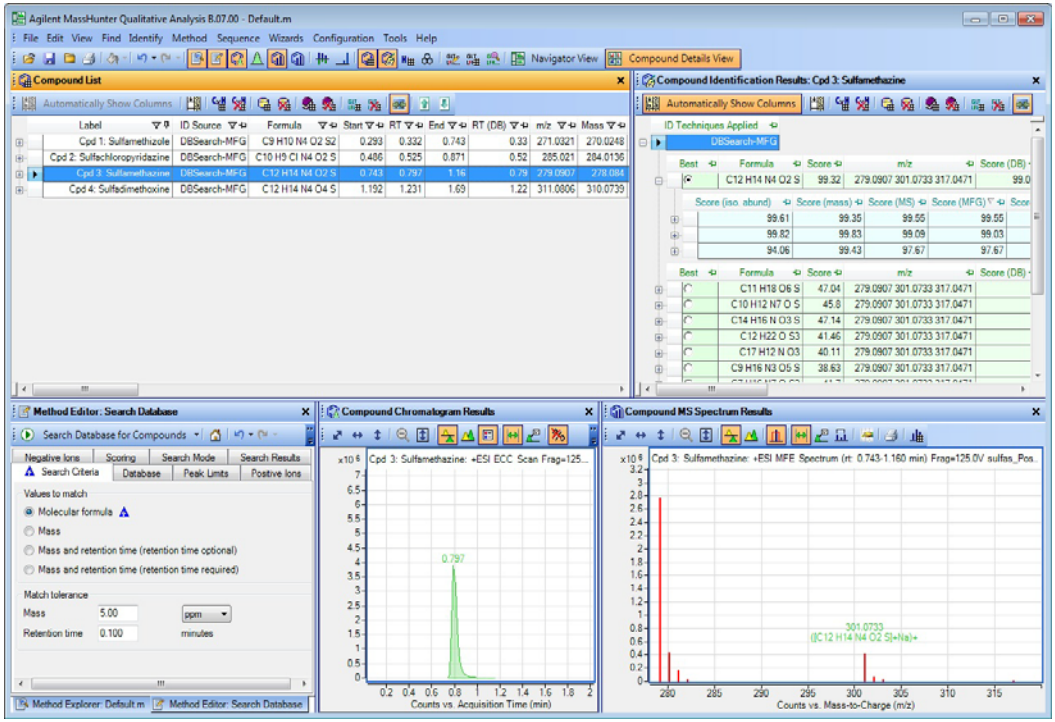
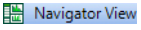


Figure 50 Reviewing Compounds 1 to 4 in sulfas_PosMS.d in Compound Details View

Task 2. Generate formulas and identify compounds (LC/MS - MS only)

Step	Detailed Instructions	Comments
5 Switch back to Navigator View.	• Click the  button in the main toolbar	• You can switch between Compound Details View and Navigator View anytime. If you have multiple data files open at the same time, you use the Navigator View. If you have only one data file open, then you can use either view.

Task 3. Print a compound report (LC/MS - MS only)

You generate a report for each of those compounds found in **"Task 1. Find compounds by molecular feature (LC/MS - MS only)"** on page 70 and identified in **"Task 2. Generate formulas and identify compounds (LC/MS - MS only)"** on page 74.

Task 3. Print a compound report (LC/MS - MS only)

Step	Detailed Instructions	Comments
1 Change some of the selections in the method for compound reports: • Turn off viewing the MS spectra zoomed in on special peaks. • Turn off the MS/MS options in the report.	**a** In Method Explorer, click **Reports > Compound Report**. **b** Clear the **Show MS spectrum** check box. **c** Clear the **Show MS/MS spectrum** check box. **d** 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.

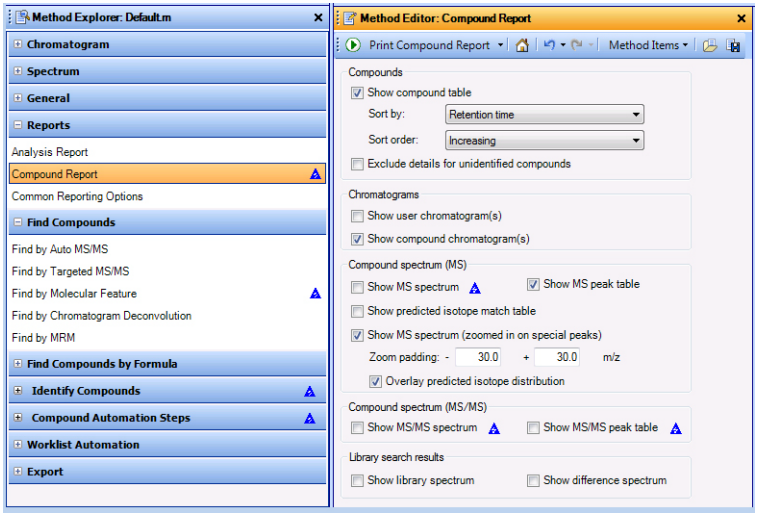
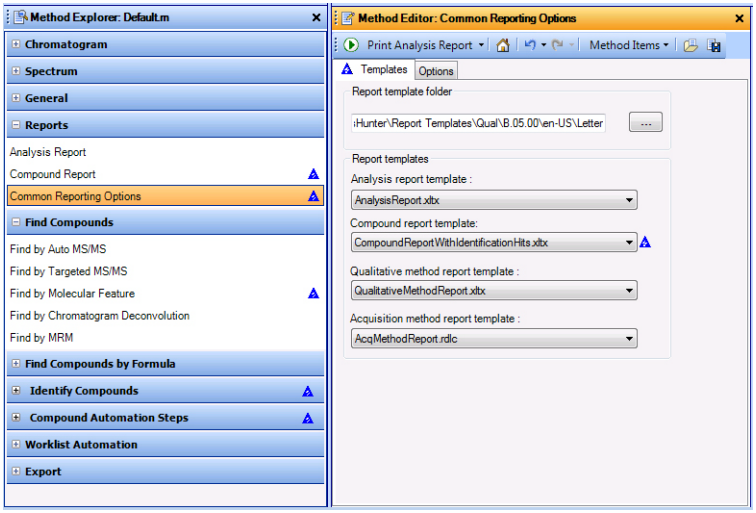


Figure 51 Compound Report section in the Method Editor

Task 3. Print a compound report (LC/MS - MS only)

Step	Detailed Instructions	Comments
2 (optional) Choose a different compound report template.	**a** In the Method Explorer window, click **Reports > Common Reporting Options.** **b** Select **CompoundReport WithIdentificationHits.xlsx** 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. See "Customize a report template" on page 171.



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 52 Common Reporting Options section in the Method Editor

3 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. **b** Mark the **Print preview** check box. **c** Click **OK**. Examine the report. **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. • See the online Help or the Report Designer Training DVD for additional information.
4 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked if you want to save the results.	

Task 4. Find compounds by formula and calculate sample purity (LC/MS - MS only)

The Find Compounds algorithms find compounds in data and create averaged MS spectra for each compound. This functionality is an easy way to "mine" information from complex data. You can also compute sample purity. Before calculating sample purity, you align the different chromatograms.

Task 4. Find compounds by formula (LC/MS - MS only)

Step	Detailed Instructions	Comments
1 Open the **sulfas_PosMS.d** chromatogram. • Use the General workflow. • Select a range between 0.2 and 1.5 minutes.	**a** Click **File > Open Data File**. **b** Select **sulfas_PosMS.d** and click **OK**. **c** Click **Configuration > Configure for Workflow > General**. See "Open the sulfas_PosMS.d chromatogram." on page 70 for more information. **d** Click the **Autoscale Y-axis during Zoom** icon,  , in the Chromatogram Results toolbar. **e** Click the **Range Select** tool, and select the region from 0.2 to 1.5 minutes.	• If you switch to the Formula Confirmation and Sample Purity workflow, the Compound List table automatically shows the sample purity columns. • The Find by Formula sections are included in the **Formula Confirmation and Sample Purity Workflow** section.
2 Set the parameters to run the Find by Formula algorithm within the specified range on the chromatogram. • Enable sample purity calculations. • Calculate the TIC %, ADC %, and UV A% purity values. • Use the maximum value as the purity value. • Specify a delay time of 0.045 minutes. • Add a **Relative height** filter.	**a** In the Method Explorer window, click the **Find Compounds by Formula > Find by Formula - Options** section. **b** Click **Database/Library** as the **Source of formulas to confirm** and select default.csv. **c** In the Method Explorer window, click **Find Compounds by Formula > Find by Formula - Sample Purity** section. **d** Mark the **Compute sample purity** check box. **e** Mark the **TIC %**, **ADC %**, and **UV A%** check boxes. **f** Click **Maximum of all selected algorithms**. **g** In the **Minimum acceptable purity** box, type `20`.	• You double-click the title bar to anchor a window that is floating. By default in the General workflow, the Method Editor window is floating. You can also right-click the title of the window, and then click **Floating**. • The blue triangle appears when you change a setting from the value that is saved in the current method. When you save the method, the triangles disappear. • This data file contains multiple sulfa drugs which is why the expected purity is 20%.

Task 4. Find compounds by formula (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments
• Adjust the delay time for the MS signal by 0.045 minutes.	**h** Click the **Find by Formula - Sample Purity > Delay Time** section in the Method Explorer window. **i** Mark the **Use Delay** check box. **j** Type `0.045` in the **Time delay (min)** parameter.	• When you calculate the sample purity with multiple signals, the software matches peaks from the different signals, so it is better to line up the signals before running the algorithm.
• Add a relative height filter to the chromatograms.	**k** Click the **Find by Formula - Chromatograms > EIC Peak Filters** section in the Method Explorer window. **l** Mark the **Relative height** check box. **m** Type `5` in the **% of largest peak** parameter. In the default method, this is the default.	• You can limit which peaks are returned from the integration algorithm.

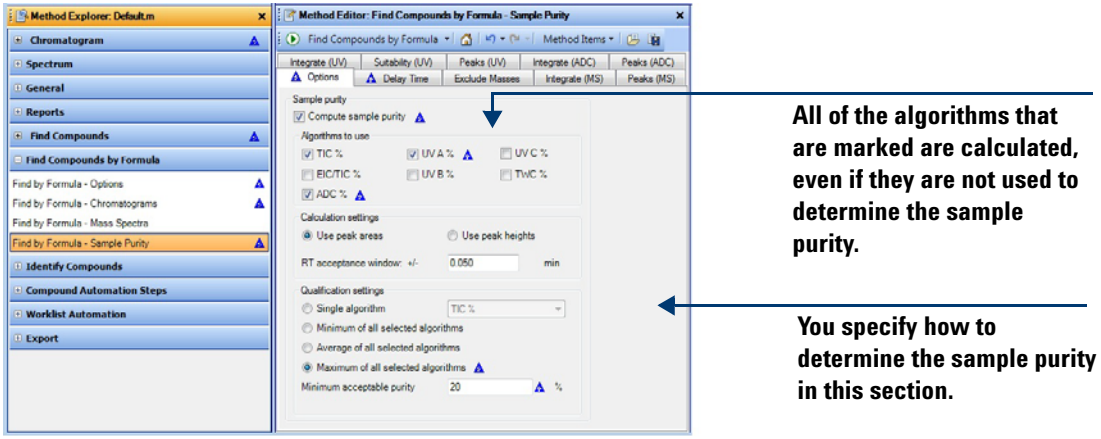


Figure 53 Setting sample purity options for the Find Compounds by Formula algorithm

Task 4. Find compounds by formula (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments
3 Run the Find by Formula algorithm within the specified range, and review the results.	**a** Click the **Find Compounds by Formula > Find by Formula - Options** tab. **b** Click  to run the **Find Compounds by Formula** algorithm on the data file. **c** Change the Maximum number of list panes to 3 in the MS Spectrum Results windows. **d** Click **View > Compound List** to open the Compound List window. **e** In the Compound List window, if the **Automatically Show Columns** icon in the toolbar is not on, click the icon. **f** Click the Hide Empty Columns () button in the Compound List window. **g** In the Compound List window, click the Automatically Show Columns icon. **h** Remove columns from the table that you don't want to include.	• The Qualitative Analysis program finds 4 compounds in the selected range. • When you click the Category column, the columns are shown with columns from the same algorithm together. They are shown alphabetically within each section. • The Compound List was docked at the top of the Qualitative Analysis window so that more columns are visible. See "Task 4. Change window layouts" on page 19 for more information on moving windows.
4 Display Sample Purity columns. • If the Automatically Show Columns icon is not on, then you can manually display the Purity Columns.	**a** Right-click a column, and click **Add/Remove Columns** to open the Compound Columns dialog box. **b** Click the **Category** column header to sort the possible columns. **c** Mark the **Purity Value** column, the **Purity Result** column, the **ADC% Area** column, the **TIC% Area** column, the **UVA% Area** column, and the **UVB% Area** column. **d** Click the **OK** button.	

Task 4. Find compounds by formula (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments

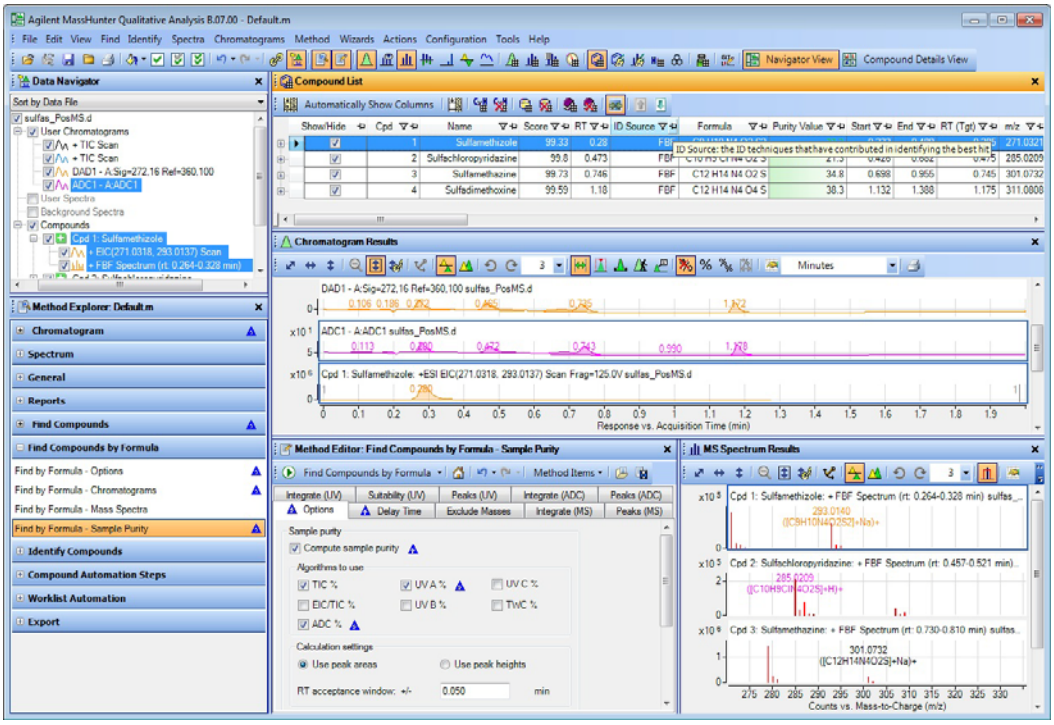


Figure 54 Finding all four compounds in the sulfa drug mix

- The icon for the Compound in the Data Navigator indicates whether the Compound passed the Sample Purity test.

- The Purity Value column is color coded:
  - Green - Pass
  - Yellow - Fail
  - Red - Cannot measure

5 Review results in the Compound Details View.

a Click the Compound Details View button in the main toolbar.

b Close any other window that does not contain data.

c In the Compound List window, switch between different compounds.

- The Compound Fragment Spectrum Results window is empty unless you are analyzing All Ions MS/MS data. See "Analyzing Data Files acquired in All Ions MS/MS Mode" on page 137.

Task 4. Find compounds by formula (LC/MS - MS only) (continued)

Step	Detailed Instructions	Comments

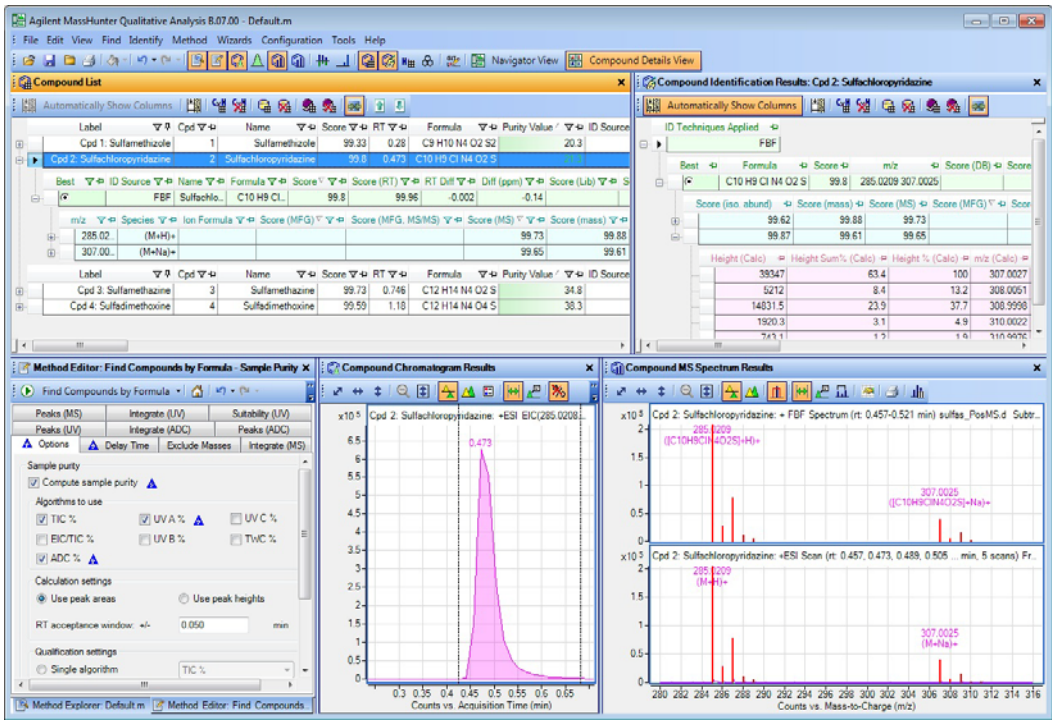


Figure 55 Finding all four compounds in the sulfa drug mix

6 Return to Navigator View and close the data file without saving results.

a Click the Navigator View button in the main toolbar

b Click **File > Close Data File**.

c Click **No** when asked if you want to save the results.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS only)

In this task, you do molecular feature extraction on a protein digest using only MS data.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS only)

Step	Detailed Instructions	Comments
1 Enable Peptide Sequence Editor features.	**a** Click **Configuration > Configure for Workflows > General**. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click the **OK** button. **d** Click **Configuration > User Interface Configuration**. **e** Mark the **Peptide Sequence Editor** check box. **f** Click **OK**.	• The Peptides option in the Charge State tab is not available unless the **Peptide Sequence Editor** or the **BioConfirm** check box is marked. • You switch to the General workflow to change the layout and the visible Compound columns back to the defaults.
2 Do a molecular feature extraction for the data file **peptide-ms-only.d** with these parameters: • Time range is 2.5 to 4 minutes. • Specify that the Isotope model is peptides. • Filter to show only the largest 20 compounds in abundance. • Change the window layout to match that of Figure 56 (next page).	**a** Open the **peptide-ms-only.d** data file. **b** In the Method Explorer window, click **Find Compounds > Find by Molecular Feature** to display the parameters in the Method Explorer window. **c** In the **Extraction** tab, mark the **Restrict retention time to** check box. **d** Type `2.5 - 4`. **e** Clear the **Restrict m/z to** check box, if necessary. **f** On the **Charge State** tab, select **Peptides** in the **Isotope model** list. **g** On the **Compound Filters** tab, mark the **Limit to the largest** check box and type `20` for the number of compounds. **h** On the **Results** tab, mark the **Extract MFE spectrum** and **Extract ECC** check boxes. **i** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file.	• The **Limit to the largest** filter does not limit the number of features extracted. It just limits the number of compounds displayed in Qualitative Analysis. • You extract features using the Qualitative Analysis Molecular Feature algorithm. Then, you can compare sets of different compounds using Agilent Mass Profiler Professional software. • You export the compounds to a CEF file using the **File > Export > Export CEF Options** command. • If you are going to use the Match Sequences algorithm, you also mark the **Extract MS/MS** check box. If you do not, the columns are not displayed in the Compound List window and the Compound Identification Results window.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS only)

Step	Detailed Instructions	Comments
3 Find the compound spectrum for the *m/z* 570.7362 ion and determine the charge state, mass and ion species.	**a** In the MS Spectrum Results window, scroll to find the spectrum containing the *m/z* 570.7362 ion. **b** Find the charge state. **c** Find the ion species. **d** Find this compound in the Compound List window. **e** Find the mass.	• Compound 3 has a spectrum containing this ion with a charge state of +2. • The mass is 1139.4578. The ion species is (M+2H)+2. You can see the ion species in the MS Spectrum Results window and also in the Spectrum Peak List window in the column labeled **Ion Species**.

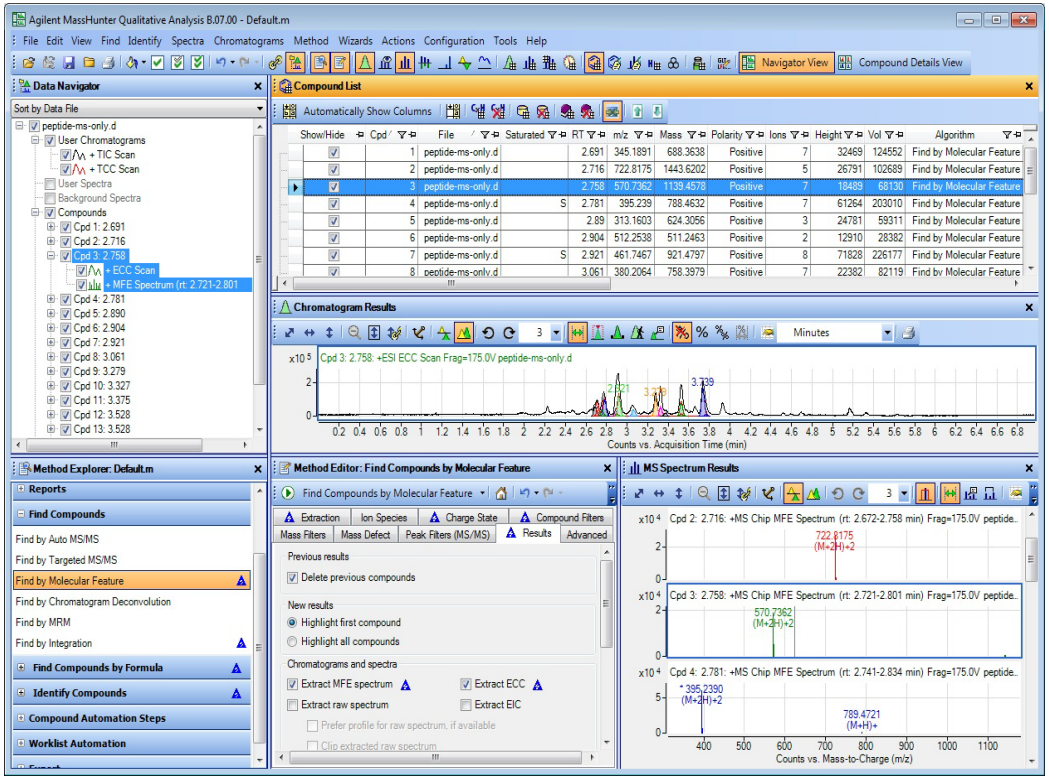
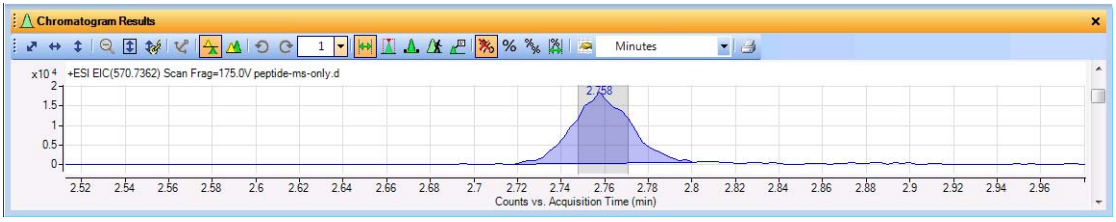
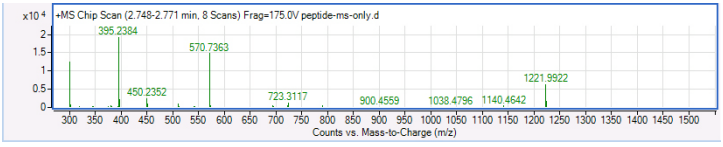


Figure 56 Find Compounds by Molecular Feature with Qualitative Analysis

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS only)

Step	Detailed Instructions	Comments
4 Extract an integrated EIC for this peptide. • Use 570.7362 as the m/z value.	**a** Right-click the TIC for the data file, and click **Extract Chromatograms**. **b** From the **Type** list, select **EIC**. **c** Mark the **Integrate when extracted** check box. **d** Type `570.7362` as the *m/z* value. **e** Click the **Advanced** tab. **f** Select **Symmetric (ppm)** and click **OK**.	It is important that the Single m/z expansion value is set appropriately for the data file. For this Q-TOF data file, an extraction range of +/- 100 ppm is more appropriate.
5 Extract an averaged spectrum for the first integrated peak in the EIC. • Zoom into what appears to be the first integrated peak. • Select a range from the halfway point across the highest peak.	**a** Click the List mode icon in the Chromatogram Results toolbar. **b** Right-click the EIC and drag the cursor to zoom in around the peak at 2.76 minutes. **c** Make sure that the **Range Select** tool has been selected, and select a range across the peak at the midpoint.	
		
	d Double-click within the shaded region of the peak to extract an averaged spectrum.	
		
6 Close the data file.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save results.	

Tasks for MS/MS Data (LC/MS - Q-TOF or Triple Quad)

Task 1. Find compounds (LC/MS - MS and MS/MS)

The FindCompounds algorithms identify compounds in MS/MS data and create averaged MS and MS/MS spectra for each compound. This functionality is an easy way to "mine" information from complex data.

Task 1. Find compounds (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments
1 Open the TIC for the **sulfas_PosAutoMSMS.d** data file and select a range from 0.2 to 1.3 minutes. • Use the General workflow. • Highlight a range from 0.2 to 1.3 minutes.	**a** If the program is not open, double-click the **Mass Hunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**. **b** Click the **sulfas_PosAutoMSMS.d** data file in the example data file directory, and click **Open**. **c** Click the **Configuration > Configure for Workflow > General** command. **d** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **e** Click the **OK** button. **f** Click **Configuration > User Interface Configuration**. **g** Clear the **GC** check box. **h** Click **OK**. **i** Click the **Range Select** tool in the Chromatogram Results toolbar, if necessary. **j** Click the **Auto-scale Y-axis during Zoom** tool in the Chromatogram Results toolbar, if necessary. **k** Click and drag to select the range from 0.2 to 1.3 minutes.	• The method default.m is automatically opened. To open a different method, click **Method > Open**, select the method, and click **Open**. • A blue triangle is automatically shown in the Adjust Delay Time tabs in the Method Explorer when you open this data file. This data file also contains DAD and ADC data. You may ignore these blue triangles unless you want to enter a delay time. • Some of the Find Compounds algorithms only work with GC/MS data files. If you clear the GC check box, those algorithms are not shown.

Task 1. Find compounds (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments

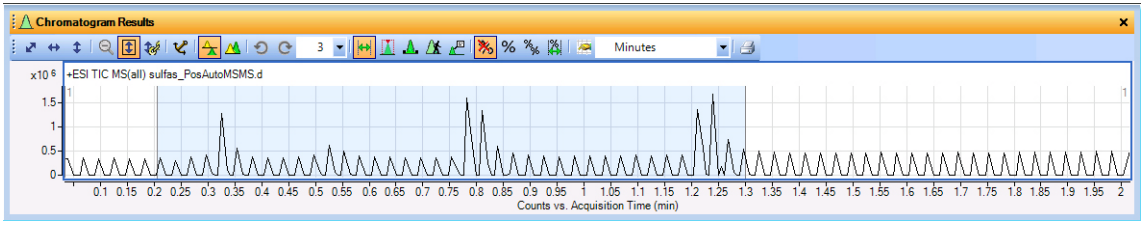


Figure 57 Zoomed range for TIC chromatogram of sulfas_PosAutoMSMS.d data file

2 Find compounds from 0.2 to 1.3 minutes on the chromatogram.
- Enter a Positive MS/MS TIC threshold of 100000.
- Exclude masses 121.0504 and 922.0097.

a In Method Explorer click **Find Compounds > Find by Auto MS/MS.**
b On the Processing tab in the **Positive MS/MS TIC threshold**, type `100000`.
c Click the **Excluded Masses** tab.
d Click **Exclude masses (or m/z ranges) from all new chromatograms.**
e Type `121.0504, 922.0097`
f Select **Symmetric (ppm)**.
g Select **20**.

- You can choose the region of the chromatogram from which you intend to find compounds. See Figure 57.
- 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.

NOTE: Blue triangles are also displayed in the Chromatogram, Find Compounds by Formula, and Compound Automation Steps sections in the Method Explorer when you change the Excluded Masses tab. These same values are also used in these other sections of the method.

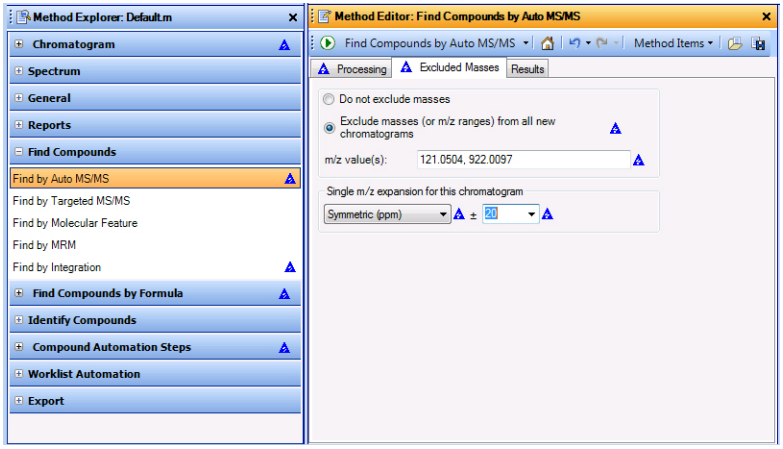


Figure 58 Excluded Masses tab of Find Compounds by AutoMS/MS

Task 1. Find compounds (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments
• Select to extract EIC, MS spectra and MS/MS spectra.	**h** Click the **Results** tab. **i** Mark the **Extract EIC**, **Extract MS**, and **Extract MS/MS** check boxes. **j** Clear the **Extract ECC** check box. **k** Click to run the **Find Compounds by Auto MS/MS** algorithm on the data file.	• You can also click **Find > Find Compounds by Auto MS/MS > Over Selected Ranges**. • The Qualitative Analysis program will find 4 compounds in the selected range under these conditions. • In the next task you identify which compounds are the sulfa drugs.
3 Display both spectra for Compound 4 only. See Figure 59.	**a** Highlight Compound 4 only. **b** Click the **Show only the highlighted items** tool in the main toolbar. **c** Expand Compound 4 to see the chromatogram and two spectra. You click the Plus sign next to the compound in the Data Navigator window to see the labels for the chromatogram and spectra.	• Showing both spectra is a convenient way to display all the information for a single compound. • Note that both the precursor and product spectra are extracted for each compound. The diamond represents the precursor ion. You can change the color to use for the MS/MS precursor ion symbol in the MS and MS/MS Spectra Display Options dialog box.

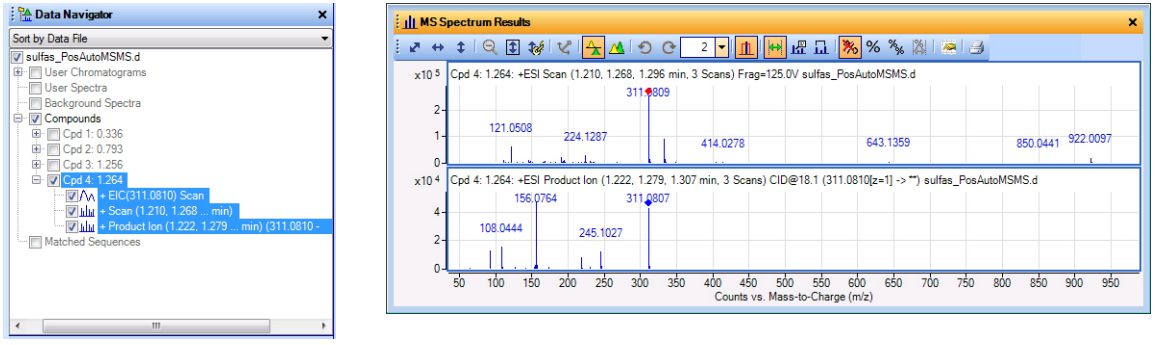


Figure 59 Data Navigator window and MS Spectrum Results window showing MS and MS/MS spectra for Compound 4

Task 2. Identify compounds and generate formulas (LC/MS - MS and MS/MS)

In this task, you identify and generate formulas for the compounds found in Task 1.

Task 2. Identify compounds and generate formulas (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments
1 Do a database search of Compounds 1 to 4 based on masses. See Figure 60 on page 93.	**a** Highlight all compounds in the Data Navigator window. **b** In Method Explorer, click **Identify Compounds > Search Database**. **c** In the Method Editor window in the Search Criteria tab, click **Mass**. **d** Click the **Database** tab. **e** Verify that **Database path** is **default.csv**. **f** Click **Identify > Search Database for Compounds** from the main menu. You can instead click the **Search Database for Compounds** icon to run the algorithm. **g** If the Compound List is not showing, click **View > Compound List**. **h** If the Compound Identification Results window is not showing, click **View > Compound Identification Results**. **i** Mark the **Show/Hide** check boxes for compounds 1 to 3 in the Compound List. Compounds 1 to 3 were hidden in the last task. You can also click the **Show all highlighted items** tool in the main toolbar.	• Note that three of the sulfa drugs have been identified in the Compound List (See Figure 61 on page 94). • Note that no compound name was found for Compound 3 after running the database search algorithm.

Task 2. Identify compounds and generate formulas (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments

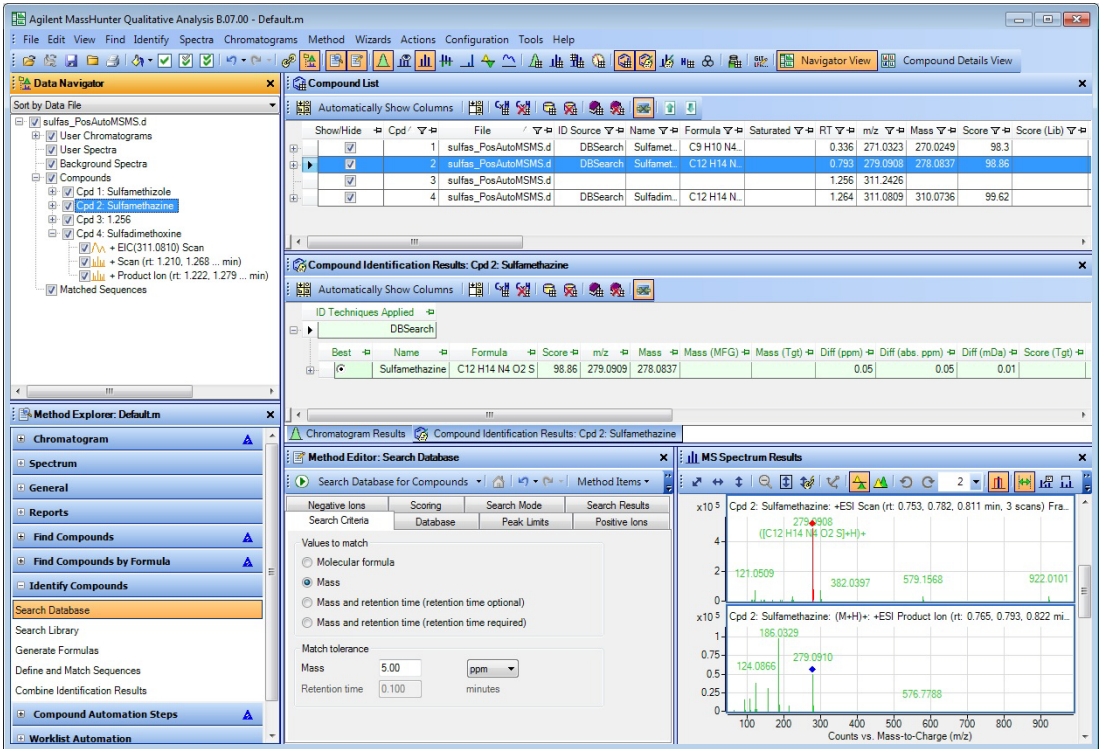
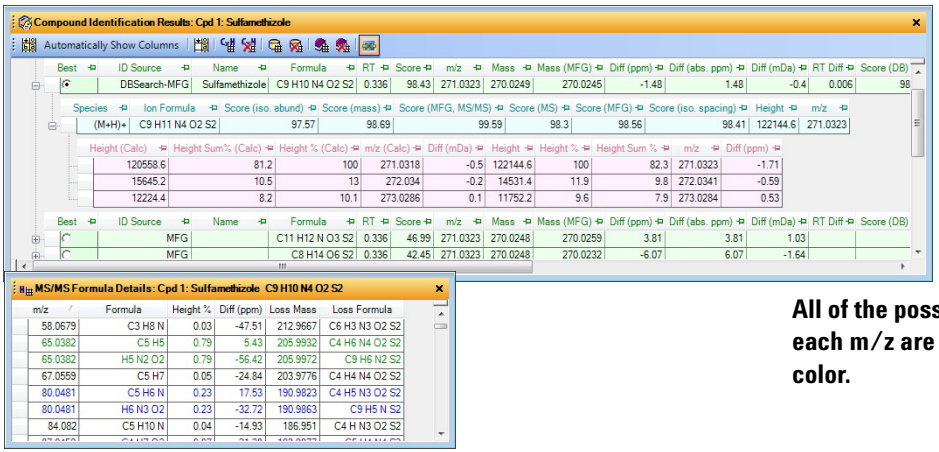


Figure 60 Compounds in sulfas_PosAutoMSMS.d data file identified by searching a database

Task 2. Identify compounds and generate formulas (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments
2 Generate formulas for Compounds 1 to 4. • View the Compound List. • View the Compound Identification Results list. • Close the MS Spectrum Results window. Hint: To obtain the same results as in Figure 61, make sure you have selected **Common organic molecules** for the Isotope model.	**a** In the Method Explorer window, click **Identify Compounds > Generate Formulas**. **b** Click the **Charge State** tab, and select **Common organic molecules**. **c** Highlight all four compounds. **d** Click the **Generate Formulas from Compound** tool ( ) to run the algorithm or click the **Identify > Generate Formulas from Compounds** command. **e** In the Data Navigator window, highlight the compound that you want to see. **f** Use the scroll bar in the Compound Identification Results window to see the Generate Formulas results (MFG). The second level of the table shows several Score columns. The ID Source column shows that the result was found by both the Database Search (DB) algorithm and the Generate Formulas algorithm (MFG).	• By default, the Compound Identification Results window is tabbed with the Chromatogram Results window. Click on the tab at the bottom of the window to switch between windows. • You see the predicted isotope abundance ratios on the spectrum plot when you zoom in at the appropriate m/z. • Note that one or more formula were found for all compounds. • Click the Hide Empty Columns icon to automatically hide empty columns. You can also use the Remove column shortcut command. • Note that the formula from the database search is the same as the formula determined by the Generate Formulas algorithm. • Click **Configuration > Compound Label Configuration** to change the compound label.



All of the possible formulas for each m/z are shown in the same color.

Figure 61 Compound Identification Results window and MS/MS Details window for Compound 1

Task 3. Print a compound report (LC/MS - MS/MS)

In this task, you generate a report for each of those compounds found in Task 1 and identified in Task 2.

Task 3. Print a compound report (LC/MS - MS/MS)

Step	Detailed Instructions	Comments
1 Change some of the selections in the method for compound reports: • Turn off viewing the MS spectra zoomed in on special peaks, if necessary. • Turn on the MS/MS options in the report.	**a** In the Method Explorer window, click **Reports > Compound Report**. **b** Clear the **Show MS spectrum (zoomed in on special peaks)** check box, if necessary. **c** Mark the **Show MS/MS spectrum** check box and the **Show MS/MS peak table** check box.	• Only sections that are marked in this tab are included in the report. • To change the template that is used to print the report, click the **Reports > Common Reporting Options** line in the Method Explorer window. Select a different template to use for the report.

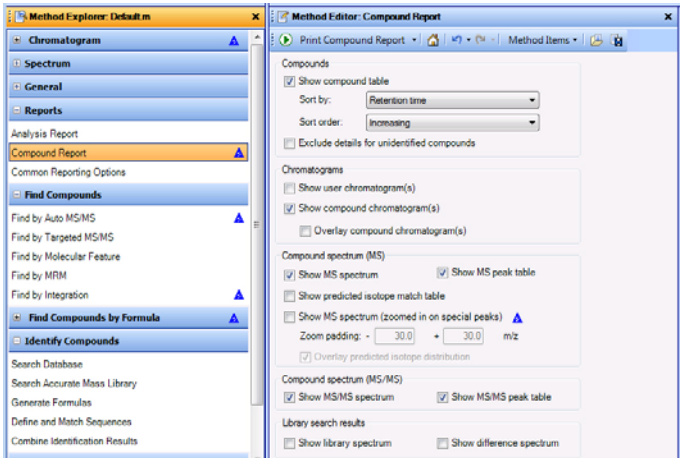
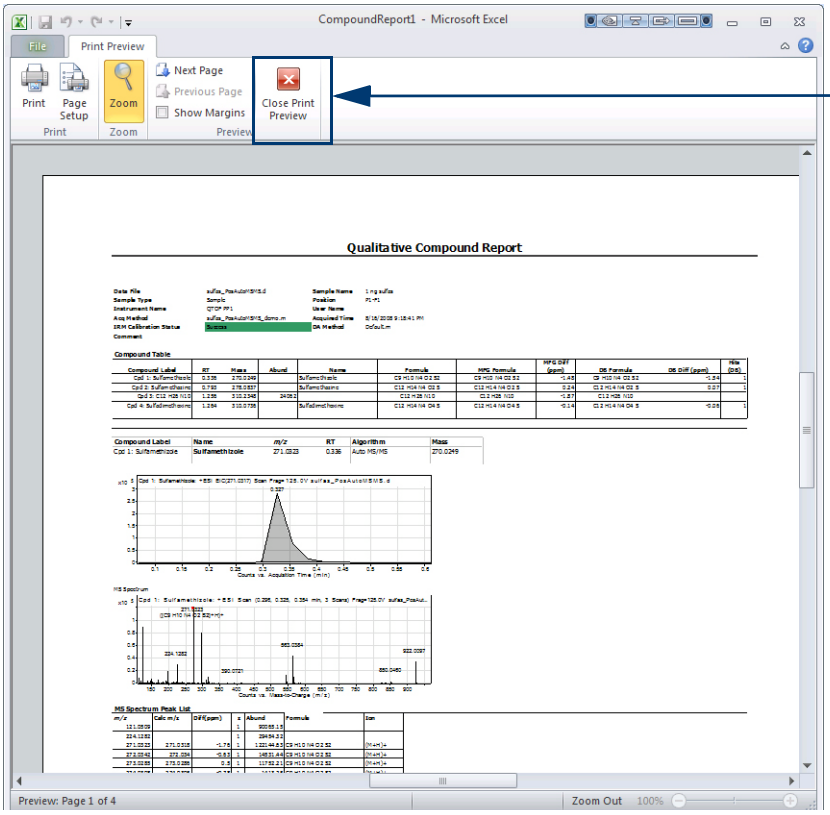


Figure 62 Compound Report window in the Method Editor

2 Print the report. • Preview the report.	**a** Click the **Print Compound Report** icon to print the report. **b** In the Print Compound Report dialog box, click the **All results** button. **c** Mark **Print report**. **d** Select a printer. **e** Mark **Print preview**. **f** Click **OK**.	• You create a PDF file when you mark the **Save report as PDF file** check box. This option only works if you installed the Microsoft Excel PDF add-in after installing Excel.

Task 3. Print a compound report (LC/MS - MS/MS)

Step	Detailed Instructions	Comments



This button closes the Print Preview window without sending the report to the printer.

Figure 63 Print Preview of a Compound Report

3 Close the Print Preview window.

a Click **Close Print Preview** in the toolbar.

- If you want to print the report, click the Print button. The report is printed on the printer selected in step 2 in the Print Compound Report dialog box.

4 Close the data file without saving results.

a Click **File > Close Data File**.
b Click **No** when asked if you want to save the results.

Task 4. Find Compounds and Search Library (LC/MS - MS/MS)

The Find Compounds by Targeted MS/MS algorithm identifies compounds in MS/MS data and can extract an MS and MS/MS spectra for each compound. If MS/MS spectra from multiple collision energies are used, you can either extract an average MS/MS spectrum for all collision energies or a separate MS/MS spectrum for each Collision Energy.

The Search Accurate Mass Library algorithm searches a library file (CDB) for a Product Ion spectrum. Only centroid spectra can be searched, so any profile spectrum needs to be converted to a centroid spectrum first.

Task 4. Find compounds and Search Accurate Mass Library (LC/MS - MS/MS)

Step	Detailed Instructions	Comments
1 Open the TIC for the **sulfas_PosTargetedMSMS.d** data file. • Use the General workflow.	**a** If the program is not open, double-click the **Mass Hunter Qualitative Analysis icon**. Click **Cancel** in the Open Data File dialog box. **b** Click the **Configuration > Configure for Workflow > General** command. **c** Click **OK**. **d** Click **File > Open Data File**. **e** Click **sulfas_PosTargetedMSMS.d**, and click **Open**. **f** Click the **Range Select** icon in the Chromatogram Results toolbar, if necessary. **g** Click the **Auto-scale Y-axis during Zoom** icon in the Chromatogram Results toolbar, if necessary.	• Click the **Load workflow's default method** and the **Load workflow's default layout** buttons. • To open a different method, click **Method > Open**, select the method, and click **Open**. • A blue triangle is automatically shown in the Adjust Delay Time tabs in the Method Explorer. This data file also contains DAD and ADC data. You may ignore these blue triangles unless you want to enter a delay time.

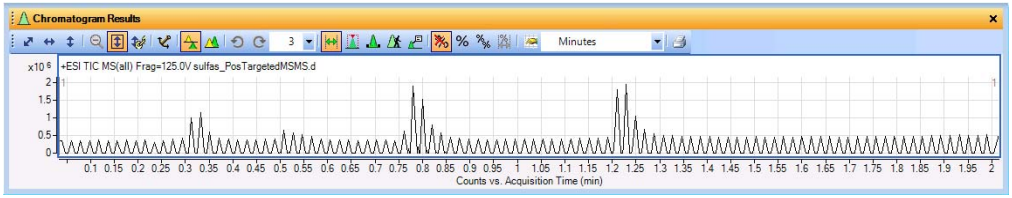


Figure 64 The TIC chromatogram for sulfas_PosTargetedMSMS.d data file

Task 4. Find compounds and Search Accurate Mass Library (LC/MS - MS/MS)

Step	Detailed Instructions	Comments
2 Find compounds using the Targeted MS/MS algorithm. • Select to extract an MS/MS chromatogram and MS/MS spectra.	**a** In the Method Explorer window, click **Find Compounds > Find by Targeted MS/MS.** **b** Click the **Results** tab. **c** Mark the **Extract MS/MS chromatogram** and **Extract MS/MS spectrum** check boxes. **d** Click **Find > Find Compounds by Targeted MS/MS**.	• 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. • The Qualitative Analysis program will find 4 compounds under these conditions.
3 Search each compound using the Search Accurate Mass Library algorithm. • Select the Sulfas_AM_PCDL.cdb library. • If this library is not available, then install the Personal Compound Database and Library (PCDL) program. • Lower the minimum match score to 50.	**a** In the Method Explorer window, click **Identify Compounds > Search Library.** **b** Click the **Add Library** button. **c** Select **Sulfas_AM_PCDL.cdb**. **d** Click the **Open** button. **e** Type 20 as the **Score (fwd)**. **f** Review the other parameters on this tab. **g** Review the parameters on the Search Criteria tab. **h** Highlight all compounds in the Data Navigator window. **i** Click **Identify > Search Library for Compounds**. **j** If not showing, click **View > Compound List**. **k** If not showing, click **View > Compound Identification Results**.	• If the selected library has the *CDB* extension, then the Search Accurate Mass Library algorithm is run when you search a library. If the selected library has the *L* extension, then the Search Unit Mass Library algorithm is run when you search a library. • You can also right-click the Compounds line in the Data Navigator window and then click **Search Library for Compounds**. • The Search Criteria tab allows you to filter the library entries that are searched on **Ionization mode**, **Instrument type**, and **Collision Energy**. • If available, the structures are automatically shown in the MS Spectrum Results window.

Task 4. Find compounds and Search Accurate Mass Library (LC/MS - MS/MS)

Step	Detailed Instructions	Comments

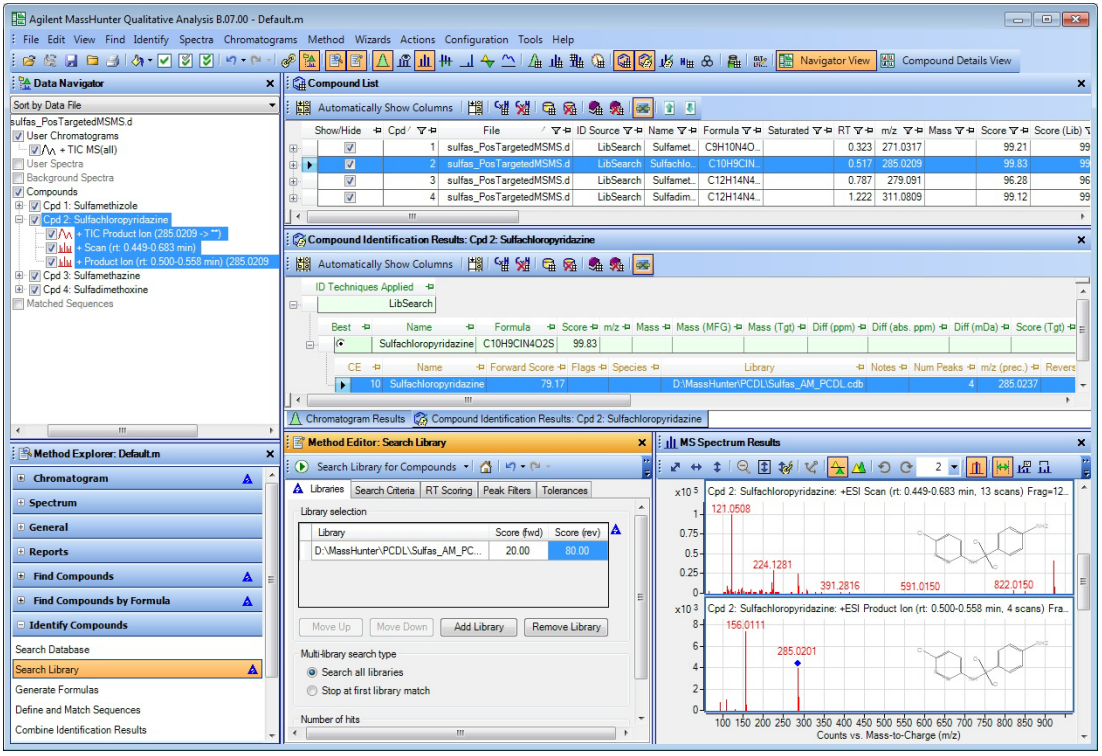


Figure 65 Results after running the Search Accurate Mass Library algorithm.

4	Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked if you want to save the results.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS and MS/MS)

In this task, you do molecular feature extraction on protein digest data obtained on a Q-TOF in Auto MS/MS mode.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments
1 Do a molecular feature extraction in the data file **peptide-auto.d** with these parameters: • Make sure the layout is returned to the Default Layout. • Time range is 2.5 to 4 minutes. • Set the isotope model to peptides. • Filter to show only the largest 20 compounds in abundance. • Change the window layout to match that of Figure 66 (next page).	**a** Open the **peptide-auto.d** data file. **b** Click the **Configuration > Configure for Workflow > General** command. **c** Click **OK**. **d** Click **Find Compounds > Find by Molecular Feature** in the Method Explorer to display the parameters in the Method Editor window. **e** In the **Extraction** tab, select **Small molecules (chromatographic)** as the Target data type. **f** Mark the **Restrict retention time to** check box. Then, type `2.5 - 4`. **g** On the **Charge State** tab, select **Peptides** as the **Isotope model**. **h** On the **Compound Filters** tab, mark the **Limit to the largest** check box and type `20` for the number of compounds. **i** On the Results tab, mark the **Extract MFE spectrum** and **Extract ECC** check boxes. **j** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file. **k** Click the List Mode tool in the Chromatogram Results toolbar. **l** If necessary, select **1** in the **Maximum number of list panes** box in the Chromatogram Results toolbar.	• To return the layout to the default layout, click **Configuration > Window Layouts > Restore Default Layout**. • The **Limit to the largest** filter does not limit the number of features extracted. It just limits the number of compounds displayed in Qualitative Analysis. • If Peptides is not one of the options for the **Isotope model**, you enable this feature by marking the **Peptide Sequence Editor** check box in the User Interface Configuration dialog box. Click **Configuration > User Interface Configuration** to display this dialog box. To display the Advanced tabs for the Find by Molecular Feature section, you mark the **Advanced** check box. To display the LMFE tab, you mark the **BioConfirm** check box. • You extract features using the Molecular Feature algorithm. Then, you can compare sets of data from different extractions using Agilent MassHunter Profiler software or GeneSpring MS software.
2 Find the compound spectrum for the *m/z* 625.31585 ion and determine the charge state.	**a** In the MS Spectrum Results window, scroll to find the spectrum containing the *m/z* 625.3166 ion. **b** Find the charge state.	• Compound 7 has a spectrum containing this ion with a charge state of +1.

Task 5. Do molecular feature extraction on a protein digest (LC/MS - MS and MS/MS)

Step	Detailed Instructions	Comments

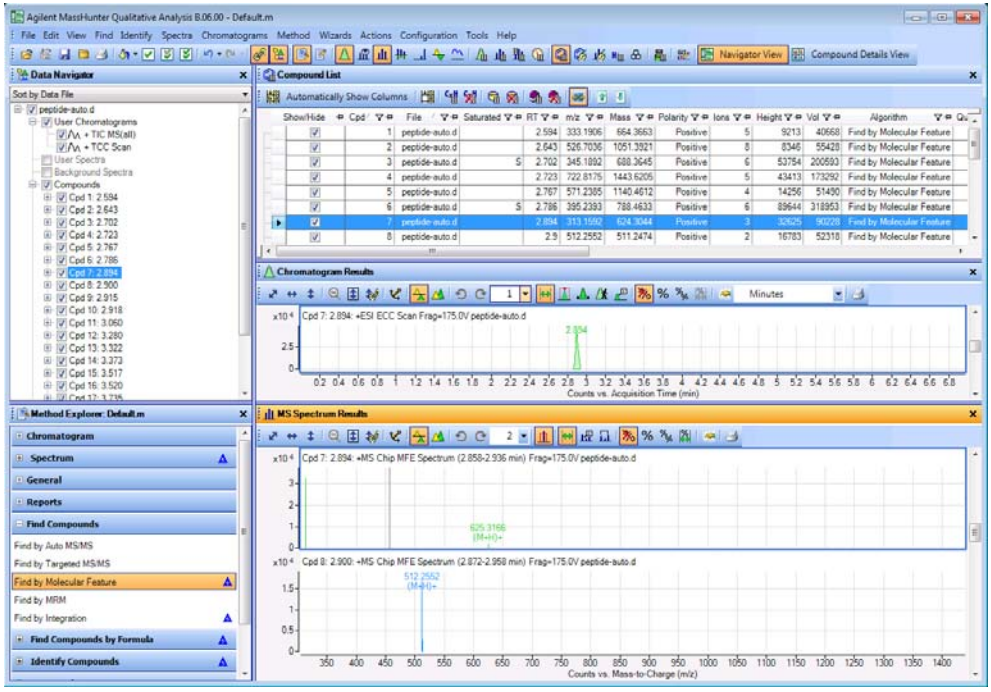


Figure 66 Find Compounds by Molecular Feature for a protein digest with auto MS/MS data

3	Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save the results.

Exercise 3
Set up and run qualitative analysis methods using different workflows

In these tasks, you learn to set up and run any qualitative analysis method. You also learn to edit a method to automate the analysis and/or compound identification. Then you run the actions within the automated method when you open a data file. You also learn to create a method to perform automated actions with a worklist.

You learn to create the worklist method with qualitative analysis parameters only or with both acquisition and qualitative analysis parameters.

An MS-only data file (Q-TOF) is used for illustration, although all of these tasks apply to MS/MS data from either a Q-TOF or Triple Quad as well.

Different workflows are used for these examples. You can explore these different workflows before deciding which one best matches your tasks. See “Workflows” on page 166 for more information.

The General workflow supports both GC/MS and LC/MS data. The GC/Q-TOF Compound Screening workflow supports GC/Q-TOF data. The other workflows only support LC/MS data.

The BioConfirm workflows are described in the online Help and in the BioConfirm Quick Start Guide and BioConfirm Familiarization Guide.

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. 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 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments

1 Open the **sulfas_PosMS.d** data file.
• Make sure that the program will not run any file actions when the data file is open.
• Make sure the method is Default.m.
• Make sure the window layout is the default layout.
	a Double-click the **Qualitative Analysis** icon on your desktop.
	b In the Open Data File dialog box, select **sulfas_PosMS.d**,
	c If necessary, clear the **Run 'File Open' actions from selected method** check box.
	d If necessary, clear the **Load result data** check box.
	e Click **Open**.
	f Click the **Configuration > Configure for Workflow > General** command.
	g Click the **Load workflow's default method** button and the **Load workflow's default layout** button.
	h Click **OK**.
	i Click **Configuration > User Interface Configuration**.
	j Mark all of the check boxes so all options are available.
	k Click the **OK** button.
		• The default layout for the General workflow is automatically loaded. If you want to return to this default layout, click **View > Window Layouts > Restore Default Layout**. This command always restores the layout that is used with the General workflow.
		• To load a method, do this:
		  • Click **Method > Open**.
		  • Select the method
		  • Click **Open**.
		• As you noticed in the last exercise, every time a change is made to a method, a blue triangle appears next to the change and in the Method Explorer next to the section which has changed.

2 Set up the method to extract a TIC chromatogram. • Define a TIC chromatogram for MS data. • Turn off cycle sum since this is an MS-only data file.	**a** In the Method Explorer window, select **Chromatogram > Define Chromatograms**. **b** Delete the **BPC** chromatogram. **c** Select **TIC** as the **Type**. **d** Make sure the MS Level is **MS**. **e** Clear the **Do cycle sum** check box. **f** Click **Add**.	
3 Edit the method to integrate the data. • Limit the integration to the four highest peaks.	**a** In the Method Explorer window, click **Chromatogram > Integrate (MS)**. **b** Click the **Peak Filters** tab. **c** In the Maximum number of peaks section, mark the **Limit (by height) to the largest** check box. **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.

Task 1. Set up and run a qualitative analysis method

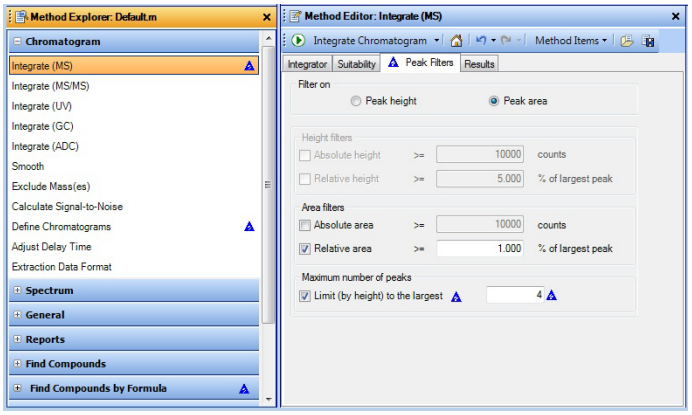
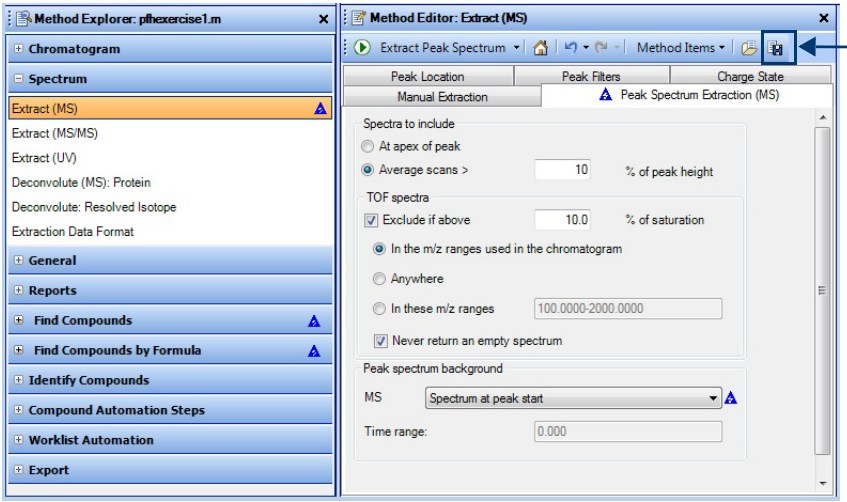
Steps	Detailed Instructions	Comments
		**You can click the Save Method icon to save the current method.**

Figure 67 The Chromatogram > Integrate (MS) > Peak Filters tab

4 Test the integration to make sure that only 4 integrated peaks appear.	• Click the **Integrate Chromatogram** icon to integrate the data file.	
5 Save the method to *iii*exercise1, where "*iii*" are your initials.	**a** From the top menu, click **Method > Save As**. **b** Type **iiiexercise1**. **c** Click the **Save** button.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.
6 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)**. **b** Click the **Peak Spectrum Extraction (MS)** tab. **c** For the Peak spectrum background, select **Spectrum at peak start**.	• If you make any changes after saving the method, then the blue triangles are added to show that the current method is different than the method saved on the computer.

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments



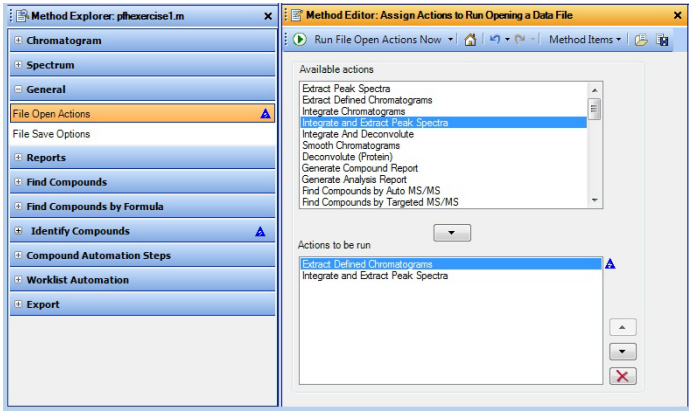
You can click the Save Method icon to save the current method.

Figure 68 The Spectrum > Extract (MS) > Peak Spectrum Extraction (MS) tab

7 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.	• The label for the spectrum contains "Sub" to indicate that it is a spectrum with something subtracted from it.
8 Save the method.	• Save the method in one of three ways: ◦Click the **Save Method** icon in the Method Editor. ◦Right-click the Method Editor, and click **Save Method**. • From the top menu click **Method > Save**.	• The Save Method icon is shown in Figure 68 on page 107

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
9 Set up the method to automate the actions whose parameters you just changed when you open a data file. • List the actions to be performed when this or another data file is opened. Hint: Look under General in Method Explorer.	**a** In the Method Explorer window, select **General > File Open Actions**. **b** Select **Integrate and Extract Peak Spectra** from the **Available actions** list. **c** Click the **Add** button, , to move the selected action to the **Actions to be run** list. You can also double-click on the selected action to move it to the other list.	• The actions in the Actions to be run list are executed in the order that they are listed. First, you need to extract the defined chromatograms, so **Extract Defined Chromatograms** is the first action in the list. Then, you integrate and extract peak spectra, so **Integrate and Extract Peak Spectra** is second in the list..
10 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.



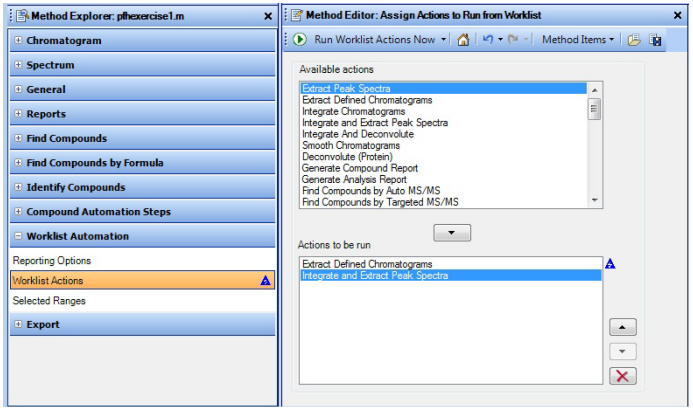
Two different actions are part of the Actions to be run list. The first action is to extract the defined chromatograms. Then, that chromatogram is integrated and peaks are extracted.

Figure 69 The General > File Open Actions section in the Method Editor

11 Save the method.	• Click the **Save Method** icon in Method Editor,	

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
12 Set up the method to automate the actions when the method is run during a worklist. • List the actions to be performed when this or another data file is opened. Hint: Look under Worklist Automation in the Method Explorer window	**a** In the Method Explorer window, select **Worklist Automation > Worklist Actions**. **b** Remove **Generate Analysis Report** from the **Actions to be run** list.	• When you are running a method during a worklist, you often generate a report or export the results. You are removing this option just for this example.
13 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.



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 as part of a worklist.

Figure 70 The Worklist Automation > Worklist Actions section in the Method Editor

14 Undo the method change in the Worklist Actions section.	**a** Click the icon in Method Editor,	• The blue triangles are removed when you undo the changes on this tab.
15 Save the method and close the data file without saving results.	**a** Click the **Save Method** icon in Method Editor, **b** Click **File > Close Data File,** and click **No** when asked to save results.	

Task 2. Set up and run a method to automate an analysis using the Chromatogram Peak Survey 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 database for peak spectra, generating formulas for spectra and printing an analysis report.

You switch to the Chromatogram Peak Survey workflow to set up this method. You will also set up to run this automated analysis in the method when you open a data file.

The Chromatogram Peak Survey workflow can only be used with LC/MS data files.

Task 2. Set up and run a method to automate an analysis

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS**.d again. • Make sure that the method will not perform any actions on the data file when opening the file. • Make sure the method is *iii*exercise1.m.	**a** Click the **Configuration > Configure for Workflow > Chromatogram Peak Survey** command. **b** Click the **Reload current method** button and the **Load workflow's default layout** button. **c** Click **OK**. **d** Click **Configuration > User Interface Configuration**. **e** Mark all of the check boxes so all options are available. **f** Click the **OK** button. **g** Click **File > Open Data File**. **h** In the Open Data File dialog box, select **sulfas_PosMS.d**. **i** Clear the **Run 'File Open' actions from selected method** check box. **j** Click **Open**. **k** Click **Method > Open**, select the iiiexercise1.m method, then click **Open**.	• Make sure the **Load result data** check box is either clear or grayed out. • When you switch to a different workflow, a new method can be loaded, a new window layout can be loaded, and a new section is added to the Method Explorer. • If you are prompted to save changes to the method, you decide whether or not you want to save the changes that you had made. • The method may load with red exclamation marks. These errors may be caused if the MassHunter folder is not on the D: drive. You can fix these errors by changing the folder that is specified for the database and library.

Task 2. Set up and run a method to automate an analysis

Steps	Detailed Instructions	Comments
2 Look at the sections for the Chromatogram Peak Survey algorithm.	• In the Method Explorer window, click **Chromatogram Peak Survey Workflow**.	• Note the eleven sections in this workflow. Most of these sections are duplicates of sections in the General workflow. • A workflow is designed for you to review each section.
3 Make sure that new results will overwrite previous results.	**a** In the Method Explorer window, select **Previous Results**. **b** Mark the **Delete all previous results** check box.	• Note that blue triangles may appear in other sections of Method Explorer window when you make changes. These indicate that the same parameter values have been changed elsewhere as well.
4 Make sure that a TIC will be extracted, and the four largest peaks integrated.	**a** Select **Chromatogram Extraction**. **b** Click the **Chromatograms** tab. **c** Make sure that **TIC** has been selected as the Chromatogram used to find mass spectra. **d** Mark **Signal A** under Additional chromatograms to extract. **e** Select **DAD** from the **Get Signal A from** list. **f** Select the **Chromatogram Integration** section in the Method Explorer. **g** Click the **Peaks (MS)** tab, and mark **Limit (by height) to the largest** and type `4`.	• Note that the **Chromatogram Extraction** section is unique. You cannot enter this information anywhere else in the Method Editor. It is only available when this workflow is selected.
5 Set up to extract MS spectra and subtract a peak spectrum background of the average of spectra before and after the peak.	**a** Select **Mass Spectrum Extraction**. **b** Click the **Peak Spectrum** tab. **c** For **Peak spectrum background** select **Average of spectra at peak start and end**.	
6 Choose to search a database and generate formulas for all spectrum peaks. • Don't change the Molecular Formula Generation nor the Database Search parameter values.	**a** Select **Spectrum Peak Identification** in the Method Explorer. **b** Mark the **Search a database for each peak** check box. **c** Mark the **Generate formula for each peak** check box. **d** Click the **All peaks** button.	• Note that the **Spectrum Peak Identification** section is unique. You cannot enter this information anywhere else in the Method Editor.

Task 2. Set up and run a method to automate an analysis

Steps	Detailed Instructions	Comments
7 Test the automated analysis process up to this point.	• Click the **Run Chromatogram Peak Survey** icon from the Spectrum Peak Identification section.	• If you click the icon from the Molecular Formula Generation section, you click the arrow first and select **Run Chromatogram Peak Survey** from the list of possible action. By default, the action that is run in this section is **Generate Formulas from Spectrum Peaks**. Several other sections also have different default actions.
8 Open the Spectrum Identification Results window for viewing: • This window is tabbed with the Chromatogram Results window as in Figure 71.	**a** If necessary, click **View > Spectrum Identification Results**. **b** Review the results for each MS scan to make sure that all actions in the Chromatogram Peak Survey algorithm were performed.	• See"Task 4. Change window layouts" on page 19 to learn how to move windows on the main screen. • The Spectrum Identification Results window is tabbed with the Chromatogram Results window. You can click on the tab if the Spectrum Identification Results window is not visible. • You can also use the icons in the main toolbar to display these windows.

Task 2. Set up and run a method to automate an analysis

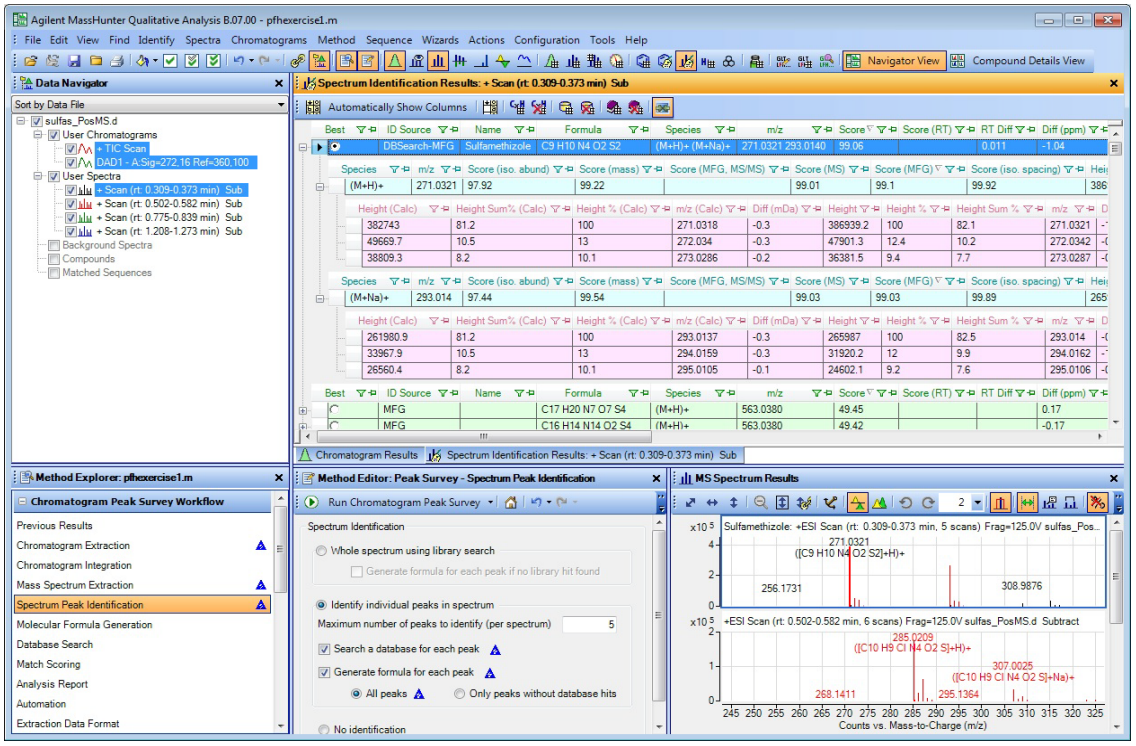
Steps	Detailed Instructions	Comments
		

Figure 71 Tabbed results from running automated analysis steps

9 Save the method to *iii*exercise2, where "*iii*" are your initials.	**a** From the menu, click **Method > Save As**. **b** Type **iiiexercise2**. **c** Click **Save**.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.
10 Set up the Analysis Report and indicate what sections to print for this exercise. • Save the method.	**a** Select **Analysis Report** in the Method Explorer. **b** Click the **Contents** tab. **c** Make any changes you want. **d** Click the **Print Analysis Report** icon. **e** If necessary, click the **Save Method** icon in Method Editor.	• You select whether or not to print the report when you select the action that you want to run. • When you click the Print Analysis Report icon, the **Print Analysis Report** dialog box is opened.

Task 2. Set up and run a method to automate an analysis

Steps	Detailed Instructions	Comments
11 Set up the method to run the automated analysis when the data file is opened • Save the method.	**a** Select **Automation** in the Method Explorer. **b** Click the **File Open Actions** tab. **c** Select each item in the **Actions to run** list, and click the **Remove** icon,  . **d** Select **Chromatogram Peak Survey without Analysis Report** in the **Available Actions** list, and click the **Add** button,  . **e** Click the **Save Method** icon in Method Editor.	• You can also test these actions if you want. • The **Chromatogram Peak Survey without Analysis Report** and **Chromatogram Peak Survey with Analysis Report** both work without you needing to separately extract chromatograms. Most other actions require you to extract chromatograms first.
12 Close the Method Editor, Method Explorer and Data Navigator windows. • Move the windows so they look like the layout in Figure 72. • Close the data file, and do not save results.	**a** Click the **Close** button for the Method Editor, Method Explorer and Data Navigator window. **b** Move the windows so they look like Figure 72. **c** Click **File > Close Data File**. **d** Click **No** when asked to save results.	• Note that the window layout that appears when you open a new data file is the same as the last window layout used.
13 Open the **sulfas_PosMS.d** data file again to run the automated analysis. • The results should look like the results in Figure 72.	**a** Click **File > Open Data File.** **b** Select sulfas_PosMS.d **c** Mark the **Run 'File Open' actions from selected method** check box. **d** Click **Open**. **e** Mark the **Best** result by clicking the radio button in the line in the Spectrum Identification Results window.	

Task 2. Set up and run a method to automate an analysis

Steps	Detailed Instructions	Comments

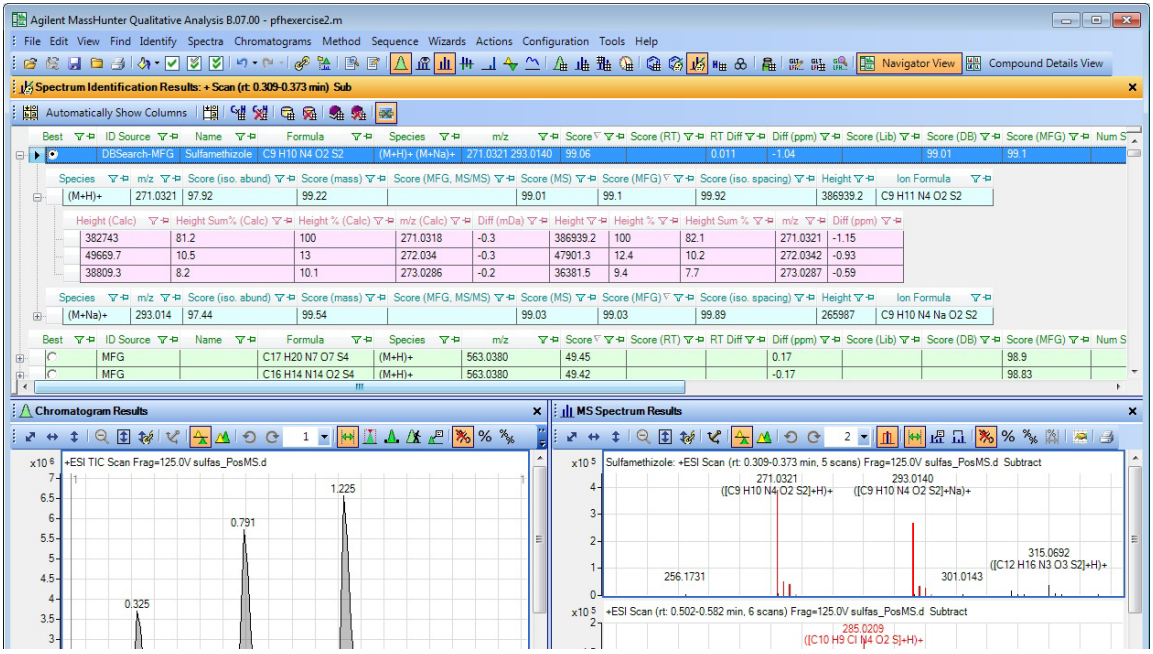


Figure 72 Results of Chromatogram Peak Survey action when opening the sulfas_PosMS.d data file

14 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save results.	

Task 3. Set up and run a method to automate compound identification using the MS Target Compound Screening workflow

In this task you set up a qualitative analysis method that contains a list of actions to find and identify compounds. These include finding compounds based on a selected algorithm, searching the database for compounds, generating formulas for specific compounds and printing the compound report.

You switch to the MS Target Compound Screening workflow to set up this method. You can also set up this method using the Compound Automation Steps section. You will also set up to run the compound automation in the method when you open a data file.

The MS Target Compounds Screening workflow can only be used with LC/MS data files.

Task 3. Set up and run a method to automate compound identification

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS**.d again. • Make sure that the method will not perform any actions on the data file when opening the file. • Make sure the method is *iii*exercise2.m. • Start with the MS Target Compound Screening workflow.	**a** Click **Configuration > Configure for Workflow > MS Target Compound Screening**. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click the **OK** button. **d** Click **Configuration > User Interface Configuration**. **e** Mark all of the check boxes, so all options are available. **f** Click the **OK** button. **g** Click **File > Open Data File**. **h** In the Open Data File dialog box, select **sulfas_PosMS.d**. **i** Clear the **Run 'File Open' actions from selected method** and the **Load Result Data** check boxes and click **Open**. **j** Click **Method > Open.** Select the *iii*exercise2.m method. **k** Click **Open.** **l** Click **No** to save method changes.	• Make sure the **Load result data** check box is either clear or grayed out. • The method Screening-Default.m is loaded when you switch to the MS Target Compound Screening workflow. • If the MassHunter folder is not in the default location in the D: drive, you will have errors in the method when you switch to this workflow. You can change the folder for the database to the appropriate location.

Task 3. Set up and run a method to automate compound identification

Steps	Detailed Instructions	Comments
2 Look at the automation steps for finding and identifying compounds. • Tab the Method Editor window in a convenient location.	**a** In the Method Explorer window, click **MS Target Compound Screening Workflow > Automation**. **b** (optional) Tab the Method Editor window with the Data Navigator window. **c** Close the **Compound List** window.	• In this workflow, the Method Editor is a floating window. You can either leave it as a floating window or tab it with another window, such as the Data Navigator window.
3 Choose to search a database and generate formulas for all compounds. • Make sure you are finding compounds by molecular feature.	**a** Click the **Analysis Options** tab. **b** Select **Find by Molecular Feature**. **c** Mark the **Search a database for each compound** check box. **d** Mark the **Generate formulas for each compound** check box. **e** Click **All compounds**. **f** Mark the **Show only identified compounds** check box.	• A compound can be identified by the Search Database algorithm, the Generate Formulas algorithm, the Search library algorithm or if the compound was found using the Find by Formula algorithm. If MassHunter BioConfirm software is installed, then a compound can also be identified by the Match Sequences algorithm.
4 Make sure that new results will overwrite previous results.	**a** Click the **Results** tab. **b** Mark the **Delete all previous results** check box.	
5 Test the automation process up to this point.	• Click the **Run Compound Automation Steps** icon  from any of the MS Target Compound Screening Workflow > Automation sections.	
6 Open these windows for viewing: • Compound List • Compound Identification Results • Make sure the windows are displayed as in Figure 73 • Review each list for each compound (except for Compounds 1 and 2).	**a** (if necessary) Click **View > Compound List**. **b** (if necessary) Click **View > Compound Identification Results**. **c** Clear the Compound 1 and Compound 2 check boxes in the Data Navigator. Or, you can clear the check boxes for Compound 1 and Compound 2 in the **Show/Hide** column in the Compound List window **d** Review each table for each identified compound to make sure that all actions in the Compound Automation Steps were performed.	• See "Task 4. Change window layouts" on page 19 to learn how to move windows on the main screen. • The Compound Identification Results window is tabbed with the Chromatogram Results window in Figure 73.

Task 3. Set up and run a method to automate compound identification

Steps	Detailed Instructions	Comments
7 Save the method to *iii*exercise3, where "*iii*" are your initials.	**a** From the top menu, click **Method > Save As**. **b** Type **iiiexercise3**. **c** Click **Save**.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.

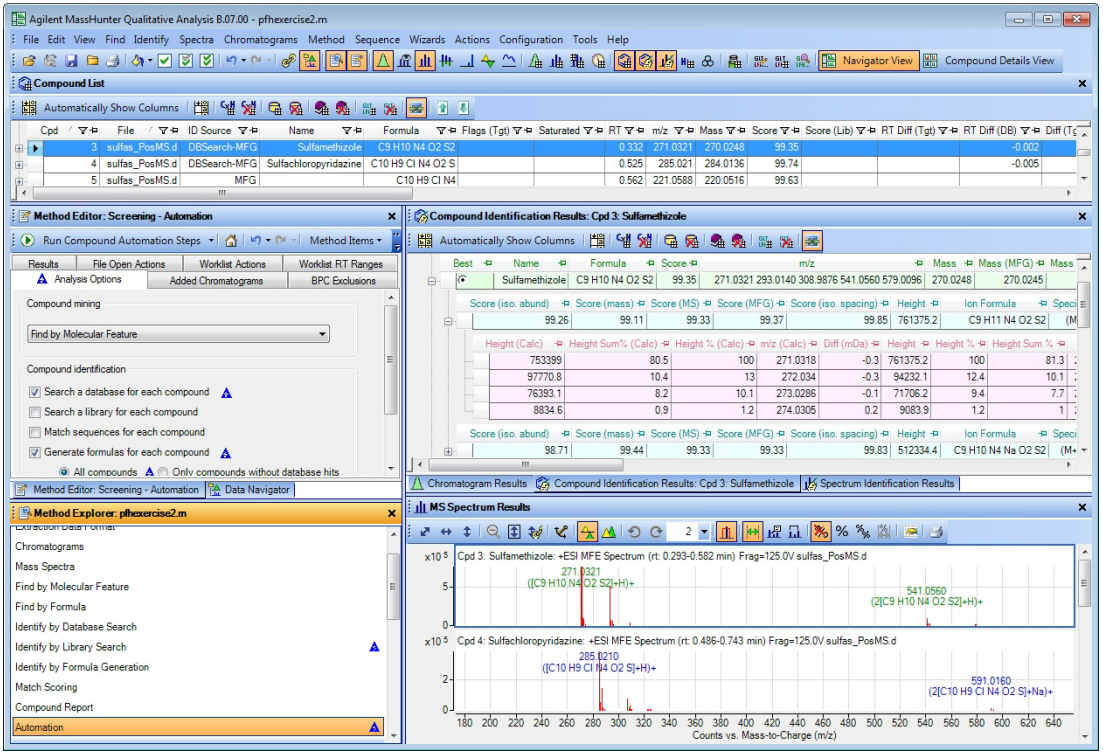


Figure 73 Tabbed results from running compound automation identification steps

8 Set up the Compound Report for this exercise. • If necessary, save the method.	**a** Select **Compound Report**. **b** Make any changes you want. **c** Click the **Templates** tab. **d** (optional) Select *TargetCompoundScreeningReport.xltx* for the **Compound report template**. **e** If necessary, click the **Save Method** icon in Method Editor.	• The default compound report template for this workflow is the "TargetCompoundScreeningReport.xltx". The *iiiExercise2.m* method that you loaded was started from the default method for the General workflow. You can select either report template.

Task 3. Set up and run a method to automate compound identification

Steps	Detailed Instructions	Comments
9 Set up the method to run the automated compound identification when the data file is opened • Save the method.	**a** Select **MS Target Compound Screening Workflow > Automation > File Open Actions**. **b** Select all actions in the **Actions to run** list, and click the **Remove** icon,  . **c** Select **Compound Automation without Report** in the **Available Actions** list, and click the **Add** button,  . **d** Click the **Save Method** icon in Method Editor.	• You can also test these actions if you want.
10 Close Method Editor, Method Explorer and Data Navigator. • Move the windows so they look like the layout in Figure 74. • Close the data file, and do not save results.	**a** Click the **Close** button for Method Editor, Method Explorer and Data Navigator. **b** Move the windows so they look like Figure 74. **c** Click **File > Close Data File**. **d** Click **No** when asked to save results.	• See Exercise 1 Task 4 to learn how to move windows.
11 Open the **sulfas_PosMS.d** data file again to run the automated compound identification. • The results should look like the results in Figure 74. • Hide Compounds 1 and 2 in the Compound List.	**a** Click **File > Open Data File**. **b** Select the sulfas_PosMS.d data file. **c** Mark the **Run 'File Open' actions from selected method** check box. **d** Click **Open**. **e** Clear the **Show/Hide** check boxes for Compounds 1 and 2 in the Compound List.	• Compounds 1, 2, 5, 6, and 8 are not found by the database search algorithm, but they do have formulas generated by the formula generation algorithm.

Task 3. Set up and run a method to automate compound identification

Steps	Detailed Instructions	Comments

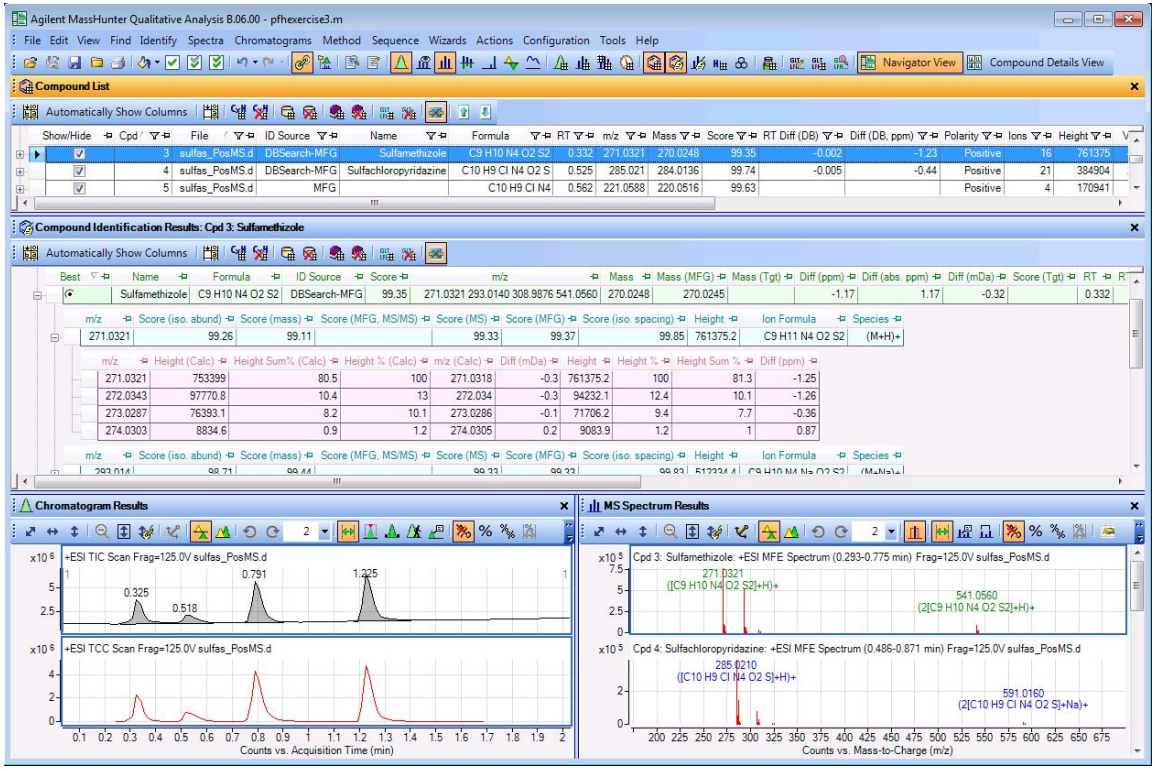


Figure 74 Results of automated compound identification when opening the sulfas_PosMS.d data file

12 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save results.	

Task 4. Set up a qualitative method to run with a worklist

In this task you set up a qualitative analysis method that contains a list of actions to execute when you run the worklist. You learn to save the method with both acquisition and qualitative analysis parameters, although you will not actually do this in this task.

Starting in revision B.05.00 of the Data Acquisition software, you can use the Data Acquisition software to automatically run a qualitative method from an existing data acquisition method when you are running the data acquisition method. See the online Help for Data Acquisition for more information.

Task 4. Set up a qualitative method to run with worklist

Steps	Detailed Instructions	Comments
1 Load the sulfas_PosMS.d data file. • Open the method you saved in Task 2. • Make sure actions are not run when you open the file. • Restore the default window layout.	**a** To restore the default workflow, click **Configuration > Configure for Workflow > General**. **b** Click **OK** to continue. **c** Click **File > Open Data File**. **d** In the Open Data File dialog box, select **sulfas_PosMS.d**. **e** Clear the **Run 'File Open' actions from selected method** check box. **f** Clear the **Load result data** check box. **g** Click **Open**. **h** Load the method ***iii*Exercise2.m**.	• In this task you are creating a method that contains only qualitative analysis parameters. • To create a worklist method from this method, you must add acquisition parameters to this method in the acquisition program. • If you select **Load worklist method** (assuming it's available) in the Open Data File dialog box, the program opens the data file using the qualitative analysis part of the acquisition method in the worklist that produced the data file. • You can create a worklist method with both acquisition and qualitative analysis parameters by saving the qualitative analysis parameters to an existing acquisition method. • You can also set up the method for a complete analysis with the Analysis Automation Steps. Then you would remove these actions and add on the Analysis Automation action. • You can do the same with Compound Automation.

Task 4. Set up a qualitative method to run with worklist

Steps	Detailed Instructions	Comments
2 Set up a method to automatically execute upon completion of every run in the worklist. Set up the method to perform the following tasks: • Extract the defined chromatogram • Integrate and extract peak spectra • Generate Analysis Report Hint: Look under Worklist Automation in Method Explorer.	**a** In the Method Explorer, select **Worklist Automation > Worklist Actions** to display the **Assign Actions to Run from Worklist** section. **b** Make sure that the following actions are in the **Actions to be run** list in this order: • Extract Defined Chromatograms • Integrate and Extract Peak Spectra • Generate Analysis Report **c** If necessary, select each of these actions from the **Available actions** list, and click the **Add** button, , to move the selected action to the **Actions to be run** list. You can also double-click on the selected action to copy it to the other list. **d** If necessary, select any actions in the **Actions to be run** list that are not in the list of actions mentioned, and click the **Remove** icon .	

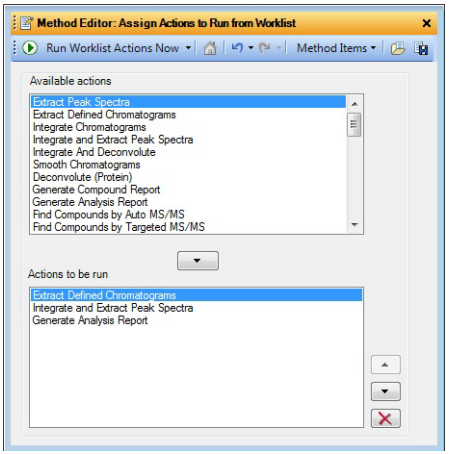


Figure 75 Method Editor with Worklist Actions section displayed

Task 4. Set up a qualitative method to run with worklist

Steps	Detailed Instructions	Comments
3 Save the method to ***iii*exercise 2worklist.m**, where "*iii*" is your initials. • Close the program and do not save results.	**a** To save the method, click **Method > Save As**. **b** Type ***iii*exercise2worklist.m**. **c** Click **Save**. **d** Click **File > Exit**. **e** Click **No** when asked if you want to save the results.	• After the acquisition parameters have been added to this method in the acquisition program, you can save it to the same name or a different one. • When run from the worklist, this method (with acquisition parameters added) will acquire and analyze data sequentially and automatically. The actions in the **Actions to be run** list in the Worklist Actions section are run automatically.

Exercise 4
Qualitative Analysis Wizards

Several wizards are included in the Qualitative Analysis program. These wizards lead you through the steps necessary to do certain tasks.

- **Identify Chromatogram Peaks wizard** - This wizard shows you the different method editor sections and tabs that you modify before running the **Chromatogram Peak Survey without Analysis Report** action.
- **Find Targets by: MFE + Database Search wizard** - This wizard shows you the different method editor sections and tabs that you modify before running the **Find by Molecular Feature algorithm** and the **Database Search algorithm**.

You can also update these method editor sections in the Method Editor window.

If you install BioConfirm, several other wizards are available. These other wizards are discussed in the **BioConfirm Familiarization Guide**.

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. Run the Identify Chromatogram Peaks Wizard

When you run this wizard, the program shows you all of the method editor sections and other pages which affect the **Chromatogram Peak Survey without Analysis Report** action. Then, when you click the **Finish** button, the changes to the method are saved, and the **Chromatogram Peak Survey without analysis report** action is performed.

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS.d** data file. • Make sure that the program will not run any file actions when the data file is open. • Make sure the method is Default.m. • Make sure the window layout is the default layout.	**a** Double-click the **Qualitative Analysis** icon on your desktop. **b** In the Open Data File dialog box, select **sulfas_PosMS.d**, **c** If necessary, clear the **Run 'File Open' actions from selected method** check box. **d** If necessary, clear the **Load result data** check box. **e** Click **Open**. **f** Click the **Configuration > Configure for Workflow > General** command. **g** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **h** Click the **OK** button. **i** Click **Configuration > User Interface Configuration**. **j** Mark all of the check boxes so all options are available. **k** Click the **OK** button.	• The default layout for the General workflow is automatically loaded. If you want to return to this default layout, click **Configuration > Window Layouts > Restore Default Layout**. This command always restores the layout that is used with the General workflow. • As you noticed in the previous tasks, every time a change is made to a method, a blue triangle appears next to the change and in the Method Explorer next to the section which has changed.
2 Start the Identify Chromatogram Peaks wizard. Change the parameters to delete previous results.	**a** Click the **Wizards > Identify Chromatogram Peaks** command. **b** In the Previous Results page, mark the **Delete all previous results** check box. **c** Click **Next**.	• The wizard leads you through a series of pages. You set the parameters for the task on these pages. Many of these pages are duplicates of the sections and tabs in the Method Editor window. • Chromatograms, spectra, and compounds are deleted.

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments
3 Edit the Chromatogram Extraction page. Change the parameters to extract the BPC and the Signal A chromatogram.	**a** In the Chromatogram Extraction page under Additional chromatograms to extract, mark the **BPC** check box and the **Signal A** check box. **b** Select DAD1 from the **Get Signal A from** list. **c** Click **Next**.	• The current method is changed when you click **Finish**. In the Method Editor, a blue triangle is displayed when you make a change from the values that are saved with the method. However, a blue triangle in the wizard means that you changed the value in the wizard from the current values in the method.

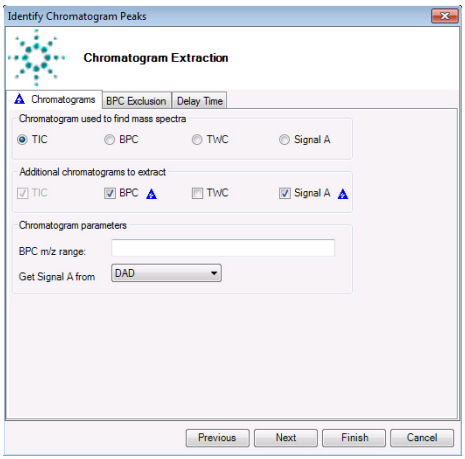


Figure 76 The Chromatogram Extraction page in the Identify Chromatogram Peaks wizard

4 Edit the Chromatogram Integration page. Change the parameters to only integrate the four largest MS peaks.	**a** In the Chromatogram Integration page, click the **Peaks (MS)** tab. **b** Mark the **Limit (by height) to the largest** check box and enter 4. **c** Click **Next**.	• You can click the **Finish** button on any of the pages in the wizard. The current values in the method are used when the wizard is run.

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

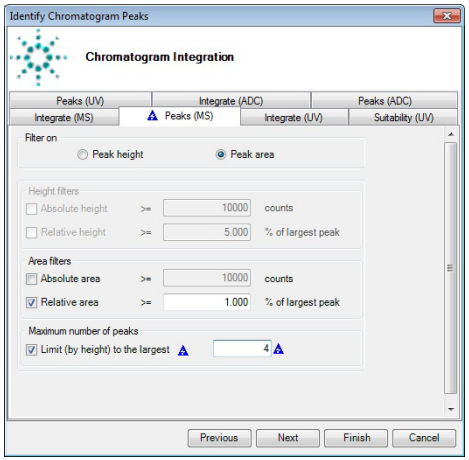


Figure 77 The Chromatogram Integration page in the Identify Chromatogram Peaks wizard

5 Review the parameters on the Extraction Data Format page.

a In the Extraction Data Format page, review the parameters.

b Click **Next**.

- On the last page of any wizard, the **Next** button is grayed out. You can either finish the wizard or return to a previous page.

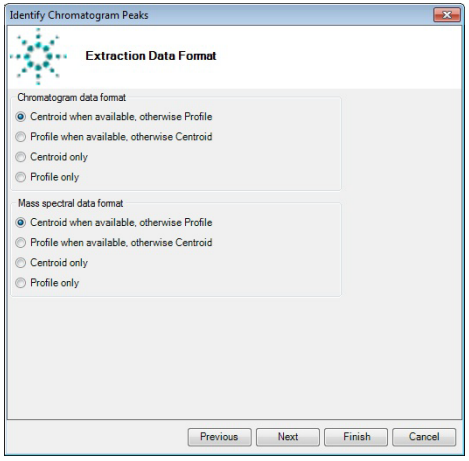


Figure 78 The Extraction Data Format page in the Identify Chromatogram Peaks wizard

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments
6 Edit the Mass Spectrum Extraction page to change the spectrum to subtract from each peak to the spectrum at the peak start.	**a** In the Mass Spectrum Extraction page, select **Spectrum at peak start** for the MS Peak spectrum background. **b** Click **Next**.	

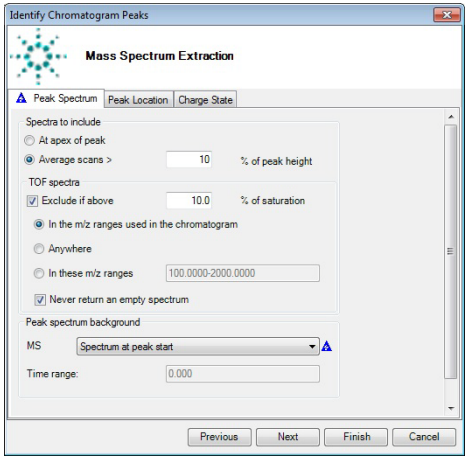


Figure 79 The Mass Spectrum Extraction page in the Identify Chromatogram Peaks wizard

7 Edit the Spectrum Peak Identification page. Change the parameters to search the database and generate formulas for all peaks.	**a** In the Spectrum Peak Identification page, click **Identify individual peaks in spectrum**. **b** Mark the **Search a database for each peak** check box. **c** Mark the **Generate formula for each peak** check box. **d** Click the **All peaks** button. **e** Click **Next**.	

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

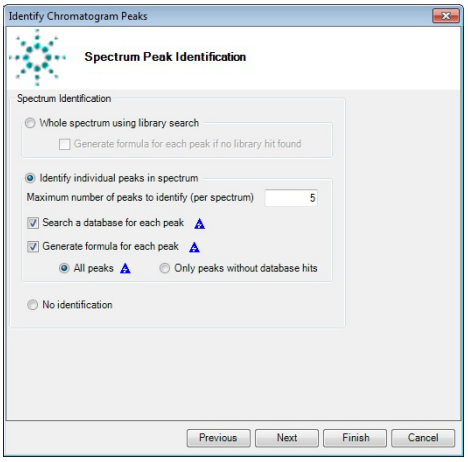


Figure 80 The Spectrum Peak Identification page in the Identify Chromatogram Peaks wizard

8 Review the parameters on the Database Search page.

a In the Database Search page, review the parameters.
b Click the **Next** button.

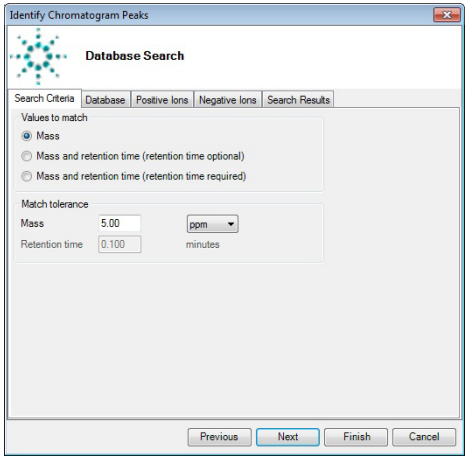


Figure 81 The Database Search page in the Identify Chromatogram Peaks wizard

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments
9 Edit the Molecular Formula Generation page. Change the minimum overall score to 25.	**a** In the Molecular Formula Generation page, click the **Limits** tab. **b** Mark the **Minimum overall score** check box. **c** Enter **25** for the Minimum overall score. **d** Click the **Next** button.	

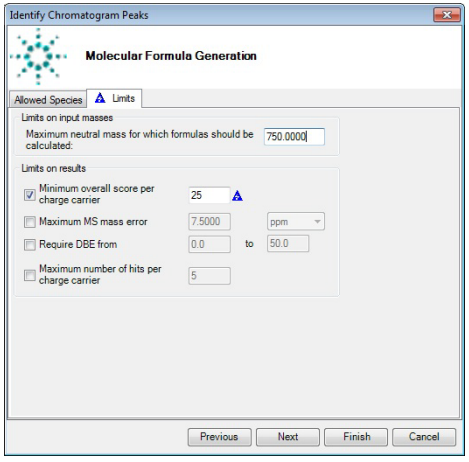


Figure 82 The Molecular Formula Generation page in the Identify Chromatogram Peaks wizard

10 Review the parameters on the Match Scoring page.	**a** In the Match Scoring page, review the parameters. **b** Click the **Finish** button.	

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

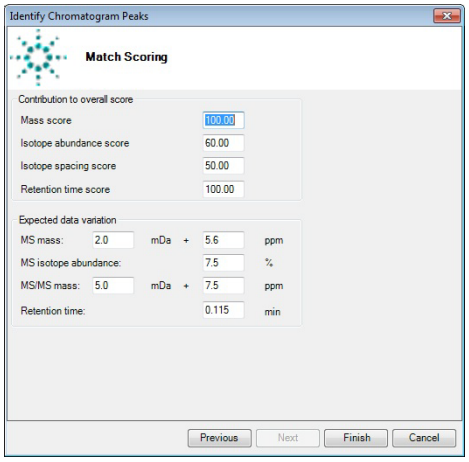


Figure 83 The Match Scoring page in the Identify Chromatogram Peaks wizard

11 Review the results.	• First, the changes to the method are made to the current method. These changes are not automatically saved to the method on the disk. • Then, the chromatogram Peak Survey action is done.	• Note that when you click **Finish**, a blue triangle is added to the Method Explorer window section and to the Method Editor window if the changes from the wizard are different from the changes on the disk.
12 Save the method to *iii*exercise4, where "*iii*" are your initials and close the data file without saving results.	**a** From the top menu, click **Method > Save As**. **b** Type **iiiexercise4.m**. **c** Click the **Save** button. **d** Click **File > Close Data File,** and click **No** when asked to save results.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.

Task 2. Run Find Targets by: MFE + Database Search Wizard

This wizard shows you the different method editor sections and tabs that you modify before running the **Find by Molecular Feature algorithm** and the **Database Search algorithm**.

Task 2. Run Find Targets by: MFE + Database Search

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS**.d again. • Make sure that the method will not perform any actions on the data file when opening the file. • Make sure the method is *iii*exercise1.m.	**a** Click the **Configuration > Configure for Workflow > General** command. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click **OK**. **d** Click **Configuration > User Interface Configuration**. **e** Mark all of the check boxes so all options are available. **f** Click the **OK** button. **g** Click **File > Open Data File**. **h** In the Open Data File dialog box, select **sulfas_PosMS.d**. **i** Clear the **Run 'File Open' actions from selected method** check box. **j** Click **Open**. **k** Click **Method > Open**, select the iiiexercise1.m method, then click **Open**.	• Make sure the **Load result data** check box is either clear or grayed out. • When you switch to a different workflow, a new method is loaded, a new window layout is loaded and a new section is added to the Method Explorer. • If you are prompted to save changes to the method, click **No**. • This wizard can also run with other workflows loaded.
2 Start the **Find Targets by: MFE + Database Search** wizard. Change the parameters to use the small chromatographic molecules algorithm.	**a** Click **Wizards > Find Targets by: MFE + Database Search + MFG**. **b** In the Find by Molecular Feature page, select **Small molecules (chromatographic)** as the **Target data type**. **c** Click the **Next** button.	• The MFE algorithm is modified depending on the **Target data type** that you select.

Task 2. Run Find Targets by: MFE + Database Search

Steps	Detailed Instructions	Comments

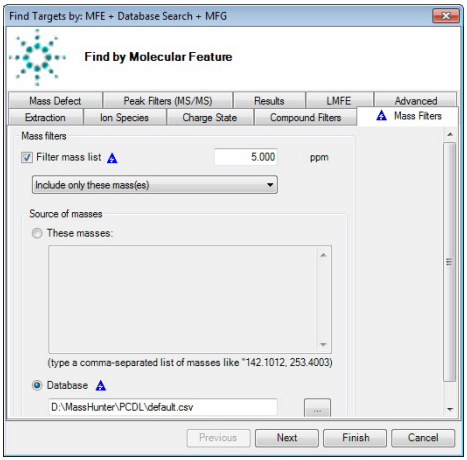


Figure 84 The Find by Molecular Feature page in the Find Targets by: MFE + Database Search + MFG wizard

3 Edit the Filter by Mass List page. Change the minimum overall score to 25.	**a** In the Filter by Mass List page, mark the **Filter mass list** check box. **b** Select **Include only these mass(es)**. **c** Click the **Database** button. **d** Select the *default.csv* file. **e** Click the **Next** button.	• This page of the wizard contains a single tab from the previous page of the wizard. In this task, it is very important to filter the mass list. • You can instead select the example database, *default.csv*.

Task 2. Run Find Targets by: MFE + Database Search

Steps	Detailed Instructions	Comments

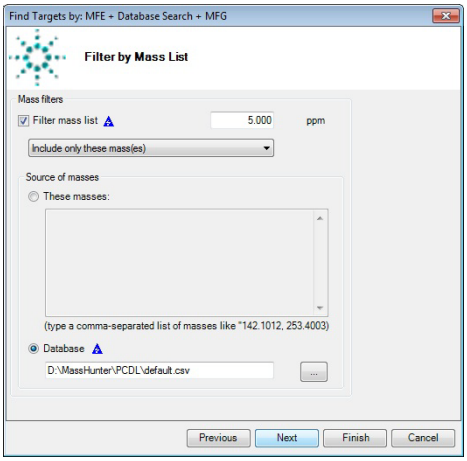


Figure 85 The Filter by Mass List page in the Find Targets by: MFE + Database Search + MFG wizard

4 Review the parameters on the Search Database page.	**a** Review the parameters. **b** Click the **Next** button.	

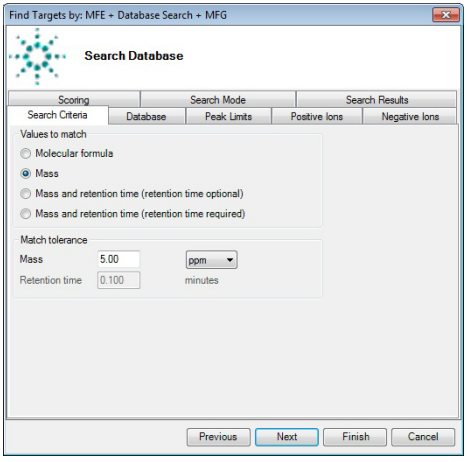


Figure 86 The Search Database page in the Find Targets by: MFE + Database Search + MFG wizard

Task 2. Run Find Targets by: MFE + Database Search

Steps	Detailed Instructions	Comments
5 Edit the Generate Formulas page. Change the minimum overall score to 25.	**a** Click the **Limits** tab. **b** Type 25 as the **Minimum overall score**. **c** Click the **Finish** button.	

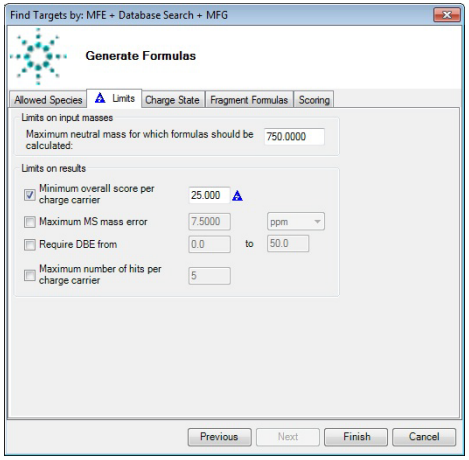


Figure 87 The Generate Formulas page in the Find Targets by: MFE + Database Search + MFG wizard

6 Review the results in the Qualitative Analysis program.	• A report is not generated. • You can review the results in the Compound List window and in the Compound Identification Results window.	
7 Save the method to *iii*exercise5, where "*iii*" are your initials.	**a** From the menu, click **Method > Save As**. **b** Type **iiiexercise5.m**. **c** Click **Save**.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.
8 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save results.	

Exercise 5
Analyzing Data Files acquired in All Ions MS/MS Mode

The program can qualify fragment ions when running the Find Compounds by Formula algorithm if the data file is acquired in All Ions MS/MS mode.

Task 1 shows you how to generate results when you have structural isomers. One way to distinguish structural isomers is using retention times.

Task 2 shows you how to generate results when you have a data file that was acquired in All Ions MS/MS mode. You acquire data with a low Fragmentor or Collision Energy voltage and then one or more higher Fragmentor or Collision Energy voltages. The MS information is used with the Find by Formula algorithm. This MS/MS information is used to qualify fragment ions.

Task 3 shows you how to use the Compound Details View to review the results after running the Find by Formula algorithm with fragment confirmation enabled. One of the new features is the Coelution plot which is part of the Compound Chromatogram Results window.

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. Run Find by Formula on data file with structural isomers

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments
1 Open the **AIM_3CE(0-20-40).d** data file. • Use the General workflow.	**a** Click **File > Open Data File**. **b** Select **AIM_3CE(0-20-40).d** and click **OK**. **c** Click **View > Configure for Workflow > General**. **d** Click the **Autoscale Y-axis during Zoom** icon,  , in the Chromatogram Results toolbar. **e** Click the **Range Select** tool.	• The Find by Formula sections are included in the **Formula Confirmation and Sample Purity Workflow** section. • Fragment Confirmation is only possible on data files that are acquired in All Ions MS/MS mode. • **AIM_3CE(0-20-40).d** is an All Ions MS/MS data file.

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments
2 Review the Find by Formula - Options section. • For this data file, select the PestMix_AIM_PCDL_SP1.cdb library.	**a** In the Method Explorer window, click the **Find Compounds by Formula > Find by Formula - Options** section. **b** Click the **Formula Source** tab. **c** Click **Database** as the **Source of formulas to confirm** and select `PestMix_AIM_PCDL_SP1.cdb`. **d** Mark the **Automatically increase for isomeric compounds** check box. **e** Click the **Positive Ions** tab. **f** Mark the **+H** and **+Na** check boxes. **g** Click the **Results** tab. **h** Mark the **Extract EIC** check box. **i** Mark the **Extract cleaned spectrum** check box. **j** Mark the **Include structure** check box. **k** Click the **Result Filters** tab. **l** Clear the **Only generate compounds for matched formulas** check box. **m** Click the **Fragment Confirmation** tab. **n** Clear the **Confirm with fragment ions** check box.	• As you noticed in the previous tasks, every time a change is made to a method, a blue triangle appears next to the change and in the Method Explorer next to the section which has changed. • If you do not mark the Automatically increase for isomeric compounds check box, then the Compound List table does not contain information on all isomers for each compound in the table. • For this first time that you find compounds by formula algorithm, do not confirm with fragment ions.
3 Run the Find Compounds by Formula algorithm.	**a** Click the **Find > Find Compounds by Formula** command. **b** Close the Method Explorer and Method Editor windows. **c** Close the Chromatogram Results window.	• Two of the formula could not be confirmed. The Name for each of these formula is shown in angle brackets. “Cpd 10: < Chloropropham (Chlorpropham)>” and “Cpd 11:< Monolinuron (phenylurea)>” were not confirmed with the current parameters.

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments
4 Review **Cpd 10: <Chloropropham>**. • This compound was not found. • **Cpd 11: < Monolinuron (phenylurea)>** also was not found.	**a** Click on **Cpd 10: <Chloropropham> (Chlorpropham)**. **b** Expand the Compound List table to show two levels of the table for **Cpd 10**. . **c** Click on **Cpd 11: <Monolinuron (phenylurea)>**. **d** Expand the Compound List table to show three levels of the table for **Cpd 11**.	• For Cpd 11, the **Score (mass)** is good (over 95%), but the **Score (iso. abund)** and **Score (iso. spacing)** are zero. So, the **Score (MS)** is lower than the limit set on the Result Filters tab. Thus, the compound was not qualified. • This compound does not have any spectra associated with it, so the **MS Spectrum Results** window still shows the results for the previously selected compound.

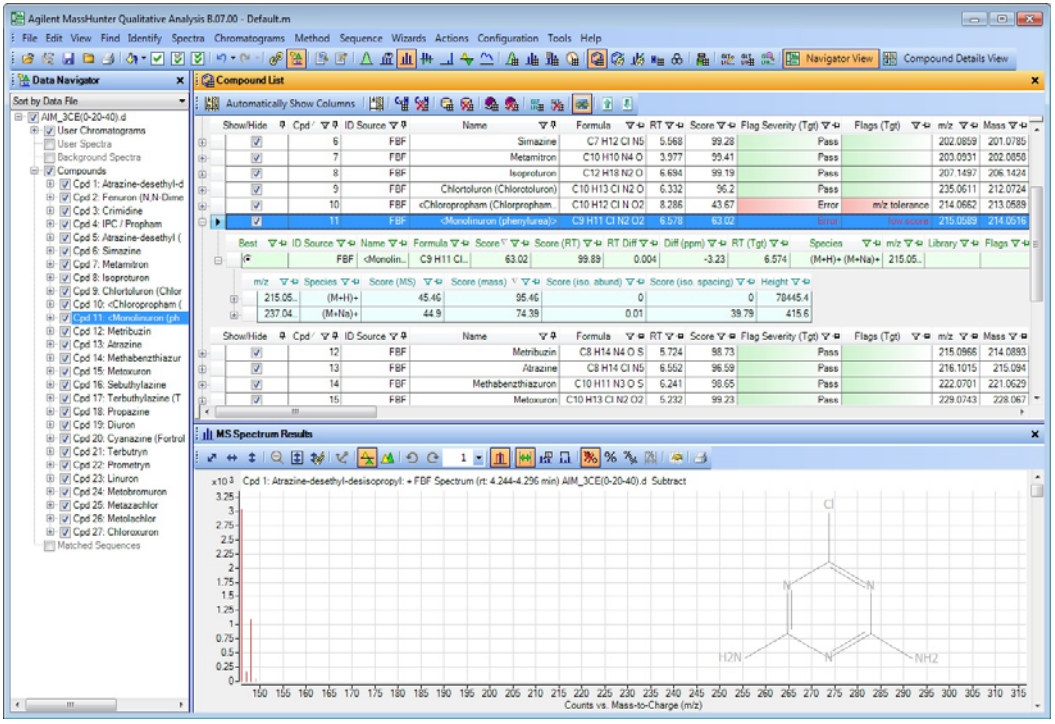


Figure 88 Review results when compound not found

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments
5 Review the results for **Cpd 7: Metamitron**. • This compound was found.	**a** In the Data Navigator window, click **Cpd 7: Metamitron**. **b** Expand the results in the Compound List window to show the first four layers in the Compound List table. **c** Review the **Score (Tgt)** value.	• You can change the columns that are shown by using the shortcut menu. • The overall **Score (Tgt)** value is composed of the **Score (MS)** and the **Score (RT)**. • **Score (MS)** is composed of the **Score (mass)**, **Score (iso. abund)**, and **Score (iso. spacing)** values.

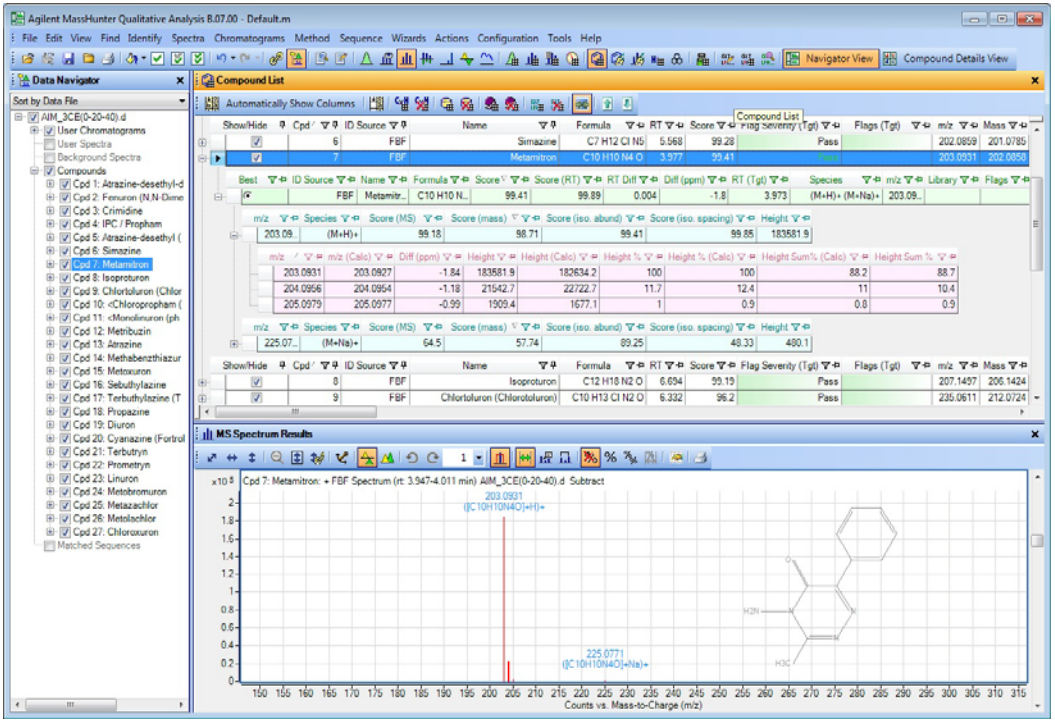


Figure 89 Find by Formula results in the Data Navigator, Compound List, and MS Spectrum Results windows.

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments
6 Review the results for **Cpd 18: Propazine**. • Propazine has the same formulas as **Cpd 16: Sebuthylazine** and **Cpd 19: Diuron**, so they are structural isomers. • You can manually select a different isomer.	**a** In the Data Navigator window, click **Cpd 18: Propazine**. **b** Expand the results in the Compound List window to show the first two levels in the Compound List table. **c** Click **View > Chromatogram Results**. **d** Review the **Score** and the **Score (RT)** values for this compound. **e** Review the **Flags (Tgt)** value. For structural isomers, it is set to "**multiple IDs**". The **Flag Severity (Tgt)** value is set to **Information**.	• The Find by Formula algorithm can distinguish between structural isomers if the mass and retention time are available in the database and used in the algorithm. • MS/MS information can help distinguish between structural isomers, also. • The **Score (RT)** value is clearly higher for the compound Propazine, so that is the reason that Propazine was selected as the **Best** hit.

Task 1. Run Find by Formula on data file with structural isomers

Steps	Detailed Instructions	Comments

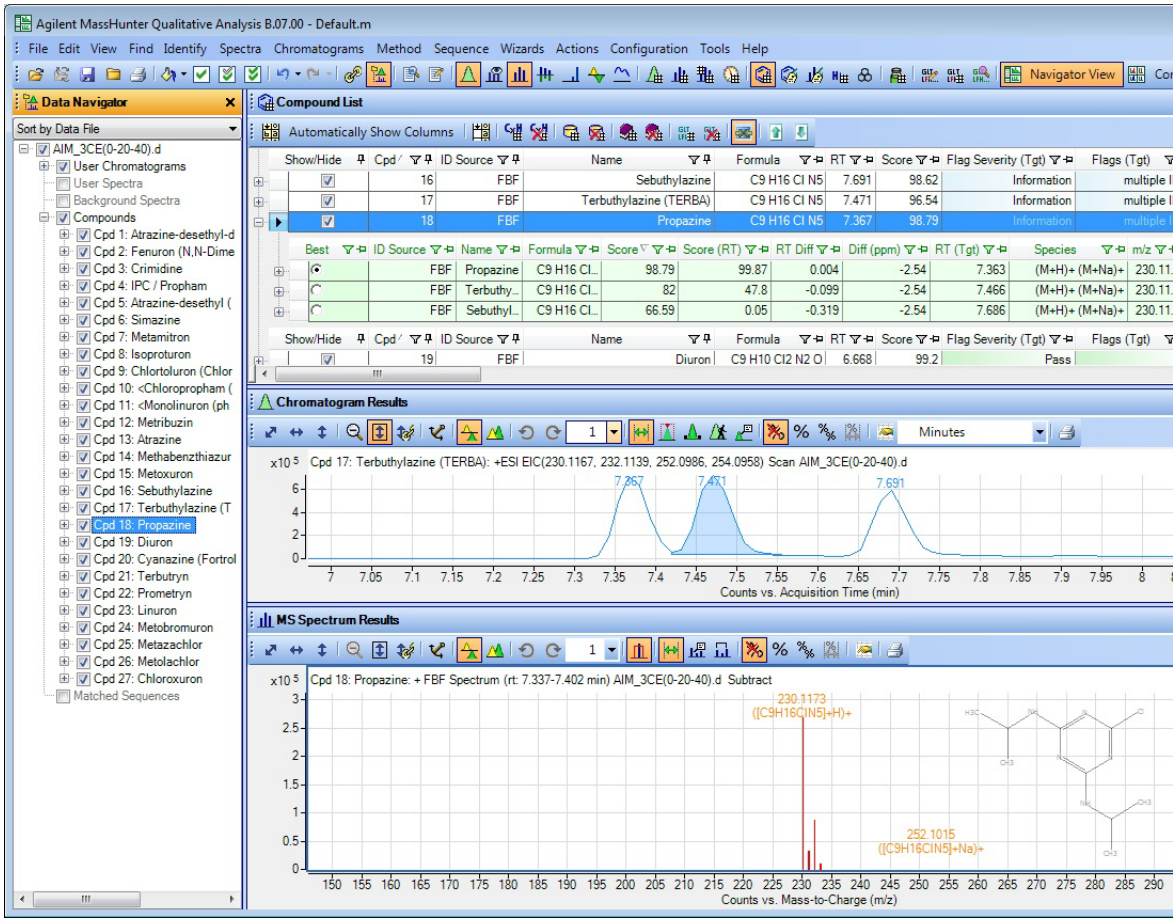


Figure 90 Reviewing results for structural isomers such as Cpd 18: Propazine.

7 Save the method to *iii*All_Ions1, where "*iii*" are your initials and close the data file without saving results.

a From the top menu, click **Method > Save As**.
b Type **iiiAll_Ions1.m**.
c Click the **Save** button.

- Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.

Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode

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 0V, 20V and 40V. The 0V spectra is considered the “low energy channel” which predominantly shows the precursor ions of the eluting compounds, while the 20V and 40V spectra are considered the “high energy channel(s)”, which will 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, 125V, 200V and 275V). 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 will 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 mode needs to be turned on. 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 be annotated with their sub formulas.

Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode

Steps	Detailed Instructions	Comments
1 Run the Find Compounds by Formula algorithm with Fragment Confirmation. • Fragment Confirmation uses information from the high energy (High-E) channels	**a** Complete the steps in "Task 1. Run Find by Formula on data file with structural isomers" on page 138. **b** Click the **Fragment Confirmation** tab in the Find by Formula - Options section in the Method Explorer. **c** Mark the **Confirm with fragment ions** check box. **d** Click the **Use average fragment spectrum if spectral library not available** button. **e** Review the parameters for the **Fragment ion EIC qualification settings**. Enter `90` for the **Coelution score**. **f** Review the parameters for the **Fragment ion confirmation criteria**. Click the **Minimum number of qualified fragments** and enter `1`.	• The Fragment ion source can be either the MS/MS spectra in the PCDL library or the fragment spectrum from the high energy channels in the data file. • If it is the fragment spectrum, it is the average fragment spectrum across the elution profile of the precursor ion. • The **S/N ratio** is supposed to be at least 5. • If you click the **Minimum percent of qualified fragments** button and the **Number of most abundant ions from spectral library** is 5, then if the minimum percent is 75%, then 4 of the ions need to be qualified. (4/5 is 80% which is greater than 75%)

Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode

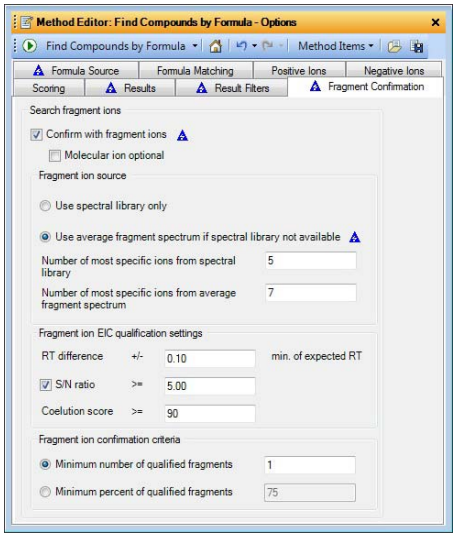
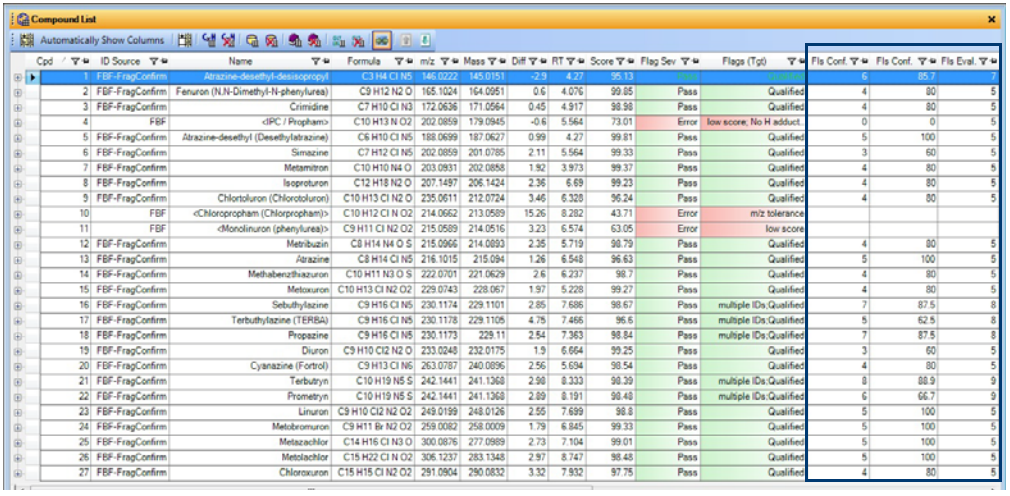
Steps	Detailed Instructions	Comments
		

Figure 91 The Find Compounds by Formula > Fragment Confirmation tab

2 Run the Find Compounds by Formula algorithm.	• Click **Find > Find Compounds by Formula**. • Click the button in the Method Editor window. • Right-click the Method Editor window and click **Find Compounds by Formula**.	• Fragment confirmation looks at overall retention time, signal-to-noise and coelution score.

Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode

Steps	Detailed Instructions	Comments
3 Review results in the Compound List window.	**a** Right-click the title of the Compound List window and click **Floating**. **b** Use the shortcut menu to add and remove columns from the table. **c** Review the **FIs Eval.** column. **d** Review the **FIs Conf.** column. **e** Review the **FIs Conf. %** column. **f** Review the values in the **Flags (Tgt)** column.	• **FIs** means Fragment Ions. • The first compound does not have a PCDL spectrum, so it uses the average fragment spectrum from the data file. The number of fragment ions evaluated is 7. • The number of fragment ions evaluated for the structural isomers is also greater than 5. • The **Flags (Tgt)** column shows the combined result from both the information from the low energy channel and from the MS/MS information from the high energy channel.



Compound List

Automatically Show Columns

Cpd	ID Source	Name	Formula	m/z	Mass	Diff	RT	Score	Flag Sev	Flags (Tgt)	FIs Conf.	FIs Conf.	FIs Eval.
1	FBF-FragConfirm	Atrazine-desethyl-desisopropyl	C3 H4 Cl N5	146.0222	145.0151	-2.9	4.27	95.13			6	85.7	7
2	FBF-FragConfirm	Fenuron (N,N-Dimethyl-N-phenylurea)	C9 H12 N2 O	165.1024	164.0951	0.6	4.076	99.85	Pass	Qualified	4	80	5
3	FBF-FragConfirm	Crimidine	C7 H10 Cl N3	172.0636	171.0564	0.45	4.917	98.98	Pass	Qualified	4	80	5
4	FBF	<IPC / Propham>	C10 H13 N O2	202.0859	179.0945	-0.6	5.564	73.01	Error	low score; No H adduct.	0	0	5
5	FBF-FragConfirm	Atrazine-desethyl (Desethylatrazine)	C6 H10 Cl N5	188.0699	187.0627	0.99	4.27	99.81	Pass	Qualified	5	100	5
6	FBF-FragConfirm	Simazine	C7 H12 Cl N5	202.0859	201.0785	2.11	5.564	99.33	Pass	Qualified	3	60	5
7	FBF-FragConfirm	Metamitron	C10 H10 N4 O	203.0931	202.0858	1.92	3.973	99.37	Pass	Qualified	4	80	5
8	FBF-FragConfirm	Isoproturon	C12 H18 N2 O	207.1497	206.1424	2.36	6.69	99.23	Pass	Qualified	4	80	5
9	FBF-FragConfirm	Chlortoluron (Chlorotoluron)	C10 H13 Cl N2 O	235.0611	212.0724	3.46	6.328	96.24	Pass	Qualified	4	80	5
10	FBF	<Chloropropham (Chlorpropham)>	C10 H12 Cl N O2	214.0662	213.0589	15.26	8.282	43.71	Error	m/z tolerance			
11	FBF	<Monolinuron (phenylurea)>	C9 H11 Cl N2 O2	215.0589	214.0516	3.23	6.574	63.05	Error	low score			
12	FBF-FragConfirm	Metribuzin	C8 H14 N4 O S	215.0966	214.0893	2.35	5.719	98.79	Pass	Qualified	4	80	5
13	FBF-FragConfirm	Atrazine	C8 H14 Cl N5	216.1015	215.094	1.26	6.548	96.63	Pass	Qualified	5	100	5
14	FBF-FragConfirm	Methabenzthiazuron	C10 H11 N3 O S	222.0701	221.0629	2.6	6.237	98.7	Pass	Qualified	4	80	5
15	FBF-FragConfirm	Metoxuron	C10 H13 Cl N2 O2	229.0743	228.067	1.97	5.228	99.27	Pass	Qualified	4	80	5
16	FBF-FragConfirm	Sebuthylazine	C9 H16 Cl N5	230.1174	229.1101	2.85	7.686	98.67	Pass	multiple IDs;Qualified	7	87.5	8
17	FBF-FragConfirm	Terbuthylazine (TERBA)	C9 H16 Cl N5	230.1178	229.1105	4.75	7.466	96.6	Pass	multiple IDs;Qualified	5	62.5	8
18	FBF-FragConfirm	Propazine	C9 H16 Cl N5	230.1173	229.11	2.54	7.363	98.84	Pass	multiple IDs;Qualified	7	87.5	8
19	FBF-FragConfirm	Diuron	C9 H10 Cl2 N2 O	233.0248	232.0175	1.9	6.664	99.25	Pass	Qualified	3	60	5
20	FBF-FragConfirm	Cyanazine (Fortrol)	C9 H13 Cl N6	263.0787	240.0896	2.56	5.694	98.54	Pass	Qualified	4	80	5
21	FBF-FragConfirm	Terbutryn	C10 H19 N5 S	242.1441	241.1368	2.98	8.333	98.39	Pass	multiple IDs;Qualified	8	88.9	9
22	FBF-FragConfirm	Prometryn	C10 H19 N5 S	242.1441	241.1368	2.89	8.191	98.48	Pass	multiple IDs;Qualified	6	66.7	9
23	FBF-FragConfirm	Linuron	C9 H10 Cl2 N2 O2	249.0199	248.0126	2.55	7.699	98.8	Pass	Qualified	5	100	5
24	FBF-FragConfirm	Metobromuron	C9 H11 Br N2 O2	259.0082	258.0009	1.79	6.845	99.33	Pass	Qualified	5	100	5
25	FBF-FragConfirm	Metazachlor	C14 H16 Cl N3 O	300.0876	277.0989	2.73	7.104	99.01	Pass	Qualified	5	100	5
26	FBF-FragConfirm	Metolachlor	C15 H22 Cl N O2	306.1237	283.1348	2.97	8.747	98.48	Pass	Qualified	5	100	5
27	FBF-FragConfirm	Chloroxuron	C15 H15 Cl N2 O2	291.0904	290.0832	3.32	7.932	97.75	Pass	Qualified	4	80	5

Figure 92 Compound List window with three new columns for fragment ions

Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode

Steps	Detailed Instructions	Comments
4 Review the other levels in the Compound List table.	• Expand the Compound List table for compound 19.	• Three fragment ions Qualified. The **Flags (FIs)** column shows if the fragment ion Qualified. If the fragment ion did not qualify, then the **Flags (FIs)** column shows the reason why the Fragment Ion did not qualify.

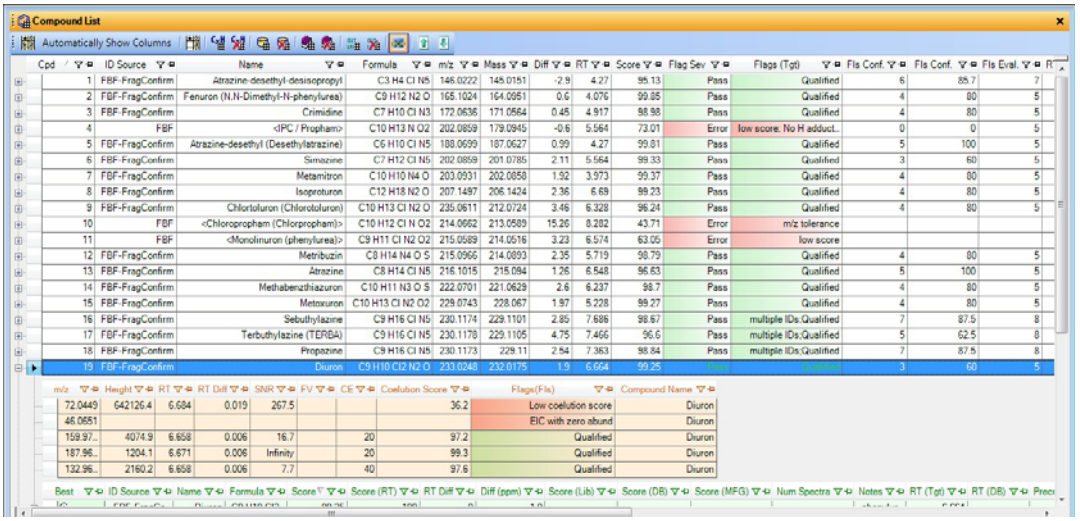


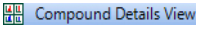
Figure 93 Compound List table including the Fragment Ion table

5 Save the method to *iii*All_Ions2, where "*iii*" are your initials.	**a** From the menu, click **Method > Save As**. **b** Type **iiiAll_Ions2.m**. **c** Click **Save**.	

Task 3. Review results in Compound Details View

The Compound Details View allows you to look at just one data file and to look at the individual compounds in that data file. In the Compound Details View, you can visually see how well fragment ions coeluted in the Coelution Plot.

Task 2. Review Fragment Confirmation results in Compound Details View

Steps	Detailed Instructions	Comments
1 Switch to **Compound Details View**.	**a** Complete the steps in "Task 2. Run Find by Formula on data acquired in All Ions MS/MS mode" on page 144. **b** Click the  button in the main toolbar. **c** Switch to different compounds in the Compound List window. You can click the arrow buttons in the Compound List toolbar, or click each row in the Compound List window, or press the arrow keys on the keyboard.	• Compound Chromatogram Results window shows individual ion traces for each fragment ion and the Coelution Plot. • Compound MS Spectrum Results shows the spectrum from the low energy channel. • Compound Fragment Spectrum Results window shows the average spectrum across all of the high energy channels.

Task 2. Review Fragment Confirmation results in Compound Details View

Steps	Detailed Instructions	Comments

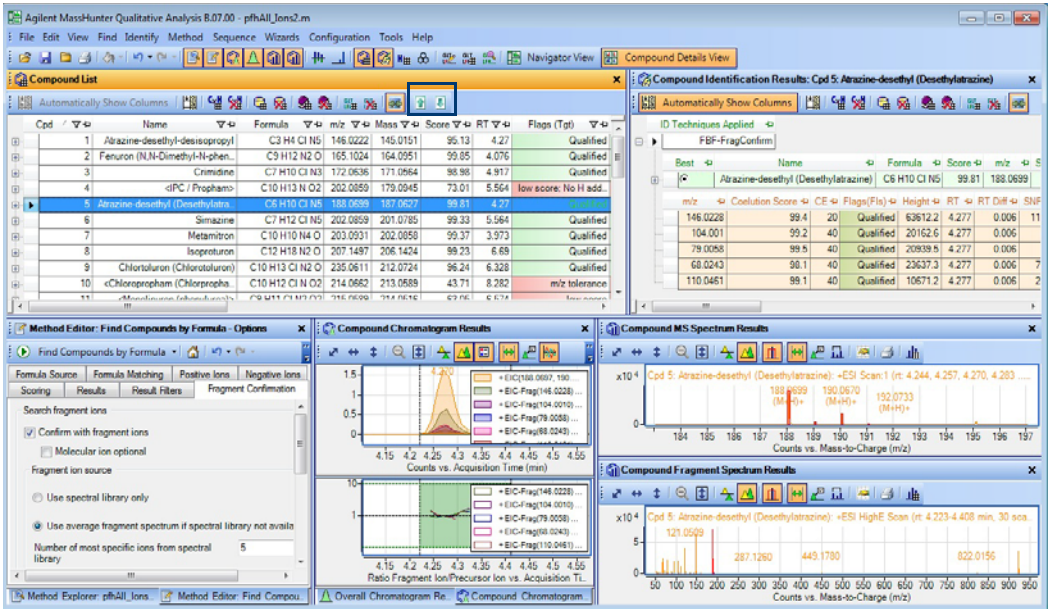


Figure 94 The Compound Details View

Task 2. Review Fragment Confirmation results in Compound Details View

Steps	Detailed Instructions	Comments
2 Review the results for **Cpd 3: Crimidine**.	**a** Click **Cpd 3: Crimidine** in the Compound List window. **b** Review the **Coelution Score**, the **CE,** the **Flags (Fls)**, and the **SNR** columns in the Compound Identification Results window. **c** Review the MS spectra in the Compound MS Spectrum Results window.	• Four out of five fragment ions are qualified for this compound. • The signal-to-noise (SNR) values for four of the five fragment ions is greater than 5. • The fragment ions do not all have the same Collision Energy. • The Compound MS Spectrum Results window contains the cleaned Find by Formula spectrum (annotated with the formula and the adduct ion) and the raw spectrum.

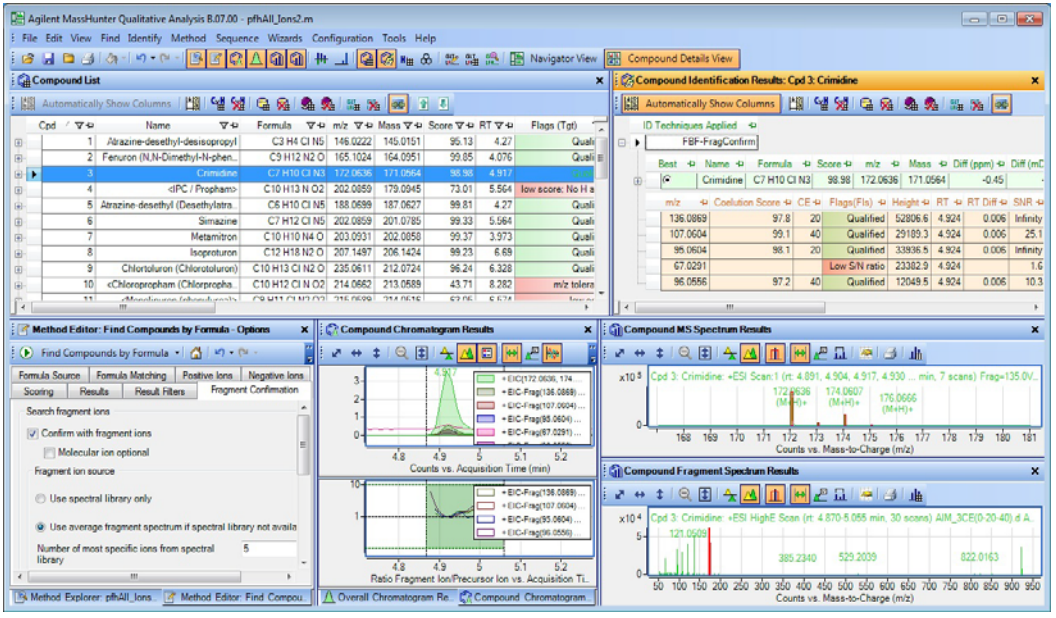


Figure 95 Compound 3 has one fragment ion with a low SNR

Task 2. Review Fragment Confirmation results in Compound Details View

Steps	Detailed Instructions	Comments
3 Review the other levels in the Compound Identification Results window.	**a** Right-click the title of the Compound Identification Results window and click **Floating**. **b** Expand the Compound Identification Results window for compound 2. **c** Review the result for **Score (MS)**. **d** Review the **Coelution Score** values which are over 99 % for the two Qualified fragment ions. The **Coelution Score** does not directly affect the **Score (Tgt)** value.	• The **Score (MS)** is determined from the **Score (mass)**, the **Score (iso.abund.)**, and the **Score (iso. spacing)** values. • The **Score (Tgt)** is 99.85 based on the **Score (RT)** and the **Score (MS)**. • This compound is qualified to be set up for a targeted analysis on a TOF or Q-TOF system with fragment ion confirmation in the Quantitative Analysis program.

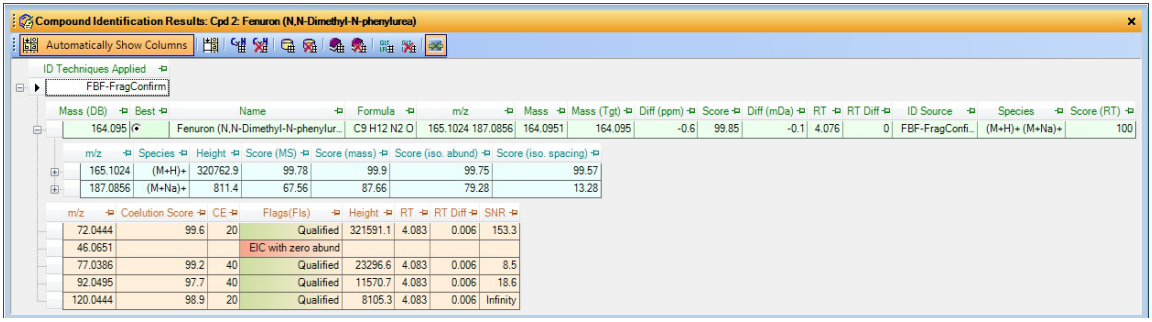


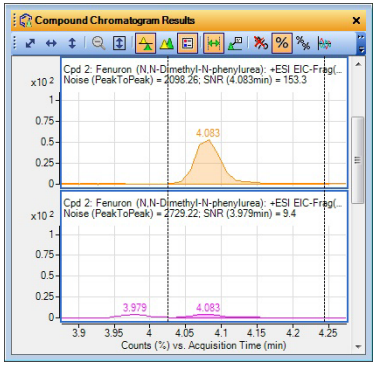
Figure 96 Compound List table including the Fragment Ion table

4 Review the results in the Compound Fragment Spectrum Results window.	**a** Click the button in the Compound Fragment Spectrum Results window. **b** Zoom in to the *m/z* range 30 to 190. **c** Observe the qualified fragment ions are annotated in green.	• Some fragment ions are not labeled because they are close to other labeled peaks. • The spectrum is an averaged spectrum which is composed of the two CE values: 20 and 40 volts.

Figure 97 Compound Fragment Spectrum Results window

Task 2. Review Fragment Confirmation results in Compound Details View

Steps	Detailed Instructions	Comments
5 Review the results in the Compound Chromatogram Results window.	**a** Click the button in the Compound Chromatogram Results toolbar. **b** Click the button. **c** Scroll through the EIC for each of the fragment ions and the EIC from the precursor ions. **d** Click the button in the Compound Chromatogram Results toolbar to show the Coelution Plot pane. **e** Click the button to overlay the chromatograms. **f** Click the % button to scale the chromatograms. **g** In the legend, find the color that identifies each of the fragment ions and the EIC for the precursor ions in the Coelution Plot pane.	• EIC-Frag is included in the title for each of the fragment ion chromatograms; these EICs are extracted from the best Collision Energy voltage channel. • In the graph of the overlaid chromatograms, you can visually see that the chromatograms are coeluting which is also reflected in the high Coelution Scores. • The Coelution Score compares retention times of the fragment ions and the precursor ion, the peak width and the peak symmetry. • The ion ratio across the majority of the chromatographic peak should be close to 1 if the fragments are coeluting. • At the start and end of the Coelution Plot, you see some larger values because some of the noise gets exaggerated.



In the Coelution Plot, the black line drawn at 1 shows the value that a perfectly coeluting peak has. Each of the other five lines shows the abundance ratio of the normalized fragment ion to the precursor across the time range of the precursor. The green area shows you the confidence area.

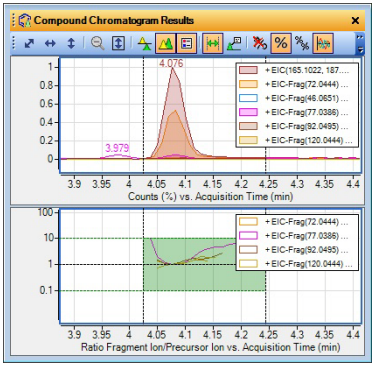


Figure 98 Compound Chromatogram Results window

6 Close the data file and return to Navigator View.	**a** Click **File > Close Data File**. **b** Click the Navigator View button in the main toolbar.	

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.

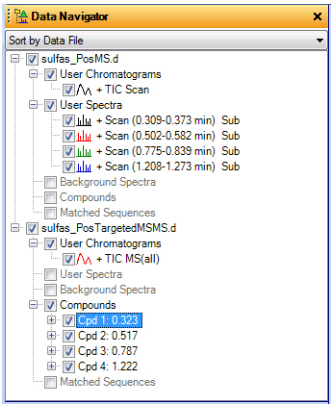
Toolbar Icon	Window
	Data Navigator window Method Explorer window Method Editor window
	Chromatogram Results window Spectrum Preview window MS Spectrum Results window Different Resutls window Deconvolution Results window Deconvolution Mirror Plot window UV Spectrum Results window
	Integration Peak List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

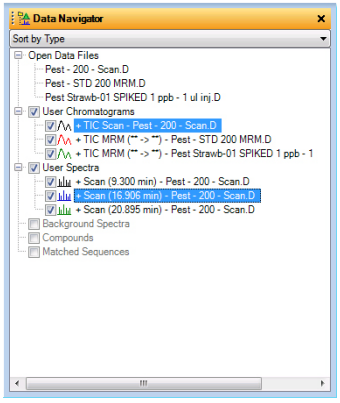
Toolbar Icon	Window
	Compound List window Compound Identification Results window Spectrum Identification Results window MS/MS Formula Details window Structure Viewer window Sample Information window Sequence Editor window

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

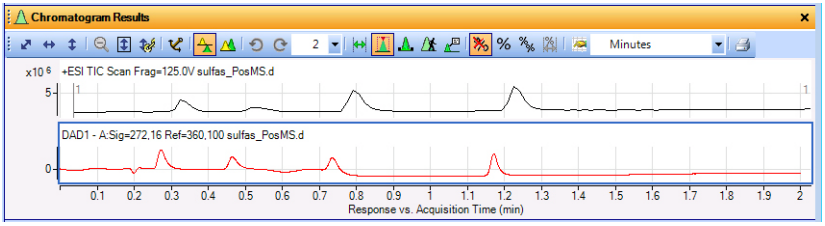
You can perform the following operations on an MS or MS/MS spectrum or on a selected region of an MS or MS/MS spectrum by using the menu items:

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)
Use the Deconvolute (Resolved Isotope) algorithm	Spectra > Deconvolute (Resolved Isotope)

Action	Menu Item
Search Library	Identify > Search Library for Spectra or Spectra > Search Library for Spectra

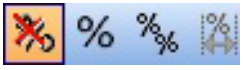
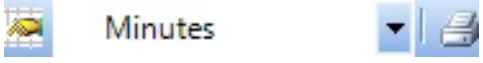
Work with chromatographic visual data

Chromatogram Results Window



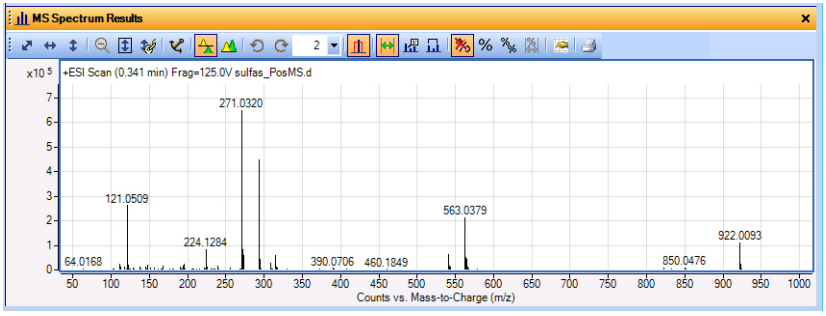
Chromatogram Results Tools

Toolbar Icon	Action
Zoom tools 	• Autoscale X-axis and Y-axis • Autoscale X-axis • Autoscale Y-axis • Unzoom • Autoscale Y-axis during Zoom • Linked Y-axis mode

Toolbar Icon	Action
	• **Anchor chromatogram** - the current chromatogram is always visible until you click the **Clear Anchor** command. • **List mode** - chromatograms are drawn with each chromatogram having a separate Y-axis. • **Overlay mode** - chromatograms are drawn with the same X-axis and the same Y-axis • **Switches to previous plot**. This button is only available in Overlay mode. • **Switches to next plot**. This button is only available in Overlay mode. • **Number of spectra to show** at the same time before adding a scroll bar.
Select tools in order  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. • **Peak Select** – When On, you can select spectrum of an integrated peak at apex. • **Manual Integration** – When On, you can integrate interactively. • **Walk Chromatogram** – When On, you can see individual spectra as you click each point or use the left and right arrows on the keyboard. • **Annotation** – When On, you can add image and text annotations to the chromatograms.
Normalization tools 	• Stops normalizing chromatograms • Normalizes all chromatograms to the largest peak in any of the chromatograms • Normalizes all chromatograms to the largest peak in itself • Normalizes each chromatogram to the highest peak within the selected range
Other tools 	• Opens **Chromatogram Display Options** dialog box • Sets the units used to display the chromatograms • Prints the displayed chromatograms

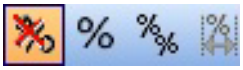
Work with spectral visual data

MS Spectrum Results Window



MS Spectrum Results Tools

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

Toolbar Icon	Action
Select tools in order  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 • **Annotation** – When **On**, you can add image and text annotations to the spectra. • **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 	• Stops normalizing spectra • Normalizes all spectra to the largest peak in any of the spectra • Normalizes all spectra to the largest peak in itself • Normalizes each spectra to the highest peak within the selected range
Other tools 	• Opens **MS and MS/MS Spectra Display Options** dialog box • 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.

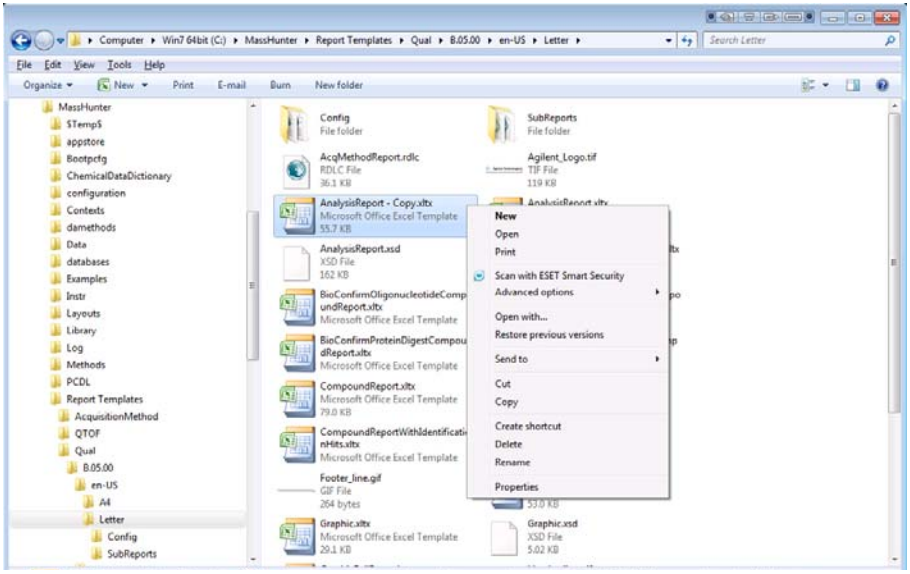
Workflow	Method	Layout	Method Editor Section
General	default.m	Default.xml	None
BioConfirm Intact Protein	BioConfirm IntactProtein-Default.m	BioConfirm-IntactProtein-MaximumEntropy-Default.xml	BioConfirm Workflow
BioConfirm High Mass Intact Protein	BioConfirm IntactProtein HighMass Default.m	BioConfirm IntactProtein LMFE.xml	BioConfirm Workflow
BioConfirm Small Oligonucleotides	BioConfirmOligo nucleotideSmall.m	BioConfirmOligo-nucleotide.xml	BioConfirm Workflow
BioConfirm Large Oligonucleotides	BioConfirmOligo nucleotideLarge-Default.m	BioConfirmOligo-nucleotide.xml	BioConfirm Workflow
BioConfirm Protein Digest	BioConfirmProtein Digest-Default.m	BioConfirm ProteinDigest.xml	BioConfirm Workflow
BioConfirm Synthetic Peptide	BioConfirmSynthetic Peptide-Default.m	BioConfirm SyntheticPeptide.xml	BioConfirm Workflow
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

Workflow	Method	Layout	Method Editor Section
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

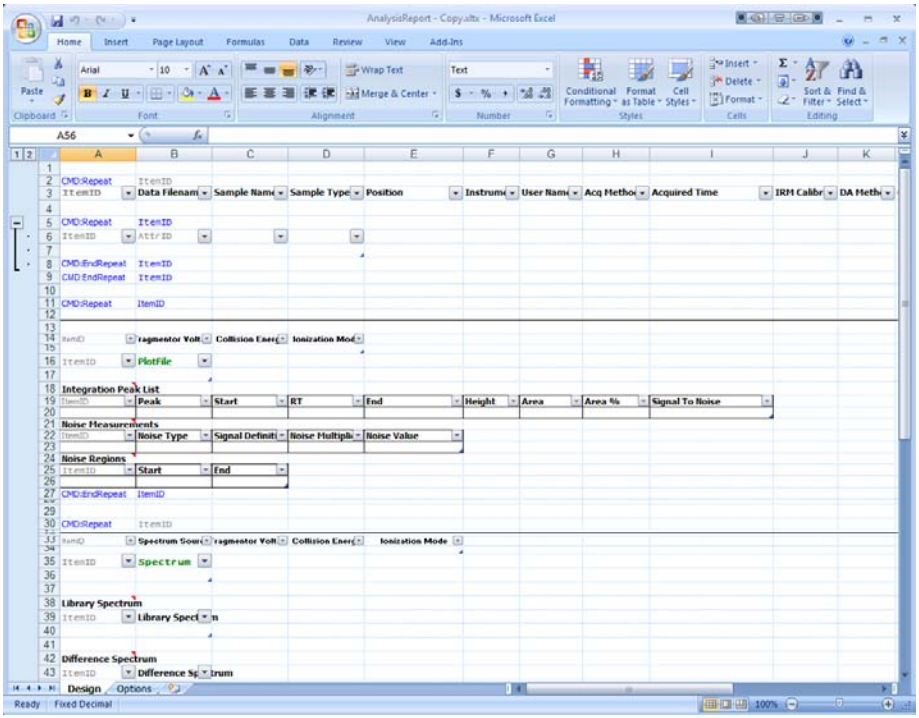
Please refer to either the online Help for the MassHunter Report Designer Add-in, the Report Designer Familiarization Guide or the Reporting Training DVD for detailed information on how to modify a report template. The following steps give you a quick look at what it means to customize a template.

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 LC/MS data.

Revision A, September 2014

G3336-90023

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